# **NISTIR 8237**

# Health Assessment Measurements Quality Assurance Program: Exercise 1 Final Report

Charles A. Barber Bruce A. Benner, Jr. Jeanice Brown Thomas Carolyn Q. Burdette Johanna Camara Stephen Long Jacolin A. Murray Melissa M. Phillips Benjamin J. Place Catherine A. Rimmer Laura J. Wood Lee Yu Siva K.R. Chinthalapati Susan S.-C. Tai

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#### ABSTRACT

The NIST Health Assessment Measurements Quality Assurance Program (HAMQAP) was launched in collaboration with the National Institutes of Health (NIH) Office of Dietary Supplements (ODS) in 2017. HAMQAP was established to enable laboratories to improve the accuracy of measurements for demonstration of compliance with various regulations by measuring samples that represent human intake (e.g., foods, dietary supplements, tobacco) and samples that represent human output (e.g., blood, serum, plasma, urine). Analytes are paired where possible to represent the full spectrum of health assessment. Exercise 1 of this program offered the opportunity for laboratories to assess their in-house measurements of nutritional elements (iron and transferrin), contaminants (arsenic species and mycotoxins), water-soluble vitamins (vitamin B<sub>12</sub>), fat-soluble vitamins (vitamin D and metabolites), fatty acids, and botanical marker compounds (actein and 27-deoxyactein) in foods, botanical or dietary supplement ingredients, and human serum, plasma, or urine.

### **INTRODUCTION**

The Health Assessment Measurements Quality Assurance Program (HAMQAP) was formed in 2017, in part as a collaboration with the National Institutes of Health (NIH) Office of Dietary Supplements (ODS) and represents ongoing efforts at NIST that were supported previously via historical quality assurance programs (QAPs), including the Dietary Supplements QAP (DSQAP), Micronutrients Measurement QAP (MMQAP), Fatty Acids in Human Serum QAP (FAQAP), and Vitamin D Metabolites QAP (VitDQAP).

HAMQAP offers the opportunity for laboratories to assess their in-house measurements of nutritional and toxic elements, fat- and water-soluble vitamins, fatty acids, active and/or marker compounds, and contaminants in samples distributed by NIST. Samples that represent human intake (e.g., food, dietary supplements, tobacco) are paired with samples that represent human output (e.g., blood, serum, plasma, urine)<sup>1</sup>, where possible, to represent the full spectrum of intake and metabolism for health assessment. Reports and certificates of participation are provided and may be used to demonstrate compliance with the current good manufacturing practices (cGMPs) or to fulfill requirements established by related accreditation bodies. In addition, NIST and the HAMQAP assist the ODS Analytical Methods and Reference Materials program (AMRM) at the NIH in supporting the development and dissemination of analytical tools and reference materials. In the future, results from HAMQAP exercises could be used by ODS and NIST to identify problematic matrices and analytes for which official methods of analysis would benefit the dietary supplements and clinical communities.

NIST has decades of experience in the administration of QAPs, and the HAMQAP builds on the approach taken by the previous DSQAP by providing a wide range of matrices and analytes. The

<sup>&</sup>lt;sup>1</sup> Human intake samples were intended for research use only and not for human consumption. Human output samples were humansource biohazardous materials capable of transmitting infectious disease. Participants were advised to handle these materials at the Biosafety Level 2 or higher as recommended for any potentially infectious human source materials by the Centers for Disease Control and Prevention (CDC) Office of Safety, Health, and Environment and the National Institutes of Health (NIH). The supplier of the source materials for the blood, serum, and/or plasma used to prepare the sample materials found the materials to be nonreactive when tested for hepatitis B surface antigen (HBsAg), human immunodeficiency virus (HIV), hepatitis C virus (HCV), and human immunodeficiency virus 1 antigen (HIV-1Ag) by Food and Drug Administration (FDA) licensed tests.

HAMQAP design emphasizes emerging and challenging measurements in the dietary supplement, food, and clinical matrix categories. Participating laboratories are interested in testing in-house methods on a wide variety of challenging, real-world matrices to demonstrate that their performance is comparable to that of the community and that their methods provide accurate results. In areas where few standard methods have been recognized, the HAMQAP offers a unique tool for assessment of the quality of measurements and provides feedback about performance that can assist participants in improving laboratory operations.

This report summarizes the results from the first exercise of the HAMQAP. Seventy-one laboratories responded to the dietary intake portion and forty-seven laboratories responded to the human metabolites portion of the call for participants distributed in November 2017 (see table below). The human metabolites contaminants study was cancelled prior to shipment due to low enrollment. Samples were shipped to participants in February 2018 and results were returned to NIST by May 2018. This report contains the final data and information that was disseminated to the participants in September 2018.

| Study Group   | <b>Dietary Intake Study</b>           | Human Metabolites Study          |  |  |  |  |
|---------------|---------------------------------------|----------------------------------|--|--|--|--|
| Nutritional   | Iron                                  | Iron, Transferrins               |  |  |  |  |
| Elements      | Multivitamin, Cereal                  | Human Serum                      |  |  |  |  |
| Toxic         | Arsenic, Arsenic Species              | Arsenic, Arsenic Species         |  |  |  |  |
| Elements      | Tobacco <sup>†</sup> , Kelp           | Human Urine                      |  |  |  |  |
| Water-Soluble | Vitamin B <sub>12</sub>               | Vitamin B <sub>12</sub>          |  |  |  |  |
| Vitamins      | Multivitamin, Cereal                  | Human Serum                      |  |  |  |  |
| Fat-Soluble   | Vitamin D, Vitamin D Metabolites      | Vitamin D, Vitamin D Metabolites |  |  |  |  |
| Vitamins      | Liver, Cod Liver Oil                  | Human Serum                      |  |  |  |  |
| Eatty A aida  | Fatty Acids                           | Fatty Acids                      |  |  |  |  |
| Fatty Actus   | Solution, Fish Oil, Cod Liver Oil     | Human Plasma                     |  |  |  |  |
| Natural       | Actein, 27-Deoxyactein                | Not offered                      |  |  |  |  |
| Products      | Black Cohosh Rhizome, Leaves, Extract | Not offered                      |  |  |  |  |
| Contominants  | Mycotoxins                            | <b>VOC Metabolites</b> *         |  |  |  |  |
| Containmants  | Corn                                  | Human Urine                      |  |  |  |  |

<sup>†</sup> Study on tobacco was not funded by the NIH-ODS

\* Cancelled due to low enrollment.

Each study group is summarized in a series of tables, figures, and text, and reported by section. Within the section, each study is summarized individually, and then conclusions are drawn for the entire study group when possible.

#### **OVERVIEW OF DATA TREATMENT AND REPRESENTATION**

Individualized data tables and certificates are provided to the participants that have submitted data in each study, in addition to this report. Examples of the data tables using NIST data are also included in each section of this report. Community tables and graphs are provided using randomized laboratory codes, with identities known only to NIST and individual laboratories. The statistical approaches are outlined below for each type of data representation.

#### **Statistics**

Data tables and graphs throughout this report contain information about the performance of each laboratory relative to that of the other participants in this study and relative to a target around the expected result, if available. All calculations are performed in PROLab Plus (QuoData GmbH, Dresden, Germany).<sup>2</sup> The consensus mean and standard deviation are calculated according to the robust Q/Hampel method outlined in ISO 13528:2015(E), Annex C.<sup>3</sup>

#### Individualized Data Table

The data in this table is individualized to each participating laboratory and is provided to allow participants to directly compare their data to the summary statistics (consensus or community data as well as NIST certified, reference, or estimated values, when available). The upper left of the data table includes the randomized laboratory code. Example individualized data tables are included in this report; participating laboratories received uniquely coded individualized data tables in a separate distribution.

Section 1 of the data table (*Your Results*) contains the laboratory results as reported, including the mean and standard deviation when multiple values were reported. A blank indicates that NIST does not have data on file for that laboratory for a particular analyte or matrix. An empty box for standard deviation indicates that a single value or a value below the limit of quantification (LOQ) for the participant was reported and therefore that value was not included in the calculation of the consensus data.<sup>3</sup> Example individualized data tables are included in this report using NIST data in Section 1 to protect the identity and performance of participants.

Also included in Section 1 are two Z-scores. The first Z-score,  $Z'_{comm}$ , is calculated with respect to the community consensus value, taking into consideration bias that may result from the uncertainty in the assigned consensus value, using the consensus mean (x\*), consensus standard deviation (s\*), and standard deviation for proficiency assessment (SDPA,  $\sigma_{PT}^2$ ) determined from the Q/Hampel estimator:

$$Z'_{\rm comm} = \frac{x_i - x^*}{\sqrt{\sigma_{PT}^2 + s^*}}$$

The second Z-score,  $Z_{\text{NIST}}$ , is calculated with respect to the target value (NIST certified, reference, or estimated value, when available), using  $x_{\text{NIST}}$  and  $2^*U_{95}$  (the expanded uncertainty on the certified or reference value,  $U_{95}$ , or twice the standard deviation of NIST or other measurements):

$$Z_{\text{NIST}} = \frac{x_i - x_{\text{NIST}}}{2 \cdot U_{95}}$$

or

$$Z_{\text{NIST}} = \frac{x_i - x_{\text{NIST}}}{2 \cdot U_{\text{NIST}}}.$$

<sup>&</sup>lt;sup>2</sup> Certain commercial equipment, instruments or materials are identified in this certificate to adequately specify the experimental procedure. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that the materials or equipment identified are necessarily the best available for the purpose.

<sup>&</sup>lt;sup>3</sup> ISO 13528:2015(E), Statistical methods for use in proficiency testing by interlaboratory comparisons, pp. 53-54.

The significance of the *Z*-score and *Z*'-score is as follows:

- |Z| < 2 indicates that the laboratory result is considered to be within the community consensus range (for  $Z'_{\text{comm}}$ ) or NIST target range (for  $Z_{\text{NIST}}$ ).
- 2 < |Z| < 3 indicates that the laboratory result is considered to be marginally different from the community consensus value (for  $Z'_{comm}$ ) or NIST target value (for  $Z_{NIST}$ ).
- |Z| > 3 indicates that the laboratory result is considered to be significantly different from the community consensus value (for  $Z'_{comm}$ ) or NIST target value (for  $Z_{NIST}$ ).

Section 2 of the data table (*Community Results*) contains the consensus results, including the number of laboratories reporting more than a single quantitative value for a given analyte, the mean value determined for each analyte, and a robust estimate of the standard deviation of the reported values.<sup>3</sup> Consensus means and standard deviations are calculated using the laboratory means; if a laboratory reported a single value, the reported value is not included.<sup>3</sup> Additional information on calculation of the consensus mean and standard deviation can be found in the previous section.

Section 3 of the data table (*Target*) contains the target values for each analyte, when available. When possible, the target value is a certified value, a reference value, or a value determined at NIST. Certified values and the associated expanded uncertainty  $(U_{95})$  have been determined with two independent analytical methods at NIST, one Joint Committee for Traceability in Laboratory Medicine (JCTLM)-recognized Reference Measurement Procedure (RMP) at NIST, or by combination of a single method at NIST and results from collaborating laboratories. Reference values are assigned using NIST values obtained from the average and standard deviation of measurements made using a single analytical method at NIST or by measurements obtained from collaborating laboratories. For both certified and reference values, at least six samples have been tested and duplicate preparations from the sample package have been included, allowing the uncertainty to encompass variability due to inhomogeneity within and between packages. For samples in which a NIST certified or reference value is not available, the analytes may be measured at NIST using a validated method or data from a partner laboratory to establish a NIST-assessed value. The NIST-assessed value represents the mean of at least three replicates. For materials acquired from another interlaboratory study or proficiency testing program, the consensus value and uncertainty from the completed round is used as the target range. Within each section of this report, the exact methods for determination of the study target values are outlined in detail.

#### Summary Data Table

This data table includes a summary of all reported data for a particular analyte in a particular study. Participants can compare the raw data for their laboratory to data reported by the other participating laboratories or to the consensus data. A blank indicates that the laboratory signed up and received samples for that particular analyte and matrix, but NIST does not have data on file for that laboratory. Data points highlighted in red have been flagged as potential outliers (e.g., difference from reference value, Grubb and/or Cochran) by the NIST software package. The standard deviation (SD) for the target value in this table is the uncertainty ( $U_{NIST}$ ) around the target value.

#### Graphs

#### Data Summary View (Method Comparison Data Summary View)

In this view, individual laboratory data (circles) are plotted with the individual laboratory standard deviation (rectangles). Laboratories reporting values below the method quantitation limit are shown in this view as downward triangles beginning at the limit of quantitation (LOQ), reported as quantitation limit (QL) on the graphs. Laboratories reporting values as "below LOQ" can still be successful in the study if the target value is also below the laboratory LOQ. The black solid line represents the consensus mean, and the green shaded area represents the 95 % confidence interval for the consensus mean, based on the standard error of the consensus mean. The uncertainty in the consensus mean is calculated using the equation below, based on the repeatability standard deviation ( $s_r$ ), the reproducibility standard deviation ( $s_R$ ), the number of participants reporting data, and the average number of replicates reported by each participant. The uncertainty about the consensus mean is independent of the range of tolerance. Where appropriate, two consensus means may be calculated for the same sample if bimodality is identified in the data. In this case, two consensus means and ranges will be displayed in the data summary view.

$$u_{mean} = \sqrt{\frac{s_R^2 - s_r^2}{n_{participants}} + \frac{s_R^2}{n_{participants} \times n_{Average \ Number \ of \ Replicates \ per \ Participant}}}$$

The red shaded region represents the target zone for "acceptable" performance, which encompasses the NIST target value bounded by twice its uncertainty ( $U_{95}$  or  $U_{\text{NIST}}$ ). The solid red lines represent the range of tolerance (values that result in an acceptable Z' score,  $|Z'| \leq 2$ ). If the lower limit is below zero, the lower limit has been set to zero. In this view, the relative locations of individual laboratory data and consensus zones with respect to the target zone can be compared easily. In most cases, the target zone and the consensus zone overlap, which is the expected result. The major program goals are to reduce the size of the consensus zone and center the consensus zone about the target value. Analysis of an appropriate reference material as part of a quality control scheme can help to identify sources of bias for laboratories reporting results that are significantly different from the target zone. In the case in which a method comparison is relevant, different colored data points may be used to indicate laboratories that used a specific approach to sample preparation, analysis, or quantitation.

#### Sample/Sample Comparison View

In this view, the individual laboratory results for one sample (NIST SRM with a certified, reference, or NIST-determined value) are compared to the results for another sample (another NIST SRM with a more challenging matrix, a commercial sample, etc.). The solid red box represents the target zone for the first sample (x-axis) and the second sample (y-axis). The dotted blue box represents the consensus zone for the first sample (x-axis) and the second sample (y-axis). The dotted blue box represents the consensus zone for the first sample (x-axis) and the second sample (y-axis). The axes of this graph are centered about the consensus mean values for each sample or control, to a limit of twice the range of tolerance (values that result in an acceptable Z' score,  $|Z'| \leq 2$ ). Depending on the variability in the data, the axes may be scaled proportionally to better display the individual data points for each laboratory. In some cases, when the consensus and target ranges have limited overlap, the solid red box may only appear partially on the graph. If the variability in the data is high (greater than 100 % relative standard deviation (RSD)), the dotted blue box may also only appear partially on the graph. These views emphasize trends in the data that may indicate

potential calibration issues or method biases. One program goal is to identify such calibration or method biases and assist participants in improving analytical measurement capabilities. In some cases, when two equally challenging materials are provided, the same view (sample/sample comparison) can be helpful in identifying commonalities or differences in the analysis of the two materials.

#### **SECTION 1: NUTRITIONAL ELEMENTS (Iron and/or Transferrins)**

#### Study Overview

In this study, participants were provided with four NIST SRMs, SRM 3280 Multivitamin/Multielement Tablets and SRM 3233 Fortified Breakfast Cereal for dietary intake, and SRM 909c Frozen Human Serum and SRM 968d Fat-Soluble Vitamins, Carotenoids, and Cholesterol in Human Serum for human metabolites. Participants were asked to use in-house analytical methods to determine the mass fraction of iron in each of the dietary intake matrices, and iron and transferrin (Tf) in each of the human metabolites matrices. Transferrins are iron-binding proteins that control the level of free iron in biological fluids, therefore measurement of transferrin can inform interpretation of human iron health status.

#### **Dietary Intake Sample Information**

Multivitamin. Participants provided were with three bottles, each containing 30 multivitamin/multielement tablets. Before use, participants were instructed to grind all tablets within a bottle, mix the resulting powder thoroughly, and to use a sample size of at least 0.2 g. Participants were asked to store the material at controlled room temperature, 10 °C to 30 °C, and to prepare one sample and report one value from each bottle provided. The approximate analyte level was not reported to participants prior to the study. The certified value for iron in SRM 3280 was determined at NIST using prompt gamma activation analysis (PGAA), instrumental neutron activation analysis (INAA), X-ray fluorescence spectrometry (XRF), and results from collaborating laboratories. The certified value and uncertainty for iron is provided in the table below, both on a dry-mass basis and on an as-received basis accounting for moisture of the material (1.37 %).

|           | Certified Mass Fraction in SRM 3280 (mg/g) |                     |  |  |  |  |
|-----------|--|---------------------|--|--|--|--|
| Analyte   | <u>(dry-mass basis)</u>                    | (as-received basis) |  |  |  |  |
| Iron (Fe) | $12.35 \pm 0.91$                           | $12.18 ~\pm~ 0.90$  |  |  |  |  |

*Cereal.* Participants were provided with one bottle containing 60 g of ground cereal. Before use, participants were instructed to thoroughly mix the contents by rotating and/or rolling the bottle prior to removal of a test sample for analysis, and to use a sample size of at least 0.5 g. Participants were asked to store the material at controlled room temperature, 10 °C to 30 °C, and to prepare three samples and report three values from the single bottle provided. The approximate analyte level was not reported to participants prior to the study. The certified value for iron in SRM 3233 was determined at NIST using inductively coupled plasma optical emission spectrometry (ICP-OES), INAA, and results from collaborating laboratories. The certified value and uncertainty for iron is provided in the table below, both on a dry-mass basis and on an as-received basis accounting for moisture of the material (1.7 %).

|           | Certified Mass Fractio  | <u>n in SRM 3233 (mg/g)</u> |
|-----------|-------------------------|-----------------------------|
| Analyte   | <u>(dry-mass basis)</u> | (as-received basis)         |
| Iron (Fe) | $0.766 ~\pm~ 0.036$     | $0.753 ~\pm~ 0.035$         |

## Dietary Intake Study Results

- Forty-five laboratories enrolled in this study and received samples to measure iron. Thirtythree laboratories reported results for the multivitamin/multielement tablets (73 % participation) and 32 laboratories reported results for the cereal (71 % participation).
- The consensus mean for iron in the multivitamin was within the target range, while the consensus mean for iron in cereal was below the target range. The between-laboratory variability was good for both the multivitamin and the cereal (13 % and 16 % relative standard deviation (RSD), respectively).
- Most laboratories did not specify sample preparation methods or analytical methods used.

## Dietary Intake Technical Recommendations

The following recommendations are based on results obtained from the participants in this study.

- As shown in **Figure 1-3**, many laboratories reported data for iron that was within the NIST target range for the multivitamin sample but was lower than the NIST target range for cereal. **Figures 1-1 and 1-2** also show that over half of the laboratories reported values within the target range for the multivitamin but fewer laboratories overlap the NIST target range for the cereal.
  - For laboratories whose results follow this trend (i.e., reported the correct value for one sample but a low or high value for the second sample), the cause may be differences in digestion difficultly between the two samples. The use of a small amount of HF and an increased temperature may be needed to achieve a complete digestion.
  - A matrix interference may be present in either one or both samples. The use of an internal standard may reduce the impact of matrix interferences.
  - A linear calibration curve which encompasses the expected sample concentration values should be used for calculations. This curve should include both the lowest and highest expected concentration values of the sample solutions. Extrapolation of results beyond calibration curves may result in the low values seen in cereal.
  - Since the levels of iron are lower in the cereal, it is important to know the LOQ, and prepare enough calibration standards close to the low levels expected in the solutions for the cereal.
  - Most laboratories had good sample-to-sample variability (< 6 % RSD for both the multivitamin and cereal). Difficulty in the digestion of samples typically will cause increased variability between samples.
- For both the multivitamin and cereal, a few laboratories reported data significantly outside of the target and consensus ranges. The use of appropriate quality assurance samples to establish that a method is in control and performing correctly prior to running unknown samples may reduce the likelihood of outlying data. Quality assurance samples can be commercially available reference materials (CRMs, SRMs, or RMs) or materials prepared in-house.
- All results should be checked closely for calculation errors and to be sure that results are reported in the requested units.

**Table 1-1.** Individualized data summary table (NIST) for iron in cereal and multivitamin.

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|   |  | 1 1000000         | 1                                    |                | (210000) 10     |                   |               |                   |            |                   |             |         |
|---|--|-------------------|--------------------------------------|----------------|-----------------|-------------------|---------------|-------------------|------------|-------------------|-------------|---------|
| Lab Code:       NIST       1. Your Results       2. Community Results |  |                   |                                      |                |                 |                   |               |                   | 3. Ta      | get               |             |         |
| Analyte   | Sample                                     | Units             | x <sub>i</sub>                       | s <sub>i</sub> | $Z'_{\rm comm}$ | $Z_{\text{NIST}}$ | N             | <i>x</i> *        | <i>s</i> * | x <sub>NIST</sub> | U           |         |
| Iron  | SRM 3233 Fortified Breakfast Cereal        | mg/g              | 0.753                                | 0.018          |                 |                   | 34            | 0.70              | 0.11       | 0.753             | 0.035       |         |
| Iron  | SRM 3280 Multivitamin/Multielement Tablets | mg/g              | 12.20                                | 0.45           |                 |                   | 34            | 12.1              | 1.6        | 12.20             | 0.90        |         |
| $x_i$ Mean of reported values   |  |                   |                                      |                | Ν               | Number of q       | uantitative   | x <sub>NIST</sub> | NIST-asses | sed value         |             |         |
| $s_i$ Standard deviation of reported values                           |  |                   |                                      |                |                 | s                 | values report | ed                | U          | expanded un       | certainty   |         |
|   |  | $Z'_{\rm comm}$   | m Z'-score with respect to community |                |                 | <i>x</i> *        | Robust mean   | of reported       |            | about the NI      | ST-assessed | i value |
| consensus   |  |                   |                                      |                |                 | values            |               |                   |            |                   |             |         |
|   |  | $Z_{\text{NIST}}$ | Z-score with                         | respect to     | NIST value      | <i>s</i> *        | Robust stand  | lard deviation    |            |                   |             |         |

#### HAMQAP Exercise 1 - Nutritional Elements (Dietary Intake)

**Table 1-2.** Data summary table for iron in multivitamin and cereal. Data points highlighted in red have been flagged as potential outliers (e.g., difference from reference value, Grubb and/or Cochran) by the NIST software package.

|            |        | Iron        |                     |             |  |              |          |            |             |         |       |
|------------|--------|-------------|---------------------|-------------|--|--------------|----------|------------|-------------|---------|-------|
|            |        | SRM 328     | itamin/Mu<br>(mg/g) | ıltielement | s SRM 3233 Fortified Breakfast Cereal (mg/g) |              |          |            |             |         |       |
|            | Lab    | A B C       |                     |             | Avg  | SD           | А        | В          | C           | Avg     | SD    |
|            | Target |             |                     |             | 12.18  | 0.90         |          |            |             | 0.753   | 0.035 |
|            | A001   |             |                     |             |  |              |          |            |             |         |       |
|            | A002   |             |                     |             |  |              |          |            |             |         |       |
|            | A003   |             |                     |             |  |              |          |            |             |         |       |
|            | A006   | 11.20       | 10.90               | 10.60       | 10.90  | 0.30         | 0.558    | 0.429      | 0.440       | 0.476   | 0.072 |
|            | A007   | 14.04       | 14.31               | 14.20       | 14.18  | 0.14         | 0.923    | 0.925      | 0.872       | 0.907   | 0.030 |
|            | A008   | 12.49       | 11.75               | 12.13       | 12.12  | 0.37         | 0.691    | 0.702      | 0.702       | 0.698   | 0.006 |
|            | A010   | 11.07       | 10.98               | 11.21       | 11.09  | 0.12         | 0.755    | 0.716      | 0.698       | 0.723   | 0.029 |
|            | A011   |             |                     |             |  |              |          |            |             |         |       |
|            | A014   |             |                     |             |  |              |          |            |             |         |       |
|            | A015   | 10.04       | 12.20               | 12 - 60     | 10.01  | 0.00         | 0 7 10   | 0 7 40     | 0 7 10      | 0 = 1 = | 0.010 |
|            | A025   | 12.06       | 12.20               | 12.68       | 12.31  | 0.33         | 0.740    | 0.760      | 0.740       | 0.747   | 0.012 |
|            | A026   | 13.00       | 13.40               | 13.90       | 13.43  | 0.45         | 0.797    | 0.792      | 0.796       | 0.795   | 0.003 |
|            | A027   | 8.26        | 8.34                | 8.44        | 8.35   | 0.09         | 0.770    | 0.700      | 0.702       | 0.770   | 0.007 |
|            | A028   | 12.50       | 12.40               | 12.00       | 12.30  | 0.27         | 0.770    | 0.782      | 0.782       | 0.778   | 0.007 |
|            | A029   | 15.00       | 15.00               | 15.20       | 15.27  | 0.51         | 0.550    | 0.300      | 0.550       | 0.327   | 0.025 |
|            | A030   | 12.86       | 12 99               | 12.84       | 12.90  | 0.08         | 0.780    | 0 770      | 0.770       | 0 773   | 0.006 |
|            | A035   | 12.00       | 14.75               | 13.18       | 13.51  | 1.12         | 0.734    | 0.770      | 0.770       | 0.773   | 0.000 |
|            | A039   | 11.48       | 11 59               | 11.61       | 11.56  | 0.07         | 0.652    | 0.636      | 0.631       | 0.640   | 0.014 |
| lts        | A041   | 11110       | 11107               | 11101       | 11.00  | 0.07         | 0.002    | 0.000      | 0.001       | 0.0.0   | 0.011 |
| esu        | A043   | 11.70       | 12.10               | 10.80       | 11.53  | 0.67         | 0.720    | 0.704      | 0.644       | 0.689   | 0.040 |
| ıl R       | A044   | 7.80        | 6.82                | 9.10        | 7.91   | 1.14         | 0.774    | 0.747      | 0.748       | 0.756   | 0.015 |
| du£        | A047   | 11.17       | 11.56               | 11.08       | 11.27  | 0.26         | 0.375    | 0.376      | 0.372       | 0.374   | 0.002 |
| livi       | A049   | 11.08       | 11.65               | 11.59       | 11.44  | 0.32         | 0.730    | 0.720      | 0.700       | 0.717   | 0.015 |
| Inc        | A050   | 816.57      | 796.03              | 802.24      | 804.9  | 10.5         | 0.768    | 0.750      | 0.763       | 0.760   | 0.009 |
|            | A051   | 12.60       | 12.58               | 12.75       | 12.64  | 0.09         | 0.534    | 0.555      | 0.533       | 0.541   | 0.012 |
|            | A055   |             |                     |             |  |              |          |            |             |         |       |
|            | A058   | 12.51       | 12.48               | 12.33       | 12.44  | 0.10         | 0.700    | 0.660      | 0.740       | 0.700   | 0.040 |
|            | A060   | 10.98       | 10.56               | 10.85       | 10.80  | 0.22         | 0.395    | 0.387      | 0.390       | 0.391   | 0.004 |
|            | A061   | 14.16       | 13.97               | 14.73       | 14.29  | 0.40         | 0.477    | 0.471      | 0.482       | 0.477   | 0.006 |
|            | A062   | 11.01       | 10.75               | 12.27       | 11.34  | 0.81         | 0.940    | 1.020      | 0.990       | 0.983   | 0.040 |
|            | A063   | 12.98       | 12.63               | 12.72       | 12.78  | 0.18         | 0.790    | 0.780      | 0.790       | 0.787   | 0.006 |
|            | A064   | 9.63        | 11.20               | 11.40       | 10.74  | 0.97         | 0./4/    | 0.701      | 0.751       | 0.733   | 0.028 |
|            | A060   | 12.00       | 12.02               | 12.06       | 12.00  | 0.01         | 4.151    | 4.105      | 4.154       | 4.14    | 0.028 |
|            | A009   | 12.99       | 13.25               | 13.00       | 13.09  | 0.12         | 0.849    | 0.840      | 0.651       | 0.649   | 0.005 |
|            | A070   | 13.30       | 13.90               | 13.30       | 13.50  | 0.35         | 0.570    | 0.400      | 0.442       | 0.493   | 0.008 |
|            | A072   | 15.50       | 15.70               | 15.50       | 15.50  | 0.55         | 0.027    | 0.075      | 0.050       | 0.054   | 0.050 |
|            | A074   | 13.30       | 13.10               | 13.10       | 13.17  | 0.12         | 0.710    | 0.730      | 0.740       | 0.727   | 0.015 |
|            | A076   | 12.97       | 12.43               | 12.52       | 12.64  | 0.29         | 0.732    | 01700      | 017.10      | 0.732   | 0.010 |
|            | A077   | 11.99       | 12.09               | 12.00       | 12.03  | 0.06         | 0.550    | 0.540      | 0.550       | 0.547   | 0.006 |
|            | A078   |             |                     |             |  |              |          |            |             |         |       |
|            | A079   | 13.00       | 12.00               | 12.00       | 12.33  | 0.58         | 0.713    | 0.746      | 0.756       | 0.738   | 0.023 |
|            | A080   |             |                     |             |  |              | 0.720    | 0.754      | 0.783       | 0.753   | 0.032 |
|            | A084   | 7.05        | 7.83                | 5.14        | 6.67   | 1.38         | 0.772    | 0.733      | 0.753       | 0.753   | 0.020 |
| ity        |        | Consensu    | s Mean              |             | 12.15  |              | Consensu | s Mean     |             | 0.695   |       |
| uni<br>lts |        | Consensu    | s Standard          | Deviation   | 1.63   |              | Consensu | s Standard | l Deviation | 0.11    |       |
| mm<br>esu  |        | Maximum 805 |                     |             |  | Maximum 4.14 |          |            |             |         |       |
| C OI       |        | Minimum     |                     |             | 1.61   |              | Minimum  |            |             | 0.327   |       |
| -          |        | Ν           |                     |             | 33   |              | Ν        |            |             | 32      |       |



**Figure 1-1.** Iron in SRM 3280 Multivitamin/Multielement Tablets (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The red solid lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \leq 2$ . The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by its uncertainty ( $U_{NIST}$ ) and represents the range that results in an acceptable  $Z_{NIST}$  score,  $|Z_{NIST}| \leq 2$ .



**Figure 1-2.** Iron in SRM 3233 Fortified Breakfast Cereal (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \leq 2$ . The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by its uncertainty ( $U_{NIST}$ ) and represents the range that results in an acceptable  $Z_{NIST}$  score,  $|Z_{NIST}| \leq 2$ .



**Figure 1-3.** Laboratory means for iron in SRM 3233 Fortified Breakfast Cereal and SRM 3280 Multivitamin/Multielement Tablets (sample/sample comparison view). In this view, the individual laboratory mean for one sample (SRM 3233) is compared to the individual laboratory mean for a second sample (SRM 3280). The solid red box represents the NIST range of tolerance for the two samples, cereal (x-axis) and multivitamin (y-axis), which encompasses the target values bounded by their uncertainties ( $U_{\text{NIST}}$ ) and represents the range that results in an acceptable  $Z_{\text{NIST}}$  score,  $|Z_{\text{NIST}}| \leq 2$ . The dotted blue box represents the consensus range of tolerance for SRM 3233 (x axis) and SRM 3280 (y-axis), calculated as the values above and below the consensus means that result in an acceptable  $Z'_{\text{comm}}$  score,  $|Z'_{\text{comm}}| \leq 2$ .

#### Human Metabolites Sample Information

Human Serum A. Participants were provided with three vials, each containing 2 mL of frozen human serum. The material was prepared from "off-the-shelf" plasma (no age or gender requirements for donors) that was then converted to serum. Before use, participants were instructed to allow the material to thaw at room temperature for at least 30 min prior to sampling, use the material immediately after thawing, gently mix the contents prior to removal of a test portion for analysis, and use a sample size appropriate for their usual in-house method of analysis. Participants were asked to avoid exposing the material to direct UV light, to store the material between -20 °C and -80 °C, and to prepare one sample and report one value from each vial provided. The approximate analyte levels were not reported to participants prior to the study. The certified value for iron in SRM 909c was determined at NIST using isotope dilution inductively coupled plasma mass spectrometry (ID ICP-MS). The reference value for total protein in SRM 909c was determined at NIST by using the JCTLM-approved reference method for total serum protein<sup>4</sup>. The certified value for total transferrin (Tf) and the reference values for Tf glycoforms in SRM 909c were determined at NIST by ID ICP-MS. The NIST-determined values and uncertainties for iron, total protein, total Tf, disialo Tf, trisialo Tf, tetrasialo Tf, pentasialo Tf, and hexasialo Tf in SRM 909c are provided in the table below.

|               | NIST-Determined Mass Fraction in SRM 909c |                 |  |  |  |  |  |  |
|---------------|---|-----------------|--|--|--|--|--|--|
| Analyte       | Value                                     | <u>Units</u>    |  |  |  |  |  |  |
| Iron (Fe)     | $90.3 \pm 3.9$                            | μg/dL           |  |  |  |  |  |  |
| Total Protein | $69.0  \pm  2.0$                          | g/L             |  |  |  |  |  |  |
| Disialo Tf    | $1.61 \pm 0.10$                           | % total protein |  |  |  |  |  |  |
| Trisialo Tf   | $3.93 \pm 0.21$                           | % total protein |  |  |  |  |  |  |
| Tetrasialo Tf | $77 \pm 5.1$                              | % total protein |  |  |  |  |  |  |
| Pentasialo Tf | $16.0 \pm 4.8$                            | % total protein |  |  |  |  |  |  |
| Hexasialo Tf  | $1.71 \pm 0.14$                           | % total protein |  |  |  |  |  |  |
| Total Tf      | $2.28 \pm 0.11$                           | g/L             |  |  |  |  |  |  |

*Human Serum B.* Participants were provided with three vials, each containing 1 mL of frozen human serum. The material was prepared from source plasma; bovine thrombin and calcium chloride were added to convert the plasma to serum. The serum was dialyzed to remove bovine thrombin, calcium chloride, and anticoagulants, and salts were added back into the serum. The serum was pooled along with isotonic saline, blended, bottled, and stored at -80 °C. Before use, participants were instructed to allow the material to thaw at room temperature for at least 30 min prior to sampling, use the material immediately after thawing, gently mix the contents prior to removal of a test portion for analysis, and use a sample size appropriate for their usual in-house method of analysis. Participants were asked to avoid exposing the material to direct UV light, to store the material between -20 °C and -80 °C, and to prepare one sample and report one value from each vial provided. The approximate analyte levels were not reported to participants prior to the study, and target values for iron, total protein, total Tf, disialo Tf, trisialo Tf, tetrasialo Tf, pentasialo Tf, and hexasialo Tf in SRM 968d Level 3 have not been determined at NIST.

<sup>&</sup>lt;sup>4</sup> Doumas, B.T.; Bayse, D.D.; Carter, R.J.; Peters, Jr., T.; Schaffer, R.; Clin Chem 27, pp. 1642–1650 (1981).

## Human Metabolites Study Results

- Thirteen laboratories enrolled in this study and received samples to measure iron. Three laboratories reported results for SRM 909c and SRM 968d (23 % participation).
- The consensus mean for iron in SRM 909c was just below the target range with very good between-laboratory variability (13 % RSD).
- The between-laboratory variability for iron was poor for SRM 968d (75 % RSD). No target value has been established for iron in SRM 968d.
- Five laboratories enrolled in this study and received samples to measure total protein. One laboratory reported results for both serum samples (20 % participation).
- The single reported value for total protein in SRM 909c was below the target range, and no target value has been established for total protein in SRM 968d.
- Five laboratories enrolled in this study and received samples to measure total Tf. Two laboratories reported results for both serum samples (40 % participation).
- The consensus mean for total Tf in SRM 909c was above the target range. No target value has been established for total Tf in SRM 968d.
- Three laboratories enrolled in this study and received samples to measure Tf glycoforms. No laboratories reported results for these analytes.
- Each laboratory reporting results for total iron used a unique method. Two laboratories reported using colorimetric methods (one using ferene and one using TPTZ), and the third laboratory reported using ICP-MS with a collision cell.

## Human Metabolites Technical Recommendations

Because of the low participation in this study, strong recommendations cannot be made based on results obtained from the participants.

- Overall, the small number of laboratories reporting values for iron in serum performed well.
  - In **Figure 1-4**, all three of the reported values overlapped the NIST range of tolerance for iron in SRM 909c.
  - One reported result had a large within laboratory variability, indicating either the sample or analyte was a challenge.
- The low participation rate in this study may indicate that the serum matrices were particularly challenging for the determination of iron and Tf. The complexity of the matrix and concomitant element interferences will be extremely challenging for laboratories using ICP-MS, even in energy discrimination or collision mode.
- The low participation rate may also indicate that established methods or protocols do not exist for some of the analytes requested in this study (such as Tf and the sialoforms).
  - Interested laboratories may need to look for workshops or training available in the measurement of Tf.
  - Laboratories with existing measurement procedures should publish in the peer-reviewed literature to promote knowledge exchange with other laboratories.
- Using a known quality assurance sample (CRM, SRM, RM, or in-house control) may assist with method development and method validation.

## Table 1-3. Individualized data summary table (NIST) for iron and transferrins in human serum.

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|                        | Lab Code: NIST                              |                              |   | 1. Your Results |                 |                                 |               | 2. Community Results                  |   |                   | 3. Target |  |
|------------------------|---|------------------------------|---|-----------------|-----------------|---------------------------------|---------------|---------------------------------------|---|-------------------|-----------|--|
| Analyte                | Sample                                      | Units                        | <i>x</i> <sub><i>i</i></sub>  | s <sub>i</sub>  | $Z'_{\rm comm}$ | Z <sub>NIST</sub>               | Ν             | <i>x</i> *                            | <i>s</i> *  | x <sub>NIST</sub> | U         |  |
| Iron                   | SRM 909c Frozen Human Serum                 | µg/dL                        | 90.3  | 1.9             |                 |                                 | 4             | 90                                    | 11  | 90.3              | 3.9       |  |
| Iron                   | SRM 968d Fat-Soluble Vitamins, Carotenoids, | µg/dL                        |   |                 |                 |                                 | 3             | 70                                    | 43  |                   |           |  |
|                        | and Cholesterol in Human Serum (Level 3)    |                              |   |                 |                 |                                 |               |                                       |   |                   |           |  |
| Total Protein          | SRM 909c Frozen Human Serum                 | g/L                          | 69.0  | 1.0             |                 |                                 | 2             |                                       |   | 69.0              | 2.0       |  |
| Total Protein          | SRM 968d Fat-Soluble Vitamins, Carotenoids, | g/L                          |   |                 |                 |                                 | 1             |                                       |   |                   |           |  |
|                        | and Cholesterol in Human Serum (Level 3)    |                              |   |                 |                 |                                 |               |                                       |   |                   |           |  |
| Disialo Transferrin    | SRM 909c Frozen Human Serum                 | % total protein              | 1.61  | 0.05            |                 |                                 | 1             |                                       |   | 1.61              | 0.10      |  |
| Disialo Transferrin    | SRM 968d Fat-Soluble Vitamins, Carotenoids, | % total protein              |   |                 |                 |                                 | 0             |                                       |   |                   |           |  |
|                        | and Cholesterol in Human Serum (Level 3)    |                              |   |                 |                 |                                 |               |                                       |   |                   |           |  |
| Hexasialo Transferrin  | SRM 909c Frozen Human Serum                 | % total protein              | 1.71  | 0.07            |                 |                                 | 1             |                                       |   | 1.71              | 0.14      |  |
| Hexasialo Transferrin  | SRM 968d Fat-Soluble Vitamins, Carotenoids, | % total protein              |   |                 |                 |                                 | 0             |                                       |   |                   |           |  |
|                        | and Cholesterol in Human Serum (Level 3)    |                              |   |                 |                 |                                 |               |                                       |   |                   |           |  |
| Pentasialo Transferrin | SRM 909c Frozen Human Serum                 | % total protein              | 16.0  | 2.4             |                 |                                 | 1             |                                       |   | 16.0              | 4.8       |  |
| Pentasialo Transferrin | SRM 968d Fat-Soluble Vitamins, Carotenoids, | % total protein              |   |                 |                 |                                 | 0             |                                       |   |                   |           |  |
|                        | and Cholesterol in Human Serum (Level 3)    |                              |   |                 |                 |                                 |               |                                       |   |                   |           |  |
| Tetrasialo Transferrin | SRM 909c Frozen Human Serum                 | % total protein              | 76.9  | 2.5             |                 |                                 | 1             |                                       |   | 76.9              | 5.1       |  |
| Tetrasialo Transferrin | SRM 968d Fat-Soluble Vitamins, Carotenoids, | % total protein              |   |                 |                 |                                 | 0             |                                       |   |                   |           |  |
|                        | and Cholesterol in Human Serum (Level 3)    |                              |   |                 |                 |                                 |               |                                       |   |                   |           |  |
| Trisialo Transferrin   | SRM 909c Frozen Human Serum                 | % total protein              | 3.93  | 0.11            |                 |                                 | 1             |                                       |   | 3.93              | 0.21      |  |
| Trisialo Transferrin   | SRM 968d Fat-Soluble Vitamins, Carotenoids, | % total protein              |   |                 |                 |                                 | 0             |                                       |   |                   |           |  |
|                        | and Cholesterol in Human Serum (Level 3)    |                              |   |                 |                 |                                 |               |                                       |   |                   |           |  |
| Total Transferrin (Tf) | SRM 909c Frozen Human Serum                 | g/L                          | 2.28  | 0.06            |                 |                                 | 3             | 150                                   | 670   | 2.28              | 0.11      |  |
| Total Transferrin (Tf) | SRM 968d Fat-Soluble Vitamins, Carotenoids, | g/L                          |   |                 |                 |                                 | 2             | 100                                   | 460   |                   |           |  |
|                        | and Cholesterol in Human Serum (Level 3)    |                              |   |                 |                 |                                 |               |                                       |   |                   |           |  |
|                        |   | <i>x</i> <sub><i>i</i></sub> | <ul> <li><i>i</i> Mean of reported values</li> <li><i>i</i> Standard deviation of reported values</li> <li><i>m</i> Z'-score with respect to community</li> </ul> |                 | Ν               | <i>N</i> Number of quantitative |               | $x_{\text{NIST}}$ NIST-assessed value |   |                   |           |  |
|                        |   | s <sub>i</sub>               |   |                 | s               | x* Robust mean of reported      |               |                                       | U expanded uncertainty<br>about the NIST-assessed value |                   |           |  |
|                        |   | $Z'_{comm}$                  |   |                 | <i>x</i> *      |                                 |               |                                       |   |                   |           |  |
|                        |   |                              | consensus   |                 |                 | values                          |               |                                       |   |                   |           |  |
|                        |   | $Z_{\text{NIST}}$            | T Z-score with respect to NIST value  |                 | <i>s</i> *      | Robust standa                   | ard deviation |                                       |   |                   |           |  |

#### HAMOAP Exercise 1 - Nutritional Elements (Human Metabolites)

|                      |        | Iron                                |    |     |       |       |  |    |     |     |    |  |
|----------------------|--------|-------------------------------------|----|-----|-------|-------|--|----|-----|-----|----|--|
|                      |        | SRM 909c Frozen Human Serum (µg/dL) |    |     |       |       | SRM 968d Fat-Soluble Vitamins, Carotenoids,<br>and Cholesterol in Human Serum (Level 3)<br>(µg/dL) |    |     |     |    |  |
|                      | Lab    | Α                                   | В  | С   | Avg   | SD    | Α  | В  | С   | Avg | SD |  |
|                      | Target |                                     |    |     | 90.34 | 3.89  |  |    |     |     |    |  |
|                      | A002   |                                     |    |     |       |       |  |    |     |     |    |  |
|                      | A030   |                                     |    |     |       |       |  |    |     |     |    |  |
|                      | A034   | 78                                  | 74 | 108 | 86.67 | 18.58 | 73   | 85 | 150 | 103 | 41 |  |
| ults                 | A049   |                                     |    |     |       |       |  |    |     |     |    |  |
| al Resu              | A050   |                                     |    |     |       |       |  |    |     |     |    |  |
|                      | A070   |                                     |    |     |       |       |  |    |     |     |    |  |
| npi                  | A079   |                                     |    |     |       |       |  |    |     |     |    |  |
| livi                 | A080   |                                     |    |     |       |       |  |    |     |     |    |  |
| Inc                  | A083   |                                     |    |     |       |       |  |    |     |     |    |  |
|                      | A086   |                                     |    |     |       |       |  |    |     |     |    |  |
|                      | A101   | 88                                  | 86 | 89  | 87.67 | 1.53  | 46   | 54 | 40  | 47  | 7  |  |
|                      | A103   | 82                                  | 83 | 87  | 84.00 | 2.65  | 78   | 74 | 74  | 75  | 2  |  |
|                      | A104   |                                     |    |     |       |       |  |    |     |     |    |  |
| Community<br>Results |        | Consensus Mean                      |    |     | 86.11 |       | Consensus Mean 75  |    |     |     |    |  |
|                      |        | Consensus Standard Deviation        |    |     | 10.80 |       | Consensus Standard Deviation 43  |    |     |     |    |  |
|                      |        | Maximum                             |    |     | 87.67 |       | Maximun  | ı  | 103 |     |    |  |
|                      |        | Minimum                             |    |     | 84    |       | Minimum  |    |     | 47  |    |  |
|                      |        | Ν                                   |    |     | 3     |       | Ν  |    |     | 3   |    |  |

**Table 1-4.** Data summary table for iron in human serum.



Exercise HAMQAP Exercise 1 - Human Metabolites Sample: SRM 909c Frozen Human Serum Measurand: Iron

**Figure 1-4.** Iron in SRM 909c Frozen Human Serum (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \leq 2$ . The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by its uncertainty ( $U_{NIST}$ ) and represents the range that results in an acceptable  $Z_{NIST}$  score,  $|Z_{NIST}| \leq 2$ .


Exercise HAMQAP Exercise 1 - Human Metabolites Sample: SRM 968d Fat-Soluble Vitamins,Carotenoids and Cholesterol in Human Serum (Level 3) Measurand: Iron

**Figure 1-5.** Iron in SRM 968d Fat-Soluble Vitamins, Carotenoids, and Cholesterol in Human Serum (Level 3) (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \le 2$ . A NIST value has not been determined in this material.



**Figure 1-6.** Laboratory means for iron in SRM 909c Frozen Human Serum and SRM 968d Fat-Soluble Vitamins, Carotenoids, and Cholesterol in Human Serum (Level 3) (sample/sample comparison view). In this view, the individual laboratory mean for one sample (SRM 909c) is compared to the individual laboratory mean for a second sample (SRM 968d). The dotted blue box represents the consensus range of tolerance for SRM 909c (x-axis) and SRM 968d (y-axis), calculated as the values above and below the consensus means that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \leq 2$ .

|            |        |          |            |           |            | Total | Protein  |            |             |     |     |  |  |
|------------|--------|----------|------------|-----------|------------|-------|--|------------|-------------|-----|-----|--|--|
|            |        | SRM      | [ 909c Fro | zen Hum   | an Serum ( | (g/L) | SRM 968d Fat-Soluble Vitamins, Carotenoid<br>and Cholesterol in Human Serum (Level 3)<br>(g/L) |            |             |     |     |  |  |
|            | Lab    | Α        | В          | С         | Avg        | SD    | Α  | В          | С           | Avg | SD  |  |  |
| ults       | Target |          |            |           | 69.00      | 2.00  |  |            |             |     |     |  |  |
| tesı       | A002   |          |            |           |            |       |  |            |             |     |     |  |  |
| idual R    | A070   |          |            |           |            |       |  |            |             |     |     |  |  |
|            | A079   |          |            |           |            |       |  |            |             |     |     |  |  |
| divi       | A083   |          |            |           |            |       |  |            |             |     |     |  |  |
| Inc        | A101   | 6.9      | 6.9        | 6.8       | 6.9        | 0.1   | 4.6  | 4.7        | 4.7         | 4.7 | 0.1 |  |  |
| ty         |        | Consensu | s Mean     |           |            |       | Consensu   | s Mean     |             |     |     |  |  |
| uni<br>lts |        | Consensu | s Standard | Deviation |            |       | Consensu   | s Standard | l Deviation |     |     |  |  |
| nm<br>esu  |        | Maximum  | ı          |           | 6.9        |       | Maximum  | l          |             | 4.7 |     |  |  |
| Con<br>R   |        | Minimum  |            |           | 6.9        |       | Minimum  |            |             | 4.7 |     |  |  |
| •          | N 1    |          |            |           |            |       | Ν  |            | 1           |     |     |  |  |

 Table 1-5. Data summary table for total protein in human serum.

**Table 1-6.** Data summary table for total Tf in human serum.

|                         |        |          |             |             | 1        | otal Trar | sferrin (T  | f)          |             |      |    |  |  |
|-------------------------|--------|----------|-------------|-------------|----------|-----------|---|-------------|-------------|------|----|--|--|
|                         | _      | SRM      | I 909c Fro  | ozen Huma   | an Serum | (g/L)     | SRM 968d Fat-Soluble Vitamins, Carota<br>and Cholesterol in Human Serum (Lev<br>(g/L) |             |             |      |    |  |  |
|                         | Lab    | Α        | В           | С           | Avg      | SD        | Α   | В           | С           | Avg  | SD |  |  |
| ılts                    | Target |          |             |             | 2.28     | 0.11      |   |             |             |      |    |  |  |
| test                    | A070   |          |             |             |          |           |   |             |             |      |    |  |  |
| idual R                 | A086   |          |             |             |          |           |   |             |             |      |    |  |  |
|                         | A101   | 302      | 302         | 306         | 303      | 2         | 208   | 211         | 211         | 210  | 2  |  |  |
| divi                    | A103   | 0.029    | 0.029       | 0.028       | 0.029    | 0.001     | 0.02  | 0.02        | 0.02        | 0.02 | 0  |  |  |
| In                      | A104   |          |             |             |          |           |   |             |             |      |    |  |  |
| ty                      |        | Consensu | ıs Mean     |             | 152      |           | Consensu  | is Mean     |             | 105  |    |  |  |
| uni <sup>,</sup><br>lts |        | Consensu | is Standard | l Deviation | 670      |           | Consensu  | is Standard | l Deviation | 462  |    |  |  |
| nmı                     |        | Maximun  | 1           |             | 303      |           | Maximun   | 1           | 210         |      |    |  |  |
| Con<br>R                |        | Minimum  | L           |             | 0        |           | Minimum   |             |             | 0.02 |    |  |  |
| ,                       |        | Ν        |             |             | 2        |           | Ν   |             |             | 2    |    |  |  |

## Nutritional Elements Overall Study Comparison

The following recommendations are based on results obtained from the participants in both portions of this study (dietary intake and human metabolism).

- Generally, laboratories are capable of measuring iron in food, dietary supplement, and human serum samples.
  - For the intake samples, the lower level of iron in the cereal matrix compared to the multivitamin may have resulted in increased measurement difficulty for laboratories.
  - The limited number of laboratories that measured iron in the serum samples had good agreement with one another in both materials and with the NIST certified value for SRM 909c.
  - A well-characterized quality assurance sample with a similar matrix composition and similar analyte levels will help assure that a method is in control.
  - A calibration curve surrounding the lower levels of the expected test solution values will ensure that measured samples are above the LOQ for the instrumentation used.
- A limited number of laboratories reported values for Tf glycoforms. However, because the bioavailability of iron is limited, measurement of human serum Tf is critical in understanding iron homeostasis.
  - Additional laboratories working in the area of serum Tf measurements should be recruited for future studies so that data collected can inform NIST and the community about measurement needs.
  - If a community need is revealed, new reference materials for serum Tf should be developed.

#### **SECTION 2: TOXIC ELEMENTS (Arsenic, Arsenic Species)**

#### Study Overview

In this study, participants were provided with Kelp and SRM 3222 Cigarette Tobacco Filler for dietary intake, and non-smokers' urine and smokers' urine for human metabolites. Participants were asked to use in-house analytical methods to determine the mass fraction of total arsenic (tAs), inorganic arsenic (iAs), arsenous acid (AsIII), arsenic acid (AsV), monomethylarsonic acid (MMA), dimethylarsinic acid (DMA), and arsenobetaine (AB) in each of the matrices. Inorganic arsenic in food is regulated based on known toxicity to humans, and dietary intake of or other exposure to arsenic can be evaluated clinically through measurement of arsenic and arsenic species in urine.

#### **Dietary Intake Sample Information**

*Kelp.* Participants were provided with three packets, each containing approximately 3 g of powdered kelp. The kelp was blended, aliquoted, and heat-sealed inside 4 mil polyethylene bags, which were then sealed inside nitrogen-flushed aluminized plastic bags along with two packets of silica gel. Before use, participants were instructed to thoroughly mix the contents of each packet and to use a sample size of at least 0.5 g. Participants were asked to store the material at controlled room temperature, 10 °C to 30 °C, and to prepare a single sample and to report a single value from each packet provided. Approximate analyte levels were not reported to participants prior to the study. A target value for tAs in Kelp was determined at NIST using inductively coupled plasma mass spectrometry (ICP-MS). The NIST-determined value and uncertainty for tAs in Kelp is provided in the table below. Target values for arsenic species in the kelp have not been determined at NIST.

| <u>Analyte</u> | NIST-Determined Value | for Mass Fraction in kelp (ng/g) |
|----------------|-----------------------|----------------------------------|
| tAs            | 26400                 | ± 1040                           |

*Tobacco*. Participants were provided with one jar containing approximately 10 g of cigarette tobacco filler. Before use, participants were instructed to grind the entire contents of the jar, mix the resulting powder thoroughly, and to use a sample size of at least 1 g. Participants were asked to store the material at controlled room temperature, 10 °C to 30 °C, and to prepare three samples and report three values from the single jar provided. Approximate analyte levels were not reported to participants prior to the study. The target value for tAs in SRM 3222 was determined by NIST and a collaborating laboratory using inductively coupled plasma mass spectrometry (ICP-MS). The NIST-determined value and uncertainty for tAs in SRM 3222 is provided in the table below. Target values for arsenic species in SRM 3222 have not been determined at NIST.

|                | NIST-Determined Value for Mass Fraction in SRM 3222 |
|----------------|---|
| <u>Analyte</u> | <u>(ng/g)</u>                                       |
| tAs            | $200 \pm 8$   |

## **Dietary Intake Study Results**

• The enrollment and reporting statistics for the arsenic speciation studies are described in the table below. Some of the reported values were non-quantitative (zero or below LOQ) but are included in the participation and reporting statistics.

|                | <u>Number of</u><br>Laboratories | Number of Laborato<br>(Percent P | ries Reporting Results<br>articipation) |
|----------------|----------------------------------|----------------------------------|---|
|                | Requesting                       |                                  | <u>SRM 3222</u>                         |
| <u>Analyte</u> | Samples                          | <u>Kelp</u>                      | Cigarette Tobacco Filler                |
| tAs            | 37                               | 25 (71 %)                        | 27 (73 %)                               |
| iAs            | 16                               | 8 (50%)                          | 6 (38%)                                 |
| AsIII          | 15                               | 2 (13 %)                         | 3 (20 %)                                |
| AsV            | 15                               | 2 (13 %)                         | 3 (20 %)                                |
| MMA            | 10                               | 2 (20 %)                         | 1 (10 %)                                |
| DMA            | 10                               | 2 (20 %)                         | 1 (10 %)                                |
| AB             | 8                                | 2 (25%)                          | 1 (12 %)                                |

- The consensus means for tAs in kelp and tobacco were above the target ranges.
  - The between-laboratory variability for kelp was good (14 % RSD).
  - The between-laboratory variability for tobacco was reasonable (30 % RSD).
- Most laboratories did not specify sample preparation methods or analytical methods used.

## **Dietary Intake Technical Recommendations**

The following recommendations are based on results obtained from the participants in this study.

- As shown in **Figures 2-1 and 2-2**, many laboratories reported data for tAs that were outside the NIST target range for one or both samples.
- Difficulty in the digestion of samples can cause bias and/or increased variability between samples.
  - The tobacco material is less homogeneous than the kelp material, which may contribute to the greater between-laboratory variability between the two materials (< 14 % RSD for the kelp, > 30 % RSD for tobacco).
  - Kelp and tobacco can be difficult to digest, requiring higher temperatures or the use of a small amount of HF in addition to oxidizing reagents to ensure complete digestion of the materials prior to analysis.
  - Arsenic is volatile and can be lost during sample preparation. The high temperatures of a vigorous microwave digestion should convert all volatile organoarsenic species to arsenic acid (AsV), at which point subsequent heating will not result in loss of arsenic.
  - Open-beaker digestion should not be used for As sample preparation. Closed-vessel digestions should be opened with care ensuring that no As is lost as a result of inadvertent venting.
  - Collision cell technology can be used to minimize many of the molecular interferences that may be found when analyzing As in these two materials.
  - Calibration curves must be linear and include the lowest and highest values expected to be measured in the sample solutions. Extrapolation of the curve may cause incorrect results.

- Calculation errors may be a cause for incorrect results. Using a quality assurance material (CRM, SRM, RM), or in-house prepared material, to establish that a method is in control will help identify calculation errors. Be sure results are reported in the correct units.
- Inorganic arsenic (iAs) is typically determined by combining the measured values for arsenous acid (AsIII) and AsV. Only two or three laboratories provided values for either AsIII or AsV, indicating that many laboratories may need guidance with measurement procedures for iAs. Training or workshops would be recommended for interested laboratories.
- The number of reported results for AsIII, AsV, MMA, DMA, and AB are insufficient to result in an informed recommendation for these analytes in the kelp and tobacco matrices.

# Table 2-1. Individualized data summary table (NIST) for arsenic in kelp and tobacco.

|                               | HAMQAP                            | Exercise          | e 1 - Toxic El          | ements (l      | Dietary Inta    | ke)               |                      |                |                   |                   |                   |
|-------------------------------|-----------------------------------|-------------------|-------------------------|----------------|-----------------|-------------------|----------------------|----------------|-------------------|-------------------|-------------------|
|                               | Lab Code:                         | NIST              | _                       | 1. You         | r Results       |                   | 2. (                 | Community F    | Results           | 3. Ta             | arget             |
| Analyte                       | Sample                            | Units             | x <sub>i</sub>          | s <sub>i</sub> | $Z'_{\rm comm}$ | Z <sub>NIST</sub> | N                    | <i>x</i> *     | <i>s</i> *        | x <sub>NIST</sub> | U                 |
| Total Arsenic                 | Kelp                              | ng/g              | 26000                   | 520            |                 |                   | 28                   | 27800          | 3800              | 26000             | 1040              |
| Total Arsenic                 | SRM 3222 Cigarette Tobacco Filler | ng/g              | 200                     | 4              |                 |                   | 27                   | 210            | 65                | 200               | 8                 |
| Total Inorganic Arsenic (iAs) | Kelp                              | ng/g              |                         |                |                 |                   | 7                    | 140            | 450               |                   |                   |
| Total Inorganic Arsenic (iAs) | SRM 3222 Cigarette Tobacco Filler | ng/g              |                         |                |                 |                   | 6                    | 130            | 51                |                   |                   |
| Arsenic III                   | Kelp                              | ng/g              |                         |                |                 |                   | 2                    | 40             | 190               |                   |                   |
| Arsenic III                   | SRM 3222 Cigarette Tobacco Filler | ng/g              |                         |                |                 |                   | 3                    | 1.1            | 5.3               |                   |                   |
| Arsenic V                     | Kelp                              | ng/g              |                         |                |                 |                   | 1                    |                |                   |                   |                   |
| Arsenic V                     | SRM 3222 Cigarette Tobacco Filler | ng/g              |                         |                |                 |                   | 2                    | 80             | 180               |                   |                   |
| Monomethylarsonic acid (MMA)  | Kelp                              | ng/g              |                         |                |                 |                   | 1                    |                |                   |                   |                   |
| Monomethylarsonic acid (MMA)  | SRM 3222 Cigarette Tobacco Filler | ng/g              |                         |                |                 |                   | 0                    |                |                   |                   |                   |
| Dimethylarsinic acid (DMA)    | Kelp                              | ng/g              |                         |                |                 |                   | 2                    | 450            | 250               |                   |                   |
| Dimethylarsinic acid (DMA)    | SRM 3222 Cigarette Tobacco Filler | ng/g              |                         |                |                 |                   | 1                    |                |                   |                   |                   |
| Arsenobetaine (AB)            | Kelp                              | ng/g              |                         |                |                 |                   | 2                    | 22200          | 1200              |                   |                   |
| Arsenobetaine (AB)            | SRM 3222 Cigarette Tobacco Filler | ng/g              |                         |                |                 |                   | 1                    |                |                   |                   |                   |
|                               |                                   | x <sub>i</sub>    | Mean of repo            | orted value    | s               | Ν                 | Number of            | quantitative   | x <sub>NIST</sub> | NIST-assess       | ed value          |
|                               |                                   | S <sub>i</sub>    | Standard dev            | iation of re   | ported value    | s                 | values repor         | ted            | U                 | expanded unc      | certainty         |
|                               |                                   | $Z'_{\rm comm}$   | Z'-score with consensus | respect to     | community       | <i>x</i> *        | Robust mea<br>values | n of reported  |                   | about the NIS     | ST-assessed value |
|                               |                                   | $Z_{\text{NIST}}$ | Z-score with            | respect to     | NIST value      | <i>s</i> *        | Robust stan          | dard deviation | 1                 |                   |                   |

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|            | P      | Total Arsenic (tAs) |             |             |       |      |          |             |             |           |          |  |  |
|------------|--------|---------------------|-------------|-------------|-------|------|----------|-------------|-------------|-----------|----------|--|--|
|            |        |                     | I           | Kelp (ng/g  | )     |      | SRM 32   | 22 Cigare   | ette Tobac  | co Filler | • (ng/g) |  |  |
|            | Lab    | A                   | В           | С           | Avg   | SD   | A        | В           | С           | Avg       | SD       |  |  |
|            | Target |                     |             |             | 26000 | 1040 |          |             |             | 200       | 8        |  |  |
|            | A001   |                     |             |             |       |      |          |             |             |           |          |  |  |
|            | A002   |                     |             |             | L     |      |          |             |             | I         |          |  |  |
|            | A003   | 25174               | 24788       | 27278       | 25747 | 1340 | 206      | 219         | 201         | 209       | 9        |  |  |
|            | A006   | 26589               | 26694       | 26814       | 26699 | 112  | 161.1    | 174.4       | 169.5       | 168       | 7        |  |  |
|            | A007   | 28737               | 28430       | 28453       | 28540 | 171  | 200      | 200         | 200         | 200       | 0        |  |  |
|            | A008   | 33095               | 33052       | 32846       | 32998 | 133  | 292      | 328         | 260         | 293       | 34       |  |  |
|            | A011   |                     |             |             |       |      |          |             |             |           |          |  |  |
|            | A015   |                     |             |             | L     |      |          |             |             | L         |          |  |  |
|            | A026   | 30755               | 30360       | 30010       | 30375 | 373  | 157      | 156         | 176         | 163       | 11       |  |  |
|            | A027   | 6                   | 6           | 6           | 6     | 0    |          |             |             | L         |          |  |  |
|            | A028   | 30900               | 32500       | 32300       | 31900 | 872  | 240      | 240         | 240         | 240       | 0        |  |  |
|            | A029   | 23352               | 23026       | 23033       | 23137 | 186  | 252.9    | 269.9       | 263.94      | 262       | 9        |  |  |
|            | A030   |                     |             |             |       |      |          |             |             |           |          |  |  |
|            | A034   | 27000               | 27000       | 29000       | 27667 | 1155 | 180      | 200         | 210         | 197       | 15       |  |  |
| ~          | A039   | 24409               | 24478       | 24801       | 24563 | 209  | 382      | 389         | 348         | 373       | 22       |  |  |
| ults       | A041   | 24996               | 25532       | 27099       | 25876 | 1093 | 203      | 202         | 206         | 204       | 2        |  |  |
| Res        | A043   | 28160               | 28380       | 29800       | 28780 | 890  | 174      | 183         | 202         | 186       | 14       |  |  |
| al J       | A044   | 27524               | 27211       | 26735       | 27157 | 397  | 173      | 169         | 184         | 175       | 8        |  |  |
| idu        | A047   | 27830               | 27740       | 27910       | 27827 | 85   | 735      | 732         | 730         | 732       | 3        |  |  |
| div        | A049   | 23576               | 24063       | 24213       | 23951 | 333  | 179      | 167         | 198         | 181       | 16       |  |  |
| In         | A051   | 25749               | 25262       | 27123       | 26045 | 965  | 213      | 224         | 244         | 227       | 16       |  |  |
|            | A055   | L                   |             |             | L     |      |          |             |             | L         |          |  |  |
|            | A058   |                     |             |             |       |      |          |             |             |           |          |  |  |
|            | A059   | 27700               | 28000       | 26600       | 27433 | 737  | 210      | 223         | 218         | 217       | 7        |  |  |
|            | A060   | 27910               | 27840       | 27870       | 27873 | 35   | 776      | 756         | 765         | 766       | 10       |  |  |
|            | A061   | 28                  | 28          | 27          | 27    | 0    | 405      | 393         | 387         | 395       | 9        |  |  |
|            | A062   | 28867               | 31200       | 29419       | 29829 | 1219 | 114.2    | 115.5       | 112.5       | 114       | 2        |  |  |
|            | A064   | 30900               | 30400       | 30900       | 30733 | 289  | 230      | 220         | 180         | 210       | 26       |  |  |
|            | A065   |                     |             |             |       |      |          |             |             |           |          |  |  |
|            | A068   | 33700               | 33900       | 35600       | 34400 | 1044 | 267      | 241         | 224         | 244       | 22       |  |  |
|            | A069   | 30852               | 31507       | 31623       | 31327 | 416  | 354      | 342         | 341         | 346       | 7        |  |  |
|            | A070   | 22839               | 22974       | 23076       | 22963 | 119  | 134.16   | 135.47      | 136.28      | 135       | 1        |  |  |
|            | A074   |                     |             |             |       |      |          |             |             |           |          |  |  |
|            | A076   |                     |             |             | L     |      |          |             |             | I         |          |  |  |
|            | A079   | 27612               | 26973       | 27926       | 27504 | 486  | 196      | 190         | 178         | 188       | 9        |  |  |
|            | A080   | 27520               | 27290       | 27760       | 27523 | 235  | 168      | 176         | 199         | 181       | 16       |  |  |
|            | A083   | 25360               | 25000       | 22900       | 24420 | 1329 | 210      |             |             |           |          |  |  |
| ity        |        | Consensu            | ıs Mean     |             | 27776 | I    | Consensu | is Mean     |             | 214       |          |  |  |
| uni<br>lts | ļ      | Consensu            | is Standard | I Deviation | 3840  | l    | Consensu | is Standard | l Deviation | 65        |          |  |  |
| mm<br>esu  | ļ      | Maximum             | 1           |             | 34400 | l    | Maximum  |             |             | 766       |          |  |  |
| Cor<br>R   |        | Minimum             |             |             | 6     | I    | Minimum  |             |             | 114       |          |  |  |
| •          | , I    | N                   |             |             | 27    |      | N        |             |             | 25        |          |  |  |

**Table 2-2.** Data summary table for total arsenic (tAs) in kelp and tobacco. Data points highlighted in red have been flagged as potential outliers (e.g., difference from reference value, Grubb and/or Cochran) by the NIST software package.



Figure 2-1. Total arsenic (tAs) in Kelp (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \leq 2$ . The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by its uncertainty ( $U_{NIST}$ ) and represents the range that results in an acceptable  $Z_{NIST}$  score,  $|Z_{NIST}| \leq 2$ .



**Figure 2-2.** Total arsenic (tAs) in SRM 3222 Cigarette Tobacco Filler (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \leq 2$ . The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by its uncertainty ( $U_{NIST}$ ) and represents the range that results in an acceptable  $Z_{NIST}$  score,  $|Z_{NIST}| \leq 2$ .



Figure 2-3. Laboratory means for total arsenic (tAs) in Kelp and SRM 3222 Cigarette Tobacco Filler (sample/sample comparison view). In this view, the individual laboratory mean for one sample (Kelp) is compared to the mean for a second sample (SRM 3222). The solid red box represents the NIST range of tolerance for the two samples, Kelp (x-axis) and SRM 3222 (y-axis), which encompasses the NIST-determined values bounded by their uncertainties ( $U_{\text{NIST}}$ ) and represents the range that results in an acceptable  $Z_{\text{NIST}}$  score,  $|Z_{\text{NIST}}| \leq 2$ . The dotted blue box represents the consensus range of tolerance for Kelp (x-axis) and SRM 3222 (y-axis), calculated as the values above and below the consensus means that result in an acceptable  $Z'_{\text{comm}}$  score,  $|Z'_{\text{comm}}| \leq 2$ .

**Table 2-3.** Data summary table for total inorganic arsenic (iAs) in kelp and tobacco. Data points highlighted in red have been flagged as potential outliers (e.g., difference from reference value, Grubb and/or Cochran) by the NIST software package.

|            |        |                     |             |             | Total | Inorgani | ic Arsenic | (iAs)       |             |             |        |  |
|------------|--------|---------------------|-------------|-------------|-------|----------|------------|-------------|-------------|-------------|--------|--|
|            |        |                     | ]           | Kelp (ng/g  | )     |          | SRM 3      | 3222 Ciga   | rette Toba  | acco Filler | (ng/g) |  |
|            | Lab    | Α                   | В           | С           | Avg   | SD       | А          | В           | С           | Avg         | SD     |  |
|            | Target |                     |             |             |       |          |            |             |             |             |        |  |
|            | A015   |                     |             |             |       |          |            |             |             |             |        |  |
|            | A028   | 202                 | 204         | 208         | 204.7 | 3.1      | 128        | 125         | 132         | 128.3       | 3.5    |  |
|            | A034   |                     |             |             |       |          |            |             |             |             |        |  |
|            | A039   |                     |             |             |       |          |            |             |             |             |        |  |
| ts         | A043   |                     |             |             |       |          |            |             |             |             |        |  |
| lus        | A044   |                     |             |             |       |          |            |             |             |             |        |  |
| vidual Res | A049   | 124                 | 0           | 0           | 41    | 72       | 141        | 103         | 152         | 132         | 26     |  |
|            | A051   |                     |             |             |       |          |            |             |             |             |        |  |
|            | A059   | 226                 | 204         | 179         | 203   | 24       | 161        | 142         | 152         | 151.7       | 9.5    |  |
| ndi        | A060   |                     |             |             |       |          |            |             |             |             |        |  |
| Π          | A061   | < 0.001             | < 0.001     | < 0.001     |       |          | < 0.001    | < 0.001     | < 0.001     |             |        |  |
|            | A064   | 8590                | 8630        | 8760        | 8660  | 89       |            |             |             |             |        |  |
|            | A065   |                     |             |             |       |          |            |             |             |             |        |  |
|            | A068   | 101                 | 91          | 95          | 96    | 5        | 128        | 123         | 125         | 125.3       | 2.5    |  |
|            | A070   | 2800                | 2560        | 1320        | 2227  | 790      | 2980       | 2980        | 2980        | 2980        | 0      |  |
|            | A076   | 30720               | 30700       | 30720       | 30713 | 12       | 29460      |             |             |             |        |  |
| ty         |        | Consensu            | ıs Mean     |             | 136   |          | Consensu   | ıs Mean     |             | 134         |        |  |
| uni<br>lts |        | Consensu            | is Standard | l Deviation | 450   |          | Consensu   | is Standard | l Deviation | 51          |        |  |
| nm         |        | Maximun             | 1           |             | 30713 |          | Maximum    |             |             | 2980        |        |  |
| R. Cor     |        | Minimum             | l           |             | 41    |          | Minimum    |             | 125.3       |             |        |  |
| •          |        | Minimum41MinimumN7N |             |             |       |          |            |             |             | 5           |        |  |





**Figure 2-4.** Total inorganic arsenic (iAs) in Kelp (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red line represents the upper consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \leq 2$ , with the lower range set at zero. A NIST value has not been determined in this material.



**Figure 2-5.** Total inorganic arsenic (iAs) in SRM 3222 Cigarette Tobacco Filler (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \leq 2$ . A NIST value has not been determined in this material.



Figure 2-6. Laboratory means for total inorganic arsenic (iAs) in Kelp and SRM 3222 Cigarette Tobacco Filler (sample/sample comparison view). In this view, the individual laboratory mean for one sample (Kelp) is compared to the mean for a second sample (SRM 3222). The dotted blue box represents the consensus range of tolerance for Kelp (x-axis) and SRM 3222 (y-axis), calculated as the values above and below the consensus means that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \le 2$ .

**Table 2-4.** Data summary table for arsenous acid (AsIII) in kelp and tobacco. Data points highlighted in red have been flagged as potential outliers (e.g., difference from reference value, Grubb and/or Cochran) by the NIST software package.

|            |                              |          | Arsenous Acid (AsIII) |             |     |    |          |             |             |             |        |  |  |  |  |
|------------|------------------------------|----------|-----------------------|-------------|-----|----|----------|-------------|-------------|-------------|--------|--|--|--|--|
|            |                              |          | ŀ                     | Kelp (ng/g) | )   |    | SRM 3    | 3222 Ciga   | rette Toba  | acco Filler | (ng/g) |  |  |  |  |
|            | Lab                          | Α        | В                     | С           | Avg | SD | Α        | В           | С           | Avg         | SD     |  |  |  |  |
|            | Target                       |          |                       |             |     |    |          |             |             |             |        |  |  |  |  |
|            | A007                         |          |                       |             |     |    |          |             |             |             |        |  |  |  |  |
|            | A015                         |          |                       |             |     |    |          |             |             |             |        |  |  |  |  |
|            | A028                         |          |                       |             |     |    |          |             |             |             |        |  |  |  |  |
|            | A034                         |          |                       |             |     |    |          |             |             |             |        |  |  |  |  |
| ults       | A039                         |          |                       |             |     |    |          |             |             |             |        |  |  |  |  |
| Res        | A043                         |          |                       |             |     |    |          |             |             |             |        |  |  |  |  |
| dividual R | A044                         |          |                       |             |     |    |          |             |             |             |        |  |  |  |  |
|            | A049                         | 0        | 0                     | 0           | 0   | 0  | 0        | 0           | 0           | 0           | 0      |  |  |  |  |
|            | A059                         |          |                       |             |     |    |          |             |             |             |        |  |  |  |  |
| In         | A060                         |          |                       |             |     |    |          |             |             |             |        |  |  |  |  |
|            | A061                         |          |                       |             |     |    |          |             |             |             |        |  |  |  |  |
|            | A064                         |          |                       |             |     |    |          |             |             |             |        |  |  |  |  |
|            | A065                         |          |                       |             |     |    | 2.341    | 2.124       | 2.387       | 2.28        | 0.14   |  |  |  |  |
|            | A068                         | 91       | 83                    | 88          | 87  | 4  | 102      | 99          | 102         | 101         | 1.73   |  |  |  |  |
|            | A070                         |          |                       |             |     |    |          |             |             |             |        |  |  |  |  |
| ity        |                              | Consensu | s Mean                |             | 44  |    | Consensu | is Mean     |             | 1.14        |        |  |  |  |  |
| un<br>ilts | Consensus Standard Deviation |          |                       |             |     |    | Consensu | is Standard | l Deviation | 5.27        |        |  |  |  |  |
| mm<br>esu  |                              | Maximum  | l                     |             | 87  |    | Maximun  | 1           |             | 101         |        |  |  |  |  |
| C01<br>R   |                              | Minimum  |                       |             | 0   |    | Minimum  |             |             | 0           |        |  |  |  |  |
| -          |                              | Ν        |                       |             | 2   |    | Ν        |             |             | 3           |        |  |  |  |  |

|            |        |          | Arsenic V  |             |     |    |  |            |             |      |     |  |  |  |  |
|------------|--------|----------|------------|-------------|-----|----|--|------------|-------------|------|-----|--|--|--|--|
|            |        |          | ]          | Kelp (ng/g  | )   |    | SRM 3222 Cigarette Tobacco Filler (ng/g) |            |             |      |     |  |  |  |  |
|            | Lab    | Α        | В          | С           | Avg | SD | Α  | В          | С           | Avg  | SD  |  |  |  |  |
|            | Target |          |            |             |     |    |  |            |             |      |     |  |  |  |  |
|            | A007   |          |            |             |     |    |  |            |             |      |     |  |  |  |  |
|            | A015   |          |            |             |     |    |  |            |             |      |     |  |  |  |  |
|            | A028   |          |            |             |     |    |  |            |             |      |     |  |  |  |  |
|            | A034   |          |            |             |     |    |  |            |             |      |     |  |  |  |  |
| ults       | A039   |          |            |             |     |    |  |            |             |      |     |  |  |  |  |
| kesi       | A043   |          |            |             |     |    |  |            |             |      |     |  |  |  |  |
| idual Ro   | A044   |          |            |             |     |    |  |            |             |      |     |  |  |  |  |
|            | A049   | 124      | 0          | 0           | 41  | 72 | 141                                      | 103        | 152         | 132  | 26  |  |  |  |  |
| div        | A059   |          |            |             |     |    |  |            |             |      |     |  |  |  |  |
| In         | A060   |          |            |             |     |    |  |            |             |      |     |  |  |  |  |
|            | A061   |          |            |             |     |    |  |            |             |      |     |  |  |  |  |
|            | A064   |          |            |             |     |    |  |            |             |      |     |  |  |  |  |
|            | A065   |          |            |             |     |    |  |            |             |      |     |  |  |  |  |
|            | A068   | < 14.0   | <13.2      | < 14.5      |     |    | 26.3                                     | 23.8       | 22.3        | 24.1 | 2.0 |  |  |  |  |
|            | A070   |          |            |             |     |    |  |            |             |      |     |  |  |  |  |
| ty         |        | Consensu | s Mean     |             |     |    | Consensu                                 | s Mean     |             | 78   |     |  |  |  |  |
| uni<br>lts |        | Consensu | s Standard | l Deviation |     |    | Consensu                                 | s Standard | l Deviation | 178  |     |  |  |  |  |
| mm<br>esu  |        | Maximun  | 1          |             | 41  |    | Maximum                                  | 1          |             | 132  |     |  |  |  |  |
| C01<br>R   |        | Minimum  |            |             | 41  |    | Minimum                                  |            |             | 24   |     |  |  |  |  |
| 5          |        | Ν        |            |             | 1   |    | Ν  |            |             | 2    |     |  |  |  |  |

 Table 2-5. Data summary table for arsenic V (AsV) in kelp and tobacco.

|            |        |           |            |            | Monome | thylarsor | nic acid (M | (MA)        |             |             |        |
|------------|--------|-----------|------------|------------|--------|-----------|-------------|-------------|-------------|-------------|--------|
|            |        |           | ]          | Kelp (ng/g | )      |           | SRM 3       | 3222 Ciga   | rette Tob   | acco Filler | (ng/g) |
|            | Lab    | Α         | В          | С          | Avg    | SD        | Α           | В           | С           | Avg         | SD     |
|            | Target |           |            |            |        |           |             |             |             |             |        |
|            | A015   |           |            |            |        |           |             |             |             |             |        |
| ts         | A028   | < 3.39    | < 3.41     | < 3.42     |        |           | < 3.44      | <3.42       | < 3.40      |             |        |
| Ins        | A039   |           |            |            |        |           |             |             |             |             |        |
| I Re       | A043   |           |            |            |        |           |             |             |             |             |        |
| ual        | A044   |           |            |            |        |           |             |             |             |             |        |
| ivid       | A059   |           |            |            |        |           |             |             |             |             |        |
| ipu        | A064   | 12000     | 12400      | 12400      | 12267  | 230       |             |             |             |             |        |
| Ι          | A065   |           |            |            |        |           |             |             |             |             |        |
|            | A068   |           |            |            |        |           |             |             |             |             |        |
|            | A070   |           |            |            |        |           |             |             |             |             |        |
| ity        |        | Consensus | s Mean     |            |        |           | Consensu    | is Mean     |             |             |        |
| uni<br>lts |        | Consensus | s Standard | Deviation  |        |           | Consensu    | is Standard | 1 Deviation |             |        |
| mm<br>esu  |        | Maximum   |            |            | 12267  |           | Maximum     | 1           |             |             |        |
| COL<br>R   |        | Minimum   |            |            | 12267  |           | Minimum     |             |             |             |        |
| •          |        | Ν         |            |            | 1      |           | Ν           |             |             | 0           |        |

Table 2-6. Data summary table for monomethylarsonic acid (MMA) in kelp and tobacco.

**Table 2-7.** Data summary table for dimethylarsinic acid (DMA) in kelp and tobacco.

|            |        |          |            |             | Dime | ethylarsir | nic acid (D                              | MA)         |             |      |      |  |  |
|------------|--------|----------|------------|-------------|------|------------|--|-------------|-------------|------|------|--|--|
|            |        |          | I          | Kelp (ng/g) | 1    |            | SRM 3222 Cigarette Tobacco Filler (ng/g) |             |             |      |      |  |  |
|            | Lab    | Α        | В          | С           | Avg  | SD         | Α  | В           | С           | Avg  | SD   |  |  |
|            | Target |          |            |             |      |            |  |             |             |      |      |  |  |
|            | A015   |          |            |             |      |            |  |             |             |      |      |  |  |
| ts         | A028   | 527      | 495        | 508         | 510  | 16         | 6.88                                     | 6.78        | 6.70        | 6.79 | 0.09 |  |  |
| Inse       | A039   |          |            |             |      |            |  |             |             |      |      |  |  |
| R          | A043   |          |            |             |      |            |  |             |             |      |      |  |  |
| ividual    | A044   |          |            |             |      |            |  |             |             |      |      |  |  |
|            | A059   |          |            |             |      |            |  |             |             |      |      |  |  |
| ndi        | A064   | 370      | 410        | 370         | 383  | 23         |  |             |             |      |      |  |  |
| Ι          | A065   |          |            |             |      |            |  |             |             |      |      |  |  |
|            | A068   |          |            |             |      |            |  |             |             |      |      |  |  |
|            | A070   |          |            |             |      |            |  |             |             |      |      |  |  |
| ty         |        | Consensu | s Mean     |             | 447  |            | Consensu                                 | ıs Mean     |             |      |      |  |  |
| uni<br>lts |        | Consensu | s Standard | l Deviation | 249  |            | Consensu                                 | is Standard | l Deviation |      |      |  |  |
| nm<br>esu  |        | Maximum  |            |             | 510  |            | Maximun                                  | 1           |             | 6.79 |      |  |  |
| R          |        | Minimum  |            |             | 383  |            | Minimum                                  |             |             | 6.79 |      |  |  |
| •          |        | Ν        |            |             | 2    |            | Ν  |             | 1           |      |      |  |  |

|             |        |          |             |             | I     | Arsenobe | taine (AB)                               | )            |             |      |     |  |  |
|-------------|--------|----------|-------------|-------------|-------|----------|--|--------------|-------------|------|-----|--|--|
|             |        |          | I           | Kelp (ng/g  | )     |          | SRM 3222 Cigarette Tobacco Filler (ng/g) |              |             |      |     |  |  |
|             | Lab    | Α        | В           | С           | Avg   | SD       | Α  | В            | С           | Avg  | SD  |  |  |
|             | Target |          |             |             |       |          |  |              |             |      |     |  |  |
| ts          | A015   |          |             |             |       |          |  |              |             |      |     |  |  |
| lus         | A028   | 22000    | 23000       | 23500       | 22833 | 764      | 17.9                                     | 16.7         | 15.4        | 16.7 | 1.3 |  |  |
| vidual Re   | A039   |          |             |             |       |          |  |              |             |      |     |  |  |
|             | A043   |          |             |             |       |          |  |              |             |      |     |  |  |
|             | A059   |          |             |             |       |          |  |              |             |      |     |  |  |
| ndi         | A064   | 21900    | 21700       | 21400       | 21667 | 252      |  |              |             |      |     |  |  |
| I           | A065   |          |             |             |       |          |  |              |             |      |     |  |  |
|             | A070   |          |             |             |       |          |  |              |             |      |     |  |  |
| ty          |        | Consensu | ıs Mean     |             | 22250 |          | Consensu                                 | ıs Mean      |             |      |     |  |  |
| umi<br>lts  |        | Consensu | is Standard | l Deviation | 1170  |          | Consensu                                 | is Standard  | l Deviation |      |     |  |  |
| nmu<br>sult |        | Maximum  | ı           |             | 22833 |          | Maximum                                  |              |             | 16.7 |     |  |  |
| R.          |        | Minimum  |             |             | 21667 |          | Minimum                                  | Iinimum 16.7 |             |      |     |  |  |
| •           |        | Ν        |             |             | 2     |          | Ν  |              | 1           |      |     |  |  |

*Human Urine A.* Participants were provided with three vials, each containing approximately 10 mL of smokers' urine. The pooled urine was collected from a minimum of 10 donors who were smokers (no age or gender constraints). Before use, participants were asked to allow the material to thaw at room temperature for at least 30 min prior to sampling, to use the thawed material within 4 h, to gently invert the vial several times before a test portion was removed to ensure homogenization, and to use a sample size appropriate for their usual in-house method of analysis. Participants were asked to store the material between -20 °C and -80 °C and to prepare one sample and report one value from each vial provided. Approximate analyte levels were not reported to participants prior to the study. The target values for AsV, DMA, MMA, and AB in Human Urine A were determined at NIST using liquid chromatography with inductively coupled plasma mass spectrometry (LC-ICP-MS). The NIST-determined values and uncertainties for AsV, DMA, MMA, and AB in Human Urine A are provided in the table below. Target values for iAs and AsIII in Human Urine A have not been determined at NIST.

|                | NIST-Determined Mass Fractions in |
|----------------|-----------------------------------|
| <u>Analyte</u> | <u>Human Urine A (µg/L)</u>       |
| tAs            | $13.4 \pm 1.4$                    |
| AsV            | $0.35$ $\pm$ $0.15$               |
| DMA            | $3.15 \pm 0.85$                   |
| MMA            | $0.56$ $\pm$ $0.14$               |
| AB             | $11.05 \pm 0.62$                  |

*Human Urine B.* Participants were provided with three vials, each containing approximately 3 mL of non-smokers' urine. The pooled urine was collected from a minimum of 10 donors who were non-smokers and were not exposed to secondhand cigarette smoke (no age or gender constraints). Before use, participants were asked to allow the material to thaw at room temperature for at least 30 min prior to sampling, to use the thawed material within 4 h, to gently invert the vial several times before a test portion was removed to ensure homogenization, and to use a sample size appropriate for their usual in-house method of analysis. Participants were asked to store the material between -20 °C and -80 °C and to prepare one sample and report one value from each vial provided. Approximate analyte levels were not reported to participants prior to the study, and target values for arsenic species in Human Urine B have not been determined at NIST.

#### Human Metabolites Study Results

- Fourteen laboratories enrolled in this exercise and received samples to measure tAs in the human urine samples. Five laboratories reported results for tAs in both samples (36 % participation).
  - The consensus range for tAs in Human Urine A overlapped with the target range with excellent between-laboratory variability (14 % RSD).
  - The between-laboratory variability for tAs in Human Urine B was unacceptable (> 100 % RSD).
- Eight laboratories enrolled in this exercise and received samples to measure AsIII, AsV, and iAs in the human urine samples. Three laboratories reported results for these species in both samples (38 % participation).

- Of the three laboratories reporting values for AsIII, AsV, and iAs, one laboratory reported zero for the analytes, another lab reported results below the detection limit, and only the third laboratory reported results of detectable levels of analytes.
- For the data analyses and recommendations, a reported result of zero is treated as a result below the detection limit.
- Six laboratories enrolled in this exercise and received samples to measure MMA, DMA, and AB in the human urine samples.
  - Three laboratories reported results for AB in both samples (50 % participation).
    - The consensus mean for AB in Human Urine A was below the target range, and only the upper portion of the consensus range overlaps the target range. The consensus variability for AB in Human Urine A was excellent (12 % RSD).
    - The consensus variability was moderately high for AB in Human Urine B (29 % RSD).
  - Two laboratories reported results for DMA in both samples (33 % participation).
  - One laboratory reported results for MMA in both samples (17 % participation).
- Reported methods for the total arsenic measurements included isotope dilution inductively coupled plasma mass spectrometry (ID-ICP-MS), ICP-MS in kinetic energy discrimination (KED) mode, EPA method 6010B that uses ICP optical emission spectrometry (OES), and liquid chromatography mass spectrometry (LC-MS).
- Reported methods for arsenic speciation included LC-MS and ID-ICP-MS. One laboratory did not report the method of analysis used.

## Human Metabolites Technical Recommendations

Due to the limited number of participants in the metabolites study, a sound statistical analysis of the results cannot be performed, especially for arsenic species. General recommendations are provided for tAs and arsenic speciation studies that had five and three participants, respectively.

- tAs is four times higher in Human Urine A (double digit ng/mL) than in Human Urine B (single digit ng/mL). The number of laboratories that reported results below the detection limit is one and three for Human Urine A and Human Urine B, respectively, indicating a greater challenge in measuring arsenic at the single ng/g level in Urine B. The larger consensus variability for tAs in Urine B (> 100 % RSD) compared to Urine A (14 % RSD) is also a result of lower tAs concentration in Human Urine B.
- **Figure 2-12** shows that tAs results of all participants overlap the band of the target values and the results are within the band of consensus, demonstrating the proficiency of participating in the determination of tAs in biological fluids such as urine. However, the measurement of tAs at single ng/g level is still a challenge, since only two laboratories submitted results that are above the detection limit for Human Urine B.
  - In view of the challenges for the detection of low levels of arsenic in metabolites such as urine, sample dilution should be minimized to increase the analyte contents in measurement samples.
  - With minimum sample dilution, however, matrix effects may become significant for urine analyses. A matrix-matched calibration curve can be used to alleviate interferences from the matrix.

- The U.S. Centers for Disease Control and Prevention (CDC) method of measurement of urine should be considered as a template in developing an in-house method for the determination of total arsenic in urine.<sup>5</sup>
- AsIII, AsV, and iAs are related analytes, as iAs is the sum of AsIII and AsV. Since the laboratories reported iAs also reported AsIII and AsV, iAs values are assumed to be determined from the sum of measured AsIII and AsV. Only one laboratory reported results that are above detection limits for iAs, AsIII, and AsV in both urine samples.
  - Because the iAs in the two urine samples is below 1 ng/mL, the challenge in detecting AsIII, AsV, and iAs is obvious in light of the challenge in detecting tAs at single ng/mL levels discussed above.
  - When LC-ICP-MS is used for arsenic speciation measurements, addition of organic solvent to the mobile phase can increase the sensitivity of the measurement, resulting in a lower detection limit for the measurand.
- Of the three organic arsenic species (AB, MMA, and DMA), only AB in Human Urine A is present at double digit ng/mL level. The mass fractions of MMA and DMA in both urine samples and AB in Human Urine B are found at or below single digit ng/mL levels as arsenic, and only two laboratories reported results above detection limits. A sound analysis of the results cannot be made because of the limited number of participants in the study. However, the above recommendation on using organic solvent in the mobile phase to enhance sensitivity for improvement of the detection limit applies to organo-arsenic measurements as well.
- ID-ICP-MS is not a practical method for tAs or arsenic speciation because arsenic is monoisotopic. Similarly, LC-MS for arsenic speciation is likely a mistake in reporting by the participant. The limited number of participants, however, does not permit evaluation of the results based on analytical method.

<sup>&</sup>lt;sup>5</sup> U.S. Centers for Disease Control and Prevention (CDC) Laboratory Procedure Manual.

https://wwwn.cdc.gov/nchs/data/nhanes/2013-2014/labmethods/UM\_UMS\_UTAS\_UTASS\_H\_MET.pdf (accessed Aug. 2018).

| National Institute of Standards & Technolog | gy |
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|                               | HAMQAP Ex     | ercise 1          | - Toxic Elem   | ents (Hu   | nan Metab       | olites)           |              |                |                |                   |                   |
|-------------------------------|---------------|-------------------|----------------|--|-----------------|-------------------|--------------|----------------|----------------|-------------------|-------------------|
|                               | Lab Code:     | NIST              | _              | 1. You   | Results         |                   | 2. 0         | Community R    | esults         | 3. Ta             | arget             |
| Analyte                       | Sample        | Units             | x <sub>i</sub> | s <sub>i</sub>   | $Z'_{\rm comm}$ | $Z_{\text{NIST}}$ | N            | <i>x</i> *     | <i>s</i> *     | x <sub>NIST</sub> | U                 |
| Total Arsenic                 | Human Urine A | µg/L              | 13.4           | 0.7  |                 |                   | 5            | 13.2           | 1.9            | 13.4              | 1.4               |
| Total Arsenic                 | Human Urine B | µg/L              |                |  |                 |                   | 2            | 2.2            | 2.3            |                   |                   |
| Total Inorganic Arsenic (iAs) | Human Urine A | µg/L              |                |  |                 |                   | 2            | 0.14           | 0.59           |                   |                   |
| Total Inorganic Arsenic (iAs) | Human Urine B | µg/L              |                |  |                 |                   | 2            | 0.2            | 0.6            |                   |                   |
| Arsenic III                   | Human Urine A | µg/L              |                |  |                 |                   | 2            | 0.06           | 0.23           |                   |                   |
| Arsenic III                   | Human Urine B | µg/L              |                |  |                 |                   | 2            | 0.07           | 0.29           |                   |                   |
| Arsenic V                     | Human Urine A | µg/L              | 0.346          | 0.076  |                 |                   | 3            | 0.08           | 0.35           | 0.346             | 0.151             |
| Arsenic V                     | Human Urine B | µg/L              |                |  |                 |                   | 2            | 0.1            | 0.5            |                   |                   |
| Monomethylarsonic acid (MMA)  | Human Urine A | µg/L              | 0.559          | 0.071  |                 |                   | 3            | 0.12           | 0.45           | 0.559             | 0.141             |
| Monomethylarsonic acid (MMA)  | Human Urine B | µg/L              |                |  |                 |                   | 2            | 0.14           | 0.61           |                   |                   |
| Dimethylarsinic acid (DMA)    | Human Urine A | µg/L              | 3.15           | 0.43   |                 |                   | 3            | 1.9            | 0.2            | 3.15              | 0.85              |
| Dimethylarsinic acid (DMA)    | Human Urine B | µg/L              |                |  |                 |                   | 2            | 1.34           | 0.28           |                   |                   |
| Arsenobetaine (AB)            | Human Urine A | µg/L              | 11.00          | 0.31   |                 |                   | 4            | 10.4           | 1.2            | 11.00             | 0.62              |
| Arsenobetaine (AB)            | Human Urine B | µg/L              |                |  |                 |                   | 2            | 0.342          | 0.099          |                   |                   |
|                               |               | $x_i$             | Mean of repo   | orted value  | s               | N                 | Number of a  | quantitative   | $x_{\rm NIST}$ | NIST-assess       | ed value          |
|                               |               | s <sub>i</sub>    | Standard dev   | Standard deviation of reported values Z'-score with respect to community |                 |                   | values repor | ted            | U              | expanded unc      | certainty         |
|                               |               | $Z'_{\rm comm}$   | Z'-score with  |  |                 |                   | Robust mean  | n of reported  |                | about the NIS     | ST-assessed value |
|                               |               |                   | consensus      |  |                 |                   | values       |                |                |                   |                   |
|                               |               | $Z_{\text{NIST}}$ | Z-score with   | respect to   | NIST value      | <i>s</i> *        | Robust stand | dard deviation |                |                   |                   |

|            |        |          |             |             | ,      | Total Ars | enic (tAs)           |             |        |      |      |  |  |
|------------|--------|----------|-------------|-------------|--------|-----------|----------------------|-------------|--------|------|------|--|--|
|            |        |          | Huma        | n Urine A   | (µg/L) |           | Human Urine B (µg/L) |             |        |      |      |  |  |
|            | Lab    | Α        | В           | С           | Avg    | SD        | Α                    | В           | С      | Avg  | SD   |  |  |
|            | Target |          |             |             | 13.37  | 1.40      |                      |             |        |      |      |  |  |
|            | A002   |          |             |             |        |           |                      |             |        |      |      |  |  |
|            | A030   |          |             |             |        |           |                      |             |        |      |      |  |  |
|            | A034   | 15       | 15          | 14          | 14.67  | 0.58      | < 12.5               | < 12.5      | < 12.5 |      |      |  |  |
| ts         | A039   | < 1000   | < 1000      | < 1000      |        |           | < 1000               | < 1000      | < 1000 |      |      |  |  |
| Ins        | A049   |          |             |             |        |           |                      |             |        |      |      |  |  |
| ividual Re | A059   | 12.81    | 10.98       | 13.58       | 12.46  | 1.34      | 2.72                 | 2.7         | 2.75   | 2.72 | 0.03 |  |  |
|            | A070   |          |             |             |        |           |                      |             |        |      |      |  |  |
|            | A079   |          |             |             |        |           |                      |             |        |      |      |  |  |
| ndi        | A080   |          |             |             |        |           |                      |             |        |      |      |  |  |
| I          | A083   |          |             |             |        |           |                      |             |        |      |      |  |  |
|            | A086   |          |             |             |        |           |                      |             |        |      |      |  |  |
|            | A088   | 12.22    | 11.96       | 11.9        | 12.03  | 0.17      | 1.67                 | 1.64        | 1.59   | 1.64 | 0.04 |  |  |
|            | A091   |          |             |             |        |           |                      |             |        |      |      |  |  |
|            | A092   | 12.7     | 14.4        | 13.4        | 13.5   | 0.85      | < 10.0               | < 10.0      | < 10.0 |      |      |  |  |
| ty         |        | ıs Mean  |             | 13.16       |        | Consensu  | ıs Mean              |             | 2.18   |      |      |  |  |
| uni<br>lts |        | Consensu | is Standard | l Deviation | 1.89   |           | Consensu             | is Standard | 2.34   |      |      |  |  |
| nm<br>esu  |        | Maximun  | 1           |             | 14.67  |           | Maximun              | 1           | 2.72   |      |      |  |  |
| R          |        | Minimum  | L           |             | 12.03  |           | Minimum              |             |        | 1.64 |      |  |  |
| •          |        | Ν        |             |             | 4      |           | Ν                    |             |        | 2    |      |  |  |

 Table 2-10.
 Data summary table for total arsenic (tAs) in human urine.

Exercise HAMQAP Exercise 1 - Human Metabolites Sample: Human Urine A Measurand: Total Arsenic



**Figure 2-7.** Total arsenic (tAs) in Human Urine A (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \le 2$ . The red shaded region represents the NIST range of tolerance, which encompasses the NIST-determined value bounded by its uncertainty ( $U_{NIST}$ ) and represents the range that results in an acceptable  $Z_{NIST}| \le 2$ .

|             |        |          |             |             | Tota   | l Inorgani | c Arsenic            | (iAs)       |             |       |       |  |  |
|-------------|--------|----------|-------------|-------------|--------|------------|----------------------|-------------|-------------|-------|-------|--|--|
|             |        |          | Humai       | n Urine A   | (µg/L) |            | Human Urine B (µg/L) |             |             |       |       |  |  |
|             | Lab    | Α        | В           | С           | Avg    | SD         | Α                    | В           | С           | Avg   | SD    |  |  |
|             | Target |          |             |             |        |            |                      |             |             |       |       |  |  |
| ts          | A034   |          |             |             |        |            |                      |             |             |       |       |  |  |
| sul         | A039   |          |             |             |        |            |                      |             |             |       |       |  |  |
| Re          | A049   |          |             |             |        |            |                      |             |             |       |       |  |  |
| vidual      | A059   | 0.311    | 0.27        | 0.265       | 0.282  | 0.025      | 0.363                | 0.363       | 0.417       | 0.381 | 0.031 |  |  |
|             | A070   |          |             |             |        |            |                      |             |             |       |       |  |  |
| ndi         | A088   | 0        | 0           | 0           | 0      | 0          | 0                    | 0           | 0           | 0     | 0     |  |  |
| I           | A091   |          |             |             |        |            |                      |             |             |       |       |  |  |
|             | A092   | < 10.0   | < 10.0      | < 10.0      |        |            | < 10.0               | < 10.0      | < 10.0      |       |       |  |  |
| ty          |        | Consensu | is Mean     |             | 0.141  |            | Consensu             | ıs Mean     |             | 0.191 |       |  |  |
| unit<br>lts |        | Consensu | is Standard | l Deviation | 0.591  |            | Consensu             | is Standard | l Deviation | 0.604 |       |  |  |
| um          |        | Maximum  | ı           |             | 0.282  |            | Maximun              | n           | 0.381       |       |       |  |  |
| Ron         |        | Minimum  |             |             | 0      |            | Minimum              | l           | 0           |       |       |  |  |
| )           |        | Ν        |             |             | 2 N    |            |                      | Ν           |             |       |       |  |  |

**Table 2-11.** Data summary table for total inorganic arsenic (iAs) in human urine.

 Table 2-12.
 Data summary table for arsenous acid (AsIII) in human urine.

|             |        |          |             |             | Α      | rsenous A | Acid (AsIII          | [)          |             |       |       |  |  |
|-------------|--------|----------|-------------|-------------|--------|-----------|----------------------|-------------|-------------|-------|-------|--|--|
|             |        |          | Huma        | n Urine A   | (µg/L) |           | Human Urine B (µg/L) |             |             |       |       |  |  |
|             | Lab    | Α        | В           | С           | Avg    | SD        | Α                    | В           | С           | Avg   | SD    |  |  |
|             | Target |          |             |             |        |           |                      |             |             |       |       |  |  |
| ts          | A034   |          |             |             |        |           |                      |             |             |       |       |  |  |
| sul         | A039   |          |             |             |        |           |                      |             |             |       |       |  |  |
| vidual Re   | A049   |          |             |             |        |           |                      |             |             |       |       |  |  |
|             | A059   | 0.132    | 0.098       | 0.113       | 0.114  | 0.017     | 0.127                | 0.14        | 0.157       | 0.141 | 0.015 |  |  |
|             | A070   |          |             |             |        |           |                      |             |             |       |       |  |  |
| ndi         | A088   | 0        | 0           | 0           | 0      | 0         | 0                    | 0           | 0           | 0     | 0     |  |  |
| I           | A091   |          |             |             |        |           |                      |             |             |       |       |  |  |
|             | A092   | < 5.0    | < 5.0       | < 5.0       |        |           | < 5.0                | < 5.0       | < 5.0       |       |       |  |  |
| ty          |        | Consensu | ıs Mean     |             | 0.057  |           | Consensu             | is Mean     |             | 0.071 |       |  |  |
| umi<br>lts  |        | Consensu | is Standard | l Deviation | 0.226  |           | Consensu             | is Standard | l Deviation | 0.289 |       |  |  |
| nmu<br>ssul |        | Maximum  | ı           |             | 0.114  |           | Maximun              | ı           | 0.141       |       |       |  |  |
| R O         |        | Minimum  |             |             | 0      |           | Minimum              |             | 0           |       |       |  |  |
| •           |        | Ν        |             |             | 2      |           | Ν                    |             |             | 2     |       |  |  |

|            |           |          |             |           |        | Arsenic A | Acid (AsV)           |            |             |       |       |  |  |
|------------|-----------|----------|-------------|-----------|--------|-----------|----------------------|------------|-------------|-------|-------|--|--|
|            |           |          | Huma        | n Urine A | (µg/L) |           | Human Urine B (µg/L) |            |             |       |       |  |  |
|            | Lab       | Α        | В           | С         | Avg    | SD        | Α                    | В          | С           | Avg   | SD    |  |  |
|            | Target    |          |             |           | 0.35   | 0.15      |                      |            |             |       |       |  |  |
| ţ          | A034      |          |             |           |        |           |                      |            |             |       |       |  |  |
| sul        | A039      |          |             |           |        |           |                      |            |             |       |       |  |  |
| Re         | A049      |          |             |           |        |           |                      |            |             |       |       |  |  |
| vidual     | A059      | 0.178    | 0.172       | 0.153     | 0.168  | 0.013     | 0.236                | 0.223      | 0.260       | 0.240 | 0.019 |  |  |
|            | A070      |          |             |           |        |           |                      |            |             |       |       |  |  |
| ndi        | A088      | 0        | 0           | 0         | 0      | 0         | 0                    | 0          | 0           | 0     | 0     |  |  |
| I          | A091      |          |             |           |        |           |                      |            |             |       |       |  |  |
|            | A092      | < 5.0    | < 5.0       | < 5.0     |        |           | < 5.0                | < 5.0      | < 5.0       |       |       |  |  |
| ty         |           | Consensu | ıs Mean     |           | 0.08   |           | Consensu             |            | 0.120       |       |       |  |  |
| uni<br>lts |           | Consensu | is Standard | Deviation | 0.35   |           | Consensu             | s Standard | l Deviation | 0.502 |       |  |  |
| nmi        |           | Maximun  | ı           |           | 0.17   |           | Maximun              | ı          |             | 0.24  |       |  |  |
| R.         |           | Minimum  |             |           | 0      |           | Minimum              |            |             | 0     |       |  |  |
| •          | N Minimum |          |             |           |        |           | Ν                    |            |             | 2     |       |  |  |

Table 2-13. Data summary table for arsenic acid (AsV) in human urine.

 Table 2-14.
 Data summary table for monomethylarsinic acid (MMA) in human urine.

|            |                |          |             |             | Monor  | nethylars | onic acid (          | MMA)        |             |      |      |  |  |
|------------|----------------|----------|-------------|-------------|--------|-----------|----------------------|-------------|-------------|------|------|--|--|
|            |                |          | Huma        | n Urine A   | (µg/L) |           | Human Urine B (µg/L) |             |             |      |      |  |  |
|            | Lab            | Α        | В           | С           | Avg    | SD        | Α                    | В           | С           | Avg  | SD   |  |  |
| ts         | Target         |          |             |             | 0.56   | 0.14      |                      |             |             |      |      |  |  |
| sul        | A039           |          |             |             |        |           |                      |             |             |      |      |  |  |
| lual Re    | A059           | 0.254    | 0.187       | 0.252       | 0.23   | 0.04      | 0.304                | 0.288       | 0.271       | 0.29 | 0.02 |  |  |
|            | A070           |          |             |             |        |           |                      |             |             |      |      |  |  |
| vid        | A088           | 0        | 0           | 0           | 0      | 0         | 0                    | 0           | 0           | 0    | 0    |  |  |
| ndi        | A091           |          |             |             |        |           |                      |             |             |      |      |  |  |
| I          | A092           | < 5.0    | < 5.0       | < 5.0       |        |           | < 5.0                | < 5.0       | < 5.0       |      |      |  |  |
| ty         | Consensus Mean |          |             |             | 0.12   |           | Consensu             | ıs Mean     | 0.14        |      |      |  |  |
| uni<br>lts |                | Consensu | is Standard | l Deviation | 0.45   |           | Consensu             | is Standard | l Deviation | 0.61 |      |  |  |
| nmı        |                | Maximun  | Maximum     |             |        |           | Maximun              | 1           | 0.29        |      |      |  |  |
| R          |                | Minimum  |             |             | 0      |           | Minimum              |             |             | 0    |      |  |  |
| •          |                | Ν        |             |             | 2      |           | Ν                    |             |             | 2    |      |  |  |

|            |        |          |             |             | Dim    | ethylarsin | nic acid (D          | MA)        |             |       |       |  |  |
|------------|--------|----------|-------------|-------------|--------|------------|----------------------|------------|-------------|-------|-------|--|--|
|            |        |          | Human       | n Urine A   | (µg/L) |            | Human Urine B (µg/L) |            |             |       |       |  |  |
|            | Lab    | Α        | В           | С           | Avg    | SD         | Α                    | В          | С           | Avg   | SD    |  |  |
| ts         | Target |          |             |             | 3.15   | 0.85       |                      |            |             |       |       |  |  |
| sul        | A039   |          |             |             |        |            |                      |            |             |       |       |  |  |
| vidual Re  | A059   | 2.026    | 1.54        | 2.008       | 1.86   | 0.28       | 1.404                | 1.402      | 1.415       | 1.407 | 0.007 |  |  |
|            | A070   |          |             |             |        |            |                      |            |             |       |       |  |  |
|            | A088   | 1.936    | 1.911       | 1.925       | 1.92   | 0.01       | 1.287                | 1.251      | 1.252       | 1.263 | 0.021 |  |  |
| ndi        | A091   |          |             |             |        |            |                      |            |             |       |       |  |  |
| I          | A092   | < 5.0    | < 5.0       | < 5.0       |        |            | < 5.0                | < 5.0      | < 5.0       |       |       |  |  |
| ty         |        | Consensu | is Mean     |             | 1.89   |            | Consensu             | s Mean     |             | 1.335 |       |  |  |
| uni<br>lts |        | Consensu | is Standard | l Deviation | 0.20   |            | Consensu             | s Standard | l Deviation | 0.278 |       |  |  |
| nm         |        | Maximun  | ı           |             | 1.92   |            | Maximum              | ı          |             | 1.407 |       |  |  |
| R          |        | Minimum  |             |             | 1.86   |            | Minimum              |            |             | 1.263 |       |  |  |
| •          |        | N        |             |             |        |            | Ν                    |            |             | 2     |       |  |  |

**Table 2-15.** Data summary table for dimethylarsinic acid (DMA) in human urine.

Table 2-16. Data summary table for arsenobetaine (AB) in human urine.

|            |        |          |            |             | I      | Arsenobe | etaine (AB)          |             |             |       |       |  |  |
|------------|--------|----------|------------|-------------|--------|----------|----------------------|-------------|-------------|-------|-------|--|--|
|            |        |          | Huma       | n Urine A   | (µg/L) |          | Human Urine B (µg/L) |             |             |       |       |  |  |
|            | Lab    | Α        | В          | С           | Avg    | SD       | Α                    | В           | С           | Avg   | SD    |  |  |
| ts         | Target |          |            |             | 11.05  | 0.62     |                      |             |             |       |       |  |  |
| sul        | A039   |          |            |             |        |          |                      |             |             |       |       |  |  |
| vidual Re  | A059   | 10.74    | 8.12       | 10.49       | 9.78   | 1.45     | 0.332                | 0.292       | 0.309       | 0.311 | 0.02  |  |  |
|            | A070   |          |            |             |        |          |                      |             |             |       |       |  |  |
|            | A088   | 10.29    | 10.05      | 9.97        | 10.10  | 0.16     | 0.384                | 0.394       | 0.341       | 0.373 | 0.028 |  |  |
| ndi        | A091   |          |            |             |        |          |                      |             |             |       |       |  |  |
| I          | A092   | 11.3     | 11.3       | 11.0        | 11.20  | 0.17     | < 5.0                | < 5.0       | < 5.0       |       |       |  |  |
| ty         |        | Consensu | s Mean     |             | 10.36  |          | Consensu             | ıs Mean     |             | 0.342 |       |  |  |
| uni<br>lts |        | Consensu | s Standard | l Deviation | 1.23   |          | Consensu             | is Standard | 1 Deviation | 0.099 |       |  |  |
| nm<br>esu  |        | Maximum  | 1          |             | 11.2   |          | Maximum              |             |             | 0.373 |       |  |  |
| Cor<br>R   |        | Minimum  |            |             | 9.78   |          | Minimum              |             |             | 0.311 |       |  |  |
| •          |        | Ν        |            |             | 3      |          | Ν                    |             |             | 2     |       |  |  |

Exercise HAMQAP Exercise 1 - Human Metabolites Sample: Human Urine A Measurand: Arsenobetaine (AB)



**Figure 2-8.** Arsenobetaine (AB) in Human Urine A (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \leq 2$ . The red shaded region represents the NIST range of tolerance, which encompasses the NIST-determined value bounded by its uncertainty ( $U_{NIST}$ ) and represents the range that results in an acceptable  $Z_{NIST}$  score,  $|Z_{NIST}| \leq 2$ .

## Toxic Elements Overall Study Comparison

The following recommendations are based on results obtained from the participants in both portions of this study (dietary intake and human metabolism).

- The low participation rate and the large number of results reported above or below both the consensus and target ranges suggest that the participating laboratories may not have adequate methods for the matrices and/or the arsenic species probed in this study.
  - Sample preparation methods should be well established before analyzing unknown samples. Use established quality control materials (SRM, CRM, RM and in-house materials) and find established methods of analyses.
  - Calibration curves must be linear and include the lowest and highest values expected to be measured in the sample solutions. When concentrations in sample solutions are low, use of an organic solvent in the mobile phase may enhance sensitivity for improvement of the detection limit for arsenic.
- Matrix matched standards used for calibration curves and minimal dilutions of samples may also be applied for measurement of unknown samples to improve the detection of the analyte in the sample.
  - Calculation errors are often a cause for incorrect results. Using a quality assurance material (CRM, SRM, RM), or in-house prepared material, to establish that a method is in control will also help find calculation errors. Once a method and quality assurance material appear to be in control, be sure results are reported in the correct units.
- Measurement methods must be correctly reported.

#### **SECTION 3: WATER-SOLUBLE VITAMINS (Vitamin B<sub>12</sub>)**

#### Study Overview

In this study, participants were provided with SRM 3233 Fortified Breakfast Cereal and SRM 3280 Multivitamin/Multielement Tablets for dietary intake, and Human Serum C and SRM 968d Fat-Soluble Vitamins, Carotenoids, and Cholesterol in Human Serum (Level 2). Participants were asked to use in-house analytical methods to determine the mass fraction of vitamin  $B_{12}$  (as cyanocobalamin) in each of the matrices and report values in mg/g, on an as-received basis. Deficiency of vitamin  $B_{12}$  is linked to a variety of health problems including hematological disorders and neurological disorders. The human body cannot synthesize vitamin  $B_{12}$ , and therefore, dietary intake is essential for maintaining adequate health. One mechanism for understanding vitamin  $B_{12}$  health status is through measurement of vitamin  $B_{12}$  in serum.

#### **Dietary Intake Sample Information**

*Cereal.* Participants were provided with one bottle containing 60 g of ground cereal. Before use, participants were instructed to thoroughly mix the contents by rotating and/or rolling the bottle prior to removal of a test sample for analysis, and to use a sample size of at least 2 g. Participants were asked to store the material at controlled room temperature, 10 °C to 30 °C, and to prepare three samples and report three values from the single bottle provided. The approximate analyte level was not reported to participants prior to the study. The target value for vitamin B<sub>12</sub> in SRM 3233 was determined at NIST using LC-ICP-MS and results from collaborating laboratories. The NIST-determined value and uncertainty for vitamin B<sub>12</sub> in SRM 3233 are provided in the table below, both on a dry-mass basis and on an as-received basis accounting for moisture of the material (1.7 %).

|   | NIST-Determined Mass Fra | <u>ction in SRM 3233 (mg/kg)</u> |
|---|--------------------------|----------------------------------|
| Analyte                                     | (dry-mass basis)         | (as-received basis)              |
| Vitamin B <sub>12</sub><br>(Cyanocobalamin) | $0.210\pm0.040$          | $0.206\pm0.039$                  |

*Multivitamin.* Participants were provided with three bottles, each containing 30 multivitamin/multielement tablets. Before use, participants were instructed to grind all tablets within a bottle, mix the resulting powder thoroughly, and to use a sample size of at least 4.5 g. Participants were asked to store the material at controlled room temperature, 10 °C to 30 °C, and to prepare one sample and report one value from each bottle provided. The approximate analyte level was not reported to participants prior to the study. The certified value for vitamin B<sub>12</sub> in SRM 3280 was determined at NIST using LC-ICP-MS and data from collaborating laboratories. The certified value and uncertainty for vitamin B<sub>12</sub> in SRM 3280 is provided in the table below, both on a dry-mass basis and on an as-received basis accounting for moisture of the material (1.37 %).

|   | NIST-Determined Mass Fraction in SRM 3280 (mg/kg) |                     |  |  |  |  |  |
|---|---|---------------------|--|--|--|--|--|
| Analyte                                     | (dry-mass basis)                                  | (as-received basis) |  |  |  |  |  |
| Vitamin B <sub>12</sub><br>(Cyanocobalamin) | 4.8 ±1.0  | $4.73\pm0.99$       |  |  |  |  |  |

## **Dietary Intake Study Results**

- Twenty-nine laboratories enrolled in this exercise and received samples to measure vitamin B<sub>12</sub> (as cyanocobalamin). Thirteen laboratories reported results for the cereal (45 % participation) and 17 laboratories reported results for the multivitamin (41 % participation).
  - The consensus mean for vitamin  $B_{12}$  in the cereal was above the target range, while the consensus mean for vitamin  $B_{12}$  in the multivitamin was within the target range. The between-laboratory variability was very high for both the cereal and the multivitamin (120 % RSD and 69 % RSD, respectively).
  - For the cereal measurements, two laboratories reported using liquid chromatography with absorbance detection (LC-UV), one laboratory reported using a microbiological assay (MBA), and 10 laboratories did not report the measurement method.
  - For the multivitamin measurements, three laboratories reported using LC-UV, one laboratory reported MBA, and 13 laboratories did not report the measurement method.

## Dietary Intake Technical Recommendations

The following recommendations are based on results obtained from the participants in this study. Because most laboratories did not report the method used for analysis, only general recommendations are provided.

- As shown in **Figure 3-3**, many laboratories reported data for vitamin B<sub>12</sub> that was within the NIST target range for the multivitamin sample but was higher than the NIST target range for the cereal. **Figures 3-1 and 3-2** also show that fewer laboratories overlap the NIST uncertainty range for the cereal than for the multivitamin.
  - The large between-laboratory variability (69 % RSD and 120 % RSD for multivitamin and cereal, respectively), shows that the determination of cyanocobalamin in dietary intake samples is a challenge.
  - The cyanocobalamin standard used for calibration must be characterized for purity, including evaluation of moisture content.
  - The extraction efficiency of cyanocobalamin must be evaluated to ensure quantitative extraction of the analyte from the sample matrix.
  - Cyanocobalamin may decompose upon exposure to ultraviolet light. Samples and standards should be prepared in a room with amber or attenuated lighting.
  - The calculations and reporting units must be verified prior to submission. For example, three laboratories reported results that are either 1000 times higher or lower than the target, which indicates that a dilution factor may have been forgotten during the calculation of the final result, or results were reported in the wrong units.
- Use of matrix CRMs for method validation and quality assurance of the measurement process is recommended.

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| HAMQAP Exercise 1 - Water-Soluble Vitamins (Dietary Intake) |                                   |                   |                                       |                 |                 |                   |                           |                |                        |                                       |       |  |
|---|-----------------------------------|-------------------|---------------------------------------|-----------------|-----------------|-------------------|---------------------------|----------------|------------------------|---------------------------------------|-------|--|
| Lab Code:   |                                   |                   |                                       | 1. Your Results |                 | 2. C              | ommunity Re               | esults 3.      |                        | Target                                |       |  |
| Analyte   | Sample                            | Units             | x <sub>i</sub>                        | s <sub>i</sub>  | $Z'_{\rm comm}$ | Z <sub>NIST</sub> | N                         | <i>x</i> *     | <i>s</i> *             | x <sub>NIST</sub>                     | U     |  |
| Total Vitamin B12   | SRM 3233                          |                   |                                       |                 |                 |                   | _                         |                |                        |                                       |       |  |
| (as Cyanocobalamin)   | Fortified Breakfast Cereal        | mg/kg             | 0.206                                 | 0.020           |                 |                   | 14                        | 0.5            | 0.6                    | 0.206                                 | 0.039 |  |
| Total Vitamin B12   | SRM 3280                          |                   |                                       |                 |                 |                   |                           |                |                        |                                       |       |  |
| (as Cyanocobalamin)   | Multivitamin/Multielement Tablets | mg/kg             | 4.73                                  | 0.50            |                 |                   | 17                        | 3.9            | 2.7                    | 4.73                                  | 0.99  |  |
|   |                                   | $x_i$             | Mean of reported values               |                 |                 | Ν                 | Number of quantitative    |                | x <sub>NIST</sub>      | x <sub>NIST</sub> NIST-assessed value |       |  |
|   |                                   | s <sub>i</sub>    | Standard deviation of reported values |                 |                 |                   | values report             | ted            | U expanded uncertainty |                                       |       |  |
|   |                                   | $Z'_{\rm comm}$   | Z'-score with respect to community    |                 |                 | <i>x</i> *        | * Robust mean of reported |                |                        | about the NIST-assessed value         |       |  |
|   |                                   |                   | consensus                             |                 |                 | values            |                           |                |                        |                                       |       |  |
|   |                                   | $Z_{\text{NIST}}$ | Z-score with respect to NIST value    |                 |                 | <i>s</i> *        | Robust stand              | lard deviation |                        |                                       |       |  |

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**Table 3-2.** Data summary table for vitamin  $B_{12}$  in cereal and multivitamin. Data points highlighted in red have been flagged as potential outliers (e.g., difference from reference value, Grubb and/or Cochran) by the NIST software package.

|              |        | Total Vitamin B12 (as Cyanocobalamin)       |          |          |       |               |   |                             |         |        |          |  |  |
|--------------|--------|---|----------|----------|-------|---------------|---|-----------------------------|---------|--------|----------|--|--|
|              |        | SRM 3233 Fortified Breakfast Cereal (mg/kg) |          |          |       |               | SRM 3280 Multivitamin/Multielement Tablets<br>(mg/kg) |                             |         |        |          |  |  |
|              | Lab    | Α   | В        | С        | Avg   | SD            | Α   | В                           | С       | Avg    | SD       |  |  |
|              | Target |   |          |          | 0.21  | 0.04          |   |                             |         | 4.73   | 0.99     |  |  |
|              | A001   |   |          |          |       |               |   |                             |         |        |          |  |  |
|              | A007   | 3.04  | 2.94     | 2.91     | 2.96  | 0.07          | 5.28  | 5.04                        | 5.11    | 5.14   | 0.12     |  |  |
|              | A008   | 188   | 202      | 190      | 193   | 8             | 4.31  | 4.63                        | 4.77    | 4.57   | 0.24     |  |  |
|              | A010   |   |          |          |       |               | 5.14  | 4.29                        | 4.47    | 4.63   | 0.45     |  |  |
|              | A012   |   |          |          |       |               |   |                             |         |        |          |  |  |
|              | A013   | 0.16  | 0.169    | 0.168    | 0.166 | 0.005         | 4.49  | 4.74                        | 4.92    | 4.72   | 0.22     |  |  |
|              | A026   |   |          |          |       |               |   |                             |         |        |          |  |  |
|              | A027   |   |          |          |       |               |   |                             |         |        |          |  |  |
|              | A029   | 1.6   | 1.4      | 2        | 1.667 | 0.306         | 3.8   | 3.8                         | 3.9     | 3.83   | 0.06     |  |  |
|              | A030   |   |          |          |       |               |   |                             |         |        |          |  |  |
| s            | A037   |   |          |          |       |               |   |                             |         |        |          |  |  |
| sult         | A041   |   |          |          |       | _             |   |                             |         |        |          |  |  |
| Re           | A043   | 0.105                                       | <u> </u> | <u> </u> | 0.105 | a aa <b>a</b> | 1.40  | - 15                        | 1.40    |        | <u> </u> |  |  |
| ual          | A047   | 0.187                                       | 0.184    | 0.189    | 0.187 | 0.003         | 1.48  | 1.45                        | 1.42    | 1.45   | 0.03     |  |  |
| vidı         | A049   | 2.13  | 2.15     | 2.09     | 2.123 | 0.031         | 15.51   | 15.25                       | 15.1    | 15.29  | 0.21     |  |  |
| ndi          | A050   | 140   | 141      | 129      | 13/   | -0.001        | 0   | 0                           | 0.01    | 0.01   | 0        |  |  |
| IJ           | A051   | 0.145                                       | 0.141    | 0.14     | 0.141 | 0.001         | 3.89  | 3.94                        | 4.07    | 3.97   | 0.1      |  |  |
|              | A055   |   |          |          |       |               |   |                             |         |        |          |  |  |
|              | A050   | 0.42  | 0.30     | 0.44     | 0.417 | 0.025         | 3.07  | 2.73                        | 2.67    | 2 82   | 0.22     |  |  |
|              | A058   | 0.42  | 0.39     | 0.44     | 0.417 | 0.025         | 1.25  | -1.34                       | 2.07    | 2.02   | 0.22     |  |  |
|              | A061   | < 0.210                                     | < 0.200  | < 0.221  | 0.215 | 0.007         | < 0.300   | <ul> <li>- 0.300</li> </ul> | - 0.300 | 1.5    | 0.04     |  |  |
|              | A062   | < 0.500                                     | < 0.500  | < 0.500  |       |               | 5.66  | 5.23                        | 4 87    | 5.25   | 0.4      |  |  |
|              | A070   | 25.53                                       | 16.17    | 25.68    | 22.46 | 5 448         | 8 58  | 8.02                        | 8 46    | 8 35   | 0.29     |  |  |
|              | A072   | 20.00                                       | 10.17    | 20.00    | 22.10 |               | 0.00  | 0.02                        | 0.10    | 0.00   | 0.27     |  |  |
|              | A074   |   |          |          |       |               | 4.36  | 4.42                        | 4.19    | 4.32   | 0.12     |  |  |
|              | A075   |   |          |          |       |               |   |                             |         |        |          |  |  |
|              | A076   | 0   |          |          | 0     |               | 0.004   | 0.005                       | 0.005   | 0.0047 | 0.00027  |  |  |
|              | A079   | 0.17  | 0.19     | 0.21     | 0.19  | 0.02          | 8   | 9                           | 9       | 8.67   | 0.58     |  |  |
| ly .         |        | Consensus Mean 0.479                        |          |          |       |               | Consensus Mean 3.94                                   |                             |         |        |          |  |  |
| u nit<br>lts |        | Consensus Standard Deviation 0.603          |          |          |       |               | Consensus Standard Deviation 2.74                     |                             |         |        |          |  |  |
| Ins          |        | Maximum                                     |          |          | 193.3 |               | Maximum   | ı                           |         | 15.29  |          |  |  |
| Re           |        | Minimum                                     |          |          | 0     |               | Minimum   |                             |         | 0      |          |  |  |
| 5            |        | Ν   |          |          | 12    |               | Ν   |                             |         | 16     |          |  |  |





**Figure 3-1.** Total vitamin B<sub>12</sub> (as cyanocobalamin) in SRM 3233 Fortified Breakfast Cereal (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents the consensus mean, and the green shaded region represents the consensus mean bounded by twice the consensus standard error. The solid red line represents the upper consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \le 2$ , with the lower range set at zero. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by its uncertainty ( $U_{NIST}$ ) and represents the range that results in an acceptable  $Z'_{NIST} | \le 2$ .




**Figure 3-2.** Total vitamin B<sub>12</sub> (as cyanocobalamin) in SRM 3280 Multivitamin/Multielement Tablets (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents the consensus mean, and the green shaded region represents the consensus mean bounded by twice the consensus standard error. The solid red line represents the upper consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \le 2$  with the lower range set at zero. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by its uncertainty ( $U_{NIST}$ ) and represents the range that results in an acceptable  $Z_{NIST}| \le 2$ .



HAMQAP Exercise 1 - Dietary Intake, Measurand: Total Vitamin B12 (as Cyanocobalamin) No. of laboratories: 13

**Figure 3-3.** Laboratory means for total vitamin B<sub>12</sub> (as cyanocobalamin) in SRM 3233 Fortified Breakfast Cereal and SRM 3280 Multivitamin/Multielement Tablets (sample/sample comparison view). In this view, the individual laboratory mean for one sample (SRM 3233) is compared to the mean for a second sample (SRM 3280). The solid red box represents the NIST range of tolerance for the two samples, cereal (x-axis) and multivitamin (y-axis), which encompasses the target values bounded by their uncertainties ( $U_{\text{NIST}}$ ) and represents the range that results in an acceptable  $Z_{\text{NIST}}$  score,  $|Z_{\text{NIST}}| \leq 2$ . The dotted blue box represents the consensus range of tolerance for SRM 3233 (x-axis) and SRM 3280 (y-axis), calculated as the values above and below the consensus means that result in an acceptable  $Z'_{\text{comm}}$  score,  $|Z'_{\text{comm}}| \leq 2$ .

#### Human Metabolites Sample Information

*Human Serum C.* Participants were provided with three vials, each containing 1 mL of frozen serum. The material was prepared from "off the shelf" serum and diluted with stripped serum to achieve a mid-level target for vitamin  $B_{12}$ . The serum was bottled in 1 mL aliquots and stored at -80 °C. Before use, participants were instructed to allow the material to thaw at room temperature for at least 30 min prior to sampling, use the material immediately after thawing, gently mix the contents prior to removal of a test portion for analysis, and use a sample size appropriate for their usual in-house method of analysis. Participants were asked to avoid exposing the material to direct UV light, to store the material between -20 °C and -80 °C, and to prepare one sample and report one value from each vial provided. The approximate analyte levels were not reported to participants prior to the study, and a target value for cyanocobalamin in Human Serum C has not been determined at NIST.

*Human Serum D.* Participants were provided with three vials, each containing 1 mL of frozen serum. Bovine thrombin and calcium chloride were added to convert the plasma to serum. The serum was dialyzed to remove bovine thrombin, calcium chloride, and anticoagulants. Salts were added back into the serum, and the material was pooled along with isotonic saline, blended, bottled in 1 mL aliquots, and stored at -80 °C. Before use, participants were instructed to allow the material to thaw at room temperature for at least 30 min prior to sampling, use the material immediately after thawing, gently mix the contents prior to removal of a test portion for analysis, and use a sample size appropriate for their usual in-house method of analysis. Participants were asked to avoid exposing the material to direct UV light, to store the material between -20 °C and -80 °C, and to prepare one sample and report one value from each vial provided. The approximate analyte levels were not reported to participants prior to the study, and a target value for cyanocobalamin in SRM 968d Level 2 has not been determined at NIST.

#### Human Metabolites Study Results

- Thirteen laboratories enrolled in this exercise and received samples to measure vitamin B<sub>12</sub> (as cyanocobalamin). Four laboratories reported results for both samples (31 % participation).
- The between-laboratory variability for both samples was extremely high (70 % RSD and > 100 % for Human Serum C and SRM 968d, respectively).
- Two laboratories reported using immunoassay for determination of vitamin B<sub>12</sub> in both samples (50 %). The immunoassay results were close to the consensus mean with low within-laboratory variation. One laboratory using immunoassay reported results below an LOQ of 150 pg/mL for SRM 968d.
- One laboratory reported using a microbiological assay for determination of vitamin B<sub>12</sub> in both samples (25 %). The results from this single laboratory were above consensus range of tolerance and had high within-laboratory variability for the sample of Human Serum C.
- One laboratory reported using mass spectrometry (MS) for determination of vitamin B<sub>12</sub> in both samples (liquid chromatography with MS for Human Serum C and isotope dilution inductively coupled plasma MS for SRM 968d) (25 %). This laboratory reported values of 0.0 pg/mL for all measurements.

### Human Metabolites Technical Recommendations

The following recommendations are based on results obtained from the participants in this study. The low response rate, however, does not enable a robust statistical analysis of the results reported for vitamin  $B_{12}$  in the human serum materials.

- Measurement of vitamin  $B_{12}$  (as cyanocobalamin) in human serum is challenging, due partly to the low pg/mL levels found in typical samples.
  - The low participation in this study may be a reflection on the difficulty of the measurement of vitamin B<sub>12</sub> in human serum. Many laboratories rely on measurements of homocysteine or methylmalonic acid for information about vitamin B<sub>12</sub> human health status.
  - The MS methods used are likely not sensitive enough to detect the typical levels of vitamin  $B_{12}$  in human serum.
  - Currently, immunoassay and microbiological assay approaches are more widely applied to measurement of vitamin B<sub>12</sub> in serum than MS methods. These approaches, however, require rigorous validation and use of appropriate controls to ensure accurate and precise measurements.
- The upward trend in **Figure 3-6** indicates potential bias of the methods used. Laboratories consistently measure in the same direction (high or low) when analyzing serum samples, which indicates that an independent control material (such as a certified reference material) could greatly benefit this community.

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|                     | Lab Code:                                   | Lab Code: NIST    |                |                | 1. Your Results |                   |    | 2. Cor        | nmunity Re  | 3. Target      |                |                  |
|---------------------|---|-------------------|----------------|----------------|-----------------|-------------------|----|---------------|-------------|----------------|----------------|------------------|
| Analyte             | Sample                                      | Units             | x <sub>i</sub> | s <sub>i</sub> | $Z'_{\rm comm}$ | $Z_{\text{NIST}}$ |    | Ν             | <i>x</i> *  | <i>s</i> *     | $x_{\rm NIST}$ | U                |
| Total Vitamin B12   |   |                   |                |                |                 |                   |    |               |             |                |                |                  |
| (as Cyanocobalamin) | Human Serum C                               | pg/mL             |                |                |                 |                   |    | 4             | 280         | 190            |                |                  |
| Total Vitamin B12   | SRM 968d Fat-Soluble Vitamins, Carotenoids, |                   |                |                |                 |                   |    |               |             |                |                |                  |
| (as Cyanocobalamin) | and Cholesterol in Human Serum (Level 2)    | pg/mL             |                |                |                 |                   |    | 3             | 110         | 180            |                |                  |
|                     |   | $x_i$             | Mean of rep    | orted value    | es              | N                 | Nι | umber of qua  | ntitative   | $x_{\rm NIST}$ | NIST-assesse   | ed value         |
|                     |   | s <sub>i</sub>    | Standard dev   | viation of re  | eported values  | 5                 | va | lues reported | ł           | U              | expanded unco  | ertainty         |
|                     |   | $Z'_{\rm comm}$   | Z'-score with  | n respect to   | o community     | <i>x</i> *        | Ro | obust mean o  | f reported  |                | about the NIS' | T-assessed value |
|                     |   |                   | consensus      |                |                 |                   | va | alues         |             |                |                |                  |
|                     |   | $Z_{\text{NIST}}$ | Z-score with   | respect to     | NIST value      | <i>s</i> *        | Ro | obust standar | d deviation |                |                |                  |

HAMQAP Exercise 1 - Water-Soluble Vitamins (Human Metabolites)

|            |        |          |            |           | Total Vita | min B12 | (as Cyanoc  | obalamin)  |           |     |    |  |  |
|------------|--------|----------|------------|-----------|------------|---------|---|------------|-----------|-----|----|--|--|
|            |        |          | Human      | Serum C ( | pg/mL)     |         | SRM 968d Fat-Soluble Vitamins, Carotenoids a<br>Cholesterol in Human Serum (Level 2) (pg/mL |            |           |     |    |  |  |
|            | Lab    | Α        | В          | С         | Avg        | SD      | Α   | В          | С         | Avg | SD |  |  |
|            | Target |          |            |           |            |         |   |            |           |     |    |  |  |
|            | A002   |          |            |           |            |         |   |            |           |     |    |  |  |
|            | A012   | 450      | 365        | 749       | 522        | 202     | 204   | 208        | 207       | 206 | 2  |  |  |
| s          | A030   |          |            |           |            |         |   |            |           |     |    |  |  |
| ult        | A049   |          |            |           |            |         |   |            |           |     |    |  |  |
| kes        | A050   |          |            |           |            |         |   |            |           |     |    |  |  |
| dual R     | A070   |          |            |           |            |         |   |            |           |     |    |  |  |
|            | A079   |          |            |           |            |         |   |            |           |     |    |  |  |
| livi       | A083   |          |            |           |            |         |   |            |           |     |    |  |  |
| Ind        | A086   |          |            |           |            |         |   |            |           |     |    |  |  |
|            | A101   | 292      | 310        | 312       | 305        | 11      | < 150   | < 150      | < 150     |     |    |  |  |
|            | A103   | 282      | 277        | 278       | 279        | 3       | 133   | 139        | 123       | 132 | 8  |  |  |
|            | A104   |          |            |           |            |         |   |            |           |     |    |  |  |
|            | A105   | 0        | 0          | 0         | 0          | 0       | 0   | 0          | 0         | 0   | 0  |  |  |
| ty         |        | Consensu | s Mean     |           | 276        |         | Consensu  | s Mean     |           | 113 |    |  |  |
| uni<br>Its |        | Consensu | s Standard | Deviation | 194        |         | Consensu  | s Standard | Deviation | 181 |    |  |  |
| nsa        |        | Maximum  | ı          |           | 522        |         | Maximum   | ı          | 206       |     |    |  |  |
| on<br>Re   |        | Minimum  |            |           | 0          |         | Minimum   |            | 0         |     |    |  |  |
| C          |        | Ν        |            |           | 4          |         | Ν   |            |           | 3   |    |  |  |

**Table 3-4.** Data summary table for vitamin  $B_{12}$  in human serum.

Exercise HAMQAP Exercise 1 - Human Metabolites Sample: Human Serum C Measurand: Total Vitamin B12 (as Cyanocobalamin)



**Figure 3-4.** Total vitamin B<sub>12</sub> (as cyanocobalamin) in Human Serum C (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents the consensus mean, and the green shaded region represents the consensus mean bounded by twice the consensus standard error. The solid red line represents the upper consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \le 2$ , with the lower range set at zero. A NIST value for has not been determined in this material.





**Figure 3-5.** Total vitamin B<sub>12</sub> (as cyanocobalamin) in SRM 968d Fat-Soluble Vitamins, Carotenoids, and Cholesterol in Human Serum (Level 2) (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents the consensus mean, and the green shaded region represents the consensus mean bounded by twice the consensus standard error. The solid red line represents the upper consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \le 2$ , with the lower range set at zero. A NIST value has not been determined in this material.



HAMQAP Exercise 1 - Human Metabolites, Measurand: Total Vitamin B12 (as Cyanocobalamin) No. of laboratories: 3

**Figure 3-6.** Laboratory means for total vitamin B<sub>12</sub> (as cyanocobalamin) in Human Serum C and SRM 968d Fat-Soluble Vitamins, Carotenoids, and Cholesterol in Human Serum (Level 2) (sample/sample comparison view). In this view, the individual laboratory mean for one sample (Human Serum C) is compared to the mean for a second sample (SRM 968d). The dotted blue box represents the consensus range of tolerance for Human Serum C (x-axis) and SRM 968d (y-axis), calculated as the values above and below the consensus means that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \le 2$ .

#### Water-Soluble Vitamins Overall Study Comparison

Overall, laboratories measuring vitamin  $B_{12}$  (as cyanocobalamin) in the cereal and multivitamin matrices were more successful than those few laboratories that reported values for human serum.

- The significantly lower levels of cyanocobalamin in serum versus intake samples may be the main reason for the discrepancy.
- Assays for cyanocobalamin in serum may react differently to unknown matrix interferences, leading to wide between laboratory variation.
- While laboratories have access to a variety of matrix-based intake materials for vitamin B<sub>12</sub> quality assurance (i.e., RMs, CRMs), such materials are not widely available for serum. Assignment of a meaningful consensus value to a QA material is difficult due to the wide variation among assays and laboratories.
- Higher-order methods, such as LC-MS/MS, do not possess the sensitivity to measure cyanocobalamin in the pg/mL ranges that exist in human serum. As a result, reference material producers with traceability requirements are challenged to provide an accuracy-based target for these communities.

#### SECTION 4: FAT-SOLUBLE VITAMINS (Vitamin D and Vitamin D Metabolites)

#### Study Overview

In this study, participants were provided with SRM 1577c Bovine Liver and commercial cod liver oil for dietary intake, and SRM 968d Fat-Soluble Vitamins, Carotenoids, and Cholesterol in Human Serum and SRM 2973 Vitamin D Metabolites in Frozen Human Serum (High Level) for human metabolites. Participants were asked to use in-house analytical methods to determine and report as many forms of vitamin D and vitamin D metabolites as possible. Vitamin D is well known for its essential role in bone health and other important health functions. Obtained from sun exposure or dietary intake (food and/or supplements), vitamin D is biologically inert and must be metabolized in the body to become bioactive. The vitamin undergoes hydroxylation to become 25-hydroxyvitamin D [25(OH)D], and then a second hydroxylation to become the physiologically active 1,25-dihydroxyvitamin D [1,25(OH)<sub>2</sub>D]. To fully understand the significance of vitamin D health status and health outcomes, confident measurement of vitamin D and the metabolites in both dietary intake and human metabolism matrices is critical.

#### **Dietary Intake Sample Information**

*Cod Liver Oil.* Participants were provided three ampoules, each containing 5 mL of commercial cod liver oil. The oil was fortified with vitamin  $D_3$  and packaged in amber glass ampoules that were flushed with argon. Before use, participants were instructed to thoroughly mix the ampoule prior to removal of a test sample, and to use a sample size of at least 0.5 g. Participants were asked to store the material under refrigeration at 2 °C to 8 °C, and to prepare one sample and report one value from each ampoule provided. The approximate analyte levels were not reported to participants prior to the study, and target values for vitamin D and vitamin D metabolites in Cod Liver Oil have not been determined at NIST.

*Bovine Liver*. Participants were provided with one bottle containing 20 g of powdered liver. Before use, participants were instructed to thoroughly mix the contents of the bottle by rotating and/or rolling prior to removal of a test sample for analysis, and to use a sample size of at least 2 g. Participants were instructed to store the sample in the dark at temperatures between 10 °C and 30 °C, and to prepare three samples and report three values from the single bottle provided. The approximate analyte level was not reported to participants prior to the study. The target values for vitamin D<sub>3</sub> and 25-hydroxyvitamin D<sub>3</sub> in SRM 1577c were determined at NIST using isotope-dilution liquid chromatography with tandem mass spectrometry (ID-LC-MS/MS). Results from collaborating laboratories were also used in the determination of the target value for vitamin D<sub>3</sub> in SRM 1577c. The NIST-determined values and uncertainties for vitamin D<sub>3</sub> and 25-hydroxyvitamin D<sub>3</sub> in SRM 1577c are provided in the table below on an as-received basis.

| Analyte                | NIST-Determined Mass Fraction in SRM 1577c (mg/kg) |
|------------------------|--|
| Vitamin D <sub>3</sub> | $0.0005 \pm 0.0004$                                |
| 25-Hydroxyvitamin D3   | $0.0137 \pm 0.0031$                                |

#### **Dietary Intake Study Results**

• The table below summarizes the participation statistics for the study of vitamin D and vitamin D metabolites in bovine liver and cod liver oil.

|  | <u>Number of</u><br>Laboratories | Number of Laborat<br>(Percent) | ories Reporting Results<br>Participation) |
|--|----------------------------------|--------------------------------|---|
|  | <u>Requesting</u>                |                                | <u>SRM 1577c</u>                          |
| <u>Analyte</u>                           | Samples <b>Samples</b>           | Cod Liver Oil                  | Bovine Liver                              |
| Vitamin D <sub>2</sub> (Ergocalciferol)  | 25                               | 6 (24 %)                       | 6 (24 %)                                  |
| Vitamin D <sub>3</sub> (Cholecalciferol) | 22                               | 12 (55 %)                      | 7 (32 %)                                  |
| 25-Hydroxyvitamin D <sub>2</sub>         | 9                                | 0 (0 %)                        | 1 (11 %)                                  |
| 25-Hydroxyvitamin D3                     | 9                                | 0 (0 %)                        | 1 (11 %)                                  |
| 25-Hydroxyvitamin D                      | 12                               | 0 (0 %)                        | 1 (8 %)                                   |

- The between-laboratory variability for vitamin D<sub>3</sub> in Cod Liver Oil was good at 22.5 % RSD. Sufficient participation was not attained for any other sample/measurand combination to allow discussion of performance.
- One laboratory reported using LC-absorbance as their analytical method for the determination of vitamin D<sub>3</sub> in Cod Liver Oil. The remaining laboratories did not specify an analytical method.

## **Dietary Intake Technical Recommendations**

Due to the low number of results received for the determination of vitamin D and metabolites in Cod Liver Oil and SRM 1577c Bovine Liver, specific recommendations for improvement cannot be provided.

- The overall results for vitamin D<sub>3</sub> in Cod Liver Oil indicate that laboratories are performing well for this sample and measurand combination. The disagreement between laboratories is minor, but the following considerations may improve results for any laboratory.
  - The vitamin  $D_3$  in Cod Liver Oil was fortified at approximately  $3 \mu g/g$  according to the manufacturer, while the other forms of the vitamins were endogenous. Analysis of endogenous vitamins, which are usually present at lower concentrations, is typically more challenging regardless of the sample type.
  - The concentration of calibration solutions for vitamin D can be determined in several ways (e.g., gravimetrically, spectrophotometrically). In addition, the purity of the neat calibration material should be carefully evaluated to prevent bias in the final analytical result. For future studies on vitamin D, information about the calibration approach by participants will be collected to allow for related conclusions to be drawn.
  - In many analytical approaches for the determination of vitamin D, a saponification step is necessary to remove fat from the sample. An adequate amount of acid or base must be added, based on the fat content of the sample, to ensure that the fat removal is exhaustive. Often, post-extraction cleanup is also necessary (e.g., liquid-liquid extraction, solid-phase extraction) and can affect the overall analytical result, particularly if the cleanup results in analyte loss (even when a recovery calculation is used). For future studies on vitamin D, information about the sample preparation by participants will be collected to allow for related conclusions to be drawn.

|                              | HA                     | MQAP E             | xercise 1 - F                      | at-Soluble                            | Vitamins (1     | Dietary In     | itake)                  |                |                   |                   |                   |
|------------------------------|------------------------|--------------------|------------------------------------|---------------------------------------|-----------------|----------------|-------------------------|----------------|-------------------|-------------------|-------------------|
|                              | Lab Code:              | NIST               |                                    | 1. Your                               | Results         |                | 2. C                    | ommunity Re    | esults            | ults 3. Target    |                   |
| Analyte                      | Sample                 | Units              | <i>x</i> <sub><i>i</i></sub>       | s <sub>i</sub>                        | $Z'_{\rm comm}$ | $Z_{\rm NIST}$ | Ν                       | <i>x</i> *     | <i>s*</i>         | x <sub>NIST</sub> | U                 |
| Vitamin D2 (Ergocalciferol)  | Cod Liver Oil          | mg/kg              |                                    |                                       |                 |                | 3                       | 0.1            | 0.3               |                   |                   |
| Vitamin D2 (Ergocalciferol)  | SRM 1577c Bovine Liver | mg/kg              |                                    |                                       |                 |                | 3                       | 1.6            | 3.8               |                   |                   |
| Vitamin D3 (Cholecalciferol) | Cod Liver Oil          | mg/kg              |                                    |                                       |                 |                | 12                      | 3.29           | 0.72              |                   |                   |
| Vitamin D3 (Cholecalciferol) | SRM 1577c Bovine Liver | mg/kg              | 0.0005                             | 0.0002                                |                 |                | 6                       | 0.6            | 1.6               | 0.0005            | 0.0004            |
| 25-Hydroxyvitamin D2         | Cod Liver Oil          | mg/kg              |                                    |                                       |                 |                | 0                       |                |                   |                   |                   |
| 25-Hydroxyvitamin D2         | SRM 1577c Bovine Liver | mg/kg              |                                    |                                       |                 |                | 1                       |                |                   |                   |                   |
| 25-Hydroxyvitamin D3         | Cod Liver Oil          | mg/kg              |                                    |                                       |                 |                | 0                       |                |                   |                   |                   |
| 25-Hydroxyvitamin D3         | SRM 1577c Bovine Liver | mg/kg              | 0.0137                             | 0.0016                                |                 |                | 2                       |                |                   | 0.0137            | 0.0031            |
| Total 25-Hydroxyvitamin D    | Cod Liver Oil          | mg/kg              |                                    |                                       |                 |                | 0                       |                |                   |                   |                   |
| Total 25-Hydroxyvitamin D    | SRM 1577c Bovine Liver | mg/kg              |                                    |                                       |                 |                | 1                       |                |                   |                   |                   |
|                              |                        | $x_i$              | Mean of rep                        | Mean of reported values               |                 |                | Number of q             | uantitative    | X <sub>NIST</sub> | г NIST-assess     | sed value         |
|                              |                        | s <sub>i</sub>     | Standard dev                       | Standard deviation of reported values |                 | s              | values report           | ed             | U                 | v expanded und    | certainty         |
|                              |                        | $Z'_{\text{comm}}$ | Z'-score with                      | Z'-score with respect to community    |                 | <i>x</i> *     | Robust mean of reported |                |                   | about the NIS     | ST-assessed value |
|                              |                        |                    | consensus                          |                                       |                 | values         |                         |                |                   |                   |                   |
|                              |                        | $Z_{\text{NIST}}$  | Z-score with respect to NIST value |                                       |                 | <i>s</i> *     | Robust stand            | lard deviation |                   |                   |                   |

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|            |        |           |            |            | Vita      | min D2 (l | Ergocalcife                  | ol)              |            |           |       |  |
|------------|--------|-----------|------------|------------|-----------|-----------|------------------------------|------------------|------------|-----------|-------|--|
|            |        |           | Cod Li     | ver Oil (m | g/kg)     |           | SR                           | <b>M 1577c</b> ] | Bovine Liv | ver (mg/k | g)    |  |
|            | Lab    | Α         | В          | С          | Avg       | SD        | Α                            | В                | С          | Avg       | SD    |  |
|            | Target |           |            |            |           |           |                              |                  |            |           |       |  |
|            | A007   | 0.36      | 0.32       | 0.34       | 0.34      | 0.02      |                              |                  |            |           |       |  |
|            | A008   |           |            |            |           |           |                              |                  |            |           |       |  |
|            | A012   |           |            |            |           |           | 6.791                        | 4.784            | 3.252      | 4.942     | 1.775 |  |
|            | A027   |           |            |            |           |           |                              |                  |            |           |       |  |
|            | A030   |           |            |            |           |           |                              |                  |            |           |       |  |
|            | A038   |           |            |            |           |           |                              |                  |            |           |       |  |
|            | A041   |           |            |            |           |           |                              |                  |            |           |       |  |
| ts         | A043   | 0         | 0          | 0          | 0         | 0         | 0                            | 0                | 0          | 0         | 0     |  |
| Ins        | A047   |           |            |            |           |           |                              |                  |            |           |       |  |
| vidual Re  | A049   |           |            |            |           |           |                              |                  |            |           |       |  |
|            | A055   |           |            |            |           |           |                              |                  |            |           |       |  |
|            | A058   |           |            |            |           |           |                              |                  |            |           |       |  |
| ibu        | A060   |           |            |            |           |           |                              |                  |            |           |       |  |
| Ι          | A061   | < 0.150   | < 0.150    | < 0.150    |           |           | < 0.150                      | < 0.150          | < 0.150    |           |       |  |
|            | A062   |           |            |            |           |           |                              |                  |            |           |       |  |
|            | A070   | 0.054     | 0.054      | 0.054      | 0.054     | 0         | 0                            | 0                | 0          | 0         | 0     |  |
|            | A074   |           |            |            |           |           |                              |                  |            |           |       |  |
|            | A076   |           |            |            |           |           |                              |                  |            |           |       |  |
|            | A077   | < 0.010   | < 0.010    | < 0.010    |           |           | < 0.010                      | < 0.010          | < 0.010    |           |       |  |
|            | A078   |           |            |            |           |           |                              |                  |            |           |       |  |
|            | A079   |           |            |            |           |           |                              |                  |            |           |       |  |
|            | A084   | < 0.010   | < 0.010    | < 0.010    |           |           | < 0.010                      | < 0.010          | < 0.010    |           |       |  |
| ty         |        | Consensus | Mean       |            | 0.131     |           | Consensus                    | Mean             |            | 1.647     |       |  |
| uni<br>lts |        | Consensus | Standard I | Deviation  | 0.296     |           | Consensus Standard Deviation |                  |            | 3.811     |       |  |
| nm<br>esu  |        | Maximum   |            |            | 0.34      |           | Maximum                      |                  |            | 4.942     |       |  |
| R          |        | Minimum   |            |            | 0 Minimum |           |                              |                  | 0          |           |       |  |
| •          |        | Ν         |            |            | 3         |           | Ν                            |                  |            | 3         |       |  |

**Table 4-2.** Data summary table for vitamin  $D_2$  in cod liver oil and bovine liver.

**Table 4-3.** Data summary table for vitamin  $D_3$  in cod liver oil and bovine liver. Data points highlighted in red have been flagged as potential outliers (e.g., difference from reference value, Grubb and/or Cochran) by the NIST software package.

|            |        |           |            |            | Vita  | min D3 (C | Cholecalci | erol)      |              |            |       |
|------------|--------|-----------|------------|------------|-------|-----------|------------|------------|--------------|------------|-------|
|            |        |           | Cod Li     | ver Oil (m | g/kg) |           | S          | RM 1577c   | e Bovine Liv | ver (mg/kg | g)    |
|            | Lab    | Α         | В          | С          | Avg   | SD        | Α          | В          | С            | Avg        | SD    |
|            | Target |           |            |            |       |           |            |            |              | 0.001      | 0.000 |
|            | A007   | 1.87      | 2.12       | 2.31       | 2.10  | 0.22      |            |            |              |            |       |
|            | A008   |           |            |            |       |           |            |            |              |            |       |
|            | A012   | 2.892     | 2.898      | 2.863      | 2.884 | 0.019     | 2.889      | 1.683      | 1.272        | 1.948      | 0.840 |
|            | A027   |           |            |            |       |           |            |            |              |            |       |
|            | A030   |           |            |            |       |           |            |            |              |            |       |
|            | A038   |           |            |            |       |           |            |            |              |            |       |
|            | A041   |           |            |            |       |           |            |            |              |            |       |
|            | A043   | 2.96      | 3.187      | 3.131      | 3.093 | 0.118     | 0          | 0          | 0            | 0          | 0     |
|            | A044   | 3.85      | 3.84       | 3.86       | 3.85  | 0.01      | 0          | 0          | 0            | 0          | 0     |
| ults       | A047   |           |            |            |       |           |            |            |              |            |       |
| idual Res  | A049   | 3.4       | 3.6        | 3.7        | 3.6   | 0.2       |            |            |              |            |       |
|            | A050   | 3.21      | 3.1        | 2.97       | 3.09  | 0.12      |            |            |              |            |       |
|            | A055   |           |            |            |       |           |            |            |              |            |       |
| div        | A058   |           |            |            |       |           |            |            |              |            |       |
| In         | A060   |           |            |            |       |           |            |            |              |            |       |
|            | A061   | 14.3      | 16.6       | 15.5       | 15.5  | 1.2       | < 0.150    | < 0.150    | < 0.150      |            |       |
|            | A062   | 3.416     | 3.061      | 2.874      | 3.117 | 0.275     |            |            |              |            |       |
|            | A070   | 3.09      | 3.09       | 3.09       | 3.09  | 0         | 0.369      | 0.369      | 0.369        | 0.369      | 0     |
|            | A072   |           |            |            |       |           |            |            |              |            |       |
|            | A074   | 3.53      | 3.28       | 3.34       | 3.38  | 0.13      |            |            |              |            |       |
|            | A076   |           |            |            |       |           |            |            |              |            |       |
|            | A077   | 5.54      | 5.65       | 5.7        | 5.63  | 0.08      | 14.61      | 15.06      | 15.08        | 14.92      | 0.27  |
|            | A078   |           |            |            |       |           |            |            |              |            |       |
|            | A079   |           |            |            |       |           |            |            |              |            |       |
|            | A084   | 3.77      | 3.58       | 3.84       | 3.73  | 0.13      | < 0.010    | < 0.010    | < 0.010      |            |       |
| ity        |        | Consensus | Mean       |            | 3.29  |           | Consensu   | s Mean     |              | 0.579      |       |
| aun        |        | Consensus | Standard I | Deviation  | 0.72  |           | Consensu   | s Standard | Deviation    | 1.627      |       |
| mn<br>test |        | Maximum   |            |            | 15.5  |           | Maximum    | 1          |              | 14.92      |       |
| R Co       |        | Minimum   |            |            | 2.1   |           | Minimum    |            |              | 0          |       |
|            |        | Ν         |            |            | 12    |           | Ν          |            |              | 5          |       |



Exercise HAMQAP Exercise 1 - Dietary Intake Sample: Cod Liver Oil Measurand: Vitamin D3 (Cholecalciferol)

**Figure 4-1.** Vitamin D<sub>3</sub> (cholecalciferol) in Cod Liver Oil (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \leq 2$ . A NIST value has not been determined in this material.



**Figure 4-2.** Laboratory means for vitamin D<sub>3</sub> (cholecalciferol) in SRM 1577c Bovine Liver and Cod Liver Oil (sample/sample comparison view). In this view, the individual laboratory mean for one sample (SRM 1577c) is compared to the mean for a second sample (Cod Liver Oil). The dotted blue box represents the consensus range of tolerance for SRM 1577c (x-axis) and Cod Liver Oil (y-axis), calculated as the values above and below the consensus means that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \le 2$ .

#### Human Metabolites Sample Information

Human Serum E. Participants were provided with three vials, each containing 1 mL of frozen serum. Bovine thrombin and calcium chloride were added to convert the plasma to serum. The serum was dialyzed to remove bovine thrombin, calcium chloride, and anticoagulants. Salts were added back into the serum, and the material was pooled along with isotonic saline, blended, bottled in 1 mL aliquots, and stored at -80 °C. Before use, participants were instructed to allow the material to thaw at room temperature for at least 30 min prior to sampling, use the material immediately after thawing, gently mix the contents prior to removal of a test portion for analysis, and use a sample size appropriate for their usual in-house method of analysis. Participants were asked to avoid exposing the material to direct UV light, to store the material at or below -80 °C, and to prepare one sample and report one value from each vial provided. The approximate analyte levels were not reported to participants prior to the study. The target value for 25-hydroxyvitamin D<sub>3</sub> in SRM 968d Level 2 was determined at NIST using isotope-dilution liquid chromatography tandem mass spectroscopy (ID-LC-MS/MS). The NIST-determined value and uncertainty for 25-hydroxyvitamin D<sub>3</sub> in SRM 968d Level 2 are provided in the table below. Target values for vitamin D<sub>2</sub>, vitamin D<sub>3</sub>, 25-hydroxyvitamin D<sub>2</sub>, total 25-hydroxyvitamin D, 3-epi-25-hydroxyvitamin D<sub>3</sub>, 3-epi-25-hydroxyvitamin D<sub>2</sub>, 24,25-dihydroxyvitamin D<sub>3</sub>, 24,25-dihydroxyvitamin D<sub>2</sub>, 1,25-dihydroxyvitamin D<sub>2</sub>, and 1,25-dihydroxyvitamin D<sub>3</sub> in SRM 968d Level 2 have not been determined at NIST.

|                                  | NIST-Determined Mass Concentration in |
|----------------------------------|---------------------------------------|
| <u>Analyte</u>                   | <u>SRM 968d Level 2 (ng/mL)</u>       |
| 25-Hydroxyvitamin D <sub>3</sub> | $10.42 \pm 0.12$                      |

Human Serum F. Participants were provided with three vials, each containing 1 mL of frozen serum. The material was prepared from source donation units aiming to achieve a high vitamin D metabolite level in the pool. Units were drawn according to CLSI C37-A guidelines before pooling, blending, and filtering. The serum was bottled and stored at -80 °C. Before use, participants were instructed to allow the material to thaw at room temperature for at least 30 min prior to sampling, use the material immediately after thawing, gently mix the contents prior to removal of a test portion for analysis, and use a sample size appropriate for their usual in-house method of analysis. Participants were asked to avoid exposing the material to direct UV light, to store the material at or below -80 °C, and to prepare one sample and report one value from each vial provided. The approximate analyte levels were not reported to participants prior to the study. Certified values for 25-hydroxyvitamin  $D_3$  and 24,25-dihydroxyvitamin  $D_3$  and reference values for 25-hydroxyvitamin D<sub>2</sub>, total 25-hydroxyvitamin D, and 3-epi-25-hydroxyvitamin D<sub>3</sub> in SRM 2973 were determined at NIST using ID-LC-MS/MS. The NIST-determined values and uncertainties for 25-hydroxyvitamin D<sub>2</sub>, 25-hydroxyvitamin D<sub>3</sub>, total 25-hydroxyvitamin D, 3-epi-25-hydroxyvitamin D<sub>3</sub>, and 24,25-dihydroxyvitamin D<sub>3</sub> in SRM 2973 are provided in the table below. Target values for vitamin D<sub>2</sub>, vitamin D<sub>3</sub>, 3-epi-25-hydroxyvitamin D<sub>2</sub>, 24,25-dihydroxyvitamin D<sub>2</sub>, 1,25-dihydroxyvitamin D<sub>2</sub>, and 1,25-dihydroxyvitamin D<sub>3</sub> in SRM 2973 have not been determined at NIST.

|  | NIST-Determined Mass Concentration in |
|--|---------------------------------------|
| <u>Analyte</u>                         | <u>SRM 2973 (ng/mL)</u>               |
| 25-Hydroxyvitamin D2                   | $0.65 \pm 0.02$                       |
| 25-Hydroxyvitamin D <sub>3</sub>       | $39.4 \pm 0.8$                        |
| Total 25-Hydroxyvitamin D              | $40.1 \pm 0.8$                        |
| 3-epi-25-Hydroxyvitamin D <sub>3</sub> | $2.10 \pm 0.08$                       |
| 24,25-Dihydroxyvitamin D3              | $3.13 \pm 0.11$                       |
|  |                                       |

### Human Metabolites Study Results

- Twenty-nine laboratories enrolled in this exercise and received samples for vitamin D and the vitamin D metabolites. Not all laboratories measured and reported results for every analyte in the study.
- The highest participation rates were for 25-hydroxyvitamin D<sub>3</sub>, 25-hydroxyvitamin D<sub>2</sub>, and total 25-hydroxyvitamin D, with 40 % to 50 % of laboratories returning results.
  - The consensus means for total 25-hydroxyvitamin D and 25-hydroxvitamin D<sub>3</sub> were within the target ranges for SRM 2973, with good between-laboratory variability of 6.2 % RSD and 5.2 % RSD, respectively.
  - For SRM 968d, the consensus mean for 25-hydroxyvitamin D<sub>3</sub> was within the target range with a moderate between-laboratory variability (18 % RSD).
  - Laboratories measured 25-hydroxyvitamin D in SRM 968d with moderate variability (20 % RSD).
  - About half of the participants returned results for 3-epi-25-hydroxyvitamin D<sub>3</sub> in SRM 2973, and the consensus mean was within the target value. The between-laboratory variability was high at 37 % RSD.
- The participation for 24,25-dihydroxyvitamin  $D_3$  and 1,2-dihydroxyvitamin  $D_3$  was significantly lower, with only three of ten laboratories returning results (30 % participation). Even with the small number of results, two of the three reporting laboratories were within the target range for 24,25-dihydroxyvitamin  $D_3$  in SRM 2973.

### Human Metabolites Technical Recommendations

The following recommendations are based on results obtained from the participants in this study. In some cases, there were too few data to obtain a consensus mean or to draw meaningful conclusions.

- For both serum samples, several laboratories reported data significantly outside of the target and consensus ranges. The use of appropriate calibration materials and quality assurance samples to establish that a method is in control and performing correctly may reduce the likelihood of outlying data. Quality assurance samples can be commercially available reference materials (CRMs, SRMs, or RMs) or prepared in-house.
  - The analyte "total 25-hydroxyvitamin D" is defined in this measurement community as the sum of 25-hydroxyvitamin D<sub>3</sub> and 25-hydroxyvitamin D<sub>2</sub>; the total should not include the 3-epi-isomers or other similar metabolites. Most laboratories were within the consensus range of tolerance for total 25-hydroxyvitamin D in SRM 968d.

- Values from laboratories that reported using immunoassay appeared to trend lower than from those that reported using LC-MS and LC-MS/MS for SRM 968d.
  - Immunoassay methods may not be detecting any or all of the 25-hydroxyvitamin D<sub>2</sub> that is present in the sample, and therefore underestimating the total 25-hydroxyvitamin D level.
  - The LC-MS and LC-MS/MS methods may not be separating the 3-epimers from the 25-hydroxyvitamin D forms, resulting in an overestimation of the level.
- In SRM 2973, the level of 25-hydroxvitamin  $D_2$  is much lower than the level of 25-hydroxyvitamin  $D_3$ . The difference in results between laboratories reporting using immunoassay and MS-based methods is not as large in SRM 2973 compared to SRM 968d.
  - Undetected 25-hydroxyvitamin D<sub>2</sub> will have less impact on immunoassay method results for total 25-hydroxyvitamin D in SRM 2973 compared to SRM 968d.
  - One laboratory using LC-MS/MS had a strong positive bias for SRM 2973, indicating a potential calibration error or a bias from unresolved metabolites.
- Immunoassay users should understand the fitness-for-purpose of the assay for analytes being detected. Assay developers should be diligent and indicate whether the platform can or cannot detect the different target metabolites of vitamin D.
- LC-MS and LC-MS/MS users should ensure that the method has acceptable separation of metabolite isomers (e.g., 25-hydroxyvitamin D<sub>3</sub> and 3-epi-25-hydroxyvitamin D<sub>3</sub>). Monitoring qualifier ions, when possible, can help detect and remove other interferences.
- The use of an internal standard may reduce the impact of any matrix interferences present in the samples. Also, metabolite isomers need to be separated from the target analytes when using LC-MS and LC-MS/MS methods to prevent overestimation of results.
- A linear calibration curve which surrounds the expected sample concentration values should be used for calculations based on LC-MS methods. This curve should include both the lowest and highest expected concentration values of the sample solutions. Extrapolation of results beyond calibration curves may result in incorrect values.
- In general, all results should be checked closely to avoid calculation errors and to be sure that results are reported in the requested units.

## Table 4-4. Individualized data summary table (NIST) for vitamin D and vitamin D metabolites in human serum.

## National Institute of Standards & Technology

|                               | HAMQAP Exe<br>Lab Code:  | ercise 1 -<br>NIST                                   | Fat-Soluble   | Vitamins    | (Human Me<br>r Results | tabolites)        | 2 0                        | ommunity R     | 3 т              | 3 Target                        |          |  |
|-------------------------------|--|--|---|-------------|------------------------|-------------------|----------------------------|----------------|------------------|---------------------------------|----------|--|
| A nabite                      | Sample   | Unite  | r   | 1. IU       | 7'                     | 7                 | N                          | v*             | .suits<br>*      | <u></u>                         | II       |  |
| Analyte                       | SRM 2973 Vitamin D Metabolites in Frozen   | Onits  | X_i   | 3 i         | L comm                 | <sup>2</sup> NIST | 14                         | л              | 3                | ~ NIST                          | 0        |  |
| Vitamin D2 (Ergocalciferol)   | Human Serum (High Level)   | ng/mI.   |   |             |                        |                   | 1                          |                |                  |                                 |          |  |
| Vitalinin D2 (Engoeaterieron) | SRM 968d Fat-Soluble Vitamins Carotenoids  | 115/1112   |   |             |                        |                   | 1                          |                |                  |                                 |          |  |
| Vitamin D2 (Ergocalciferol)   | and Cholesterol in Human Serum (Level 2)   | ng/mL  |   |             |                        |                   | 0                          |                |                  |                                 |          |  |
|                               | SRM 2973 Vitamin D Metabolites in Frozen   |  |   |             |                        |                   |                            |                |                  |                                 |          |  |
| Vitamin D3 (Cholecalciferol)  | Human Serum (High Level)   | ng/mL  |   |             |                        |                   | 2                          | 30             | 69               |                                 |          |  |
|                               | SRM 968d Fat-Soluble Vitamins, Carotenoids,  | 0  |   |             |                        |                   |                            |                |                  |                                 |          |  |
| Vitamin D3 (Cholecalciferol)  | and Cholesterol in Human Serum (Level 2)   | ng/mL  |   |             |                        |                   | 2                          | 1              | 3.9              |                                 |          |  |
|                               | SRM 2973 Vitamin D Metabolites in Frozen   | 0  |   |             |                        |                   |                            |                |                  |                                 |          |  |
| 25-Hydroxyvitamin D2          | Human Serum (High Level)   | ng/mL  | 0.65  | 0.01        |                        |                   | 9                          | 0.81           | 0.47             | 0.65                            | 0.02     |  |
|                               | SRM 968d Fat-Soluble Vitamins, Carotenoids,  |  |   |             |                        |                   |                            |                |                  |                                 |          |  |
| 25-Hydroxyvitamin D2          | and Cholesterol in Human Serum (Level 2)   | ng/mL  |   |             |                        |                   | 8                          | 0.76           | 0.28             |                                 |          |  |
|                               | SRM 2973 Vitamin D Metabolites in Frozen   |  |   |             |                        |                   |                            |                |                  |                                 |          |  |
| 25-Hydroxyvitamin D3          | Human Serum (High Level)   | ng/mL  | 39.4  | 0.4         |                        |                   | 12                         | 40.1           | 2.1              | 39.4                            | 0.8      |  |
|                               | SRM 968d Fat-Soluble Vitamins, Carotenoids,  |  |   |             |                        |                   |                            |                |                  |                                 |          |  |
| 25-Hydroxyvitamin D3          | and Cholesterol in Human Serum (Level 2)   | ng/mL  | 10.4  | 0.06        |                        |                   | 12                         | 11.6           | 2.1              | 10.4                            | 0.12     |  |
|                               | SRM 2973 Vitamin D Metabolites in Frozen   |  |   |             |                        |                   |                            |                |                  |                                 |          |  |
| Total 25-Hydroxyvitamin D     | Human Serum (High Level)   | ng/mL  | 40.1  | 0.4         |                        |                   | 13                         | 40.1           | 2.5              | 40.1                            | 0.8      |  |
|                               | SRM 968d Fat-Soluble Vitamins, Carotenoids,  |  |   |             |                        |                   |                            |                |                  |                                 |          |  |
| Total 25-Hydroxyvitamin D     | and Cholesterol in Human Serum (Level 2)<br>SRM 2973 Vitamin D Metabolites in Frozen | ng/mL  |   |             |                        |                   | 12                         | 10.5           | 2.2              |                                 |          |  |
| 3-epi-25-Hydroxvitamin D2     | Human Serum (High Level)   | ng/mL  |   |             |                        |                   | 0                          |                |                  |                                 |          |  |
|                               | SRM 968d Fat-Soluble Vitamins, Carotenoids,  |  |   |             |                        |                   |                            |                |                  |                                 |          |  |
| 3-epi-25-Hydroxvitamin D2     | and Cholesterol in Human Serum (Level 2)   | ng/mL  |   |             |                        |                   | 0                          |                |                  |                                 |          |  |
|                               | SRM 2973 Vitamin D Metabolites in Frozen   |  |   |             |                        |                   |                            |                |                  |                                 |          |  |
| 3-epi-25-Hydroxvitamin D3     | Human Serum (High Level)   | ng/mL  | 2.1   | 0.04        |                        |                   | 7                          | 2.66           | 0.95             | 2.1                             | 0.08     |  |
|                               | SRM 968d Fat-Soluble Vitamins, Carotenoids,  |  |   |             |                        |                   |                            |                |                  |                                 |          |  |
| 3-epi-25-Hydroxvitamin D3     | and Cholesterol in Human Serum (Level 2)<br>SRM 2973 Vitamin D Metabolites in Frozen | ng/mL  |   |             |                        |                   | 4                          | 0.8            | 0.3              |                                 |          |  |
| 1,25-Dihydroxyvitamin D3      | Human Serum (High Level)   | ng/mL  |   |             |                        |                   | 3                          | 0.047          | 0.016            |                                 |          |  |
|                               | SRM 968d Fat-Soluble Vitamins, Carotenoids,  |  |   |             |                        |                   |                            |                |                  |                                 |          |  |
| 1,25-Dihydroxyvitamin D3      | and Cholesterol in Human Serum (Level 2)   | ng/mL  |   |             |                        |                   | 2                          | 0.242          | 0.044            |                                 |          |  |
|                               | SRM 2973 Vitamin D Metabolites in Frozen   |  |   |             |                        |                   |                            |                |                  |                                 |          |  |
| 24,25-Dihydroxyvitamin D3     | Human Serum (High Level)   | ng/mL  | 3.13  | 0.055       |                        |                   | 4                          | 3.0            | 1.3              | 3.13                            | 0.11     |  |
|                               | SRM 968d Fat-Soluble Vitamins, Carotenoids,  |  |   |             |                        |                   |                            |                |                  |                                 |          |  |
| 24,25-Dihydroxyvitamin D3     | and Cholesterol in Human Serum (Level 2)   | ng/mL  |   |             |                        |                   | 2                          | 0.48           | 0.02             |                                 |          |  |
|                               |  | x <sub>i</sub>                                       | Mean of rep   | orted value | s                      | Ν                 | Number of c                | uantitative    | x <sub>NIS</sub> | T NIST-assess                   | ed value |  |
|                               |  | s <sub>i</sub> Standard deviation of reported values |   | s           | values reported        |                   | L                          | v expanded unc | ertainty         |                                 |          |  |
|                               |  | $Z'_{\rm comm}$                                      | $x_{\rm comm}$ Z'-score with respect to community $x^*$ |             |                        | <i>x</i> *        | x* Robust mean of reported |                |                  | d about the NIST-assessed value |          |  |
|                               |  |  | consensus   |             |                        |                   | values                     |                |                  |                                 |          |  |
|                               |  | Z-score with   | respect to  | NIST value  | <i>s</i> *             | Robust stand      | lard deviation             |                |                  |                                 |          |  |

25-Hydroxyvitamin D2 SRM 968d Fat-Soluble Vitamins, Carotenoids SRM 2973 Vitamin D Metabolites in Frozen and Cholesterol in Human Serum (Level 2) Human Serum (High Level) (ng/mL) (ng/mL) Lab A A С B С Avg SD B Avg SD 0.02 Target 0.65 1.07 1.29 0.12 1.39 1.25 A012 1.1 1.15 1.18 1.27 0.11 A016 A022 A024 < 5.000 < 5.000 A038 A070 A079 Individual Results 0.98 0.94 1.02 0.04 0.84 A088 0.98 0.86 0.84 0.85 0.01 A089 0.772 0.698 0.617 0.078 0.652 0.41 0.387 0.483 0.147 0.696 A091 0.49 < 0.100 0.78 0.21 0.97 0.33 0.26 0.39 A093 0.64 0.52 A094 0.64 0.58 0.72 0.65 0.07 0.64 0.64 0.7 0.66 0.03 A095 A096 A098 < 5.000 < 5.000 < 5.000< 5.000 < 5.000 < 5.000 3.3 A101 2.4 1.6 2.4 0.9 1.4 2.6 2.8 2.3 0.8 A102 A103 A104 A106 0.631 0.615 0.643 0.630 0.014 0.626 0.627 < 0.000 0.627 0.001 0.6 0 0.6 0.6 0.6 0 A107 0.6 0.6 0.6 0.6 Consensus Mean 0.76 Consensus Mean 0.81 Community Results Consensus Standard Deviation 0.28 Consensus Standard Deviation 0.47 Maximum 2.4 Maximum 2.3 0.6 0.483 Minimum Minimum Ν 8 N 8

**Table 4-5.** Data summary table for 25-Hydroxyvitamin  $D_2$  in human serum. Data points highlighted in red have been flagged as potential outliers (e.g., difference from reference value, Grubb and/or Cochran) by the NIST software package.





**Figure 4-3.** 25-Hydroxyvitamin D<sub>2</sub> in SRM 968d Fat-Soluble Vitamins, Carotenoids, and Cholesterol in Human Serum (Level 2) (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \leq 2$ . A NIST value has not been determined in this material.

Exercise HAMQAP Exercise 1 - Human Metabolites Sample: SRM 2973 Vitamin D Metabolites in Frozen Human Serum (High Level) Measurand: 25-Hydroxyvitamin D2



**Figure 4-4.** 25-Hydroxyvitamin D<sub>2</sub> in SRM 2973 Vitamin D Metabolites in Frozen Human Serum (High Level) (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red line represents the upper consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \le 2$ , with the lower range set at zero. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by its uncertainty ( $U_{NIST}$ ) and represents the range that results in an acceptable  $Z'_{NIST} | \le 2$ .



HAMQAP Exercise 1 - Human Metabolites, Measurand: 25-Hydroxyvitamin D2 No. of laboratories: 8

**Figure 4-5.** Laboratory means for 25-Hydroxyvitamin D<sub>2</sub> in SRM 968d Fat-Soluble Vitamins, Carotenoids, and Cholesterol in Human Serum (Level 2) and SRM 2973 Vitamin D Metabolites in Frozen Human Serum (High Level) (sample/sample comparison view). In this view, the individual laboratory mean for one sample (SRM 968d) is compared to the mean for a second sample (SRM 2973). The dotted blue box represents the consensus range of tolerance for SRM 968d (x-axis) and SRM 2973 (y-axis), calculated as the values above and below the consensus means that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \leq 2$ .

**Table 4-6.** Data summary table for 25-Hydroxyvitamin  $D_3$  in human serum. Data points highlighted in red have been flagged as potential outliers (e.g., difference from reference value, Grubb and/or Cochran) by the NIST software package.

|              |        |           |             |            | 25         | -Hydroxy | vitamin D3 | 3          |            |            |        |  |
|--------------|--------|-----------|-------------|------------|------------|----------|------------|------------|------------|------------|--------|--|
|              |        | SRM 968   | 8d Fat-Sol  | uble Vitar | nins, Caro | tenoids  | SRM 20     | 73 Vitami  | n D Moto   | bolites in | Frozen |  |
|              |        | and Ch    | olesterol i | n Human S  | Serum (Le  | vel 2)   | BKW 27     | nan Serum  | n (High Le | vel) (ng/m | L)     |  |
| i            |        |           |             | (ng/mL)    |            | ~~~      |            |            | r (High De | · · · · ·  |        |  |
|              | Lab    | A         | В           | С          | Avg        | SD       | A          | В          | С          | Avg        | SD     |  |
|              | Target |           |             |            | 10.42      | 0.12     |            |            |            | 39.40      | 0.80   |  |
|              | A012   | 12.48     | 13.02       | 13.28      | 12.93      | 0.41     | 56.24      | 53.00      | 52.26      | 53.83      | 2.12   |  |
|              | A016   |           |             |            |            |          |            |            |            |            |        |  |
|              | A022   |           |             |            |            |          |            |            |            |            |        |  |
|              | A024   |           |             |            |            |          |            |            |            |            |        |  |
|              | A038   | 12        |             |            | 12         |          | 40         |            |            | 40         |        |  |
|              | A070   |           |             |            |            |          |            |            |            |            |        |  |
| s            | A079   |           |             |            |            |          |            |            |            |            |        |  |
| ial Results  | A088   | 11.8      | 11.6        | 12.2       | 11.9       | 0.3      | 39.5       | 39.9       | 39.9       | 39.8       | 0.2    |  |
|              | A089   | 14.3      | 18.4        | 13.5       | 15.4       | 2.6      | 44.3       | 42.6       | 42.5       | 43.1       | 1.0    |  |
|              | A091   |           |             |            |            |          |            |            |            |            |        |  |
| idu          | A093   | 10.5      | 10.8        | 7.95       | 9.8        | 1.6      | 42.3       | 39.2       | 42.6       | 41.4       | 1.9    |  |
| div          | A094   | 10.49     | 10.27       | 10.3       | 10.35      | 0.12     | 40.07      | 39.58      | 39.92      | 39.86      | 0.25   |  |
| In           | A095   |           |             |            |            |          |            |            |            |            |        |  |
|              | A096   | 10.6      | 10.9        | 10         | 10.5       | 0.5      | 37.5       | 38.9       | 39.5       | 38.6       | 1.0    |  |
|              | A098   | 10.9      | 10.9        | 9.9        | 10.6       | 0.6      | 37.5       | 41.9       | 40         | 39.8       | 2.2    |  |
|              | A101   | 15        | 18.1        | 13.9       | 15.7       | 2.2      | 56.2       | 51.5       | 44.9       | 50.9       | 5.7    |  |
|              | A102   |           |             |            |            |          |            |            |            |            |        |  |
|              | A103   |           |             |            |            |          |            |            |            |            |        |  |
|              | A104   |           |             |            |            |          |            |            |            |            |        |  |
|              | A106   | 10.9      | 10.9        | 11         | 10.9       | 0.1      | 39.9       | 39.8       | 40         | 39.9       | 0.1    |  |
|              | A107   | 9.26      | 9.66        | 9.86       | 9.59       | 0.31     | 39.3       | 38.5       | 36.9       | 38.2       | 1.2    |  |
| ity          |        | Consensus | Mean        |            | 11.6       |          | Consensus  | Mean       |            | 40.1       |        |  |
| un<br>lts    |        | Consensus | Standard I  | Deviation  | 2.1        |          | Consensus  | Standard I | Deviation  | 2.1        |        |  |
| nm<br>esu    |        | Maximum   |             |            | 15.7       |          | Maximum    |            |            | 53.8       |        |  |
| <b>R</b> C01 |        | Minimum   |             |            | 9.6        |          | Minimum    |            |            | 38.2       |        |  |
| •            |        | Ν         |             |            | 10         |          | Ν          |            | 10         |            |        |  |





**Figure 4-6.** 25-Hydroxyvitamin D<sub>3</sub> in SRM 968d Fat-Soluble Vitamins, Carotenoids, and Cholesterol in Human Serum (Level 2) (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \le 2$ . The red shaded region represents the NIST range of tolerance, which encompasses the NIST-determined value bounded by its uncertainty ( $U_{NIST}$ ) and represents the range that results in an acceptable  $Z_{NIST}$  score,  $|Z_{NIST}| \le 2$ .



Exercise HAMQAP Exercise 1 - Human Metabolites Sample: SRM 2973 Vitamin D Metabolites in Frozen Human Serum (High Level) Measurand: 25-Hydroxyvitamin D3

**Figure 4-7.** 25-Hydroxyvitamin D<sub>3</sub> in SRM 2973 Vitamin D Metabolites in Frozen Human Serum (High Level) (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \le 2$ . The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by its uncertainty ( $U_{NIST}$ ) and represents the range that results in an acceptable  $Z'_{NIST} | \le 2$ .



**Figure 4-8.** Laboratory means for 25-Hydroxyvitamin D<sub>3</sub> in SRM 968d Fat-Soluble Vitamins, Carotenoids, and Cholesterol in Human Serum (Level 2) and SRM 2973 Vitamin D Metabolites in Frozen Human Serum (High Level) (sample/sample comparison view). In this view, the individual laboratory mean for one sample (SRM 968d) is compared to the mean for a second sample (SRM 2973). The solid red box represents the NIST range of tolerance for the two samples, SRM 968d (x-axis) and SRM 2973 (y-axis), which encompasses the target values bounded by their uncertainties ( $U_{\text{NIST}}$ ) and represents the range that results in an acceptable  $Z_{\text{NIST}}$  score,  $|Z_{\text{NIST}}| \leq 2$ . The dotted blue box represents the consensus range of tolerance for SRM 968d (x-axis) SRM 2973 (y-axis), calculated as the values above and below the consensus means that result in an acceptable  $Z'_{\text{comm}} = 2$ .

**Table 4-7.** Data summary table for total 25-Hydroxyvitamin D in human serum. Data points highlighted in red have been flagged as potential outliers (e.g., difference from reference value, Grubb and/or Cochran) by the NIST software package.

|             |        |                   |                           |                                    | Tota                    | al 25-Hydi          | roxyvitamiı  | n D        |           |          |       |  |  |
|-------------|--------|-------------------|---------------------------|------------------------------------|-------------------------|---------------------|--|------------|-----------|----------|-------|--|--|
|             |        | SRM 968<br>and Ch | 8d Fat-Sol<br>olesterol i | uble Vitar<br>n Human S<br>(ng/mL) | nins, Caro<br>Serum (Le | otenoids<br>evel 2) | SRM 2973 Vitamin D Metabolites in Frozen<br>Human Serum (High Level) (ng/mL) |            |           |          |       |  |  |
|             | Lab    | Α                 | В                         | С                                  | Avg                     | SD                  | Α  | В          | С         | Avg      | SD    |  |  |
|             | Target |                   |                           |                                    |                         |                     |  |            |           | 40.10    | 0.80  |  |  |
|             | A002   |                   |                           |                                    |                         |                     |  |            |           |          |       |  |  |
|             | A012   |                   |                           |                                    |                         |                     |  |            |           |          |       |  |  |
|             | A016   |                   |                           |                                    |                         |                     |  |            |           |          |       |  |  |
|             | A022   |                   |                           |                                    |                         |                     |  |            |           |          |       |  |  |
|             | A024   |                   |                           |                                    |                         |                     |  |            |           |          |       |  |  |
|             | A033   |                   |                           |                                    |                         |                     |  |            |           |          |       |  |  |
|             | A038   | 12                |                           |                                    | 12                      |                     | 40   |            |           | 40       |       |  |  |
|             | A046   | 9.63              | 9.44                      | 9.84                               | 9.64                    | 0.20                | 36.6   | 36.5       | 37.6      | 36.9     | 0.6   |  |  |
|             | A070   |                   |                           |                                    |                         |                     |  |            |           |          |       |  |  |
| lts         | A079   |                   |                           |                                    |                         |                     |  |            |           |          |       |  |  |
| dual Resu   | A086   |                   |                           |                                    |                         |                     |  |            |           |          |       |  |  |
|             | A088   | 12.8              | 12.5                      | 13.2                               | 12.8                    | 0.4                 | 40.4   | 40.7       | 40.7      | 40.6     | 0.2   |  |  |
|             | A089   |                   |                           |                                    |                         |                     |  |            |           |          |       |  |  |
| ivid        | A090   | 8.8               | 9.2                       | 8.1                                | 8.7                     | 0.6                 | 42   | 43.8       | 41.4      | 42.4     | 1.2   |  |  |
| pu          | A091   |                   |                           |                                    |                         |                     |  |            |           |          |       |  |  |
| -           | A093   | 10.99             | 10.9                      | 8.73                               | 10.21                   | 1.28                | 43.27  | 39.53      | 42.86     | 41.89    | 2.05  |  |  |
|             | A094   | 11.13             | 10.86                     | 11.02                              | 11.00                   | 0.14                | 40.71  | 40.21      | 40.62     | 40.51    | 0.27  |  |  |
|             | A095   |                   |                           |                                    |                         |                     |  |            |           |          |       |  |  |
|             | A096   | 10.6              | 10.9                      | 10                                 | 10.5                    | 0.5                 | 37.5   | 38.9       | 39.5      | 38.6     | 1.0   |  |  |
|             | A097   | 7.551             | 8.677                     | 8.693                              | 8.307                   | 0.655               | 40.608   | 40.466     | 37.68     | 39.585   | 1.651 |  |  |
|             | A098   | 10.9              | 10.9                      | 9.9                                | 10.6                    | 0.6                 | 37.5   | 41.9       | 40        | 39.8     | 2.2   |  |  |
|             | A101   | 17.4              | 21.3                      | 15.5                               | 18.1                    | 3.0                 | 57.6   | 54         | 47.7      | 53.1     | 5.0   |  |  |
|             | A102   |                   |                           |                                    |                         |                     |  |            |           |          |       |  |  |
|             | A103   | 8.5               | 8.5                       | 9.1                                | 8.7                     | 0.3                 | 41.9   | 43.5       | 41.7      | 42.4     | 1.0   |  |  |
|             | A104   |                   |                           |                                    |                         |                     |  |            |           |          |       |  |  |
|             | A107   | 9.86              | 10.3                      | 10.5                               | 10.2                    | 0.3                 | 39.9   | 39.1       | 37.5      | 38.8     | 1.2   |  |  |
| iity        |        | Consensus         | Mean                      |                                    | 10.46                   |                     | Consensus  | Mean       |           | 40.14    |       |  |  |
| nun<br>alts |        | Consensus         | Standard I                | Deviation                          | 2.21                    |                     | Consensus  | Standard I | Deviation | ion 2.54 |       |  |  |
| mn<br>kest  |        | Maximum           |                           |                                    | 18.1                    |                     | Maximum  |            |           | 53.1     |       |  |  |
| C0<br>F     |        | Minimum           |                           |                                    | 8.3                     |                     | Minimum  |            |           | 36.9     |       |  |  |
|             |        | N                 |                           |                                    | 11                      |                     | N  |            | 11        |          |       |  |  |





**Figure 4-9.** Total 25-Hydroxyvitamin D in SRM 968d Fat-Soluble Vitamins, Carotenoids, and Cholesterol in Human Serum (Level 2) (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable  $Z'_{\text{comm}} | \leq 2$ . A NIST value has not been determined in this material.

Exercise HAMQAP Exercise 1 - Human Metabolites Sample: SRM 2973 Vitamin D Metabolites in Frozen Human Serum (High Level) Measurand: Total 25-Hydroxyvitamin D



**Figure 4-10.** Total 25-Hydroxyvitamin D in SRM 2973 Vitamin D Metabolites in Frozen Human Serum (High Level) (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \le 2$ . The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by its uncertainty ( $U_{NIST}$ ) and represents the range that results in an acceptable  $Z_{NIST}$  score,  $|Z_{NIST}| \le 2$ .



HAMQAP Exercise 1 - Human Metabolites, Measurand: Total 25-Hydroxyvitamin D No. of laboratories: 12

**Figure 4-11.** Laboratory means for Total 25-Hydroxyvitamin D in SRM 968d Fat-Soluble Vitamins, Carotenoids, and Cholesterol in Human Serum (Level 2) and SRM 2973 Vitamin D Metabolites in Frozen Human Serum (High Level) (sample/sample comparison view). In this view, the individual laboratory mean for one sample (SRM 968d) is compared to the mean for a second sample (SRM 2973). The dotted blue box represents the consensus range of tolerance for SRM 968d (x-axis) SRM 2973 (y-axis), calculated as the values above and below the consensus means that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \le 2$ .

|        | 3-epi-25-Hydroxvitamin D3   |         |         |      |      |  |      |      |      |      |
|--------|---|---------|---------|------|------|--|------|------|------|------|
|        | SRM 968d Fat-Soluble Vitamins, Carotenoids<br>and Cholesterol in Human Serum (Level 2)<br>(ng/mL) |         |         |      |      | SRM 2973 Vitamin D Metabolites in Frozen<br>Human Serum (High Level) (ng/mL) |      |      |      |      |
| Lab    | Α   | В       | С       | Avg  | SD   | Α  | В    | С    | Avg  | SD   |
| Target |   |         |         |      |      |  |      |      | 2.10 | 0.08 |
| A012   | 1.05  | 0.98    | 0.92    | 0.98 | 0.07 | 2.74   | 3.06 | 2.98 | 2.93 | 0.17 |
| A016   |   |         |         |      |      |  |      |      |      |      |
| A038   |   |         |         |      |      |  |      |      |      |      |
| A070   |   |         |         |      |      |  |      |      |      |      |
| A079   |   |         |         |      |      |  |      |      |      |      |
| A089   | 1.01  | 1.03    | 1.11    | 1.05 | 0.05 | 3.56   | 3.31 | 3.36 | 3.41 | 0.13 |
| A094   | 0.75  | 0.88    | 0.74    | 0.79 | 0.08 | 2.18   | 2.36 | 2.44 | 2.33 | 0.13 |
| A096   |   |         |         |      |      | 2.44   | 2.58 | 2.56 | 2.53 | 0.08 |
| A098   | < 1.000   | < 1.000 | < 1.000 |      |      | 1.3  | 1.7  | 1.5  | 1.5  | 0.2  |
| A102   |   |         |         |      |      |  |      |      |      |      |
| A103   |   |         |         |      |      |  |      |      |      |      |
| A104   |   |         |         |      |      |  |      |      |      |      |
| A107   | 0.46  | 0.78    | 0.46    | 0.57 | 0.18 | 3.43   | 3.02 | 3.3  | 3.25 | 0.21 |
|        | Consensus   | Mean    |         | 0.85 |      | Consensus Mean   |      |      | 2.66 |      |
|        | Consensus Standard Deviation  |         |         | 0.30 |      | Consensus Standard Deviation   |      |      | 0.95 |      |
|        | Maximum   |         |         | 1.05 |      | Maximum  |      |      | 3.41 |      |
|        | Minimum   |         |         | 0.57 |      | Minimum  |      |      | 1.5  |      |
|        | Ν   |         |         | 4    |      | Ν  |      |      | 6    |      |

**Table 4-8.** Data summary table for total 3-epi-25-Hydroxyvitamin  $D_3$  in human serum.





**Figure 4-12.** 3-epi-25-Hydroxyvitamin D<sub>3</sub> in SRM 968d Fat-Soluble Vitamins, Carotenoids, and Cholesterol in Human Serum (Level 2) (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \leq 2$ . A NIST value has not been determined in this material.





**Figure 4-13.** 3-epi-25-Hydroxyvitamin D<sub>3</sub> in SRM 2973 Vitamin D Metabolites in Frozen Human Serum (High Level) (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \le 2$ . The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by its uncertainty ( $U_{NIST}$ ) and represents the range that results in an acceptable  $Z_{NIST}$  score,  $|Z_{NIST}| \le 2$ .
**Table 4-9.** Data summary table for 1,25-Dihydroxyvitamin  $D_3$  in human serum. Data points highlighted in red have been flagged as potential outliers (e.g., difference from reference value, Grubb and/or Cochran) by the NIST software package.

|            | ŗ      |                  |                          |                                   | 1,2                      | 5-Dihydro           | xyvitamin     | 1 D3   |             |         | !       |  |
|------------|--------|------------------|--------------------------|-----------------------------------|--------------------------|---------------------|---------------|--|-------------|---------|---------|--|
|            |        | SRM 96<br>and Cł | 68d Fat-So<br>holesterol | luble Vita<br>in Humar<br>(ng/mL) | ımins,Caro<br>1 Serum (I | otenoids<br>Level2) | SRM 29<br>Hui | M 2973 Vitamin D Metabolites in Fr<br>Human Serum (High Level) (ng/mL) |             |         |         |  |
|            | Lab    | Α                | В                        | С                                 | Avg                      | SD                  | A             | В  | С           | Avg     | SD      |  |
|            | Target |                  |                          |                                   |                          |                     |               |  |             |         |         |  |
|            | A012   |                  |                          |                                   |                          |                     | 1.15          | 1.21   | 1.32        | 1.227   | 0.086   |  |
| ts         | A016   |                  |                          |                                   |                          |                     |               |  |             |         |         |  |
| sult       | A038   |                  |                          |                                   |                          |                     |               |  |             |         |         |  |
| Re         | A046   | 0.053            | 0.056                    | 0.054                             | 0.0544                   | 0.0015              | 0.049         | 0.051  | 0.05        | 0.04977 | 0.00095 |  |
| ual        | A070   |                  |                          |                                   |                          |                     |               |  |             |         |         |  |
| vid        | A079   |                  |                          |                                   |                          |                     |               |  |             |         |         |  |
| ndi        | A089   |                  |                          |                                   |                          |                     |               |  |             |         |         |  |
| Ĥ          | A101   | 0.56             | 0.48                     | 0.066                             | 0.37                     | 0.27                | 0.044         |  | 0.043       | 0.0435  | 0.00071 |  |
|            | A103   |                  |                          |                                   |                          |                     |               |  |             |         |         |  |
|            | A104   |                  |                          |                                   |                          |                     |               |  |             |         |         |  |
| ţy         | ·      | Consensu         | ls Mean                  |                                   | 0.242                    | I                   | Consensu      | ıs Mean  |             | 0.047   |         |  |
| umi<br>Its | 1      | Consensu         | is Standard              | I Deviation                       | ı 0.044                  | ļ                   | Consensu      | is Standard  | d Deviation | ı 0.016 | ļ       |  |
| nmi        | 1      | Maximun          | a                        |                                   | 0.37                     | P                   | Maximur       | .1   |             | 1.227   |         |  |
| No.        | 1      | Minimum          | 1                        |                                   | 0.0544                   | P                   | Minimum       | 1  |             | 0.0435  | ļ       |  |
| $\sim$     | 1      | Ν                |                          |                                   | 2                        | ľ                   | Ν             |  |             | 3       |         |  |

**Table 4-10.** Data summary table for 24,25-Dihydroxyvitamin  $D_3$  in human serum. Data points highlighted in red have been flagged as potential outliers (e.g., difference from reference value, Grubb and/or Cochran) by the NIST software package.

|            |        |                  |                           |                                   | 24,25                   | 5-Dihydro         | xyvitamin l   | D3         |           |       |      |  |
|------------|--------|------------------|---------------------------|-----------------------------------|-------------------------|-------------------|---|------------|-----------|-------|------|--|
|            |        | SRM 96<br>and Ch | 8d Fat-Sol<br>olesterol i | luble Vitan<br>n Human<br>(ng/mL) | nins, Caro<br>Serum (Le | tenoids<br>vel 2) | SRM 2973 Vitamin D Metabolites in Fro<br>Human Serum (High Level) (ng/mL) |            |           |       |      |  |
|            | Lab    | Α                | В                         | С                                 | Avg                     | SD                | Α   | В          | С         | Avg   | SD   |  |
|            | Target |                  |                           |                                   |                         |                   |   |            |           | 3.13  | 0.11 |  |
|            | A012   |                  |                           |                                   |                         |                   | 12.31   | 10.37      | 10.16     | 10.95 | 1.19 |  |
| ts         | A016   |                  |                           |                                   |                         |                   |   |            |           |       |      |  |
| ual Resul  | A038   |                  |                           |                                   |                         |                   |   |            |           |       |      |  |
|            | A070   |                  |                           |                                   |                         |                   |   |            |           |       |      |  |
|            | A079   |                  |                           |                                   |                         |                   |   |            |           |       |      |  |
| vid        | A089   | 0.501            | 0.447                     | 0.452                             | 0.467                   | 0.030             | 2.75  | 2.72       | 2.65      | 2.71  | 0.05 |  |
| ndi        | A096   |                  |                           |                                   |                         |                   |   |            |           |       |      |  |
| I          | A103   |                  |                           |                                   |                         |                   |   |            |           |       |      |  |
|            | A104   |                  |                           |                                   |                         |                   |   |            |           |       |      |  |
|            | A106   | 0.51             | 0.5                       | 0.5                               | 0.50                    | 0.01              | 3.31  | 3.23       | 3.15      | 3.23  | 0.08 |  |
| ty         |        | Consensus        | Mean                      |                                   | 0.485                   |                   | Consensus   | Mean       |           | 2.97  |      |  |
| uni<br>lts |        | Consensus        | Standard I                | Deviation                         | 0.020                   |                   | Consensus   | Standard I | Deviation | 1.29  |      |  |
| nm         |        | Maximum          |                           |                                   | 0.50                    |                   | Maximum   |            | 10.95     |       |      |  |
| <b>R</b>   |        | Minimum          |                           |                                   | 0.467                   |                   | Minimum   |            | 2.71      |       |      |  |
| •          |        | Ν                |                           |                                   | 2                       |                   | Ν   |            |           | 3     |      |  |





**Figure 4-14.** 24,25-Dihydroxyvitamin D<sub>3</sub> in SRM 968d Fat-Soluble Vitamins, Carotenoids, and Cholesterol in Human Serum (Level 2) (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable  $Z'_{\text{comm}} | \leq 2$ . A NIST value has not been determined in this material.





**Figure 4-15.** 24,25-Dihydroxyvitamin D<sub>3</sub> in SRM 2973 Vitamin D Metabolites in Frozen Human Serum (High Level) (data summary view –analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \le 2$ . The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by its uncertainty ( $U_{NIST}$ ) and represents the range that results in an acceptable  $Z'_{NIST} | \le 2$ .

### Fat-Soluble Vitamins Overall Study Comparison

The following recommendations are based on results obtained from the participants in this study.

- Food laboratories have more experience measuring fortified vitamin D than endogenous vitamin D metabolites. As requirements for food labeling change and vitamin D becomes a mandated declared nutrient, food companies may see increased demand for measurement of 25-hydroxyvitamin D, in particular. In turn, this increased demand will lead to greater need for interlaboratory comparisons and reference materials with values assigned for vitamin D metabolites in food.<sup>6,7</sup>
- Clinical laboratories demonstrated good performance in the determination of 25-hydroxyvitamin D in serum. Better performance of clinical laboratories in this measurement is expected given the longer history of vitamin D metabolites measurements and the existence of numerous interlaboratory comparisons, proficiency tests, and reference materials with values assigned for vitamin D metabolites in human serum.
- Beyond 25-hydroxyvitamin D, clinical laboratories had lower participation, indicating that newer analytes such as the 1,25-dihydroxyvitamin D and 24,25-dihydroxyvitamin D metabolites will be a key area for future interlaboratory comparisons and reference material development.

<sup>&</sup>lt;sup>6</sup> Ovesen, L.; Brot, C.; Jakobsen, J.; Ann Nutr Metab 47, pp. 107–113 (2003).

<sup>&</sup>lt;sup>7</sup> Taylor, C.L.; Roseland, J.M.; Coates, P.M.; Pehrsson, P.R.; J Nutr 146(4), pp. 855–856 (2016).

#### **SECTION 5: FATTY ACIDS**

#### **Study Overview**

In this study, participants were asked to measure 10 fatty acids (both total and free) in three dietary intake samples and in three human metabolite samples. Total fatty acids are defined as the fatty acid content after acid and/or base hydrolysis of the sample that converts the lipids into their individual fatty acid constituents (typically as fatty acid methyl esters). Free fatty acids are defined as those present as unconjugated, endogenous free acids. For the dietary intake study, participants were provided with three materials, a solution of mixed free fatty acids (Fatty Acids Solution A), NIST SRM 3275-II Omega-3 and Omega-6 Fatty Acids in Fish Oil, and Cod Liver Oil. For the human metabolite study, participants were provided with three materials, a solution of mixed free fatty acids (Fatty Acids Solution B), SRM 1950 Metabolites in Frozen Human Plasma, and an additional human plasma sample (Human Plasma B). Participants were asked to use in-house analytical methods to determine the mass fractions of total fatty acids and free fatty acids in mg/g, on an as-received basis. Fatty acids from the diet can have beneficial and detrimental effects on human health depending on the degree and type of saturation. Thus, the fatty acid content of human plasma and serum can serve as indicators of health status and diet quality. A recent paper demonstrated the link between fatty acid supplements (e.g., flaxseed oil and fish oil) and enrichments of specific fatty acids in human blood serum.<sup>8</sup> Additionally, levels of fatty acids measured in their free form in serum or plasma, prior to any acid or base hydrolysis, versus conjugated as triglycerides and phospholipids, have been proposed as possible health markers for metabolic,<sup>9</sup> neurological,<sup>10</sup> and cardiovascular<sup>11,12</sup> diseases. This interlaboratory study provided the opportunity for measurement of fatty acids in both foods (intake) and serum (metabolites).

### **Dietary Intake Sample Information**

Fatty Acids Solution A. Participants were provided with one amber glass ampoule containing 1.2 mL of a toluene solution containing mixed free fatty acids. Before use, participants were instructed to invert the ampoule to mix the solution and withdraw subsamples at 20 °C to 25 °C immediately after opening the ampoule. Participants were asked to store the solution in the dark at temperatures between -20 °C and -80 °C, to process the samples without delay, and to prepare three samples and report three values from the single ampoule provided. The approximate analyte levels were not reported to the participants prior to the study. The target values for the fatty acids in Solution A were determined by gravimetric preparation using neat fatty acid standards with an uncertainty approximated as 5 % of the mass fraction. Purity of the neat fatty acids used to prepare Fatty Acids Solution A was not determined. The NIST-determined mass fractions and uncertainties for free stearic acid, free cis-vaccenic acid, free oleic acid, free linoleic acid, free α-linolenic acid, free arachidonic acid, free eicosapentaenoic acid (EPA), free docosapentaenoic acid (DPA), and free docosahexaenoic acid (DHA) in Fatty Acids Solution A are provided in the table below. Free  $\gamma$ -linolenic acid was not added to Fatty Acids Solution A. Since only free fatty acids were used in the solution preparation, the target values for total and free fatty acids are the same (i.e., a method to determine total fatty acids should get the same value as for a method to determine free fatty acids in this solution).

 <sup>&</sup>lt;sup>8</sup> Benner, B.A.; Schantz, M.M.; Powers, C.D.; Schleicher, R.L.; Camara, J.; et al.; *Anal Bioanal Chem* 410; pp. 2321–2329 (2018).
<sup>9</sup> Chu, X.; He, X.; Shi, Z.; Li, C.; Guo, F.; et al.; *Mol Nutr Food Res* 59(8); pp. 1491–1503 (2015).

<sup>&</sup>lt;sup>10</sup> Nishikiori, M.; Iizuka, H.; Ichiba, H.; Sadamoto, K.; Fukushima, T.; J Chromatogr Sci 53(4); pp. 537–541 (2014).

<sup>&</sup>lt;sup>11</sup> Guo, S.-X.; Yan, Y.-Z.; Mu, L.-T.; Niu, Q.; He, J.; et al.; Int J Environ Res Public Health 12(6); pp. 6582–6590 (2015).

<sup>&</sup>lt;sup>12</sup> Salerno, A.; Fragasso, G.; Esposito, A.; Canu, T.; Lattuada, G.; et al.; Acta Diabetologica 52(4); pp. 753–761 (2015).

|                               | <b>NIST-Determined Mass Fraction</b> |
|-------------------------------|--------------------------------------|
| Analyte (Total and Free)      | in Fatty Acids Solution A (mg/g)     |
| Stearic Acid (C18:0)          | $0.180 \pm 0.0090$                   |
| cis-Vaccenic Acid (C18:1 n-7) | $0.037 \pm 0.00185$                  |
| Oleic Acid (C18:1 n-9)        | $0.428 \pm 0.0214$                   |
| Linoleic Acid (C18:2 n-6)     | $0.802 \pm 0.0401$                   |
| α-Linolenic Acid (C18:3 n-3)  | $0.014 \pm 0.0007$                   |
| Arachidonic Acid (C20:4 n-6)  | $0.282 \pm 0.0141$                   |
| EPA (C20:5 n-3)               | $0.013 \pm 0.00065$                  |
| DPA (C22:5 n-3)               | $0.015 \pm 0.00075$                  |
| DHA (C22:6 n-3)               | $0.037 \pm 0.00185$                  |

Fish Oil. Participants were provided with three ampoules, each containing 1.2 mL of anchovy oil. The commercial fish oil was combined with mixed natural tocopherols by the supplier and packaged in amber glass ampoules that were flushed with argon. Before use, participants were instructed to thoroughly mix the ampoule prior to removal of a test sample, and to use a sample size of at least 0.5 g. Participants were asked to store the material under refrigeration at 2 °C to 8 °C, and to prepare one sample and report one value from each ampoule provided. The approximate analyte levels were not reported to participants prior to the study. Certified values for total stearic acid, total cis-vaccenic acid, total oleic acid, and total linoleic acid in SRM 3275-II were determined at NIST using gas chromatography with flame ionization detection (GC-FID) and gas chromatography mass spectrometry (GC-MS) using different transesterification methods. Reference values for total  $\alpha$ -linolenic acid, total  $\gamma$ -linolenic acid, total arachidonic acid, total EPA, total DPA, and total DHA in SRM 3275-II were determined at NIST using GC-FID. The NISTdetermined mass fractions and uncertainties for total stearic acid, total cis-vaccenic acid, total oleic acid, and total linoleic acid, total  $\alpha$ -linolenic acid, total  $\gamma$ -linolenic acid, total arachidonic acid, total EPA, total DPA, and total DHA in SRM 3275-II are provided in the table below. Target values for free fatty acids in SRM 3275-II have not been determined at NIST.

|                                     | NIST-Determined Mass Fractio |  |  |  |  |  |  |  |
|-------------------------------------|------------------------------|--|--|--|--|--|--|--|
| Analyte                             | <u>in SRM 3275-II (mg/g)</u> |  |  |  |  |  |  |  |
| Total Stearic Acid (C18:0)          | $12.94 \pm 0.62$             |  |  |  |  |  |  |  |
| Total cis-Vaccenic Acid (C18:1 n-7) | $9.24 \pm 0.77$              |  |  |  |  |  |  |  |
| Total Oleic Acid (C18:1 n-9)        | $22.1 \pm 1.6$               |  |  |  |  |  |  |  |
| Total Linoleic Acid (C18:2 n-6)     | $3.00 \pm 0.42$              |  |  |  |  |  |  |  |
| Total α-Linolenic Acid (C18:3 n-3)  | $1.42 \pm 0.12$              |  |  |  |  |  |  |  |
| Total γ-Linolenic Acid (C18:3 n-3)  | $0.507 \pm 0.043$            |  |  |  |  |  |  |  |
| Total Arachidonic Acid (C20:4 n-6)  | $22.9 \pm 1.0$               |  |  |  |  |  |  |  |
| Total EPA (C20:5 n-3)               | 394 ± 17                     |  |  |  |  |  |  |  |
| Total DPA (C22:5 n-3)               | $67.6 \pm 2.3$               |  |  |  |  |  |  |  |
| Total DHA (C22:6 n-3)               | $187 \pm 8$                  |  |  |  |  |  |  |  |

*Cod Liver Oil.* Participants were provided three ampoules, each containing 5 mL of commercial cod liver oil. The oil was packaged in amber glass ampoules that were flushed with argon. Before use, participants were instructed to thoroughly mix the ampoule prior to removal of a test sample, and to use a sample size of at least 0.5 g. Participants were asked to store the material under refrigeration at 2  $^{\circ}$ C to 8  $^{\circ}$ C, and to prepare one sample and report one value from each ampoule provided. The approximate analyte levels were not reported to participants prior to the study, and target values for free fatty acids or total fatty acids in Cod Liver Oil have not been determined at NIST.

### **Dietary Intake Study Results**

• Twenty-five to twenty-nine laboratories enrolled in this exercise to measure total fatty acids. The table below lists the participation statistics for total fatty acids in the dietary intake samples.

|                                     | Number of <u>Number of Laboratories Reporting Re</u> |             |                    |            |  |  |  |
|-------------------------------------|--|-------------|--------------------|------------|--|--|--|
|                                     | Laboratories   | <u>(Pe</u>  | rcent Participatio | <u>on)</u> |  |  |  |
|                                     | <b>Requesting</b>                                    | Fatty Acids | <u>SRM 3275-II</u> | Cod Liver  |  |  |  |
| Analyte                             | Samples 1  | Solution A  | (Fish Oil)         | <u>Oil</u> |  |  |  |
| Total Stearic Acid (C18:0)          | 27   | 12 (44 %)   | 15 (56 %)          | 15 (56 %)  |  |  |  |
| Total cis-Vaccenic Acid (C18:1 n-7) | 25   | 6 (24 %)    | 10 (40 %)          | 9 (36 %)   |  |  |  |
| Total Oleic Acid (C18:1 n-9)        | 27   | 11 (41 %)   | 14 (52 %)          | 15 (56 %)  |  |  |  |
| Total Linoleic Acid (C18:2 n-6)     | 27   | 12 (44 %)   | 14 (52 %)          | 14 (52 %)  |  |  |  |
| Total α-Linolenic Acid (C18:3 n-3)  | 27   | 9 (33 %)    | 13 (48 %)          | 13 (48 %)  |  |  |  |
| Total γ-Linolenic Acid (C18:3 n-3)  | 27   | 8 (30 %)    | 13 (48 %)          | 10 (37 %)  |  |  |  |
| Total Arachidonic Acid (C20:4 n-6)  | 26   | 11 (42 %)   | 15 (58 %)          | 14 (54 %)  |  |  |  |
| Total EPA (C20:5 n-3)               | 29   | 9 (31 %)    | 15 (52 %)          | 15 (52 %)  |  |  |  |
| Total DPA (C22:5 n-3)               | 27   | 8 (30 %)    | 13 (48 %)          | 11 (41 %)  |  |  |  |
| Total DHA (C22:6 n-3)               | 29   | 8 (28 %)    | 15 (52 %)          | 15 (52 %)  |  |  |  |

The consensus means for most of the fatty acids in Fatty Acids Solution A were below the • target ranges and the consensus ranges overlapped with the target ranges (see table below). The exceptions included total stearic acid and total linoleic acid, with consensus values within the target ranges. The between-laboratory variability is also summarized below and ranged from good for total linoleic acid (17%) to very high for total cis-vaccenic acid and total EPA (> 200 %). For most fatty acids in this sample, the between-laboratory variability was over 75 %.

|                               | Performance Summary for Fatty Acids Solu  | ution A            |
|-------------------------------|---|--------------------|
|                               |   | Between-           |
|                               | Relative Position of  | Laboratory         |
| Analyte (Total and Free)      | Consensus and Target Ranges   | <u>Variability</u> |
| Stearic Acid (C18:0)          | Consensus mean <i>within</i> target range<br>Consensus range <i>overlaps</i> target range         | 112 %              |
| cis-Vaccenic Acid (C18:1 n-7) | Consensus mean <i>below</i> target range<br>Consensus range <i>overlaps</i> target range          | 217 %              |
| Oleic Acid (C18:1 n-9)        | Consensus mean <i>slightly below</i> target range<br>Consensus range <i>overlaps</i> target range | 102 %              |
| Linoleic Acid (C18:2 n-6)     | Consensus mean <i>within</i> target range<br>Consensus range <i>overlaps</i> target range         | 17 %               |
| α-Linolenic Acid (C18:3 n-3)  | Consensus mean <i>below</i> target range<br>Consensus range <i>does not overlap</i> target range  | 100 %              |
| Arachidonic Acid (C20:4 n-6)  | Consensus mean <i>slightly below</i> target<br>Consensus range <i>overlaps</i> target range       | 76 %               |
| EPA (C20:5 n-3)               | Consensus mean <i>below</i> target range<br>Consensus range <i>slightly overlaps</i> target range | 200 %              |
| DPA (C22:5 n-3)               | Consensus mean <i>below</i> target range<br>Consensus range <i>does not overlap</i> target range  | 160 %              |
| DHA (C22:6 n-3)               | Consensus mean <i>below</i> target range<br>Consensus range <i>does not overlap</i> target range  | 74 %               |

The consensus means for most of the fatty acids in SRM 3275-II were within the target ranges and the consensus ranges overlapped with the target ranges (see table below). The exceptions included total γ-linolenic acid and total DHA, with consensus values above and below the target ranges, respectively. The between-laboratory variability is also summarized below and was good for most fatty acids (less than 25 % RSD), with the exception of total α-linolenic acid (41 %) and γ-linolenic acid (99 %).

|                               | Performance Summary for SRM 3275-II ()  | Fish Oil)          |
|-------------------------------|---|--------------------|
|                               |   | Between-           |
|                               | Relative Position of Consensus and Target   | Laboratory         |
| Analyte (Total)               | Ranges  | <u>Variability</u> |
| Stearic Acid (C18:0)          | Consensus mean <i>within</i> target range   | 10 %               |
| cis-Vaccenic Acid (C18:1 n-7) | Consensus range overlaps target range<br>Consensus range overlaps target range                    | 9 %                |
| Oleic Acid (C18:1 n-9)        | Consensus mean <i>within</i> target range<br>Consensus range <i>overlaps</i> target range         | 20 %               |
| Linoleic Acid (C18:2 n-6)     | Consensus mean <i>within</i> target range<br>Consensus range <i>overlaps</i> target range         | 22 %               |
| α-Linolenic Acid (C18:3 n-3)  | Consensus mean <i>within</i> target range<br>Consensus range <i>overlaps</i> target range         | 41 %               |
| γ-Linolenic Acid (C18:3 n-3)  | Consensus mean <i>above</i> target range<br>Consensus range <i>overlaps</i> target range          | 99 %               |
| Arachidonic Acid (C20:4 n-6)  | Consensus mean <i>overlaps</i> target<br>Consensus range <i>overlaps</i> target range             | 24 %               |
| EPA (C20:5 n-3)               | Consensus mean <i>overlaps</i> target range<br>Consensus range <i>overlaps</i> target range       | 13 %               |
| DPA (C22:5 n-3)               | Consensus mean <i>overlaps</i> target range<br>Consensus range <i>overlaps</i> target range       | 10 %               |
| DHA (C22:6 n-3)               | Consensus mean slightly <i>below</i> target range<br>Consensus range <i>overlaps</i> target range | 21 %               |

 The between-laboratory variability for Cod Liver Oil is summarized below and was good for most fatty acids (less than 25 % RSD), with the exception of γ-linolenic acid (74 %) and total arachidonic acid (48 %).

|                               | Between-Laboratory Variability |
|-------------------------------|--------------------------------|
| Analyte (Total)               | in Cod Liver Oil               |
| Stearic Acid (C18:0)          | 16 %                           |
| cis-Vaccenic Acid (C18:1 n-7) | 6 %                            |
| Oleic Acid (C18:1 n-9)        | 30 %                           |
| Linoleic Acid (C18:2 n-6)     | 21 %                           |
| α-Linolenic Acid (C18:3 n-3)  | 25 %                           |
| γ-Linolenic Acid (C18:3 n-3)  | 74 %                           |
| Arachidonic Acid (C20:4 n-6)  | 48 %                           |
| EPA (C20:5 n-3)               | 12 %                           |
| DPA (C22:5 n-3)               | 9 %                            |
| DHA (C22:6 n-3)               | 19 %                           |
|                               |                                |

- Three laboratories reported using GC-FID for determination of total fatty acids. No other laboratories specified the analytical methods used.
- Nineteen to twenty-one laboratories enrolled in this exercise to measure free fatty acids. The table below lists the participation statistics for free fatty acids.

|                               | Number of         | Number of Laboratories Reporting Result |                    |               |  |  |  |  |  |
|-------------------------------|-------------------|---|--------------------|---------------|--|--|--|--|--|
|                               | Laboratories      | <u>(Pe</u>                              | ercent Participati | <u>on)</u>    |  |  |  |  |  |
|                               | <u>Requesting</u> | Fatty Acids                             | <u>SRM 3275-II</u> |               |  |  |  |  |  |
| Analyte (Free)                | Samples           | Solution A                              | (Fish Oil)         | Cod Liver Oil |  |  |  |  |  |
| Stearic Acid (C18:0)          | 21                | 4 (19 %)                                | 2 (10 %)           | 2 (10 %)      |  |  |  |  |  |
| cis-Vaccenic Acid (C18:1 n-7) | 19                | 1 (5 %)                                 | 0 (0 %)            | 0 (0 %)       |  |  |  |  |  |
| Oleic Acid (C18:1 n-9)        | 21                | 4 (19 %)                                | 2 (10 %)           | 2 (10 %)      |  |  |  |  |  |
| Linoleic Acid (C18:2 n-6)     | 21                | 4 (19 %)                                | 2 (10 %)           | 2 (10 %)      |  |  |  |  |  |
| α-Linolenic Acid (C18:3 n-3)  | 21                | 3 (14 %)                                | 2 (10 %)           | 2 (10 %)      |  |  |  |  |  |
| γ-Linolenic Acid (C18:3 n-3)  | 21                | 3 (14 %)                                | 2 (10 %)           | 2 (10 %)      |  |  |  |  |  |
| Arachidonic Acid (C20:4 n-6)  | 20                | 4 (20 %)                                | 2 (10 %)           | 2 (10 %)      |  |  |  |  |  |
| EPA (C20:5 n-3)               | 23                | 3 (13 %)                                | 3 (13 %)           | 3 (13 %)      |  |  |  |  |  |
| DPA (C22:5 n-3)               | 21                | 3 (14 %)                                | 2 (10 %)           | 2 (10 %)      |  |  |  |  |  |
| DHA (C22:6 n-3)               | 23                | 3 (13 %)                                | 3 (13 %)           | 3 (13 %)      |  |  |  |  |  |
|                               |                   |   |                    |               |  |  |  |  |  |

• The percentage of laboratories that returned data for free fatty acids ranged from 0 % to 13 %. Due to the low participation rate, results for free fatty acids are only presented in table format in the following section (no graphical representation).

## Dietary Intake Technical Recommendations

The following recommendations are based on results obtained from the participants in this study.

- Most laboratories reported values below the NIST-determined value for free and total fatty acids in Fatty Acids Solution A.
  - The mass fractions of the free fatty acids in Fatty Acids Solution A were orders of magnitude below those in SRM 3275-II and Cod Liver Oil. For example, the target value for total stearic acid in Fatty Acids Solution A is 0.18 mg/g, while the target values for total stearic acid in SRM 3275-II and Cod Liver Oil are 12.7 mg/g and 18.1 mg/g, respectively.
  - Methods for determination of fatty acids in oils and foods typically focus on relatively high concentrations (e.g., the oil samples ranged from 0.88 mg/g to 394 mg/g for individual fatty acids), so the calibration range employed by the participants may not have been appropriate for low-level concentrations of fatty acids in solution.
  - The target values for the fatty acids in Fatty Acids Solution A were determined based on the gravimetric preparation. The purity of the neat fatty acids used to prepare the solution was not evaluated; impurities in these neat materials may be the cause of the apparent low bias in the consensus values.
  - Although all possible precautions were taken to ensure stability, the fatty acids in Fatty Acids Solution A may have degraded since the preparation.
  - Laboratories that are unable to detect fatty acids should report the method detection limit; zero values should not be reported.
- For the two oil-based samples (SRM 3275-II and Cod Liver Oil), the between-laboratory variability about the consensus means were good for most of the total fatty acids. The accuracy for SRM 3275-II, as determined by the consensus means compared to the target range, was good for most of the total fatty acids.
  - Greater differences between the consensus mean and the target range were observed in individual fatty acids with lower concentrations, such as total  $\gamma$ -linolenic acid (0.88 mg/g), which generally corresponds with the results for Fatty Acids Solution A.
  - Chromatographic coelution can cause a high bias in reported results. Adequate separation of fatty acids is necessary for accurate measurements.
- Generally, fewer participants reported results for free fatty acids in comparison to the total fatty acids, although the number of enrolled laboratories was similar.
  - Participating laboratories may not have analytical methods to measure free fatty acids, or the measurement of free fatty acids is not a current concern of the participating laboratories.
  - Future studies will focus on measurement of total fatty acids, unless a need for reporting of free fatty acids is indicated by participating laboratories.
- All results should be checked closely to avoid calculation errors and to be sure that results are reported in the requested units.

## Table 5-1. Individualized data summary table (NIST) for total fatty acids in a solution, fish oil, and cod liver oil.

#### National Institute of Standards & Technology

|  | Lab Code:  | NIST  | - Iotai Fatty         | 1. Your       | Results            | .)                | 2. C          | ommunity I  | Results           | 3. T         | Farget |  |
|--|--|-------|-----------------------|---------------|--------------------|-------------------|---------------|-------------|-------------------|--------------|--------|--|
| Analyte                                | Sample   | Units | <i>x</i> <sub>i</sub> | S i           | Z' <sub>comm</sub> | Z <sub>NIST</sub> | N             | x*          | s*                | X NIST       | U      |  |
| Total Stearic Acid (C18:0)             | Fatty Acids Solution A                                     | mg/g  | 0.18                  | 0.0045        |                    |                   | 11            | 0.2         | 0.2               | 0.18         | 0.00   |  |
| Total Stearic Acid (C18:0)             | SRM 3275-II Omega-3 and Omega-6                            | mg/g  | 12.9                  | 0.31          |                    |                   | 16            | 12.7        | 1.3               | 12.9         | 0.62   |  |
| Total Stearic Acid (C18:0)             | Fatty Acids in Fish Oil<br>Cod Liver Oil                   | mg/g  |                       |               |                    |                   | 15            | 18.1        | 2.9               |              |        |  |
| Total cis-Vaccenic Acid (C18:1 n-7)    | Fatty Acids Solution A                                     | mg/g  | 0.037                 | 0.001         |                    |                   | 5             | 0.018       | 0.039             | 0.037        | 0.00   |  |
| Total cis-Vaccenic Acid (C18:1 n-7)    | SRM 3275-II Omega-3 and Omega-6<br>Fatty Acids in Fish Oil | mg/g  | 9.24                  | 0.385         |                    |                   | 10            | 8.99        | 0.83              | 9.24         | 0.7    |  |
| Total cis-Vaccenic Acid (C18:1 n-7)    | Cod Liver Oil  | mg/g  |                       |               |                    |                   | 8             | 40.8        | 2.5               |              |        |  |
| Total Oleic Acid (C18:1 n-9)           | Fatty Acids Solution A                                     | mg/g  | 0.428                 | 0.0105        |                    |                   | 11            | 0.38        | 0.39              | 0.428        | 0.02   |  |
| Total Oleic Acid (C18:1 n-9)           | SRM 3275-II Omega-3 and Omega-6<br>Fatty Acids in Fish Oil | mg/g  | 22.1                  | 0.8           |                    |                   | 15            | 23          | 4.7               | 22.1         | 1.6    |  |
| Total Oleic Acid (C18:1 n-9)           | Cod Liver Oil  | mg/g  |                       |               |                    |                   | 15            | 140         | 42                |              |        |  |
| Total Linoleic Acid (C18:2 n-6)        | Fatty Acids Solution A                                     | mg/g  | 0.802                 | 0.02          |                    |                   | 12            | 0.77        | 0.13              | 0.802        | 0.0    |  |
| Total Linoleic Acid (C18:2 n-6)        | SRM 3275-II Omega-3 and Omega-6<br>Fatty Acids in Fish Oil | mg/g  | 3                     | 0.21          |                    |                   | 14            | 2.93        | 0.64              | 3            | 0.4    |  |
| Total Linoleic Acid (C18:2 n-6)        | Cod Liver Oil  | mg/g  |                       |               |                    |                   | 14            | 20.2        | 4.3               |              |        |  |
| Total alpha-Linolenic Acid (C18:3 n-3) | Fatty Acids Solution A                                     | mg/g  | 0.014                 | 0.0005        |                    |                   | 8             | 0.007       | 0.007             | 0.014        | 0.0    |  |
| Total alpha-Linolenic Acid (C18:3 n-3) | SRM 3275-II Omega-3 and Omega-6<br>Fatty Acids in Fish Oil | mg/g  | 1.42                  | 0.06          |                    |                   | 13            | 1.5         | 0.61              | 1.42         | 0.1    |  |
| Total alpha-Linolenic Acid (C18:3 n-3) | Cod Liver Oil  | mg/g  |                       |               |                    |                   | 12            | 8.8         | 2.2               |              |        |  |
| otal gamma-Linolenic Acid (C18:3 n-6)  | Fatty Acids Solution A                                     | mg/g  |                       |               |                    |                   | 6             | 0           | 0.002             |              |        |  |
| 'otal gamma-Linolenic Acid (C18:3 n-6) | SRM 3275-II Omega-3 and Omega-6<br>Fatty Acids in Fish Oil | mg/g  | 0.507                 | 0.0215        |                    |                   | 14            | 0.88        | 0.87              | 0.507        | 0.0    |  |
| Cotal gamma-Linolenic Acid (C18:3 n-6) | Cod Liver Oil  | mg/g  |                       |               |                    |                   | 9             | 1.9         | 1.4               |              |        |  |
| Total Arachidonic Acid (C20:4 n-6)     | Fatty Acids Solution A                                     | mg/g  | 0.282                 | 0.007         |                    |                   | 10            | 0.3         | 0.2               | 0.282        | 0.0    |  |
| Total Arachidonic Acid (C20:4 n-6)     | SRM 3275-II Omega-3 and Omega-6<br>Fatty Acids in Fish Oil | mg/g  | 22.9                  | 0.5           |                    |                   | 15            | 22.7        | 5.5               | 22.9         | 1      |  |
| Total Arachidonic Acid (C20:4 n-6)     | Cod Liver Oil  | mg/g  |                       |               |                    |                   | 13            | 2.9         | 1.4               |              |        |  |
| Total EPA (C20:5 n-3)                  | Fatty Acids Solution A                                     | mg/g  | 0.013                 | 0.0005        |                    |                   | 8             | 0           | 0.01              | 0.013        | 0.0    |  |
| Total EPA (C20:5 n-3)                  | SRM 3275-II Omega-3 and Omega-6<br>Fatty Acids in Fish Oil | mg/g  | 394                   | 8.5           |                    |                   | 16            | 390         | 50                | 394          | 1'     |  |
| Total EPA (C20:5 n-3)                  | Cod Liver Oil  | mg/g  |                       |               |                    |                   | 15            | 76.5        | 8.9               |              |        |  |
| Total DPA (C22:5 n-3)                  | Fatty Acids Solution A                                     | mg/g  | 0.015                 | 0.0005        |                    |                   | 7             | 0.005       | 0.008             | 0.015        | 0.0    |  |
| Total DPA (C22:5 n-3)                  | SRM 3275-II Omega-3 and Omega-6<br>Fatty Acids in Fish Oil | mg/g  | 67.6                  | 1.15          |                    |                   | 14            | 66.7        | 6.7               | 67.6         | 2.     |  |
| Total DPA (C22:5 n-3)                  | Cod Liver Oil  | mg/g  |                       |               |                    |                   | 11            | 10.5        | 0.93              |              |        |  |
| Total DHA (C22:6 n-3)                  | Fatty Acids Solution A                                     | mg/g  | 0.037                 | 0.001         |                    |                   | 7             | 0.019       | 0.014             | 0.037        | 0.0    |  |
| Total DHA (C22:6 n-3)                  | SRM 3275-II Omega-3 and Omega-6<br>Fatty Acids in Fish Oil | mg/g  | 187                   | 4             |                    |                   | 16            | 180         | 37                | 187          | 8      |  |
| Total DHA (C22:6 n-3)                  | Cod Liver Oil  | mg/g  |                       |               |                    |                   | 15            | 110         | 21                |              |        |  |
|  |  | x     | Mean of rep           | ported value  | s                  | Ν                 | Number of q   | uantitative | x <sub>NIST</sub> | NIST-assess  | sed va |  |
|  |  | S     | Standard de           | viation of re | ported value       | s                 | values report | ted         | U                 | expanded und | ertain |  |

values reported  $Z'_{\text{comm}}$  Z'-score with respect to community

x\* Robust mean of reported about the NIST-assessed value

consensus

values  $Z_{\rm NIST}~$  Z-score with respect to NIST value s\* Robust standard deviation

# Table 5-2. Individualized data summary table (NIST) for free fatty acids in a solution, fish oil, and cod liver oil.

### National Institute of Standards & Technology

|                                       | Lab Code:  | NIST            |              | 1. Your        | Results      | ·                 | 2. C         | Community I   | Results           | sults 3. Target |             |  |
|---------------------------------------|--|-----------------|--------------|----------------|--------------|-------------------|--------------|---------------|-------------------|-----------------|-------------|--|
| Analyte                               | Sample   | Units           | $x_i$        | S <sub>i</sub> | $Z'_{comm}$  | Z <sub>NIST</sub> | N            | <i>x</i> *    | <i>s</i> *        | X NIST          | U           |  |
| Free Stearic Acid (C18:0)             | Fatty Acids Solution A                                     | mg/g            | 0.18         | 0.0045         |              |                   | 4            | 0.16          | 0.011             | 0.18            | 0.009       |  |
| Free Stearic Acid (C18:0)             | SRM 3275-II Omega-3 and Omega-6                            | mg/g            |              |                |              |                   | 2            | 2.2           | 4.5               |                 |             |  |
|                                       | Fatty Acids in Fish Oil                                    |                 |              |                |              |                   |              |               |                   |                 |             |  |
| Free Stearic Acid (C18:0)             | Cod Liver Oil  | mg/g            |              |                |              |                   | 2            | 0.5           | 1.3               |                 |             |  |
| Free cis-Vaccenic Acid (C18:1 n-7)    | Fatty Acids Solution A                                     | mg/g            | 0.037        | 0.001          |              |                   | 2            |               |                   | 0.037           | 0.002       |  |
| Free cis-Vaccenic Acid (C18:1 n-7)    | SRM 3275-II Omega-3 and Omega-6                            | mg/g            |              |                |              |                   | 1            |               |                   |                 |             |  |
|                                       | Fatty Acids in Fish Oil                                    |                 |              |                |              |                   |              |               |                   |                 |             |  |
| Free cis-Vaccenic Acid (C18:1 n-7)    | Cod Liver Oil  | mg/g            |              |                |              |                   | 1            |               |                   |                 |             |  |
| Free Oleic Acid (C18:1 n-9)           | Fatty Acids Solution A                                     | mg/g            | 0.428        | 0.0105         |              |                   | 4            | 0.408         | 0.038             | 0.428           | 0.021       |  |
| Free Oleic Acid (C18:1 n-9)           | SRM 3275-II Omega-3 and Omega-6                            | mg/g            |              |                |              |                   | 2            | 4.11          | 9.2               |                 |             |  |
|                                       | Fatty Acids in Fish Oil                                    |                 |              |                |              |                   |              |               |                   |                 |             |  |
| Free Oleic Acid (C18:1 n-9)           | Cod Liver Oil  | mg/g            |              |                |              |                   | 2            | 10            | 17                |                 |             |  |
| Free Linoleic Acid (C18:2 n-6)        | Fatty Acids Solution A                                     | mg/g            | 0.802        | 0.02           |              |                   | 4            | 0.716         | 0.074             | 0.802           | 0.04        |  |
| Free Linoleic Acid (C18:2 n-6)        | SRM 3275-II Omega-3 and Omega-6                            | mg/g            |              |                |              |                   | 2            | 0.5           | 1.1               |                 |             |  |
|                                       | Fatty Acids in Fish Oil                                    |                 |              |                |              |                   |              |               |                   |                 |             |  |
| Free Linoleic Acid (C18:2 n-6)        | Cod Liver Oil  | mg/g            |              |                |              |                   | 2            | 0.8           | 2.5               |                 |             |  |
| Free alpha-Linolenic Acid (C18:3 n-3) | Fatty Acids Solution A                                     | mg/g            | 0.014        | 0.0005         |              |                   | 3            | 0.005         | 0.018             | 0.014           | 0.001       |  |
| Free alpha-Linolenic Acid (C18:3 n-3) | SRM 3275-II Omega-3 and Omega-6                            | mg/g            |              |                |              |                   | 2            | 0.34          | 0.29              |                 |             |  |
|                                       | Fatty Acids in Fish Oil                                    |                 |              |                |              |                   |              |               |                   |                 |             |  |
| Free alpha-Linolenic Acid (C18:3 n-3) | Cod Liver Oil  | mg/g            |              |                |              |                   | 2            | 0.3           | 1.1               |                 |             |  |
| Free gamma-Linolenic Acid (C18:3 n-6) | Fatty Acids Solution A                                     | mg/g            |              |                |              |                   | 2            | 0             | 0                 |                 |             |  |
| Free gamma-Linolenic Acid (C18:3 n-6) | SRM 3275-II Omega-3 and Omega-6<br>Fatty Acids in Fish Oil | mg/g            |              |                |              |                   | 2            | 0.05          | 0.06              |                 |             |  |
| Free gamma-Linolenic Acid (C18:3 n-6) | Cod Liver Oil  | mg/g            |              |                |              |                   | 2            | 0.031         | 0.079             |                 |             |  |
| Free Arachidonic Acid (C20:4 n-6)     | Fatty Acids Solution A                                     | mg/g            | 0.282        | 0.007          |              |                   | 4            | 0.253         | 0.022             | 0.282           | 0.014       |  |
| Free Arachidonic Acid (C20:4 n-6)     | SRM 3275-II Omega-3 and Omega-6<br>Fatty Acids in Fish Oil | mg/g            |              |                |              |                   | 2            | 0             | 11                |                 |             |  |
| Free Arachidonic Acid (C20:4 n-6)     | Cod Liver Oil  | mg/g            |              |                |              |                   | 2            | 0.1           | 0.36              |                 |             |  |
| Free EPA (C20:5 n-3)                  | Fatty Acids Solution A                                     | mg/g            | 0.013        | 0.0005         |              |                   | 3            | 0.005         | 0.021             | 0.013           | 0.001       |  |
| Free EPA (C20:5 n-3)                  | SRM 3275-II Omega-3 and Omega-6<br>Fatty Acids in Fish Oil | mg/g            |              |                |              |                   | 3            | 200           | 220               |                 |             |  |
| Free EPA (C20:5 n-3)                  | Cod Liver Oil  | mg/g            |              |                |              |                   | 3            | 0             | 11                |                 |             |  |
| Free DPA (C22:5 n-3)                  | Fatty Acids Solution A                                     | mg/g            | 0.015        | 0.0005         |              |                   | 3            | 0.006         | 0.025             | 0.015           | 0.001       |  |
| Free DPA (C22:5 n-3)                  | SRM 3275-II Omega-3 and Omega-6<br>Fatty Acids in Fish Oil | mg/g            |              |                |              |                   | 2            | 10            | 31                |                 |             |  |
| Free DPA (C22:5 n-3)                  | Cod Liver Oil  | mg/g            |              |                |              |                   | 2            | 0.3           | 1.1               |                 |             |  |
| Free DHA (C22:6 n-3)                  | Fatty Acids Solution A                                     | mg/g            | 0.037        | 0.001          |              |                   | 3            | 0.027         | 0.002             | 0.037           | 0.002       |  |
| Free DHA (C22:6 n-3)                  | SRM 3275-II Omega-3 and Omega-6                            | mg/g            |              |                |              |                   | 3            | 80            | 100               |                 |             |  |
|                                       | Fatty Acids in Fish Oil                                    | 00              |              |                |              |                   |              |               |                   |                 |             |  |
| Free DHA (C22:6 n-3)                  | Cod Liver Oil  | mg/g            |              |                |              |                   | 3            | 0             | 13                |                 |             |  |
|                                       |  | <i>x i</i>      | Mean of rep  | orted value    | s            | Ν                 | Number of c  | quantitative  | x <sub>NIST</sub> | NIST-assess     | ed value    |  |
|                                       |  | s <sub>i</sub>  | Standard de  | viation of re  | ported value | s                 | values repor | ted           | U                 | expanded und    | ertainty    |  |
|                                       |  | $Z'_{\rm comm}$ | Z'-score wit | h respect to   | community    | <i>x</i> *        | Robust mean  | n of reported |                   | about the NIS   | ST-assessed |  |

consensus

 $Z_{\rm NIST}$  Z-score with respect to NIST value

values s\* Robust standard deviation value

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**Table 5-3.** Data summary table for total stearic acid in a fatty acids solution, fish oil, and cod liver oil. Data points highlighted in red have been flagged as potential outliers (e.g., difference from reference value, Grubb and/or Cochran) by the NIST software package.

|        |        |           |            |             |             |         | ]        | Fotal Stea            | ric Acid (               | C18:0)                 |       |                              |                           |             |        |       |
|--------|--------|-----------|------------|-------------|-------------|---------|----------|-----------------------|--------------------------|------------------------|-------|------------------------------|---------------------------|-------------|--------|-------|
|        |        |           | Fatty Ac   | ids Solutio | on A (mg/g) |         | SRM 3    | 3275-II Oı<br>Acids i | nega-3 an<br>In Fish Oil | nd Omega-6<br>l (mg/g) | Fatty |                              | Cod I                     | Liver Oil ( | mg/g)  |       |
|        | Lab    | Α         | В          | С           | Avg         | SD      | Α        | В                     | С                        | Avg                    | SD    | Α                            | В                         | С           | Avg    | SD    |
|        | Target |           |            |             | 0.180       | 0.0090  |          |                       |                          | 12.94                  | 0.62  |                              |                           |             |        |       |
|        | A004   | 0.084     | 0.089      | 0.083       | 0.085       | 0.0032  | 6.82     | 6.22                  | 7.56                     | 6.87                   | 0.67  | 9.8                          | 6.5                       | 8.1         | 8.1    | 1.7   |
|        | A007   | 0         | 0          | 0           | 0           | 0       | 15.00    | 15.06                 | 15.29                    | 15.12                  | 0.15  | 21.11                        | 21.27                     | 21.22       | 21.20  | 0.082 |
|        | A008   |           |            |             |             |         |          |                       |                          |                        |       |                              |                           |             |        |       |
|        | A010   |           |            |             |             |         | 11.99    | 12.36                 | 12.22                    | 12.19                  | 0.19  |                              |                           |             |        |       |
|        | A012   | 0.372     | 0.279      | 0.275       | 0.309       | 0.055   | 12.691   | 12.643                | 12.771                   | 12.702                 | 0.065 | 17.371                       | 17.211                    | 16.932      | 17.171 | 0.22  |
|        | A013   |           |            |             |             |         |          |                       |                          |                        |       |                              |                           |             |        |       |
|        | A014   | < 2.000   | < 2.000    | < 2.000     |             |         | 13       | 13                    | 13                       | 13                     | 0     | 18                           | 18                        | 18          | 18     | 0     |
|        | A015   |           |            |             |             |         |          |                       |                          |                        |       |                              |                           |             |        |       |
|        | A016   |           |            |             |             |         |          |                       |                          |                        |       |                              |                           |             |        |       |
| s      | A019   | 0.18      | 0.19       | 0.18        | 0.18        | 0.0058  | 11.92    | 11.45                 | 10.98                    | 11.45                  | 0.47  | 16.43                        | 16.93                     | 16.72       | 16.69  | 0.25  |
| sult   | A024   | 0.178     | 0.178      | 0.18        | 0.18        | 0.00095 | 12.765   | 12.771                | 12.312                   | 12.616                 | 0.26  | 19.253                       | 19.256                    | 18.946      | 19.152 | 0.18  |
| Re     | A027   |           |            |             |             |         |          |                       |                          |                        |       | 22.4                         | 22.7                      | 22.7        | 22.6   | 0.17  |
| lai    | A028   |           |            |             |             |         |          |                       |                          |                        |       |                              |                           |             |        |       |
| vidı   | A040   |           |            |             |             |         | 12.670   | 12.725                | 12.705                   | 12.700                 | 0.028 | 17.873                       | 17.97                     | 18.086      | 17.976 | 0.11  |
| vibr   | A047   | 0.66      | 0.67       | 0.62        | 0.65        | 0.026   | 12.2     | 13.3                  | 12.5                     | 12.7                   | 0.57  | 12.3                         | 13.3                      | 13.4        | 13.0   | 0.61  |
| Ц      | A049   | 132.664   | 119.055    | 124.748     | 125.489     | 6.8     | 19.986   | 19.854                | 20.727                   | 20.18903               | 0.47  | 25.134                       | 24.786                    | 25.435      | 25.118 | 0.32  |
|        | A050   |           |            |             |             |         |          |                       |                          |                        |       |                              |                           |             |        |       |
|        | A058   | 0.00      | 0.00       | 0.00        | 0.00        | 0.00    | 12.2     | 12.1                  | 12.64                    | 12.3                   | 0.29  | 17.53                        | 17.94                     | 17.8        | 17.76  | 0.21  |
|        | A061   | < 0.001   | < 0.001    | < 0.001     |             |         | 12.9     | 12.8                  | 12.8                     | 12.8                   | 0.058 | 17.1                         | 17.1                      | 17.1        | 17.1   | 0.0   |
|        | A065   |           |            |             |             |         | 32.46    | 32.79                 | 32.60                    | 32.62                  | 0.17  | 2.84                         | 2.88                      | 2.94        | 2.89   | 0.050 |
|        | A070   | 0.205     | 0.175      | 0.185       | 0.188       | 0.015   | 14.0     | 13.965                | 13.21                    | 13.725                 | 0.45  | 17.750                       | 18.145                    | 18.385      | 18.093 | 0.32  |
|        | A0/1   | 0.400     | 0.488      | 0.400       | 0.404       | 0.0000  |          |                       | 11.0                     | 44.0                   |       | 17.0                         | 4.5.0                     | 110         |        |       |
|        | A074   | 0.182     | 0.177      | 0.183       | 0.181       | 0.0032  | 11.9     | 11.7                  | 11.8                     | 11.8                   | 0.1   | 17.3                         | 15.9                      | 16.3        | 16.5   | 0.72  |
|        | A076   |           |            |             |             |         |          |                       |                          |                        |       |                              |                           |             |        |       |
|        | A078   |           |            |             |             |         |          |                       |                          |                        |       |                              |                           |             |        |       |
|        | A079   |           |            |             |             |         |          |                       |                          |                        |       |                              |                           |             |        |       |
|        | A085   | Consersu  | Maan       |             | 0.178       |         | Conseren | e Maan                |                          | 12.7                   |       | Consersu                     | ic Moon                   |             | 18.1   |       |
| s nity |        | Consensus | s Standard | Deviation   | 0.178       |         | Consensu | s wean                | Deviation                | 12.7                   |       | Consensu                     | is ivicali<br>is Standard | Deviation   | 2.0    |       |
| ult;   |        | Movimum   | sianuaru   | Deviation   | 125 4901    |         | Mavimum  | s standard            | Deviation                | 1.5                    |       | Consensus Standard Deviation |                           |             | 2.9    |       |
| Res    |        | Minimum   |            |             | 123.4691    |         | Minimum  | 1                     |                          | 52.62                  |       | Maximum                      |                           |             | 23.110 |       |
| ర్     |        | Minimum   |            |             | 10          |         | N        |                       |                          | 15                     |       | N                            |                           |             | 2.09   |       |
|        |        | IN        |            |             | 10          |         | 1N       |                       |                          | 13                     |       | IN                           |                           |             | 13     |       |



**Figure 5-1.** Total stearic acid (C18:0) in Fatty Acids Solution A (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red line represents the upper consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \leq 2$ , with the lower range set at zero. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by its uncertainty ( $U_{NIST}$ ) and represents the range that results in an acceptable  $Z'_{NIST} | \leq 2$ .



**Figure 5-2.** Total stearic acid (C18:0) in SRM 3275-II Omega-3 and Omega-6 Fatty Acids in Fish Oil (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by its uncertainty ( $U_{\text{NIST}}$ ) and represents the range that results in an acceptable  $Z_{\text{NIST}} | \leq 2$ .

Exercise HAMQAP Exercise 1 - Dietary Intake Sample: Cod Liver Oil Measurand: Total Stearic Acid (C18:0)



**Figure 5-3.** Total stearic acid (C18:0) in Cod Liver Oil (data summary view –analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \leq 2$ . A NIST value has not been determined in this material.



HAMQAP Exercise 1 - Dietary Intake, Measurand: Total Stearic Acid (C18:0) No. of laboratories: 10

**Figure 5-4.** Laboratory means for total stearic acid in SRM 3275-II Omega-3 and Omega-6 Fatty Acids in Fish Oil and Fatty Acids Solution A (sample/sample comparison view). In this view, the individual laboratory mean for one sample (SRM 3275-II) is compared to the mean for a second sample (Fatty Acids Solution A). The solid red box represents the NIST range of tolerance for the two samples, SRM 3275-II (x-axis) and Fatty Acids Solution A (y-axis), which encompasses the target values bounded by their uncertainties ( $U_{\text{NIST}}$ ) and represents the range that results in an acceptable  $Z_{\text{NIST}}$  score,  $|Z_{\text{NIST}}| \leq 2$ . The dotted blue box represents the consensus range of tolerance for SRM 3275-II (x-axis) and Fatty Acids Solution A (y-axis), calculated as the values above and below the consensus means that result in an acceptable  $Z'_{\text{comm}} \leq 2$ .

**Table 5-4.** Data summary table for total cis-vaccenic acid in a fatty acids solution, fish oil, and cod liver oil. Data points highlighted in red have been flagged as potential outliers (e.g., difference from reference value, Grubb and/or Cochran) by the NIST software package.

|            |        |           |            |             |               |           | (C18:1 n-7)                  |              |             |          |         |                              |         |         |        |       |  |  |
|------------|--------|-----------|------------|-------------|---------------|-----------|------------------------------|--------------|-------------|----------|---------|------------------------------|---------|---------|--------|-------|--|--|
|            |        |           | Fotty Ac   | ide Soluti  | n A (mala     | <b>`</b>  | SRM 3                        | 3275-II Oı   | nega-3 an   | d Omega- | 6 Fatty | Cod Liver Oil (mg/g)         |         |         |        |       |  |  |
|            |        |           | Fatty At   | ius solutio | JII A (IIIg/g | ,         |                              | Acids i      | in Fish Oil | (mg/g)   |         | Cou Laver Oli (ling/g)       |         |         |        |       |  |  |
|            | Lab    | Α         | В          | С           | Avg           | SD        | Α                            | В            | С           | Avg      | SD      | Α                            | В       | С       | Avg    | SD    |  |  |
|            | Target |           |            |             | 0.0370        | 0.0020    |                              |              |             | 9.24     | 0.77    |                              |         |         |        |       |  |  |
|            | A004   |           |            |             |               |           |                              |              |             |          |         |                              |         |         |        |       |  |  |
|            | A007   | 0         | 0          | 0           | 0             | 0         | 8.81                         | 8.75         | 8.8         | 8.79     | 0.032   | 41.18                        | 41.17   | 41.27   | 41.21  | 0.055 |  |  |
|            | A010   |           |            |             |               |           | 10.75                        | 10.11        | 9.58        | 10.15    | 0.59    |                              |         |         |        |       |  |  |
|            | A012   |           |            |             |               |           | 8.755                        | 8.682        | 8.779       | 8.739    | 0.051   | 41.654                       | 41.086  | 40.601  | 41.114 | 0.53  |  |  |
|            | A013   |           |            |             |               |           | _                            | -            |             |          | -       |                              |         |         |        | -     |  |  |
|            | A014   | < 2.000   | < 2.000    | < 2.000     |               |           | 9                            | 9            | 9           | 9        | 0       | 41                           | 41      | 41      | 41     | 0     |  |  |
|            | A015   |           |            |             |               |           |                              |              |             |          |         |                              |         |         |        |       |  |  |
|            | A016   |           |            |             |               |           | 6.60                         | < <b>2</b> 0 | 6.1.5       | 6.00     | 0.05    | 07.60                        | 20.02   | 26.70   | 07.47  | 0.60  |  |  |
| s          | A019   | 0.0270    | 0.0295     | 0.0295      | 0.0292        | 0.00040   | 0.07                         | 6.20         | 6.15        | 6.32     | 0.25    | 27.68                        | 28.02   | 26.70   | 21.47  | 0.69  |  |  |
| sult       | A024   | 0.0378    | 0.0385     | 0.0385      | 0.0383        | 0.00040   | 9.37                         | 9.387        | 8.96        | 9.239    | 0.24    | 39.662                       | 39.596  | 38.755  | 39.338 | 0.51  |  |  |
| Re         | A027   |           |            |             |               |           |                              |              |             |          |         |                              |         |         |        |       |  |  |
| ual        | A028   |           |            |             |               |           | 8 614                        | 8 7 7 3      | 8 606       | 8 678    | 0.057   | 30.648                       | 30 883  | 40.001  | 30 874 | 0.22  |  |  |
| vid        | A040   |           |            |             |               |           | 0.014                        | 0.725        | 0.090       | 8.078    | 0.037   | 39.040                       | 37.005  | 40.091  | 37.074 | 0.22  |  |  |
| ipu        | A049   |           |            |             |               |           |                              |              |             |          |         |                              |         |         |        |       |  |  |
| -          | A058   | 0.00      | 0.00       | 0.00        | 0.00          | 0.00      | 10.19                        | 8 72         | 13.18       | 10.70    | 2 27    | 42.76                        | 45.04   | 44.9    | 44.2   | 13    |  |  |
|            | A061   | < 0.001   | < 0.001    | < 0.001     | 0.00          | 0.00      | < 0.001                      | < 0.001      | < 0.001     | 10.70    | 2.27    | < 0.001                      | < 0.001 | < 0.001 | 11.2   | 1.5   |  |  |
|            | A065   | 0.001     | 10.001     | 10.001      |               |           | 10.001                       | 0.001        | . 0.001     |          |         | 10.001                       | . 0.001 | . 0.001 |        |       |  |  |
|            | A070   |           |            |             |               |           |                              |              |             |          |         |                              |         |         |        |       |  |  |
|            | A071   |           |            |             |               |           |                              |              |             |          |         |                              |         |         |        |       |  |  |
|            | A074   | 0.032     | 0.031      | 0.036       | 0.033         | 0.0024007 | 8.21                         | 8.12         | 8.10        | 8.14     | 0.059   | 40.5                         | 37.3    | 38.1    | 38.6   | 1.7   |  |  |
|            | A076   |           |            |             |               |           |                              |              |             |          |         |                              |         |         |        |       |  |  |
|            | A078   |           |            |             |               |           |                              |              |             |          |         |                              |         |         |        |       |  |  |
|            | A079   |           |            |             |               |           |                              |              |             |          |         |                              |         |         |        |       |  |  |
|            | A083   |           |            |             |               |           |                              |              |             |          |         |                              |         |         |        |       |  |  |
| ty         |        | Consensus | s Mean     |             | 0.018         |           | Consensus Mean               |              |             | 8.99     |         | Consensu                     | is Mean |         | 40.8   |       |  |  |
| uni<br>Its |        | Consensus | s Standard | Deviation   | 0.039         |           | Consensus Standard Deviation |              |             | 0.83     |         | Consensus Standard Deviation |         |         | 2.5    |       |  |  |
| esu        |        | Maximum   |            |             | 0.0383        |           | Maximum                      |              |             | 10.70    |         | Maximum                      |         |         | 44.2   |       |  |  |
| <b>B B</b> |        | Minimum   |            |             | 0             |           | Minimum                      | i i          |             | 6.32     |         | Minimum                      |         |         | 27.47  |       |  |  |
| •          |        | Ν         |            |             | 4             |           | Ν                            |              |             | 9        |         | N                            |         |         | 8      |       |  |  |

Exercise HAMQAP Exercise 1 - Dietary Intake Sample: Fatty Acids Solution A Measurand: Total cis-Vaccenic Acid (C18:1 n-7)



**Figure 5-5.** Total cis-vaccenic acid (C18:1 n-7) in Fatty Acids Solution A (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red line represents the upper consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \leq 2$ , with the lower range set at zero. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by its uncertainty ( $U_{NIST}$ ) and represents the range that results in an acceptable  $Z'_{NIST}| \leq 2$ .



Exercise HAMQAP Exercise 1 - Dietary Intake Sample: SRM 3275-II Omega-3 and Omega-6 Fatty Acids in Fish Oil Measurand: Total cis-Vaccenic Acid (C18:1 n-7)

**Figure 5-6.** Total cis-vaccenic acid (C18:1 n-7) in SRM 3275-II Omega-3 and Omega-6 Fatty Acids in Fish Oil (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \le 2$ . The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by its uncertainty ( $U_{NIST}$ ) and represents the range that results in an acceptable  $Z'_{NIST} | \le 2$ .

Exercise HAMQAP Exercise 1 - Dietary Intake Sample: Cod Liver Oil Measurand: Total cis-Vaccenic Acid (C18:1 n-7)



**Figure 5-7.** Total cis-vaccenic acid (C18:1 n-7) in Cod Liver Oil (data summary view –analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \leq 2$ . A NIST value has not been determined in this material.



HAMQAP Exercise 1 - Dietary Intake, Measurand: Total cis-Vaccenic Acid (C18:1 n-7) No. of laboratories: 4

**Figure 5-8.** Laboratory means for total cis-vaccenic acid in SRM 3275-II Omega-3 and Omega-6 Fatty Acids in Fish Oil and Fatty Acids Solution A (sample/sample comparison view). In this view, the individual laboratory mean for one sample (SRM 3275-II) is compared to the mean for a second sample (Fatty Acids Solution A). The solid red box represents the NIST range of tolerance for the two samples, SRM 3275-II (x-axis) and Fatty Acids Solution A (y-axis), which encompasses the target values bounded by their uncertainties ( $U_{\text{NIST}}$ ) and represents the range that results in an acceptable  $Z_{\text{NIST}}$  score,  $|Z_{\text{NIST}}| \leq 2$ . The dotted blue box represents the consensus range of tolerance for SRM 3275-II (x-axis) and Fatty Acids Solution A (y-axis), calculated as the values above and below the consensus means that result in an acceptable  $Z'_{\text{comm}} \leq 2$ .

**Table 5-5.** Data summary table for total oleic acid in a fatty acids solution, fish oil, and cod liver oil. Data points highlighted in red have been flagged as potential outliers (e.g., difference from reference value, Grubb and/or Cochran) by the NIST software package.

|        |        | Total Oleic Acid (C18:1 n-9) |            |              |                   |        |                              |            |            |          |         |                              |         |         |          |       |  |  |
|--------|--------|------------------------------|------------|--------------|-------------------|--------|------------------------------|------------|------------|----------|---------|------------------------------|---------|---------|----------|-------|--|--|
|        |        |                              | Fatty Ac   | rids Solutio | n A (mg/g)        |        | SRM 3                        | 3275-II Oı | nega-3 an  | d Omega- | 6 Fatty | Cod Liver Oil (mg/g)         |         |         |          |       |  |  |
| -      |        |                              | Tutty IN   | ius solutio  | , in . in (ing/g) |        |                              | Acids i    | n Fish Oil | (mg/g)   |         | Coulding on (ing/g)          |         |         |          |       |  |  |
|        | Lab    | Α                            | В          | С            | Avg               | SD     | Α                            | В          | С          | Avg      | SD      | Α                            | В       | С       | Avg      | SD    |  |  |
|        | Target |                              |            |              | 0.428             | 0.021  |                              |            |            | 22.1     | 1.6     |                              |         |         |          |       |  |  |
|        | A004   | 0.23                         | 0.23       | 0.23         | 0.23              | 0      | 10.7                         | 11.8       | 11.8       | 11.4     | 0.64    | 79.8                         | 59.7    | 64.4    | 68.0     | 11    |  |  |
|        | A007   | 0                            | 0          | 0            | 0                 | 0      | 23.52                        | 23.41      | 23.59      | 23.51    | 0.091   | 138.95                       | 138.03  | 139.06  | 138.68   | 0.57  |  |  |
|        | A008   |                              |            |              |                   |        |                              |            |            |          |         |                              |         |         |          |       |  |  |
|        | A010   |                              |            |              |                   |        | 19.98                        | 20.76      | 21.47      | 20.74    | 0.75    |                              |         |         |          |       |  |  |
|        | A012   |                              |            |              |                   |        |                              |            |            |          |         | 0.387                        | 0.387   | 0.486   | 0.420    | 0.057 |  |  |
|        | A013   |                              |            |              |                   |        |                              |            |            |          |         |                              |         |         |          |       |  |  |
|        | A014   | < 2.000                      | < 2.000    | < 2.000      |                   |        | 22                           | 22         | 22         | 22       | 0       | 132                          | 132     | 133     | 132      | 0.58  |  |  |
|        | A015   |                              |            |              |                   |        |                              |            |            |          |         |                              |         |         |          |       |  |  |
|        | A016   |                              |            |              |                   |        |                              |            |            |          |         |                              |         |         |          |       |  |  |
|        | A019   | 0.36                         | 0.40       | 0.35         | 0.37              | 0.026  | 20.29                        | 20.01      | 19.29      | 19.86    | 0.52    | 125.79                       | 124.04  | 122.16  | 124.00   | 1.82  |  |  |
| fi     | A024   | 0.429                        | 0.428      | 0.431        | 0.4291            | 0.0017 | 20.645                       | 20.628     | 19.754     | 20.342   | 0.51    | 121.975                      | 122.994 | 120.14  | 121.703  | 1.4   |  |  |
| Ses    | A027   |                              |            |              |                   |        |                              |            |            |          |         | 240                          | 240     | 240     | 240      | 0     |  |  |
| all    | A028   |                              |            |              |                   |        |                              |            |            |          |         |                              |         |         |          |       |  |  |
| idu    | A040   |                              |            |              |                   |        | 22.826                       | 22.973     | 22.934     | 22.911   | 0.076   | 127.498                      | 128.384 | 128.923 | 128.2683 | 0.72  |  |  |
| div    | A047   | 1.21                         | 1.32       | 1.27         | 1.27              | 0.055  | 31.2                         | 32.1       | 31.8       | 31.7     | 0.46    | 197.1                        | 200.5   | 199.3   | 199.0    | 1.7   |  |  |
| Ч      | A049   | 266.072                      | 255.408    | 253.585      | 258.355           | 6.7    | 29.233                       | 27.393     | 27.838     | 28.155   | 0.96    | 145.27                       | 146.238 | 146.076 | 145.8616 | 0.52  |  |  |
|        | A050   |                              |            |              |                   |        |                              |            |            |          |         |                              |         |         |          |       |  |  |
|        | A058   | 0.00                         | 0.00       | 0.00         | 0.00              | 0.00   | 0.00                         | 0.00       | 0.00       | 0.00     | 0.00    | 153.19                       | 160.71  | 160.65  | 158.18   | 4.3   |  |  |
|        | A061   | 0.6                          | 0.5        | 0.5          | 0.53              | 0.058  | 24.7                         | 24.6       | 24.6       | 24.6     | 0.058   | 153.9                        | 153.6   | 152.9   | 153.5    | 0.51  |  |  |
|        | A065   |                              |            |              |                   |        | 24.96                        | 24.90      | 24.95      | 24.94    | 0.032   | 33.30                        | 33.41   | 33.65   | 33.45    | 0.18  |  |  |
|        | A070   | 0.53                         | 0.47       | 0.44         | 0.48              | 0.046  | 24.945                       | 25.04      | 23.68      | 24.56    | 0.76    | 163.325                      | 168.465 | 170.08  | 167.290  | 3.53  |  |  |
|        | A071   |                              |            |              |                   |        |                              |            |            |          |         |                              |         |         |          |       |  |  |
|        | A074   | 0.422                        | 0.411      | 0.429        | 0.42              | 0.0091 | 21.3                         | 20.8       | 21.0       | 21.0     | 0.25    | 151                          | 139     | 143     | 144      | 6.1   |  |  |
|        | A076   |                              |            |              |                   |        |                              |            |            |          |         |                              |         |         |          |       |  |  |
|        | A078   |                              |            |              |                   |        |                              |            |            |          |         |                              |         |         |          |       |  |  |
|        | A079   |                              |            |              |                   |        |                              |            |            |          |         |                              |         |         |          |       |  |  |
|        | A083   |                              |            |              |                   |        |                              |            |            |          |         |                              |         |         |          |       |  |  |
| ity    |        | Consensu                     | s Mean     |              | 0.382             |        | Consensus Mean               |            |            | 23.0     |         | Consensus Mean               |         |         | 136      |       |  |  |
| un sti |        | Consensu                     | s Standard | Deviation    | 0.39              |        | Consensus Standard Deviation |            |            | 4.7      |         | Consensus Standard Deviation |         |         | 42       |       |  |  |
| esu    |        | Maximum                      | L          |              | 258.355           |        | Maximum                      |            |            | 31.7     |         | Maximum                      |         |         | 240      |       |  |  |
| ъ с    |        | Minimum                      |            |              | 0                 |        | Minimum                      |            |            | 0.00     | Minimum |                              |         | 0.420   |          |       |  |  |
| -      |        | Ν                            |            |              | 10                |        | Ν                            |            |            | 14       |         | Ν                            |         |         | 15       |       |  |  |



**Figure 5-9.** Total oleic acid (C18:1 n-9) in Fatty Acids Solution A (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red line represents the upper consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \leq 2$ , with the lower range set at zero. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by its uncertainty ( $U_{NIST}$ ) and represents the range that results in an acceptable  $Z_{NIST}$  score,  $|Z_{NIST}| \leq 2$ .



Exercise HAMQAP Exercise 1 - Dietary Intake Sample: SRM 3275-II Omega-3 and Omega-6 Fatty Acids in Fish Oil Measurand: Total Oleic Acid (C18:1 n-9)

**Figure 5-10.** Total oleic acid (C18:1 n-9) in SRM 3275-II Omega-3 and Omega-6 Fatty Acids in Fish Oil (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \le 2$ . The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by its uncertainty ( $U_{NIST}$ ) and represents the range that results in an acceptable  $Z'_{NIST} | \le 2$ .

Exercise HAMQAP Exercise 1 - Dietary Intake Sample: Cod Liver Oil Measurand: Total Oleic Acid (C18:1 n-9)



**Figure 5-11.** Total oleic acid (C18:1 n-9) in Cod Liver Oil (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \leq 2$ . A NIST value has not been determined in this material.



**Figure 5-12.** Laboratory means for total oleic acid in SRM 3275-II Omega-3 and Omega-6 Fatty Acids in Fish Oil and Fatty Acids Solution A (sample/sample comparison view). In this view, the individual laboratory mean for one sample (SRM 3275-II) is compared to the mean for a second sample (Fatty Acids Solution A). The solid red box represents the NIST range of tolerance for the two samples, SRM 3275-II (x-axis) and Fatty Acids Solution A (y-axis), which encompasses the target values bounded by their uncertainties ( $U_{\text{NIST}}$ ) and represents the range that results in an acceptable  $Z_{\text{NIST}}$  score,  $|Z_{\text{NIST}}| \leq 2$ . The dotted blue box represents the consensus range of tolerance for SRM 3275-II (x-axis) and Fatty Acids Solution A (y-axis), calculated as the values above and below the consensus means that result in an acceptable  $Z'_{\text{comm}} \leq 2$ .

**Table 5-6.** Data summary table for total linoleic acid in a fatty acids solution, fish oil, and cod liver oil. Data points highlighted in red have been flagged as potential outliers (e.g., difference from reference value, Grubb and/or Cochran) by the NIST software package.

|       |        |           | Total Linoleic Acid (C18:2 n-6) |             |             |        |           |                           |                          |                    |         |                      |                              |           |            |       |  |
|-------|--------|-----------|---------------------------------|-------------|-------------|--------|-----------|---------------------------|--------------------------|--------------------|---------|----------------------|------------------------------|-----------|------------|-------|--|
|       |        |           | Fatty Ac                        | ids Solutio | on A (mg/g) |        | SRM 3     | 3275-II O<br>Acids        | mega-3 an<br>in Fish Oil | d Omega-<br>(mg/g) | 6 Fatty | Cod Liver Oil (mg/g) |                              |           |            |       |  |
|       | Lab    | Α         | В                               | С           | Avg         | SD     | A         | В                         | С                        | Avg                | SD      | Α                    | В                            | С         | Avg        | SD    |  |
|       | Target |           |                                 |             | 0.802       | 0.040  |           |                           |                          | 3.00               | 0.42    |                      |                              |           |            |       |  |
|       | A004   | 0.52      | 0.48                            | 0.49        | 0.50        | 0.021  | 2.15      | 2.59                      | 2.1                      | 2.28               | 0.27    | 11.6                 | 8.5                          | 11.4      | 10.5       | 1.73  |  |
|       | A007   | 0.84      | 0.84                            | 0.83        | 0.84        | 0.0058 | 3.13      | 3.12                      | 3.13                     | 3.13               | 0.0058  | 21.22                | 21.23                        | 21.27     | 21.24      | 0.026 |  |
|       | A008   |           |                                 |             |             |        |           |                           |                          |                    |         |                      |                              |           |            |       |  |
|       | A010   |           |                                 |             |             |        | 2.83      | 3.14                      | 3.09                     | 3.02               | 0.17    |                      |                              |           |            |       |  |
|       | A012   | 0.774     | 0.836                           | 0.834       | 0.815       | 0.035  | 2.744     | 2.699                     | 2.653                    | 2.699              | 0.046   | 19.803               | 19.556                       | 19.228    | 19.529     | 0.29  |  |
|       | A013   |           |                                 |             |             |        |           |                           |                          |                    |         |                      |                              |           |            |       |  |
|       | A014   | < 2.000   | < 2.000                         | < 2.000     |             |        | 3         | 3                         | 3                        | 3                  | 0.0     | 21                   | 21                           | 21        | 21         | 0.0   |  |
|       | A015   |           |                                 |             |             |        |           |                           |                          |                    |         |                      |                              |           |            |       |  |
|       | A016   |           |                                 |             |             |        |           |                           |                          |                    |         |                      |                              |           |            |       |  |
| s     | A019   | 0.62      | 0.62                            | 0.62        | 0.62        | 0.00   | 2.49      | 2.41                      | 2.35                     | 2.42               | 0.070   | 16.27                | 16.12                        | 15.77     | 16.05      | 0.26  |  |
| ult   | A024   | 0.7928    | 0.7904                          | 0.7951      | 0.7928      | 0.0024 | 2.965     | 2.957                     | 2.92                     | 2.947              | 0.024   | 19.515               | 19.693                       | 19.083    | 19.430     | 0.31  |  |
| Res   | A027   |           |                                 |             |             |        |           |                           |                          |                    |         | 26.5                 | 26.6                         | 26.6      | 26.6       | 0.058 |  |
| [a]   | A028   |           |                                 |             |             |        |           |                           |                          |                    |         |                      |                              |           |            |       |  |
| idu   | A040   |           |                                 |             |             |        | 4.761     | 4.794                     | 4.763                    | 4.773              | 0.019   | 21.646               | 21.818                       | 21.873    | 21.779     | 0.12  |  |
| div   | A047   | 1.87      | 1.81                            | 1.82        | 1.83        | 0.032  |           |                           |                          |                    |         |                      |                              |           |            |       |  |
| Ц     | A049   | 443.265   | 461.347                         | 467.285     | 457.299     | 13     | 6.092     | 6.098                     | 6.146                    | 6.112              | 0.029   | 26.149               | 26.11                        | 26.208    | 26.156     | 0.049 |  |
|       | A050   |           |                                 |             |             |        |           |                           |                          |                    |         |                      |                              |           |            |       |  |
|       | A058   | 0.74      | 0.77                            | 0.88        | 0.80        | 0.074  | 6.35      | 6.81                      | 6.06                     | 6.41               | 0.38    | 21.44                | 22.23                        | 22.32     | 22.00      | 0.48  |  |
|       | A061   | 0.8       | 0.8                             | 0.8         | 0.8         | 0.00   | < 0.001   | < 0.001                   | < 0.001                  | 40.50              | 0.001   | 17.0                 | 17.0                         | 16.9      | 17.0       | 0.058 |  |
|       | A065   | 0.045     | 0.070                           | 0.020       | 0.00        | 0.070  | 40.53     | 40.55                     | 40.49                    | 40.52              | 0.031   | 3.63                 | 3.65                         | 3.64      | 3.64       | 0.010 |  |
|       | A070   | 0.965     | 0.870                           | 0.830       | 0.89        | 0.069  | 3.105     | 3.12                      | 2.97                     | 3.07               | 0.083   | 20.765               | 21.415                       | 21.70     | 21.293     | 0.48  |  |
|       | A071   | 0.702     | 0.7/2                           | 0.707       | 0.701       | 0.010  | 2.02      | 0.00                      | 0.00                     | 2.00               | 0.020   | 20.7                 | 10.0                         | 10.6      | 10.0       | 0.96  |  |
|       | A074   | 0.785     | 0.762                           | 0.797       | 0.781       | 0.018  | 2.95      | 2.88                      | 2.88                     | 2.90               | 0.029   | 20.7                 | 19.0                         | 19.0      | 19.8       | 0.80  |  |
|       | A070   |           |                                 |             |             |        |           |                           |                          |                    |         |                      |                              |           |            |       |  |
|       | A078   |           |                                 |             |             |        |           |                           |                          |                    |         |                      |                              |           |            |       |  |
|       | A079   |           |                                 |             |             |        |           |                           |                          |                    |         |                      |                              |           |            |       |  |
|       | A085   | Consersu  | Maan                            |             | 0.768       |        | Conserve  | Community Marine 2.02     |                          |                    |         |                      | ic Maan                      |           | 20.2       |       |  |
| s int |        | Consensus | s Standard                      | Deviation   | 0.700       |        | Consense  | is ivicali<br>is Standary | Deviation                | 2.93               |         | Consersu             | is ivicali<br>is Standard    | Deviation | 4.3        |       |  |
| alt   |        | Maximum   | stanualu                        | Deviation   | 457 200     |        | Maximum 4 |                           |                          | 40.52              |         | Maximum              | Consensus Standard Deviation |           |            |       |  |
| Res   |        | Minimum   |                                 |             | 0.50        |        | Minimum   |                           |                          | 2.28               |         | Minimum              |                              |           | 3 64       |       |  |
| 5 · · |        | N         |                                 |             | 11          |        | N         |                           |                          | 13                 |         | N                    |                              |           | 3.04<br>14 |       |  |
|       |        | 11        |                                 |             | 11          |        | 11        |                           |                          | 15                 |         | 11                   |                              |           | 17         |       |  |



**Figure 5-13.** Total linoleic acid (C18:2 n-6) in Fatty Acids Solution A (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \leq 2$ . The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by its uncertainty ( $U_{NIST}$ ) and represents the range that results in an acceptable  $Z_{NIST}$  score,  $|Z_{NIST}| \leq 2$ .



Exercise HAMQAP Exercise 1 - Dietary Intake Sample: SRM 3275-II Omega-3 and Omega-6 Fatty Acids in Fish Oil Measurand: Total Linoleic Acid (C18:2 n-6)

**Figure 5-14.** Total linoleic acid (C18:2 n-6) in SRM 3275-II Omega-3 and Omega-6 Fatty Acids in Fish Oil (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \le 2$ . The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by its uncertainty ( $U_{NIST}$ ) and represents the range that results in an acceptable  $Z'_{NIST} | \le 2$ .

Exercise HAMQAP Exercise 1 - Dietary Intake Sample: Cod Liver Oil Measurand: Total Linoleic Acid (C18:2 n-6)



**Figure 5-15.** Total linoleic acid (C18:2 n-6) in Cod Liver Oil (data summary view –analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \leq 2$ . A NIST value has not been determined in this material.



HAMQAP Exercise 1 - Dietary Intake, Measurand: Total Linoleic Acid (C18:2 n-6) No. of laboratories: 9

**Figure 5-16.** Laboratory means for total linoleic acid in SRM 3275-II Omega-3 and Omega-6 Fatty Acids in Fish Oil and Fatty Acids Solution A (sample/sample comparison view). In this view, the individual laboratory mean for one sample (SRM 3275-II) is compared to the mean for a second sample (Fatty Acids Solution A). The solid red box represents the NIST range of tolerance for the two samples, SRM 3275-II (x-axis) and Fatty Acids Solution A (y-axis), which encompasses the target values bounded by their uncertainties ( $U_{\text{NIST}}$ ) and represents the range that results in an acceptable  $Z_{\text{NIST}}$  score,  $|Z_{\text{NIST}}| \leq 2$ . The dotted blue box represents the consensus range of tolerance for SRM 3275-II (x-axis) and Fatty Acids Solution A (y-axis), calculated as the values above and below the consensus means that result in an acceptable  $Z'_{\text{comm}} \leq 2$ .

**Table 5-7.** Data summary table for total  $\alpha$ -linolenic acid in a fatty acids solution, fish oil, and cod liver oil. Data points highlighted in red have been flagged as potential outliers (e.g., difference from reference value, Grubb and/or Cochran) by the NIST software package.

|            |              | Total alpha-Linolenic Acid (C18:3 n-3) |            |              |            |           |                              |                       |                          |                      |              |                              |         |         |         |       |  |
|------------|--------------|--|------------|--------------|------------|-----------|------------------------------|-----------------------|--------------------------|----------------------|--------------|------------------------------|---------|---------|---------|-------|--|
| _          |              |  | Fatty Ac   | cids Solutio | on A (mg/g | )         | SRM 3                        | 3275-II Or<br>Acids i | nega-3 an<br>in Fish Oil | d Omega-<br>l (mg/g) | 6 Fatty      | Cod Liver Oil (mg/g)         |         |         |         |       |  |
|            | Lab          | Α                                      | В          | С            | Avg        | SD        | A                            | В                     | С                        | Avg                  | SD           | Α                            | В       | С       | Avg     | SD    |  |
|            | Target       |  |            |              | 0.0140     | 0.0010    |                              |                       |                          | 1.42                 | 0.12         |                              |         |         |         |       |  |
|            | A004         | 0.011                                  | 0.011      | 0.0076       | 0.0099     | 0.0020    | 1.18                         | 1.06                  | 1.03                     | 1.09                 | 0.079        | 5.3                          | 3.3     | 3.7     | 4.1     | 1.1   |  |
|            | A007         | 0                                      | 0          | 0            | 0          | 0         | 1.60                         | 1.60                  | 1.61                     | 1.60                 | 0.0058       | 9.03                         | 9.02    | 9.06    | 9.04    | 0.021 |  |
|            | A008         |  |            |              |            |           |                              |                       |                          |                      |              |                              |         |         |         |       |  |
|            | A010         |  |            |              |            |           | 1.19                         | 1.39                  | 1.26                     | 1.28                 | 0.10         |                              |         |         |         |       |  |
|            | A012         |  |            |              |            |           | 1.342                        | 1.5                   | 1.317                    | 1.386                | 0.099        | 8.568                        | 8.397   | 8.264   | 8.410   | 0.15  |  |
|            | A013         |  |            |              |            |           |                              |                       |                          | _                    |              |                              | -       |         |         |       |  |
|            | A014         | < 2.000                                | < 2.000    | < 2.000      |            |           | 6                            | 6                     | 3                        | 5                    | 1.7          | 12                           | 9       | 9       | 10      | 1.7   |  |
|            | A015         |  |            |              |            |           |                              |                       |                          |                      |              |                              |         |         |         |       |  |
|            | A016         |  |            |              |            |           |                              |                       |                          |                      |              |                              |         | 1.00    |         |       |  |
| s          | A019         | 0.014                                  | 0.011      | 0.014        | 0.044      | 0.0000444 | 1.20                         | 1.17                  | 1.13                     | 1.17                 | 0.035        | 1.31                         | 1.29    | 1.33    | 1.31    | 0.02  |  |
| sult       | A024         | 0.014                                  | 0.014      | 0.014        | 0.014      | 0.0003464 | 1.//6                        | 1.891                 | 1.695                    | 1./8/                | 0.098        | 8.047                        | 8.085   | 1.83    | /.98/   | 0.14  |  |
| Re         | A027         |  |            |              |            |           |                              |                       |                          |                      |              |                              |         |         |         |       |  |
| lal        | A028         |  |            |              |            |           | 1 171                        | 1 202                 | 1 157                    | 1 1 7 7              | 0.024        | 9 5 4 1                      | 0.050   | 0.045   | 0 7 4 0 | 0.19  |  |
| vid        | A040         |  |            |              |            |           | 1.1/1                        | 1.205                 | 1.157                    | 1.177                | 0.024        | 8.341                        | 8.838   | 8.845   | 8.748   | 0.18  |  |
| ibu        | A047<br>A049 | 0                                      | 0          | 0            | 0          | 0         | 1.857                        | 1.802                 | 1.806                    | 1 822                | 0.030        | 10.048                       | 9.98/   | 10.038  | 10.023  | 0.035 |  |
| Ι          | A050         | 0                                      | 0          | 0            | 0          | 0         | 1.057                        | 1.002                 | 1.000                    | 1.022                | 0.050        | 10.040                       | 7.704   | 10.050  | 10.025  | 0.055 |  |
|            | A058         | 0.00                                   | 0.00       | 0.00         | 0.00       | 0.00      | 9.21                         | 9 14                  | 9 55                     | 9 30                 | 0.22         | 12.30                        | 12.75   | 13 13   | 12.73   | 0.42  |  |
|            | A061         | < 0.001                                | < 0.001    | < 0.001      | 0.00       | 0.00      | < 0.001                      | < 0.001               | < 0.001                  | 7.00                 | 0.22         | < 0.001                      | < 0.001 | < 0.001 | 12.70   | 0112  |  |
|            | A065         |  |            |              |            |           | 2.06                         | 2.10                  | 2.09                     | 2.08                 | 0.021        | 22.12                        | 22.13   | 22.16   | 22.14   | 0.021 |  |
|            | A070         | 0.01                                   | 0.01       | 0.01         | 0.01       | 0.00      | 1.485                        | 1.625                 | 1.59                     | 1.567                | 0.073        | 8.505                        | 8.85    | 9.04    | 8.798   | 0.27  |  |
|            | A071         |  |            |              |            |           |                              |                       |                          |                      |              |                              |         |         |         |       |  |
|            | A074         | 0.0134                                 | 0.0135     | 0.0158       | 0.0142     | 0.0014    |                              |                       |                          |                      |              | 9.68                         | 10.2    | 9.46    | 9.78    | 0.38  |  |
|            | A076         |  |            |              |            |           |                              |                       |                          |                      |              |                              |         |         |         |       |  |
|            | A078         |  |            |              |            |           |                              |                       |                          |                      |              |                              |         |         |         |       |  |
|            | A079         |  |            |              |            |           |                              |                       |                          |                      |              |                              |         |         |         |       |  |
|            | A083         |  |            |              |            |           |                              |                       |                          |                      |              |                              |         |         |         |       |  |
| ty         |              | Consensu                               | s Mean     |              | 0.007      |           | Consensus Mean               |                       |                          | 1.50                 |              | Consensu                     | ıs Mean |         | 8.80    |       |  |
| uni<br>lts |              | Consensu                               | s Standard | Deviation    | 0.007      |           | Consensus Standard Deviation |                       |                          | 0.61                 |              | Consensus Standard Deviation |         |         | 2.2     |       |  |
| nm<br>esu  |              | Maximum                                |            |              | 0.0142     |           | Maximum                      |                       |                          | 9.30                 |              | Maximum                      |         |         | 22.14   |       |  |
| R          |              | Minimum                                |            |              | 0          |           | Minimum                      |                       |                          | 1.09                 | 1.09 Minimum |                              |         | 1.31    |         |       |  |
| -          |              | Ν                                      |            |              | 7          |           | Ν                            |                       |                          | 12                   |              | Ν                            |         |         | 12      |       |  |





Figure 5-17. Total  $\alpha$ -linolenic acid (C18:3 n-3) in Fatty Acids Solution A (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red line represents the upper consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \leq 2$ , with the lower range set at zero. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by its uncertainty ( $U_{NIST}$ ) and represents the range that results in an acceptable  $Z'_{NIST}$  score,  $|Z_{NIST}| \leq 2$ .




Figure 5-18. Total  $\alpha$ -linolenic acid (C18:3 n-3) in SRM 3275-II Omega-3 and Omega-6 Fatty Acids in Fish Oil (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \leq 2$ . The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by its uncertainty ( $U_{NIST}$ ) and represents the range that results in an acceptable  $Z'_{NIST} | \leq 2$ .





Figure 5-19. Total  $\alpha$ -linolenic acid (C18:3 n-3) in Cod Liver Oil (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \leq 2$ . A NIST value has not been determined in this material.



**Figure 5-20.** Laboratory means for total  $\alpha$ -linolenic acid in SRM 3275-II Omega-3 and Omega-6 Fatty Acids in Fish Oil and Fatty Acids Solution A and (sample/sample comparison view). In this view, the individual laboratory mean for one sample (SRM 3275-II) is compared to the mean for a second sample (Fatty Acids Solution A). The solid red box represents the NIST range of tolerance for the two samples, SRM 3275-II (x-axis) and Fatty Acids Solution A (y-axis), which encompasses the target values bounded by their uncertainties ( $U_{\text{NIST}}$ ) and represents the range that results in an acceptable  $Z_{\text{NIST}}$  score,  $|Z_{\text{NIST}}| \leq 2$ . The dotted blue box represents the consensus range of tolerance for SRM 3275-II (x-axis) and Fatty Acids Solution A (y-axis), calculated as the values above and below the consensus means that result in an acceptable  $Z'_{\text{comm}} \leq 2$ .

**Table 5-8.** Data summary table for total  $\gamma$ -linolenic acid in a fatty acids solution, fish oil, and cod liver oil. Data points highlighted in red have been flagged as potential outliers (e.g., difference from reference value, Grubb and/or Cochran) by the NIST software package.

|        |        |  |            |              |             |         | Total g  | amma-Lir           | olenic Aci               | d (C18:3 1         | <b>1-6</b> ) |          |             |             |               |         |
|--------|--------|--|------------|--------------|-------------|---------|----------|--------------------|--------------------------|--------------------|--------------|----------|-------------|-------------|---------------|---------|
|        |        |  | Fatty Ac   | cids Solutio | on A (mg/g) |         | SRM 3    | 3275-II O<br>Acids | mega-3 an<br>in Fish Oil | d Omega-<br>(mg/g) | 6 Fatty      |          | Cod         | Liver Oil ( | mg/g)         |         |
|        | Lab    | Α  | В          | С            | Avg         | SD      | Α        | В                  | С                        | Avg                | SD           | Α        | В           | С           | Avg           | SD      |
|        | Target |  |            |              |             |         |          |                    |                          | 0.507              | 0.043        |          |             |             |               |         |
|        | A004   |  |            |              |             |         |          |                    |                          |                    |              |          |             |             |               |         |
|        | A007   | 0  | 0          | 0            | 0           | 0       | 2.19     | 2.20               | 2.22                     | 2.20               | 0.015        | 2.87     | 2.98        | 3.00        | 2.95          | 0.07    |
|        | A008   |  |            |              |             |         |          |                    |                          |                    |              |          |             |             |               |         |
|        | A010   |  |            |              |             |         | 0.32     | 0.45               | 0.42                     | 0.40               | 0.068        |          |             |             |               |         |
|        | A012   |  |            |              |             |         | 0.536    | 0.557              | 0.610                    | 0.568              | 0.038        | 1.378    | 1.407       | 1.228       | 1.338         | 0.096   |
|        | A013   |  |            |              |             |         |          |                    |                          |                    |              |          |             |             |               |         |
|        | A014   | < 2.000  | < 2.000    | < 2.000      |             |         | 4        | 4                  | 4                        | 4                  | 0            | < 2.000  | < 2.000     | < 2.000     |               |         |
|        | A015   |  |            |              |             |         |          |                    |                          |                    |              |          |             |             |               |         |
|        | A016   |  |            |              |             |         |          |                    |                          |                    |              |          |             |             |               |         |
|        | A019   | 0.23   | 0.21       | 0.22         | 0.22        | 0.010   | 1.29     | 1.27               | 1.24                     | 1.27               | 0.025        | 6.81     | 6.77        | 6.62        | 6.73          | 0.10    |
| alts   | A024   | 0.0002   | 0.0003     | 0.0004       | 0.0003      | 0.00010 | 0.336    | 0.353              | 0.33                     | 0.340              | 0.012        | 1.89     | 1.833       | 1.906       | 1.876         | 0.038   |
| Res    | A027   |  |            |              |             |         | 0.4      | 0.4                | 0.4                      | 0.4                | 0            |          |             |             |               |         |
| all    | A028   |  |            |              |             |         |          |                    |                          |                    |              |          |             |             |               |         |
| idu    | A040   |  |            |              |             |         | 0.394    | 0.388              | 0.402                    | 0.395              | 0.0070       | 1.221    | 1.166       | 1.188       | 1.192         | 0.028   |
| div    | A047   | 0 0 0 0  |            |              |             |         |          |                    |                          |                    |              |          |             |             |               |         |
| Ч      | A049   | 0  | 0          | 0            | 0           | 0       | 0        | 0                  | 0                        | 0                  | 0            | 1.615    | 1.615       | 1.614       | 1.615         | 0.00040 |
|        | A050   |  |            |              |             |         |          |                    |                          |                    |              |          |             |             |               |         |
|        | A058   | 0.00   | 0.00       | 0.00         | 0.00        | 0.00    | 2.68     | 2.60               | 2.71                     | 2.66               | 0.057        | 1.25     | 3.35        | 2.32        | 2.31          | 1.1     |
|        | A061   | < 0.001  | < 0.001    | < 0.001      |             |         | 5.2      | 5.1                | 5.1                      | 5.1                | 0.058        | 24.5     | 24.5        | 24.3        | 24.4          | 0.12    |
|        | A065   |  |            |              |             |         |          |                    |                          |                    |              |          |             |             |               |         |
|        | A070   | 0.000  | 0.000      | 0.000        | 0.000       | 0.000   | 0.215    | 0.25               | 0.25                     | 0.238              | 0.020        | 0.895    | 0.885       | 0.945       | 0.908         | 0.032   |
|        | A071   |  |            |              |             |         | 0.046    | 0.024              | 0.020                    | 0.022              | 0.011        |          |             |             |               |         |
|        | A0/4   |  |            |              |             |         | 0.946    | 0.924              | 0.930                    | 0.933              | 0.011        |          |             |             |               |         |
|        | A076   |  |            |              |             |         |          |                    | _                        |                    |              |          |             |             |               |         |
|        | A078   |  |            |              |             |         |          |                    |                          |                    |              |          |             |             |               |         |
|        | A079   |  |            |              |             |         |          |                    |                          |                    |              |          |             |             |               |         |
|        | A085   | Comment  | Maar       |              | 0           |         | Comme    | Maar               |                          | 0.991              |              | Com      | Maar        |             | 1.02          |         |
| nity . |        | Consensus Mean 0<br>Consensus Standard Deviation 0.002 |            |              |             |         | Consense | s iviean           | Deviation                | 0.881              |              | Consense | is iviean   | Deviation   | 1.92          |         |
| n fin  |        | Moving   | s standard | Deviation    | 0.002       |         | Moving   | s standard         | Deviation                | 5.1                |              | Moving   | is standard | Deviation   | 1.4           |         |
| Res    |        | Minimum  | 1          |              | 0.22        |         | Minimum  | 1                  |                          | 0.1                |              | Minimum  | 1           |             | 24.4<br>0.009 |         |
| ŭ T    |        | N  |            |              | 6           |         | N        |                    |                          | 13                 |              | N        |             |             | 0.908         |         |
|        |        | 11   |            |              | 0           |         | 14       |                    |                          | 15                 |              | 1.4      |             |             | 7             |         |



Figure 5-21. Total  $\gamma$ -linolenic acid (C18:3 n-6) in Fatty Acids Solution A (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red line represents the upper consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \leq 2$ , with the lower range set at zero. A NIST value has not been determined in this material.



**Figure 5-22.** Total  $\gamma$ -linolenic acid (C18:3 n-6) in SRM 3275-II Omega-3 and Omega-6 Fatty Acids in Fish Oil (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red line represents the upper consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \leq 2$ , with the lower range set at zero. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by its uncertainty ( $U_{NIST}$ ) and represents the range that results in an acceptable  $Z_{NIST}$  score,  $|Z_{NIST}| \leq 2$ .





Figure 5-23. Total  $\gamma$ -linolenic acid (C18:3 n-6) in Cod Liver Oil (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red line represents the upper consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \leq 2$ , with the lower range set at zero. A NIST value has not been determined in this material.



HAMQAP Exercise 1 - Dietary Intake, Measurand: Total gamma-Linolenic Acid (C18:3 n-6) No. of laboratories: 6

**Figure 5-24.** Laboratory means for total  $\gamma$ -linolenic acid in SRM 3275-II Omega-3 and Omega-6 Fatty Acids in Fish Oil and Fatty Acids Solution A (sample/sample comparison view). In this view, the individual laboratory mean for one sample (SRM 3275-II) is compared to the mean for a second sample (Fatty Acids Solution A). The dotted blue box represents the consensus range of tolerance for SRM 3275-II (x-axis) and Fatty Acids Solution A (y-axis), calculated as the values above and below the consensus means that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \leq 2$ .

**Table 5-9.** Data summary table for total arachidonic acid in a fatty acids solution, fish oil, and cod liver oil. Data points highlighted in red have been flagged as potential outliers (e.g., difference from reference value, Grubb and/or Cochran) by the NIST software package.

|            |        |  |          |              |            |           | Tota     | l Arachido            | onic Acid (              | C20:4 n-6)            |         |          |             |             |       |       |
|------------|--------|--|----------|--------------|------------|-----------|----------|-----------------------|--------------------------|-----------------------|---------|----------|-------------|-------------|-------|-------|
| _          |        |  | Fatty Ac | rids Solutio | on A (mg/g | )         | SRM 3    | 3275-II Or<br>Acids i | nega-3 an<br>in Fish Oil | d Omega-0<br>l (mg/g) | 6 Fatty |          | Cod 1       | Liver Oil ( | mg/g) |       |
|            | Lab    | Α  | В        | С            | Avg        | SD        | Α        | В                     | С                        | Avg                   | SD      | Α        | В           | С           | Avg   | SD    |
|            | Target |  |          |              | 0.282      | 0.014     |          |                       |                          | 22.9                  | 1.0     |          |             |             |       |       |
|            | A004   | 0.20   | 0.18     | 0.18         | 0.19       | 0.012     | 16.5     | 14.2                  | 21.2                     | 17.3                  | 3.6     | 3.6      | 2.9         | 2.8         | 3.1   | 0.44  |
|            | A007   | 0.3  | 0.3      | 0.3          | 0.3        | 0.0       | 24.51    | 24.39                 | 24.52                    | 24.47                 | 0.072   | 3.26     | 3.24        | 3.25        | 3.25  | 0.010 |
|            | A008   |  |          |              |            |           |          |                       |                          |                       |         |          |             |             |       |       |
|            | A010   |  |          |              |            |           | 21.77    | 22.41                 | 22.06                    | 22.08                 | 0.32    |          |             |             |       |       |
|            | A012   | 0.395  | 0.567    | 0.388        | 0.450      | 0.10      | 24.685   | 24.598                | 24.750                   | 24.678                | 0.076   | 3.4      | 3.352       | 3.302       | 3.351 | 0.049 |
|            | A013   |  |          |              |            |           |          |                       |                          |                       |         |          |             |             |       |       |
|            | A014   | < 2.000  | < 2.000  | < 2.000      |            |           | 27       | 27                    | 27                       | 27                    | 0       | 10       | 10          | 10          | 10    | 0     |
|            | A015   |  |          |              |            |           |          |                       |                          |                       |         |          |             |             |       |       |
|            | A016   |  |          |              |            |           |          |                       |                          |                       |         |          |             |             |       |       |
| \$         | A019   |  |          |              |            |           | 1.19     | 1.16                  | 1.11                     | 1.15                  | 0.040   | 0.91     | 1.45        | 1.43        | 1.26  | 0.31  |
| Ins        | A024   | 0.287  | 0.285    | 0.288        | 0.287      | 0.0011015 | 21.601   | 21.622                | 20.711                   | 21.311                | 0.52    | 2.917    | 2.922       | 2.825       | 2.888 | 0.055 |
| R          | A027   |  |          |              |            |           | 31.5     | 30.4                  | 31.2                     | 31.0                  | 0.57    |          |             |             |       |       |
| ual        | A028   |  |          |              |            |           |          |                       |                          |                       |         |          |             |             |       |       |
| vid        | A040   |  |          |              |            |           | 21.950   | 22.074                | 22.038                   | 22.021                | 0.064   | 2.897    | 2.910       | 2.918       | 2.908 | 0.011 |
| ipu        | A047   | 0.52 0.55 0.51 0.53 0.0<br>0 0 0 0 0 0<br>0.00 0 0 0 |          |              | 0.021      | 7.5       | 7.8      | 7.7                   | 7.7                      | 0.15                  | 6.9     | 6.3      | 6.1         | 6.4         | 0.42  |       |
| Ξ          | A049   | 0  | 0        | 0            | 0          | 0         | 0        | 0                     | 0                        | 0                     | 0       | 0        | 0           | 0           | 0     | 0     |
|            | A058   | 0.00   | 0.00     | 0.00         | 0.00       | 0.00      | 24.64    | 22.54                 | 26.28                    | 24.49                 | 1.9     | 2.41     | 2.41        | 2.46        | 2.43  | 0.029 |
|            | A061   | < 0.001  | < 0.001  | < 0.001      |            |           | < 0.001  | < 0.001               | < 0.001                  |                       |         | < 0.001  | < 0.001     | < 0.001     |       |       |
|            | A065   |  |          |              |            |           |          |                       |                          |                       |         | 10.38    | 10.4        | 10.41       | 10.40 | 0.015 |
|            | A070   | 0.34   | 0.305    | 0.310        | 0.318      | 0.019     | 25.74    | 26.695                | 25.315                   | 25.917                | 0.71    | 3.195    | 3.28        | 3.435       | 3.303 | 0.12  |
|            | A071   |  |          |              |            |           |          |                       |                          |                       |         |          |             |             |       |       |
|            | A074   | 0.290  | 0.285    | 0.296        | 0.290      | 0.0055    | 23.4     | 22.9                  | 23.1                     | 23.1                  | 0.25    | 3.94     | 4.11        | 3.85        | 3.97  | 0.13  |
|            | A076   |  |          |              |            |           |          |                       |                          |                       |         |          |             |             |       |       |
|            | A078   |  |          |              |            |           |          |                       |                          |                       |         |          |             |             |       |       |
|            | A079   |  |          |              |            |           |          |                       |                          |                       |         |          |             |             |       |       |
|            | A083   |  |          |              |            |           |          |                       |                          |                       |         |          |             |             |       |       |
| ty         |        | Consensus Mean 0.262                                 |          |              |            |           | Consensu | ıs Mean               |                          | 22.7                  |         | Consense | is Mean     |             | 2.94  |       |
| uni<br>Its |        | Consensus Standard Deviation 0.20                    |          |              |            |           | Consensu | is Standard           | l Deviation              | 5.5                   |         | Consense | is Standard | l Deviation | 1.4   |       |
| nm<br>esu  |        | Maximum  |          |              | 0.53       |           | Maximun  | 1                     |                          | 31.0                  |         | Maximur  | n           |             | 10.40 |       |
| R          |        | Minimum  |          |              | 0          |           | Minimum  |                       |                          | 0                     |         | Minimum  | 1           |             | 0     |       |
| 5          |        | Ν  |          |              | 9          |           | Ν        |                       |                          | 14                    |         | Ν        |             |             | 13    |       |

Exercise HAMQAP Exercise 1 - Dietary Intake Sample: Fatty Acids Solution A Measurand: Total Arachidonic Acid (C20:4 n-6)



Figure 5-25. Total arachidonic acid (C20:4 n-6) in Fatty Acids Solution A (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red line represents the upper consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \leq 2$ , with the lower range set at zero. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by its uncertainty  $(U_{NIST})$  and represents the range that results in an acceptable  $Z_{NIST}$  score,  $|Z_{NIST}| \leq 2$ .



Figure 5-26. Total arachidonic acid (C20:4 n-6) in SRM 3275-II Omega-3 and Omega-6 Fatty Acids in Fish Oil (data summary view -analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \le 2$ . The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by its uncertainty ( $U_{\text{NIST}}$ ) and represents the range that results in an acceptable  $Z_{\text{NIST}}$  score,  $|Z_{\text{NIST}}| \leq 2$ .

Exercise





**Figure 5-27.** Total arachidonic acid (C20:4 n-6) in Cod Liver Oil (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \leq 2$ . A NIST value has not been determined in this material.



HAMQAP Exercise 1 - Dietary Intake, Measurand: Total Arachidonic Acid (C20:4 n-6) No. of laboratories: 9

**Figure 5-28.** Laboratory means for total arachidonic acid in SRM 3275-II Omega-3 and Omega-6 Fatty Acids in Fish Oil and Fatty Acids Solution A (sample/sample comparison view). In this view, the individual laboratory mean for one sample (SRM 3275-II) is compared to the mean for a second sample (Fatty Acids Solution A). The solid red box represents the NIST range of tolerance for the two samples, SRM 3275-II (x-axis) and Fatty Acids Solution A (y-axis), which encompasses the target values bounded by their uncertainties ( $U_{\text{NIST}}$ ) and represents the range that results in an acceptable  $Z_{\text{NIST}}$  score,  $|Z_{\text{NIST}}| \leq 2$ . The dotted blue box represents the consensus range of tolerance for SRM 3275-II (x-axis) and Fatty Acids Solution A (y-axis), calculated as the values above and below the consensus means that result in an acceptable  $Z'_{\text{comm}} \leq 2$ .

**Table 5-10.** Data summary table for total EPA in a fatty acids solution, fish oil, and cod liver oil. Data points highlighted in red have been flagged as potential outliers (e.g., difference from reference value, Grubb and/or Cochran) by the NIST software package.

|                  |        |                                    |          |              |             |         |          | Total El              | PA (C20:5                | n-3)                   |       |          |             |             |        |       |
|------------------|--------|------------------------------------|----------|--------------|-------------|---------|----------|-----------------------|--------------------------|------------------------|-------|----------|-------------|-------------|--------|-------|
|                  |        |                                    | Fatty Ac | cids Solutio | on A (mg/g) |         | SRM 3    | 3275-II Or<br>Acids i | nega-3 an<br>in Fish Oil | nd Omega-6<br>l (mg/g) | Fatty |          | Cod         | Liver Oil ( | mg/g)  |       |
|                  | Lab    | Α                                  | В        | С            | Avg         | SD      | Α        | В                     | С                        | Avg                    | SD    | Α        | В           | С           | Avg    | SD    |
|                  | Target |                                    |          |              | 0.0130      | 0.0010  |          |                       |                          | 394                    | 17    |          |             |             |        |       |
|                  | A004   | 0.0067                             | 0.0045   | 0.0047       | 0.0053      | 0.0012  | 126.5    | 76.0                  | 84.9                     | 95.8                   | 27    | 20.1     | 14.0        | 14.9        | 16.3   | 3.3   |
|                  | A007   | 0                                  | 0        | 0            | 0           | 0       | 395.43   | 393.29                | 394.91                   | 394.54                 | 1.12  | 80.64    | 80.64       | 80.77       | 80.68  | 0.075 |
|                  | A008   |                                    |          |              |             |         |          |                       |                          |                        |       |          |             |             |        |       |
|                  | A010   |                                    |          |              |             |         | 404.2    | 391.2                 | 381.7                    | 392.4                  | 11    |          |             |             |        |       |
|                  | A012   |                                    |          |              |             |         | 406.65   | 404.7                 | 409.3                    | 406.9                  | 2.3   | 85.7     | 84.5        | 83.4        | 84.5   | 1.2   |
|                  | A013   |                                    |          |              |             |         |          |                       |                          |                        |       |          |             |             |        |       |
|                  | A014   | < 2.000                            | < 2.000  | < 2.000      |             |         | 378      | 378                   | 372                      | 376                    | 3.5   | 80       | 80          | 81          | 80     | 0.58  |
|                  | A015   |                                    |          |              |             |         |          |                       |                          |                        |       |          |             |             |        |       |
|                  | A016   |                                    |          |              |             |         |          |                       |                          |                        |       |          |             |             |        |       |
|                  | A019   |                                    |          |              |             |         |          |                       |                          |                        |       |          |             |             |        |       |
|                  | A024   | 0.012                              | 0.013    | 0.013        | 0.013       | 0.00026 | 344.253  | 346.165               | 331.021                  | 340.480                | 8.2   | 71.606   | 72.033      | 70.115      | 71.251 | 1.0   |
| ults             | A027   |                                    |          |              |             |         | 506      | 506                   | 505                      | 506                    | 0.58  |          |             |             |        |       |
| Res              | A028   |                                    |          |              |             |         |          |                       |                          |                        |       |          |             |             |        |       |
| all              | A040   |                                    |          |              |             |         | 343.219  | 344.982               | 344.679                  | 344.293                | 0.94  | 71.918   | 72.447      | 72.803      | 72.389 | 0.45  |
| idu              | A047   | 0.31                               | 0.34     | 0.32         | 0.32        | 0.015   | 400.1    | 395.2                 | 397.6                    | 397.6                  | 2.5   | 67.9     | 68.3        | 68.1        | 68.1   | 0.20  |
| div              | A049   |                                    |          |              |             |         | 334      | 333                   | 337                      | 335                    | 2.1   | 71       | 69          | 69          | 70     | 1.2   |
| ĥ                | A050   |                                    |          |              |             |         |          |                       |                          |                        |       |          |             |             |        |       |
|                  | A051   |                                    |          |              |             |         |          |                       |                          |                        |       | 80.69    | 80.48       | 81.07       | 80.75  | 0.30  |
|                  | A058   | 0.00                               | 0.00     | 0.00         | 0.00        | 0.00    | 381.56   | 375.34                | 393.40                   | 383.43                 | 9.2   | 77.38    | 80.91       | 81.28       | 79.86  | 2.2   |
|                  | A061   | < 0.001                            | < 0.001  | < 0.001      |             |         | 382.0    | 380.4                 | 375.4                    | 379.3                  | 3.4   | 71.2     | 71.0        | 70.9        | 71.0   | 0.15  |
|                  | A065   |                                    |          |              |             |         |          |                       |                          |                        |       | 12.13    | 12.16       | 12.1        | 12.13  | 0.030 |
|                  | A070   | 0.000                              | 0.000    | 0.000        | 0.000       | 0.000   | 441.58   | 445.735               | 428.125                  | 438.48                 | 9.2   | 79.595   | 82.715      | 85.35       | 82.553 | 2.9   |
|                  | A071   |                                    |          |              |             |         |          |                       |                          |                        |       |          |             |             |        |       |
|                  | A074   | 0.014                              | 0.011    | 0.013        | 0.012       | 0.0014  | 402      | 389                   | 383                      | 391                    | 9.7   | 79.2     | 74.8        | 76.2        | 76.7   | 2.2   |
|                  | A076   |                                    |          |              |             |         |          |                       |                          |                        |       |          |             |             |        |       |
|                  | A078   |                                    |          |              |             |         |          |                       |                          |                        |       |          |             |             |        |       |
|                  | A079   |                                    |          |              |             |         |          |                       |                          |                        |       |          |             |             |        |       |
|                  | A082   |                                    |          |              |             |         | 132.52   | 133.24                | 147.45                   | 137.74                 | 8.4   | 15.24    | 14.3        | 14.37       | 14.6   | 0.52  |
|                  | A083   |                                    |          |              |             |         |          |                       |                          |                        |       |          |             |             |        |       |
| ity              |        | Consensu                           | s Mean   |              | 0.00500     |         | Consensu | ıs Mean               |                          | 388                    |       | Consensu | is Mean     |             | 76.5   |       |
| un<br>Its        |        | Consensus Standard Deviation 0.010 |          |              |             |         | Consensu | is Standard           | Deviation                | 50                     |       | Consensu | is Standard | Deviation   | 8.89   |       |
| esu              |        | Maximum                            |          |              | 0.32        |         | Maximun  | 1                     |                          | 506                    |       | Maximun  | 1           |             | 84.5   |       |
| <sup>10</sup> Co |        | Minimum                            |          |              | 0           |         | Minimum  |                       |                          | 95.8                   |       | Minimum  |             |             | 12.13  |       |
| -                |        | Ν                                  |          |              | 7           |         | Ν        |                       |                          | 15                     |       | Ν        |             |             | 15     |       |



**Figure 5-29.** Total EPA (C20:5 n-3) in Fatty Acids Solution A (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red line represents the upper consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \leq 2$ , with the lower range set at zero. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by its uncertainty ( $U_{NIST}$ ) and represents the range that results in an acceptable  $Z'_{NIST} | \leq 2$ .



Exercise HAMQAP Exercise 1 - Dietary Intake Sample: SRM 3275-II Omega-3 and Omega-6 Fatty Acids in Fish Oil Measurand: Total EPA (C20:5 n-3)

**Figure 5-30.** Total EPA (C20:5 n-3) in SRM 3275-II Omega-3 and Omega-6 Fatty Acids in Fish Oil (data summary view –analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \le 2$ . The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by its uncertainty ( $U_{NIST}$ ) and represents the range that results in an acceptable  $Z'_{NIST} | \le 2$ .

Exercise HAMQAP Exercise 1 - Dietary Intake Sample: Cod Liver Oil Measurand: Total EPA (C20:5 n-3)



**Figure 5-31.** Total EPA (C20:5 n-3) in Cod Liver Oil (data summary view –analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \leq 2$ . The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by its uncertainty ( $U_{NIST}$ ) and represents the range that results in an acceptable  $Z_{NIST}$  score,  $|Z_{NIST}| \leq 2$ . A NIST value has not been determined in this material.



**Figure 5-32.** Laboratory means for total EPA in SRM 3275-II Omega-3 and Omega-6 Fatty Acids in Fish Oil and Fatty Acids Solution A (sample/sample comparison view). In this view, the individual laboratory mean for one sample (SRM 3275-II) is compared to the mean for a second sample (Fatty Acids Solution A). The solid red box represents the NIST range of tolerance for the two samples, SRM 3275-II (x-axis) and Fatty Acids Solution A (y-axis), which encompasses the target values bounded by their uncertainties ( $U_{\text{NIST}}$ ) and represents the range that results in an acceptable  $Z_{\text{NIST}}$  score,  $|Z_{\text{NIST}}| \leq 2$ . The dotted blue box represents the consensus range of tolerance for SRM 3275-II (x-axis) and Fatty Acids Solution A (y-axis), calculated as the values above and below the consensus means that result in an acceptable  $Z'_{\text{comm}} \leq 2$ .

Table 5-11. Data summary table for total DPA in a fatty acids solution, fish oil, and cod liver oil. Data points highlighted in red have been flagged as potential outliers (e.g., difference from reference value, Grubb and/or Cochran) by the NIST software package.

|      |                      |                         |             |              |            |           |          | Total D            | PA (C22:5                | n-3)                   |         |          |            |             |        |       |
|------|----------------------|-------------------------|-------------|--------------|------------|-----------|----------|--------------------|--------------------------|------------------------|---------|----------|------------|-------------|--------|-------|
|      |                      |                         | Fatty Ac    | cids Solutio | on A (mg/g | )         | SRM 3    | 3275-II O<br>Acids | nega-3 an<br>in Fish Oil | nd Omega-(<br>l (mg/g) | 6 Fatty |          | Cod I      | Liver Oil ( | mg/g)  |       |
|      | Lab                  | Α                       | В           | С            | Avg        | SD        | Α        | В                  | С                        | Avg                    | SD      | Α        | В          | С           | Avg    | SD    |
|      | Target               |                         |             |              | 0.0150     | 0.0010    |          |                    |                          | 67.6                   | 2.3     |          |            |             |        |       |
|      | A004                 | 0.007                   | 0.004       | 0.004        | 0.005      | 0.0017    | 20.8     | 24.1               | 24.5                     | 23.1                   | 2.0     | 3.8      | 3.6        | 3.2         | 3.5    | 0.31  |
|      | A007                 | 0                       | 0           | 0            | 0          | 0         | 68.92    | 68.59              | 68.87                    | 68.79                  | 0.18    | 11.25    | 11.26      | 11.28       | 11.26  | 0.015 |
|      | A008                 |                         |             |              |            |           |          |                    |                          |                        |         |          |            |             |        |       |
|      | A010                 |                         |             |              |            |           | 70.26    | 66.14              | 65.17                    | 67.19                  | 2.7     |          |            |             |        |       |
|      | A012                 |                         |             |              |            |           | 66.9     | 66.5               | 67.1                     | 66.8                   | 0.31    | 11.3     | 11.1       | 11          | 11.1   | 0.15  |
|      | A013                 |                         |             |              |            |           |          |                    |                          |                        |         |          |            |             |        |       |
|      | A014                 | < 2.000                 | < 2.000     | < 2.000      |            |           | 68       | 68                 | 68                       | 68                     | 0       | 12       | 11         | 11          | 11.3   | 0.58  |
|      | A015                 |                         |             |              |            |           |          |                    |                          |                        |         |          |            |             |        |       |
|      | A016                 |                         |             |              |            |           |          |                    |                          |                        |         |          |            |             |        |       |
|      | A019                 |                         |             |              |            |           |          |                    |                          |                        |         |          |            |             |        |       |
| ults | A024                 | 0.013                   | 0.013       | 0.015        | 0.014      | 0.0006807 | 59.464   | 59.059             | 56.537                   | 58.353                 | 1.6     | 9.669    | 9.761      | 9.558       | 9.663  | 0.10  |
| Res  | A027                 |                         |             |              |            |           | 86.7     | 85.2               | 86.6                     | 86.2                   | 0.84    |          |            |             |        |       |
| all  | A028                 |                         |             |              |            |           |          |                    |                          |                        |         |          |            |             |        |       |
| idu  | A040                 |                         |             |              |            |           | 60.822   | 61.176             | 61.035                   | 61.011                 | 0.18    | 10.170   | 10.223     | 10.226      | 10.206 | 0.032 |
| div  | A047                 |                         |             |              |            |           | 62.9     | 65.6               | 66.1                     | 64.9                   | 1.7     | 9.5      | 9.7        | 9.3         | 9.5    | 0.2   |
| Ч    | A049                 |                         |             |              |            |           |          |                    |                          |                        |         |          |            |             |        |       |
|      | A050                 |                         |             |              |            |           |          |                    |                          |                        |         |          |            |             |        |       |
|      | A058                 | 0.00                    | 0.00        | 0.00         | 0.00       | 0.00      | 65.13    | 64.10              | 66.96                    | 65.40                  | 1.4     | 10.41    | 10.88      | 10.93       | 10.74  | 0.29  |
|      | A061                 | < 0.001                 | < 0.001     | < 0.001      |            |           | 61.0     | 60.8               | 62.9                     | 61.6                   | 1.2     | 10.0     | 10.0       | 9.9         | 10.0   | 0.058 |
|      | A065                 |                         |             |              |            |           |          |                    |                          |                        |         |          |            |             |        |       |
|      | A070                 | 0.000 0.000 0.000 0.00  |             | 0.000        | 0.000      | 72.42     | 74.055   | 71.08              | 72.518                   | 1.5                    | 10.57   | 10.895   | 11.345     | 10.937      | 0.39   |       |
|      | A071                 |                         |             |              |            |           |          |                    |                          |                        |         |          |            |             |        |       |
|      | A074                 | 0.014 0.013 0.013 0.013 |             |              | 0.013      | 0.0005508 | 71.4     | 68.1               | 67.6                     | 69.0                   | 2.1     | 10.9     | 10.5       | 10.6        | 10.7   | 0.21  |
|      | A076                 |                         |             |              |            |           |          |                    |                          |                        |         |          |            |             |        |       |
|      | A078                 | )78<br>)79              |             |              |            |           |          |                    |                          |                        |         |          |            |             |        |       |
|      | A079                 | .079                    |             |              |            |           |          |                    |                          |                        |         |          |            |             |        |       |
|      | A083                 | -                       |             |              |            |           | _        |                    |                          |                        |         | -        |            |             |        |       |
| lity | Consensus Mean 0.005 |                         |             |              |            |           | Consensu | is Mean            |                          | 66.7                   |         | Consensu | s Mean     |             | 10.5   |       |
| aun  |                      | Consensu                | is Standard | Deviation    | 0.008      |           | Consensu | is Standard        | Deviation                | 6.7                    |         | Consensu | s Standard | Deviation   | 0.93   |       |
| Rest |                      | Maximum                 | 1           |              | 0.014      |           | Maximun  | 1                  |                          | 86.2                   |         | Maximum  | 1          |             | 11.3   |       |
| Co   |                      | Minimum                 |             |              | 0          |           | Minimum  |                    |                          | 23.1                   |         | Minimum  |            |             | 3.5    |       |
|      |                      | N                       |             |              | 6          |           | N        |                    |                          | 13                     |         | N        |            |             | 11     |       |

Sample: Fatty Acids Solution A Measurand: Total DPA (C22:5 n-3) 0.040<sub>f</sub> Gas Chromatography with Flame Ionization Detection not specified - Mean line 0.035 - Limit of tolerance 0.030 0.025 mg/g 0.020 0.015 G 0.010 0.005 A061-40.001 (C 8 0.000 A070-A007 A058-A004-A074-A024-A014-Laboratory

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Exercise

**Figure 5-33.** Total DPA (C22:5 n-3) in Fatty Acids Solution A (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red line represents the upper consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \le 2$ , with the lower range set at zero. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by its uncertainty ( $U_{NIST}$ ) and represents the range that results in an acceptable  $Z_{NIST} | \le 2$ .



Exercise HAMQAP Exercise 1 - Dietary Intake Sample: SRM 3275-II Omega-3 and Omega-6 Fatty Acids in Fish Oil Measurand: Total DPA (C22:5 n-3)

**Figure 5-34.** Total DPA (C22:5 n-3) in SRM 3275-II Omega-3 and Omega-6 Fatty Acids in Fish Oil (data summary view –analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \leq 2$ . The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by its uncertainty ( $U_{NIST}$ ) and represents the range that results in an acceptable  $Z'_{NIST} | \leq 2$ .

Exercise HAMQAP Exercise 1 - Dietary Intake Sample: Cod Liver Oil Measurand: Total DPA (C22:5 n-3)



**Figure 5-35.** Total DPA (C22:5 n-3) in Cod Liver Oil (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \leq 2$ . A NIST value has not been determined in this material.



**Figure 5-36.** Laboratory means for total DPA in SRM 3275-II Omega-3 and Omega-6 Fatty Acids in Fish Oil and Fatty Acids Solution A (sample/sample comparison view). In this view, the individual laboratory mean for one sample (SRM 3275-II) is compared to the mean for a second sample (fish oil). The solid red box represents the NIST range of tolerance for the two samples, SRM 3275-II (x-axis) and Fatty Acids Solution A (y-axis), which encompasses the target values bounded by their uncertainties ( $U_{\text{NIST}}$ ) and represents the range that results in an acceptable  $Z_{\text{NIST}}$  score,  $|Z_{\text{NIST}}| \leq 2$ . The dotted blue box represents the consensus range of tolerance for SRM 3275-II (x-axis) and Fatty Acids Solution A (y-axis), calculated as the values above and below the consensus means that result in an acceptable  $Z'_{\text{comm}} | \leq 2$ .

**Table 5-12.** Data summary table for total DHA in a fatty acids solution, fish oil, and cod liver oil. Data points highlighted in red have been flagged as potential outliers (e.g., difference from reference value, Grubb and/or Cochran) by the NIST software package.

|             |                                    |               |          |              |             |        |          | Total DI              | HA (C22:6                | 5 n-3)                 |       |          |             |             |         |      |
|-------------|------------------------------------|---------------|----------|--------------|-------------|--------|----------|-----------------------|--------------------------|------------------------|-------|----------|-------------|-------------|---------|------|
|             |                                    |               | Fatty Ac | cids Solutio | on A (mg/g) |        | SRM 3    | 3275-II Or<br>Acids i | mega-3 an<br>in Fish Oil | nd Omega-6<br>l (mg/g) | Fatty |          | Cod I       | Liver Oil ( | mg/g)   |      |
|             | Lab                                | Α             | В        | С            | Avg         | SD     | Α        | В                     | С                        | Avg                    | SD    | Α        | В           | С           | Avg     | SD   |
|             | Target                             |               |          |              | 0.0370      | 0.0020 |          |                       |                          | 187                    | 8     |          |             |             |         |      |
|             | A004                               | 0.015         | 0.0054   | 0.0048       | 0.0084      | 0.0057 | 44.0     | 37.3                  | 37.4                     | 39.6                   | 3.8   | 27.9     | 16.5        | 19.0        | 21.1    | 6.0  |
|             | A007                               | 0             | 0        | 0            | 0           | 0      | 198.06   | 197.05                | 197.83                   | 197.65                 | 0.53  | 121.65   | 121.65      | 121.92      | 121.74  | 0.16 |
|             | A008                               |               |          |              |             |        |          |                       |                          |                        |       |          |             |             |         |      |
|             | A010                               |               |          |              |             |        | 182.6    | 194.2                 | 190.7                    | 189.2                  | 6.0   |          |             |             |         |      |
|             | A012                               |               |          |              |             |        | 200.1    | 199.2                 | 201.5                    | 200.3                  | 1.2   | 127.4    | 125.7       | 124.2       | 125.8   | 1.6  |
|             | A013                               |               |          |              |             |        |          |                       |                          |                        |       |          |             |             |         |      |
|             | A014                               | < 2.000       | < 2.000  | < 2.000      |             |        | 180      | 180                   | 178                      | 179                    | 1.2   | 115      | 115         | 116         | 115     | 0.58 |
|             | A015                               |               |          |              |             |        |          |                       |                          |                        |       |          |             |             |         |      |
|             | A016                               |               |          |              |             |        |          |                       |                          |                        |       |          |             |             |         |      |
|             | A019                               |               |          |              |             |        |          |                       |                          |                        |       |          |             |             |         |      |
| s           | A024                               | 0.038         | 0.035    | 0.036        | 0.037       | 0.0016 | 164.184  | 164.727               | 157.8                    | 162.237                | 3.9   | 103.757  | 104.251     | 101.502     | 103.170 | 1.5  |
| sult        | A027                               |               |          |              |             |        | 229      | 229                   | 228                      | 229                    | 0.58  |          |             |             |         |      |
| Re          | A028                               |               |          |              |             |        | 157.055  | 150.000               | 150.205                  | 150.050                | 0.40  | 00.422   | 100.020     | 100.460     | 00.074  | 0.53 |
| lal         | A040                               |               |          |              |             |        | 157.855  | 158.809               | 158.396                  | 158.353                | 0.48  | 99.432   | 100.029     | 100.460     | 99.974  | 0.52 |
| vidı        | A047                               |               |          |              |             |        | 158.1    | 157.8                 | 150.5                    | 155.4                  | 4.4   | 84.5     | 85.5        | 85.1        | 85.0    | 0.42 |
| ndř         | A049                               |               |          |              |             |        | 155      | 152                   | 155                      | 155                    | 1.5   | 100      | 97          | 97          | 98      | 1.7  |
| 1           | A051                               |               |          |              |             |        |          |                       |                          |                        |       | 116.62   | 116 70      | 117.20      | 116.01  | 0.42 |
|             | A051                               | 0.00          | 0.00     | 0.00         | 0.00        | 0.00   | 182.68   | 175.06                | 185.04                   | 181.23                 | 5.6   | 110.05   | 115.60      | 116.04      | 114.00  | 3.1  |
|             | A061                               | < 0.001       | < 0.001  | < 0.001      | 0.00        | 0.00   | 171.0    | 171.3                 | 171.3                    | 171.5                  | 0.35  | 10.55    | 00.8        | 00.5        | 00.8    | 0.25 |
|             | A065                               | < 0.001       | < 0.001  | < 0.001      |             |        | 1/1.9    | 171.5                 | 171.5                    | 171.5                  | 0.55  | 15 59    | 15.61       | 15.60       | 15.60   | 0.25 |
|             | A070                               | 0.05          | 0.035    | 0.03         | 0.038       | 0.010  | 208.15   | 209.05                | 202.865                  | 206.69                 | 3 34  | 117 270  | 120 765     | 125 605     | 121 213 | 4.2  |
|             | A071                               | 0.05          | 01000    | 0105         | 01050       | 0.010  | 200110   | 207100                | 202.000                  | 200107                 | 5151  | 11/12/0  | 1201700     | 1201000     | 1211210 |      |
|             | A074                               | 0.033         | 0.032    | 0.035        | 0.033       | 0.0013 | 190      | 181                   | 179                      | 183                    | 5.9   | 110      | 107         | 109         | 109     | 1.5  |
|             | A076                               |               |          |              |             |        |          | -                     |                          |                        |       | -        |             |             |         |      |
|             | A078                               |               |          |              |             |        |          |                       |                          |                        |       |          |             |             |         |      |
|             | A079                               |               |          |              |             |        |          |                       |                          |                        |       |          |             |             |         |      |
|             | A082                               |               |          |              |             |        | 96.21    | 88.24                 | 89.33                    | 91.26                  | 4.3   | 23.72    | 22.05       | 24.89       | 23.6    | 1.4  |
|             | A083                               |               |          |              |             |        |          |                       |                          |                        |       |          |             |             |         |      |
| ţ           |                                    | Consensus     | s Mean   |              | 0.019       |        | Consensu | s Mean                |                          | 176                    |       | Consensu | is Mean     |             | 107     |      |
| unit<br>Its | Consensus Standard Deviation 0.014 |               |          |              |             |        | Consensu | s Standard            | l Deviation              | 37                     |       | Consensu | is Standard | Deviation   | 21      |      |
| nm          |                                    | Maximum 0.038 |          |              |             |        | Maximum  | ı                     |                          | 229                    |       | Maximun  | 1           |             | 125.8   |      |
| R           |                                    | Minimum       |          |              | 0           |        | Minimum  |                       |                          | 39.6                   |       | Minimum  |             |             | 15.60   |      |
| 5           |                                    | Ν             |          |              | 6           |        | Ν        |                       |                          | 15                     |       | Ν        |             |             | 15      |      |



**Figure 5-37.** Total DHA (C22:6 n-3) in Fatty Acids Solution A (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red line represents the upper consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \leq 2$ , with the lower range set at zero. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by its uncertainty ( $U_{NIST}$ ) and represents the range that results in an acceptable  $Z'_{NIST}| \leq 2$ .



Exercise HAMQAP Exercise 1 - Dietary Intake Sample: SRM 3275-II Omega-3 and Omega-6 Fatty Acids in Fish Oil Measurand: Total DHA (C22:6 n-3)

**Figure 5-38.** Total DHA (C22:6 n-3) in SRM 3275-II Omega-3 and Omega-6 Fatty Acids in Fish Oil (data summary view –analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \le 2$ . The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by its uncertainty ( $U_{NIST}$ ) and represents the range that results in an acceptable  $Z'_{NIST} | \le 2$ .

Exercise HAMQAP Exercise 1 - Dietary Intake Sample: Cod Liver Oil Measurand: Total DHA (C22:6 n-3)



Figure 5-39. Total DHA (C22:6 n-3) in Cod Liver Oil (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \leq 2$ . A NIST value has not been determined in this material.



HAMQAP Exercise 1 - Dietary Intake, Measurand: Total DHA (C22:6 n-3) No. of laboratories: 6

**Figure 5-40.** Laboratory means for total DHA in SRM 3275-II Omega-3 and Omega-6 Fatty Acids in Fish Oil and Fatty Acids Solution A (sample/sample comparison view). In this view, the individual laboratory mean for one sample (SRM 3275-II) is compared to the mean for a second sample (Fatty Acids Solution A). The solid red box represents the NIST range of tolerance for the two samples, SRM 3275-II (x-axis) and Fatty Acids Solution A (y-axis), which encompasses the target values bounded by their uncertainties ( $U_{\text{NIST}}$ ) and represents the range that results in an acceptable  $Z_{\text{NIST}}$  score,  $|Z_{\text{NIST}}| \leq 2$ . The dotted blue box represents the consensus range of tolerance for SRM 3275-II (x-axis) and Fatty Acids Solution A (y-axis), calculated as the values above and below the consensus means that result in an acceptable  $Z'_{\text{comm}} \leq 2$ .

|            |              |          |            |              |            |           |          | Free Stea          | ric Acid (O              | C18:0)             |         |          |             |             |       |        |
|------------|--------------|----------|------------|--------------|------------|-----------|----------|--------------------|--------------------------|--------------------|---------|----------|-------------|-------------|-------|--------|
|            |              |          | Fatty Ac   | cids Solutio | on A (mg/g | )         | SRM 3    | 3275-II O<br>Acids | mega-3 an<br>in Fish Oil | d Omega-<br>(mg/g) | 6 Fatty |          | Cod         | Liver Oil ( | mg/g) |        |
|            | Lab          | Α        | В          | С            | Avg        | SD        | Α        | В                  | С                        | Avg                | SD      | Α        | В           | С           | Avg   | SD     |
|            | Target       |          |            |              | 0.180      | 0.0090    |          |                    |                          |                    |         |          |             |             |       |        |
|            | A004         |          |            |              |            |           |          |                    |                          |                    |         |          |             |             |       |        |
|            | A008         |          |            |              |            |           |          |                    |                          |                    |         |          |             |             |       |        |
|            | A010         |          |            |              |            |           |          |                    |                          |                    |         |          |             |             |       |        |
|            | A012         |          |            |              |            |           |          |                    |                          |                    |         |          |             |             |       |        |
|            | A013         |          |            |              |            |           |          |                    |                          |                    |         |          |             |             |       |        |
|            | A014         | < 2.000  | < 2.000    | < 2.000      |            |           |          |                    |                          |                    |         |          |             |             |       |        |
|            | A015         |          |            |              |            |           |          |                    |                          |                    |         |          |             |             |       |        |
| lts        | A019         |          |            |              |            |           |          |                    |                          |                    |         |          |             |             |       |        |
| lesi       | A024         | 0.171    | 0.171      | 0.171        | 0.171      | 0.0004619 | 1.153    | 1.149              | 1.127                    | 1.143              | 0.014   | 0.223    | 0.220       | 0.218       | 0.220 | 0.0025 |
| al B       | A028         |          |            |              |            |           |          |                    |                          |                    |         |          |             |             |       |        |
| dus        | A040         |          |            |              |            |           |          |                    |                          |                    |         |          |             |             |       |        |
| livi       | A047         |          |            |              |            |           |          |                    |                          |                    |         |          |             |             |       |        |
| Inc        | A049         |          |            |              |            |           |          |                    |                          |                    |         |          |             |             |       |        |
|            | A050         |          |            |              |            |           |          |                    |                          |                    |         |          |             |             |       |        |
|            | A065         |          |            |              |            |           |          |                    |                          |                    |         |          |             |             |       |        |
|            | A070         | 0.14     | 0.16       | 0.13         | 0.14       | 0.015     | 3.335    | 3.175              | 3.360                    | 3.290              | 0.10    | 0.875    | 0.865       | 0.820       | 0.853 | 0.029  |
|            | A071         |          |            |              |            |           |          |                    |                          |                    |         |          |             |             |       |        |
|            | A074         | 0.167    | 0.167      | 0.165        | 0.166      | 0.0012    |          |                    |                          |                    |         |          |             |             |       |        |
|            | A074<br>A078 |          |            |              |            |           |          |                    |                          |                    |         |          |             |             |       |        |
|            | A079         |          |            |              |            |           |          |                    |                          |                    |         |          |             |             |       |        |
|            | A083         |          |            |              |            |           |          |                    |                          |                    |         |          |             |             |       |        |
| ty         |              | Consensu | s Mean     |              | 0.16       |           | Consensu | ıs Mean            |                          | 2.22               |         | Consensu | ıs Mean     |             | 0.537 |        |
| uni<br>Its |              | Consensu | s Standard | Deviation    | 0.011      |           | Consensu | is Standard        | l Deviation              | 4.5                |         | Consensu | is Standard | Deviation   | 1.3   |        |
| nm<br>esul |              | Maximum  |            |              | 0.171      |           | Maximun  | 1                  |                          | 3.290              |         | Maximun  | 1           |             | 0.853 |        |
| R. G       |              | Minimum  |            |              | 0.14       |           | Minimum  |                    |                          | 1.143              |         | Minimum  |             |             | 0.220 |        |
| 5          |              | Ν        |            |              | 3          |           | Ν        |                    |                          | 2                  |         | Ν        |             |             | 2     |        |

**Table 5-13.** Data summary table for free stearic acid in a fatty acids solution, fish oil, and cod liver oil.

**Table 5-14.** Data summary table for free cis-vaccenic acid in a fatty acids solution, fish oil, and cod liver oil.

|         |                              |          |          |              |            |           | Free     | cis-Vacce            | nic Acid (              | (C18:1 n-7            | )       |          |             |             |       |       |
|---------|------------------------------|----------|----------|--------------|------------|-----------|----------|----------------------|-------------------------|-----------------------|---------|----------|-------------|-------------|-------|-------|
| _       |                              |          | Fatty Ac | cids Solutio | on A (mg/g | )         | SRM 3    | 275-II On<br>Acids i | nega-3 an<br>n Fish Oil | nd Omega-<br>l (mg/g) | 6 Fatty |          | Cod I       | Liver Oil ( | mg/g) |       |
|         | Lab                          | Α        | В        | С            | Avg        | SD        | Α        | В                    | С                       | Avg                   | SD      | Α        | В           | С           | Avg   | SD    |
|         | Target                       |          |          |              | 0.0370     | 0.0020    |          |                      |                         |                       |         |          |             |             |       |       |
|         | A004                         |          |          |              |            |           |          |                      |                         |                       |         |          |             |             |       |       |
|         | A010                         |          |          |              |            |           |          |                      |                         |                       |         |          |             |             |       |       |
|         | A012                         |          |          |              |            |           |          |                      |                         |                       |         |          |             |             |       |       |
|         | A013                         |          |          |              |            |           |          |                      |                         |                       |         |          |             |             |       |       |
|         | A014                         | < 2.000  | < 2.000  | < 2.000      |            |           |          |                      |                         |                       |         |          |             |             |       |       |
|         | A015                         |          |          |              |            |           |          |                      |                         |                       |         |          |             |             |       |       |
| ults    | A019                         |          |          |              |            |           |          |                      |                         |                       |         |          |             |             |       |       |
| Res     | A024                         | 0.034    | 0.035    | 0.035        | 0.034      | 0.0003055 | 0.833    | 0.834                | 0.819                   | 0.829                 | 0.0084  | 0.368    | 0.362       | 0.365       | 0.365 | 0.003 |
| al l    | A028                         |          |          |              |            |           |          |                      |                         |                       |         |          |             |             |       |       |
| idu     | A040                         |          |          |              |            |           |          |                      |                         |                       |         |          |             |             |       |       |
| div     | A047                         |          |          |              |            |           |          |                      |                         |                       |         |          |             |             |       |       |
| Ц       | A049                         |          |          |              |            |           |          |                      |                         |                       |         |          |             |             |       |       |
|         | A065                         |          |          |              |            |           |          |                      |                         |                       |         |          |             |             |       |       |
|         | A070                         |          |          |              |            |           |          |                      |                         |                       |         |          |             |             |       |       |
|         | A071                         |          |          |              |            |           |          |                      |                         |                       |         |          |             |             |       |       |
|         | A074                         |          |          |              |            |           |          |                      |                         |                       |         |          |             |             |       |       |
|         | A078                         |          |          |              |            |           |          |                      |                         |                       |         |          |             |             |       |       |
|         | A079                         |          |          |              |            |           |          |                      |                         |                       |         |          |             |             |       |       |
|         | A083                         | _        |          |              |            |           | -        |                      |                         |                       |         | -        |             |             |       |       |
| ity     |                              | Consensu | s Mean   |              |            |           | Consensu | s Mean               |                         |                       |         | Consensu | is Mean     |             |       |       |
| un      | Consensus Standard Deviation |          |          |              |            |           | Consensu | s Standard           | Deviation               |                       |         | Consensu | is Standard | Deviation   |       |       |
| un Kest |                              | Maximum  | 1        |              | 0.034      |           | Maximum  | I                    |                         | 0.829                 |         | Maximun  | 1           |             | 0.365 |       |
| Co.     |                              | Minimum  |          |              | 0.034      |           | Minimum  |                      |                         | 0.829                 |         | Minimum  |             |             | 0.365 |       |
|         |                              | N        |          |              | 1          |           | Ν        |                      |                         | 1                     |         | N        |             |             | 1     |       |

|                 |        |                   |  |              |             |        | F        | ree Oleic             | Acid (C18               | 8:1 n-9)              |         |          |             |             |       |       |
|-----------------|--------|-------------------|--|--------------|-------------|--------|----------|-----------------------|-------------------------|-----------------------|---------|----------|-------------|-------------|-------|-------|
|                 |        |                   | Fatty Ac   | cids Solutio | on A (mg/g) |        | SRM 3    | 3275-II Or<br>Acids i | mega-3 ar<br>in Fish Oi | nd Omega-<br>l (mg/g) | 6 Fatty |          | Cod         | Liver Oil ( | mg/g) |       |
|                 | Lab    | Α                 | В  | С            | Avg         | SD     | Α        | В                     | С                       | Avg                   | SD      | Α        | В           | С           | Avg   | SD    |
|                 | Target |                   |  |              | 0.428       | 0.021  |          |                       |                         |                       |         |          |             |             |       |       |
|                 | A004   |                   |  |              |             |        |          |                       |                         |                       |         |          |             |             |       |       |
|                 | A008   |                   |  |              |             |        |          |                       |                         |                       |         |          |             |             |       |       |
|                 | A010   |                   |  |              |             |        |          |                       |                         |                       |         |          |             |             |       |       |
|                 | A012   |                   |  |              |             |        |          |                       |                         |                       |         |          |             |             |       |       |
|                 | A013   |                   |  |              |             |        |          |                       |                         |                       |         |          |             |             |       |       |
|                 | A014   | < 2.000           | < 2.000  | < 2.000      |             |        |          |                       |                         |                       |         |          |             |             |       |       |
|                 | A015   |                   |  |              |             |        |          |                       |                         |                       |         |          |             |             |       |       |
| alts            | A019   |                   |  |              |             |        |          |                       |                         |                       |         |          |             |             |       |       |
| Res             | A024   | 0.3881            | 0.3871   | 0.3861       | 0.3871      | 0.0010 | 1.898    | 1.891                 | 1.871                   | 1.887                 | 0.014   | 1.105    | 1.080       | 1.067       | 1.084 | 0.019 |
| []              | A028   |                   |  |              |             |        |          |                       |                         |                       |         |          |             |             |       |       |
| idu             | A040   |                   |  |              |             |        |          |                       |                         |                       |         |          |             |             |       |       |
| div             | A047   |                   |  |              |             |        |          |                       |                         |                       |         |          |             |             |       |       |
| Б               | A049   |                   |  |              |             |        |          |                       |                         |                       |         |          |             |             |       |       |
|                 | A050   |                   |  |              |             |        |          |                       |                         |                       |         |          |             |             |       |       |
|                 | A065   |                   |  |              |             |        |          |                       |                         |                       |         |          |             |             |       |       |
|                 | A070   | 0.405             | 0.400  | 0.375        | 0.393       | 0.016  | 6.470    | 6.020                 | 6.485                   | 6.325                 | 0.26    | 9.325    | 9.125       | 8.715       | 9.055 | 0.31  |
|                 | A071   |                   |  |              |             |        |          |                       |                         |                       |         |          |             |             |       |       |
|                 | A074   | 0.433             | 0.440  | 0.454        | 0.442       | 0.011  |          |                       |                         |                       |         |          |             |             |       |       |
|                 | A078   | 0.455 0.440 0.454 |  |              |             |        |          |                       |                         |                       |         |          |             |             |       |       |
|                 | A079   |                   |  |              |             |        |          |                       |                         |                       |         |          |             |             |       |       |
|                 | A083   |                   |  |              |             |        |          |                       |                         |                       |         |          |             |             |       |       |
| ţ,              |        | Consensus         | s Mean   |              | 0.408       |        | Consensu | is Mean               |                         | 4.11                  |         | Consensu | is Mean     |             | 5.07  |       |
| utra lits       |        | Consensus         | s Standard   | Deviation    | 0.038       |        | Consensu | is Standard           | 1 Deviation             | 9.2                   |         | Consensu | is Standard | Deviation   | 17.0  |       |
| un n            |        | Maximum 0.442     |  |              |             |        | Maximun  | 1                     |                         | 6.325                 |         | Maximun  | 1           |             | 9.055 |       |
| <sup>B</sup> Co |        | Minimum           |  |              | 0.3871      |        | Minimum  |                       |                         | 1.887                 |         | Minimum  |             |             | 1.084 |       |
| -               |        | N                 | s Mean 0.408<br>s Standard Deviation 0.038<br>n 0.442<br>0.3871<br>3 |              |             |        | Ν        |                       |                         | 2                     |         | Ν        |             |             | 2     |       |

**Table 5-15.** Data summary table for free oleic acid in a fatty acids solution, fish oil, and cod liver oil.

**Table 5-16.** Data summary table for free linoleic acid in a fatty acids solution, fish oil, and cod liver oil.

|            |        |                                    |          |              |             |          | Fr          | ee Linolei            | c Acid (Cl               | 18:2 n-6)          |          |            |             |             |       |        |
|------------|--------|------------------------------------|----------|--------------|-------------|----------|-------------|-----------------------|--------------------------|--------------------|----------|------------|-------------|-------------|-------|--------|
| _          |        |                                    | Fatty Ac | cids Solutio | on A (mg/g) |          | SRM 3       | 3275-II Or<br>Acids i | nega-3 an<br>in Fish Oil | d Omega-<br>(mg/g) | 6 Fatty  |            | Cod         | Liver Oil ( | mg/g) |        |
|            | Lab    | Α                                  | В        | С            | Avg         | SD       | Α           | В                     | С                        | Avg                | SD       | Α          | В           | С           | Avg   | SD     |
|            | Target |                                    |          |              | 0.802       | 0.040    |             |                       |                          |                    |          |            |             |             |       |        |
|            | A004   |                                    |          |              |             |          |             |                       |                          |                    |          |            |             |             |       |        |
|            | A008   |                                    |          |              |             |          |             |                       |                          |                    |          |            |             |             |       |        |
|            | A010   |                                    |          |              |             |          |             |                       |                          |                    |          |            |             |             |       |        |
|            | A012   |                                    |          |              |             |          |             |                       |                          |                    |          |            |             |             |       |        |
|            | A013   |                                    |          |              |             |          |             |                       |                          |                    |          |            |             |             |       |        |
|            | A014   | < 2.000                            | < 2.000  | < 2.000      |             |          |             |                       |                          |                    |          |            |             |             |       |        |
|            | A015   |                                    |          |              |             |          |             |                       |                          |                    |          |            |             |             |       |        |
| ılts       | A019   |                                    |          |              |             |          |             |                       |                          |                    |          |            |             |             |       |        |
| test       | A024   | 0.669                              | 0.668    | 0.667        | 0.668       | 0.0010   | 0.225       | 0.223                 | 0.220                    | 0.223              | 0.0025   | 0.178      | 0.174       | 0.172       | 0.175 | 0.0031 |
| 1 B        | A028   |                                    |          |              |             |          |             |                       |                          |                    |          |            |             |             |       |        |
| duź        | A040   |                                    |          |              |             |          |             |                       |                          |                    |          |            |             |             |       |        |
| livi       | A047   |                                    |          |              |             |          |             |                       |                          |                    |          |            |             |             |       |        |
| Inc        | A049   |                                    |          |              |             |          |             |                       |                          |                    |          |            |             |             |       |        |
|            | A050   |                                    |          |              |             |          |             |                       |                          |                    |          |            |             |             |       |        |
|            | A065   |                                    |          |              |             |          |             |                       |                          |                    |          |            |             |             |       |        |
|            | A070   | 0.735                              | 0.735    | 0.675        | 0.715       | 0.035    | 0.745       | 0.695                 | 0.745                    | 0.728              | 0.029    | 1.375      | 1.315       | 1.285       | 1.325 | 0.046  |
|            | A071   |                                    |          |              |             |          |             |                       |                          |                    |          |            |             |             |       |        |
|            | A074   | 0.761                              | 0.753    | 0.781        | 0.765       | 0.014    |             |                       |                          |                    |          |            |             |             |       |        |
|            | A078   |                                    |          |              |             |          |             |                       |                          |                    |          |            |             |             |       |        |
|            | A079   |                                    |          |              |             |          |             |                       |                          |                    |          |            |             |             |       |        |
|            | A083   |                                    |          |              |             |          |             |                       |                          |                    |          |            |             |             |       |        |
| ţy         |        | Consensus Mean 0.716               |          |              | 0.716       |          | Consensu    | is Mean               |                          | 0.475              |          | Consensu   | s Mean      |             | 0.75  |        |
| uni<br>Its |        | Consensus Standard Deviation 0.074 |          | 0.074        |             | Consensu | is Standard | l Deviation           | 1.1                      |                    | Consensu | s Standard | l Deviation | 2.5         |       |        |
| un sa      |        | Maximum                            |          |              | 0.765       |          | Maximum     | 1                     |                          | 0.728              |          | Maximum    | ı           |             | 1.325 |        |
| E Z        |        | Minimum                            |          |              | 0.668       |          | Minimum     |                       |                          | 0.223              |          | Minimum    |             |             | 0.175 |        |
| 5          |        | Ν                                  |          |              | 3           |          | Ν           |                       |                          | 2                  |          | Ν          |             |             | 2     |        |

|            |              |           |            |              |             |          | Free a   | lpha-Lino          | lenic Acid               | l (C18:3 n            | -3)     |          |            |             |       |        |
|------------|--------------|-----------|------------|--------------|-------------|----------|----------|--------------------|--------------------------|-----------------------|---------|----------|------------|-------------|-------|--------|
|            |              |           | Fatty Ac   | cids Solutio | on A (mg/g) | )        | SRM 3    | 3275-II O<br>Acids | mega-3 an<br>in Fish Oil | nd Omega-<br>l (mg/g) | 6 Fatty |          | Cod        | Liver Oil ( | mg/g) |        |
|            | Lab          | Α         | В          | С            | Avg         | SD       | Α        | В                  | С                        | Avg                   | SD      | Α        | В          | С           | Avg   | SD     |
|            | Target       |           |            |              | 0.0140      | 0.0010   |          |                    |                          |                       |         |          |            |             |       |        |
|            | A004         |           |            |              |             |          |          |                    |                          |                       |         |          |            |             |       |        |
|            | A008         |           |            |              |             |          |          |                    |                          |                       |         |          |            |             |       |        |
|            | A010         |           |            |              |             |          |          |                    |                          |                       |         |          |            |             |       |        |
|            | A012         |           |            |              |             |          |          |                    |                          |                       |         |          |            |             |       |        |
|            | A013         |           |            |              |             |          |          |                    |                          |                       |         |          |            |             |       |        |
|            | A014         | < 2.000   | < 2.000    | < 2.000      |             |          |          |                    |                          |                       |         |          |            |             |       |        |
|            | A015         |           |            |              |             |          |          |                    |                          |                       |         |          |            |             |       |        |
| ılts       | A019         |           |            |              |             |          |          |                    |                          |                       |         |          |            |             |       |        |
| test       | A024         | 0.0109    | 0.0108     | 0.0108       | 0.0108      | 0.000058 | 0.268    | 0.263              | 0.259                    | 0.263                 | 0.0045  | 0.074    | 0.074      | 0.067       | 0.072 | 0.0040 |
| al R       | A028         |           |            |              |             |          |          |                    |                          |                       |         |          |            |             |       |        |
| qui        | A040         |           |            |              |             |          |          |                    |                          |                       |         |          |            |             |       |        |
| livi       | A047         |           |            |              |             |          |          |                    |                          |                       |         |          |            |             |       |        |
| Ind        | A049         |           |            |              |             |          |          |                    |                          |                       |         |          |            |             |       |        |
|            | A050         |           |            |              |             |          |          |                    |                          |                       |         |          |            |             |       |        |
|            | A065         |           |            |              |             |          |          |                    |                          |                       |         |          |            |             |       |        |
|            | A070         | 0.000     | 0.000      | 0.000        | 0.000       | 0.000    | 0.44     | 0.39               | 0.44                     | 0.42                  | 0.029   | 0.615    | 0.585      | 0.575       | 0.592 | 0.021  |
|            | A071         |           |            |              |             |          |          |                    |                          |                       |         |          |            |             |       |        |
|            | A074         |           |            |              |             |          |          |                    |                          |                       |         |          |            |             |       |        |
|            | A074<br>A078 |           |            |              |             |          |          |                    |                          |                       |         |          |            |             |       |        |
|            | A079         |           |            |              |             |          |          |                    |                          |                       |         |          |            |             |       |        |
|            | A083         |           |            |              |             |          |          |                    |                          |                       |         |          |            |             |       |        |
| ty.        |              | Consensus | s Mean     |              | 0.005       |          | Consensu | s Mean             |                          | 0.343                 |         | Consensu | is Mean    |             | 0.332 |        |
| uni<br>lts |              | Consensus | s Standard | Deviation    | 0.018       |          | Consensu | s Standard         | l Deviation              | 0.29                  |         | Consensu | s Standard | l Deviation | 1.125 |        |
| nnu        |              | Maximum   |            |              | 0.0108      |          | Maximum  | ı                  |                          | 0.42                  |         | Maximun  | 1          |             | 0.592 |        |
| R.         |              | Minimum   |            |              | 0.000       |          | Minimum  |                    |                          | 0.263                 |         | Minimum  |            |             | 0.072 |        |
| )          |              | Ν         |            |              | 2           |          | Ν        |                    |                          | 2                     |         | Ν        |            |             | 2     |        |

**Table 5-17.** Data summary table for free  $\alpha$ -linolenic acid in a fatty acids solution, fish oil, and cod liver oil.

**Table 5-18.** Data summary table for free  $\gamma$ -linolenic acid in a fatty acids solution, fish oil, and cod liver oil.

|      | 1      |           |            |              |             |        | Free ga  | mma-Lin              | olenic Aci               | d (C18:3 r            | <b>1-6</b> ) |          |             |             |       |        |
|------|--------|-----------|------------|--------------|-------------|--------|----------|----------------------|--------------------------|-----------------------|--------------|----------|-------------|-------------|-------|--------|
|      |        |           | Fatty Ac   | cids Solutio | on A (mg/g) |        | SRM 3    | 275-II Or<br>Acids i | nega-3 an<br>in Fish Oil | nd Omega-<br>l (mg/g) | 6 Fatty      |          | Cod I       | Liver Oil ( | mg/g) |        |
|      | Lab    | Α         | В          | С            | Avg         | SD     | Α        | В                    | С                        | Avg                   | SD           | Α        | В           | С           | Avg   | SD     |
|      | Target |           |            |              |             |        |          |                      |                          |                       |              |          |             |             |       |        |
|      | A004   |           |            |              |             |        |          |                      |                          |                       |              |          |             |             |       |        |
|      | A008   |           |            |              |             |        |          |                      |                          |                       |              |          |             |             |       |        |
|      | A010   |           |            |              |             |        |          |                      |                          |                       |              |          |             |             |       |        |
|      | A012   |           |            |              |             |        |          |                      |                          |                       |              |          |             |             |       |        |
|      | A013   |           |            |              |             |        |          |                      |                          |                       |              |          |             |             |       |        |
|      | A014   | < 2.000   | < 2.000    | < 2.000      |             |        |          |                      |                          |                       |              |          |             |             |       |        |
|      | A015   |           |            |              |             |        |          |                      |                          |                       |              |          |             |             |       |        |
| ılts | A019   |           |            |              |             |        |          |                      |                          |                       |              |          |             |             |       |        |
| esu  | A024   | 0.0001    | 0.0001     | 0.0001       | 0.0001      | 0.0000 | 0.033    | 0.033                | 0.036                    | 0.034                 | 0.0017       | 0.009    | 0.010       | 0.013       | 0.011 | 0.0021 |
| IR   | A028   |           |            |              |             |        |          |                      |                          |                       |              |          |             |             |       |        |
| dua  | A040   |           |            |              |             |        |          |                      |                          |                       |              |          |             |             |       |        |
| livi | A047   |           |            |              |             |        |          |                      |                          |                       |              |          |             |             |       |        |
| Ind  | A049   |           |            |              |             |        |          |                      |                          |                       |              |          |             |             |       |        |
|      | A050   |           |            |              |             |        |          |                      |                          |                       |              |          |             |             |       |        |
|      | A065   |           |            |              |             |        |          |                      |                          |                       |              |          |             |             |       |        |
|      | A070   | 0.000     | 0.000      | 0.000        | 0.000       | 0.000  | 0.065    | 0.060                | 0.065                    | 0.063                 | 0.0029       | 0.055    | 0.045       | 0.055       | 0.052 | 0.0058 |
|      | A071   |           |            |              |             |        |          |                      |                          |                       |              |          |             |             |       |        |
|      | A074   |           |            |              |             |        |          |                      |                          |                       |              |          |             |             |       |        |
|      | A078   |           |            |              |             |        |          |                      |                          |                       |              |          |             |             |       |        |
|      | A079   |           |            |              |             |        |          |                      |                          |                       |              |          |             |             |       |        |
|      | A083   |           |            |              |             |        |          |                      |                          |                       |              |          |             |             |       |        |
| ty.  |        | Consensus | s Mean     |              | 0           |        | Consensu | s Mean               |                          | 0.049                 |              | Consensu | ıs Mean     |             | 0.031 |        |
| ts t |        | Consensus | s Standard | Deviation    | 0           |        | Consensu | s Standard           | l Deviation              | 0.06                  |              | Consensu | is Standard | Deviation   | 0.079 |        |
| lus  |        | Maximum   |            |              | 0.0001      |        | Maximum  | ı                    |                          | 0.063                 |              | Maximun  | 1           |             | 0.052 |        |
| R N  |        | Minimum   |            |              | 0.000       |        | Minimum  |                      |                          | 0.034                 |              | Minimum  |             |             | 0.011 |        |
| 0    |        | Ν         |            |              | 2           |        | Ν        |                      |                          | 2                     |              | Ν        |             |             | 2     |        |

|           |        |          |             |              |             |         | Free     | Arachido          | onic Acid (              | C20:4 n-6          | ,       |          |             |             |       |        |
|-----------|--------|----------|-------------|--------------|-------------|---------|----------|-------------------|--------------------------|--------------------|---------|----------|-------------|-------------|-------|--------|
|           |        |          | Fatty Ac    | cids Solutio | on A (mg/g) |         | SRM 3    | 275-II O<br>Acids | mega-3 an<br>in Fish Oil | d Omega-<br>(mg/g) | 6 Fatty |          | Cod         | Liver Oil ( | mg/g) |        |
|           | Lab    | Α        | В           | С            | Avg         | SD      | Α        | В                 | С                        | Avg                | SD      | Α        | В           | С           | Avg   | SD     |
|           | Target |          |             |              | 0.282       | 0.014   |          |                   |                          |                    |         |          |             |             |       |        |
|           | A004   |          |             |              |             |         |          |                   |                          |                    |         |          |             |             |       |        |
|           | A008   |          |             |              |             |         |          |                   |                          |                    |         |          |             |             |       |        |
|           | A010   |          |             |              |             |         |          |                   |                          |                    |         |          |             |             |       |        |
|           | A012   |          |             |              |             |         |          |                   |                          |                    |         |          |             |             |       |        |
|           | A013   |          |             |              |             |         |          |                   |                          |                    |         |          |             |             |       |        |
|           | A014   | < 2.000  | < 2.000     | < 2.000      |             |         |          |                   |                          |                    |         |          |             |             |       |        |
| ts        | A015   |          |             |              |             |         |          |                   |                          |                    |         |          |             |             |       |        |
| lus       | A019   |          |             |              |             |         |          |                   |                          |                    |         |          |             |             |       |        |
| Re        | A024   | 0.235    | 0.235       | 0.235        | 0.235       | 0.00032 | 1.888    | 1.872             | 1.857                    | 1.872              | 0.016   | 0.012    | 0.017       | 0.020       | 0.016 | 0.0040 |
| ual       | A028   |          |             |              |             |         |          |                   |                          |                    |         |          |             |             |       |        |
| vid       | A040   |          |             |              |             |         |          |                   |                          |                    |         |          |             |             |       |        |
| ibu       | A047   |          |             |              |             |         |          |                   |                          |                    |         |          |             |             |       |        |
| I         | A049   |          |             |              |             |         |          |                   |                          |                    |         |          |             |             |       |        |
|           | A065   |          |             |              |             |         |          |                   |                          |                    |         |          |             |             |       |        |
|           | A070   | 0.27     | 0.265       | 0.245        | 0.260       | 0.013   | 7.345    | 6.85              | 7.355                    | 7.183              | 0.29    | 0.195    | 0.175       | 0.185       | 0.185 | 0.010  |
|           | A071   |          |             |              |             |         |          |                   |                          |                    |         |          |             |             |       |        |
|           | A074   | 0.263    | 0.258       | 0.271        | 0.264       | 0.0066  |          |                   |                          |                    |         |          |             |             |       |        |
|           | A078   |          |             |              |             |         |          |                   |                          |                    |         |          |             |             |       |        |
|           | A079   |          |             |              |             |         |          |                   |                          |                    |         |          |             |             |       |        |
|           | A083   |          |             |              |             |         |          |                   |                          |                    |         |          |             |             |       |        |
| ty.       |        | Consensu | is Mean     |              | 0.253       |         | Consensu | s Mean            |                          | 4.53               |         | Consensu | ıs Mean     |             | 0.101 |        |
| tts mi    |        | Consensu | is Standard | Deviation    | 0.022       |         | Consensu | s Standard        | d Deviation              | 11                 |         | Consensu | is Standard | Deviation   | 0.36  |        |
| um<br>esu |        | Maximum  | 1           |              | 0.264       |         | Maximum  | ı                 |                          | 7.183              |         | Maximun  | 1           |             | 0.185 |        |
| ē ž       |        | Minimum  |             |              | 0.235       |         | Minimum  |                   |                          | 1.872              |         | Minimum  |             |             | 0.016 |        |
| <b>~</b>  |        | Ν        |             |              | 3           |         | Ν        |                   |                          | 2                  |         | Ν        |             |             | 2     |        |

**Table 5-19.** Data summary table for free arachidonic acid in a fatty acids solution, fish oil, and cod liver oil.

**Table 5-20.** Data summary table for free EPA in a fatty acids solution, fish oil, and cod liver oil. Data points highlighted in red have been flagged as potential outliers (e.g., difference from reference value, Grubb and/or Cochran) by the NIST software package.

|               |        | Free EPA (C20:5 n-3)          |         |         |        |         |                              |   |         |         |       |                              |       |       |       |       |  |
|---------------|--------|-------------------------------|---------|---------|--------|---------|------------------------------|---|---------|---------|-------|------------------------------|-------|-------|-------|-------|--|
|               |        | Fatty Acids Solution A (mg/g) |         |         |        |         |                              | SRM 3275-II Omega-3 and Omega-6 Fatty<br>Acids in Fish Oil (mg/g) |         |         |       | Cod Liver Oil (mg/g)         |       |       |       |       |  |
|               | Lab    | Α                             | В       | С       | Avg    | SD      | Α                            | В   | С       | Avg     | SD    | Α                            | В     | С     | Avg   | SD    |  |
|               | Target |                               |         |         | 0.0130 | 0.0010  |                              |   |         |         |       |                              |       |       |       |       |  |
|               | A004   |                               |         |         |        |         |                              |   |         |         |       |                              |       |       |       |       |  |
|               | A008   |                               |         |         |        |         |                              |   |         |         |       |                              |       |       |       |       |  |
|               | A010   |                               |         |         |        |         |                              |   |         |         |       |                              |       |       |       |       |  |
|               | A012   |                               |         |         |        |         |                              |   |         |         |       |                              |       |       |       |       |  |
|               | A013   |                               |         |         |        |         |                              |   |         |         |       |                              |       |       |       |       |  |
| ults          | A014   | < 2.000                       | < 2.000 | < 2.000 |        |         |                              |   |         |         |       |                              |       |       |       |       |  |
|               | A015   |                               |         |         |        |         |                              |   |         |         |       |                              |       |       |       |       |  |
|               | A019   |                               |         |         |        |         | 446.7                        | 454.2   | 455.9   | 452.3   | 4.9   | 90.6                         | 86.6  | 92.1  | 89.8  | 2.8   |  |
|               | A024   | 0.0095                        | 0.0094  | 0.0093  | 0.0094 | 0.00010 | 32.439                       | 32.343  | 32.256  | 32.346  | 0.092 | 0.528                        | 0.551 | 0.547 | 0.542 | 0.012 |  |
| Res           | A028   |                               |         |         |        |         |                              |   |         |         |       |                              |       |       |       |       |  |
| al I          | A040   |                               |         |         |        |         |                              |   |         |         |       |                              |       |       |       |       |  |
| idu           | A047   |                               |         |         |        |         |                              |   |         |         |       |                              |       |       |       |       |  |
| div           | A049   |                               |         |         |        |         |                              |   |         |         |       |                              |       |       |       |       |  |
| Ч             | A050   |                               |         |         |        |         |                              |   |         |         |       |                              |       |       |       |       |  |
|               | A051   |                               |         |         |        |         |                              |   |         |         |       |                              |       |       |       |       |  |
|               | A065   |                               |         |         |        |         |                              |   |         |         |       |                              |       |       |       |       |  |
|               | A070   | 0.000                         | 0.000   | 0.000   | 0.000  | 0.000   | 131.720                      | 124.045   | 131.710 | 129.158 | 4.4   | 5.675                        | 5.545 | 5.290 | 5.503 | 0.20  |  |
|               | A071   |                               |         |         |        |         |                              |   |         |         |       |                              |       |       |       |       |  |
|               | A074   |                               |         |         |        |         |                              |   |         |         |       |                              |       |       |       |       |  |
|               | A078   |                               |         |         |        |         |                              |   |         |         |       |                              |       |       |       |       |  |
|               | A079   |                               |         |         |        |         |                              |   |         |         |       |                              |       |       |       |       |  |
|               | A082   |                               |         |         |        |         |                              |   |         |         |       |                              |       |       |       |       |  |
|               | A083   |                               |         |         |        |         |                              |   |         |         |       |                              |       |       |       |       |  |
| unity<br>Ilts |        | Consensus                     | s Mean  |         | 0.005  |         | Consensu                     | s Mean  |         | 205     |       | Consensus Mean               |       |       | 3.02  |       |  |
|               |        | Consensus Standard Deviation  |         |         | 0.021  |         | Consensus Standard Deviation |   |         | 221     |       | Consensus Standard Deviation |       |       | 11    |       |  |
| lest          |        | Maximum                       |         |         | 0.0094 |         | Maximum                      | 1   |         | 452.3   |       | Maximun                      | 1     |       | 89.8  |       |  |
| °° C          |        | Minimum                       |         |         | 0.000  |         | Minimum                      |   |         | 32.346  |       | Minimum                      |       |       | 0.542 |       |  |
|               |        | Ń                             |         |         | 2      |         | N                            |   |         | 3       |       | N                            |       |       | 3     |       |  |

|             |        | Free DPA (C22:5 n-3) |            |             |             |          |   |             |           |        |                      |                              |       |       |       |        |
|-------------|--------|----------------------|------------|-------------|-------------|----------|---|-------------|-----------|--------|----------------------|------------------------------|-------|-------|-------|--------|
|             |        |                      | Fatty Ac   | ids Solutic | on A (mg/g) | )        | SRM 3275-II Omega-3 and Omega-6 Fatty<br>Acids in Fish Oil (mg/g) |             |           |        | Cod Liver Oil (mg/g) |                              |       |       |       |        |
|             | Lab    | Α                    | В          | С           | Avg         | SD       | Α   | В           | С         | Avg    | SD                   | Α                            | В     | С     | Avg   | SD     |
|             | Target | í                    |            |             | 0.0150      | 0.0010   |   |             |           |        |                      |                              |       |       |       |        |
|             | A004   |                      |            |             |             |          |   |             |           |        |                      |                              |       |       |       |        |
|             | A008   | 1                    |            |             | 1           |          |   |             |           |        |                      |                              |       |       |       |        |
|             | A010   |                      |            |             |             |          |   |             |           |        |                      |                              |       |       |       |        |
|             | A012   | 1                    |            |             | 1           |          |   |             |           |        |                      |                              |       |       |       |        |
|             | A013   |                      |            |             |             |          |   |             |           |        |                      |                              |       |       |       |        |
|             | A014   | < 2.000              | < 2.000    | < 2.000     | 1           |          |   |             |           |        |                      |                              |       |       |       |        |
|             | A015   |                      |            |             |             |          |   |             |           |        |                      |                              |       |       |       |        |
| ults        | A019   | 1                    |            |             | 1           |          |   |             |           |        |                      |                              |       |       |       |        |
| Kesı        | A024   | 0.0115               | 0.0115     | 0.0114      | 0.0115      | 0.000058 | 6.074   | 5.946       | 5.984     | 6.001  | 0.066                | 0.069                        | 0.067 | 0.068 | 0.068 | 0.0010 |
| alF         | A028   |                      |            |             |             |          |   |             |           |        |                      |                              |       |       |       |        |
| qui         | A040   |                      |            |             |             |          |   |             |           |        |                      |                              |       |       |       |        |
| livi        | A047   | 1                    |            |             | 1           |          |   |             |           |        |                      |                              |       |       |       |        |
| Inc         | A049   |                      |            |             |             |          |   |             |           |        |                      |                              |       |       |       |        |
|             | A050   | 1                    |            |             | 1           |          |   |             |           |        |                      |                              |       |       |       |        |
|             | A065   |                      |            |             |             |          |   |             |           |        |                      |                              |       |       |       |        |
|             | A070   | 0.000                | 0.000      | 0.000       | 0.000       | 0.000    | 21.040  | 19.730      | 21.085    | 20.618 | 0.77                 | 0.620                        | 0.595 | 0.580 | 0.598 | 0.020  |
|             | A071   |                      |            |             |             |          |   |             |           |        |                      |                              |       |       |       |        |
|             | A074   | 1                    |            |             | 1           |          |   |             |           |        |                      |                              |       |       |       |        |
|             | A078   |                      |            |             |             |          |   |             |           |        |                      |                              |       |       |       |        |
|             | A079   | 1                    |            |             | 1           |          |   |             |           |        |                      |                              |       |       |       |        |
|             | A083   |                      |            |             |             |          |   |             |           |        |                      |                              |       |       |       |        |
| ty.         |        | Consensur            | s Mean     |             | 0.006       |          | Consensu  | ıs Mean     |           | 13.3   |                      | Consensus Mean               |       |       | 0.333 |        |
| unit<br>Its | 1      | Consensur            | s Standard | Deviation   | 0.025       | ļ        | Consensu  | is Standard | Deviation | n 31   |                      | Consensus Standard Deviation |       |       | 1.1   |        |
| Inte        | 1      | Maximum              | ı          |             | 0.0115      | ļ        | Maximur   | a           |           | 20.618 |                      | Maximum                      |       |       | 0.598 |        |
| N N         | 1      | Minimum              |            |             | 0.000       | ļ        | Minimum   | i           |           | 6.001  | I                    | Minimum                      | i -   |       | 0.068 |        |
| 0           | 1 1    | Ν                    |            |             | 2           | ŀ        | Ν   |             |           | 2      |                      | Ν                            |       |       | 2     |        |

Table 5-21. Data summary table for free DPA in a fatty acids solution, fish oil, and cod liver oil.

**Table 5-22.** Data summary table for free DHA in a fatty acids solution, fish oil, and cod liver oil. Data points highlighted in red have been flagged as potential outliers (e.g., difference from reference value, Grubb and/or Cochran) by the NIST software package.

|            |        | Free DHA (C22:6 n-3)          |            |           |        |         |                              |   |        |        |         |                              |       |       |       |       |  |
|------------|--------|-------------------------------|------------|-----------|--------|---------|------------------------------|---|--------|--------|---------|------------------------------|-------|-------|-------|-------|--|
|            |        | Fatty Acids Solution A (mg/g) |            |           |        |         |                              | SRM 3275-II Omega-3 and Omega-6 Fatty<br>Acids in Fish Oil (mg/g) |        |        |         | Cod Liver Oil (mg/g)         |       |       |       |       |  |
|            | Lab    | Α                             | В          | С         | Avg    | SD      | Α                            | В   | С      | Avg    | SD      | Α                            | В     | С     | Avg   | SD    |  |
|            | Target |                               |            |           | 0.0370 | 0.0020  |                              |   |        |        |         |                              |       |       |       |       |  |
|            | A004   |                               |            |           |        |         |                              |   |        |        |         |                              |       |       |       |       |  |
|            | A008   |                               |            |           |        |         |                              |   |        |        |         |                              |       |       |       |       |  |
|            | A010   |                               |            |           |        |         |                              |   |        |        |         |                              |       |       |       |       |  |
|            | A012   |                               |            |           |        |         |                              |   |        |        |         |                              |       |       |       |       |  |
|            | A013   |                               |            |           |        |         |                              |   |        |        |         |                              |       |       |       |       |  |
|            | A014   | < 2.000                       | < 2.000    | < 2.000   |        |         |                              |   |        |        |         |                              |       |       |       |       |  |
|            | A015   |                               |            |           |        |         |                              |   |        |        |         |                              |       |       |       |       |  |
| ılts       | A019   |                               |            |           |        |         | 174.1                        | 176.7   | 177.3  | 176.0  | 1.7     | 109.7                        | 105.6 | 111.3 | 108.9 | 2.9   |  |
|            | A024   | 0.0288                        | 0.0287     | 0.0285    | 0.0287 | 0.00015 | 15.747                       | 15.672  | 15.593 | 15.671 | 0.077   | 0.587                        | 0.587 | 0.567 | 0.580 | 0.012 |  |
| fesi       | A028   |                               |            |           |        |         |                              |   |        |        |         |                              |       |       |       |       |  |
| al E       | A040   |                               |            |           |        |         |                              |   |        |        |         |                              |       |       |       |       |  |
| np         | A047   |                               |            |           |        |         |                              |   |        |        |         |                              |       |       |       |       |  |
| livi       | A049   |                               |            |           |        |         |                              |   |        |        |         |                              |       |       |       |       |  |
| Γ          | A050   |                               |            |           |        |         |                              |   |        |        |         |                              |       |       |       |       |  |
|            | A051   |                               |            |           |        |         |                              |   |        |        |         |                              |       |       |       |       |  |
|            | A065   |                               |            |           |        |         |                              |   |        |        |         |                              |       |       |       |       |  |
|            | A070   | 0.028                         | 0.02       | 0.025     | 0.024  | 0.0040  | 60.715                       | 56.27   | 61.125 | 59.37  | 2.69249 | 6.41                         | 6.41  | 6.02  | 6.28  | 0.23  |  |
|            | A071   |                               |            |           |        |         |                              |   |        |        |         |                              |       |       |       |       |  |
|            | A074   |                               |            |           |        |         |                              |   |        |        |         |                              |       |       |       |       |  |
|            | A078   |                               |            |           |        |         |                              |   |        |        |         |                              |       |       |       |       |  |
|            | A079   |                               |            |           |        |         |                              |   |        |        |         |                              |       |       |       |       |  |
|            | A082   |                               |            |           |        |         |                              |   |        |        |         |                              |       |       |       |       |  |
|            | A083   |                               |            |           |        |         |                              |   |        |        |         |                              |       |       |       |       |  |
| ty         |        | Consensus Mean                |            |           | 0.027  |         | Consensus Mean               |   |        | 83.7   |         | Consensus Mean               |       |       | 3.43  |       |  |
| uni<br>Its |        | Consensus                     | s Standard | Deviation | 0.002  |         | Consensus Standard Deviation |   |        | 101    |         | Consensus Standard Deviation |       |       | 13    |       |  |
| esu        |        | Maximum                       |            |           | 0.0287 |         | Maximum                      | ı   |        | 176.0  |         | Maximum                      |       |       | 109   |       |  |
| R G        |        | Minimum                       |            |           | 0.024  |         | Minimum                      |   |        | 15.671 |         | Minimum                      |       |       | 0.580 |       |  |
| J          |        | Ν                             |            |           | 2      |         | Ν                            |   |        | 3      |         | Ν                            |       |       | 3     |       |  |

## Human Metabolites Sample Information

Fatty Acids Solution B. Participants were provided with one amber glass ampoule containing 1.2 mL of a toluene solution containing mixed free fatty acids. Before use, participants were instructed to invert the ampoule to mix the solution and withdraw subsamples at 20 °C to 25 °C immediately after opening the ampoule. Participants were asked to store the solution in the dark at temperatures between -20 °C and -80 °C, to process the samples without delay, and to prepare three samples and report three values from the single ampoule provided. The approximate analyte levels were not reported to the participants prior to the study. The target values for the fatty acids in Solution B were determined by gravimetric preparation using neat fatty acid standards with an uncertainty approximated as 5 % of the mass fraction. Purity of the neat fatty acids used to prepare Fatty Acids Solution B was not determined. The NIST-determined mass fractions and uncertainties for free stearic acid, free cis-vaccenic acid, free oleic acid, free linoleic acid, free α-linolenic acid, free arachidonic acid, free EPA, free DPA, and free DHA in Fatty Acids Solution B are provided in the table below. Free  $\gamma$ -linolenic acid was not added to Fatty Acids Solution B. Since only free fatty acids were used in the solution preparation, the target values for total and free fatty acids are the same (i.e., a method to determine total fatty acids should get the same value as for a method to determine free fatty acids in this solution).

|                               | NIST-Determined Mass Fractic |     |                     |  |  |  |  |
|-------------------------------|------------------------------|-----|---------------------|--|--|--|--|
| Analyte (Total and Free)      | Fatty Acids Solu             | tio | <u>n B (µmol/L)</u> |  |  |  |  |
| Stearic Acid (C18:0)          | 550                          | ±   | 28                  |  |  |  |  |
| cis-Vaccenic Acid (C18:1 n-7) | 115                          | ±   | 6                   |  |  |  |  |
| Oleic Acid (C18:1 n-9)        | 1318                         | ±   | 66                  |  |  |  |  |
| Linoleic Acid (C18:2 n-6)     | 2488                         | ±   | 124                 |  |  |  |  |
| α-Linolenic Acid (C18:3 n-3)  | 44.7                         | ±   | 2.2                 |  |  |  |  |
| Arachidonic Acid (C20:4 n-6)  | 806                          | ±   | 40                  |  |  |  |  |
| EPA (C20:5 n-3)               | 36.8                         | ±   | 1.8                 |  |  |  |  |
| DPA (C22:5 n-3)               | 38.2                         | ±   | 1.9                 |  |  |  |  |
| DHA (C22:6 n-3)               | 98.8                         | ±   | 4.9                 |  |  |  |  |

*Human Plasma A.* Participants were provided with three ampoules, each containing 1 mL of frozen plasma. The material was prepared from normal human source plasma from individuals that had undergone an overnight fast. The plasma was pooled from an equal number of male and female donors, age 40 to 50 years, and reflecting the racial distribution of the United States population. Lithium heparin was added to the serum as an anticoagulant and the material was stored at -80 °C. Before use, participants were instructed to allow the material to thaw at room temperature for at least 30 min prior to sampling, use the material immediately after thawing, gently mix the contents prior to removal of a test portion for analysis, and to use a sample size appropriate for their usual in-house method of analysis. Participants were asked to avoid exposing the material to direct UV light, to store the material at or below -80 °C, and to prepare one sample and report one value from each vial provided. The approximate analyte levels were not reported to participants prior to the study. The certified values for total stearic acid, total oleic acid, and total  $\alpha$ -linolenic acid in SRM 1950 were determined at NIST using gas chromatography with flame ionization detection (GC-FID) and gas chromatography with mass spectrometry detection
(GC-MS), and results from the Centers for Disease Control and Prevention (CDC) using GC-MS. The certified value for total linoleic acid in SRM 1950 was determined at NIST using GC-FID and results from the CDC using GC-MS. The reference values for total  $\gamma$ -linolenic acid, total arachidonic acid, and total DHA in SRM 1950 were determined at NIST using GC-FID and GC-MS, and results from the CDC using GC-MS. The reference values for total cis-vaccenic acid, total EPA, and total DPA in SRM 1950 were determined at the CDC using GC-MS. The NIST-determined values and uncertainties for total stearic acid, total cis-vaccenic acid, total oleic acid, total linoleic acid, total  $\alpha$ -linolenic acid, total  $\gamma$ -linolenic acid, total arachidonic acid, total EPA, total DPA in SRM 1950 are provided in the table below.

| Analyta                             | NIST-Determined Mass Fraction |
|-------------------------------------|-------------------------------|
| Allaryte                            | <u>in SRM 1950 (µmol/L)</u>   |
| Total Stearic Acid (C18:0)          | $644 \pm 41$                  |
| Total cis-Vaccenic Acid (C18:1 n-7) | $136 \pm 3$                   |
| Total Oleic Acid (C18:1 n-9)        | $1614  \pm \ 154$             |
| Total Linoleic Acid (C18:2 n-6)     | $2838  \pm \ 143$             |
| Total α-Linolenic Acid (C18:3 n-3)  | $54.6 \pm 3.6$                |
| Total γ-Linolenic Acid (C18:3 n-3)  | $39.9 \pm 4.3$                |
| Total Arachidonic Acid (C20:4 n-6)  | $984  \pm \ 180$              |
| Total EPA (C20:5 n-3)               | $38.6 \pm 0.5$                |
| Total DPA (C22:5 n-3)               | $38.5 \pm 0.7$                |
| Total DHA (C22:6 n-3)               | $118 \pm 21$                  |

*Human Plasma B.* Participants were provided with three ampoules, each containing 1 mL of frozen plasma. Before use, participants were instructed to allow the material to thaw at room temperature for at least 30 min prior to sampling, use the material immediately after thawing, gently mix the contents prior to removal of a test portion for analysis, and to use a sample size appropriate for their usual in-house method of analysis. Participants were asked to avoid exposing the material to direct UV light, to store the material at or below -80 °C, and to prepare one sample and report one value from each vial provided. The approximate analyte levels were not reported to participants prior to the study, and target values for free or total fatty acids in Human Serum B have not been determined at NIST.

# Human Metabolites Study Results

• Fifteen to eighteen laboratories enrolled in this exercise to measure total fatty acids. The table below lists the participation statistics for total fatty acids in the dietary intake samples.

|                               | Number of           | Number of L | of Laboratories Reporting |                 |  |  |  |  |
|-------------------------------|---------------------|-------------|---------------------------|-----------------|--|--|--|--|
|                               | <b>Laboratories</b> | <u>(Pe</u>  | ercent Participatio       | <u>on)</u>      |  |  |  |  |
|                               | <u>Requesting</u>   | Fatty Acids |                           | <u>Human</u>    |  |  |  |  |
| Analyte (Total)               | <u>Samples</u>      | Solution B  | <u>SRM 1950</u>           | <u>Plasma B</u> |  |  |  |  |
| Stearic Acid (C18:0)          | 18                  | 9 (50 %)    | 9 (50 %)                  | 9 (50 %)        |  |  |  |  |
| cis-Vaccenic Acid (C18:1 n-7) | 15                  | 6 (40 %)    | 6 (40 %)                  | 6 (40 %)        |  |  |  |  |
| Oleic Acid (C18:1 n-9)        | 18                  | 9 (50 %)    | 9 (50 %)                  | 9 (50 %)        |  |  |  |  |
| Linoleic Acid (C18:2 n-6)     | 18                  | 9 (50 %)    | 9 (50 %)                  | 9 (50 %)        |  |  |  |  |
| α-Linolenic Acid (C18:3 n-3)  | 18                  | 9 (50 %)    | 9 (50 %)                  | 9 (50 %)        |  |  |  |  |
| γ-Linolenic Acid (C18:3 n-3)  | 18                  | 6 (33 %)    | 8 (44 %)                  | 8 (44 %)        |  |  |  |  |
| Arachidonic Acid (C20:4 n-6)  | 17                  | 9 (53 %)    | 9 (53 %)                  | 9 (53 %)        |  |  |  |  |
| EPA (C20:5 n-3)               | 18                  | 9 (50 %)    | 9 (50 %)                  | 9 (50 %)        |  |  |  |  |
| DPA (C22:5 n-3)               | 17                  | 7 (41 %)    | 7 (41 %)                  | 7 (41 %)        |  |  |  |  |
| DHA (C22:6 n-3)               | 18                  | 9 (50 %)    | 9 (50 %)                  | 9 (50 %)        |  |  |  |  |

The consensus means for most of the fatty acids in Fatty Acids Solution B were below the target ranges and the consensus ranges overlapped with the target ranges (see table below). The exceptions included total cis-vaccenic acid and total DHA, with consensus values within the target ranges. The between-laboratory variability is also summarized below and ranged from good for total cis-vaccenic acid (15%) and total DHA (12%) to high for total α-linolenic acid and total EPA (42% and 43%, respectively). For most fatty acids in this sample, the between-laboratory variability was under 30%.

|                               | Performance Summary for Fatty Acids Solu  | ution B            |
|-------------------------------|---|--------------------|
|                               |   | Between-           |
|                               | Relative Position of Consensus  | Laboratory         |
| Analyte                       | and Target Ranges   | <u>Variability</u> |
| Stearic Acid (C18:0)          | Consensus mean <i>below</i> target range<br>Consensus range <i>slightly overlaps</i> target range | 27 %               |
| cis-Vaccenic Acid (C18:1 n-7) | Consensus mean <i>within</i> target range<br>Consensus range <i>overlaps</i> target range         | 15 %               |
| Oleic Acid (C18:1 n-9)        | Consensus mean <i>below</i> target range<br>Consensus range <i>slightly overlaps</i> target range | 30 %               |
| Linoleic Acid (C18:2 n-6)     | Consensus mean <i>below</i> target range<br>Consensus range <i>slightly overlaps</i> target range | 21 %               |
| α-Linolenic Acid (C18:3 n-3)  | Consensus mean <i>below</i> target range<br>Consensus range <i>slightly overlaps</i> target range | 42 %               |
| Arachidonic Acid (C20:4 n-6)  | Consensus mean <i>slightly below</i> target<br>Consensus range <i>overlaps</i> target range       | 29 %               |
| EPA (C20:5 n-3)               | Consensus mean <i>below</i> target range<br>Consensus range <i>overlaps</i> target range          | 43 %               |
| DPA (C22:5 n-3)               | Consensus mean <i>below</i> target range<br>Consensus range <i>overlaps with</i> target range     | 35 %               |
| DHA (C22:6 n-3)               | Consensus mean <i>within</i> target range<br>Consensus range <i>overlaps</i> target range         | 12 %               |

• The consensus means for total linoleic acid, total  $\gamma$ -linolenic acid, and total DHA in SRM 1950 were within the target ranges and the consensus ranges overlapped with the target ranges (see table below). The consensus means for total stearic acid, total cis-vaccenic acid, total  $\alpha$ -linolenic acid, total arachidonic acid, and total DPA were below the target ranges. The consensus means for total oleic acid and total EPA were above the target ranges. The between-laboratory variability is also summarized below and ranged from good for total oleic acid (13 %), total DHA (14 %), and total linoleic acid (16 %) to poor for total cis-vaccenic acid (93 %). The between-laboratory variability for most fatty acids ranged from 26 % to 38 %.

|                               | Performance Summary for SRM 1950  | 1                  |
|-------------------------------|---|--------------------|
|                               |   | Between-           |
|                               | Relative Position of Consensus  | Laboratory         |
| Analyte (Total)               | and Target Ranges   | <u>Variability</u> |
| Stearic Acid (C18:0)          | Consensus mean <i>below</i> target range<br>Consensus range <i>overlaps</i> target range                    | 38 %               |
| cis-Vaccenic Acid (C18:1 n-7) | Consensus mean <i>slightly below</i> target range<br>Consensus range <i>overlaps</i> target range           | 93 %               |
| Oleic Acid (C18:1 n-9)        | Consensus mean <i>slightly above</i> target range<br>Consensus range <i>partially overlaps</i> target range | 13 %               |
| Linoleic Acid (C18:2 n-6)     | Consensus mean <i>within</i> target range<br>Consensus range <i>overlaps</i> target range                   | 16 %               |
| α-Linolenic Acid (C18:3 n-3)  | Consensus mean <i>below</i> target range<br>Consensus range slightly <i>overlaps</i> target range           | 31 %               |
| γ-Linolenic Acid (C18:3 n-3)  | Consensus mean <i>within</i> target range<br>Consensus range <i>overlaps</i> target range                   | 33 %               |
| Arachidonic Acid (C20:4 n-6)  | Consensus mean <i>below</i> target<br>Consensus range <i>slightly overlaps</i> target range                 | 18 %               |
| EPA (C20:5 n-3)               | Consensus mean <i>slightly above</i> target range<br>Consensus range <i>overlaps</i> target range           | 38 %               |
| DPA (C22:5 n-3)               | Consensus mean <i>below</i> target range<br>Consensus range <i>overlaps with</i> target range               | 26 %               |
| DHA (C22:6 n-3)               | Consensus mean <i>within</i> target range<br>Consensus range <i>overlaps</i> target range                   | 14 %               |
|                               |   |                    |

• The between-laboratory variability for Human Plasma B is summarized below and was good for most fatty acids (less than 30 % RSD), with the exception of total EPA (38 %) and total cis-vaccenic acid (51 %).

|                               | Between-Laboratory Variability in |
|-------------------------------|-----------------------------------|
| Analyte (Total)               | <u>Human Plasma B</u>             |
| Stearic Acid (C18:0)          | 37 %                              |
| cis-Vaccenic Acid (C18:1 n-7) | 51 %                              |
| Oleic Acid (C18:1 n-9)        | 19 %                              |
| Linoleic Acid (C18:2 n-6)     | 20 %                              |
| α-Linolenic Acid (C18:3 n-3)  | 23 %                              |
| γ-Linolenic Acid (C18:3 n-3)  | 24 %                              |
| Arachidonic Acid (C20:4 n-6)  | 24 %                              |
| EPA (C20:5 n-3)               | 38 %                              |
| DPA (C22:5 n-3)               | 26 %                              |
| DHA (C22:6 n-3)               | 24 %                              |

- Up to four laboratories reported using GC-FID and five laboratories reported using GC-MS for determination of total fatty acids.
- Nine to eleven laboratories enrolled in this exercise to measure free fatty acids. The table below lists the participation statistics for free fatty acids.

|                               | Number of         | Number of La | aboratories Repo    | orting Results  |
|-------------------------------|-------------------|--------------|---------------------|-----------------|
|                               | Laboratories      | (Per         | rcent Participation | <u>on)</u>      |
|                               | <u>Requesting</u> | Fatty Acids  |                     | <u>Human</u>    |
| Analyte (Free)                | Samples 1         | Solution B   | <u>SRM 1950</u>     | <u>Plasma B</u> |
| Stearic Acid (C18:0)          | 11                | 1 (9 %)      | 1 (9 %)             | 1 (9 %)         |
| cis-Vaccenic Acid (C18:1 n-7) | 9                 | 1 (11 %)     | 1 (11 %)            | 1 (11 %)        |
| Oleic Acid (C18:1 n-9)        | 11                | 1 (9 %)      | 1 (9 %)             | 1 (9 %)         |
| Linoleic Acid (C18:2 n-6)     | 11                | 1 (9 %)      | 1 (9 %)             | 1 (9 %)         |
| α-Linolenic Acid (C18:3 n-3)  | 11                | 1 (9 %)      | 1 (9 %)             | 1 (9 %)         |
| γ-Linolenic Acid (C18:3 n-3)  | 11                | 1 (9 %)      | 1 (9 %)             | 1 (9 %)         |
| Arachidonic Acid (C20:4 n-6)  | 10                | 1 (10 %)     | 1 (10 %)            | 1 (10 %)        |
| EPA (C20:5 n-3)               | 11                | 1 (9 %)      | 1 (9 %)             | 1 (9 %)         |
| DPA (C22:5 n-3)               | 11                | 1 (9 %)      | 1 (9 %)             | 1 (9 %)         |
| DHA (C22:6 n-3)               | 11                | 1 (9 %)      | 1 (9 %)             | 1 (9 %)         |
|                               |                   |              |                     |                 |

• Only one laboratory (9 % to 11 % participation) reported results for free fatty acids. Due to the low participation rate, results for free fatty acids are only presented in table format in the following sections (no graphical representation).

## Human Metabolites Technical Recommendations

The following recommendations are based on results obtained from the participants in this study.

- The consensus means for most total fatty acids in Fatty Acids Solution B were below the NISTdetermined (gravimetric) values.
  - For three analytes (total  $\alpha$ -linolenic acid, total stearic acid, and total DPA), laboratories reported low values for both Fatty Acids Solution B and SRM 1950. Because this trend was not observed for all analytes in both materials, the low bias appears to be isolated to the fatty acids solution.
  - Two to three laboratories reported consistently lower results compared to the other laboratories and to the NIST target values. Use of a quality control material (CRM, SRM, RM or in-house material) can assist in verification of methods and results.
  - The target values for the fatty acids in Fatty Acids Solution B were determined based on the gravimetric preparation. The purity of the neat fatty acids used to prepare the solution was not evaluated; impurities in these neat materials may be the cause of the apparent low bias in the consensus values.
- The between-laboratory variability was only good (less than 20 % RSD) for two analytes in the solution (total cis-vaccenic acid and total DHA), for three analytes in SRM 1950 (total oleic acid, total linoleic acid, and total arachidonic acid), and one analyte for Human Plasma B (total oleic acid).
- All results should be checked closely to avoid calculation errors and to ensure that results are reported in the requested units.
- Despite the small number of laboratories reporting methods of analysis, one possible trend was observed. For SRM 1950, three of the four laboratories using GC-FID reported values that were higher than those from all laboratories using GC-MS for total stearic acid, total linoleic acid, total  $\alpha$ -linolenic acid, and total DHA. A similar trend was observed for total stearic acid and total linoleic acid results in Human Plasma B. Further studies will be required to confirm the significance of this trend.
- Generally, fewer participants reported results for free fatty acids in comparison to the total fatty acids, although the number of enrolled laboratories was similar.
  - Participating laboratories may not have analytical methods to measure free fatty acids, or the measurement of free fatty acids is not a current concern of the participating laboratories.
  - Future studies will focus on measurement of total fatty acids, unless a need for reporting of free fatty acids is indicated by participating laboratories.

## Table 5-23. Individualized data summary table (NIST) for total fatty acids in a solution and human plasma.

#### National Institute of Standards & Technology

|  | Lab Code:                            | NIST                         |              | 1. You       | r Results     |       | 2, C          | ommunitv F  | 3. Target              |             |       |
|--|--------------------------------------|------------------------------|--------------|--------------|---------------|-------|---------------|-------------|------------------------|-------------|-------|
| Analyte                                | Sample                               | Units                        | <i>x</i> ,   | <i>s</i> :   | Z'aamm        | ZNIST | N             | x*          | s*                     | X NIST      | U     |
| Total Stearic Acid (C18:0)             | Fatty Acids Solution B               | umol/L                       | 550          | 14           | comm          | 14151 | 10            | 460         | 120                    | 550         | 28    |
| Total Stearic Acid (C18:0)             | SRM 1950 Metabolites in Frozen Human | ,<br>µmol/L                  | 644          | 21           |               |       | 10            | 540         | 210                    | 644         | 4     |
|  | Plasma                               | •                            |              |              |               |       |               |             |                        |             |       |
| Total Stearic Acid (C18:0)             | Human Plasma B                       | µmol/L                       |              |              |               |       | 9             | 780         | 290                    |             |       |
| Total cis-Vaccenic Acid (C18:1 n-7)    | Fatty Acids Solution B               | µmol/L                       | 115          | 3            |               |       | 7             | 110         | 17                     | 115         | 6     |
| Total cis-Vaccenic Acid (C18:1 n-7)    | SRM 1950 Metabolites in Frozen Human | μmol/L                       | 136          | 1.5          |               |       | 7             | 130         | 120                    | 136         | 3     |
|  | Plasma                               |                              |              |              |               |       |               |             |                        |             |       |
| Total cis-Vaccenic Acid (C18:1 n-7)    | Human Plasma B                       | µmol/L                       |              |              |               |       | 6             | 180         | 92                     |             |       |
| Total Oleic Acid (C18:1 n-9)           | Fatty Acids Solution B               | µmol/L                       | 1320         | 33           |               |       | 10            | 1080        | 320                    | 1320        | 66    |
| Total Oleic Acid (C18:1 n-9)           | SRM 1950 Metabolites in Frozen Human | µmol/L                       | 1610         | 77           |               |       | 10            | 1780        | 230                    | 1610        | 15    |
|  | Plasma                               |                              |              |              |               |       |               |             |                        |             |       |
| Total Oleic Acid (C18:1 n-9)           | Human Plasma B                       | µmol/L                       |              |              |               |       | 9             | 3170        | 590                    |             |       |
| Total Linoleic Acid (C18:2 n-6)        | Fatty Acids Solution B               | µmol/L                       | 2490         | 62           |               |       | 10            | 2150        | 460                    | 2490        | 12    |
| Total Linoleic Acid (C18:2 n-6)        | SRM 1950 Metabolites in Frozen Human | µmol/L                       | 2840         | 72           |               |       | 10            | 2830        | 440                    | 2840        | 14    |
|  | Plasma                               |                              |              |              |               |       |               |             |                        |             |       |
| Total Linoleic Acid (C18:2 n-6)        | Human Plasma B                       | µmol/L                       |              |              |               |       | 9             | 3680        | 720                    |             |       |
| Total alpha-Linolenic Acid (C18:3 n-3) | Fatty Acids Solution B               | µmol/L                       | 44.7         | 1.1          |               |       | 10            | 40          | 15                     | 44.7        | 2.    |
| Total alpha-Linolenic Acid (C18:3 n-3) | SRM 1950 Metabolites in Frozen Human | µmol/L                       | 54.6         | 1.8          |               |       | 10            | 50          | 14                     | 54.6        | 3.    |
|  | Plasma                               |                              |              |              |               |       |               |             |                        |             |       |
| Total alpha-Linolenic Acid (C18:3 n-3) | Human Plasma B                       | µmol/L                       |              |              |               |       | 9             | 110         | 26                     |             |       |
| Total gamma-Linolenic Acid (C18:3 n-6) | Fatty Acids Solution B               | µmol/L                       |              |              |               |       | 5             | 0.7         | 0.94                   |             |       |
| Total gamma-Linolenic Acid (C18:3 n-6) | SRM 1950 Metabolites in Frozen Human | µmol/L                       | 39.9         | 4.3          |               |       | 9             | 50          | 15                     | 39.9        | 8.    |
|  | Plasma                               |                              |              |              |               |       |               |             |                        |             |       |
| Total gamma-Linolenic Acid (C18:3 n-6) | Human Plasma B                       | µmol/L                       |              |              |               |       | 8             | 80          | 20                     |             |       |
| Total Arachidonic Acid (C20:4 n-6)     | Fatty Acids Solution B               | µmol/L                       | 806          | 20           |               |       | 10            | 760         | 220                    | 806         | 40    |
| Total Arachidonic Acid (C20:4 n-6)     | SRM 1950 Metabolites in Frozen Human | µmol/L                       | 984          | 90           |               |       | 10            | 750         | 140                    | 984         | 18    |
|  | Plasma                               |                              |              |              |               |       |               |             |                        |             |       |
| Total Arachidonic Acid (C20:4 n-6)     | Human Plasma B                       | µmol/L                       |              |              |               |       | 9             | 870         | 210                    |             |       |
| Total EPA (C20:5 n-3)                  | Fatty Acids Solution B               | µmol/L                       | 36.8         | 0.9          |               |       | 10            | 30          | 14                     | 36.8        | 1.    |
| Total EPA (C20:5 n-3)                  | SRM 1950 Metabolites in Frozen Human | µmol/L                       | 38.6         | 0.3          |               |       | 10            | 40          | 15                     | 38.6        | 0.    |
|  | Plasma                               |                              |              |              |               |       |               |             |                        |             |       |
| Total EPA (C20:5 n-3)                  | Human Plasma B                       | µmol/L                       |              |              |               |       | 9             | 90          | 33                     |             |       |
| Total DPA (C22:5 n-3)                  | Fatty Acids Solution B               | µmol/L                       | 38.2         | 1.0          |               |       | 8             | 30          | 11                     | 38.2        | 1.    |
| Total DPA (C22:5 n-3)                  | SRM 1950 Metabolites in Frozen Human | µmol/L                       | 38.5         | 0.4          |               |       | 8             | 40          | 9                      | 38.5        | 0.    |
|  | Plasma                               |                              |              |              |               |       |               |             |                        |             |       |
| Total DPA (C22:5 n-3)                  | Human Plasma B                       | µmol/L                       |              |              |               |       | 7             | 70          | 17                     |             |       |
| Total DHA (C22:6 n-3)                  | Fatty Acids Solution B               | µmol/L                       | 99           | 2            |               |       | 10            | 100         | 12                     | 99          | 5     |
| Total DHA (C22:6 n-3)                  | SRM 1950 Metabolites in Frozen Human | µmol/L                       | 118          | 11           |               |       | 10            | 110         | 15                     | 118         | 2     |
|  | Plasma                               |                              |              |              |               |       |               |             |                        |             |       |
| Total DHA (C22:6 n-3)                  | Human Plasma B                       | µmol/L                       |              |              |               |       | 9             | 140         | 35                     |             |       |
|  |                                      | <i>x</i> <sub><i>i</i></sub> | Mean of rep  | orted value  | es            | Ν     | Number of q   | uantitative | $x_{\text{NIST}}$      | NIST-assess | ed va |
|  |                                      | <i>s</i> :                   | Standard dev | viation of r | eported value | es    | values report | ed          | U expanded uncertainty |             |       |

U expanded uncertainty

about the NIST-assessed value x\* Robust mean of reported

 $Z'_{\text{comm}}$  Z'-score with respect to community consensus

 $Z_{\rm NIST}~$  Z-score with respect to NIST value

values s\* Robust standard deviation

# **Table 5-24.** Individualized data summary table (NIST) for free fatty acids in a solution and human plasma.

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|                                       | Lab Code:                            | Lab Code: NIST    |   |  |                    |                   | 2.                              | Community R     | 3. Target |               |                        |  |  |
|---------------------------------------|--------------------------------------|-------------------|---|--|--------------------|-------------------|---------------------------------|-----------------|-----------|---------------|------------------------|--|--|
| Analyte                               | Sample                               | Units             | x,  | <i>s</i> ;   | Z' <sub>comm</sub> | Z <sub>NIST</sub> | N                               | x*              | s*        | X NIST        | U                      |  |  |
| Free Stearic Acid (C18:0)             | Fatty Acids Solution B               | µmol/L            | 550   | 14   | comm               |                   | 2                               |                 |           | 550           | 28                     |  |  |
| . ,                                   | SRM 1950 Metabolites in Frozen Human | •                 |   |  |                    |                   |                                 |                 |           |               |                        |  |  |
| Free Stearic Acid (C18:0)             | Plasma                               | µmol/L            |   |  |                    |                   | 1                               |                 |           |               |                        |  |  |
| Free Stearic Acid (C18:0)             | Human Plasma B                       | μmol/L            |   |  |                    |                   | 1                               |                 |           |               |                        |  |  |
| Free cis-Vaccenic Acid (C18:1 n-7)    | Fatty Acids Solution B               | μmol/L            | 115   | 3  |                    |                   | 2                               |                 |           | 115           | 6                      |  |  |
|                                       | SRM 1950 Metabolites in Frozen Human |                   |   |  |                    |                   |                                 |                 |           |               |                        |  |  |
| Free cis-Vaccenic Acid (C18:1 n-7)    | Plasma                               | µmol/L            |   |  |                    |                   | 1                               |                 |           |               |                        |  |  |
| Free cis-Vaccenic Acid (C18:1 n-7)    | Human Plasma B                       | µmol/L            |   |  |                    |                   | 1                               |                 |           |               |                        |  |  |
| Free Oleic Acid (C18:1 n-9)           | Fatty Acids Solution B               | µmol/L            | 1320  | 33   |                    |                   | 2                               |                 |           | 1320          | 66                     |  |  |
|                                       | SRM 1950 Metabolites in Frozen Human |                   |   |  |                    |                   |                                 |                 |           |               |                        |  |  |
| Free Oleic Acid (C18:1 n-9)           | Plasma                               | µmol/L            |   |  |                    |                   | 1                               |                 |           |               |                        |  |  |
| Free Oleic Acid (C18:1 n-9)           | Human Plasma B                       | µmol/L            |   |  |                    |                   | 1                               |                 |           |               |                        |  |  |
| Free Linoleic Acid (C18:2 n-6)        | Fatty Acids Solution B               | µmol/L            | 2490  | 62   |                    |                   | 2                               |                 |           | 2490          | 124                    |  |  |
|                                       | SRM 1950 Metabolites in Frozen Human |                   |   |  |                    |                   |                                 |                 |           |               |                        |  |  |
| Free Linoleic Acid (C18:2 n-6)        | Plasma                               | µmol/L            |   |  |                    |                   | 1                               |                 |           |               |                        |  |  |
| Free Linoleic Acid (C18:2 n-6)        | Human Plasma B                       | µmol/L            |   |  |                    |                   | 1                               |                 |           |               |                        |  |  |
| Free alpha-Linolenic Acid (C18:3 n-3) | Fatty Acids Solution B               | µmol/L            | 44.7  | 1.1  |                    |                   | 2                               |                 |           | 44.7          | 2.2                    |  |  |
|                                       | SRM 1950 Metabolites in Frozen Human |                   |   |  |                    |                   |                                 |                 |           |               |                        |  |  |
| Free alpha-Linolenic Acid (C18:3 n-3) | Plasma                               | µmol/L            |   |  |                    |                   | 1                               |                 |           |               |                        |  |  |
| Free alpha-Linolenic Acid (C18:3 n-3) | Human Plasma B                       | µmol/L            |   |  |                    |                   | 1                               |                 |           |               |                        |  |  |
| ree gamma-Linolenic Acid (C18:3 n-6)  | Fatty Acids Solution B               | µmol/L            |   |  |                    |                   | 1                               |                 |           |               |                        |  |  |
|                                       | SRM 1950 Metabolites in Frozen Human | 10                |   |  |                    |                   |                                 |                 |           |               |                        |  |  |
| Pree gamma-Linolenic Acid (C18:3 n-6) | Plasma                               | µmol/L            |   |  |                    |                   | 1                               |                 |           |               |                        |  |  |
| Free gamma-Linolenic Acid (C18:3 n-6) | Human Plasma B                       | µmol/L            |   |  |                    |                   | 1                               |                 |           |               |                        |  |  |
| Free Arachidonic Acid (C20:4 n-6)     | Fatty Acids Solution B               | µmol/L            | 806   | 20   |                    |                   | 2                               |                 |           | 806           | 40                     |  |  |
|                                       | SRM 1950 Metabolites in Frozen Human |                   |   |  |                    |                   |                                 |                 |           |               |                        |  |  |
| Free Arachidonic Acid (C20:4 n-6)     | Plasma                               | µmol/L            |   |  |                    |                   | 1                               |                 |           |               |                        |  |  |
| Free Arachidonic Acid (C20:4 n-6)     | Human Plasma B                       | µmol/L            |   |  |                    |                   | 1                               |                 |           |               |                        |  |  |
| Free EPA (C20:5 n-3)                  | Fatty Acids Solution B               | µmol/L            | 36.8  | 0.9  |                    |                   | 2                               |                 |           | 36.8          | 1.8                    |  |  |
|                                       | SRM 1950 Metabolites in Frozen Human |                   |   |  |                    |                   |                                 |                 |           |               |                        |  |  |
| Free EPA (C20:5 n-3)                  | Plasma                               | µmol/L            |   |  |                    |                   | 1                               |                 |           |               |                        |  |  |
| Free EPA (C20:5 n-3)                  | Human Plasma B                       | µmol/L            |   |  |                    |                   | 1                               |                 |           |               |                        |  |  |
| Free DPA (C22:5 n-3)                  | Fatty Acids Solution B               | µmol/L            | 38.2  | 1.0  |                    |                   | 2                               |                 |           | 38.2          | 1.9                    |  |  |
|                                       | SRM 1950 Metabolites in Frozen Human | 1.7               |   |  |                    |                   |                                 |                 |           |               |                        |  |  |
| Free DPA (C22:5 n-3)                  | Plasma                               | µmol/L            |   |  |                    |                   | 1                               |                 |           |               |                        |  |  |
| Free DPA (C22:5 n-3)                  | Human Plasma B                       | µmol/L            | 00.0  | 2.5  |                    |                   | 1                               |                 |           | 00.0          | 4.0                    |  |  |
| Free DHA (C22:6 n-3)                  | Fatty Acids Solution B               | µmol/L            | 98.8  | 2.5  |                    |                   | 2                               |                 |           | 98.8          | 4.9                    |  |  |
| Erro DUA (COD( = 2)                   | SRM 1950 Metabolites in Frozen Human |                   |   |  |                    |                   | 1                               |                 |           |               |                        |  |  |
| Free DHA (C22:6 II-5)                 | Plasma                               | µmorL             |   |  |                    |                   | 1                               |                 |           |               |                        |  |  |
| Free DHA (C22:0 fi-3)                 | Human Plasma B                       | µmorL             |   |  |                    | N                 | 1                               |                 |           | NICT          |                        |  |  |
|                                       |                                      | $x_i$             | Mean of rep   | orted valu   | es                 | 10                | Number of                       | quantitative    | X NIS     | INIST-assess  | ed value               |  |  |
|                                       |                                      |                   |   | s <sub>i</sub> Standard deviation of reported values |                    |                   | es values reported              |                 |           |               | U expanded uncertainty |  |  |
|                                       |                                      | Z' comm           | Z <sub>comm</sub> Z-score with respect to community |  |                    | X*                | Robust mea                      | an of reported  |           | about the NIS | 1-assessed             |  |  |
|                                       |                                      | _                 | consensus   |  |                    |                   | values                          |                 |           |               |                        |  |  |
|                                       |                                      | Z <sub>NIST</sub> | Z-score with  | respect to   | o NIST value       | 5 *               | <ul> <li>Robust star</li> </ul> | ndard deviation |           |               |                        |  |  |

**Table 5-25.** Data summary table for total stearic acid in a fatty acids solution and human plasma. Data points highlighted in red have been flagged as potential outliers (e.g., difference from reference value, Grubb and/or Cochran) by the NIST software package.

|            |             |                                  |             |            |            |             |          | Total Ste   | aric Acid   | (C18:0)    |                |          |             |           |          |       |
|------------|-------------|----------------------------------|-------------|------------|------------|-------------|----------|-------------|-------------|------------|----------------|----------|-------------|-----------|----------|-------|
|            |             | ŀ                                | Fatty Acids | s Solution | B (µmol/L) |             | SRM      | 1950 Met    | abolites ii | ı Frozen H | luman          |          | Human l     | 'lasma B  | (µmol/L) |       |
|            | Lab         | Α                                | В           | С          | Avg        | SD          | Α        | В           | С           | Avg        | SD             | Α        | В           | С         | Avg      | SD    |
|            | Target      |                                  |             |            | 550        | 28          |          |             |             | 644        | 41             |          |             |           |          |       |
|            | A004        | 278.3                            | 262.9       | 269.4      | 270.2      | 7.7         | 674.1    | 683.7       | 690.7       | 682.8      | 8.3            | 1059.8   | 1144.2      | 1058.3    | 1087.4   | 49    |
|            | A012        | 0.585                            | 0.547       | 0.571      | 0.568      | 0.019       | 0.681    | 0.594       | 0.582       | 0.619      | 0.054          | 0.846    | 0.949       | 0.937     | 0.911    | 0.056 |
|            | A016        |                                  |             |            |            |             |          |             |             |            |                |          |             |           |          |       |
|            | A024        | 539.74                           | 545.93      | 544.25     | 543.31     | 3.2         | 759.36   | 674.35      | 701.90      | 711.87     | 43             | 966.12   | 1027.43     | 985.87    | 993.14   | 31    |
|            | A028        |                                  |             |            |            |             |          |             |             |            |                |          |             |           |          |       |
| ts         | A049        |                                  |             |            |            |             |          |             |             |            |                |          |             |           |          |       |
| Ins        | A050        |                                  |             |            |            |             |          |             |             |            |                |          |             |           |          |       |
| R.         | A052        |                                  |             |            |            |             |          |             |             |            |                |          |             |           |          |       |
| ual        | A070        |                                  |             |            |            |             |          |             |             |            |                |          |             |           |          |       |
| ivid       | A079        |                                  |             |            |            |             |          |             |             |            |                |          |             |           |          |       |
| ibu        | A083        |                                  |             |            |            |             |          |             |             |            |                |          |             |           |          |       |
| -          | A085        |                                  |             |            |            |             |          |             |             |            |                |          |             |           |          |       |
|            | A087        | 497.966                          | 499.844     | 490.227    | 496.0123   | 5.1         | 643.537  | 646.363     | 698.855     | 662.918    | 31             | 937.297  | 934.357     | 945.169   | 938.941  | 5.6   |
|            | A090        | 460                              | 464         | 464        | 463        | 2.3         | 609      | 580         | 598         | 596        | 15             | 889      | 903         | 884       | 892      | 9.8   |
|            | A097        | 565.5                            | 493.5       | 517.5      | 525.5      | 36.7        | 425.9    | 465.5       | 456.6       | 449        | 21             | 576.1    | 570.0       | 589.2     | 578.4    | 9.8   |
|            | A099        | 580                              | 500         | 490        | 523        | 49          | 720      | 750         | 700         | 723        | 25             | 1050     | 970         | 990       | 1003     | 42    |
|            | A100        | 381.13                           | 384.6       | 381        | 382        | 2.0         | 263.01   | 273.56      | 254.65      | 263.74     | 9.5            | 377.65   | 383.5       | 365.28    | 375.48   | 9.3   |
|            | A107        | 555                              | 593         | 588        | 579        | 21          | 577      | 537         | 545         | 553        | 21             | 786      | 798         | 831       | 805      | 23    |
| ŝ          |             | Consensus                        | s Mean      |            | 461        |             | Consensu | is Mean     |             | 542        |                | Consensu | is Mean     |           | 780      |       |
| uni<br>Its |             | Consensus Standard Deviation 124 |             |            |            |             | Consensu | is Standard | l Deviation | 206        |                | Consensu | is Standard | Deviation | 289      |       |
| nsa        | Maximum 579 |                                  |             |            |            | Maximum 723 |          |             |             |            | Maximum 1087.4 |          |             |           |          |       |
| N N        |             | Minimum                          |             |            | 0.568      |             | Minimum  |             |             | 0.62       |                | Minimum  |             |           | 0.911    |       |
| -          |             | Ν                                |             |            | 9          |             | Ν        |             |             | 9          |                | Ν        |             |           | 9        |       |

Exercise HAMQAP Exercise 1 - Human Metabolites Sample: Fatty Acids Solution B Measurand: Total Stearic Acid (C18:0)



**Figure 5-41.** Total stearic acid (C18:0) in Fatty Acids Solution B (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \leq 2$ . The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by its uncertainty ( $U_{NIST}$ ) and represents the range that results in an acceptable  $Z_{NIST}$  score,  $|Z_{NIST}| \leq 2$ .



**Figure 5-42.** Total stearic acid (C18:0) in SRM 1950 Metabolites in Frozen Human Serum (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \le 2$ . The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by its uncertainty ( $U_{NIST}$ ) and represents the range that results in an acceptable  $Z'_{NIST} | \le 2$ .

Exercise HAMQAP Exercise 1 - Human Metabolites Sample: Human Plasma B Measurand: Total Stearic Acid (C18:0)



Figure 5-43. Total stearic acid (C18:0) in Human Plasma B (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \leq 2$ . A NIST value has not been determined in this material.



HAMQAP Exercise 1 - Human Metabolites, Measurand: Total Stearic Acid (C18:0) No. of laboratories: 9

**Figure 5-44.** Laboratory means for total stearic acid in Fatty Acids Solution B and SRM 1950 Metabolites in Frozen Human Plasma (sample/sample comparison view). In this view, the individual laboratory mean for one sample (Fatty Acids Solution B) is compared to the mean for a second sample (SRM 1950). The solid red box represents the NIST range of tolerance for the two samples, Fatty Acids Solution B (x-axis) and SRM 1950 (y-axis), which encompasses the target values bounded by their uncertainties ( $U_{\text{NIST}}$ ) and represents the range that results in an acceptable  $Z_{\text{NIST}}$  score,  $|Z_{\text{NIST}}| \leq 2$ . The dotted blue box represents the consensus range of tolerance for Fatty Acids Solution B (x-axis) and SRM 1950 (y-axis), calculated as the values above and below the consensus means that result in an acceptable  $Z'_{\text{comm}} \leq 2$ .

**Table 5-26.** Data summary table for total cis-vaccenic acid in a fatty acids solution and human plasma. Data points highlighted in red have been flagged as potential outliers (e.g., difference from reference value, Grubb and/or Cochran) by the NIST software package.

|            |        |                      |             |           |            |         | Tota     | al cis-Vaco | cenic Acid  | (C18:1 n-  | 7)      |          |             |           |          |       |
|------------|--------|----------------------|-------------|-----------|------------|---------|----------|-------------|-------------|------------|---------|----------|-------------|-----------|----------|-------|
|            |        | I                    | Fatty Acids | Solution  | B (µmol/L) |         | SRM      | 1950 Met    | abolites ii | ı Frozen H | uman    |          | Human I     | Plasma B  | (µmol/L) |       |
|            | Lab    | Α                    | В           | С         | Avg        | SD      | Α        | В           | С           | Avg        | SD      | Α        | В           | С         | Avg      | SD    |
|            | Target |                      |             |           | 115        | 5.8     |          |             |             | 136        | 3       |          |             |           |          |       |
|            | A004   |                      |             |           |            |         |          |             |             |            |         |          |             |           |          |       |
|            | A012   | 0.127                | 0.120       | 0.125     | 0.124      | 0.0036  | 0.160    | 0.142       | 0.140       | 0.147      | 0.011   | 0.212    | 0.235       | 0.229     | 0.225    | 0.012 |
|            | A016   |                      |             |           |            |         |          |             |             |            |         |          |             |           |          |       |
|            | A024   | 118.839              | 117.135     | 117.661   | 117.8783   | 0.87    | 174.234  | 149.481     | 157.422     | 160.379    | 13      | 237.907  | 248.672     | 237.955   | 241.511  | 6.2   |
| ults       | A028   |                      |             |           |            |         |          |             |             |            |         |          |             |           |          |       |
| tesi       | A049   |                      |             |           |            |         |          |             |             |            |         |          |             |           |          |       |
| al B       | A052   |                      |             |           |            |         |          |             |             |            |         |          |             |           |          |       |
| Individua  | A070   |                      |             |           |            |         |          |             |             |            |         |          |             |           |          |       |
|            | A079   |                      |             |           |            |         |          |             |             |            |         |          |             |           |          |       |
|            | A083   |                      |             |           |            |         |          |             |             |            |         |          |             |           |          |       |
|            | A085   |                      |             |           |            |         |          |             |             |            |         |          |             |           |          |       |
|            | A087   | 108.401              | 108.367     | 107.677   | 108.1483   | 0.41    | 147.627  | 147.133     | 158.478     | 151.079    | 6.4     | 232.874  | 228.708     | 231.63    | 231.071  | 2.1   |
|            | A090   | 115                  | 116         | 114       | 115        | 1.0     | 274      | 260         | 271         | 268        | 7.4     | 271      | 290         | 290       | 284      | 11    |
|            | A100   | 104.8                | 104.1       | 103.58    | 104.2      | 0.61    | 45.8     | 53.97       | 52.77       | 50.85      | 4.4     | 71.97    | 69.47       | 68.92     | 70.12    | 1.63  |
|            | A107   | 129                  | 116         | 125       | 123        | 6.7     | 137      | 146         | 135         | 139        | 5.9     | 230      | 223         | 207       | 220      | 12    |
| ţ,         |        | Consensus Mean 114   |             |           |            |         | Consensu | s Mean      |             | 128        |         | Consensu | ıs Mean     |           | 182      |       |
| lts l      |        | Consensus            | s Standard  | Deviation | 17         |         | Consensu | s Standard  | Deviation   | 119        |         | Consensu | is Standard | Deviation | 92       |       |
| esu        |        | Maximum              |             |           | 123        |         | Maximum  | ı           |             | 268        |         | Maximum  | 1           |           | 284      |       |
| <u>B</u> A |        | Minimum              |             | 0.124     |            | Minimum |          |             | 0.147       |            | Minimum |          |             | 0.225     |          |       |
| J          |        | Minimum 0.124<br>N 6 |             |           |            |         | Ν        |             |             | 6          |         | N 6      |             |           |          |       |

Exercise HAMQAP Exercise 1 - Human Metabolites Sample: Fatty Acids Solution B Measurand: Total cis-Vaccenic Acid (C18:1 n-7)



**Figure 5-45.** Total cis-vaccenic acid (C18:1 n-7) in Fatty Acids Solution B (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \le 2$ . The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by its uncertainty ( $U_{NIST}$ ) and represents the range that results in an acceptable  $Z'_{NIST}$  score,  $|Z_{NIST}| \le 2$ .

Exercise HAMQAP Exercise 1 - Human Metabolites Sample: SRM 1950 Metabolites in Frozen Human Plasma Measurand: Total cis-Vaccenic Acid (C18:1 n-7)



**Figure 5-46.** Total cis-vaccenic acid (C18:1 n-7) in SRM 1950 Metabolites in Frozen Human Serum (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red line represents the upper consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \le 2$ , with the lower range set at zero. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by its uncertainty ( $U_{NIST}$ ) and represents the range that results in an acceptable  $Z'_{NIST}| \le 2$ .

Exercise HAMQAP Exercise 1 - Human Metabolites Sample: Human Plasma B Measurand: Total cis-Vaccenic Acid (C18:1 n-7)



**Figure 5-47.** Total cis-vaccenic acid (C18:1 n-7) in Human Plasma B (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red line represents the upper consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \le 2$ , with the lower range set at zero. A NIST value has not been determined in this material.



HAMQAP Exercise 1 - Human Metabolites, Measurand: Total cis-Vaccenic Acid (C18:1 n-7) No. of laboratories: 6

**Figure 5-48.** Laboratory means for total cis-vaccenic acid in Fatty Acids Solution B and SRM 1950 Metabolites in Frozen Human Plasma (sample/sample comparison view). In this view, the individual laboratory mean for one sample (Fatty Acids Solution B) is compared to the mean for a second sample (SRM 1950). The solid red box represents the NIST range of tolerance for the two samples, Fatty Acids Solution B (x-axis) and SRM 1950 (y-axis), which encompasses the target values bounded by their uncertainties ( $U_{\text{NIST}}$ ) and represents the range that results in an acceptable  $Z_{\text{NIST}}$  score,  $|Z_{\text{NIST}}| \leq 2$ . The dotted blue box represents the consensus range of tolerance for Fatty Acids Solution B (x-axis) and SRM 1950 (y-axis), calculated as the values above and below the consensus means that result in an acceptable  $Z'_{\text{comm}} \leq 2$ .

**Table 5-27.** Data summary table for total oleic in a fatty acids solution and human plasma. Data points highlighted in red have been flagged as potential outliers (e.g., difference from reference value, Grubb and/or Cochran) by the NIST software package.

|           |              |                                  |             |          |            |       |          | Fotal Olei | c Acid (C   | 18:1 n-9)      |      |          |             |           |          |      |
|-----------|--------------|----------------------------------|-------------|----------|------------|-------|----------|------------|-------------|----------------|------|----------|-------------|-----------|----------|------|
|           |              | ŀ                                | Fatty Acids | Solution | B (µmol/L) |       | SRM      | 1950 Met   | abolites ii | n Frozen H     | uman |          | Human I     | lasma B ( | (µmol/L) |      |
|           | Lab          | Α                                | В           | С        | Avg        | SD    | Α        | В          | С           | Avg            | SD   | Α        | В           | С         | Avg      | SD   |
|           | Target       |                                  |             |          | 1318       | 66    |          |            |             | 1614           | 154  |          |             |           |          |      |
|           | A004         | 767.5                            | 697.4       | 672.2    | 712.4      | 49    | 1804.6   | 1923.9     | 1888.9      | 1872.5         | 61   | 3798.8   | 3843.1      | 3797.9    | 3813.3   | 26   |
|           | A012         | 1.43                             | 1.34        | 1.40     | 1.39       | 0.046 | 1.98     | 1.72       | 1.68        | 1.79           | 0.16 | 3.04     | 3.40        | 3.31      | 3.25     | 0.19 |
|           | A016         |                                  |             |          |            |       |          |            |             |                |      |          |             |           |          |      |
|           | A024         | 1300.41                          | 1320.69     | 1319.35  | 1313.483   | 11    | 2068.23  | 1833.14    | 1909.4      | 1936.92        | 120  | 3352.02  | 3571.66     | 3409.47   | 3444.38  | 114  |
|           | A028         |                                  |             |          |            |       |          |            |             |                |      |          |             |           |          |      |
| ts        | A049         |                                  |             |          |            |       |          |            |             |                |      |          |             |           |          |      |
| lus       | A050         |                                  |             |          |            |       |          |            |             |                |      |          |             |           |          |      |
| R         | A052         |                                  |             |          |            |       |          |            |             |                |      |          |             |           |          |      |
| ual       | A070         |                                  |             |          |            |       |          |            |             |                |      |          |             |           |          |      |
| ivid      | A079         |                                  |             |          |            |       |          |            |             |                |      |          |             |           |          |      |
| ipu       | A083         |                                  |             |          |            |       |          |            |             |                |      |          |             |           |          |      |
| Ξ         | A085         |                                  |             |          |            |       |          |            |             |                |      |          |             |           |          |      |
|           | A087         | 1216.52                          | 1214.18     | 1207.54  | 1212.746   | 4.7   | 1719.36  | 1726.44    | 1863.00     | 1769.60        | 81   | 3236.15  | 3203.84     | 3249.52   | 3229.83  | 23   |
|           | A090         | 1346                             | 1340        | 1342     | 1343       | 3.1   | 1880     | 1788       | 1869        | 1846           | 50   | 3341     | 3329        | 3306      | 3325     | 18   |
|           | A097         | 841.5                            | 787.2       | 885.3    | 838.0      | 49    | 1432     | 1420       | 1410        | 1421           | 11   | 2120     | 2000        | 2010      | 2043     | 67   |
|           | A099         | 1360                             | 1190        | 1180     | 1243       | 101   | 1850     | 1870       | 1790        | 1837           | 42   | 3440     | 3100        | 3220      | 3253     | 172  |
|           | A100         | 999.95                           | 1000.58     | 988.82   | 996.45     | 6.6   | 481.87   | 480.46     | 494.02      | 485.45         | 7.5  | 832.7    | 845.63      | 852.78    | 843.70   | 10   |
|           | A107         | 1398                             | 1360        | 1352     | 1370       | 25    | 1704     | 1851       | 1751        | 1769           | 75   | 3171     | 3292        | 3146      | 3203     | 78   |
| ty.       |              | Consensus                        | s Mean      |          | 1083       |       | Consensu | s Mean     |             | 1781           |      | Consensu | is Mean     |           | 3173     |      |
| um<br>Its |              | Consensus Standard Deviation 322 |             |          |            |       | Consensu | s Standard | Deviation   | 228            |      | Consensu | is Standard | Deviation | 593      |      |
| esu       | Maximum 1370 |                                  |             |          | Maximum    | 1     |          | 1936.92    |             | Maximum 3813.3 |      |          |             |           |          |      |
| R R       |              | Minimum                          |             |          | 1.39       |       | Minimum  |            |             | 1.79           |      | Minimum  |             |           | 3.25     |      |
|           |              | Ν                                |             |          | 9          |       | Ν        |            |             | 9              |      | Ν        |             |           | 9        |      |

Exercise HAMQAP Exercise 1 - Human Metabolites Sample: Fatty Acids Solution B Measurand: Total Oleic Acid (C18:1 n-9)



**Figure 5-49.** Total oleic acid (C18:1 n-9) in Fatty Acids Solution B (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \leq 2$ . The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by its uncertainty ( $U_{NIST}$ ) and represents the range that results in an acceptable  $Z_{NIST}$  score,  $|Z_{NIST}| \leq 2$ .

Exercise HAMQAP Exercise 1 - Human Metabolites Sample: SRM 1950 Metabolites in Frozen Human Plasma Measurand: Total Oleic Acid (C18:1 n-9)



**Figure 5-50.** Total oleic acid (C18:1 n-9) in SRM 1950 Metabolites in Frozen Human Serum (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \leq 2$ . The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by its uncertainty ( $U_{NIST}$ ) and represents the range that results in an acceptable  $Z_{NIST} | \leq 2$ .

Exercise HAMQAP Exercise 1 - Human Metabolites Sample: Human Plasma B Measurand: Total Oleic Acid (C18:1 n-9)



**Figure 5-51.** Total oleic acid (C18:1 n-9) in Human Plasma B (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \leq 2$ . A NIST value has not been determined in this material.



HAMQAP Exercise 1 - Human Metabolites, Measurand: Total Oleic Acid (C18:1 n-9) No. of laboratories: 9

**Figure 5-52.** Laboratory means for total oleic acid in Fatty Acids Solution B and SRM 1950 Metabolites in Frozen Human Plasma (sample/sample comparison view). In this view, the individual laboratory mean for one sample (Fatty Acids Solution B) is compared to the mean for a second sample (SRM 1950). The solid red box represents the NIST range of tolerance for the two samples, Fatty Acids Solution B (x-axis) and SRM 1950 (y-axis), which encompasses the target values bounded by their uncertainties ( $U_{\text{NIST}}$ ) and represents the range that results in an acceptable  $Z_{\text{NIST}}$  score,  $|Z_{\text{NIST}}| \leq 2$ . The dotted blue box represents the consensus range of tolerance for Fatty Acids Solution B (x-axis) and SRM 1950 (y-axis), calculated as the values above and below the consensus means that result in an acceptable  $Z'_{\text{comm}} \leq 2$ .

**Table 5-28.** Data summary table for total linoleic acid in a fatty acids solution and human plasma. Data points highlighted in red have been flagged as potential outliers (e.g., difference from reference value, Grubb and/or Cochran) by the NIST software package.

|            |                     |           |             |            |            |          | Т        | otal Linol  | eic Acid (  | C18:2 n-6) |          |          |            |           |          |       |
|------------|---------------------|-----------|-------------|------------|------------|----------|----------|-------------|-------------|------------|----------|----------|------------|-----------|----------|-------|
| -          |                     | ŀ         | Fatty Acids | s Solution | B (µmol/L) |          | SRM      | 1950 Met    | abolites ii | n Frozen H | uman     |          | Human I    | Plasma B  | (µmol/L) |       |
|            | Lab                 | Α         | В           | С          | Avg        | SD       | Α        | В           | С           | Avg        | SD       | Α        | В          | С         | Avg      | SD    |
|            | Target              |           |             |            | 2488       | 124      |          |             |             | 2838       | 143      |          |            |           |          |       |
|            | A004                | 1596.4    | 1504.2      | 1557.8     | 1552.8     | 46       | 2991.6   | 2963.6      | 3150.4      | 3035.2     | 101      | 4364.7   | 4347.0     | 4562.7    | 4424.8   | 120   |
|            | A012                | 2.52      | 2.36        | 2.47       | 2.45       | 0.082    | 2.88     | 2.46        | 2.44        | 2.59       | 0.25     | 3.24     | 3.58       | 3.51      | 3.44     | 0.18  |
|            | A016                |           |             |            |            |          |          |             |             |            |          |          |            |           |          |       |
|            | A024                | 2418.86   | 2454.31     | 2449.93    | 2441.033   | 19       | 3259.7   | 2902.44     | 3005.87     | 3056.00    | 184      | 3777.66  | 4016.83    | 3845.55   | 3880.01  | 123   |
|            | A028                |           |             |            |            |          |          |             |             |            |          |          |            |           |          |       |
| ts         | A049                |           |             |            |            |          |          |             |             |            |          |          |            |           |          |       |
| Ins        | A050                |           |             |            |            |          |          |             |             |            |          |          |            |           |          |       |
| R          | A052                |           |             |            |            |          |          |             |             |            |          |          |            |           |          |       |
| ndividual  | A070                |           |             |            |            |          |          |             |             |            |          |          |            |           |          |       |
|            | A079                |           |             |            |            |          |          |             |             |            |          |          |            |           |          |       |
|            | A083                |           |             |            |            |          |          |             |             |            |          |          |            |           |          |       |
| Π          | A085                |           |             |            |            |          |          |             |             |            |          |          |            |           |          |       |
|            | A087                | 2288.18   | 2292.63     | 2270.03    | 2283.612   | 12       | 2760.98  | 2777.75     | 3004.66     | 2847.80    | 136      | 3726.24  | 3669.00    | 3734.34   | 3709.86  | 35.62 |
|            | A090                | 2363      | 2366        | 2376       | 2368       | 6.8      | 2860     | 2769        | 2860        | 2830       | 53       | 3707     | 3698       | 3737      | 3714     | 20    |
|            | A097                | 1584      | 1519        | 1650       | 1584       | 66       | 2232     | 2158        | 2150        | 2180       | 45       | 2960     | 3005       | 2903      | 2956     | 51    |
|            | A099                | 2620      | 2310        | 2260       | 2397       | 195      | 3060     | 3100        | 2970        | 3043       | 67       | 4140     | 3800       | 3900      | 3947     | 175   |
|            | A100                | 2063.22   | 2074.83     | 2049.18    | 2062.41    | 13       | 823.58   | 819.33      | 837.86      | 826.92     | 9.7      | 1060.77  | 1092.09    | 1080.34   | 1077.73  | 16    |
|            | A107                | 2496      | 2528        | 2599       | 2541       | 53       | 2747     | 2789        | 2833        | 2790       | 43       | 3758     | 3783       | 3734      | 3758     | 25    |
| ty         | Consensus Mean 2154 |           |             |            |            | Consensu | is Mean  |             | 2826        |            | Consensu | s Mean   |            | 3679      |          |       |
| uni<br>Its |                     | Consensus | s Standard  | Deviation  | 460        |          | Consensu | is Standard | Deviation   | 444        |          | Consensu | s Standard | Deviation | 720      |       |
| nm<br>esu  |                     | Maximum   | L           |            | 2541       |          | Maximun  | 1           |             | 3056.00    |          | Maximum  | 1          |           | 4424.8   |       |
| R          |                     | Minimum   |             |            | 2.45       |          | Minimum  |             | 2.59        |            |          | Minimum  |            |           | 3.44     |       |
| -          |                     | Ν         |             |            | 9          |          | Ν        |             |             | 9          |          | Ν        |            |           | 9        |       |

Exercise HAMQAP Exercise 1 - Human Metabolites Sample: Fatty Acids Solution B Measurand: Total Linoleic Acid (C18:2 n-6)



**Figure 5-53.** Total linoleic acid (C18:2 n-6) in Fatty Acids Solution B (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \leq 2$ . The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by its uncertainty ( $U_{NIST}$ ) and represents the range that results in an acceptable  $Z_{NIST}$  score,  $|Z_{NIST}| \leq 2$ .

Exercise HAMQAP Exercise 1 - Human Metabolites Sample: SRM 1950 Metabolites in Frozen Human Plasma Measurand: Total Linoleic Acid (C18:2 n-6)



**Figure 5-54.** Total linoleic acid (C18:2 n-6) in SRM 1950 Metabolites in Frozen Human Serum (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \leq 2$ . The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by its uncertainty ( $U_{NIST}$ ) and represents the range that results in an acceptable  $Z_{NIST} | \leq 2$ .

Exercise HAMQAP Exercise 1 - Human Metabolites Sample: Human Plasma B Measurand: Total Linoleic Acid (C18:2 n-6)



**Figure 5-55.** Total linoleic acid (C18:2 n-6) in Human Plasma B (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \leq 2$ . A NIST value has not been determined in this material.



HAMQAP Exercise 1 - Human Metabolites, Measurand: Total Linoleic Acid (C18:2 n-6) No. of laboratories: 9

**Figure 5-56.** Laboratory means for total linoleic acid in Fatty Acids Solution B and SRM 1950 Metabolites in Frozen Human Plasma (sample/sample comparison view). In this view, the individual laboratory mean for one sample (Fatty Acids Solution B) is compared to the mean for a second sample (SRM 1950). The solid red box represents the NIST range of tolerance for the two samples, Fatty Acids Solution B (x-axis) and SRM 1950 (y-axis), which encompasses the target values bounded by their uncertainties ( $U_{\text{NIST}}$ ) and represents the range that results in an acceptable  $Z_{\text{NIST}}$  score,  $|Z_{\text{NIST}}| \leq 2$ . The dotted blue box represents the consensus range of tolerance for Fatty Acids Solution B (x-axis) and SRM 1950 (y-axis), calculated as the values above and below the consensus means that result in an acceptable  $Z'_{\text{comm}} \leq 2$ .

**Table 5-29.** Data summary table for total  $\alpha$ -linolenic acid in a fatty acids solution and human plasma. Data points highlighted in red have been flagged as potential outliers (e.g., difference from reference value, Grubb and/or Cochran) by the NIST software package.

|                    | 1      | Total alpha-Linolenic Acid (C18:3 n-3) |                                      |        |        |                     |                              |                         |        |        |                    |                              |         |        |        |        |
|--------------------|--------|--|--------------------------------------|--------|--------|---------------------|------------------------------|-------------------------|--------|--------|--------------------|------------------------------|---------|--------|--------|--------|
|                    |        | I                                      | SRM 1950 Metabolites in Frozen Human |        |        |                     |                              | Human Plasma B (µmol/L) |        |        |                    |                              |         |        |        |        |
|                    | Lab    | Α                                      | В                                    | С      | Avg    | SD                  | Α                            | В                       | С      | Avg    | SD                 | Α                            | В       | С      | Avg    | SD     |
| Individual Results | Target |  |                                      |        | 44.7   | 2.2                 |                              |                         |        | 54.6   | 3.6                |                              |         |        |        |        |
|                    | A004   | 25.0                                   | 23.5                                 | 24.0   | 24.2   | 0.76                | 65.6                         | 55.4                    | 63.6   | 61.5   | 5.4                | 135.4                        | 137.2   | 127.5  | 133.4  | 5.2    |
|                    | A012   | 0.0488                                 | 0.0458                               | 0.0483 | 0.0476 | 0.0016              | 0.0554                       | 0.0473                  | 0.0463 | 0.0497 | 0.0050             | 0.112                        | 0.121   | 0.120  | 0.118  | 0.0049 |
|                    | A016   |  |                                      |        |        |                     |                              |                         |        |        |                    |                              |         |        |        |        |
|                    | A024   | 41.75                                  | 45.06                                | 43.93  | 43.58  | 1.68                | 55.53                        | 52.62                   | 55.73  | 54.63  | 1.7                | 118.95                       | 125.33  | 119.83 | 121.37 | 3.5    |
|                    | A028   |  |                                      |        |        |                     |                              |                         |        |        |                    |                              |         |        |        |        |
|                    | A049   |  |                                      |        |        |                     |                              |                         |        |        |                    |                              |         |        |        |        |
|                    | A050   |  |                                      |        |        |                     |                              |                         |        |        |                    |                              |         |        |        |        |
|                    | A052   |  |                                      |        |        |                     |                              |                         |        |        |                    |                              |         |        |        |        |
|                    | A070   |  |                                      |        |        |                     |                              |                         |        |        |                    |                              |         |        |        |        |
|                    | A079   |  |                                      |        |        |                     |                              |                         |        |        |                    |                              |         |        |        |        |
|                    | A083   |  |                                      |        |        |                     |                              |                         |        |        |                    |                              |         |        |        |        |
|                    | A085   |  |                                      |        |        |                     |                              |                         |        |        |                    |                              |         |        |        |        |
|                    | A087   | 41.786                                 | 41.608                               | 41.49  | 41.628 | 0.14901             | 50.097                       | 49.936                  | 53.474 | 51.169 | 2.0                | 116.96                       | 114.308 | 115.61 | 115.63 | 1.3    |
|                    | A090   | 44                                     | 44                                   | 44     | 44     | 0                   | 49                           | 47                      | 49     | 48     | 1.2                | 115                          | 115     | 119    | 116    | 2.3    |
|                    | A097   | 22.52                                  | 24.56                                | 21.82  | 22.97  | 1.4                 | 30.8                         | 31.3                    | 28.4   | 30.2   | 1.6                | 42.6                         | 45.7    | 48.1   | 45.5   | 2.8    |
|                    | A099   | 51                                     | 47                                   | 46     | 48     | 2.6                 | 57                           | 57                      | 55     | 56     | 1.2                | 138                          | 124     | 130    | 131    | 7.0    |
|                    | A100   | 34.81                                  | 34.9                                 | 34.67  | 34.79  | 0.12                | 15.19                        | 14.49                   | 14.95  | 14.88  | 0.36               | 32.15                        | 33.47   | 33.91  | 33.18  | 0.92   |
|                    | A107   | 50.2                                   | 49.6                                 | 50.5   | 50.1   | 0.46                | 53.9                         | 54.4                    | 54.8   | 54.4   | 0.45               | 132                          | 134     | 130    | 132    | 2      |
| ty                 |        | Consensus Mean 35.9                    |                                      |        |        | Consensus Mean 45.7 |                              |                         |        |        | Consensus Mean 111 |                              |         |        |        |        |
| mmuni<br>esults    |        | Consensus Standard Deviation           |                                      |        | 15     |                     | Consensus Standard Deviation |                         |        | 14     |                    | Consensus Standard Deviation |         |        | 26     |        |
|                    |        | Maximum                                |                                      |        | 50.1   |                     | Maximun                      | 1                       |        | 61.5   |                    | Maximun                      | 1       |        | 133.4  |        |
| R                  |        | Minimum                                |                                      |        | 0.0476 |                     | Minimum                      |                         |        | 0.0497 |                    | Minimum                      |         |        | 0.118  |        |
| 5                  |        | Ν                                      |                                      |        | 9      |                     | Ν                            |                         |        | 9      |                    | Ν                            |         |        | 9      |        |

Exercise HAMQAP Exercise 1 - Human Metabolites Sample: Fatty Acids Solution B Measurand: Total alpha-Linolenic Acid (C18:3 n-3)



Figure 5-57. Total  $\alpha$ -linolenic acid (C18:3 n-3) in Fatty Acids Solution B (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \leq 2$ . The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by its uncertainty ( $U_{NIST}$ ) and represents the range that results in an acceptable  $Z'_{NIST}| \leq 2$ .

Exercise HAMQAP Exercise 1 - Human Metabolites Sample: SRM 1950 Metabolites in Frozen Human Plasma Measurand: Total alpha-Linolenic Acid (C18:3 n-3)



Figure 5-58. Total  $\alpha$ -linolenic acid (C18:3 n-3) in SRM 1950 Metabolites in Frozen Human Serum (data summary view –analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \leq 2$ . The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by its uncertainty ( $U_{NIST}$ ) and represents the range that results in an acceptable  $Z'_{NIST}| \leq 2$ .

Exercise HAMQAP Exercise 1 - Human Metabolites Sample: Human Plasma B Measurand: Total alpha-Linolenic Acid (C18:3 n-3)



Figure 5-59. Total  $\alpha$ -linolenic acid (C18:3 n-3) in Human Plasma B (data summary view –analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \leq 2$ . A NIST value has not been determined in this material.



HAMQAP Exercise 1 - Human Metabolites, Measurand: Total alpha-Linolenic Acid (C18:3 n-3) No. of laboratories: 9

Figure 5-60. Laboratory means for total  $\alpha$ -linolenic acid in Fatty Acids Solution B and SRM 1950 Metabolites in Frozen Human Plasma (sample/sample comparison view). In this view, the individual laboratory mean for one sample (Fatty Acids Solution B) is compared to the mean for a second sample (SRM 1950). The solid red box represents the NIST range of tolerance for the two samples, Fatty Acids Solution B (x-axis) and SRM 1950 (y-axis), which encompasses the target values bounded by their uncertainties ( $U_{\text{NIST}}$ ) and represents the range that results in an acceptable  $Z_{\text{NIST}}$  score,  $|Z_{\text{NIST}}| \leq 2$ . The dotted blue box represents the consensus range of tolerance for Fatty Acids Solution B (x-axis) and SRM 1950 (y-axis), calculated as the values above and below the consensus means that result in an acceptable  $Z'_{\text{comm}} \leq 2$ .

**Table 5-30.** Data summary table for total  $\gamma$ -linolenic acid in a fatty acids solution and human plasma. Data points highlighted in red have been flagged as potential outliers (e.g., difference from reference value, Grubb and/or Cochran) by the NIST software package.

|                     |        | Total gamma-Linolenic Acid (C18:3 n-6) |                                      |         |       |         |                              |                         |        |        |         |                              |        |        |        |        |
|---------------------|--------|--|--------------------------------------|---------|-------|---------|------------------------------|-------------------------|--------|--------|---------|------------------------------|--------|--------|--------|--------|
| -                   |        | F                                      | SRM 1950 Metabolites in Frozen Human |         |       |         |                              | Human Plasma B (µmol/L) |        |        |         |                              |        |        |        |        |
|                     | Lab    | Α                                      | В                                    | С       | Avg   | SD      | Α                            | В                       | С      | Avg    | SD      | Α                            | В      | С      | Avg    | SD     |
| ndividual Results   | Target |  |                                      |         |       |         |                              |                         |        | 39.9   | 8.5     |                              |        |        |        |        |
|                     | A004   |  |                                      |         |       |         |                              |                         |        |        |         |                              |        |        |        |        |
|                     | A012   |  |                                      |         |       |         | 0.0514                       | 0.0433                  | 0.0437 | 0.0461 | 0.0046  | 0.0744                       | 0.0804 | 0.0803 | 0.0784 | 0.0034 |
|                     | A016   |  |                                      |         |       |         |                              |                         |        |        |         |                              |        |        |        |        |
|                     | A024   | 0.055                                  | 0.45                                 | 0.28    | 0.26  | 0.20    | 60.39                        | 54.72                   | 56.13  | 57.08  | 3.0     | 91.17                        | 97.61  | 91.92  | 93.57  | 3.5    |
|                     | A028   |  |                                      |         |       |         |                              |                         |        |        |         |                              |        |        |        |        |
|                     | A049   |  |                                      |         |       |         |                              |                         |        |        |         |                              |        |        |        |        |
|                     | A050   |  |                                      |         |       |         |                              |                         |        |        |         |                              |        |        |        |        |
|                     | A052   |  |                                      |         |       |         |                              |                         |        |        |         |                              |        |        |        |        |
|                     | A070   |  |                                      |         |       |         |                              |                         |        |        |         |                              |        |        |        |        |
|                     | A079   |  |                                      |         |       |         |                              |                         |        |        |         |                              |        |        |        |        |
|                     | A083   |  |                                      |         |       |         |                              |                         |        |        |         |                              |        |        |        |        |
| I                   | A085   |  |                                      |         |       |         |                              |                         |        |        |         |                              |        |        |        |        |
|                     | A087   | 0                                      | 0                                    | 0       | 0     | 0       | 51.593                       | 50.982                  | 55.400 | 52.658 | 2.4     | 87.135                       | 85.495 | 86.592 | 86.407 | 0.84   |
|                     | A090   | < 1.000                                | 1                                    | < 1.000 | 1     |         | 59                           | 56                      | 58     | 58     | 1.5     | 96                           | 100    | 99     | 98     | 2.1    |
|                     | A097   | 2.08                                   | 1.52                                 | 1.33    | 1.64  | 0.39    | 39.1                         | 38.5                    | 38.2   | 38.6   | 0.46    | 60.2                         | 55.9   | 63.1   | 59.7   | 3.6    |
|                     | A099   |  |                                      |         |       |         | 61                           | 61                      | 58     | 60     | 1.7     | 104                          | 94     | 97     | 98     | 5.1    |
|                     | A100   | < 1.000                                | < 1.000                              | < 1.000 |       |         | 15.48                        | 14.97                   | 15.94  | 15.46  | 0.49    | 25.58                        | 26.65  | 26.85  | 26.36  | 0.68   |
|                     | A107   | < 0.000                                | 0.605                                | < 0.000 | 0.605 |         | 50.3                         | 51.0                    | 51.9   | 51.1   | 0.80    | 91.0                         | 91.4   | 88.3   | 90.2   | 1.7    |
| ommunity<br>Results |        | Consensus Mean                         |                                      |         | 0.702 |         | Consensus Mean               |                         |        | 46.0   |         | Consensus Mean               |        |        | 81.7   |        |
|                     |        | Consensus Standard Deviation           |                                      |         | 0.94  |         | Consensus Standard Deviation |                         |        | 15     |         | Consensus Standard Deviation |        |        | 20     |        |
|                     |        | Maximum                                |                                      | 1.64    |       | Maximun | 1                            | 60                      |        |        | Maximum |                              |        | 98     |        |        |
|                     |        | Minimum                                |                                      |         | 0     |         | Minimum                      |                         |        | 0.0461 |         | Minimum                      |        |        | 0.0784 |        |
| 2                   |        | Ν                                      |                                      |         | 3     |         | Ν                            |                         |        | 8      |         | Ν                            |        |        | 8      |        |

Exercise HAMQAP Exercise 1 - Human Metabolites Sample: Fatty Acids Solution B Measurand: Total gamma-Linolenic Acid (C18:3 n-6)



Figure 5-61. Total  $\gamma$ -linolenic acid (C18:3 n-6) in Fatty Acids Solution B (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red line represents the upper consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \leq 2$ , with the lower range set at zero. A NIST value has not been determined in this material.



Figure 5-62. Total  $\gamma$ -linolenic acid (C18:3 n-6) in SRM 1950 Metabolites in Frozen Human Serum (data summary view –analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \leq 2$ . The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by its uncertainty ( $U_{NIST}$ ) and represents the range that results in an acceptable  $Z_{NIST} | \leq 2$ .

Exercise

HAMQAP Exercise 1 - Human Metabolites
Exercise HAMQAP Exercise 1 - Human Metabolites Sample: Human Plasma B Measurand: Total gamma-Linolenic Acid (C18:3 n-6)



**Figure 5-63.** Total  $\gamma$ -linolenic acid (C18:3 n-6) in Human Plasma B (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \leq 2$ . A NIST value has not been determined in this material.



HAMQAP Exercise 1 - Human Metabolites, Measurand: Total gamma-Linolenic Acid (C18:3 n-6) No. of laboratories: 5

**Figure 5-64.** Laboratory means for total  $\gamma$ -linolenic acid in Fatty Acids Solution B and SRM 1950 Metabolites in Frozen Human Plasma (sample/sample comparison view). In this view, the individual laboratory mean for one sample (Fatty Acids Solution B) is compared to the mean for a second sample (SRM 1950). The dotted blue box represents the consensus range of tolerance for Fatty Acids Solution B (x-axis) and SRM 1950 (y-axis), calculated as the values above and below the consensus means that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \leq 2$ .

**Table 5-31.** Data summary table for total arachidonic acid in a fatty acids solution and human plasma. Data points highlighted in red have been flagged as potential outliers (e.g., difference from reference value, Grubb and/or Cochran) by the NIST software package.

|            | 1      |          |             |            |            |       | Tot      | al Arachic  | lonic Acid  | (C20:4 n-  | 6)    |          |             |            |          |       |
|------------|--------|----------|-------------|------------|------------|-------|----------|-------------|-------------|------------|-------|----------|-------------|------------|----------|-------|
|            |        | F        | Fatty Acids | s Solution | B (µmol/L) |       | SRM      | 1950 Met    | abolites ir | n Frozen H | luman |          | Human I     | Plasma B ( | (µmol/L) |       |
|            | Lab    | A        | В           | С          | Avg        | SD    | A        | В           | С           | Avg        | SD    | A        | В           | С          | Avg      | SD    |
|            | Target |          |             |            | 806        | 40    |          |             |             | 984        | 180   |          |             |            |          |       |
|            | A004   | 537.2    | 482.9       | 511.6      | 510.6      | 27    | 725.2    | 755         | 739.5       | 739.9      | 15    | 978.1    | 1056.7      | 1048.5     | 1027.8   | 43    |
|            | A012   | 0.92     | 0.86        | 0.904      | 0.89       | 0.031 | 0.770    | 0.660       | 0.652       | 0.694      | 0.066 | 0.787    | 0.86        | 0.845      | 0.831    | 0.039 |
|            | A016   |          |             |            |            |       |          |             |             |            |       |          |             |            |          |       |
|            | A024   | 807.31   | 815.33      | 816.27     | 812.97     | 4.9   | 794.46   | 708.43      | 724.7       | 742.53     | 46    | 825.49   | 878.79      | 840.85     | 848.38   | 27    |
|            | A028   |          |             |            |            |       |          |             |             |            |       |          |             |            |          |       |
| ults       | A049   |          |             |            | L          |       |          |             |             | L          |       |          |             | /          |          | /     |
| Ses        | A052   |          |             |            |            |       |          |             |             |            |       |          |             |            |          |       |
| al I       | A070   |          |             | ]          | L          |       |          |             |             | L          |       |          |             | !          |          | !     |
| iĝ         | A079   |          |             |            |            |       |          |             |             |            |       |          |             |            |          |       |
| divi       | A083   |          |             | ]          | L          |       |          |             |             | L          |       |          |             | !          |          | !     |
| Ē          | A085   |          |             |            |            |       |          |             |             |            |       |          |             |            |          |       |
|            | A087   | 763.614  | 764.126     | 761.870    | 763.203    | 1.2   | 674.751  | 678.686     | 734.263     | 695.900    | 33    | 811.469  | 799.792     | 815.033    | 808.765  | 8.0   |
|            | A090   | 807      | 851         | 820        | 826        | 22.6  | 771      | 751         | 771         | 764        | 12    | 920      | 912         | 925        | 919      | 6.6   |
|            | A097   | 1048     | 975         | 964        | 996        | 46    | 909      | 915         | 910         | 911        | 3.2   | 1100     | 1052        | 1023       | 1058     | 39    |
|            | A099   | 940      | 840         | 826        | 869        | 62    | 810      | 820         | 785         | 805        | 18    | 997      | 920         | 935        | 951      | 41    |
| 1          | A100   | 655      | 660.19      | 645.79     | 653.66     | 7.3   | 202.99   | 212.01      | 199.28      | 204.76     | 6.5   | 254.94   | 261.5       | 251.66     | 256.0    | 5.0   |
|            | A107   | 847      | 909         | 854        | 870        | 34    | 695      | 694         | 664         | 684        | 18    | 861      | 858         | 862        | 860      | 2.1   |
| ty.        | ,      | Consensu | s Mean      | _          | 761        |       | Consensu | is Mean     |             | 752        |       | Consensu | is Mean     |            | 871      | _     |
| uni<br>Its | , I    | Consensu | s Standard  | Deviation  | 217        | I     | Consensu | is Standard | Deviation   | 139        | I     | Consensu | is Standard | Deviation  | 208      | 1     |
| esu        | , 1    | Maximum  | 1           |            | 996        | 1     | Maximum  | .1          |             | 911        | 1     | Maximum  | .1          |            | 1058     | 1     |
| 5 ×        | , I    | Minimum  |             |            | 0.89       | l     | Minimum  |             |             | 0.694      | l     | Minimum  |             |            | 0.831    | l     |
| ~ I        | , I    | Ν        |             |            | 9          | 1     | Ν        |             |             | 9          | 1     | Ν        |             |            | 9        |       |

Exercise HAMQAP Exercise 1 - Human Metabolites Sample: Fatty Acids Solution B Measurand: Total Arachidonic Acid (C20:4 n-6)



**Figure 5-65.** Total arachidonic acid (C20:4 n-6) in Fatty Acids Solution B (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \le 2$ . The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by its uncertainty ( $U_{NIST}$ ) and represents the range that results in an acceptable  $Z_{NIST}| \le 2$ .

Exercise HAMOAP Exercise 1 - Human Metabolites Sample: SRM 1950 Metabolites in Frozen Human Plasma Measurand: Total Arachidonic Acid (C20:4 n-6)



**Figure 5-66.** Total arachidonic acid (C20:4 n-6) in SRM 1950 Metabolites in Frozen Human Serum (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \leq 2$ . The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by its uncertainty ( $U_{NIST}$ ) and represents the range that results in an acceptable  $Z'_{NIST} | \leq 2$ .

Exercise HAMQAP Exercise 1 - Human Metabolites Sample: Human Plasma B Measurand: Total Arachidonic Acid (C20:4 n-6)



**Figure 5-67.** Total arachidonic acid (C20:4 n-6) in Human Plasma B analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \leq 2$ . A NIST value has not been determined in this material.



HAMQAP Exercise 1 - Human Metabolites, Measurand: Total Arachidonic Acid (C20:4 n-6) No. of laboratories: 9

**Figure 5-68.** Laboratory means for total arachidonic acid in Fatty Acids Solution B and SRM 1950 Metabolites in Frozen Human Plasma (sample/sample comparison view). In this view, the individual laboratory mean for one sample (Fatty Acids Solution B) is compared to the mean for a second sample (SRM 1950). The solid red box represents the NIST range of tolerance for the two samples, Fatty Acids Solution B (x-axis) and SRM 1950 (y-axis), which encompasses the target values bounded by their uncertainties ( $U_{\text{NIST}}$ ) and represents the range that results in an acceptable  $Z_{\text{NIST}}$  score,  $|Z_{\text{NIST}}| \leq 2$ . The dotted blue box represents the consensus range of tolerance for Fatty Acids Solution B (x-axis) and SRM 1950 (y-axis), calculated as the values above and below the consensus means that result in an acceptable  $Z'_{\text{comm}} | \leq 2$ .

**Table 5-32.** Data summary table for total EPA in a fatty acids solution and human plasma. Data points highlighted in red have been flagged as potential outliers (e.g., difference from reference value, Grubb and/or Cochran) by the NIST software package.

|            |        |          |             |            |            |        |          | Total E     | PA (C20:    | 5 n-3)     |        |          |             |            |          |        |
|------------|--------|----------|-------------|------------|------------|--------|----------|-------------|-------------|------------|--------|----------|-------------|------------|----------|--------|
|            |        | I        | Fatty Acids | s Solution | B (µmol/L) | )      | SRM      | 1950 Met    | abolites ii | n Frozen H | Iuman  |          | Human l     | Plasma B ( | (µmol/L) |        |
|            | Lab    | Α        | В           | С          | Avg        | SD     | Α        | В           | С           | Avg        | SD     | Α        | В           | С          | Avg      | SD     |
|            | Target |          |             |            | 36.8       | 1.8    |          |             |             | 38.6       | 0.5    |          |             |            |          |        |
|            | A004   | 15.6     | 14.6        | 15.5       | 15.2       | 0.55   | 70.1     | 48.4        | 53.1        | 57.2       | 11     | 128.0    | 103.2       | 84.3       | 105.2    | 22     |
|            | A012   | 0.042    | 0.0406      | 0.0428     | 0.0418     | 0.0011 | 0.0445   | 0.0383      | 0.0382      | 0.0403     | 0.0036 | 0.0911   | 0.0999      | 0.0991     | 0.0967   | 0.0049 |
|            | A016   |          |             |            |            |        |          |             |             |            |        |          |             |            |          |        |
|            | A024   | 35.94    | 36.28       | 36.24      | 36.15      | 0.19   | 44.32    | 40.18       | 39.92       | 41.47      | 2.5    | 95.26    | 100.52      | 95.83      | 97.20    | 2.9    |
|            | A028   |          |             |            |            |        |          |             |             |            |        |          |             |            |          |        |
| ts         | A049   |          |             |            |            |        |          |             |             |            |        |          |             |            |          |        |
| Ins        | A050   |          |             |            |            |        |          |             |             |            |        |          |             |            |          |        |
| R          | A052   |          |             |            |            |        |          |             |             |            |        |          |             |            |          |        |
| ual        | A070   |          |             |            |            |        |          |             |             |            |        |          |             |            |          |        |
| vid        | A079   |          |             |            |            |        |          |             |             |            |        |          |             |            |          |        |
| ibu        | A083   |          |             |            |            |        |          |             |             |            |        |          |             |            |          |        |
| I          | A085   |          |             |            |            |        |          |             |             |            |        |          |             |            |          |        |
|            | A087   | 33.833   | 33.636      | 33.475     | 33.648     | 0.18   | 39.427   | 39.559      | 42.44       | 40.475     | 1.7    | 93.286   | 92.218      | 94.006     | 93.170   | 0.90   |
|            | A090   | 54       | 53          | 54         | 54         | 0.58   | 60       | 57          | 61          | 59         | 2      | 133      | 131         | 133        | 132      | 1.2    |
|            | A097   | 34.3     | 37.5        | 40.1       | 37.3       | 2.9    | 35.2     | 36.4        | 36.1        | 35.9       | 0.62   | 80.1     | 78.4        | 79.3       | 79.3     | 0.85   |
|            | A099   | 44       | 37          | 39         | 40         | 3.6    | 45       | 47          | 41          | 44         | 3.1    | 110      | 106         | 97.5       | 105      | 6.4    |
|            | A100   | 27.74    | 27.72       | 27.43      | 27.63      | 0.17   | 10.82    | 11.35       | 11.04       | 11.07      | 0.27   | 26.8     | 27.86       | 27.15      | 27.27    | 0.54   |
|            | A107   | 40.5     | 39.0        | 41.0       | 40.2       | 1.0    | 42.8     | 42.4        | 42.8        | 42.7       | 0.23   | 103      | 104         | 100        | 102      | 2.1    |
| ty         |        | Consensu | s Mean      |            | 32.8       |        | Consensu | is Mean     |             | 39.5       |        | Consensu | is Mean     |            | 88.0     |        |
| uni<br>Its |        | Consensu | s Standard  | Deviation  | 14.1       |        | Consensu | is Standard | Deviation   | 15         |        | Consensu | is Standard | Deviation  | 33       |        |
| esu        |        | Maximum  | l I         |            | 54         |        | Maximun  | ı           |             | 59         |        | Maximun  | ı           |            | 132      |        |
| N N        |        | Minimum  |             |            | 0.0418     |        | Minimum  |             |             | 0.0403     |        | Minimum  |             |            | 0.0967   |        |
| -          |        | Ν        |             |            | 9          |        | Ν        |             |             | 9          |        | Ν        |             |            | 9        |        |

Exercise HAMQAP Exercise 1 - Human Metabolites Sample: Fatty Acids Solution B Measurand: Total EPA (C20:5 n-3)



**Figure 5-69.** Total EPA (C20:5 n-3) in Fatty Acids Solution B (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \leq 2$ . The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by its uncertainty ( $U_{NIST}$ ) and represents the range that results in an acceptable  $Z_{NIST}$  score,  $|Z_{NIST}| \leq 2$ .

Exercise HAMQAP Exercise 1 - Human Metabolites Sample: SRM 1950 Metabolites in Frozen Human Plasma Measurand: Total EPA (C20:5 n-3)



**Figure 5-70.** Total EPA (C20:5 n-3) in SRM 1950 Metabolites in Frozen Human Serum (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \leq 2$ . The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by its uncertainty ( $U_{NIST}$ ) and represents the range that results in an acceptable  $Z_{NIST}$  score,  $|Z_{NIST}| \leq 2$ .

Exercise HAMQAP Exercise 1 - Human Metabolites Sample: Human Plasma B Measurand: Total EPA (C20:5 n-3)



**Figure 5-71.** Total EPA (C20:5 n-3) in Human Plasma B (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \leq 2$ . A NIST value has not been determined in this material.



#### HAMQAP Exercise 1 - Human Metabolites, Measurand: Total EPA (C20:5 n-3) No. of laboratories: 9

**Figure 5-72.** Laboratory means for total EPA in Fatty Acids Solution B and SRM 1950 Metabolites in Frozen Human Plasma (sample/sample comparison view). In this view, the individual laboratory mean for one sample (Fatty Acids Solution B) is compared to the mean for a second sample (SRM 1950). The solid red box represents the NIST range of tolerance for the two samples, Fatty Acids Solution B (x-axis) and SRM 1950 (y-axis), which encompasses the target values bounded by their uncertainties ( $U_{\text{NIST}}$ ) and represents the range that results in an acceptable  $Z_{\text{NIST}}$  score,  $|Z_{\text{NIST}}| \leq 2$ . The dotted blue box represents the consensus range of tolerance for Fatty Acids Solution B (x-axis) and SRM 1950 (y-axis), calculated as the values above and below the consensus means that result in an acceptable  $Z'_{\text{comm}} \leq 2$ .

**Table 5-33.** Data summary table for total DPA in a fatty acids solution and human plasma. Data points highlighted in red have been flagged as potential outliers (e.g., difference from reference value, Grubb and/or Cochran) by the NIST software package.

|            | 1      |          |            |            |           | · · · · · · · · · · · · · · · · · · · | · · · · · · · · · · · · · · · · · · · | Total D     | PA (C22:    | 5 n-3)     | · · · · · · · · · · · · · · · · · · · |          |             | · · · · · · · · · · · · · · · · · · · |          |        |
|------------|--------|----------|------------|------------|-----------|---------------------------------------|---------------------------------------|-------------|-------------|------------|---------------------------------------|----------|-------------|---------------------------------------|----------|--------|
| _          |        | I        | atty Acid  | s Solution | B (µmol/L | .)                                    | SRM                                   | 1950 Met    | abolites ir | a Frozen F | Iuman                                 |          | Human I     | Plasma B (                            | (µmol/L) |        |
|            | Lab    | Α        | В          | С          | Avg       | SD                                    | A                                     | В           | С           | Avg        | SD                                    | Α        | В           | С                                     | Avg      | SD     |
|            | Target |          |            |            | 38.2      | 1.9                                   |                                       |             |             | 38.5       | 0.7                                   |          |             |                                       |          |        |
|            | A004   | 9.0      | 8.9        | 7.3        | 8.4       | 0.95                                  | 27.1                                  | 36.6        | 38.3        | 34.0       | 6.0                                   | 63.9     | 63.4        | 75.2                                  | 67.5     | 6.7    |
|            | A012   | 0.0375   | 0.0361     | 0.0376     | 0.0371    | 0.00084                               | 0.0378                                | 0.0336      | 0.0325      | 0.0346     | 0.0028                                | 0.0625   | 0.0681      | 0.0674                                | 0.0660   | 0.0031 |
|            | A016   |          |            |            |           |                                       |                                       |             |             |            |                                       |          |             |                                       |          |        |
|            | A024   | 31.43    | 33.82      | 33.74      | 33.0      | 1.4                                   | 41.91                                 | 38.27       | 39.79       | 39.99      | 1.8                                   | 70.53    | 74.96       | 71.28                                 | 72.26    | 2.4    |
|            | A028   |          |            |            |           |                                       |                                       |             |             |            |                                       |          |             |                                       |          |        |
| ults       | A049   |          |            |            |           |                                       | L                                     |             |             | 1          | /                                     |          |             |                                       | L        |        |
| Resi       | A050   |          |            |            |           |                                       |                                       |             |             |            |                                       |          |             |                                       |          |        |
| al F       | A052   |          |            |            |           |                                       | 1                                     |             |             | 1          | I                                     |          |             |                                       |          |        |
| inpi       | A070   |          |            |            |           |                                       |                                       |             |             |            |                                       |          |             |                                       |          |        |
| divi       | A079   |          |            |            |           |                                       | 1                                     |             |             | 1          | !                                     |          |             |                                       | L        |        |
| In         | A083   |          |            |            |           |                                       |                                       |             |             |            |                                       |          |             |                                       |          |        |
|            | A085   |          |            |            |           |                                       | I                                     |             |             | 1          | !                                     |          |             |                                       |          |        |
|            | A087   | 35.175   | 35.411     | 34.765     | 35.117    | 0.33                                  | 37.869                                | 37.813      | 40.885      | 38.856     | 1.8                                   | 72.611   | 71.158      | 74.012                                | 72.594   | 1.4    |
|            | A090   | 43       | 43         | 43         | 43        | 0                                     | 45                                    | 43          | 44          | 44         | 1                                     | 83       | 83          | 84                                    | 83       | 0.58   |
|            | A099   |          |            |            |           |                                       |                                       |             |             |            |                                       |          |             |                                       |          |        |
|            | A100   | 37.82    | 38.06      | 37.77      | 37.88     | 0.16                                  | 12.08                                 | 13.47       | 11.78       | 12.44      | 0.90                                  | 24.99    | 26.04       | 24.16                                 | 25.06    | 0.94   |
|            | A107   | 40.4     | 39.7       | 39.9       | 40.0      | 0.36                                  | 38.9                                  | 36.6        | 37.8        | 37.8       | 1.2                                   | 76.3     | 73.8        | 76.4                                  | 75.5     | 1.5    |
| ty         |        | Consensu | s Mean     |            | 31.2      | — I                                   | Consensu                              | is Mean     |             | 35.2       |                                       | Consensu | ıs Mean     |                                       | 66.9     |        |
| uni<br>Its |        | Consensu | s Standard | Deviation  | 11        |                                       | Consensu                              | is Standard | Deviation   | 9.0        | 1                                     | Consensu | ıs Standard | Deviation                             | 17.3     |        |
| nm         |        | Maximum  | L          |            | 43        |                                       | Maximur                               | .1          |             | 44         | ļ                                     | Maximum  | 1           |                                       | 83       |        |
| R G        |        | Minimum  |            |            | 0.0371    |                                       | Minimum                               |             |             | 0.0346     | 1                                     | Minimum  |             |                                       | 0.0660   |        |
| 9          |        | Ν        |            |            | 7         |                                       | Ν                                     |             |             | 7          |                                       | Ν        |             |                                       | 7        |        |

Exercise HAMQAP Exercise 1 - Human Metabolites Sample: Fatty Acids Solution B Measurand: Total DPA (C22:5 n-3)



**Figure 5-73.** Total DPA (C22:5 n-3) in Fatty Acids Solution B (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \leq 2$ . The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by its uncertainty ( $U_{NIST}$ ) and represents the range that results in an acceptable  $Z_{NIST}$  score,  $|Z_{NIST}| \leq 2$ .

Exercise HAMQAP Exercise 1 - Human Metabolites Sample: SRM 1950 Metabolites in Frozen Human Plasma Measurand: Total DPA (C22:5 n-3)



**Figure 5-74.** Total DPA (C22:5 n-3) in SRM 1950 Metabolites in Frozen Human Serum (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \leq 2$ . The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by its uncertainty ( $U_{NIST}$ ) and represents the range that results in an acceptable  $Z_{NIST}| \leq 2$ .

Exercise HAMQAP Exercise 1 - Human Metabolites Sample: Human Plasma B Measurand: Total DPA (C22:5 n-3)



**Figure 5-75.** Total DPA (C22:5 n-3) in Human Plasma B (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \leq 2$ . A NIST value has not been determined in this material.



#### HAMQAP Exercise 1 - Human Metabolites, Measurand: Total DPA (C22:5 n-3) No. of laboratories: 7

**Figure 5-76.** Laboratory means for total DPA in Fatty Acids Solution B and SRM 1950 Metabolites in Frozen Human Plasma (sample/sample comparison view). In this view, the individual laboratory mean for one sample (Fatty Acids Solution B) is compared to the mean for a second sample (SRM 1950). The solid red box represents the NIST range of tolerance for the two samples, Fatty Acids Solution B (x-axis) and SRM 1950 (y-axis), which encompasses the target values bounded by their uncertainties ( $U_{\text{NIST}}$ ) and represents the range that results in an acceptable  $Z_{\text{NIST}}$  score,  $|Z_{\text{NIST}}| \leq 2$ . The dotted blue box represents the consensus range of tolerance for Fatty Acids Solution B (x-axis) and SRM 1950 (y-axis), calculated as the values above and below the consensus means that result in an acceptable  $Z'_{\text{comm}} \leq 2$ .

**Table 5-34.** Data summary table for total DHA in a fatty acids solution and human plasma. Data points highlighted in red have been flagged as potential outliers (e.g., difference from reference value, Grubb and/or Cochran) by the NIST software package.

|            | 1      |           |            |           |            |        |          | Total D     | HA (C22:    | 6 n-3)     |        |          |             |            |          |        |
|------------|--------|-----------|------------|-----------|------------|--------|----------|-------------|-------------|------------|--------|----------|-------------|------------|----------|--------|
|            |        | I         | atty Acids | Solution  | B (µmol/L) | )      | SRM      | 1950 Met    | abolites ir | ı Frozen H | Iuman  |          | Human I     | Plasma B ( | (µmol/L) |        |
|            | Lab    | Α         | В          | С         | Avg        | SD     | Α        | В           | С           | Avg        | SD     | Α        | В           | С          | Avg      | SD     |
|            | Target |           |            |           | 98.8       | 4.9    |          |             |             | 118        | 21     |          |             |            |          |        |
|            | A004   | 25.5      | 21.0       | 22.8      | 23.1       | 2.3    | 126.7    | 112.6       | 105.0       | 114.8      | 11     | 205.7    | 182.5       | 174.1      | 187.4    | 16.4   |
|            | A012   | 0.109     | 0.102      | 0.107     | 0.106      | 0.0036 | 0.114    | 0.0995      | 0.0982      | 0.104      | 0.0088 | 0.137    | 0.149       | 0.147      | 0.144    | 0.0064 |
|            | A016   |           |            |           |            |        |          |             |             |            |        |          |             |            |          |        |
|            | A024   | 93.63     | 91.39      | 93.49     | 92.84      | 1.3    | 116.13   | 105.75      | 111.07      | 110.98     | 5.2    | 145.70   | 156.53      | 151.00     | 151.08   | 5.4    |
|            | A028   |           |            |           |            |        |          |             |             |            |        |          |             |            |          |        |
| lts        | A049   |           |            |           |            |        |          |             |             |            |        |          |             |            |          |        |
| Inse       | A050   |           |            |           |            |        |          |             |             |            |        |          |             |            |          |        |
| Re         | A052   |           |            |           |            |        |          |             |             |            |        |          |             |            |          |        |
| ual        | A070   |           |            |           |            |        |          |             |             |            |        |          |             |            |          |        |
| ivid       | A079   |           |            |           |            |        |          |             |             |            |        |          |             |            |          |        |
| ibu        | A083   |           |            |           |            |        |          |             |             |            |        |          |             |            |          |        |
| I          | A085   |           |            |           |            |        |          |             |             |            |        |          |             |            |          |        |
|            | A087   | 90.101    | 89.549     | 89.804    | 89.818     | 0.28   | 100.946  | 100.437     | 108.767     | 103.383    | 4.7    | 141.242  | 139.517     | 141.786    | 140.848  | 1.2    |
|            | A090   | 96        | 93         | 96        | 95         | 1.7    | 97       | 96          | 98          | 97         | 1      | 130      | 133         | 134        | 132      | 2.1    |
|            | A097   | 98.3      | 90.6       | 97.2      | 95.4       | 4.2    | 110.2    | 109.3       | 109.4       | 109.6      | 0.49   | 130.4    | 129.4       | 156.1      | 138.6    | 15.1   |
|            | A099   | 110       | 104        | 100       | 105        | 5.0    | 110      | 125         | 110         | 115        | 8.7    | 165      | 160         | 160        | 162      | 2.9    |
|            | A100   | 92.74     | 91.83      | 91.52     | 92.03      | 0.63   | 35       | 38.22       | 34.79       | 36.00      | 1.9    | 54.24    | 55.59       | 51.83      | 53.89    | 1.9    |
|            | A107   | 105       | 106        | 109       | 107        | 2.1    | 102      | 101         | 107         | 103        | 3.2    | 151      | 154         | 152        | 152      | 1.5    |
| ty         |        | Consensus | s Mean     |           | 96.6       | I      | Consensu | ıs Mean     |             | 108        | 1      | Consensu | is Mean     |            | 143      |        |
| uni<br>lts |        | Consensus | s Standard | Deviation | 12         | I      | Consensu | is Standard | Deviation   | 15         | 1      | Consensu | is Standard | Deviation  | 35       |        |
| nm<br>esu  |        | Maximum   | 1          |           | 107        | I      | Maximur  | 1           |             | 115        | 1      | Maximum  | 1           |            | 187.4    |        |
| R G        |        | Minimum   |            |           | 0.106      | I      | Minimum  |             |             | 0.104      | 1      | Minimum  |             |            | 0.144    |        |
| 0          |        | Ν         |            |           | 9          | ł      | Ν        |             |             | 9          |        | Ν        |             |            | 9        |        |

Exercise HAMQAP Exercise 1 - Human Metabolites Sample: Fatty Acids Solution B Measurand: Total DHA (C22:6 n-3)



**Figure 5-77.** Total DHA (C22:6 n-3) in Fatty Acids Solution B (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \leq 2$ . The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by its uncertainty ( $U_{NIST}$ ) and represents the range that results in an acceptable  $Z_{NIST}$  score,  $|Z_{NIST}| \leq 2$ .

Exercise HAMQAP Exercise 1 - Human Metabolites Sample: SRM 1950 Metabolites in Frozen Human Plasma Measurand: Total DHA (C22:6 n-3)



**Figure 5-78.** Total DHA (C22:6 n-3) in SRM 1950 Metabolites in Frozen Human Serum (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \leq 2$ . The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by its uncertainty ( $U_{NIST}$ ) and represents the range that results in an acceptable  $Z_{NIST}| \leq 2$ .

Exercise HAMQAP Exercise 1 - Human Metabolites Sample: Human Plasma B Measurand: Total DHA (C22:6 n-3)



**Figure 5-79.** Total DHA (C22:6 n-3) in Human Plasma B (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \leq 2$ . A NIST value has not been determined in this material.



HAMQAP Exercise 1 - Human Metabolites, Measurand: Total DHA (C22:6 n-3) No. of laboratories: 9

**Figure 5-80.** Laboratory means for total DHA in Fatty Acids Solution B and SRM 1950 Metabolites in Frozen Human Plasma (sample/sample comparison view). In this view, the individual laboratory mean for one sample (Fatty Acids Solution B) is compared to the mean for a second sample (SRM 1950). The solid red box represents the NIST range of tolerance for the two samples, Fatty Acids Solution B (x-axis) and SRM 1950 (y-axis), which encompasses the target values bounded by their uncertainties ( $U_{\text{NIST}}$ ) and represents the range that results in an acceptable  $Z_{\text{NIST}}$  score,  $|Z_{\text{NIST}}| \leq 2$ . The dotted blue box represents the consensus range of tolerance for Fatty Acids Solution B (x-axis) and SRM 1950 (y-axis), calculated as the values above and below the consensus means that result in an acceptable  $Z'_{\text{comm}} \leq 2$ .

|            |        |          |            |            |            |     |          | Free Stea   | aric Acid  | (C18:0)    |      |          |             |           |          |     |
|------------|--------|----------|------------|------------|------------|-----|----------|-------------|------------|------------|------|----------|-------------|-----------|----------|-----|
|            |        | I        | Fatty Acid | s Solution | B (µmol/L) |     | SRM      | 1950 Met    | abolites i | n Frozen H | uman |          | Human I     | lasma B   | (µmol/L) |     |
|            | Lab    | Α        | В          | С          | Avg        | SD  | Α        | В           | С          | Avg        | SD   | Α        | В           | С         | Avg      | SD  |
|            | Target |          |            |            | 550        | 28  |          |             |            |            |      |          |             |           |          |     |
|            | A004   |          |            |            |            |     |          |             |            |            |      |          |             |           |          |     |
|            | A012   |          |            |            |            |     |          |             |            |            |      |          |             |           |          |     |
| ılts       | A024   | 535.068  | 537.865    | 537.995    | 536.976    | 1.7 | 113.530  | 111.389     | 109.640    | 111.520    | 1.9  | 103.699  | 100.904     | 99.694    | 101.432  | 2.1 |
| tesı       | A028   |          |            |            |            |     |          |             |            |            |      |          |             |           |          |     |
| al B       | A049   |          |            |            |            |     |          |             |            |            |      |          |             |           |          |     |
| inp        | A050   |          |            |            |            |     |          |             |            |            |      |          |             |           |          |     |
| livi       | A070   |          |            |            |            |     |          |             |            |            |      |          |             |           |          |     |
| Inc        | A079   |          |            |            |            |     |          |             |            |            |      |          |             |           |          |     |
|            | A083   |          |            |            |            |     |          |             |            |            |      |          |             |           |          |     |
|            | A085   |          |            |            |            |     |          |             |            |            |      |          |             |           |          |     |
|            | A099   |          |            |            |            |     |          |             |            |            |      |          |             |           |          |     |
| ty.        |        | Consensu | s Mean     |            |            |     | Consensu | is Mean     |            |            |      | Consensu | is Mean     |           |          |     |
| uni<br>Its |        | Consensu | s Standard | Deviation  |            |     | Consensu | is Standard | Deviation  | l          |      | Consensu | is Standard | Deviation |          |     |
| Inni       |        | Maximum  | i i        |            | 536.976    |     | Maximum  | 1           |            | 111.52     |      | Maximun  | 1           |           | 101.432  |     |
| R          |        | Minimum  |            |            | 536.976    |     | Minimum  |             |            | 111.52     |      | Minimum  |             |           | 101.432  |     |
| <u> </u>   |        | Ν        |            |            | 1          |     | Ν        |             |            | 1          |      | Ν        |             |           | 1        |     |

Table 5-35. Data summary table for free stearic acid in a fatty acids solution and human plasma.

**Table 5-36.** Data summary table for free cis-vaccenic acid in a fatty acids solution and human plasma.

|            |        |          |             |            |            |      | Fre      | e cis-Vaco  | enic Acid   | (C18:1 n-  | 7)    |          |             |           |          |       |
|------------|--------|----------|-------------|------------|------------|------|----------|-------------|-------------|------------|-------|----------|-------------|-----------|----------|-------|
|            |        | I        | Fatty Acids | s Solution | B (µmol/L) |      | SRM      | 1950 Met    | abolites i  | n Frozen H | luman |          | Human l     | Plasma B  | (µmol/L) |       |
|            | Lab    | Α        | В           | С          | Avg        | SD   | Α        | В           | С           | Avg        | SD    | Α        | В           | С         | Avg      | SD    |
|            | Target |          |             |            | 115        | 5.8  |          |             |             |            |       |          |             |           |          |       |
|            | A004   |          |             |            |            |      |          |             |             |            |       |          |             |           |          |       |
| ults       | A012   |          |             |            |            |      |          |             |             |            |       |          |             |           |          |       |
| Kesi       | A024   | 110.170  | 108.409     | 108.694    | 109.091    | 0.95 | 15.375   | 15.196      | 14.884      | 15.152     | 0.25  | 16.767   | 16.708      | 16.295    | 16.590   | 0.257 |
| alF        | A028   |          |             |            |            |      |          |             |             |            |       |          |             |           |          |       |
| np         | A049   |          |             |            |            |      |          |             |             |            |       |          |             |           |          |       |
| divi       | A070   |          |             |            |            |      |          |             |             |            |       |          |             |           |          |       |
| ľ          | A079   |          |             |            |            |      |          |             |             |            |       |          |             |           |          |       |
|            | A083   |          |             |            |            |      |          |             |             |            |       |          |             |           |          |       |
|            | A085   |          |             |            |            |      |          |             |             |            |       |          |             |           |          |       |
| ty         |        | Consensu | s Mean      |            |            |      | Consensu | ıs Mean     |             |            |       | Consensu | ıs Mean     |           |          |       |
| uni<br>Its |        | Consensu | s Standard  | Deviation  |            |      | Consensu | is Standard | l Deviation | I          |       | Consensu | is Standard | Deviation |          |       |
| nsa        |        | Maximum  | L           |            | 109.091    |      | Maximun  | n           |             | 15.152     |       | Maximun  | 1           |           | 16.590   |       |
| 5 Å        |        | Minimum  |             |            | 109.091    |      | Minimum  |             |             | 15.152     |       | Minimum  |             |           | 16.590   |       |
| <u> </u>   |        | Ν        |             |            | 1          |      | Ν        |             |             | 1          |       | Ν        |             |           | 1        |       |

|            |        |          |            |            |            |     |          | Free Oleio  | e Acid (Cl | 18:1 n-9)  |      |          |             |           |          |     |
|------------|--------|----------|------------|------------|------------|-----|----------|-------------|------------|------------|------|----------|-------------|-----------|----------|-----|
|            |        | 1        | Fatty Acid | s Solution | B (µmol/L) |     | SRM      | 1950 Met    | abolites i | n Frozen H | uman |          | Human I     | Plasma B  | (µmol/L) |     |
|            | Lab    | Α        | В          | С          | Avg        | SD  | Α        | В           | С          | Avg        | SD   | Α        | В           | С         | Avg      | SD  |
|            | Target |          |            |            | 1318       | 66  |          |             |            |            |      |          |             |           |          |     |
|            | A004   |          |            |            |            |     |          |             |            |            |      |          |             |           |          |     |
|            | A012   |          |            |            |            |     |          |             |            |            |      |          |             |           |          |     |
| ılts       | A024   | 1232.44  | 1237.448   | 1235.854   | 1235.247   | 2.6 | 193.747  | 188.804     | 186.358    | 189.636    | 3.8  | 262.295  | 256.130     | 251.360   | 256.595  | 5.5 |
| tesı       | A028   |          |            |            |            |     |          |             |            |            |      |          |             |           |          |     |
| al B       | A049   |          |            |            |            |     |          |             |            |            |      |          |             |           |          |     |
| inp        | A050   |          |            |            |            |     |          |             |            |            |      |          |             |           |          |     |
| livi       | A070   |          |            |            |            |     |          |             |            |            |      |          |             |           |          |     |
| Inc        | A079   |          |            |            |            |     |          |             |            |            |      |          |             |           |          |     |
|            | A083   |          |            |            |            |     |          |             |            |            |      |          |             |           |          |     |
|            | A085   |          |            |            |            |     |          |             |            |            |      |          |             |           |          |     |
|            | A099   |          |            |            |            |     |          |             |            |            |      |          |             |           |          |     |
| ţy         |        | Consensu | s Mean     |            |            |     | Consensu | is Mean     |            |            |      | Consensu | ıs Mean     |           |          |     |
| uni<br>Its |        | Consensu | s Standard | Deviation  |            |     | Consensu | is Standard | Deviation  | I          |      | Consensu | is Standard | Deviation |          |     |
| Imi        |        | Maximum  | 1          |            | 1235.247   |     | Maximum  | 1           |            | 189.636    |      | Maximun  | 1           |           | 256.595  |     |
| R          |        | Minimum  |            |            | 1235.247   |     | Minimum  |             |            | 189.636    |      | Minimum  |             |           | 256.595  |     |
| <u> </u>   |        | Ν        |            |            | 1          |     | Ν        |             |            | 1          |      | Ν        |             |           | 1        |     |

 Table 5-37. Data summary table for free oleic acid in a fatty acids solution and human plasma.

**Table 5-38.** Data summary table for free linoleic acid in a fatty acids solution and human plasma.

|            |        |          |            |            |            |     | F        | ree Linole | ic Acid (O  | C18:2 n-6) |      |          |             |            |          |     |
|------------|--------|----------|------------|------------|------------|-----|----------|------------|-------------|------------|------|----------|-------------|------------|----------|-----|
| _          |        | I        | Fatty Acid | s Solution | B (µmol/L) |     | SRM      | 1950 Met   | abolites ii | n Frozen H | uman |          | Human I     | Plasma B ( | (µmol/L) |     |
|            | Lab    | Α        | В          | С          | Avg        | SD  | Α        | В          | С           | Avg        | SD   | Α        | В           | С          | Avg      | SD  |
|            | Target |          |            |            | 2488       | 124 |          |            |             |            |      |          |             |            |          |     |
|            | A004   |          |            |            |            |     |          |            |             |            |      |          |             |            |          |     |
|            | A012   |          |            |            |            |     |          |            |             |            |      |          |             |            |          |     |
| ılts       | A024   | 2144.852 | 2148.868   | 2143.009   | 2145.576   | 3.0 | 141.912  | 138.102    | 134.462     | 138.159    | 3.7  | 146.753  | 142.647     | 140.652    | 143.351  | 3.1 |
| tesı       | A028   |          |            |            |            |     |          |            |             |            |      |          |             |            |          |     |
| al F       | A049   |          |            |            |            |     |          |            |             |            |      |          |             |            |          |     |
| mp         | A050   |          |            |            |            |     |          |            |             |            |      |          |             |            |          |     |
| divi       | A070   |          |            |            |            |     |          |            |             |            |      |          |             |            |          |     |
| ľ          | A079   |          |            |            |            |     |          |            |             |            |      |          |             |            |          |     |
|            | A083   |          |            |            |            |     |          |            |             |            |      |          |             |            |          |     |
|            | A085   |          |            |            |            |     |          |            |             |            |      |          |             |            |          |     |
|            | A099   |          |            |            |            |     |          |            |             |            |      |          |             |            |          |     |
| ty.        |        | Consensu | s Mean     |            |            |     | Consensu | s Mean     |             |            |      | Consensu | is Mean     |            |          |     |
| uni<br>Its |        | Consensu | s Standard | Deviation  |            |     | Consensu | s Standard | Deviation   | I          |      | Consensu | is Standard | Deviation  |          |     |
| nm         |        | Maximum  | I          |            | 2145.576   |     | Maximum  | ı          |             | 138.159    |      | Maximun  | 1           |            | 143.351  |     |
| R. G       |        | Minimum  |            |            | 2145.576   |     | Minimum  |            |             | 138.159    |      | Minimum  |             |            | 143.351  |     |
| 5          |        | Ν        |            |            | 1          |     | Ν        |            |             | 1          |      | Ν        |             |            | 1        |     |

|            |        |           |             |           |            |      | Free     | alpha-Lin   | olenic Aci  | id (C18:3 1 | <b>1-3</b> ) |          |            |           |          |      |
|------------|--------|-----------|-------------|-----------|------------|------|----------|-------------|-------------|-------------|--------------|----------|------------|-----------|----------|------|
| _          |        | F         | Fatty Acids | Solution  | B (µmol/L) |      | SRM      | 1950 Met    | abolites ii | n Frozen H  | luman        |          | Human F    | lasma B ( | (µmol/L) |      |
|            | Lab    | Α         | В           | С         | Avg        | SD   | Α        | В           | С           | Avg         | SD           | Α        | В          | С         | Avg      | SD   |
|            | Target |           |             |           | 44.7       | 2.2  |          |             |             |             |              |          |            |           |          |      |
|            | A004   |           |             |           |            |      |          |             |             |             |              |          |            |           |          |      |
|            | A012   |           |             |           |            |      |          |             |             |             |              |          |            |           |          |      |
| ults       | A024   | 35.367    | 34.918      | 34.945    | 35.077     | 0.25 | 6.232    | 5.929       | 5.697       | 5.953       | 0.27         | 10.922   | 10.824     | 9.907     | 10.551   | 0.56 |
| tesı       | A028   |           |             |           |            |      |          |             |             |             |              |          |            |           |          |      |
| al F       | A049   |           |             |           |            |      |          |             |             |             |              |          |            |           |          |      |
| np         | A050   |           |             |           |            |      |          |             |             |             |              |          |            |           |          |      |
| divi       | A070   |           |             |           |            |      |          |             |             |             |              |          |            |           |          |      |
| Inc        | A079   |           |             |           |            |      |          |             |             |             |              |          |            |           |          |      |
|            | A083   |           |             |           |            |      |          |             |             |             |              |          |            |           |          |      |
|            | A085   |           |             |           |            |      |          |             |             |             |              |          |            |           |          |      |
|            | A099   |           |             |           |            |      |          |             |             |             |              |          |            |           |          |      |
| ty         |        | Consensus | s Mean      |           |            |      | Consensu | ıs Mean     |             |             |              | Consensu | s Mean     |           |          |      |
| uni<br>Its |        | Consensus | s Standard  | Deviation |            |      | Consensu | is Standard | l Deviation | I           |              | Consensu | s Standard | Deviation |          |      |
| mm         |        | Maximum   |             |           | 35.077     |      | Maximun  | 1           |             | 5.953       |              | Maximum  | ı          |           | 10.551   |      |
| <u>6</u>   |        | Minimum   |             |           | 35.077     |      | Minimum  |             |             | 5.953       |              | Minimum  |            |           | 10.551   |      |
| 9          |        | Ν         |             |           | 1          |      | Ν        |             |             | 1           |              | Ν        |            |           | 1        |      |

**Table 5-39.** Data summary table for free  $\alpha$ -linolenic acid in a fatty acids solution and human plasma.

**Table 5-40.** Data summary table for free  $\gamma$ -linolenic acid in a fatty acids solution and human plasma.

|            |        |           |             |            |            |      | Free g   | gamma-Lii   | nolenic Ac  | cid (C18:3 | <b>n-6</b> ) |          |             |           |          |      |
|------------|--------|-----------|-------------|------------|------------|------|----------|-------------|-------------|------------|--------------|----------|-------------|-----------|----------|------|
|            |        | I         | Fatty Acids | s Solution | B (µmol/L) |      | SRM      | 1950 Met    | abolites ii | n Frozen H | Iuman        |          | Human I     | Plasma B  | (µmol/L) |      |
|            | Lab    | Α         | В           | С          | Avg        | SD   | Α        | В           | С           | Avg        | SD           | Α        | В           | С         | Avg      | SD   |
|            | Target |           |             |            |            |      |          |             |             |            |              |          |             |           |          |      |
|            | A004   |           |             |            |            |      |          |             |             |            |              |          |             |           |          |      |
|            | A012   |           |             |            |            |      |          |             |             |            |              |          |             |           |          |      |
| ılts       | A024   | 0.225     | 0.228       | 0.457      | 0.303      | 0.13 | 1.768    | 1.582       | 1.812       | 1.721      | 0.12         | 2.548    | 2.324       | 2.592     | 2.488    | 0.14 |
| est        | A028   |           |             |            |            |      |          |             |             |            |              |          |             |           |          |      |
| al R       | A049   |           |             |            |            |      |          |             |             |            |              |          |             |           |          |      |
| qui        | A050   |           |             |            |            |      |          |             |             |            |              |          |             |           |          |      |
| livi       | A070   |           |             |            |            |      |          |             |             |            |              |          |             |           |          |      |
| Inc        | A079   |           |             |            |            |      |          |             |             |            |              |          |             |           |          |      |
|            | A083   |           |             |            |            |      |          |             |             |            |              |          |             |           |          |      |
|            | A085   |           |             |            |            |      |          |             |             |            |              |          |             |           |          |      |
|            | A099   |           |             |            |            |      |          |             |             |            |              |          |             |           |          |      |
| ty         |        | Consensus | s Mean      |            |            |      | Consensu | ıs Mean     |             |            |              | Consensu | ıs Mean     |           |          |      |
| umi<br>lts |        | Consensu  | s Standard  | Deviation  |            |      | Consensu | is Standard | l Deviation | I          |              | Consensu | is Standard | Deviation |          |      |
| Inni       |        | Maximum   | L           |            | 0.303      |      | Maximun  | n           |             | 1.721      |              | Maximun  | 1           |           | 2.488    |      |
| R Q        |        | Minimum   |             |            | 0.303      |      | Minimum  |             |             | 1.721      |              | Minimum  |             |           | 2.488    |      |
| 5          |        | Ν         |             |            | 1          |      | Ν        |             |             | 1          |              | Ν        |             |           | 1        |      |

|       |        |          |             |           |            |      | Fre      | e Arachid   | onic Acid   | (C20:4 n-6 | 6)    |          |             |           |          |      |
|-------|--------|----------|-------------|-----------|------------|------|----------|-------------|-------------|------------|-------|----------|-------------|-----------|----------|------|
|       |        | ŀ        | Fatty Acids | Solution  | B (µmol/L) |      | SRM      | 1950 Met    | abolites i  | n Frozen H | luman |          | Human l     | Plasma B  | (µmol/L) |      |
|       | Lab    | Α        | В           | С         | Avg        | SD   | Α        | В           | С           | Avg        | SD    | Α        | В           | С         | Avg      | SD   |
|       | Target |          |             |           | 806        | 40   |          |             |             |            |       |          |             |           |          |      |
|       | A004   |          |             |           |            |      |          |             |             |            |       |          |             |           |          |      |
| ts    | A012   |          |             |           |            |      |          |             |             |            |       |          |             |           |          |      |
| sul   | A024   | 688.321  | 688.186     | 687.95    | 688.152    | 0.19 | 30.460   | 29.484      | 28.995      | 29.646     | 0.75  | 23.193   | 22.225      | 22.307    | 22.575   | 0.54 |
| Re    | A028   |          |             |           |            |      |          |             |             |            |       |          |             |           |          |      |
| ual   | A049   |          |             |           |            |      |          |             |             |            |       |          |             |           |          |      |
| vid   | A070   |          |             |           |            |      |          |             |             |            |       |          |             |           |          |      |
| ndi   | A079   |          |             |           |            |      |          |             |             |            |       |          |             |           |          |      |
| 1     | A083   |          |             |           |            |      |          |             |             |            |       |          |             |           |          |      |
|       | A085   |          |             |           |            |      |          |             |             |            |       |          |             |           |          |      |
|       | A099   |          |             |           |            |      |          |             |             |            |       |          |             |           |          |      |
| ty    |        | Consensu | s Mean      |           |            |      | Consensu | ıs Mean     |             |            |       | Consensu | ıs Mean     |           |          |      |
| lts I |        | Consensu | s Standard  | Deviation |            |      | Consensu | is Standard | l Deviation | I          |       | Consensu | is Standard | Deviation |          |      |
| nsa   |        | Maximum  |             |           | 688.152    |      | Maximun  | 1           |             | 29.646     |       | Maximun  | 1           |           | 22.575   |      |
| E A   |        | Minimum  |             |           | 688.152    |      | Minimum  |             |             | 29.646     |       | Minimum  |             |           | 22.575   |      |
| •     |        | Ν        |             |           | 1          |      | Ν        |             |             | 1          |       | Ν        |             |           | 1        |      |

**Table 5-41.** Data summary table for free arachidonic acid in a fatty acids solution and human plasma.

Table 5-42. Data summary table for free EPA in a fatty acids solution and human plasma.

|            | ĺ      |          |            |            |            |      |          | Free E     | PA (C20:    | 5 n-3)     |       |          |             |           |          |       |
|------------|--------|----------|------------|------------|------------|------|----------|------------|-------------|------------|-------|----------|-------------|-----------|----------|-------|
|            |        | I        | Fatty Acid | s Solution | B (µmol/L) |      | SRM      | 1950 Met   | abolites i  | n Frozen H | Iuman |          | Human I     | Plasma B  | (µmol/L) |       |
|            | Lab    | Α        | В          | С          | Avg        | SD   | Α        | В          | С           | Avg        | SD    | Α        | В           | С         | Avg      | SD    |
|            | Target |          |            |            | 36.8       | 1.8  |          |            |             |            |       |          |             |           |          |       |
|            | A004   |          |            |            |            |      |          |            |             |            |       |          |             |           |          |       |
|            | A012   |          |            |            |            |      |          |            |             |            |       |          |             |           |          |       |
| ılts       | A024   | 27.857   | 27.664     | 27.965     | 27.829     | 0.15 | 1.720    | 1.480      | 1.442       | 1.547      | 0.15  | 2.751    | 2.677       | 2.658     | 2.695    | 0.049 |
| tesı       | A028   |          |            |            |            |      |          |            |             |            |       |          |             |           |          |       |
| al F       | A049   |          |            |            |            |      |          |            |             |            |       |          |             |           |          |       |
| mp         | A050   |          |            |            |            |      |          |            |             |            |       |          |             |           |          |       |
| livi       | A070   |          |            |            |            |      |          |            |             |            |       |          |             |           |          |       |
| Inc        | A079   |          |            |            |            |      |          |            |             |            |       |          |             |           |          |       |
|            | A083   |          |            |            |            |      |          |            |             |            |       |          |             |           |          |       |
|            | A085   |          |            |            |            |      |          |            |             |            |       |          |             |           |          |       |
|            | A099   |          |            |            |            |      |          |            |             |            |       |          |             |           |          |       |
| ty         |        | Consensu | s Mean     |            |            |      | Consensu | s Mean     |             |            |       | Consensu | ıs Mean     |           |          |       |
| uni<br>lts |        | Consensu | s Standard | Deviation  |            |      | Consensu | s Standard | l Deviation | L          |       | Consensu | is Standard | Deviation |          |       |
| Inne       |        | Maximum  | L          |            | 27.829     |      | Maximum  | ı          |             | 1.547      |       | Maximun  | n           |           | 2.695    |       |
| R          |        | Minimum  |            |            | 27.829     |      | Minimum  |            |             | 1.547      |       | Minimum  |             |           | 2.695    |       |
| 5          |        | Ν        |            |            | 1          |      | Ν        |            |             | 1          |       | Ν        |             |           | 1        |       |

|            |        |                              |            |          | Free DPA (C22:5 n-3) |          |             |             |            |            |          |                         |           |       |       |      |  |  |
|------------|--------|------------------------------|------------|----------|----------------------|----------|-------------|-------------|------------|------------|----------|-------------------------|-----------|-------|-------|------|--|--|
|            |        | I                            | atty Acids | Solution | B (µmol/L)           |          | SRM         | 1950 Met    | abolites i | n Frozen H | Iuman    | Human Plasma B (µmol/L) |           |       |       |      |  |  |
|            | Lab    | Α                            | В          | С        | Avg                  | SD       | Α           | В           | С          | Avg        | SD       | Α                       | В         | С     | Avg   | SD   |  |  |
|            | Target |                              |            |          | 38.2                 | 1.9      |             |             |            |            |          |                         |           |       |       |      |  |  |
|            | A004   |                              |            |          |                      |          |             |             |            |            |          |                         |           |       |       |      |  |  |
|            | A012   |                              |            |          |                      |          |             |             |            |            |          |                         |           |       |       |      |  |  |
| ults       | A024   | 30.728                       | 30.553     | 30.710   | 30.664               | 0.096    | 2.494       | 2.574       | 2.322      | 2.463      | 0.13     | 3.090                   | 3.008     | 2.786 | 2.961 | 0.16 |  |  |
| tesı       | A028   |                              |            |          |                      |          |             |             |            |            |          |                         |           |       |       |      |  |  |
| I B        | A049   |                              |            |          |                      |          |             |             |            |            |          |                         |           |       |       |      |  |  |
| np         | A050   |                              |            |          |                      |          |             |             |            |            |          |                         |           |       |       |      |  |  |
| livi       | A070   |                              |            |          |                      |          |             |             |            |            |          |                         |           |       |       |      |  |  |
| Ъ          | A079   |                              |            |          |                      |          |             |             |            |            |          |                         |           |       |       |      |  |  |
|            | A083   |                              |            |          |                      |          |             |             |            |            |          |                         |           |       |       |      |  |  |
|            | A085   |                              |            |          |                      |          |             |             |            |            |          |                         |           |       |       |      |  |  |
|            | A099   |                              |            |          |                      |          |             |             |            |            |          |                         |           |       |       |      |  |  |
| ţy         |        | Consensus                    | s Mean     |          |                      |          | Consensu    | ıs Mean     |            |            |          | Consensus Mean          |           |       |       |      |  |  |
| ini<br>Its |        | Consensus Standard Deviation |            |          |                      | Consensu | is Standard | l Deviation | ı          |            | Consensu | is Standard             | Deviation |       |       |      |  |  |
| nu [ns     |        | Maximum                      |            |          | 30.664               |          | Maximun     | 1           |            | 2.463      |          | Maximum                 | 1         |       | 2.961 |      |  |  |
| R G        |        | Minimum                      |            |          | 30.664               |          | Minimum     |             |            | 2.463      |          | Minimum                 |           |       | 2.961 |      |  |  |
| 5          |        | Ν                            |            |          | 1                    |          | Ν           |             |            | 1          |          | Ν                       |           |       | 1     |      |  |  |

 Table 5-43. Data summary table for free DPA in a fatty acids solution and human plasma.

 Table 5-44.
 Data summary table for free DHA in a fatty acids solution and human plasma.

|            |        | Free DHA (C22:6 n-3)         |             |          |            |          |             |           |            |            |          |                         |           |         |         |    |  |
|------------|--------|------------------------------|-------------|----------|------------|----------|-------------|-----------|------------|------------|----------|-------------------------|-----------|---------|---------|----|--|
|            |        | ]                            | Fatty Acids | Solution | B (µmol/L) |          | SRM         | 1950 Met  | abolites i | n Frozen H | uman     | Human Plasma B (µmol/L) |           |         |         |    |  |
|            | Lab    | Α                            | В           | С        | Avg        | SD       | Α           | В         | С          | Avg        | SD       | Α                       | В         | С       | Avg     | SD |  |
|            | Target |                              |             |          | 98.8       | 4.9      |             |           |            |            |          |                         |           |         |         |    |  |
|            | A004   |                              |             |          |            |          |             |           |            |            |          |                         |           |         |         |    |  |
|            | A012   |                              |             |          |            |          |             |           |            |            |          |                         |           |         |         |    |  |
| ults       | A024   | 4901.015                     | 4911.133    | 4903.633 | 4905.260   | 5.3      | 873.269     | 853.276   | 842.077    | 856.207    | 16       | 965.057                 | 937.595   | 923.281 | 941.978 | 21 |  |
| tesı       | A028   |                              |             |          |            |          |             |           |            |            |          |                         |           |         |         |    |  |
| al F       | A049   |                              |             |          |            |          |             |           |            |            |          |                         |           |         |         |    |  |
| np         | A050   |                              |             |          |            |          |             |           |            |            |          |                         |           |         |         |    |  |
| livi       | A070   |                              |             |          |            |          |             |           |            |            |          |                         |           |         |         |    |  |
| Inc        | A079   |                              |             |          |            |          |             |           |            |            |          |                         |           |         |         |    |  |
|            | A083   |                              |             |          |            |          |             |           |            |            |          |                         |           |         |         |    |  |
|            | A085   |                              |             |          |            |          |             |           |            |            |          |                         |           |         |         |    |  |
|            | A099   |                              |             |          |            |          |             |           |            |            |          |                         |           |         |         |    |  |
| ty         |        | Consensu                     | ıs Mean     |          |            |          | Consensu    | ıs Mean   |            |            |          | Consensu                | ıs Mean   |         |         |    |  |
| uni<br>lts |        | Consensus Standard Deviation |             |          |            | Consensu | is Standard | Deviation | 1          |            | Consensu | is Standard             | Deviation |         |         |    |  |
| Inne       |        | Maximun                      | 1           |          | 4905.260   |          | Maximun     | 1         |            | 856.207    |          | Maximun                 | 1         |         | 941.978 |    |  |
| R          |        | Minimum                      |             |          | 4905.260   |          | Minimum     |           |            | 856.207    |          | Minimum                 |           |         | 941.978 |    |  |
| •          |        | Ν                            |             |          | 1          |          | Ν           |           |            | 1          |          | Ν                       |           |         | 1       |    |  |

## Fatty Acids Overall Samples Comparison

- The consensus means for the fatty acids in the solutions for both the dietary intake and human metabolite studies were lower for most fatty acids compared to the target values.
  - For the dietary intake study, the fatty acids levels were much lower in the solution compared to the fish oil and cod liver oil, which may have caused difficulty for some laboratories.
  - The target values for the fatty acids in both solutions were determined based on the gravimetric preparation. The purity of the neat fatty acids used to prepare the solution was not evaluated; impurities in these neat materials may be the cause of the apparent low bias in the consensus values.
- Participation was low for the free fatty acids in both the dietary intake and human metabolites studies. Participating laboratories may not have analytical methods to measure free fatty acids, or the measurement of free fatty acids is not a current concern of the participating laboratories. Future studies will focus on measurement of total fatty acids, unless a need for reporting of free fatty acids is indicated by participating laboratories.

### SECTION 6: BOTANICALS (Actein, 27-Deoxyactein)

#### Study Overview

In this study, participants were provided with candidate NIST SRM 3295 Actaea racemosa (Black Cohosh) Rhizome, candidate NIST RM 8657 Actaea racemosa (Black Cohosh) Leaves, and candidate NIST RM 8658 Actaea racemosa (Black Cohosh) Extract. Participants were asked to use in-house analytical methods to determine the mass fraction of actein and 27-deoxyactein in each of the matrices and report values in mg/g, on an as-received basis. Black cohosh dietary supplements are most often used to relieve menopausal symptoms. Consensus has not been reached regarding the compounds in black cohosh that provide relieve from menopausal symptoms, but triterpene glycosides such as actein and 27-deoxyactein are commonly used to standardize black cohosh products and may contribute to bioactivity.

## **Dietary Intake Sample Information**

*Black Cohosh Rhizome*. Participants were provided with three packets, each containing 3 grams of black cohosh rhizome powder. The plant material was ground, homogenized, and heat-sealed inside 4 mil polyethylene bags, which were then sealed inside nitrogen-flushed aluminized plastic bags along with two packets of silica gel. Before use, participants were instructed to mix the contents of the packet thoroughly, and to use a sample size of at least 0.5 g. Participants were asked to store the material at controlled room temperature, 10  $^{\circ}$ C to 30  $^{\circ}$ C, and to prepare one sample and report one value from each packet provided. The approximate analyte levels were not reported to participants prior to the study, and target values for actein and 27-deoxyactein in SRM 3295 have not been determined at NIST.

*Black Cohosh Leaves.* Participants were provided with three packets, each containing 3 grams of powdered black cohosh leaves. The dried leaves were ground, homogenized, and heat-sealed inside 4 mil polyethylene bags, which were then sealed inside nitrogen-flushed aluminized plastic bags along with two packets of silica gel. Before use, participants were instructed to mix the contents of the packet thoroughly, and to use a sample size of at least 0.5 g. Participants were asked to store the material at controlled room temperature, 10  $^{\circ}$ C to 30  $^{\circ}$ C, and to prepare one sample and report one value from each packet provided. The approximate analyte levels were not reported to participants prior to the study, and target values for actein and 27-deoxyactein in RM 8657 have not been determined at NIST.

*Black Cohosh Rhizome Extract.* Participants were provided with three packets, each containing 3 grams of black cohosh rhizome extract. The dried extract was ground, homogenized, and heat-sealed inside 4 mil polyethylene bags, which were then sealed inside nitrogen-flushed aluminized plastic bags along with two packets of silica gel. Before use, participants were instructed to mix the contents of the packet thoroughly, and to use a sample size of at least 0.5 g. Participants were asked to store the material at controlled room temperature, 10 °C to 30 °C, and to prepare one sample and report one value from each packet provided. The approximate analyte levels were not reported to participants prior to the study, and target values for actein and 27-deoxyactein in RM 8658 have not been determined at NIST.

## **Dietary Intake Study Results**

- Nine laboratories enrolled in this exercise and received samples to measure actein. Two laboratories reported results for each sample (22 % participation).
  - The between-laboratory variability was high for actein in the black cohosh extract (45 % RSD) and unsatisfactory in the black cohosh leaves and rhizome (> 100 % RSD).
  - None of the participating laboratories reported their analytical methodology.
- Ten laboratories enrolled in this exercise and received samples to measure 27-deoxyactein. Three laboratories reported results for each sample (30 % participation).
  - The between-laboratory variability was acceptable for 27-deoxyactein in the black cohosh leaves (38 % RSD), and unsatisfactory in the black cohosh extract and rhizome (> 100 % RSD).
  - None of the participating laboratories reported their analytical methodology.

# Dietary Intake Technical Recommendations

The following recommendations are based on results obtained from the participants in this study.

- Extremely low participation in this study does not permit any technical recommendations to be made.
  - Low rates of data return (22 % and 30 % for actein and 27-deoxyactein, respectively) may indicate that analysis of these compounds is challenging to dietary supplement testing laboratories, or that laboratories are not capable of measuring actein and 27-deoxyactein in black cohosh matrices.
  - The low number of laboratories requesting samples (9 and 10 laboratories for actein and 27-deoxyactein, respectively) may indicate that measurement of these compounds may not be a priority for program participants.
- Laboratories did not report sample preparation or analytical methodology.
  - If using an extraction method, the extraction procedure should be optimized for the extraction solvent and the number of repeated extraction cycles to ensure exhaustive extraction from the matrix.
  - The optimum number of extraction cycles must be determined by sequential extraction until no further increase in yield is observed. Sequential extractions may be important if the extraction solvent becomes saturated during the extraction.
- Each triterpene glycoside should be quantified based on a matched calibration material for maximum accuracy.
- The purity of all calibrant materials should be rigorously determined using multiple techniques, and the final sample result corrected for any impurities.

Table 6-1. Individualized data summary table (NIST) for actein and 27-deoxyactein in black cohosh extract, leaves, and rhizome.

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|                | Lab Code:                                       | NIST              |                                    | 1. You         | r Results       |                   | 2. 0                   | Community Re   | 3. Target         |                               |
|----------------|---|-------------------|------------------------------------|----------------|-----------------|-------------------|------------------------|----------------|-------------------|-------------------------------|
| Analyte        | Sample  | Units             | x <sub>i</sub>                     | s <sub>i</sub> | $Z'_{\rm comm}$ | $Z_{\text{NIST}}$ | N                      | <i>x</i> *     | <i>s</i> *        | x <sub>NIST</sub> U           |
| 27-Deoxyactein | RM 8658 Actaea racemosa (Black Cohosh) Extract  | mg/g              |                                    |                |                 |                   | 3                      | 1.4            | 1.7               |                               |
| 27-Deoxyactein | RM 8657 Actaea racemosa (Black Cohosh) Leaves   | mg/g              |                                    |                |                 |                   | 3                      | 0.4            | 0.2               |                               |
| 27-Deoxyactein | SRM 3295 Actaea racemosa (Black Cohosh) Rhizome | mg/g              |                                    |                |                 |                   | 3                      | 6.8            | 8.9               |                               |
| Actein         | RM 8658 Actaea racemosa (Black Cohosh) Extract  | mg/g              |                                    |                |                 |                   | 2                      | 10             | 13                |                               |
| Actein         | RM 8657 Actaea racemosa (Black Cohosh) Leaves   | mg/g              |                                    |                |                 |                   | 2                      | 1              | 4                 |                               |
| Actein         | SRM 3295 Actaea racemosa (Black Cohosh) Rhizome | mg/g              |                                    |                |                 |                   | 2                      | 13             | 6                 |                               |
|                |   | x <sub>i</sub>    | Mean of rep                        | orted valu     | s               | Ν                 | Number of a            | quantitative   | x <sub>NIST</sub> | NIST-assessed value           |
|                |   | S <sub>i</sub>    | Standard dev                       | viation of r   | eported value   | es                | values repor           | ted            | U                 | expanded uncertainty          |
|                |   | $Z'_{\rm comm}$   | Z'-score with respect to community |                |                 | <i>x</i> *        | Robust mean of reporte |                |                   | about the NIST-assessed value |
|                |   |                   | consensus                          |                |                 |                   | values                 |                |                   |                               |
|                |   | Z <sub>NIST</sub> | Z-score with                       | n respect to   | NIST value      | <i>s</i> *        | Robust stand           | dard deviation |                   |                               |

#### HAMQAP Exercise 1 - Natural Products (Dietary Intake)

|            |        | Actein   |      |      |      |         |                |                 |                       |                   |         |   |             |           |       |      |
|------------|--------|--|------|------|------|---------|----------------|-----------------|-----------------------|-------------------|---------|---|-------------|-----------|-------|------|
|            |        | RM 8658 Actaea racemosa (Black Cohosh)<br>Extract (mg/g) |      |      |      |         |                | 57 Actaea<br>Le | racemosa<br>aves (mg/ | a (Black (<br>′g) | Cohosh) | SRM 3295 Actaea racemosa (Black Cohosh)<br>Rhizome (mg/g) |             |           |       |      |
|            | Lab    | Α  | В    | С    | Avg  | SD      | Α              | В               | С                     | Avg               | SD      | Α   | В           | С         | Avg   | SD   |
|            | Target |  |      |      |      |         |                |                 |                       |                   |         |   |             |           |       |      |
|            | A003   | 8.6  | 8.1  | 8.9  | 8.5  | 0.4     | 2.0            | 2.2             | 1.6                   | 1.93              | 0.31    | 15.1  | 14.6        | 14.4      | 14.7  | 0.36 |
| ults       | A037   |  |      |      |      |         |                |                 |                       |                   |         |   |             |           |       |      |
| Res        | A041   |  |      |      |      |         |                |                 |                       |                   |         |   |             |           |       |      |
| al I       | A056   |  |      |      |      |         |                |                 |                       |                   |         |   |             |           |       |      |
| idu        | A060   |  |      |      |      |         |                |                 |                       |                   |         |   |             |           |       |      |
| div        | A070   |  |      |      |      |         |                |                 |                       |                   |         |   |             |           |       |      |
| In         | A071   | 2.32   | 2.3  | 2.27 | 2.3  | 0       | 0              | 0               | 0                     | 0                 | 0       | 11.7  | 11.7        | 11.9      | 11.77 | 0.12 |
|            | A074   |  |      |      |      |         |                |                 |                       |                   |         |   |             |           |       |      |
|            | A079   |  |      |      |      |         |                |                 |                       |                   |         |   |             |           |       |      |
| ty         |        | Consensus  | Mean |      | 5.4  |         | Consensus Mean |                 |                       | 0.97              |         | Consensu  | ıs Mean     |           | 13.23 |      |
| uni<br>lts |        | Consensus Standard Deviation                             |      |      | 12.9 |         | Consensu       | is Standard     | l Deviation           | 3.77              |         | Consensu  | is Standard | Deviation | 5.94  |      |
| mm<br>esu  |        | Maximum  |      | 8.5  |      | Maximum |                |                 | 1.93                  |                   | Maximum |   |             | 14.7      |       |      |
| R          |        | Minimum  |      |      | 2.3  | 2.3     |                | Minimum         |                       |                   |         | Minimum   |             |           | 11.77 |      |
| •          |        | N  |      |      | 2    |         | Ν              | I               |                       |                   |         | N   |             |           | 2     |      |

**Table 6-2.** Data summary table for actein in black cohosh extract, leaves, and rhizome.

|           |        |  |            |             |      |      |          | 27-             | Deoxyact                 | ein               |         |   |             |           |       |      |
|-----------|--------|--|------------|-------------|------|------|----------|-----------------|--------------------------|-------------------|---------|---|-------------|-----------|-------|------|
|           |        | RM 8658 Actaea racemosa (Black Cohosh)<br>Extract (mg/g) |            |             |      |      |          | 57 Actaea<br>Le | a racemosa<br>eaves (mg/ | a (Black (<br>/g) | Cohosh) | SRM 3295 Actaea racemosa (Black Cohosh)<br>Rhizome (mg/g) |             |           |       |      |
|           | Lab    | А  | В          | С           | Avg  | SD   | Α        | В               | С                        | Avg               | SD      | Α   | В           | С         | Avg   | SD   |
|           | Target |  |            |             |      |      |          |                 |                          |                   |         |   |             |           |       |      |
|           | A003   | 2.1  | 2          | 2.2         | 2.1  | 0.1  | 2        | 2.2             | 1.6                      | 1.933             | 0.306   | 11.3  | 11          | 10.8      | 11.03 | 0.25 |
| ts        | A007   | 0.64   | 0.66       | 0.63        | 0.64 | 0.02 | 0.454    | 0.4             | 0.419                    | 0.424             | 0.027   | 2.35  | 2.49        | 1.95      | 2.26  | 0.28 |
| ual Resul | A037   |  |            |             |      |      |          |                 |                          |                   |         |   |             |           |       |      |
|           | A041   |  |            |             |      |      |          |                 |                          |                   |         |   |             |           |       |      |
|           | A056   |  |            |             |      |      |          |                 |                          |                   |         |   |             |           |       |      |
| vid       | A060   |  |            |             |      |      |          |                 |                          |                   |         |   |             |           |       |      |
| ibu       | A070   |  |            |             |      |      |          |                 |                          |                   |         |   |             |           |       |      |
| Ι         | A071   | 1.43   | 1.42       | 1.43        | 1.43 | 0.01 | 0.385    | 0.384           | 0.389                    | 0.386             | 0.003   | 7.26  | 7.02        | 7.26      | 7.18  | 0.14 |
|           | A074   |  |            |             |      |      |          |                 |                          |                   |         |   |             |           |       |      |
|           | A079   |  |            |             |      |      |          |                 |                          |                   |         |   |             |           |       |      |
| Ś         |        | Consensus  | s Mean     |             | 1.39 |      | Consense | us Mean         |                          | 0.405             |         | Consensu  | is Mean     |           | 6.83  |      |
| lts       |        | Consensus  | s Standard | l Deviation | 1.68 |      | Consense | us Standard     | l Deviation              | 0.146             |         | Consensu  | is Standard | Deviation | 8.94  |      |
| esu       |        | Maximum  |            | 2.1         | 2.1  |      | Maximum  |                 |                          | 1.933             |         | Maximum   |             |           |       |      |
|           |        | Minimum  |            |             | 0.64 |      | Minimum  | ı               |                          | 0.386             |         | Minimum   |             |           | 2.26  |      |
| •         |        | N  |            |             | 3    |      | Ν        |                 |                          | 3                 |         | Ν   |             |           | 3     |      |

**Table 6-3.** Data summary table for 27-deoxyactein in black cohosh extract, leaves, and rhizome. Data points highlighted in red have been flagged as potential outliers (e.g., difference from reference value, Grubb and/or Cochran) by the NIST software package.

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**Figure 6-1.** 27-Deoxyactein in RM 8658 *Actaea racemosa* (Black Cohosh) Extract (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The solid black line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red line represents the upper consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \le 2$ , with the lower range set at zero. A NIST value has not been determined in this material.

Exercise HAMQAP Exercise 1 - Dietary Intake Sample: Black Cohosh Extract Measurand: 27-Deoxyactein



**Figure 6-2.** 27-Deoxyactein in RM 8657 *Actaea racemosa* (Black Cohosh) Leaves (data summary view –analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The solid black line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red line represents the upper consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \le 2$ , with the lower range set at zero. A NIST value has not been determined in this material.



**Figure 6-3.** 27-Deoxyactein in candidate SRM 3295 *Actaea racemosa* (Black Cohosh) Rhizome (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The solid black line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red line represents the upper consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \leq 2$ , with the lower range set at zero. A NIST value has not been determined in this material.
### **SECTION 7: CONTAMINANTS (Mycotoxins)**

#### Study Overview

In this study, participants were provided with one NIST candidate RM, RM 8427 Mycotoxins in Corn containing two parts (Blank and Incurred), and one external quality control (QC) corn material. Participants were asked to use in-house analytical methods to determine the mass fraction of mycotoxins (aflatoxin B1, aflatoxin B2, aflatoxin G1, aflatoxin G2, total aflatoxins, fumonisin B1, fumonisin B2, fumonisin B3, total fumonisins, deoxynivalenol, ochratoxin A, T-2 toxin, HT2 toxin, and zearalenone) in each of the matrices and report values in ng/g, on an asreceived basis. Certain types of molds (fungus) can grow on numerous foodstuffs under warm, moist conditions, and the toxins that they produce can be harmful to animals and humans. Mycotoxin contamination is commonly found in cereals, dried fruits, nuts, and spices, as well as in milk produced by animals consuming contaminated feed.

## **Dietary Intake Sample Information**

*Blank Corn.* Participants were provided with one bottle containing 60 g of ground corn. Commercial corns were cryo-milled, blended, and aliquoted into amber bottles, which were then sealed inside aluminized plastic bags. Before use, participants were instructed to thoroughly mix the contents of the bottle by rotating and/or rolling prior to removal of a test sample for analysis, and to use a sample size of at least 5 g. Participants were asked to store the material at controlled room temperature, 10 °C to 30 °C, and to prepare three samples and report three values from the single bottle provided. The approximate analyte levels were not reported to participants prior to the study. The target values in candidate RM 8427 (Blank) reflect measurements conducted by the US Food and Drug Administration (FDA) using isotope dilution liquid chromatography with tandem mass spectrometry detection (ID-LC-MS/MS). The target values and standard deviations for deoxynivalenol and fumonisin B1 are provided in the table below on an as-received basis. Levels of other mycotoxins in RM 8427 (Blank) were below the limit of quantitation of the ID-LC-MS/MS method, as described in the table below.

|                | Target Mass Fraction in candidate         |
|----------------|---|
|                | RM 8427 Mycotoxins in Corn (Blank) (ng/g) |
| <u>Analyte</u> | (as-received basis)                       |
| Aflatoxin B1   | < 0.6                                     |
| Aflatoxin B2   | < 0.8                                     |
| Aflatoxin G1   | < 0.7                                     |
| Aflatoxin G2   | < 0.8                                     |
| Fumonisin B1   | $10.5 \pm 2.5$                            |
| Fumonisin B2   | < 6.5                                     |
| Fumonisin B3   | < 9                                       |
| Deoxynivalenol | $146 \pm 17$                              |
| Ochratoxin A   | < 0.9                                     |
| T-2 Toxin      | < 3.9                                     |
| HT-2 Toxin     | < 18.8                                    |
| Zearalenone    | < 9.6                                     |

*Incurred Corn A.* Participants were provided with one bottle containing 60 g of ground corn. Commercial corns were cryo-milled, blended, and aliquoted into amber bottles, which were then sealed inside aluminized plastic bags. Before use, participants were instructed to thoroughly mix the contents of the bottle by rotating and/or rolling prior to removal of a test sample for analysis, and to use a sample size of at least 5 g. Participants were asked to store the material at controlled room temperature, 10 °C to 30 °C, and to prepare three samples and report three values from the single bottle provided. The approximate analyte levels were not reported to participants prior to the study. The target values in candidate RM 8427 (Incurred) reflect measurements conducted by the US FDA using ID-LC-MS/MS. The target values and standard deviations for aflatoxin B1, aflatoxin B2, aflatoxin G1, total aflatoxins, fumonisin B1, fumonisin B2, fumonisin B3, total fumonisins, deoxynivalenol, ochratoxin A, T-2 toxin, HT-2 toxin, and zearalenone are provided in the table below on an as-received basis. The level of aflatoxin G2 in RM 8427 (Incurred) was below the limit of quantitation of the ID-LC-MS/MS method and did not meet FDA criteria for identification.

|                  | Target Mass Fraction in candidate            |
|------------------|--|
|                  | RM 8427 Mycotoxins in Corn (Incurred) (ng/g) |
| <u>Analyte</u>   | (as-received basis)                          |
| Aflatoxin B1     | $7.8 \pm 2.4$                                |
| Aflatoxin B2     | $1.55 \pm 0.35$                              |
| Aflatoxin G1     | $1.05 \pm 0.20$                              |
| Aflatoxin G2     | < 0.8  |
| Total Aflatoxins | $10.4 \pm 2.5$                               |
| Fumonisin B1     | $869 \pm 79$                                 |
| Fumonisin B2     | $226 \pm 25$                                 |
| Fumonisin B3     | $98.0 \pm 9.2$                               |
| Total Fumonisins | $1193 \pm 83$                                |
| Deoxynivalenol   | $468 \pm 24$                                 |
| Ochratoxin A     | $10.6 \pm 5.3$                               |
| T-2 Toxin        | $19.8 \pm 3.9$                               |
| HT-2 Toxin       | $37.8 \pm 5.8$                               |
| Zearalenone      | $75 \pm 11$                                  |

*Incurred Corn B.* Participants were provided with one packet containing 50 g of ground corn. The commercial corn was aliquoted and sealed into plastic bags, which were then sealed inside aluminized plastic bags. Before use, participants were instructed to thoroughly mix the contents of the packet prior to removal of a test sample for analysis, and to use a sample size of at least 5 g. Participants were asked to store the material at controlled room temperature, 10 °C to 30 °C, and to prepare three samples and report three values from the single packet provided. The approximate analyte levels were not reported to participants prior to the study. The target values in Incurred Corn B reflect the results of a proficiency testing scheme conducted by an ISO 17043 accredited provider and contain at least 40 data points. The target values and uncertainties for aflatoxin B1,

fumonisin B1, fumonisin B2, total fumonisins, deoxynivalenol, ochratoxin A, T-2 toxin, HT-2 toxin, and zearalenone are provided in the table below on an as-received basis. The levels of aflatoxin B2, aflatoxin G1, aflatoxin G2, and fumonisin B3 were not reported on the material data sheet from the supplier.

|                              | Target Mass Fraction in |
|------------------------------|-------------------------|
|                              | Incurred Corn B (ng/g)  |
| Analyte                      | (as-received basis)     |
| Aflatoxin B1                 | $3.07 \pm 1.35$         |
| Fumonisin B1                 | $463  \pm \ 166$        |
| Fumonisin B2                 | $108 \pm 48$            |
| Total Fumonisins (B1 and B2) | 555 ± 195               |
| Deoxynivalenol               | $731  \pm \ 245$        |
| Ochratoxin A                 | $2.18~\pm 0.96$         |
| T-2 Toxin                    | $109 \pm 48$            |
| HT-2 Toxin                   | $120 \pm 53$            |
| Zearalenone                  | $141 \pm 60$            |

# **Dietary Intake Study Results**

• Thirty-eight laboratories enrolled in this exercise and received samples to measure at least one mycotoxin or group of mycotoxins in these samples.

|                  | Number of         | Number of      | Laboratories Repo       | orting Results  |  |  |  |  |  |  |
|------------------|-------------------|----------------|-------------------------|-----------------|--|--|--|--|--|--|
|                  | Laboratories      | <u>(</u> ]     | (Percent Participation) |                 |  |  |  |  |  |  |
|                  | <u>Requesting</u> | <u>RM 8427</u> | <u>RM 8427</u>          |                 |  |  |  |  |  |  |
| <u>Analyte</u>   | Samples           | (Blank)        | (Incurred)              | Incurred Corn B |  |  |  |  |  |  |
| Aflatoxin B1     | 33                | 21 (64 %)      | 22 (67 %)               | 22 (67 %)       |  |  |  |  |  |  |
| Aflatoxin B2     | 33                | 22 (67 %)      | 22 (67 %)               | 23 (70 %)       |  |  |  |  |  |  |
| Aflatoxin G1     | 32                | 22 (69 %)      | 23 (72 %)               | 22 (69 %)       |  |  |  |  |  |  |
| Aflatoxin G2     | 32                | 21 (66 %)      | 22 (69 %)               | 21 (66 %)       |  |  |  |  |  |  |
| Total Aflatoxins | 37                | 21 (57 %)      | 21 (57 %)               | 21 (57 %)       |  |  |  |  |  |  |
| Fumonisin B1     | 24                | 15 (63 %)      | 16 (67 %)               | 16 (63 %)       |  |  |  |  |  |  |
| Fumonisin B2     | 24                | 14 (58 %)      | 16 (67 %)               | 16 (67 %)       |  |  |  |  |  |  |
| Fumonisin B3     | 21                | 10 (48 %)      | 11 (52 %)               | 11 (52 %)       |  |  |  |  |  |  |
| Total Fumonisins | 24                | 11 (46 %)      | 10 (42 %)               | 10 (42 %)       |  |  |  |  |  |  |
| Deoxynivalenol   | 25                | 15 (60 %)      | 17 (68 %)               | 17 (68 %)       |  |  |  |  |  |  |
| Ochratoxin A     | 31                | 19 (61 %)      | 20 (65 %)               | 20 (65 %)       |  |  |  |  |  |  |
| T-2 Toxin        | 22                | 12 (55 %)      | 13 (59 %)               | 13 (59 %)       |  |  |  |  |  |  |
| HT-2 Toxin       | 21                | 11 (52 %)      | 11 (52 %)               | 12 (57 %)       |  |  |  |  |  |  |
| Zearalenone      | 27                | 15 (56 %)      | 15 (56 %)               | 16 (59 %)       |  |  |  |  |  |  |
|                  |                   |                |                         |                 |  |  |  |  |  |  |

Measurable levels of fumonisin B1, total fumonisins, and deoxynivalenol were expected in • RM 8427 (Blank). The consensus means for these mycotoxins were within the target ranges and the consensus ranges overlapped with the target ranges (see table below). The betweenlaboratory variability is also summarized below and ranged from high for deoxynivalenol (46 %) to very high for fumonisin B1 and total fumonisins (84 % and > 100 %, respectively).

|                  |   | Between-                         |
|------------------|---|----------------------------------|
| Analyte          | Relative Position of Consensus and Target Ranges  | <u>Laboratory</u><br>Variability |
| Aflatoxin B1     | 3 laboratories reported results above LOQ<br>Measurable levels not expected               | NA                               |
| Aflatoxin B2     | 2 laboratories reported results above LOQ<br>Measurable levels not expected               | NA                               |
| Aflatoxin G1     | 1 laboratory reported results above LOQ<br>Measurable levels not expected                 | NA                               |
| Aflatoxin G2     | 1 laboratory reported results above LOQ<br>Measurable levels not expected                 | NA                               |
| Total Aflatoxins | 5 laboratories reported results above LOQ<br>Measurable levels not expected               | NA                               |
| Fumonisin B1     | Consensus mean <i>within</i> target range<br>Consensus range <i>overlaps</i> target range | 84 %                             |
| Fumonisin B2     | 3 laboratories reported results above LOQ<br>Measurable levels not expected               | NA                               |
| Fumonisin B3     | 2 laboratories reported results above LOQ<br>Measurable levels not expected               | NA                               |
| Total Fumonisins | Consensus mean <i>within</i> target range<br>Consensus range <i>overlaps</i> target range | > 100 %                          |
| Deoxynivalenol   | Consensus mean <i>within</i> target range<br>Consensus range <i>overlaps</i> target range | 46 %                             |
| Ochratoxin A     | 3 laboratories reported results above LOQ<br>Measurable levels not expected               | NA                               |
| T-2 Toxin        | No laboratories reported results above LOQ<br>Measurable levels not expected              | NA                               |
| HT-2 Toxin       | 1 laboratory reported results above LOQ<br>Measurable levels not expected                 | NA                               |
| Zearalenone      | 4 laboratories reported results above LOQ<br>Measurable levels not expected               | NA                               |

## Performance Summary for RM 8427 (Blank)

Measurable levels of aflatoxin B1, aflatoxin B2, aflatoxin G1, total aflatoxins, fumonisin B1, fumonisin B2, fumonisin B3, total fumonisins, deoxynivalenol, ochratoxin A, T-2 toxin, HT-2 toxin, and zearalenone were expected in RM 8427 (Incurred). The consensus means for most of these mycotoxins were within the target ranges and the consensus ranges overlapped with the target ranges (see table below). The exceptions included fumonisin B1, ochratoxin A, and T-2 toxin, with consensus values biased low with respect to the target values, and HT-2 toxin and zearalenone, with consensus values biased high with respect to the target values. The between-laboratory variability is also summarized below and ranged from very good for total fumonisins (16 %) to very high for aflatoxin G2 (82 %). For most mycotoxins in this sample, the between-laboratory variability was between 20 % and 50 %.

#### Performance Summary for RM 8427 (Incurred)

|                  |  | Between-                         |
|------------------|--|----------------------------------|
| Analyte          | Relative Position of Consensus and Target Ranges   | <u>Laboratory</u><br>Variability |
| Aflatoxin B1     | Consensus mean <i>within</i> target range<br>Consensus range <i>overlaps</i> target range                          | <u>44 %</u>                      |
| Aflatoxin B2     | Consensus mean <i>within</i> target range<br>Consensus range <i>overlaps</i> target range                          | 41 %                             |
| Aflatoxin G1     | Consensus mean <i>within</i> target range<br>Consensus range <i>overlaps</i> target range                          | 42 %                             |
| Aflatoxin G2     | Measurable levels not expected   | 82 %                             |
| Total Aflatoxins | Consensus mean <i>within</i> target range<br>Consensus range <i>overlaps</i> target range                          | 30 %                             |
| Fumonisin B1     | Consensus mean <i>within</i> target range, at lower limit<br>Consensus range <i>slightly overlaps</i> target range | 22 %                             |
| Fumonisin B2     | Consensus mean <i>within</i> target range<br>Consensus range <i>overlaps</i> target range                          | 23 %                             |
| Fumonisin B3     | Consensus mean <i>within</i> target range<br>Consensus range <i>overlaps</i> target range                          | 20 %                             |
| Total Fumonisins | Consensus mean <i>within</i> target range<br>Consensus range <i>overlaps</i> target range                          | 16 %                             |
| Deoxynivalenol   | Consensus mean <i>within</i> target range<br>Consensus range <i>overlaps</i> target range                          | 24 %                             |
| Ochratoxin A     | Consensus mean <i>below</i> target range<br>Consensus range <i>below</i> target range                              | 41 %                             |
| T-2 Toxin        | Consensus mean <i>below</i> target range<br>Consensus range <i>below</i> target range                              | 25 %                             |
| HT-2 Toxin       | Consensus mean <i>above</i> target range<br>Consensus range <i>above</i> target range                              | 49 %                             |
| Zearalenone      | Consensus mean <i>above</i> target range<br>Consensus range <i>above</i> target range                              | 45 %                             |

Measurable levels of aflatoxin B1, fumonisin B1, fumonisin B2, deoxynivalenol, ochratoxin A, T-2 toxin, HT 2 toxin, and zearalenone were expected in Incurred Corn B. The consensus means for these mycotoxins were within the target ranges and the consensus ranges overlapped with the target ranges (see table below). Consensus data for HT-2 toxin and zearalenone were skewed to the higher end of the target range. The between-laboratory variability is also summarized below and was good for most mycotoxins, ranging from very good for deoxynivalenol (17%) to high for ochratoxin A (50%).

## Performance Summary for Incurred Corn B

|                  |  | Between-                  |
|------------------|--|---------------------------|
| Analyta          | Palative Position of Consensus and Target Panges         | Laboratory<br>Variability |
| Analyte          | <u>Consensus meen within tenget renge</u>                | <u>v arraunity</u>        |
| Aflatoxin B1     | Consensus mean <i>within</i> target range                | 29 %                      |
|                  | Consensus range <i>overlaps</i> target range             |                           |
| Aflatoxin B2     | 3 laboratories reported results above LOQ                | NA                        |
|                  | Measurable levels not expected                           |                           |
| Aflatoxin G1     | I laboratory reported results above LOQ                  | NA                        |
|                  | Measurable levels not expected                           |                           |
| Aflatoxin G2     | 1 laboratory reported results above LOQ                  | NA                        |
|                  | Measurable levels not expected                           |                           |
| Total Aflatoxins | Measurable levels not expected                           | 26 %                      |
|                  | Consensus mean within target range                       | 21.0/                     |
| Fumonisin B1     | Consensus range <i>overlaps</i> target range             | 31 %                      |
| En en la in D2   | Consensus mean within target range                       | 20.0/                     |
| Fumonisin B2     | Consensus range overlaps target range                    | 29 %                      |
| Fumonisin B3     | Measurable levels not expected                           | 29 %                      |
|                  | Only EB1 and EB2 values reported on PT sample, so        |                           |
| Total Fumonisins | total FB target value not established                    | 24 %                      |
|                  | Consensus mean within target range                       |                           |
| Deoxynivalenol   | Consensus range overlans target range                    | 17 %                      |
|                  | Consensus mean within target range                       |                           |
| Ochratoxin A     | Consensus range overlans target range                    | 50 %                      |
|                  | Consensus mean within target range                       |                           |
| T-2 Toxin        | Consensus range <i>ovarlans</i> target range             | 39 %                      |
|                  | Consensus mean within target range at upper limit        |                           |
| HT-2 Toxin       | Consensus mean <i>winin</i> target range, at upper mint  | 36 %                      |
|                  | Consensus mean <i>within</i> target range at upper limit |                           |
| Zearalenone      | Consensus mean winni target range, at upper mint         | 24 %                      |
|                  | Consensus range parmany overraps target range            |                           |

 Four laboratories reported using liquid chromatography with tandem mass spectrometry detection (LC-MS/MS) and two laboratories reported using LC with fluorescence detection (LC-FLD) for determination of mycotoxins. One laboratory reported using an enzyme-linked immunosorbent assay (ELISA) approach for determination of total aflatoxins. One laboratory reported using gas chromatography with electron capture detection (GC-ECD), and one laboratory reported using LC with absorbance detection (LC-abs) for determination of deoxynivalenol and ochratoxin A. Most laboratories did not indicate the analytical method used for determination of mycotoxins.

# **Dietary Intake Technical Recommendations**

The following recommendations are based on results obtained from the participants in this study.

- Overall, most results for the incurred corns were good. No methods were presented as significantly better or worse than any other, although many laboratories did not report their methodology as a part of the study. No systematic biases were noted.
- A general trend among laboratory means can be observed in the sample/sample comparison views for aflatoxin B1, total aflatoxins, fumonisin B1, fumonisin B2, fumonisin B3, total fumonisins, deoxynivalenol, ochratoxin A, and zearalenone, indicating that laboratories reporting data below the consensus and/or target range for one incurred sample also reported data below the consensus and/or target range for the second incurred sample. Similarly, laboratories reporting data above the consensus and/or target range for the second incurred sample also reported sample also reported data above the consensus and/or target range for the second incurred sample.
  - An increasing trend in the sample/sample comparison view can be attributed to a potential calibration bias or error, in which a common calibration curve or scheme affects both samples equally.
  - The use of appropriate quality assurance samples to establish that a method is in control and performing correctly may reduce the likelihood of persistent calibration errors. Quality assurance samples can be commercially available reference materials (CRMs, SRMs, or RMs) or materials prepared in-house.
- For ochratoxin A and T-2 toxin, the consensus ranges in the candidate RM 8427 Mycotoxins in Corn (Incurred) were at the low end of the target ranges. These low values could be a result of incomplete extraction of these components from the corn matrix.
  - A re-extraction of a sample material (i.e., adding fresh extraction solvent to the solid material for a subsequent extraction step) can demonstrate whether additional analyte is extractable. Even when using isotopically labeled internal standards, the spiked internal standard does not completely reflect the incorporation of the incurred mycotoxin into the matrix.
  - Use caution when using a recovery factor to correct for incomplete extraction, as the perceived recovery of spiked samples does not completely reflect the incorporation of the incurred mycotoxin into the matrix and may result in low results.
  - Multi-mycotoxin methods based on aqueous solvent mixtures may underestimate T-2 toxin, which has limited water solubility.
- Several laboratories reported values of zero for measurements in all materials. "Zero" is not a quantity that can be measured, and therefore a more appropriate result would be to report that a value is below the MDL, LOQ, or quantitation limit.
- All results should be checked closely to avoid calculation errors and to be sure that results are reported in the requested units.

# Table 7-1. Individualized data summary table (NIST) for mycotoxins in blank corn and incurred corns.

| National Institute of St | tandards & | <b>Technology</b> |
|--------------------------|------------|-------------------|
|--------------------------|------------|-------------------|

|                                       | Lab Code:                                 | NIST  |             | 1. You         | r Results   |                   | 2. C | ommunity l | Results    | 3. T              | arget |
|---------------------------------------|---|-------|-------------|----------------|-------------|-------------------|------|------------|------------|-------------------|-------|
| Analyte                               | Sample                                    | Units | xi          | s <sub>i</sub> | $Z'_{comm}$ | Z <sub>NIST</sub> | Ν    | <i>x</i> * | <i>s</i> * | x <sub>NIST</sub> | L     |
| Aflatoxin B1                          | RM 8427 Mycotoxins in Corn (Blank)        | ng/g  |             |                |             |                   | 9    | 0.03       | 0.053      |                   |       |
| Aflatoxin B1                          | RM 8427 Mycotoxins in Corn (Incurred)     | ng/g  | 7.8         | 2.4            |             |                   | 24   | 6.3        | 2.8        | 7.8               | 4     |
| Aflatoxin B1                          | Incurred Corn B                           | ng/g  | 3.1         | 0.7            |             |                   | 24   | 2.9        | 0.83       | 3.1               | 1     |
| Aflatoxin B2                          | RM 8427 Mycotoxins in Corn (Blank)        | ng/g  |             |                |             |                   | 8    | 0.016      | 0.042      |                   |       |
| Aflatoxin B2                          | RM 8427 Mycotoxins in Corn (Incurred)     | ng/g  | 1.55        | 0.35           |             |                   | 24   | 1.16       | 0.47       | 1.55              | 0.    |
| Aflatoxin B2                          | Incurred Corn B                           | ng/g  |             |                |             |                   | 10   | 0.04       | 0.076      |                   |       |
| Aflatoxin G1                          | RM 8427 Mycotoxins in Corn (Blank)        | ng/g  |             |                |             |                   | 8    | 0.02       | 0.08       |                   |       |
| Aflatoxin G1                          | RM 8427 Mycotoxins in Corn (Incurred)     | ng/g  | 1.05        | 0.20           |             |                   | 25   | 1.01       | 0.42       | 1.05              | 0     |
| Aflatoxin G1                          | Incurred Corn B                           | ng/g  |             |                |             |                   | 9    | 0.03       | 0.12       |                   |       |
| Aflatoxin G2                          | RM 8427 Mycotoxins in Corn (Blank)        | ng/g  |             |                |             |                   | 10   | 0.06       | 0.33       |                   |       |
| Aflatoxin G2                          | RM 8427 Mycotoxins in Corn (Incurred)     | ng/g  |             |                |             |                   | 20   | 0.78       | 0.64       |                   |       |
| Aflatoxin G2                          | Incurred Corn B                           | ng/g  |             |                |             |                   | 9    | 0.4        | 1.9        |                   |       |
| Total Aflatoxins                      | RM 8427 Mycotoxins in Corn (Blank)        | ng/g  |             |                |             |                   | 11   | 0.05       | 0.12       |                   |       |
| Total Aflatoxins                      | RM 8427 Mycotoxins in Corn (Incurred)     | ng/g  | 10.4        | 2.5            |             |                   | 23   | 9.4        | 2.8        | 10.4              | 4     |
| Total Aflatoxins                      | Incurred Corn B                           | ng/g  |             |                |             |                   | 21   | 2.99       | 0.78       |                   |       |
| Fumonisin B1                          | RM 8427 Mycotoxins in Corn (Blank)        | ng/g  | 10.5        | 2.5            |             |                   | 12   | 9.2        | 7.7        | 10.5              | -     |
| Fumonisin B1                          | RM 8427 Mycotoxins in Corn (Incurred)     | ng/g  | 869         | 79             |             |                   | 17   | 740        | 160        | 869               | 1     |
| Fumonisin B1                          | Incurred Corn B                           | ng/g  | 463         | 83             |             |                   | 17   | 450        | 140        | 463               | 1     |
| Fumonisin B2                          | RM 8427 Mycotoxins in Corn (Blank)        | ng/g  |             |                |             |                   | 7    | 1.8        | 5.4        |                   |       |
| Fumonisin B2                          | RM 8427 Mycotoxins in Corn (Incurred)     | ng/g  | 226         | 26             |             |                   | 17   | 210        | 48         | 226               |       |
| Fumonisin B2                          | Incurred Corn B                           | ng/g  | 108         | 24             |             |                   | 17   | 100        | 30         | 108               |       |
| Fumonisin B3                          | RM 8427 Mycotoxins in Corn (Blank)        | ng/g  |             |                |             |                   | 6    | 0.5        | 2.2        |                   |       |
| Fumonisin B3                          | RM 8427 Mycotoxins in Corn (Incurred)     | ng/g  | 98          | 9              |             |                   | 12   | 100        | 20         | 98                |       |
| Fumonisin B3                          | Incurred Corn B                           | ng/g  |             | ·              |             |                   | 11   | 90         | 27         |                   |       |
| Total Fumonisins                      | RM 8427 Mycotoxins in Corn (Blank)        | ng/g  | 10.5        | 25             |             |                   | 10   | 20         | 20         | 10.5              | 4     |
| Total Fumonisins                      | RM 8427 Mycotoxins in Corn (Incurred)     | ng/g  | 1190        | 83             |             |                   | 11   | 1130       | 180        | 1190              | 1     |
| Total Fumonisins                      | Incurred Corn B                           | ng/g  |             |                |             |                   | 10   | 710        | 170        |                   | -     |
| Deoxynivalenol                        | RM 8427 Mycotoxins in Corn (Blank)        | ng/g  | 146         | 17             |             |                   | 17   | 150        | 67         | 146               |       |
| Deoxynivalenol                        | RM 8427 Mycotoxins in Corn (Incurred)     | ng/g  | 468         | 24             |             |                   | 18   | 490        | 120        | 468               |       |
| Deoxynivalenol                        | Incurred Corn B                           | ng/g  | 731         | 123            |             |                   | 18   | 760        | 130        | 731               | 2     |
| Ochratovin A                          | RM 8427 Mycotoxins in Corn (Blank)        | ng/g  | ,51         | 120            |             |                   | 8    | 0.26       | 0.76       | ,51               | -     |
| Ochratovin A                          | RM 8427 Mycotoxins in Corn (Incurred)     | ng/g  | 11          | 5              |             |                   | 21   | 9.6        | 3.9        | 11                |       |
| Ochratoxin A                          | Incurred Corn B                           | ng/g  | 2.18        | 0.48           |             |                   | 20   | 2.1        | 1.1        | 2.18              | 0     |
| T 2 Toxin                             | PM 8427 Mucatovins in Corn (Blank)        | ng/g  | 2.10        | 0.40           |             |                   | 20   | 0          | 0          | 2.10              | 0     |
| T 2 Toxin                             | PM 8427 Mycotoxins in Corn (Incurred)     | ng/g  | 10.8        | 3.0            |             |                   | 13   | 20         | 4          | 10.8              | -     |
| T 2 Toxin                             | Incurred Corn B                           | ng/g  | 100         | 24             |             |                   | 13   | 110        | 4          | 100               |       |
| UT 2 Toxin                            | PM 8427 Mugatoring in Corn (Plank)        | ng/g  | 109         | 24             |             |                   | 5    | 0          | 45         | 109               |       |
| HT-2 TOXIII                           | RM 8427 Mycotoxins in Corn (Insurred)     | ng/g  | 29          | 6              |             |                   | 12   | 40         | 13         | 29                |       |
| HT 2 Toxin                            | Incurred Corn B                           | ng/g  | - 30<br>120 | 27             |             |                   | 12   | 40         | 10         | - 30<br>120       |       |
| Zaaralanona                           | DM 8427 Mugatoving in Corr (Direct)       | ng/g  | 120         | 27             |             |                   | 15   | 100        | 10         | 120               |       |
| Zearalenone                           | Rivi 8427 Mycotoxins in Corn (Blank)      | ng/g  | 75          | 11             |             |                   | 10   | 60         | 10         | 75                |       |
| Zearaienone                           | Kivi 842/ iviycoloxins in Corn (incurred) | ng/g  | /5          | 11             |             |                   | 10   | 150        | 21         | /5                |       |
| · · · · · · · · · · · · · · · · · · · | Inclusion of Loren M                      | 00/0  | 141         | 50             |             |                   | 1/   | 170        | 17         | 141               |       |

U expanded uncertainty x\* Robust mean of reported about the NIST-assessed value

 $Z'_{\rm comm}$  Z'-score with respect to community consensus

values  $Z_{\rm NIST}$  Z-score with respect to NIST value s\* Robust standard deviation

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**Table 7-2.** Data summary table for aflatoxin B1 in blank and incurred corns. Data points highlighted in red have been flagged as potential outliers (e.g., difference from reference value, Grubb and/or Cochran) by the NIST software package.

|         |        | Aflatoxin B1 |            |             |            |           |          |            |             |                                    |            |          |        |           |        |       |
|---------|--------|--------------|------------|-------------|------------|-----------|----------|------------|-------------|------------------------------------|------------|----------|--------|-----------|--------|-------|
| -       |        | RM 842       | 27 Mycoto  | oxins in Co | orn (Blanl | k) (ng/g) | RM 8427  | ' Mycotor  | kins in Cor | n (Incurre                         | ed) (ng/g) |          | Incurr | ed Corn B | (ng/g) |       |
|         | Lab    | Α            | В          | С           | Avg        | SD        | Α        | В          | С           | Avg                                | SD         | Α        | В      | С         | Avg    | SD    |
|         | Target |              |            |             |            |           |          |            |             | 7.80                               | 4.80       |          |        |           | 3.07   | 1.35  |
|         | A005   | < 1.000      | < 1.000    | < 1.000     |            |           | 8        | 7.9        | 8           | 7.97                               | 0.06       | 3.1      | 3.1    | 3.1       | 3.1    | 0     |
|         | A013   | < 0.120      | < 0.120    | < 0.120     |            |           | 5.84     | 5.58       | 5.57        | 5.66                               | 0.15       | 2.38     | 2.45   | 2.47      | 2.433  | 0.047 |
|         | A017   | 0            | 0          | 0           | 0          | 0         | 8.2      | 6.09       | 6           | 6.76                               | 1.25       | 1.82     | 1.89   | 1.78      | 1.83   | 0.056 |
|         | A020   |              |            |             |            |           |          |            |             |                                    |            |          |        |           |        |       |
|         | A021   | 0.11         | 0.18       |             | 0.145      | 0.049     | 5.5      | 6.7        | 7.1         | 6.43                               | 0.83       | 3.4      | 3.4    | 3.2       | 3.333  | 0.115 |
|         | A025   | < 0.300      | < 0.300    | < 0.300     |            |           | 7.59     | 5.49       | 7.12        | 6.73                               | 1.1        | 3.25     | 3.1    | 3.38      | 3.243  | 0.14  |
|         | A026   |              |            |             |            |           | 4.6      |            |             | 4.6                                |            | 2.7      |        |           | 2.7    |       |
|         | A027   |              |            |             |            |           |          |            |             |                                    |            |          |        |           |        |       |
|         | A028   | < 0.500      | < 0.500    | < 0.500     |            |           | 6.9      | 7.7        | 7.5         | 7.37                               | 0.42       | 3.2      | 3.1    | 3.4       | 3.233  | 0.153 |
|         | A031   | 0.18         | 0.18       | 0.18        | 0.18       | 0         | 7.56     | 5.05       | 7.9         | 6.84                               | 1.56       | 3.01     | 3.15   | 3.23      | 3.13   | 0.111 |
|         | A035   |              |            |             |            |           |          |            |             |                                    |            |          |        |           |        |       |
|         | A036   | < 0.075      | < 0.075    | < 0.075     |            |           | 6.57     | 5.04       | 5.82        | 5.81                               | 0.77       | 3.65     | 3.5    | 3.57      | 3.573  | 0.075 |
|         | A039   |              |            |             |            |           |          |            |             |                                    |            |          |        |           |        |       |
| ault    | A042   |              |            |             |            |           |          |            |             |                                    |            |          |        |           |        |       |
| Res     | A043   | 0            | 0          | 0           | 0          | 0         | 3.94     | 3.9        | 6.05        | 4.63                               | 1.23       | 3.09     | 2.91   | 2.6       | 2.867  | 0.248 |
| lal     | A045   |              |            |             |            |           | 10.55    | 9.55       | 11.08       | 10.39                              | 0.78       | 3.51     | 3.72   | 3.85      | 3.693  | 0.172 |
| ubi     | A047   | < 0.100      | < 0.100    | < 0.100     |            |           | 2.63     | 2.19       | 2.33        | 2.38                               | 0.22       | 1.95     | 1.85   | 1.8       | 1.867  | 0.076 |
| div     | A048   | 0            | 0          | 0           | 0          | 0         | 8.1      | 7.8        | 11          | 8.97                               | 1.77       | 3.7      | 3.8    | 3.6       | 3.7    | 0.1   |
| Ц       | A052   | 0.05         | 0.049      | 0.06        | 0.053      | 0.006     | 5.3      | 3.62       | 4.41        | 4.44                               | 0.84       | 3.5      | 3.2    | 3.3       | 3.333  | 0.153 |
|         | A053   | < 0.500      | < 0.500    |             |            |           | 5        | 5.05       | 4.3         | 4.78                               | 0.42       | 2.97     | 3.1    | 2.84      | 2.97   | 0.13  |
|         | A054   | 0            | 0          | 0           | 0          | 0         | 11.4     | 8.32       | 7.79        | 9.17                               | 1.95       | 3.34     | 3.35   | 3.32      | 3.337  | 0.015 |
|         | A060   | < 0.100      | < 0.100    | < 0.100     |            |           | 3.33     | 3.17       | 3.04        | 3.18                               | 0.15       | 1.82     | 1.78   | 1.88      | 1.827  | 0.05  |
|         | A065   |              |            |             |            |           |          |            |             |                                    |            |          |        |           |        |       |
|         | A066   | 0            | 0          | 0           | 0          | 0         | 11       | 8          | 5           | 8                                  | 3          | 4        | 4      | 3         | 3.667  | 0.577 |
|         | A067   | < 1.000      | < 1.000    | < 1.000     |            |           | 10.2     | 9          | 9.7         | 9.63                               | 0.6        | 3.9      | 3.9    | 3.9       | 3.9    | 0     |
|         | A070   |              |            | 0           |            |           |          |            |             |                                    |            |          |        |           |        |       |
|         | A071   | 0            | 0          | 0           | 0          | 0         | 5.14     | 5.14       | 5.66        | 5.31                               | 0.3        | 2.72     | 2.6    | 2.63      | 2.65   | 0.062 |
|         | A073   | < 0.300      |            |             |            |           | 2.85     | 4.48       | 2.21        | 3.18                               | 1.17       | 1.47     | 1.42   | 1.64      | 1.51   | 0.115 |
|         | A074   |              |            |             |            |           |          |            |             |                                    |            |          |        |           |        |       |
|         | A075   |              |            |             |            |           |          |            |             |                                    |            |          |        |           |        |       |
|         | A077   | < 1.000      | < 1.000    | < 1.000     |            |           | 7.42     | 7.22       | 6.77        | 7.14                               | 0.33       | 1.77     | 1.84   | 1.81      | 1.807  | 0.035 |
|         | A079   | < 4.000      | < 4.000    | < 4.000     |            |           | 40.5     | 16.3       | 14.2        | 23.67                              | 14.62      | 8.55     | 8.52   | 10.84     | 9.303  | 1.331 |
|         | A083   | G            |            |             | 0.02       |           | G        |            |             | ( )                                |            | G        |        |           | 2.00   |       |
| uity    |        | Consensu     | s Mean     | 10          | 0.03       |           | Consensu | s Mean     |             | 6.3                                |            | Consensu | s Mean |           | 2.90   |       |
| ults    |        | Consensu     | s Standard | 1 Deviation | 0.053      |           | Consensu | s Standard | 1 Deviation | a 2.8 Consensus Standard Deviation |            |          |        | Deviation | 0.83   |       |
| Ses     |        | Maximun      | 1          |             | 0.18       |           | Maximum  | 1          |             | 23.7                               |            | Maximum  | 1      |           | 9.30   |       |
| °C<br>C |        | Minimum      |            |             | 0          |           | Minimum  |            |             | 2.4                                |            | Minimum  |        |           | 1.51   |       |
|         |        | N            |            |             | 9          |           | N        |            |             | 22                                 |            | Ν        |        |           | 22     |       |



Figure 7-1. Aflatoxin B1 in candidate RM 8427 Mycotoxins in Corn (Blank) (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red line represents the upper consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \leq 2$ , with the lower range set at zero. A NIST value has not been determined in this material.



**Figure 7-2.** Aflatoxin B1 in candidate RM 8427 Mycotoxins in Corn (Incurred) (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \leq 2$ . The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by its uncertainty ( $U_{NIST}$ ) and represents the range that results in an acceptable  $Z_{NIST}| \leq 2$ .



**Figure 7-3.** Aflatoxin B1 in Incurred Corn B (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \leq 2$ . The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by its uncertainty ( $U_{NIST}$ ) and represents the range that results in an acceptable  $Z_{NIST}$  score,  $|Z_{NIST}| \leq 2$ .



**Figure 7-4.** Laboratory means for aflatoxin B1 in candidate RM 8427 Mycotoxins in Corn (Incurred) and Incurred Corn B (sample/sample comparison view). In this view, the individual laboratory mean for one sample (RM 8427) is compared to the mean for a second sample (Incurred Corn B). The solid red box represents the NIST range of tolerance for the two samples, RM 8427 Mycotoxins in Corn (Incurred) (x-axis) and Incurred Corn B (y-axis), which encompasses the target values bounded by their uncertainties ( $U_{\text{NIST}}$ ) and represents the range that results in an acceptable  $Z_{\text{NIST}}$  score,  $|Z_{\text{NIST}}| \leq 2$ . The dotted blue box represents the consensus range of tolerance for RM 8427 Mycotoxins in Corn (Incurred) (x-axis) and Incurred (x-axis) and Incurred Corn B (y-axis), calculated as the values above and below the consensus means that result in an acceptable  $Z'_{\text{comm}}$  score,  $|Z'_{\text{comm}}| \leq 2$ .

**Table 7-3.** Data summary table for aflatoxin B2 in blank and incurred corns. Data points highlighted in red have been flagged as potential outliers (e.g., difference from reference value, Grubb and/or Cochran) by the NIST software package.

|            |        | Aflatoxin B2 |               |             |            |           |          |            |             |              |            |          |             |           |        |       |
|------------|--------|--------------|---------------|-------------|------------|-----------|----------|------------|-------------|--------------|------------|----------|-------------|-----------|--------|-------|
|            |        | RM 842       | 27 Mycoto     | oxins in Co | orn (Blanl | k) (ng/g) | RM 8427  | Mycoto     | xins in Cor | n (Incurre   | ed) (ng/g) |          | Incurr      | ed Corn B | (ng/g) |       |
|            | Lab    | Α            | В             | С           | Avg        | SD        | Α        | В          | С           | Avg          | SD         | Α        | В           | С         | Avg    | SD    |
|            | Target |              |               |             |            |           |          |            |             | 1.55         | 0.70       |          |             |           |        |       |
|            | A005   | < 1.000      | < 1.000       | < 1.000     |            |           | 1.6      | 1.6        | 1.6         | 1.6          | 0          | < 1.000  | < 1.000     | < 1.000   |        |       |
|            | A013   | < 0.120      | < 0.120       | < 0.120     |            |           | 1.31     | 1.26       | 1.27        | 1.28         | 0.026      | < 0.120  | < 0.120     | < 0.120   |        |       |
|            | A017   | 0            | 0             | 0           | 0          | 0         | 0.79     | 0.78       | 0.75        | 0.773        | 0.021      | 0        | 0           | 0         | 0      | 0     |
|            | A020   |              |               |             |            |           |          |            |             |              |            |          |             |           |        |       |
|            | A021   | 0.12         | 0.19          |             | 0.155      | 0.049     | 1.54     | 1.48       | 1.52        | 1.513        | 0.031      | 0.18     | 0.15        | 0.14      | 0.157  | 0.021 |
|            | A025   | < 0.150      | < 0.150       | < 0.150     |            |           | 1.2      | 1.34       | 1.32        | 1.287        | 0.076      | 0.22     | 0.23        | 0.21      | 0.22   | 0.01  |
|            | A026   |              |               |             |            |           | 0.8      |            |             | 0.8          |            |          |             |           |        |       |
|            | A027   |              |               |             |            |           |          |            |             |              |            |          |             |           |        |       |
|            | A028   | < 0.300      | < 0.300       | < 0.300     |            |           | 1.5      | 1.6        | 1.4         | 1.5          | 0.1        | < 0.300  | < 0.300     | < 0.300   |        |       |
|            | A031   | < 0.000      | < 0.000       | < 0.000     |            |           | 1.56     | 1.22       | 1.31        | 1.363        | 0.176      | < 0.000  | < 0.000     | < 0.000   |        |       |
|            | A035   |              |               |             |            |           |          |            |             |              |            |          |             |           |        |       |
|            | A036   | < 0.045      | < 0.045       | < 0.045     |            |           | 1.64     | 1.22       | 1.14        | 1.333        | 0.269      | < 0.045  | < 0.045     | < 0.045   |        |       |
|            | A039   | < 2.000      | < 2.000       | < 2.000     |            |           | < 2.000  | 7.5        | 5.7         | 6.6          | 1.273      | < 2.000  | < 2.000     | < 2.000   |        |       |
| ults       | A042   |              |               |             |            |           |          |            |             |              |            |          |             |           |        |       |
| kes        | A043   | 0            | 0             | 0           | 0          | 0         | 0.887    | 0.871      | 0.896       | 0.885        | 0.013      | 0        | 0           | 0         | 0      | 0     |
| al F       | A045   |              |               |             |            |           | 1.73     | 1.76       | 1.77        | 1.753        | 0.021      | 0.09     | 0.09        | 0.11      | 0.097  | 0.012 |
| np         | A047   | < 0.100      | < 0.100       | < 0.100     |            |           | 0.34     | 0.34       | 0.35        | 0.343        | 0.006      | < 0.100  | < 0.100     | < 0.100   |        |       |
| livid      | A048   | 0            | 0             | 0           | 0          | 0         | 1.7      | 1.7        | 1.5         | 1.633        | 0.115      | 0        | 0           | 0         | 0      | 0     |
| Inc        | A052   | 0.055        | 0.063         | 0.056       | 0.058      | 0.004     | 0.81     | 0.61       | 0.6         | 0.673        | 0.118      | 0        | 0           | 0         | 0      | 0     |
|            | A053   | < 0.500      | < 0.500       |             |            |           | 1.13     | 1.01       | 0.99        | 1.043        | 0.076      | < 0.500  | < 0.500     | < 0.500   |        |       |
|            | A054   | 0            | 0             | 0           | 0          | 0         | 1.67     | 1.4        | 1.44        | 1.503        | 0.146      | 0        | 0           | 0         | 0      | 0     |
|            | A060   | < 0.100      | < 0.100       | < 0.100     |            |           | 0.55     | 0.47       | 0.52        | 0.513        | 0.04       | < 0.100  | < 0.100     | < 0.100   |        |       |
|            | A065   |              |               |             |            |           |          |            |             |              |            |          |             |           |        |       |
|            | A066   | 0            | 0             | 0           | 0          | 0         | 1        | 1          | 1           | 1            | 0          | 0        | 0           | 0         | 0      | 0     |
|            | A067   | < 1.000      | < 1.000       | < 1.000     |            |           | 1.4      | 1.4        | 1.5         | 1.433        | 0.058      | < 1.000  | < 1.000     | < 1.000   |        |       |
|            | A070   |              |               |             |            |           |          |            |             |              |            |          |             |           |        |       |
|            | A071   | 0            | 0             | 0           | 0          | 0         | 0.959    | 0.86       | 1.16        | 0.993        | 0.153      | 0        | 0           | 0         | 0      | 0     |
|            | A073   | < 0.300      |               |             |            |           | 1.41     | 1.51       | 1.35        | 1.423        | 0.081      | < 0.300  | < 0.300     | < 0.300   |        |       |
|            | A074   |              |               |             |            |           |          |            |             |              |            |          |             |           |        |       |
|            | A075   |              |               |             |            |           |          |            |             |              |            |          |             |           |        |       |
|            | A077   | < 0.500      | < 0.500       | < 0.500     |            |           | 0.76     | 0.73       | 0.76        | 0.75         | 0.017      | < 0.500  | < 0.500     | < 0.500   |        |       |
|            | A079   | < 4.000      | < 4.000       | < 4.000     |            |           | < 4.000  | < 4.000    | < 4.000     |              |            | < 4.000  | < 4.000     | < 4.000   |        |       |
|            | A083   |              |               |             |            |           |          |            |             |              |            |          |             |           |        |       |
| ţ          |        | Consensu     | s Mean        |             | 0.016      |           | Consensu | s Mean     |             | 1.16         |            | Consensu | ıs Mean     |           | 0.040  |       |
| uni<br>lts |        | Consensu     | s Standard    | 1 Deviation | 0.042      |           | Consensu | s Standard | 1 Deviation | 0.47         |            | Consensu | is Standard | 0.076     |        |       |
| nm         |        | Maximum      | faximum 0.155 |             |            |           | Maximum  |            |             | 6.6 Maximun  |            |          | n 0.2       |           |        |       |
| R          |        | Minimum      |               |             | 0          |           | Minimum  |            |             | 0.34 Minimum |            |          |             |           | 0      |       |
| 5          |        | Ν            |               |             | 8          |           | Ν        |            |             | 22           |            | Ν        |             |           | 10     |       |



Figure 7-5. Aflatoxin B2 in candidate RM 8427 Mycotoxins in Corn (Blank) (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red line represents the upper consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \le 2$ , with the lower range set at zero. A NIST value has not been determined in this material.

Exercise

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**Figure 7-6.** Aflatoxin B2 in candidate RM 8427 Mycotoxins in Corn (Incurred) (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \leq 2$ . The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by its uncertainty ( $U_{NIST}$ ) and represents the range that results in an acceptable  $Z_{NIST}$  score,  $|Z_{NIST}| \leq 2$ .



**Figure 7-7.** Aflatoxin B2 in Incurred Corn B (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red line represents the upper consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \leq 2$ , with the lower range set at zero. A NIST value has not been determined in this material.



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**Figure 7-8.** Laboratory means for aflatoxin B2 in candidate RM 8427 Mycotoxins in Corn (Incurred) and Incurred Corn B (sample/sample comparison view). In this view, the individual laboratory mean for one sample (RM 8427) is compared to the mean for a second sample (Incurred Corn B). The dotted blue box represents the consensus range of tolerance for RM 8427 Mycotoxins in Corn (Incurred) (x-axis) and Incurred Corn B (y-axis), calculated as the values above and below the consensus means that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \le 2$ .

**Table 7-4.** Data summary table for aflatoxin G1 in blank and incurred corns. Data points highlighted in red have been flagged as potential outliers (e.g., difference from reference value, Grubb and/or Cochran) by the NIST software package.

|            |        | Aflatoxin G1 |             |             |            |                    |          |             |             |               |       |                                   |           |         |       |       |
|------------|--------|--------------|-------------|-------------|------------|--------------------|----------|-------------|-------------|---------------|-------|-----------------------------------|-----------|---------|-------|-------|
|            |        | RM 842       | 27 Mycoto   | oxins in Co | orn (Blank | x) ( <b>ng/g</b> ) | RM 8427  | tins in Cor | n (Incurre  | ed) (ng/g)    |       | Incurr                            | ed Corn B | (ng/g)  |       |       |
|            | Lab    | Α            | В           | С           | Avg        | SD                 | Α        | В           | С           | Avg           | SD    | Α                                 | В         | С       | Avg   | SD    |
|            | Target |              |             |             |            |                    |          |             |             | 1.05          | 0.40  |                                   |           |         |       |       |
|            | A005   | < 1.000      | < 1.000     | < 1.000     |            |                    | 2        | 2.2         | 1.8         | 2             | 0.2   | < 1.000                           | < 1.000   | < 1.000 |       |       |
|            | A013   | < 0.120      | < 0.120     | < 0.120     |            |                    | 1.46     | 1.39        | 1.41        | 1.42          | 0.036 | < 0.120                           | < 0.120   | < 0.120 |       |       |
|            | A017   | 0            | 0           | 0           | 0          | 0                  | 0.67     | 0.65        | 0.64        | 0.653         | 0.015 | 0                                 | 0         | 0       | 0     | 0     |
|            | A020   |              |             |             |            |                    |          |             |             |               |       |                                   |           |         |       |       |
|            | A021   | 0.16         | 0.16        |             | 0.16       | 0                  | 1.23     | 1.16        | 1.49        | 1.293         | 0.174 | 0.14                              | 0.45      | 0.11    | 0.233 | 0.188 |
|            | A025   | < 0.300      | < 0.300     | < 0.300     |            |                    | 1.42     | 1.1         | 1.13        | 1.217         | 0.177 | < 0.300                           | < 0.300   | < 0.300 |       |       |
|            | A026   |              |             |             |            |                    | 0.84     |             |             | 0.84          |       |                                   |           |         |       |       |
|            | A027   |              |             |             |            |                    |          |             |             |               |       |                                   |           |         |       |       |
|            | A028   | < 0.700      | < 0.700     | < 0.700     |            |                    | 0.9      | 0.9         | 1           | 0.933         | 0.058 | < 0.700                           | < 0.700   | < 0.700 |       |       |
|            | A031   | < 0.000      | < 0.000     | < 0.000     |            |                    | 1.03     | 1.1         | 1.05        | 1.06          | 0.036 | < 0.000                           | < 0.000   | < 0.000 |       |       |
|            | A036   | < 0.045      | < 0.045     | < 0.045     |            |                    | 0.82     | 0.94        | 0.83        | 0.863         | 0.067 | < 0.045                           | < 0.045   | < 0.045 |       |       |
|            | A039   | < 2.000      | < 2.000     | < 2.000     |            |                    | < 2.000  | 7.05        | 6.55        | 6.8           | 0.354 | 4.15                              | < 2.000   | < 2.000 | 4.15  |       |
| 2          | A042   |              |             |             |            |                    |          |             |             |               |       |                                   |           |         |       |       |
| sul        | A043   | 0            | 0           | 0           | 0          | 0                  | 1.02     | 0.855       | 0.786       | 0.887         | 0.12  | 0                                 | 0         | 0       | 0     | 0     |
| Re         | A045   |              |             |             |            |                    | 0.92     | 0.88        | 1           | 0.933         | 0.061 |                                   |           |         |       |       |
| ual        | A047   | < 0.100      | < 0.100     | < 0.100     |            |                    | 0.41     | 0.35        | 0.46        | 0.407         | 0.055 | < 0.100                           | < 0.100   | < 0.100 |       |       |
| vid        | A048   | 0            | 0           | 0           | 0          | 0                  | 1        | 1.2         | 1.1         | 1.1           | 0.1   | 0                                 | 0         | 0       | 0     | 0     |
| ibi        | A052   | 0            | 0           | 0           | 0          | 0                  | 0.52     | 0.59        | 0.53        | 0.547         | 0.038 | 0                                 | 0         | 0       | 0     | 0     |
| 4          | A053   | < 0.500      | < 0.500     |             |            |                    | 0.97     | 0.8         | 0.83        | 0.867         | 0.091 | < 0.500                           | < 0.500   | < 0.500 |       |       |
|            | A054   | 0            | 0           | 0           | 0          | 0                  | 1.1      | 1.03        | 1.24        | 1.123         | 0.107 | 0                                 | 0         | 0       | 0     | 0     |
|            | A060   | < 0.100      | < 0.100     | < 0.100     |            |                    | 0.76     | 0.67        | 0.6         | 0.677         | 0.08  | < 0.100                           | < 0.100   | < 0.100 |       |       |
|            | A065   |              |             |             |            |                    |          |             |             |               |       |                                   |           |         |       |       |
|            | A066   | 0            | 0           | 0           | 0          | 0                  | 1        | 3           | 1           | 1.667         | 1.155 | 0                                 | 0         | 0       | 0     | 0     |
|            | A067   | < 1.000      | < 1.000     | < 1.000     |            |                    | 1.2      | 1.2         | 1.2         | 1.2           | 0     | < 1.000                           | < 1.000   | < 1.000 |       |       |
|            | A070   |              |             |             |            |                    |          |             |             |               |       |                                   |           |         |       |       |
|            | A071   | 0            | 0           | 0           | 0          | 0                  | 1.66     | 0.776       | 1.03        | 1.155         | 0.455 | 0                                 | 0         | 0       | 0     | 0     |
|            | A073   | < 0.300      |             |             |            |                    | 1.1      | 1.14        | 1.12        | 1.12          | 0.02  | < 0.300                           | < 0.300   | < 0.300 |       |       |
|            | A074   |              |             |             |            |                    |          |             |             |               |       |                                   |           |         |       |       |
|            | A075   |              |             |             |            |                    |          |             |             |               |       |                                   |           |         |       |       |
|            | A077   | < 1.000      | < 1.000     | < 1.000     |            |                    | 0.6      | 0.66        | 0.69        | 0.65          | 0.046 | < 1.000                           | < 1.000   | < 1.000 |       |       |
|            | A079   | < 4.000      | < 4.000     | < 4.000     |            |                    | 15.4     | 13.7        | 11.6        | 13.567        | 1.904 | < 4.000                           | < 4.000   | < 4.000 |       |       |
|            | A083   |              |             |             |            |                    |          |             |             |               |       |                                   |           |         |       |       |
| ţ          |        | Consensu     | is Mean     |             | 0.02       |                    | Consensu | s Mean      |             | 1.01          |       | Consensu                          | is Mean   |         | 0.03  |       |
| uni<br>Its |        | Consensu     | is Standard | 1 Deviation | 0.08       |                    | Consensu | s Standard  | l Deviation | 0.42          |       | Consensus Standard Deviation 0.12 |           |         |       |       |
| am         |        | Maximun      | ı           |             | 0.16       |                    | Maximum  |             |             | 13.57 Maximum |       |                                   |           | 4.15    |       |       |
| R. Con     |        | Minimum      |             |             | 0          |                    | Minimum  |             |             | 0.41 Minimum  |       |                                   |           |         | 0     |       |
| •          |        | Ν            |             |             | 8          |                    | Ν        |             |             | 23            |       | Ν                                 | 8         |         |       |       |



Exercise HAMQAP Exercise 1 - Dietary Intake Sample: RM 8427 Mycotoxins in Corn (Blank) Measurand: Aflatoxin G1

**Figure 7-9.** Aflatoxin G1 in candidate RM 8427 Mycotoxins in Corn (Blank) (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red line represents the upper consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \leq 2$ , with the lower range set at zero. A NIST value has not been determined in this material.



**Figure 7-10.** Aflatoxin G1 in candidate RM 8427 Mycotoxins in Corn (Incurred) (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \leq 2$ . The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by its uncertainty ( $U_{NIST}$ ) and represents the range that results in an acceptable  $Z_{NIST}$  score,  $|Z_{NIST}| \leq 2$ .



**Figure 7-11.** Aflatoxin G1 in Incurred Corn B (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red line represents the upper consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \leq 2$ , with the lower range set at zero. A NIST value has not been determined in this material.



**Figure 7-12.** Laboratory means for aflatoxin G1 in candidate RM 8427 Mycotoxins in Corn (Incurred) and Incurred Corn B (sample/sample comparison view). In this view, the individual laboratory mean for one sample (RM 8427) is compared to the mean for a second sample (Incurred Corn B). The dotted blue box represents the consensus range of tolerance for RM 8427 Mycotoxins in Corn (Incurred) (x-axis) and Incurred Corn B (y-axis), calculated as the values above and below the consensus means that result in an acceptable  $Z'_{\text{comm}}$  score,  $|Z'_{\text{comm}}| \le 2$ .

**Table 7-5.** Data summary table for aflatoxin G2 in blank and incurred corns. Data points highlighted in red have been flagged as potential outliers (e.g., difference from reference value, Grubb and/or Cochran) by the NIST software package.

|             |       | Aflatoxin G2                      |           |             |            |          |                                   |           |             |            |            |                        |             |         |      |    |  |
|-------------|-------|-----------------------------------|-----------|-------------|------------|----------|-----------------------------------|-----------|-------------|------------|------------|------------------------|-------------|---------|------|----|--|
|             |       | RM 842                            | 27 Mycoto | oxins in Co | orn (Blank | ) (ng/g) | RM 8427                           | / Mycotox | tins in Cor | n (Incurre | ed) (ng/g) | Incurred Corn B (ng/g) |             |         |      |    |  |
| L           | Lab   | Α                                 | В         | С           | Avg        | SD       | Α                                 | В         | С           | Avg        | SD         | Α                      | В           | С       | Avg  | SD |  |
| Ta          | arget |                                   |           |             |            |          |                                   |           |             |            |            |                        |             |         |      |    |  |
| A           | 4005  | < 1.000                           | < 1.000   | < 1.000     |            |          | 1.4                               | 1.3       | 1.4         | 1.367      | 0.058      | < 1.000                | < 1.000     | < 1.000 |      |    |  |
| A           | 013   | < 0.120                           | < 0.120   | < 0.120     |            |          | 1.22                              | 0.84      | 0.72        | 0.927      | 0.261      | < 0.120                | < 0.120     | < 0.120 |      |    |  |
| A           | 017   | 0                                 | 0         | 0           | 0          | 0        | 0.36                              | 0.43      | 0.43        | 0.407      | 0.04       | 0                      | 0           | 0       | 0    | 0  |  |
| A           | 020   |                                   |           |             |            |          |                                   |           |             |            |            |                        |             |         |      |    |  |
| A           | A021  | 0                                 | 0         |             | 0          | 0        | 1                                 | 1.1       | 1           | 1.033      | 0.058      | 0                      | 0           | 0       | 0    | 0  |  |
| A           | A025  | < 0.150                           | < 0.150   | < 0.150     |            |          | 1.23                              | 1.1       | 1.26        | 1.197      | 0.085      | < 0.150                | < 0.150     | < 0.150 |      |    |  |
| A           | A026  |                                   |           |             |            |          | 0.74                              |           |             | 0.74       |            |                        |             |         |      |    |  |
| A           | 027   |                                   |           |             |            |          |                                   |           |             |            |            |                        |             |         |      |    |  |
| A           | A028  | < 0.900                           | < 0.900   | < 0.900     |            |          | < 0.900                           | < 0.900   | < 0.900     |            |            | < 0.900                | < 0.900     | < 0.900 |      |    |  |
| A           | A031  | < 0.000                           | < 0.000   | < 0.000     |            |          | < 0.000                           | < 0.000   | < 0.000     |            |            | < 0.000                | < 0.000     | < 0.000 |      |    |  |
| A           | A036  | 0.47                              | 0.54      | 0.47        | 0.493      | 0.04     | 1.65                              | 1.76      | 1.69        | 1.7        | 0.056      | < 0.160                | < 0.160     | < 0.160 |      |    |  |
| A           | 1039  | < 2.000                           | < 2.000   | 3.34        | 3.34       |          | 2.96                              | 4.41      | 6.26        | 4.543      | 1.654      | 3.89                   | < 2.000     | < 2.000 | 3.89 |    |  |
| <u>a</u> A( | A042  |                                   |           |             |            |          |                                   |           |             |            |            |                        |             |         |      |    |  |
| B A         | A043  | 0                                 | 0         | 0           | 0          | 0        | 0                                 | 0         | 0           | 0          | 0          | 0                      | 0           | 0       | 0    | 0  |  |
| A A         | A045  |                                   |           |             |            |          |                                   |           |             |            |            |                        |             |         |      |    |  |
| A A         | A047  | < 0.100                           | < 0.100   | < 0.100     |            |          | 0.38                              | 0.52      | 0.46        | 0.453      | 0.07       | < 0.100                | < 0.100     | < 0.100 |      |    |  |
| ivi V       | 4048  | 0                                 | 0         | 0           | 0          | 0        | 0                                 | 0         | 0           | 0          | 0          | 0                      | 0           | 0       | 0    | 0  |  |
| A A         | \$052 | 0                                 | 0         | 0           | 0          | 0        | 0.59                              | 0.48      | 0.54        | 0.537      | 0.055      | 0                      | 0           | 0       | 0    | 0  |  |
| A           | 4053  | < 0.500                           | < 0.500   |             |            |          | 1.07                              | 0.8       | 0.63        | 0.833      | 0.222      | < 0.500                | < 0.500     | < 0.500 |      |    |  |
| A           | A054  | 0                                 | 0         | 0           | 0          | 0        | 0.93                              | 0.94      | 0.97        | 0.947      | 0.021      | 0                      | 0           | 0       | 0    | 0  |  |
| A           | 4060  | < 0.100                           | < 0.100   | < 0.100     |            |          | 0.86                              | 0.78      | 0.8         | 0.813      | 0.042      | < 0.100                | < 0.100     | < 0.100 |      |    |  |
| A           | 4065  |                                   |           | 0           |            |          |                                   |           |             |            | 0          |                        |             | 0       |      |    |  |
| A           | 4066  | 0                                 | 0         | 0           | 0          | 0        | 1                                 | 1         | 1           | 1          | 0          | 0                      | 0           | 0       | 0    | 0  |  |
| A           | 4067  | < 1.000                           | < 1.000   | < 1.000     |            |          | 1.1                               | 1.2       | 1.1         | 1.133      | 0.058      | < 1.000                | < 1.000     | < 1.000 |      |    |  |
| A           | 070   | 0                                 | 0         | 0           | 0          | 0        | 0.501                             | 0.246     | 0.706       | 0.510      | 0.101      | 0                      | 0           | 0       | 0    | 0  |  |
| A           | 072   | 0                                 | 0         | 0           | 0          | 0        | 0.501                             | 0.346     | 0.706       | 0.518      | 0.181      | 0                      | 0           | 0       | 0    | 0  |  |
| A           | 074   |                                   |           |             |            |          |                                   |           |             |            |            |                        |             |         |      |    |  |
| A           | 075   |                                   |           |             |            |          |                                   |           |             |            |            |                        |             |         |      |    |  |
|             | 077   | < 0.500                           | < 0.500   | < 0.500     |            |          | 0.48                              | 0.40      | 0.5         | 0.40       | 0.01       | < 0.500                | < 0.500     | < 0.500 |      |    |  |
| A           | 070   | < 4.000                           | < 0.300   | < 0.300     |            |          | 0.48                              | 4.16      | 0.5         | 4 5 4 5    | 0.01       | < 4.000                | < 4.000     | < 1.000 |      |    |  |
|             | 083   | < 4.000                           | < 4.000   | < 4.000     |            |          | 4.93                              | 4.10      | < 4.000     | 4.545      | 0.544      | < 4.000                | < 4.000     | < 4.000 |      |    |  |
|             | 1005  | Consensus Mean 0.06               |           |             |            |          | Consensu                          | s Mean    |             | 0.78       |            | Consensi               | is Mean     | 0.4     |      |    |  |
| s           |       | Consensus Standard Deviation 0.33 |           |             |            |          | Consensus Standard Deviation 0.64 |           |             |            |            | Consense               | is Standard | 19      |      |    |  |
|             |       | Maximum                           |           |             | 3 34       |          | Maximum                           |           |             | 4 55       |            | Maximum                | Maximum     |         |      |    |  |
| Re          |       | Minimum                           |           |             | 0          |          | Minimum                           |           |             | 0          |            | Minimum                |             |         | 0    |    |  |
|             |       | N                                 |           |             | 9          |          | N                                 |           |             | 19         |            | N                      |             |         | 8    |    |  |



**Figure 7-13.** Aflatoxin G2 in candidate RM 8427 Mycotoxins in Corn (Blank) (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red line represents the upper consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \le 2$ , with the lower range set at zero. A NIST value has not been determined in this material.

Exercise

HAMQAP Exercise 1 - Dietary Intake



**Figure 7-14.** Aflatoxin G2 in candidate RM 8427 Mycotoxins in Corn (Incurred) (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red line represents the upper consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \le 2$ , with the lower range set at zero. A NIST value has not been determined in this material.



Figure 7-15. Aflatoxin G2 in Incurred Corn B (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red line represents the upper consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \leq 2$ , with the lower range set at zero. A NIST value has not been determined in this material.

Exercise

HAMQAP Exercise 1 - Dietary Intake



**Figure 7-16.** Laboratory means for aflatoxin G2 in candidate RM 8427 Mycotoxins in Corn (Incurred) and Incurred Corn B (sample/sample comparison view). In this view, the individual laboratory mean for one sample (RM 8427) is compared to the mean for a second sample (Incurred Corn B). The dotted blue box represents the consensus range of tolerance for RM 8427 Mycotoxins in Corn (Incurred) (x-axis) and Incurred Corn B (y-axis), calculated as the values above and below the consensus means that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \le 2$ .

**Table 7-6.** Data summary table for total aflatoxins in blank and incurred corns. Data points highlighted in red have been flagged as potential outliers (e.g., difference from reference value, Grubb and/or Cochran) by the NIST software package.

|                |        | Total Aflatoxins             |           |             |            |           |  |       |       |       |             |                        |             |         |       |       |  |  |
|----------------|--------|------------------------------|-----------|-------------|------------|-----------|--|-------|-------|-------|-------------|------------------------|-------------|---------|-------|-------|--|--|
|                |        | RM 842                       | 27 Mycoto | oxins in Co | orn (Blanl | k) (ng/g) | RM 8427 Mycotoxins in Corn (Incurred) (ng/g) |       |       |       |             | Incurred Corn B (ng/g) |             |         |       |       |  |  |
|                | Lab    | Α                            | В         | С           | Avg        | SD        | Α  | В     | С     | Avg   | SD          | Α                      | В           | С       | Avg   | SD    |  |  |
|                | Target |                              |           |             |            |           |  |       |       | 10.40 | 4.90        |                        |             |         |       |       |  |  |
|                | A013   | < 0.120                      | < 0.120   | < 0.120     |            |           | 9.82   | 9.08  | 8.96  | 9.29  | 0.47        | 2.38                   | 2.45        | 2.47    | 2.433 | 0.047 |  |  |
|                | A017   | 0                            | 0         | 0           | 0          | 0         | 10.02  | 7.95  | 7.82  | 8.6   | 1.23        | 1.82                   | 1.89        | 1.78    | 1.83  | 0.056 |  |  |
|                | A020   | 0.00                         | 0.50      |             | 0.44       | 0.000     | 0.05   | 10.04 |       | 10.01 | 0.02        | 0.50                   |             | 0.45    |       | 0.075 |  |  |
|                | A021   | 0.39                         | 0.53      | .0.150      | 0.46       | 0.099     | 9.27   | 10.34 | 10.82 | 10.24 | 0.92        | 3.72                   | 4           | 3.45    | 3.723 | 0.275 |  |  |
|                | A025   | < 0.150                      | < 0.150   | < 0.150     |            |           | 11.44  | 9.03  | 10.85 | 10.45 | 1.25        | 3.40                   | 5.54        | 3.39    | 3.465 | 0.125 |  |  |
|                | A020   |                              |           |             |            |           | /  |       |       | /     |             | 2.7                    |             |         | 2.7   |       |  |  |
|                | A027   | < 0.900                      | < 0.900   | < 0.900     |            |           | 93   | 10.2  | 9.9   | 9.8   | 0.46        | 3.2                    | 3.1         | 3.4     | 3 233 | 0.153 |  |  |
|                | A031   | < 0.000                      | < 0.000   | < 0.000     |            |           | 10.15  | 7 37  | 10.27 | 9.26  | 1.64        | 2.97                   | 3.11        | 3 19    | 3.09  | 0.133 |  |  |
|                | A036   | 0.47                         | 0.54      | 0.47        | 0.493      | 0.04      | 10.68  | 8.96  | 9.49  | 9.71  | 0.88        | 3.65                   | 3.5         | 3.57    | 3.573 | 0.075 |  |  |
|                | A039   |                              |           |             |            |           |  |       |       |       |             |                        |             |         |       |       |  |  |
| vidual Results | A041   |                              |           |             |            |           |  |       |       |       |             |                        |             |         |       |       |  |  |
|                | A042   |                              |           |             |            |           |  |       |       |       |             |                        |             |         |       |       |  |  |
|                | A043   |                              |           |             |            |           |  |       |       |       |             |                        |             |         |       |       |  |  |
|                | A044   | 0                            | 0         | 0           | 0          | 0         | 11.7   | 11.4  | 12.2  | 11.77 | 0.4         | 3.5                    | 3.7         | 3.9     | 3.7   | 0.2   |  |  |
|                | A045   |                              |           |             |            |           | 13.2   | 12.19 | 13.85 | 13.08 | 0.84        | 3.6                    | 3.81        | 3.96    | 3.79  | 0.181 |  |  |
|                | A047   | < 0.100                      | < 0.100   | < 0.100     |            |           | 3.76   | 3.4   | 3.6   | 3.59  | 0.18        | 2.25                   | 2.15        | 2.1     | 2.167 | 0.076 |  |  |
|                | A048   | 0                            | 0         | 0           | 0          | 0         | 10.8   | 10.7  | 13.6  | 11.7  | 1.65        | 3.7                    | 3.8         | 3.6     | 3.7   | 0.1   |  |  |
|                | A052   | 0.105                        | 0.112     | 0.116       | 0.111      | 0.006     | 7.27   | 5.3   | 6.08  | 6.22  | 0.99        | 3.5                    | 3.2         | 3.3     | 3.333 | 0.153 |  |  |
| ibu            | A053   | < 0.500                      | < 0.500   | 0           | 0          | 0         | 8.17   | /.66  | 6.75  | 10.72 | 0.72        | 2.97                   | 3.1         | 2.84    | 2.97  | 0.13  |  |  |
| Т              | A054   | 0                            | 0         | 0           | 0          | 0         | 13.1   | 11./  | 11.4  | 12.75 | 2.00        | 5.54                   | 5.55        | 5.52    | 5.557 | 0.015 |  |  |
|                | A055   |                              |           |             |            |           |  |       |       |       |             |                        |             |         |       |       |  |  |
|                | A060   |                              |           |             |            |           |  |       |       |       |             |                        |             |         |       |       |  |  |
|                | A062   | 0.01                         | 0.01      | 0.01        | 0.01       | 0         | 8.8  | 8.1   | 8.1   | 8.33  | 0.4         | 2.6                    | 2.4         | 2.5     | 2.5   | 0.1   |  |  |
|                | A063   | 0.6                          | 0.6       | 0.7         | 0.633      | 0.058     | 9.1  | 7.9   | 7.3   | 8.1   | 0.92        | 3                      | 2.9         | 3.8     | 3.233 | 0.493 |  |  |
|                | A065   |                              |           |             |            |           |  |       |       |       |             |                        |             |         |       |       |  |  |
|                | A066   | 0                            | 0         | 0           | 0          | 0         | 14   | 13    | 8     | 11.67 | 3.21        | 4                      | 4           | 3       | 3.667 | 0.577 |  |  |
|                | A067   | < 4.000                      | < 4.000   | < 4.000     |            |           | 13.9   | 12.8  | 13.5  | 13.4  | 0.56        | < 4.000                | < 4.000     | < 4.000 |       |       |  |  |
|                | A070   |                              |           |             |            |           |  |       |       |       |             |                        |             |         |       |       |  |  |
|                | A071   | 0                            | 0         | 0           | 0          | 0         | 8.26   | 7.12  | 8.56  | 7.98  | 0.76        | 2.72                   | 2.6         | 2.63    | 2.65  | 0.062 |  |  |
|                | A073   | < 0.300                      |           |             |            |           | 5.36   | 7.13  | 4.68  | 5.72  | 1.26        | 1.47                   | 1.42        | 1.64    | 1.51  | 0.115 |  |  |
|                | A074   |                              |           |             |            |           |  |       |       |       |             |                        |             |         |       |       |  |  |
|                | A075   | < 1.000                      | < 1.000   | < 1.000     |            |           | 0.26   | 0.1   | 0 72  | 0.02  | 0.29        | 1 77                   | 1.9.4       | 1.01    | 1 207 | 0.025 |  |  |
|                | A077   | < 1.000                      | < 16,000  | < 16,000    |            |           | 9.20   | 9.1   | 0.72  | 9.05  | 0.28        | 1.//                   | 1.64        | 1.61    | 1.607 | 0.055 |  |  |
|                | A083   | 10.000                       | 10.000    | 10.000      |            |           |  |       |       |       | _           |                        |             |         |       |       |  |  |
| Ŷ              |        | Consensu                     | is Mean   |             | 0.05       |           | Consensus Mean                               |       |       | 9.4   |             | Consensu               | is Mean     |         | 2.99  |       |  |  |
| unit<br>Its    |        | Consensus Standard Deviation |           |             | 0.12       |           | Consensus Standard Deviation                 |       |       | 2.8   |             | Consensu               | is Standard | 0.78    |       |       |  |  |
| esul           |        | Maximum                      |           |             | 0.63       |           | Maximum                                      |       |       | 13.4  |             | Maximum                |             |         | 3.79  |       |  |  |
| R. Con         |        | Minimum                      |           |             | 0          |           | Minimum                                      |       |       |       | 3.6 Minimum |                        |             |         |       | 1.51  |  |  |
| -              |        | Ν                            |           |             | 11         |           | Ν  |       |       | 21    |             | Ν                      |             |         | 20    |       |  |  |



**Figure 7-17.** Total aflatoxins in candidate RM 8427 Mycotoxins in Corn (Blank) (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red line represents the upper consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \leq 2$ , with the lower range set at zero. A NIST value has not been determined in this material.



**Figure 7-18.** Total aflatoxins in candidate RM 8427 Mycotoxins in Corn (Incurred) (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \le 2$ . The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by its uncertainty ( $U_{NIST}$ ) and represents the range that results in an acceptable  $Z_{NIST}$  score,  $|Z_{NIST}| \le 2$ .





**Figure 7-19.** Total aflatoxins in Incurred Corn B (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \leq 2$ . A NIST value has not been determined in this material.



HAMQAP Exercise 1 - Dietary Intake, Measurand: Total Aflatoxins No. of laboratories: 21

**Figure 7-20.** Laboratory means for total aflatoxins in candidate RM 8427 Mycotoxins in Corn (Incurred) and Incurred Corn B (sample/sample comparison view). In this view, the individual laboratory mean for one sample (RM 8427) is compared to the mean for a second sample (Incurred Corn B). The dotted blue box represents the consensus range of tolerance for RM 8427 Mycotoxins in Corn (Incurred) (x-axis) and Incurred Corn B (y-axis), calculated as the values above and below the consensus means that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \le 2$ .

**Table 7-7.** Data summary table for fumonisin B1 in blank and incurred corns. Data points highlighted in red have been flagged as potential outliers (e.g., difference from reference value, Grubb and/or Cochran) by the NIST software package.

|                 |        | Fumonisin B1                 |           |             |            |           |  |        |      |      |     |                              |     |     |     |     |  |
|-----------------|--------|------------------------------|-----------|-------------|------------|-----------|--|--------|------|------|-----|------------------------------|-----|-----|-----|-----|--|
|                 |        | RM 842                       | 27 Mycoto | oxins in Co | orn (Blank | x) (ng/g) | RM 8427 Mycotoxins in Corn (Incurred) (ng/g) |        |      |      |     | Incurred Corn B (ng/g)       |     |     |     |     |  |
|                 | Lab    | Α                            | В         | С           | Avg        | SD        | Α  | В      | С    | Avg  | SD  | Α                            | В   | С   | Avg | SD  |  |
|                 | Target |                              |           |             | 10.5       | 5.0       |  |        |      | 869  | 157 |                              |     |     | 463 | 166 |  |
|                 | A005   | < 80.000 < 80.000 < 80.000   |           |             |            |           |  | 686    | 705  | 705  | 19  | 524                          | 574 | 542 | 547 | 25  |  |
|                 | A013   | < 30.000                     | < 30.000  | < 30.000    |            |           | 508  | 538    |      | 523  | 21  | 228                          | 227 | 212 | 222 | 9   |  |
|                 | A020   |                              |           |             |            |           |  |        |      |      |     |                              |     |     |     |     |  |
|                 | A021   | 21                           | 22        |             | 21.5       | 0.71      | 713  | 872    | 799  | 795  | 80  | 648                          | 566 | 372 | 529 | 142 |  |
|                 | A025   | 9.1                          | 11.4      | 10.6        | 10.4       | 1.2       | 842  | 809    | 772  | 808  | 35  | 489                          | 482 | 471 | 480 | 9   |  |
| ividual Results | A027   |                              |           |             |            |           |  |        |      |      |     |                              |     |     |     |     |  |
|                 | A028   | < 4.000                      | < 4.000   | < 4.000     |            |           | 723  | 623    | 641  | 662  | 53  | 442                          | 399 | 447 | 429 | 26  |  |
|                 | A031   | < 0.000                      | < 0.000   | < 0.000     |            |           | 712  | 578    | 591  | 627  | 74  | 388                          | 561 | 382 | 444 | 102 |  |
|                 | A036   | 6.55                         | 7.41      | 6.55        | 6.84       | 0.5       | 603  | 604    | 614  | 607  | 6   | 424                          | 403 | 419 | 415 | 11  |  |
|                 | A042   |                              |           |             |            |           |  |        |      |      |     |                              |     |     |     |     |  |
|                 | A043   | 0                            | 0         | 0           | 0          | 0         | 631  | 639    | 633  | 634  | 4   | 302                          | 303 | 296 | 300 | 4   |  |
|                 | A045   | 9                            | 12        | 13          | 11         | 2         | 994  | 970    | 877  | 947  | 62  | 571                          | 613 | 582 | 589 | 22  |  |
|                 | A047   |                              |           |             |            |           |  |        |      |      |     |                              |     |     |     |     |  |
| pu              | A048   | 10.3                         | 9.5       | 11.3        | 10.4       | 0.9       | 965  | 835    | 1012 | 937  | 92  | 516                          | 578 | 618 | 570 | 51  |  |
| -               | A052   | 0                            | 0         | 0           | 0          | 0         | 783  | 763    | 787  | 777  | 13  | 463                          | 408 | 448 | 440 | 28  |  |
|                 | A053   | 12.42                        | 9.11      |             | 10.77      | 2.34      | 806  | 710    | 836  | 784  | 66  | 446                          | 528 | 522 | 499 | 46  |  |
|                 | A054   | 10.87                        | 8.22      | 11.71       | 10.27      | 1.82      | 756  | 813    | 824  | 798  | 36  | 397                          | 413 | 417 | 409 | 10  |  |
|                 | A065   |                              |           |             |            |           |  |        |      |      |     |                              |     |     |     |     |  |
|                 | A066   | 0                            | 0         | 0           | 0          | 0         | 661  | 705    | 678  | 681  | 22  | 387                          | 387 | 375 | 383 | 7   |  |
|                 | A067   |                              |           |             |            |           | 645  | 665    | 623  | 644  | 21  | 323                          | 368 | 393 | 361 | 35  |  |
|                 | A070   |                              |           |             |            |           |  |        |      |      |     |                              |     |     |     |     |  |
|                 | A073   | 25.2                         |           |             | 25.2       |           | 1430   | 1320   | 1310 | 1353 | 67  | 961                          | 901 | 860 | 907 | 51  |  |
|                 | A074   |                              |           |             |            |           |  |        |      |      |     |                              |     |     |     |     |  |
|                 | A079   |                              |           |             |            |           |  |        |      |      |     |                              |     |     |     |     |  |
| ity             |        | Consensus Mean 9.            |           |             | 9.2        |           | Consensu                                     | s Mean |      | 740  |     | Consensus Mean               |     |     | 450 |     |  |
| uni<br>Its      |        | Consensus Standard Deviation |           |             | 7.7        |           | Consensus Standard Deviation                 |        |      | 160  |     | Consensus Standard Deviation |     |     | 140 |     |  |
| mr<br>tesu      |        | Maximum                      |           |             | 25.2       |           | Maximum                                      |        |      | 1353 |     | Maximum                      |     |     | 907 |     |  |
| °C<br>B         |        | Minimum                      |           |             | 0          |           | Minimum                                      |        |      | 523  |     | Minimum                      |     |     | 222 |     |  |
|                 |        | Ν                            |           |             | 10         |           | Ν  |        |      | 16   |     | Ν                            |     |     | 16  |     |  |



**Figure 7-21.** Fumonisin B1 in candidate RM 8427 Mycotoxins in Corn (Blank) (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red line represents the upper consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \le 2$ , with the lower range set at zero. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by its uncertainty ( $U_{NIST}$ ) and represents the range that results in an acceptable  $Z_{NIST}$  score,  $|Z_{NIST}| \le 2$ .



**Figure 7-22.** Fumonisin B1 in candidate RM 8427 Mycotoxins in Corn (Incurred) (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \leq 2$ . The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by its uncertainty ( $U_{NIST}$ ) and represents the range that results in an acceptable  $Z_{NIST}$  score,  $|Z_{NIST}| \leq 2$ .


**Figure 7-23.** Fumonisin B1 in Incurred Corn B (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \leq 2$ . The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by its uncertainty ( $U_{NIST}$ ) and represents the range that results in an acceptable  $Z_{NIST}| \leq 2$ .



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**Figure 7-24.** Laboratory means for fumonisin B1 in candidate RM 8427 Mycotoxins in Corn (Incurred) and Incurred Corn B (sample/sample comparison view). In this view, the individual laboratory mean for one sample (RM 8427) is compared to the mean for a second sample (Incurred Corn B). The solid red box represents the NIST range of tolerance for the two samples, RM 8427 Mycotoxins in Corn (Incurred) (x-axis) and Incurred Corn B (y-axis), which encompasses the target values bounded by their uncertainties ( $U_{\text{NIST}}$ ) and represents the range that results in an acceptable  $Z_{\text{NIST}}$  score,  $|Z_{\text{NIST}}| \leq 2$ . The dotted blue box represents the consensus range of tolerance for RM 8427 Mycotoxins in Corn (Incurred) (x-axis) and Incurred Corn B (y-axis), calculated as the values above and below the consensus means that result in an acceptable  $Z'_{\text{comm}}$  score,  $|Z'_{\text{comm}}| \leq 2$ .

**Table 7-8.** Data summary table for fumonisin B2 in blank and incurred corns. Data points highlighted in red have been flagged as potential outliers (e.g., difference from reference value, Grubb and/or Cochran) by the NIST software package.

|               |        |                  |            |             |            |          |          | Fu          | imonisin H | 32          |           |          |            |           |        |      |
|---------------|--------|------------------|------------|-------------|------------|----------|----------|-------------|------------|-------------|-----------|----------|------------|-----------|--------|------|
|               |        | RM 842           | 27 Mycoto  | oxins in Co | orn (Blank | ) (ng/g) | RM 8427  | / Mycotox   | ins in Co  | rn (Incurre | d) (ng/g) |          | Incurre    | ed Corn B | (ng/g) |      |
|               | Lab    | Α                | В          | С           | Avg        | SD       | Α        | В           | С          | Avg         | SD        | Α        | В          | С         | Avg    | SD   |
|               | Target |                  |            |             |            |          |          |             |            | 226         | 51        |          |            |           | 108    | 48   |
|               | A005   | < 80.000         | < 80.000   | < 80.000    |            |          | 156      | 164         | 166        | 162         | 5         | 78       | 72         | 78        | 76     | 3    |
|               | A013   | < 10.000         | < 10.000   | < 10.000    |            |          | 318      | 347         | 238        | 301         | 56        | 120      | 125        | 109       | 118    | 8    |
|               | A020   |                  |            |             |            |          |          |             |            |             |           |          |            |           |        |      |
|               | A021   | 0                | 0          |             | 0          | 0        | 208      | 203         | 210        | 207         | 4         | 117      | 175        | 68        | 120    | 54   |
|               | A025   | < 4.500          | < 4.500    | < 4.500     |            |          | 224.88   | 208.06      | 200.75     | 211.23      | 12.37     | 108.52   | 103.11     | 101.98    | 104.54 | 3.50 |
|               | A027   |                  |            |             |            |          |          |             |            |             |           |          |            |           |        |      |
|               | A028   | < 10.000         | < 10.000   | < 10.000    |            |          | 220      | 180         | 210        | 203         | 21        | 130      | 110        | 130       | 123    | 12   |
|               | A031   | < 0.000          | < 0.000    | < 0.000     |            |          | 167.2    | 140.4       | 157.8      | 155.1       | 13.6      | 87.06    | 81.51      | 86.68     | 85.08  | 3.10 |
| 2             | A036   | 0.94             | 1.16       | 1.07        | 1.06       | 0.11     | 189.4    | 186.8       | 186.6      | 187.6       | 1.6       | 108.3    | 106        | 107.8     | 107.37 | 1.21 |
| sul           | A043   | 0                | 0          | 0           | 0          | 0        | 159      | 151         | 153        | 154         | 4         | 78.1     | 77.1       | 69.6      | 74.9   | 4.6  |
| Individual Re | A045   |                  |            |             |            |          | 182      | 286         | 218        | 229         | 53        | 125      | 139        | 134       | 133    | 7    |
|               | A047   |                  |            |             |            |          |          |             |            |             |           |          |            |           |        |      |
|               | A048   | 0                | 0          | 0           | 0          | 0        | 245.8    | 245         | 234.7      | 241.8       | 6.2       | 119.6    | 125.8      | 122.2     | 122.5  | 3.1  |
|               | A052   | 60.57            | 63.58      | 62.13       | 62.09      | 1.51     | 215.48   | 221.76      | 218.06     | 218.43      | 3.16      | 98.65    | 104.58     | 97.22     | 100.15 | 3.90 |
|               | A053   | < 10.000         | < 10.000   |             |            |          | 241      | 213         | 205        | 220         | 19        | 118      | 92         | 102       | 104    | 13   |
|               | A054   | 11.35            | 11.44      | 8.46        | 10.42      | 1.7      | 223.79   | 238.02      | 238.02     | 233.28      | 8.22      | 99.69    | 92.27      | 99.18     | 97.05  | 4.14 |
|               | A060   |                  |            |             |            |          |          |             |            |             |           |          |            |           |        |      |
|               | A065   |                  |            |             |            |          |          |             |            |             |           |          |            |           |        |      |
|               | A066   | 0                | 0          | 0           | 0          | 0        | 171      | 189         | 172        | 177         | 10        | 83       | 86         | 82        | 84     | 2    |
|               | A067   |                  |            |             |            |          | 156      | 134         | 152        | 147         | 12        | 64       | 63         | 52        | 60     | 7    |
|               | A070   |                  |            |             |            |          |          |             |            |             |           |          |            |           |        |      |
|               | A073   | < 5.000          |            |             |            |          | 304      | 289         | 310        | 301         | 11        | 139      | 140        | 135       | 138    | 3    |
|               | A074   |                  |            |             |            |          |          |             |            |             |           |          |            |           |        |      |
|               | A079   |                  |            |             |            |          |          |             |            |             |           |          |            |           |        |      |
| ty            |        | Consensu         | s Mean     |             | 1.82       |          | Consensu | is Mean     |            | 207         |           | Consensu | s Mean     |           | 103    |      |
| uni<br>lts    |        | Consensu         | s Standard | l Deviation | 5.37       |          | Consensu | is Standard | Deviation  | 48          |           | Consensu | s Standard | Deviation | 30     |      |
| Inne          |        | Maximum          | ı          |             | 62.09      |          | Maximun  | 1           |            | 301         |           | Maximum  | 1          |           | 138    |      |
| R. O          |        | Minimum          |            |             | 0          |          | Minimum  |             |            | 147         |           | Minimum  |            |           | 60     |      |
| 5             |        | Minimum 0<br>N 7 |            |             |            |          | Ν        |             |            | 16          |           | Ν        |            |           | 16     |      |



Exercise HAMQAP Exercise 1 - Dietary Intake Sample: RM 8427 Mycotoxins in Corn (Blank) Measurand: Fumonisin B2

**Figure 7-25.** Fumonisin B2 in candidate RM 8427 Mycotoxins in Corn (Blank) (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red line represents the upper consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \leq 2$ , with the lower range set at zero. A NIST value has not been determined in this material.



**Figure 7-26.** Fumonisin B2 in candidate RM 8427 Mycotoxins in Corn (Incurred) (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \leq 2$ . The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by its uncertainty ( $U_{NIST}$ ) and represents the range that results in an acceptable  $Z_{NIST}$  score,  $|Z_{NIST}| \leq 2$ .



**Figure 7-27.** Fumonisin B2 in Incurred Corn B (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \leq 2$ . The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by its uncertainty ( $U_{NIST}$ ) and represents the range that results in an acceptable  $Z'_{NIST} | \leq 2$ .



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**Figure 7-28.** Laboratory means for fumonisin B2 in candidate RM 8427 Mycotoxins in Corn (Incurred) and Incurred Corn B (sample/sample comparison view). In this view, the individual laboratory mean for one sample (RM 8427) is compared to the mean for a second sample (Incurred Corn B). The solid red box represents the NIST range of tolerance for the two samples, RM 8427 Mycotoxins in Corn (Incurred) (x-axis) and Incurred Corn B (y-axis), which encompasses the target values bounded by their uncertainties ( $U_{\text{NIST}}$ ) and represents the range that results in an acceptable  $Z_{\text{NIST}}$  score,  $|Z_{\text{NIST}}| \leq 2$ . The dotted blue box represents the consensus range of tolerance for RM 8427 Mycotoxins in Corn (Incurred) (x-axis) and Incurred (x-axis) and Incurred Corn B (y-axis), calculated as the values above and below the consensus means that result in an acceptable  $Z'_{\text{comm}}$  score,  $|Z'_{\text{comm}}| \leq 2$ .

**Table 7-9.** Data summary table for fumonisin B3 in blank and incurred corn. Data points highlighted in red have been flagged as potential outliers (e.g., difference from reference value, Grubb and/or Cochran) by the NIST software package.

|                |        |          |             |             |            |           |          | Fu          | ımonisin I | 33          |           |          |             |           |        |      |
|----------------|--------|----------|-------------|-------------|------------|-----------|----------|-------------|------------|-------------|-----------|----------|-------------|-----------|--------|------|
|                |        | RM 842   | 27 Mycoto   | oxins in Co | orn (Blank | x) (ng/g) | RM 842   | 7 Mycotox   | tins in Co | rn (Incurre | d) (ng/g) |          | Incurre     | ed Corn B | (ng/g) |      |
|                | Lab    | Α        | В           | С           | Avg        | SD        | Α        | В           | С          | Avg         | SD        | Α        | В           | С         | Avg    | SD   |
|                | Target |          |             |             |            |           |          |             |            | 98          | 18        |          |             |           |        |      |
|                | A020   |          |             |             |            |           |          |             |            |             |           |          |             |           |        |      |
|                | A021   | 11.1     | 12          |             | 11.6       | 0.6       | 97       | 98          | 109        | 101         | 7         | 116      | 102         | 99        | 106    | 9    |
|                | A025   | < 9.000  | < 9.000     | < 9.000     |            |           | 104      | 102.46      | 92.9       | 99.79       | 6.01      | 98.71    | 93.46       | 90.98     | 94.38  | 3.95 |
|                | A027   |          |             |             |            |           |          |             |            |             |           |          |             |           |        |      |
|                | A028   | < 8.000  | < 8.000     | < 8.000     |            |           | 93       | 100         | 94         | 96          | 4         | 90       | 94          | 113       | 99     | 12   |
|                | A031   |          |             |             |            |           |          |             |            |             |           |          |             |           |        |      |
| ts             | A043   | 0        | 0           | 0           | 0          | 0         | 65.3     | 66.1        | 73.9       | 68.4        | 4.8       | 60.3     | 56.1        | 53.7      | 56.7   | 3.3  |
| sul            | A045   |          |             |             |            |           | 92       | 112         | 103        | 102         | 10        | 105      | 118         | 108       | 110    | 7    |
| Individual Res | A047   |          |             |             |            |           |          |             |            |             |           |          |             |           |        |      |
|                | A048   | 0        | 0           | 0           | 0          | 0         | 112.4    | 127.3       | 107.8      | 115.8       | 10.2      | 104.6    | 113.2       | 110.4     | 109.4  | 4.4  |
|                | A052   | 2.12     | 2.3         | 2.39        | 2.27       | 0.14      | 90.55    | 80.31       | 85.06      | 85.31       | 5.12      | 77.23    | 71.63       | 66.55     | 71.80  | 5.34 |
|                | A053   | < 10.000 | < 10.000    |             |            |           | 107.8    | 106.2       | 95.6       | 103.2       | 6.6       | 96.8     | 104.5       | 94.1      | 98.5   | 5.4  |
|                | A054   | 0        | 0           | 0           | 0          | 0         | 101      | 107.74      | 113.75     | 107.50      | 6.38      | 82.01    | 83.99       | 78.32     | 81.44  | 2.88 |
|                | A065   |          |             |             |            |           |          |             |            |             |           |          |             |           |        |      |
|                | A066   | 0        | 0           | 0           | 0          | 0         | 70       | 80          | 73         | 74          | 5         | 65       | 66          | 66        | 66     | 1    |
|                | A067   |          |             |             |            |           |          |             |            |             |           |          |             |           |        |      |
|                | A070   |          |             |             |            |           |          |             |            |             |           |          |             |           |        |      |
|                | A073   | < 5.000  |             |             |            |           | 172      | 146         | 163        | 160         | 13        | 149      | 153         | 143       | 148    | 5    |
|                | A074   |          |             |             |            |           |          |             |            |             |           |          |             |           |        |      |
|                | A079   |          |             |             |            |           |          |             |            |             |           |          |             |           |        |      |
| <u>,</u>       |        | Consensu | ıs Mean     |             | 0.5        |           | Consensu | ıs Mean     |            | 98          |           | Consensu | is Mean     |           | 93     |      |
| lts            |        | Consensu | is Standard | l Deviation | 2.2        |           | Consensu | is Standard | Deviation  | 20          |           | Consensu | is Standard | Deviation | 27     |      |
| esu            |        | Maximun  | ı           |             | 11.6       |           | Maximun  | n           |            | 160         |           | Maximun  | 1           |           | 148    |      |
| 2 2            |        | Minimum  |             |             | 0          |           | Minimum  |             |            | 68          |           | Minimum  |             |           | 57     |      |
| •              |        | Ν        |             |             | 6          |           | Ν        |             |            | 11          |           | Ν        |             |           | 11     |      |

|     | 9.5  | Liquid Chromatography with Tandem Mass Spectrometry |      |                  |      |      | 000  |       | 1    |
|-----|------|---|------|------------------|------|------|------|-------|------|
|     | 9.0  | not specified                                       |      |                  |      |      | 0.6M |       | 20   |
|     | 8.5  | - Limit of tolerance                                |      |                  |      |      |      |       | 11.5 |
|     | 8.0  |   |      |                  |      | 0.88 |      |       |      |
|     | 7.5- |   |      |                  |      | *    | Ų    |       |      |
|     | 7.0- |   |      |                  |      |      |      |       |      |
|     | 6.5  |   |      |                  |      |      |      |       |      |
|     | 6.0  |   |      |                  | Î    |      |      |       |      |
|     | 6.0- |   |      |                  | 0)0  |      |      |       |      |
| _   | 5.5  |   |      |                  | 0    |      |      |       |      |
| )ĝu | 5.0  |   |      |                  | Ň    | -    |      | 1     |      |
| _   | 4.5  |   |      |                  | 0    |      |      |       |      |
|     | 4.0  |   |      |                  |      |      |      |       |      |
|     | 3.5  |   |      |                  | -    |      |      |       |      |
|     | 3.0  |   |      |                  |      |      |      |       |      |
|     | 2.5  |   |      | <b>9</b> 1       |      | -    |      |       |      |
|     | 2.0  |   | -    | õ <b>-</b>       |      |      |      |       |      |
|     | 1.5  |   |      |                  |      |      |      |       |      |
|     | 1.0  |   |      |                  |      |      |      |       |      |
|     | 0.5  |   |      |                  |      |      |      |       |      |
|     | 0.0  |   |      |                  |      |      |      |       |      |
|     |      | 043<br>048-   | 066- | 052-             | 0/3- | 028  | 025- | - con | 021- |
|     |      | AC AC   | Ā    | کر<br>Laboratory | Ā .  | Ā    | Ā Š  | ζ.    | Ā    |
|     |      |   |      | Laboratory       |      |      |      |       |      |

Exercise HAMQAP Exercise 1 - Dietary Intake Sample: RM 8427 Mycotoxins in Corn (Blank) Measurand: Fumonisin B3

**Figure 7-29.** Fumonisin B3 in candidate RM 8427 Mycotoxins in Corn (Blank) (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red line represents the upper consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \leq 2$ , with the lower range set at zero. A NIST value has not been determined in this material.



HAMQAP Exercise 1 - Dietary Intake

Exercise

**Figure 7-30.** Fumonisin B3 in candidate RM 8427 Mycotoxins in Corn (Incurred) (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \leq 2$ . The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by its uncertainty ( $U_{NIST}$ ) and represents the range that results in an acceptable  $Z_{NIST}$  score,  $|Z_{NIST}| \leq 2$ .



Exercise HAMQAP Exercise 1 - Dietary Intake Sample: Incurred Corn B Measurand: Fumonisin B3

**Figure 7-31.** Function B3 in Incurred Corn B (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \leq 2$ . A NIST value has not been determined in this material.



## HAMQAP Exercise 1 - Dietary Intake, Measurand: Fumonisin B3 No. of laboratories: 11

**Figure 7-32.** Laboratory means for fumonisin B3 in candidate RM 8427 Mycotoxins in Corn (Incurred) and Incurred Corn B (sample/sample comparison view). In this view, the individual laboratory mean for one sample (RM 8427) is compared to the mean for a second sample (Incurred Corn B). The dotted blue box represents the consensus range of tolerance for RM 8427 Mycotoxins in Corn (Incurred) (x-axis) and Incurred Corn B (y-axis), calculated as the values above and below the consensus means that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \le 2$ .

**Table 7-10.** Data summary table for total fumonisins in blank and incurred corns. Data points highlighted in red have been flagged as potential outliers (e.g., difference from reference value, Grubb and/or Cochran) by the NIST software package.

|                      |        |                   |            |             |            |                   |          | Tota       | l Fumonis   | ins        |           |          |            |           |        |     |
|----------------------|--------|-------------------|------------|-------------|------------|-------------------|----------|------------|-------------|------------|-----------|----------|------------|-----------|--------|-----|
|                      |        | RM 842            | 27 Mycot   | oxins in C  | orn (Blank | ) ( <b>ng/g</b> ) | RM 8427  | Mycotox    | tins in Cor | n (Incurre | d) (ng/g) |          | Incurre    | ed Corn B | (ng/g) |     |
|                      | Lab    | Α                 | В          | С           | Avg        | SD                | Α        | В          | С           | Avg        | SD        | Α        | В          | С         | Avg    | SD  |
|                      | Target |                   |            |             | 10.5       | 5.0               |          |            |             | 1193       | 166       |          |            |           |        |     |
|                      | A020   |                   |            |             |            |                   |          |            |             |            |           |          |            |           |        |     |
|                      | A021   | 32                | 34         |             | 33         | 1                 | 1018     | 1173       | 1118        | 1103       | 79        | 881      | 843        | 539       | 754    | 187 |
|                      | A025   | 9.1               | 11.4       | 10.6        | 10.4       | 1.2               | 1171     | 1119       | 1066        | 1119       | 53        | 696      | 678        | 664       | 679    | 16  |
|                      | A027   |                   |            |             |            |                   |          |            |             |            |           |          |            |           |        |     |
|                      | A028   | < 10.000          | < 10.000   | < 10.000    |            |                   | 1036     | 903        | 945         | 961        | 68        | 662      | 603        | 690       | 652    | 44  |
|                      | A031   |                   |            |             |            |                   |          |            |             |            |           |          |            |           |        |     |
|                      | A043   |                   |            |             |            |                   |          |            |             |            |           |          |            |           |        |     |
| 2                    | A045   | 9                 | 12         | 13          | 11         | 2                 | 1268     | 1368       | 1198        | 1278       | 85        | 801      | 870        | 825       | 832    | 35  |
| sul                  | A047   |                   |            |             |            |                   |          |            |             |            |           |          |            |           |        |     |
| Individual Re        | A048   | 10.3              | 9.5        | 11.3        | 10.4       | 0.9               | 1323     | 1207       | 1354        | 1295       | 78        | 740      | 817        | 850       | 802    | 57  |
|                      | A052   | 62.685            | 65.88      | 64.515      | 64.36      | 1.60              | 1089     | 1065       | 1090        | 1081       | 14        | 638      | 584        | 612       | 611    | 27  |
|                      | A053   | 12.42             | 9.11       |             | 10.77      | 2.34              | 1155     | 1029       | 1137        | 1107       | 68        | 661      | 725        | 718       | 701    | 35  |
|                      | A054   | 22.23             | 19.66      | 20.17       | 20.69      | 1.36              | 1081     | 1159       | 1176        | 1139       | 50        | 579      | 589        | 594       | 587    | 8   |
|                      | A058   |                   |            |             |            |                   |          |            |             |            |           |          |            |           |        |     |
|                      | A060   |                   |            |             |            |                   |          |            |             |            |           |          |            |           |        |     |
| A060<br>A065<br>A066 | A065   |                   |            |             |            |                   |          |            |             |            |           |          |            |           |        |     |
|                      | A066   | 0                 | 0          | 0           | 0          | 0                 | 902      | 974        | 923         | 933        | 37        | 535      | 539        | 523       | 532    | 8   |
|                      | A067   | < 300             | < 300      | < 300       |            |                   |          |            |             |            |           |          |            |           |        |     |
|                      | A070   |                   |            |             |            |                   |          |            |             |            |           |          |            |           |        |     |
|                      | A073   | 25.2              |            |             | 25.2       |                   | 1910     | 1750       | 1780        | 1813       | 85        | 1250     | 1190       | 1140      | 1193   | 55  |
|                      | A074   |                   |            |             |            |                   |          |            |             |            |           |          |            |           |        |     |
|                      | A079   |                   |            |             |            |                   |          |            |             |            |           |          |            |           |        |     |
| ty                   |        | Consensus Mean 19 |            |             | Consensu   | s Mean            |          | 1130       |             | Consensu   | s Mean    |          | 710        |           |        |     |
| uni<br>Its           |        | Consensu          | s Standard | d Deviation | 20         |                   | Consensu | s Standard | Deviation   | 180        |           | Consensu | s Standard | Deviation | 170    |     |
| nm                   |        | Maximum           | ı          |             | 64         |                   | Maximum  | I          |             | 1813       |           | Maximum  | I          |           | 1193   |     |
| R. G                 |        | Minimum           |            |             | 0          |                   | Minimum  |            |             | 933        |           | Minimum  |            |           | 532    |     |
| -                    |        | Ν                 |            |             | 8          |                   | Ν        |            |             | 10         |           | Ν        |            |           | 10     |     |



**Figure 7-33.** Total fumonisins in candidate RM 8427 Mycotoxins in Corn (Blank) (data summary view –analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red line represents the upper consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \le 2$ , with the lower range set at zero. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by its uncertainty ( $U_{NIST}$ ) and represents the range that results in an acceptable  $Z'_{NIST}| \le 2$ .

Liquid Chromatography with Tandem Mass Spectrometry 1800 not specified 8 – Mean line 1700 - Limit of tolerance 1600 1500 1400 8 1300 σ 1200 8 b/gu -0-1100 8 1000 Ŕ 0 900 800 700 600 500 400 A066-A028-A052-A053-A025-A045-A048-A073-A021-A054-Laboratory

**Figure 7-34.** Total fumonisins in candidate RM 8427 Mycotoxins in Corn (Incurred) (data summary view –analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \le 2$ . The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by its uncertainty ( $U_{NIST}$ ) and represents the range that results in an acceptable  $Z_{NIST}$  score,  $|Z_{NIST}| \le 2$ .

Exercise HAMQAP Exercise 1 - Dietary Intake Sample: Incurred Corn B Measurand: Total Fumonisins



Figure 7-35. Total fumonisins in Incurred Corn B (data summary view –analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \leq 2$ . A NIST value has not been determined in this material.



## HAMQAP Exercise 1 - Dietary Intake, Measurand: Total Fumonisins No. of laboratories: 10

**Figure 7-36.** Laboratory means for total fumonisins in candidate RM 8427 Mycotoxins in Corn (Incurred) and Incurred Corn B (sample/sample comparison view). In this view, the individual laboratory mean for one sample (RM 8427) is compared to the mean for a second sample (Incurred Corn B). The dotted blue box represents the consensus range of tolerance for RM 8427 Mycotoxins in Corn (Incurred) (x-axis) and Incurred Corn B (y-axis), calculated as the values above and below the consensus means that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \le 2$ .

**Table 7-11.** Data summary table for deoxynivalenol in blank and incurred corns. Data points highlighted in red have been flagged as potential outliers (e.g., difference from reference value, Grubb and/or Cochran) by the NIST software package.

|               |        |          |            |             |            |                    |          | De          | oxynivale  | nol         |            |          |             |           |        |     |
|---------------|--------|----------|------------|-------------|------------|--------------------|----------|-------------|------------|-------------|------------|----------|-------------|-----------|--------|-----|
|               |        | RM 842   | 27 Mycoto  | oxins in Co | orn (Blank | :) ( <b>ng/g</b> ) | RM 8427  | / Mycotox   | tins in Co | rn (Incurre | ed) (ng/g) |          | Incurr      | ed Corn B | (ng/g) |     |
|               | Lab    | Α        | В          | С           | Avg        | SD                 | Α        | В           | С          | Avg         | SD         | Α        | В           | С         | Avg    | SD  |
|               | Target |          |            |             | 146        | 33                 |          |             |            | 468         | 48         |          |             |           | 731    | 245 |
|               | A005   | 182      | 202        | 215         | 200        | 17                 | 583      | 574         | 592        | 583         | 9          | 943      | 902         | 923       | 923    | 21  |
|               | A013   | 165      | 171        | 146         | 161        | 13                 | 630      | 648         | 598        | 625         | 25         | 881      | 864         | 937       | 894    | 38  |
|               | A020   |          |            |             |            |                    |          |             |            |             |            |          |             |           |        |     |
|               | A021   | 304      | 292        |             | 298        | 8                  | 617      | 661         | 610        | 629         | 28         | 984      | 895         | 966       | 948    | 47  |
|               | A025   | 220      | 163        | 175         | 186        | 30                 | 524      | 525         | 555        | 535         | 18         | 749      | 748         | 747       | 748    | 1   |
|               | A027   |          |            |             |            |                    |          |             |            |             |            |          |             |           |        |     |
|               | A028   | 170      | 150        | 170         | 163        | 12                 | 580      | 570         | 530        | 560         | 26         | 750      | 750         | 760       | 753    | 6   |
|               | A031   | < 0.000  | < 0.000    | < 0.000     |            |                    | 437      | 448         | 445        | 443         | 6          | 707      | 702         | 722       | 711    | 11  |
| s             | A036   | 148      | 156        | 160         | 155        | 7                  | 425      | 409         | 412        | 415         | 9          | 896      | 850         | 873       | 873    | 23  |
| sult          | A042   |          |            |             |            |                    |          |             |            |             |            |          |             |           |        |     |
| ndividual Res | A043   | 89       | 75         | 93          | 86         | 10                 | 325      | 326         | 300        | 317         | 15         | 431      | 421         | 422       | 425    | 6   |
|               | A044   | 209      | 208        | 227         | 215        | 11                 | 610      | 604         | 569        | 594         | 22         | 876      | 885         | 875       | 879    | 6   |
|               | A045   | 120      | 135        | 144         | 133        | 12                 | 474      | 517         | 507        | 499         | 23         | 784      | 790         | 779       | 784    | 6   |
|               | A047   | 104      | 201        | 102         | 102        | 0                  | 660      | (2)(        | (22)       | 640         | 17         | 701      | 775         | 7.02      | 770    | 0   |
| Р             | A048   | 184      | 201        | 192         | 193        | 8                  | 660      | 636         | 633        | 643         | 15         | /81      | 7/5         | /63       | 113    | 9   |
|               | A052   | 102      | 145        | 0           | 124        | 20                 | 452      | 4/5         | 459        | 462         | 12         | /00      | /01         | 087       | 501    | 8   |
|               | A053   | 105      | 145        | 150         | 124        | 30                 | 307      | 290         | 407        | 298         | 12         | 480      | 088         | 005       | 591    | 105 |
|               | A054   | 135      | 142        | 152         | 145        | 8                  | 4/1      | 500         | 487        | 480         | 14         | /15      | 115         | /45       | /44    | 29  |
|               | A065   | 0        | 0          | 0           | 0          | 0                  | 410      | 440         | 420        | 422         | 15         | 670      | 660         | 650       | 660    | 10  |
|               | A000   | 125      | 120        | 124         | 123        | 3                  | 410      | 440         | 420        | 423         | 0          | 662      | 661         | 651       | 658    | 6   |
|               | A007   | 123      | 120        | 124         | 123        | 5                  | 401      | 400         | 445        | 455         | ,          | 002      | 001         | 0.51      | 058    | 0   |
|               | A073   | 114      |            |             | 114        |                    | 405      | 406         | 378        | 396         | 16         | 660      | 652         | 654       | 655    | 4   |
|               | A074   | 114      |            |             | 114        |                    | 405      | 400         | 570        | 570         | 10         | 000      | 032         | 0.54      | 055    | -   |
|               | A079   |          |            |             |            |                    |          |             |            |             |            |          |             |           |        |     |
| 'n            |        | Consensu | s Mean     |             | 146        |                    | Consensu | is Mean     |            | 490         |            | Consensu | ıs Mean     |           | 760    |     |
| ts I          |        | Consensu | s Standard | Deviation   | 67         |                    | Consensu | is Standard | Deviation  | 120         |            | Consensu | is Standard | Deviation | 130    |     |
| sul           |        | Maximum  | ı          |             | 298        |                    | Maximum  | ı           |            | 643         |            | Maximun  | 1           |           | 948    |     |
| Ř             |        | Minimum  |            |             | 0          |                    | Minimum  |             |            | 298         |            | Minimum  |             |           | 425    |     |
|               |        | Ν        |            |             | 15         |                    | Ν        |             |            | 17          |            | Ν        |             |           | 17     |     |



**Figure 7-37.** Deoxynivalenol in candidate RM 8427 Mycotoxins in Corn (Blank) (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \leq 2$ . The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by its uncertainty ( $U_{NIST}$ ) and represents the range that results in an acceptable  $Z_{NIST}$  score,  $|Z_{NIST}| \leq 2$ .



**Figure 7-38.** Deoxynivalenol in candidate RM 8427 Mycotoxins in Corn (Incurred) (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \leq 2$ . The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by its uncertainty ( $U_{NIST}$ ) and represents the range that results in an acceptable  $Z_{NIST}$  score,  $|Z_{NIST}| \leq 2$ .



Figure 7-39. Deoxynivalenol in Incurred Corn B (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \leq 2$ . The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by its uncertainty ( $U_{NIST}$ ) and represents the range that results in an acceptable  $Z_{NIST}$  score,  $|Z_{NIST}| \leq 2$ .



HAMQAP Exercise 1 - Dietary Intake, Measurand: Deoxynivalenol No. of laboratories: 17

**Figure 7-40.** Laboratory means for deoxynivalenol in candidate RM 8427 Mycotoxins in Corn (Incurred) and Incurred Corn B (sample/sample comparison view). In this view, the individual laboratory mean for one sample (RM 8427) is compared to the mean for a second sample (Incurred Corn B). The solid red box represents the NIST range of tolerance for the two samples, RM 8427 Mycotoxins in Corn (Incurred) (x-axis) and Incurred Corn B (y-axis), which encompasses the target values bounded by their uncertainties ( $U_{\text{NIST}}$ ) and represents the range that results in an acceptable  $Z_{\text{NIST}}$  score,  $|Z_{\text{NIST}}| \leq 2$ . The dotted blue box represents the consensus range of tolerance for RM 8427 Mycotoxins in Corn (Incurred) (x-axis) and Incurred (x-axis) and Incurred Corn B (y-axis), calculated as the values above and below the consensus means that result in an acceptable  $Z'_{\text{comm}}$  score,  $|Z'_{\text{comm}}| \leq 2$ .

**Table 7-12.** Data summary table for ochratoxin A in blank and incurred corns. Data points highlighted in red have been flagged as potential outliers (e.g., difference from reference value, Grubb and/or Cochran) by the NIST software package.

|                |        | Ochratoxin A |                            |             |            |                    |          |            |             |            |           |          |             |           |        |      |
|----------------|--------|--------------|----------------------------|-------------|------------|--------------------|----------|------------|-------------|------------|-----------|----------|-------------|-----------|--------|------|
|                |        | RM 842       | 27 Mycoto                  | oxins in Co | orn (Blank | k) ( <b>ng/g</b> ) | RM 8427  | Mycotox    | tins in Cor | n (Incurre | d) (ng/g) |          | Incurr      | ed Corn B | (ng/g) |      |
|                | Lab    | Α            | В                          | С           | Avg        | SD                 | Α        | В          | С           | Avg        | SD        | Α        | В           | С         | Avg    | SD   |
|                | Target |              |                            |             |            |                    |          |            |             | 10.6       | 10.6      |          |             |           | 2.18   | 0.96 |
|                | A005   | < 1.500      | < 1.500                    | < 1.500     |            |                    | 12       | 12.7       | 11.7        | 12.1       | 0.5       | 2.2      | 2           | 2.7       | 2.3    | 0.36 |
|                | A013   | < 1.000      | < 1.000                    | < 1.000     |            |                    | 7.8      | 7.8        | 7.6         | 7.8        | 0.1       | 1.78     | 1.67        | 1.46      | 1.64   | 0.16 |
|                | A017   | 0            | 0                          | 0           | 0          | 0                  | 10.4     | 12.4       | 10.4        | 11.1       | 1.2       | 0        | 0           | 0         | 0      | 0    |
|                | A020   |              |                            |             |            |                    |          |            |             |            |           |          |             |           |        |      |
|                | A021   | 1.8          | 1.7                        |             | 1.8        | 0.1                | 7.5      | 6.5        | 6.9         | 7.0        | 0.5       | 3.1      | 3.7         | 3         | 3.3    | 0.4  |
|                | A025   | < 0.600      | < 0.600                    | < 0.600     |            |                    | 12.2     | 9.9        | 7.3         | 9.8        | 2.5       | 1.59     | 1.65        | 1.76      | 1.67   | 0.09 |
|                | A026   |              |                            |             |            |                    | 5.3      |            |             | 5.3        |           | 0.41     |             |           | 0.41   |      |
|                | A027   |              |                            |             |            |                    |          |            |             |            |           |          |             |           |        |      |
|                | A028   | < 0.800      | < 0.800                    | < 0.800     |            |                    | 12       | 8.9        | 12          | 11.0       | 1.8       | 2.6      | 2.3         | 2.4       | 2.4    | 0.2  |
|                | A031   | 0.49         | 0.25                       | 0.5         | 0.41       | 0.14               | 6.9      | 8.8        | 12.8        | 9.5        | 3.0       | 2.01     | 2.07        | 2.05      | 2.04   | 0.03 |
|                | A035   |              |                            |             |            |                    |          |            |             |            |           |          |             |           |        |      |
|                | A036   | < 0.130      | < 0.130                    | < 0.130     |            |                    | 9.4      | 11.5       | 8.5         | 9.8        | 1.5       | 2.4      | 2.46        | 2.47      | 2.44   | 0.04 |
| sult           | A039   |              |                            |             |            |                    |          |            |             |            |           |          |             |           |        |      |
| Individual Res | A042   |              |                            |             |            |                    |          |            |             |            |           |          |             |           |        |      |
|                | A043   | 0            | 0                          | 0           | 0          | 0                  | 4.3      | 3.7        | 4.9         | 4.3        | 0.6       | 1.16     | 1.02        | 1.07      | 1.08   | 0.07 |
|                | A044   | 0            | 0                          | 0           | 0          | 0                  | 17.9     | 17.2       | 16          | 17.1       | 1.0       | 3.8      | 3.74        | 3.7       | 3.75   | 0.05 |
|                | A045   |              |                            |             |            |                    | 9.4      | 10         | 11.2        | 10.2       | 0.9       | 2.21     | 2.28        | 2.41      | 2.30   | 0.10 |
|                | A047   | < 1.000      | < 1.000                    | < 1.000     |            |                    | 46.7     | 41.6       | 43.5        | 44.0       | 2.5       | 9.1      | 11.43       | 10.15     | 10.23  | 1.17 |
|                | A048   | 0            | 0                          | 0           | 0          | 0                  | 12.6     | 11.6       | 13.8        | 12.7       | 1.1       | 2.4      | 2.5         | 2.2       | 2.4    | 0.2  |
|                | A052   | 8.252        | 8.26                       | 8.207       | 8.240      | 0.029              | 8        | 9          | 8.8         | 8.6        | 0.5       | 3.8      | 3.83        | 3.87      | 3.83   | 0.04 |
|                | A053   | < 0.500      | < 0.500                    |             |            |                    | 9.4      | 8.9        | 8.8         | 9.0        | 0.3       | 2.2      | 2.24        | 2.03      | 2.16   | 0.11 |
|                | A054   |              |                            |             |            |                    |          |            |             |            |           |          |             |           |        |      |
|                | A060   | < 1.000      | < 1.000                    | < 1.000     |            |                    | 33.5     | 38.5       | 35.4        | 35.8       | 2.5       | 11.43    | 10.8        | 10.95     | 11.06  | 0.33 |
|                | A065   |              |                            |             |            |                    |          |            |             |            |           |          |             |           |        |      |
|                | A066   | 0            | 0                          | 0           | 0          | 0                  | 9        | 14         | 6           | 9.7        | 4.0       | 3        | 2           | 2         | 2.3    | 0.6  |
|                | A067   | < 10.000     | < 10.000 < 10.000 < 10.000 |             |            |                    | < 10.000 | < 10.000   | < 10.000    |            |           | < 10.000 | < 10.000    | < 10.000  |        |      |
|                | A0/0   | 0.500        |                            |             |            |                    | 0        |            | 10.4        | 0.5        | 0.0       | 2.00     | 2.10        | 1.0.1     | 2.05   | 0.12 |
|                | A073   | < 0.500      |                            |             |            |                    | 9        | 9.2        | 10.4        | 9.5        | 0.8       | 2.09     | 2.19        | 1.94      | 2.07   | 0.13 |
|                | A074   | 1.000        | 1.000                      | 1.000       |            |                    | 0.7      | 0.5        | 0.6         | 0.6        | 0.1       | 1.000    | 1.000       | 1.000     |        |      |
|                | A0/7   | < 1.000      | < 1.000                    | < 1.000     |            |                    | 9.7      | 9.5        | 9.6         | 9.6        | 0.1       | < 1.000  | < 1.000     | < 1.000   |        |      |
|                | A079   | G            |                            |             | 0.26       |                    | G        |            |             | 0.6        |           | G        |             |           | 2.2    |      |
| uity<br>i      |        | Consensu     | is Mean                    | Destation   | 0.26       |                    | Consensu | s Mean     | Desisten    | 9.6        |           | Consensu | is Mean     | Destation | 2.2    |      |
| ults           |        | Monim        | is standard                | Deviation   | 0.70       |                    | Consensu | s standard | Deviation   | 3.9        |           | Consensu | is standard | Deviation | 1.1    |      |
| Res            |        | Minimum      | 1                          |             | 8.24       |                    | Minimum  | l          |             | 44.0       |           | Minimum  | 1           |           | 11.1   |      |
| ŭΓ             |        | N            |                            |             | 0          |                    | N        |            |             | 4.5        |           | N        |             |           | 10     |      |
|                |        | IN           |                            |             | 8          |                    | IN       |            |             | 19         |           | IN       |             |           | 18     |      |



Figure 7-41. Ochratoxin A in candidate RM 8427 Mycotoxins in Corn (Blank) (data summary view –analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red line represents the upper consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \leq 2$ , with the lower range set at zero. A NIST value has not been determined in this material.



**Figure 7-42.** Ochratoxin A in candidate RM 8427 Mycotoxins in Corn (Incurred) (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \leq 2$ . The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by its uncertainty ( $U_{NIST}$ ) and represents the range that results in an acceptable  $Z_{NIST}$  score,  $|Z_{NIST}| \leq 2$ .



**Figure 7-43.** Ochratoxin A in Incurred Corn B (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \leq 2$ . The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by its uncertainty ( $U_{NIST}$ ) and represents the range that results in an acceptable  $Z_{NIST}$  score,  $|Z_{NIST}| \leq 2$ .



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**Figure 7-44.** Laboratory means for ochratoxin A in candidate RM 8427 Mycotoxins in Corn (Incurred) and Incurred Corn B (sample/sample comparison view). In this view, the individual laboratory mean for one sample (RM 8427) is compared to the mean for a second sample (Incurred Corn B). The solid red box represents the NIST range of tolerance for the two samples, RM 8427 Mycotoxins in Corn (Incurred) (x-axis) and Incurred Corn B (y-axis), which encompasses the target values bounded by their uncertainties ( $U_{\text{NIST}}$ ) and represents the range that results in an acceptable  $Z_{\text{NIST}}$  score,  $|Z_{\text{NIST}}| \leq 2$ . The dotted blue box represents the consensus range of tolerance for RM 8427 Mycotoxins in Corn (Incurred) (x-axis) and Incurred (x-axis) and Incurred Corn B (y-axis), ealculated as the values above and below the consensus means that result in an acceptable  $Z'_{\text{comm}}$  score,  $|Z'_{\text{comm}}| \leq 2$ .

**Table 7-13.** Data summary table for T-2 toxin in blank and incurred corns. Data points highlighted in red have been flagged as potential outliers (e.g., difference from reference value, Grubb and/or Cochran) by the NIST software package.

|             |        |                  |             |             |            |           |          |             | T-2 Toxin   |            |            |          |             |             |        |       |
|-------------|--------|------------------|-------------|-------------|------------|-----------|----------|-------------|-------------|------------|------------|----------|-------------|-------------|--------|-------|
|             |        | RM 842           | 27 Mycoto   | oxins in Co | orn (Blank | s) (ng/g) | RM 8427  | 7 Mycotox   | cins in Cor | n (Incurre | ed) (ng/g) |          | Incurr      | ed Corn B   | (ng/g) |       |
|             | Lab    | Α                | В           | С           | Avg        | SD        | Α        | В           | С           | Avg        | SD         | Α        | В           | С           | Avg    | SD    |
|             | Target |                  |             |             |            |           |          |             |             | 19.8       | 7.8        |          |             |             | 109    | 48    |
|             | A005   | < 50.000         | < 50.000    | < 50.000    |            |           | < 50.000 | < 50.000    | < 50.000    |            |            | 110      | 125         | 120         | 118    | 8     |
|             | A013   |                  |             |             |            |           |          |             |             |            |            |          |             |             |        |       |
|             | A020   |                  |             |             |            |           |          |             |             |            |            |          |             |             |        |       |
|             | A021   | 0                | 0           |             | 0          | 0         | 0        | 0           | 11          | 3.7        | 6.4        | 0        | 0           | 0           | 0      | 0     |
|             | A025   | < 0.600          | < 0.600     | < 0.600     |            |           | 17.36    | 16.17       | 17.69       | 17.07      | 0.8        | 107.22   | 106.77      | 107.43      | 107.14 | 0.34  |
|             | A027   |                  |             |             |            |           |          |             |             |            |            |          |             |             |        |       |
|             | A028   | < 9.000          | < 9.000     | < 9.000     |            |           | 16       | 12          | 19          | 16         | 4          | 134      | 133         | 129         | 132    | 3     |
| lts         | A031   | < 0.000          | < 0.000     | < 0.000     | _          | _         | 16.22    | 17.84       | 15.04       | 16.37      | 1.41       | 99.34    | 92.67       | 231.64      | 141.22 | 78.38 |
| nsə         | A042   |                  |             |             |            |           |          |             |             |            |            |          |             |             |        |       |
| dual Res    | A043   | 0                | 0           | 0           | 0          | 0         | 0        | 0           | 0           | 0          | 0          | 184      | 175         | 170         | 176    | 7     |
|             | A045   |                  |             |             |            |           | 18       | 20          | 20          | 19         | 1          | 129      | 130         | 123         | 127    | 4     |
| ivid        | A047   |                  |             |             | _          | _         |          |             |             |            |            |          |             |             |        |       |
| ipu         | A048   | 0                | 0           | 0           | 0          | 0         | 20.5     | 20.4        | 20.8        | 20.6       | 0.2        | 128.1    | 128.2       | 131.1       | 129.1  | 1.7   |
| -           | A052   | 0                | 0           | 0           | 0          | 0         | 0        | 0           | 0           | 0          | 0          | 0        | 0           | 0           | 0      | 0     |
|             | A053   | < 1.000          | < 1.000     |             |            |           | 15.9     | 17.27       | 16.35       | 16.51      | 0.7        | 37.5     | 31.1        | 37.6        | 35.4   | 3.7   |
|             | A065   |                  |             |             |            |           |          |             |             |            |            |          |             |             |        |       |
|             | A066   | 0                | 0           | 0           | 0          | 0         | 10       | 20          | 10          | 13         | 6          | 180      | 220         | 150         | 183    | 35    |
|             | A067   | < 10.000         | < 10.000    | < 10.000    |            |           | 18       | 18          | 18          | 18         | 0          | 121      | 126         | 128         | 125    | 4     |
|             | A070   |                  |             |             |            |           |          |             |             |            |            |          |             |             |        |       |
|             | A073   | < 0.800          |             |             |            |           | 17.1     | 16.2        | 16.7        | 16.7       | 0.5        | 111      | 108         | 104         | 108    | 4     |
|             | A074   |                  |             |             |            |           |          |             |             |            |            |          |             |             |        |       |
|             | A079   |                  |             |             |            |           |          |             |             |            |            |          |             |             | L      |       |
| ity         |        | Consensus Mean 0 |             |             |            |           | Consensu | ıs Mean     |             | 16.0       |            | Consensu | ıs Mean     |             | 114    |       |
| uni<br>lts  |        | Consensu         | is Standard | 1 Deviation | 0          |           | Consensu | is Standard | I Deviation | 4.0        |            | Consensu | is Standard | 1 Deviation | 45     |       |
| esu         |        | Maximun          | 1           |             | 0          |           | Maximum  | 1           |             | 20.6       |            | Maximun  | 1           |             | 183    |       |
| <b>R</b> C0 |        | Minimum          |             |             | 0          |           | Minimum  | L.          |             | 0          |            | Minimum  |             |             | 0      |       |
| Ŭ           |        | Ν                |             |             | 5          |           | Ν        |             |             | 12         |            | N        |             |             | 13     |       |

|       | quid Chromatogra | aphy with Tander   | n Mass Spectrom  | etry  |  |  |   |   |   |  |  |   |
|-------|------------------|--|--|---|--|--|---|---|---|--|--|---|
|       | ean line         |  |  |   |  |  |   |   |   |  |  |   |
| - Lir | nit of tolerance |  |  |   |  |  |   |   |   |  |  |   |
|       |                  |  |  |   |  |  |   |   |   |  |  |   |
| 200   |                  |  |  |   |  |  |   |   |   |  |  |   |
| 200   |                  |  |  |   |  |  |   |   |   |  |  |   |
|       |                  |  |  |   |  |  |   |   |   |  |  |   |
| 00    |                  |  |  |   |  |  |   |   |   |  |  |   |
| 200   |                  |  |  |   |  |  |   |   |   |  |  |   |
| 000   |                  |  |  |   |  |  |   |   |   |  |  |   |
| 00    |                  |  |  |   |  |  |   |   |   |  |  |   |
| 00    |                  |  |  |   |  |  |   |   |   |  |  |   |
| 00    |                  |  |  |   |  |  |   |   |   |  |  |   |
| 200   |                  |  |  |   |  |  |   |   |   |  |  |   |
| 000   |                  |  |  |   |  |  |   |   |   |  |  |   |
| 00    |                  |  |  |   |  |  |   |   |   |  |  |   |
| 00    |                  |  |  |   |  |  |   |   |   |  |  |   |
| 00    |                  |  |  |   |  | (0F)   |   | (GF)  |   | Ū.   | <u>o</u>   |   |
| 200   |                  |  |  |   |  |  | 000   | 00  | 000   | 000  |  |   |
| 0     |                  |  |  |   |  | <u> </u>   | <u>0</u>  | <u>,</u>  | ); <u>6</u>   | <u><u> </u></u>  | 99   |   |
|       | 021              | 043  | 048  | 052   | 066  | 025  | 073   | 053   | 028   | 067  | 005  |   |
|       |                  | 00         Eliquid Chromatogra           1         Liquid Chromatogra           00         — Mean line           00         — Limit of tolerance           00         — Mean line           00         — Limit of tolerance           00         — Mean line           00         — Limit of tolerance           00         — Mean line           00 | Liquid Chromatography with Absorb<br>Liquid Chromatography with Tander<br>Liquid Chromatography with Tander<br>— Mean line<br>— Limit of tolerance<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00<br>00 | 00       Eliquid Chromatography with Absorbance Detection or         01       Liquid Chromatography with Tandem Mass Spectrom         00       - Mean line         - Limit of tolerance       - Limit of tolerance         00       - Limit of tolerance </th <th>00       Eliquid Chromatography with Absorbance Detection of PDA         1       Liquid Chromatography with Tandem Mass Spectrometry         00       - Mean line         -       Limit of tolerance         00       - Mean line         -       - Limit of tolerance         00       - Mean line         -       - Limit of tolerance         00       - Mean line         00       - Mean line</th> <th>Liquid Chromatography with Absorbance Detection or PDA   Liquid Chromatography with Tandem Mass Spectrometry   O   Inot specified   - Mean line   - Limit of tolerance</th> <th>Icluid Chromatography with Absorbance Detection or PDA   Icluid Chromatography with Tandem Mass Spectrometry   Individe Chromatography with Tandem Mass Spectrometry   Image: Individe Chromatography w</th> <th>Idjuid Chromatography with Absorbance Detection of PDA       Liquid Chromatography with Tandem Mass Spectrometry       Mean line       Limit of tolerance       Limit of tolerance       Image: Spectrometry of the spectro</th> <th>Icliquid Chromatography with Absorbance Detection or PDA       Icliquid Chromatography with Tandem Mass Spectrometry       Mean line       Linit of tolerance       Image: Spectrometry       I</th> <th>Liquid Chromatography with Absorbance Detection of PDA<br/>Liquid Chromatography with Tandem Mass Spectrometry<br/>not specified<br/>Limit of tolerance<br/>Limit of tol</th> <th>liquid Chromatography with Absorbance Detection of PDA<br/>Liquid Chromatography with Tandem Mass Spectrometry<br/>not specified<br/></th> <th>- Uquid Chromatography with Absorbance Detection of PDA           - Uquid Chromatography with Tandem Mass Spectrometry           - Maan line           - Limit of tolerance           - Maan line           - Uquid Chromatography with Tandem Mass Spectrometry           - Maan line           - Umit of tolerance           - Maan line           - Maan line      &lt;</th> | 00       Eliquid Chromatography with Absorbance Detection of PDA         1       Liquid Chromatography with Tandem Mass Spectrometry         00       - Mean line         -       Limit of tolerance         00       - Mean line         -       - Limit of tolerance         00       - Mean line         -       - Limit of tolerance         00       - Mean line         00       - Mean line | Liquid Chromatography with Absorbance Detection or PDA   Liquid Chromatography with Tandem Mass Spectrometry   O   Inot specified   - Mean line   - Limit of tolerance | Icluid Chromatography with Absorbance Detection or PDA   Icluid Chromatography with Tandem Mass Spectrometry   Individe Chromatography with Tandem Mass Spectrometry   Image: Individe Chromatography w | Idjuid Chromatography with Absorbance Detection of PDA       Liquid Chromatography with Tandem Mass Spectrometry       Mean line       Limit of tolerance       Limit of tolerance       Image: Spectrometry of the spectro | Icliquid Chromatography with Absorbance Detection or PDA       Icliquid Chromatography with Tandem Mass Spectrometry       Mean line       Linit of tolerance       Image: Spectrometry       I | Liquid Chromatography with Absorbance Detection of PDA<br>Liquid Chromatography with Tandem Mass Spectrometry<br>not specified<br>Limit of tolerance<br>Limit of tol | liquid Chromatography with Absorbance Detection of PDA<br>Liquid Chromatography with Tandem Mass Spectrometry<br>not specified<br> | - Uquid Chromatography with Absorbance Detection of PDA           - Uquid Chromatography with Tandem Mass Spectrometry           - Maan line           - Limit of tolerance           - Maan line           - Uquid Chromatography with Tandem Mass Spectrometry           - Maan line           - Umit of tolerance           - Maan line           - Maan line      < |

Figure 7-45. T-2 Toxin in candidate RM 8427 Mycotoxins in Corn (Blank) (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid red line represents the upper consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \leq 2$ , with the lower range set at zero. A NIST value has not been determined in this material.

Exercise HAMQAP Exercise 1 - Dietary Intake Sample: RM 8427 Mycotoxins in Corn (Blank)

Measurand: T-2 Toxin



Figure 7-46. T-2 Toxin in candidate RM 8427 Mycotoxins in Corn (Incurred) (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \leq 2$ . The partially visible red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by its uncertainty ( $U_{NIST}$ ) and represents the range that results in an acceptable  $Z_{NIST}| \leq 2$ .



**Figure 7-47.** T-2 Toxin in Incurred Corn B (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \leq 2$ . The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by its uncertainty ( $U_{NIST}$ ) and represents the range that results in an acceptable  $Z'_{NIST} | \leq 2$ .



**Figure 7-48.** Laboratory means for T-2 toxin in candidate RM 8427 Mycotoxins in Corn (Incurred) and Incurred Corn B (sample/sample comparison view). In this view, the individual laboratory mean for one sample (RM 8427) is compared to the mean for a second sample (Incurred Corn B). The solid red box represents the NIST range of tolerance for the two samples, RM 8427 Mycotoxins in Corn (Incurred) (x-axis) and Incurred Corn B (y-axis), which encompasses the target values bounded by their uncertainties ( $U_{\text{NIST}}$ ) and represents the range that results in an acceptable  $Z_{\text{NIST}}$  score,  $|Z_{\text{NIST}}| \leq 2$ . The dotted blue box represents the consensus range of tolerance for RM 8427 Mycotoxins in Corn (Incurred) (x-axis) and Incurred Corn B (y-axis), calculated as the values above and below the consensus means that result in an acceptable  $Z'_{\text{comm}}$  score,  $|Z'_{\text{comm}}| \leq 2$ .

**Table 7-14.** Data summary table for HT-2 toxin in blank and incurred corns. Data points highlighted in red have been flagged as potential outliers (e.g., difference from reference value, Grubb and/or Cochran) by the NIST software package.

|             |        |          |            |             |            |                    |          | I           | IT-2 Toxi   | n          |            |          |             |           |        |       |
|-------------|--------|----------|------------|-------------|------------|--------------------|----------|-------------|-------------|------------|------------|----------|-------------|-----------|--------|-------|
|             |        | RM 842   | 7 Mycoto   | oxins in Co | orn (Blank | .) ( <b>ng/g</b> ) | RM 8427  | / Mycotox   | tins in Cou | n (Incurre | ed) (ng/g) |          | Incurre     | ed Corn B | (ng/g) |       |
|             | Lab    | Α        | В          | С           | Avg        | SD                 | Α        | В           | С           | Avg        | SD         | Α        | В           | С         | Avg    | SD    |
|             | Target |          |            |             |            |                    |          |             |             | 38         | 12         |          |             |           | 120    | 53    |
|             | A013   |          |            |             |            |                    |          |             |             |            |            |          |             |           |        |       |
|             | A020   |          |            |             |            |                    |          |             |             |            |            |          |             |           |        |       |
|             | A021   | 0        | 0          |             | 0          | 0                  | 63       | 72          | 128         | 88         | 35         | 252      | 367         | 265       | 295    | 63    |
|             | A025   | < 3.000  | < 3.000    | < 3.000     |            |                    | 39.89    | 42.79       | 46.03       | 42.90      | 3.07       | 124.78   | 124.86      | 122.79    | 124.14 | 1.17  |
|             | A028   | < 8.000  | < 8.000    | < 8.000     |            |                    | 24       | 31          | 24          | 26         | 4          | 143      | 90          | 126       | 120    | 27    |
|             | A031   | < 0.000  | < 0.000    | < 0.000     |            |                    | < 0.000  | < 0.000     | < 0.000     |            |            | 50.1     | 19.7        | 160.3     | 76.7   | 74.0  |
| ts          | A042   |          |            |             |            |                    |          |             |             |            |            |          |             |           |        |       |
| sul         | A043   | 0        | 0          | 0           | 0          | 0                  | 45.1     | 52.1        | 60.1        | 52.4       | 7.5        | 210      | 196         | 199       | 202    | 7     |
| ividual Res | A045   |          |            |             |            |                    | 43       | 50          | 47          | 47         | 4          | 124      | 99          | 116       | 113    | 13    |
|             | A047   |          |            |             |            |                    |          |             |             |            |            |          |             |           |        |       |
|             | A048   | 0        | 0          | 0           | 0          | 0                  | 38.5     | 38.5        | 36.3        | 37.8       | 1.3        | 123.6    | 126.1       | 117.5     | 122.4  | 4.4   |
| ibu         | A052   | 21.789   | 22.151     | 21.492      | 21.811     | 0.330              | 0        | 0           | 0           | 0          | 0          | 270.65   | 240.46      | 266.87    | 259.33 | 16.45 |
| н           | A053   | < 1.000  | < 1.000    |             |            |                    | 39.5     | 23.4        |             | 31.5       | 11.4       | 186.8    | 148.5       | 185.5     | 173.6  | 21.7  |
|             | A065   |          |            |             |            |                    |          |             |             |            |            |          |             |           |        |       |
|             | A066   | 0        | 0          | 0           | 0          | 0                  | 20       | 30          | 20          | 23         | 6          | 220      | 250         | 240       | 237    | 15    |
|             | A067   | < 15.000 | < 15.000   | < 15.000    |            |                    | 38       | 39          | 41          | 39         | 2          | 116      | 119         | 116       | 117    | 2     |
|             | A070   |          |            |             |            |                    |          |             |             |            |            |          |             |           |        |       |
|             | A073   | < 5.000  |            |             |            |                    | 34.7     | 30.8        | 29.1        | 31.5       | 2.9        | 138      | 122         | 111       | 124    | 14    |
|             | A074   |          |            |             |            |                    |          |             |             |            |            |          |             |           |        |       |
|             | A079   |          |            |             |            |                    |          |             |             |            |            |          |             |           |        |       |
| \$          |        | Consensu | s Mean     |             | 4          |                    | Consensu | is Mean     |             | 37         |            | Consensu | is Mean     |           | 158    |       |
| lts         |        | Consensu | s Standard | Deviation   | 15         |                    | Consensu | is Standard | Deviation   | 18         |            | Consensu | is Standard | Deviation | 57     |       |
| esu         |        | Maximum  | ı          |             | 22         |                    | Maximun  | 1           |             | 88         |            | Maximun  | 1           |           | 295    |       |
| S M         |        | Minimum  |            |             | 0          |                    | Minimum  |             |             | 0          |            | Minimum  |             |           | 77     |       |
|             |        | Ν        |            |             | 5          |                    | Ν        |             |             | 11         |            | Ν        |             |           | 12     |       |

| Liquid Chro | omatography with | h Tandem Mass             | Spectrometry   |   |  |   |   |  |   |  |
|-------------|------------------|---------------------------|--|---|--|---|---|--|---|--|
| - Mean line | erance           |                           |  |   |  |   |   |  |   |  |
|             |                  |                           |  |   |  |   |   |  |   |  |
|             |                  |                           |  |   |  |   |   |  |   |  |
|             |                  |                           |  |   |  |   |   |  |   |  |
|             |                  |                           |  |   |  |   |   |  |   |  |
|             |                  |                           |  |   |  |   |   |  |   |  |
|             |                  |                           |  |   |  |   |   |  |   |  |
|             |                  |                           |  |   |  |   |   |  |   |  |
|             |                  |                           |  |   |  |   |   |  |   |  |
|             |                  |                           |  |   |  |   |   |  | OL)   | A-   |
|             |                  |                           |  |   |  |   |   |  | 000   |  |
|             |                  |                           |  |   |  |   |   | (OL)   | ui<br>N   |  |
|             |                  |                           |  |   | <u> </u>   | σF)   | 0 (01   | 000  |   |  |
|             |                  |                           |  |   | 0 (01  | 000   | 22.00   | й<br>  |   |  |
|             |                  |                           |  |   | ₹1.00  | 3   |   |  |   |  |
|             |                  | 043                       | 048  | 066   | 053-   | 025   | 073-  | 028  | 067   | 052  |
| <           | τ.               | <                         | 4  | ∢   | <ul> <li>≺</li> <li>Laboratory</li> </ul>  | <<br>∕  | 4   | 4  | 4   | ∢  |
|             | Liquid Chro      | Liquid Chromatography wit | Liquid Chromatography with Tandem Mass Ont specified Otherance Comparison Com | Liquid Chromatography with Tandem Mass Spectrometry | Liquid Chromatography with Tandem Mass Spectrometry Mean line Limit of tolerance | Liquid Chromatography with Tandem Mass Spectrometry  Mean line Limit of tolerance | Liquid Chromatography with Tandem Mass Spectrometry<br>Mean line<br>Limit of tolerance<br>I I I I I I I I I I I I I I I I I I I | Liquid Chromatography with Tandem Mass Spectrometry<br>not specified<br>- Mean line<br>- Limit of tolerance<br>- Limit | Liquid Chromatography with Tandem Mass Spectrometry<br>of specified<br>- Limit of tolerance<br>- Limit of tolerance | Liquid Chromatography with Tandem Mass Spectrometry<br>- Lonit of tolerance - Limit of toleranc |

Exercise HAMQAP Exercise 1 - Dietary Intake Sample: RM 8427 Mycotoxins in Corn (Blank) Measurand: HT-2 Toxin

**Figure 7-49.** HT-2 Toxin in candidate RM 8427 Mycotoxins in Corn (Blank) (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red line represents the upper consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \leq 2$ , with the lower range set at zero. A NIST value has not been determined in this material.

110 Liquid Chromatography with Tandem Mass Spectrometry not specified 100 Mean line - Limit of tolerance 90 80 70 60 b/gu -0-50 ß Ê F\$A 40 гġл ß 30 0  $^{\circ}$ 20 10 0 A052-A066-A028-A053-A073-A048-A025-A045-A043-A067-A021-Laboratory

**Figure 7-50.** HT-2 Toxin in candidate RM 8427 Mycotoxins in Corn (Incurred) (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \leq 2$ . The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by its uncertainty ( $U_{NIST}$ ) and represents the range that results in an acceptable  $Z_{NIST}$  score,  $|Z_{NIST}| \leq 2$ .

Exercise HAMQAP Exercise 1 - Dietary Intake Sample: RM 8427 Mycotoxins in Corn (Incurred) Measurand: HT-2 Toxin



**Figure 7-51.** HT-2 Toxin in Incurred Corn B (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \leq 2$ . The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by its uncertainty ( $U_{NIST}$ ) and represents the range that results in an acceptable  $Z_{NIST}| \leq 2$ .


## HAMQAP Exercise 1 - Dietary Intake, Measurand: HT-2 Toxin No. of laboratories: 11

**Figure 7-52.** Laboratory means for HT-2 toxin in candidate RM 8427 Mycotoxins in Corn (Incurred) and Incurred Corn B (sample/sample comparison view). In this view, the individual laboratory mean for one sample (RM 8427) is compared to the mean for a second sample (Incurred Corn B). The solid red box represents the NIST range of tolerance for the two samples, RM 8427 Mycotoxins in Corn (Incurred) (x-axis) and Incurred Corn B (y-axis), which encompasses the target values bounded by their uncertainties ( $U_{\text{NIST}}$ ) and represents the range that results in an acceptable  $Z_{\text{NIST}}$  score,  $|Z_{\text{NIST}}| \leq 2$ . The dotted blue box represents the consensus range of tolerance for RM 8427 Mycotoxins in Corn (Incurred) (x-axis) and Incurred (x-axis) and Incurred Corn B (y-axis), calculated as the values above and below the consensus means that result in an acceptable  $Z'_{\text{comm}}$  score,  $|Z'_{\text{comm}}| \leq 2$ .

**Table 7-15.** Data summary table for zearalenone in blank and incurred corns. Data points highlighted in red have been flagged as potential outliers (e.g., difference from reference value, Grubb and/or Cochran) by the NIST software package.

|                    |        | Zearalenone                               |         |         |       |      |  |         |         |       |                |                              |        |        |        |      |
|--------------------|--------|---|---------|---------|-------|------|--|---------|---------|-------|----------------|------------------------------|--------|--------|--------|------|
|                    |        | RM 8427 Mycotoxins in Corn (Blank) (ng/g) |         |         |       |      | RM 8427 Mycotoxins in Corn (Incurred) (ng/g) |         |         |       |                | Incurred Corn B (ng/g)       |        |        |        |      |
|                    | Lab    | Α   | В       | С       | Avg   | SD   | Α  | В       | С       | Avg   | SD             | Α                            | В      | С      | Avg    | SD   |
| Individual Results | Target |   |         |         |       |      |  |         |         | 75    | 21             |                              |        |        | 141    | 60   |
|                    | A005   | 34  | 34      | 38      | 35    | 2    | 96   | 114     | 100     | 103   | 9              | 163                          | 165    | 162    | 163    | 2    |
|                    | A013   |   |         |         |       |      |  |         |         |       |                |                              |        |        |        |      |
|                    | A020   |   |         |         |       |      |  |         |         |       |                |                              |        |        |        |      |
|                    | A021   | 91  | 82      |         | 87    | 6    | 211  | 157     | 146     | 171   | 35             | 255                          | 216    | 93     | 188    | 85   |
|                    | A025   | < 6.000                                   | < 6.000 | < 6.000 |       |      | 72.13  | 66.59   | 75.62   | 71.45 | 4.55           | 145.83                       | 142.05 | 142.52 | 143.47 | 2.06 |
|                    | A027   |   |         |         |       |      |  |         |         |       |                |                              |        |        |        |      |
|                    | A028   | < 7.000                                   | < 7.000 | < 7.000 |       |      | 70   | 71      | 93      | 78    | 13             | 177                          | 179    | 172    | 176    | 4    |
|                    | A031   | < 0.000                                   | < 0.000 | < 0.000 |       |      | 40.5   | 20.5    | 23.1    | 28.0  | 10.9           | 112.9                        | 113.1  | 118.4  | 114.8  | 3.1  |
|                    | A035   |   |         |         |       |      |  |         |         |       |                |                              |        |        |        |      |
|                    | A036   | < 5.370                                   | < 5.370 | < 5.370 |       |      | 63.36  | 65.72   | 67.52   | 65.53 | 2.09           | 198.3                        | 196.4  | 198.9  | 197.9  | 1.3  |
|                    | A042   |   |         |         |       |      |  |         |         |       |                |                              |        |        |        |      |
|                    | A043   |   |         |         |       |      |  |         |         |       |                |                              |        |        |        |      |
|                    | A044   | 0   | 0       | 0       | 0     | 0    | 66.06  | 63.73   | 68.52   | 66.10 | 2.40           | 124.66                       | 130.37 | 127.43 | 127.49 | 2.86 |
|                    | A045   |   |         |         |       |      | 70   | 77      | 79      | 75    | 5              | 163                          | 181    | 157    | 167    | 12   |
|                    | A047   | 7.1                                       | 8.5     | 8.4     | 8.0   | 0.8  | 37.5   | 39.8    | 37.2    | 38.2  | 1.4            | 123.4                        | 134.6  | 129.1  | 129.0  | 5.6  |
|                    | A048   | 0   | 0       | 0       | 0     | 0    | 70.9   | 77.1    | 79.5    | 75.8  | 4.4            | 165.3                        | 166.4  | 167    | 166    | 1    |
|                    | A052   | 0   | 0       | 0       | 0     | 0    | 32.92  | 35.71   | 36.61   | 35.08 | 1.92           | 110.3                        | 112.38 | 112.93 | 111.87 | 1.39 |
|                    | A053   | 10.26                                     | 13.85   |         | 12.06 | 2.54 | 51.6   | 43.5    |         | 47.6  | 5.7            | 153.4                        | 124.2  | 132.1  | 136.6  | 15.1 |
|                    | A054   | 0   | 0       | 0       | 0     | 0    | 64.2   | 66.7    | 67      | 66.0  | 1.5            | 149.6                        | 140    | 146.1  | 145.2  | 4.9  |
|                    | A058   |   |         |         |       |      |  |         |         |       |                |                              |        |        |        |      |
|                    | A065   |   |         |         |       |      |  |         |         |       |                |                              |        |        |        |      |
|                    | A066   | 0   | 0       | 0       | 0     | 0    | 50   | 50      | 40      | 47    | 6              | 130                          | 140    | 130    | 133    | 6    |
|                    | A067   | < 100.0                                   | < 100.0 | < 100.0 |       |      | < 100.0                                      | < 100.0 | < 100.0 |       |                | 141                          | 143    | 144    | 143    | 2    |
|                    | A070   |   |         |         |       |      |  |         |         |       |                |                              |        |        |        |      |
|                    | A073   | 2.1                                       |         |         | 2.1   |      | 33.9   | 44.2    | 38.1    | 38.7  | 5.2            | 110                          | 104    | 97     | 104    | 7    |
|                    | A074   |   |         |         |       |      |  |         |         |       |                |                              |        |        |        |      |
|                    | A079   |   |         |         |       |      |  |         |         |       |                |                              |        |        |        |      |
| amunity<br>ssults  |        | Consensus Mean 5                          |         |         | 5     |      | Consensus Mean                               |         |         | 60    | Consensus Mean |                              |        |        | 147    |      |
|                    |        | Consensus Standard Deviation              |         |         | 10    |      | Consensus Standard Deviation                 |         |         | 27    |                | Consensus Standard Deviation |        |        | 35     |      |
|                    |        | Maximum                                   |         |         | 87    |      | Maximum                                      |         |         | 171   |                | Maximum                      |        |        | 198    |      |
| Ré N               |        | Minimum                                   |         |         | 0     |      | Minimum                                      |         |         | 28    |                | Minimum                      |        |        | 104    |      |
| 5                  |        | Ν   |         |         | 9     |      | Ν  |         |         | 15    |                | Ν                            |        |        | 16     |      |



Exercise HAMQAP Exercise 1 - Dietary Intake Sample: RM 8427 Mycotoxins in Corn (Blank) Measurand: Zearalenone

Figure 7-53. Zearalenone in candidate RM 8427 Mycotoxins in Corn (Blank) (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red line represents the upper consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \leq 2$ , with the lower range set at zero. A NIST value has not been determined in this material.



**Figure 7-54.** Zearalenone in candidate RM 8427 Mycotoxins in Corn (Incurred) (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \leq 2$ . The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by its uncertainty ( $U_{NIST}$ ) and represents the range that results in an acceptable  $Z_{NIST}$  score,  $|Z_{NIST}| \leq 2$ .



Figure 7-55. Zearalenone in Incurred Corn B (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \leq 2$ . The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by its uncertainty ( $U_{NIST}$ ) and represents the range that results in an acceptable  $Z_{NIST} | \leq 2$ .



Figure 7-56. Laboratory means for zearalenone in candidate RM 8427 Mycotoxins in Corn (Incurred) and Incurred Corn B (sample/sample comparison view). In this view, the individual laboratory mean for one sample (RM 8427) is compared to the mean for a second sample (Incurred Corn B). The solid red box represents the NIST range of tolerance for the two samples, RM 8427 Mycotoxins in Corn (Incurred) (x-axis) and Incurred Corn B (y-axis), which encompasses the target values bounded by their uncertainties ( $U_{\text{NIST}}$ ) and represents the range that results in an acceptable  $Z_{\text{NIST}}$  score,  $|Z_{\text{NIST}}| \leq 2$ . The dotted blue box represents the consensus range of tolerance for RM 8427 Mycotoxins in Corn (Incurred) (x-axis) and Incurred Corn B (y-axis), calculated as the values above and below the consensus means that result in an acceptable  $Z'_{\text{comm}}$  score,  $|Z'_{\text{comm}}| \leq 2$ .