**NISTIR 8394**

# Health Assessment Measurements Quality Assurance Program: Exercise 6 Final Report


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National Institute of
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## LIST OF ACRONYMS

| | |
|---|---|
| AAS | Atomic Absorption Spectroscopy |
| ALA | Alpha (α) linolenic acid |
| AMPA | Aminomethylphosphonic acid |
| AMRM | Analytical Methods and Reference Materials |
| ARA | Arachidonic Acid |
| CDC | US Centers for Disease Control and Prevention |
| cGMP | current Good Manufacturing Practice |
| COA | Certificate of Analysis |
| CRM | Certified Reference Material |
| CV AAS | Cold Vapor Atomic Absorption Spectroscopy |
| CV-ICP-MS | Cold Vapor Inductively Coupled Plasma Mass Spectrometry |
| DHA | Docosahexaenoic Acid |
| DSQAP | Dietary Supplements Quality Assurance Program |
| EPA | Eicosapentaenoic Acid |
| FAQAP | Fatty Acids in Human Serum Quality Assurance Program |
| FAMEs | Fatty Acid Methyl Esters |
| FDA | US Food and Drug Administration |
| GC-FID | Gas Chromatography with Flame Ionization Detection |
| GC-MS | Gas Chromatography Mass Spectrometry |
| HAMQAP | Health Assessment Measurements Quality Assurance Program |
| IC | Ion Chromatography |
| IC-CD | Ion Chromatography with Conductivity Detection |
| IC-MS | Ion Chromatography Mass Spectrometry |
| ICP-MS | Inductively Coupled Plasma Mass Spectrometry |
| ICP-OES | Inductively Coupled Plasma Optical Emission Spectrometry |
| ID-GC-MS | Isotope Dilution Gas Chromatography Mass Spectrometry |
| ID ICP-MS | Isotope Dilution Inductively Coupled Plasma Mass Spectrometry |
| INAA | Instrumental Neutron Activation Analysis |
| ISE | Ion-Selective Electrode |
| JCTLM | Joint Committee for Traceability in Laboratory Medicine |
| LC-Abs | Liquid Chromatography with Absorbance Detection |
| LC-FLD | Liquid Chromatography with Fluorescence Detection |
| LC-HRMS | Liquid Chromatography with High Mass Resolution Spectrometry |
| LC-MS | Liquid Chromatography Mass Spectrometry |
| LC-MS/MS | Liquid Chromatography with Tandem Mass Spectrometry |
| LOQ | Limit of Quantification |
| MMQAP | Micronutrients Measurement Quality Assurance Program |
| NIST | National Institute of Standards and Technology |
| NIH | National Institutes of Health |
| ODS | Office of Dietary Supplements |
| PDA | Photodiode-Array Detection |
| PGAA | Prompt-gamma Neutron Activation Analysis |
| QAP | Quality Assurance Program |
| QL | Quantification Limit |

| | |
|---|---|
| RBC | Red Blood Cell |
| RM | Reference Material |
| RSD | Relative Standard Deviation |
| RMP | Reference Measurement Procedure |
| SD | Standard Deviation |
| SODF | Solid Oral Dosage Form |
| SRM | Standard Reference Material |
| TGA | Thermogravimetric Analysis |
| VitDQAP | Vitamin D Metabolites Quality Assurance Program |
| XRF | X-ray Fluorescence Spectrometry |

## ABSTRACT

The 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 in samples that represent human intake (e.g., foods, dietary supplements, tobacco) and samples that represent human metabolism (e.g., blood, serum, plasma, urine) for demonstration of proficiency and/or compliance with various regulations. Analytes are paired where possible to represent the full spectrum of health assessment. Exercise 6 of this program offered the opportunity for laboratories to assess their in-house measurements of nutritional elements (chlorine, iodine, chromium, molybdenum, and selenium), toxic elements (arsenic, cadmium, lead, and mercury), contaminants (chlorate and perchlorate, glyphosate and aminomethylphosphonic acid (AMPA)), water-soluble vitamins (biotin and vitamin C), fat-soluble vitamins (vitamins A and E), fatty acids (select omega-3 and omega-6 fatty acids), botanicals (anthocyanidins), natural products (caffeine, theobromine, and theophylline), and proximates (fat, protein, carbohydrates, solids, ash, and calories) in foods and dietary supplements, and corresponding biomarkers/metabolites in clinical specimens (human red blood cells).

## INTRODUCTION

HAMQAP was formed in 2017, in part as a collaboration with the NIH-ODS and represents ongoing efforts at NIST that were supported previously via historical quality assurance programs (QAPs), including the Dietary Supplements Laboratory QAP (DSQAP), Fatty Acids in Human Serum QAP (FAQAP), Micronutrients Measurement QAP (MMQAP), 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, natural products) are paired with samples that represent human metabolism (e.g., blood, serum, plasma, urine)[1], 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 Practice (cGMPs) or to fulfill proficiency requirements established by related accreditation bodies. In addition, NIST and HAMQAP assist the ODS Analytical Methods and Reference Materials (AMRM) program at the NIH in supporting the development and dissemination of analytical tools and reference materials (RMs). In the future, results from HAMQAP exercises could be used by ODS and NIST to identify problematic matrices and analytes for which consensus-based methods of analysis would benefit the dietary supplements and clinical communities.

NIST has decades of experience in the administration of QAPs, and HAMQAP builds on the approach taken by the former DSQAP by providing a wide range of matrices and analytes. The HAMQAP design combines activities of DSQAP, FAQAP, MMQAP, and VitDQAP, and emphasizes emerging and challenging measurements in the dietary supplement, food, and clinical matrix categories. Participating laboratories are interested in evaluating 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, 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 sixth exercise of HAMQAP. Eighty-eight laboratories responded to the dietary intake portion and sixteen laboratories responded to the human metabolites portion of the call for participants distributed in August 2020 (see table below). Seven human metabolites studies were cancelled prior to shipment due to low enrollment. Samples were shipped to participants in January 2021 and results were returned to NIST by March 2021. This report contains the final data and information that was disseminated to the participants in September 2021.

[1] Human intake samples were intended for research use only and not for human consumption. Human output samples were human-source 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 non-reactive 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 FDA licensed tests.

| Study Group | Dietary Intake Study | Human Metabolites Study |
|---|---|---|
| **Nutritional Elements** | CL, I, Cr, Mo, Se in: Multivitamin, Infant Formula | ~~CL, I, Cr, Mo, Se in:~~** ~~Human and Animal Serum~~ |
| **Toxic Elements** | As, Cd, Pb, Hg in: Rice Flour, Green Tea | ~~As, Cd, Pb, Hg in:~~** ~~Human and Animal Serum~~ |
| **Water-Soluble Vitamins** | Biotin, Vitamin C in: Multivitamin, Infant Formula | ~~Biotin, Vitamin C in:~~** ~~Human Serum~~ |
| **Fat-Soluble Vitamins** | Vitamins A and E in: Multivitamin, Infant Formula | ~~Vitamins A and E in~~:** ~~Human Serum~~ |
| **Fatty Acids** | Omega-3, Omega-6 Fatty Acids Fish and Fish Oil | Omega-3, Omega-6 Fatty Acids in: Human Red Blood Cells |
| **Botanicals** | Anthocyanidins in: Cranberries, Blueberries, Bilberry Extract | Not Offered |
| **Natural Products** | Caffeine, Theobromine, Theophylline in: Protein Supplements | ~~Caffeine, Theobromine, Theophylline in:~~** ~~Human Urine~~ |
| **Contaminants I** | Chlorate, Perchlorate in: Infant Formula Ingredients and Finished Products | ~~Chlorate, Perchlorate in:~~** ~~Human Urine~~ |
| **Contaminants II** | Glyphosate, AMPA in:* Oats | ~~Glyphosate, AMPA in:~~** ~~Human Urine~~ |
| **Proximates** | Proximates in:* Infant Formula, Rice Flour | Not Offered |

* Study not sponsored by the NIH ODS.
** Cancelled due to low enrollment (less than 10 laboratories registered).

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 figures 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 figures 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).[2] The consensus means and standard deviations are calculated according to the robust Q/Hampel method outlined in ISO 13528:2015, Annex C.[3]

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 using sample NIST data; 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 ($x_i$) and standard deviation ($s_i$) when multiple values were reported. A blank indicates that NIST does not have data on file for that laboratory for the corresponding analyte or matrix. An empty box for standard deviation indicates that the participant reported a single value or a value below the limit of quantification (LOQ) and therefore that value was not included in the calculation of the consensus data.[3] 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, $Z'_{\text{comm}}$, 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:

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

[2] 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.

[3] ISO 13528:2015, *Statistical methods for use in proficiency testing by interlaboratory comparisons*, pp. 53–54.

The second Z-score, $Z_{\mathrm{NIST}}$, is calculated with respect to the target value (NIST certified, reference, or estimated value, when available), using $x_{\mathrm{NIST}}$ 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):

$$Z_{\mathrm{NIST}} = \frac{x_i - x_{\mathrm{NIST}}}{2{*}U_{95}}$$

or

$$Z_{\mathrm{NIST}} = \frac{x_i - x_{\mathrm{NIST}}}{2{*}U_{\mathrm{NIST}}}.$$

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

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 each analyte (N), the consensus mean value determined for each analyte ($x^*$), and a consensus robust estimate of the standard deviation of the reported values ($s^*$).[3] Consensus means and standard deviations are calculated using the laboratory means; if a laboratory reported a single value, the reported value is not included in determination of the consensus values.[3] 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 ($x_{\mathrm{NIST}}$) 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, by measurements obtained from collaborating laboratories, or a combination of NIST and collaborator data. 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 packaged units. For samples in which a NIST certified or reference value is not available, a NIST-determined value may be assessed using a validated method or data from a collaborating laboratory. The NIST-determined values of this type represent 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 and to the consensus data. A blank indicates that the laboratory signed up and received samples for that analyte and matrix, but NIST does not have data on file for that laboratory. Data points highlighted in blue have been identified as outside the consensus tolerance limits and would be estimated to result in an unacceptable $Z'_{\mathrm{comm}}$ score, $|Z'_{\mathrm{comm}}| \geq 2$. Data points highlighted in red have a zero or non-numeric data point. The standard deviation (SD) for the target value in this table is the uncertainty ($U_{\mathrm{NIST}}$) around the target value.

Figures

*Data Summary View (Method Comparison Data Summary View)*

In this view, individual laboratory data (circles) are plotted with the individual laboratory standard deviation (rectangle). Laboratories reporting values below the LOQ are shown in this view as downward triangles beginning at the LOQ, reported as quantification limit (QL) on the figures. Laboratories reporting values as "below LOQ" can still be successful in the study if the target value is also below the laboratory LOQ. The blue 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 ($s_{\mathrm{r}}$), the reproducibility standard deviation ($s_{\mathrm{R}}$), 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.

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

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 $U_{\mathrm{NIST}}$). 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. Major program goals include both reducing the size of the consensus zone and centering 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 identify 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 (e.g., NIST Standard Reference Material (SRM) with a certified, reference, or NIST-determined value; a less challenging matrix) are compared to the results for another sample (e.g., NIST SRM with a more challenging matrix; a commercial sample). The solid red box represents the target zone for the first sample (x-axis) and the second sample (y-axis), if available. 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 (Chlorine, Iodine, Chromium, Molybdenum, Selenium)**

Study Overview

In this study, participants were provided with samples of SRM 3280 Multivitamin/Multielement Tablets and Infant Formula A for dietary intake. Participants were asked to use in-house analytical methods to determine the mass fractions (mg/kg) of chlorine (Cl), iodine (I), chromium (Cr), molybdenum (Mo), and selenium (Se) in the samples. In the US, updated US Food and Drug Administration (FDA) regulations require all nutrient levels claimed on Nutrition Facts labels on packaged foods to be accurate. Consumers expect labeling information to be accurate on food and dietary supplement products in order to make informed purchasing choices. Supplements and foods used for sole-source nutrition are often fortified with trace minerals for a well-rounded nutrient profile. These trace minerals are essential for the body to function properly, and deficiencies can lead to negative health outcomes. Testing of these minerals in foods and supplements helps ensure accurate product labeling.

Dietary Intake Sample Information

*Multivitamin A.* Participants were provided with three bottles of SRM 3280 Multivitamin/Multielement Tablets, each containing 30 tablets. Participants were asked to store the material at controlled room temperature (20 °C to 25 °C) in the original unopened bottles and to prepare one sample and report one value from each bottle provided. Before use, participants were instructed to grind all 30 tablets and to mix the resulting powder thoroughly prior to removal of a test portion for analysis and to use a sample size of at least 0.2 g for the determination of I, Cr, Mo, and Se and 0.75 g for the determination of Cl. After grinding, participants were instructed to store the resulting powder at controlled room temperature, 20 °C to 25 °C, and to analyze the material within two days for analytes in this study. Approximate analyte levels were not reported to participants prior to the study. The certified value for chlorine in SRM 3280 was determined by prompt-gamma neutron activation analysis (PGAA) and collaborating laboratories; for iodine and selenium by inductively coupled plasma mass spectrometry (ICP-MS) and instrumental neutron activation analysis (INAA); for chromium by ICP-MS and X-ray fluorescence spectrometry (XRF); and for molybdenum by ICP-MS, inductively coupled plasma optical emission spectrometry (ICP-OES), and XRF. The certified values and uncertainties are provided in the table below, both on a dry-mass basis, as shown on the certificate of analysis (COA), and on an as-received basis accounting for moisture of the material (1.4 %).

| | NIST-Determined Mass Fractions in SRM 3280 (mg/kg) | |
|---|---|---|
| Analyte | (dry-mass basis) | (as-received basis) |
| Chlorine (Cl) | 53000 ± 2300 | 52270 ± 2270 |
| Iodine (I) | 132.7 ± 6.6 | 130.9 ± 6.5 |
| Chromium (Cr) | 93.7 ± 2.7 | 92.4 ± 2.7 |
| Molybdenum (Mo) | 70.7 ± 4.5 | 69.7 ± 4.4 |
| Selenium (Se) | 17.42 ± 0.45 | 17.18 ± 0.44 |

*Infant Formula A.* Participants were provided with three packets of Infant Formula A, each containing approximately 10 g of material. Participants were asked to store the material at –20 °C in the original unopened packets and to prepare one sample and report one value from each packet provided. Before use, participants were instructed to thoroughly mix the contents of the packets prior to removal of a test portion for analysis, and to use a sample size of at least 0.5 g for the determination of Cl, I, Cr, Mo, and Se. The approximate analyte levels were not reported to participants prior to the study. The NIST-determined values for nutritional elements were assigned using results from the manufacturer of the material. The NIST-determined values and uncertainties are provided in the table below on an as-received basis.

| Analyte | NIST-Determined Mass Fraction in Infant Formula A (mg/kg) | | |
|---|---|---|---|
| Chlorine (Cl) | 6609.1 | ± | 5.4 |
| Iodine (I) | 2.095 | ± | 0.038 |
| Chromium (Cr) | 1.044 | ± | 0.024 |
| Molybdenum (Mo) | 1.795 | ± | 0.037 |
| Selenium (Se) | 0.836 | ± | 0.018 |

Dietary Intake Study Results

- The enrollment and reporting statistics for the dietary intake study are described in the table below. Reported values may include non-quantitative results (zero or below LOQ) but are included in the participation statistics.

| | | Number of Laboratories Reporting Results (Percent Participation) | |
|---|---|---|---|
| Analyte | Number of Laboratories Requesting Samples | Multivitamin | Infant Formula |
| Chlorine (Cl) | 15 | 5 (33 %) | 5 (33 %) |
| Iodine (I) | 18 | 11 (61 %) | 9 (50 %) |
| Chromium (Cr) | 35 | 24 (69 %) | 21 (60 %) |
| Molybdenum (Mo) | 32 | 21 (66 %) | 20 (62 %) |
| Selenium (Se) | 34 | 21 (62 %) | 20 (59 %) |

- The between-laboratory variabilities were good for most analytes in both materials. Iodine in infant formula was an exception. (see table below).

| Analyte | Between-Laboratory Variability (% RSD) Multivitamin | Infant Formula |
|---|---|---|
| Chlorine (Cl) | 3 % | 6 % |
| Iodine (I) | 18 % | 40 % |
| Chromium (Cr) | 12 % | 7 % |
| Molybdenum (Mo) | 16 % | 12 % |
| Selenium (Se) | 12 % | 15 % |

- Most laboratories reported using microwave digestion for their sample preparation of Cr, Mo, and Se (see table below). The sample preparation methods are also depicted graphically in **Figures 1-1** and **1-2**, **1-6** and **1-7**, **1-11** and **1-12, 1-16** and **1-17**, and **1-21** and **1-22**, for Cl, I, Cr, Mo, and Se, respectively. The values shown below are the combined (as an average) reported sample preparations for both samples.

| Reported Sample Preparation Method | Percent Reporting (Averaged for both samples types) | | | | |
|---|---|---|---|---|---|
| | Cl | I | Cr | Mo | Se |
| Microwave Digestion | - | 15 % | 76 % | 76 % | 73 % |
| Hot Block | - | 10 % | 16 % | 15 % | 15 % |
| Dilution | 18 % | 10 % | - | - | - |
| Solvent Extraction | 18 % | 10 % | - | - | - |
| Thermal Decomposition | - | 10 % | - | - | 5 % |
| Acid Hydrolysis | - | - | 4 % | 5 % | 5 % |
| Base Hydrolysis | - | 20 % | - | - | - |
| Other or None Reported | 64 % | 25 % | 4 % | 5 % | 2 % |

- Most laboratories reported using ICP-MS for determination of Cr, I, Mo, and Se (see table below). The analytical methods reported by participating laboratories are also depicted graphically in **Figures 1-3** and **1-4**, **1-8** and **1-9**, **1-13** and **1-14, 1-18** and **1-19**, and **1-23** and **1-24**, for Cl, I, Cr, Mo, and Se, respectively. The values shown below are the combined (as an average) reported analytical methods for both samples.

| Reported Analytical Method | Percent Reporting (Averaged for both samples types) | | | | |
|---|---|---|---|---|---|
| | Cl | I | Cr | Mo | Se |
| ICP-MS | - | 50 % | 74 % | 69 % | 69 % |
| ID ICP-MS | - | 25 % | 19 % | 21 % | 26 % |
| ICP-OES | - | 5 % | 7 % | 8 % | 5 % |
| ISE | 40 % | 10 % | - | - | - |
| LC-MS | - | - | - | 3 % | - |
| Potentiometry | 20 % | - | - | - | - |
| Other or None Reported | 40 % | 5 % | - | - | - |

- For SRM 3280 Multivitamin/Multielement Tablets, the consensus means lie within the target ranges for all five elements (**Figures 1-1, 1-3**, **1-6, 1-8**, **1-11, 1-13, 1-16, 1-18**, **1-21, 1-23**).
- For Infant Formula A, the consensus means lie within the target ranges for I, Cr, Mo, and Se (**Figures 1-7, 1-9**, **1-12, 1-14, 1-17, 1-19**, **1-22, 1-24**). The consensus mean lies slightly above the target range for Cl, however, the consensus range for Cl overlaps the target range (**Figures 1-2,** and **1-4**).

Dietary Intake Technical Recommendations

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

- For chlorine, the low participation rate could be a result of a lack of interest in chlorine measurements, a lack of established protocols for chlorine measurements, or a greater challenge posed by measurement of chlorine.
  - Too few results were received to make any meaningful conclusions on potential bias in current sample preparation approaches or analytical methodology used.
  - Where within-sample variability is large, laboratories may want to check for calculation errors.
- For iodine, **Figure 1-10** shows a few laboratories have reported low values compared to the target values for one or both sample matrices.
  - Iodine can form volatile hydrogen iodide (HI) during acid digestion so care must be taken to retain iodine during sample preparation. For laboratories reporting low values, the addition of an extraction solvent for iodine in the sample preparation step may be necessary.
  - Many protocols call for the use of tetramethylammonium hydroxide (TMAH). TMAH is a very effective solvent for iodine sample preparation, however, TMAH is a strong base with high toxicity and extreme caution must be taken when used. A safer alternative is to use an acid digestion followed by the neutralization of sample solutions with a base such as ammonium hydroxide before analysis.

    - When using ICP-MS as the analytical methodology for iodine, carryover between analyses may be observed for samples prepared in an acidic solution. The addition of a surfactant to sample solutions (e.g., Triton X-100) will improve washout of iodine. The rinse solution used between sample readings should be slightly basic, above pH 7, and contain Triton X-100.
    - During sample preparation, iodine can adhere to tetrafluoroethylene (TFM) vessels, so perfluoroalkoxy (PFA) vessels or quartz vessels are recommended to improve repeatability.
- For chromium, **Figures 1-12, 1-14,** and **1-15** indicate that most laboratories are within the NIST target range for infant formula indicating that the sample preparation and analytical methodology for chromium in this matrix is valid.
    - For the infant formula, the few values that were outside the consensus tolerance limits may need to be confirmed they are reported in the correct units or calculations may need to be checked.
    - Incomplete digestion of either sample matrix may have resulted in results with a large variability and/or values below the target range.
    - Use of collision cell gas is recommended since polyatomic interferences can occur from the plasma, $^{35}Cl^{16}O^{1}H^{+}$, $^{40}Ar^{12}C^{+}$, $^{37}Cl^{15}N^{+}$. The collision gas flow rate may need to be adjusted to reduce polyatomic interferences from the matrix itself, such as in the multivitamin/multielement samples.
    - For matrices with high total dissolved solids such as the multivitamin/multielement tablets, samples may need to be diluted appropriately in order to achieve accurate results.
    - Sample solutions should be stored in dilute nitric acid, at 1.5% or higher, to maintain stability.
    - Preparation of procedural blanks is a key step to identify sources of bias such as contamination from autosampler vials.
- For molybdenum, the sample/sample comparison view (**Figure 1-20)** shows a linear trend for both samples except for some participants reporting results outside the consensus tolerance limits.
    - Trends of this type often indicate calibration errors. Laboratories should check that all sample solution concentrations are within the linear calibration range.
    - Laboratories reporting results that are either extremely low or have high sample-to-sample variability may have incompletely digested their samples.
    - Polyatomic interferences may occur, $^{40}Ar^{39}K^{16}O^{+}$, $^{39}K^{41}K^{16}O^{+}$, $^{41}K_2{}^{16}O^{+}$, but the use of collision cell technology with either He or H gas should improve or eliminate these interferences.
    - Isobaric interferences could be caused by Zr or Ru but should be negligible since the concentration of these elements is low in these two matrices.
    - Preparation of procedural blanks is a key step to identify sources of bias such as reagent impurities.
- For selenium, the sample/sample comparison view (**Figure 1-25)** shows a slight linear trend for much of the sample data indicating a possible calibration error. Values that are low may indicate matrix-induced signal suppression, which may be avoided with the use of an internal standard.
    - The digestion procedure is critical to the accuracy of selenium determination. Digestion of the multivitamin/multielement tablets is difficult in comparison to the infant formula due

to the film coating found on the tablets. Even when ground, this coating is difficult to digest.

- To breakdown the organoselenium compounds, mixtures of nitric, hydrofluoric, and perchloric acids with temperatures of up to 200 °C are recommended for open beaker digestion techniques. For microwave digestion, nitric acid and a small amount of HF with high pressure and high temperature are recommended. A small amount of HF ensures complete digestion and more accurate selenium determination.
- When using ICP-MS, collision cell technology can be used to minimize polyatomic interferences caused by molecular ions that have the same mass-to-charge ratio as selenium, such as $^{40}Ar^{38}Ar^{+}$, $^{40}Ar^{37}Cl^{+}$, and $^{40}Ar_2^{+}$.

- Validation tools, (e.g. Certified Reference Materials (CRMs)) are available and should be used to confirm accuracy of measurement techniques. When selecting a CRM, choose matrix-matched materials that have the analytes of interest, where possible.
- All results should be reported accurately.
    - Zero is not a quantity that can be measured. If measured values are below quantitation limits, results should be reported as such. A more appropriate result would be to report that a value is below the LOQ or QL.
    - Laboratories reporting results outside the consensus tolerance levels should check for calculation errors. One example is to confirm that factors for all dilutions have been properly tabulated and that results are reported in correct reporting units.

**Table 1-1.** Individualized data summary table (NIST) for nutritional elements in SRM 3280 Multivitamin/Multielement Tablets and Infant Formula A.

***National Institute of Standards and Technology***

**HAMQAP Exercise 6 - Nutritional Elements**

| | **Lab Code:** | **NIST** | **1. Your Results** | | | | **2. Community Results** | | | **3. Target** | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Analyte | Sample | Units | $x_i$ | $s_i$ | $Z'_{comm}$ | $Z_{NIST}$ | N | x* | s* | $x_{NIST}$ | $U$ |
| Chlorine | Infant Formula A | mg/kg | 6610 | 10.8 | | | 5 | 6640 | 170 | 6610 | 10.8 |
| Chlorine | SRM 3280 Multivitamin/Multielement Tablets | mg/kg | 52300 | 2270 | | | 5 | 50800 | 3100 | 52300 | 2270 |
| Chromium | Infant Formula A | mg/kg | 1.04 | 0.0484 | | | 21 | 1.02 | 0.076 | 1.04 | 0.0484 |
| Chromium | SRM 3280 Multivitamin/Multielement Tablets | mg/kg | 92.4 | 2.7 | | | 24 | 100 | 11 | 92.4 | 2.7 |
| Iodine | Infant Formula A | mg/kg | 2.1 | 0.076 | | | 9 | 2.08 | 0.84 | 2.1 | 0.076 |
| Iodine | SRM 3280 Multivitamin/Multielement Tablets | mg/kg | 131 | 6.5 | | | 11 | 120 | 23 | 131 | 6.5 |
| Molybdenum | Infant Formula A | mg/kg | 1.8 | 0.0745 | | | 20 | 1.7 | 0.21 | 1.8 | 0.0745 |
| Molybdenum | SRM 3280 Multivitamin/Multielement Tablets | mg/kg | 69.7 | 4.4 | | | 21 | 70 | 12 | 69.7 | 4.4 |
| Selenium | Infant Formula A | mg/kg | 0.836 | 0.0358 | | | 20 | 0.79 | 0.12 | 0.836 | 0.0358 |
| Selenium | SRM 3280 Multivitamin/Multielement Tablets | mg/kg | 17.2 | 0.44 | | | 21 | 20 | 2 | 17.2 | 0.44 |

$x_i$ Mean of reported values
$s_i$ Standard deviation of reported values
$Z'_{comm}$ Z'-score with respect to community consensus
$Z_{NIST}$ Z-score with respect to NIST value

N Number of quantitative values reported
x* Robust mean of reported values
s* Robust standard deviation

$x_{NIST}$ NIST-assessed value
$U$ expanded uncertainty about the NIST-assessed value

**Table 1-2.** Data summary table for chlorine in 3280 Multivitamin/Multielement Tablets and Infant Formula A. Data points highlighted in blue have been identified as outside the consensus tolerance limits and would be estimated to result in an unacceptable $Z'_{comm}$ score, $|Z'_{comm}| \geq 2$.

| | | Chlorine | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | SRM 3280 Multivitamin/Multielement Tablets (mg/kg) | | | | | Infant Formula A (mg/kg) | | | | |
| | Lab | A | B | C | Avg | SD | A | B | C | Avg | SD |
| Individual Results | Target | | | | 52274 | 2268 | | | | 6609 | 10.8 |
| | F004 | | | | | | | | | | |
| | F005 | | | | | | | | | | |
| | F021 | | | | | | | | | | |
| | F026 | | | | | | | | | | |
| | F030 | 53600 | 52600 | 53400 | 53200 | 529 | 6640 | 6650 | 6620 | 6637 | 15 |
| | F031 | | | | | | | | | | |
| | F034 | 51600 | 44050 | 53110 | 49587 | 4854 | 6520 | 6560 | 6300 | 6460 | 140 |
| | F035 | | | | | | | | | | |
| | F039 | 52510 | 52030 | 51670 | 52070 | 421 | 6640 | 6650 | 6640 | 6643 | 5.8 |
| | F042 | | | | | | | | | | |
| | F056 | | | | | | | | | | |
| | F061 | 49500 | 50600 | 50600 | 50233 | 635 | 6540 | 6550 | 661 | 4584 | 3397 |
| | F062 | | | | | | | | | | |
| | F067 | 48635 | 49198 | 48751 | 48861 | 297 | 6651 | 6843 | 6952 | 6815 | 152 |
| | F074 | | | | | | | | | | |
| Community Results | | Consensus Mean | | | 50790 | | Consensus Mean | | | 6639 | |
| | | Consensus Standard Deviation | | | 3088 | | Consensus Standard Deviation | | | 169 | |
| | | Maximum | | | 53200 | | Maximum | | | 6815 | |
| | | Minimum | | | 48861 | | Minimum | | | 4584 | |
| | | N | | | 5 | | N | | | 5 | |

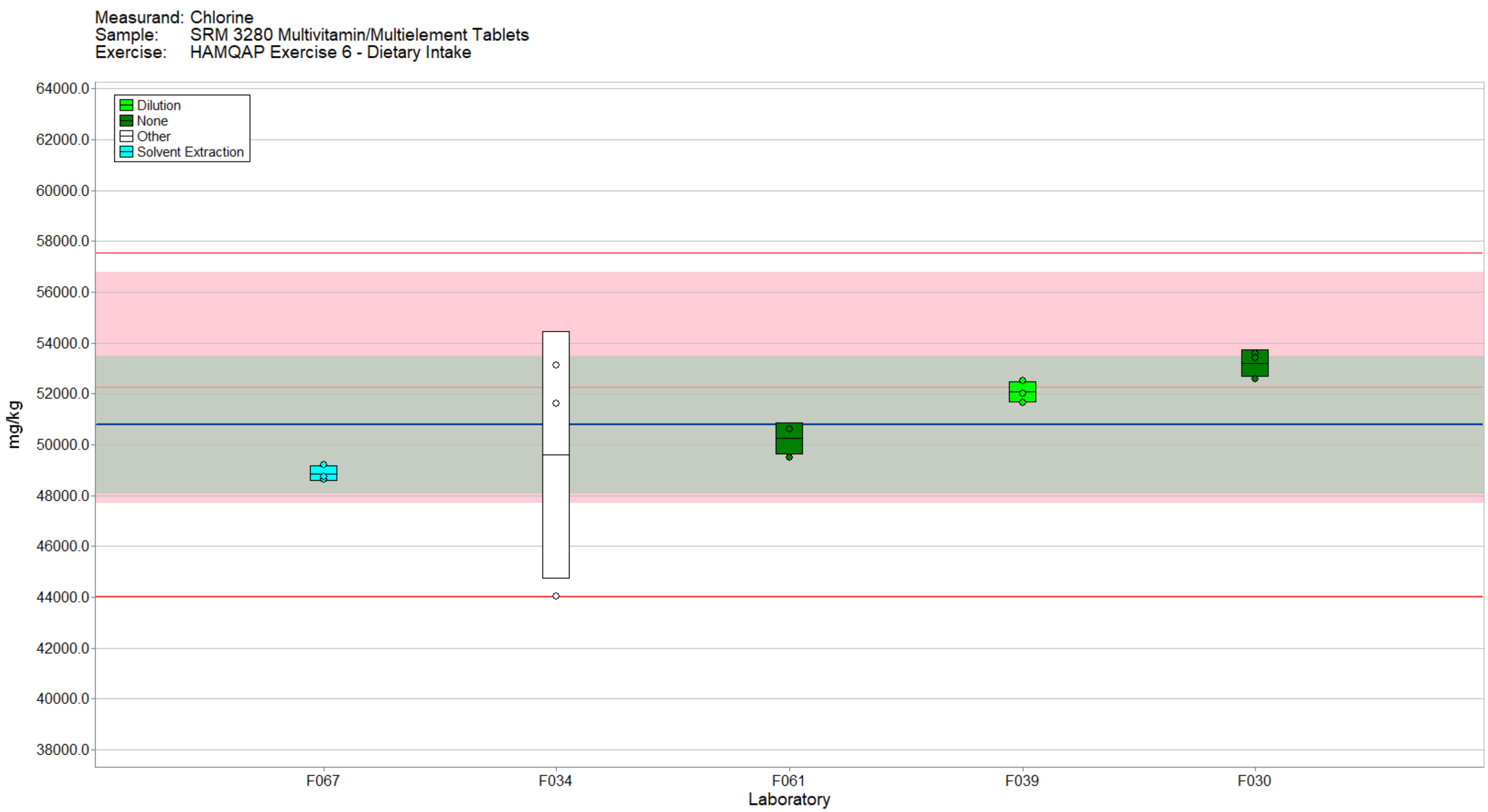


**Figure 1-1.** Chlorine in SRM 3280 Multivitamin/Multielement Tablets (data summary view – sample preparation method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The solid blue 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 $Z'_{\text{comm}}$ score, $|Z'_{\text{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty ($U_{\text{NIST}}$) and represents the range that results in an acceptable $Z_{\text{NIST}}$ score, $|Z_{\text{NIST}}| \leq 2$.

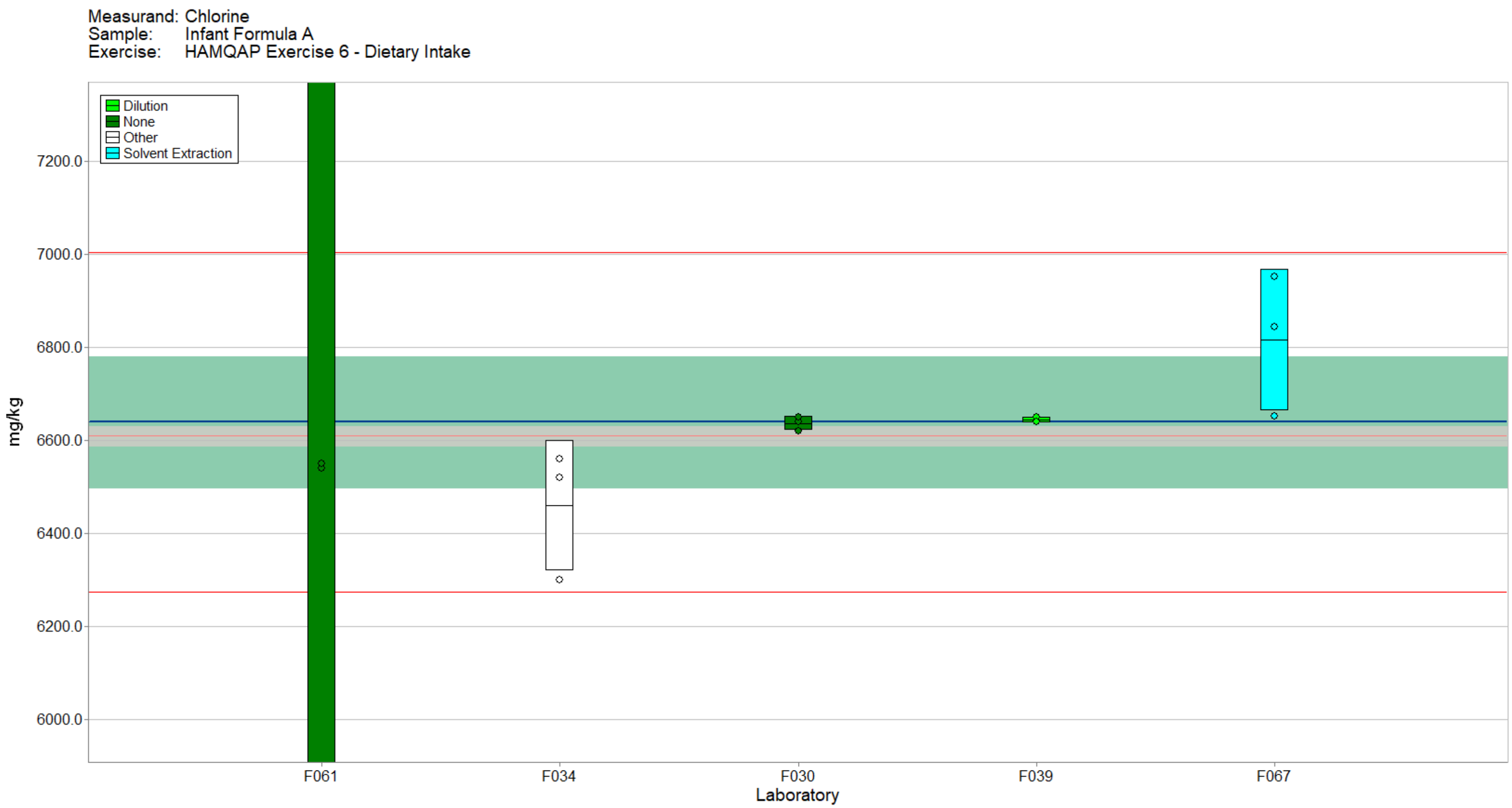


**Figure 1-2.** Chlorine in Infant Formula A (data summary view – sample preparation method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The solid blue 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 $Z'_{\text{comm}}$ score, $|Z'_{\text{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty ($U_{\text{NIST}}$) and represents the range that results in an acceptable $Z_{\text{NIST}}$ score, $|Z_{\text{NIST}}| \leq 2$.

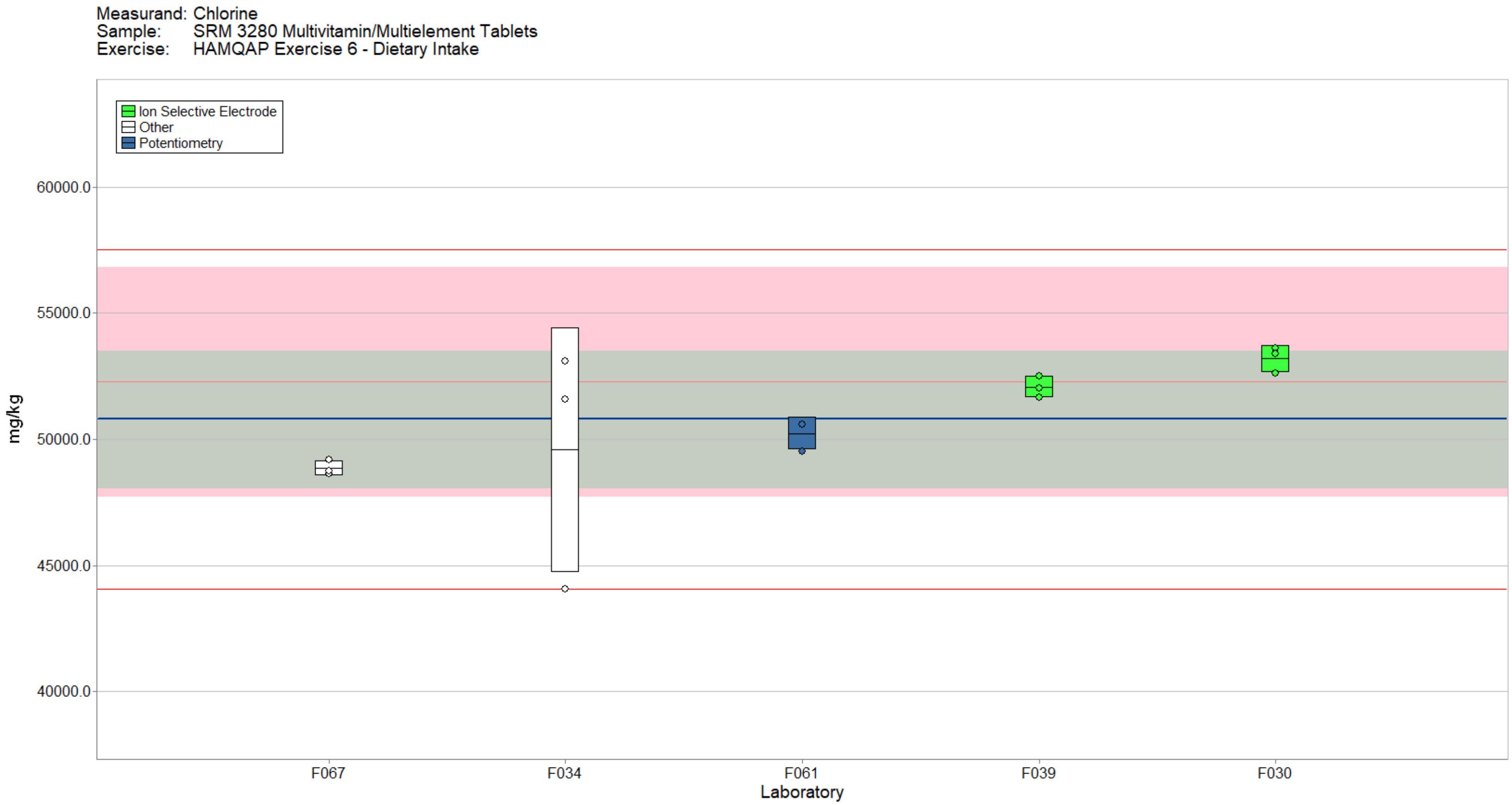


**Figure 1-3.** Chlorine in SRM 3280 Multivitamin/Multielement (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue 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 $Z'_{\text{comm}}$ score, $|Z'_{\text{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty ($U_{\text{NIST}}$) and represents the range that results in an acceptable $Z_{\text{NIST}}$ score, $|Z_{\text{NIST}}| \leq 2$.

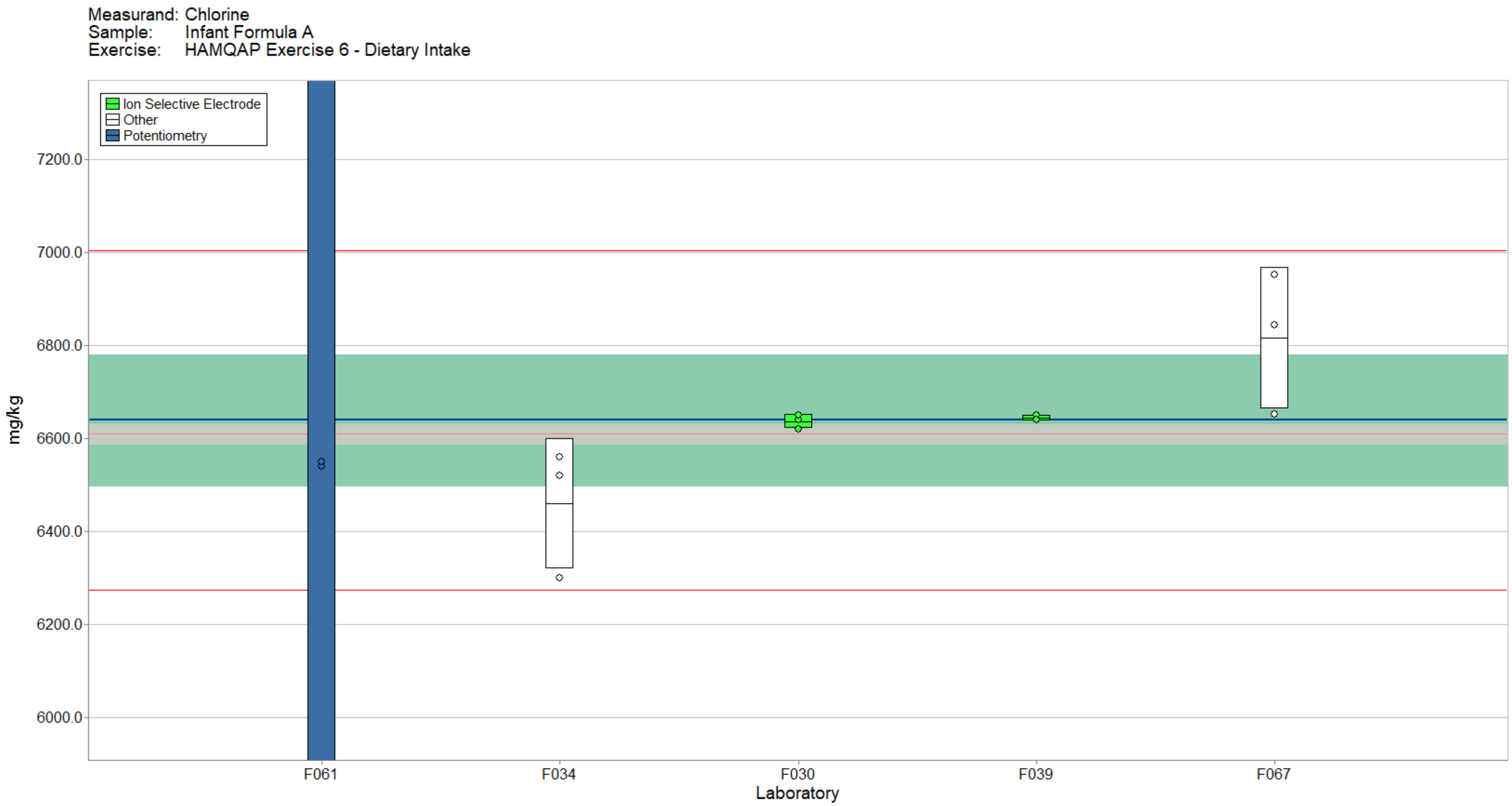


**Figure 1-4.** Chlorine in Infant Formula A (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line 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 $Z'_{\text{comm}}$ score, $|Z'_{\text{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty ($U_{\text{NIST}}$) and represents the range that results in an acceptable $Z_{\text{NIST}}$ score, $|Z_{\text{NIST}}| \leq 2$.

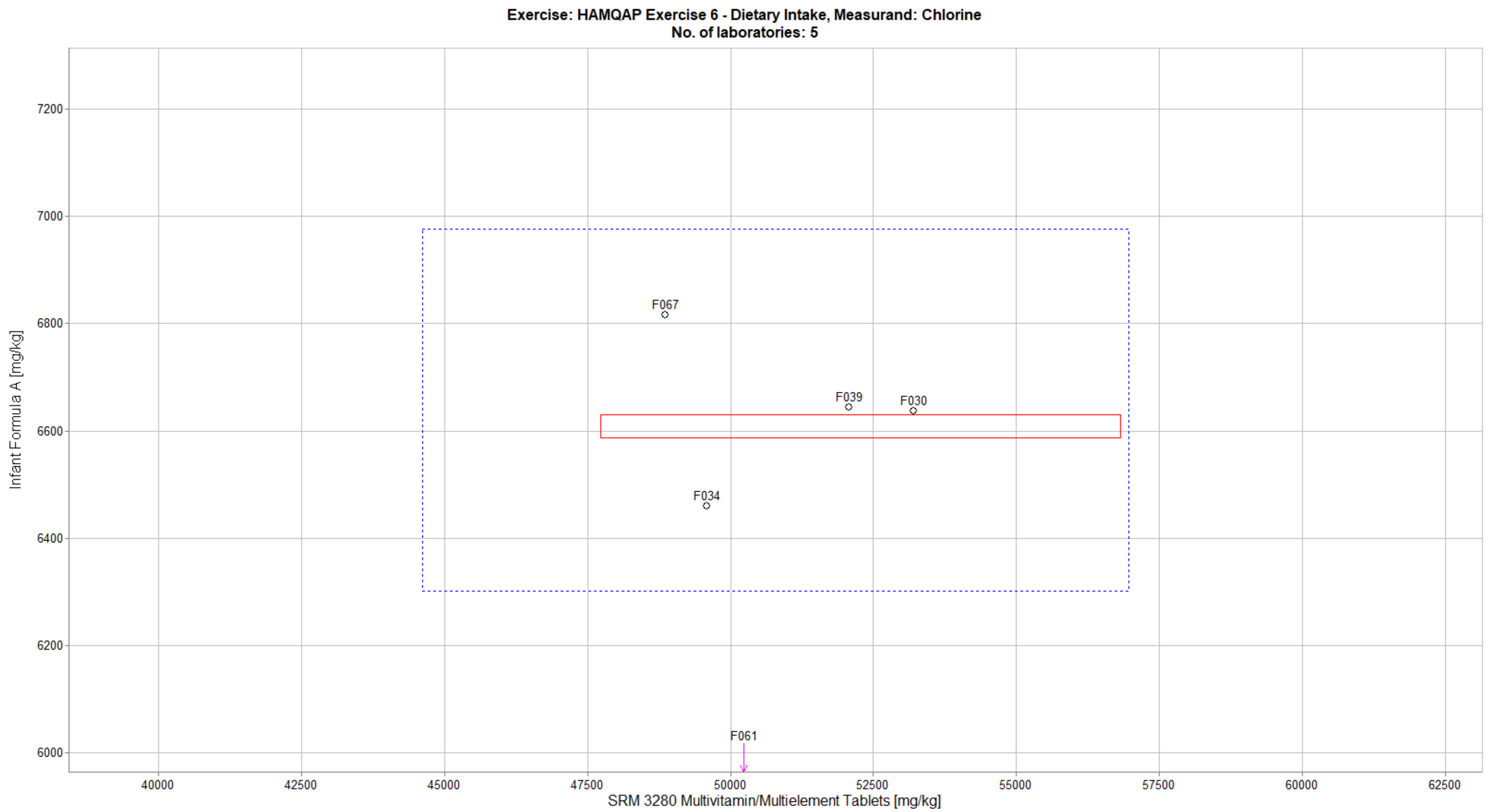


**Figure 1-5.** Laboratory means for chlorine in SRM 3280 Multivitamin/Multielement Tablets and Infant Formula A (sample/sample comparison view). In this view, the individual laboratory mean for one sample (SRM 3280) is compared to the individual laboratory mean for a second sample (Infant Formula A). The solid red box represents the NIST range of tolerance for the two samples, SRM 3280 (x-axis) and Infant Formula A (y-axis), which encompasses the target values bounded by their uncertainties ($U_{NIST}$) and represents the range that results in an acceptable $Z_{NIST}$ score, $|Z_{NIST}| \leq 2$. The dotted blue box represents the consensus range of tolerance for SRM 3280 (x-axis) and Infant Formula 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 1-3.** Data summary table for iodine in 3280 Multivitamin/Multielement Tablets and Infant Formula A. Data points highlighted in blue have been identified as outside the consensus tolerance limits and would be estimated to result in an unacceptable $Z'_{comm}$ score, $|Z'_{comm}| \geq 2$.

| | | Iodine | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | SRM 3280 Multivitamin/Multielement Tablets (mg/kg) | | | | | Infant Formula A (mg/kg) | | | | |
| | Lab | A | B | C | Avg | SD | A | B | C | Avg | SD |
| Individual Results | Target | | | | 130.9 | 6.5 | | | | 2.10 | 0.08 |
| | F004 | | | | | | | | | | |
| | F005 | 112.75 | 115.3 | 113.59 | 113.9 | 1.30 | 3.55 | 3.56 | 3.58 | 3.56 | 0.015 |
| | F017 | 155 | 163 | 160 | 159.3 | 4.04 | 2.52 | 2.63 | 2.53 | 2.56 | 0.061 |
| | F021 | | | | | | | | | | |
| | F026 | 113.671 | 125.173 | 119.691 | 119.5 | 5.75 | 1.872 | 1.95 | 1.9 | 1.91 | 0.040 |
| | F031 | 129.56 | 128.43 | 122.86 | 127.0 | 3.59 | 1.62 | 1.55 | 1.55 | 1.57 | 0.040 |
| | F033 | 154 | 148 | 147 | 149.7 | 3.79 | 2.26 | 2.29 | 2.32 | 2.29 | 0.030 |
| | F034 | 146 | 132 | 120 | 132.7 | 13.0 | 1.42 | 1.45 | | 1.44 | 0.021 |
| | F035 | | | | | | | | | | |
| | F042 | | | | | | | | | | |
| | F056 | 105 | 96.6 | 107 | 102.9 | 5.52 | | | | | |
| | F061 | 111 | 118 | 115 | 114.7 | 3.51 | | | | | |
| | F062 | 95.215 | 98.44 | 105.055 | 99.6 | 5.02 | 1.575 | 1.61 | 1.655 | 1.61 | 0.040 |
| | F067 | 109.1 | 108.4 | 112.7 | 110.1 | 2.31 | 1.84 | 1.94 | 2.02 | 1.93 | 0.090 |
| | F070 | 197.75 | 73.38 | 95.61 | 122.2 | 66.3 | 13.52 | 10.3 | 6.61 | 10.14 | 3.46 |
| | F073 | | | | | | | | | | |
| | F077 | | | | | | | | | | |
| | F088 | | | | | | | | | | |
| Community Results | | Consensus Mean | | | 122.6 | | Consensus Mean | | | 2.08 | |
| | | Consensus Standard Deviation | | | 22.6 | | Consensus Standard Deviation | | | 0.84 | |
| | | Maximum | | | 159.3 | | Maximum | | | 10.14 | |
| | | Minimum | | | 99.6 | | Minimum | | | 1.44 | |
| | | N | | | 11 | | N | | | 9 | |

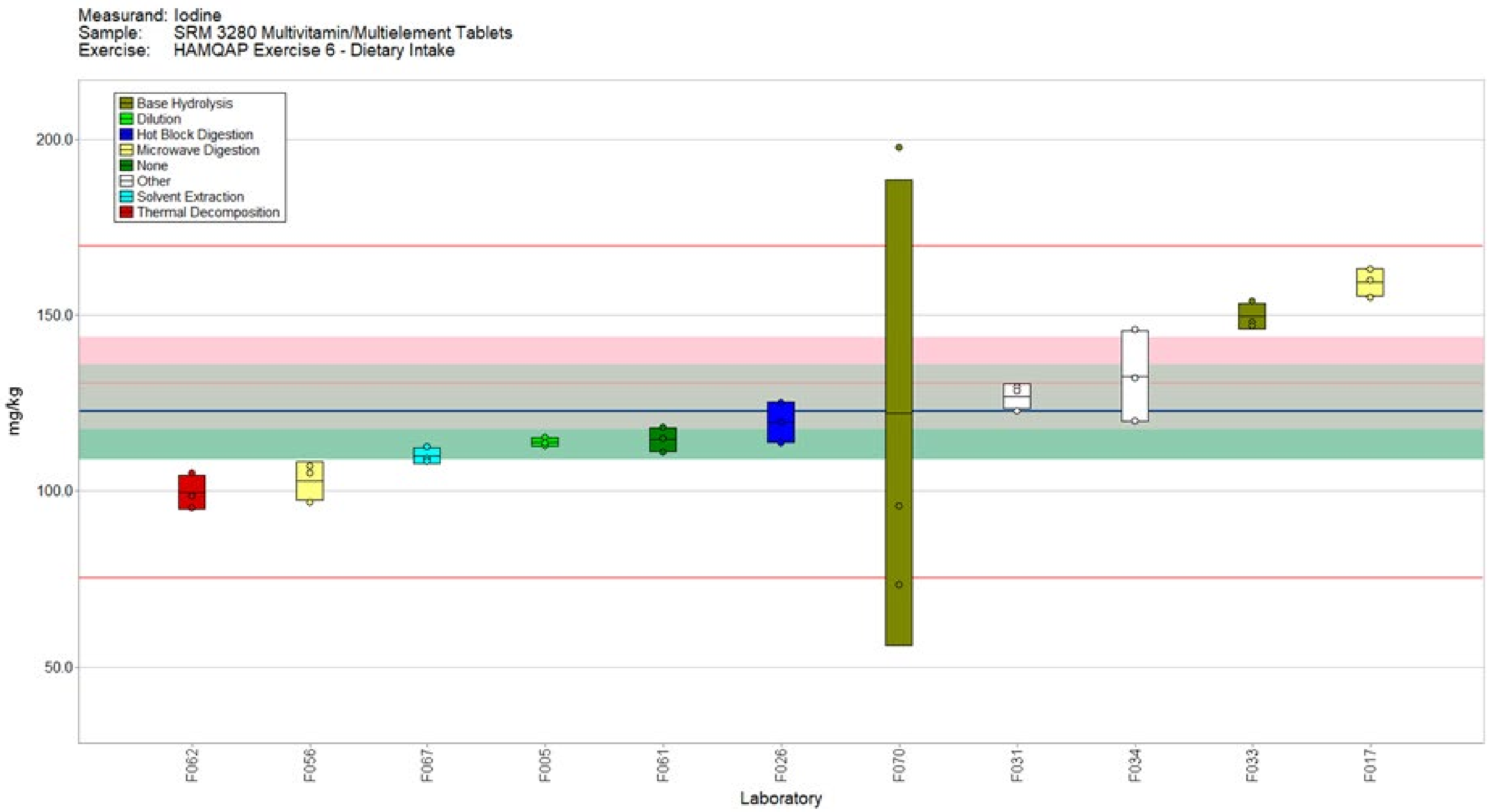


**Figure 1-6.** Iodine in SRM 3280 Multivitamin/Multielement Tablets (data summary view – sample preparation method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The solid blue 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 $Z'_{\text{comm}}$ score, $|Z'_{\text{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty ($U_{\text{NIST}}$) and represents the range that results in an acceptable $Z_{\text{NIST}}$ score, $|Z_{\text{NIST}}| \leq 2$.

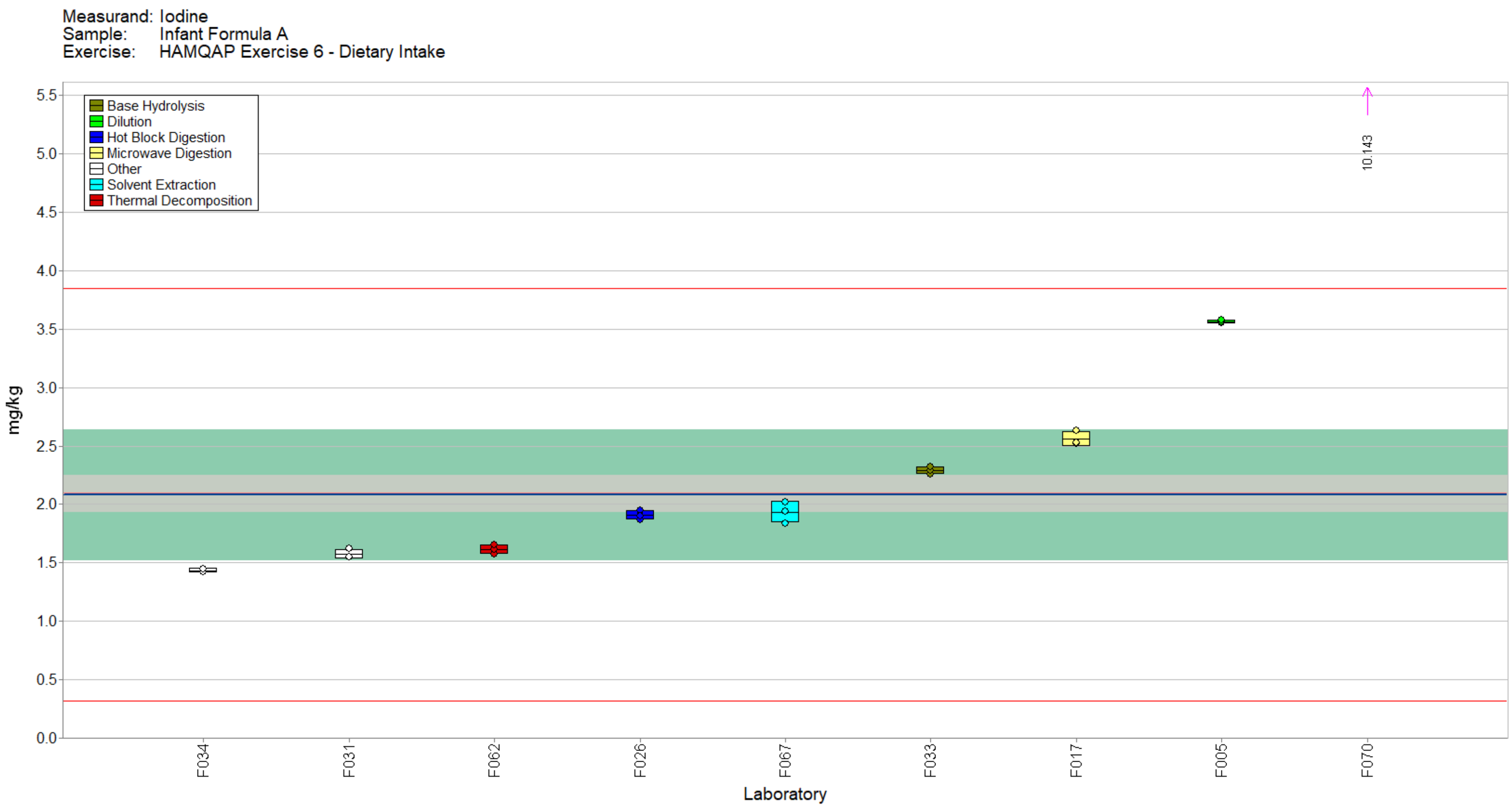


**Figure 1-7.** Iodine in Infant Formula A (data summary view – sample preparation method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The solid blue 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 $Z'_{\mathrm{comm}}$ score, $|Z'_{\mathrm{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty ($U_{\mathrm{NIST}}$) and represents the range that results in an acceptable $Z_{\mathrm{NIST}}$ score, $|Z_{\mathrm{NIST}}| \leq 2$.

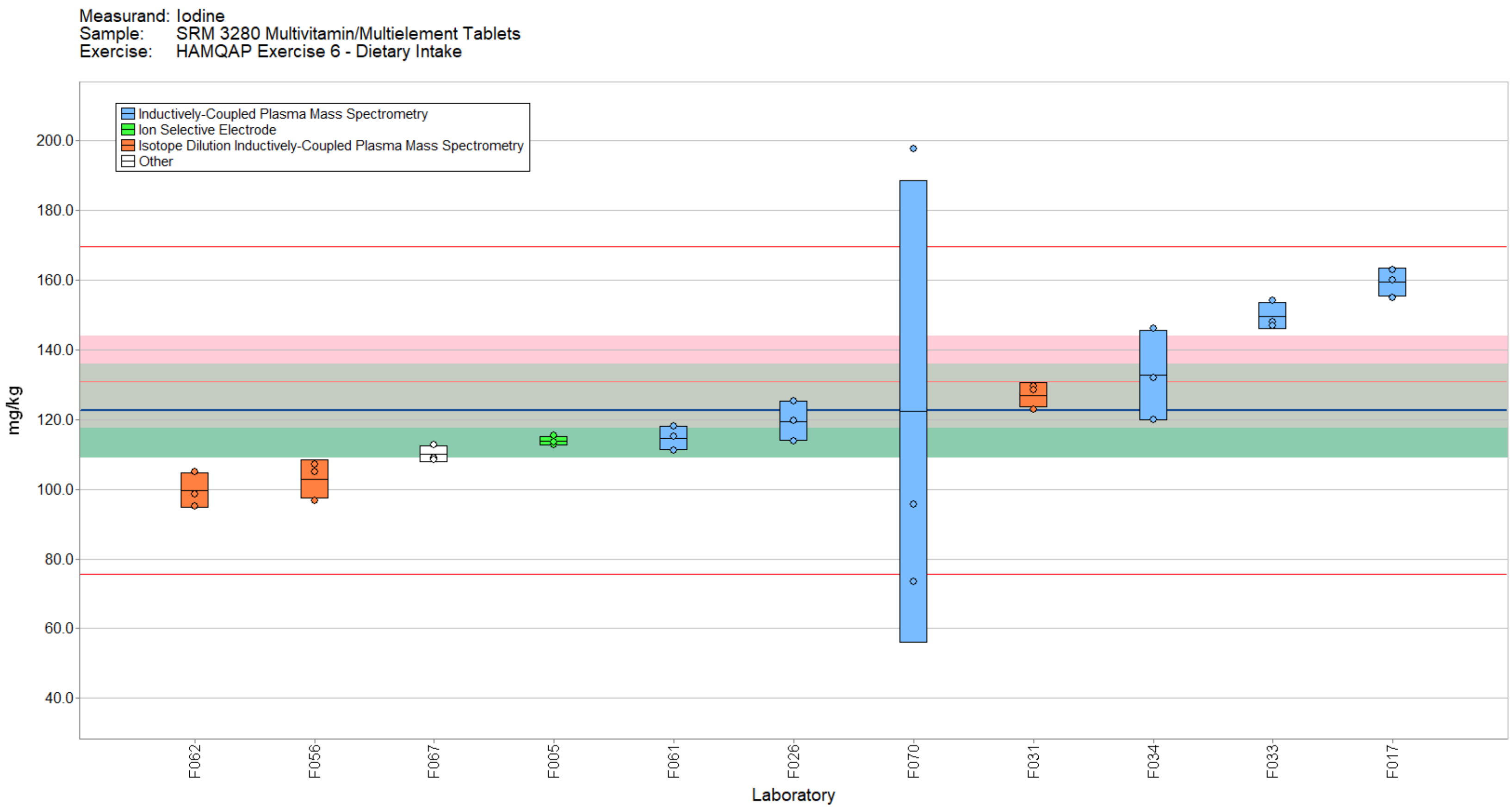


**Figure 1-8.** Iodine in SRM 3280 Multivitamin/Multielement Tablets (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue 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 $Z'_{\mathrm{comm}}$ score, $|Z'_{\mathrm{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty ($U_{\mathrm{NIST}}$) and represents the range that results in an acceptable $Z_{\mathrm{NIST}}$ score, $|Z_{\mathrm{NIST}}| \leq 2$.

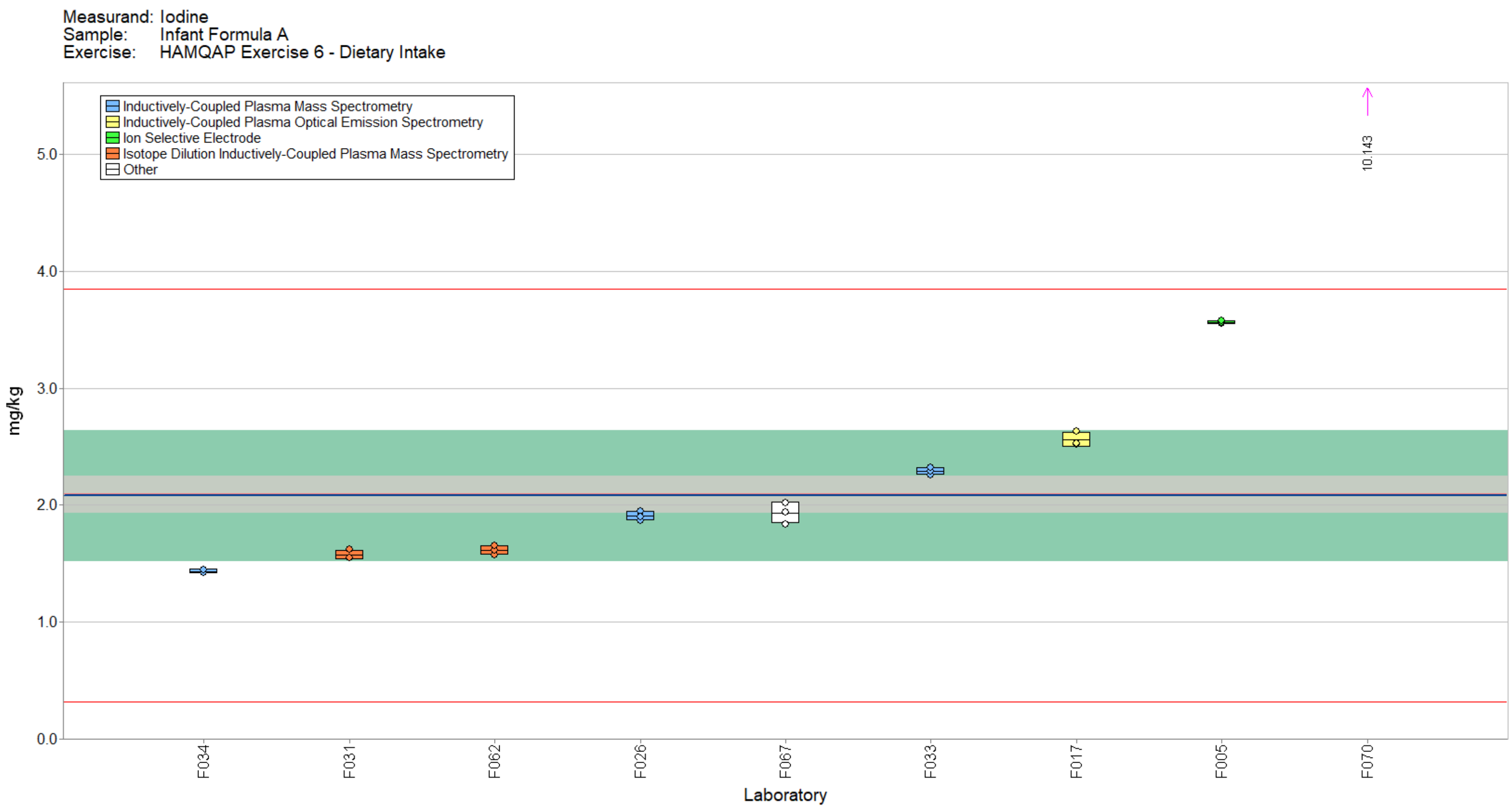


**Figure 1-9.** Iodine in Infant Formula A (data summary view –analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line represents 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 $Z'_{\mathrm{comm}}$ score, $|Z'_{\mathrm{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty ($U_{\mathrm{NIST}}$) and represents the range that results in an acceptable $Z_{\mathrm{NIST}}$ score, $|Z_{\mathrm{NIST}}| \leq 2$.

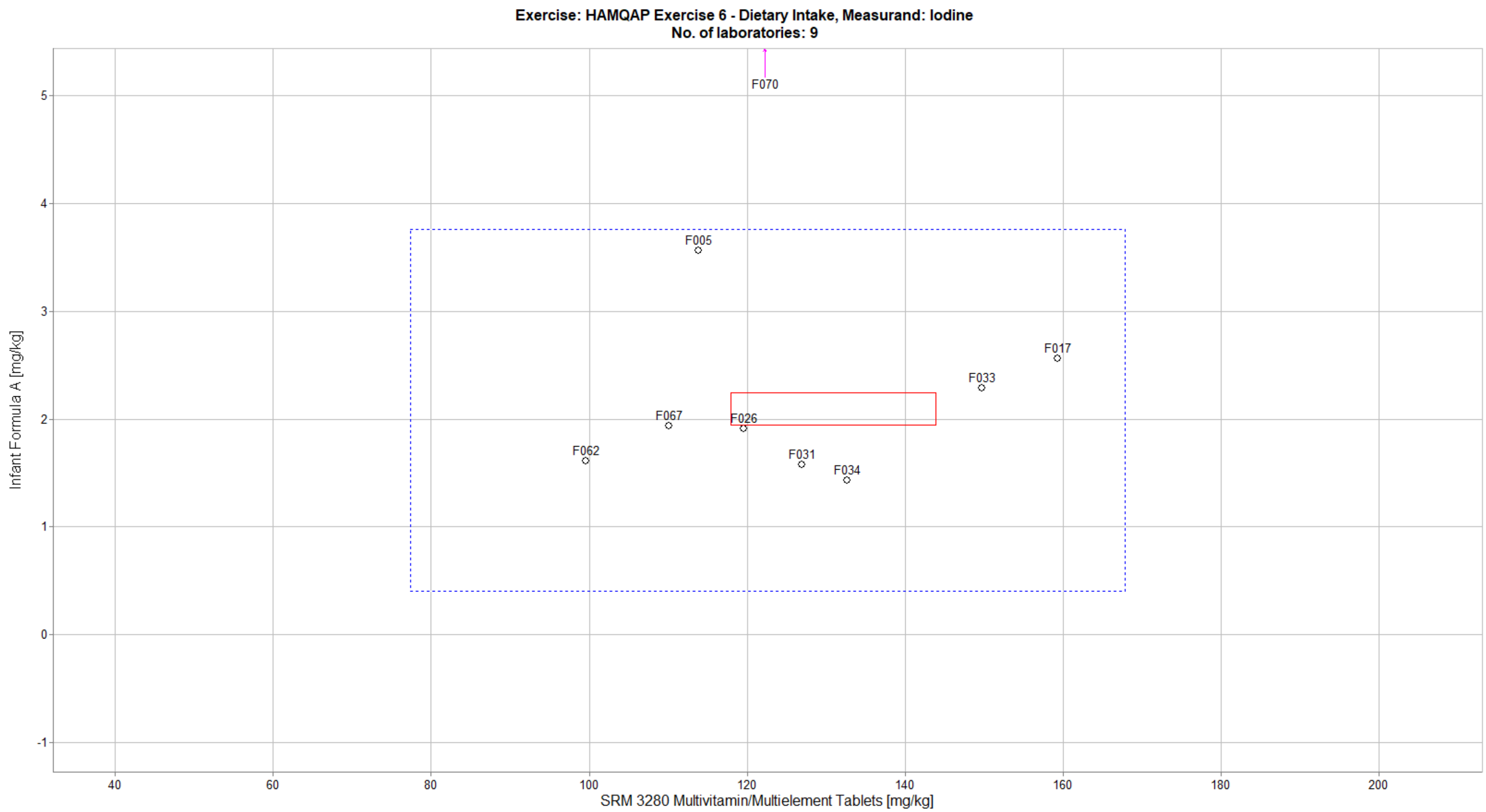


**Figure 1-10.** Laboratory means for iodine in SRM 3280 Multivitamin/Multielement Tablets and Infant Formula A (sample/sample comparison view). In this view, the individual laboratory mean for one sample (SRM 3280) is compared to the individual laboratory mean for a second sample (Infant Formula A). The solid red box represents the NIST range of tolerance for the two samples, SRM 3280 (x-axis) and Infant Formula A (y-axis), which encompasses the target values bounded by their uncertainties ($U_{NIST}$) and represents the range that results in an acceptable $Z_{NIST}$ score, $|Z_{NIST}| \leq 2$. The dotted blue box represents the consensus range of tolerance for SRM 3280 (x-axis) and Infant Formula 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 1-4.** Data summary table for chromium in 3280 Multivitamin/Multielement Tablets and Infant Formula A. Data points highlighted in blue have been identified as outside the consensus tolerance limits and would be estimated to result in an unacceptable $Z'_{comm}$ score, $|Z'_{comm}| \geq 2$.

| | | Chromium | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | SRM 3280 Multivitamin/Multielement Tablets (mg/kg) | | | | | Infant Formula A (mg/kg) | | | | |
| | Lab | A | B | C | Avg | SD | A | B | C | Avg | SD |
| Individual Results | Target | | | | 92.4 | 2.7 | | | | 1.04 | 0.05 |
| | F004 | | | | | | | | | | |
| | F005 | 73.449 | 65.396 | 66.139 | 68.33 | 4.45 | 0.62 | 0.629 | 0.582 | 0.61 | 0.02 |
| | F011 | 104.72 | 93.36 | 91 | 96.36 | 7.34 | 1.04 | 1.06 | 1.06 | 1.05 | 0.01 |
| | F014 | | | | | | | | | | |
| | F017 | 90.1 | 86.4 | 88.7 | 88.40 | 1.87 | 1.08 | 1.07 | 0.994 | 1.05 | 0.05 |
| | F018 | | | | | | | | | | |
| | F020 | 99.08 | 106.2 | 87.99 | 97.76 | 9.18 | 1.05 | 1.01 | 1 | 1.02 | 0.03 |
| | F021 | | | | | | | | | | |
| | F022 | 102.41 | 106.32 | 103.85 | 104.2 | 1.98 | 1.1 | 1.18 | 1.06 | 1.11 | 0.06 |
| | F026 | 106.344 | 103.488 | 107.71 | 105.8 | 2.15 | 1.033 | 1.015 | 1.003 | 1.02 | 0.02 |
| | F030 | 105 | 106 | 104 | 105.0 | 1.00 | 1.05 | 1.07 | 1.1 | 1.07 | 0.03 |
| | F031 | 86.58 | 92.09 | 91.87 | 90.18 | 3.12 | 1.08 | 1.02 | 1.02 | 1.04 | 0.03 |
| | F032 | | | | | | | | | | |
| | F033 | 92.9 | 93.6 | 93.2 | 93.23 | 0.35 | 1.04 | 1.03 | 1.05 | 1.04 | 0.01 |
| | F034 | 94.4 | 98.3 | 94 | 95.57 | 2.38 | 1.019 | 1.015 | 1.008 | 1.01 | 0.01 |
| | F035 | | | | | | | | | | |
| | F039 | 93.5 | 91.1 | 87.9 | 90.83 | 2.81 | 1.06 | 1.09 | 1.04 | 1.06 | 0.03 |
| | F041 | 90.9 | 81.9 | 93.1 | 88.63 | 5.93 | 0.96 | 0.93 | 0.97 | 0.95 | 0.02 |
| | F042 | 111 | 104 | 97.6 | 104.2 | 6.70 | 0.933 | 0.974 | 0.97 | 0.96 | 0.02 |
| | F046 | 97.8 | 114 | 99.2 | 103.7 | 8.98 | 1.007 | 1.083 | 1.116 | 1.07 | 0.06 |
| | F051 | | | | | | | | | | |
| | F056 | 94.7 | 97.5 | 87.4 | 93.20 | 5.21 | | | | | |
| | F060 | 100.596 | 128.147 | 99.269 | 109.3 | 16.3 | | | | | |
| | F061 | 31.87 | 34.13 | 34.49 | 33.50 | 1.42 | 80.5 | 80.6 | 83.5 | 81.5 | 1.70 |
| | F062 | 96.73 | 93.468 | 94.95 | 95.05 | 1.63 | 0.967 | 0.945 | 0.942 | 0.95 | 0.01 |
| | F067 | | | | | | | | | | |
| | F069 | 92 | 96.3 | 88.7 | 92.33 | 3.81 | 1.5 | 1.5 | 1.5 | 1.50 | 0.00 |
| | F070 | 147.19 | 188.09 | 193.34 | 176.2 | 25.3 | 1.07 | 1 | 1.02 | 1.03 | 0.04 |
| | F073 | 178 | 165 | 175 | 172.7 | 6.81 | | | | | |
| | F074 | 99.63 | 94.45 | 95.24 | 96.44 | 2.79 | 1.058 | 0.715 | 0.633 | 0.80 | 0.23 |
| | F077 | | | | | | | | | | |
| | F079 | 92 | 91.1 | 80.9 | 88.00 | 6.17 | 1.04 | 1.08 | 1.03 | 1.05 | 0.03 |
| | F085 | 107.17 | 96.411 | 95.154 | 99.58 | 6.60 | 1.004 | 1.03 | 1.04 | 1.02 | 0.02 |
| | F088 | | | | | | | | | | |
| | F089 | | | | | | | | | | |
| Community Results | | Consensus Mean | | | 96.05 | | Consensus Mean | | | 1.02 | |
| | | Consensus Standard Deviation | | | 11.25 | | Consensus Standard Deviation | | | 0.08 | |
| | | Maximum | | | 176.21 | | Maximum | | | 81.53 | |
| | | Minimum | | | 33.50 | | Minimum | | | 0.61 | |
| | | N | | | 24 | | N | | | 21 | |

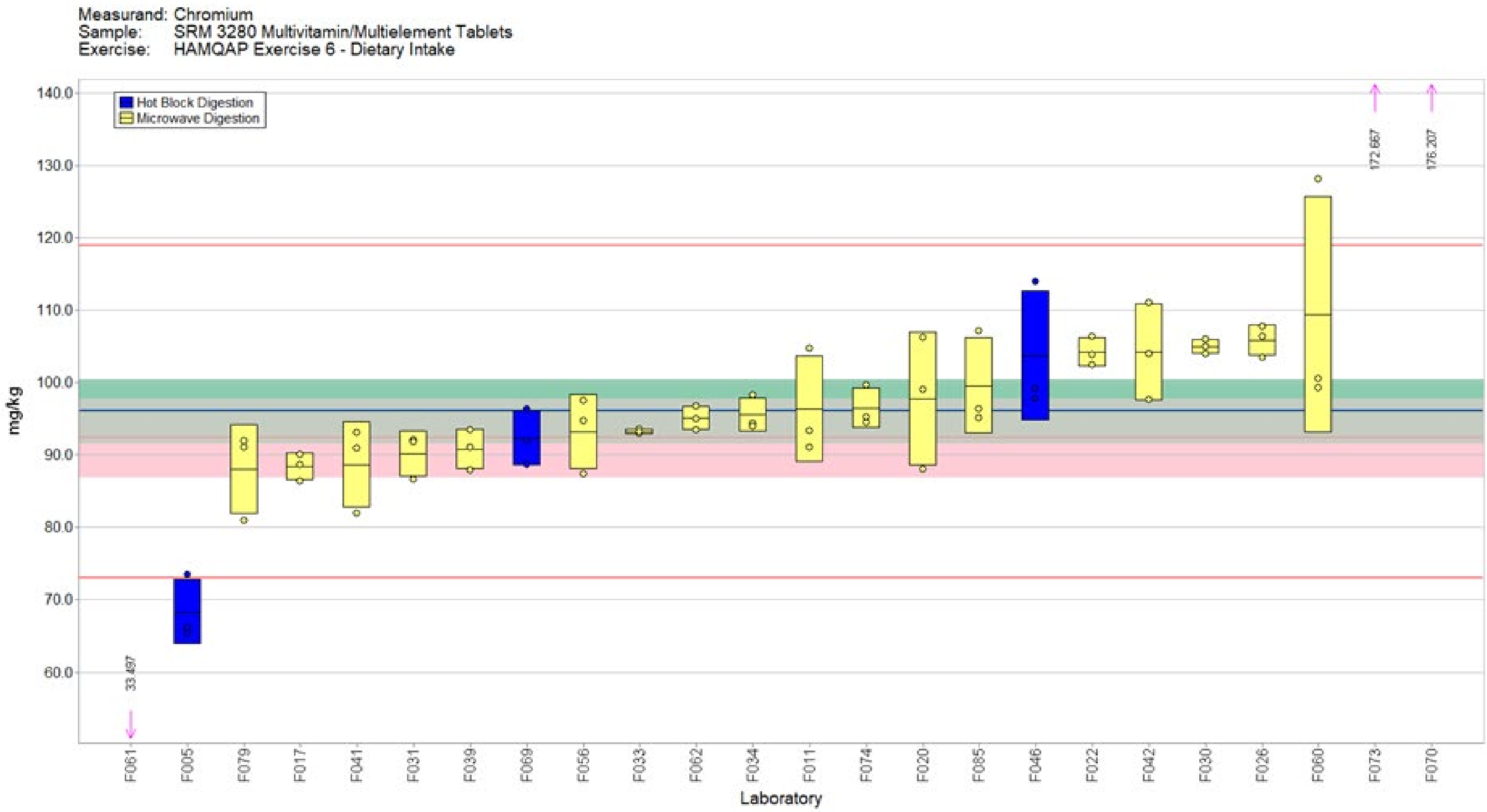


**Figure 1-11.** Chromium in SRM 3280 Multivitamin/Multielement Tablets (data summary view – sample preparation method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The solid blue 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 $Z'_{\text{comm}}$ score, $|Z'_{\text{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty ($U_{\text{NIST}}$) and represents the range that results in an acceptable $Z_{\text{NIST}}$ score, $|Z_{\text{NIST}}| \leq 2$.

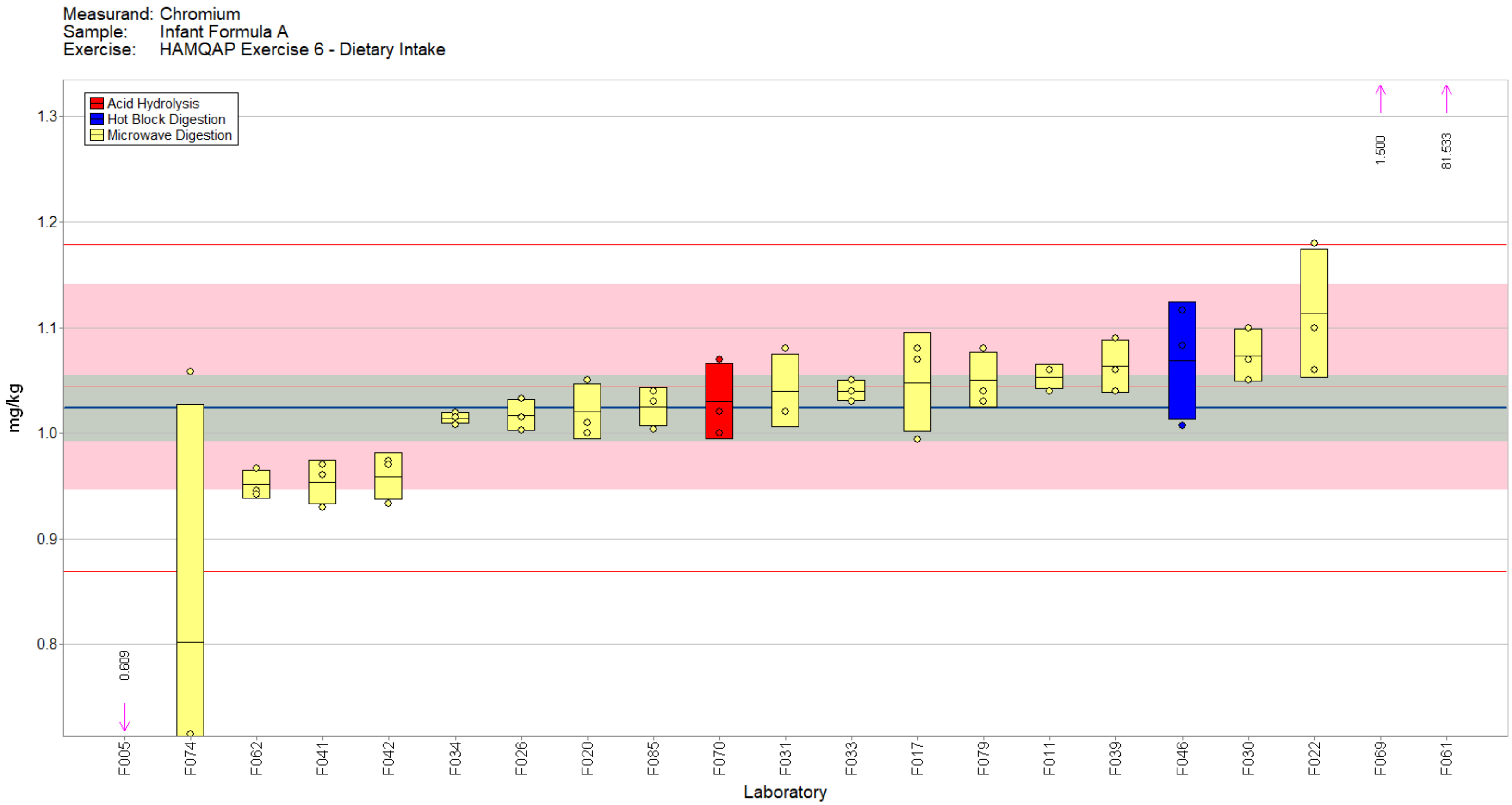


**Figure 1-12.** Chromium in Infant Formula A (data summary view – sample preparation method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The solid blue 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 $Z'_{\text{comm}}$ score, $|Z'_{\text{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty ($U_{\text{NIST}}$) and represents the range that results in an acceptable $Z_{\text{NIST}}$ score, $|Z_{\text{NIST}}| \leq 2$.

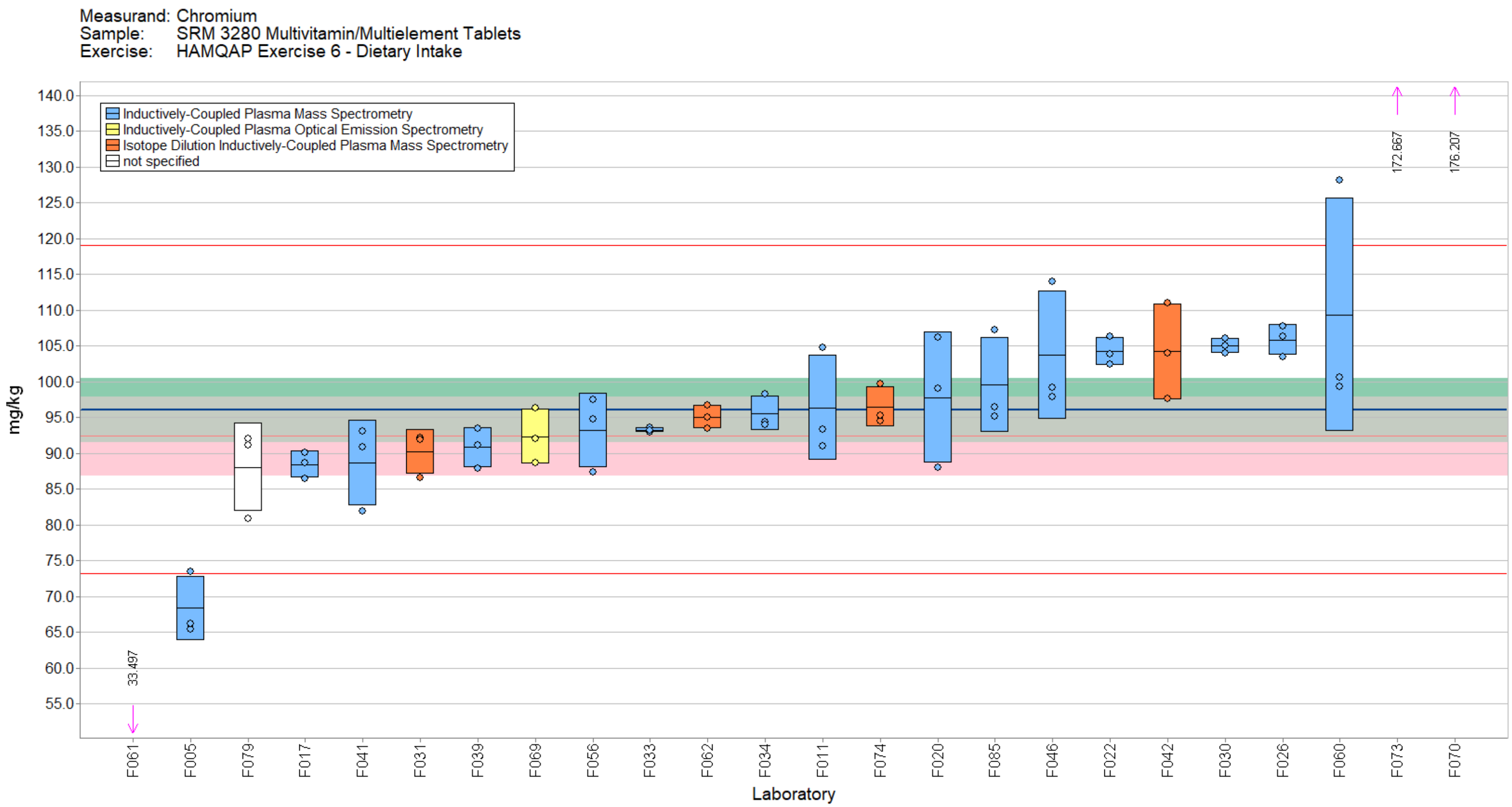


**Figure 1-13.** Chromium in SRM 3280 Multivitamin/Multielement Tablets (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue 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 $Z'_{\text{comm}}$ score, $|Z'_{\text{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty ($U_{\text{NIST}}$) and represents the range that results in an acceptable $Z_{\text{NIST}}$ score, $|Z_{\text{NIST}}| \leq 2$.

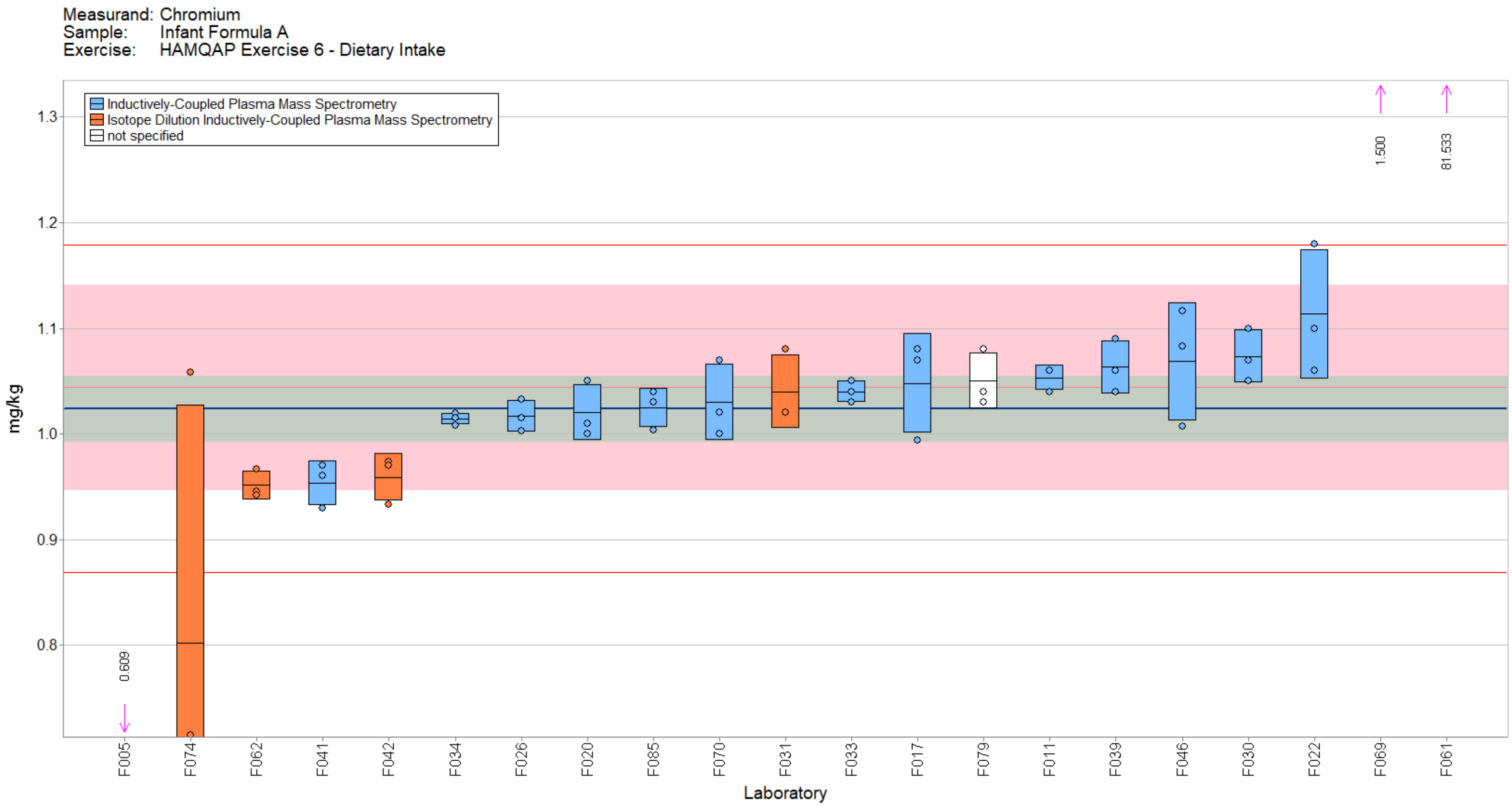


**Figure 1-14.** Chromium in Infant Formula A (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line 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 $Z'_{\text{comm}}$ score, $|Z'_{\text{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty ($U_{\text{NIST}}$) and represents the range that results in an acceptable $Z_{\text{NIST}}$ score, $|Z_{\text{NIST}}| \leq 2$.

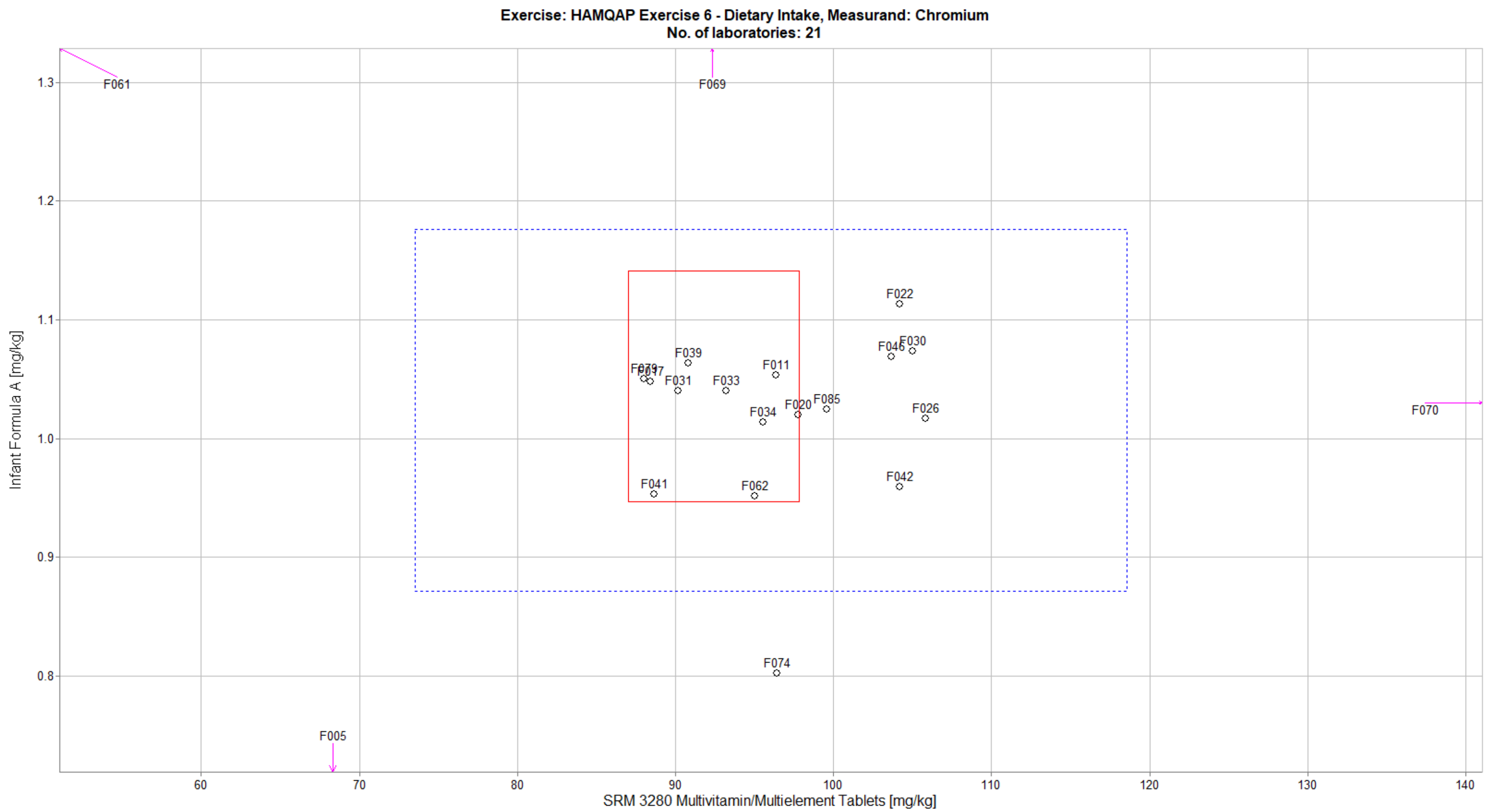


**Figure 1-15.** Laboratory means for chromium in SRM 3280 Multivitamin/Multielement Tablets and Infant Formula A (sample/sample comparison view). In this view, the individual laboratory mean for one sample (SRM 3280) is compared to the individual laboratory mean for a second sample (Infant Formula A). The solid red box represents the NIST range of tolerance for the two samples, SRM 3280 (x-axis) and Infant Formula A (y-axis), which encompasses the target values bounded by their uncertainties ($U_{\mathrm{NIST}}$) and represents the range that results in an acceptable $Z_{\mathrm{NIST}}$ score, $|Z_{\mathrm{NIST}}| \leq 2$. The dotted blue box represents the consensus range of tolerance for SRM 3280(x-axis) and Infant Formula A (y-axis), calculated as the values above and below the consensus means that result in an acceptable $Z'_{\mathrm{comm}}$ score, $|Z'_{\mathrm{comm}}| \leq 2$.

**Table 1-5.** Data summary table for molybdenum in 3280 Multivitamin/Multielement Tablets and Infant Formula A. Data points highlighted in blue have been identified as outside the consensus tolerance limits and would be estimated to result in an unacceptable $Z'_{\mathrm{comm}}$ score, $|Z'_{\mathrm{comm}}| \geq 2$.

| | | Molybdenum | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | SRM 3280 Multivitamin/Multielement Tablets (mg/kg) | | | | | Infant Formula A (mg/kg) | | | | |
| | Lab | A | B | C | Avg | SD | A | B | C | Avg | SD |
| Individual Results | Target | | | | 69.7 | 4.4 | | | | 1.80 | 0.07 |
| | F004 | | | | | | | | | | |
| | F005 | 70.022 | 77.326 | 84.668 | 77.34 | 7.32 | 5.265 | 4.673 | 4.445 | 4.79 | 0.42 |
| | F011 | 77.38 | 65.51 | 64.63 | 69.17 | 7.12 | 1.54 | 1.52 | 1.5 | 1.52 | 0.02 |
| | F014 | | | | | | | | | | |
| | F017 | 75.6 | 89 | 76 | 80.20 | 7.62 | 1.77 | 1.87 | 1.82 | 1.82 | 0.05 |
| | F018 | | | | | | | | | | |
| | F020 | 50.405 | 48.85 | 53.64 | 50.97 | 2.44 | 1.31 | 1.329 | 1.309 | 1.32 | 0.01 |
| | F021 | | | | | | | | | | |
| | F026 | 59.453 | 59.383 | 67.83 | 62.22 | 4.86 | 1.506 | 1.53 | 1.388 | 1.47 | 0.08 |
| | F030 | 79.9 | 83.7 | 85.7 | 83.10 | 2.95 | 1.92 | 1.96 | 1.93 | 1.94 | 0.02 |
| | F031 | 69.85 | 73.7 | 67.91 | 70.49 | 2.95 | 1.72 | 1.79 | 1.73 | 1.75 | 0.04 |
| | F032 | | | | | | | | | | |
| | F033 | 71.4 | 71.5 | 70.8 | 71.23 | 0.38 | 1.78 | 1.76 | 1.8 | 1.78 | 0.02 |
| | F034 | 60 | 58.9 | 58.9 | 59.27 | 0.64 | 1.727 | 1.71 | 1.721 | 1.72 | 0.01 |
| | F035 | | | | | | | | | | |
| | F039 | 72.7 | 86.5 | 73.5 | 77.57 | 7.75 | 1.82 | 1.85 | 1.78 | 1.82 | 0.04 |
| | F042 | 69.5 | 75.6 | 79.6 | 74.90 | 5.09 | 1.67 | 1.71 | 1.67 | 1.68 | 0.02 |
| | F046 | 88.1 | 75.5 | 75.1 | 79.57 | 7.39 | 2.774 | 2.135 | 2.014 | 2.31 | 0.41 |
| | F051 | | | | | | | | | | |
| | F056 | 74.4 | 76.9 | 71.5 | 74.27 | 2.70 | | | | | |
| | F060 | 84.362 | 77.958 | 62.419 | 74.91 | 11.3 | | | | | |
| | F061 | 42.78 | 46.82 | 47.67 | 45.76 | 2.61 | 1.419 | 1.43 | 1.47 | 1.44 | 0.03 |
| | F062 | 80.815 | 77.048 | 75.075 | 77.65 | 2.92 | 1.695 | 1.719 | 1.706 | 1.71 | 0.01 |
| | F067 | | | | | | | | | | |
| | F069 | 79.2 | 62.7 | 76.9 | 72.93 | 8.94 | 0.5 | 0.5 | 0.5 | 0.50 | 0 |
| | F070 | 115.91 | 82.11 | 54.52 | 84.18 | 30.7 | 1.7 | 1.71 | 1.67 | 1.69 | 0.02 |
| | F073 | | | | | | 1.679 | 1.673 | 1.656 | 1.67 | 0.01 |
| | F074 | 63.7 | 63.57 | 58.26 | 61.84 | 3.10 | 1.857 | 1.738 | 1.529 | 1.71 | 0.17 |
| | F077 | | | | | | | | | | |
| | F079 | 80.8 | 65.1 | 80.2 | 75.37 | 8.90 | 1.8 | 1.75 | 1.74 | 1.76 | 0.03 |
| | F085 | 79.624 | 68.029 | 69.487 | 72.38 | 6.32 | 1.619 | 1.671 | 1.7 | 1.66 | 0.04 |
| | F089 | | | | | | | | | | |
| Community Results | | Consensus Mean | | | 71.79 | | Consensus Mean | | | 71.79 | |
| | | Consensus Standard Deviation | | | 11.55 | | Consensus Standard Deviation | | | 11.55 | |
| | | Maximum | | | 84.18 | | Maximum | | | 84.18 | |
| | | Minimum | | | 45.76 | | Minimum | | | 45.76 | |
| | | N | | | 21 | | N | | | 21 | |

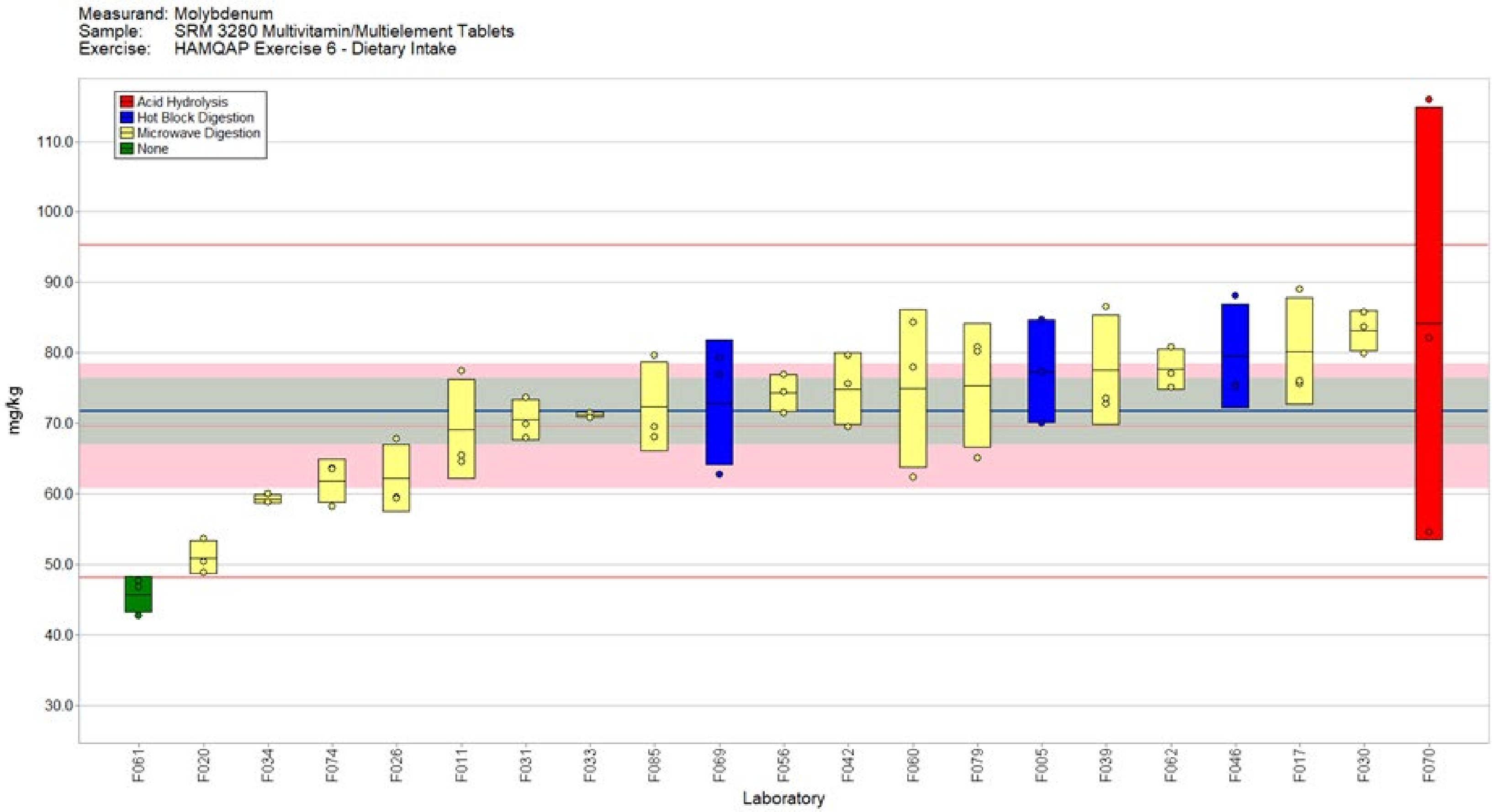


**Figure 1-16.** Molybdenum in SRM 3280 Multivitamin/Multielement Tablets (data summary view – sample preparation method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The solid blue 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 $Z'_{\mathrm{comm}}$ score, $|Z'_{\mathrm{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty ($U_{\mathrm{NIST}}$) and represents the range that results in an acceptable $Z_{\mathrm{NIST}}$ score, $|Z_{\mathrm{NIST}}| \leq 2$.

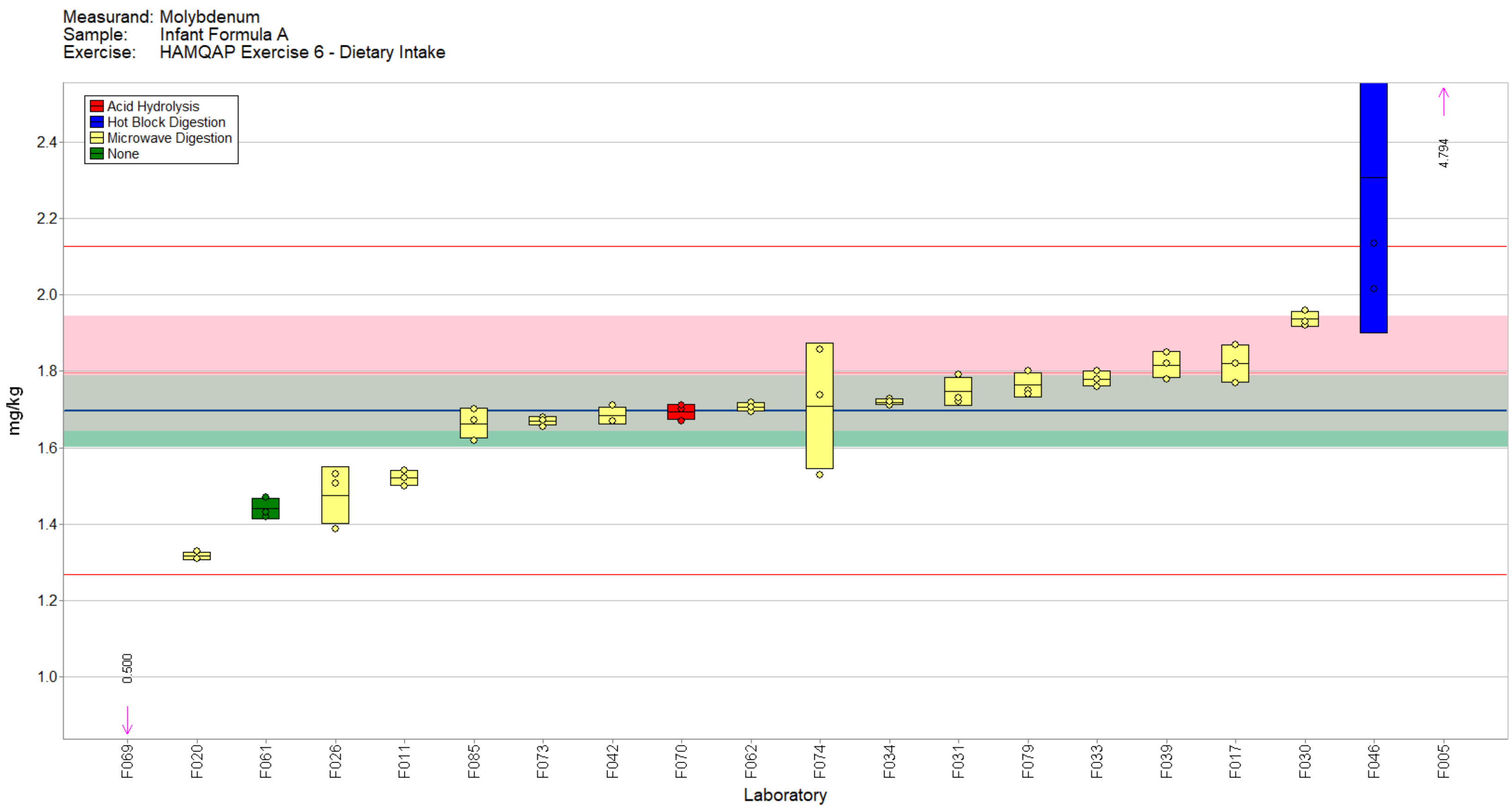


**Figure 1-17.** Molybdenum in Infant Formula A (data summary view – sample preparation method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The solid blue 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 $Z'_{\text{comm}}$ score, $|Z'_{\text{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty ($U_{\text{NIST}}$) and represents the range that results in an acceptable $Z_{\text{NIST}}$ score, $|Z_{\text{NIST}}| \leq 2$.

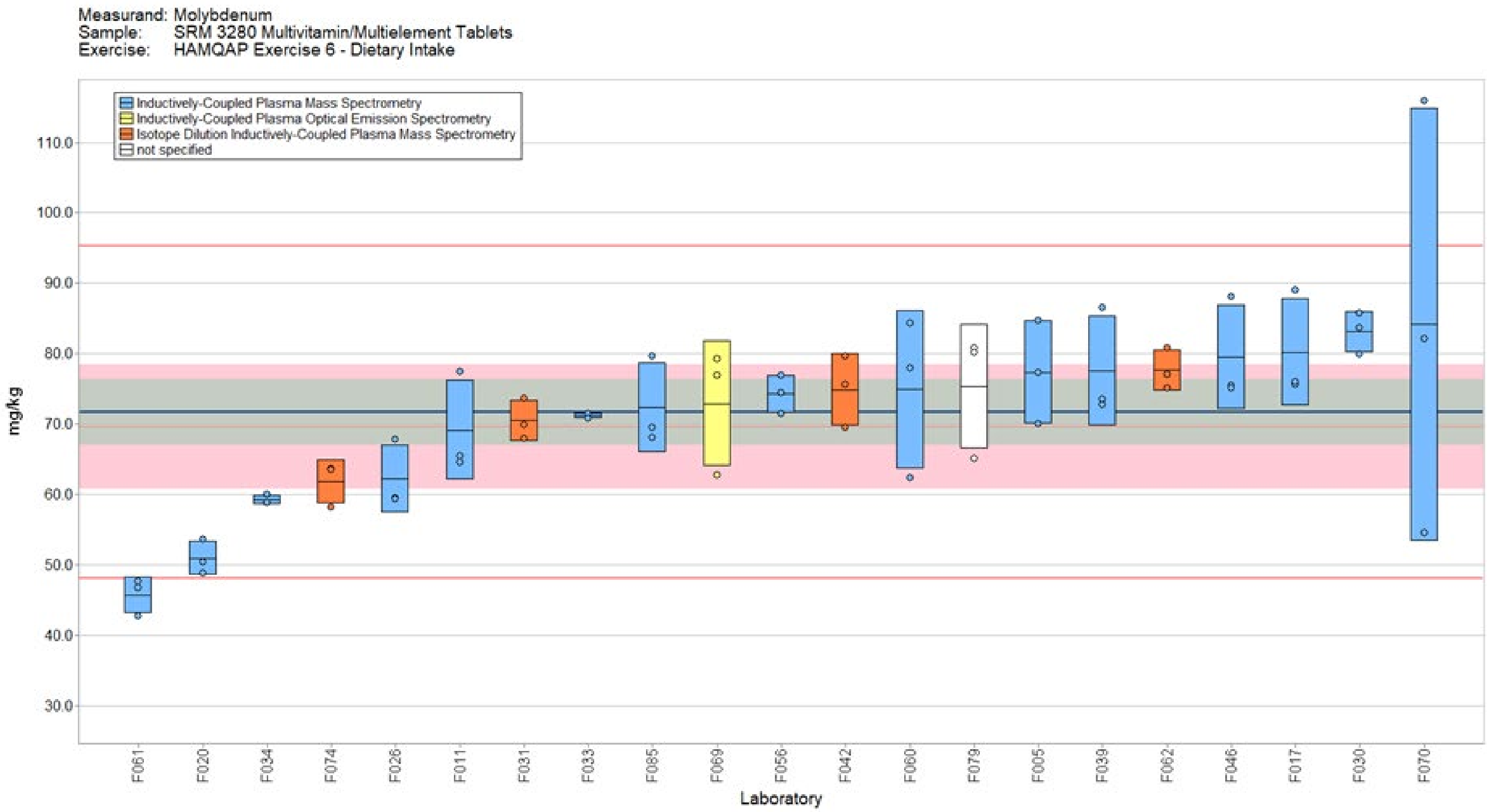


**Figure 1-18.** Molybdenum in SRM 3280 Multivitamin/Multielement Tablets (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue 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 $Z'_{\text{comm}}$ score, $|Z'_{\text{comm}}| \leq 2$. 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 $Z'_{\text{comm}}$ score, $|Z'_{\text{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty ($U_{\text{NIST}}$) and represents the range that results in an acceptable $Z_{\text{NIST}}$ score, $|Z_{\text{NIST}}| \leq 2$.

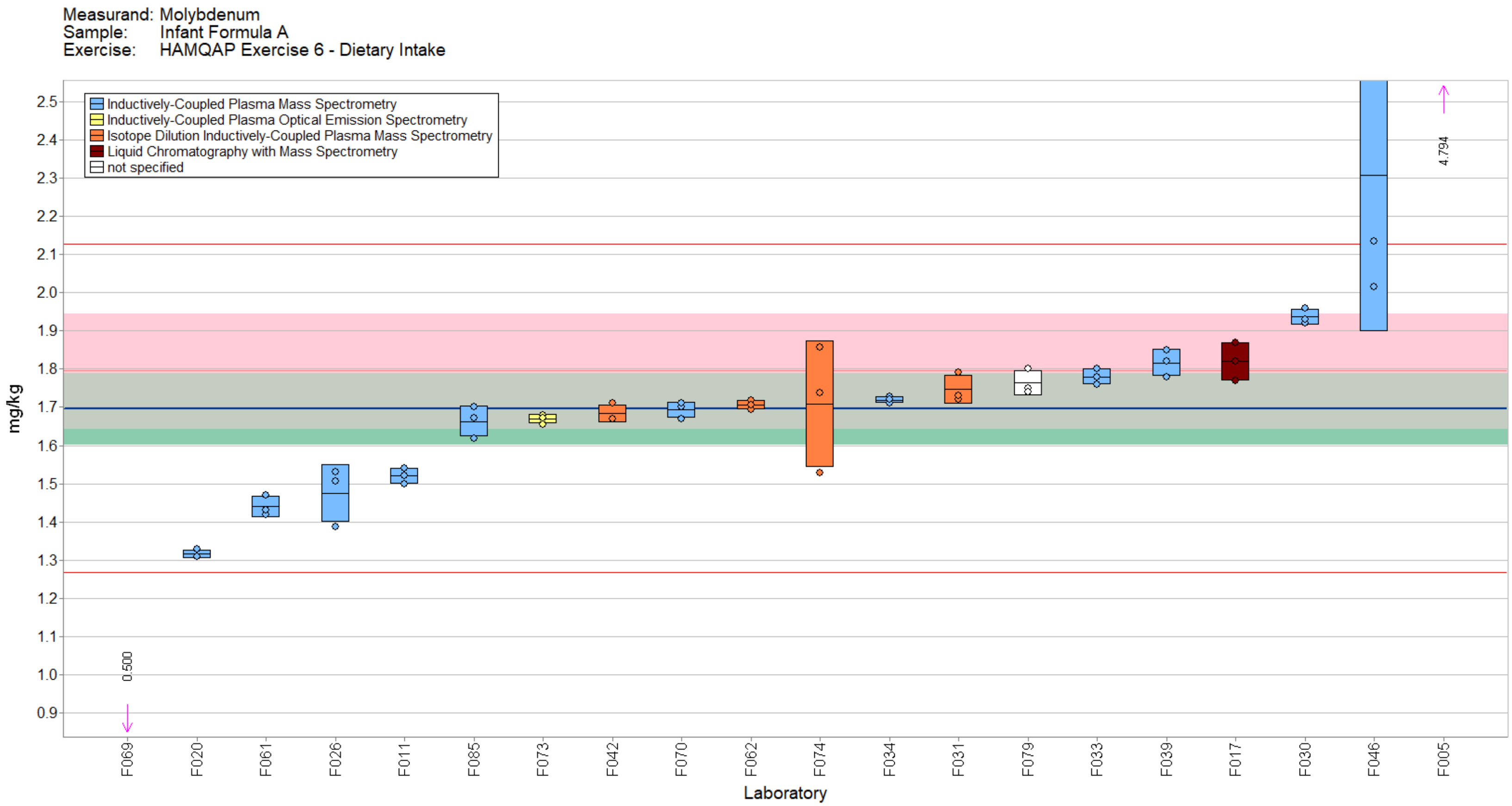


**Figure 1-19.** Molybdenum in Infant Formula A (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue 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 $Z'_{\text{comm}}$ score, $|Z'_{\text{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty ($U_{\text{NIST}}$) and represents the range that results in an acceptable $Z_{\text{NIST}}$ score, $|Z_{\text{NIST}}| \leq 2$.

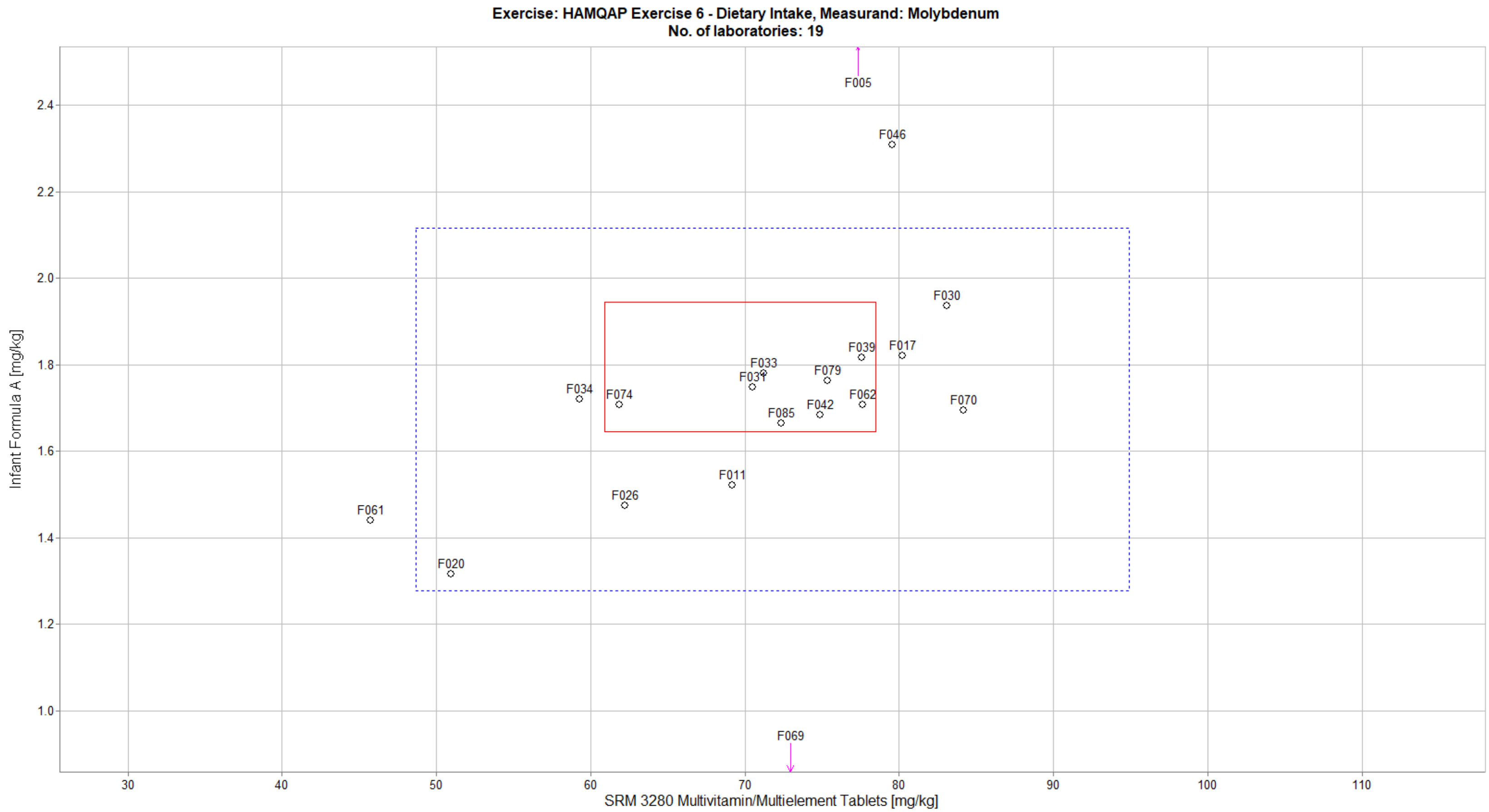


**Figure 1-20.** Laboratory means for molybdenum in SRM 3280 Multivitamin/Multielement Tablets and Infant Formula A (sample/sample comparison view). In this view, the individual laboratory mean for one sample (SRM 3280) is compared to the individual laboratory mean for a second sample (Infant Formula A). The solid red box represents the NIST range of tolerance for the two samples, SRM 3280 (x-axis) and Infant Formula 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 3280 (x-axis) and Infant Formula A (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 1-6.** Data summary table for selenium in 3280 Multivitamin/Multielement Tablets and Infant Formula A. Data points highlighted in blue have been identified as outside the consensus tolerance limits and would be estimated to result in an unacceptable $Z'_{comm}$ score, $|Z'_{comm}| \geq 2$.

| | | Selenium | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | SRM 3280 Multivitamin/Multielement Tablets (mg/kg) | | | | | Infant Formula A (mg/kg) | | | | |
| | Lab | A | B | C | Avg | SD | A | B | C | Avg | SD |
| Individual Results | Target | | | | 17.18 | 0.44 | | | | 0.84 | 0.04 |
| | F004 | | | | | | | | | | |
| | F005 | 13.666 | 11.878 | 13.792 | 13.11 | 1.07 | 0.984 | 1.077 | 0.964 | 1.01 | 0.06 |
| | F011 | 16.84 | 17.59 | 15.13 | 16.52 | 1.26 | 0.6 | 0.62 | 0.65 | 0.62 | 0.03 |
| | F014 | | | | | | | | | | |
| | F017 | 19.5 | 19.1 | 17.3 | 18.63 | 1.17 | 0.774 | 0.754 | 0.79 | 0.77 | 0.02 |
| | F018 | | | | | | | | | | |
| | F020 | 14.698 | 17.773 | 13.413 | 15.29 | 2.24 | 0.737 | 0.778 | 0.719 | 0.74 | 0.03 |
| | F021 | | | | | | | | | | |
| | F022 | 16.82 | 18.15 | 19.56 | 18.18 | 1.37 | 0.77 | 0.78 | 0.89 | 0.81 | 0.07 |
| | F026 | 16.379 | 17.542 | 13.662 | 15.86 | 1.99 | 0.828 | 0.84 | 0.81 | 0.83 | 0.02 |
| | F030 | 18.1 | 18.3 | 18.6 | 18.33 | 0.25 | 0.86 | 0.91 | 0.9 | 0.89 | 0.03 |
| | F031 | 16.31 | 16.76 | 17.61 | 16.89 | 0.66 | 0.84 | 0.92 | 0.8 | 0.85 | 0.06 |
| | F032 | | | | | | | | | | |
| | F033 | 15.6 | 15.7 | 15.8 | 15.70 | 0.10 | 0.77 | 0.766 | 0.779 | 0.77 | 0.01 |
| | F034 | 16.1 | 17.4 | 16.1 | 16.53 | 0.75 | 0.605 | 0.606 | 0.607 | 0.61 | 0.00 |
| | F035 | | | | | | | | | | |
| | F039 | 16.7 | 18 | 17.3 | 17.33 | 0.65 | 0.832 | 0.874 | 0.858 | 0.85 | 0.02 |
| | F042 | 16.3 | 17.3 | 16.3 | 16.63 | 0.58 | 0.831 | 0.783 | 0.791 | 0.80 | 0.03 |
| | F046 | 16.2 | 18.4 | 15.4 | 16.67 | 1.55 | 0.84 | 0.711 | 0.503 | 0.68 | 0.17 |
| | F051 | | | | | | | | | | |
| | F056 | 18.5 | 18.3 | 17.6 | 18.13 | 0.47 | | | | | |
| | F060 | | | | | | | | | | |
| | F061 | 16.01 | 16.46 | 16.59 | 16.35 | 0.30 | 0.864 | 0.869 | 0.872 | 0.87 | 0.00 |
| | F062 | 16.985 | 17.768 | 18.173 | 17.64 | 0.60 | 0.894 | 0.867 | 0.906 | 0.89 | 0.02 |
| | F067 | | | | | | | | | | |
| | F069 | 17.06 | 15.45 | 15.46 | 15.99 | 0.93 | 0.81 | 0.72 | 0.78 | 0.77 | 0.05 |
| | F070 | 13.45 | 16.32 | 19.35 | 16.37 | 2.95 | 0.82 | 0.69 | 0.98 | 0.83 | 0.15 |
| | F073 | | | | | | | | | | |
| | F074 | 20.8 | 19.8 | 18.7 | 19.77 | 1.05 | 1.86 | 1.391 | 1.487 | 1.58 | 0.25 |
| | F077 | | | | | | | | | | |
| | F079 | 15.1 | 13.2 | 14.5 | 14.27 | 0.97 | 0.82 | 0.7 | 0.68 | 0.73 | 0.08 |
| | F085 | 19.749 | 13.38 | 16.208 | 16.45 | 3.19 | 0.746 | 0.787 | 0.765 | 0.77 | 0.02 |
| | F088 | | | | | | | | | | |
| | F089 | | | | | | | | | | |
| Community Results | | Consensus Mean | | | 16.73 | | Consensus Mean | | | 0.79 | |
| | | Consensus Standard Deviation | | | 1.95 | | Consensus Standard Deviation | | | 0.12 | |
| | | Maximum | | | 19.77 | | Maximum | | | 1.58 | |
| | | Minimum | | | 13.11 | | Minimum | | | 0.61 | |
| | | N | | | 21 | | N | | | 20 | |

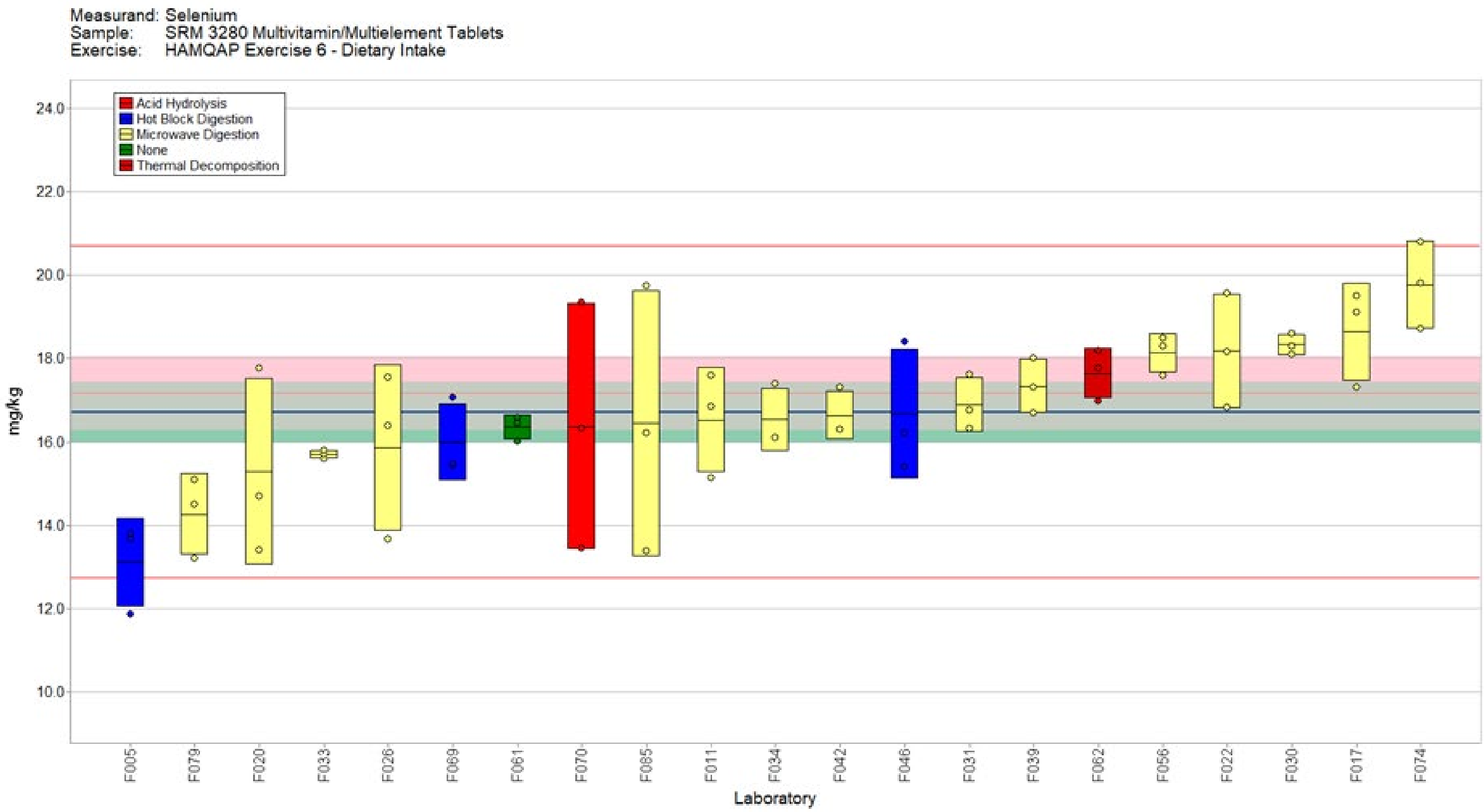


**Figure 1-21.** Selenium in SRM 3280 Multivitamin/Multielement Tablets (data summary view – sample preparation method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The solid blue 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 $Z'_{\text{comm}}$ score, $|Z'_{\text{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty ($U_{\text{NIST}}$) and represents the range that results in an acceptable $Z_{\text{NIST}}$ score, $|Z_{\text{NIST}}| \leq 2$.

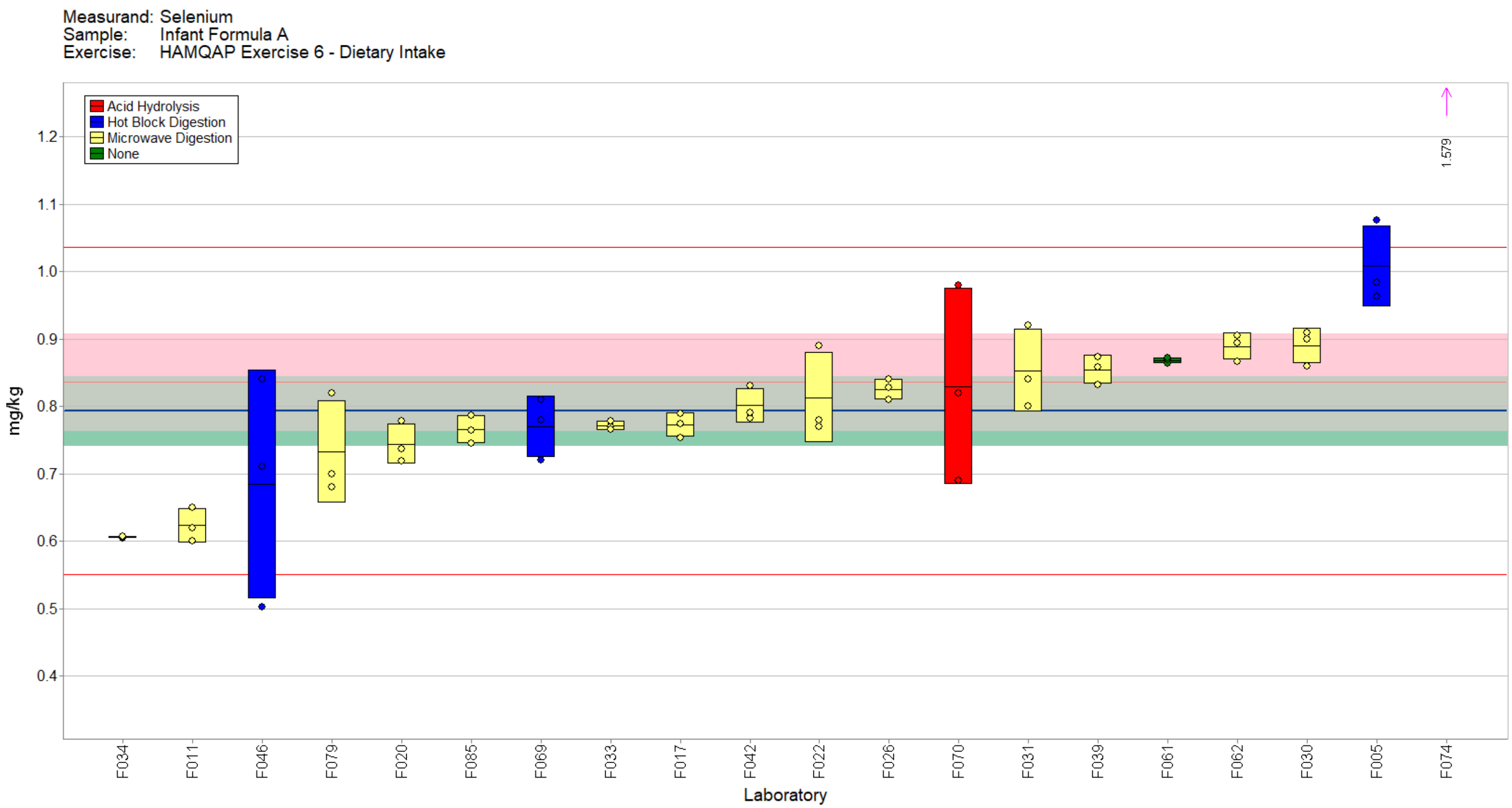


**Figure 1-22.** Selenium in Infant Formula A (data summary view – sample preparation method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The solid blue 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 $Z'_{\mathrm{comm}}$ score, $|Z'_{\mathrm{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty ($U_{\mathrm{NIST}}$) and represents the range that results in an acceptable $Z_{\mathrm{NIST}}$ score, $|Z_{\mathrm{NIST}}| \leq 2$.

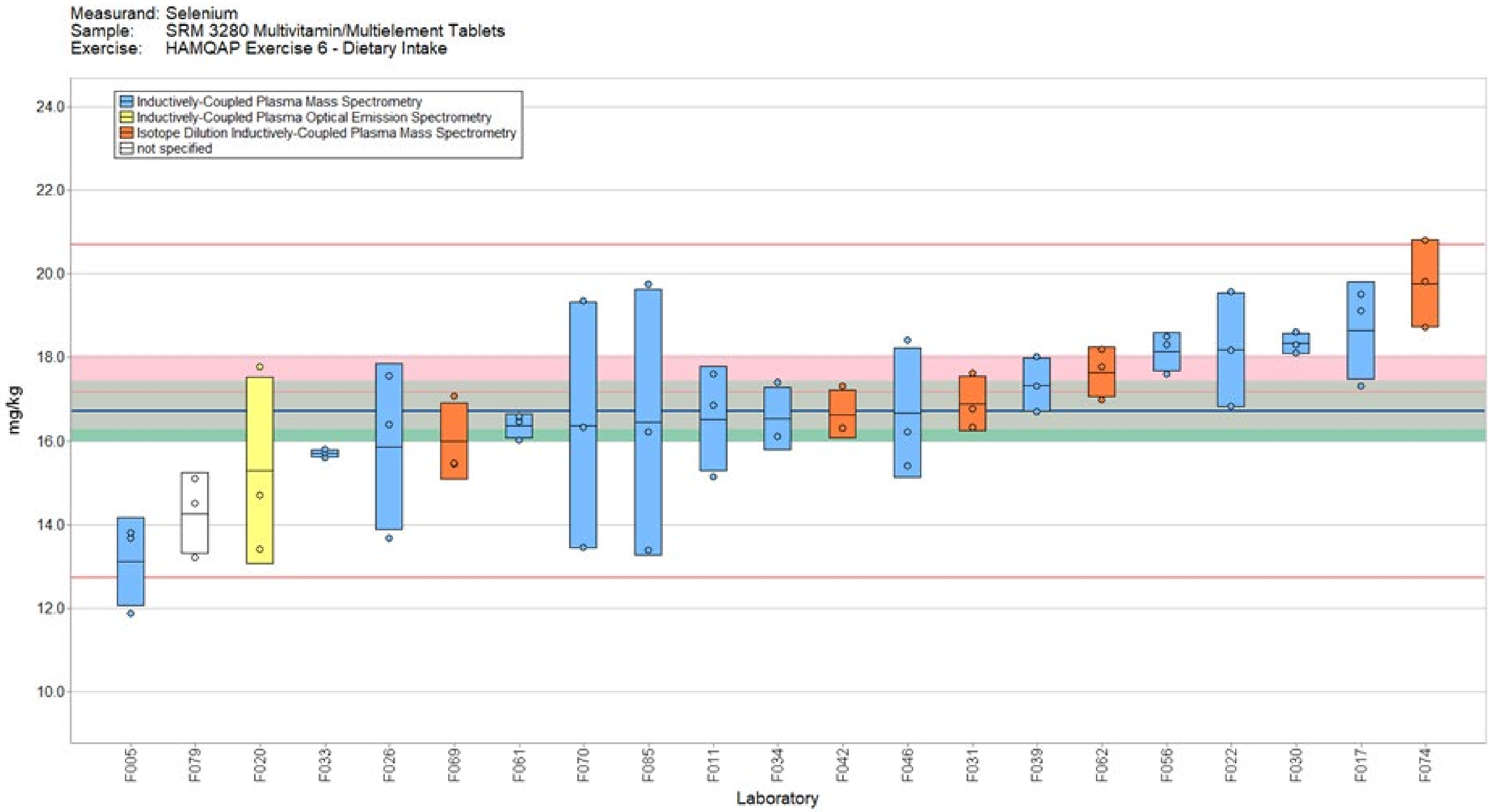


**Figure 1-23.** Selenium in SRM 3280 Multivitamin/Multielement Tablets (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue 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 $Z'_{\text{comm}}$ score, $|Z'_{\text{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty ($U_{\text{NIST}}$) and represents the range that results in an acceptable $Z_{\text{NIST}}$ score, $|Z_{\text{NIST}}| \leq 2$.

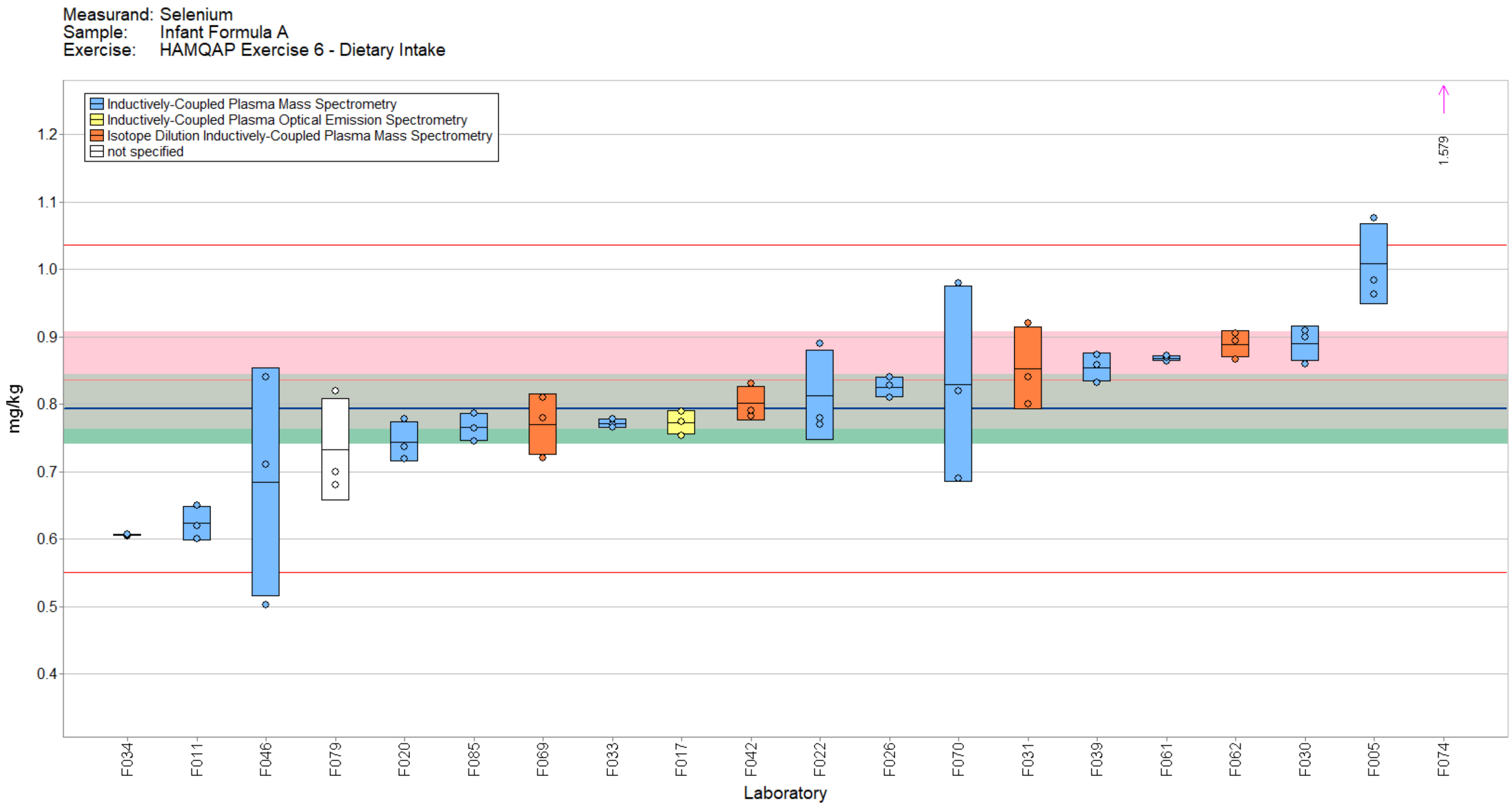


**Figure 1-24.** Selenium in Infant Formula A (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line 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 $Z'_{\text{comm}}$ score, $|Z'_{\text{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty ($U_{\text{NIST}}$) and represents the range that results in an acceptable $Z_{\text{NIST}}$ score, $|Z_{\text{NIST}}| \leq 2$.

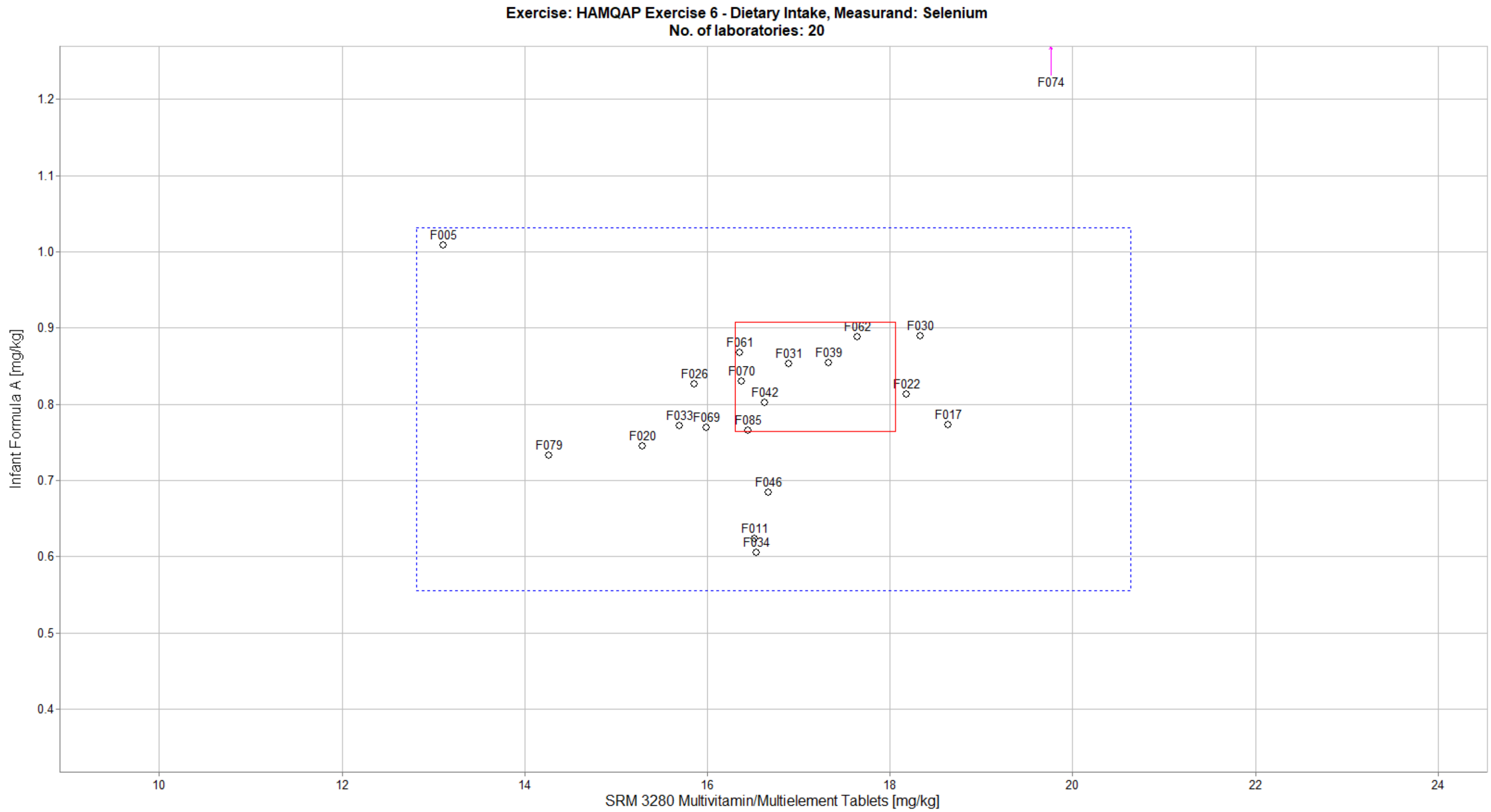


**Figure 1-25.** Laboratory means for selenium in SRM 3280 Multivitamin/Multielement Tablets and Infant Formula A (sample/sample comparison view). In this view, the individual laboratory mean for one sample (SRM 3280) is compared to the individual laboratory mean for a second sample (Infant Formula A). The solid red box represents the NIST range of tolerance for the two samples, SRM 3280 (x-axis) and Infant Formula 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 3280 (x-axis) and Infant Formula A (y-axis), calculated as the values above and below the consensus means that result in an acceptable $Z'_{\text{comm}}$ score, $|Z'_{\text{comm}}| \leq 2$.

## SECTION 2: TOXIC ELEMENTS (Arsenic, Cadmium, Lead, and Mercury)

Study Overview

In this study, participants were provided with samples of Rice Flour and SRM 3256 Green Tea-Containing Solid Oral Dosage Form (SODF) and asked to use in-house analytical methods to determine the mass fractions (mg/kg) of arsenic (As), cadmium (Cd), lead (Pb), and mercury (Hg) in each matrix. Plant uptake of toxic elements from air, water, or soil may result in contamination of certain foods and supplements, and consumption of these contaminated foods can cause illness, impairment, or, at high doses, death. Testing of these toxins in foods and supplements helps ensure product safety for consumers.

Dietary Intake Sample Information

*Rice Flour.* Participants were provided with one bottle containing approximately 50 g of rice flour. Participants were asked to store the material at controlled room temperature (20 °C to 25 °C) in the original unopened bottle and to prepare three samples and report three values from the single bottle provided. Before use, participants were instructed to mix the contents of the bottle thoroughly and to use a sample size of at least 0.5 g. Approximate analyte levels were not reported to participants prior to the study. The NIST-determined values for As, Cd, Pb, and Hg in Rice Flour were assigned using results from NIST by ICP-MS, cold vapor inductively coupled plasma mass spectrometry (CV-ICP-MS), and INAA. The NIST-determined values and expanded uncertainties are provided in the table below, both on a dry-mass basis and on an as-received basis accounting for moisture of the material (9.6 %).

| | NIST-Determined Mass Fractions in Rice Flour (mg/kg) | |
|---|---|---|
| Analyte | (dry-mass basis) | (as-received basis) |
| Arsenic (As) | 0.356 ± 0.036 | 0.322 ± 0.033 |
| Cadmium (Cd) | 0.00975 ± 0.00082 | 0.00881 ± 0.00074 |
| Lead (Pb) | 0.0186 ± 0.0050 | 0.0168 ± 0.0045 |
| Mercury (Hg) | 0.00178 ± 0.00006 | 0.00161 ± 0.00005 |

*Green Tea Tablets.* Participants were provided with three packets of SRM 3256 Green Tea-Containing Solid Oral Dosage Form (SODF), each packet containing approximately 2.5 g of ground material. Participants were asked to store the material at controlled room temperature (20 °C to 25 °C) in the original unopened packets and to prepare one sample and report one value from each packet provided. Before use, participants were instructed to mix the contents of the packets thoroughly and to use a sample size of at least 0.5 g. Approximate analyte levels were not reported to participants prior to the study. The certified values for As, Cd, Pb, and Hg in SRM 3256 were determined at NIST using ICP-MS and by results from collaborating laboratories. The certified values and uncertainties are provided in the table below, both on a dry-mass basis, as shown on the COA, and on an as-received basis accounting for moisture of the material (2.36 %).

| Analyte | NIST-Determined Mass Fractions in SRM 3256 Green Tea-Containing SODF (mg/kg) (dry-mass basis) | (as-received basis) |
|---|---|---|
| Arsenic (As) | 0.269 ± 0.019 | 0.263 ± 0.019 |
| Cadmium (Cd) | 0.025 ± 0.002 | 0.024 ± 0.002 |
| Lead (Pb) | 0.316 ± 0.030 | 0.309 ± 0.029 |
| Mercury (Hg) | 0.014 ± 0.002 | 0.014 ± 0.002 |

Dietary Intake Study Results

- The enrollment and reporting statistics for the toxic element studies are described in the table below. Reported values may include non-quantitative results (zero or below LOQ) but are included in the participation statistics.

| Analyte | Number of Laboratories Requesting Samples | Number of Laboratories Reporting Results (Percent Participation) | |
|---|---|---|---|
| | | Rice Flour | SRM 3256 |
| Arsenic (As) | 41 | 31 (76 %) | 34 (83 %) |
| Cadmium (Cd) | 41 | 28 (68 %) | 33 (80 %) |
| Lead (Pb) | 43 | 31 (72 %) | 36 (84 %) |
| Mercury (Hg) | 40 | 29 (73 %) | 33 (82 %) |

- The between-laboratory variabilities were very good or good for arsenic and cadmium in both materials. The between-laboratory variabilities for lead and mercury in green tea SODF were very good to moderate, respectively, but the between-laboratory variabilities were not good in the Rice Flour for lead and mercury. See table below.

| Analyte | Between-Laboratory Variability (% RSD) | |
|---|---|---|
| | Rice Flour | SRM 3256 |
| Arsenic (As) | 16 % | 14 % |
| Cadmium (Cd) | 22 % | 18 % |
| Lead (Pb) | 50 % | 15 % |
| Mercury (Hg) | >100 % | 31 % |

- Most laboratories reported using microwave digestion as their sample preparation method for both Rice Flour and SRM 3256. The reported sample preparation methods are listed below. The values shown below are the combined (as an average) reported sample preparations for both samples.

| Sample Preparation Method | Percent Reporting (Averaged for both samples types) | | | |
|---|---|---|---|---|
| | As | Cd | Pb | Hg |
| Microwave Digestion | 72 % | 73 % | 76 % | 76 % |
| Hot Block Digestion | 20 % | 17 % | 16 % | 13 % |
| Acid Hydrolysis | 3 % | 3 % | 3 % | 3 % |
| Open Beaker Digestion | 2 % | 2 % | 1 % | 2 % |
| Dilution | - | 2 % | - | - |
| None | 3 % | 3 % | 3 % | 6 % |

- Most laboratories reported using ICP-MS or isotope dilution inductively coupled plasma mass spectrometry (ID ICP-MS) as the analytical method used for both Rice Flour and SRM 3256. The reported analytical methods are listed below. Cold vapor atomic absorption spectroscopy (CV AAS) was reported for mercury determination as opposed to atomic absorption spectroscopy (AAS).

| Analytical Method | Percent Reporting (Averaged for both samples types) | | | |
|---|---|---|---|---|
| | As | Cd | Pb | Hg |
| ICP-MS | 68 % | 64 % | 70 % | 62 % |
| ID ICP-MS | 16 % | 16 % | 15 % | 17 % |
| ICP-OES | 11 % | 11 % | 9 % | 10 % |
| AAS (CV AAS) | 5 % | 8 % | 6 % | 7 % |
| Other/None | - | - | - | 5 % |

- For SRM 3256 Green Tea-Containing SODF it was noted that: The consensus mean lies within the target range for all four elements measured, As, Cd, Pb, and Hg (**Figures 2-2, 2-4, 2-7, 2-9, 2-12, 2-14, 2-17, 2-19**).
- For Rice Flour, the consensus means lie within the target ranges for As, Cd, and Pb (**Figures 2-1, 2-3, 2-6, 2-8, 2-11, 2-13**). Both the consensus mean and consensus range lie above the target range for Hg (**Figures 2-16** and **2-18**).
- For SRM 3256 Green Tea-Containing SODF, the consensus means lies within the target range for all four elements measured (**Figures 2-2, 2-4, 2-7, 2-9, 2-12, 2-14, 2-17, 2-19**).

Dietary Intake Technical Recommendations

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

- For all analytes, no significant bias or pattern was observed between the results obtained by different sample preparation techniques or instrumental techniques in either sample (**Figures 2-5, 2-10, 2-15, 2-20**).
- The levels of these contaminants are extremely low in these samples, especially cadmium, lead, and mercury in the Rice Flour.
  - The low levels of contaminants in Rice Flour may have resulted in higher between-laboratory variability for the lead and for the mercury which was over 100 %. Outliers have also increased the variability in some instances.
  - Because of the very low concentrations, detection of the analytes in the sample may be improved by limiting the number of dilutions performed, however matrix effects may become more significant.
  - A better alternative may be to perform standard additions; however, this option is more time consuming.
  - The determination of the LOQ is important when concentrations are low. Analysis of an appropriate number of procedural blanks can be critical in the determination of LOQ or when trying to reduce sample-to-sample variability. Analysis of many blanks can provide information about whether the variability is arising from the sample preparation method itself. The suggested minimum number of blanks to prepare is equal to the number of samples being prepared, or often 10 when determining LOQ.
- Sample preparation methods and analytical techniques should be well established by using quality control materials (CRMs, SRMs, RMs, and in-house materials) before analyzing unknown materials.
- The high temperatures of a microwave digestion system should ensure complete digestion of the materials prior to analysis.
- For arsenic (**Table 2-2**), most of the laboratories reporting data were within the NIST target range for both materials (**Figure 2-5**).
  - Where laboratories reported results closer to the target range for one material than for the other, the differences in the two matrices or the concentration levels may have resulted from difficulties in preparation and analysis.
    - Calibration curves must be linear and include standards that encompass the lowest and highest values expected to be measured in the sample solutions and include several standards in between these two standards.
    - Difficulty in the digestion of samples can cause bias and/or increased variability between samples.
  - Results produced by microwave digestion were most consistent with the target ranges, especially for the Rice Flour.
    - Arsenic is volatile and can be lost during sample preparation. A vigorous microwave digestion should convert all volatile organoarsenic species to arsenic acid (AsV). At this point subsequent heating will not result in loss of arsenic.
    - Open beaker digestion may not be the best choice for arsenic sample preparation and may lead to low results due to loss of arsenic.
  - Failure to eliminate the organic constituents by incomplete sample digestion may produce interferences that cause signal enhancement or suppression and thereby introducing measurement bias in one of the matrices. Collision cell technology can be used to minimize

the molecular ion interferences that may be found when analyzing arsenic in these materials.
    - Some laboratories reported using ID ICP-MS as the analytical method. ID ICP-MS is not a practicable method for arsenic measurement because arsenic is monoisotopic. Measurement methods should be reported correctly and completely.
- For cadmium, **Figure 2-10** shows that most laboratories were able to measure both samples well and most laboratories reporting data were within the NIST target range for both materials.
    - Several laboratories reported values of below LOQ for cadmium in Rice Flour.
    - The boiling point of Cd is high and volatile loss of Cd should not be a concern.
    - Spectral/isobaric interferences can make Cd difficult to measure accurately by ICP-MS.
        - High concentrations of certain elements, mainly Mo, Sn, or Zr, are known to cause interferences in the analysis of Cd by ICP-MS. A scan of the sample before analysis will indicate any potential interferences in the sample that will need to be addressed.
        - Anion exchange separation of matrix elements prior to ICP-MS can reduce interferences.
        - Collision cell technology can be used to minimize molecular interferences that may be found in these two materials.
        - The use of ID ICP-MS is a good choice for analytical measurements of Cd.
    - Some recommendations made above for arsenic are applicable to the measurement of cadmium, such as limiting the number of dilutions; ensuring linearity of calibration curves and inclusion of lowest and highest points of interest; and preparing an appropriate number of procedural blanks.
- For lead, **Figures 2-12, 2-14,** and **2-15** show that several laboratories were below the NIST target range in the green tea tablets.
    - Lead is easily digested, and volatile loss of lead is not a concern. However, digestion with HCl may form insoluble $PbCl_2$ precipitate so digestion with $HNO_3$ is recommended. Because the level of lead in the Green Tea-Containing SODF is approximately 10 times greater than in the Rice Flour, $PbCl_2$ precipitation may have resulted in low results being reported if the sample digestion was conducted consistently between materials.
    - Since no linear trend was observed in **Figure 2-15** between the reported results for lead in the two materials, the sample preparation or analysis of green tea material may have caused a greater difficulty compared to the Rice Flour.
    - Some laboratories reported high sample-to-sample variability in either one or both materials. This may be due to the low lead concentrations in the material, difficulties in sample preparation, incomplete sample digestion, or calibration curves which do not encompass all sample solutions measured. Sample solutions which fall above the upper limit of the calibration curve will usually give an erroneous value.
    - Analysis of an appropriate number of procedural blanks is always important and can be critical when sample concentrations are near the LOQs or, as is the case for lead in the Rice Flour material, when trying to determine the cause of sample-to-sample variability. Analysis of many blanks can provide information about whether the variability is arising from the sample preparation method itself.
- For mercury, **Figures 2-16** through **2-20** show that many laboratories reported results outside of the NIST target range or that were below the laboratory LOQ.

- Mercury is volatile so care must be taken to not lose mercury during sample preparation. Microwave digestion is the best sample preparation method for mercury analysis. Laboratories that reported using hot block or open beaker digestion had a greater sample-to-sample variability.
- The low levels of mercury in the Rice Flour may be close to the LOQ for some techniques.
  - Since levels in blanks and backgrounds for mercury measurements may be large, leading to high detection limits and making determination of low-level samples difficult, a sufficient number of procedural blanks should be used to determine an accurate LOQ.
  - Low concentrations of mercury are not stable in solution over time. Samples should be prepared as near as possible to the time of analysis. Samples containing low concentrations of Hg may be more stable in dilute HCl than in dilute $HNO_3$.
  - Acidification of sample solutions will help prevent loss of Hg by adsorption. The addition of dichromate will help prevent loss of Hg through volatilization.
- The sensitivity of ICP-MS is low for Hg. Using cold vapor Hg generation increases sensitivity of ICP-MS and allows lower levels of Hg to be measured.
- Mercury carryover between samples is common and can lead to erratic results. Adequate washout time is needed after each measurement. The use of dilute HCl in the rinse solution may decrease the length of the washout time needed.
- Laboratories reporting measured values at or above the upper limit of the range of tolerance also reported larger within-laboratory variability indicating a potential calibration issue.

- All results should be reported accurately.
  - Zero is not a quantity that can be measured. If measured values are below detection limits, results should be reported as such. A more appropriate result would be to report that a value is below the LOQ or QL.
  - Laboratories reporting results outside the consensus tolerance levels should check for calculation errors. One example is to confirm that factors for all dilutions have been properly tabulated and that results are reported in correct reporting units.

**Table 2-1.** Individualized data summary table (NIST) for toxic elements in Rice Flour and SRM 3256 Green Tea-Containing SODF.

***National Institute of Standards and Technology***

**HAMQAP Exercise 6 - Toxic Elements**

| | **Lab Code:** | **NIST** | **1. Your Results** | | | | **2. Community Results** | | | **3. Target** | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Analyte | Sample | Units | $x_i$ | $s_i$ | $Z'_{comm}$ | $Z_{NIST}$ | N | x* | s* | $x_{NIST}$ | $U$ |
| Arsenic | SRM 3256 Green Tea-Containing SODF | mg/kg | 0.263 | 0.019 | | | 34 | 0.266 | 0.038 | 0.263 | 0.019 |
| Arsenic | Rice Flour | mg/kg | 0.32 | 0.033 | | | 31 | 0.341 | 0.053 | 0.32 | 0.033 |
| Cadmium | SRM 3256 Green Tea-Containing SODF | mg/kg | 0.024 | 0.002 | | | 32 | 0.022 | 0.004 | 0.024 | 0.002 |
| Cadmium | Rice Flour | mg/kg | 0.009 | 0.00074 | | | 21 | 0.009 | 0.002 | 0.009 | 0.00074 |
| Mercury | SRM 3256 Green Tea-Containing SODF | mg/kg | 0.014 | 0.002 | | | 28 | 0.013 | 0.004 | 0.014 | 0.002 |
| Mercury | Rice Flour | mg/kg | 0.002 | 0.00005 | | | 14 | 0.004 | 0.004 | 0.002 | 0.00005 |
| Lead | SRM 3256 Green Tea-Containing SODF | mg/kg | 0.309 | 0.029 | | | 36 | 0.281 | 0.043 | 0.309 | 0.029 |
| Lead | Rice Flour | mg/kg | 0.017 | 0.0045 | | | 26 | 0.018 | 0.009 | 0.017 | 0.0045 |

$x_i$ Mean of reported values
$s_i$ Standard deviation of reported values
$Z'_{comm}$ Z'-score with respect to community consensus
$Z_{NIST}$ Z-score with respect to NIST value

N Number of quantitative values reported
x* Robust mean of reported values
s* Robust standard deviation

$x_{NIST}$ NIST-assessed value
$U$ expanded uncertainty about the NIST-assessed value

**Table 2-2.** Data summary table for arsenic in Rice Flour and SRM 3256 Green Tea-Containing SODF. Data points highlighted in blue have been identified as outside the consensus tolerance limits and would be estimated to result in an unacceptable $Z'_{comm}$ score, $|Z'_{comm}| \geq 2$.

| | | Arsenic | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Rice Flour (mg/kg) | | | | | SRM 3256 Green Tea Containing SODF (mg/kg) | | | | |
| | Lab | A | B | C | Avg | SD | A | B | C | Avg | SD |
| Individual Results | Target | | | | 0.32 | 0.03 | | | | 0.26 | 0.02 |
| | F001 | 0.36 | 0.356 | 0.365 | 0.360 | 0.005 | 0.255 | 0.241 | 0.249 | 0.248 | 0.007 |
| | F002 | 0.323 | 0.321 | 0.322 | 0.322 | 0.001 | 0.33 | 0.33 | 0.315 | 0.325 | 0.009 |
| | F004 | | | | | | | | | | |
| | F005 | 0.5626 | 0.5681 | 0.6079 | 0.580 | 0.025 | 0.2323 | 0.2367 | 0.2356 | 0.235 | 0.002 |
| | F011 | 0.338 | 0.343 | 0.325 | 0.335 | 0.009 | 0.224 | 0.233 | 0.247 | 0.235 | 0.012 |
| | F014 | 0.2306 | 0.2244 | 0.2203 | 0.225 | 0.005 | 0.29 | 0.28 | 0.32 | 0.297 | 0.021 |
| | F015 | 0.3613 | 0.3805 | 0.3727 | 0.372 | 0.010 | 0.2645 | 0.2767 | 0.2783 | 0.273 | 0.008 |
| | F017 | 0.313 | 0.31 | 0.324 | 0.316 | 0.007 | 0.262 | 0.255 | 0.251 | 0.256 | 0.006 |
| | F018 | | | | | | | | | | |
| | F019 | 0.37 | 0.37 | 0.4 | 0.380 | 0.017 | 0.17 | 0.18 | 0.18 | 0.177 | 0.006 |
| | F020 | 0.36 | 0.35 | 0.34 | 0.350 | 0.010 | 0.25 | 0.27 | 0.25 | 0.257 | 0.012 |
| | F021 | 0.37 | 0.38 | 0.39 | 0.380 | 0.010 | 0.3 | 0.31 | 0.33 | 0.313 | 0.015 |
| | F026 | 287 | 292 | 287 | 289 | 2.9 | 228 | 221 | 217 | 222 | 5.6 |
| | F027 | 0.3105 | 0.29543 | 0.2996 | 0.302 | 0.008 | 0.26589 | 0.24861 | 0.24445 | 0.253 | 0.011 |
| | F030 | 0.369 | 0.378 | 0.377 | 0.375 | 0.005 | 0.268 | 0.276 | 0.281 | 0.275 | 0.007 |
| | F031 | 0.313 | 0.312 | 0.296 | 0.307 | 0.010 | 0.267 | 0.281 | 0.284 | 0.277 | 0.009 |
| | F032 | | | | | | | | | | |
| | F033 | 0.326 | 0.322 | 0.337 | 0.328 | 0.008 | 0.247 | 0.236 | 0.245 | 0.243 | 0.006 |
| | F034 | 0.285 | 0.286 | 0.287 | 0.286 | 0.001 | 0.206 | 0.205 | 0.207 | 0.206 | 0.001 |
| | F039 | 0.368 | 0.358 | 0.375 | 0.367 | 0.009 | 0.276 | 0.281 | 0.282 | 0.280 | 0.003 |
| | F042 | 0.338 | 0.338 | 0.336 | 0.337 | 0.001 | 0.252 | 0.268 | 0.249 | 0.256 | 0.010 |
| | F045 | | | | | | | | | | |
| | F046 | 0.34 | 0.31 | 0.32 | 0.323 | 0.015 | 0.24 | 0.23 | 0.24 | 0.237 | 0.006 |
| | F051 | | | | | | | | | | |
| | F052 | 0.04 | 0.036 | 0.04 | 0.039 | 0.002 | 0.3 | 0.33 | 0.32 | 0.317 | 0.015 |
| | F056 | | | | | | 0.2438 | 0.2661 | 0.2636 | 0.258 | 0.012 |
| | F057 | | | | | | 0.365187 | 0.313686 | 0.303506 | 0.327 | 0.033 |
| | F059 | 0.43 | 0.43 | 0.4 | 0.420 | 0.017 | 0.3 | 0.29 | 0.31 | 0.300 | 0.010 |
| | F060 | | | | | | 0.248 | 0.267 | 0.256 | 0.257 | 0.010 |
| | F061 | 0.32 | 0.33 | 0.34 | 0.330 | 0.010 | 0.25 | 0.28 | 0.3 | 0.277 | 0.025 |
| | F062 | 0.319 | 0.344 | 0.335 | 0.333 | 0.013 | 0.219 | 0.234 | 0.227 | 0.227 | 0.008 |
| | F063 | 0.324 | 0.338 | 0.331 | 0.331 | 0.007 | 0.259 | 0.28 | 0.25 | 0.263 | 0.015 |
| | F066 | 0.306 | 0.308 | 0.304 | 0.306 | 0.002 | 0.22 | 0.208 | 0.218 | 0.215 | 0.006 |
| | F069 | 0.34 | 0.35 | 0.36 | 0.350 | 0.010 | 0.28 | 0.27 | 0.28 | 0.277 | 0.006 |
| | F070 | 0.363 | 0.351 | 0.344 | 0.353 | 0.010 | 0.26 | 0.28 | 0.25 | 0.263 | 0.015 |
| | F073 | 0.395 | 0.38 | 0.392 | 0.389 | 0.008 | 0.265 | 0.273 | 0.281 | 0.273 | 0.008 |
| | F074 | 0.503 | | | 0.503 | | 0.352 | 0.342 | 0.311 | 0.335 | 0.021 |
| | F077 | | | | | | | | | | |
| | F079 | 0.34 | 0.34 | 0.33 | 0.337 | 0.006 | 0.28 | 0.26 | 0.28 | 0.273 | 0.012 |
| | F088 | 0.282 | 0.279 | 0.271 | 0.277 | 0.006 | 0.249 | 0.245 | 0.246 | 0.247 | 0.002 |
| | F089 | | | | | | | | | | |
| Community Results | | Consensus Mean | | | 0.341 | | Consensus Mean | | | 0.266 | |
| | | Consensus Standard Deviation | | | 0.053 | | Consensus Standard Deviation | | | 0.038 | |
| | | Maximum | | | 289 | | Maximum | | | 222 | |
| | | Minimum | | | 0.039 | | Minimum | | | 0.18 | |
| | | N | | | 30 | | N | | | 34 | |

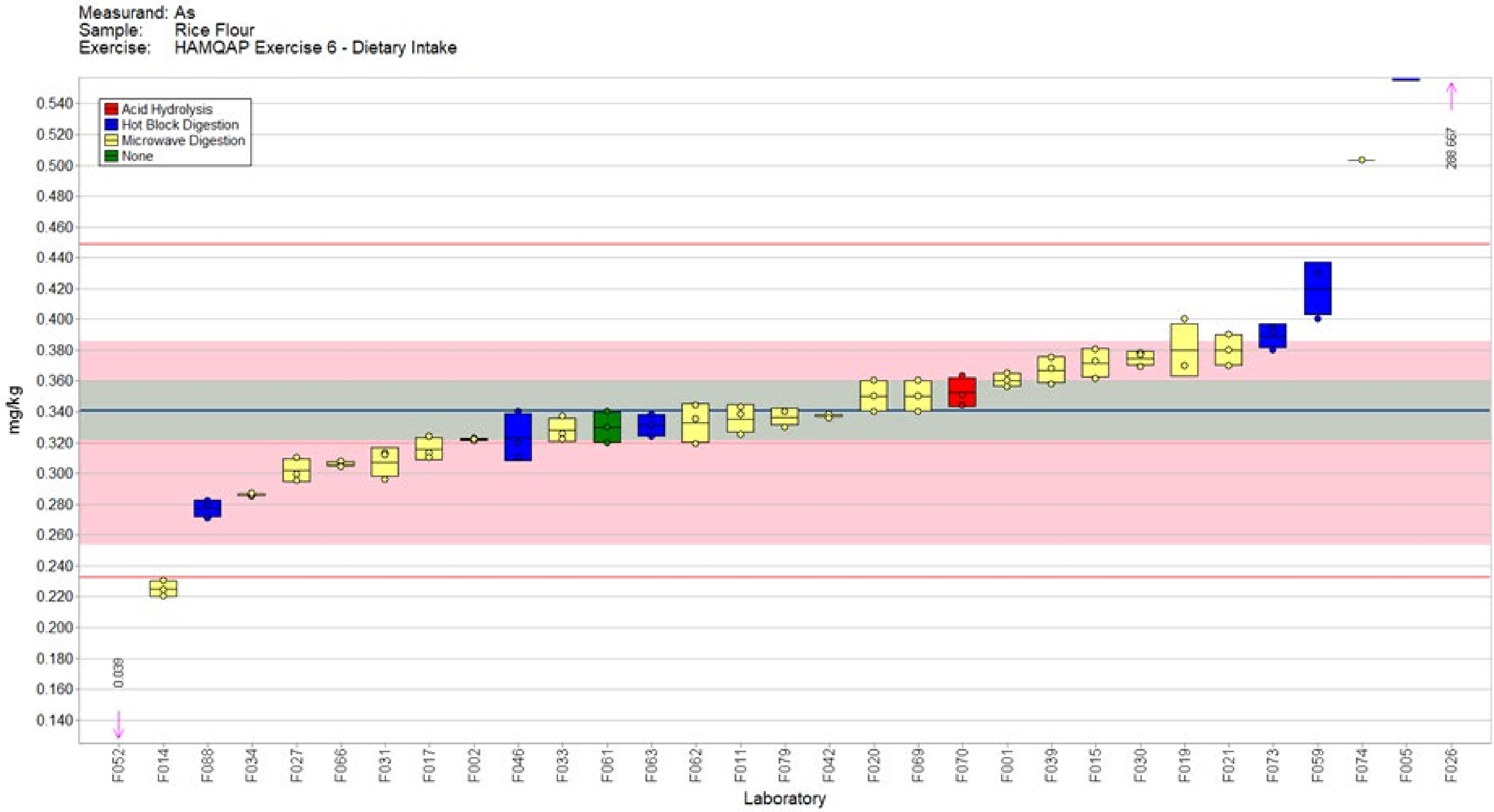


**Figure 2-1.** Arsenic in Rice Flour (data summary view –sample preparation method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The solid blue 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 $Z'_{\text{comm}}$ score, $|Z'_{\text{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty ($U_{\text{NIST}}$) and represents the range that results in an acceptable $Z_{\text{NIST}}$ score, $|Z_{\text{NIST}}| \leq 2$.

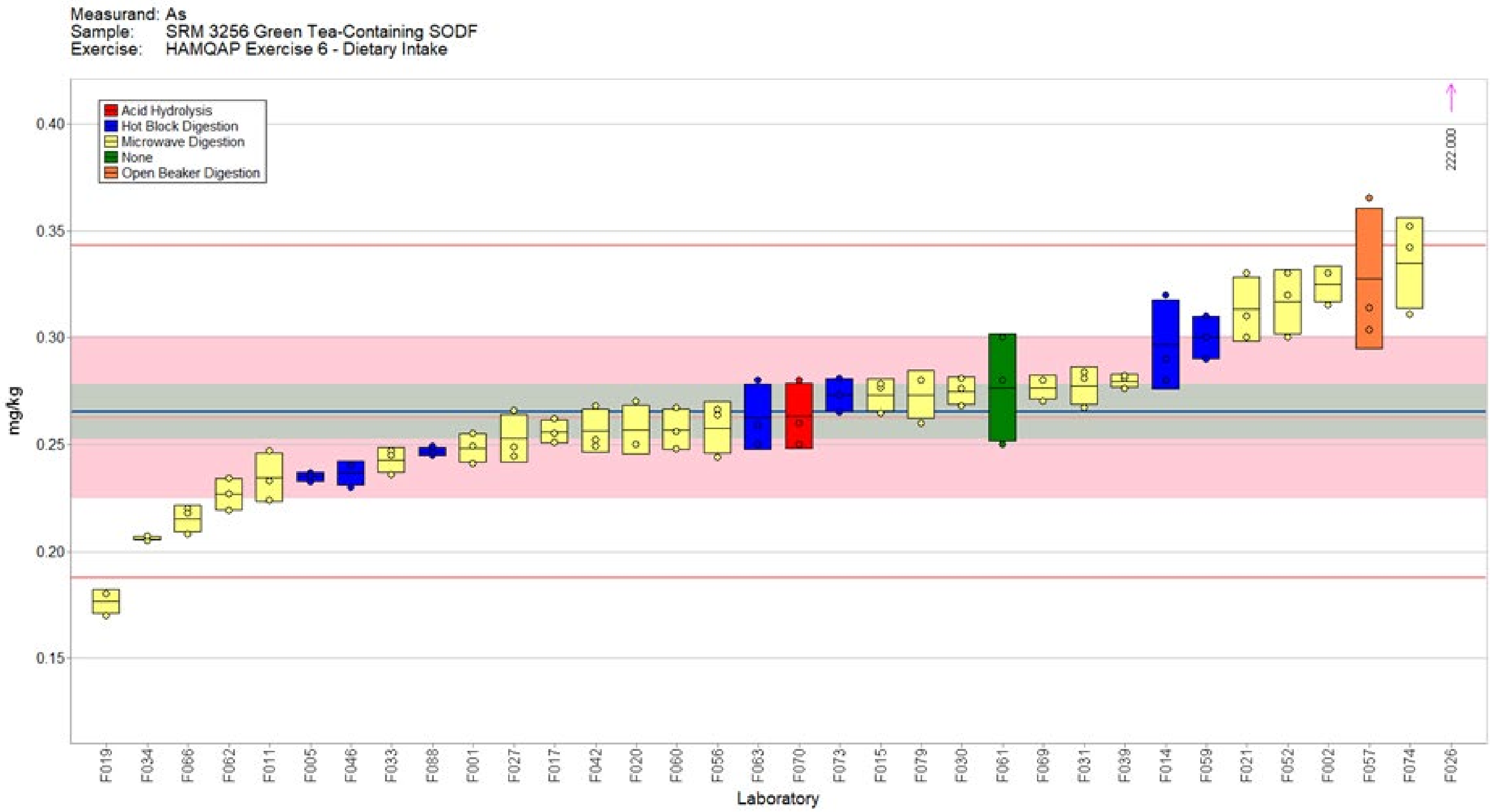


**Figure 2-2.** Arsenic in SRM 3256 Green Tea-Containing SODF (data summary view –sample preparation method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The solid blue 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 $Z'_{\mathrm{comm}}$ score, $|Z'_{\mathrm{comm}}| \leq 2$ . The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty ($U_{\mathrm{NIST}}$) and represents the range that results in an acceptable $Z_{\mathrm{NIST}}$ score, $|Z_{\mathrm{NIST}}| \leq 2$.

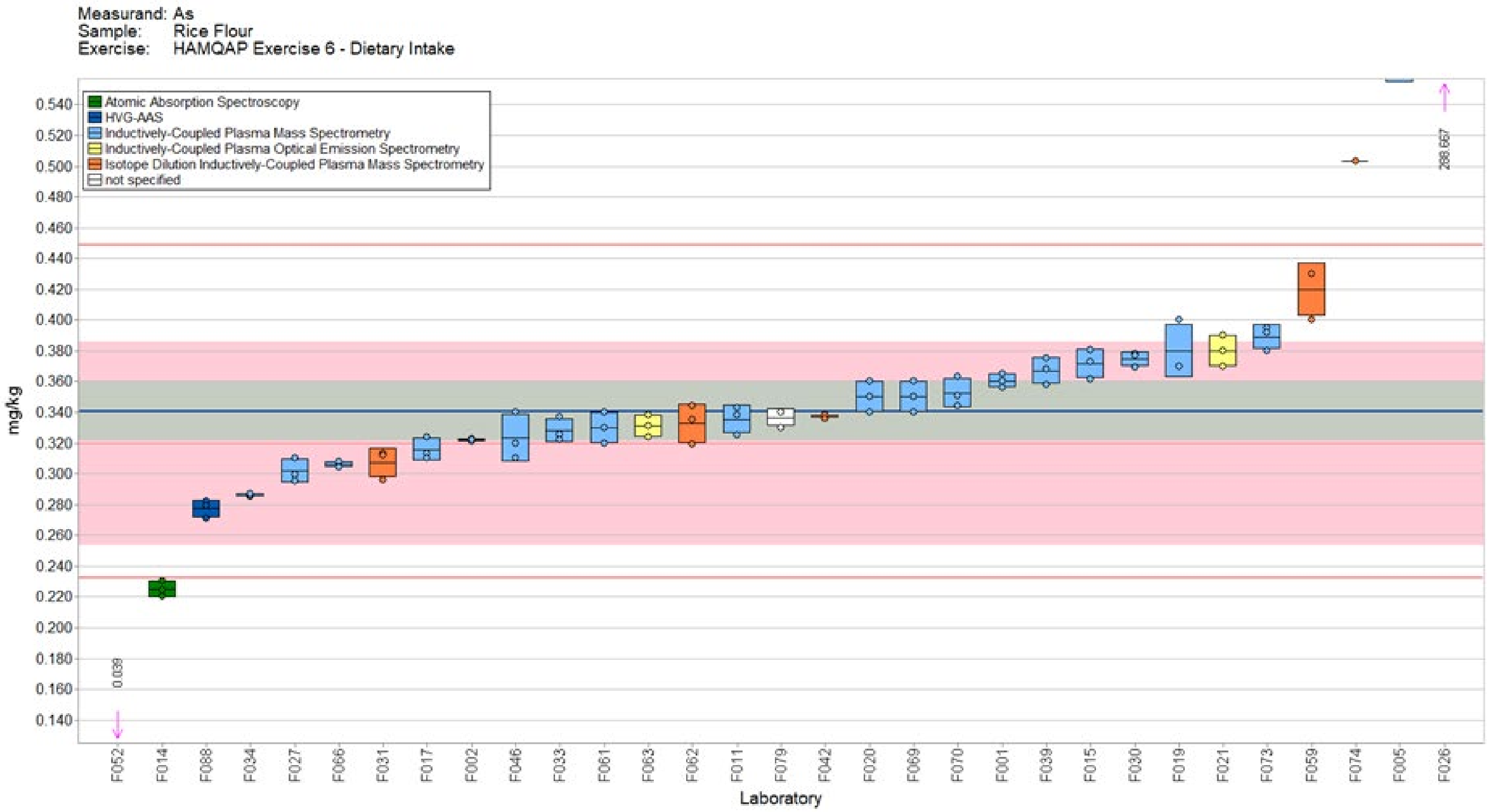


**Figure 2-3.** Arsenic in Rice Flour (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line represents 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 $Z'_{\text{comm}}$ score, $|Z'_{\text{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty ($U_{\text{NIST}}$) and represents the range that results in an acceptable $Z_{\text{NIST}}$ score, $|Z_{\text{NIST}}| \leq 2$.

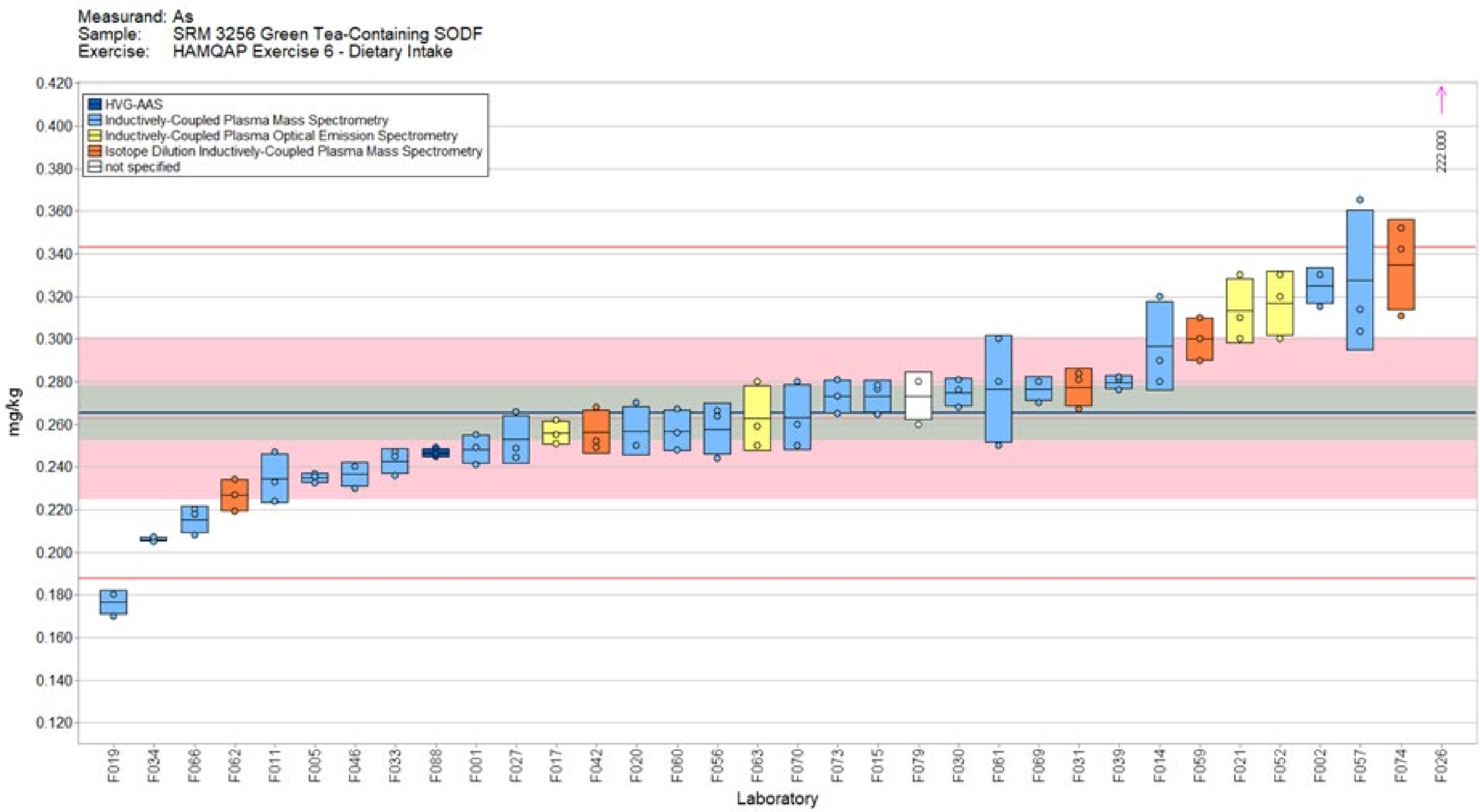


**Figure 2-4.** Arsenic in SRM 3256 Green Tea-Containing SODF (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue 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 $Z'_{\mathrm{comm}}$ score, $|Z'_{\mathrm{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty ($U_{\mathrm{NIST}}$) and represents the range that results in an acceptable $Z_{\mathrm{NIST}}$ score, $|Z_{\mathrm{NIST}}| \leq 2$.

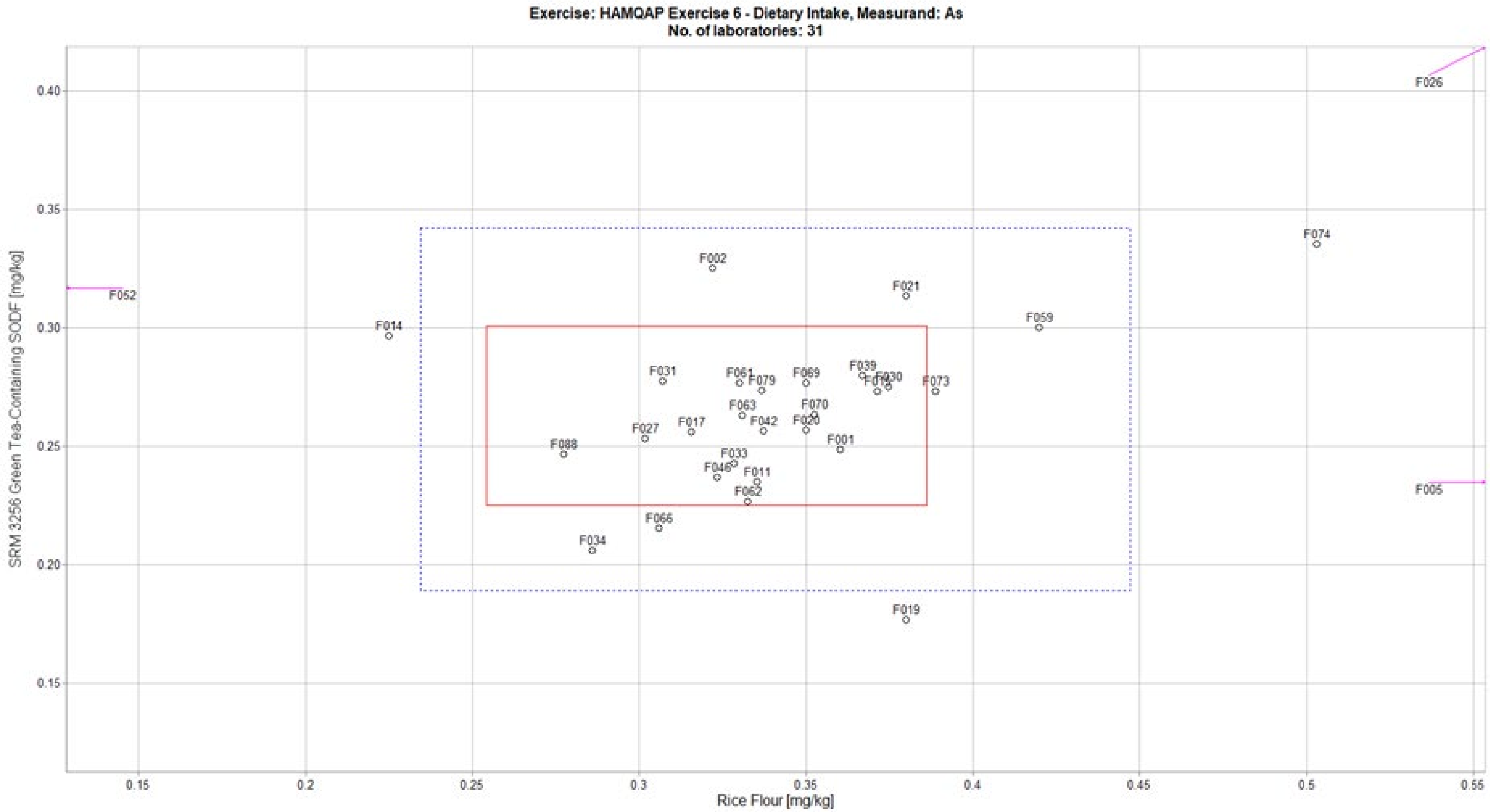


**Figure 2-5.** Laboratory means for arsenic in Rice Flour and SRM 3256 Green Tea-Containing SODF (sample/sample comparison view). In this view, the individual laboratory mean for one sample (Rice Flour) is compared to the mean for a second sample (SRM 3256). The solid red box represents the NIST range of tolerance for the two samples, Rice Flour (x-axis) and SRM 3256 (y-axis), which encompasses the target values bounded by their uncertainties ($U_{\mathrm{NIST}}$) and represents the range that results in an acceptable $Z_{\mathrm{NIST}}$ score, $|Z_{\mathrm{NIST}}| \leq 2$. The dotted blue box represents the consensus range of tolerance for Rice Flour (x-axis) and SRM 3256 (y-axis), calculated as the values above and below the consensus means that result in an acceptable $Z'_{\mathrm{comm}}$ score, $|Z'_{\mathrm{comm}}| \leq 2$.

**Table 2-3.** Data summary table for cadmium in Rice Flour and SRM 3256 Green Tea-Containing SODF. Data points highlighted in blue have been identified as outside the consensus tolerance limits and would be estimated to result in an unacceptable $Z'_{comm}$ score, $|Z'_{comm}| \geq 2$.

| | | Cadmium | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Rice Flour (mg/kg) | | | | | SRM 3256 Green Tea Containing SODF (mg/kg) | | | | |
| | Lab | A | B | C | Avg | SD | A | B | C | Avg | SD |
| Individual Results | Target | | | | 0.009 | 0.001 | | | | 0.024 | 0.002 |
| | F001 | 0.0082 | 0.00885 | 0.00965 | 0.0089 | 0.0007 | 0.0189 | 0.0178 | 0.0212 | 0.019 | 0.002 |
| | F002 | 0.011 | 0.012 | 0.011 | 0.011 | 0.001 | 0.023 | 0.023 | 0.023 | 0.023 | 0.000 |
| | F004 | | | | | | | | | | |
| | F005 | 0.0088 | 0.0084 | 0.0088 | 0.0087 | 0.0002 | 0.0113 | 0.0113 | 0.0118 | 0.011 | 0.000 |
| | F011 | 0.007 | 0.009 | 0.007 | 0.0077 | 0.0012 | 0.021 | 0.019 | 0.02 | 0.020 | 0.001 |
| | F014 | | | | | | | | | | |
| | F015 | 0.0091 | 0.0091 | 0.0088 | 0.0090 | 0.0002 | 0.0234 | 0.024 | 0.0231 | 0.024 | 0.000 |
| | F017 | < 0.010 | < 0.010 | < 0.010 | | | 0.0255 | 0.0237 | 0.0281 | 0.026 | 0.002 |
| | F018 | | | | | | | | | | |
| | F019 | < 0.040 | < 0.040 | < 0.040 | | | < 0.040 | < 0.040 | < 0.040 | | |
| | F020 | 0.009 | 0.01 | 0.009 | 0.0093 | 0.0006 | 0.026 | 0.025 | 0.026 | 0.026 | 0.001 |
| | F021 | 0.01 | 0.01 | 0.01 | 0.01 | 0 | 0.02 | 0.02 | 0.02 | 0.020 | 0.000 |
| | F026 | 7 | 9 | 7 | 7.67 | 1.15 | 17 | 20 | 23 | 20.0 | 3.0 |
| | F027 | 0.00824 | 0.00743 | 0.00821 | 0.0080 | 0.0005 | 0.02044 | 0.01943 | 0.01815 | 0.019 | 0.001 |
| | F030 | 0.0089 | 0.009 | 0.0093 | 0.0091 | 0.0002 | 0.0233 | 0.0238 | 0.23 | 0.092 | 0.119 |
| | F031 | < 0.010 | < 0.010 | < 0.010 | | | 0.026 | 0.023 | 0.023 | 0.024 | 0.002 |
| | F032 | | | | | | | | | | |
| | F033 | 0.00963 | 0.0095 | 0.00937 | 0.0095 | 0.0001 | 0.0221 | 0.0219 | 0.0224 | 0.022 | 0.000 |
| | F034 | < 0.010 | < 0.010 | < 0.010 | | | 0.016 | 0.016 | 0.016 | 0.016 | 0.000 |
| | F039 | < 0.010 | < 0.010 | < 0.010 | | | 0.02 | 0.026 | 0.023 | 0.023 | 0.003 |
| | F042 | 0.0083 | 0.0079 | 0.0088 | 0.0083 | 0.0005 | 0.0213 | 0.0211 | 0.0249 | 0.022 | 0.002 |
| | F045 | | | | | | | | | | |
| | F046 | < 0.010 | < 0.010 | < 0.010 | | | < 0.020 | 0.02 | < 0.020 | 0.020 | |
| | F051 | | | | | | | | | | |
| | F052 | 0.012 | 0.01 | 0.013 | 0.012 | 0.002 | 0.01 | 0.011 | 0.01 | 0.010 | 0.001 |
| | F056 | 0.01102 | | | 0.011 | | 0.0259 | 0.0218 | 0.0215 | 0.023 | 0.002 |
| | F057 | | | | | | 0.007078 | 0.007947 | 0.006393 | 0.0071 | 0.0008 |
| | F059 | 0.009 | 0.009 | 0.009 | 0.009 | 0 | 0.022 | 0.02 | 0.022 | 0.021 | 0.001 |
| | F060 | | | | | | 0.018 | 0.019 | 0.018 | 0.018 | 0.001 |
| | F061 | | | | | | 0.006 | 0.007 | 0.008 | 0.007 | 0.001 |
| | F062 | < 0.040 | < 0.040 | < 0.040 | | | 0.022 | 0.023 | 0.023 | 0.023 | 0.001 |
| | F063 | < 0.020 | < 0.020 | < 0.020 | 0.01 | | 0.023 | 0.024 | 0.028 | 0.025 | 0.003 |
| | F066 | 0.01 | < 0.010 | < 0.010 | 0.0087 | 0.0006 | 0.02 | 0.021 | 0.02 | 0.020 | 0.001 |
| | F069 | 0.01 | 0.01 | 0.01 | 0.01 | 0 | 0.02 | 0.03 | 0.03 | 0.027 | 0.006 |
| | F070 | < 0.020 | < 0.020 | < 0.020 | | | 0.025 | 0.024 | 0.025 | 0.025 | 0.001 |
| | F073 | | | | | | 0.027 | 0.027 | 0.028 | 0.027 | 0.001 |
| | F074 | 0.00768 | | | 0.0077 | | 0.022 | 0.022 | 0.018 | 0.021 | 0.002 |
| | F077 | | | | | | | | | | |
| | F079 | 0.01 | 0.009 | 0.009 | 0.0093 | 0.0006 | 0.023 | 0.022 | 0.022 | 0.022 | 0.001 |
| | F088 | 0.015 | 0.017 | 0.016 | 0.016 | 0.001 | 0.025 | 0.026 | 0.029 | 0.027 | 0.002 |
| | F089 | | | | | | | | | | |
| Community Results | | Consensus Mean | | | 0.009 | | Consensus Mean | | | 0.022 | |
| | | Consensus Standard Deviation | | | 0.002 | | Consensus Standard Deviation | | | 0.004 | |
| | | Maximum | | | 7.67 | | Maximum | | | 20 | |
| | | Minimum | | | 0.0077 | | Minimum | | | 0.007 | |
| | | N | | | 18 | | N | | | 31 | |

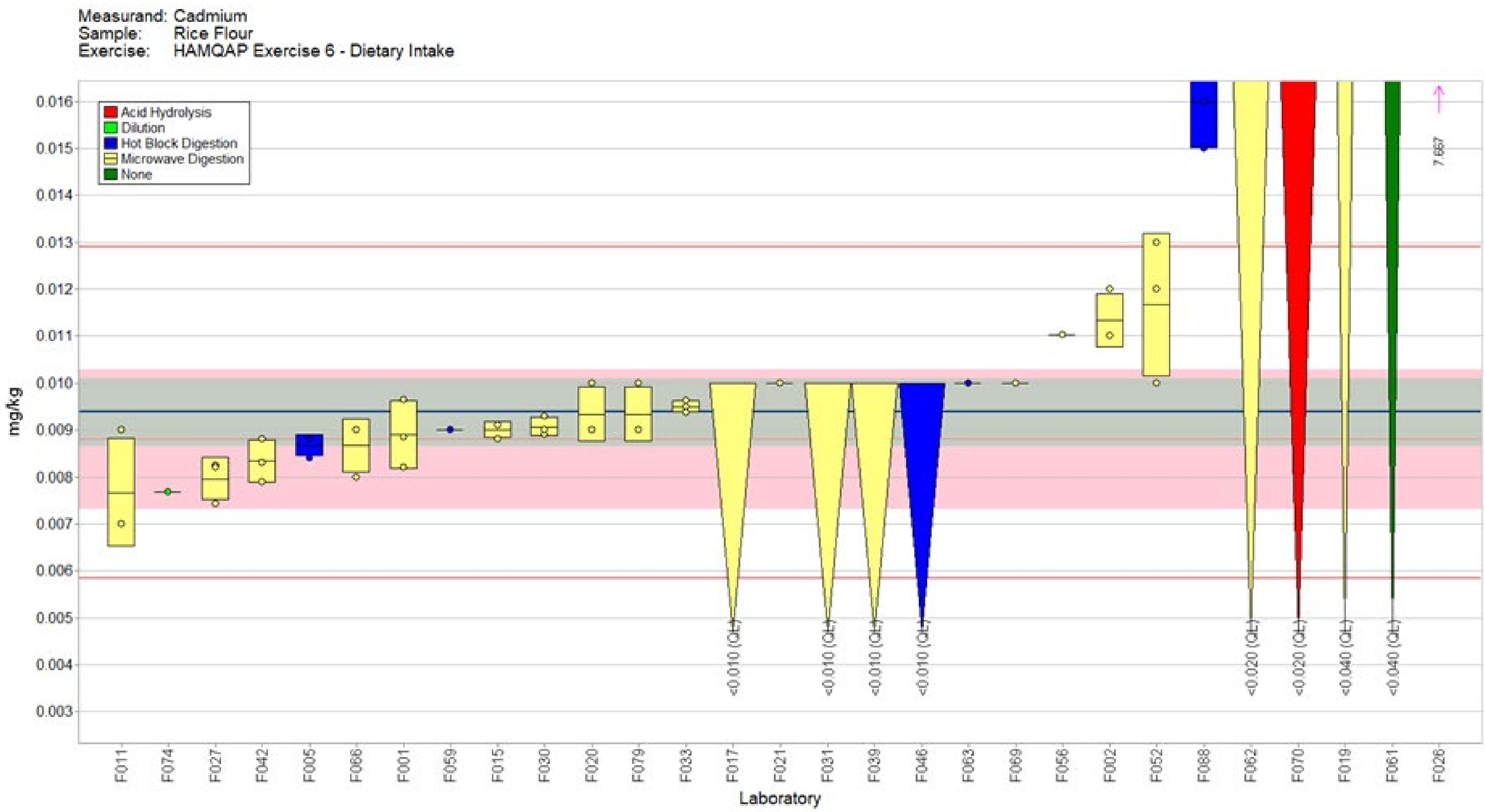


**Figure 2-6.** Cadmium in Rice Flour (data summary view –sample preparation method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The solid blue 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 $Z'_{\text{comm}}$ score, $|Z'_{\text{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty ($U_{\text{NIST}}$) and represents the range that results in an acceptable $Z_{\text{NIST}}$ score, $|Z_{\text{NIST}}| \leq 2$.

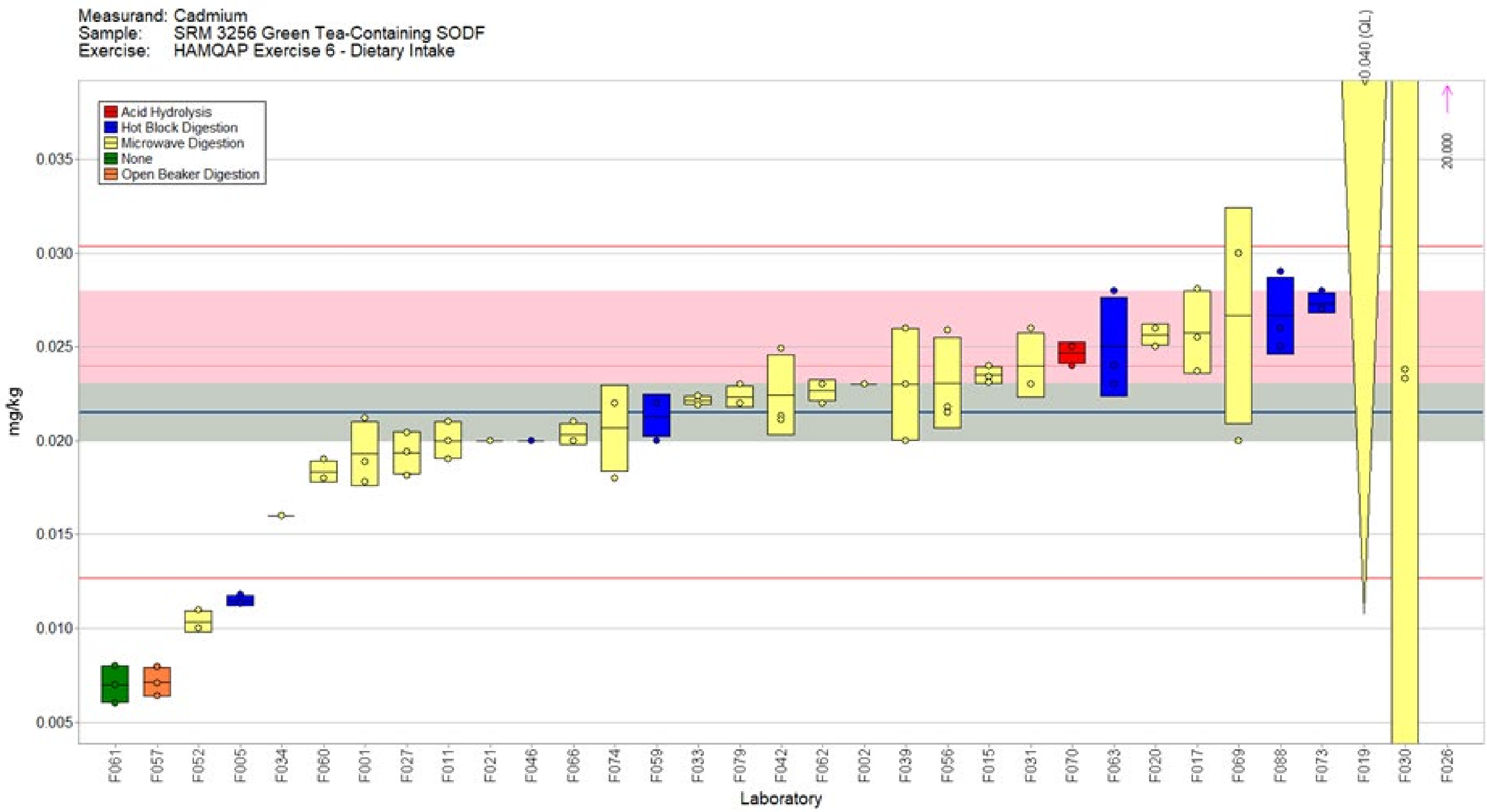


**Figure 2-7.** Cadmium in SRM 3256 Green Tea-Containing SODF (data summary view –sample preparation method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The solid blue 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 $Z'_{\mathrm{comm}}$ score, $|Z'_{\mathrm{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty ($U_{\mathrm{NIST}}$) and represents the range that results in an acceptable $Z_{\mathrm{NIST}}$ score, $|Z_{\mathrm{NIST}}| \leq 2$.

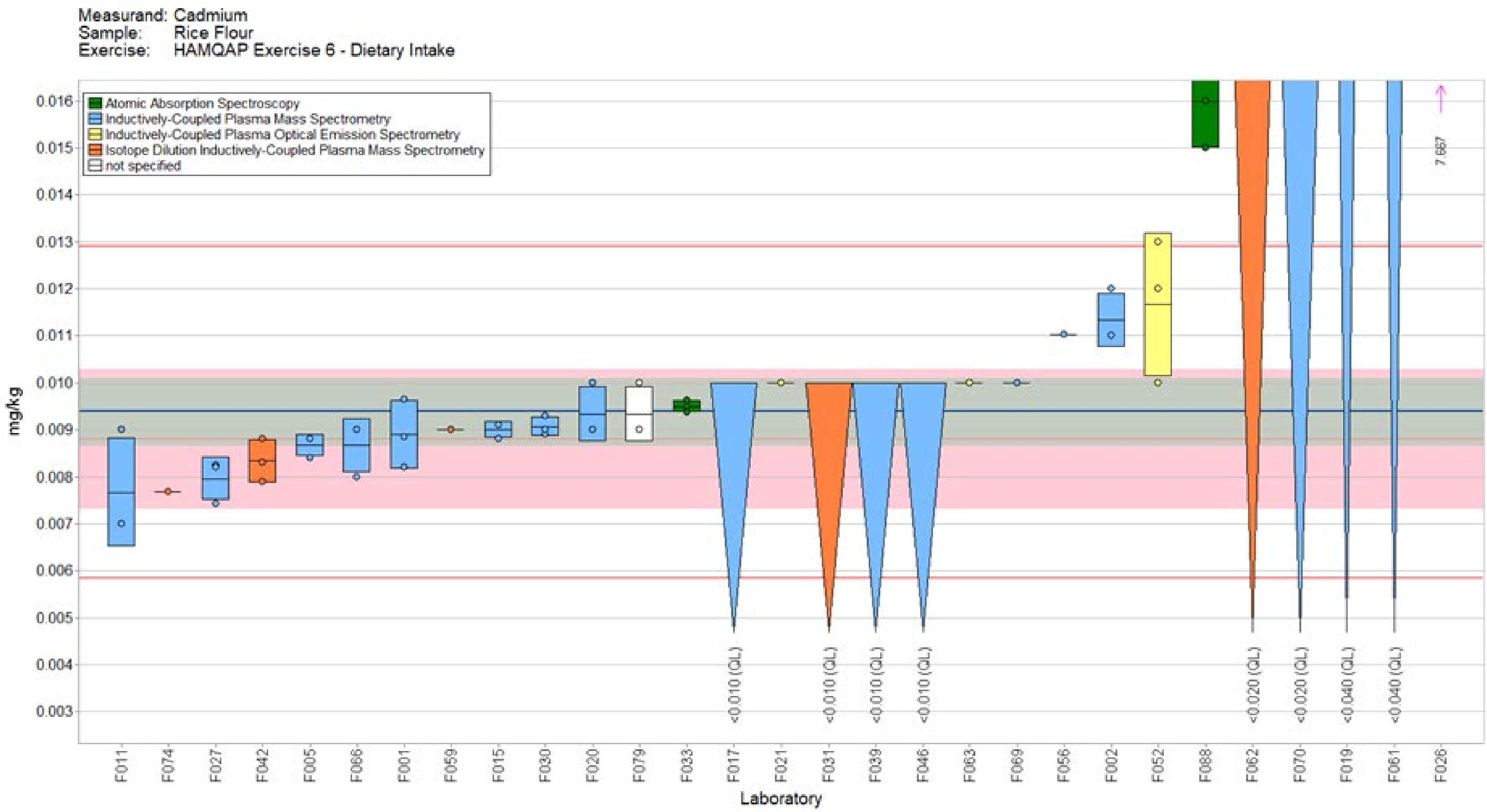


**Figure 2-8.** Cadmium in Rice Flour (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line represents 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 $Z'_{\text{comm}}$ score, $|Z'_{\text{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty ($U_{\text{NIST}}$) and represents the range that results in an acceptable $Z_{\text{NIST}}$ score, $|Z_{\text{NIST}}| \leq 2$.

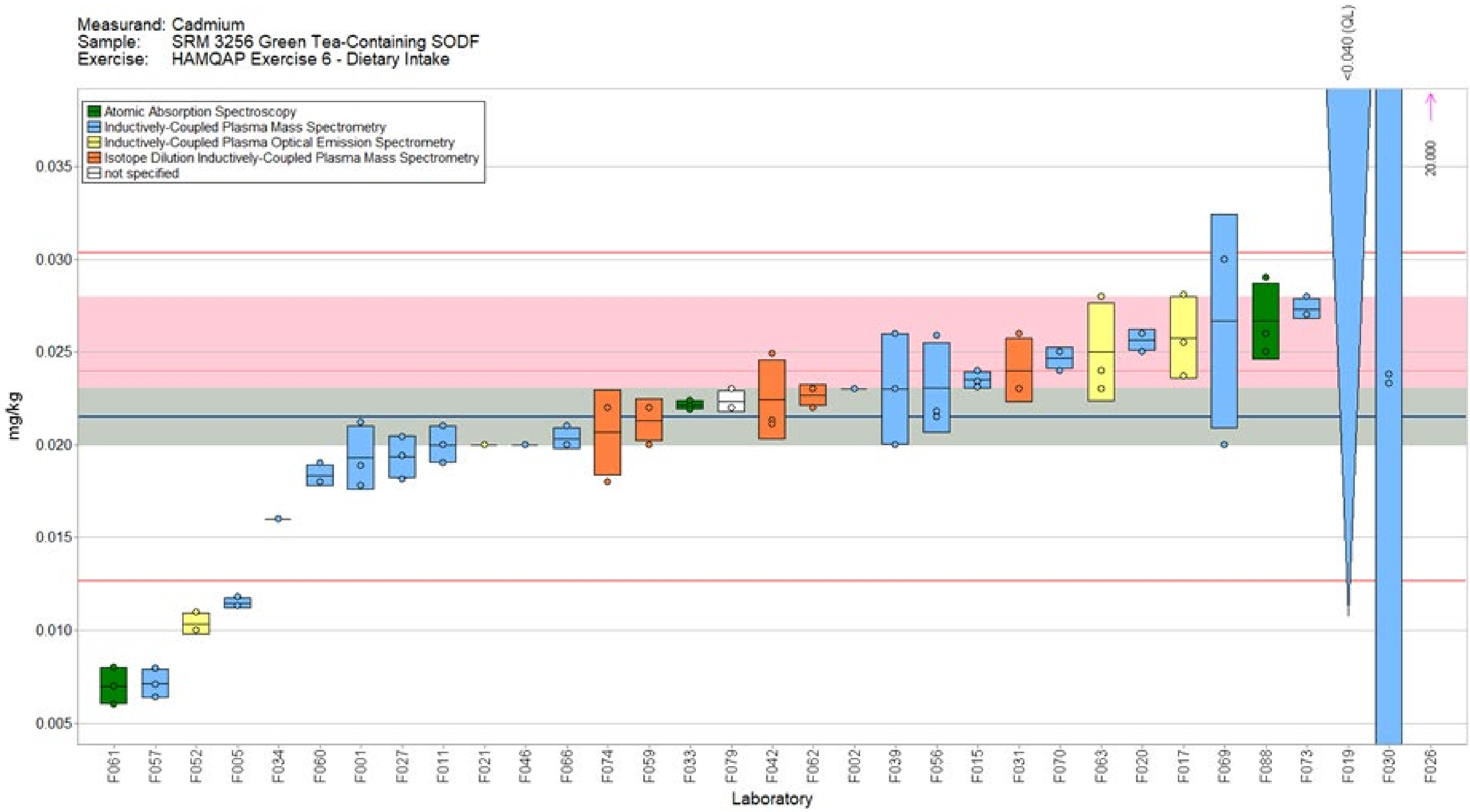


**Figure 2-9.** Cadmium in SRM 3256 Green Tea-Containing SODF (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue 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 $Z'_{\text{comm}}$ score, $|Z'_{\text{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty ($U_{\text{NIST}}$) and represents the range that results in an acceptable $Z_{\text{NIST}}$ score, $|Z_{\text{NIST}}| \leq 2$.

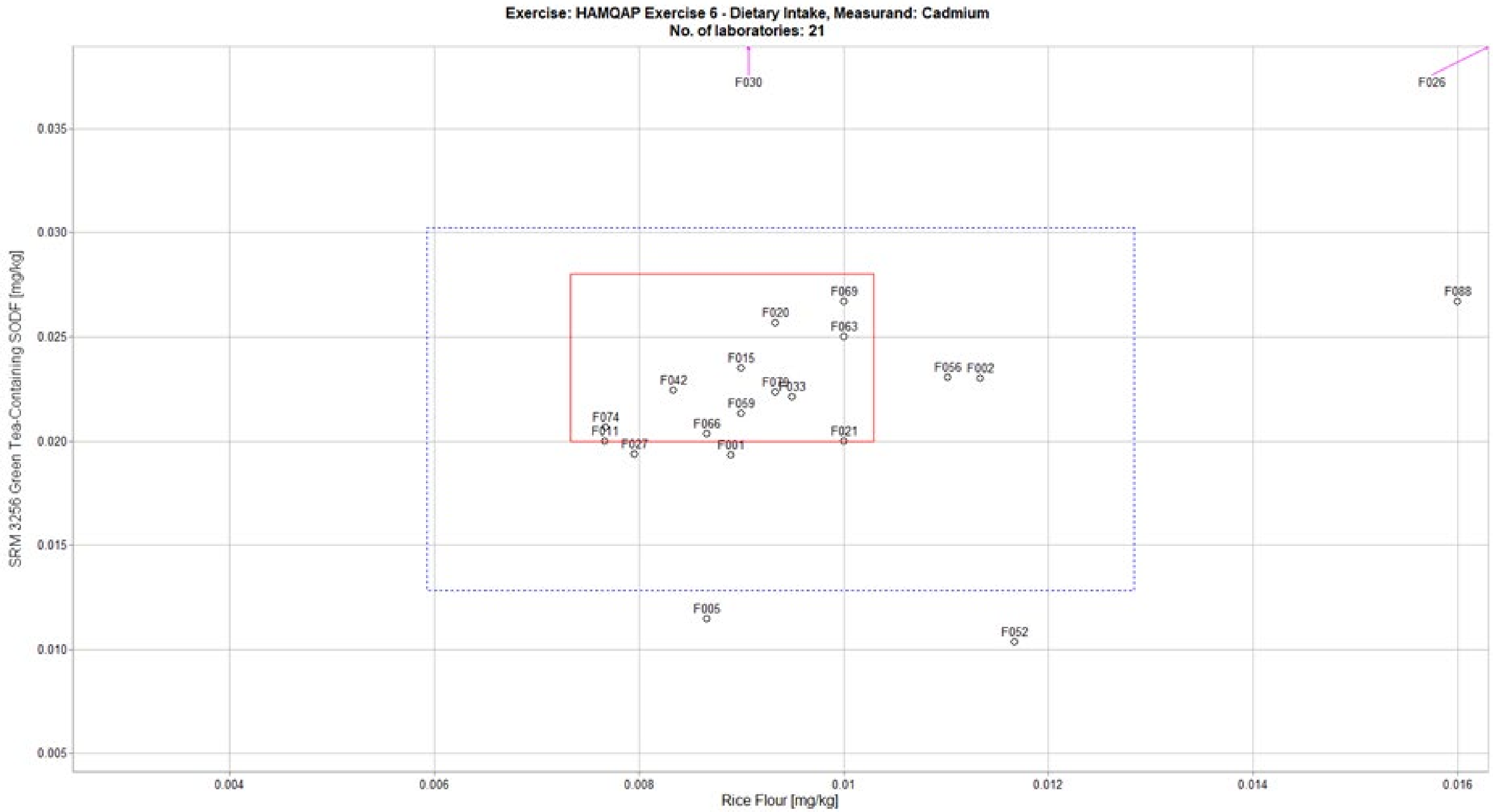


**Figure 2-10.** Laboratory means for cadmium in Rice Flour and SRM 3256 Green Tea-Containing SODF (sample/sample comparison view). In this view, the individual laboratory mean for one sample (Rice Flour) is compared to the mean for a second sample (SRM 3256). The solid red box represents the NIST range of tolerance for the two samples, Rice Flour (x-axis) and SRM 3256 (y-axis), which encompasses the target values bounded by their uncertainties ($U_{\mathrm{NIST}}$) and represents the range that results in an acceptable $Z_{\mathrm{NIST}}$ score, $|Z_{\mathrm{NIST}}| \leq 2$. The dotted blue box represents the consensus range of tolerance for Rice Flour (x-axis) and SRM 3256 (y-axis), calculated as the values above and below the consensus means that result in an acceptable $Z'_{\mathrm{comm}}$ score, $|Z'_{\mathrm{comm}}| \leq 2$

**Table 2-4.** Data summary table for lead in Rice Flour and SRM 3256 Green Tea-Containing SODF. Data points highlighted in blue have been identified as outside the consensus tolerance limits and would be estimated to result in an unacceptable $Z'_{comm}$ score, $|Z'_{comm}| \geq 2$.

| | | Lead | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Rice Flour (mg/kg) | | | | | SRM 3256 Green Tea Containing SODF (mg/kg) | | | | |
| | Lab | A | B | C | Avg | SD | A | B | C | Avg | SD |
| Individual Results | Target | | | | 0.017 | 0.005 | | | | 0.309 | 0.029 |
| | F001 | 0.0115 | 0.0124 | 0.0119 | 0.012 | 0.000 | 0.294 | 0.324 | 0.296 | 0.305 | 0.017 |
| | F002 | 0.013 | 0.012 | 0.013 | 0.013 | 0.001 | 0.256 | 0.252 | 0.27 | 0.259 | 0.009 |
| | F004 | | | | | | | | | | |
| | F005 | 0.0239 | 0.0176 | 0.019 | 0.020 | 0.003 | 0.1809 | 0.1969 | 0.1845 | 0.187 | 0.008 |
| | F011 | 0.015 | 0.014 | 0.009 | 0.013 | 0.003 | 0.312 | 0.323 | 0.293 | 0.309 | 0.015 |
| | F014 | | | | | | 0.53 | 0.49 | 0.58 | 0.533 | 0.045 |
| | F015 | 0.0158 | 0.0137 | 0.0143 | 0.015 | 0.001 | 0.2765 | 0.2764 | 0.2832 | 0.279 | 0.004 |
| | F017 | 0.0252 | 0.021 | 0.0255 | 0.024 | 0.003 | 0.276 | 0.301 | 0.292 | 0.290 | 0.013 |
| | F018 | | | | | | | | | | |
| | F019 | < 0.040 | < 0.040 | < 0.040 | | | 0.23 | 0.24 | 0.26 | 0.243 | 0.015 |
| | F020 | 0.04 | 0.04 | 0.05 | 0.043 | 0.006 | 0.3 | 0.31 | 0.29 | 0.300 | 0.010 |
| | F021 | 0.04 | 0.03 | 0.04 | 0.037 | 0.006 | 0.25 | 0.22 | 0.22 | 0.230 | 0.017 |
| | F022 | 0.025 | 0.027 | 0.025 | 0.026 | 0.001 | 0.31 | 0.29 | 0.27 | 0.290 | 0.020 |
| | F026 | 13 | 14 | 13 | 13.3 | 0.58 | 266 | 258 | 253 | 259 | 6.6 |
| | F027 | 0.03332 | 0.02479 | 0.02248 | 0.027 | 0.006 | 0.29219 | 0.29985 | 0.26708 | 0.286 | 0.017 |
| | F030 | 0.122 | 0.126 | 0.145 | 0.131 | 0.012 | 0.291 | 0.303 | 0.295 | 0.296 | 0.006 |
| | F031 | 0.024 | 0.024 | 0.028 | 0.025 | 0.002 | 0.315 | 0.316 | 0.296 | 0.309 | 0.011 |
| | F032 | | | | | | | | | | |
| | F033 | 0.0144 | 0.0142 | 0.0146 | 0.014 | 0.000 | 0.312 | 0.309 | 0.315 | 0.312 | 0.003 |
| | F034 | < 0.020 | < 0.020 | < 0.020 | | | 0.2 | 0.193 | 0.195 | 0.196 | 0.004 |
| | F039 | 0.016 | 0.013 | 0.015 | 0.015 | 0.002 | 0.259 | 0.277 | 0.27 | 0.269 | 0.009 |
| | F041 | 0.015 | 0.012 | 0.013 | 0.013 | 0.002 | 0.298 | 0.294 | 0.291 | 0.294 | 0.004 |
| | F042 | 0.0117 | 0.0109 | 0.0108 | 0.011 | 0.000 | 0.284 | 0.292 | 0.274 | 0.283 | 0.009 |
| | F045 | | | | | | | | | | |
| | F046 | | 0.03 | 0.03 | 0.030 | 0 | 0.27 | 0.27 | 0.3 | 0.280 | 0.017 |
| | F051 | | | | | | | | | | |
| | F052 | 0.008 | 0.0082 | 0.0079 | 0.008 | 0.000 | 0.077 | 0.08 | 0.076 | 0.078 | 0.002 |
| | F056 | 0.01903 | | | 0.019 | | 0.3055 | 0.279 | 0.264 | 0.283 | 0.021 |
| | F057 | | | | | | 0.358141 | 0.316359 | 0.296551 | 0.324 | 0.031 |
| | F059 | 0.01 | 0.01 | 0.01 | 0.010 | 0 | 0.26 | 0.26 | 0.27 | 0.263 | 0.006 |
| | F060 | | | | | | 0.245 | 0.25 | 0.25 | 0.248 | 0.003 |
| | F061 | < 0.050 | < 0.050 | < 0.050 | | | 0.206 | 0.223 | 0.227 | 0.219 | 0.011 |
| | F062 | < 0.020 | < 0.020 | < 0.020 | | | 0.316 | 0.321 | 0.308 | 0.315 | 0.007 |
| | F063 | | | | | | 0.241 | 0.259 | 0.256 | 0.252 | 0.010 |
| | F066 | 0.013 | 0.013 | 0.013 | 0.013 | 0 | 0.25 | 0.264 | 0.272 | 0.262 | 0.011 |
| | F069 | 0.01 | 0.01 | 0.01 | 0.010 | 0 | 0.32 | 0.32 | 0.33 | 0.323 | 0.006 |
| | F070 | < 0.030 | < 0.030 | < 0.030 | | | 0.287 | 0.347 | 0.302 | 0.312 | 0.031 |
| | F073 | | | | | | 0.315 | 0.336 | 0.323 | 0.325 | 0.011 |
| | F074 | 0.0135 | | | 0.014 | | 0.253 | 0.26 | 0.252 | 0.255 | 0.004 |
| | F077 | | | | | | | | | | |
| | F079 | 0.014 | 0.014 | 0.013 | 0.014 | 0.001 | 0.31 | 0.31 | 0.35 | 0.323 | 0.023 |
| | F088 | 0.291 | 0.272 | 0.281 | 0.281 | 0.010 | 0.307 | 0.31 | 0.281 | 0.299 | 0.016 |
| | F089 | | | | | | | | | | |
| Community Results | | Consensus Mean | | | 0.018 | | Consensus Mean | | | 0.281 | |
| | | Consensus Standard Deviation | | | 0.009 | | Consensus Standard Deviation | | | 0.043 | |
| | | Maximum | | | 13.3 | | Maximum | | | 259 | |
| | | Minimum | | | 0.008 | | Minimum | | | 0.078 | |
| | | N | | | 24 | | N | | | 36 | |

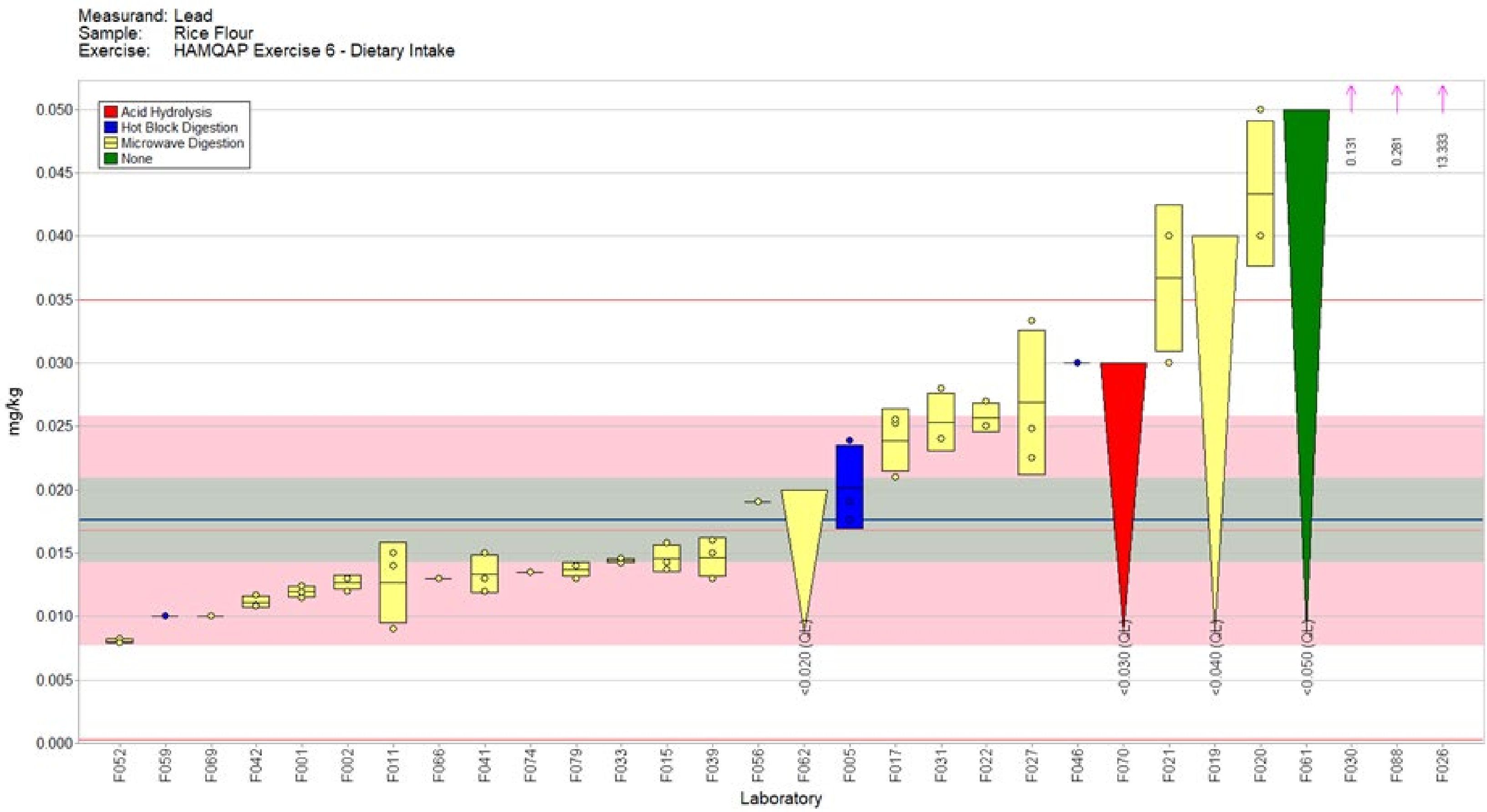


**Figure 2-11.** Lead in Rice Flour (data summary view –sample preparation method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The solid blue 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 $Z'_{\text{comm}}$ score, $|Z'_{\text{comm}}| \leq 2$, with the lower range set at zero. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty ($U_{\text{NIST}}$) and represents the range that results in an acceptable $Z_{\text{NIST}}$ score, $|Z_{\text{NIST}}| \leq 2$.

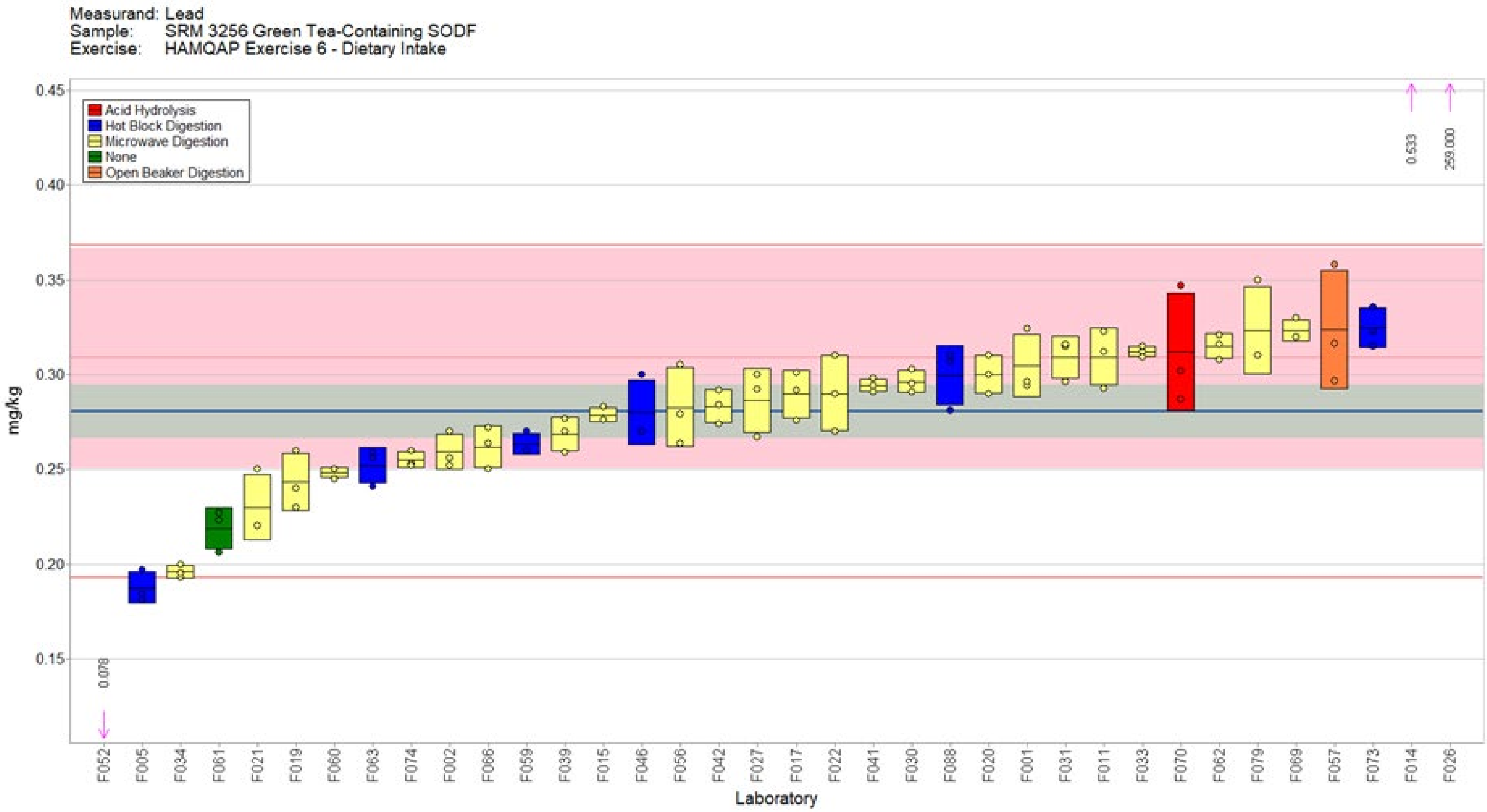


**Figure 2-12.** Lead in SRM 3256 Green Tea-Containing SODF (data summary view –sample preparation method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The solid blue 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 $Z'_{\mathrm{comm}}$ score, $|Z'_{\mathrm{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty ($U_{\mathrm{NIST}}$) and represents the range that results in an acceptable $Z_{\mathrm{NIST}}$ score, $|Z_{\mathrm{NIST}}| \leq 2$.

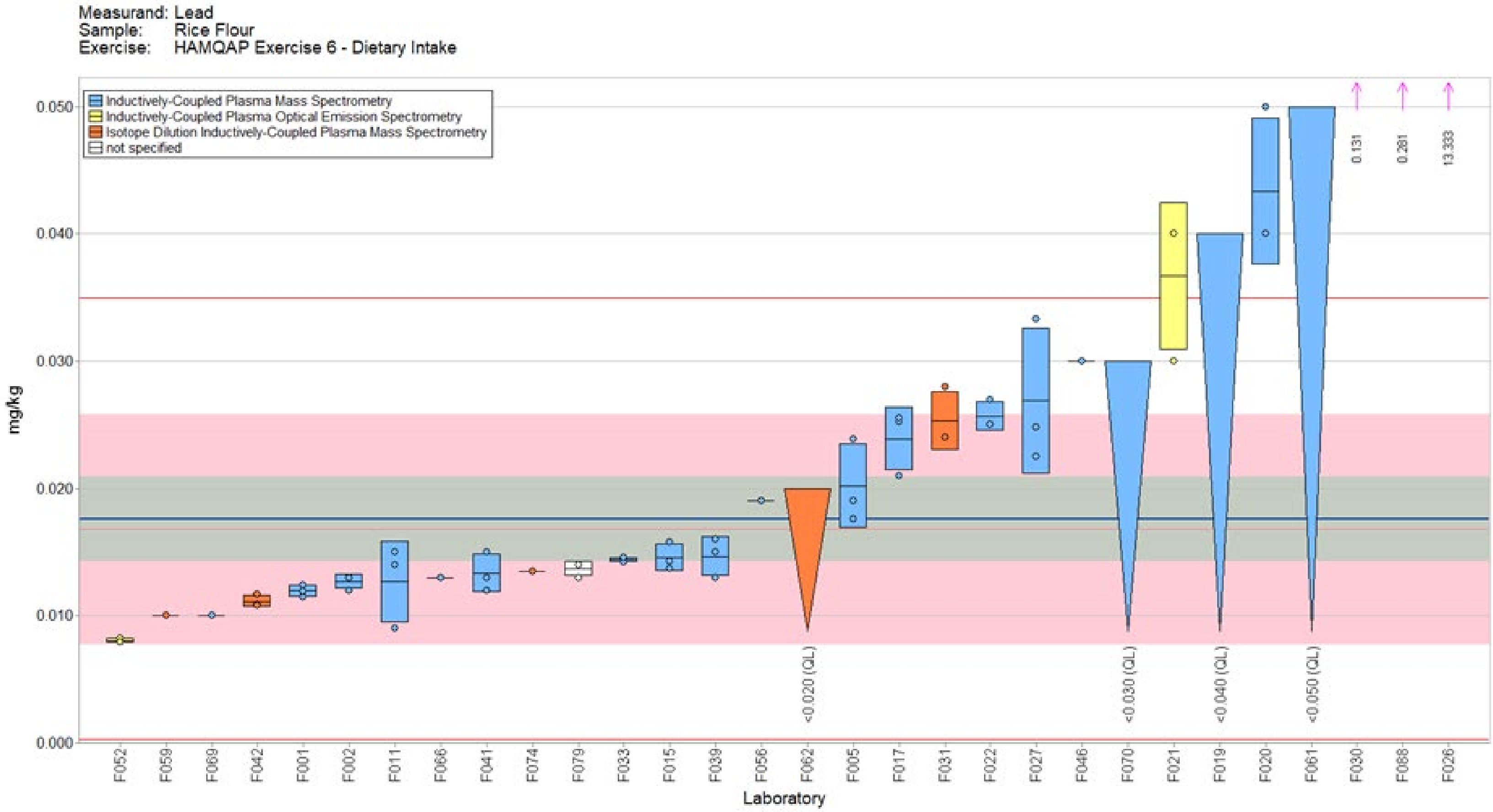


**Figure 2-13.** Lead in Rice Flour (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line represents 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 $Z'_{\mathrm{comm}}$ score, $|Z'_{\mathrm{comm}}| \leq 2$, with the lower range set to zero. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty ($U_{\mathrm{NIST}}$) and represents the range that results in an acceptable $Z_{\mathrm{NIST}}$ score, $|Z_{\mathrm{NIST}}| \leq 2$.

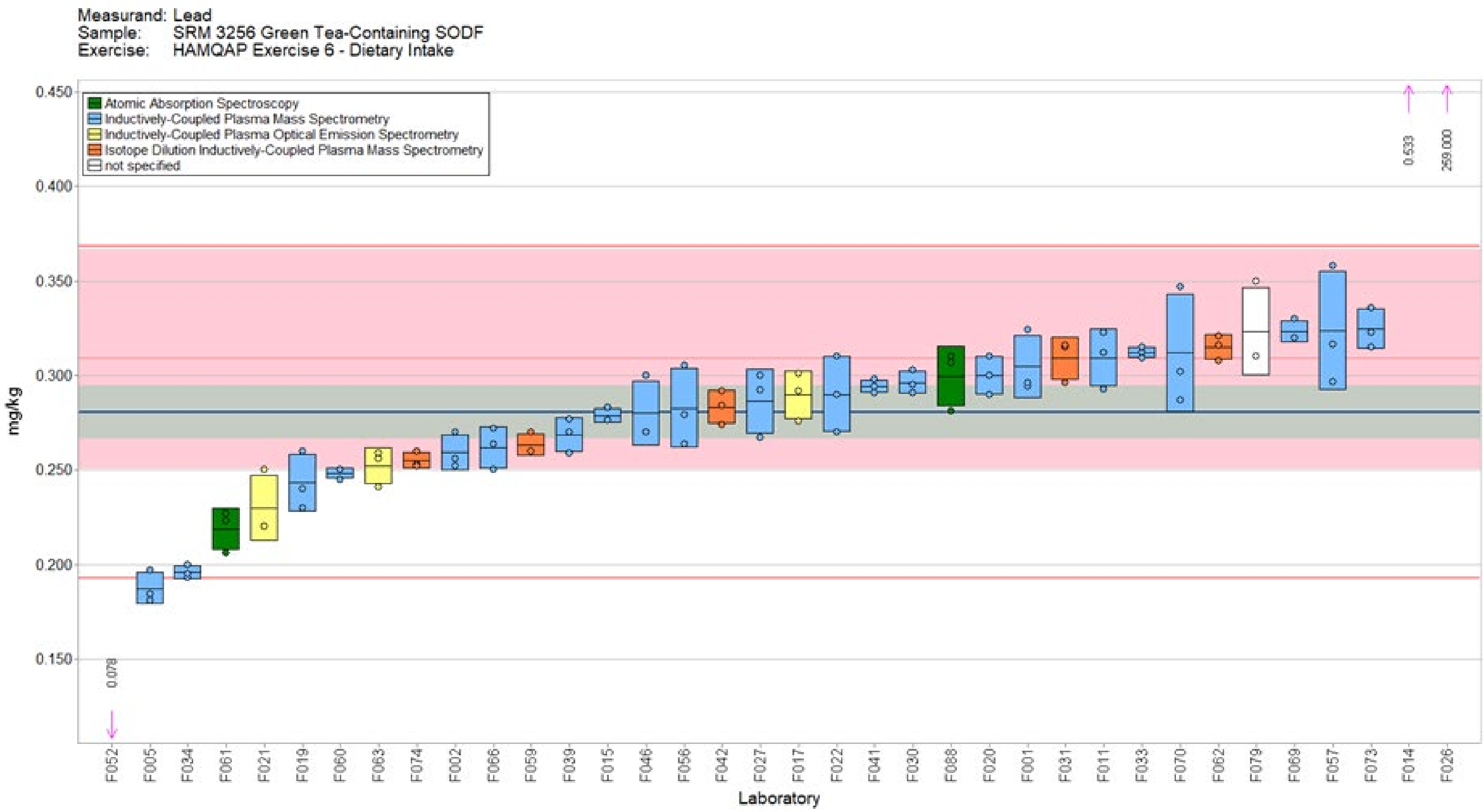


**Figure 2-14.** Lead in SRM 3256 Green Tea-Containing SODF (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue 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 $Z'_{\text{comm}}$ score, $|Z'_{\text{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty ($U_{\text{NIST}}$) and represents the range that results in an acceptable $Z_{\text{NIST}}$ score, $|Z_{\text{NIST}}| \leq 2$.

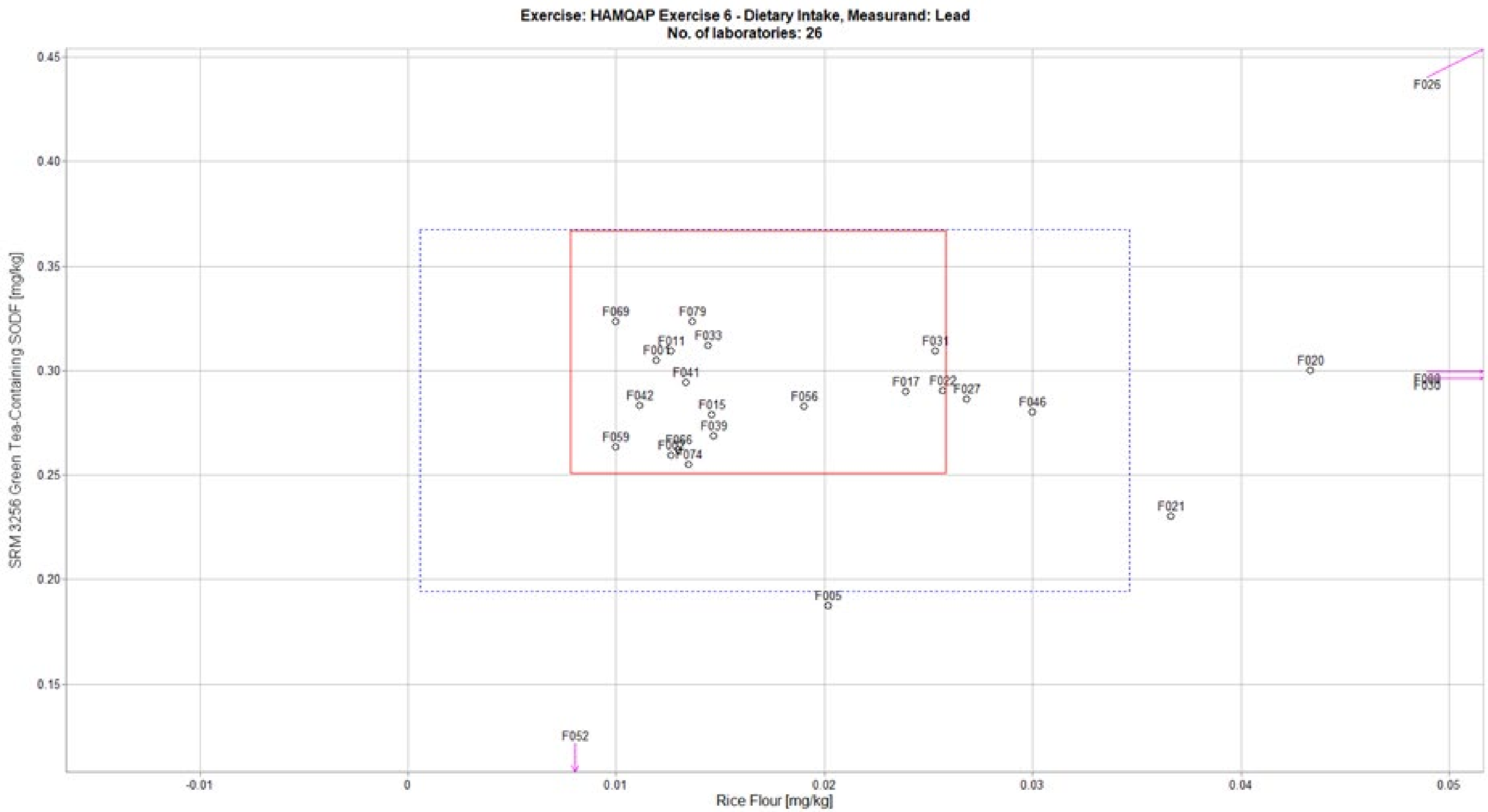


**Figure 2-15.** Laboratory means for lead in Rice Flour and SRM 3256 Green Tea-Containing SODF (sample/sample comparison view). In this view, the individual laboratory mean for one sample (Rice Flour) is compared to the mean for a second sample (SRM 3256). The solid red box represents the NIST range of tolerance for the two samples, Rice Flour (x-axis) and SRM 3256 (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 Rice Flour (x-axis) and SRM 3256 (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 2-5.** Data summary table for mercury in Rice Flour and SRM 3256 Green Tea-Containing SODF. Data points highlighted in blue have been identified as outside the consensus tolerance limits and would be estimated to result in an unacceptable $Z'_{comm}$ score, $|Z'_{comm}| \geq 2$. Data points highlighted in red have a zero or non-numeric data point.

| | | Mercury | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Rice Flour (mg/kg) | | | | | SRM 3256 Green Tea Containing SODF (mg/kg) | | | | |
| | Lab | A | B | C | Avg | SD | A | B | C | Avg | SD |
| Individual Results | Target | | | | 0.0020 | 0.0001 | | | | 0.014 | 0.002 |
| | F001 | 0.00031 | 2.00E-04 | 0.00051 | 0.00034 | 0.0001572 | 0.0124 | 0.0114 | 0.011 | 0.0116 | 0.0007 |
| | F002 | 0 | 0 | 0 | 0 | 0 | 0.012 | 0.013 | 0.012 | 0.012 | 0.001 |
| | F004 | | | | | | | | | | |
| | F005 | 0.002 | 0.003 | 0.002 | 0.00233 | 0.00058 | 0.0131 | 0.0138 | 0.0114 | 0.0128 | 0.0012 |
| | F011 | < 0.003 | < 0.003 | < 0.003 | | | 0.004 | < 0.003 | < 0.003 | 0.0040 | |
| | F014 | 0.0034 | 0.0045 | 0.0037 | 0.00387 | 0.00057 | 0.023 | 0.021 | 0.019 | 0.0210 | 0.0020 |
| | F015 | 0.0014 | 0.0012 | 0.0012 | 0.00127 | 0.00012 | 0.02 | 0.013 | 0.018 | 0.017 | 0.004 |
| | F017 | < 0.010 | < 0.010 | < 0.010 | | | 0.0132 | 0.0133 | 0.0151 | 0.0139 | 0.0011 |
| | F018 | | | | | | | | | | |
| | F019 | < 0.040 | < 0.040 | < 0.040 | | | < 0.040 | < 0.040 | < 0.040 | | |
| | F020 | < 0.050 | < 0.050 | < 0.050 | | | < 0.050 | < 0.050 | < 0.050 | | |
| | F021 | < 0.010 | < 0.010 | < 0.010 | | | 0.01 | 0.01 | 0.01 | 0.01 | 0 |
| | F026 | 1 | 2 | 2 | 1.67 | 0.58 | 15 | 14 | 14 | 14.33 | 0.58 |
| | F027 | 0.00652 | 0.0149 | 0.00931 | 0.01024 | 0.00427 | 0.01571 | 0.01599 | 0.01242 | 0.0147 | 0.0020 |
| | F030 | < 0.010 | < 0.010 | < 0.010 | | | 0.0106 | 0.0111 | 0.012 | 0.0112 | 0.0007 |
| | F031 | < 0.010 | < 0.010 | < 0.010 | | | < 0.010 | < 0.010 | < 0.010 | | |
| | F032 | | | | | | | | | | |
| | F033 | 0.00173 | 0.00169 | 0.00177 | 0.00173 | 0.00004 | 0.0111 | 0.0113 | 0.0115 | 0.0113 | 0.0002 |
| | F034 | | | | | | 0.013 | 0.012 | 0.012 | 0.012 | 0.0006 |
| | F039 | < 0.010 | < 0.010 | < 0.010 | | | 0.016 | 0.015 | 0.018 | 0.016 | 0.002 |
| | F042 | 0.0016 | 0.0014 | 0.0016 | 0.00153 | 0.00012 | 0.011 | 0.0122 | 0.0108 | 0.0113 | 0.0008 |
| | F045 | | | | | | | | | | |
| | F046 | 0.03 | 0.04 | 0.02 | 0.03 | 0.01 | 0.03 | 0.03 | 0.01 | 0.023 | 0.012 |
| | F051 | | | | | | | | | | |
| | F052 | 0.0101 | 0.01 | 0.0105 | 0.0102 | 0.0003 | 0.01 | 0.0103 | 0.0102 | 0.0102 | 0.0002 |
| | F056 | < 0.001 | | | | | < 0.001 | < 0.001 | < 0.001 | | |
| | F057 | | | | | | 0.015129 | 0.01202 | 0.011792 | 0.013 | 0.002 |
| | F059 | < 0.005 | < 0.005 | < 0.005 | | | 0.013 | 0.015 | 0.013 | 0.014 | 0.001 |
| | F060 | | | | | | 0.012 | 0.012 | 0.01 | 0.011 | 0.001 |
| | F061 | < 0.010 | < 0.010 | < 0.010 | | | 0.01 | 0.01 | 0.01 | 0.01 | 0 |
| | F062 | < 0.005 | < 0.005 | < 0.005 | | | 0.018 | 0.019 | 0.016 | 0.018 | 0.002 |
| | F063 | | | | | | 0.012 | 0.009 | 0.013 | 0.011 | 0.002 |
| | F066 | < 0.007 | < 0.007 | < 0.007 | | | 0.011 | 0.011 | 0.01 | 0.011 | 0.001 |
| | F069 | 0.01 | 0.01 | 0.01 | 0.01 | 0 | 0.03 | 0.03 | 0.03 | 0.03 | 0 |
| | F070 | < 0.030 | < 0.030 | < 0.030 | | | < 0.030 | < 0.030 | < 0.030 | | |
| | F073 | | | | | | 0.009 | 0.011 | 0.013 | 0.011 | 0.002 |
| | F074 | 0.00347 | | | 0.00347 | | 0.003 | 0.001 | 0.001 | 0.0017 | 0.001 |
| | F077 | | | | | | | | | | |
| | F079 | 0.0013 | 0.0012 | 0.00068 | 0.00106 | 0.00033 | 0.013 | 0.013 | 0.012 | 0.013 | 0.001 |
| | F089 | | | | | | | | | | |
| Community Results | | Consensus Mean | | | 0.004 | | Consensus Mean | | | 0.013 | |
| | | Consensus Standard Deviation | | | 0.004 | | Consensus Standard Deviation | | | 0.004 | |
| | | Maximum | | | 1.67 | | Maximum | | | 14.33 | |
| | | Minimum | | | 0 | | Minimum | | | 0.002 | |
| | | N | | | 13 | | N | | | 27 | |

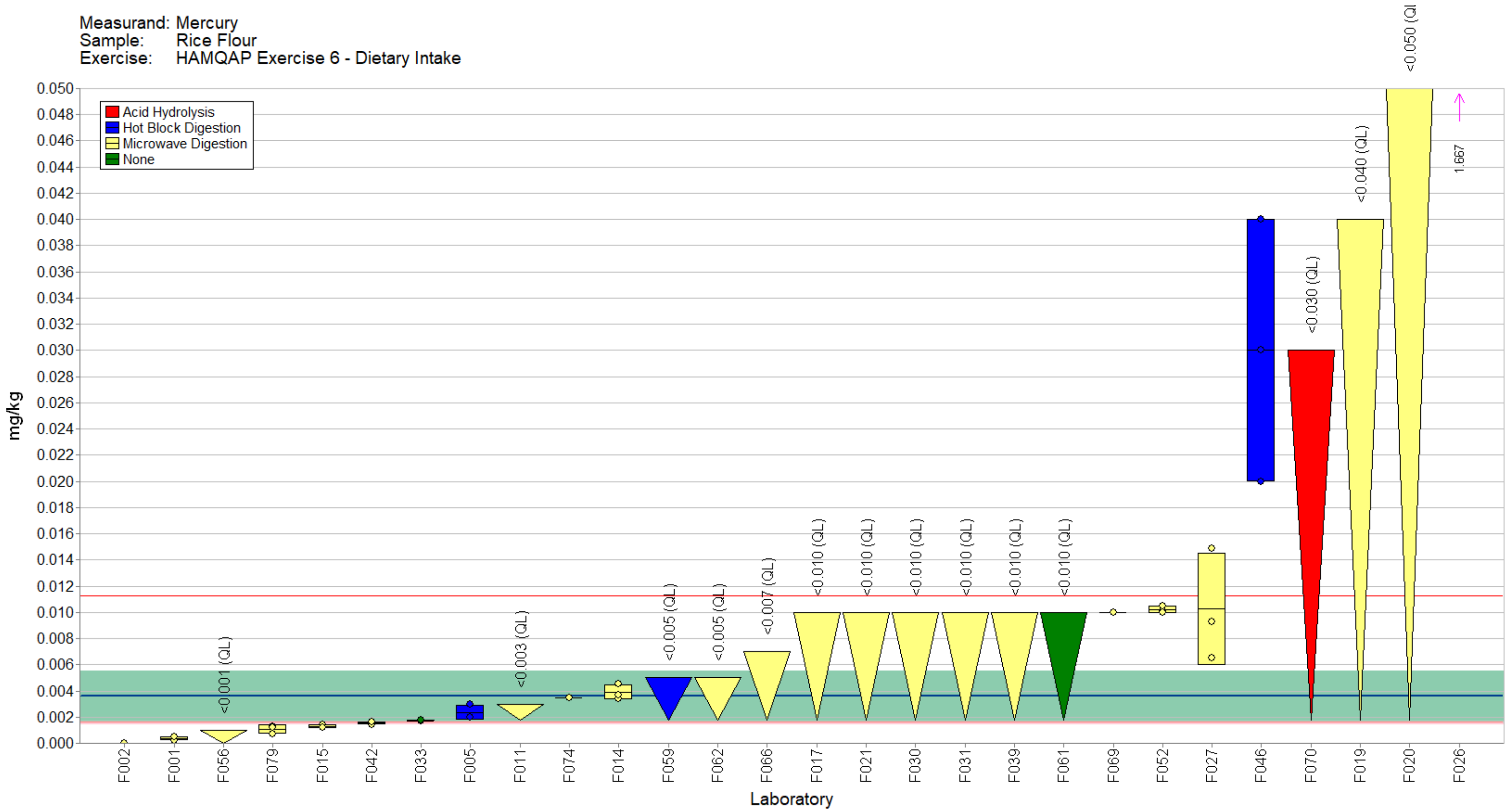


**Figure 2-16.** Mercury in Rice Flour (data summary view –sample preparation method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The solid blue 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 $Z'_{\text{comm}}$ score, $|Z'_{\text{comm}}| \leq 2$, with the lower range set at zero. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty ($U_{\text{NIST}}$) and represents the range that results in an acceptable $Z_{\text{NIST}}$ score, $|Z_{\text{NIST}}| \leq 2$.

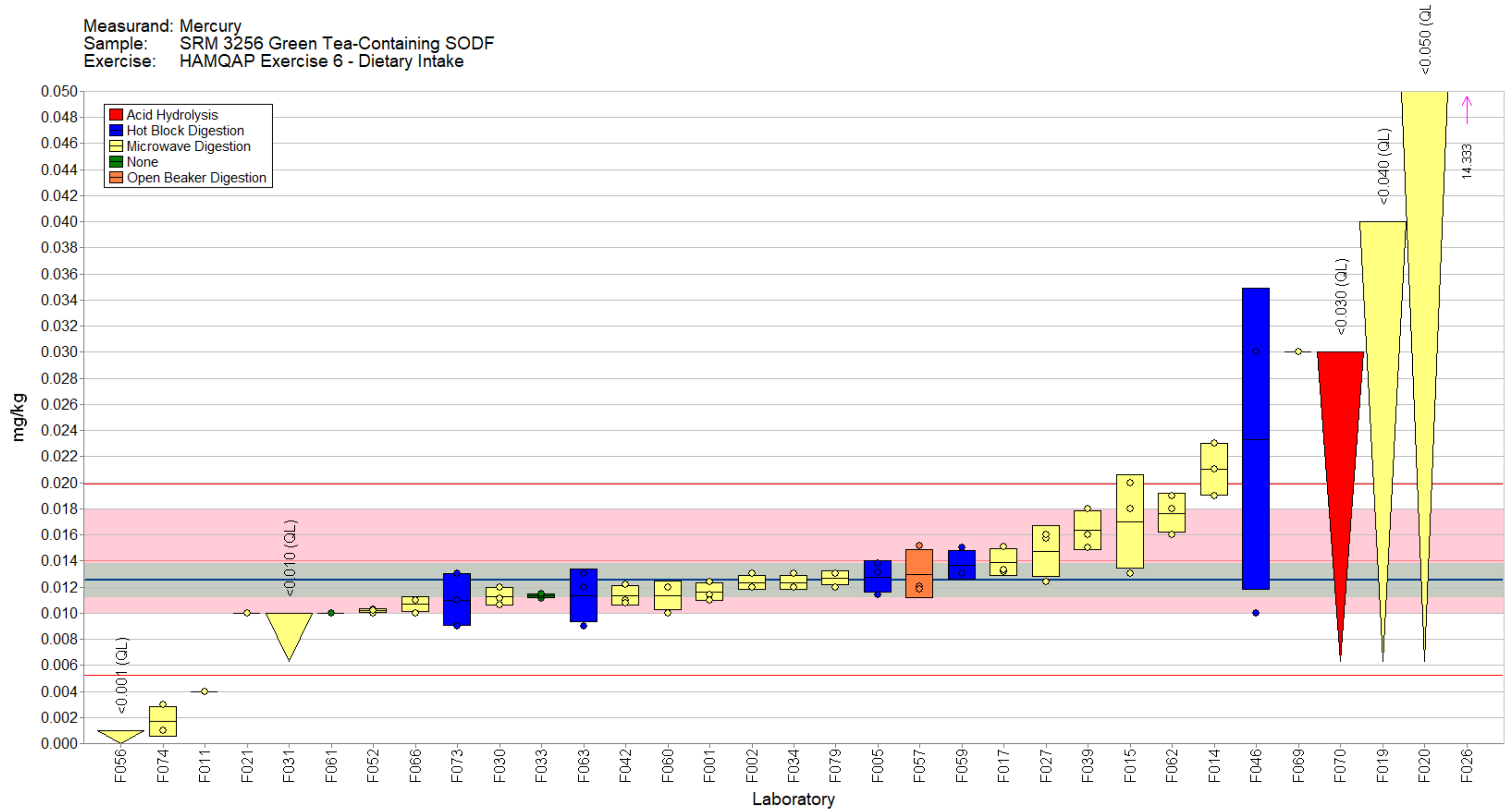


**Figure 2-17.** Mercury in SRM 3256 Green Tea-Containing SODF (data summary view –sample preparation method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The solid blue 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 $Z'_{\text{comm}}$ score, $|Z'_{\text{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty ($U_{\text{NIST}}$) and represents the range that results in an acceptable $Z_{\text{NIST}}$ score, $|Z_{\text{NIST}}| \leq 2$.

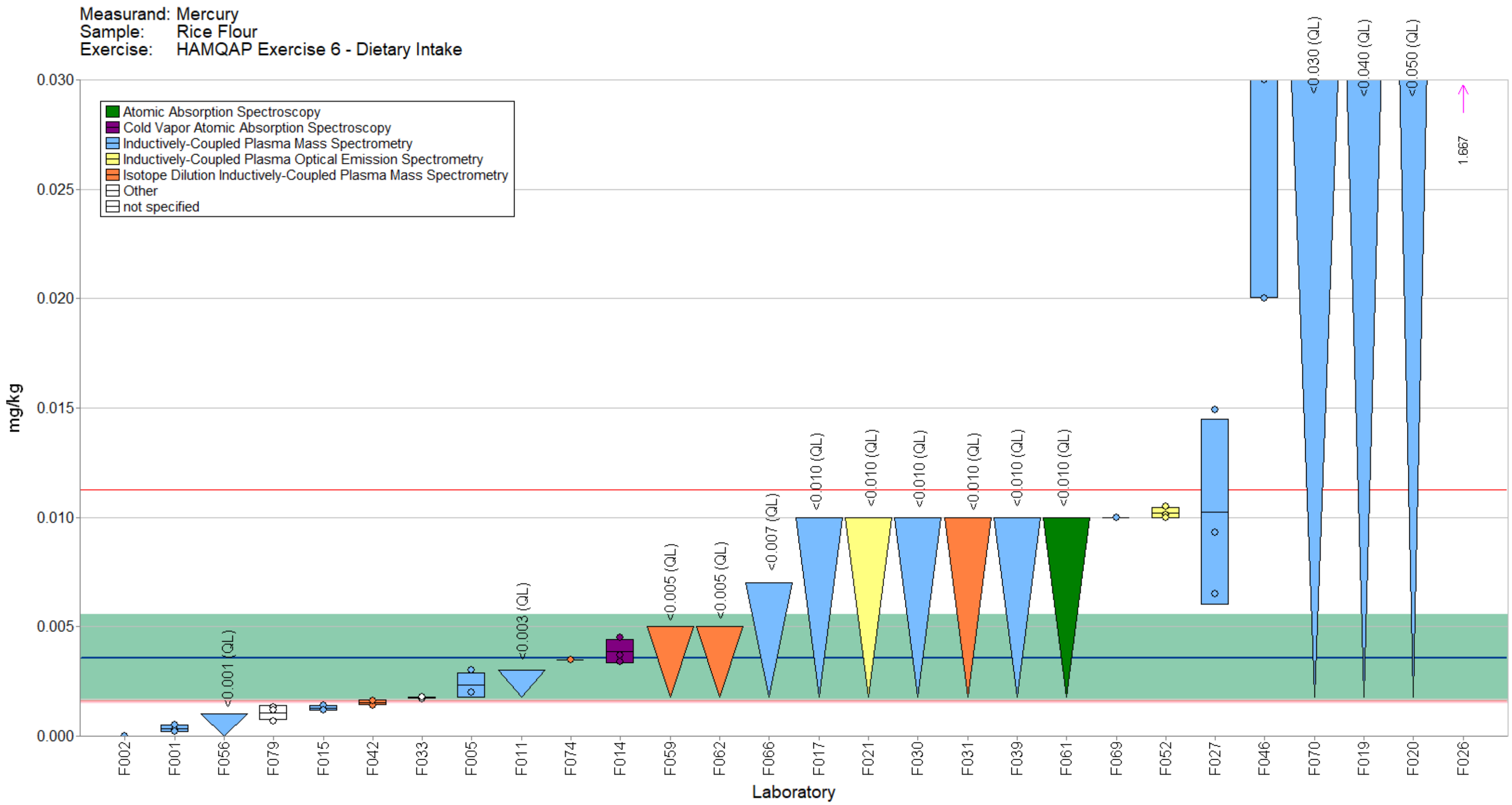


**Figure 2-18.** Mercury in Rice Flour (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line represents 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 $Z'_{\text{comm}}$ score, $|Z'_{\text{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty ($U_{\text{NIST}}$) and represents the range that results in an acceptable $Z_{\text{NIST}}$ score, $|Z_{\text{NIST}}| \leq 2$.

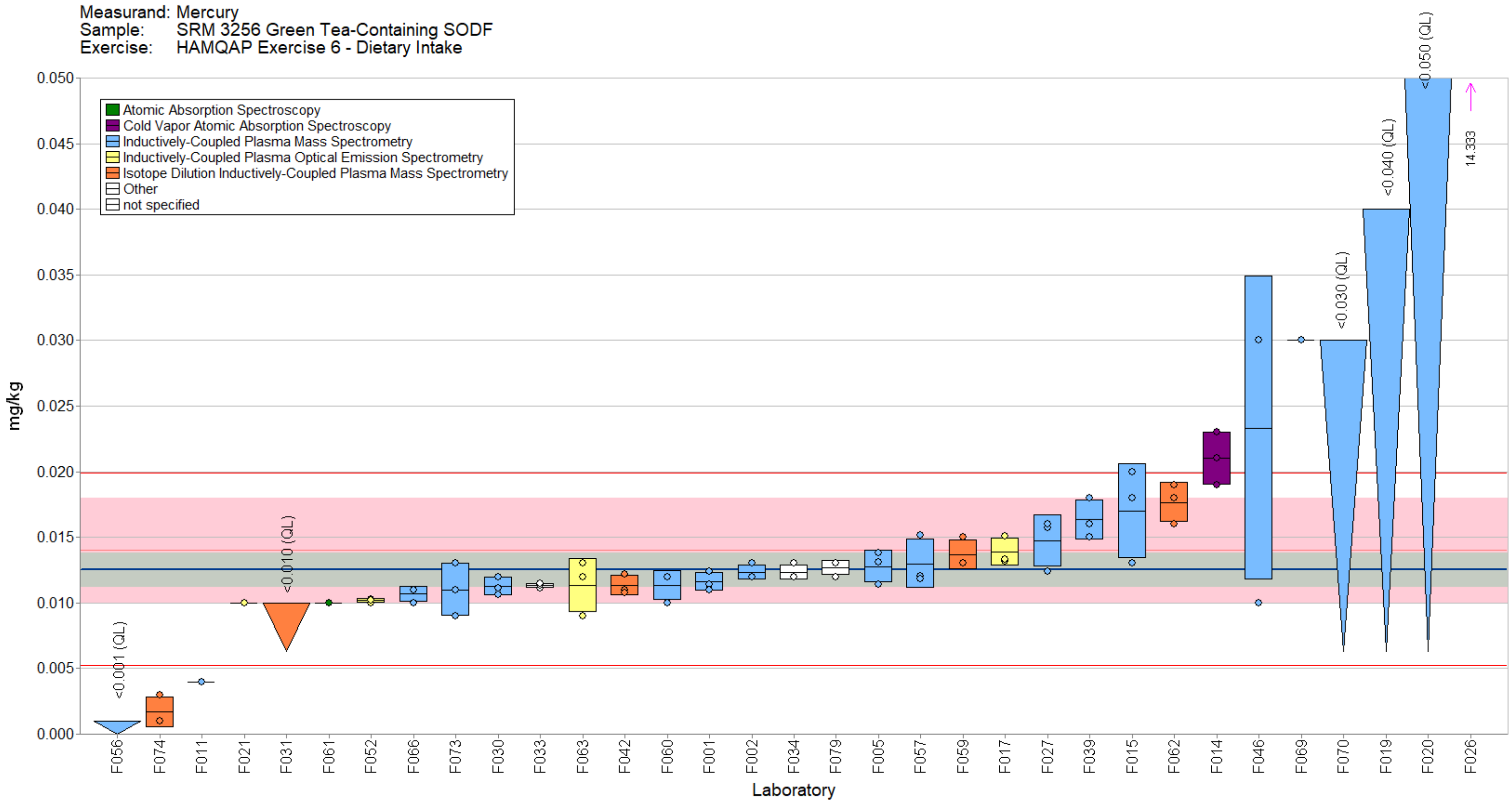


**Figure 2-19.** Mercury in SRM 3256 Green Tea-Containing SODF (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue 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 $Z'_{\mathrm{comm}}$ score, $|Z'_{\mathrm{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty ($U_{\mathrm{NIST}}$) and represents the range that results in an acceptable $Z_{\mathrm{NIST}}$ score, $|Z_{\mathrm{NIST}}| \leq 2$.

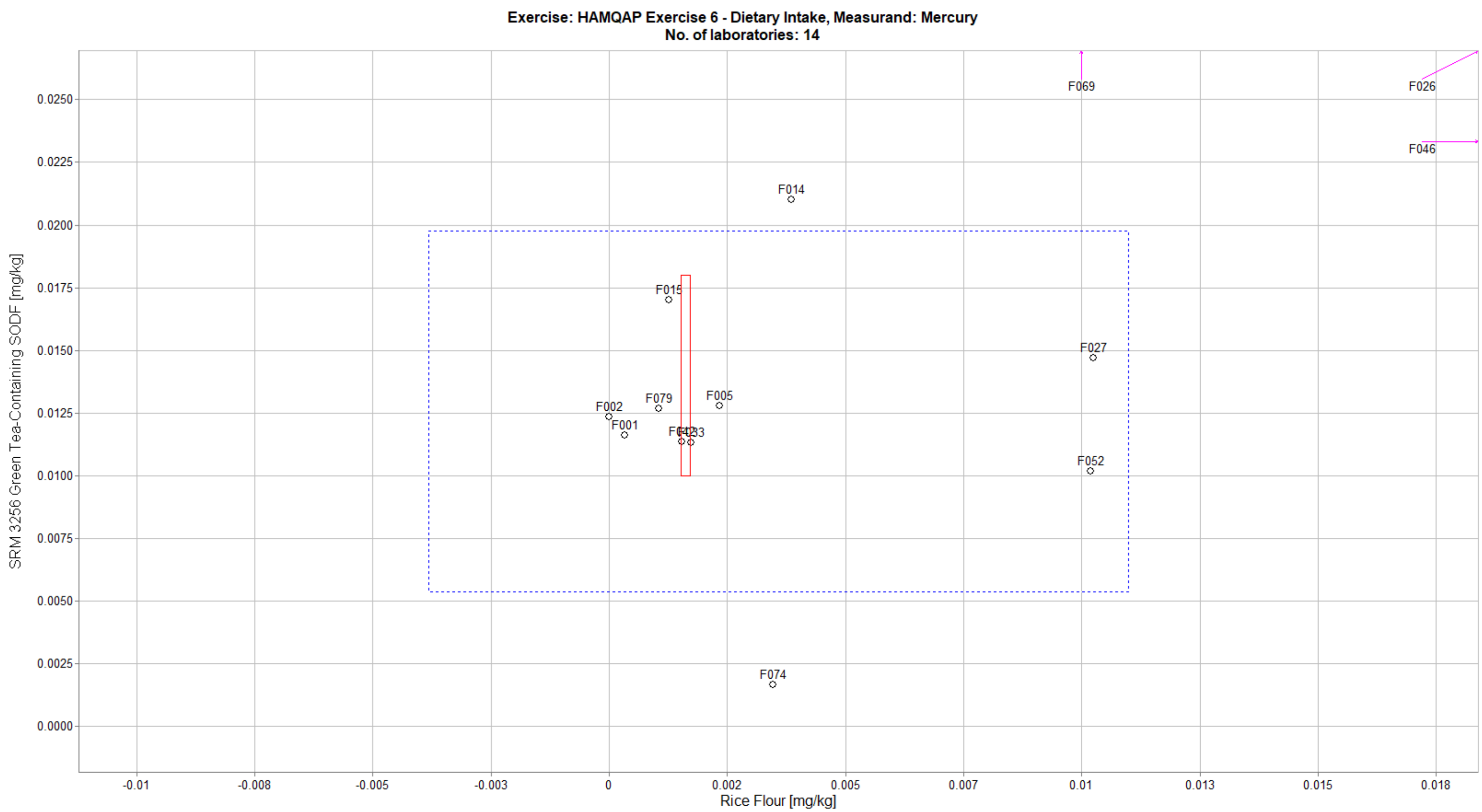


**Figure 2-20.** Laboratory means for mercury in Rice Flour and SRM 3256 Green Tea-Containing SODF (sample/sample comparison view). In this view, the individual laboratory mean for one sample (Rice Flour) is compared to the mean for a second sample (SRM 3256). The solid red box represents the NIST range of tolerance for the two samples, Rice Flour (x-axis) and SRM 3256 (y-axis), which encompasses the target values bounded by their uncertainties ($U_{\mathrm{NIST}}$) and represents the range that results in an acceptable $Z_{\mathrm{NIST}}$ score, $|Z_{\mathrm{NIST}}| \leq 2$. The dotted blue box represents the consensus range of tolerance for Rice Flour (x-axis) and SRM 3256 (y-axis), calculated as the values above and below the consensus means that result in an acceptable $Z'_{\mathrm{comm}}$ score, $|Z'_{\mathrm{comm}}| \leq 2$.

## SECTION 3: WATER-SOLUBLE VITAMINS (Biotin and Vitamin C (Ascorbic Acid))

Study Overview

In this study, participants were provided with samples of Infant Formula A and Multivitamin B for dietary intake. Participants were asked to use in-house analytical methods to determine the mass fraction (mg/kg) of biotin and vitamin C in each matrix. Biotin and vitamin C are essential vitamins commonly found in certain foods and dietary supplements. Biotin is critical for the metabolism of fatty acids, glucose, and amino acids, and is also involved in gene regulation and cell signaling. Vitamin C is an important antioxidant, required for the biosynthesis of collagen, L-carnitine, and some neurotransmitters, and is also involved in protein metabolism and immune function. Accurate measurement of water-soluble vitamins in foods provides confidence for both food labeling and dietary intake studies.

Dietary Intake Sample Information

*Infant Formula A.* Participants were provided with three packets, each containing approximately 10 g of powdered infant formula. Participants were asked to store the material at –20 °C in the original unopened packet and to prepare one sample and report one value from each packet provided. Before use, participants were instructed to thoroughly mix the contents of the packet prior to removal of a test portion for analysis. Sample sizes of at least 1 g and 2 g were suggested for the determination of biotin and vitamin C, respectively. The approximate analyte levels were not reported to participants prior to the study. The NIST-determined values for biotin and vitamin C in the infant formula sample were assigned using only the results from the manufacturer of the material. The NIST-determined values and uncertainties for biotin and vitamin C are provided in the table below on an as-received basis.

| Analyte | NIST-Determined Mass Fraction in Infant Formula A (mg/kg) |
|---|---|
| Biotin | 2.14 ± 0.14 |
| Vitamin C (Ascorbic Acid) | 22.04 ± 1.66 |

*Multivitamin B.* Participants were provided with three bottles, each containing 30 multivitamin tablets. Participants were asked to store the material at controlled room temperature (20 °C to 25 °C) in the original unopened bottles and to prepare one sample and report one value from each bottle provided. Before use, participants were instructed to grind all 30 tablets, mix the resulting powder thoroughly prior to removal of a test portion for analysis, and to use a sample size of at least 1.5 g and 2 g, respectively, for determination of biotin and vitamin C. After grinding, participants were instructed to store the resulting powder at –20 °C or colder and to analyze the material within two days for analytes in this study. Approximate analyte levels were not reported to participants prior to the study. The NIST-determined values for biotin and vitamin C in the multivitamin sample were assigned using results from the manufacturer of the material. The NIST-determined values and uncertainties for the biotin and vitamin C are provided in the table below, on an as-received basis.

| Analyte | NIST-Determined Mass Fraction in Multivitamin B (mg/kg) |
|---|---|
| Biotin | 946 ± 36 |
| Vitamin C (Ascorbic Acid) | 46700 ± 2600 |

Dietary Intake Study Results

- The enrollment and reporting statistics for the dietary intake study are described in the table below. Reported values may include non-quantitative results (zero or below LOQ) but are included in the participation statistics.

| Analyte | Number of Laboratories Requesting Samples | Number of Laboratories Reporting Results (Percent Participation) | |
|---|---|---|---|
| | | Infant Formula | Multivitamin |
| Biotin | 29 | 14 (48 %) | 15 (55 %) |
| Vitamin C (Ascorbic Acid) | 38 | 21 (55 %) | 25 (66 %) |

- The between-laboratory variabilities were good for biotin in infant formula and for vitamin C in both samples (see table below).

| Analyte | Between-Laboratory Variability (% RSD) | |
|---|---|---|
| | Infant Formula | Multivitamin |
| Biotin | 14 % | 39 % |
| Vitamin C (Ascorbic Acid) | 17 % | 12 % |

- Most laboratories reported using solvent extraction as the sample preparation method for determination of biotin and vitamin C in infant formula and multivitamin (see table below).

| Reported Sample Preparation Method | Percent Reporting | | | |
|---|---|---|---|---|
| | Biotin | | Vitamin C | |
| | Infant Formula | Multivitamin | Infant Formula | Multivitamin |
| Solvent Extraction | 53 % | 44 % | 48 % | 56 % |
| Dilution | 20 % | 19 % | 14 % | 16 % |
| Enzymatic Hydrolysis | 7 % | - | - | - |
| Solid Phase Extraction (SPE) | - | 6 % | 5 % | 4 % |
| Solvent Extraction & SPE | - | 6 % | - | - |
| Base Hydrolysis | - | 6 % | - | - |
| Protein Precipitation | - | - | 5 % | 4 % |
| Other/None Reported | 20 % | 19 % | 29 % | 20 % |

- Most laboratories reported using either liquid chromatography mass spectrometry (LC-MS) or liquid chromatography with tandem mass spectrometry (LC-MS/MS) as their analytical method for determination of biotin and liquid chromatography with absorbance detection (LC-Abs) or photodiode-array detection (PDA) as their analytical method for determination of vitamin C in infant formula and multivitamin (see table below).

| Reported Analytical Method | Percent Reporting | | | |
|---|---|---|---|---|
| | Biotin | | Vitamin C | |
| | Infant Formula | Multivitamin | Infant Formula | Multivitamin |
| LC with Absorbance Detection or PDA | 7 % | 19 % | 71 % | 80 % |
| Spectrophotometry | - | - | 5 % | 4 % |
| LC-MS | 36 % | 44 % | - | - |
| LC-MS/MS | 21 % | 12 % | - | - |
| Microbiological Assay | 21 % | 19 % | - | - |
| LC-FLD | 7 % | | - | - |
| Other/None Reported | 14 % | 12 % | 19 % | 16 % |

- For both infant formula and multivitamin, the consensus mean for biotin was inside the target range (**Figures 3-1, 3-2, 3-3, 3-4**); however, the between-laboratory variability for biotin in the multivitamin was high.
- For both infant formula and multivitamin, the consensus mean for vitamin C was inside the target range with only two laboratories reporting values below the consensus range of tolerance for both materials (**Figures 3-6, 3-7, 3-8, 3-9**).

Dietary Intake Technical Recommendations

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

- For biotin, the consensus mean was very close to the target value in the infant formula with low between-laboratory variability despite the various analytical techniques used. Alternatively, higher between-laboratory variability was observed in the multivitamin results and could be a result of the variability in sample preparation techniques employed.
  - These trends indicate that a well-accepted sample preparation approach may be required for accurate determination of biotin in these matrices to eliminate methodology-caused variabilities.
  - No additional trends were noted for other sample preparation techniques or analytical methods.
- For vitamin C, the consensus mean was close to the target value in both materials.
  - Two laboratories reported results below the consensus range of tolerance for vitamin C in both materials which could indicate the need for improved sample preparation techniques.
  - The multivitamin material requires proper grinding and homogenization of the entire bottle of tablets prior to subsampling for analysis. This practice helps reduce variability due to between-tablet differences and improves repeatability.
- Analytes may decompose in light; therefore, samples and standards should be prepared under amber or attenuated lighting.

- Calculations and reporting units must be verified prior to submission of results. Laboratories often report results in the wrong units or forget a dilution factor during the calculation of the final results, resulting in poor performance for the study.
- The use of appropriate calibration materials and quality assurance samples to establish that a method is in control and being performed correctly 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.

**Table 3-1.** Individualized data summary table (NIST) for biotin and vitamin C in Infant Formula A and Multivitamin B.

*National Institute of Standards and Technology*

**HAMQAP Exercise 6 - Water-Soluble Vitamins**

| | **Lab Code:** | **NIST** | **1. Your Results** | | | | **2. Community Results** | | | **3. Target** | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Analyte | Sample | Units | $x_i$ | $s_i$ | $Z'_{comm}$ | $Z_{NIST}$ | N | x* | s* | $x_{NIST}$ | *U* |
| Biotin | Infant Formula A | mg/kg | 2.13 | 0.142 | | | 14 | 2.06 | 0.28 | 2.13 | 0.142 |
| Biotin | Multivitamin B | mg/kg | 22 | 1.66 | | | 16 | 23.6 | 9.2 | 22 | 1.66 |
| Vitamin C (Ascorbic Acid) | Infant Formula A | mg/kg | 946 | 36 | | | 21 | 970 | 170 | 946 | 36 |
| Vitamin C (Ascorbic Acid) | Multivitamin B | mg/kg | 46700 | 2600 | | | 25 | 42800 | 5100 | 46700 | 2600 |

$x_i$ Mean of reported values
$s_i$ Standard deviation of reported values
$Z'_{comm}$ Z'-score with respect to community consensus
$Z_{NIST}$ Z-score with respect to NIST value

N Number of quantitative values reported
x* Robust mean of reported values
s* Robust standard deviation

$x_{NIST}$ NIST-assessed value
*U* expanded uncertainty about the NIST-assessed value

**Table 3-2.** Data summary table for biotin in Infant Formula A and Multivitamin B. Data points highlighted in blue have been identified as outside the consensus tolerance limits and would be estimated to result in an unacceptable $Z'_{comm}$ score, $|Z'_{comm}| \geq 2$.

| | | Biotin | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Infant Formula A (mg/kg) | | | | | Multivitamin B (mg/kg) | | | | |
| | **Lab** | **A** | **B** | **C** | **Avg** | **SD** | **A** | **B** | **C** | **Avg** | **SD** |
| Individual Results | Target | | | | 2.14 | 0.14 | | | | 22.04 | 1.66 |
| | F005 | 1.89 | 1.96 | 2.04 | 1.963 | 0.075 | | | | | |
| | F011 | | | | | | | | | | |
| | F017 | < 135.000 | < 135.000 | < 135.000 | | | 19.8 | 21.6 | 24.3 | 21.9 | 2.3 |
| | F018 | | | | | | | | | | |
| | F021 | | | | | | | | | | |
| | F026 | 2.3414 | | | 2.34 | | 35.0497 | | | 35.0 | |
| | F030 | 2.06 | 1.94 | 2.07 | 2.02 | 0.072 | 20.53 | 19.35 | 21.3 | 20.4 | 0.98 |
| | F031 | 2.49 | 2.17 | 2.11 | 2.26 | 0.204 | 18.83 | 19.57 | 19.78 | 19.4 | 0.50 |
| | F034 | 2.23 | 2.19 | 2.19 | 2.20 | 0.023 | 42.8 | 44.9 | 44.4 | 44.0 | 1.1 |
| | F035 | | | | | | | | | | |
| | F036 | 1.66 | 1.87 | 2.05 | 1.86 | 0.20 | 17.93 | 20.55 | 20.53 | 19.7 | 1.5 |
| | F039 | 1.72 | 1.67 | 1.68 | 1.69 | 0.026 | 13.5 | 14.2 | 12.5 | 13.4 | 0.85 |
| | F040 | | | | | | 31.7 | 29.7 | 30.5 | 30.6 | 1.01 |
| | F045 | | | | | | | | | | |
| | F046 | | | | | | 28.58 | 24.75 | 24.8 | 26.0 | 2.2 |
| | F051 | | | | | | | | | | |
| | F056 | | | | | | | | | | |
| | F059 | | | | | | 18.6 | 19.5 | 21.4 | 19.8 | 1.4 |
| | F060 | | | | | | | | | | |
| | F061 | 2.225 | 2.236 | 2.238 | 2.23 | 0.007 | 20.71 | 20.75 | 20.91 | 20.8 | 0.11 |
| | F062 | 2.0551 | 2.0786 | 2.1312 | 2.09 | 0.039 | | | | | |
| | F069 | 61 | 57 | 62 | 60 | 2.6 | 130 | 127 | 89 | 115 | 23 |
| | F073 | 1.95 | 2.04 | 2.11 | 2.03 | 0.080 | 28.2 | 27.4 | 28.5 | 28.0 | 0.6 |
| | F074 | 78426 | 79938 | 75709 | 78024 | 2143 | 27986 | 27952 | 27311 | 27750 | 380 |
| | F075 | 2 | 1.96 | 1.99 | 1.98 | 0.021 | 18.6 | 23.8 | 18.8 | 20.4 | 2.9 |
| | F079 | | | | | | | | | | |
| | F080 | 2.1 | 1.94 | 2 | 2.01 | 0.081 | 16.66 | 16.89 | 16.81 | 16.8 | 0.12 |
| | F081 | | | | | | | | | | |
| | F089 | | | | | | | | | | |
| Community Results | | Consensus Mean | | | 2.06 | | Consensus Mean | | | 23.55 | |
| | | Consensus Standard Deviation | | | 0.28 | | Consensus Standard Deviation | | | 9.2 | |
| | | Maximum | | | 78024 | | Maximum | | | 27750 | |
| | | Minimum | | | 1.69 | | Minimum | | | 13.4 | |
| | | N | | | 13 | | N | | | 15 | |

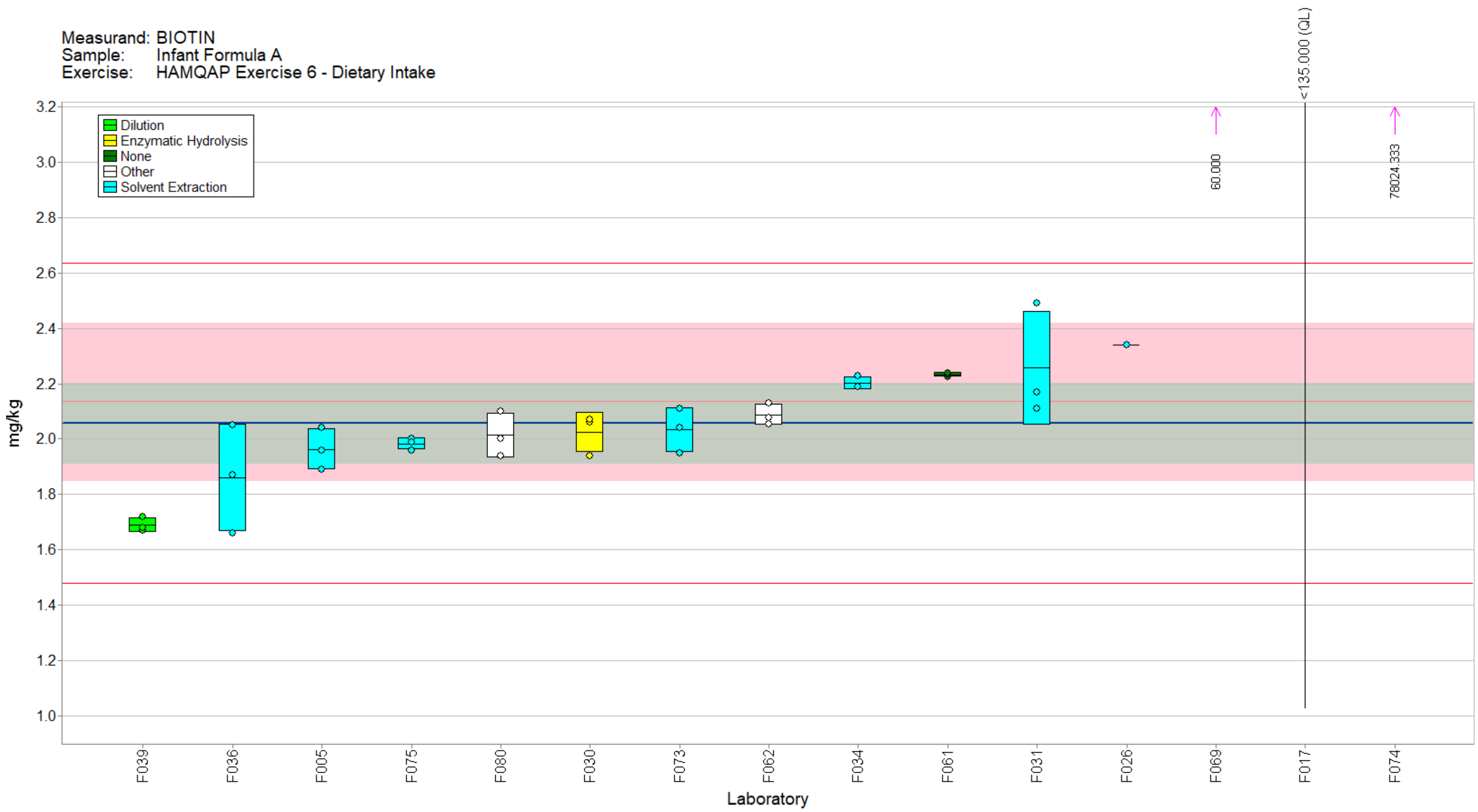


**Figure 3-1.** Biotin in Infant Formula A (data summary view – sample preparation). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The solid blue 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 $Z'_{\text{comm}}$ score, $|Z'_{\text{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty ($U_{\text{NIST}}$) and represents the range that results in an acceptable $Z_{\text{NIST}}$ score, $|Z_{\text{NIST}}| \leq 2$.

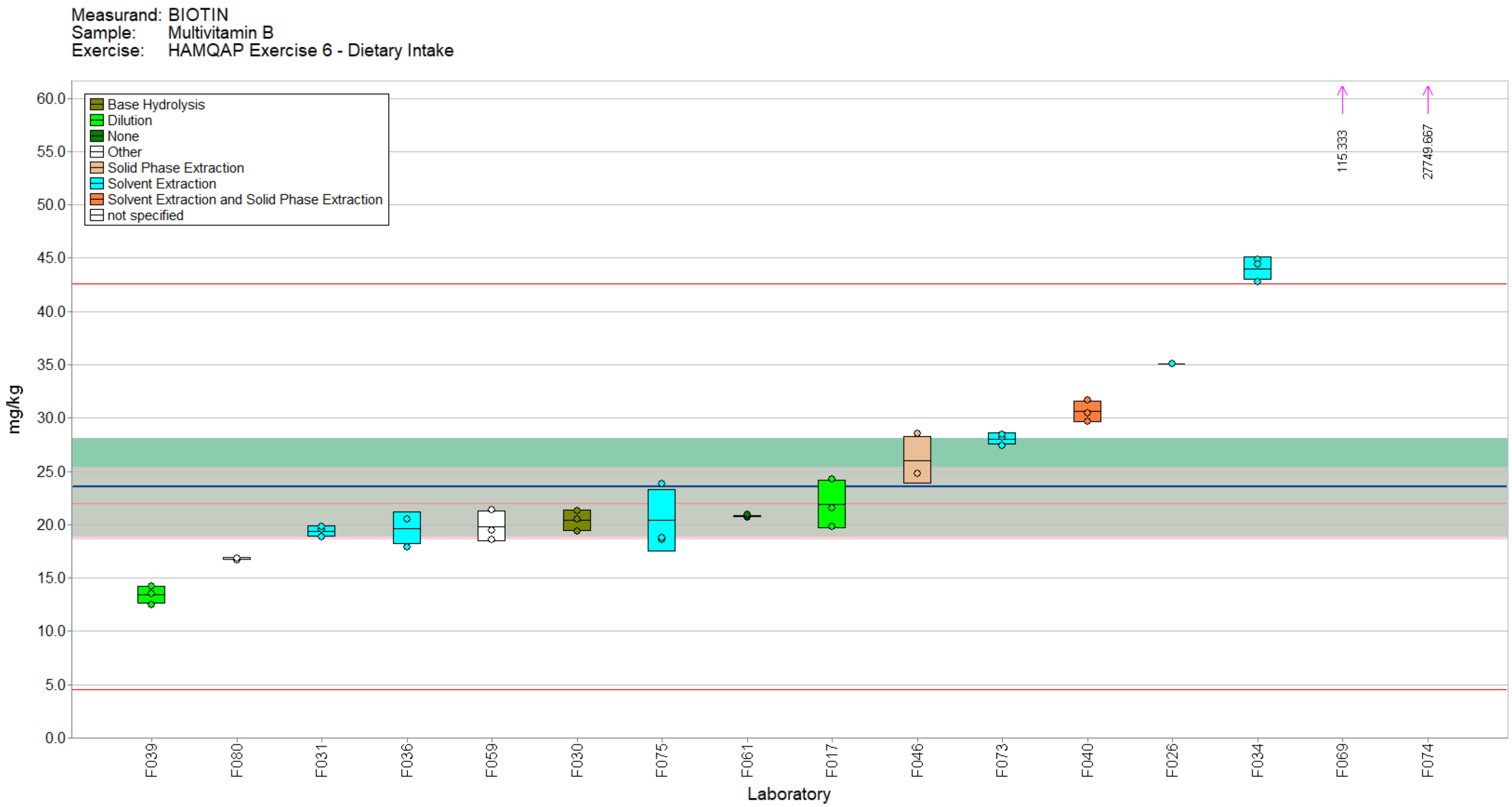


**Figure 3-2.** Biotin in Multivitamin B (data summary view – sample preparation). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The solid blue 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 $Z'_{\text{comm}}$ score, $|Z'_{\text{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty ($U_{\text{NIST}}$) and represents the range that results in an acceptable $Z_{\text{NIST}}$ score, $|Z_{\text{NIST}}| \leq 2$.

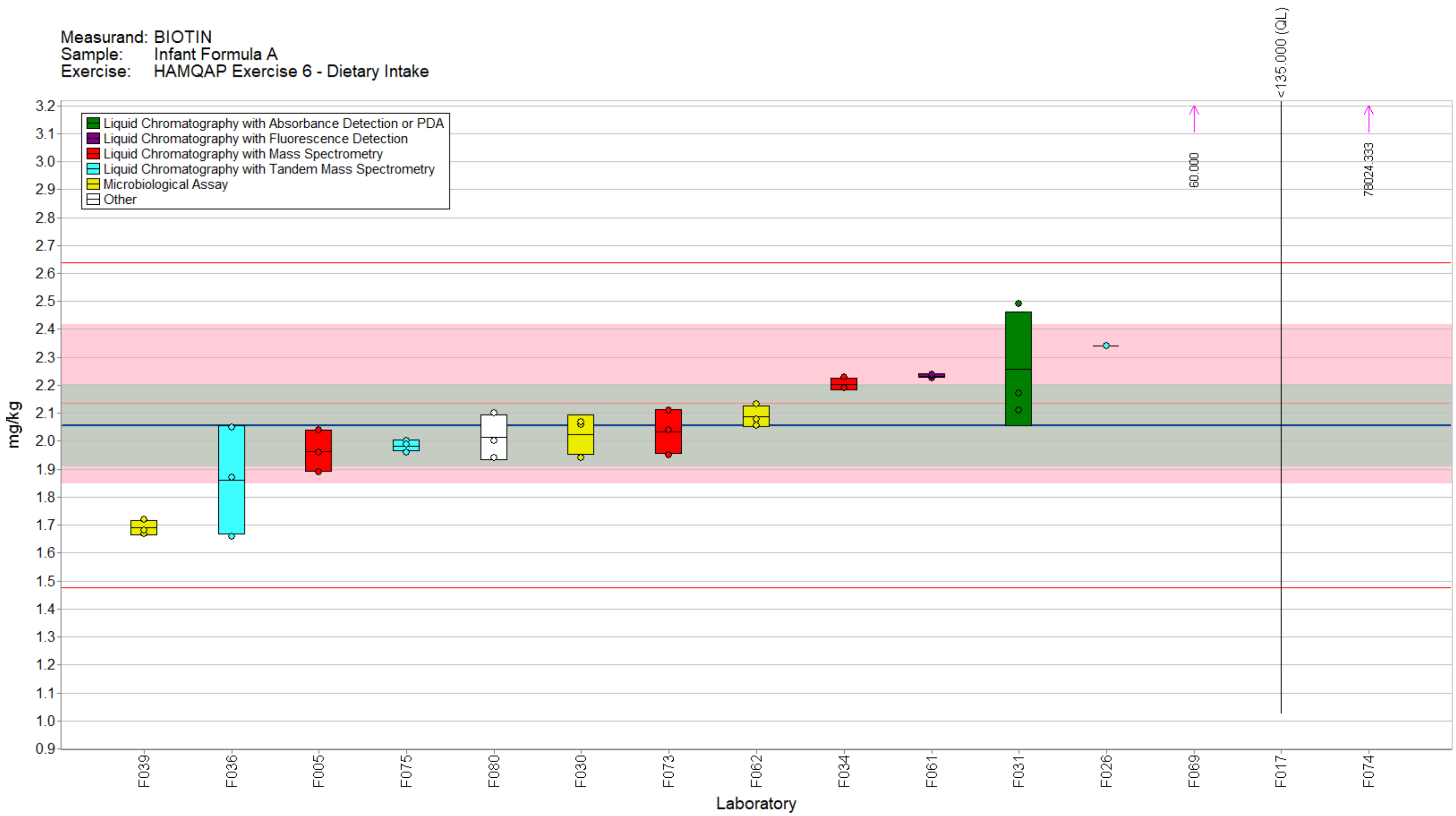


**Figure 3-3.** Biotin in Infant Formula A (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line 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 $Z'_{\mathrm{comm}}$ score, $|Z'_{\mathrm{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty ($U_{\mathrm{NIST}}$) and represents the range that results in an acceptable $Z_{\mathrm{NIST}}$ score, $|Z_{\mathrm{NIST}}| \leq 2$.

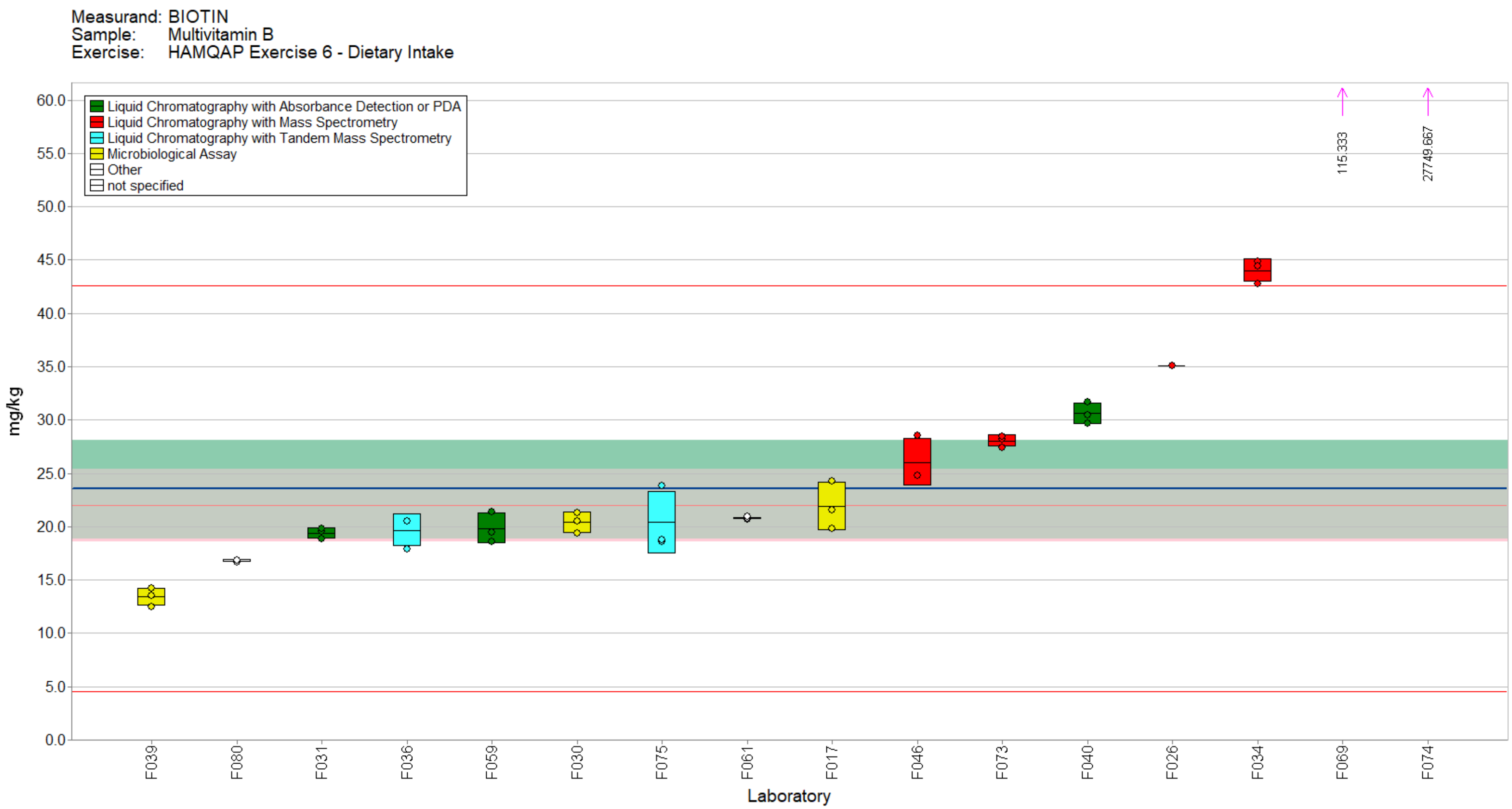


**Figure 3-4.** Biotin in Multivitamin B (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line represents 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 $Z'_{\text{comm}}$ score, $|Z'_{\text{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty ($U_{\text{NIST}}$) and represents the range that results in an acceptable $Z_{\text{NIST}}$ score, $|Z_{\text{NIST}}| \leq 2$.

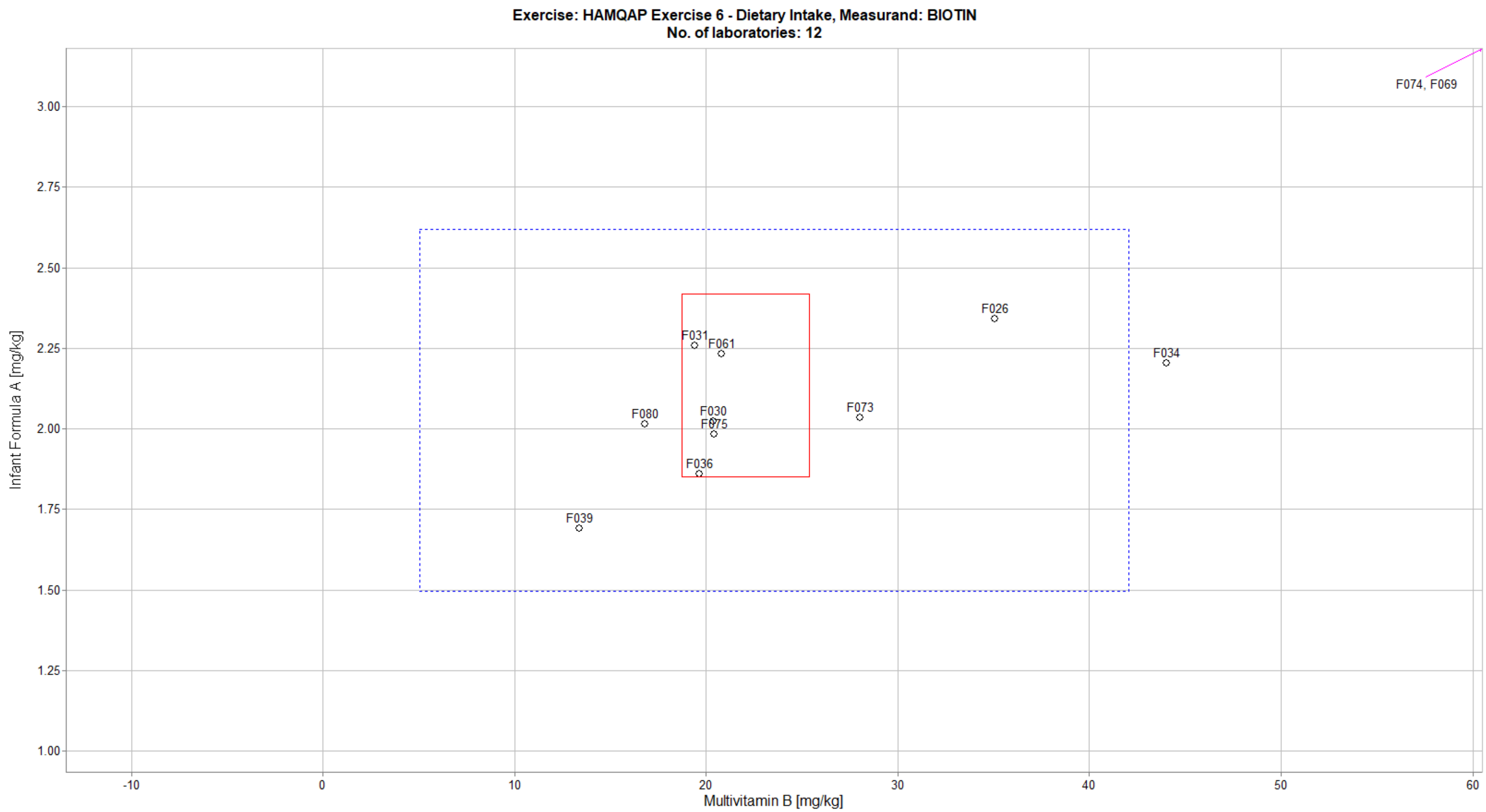


**Figure 3-5.** Laboratory means for biotin in Infant Formula A and Multivitamin B (sample/sample comparison view). In this view, the individual laboratory mean for one sample (Infant Formula A) is compared to the mean for a second sample (multivitamin). The solid red box represents the NIST range of tolerance for the two samples, Multivitamin B (x-axis) and Infant Formula A (y-axis), which encompasses the target values bounded by their uncertainties ($U_{\mathrm{NIST}}$) and represents the range that results in an acceptable $Z_{\mathrm{NIST}}$ score, $|Z_{\mathrm{NIST}}| \leq 2$. The dotted blue box represents the consensus range of tolerance for Multivitamin B (x-axis) and Infant Formula A (y--axis), calculated as the values above and below the consensus means that result in an acceptable $Z'_{\mathrm{comm}}$ score, $|Z'_{\mathrm{comm}}| \leq 2$.

**Table 3-3.** Data summary table for vitamin C (ascorbic acid) in Infant Formula A and Multivitamin B. Data points highlighted in blue have been identified as outside the consensus tolerance limits and would be estimated to result in an unacceptable $Z'_{comm}$ score, $|Z'_{comm}| \geq 2$.

| | | Vitamin C (Ascorbic Acid) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Infant Formula A (mg/kg) | | | | | Multivitamin B (mg/kg) | | | | |
| | Lab | A | B | C | Avg | SD | A | B | C | Avg | SD |
| Individual Results | Target | | | | 946 | 36 | | | | 46700 | 2600 |
| | F004 | | | | | | | | | | |
| | F005 | 342.83 | 342.8 | 374.77 | 353 | 18 | 30116.51 | 23891.23 | 21375.65 | 25128 | 4500 |
| | F011 | | | | | | 45203.6 | 45736 | 45489.4 | 45476 | 266 |
| | F013 | 1182 | 1104.2 | 1123.4 | 1137 | 41 | 48075 | 49824 | 48678 | 48859 | 888 |
| | F014 | 872 | 869 | 875 | 872 | 3.0 | 46600 | 44400 | 48700 | 46567 | 2150 |
| | F017 | 1300 | 1300 | 1300 | 1300 | 0 | 48000 | 45000 | 47900 | 46967 | 1704 |
| | F018 | | | | | | | | | | |
| | F021 | | | | | | 52.43 | 46.94 | 41.69 | 47 | 5.4 |
| | F022 | 991 | 1050 | 1017 | 1019 | 30 | 42373 | 41786 | 38559 | 40906 | 2054 |
| | F026 | 1023.7 | | | 1024 | | 43867 | | | 43867 | |
| | F030 | 1080 | 935 | 905 | 973 | 94 | 48900 | 47200 | 47500 | 47867 | 907 |
| | F031 | 865.75 | 834 | 834.85 | 845 | 18 | 46181.46 | 45979.86 | 42375.18 | 44846 | 2142 |
| | F032 | | | | | | | | | | |
| | F034 | 935 | 930 | 937 | 934 | 3.6 | 38621 | 38887 | 38018 | 38509 | 445 |
| | F035 | | | | | | | | | | |
| | F036 | 1127 | 1116 | 1103 | 1115 | 12 | 46018 | 42284 | 42346 | 43549 | 2138 |
| | F039 | 883 | 892 | 872 | 882 | 10 | 44600 | 44900 | 43800 | 44433 | 569 |
| | F040 | | | | | | 41064 | 42802 | 40701 | 41522 | 1123 |
| | F041 | | | | | | | | | | |
| | F045 | | | | | | | | | | |
| | F046 | 1075.46 | 969.22 | 1063.09 | 1036 | 58 | 47101.25 | 42960.1 | 43955.2 | 44672 | 2162 |
| | F051 | | | | | | | | | | |
| | F056 | | | | | | | | | | |
| | F057 | | | | | | 36774.4 | 37843.1 | 36940.2 | 37186 | 575 |
| | F059 | 1030 | 1000 | 1000 | 1010 | 17 | 44300 | 44800 | 45000 | 44700 | 361 |
| | F060 | | | | | | 49780 | 49330 | 48190 | 49100 | 820 |
| | F061 | 930 | 930 | 930 | 930 | 0 | | | | | |
| | F062 | 934.3 | 939 | 914.7 | 929 | 13 | | | | | |
| | F069 | 1060 | 1030 | 1040 | 1043 | 15 | 40090 | 41710 | 45560 | 42453 | 2810 |
| | F070 | 514 | 734 | 575 | 608 | 114 | 36410 | 34630 | 33750 | 34930 | 1355 |
| | F073 | | | | | | 45090 | 43867 | 44179 | 44379 | 635 |
| | F074 | 1099 | 1099 | 1099 | 1099 | 0 | 36100 | 37900 | 38300 | 37433 | 1172 |
| | F075 | 968.6 | 940.7 | 936.1 | 948 | 18 | 44200 | 42300 | 42100 | 42867 | 1159 |
| | F077 | | | | | | | | | | |
| | F079 | 335 | < 97.0 | 97.5 | 216 | 168 | 46600 | 42000 | 42200 | 43600 | 2600 |
| | F080 | 853.13 | 836.19 | 826.34 | 839 | 14 | 34993.4 | 34829.4 | 34552.5 | 34792 | 223 |
| | F088 | | | | | | | | | | |
| | F089 | | | | | | | | | | |
| Community Results | | Consensus Mean | | | 970 | | Consensus Mean | | | 42828 | |
| | | Consensus Standard Deviation | | | 168 | | Consensus Standard Deviation | | | 5077 | |
| | | Maximum | | | 1300 | | Maximum | | | 49100 | |
| | | Minimum | | | 216 | | Minimum | | | 47 | |
| | | N | | | 20 | | N | | | 24 | |

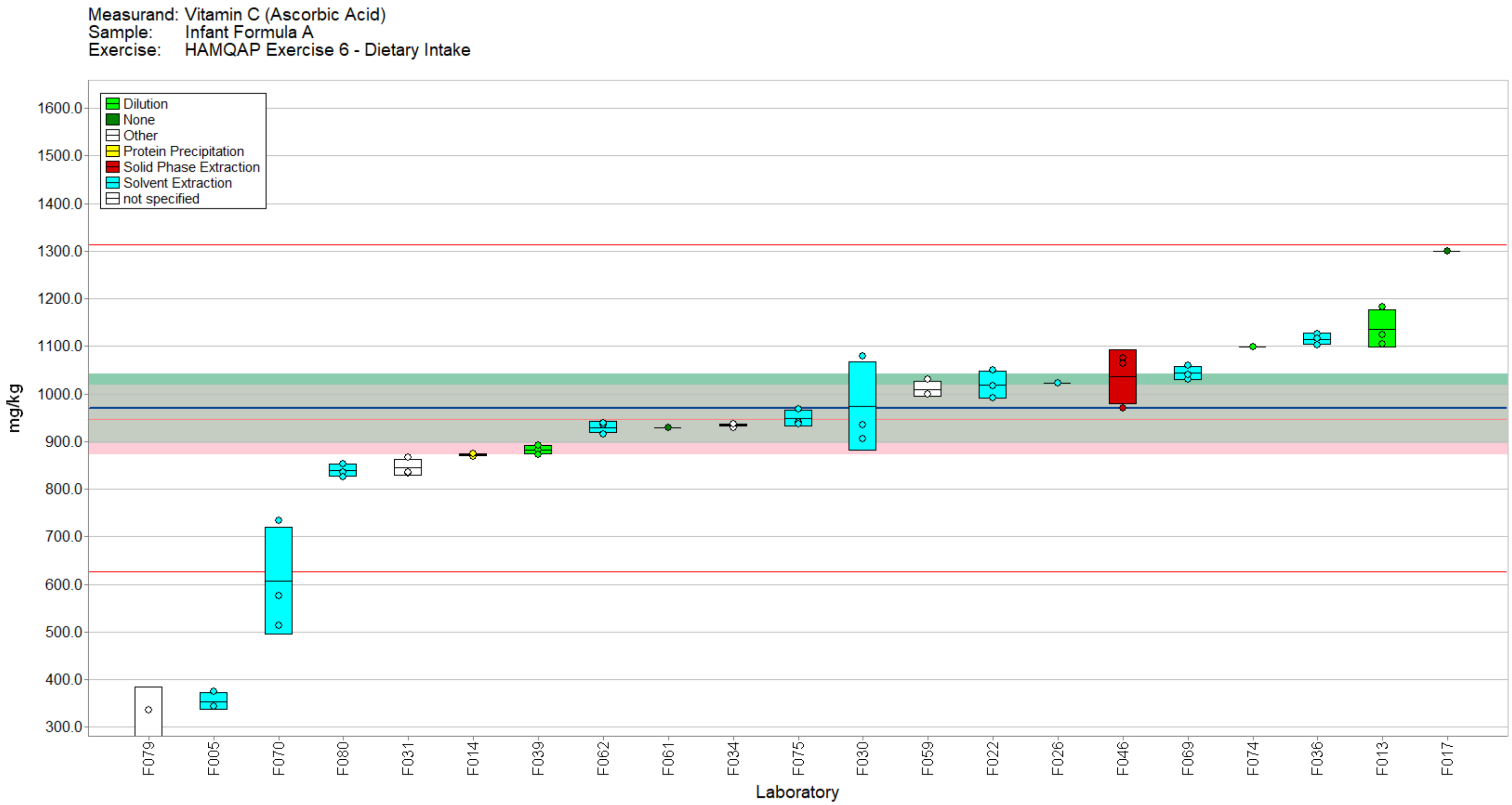


**Figure 3-6.** Vitamin C (ascorbic acid) in Infant Formula A (data summary view – sample preparation). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The solid blue 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 $Z'_{\text{comm}}$ score, $|Z'_{\text{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty ($U_{\text{NIST}}$) and represents the range that results in an acceptable $Z_{\text{NIST}}$ score, $|Z_{\text{NIST}}| \leq 2$.

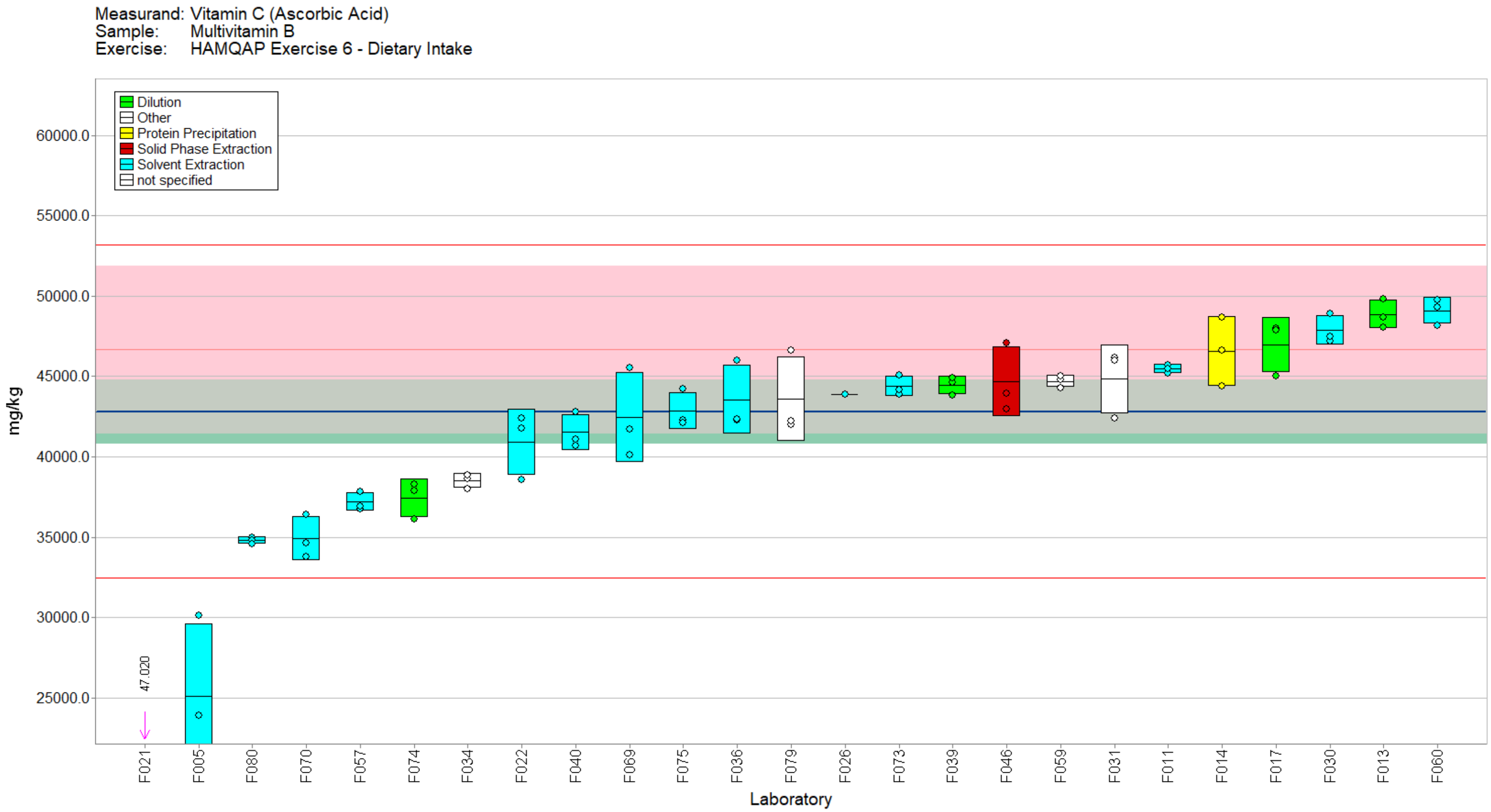


**Figure 3-7.** Vitamin C (ascorbic acid) in Multivitamin B (data summary view – sample preparation). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The solid blue 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 $Z'_{\text{comm}}$ score, $|Z'_{\text{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty ($U_{\text{NIST}}$) and represents the range that results in an acceptable $Z_{\text{NIST}}$ score, $|Z_{\text{NIST}}| \leq 2$.

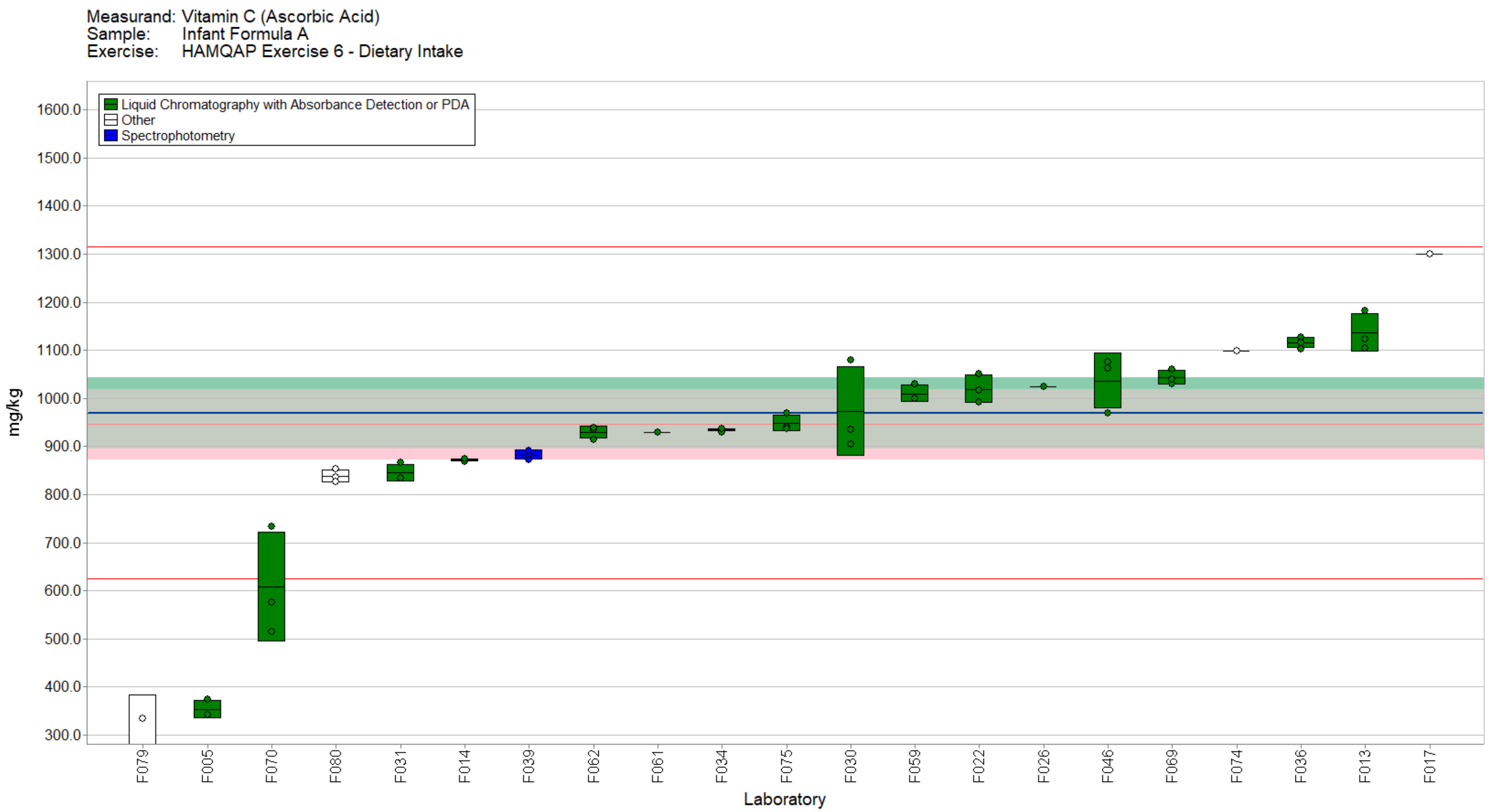


**Figure 3-8.** Vitamin C (ascorbic acid) in Infant Formula A (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue 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 $Z'_{\mathrm{comm}}$ score, $|Z'_{\mathrm{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty ($U_{\mathrm{NIST}}$) and represents the range that results in an acceptable $Z_{\mathrm{NIST}}$ score, $|Z_{\mathrm{NIST}}| \leq 2$.

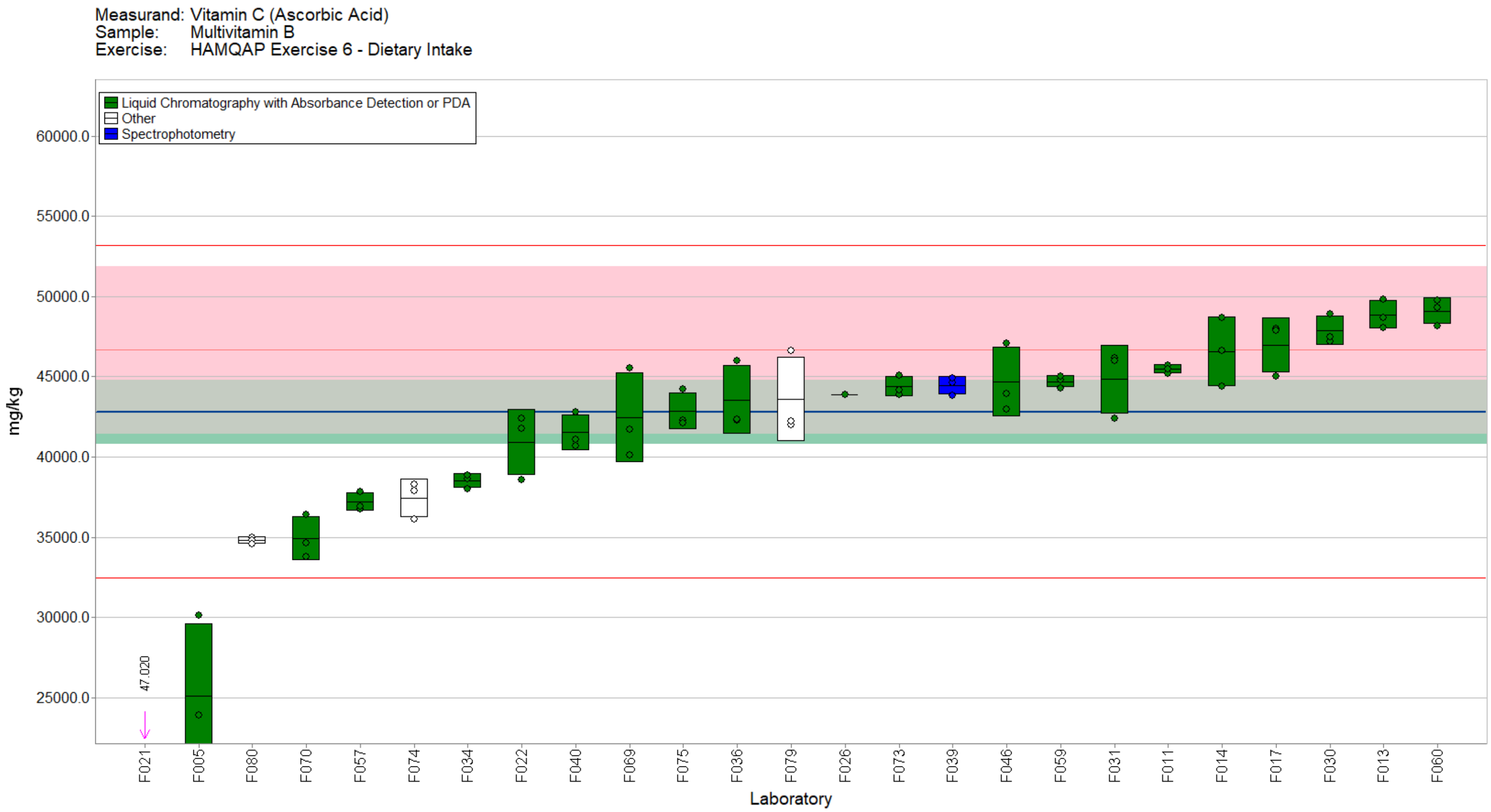


**Figure 3-9.** Vitamin C (ascorbic acid) in Multivitamin B (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue 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 $Z'_{\mathrm{comm}}$ score, $|Z'_{\mathrm{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty ($U_{\mathrm{NIST}}$) and represents the range that results in an acceptable $Z_{\mathrm{NIST}}$ score, $|Z_{\mathrm{NIST}}| \leq 2$.

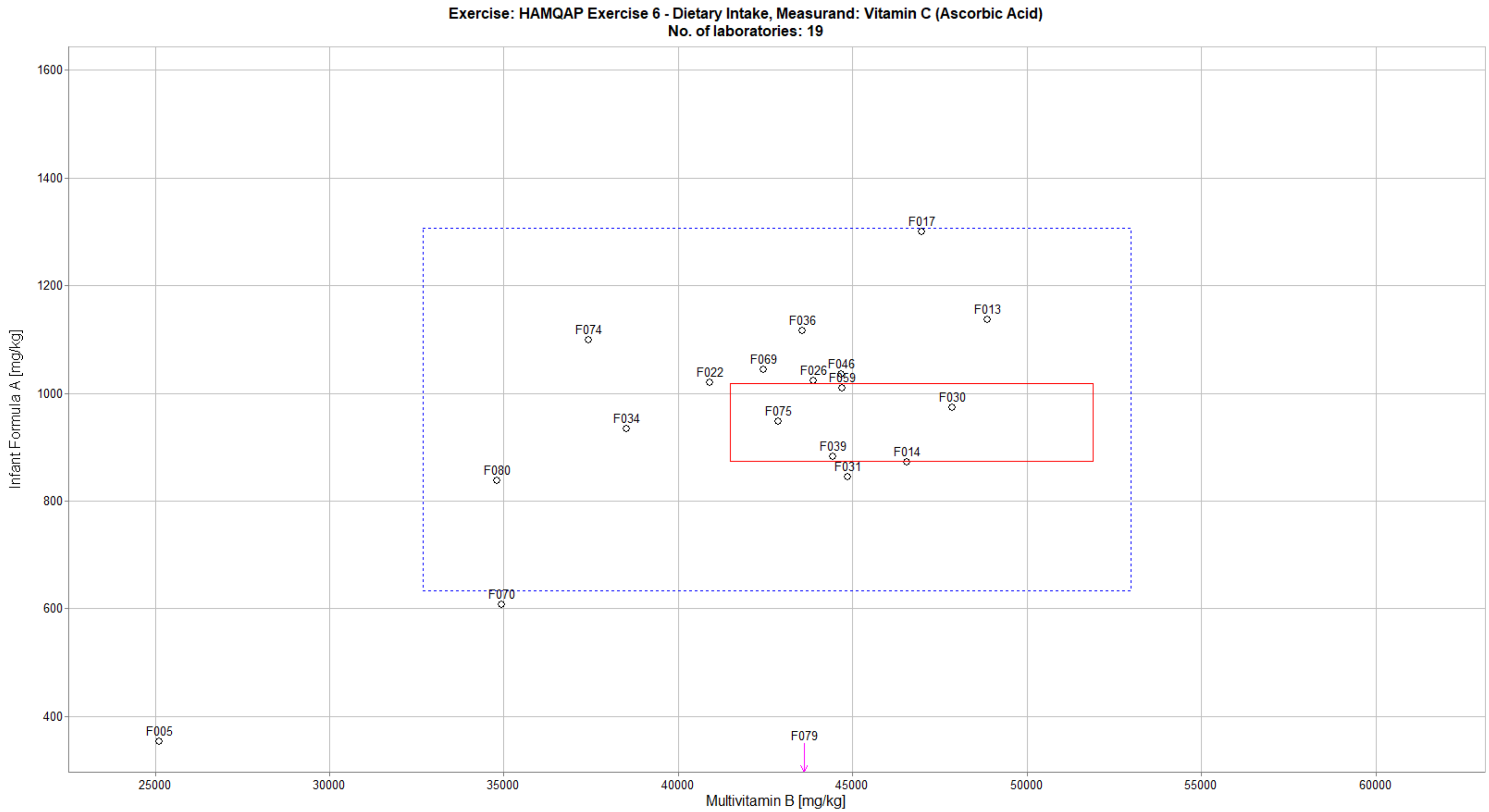


**Figure 3-10.** Laboratory means for vitamin C (ascorbic acid) in Infant Formula A and Multivitamin B (sample/sample comparison view). In this view, the individual laboratory mean for one sample (Infant Formula A) is compared to the mean for a second sample (multivitamin). The solid red box represents the NIST range of tolerance for the two samples, Multivitamin B (x-axis) and Infant Formula 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 Multivitamin B (x-axis) and Infant Formula A (y-axis), calculated as the values above and below the consensus means that result in an acceptable $Z'_{\text{comm}}$ score, $|Z'_{\text{comm}}| \leq 2$.

## SECTION 4: FAT-SOLUBLE VITAMINS (Vitamins A and E)

<u>Study Overview</u>

In this study, participants were provided with samples of Infant Formula A and Multivitamin B for dietary intake. Participants were asked to use in-house analytical methods to determine and report the mass fraction (mg/kg) of vitamin A and vitamin E related compounds in the infant formula and multivitamin samples. Accurate measurements of vitamins A and vitamin E are important for both the food industry and clinical communities. Both vitamin groups are composed of chemically related compounds, i.e., retinol, retinal, and retinyl esters for vitamin A and tocopherols and tocotrienol for vitamin E. These different vitamin A and vitamin E compounds are known to have distinct biological activities in humans, so it is important for testing labs to use fit-for-purpose methods, standards, and conversion techniques that can support reliable and accurate measurements for appropriate nutritional labelling.

<u>Dietary Intake Sample Information</u>

*Infant Formula A.* Participants were provided with three packets, each containing approximately 10 g of powdered infant formula. Participants were asked to store the material at –20 °C in the original unopened packets and to prepare one sample and report one value from each packet provided. Before use, participants were instructed to thoroughly mix the contents of the packet prior to removal of a test portion for analysis, and to use a sample size of at least 3 g for the determination of vitamin A related compounds and 2 g for the determination of vitamin E related compounds. Approximate analyte levels were not reported to participants prior to the study. The NIST-determined values for vitamins A and E related compounds in the infant formula sample were assigned using results from the manufacturer of the material and are provided in the table below on an as-received basis.

| Analyte | NIST-Determined Mass Fraction in Infant Formula A (mg/kg) | | |
|---|---|---|---|
| *Vitamin A* | | | |
| Total Retinol | 10.13 | ± | 0.23 |
| Retinyl Acetate | 6.73 | ± | 0.23 |
| Retinyl Palmitate | 7.80 | ± | 0.23 |
| *Vitamin E* | | | |
| Total alpha-Tocopherol | 250.7 | ± | 5.9 |
| alpha-Tocopherol | 58.39 | ± | 3.88 |
| alpha-Tocopheryl Acetate | 161.6 | ± | 4.9 |
| beta-Tocopherol | 4.94 | ± | 0.10 |
| delta-Tocopherol | 37.88 | ± | 1.02 |
| gamma-Tocopherol | 114.0 | ± | 2.2 |

*Multivitamin B.* Participants were provided with three bottles, each containing 30 multivitamin tablets. Participants were asked to store the material at controlled room temperature (20 °C to 25 °C) in the original unopened bottles and to prepare one sample and report one value from each bottle provided. Before use, participants were instructed to grind all 30 tablets, mix the resulting powder thoroughly prior to removal of a test portion for analysis, and to use a sample size of at least 2 g for the determination of both vitamin A and vitamin E compounds. After grinding, participants were instructed to store the resulting powder at –20 °C or colder and analyze the material within two days for analytes in this study. Approximate analyte levels were not reported to participants prior to the study. The NIST-determined values for vitamins A and E compounds in the multivitamin sample were assigned using results from the manufacturer of the material and are provided in the table below on an as-received basis.

| Analyte | NIST-Determined Mass Fraction in Multivitamin B (mg/kg) |
|---|---|
| *Vitamin A* | |
| Retinyl Acetate | 895 ± 42 |
| *Vitamin E* | |
| alpha-Tocopheryl Acetate | 17931 ± 430 |

Dietary Intake Study Results

- The enrollment and reporting statistics for the dietary intake study is described in the table below. Reported values may include non-quantitative results (zero or below LOQ) but are included in the participation statistics.

| Analyte | Number of Laboratories Requesting Samples | Number of Laboratories Reporting Results (Percent Participation) | |
|---|---|---|---|
| | | Infant Formula | Multivitamin |
| Total Retinol | 30 | 17 (57 %) | 14 (47 %) |
| Retinyl Acetate | 27 | 7 (26 %) | 12 (44 %) |
| Retinyl Palmitate | 26 | 9 (35 %) | 6 (23 %) |
| Total alpha-Tocopherol | 33 | 12 (36 %) | 12 (36 %) |
| alpha-Tocopherol | 31 | 12 (39 %) | 10 (32 %) |
| alpha-Tocopheryl Acetate | 29 | 9 (31 %) | 15 (52%) |
| beta-Tocopherol | 21 | 7 (33 %) | 5 (24 %) |
| delta-Tocopherol | 23 | 10 (43 %) | 7 (30 %) |
| gamma-Tocopherol | 22 | 8 (36 %) | 5 (23%) |

- The between-laboratory variabilities ranged from good (15 %) to needs more improvement (74 %) in the infant formula and good (7%) to unacceptable (>100 %) in the multivitamin (see table below). More discussion can be found in the following Technical Recommendations section.

| Analyte | Between-Laboratory Variability (% RSD) | |
|---|---|---|
| | Infant Formula | Multivitamin |
| Total Retinol | 17 % | 23 % |
| Retinyl Acetate | 36 % | 16 % |
| Retinyl Palmitate | 61 % | > 100 % |
| Total alpha-Tocopherol | 56 % | 7 % |
| alpha-Tocopherol | 48 % | > 100 % |
| alpha-Tocopheryl Acetate | 74 % | 8 % |
| beta-Tocopherol | 49 % | > 100 % |

| | | |
|---|---|---|
| delta-Tocopherol | 53 % | 100 % |
| gamma-Tocopherol | 15 % | > 100 % |

- The sample preparation methods reported were similar for the determination of vitamin A and vitamin E in both the infant formula and the multivitamin samples. Sample preparations reported below are based on total retinal and total alpha-tocopherol to give an idea of the spread across vitamin A and vitamin E, respectively.

| Reported Sample Preparation | Infant Formula and Multivitamin | |
|---|---|---|
| | Vitamin A | Vitamin E |
| Base hydrolysis/saponification | 41 % | 25 % |
| Solvent extraction | 6 % | 42 % |
| Enzymatic hydrolysis | 6 % | 8 % |
| Solid phase extraction | 24 % | 8 % |
| Dilution | - | 8 % |
| Other/Not Specified | - | 17 % |

- Most laboratories reported using either LC-Abs or liquid chromatography with fluorescence detection (LC-FLD) methods for the determination of vitamin A and vitamin E in infant formula and in multivitamin (see table below for total retinol and total alpha-tocopherol). The analytical methods reported below are based on total retinal and total alpha-tocopherol to give an idea of the spread across vitamin A and vitamin E, respectively.

| Reported Analytical Method | Percent Reporting | | | |
|---|---|---|---|---|
| | Infant Formula | | Multivitamin | |
| | Vitamin A | Vitamin E | Vitamin A | Vitamin E |
| LC-Abs | 65 % | 71 % | 58 % | 74 % |
| LC-FLD | 24 % | 29 % | 25 % | 27 % |
| LC-MS | 6 % | - | - | - |
| HPLC | 6 % | - | 8 %- | - |
| Other/Not Specified | - | - | 8 % | - |

- For vitamin A in Infant Formula A, the total retinol consensus mean and confidence interval were above the target range, with one lab in the target range (**Figure 4-1**). For retinyl acetate, the consensus mean was above the target range, however, the consensus confidence interval overlapped with the target range. Two laboratories were within or close to the target range (**Figure 4-4**). For retinyl palmitate, the consensus mean and confidence interval for retinyl palmitate data just overlap with the upper portion of the target range, with one lab very close to the target range (**Figure 4-7**).
- For vitamin E in Infant Formula A, the total alpha-tocopherol consensus mean was very close to the target value (**Figure 4-9**). The alpha tocopherol consensus mean and confidence range were above the target range, with two grouping of reported values **(Figures 4-12 and 4-14)**. For alpha-tocopheryl acetate, beta-tocopherol, and delta-tocopherol, the consensus means were lower than the target, but their consensus confidence intervals overlapped with their respective target ranges (**Figures 4-17, 4-20, 4-22**). The gamma-tocopherol consensus mean was close to the target range (**Figure 4-24**).

- For vitamin A in Multivitamin B, most labs were within the consensus range of tolerance for total retinol, and many were within the confidence interval for the consensus mean. One laboratory was above the consensus range of tolerance and one lab reported zero. (**Figure 4-2**). The retinyl acetate consensus mean and confidence interval were lower than the target range (**Figure 4-5**). For retinyl palmitate, and the participation rate was low. Several labs reported below their LOQ, some reporting zero. One laboratory was significantly above the consensus range of tolerance (**Figure 4-8**).
- For vitamin E in Multivitamin B, most labs were within the consensus range of tolerance for total alpha-tocopherol, and many were within the confidence interval for the consensus mean. One laboratory reported below their LOQ and one laboratory was below the consensus range of tolerance (**Figure 4-10**). For the alpha tocopherol there were two significantly different groupings of reported values **(Figures 4-13 and 4-15)**. The alpha-tocopheryl acetate consensus mean and confidence interval just overlapped with the upper portion of the target range (**Figure 4-18**). For beta-tocopherol, delta-tocopherol, and gamma-tocopherol. Most labs reported below their LOQ, some reporting zero (**Figures 4-21, 4-23, 4-25).**

<u>Dietary Intake Technical Recommendations</u>

The following recommendations are based on results obtained from the participants in this study. Figures were chosen to show results according to analytical method for all measurands, plus additional figures to show sample preparation for alpha-tocopherol.

- In Infant Formula A, many of the results reported for vitamin A (total retinol, retinyl acetate, and retinyl palmitate) were within the consensus range of tolerances and several near the target values, providing support that the participants are able to measure these analytes in infant formula matrices. In some cases, bias may arise from improper calibrant characterization and preparation.
- In Infant Formula A, many of the results reported for vitamin E (total alpha-tocopherol, alpha-tocopherol, alpha-tocopheryl acetate, beta-tocopherol, delta-tocopherol, and gamma-tocopherol) were within the consensus range of tolerances and several near the target value, providing support that the participants are able to measure these analytes in infant formula matrices. The 95 % confidence interval for the consensus mean for alpha-tocopherol was above the target range of tolerance. Two groups of reported values were identified based on sample preparation approach.
  - Laboratories that reported using saponification/base hydrolysis reported higher values than laboratories reporting other preparation techniques. Laboratories using hydrolysis techniques likely converted other forms present (i.e., alpha-tocopherol acetate) which biased the results. A few labs that reported using solvent extraction reported values very close to the target (**Figures 4-12 and 4-14**).
- In Multivitamin B, many of the results reported for vitamin A (total retinol and retinyl palmitate) were within the consensus range of tolerances, providing support that the participants are able to measure these analytes in multivitamin matrices.
  - For retinyl acetate, the consensus range as below the target range. Incomplete extraction of vitamins from encapsulated formulas could lead to biased results.
- In Multivitamin B, many of the results reported for vitamin E (total alpha-tocopherol, alpha-tocopherol, alpha-tocopheryl acetate, beta-tocopherol, delta-tocopherol, and gamma-tocopherol) were within the consensus range of tolerances and several near the target

value, providing support that the participants are able to measure these analytes in infant formula matrices.

- For alpha-tocopherol, two significantly different groups of reported values were identified based on sample preparation.
  - Laboratories that reported using saponification/base hydrolysis reported higher values than laboratories reporting other preparation techniques. Laboratories using hydrolysis techniques likely converted other forms present (i.e., alpha-tocopherol acetate) to alpha-tocopherol which resulted in a high bias for this analyte.
  - A few laboratories that reported using solvent extraction reported values very close to the target (**Figures 4-13 and 4-15**).
- For alpha-tocopheryl acetate, the 95 % confidence interval for the consensus mean was just overlapped with the upper portion of the target range of tolerance. Three reported that values significantly lower than the consensus range (**Figures 4-18**) should double check calibrant preparations, final calculations, and reporting units.

- Zero is not a measurable value and should not be reported.
- Overall, for fat-soluble vitamins, especially those with different chemical forms, it is important to understand what analytes are being measured and reported, and to use appropriate, high quality, and well characterized calibrants.
- Vitamin A or vitamin E compounds can be reported as totals, or as equivalents. Laboratories should choose appropriate techniques; measuring a total (by chemically converting prior to analysis) or reporting a total (by mathematically converting and combining the separately measured forms).
- Sample preparation techniques must be able to fully extract the analytes from the sample matrix, while also being mindful of analyte degradation and/or conversion. The use of reduced lighting/yellow lighting when conducting preparation techniques and storing samples in the dark or in amber colored vials can significantly reduce UV induced analyte degradation.
- In the case of tablets, sample preparation and storage are also important, being mindful of the grinding and homogenizing methods and as well as storage of ground material. Grinding shortly before sample extract and the use of cold storage when necessary can reduce the potential of analyte degradation due to the change in encapsulation.
- The use of appropriate calibration materials and quality assurance samples to establish that a method is in control and being performed correctly 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.

**Table 4-1.** Individualized data summary table (NIST) for vitamin A and vitamin E in Infant Formula A and Multivitamin B.

## *National Institute of Standards and Technology*

**HAMQAP Exercise 6 - Fat-Soluble Vitamins**

| | **Lab Code:** | **NIST** | **1. Your Results** | | | | **2. Community Results** | | | **3. Target** | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Analyte | Sample | Units | $x_i$ | $s_i$ | $Z'_{comm}$ | $Z_{NIST}$ | N | x* | s* | $x_{NIST}$ | $U$ |
| alpha-Tocopherol | Infant Formula A | mg/kg | 58.4 | 3.88 | | | 12 | 120 | 58 | 58.4 | 3.88 |
| alpha-Tocopherol | Multivitamin B | mg/kg | | | | | 9 | 160 | 200 | | |
| alpha-Tocopheryl Acetate | Infant Formula A | mg/kg | 162 | 4.92 | | | 9 | 120 | 89 | 162 | 4.92 |
| alpha-Tocopheryl Acetate | Multivitamin B | mg/kg | 17900 | 430 | | | 15 | 19500 | 1600 | 17900 | 430 |
| beta-Tocopherol | Infant Formula A | mg/kg | 4.94 | 0.102 | | | 5 | 3.7 | 1.8 | 4.94 | 0.102 |
| beta-Tocopherol | Multivitamin B | mg/kg | | | | | 2 | 30 | 140 | | |
| delta-Tocopherol | Infant Formula A | mg/kg | 37.9 | 1.02 | | | 10 | 30 | 16 | 37.9 | 1.02 |
| delta-Tocopherol | Multivitamin B | mg/kg | | | | | 3 | 200 | 200 | | |
| gamma-Tocopherol | Infant Formula A | mg/kg | 114 | 2.19 | | | 8 | 120 | 18 | 114 | 2.19 |
| gamma-Tocopherol | Multivitamin B | mg/kg | | | | | 2 | 20 | 80 | | |
| Retinyl Acetate | Infant Formula A | mg/kg | 6.73 | 0.226 | | | 7 | 8.7 | 3.1 | 6.73 | 0.226 |
| Retinyl Acetate | Multivitamin B | mg/kg | 895 | 42 | | | 12 | 700 | 110 | 895 | 42 |
| Retinyl Palmitate | Infant Formula A | mg/kg | 7.8 | 0.232 | | | 9 | 13 | 7.9 | 7.8 | 0.232 |
| Retinyl Palmitate | Multivitamin B | mg/kg | | | | | 4 | 40 | 230 | | |
| Total alpha-Tocopherol | Infant Formula A | mg/kg | 206 | 5.93 | | | 12 | 180 | 100 | 206 | 5.93 |
| Total alpha-Tocopherol | Multivitamin B | mg/kg | | | | | 11 | 18700 | 1400 | | |
| Total Retinol | Infant Formula A | mg/kg | 10.1 | 0.234 | | | 17 | 14.3 | 2.4 | 10.1 | 0.234 |
| Total Retinol | Multivitamin B | mg/kg | | | | | 14 | 620 | 140 | | |

$x_i$ Mean of reported values
$s_i$ Standard deviation of reported values
$Z'_{comm}$ Z'-score with respect to community consensus
$Z_{NIST}$ Z-score with respect to NIST value

N Number of quantitative values reported
x* Robust mean of reported values
s* Robust standard deviation

$x_{NIST}$ NIST-assessed value
$U$ expanded uncertainty about the NIST-assessed value

**Table 4-2.** Data summary table for total retinol in Infant Formula A and Multivitamin B. Data points highlighted in blue have been identified as outside the consensus tolerance limits and would be estimated to result in an unacceptable $Z'_{\text{comm}}$ score, $|Z'_{\text{comm}}| \geq 2$. Data points highlighted in red have a zero or non-numeric data point.

| | | Total Retinol | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Infant Formula A (mg/kg) | | | | | Multivitamin B (mg/kg) | | | | |
| | **Lab** | **A** | **B** | **C** | **Avg** | **SD** | **A** | **B** | **C** | **Avg** | **SD** |
| **Individual Results** | Target | | | | 10.13 | 0.23 | | | | | |
| | F004 | | | | | | | | | | |
| | F005 | 19.33 | 16.72 | 17.73 | 17.93 | 1.32 | 486.06 | 525.15 | 521.39 | 510.9 | 21.6 |
| | F011 | | | | | | | | | | |
| | F013 | 15.1 | 14.7 | 16.1 | 15.30 | 0.72 | 646 | 702 | 660 | 669.3 | 29.1 |
| | F014 | 14 | 14 | 13.6 | 13.87 | 0.23 | 578 | 608 | 586 | 590.7 | 15.5 |
| | F018 | | | | | | | | | | |
| | F020 | 11.5 | 12.1 | 12.1 | 11.90 | 0.35 | 630 | 774 | 598 | 667.3 | 93.8 |
| | F021 | | | | | | | | | | |
| | F026 | | | | | | | | | | |
| | F030 | 14.5 | 13.4 | 13.3 | 13.73 | 0.67 | 544 | 497 | 476 | 505.7 | 34.8 |
| | F031 | 13.02 | 13.43 | 15.91 | 14.12 | 1.56 | 660.61 | 620.97 | 662.62 | 648.1 | 23.5 |
| | F032 | | | | | | | | | | |
| | F033 | 13.1 | 12.9 | 13.3 | 13.10 | 0.20 | 529 | 520 | 554 | 534.3 | 17.6 |
| | F034 | 13.08 | 13.29 | 13.16 | 13.18 | 0.11 | | | | | |
| | F035 | | | | | | | | | | |
| | F039 | 13.9 | 13.8 | 13.6 | 13.77 | 0.15 | 585 | 626 | 649 | 620.0 | 32.4 |
| | F041 | 13.225 | 12.473 | 12.865 | 12.85 | 0.38 | | | | | |
| | F046 | 18.66 | 23.51 | 23.22 | 21.80 | 2.72 | 1284.78 | 1123.4 | 884.59 | 1097.6 | 201.3 |
| | F056 | | | | | | | | | | |
| | F057 | | | | | | | | | | |
| | F059 | 15.1 | 15.1 | 13.9 | 14.70 | 0.69 | | | | | |
| | F060 | | | | | | 637.7 | 653 | 645.8 | 645.5 | 7.65 |
| | F061 | 15.52 | 15.53 | 15.83 | 15.63 | 0.18 | | | | | |
| | F062 | 10.069 | 10.046 | 9.913 | 10.01 | 0.08 | 476.33 | 435.95 | 440.55 | 450.9 | 22.1 |
| | F069 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | F073 | | | | | | | | | | |
| | F075 | 14.6 | 14.4 | 14.4 | 14.47 | 0.12 | 610 | 601 | 660 | 623.7 | 31.8 |
| | F079 | | | | | | | | | | |
| | F088 | 14.558 | 16.653 | 15.147 | 15.45 | 1.08 | 788.883 | 853.168 | 777.075 | 806.4 | 41.0 |
| **Community Results** | | Consensus Mean | | | 14.25 | | Consensus Mean | | | 617.5 | |
| | | Consensus Standard Deviation | | | 2.40 | | Consensus Standard Deviation | | | 142.8 | |
| | | Maximum | | | 21.80 | | Maximum | | | 1097.6 | |
| | | Minimum | | | 0 | | Minimum | | | 0 | |
| | | N | | | 16 | | N | | | 13 | |

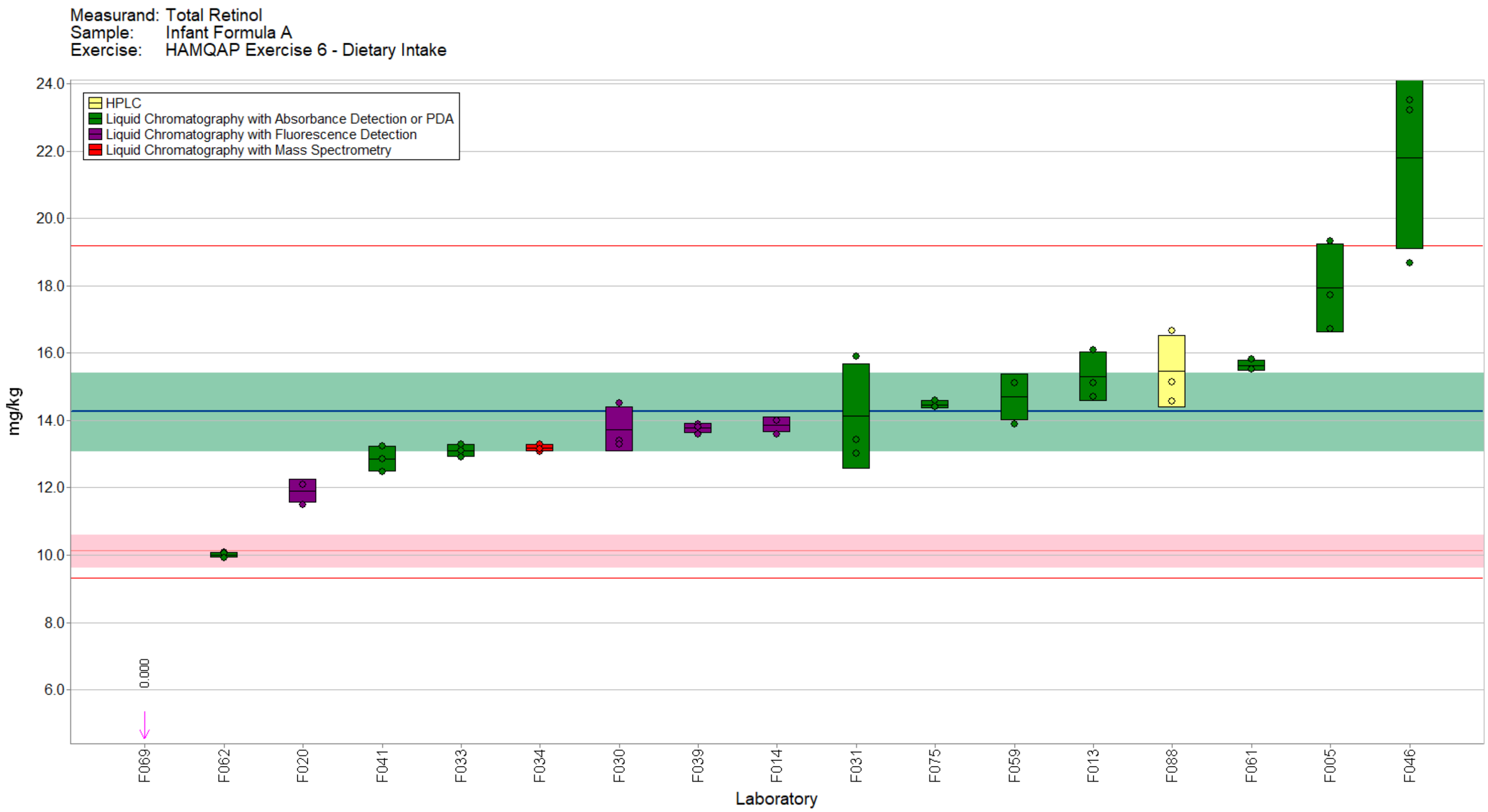


**Figure 4-1.** Total retinol in Infant Formula A (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue 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 $Z'_{\text{comm}}$ score, $|Z'_{\text{comm}}| \leq 2$. 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, $|Z_{\text{NIST}}| \leq 2$.

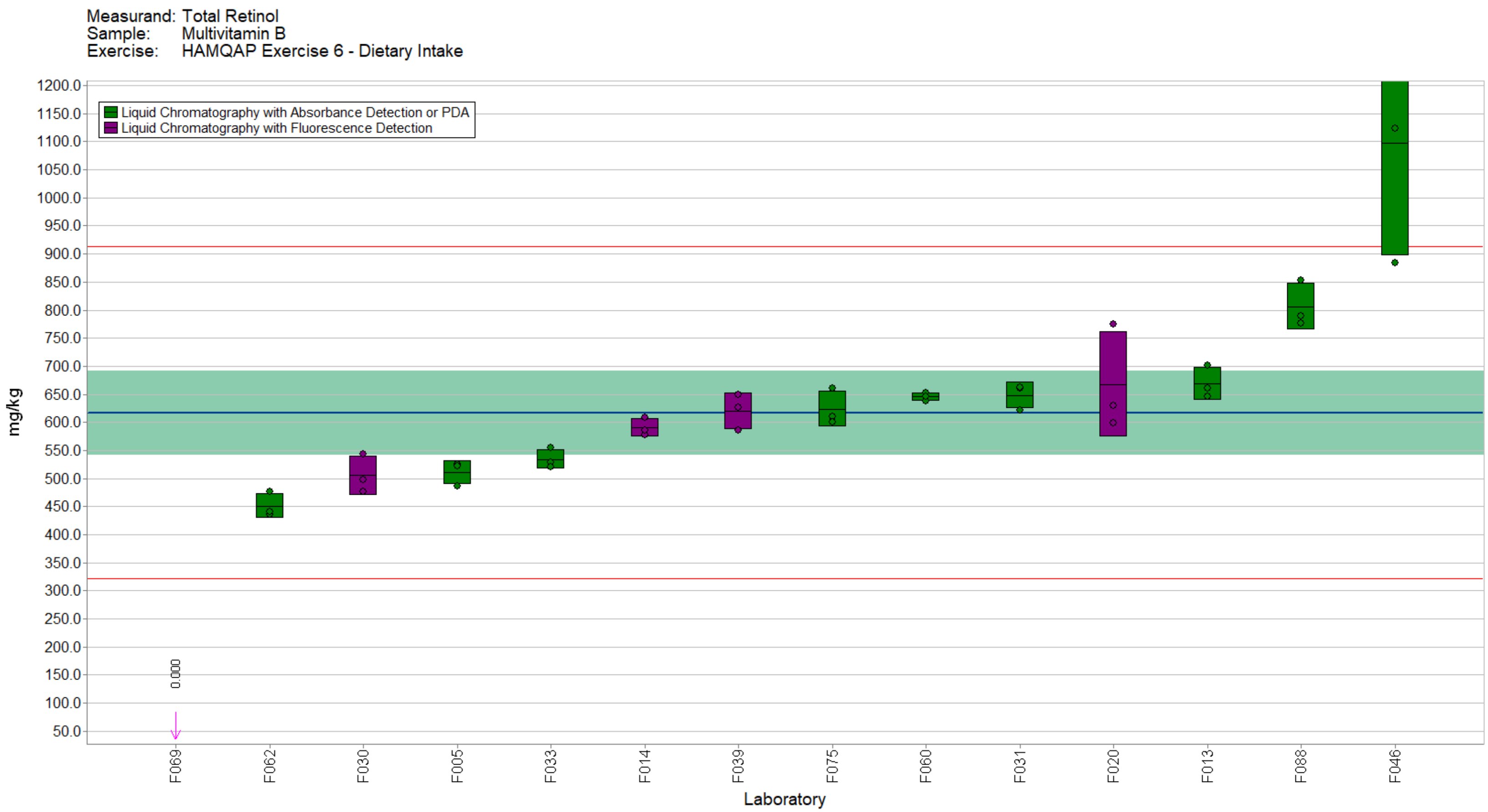


**Figure 4-2.** Total retinol in Multivitamin B (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue 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 $Z'_{\mathrm{comm}}$ score, $|Z'_{\mathrm{comm}}| \leq 2$. A NIST value has not been determined in this material.

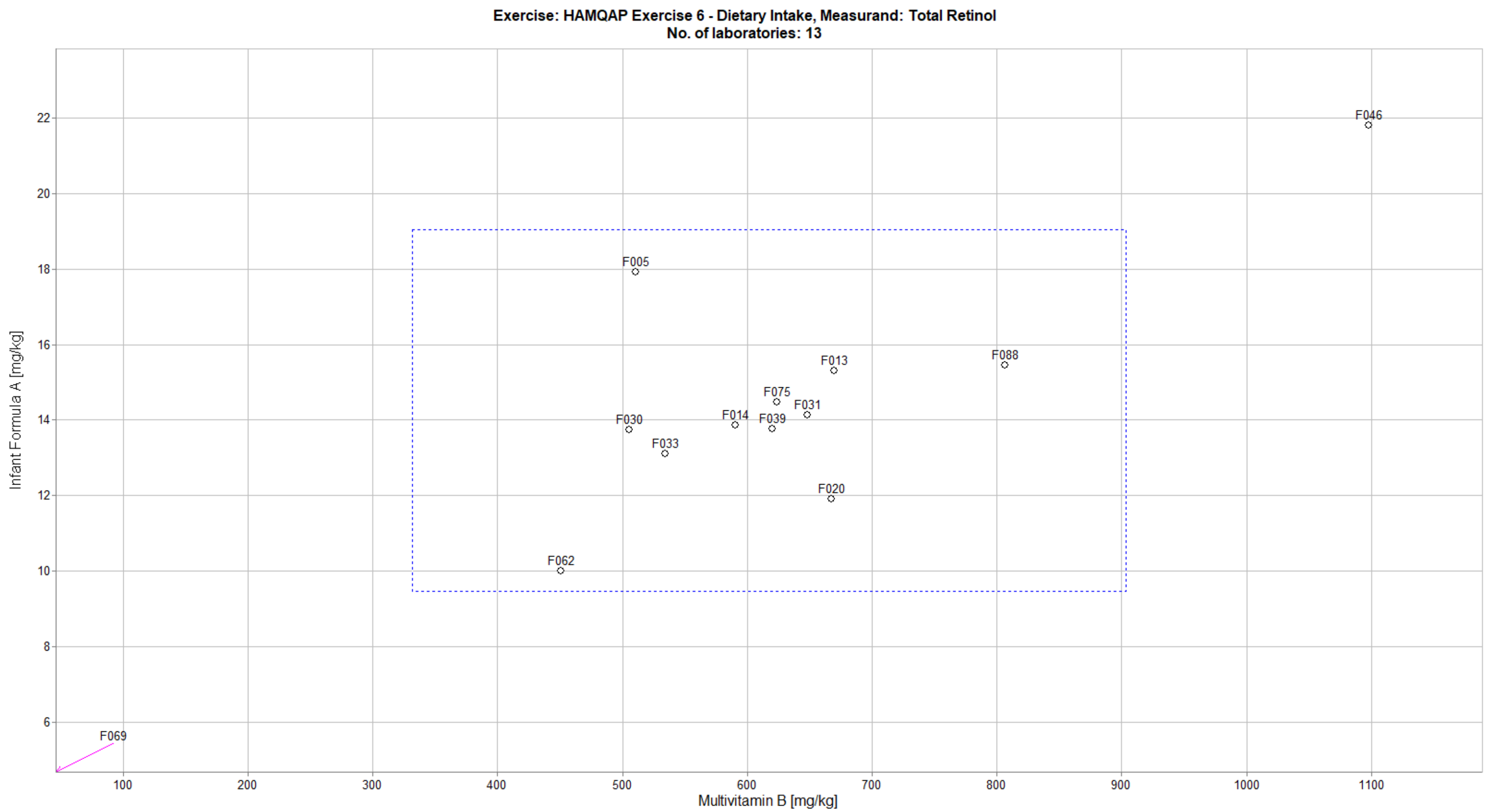


**Figure 4-3.** Laboratory means for total retinol in Infant Formula A and Multivitamin B (sample/sample comparison view). In this view, the individual laboratory mean for one sample (Multivitamin B) is compared to the individual laboratory mean for a second sample (Infant Formula A). The dotted blue box represents the consensus range of tolerance for Multivitamin B (x-axis) and Infant Formula A (y-axis), calculated as the values above and below the consensus means that result in an acceptable $Z'_{\mathrm{comm}}$ score, $|Z'_{\mathrm{comm}}| \leq 2$.

**Table 4-3.** Data summary table for retinyl acetate in Infant Formula A and Multivitamin B. Data points highlighted in blue have been identified as outside the consensus tolerance limits and would be estimated to result in an unacceptable $Z'_{\mathrm{comm}}$ score, $|Z'_{\mathrm{comm}}| \geq 2$.

| | | Retinyl Acetate | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Infant Formula A (mg/kg) | | | | | Multivitamin B (mg/kg) | | | | |
| | Lab | A | B | C | Avg | SD | A | B | C | Avg | SD |
| Individual Results | Target | | | | 6.73 | 0.23 | | | | 895 | 42 |
| | F004 | | | | | | | | | | |
| | F005 | 7.3 | 6.78 | 7.18 | 7.09 | 0.27 | 486.06 | 525.15 | 521.39 | 511 | 22 |
| | F011 | | | | | | 746.4 | 818.2 | 758.6 | 774 | 38 |
| | F018 | | | | | | | | | | |
| | F021 | | | | | | | | | | |
| | F022 | | | | | | 774 | 757 | 722 | 751 | 27 |
| | F026 | | | | | | | | | | |
| | F031 | | | | | | | | | | |
| | F033 | | | | | | | | | | |
| | F034 | | | | | | 789 | 830 | 787 | 802 | 24 |
| | F035 | | | | | | | | | | |
| | F039 | 15.9 | 15.9 | 15.6 | 15.80 | 0.17 | 682 | 718 | 744 | 715 | 31 |
| | F041 | 6.12 | 5.65 | 5.92 | 5.90 | 0.24 | | | | | |
| | F046 | 10.68 | 12.3 | 12.34 | 11.77 | 0.95 | 1120.35 | 979.63 | 771.38 | 957 | 176 |
| | F056 | | | | | | | | | | |
| | F057 | | | | | | | | | | |
| | F059 | | | | | | 607 | 583 | 623 | 604 | 20 |
| | F060 | | | | | | 731.3 | 748.9 | 740.6 | 740 | 8.8 |
| | F061 | | | | | | 590 | 599 | 615 | 601 | 13 |
| | F062 | | | | | | | | | | |
| | F069 | 7 | 8 | 13 | 9.33 | 3.21 | 666 | 638 | 658 | 654 | 14 |
| | F073 | | | | | | | | | | |
| | F075 | 7.86 | 7.7 | 7.66 | 7.74 | 0.11 | 700 | 689 | 757 | 715 | 37 |
| | F079 | | | | | | | | | | |
| | F088 | 5.791 | 4.701 | 6.021 | 5.50 | 0.71 | 737.78 | 762.098 | 561.3867 | 687 | 110 |
| | F089 | | | | | | | | | | |
| Community Results | | Consensus Mean | | | 8.66 | | Consensus Mean | | | 704 | |
| | | Consensus Standard Deviation | | | 3.08 | | Consensus Standard Deviation | | | 108 | |
| | | Maximum | | | 15.80 | | Maximum | | | 957 | |
| | | Minimum | | | 5.50 | | Minimum | | | 511 | |
| | | N | | | 7 | | N | | | 12 | |

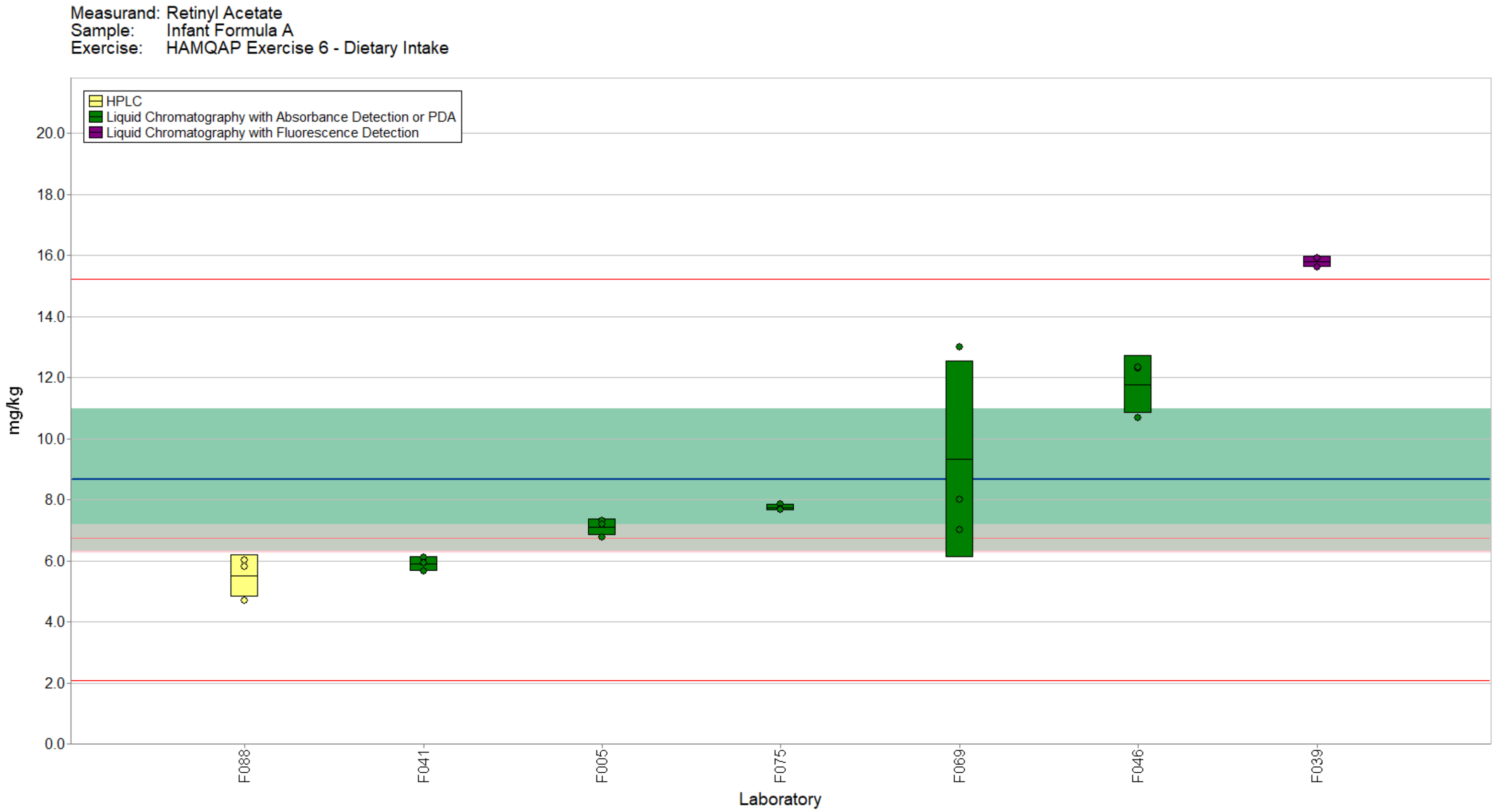


**Figure 4-4.** Retinyl acetate in Infant Formula A (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue 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 $Z'_{\mathrm{comm}}$ score, $|Z'_{\mathrm{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by its uncertainty ($U_{\mathrm{NIST}}$) and represents the range that results in an acceptable $Z_{\mathrm{NIST}}$ score, $|Z_{\mathrm{NIST}}| \leq 2$.

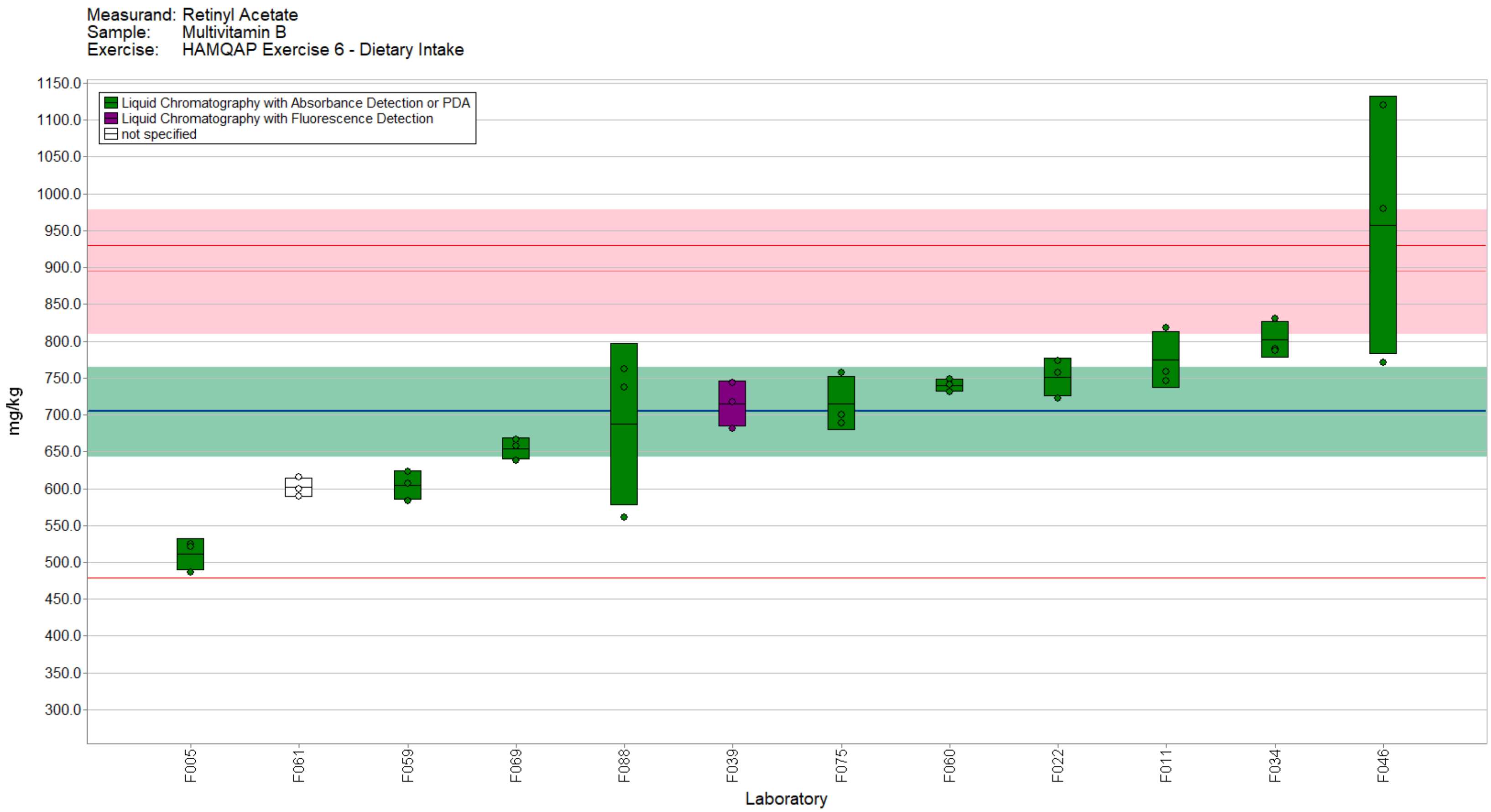


**Figure 4-5.** Retinyl acetate in Multivitamin B (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue 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 $Z'_{\text{comm}}$ score, $|Z'_{\text{comm}}| \leq 2$. 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, $|Z_{\text{NIST}}| \leq 2$.

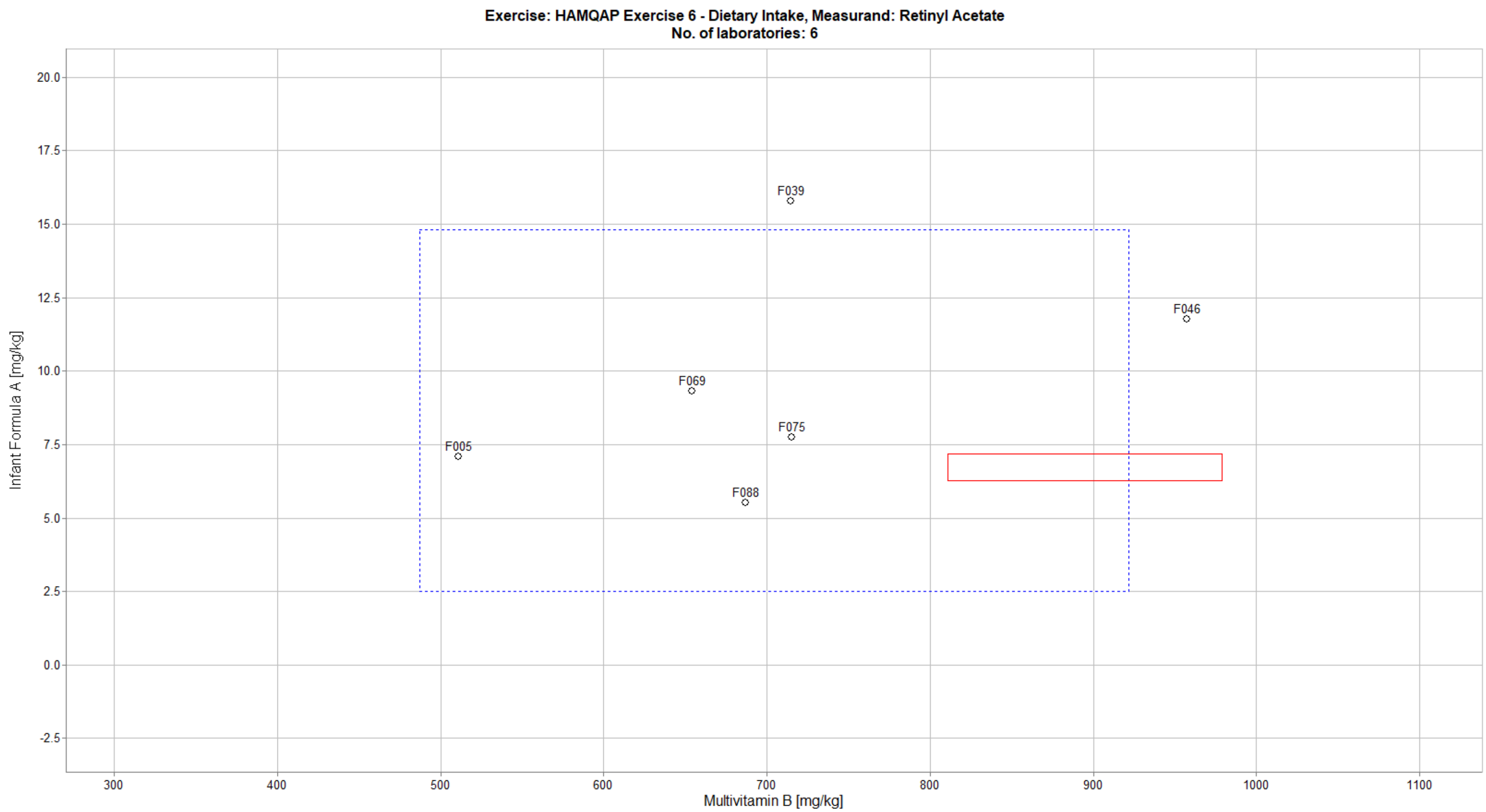


**Figure 4-6.** Laboratory means for retinyl acetate in Infant Formula A and Multivitamin B (sample/sample comparison view). In this view, the individual laboratory mean for one sample (multivitamin) is compared to the individual laboratory mean for a second sample (Infant Formula A). The solid red box represents the NIST range of tolerance for the two samples, Multivitamin B (x-axis) and Infant Formula 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 Multivitamin B (x-axis) and Infant Formula A (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 4-4.** Data summary table for retinyl palmitate in Infant Formula A and Multivitamin B. Data points highlighted in blue have been identified as outside the consensus tolerance limits and would be estimated to result in an unacceptable $Z'_{\mathrm{comm}}$ score, $|Z'_{\mathrm{comm}}| \geq 2$. Data points highlighted in red have a zero or non-numeric data point.

| | | Retinyl Palmitate | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Infant Formula A (mg/kg) | | | | | Multivitamin B (mg/kg) | | | | |
| | Lab | A | B | C | Avg | SD | A | B | C | Avg | SD |
| Individual Results | Target | | | | 7.80 | 0.23 | | | | | |
| | F004 | | | | | | | | | | |
| | F005 | 12.03 | 9.94 | 10.55 | 10.84 | 1.07 | 0 | 0 | 0 | 0 | 0 |
| | F011 | | | | | | | | | | |
| | F018 | | | | | | | | | | |
| | F021 | | | | | | | | | | |
| | F026 | 13.0103 | | | 13.01 | | | | | | |
| | F031 | | | | | | | | | | |
| | F033 | | | | | | | | | | |
| | F034 | | | | | | | | | | |
| | F035 | | | | | | | | | | |
| | F039 | 25.4 | 25.3 | 25 | 25.23 | 0.21 | 1090 | 1150 | 1190 | 1143 | 50 |
| | F041 | 14.46 | 13.83 | 14.12 | 14.14 | 0.32 | | | | | |
| | F045 | | | | | | | | | | |
| | F046 | 11.74 | 17.23 | 16.62 | 15.20 | 3.01 | | | | | |
| | F056 | | | | | | | | | | |
| | F057 | | | | | | | | | | |
| | F059 | | | | | | | | | | |
| | F060 | | | | | | < 562.000 | < 562.000 | < 562.000 | | |
| | F061 | | | | | | < 40.000 | < 40.000 | < 40.000 | | |
| | F062 | | | | | | | | | | |
| | F069 | 36 | 44 | 72 | 50.67 | 18.90 | 116 | 104 | 146 | 122 | 22 |
| | F073 | | | | | | | | | | |
| | F075 | 14.1 | 14.2 | 14.2 | 14.17 | 0.06 | | | | | |
| | F079 | 6.25 | 4.8 | 4.57 | 5.21 | 0.91 | 1.36 | 1.29 | 1.13 | 1.26 | 0.12 |
| | F088 | 7.312 | 7.119 | 6.13 | 6.85 | 0.63 | | | | | |
| Community Results | | Consensus Mean | | | 13.04 | | Consensus Mean | | | 41 | |
| | | Consensus Standard Deviation | | | 7.90 | | Consensus Standard Deviation | | | 231 | |
| | | Maximum | | | 50.67 | | Maximum | | | 1143 | |
| | | Minimum | | | 5.21 | | Minimum | | | 0 | |
| | | N | | | 8 | | N | | | 3 | |

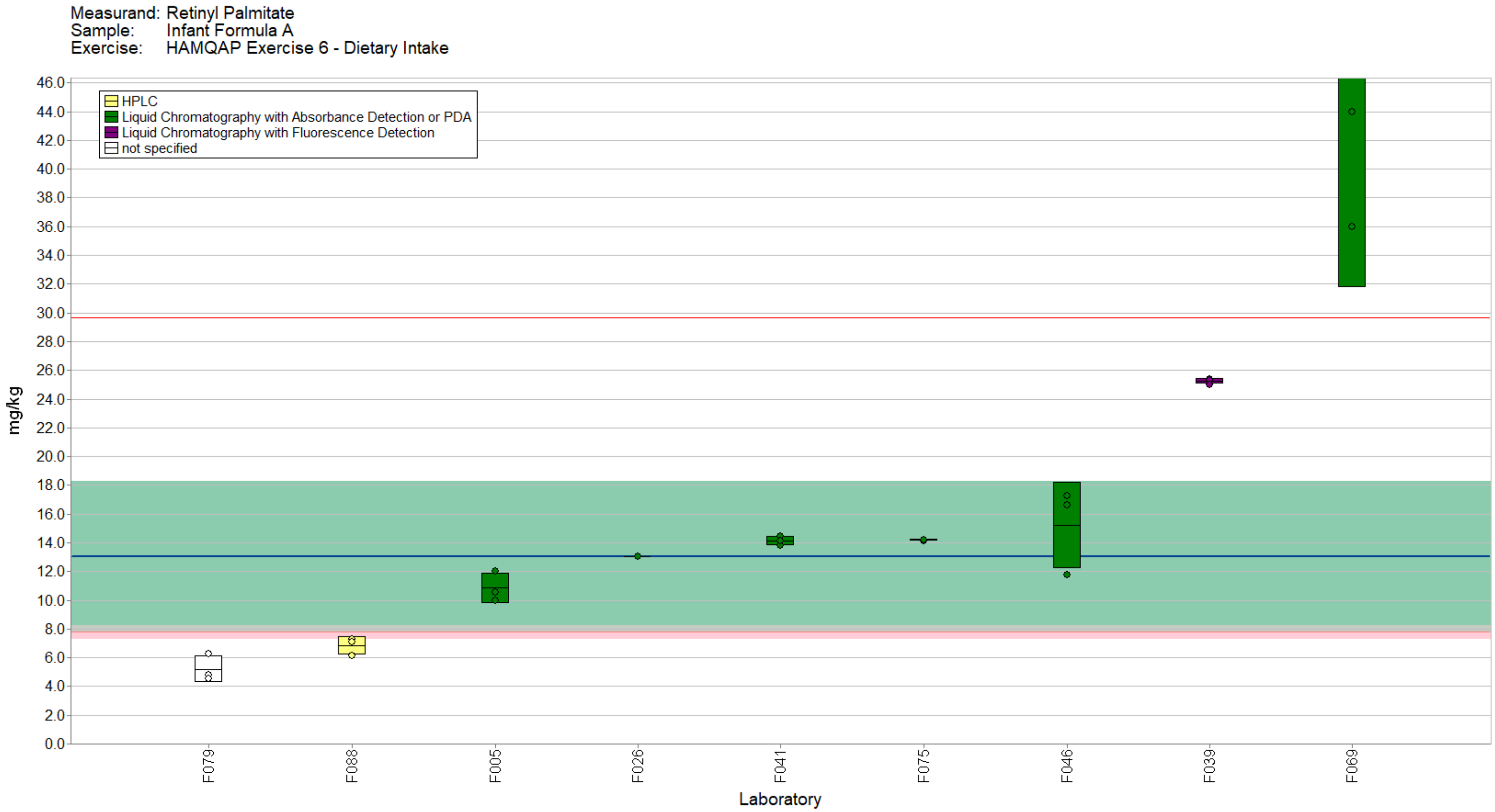


**Figure 4-7.** Retinyl palmitate in Infant Formula A (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue 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 $Z'_{\text{comm}}$ score, $|Z'_{\text{comm}}| \leq 2$. 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, $|Z_{\text{NIST}}| \leq 2$.

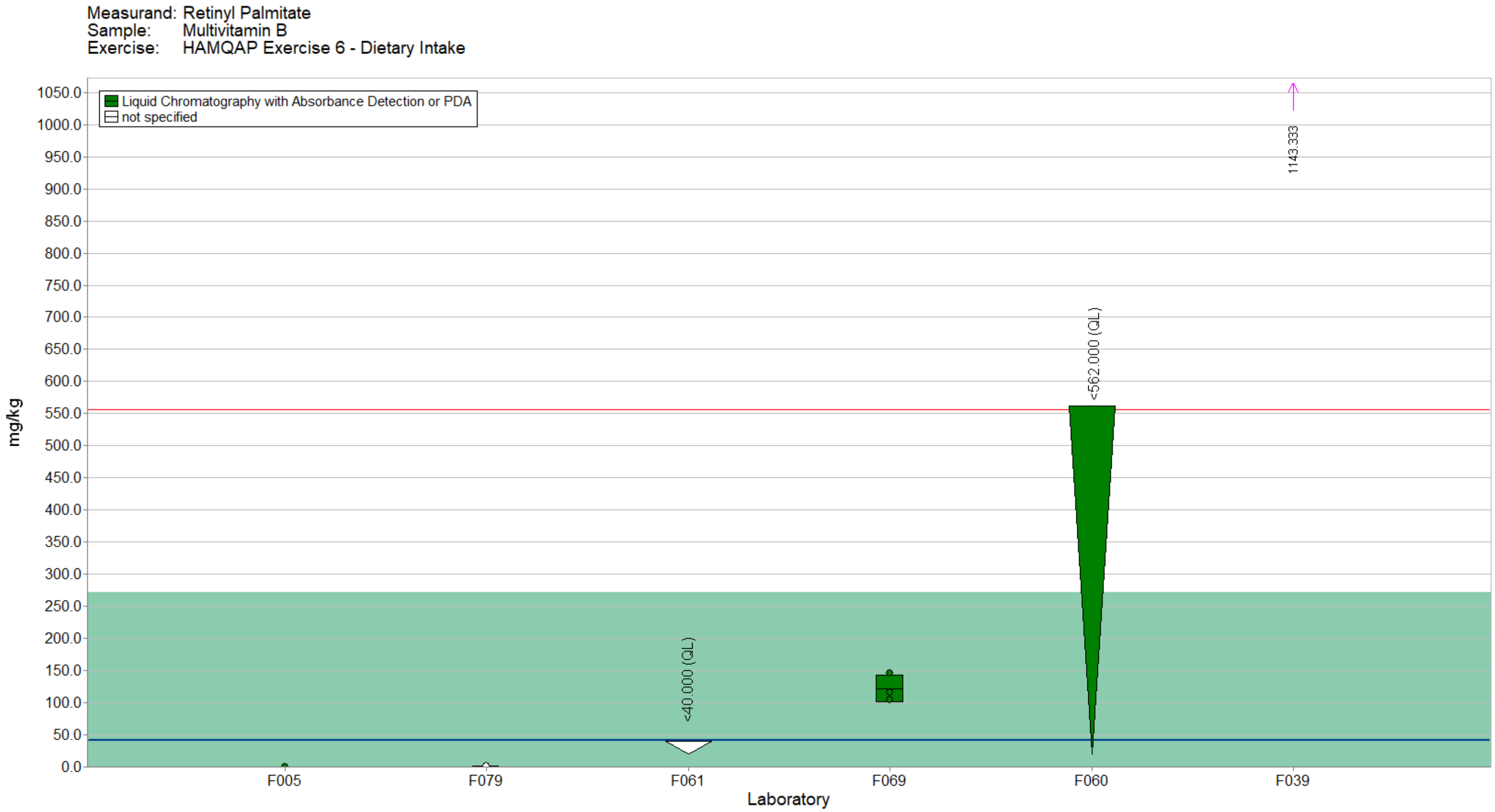


**Figure 4-8.** Retinyl palmitate in Multivitamin B (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue 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 $Z'_{\mathrm{comm}}$ score, $|Z'_{\mathrm{comm}}| \leq 2$. A NIST value has not been determined in this material.

**Table 4-5.** Data summary table for total alpha-tocopherol in Infant Formula A and Multivitamin B. Data points highlighted in blue have been identified as outside the consensus tolerance limits and would be estimated to result in an unacceptable $Z'_{\mathrm{comm}}$ score, $|Z'_{\mathrm{comm}}| \geq 2$. Data points highlighted in red have a zero or non-numeric data point.

| | | Total alpha-Tocopherol | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Infant Formula A (mg/kg) | | | | | Multivitamin B (mg/kg) | | | | |
| | Lab | A | B | C | Avg | SD | A | B | C | Avg | SD |
| Individual Results | Target | | | | 205.66 | 5.93 | | | | | |
| | F004 | | | | | | | | | | |
| | F005 | 183.3 | 195.84 | 209.85 | 196.3 | 13.3 | 19114.12 | 19207.86 | 19392.93 | 19238 | 142 |
| | F011 | | | | | | | | | | |
| | F014 | 211 | 196 | 190 | 199.0 | 10.8 | 20600 | 19800 | 19500 | 19967 | 569 |
| | F017 | 26.36 | 26.81 | 29.86 | 27.7 | 1.9 | < 10.000 | < 10.000 | < 10.000 | | |
| | F018 | | | | | | | | | | |
| | F020 | 193 | 206 | 204 | 201.0 | 7.0 | 18200 | 22700 | 18300 | 19733 | 2570 |
| | F021 | | | | | | | | | | |
| | F022 | 435 | 437 | 426 | 432.7 | 5.9 | 20055 | 18747 | 19164 | 19322 | 668 |
| | F026 | | | | | | 19294 | | | 19294 | |
| | F030 | | | | | | | | | | |
| | F031 | 212.92 | 215.66 | 225.79 | 218.1 | 6.8 | 18563.68 | 18047.61 | 18556.67 | 18389 | 296 |
| | F032 | | | | | | | | | | |
| | F033 | 364 | 361 | 356 | 360.3 | 4.0 | 17117 | 17362 | 17485 | 17321 | 187 |
| | F034 | | | | | | | | | | |
| | F035 | | | | | | | | | | |
| | F039 | | | | | | | | | | |
| | F046 | 149.59 | 123.86 | 126.5 | 133.3 | 14.2 | 17876.16 | 17755.56 | 18279.84 | 17971 | 275 |
| | F056 | | | | | | | | | | |
| | F057 | | | | | | | | | | |
| | F059 | | | | | | | | | | |
| | F060 | | | | | | | | | | |
| | F061 | | | | | | | | | | |
| | F062 | | | | | | | | | | |
| | F069 | 0 | 0 | 0 | 0 | 0 | 17637 | 17209 | 17817 | 17554 | 312 |
| | F073 | | | | | | | | | | |
| | F075 | 197 | 200 | 199 | 198.7 | 1.5 | 18100 | 18000 | 17700 | 17933 | 208 |
| | F077 | | | | | | | | | | |
| | F079 | 96 | 71 | 46 | 71.0 | 25.0 | | | | | |
| | F088 | 217.97197 | 247.007 | 210.25132 | 225.1 | 19.4 | 7193.814 | 9285.496 | 11126.592 | 9202 | 1968 |
| | F089 | | | | | | | | | | |
| Community Results | | Consensus Mean | | | 180.4 | | Consensus Mean | | | 18672 | |
| | | Consensus Standard Deviation | | | 102.1 | | Consensus Standard Deviation | | | 1430 | |
| | | Maximum | | | 432.7 | | Maximum | | | 19967 | |
| | | Minimum | | | 0 | | Minimum | | | 9202 | |
| | | N | | | 11 | | N | | | 10 | |

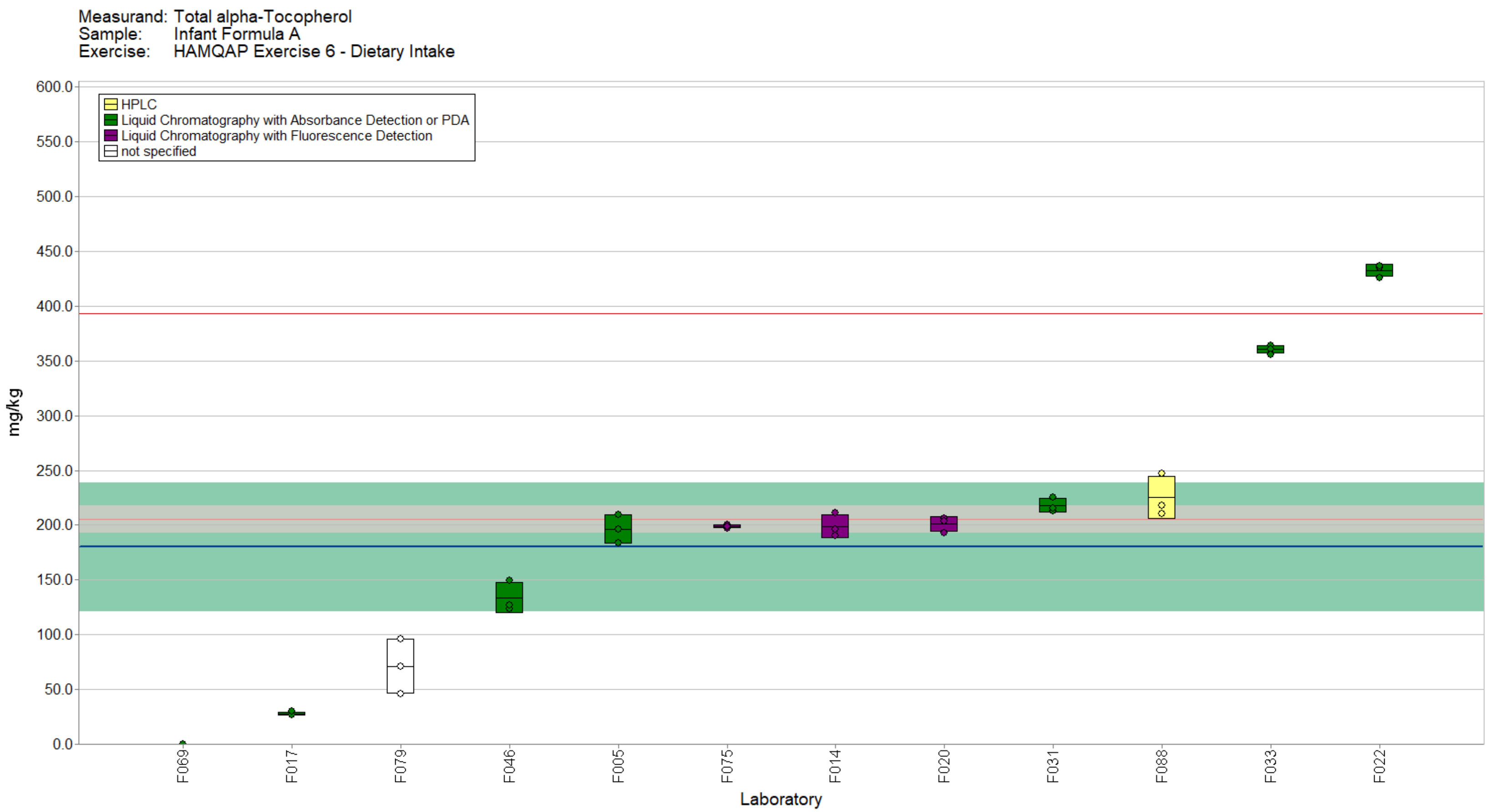


**Figure 4-9.** Total alpha-tocopherol in Infant Formula A (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue 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 $Z'_{\mathrm{comm}}$ score, $|Z'_{\mathrm{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by its uncertainty ($U_{\mathrm{NIST}}$) and represents the range that results in an acceptable $Z_{\mathrm{NIST}}$ score, $|Z_{\mathrm{NIST}}| \leq 2$.

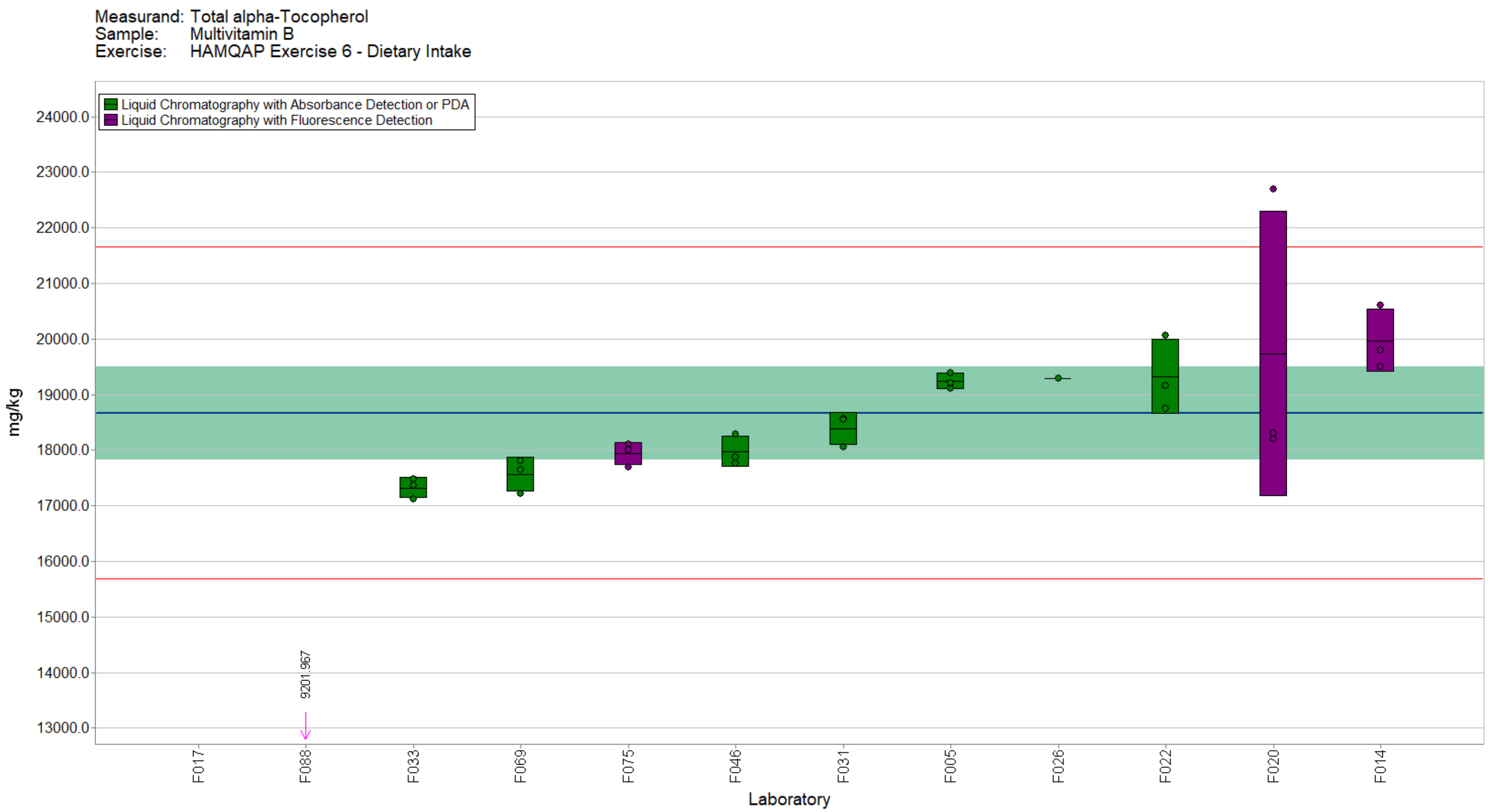


**Figure 4-10.** Total alpha-tocopherol in Multivitamin B (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue 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 $Z'_{\mathrm{comm}}$ score, $|Z'_{\mathrm{comm}}| \leq 2$. A NIST value has not been determined in this material.

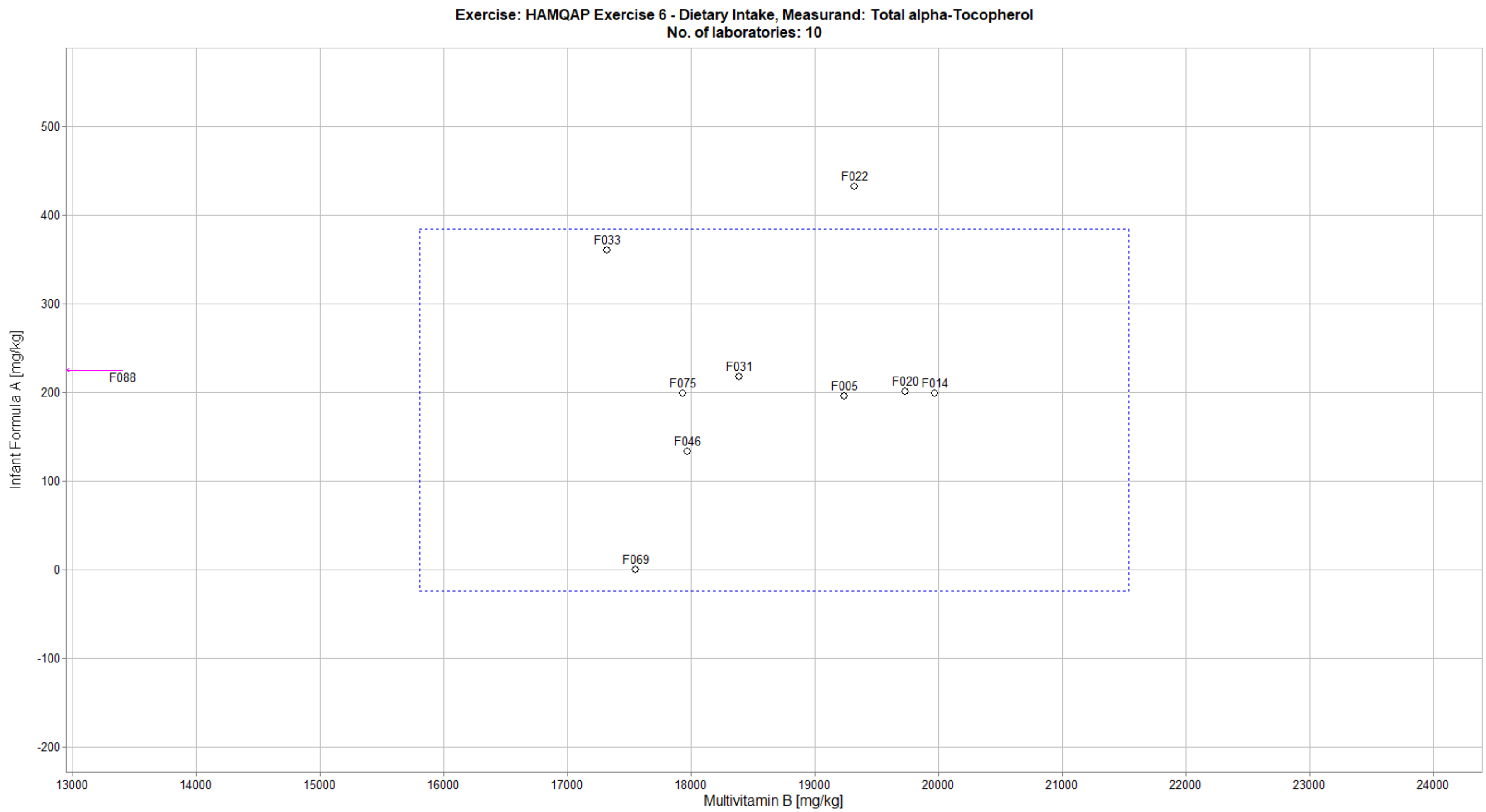


**Figure 4-11.** Laboratory means for total alpha-tocopherol in Infant Formula A and Multivitamin B (sample/sample comparison view). In this view, the individual laboratory mean for one sample (multivitamin) is compared to the individual laboratory mean for a second sample (Infant Formula A). The dotted blue box represents the consensus range of tolerance for Multivitamin B (x-axis) and Infant Formula A (y-axis), calculated as the values above and below the consensus means that result in an acceptable $Z'_{\mathrm{comm}}$ score, $|Z'_{\mathrm{comm}}| \leq 2$.

**Table 4-6.** Data summary table for alpha-tocopherol in Infant Formula A and Multivitamin B. Data points highlighted in blue have been identified as outside the consensus tolerance limits and would be estimated to result in an unacceptable $Z'_{\mathrm{comm}}$ score, $|Z'_{\mathrm{comm}}| \geq 2$.

| | | alpha-Tocopherol | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Infant Formula A (mg/kg) | | | | | Multivitamin B (mg/kg) | | | | |
| | Lab | A | B | C | Avg | SD | A | B | C | Avg | SD |
| Individual Results | Target | | | | 58.39 | 3.88 | | | | | |
| | F004 | | | | | | | | | | |
| | F005 | 46.24 | 52.6 | 60.09 | 52.98 | 6.93 | 164.73 | 172.2 | 171.2 | 169.38 | 4.06 |
| | F011 | | | | | | 143.8 | 145.5 | 142.5 | 143.93 | 1.50 |
| | F013 | 191 | 175 | 180 | 182.0 | 8.19 | | | | | |
| | F014 | | | | | | | | | | |
| | F017 | 26.36 | 26.81 | 29.86 | 27.68 | 1.90 | < 10.000 | < 10.000 | < 10.000 | | |
| | F018 | | | | | | | | | | |
| | F021 | | | | | | | | | | |
| | F026 | | | | | | | | | | |
| | F030 | 188 | 185 | 185 | 186.0 | 1.73 | 18000 | 17800 | 17800 | 17867 | 115 |
| | F031 | | | | | | | | | | |
| | F033 | 203 | 200 | 199 | 200.7 | 2.08 | 16510 | 16753 | 16860 | 16708 | 179 |
| | F034 | 71.4 | 66.3 | 71.2 | 69.63 | 2.89 | | | | | |
| | F035 | | | | | | | | | | |
| | F039 | 211 | 221 | 227 | 219.7 | 8.08 | 17100 | 18200 | 17400 | 17566.67 | 569 |
| | F041 | | | | | | | | | | |
| | F046 | 23.36 | 22.94 | 22.66 | 22.99 | 0.35 | 179.95 | 177.57 | 176.71 | 178.08 | 1.68 |
| | F051 | | | | | | | | | | |
| | F056 | | | | | | | | | | |
| | F057 | | | | | | | | | | |
| | F059 | | | | | | | | | | |
| | F060 | | | | | | | | | | |
| | F061 | 197.6 | 202.1 | 213.1 | 204.3 | 7.97 | | | | | |
| | F062 | 160.2 | 147.3 | 140.5 | 149.3 | 10.01 | 14826.9 | 13872.7 | 12539.1 | 13746 | 1149 |
| | F073 | | | | | | | | | | |
| | F075 | 59.4 | 58.5 | 59.3 | 59.07 | 0.49 | 201 | 207 | 190 | 199.33 | 8.62 |
| | F077 | | | | | | | | | | |
| | F079 | | | | | | | | | | |
| | F088 | 52.802 | 43.774 | 53.929 | 50.17 | 5.57 | 87.815 | 87.664 | 109.287 | 94.92 | 12.44 |
| | F089 | | | | | | | | | | |
| Community Results | | Consensus Mean | | | 118.6 | | Consensus Mean | | | 157.1 | |
| | | Consensus Standard Deviation | | | 57.9 | | Consensus Standard Deviation | | | 204.0 | |
| | | Maximum | | | 219.7 | | Maximum | | | 17866.7 | |
| | | Minimum | | | 23.0 | | Minimum | | | 94.9 | |
| | | N | | | 12 | | N | | | 9 | |

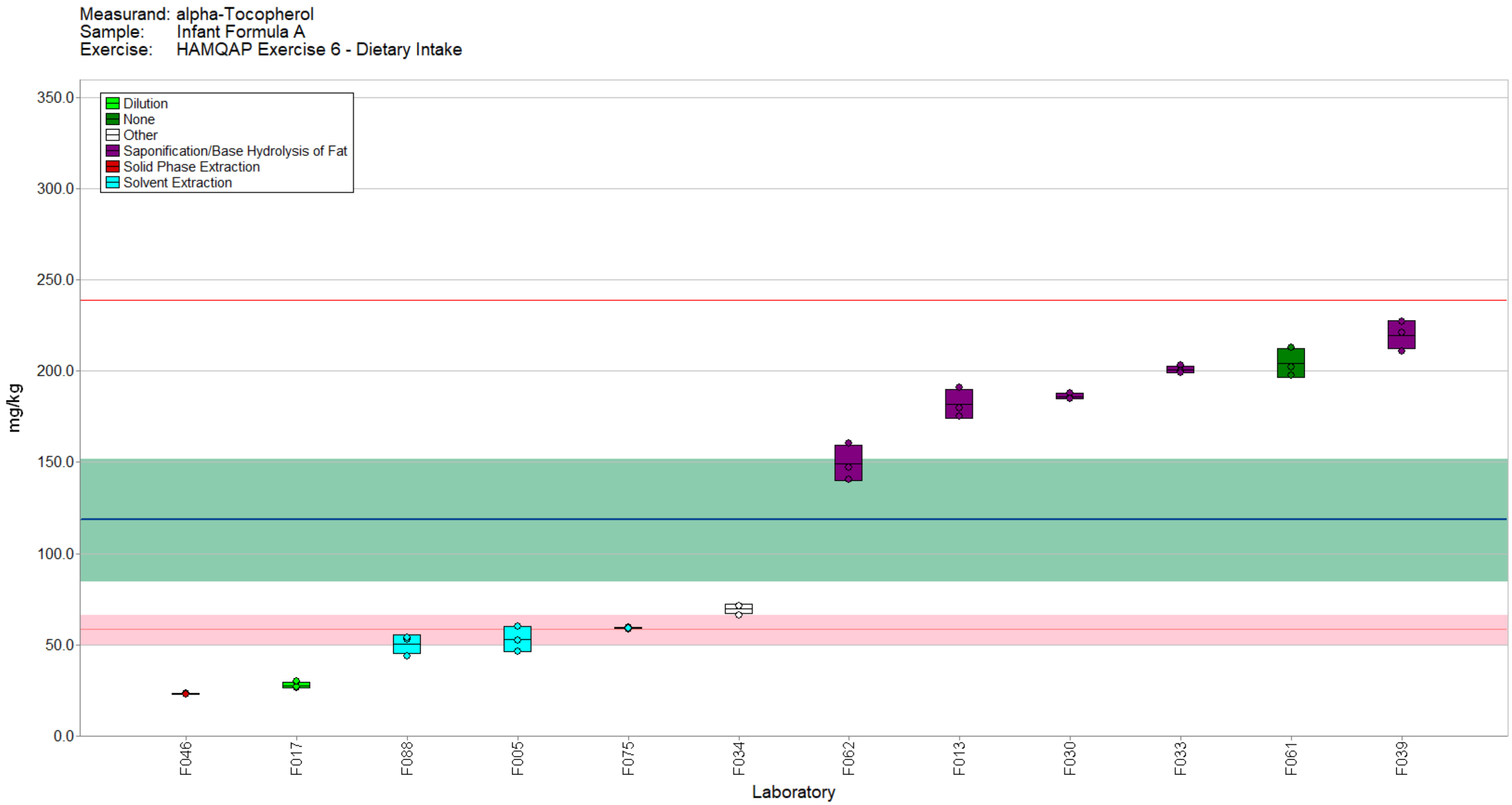


**Figure 4-12.** Alpha-tocopherol in Infant Formula A (data summary view – sample preparation). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation employed. The solid blue 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 $Z'_{\text{comm}}$ score, $|Z'_{\text{comm}}| \leq 2$. 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, $|Z_{\text{NIST}}| \leq 2$.

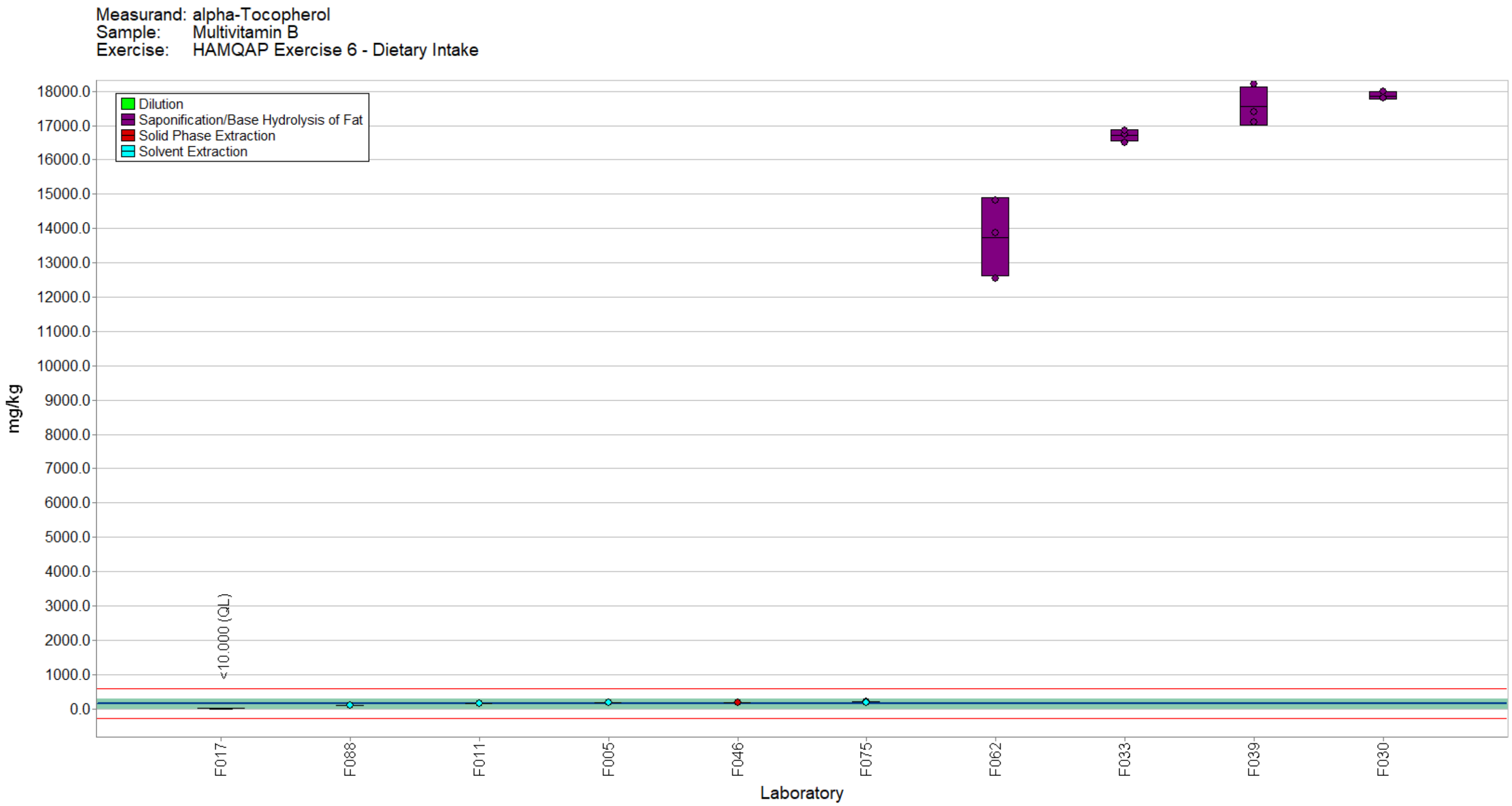


**Figure 4-13.** Alpha-tocopherol in Multivitamin B (data summary view – sample preparation). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation employed. The solid blue 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 $Z'_{\text{comm}}$ score, $|Z'_{\text{comm}}| \leq 2$. A NIST value has not been determined in this material.

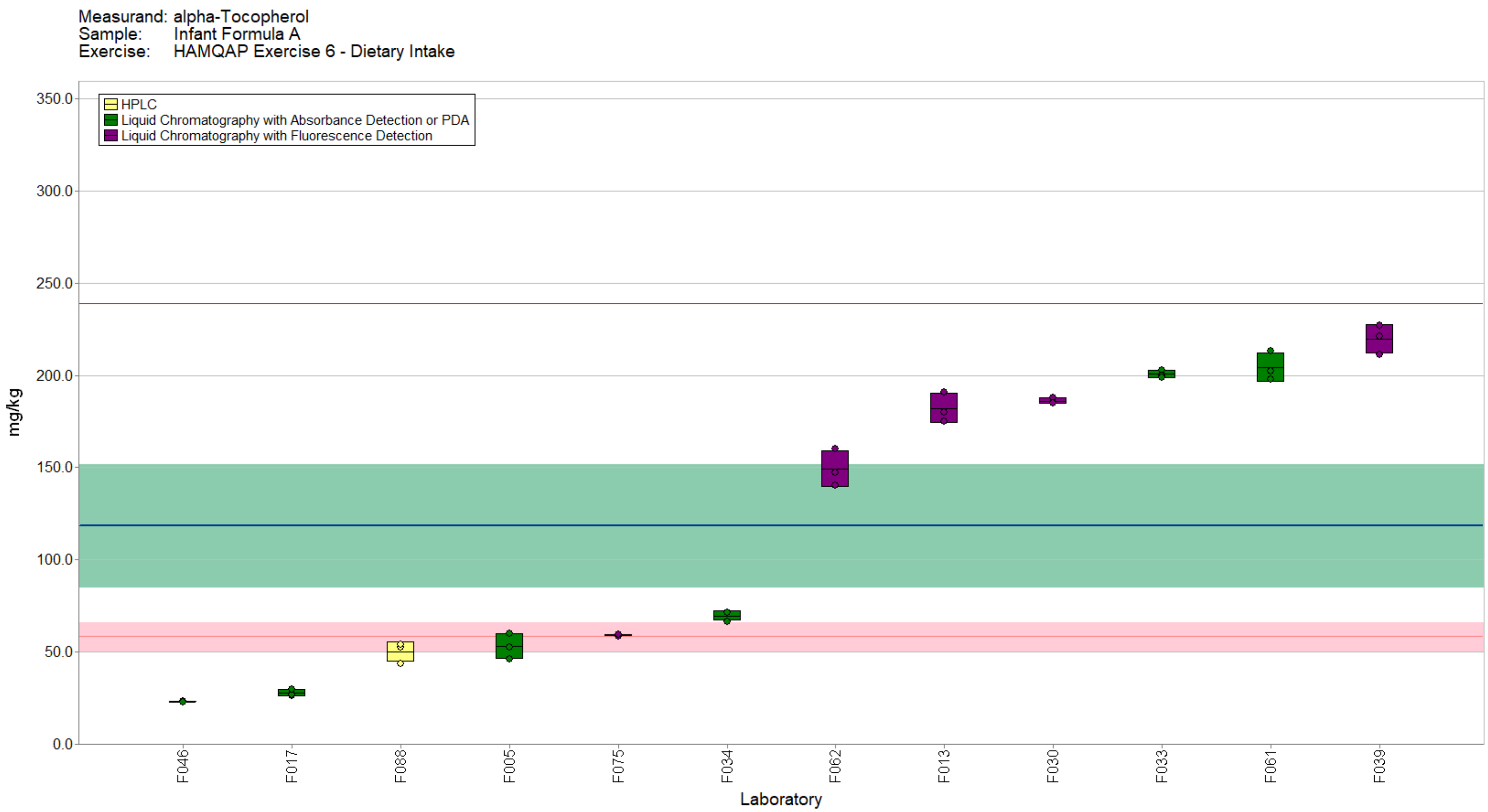


**Figure 4-14.** Alpha-tocopherol in Infant Formula A (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue 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 $Z'_{\text{comm}}$ score, $|Z'_{\text{comm}}| \leq 2$. 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, $|Z_{\text{NIST}}| \leq 2$.

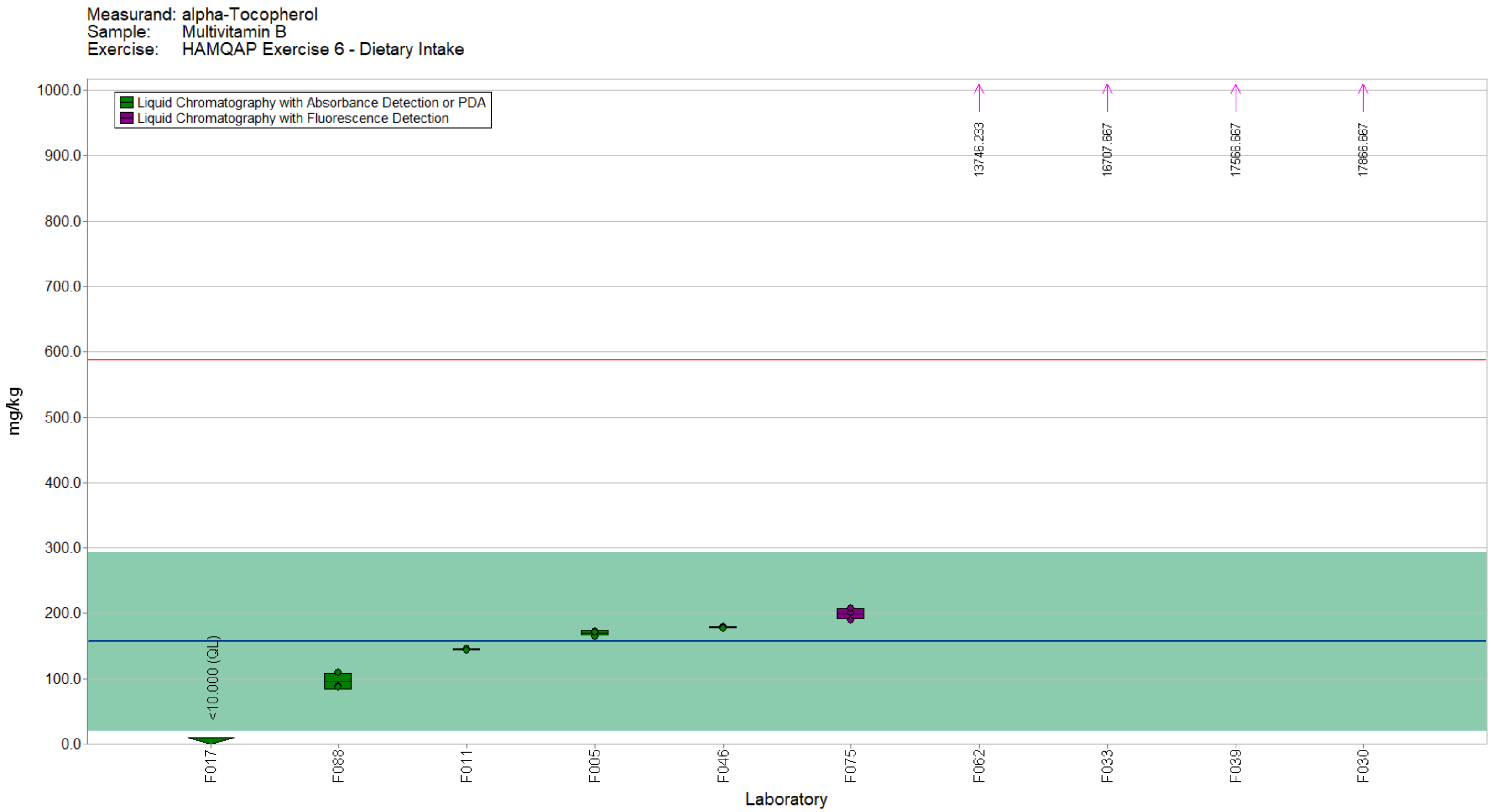


**Figure 4-15.** Alpha-tocopherol in Multivitamin B (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue 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 $Z'_{\text{comm}}$ score, $|Z'_{\text{comm}}| \leq 2$. A NIST value has not been determined in this material.

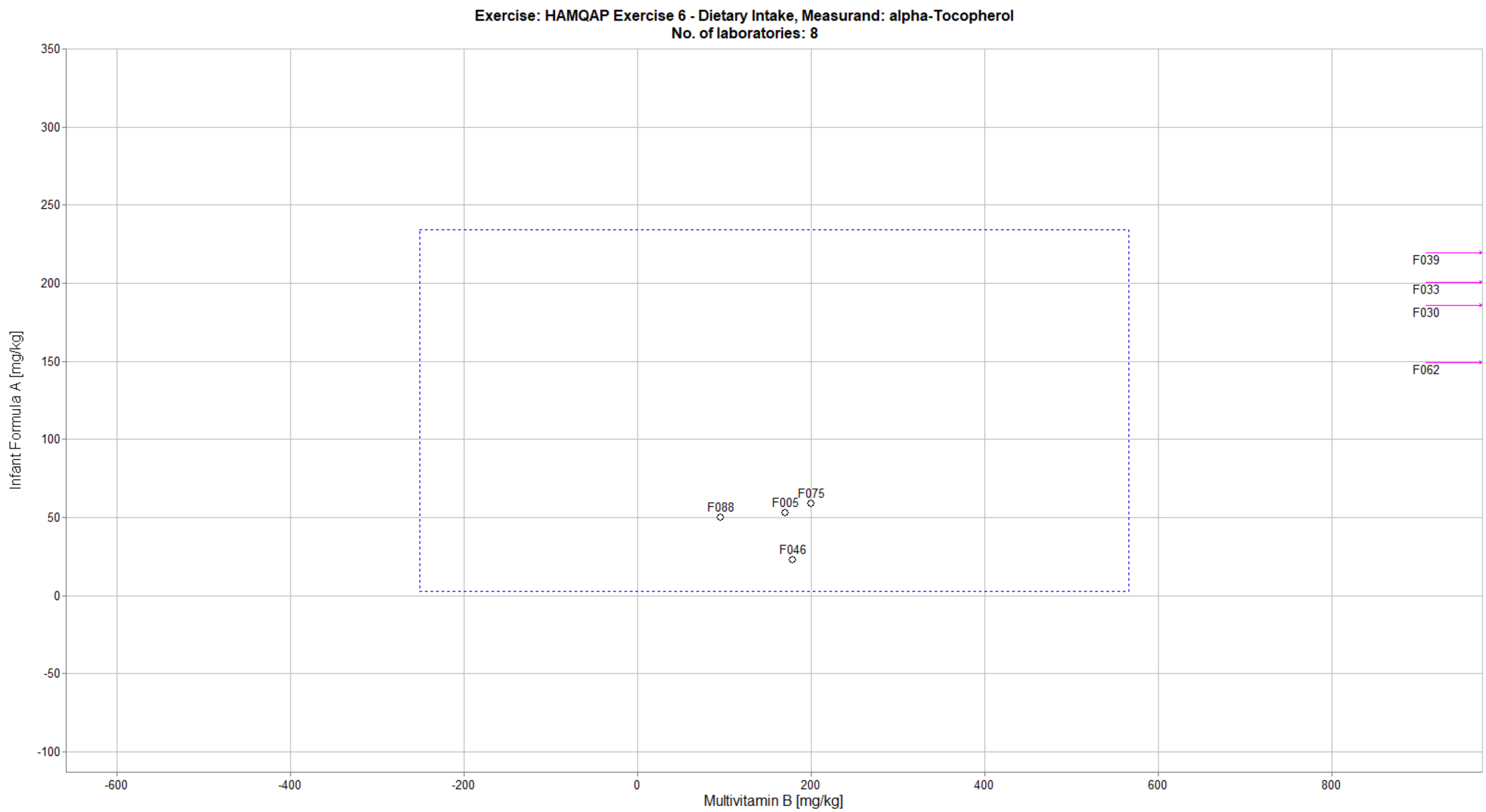


**Figure 4-16.** Laboratory means for alpha-tocopherol in Infant Formula A and Multivitamin B (sample/sample comparison view). In this view, the individual laboratory mean for one sample (multivitamin) is compared to the individual laboratory mean for a second sample (Infant Formula A). The dotted blue box represents the consensus range of tolerance for Multivitamin B (x-axis) and Infant Formula A (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 4-7.** Data summary table for alpha-tocopheryl acetate in Infant Formula A and Multivitamin B. Data points highlighted in blue have been identified as outside the consensus tolerance limits and would be estimated to result in an unacceptable $Z'_{\mathrm{comm}}$ score, $|Z'_{\mathrm{comm}}| \geq 2$.

| | | alpha-Tocopheryl Acetate | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Infant Formula A (mg/kg) | | | | | Multivitamin B (mg/kg) | | | | |
| | Lab | A | B | C | Avg | SD | A | B | C | Avg | SD |
| Individual Results | Target | | | | 161.6 | 4.9 | | | | 17931 | 430 |
| | F004 | | | | | | | | | | |
| | F005 | 137.06 | 143.24 | 149.76 | 143.4 | 6.35 | 18949.39 | 19035.66 | 19221.73 | 19069 | 139 |
| | F011 | | | | | | 19942.8 | 21584.2 | 20595.8 | 20708 | 826 |
| | F013 | | | | | | 19500 | 19540 | 19430 | 19490 | 56 |
| | F017 | 71.35 | 77.54 | 92.74 | 80.5 | 11.01 | 18696 | 18905 | 20112 | 19238 | 764 |
| | F018 | | | | | | | | | | |
| | F021 | | | | | | | | | | |
| | F026 | | | | | | | | | | |
| | F031 | | | | | | | | | | |
| | F033 | | | | | | | | | | |
| | F034 | 179 | 192 | 195 | 188.7 | 8.50 | 21300 | 21350 | 20980 | 21210 | 201 |
| | F035 | | | | | | | | | | |
| | F039 | 232 | 243 | 249 | 241.3 | 8.62 | 18800 | 20000 | 19100 | 19300 | 624 |
| | F046 | 138.54 | 110.76 | 113.96 | 121.1 | 15.20 | 19421.41 | 19291.67 | 19868.02 | 19527 | 302 |
| | F051 | | | | | | | | | | |
| | F056 | | | | | | 20485 | 20668 | 19896 | 20350 | 403 |
| | F057 | | | | | | 18323.6 | 18092 | 18096.2 | 18171 | 133 |
| | F059 | | | | | | 18400 | 18200 | 18900 | 18500 | 361 |
| | F060 | | | | | | 18690 | 18482 | 18910 | 18694 | 214 |
| | F061 | | | | | | | | | | |
| | F062 | | | | | | | | | | |
| | F073 | | | | | | | | | | |
| | F074 | 13.28 | 13.79 | 16.9 | 14.7 | 1.96 | 1111 | 1119 | 1124 | 1118 | 6.6 |
| | F075 | 151 | 156 | 153 | 153.3 | 2.52 | 19700 | 19600 | 19600 | 19633 | 58 |
| | F077 | | | | | | | | | | |
| | F079 | 132 | 111 | 50 | 97.7 | 42.59 | 6359 | 7147 | 9447 | 7651 | 1605 |
| | F088 | 81.433 | 56.389 | 80.197 | 72.7 | 14.12 | 4207.561 | 5284.536 | 4910.164 | 4801 | 547 |
| | F089 | | | | | | | | | | |
| Community Results | | Consensus Mean | | | 124 | | Consensus Mean | | | 19491 | |
| | | Consensus Standard Deviation | | | 89 | | Consensus Standard Deviation | | | 1621 | |
| | | Maximum | | | 241 | | Maximum | | | 21210 | |
| | | Minimum | | | 15 | | Minimum | | | 1118 | |
| | | N | | | 9 | | N | | | 15 | |

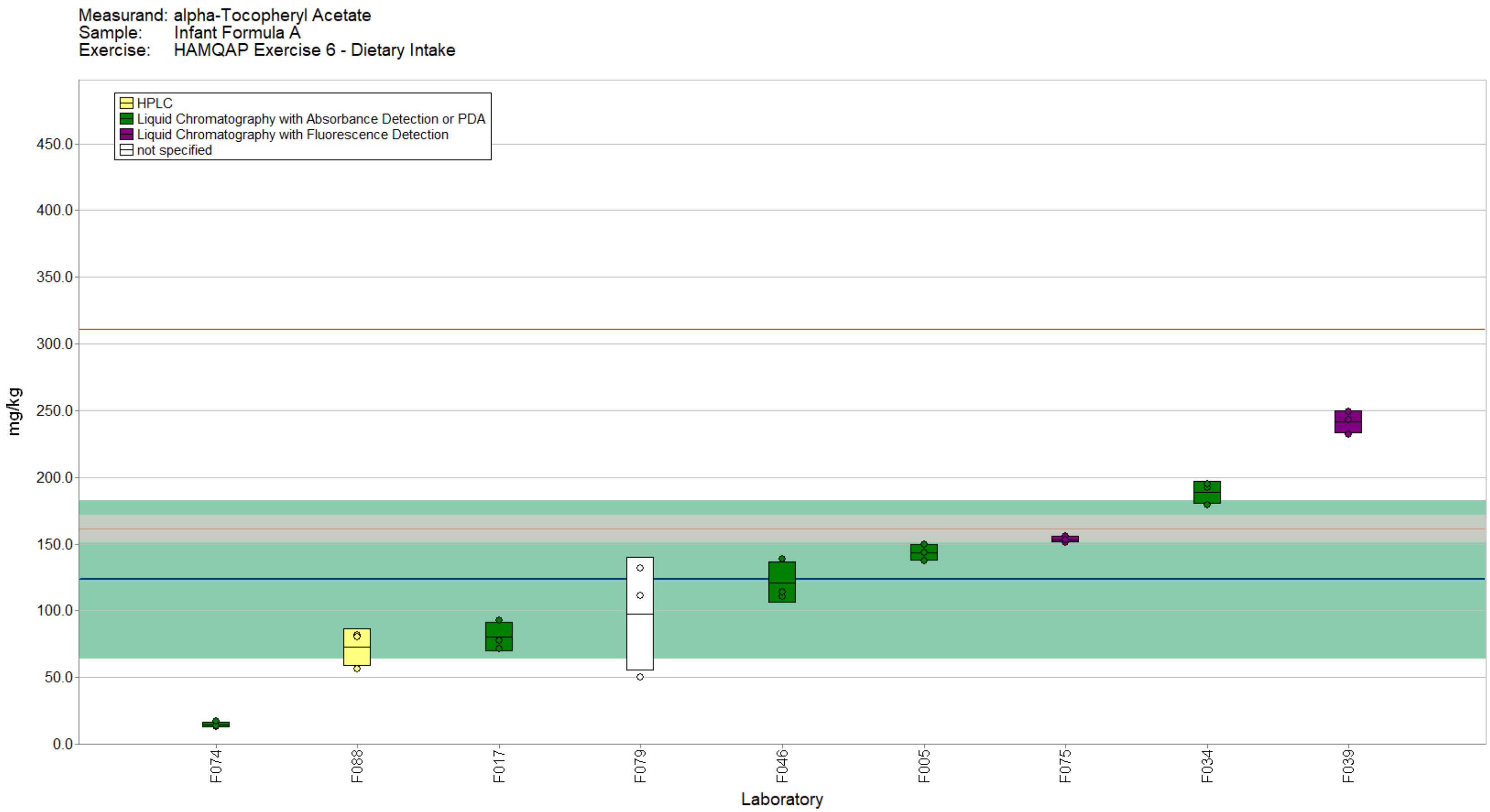


**Figure 4-17.** Alpha-tocopherol acetate in Infant Formula A (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue 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 $Z'_{\text{comm}}$ score, $|Z'_{\text{comm}}| \leq 2$. 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, $|Z_{\text{NIST}}| \leq 2$.

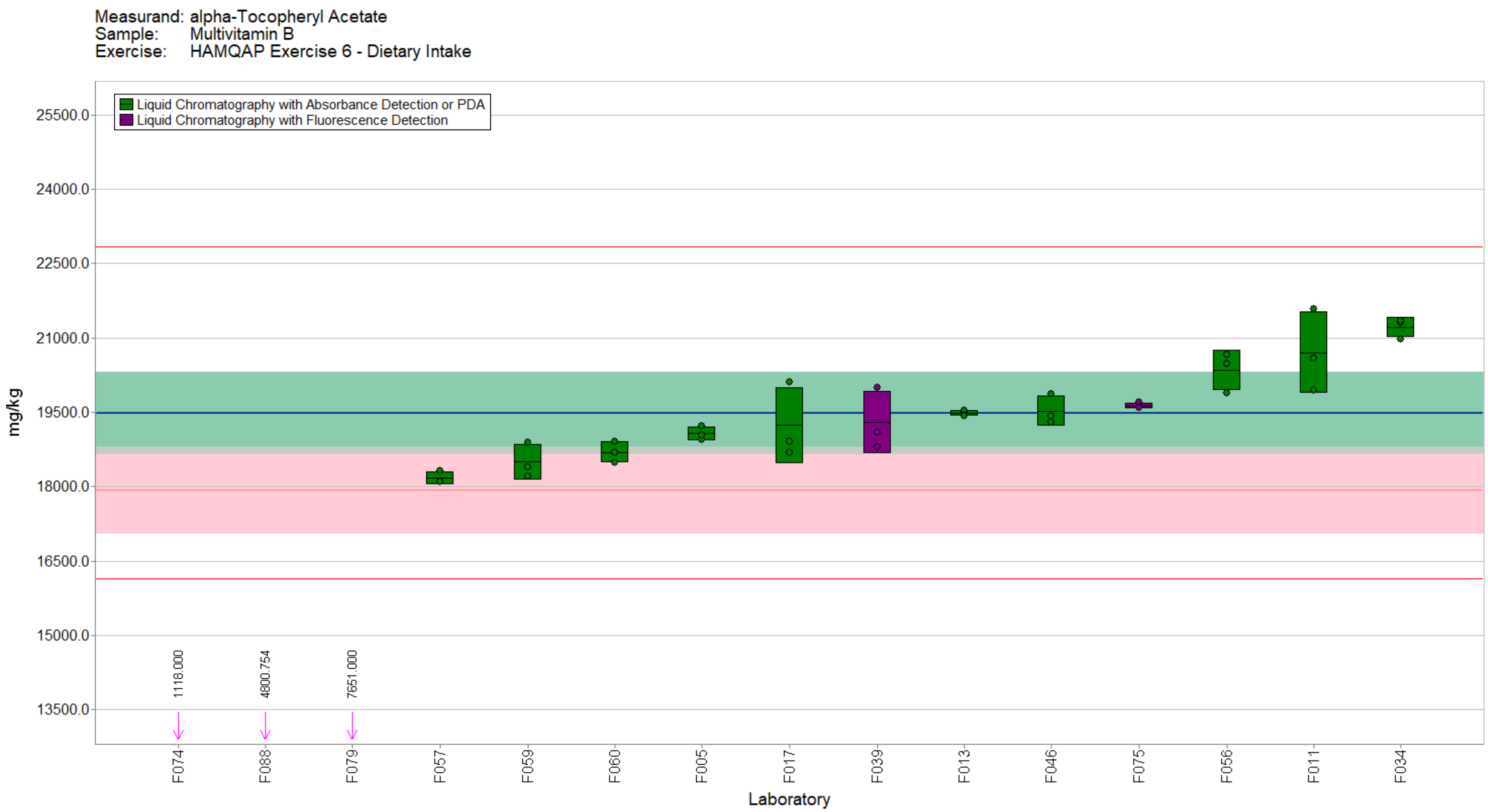


**Figure 4-18.** Alpha-tocopherol acetate in Multivitamin B (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue 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 $Z'_{\text{comm}}$ score, $|Z'_{\text{comm}}| \leq 2$. 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, $|Z_{\text{NIST}}| \leq 2$.

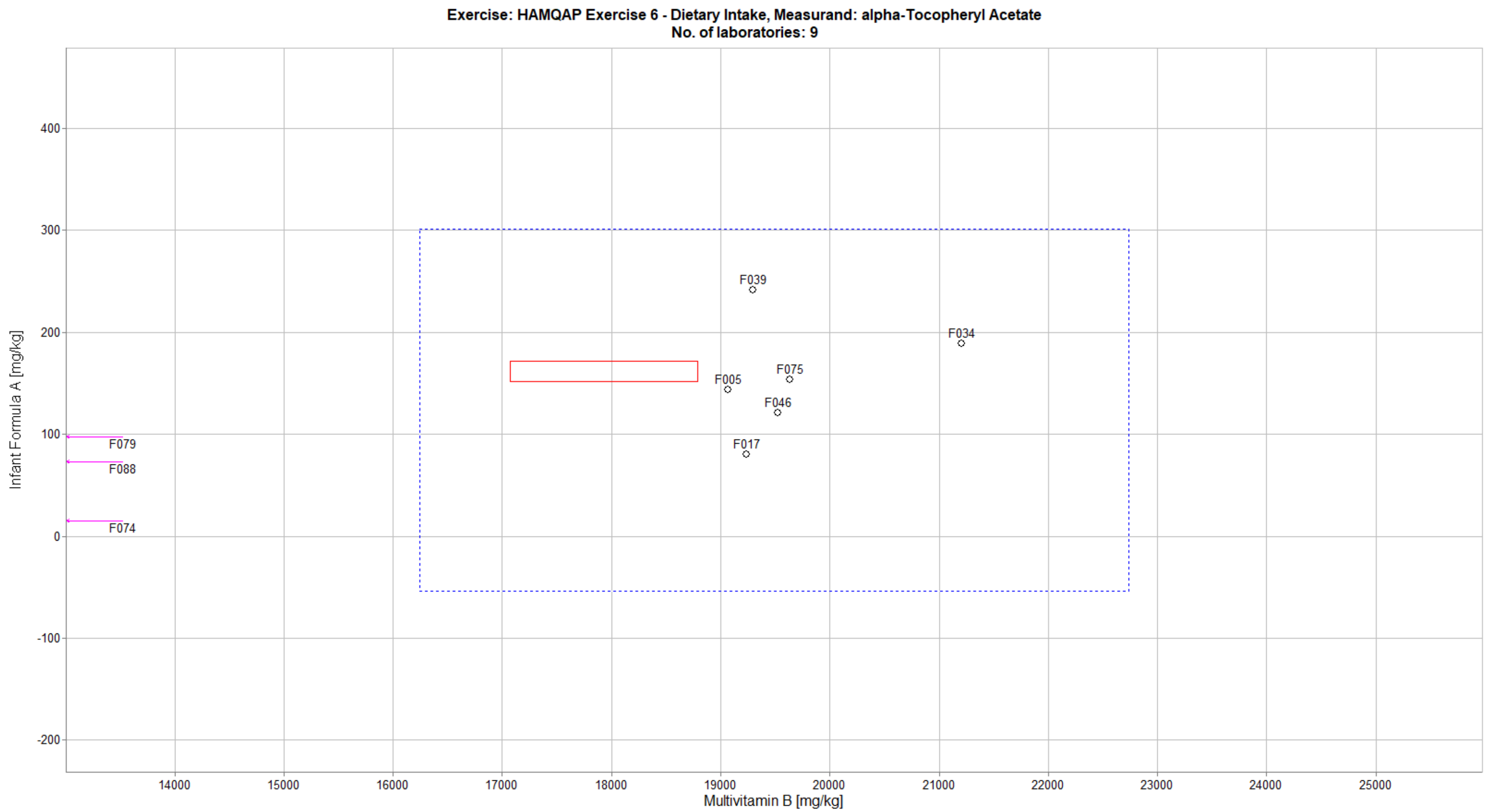


**Figure 4-19.** Laboratory means for alpha-tocopheryl acetate in Infant Formula A and Multivitamin B (sample/sample comparison view). In this view, the individual laboratory mean for one sample (multivitamin) is compared to the individual laboratory mean for a second sample (Infant Formula A). The solid red box represents the NIST range of tolerance for the two samples, Multivitamin B (x-axis) and Infant Formula A (y-axis), which encompasses the target values bounded by their uncertainties ($U_{NIST}$) and represents the range that results in an acceptable $Z_{NIST}$ score, $|Z_{NIST}| \leq 2$. The dotted blue box represents the consensus range of tolerance for Multivitamin B (x-axis) and Infant Formula 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 4-8.** Data summary table for beta-tocopherol in Infant Formula A and Multivitamin B. Data points highlighted in red have a zero or non-numeric data point.

| | | beta-Tocopherol | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Infant Formula A (mg/kg) | | | | | Multivitamin B (mg/kg) | | | | |
| | Lab | A | B | C | Avg | SD | A | B | C | Avg | SD |
| Individual Results | Target | | | | 4.94 | 0.10 | | | | | |
| | F004 | | | | | | | | | | |
| | F005 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | F011 | | | | | | | | | | |
| | F013 | 2.79 | 3.15 | 2.92 | 2.95 | 0.18 | | | | | |
| | F014 | 5.08 | 4.78 | 4.81 | 4.89 | 0.17 | | | | | |
| | F018 | | | | | | | | | | |
| | F021 | | | | | | | | | | |
| | F026 | | | | | | | | | | |
| | F030 | < 5.000 | < 5.000 | < 5.000 | | | < 50.000 | < 50.000 | < 50.000 | | |
| | F031 | | | | | | | | | | |
| | F033 | 4.6 | 4.3 | 4.3 | 4.40 | 0.17 | 64.1 | 64.6 | 65 | 64.57 | 0.45 |
| | F039 | 5.01 | 5.26 | 5.31 | 5.19 | 0.16 | < 16.600 | < 16.600 | < 16.600 | | |
| | F051 | | | | | | | | | | |
| | F056 | | | | | | | | | | |
| | F057 | | | | | | | | | | |
| | F060 | | | | | | | | | | |
| | F061 | | | | | | | | | | |
| | F062 | < 0.200 | < 0.200 | < 0.200 | | | < 0.200 | < 0.200 | < 0.200 | | |
| | F073 | | | | | | | | | | |
| | F075 | | | | | | | | | | |
| Community Results | | Consensus Mean | | | 3.67 | | Consensus Mean | | | 32.3 | |
| | | Consensus Standard Deviation | | | 1.83 | | Consensus Standard Deviation | | | 142.5 | |
| | | Maximum | | | 5.19 | | Maximum | | | 64.57 | |
| | | Minimum | | | 0 | | Minimum | | | 0 | |
| | | N | | | 5 | | N | | | 1 | |

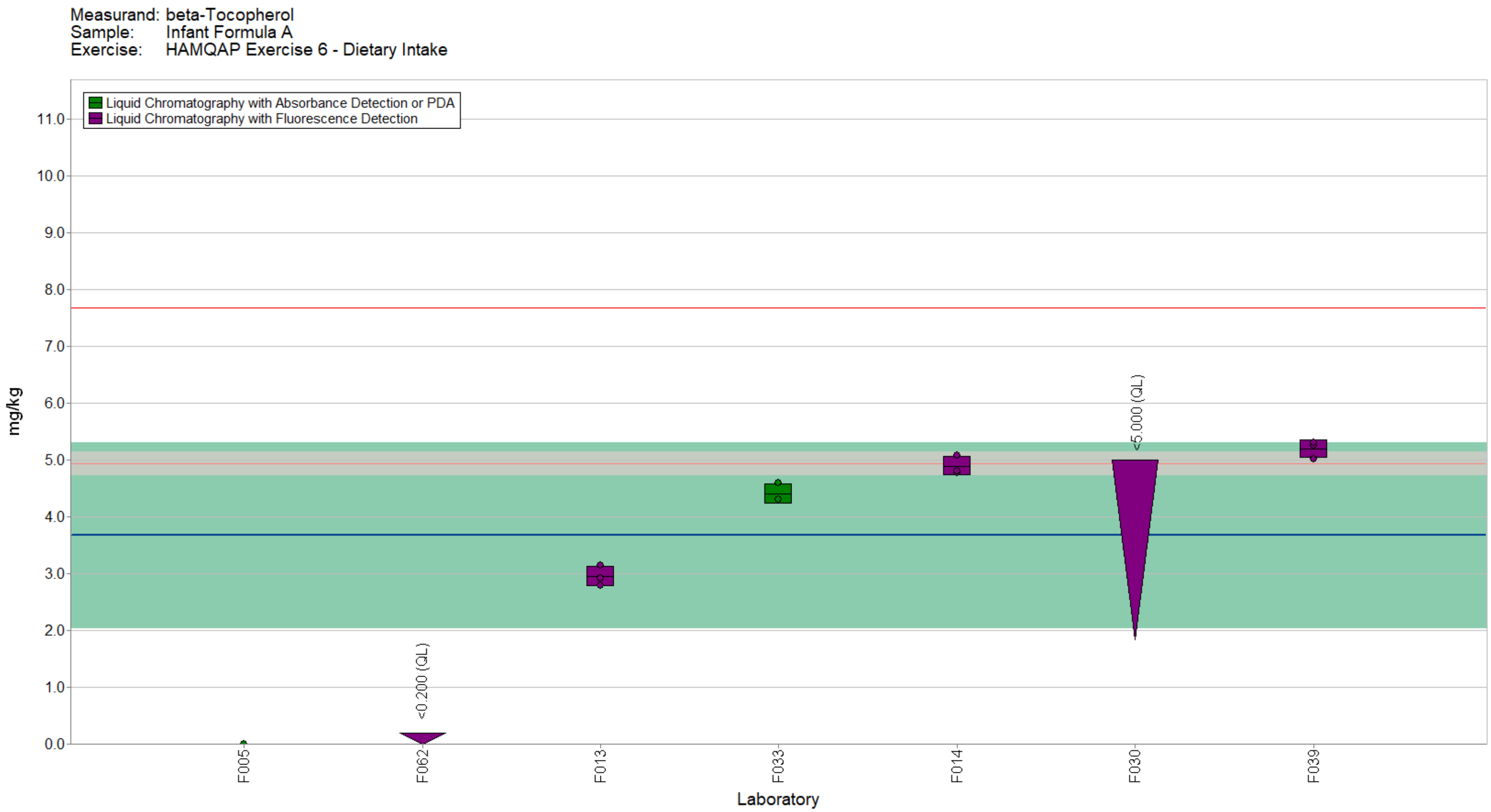


**Figure 4-20.** Beta-tocopherol in Infant Formula A (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue 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 $Z'_{\text{comm}}$ score, $|Z'_{\text{comm}}| \leq 2$. 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, $|Z_{\text{NIST}}| \leq 2$.

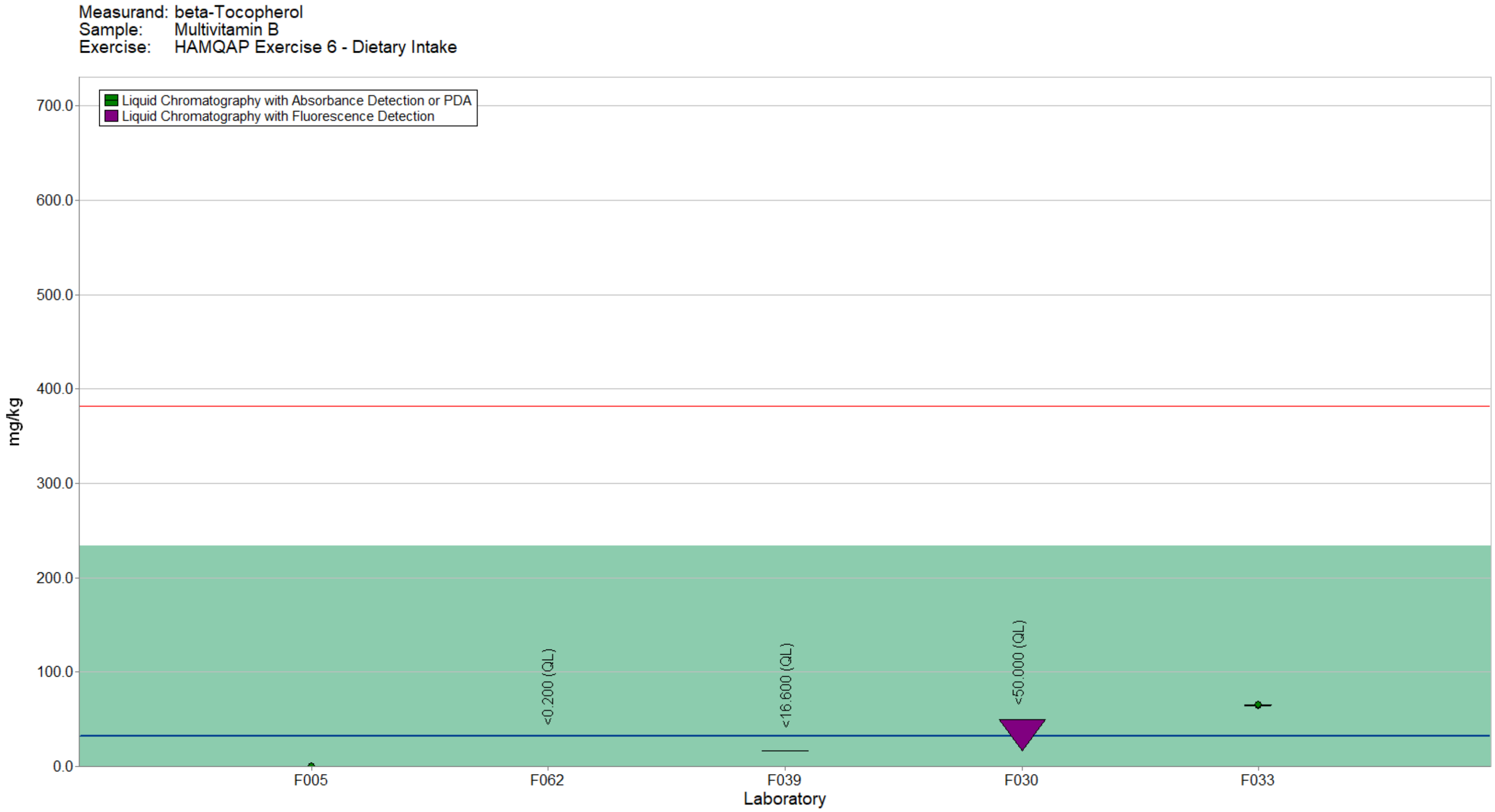


**Figure 4-21.** Beta-tocopherol in Multivitamin B (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue 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 $Z'_{\mathrm{comm}}$ score, $|Z'_{\mathrm{comm}}| \leq 2$. A NIST value has not been determined in this material.

**Table 4-9.** Data summary table for delta-tocopherol in Infant Formula A and Multivitamin B. Data points highlighted in red have a zero or non-numeric data point.

| | | delta-Tocopherol | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Infant Formula A (mg/kg) | | | | | Multivitamin B (mg/kg) | | | | |
| | Lab | A | B | C | Avg | SD | A | B | C | Avg | SD |
| Individual Results | Target | | | | 37.88 | 1.02 | | | | | |
| | F004 | | | | | | | | | | |
| | F005 | 39.88 | 52.26 | 55.37 | 49.17 | 8.19 | 0 | 0 | 0 | 0 | 0 |
| | F011 | | | | | | | | | | |
| | F013 | 28.6 | 27.7 | 27.5 | 27.93 | 0.59 | | | | | |
| | F014 | 36.6 | 33 | 34.9 | 34.83 | 1.80 | | | | | |
| | F017 | 25.29 | 21.6 | 19.75 | 22.21 | 2.82 | < 10.000 | < 10.000 | < 10.000 | | |
| | F018 | | | | | | | | | | |
| | F021 | | | | | | | | | | |
| | F026 | | | | | | | | | | |
| | F030 | 18 | 18.2 | 19.8 | 18.67 | 0.99 | < 50.000 | < 50.000 | < 50.000 | | |
| | F031 | | | | | | | | | | |
| | F033 | 40.3 | 41.8 | 40.7 | 40.93 | 0.78 | 505 | 509 | 524 | 512.7 | 10.0 |
| | F039 | 43.2 | 45.8 | 47.3 | 45.43 | 2.07 | < 16.600 | < 16.600 | < 16.600 | | |
| | F046 | 11.77 | 11.76 | 11.41 | 11.65 | 0.21 | 90.69 | 89.49 | 89.05 | 89.7 | 0.85 |
| | F056 | | | | | | | | | | |
| | F057 | | | | | | | | | | |
| | F060 | | | | | | | | | | |
| | F061 | | | | | | | | | | |
| | F062 | 31.5 | 31.1 | 29.3 | 30.63 | 1.17 | < 0.200 | < 0.200 | < 0.200 | | |
| | F073 | | | | | | | | | | |
| | F075 | 34.3 | 32.9 | 34.1 | 33.77 | 0.76 | | | | | |
| | F089 | | | | | | | | | | |
| Community Results | | Consensus Mean | | | 31.52 | | Consensus Mean | | | 195.3 | |
| | | Consensus Standard Deviation | | | 15.51 | | Consensus Standard Deviation | | | 200.6 | |
| | | Maximum | | | 49.17 | | Maximum | | | 512.7 | |
| | | Minimum | | | 11.65 | | Minimum | | | 0 | |
| | | N | | | 10 | | N | | | 2 | |

Measurand: delta-Tocopherol
Sample: Infant Formula A
Exercise: HAMQAP Exercise 6 - Dietary Intake

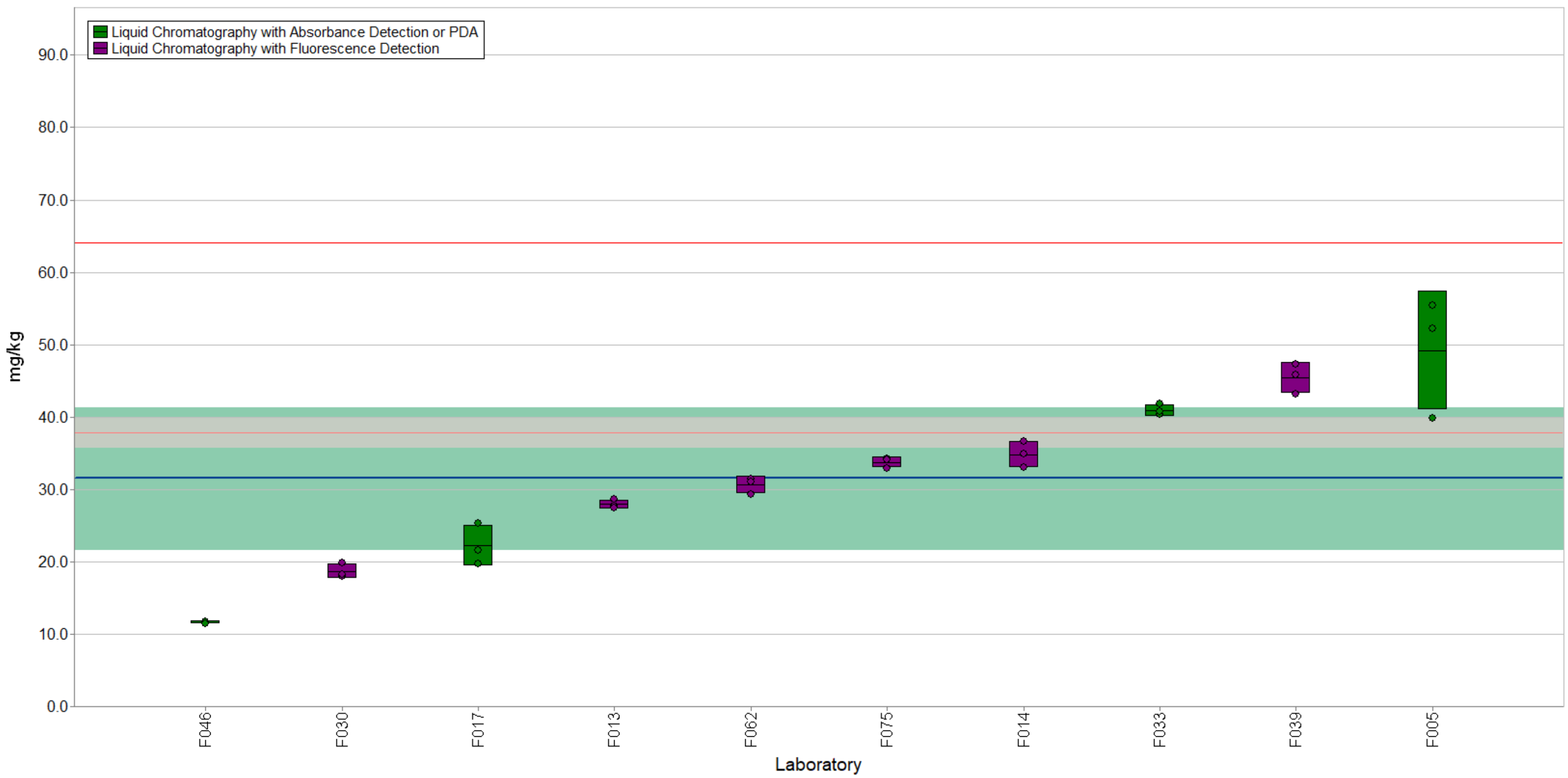


**Figure 4-22.** Delta-tocopherol in Infant Formula A (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue 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 $Z'_{\mathrm{comm}}$ score, $|Z'_{\mathrm{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by its uncertainty ($U_{\mathrm{NIST}}$) and represents the range that results in an acceptable $Z_{\mathrm{NIST}}$ score, $|Z_{\mathrm{NIST}}| \leq 2$.

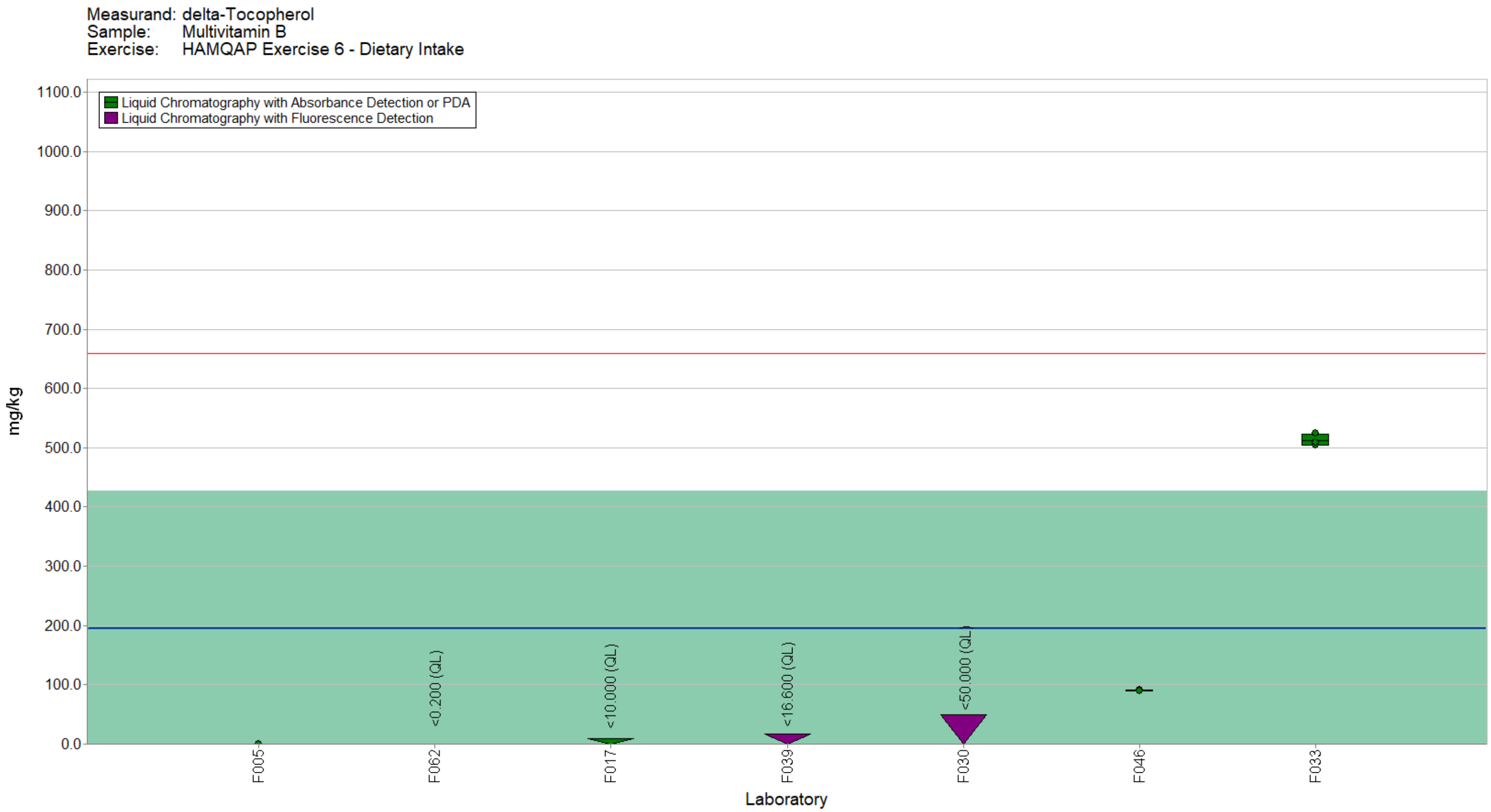


**Figure 4-23.** Delta-tocopherol in Multivitamin B (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue 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 $Z'_{\mathrm{comm}}$ score, $|Z'_{\mathrm{comm}}| \leq 2$. A NIST value has not been determined in this material.

**Table 4-10.** Data summary table for gamma-tocopherol in Infant Formula A and Multivitamin B. Data points highlighted in red have a zero or non-numeric data point.

| | | gamma-Tocopherol | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Infant Formula A (mg/kg) | | | | | Multivitamin B (mg/kg) | | | | |
| | Lab | A | B | C | Avg | SD | A | B | C | Avg | SD |
| Individual Results | Target | | | | 114 | 2.2 | | | | | |
| | F004 | | | | | | | | | | |
| | F005 | 117.72 | 149.49 | 159 | 142.1 | 21.6 | 0 | 0 | 0 | 0 | 0 |
| | F011 | | | | | | | | | | |
| | F013 | 128 | 118 | 122 | 122.7 | 5.03 | | | | | |
| | F014 | 126 | 117 | 118 | 120.3 | 4.93 | | | | | |
| | F018 | | | | | | | | | | |
| | F021 | | | | | | | | | | |
| | F026 | | | | | | | | | | |
| | F030 | 93.2 | 94.3 | 97.3 | 94.93 | 2.12 | < 50.000 | < 50.000 | < 50.000 | | |
| | F031 | | | | | | | | | | |
| | F033 | 116 | 115 | 112 | 114.3 | 2.08 | 37.7 | 35.9 | 35.8 | 36.47 | 1.07 |
| | F039 | 135 | 141 | 146 | 140.7 | 5.51 | < 16.600 | < 16.600 | < 16.600 | | |
| | F051 | | | | | | | | | | |
| | F056 | | | | | | | | | | |
| | F057 | | | | | | | | | | |
| | F060 | | | | | | | | | | |
| | F061 | | | | | | | | | | |
| | F062 | 114.2 | 104.7 | 98.5 | 105.8 | 7.91 | < 0.200 | < 0.200 | < 0.200 | | |
| | F073 | | | | | | | | | | |
| | F075 | 112 | 107 | 110 | 109.7 | 2.52 | | | | | |
| | F089 | | | | | | | | | | |
| Community Results | | Consensus Mean | | | 118.8 | | Consensus Mean | | | 18.2 | |
| | | Consensus Standard Deviation | | | 18.4 | | Consensus Standard Deviation | | | 79.5 | |
| | | Maximum | | | 142.1 | | Maximum | | | 36.5 | |
| | | Minimum | | | 94.9 | | Minimum | | | 0 | |
| | | N | | | 8 | | N | | | 1 | |

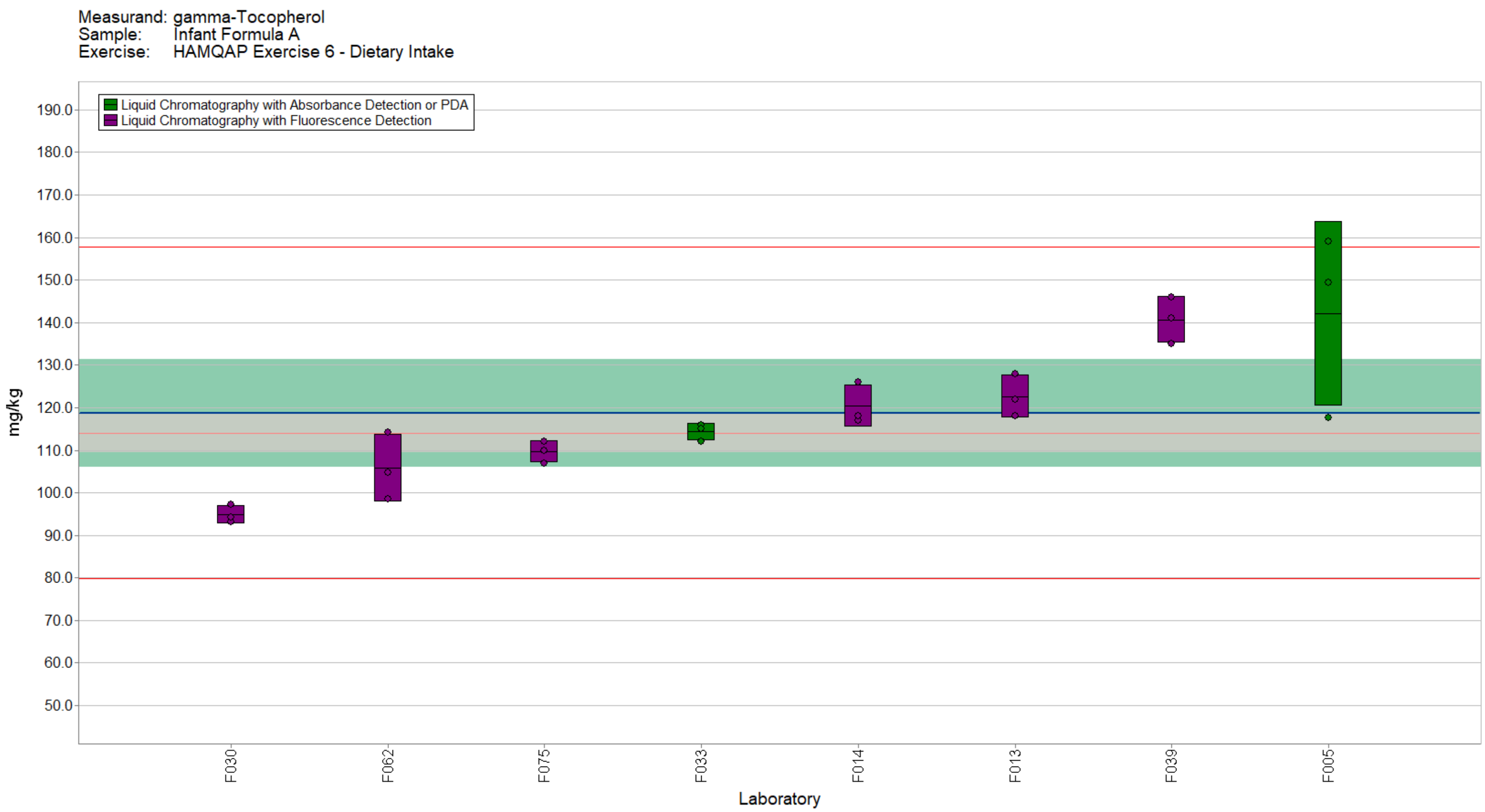


**Figure 4-24.** Gamma-tocopherol in Infant Formula A (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue 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 $Z'_{\text{comm}}$ score, $|Z'_{\text{comm}}| \leq 2$. 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, $|Z_{\text{NIST}}| \leq 2$.

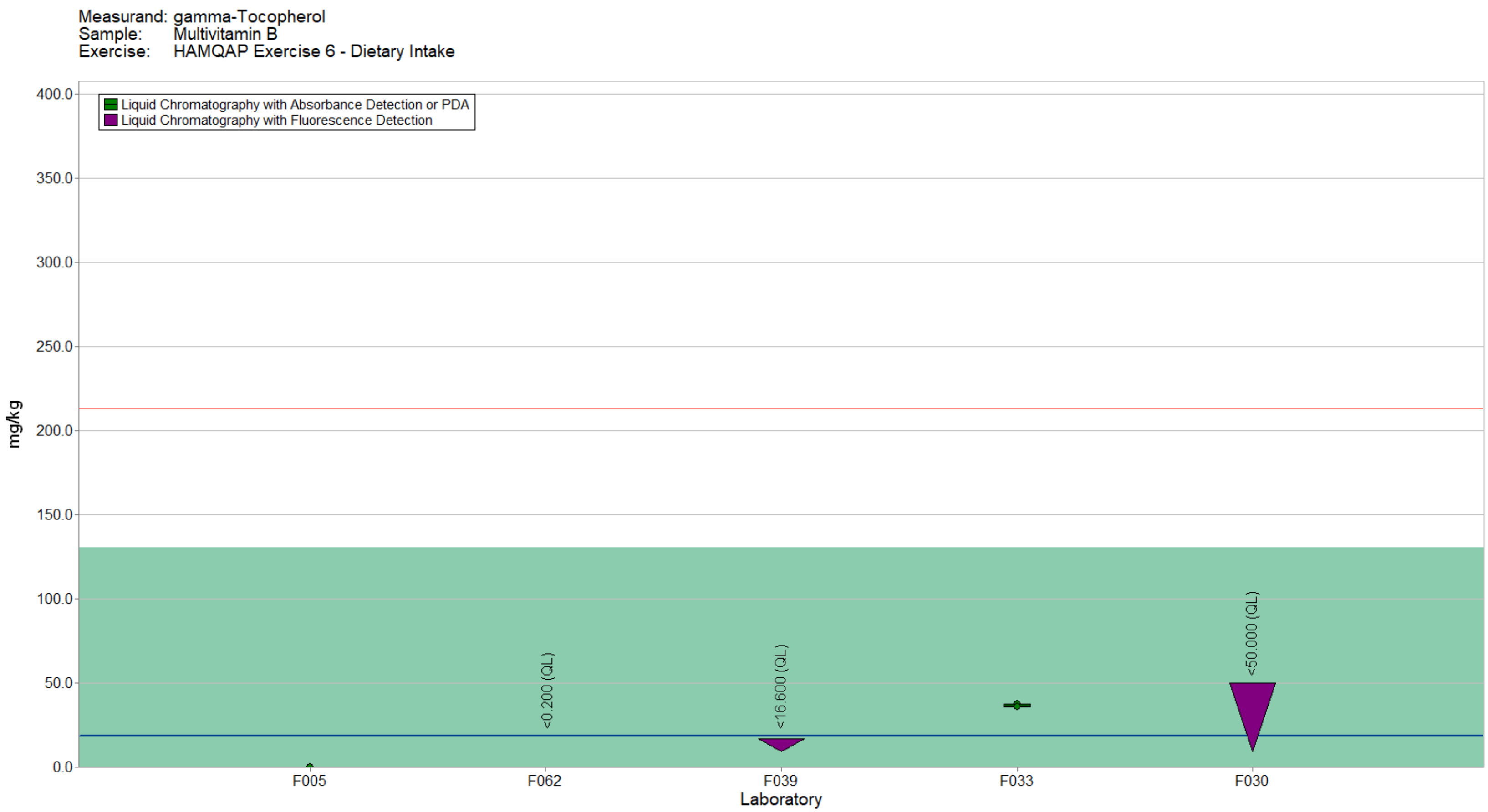


**Figure 4-25.** Gamma-tocopherol in Multivitamin B (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue 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 $Z'_{\text{comm}}$ score, $|Z'_{\text{comm}}| \leq 2$. A NIST value has not been determined in this material.

## SECTION 5: FATTY ACIDS (Omega-3 and Omega-6 Fatty Acids)



Study Overview

In this study, participants were provided with samples of SRM 3275 Omega-3 and Omega-6 Fatty Acids in Fish Oil Level 2, commercial anchovies, and commercial sardines for dietary intake, and as well as two samples of human red blood cells (RBC) for human metabolism. Participants were asked to use in-house analytical methods to determine the mass fraction (mg/g) of omega-3 and omega-6 fatty acids in each intake matrix and percentage (%) of total RBC fatty acids in each metabolism sample. Omega-3 fatty acids are important components of the phospholipids that form the structures of cell membranes.[4] In addition, omega-3 and omega-6 fatty acids provide energy for the body and are used to form eicosanoids, which are mediators of inflammation, vasoconstriction, and platelet aggregation. Some researchers propose that the relative intakes of omega-3 and omega-6 fatty acids may have important implications for the pathogenesis of chronic diseases such as cardiovascular disease and cancer, but an optimal ratio has not yet been defined. Scientific research has mostly focused on three omega-3 fatty acids (α-linolenic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA)) and two omega-6 fatty acids (linoleic acid and arachidonic acid (ARA)). Fish and fish oils are dietary sources of EPA and DHA, as fatty acids originally synthesized by microalgae further down the food chain accumulate in fish tissues. ALA and other omega-6 fatty acids can be found in plant sources such as plant oils, chia seeds, and walnuts. Omega-3 and omega-6 fatty acid health status can be evaluated by measuring individual components in plasma or serum phospholipids, but values can vary substantially based on an individual's most recent intake and as such do not reflect long-term dietary consumption. Understanding intake of omega-3 and omega-6 fatty acids and their impact on inflammation and disease can advance clinical research that investigates how manipulating the omega-6 to omega-3 ratio may yield positive health outcomes.

Dietary Intake Sample Information

*Fish Oil.* Participants were provided with three ampoules of SRM 3275 Omega-3 and Omega-6 Fatty Acids in Fish Oil Level 2, each containing 1.2 mL of anchovy oil high in DHA and EPA. Participants were asked to store the material under refrigeration (2 °C to 4 °C) in the original unopened ampoules and to prepare one sample and to report one value from each ampoule provided. Before use, participants were instructed to thoroughly mix the contents of the ampoule prior to removal of a test portion for analysis and to use a sample size of at least 0.5 g. The approximate analyte levels were not reported to participants prior to the study. A certified value for linoleic acid in SRM 3275 Level 2 was assigned using results from NIST by gas chromatography with flame ionization detection (GC-FID) and gas chromatography mass spectrometry (GC-MS). Reference values for ALA, ARA, EPA, and DHA in SRM 3275 Level 2 were assigned using results from NIST by GC-FID. The NIST-determined values and uncertainties for omega-3 and omega-6 fatty acids in SRM 3275 are provided in the table below, reported both as the fatty acid methyl esters (FAMEs), as listed on the Certificate of Analysis, and as the free fatty acids (FFAs), using standard molecular weight conversion factors, with expanded uncertainties for the purpose of determining $Z_{NIST}$ scores.[5]

[4] Omega-3 Fatty Acids Fact Sheet for Health Professionals. National Institutes of Health Office of Dietary Supplements. https://ods.od.nih.gov/factsheets/Omega3FattyAcids-HealthProfessional/ (accessed March 2020).

[5] DeVries, J.W., Kjos, L., Groff, L., Martin, B., Cernohous, K., Patel, H., Payne, H., Leichtweis, H., Shay, M., and Newcomer, L. (1999) Studies in Improvement of Official Method 996.06, *J. AOAC Int.* 82, 1146–1155.

| Analyte | NIST-Determined Mass Fractions in SRM 3275-2 (mg/g) (as FAMEs) | (as FFAs) |
|---|---|---|
| ALA | 1.42 ± 0.12 | 1.35 ± 0.11 |
| Linoleic Acid | 3.00 ± 0.42 | 2.86 ± 0.40 |
| ARA | 22.9 ± 1.0 | 21.89 ± 0.96 |
| EPA | 394 ± 17 | 377 ± 16 |
| DHA | 187 ± 8 | 179 ± 8 |

*Anchovies and Sardines.* Participants were provided one can each of commercial anchovies containing 56 g of material, and commercial sardines containing 120 g of material. Participants were asked to blend the entire can of material with either a handheld homogenizer or an immersion blender prior to sampling. Participants were also asked to store the materials at room temperature ((20 °C to 25 °C)) in the original unopened cans until ready for use and to prepare three samples and reported three values from each can. A sample size appropriate for the laboratory's usual in-house method of analysis was encouraged. The approximate analyte levels were not reported to participants prior to the study and NIST did not determine analyte levels in these materials.

Dietary Intake Study Results

- Thirty-two laboratories enrolled in this exercise and received samples to measure fatty acids in the fish oil, anchovy, and sardine samples. Between 14 and 23 laboratories reported results for each analyte, resulting in 45 % to 72 % participation. Participation statistics for each analyte are described in more detail below.

| Analyte | Number of Laboratories Requesting Samples | Number of Laboratories Reporting Results (Percent Participation) SRM 3275-2 | Anchovies | Sardines |
|---|---|---|---|---|
| α-Linolenic Acid | 31 | 21 (68 %) | 19 (61 %) | 18 (58 %) |
| Linoleic Acid | 31 | 20 (65 %) | 19 (61 %) | 18 (58 %) |
| Arachidonic Acid | 31 | 21 (68%) | 14 (45 %) | 17 (55 %) |
| EPA | 32 | 23 (72 %) | 20 (63 %) | 20 (63 %) |
| DHA | 32 | 23 (72 %) | 20 (63 %) | 21 (63 %) |

- The consensus ranges for all fatty acids overlapped the target ranges in SRM 3275 Level 2.
  - The consensus means for α-linoleic acid, linoleic acid, and arachidonic acid were above the target range, but the consensus range overlapped with the target range (**Figure 5-1**, **Figure 5-6**, and **Figure 5-11**).
  - The consensus means for EPA and DHA were near the upper limit of the target range (**Figure 5-16** and **Figure 5-21**).

- The between-laboratory variabilities were good for arachidonic acid, EPA, and DHA in SRM 3275 but were high or very high for all other analyte/sample pairs. Variabilities for each analyte/sample pair are reported in the table below.

| | Between-Laboratory Variability (RSD) | | |
|---|---|---|---|
| Analyte | SRM 3275-2 | Anchovies | Sardines |
| α-Linolenic Acid | 55 % | 45 % | 91 % |
| Linoleic Acid | 47 % | 43 % | >100 % |
| Arachidonic Acid | 11 % | 63 % | 72 % |
| EPA | 14 % | 37 % | 73 % |
| DHA | 13 % | 45 % | 60 % |

- Laboratories reported using derivatization (to fatty acid methyl esters or non-specified), hot block digestion, and saponification/base hydrolysis of fat. Two laboratories reported using a sample preparation not listed and two laboratories reported using no sample preparation method. No trends were observed based on sample preparation method used.
- All laboratories reported using GC-FID as their analytical method for determination of fatty acids in these samples.

Dietary Intake Technical Recommendations

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

- The between-laboratory variability was higher for analytes present at lower levels such as all fatty acids in anchovies and sardines, and α-linolenic acid and linoleic acid in SRM 3275 Level 2. Laboratories should evaluate their calibration at low levels. Depending on the model of the calibration curve, high-level calibrants can be weighted more compared to lower level calibrants, which could cause a bias for low level samples. The validity of the calibration model should be evaluated at all concentration ranges.
- A linear calibration curve which surrounds 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 incorrect values.
- No measurement performance trends were observed for the sample preparation approaches reported for these samples and analytes.
- A calibration bias may be present for laboratories that are consistently reporting either low or high values of an analyte in all matrices. The sample-sample comparison view can demonstrate this trend.
  - **Figure 5-4** shows one laboratory reporting higher results compared to other laboratories for α-linolenic acid in both SRM 3275 and in the commercial anchovies.
  - **Figure 5-5** shows two laboratories reporting higher results compared to other laboratories for α-linolenic acid in both SRM 3275 and in the commercial sardines.
- Several of the sample-sample comparison views indicate that some laboratories are reporting high or low responses compared to other laboratories for an analyte in one matrix but not in another (see **Figure 5-4**, **Figure 5-5**, **Figure 5-9**, **Figure 5-10**, **Figure 5-14**, **Figure 5-15**, **Figure 5-19**, **Figure 5-20**, **Figure 5-24**, **and Figure 5-25**). This indicates that the different

matrices may pose different analytical challenges. For each type of matrix analyzed, the chromatogram should be inspected carefully to ensure that there are no visible interferences. If an interference is suspected, the interference should be remedied by additional sample cleanup or changing chromatographic conditions.

- Laboratories reporting results flagged as outside the consensus tolerance limits should check for calculation errors. One example is to confirm that factors for all dilutions have been properly tabulated.
- 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 materials prepared in-house.

**Table 5-1.** Individualized data summary table (NIST) for fatty acids in SRM 3275 Omega-3 and Omega-6 Fatty Acids in Fish Oil (Level 2), commercial anchovies, and commercial sardines.

***National Institute of Standards and Technology***

**HAMQAP Exercise 6 - Fatty Acids**

| | **Lab Code:** | **NIST** | **1. Your Results** | | | | **2. Community Results** | | | **3. Target** | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Analyte | Sample | Units | $x_i$ | $s_i$ | $Z'_{comm}$ | $Z_{NIST}$ | N | x* | s* | $x_{NIST}$ | $U$ |
| Total Linoleic Acid (C18:2 n-6) | Commercial Anchovies | mg/g | | | | | 19 | 40 | 17 | | |
| Total Linoleic Acid (C18:2 n-6) | Commercial Sardines | mg/g | | | | | 18 | 0.44 | 0.57 | | |
| Total Linoleic Acid (C18:2 n-6) | SRM 3275 Omega-3 and Omega-6 Fatty Acids in Fish Oil L 2 | mg/g | 2.86 | 0.4 | | | 20 | 3.8 | 1.8 | 2.86 | 0.4 |
| Total alpha-Linolenic Acid (C18:3 n-3) | Commercial Anchovies | mg/g | | | | | 19 | 2.7 | 1.2 | | |
| Total alpha-Linolenic Acid (C18:3 n-3) | Commercial Sardines | mg/g | | | | | 18 | 0.111 | 0.096 | | |
| Total alpha-Linolenic Acid (C18:3 n-3) | SRM 3275 Omega-3 and Omega-6 Fatty Acids in Fish Oil L 2 | mg/g | 1.35 | 0.114 | | | 21 | 1.72 | 0.94 | 1.35 | 0.114 |
| Total Arachidonic Acid (C20:4 n-6) | Commercial Anchovies | mg/g | | | | | 14 | 0.163 | 0.097 | | |
| Total Arachidonic Acid (C20:4 n-6) | Commercial Sardines | mg/g | | | | | 17 | 0.32 | 0.22 | | |
| Total Arachidonic Acid (C20:4 n-6) | SRM 3275 Omega-3 and Omega-6 Fatty Acids in Fish Oil L 2 | mg/g | 21.9 | 0.956 | | | 21 | 24.9 | 2.7 | 21.9 | 0.956 |
| Total EPA (C20:5 n-3) | Commercial Anchovies | mg/g | | | | | 20 | 1.05 | 0.39 | | |
| Total EPA (C20:5 n-3) | Commercial Sardines | mg/g | | | | | 20 | 3.1 | 2.3 | | |
| Total EPA (C20:5 n-3) | SRM 3275 Omega-3 and Omega-6 Fatty Acids in Fish Oil L 2 | mg/g | 377 | 16.2 | | | 23 | 390 | 53 | 377 | 16.2 |
| Total DHA (C22:6 n-3) | Commercial Anchovies | mg/g | | | | | 20 | 3.6 | 1.6 | | |
| Total DHA (C22:6 n-3) | Commercial Sardines | mg/g | | | | | 20 | 3.6 | 2.2 | | |
| Total DHA (C22:6 n-3) | SRM 3275 Omega-3 and Omega-6 Fatty Acids in Fish Oil L 2 | mg/g | 179 | 7.67 | | | 23 | 190 | 24 | 179 | 7.67 |

$x_i$ Mean of reported values
$s_i$ Standard deviation of reported values
$Z'_{comm}$ Z'-score with respect to community consensus
$Z_{NIST}$ Z-score with respect to NIST value

N Number of quantitative values reported
x* Robust mean of reported values
s* Robust standard deviation

$x_{NIST}$ NIST-assessed value
$U$ expanded uncertainty about the NIST-assessed value

**Table 5-2.** Data summary table for total α-linolenic acid in SRM 3275 Omega-3 and Omega-6 Fatty Acids in Fish Oil (Level 2), commercial anchovies, and commercial sardines. Data points highlighted in blue have been identified as outside the consensus tolerance limits and would be estimated to result in an unacceptable $Z'_{\text{comm}}$ score, $|Z'_{\text{comm}}| \geq 2$.

| | Total alpha-Linolenic Acid (C18:3 n-3) | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | SRM 3275 Omega-3 and Omega-6 Fatty Acids in Fish Oil Level 2 (mg/g) | | | | | Commercial Anchovies (mg/g) | | | | | Commercial Sardines (mg/g) | | | | |
| | **Lab** | **A** | **B** | **C** | **Avg** | **SD** | **A** | **B** | **C** | **Avg** | **SD** | **A** | **B** | **C** | **Avg** | **SD** |
| **Individual Results** | Target | | | | 1.35 | 0.11 | | | | | | | | | | |
| | F004 | | | | | | | | | | | | | | | |
| | F005 | 1.5 | 1.5 | 1.5 | 1.5 | 0 | 2.65 | 2.25 | 2.65 | 2.52 | 0.23 | 0.1 | 0.1 | 0.1 | 0.1 | 0 |
| | F011 | 1.8 | 1.9 | 1.8 | 1.83 | 0.06 | | | | | | | | | | |
| | F014 | | | | | | | | | | | | | | | |
| | F018 | | | | | | | | | | | | | | | |
| | F021 | | | | | | | | | | | | | | | |
| | F025 | 3 | 3.08 | 3.03 | 3.04 | 0.04 | 3.22 | 3.38 | 3.05 | 3.22 | 0.17 | 0.12 | 0.13 | 0.12 | 0.12 | 0.01 |
| | F026 | 1.7202 | 1.7267 | 1.72 | 1.72 | 0.00 | | | | | | | | | | |
| | F030 | 1.746 | 1.739 | 1.749 | 1.74 | 0.01 | 6.761 | 6.728 | 6.74 | 6.74 | 0.02 | 5.865 | 5.865 | 5.853 | 5.86 | 0.01 |
| | F031 | 9.607 | 9.271 | 9.37 | 9.42 | 0.17 | 2.657 | 2.681 | 2.584 | 2.64 | 0.05 | 0.186 | 0.183 | 0.192 | 0.19 | 0.00 |
| | F032 | | | | | | | | | | | | | | | |
| | F033 | 1.17 | 1.2 | 1.15 | 1.17 | 0.03 | 2.72 | 2.75 | 2.72 | 2.73 | 0.02 | 0.0783 | 0.078 | 0.075 | 0.08 | 0.00 |
| | F035 | | | | | | | | | | | | | | | |
| | F036 | 0.41 | 0.42 | 0.41 | 0.41 | 0.01 | 1.08 | 1.2 | 1.19 | 1.16 | 0.07 | 0.02 | 0.05 | 0.02 | 0.03 | 0.02 |
| | F038 | 11.77 | 12.1 | 12.2 | 12.02 | 0.23 | 2.67 | 2.51 | 2.82 | 2.67 | 0.16 | 0.19 | 0.2 | 0.19 | 0.19 | 0.01 |
| | F039 | 1.4 | 1.4 | 1.3 | 1.37 | 0.06 | 2.5 | 2.4 | 2.4 | 2.43 | 0.06 | | | | | |
| | F041 | 1.359 | 1.332 | 1.368 | 1.35 | 0.02 | 0.787 | 0.741 | 0.734 | 0.75 | 0.03 | 0.113 | 0.113 | 0.114 | 0.11 | 0.00 |
| | F046 | | | | | | 2.6 | 5.12 | 4.38 | 4.03 | 1.30 | 0.1 | 0.1 | 0.1 | 0.10 | 0 |
| | F048 | 1.77 | | | 1.77 | | 5.99 | | | 5.99 | | 6.23 | | | 6.23 | |
| | F056 | | | | | | | | | | | | | | | |
| | F060 | 1.34 | 1.7 | 1.42 | 1.49 | 0.19 | | | | | | | | | | |
| | F061 | 9.62 | 9.72 | 9.93 | 9.76 | 0.16 | 2.3 | 2.33 | 2.46 | 2.36 | 0.09 | 0.14 | 0.14 | 0.14 | 0.14 | 0 |
| | F062 | 10.93 | 10.91 | 10.92 | 10.92 | 0.01 | 7.5 | 7.47 | 7.47 | 7.48 | 0.02 | 6.84 | 6.84 | 6.8 | 6.83 | 0.02 |
| | F064 | 2.01 | 2.6 | 2.32 | 2.31 | 0.30 | 1.58 | | | 1.58 | | 0.06 | | | 0.06 | |
| | F069 | 3.1 | 3.09 | 3.12 | 3.10 | 0.02 | 2.75 | 2.72 | 3.07 | 2.85 | 0.19 | 0.06 | 0.05 | 0.05 | 0.05 | 0.01 |
| | F070 | | | | | | | | | | | | | | | |
| | F072 | 1.95 | 1.93 | 2.03 | 1.97 | 0.05 | 3.37 | 3.27 | 3.27 | 3.30 | 0.06 | 0.21 | 0.2 | 0.21 | 0.21 | 0.01 |
| | F079 | 1.8 | 1.1 | 1.5 | 1.47 | 0.35 | 1.88 | 3.13 | 2.99 | 2.67 | 0.68 | 0.08 | 0.06 | | 0.07 | 0.01 |
| | F080 | 9.99 | 9.98 | 10.18 | 10.05 | 0.11 | 0.155 | 0.151 | 0.191 | 0.17 | 0.02 | 2.63 | 2.55 | 2.56 | 2.58 | 0.04 |
| | F081 | | | | | | | | | | | | | | | |
| | F086 | 1.341 | 1.358 | 1.312 | 1.34 | 0.02 | 2.939 | 2.778 | 3.155 | 2.96 | 0.19 | 0.0964 | 0.0948 | 0.0987 | 0.10 | 0.00 |
| **Community Results** | | Consensus Mean | | | 1.72 | | Consensus Mean | | | 2.66 | | Consensus Mean | | | 0.11 | |
| | | Consensus Standard Deviation | | | 0.95 | | Consensus Standard Deviation | | | 1.20 | | Consensus Standard Deviation | | | 0.10 | |
| | | Maximum | | | 12.02 | | Maximum | | | 7.48 | | Maximum | | | 6.83 | |
| | | Minimum | | | 0.41 | | Minimum | | | 0.17 | | Minimum | | | 0.03 | |
| | | N | | | 21 | | N | | | 19 | | N | | | 18 | |

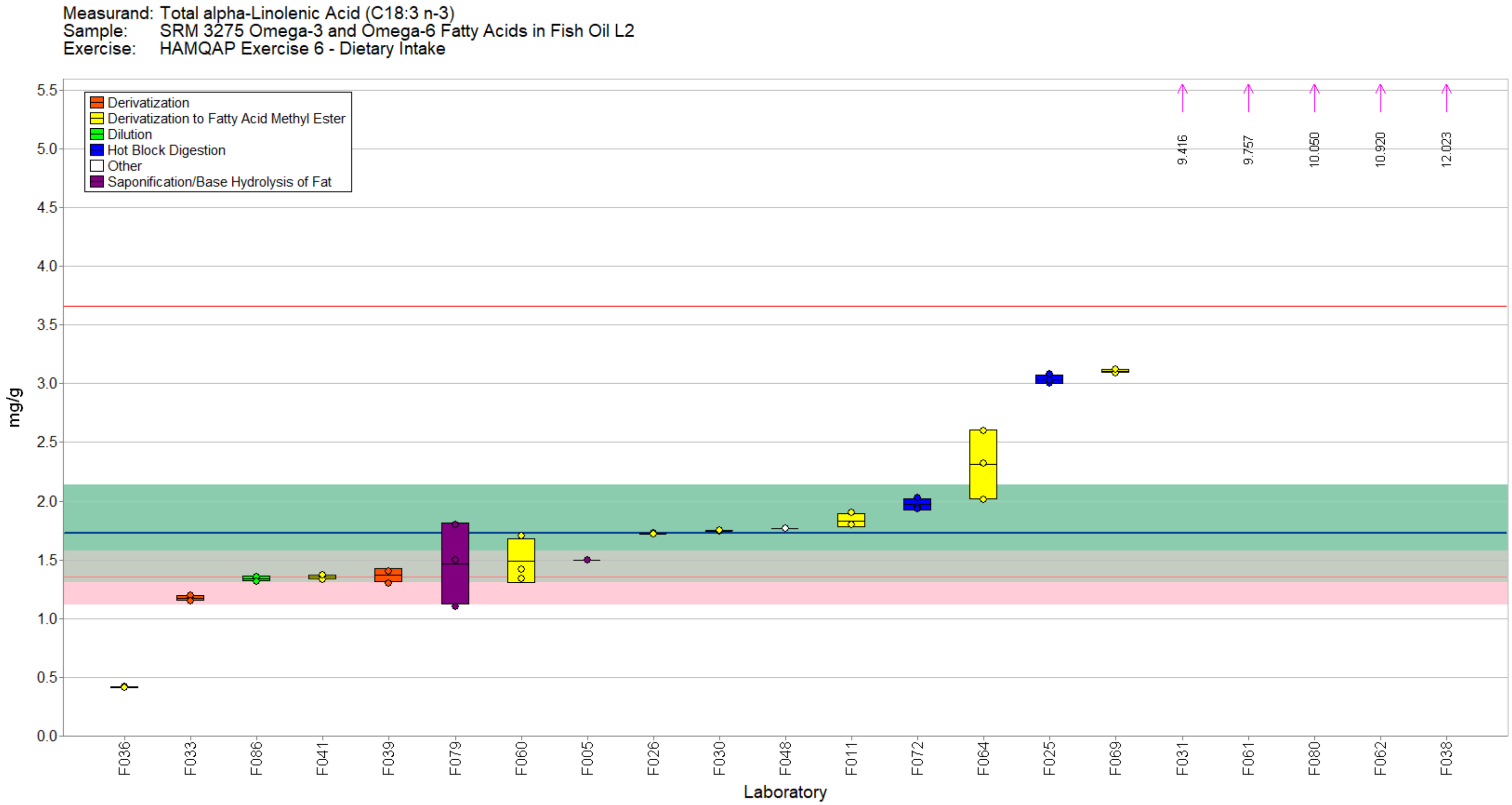


**Figure 5-1.** Total α-linolenic acid in SRM 3275 Omega-3 and Omega-6 Fatty Acids in Fish Oil (Level 2) (data summary view – sample preparation method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation employed. The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The red solid line represents the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable $Z'_{\mathrm{comm}}$ score, $|Z'_{\mathrm{comm}}| \leq 2$, with the lower limit set to zero. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by its uncertainty ($U_{\mathrm{NIST}}$) and represents the range that results in an acceptable $Z_{\mathrm{NIST}}$ score, $|Z_{\mathrm{NIST}}| \leq 2$.

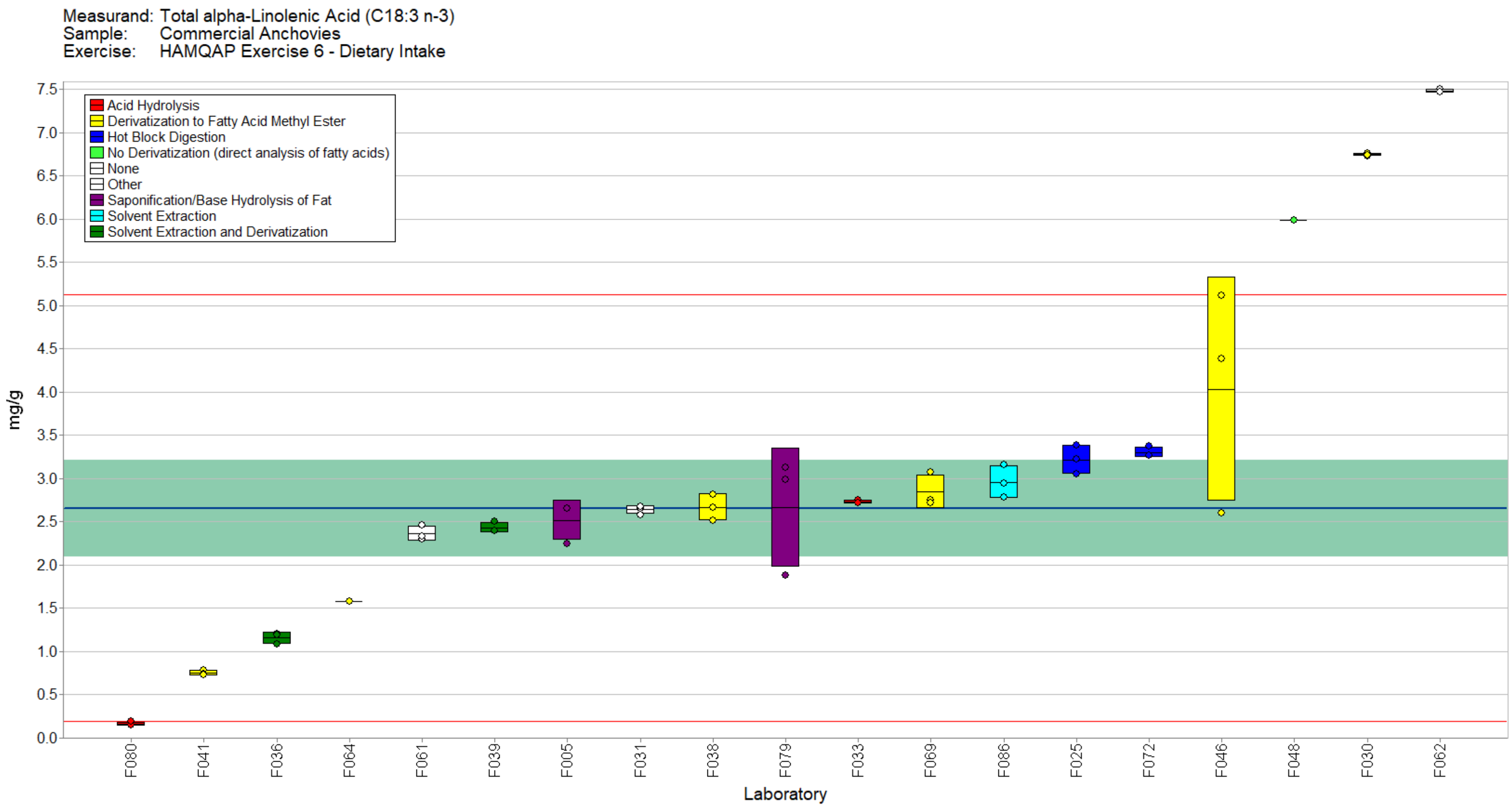


**Figure 5-2.** Total α-linolenic acid in commercial anchovies (data summary view – sample preparation method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The solid blue 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 $Z'_{\mathrm{comm}}$ score, $|Z'_{\mathrm{comm}}| \leq 2$. A NIST value has not been determined in this material.

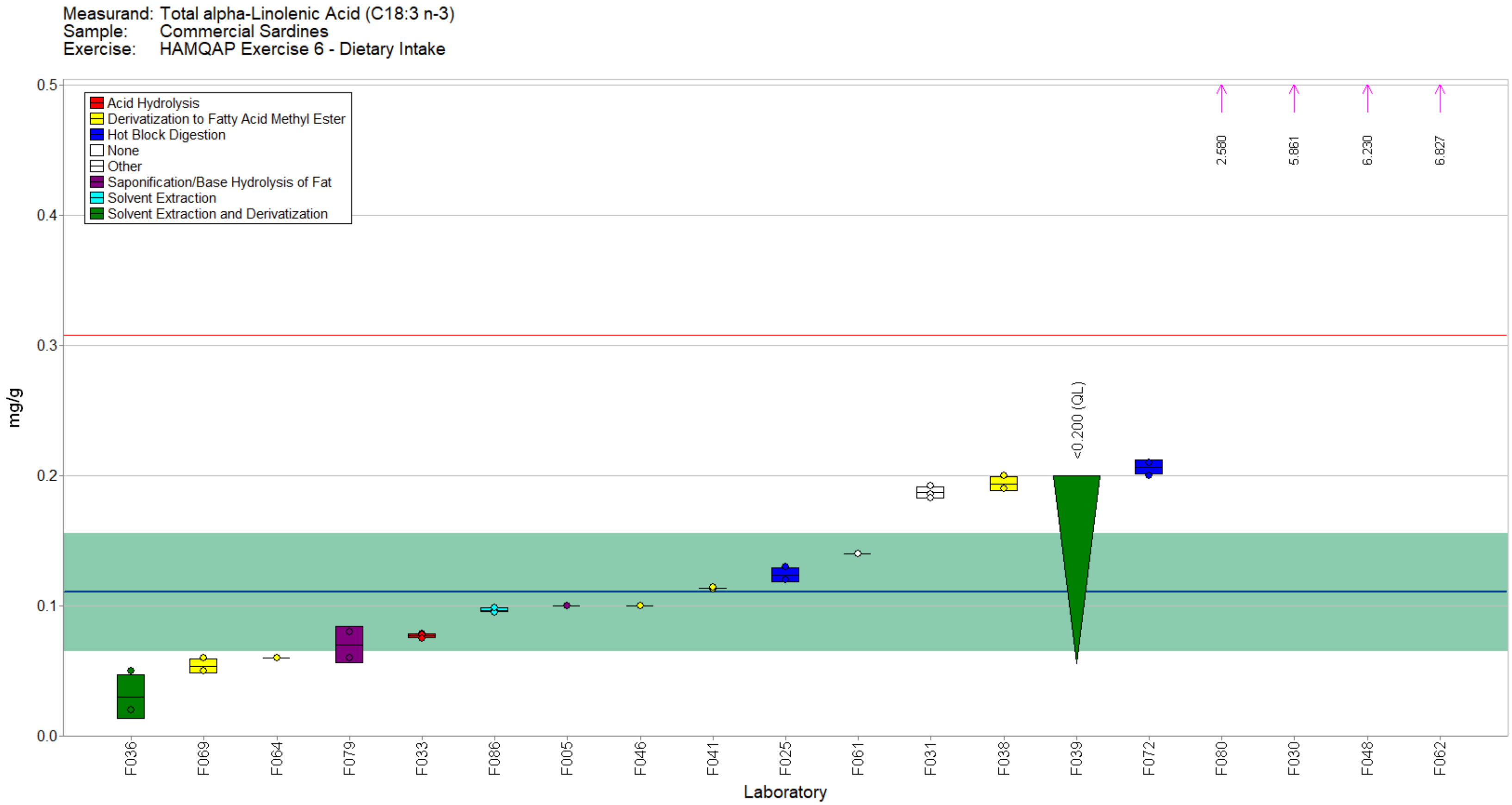


**Figure 5-3.** Total α-linolenic acid in commercial sardines (data summary view – sample preparation method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The red solid line represents the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable $Z'_{\mathrm{comm}}$ score, $|Z'_{\mathrm{comm}}| \leq 2$, with the lower limit set to zero. A NIST value has not been determined in this material.

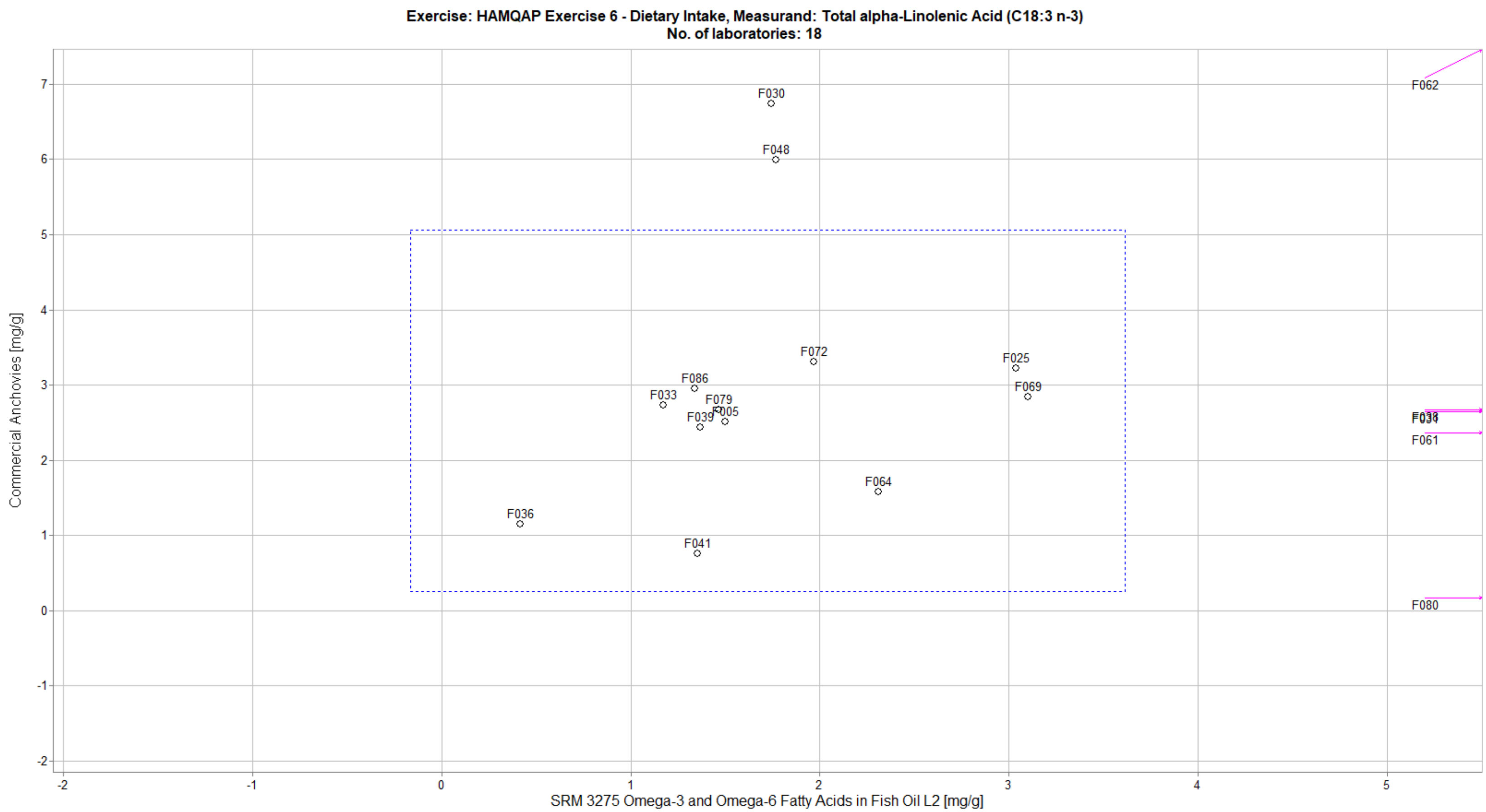


**Figure 5-4.** Laboratory means for total α-linolenic acid in SRM 3275 Omega-3 and Omega-6 Fatty Acids in Fish Oil Level 2 and commercial anchovies (sample/sample comparison view). In this view, the individual laboratory mean for one sample (SRM 3275 Level 2) is compared to the individual laboratory mean for a second sample (anchovies). The dotted blue box represents the consensus range of tolerance for SRM 3275 Level 2 (x-axis) and anchovies (y-axis), calculated as the values above and below the consensus means that result in an acceptable $Z'_{\mathrm{comm}}$ score, $|Z'_{\mathrm{comm}}| \leq 2$.

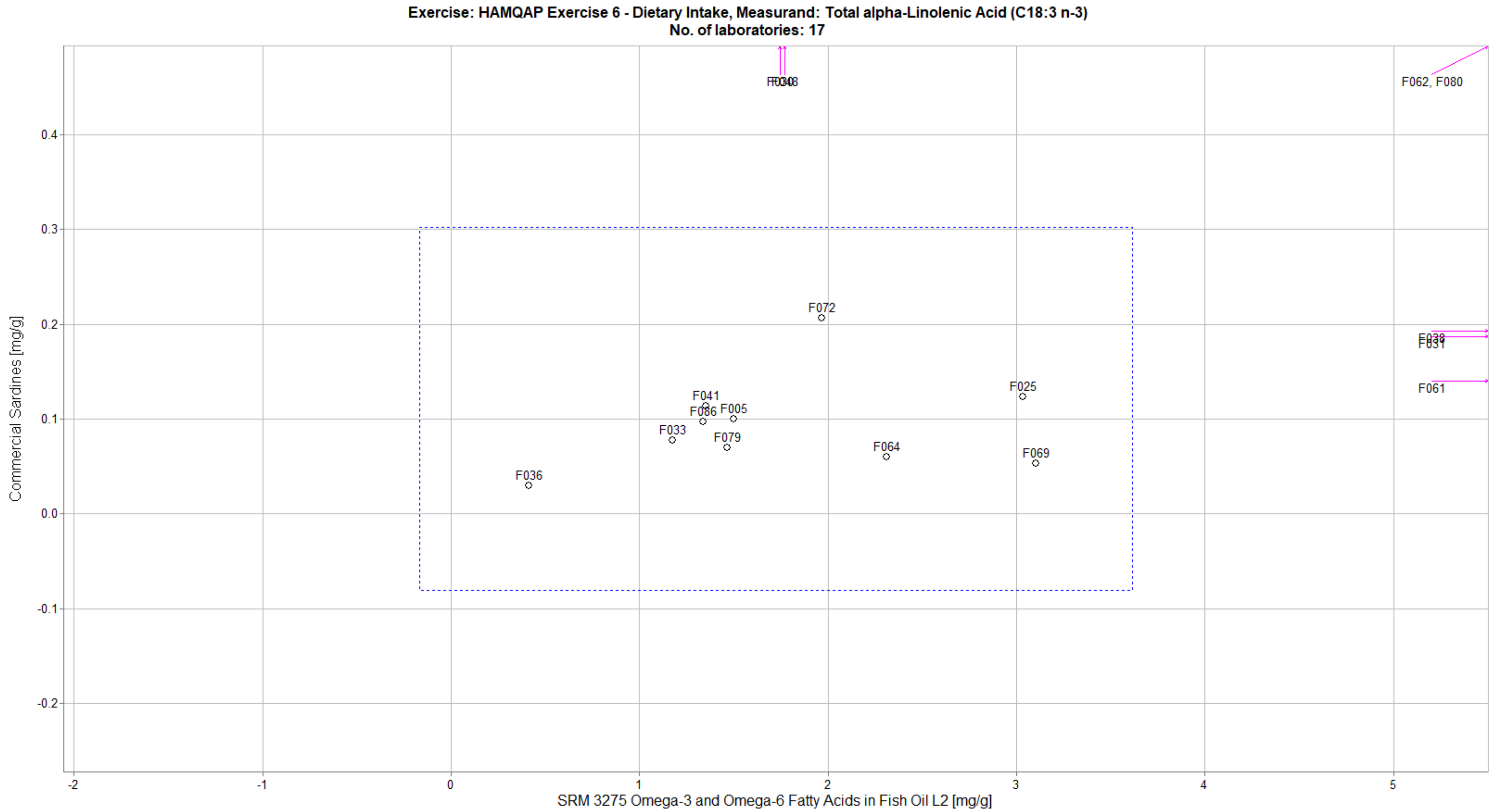


**Figure 5-5.** Laboratory means for total α-linolenic acid in SRM 3275 Omega-3 and Omega-6 Fatty Acids in Fish Oil Level 2 and commercial sardines (sample/sample comparison view). In this view, the individual laboratory mean for one sample (SRM 3275 Level 2) is compared to the individual laboratory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

**Table 5-3.** Data summary table for total linoleic acid in SRM 3275 Omega-3 and Omega-6 Fatty Acids in Fish Oil (Level 2), commercial anchovies, and commercial sardines. Data points highlighted in blue have been identified as outside the consensus tolerance limits and would be estimated to result in an unacceptable $Z'_{\mathrm{comm}}$ score, $|Z'_{\mathrm{comm}}| \geq 2$.

| | | Total Linoleic Acid (C18:2 n-6) | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | SRM 3275 Omega-3 and Omega-6 Fatty Acids in Fish Oil Level 2 (mg/g) | | | | | Commercial Anchovies (mg/g) | | | | | Commercial Sardines (mg/g) | | | | |
| | Lab | A | B | C | Avg | SD | A | B | C | Avg | SD | A | B | C | Avg | SD |
| Individual Results | Target | | | | 2.86 | 0.40 | | | | | | | | | | |
| | F004 | | | | | | | | | | | | | | | |
| | F005 | 3.5 | 3.3 | 3.4 | 3.40 | 0.10 | 30.7 | 29.1 | 37.1 | 32.30 | 4.23 | 0.2 | 0.2 | 0.2 | 0.2 | 0 |
| | F011 | | | | | | | | | | | | | | | |
| | F014 | | | | | | | | | | | | | | | |
| | F018 | | | | | | | | | | | | | | | |
| | F021 | | | | | | | | | | | | | | | |
| | F025 | 2.7 | 2.59 | 2.69 | 2.66 | 0.06 | 48.4 | 51 | 45.1 | 48.17 | 2.96 | 0.22 | 0.21 | 0.19 | 0.21 | 0.02 |
| | F026 | 3.5956 | 3.5954 | 3.8393 | 3.68 | 0.14 | | | | | | | | | | |
| | F030 | 5.8 | 5.887 | 5.862 | 5.85 | 0.04 | 93.93 | 93.74 | 93.81 | 93.83 | 0.10 | 31.64 | 31.4 | 31.46 | 31.50 | 0.12 |
| | F031 | 6.91 | 6.863 | 6.658 | 6.81 | 0.13 | 36.466 | 37.061 | 35.587 | 36.37 | 0.74 | 1.086 | 1.109 | 1.185 | 1.13 | 0.05 |
| | F032 | | | | | | | | | | | | | | | |
| | F033 | 0.652 | 0.673 | 0.678 | 0.67 | 0.01 | 35.6 | 35.9 | 35.7 | 35.73 | 0.15 | 0.099 | 0.0981 | 0.0963 | 0.10 | 0.00 |
| | F035 | | | | | | | | | | | | | | | |
| | F036 | 2.98 | 2.91 | 2.99 | 2.96 | 0.04 | 36.38 | 42.27 | 39.9 | 39.52 | 2.96 | 0.15 | 0.17 | 0.17 | 0.16 | 0.01 |
| | F038 | 6.03 | 5.6 | 5.7 | 5.78 | 0.23 | 38.59 | 36.77 | 41.91 | 39.09 | 2.61 | 0.74 | 0.75 | 0.72 | 0.74 | 0.02 |
| | F039 | 2.8 | 2.8 | 2.8 | 2.80 | 0.00 | 40.9 | 38.4 | 38.5 | 39.27 | 1.42 | 0.9 | 0.9 | 0.9 | 0.90 | 0.00 |
| | F041 | 5.728 | 6.663 | 6.46 | 6.28 | 0.49 | 13.768 | 13.044 | 12.839 | 13.22 | 0.49 | 0.464 | 0.451 | 0.457 | 0.46 | 0.01 |
| | F046 | | | | | | 40.98 | 76.16 | 54.05 | 57.06 | 17.78 | | | | | |
| | F048 | 4.57 | | | 4.57 | | 99.51 | | | 99.51 | | 24.9 | | | 24.90 | |
| | F056 | | | | | | | | | | | | | | | |
| | F060 | 3.03 | 3.2 | 3.39 | 3.21 | 0.18 | | | | | | | | | | |
| | F061 | 4.53 | 4.58 | 4.66 | 4.59 | 0.07 | 30.8 | 30.9 | 31.2 | 30.97 | 0.21 | 0.35 | 0.36 | 0.37 | 0.36 | 0.01 |
| | F062 | 3.47 | 3.51 | 3.46 | 3.48 | 0.03 | 111.99 | 111.92 | 111.82 | 111.9 | 0.09 | 10.23 | 9.81 | 9.69 | 9.91 | 0.28 |
| | F064 | 1.64 | 1.98 | 1.83 | 1.82 | 0.17 | 17.88 | | | 17.88 | | 0.14 | | | 0.14 | |
| | F069 | 1.53 | 1.53 | 1.53 | 1.53 | 0.00 | 45 | 44.34 | 50.17 | 46.50 | 3.19 | 0.16 | 0.15 | 0.13 | 0.15 | 0.02 |
| | F070 | | | | | | | | | | | | | | | |
| | F072 | 4.04 | 3.86 | 3.95 | 3.95 | 0.09 | 44.3 | 41.54 | 42.35 | 42.73 | 1.42 | 2.59 | 2.48 | 2.53 | 2.53 | 0.06 |
| | F079 | 3.1 | 3.8 | 3.8 | 3.57 | 0.40 | 60.43 | 46.3 | 60.6 | 55.78 | 8.21 | 0.38 | 0.56 | 0.39 | 0.44 | 0.10 |
| | F080 | 4.56 | 4.52 | 4.73 | 4.60 | 0.11 | 0.452 | 0.473 | 0.499 | 0.47 | 0.02 | 33.45 | 32.45 | 32.52 | 32.81 | 0.56 |
| | F081 | | | | | | | | | | | | | | | |
| | F086 | 2.758 | 2.855 | 2.874 | 2.83 | 0.06 | 39.56 | 37.31 | 42.19 | 39.69 | 2.44 | 0.241 | 0.235 | 0.231 | 0.24 | 0.01 |
| Community Results | | Consensus Mean | | | 3.75 | | Consensus Mean | | | 39.51 | | Consensus Mean | | | 0.44 | |
| | | Consensus Standard Deviation | | | 1.78 | | Consensus Standard Deviation | | | 17.03 | | Consensus Standard Deviation | | | 0.57 | |
| | | Maximum | | | 6.81 | | Maximum | | | 111.9 | | Maximum | | | 32.81 | |
| | | Minimum | | | 0.67 | | Minimum | | | 0.47 | | Minimum | | | 0.10 | |
| | | N | | | 20 | | N | | | 19 | | N | | | 18 | |

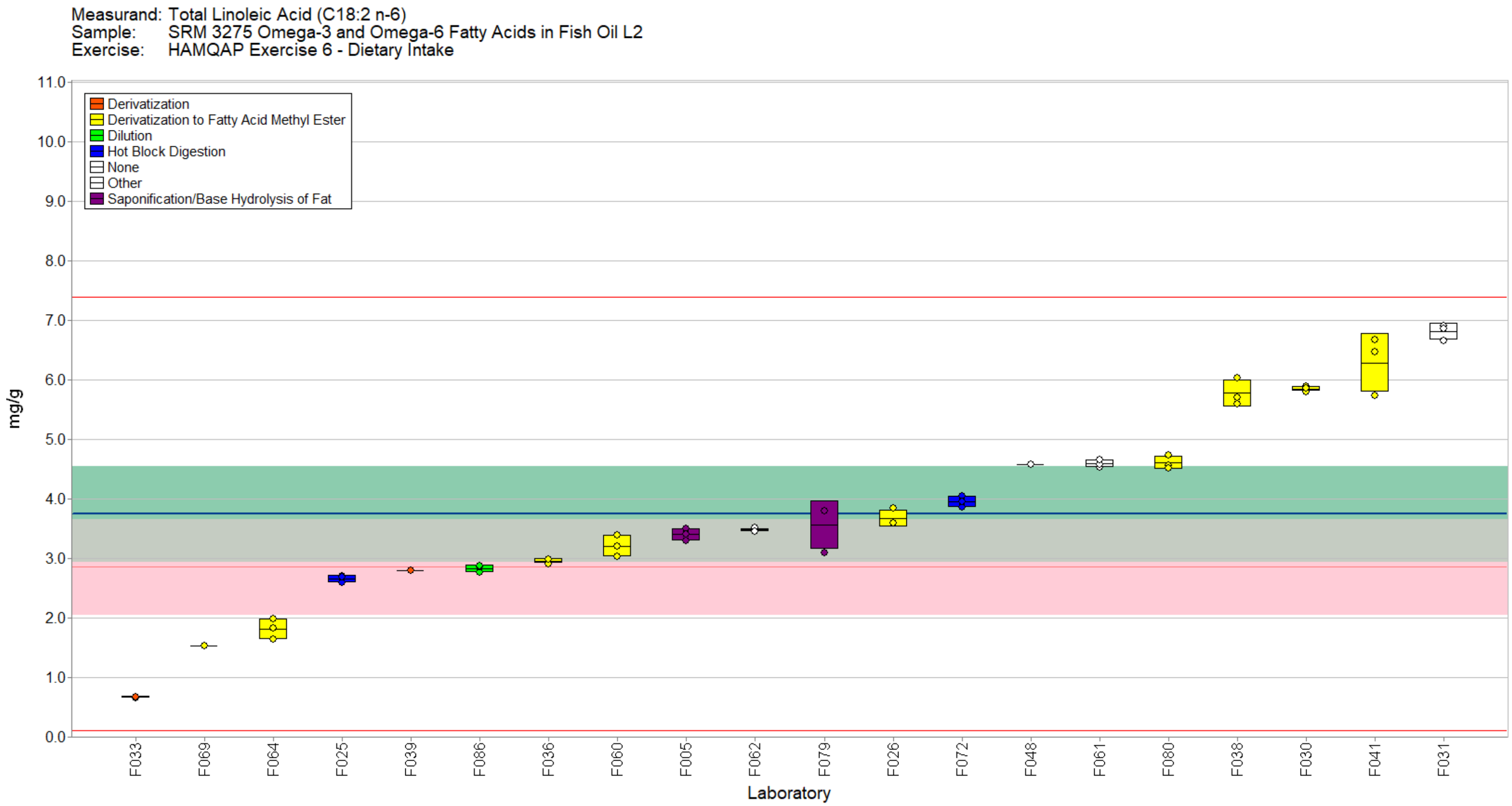


**Figure 5-6.** Total linolenic acid in SRM 3275 Omega-3 and Omega-6 Fatty Acids in Fish Oil (Level 2) (data summary view – sample preparation method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The solid blue 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 $Z'_{\text{comm}}$ score, $|Z'_{\text{comm}}| \leq 2$. 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, $|Z_{\text{NIST}}| \leq 2$.

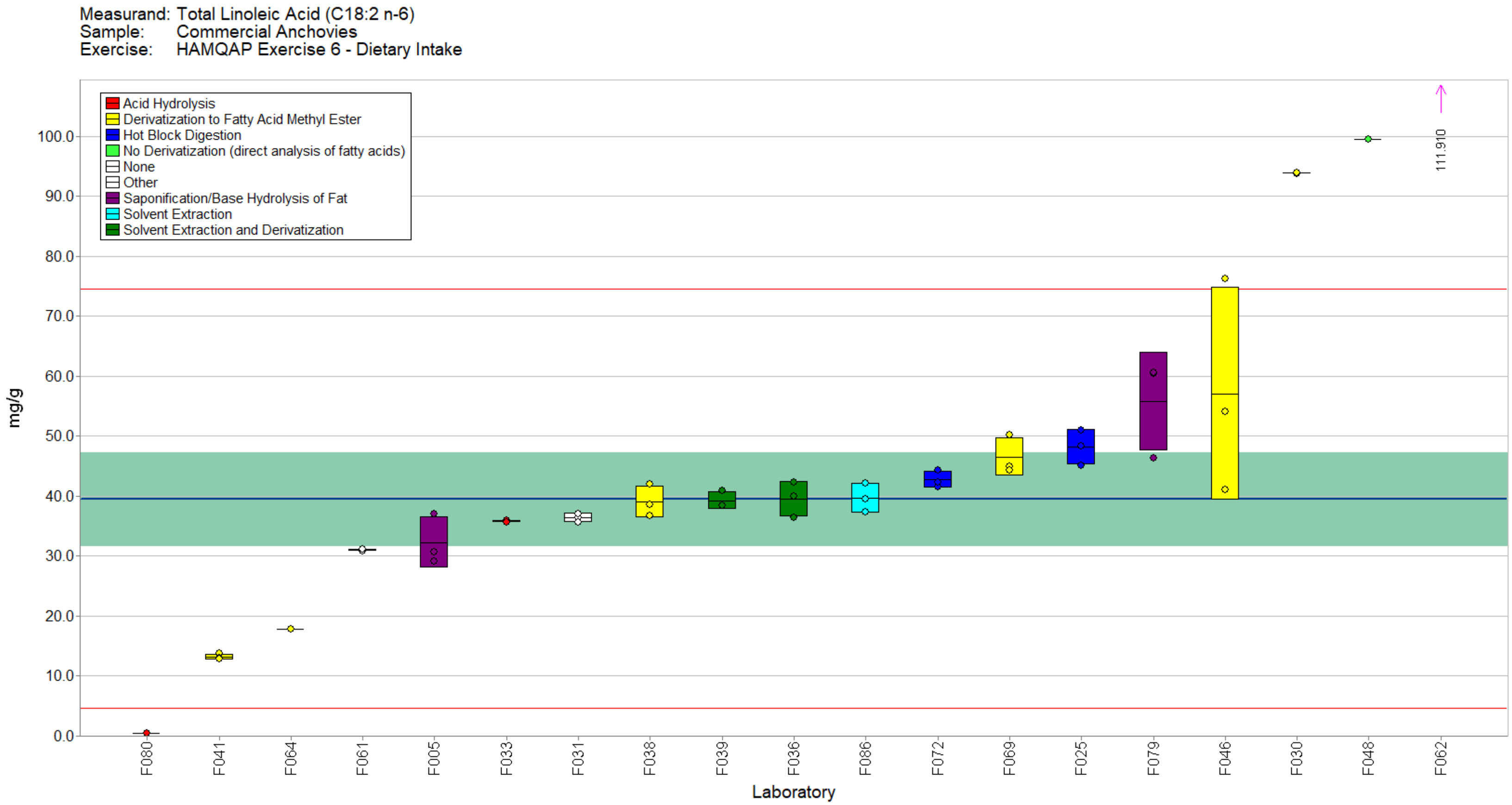


**Figure 5-7.** Total linolenic acid in commercial anchovies (data summary view – sample preparation method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The solid blue 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 $Z'_{\text{comm}}$ score, $|Z'_{\text{comm}}| \leq 2$. A NIST value has not been determined in this material.

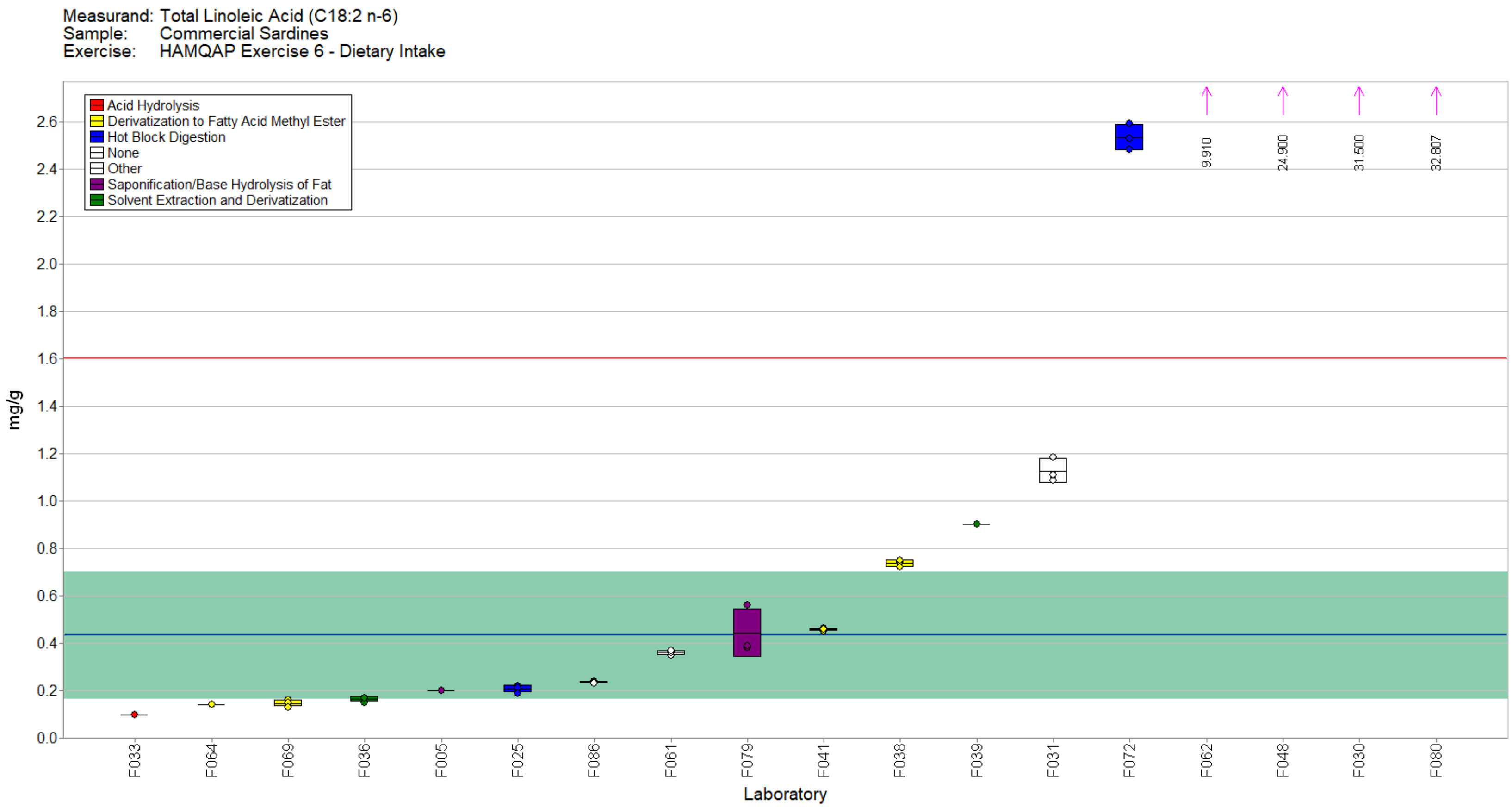


**Figure 5-8.** Total linolenic acid in commercial sardines (data summary view – sample preparation method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The red solid line represents the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable $Z'_{\text{comm}}$ score, $|Z'_{\text{comm}}| \leq 2$, with the lower limit set at zero. A NIST value has not been determined in this material.

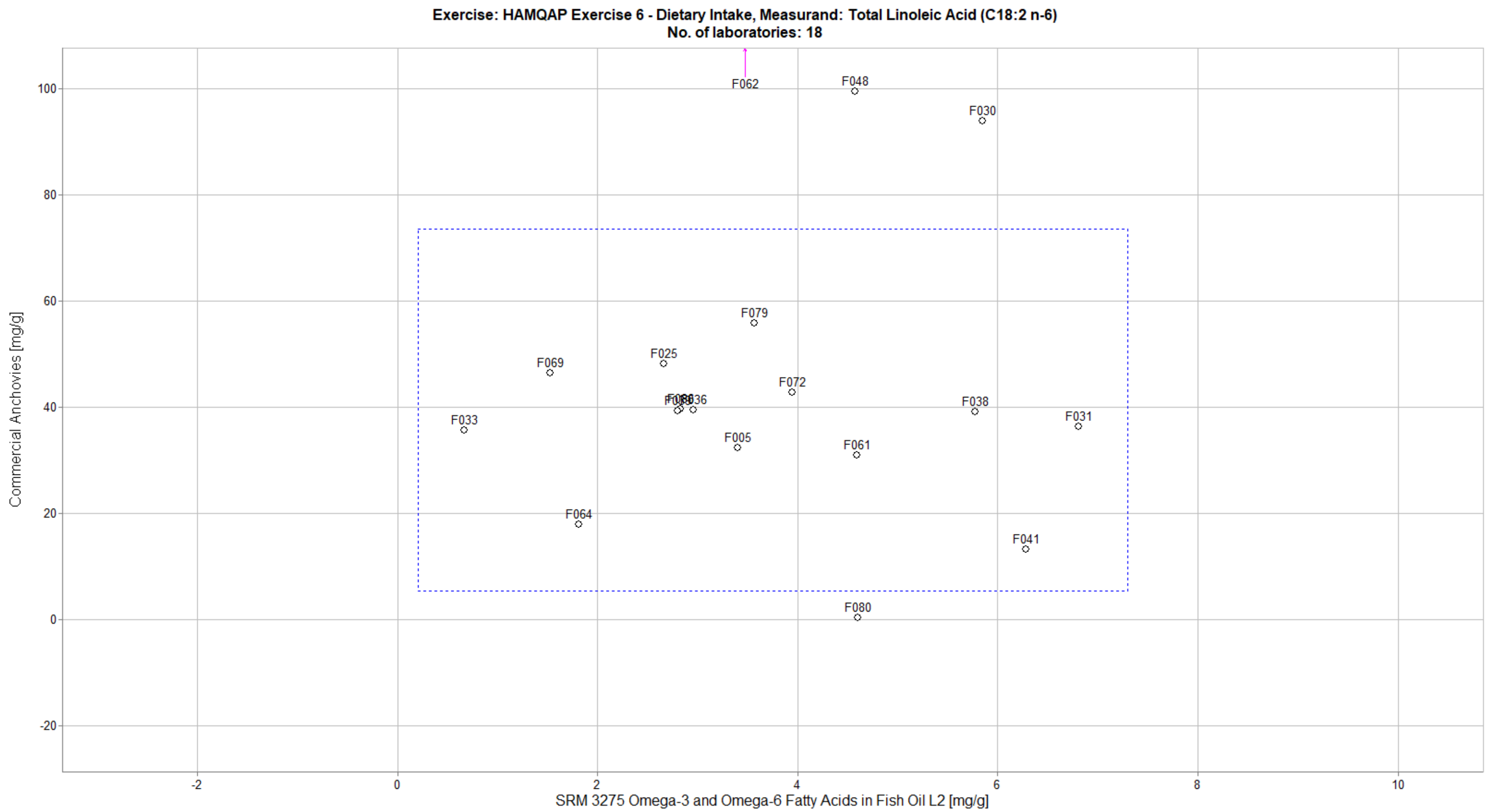


**Figure 5-9.** Laboratory means for total linolenic acid in SRM 3275 Omega-3 and Omega-6 Fatty Acids in Fish Oil Level 2 and commercial anchovies (sample/sample comparison view). In this view, the individual laboratory mean for one sample (SRM 3275 Level 2) is compared to the individual laboratory mean for a second sample (anchovies). The dotted blue box represents the consensus range of tolerance for SRM 3275 Level 2 (x-axis) and anchovies (y-axis), calculated as the values above and below the consensus means that result in an acceptable $Z'_{\mathrm{comm}}$ score, $|Z'_{\mathrm{comm}}| \leq 2$.

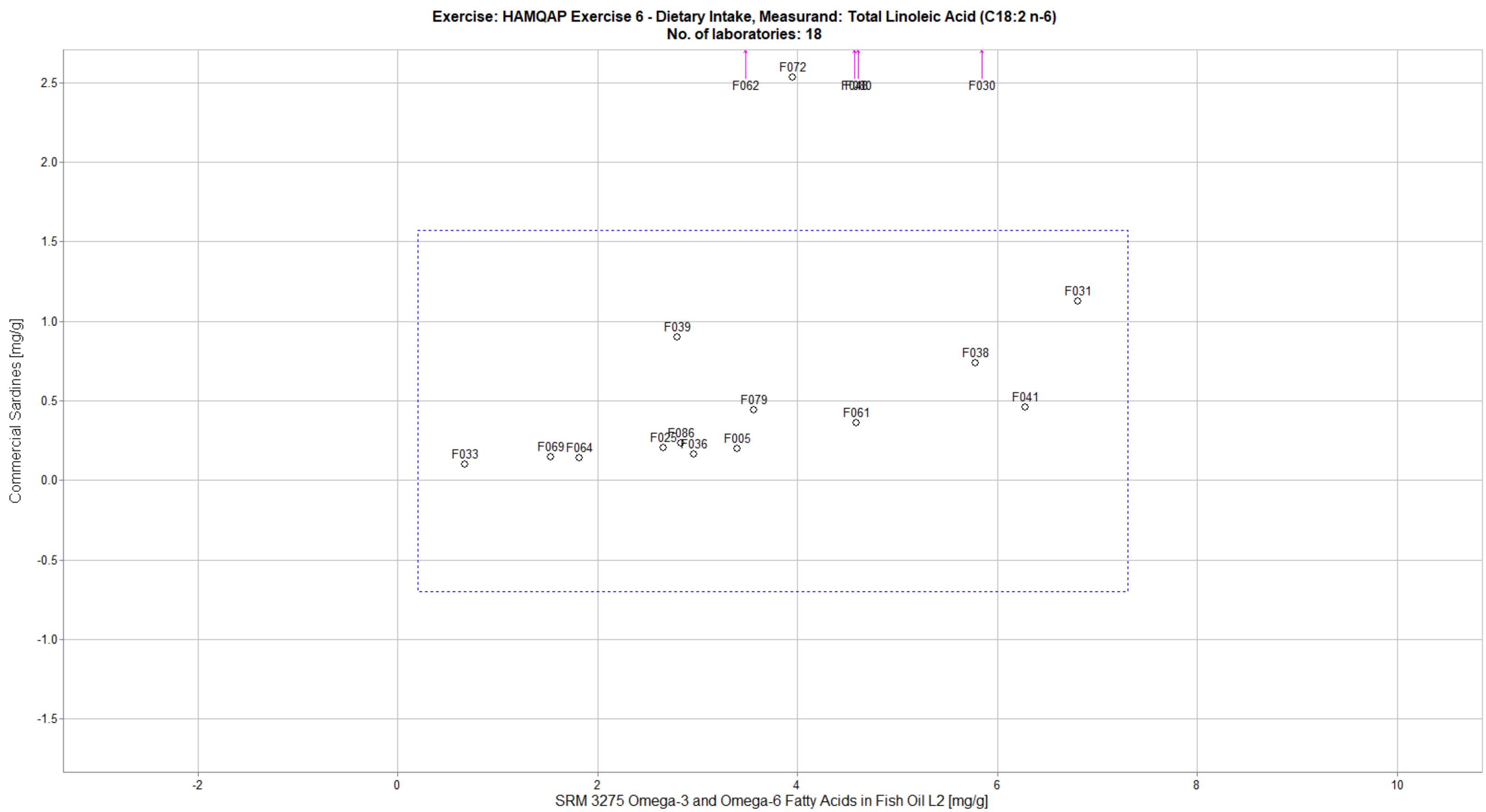


**Figure 5-10.** Laboratory means for total linolenic acid in SRM 3275 Omega-3 and Omega-6 Fatty Acids in Fish Oil Level 2 and commercial sardines (sample/sample comparison view). In this view, the individual laboratory mean for one sample (SRM 3275 Level 2) is compared to the individual laboratory mean for a second sample (sardines). The dotted blue box represents the consensus range of tolerance for SRM 3275 Level 2 (x-axis) and sardines (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 5-4.** Data summary table for total arachidonic acid in SRM 3275 Omega-3 and Omega-6 Fatty Acids in Fish Oil (Level 2), commercial anchovies, and commercial sardines. Data points highlighted in blue have been identified as outside the consensus tolerance limits and would be estimated to result in an unacceptable $Z'_{\text{comm}}$ score, $|Z'_{\text{comm}}| \geq 2$.

| | | Total Arachidonic Acid (C20:4 n-6) | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | SRM 3275 Omega-3 and Omega-6 Fatty Acids in Fish Oil Level 2 (mg/g) | | | | | Commercial Anchovies (mg/g) | | | | | Commercial Sardines (mg/g) | | | | |
| | Lab | A | B | C | Avg | SD | A | B | C | Avg | SD | A | B | C | Avg | SD |
| Individual Results | Target | | | | 21.89 | 0.96 | | | | | | | | | | |
| | F004 | | | | | | | | | | | | | | | |
| | F005 | 27.2 | 28.2 | 28 | 27.80 | 0.53 | 0.1 | 0.1 | 0.1 | 0.1 | 0 | 0.4 | 0.4 | 0.4 | 0.4 | 0 |
| | F011 | | | | | | | | | | | | | | | |
| | F014 | | | | | | | | | | | | | | | |
| | F018 | | | | | | | | | | | | | | | |
| | F021 | | | | | | | | | | | | | | | |
| | F025 | 26.4 | 26.3 | 26.7 | 26.47 | 0.21 | 0.17 | 0.16 | 0.18 | 0.170 | 0.010 | 0.5 | 0.49 | 0.49 | 0.493 | 0.006 |
| | F026 | 2.1815 | 2.3691 | 2.3994 | 2.32 | 0.12 | | | | | | | | | | |
| | F030 | 29.25 | 29.54 | 29.25 | 29.35 | 0.17 | | | | | | 12.96 | 12.89 | 12.95 | 12.9 | 0.04 |
| | F031 | 23.075 | 21.949 | 21.953 | 22.33 | 0.65 | 0.177 | 0.166 | 0.177 | 0.173 | 0.006 | 0.422 | 0.426 | 0.439 | 0.429 | 0.009 |
| | F032 | | | | | | | | | | | | | | | |
| | F033 | 24.1 | 24.2 | 24.3 | 24.20 | 0.10 | 0.0699 | 0.0735 | 0.0641 | 0.069 | 0.005 | 0.387 | 0.39 | 0.393 | 0.390 | 0.003 |
| | F035 | | | | | | | | | | | | | | | |
| | F036 | 31.46 | 30.76 | 31.73 | 31.32 | 0.50 | 0.37 | 0.42 | 0.38 | 0.390 | 0.026 | 0.3 | 0.27 | 0.32 | 0.297 | 0.025 |
| | F038 | 27.12 | 27.11 | 27.13 | 27.12 | 0.01 | 0.16 | 0.13 | 0.15 | 0.147 | 0.015 | 0.33 | 0.33 | 0.32 | 0.327 | 0.006 |
| | F039 | 22.9 | 23.2 | 23.2 | 23.10 | 0.17 | | | | | | 0.3 | 0.3 | 0.3 | 0.300 | 0.000 |
| | F041 | 23.057 | 23.582 | 23.173 | 23.27 | 0.28 | 0.116 | 0.11 | 0.115 | 0.114 | 0.003 | 0.567 | 0.564 | 0.591 | 0.574 | 0.015 |
| | F046 | 24.43 | 23.71 | 23.41 | 23.85 | 0.52 | | | | | | | | | | |
| | F048 | 23.52 | | | 23.52 | | 0.26 | | | 0.26 | | 11.34 | | | 11.34 | |
| | F056 | | | | | | | | | | | | | | | |
| | F060 | 24.15 | 24.26 | 24.27 | 24.23 | 0.07 | | | | | | | | | | |
| | F061 | 23 | 23.2 | 23.5 | 23.23 | 0.25 | 0.84 | 0.88 | 0.89 | 0.870 | 0.026 | 0.03 | 0.04 | 0.04 | 0.037 | 0.006 |
| | F062 | 26.54 | 26.52 | 26.51 | 26.52 | 0.02 | | | | | | 16.03 | 16.09 | 16.59 | 16.2 | 0.31 |
| | F064 | 12.38 | 15.15 | 13.83 | 13.79 | 1.39 | 0.16 | | | 0.16 | | 0.25 | | | 0.250 | |
| | F069 | 23.87 | 23.78 | 23.83 | 23.83 | 0.05 | 0.07 | 0.08 | 0.07 | 0.073 | 0.006 | 0.42 | 0.39 | 0.36 | 0.390 | 0.030 |
| | F070 | | | | | | | | | | | | | | | |
| | F072 | 24.75 | 24.58 | 24.29 | 24.54 | 0.23 | | | | | | | | | | |
| | F079 | 0.3 | 28 | 0.3 | 9.53 | 15.99 | 0.15 | 0.12 | 0.12 | 0.130 | 0.017 | 0.08 | 0.16 | 0.14 | 0.127 | 0.042 |
| | F080 | 24.91 | 24.85 | 25.42 | 25.06 | 0.31 | 0.326 | 0.324 | 0.372 | 0.341 | 0.027 | 0.046 | 0.049 | 0.054 | 0.050 | 0.004 |
| | F081 | | | | | | | | | | | | | | | |
| | F086 | 23.141 | 22.792 | 22.454 | 22.80 | 0.34 | 0.125 | 0.114 | 0.094 | 0.111 | 0.016 | 0.396 | 0.398 | 0.399 | 0.398 | 0.002 |
| Community Results | | Consensus Mean | | | 24.93 | | Consensus Mean | | | 0.16 | | Consensus Mean | | | 0.32 | |
| | | Consensus Standard Deviation | | | 2.71 | | Consensus Standard Deviation | | | 0.10 | | Consensus Standard Deviation | | | 0.23 | |
| | | Maximum | | | 31.32 | | Maximum | | | 0.87 | | Maximum | | | 16.24 | |
| | | Minimum | | | 2.32 | | Minimum | | | 0.07 | | Minimum | | | 0.04 | |
| | | N | | | 21 | | N | | | 14 | | N | | | 17 | |

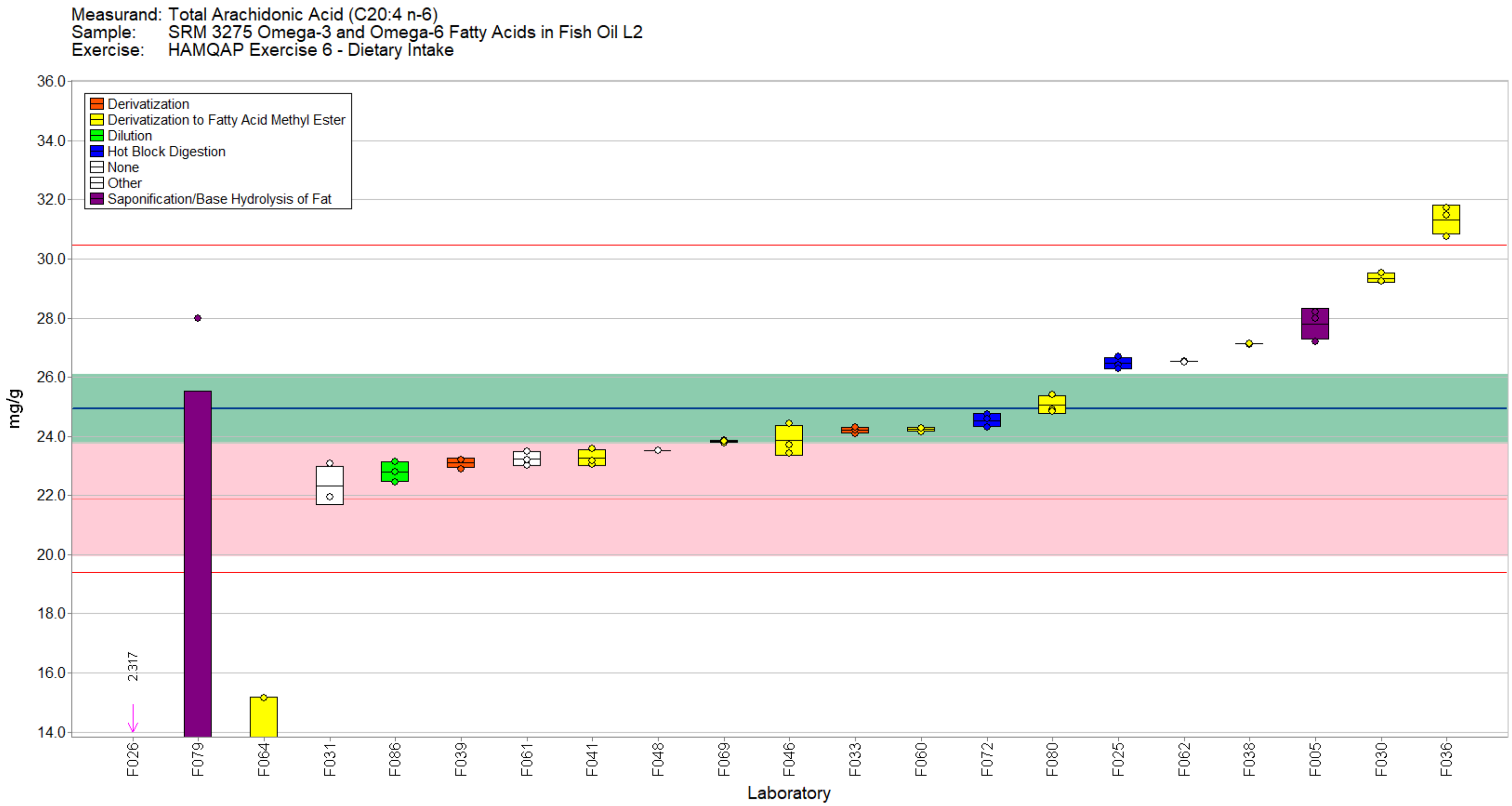


**Figure 5-11.** Total arachidonic acid in SRM 3275 Omega-3 and Omega-6 Fatty Acids in Fish Oil (Level 2) (data summary view – sample preparation method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The solid blue 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 $Z'_{\text{comm}}$ score, $|Z'_{\text{comm}}| \leq 2$. 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, $|Z_{\text{NIST}}| \leq 2$.

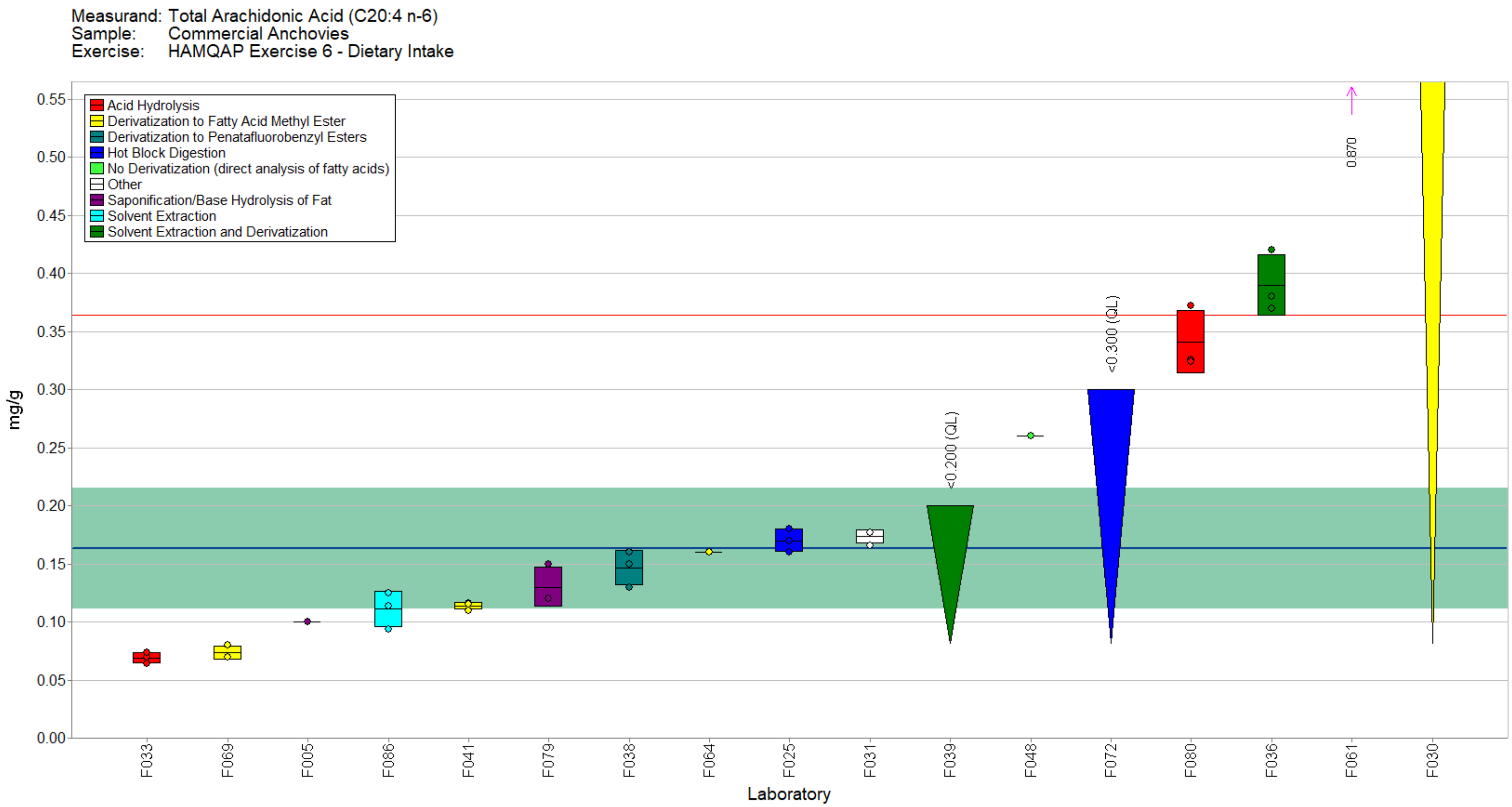


**Figure 5-12.** Total arachidonic acid in commercial anchovies (data summary view – sample preparation method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The red solid line represents the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable $Z'_{\text{comm}}$ score, $|Z'_{\text{comm}}| \leq 2$, with the lower limit set at zero. A NIST value has not been determined in this material.

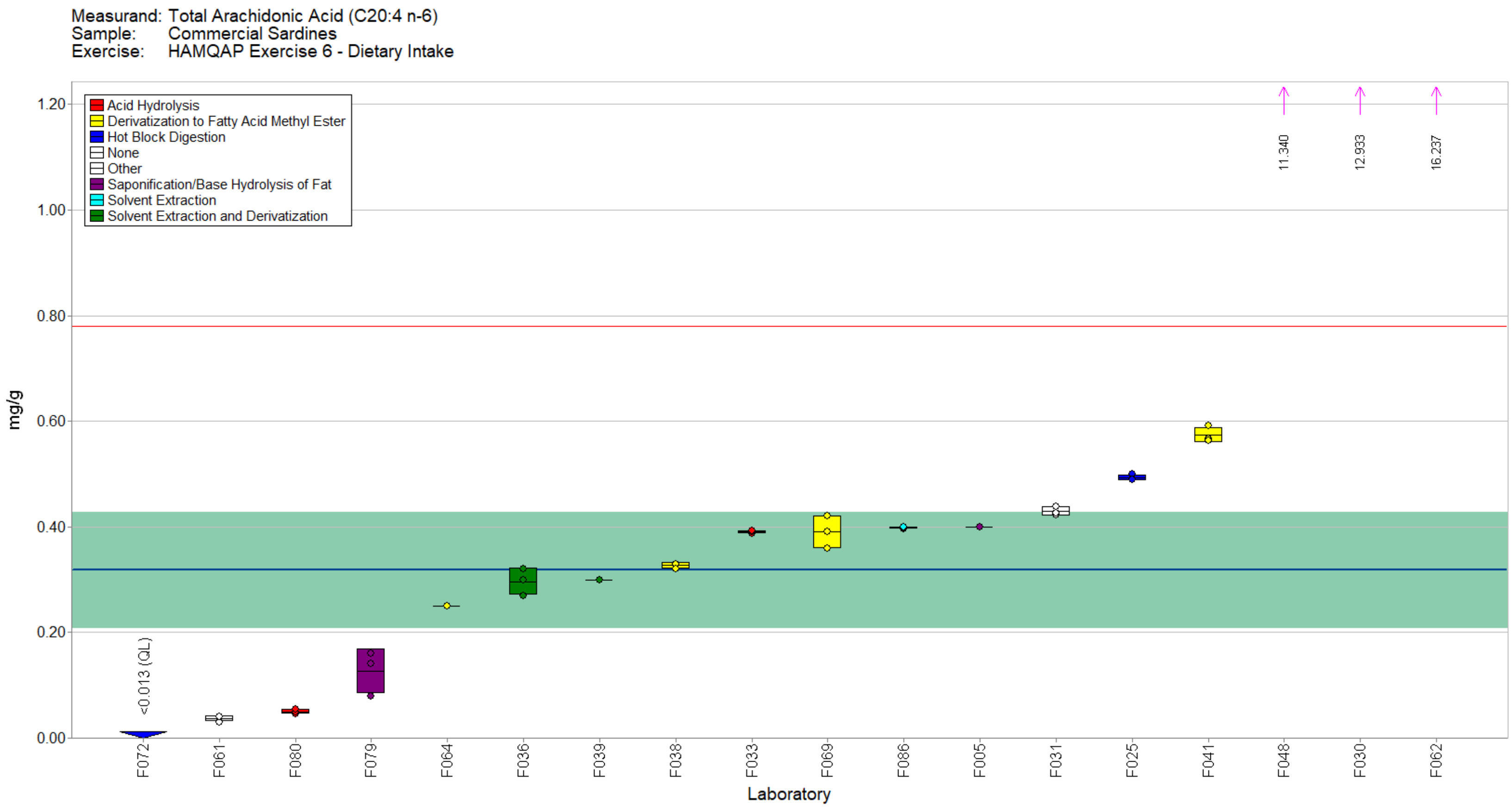


**Figure 5-13.** Total arachidonic acid in commercial sardines (data summary view – sample preparation method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The red solid line represents the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable $Z'_{\mathrm{comm}}$ score, $|Z'_{\mathrm{comm}}| \leq 2$, with the lower limit set at zero. A NIST value has not been determined in this material.

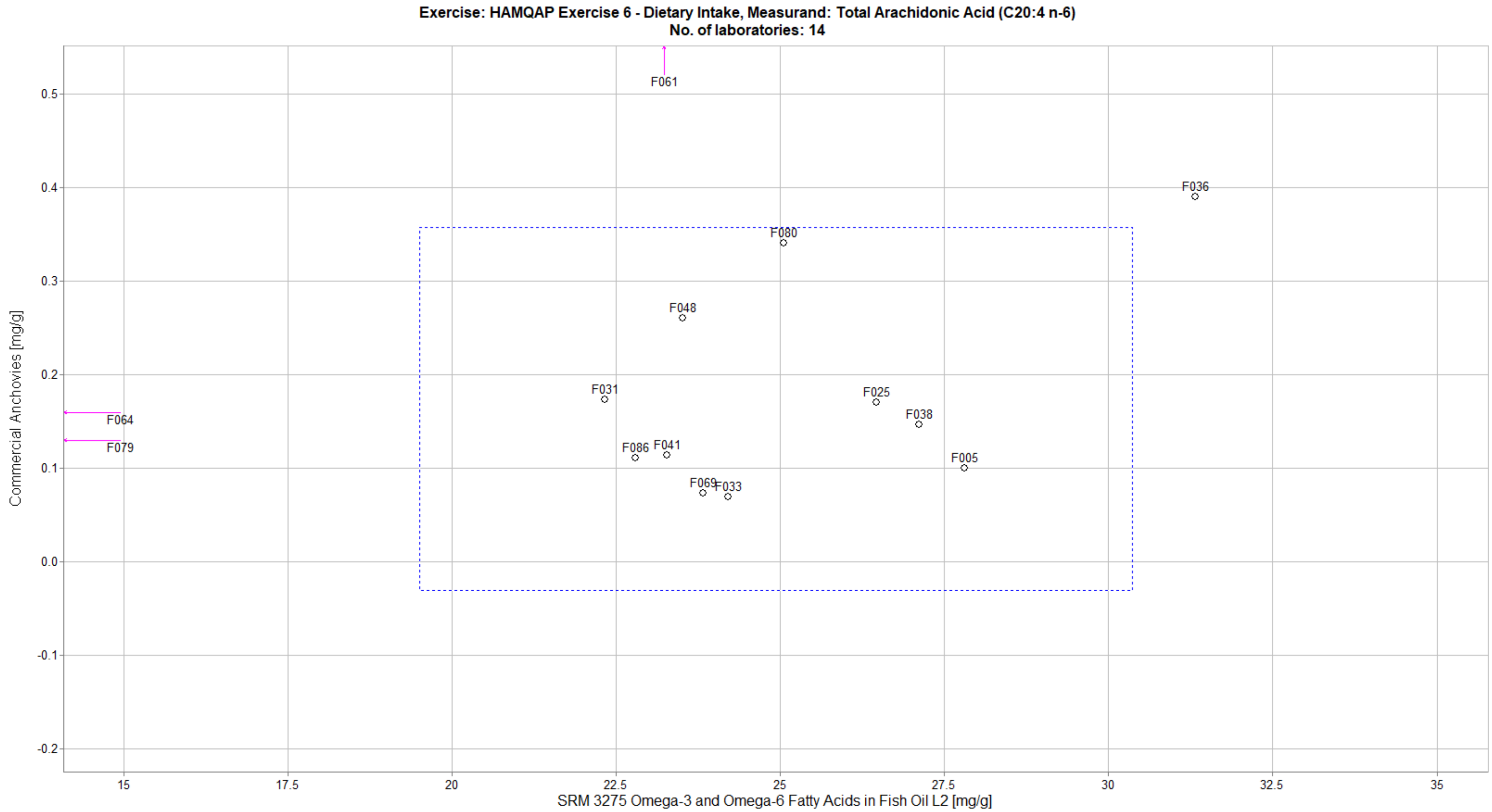


**Figure 5-14.** Laboratory means for total arachidonic acid in SRM 3275 Omega-3 and Omega-6 Fatty Acids in Fish Oil Level 2 and commercial anchovies (sample/sample comparison view). In this view, the individual laboratory mean for one sample (SRM 3275 Level 2) is compared to the individual laboratory mean for a second sample (anchovies). The dotted blue box represents the consensus range of tolerance for SRM 3275 Level 2 (x-axis) and anchovies (y-axis), calculated as the values above and below the consensus means that result in an acceptable $Z'_{\text{comm}}$ score, $|Z'_{\text{comm}}| \leq 2$.

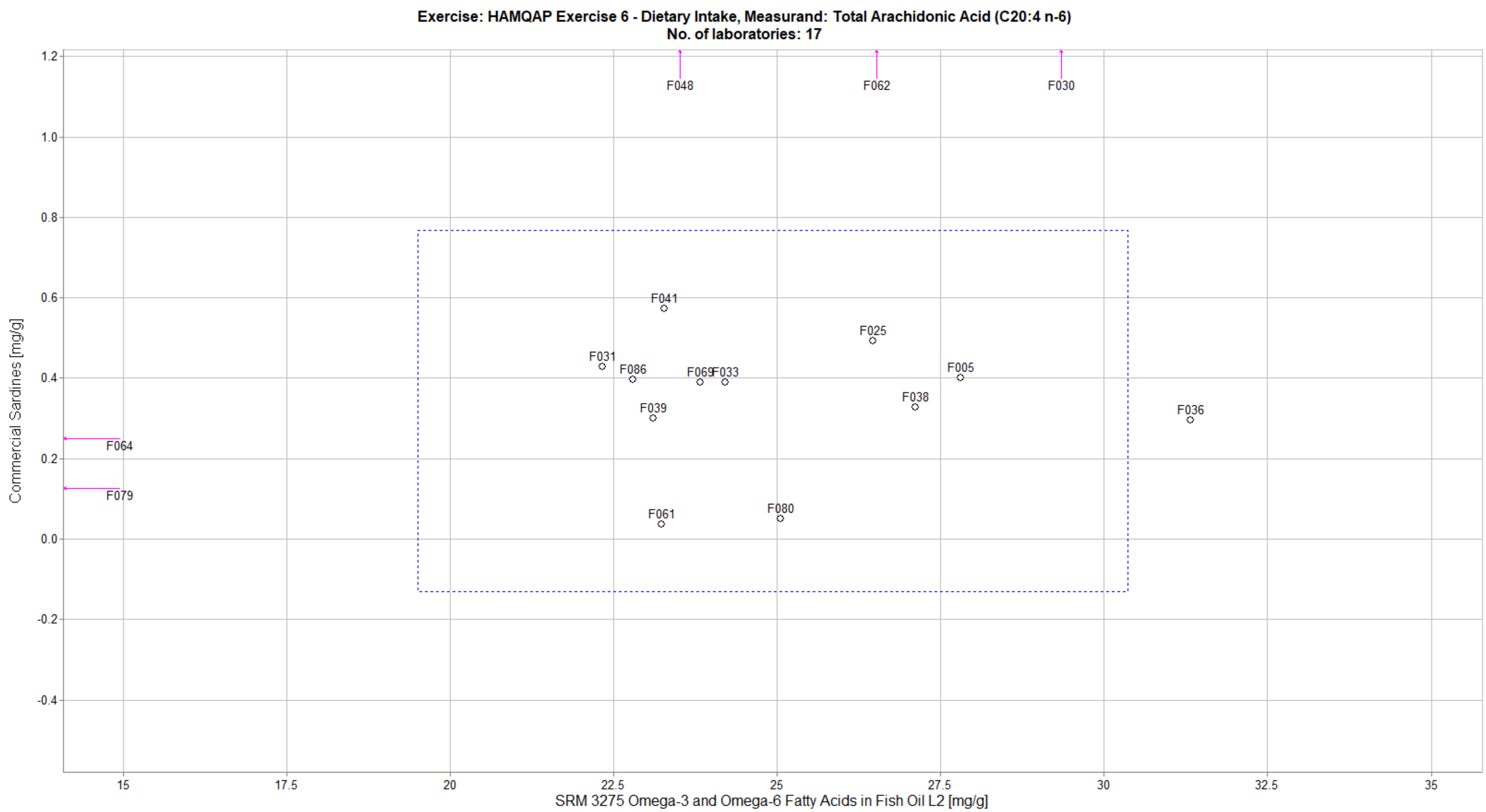


**Figure 5-15.** Laboratory means for total arachidonic acid in SRM 3275 Omega-3 and Omega-6 Fatty Acids in Fish Oil Level 2 and commercial sardines (sample/sample comparison view). In this view, the individual laboratory mean for one sample (SRM 3275 Level 2) is compared to the individual laboratory mean for a second sample (sardines). The dotted blue box represents the consensus range of tolerance for SRM 3275 Level 2 (x-axis) and sardines (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 5-5.** Data summary table for total EPA in SRM 3275 Omega-3 and Omega-6 Fatty Acids in Fish Oil (Level 2), commercial anchovies, and commercial sardines. Data points highlighted in blue have been identified as outside the consensus tolerance limits and would be estimated to result in an unacceptable $Z'_{\text{comm}}$ score, $|Z'_{\text{comm}}| \geq 2$.

| | | Total EPA (C20:5 n-3) | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | SRM 3275 Omega-3 and Omega-6 Fatty Acids in Fish Oil Level 2 (mg/g) | | | | | Commercial Anchovies (mg/g) | | | | | Commercial Sardines (mg/g) | | | | |
| | Lab | A | B | C | Avg | SD | A | B | C | Avg | SD | A | B | C | Avg | SD |
| Individual Results | Target | | | | 376.54 | 16.25 | | | | | | | | | | |
| | F004 | | | | | | | | | | | | | | | |
| | F005 | 451.8 | 469.3 | 460 | 460.4 | 8.8 | 1.15 | 1.15 | 1.2 | 1.17 | 0.03 | 4.5 | 4.6 | 4.7 | 4.60 | 0.10 |
| | F011 | 352 | 358 | 353 | 354.3 | 3.2 | | | | | | | | | | |
| | F014 | | | | | | | | | | | | | | | |
| | F018 | | | | | | | | | | | | | | | |
| | F021 | | | | | | | | | | | | | | | |
| | F025 | 343 | 342 | 347 | 344.0 | 2.6 | 0.84 | 0.84 | 0.83 | 0.84 | 0.01 | 4.95 | 4.79 | 4.78 | 4.84 | 0.10 |
| | F026 | 429.145 | 428.119 | 428.161 | 428.5 | 0.6 | | | | | | | | | | |
| | F030 | 476.1 | 477.5 | 474 | 475.9 | 1.8 | 1.109 | 1.128 | 1.117 | 1.12 | 0.01 | 175.1 | 176.4 | 175.5 | 175.7 | 0.67 |
| | F031 | 359.815 | 356.568 | 353.053 | 356.5 | 3.4 | 0.774 | 0.832 | 0.799 | 0.80 | 0.03 | 4.999 | 5.134 | 5.315 | 5.15 | 0.16 |
| | F032 | | | | | | | | | | | | | | | |
| | F033 | 363.5 | 363.6 | 365.4 | 364.2 | 1.1 | 1.05 | 1.02 | 1.02 | 1.03 | 0.02 | 3.67 | 3.69 | 3.71 | 3.69 | 0.02 |
| | F034 | 352 | 355 | 353 | 353.3 | 1.5 | 0.933 | 0.997 | 0.928 | 0.95 | 0.04 | 2.07 | 2.1 | 2.06 | 2.08 | 0.02 |
| | F035 | | | | | | | | | | | | | | | |
| | F036 | 440.63 | 443.83 | 446.73 | 443.7 | 3.1 | 0.94 | 1.11 | 1.04 | 1.03 | 0.09 | 3.87 | 4.08 | 4.12 | 4.02 | 0.13 |
| | F038 | 442.78 | 442.61 | 443.09 | 442.8 | 0.2 | 1.09 | 0.94 | 0.93 | 0.99 | 0.09 | 4.43 | 4.49 | 4.37 | 4.43 | 0.06 |
| | F039 | 353.1 | 358.5 | 355.3 | 355.6 | 2.7 | 1.2 | 1.2 | 1.2 | 1.20 | 0.00 | 3.6 | 3.6 | 3.7 | 3.63 | 0.06 |
| | F041 | 379.1 | 387.59 | 382.38 | 383.0 | 4.3 | 1.685 | 1.578 | 1.633 | 1.63 | 0.05 | 5.467 | 5.503 | 5.666 | 5.55 | 0.11 |
| | F046 | 394.05 | 369.25 | 378.69 | 380.7 | 12.5 | 2.91 | 1.75 | 1.49 | 2.05 | 0.76 | 2.5 | 3.89 | 2.45 | 2.95 | 0.82 |
| | F048 | 427.68 | | | 427.7 | | 0.52 | | | 0.52 | | 159.9 | | | 159.9 | |
| | F056 | | | | | | | | | | | | | | | |
| | F060 | 381.33 | 387.55 | 391.77 | 386.9 | 5.3 | | | | | | | | | | |
| | F061 | 333 | 336 | 341 | 336.7 | 4.0 | 0.19 | 0.2 | 0.2 | 0.20 | 0.01 | 2.22 | 2.22 | 2.26 | 2.23 | 0.02 |
| | F062 | 1.12 | 1.12 | 1.1 | 1.11 | 0.01 | 0.64 | 0.64 | 0.6 | 0.63 | 0.02 | 1.94 | 1.91 | 1.74 | 1.86 | 0.11 |
| | F064 | 173.9 | 212.1 | 193.6 | 193.2 | 19.1 | 1.2 | | | 1.20 | | 2.19 | | | 2.19 | |
| | F069 | 383.31 | 381.86 | 382.42 | 382.5 | 0.7 | 1.11 | 1.22 | 1.14 | 1.16 | 0.06 | 3.77 | 3.45 | 3.2 | 3.47 | 0.29 |
| | F070 | | | | | | | | | | | | | | | |
| | F072 | 386.52 | 383.86 | 384.95 | 385.1 | 1.3 | 1.08 | 1.15 | 1.08 | 1.10 | 0.04 | 0.06 | 0.07 | 0.08 | 0.07 | 0.01 |
| | F079 | 363 | 428 | 422 | 404.3 | 35.9 | 1.38 | 1.82 | 3.46 | 2.22 | 1.10 | 0.44 | 0.78 | 0.92 | 0.71 | 0.25 |
| | F080 | 398.98 | 391.49 | 405.83 | 398.8 | 7.2 | 3.18 | 3.25 | 3.59 | 3.34 | 0.22 | 0.948 | 0.904 | 0.931 | 0.93 | 0.02 |
| | F081 | | | | | | | | | | | | | | | |
| | F086 | 325.47 | 353.25 | 355.62 | 344.8 | 16.8 | 0.899 | 0.859 | 0.878 | 0.88 | 0.02 | 4.118 | 4.277 | 4.252 | 4.22 | 0.09 |
| Community Results | | Consensus Mean | | | 388.5 | | Consensus Mean | | | 1.05 | | Consensus Mean | | | 3.15 | |
| | | Consensus Standard Deviation | | | 52.87 | | Consensus Standard Deviation | | | 0.39 | | Consensus Standard Deviation | | | 2.30 | |
| | | Maximum | | | 475.9 | | Maximum | | | 3.34 | | Maximum | | | 175.7 | |
| | | Minimum | | | 1.11 | | Minimum | | | 0.20 | | Minimum | | | 0.07 | |
| | | N | | | 23 | | N | | | 20 | | N | | | 20 | |

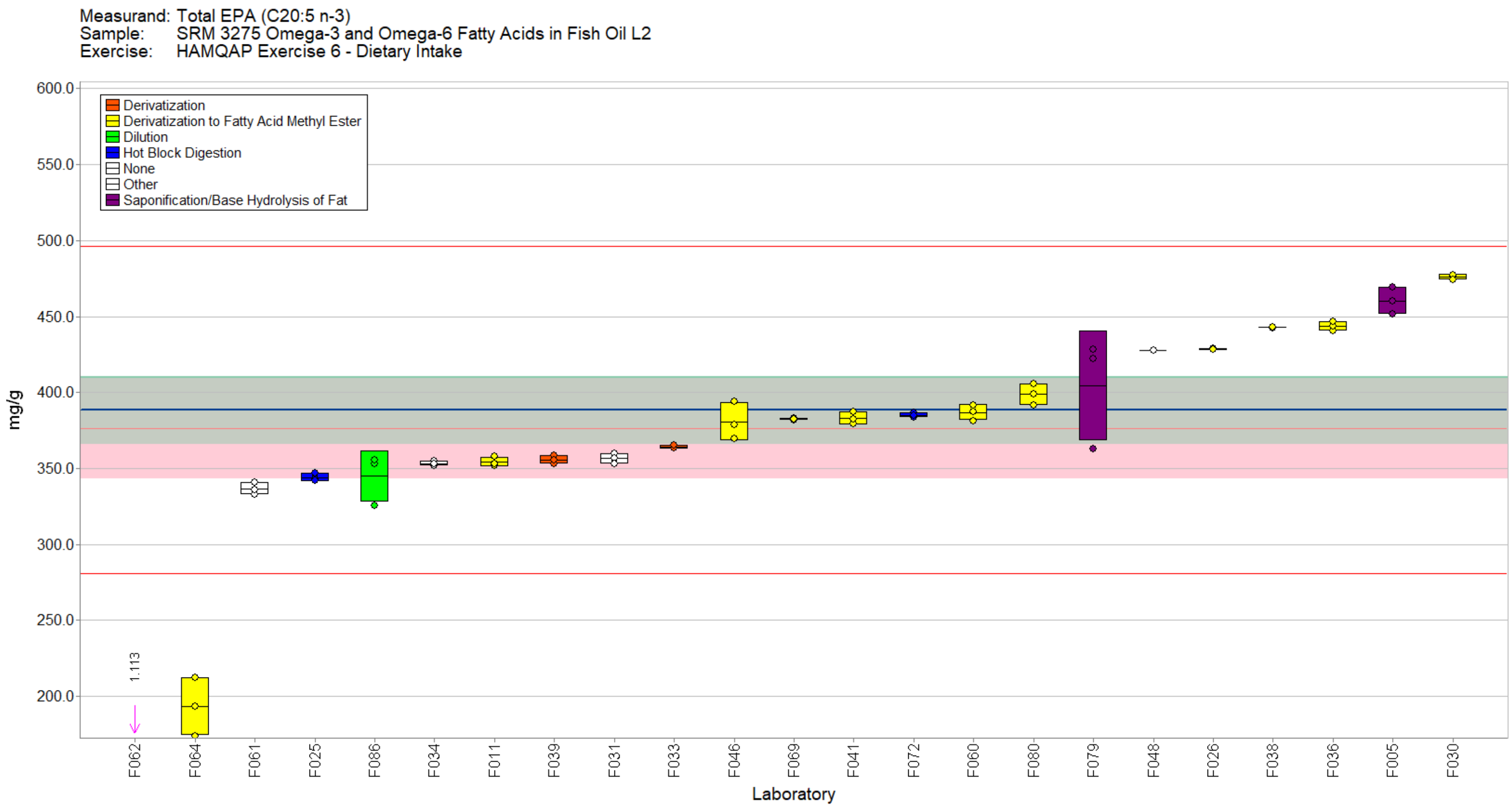


**Figure 5-16.** Total EPA in SRM 3275 Omega-3 and Omega-6 Fatty Acids in Fish Oil (Level 2) (data summary view – sample preparation method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The solid blue 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 $Z'_{\text{comm}}$ score, $|Z'_{\text{comm}}| \leq 2$. 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, $|Z_{\text{NIST}}| \leq 2$.

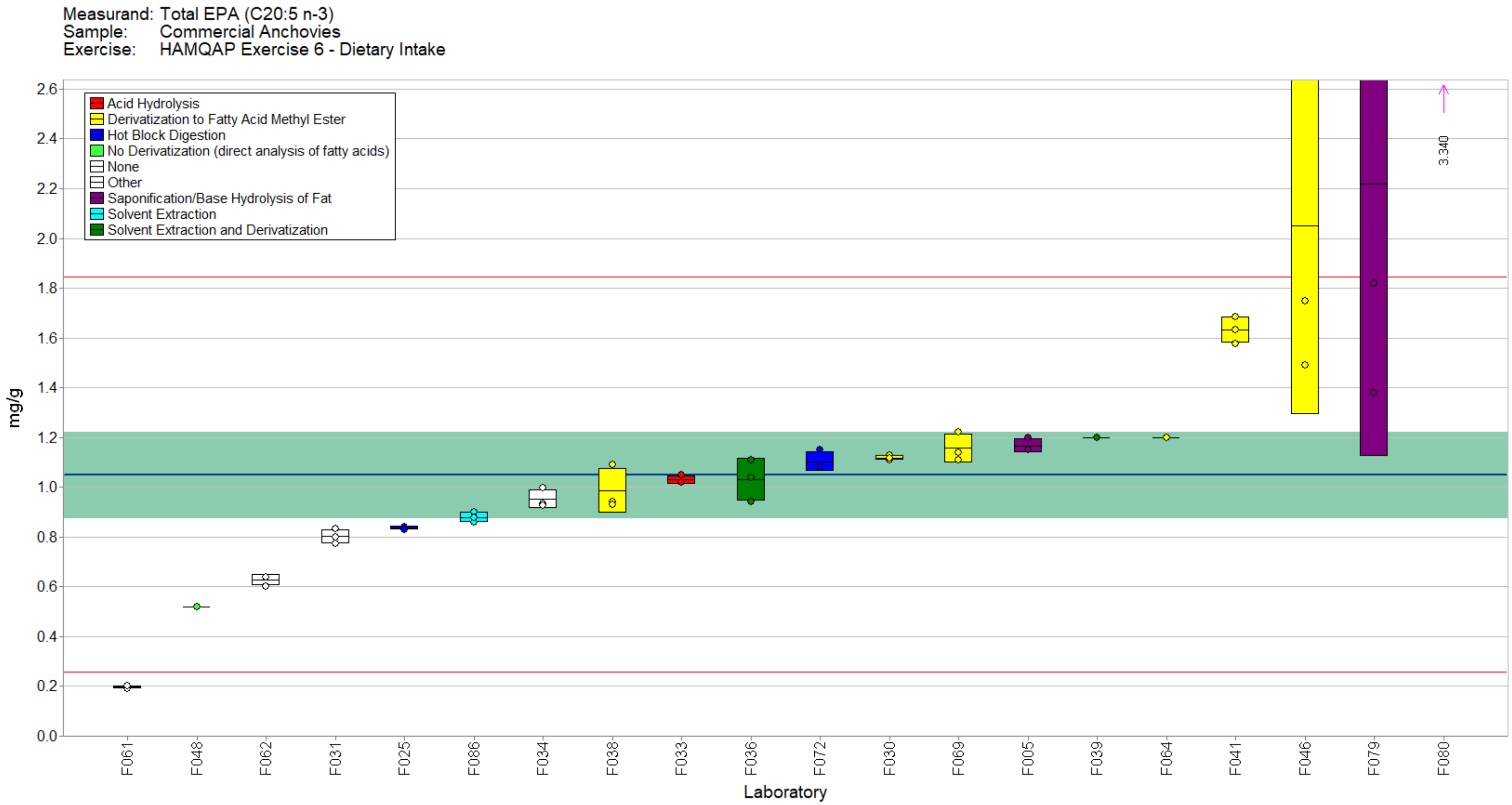


**Figure 5-17.** Total EPA in commercial anchovies (data summary view – sample preparation method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The solid blue 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 $Z'_{\text{comm}}$ score, $|Z'_{\text{comm}}| \leq 2$. A NIST value has not been determined in this material.

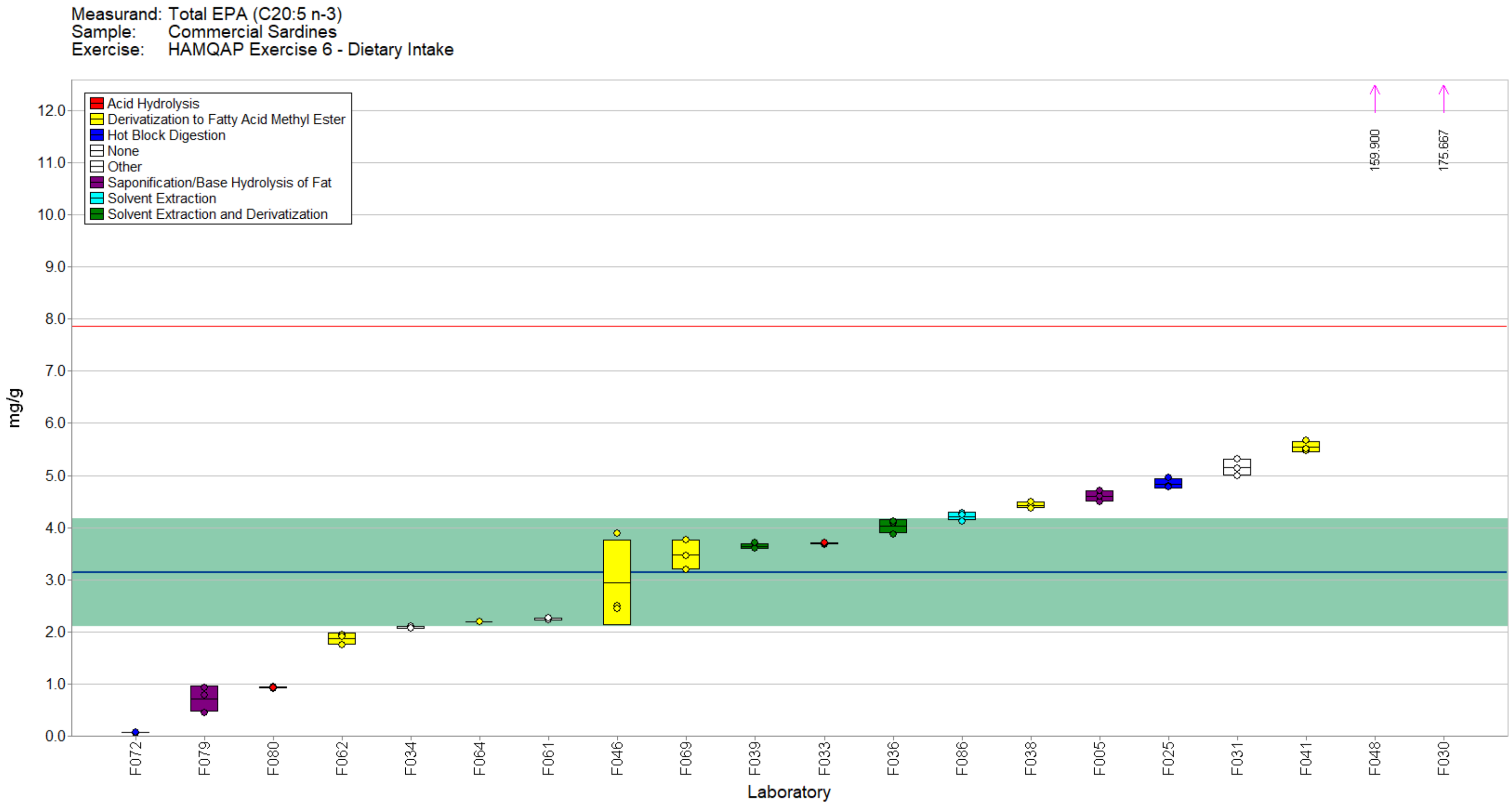


**Figure 5-18.** Total EPA in commercial sardines (data summary view – sample preparation method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The red solid line represents the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable $Z'_{\text{comm}}$ score, $|Z'_{\text{comm}}| \leq 2$, with the lower limit set at zero. A NIST value has not been determined in this material.

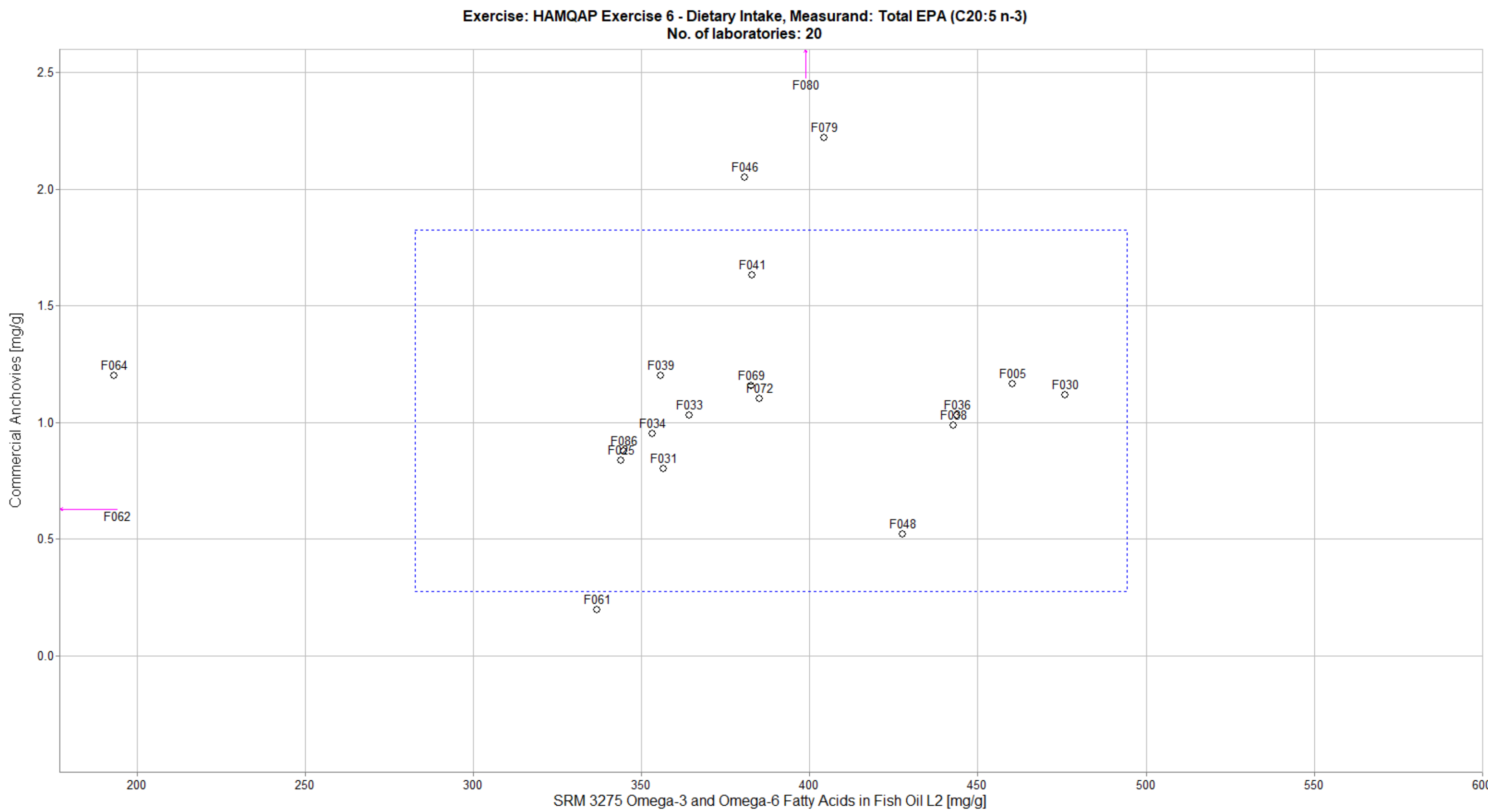


**Figure 5-19.** Laboratory means for total EPA in SRM 3275 Omega-3 and Omega-6 Fatty Acids in Fish Oil Level 2 and commercial anchovies (sample/sample comparison view). In this view, the individual laboratory mean for one sample (SRM 3275 Level 2) is compared to the individual laboratory mean for a second sample (anchovies). The dotted blue box represents the consensus range of tolerance for SRM 3275 Level 2 (x-axis) and anchovies (y-axis), calculated as the values above and below the consensus means that result in an acceptable $Z'_{\mathrm{comm}}$ score, $|Z'_{\mathrm{comm}}| \leq 2$.

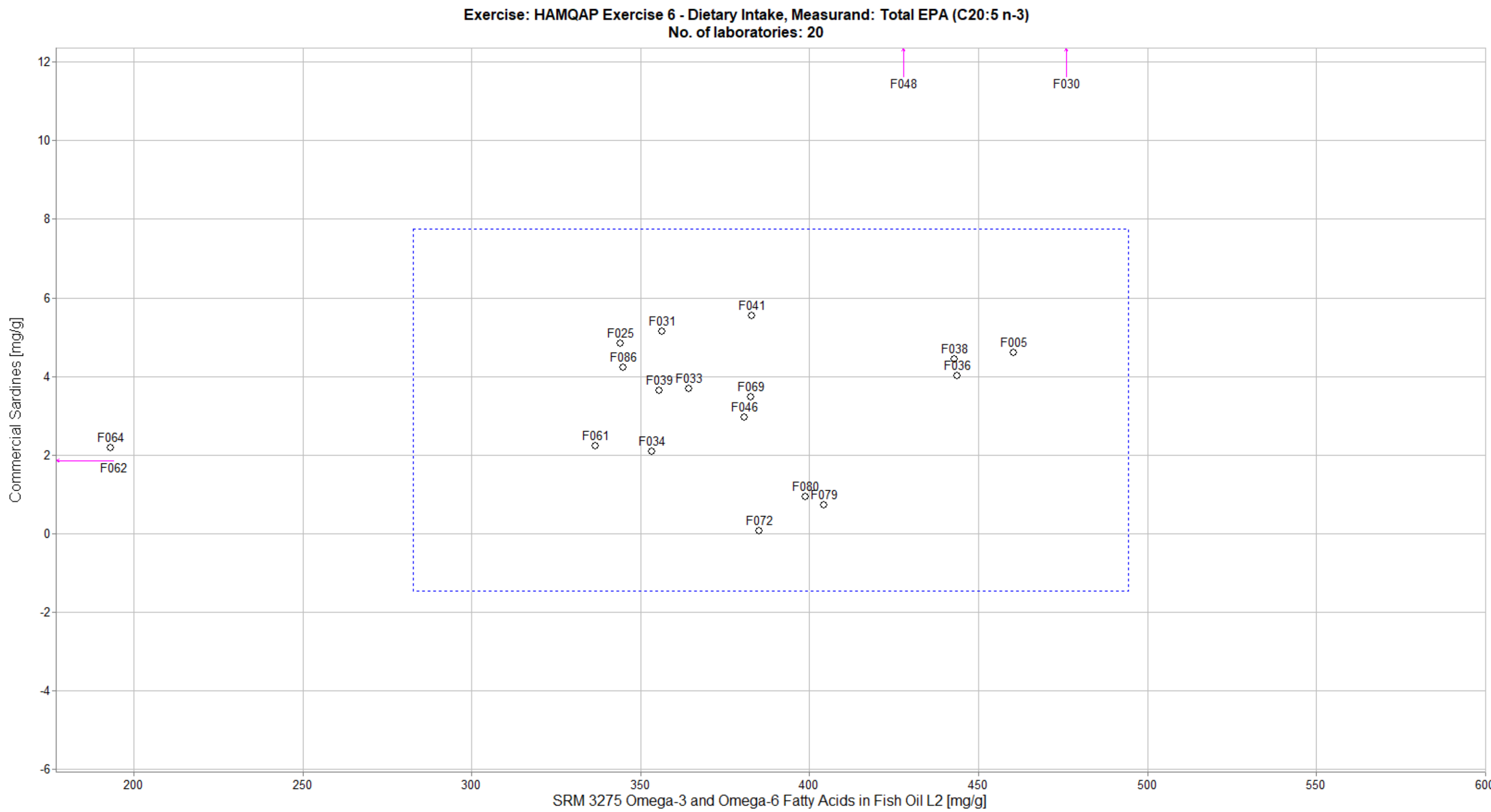


**Figure 5-20.** Laboratory means for total EPA in SRM 3275 Omega-3 and Omega-6 Fatty Acids in Fish Oil Level 2 and commercial sardines (sample/sample comparison view). In this view, the individual laboratory mean for one sample (SRM 3275 Level 2) is compared to the individual laboratory mean for a second sample (sardines). The dotted blue box represents the consensus range of tolerance for SRM 3275 Level 2 (x-axis) and sardines (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 5-6.** Data summary table for total DHA in SRM 3275 Omega-3 and Omega-6 Fatty Acids in Fish Oil (Level 2), commercial anchovies, and commercial sardines. Data points highlighted in blue have been identified as outside the consensus tolerance limits and would be estimated to result in an unacceptable $Z'_{\text{comm}}$ score, $|Z'_{\text{comm}}| \geq 2$.

| | | SRM 3275 Omega-3 and Omega-6 Fatty Acids in Fish Oil Level 2 (mg/g) | | | | | Commercial Anchovies (mg/g) | | | | | Commercial Sardines (mg/g) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Lab | A | B | C | Avg | SD | A | B | C | Avg | SD | A | B | C | Avg | SD |
| Individual Results | Target | | | | 179.34 | 7.67 | | | | | | | | | | |
| | F004 | | | | | | | | | | | | | | | |
| | F005 | 210.1 | 219.3 | 210 | 213.13 | 5.34 | 5.6 | 5.8 | 5.45 | 5.62 | 0.18 | 5.1 | 5.5 | 5.55 | 5.38 | 0.25 |
| | F011 | 173 | 175 | 173 | 173.67 | 1.15 | | | | | | | | | | |
| | F014 | | | | | | | | | | | | | | | |
| | F018 | | | | | | | | | | | | | | | |
| | F021 | | | | | | | | | | | | | | | |
| | F025 | 162 | 160 | 163 | 161.67 | 1.53 | 3.03 | 2.94 | 3.02 | 3.00 | 0.05 | 3.16 | 3.14 | 3.05 | 3.12 | 0.06 |
| | F026 | 185.198 | 184.777 | 184.909 | 184.96 | 0.22 | | | | | | | | | | |
| | F030 | 218.8 | 221 | 218.5 | 219.43 | 1.37 | 1.861 | 1.862 | 1.872 | 1.87 | 0.01 | 122.1 | 124.3 | 123 | 123.13 | 1.11 |
| | F031 | 157.963 | 157.784 | 161.123 | 158.96 | 1.88 | 2.928 | 3.096 | 3.21 | 3.08 | 0.14 | 4.454 | 4.623 | 4.809 | 4.63 | 0.18 |
| | F032 | | | | | | | | | | | | | | | |
| | F033 | 164.6 | 165.4 | 166.4 | 165.47 | 0.90 | 3.56 | 3.58 | 3.57 | 3.57 | 0.01 | 3.34 | 3.36 | 3.34 | 3.35 | 0.01 |
| | F034 | 179 | 179 | 177 | 178.33 | 1.15 | 3.38 | 3.49 | 3.37 | 3.41 | 0.07 | 2.27 | 2.29 | 2.26 | 2.27 | 0.02 |
| | F035 | | | | | | | | | | | | | | | |
| | F036 | 237.86 | 234.76 | 241.95 | 238.19 | 3.61 | 2.86 | 3.37 | 3.13 | 3.12 | 0.26 | 2.14 | 2.39 | 2.41 | 2.31 | 0.15 |
| | F038 | 207.04 | 207.08 | 207.11 | 207.08 | 0.04 | 4.63 | 3.77 | 3.64 | 4.01 | 0.54 | 4.49 | 4.52 | 4.45 | 4.49 | 0.04 |
| | F039 | 168.8 | 171.2 | 169.2 | 169.73 | 1.29 | 4.4 | 4.4 | 4.6 | 4.47 | 0.12 | 4.1 | 3.9 | 4.3 | 4.10 | 0.20 |
| | F041 | 180.67 | 184.83 | 182.34 | 182.61 | 2.09 | 6.744 | 6.662 | 6.715 | 6.71 | 0.04 | 5.83 | 5.69 | 6.099 | 5.87 | 0.21 |
| | F046 | 205.53 | 198.92 | 198.11 | 200.85 | 4.07 | 4.24 | 4.65 | 4.54 | 4.48 | 0.21 | 5.57 | 6.7 | 5.2 | 5.82 | 0.78 |
| | F048 | 197.58 | | | 197.58 | | 0.92 | | | 0.92 | | 131.07 | | | 131.07 | |
| | F056 | | | | | | | | | | | | | | | |
| | F060 | 182.03 | 184.37 | 184.67 | 183.69 | 1.45 | | | | | | | | | | |
| | F061 | 178 | 181 | 184 | 181.00 | 3.00 | 0.48 | 0.49 | 0.51 | 0.49 | 0.02 | 4.05 | 4.11 | 4.16 | 4.11 | 0.06 |
| | F062 | 201.27 | 201.16 | 201.74 | 201.39 | 0.31 | 1.65 | 1.67 | 1.64 | 1.65 | 0.02 | 76.97 | 74.81 | 75.88 | 75.89 | 1.08 |
| | F064 | 77.8 | 96.1 | 87.6 | 87.17 | 9.16 | 3.95 | | | 3.95 | | 3.49 | | | 3.49 | |
| | F069 | 190.21 | 189.64 | 189.86 | 189.90 | 0.29 | 4.11 | 4.88 | 4.6 | 4.53 | 0.39 | 4.33 | 4.22 | 4 | 4.18 | 0.17 |
| | F070 | | | | | | | | | | | | | | | |
| | F072 | 178.55 | 175.29 | 176.92 | 176.92 | 1.63 | 3.69 | 3.66 | 3.63 | 3.66 | 0.03 | 0.25 | 0.25 | 0.29 | 0.26 | 0.02 |
| | F079 | 183 | 216 | 213 | 204.00 | 18.25 | 1.27 | 4.84 | 6.31 | 4.14 | 2.59 | 0.14 | 0.36 | 0.46 | 0.32 | 0.16 |
| | F080 | 190.38 | 192.16 | 189.26 | 190.60 | 1.46 | 4.99 | 5.25 | 5.02 | 5.09 | 0.14 | 4.25 | 3.96 | 4.26 | 4.16 | 0.17 |
| | F081 | | | | | | | | | | | | | | | |
| | F086 | 169.34 | 167.51 | 168.83 | 168.56 | 0.94 | 3.475 | 3.406 | 3.341 | 3.41 | 0.07 | 3.904 | 3.911 | 3.937 | 3.92 | 0.02 |
| Community Results | | Consensus Mean | | | 187.49 | | Consensus Mean | | | 3.57 | | Consensus Mean | | | 3.64 | |
| | | Consensus Standard Deviation | | | 24.18 | | Consensus Standard Deviation | | | 1.60 | | Consensus Standard Deviation | | | 2.19 | |
| | | Maximum | | | 238.19 | | Maximum | | | 6.71 | | Maximum | | | 131.07 | |
| | | Minimum | | | 87.17 | | Minimum | | | 0.49 | | Minimum | | | 0.26 | |
| | | N | | | 23 | | N | | | 20 | | N | | | 20 | |

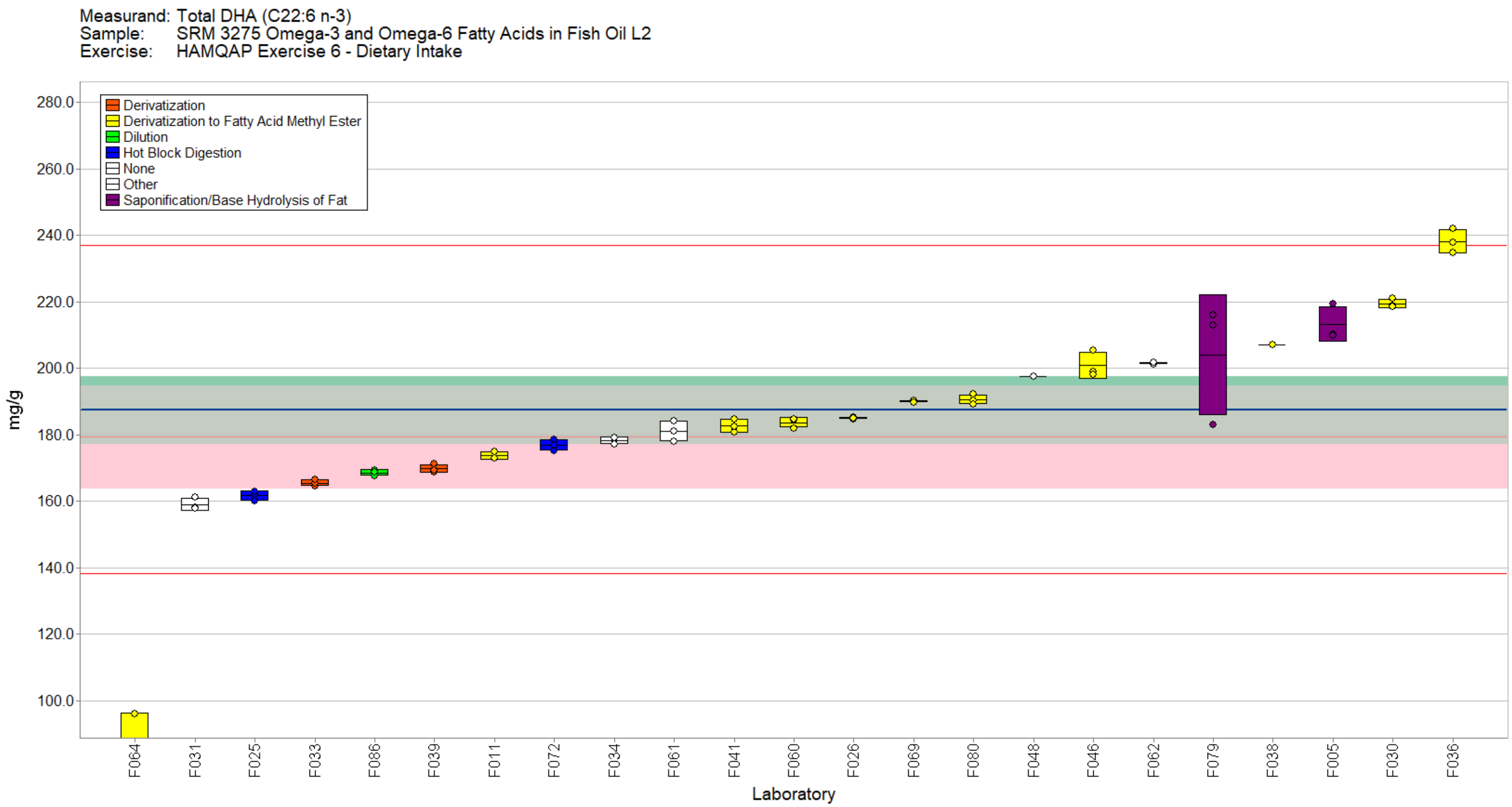


**Figure 5-21.** Total DHA in SRM 3275 Omega-3 and Omega-6 Fatty Acids in Fish Oil (Level 2) (data summary view – sample preparation method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The solid blue 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 $Z'_{\text{comm}}$ score, $|Z'_{\text{comm}}| \leq 2$. 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, $|Z_{\text{NIST}}| \leq 2$.

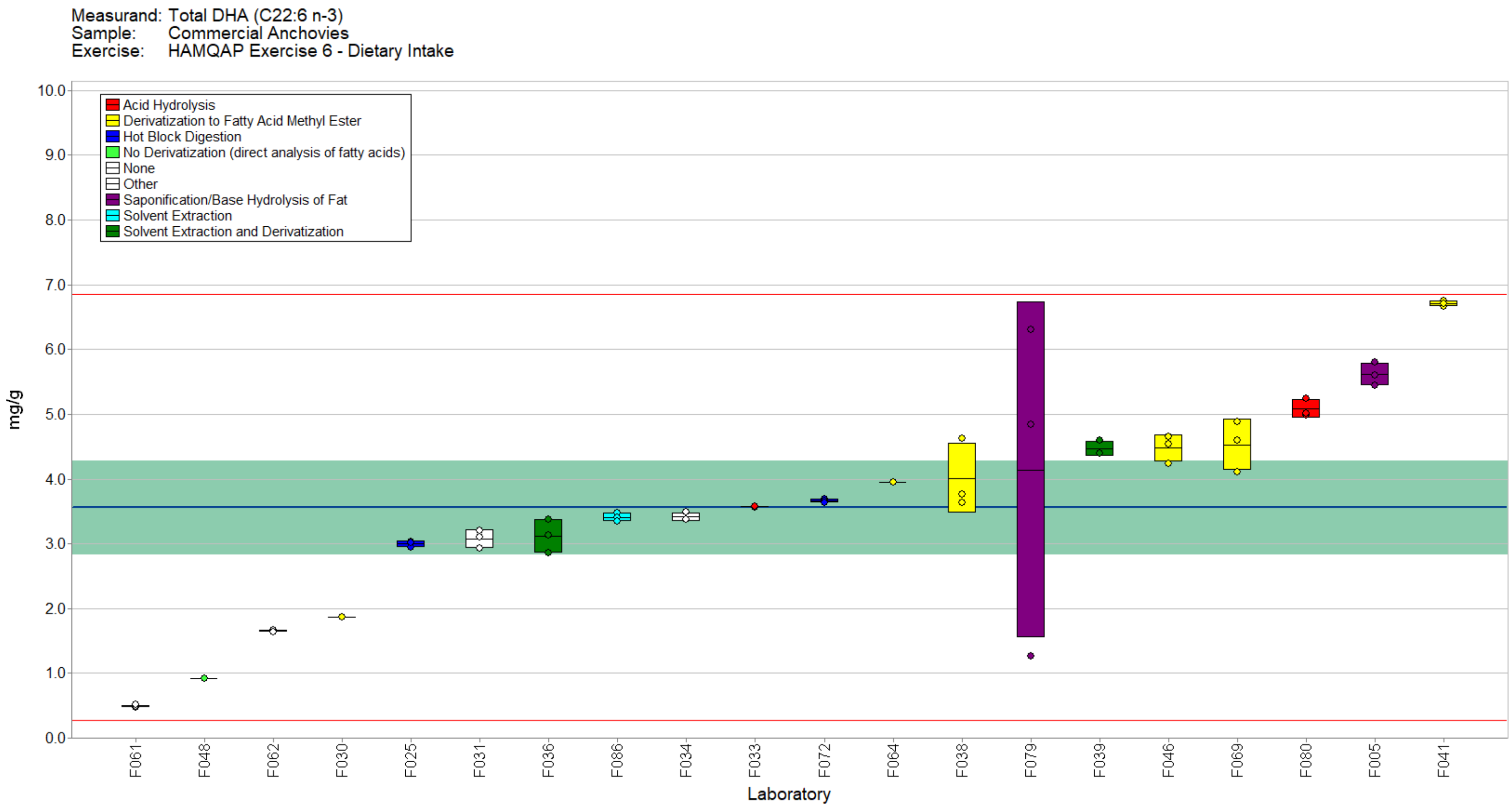


**Figure 5-22.** Total DHA in commercial anchovies (data summary view – sample preparation method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The solid blue 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 $Z'_{\text{comm}}$ score, $|Z'_{\text{comm}}| \leq 2$. A NIST value has not been determined in this material.

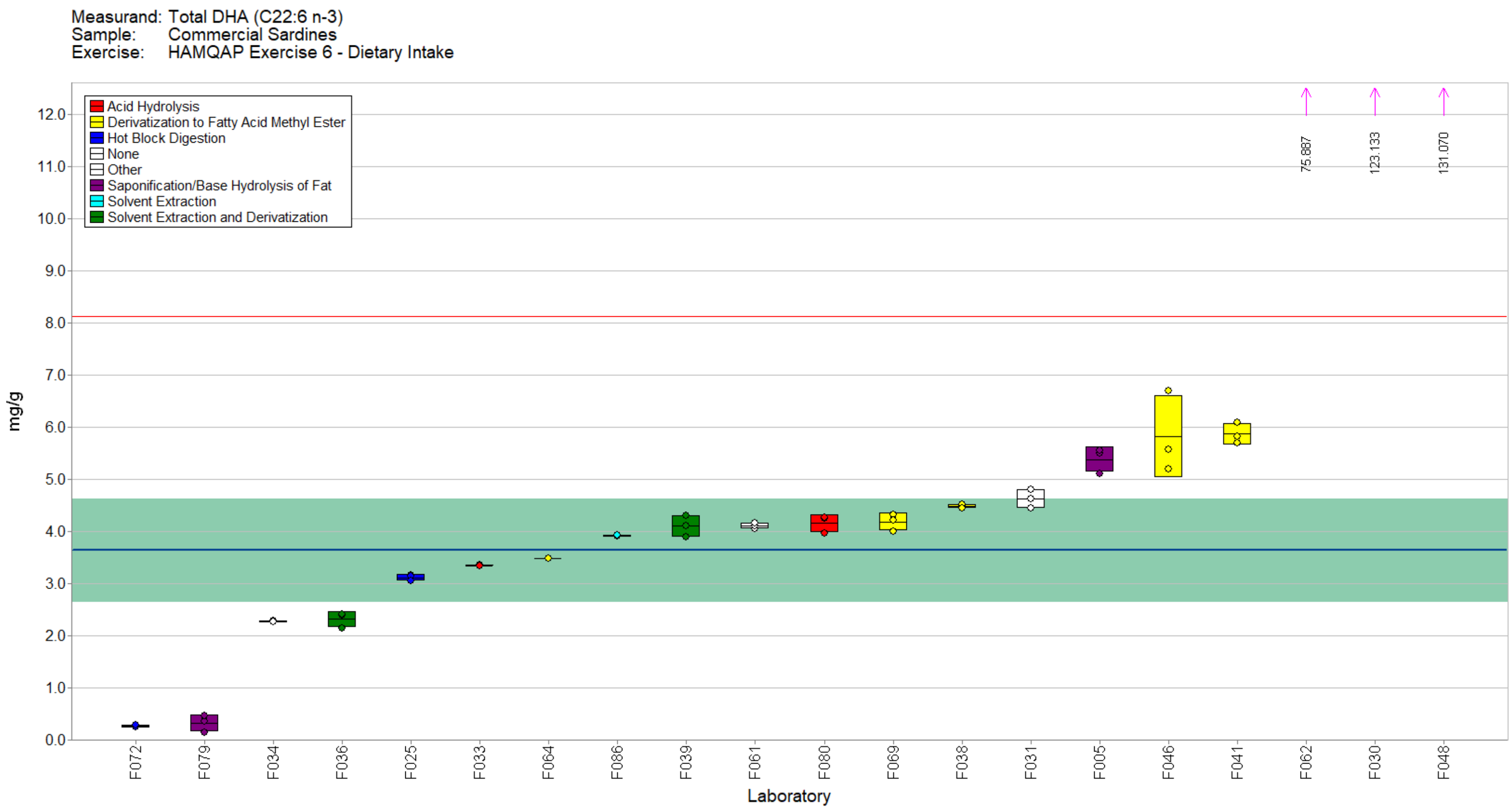


**Figure 5-23.** Total DHA in commercial sardines (data summary view – sample preparation method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The red solid line represents the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable $Z'_{\mathrm{comm}}$ score, $|Z'_{\mathrm{comm}}| \leq 2$, with the lower limit set at zero. A NIST value has not been determined in this material.

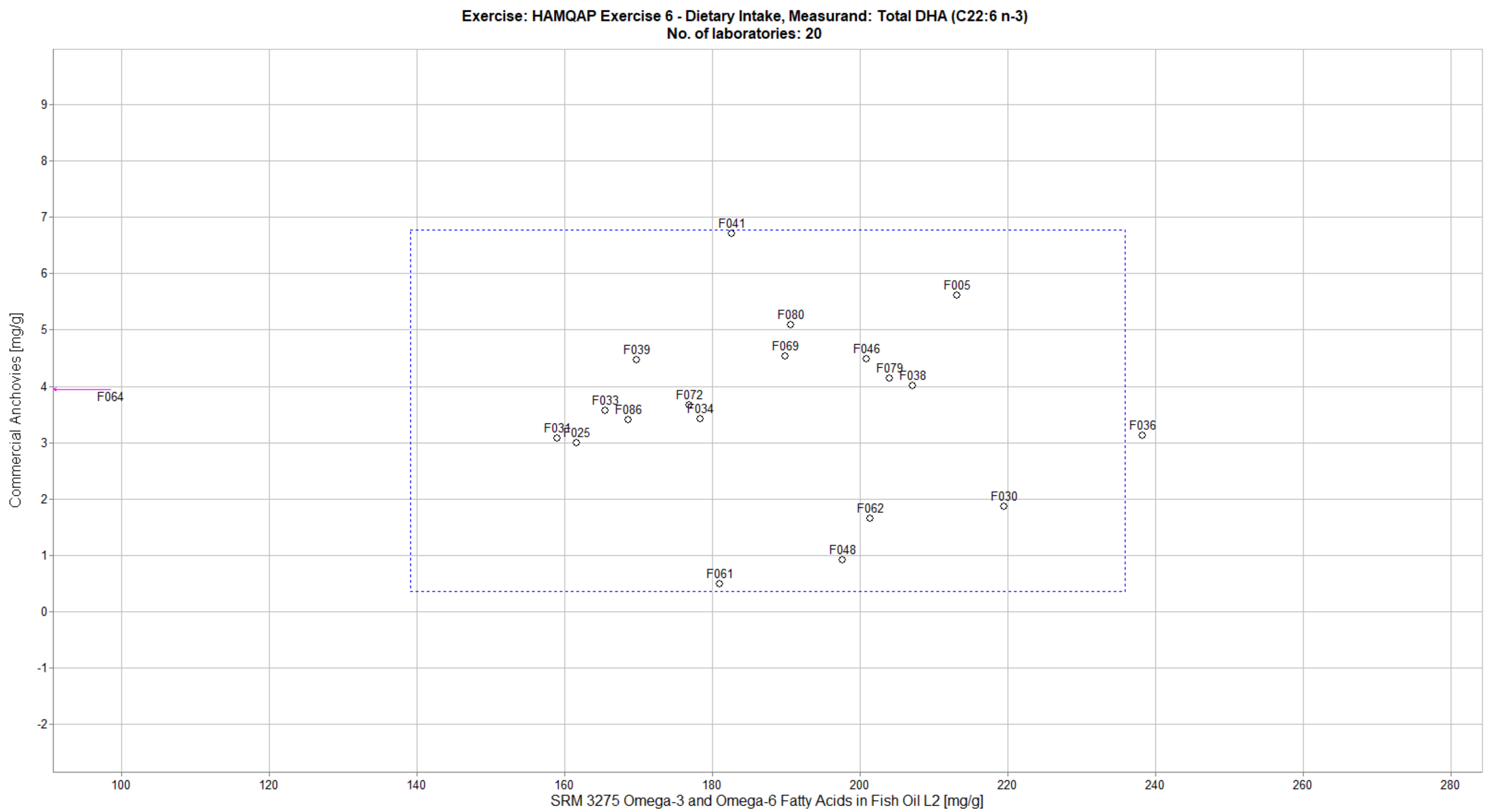


**Figure 5-24.** Laboratory means for total DHA in SRM 3275 Omega-3 and Omega-6 Fatty Acids in Fish Oil Level 2 and commercial anchovies (sample/sample comparison view). In this view, the individual laboratory mean for one sample (SRM 3275 Level 2) is compared to the individual laboratory mean for a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

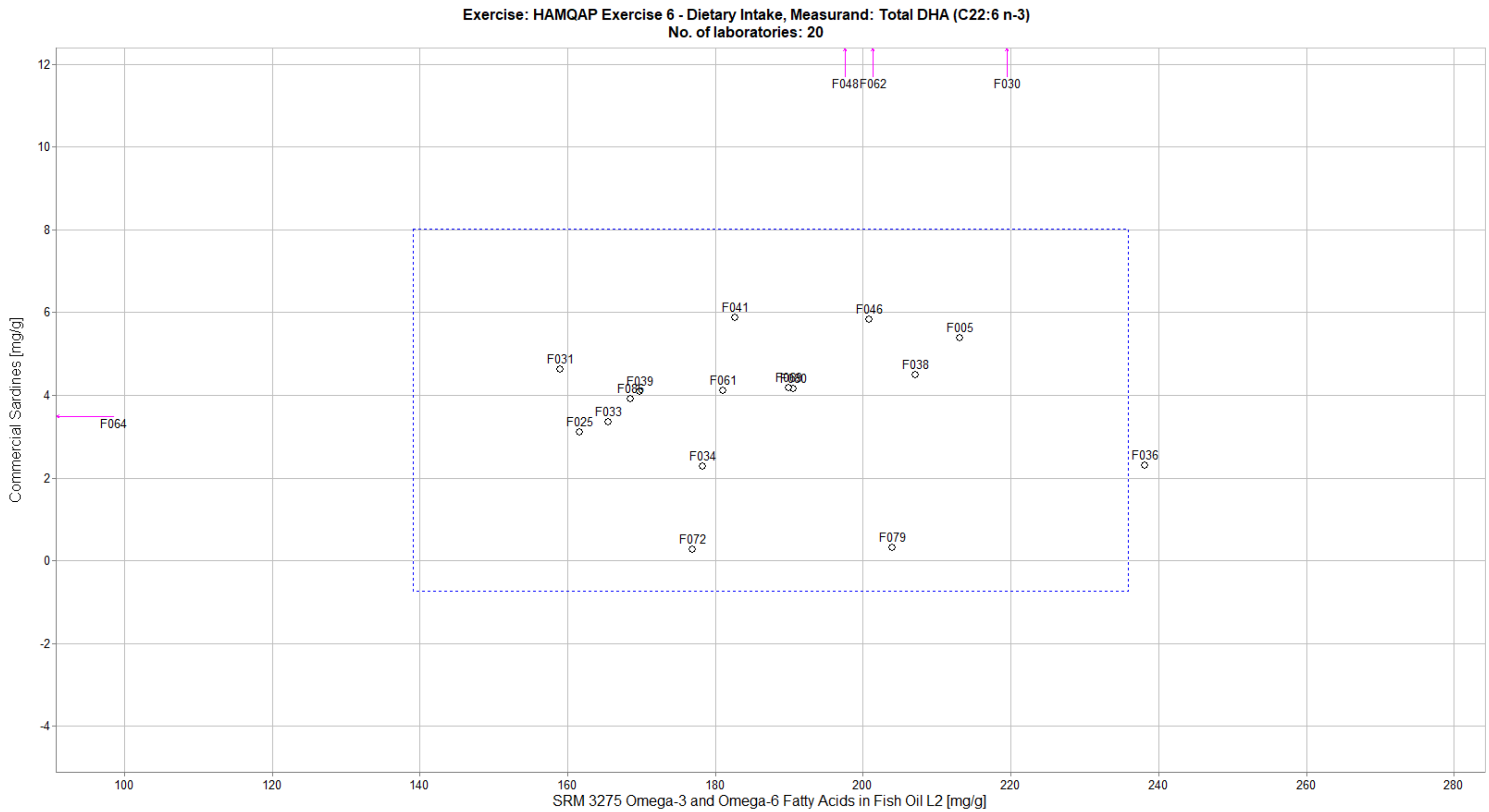


**Figure 5-25.** Laboratory means for total DHA in SRM 3275 Omega-3 and Omega-6 Fatty Acids in Fish Oil Level 2 and commercial sardines (sample/sample comparison view). In this view, the individual laboratory mean for one sample (SRM 3275 Level 2) is compared to the individual laboratory mean for a second sample (sardines). The dotted blue box represents the consensus range of tolerance for SRM 3275 Level 2 (x-axis) and sardines (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 Red Blood Cells A and B.* Participants were provided with three vials each of Human RBCs Sample A and Human RBCs Sample B, each containing 0.6 mL of frozen human red blood cells. RBC A was collected from six healthy donors and RBC B was collected from two healthy donors. Participants were asked to avoid exposing the material to direct sun or UV light, to store the material at or below –70 °C, and to prepare one sample and report one value from each vial provided. 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 their usual in-house method of analysis. Participants were also asked to report values for the individual fatty acids in units of µg/mL and for the individual fatty acids as weight percent (%) of total fatty acids. The approximate analyte levels were not reported to participants prior to the study. Target values for the weight percent each of EPA and DHA per total fatty acids were assigned using results from isotope dilution gas chromatography mass spectrometry (ID-GC-MS) analysis by the Centers for Disease Control and Prevention (CDC). The target values for EPA and DHA and their associated uncertainties are provided in the table below.

| | NIST-Determined Weight Percent (based on Total Fatty Acids) in Human RBC (%) | |
|---|---|---|
| Analyte | RBC A | RBC B |
| EPA | 0.46 ± 0.02 | 0.36 ± 0.02 |
| DHA | 2.65 ± 0.08 | 3.27 ± 0.12 |

Human Metabolites Study Results

- Eight laboratories enrolled in this exercise and received samples to measure each of the fatty acids in human red blood cells. Four laboratories reported results for EPA and DHA for both samples (50 % participation) for the individual fatty acids in units of µg/mL. Seven laboratories reported results for EPA and DHA (88 % participation) in weight percent (%) of total fatty acids.
- The consensus ranges for both fatty acids overlapped the target ranges for both materials that were reported as weight percent of total fatty acids.
  - The consensus mean for EPA was near the upper limit of the target range for RBC A (**Figure 5-27**). The consensus mean for EPA was slightly above the target range, but the consensus range overlapped the target range and was near the upper limit of the target range for RBC B (**Figure 5-29**).
  - The consensus means for DHA were near the upper limit of the target range for both samples (**Figures 5-32**, **5-34**).
- The between-laboratory variabilities for laboratories reporting values for individual fatty acids, reported in units of µg/mL, were below 35 % for sample RBC A but were higher for sample RBC B. Variabilities for each analyte/sample pair are reported in the table below.

| | Between-Laboratory Variability for laboratories reporting in units of µg/mL (% RSD) | |
|---|---|---|
| Analyte | RBC A | RBC B |
| EPA | 10 % | 70 % |
| DHA | 34 % | 42 % |

- The between-laboratory variabilities for laboratories reporting values as weight % of total fatty acids were excellent. All variabilities were below 25 %. Variabilities for each analyte/sample pair are reported in the table below.

| Analyte | Between-Laboratory Variability for laboratories reporting in units of weight % from total fatty acids (% RSD) RBC A | RBC B |
|---|---|---|
| EPA | 19 % | 24 % |
| DHA | 7.2 % | 14 % |

- Three laboratories reported using derivatization to fatty acid methyl esters as the sample preparation method. Two laboratories reported using solvent extraction. One laboratory reported using hot block digestion and one laboratory reported using base hydrolysis as the sample preparation method.
- Four laboratories reported GC-FID as their analytical method for determination of the fatty acids in these samples and three laboratories reported using GC-MS.

Human Metabolites Technical Recommendations

The following recommendations are based on results obtained from the participants in this study. For both samples, too few data were reported to allow for meaningful conclusions to be drawn.

- Sufficient data points were not available to identify trends with respect to the sample preparation or analytical methods reported by the participants. However, the laboratory that tended to report higher values compared to other laboratories reported using base hydrolysis as the sample preparation method (See **Figures 5-26** through **Figure 5-29** and **Figures 5-31** through **Figures 5-34**, laboratory data points beyond chart limits). Sample preparation methods should be checked for method biases.
- Overall, the results of all of the participants agreed well for results reported as individual fatty acids in units of µg/mL and reported as weight percent of total fatty acids with the exception of one or two laboratories reporting higher results compared to the other laboratories. These higher results could be due to a bias in calibration or a bias in the method.
- 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.
- A linear calibration curve which surrounds 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 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 and in the requested form.

**Table 5-7.** Individualized data summary table (NIST) for fatty acids in Red Blood Cells A and Red Blood Cells B.

*National Institute of Standards and Technology*

HAMQAP Exercise 6 - Fatty Acids

| | Lab Code: | NIST | 1. Your Results | | | | 2. Community Results | | | 3. Target | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Analyte | Sample | Units | $x_i$ | $s_i$ | $Z'_{comm}$ | $Z_{NIST}$ | N | x* | s* | $x_{NIST}$ | $U$ |
| Total EPA (C20:5 n-3) | Red Blood Cells B | ug/mL | | | | | 4 | 10 | 6 | | |
| Total EPA (C20:5 n-3) | Red Blood Cells A | ug/mL | | | | | 4 | 5.89 | 0.61 | | |
| Total EPA (C20:5 n-3) Weight % from total FAs | Red Blood Cells B | % | 0.36 | 0.02 | | | 7 | 0.41 | 0.095 | 0.36 | 0.02 |
| Total EPA (C20:5 n-3) Weight % from total FAs | Red Blood Cells A | % | 0.46 | 0.02 | | | 7 | 0.481 | 0.085 | 0.46 | 0.02 |
| Total DHA (C22:6 n-3) | Red Blood Cells A | ug/mL | | | | | 4 | 30 | 12 | | |
| Total DHA (C22:6 n-3) | Red Blood Cells B | ug/mL | | | | | 4 | 40 | 19 | | |
| Total DHA (C22:6 n-3) Weight % from total FAs | Red Blood Cells B | % | 3.27 | 0.12 | | | 7 | 3.4 | 0.46 | 3.27 | 0.12 |
| Total DHA (C22:6 n-3) Weight % from total FAs | Red Blood Cells A | % | 2.65 | 0.08 | | | 7 | 2.8 | 0.2 | 2.65 | 0.08 |

$x_i$ Mean of reported values
$s_i$ Standard deviation of reported values
$Z'_{comm}$ Z'-score with respect to community consensus
$Z_{NIST}$ Z-score with respect to NIST value

N Number of quantitative values reported
x* Robust mean of reported values
s* Robust standard deviation

$x_{NIST}$ NIST-assessed value
$U$ expanded uncertainty about the NIST-assessed value

**Table 5-8.** Data summary tables for total EPA in human red blood cells reported in µg/g. Data points highlighted in blue have been identified as outside the consensus tolerance limits and would be estimated to result in an unacceptable $Z'_{\text{comm}}$ score, $|Z'_{\text{comm}}| \geq 2$.

| | | Total EPA (C20:5 n-3) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Red Blood Cells A (ug/mL) | | | | | Red Blood Cells B (ug/mL) | | | | |
| | Lab | A | B | C | Avg | SD | A | B | C | Avg | SD |
| Individual Results | Target | | | | | | | | | | |
| | F064 | | | | | | | | | | |
| | F072 | 6.14 | 5.81 | 5.66 | 5.87 | 0.25 | 4.22 | 4.41 | 4.88 | 4.50 | 0.34 |
| | F081 | | | | | | | | | | |
| | F086 | 6.078 | 5.873 | 5.943 | 5.96 | 0.10 | 4.69 | 4.705 | 4.723 | 4.71 | 0.02 |
| | F091 | 24.095 | 25.53 | 25.56 | 25.06 | 0.84 | 17.065 | 17.97 | 16.935 | 17.32 | 0.56 |
| | F094 | | | | | | | | | | |
| | F097 | 5.746 | 6.351 | 5.444 | 5.85 | 0.46 | 7.864 | 7.561 | 7.259 | 7.56 | 0.30 |
| | F098 | | | | | | | | | | |
| Community Results | | Consensus Mean | | | 5.89 | | Consensus Mean | | | 8.52 | |
| | | Consensus Standard Deviation | | | 0.61 | | Consensus Standard Deviation | | | 5.95 | |
| | | Maximum | | | 25.06 | | Maximum | | | 17.32 | |
| | | Minimum | | | 5.85 | | Minimum | | | 4.50 | |
| | | N | | | 4 | | N | | | 4 | |

**Table 5-9.** Data summary tables for total EPA in human red blood cells reported in weight % of total fatty acids. Data points highlighted in blue have been identified as outside the consensus tolerance limits and would be estimated to result in an unacceptable $Z'_{\text{comm}}$ score, $|Z'_{\text{comm}}| \geq 2$.

| | | Total EPA (C20:5 n-3) Weight % from total FAs | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Red Blood Cells A (%) | | | | | Red Blood Cells B (%) | | | | |
| | Lab | A | B | C | Avg | SD | A | B | C | Avg | SD |
| Individual Results | Target | | | | 0.46 | 0.02 | | | | 0.36 | 0.02 |
| | F064 | 1.02 | 0.94 | 1.07 | 1.01 | 0.07 | 0.85 | 0.81 | 0.77 | 0.81 | 0.04 |
| | F072 | 0.49 | 0.5 | 0.52 | 0.50 | 0.02 | 0.37 | 0.41 | 0.37 | 0.38 | 0.02 |
| | F081 | | | | | | | | | | |
| | F086 | 0.483 | 0.481 | 0.483 | 0.48 | 0.00 | 0.383 | 0.387 | 0.387 | 0.39 | 0.00 |
| | F091 | 1.15 | 1.25 | 1.21 | 1.20 | 0.05 | 0.87 | 0.93 | 0.89 | 0.90 | 0.03 |
| | F094 | 0.52 | 0.52 | 0.52 | 0.52 | 0.00 | 0.43 | 0.43 | 0.43 | 0.43 | 0.00 |
| | F097 | 0.44 | 0.39 | 0.43 | 0.42 | 0.03 | 0.469 | 0.461 | 0.424 | 0.45 | 0.02 |
| | F098 | 0.461 | 0.498 | 0.479 | 0.48 | 0.02 | 0.376 | 0.367 | 0.381 | 0.37 | 0.01 |
| Community Results | | Consensus Mean | | | 0.48 | | Consensus Mean | | | 0.41 | |
| | | Consensus Standard Deviation | | | 0.09 | | Consensus Standard Deviation | | | 0.10 | |
| | | Maximum | | | 1.20 | | Maximum | | | 0.90 | |
| | | Minimum | | | 0.42 | | Minimum | | | 0.37 | |
| | | N | | | 7 | | N | | | 7 | |

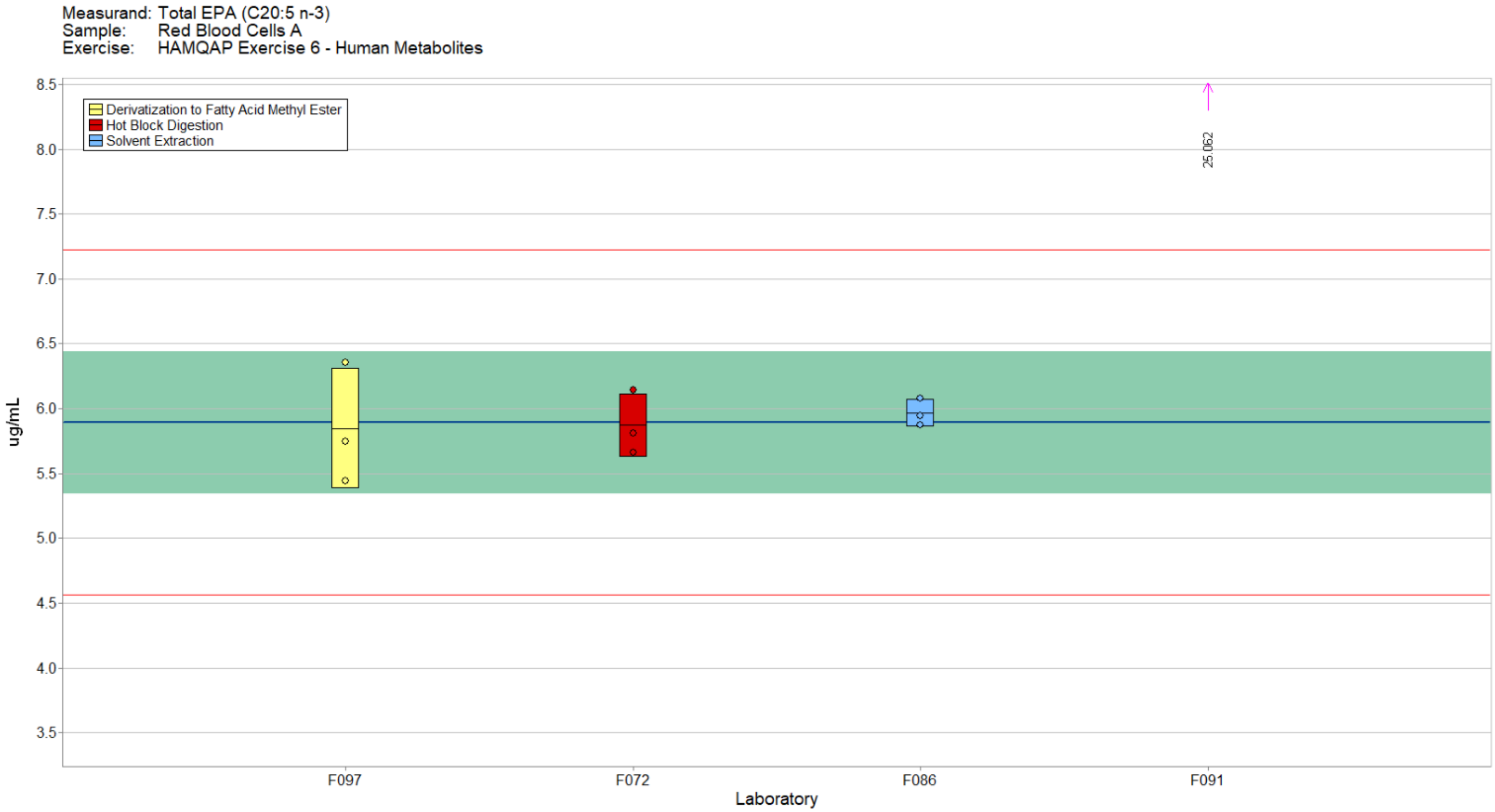


**Figure 5-26.** Total EPA in Human Red Blood Cells A reported in units of µg/mL (data summary view – sample preparation method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The solid blue 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 $Z'_{\text{comm}}$ score, $|Z'_{\text{comm}}| \leq 2$. A NIST value reported in units of µg/mL has not been established for this material.

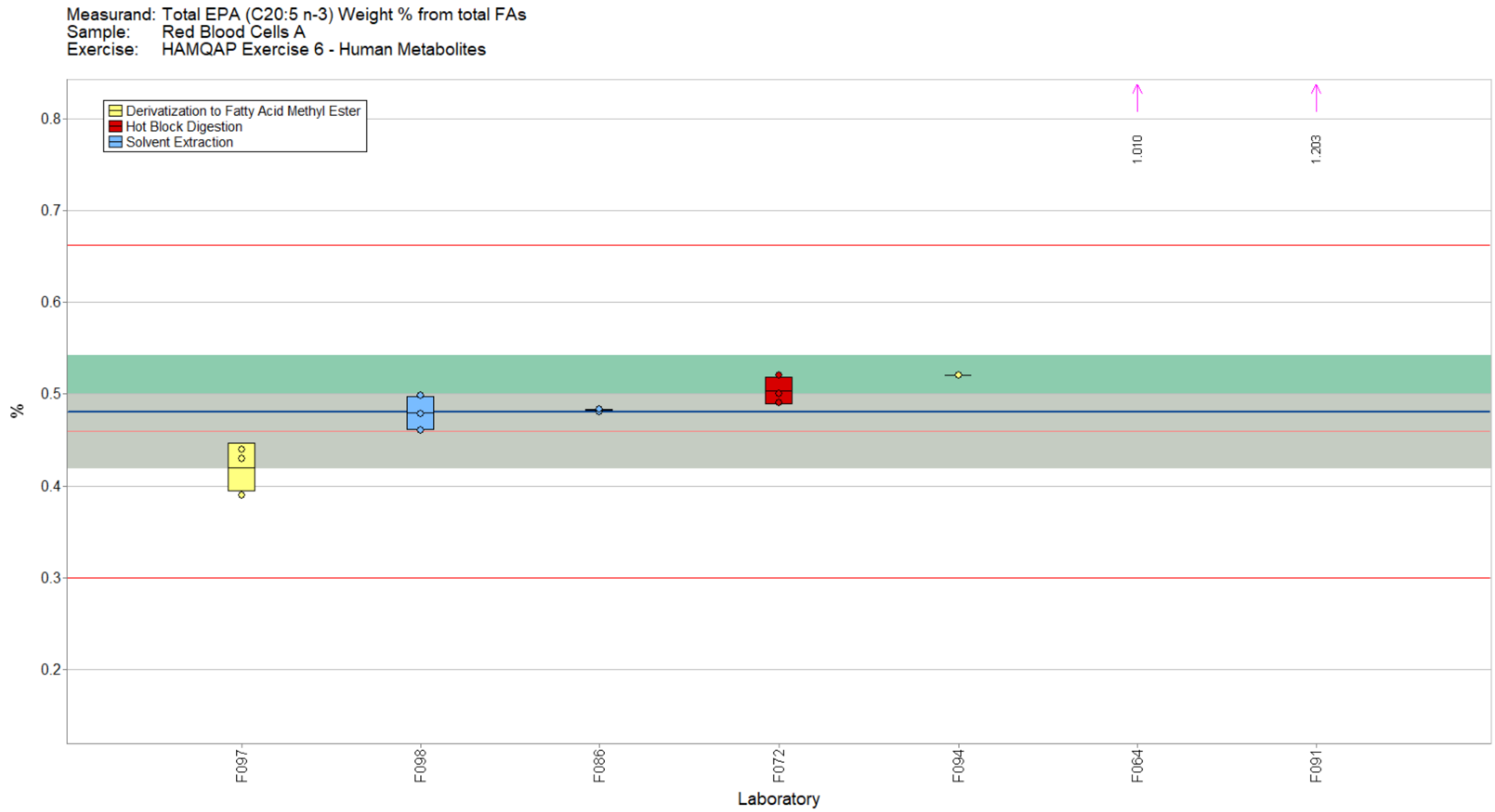


**Figure 5-27.** Total EPA in Human Red Blood Cells A reported as weight % from total fatty acids (data summary view – sample preparation method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The solid blue 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 $Z'_{\text{comm}}$ score, $|Z'_{\text{comm}}| \leq 2$. 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, $|Z_{\text{NIST}}| \leq 2$.

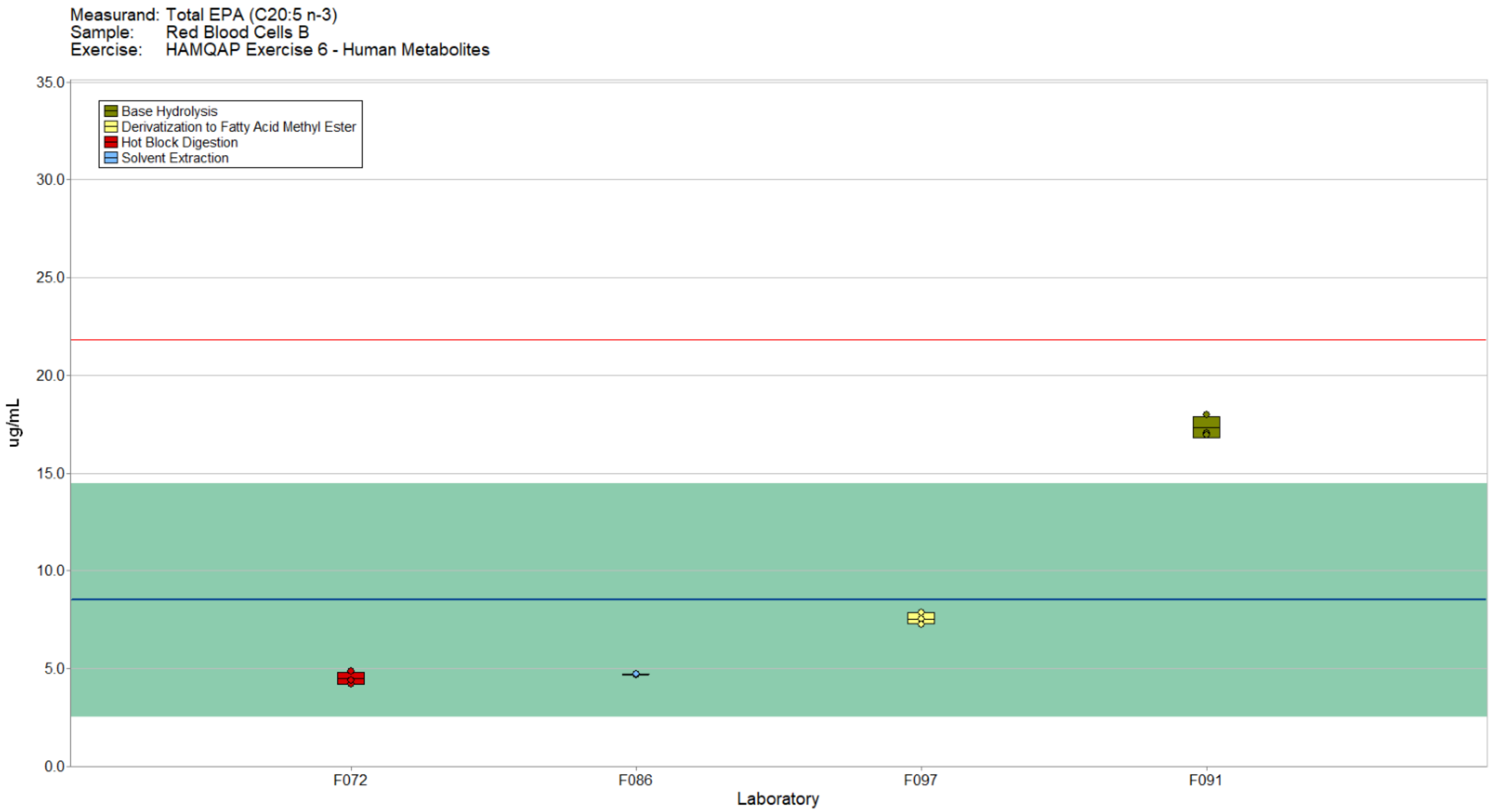


**Figure 5-28.** Total EPA in Human Red Blood Cells B reported in units of µg/mL (data summary view – sample preparation method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The red solid line represents the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable $Z'_{\mathrm{comm}}$ score, $|Z'_{\mathrm{comm}}| \leq 2$, with the lower limit set to zero. A NIST value reported in units of µg/mL has not been established for this material.

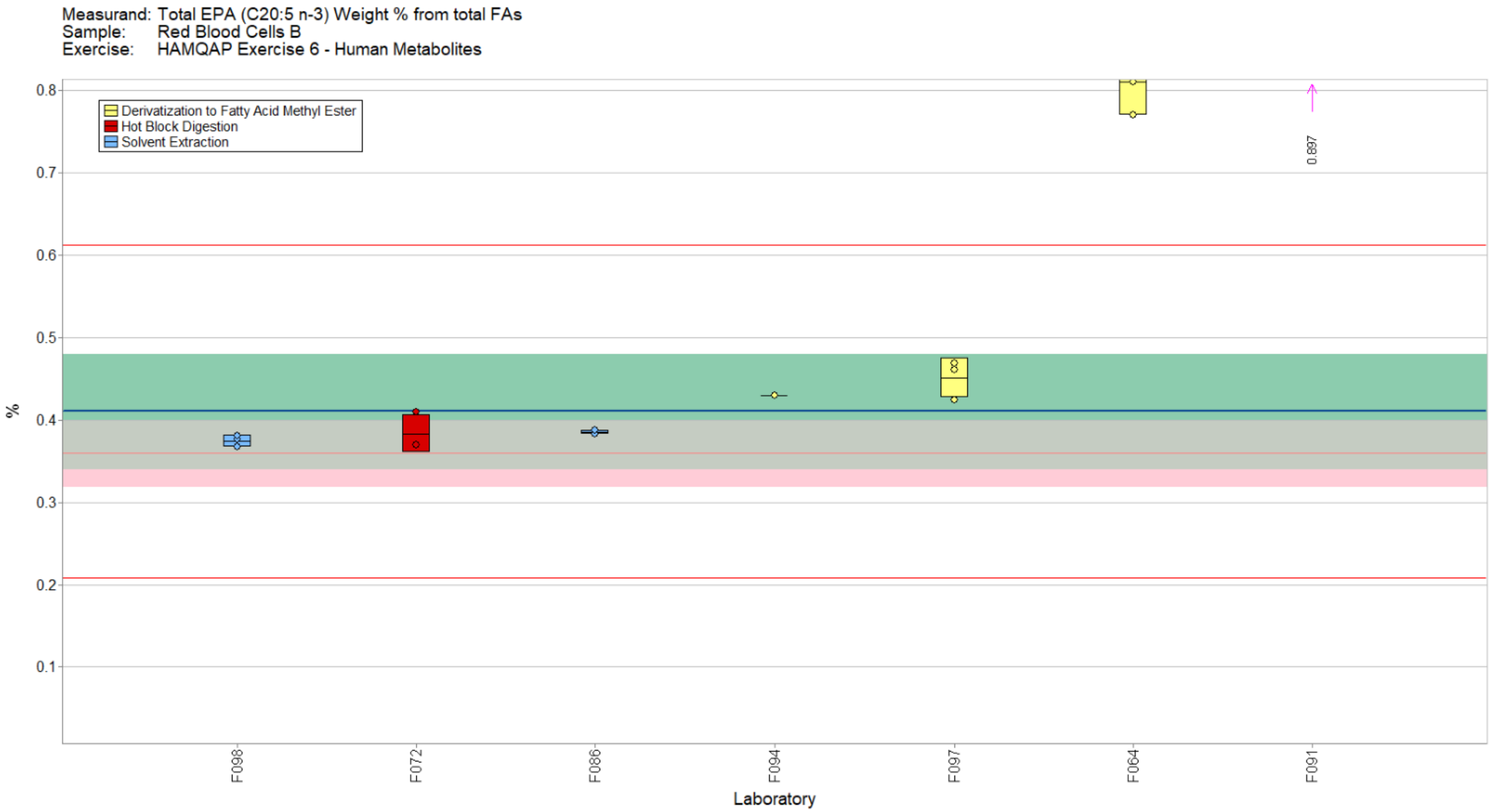


**Figure 5-29.** Total EPA in Human Red Blood Cells B reported as weight % from total fatty acids (data summary view – sample preparation method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The solid blue 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 $Z'_{\mathrm{comm}}$ score, $|Z'_{\mathrm{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by its uncertainty ($U_{\mathrm{NIST}}$) and represents the range that results in an acceptable $Z_{\mathrm{NIST}}$ score, $|Z_{\mathrm{NIST}}| \leq 2$.

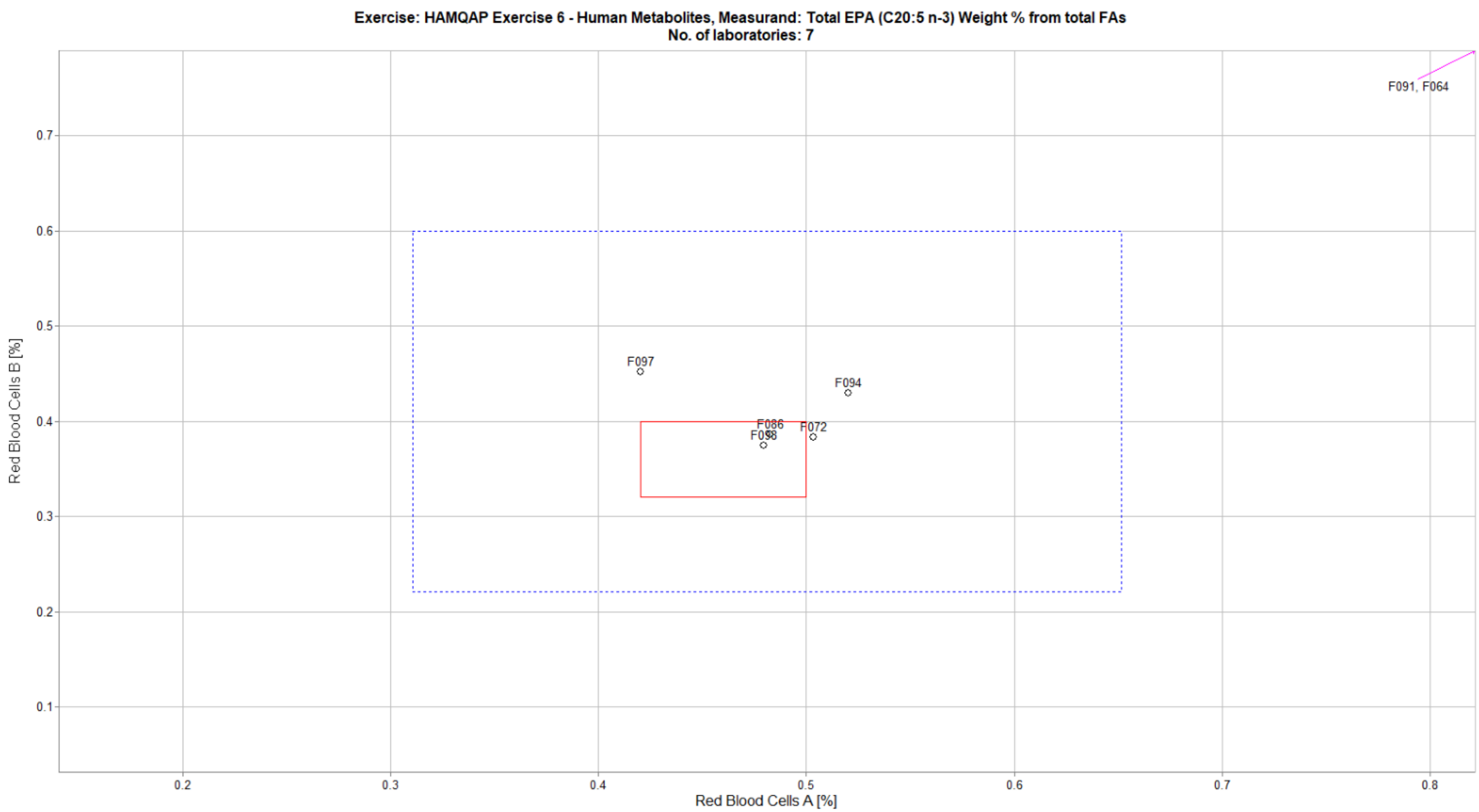


**Figure 5-30.** Laboratory means for total EPA in Human Red Blood Cells A and Human Red Blood Cells B (sample/sample comparison view) reported as weight % from total fatty acids. In this view, the individual laboratory mean for one sample (RBC A) is compared to the individual laboratory mean for a second sample (RBC B). The solid red box represents the NIST range of tolerance for the two samples, RBC A (x-axis) and RBC B (y-axis), which encompasses the target values bounded by their uncertainties ($U_{\mathrm{NIST}}$) and represents the range that results in an acceptable $Z_{\mathrm{NIST}}$ score, $|Z_{\mathrm{NIST}}| \leq 2$. The dotted blue box represents the consensus range of tolerance for RBC A (x-axis) and RBC B (y-axis), calculated as the values above and below the consensus means that result in an acceptable $Z'_{\mathrm{comm}}$ score, $|Z'_{\mathrm{comm}}| \leq 2$.

**Table 5-10.** Data summary tables for total DHA in human red blood cells reported in units of µg/mL. Data points highlighted in blue have been identified as outside the consensus tolerance limits and would be estimated to result in an unacceptable $Z'_{\mathrm{comm}}$ score, $|Z'_{\mathrm{comm}}| \geq 2$.

| | | Total DHA (C22:6 n-3) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Red Blood Cells A (ug/mL) | | | | | Red Blood Cells B (ug/mL) | | | | |
| | Lab | A | B | C | Avg | SD | A | B | C | Avg | SD |
| Individual Results | Target | | | | | | | | | | |
| | F064 | | | | | | | | | | |
| | F072 | 30.03 | 30.48 | 29.83 | 30.11 | 0.33 | 36.1 | 36.49 | 38.54 | 37.04 | 1.31 |
| | F081 | | | | | | | | | | |
| | F086 | 35.868 | 35.078 | 35.29 | 35.41 | 0.41 | 44.915 | 44.148 | 44.308 | 44.46 | 0.40 |
| | F091 | 175.6 | 183.105 | 181.67 | 180.13 | 3.98 | 180.32 | 246.645 | 199.295 | 208.75 | 34.16 |
| | F094 | | | | | | | | | | |
| | F097 | 37.78 | 37.78 | 37.12 | 37.56 | 0.38 | 51.9 | 50.92 | 55.19 | 52.67 | 2.24 |
| | F098 | | | | | | | | | | |
| Community Results | | Consensus Mean | | | 34.36 | | Consensus Mean | | | 44.72 | |
| | | Consensus Standard Deviation | | | 11.67 | | Consensus Standard Deviation | | | 18.70 | |
| | | Maximum | | | 180.13 | | Maximum | | | 208.75 | |
| | | Minimum | | | 30.11 | | Minimum | | | 37.04 | |
| | | N | | | 4 | | N | | | 4 | |

**Table 5-11.** Data summary tables for total DHA in human red blood cells reported in weight % of total fatty acids. Data points highlighted in blue have been identified as outside the consensus tolerance limits and would be estimated to result in an unacceptable $Z'_{\mathrm{comm}}$ score, $|Z'_{\mathrm{comm}}| \geq 2$.

| | | Total DHA (C22:6 n-3) Weight % from total FAs | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Red Blood Cells A (%) | | | | | Red Blood Cells B (%) | | | | |
| | Lab | A | B | C | Avg | SD | A | B | C | Avg | SD |
| Individual Results | Target | | | | 2.65 | 0.08 | | | | 3.27 | 0.12 |
| | F064 | 2.95 | 2.76 | 2.76 | 2.82 | 0.11 | 3.31 | 3.2 | 3.35 | 3.29 | 0.08 |
| | F072 | 2.65 | 2.68 | 2.69 | 2.67 | 0.02 | 3.4 | 3.41 | 3.42 | 3.41 | 0.01 |
| | F081 | | | | | | | | | | |
| | F086 | 2.85 | 2.874 | 2.867 | 2.86 | 0.01 | 3.668 | 3.627 | 3.628 | 3.64 | 0.02 |
| | F091 | 8.29 | 8.47 | 8.45 | 8.40 | 0.10 | 9.69 | 10.2 | 9.85 | 9.91 | 0.26 |
| | F094 | 2.81 | 2.81 | 2.81 | 2.81 | 0.00 | 3.62 | 3.62 | 3.62 | 3.62 | 0.00 |
| | F097 | 2.67 | 2.16 | 2.7 | 2.51 | 0.30 | 2.85 | 2.75 | 2.97 | 2.86 | 0.11 |
| | F098 | 2.91 | 2.9 | 2.8 | 2.87 | 0.06 | 3.66 | 3.52 | 3.59 | 3.59 | 0.07 |
| Community Results | | Consensus Mean | | | 2.76 | | Consensus Mean | | | 3.40 | |
| | | Consensus Standard Deviation | | | 0.20 | | Consensus Standard Deviation | | | 0.46 | |
| | | Maximum | | | 8.40 | | Maximum | | | 9.91 | |
| | | Minimum | | | 2.51 | | Minimum | | | 2.86 | |
| | | N | | | 7 | | N | | | 7 | |

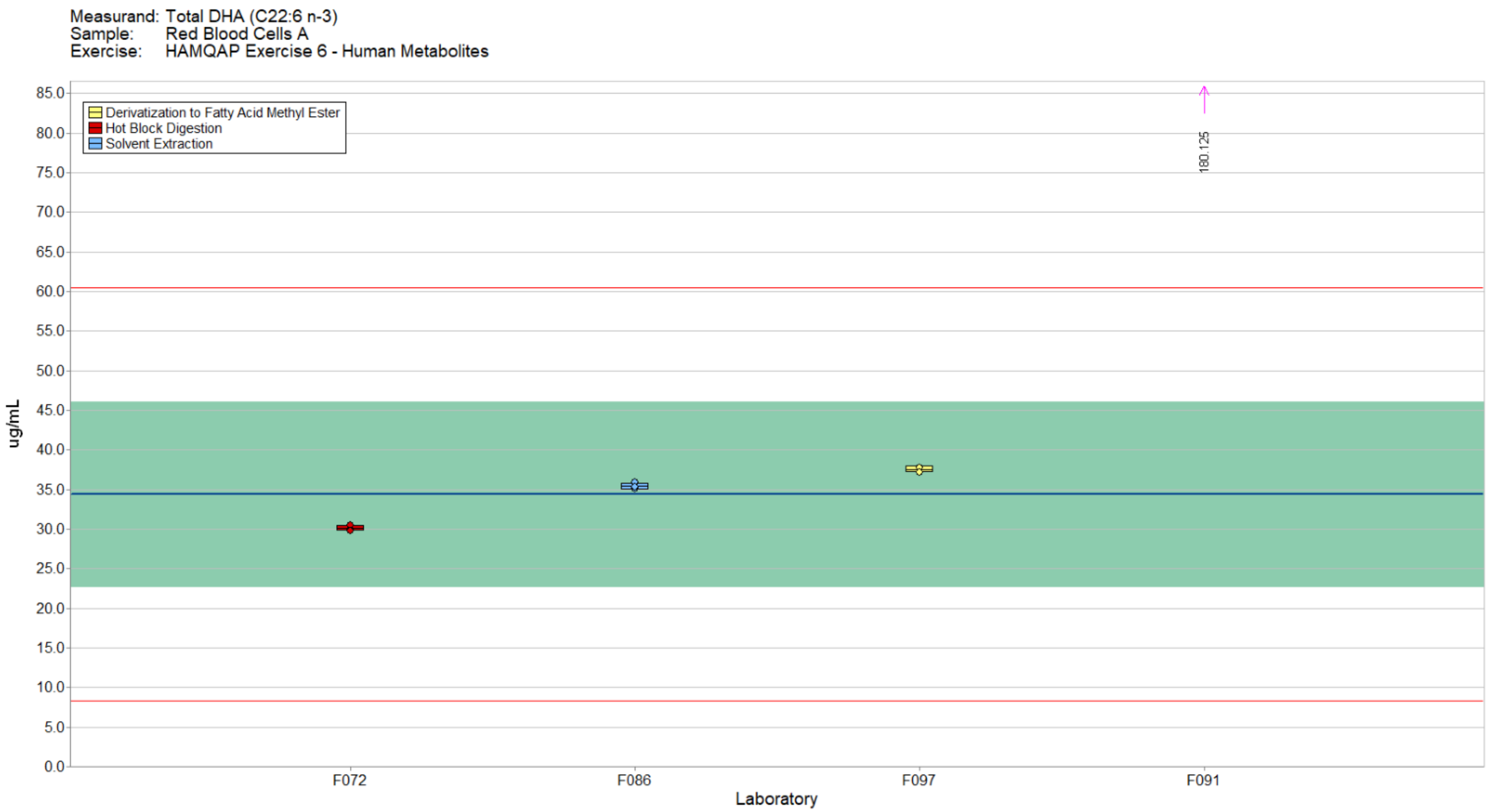


**Figure 5-31.** Total DHA in Human Red Blood Cells A reported in units of µg/mL (data summary view – sample preparation method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The solid blue 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 the consensus mean that result in an acceptable $Z'_{\text{comm}}$ score, $|Z'_{\text{comm}}| \leq 2$. A NIST value reported in units of µg/mL has not been established for this material.

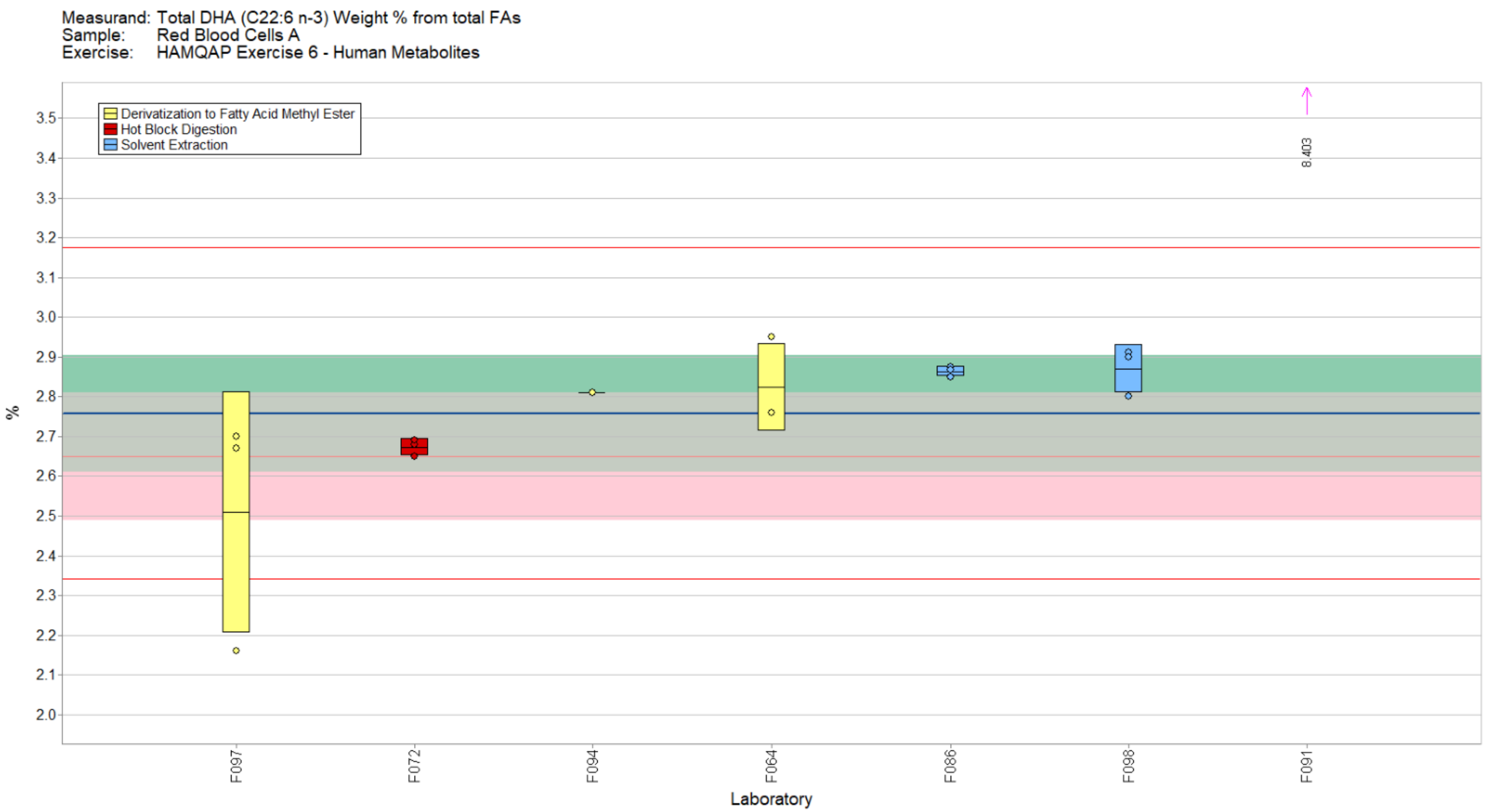


**Figure 5-32.** Total DHA in Human Red Blood Cells A reported as weight % from total fatty acids (data summary view – sample preparation method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The solid blue 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 $Z'_{\text{comm}}$ score, $|Z'_{\text{comm}}| \leq 2$. 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, $|Z_{\text{NIST}}| \leq 2$.

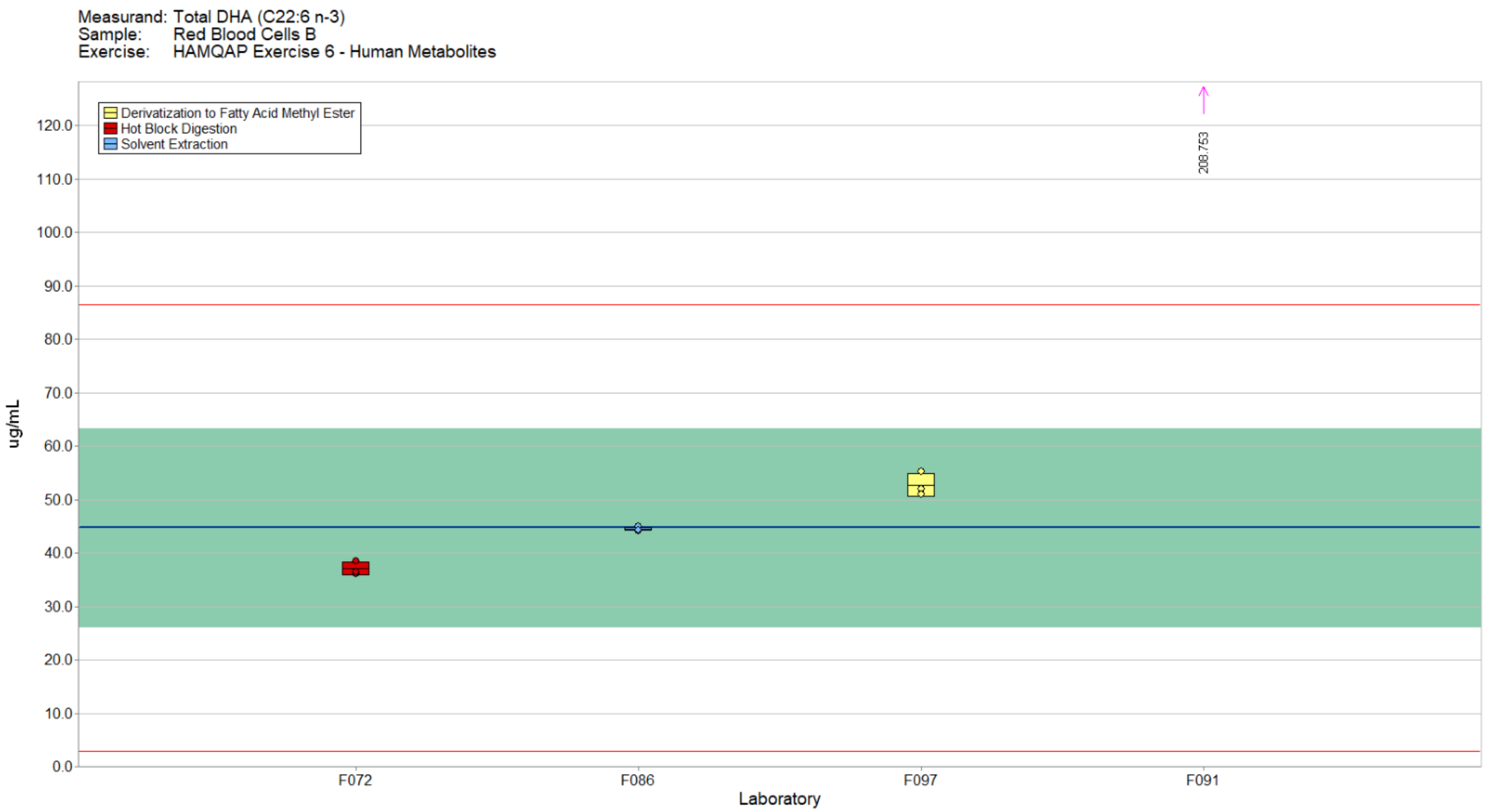


**Figure 5-33.** Total DHA in Human Red Blood Cells B reported in units of µg/mL (data summary view – sample preparation method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The solid blue 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 the consensus mean that result in an acceptable $Z'_{\text{comm}}$ score, $|Z'_{\text{comm}}| \leq 2$. A NIST value reported in units of µg/mL has not been established for this material.

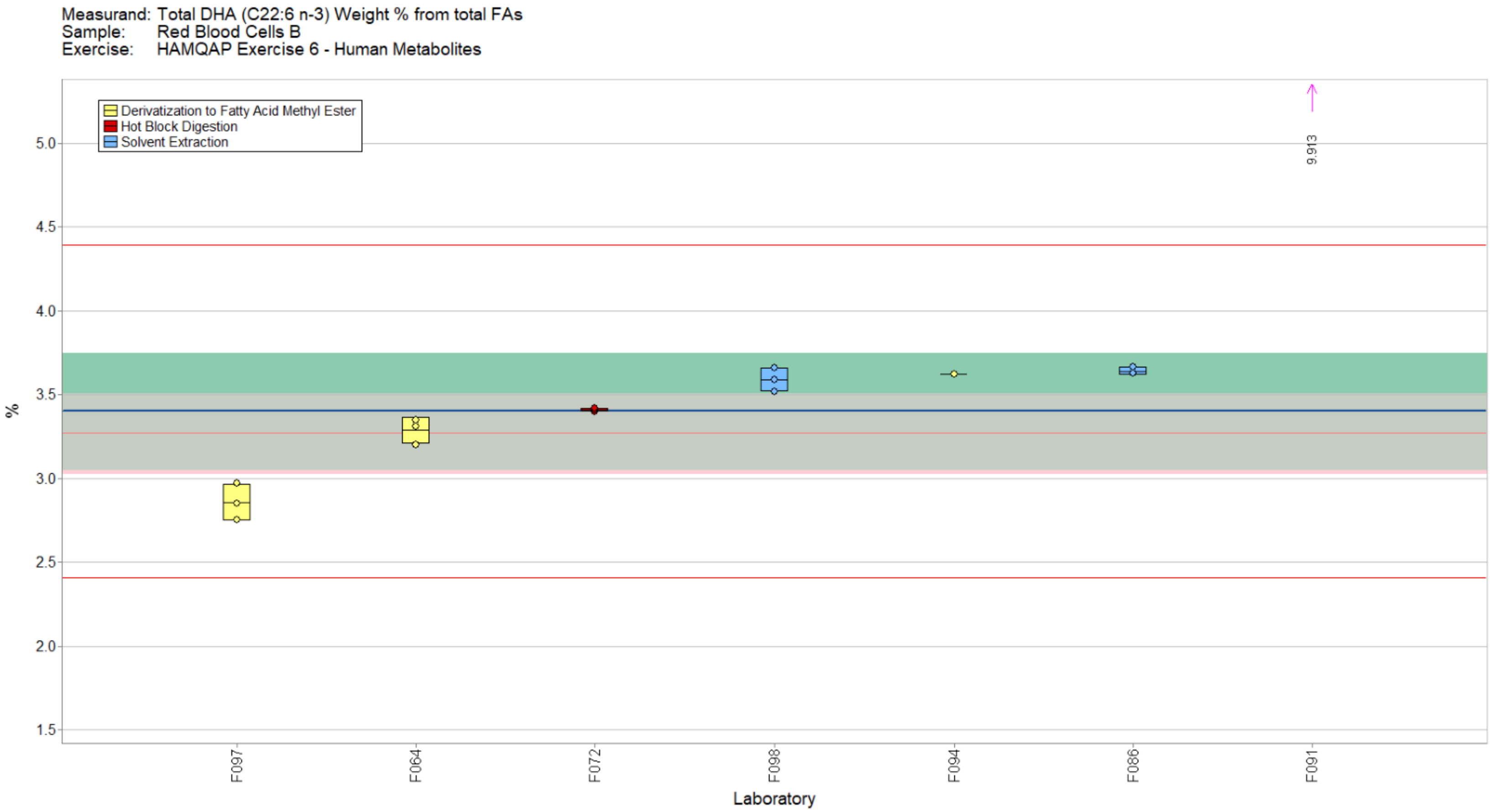


**Figure 5-34.** Total DHA in Human Red Blood Cells B reported as weight % from total fatty acids (data summary view – sample preparation method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The solid blue 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 $Z'_{\text{comm}}$ score, $|Z'_{\text{comm}}| \leq 2$. 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, $|Z_{\text{NIST}}| \leq 2$.

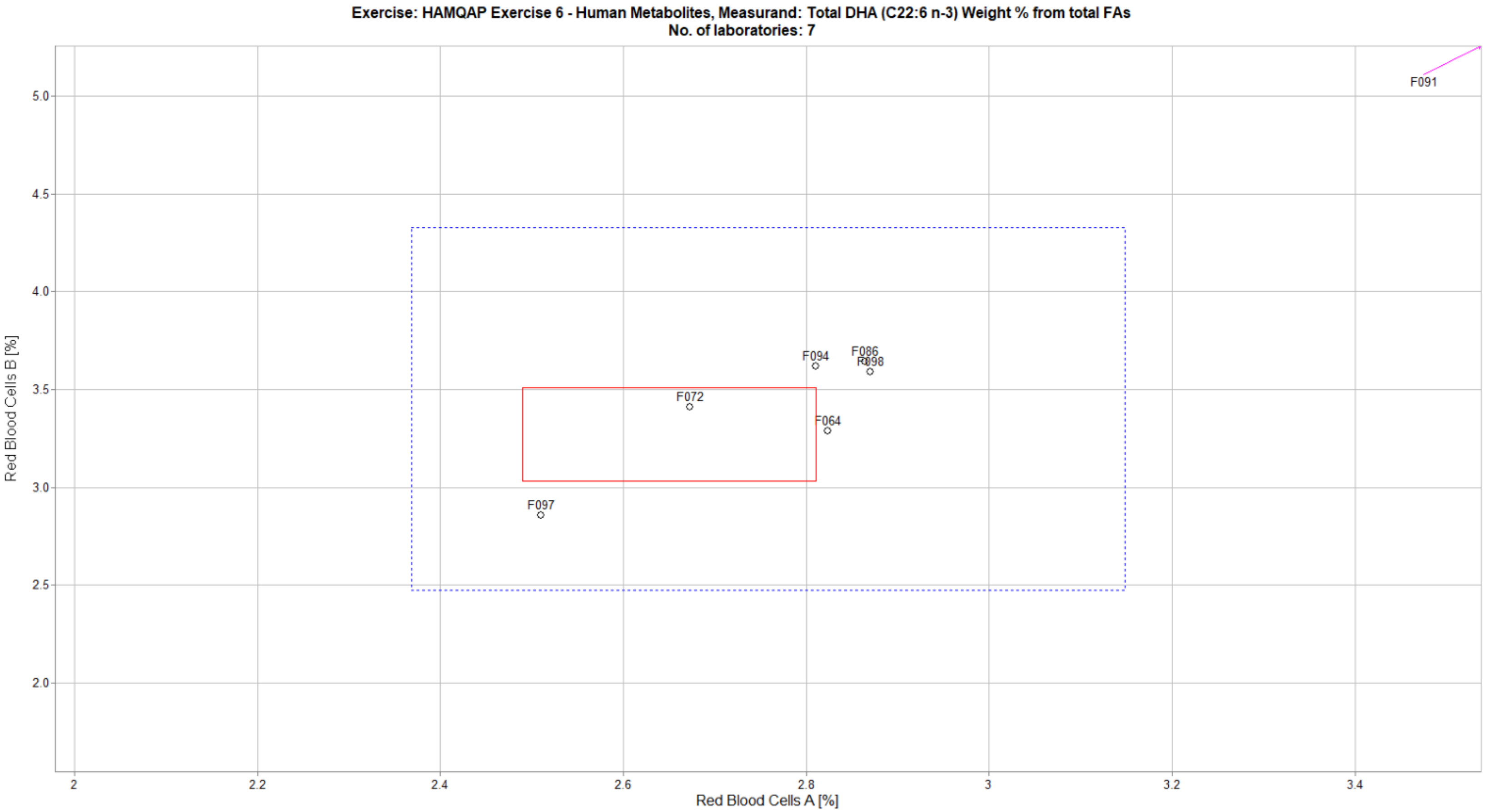


**Figure 5-35.** Laboratory means for total DHA in Human Red Blood Cells A and Human Red Blood Cells B reported as weight % from total fatty acids (sample/sample comparison view). In this view, the individual laboratory mean for one sample (RBC A) is compared to the individual laboratory mean for a second sample (RBC B). The solid red box represents the NIST range of tolerance for the two samples, RBC A (x-axis) and RBC 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 RBC A (x-axis) and RBC B (y-axis), calculated as the values above and below the consensus means that result in an acceptable $Z'_{\text{comm}}$ score, $|Z'_{\text{comm}}| \leq 2$.

Fatty Acids Overall Study Comparison

Overall, laboratories measuring fatty acids in fish oils and human red blood cells were successful based on the limited results reported.

- Between-laboratory variability were high for fatty acids that were present at low levels in the dietary intake samples.
- The different matrices in the dietary intake samples (fish oil, anchovies, and sardines) may pose different analytical challenges.
- Clinical laboratories had lower participation, but those laboratories reporting results were in good agreement. The limited number of participating laboratories could indicate the measurement is challenging or limited interest exists for a QAP in the clinical community.

## SECTION 6: BOTANICALS (Anthocyanidins)

Study Overview

In this study, participants were provided with samples of SRM 3287 Blueberry (Fruit), SRM 3281 Cranberry (Fruit), and SRM 3291 Bilberry Extract. Participants were asked to use in-house analytical methods to determine the mass fraction (mg/kg) of select anthocyanidins, either as the sum of all measured anthocyanidins calibrated to cyanidin-3-glucoside (C3G), or as individual forms (e.g., cyanidin, delphinidin) in each matrix. Anthocyanidins are a class of flavonoids that are commonly found in foods such as cranberry, blueberry, and bilberry. Anthocyanidins have strong antioxidant properties in *in-vitro* investigations and are often marketed for these effects in foods and dietary supplements. Researchers are still investigating whether they have beneficial *in vivo* effects on human health. Accurate determination of these compounds in foods or supplements is critical to ensure validity of any future health claims based on result of clinical studies. This study will allow laboratories to evaluate their individual measurement capabilities, while also providing suitability assessment of currently available reference materials.

Dietary Intake Sample Information

*Blueberry.* Participants were provided with three packets of SRM 3287 Blueberry (Fruit), each containing 5 g of material. Participants were asked to store the material at controlled room temperature (20 °C to 25 °C) and to prepare one sample and report one value from each packet provided. Before use, participants were instructed to mix the contents of the packet thoroughly, allow contents to settle for one minute prior to opening to minimize the loss of fine particles, and to use a sample size of at least 1 g. The approximate analyte levels were not reported to participants prior to the study. The reference values for cyanidin, delphinidin, malvidin, petunidin, and peonidin in SRM 3287 were assigned using results from NIST by LC-Abs. The values and uncertainties are provided in the table below, both on a dry-mass basis, as shown on the COA, and on an as-received basis accounting for moisture of the material (1.4 %).

| | NIST-Determined Mass Fraction in SRM 3287 (mg/kg) | |
|---|---|---|
| Analyte | (dry-mass basis) | (as-received basis) |
| Cyanidin | 294 ± 24 | 290 ± 24 |
| Delphinidin | 1180 ± 230 | 1163 ± 227 |
| Malvidin | 1390 ± 280 | 1370 ± 276 |
| Petunidin | 650 ± 120 | 641 ± 118 |
| Peonidin | 286 ± 51 | 282 ± 50 |

*Cranberry.* Participants were provided with three packets of SRM 3281 Cranberry (Fruit), each containing 6 g of material. Participants were asked to store the material at controlled room temperature (20 °C to 25 °C) and to prepare one sample and report one value from each packet provided. Before use, participants were instructed to mix the contents of the packet thoroughly, allow contents to settle for one minute prior to opening to minimize the loss of fine particles, and to use a sample size of at least 1 g. The approximate analyte levels were not reported to participants prior to the study. The reference values for cyanidin, delphinidin, and peonidin in SRM 3281 were assigned using results from NIST by LC-Abs. The values and uncertainties are provided in the table below, both on a dry-mass basis, as shown on the COA, and on an as-received basis accounting for moisture of the material (2.4 %). NIST values were not assigned for malvidin and petunidin.

| Analyte | NIST-Determined Mass Fraction in SRM 3281 (mg/kg) (dry-mass basis) | NIST-Determined Mass Fraction in SRM 3281 (mg/kg) (as-received basis) |
|---|---|---|
| Cyanidin | 119 ± 31 | 116 ± 30 |
| Delphinidin | 5.18 ± 0.68 | 5.06 ± 0.66 |
| Peonidin | 121 ± 22 | 118 ± 21 |

*Bilberry.* Participants were provided with three packets of SRM 3291 Bilberry Extract, each containing 5 g of material. Participants were asked to store the material at controlled room temperature (20 °C to 25 °C) and to prepare one sample and to report one value from each packet provided. Before use, participants were instructed to mix the contents of the packet thoroughly, allow contents to settle for one minute prior to opening to minimize the loss of fine particles, and to use a sample size of at least 1 g. The approximate analyte levels were not reported to participants prior to the study. The reference values for cyanidin, delphinidin, malvidin, petunidin, and peonidin in SRM 3291 were assigned using results from NIST by LC-Abs. The values and uncertainties are provided in the table below on an as-received basis.

| Analyte | NIST-Determined Mass Fraction in SRM 3291 (mg/kg) (as-received basis) |
|---|---|
| Cyanidin | 58.4 ± 5.8 |
| Delphinidin | 102.4 ± 37.6 |
| Malvidin | 42.8 ± 1.2 |
| Petunidin | 35.8 ± 0.8 |
| Peonidin | 29 ± 0.6 |

Dietary Intake Study Results

- Seventeen laboratories enrolled to measure the individual forms of the anthocyanidins, and 19 laboratories enrolled to measure the sum of all anthocyanidins. The enrollment and reporting statistics for the botanicals study is 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.

| Analyte | Number of Laboratories Requesting Samples | Number of Laboratories Reporting Results (Percent Participation) SRM 3287 | SRM 3281 | SRM 3291 |
|---|---|---|---|---|
| Total Anthocyanidins (C3G) | 19 | 10 (53 %) | 9 (47 %) | 8 (42 %) |
| Cyanidin | 17 | 3 (18 %) | 3 (18 %) | 4 (24 %) |
| Delphinidin | 17 | 3 (18 %) | 3 (18 %) | 4 (24 %) |
| Malvidin | 17 | 3 (18 %) | 3 (18 %) | 4 (24 %) |
| Petunidin | 17 | 3 (18 %) | 3 (18 %) | 4 (24 %) |
| Peonidin | 17 | 3 (18 %) | 3 (18 %) | 4 (24 %) |

- The between-laboratory variabilities were very large (over 43 %) for most analytes in SRM 3287 Blueberry (Fruit) and SRM 3281 Cranberry (Fruit). The between-laboratory variabilities for total anthocyanidins and delphinidin in SRM 3287 are better but need improvement (43 % and 54 %, respectively). The between-laboratory variabilities for most analytes in SRM 3291 Bilberry Extract ranged from very good (10 %) to needs improvement (71 %). See table below.

| Analyte | Between Laboratory Variability (% RSD) | | |
|---|---|---|---|
| | SRM 3287 | SRM 3281 | SRM 3291 |
| Total Anthocyanidins (C3G) | 43 % | >100 % | 10 % |
| Cyanidin | >100 % | 83 % | 43 % |
| Delphinidin | 54 % | - | 71 % |
| Malvidin | >100 % | - | 47 % |
| Petunidin | 82 % | >100 % | 16 % |
| Peonidin | >100 % | >100 % | 21 % |

- Most laboratories reported using solvent extraction or dilution and one lab reported using acid hydrolysis for sample preparation for the determination of anthocyanidins. Most laboratories reported using liquid chromatography with absorbance detection or PDA, and one laboratory reported using spectrophotometry for the analytical method.
- For SRM 3287 Blueberry (Fruit), the participation rate was low. Most laboratories reported values below the target values for cyanidin, delphinidin, malvidin, petunidin, and peonidin. For Total Anthocyanidins (C3G), no target value was available.
- For SRM 3281 Cranberry (Fruit), the participation rate was low. All laboratories reported values below the target value and range for cyanidin and peonidin. For delphinidin, the consensus mean was very close to the target. No target values for were available for malvidin, petunidin, or for total anthocyanidins (C3G). For C3G, two laboratories reported values that were significantly above the consensus range of tolerance.
- For SRM 3291 Bilberry Extract, the participation rate was low. Most laboratories reported values above the target values for cyanidin, delphinidin, malvidin, petunidin, and peonidin. One laboratory reported values below the target values for all. No target value was available for total anthocyanidins (C3G). For C3G, three laboratories reported values that were significantly below the consensus range of tolerance.

Dietary Intake Technical Recommendations

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

- Participation was very low for this study, making meaningful observations and recommendations difficult. It is clear that the material suitability should be further assessed, as well as increased education in the anthocyanidin testing community for measurement improvements.
- Sample preparation techniques should be chosen appropriately for the measurement of different individual forms of anthocyanidins (e.g., glycosides, aglycones) or as totals. Molar mass conversions can be used to obtain the totals for each aglycone.
  - For total anthocyanidins (C3G) in SRM 3281 Cranberry (Fruit), of the two laboratories that reported values above the consensus range of tolerance, one reported dilution + spectrophotometry and the other reported other. These were the only two labs to report these techniques for SRM 3281, thus technique trend analysis cannot be made.
  - For total anthocyanidins (C3G) in SRM 3291 Bilberry Extract, of the three laboratories that reported values below the consensus range of tolerance, two reported using solvent extraction + LC-Abs and one reported using acid hydrolysis + LC-Abs. Several other labs also reported using solvent extraction + LC-Abs, and no other labs reported using acid hydrolysis, thus technique trend analysis cannot be made.
- Appropriate and well characterized reference standards should be use in calibrant preparation, though limited availability of authentic standards may have contributed to difficulties in chromatographic peak identification and quantitation. The best solution is to acquire as many standards as possible and use retention times (and *m/z* when using MS techniques) to confirm peak identifications. Relying on literature or official methods has limitations, as variations in column chemistry, mobile phase composition, and temperature can all affect the chromatographic selectivity and therefore the retention times of all compounds.

**Table 6-1.** Individualized data summary table (NIST) for anthocyanidins in SRM 3287 Blueberry (Fruit), SRM 3281 Cranberry (Fruit), and SRM 3291 Bilberry Extract.

*National Institute of Standards and Technology*

HAMQAP Exercise 6 - Botanicals

| | Lab Code: | NIST | 1. Your Results | | | | 2. Community Results | | | 3. Target | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Analyte | Sample | Units | $x_i$ | $s_i$ | $Z'_{comm}$ | $Z_{NIST}$ | N | x* | s* | $x_{NIST}$ | $U$ |
| Total Anthocyanidins (C3G) | SRM 3291 Bilberry Extract | mg/kg | | | | | 10 | 282000 | 29000 | | |
| Total Anthocyanidins (C3G) | SRM 3287 Blueberry (Fruit) | mg/kg | | | | | 9 | 1180 | 510 | | |
| Total Anthocyanidins (C3G) | SRM 3281 Cranberry (Fruit) | mg/kg | | | | | 8 | 50 | 61 | | |
| Cyanidin | SRM 3291 Bilberry Extract | mg/kg | 58.4 | 5.8 | | | 4 | 1730 | 740 | 58.4 | 5.8 |
| Cyanidin | SRM 3287 Blueberry (Fruit) | mg/kg | 290 | 23.7 | | | 3 | 3.9 | 7.2 | 290 | 23.7 |
| Cyanidin | SRM 3281 Cranberry (Fruit) | mg/kg | 116 | 30.3 | | | 3 | 3.5 | 2.9 | 116 | 30.3 |
| Delphinidin | SRM 3291 Bilberry Extract | mg/kg | 102 | 7.6 | | | 4 | 1680 | 1200 | 102 | 7.6 |
| Delphinidin | SRM 3287 Blueberry (Fruit) | mg/kg | 1160 | 227 | | | 3 | 10.8 | 5.8 | 1160 | 227 |
| Delphinidin | SRM 3281 Cranberry (Fruit) | mg/kg | 5.06 | 0.664 | | | 3 | 0 | 10 | 5.06 | 0.664 |
| Malvidin | SRM 3291 Bilberry Extract | mg/kg | 42.8 | 1.2 | | | 4 | 760 | 360 | 42.8 | 1.2 |
| Malvidin | SRM 3287 Blueberry (Fruit) | mg/kg | 1370 | 276 | | | 3 | 10 | 11 | 1370 | 276 |
| Malvidin | SRM 3281 Cranberry (Fruit) | mg/kg | | | | | 3 | 0 | 0 | | |
| Peonidin | SRM 3291 Bilberry Extract | mg/kg | 29 | 0.6 | | | 4 | 360 | 77 | 29 | 0.6 |
| Peonidin | SRM 3287 Blueberry (Fruit) | mg/kg | 282 | 50.3 | | | 3 | 1.5 | 3.2 | 282 | 50.3 |
| Peonidin | SRM 3281 Cranberry (Fruit) | mg/kg | 118 | 21.5 | | | 3 | 0.6 | 1.8 | 118 | 21.5 |
| Petunidin | SRM 3291 Bilberry Extract | mg/kg | 35.8 | 0.8 | | | 4 | 880 | 140 | 35.8 | 0.8 |
| Petunidin | SRM 3287 Blueberry (Fruit) | mg/kg | 641 | 118 | | | 3 | 4.5 | 3.7 | 641 | 118 |
| Petunidin | SRM 3281 Cranberry (Fruit) | mg/kg | | | | | 3 | 0.18 | 0.34 | | |

$x_i$ Mean of reported values
$s_i$ Standard deviation of reported values
$Z'_{comm}$ Z'-score with respect to community consensus
$Z_{NIST}$ Z-score with respect to NIST value

N Number of quantitative values reported
x* Robust mean of reported values
s* Robust standard deviation

$x_{NIST}$ NIST-assessed value
$U$ expanded uncertainty about the NIST-assessed value

**Table 6-2.** Data summary table for total anthocyanidins (C3G) in SRM 3287 Blueberry (Fruit), SRM 3281 Cranberry (Fruit), and SRM 3291 Bilberry Extract. Data points highlighted in blue have been identified as outside the consensus tolerance limits and would be estimated to result in an unacceptable $Z'_{\text{comm}}$ score, $|Z'_{\text{comm}}| \geq 2$.

| | | Total Anthocyanidins (C3G) | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | SRM 3287 Blueberry Fruit (mg/kg) | | | | | SRM 3281 Cranberry Fruit (mg/kg) | | | | | SRM 3291 Bilberry Extract (mg/kg) | | | | |
| | Lab | A | B | C | Avg | SD | A | B | C | Avg | SD | A | B | C | Avg | SD |
| Individual Results | Target | | | | | | | | | | | | | | | |
| | F004 | | | | | | | | | | | | | | | |
| | F005 | 977.36 | 901.41 | 846.21 | 908 | 66 | 46.46 | 42.13 | 38.57 | 42.39 | 3.95 | 262717.06 | 261846.03 | 258113.61 | 260892 | 2445 |
| | F011 | 117.4 | 127.9 | 123.6 | 123 | 5.3 | | | | | | 13267.4 | 13126.5 | 13479.6 | 13291 | 178 |
| | F012 | | | | | | | | | | | 301.9921 | 300.844 | 302.416 | 302 | 0.81 |
| | F019 | 898 | 890 | 871 | 886 | 14 | 46 | 46 | 45 | 45.67 | 0.58 | 280400 | 279600 | 279100 | 279700 | 656 |
| | F026 | 910 | | | 910 | | 52 | | | 52.00 | | 268285 | | | 268285 | |
| | F031 | | | | | | | | | | | | | | | |
| | F034 | 2058 | 2190 | 2149 | 2132 | 68 | 1683 | 1505 | 1545 | 1577.67 | 93.39 | 319766 | 300753 | 273964 | 298161 | 23011 |
| | F035 | | | | | | | | | | | | | | | |
| | F036 | 1095.5 | 1199.7 | 1320.4 | 1205 | 113 | 97.5 | 91.2 | 88.5 | 92.40 | 4.62 | 329724 | 295827 | 316149 | 313900 | 17060 |
| | F037 | 2306 | 2168 | 2031 | 2168 | 138 | 1273 | 1048 | 1017 | 1113 | 140 | 283127 | 280459 | 282693 | 282093 | 1432 |
| | F040 | 904 | 958 | 1079 | 980 | 90 | 14.38 | 15.22 | 16.67 | 15.42 | 1.16 | 272295 | 275591 | 275167 | 274351 | 1793 |
| | F060 | | | | | | | | | | | | | | | |
| | F065 | | | | | | | | | | | | | | | |
| | F069 | | | | | | | | | | | | | | | |
| | F070 | 1620 | 1420 | 1190 | 1410 | 215 | 55.3 | 41.3 | 43.7 | 46.77 | 7.49 | 330 | 374 | 365 | 356 | 23 |
| | F089 | | | | | | | | | | | | | | | |
| Community Results | | Consensus Mean | | | 1177 | | Consensus Mean | | | 49 | | Consensus Mean | | | 282483 | |
| | | Consensus Standard Deviation | | | 507 | | Consensus Standard Deviation | | | 61 | | Consensus Standard Deviation | | | 29098 | |
| | | Maximum | | | 2168 | | Maximum | | | 1578 | | Maximum | | | 313900 | |
| | | Minimum | | | 123 | | Minimum | | | 15 | | Minimum | | | 302 | |
| | | N | | | 8 | | N | | | 7 | | N | | | 9 | |

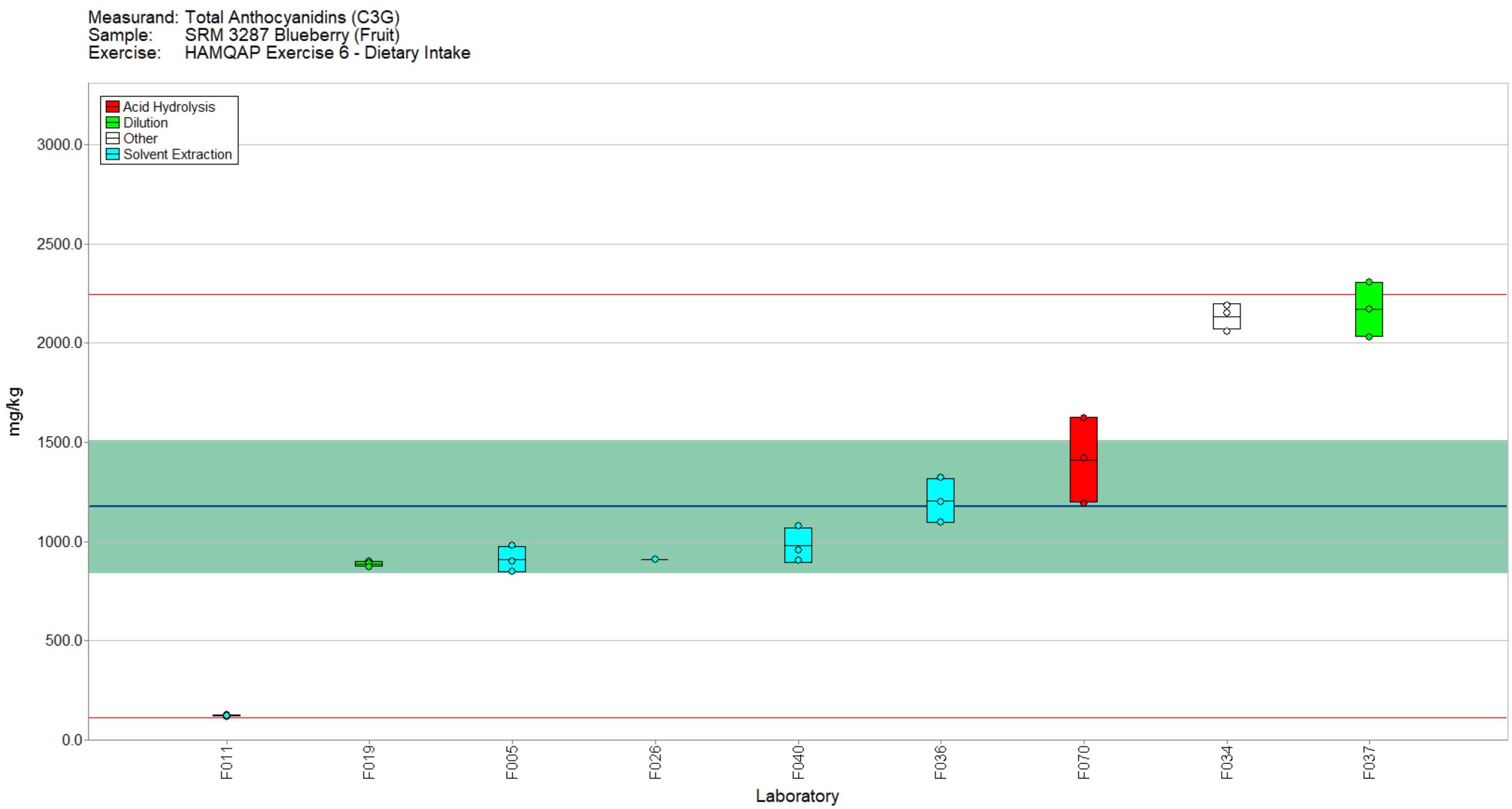


**Figure 6-1.** Total anthocyanidins (C3G) in SRM 3287 Blueberry (Fruit) (data summary view – sample preparation). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation employed. The solid blue 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 $Z'_{\mathrm{comm}}$ score, $|Z'_{\mathrm{comm}}| \leq 2$. A NIST value has not been determined in this material.

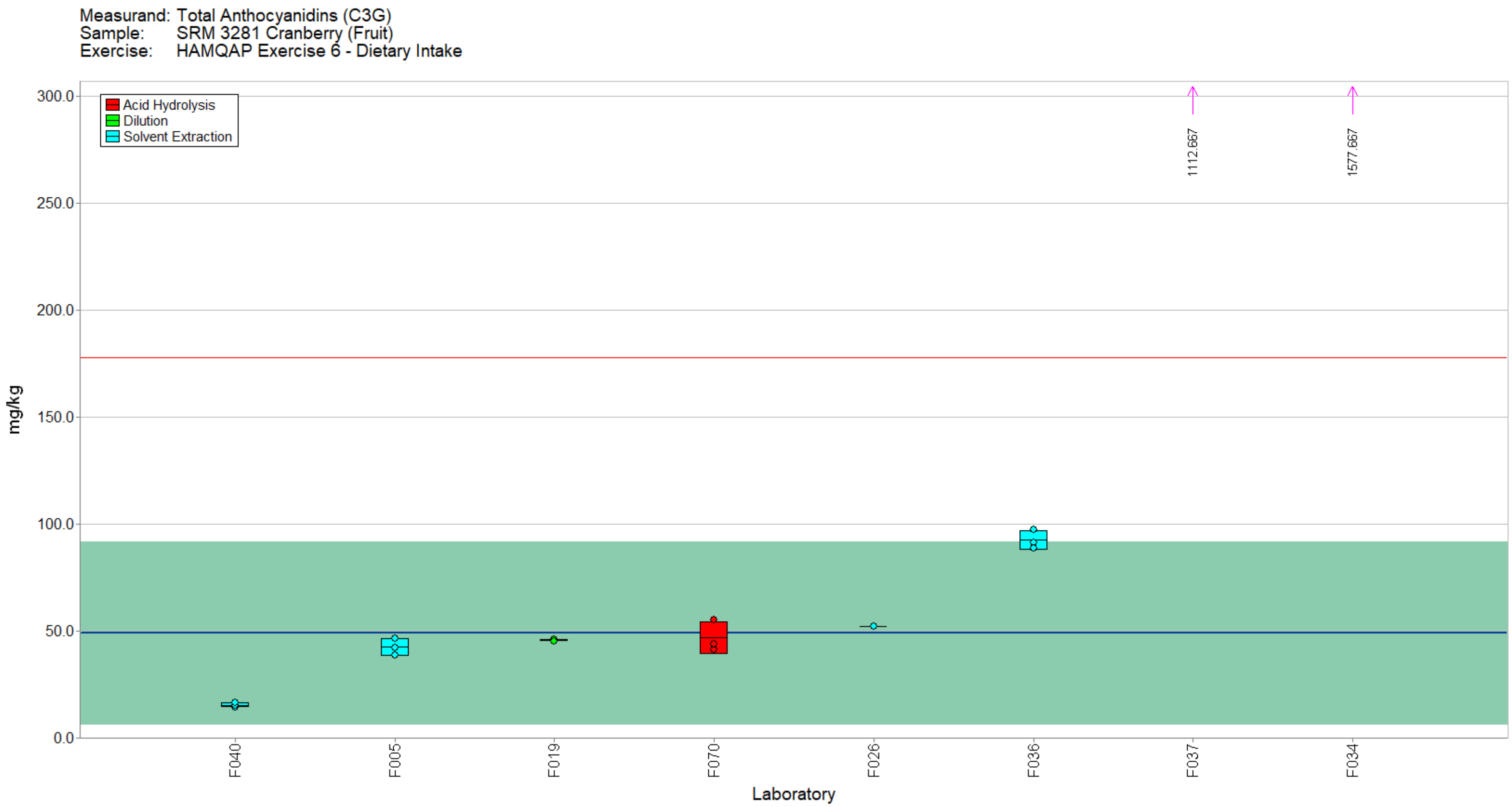


**Figure 6-2.** Total anthocyanidins (C3G) in SRM 3281 Cranberry (Fruit) (data summary view – sample preparation). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation employed. The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The red solid line represents the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable $Z'_{\text{comm}}$ score, $|Z'_{\text{comm}}| \leq 2$. A NIST value has not been determined in this material.

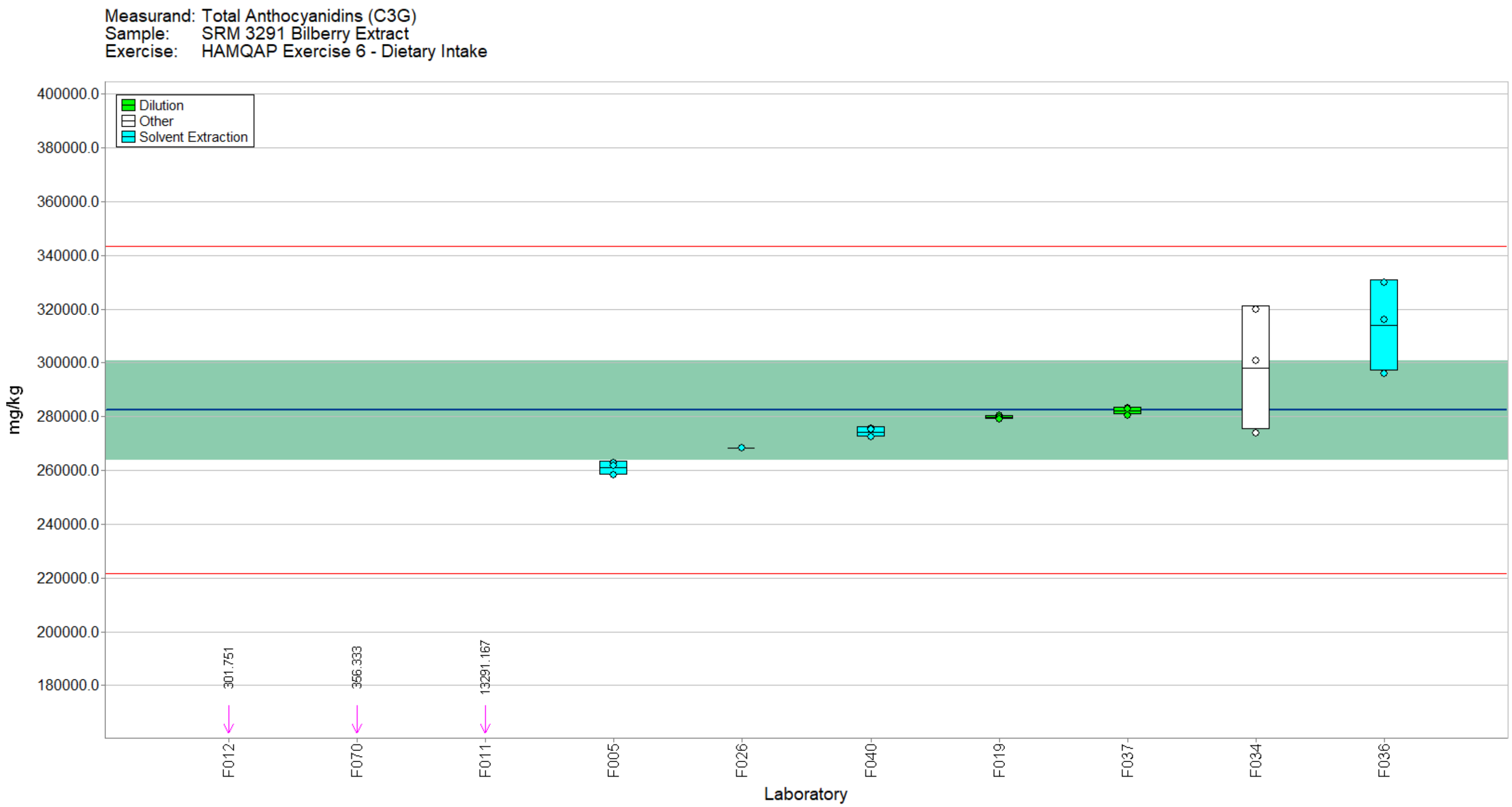


**Figure 6-3.** Total anthocyanidins (C3G) in SRM 3291 Bilberry Extract (data summary view – sample preparation). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation employed. The solid blue 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 $Z'_{\mathrm{comm}}$ score, $|Z'_{\mathrm{comm}}| \leq 2$. A NIST value has not been determined in this material.

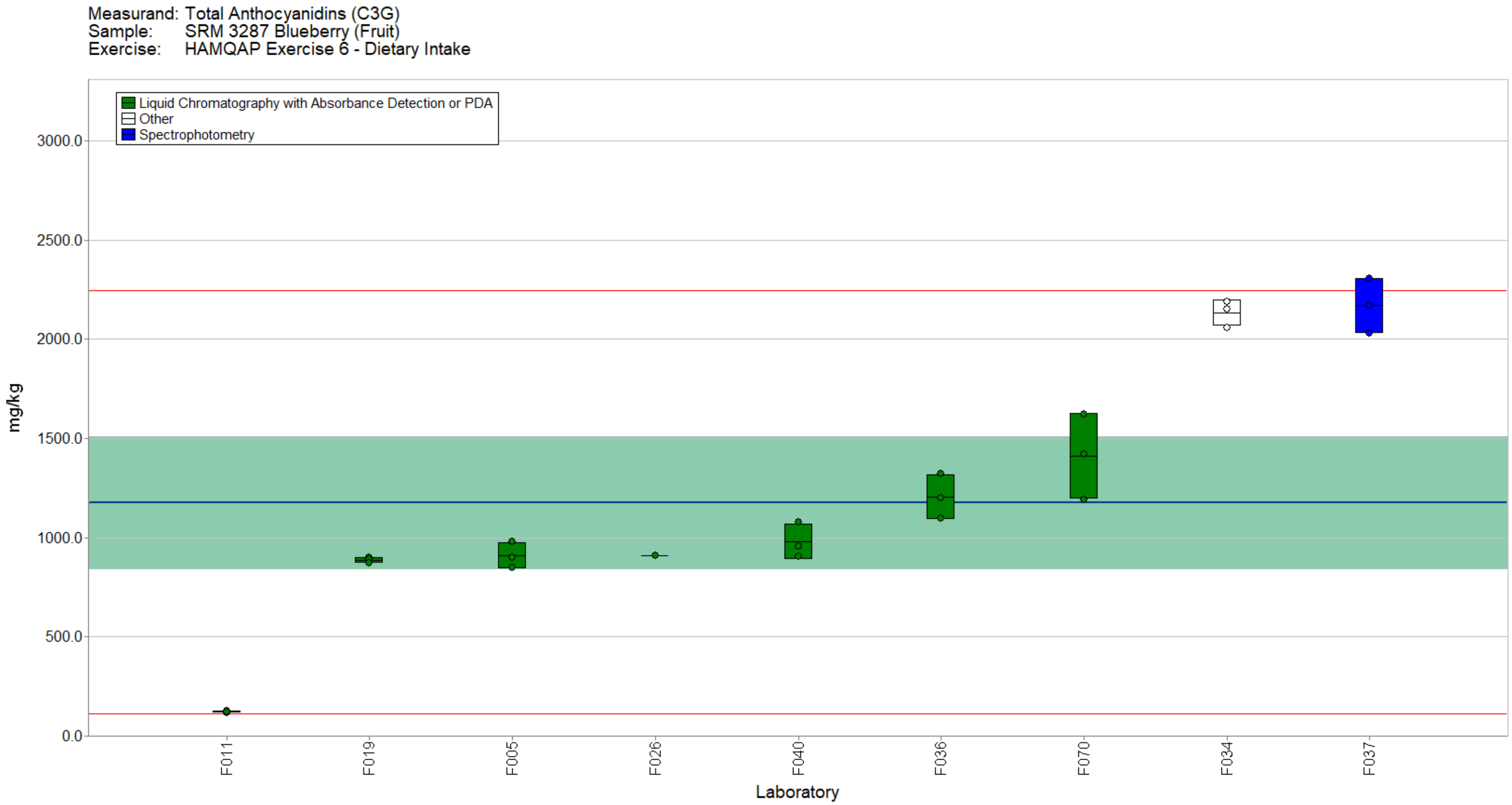


**Figure 6-4.** Total anthocyanidins (C3G) in SRM 3287 Blueberry (Fruit) (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue 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 $Z'_{\mathrm{comm}}$ score, $|Z'_{\mathrm{comm}}| \leq 2$. A NIST value has not been determined in this material.

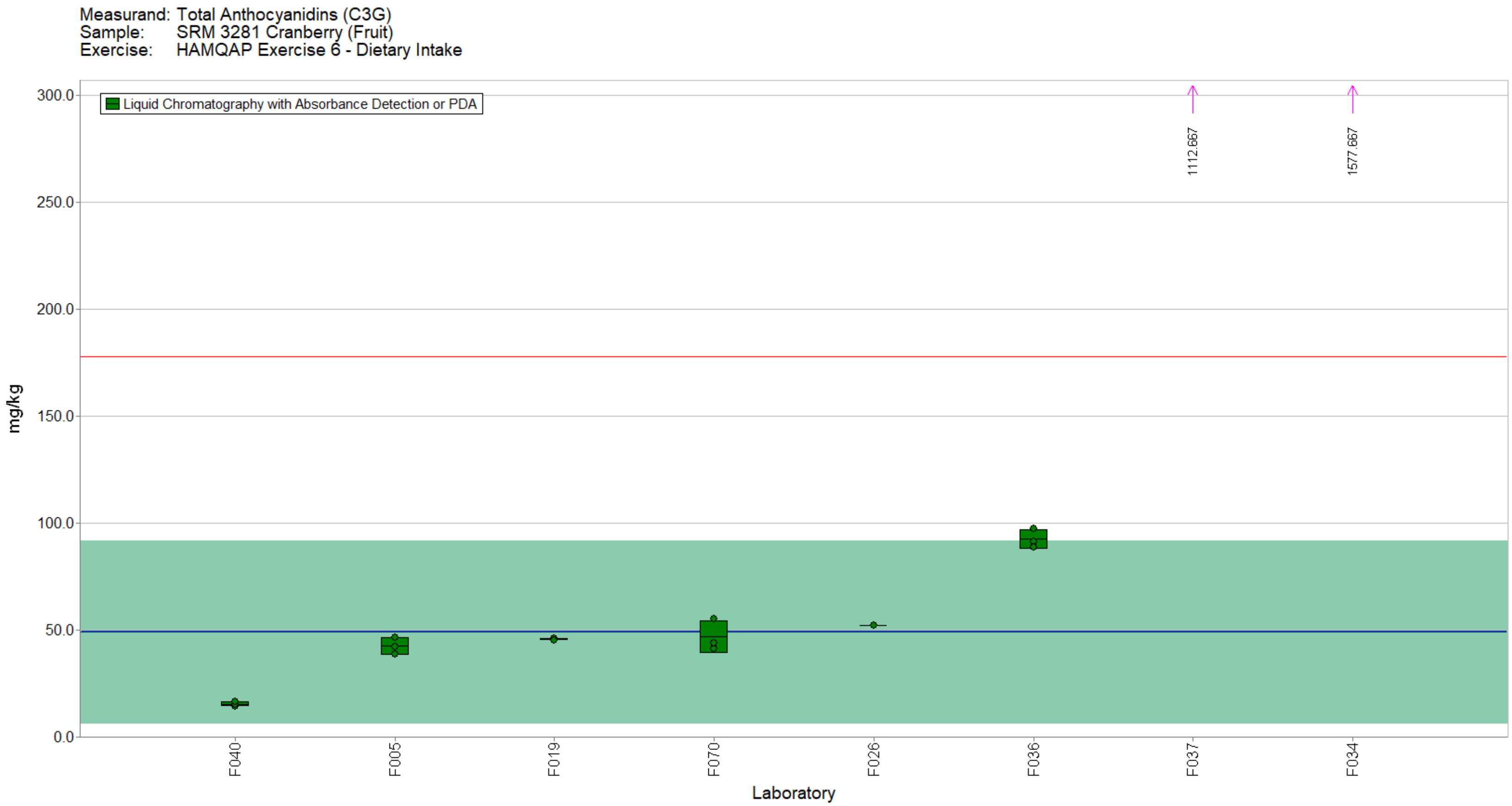


**Figure 6-5.** Total anthocyanidins (C3G) in SRM 3281 Cranberry (Fruit) (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The red solid line represents the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable $Z'_{\mathrm{comm}}$ score, $|Z'_{\mathrm{comm}}| \leq 2$. A NIST value has not been determined in this material.

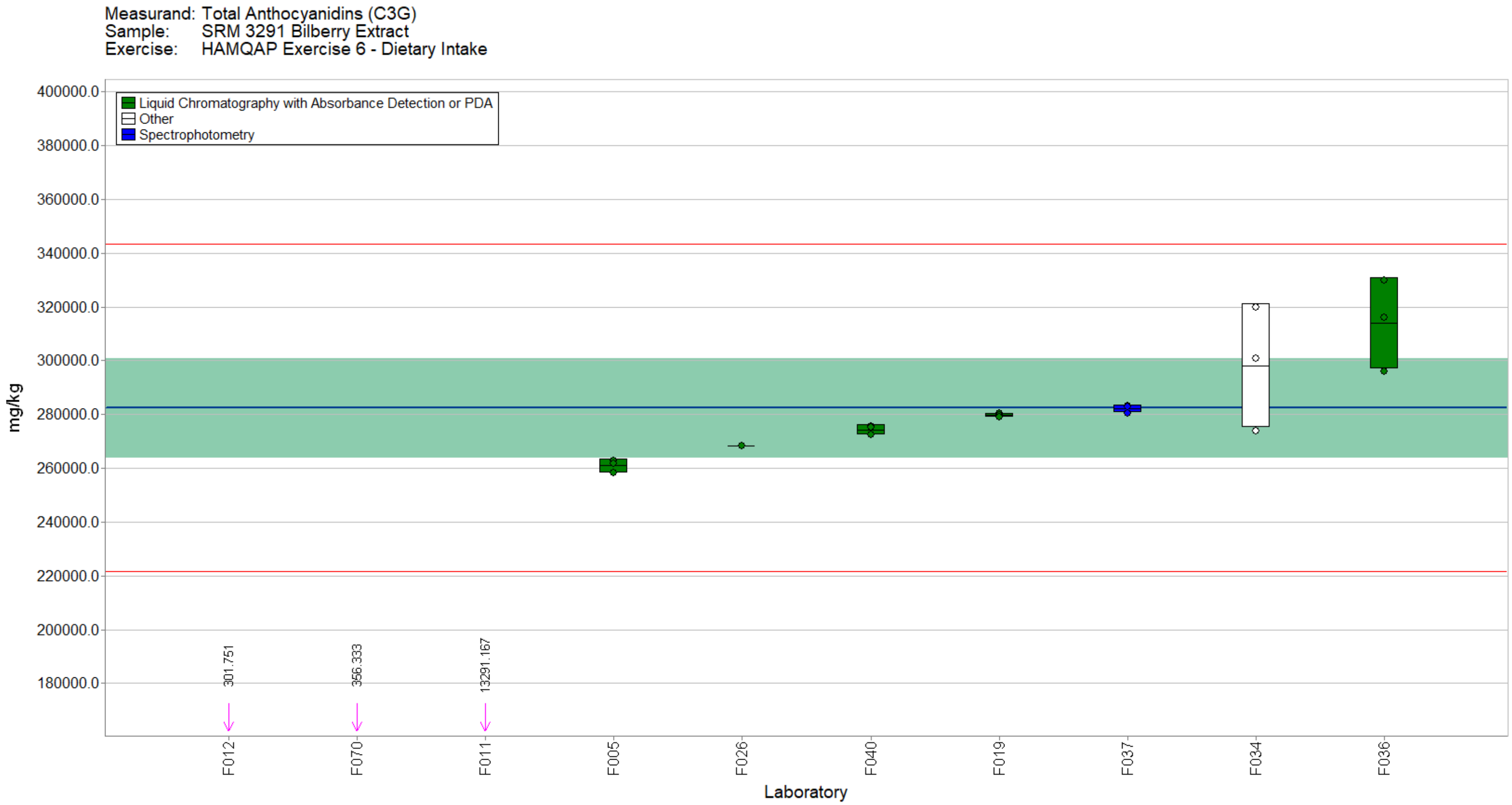


**Figure 6-6.** Total anthocyanidins (C3G) in SRM 3291 Bilberry Extract (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue 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 $Z'_{\mathrm{comm}}$ score, $|Z'_{\mathrm{comm}}| \leq 2$. A NIST value has not been determined in this material.

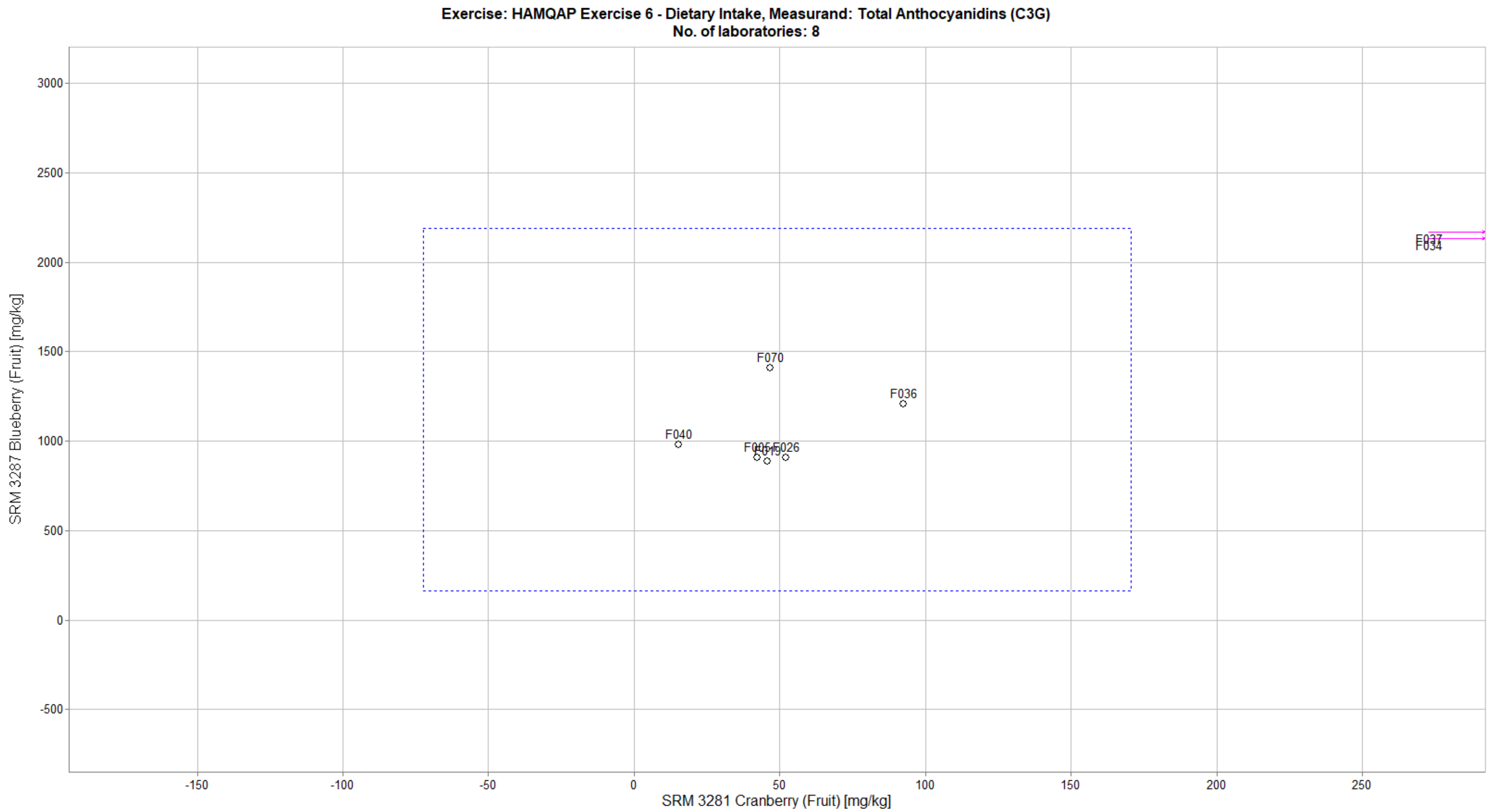


**Figure 6-7.** Laboratory means for total anthocyanidins (C3G) in SRM 3287 Blueberry (Fruit) and SRM 3281 Cranberry (Fruit) (sample/sample comparison view). In this view, the individual laboratory mean for one sample (SRM 3281) is compared to the individual laboratory mean for a second sample (SRM 3287). The dotted blue box represents the consensus range of tolerance for SRM 3281 (x-axis) and SRM 3287 (y-axis), calculated as the values above and below the consensus means that result in an acceptable $Z'_{\text{comm}}$ score, $|Z'_{\text{comm}}| \leq 2$.

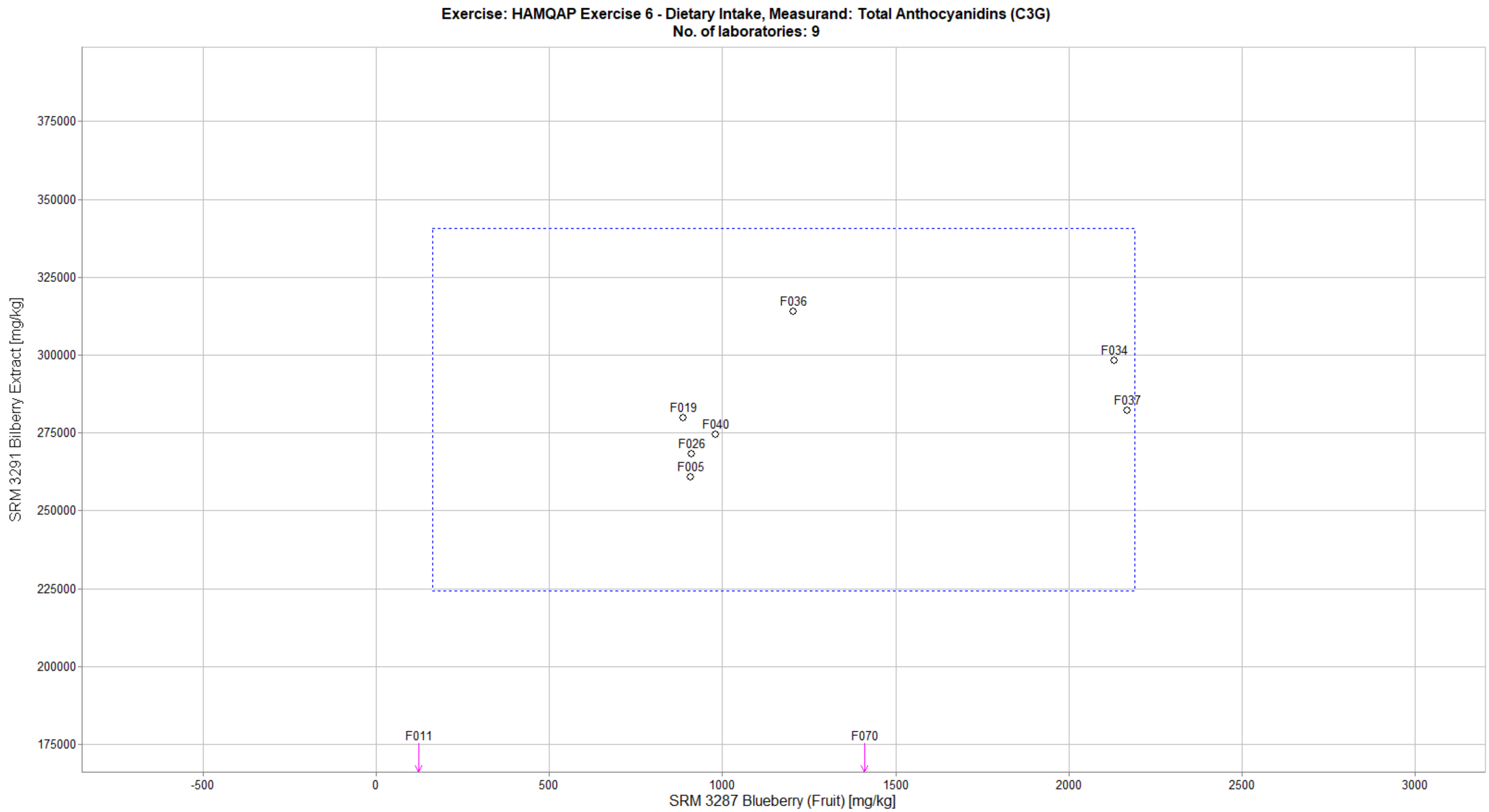


**Figure 6-8.** Laboratory means for total anthocyanidins (C3G) in SRM 3287 Blueberry (Fruit) and SRM 3291 Bilberry Extract (sample/sample comparison view). In this view, the individual laboratory mean for one sample (SRM 3287) is compared to the individual laboratory mean for a second sample (SRM 3291). The dotted blue box represents the consensus range of tolerance for SRM 3287 (x-axis) and SRM 3291 (y-axis), calculated as the values above and below the consensus means that result in an acceptable $Z'_{\text{comm}}$ score, $|Z'_{\text{comm}}| \leq 2$.

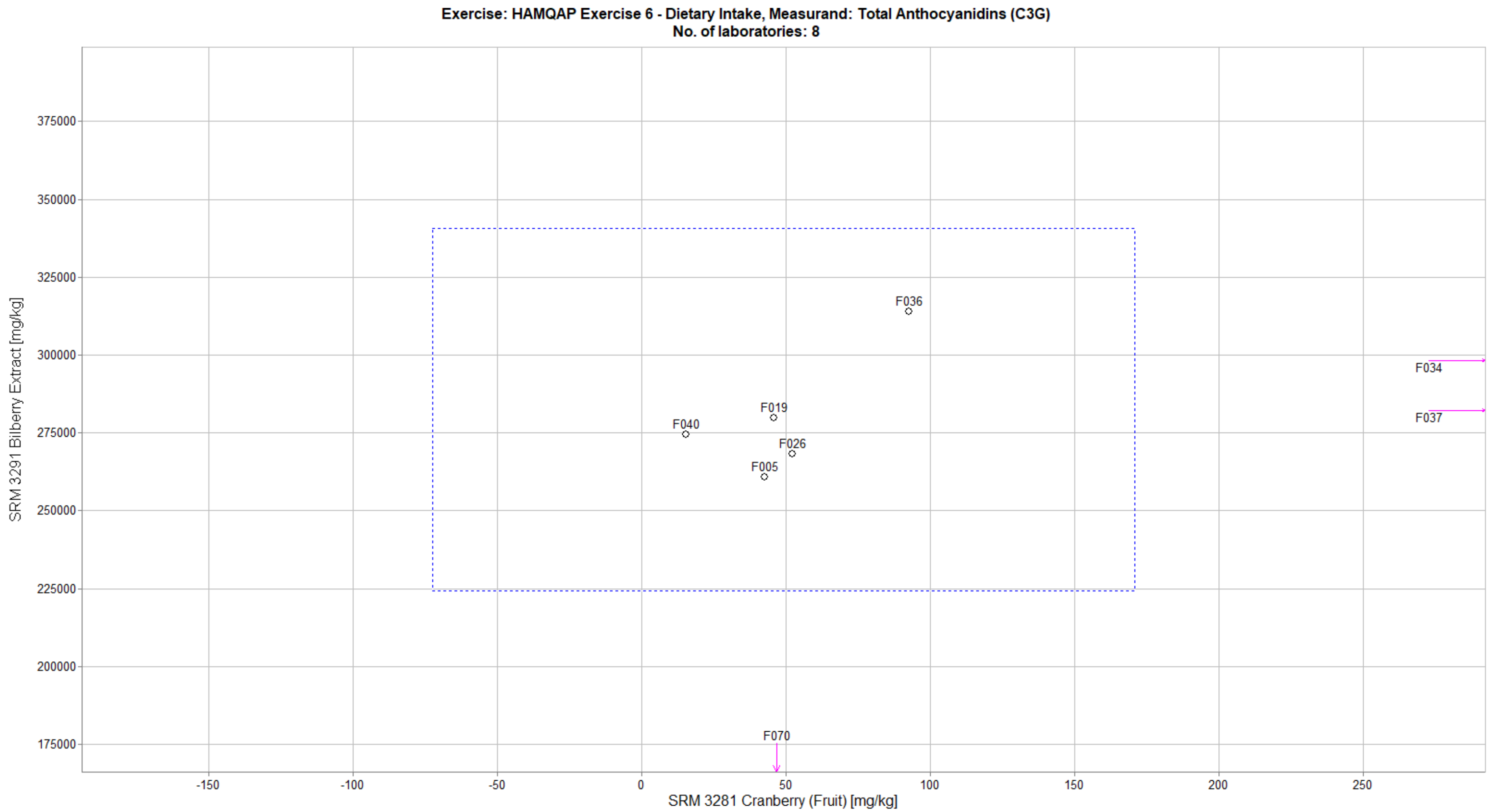


**Figure 6-9.** Laboratory means for total anthocyanidins (C3G) in SRM 3281 Cranberry (Fruit) and SRM 3291 Bilberry Extract (sample/sample comparison view). In this view, the individual laboratory mean for one sample (SRM 3281) is compared to the individual laboratory mean for a second sample (SRM 3291). The dotted blue box represents the consensus range of tolerance for SRM 3281 (x-axis) and SRM 3291 (y-axis), calculated as the values above and below the consensus means that result in an acceptable $Z'_{\mathrm{comm}}$ score, $|Z'_{\mathrm{comm}}| \leq 2$.

**Table 6-3.** Data summary table for cyanidin in SRM 3287 Blueberry (Fruit), SRM 3281 Cranberry (Fruit), and SRM 3291 Bilberry Extract. Data points highlighted in blue have been identified as outside the consensus tolerance limits and would be estimated to result in an unacceptable $Z'_{\mathrm{comm}}$ score, $|Z'_{\mathrm{comm}}| \geq 2$. Data points highlighted in red have a zero or non-numeric data point.

| | | Cyanidin | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | SRM 3287 Blueberry Fruit (mg/kg) | | | | | SRM 3281 Cranberry Fruit (mg/kg) | | | | | SRM 3291 Bilberry Extract (mg/kg) | | | | |
| | **Lab** | **A** | **B** | **C** | **Avg** | **SD** | **A** | **B** | **C** | **Avg** | **SD** | **A** | **B** | **C** | **Avg** | **SD** |
| **Individual Results** | Target | | | | 290 | 23.7 | | | | 116 | 30.3 | | | | 58.4 | 5.8 |
| | F005 | 0 | 0 | 0 | 0 | 0 | 0.85 | 0.94 | 0.81 | 0.87 | 0.07 | 2118.08 | 2200.46 | 2113.57 | 2144 | 49 |
| | F011 | | | | | | | | | | | | | | | |
| | F012 | | | | | | | | | | | 2.5059 | 2.5238 | 2.4758 | 2.50 | 0.02 |
| | F021 | | | | | | | | | | | | | | | |
| | F026 | | | | | | | | | | | | | | | |
| | F031 | | | | | | | | | | | | | | | |
| | F035 | | | | | | | | | | | | | | | |
| | F036 | 8.4 | 8.8 | 8.7 | 8.63 | 0.21 | 8.7 | 8.5 | 8.4 | 8.53 | 0.15 | 1960 | 1763 | 1786 | 1836 | 108 |
| | F040 | 2.86 | 2.74 | 3.37 | 2.99 | 0.33 | 1.56 | 1.59 | 2.24 | 1.80 | 0.38 | 2367 | 2317 | 2306 | 2330 | 33 |
| | F060 | | | | | | | | | | | | | | | |
| | F065 | | | | | | | | | | | | | | | |
| | F069 | | | | | | | | | | | | | | | |
| | F070 | | | | | | | | | | | | | | | |
| | F089 | | | | | | | | | | | | | | | |
| **Community Results** | | Consensus Mean | | | 3.87 | | Consensus Mean | | | 3.53 | | Consensus Mean | | | 1735 | |
| | | Consensus Standard Deviation | | | 7.20 | | Consensus Standard Deviation | | | 2.94 | | Consensus Standard Deviation | | | 737 | |
| | | Maximum | | | 8.63 | | Maximum | | | 8.53 | | Maximum | | | 2330 | |
| | | Minimum | | | 0 | | Minimum | | | 0.87 | | Minimum | | | 2.50 | |
| | | N | | | 3 | | N | | | 3 | | N | | | 4 | |

**Table 6-4.** Data summary table for delphinidin in SRM 3287 Blueberry (Fruit), SRM 3281 Cranberry (Fruit), and SRM 3291 Bilberry Extract. Data points highlighted in red have a zero or non-numeric data point.

| | | Delphinidin | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | SRM 3287 Blueberry Fruit (mg/kg) | | | | | SRM 3281 Cranberry Fruit (mg/kg) | | | | | SRM 3291 Bilberry Extract (mg/kg) | | | | |
| | Lab | A | B | C | Avg | SD | A | B | C | Avg | SD | A | B | C | Avg | SD |
| Individual Results | Target | | | | 5.06 | 0.66 | | | | 1163.0 | 226.8 | | | | 102.4 | 7.6 |
| | F005 | 0 | 0 | 0 | 0 | 0 | 7.27 | 7.75 | 11.28 | 8.77 | 2.19 | 2681.9 | 2753.84 | 2614.88 | 2683.5 | 69.5 |
| | F011 | | | | | | | | | | | | | | | |
| | F012 | | | | | | | | | | | 2.5081 | 2.5359 | 2.4542 | 2.5 | 0.0 |
| | F021 | | | | | | | | | | | | | | | |
| | F026 | | | | | | | | | | | | | | | |
| | F031 | | | | | | | | | | | | | | | |
| | F035 | | | | | | | | | | | | | | | |
| | F036 | 0 | 0 | 0 | 0 | 0 | 13.1 | 14 | 14.2 | 13.77 | 0.59 | 2434 | 2194 | 2246 | 2291.3 | 126.3 |
| | F040 | 9.38 | 10.07 | 10.73 | 10.06 | 0.68 | 8.1 | 10.5 | 10.7 | 9.77 | 1.45 | 1732 | 1736 | 1732 | 1733.3 | 2.3 |
| | F060 | | | | | | | | | | | | | | | |
| | F065 | | | | | | | | | | | | | | | |
| | F069 | | | | | | | | | | | | | | | |
| | F070 | | | | | | | | | | | | | | | |
| | F089 | | | | | | | | | | | | | | | |
| Community Results | | Consensus Mean | | | 3.35 | | Consensus Mean | | | 10.77 | | Consensus Mean | | | 1678 | |
| | | Consensus Standard Deviation | | | 10.01 | | Consensus Standard Deviation | | | 5.84 | | Consensus Standard Deviation | | | 1175 | |
| | | Maximum | | | 10.06 | | Maximum | | | 13.77 | | Maximum | | | 2684 | |
| | | Minimum | | | 0 | | Minimum | | | 8.77 | | Minimum | | | 2.5 | |
| | | N | | | 3 | | N | | | 3 | | N | | | 4 | |

**Table 6-5.** Data summary table for malvidin in SRM 3287 Blueberry (Fruit), SRM 3281 Cranberry (Fruit), and SRM 3291 Bilberry Extract. Data points highlighted in red have a zero or non-numeric data point.

| | | Malvidin | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | SRM 3287 Blueberry Fruit (mg/kg) | | | | | SRM 3281 Cranberry Fruit (mg/kg) | | | | | SRM 3291 Bilberry Extract (mg/kg) | | | | |
| | Lab | A | B | C | Avg | SD | A | B | C | Avg | SD | A | B | C | Avg | SD |
| Individual Results | Target | | | | 1370 | 276 | | | | 42.8 | 1.2 | | | | | |
| | F005 | 8.5 | 6.28 | 7.86 | 7.55 | 1.14 | 787.59 | 773.93 | 852.67 | 804.7 | 42.1 | 0 | 0 | 0 | 0 | 0 |
| | F011 | | | | | | | | | | | | | | | |
| | F012 | | | | | | 1.0622 | 1.0755 | 1.0442 | 1.06 | 0.02 | | | | | |
| | F021 | | | | | | | | | | | | | | | |
| | F026 | | | | | | | | | | | | | | | |
| | F031 | | | | | | | | | | | | | | | |
| | F035 | | | | | | | | | | | | | | | |
| | F036 | 0 | 0 | 0 | 0 | 0 | 1108 | 1020 | 1004 | 1044.0 | 56.0 | 0 | 0 | 0 | 0 | 0 |
| | F040 | 10.6 | 11.4 | 12.7 | 11.57 | 1.06 | 965 | 949 | 950 | 954.7 | 8.96 | 0 | 0 | 0 | 0 | 0 |
| | F060 | | | | | | | | | | | | | | | |
| | F065 | | | | | | | | | | | | | | | |
| | F069 | | | | | | | | | | | | | | | |
| | F070 | | | | | | | | | | | | | | | |
| | F089 | | | | | | | | | | | | | | | |
| Community Results | | Consensus Mean | | | 6.37 | | Consensus Mean | | | 755 | | Consensus Mean | | | 0 | |
| | | Consensus Standard Deviation | | | 10.90 | | Consensus Standard Deviation | | | 358 | | Consensus Standard Deviation | | | 0 | |
| | | Maximum | | | 11.57 | | Maximum | | | 1044 | | Maximum | | | 0 | |
| | | Minimum | | | 0 | | Minimum | | | 1.06 | | Minimum | | | 0 | |
| | | N | | | 3 | | N | | | 4 | | N | | | 3 | |

**Table 6-6.** Data summary table for petunidin in SRM 3287 Blueberry (Fruit), SRM 3281 Cranberry (Fruit), and SRM 3291 Bilberry Extract. Data points highlighted in blue have been identified as outside the consensus tolerance limits and would be estimated to result in an unacceptable $Z'_{\mathrm{comm}}$ score, $|Z'_{\mathrm{comm}}| \geq 2$. Data points highlighted in red have a zero or non-numeric data point.

| | | Petunidin | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | SRM 3287 Blueberry Fruit (mg/kg) | | | | | SRM 3281 Cranberry Fruit (mg/kg) | | | | | SRM 3291 Bilberry Extract (mg/kg) | | | | |
| | Lab | A | B | C | Avg | SD | A | B | C | Avg | SD | A | B | C | Avg | SD |
| Individual Results | Target | | | | 641.00 | 118.31 | | | | | | | | | 35.80 | 0.80 |
| | F005 | 5.95 | 6.46 | 6.48 | 6.30 | 0.30 | 0.25 | 0.86 | 0.54 | 0.55 | 0.31 | 809.27 | 864.34 | 805.82 | 826.5 | 32.8 |
| | F011 | | | | | | | | | | | | | | | |
| | F012 | | | | | | | | | | | 1.0701 | 1.0833 | 1.0546 | 1.07 | 0.01 |
| | F021 | | | | | | | | | | | | | | | |
| | F026 | | | | | | | | | | | | | | | |
| | F031 | | | | | | | | | | | | | | | |
| | F035 | | | | | | | | | | | | | | | |
| | F036 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 987 | 909.1 | 904 | 933.4 | 46.5 |
| | F040 | 6.65 | 7.01 | 8.13 | 7.26 | 0.77 | 0 | 0 | 0 | 0 | 0 | 895 | 871 | 863 | 876.3 | 16.7 |
| | F060 | | | | | | | | | | | | | | | |
| | F065 | | | | | | | | | | | | | | | |
| | F069 | | | | | | | | | | | | | | | |
| | F070 | | | | | | | | | | | | | | | |
| | F089 | | | | | | | | | | | | | | | |
| Community Results | | Consensus Mean | | | 4.52 | | Consensus Mean | | | 0.18 | | Consensus Mean | | | 879 | |
| | | Consensus Standard Deviation | | | 3.67 | | Consensus Standard Deviation | | | 0.34 | | Consensus Standard Deviation | | | 137 | |
| | | Maximum | | | 7.26 | | Maximum | | | 0.55 | | Maximum | | | 933 | |
| | | Minimum | | | 0 | | Minimum | | | 0 | | Minimum | | | 1.07 | |
| | | N | | | 3 | | N | | | 3 | | N | | | 4 | |

**Table 6-7.** Data summary table for peonidin in SRM 3287 Blueberry (Fruit), SRM 3281 Cranberry (Fruit), and SRM 3291 Bilberry Extract. Data points highlighted in blue have been identified as outside the consensus tolerance limits and would be estimated to result in an unacceptable $Z'_{\text{comm}}$ score, $|Z'_{\text{comm}}| \geq 2$. Data points highlighted in red have a zero or non-numeric data point.

| | | Peonidin | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | SRM 3287 Blueberry Fruit (mg/kg) | | | | | SRM 3281 Cranberry Fruit (mg/kg) | | | | | SRM 3291 Bilberry Extract (mg/kg) | | | | |
| | Lab | A | B | C | Avg | SD | A | B | C | Avg | SD | A | B | C | Avg | SD |
| Individual Results | Target | | | | 282.0 | 50.3 | | | | 118.0 | 21.5 | | | | 29.0 | 0.6 |
| | F005 | 1.27 | 1.48 | 0.55 | 1.100 | 0.488 | 1.97 | 1.81 | 1.67 | 1.817 | 0.150 | 349.16 | 305.3 | 369.09 | 341.2 | 32.6 |
| | F011 | | | | | | | | | | | | | | | |
| | F012 | | | | | | | | | | | 0.1981 | 0.1983 | 0.2001 | 0.199 | 0.001 |
| | F021 | | | | | | | | | | | | | | | |
| | F026 | | | | | | | | | | | | | | | |
| | F031 | | | | | | | | | | | | | | | |
| | F035 | | | | | | | | | | | | | | | |
| | F036 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 369 | 333 | 331 | 344.3 | 21.4 |
| | F040 | 3.01 | 3.53 | 3.75 | 3.430 | 0.380 | 0 | 0 | 0 | 0 | 0 | 385 | 384 | 380 | 383.0 | 2.65 |
| | F060 | | | | | | | | | | | | | | | |
| | F065 | | | | | | | | | | | | | | | |
| | F069 | | | | | | | | | | | | | | | |
| | F070 | | | | | | | | | | | | | | | |
| | F089 | | | | | | | | | | | | | | | |
| Community Results | | Consensus Mean | | | 1.51 | | Consensus Mean | | | 0.606 | | Consensus Mean | | | 356.172 | |
| | | Consensus Standard Deviation | | | 3.168 | | Consensus Standard Deviation | | | 1.787 | | Consensus Standard Deviation | | | 77.315 | |
| | | Maximum | | | 3.43 | | Maximum | | | 1.816667 | | Maximum | | | 383 | |
| | | Minimum | | | 0 | | Minimum | | | 0 | | Minimum | | | 0.198833 | |
| | | N | | | 3 | | N | | | 3 | | N | | | 4 | |

## SECTION 7: NATURAL PRODUCTS (Caffeine, Theobromine, Theophylline)

Study Overview

In this study, participants were provided with two commercial protein powders. Participants were asked to use in-house analytical methods to determine the mass fraction (mg/g) of select xanthines ((caffeine, theobromine, theophylline) in each matrix. Caffeine and other xanthine compounds such as theobromine and theophylline are included in many performance enhancing supplements.[6] Caffeine is a central nervous system stimulant that is rapidly absorbed into the bloodstream and may improve exercise performance and focus, while reducing drowsiness. Side effects of caffeine consumption include increased heart rate, insomnia, stomach discomfort, and anxiety. Accurate determination of the levels of caffeine and related xanthines in supplements can help ensure safe levels for consumers.

Dietary Intake Sample Information

*Protein Powders.* Participants were provided with two packets of protein powder, labeled **A** and **B**, each containing 10 g of material. Participants were asked to store the material at controlled room temperature (20 °C to 25 °C) in the original unopened packets, and to prepare three samples and report three values from each packet provided. Before use, participants were instructed to mix the contents of the packet thoroughly, allow contents to settle for one minute prior to opening to minimize the loss of fine particles, and to use a sample size appropriate for their usual in-house method of analysis. Approximate analyte levels were not reported to participants prior to the study and NIST did not determine analyte levels prior to the study.

Dietary Intake Study Results

- The enrollment and reporting statistics for the caffeine, theobromine, and theophylline studies are described in the table below. Reported values may include non-quantitative results (zero or below LOQ) but are included in the participation statistics.

| Analyte | Number of Laboratories Requesting Samples | Number of Laboratories Reporting Results (Percent Participation) | |
|---|---|---|---|
| | | Protein Powder A | Protein Powder B |
| Caffeine | 29 | 17 (59 %) | 18 (62 %) |
| Theobromine | 21 | 11 (52 %) | 11 (52 %) |
| Theophylline | 15 | 5 (33 %) | 5 (33 %) |

[6] Dietary Supplements for Exercise and Athletic Performance. National Institutes of Health National Center for Complementary and Integrative Health https://ods.od.nih.gov/factsheets/ExerciseAndAthleticPerformance-HealthProfessional/ (accessed June 2020).

- The between-laboratory variabilities were very good for theobromine in both protein powders while variabilities for caffeine and theophylline ranged from large to very large (see table below).

| | Between-Laboratory Variability (% RSD) | |
|---|---|---|
| Analyte | Protein Powder A | Protein Powder B |
| Caffeine | 80 % | 25 % |
| Theobromine | 1 % | 16 % |
| Theophylline | >100 % | >100 % |

- Most laboratories reported using solvent extraction for their sample preparation (see table below). The sample preparation methods reported by participating laboratories are shown in **Figures 7-1 and 7-2, 7-4 and 7-5, and 7-7 and 7-8** for caffeine, theobromine, and theophylline, respectively.

| Reported Sample Preparation Method | Percent Reporting | | |
|---|---|---|---|
| | Caffeine | Theobromine | Theophylline |
| Solvent Extraction | 44 % | 55 % | 40 % |
| Dilution | 28 % | 27 % | 40 % |
| Solid Phase Extraction | 6 % | 9 % | 20 % |
| Protein Precipitation | 6 % | | |
| Other or None Reported | 17 % | 9 % | |

- All laboratories reported using liquid chromatography with absorbance detection or PDA for their analytical method, and one laboratory did not report a method for detection of caffeine or theobromine.
- Three total data points associated with caffeine determinations were flagged as potential outliers on the low end of the reported values (1 laboratory for Protein Powder A and 2 laboratories for Protein Powder B shown in **Figures 7-1 and 7-2**, respectively).
- Six total data points associated with caffeine determinations were flagged as potential outliers on the high end of the reported values (3 laboratories for Protein Powder A and 2 laboratories for Protein Powder B shown in **Figures 7-1 and 7-2**, respectively).
- Only one data point associated with the analysis of theobromine was below the consensus range of tolerance (Protein Powder B) shown in **Figure 7-5**.

Dietary Intake Technical Recommendations

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

- Low variabilities between laboratories show the overall precision of the various in-house methods being used for the analysis of theobromine. The reported results for caffeine showed larger variabilities between laboratories.

    - These laboratories should examine the optimization of in-house methods to ensure complete extractions of caffeine and theobromine.
    - The laboratories reporting results for caffeine and/or theobromine above the consensus range of tolerance should examine preparation conditions for both samples. Extraction conditions could produce potential xanthine interferences resulting in higher reported values.
- For the analysis of theophylline, overall participation was low and limits the ability to make technical recommendations. Low participation may be the result of laboratories not having adequate in-house analytical methods for the extraction and quantification of theophylline in natural products.
- Improper calibration is a frequent source of measurement error.
    - Calibrant purity is an important consideration in analytical measurements. Where possible, calibrants should be evaluated for purity and presence of residual solvents prior to use. The measured purity should be used to correct the concentrations of the solutions used for calibration.
    - If a calibration curve is used, the calibrant concentrations should encompass the sample concentrations. No sample concentrations should be outside of the linear range.
- Laboratories reporting results flagged as outliers should check for errors in calculations or reporting units. Confirm that all dilution factors have been properly tabulated.

**Table 7-1.** Individualized data summary table (NIST) for caffeine, theobromine, and theophylline in protein powder samples.

*National Institute of Standards and Technology*

**HAMQAP Exercise 6 - Natural Products**

| | | **Lab Code: NIST** | **1. Your Results** | | | | **2. Community Results** | | | **3. Target** | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Analyte | Sample | Units | $x_i$ | $s_i$ | $Z'_{comm}$ | $Z_{NIST}$ | N | x* | s* | $x_{NIST}$ | $U$ |
| Caffeine | Protein Sample A | mg/g | | | | | 16 | 0.092 | 0.037 | | |
| Caffeine | Protein Sample B | mg/g | | | | | 18 | 0.403 | 0.092 | | |
| Theobromine | Protein Sample A | mg/g | | | | | 10 | 1.13 | 0.098 | | |
| Theobromine | Protein Sample B | mg/g | | | | | 11 | 0.98 | 0.15 | | |
| Theophylline | Protein Sample A | mg/g | | | | | 4 | 0.38 | 0.51 | | |
| Theophylline | Protein Sample B | mg/g | | | | | 4 | 0.024 | 0.042 | | |

$x_i$ Mean of reported values
$s_i$ Standard deviation of reported values
$Z'_{comm}$ Z'-score with respect to community consensus
$Z_{NIST}$ Z-score with respect to NIST value

N Number of quantitative values reported
x* Robust mean of reported values
s* Robust standard deviation

$x_{NIST}$ NIST-assessed value
$U$ expanded uncertainty about the NIST-assessed value

**Table 7-2.** Data summary table for caffeine in protein powder samples. Data points highlighted in blue have been identified as outside the consensus tolerance limits and would be estimated to result in an unacceptable $Z'_{\text{comm}}$ score, $|Z'_{\text{comm}}| \geq 2$.

| | | Caffeine | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Protein Sample A (mg/g) | | | | | Protein Sample B (mg/g) | | | | |
| | **Lab** | **A** | **B** | **C** | **Avg** | **SD** | **A** | **B** | **C** | **Avg** | **SD** |
| **Individual Results** | Target | | | | | | | | | | |
| | F004 | | | | | | | | | | |
| | F005 | 527.14 | 525.14 | 538.94 | 530.4 | 7.5 | 603.11 | 598.64 | 588.68 | 596.81 | 7.39 |
| | F011 | 0.049 | 0.044 | 0.045 | 0.046 | 0.003 | 0.421 | 0.472 | 0.449 | 0.447 | 0.026 |
| | F017 | 0.094 | 0.095 | 0.102 | 0.097 | 0.004 | 0.375 | 0.357 | 0.363 | 0.365 | 0.009 |
| | F018 | | | | | | | | | | |
| | F019 | | | | | | 0.4 | 0.4 | 0.4 | 0.4 | 0 |
| | F021 | 0.107 | 0.125 | 0.128 | 0.120 | 0.011 | 0.513 | 0.513 | 0.499 | 0.508 | 0.008 |
| | F022 | | | | | | | | | | |
| | F030 | 0.113 | 0.103 | 0.103 | 0.106 | 0.006 | 0.365 | 0.366 | 0.366 | 0.366 | 0.001 |
| | F031 | | | | | | | | | | |
| | F032 | | | | | | | | | | |
| | F033 | 0.116 | 0.118 | 0.117 | 0.117 | 0.001 | 0.417 | 0.42 | 0.425 | 0.421 | 0.004 |
| | F034 | 0.106 | 0.108 | 0.109 | 0.108 | 0.002 | 0.426 | 0.428 | 0.429 | 0.428 | 0.002 |
| | F039 | 0.113 | 0.114 | 0.109 | 0.112 | 0.003 | 0.492 | 0.471 | 0.49 | 0.484 | 0.012 |
| | F040 | 0.116 | 0.111 | 0.117 | 0.115 | 0.003 | 0.416 | 0.425 | 0.421 | 0.421 | 0.005 |
| | F045 | | | | | | | | | | |
| | F046 | 0.09 | 0.08 | 0.09 | 0.087 | 0.006 | 0.37 | 0.35 | 0.37 | 0.363 | 0.012 |
| | F051 | | | | | | | | | | |
| | F056 | | | | | | | | | | |
| | F059 | 0.095 | 0.094 | 0.096 | 0.095 | 0.001 | 0.425 | 0.431 | 0.423 | 0.426 | 0.004 |
| | F060 | 0.415 | 0.408 | 0.403 | 0.409 | 0.006 | 0.11 | 0.113 | 0.111 | 0.111 | 0.002 |
| | F062 | 0.08 | 0.1064 | 0.1042 | 0.097 | 0.015 | 0.41 | 0.429 | 0.415 | 0.418 | 0.010 |
| | F069 | 0.615 | 0.609 | 0.612 | 0.612 | 0.003 | 1.501 | 1.485 | 1.249 | 1.41 | 0.14 |
| | F070 | 0.063 | 0.054 | | 0.059 | 0.006 | 0.369 | 0.333 | | 0.351 | 0.025 |
| | F074 | 0.003 | 0.003 | 0.003 | 0.0030 | 0 | 0.019 | 0.019 | 0.019 | 0.019 | 0 |
| | F079 | | | | | | | | | | |
| | F080 | | | | | | 0.395 | 0.396 | 0.379 | 0.390 | 0.010 |
| | F088 | | | | | | | | | | |
| | F089 | | | | | | | | | | |
| **Community Results** | | Consensus Mean | | | 0.092 | | Consensus Mean | | | 0.403 | |
| | | Consensus Standard Deviation | | | 0.037 | | Consensus Standard Deviation | | | 0.092 | |
| | | Maximum | | | 530.4 | | Maximum | | | 596.8 | |
| | | Minimum | | | 0.003 | | Minimum | | | 0.019 | |
| | | N | | | 16 | | N | | | 18 | |

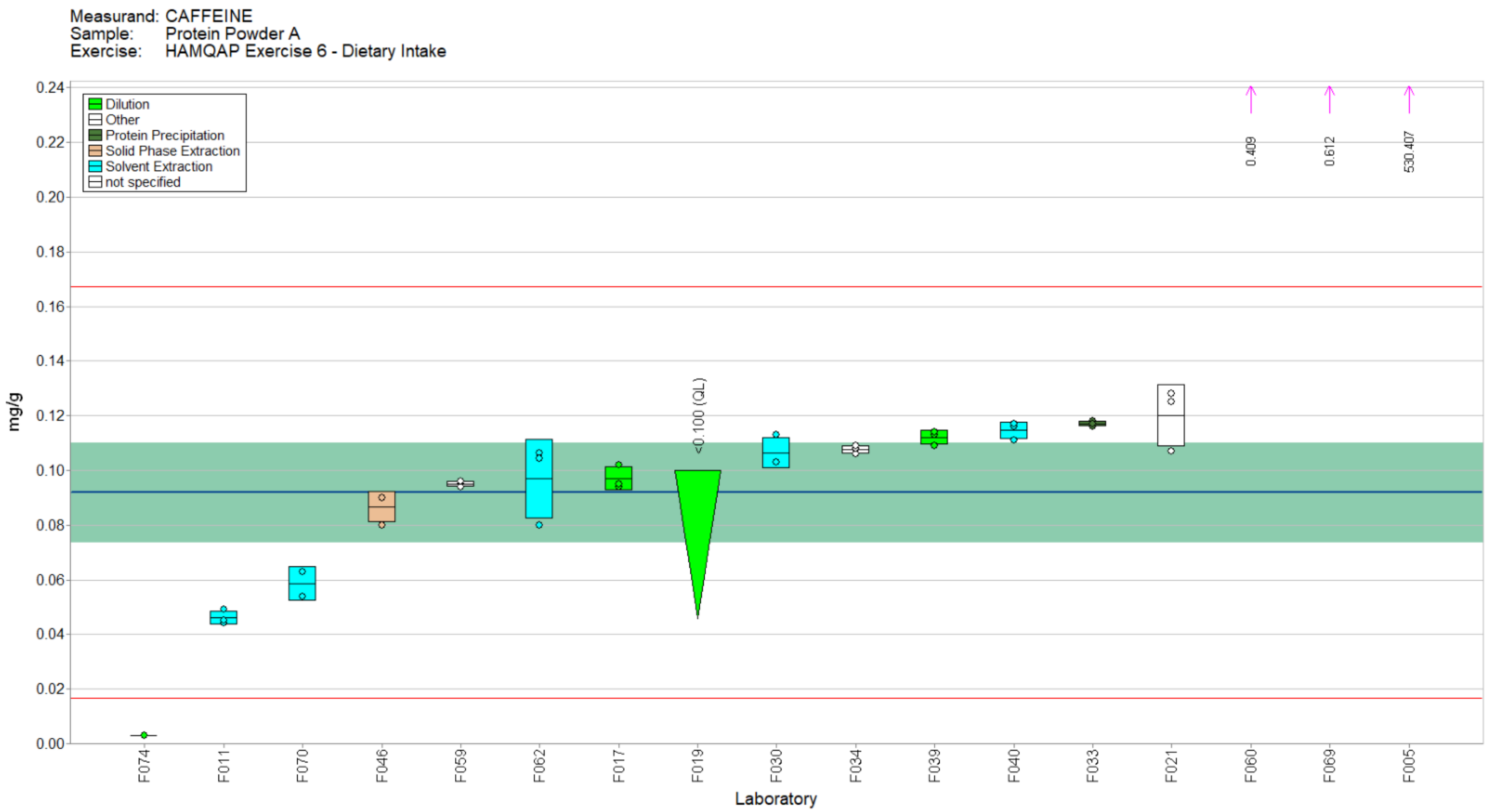


**Figure 7-1.** Caffeine in Protein Powder A (data summary view – sample preparation method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The solid blue 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 $Z'_{\text{comm}}$ score, $|Z'_{\text{comm}}| \leq 2$. A NIST value has not been determined in this material.

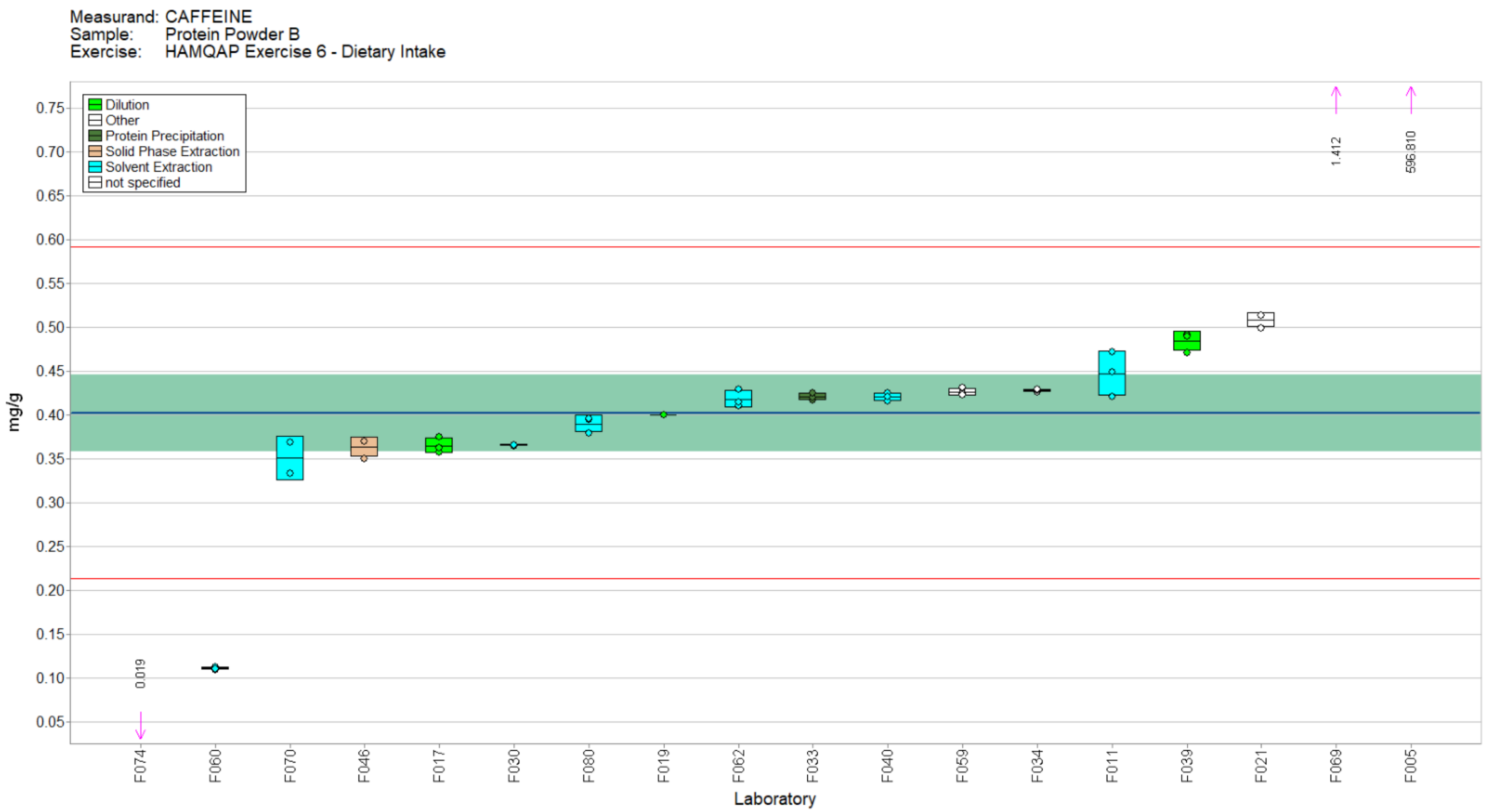


**Figure 7-2.** Caffeine in Protein Powder B (data summary view – sample preparation method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The solid blue 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 $Z'_{\text{comm}}$ score, $|Z'_{\text{comm}}| \leq 2$. A NIST value has not been determined in this material.

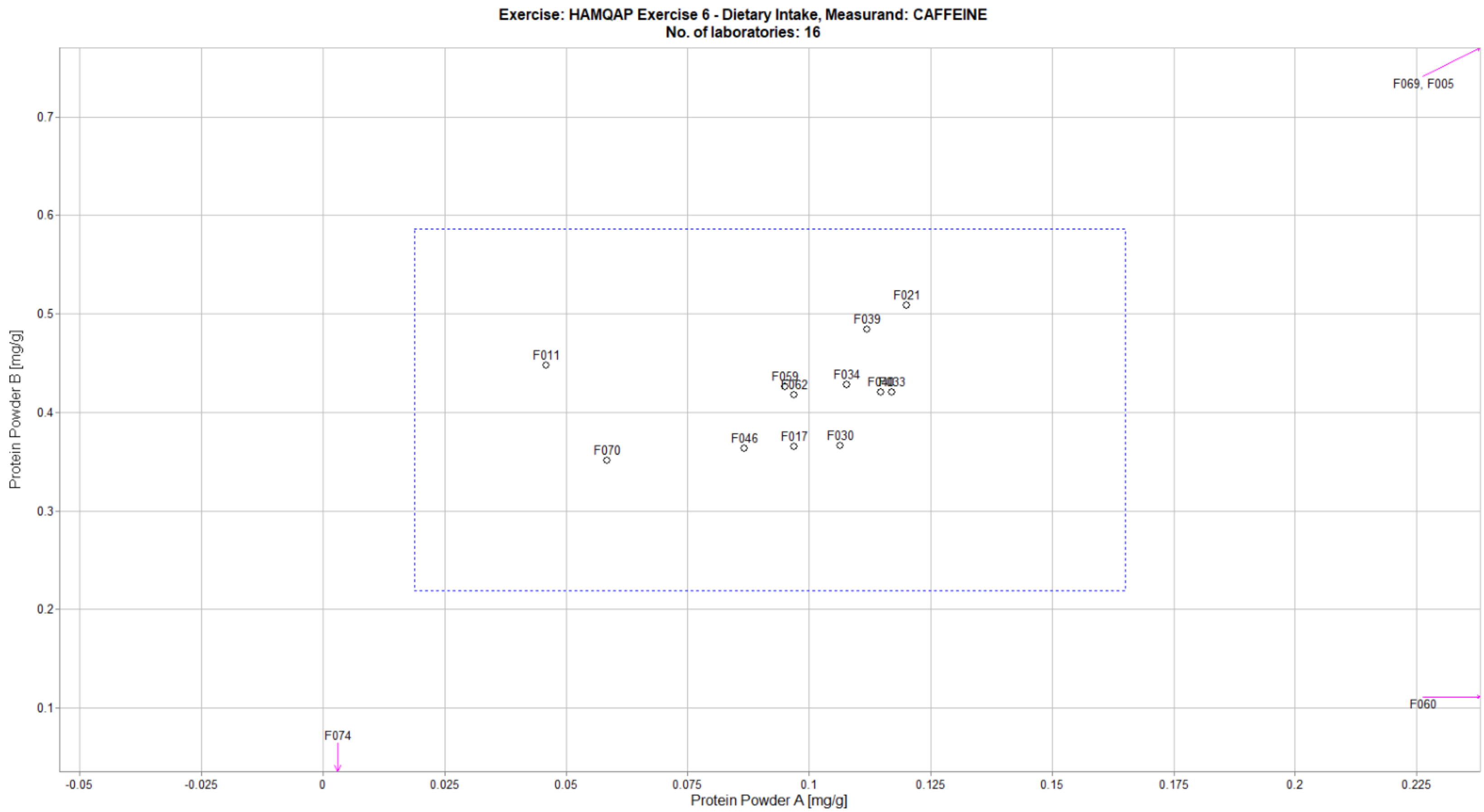


**Figure 7-3.** Laboratory means for caffeine in Protein Powder A and Protein Powder B (sample/sample comparison view). In this view, the individual laboratory mean for one sample (Protein Powder A) is compared to the mean for a second sample (Protein Powder B). The dotted blue box represents the consensus range of tolerance for Protein Powder A (x-axis) and Protein Powder B (y-axis), calculated as the values above and below the consensus means that result in an acceptable $Z'_{\mathrm{comm}}$ score, $|Z'_{\mathrm{comm}}| \leq 2$.

**Table 7-3.** Data summary table for theobromine in protein powder samples. Data points highlighted in blue have been identified as outside the consensus tolerance limits and would be estimated to result in an unacceptable $Z'_{\text{comm}}$ score, $|Z'_{\text{comm}}| \geq 2$.

| | | Theobromine | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Protein Sample A (mg/g) | | | | | Protein Sample B (mg/g) | | | | |
| | **Lab** | **A** | **B** | **C** | **Avg** | **SD** | **A** | **B** | **C** | **Avg** | **SD** |
| Individual Results | Target | | | | | | | | | | |
| | F004 | | | | | | | | | | |
| | F005 | | | | | | | | | | |
| | F011 | 1.195 | 1.171 | 1.193 | 1.186 | 0.013 | 0.928 | 1.044 | 1.017 | 0.996 | 0.061 |
| | F017 | 1.03 | 1.08 | 1.11 | 1.073 | 0.040 | 1.68 | 1.55 | 1.61 | 1.613 | 0.065 |
| | F018 | | | | | | | | | | |
| | F019 | 1.2 | 1.2 | 1.2 | 1.200 | 0.000 | 0.9 | 0.9 | 0.9 | 0.900 | 0.000 |
| | F021 | | | | | | | | | | |
| | F030 | 1.06 | 1.07 | 1.04 | 1.057 | 0.015 | 0.848 | 0.858 | 0.853 | 0.853 | 0.005 |
| | F031 | 1.16 | 1.12 | 1.09 | 1.123 | 0.035 | 0.97 | 0.94 | 0.89 | 0.933 | 0.040 |
| | F032 | | | | | | | | | | |
| | F040 | 1.144 | 1.136 | 1.128 | 1.136 | 0.008 | 0.95 | 0.956 | 0.954 | 0.953 | 0.003 |
| | F046 | 1.11 | 1.15 | 1.17 | 1.143 | 0.031 | 1.2 | 1.18 | 1.17 | 1.183 | 0.015 |
| | F051 | | | | | | | | | | |
| | F056 | | | | | | | | | | |
| | F060 | | | | | | | | | | |
| | F062 | 1.004 | 1.157 | 1.101 | 1.087 | 0.077 | 0.97 | 0.9678 | 0.9704 | 0.969 | 0.001 |
| | F069 | 1.271 | 1.281 | 1.242 | 1.265 | 0.020 | 1.098 | 1.101 | 1.022 | 1.074 | 0.045 |
| | F070 | | | | | | 0.025 | 0.017 | | 0.021 | 0.006 |
| | F079 | | | | | | | | | | |
| | F080 | 1 | 0.979 | 1 | 0.993 | 0.012 | 0.912 | 0.932 | 0.914 | 0.919 | 0.011 |
| | F089 | | | | | | | | | | |
| Community Results | | Consensus Mean | | | 1.126 | | Consensus Mean | | | 0.980 | |
| | | Consensus Standard Deviation | | | 0.098 | | Consensus Standard Deviation | | | 0.149 | |
| | | Maximum | | | 1.265 | | Maximum | | | 1.613 | |
| | | Minimum | | | 0.993 | | Minimum | | | 0.021 | |
| | | N | | | 10 | | N | | | 11 | |

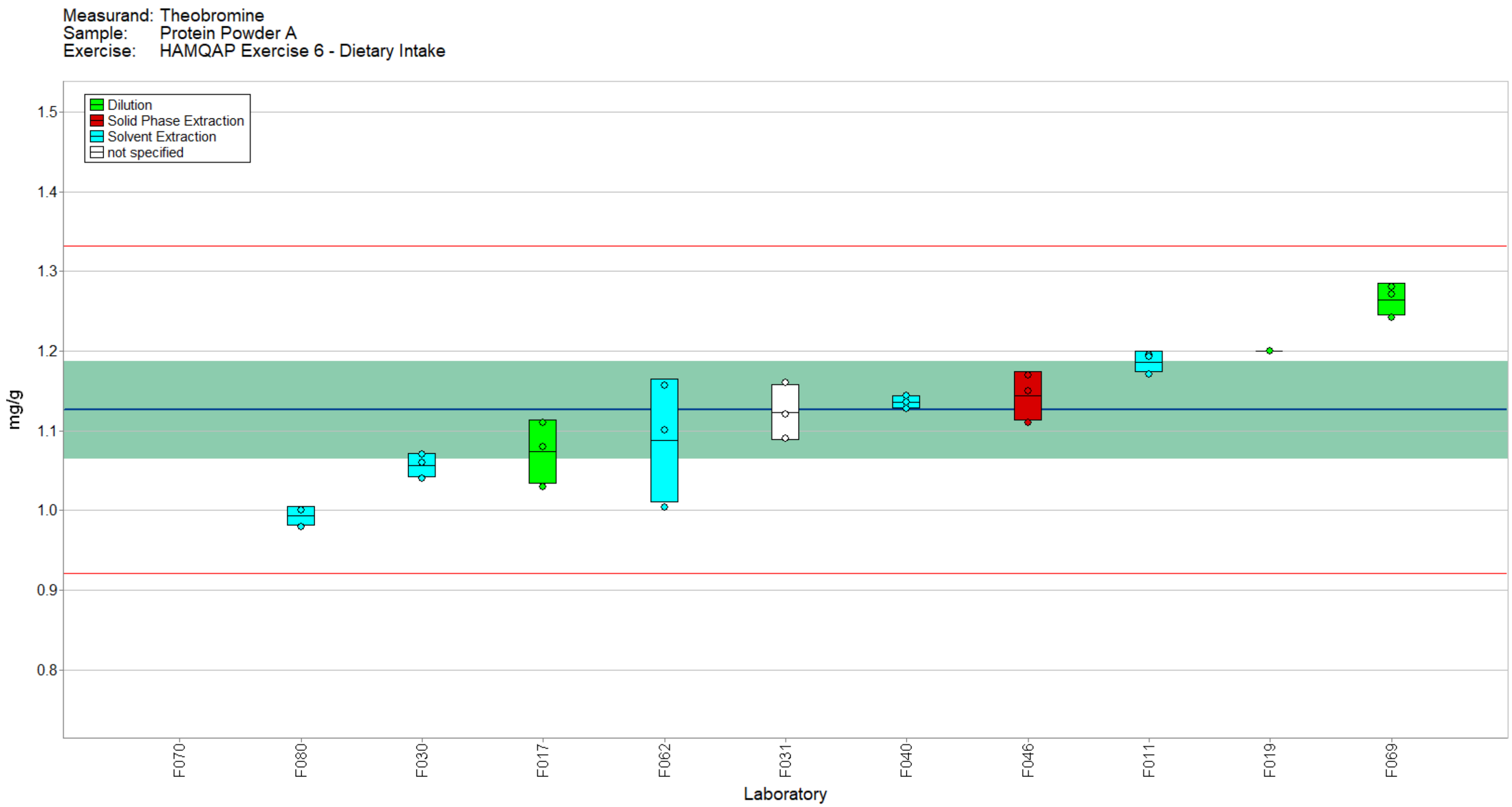


**Figure 7-4.** Theobromine in Protein Powder A (data summary view – sample preparation method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The solid blue 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 $Z'_{\mathrm{comm}}$ score, $|Z'_{\mathrm{comm}}| \le 2$. A NIST value has not been determined in this material.

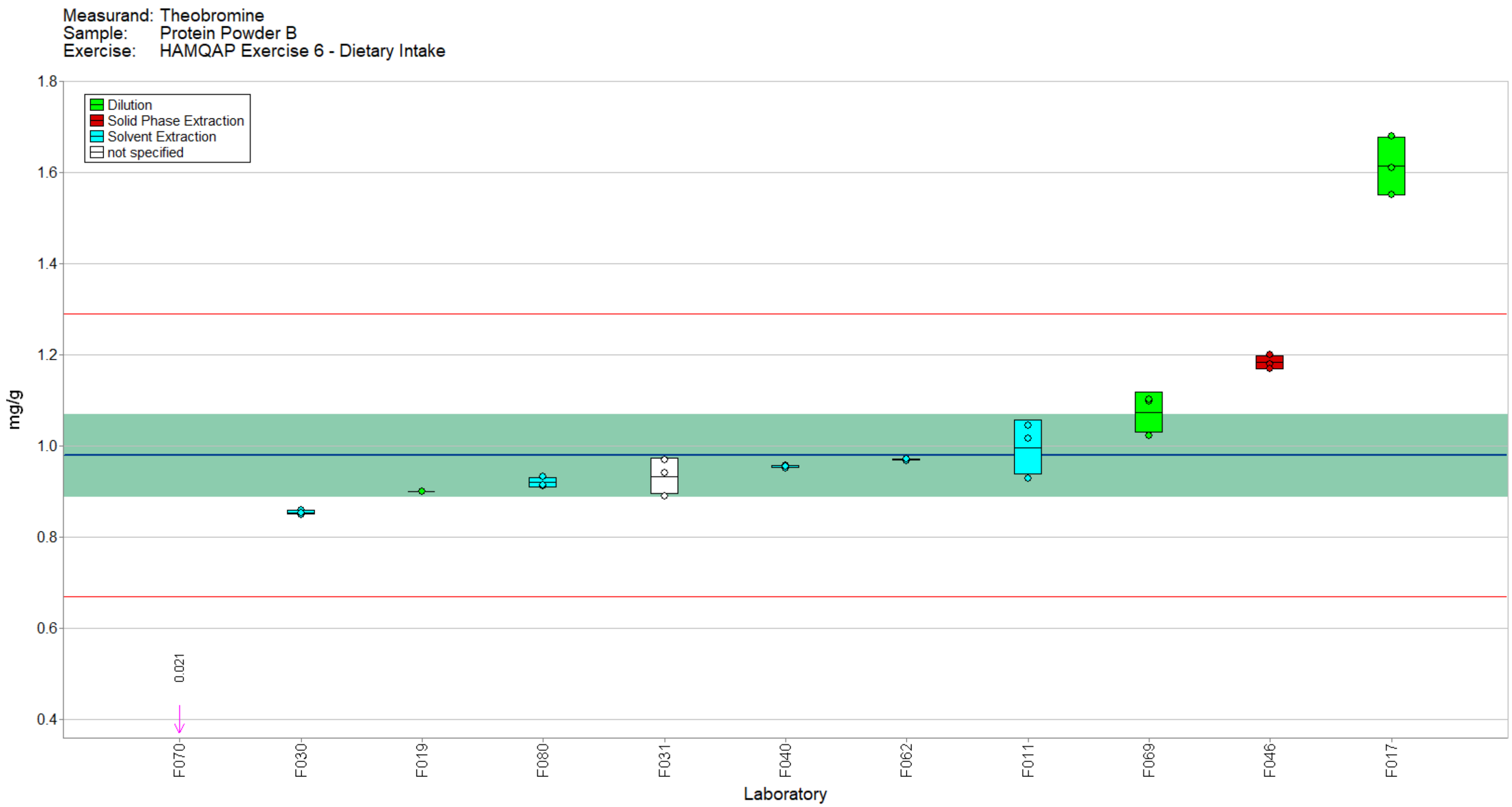


**Figure 7-5.** Theobromine in Protein Powder B (data summary view – sample preparation method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The solid blue 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 the consensus mean that result in an acceptable $Z'_{\mathrm{comm}}$ score, $|Z'_{\mathrm{comm}}| \leq 2$. A NIST value has not been determined in this material.

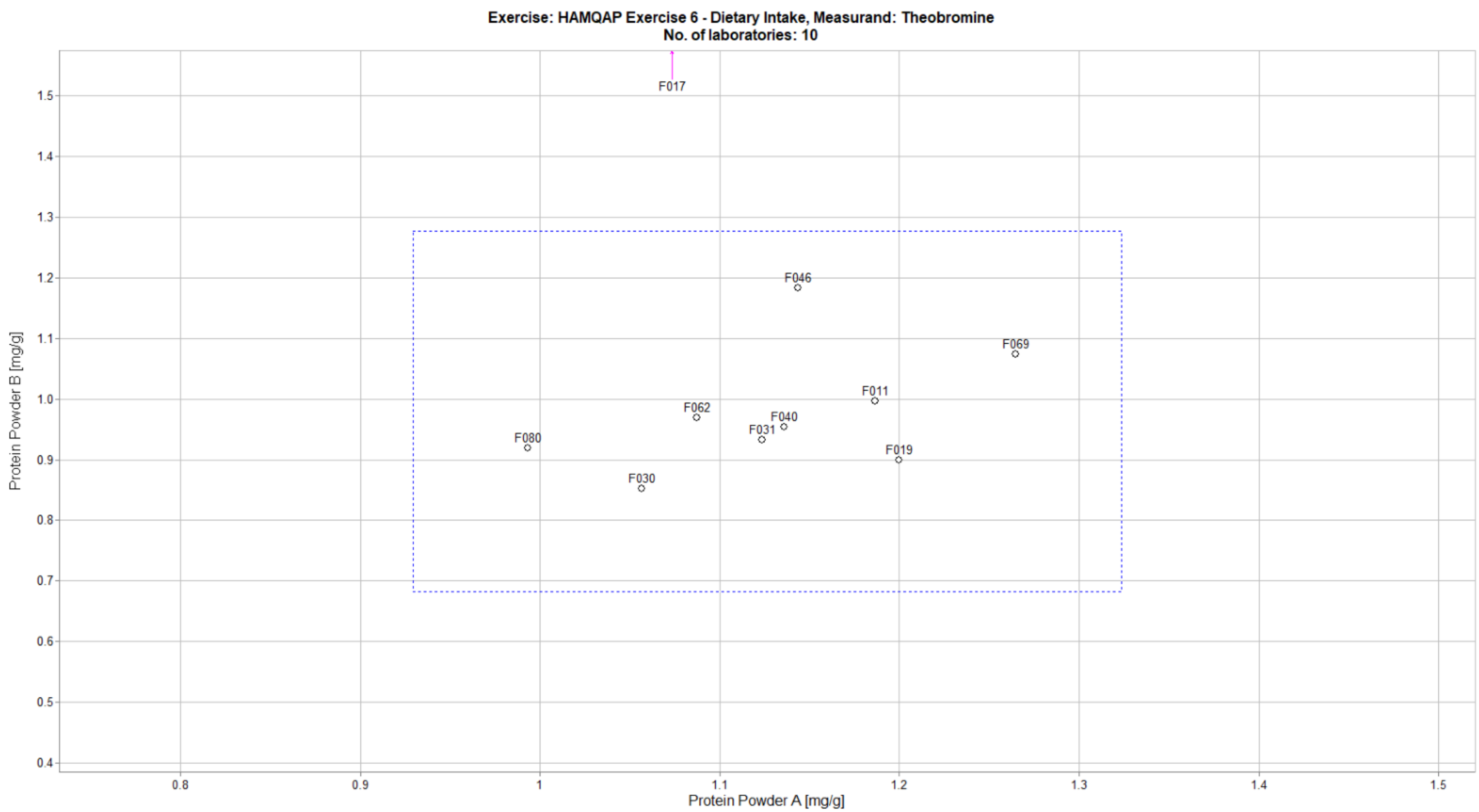


**Figure 7-6.** Laboratory means for theobromine in Protein Powder A and Protein Powder B (sample/sample comparison view). In this view, the individual laboratory mean for one sample (Protein Powder A) is compared to the mean for a second sample (Protein Powder B). The dotted blue box represents the consensus range of tolerance for Protein Powder A (x-axis) and Protein Powder B (y-axis), calculated as the values above and below the consensus means that result in an acceptable $Z'_{\mathrm{comm}}$ score, $|Z'_{\mathrm{comm}}| \leq 2$.

**Table 7-4.** Data summary table for theophylline in protein powder samples. Data points highlighted in blue have been identified as outside the consensus tolerance limits and would be estimated to result in an unacceptable $Z'_{\mathrm{comm}}$ score, $|Z'_{\mathrm{comm}}| \geq 2$.

| | | Theophylline | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Protein Sample A (mg/g) | | | | | Protein Sample B (mg/g) | | | | |
| | Lab | A | B | C | Avg | SD | A | B | C | Avg | SD |
| Individual Results | Target | | | | | | | | | | |
| | F004 | | | | | | | | | | |
| | F005 | | | | | | | | | | |
| | F011 | | | | | | | | | | |
| | F017 | 0.022 | 0.021 | 0.019 | 0.021 | 0.002 | 0.029 | 0.029 | 0.029 | 0.029 | 0 |
| | F018 | | | | | | | | | | |
| | F019 | | | | | | | | | | |
| | F021 | | | | | | | | | | |
| | F031 | | | | | | | | | | |
| | F040 | 0.13 | 0.13 | 0.128 | 0.129 | 0.001 | 0.033 | 0.035 | 0.033 | 0.034 | 0.001 |
| | F046 | 0.33 | 0.36 | 0.48 | 0.390 | 0.079 | 0.01 | 0.01 | 0.01 | 0.01 | 0 |
| | F056 | | | | | | | | | | |
| | F060 | | | | | | | | | | |
| | F062 | | | | | | | | | | |
| | F070 | 1.031 | 0.916 | | 0.974 | 0.081 | 0.852 | 0.747 | | 0.800 | 0.074 |
| | F079 | | | | | | | | | | |
| Community Results | | Consensus Mean | | | 0.378 | | Consensus Mean | | | 0.024 | |
| | | Consensus Standard Deviation | | | 0.512 | | Consensus Standard Deviation | | | 0.042 | |
| | | Maximum | | | 0.974 | | Maximum | | | 0.800 | |
| | | Minimum | | | 0.021 | | Minimum | | | 0.01 | |
| | | N | | | 4 | | N | | | 4 | |

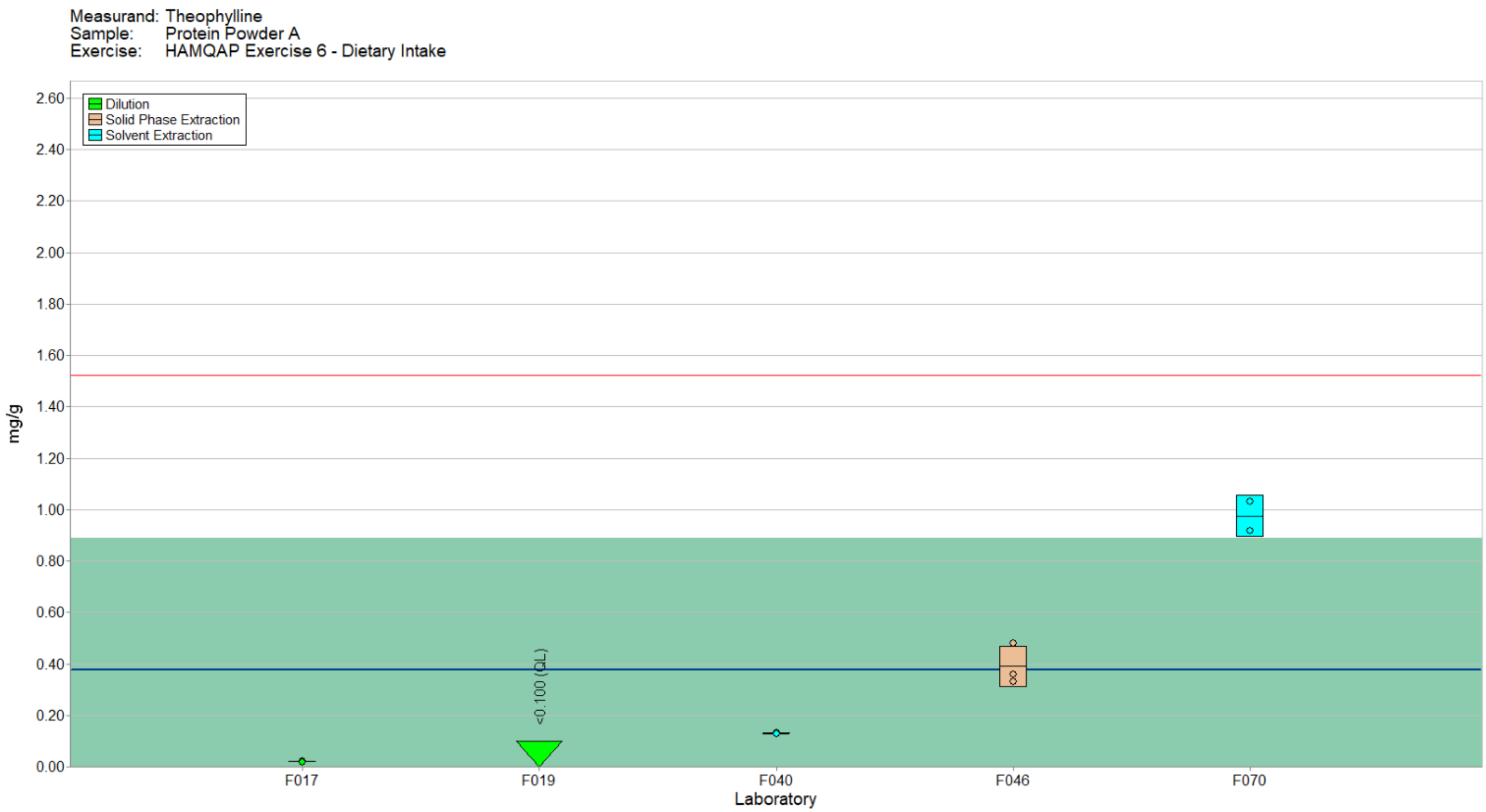


**Figure 7-7.** Theophylline in Protein Powder A (data summary view – sample preparation method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The solid blue 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 $Z'_{\mathrm{comm}}$ score, $|Z'_{\mathrm{comm}}| \leq 2$. A NIST value has not been determined in this material.

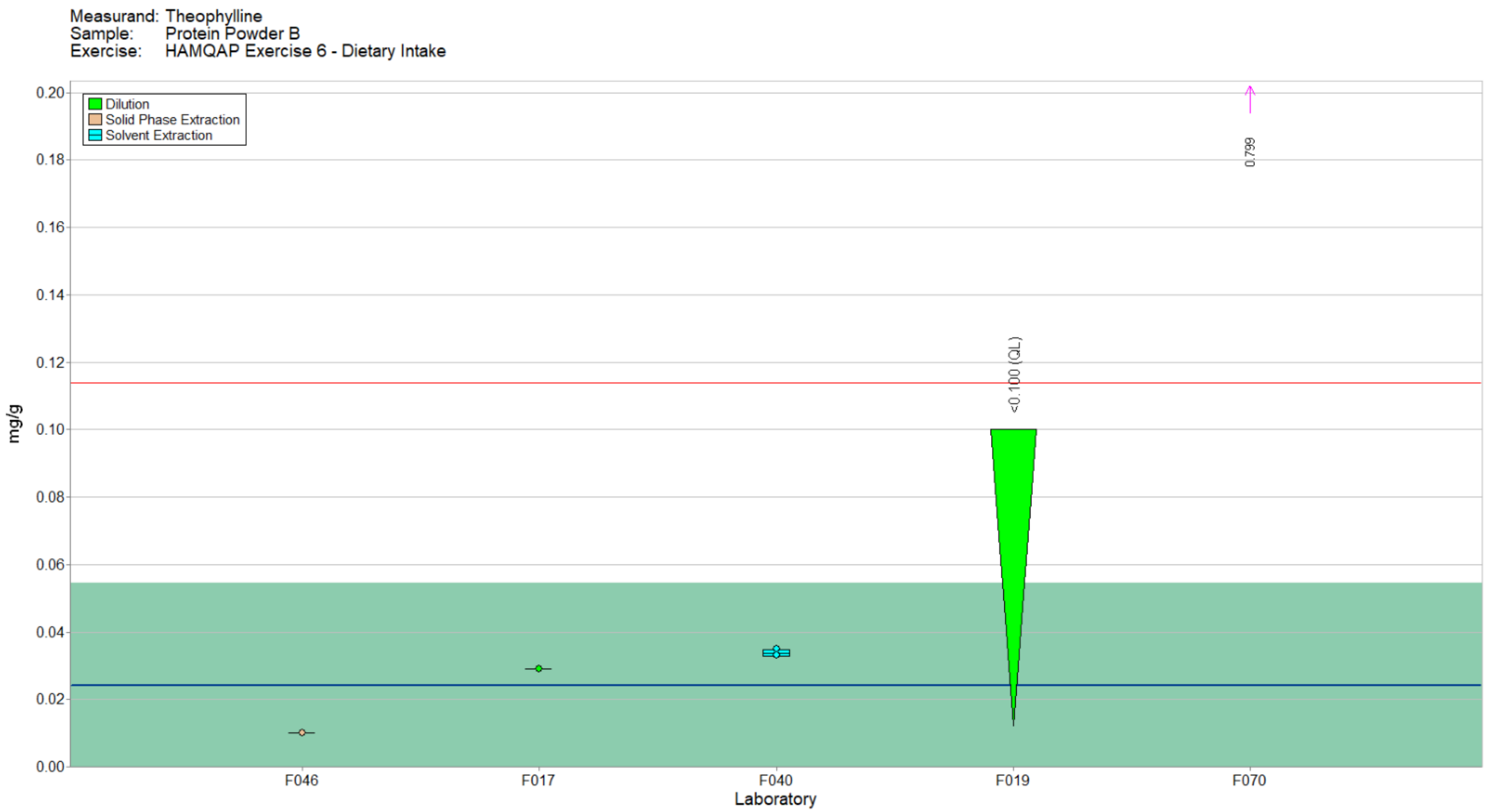


**Figure 7-8.** Theophylline in Protein Powder B (data summary view – sample preparation method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The solid blue 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 the consensus mean that result in an acceptable $Z'_{\text{comm}}$ score, $|Z'_{\text{comm}}| \leq 2$. A NIST value has not been determined in this material.

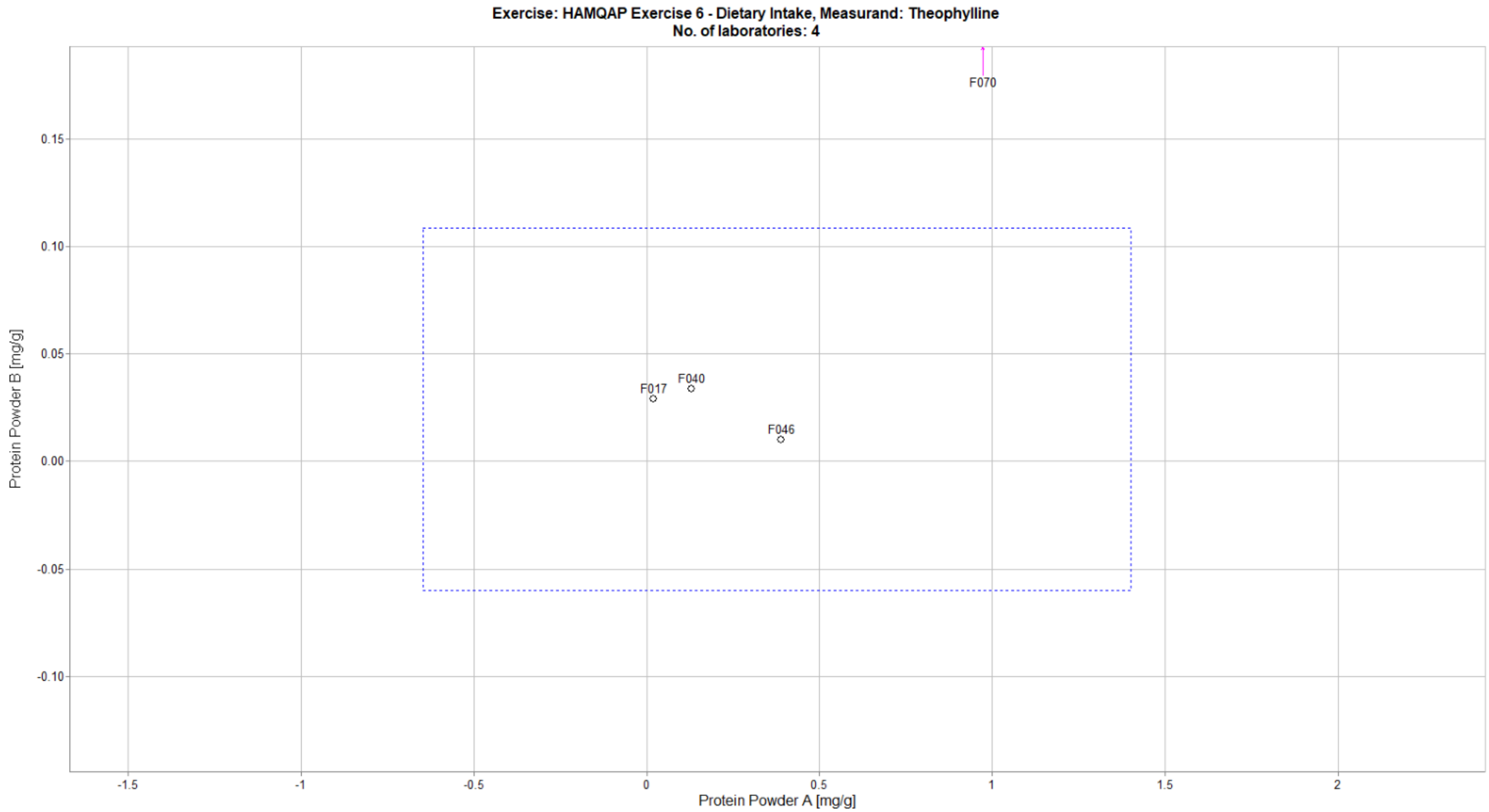


**Figure 7-9.** Laboratory means for theophylline in Protein Powder A and Protein Powder B (sample/sample comparison view). In this view, the individual laboratory mean for one sample (Protein Powder A) is compared to the mean for a second sample (Protein Powder B). The dotted blue box represents the consensus range of tolerance for Protein Powder A (x-axis) and Protein Powder B (y-axis), calculated as the values above and below the consensus means that result in an acceptable $Z'_{\text{comm}}$ score, $|Z'_{\text{comm}}| \leq 2$.

## SECTION 8: CONTAMINANTS I (Chlorate, Perchlorate)



Study Overview

In this study, participants were provided with six infant formula samples for dietary intake. Participants were asked to use in-house analytical methods to determine the mass fraction (ng/g) of chlorate and perchlorate in each matrix. Chlorine compounds possess bactericidal and sanitizing properties and therefore are commonly used in agriculture, water treatment, and industrial food manufacturing for sanitation purposes. However, the formation of chlorinated residues as by-products of their use has raised concerns with food regulatory bodies. Perchlorate is a chemical that occurs naturally in the environment and is also used in explosives, fireworks, road flares, and rocket propellant. A combination of human activity and natural sources has led to the widespread presence of perchlorate in the environment. Previous CDC studies have shown that nearly everyone in the U.S. is regularly exposed to low levels of perchlorate through eating food, as well as drinking milk and water that contain chlorate and perchlorate. Trace levels of chlorate and perchlorate have been found in both breast milk and infant formula. High levels of perchlorate (thousands of times higher than the doses estimated to result from consumption of infant formula or breast milk) affects the thyroid gland by blocking its ability to use iodine. Measurement of chlorate and perchlorate in infant formulas is critical to understand exposure of infants and to reduce risk of long-term health effects.

Dietary Intake Sample Information

*Infant Formula B.* Participants were provided three packets of SRM 1869 Infant/Adult Nutritional Formula II, a milk, whey, and soy-based infant/adult nutritional formula, each containing 10 g of material. Participants were asked to store the material at -20 °C, to thoroughly mix the contents of each packet before use, and to use a sample size appropriate for their in-house method of analysis. Participants were asked 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. The NIST-determined values for chlorate and perchlorate were assigned using results from a previous interlaboratory comparison. The NIST-determined values and uncertainties are provided in the table below on an as-received basis.

*Infant Formula C.* Participants were provided with one packet of commercial whey protein concentrate containing 100 g of material. Participants were asked to store the material at controlled room temperature (20 °C to 25 °C), thoroughly mix the contents of the packet before use, and to use a sample size appropriate for their in-house method of analysis. Participants were asked 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 NIST-determined values for chlorate and perchlorate were assigned using results from a previous interlaboratory comparison. The NIST-determined values and uncertainties are provided in the table below on an as-received basis.

*Infant Formula D.* Participants were provided with one packet of soy protein concentrate containing 20 g of material. Participants were asked to store the material at controlled room temperature (20 °C to 25 °C), to thoroughly mix the contents of the packet before use, and to use a sample size appropriate for their in-house method of analysis. Participants were asked 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, and no target values have been established for chlorate and perchlorate in this sample.

*Infant Formula E.* Participants were provided with one packet of commercial whey protein concentrate containing 100 g of material. Participants were asked to store the material at controlled room temperature (20 °C to 25 °C), to thoroughly mix the contents of the packet before use, and to use a sample size appropriate for their in-house method of analysis. Participants were asked 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 NIST-determined values for chlorate and perchlorate were assigned using results from a previous interlaboratory comparison. The NIST-determined values and uncertainties are provided in the table below on an as-received basis.

*Infant Formula F.* Participants were provided with one can of hydrolyzed soy based infant formula containing 400 g of material. Participants were asked to store the material at controlled room temperature, 20 °C to 25 °C, to thoroughly mix the contents of the can before use, and to use a sample size appropriate for their in-house method of analysis. Participants were asked to prepare three samples and report three values from the single can provided. The approximate analyte levels were not reported to participants prior to the study. The NIST-determined values for chlorate and perchlorate were assigned using results from a previous interlaboratory comparison. The NIST-determined values and uncertainties are provided in the table below on an as-received basis.

*Infant Formula G.* Participants were provided with one can of RM 8260 Infant Nutritional Formula Hydrolyzed Milk Based, containing 400 g of material. Participants were asked to store the material at controlled room temperature, 20 °C to 25 °C, to thoroughly mix the contents of the can before use, and to use a sample size appropriate for their in-house method of analysis. Participants were asked to prepare three samples and report three values from the single can provided. The approximate analyte levels were not reported to participants prior to the study. The NIST-determined values for chlorate and perchlorate were assigned using results from a previous interlaboratory comparison. The NIST-determined values and uncertainties are provided in the table below on an as-received basis.

| | NIST-Determined Mass Fractions (as-received basis) (ng/g) | |
|---|---|---|
| Analyte | Chlorate | Perchlorate |
| SRM 1869 | 104.0 ± 5.1 | |
| Infant Formula C | 1441 ± 118 | 30.0 ± 3.1 |
| Infant Formula E | 67 ± 12 | |
| Infant Formula F | 328 ± 31 | 5.75 ± 0.79 |
| RM 8260 | 265 ± 28 | |

Dietary Intake Study Results

- For both chlorate and perchlorate, 36 laboratories requested samples and 29 laboratories returned results for both analytes in all six infant formula samples (81 % participation). Reported values include non-quantitative results (zero or below LOQ) but are included in the participation statistics.
- The between-laboratory variabilities were very good or good for chlorate in all infant formulas. The between-laboratory variabilities were good for perchlorate in Infant Formula C and Infant Formula F. However, SRM 1869, Infant Formula E, and RM 8260 had between-laboratory variabilities over 100% for perchlorate (see table below).

| | Between-Laboratory Variability (% RSD) | |
|---|---|---|
| Analyte | Chlorate | Perchlorate |
| SRM 1869 | 16 % | >100 % |
| Infant Formula C | 22 % | 23 % |
| Infant Formula D | 27 % | 41 % |
| Infant Formula E | 24 % | >100 % |
| Infant Formula F | 18 % | 29 % |
| RM 8260 | 18% | >100 % |

- Most laboratories reported using either solvent extraction (41 % of laboratories reporting) or QuPPe sample preparation (24 %) for the determination of chlorate and perchlorate in the infant formula samples. Laboratories also reported use of dilution (10 %) and solvent extraction plus solid phase extraction (3 %). Six laboratories (21 %) did not report their sample preparation approach.
- Most laboratories reported using LC-MS (17 %) or liquid chromatography with tandem mass spectrometry (LC-MS/MS) (72 %) for determination of chlorate and perchlorate in infant formula. Three laboratories (10 %) did not describe the analytical approach used.

Dietary Intake Technical Recommendations

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

- Overall, laboratory performance was very good for laboratories measuring chlorate and perchlorate in these infant formula matrices. Levels of perchlorate in some matrices were below the LOQ of most common methods, leading to high between-laboratory variability for those samples.
- Analysis of chlorate and perchlorate are subject to contamination from everyday laboratory conditions.
  - Care must be taken to perform analyses in a chlorate- and perchlorate-free environment, which includes use of dedicated glassware, reagents, and other apparatuses.
  - Solvent and reagent blanks should be included with the analytical protocol to identify any potential biases that could arise from sample or instrument contamination.
- Most laboratories reported use of solvent extraction to prepare infant formula samples for analysis of chlorate and perchlorate. No trends were observed that correlated reported results with the sample preparation approach used.

- Most laboratories reported use of MS-based methodologies for determination of chlorate and perchlorate. Isotopically labeled internal standards, added at the beginning of the analytical procedure, often result in improved accuracy and precision of results.
- No trends were observed for within laboratory variability for chlorate or perchlorate.
- Any extraction procedure should be optimized to determine the most effective extraction solvent to ensure exhaustive extraction of the analyte from the matrix.
- "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 LOQ or QL.
- 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 materials prepared in-house.
- A linear calibration curve which surrounds 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 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 8-1.** Individualized data summary table (NIST) for chlorate and perchlorate in infant formulas.

## *National Institute of Standards and Technology*

**HAMQAP Exercise 6 - Contaminants I**

| | **Lab Code:** | **NIST** | **1. Your Results** | | | | **2. Community Results** | | | **3. Target** | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Analyte | Sample | Units | $x_i$ | $s_i$ | $Z'_{comm}$ | $Z_{NIST}$ | N | x* | s* | $x_{NIST}$ | *U* |
| Chlorate | SRM 1869 Infant/Adult Nutritional Formula II | ng/g | 104 | 5.12 | | | 29 | 110 | 16 | 104 | 5.12 |
| Chlorate | Infant Formula C | ng/g | 1440 | 118 | | | 29 | 1780 | 310 | 1440 | 118 |
| Chlorate | Infant Formula D | ng/g | | | | | 24 | 21.8 | 4.8 | | |
| Chlorate | Infant Formula E | ng/g | 66.8 | 12.1 | | | 28 | 50 | 11 | 66.8 | 12.1 |
| Chlorate | Infant Formula F | ng/g | 328 | 31.2 | | | 29 | 350 | 60 | 328 | 31.2 |
| Chlorate | RM 8260 Infant Nutritional Formula | ng/g | 265 | 28.4 | | | 29 | 300 | 53 | 265 | 28.4 |
| Perchlorate | SRM 1869 Infant/Adult Nutritional Formula II | ng/g | | | | | 7 | 0 | 2 | | |
| Perchlorate | Infant Formula C | ng/g | 30 | 3.1 | | | 27 | 37.4 | 7.9 | 30 | 3.1 |
| Perchlorate | Infant Formula D | ng/g | | | | | 20 | 5.9 | 1.9 | | |
| Perchlorate | Infant Formula E | ng/g | | | | | 5 | 1.3 | 2.4 | | |
| Perchlorate | Infant Formula F | ng/g | 5.75 | 0.788 | | | 20 | 6.7 | 1.7 | 5.75 | 0.788 |
| Perchlorate | RM 8260 Infant Nutritional Formula | ng/g | | | | | 9 | 0 | 2 | | |

$x_i$ Mean of reported values
$s_i$ Standard deviation of reported values
$Z'_{comm}$ Z'-score with respect to community consensus
$Z_{NIST}$ Z-score with respect to NIST value

N Number of quantitative values reported
x* Robust mean of reported values
s* Robust standard deviation

$x_{NIST}$ NIST-assessed value
*U* expanded uncertainty about the NIST-assessed value

**Table 8-2.** Data summary table for chlorate in infant formulas. Data points highlighted in blue have been identified as outside the consensus tolerance limits and would be estimated to result in an unacceptable $Z'_{\text{comm}}$ score, $|Z'_{\text{comm}}| \geq 2$. Data points highlighted in red have a zero or non-numeric data point. *Note that this table spans two pages.*

| | | Chlorate | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | SRM 1869 Infant/Adult Nutritional Formula II (ng/g) | | | | | Infant Formula C (ng/g) | | | | | Infant Formula D (ng/g) | | | | |
| | Lab | A | B | C | Avg | SD | A | B | C | Avg | SD | A | B | C | Avg | SD |
| Individual Results | Target | | | | 104 | 5.12 | | | | 1441 | 118.2 | | | | | |
| | F003 | 88.05 | 80.61 | 89.05 | 85.9 | 4.6 | 1533.8 | 1635.38 | 1558.04 | 1576 | 53 | 18.25 | 17.85 | 17.63 | 17.91 | 0.3143247 |
| | F004 | | | | | | | | | | | | | | | |
| | F005 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | F006 | 137.18 | 132.93 | 136.27 | 135.46 | 2.24 | 2144.74 | 2178.98 | 2198.45 | 2174 | 27 | 26.28 | 27.35 | 25.89 | 26.51 | 0.76 |
| | F008 | | | | | | | | | | | | | | | |
| | F009 | 123.4 | 119 | 120.2 | 120.87 | 2.27 | 1818.3 | 1394.9 | 1637.1 | 1617 | 212 | | | | | |
| | F010 | 105.8 | 108.8 | 112.8 | 109.13 | 3.51 | 1258 | 1628 | 1510 | 1465 | 189 | 23.3 | 25.3 | 22.2 | 23.60 | 1.57 |
| | F016 | 113.9 | 113 | 112.7 | 113.20 | 0.62 | 1865 | 1877 | 1880 | 1874 | 7.9 | 20.4 | 19.4 | 21.2 | 20.33 | 0.90 |
| | F021 | | | | | | | | | | | | | | | |
| | F023 | 126.2 | 125.3 | | 125.75 | 0.64 | 1434 | 1407 | | 1421 | 19 | 20.7 | 19.2 | | 19.95 | 1.06 |
| | F024 | 112.3 | 104.7 | 104.7 | 107.23 | 4.39 | 1718.7 | 1851 | 1810.6 | 1793 | 68 | 20.3 | 19.3 | 20.5 | 20.03 | 0.64 |
| | F028 | 126 | 125 | 125 | 125.33 | 0.58 | 2030 | 2050 | 2110 | 2063 | 42 | 23.8 | 22.3 | 23 | 23.03 | 0.75 |
| | F029 | 100 | 110 | 100 | 103.33 | 5.77 | 1370 | 1330 | 1240 | 1313 | 67 | 1100 | 1040 | 980 | 1040 | 60 |
| | F030 | 114 | 126 | 115 | 118.33 | 6.66 | 1973 | 1499 | 1548 | 1673 | 261 | 19.9 | 21.8 | 20.9 | 20.87 | 0.95 |
| | F031 | 119.43 | 120.17 | 118.45 | 119.35 | 0.86 | 1708.84 | 1608.92 | 1528.91 | 1616 | 90 | 30.86 | 27.76 | 21.94 | 26.85 | 4.53 |
| | F033 | 117 | 114 | 119 | 116.67 | 2.52 | 1997 | 2004 | 1986 | 1996 | 9.1 | 28.2 | 30.6 | 25.8 | 28.20 | 2.40 |
| | F034 | | | | | | | | | | | | | | | |
| | F040 | 103 | 100 | 101 | 101.33 | 1.53 | 1885 | 1871 | 1812 | 1856 | 39 | 18 | 20 | 19 | 19.00 | 1.0 |
| | F043 | 100 | 110 | 100 | 103.33 | 5.77 | 2300 | 2100 | 2200 | 2200 | 100 | 10 | 7 | 13 | 10.00 | 3.0 |
| | F044 | 109 | 111 | 104 | 108.00 | 3.61 | 1678 | 1626 | 1651 | 1652 | 26 | 21 | 22 | 20 | 21.00 | 1.0 |
| | F050 | 108 | 102 | 93 | 101.00 | 7.55 | 1657 | 1697 | 1650 | 1668 | 25 | | | | | |
| | F053 | 93 | 99 | 91 | 94.33 | 4.16 | 1562 | 1423 | 1571 | 1519 | 83 | | | | | |
| | F058 | 81.7 | 80.8 | 80.7 | 81.07 | 0.55 | 2187.7 | 1993.5 | 1940.2 | 2040 | 130 | | | | | |
| | F061 | 110 | 120 | 130 | 120.00 | 10.0 | 1900 | 1900 | 1900 | 1900 | 0 | 21 | 23 | 23 | 22.33 | 1.15 |
| | F062 | 97 | 94 | 95 | 95.33 | 1.53 | 1575 | 1562 | 1606 | 1581 | 23 | 22 | 20 | 20 | 20.67 | 1.15 |
| | F063 | 133 | 115 | 140 | 129.33 | 12.9 | 2685 | 2700 | 2510 | 2632 | 106 | 15 | 22 | 20 | 19.00 | 3.61 |
| | F066 | 101 | 100 | 102 | 101.00 | 1.00 | 1970 | 2048 | 2034 | 2017 | 42 | 21 | 19 | 19 | 19.67 | 1.15 |
| | F067 | | | | | | | | | | | | | | | |
| | F068 | 94 | 93 | 94 | 93.67 | 0.58 | 1693 | 1691 | 1697 | 1694 | 3.1 | 11 | 11 | 12 | 11.33 | 0.58 |
| | F071 | 114 | 108 | 114 | 112.00 | 3.46 | 1690 | 1730 | 1660 | 1693 | 35 | 22 | 22 | 21 | 21.67 | 0.58 |
| | F078 | | | | | | | | | | | | | | | |
| | F080 | 97 | 97 | 94 | 96.00 | 1.73 | 1630 | 1600 | 1660 | 1630 | 30 | | | | | |
| | F082 | 120 | 116 | 122 | 119.33 | 3.06 | 1894 | 2072 | 2277 | 2081 | 192 | 22 | 35 | 23 | 26.67 | 7.23 |
| | F083 | 114 | 111 | 114 | 113.00 | 1.73 | 1700 | 1780 | 1800 | 1760 | 53 | 22 | 25.1 | 25.6 | 24.23 | 1.95 |
| | F087 | 120 | 110 | 120 | 116.67 | 5.77 | 1630 | 1940 | 1810 | 1793 | 156 | 35.8 | 30.5 | 32.3 | 32.87 | 2.70 |
| | F088 | | | | | | | | | | | | | | | |
| Community Results | | Consensus Mean | | | 109.6 | | Consensus Mean | | | 1783 | | Consensus Mean | | | 21.8 | |
| | | Consensus Standard Deviation | | | 15.5 | | Consensus Standard Deviation | | | 311.6 | | Consensus Standard Deviation | | | 4.79 | |
| | | Maximum | | | 135.5 | | Maximum | | | 2632 | | Maximum | | | 1040 | |
| | | Minimum | | | 0 | | Minimum | | | 0 | | Minimum | | | 0 | |
| | | N | | | 29 | | N | | | 29 | | N | | | 24 | |

**Chlorate**

**Individual Results**

| | Infant Formula E (ng/g) | | | | | Infant Formula F (ng/g) | | | | | RM 8260 Infant Nutritional Formula (ng/g) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Lab** | **A** | **B** | **C** | **Avg** | **SD** | **A** | **B** | **C** | **Avg** | **SD** | **A** | **B** | **C** | **Avg** | **SD** |
| Target | | | | 66.8 | 12.14 | | | | 328 | 31.2 | | | | 265 | 28.4 |
| F003 | 40.8 | 41.71 | 39.12 | 40.5 | 1.3 | 281.04 | 262.28 | 252.03 | 265.1 | 14.7 | 210.71 | 228.89 | 237.52 | 225.7 | 13.7 |
| F004 | | | | | | | | | | | | | | | |
| F005 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| F006 | 54.78 | 56.34 | 54.46 | 55.19 | 1.01 | 430.93 | 417.35 | 431.01 | 426.4 | 7.9 | 371.04 | 370.68 | 372.43 | 371.4 | 0.92 |
| F008 | | | | | | | | | | | | | | | |
| F009 | 56.6 | 50.8 | 50.3 | 52.57 | 3.50 | 408.7 | 447.7 | 377.8 | 411.4 | 35.0 | 366.6 | 323.4 | 307.2 | 332.4 | 30.7 |
| F010 | 42.8 | 42.9 | 45.2 | 43.63 | 1.36 | 338.7 | 322.9 | 346.1 | 335.9 | 11.9 | 268 | 264 | 260 | 264.0 | 4.00 |
| F016 | 41 | 43 | 40.2 | 41.40 | 1.44 | 344.8 | 356.4 | 344.8 | 348.7 | 6.70 | 290 | 305.3 | 290.6 | 295.3 | 8.67 |
| F021 | | | | | | | | | | | | | | | |
| F023 | 68 | 57 | | 62.50 | 7.78 | 383 | 400 | | 391.5 | 12.0 | 339 | 330 | | 334.5 | 6.36 |
| F024 | 50.3 | 50.1 | 43.9 | 48.10 | 3.64 | 360.8 | 364.4 | 360 | 361.7 | 2.34 | 289.6 | 289.4 | 294.6 | 291.2 | 2.95 |
| F028 | 49.8 | 49.4 | 49.3 | 49.50 | 0.26 | 391 | 396 | 418 | 401.7 | 14.4 | 354 | 349 | 345 | 349.3 | 4.51 |
| F029 | 330 | 370 | 430 | 377 | 50.3 | 290 | 320 | 310 | 306.7 | 15.3 | 220 | 260 | 220 | 233.3 | 23.1 |
| F030 | 36.6 | 36.8 | 36.7 | 36.70 | 0.10 | 381.6 | 294.7 | 278.2 | 318.2 | 55.6 | 264.4 | 303.6 | 289 | 285.7 | 19.8 |
| F031 | 52 | 40.31 | 40.57 | 44.29 | 6.68 | 334.12 | 323.69 | 297.98 | 318.6 | 18.6 | 288.3 | 262.79 | 245.63 | 265.6 | 21.5 |
| F033 | 66.1 | 64.7 | 68.2 | 66.33 | 1.76 | 402 | 389 | 399 | 396.7 | 6.81 | 354 | 365 | 346 | 355.0 | 9.54 |
| F034 | | | | | | | | | | | | | | | |
| F040 | 43 | | 47 | 45.00 | 2.83 | 334 | 337 | 339 | 336.7 | 2.52 | 307 | 284 | 282 | 291.0 | 13.9 |
| F043 | 36 | 24 | 30 | 30.00 | 6.00 | 360 | 300 | 240 | 300.0 | 60.0 | 290 | 240 | 340 | 290.0 | 50.0 |
| F044 | 41 | 43 | 41 | 41.67 | 1.15 | 349 | 343 | 360 | 350.7 | 8.62 | 310 | 295 | 293 | 299.3 | 9.29 |
| F050 | | | | | | 337 | 327 | 327 | 330.3 | 5.77 | 307 | 333 | 317 | 319.0 | 13.1 |
| F053 | | 40 | 41 | 40.50 | 0.71 | 326 | 298 | 322 | 315.3 | 15.1 | 271 | 268 | 260 | 266.3 | 5.69 |
| F058 | 64.4 | 63.2 | 70.6 | 66.07 | 3.97 | 245.7 | 230.6 | 233.1 | 236.5 | 8.09 | 174.9 | 177.8 | 171.9 | 174.9 | 2.95 |
| F061 | 480 | 480 | 490 | 483.33 | 5.77 | 370 | 390 | 410 | 390.0 | 20.0 | 310 | 320 | 320 | 316.7 | 5.77 |
| F062 | 39 | 50 | 45 | 44.67 | 5.51 | 285 | 268 | 253 | 268.7 | 16.0 | 292 | 268 | 314 | 291.3 | 23.0 |
| F063 | 40 | 41 | 41 | 40.67 | 0.58 | 390 | 390 | 403 | 394.3 | 7.51 | 330 | 339 | 327 | 332.0 | 6.24 |
| F066 | 39 | 38 | 37 | 38.00 | 1.00 | 380 | 379 | 359 | 372.7 | 11.8 | 311 | 303 | 302 | 305.3 | 4.93 |
| F067 | | | | | | | | | | | | | | | |
| F068 | 38 | 31 | 35 | 34.67 | 3.51 | 279 | 281 | 290 | 283.3 | 5.86 | 260 | 252 | 264 | 258.7 | 6.11 |
| F071 | 37 | 36 | 32 | 35.00 | 2.65 | 323 | 333 | 330 | 328.7 | 5.13 | 281 | 277 | 278 | 278.7 | 2.08 |
| F078 | | | | | | | | | | | | | | | |
| F080 | 42 | 42 | 42 | 42.00 | 0.00 | 360 | 330 | 340 | 343.3 | 15.3 | 272 | 273 | 275 | 273.3 | 1.53 |
| F082 | 42 | 55 | 54 | 50.33 | 7.23 | 338 | 393 | 414 | 381.7 | 39.2 | 380 | 332 | 395 | 369.0 | 32.9 |
| F083 | 43.8 | 43.6 | 34.9 | 40.77 | 5.08 | 367 | 373 | 392 | 377.3 | 13.1 | 310 | 339 | 328 | 325.7 | 14.6 |
| F087 | 63.2 | 63.8 | 61.8 | 62.93 | 1.03 | 421 | 352 | 376 | 383.0 | 35.0 | 367 | 351 | 340 | 352.7 | 13.6 |
| F088 | | | | | | | | | | | | | | | |

**Community Results**

| | Infant Formula E (ng/g) | Infant Formula F (ng/g) | RM 8260 Infant Nutritional Formula (ng/g) |
|---|---|---|---|
| Consensus Mean | 45.6 | 346.3 | 299.8 |
| Consensus Standard Deviation | 11.4 | 59.5 | 52.7 |
| Maximum | 483.3 | 426.4 | 371.4 |
| Minimum | 0 | 0 | 0 |
| N | 28 | 29 | 29 |

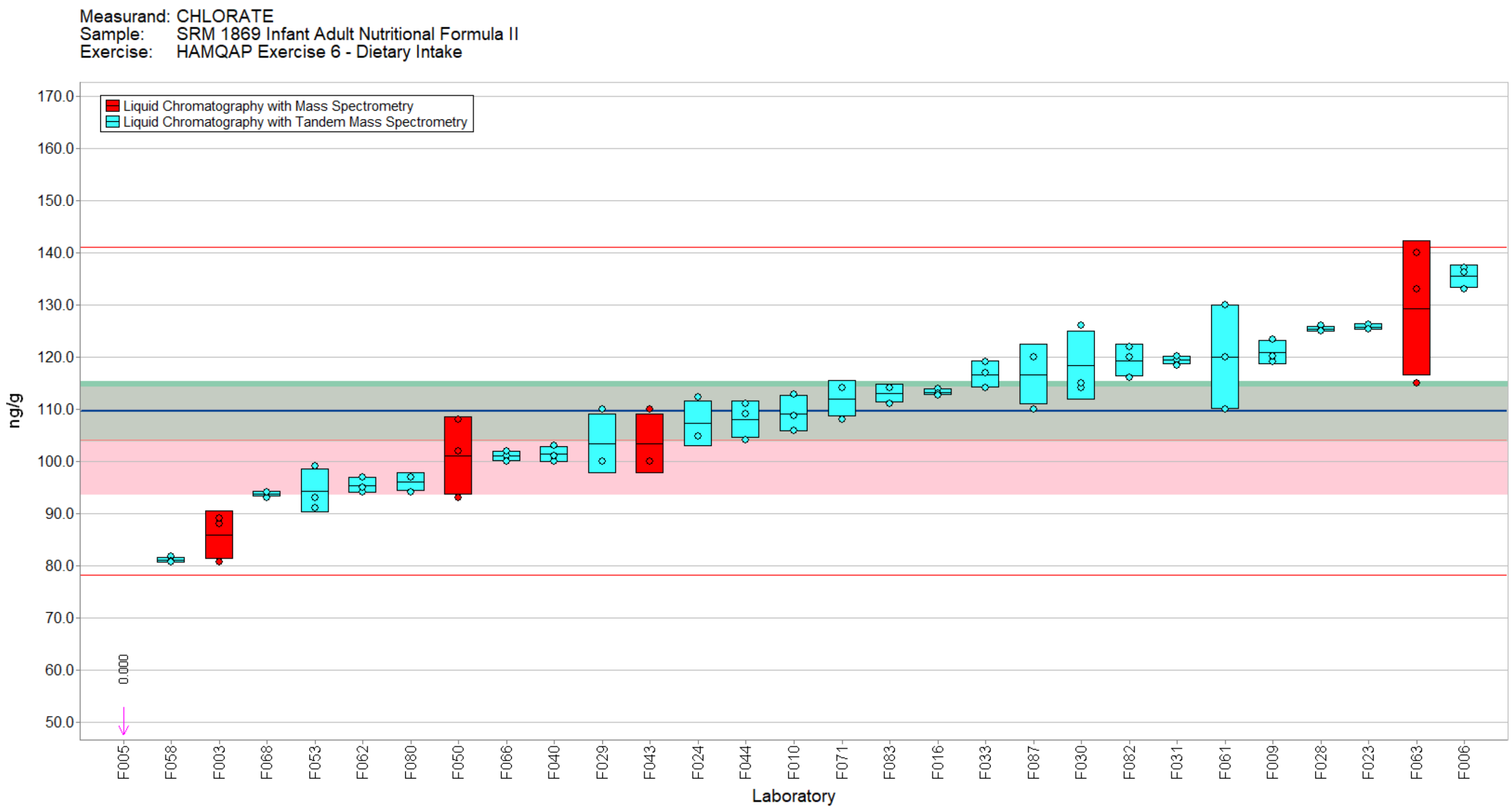


**Figure 8-1.** Chlorate in SRM 1869 Infant/Adult Nutritional Formula II (milk/whey/soy-based) (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue 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 $Z'_{\text{comm}}$ score, $|Z'_{\text{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty ($U_{\text{NIST}}$) and represents the range that results in an acceptable $Z'_{\text{NIST}}$ score, $|Z'_{\text{NIST}}| \leq 2$.

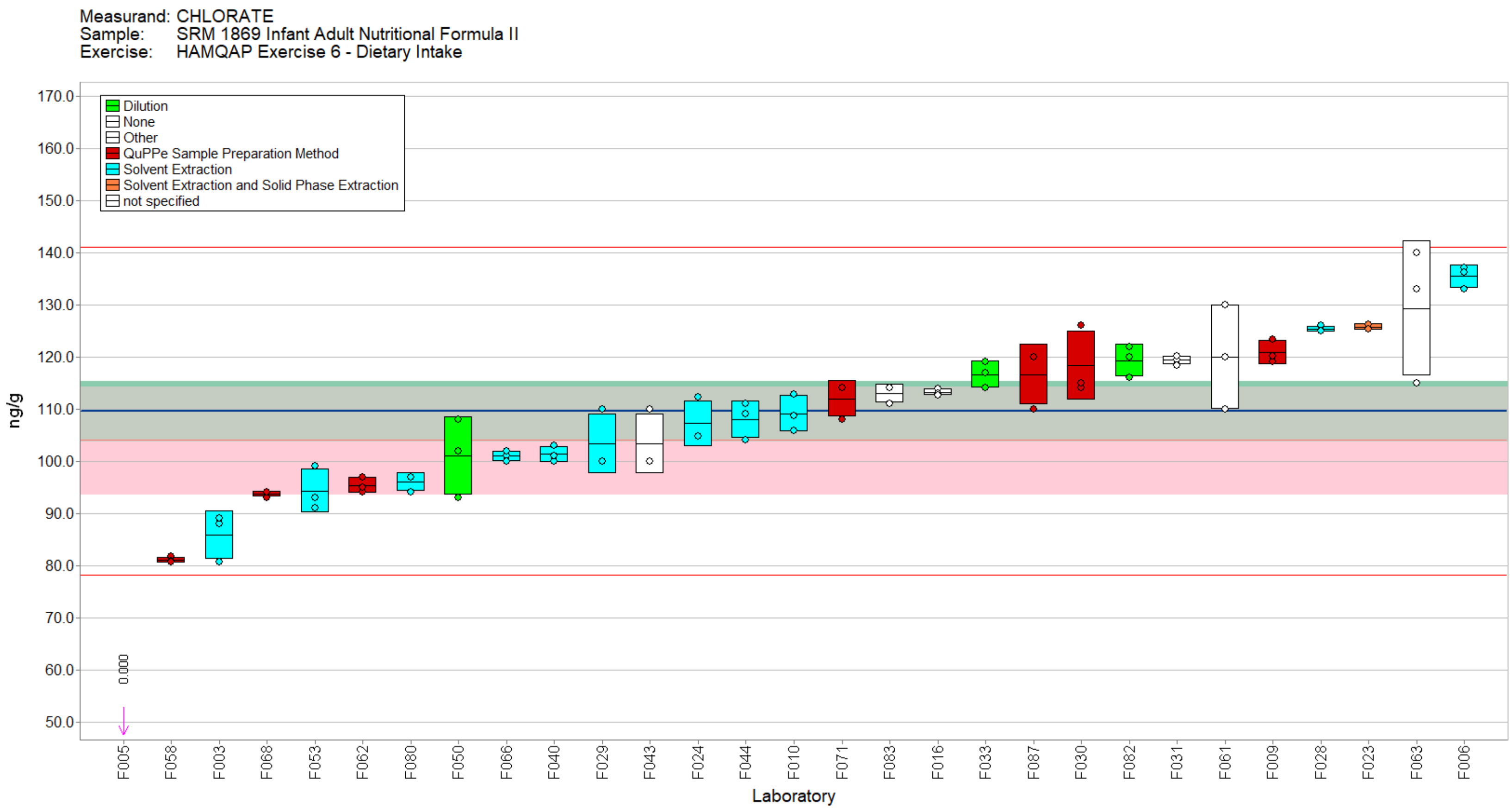


**Figure 8-2.** Chlorate in SRM 1869 Infant/Adult Nutritional Formula II (milk/whey/soy-based) (data summary view – sample preparation method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The solid blue 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 $Z'_{\mathrm{comm}}$ score, $|Z'_{\mathrm{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty ($U_{\mathrm{NIST}}$) and represents the range that results in an acceptable $Z'_{\mathrm{NIST}}$ score, $|Z'_{\mathrm{NIST}}| \leq 2$.

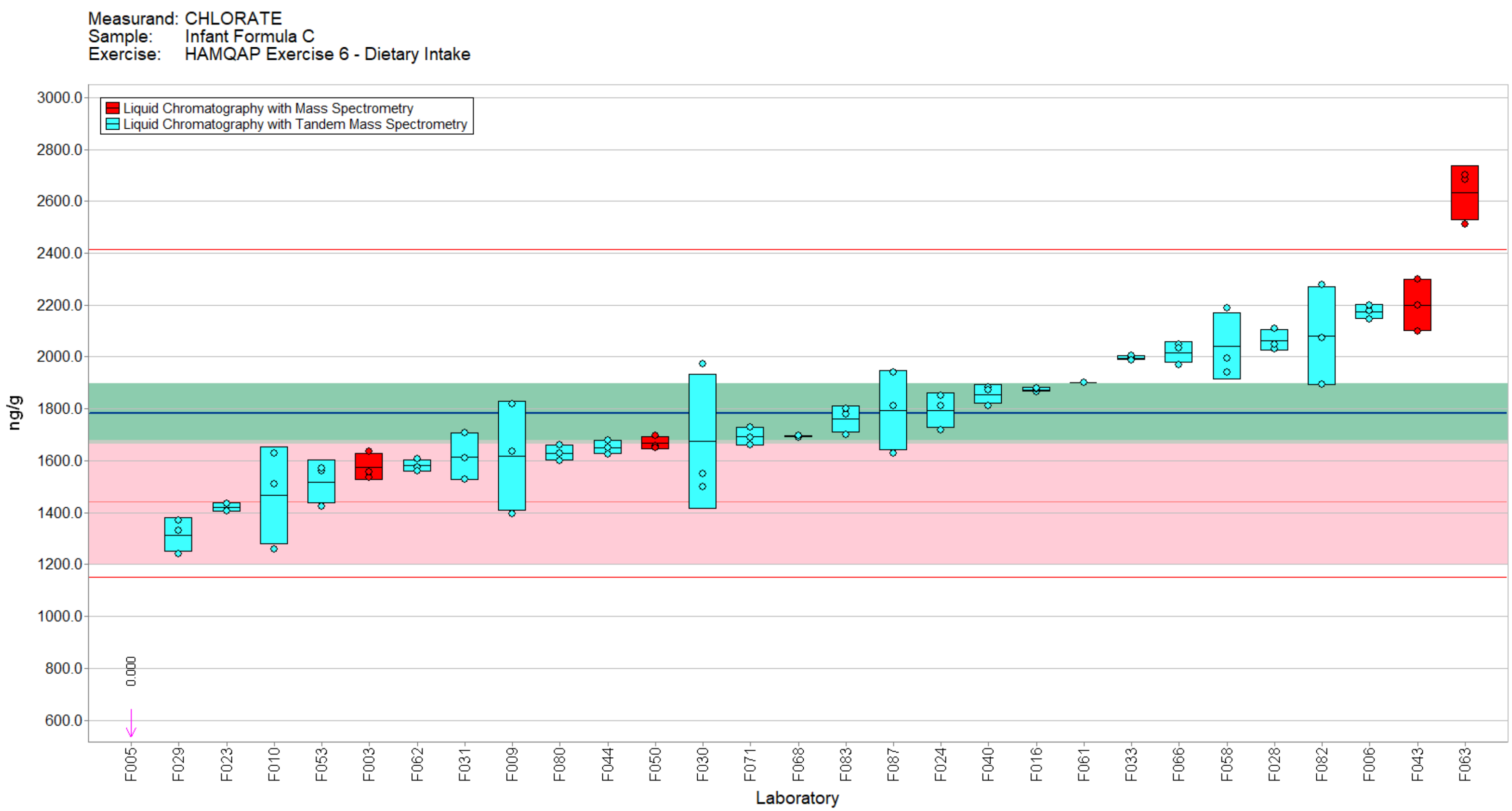


**Figure 8-3.** Chlorate in Infant Formula C (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line 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 $Z'_{\text{comm}}$ score, $|Z'_{\text{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty ($U_{\text{NIST}}$) and represents the range that results in an acceptable $Z'_{\text{NIST}}$ score, $|Z'_{\text{NIST}}| \leq 2$.

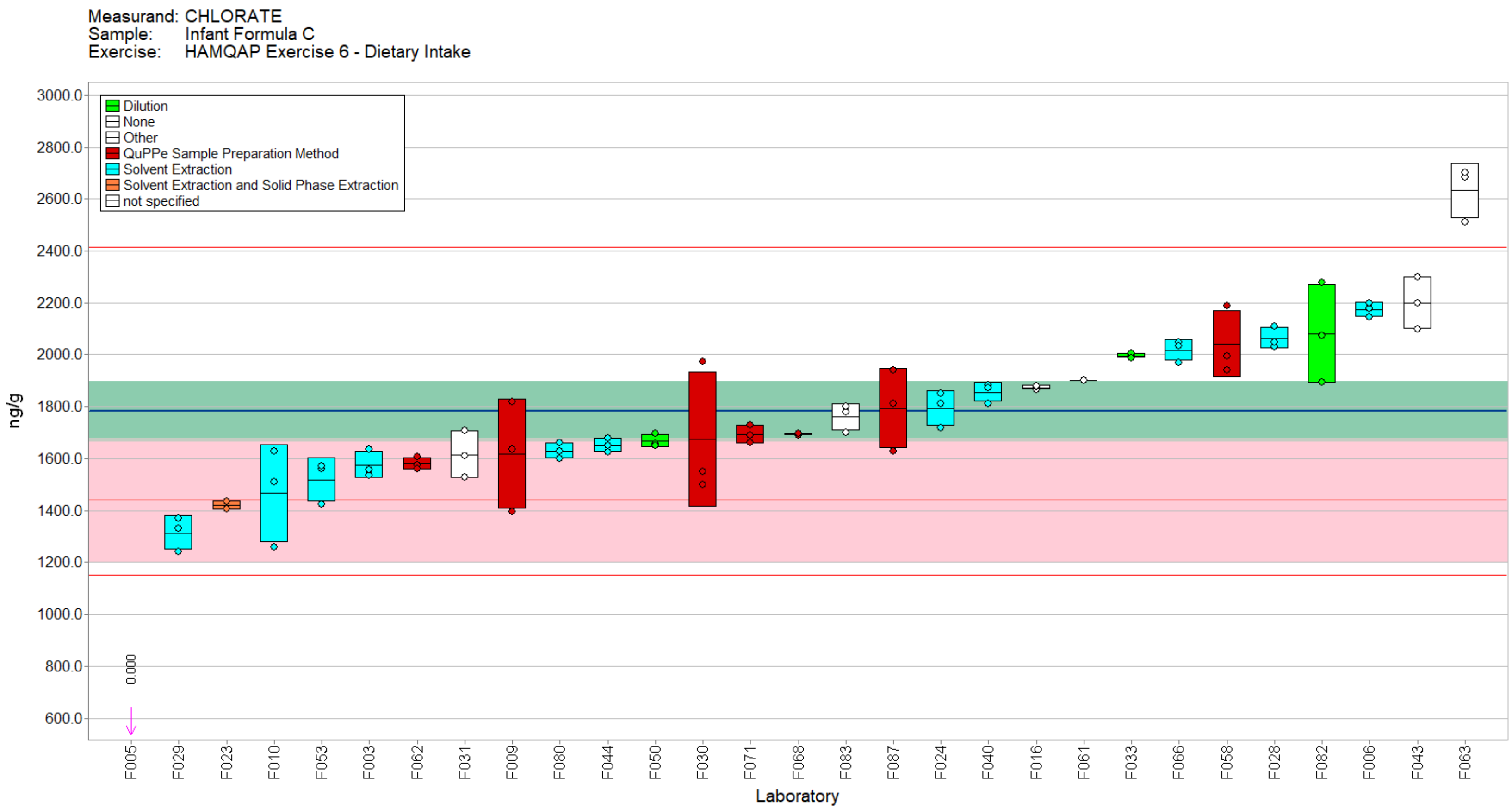


**Figure 8-4.** Chlorate in Infant Formula C (data summary view – sample preparation method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The solid blue 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 $Z'_{\text{comm}}$ score, $|Z'_{\text{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty ($U_{\text{NIST}}$) and represents the range that results in an acceptable $Z'_{\text{NIST}}$ score, $|Z'_{\text{NIST}}| \leq 2$.

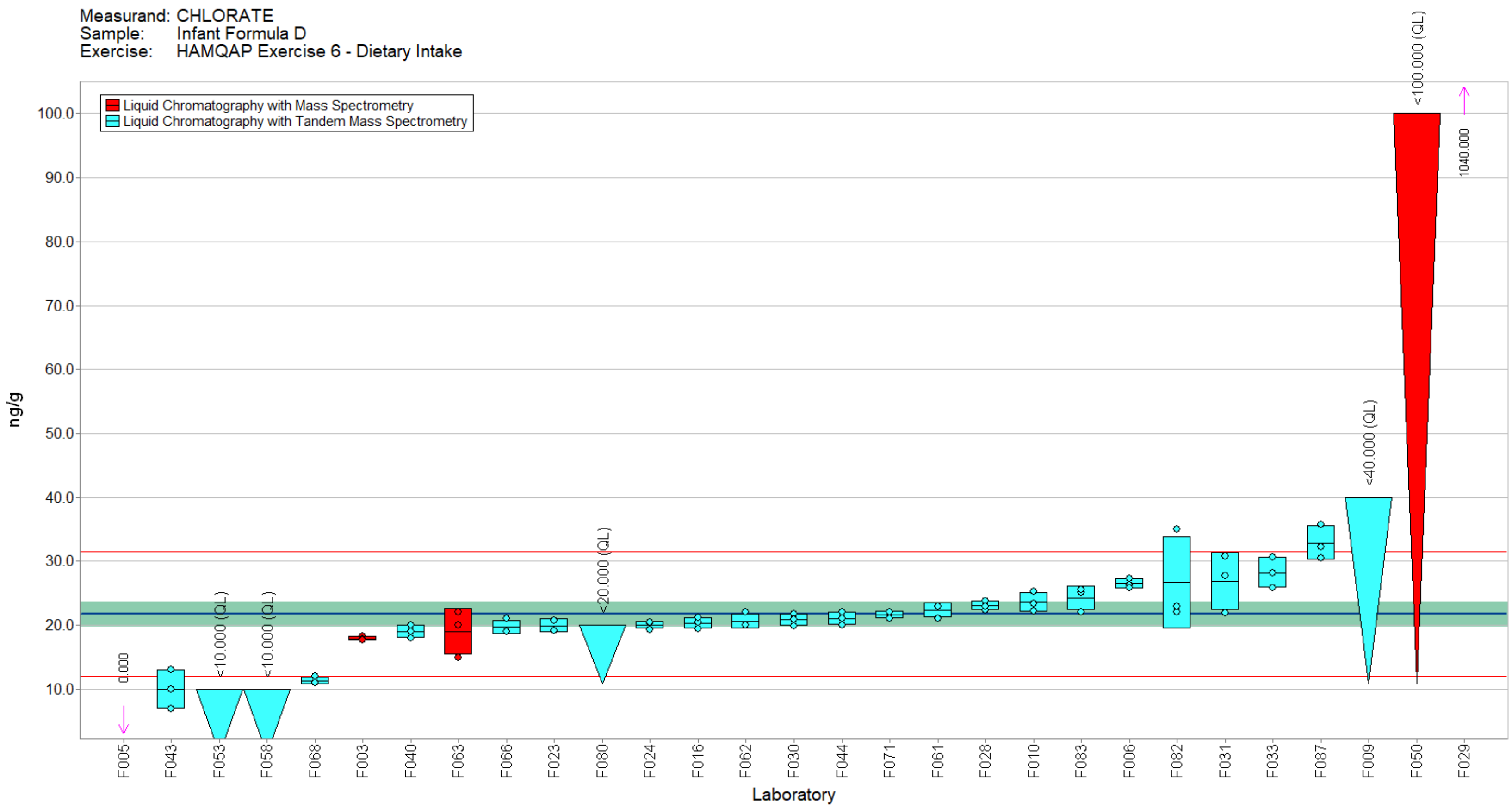


**Figure 8-5.** Chlorate in Infant Formula D (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line 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 $Z'_{\text{comm}}$ score, $|Z'_{\text{comm}}| \leq 2$. A NIST value has not been determined in this material.

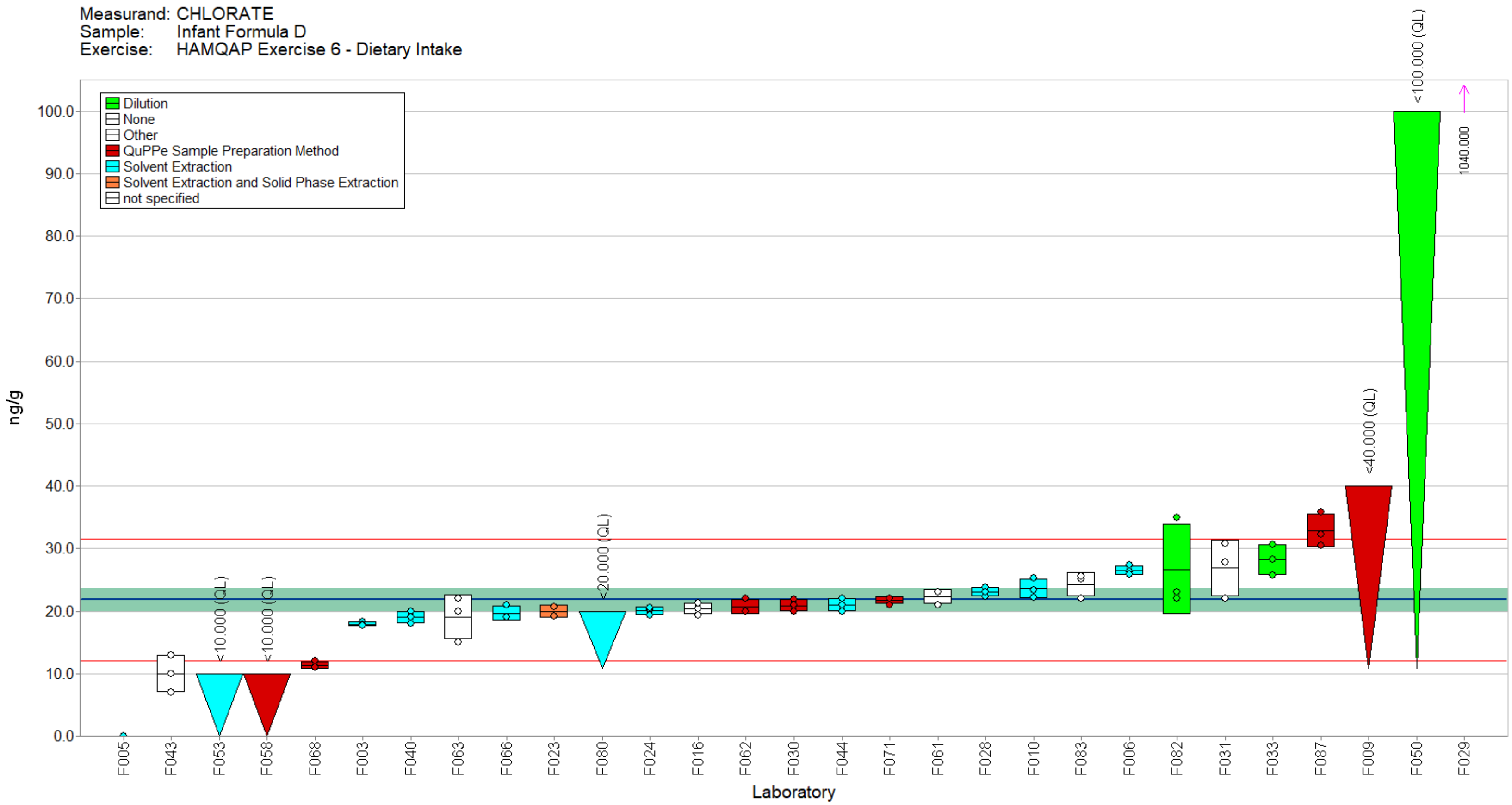


**Figure 8-6.** Chlorate in Infant Formula D (data summary view – sample preparation method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The solid blue 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 $Z'_{\mathrm{comm}}$ score, $|Z'_{\mathrm{comm}}| \leq 2$. A NIST value has not been determined in this material.

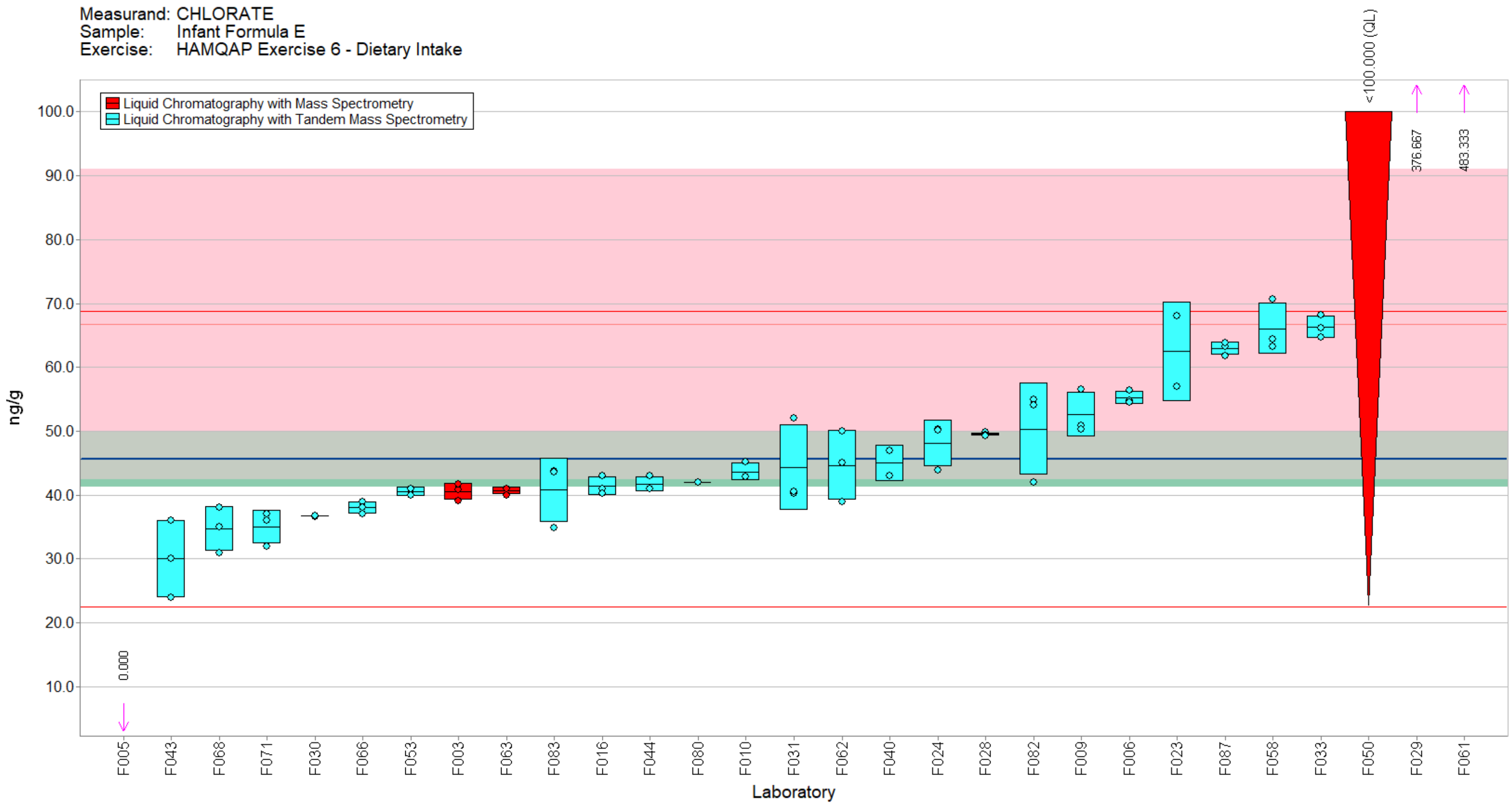


**Figure 8-7.** Chlorate in Infant Formula E (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line 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 $Z'_{\text{comm}}$ score, $|Z'_{\text{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty ($U_{\text{NIST}}$) and represents the range that results in an acceptable $Z'_{\text{NIST}}$ score, $|Z'_{\text{NIST}}| \leq 2$.

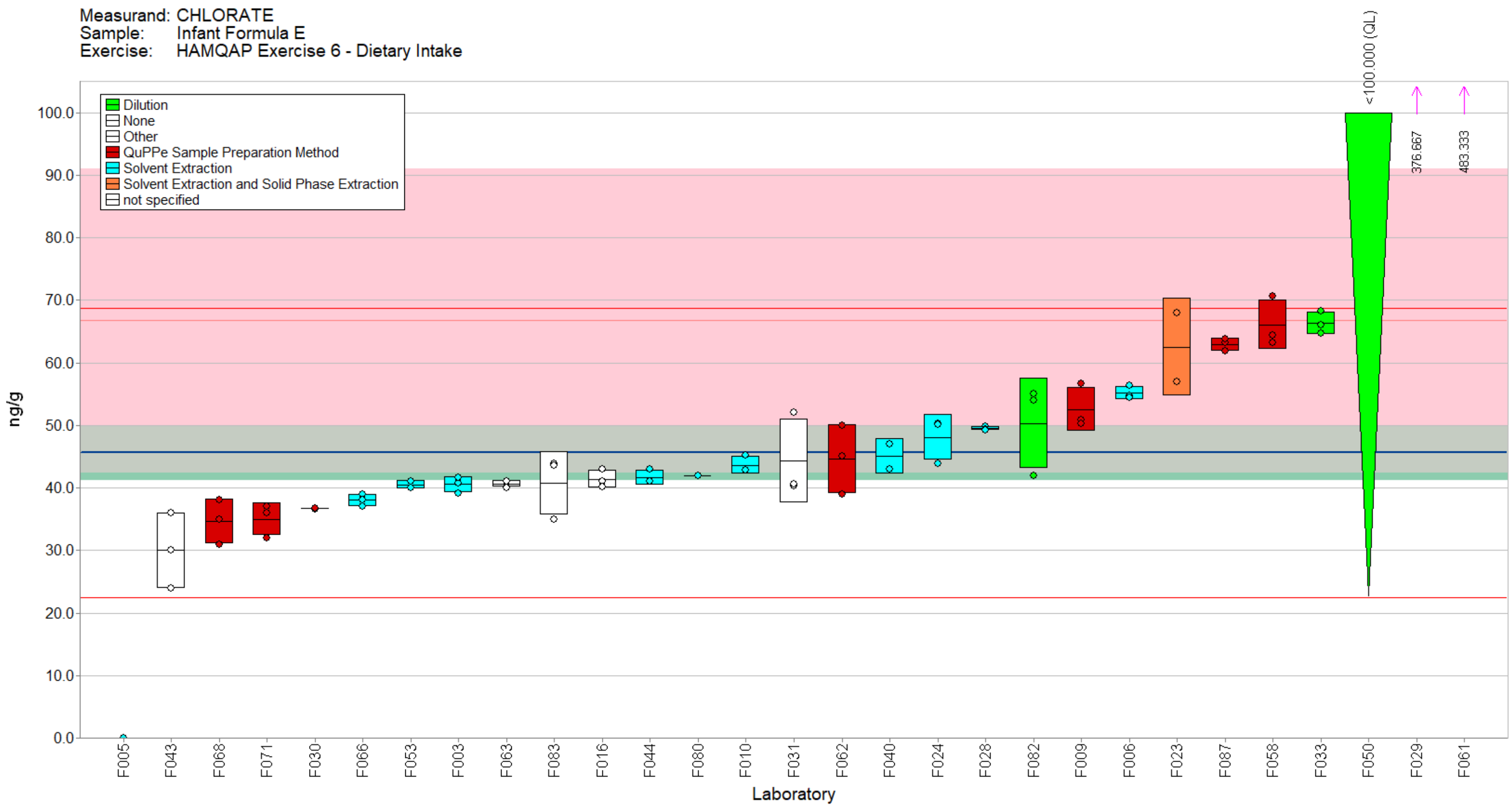


**Figure 8-8.** Chlorate in Infant Formula E (data summary view – sample preparation method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The solid blue 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 $Z'_{\text{comm}}$ score, $|Z'_{\text{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty ($U_{\text{NIST}}$) and represents the range that results in an acceptable $Z'_{\text{NIST}}$ score, $|Z'_{\text{NIST}}| \leq 2$.

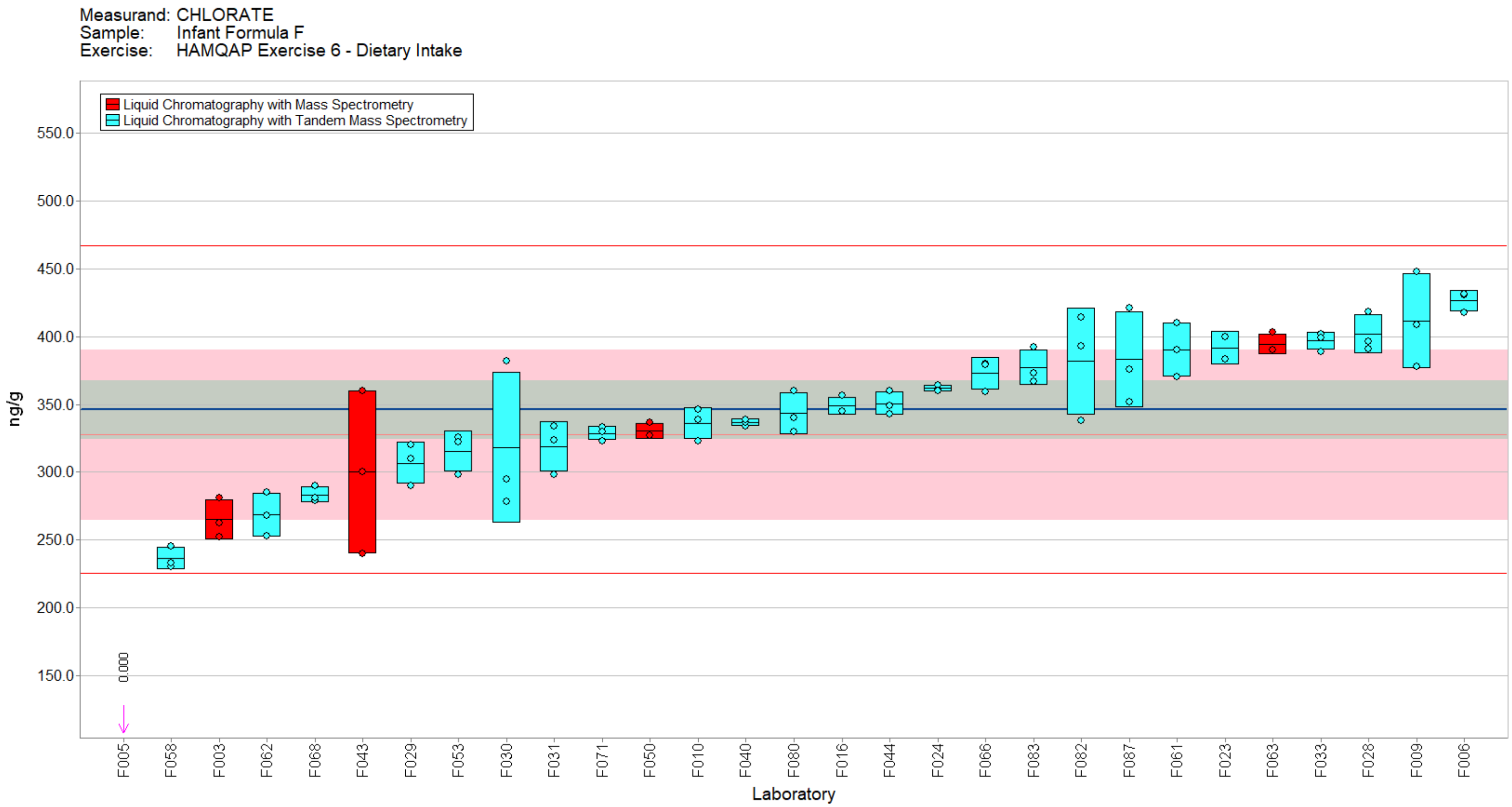


**Figure 8-9.** Chlorate in Infant Formula F (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line 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 $Z'_{\mathrm{comm}}$ score, $|Z'_{\mathrm{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty ($U_{\mathrm{NIST}}$) and represents the range that results in an acceptable $Z'_{\mathrm{NIST}}$ score, $|Z'_{\mathrm{NIST}}| \leq 2$.

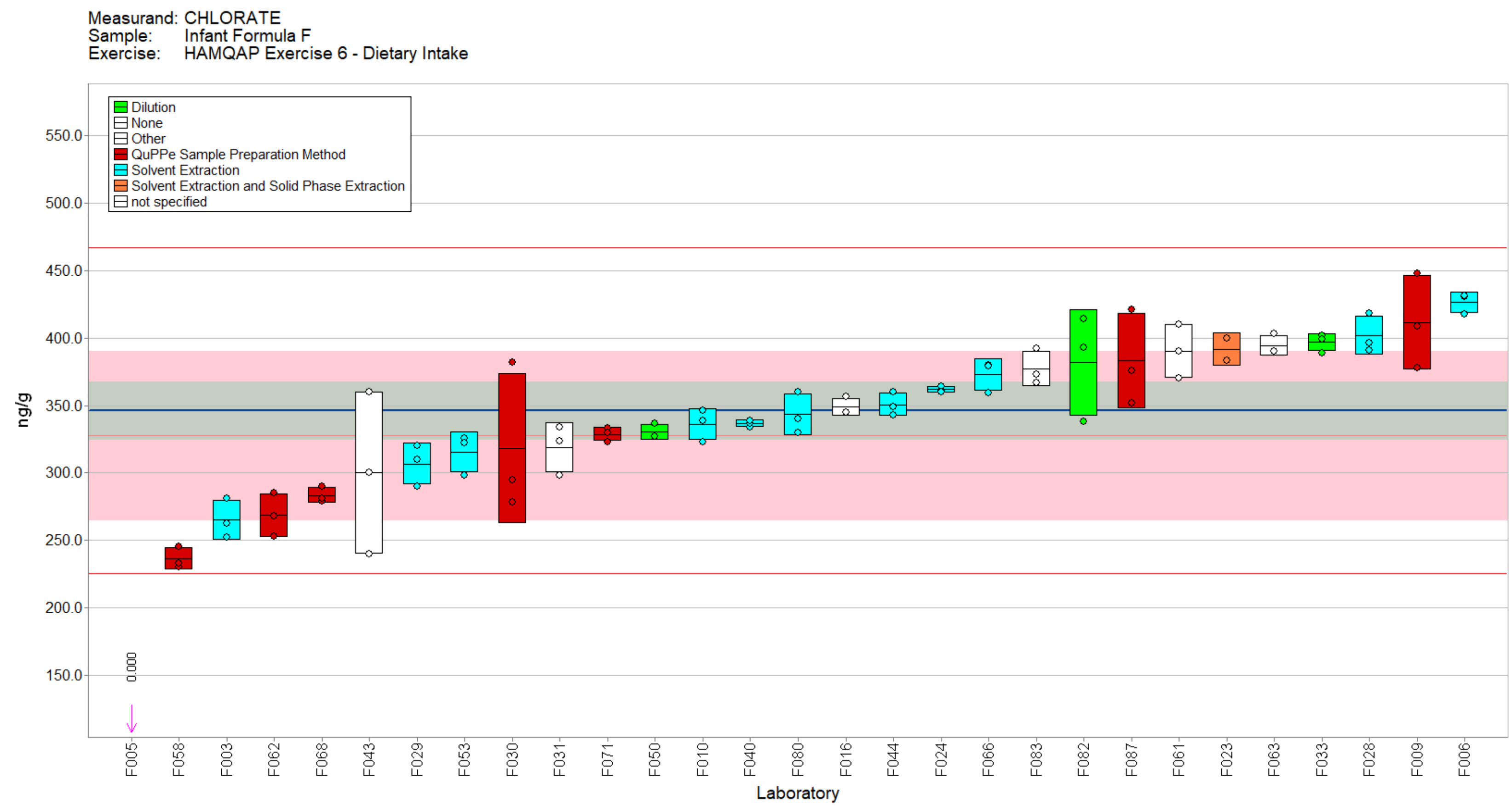


**Figure 8-10.** Chlorate in Infant Formula F (data summary view – sample preparation method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The solid blue 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 $Z'_{\mathrm{comm}}$ score, $|Z'_{\mathrm{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty ($U_{\mathrm{NIST}}$) and represents the range that results in an acceptable $Z'_{\mathrm{NIST}}$ score, $|Z'_{\mathrm{NIST}}| \leq 2$.

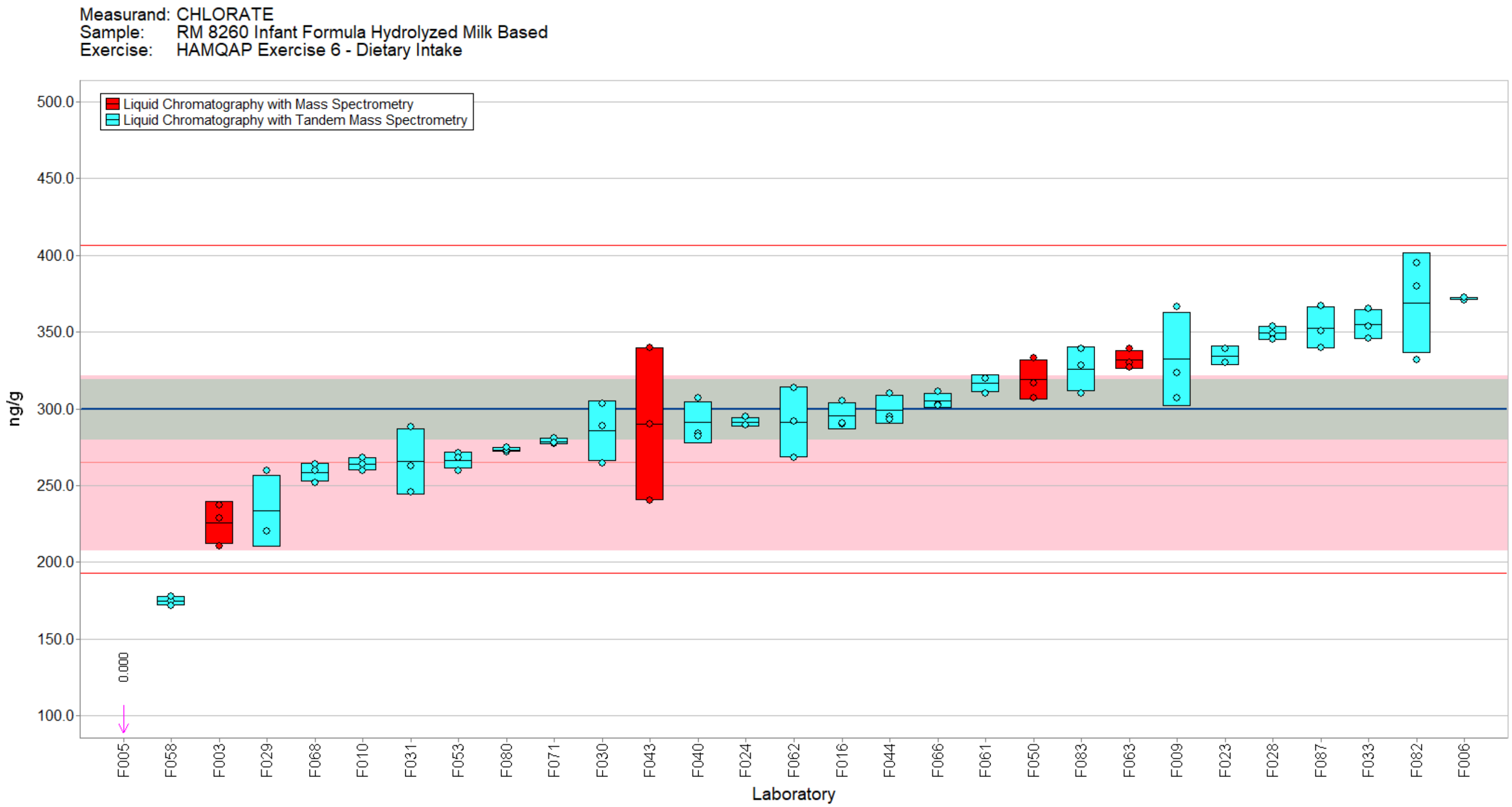


**Figure 8-11.** Chlorate in RM 8260 Infant Formula Hydrolyzed Milk Based (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue 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 $Z'_{\mathrm{comm}}$ score, $|Z'_{\mathrm{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty ($U_{\mathrm{NIST}}$) and represents the range that results in an acceptable $Z'_{\mathrm{NIST}}$ score, $|Z'_{\mathrm{NIST}}| \leq 2$.

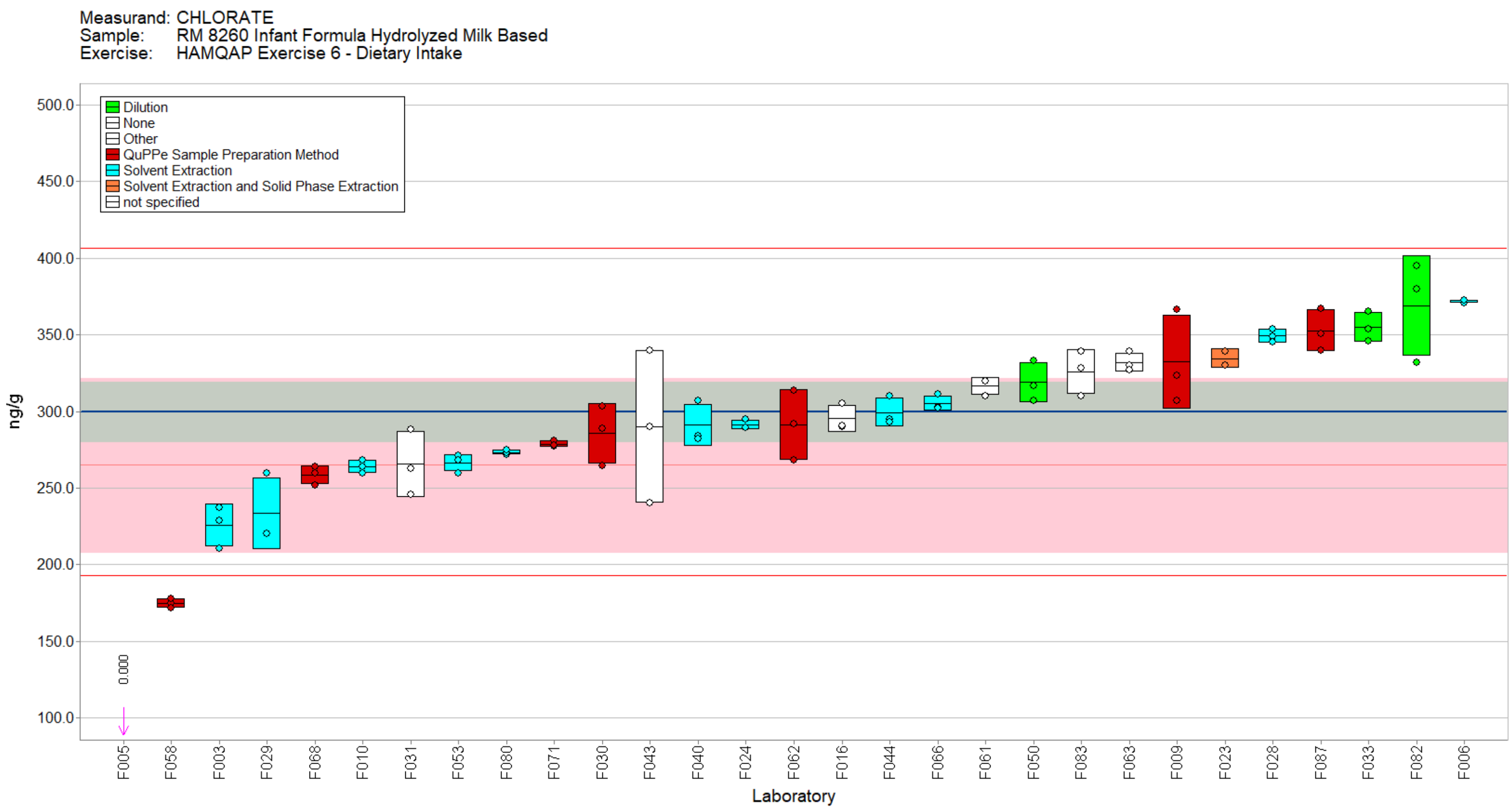


**Figure 8-12.** Chlorate in RM 8260 Infant Formula Hydrolyzed Milk Based (data summary view – sample preparation method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The solid blue 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 $Z'_{\text{comm}}$ score, $|Z'_{\text{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty ($U_{\text{NIST}}$) and represents the range that results in an acceptable $Z'_{\text{NIST}}$ score, $|Z'_{\text{NIST}}| \leq 2$.

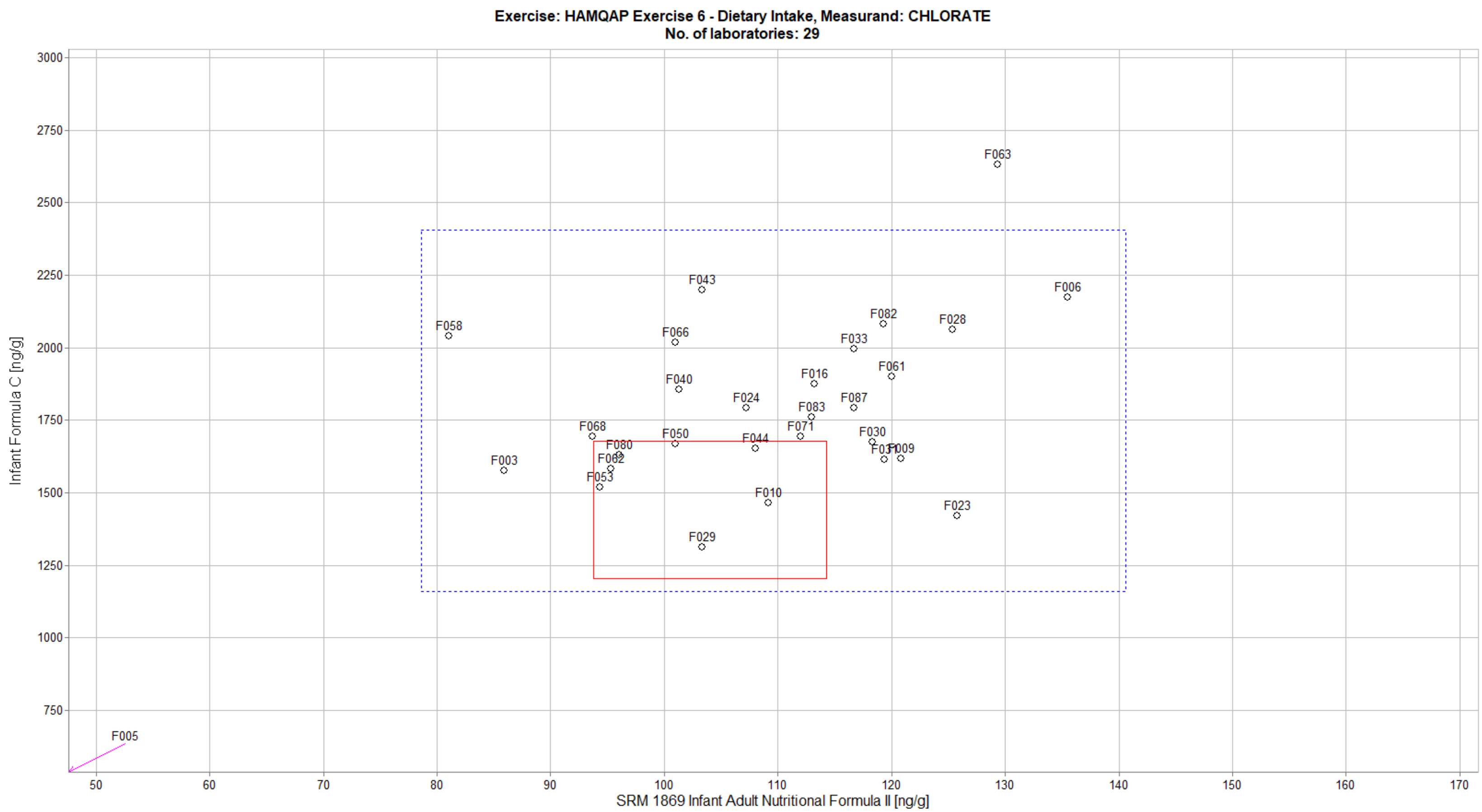


**Figure 8-13.** Laboratory means for chlorate in SRM 1869 Infant/Adult Nutritional Formula II (milk/whey/soy-based) and Infant Formula C (sample/sample comparison view). In this view, the individual laboratory mean for one sample (SRM 1869) is compared to the mean for a second sample (Infant Formula C). The solid red box represents the NIST range of tolerance for the two samples, SRM 1869 (x-axis) and Infant Formula C (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 1869 (x-axis) and Infant Formula C (y-axis), calculated as the values above and below the consensus means that result in an acceptable $Z'_{\text{comm}}$ score, $|Z'_{\text{comm}}| \leq 2$.

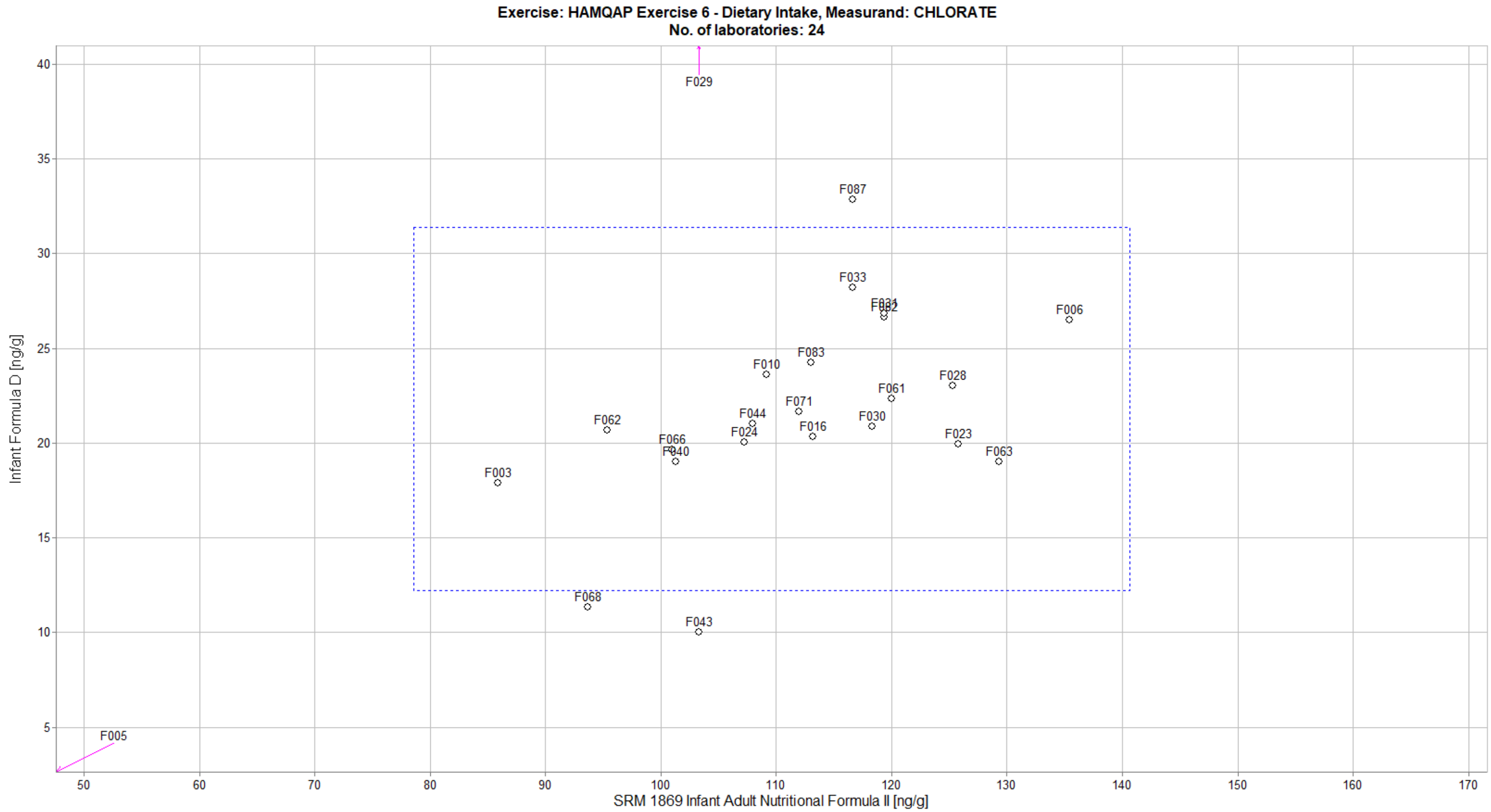


**Figure 8-14.** Laboratory means for chlorate in SRM 1869 Infant/Adult Nutritional Formula II (milk/whey/soy-based) and Infant Formula D (sample/sample comparison view). In this view, the individual laboratory mean for one sample (SRM 1869) is compared to the mean for a second sample (Infant Formula D). The dotted blue box represents the consensus range of tolerance for SRM 1869 (x-axis) and Infant Formula D (y-axis), calculated as the values above and below the consensus means that result in an acceptable $Z'_{\text{comm}}$ score, $|Z'_{\text{comm}}| \leq 2$.

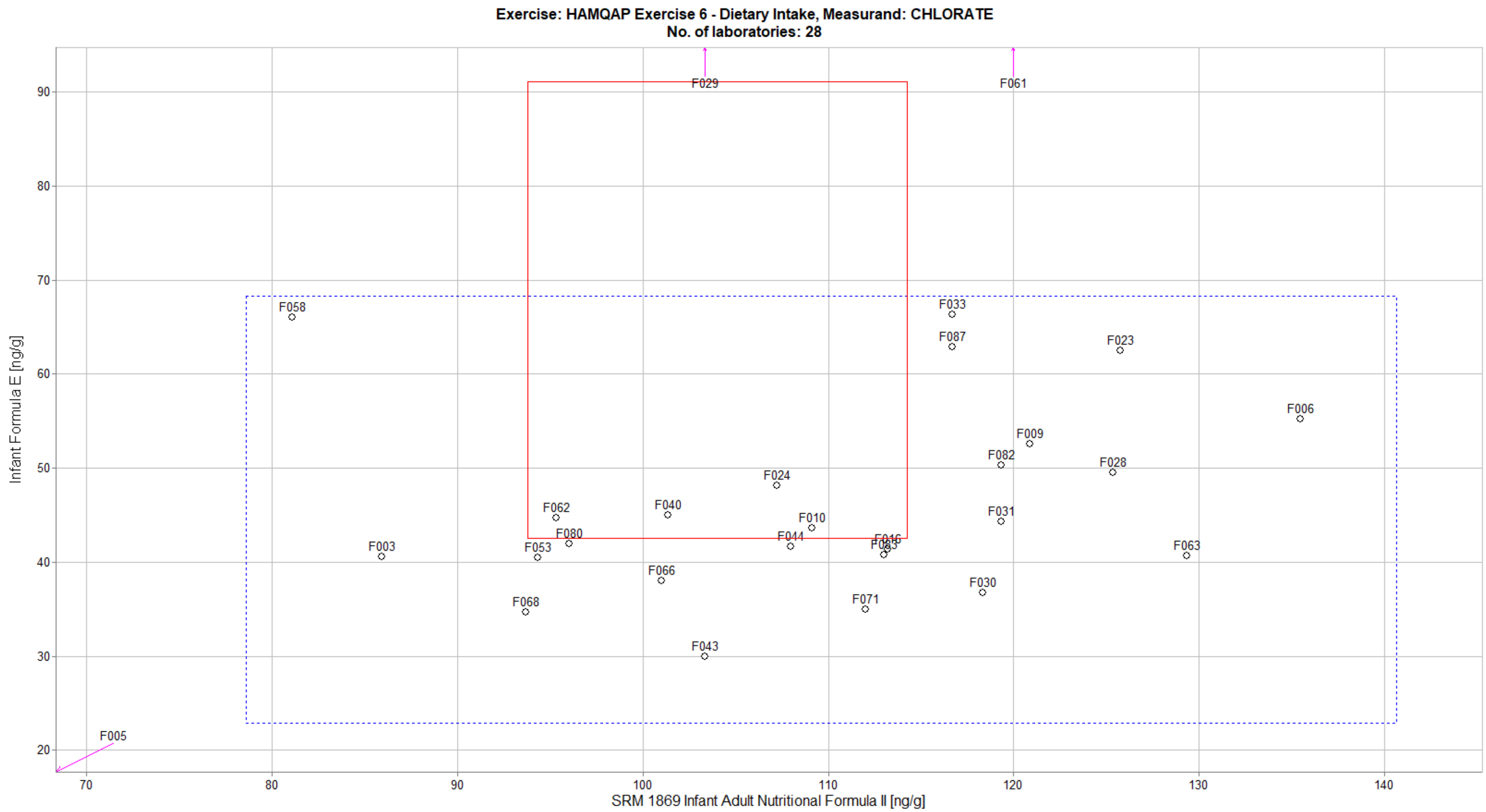


**Figure 8-15.** Laboratory means for chlorate in SRM 1869 Infant/Adult Nutritional Formula II (milk/whey/soy-based) and Infant Formula E (sample/sample comparison view). In this view, the individual laboratory mean for one sample (SRM 1869) is compared to the mean for a second sample (Infant Formula E). The solid red box represents the NIST range of tolerance for the two samples, SRM 1869 (x-axis) and Infant Formula E (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 1869 (x-axis) and Infant Formula E (y-axis), calculated as the values above and below the consensus means that result in an acceptable $Z'_{\text{comm}}$ score, $|Z'_{\text{comm}}| \leq 2$.

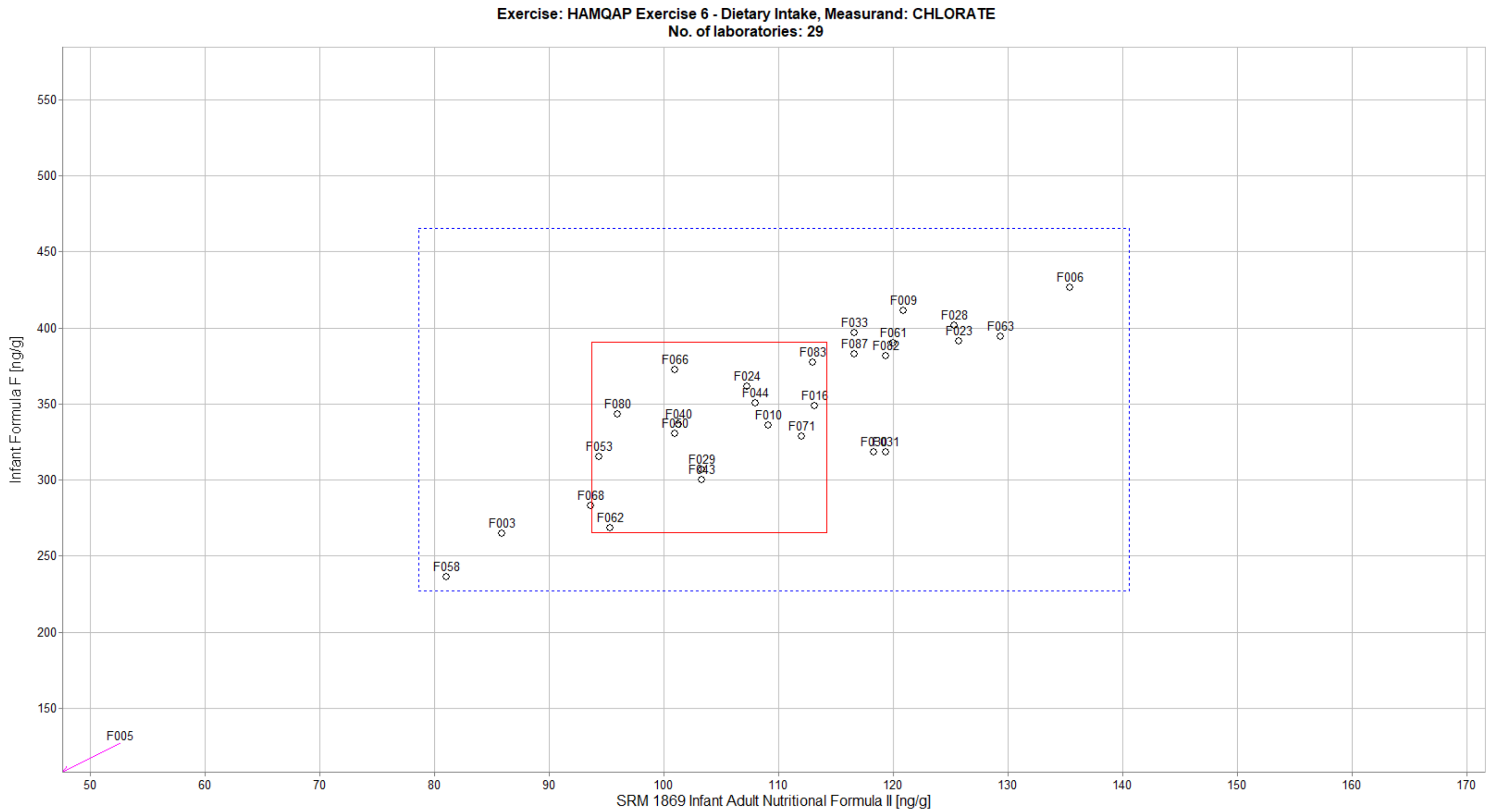


**Figure 8-16.** Laboratory means for chlorate in SRM 1869 Infant/Adult Nutritional Formula II (milk/whey/soy-based) and Infant Formula F (sample/sample comparison view). In this view, the individual laboratory mean for one sample (SRM 1869) is compared to the mean for a second sample (Infant Formula F). The solid red box represents the NIST range of tolerance for the two samples, SRM 1869 (x-axis) and Infant Formula F (y-axis), which encompasses the target values bounded by their uncertainties ($U_{\mathrm{NIST}}$) and represents the range that results in an acceptable $Z_{\mathrm{NIST}}$ score, $|Z_{\mathrm{NIST}}| \leq 2$. The dotted blue box represents the consensus range of tolerance for SRM 1869 (x-axis) and Infant Formula F (y-axis), calculated as the values above and below the consensus means that result in an acceptable $Z'_{\mathrm{comm}}$ score, $|Z'_{\mathrm{comm}}| \leq 2$.

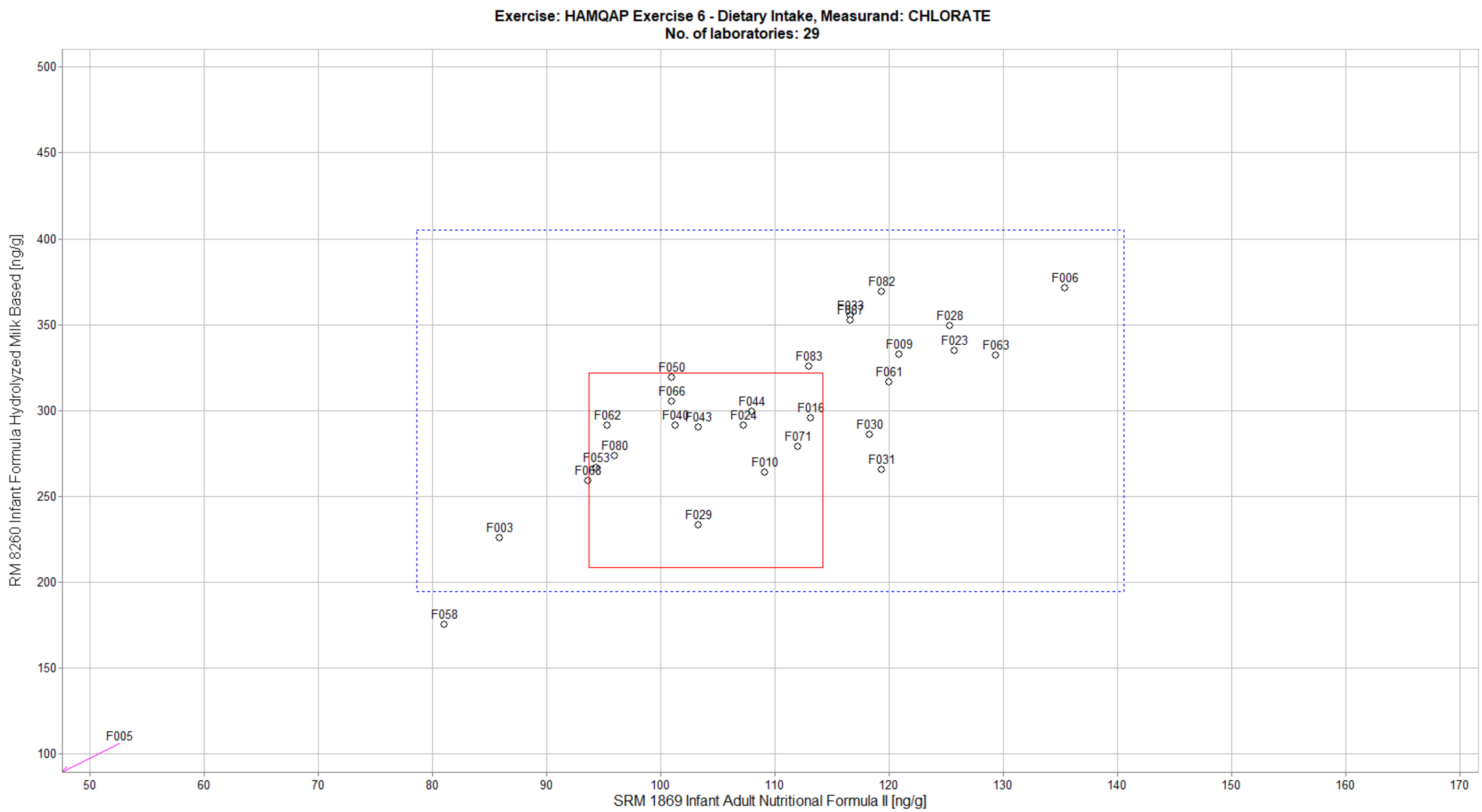


**Figure 8-17.** Laboratory means for chlorate in SRM 1869 Infant/Adult Nutritional Formula II (milk/whey/soy-based) and RM 8260 Infant Formula Hydrolyzed Milk Based (sample/sample comparison view). In this view, the individual laboratory mean for one sample (SRM 1869) is compared to the mean for a second sample (RM 8260). The solid red box represents the NIST range of tolerance for the two samples, SRM 1869 (x-axis) and RM 8260 (y-axis), which encompasses the target values bounded by their uncertainties ($U_{\mathrm{NIST}}$) and represents the range that results in an acceptable $Z_{\mathrm{NIST}}$ score, $|Z_{\mathrm{NIST}}| \leq 2$. The dotted blue box represents the consensus range of tolerance for SRM 1869 (x-axis) and RM 8260 (y-axis), calculated as the values above and below the consensus means that result in an acceptable $Z'_{\mathrm{comm}}$ score, $|Z'_{\mathrm{comm}}| \leq 2$.

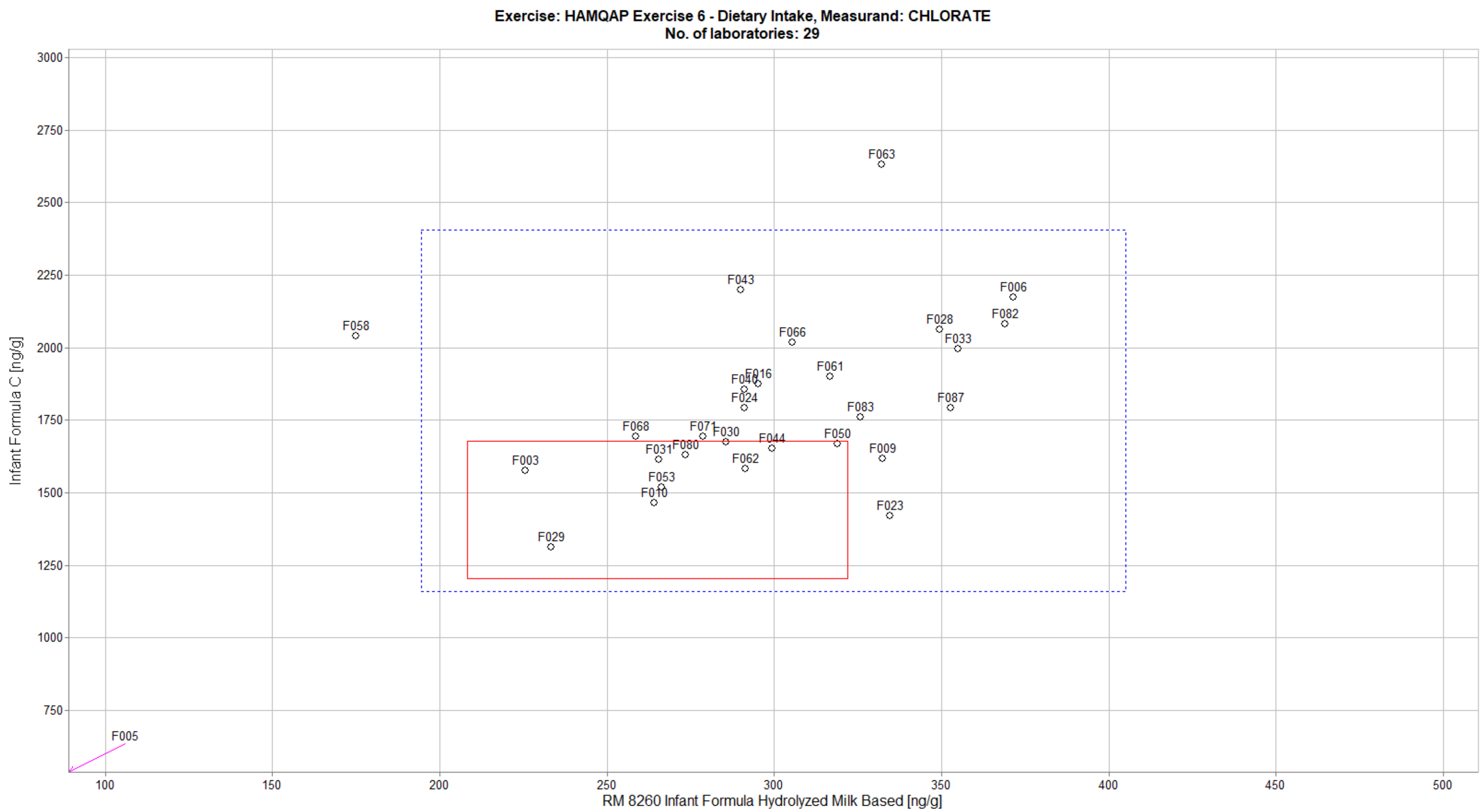


**Figure 8-18.** Laboratory means for chlorate in RM 8260 Infant Formula Hydrolyzed Milk Based and Infant Formula C (sample/sample comparison view). In this view, the individual laboratory mean for one sample (RM 8260) is compared to the mean for a second sample (Infant Formula C). The solid red box represents the NIST range of tolerance for the two samples RM 8260 (x-axis) and Infant Formula C (y-axis), which encompasses the target values bounded by their uncertainties ($U_{\mathrm{NIST}}$) and represents the range that results in an acceptable $Z_{\mathrm{NIST}}$ score, $|Z_{\mathrm{NIST}}| \leq 2$. The dotted blue box represents the consensus range of tolerance for RM 8260 (x-axis) and Infant Formula C (y-axis), calculated as the values above and below the consensus means that result in an acceptable $Z'_{\mathrm{comm}}$ score, $|Z'_{\mathrm{comm}}| \leq 2$.

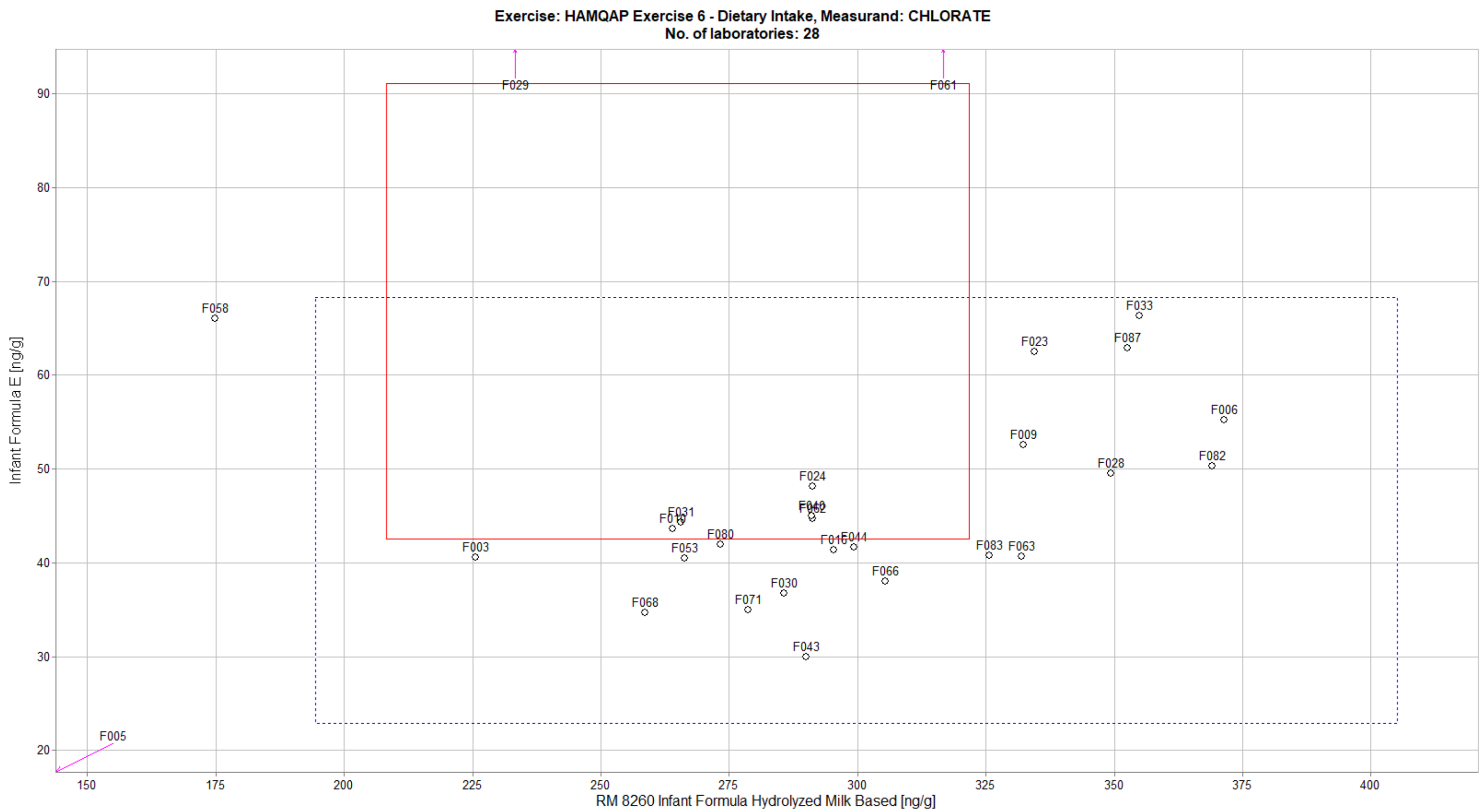


**Figure 8-19.** Laboratory means for chlorate in RM 8260 Infant Formula Hydrolyzed Milk Based and Infant Formula E (sample/sample comparison view). In this view, the individual laboratory mean for one sample (RM 8260) is compared to the mean for a second sample (Infant Formula E). The solid red box represents the NIST range of tolerance for the two samples RM 8260 (x-axis) and Infant Formula E (y-axis), which encompasses the target values bounded by their uncertainties ($U_{\mathrm{NIST}}$) and represents the range that results in an acceptable $Z_{\mathrm{NIST}}$ score, $|Z_{\mathrm{NIST}}| \leq 2$. The dotted blue box represents the consensus range of tolerance for RM 8260 (x-axis) and Infant Formula E (y-axis), calculated as the values above and below the consensus means that result in an acceptable $Z'_{\mathrm{comm}}$ score, $|Z'_{\mathrm{comm}}| \leq 2$.

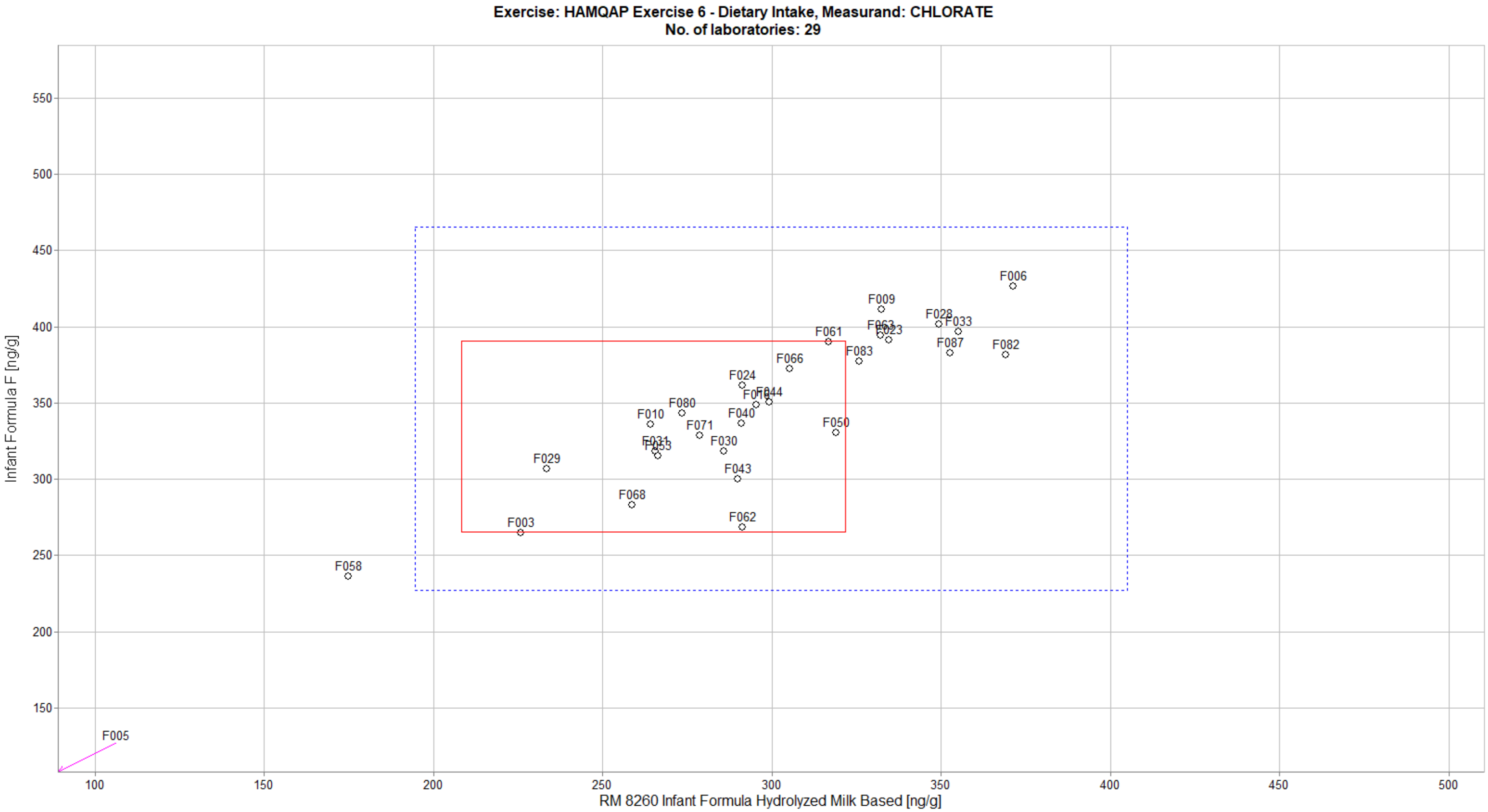


**Figure 8-20.** Laboratory means for chlorate in RM 8260 Infant Formula Hydrolyzed Milk Based and Infant Formula F (sample/sample comparison view). In this view, the individual laboratory mean for one sample (RM 8260) is compared to the mean for a second sample (Infant Formula F). The solid red box represents the NIST range of tolerance for the two samples RM 8260 (x-axis) and Infant Formula F (y-axis), which encompasses the target values bounded by their uncertainties ($U_{\mathrm{NIST}}$) and represents the range that results in an acceptable $Z_{\mathrm{NIST}}$ score, $|Z_{\mathrm{NIST}}| \leq 2$. The dotted blue box represents the consensus range of tolerance for RM 8260 (x-axis) and Infant Formula F (y-axis), calculated as the values above and below the consensus means that result in an acceptable $Z'_{\mathrm{comm}}$ score, $|Z'_{\mathrm{comm}}| \leq 2$.

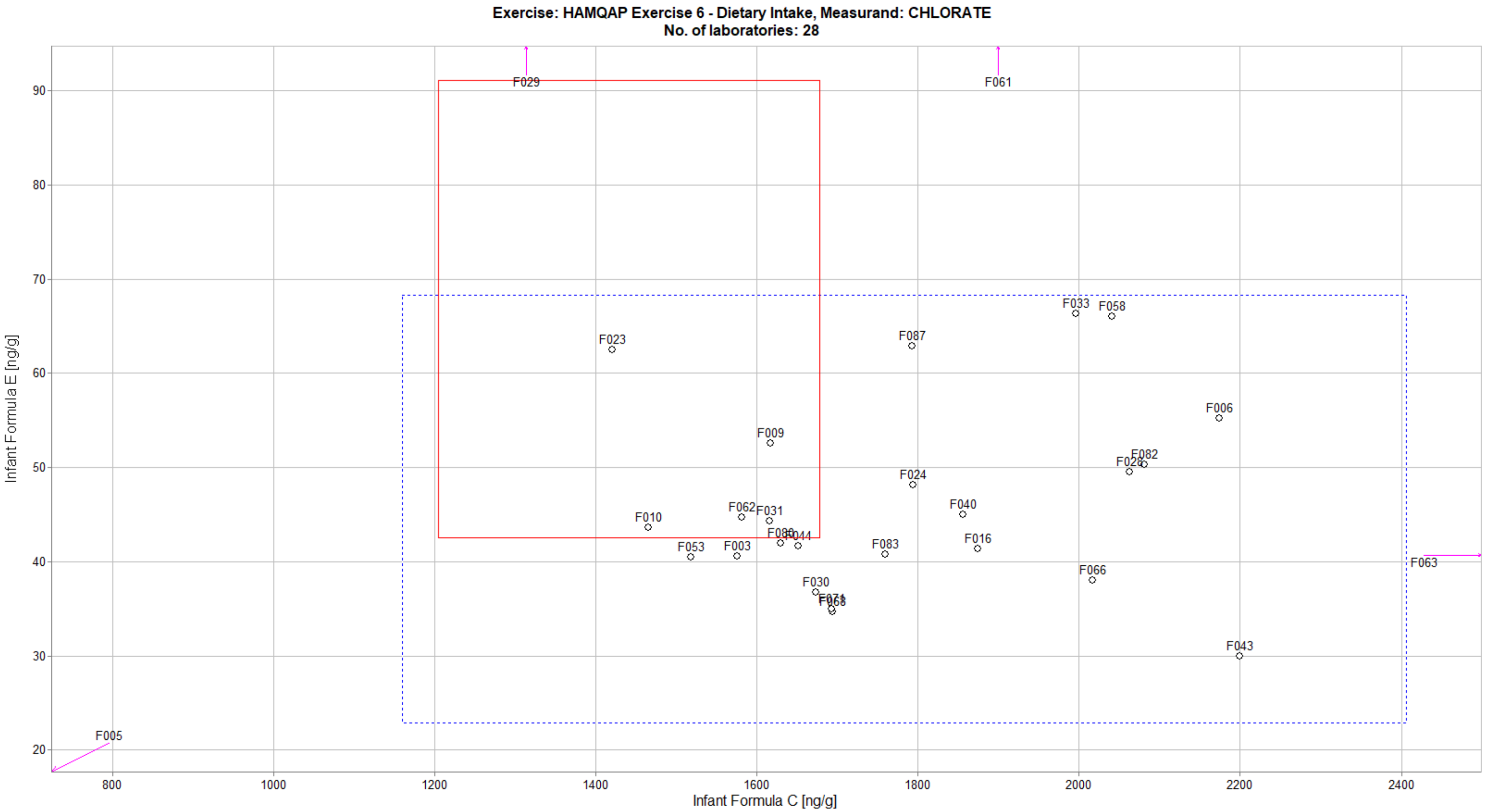


**Figure 8-21.** Laboratory means for chlorate in Infant Formula C and Infant Formula E (sample/sample comparison view). In this view, the individual laboratory mean for one sample (Infant Formula C) is compared to the mean for a second sample (Infant Formula E). The solid red box represents the NIST range of tolerance for the two samples Infant Formula C (x-axis) and Infant Formula E (y-axis), which encompasses the target values bounded by their uncertainties ($U_{\mathrm{NIST}}$) and represents the range that results in an acceptable $Z_{\mathrm{NIST}}$ score, $|Z_{\mathrm{NIST}}| \leq 2$. The dotted blue box represents the consensus range of tolerance for Infant Formula C (x-axis) and Infant Formula E (y-axis), calculated as the values above and below the consensus means that result in an acceptable $Z'_{\mathrm{comm}}$ score, $|Z'_{\mathrm{comm}}| \leq 2$.

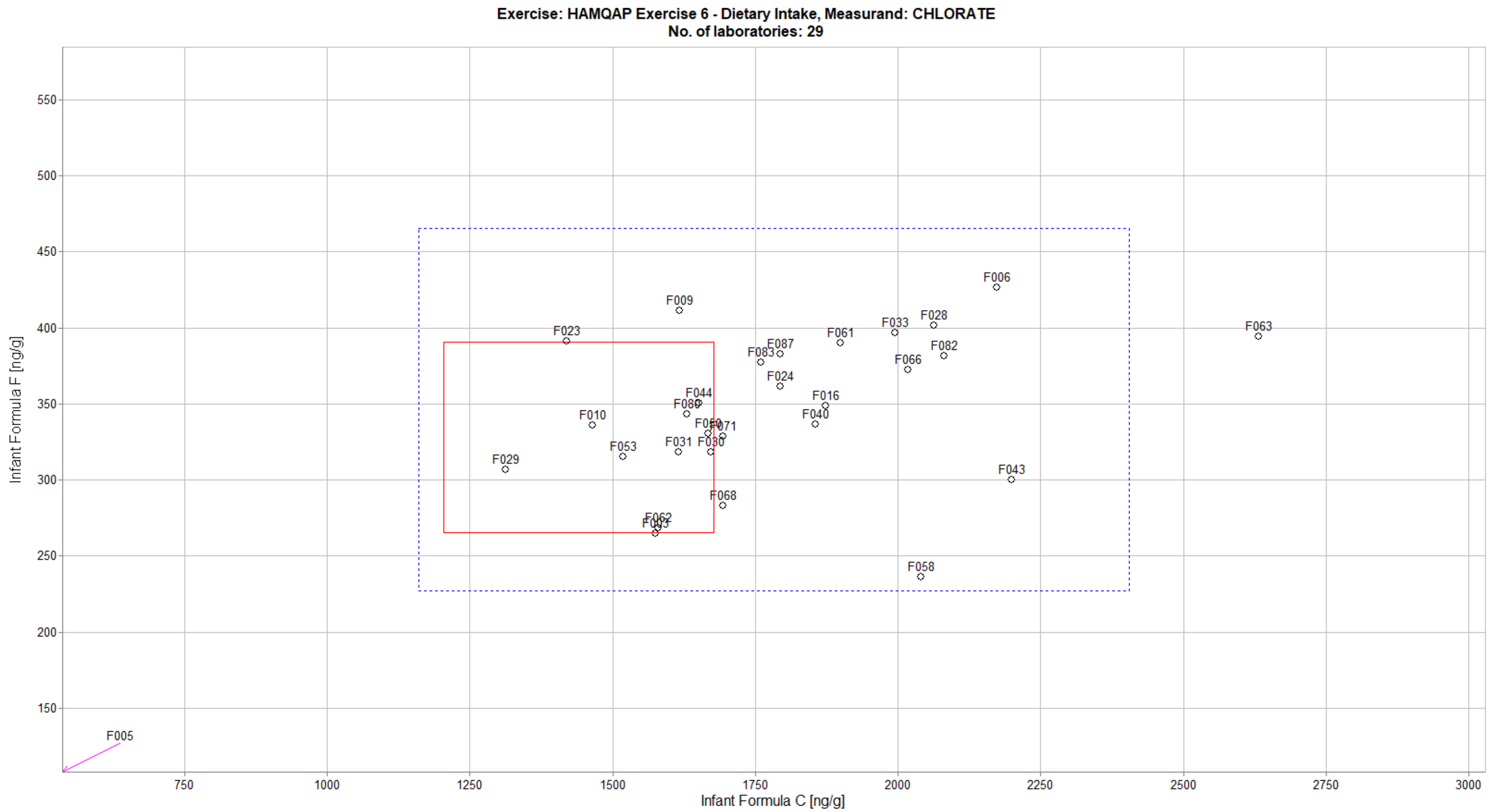


**Figure 8-22.** Laboratory means for chlorate in Infant Formula C and Infant Formula F (sample/sample comparison view). In this view, the individual laboratory mean for one sample (Infant Formula C) is compared to the mean for a second sample (Infant Formula F). The solid red box represents the NIST range of tolerance for the two samples Infant Formula C (x-axis) and Infant Formula F (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 Infant Formula C (x-axis) and Infant Formula F (y-axis), calculated as the values above and below the consensus means that result in an acceptable $Z'_{\text{comm}}$ score, $|Z'_{\text{comm}}| \leq 2$.

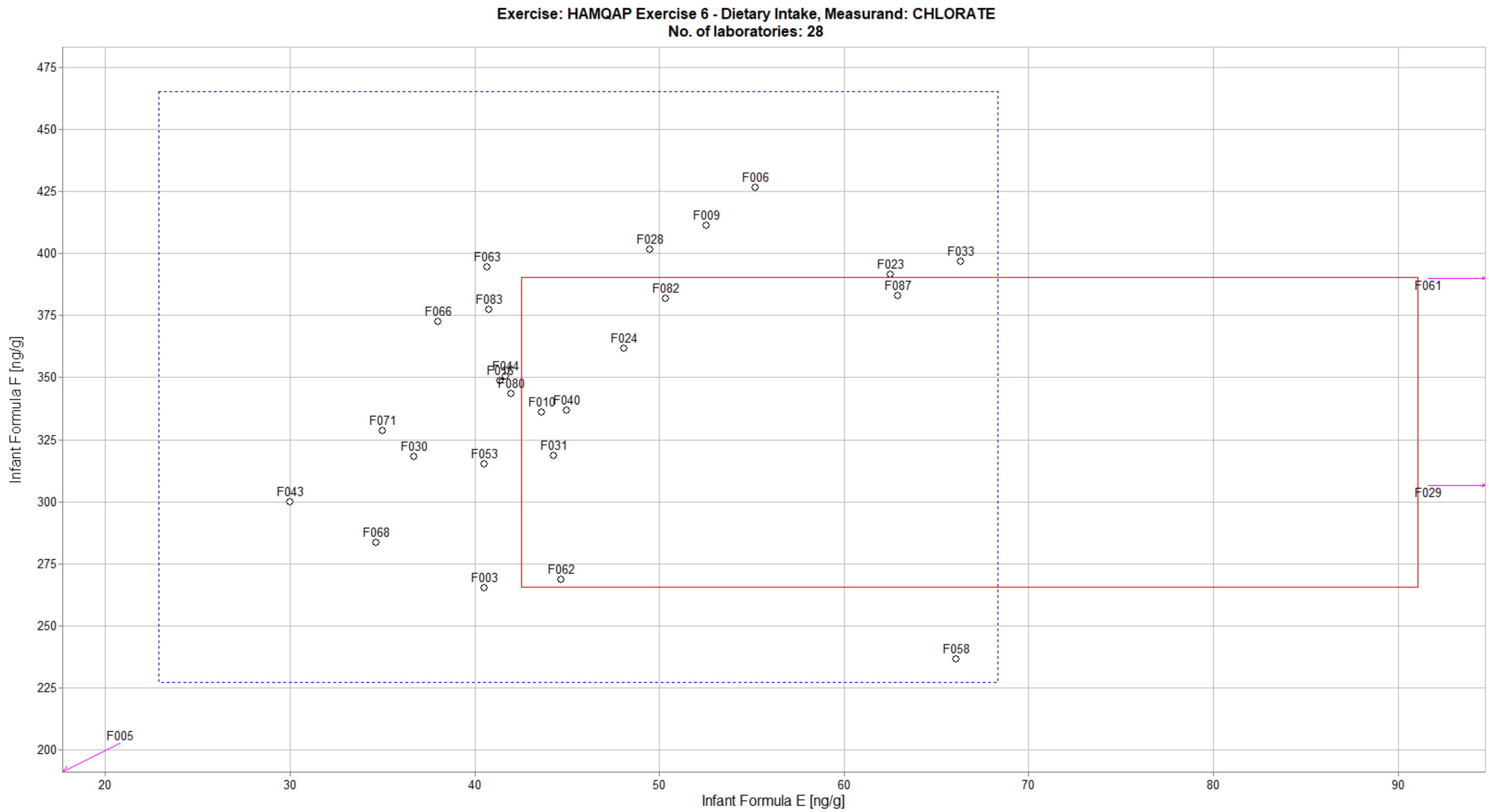


**Figure 8-23.** Laboratory means for chlorate in Infant Formula E and Infant Formula F (sample/sample comparison view). In this view, the individual laboratory mean for one sample (Infant Formula E) is compared to the mean for a second sample (Infant Formula F). The solid red box represents the NIST range of tolerance for the two samples Infant Formula E (x-axis) and Infant Formula F (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 Infant Formula E (x-axis) and Infant Formula F (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 8-3.** Data summary table for perchlorate in infant formulas. Data points highlighted in blue have been identified as outside the consensus tolerance limits and would be estimated to result in an unacceptable $Z'_{\text{comm}}$ score, $|Z'_{\text{comm}}| \geq 2$. Data points highlighted in red have a zero or non-numeric data point. *Note that this table spans two pages.*

| | | Perchlorate | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | SRM 1869 Infant/Adult Nutritional Formula II (ng/g) | | | | | Infant Formula C (ng/g) | | | | | Infant Formula D (ng/g) | | | | |
| | Lab | A | B | C | Avg | SD | A | B | C | Avg | SD | A | B | C | Avg | SD |
| Individual Results | Target | | | | | | | | | 30.00 | 3.10 | | | | | |
| | F003 | < 2.000 | < 2.000 | < 2.000 | | | 35.01 | 36.11 | 34.93 | 35.4 | 0.7 | 5.48 | 5.25 | 5.35 | 5.36 | 0.12 |
| | F004 | | | | | | | | | | | | | | | |
| | F005 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | F006 | < 2.000 | < 2.000 | < 2.000 | | | 43.74 | 44.15 | 43.79 | 43.9 | 0.2 | 6.25 | 6.38 | 5.19 | 5.94 | 0.65 |
| | F008 | | | | | | | | | | | | | | | |
| | F009 | < 10.000 | < 10.000 | < 10.000 | | | 42.3 | 29.4 | 30 | 33.9 | 7.3 | < 10.000 | < 10.000 | < 10.000 | | |
| | F010 | < 10.000 | < 10.000 | < 10.000 | | | 27 | 31 | 29 | 29.0 | 2.0 | < 10.000 | < 10.000 | < 10.000 | | |
| | F016 | 1.1 | 1 | 1 | 1.03 | 0.06 | 37.6 | 36.9 | 37 | 37.2 | 0.4 | 5.8 | 5.7 | 5.1 | 5.53 | 0.38 |
| | F021 | | | | | | | | | | | | | | | |
| | F023 | < 10.000 | < 10.000 | | | | 34.5 | 39.1 | | 36.8 | 3.3 | 6.1 | 6.5 | | 6.30 | 0.28 |
| | F024 | < 10.000 | < 10.000 | < 10.000 | | | 37.2 | 38.2 | 38 | 37.8 | 0.5 | < 10.000 | < 10.000 | < 10.000 | | |
| | F028 | < 2.000 | < 2.000 | < 2.000 | | | 38.9 | 41.9 | 40.5 | 40.4 | 1.5 | 5.91 | 5.92 | 5.62 | 5.82 | 0.17 |
| | F029 | < 2.000 | < 2.000 | < 2.000 | | | 37 | 34 | 39 | 36.7 | 2.5 | 5.3 | 4.9 | | 5.10 | 0.28 |
| | F030 | < 5.000 | < 5.000 | < 5.000 | | | 54.2 | 43.3 | 29.7 | 42.4 | 12.3 | 10.3 | 9.3 | 10.8 | 10.13 | 0.76 |
| | F031 | < 3.000 | < 3.000 | < 3.000 | | | 30.44 | 29.37 | 27.89 | 29.2 | 1.3 | 5.8 | 5.01 | 3.56 | 4.79 | 1.14 |
| | F033 | 21.1 | 19.9 | 22.3 | 21.1 | 1.2 | 50.2 | 48.6 | 51 | 49.9 | 1.2 | 23.5 | 24.6 | 25.3 | 24.47 | 0.91 |
| | F034 | | | | | | | | | | | | | | | |
| | F040 | < 10.000 | < 10.000 | < 10.000 | | | 38 | 40 | 41 | 39.7 | 1.5 | < 10.000 | < 10.000 | < 10.000 | | |
| | F043 | < 50.000 | < 50.000 | < 50.000 | | | < 50.000 | < 50.000 | < 50.000 | | | < 50.000 | < 50.000 | < 50.000 | | |
| | F044 | < 2.000 | < 2.000 | < 2.000 | | | 37 | 34 | 35 | 35.3 | 1.5 | 5 | 5 | 5 | 5 | 0 |
| | F050 | < 50.000 | < 50.000 | < 50.000 | | | < 50.000 | < 50.000 | < 50.000 | | | < 50.000 | < 50.000 | < 50.000 | | |
| | F053 | < 10.000 | < 10.000 | < 10.000 | | | 38 | 32 | 33 | 34.3 | 3.2 | < 10.000 | < 10.000 | < 10.000 | | |
| | F058 | < 10.000 | < 10.000 | < 10.000 | | | 34.1 | 33.5 | 32.9 | 33.5 | 0.6 | 11.3 | 11 | 10.5 | 10.93 | 0.40 |
| | F061 | < 2.000 | < 2.000 | < 2.000 | | | 43 | 43 | 45 | 43.7 | 1.2 | 6 | 7 | 7 | 6.67 | 0.58 |
| | F062 | < 10.000 | < 10.000 | < 10.000 | | | 28 | 28 | 30 | 28.7 | 1.2 | < 10.000 | < 10.000 | < 10.000 | | |
| | F063 | 4.1 | 3.5 | 4.3 | 3.97 | 0.42 | 4.3 | 4.3 | 4 | 4.2 | 0.2 | 8.9 | 8.5 | 8.4 | 8.60 | 0.26 |
| | F066 | < 2.000 | < 2.000 | < 2.000 | | | 31 | 33 | 32 | 32.0 | 1.0 | 5 | 5 | 5 | 5 | 0 |
| | F067 | | | | | | | | | | | | | | | |
| | F068 | < 1.000 | < 1.000 | < 1.000 | | | 44 | 43 | 42 | 43.0 | 1.0 | 1 | 1 | 1 | 1 | 0 |
| | F071 | 1 | 3 | 1 | 1.67 | 1.15 | 33 | 33 | 32 | 32.7 | 0.6 | 5 | 7 | 5 | 5.67 | 1.15 |
| | F078 | | | | | | | | | | | | | | | |
| | F080 | 1.92 | 1.48 | 1.28 | 1.56 | 0.33 | 34 | 35 | 37 | 35.3 | 1.5 | < 10.000 | < 10.000 | < 10.000 | | |
| | F082 | 2 | 2 | 1 | 1.67 | 0.58 | 58 | 45 | 51 | 51.3 | 6.5 | 6 | 4 | 7 | 5.67 | 1.53 |
| | F083 | < 2.000 | < 2.000 | < 2.000 | | | 40.7 | 39.5 | 40.6 | 40.3 | 0.7 | 5.57 | 5.35 | 5.68 | 5.53 | 0.17 |
| | F087 | < 2.000 | < 2.000 | < 2.000 | | | 34.7 | 35.9 | 44.4 | 38.3 | 5.3 | 8.02 | 7.23 | 6.63 | 7.29 | 0.70 |
| | F088 | | | | | | | | | | | | | | | |
| Community Results | | Consensus Mean | | | 1.65 | | Consensus Mean | | | 37.4 | | Consensus Mean | | | 5.89 | |
| | | Consensus Standard Deviation | | | 1.99 | | Consensus Standard Deviation | | | 7.9 | | Consensus Standard Deviation | | | 1.93 | |
| | | Maximum | | | 21.1 | | Maximum | | | 51.3 | | Maximum | | | 24.5 | |
| | | Minimum | | | 0 | | Minimum | | | 0 | | Minimum | | | 0 | |
| | | N | | | 7 | | N | | | 27 | | N | | | 20 | |

| | | Perchlorate | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Infant Formula E (ng/g) | | | | | Infant Formula F (ng/g) | | | | | RM 8260 Infant Nutritional Formula (ng/g) | | | | |
| | Lab | A | B | C | Avg | SD | A | B | C | Avg | SD | A | B | C | Avg | SD |
| Individual Results | Target | | | | | | | | | 5.75 | 0.79 | | | | | |
| | F003 | < 2.000 | < 2.000 | < 2.000 | | | 5.78 | 5.83 | 5.82 | 5.81 | 0.03 | < 2.000 | < 2.000 | < 2.000 | | |
| | F004 | | | | | | | | | | | | | | | |
| | F005 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | F006 | < 2.000 | < 2.000 | < 2.000 | | | 8.38 | 8.44 | 8.15 | 8.32 | 0.15 | < 2.000 | < 2.000 | < 2.000 | | |
| | F008 | | | | | | | | | | | | | | | |
| | F009 | < 10.000 | < 10.000 | < 10.000 | | | < 10.000 | < 10.000 | < 10.000 | | | < 10.000 | < 10.000 | < 10.000 | | |
| | F010 | < 10.000 | < 10.000 | < 10.000 | | | < 10.000 | < 10.000 | < 10.000 | | | < 10.000 | < 10.000 | < 10.000 | | |
| | F016 | 1.3 | 1.1 | 0.4 | 0.93 | 0.47 | 6.6 | 5.9 | 6.6 | 6.37 | 0.40 | 1.8 | 1 | 1 | 1.27 | 0.46 |
| | F021 | | | | | | | | | | | | | | | |
| | F023 | < 10.000 | < 10.000 | | | | 6 | 8 | | 7.00 | 1.41 | < 10.000 | < 10.000 | | | |
| | F024 | < 10.000 | < 10.000 | < 10.000 | | | < 10.000 | < 10.000 | < 10.000 | | | < 10.000 | < 10.000 | < 10.000 | | |
| | F028 | < 2.000 | < 2.000 | < 2.000 | | | 6.41 | 5.95 | 6.64 | 6.33 | 0.35 | < 2.000 | < 2.000 | < 2.000 | | |
| | F029 | < 2.000 | < 2.000 | < 2.000 | | | 6 | 6 | 5 | 5.67 | 0.58 | < 2.000 | < 2.000 | < 2.000 | | |
| | F030 | < 5.000 | < 5.000 | < 5.000 | | | 5.6 | 5.6 | 5.1 | 5.43 | 0.29 | < 5.000 | < 5.000 | < 5.000 | | |
| | F031 | < 3.000 | < 3.000 | < 3.000 | | | 5.68 | 3.63 | 5.87 | 5.06 | 1.24 | < 3.000 | < 3.000 | < 3.000 | | |
| | F033 | 20.9 | 22.4 | 20.3 | 21.2 | 1.1 | 12.7 | 11.7 | 13.1 | 12.50 | 0.72 | 14.4 | 12.2 | 13.3 | 13.3 | 1.1 |
| | F034 | | | | | | | | | | | | | | | |
| | F040 | < 10.000 | < 10.000 | < 10.000 | | | < 10.000 | < 10.000 | < 10.000 | | | < 10.000 | < 10.000 | < 10.000 | | |
| | F043 | < 50.000 | < 50.000 | < 50.000 | | | < 50.000 | < 50.000 | < 50.000 | | | < 50.000 | < 50.000 | < 50.000 | | |
| | F044 | < 2.000 | < 2.000 | < 2.000 | | | 6 | 6 | 6 | 6 | 0 | < 2.000 | < 2.000 | < 2.000 | | |
| | F050 | < 50.000 | < 50.000 | < 50.000 | | | < 50.000 | < 50.000 | < 50.000 | | | < 50.000 | < 50.000 | < 50.000 | | |
| | F053 | < 10.000 | < 10.000 | < 10.000 | | | < 10.000 | < 10.000 | < 10.000 | | | < 10.000 | < 10.000 | < 10.000 | | |
| | F058 | < 10.000 | < 10.000 | < 10.000 | | | 11.4 | 10.9 | 11.2 | 11.17 | 0.25 | < 10.000 | < 10.000 | < 10.000 | | |
| | F061 | < 2.000 | < 2.000 | < 2.000 | | | 7 | 7 | 7 | 7 | 0 | < 2.000 | < 2.000 | < 2.000 | | |
| | F062 | < 10.000 | < 10.000 | < 10.000 | | | < 10.000 | < 10.000 | < 10.000 | | | < 10.000 | < 10.000 | < 10.000 | | |
| | F063 | 10 | 11 | 10 | 10.3 | 0.58 | 8.4 | 8.7 | 8.6 | 8.57 | 0.15 | 3 | 3.6 | 4.4 | 3.67 | 0.70 |
| | F066 | < 2.000 | < 2.000 | < 2.000 | | | 5 | 5 | 5 | 5 | 0 | < 2.000 | < 2.000 | < 2.000 | | |
| | F067 | | | | | | | | | | | | | | | |
| | F068 | < 1.000 | < 1.000 | < 1.000 | | | < 1.000 | < 1.000 | < 1.000 | | | 3 | 3 | 3 | 3.00 | 0.00 |
| | F071 | < 2.000 | < 2.000 | < 2.000 | | | 5 | 6 | 6 | 5.67 | 0.58 | 1 | 1 | 1 | 1.00 | 0.00 |
| | F078 | | | | | | | | | | | | | | | |
| | F080 | < 1.000 | < 1.000 | < 1.000 | | | 5.9 | 5.9 | 6.6 | 6.13 | 0.40 | 1.57 | 1.27 | 1.44 | 1.43 | 0.15 |
| | F082 | 1 | 1 | 1 | 1 | 0 | 7 | 8 | 9 | 8.00 | 1.00 | 1 | 3 | 4 | 2.67 | 1.53 |
| | F083 | < 2.000 | < 2.000 | < 2.000 | | | 5.84 | 5.92 | 5.94 | 5.90 | 0.05 | < 2.000 | < 2.000 | < 2.000 | | |
| | F087 | < 2.000 | < 2.000 | < 2.000 | | | 8.24 | 7.95 | 7.66 | 7.95 | 0.29 | < 2.000 | < 2.000 | 2.15 | 2.15 | |
| | F088 | | | | | | | | | | | | | | | |
| Community Results | | Consensus Mean | | | 1.29 | | Consensus Mean | | | 6.68 | | Consensus Mean | | | 1.90 | |
| | | Consensus Standard Deviation | | | 2.44 | | Consensus Standard Deviation | | | 1.70 | | Consensus Standard Deviation | | | 2.00 | |
| | | Maximum | | | 21.2 | | Maximum | | | 12.5 | | Maximum | | | 13.3 | |
| | | Minimum | | | 0 | | Minimum | | | 0 | | Minimum | | | 0 | |
| | | N | | | 5 | | N | | | 20 | | N | | | 8 | |

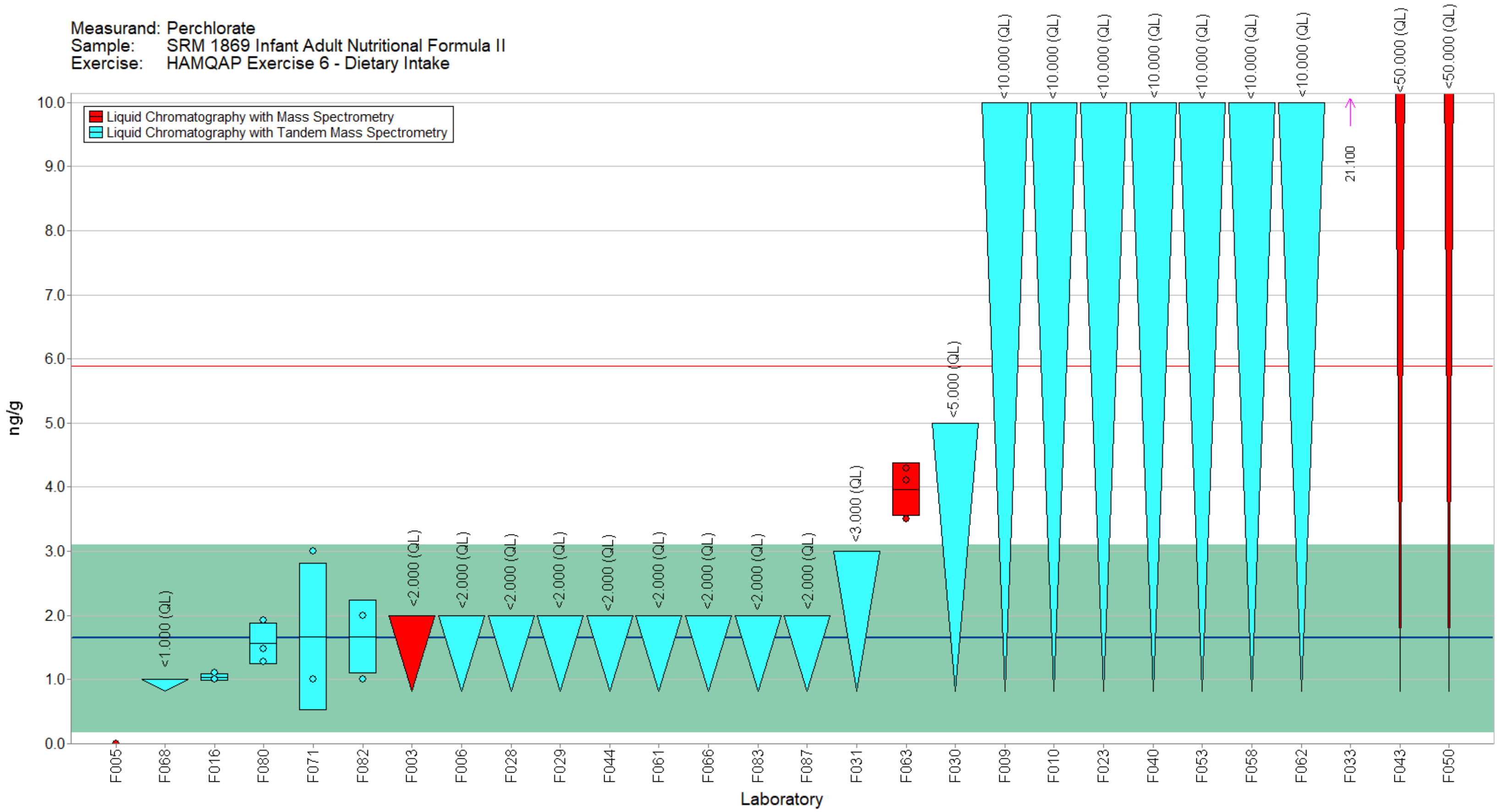


**Figure 8-24.** Perchlorate in SRM 1869 Infant/Adult Nutritional Formula II (milk/whey/soy-based) (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue 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 the consensus mean that result in an acceptable $Z'_{\text{comm}}$ score, $|Z'_{\text{comm}}| \leq 2$, with the lower limit set at zero. A NIST value has not been determined in this material.

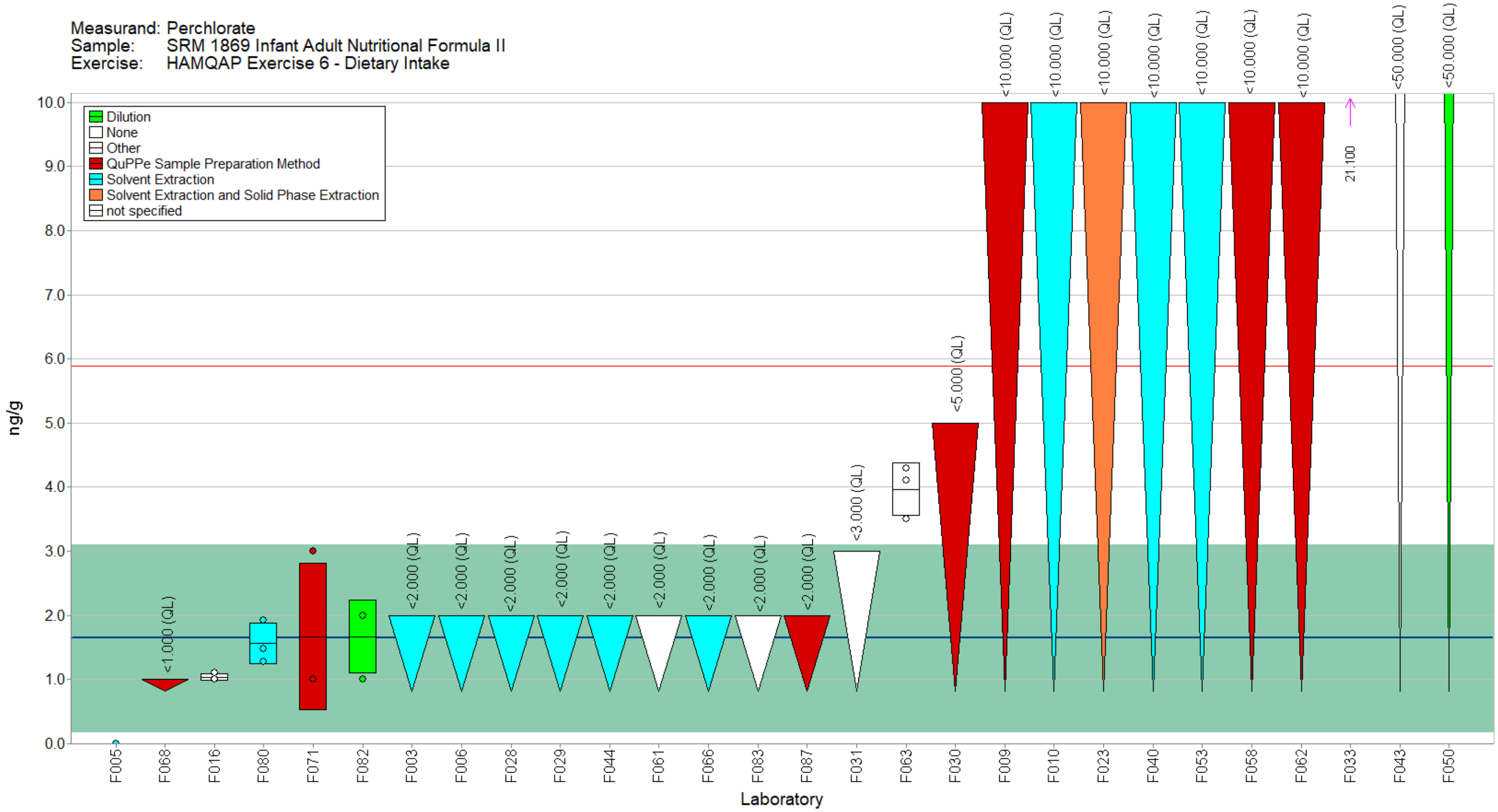


**Figure 8-25.** Perchlorate in SRM 1869 Infant/Adult Nutritional Formula II (milk/whey/soy-based) (data summary view – sample preparation method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The solid blue 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 the consensus mean that result in an acceptable $Z'_{\text{comm}}$ score, $|Z'_{\text{comm}}| \leq 2$, with the lower limit set at zero. A NIST value has not been determined in this material.

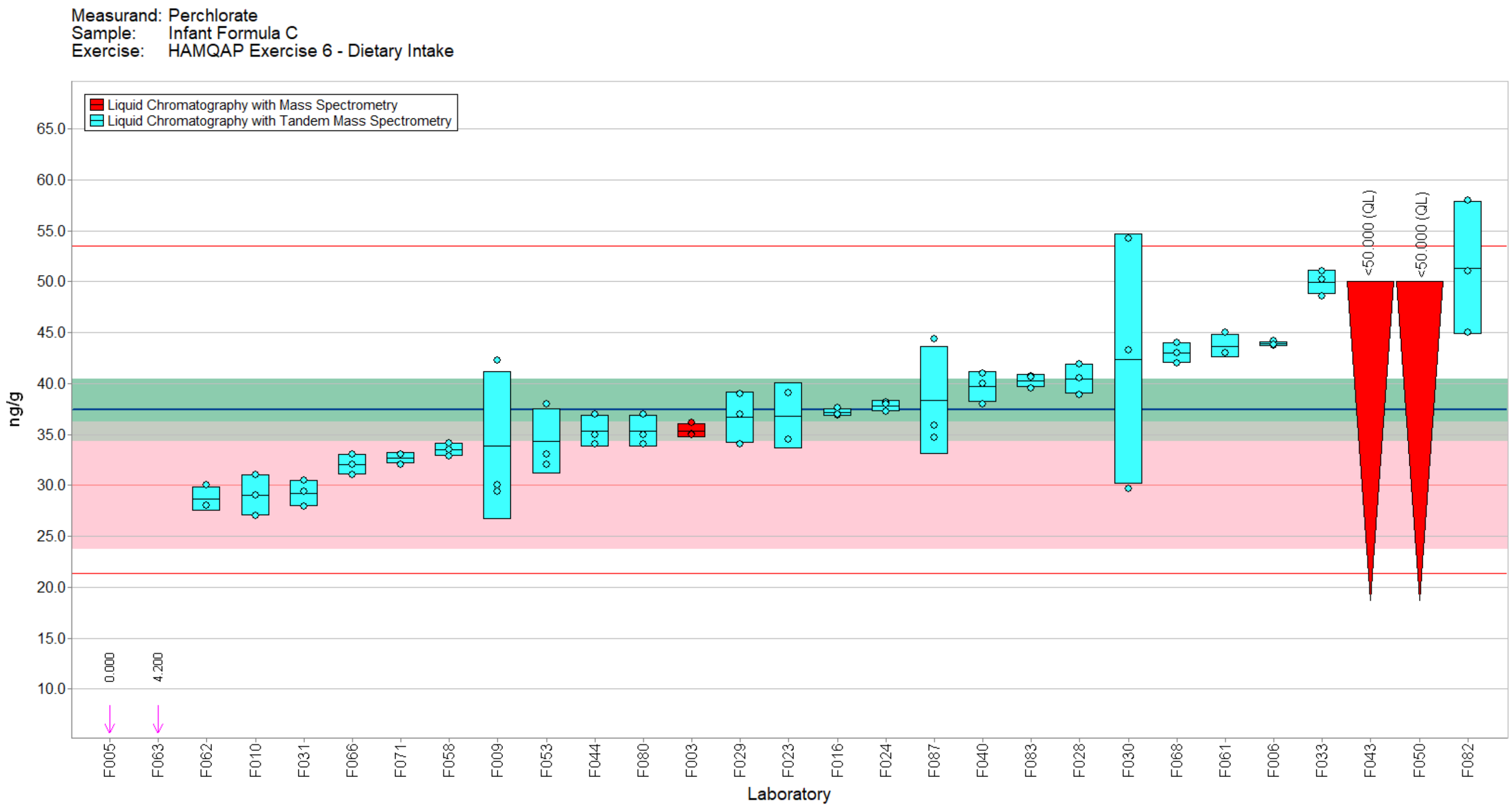


**Figure 8-26.** Perchlorate in Infant Formula C (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue 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 the consensus mean that result in an acceptable $Z'_{\text{comm}}$ score, $|Z'_{\text{comm}}| \leq 2$, with the lower limit set at zero. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty ($U_{\text{NIST}}$) and represents the range that results in an acceptable $Z'_{\text{NIST}}$ score, $|Z'_{\text{NIST}}| \leq 2$.

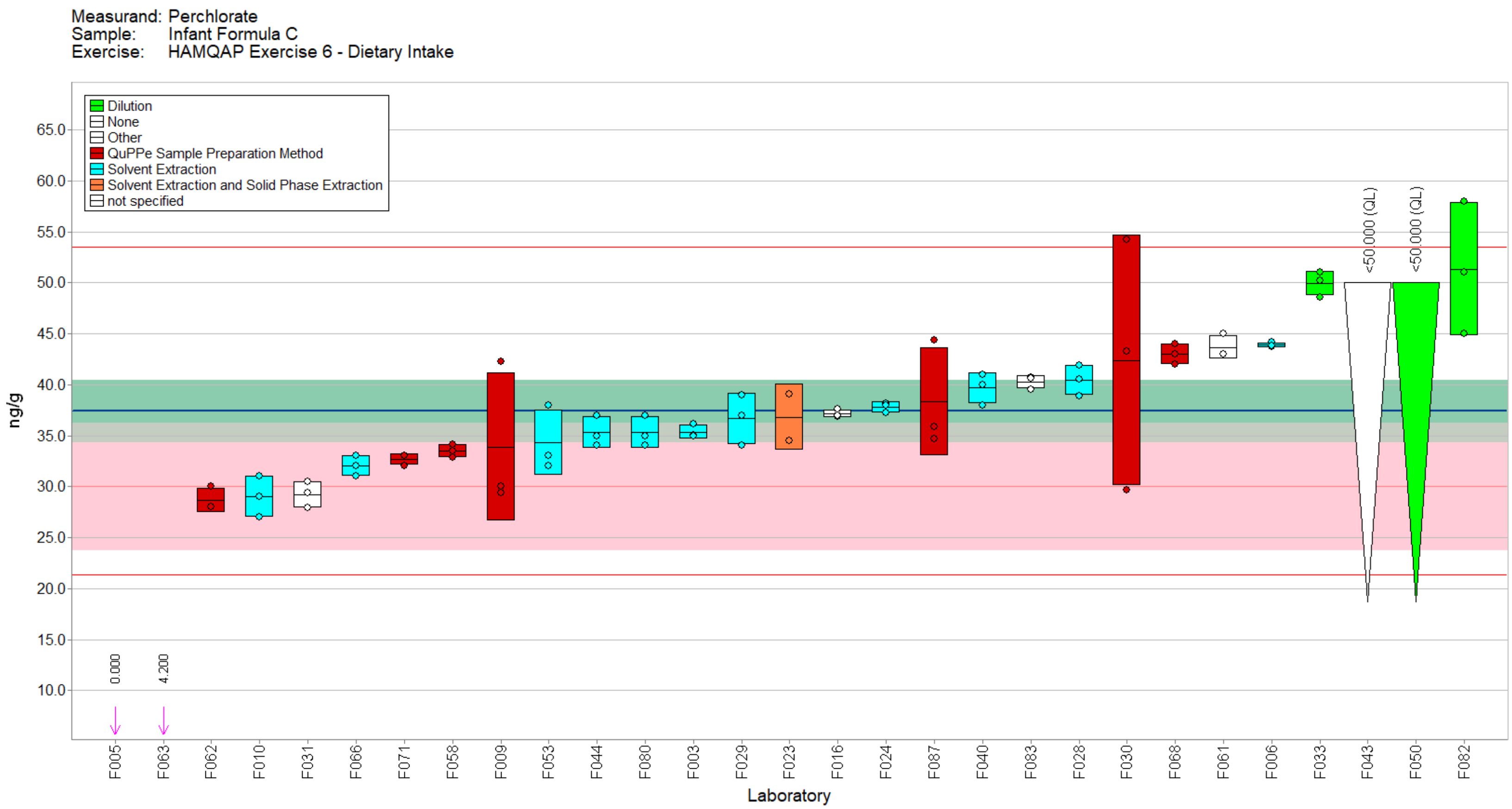


**Figure 8-27.** Perchlorate in Infant Formula C (data summary view – sample preparation method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The solid blue 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 the consensus mean that result in an acceptable $Z'_{\mathrm{comm}}$ score, $|Z'_{\mathrm{comm}}| \leq 2$, with the lower limit set at zero. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty ($U_{NIST}$) and represents the range that results in an acceptable $Z'_{\mathrm{NIST}}$ score, $|Z'_{\mathrm{NIST}}| \leq 2$.

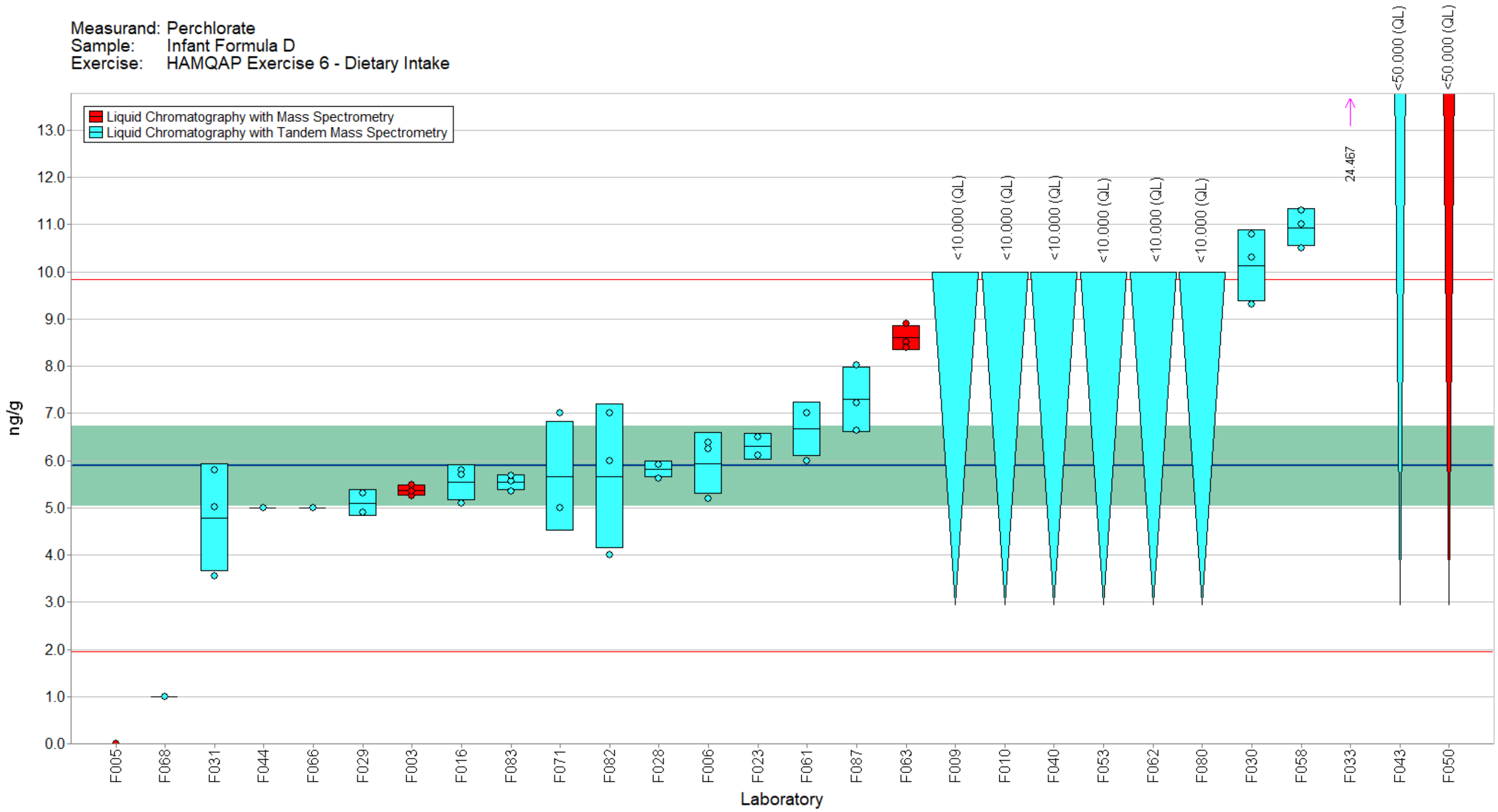


**Figure 8-28.** Perchlorate in Infant Formula D (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue 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 the consensus mean that result in an acceptable $Z'_{\text{comm}}$ score, $|Z'_{\text{comm}}| \leq 2$, with the lower limit set at zero. A NIST value has not been determined in this material.

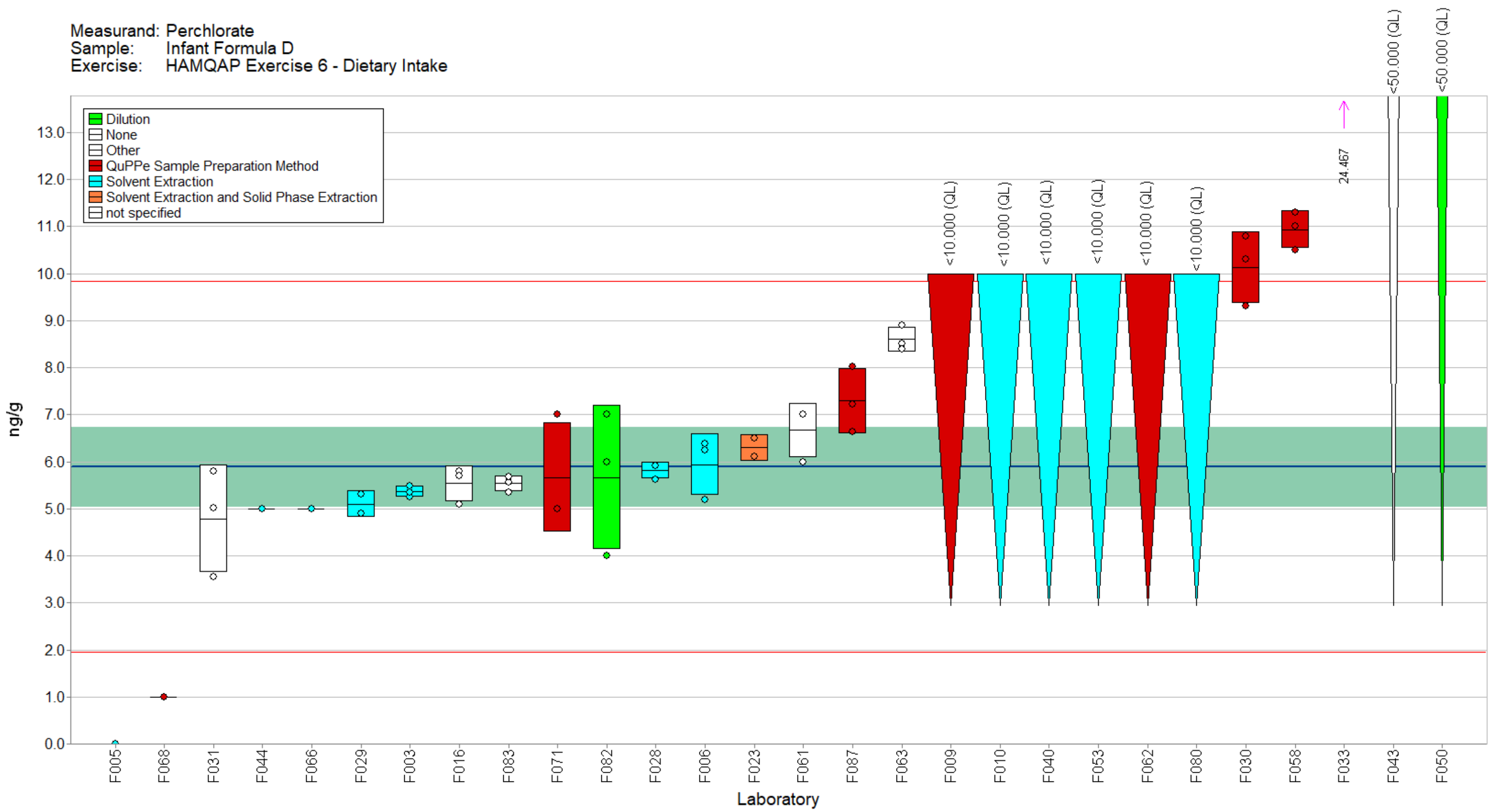


**Figure 8-29.** Perchlorate in Infant Formula D (data summary view – sample preparation method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The solid blue 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 the consensus mean that result in an acceptable $Z'_{\text{comm}}$ score, $|Z'_{\text{comm}}| \leq 2$, with the lower limit set at zero. A NIST value has not been determined in this material.

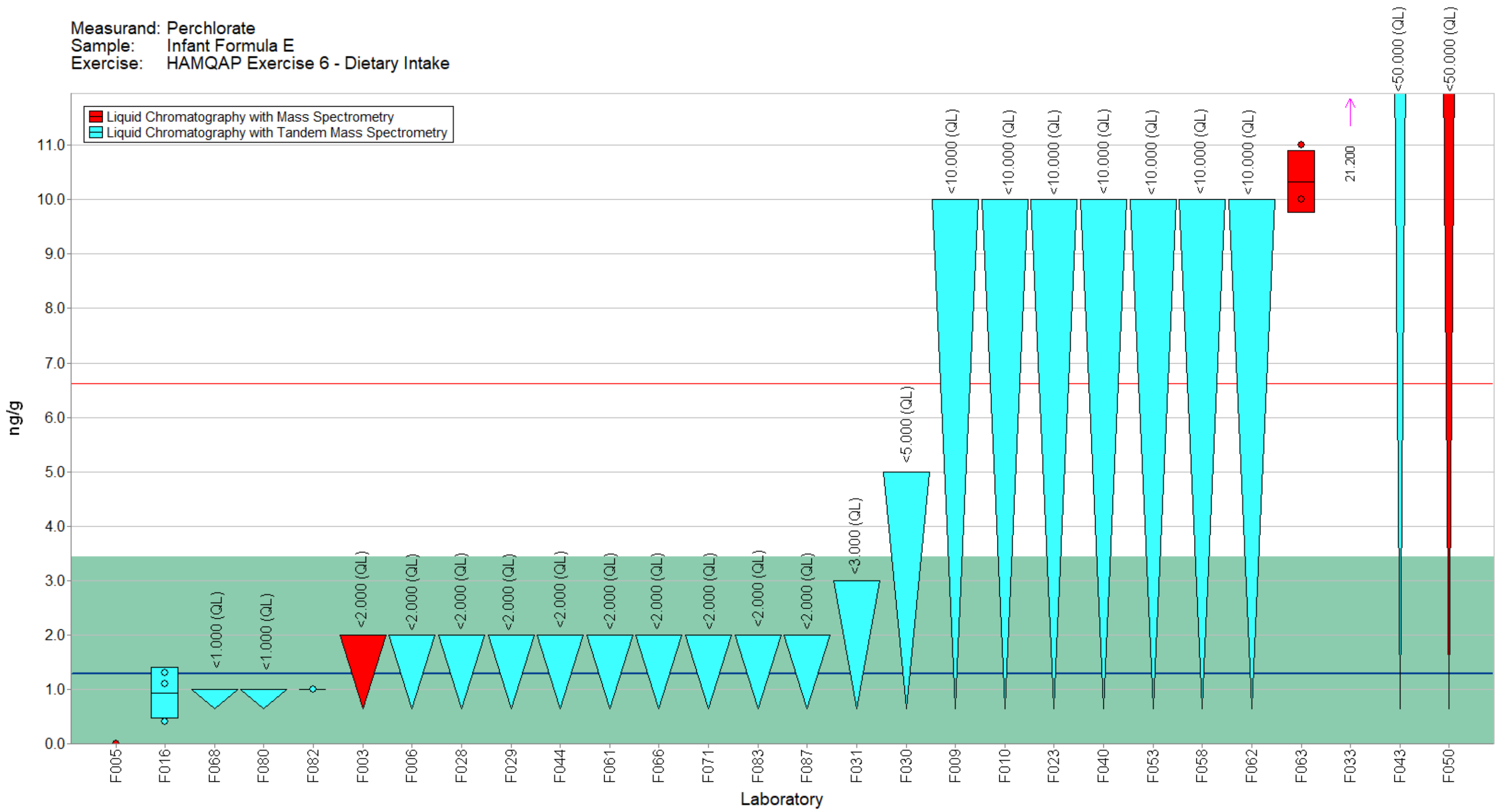


**Figure 8-30.** Perchlorate in Infant Formula E (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue 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 $Z'_{\text{comm}}$ score, $|Z'_{\text{comm}}| \leq 2$. A NIST value has not been determined in this material.

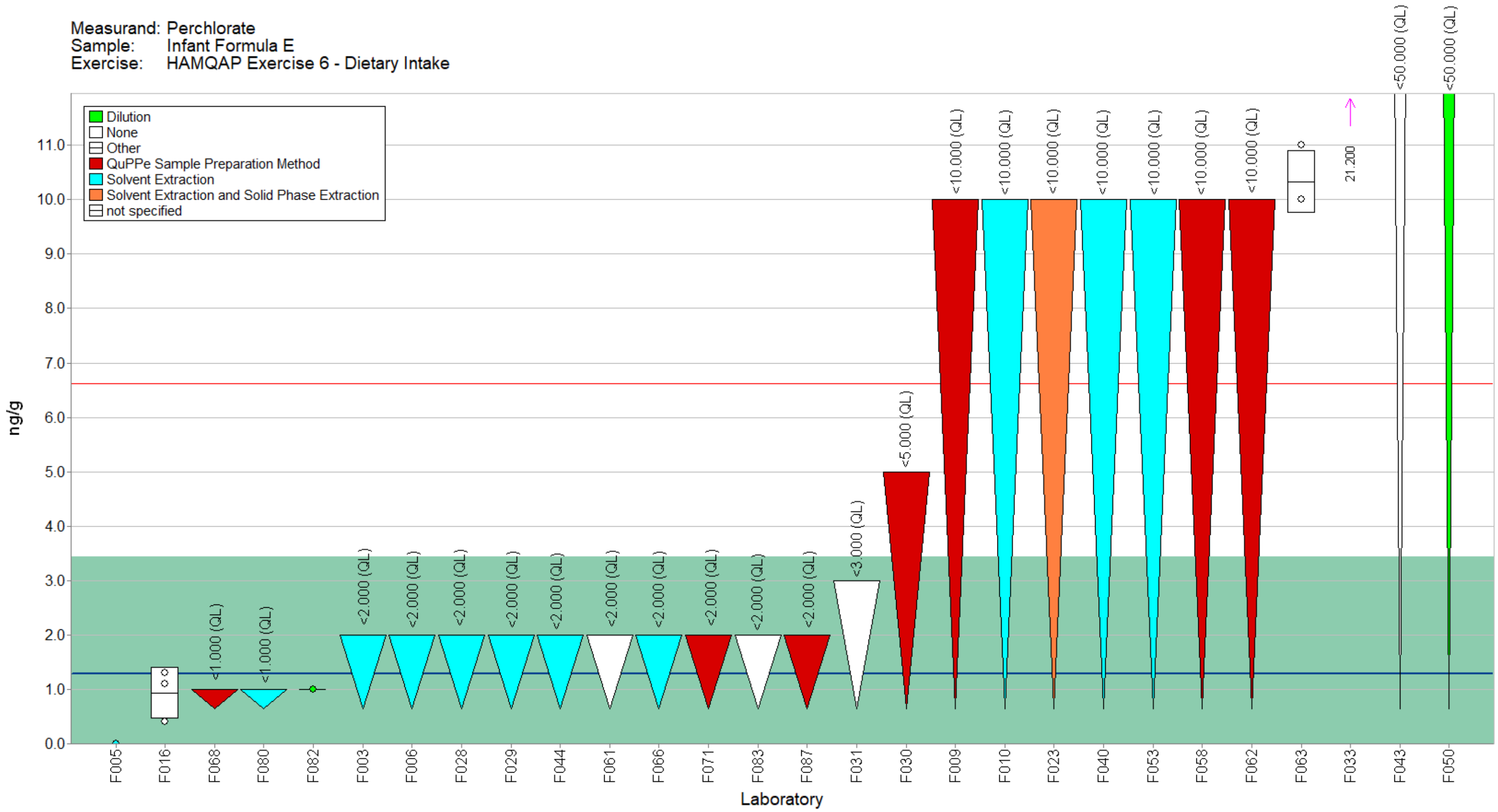


**Figure 8-31.** Perchlorate in Infant Formula E (data summary view – sample preparation method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The solid blue 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 $Z'_{\text{comm}}$ score, $|Z'_{\text{comm}}| \leq 2$. A NIST value has not been determined in this material.

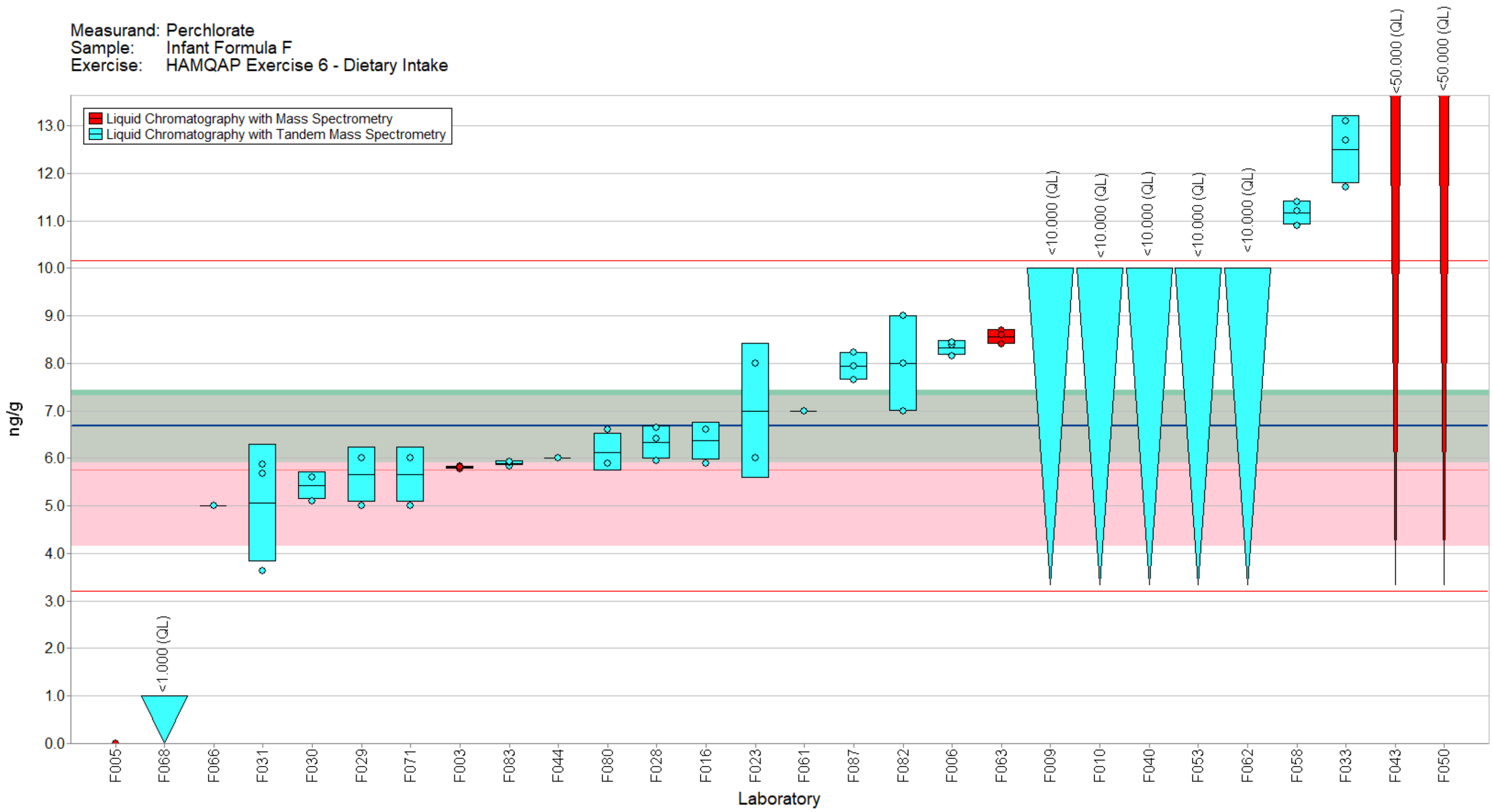


**Figure 8-32.** Perchlorate in Infant Formula F (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue 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 $Z'_{\text{comm}}$ score, $|Z'_{\text{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty ($U_{\text{NIST}}$) and represents the range that results in an acceptable $Z'_{\text{NIST}}$ score, $|Z'_{\text{NIST}}| \leq 2$.

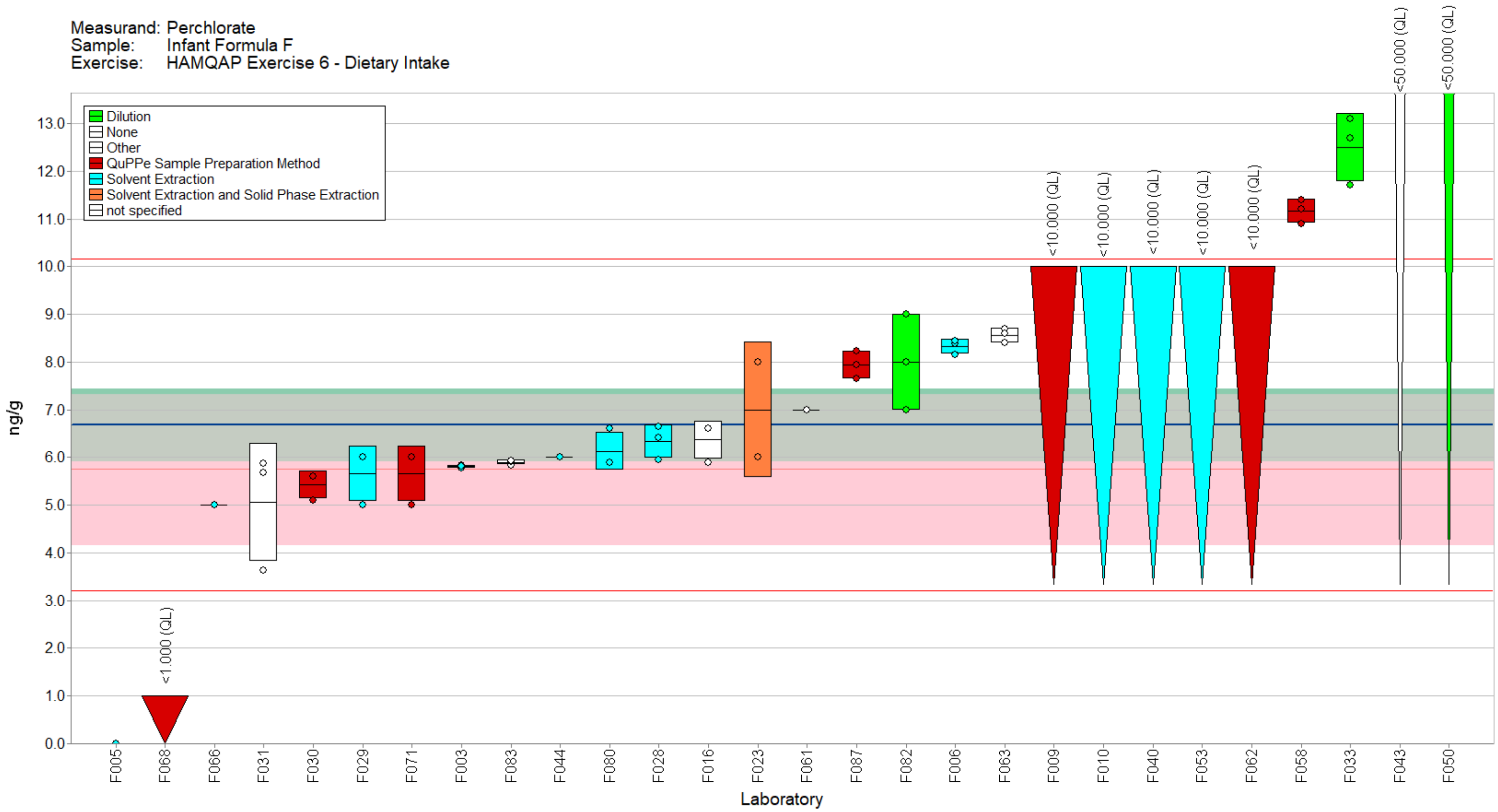


**Figure 8-33.** Perchlorate in Infant Formula F (data summary view – sample preparation method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The solid blue 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 $Z'_{\text{comm}}$ score, $|Z'_{\text{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty ($U_{\text{NIST}}$) and represents the range that results in an acceptable $Z'_{\text{NIST}}$ score, $|Z'_{\text{NIST}}| \leq 2$.

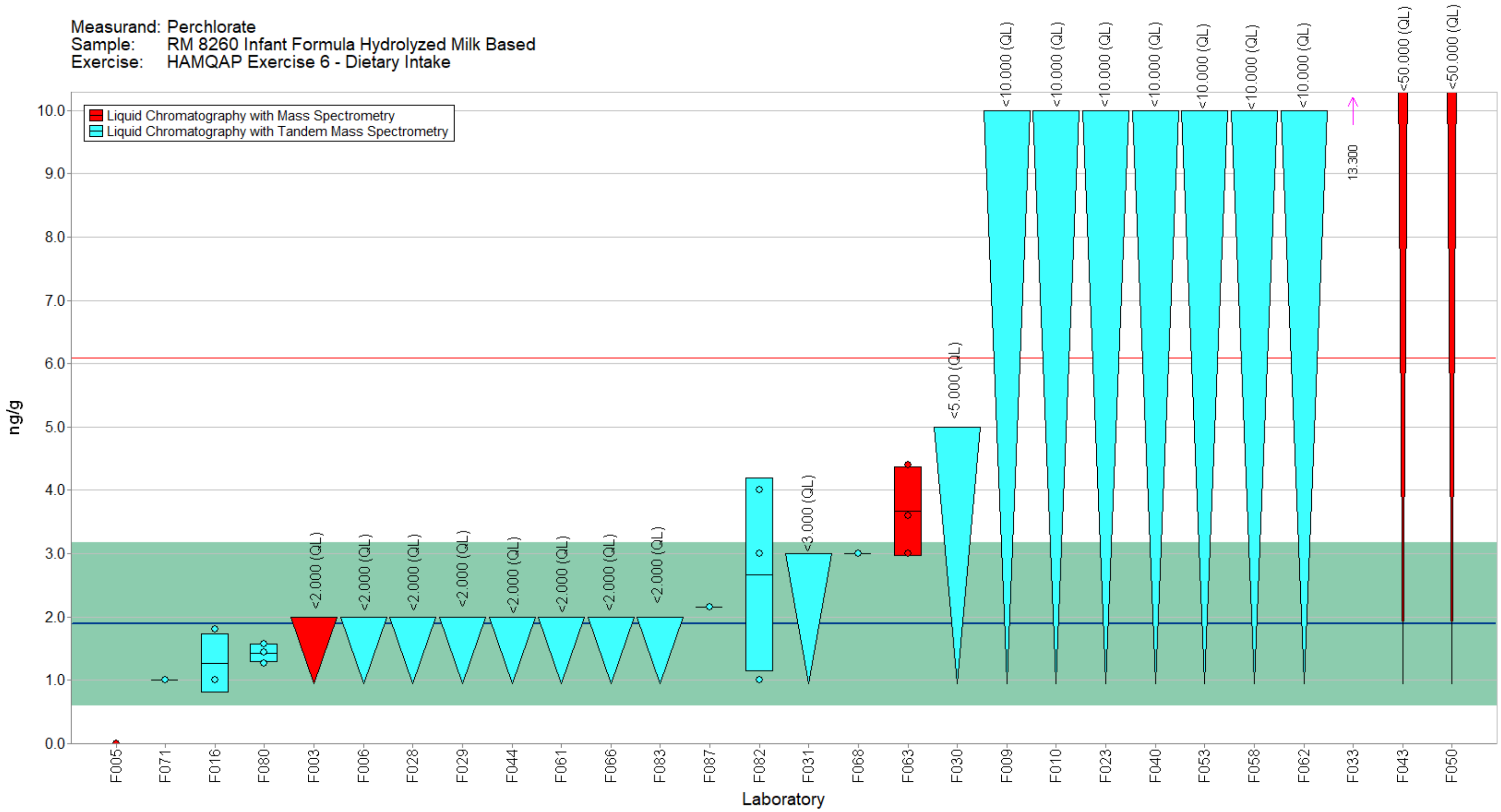


**Figure 8-34.** Perchlorate in RM 8260 Infant Formula Hydrolyzed Milk Based (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue 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 $Z'_{\mathrm{comm}}$ score, $|Z'_{\mathrm{comm}}| \leq 2$. A NIST value has not been determined in this material.

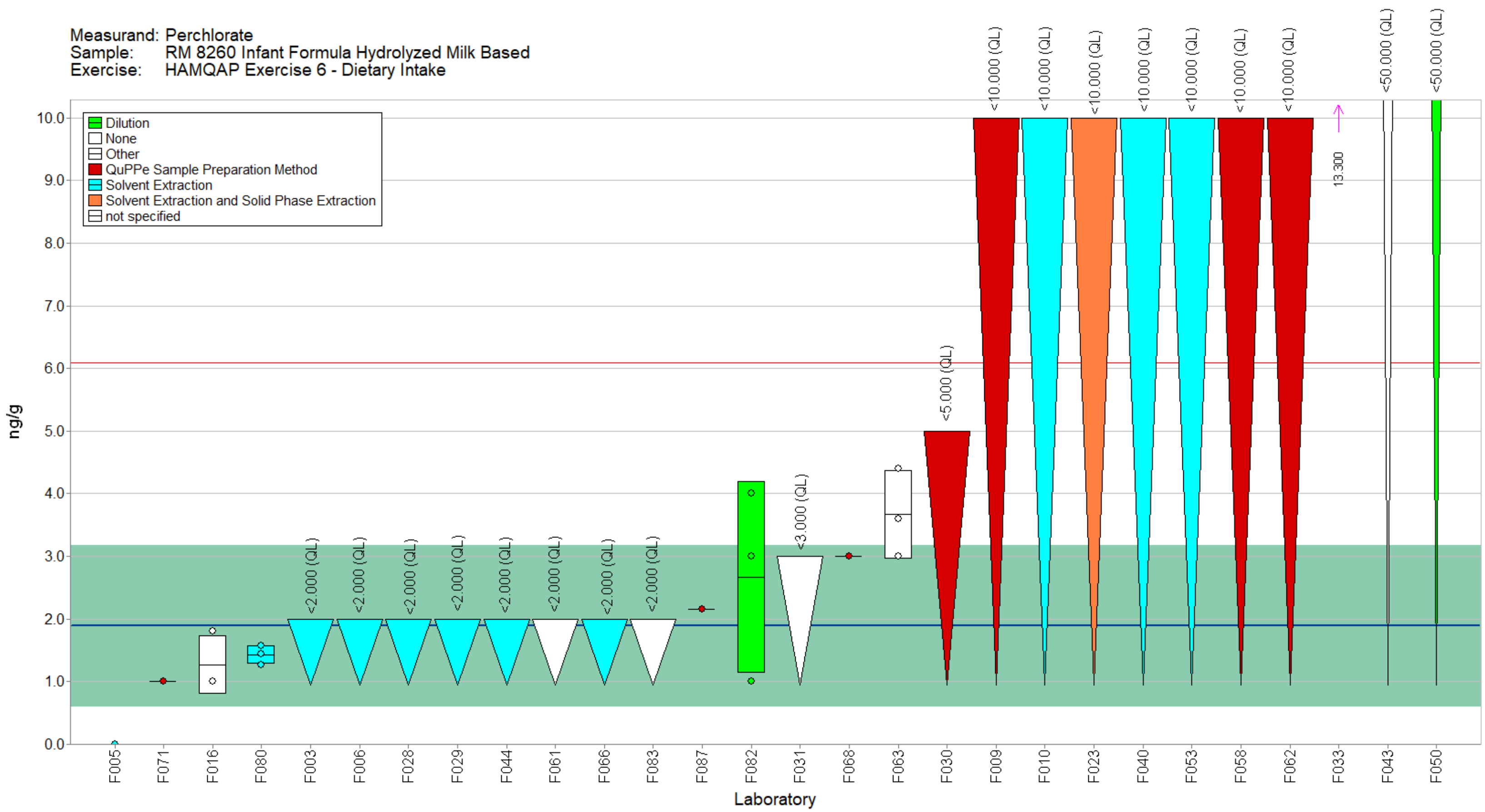


**Figure 8-35.** Perchlorate in RM 8260 Infant Formula Hydrolyzed Milk Based (data summary view – sample preparation method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The solid blue 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 $Z'_{\text{comm}}$ score, $|Z'_{\text{comm}}| \leq 2$. A NIST value has not been determined in this material.

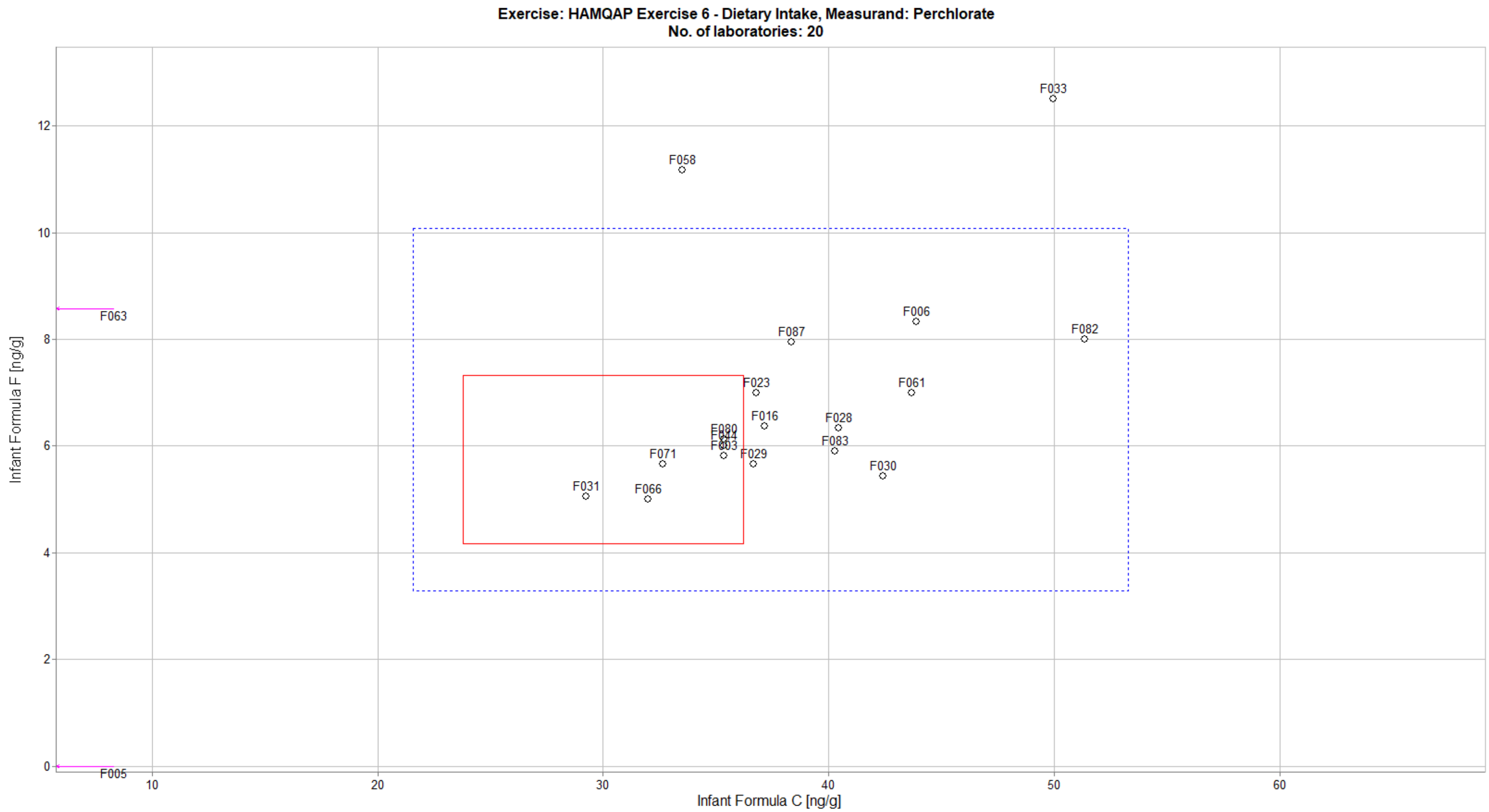


**Figure 8-36.** Laboratory means for perchlorate in Infant Formula C and Infant Formula F (sample/sample comparison view). In this view, the individual laboratory mean for one sample (Infant Formula C) is compared to the mean for a second sample (Infant Formula F). The solid red box represents the NIST range of tolerance for the two samples Infant Formula C (x-axis) and Infant Formula F (y-axis), which encompasses the target values bounded by their uncertainties ($U_{\mathrm{NIST}}$) and represents the range that results in an acceptable $Z_{\mathrm{NIST}}$ score, $|Z_{\mathrm{NIST}}| \leq 2$. The dotted blue box represents the consensus range of tolerance for Infant Formula C (x-axis) and Infant Formula F (y-axis), calculated as the values above and below the consensus means that result in an acceptable $Z'_{\mathrm{comm}}$ score, $|Z'_{\mathrm{comm}}| \leq 2$.

## SECTION 9: CONTAMINANTS II (Glyphosate, AMPA)

Study Overview

In this study, participants were provided with two jars of oat flour samples for dietary intake. Participants were asked to use in-house analytical methods to determine the mass fraction (ng/g) of glyphosate and its major metabolite, aminomethylphosphonic acid (AMPA), in each matrix. Glyphosate is a widely used broad-spectrum herbicide and crop desiccant. Worldwide experts have not agreed about the human toxicity of glyphosate, and as a result monitoring human exposure is critical to understanding population health impacts. For this reason, accurate analytical methods are needed for the determination of glyphosate in agricultural products such as oats. In addition, due to its highly polar nature, the screening for glyphosate typically requires a separate analytical method than typical methods used to screen for other pesticide residues.

Dietary Intake Sample Information

*Oat Flour A and Oat Flour B.* Participants were provided two jars of oat flour, each containing 100 g of material. Participants were asked to store the material at controlled room temperature ((20 °C to 25 °C), to use a sample size of at least 1 g, to use their in-house method of analysis, and to prepare three samples and report three values from each jar provided. Before use, participants were instructed to mix the contents of each jar thoroughly. Oat Flour A was designed to have an approximate glyphosate mass fraction of 500 ng/g and Oat Flour B was designed to have an approximate glyphosate mass fraction of 50 ng/g. The approximate analyte levels were not reported to participants prior to the study. Official target values were not assigned for glyphosate or AMPA by NIST for this study.

Dietary Intake Study Results

- Thirty-two laboratories enrolled in this exercise and received samples to measure glyphosate and AMPA. Between five and twenty-four laboratories reported quantitative results for each analyte. Ten to sixteen laboratories reported AMPA values below LOQs. The participation statistics for laboratories reporting quantitative results or reported that levels below LOQs or described in more detail below.

| Analyte | Number of Laboratories Requesting Samples | Number of Laboratories Reporting Results (Percent Participation) | |
|---|---|---|---|
| | | Oat Flour A | Oat Flour B |
| glyphosate | 32 | 24 (75 %) | 25 (72 %) |
| AMPA | 30 | 21 (70 %) | 21 (70 %) |

- The between-laboratory variability (% RSD) for glyphosate were good in both oat flours. The between-laboratory variability (% RSD) for AMPA was poor. Variabilities for each analyze/sample pair are reported below.

| Analyte | Between-Laboratory Variability (RSD) |
|---|---|

| | Oat Flour A | Oat Flour B |
|---|---|---|
| Glyphosate | 21 % | 30 % |
| AMPA | 55 % | 98 % |

- AMPA mass fractions in Oat Flour A and Oat Flour B were much lower compared to glyphosate. AMPA mass fractions levels in Oat Flour B were below the quantitation limits for most participants.
- The within laboratory repeatability of replicates was less than 12 % for all participants reporting values for glyphosate. The within laboratory repeatability of replicates was less than 25 % for AMPA for all participants who reported values.
- Majority of laboratories reporting results for glyphosate and AMPA used LC-MS/MS. LC-MS and liquid chromatography with high resolution mass spectrometry (LC-HRMS) were also reported. One or two laboratories used ion chromatography mass spectrometry (IC-MS), GC-MS, and LC-FLD. The percentage of participants using each analytical method is reported in the table below.

| Reported | Analyte | |
|---|---|---|
| Analytical Method | Glyphosate | AMPA |
| LC-MS/MS | 58 % | 59 % |
| LC-MS | 17 % | 14 % |
| LC-HRMS | 8 % | 9 % |
| IC-MS | 8 % | 5 % |
| GC-MS | 4 % | 5 % |
| LC-FLD | 4 % | 5 % |

- Due to their polar nature, glyphosate and AMPA are sometimes derivatized prior to analysis, although there are several direct methods reported in the literature. In this study for glyphosate, six laboratories out of 24 laboratories (25 %) reported that a derivatization protocol was used. For AMPA, five out of 22 laboratories (23 %) that reported either a value, 0, not detected, or below a limit of quantitation reported using a derivatization protocol.
- Three laboratories reported for both glyphosate and AMPA analysis using the Quick Polar Pesticides Method (QuPPe). Most other laboratories reported using extraction and solid phase extraction sample preparation protocols. Some laboratories did not specify a sample preparation method.

Dietary Intake Technical Recommendations

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

- Overall, the agreement between-laboratory results was good for glyphosate in both oat flours.
- More variability was observed for AMPA compared to glyphosate, likely due to the smaller amounts of AMPA in the oat flour materials.
- If using matrix-matched calibration, the blank matrix must be free of glyphosate and AMPA or the signal of the calibrants must be much higher than the blank signal.

- No trends were observed that correlated reported results with the analytical method approach used.
- No trends were observed between direct and derivatized analysis.
- Most laboratories reported use of MS-based methodologies for determination of glyphosate and AMPA. Isotopically labeled internal standards, added at the beginning of the analytical procedure, often result in improved accuracy and precision of results.
- "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 LOQ or QL.
- 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 materials prepared in-house.
- A linear calibration curve which surrounds 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 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 9-1.** Individualized data summary table (NIST) for glyphosate and AMPA in Oat Flour A and Oat Flour B.

## *National Institute of Standards and Technology*

**HAMQAP Exercise 6 - Contaminants II**

| | **Lab Code:** | **NIST** | **1. Your Results** | | | | **2. Community Results** | | | **3. Target** | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Analyte | Sample | Units | $x_i$ | $s_i$ | $Z'_{comm}$ | $Z_{NIST}$ | N | x* | s* | $x_{NIST}$ | *U* |
| AMPA | Oat Flour A | ng/g | | | | | 12 | 20 | 11 | | |
| AMPA | Oat Flour B | ng/g | | | | | 6 | 8 | 7.9 | | |
| Glyphosate | Oat Flour A | ng/g | | | | | 24 | 420 | 90 | | |
| Glyphosate | Oat Flour B | ng/g | | | | | 24 | 70 | 20 | | |

$x_i$ Mean of reported values
$s_i$ Standard deviation of reported values
$Z'_{comm}$ Z'-score with respect to community consensus
$Z_{NIST}$ Z-score with respect to NIST value

N Number of quantitative values reported
x* Robust mean of reported values
s* Robust standard deviation

$x_{NIST}$ NIST-assessed value
*U* expanded uncertainty about the NIST-assessed value

**Table 9-2.** Data summary table for glyphosate in Oat Flour A and Oat Flour B. Data points highlighted in red have a zero or non-numeric data point.

| | | Glyphosate | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Oat Flour A (ng/g) | | | | | Oat Flour B (ng/g) | | | | |
| | Lab | A | B | C | Avg | SD | A | B | C | Avg | SD |
| Individual Results | Target | | | | | | | | | | |
| | F004 | | | | | | | | | | |
| | F005 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | F007 | 276 | 312 | 305 | 297.7 | 19.1 | 63.5 | 66.5 | 43.9 | 57.97 | 12.3 |
| | F008 | | | | | | | | | | |
| | F009 | 403.6 | 407.9 | 403.9 | 405.1 | 2.40 | 48.9 | 40.7 | 53.2 | 47.60 | 6.35 |
| | F010 | 433.4 | 400.4 | 401.9 | 411.9 | 18.6 | 62 | 76.9 | 59 | 65.97 | 9.59 |
| | F014 | | | | | | | | | | |
| | F018 | | | | | | | | | | |
| | F020 | 370 | 330 | 380 | 360.0 | 26.5 | 60 | 50 | 50 | 53.33 | 5.77 |
| | F021 | | | | | | | | | | |
| | F023 | 500 | 540 | | 520.0 | 28.3 | 71 | 78 | | 74.50 | 4.95 |
| | F024 | 364.4 | 391.2 | 410.9 | 388.8 | 23.3 | 51.1 | 53.1 | 53.8 | 52.67 | 1.40 |
| | F026 | 319.66 | | | 319.7 | | 57.33 | | | 57.33 | |
| | F030 | 553 | 540 | 532 | 541.7 | 10.6 | 81 | 87 | 89 | 85.67 | 4.16 |
| | F031 | 399.59 | 439.61 | 450.01 | 429.7 | 26.6 | 76.47 | 82.05 | 83.05 | 80.52 | 3.55 |
| | F034 | 496 | 490 | 487 | 491.0 | 4.58 | 101 | 99.5 | 98.8 | 99.77 | 1.12 |
| | F040 | 300 | 351 | 329 | 326.7 | 25.6 | 44 | 49 | 43 | 45.33 | 3.21 |
| | F044 | 432 | 470 | 488 | 463.3 | 28.6 | 75 | 75 | 63 | 71.00 | 6.93 |
| | F047 | 456.1 | 446.2 | 438.8 | 447.0 | 8.68 | 82.4 | 89 | 85.7 | 85.70 | 3.30 |
| | F054 | 406.5 | 397.4 | 414.9 | 406.3 | 8.8 | 62.5 | 61.5 | 61.3 | 61.77 | 0.64 |
| | F055 | 409 | 420 | 335 | 388.0 | 46.2 | 65 | 71 | 61 | 65.67 | 5.03 |
| | F062 | 430 | 452 | 409 | 430.3 | 21.5 | 72 | 71 | 81 | 74.67 | 5.51 |
| | F063 | 420 | 450 | 410 | 426.7 | 20.8 | 86 | 79 | 96 | 87.00 | 8.54 |
| | F067 | 426 | 428 | 436 | 430.0 | 5.29 | 99.4 | 98.3 | 100 | 99.23 | 0.86 |
| | F070 | | | | | | | | | | |
| | F071 | 345 | 322 | 319 | 328.7 | 14.2 | 45 | 46 | 45 | 45.33 | 0.58 |
| | F078 | | | | | | | | | | |
| | F080 | 353 | 366 | 334 | 351.0 | 16.1 | 57 | 57 | 55 | 56.33 | 1.15 |
| | F083 | 514 | 494 | 531 | 513.0 | 18.5 | 76.9 | 88 | 73.2 | 79.37 | 7.70 |
| | F084 | 471 | 467 | 472 | 470.0 | 2.65 | 66.2 | 67.1 | 65.8 | 66.37 | 0.67 |
| | F087 | 594 | 571 | 492 | 552.3 | 53.5 | 67 | 67.3 | 63.4 | 65.90 | 2.17 |
| | F088 | | | | | | | | | | |
| Community Results | | Consensus Mean | | | 421.7 | | Consensus Mean | | | 67.48 | |
| | | Consensus Standard Deviation | | | 89.5 | | Consensus Standard Deviation | | | 20.29 | |
| | | Maximum | | | 552.3 | | Maximum | | | 99.77 | |
| | | Minimum | | | 0 | | Minimum | | | 0 | |
| | | N | | | 24 | | N | | | 24 | |

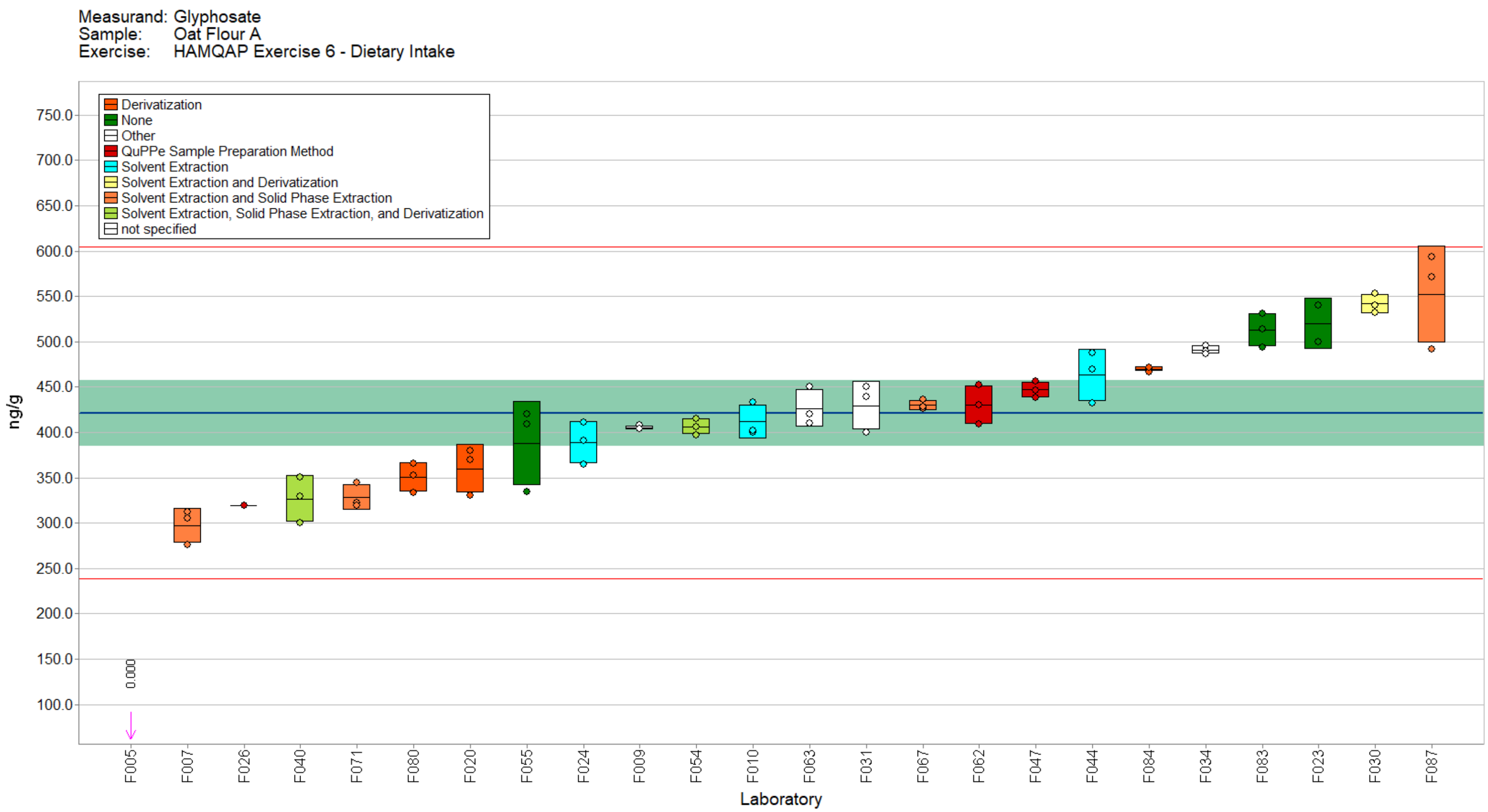


**Figure 9-1.** Glyphosate in Oat Flour A (data summary view –sample preparation). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation employed. The solid blue line 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 $Z'_{\text{comm}}$ score, $|Z'_{\text{comm}}| \leq 2$. A NIST value has not been determined in this material.

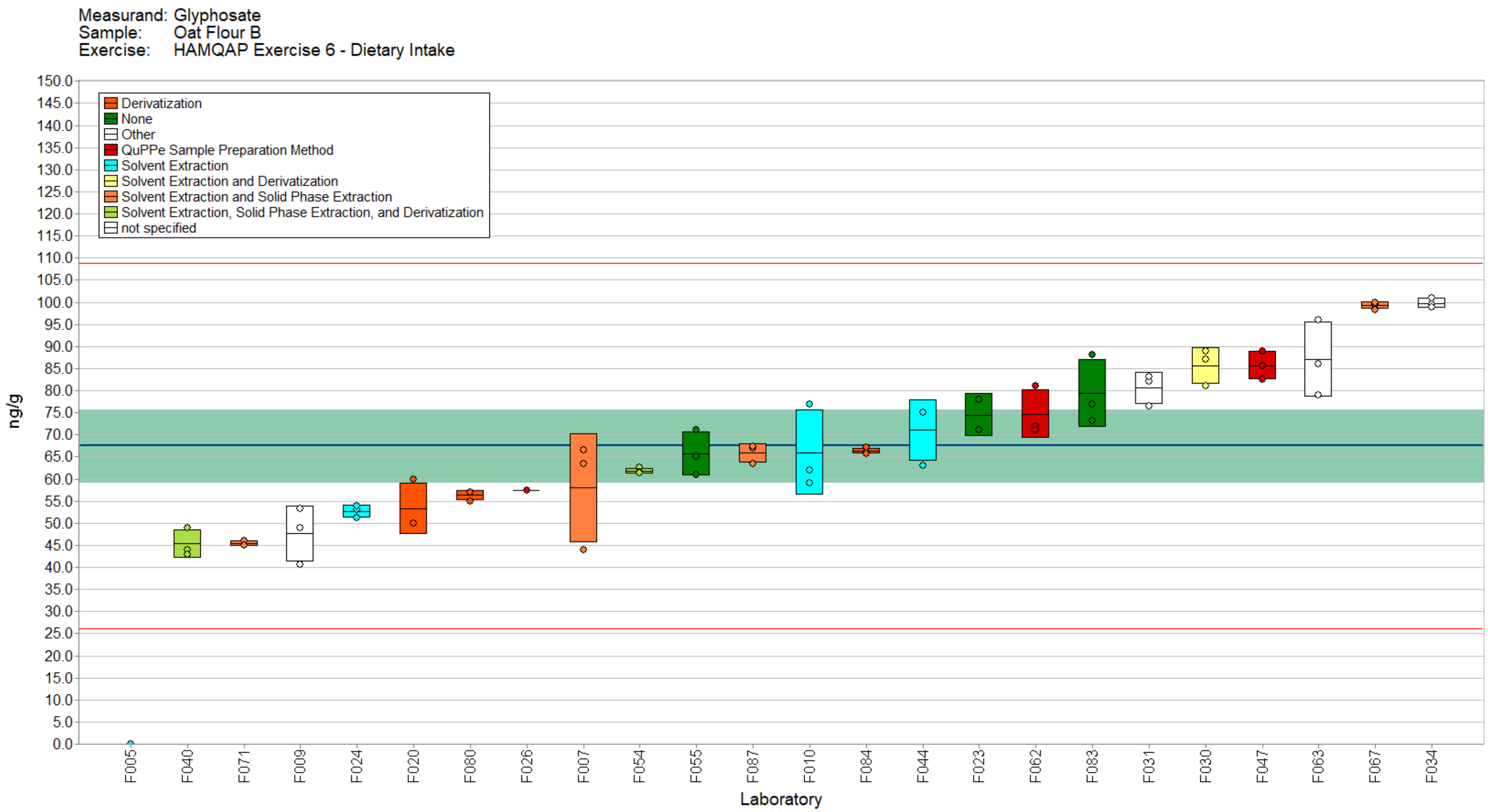


**Figure 9-2.** Glyphosate in Oat Flour B (data summary view – sample preparation). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation employed. The solid blue line 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 $Z'_{\text{comm}}$ score, $|Z'_{\text{comm}}| \leq 2$. A NIST value has not been determined in this material.

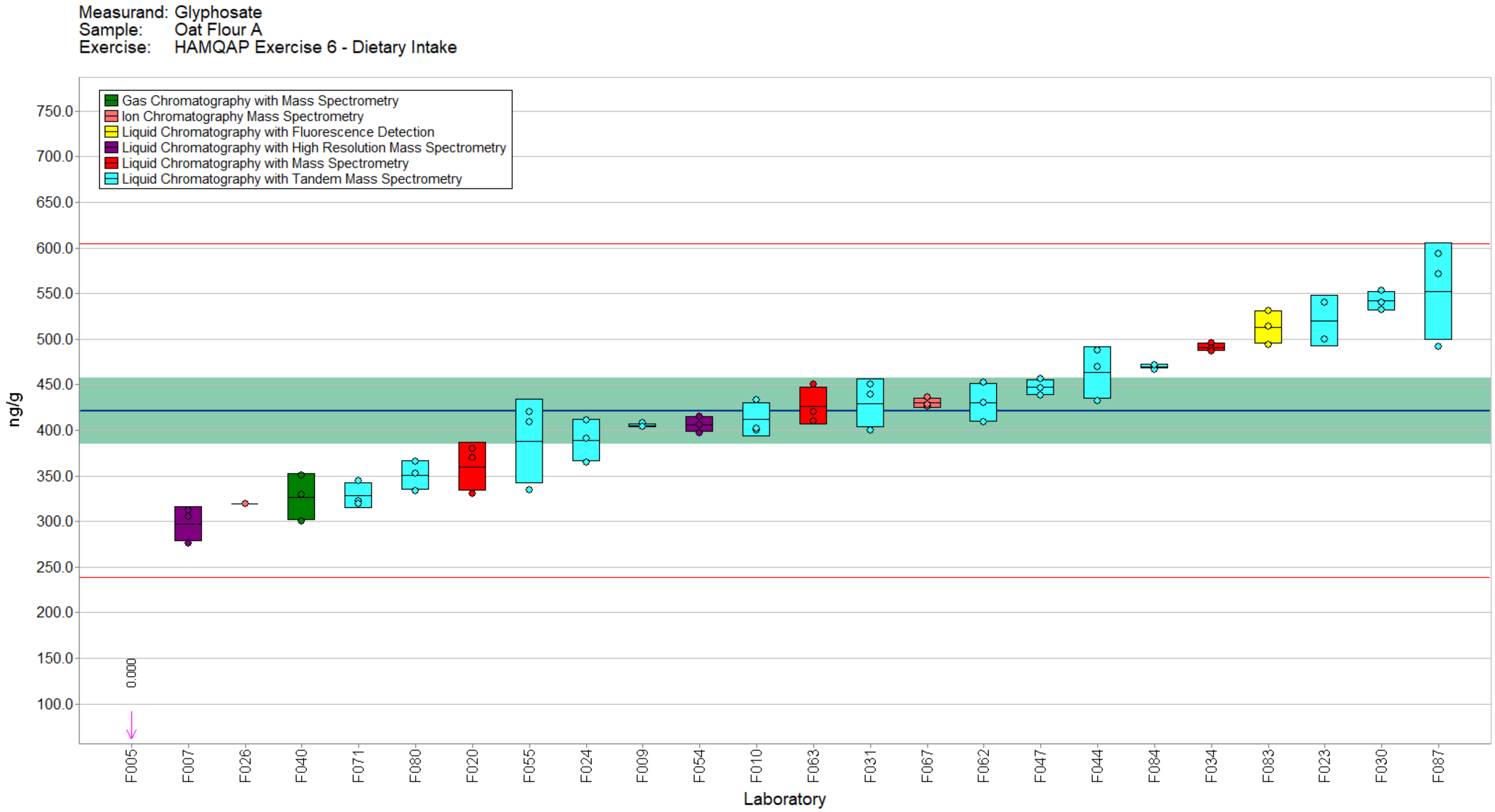


**Figure 9-3.** Glyphosate in Oat Flour A (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line represents 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 $Z'_{\text{comm}}$ score, $|Z'_{\text{comm}}| \leq 2$. A NIST value has not been determined in this material.

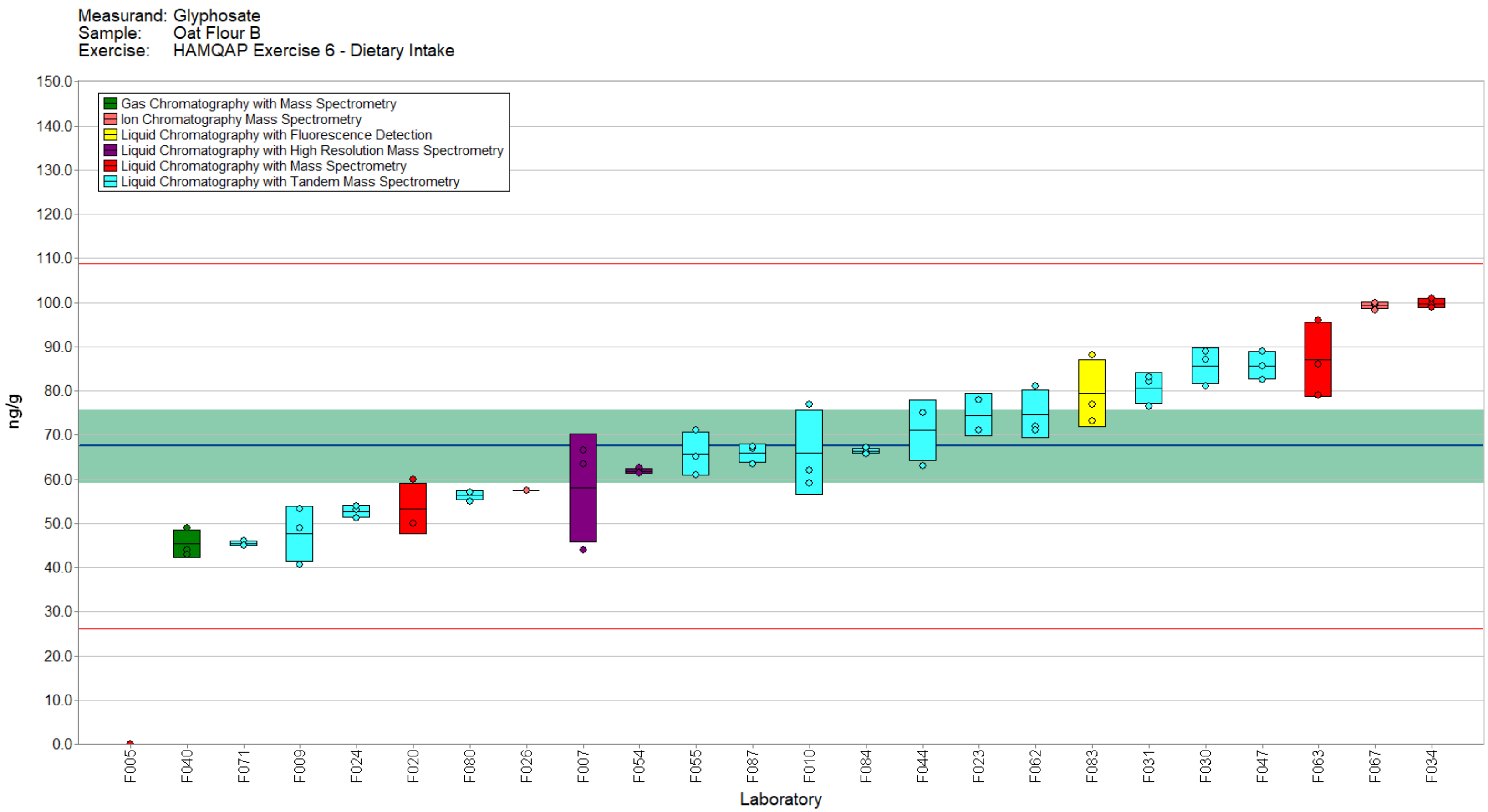


**Figure 9-4.** Glyphosate in Oat Flour B (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line represents 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 $Z'_{\text{comm}}$ score, $|Z'_{\text{comm}}| \leq 2$. A NIST value has not been determined in this material.

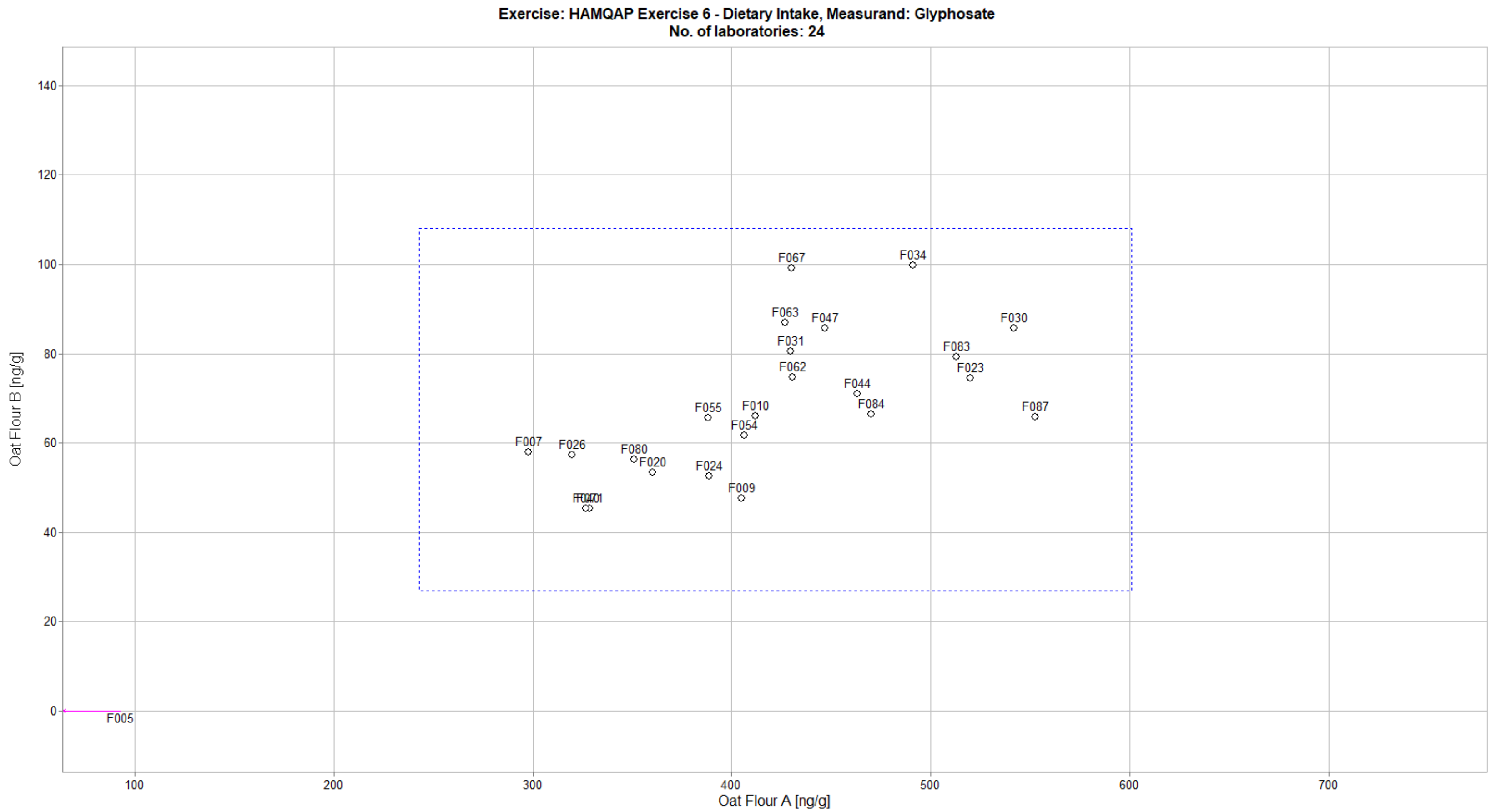


**Figure 9-5.** Laboratory means for glyphosate in Oat Flour A and Oat Flour B (sample/sample comparison view). In this view, the individual laboratory mean for one sample (Oat Flour A) is compared to the individual laboratory mean for a second sample (Oat Flour B). The dotted blue box represents the consensus range of tolerance for Oat Flour A (x-axis) and Oat Flour B (y-axis), calculated as the values above and below the consensus means that result in an acceptable $Z'_{\mathrm{comm}}$ score, $|Z'_{\mathrm{comm}}| \leq 2$.

**Table 9-3.** Data summary table for AMPA in Oat Flour A and Oat Flour B. Data points highlighted in red have a zero or non-numeric data point.

| | | AMPA | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Oat Flour A (ng/g) | | | | | Oat Flour B (ng/g) | | | | |
| | **Lab** | **A** | **B** | **C** | **Avg** | **SD** | **A** | **B** | **C** | **Avg** | **SD** |
| **Individual Results** | Target | | | | | | | | | | |
| | F004 | | | | | | | | | | |
| | F005 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | F007 | | | | | | | | | | |
| | F008 | | | | | | | | | | |
| | F009 | < 1000 | < 1000 | < 1000 | | | < 1000 | < 1000 | < 1000 | | |
| | F010 | < 50 | < 50 | < 50 | | | < 50 | < 50 | < 50 | | |
| | F014 | | | | | | | | | | |
| | F020 | < 5 | < 5 | < 5 | | | < 5 | < 5 | < 5 | | |
| | F021 | | | | | | | | | | |
| | F023 | < 40 | < 40 | | | | < 40 | < 40 | | | |
| | F024 | < 50 | < 50 | < 50 | | | < 50 | < 50 | < 50 | | |
| | F026 | 5.26 | | | 5.26 | | 6.59 | | | 6.59 | |
| | F030 | 25 | 25 | 24 | 24.67 | 0.58 | < 10 | < 10 | < 10 | | |
| | F031 | 20.36 | 17.57 | 21.94 | 19.96 | 2.2 | < 10 | < 10 | < 10 | | |
| | F040 | 27 | 20 | 33 | 26.67 | 6.5 | 24 | 19 | 15 | 19.33 | 4.5 |
| | F044 | 26 | 25 | 27 | 26.00 | 1.0 | 5 | 6 | 6 | 5.67 | 0.58 |
| | F047 | 20.1 | 20 | 19.7 | 19.93 | 0.21 | < RL | < RL | < RL | | |
| | F054 | < 100 | < 100 | < 100 | | | < 100 | < 100 | < 100 | | |
| | F055 | < 20 | < 20 | < 20 | | | < 20 | < 20 | < 20 | | |
| | F062 | < 100 | < 100 | < 100 | | | < 100 | < 100 | < 100 | | |
| | F063 | 19 | 18 | 17 | 18.00 | 1.0 | < 10 | < 10 | < 10 | | |
| | F067 | ND | ND | ND | | | ND | ND | ND | | |
| | F070 | | | | | | | | | | |
| | F071 | 13 | 14 | 14 | 13.67 | 0.58 | 3 | 3 | 3 | 3.00 | 0 |
| | F078 | | | | | | | | | | |
| | F080 | | | | | | | | | | |
| | F083 | 26.4 | 26.4 | 30.7 | 27.83 | 2.5 | 13.2 | 14.8 | 13 | 13.67 | 0.99 |
| | F084 | 19.3 | 22.7 | 21 | 21.00 | 1.7 | < 10 | < 10 | < 10 | | |
| | F087 | 35.9 | 43.4 | 39.1 | 39.47 | 3.8 | < 10 | < 10 | < 10 | | |
| | F088 | | | | | | | | | | |
| **Community Results** | | Consensus Mean | | | 20.3 | | Consensus Mean | | | 8.04 | |
| | | Consensus Standard Deviation | | | 11.2 | | Consensus Standard Deviation | | | 7.9 | |
| | | Maximum | | | 39.5 | | Maximum | | | 19.3 | |
| | | Minimum | | | 0 | | Minimum | | | 0 | |
| | | N | | | 12 | | N | | | 6 | |

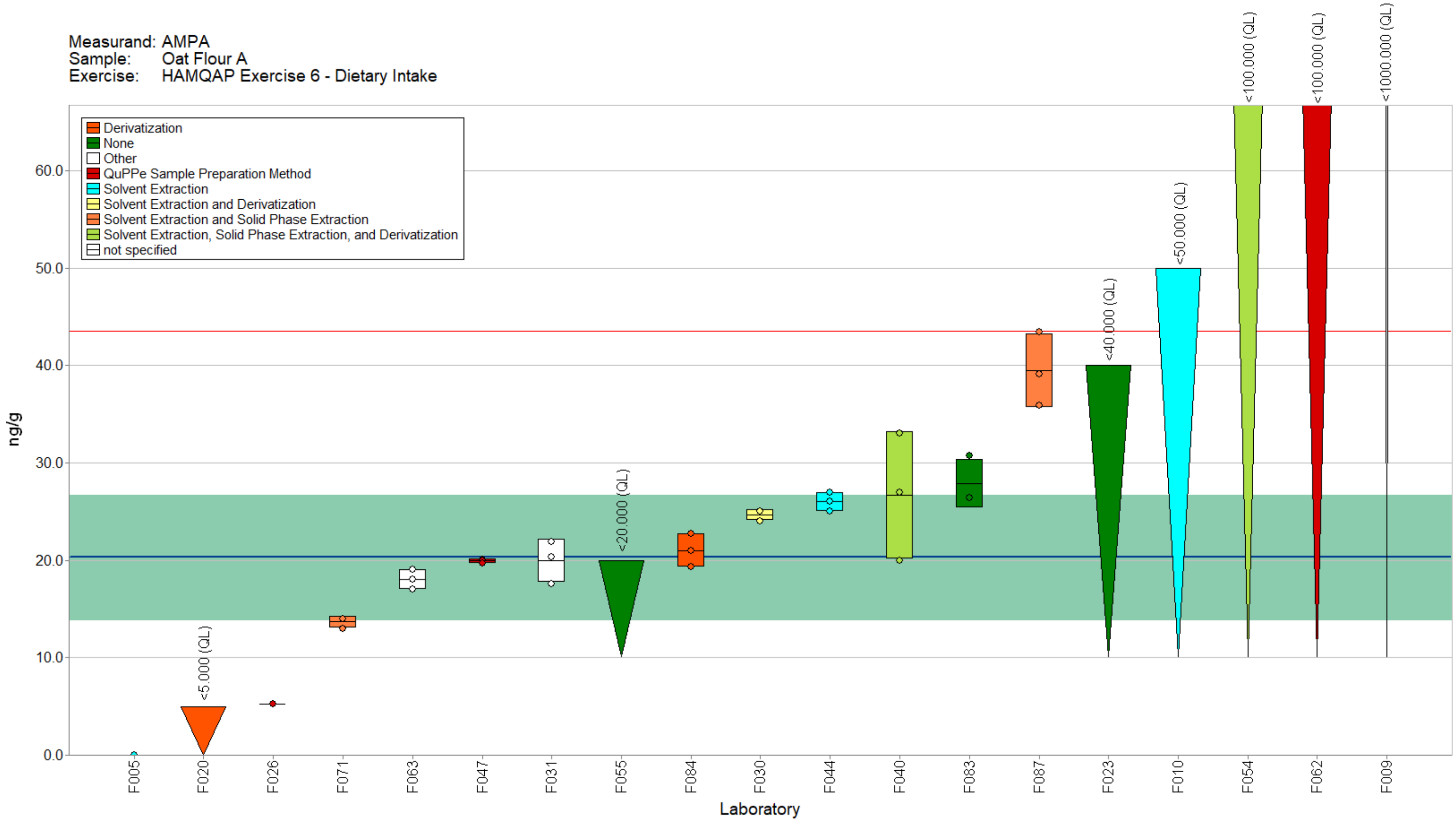


**Figure 9-6.** AMPA in Oat Flour A (data summary view – sample preparation). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation employed. The solid blue line represents 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 the consensus mean that result in an acceptable $Z'_{\text{comm}}$ score, $|Z'_{\text{comm}}| \leq 2$, with the lower limit set at zero. A NIST value has not been determined in this material.

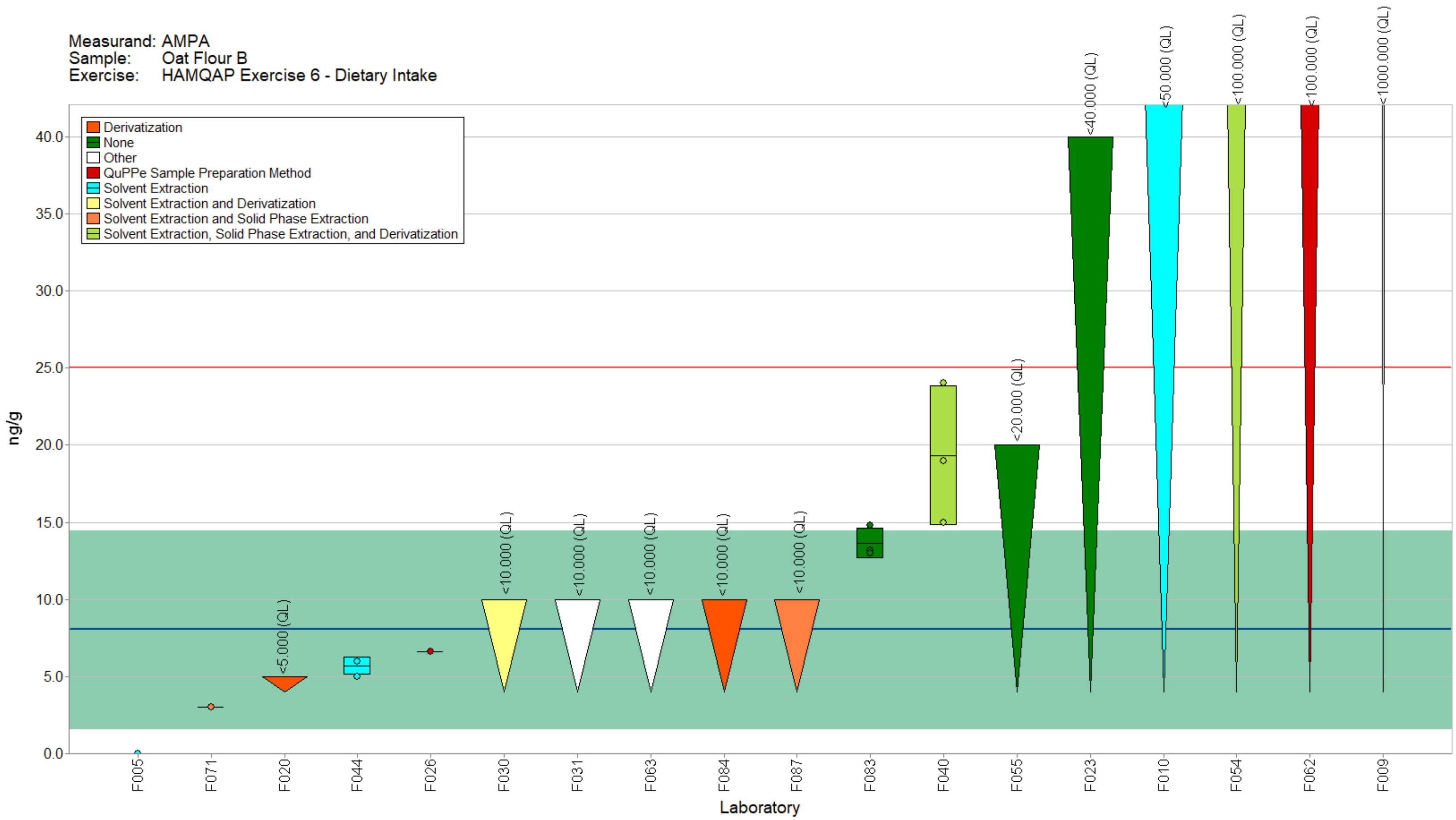


**Figure 9-7.** AMPA in Oat Flour B (data summary view – sample preparation). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation employed. The solid blue line represents 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 the consensus mean that result in an acceptable $Z'_{\text{comm}}$ score, $|Z'_{\text{comm}}| \leq 2$, with the lower limit set at zero. A NIST value has not been determined in this material.

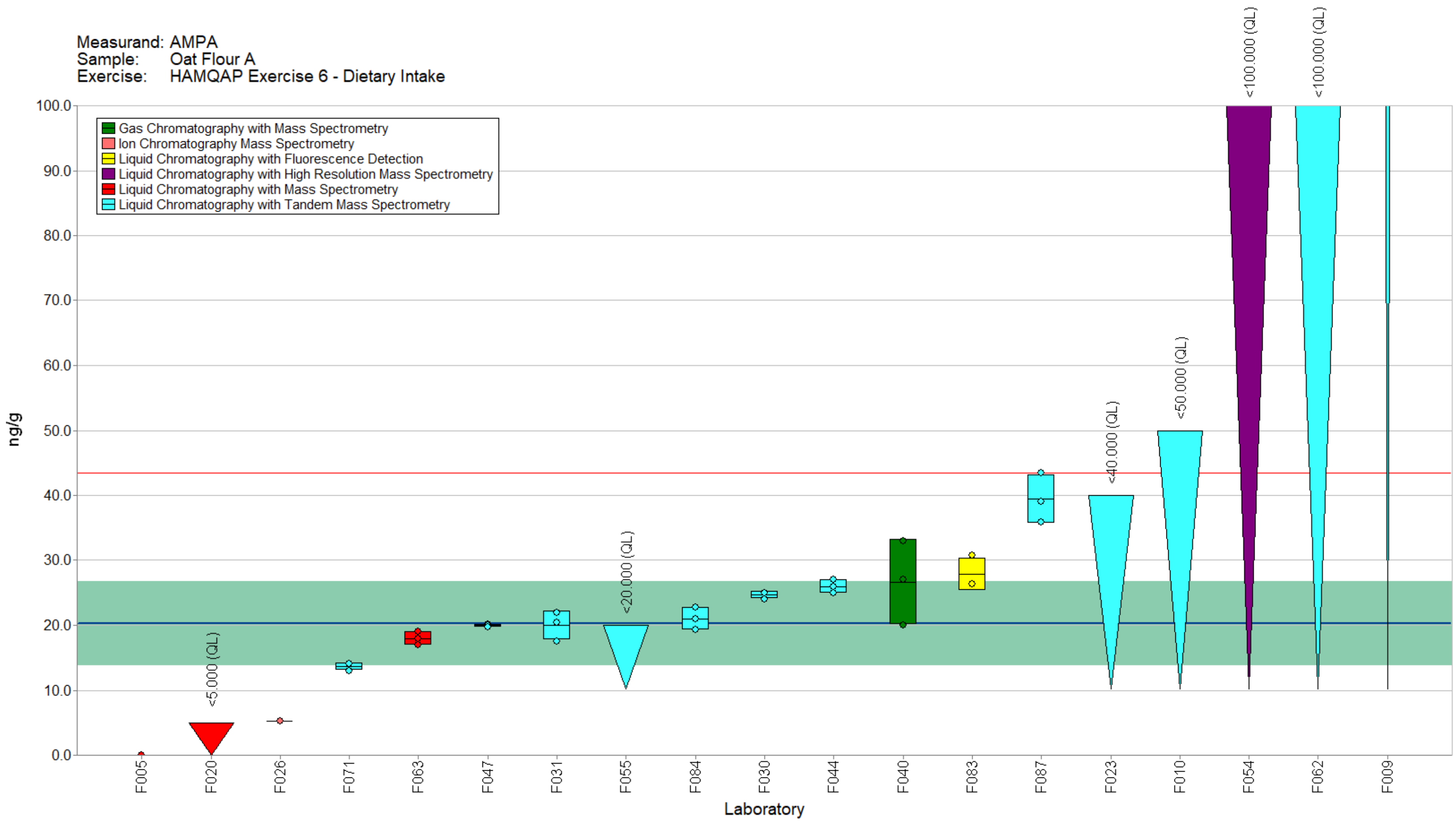


**Figure 9-8.** AMPA in Oat Flour A (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line represents 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 the consensus mean that result in an acceptable $Z'_{\text{comm}}$ score, $|Z'_{\text{comm}}| \leq 2$, with the lower limit set at zero. A NIST value has not been determined in this material.

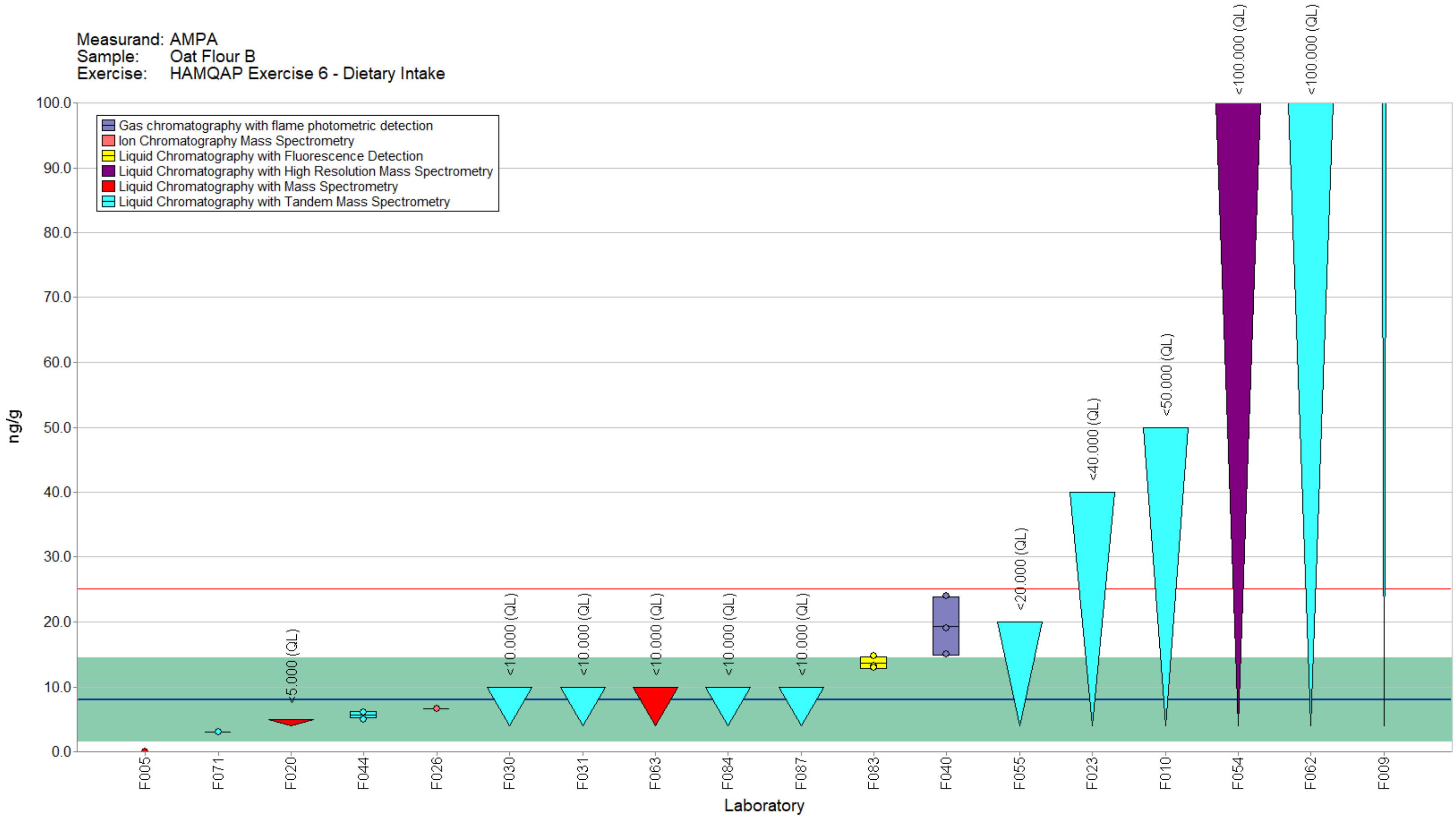


**Figure 9-9.** AMPA in Oat Flour B (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line represents 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 the consensus mean that result in an acceptable $Z'_{\text{comm}}$ score, $|Z'_{\text{comm}}| \leq 2$, with the lower limit set at zero. A NIST value has not been determined in this material.

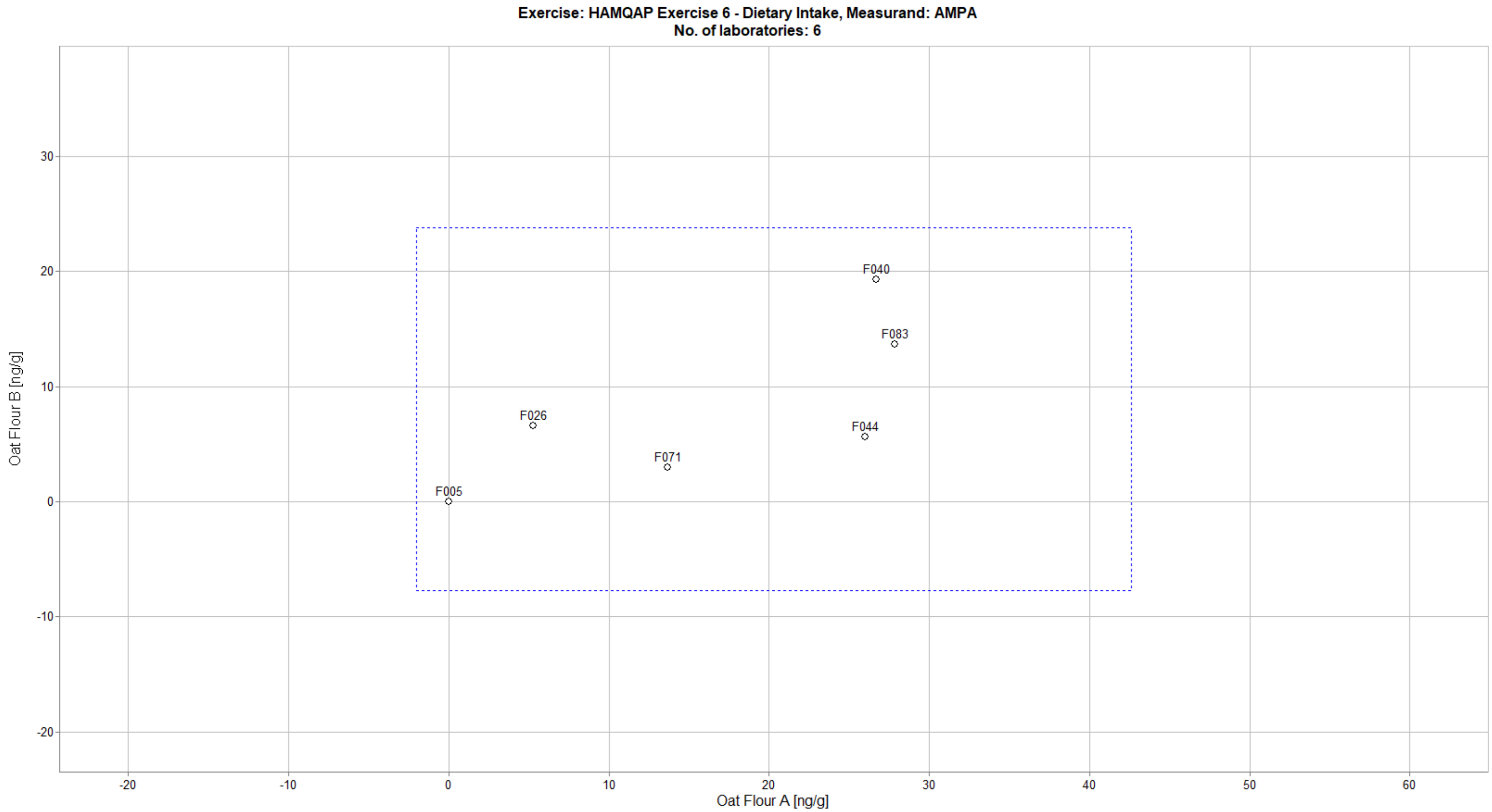


**Figure 9-10.** Laboratory means for AMPA in Oat Flour A and Oat Flour B (sample/sample comparison view). In this view, the individual laboratory mean for one sample (Oat Flour A) is compared to the individual laboratory mean for a second sample (Oat Flour B). The dotted blue box represents the consensus range of tolerance for Oat Flour A (x-axis) and Oat Flour B (y-axis), calculated as the values above and below the consensus means that result in an acceptable $Z'_{\mathrm{comm}}$ score, $|Z'_{\mathrm{comm}}| \leq 2$.

## SECTION 10: PROXIMATES

Study Overview

In this study, participants were provided with samples of infant formula and Rice Flour for dietary intake. Participants were asked to use in-house analytical methods to determine the mass fraction (percent) of proximates (fat, protein, carbohydrates, solids, and ash) as well as the energy as calories (kcal/100 g) in each matrix. As the major constituents of any food, proximates are the primary contributors to human caloric (energy) intake and are prominent on nutrition facts panels on packaged foods in the US. Proximates are also important from an analytical perspective, as the relative fat/protein/carbohydrate ratios of a food are critical factors for predicting measurement challenges and selecting appropriate control materials. Accurate measurement of proximates and calories in foods is necessary to support reliable food labeling and inform population studies that impact dietary guidelines.

Dietary Intake Sample Information

*Infant Formula A.* Participants were provided with three packets each containing 10 g of powdered infant formula. Participants were asked to store the material at -20 °C or colder, to use a sample size appropriate for their in-house method of analysis, and to prepare one sample and report one value from each packet provided. Before use, participants were instructed to mix the contents of the packet thoroughly and use a conversion factor of 6.38 for calculation of total protein from nitrogen results, as recommended in AOAC Official Method 991.20. The approximate analyte levels were not reported to participants prior to the study. The NIST-determined values for proximates were assigned using results from the manufacturer of the material. The NIST-determined values and uncertainties are provided in the table below on an as-received basis.

| | NIST-Determined Values in Infant Formula A (as-received basis) | | |
|---|---|---|---|
| Analyte | Mass Fractions (%) | | |
| Fat | 28.10 | ± | 0.14 |
| Protein | 12.89 | ± | 0.16 |
| Carbohydrates | 54.54 | ± | 0.22 |
| Solids | 97.830 | ± | 0.050 |
| Ash | 4.465 | ± | 0.027 |
| | Energy (kcal/100 g) | | |
| Calories | 522.62 | ± | 0.31 |

*Rice Flour.* Participants were provided with one bottle containing approximately 50 g of Rice Flour. Participants were asked to store the material at controlled room temperature (20 °C to 25 °C) in the original unopened bottle, to use a sample size appropriate for their in-house method of analysis, and to prepare three samples and report three values from the single bottle provided. Before use, participants were instructed to mix the contents of the packet thoroughly. The approximate analyte levels were not reported to participants prior to the study. The NIST-determined values for fat, protein, carbohydrates, and calories were assigned using information from the manufacturers of the material. The NIST-determined value for solids were assigned based on NIST results for moisture of the material after desiccator drying over magnesium perchlorate ($Mg(ClO_4)_2$) for 28 d and drying in a forced air oven for 4 h at 90 °C. The NIST-determined values are provided in the table below on an as-received basis.

| Analyte | NIST-Determined Values in Rice Flour (as-received basis) Mass Fractions (%) |
|---|---|
| Fat | 1.60 ± 0.08 |
| Protein | 8.10 ± 0.40 |
| Carbohydrates | 79.4 ± 1.9 |
| Solids | 90.4 ± 1.4 |
| | Energy (kcal/100 g) |
| Calories | 364 ± 18 |

Dietary Intake Study Results

- The enrollment and reporting statistics for the proximates study are described in the table below. Reported values may include non-quantitative results (zero or below LOQ) but are included in the participation statistics.

| | Number of Laboratories | Number of Laboratories Reporting Results (Percent Participation) | |
|---|---|---|---|
| Analyte | Requesting Samples | Infant Formula A | Rice Flour |
| Fat | 18 | 10 (56 %) | 12 (67 %) |
| Protein | 20 | 11 (55%) | 13 (65%) |
| Carbohydrates | 17 | 9 (53 %) | 9 (53 %) |
| Solids | 17 | 10 (59 %) | 11 (65 %) |
| Ash | 21 | 15 (71 %) | 16 (76 %) |
| Calories | 14 | 6 (43 %) | 6 (43 %) |

- The between-laboratory variabilities were all excellent for the proximates in both infant formula and Rice Flour, ranging from 0.4 % to 15 %. See table below.

| | Between-Laboratory Variability (% RSD) | |
|---|---|---|
| Analyte | Infant Formula A | Rice Flour |
| Fat | 1 % | 15 % |
| Protein | 5 % | 5 % |
| Carbohydrates | 2 % | 2 % |
| Solids | 0.4 % | 1 % |
| Ash | 3 % | 10 % |
| Calories | 3 % | 4 % |

- For the infant formula sample, the NIST target ranges overlap with the consensus ranges for most of the analytes.
  - The consensus range is completely within the target range for fat, protein, and ash (**Figures 10-1, 10-4, 10-5, 10-14**).
  - The consensus mean is within the target range, but the consensus range extends below the target range for solids (**Figure 10-11**).
  - The consensus ranges for carbohydrates and calories, the two analytes determined by calculation, are completely below the target ranges (**Figures 10-8, 10-17**).
- For the Rice Flour sample, the NIST target ranges overlap with the consensus ranges for most of the analytes.
  - The consensus range is completely within the target range for protein, carbohydrates, and calories (**Figures 10-6, 10-9, 10-18**).
  - The consensus mean is within the target range, but the consensus range extends below the target range for solids (**Figure 10-12**).
  - The consensus range for fat is completely above the target range (**Figure 10-2**).
  - No target range is available for ash in the Rice Flour (**Figure 10-15**).
- Due to the nature of proximates determination, different methods were reported for each analyte within the study as described below.
- Methods reported for fat determination were varied between the two matrices as described in the table below. See also **Figures 10-1** and **10-2.**

| Method for Fat Determination | Number of Participants Reporting (%) | |
|---|---|---|
| | Infant Formula A | Rice Flour |
| Röse-Gottlieb/Mojonnier/acid digestion with ether extraction (AOAC 986.25 & 945.48, 989.05) | 3 (30 %) | 3 (25 %) |
| Alkaline digestion with ether extraction | 2 (20 %) | 0 (0 %) |
| Gravimetry | 1 (10 %) | 2 (17 %) |
| Forced-air oven | 1 (10 %) | 1 (8 %) |
| Other/no method reported | 3 (30 %) | 6 (50 %) |

- Methods reported for protein determination were varied between the two matrices as described in the table below. See also **Figures 10-5** and **10-6.**

| Method for Protein Determination | Number of Participants Reporting (%) Infant Formula A | Rice Flour |
|---|---|---|
| Nitrogen by combustion (AOAC 992.15) | 2 (18 %) | 3 (23 %) |
| Nitrogen by Kjeldahl (AOAC 986.25 & 955.04, AOAC 991.20) | 5 (45 %) | 6 (46 %) |
| Other/no method reported | 3 (28 %) | 3 (23 %) |

- For determination of carbohydrates, most laboratories reported using a calculation approach (67 %). One laboratory reported using acid hydrolysis (11 %) and two laboratories did not report the method used (22 %). See also **Figures 10-8** and **10-9.**
- For determination of solids, five laboratories (50 %) reported using forced-air oven drying as described in AOAC Official Methods 986.25 and 990.20, two laboratories (20 %) reported use of vacuum oven drying, and one laboratory (10 %) reported using thermogravimetric analysis (TGA). One laboratory (9 %) reported using gravimetry for determination for solids in Rice Flour, and two laboratories (20 %) did not report the method used. See also **Figures 10-11** and **10-12.**
- For determination of ash, most laboratories reported using weight loss after ignition in a muffle furnace, e.g. AOAC Official Methods 986.25 and 945.46 (53 %) or dry ashing (33 %). One laboratory reported using TGA (7 %), and one laboratory did not report the method used (7 %). See also **Figures 10-14** and **10-15.**
- For determination of calories, four laboratories reported using a calculation approach (67 %) while two laboratories did not report the method used (33 %). See also **Figures 10-17** and **10-18.**

Dietary Intake Technical Recommendations

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

- No trends were observed based on the specific methods reported by participants.
- Overall performance, as evaluated based on a comparison of consensus means and ranges to target ranges, was good for most analytes in these matrices.
  - The fat content of the Rice Flour was very low, near 1.5 %. As a result, a larger sample size may have been needed for test methods to arrive at the correct result.
  - The consensus values for carbohydrates and calories were below the target ranges. These two analytes are typically determined by calculation based on the other proximates in the food products (i.e., fat, protein, solids).
    - In the infant formula sample, the consensus values for fat and solids were both skewed to the lower portion of the target ranges. When combined, then, to determine carbohydrates and calories, this may have resulted in the low bias of those values.
    - In the Rice Flour sample, the consensus value for solids was skewed to the lower portion of the target range, and the consensus value for fat was significantly below the target range. When combined, then, to determine carbohydrates and calories, this resulted in the low bias of those values.

- In general, all results should be checked closely to avoid calculation or other errors and to be sure that results are reported in the requested units.
  - One laboratory reported extremely high, outlying results for protein in both materials. Another laboratory reported extremely low, outlying results for protein in both materials. A third laboratory reported high, outlying results for ash in both materials. These outlying results were likely due to a miscalculation or misinterpretation of the requested data.
  - One laboratory reported using AOAC Official Methods 986.25 and 990.20 for determination of fat in the samples. This method is not appropriate for determination of fat but is instead a method used for determination of solids.
- 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 materials prepared in-house. Numerous food matrix CRMs are available with assigned values for proximates and calories.
- A linear calibration curve which surrounds 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 incorrect values.

**Table 10-1.** Individualized data summary table (NIST) for proximates in Infant Formula A and Rice Flour.

*National Institute of Standards and Technology*

**HAMQAP Exercise 6 - Proximates**

| | | Lab Code: NIST | 1. Your Results | | | | 2. Community Results | | | 3. Target | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Analyte | Sample | Units | $x_i$ | $s_i$ | $Z'_{comm}$ | $Z_{NIST}$ | N | x* | s* | $x_{NIST}$ | $U$ |
| Ash | Infant Formula A | % | 4.46 | 0.0539 | | | 15 | 4.5 | 0.14 | 4.46 | 0.0539 |
| Ash | Rice Flour | % | | | | | 16 | 1.39 | 0.12 | | |
| Calories | Infant Formula A | (kcal/100 g) | 523 | 0.616 | | | 6 | 513 | 6.4 | 523 | 0.616 |
| Calories | Rice Flour | (kcal/100 g) | 364 | 9.11 | | | 6 | 370 | 15 | 364 | 9.11 |
| Carbohydrates | Infant Formula A | % | 54.5 | 0.441 | | | 9 | 52.2 | 0.89 | 54.5 | 0.441 |
| Carbohydrates | Rice Flour | % | 79.4 | 1.99 | | | 9 | 76.5 | 1.6 | 79.4 | 1.99 |
| Fat | Infant Formula A | % | 28.1 | 0.289 | | | 10 | 27.8 | 0.36 | 28.1 | 0.289 |
| Fat | Rice Flour | % | 1.6 | 0.04 | | | 12 | 2.93 | 0.37 | 1.6 | 0.04 |
| Protein | Infant Formula A | % | 12.9 | 0.318 | | | 11 | 12.9 | 0.54 | 12.9 | 0.318 |
| Protein | Rice Flour | % | 8.1 | 0.202 | | | 13 | 8.04 | 0.36 | 8.1 | 0.202 |
| Solids | Infant Formula A | % | 97.8 | 0.101 | | | 10 | 97.7 | 0.35 | 97.8 | 0.101 |
| Solids | Rice Flour | % | 90.4 | 0.719 | | | 11 | 89 | 0.7 | 90.4 | 0.719 |

$x_i$ Mean of reported values
$s_i$ Standard deviation of reported values
$Z'_{comm}$ Z'-score with respect to community consensus
$Z_{NIST}$ Z-score with respect to NIST value

N Number of quantitative values reported
x* Robust mean of reported values
s* Robust standard deviation

$x_{NIST}$ NIST-assessed value
$U$ expanded uncertainty about the NIST-assessed value

**Table 10-2.** Data summary table for fat in Infant Formula A and Rice Flour. Data points highlighted in blue have been identified as outside the consensus tolerance limits and would be estimated to result in an unacceptable $Z'_{comm}$ score, $|Z'_{comm}| \geq 2$.

| | | Fat | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Infant Formula A (%) | | | | | Rice Flour (%) | | | | |
| | Lab | A | B | C | Avg | SD | A | B | C | Avg | SD |
| Individual Results | Target | | | | 28.10 | 0.29 | | | | 1.60 | 0.04 |
| | F004 | | | | | | | | | | |
| | F005 | 21.78 | 20.08 | 21.64 | 21.17 | 0.94 | 3.16 | 3.22 | 3.17 | 3.18 | 0.03 |
| | F009 | 28 | 26.4 | 28.1 | 27.50 | 0.95 | 3.2 | 3.1 | 3.4 | 3.23 | 0.15 |
| | F017 | 27.74 | 27.73 | 27.94 | 27.80 | 0.12 | 2.32 | 1.9 | 2.21 | 2.14 | 0.22 |
| | F020 | 27.5 | 27.4 | 27.1 | 27.33 | 0.21 | 3 | 3.1 | 2.9 | 3.00 | 0.10 |
| | F021 | | | | | | | | | | |
| | F030 | 27.35 | 27.58 | 27.7 | 27.54 | 0.18 | 2.7 | 2.67 | 2.77 | 2.71 | 0.05 |
| | F031 | 28.21 | 27.93 | 28.14 | 28.09 | 0.15 | 3.11 | 3.04 | 3 | 3.05 | 0.06 |
| | F032 | | | | | | | | | | |
| | F039 | 27.64 | 27.78 | 27.78 | 27.73 | 0.08 | 2.64 | 2.66 | 2.63 | 2.64 | 0.02 |
| | F045 | | | | | | | | | | |
| | F056 | | | | | | | | | | |
| | F059 | 27.95 | 27.96 | 27.9 | 27.94 | 0.03 | 3.05 | 3.04 | 3.18 | 3.09 | 0.08 |
| | F061 | | | | | | 2.84 | 3.08 | 3.12 | 3.01 | 0.15 |
| | F062 | | | | | | 2.83 | 2.7 | 2.67 | 2.73 | 0.09 |
| | F079 | 27.81 | 27.95 | 27.87 | 27.88 | 0.07 | 3.49 | 3.4 | 3.4 | 3.43 | 0.05 |
| | F080 | 28.01 | 28.06 | 27.9 | 27.99 | 0.08 | 2.6 | 2.74 | 2.64 | 2.66 | 0.07 |
| | F088 | | | | | | | | | | |
| Community Results | | Consensus Mean | | | 27.76 | | Consensus Mean | | | 2.93 | |
| | | Consensus Standard Deviation | | | 0.36 | | Consensus Standard Deviation | | | 0.37 | |
| | | Maximum | | | 28.09 | | Maximum | | | 3.43 | |
| | | Minimum | | | 21.17 | | Minimum | | | 2.14 | |
| | | N | | | 10 | | N | | | 12 | |

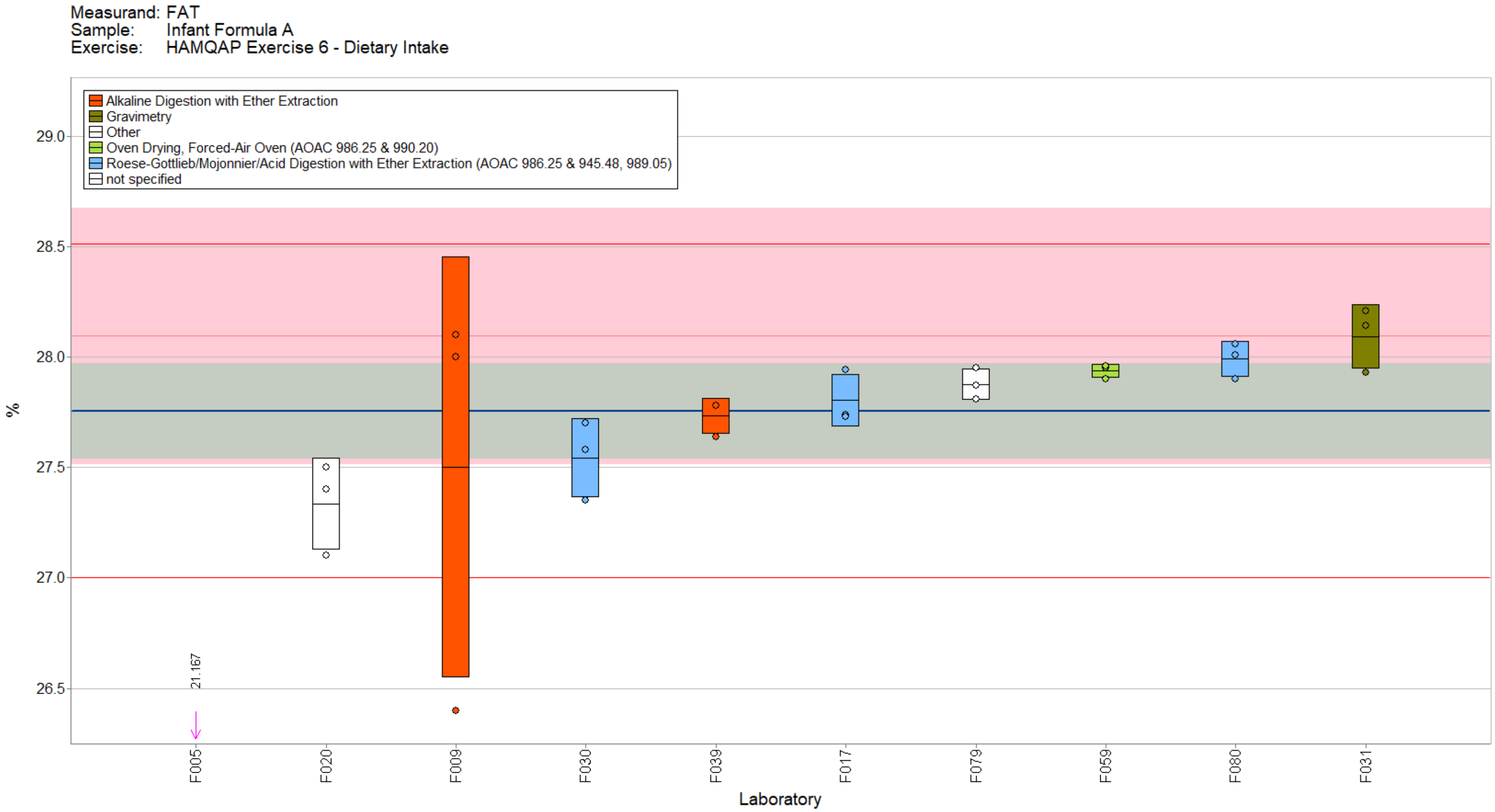


**Figure 10-1.** Fat in Infant Formula A (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line represents 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 $Z'_{\text{comm}}$ score, $|Z'_{\text{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty ($U_{\text{NIST}}$) and represents the range that results in an acceptable $Z'_{\text{NIST}}$ score, $|Z'_{\text{NIST}}| \leq 2$.

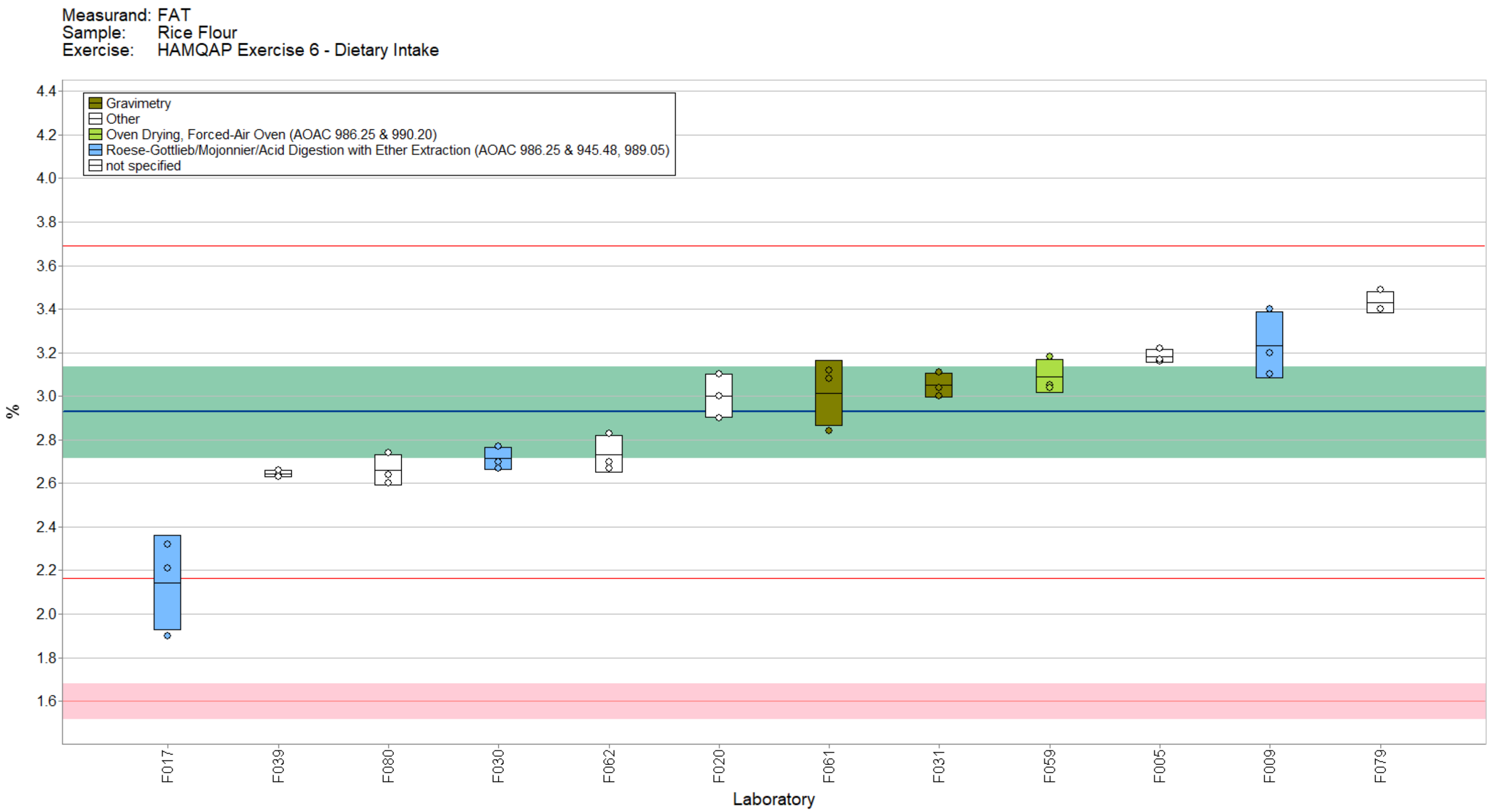


**Figure 10-2.** Fat in Rice Flour (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line represents 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 $Z'_{\text{comm}}$ score, $|Z'_{\text{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty ($U_{\text{NIST}}$) and represents the range that results in an acceptable $Z'_{\text{NIST}}$ score, $|Z'_{\text{NIST}}| \leq 2$.

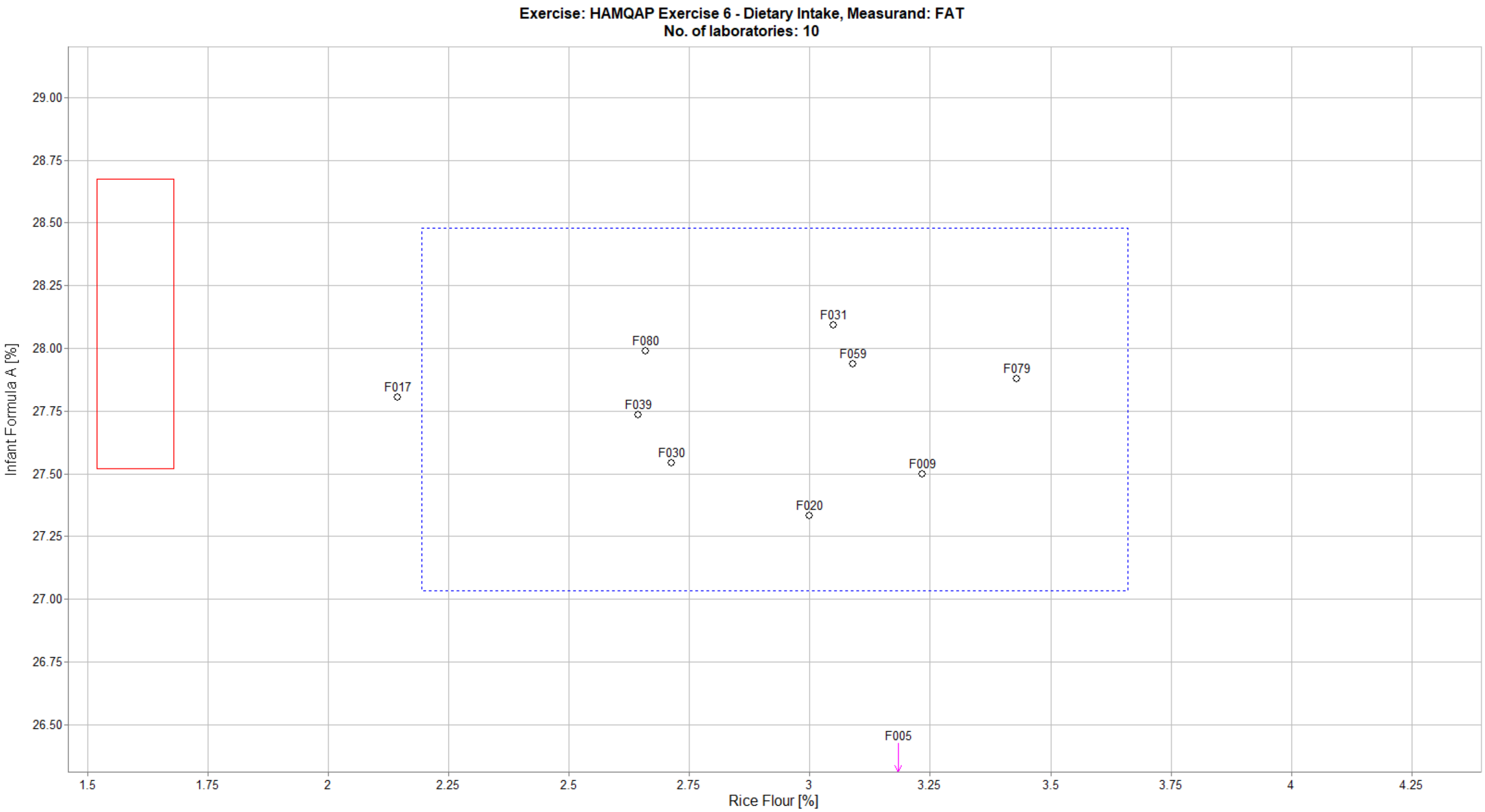


**Figure 10-3.** Laboratory means for fat in Infant Formula A and Rice Flour (sample/sample comparison view). In this view, the individual laboratory mean for one sample (Infant Formula A) is compared to the mean for a second sample (Rice Flour). The solid red box represents the NIST range of tolerance for the two samples, Rice Flour (x-axis) and Infant Formula 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 Rice Flour (x-axis) and Infant Formula A (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 10-3.** Data summary table for protein in Infant Formula A and Rice Flour. Data points highlighted in blue have been identified as outside the consensus tolerance limits and would be estimated to result in an unacceptable $Z'_{comm}$ score, $|Z'_{comm}| \geq 2$.

| | | Protein | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Infant Formula A (%) | | | | | Rice Flour (%) | | | | |
| | Lab | A | B | C | Avg | SD | A | B | C | Avg | SD |
| Individual Results | Target | | | | 12.90 | 0.32 | | | | 8.10 | 0.20 |
| | F002 | 75.3 | 75.6 | 75.5 | 75.47 | 0.15 | 62.6 | 62.7 | 62.9 | 62.73 | 0.15 |
| | F004 | | | | | | | | | | |
| | F005 | 0.0379 | 0.0128 | 0.0877 | 0.046 | 0.04 | 0.0223 | 0.029 | 0.00108 | 0.02 | 0.01 |
| | F009 | 12.6 | 12 | 13.1 | 12.57 | 0.55 | 8.2 | 8 | 8 | 8.07 | 0.12 |
| | F017 | 13.1 | 13.2 | 13.3 | 13.20 | 0.10 | 7.8 | 8.06 | 8.05 | 7.97 | 0.15 |
| | F018 | | | | | | | | | | |
| | F020 | 12.99 | 13 | 12.95 | 12.98 | 0.03 | 8 | 7.9 | 7.93 | 7.94 | 0.05 |
| | F021 | | | | | | | | | | |
| | F030 | 12.49 | 12.48 | 12.57 | 12.51 | 0.05 | 8.22 | 8.27 | 8.25 | 8.25 | 0.03 |
| | F031 | 13.07 | 13.07 | 13.05 | 13.06 | 0.01 | 8.06 | 8.16 | 7.98 | 8.07 | 0.09 |
| | F032 | | | | | | | | | | |
| | F039 | 12.7 | 12.76 | 13.02 | 12.83 | 0.17 | 7.97 | 7.97 | 7.79 | 7.91 | 0.10 |
| | F045 | | | | | | | | | | |
| | F056 | | | | | | | | | | |
| | F059 | 12.87 | 12.69 | 12.74 | 12.77 | 0.09 | 7.87 | 7.87 | 7.99 | 7.91 | 0.07 |
| | F061 | | | | | | 8.36 | 8.39 | 8.39 | 8.38 | 0.02 |
| | F062 | | | | | | 8.3 | 8.27 | 8.28 | 8.28 | 0.02 |
| | F079 | 12.58 | 12.61 | 12.43 | 12.54 | 0.10 | 7.65 | 7.64 | 7.58 | 7.62 | 0.04 |
| | F080 | 13.23 | 13.18 | 13.25 | 13.22 | 0.04 | 8.03 | 8 | 8.03 | 8.02 | 0.02 |
| | F088 | | | | | | | | | | |
| Community Results | | Consensus Mean | | | 12.85 | | Consensus Mean | | | 8.04 | |
| | | Consensus Standard Deviation | | | 0.54 | | Consensus Standard Deviation | | | 0.36 | |
| | | Maximum | | | 75.47 | | Maximum | | | 62.73 | |
| | | Minimum | | | 0.046 | | Minimum | | | 0.017 | |
| | | N | | | 11 | | N | | | 13 | |

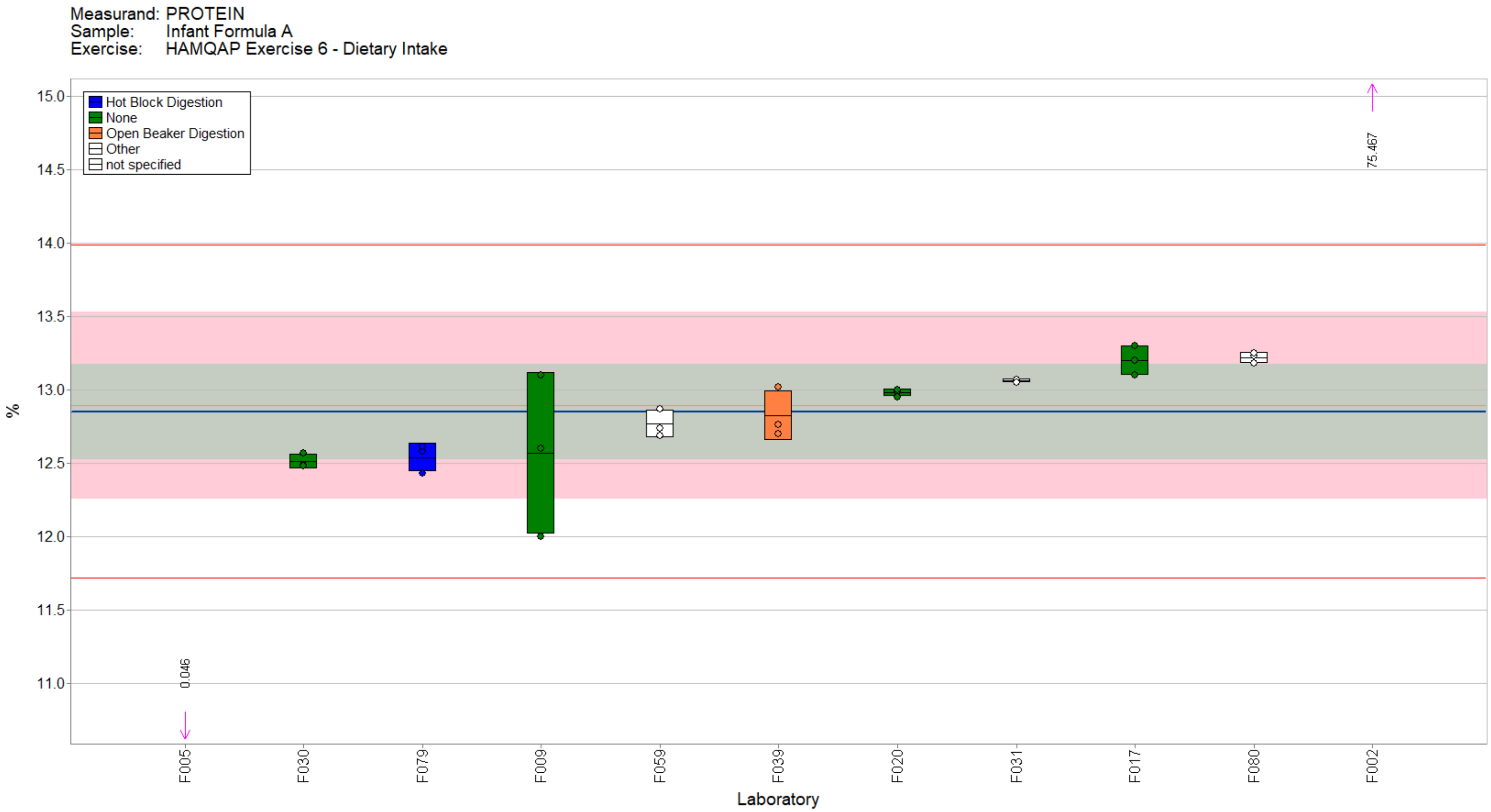


**Figure 10-4.** Protein in Infant Formula A (data summary view – sample preparation). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation employed. The solid blue line 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 $Z'_{\mathrm{comm}}$ score, $|Z'_{\mathrm{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty ($U_{\mathrm{NIST}}$) and represents the range that results in an acceptable $Z'_{\mathrm{NIST}}$ score, $|Z'_{\mathrm{NIST}}| \leq 2$.

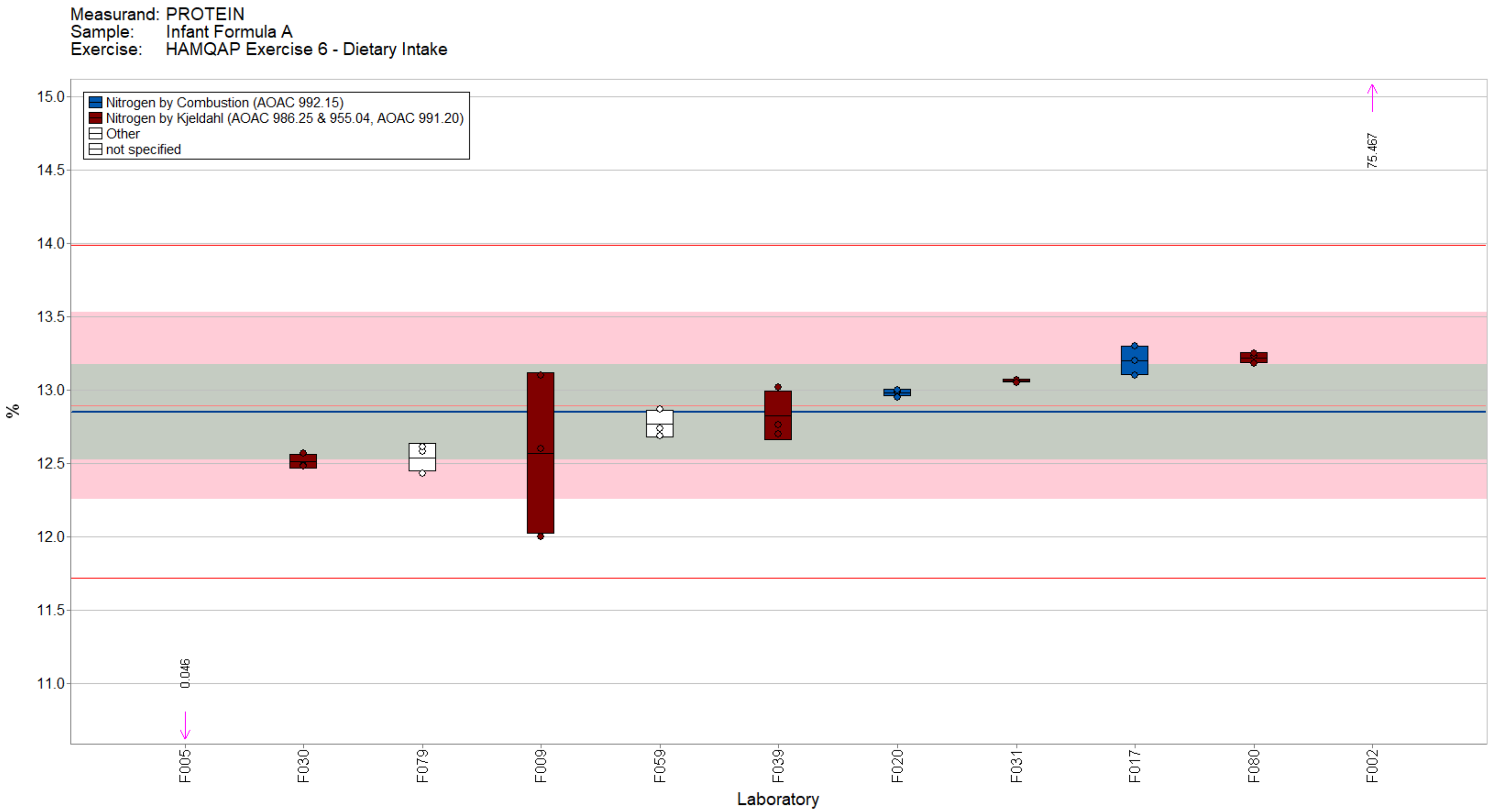


**Figure 10-5.** Protein in Infant Formula A (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line 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 $Z'_{\mathrm{comm}}$ score, $|Z'_{\mathrm{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty ($U_{\mathrm{NIST}}$) and represents the range that results in an acceptable $Z'_{\mathrm{NIST}}$ score, $|Z'_{\mathrm{NIST}}| \leq 2$.

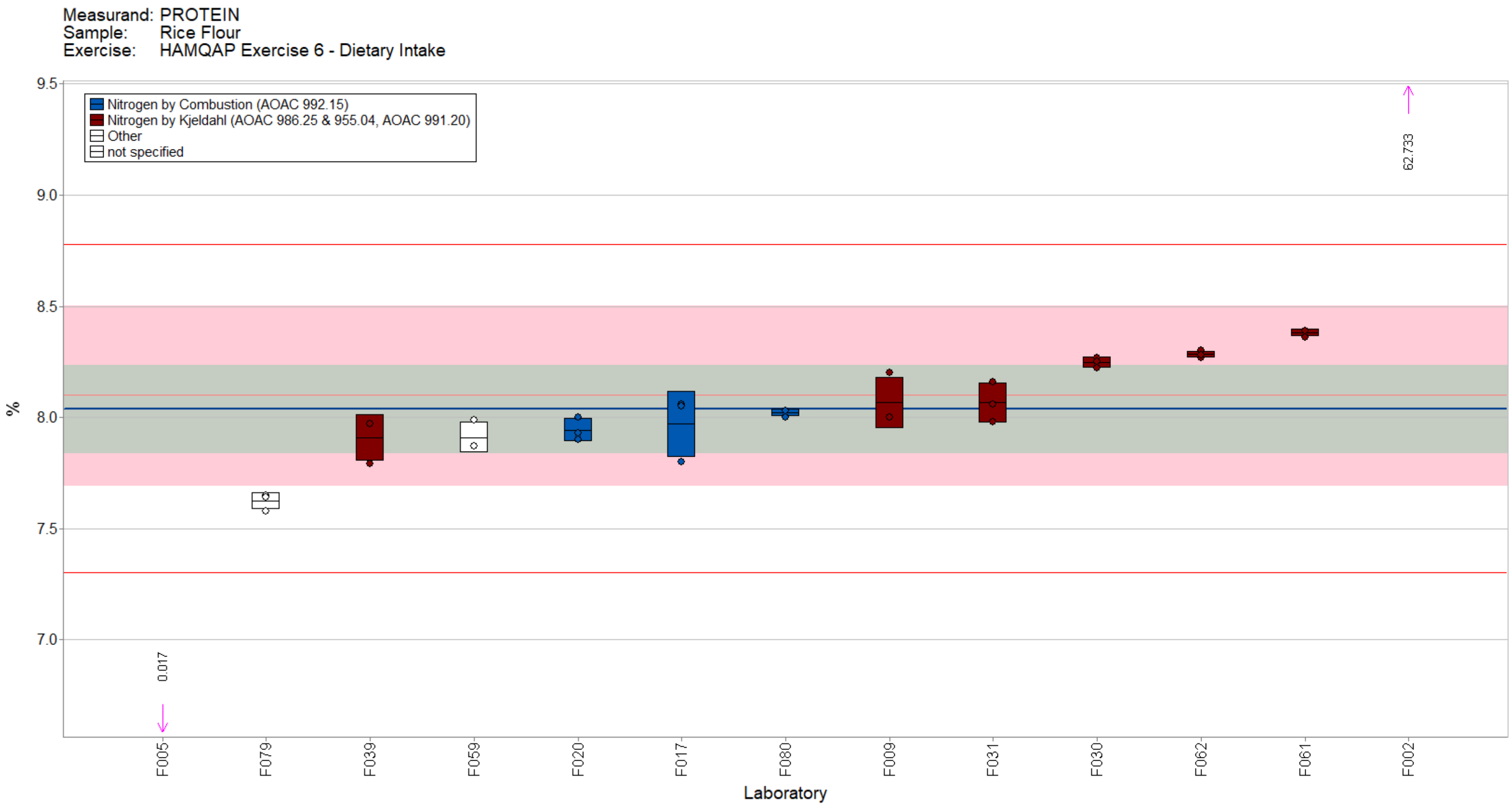


**Figure 10-6.** Protein in Rice Flour (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line represents 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 $Z'_{\text{comm}}$ score, $|Z'_{\text{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty ($U_{\text{NIST}}$) and represents the range that results in an acceptable $Z'_{\text{NIST}}$ score, $|Z'_{\text{NIST}}| \leq 2$.

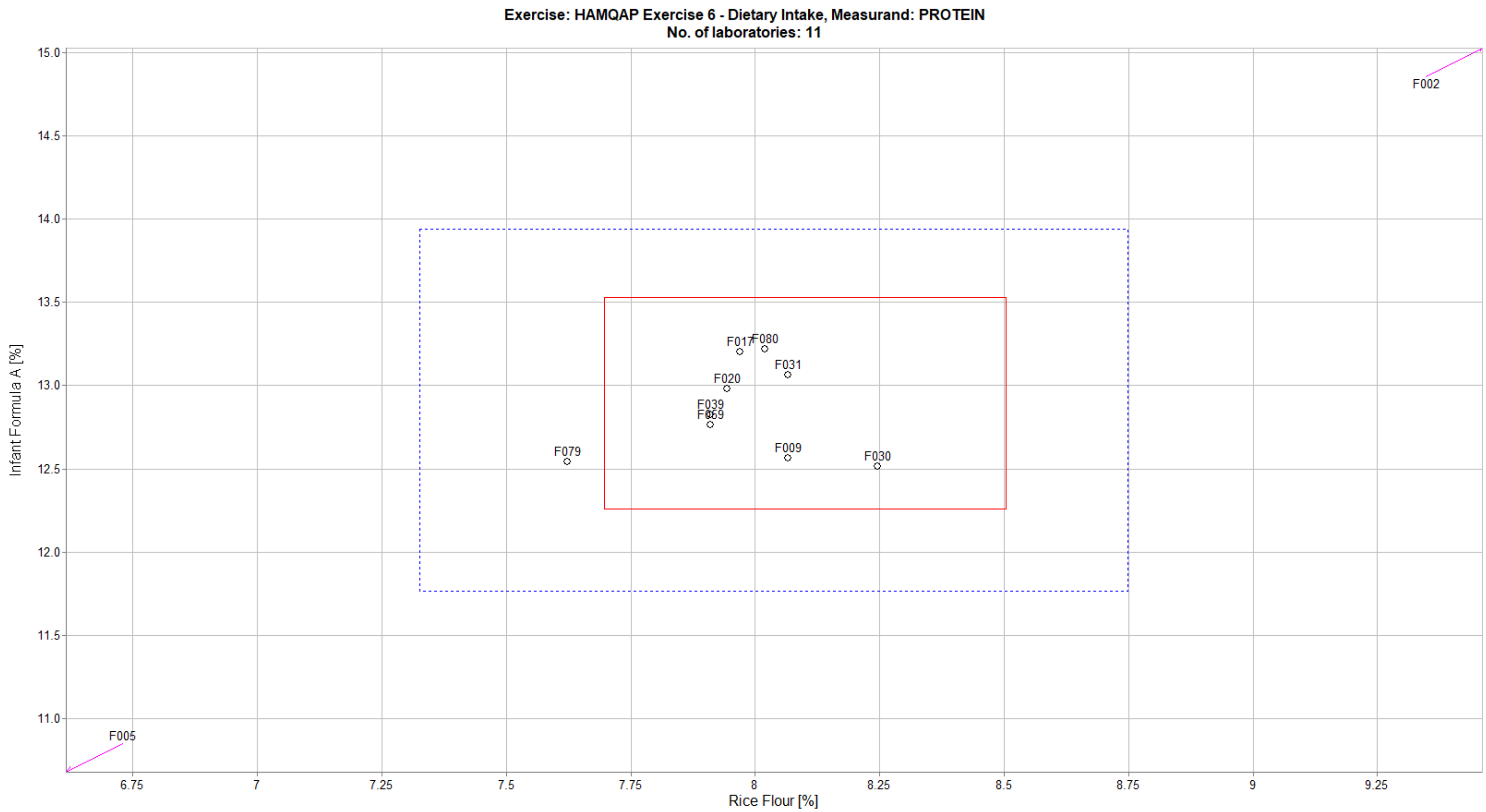


**Figure 10-7.** Laboratory means for protein in Infant Formula A and Rice Flour (sample/sample comparison view). In this view, the individual laboratory mean for one sample (Infant Formula A) is compared to the mean for a second sample (Rice Flour). The solid red box represents the NIST range of tolerance for the two samples, Rice Flour (x-axis) and Infant Formula A (y-axis), which encompasses the target values bounded by their uncertainties ($U_{\mathrm{NIST}}$) and represents the range that results in an acceptable $Z_{\mathrm{NIST}}$ score, $|Z_{\mathrm{NIST}}| \leq 2$. The dotted blue box represents the consensus range of tolerance for Rice Flour (x-axis) and Infant Formula A (y-axis), calculated as the values above and below the consensus means that result in an acceptable $Z'_{\mathrm{comm}}$ score, $|Z'_{\mathrm{comm}}| \leq 2$.

**Table 10-4.** Data summary table for carbohydrates in Infant Formula A and Rice Flour. Data points highlighted in blue have been identified as outside the consensus tolerance limits and would be estimated to result in an unacceptable $Z'_{comm}$ score, $|Z'_{comm}| \geq 2$.

| | | Carbohydrates | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Infant Formula A (%) | | | | | Rice Flour (%) | | | | |
| | Lab | A | B | C | Avg | SD | A | B | C | Avg | SD |
| Individual Results | Target | | | | 54.54 | 0.44 | | | | 79.40 | 1.99 |
| | F004 | | | | | | | | | | |
| | F005 | 47.45 | 46.55 | 43.24 | 45.75 | 2.22 | 3.17 | 3.16 | 3.15 | 3.16 | 0.01 |
| | F009 | 52.6 | 54.9 | 52.3 | 53.27 | 1.42 | 76.4 | 76.9 | 76.6 | 76.63 | 0.25 |
| | F017 | 52.65 | 52.48 | 52.17 | 52.43 | 0.24 | 80.08 | 80.08 | 79.88 | 80.01 | 0.12 |
| | F020 | 52.4 | 52.5 | 52.6 | 52.50 | 0.10 | 74.8 | 75.1 | 75.4 | 75.10 | 0.30 |
| | F021 | | | | | | | | | | |
| | F031 | 52 | 52.1 | 52.1 | 52.07 | 0.06 | 76 | 75.9 | 75.9 | 75.93 | 0.06 |
| | F032 | | | | | | | | | | |
| | F039 | 52.43 | 52.05 | 51.77 | 52.08 | 0.33 | 76.63 | 76.42 | 76.82 | 76.62 | 0.20 |
| | F045 | | | | | | | | | | |
| | F049 | 50.4 | 50.5 | 50.6 | 50.50 | 0.10 | | | | | |
| | F056 | | | | | | | | | | |
| | F061 | | | | | | 1.1 | 1.2 | 1.4 | 1.23 | 0.15 |
| | F062 | | | | | | | | | | |
| | F079 | 52.71 | 53.41 | 52.58 | 52.90 | 0.45 | 75.85 | 76.05 | 76.08 | 75.99 | 0.13 |
| | F080 | 51.93 | 51.69 | 51.7 | 51.77 | 0.14 | 76.23 | 76.39 | 76.38 | 76.33 | 0.09 |
| | F088 | | | | | | | | | | |
| Community Results | | Consensus Mean | | | 52.24 | | Consensus Mean | | | 76.51 | |
| | | Consensus Standard Deviation | | | 0.89 | | Consensus Standard Deviation | | | 1.62 | |
| | | Maximum | | | 53.27 | | Maximum | | | 80.01 | |
| | | Minimum | | | 45.75 | | Minimum | | | 1.23 | |
| | | N | | | 9 | | N | | | 9 | |

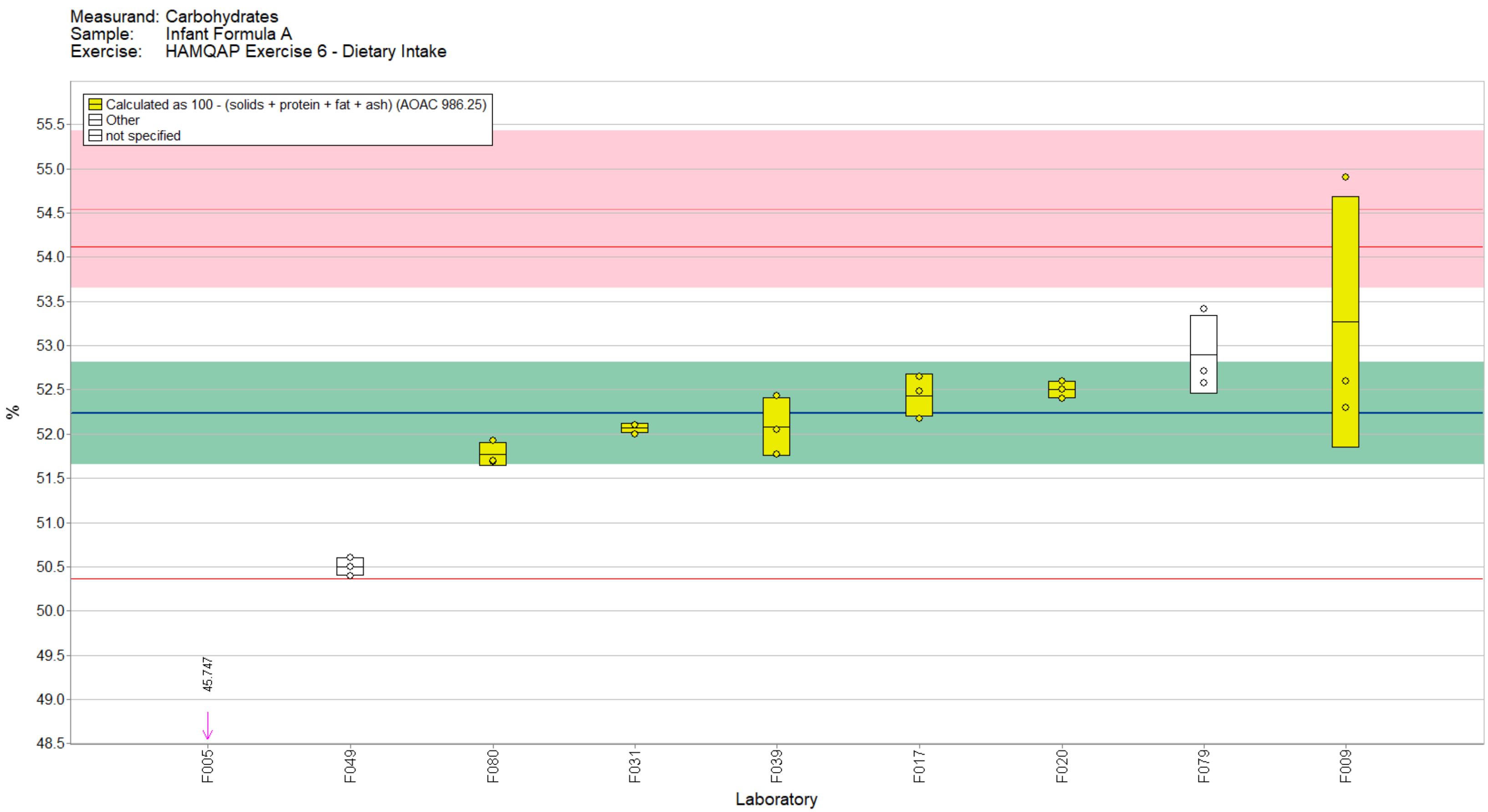


**Figure 10-8.** Carbohydrates in Infant Formula A (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue 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 the consensus mean that result in an acceptable $Z'_{\text{comm}}$ score, $|Z'_{\text{comm}}| \leq 2$, with the lower limit set at zero. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty ($U_{\text{NIST}}$) and represents the range that results in an acceptable $Z'_{\text{NIST}}$ score, $|Z'_{\text{NIST}}| \leq 2$.

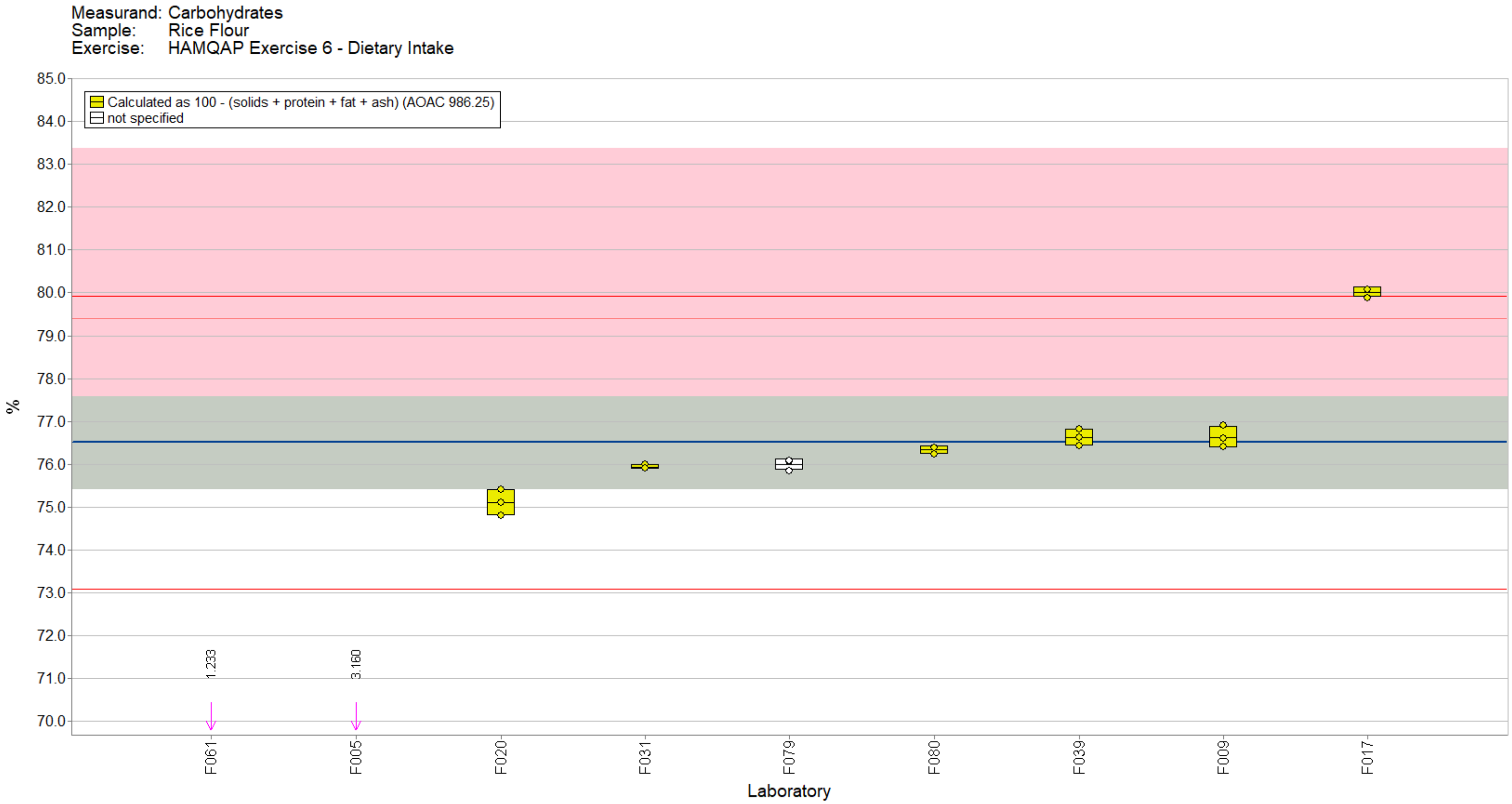


**Figure 10-9.** Carbohydrates in Rice Flour (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line 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 $Z'_{\mathrm{comm}}$ score, $|Z'_{\mathrm{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty ($U_{\mathrm{NIST}}$) and represents the range that results in an acceptable $Z'_{\mathrm{NIST}}$ score, $|Z'_{\mathrm{NIST}}| \leq 2$.

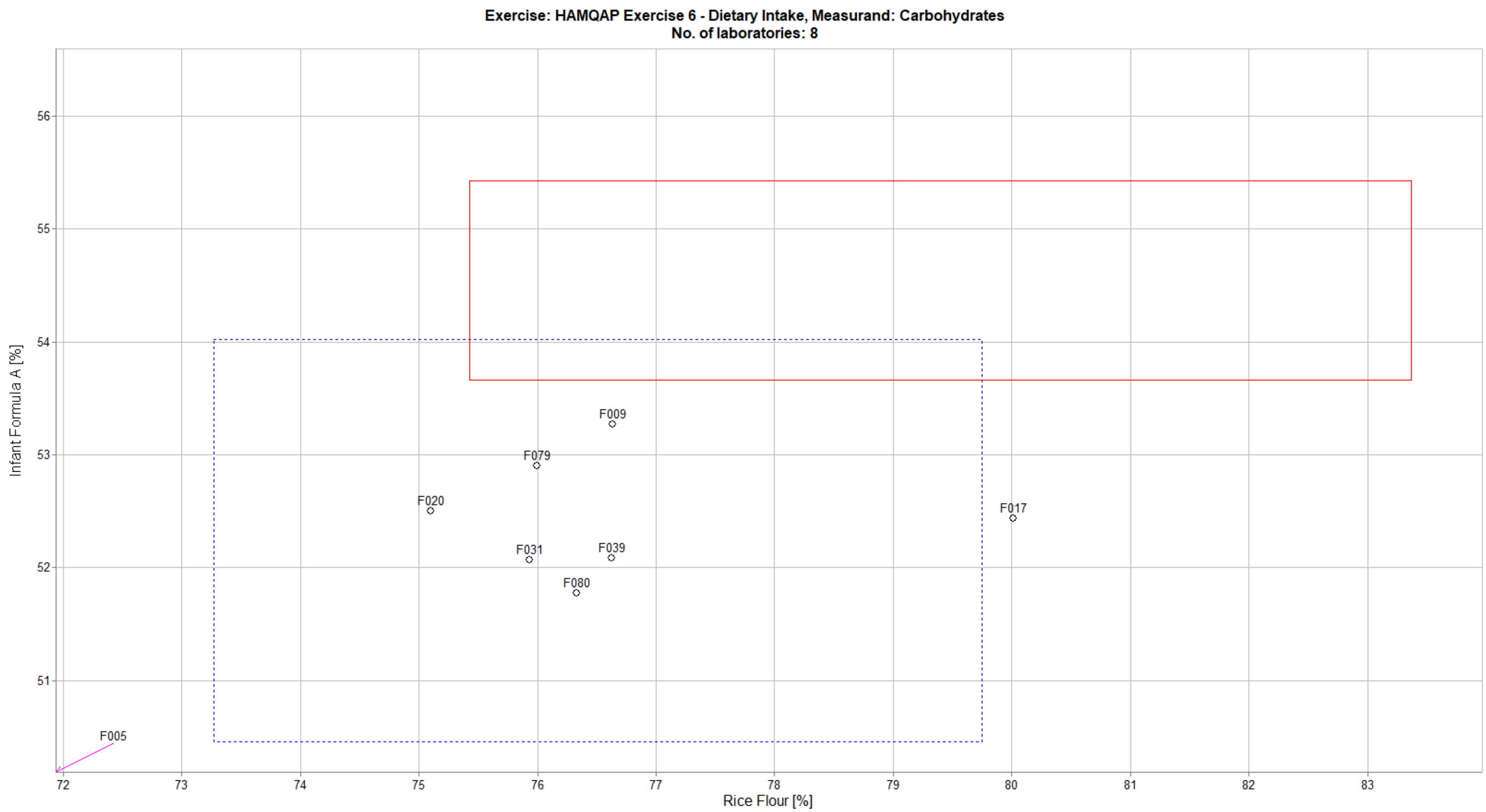


**Figure 10-10.** Laboratory means for carbohydrates in Infant Formula A and Rice Flour (sample/sample comparison view). In this view, the individual laboratory mean for one sample (Infant Formula A) is compared to the mean for a second sample (Rice Flour). The solid red box represents the NIST range of tolerance for the two samples, Rice Flour (x-axis) and Infant Formula A (y-axis), which encompasses the target values bounded by their uncertainties ($U_{\mathrm{NIST}}$) and represents the range that results in an acceptable $Z_{\mathrm{NIST}}$ score, $|Z_{\mathrm{NIST}}| \leq 2$. The dotted blue box represents the consensus range of tolerance for flour (x-axis) and Infant Formula A (y-axis), calculated as the values above and below the consensus means that result in an acceptable $Z'_{\mathrm{comm}}$ score, $|Z'_{\mathrm{comm}}| \leq 2$.

**Table 10-5.** Data summary table for solids in Infant Formula A and Rice Flour. Data points highlighted in blue have been identified as outside the consensus tolerance limits and would be estimated to result in an unacceptable $Z'_{comm}$ score, $|Z'_{comm}| \geq 2$.

| | | Solids | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Infant Formula A (%) | | | | | Rice Flour (%) | | | | |
| | Lab | A | B | C | Avg | SD | A | B | C | Avg | SD |
| Individual Results | Target | | | | 97.83 | 0.10 | | | | 90.37 | 0.72 |
| | F004 | | | | | | | | | | |
| | F005 | 84.3 | 85.2 | 97.3 | 88.93 | 7.26 | 89.6 | 90.4 | 90.5 | 90.17 | 0.49 |
| | F009 | 97.8 | 97.8 | 98.1 | 97.90 | 0.17 | 89.5 | 89.6 | 89.7 | 89.60 | 0.10 |
| | F017 | 98 | 97.99 | 98.02 | 98.00 | 0.02 | 91.65 | 91.51 | 91.71 | 91.62 | 0.10 |
| | F019 | 97.8 | 97.72 | 97.72 | 97.75 | 0.05 | 89.35 | 89.48 | 89.49 | 89.44 | 0.08 |
| | F021 | | | | | | | | | | |
| | F030 | 97.75 | 97.71 | 97.66 | 97.71 | 0.05 | 88.66 | 88.59 | 88.66 | 88.64 | 0.04 |
| | F031 | 97.85 | 97.76 | 97.89 | 97.83 | 0.07 | 88.53 | 88.49 | 88.28 | 88.43 | 0.13 |
| | F032 | | | | | | | | | | |
| | F039 | 97.3 | 97.1 | 97.1 | 97.17 | 0.12 | 88.63 | 88.45 | 88.63 | 88.57 | 0.10 |
| | F045 | | | | | | | | | | |
| | F056 | | | | | | | | | | |
| | F059 | 97.8 | 97.9 | 97.8 | 97.83 | 0.06 | 88.81 | 89.08 | 89.14 | 89.01 | 0.18 |
| | F061 | | | | | | 89.4 | 89.4 | 89.4 | 89.40 | 0.00 |
| | F062 | | | | | | | | | | |
| | F079 | 97.49 | 97.61 | 98.56 | 97.89 | 0.59 | 88.36 | 88.42 | 88.45 | 88.41 | 0.05 |
| | F080 | 97.63 | 97.55 | 97.54 | 97.57 | 0.05 | 88.15 | 88.4 | 88.33 | 88.29 | 0.13 |
| Community Results | | Consensus Mean | | | 97.74 | | Consensus Mean | | | 89.05 | |
| | | Consensus Standard Deviation | | | 0.35 | | Consensus Standard Deviation | | | 0.70 | |
| | | Maximum | | | 98.00 | | Maximum | | | 91.62 | |
| | | Minimum | | | 88.93 | | Minimum | | | 88.29 | |
| | | N | | | 10 | | N | | | 11 | |

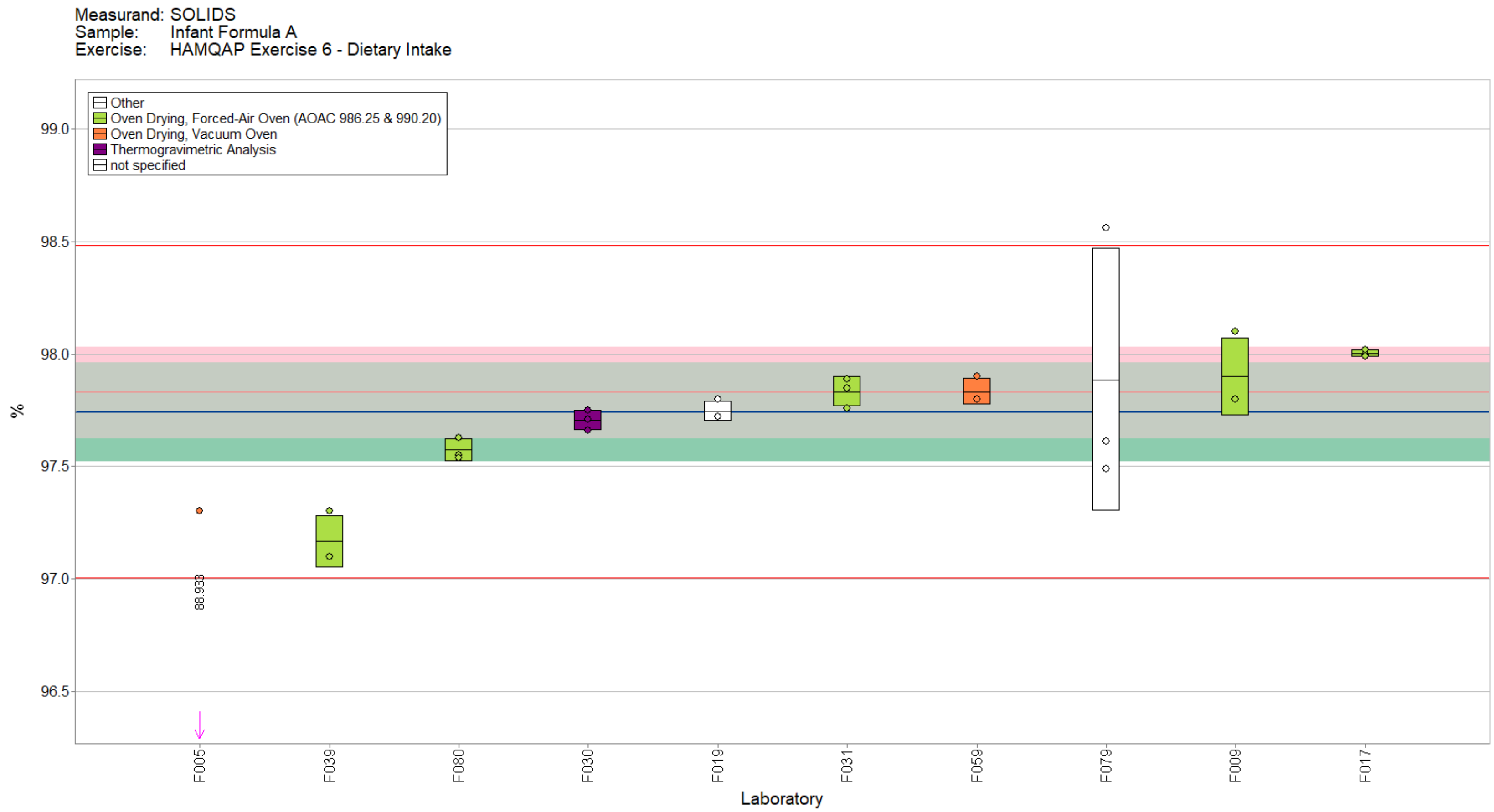


**Figure 10-11.** Solids in Infant Formula A (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line 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 $Z'_{\text{comm}}$ score, $|Z'_{\text{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty ($U_{\text{NIST}}$) and represents the range that results in an acceptable $Z'_{\text{NIST}}$ score, $|Z'_{\text{NIST}}| \leq 2$.

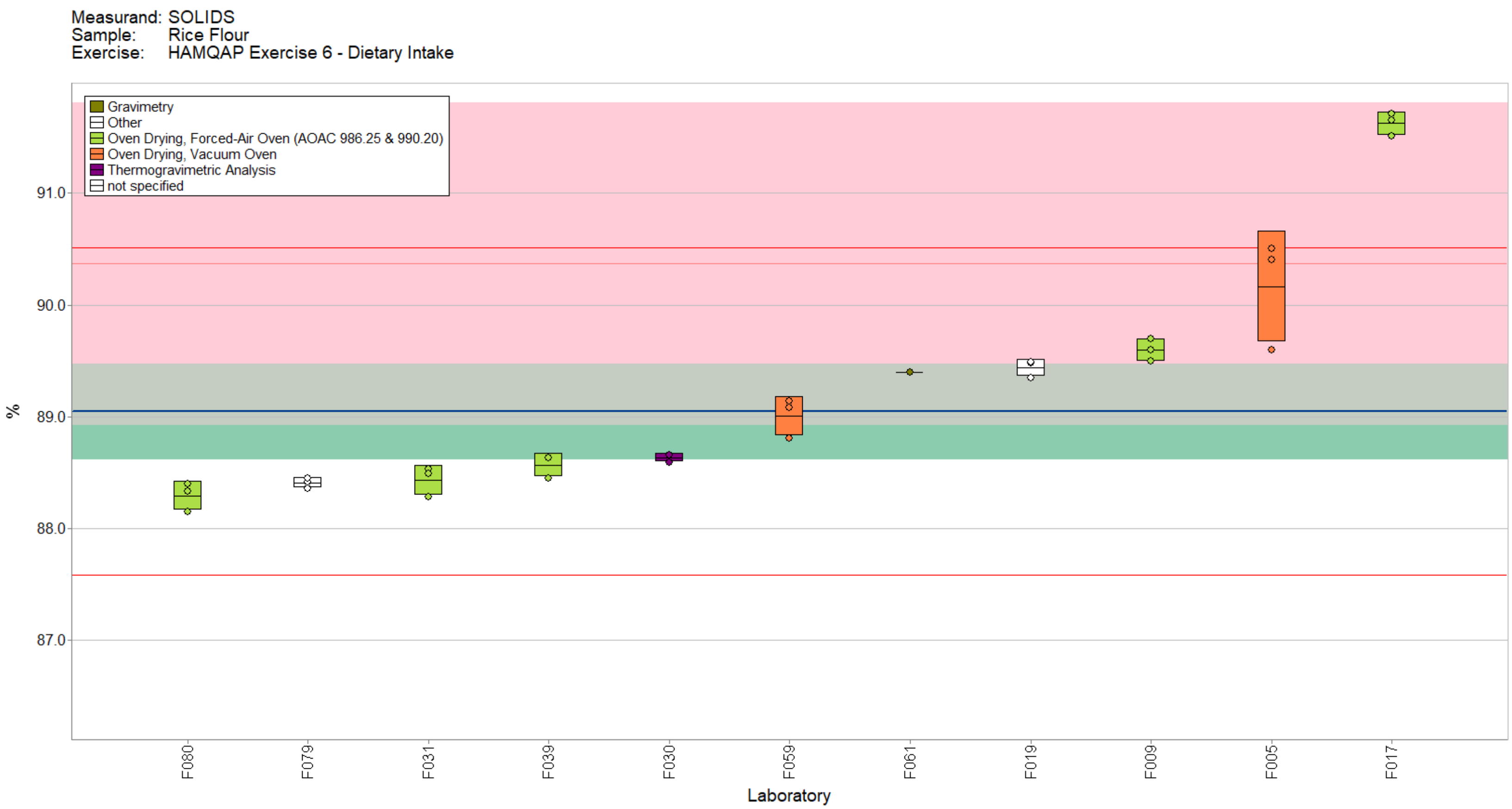


**Figure 10-12.** Solids in Rice Flour (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line represents 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 $Z'_{\text{comm}}$ score, $|Z'_{\text{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty ($U_{\text{NIST}}$) and represents the range that results in an acceptable $Z'_{\text{NIST}}$ score, $|Z'_{\text{NIST}}| \leq 2$.

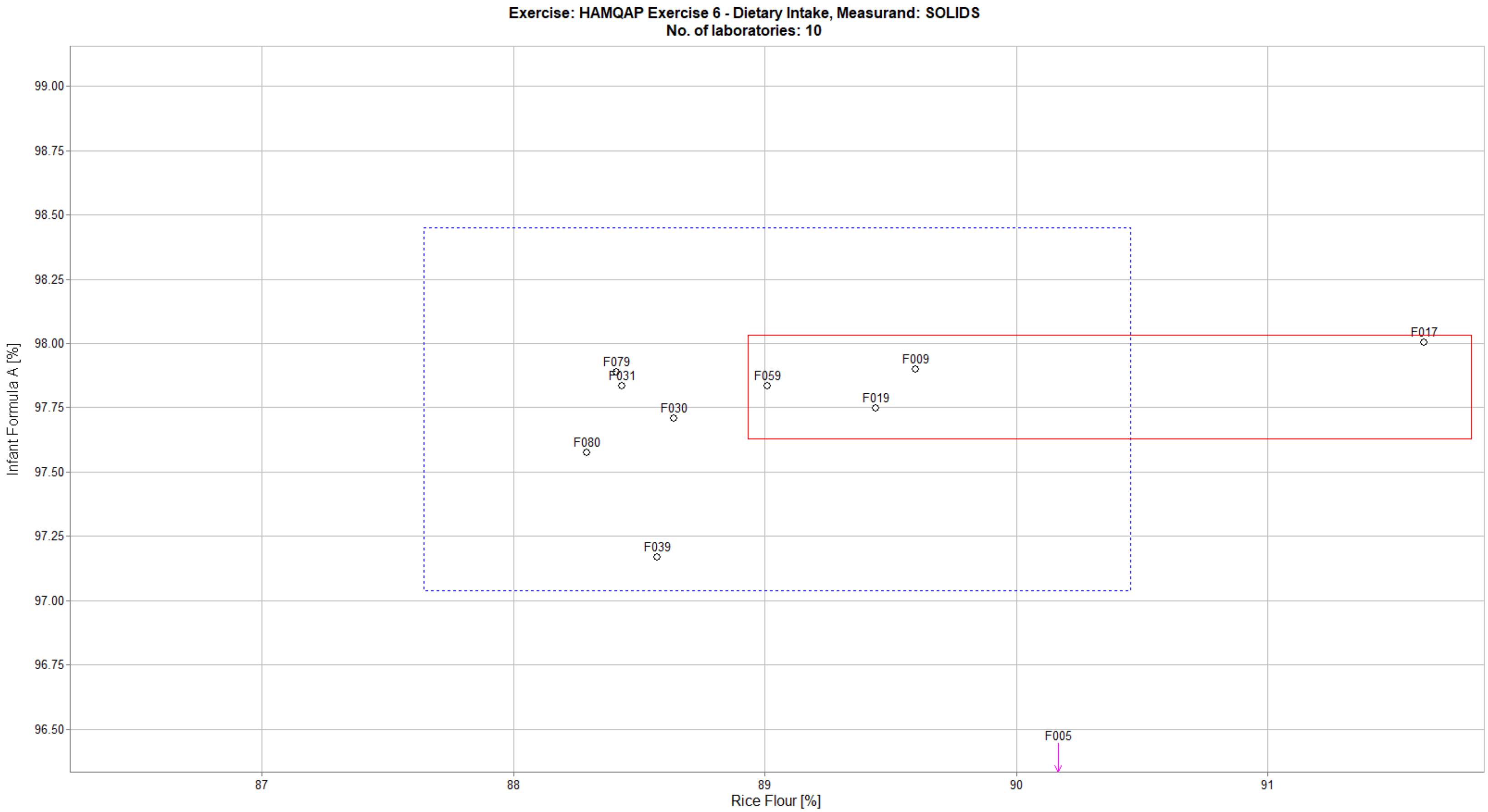


**Figure 10-13.** Laboratory means for solids in Infant Formula A and Rice Flour (sample/sample comparison view). In this view, the individual laboratory mean for one sample (Infant Formula A) is compared to the mean for a second sample (Rice Flour). The solid red box represents the NIST range of tolerance for the two samples, Rice Flour (x-axis) and Infant Formula A (y-axis), which encompasses the target values bounded by their uncertainties ($U_{\mathrm{NIST}}$) and represents the range that results in an acceptable $Z_{\mathrm{NIST}}$ score, $|Z_{\mathrm{NIST}}| \leq 2$. The dotted blue box represents the consensus range of tolerance for Rice Flour (x-axis) and Infant Formula A (y-axis), calculated as the values above and below the consensus means that result in an acceptable $Z'_{\mathrm{comm}}$ score, $|Z'_{\mathrm{comm}}| \leq 2$.

**Table 10-6.** Data summary table for ash in Infant Formula A and Rice Flour. Data points highlighted in blue have been identified as outside the consensus tolerance limits and would be estimated to result in an unacceptable $Z'_{comm}$ score, $|Z'_{comm}| \geq 2$.

| | | Ash | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Infant Formula A (%) | | | | | Rice Flour (%) | | | | |
| | Lab | A | B | C | Avg | SD | A | B | C | Avg | SD |
| Individual Results | Target | | | | 4.47 | 0.05 | | | | | |
| | F004 | | | | | | | | | | |
| | F005 | 4.29 | 4.31 | 4.33 | 4.31 | 0.020 | 1.5 | 1.46 | 1.44 | 1.47 | 0.031 |
| | F009 | 4.6 | 4.6 | 4.6 | 4.60 | 0.000 | 1.7 | 1.6 | 1.7 | 1.67 | 0.058 |
| | F011 | 4.33 | 4.38 | 4.4 | 4.37 | 0.036 | 1.14 | 1.35 | 1.3 | 1.26 | 0.11 |
| | F017 | 4.51 | 4.58 | 4.61 | 4.57 | 0.051 | 1.45 | 1.47 | 1.57 | 1.50 | 0.064 |
| | F018 | | | | | | | | | | |
| | F019 | 4.3 | 4.49 | 4.49 | 4.43 | 0.11 | 1.52 | 1.45 | 1.51 | 1.49 | 0.038 |
| | F020 | 4.5 | 4.6 | 4.5 | 4.53 | 0.058 | 1.5 | 1.5 | 1.5 | 1.50 | 0.000 |
| | F021 | 7.71 | 8.02 | 6.89 | 7.54 | 0.58 | 4.49 | 2.47 | 2.34 | 3.10 | 1.21 |
| | F030 | 4.41 | 4.45 | 4.35 | 4.40 | 0.050 | 1.36 | 1.36 | 1.32 | 1.35 | 0.023 |
| | F031 | 4.58 | 4.61 | 4.62 | 4.60 | 0.021 | 1.41 | 1.39 | 1.37 | 1.39 | 0.020 |
| | F032 | | | | | | | | | | |
| | F039 | 4.53 | 4.51 | 4.53 | 4.52 | 0.012 | 1.39 | 1.4 | 1.39 | 1.39 | 0.006 |
| | F045 | | | | | | | | | | |
| | F056 | | | | | | | | | | |
| | F059 | 4.46 | 4.59 | 4.42 | 4.49 | 0.089 | 1.31 | 1.28 | 1.28 | 1.29 | 0.017 |
| | F061 | | | | | | 1.35 | 1.37 | 1.39 | 1.37 | 0.020 |
| | F062 | 4.48 | 4.53 | 4.53 | 4.51 | 0.029 | 1.38 | 1.33 | 1.38 | 1.36 | 0.029 |
| | F079 | 4.54 | 4.58 | 4.59 | 4.57 | 0.026 | 1.37 | 1.36 | 1.36 | 1.36 | 0.006 |
| | F080 | 4.46 | 4.62 | 4.69 | 4.59 | 0.12 | 1.29 | 1.27 | 1.28 | 1.28 | 0.010 |
| | F088 | 4.457 | 4.45 | 4.448 | 4.45 | 0.005 | 1.334 | 1.326 | 1.304 | 1.32 | 0.016 |
| Community Results | | Consensus Mean | | | 4.50 | | Consensus Mean | | | 1.39 | |
| | | Consensus Standard Deviation | | | 0.14 | | Consensus Standard Deviation | | | 0.12 | |
| | | Maximum | | | 7.54 | | Maximum | | | 3.1 | |
| | | Minimum | | | 4.31 | | Minimum | | | 1.26 | |
| | | N | | | 15 | | N | | | 16 | |

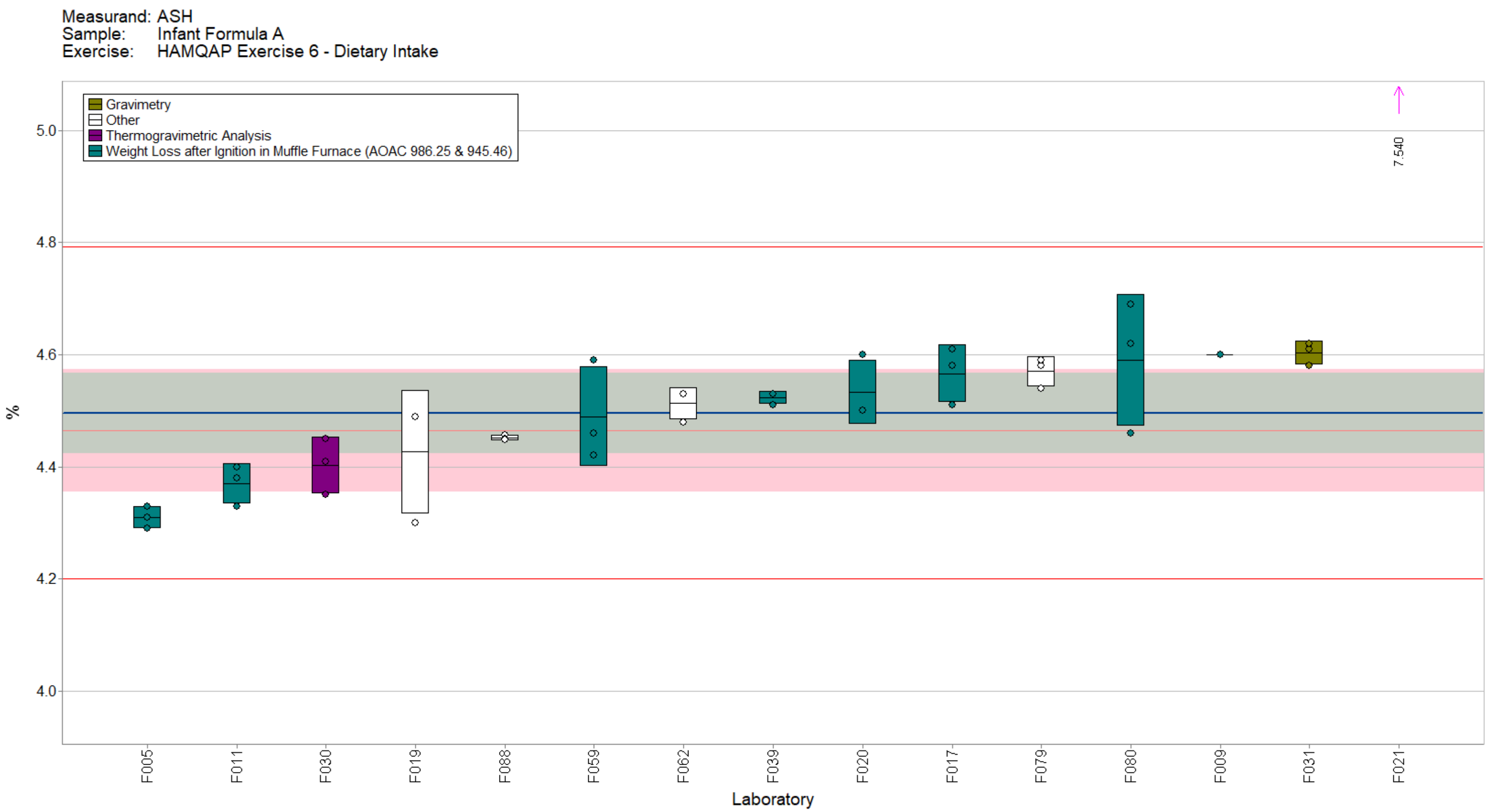


**Figure 10-14.** Ash in Infant Formula A (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line represents 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 $Z'_{\mathrm{comm}}$ score, $|Z'_{\mathrm{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty ($U_{\mathrm{NIST}}$) and represents the range that results in an acceptable $Z'_{\mathrm{NIST}}$ score, $|Z'_{\mathrm{NIST}}| \leq 2$.

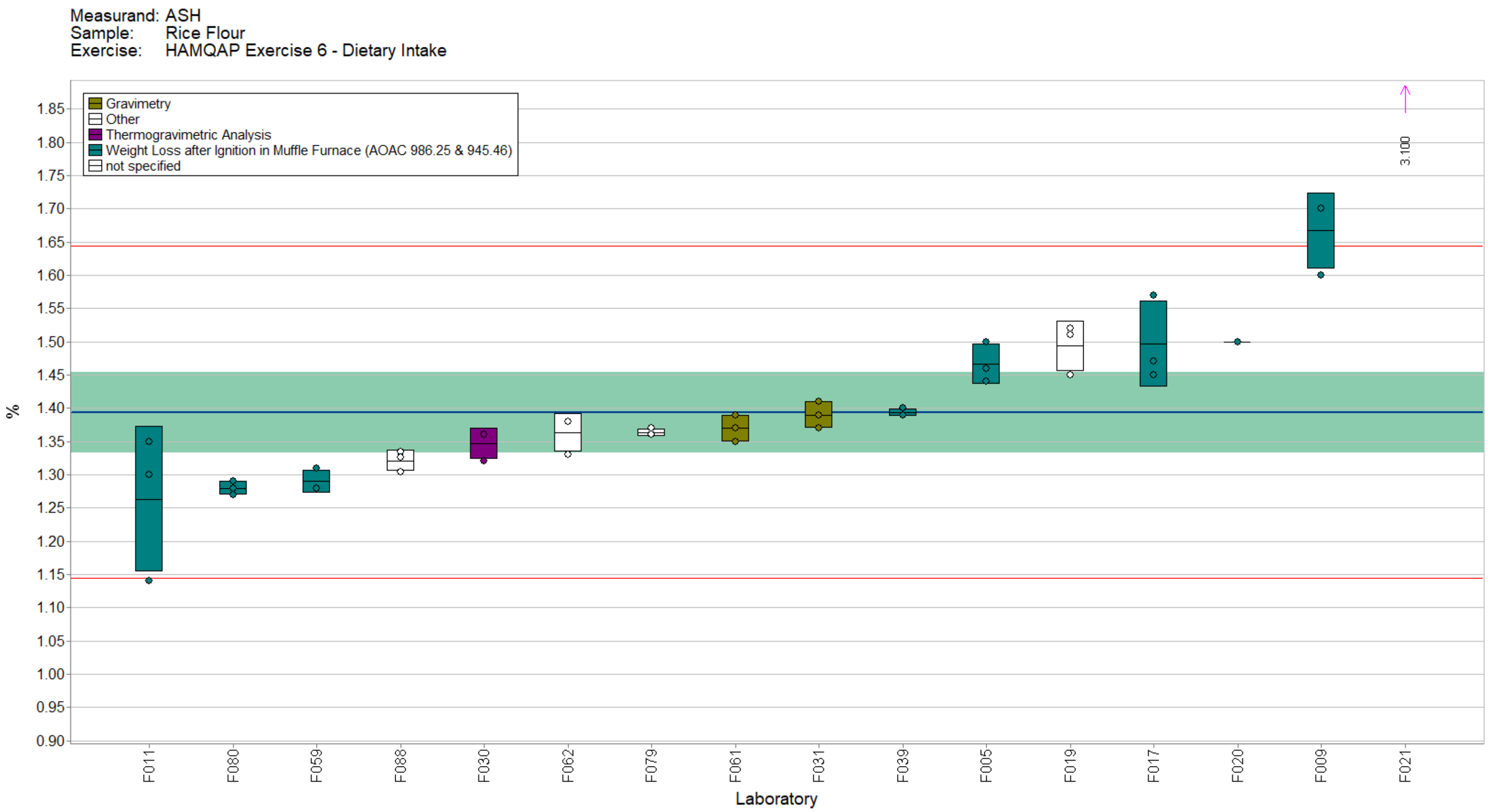


**Figure 10-15.** Ash in Rice Flour (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line represents 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 $Z'_{\mathrm{comm}}$ score, $|Z'_{\mathrm{comm}}| \leq 2$. A NIST value has not been determined in this material.

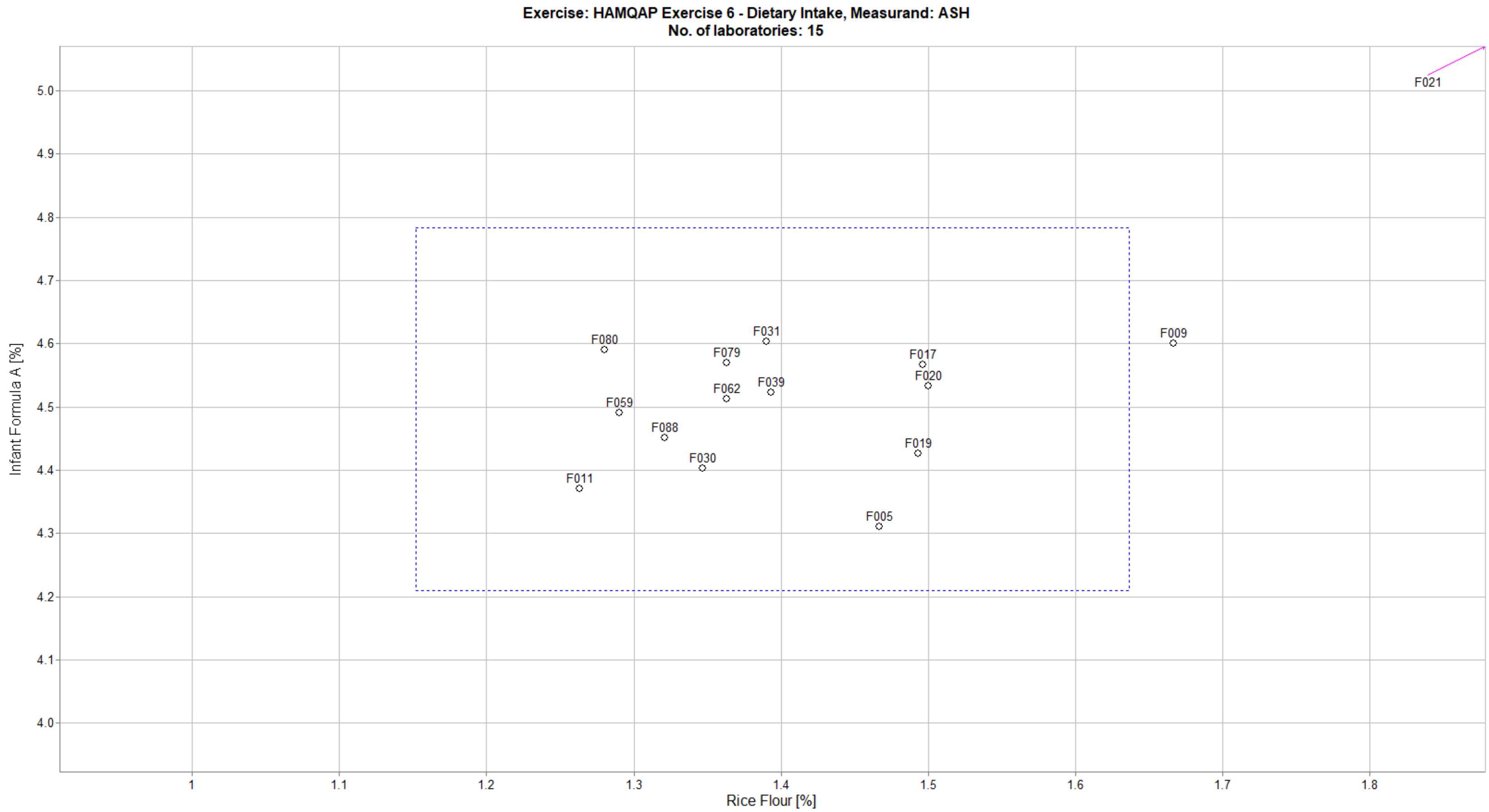


**Figure 10-16.** Laboratory means for ash in Infant Formula A and Rice Flour (sample/sample comparison view). In this view, the individual laboratory mean for one sample (Infant Formula A) is compared to the mean for a second sample (Rice Flour). The dotted blue box represents the consensus range of tolerance for Rice Flour (x-axis) and Infant Formula A (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 10-7.** Data summary table for calories in Infant Formula A and Rice Flour. Data points highlighted in blue have been identified as outside the consensus tolerance limits and would be estimated to result in an unacceptable $Z'_{comm}$ score, $|Z'_{comm}| \geq 2$.

| | | Calories | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Infant Formula A (kcal/100g) | | | | | Rice Flour (kcal/100 g) | | | | |
| | Lab | A | B | C | Avg | SD | A | B | C | Avg | SD |
| Individual Results | Target | | | | 522.6 | 0.62 | | | | 364.5 | 9.1 |
| | F004 | | | | | | | | | | |
| | F005 | 385.97 | 366.97 | 368.07 | 373.7 | 10.7 | 41.21 | 41.74 | 41.13 | 41.36 | 0.33 |
| | F009 | 513 | 505 | 514 | 510.7 | 4.93 | 367 | 368 | 369 | 368 | 1 |
| | F021 | | | | | | | | | | |
| | F031 | 514 | 512 | 514 | 513.3 | 1.15 | 373 | 372 | 371 | 372 | 1 |
| | F032 | | | | | | | | | | |
| | F039 | 540 | 536 | 538 | 538 | 2 | 392 | 392 | 392 | 392 | 0 |
| | F045 | | | | | | | | | | |
| | F056 | | | | | | | | | | |
| | F061 | | | | | | | | | | |
| | F062 | | | | | | | | | | |
| | F079 | 511 | 516 | 511 | 512.7 | 2.89 | 365 | 365 | 365 | 365 | 0 |
| | F080 | 512.73 | 512.02 | 510.9 | 511.9 | 0.92 | 360.44 | 362.22 | 361.4 | 361.35333 | 0.8909171 |
| | F088 | | | | | | | | | | |
| Community Results | | Consensus Mean | | | 513.111 | | Consensus Mean | | | 371.7 | |
| | | Consensus Standard Deviation | | | 6.40 | | Consensus Standard Deviation | | | 14.6 | |
| | | Maximum | | | 538.0 | | Maximum | | | 392.0 | |
| | | Minimum | | | 373.7 | | Minimum | | | 41.4 | |
| | | N | | | 6 | | N | | | 6 | |

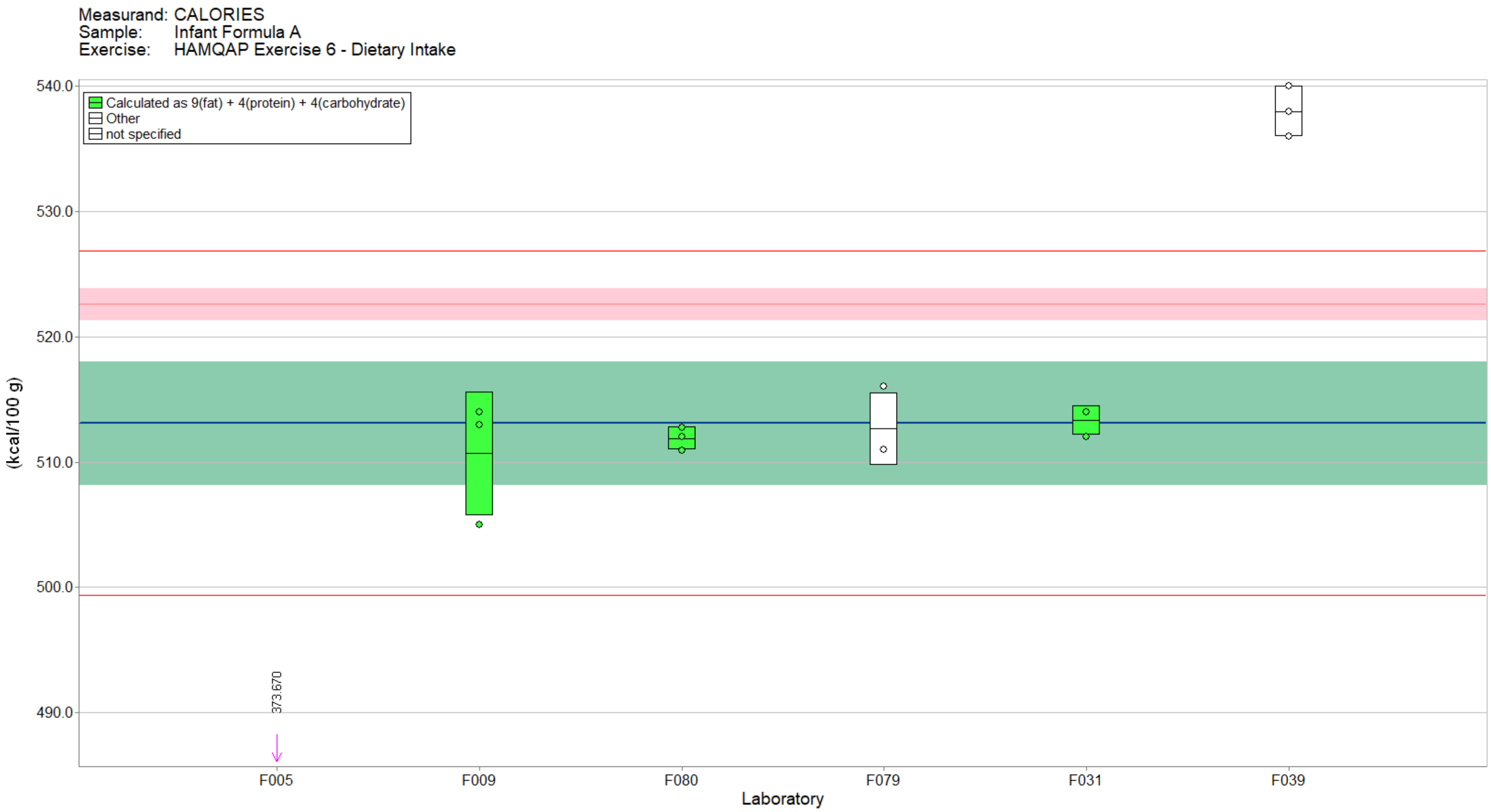


**Figure 10-17.** Calories in Infant Formula A (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line 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 $Z'_{\mathrm{comm}}$ score, $|Z'_{\mathrm{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty ($U_{\mathrm{NIST}}$) and represents the range that results in an acceptable $Z'_{\mathrm{NIST}}$ score, $|Z'_{\mathrm{NIST}}| \leq 2$.

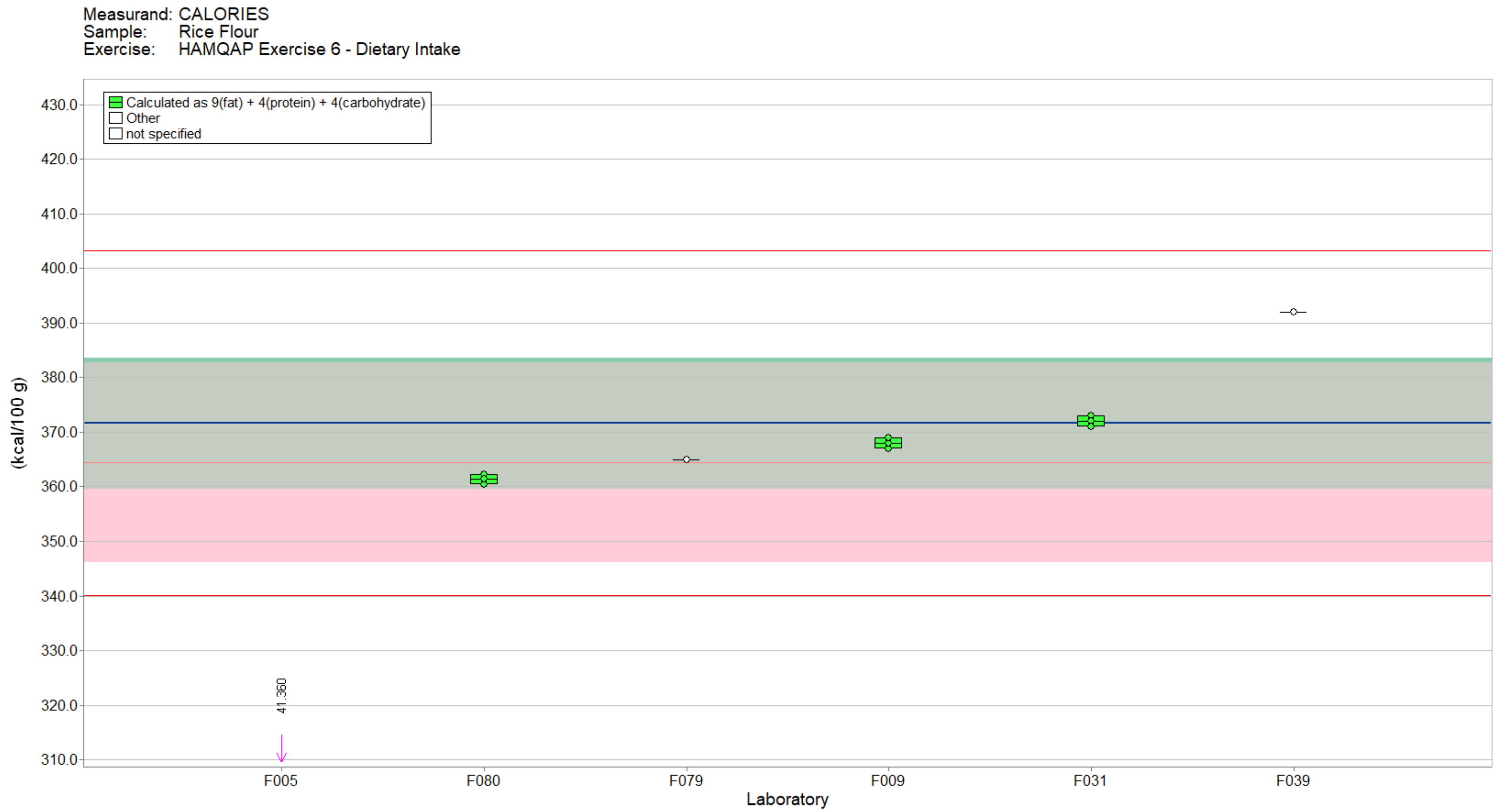


**Figure 10-18.** Calories in Rice Flour (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line represents 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 $Z'_{\text{comm}}$ score, $|Z'_{\text{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty (U$_{\text{NIST}}$) and represents the range that results in an acceptable $Z'_{\text{NIST}}$ score, $|Z'_{\text{NIST}}| \leq 2$.

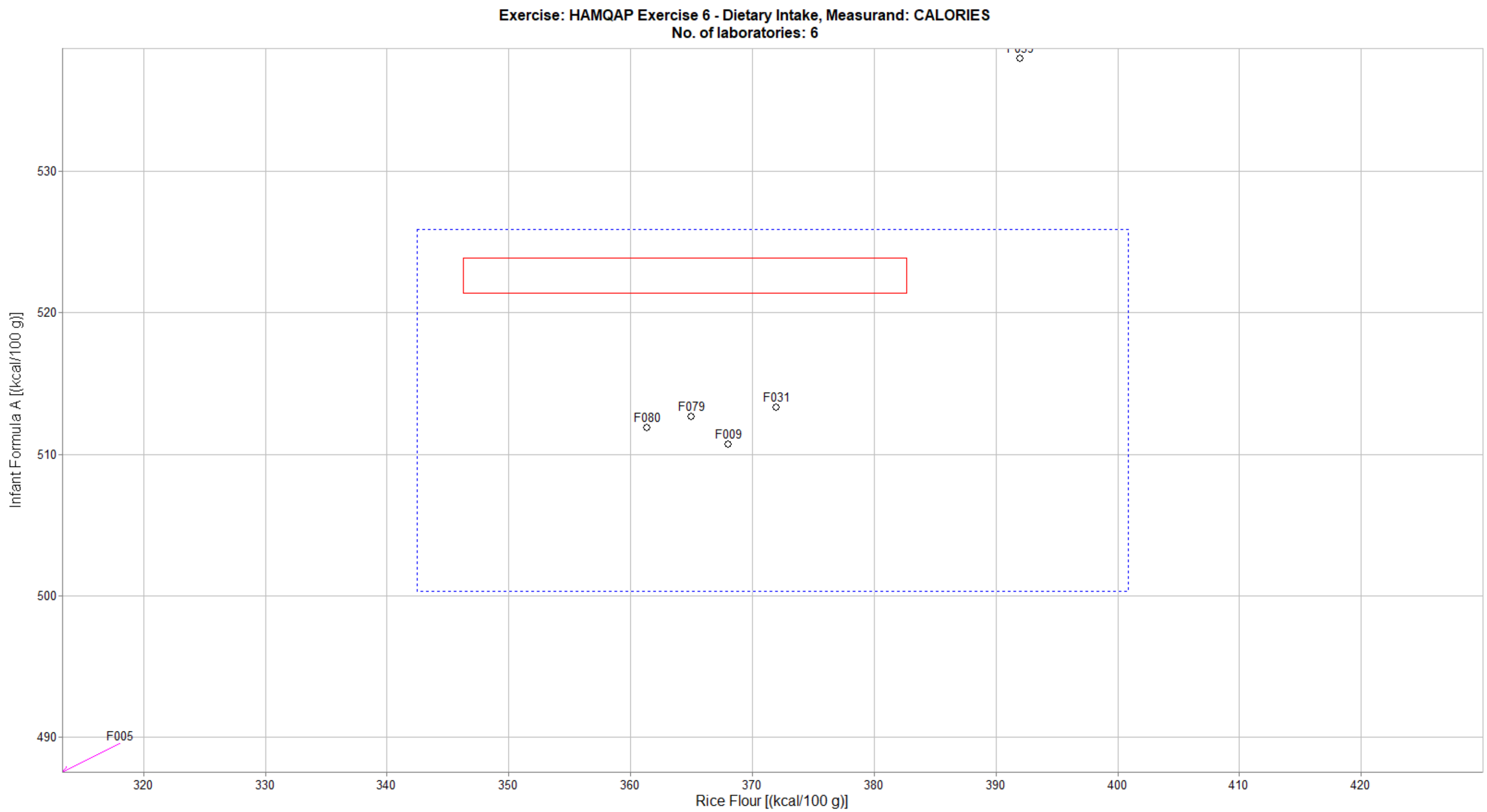


**Figure 10-19.** Laboratory means for calories in Infant Formula A and Rice Flour (sample/sample comparison view). In this view, the individual laboratory mean for one sample (Infant Formula A) is compared to the mean for a second sample (Rice Flour). The solid red box represents the NIST range of tolerance for the two samples, Rice Flour (x-axis) and Infant Formula A (y-axis), which encompasses the target values bounded by their uncertainties ($U_{NIST}$) and represents the range that results in an acceptable $Z_{NIST}$ score, $|Z_{NIST}| \leq 2$. The dotted blue box represents the consensus range of tolerance for Rice Flour (x-axis) and Infant Formula A (y-axis), calculated as the values above and below the consensus means that result in an acceptable $Z'_{comm}$ score, $|Z'_{comm}| \leq 2$.