NISTIR 8285

Health Assessment Measurements Quality Assurance Program: Exercise 3 Final Report

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https://doi.org/10.6028/NIST.IR.8285

National Institute of Standards and Technology
U.S. Department of Commerce

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Chemical Sciences Division
Material Measurement Laboratory

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February 2020

U.S. Department of Commerce
Wilbur L. Ross, Jr., Secretary

National Institute of Standards and Technology
Walter Copan, NIST Director and Undersecretary of Commerce for Standards and Technology

National Institute of Standards and Technology Interagency 8285
Natl. Inst. Stand. Technol. Interag. Rep. 8285, 221 pages (February 2020)

This publication is available free of charge from:
https://doi.org/10.6028/NIST.IR.8285

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LIST OF ACRONYMS

AAS	Atomic Absorption Spectroscopy
CDC	US Centers for Disease Control and Prevention
cGMP	current Good Manufacturing Practice
COA	Certificate of Analysis
CRM	Certified Reference Material
CV ID ICP-MS	Cold Vapor Isotope Dilution Inductively Coupled Plasma Mass Spectrometry
FDA	US Food and Drug Administration
GC-MS	Gas Chromatography-Mass Spectrometry
HAMQAP	Health Assessment Measurements Quality Assurance Program
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
GC-MS	Gas Chromatography-Mass Spectrometry
ID ICP-MS	Isotope Dilution Inductively Coupled Plasma-Mass Spectrometry
ID-LC/MS	Isotope Dilution Liquid Chromatography Mass Spectrometry
ID-LC-MS/MS	Isotope Dilution Liquid Chromatography-Tandem Mass Spectrometry
INAA	Instrumental Neutron Activation Analysis
JCTLM	Joint Committee for Traceability in Laboratory Medicine
LC-absorbance	Liquid Chromatography-Absorbance
LC-MS/MS	Liquid Chromatography-Tandem Mass Spectrometry
LOQ	Limit of Quantification
MDL	Method Detection Limit
NIST	National Institute of Standards and Technology
NIH	National Institutes of Health
NIH ODS	NIH Office of Dietary Supplements
ODS AMRM	ODS Analytical Methods and Reference Materials
PDA	Photodiode Array
RMP	Reference Measurement Procedure
QAP	Quality Assurance Program
QL	Quantification Limit
RM	Reference Material
RSD	Relative Standard Deviation
SD	Standard Deviation
SRM	Standard Reference Material

ABSTRACT

HAMQAP was launched in collaboration with the NIH 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 3 of this program offered the opportunity for laboratories to assess their in-house measurements of nutritional elements (iodine and selenium), contaminants (arsenic, cadmium, lead, mercury, and furans), water-soluble vitamins (folates), fat-soluble vitamins (carotenoids), natural product compounds (ubiquinone), and botanicals (isoflavones) in foods and dietary supplements, and corresponding biomarkers/metabolites in clinical specimens (including human milk, serum, blood, plasma, and urine).

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 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, hemp) 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 cGMPs or to fulfill proficiency requirements established by related accreditation bodies. In addition, NIST and HAMQAP assist the ODS AMRM program at the NIH in supporting the development and dissemination of analytical tools and reference materials. In the future, results from HAMQAP exercises could be used by ODS and NIST to identify problematic matrices and analytes for which 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 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

[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.

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 third exercise of HAMQAP. Fifty-four laboratories responded to the dietary intake portion and twenty-six laboratories responded to the human metabolites portion of the call for participants distributed in October 2018 (see table below). Three human metabolites studies were cancelled prior to shipment due to low enrollment. Samples were shipped to participants in February 2019 and results were returned to NIST by March 2019. This report contains the final data and information that was disseminated to the participants in February 2020.

Study Group	Dietary Intake Study	Human Metabolites Study
Nutritional Elements	Iodine, Selenium Table Salt, Cat Food*, Protein Drink	Iodine, Selenium, Thyroid Hormones Human Milk, Serum
Toxic Elements	As, Cd, Pb, Hg Black Cohosh, Hemp*	As, Cd, Pb, Hg Whole Human Blood
Water-Soluble Vitamins	Folates Multivitamin, Infant Formula	Folates Human Serum
Fat-Soluble Vitamins	Carotenoids Multivitamin, Saw Palmetto Extract	Carotenoids Human Serum, Bovine Serum
Natural Products	Ubiquinone Commercial Supplements	Ubiquinone Human Serum, Bovine Serum
Botanicals	Isoflavones Soy, Red Clover	~~Daidzein, Daidzin, Equol~~** ~~Human Urine~~
Contaminants	Furans, Alkyl furans Coffee, Baby Food, Cereal	~~Furan Metabolites~~** ~~Human Urine~~
Inflammation Markers	Not offered	~~Calprotectin, Zonulin~~** ~~Human Plasma~~

* 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(E), 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 and standard deviation 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 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'_{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, σ_{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(E), *Statistical methods for use in proficiency testing by interlaboratory comparisons*, pp. 53–54.

The second Z-score, Z_{NIST}, is calculated with respect to the target value (NIST certified, reference, or estimated value, when available), using x_{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'_{comm}) or NIST target range (for Z_{NIST}).
- $2 < |Z| < 3$ indicates that the laboratory result is considered to be marginally different from the community consensus value (for Z'_{comm}) or NIST target value (for Z_{NIST}).
- $|Z| > 3$ indicates that the laboratory result is considered to be significantly different from the community consensus value (for Z'_{comm}) or NIST target value (for Z_{NIST}).

Section 2 of the data table (*Community Results*) contains the consensus results, including the number of laboratories reporting more than a single quantitative value for each analyte, the mean value determined for each analyte, and a robust estimate of the standard deviation of the reported values.[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 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 JCTLM-recognized 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-assessed value may be determined at NIST using a validated method or data from a collaborating laboratory. The NIST-assessed value represents the mean of at least three replicates. For materials acquired from another interlaboratory study or proficiency testing program, the consensus value and uncertainty from the completed round is used as the target range. Within each section of this report, the exact methods for determination of the study target values are outlined in detail.

Summary Data Table

This data table includes a summary of all reported data for a particular analyte in a particular study. Participants can compare the raw data for their laboratory to data reported by the other participating laboratories 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 red have been flagged as potential outliers (e.g., difference from reference value, Grubb and/or Cochran) by the NIST software package. The SD for the target value in this table is the uncertainty (U_{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 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_{r}), the reproducibility standard deviation (s_{R}), the number of participants reporting data, and the average number of replicates reported by each participant. The uncertainty about the consensus mean is independent of the range of tolerance. Where appropriate, two consensus means may be calculated for the same sample if bimodality is identified in the data. In this case, two consensus means and ranges will be displayed in the data summary view.

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

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_{NIST}). The solid red lines represent the range of tolerance (values that result in an acceptable Z' score, $|Z'| \leq 2$). If the lower limit is below zero, the lower limit has been set to zero. In this view, the relative locations of individual laboratory data and consensus zones with respect to the target zone can be compared easily. In most cases, the target zone and the consensus zone overlap, which is the expected result. The major program goals are to reduce the size of the consensus zone and center the consensus zone about the target value. Analysis of an appropriate reference material as part of a quality control scheme can help to identify sources of bias for laboratories reporting results that are significantly different from the target zone. In the case in which a method comparison is relevant, different colored data points may be used to 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 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 % 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 (Iodine and Selenium)

Study Overview

In this study, participants were provided with three (3) NIST SRMs for dietary intake, SRM 3530 Iodized Table Salt (Iodide), SRM 3290 Dry Cat Food, and SRM 3252 Protein Drink Mix, and one (1) NIST SRM for human metabolites, SRM 1953 Organic Contaminants in Non-Fortified Human Milk, plus two (2) human serum samples, one sample from a healthy male and one sample from a premenopausal, healthy female. Participants were asked to use in-house analytical methods to determine the mass fraction (mg/kg) of iodine (I) in SRM 3530 and to determine the mass fractions (mg/kg) of iodine (I) and selenium (Se) in SRM 3290 and SRM 3252. Participants were asked to use in-house analytical methods to determine the mass fractions (mg/kg) of iodine (I) and the thyroid hormones triiodothyronine (T3), thyroxine (T4), and thyroid stimulating hormone (TSH) in SRM 1953 and the human serum samples. Iodine and selenium are essential minerals required in the synthesis of thyroid hormones that regulate metabolism.[4,5] Selenium deficiency decreases the selenoprotein-mediated synthesis of thyroid hormones. Iodine deficiency decreases the levels of two thyroid hormones, T3 and T4, leading to increased production of TSH. Thyroid function is regulated by TSH and is critical to the body's metabolism and the development of bones and the nervous system. Infants are more sensitive to iodine deficiency, as changes in their thyroid hormone levels respond significantly to mild changes in iodine intake, indicating a need for sufficient ability to measure iodine and related biomarkers in clinical samples. An accurate assessment of these elements in foods, supplement samples, and biological samples has been challenging for laboratories in the past, from sample preparation to instrumental measurement. Accurate measurement of iodine and selenium in foods and supplements is necessary for understanding daily intake of iodine and selenium and related health outcomes.

Dietary Intake Sample Information

Cat Food. Participants were provided with three packets, each containing 10 g of powdered cat food. Before use, participants were instructed to mix the contents of each packet thoroughly and to use a sample size of at least 0.5 g. Participants were asked to store the material at controlled room temperature, 20 °C to 25 °C, and to prepare one sample and report one value from each packet provided. The approximate analyte levels were not reported to participants prior to the study. The mass fraction values for iodine and selenium in SRM 3290 were assigned using results from NIST by ICP-MS and INAA. The NIST-determined values and uncertainties for iodine and selenium 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 (4.36 %).

	NIST-Determined Mass Fraction in SRM 3290 (mg/kg)	
Analyte	(dry-mass basis)	(as-received basis)
Iodine (I)	3.38 ± 0.54	3.23 ± 0.52
Selenium (Se)	0.548 ± 0.048	0.524 ± 0.046

[4] Iodine Fact Sheet for Health Professionals. National Institutes of Health Office of Dietary Supplements. https://ods.od.nih.gov/factsheets/Iodine-HealthProfessional/ (accessed November 2019).
[5] Selenium Fact Sheet for Health Professionals. National Institutes of Health Office of Dietary Supplements. https://ods.od.nih.gov/factsheets/Selenium-HealthProfessional/ (accessed November 2019).

Protein Drink Mix. Participants were provided with three packets, each containing 10 g of powdered protein drink mix. Before use, participants were instructed to mix the contents of each packet thoroughly and to use a sample size of at least 0.5 g. Participants were asked to store the material at controlled room temperature, 20 °C to 25 °C, and to prepare one sample and report one value from each packet provided. The approximate analyte levels were not reported to participants prior to the study. The mass fraction values for iodine and selenium in SRM 3252 were assigned using results from NIST by ICP-MS and INAA. The NIST-determined values and uncertainties for iodine and selenium 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 (4.98 %).

	NIST-Determined Mass Fraction in SRM 3252 (mg/kg)	
Analyte	(dry-mass basis)	(as-received basis)
Iodine (I)	1.84 ± 0.20	1.75 ± 0.19
Selenium (Se)	0.596 ± 0.037	0.566 ± 0.035

Table Salt. Participants were provided with one bottle containing 200 g of table salt. Participants were instructed to thoroughly mix the contents by rotating and/or rolling the unopened bottle prior to removal of a test sample for analysis, and to use a sample size of at least 0.25 g. Participants were asked to store the material in the aluminized pouch at room temperature, 20 °C to 25 °C, with maximum humidity of 65 %, and to prepare three samples and report three values from the bottle provided. The approximate analyte level was not reported to participants prior to the study. The certified value for iodine in SRM 3530 was assigned using results from NIST by gravimetric titrimetry, ICP-MS, and by linear voltammetry performed at Centro Nacional De Metrología, Mexico (CENAM). The certified value and uncertainty for iodine is provided in the table below on an as-received basis.

	Certified Mass Fraction in SRM 3530 (mg/kg)
Analyte	(as-received basis)
Iodine (as Iodide)	52.2 ± 4.2

Dietary Intake Study Results

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

		Number of Laboratories Reporting Results (Percent Participation)		
Analyte	Number of Laboratories Requesting Samples	Cat Food	Protein Drink Mix	Table Salt
Iodine (I)	28	8 (29 %)	8 (29 %)	8 (29 %)
Selenium (Se)	31	16 (52 %)	17 (55 %)	-

- The target range and the consensus range for iodine and selenium overlapped for all materials except for iodine in the protein drink mix.
- The consensus mean for iodine in the protein drink mix was above the target range with no overlap of the target range and the consensus range.
- The between-laboratory variabilities are reported below.

	Between-Laboratory Variability (Percent RSD)		
Analyte	Cat Food	Protein Drink Mix	Table Salt
Iodine (I)	16 %	19 %	31 %
Selenium (Se)	27 %	32 %	-

- Most laboratories reported using ICP-MS (75 % to 100 %) as their analytical method for both analytes. One laboratory reported using ID ICP-MS for the measurement of selenium. For the measurement of iodine in table salt, one laboratory used ion chromatography (IC) with electrochemical detection (ECD) and one laboratory did not specify a method used.
- The sample preparation methods reported by participating laboratories are shown for iodine and selenium in **Figures 1-1 to 1-3 and 1-7 to 1-8**, respectively**.**

Dietary Intake Technical Recommendations

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

- The low participation for iodine in this study could be a result of the greater challenge posed by analysis of iodine compared to other nutritional elements, a lack of interest in iodine measurements, or a lack of established protocols for iodine measurements.
- Some general suggestions regarding iodine sample preparation and analysis are provided below.
  - Iodide can form volatile hydrogen iodide (HI) during acid digestion so care must be taken to retain iodine during sample preparation.
  - Calibration may be an issue for laboratories reporting data biased high for both samples (**Figure 1-4)**. Standards used for calibration should be of known purity and traceability.
  - When using ICP-MS, 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 wash solution used between sample readings should be slightly basic, above pH 7, and also contain Triton X-100.
  - Tetramethylammonium hydroxide (TMAH) is a very effective solvent for iodine sample preparation, and many protocols call for the use of TMAH. 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.
  - During sample preparation, iodine can adhere to tetrafluoroethylene (TFM) vessels, so perfluoroalkoxy (PFA) vessels or quartz vessels are recommended to improve repeatability.
- Some general suggestions regarding selenium sample preparation and analysis are provided below.

- The majority of laboratories were within target range for selenium for both NIST materials **(Figure 1-9)** indicating laboratories are using good sample preparation techniques.
- Calibration may be an issue for laboratories reporting data biased high for both samples (**Figure 1-9)**. Standards used for calibration should be of known purity and traceability.
- The digestion procedure is critical to the accuracy of selenium determination with conditions requiring the breakdown of the organoselenium compounds.
    - Recommended digestion procedures include mixtures of nitric, hydrofluoric, and perchloric acids with temperatures of up to 200 °C for open beaker techniques.
    - Nitric acid and a small amount of HF should be sufficient with the high temperature and high pressure of a microwave system.
        - A small amount of hydrofluoric acid is useful to perform a complete digestion, required for a more accurate determination of selenium.
- Selenium contamination from the environment does not normally impact analytical testing.
- CRMs are available and may be used for assay validation.
- 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 $^{40}Ar_2^{+}$, among others.

- For both iodine and selenium measurements, calculations errors can be a cause for incorrect results. Using a quality assurance material (CRM, SRM, RM), or in-house prepared material, to establish that a method is in control will also help find calculation errors.

Table 1-1. Individualized data summary table (NIST) for nutritional elements in cat food, protein drink mix, and table salt.

National Institute of Standards and Technology

HAMQAP Exercise 3 - 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*
Total Iodine	SRM 3290 Dry Cat Food		mg/kg	3.23	0.52		0	8	3.72	0.58	3.23	0.516
Total Iodine	SRM 3252 Protein Drink Mix		mg/kg	1.75	0.19		0	8	2.5	0.47	1.75	0.19
Total Iodine	SRM 3530 Iodized Table Salt (Iodide)		mg/kg	52.2	4.2		0	8	50	17	52.2	4.2
Total Selenium	SRM 3290 Dry Cat Food		mg/kg	0.524	0.046		0	16	0.6	0.17	0.524	0.0459
Total Selenium	SRM 3252 Protein Drink Mix		mg/kg	0.566	0.035		0	17	0.6	0.2	0.566	0.0352

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 total iodine in cat food, protein drink mix, and table salt. Data points highlighted in red have been flagged as potential outliers (e.g., Grubb and/or Cochran) by the NIST software package.

		Total Iodine														
		SRM 3290 Dry Cat Food (mg/kg)					SRM 3252 Protein Drink Mix (mg/kg)					SRM 3530 Iodized Table Salt (Iodide) (mg/kg)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target				3.23	0.52				1.75	0.19				52.2	4.2
	C002															
	C005											49.1	48.4	49.5	49.0	0.6
	C006															
	C008															
	C011	3.83	3.92	4.12	3.96	0.15	2.41	2.46	2.11	2.33	0.19					
	C012															
	C013	2.5	2.39	2.43	2.44	0.06	2.77	2.1	2.36	2.41	0.34	46.4	45.7	49.4	47.2	2.0
	C014	3.512	3.399	3.665	3.53	0.13	2.199	2.073	2.425	2.23	0.18	57.291	57.858	57.052	57.4	0.4
	C016															
	C017	3.86	3.86	3.48	3.73	0.22	2.33	2.56	2.24	2.38	0.17	35.8	35.77	36.2	35.9	0.2
	C025	4.515	3.616	3.857	4.00	0.47	2.213	2.364	3.713	2.76	0.83	48.84			48.8	
	C026															
	C027	10.04	10.17	9.9	10.04	0.14	15.08	15.02	15.29	15.13	0.14	70.93	69.77	71.04	70.6	0.7
	C031															
	C032															
	C033															
	C037															
	C039															
	C043															
	C044	3.71	3.95	3.41	3.69	0.27	2.54	2.69	1.87	2.37	0.44	63.52	68.19	51.14	61.0	8.8
	C045															
	C047															
	C048	3.8	5.2	3.8	4.27	0.81	3.5	2.6	2.9	3.00	0.46	60	58	58	58.7	1.2
	C049															
	C050															
	C051															
	C054															
	C055															
Community Results		Consensus Mean			3.72		Consensus Mean			2.50		Consensus Mean			53.6	
		Consensus Standard Deviation			0.58		Consensus Standard Deviation			0.47		Consensus Standard Deviation			16.9	
		Maximum			10.04		Maximum			15.13		Maximum			70.6	
		Minimum			2.44		Minimum			2.23		Minimum			35.9	
		N			8		N			8		N			7	

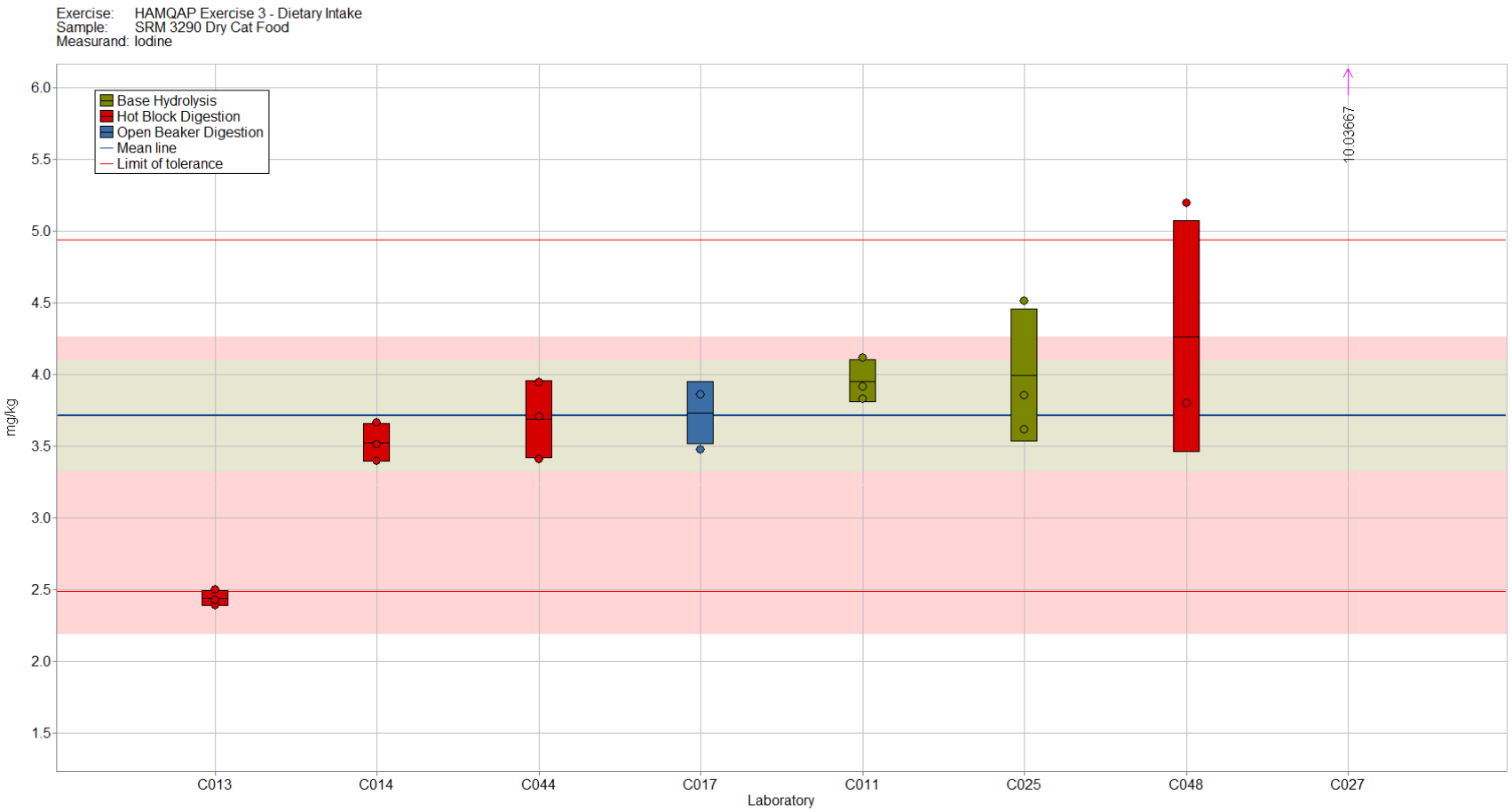

Figure 1-1. Iodine in SRM 3290 Dry Cat Food (data summary view – sample preparation method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The 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'_{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_{NIST}) and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$.

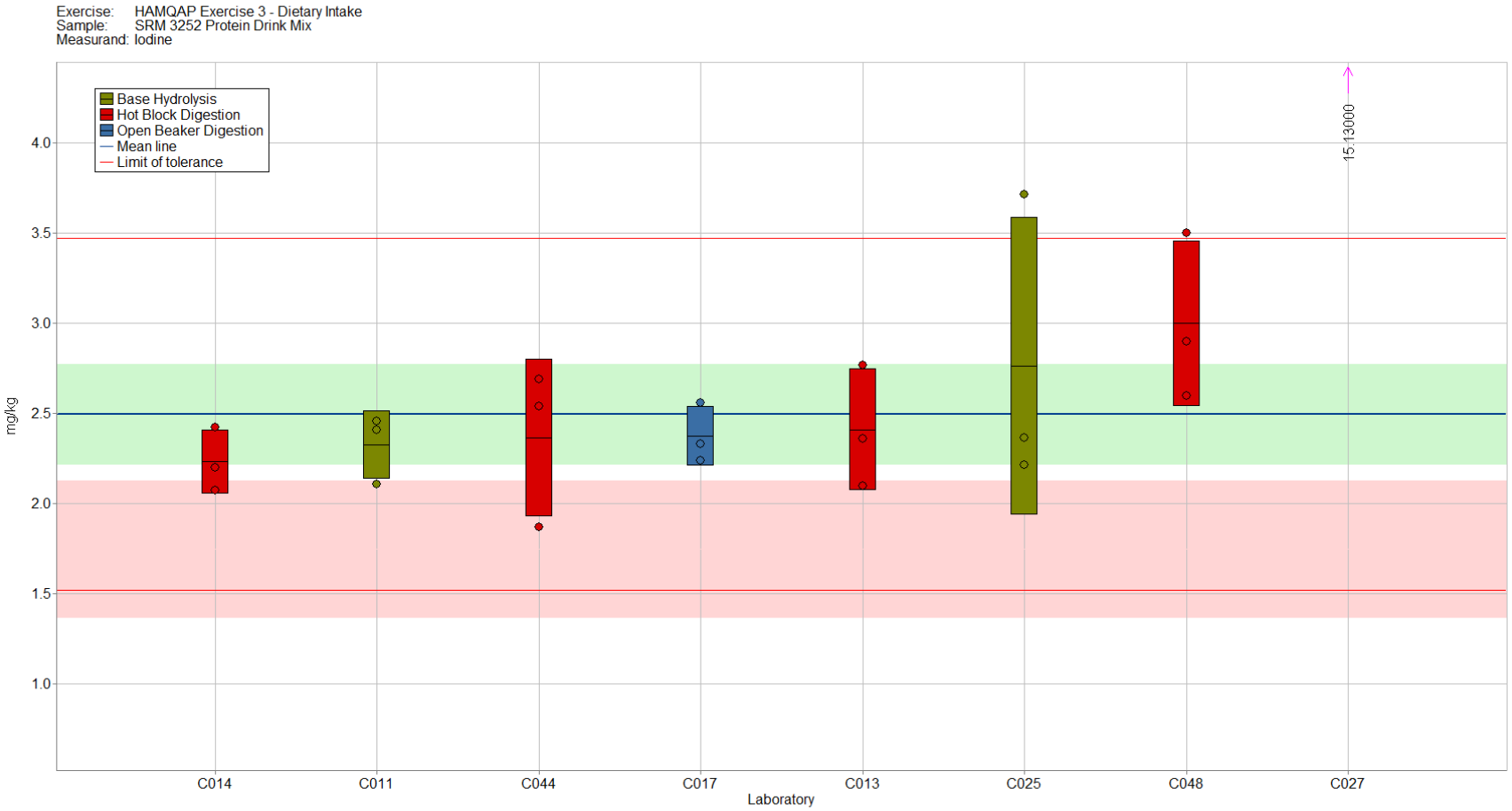

Figure 1-2. Iodine in SRM 3252 Protein Drink Mix (data summary view – sample preparation method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. 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'_{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_{NIST}) and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$.

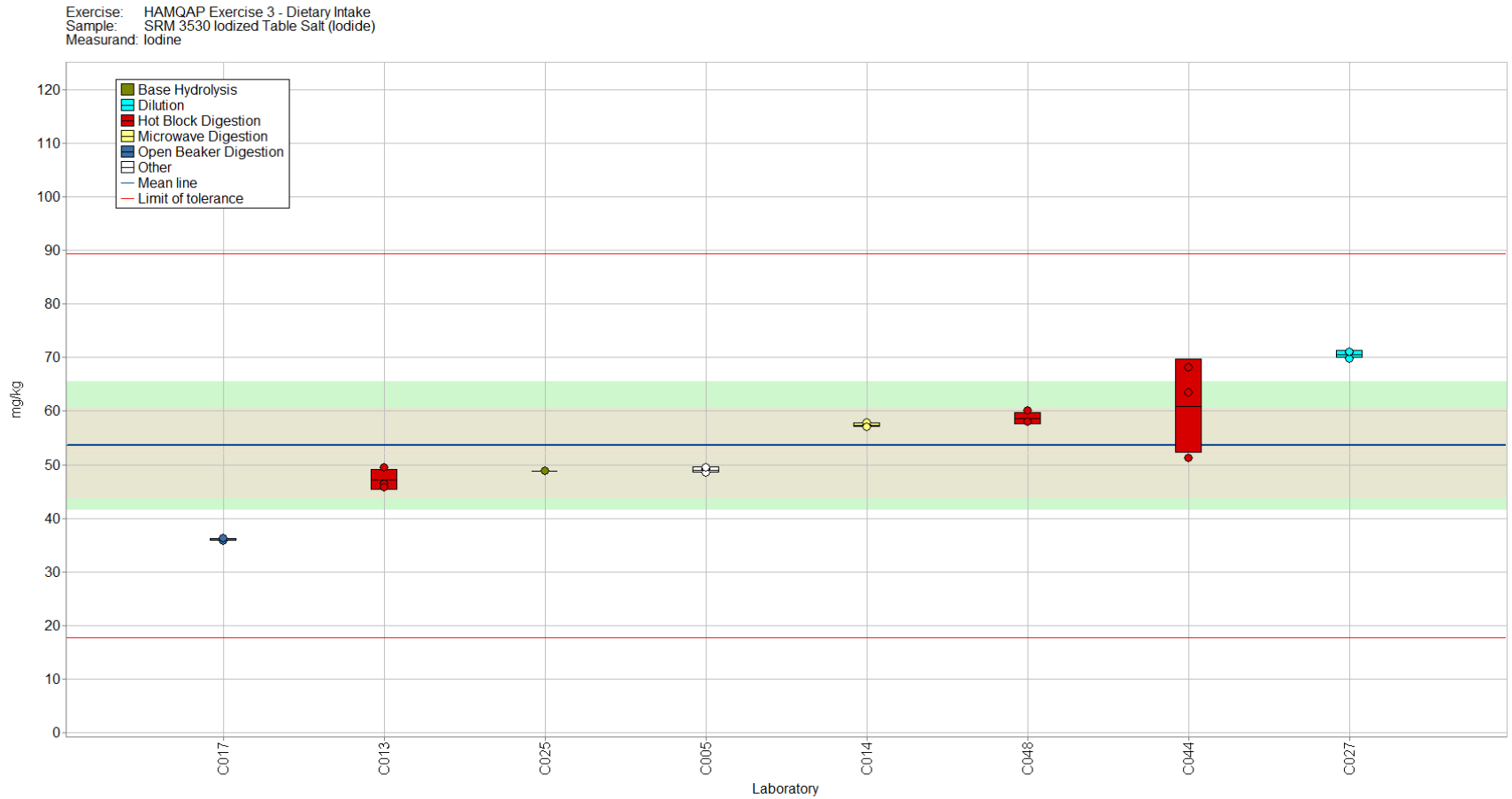

Figure 1-3. Iodine in SRM 3530 Iodized Table Salt (Iodide) (data summary view – sample preparation method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method 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'_{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_{NIST}) and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$.

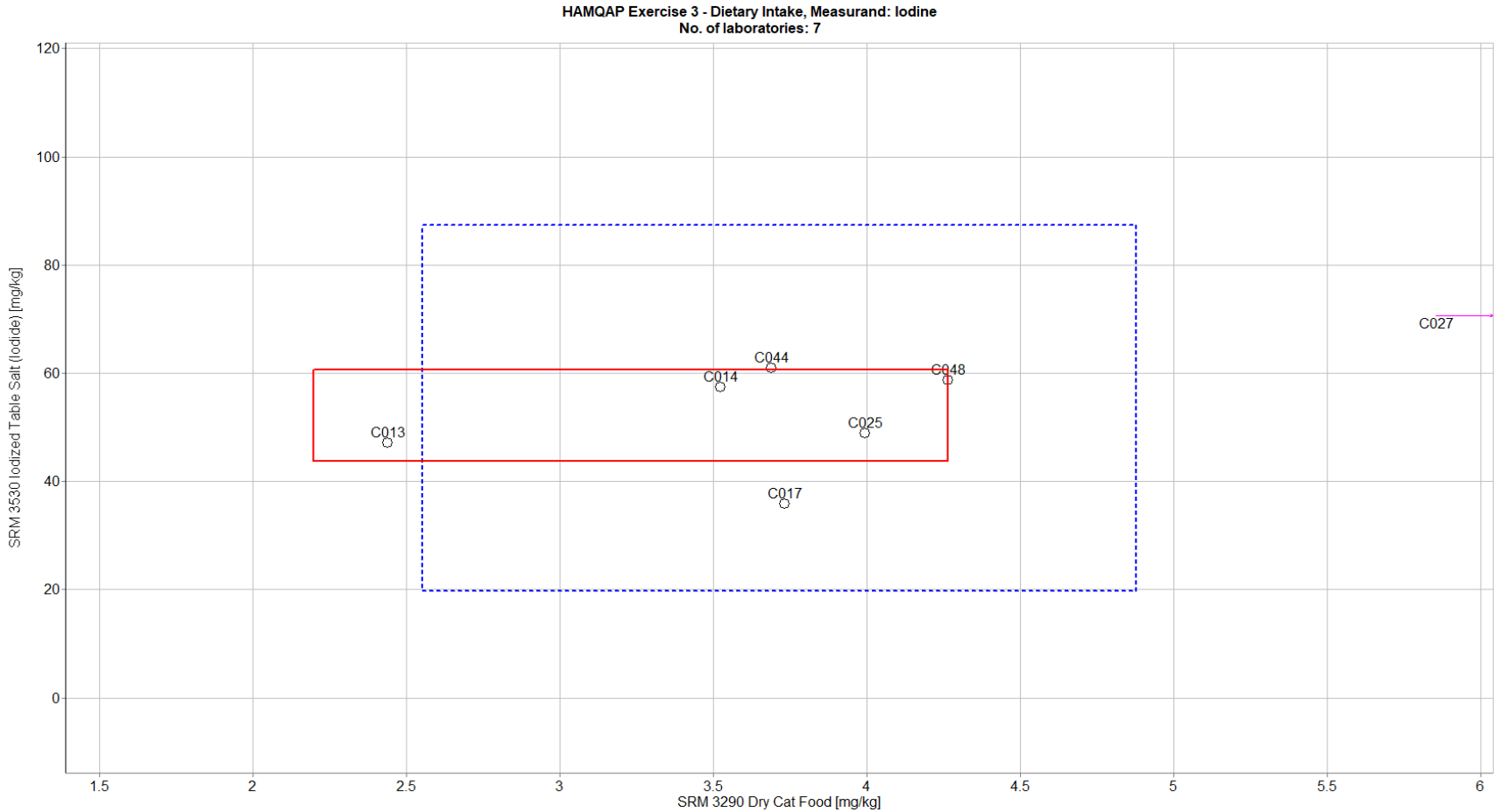

Figure 1-4. Laboratory means for iodine in SRM 3290 Dry Cat Food and SRM 3530 Iodized Table Salt (Iodide) (sample/sample comparison view). In this view, the individual laboratory mean for one sample (SRM 3290) is compared to the individual laboratory mean for a second sample (SRM 3530). The solid red box represents the NIST range of tolerance for the two samples, SRM 3290 (x-axis) and SRM 3530 (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, $|Z_{\mathrm{NIST}}| \leq 2$. The dotted blue box represents the consensus range of tolerance for SRM 3290 (x-axis) and SRM 3530 (y-axis), calculated as the values above and below the consensus means that result in an acceptable Z_{NIST} score, $|Z_{\mathrm{NIST}}| \leq 2$.

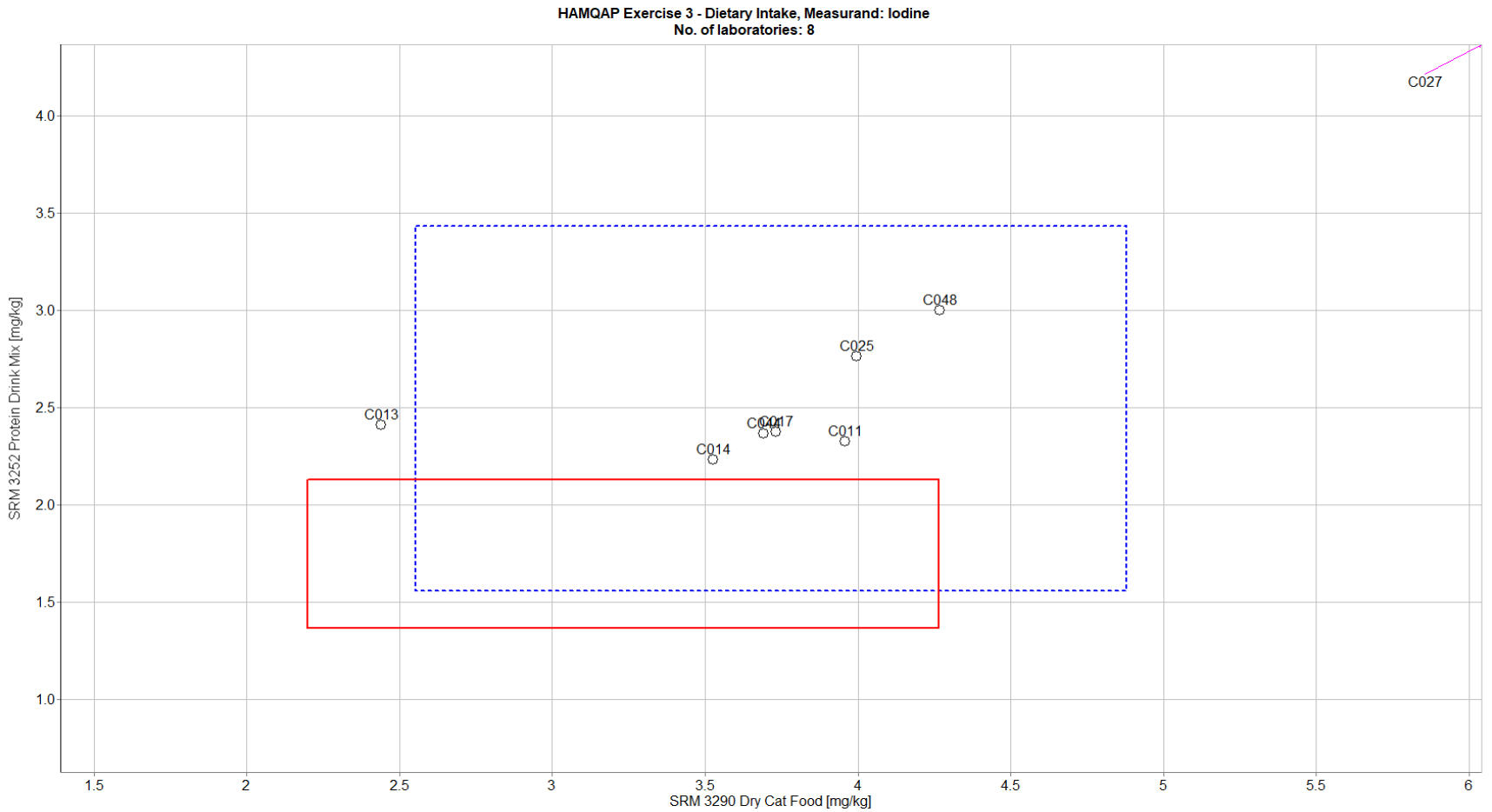

Figure 1-5. Laboratory means for iodine in SRM 3290 Dry Cat Food and SRM 3252 Protein Drink Mix (sample/sample comparison view). In this view, the individual laboratory mean for one sample (SRM 3290) is compared to the individual laboratory mean for a second sample (SRM 3252). The solid red box represents the NIST range of tolerance for the two samples, SRM 3290 (x-axis) and SRM 3252 (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, $|Z_{\mathrm{NIST}}| \leq 2$. The dotted blue box represents the consensus range of tolerance for SRM 3290 (x-axis) and SRM 3252 (y-axis), calculated as the values above and below the consensus means that result in an acceptable Z'_{comm} score, $|Z'_{\mathrm{comm}}| \leq 2$.

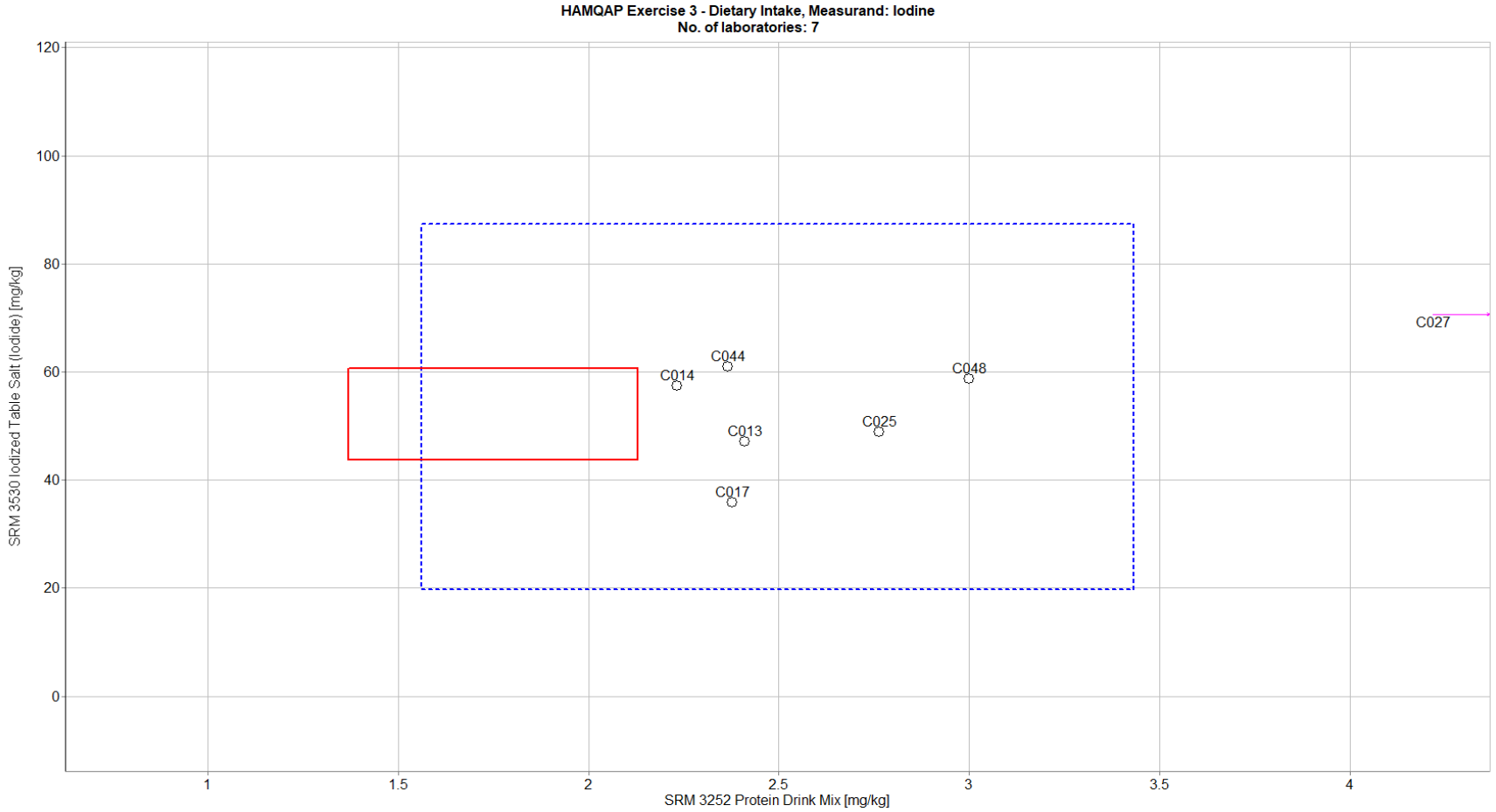

Figure 1-6. Laboratory means for iodine in SRM 3252 Protein Drink Mix and SRM 3530 Iodized Table Salt (Iodide) (sample/sample comparison view). In this view, the individual laboratory mean for one sample (SRM 3252) is compared to the individual laboratory mean for a second sample (SRM 3530). The solid red box represents the NIST range of tolerance for the two samples, SRM 3252 (x-axis) and SRM 3530 (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, $|Z_{\mathrm{NIST}}| \leq 2$. The dotted blue box represents the consensus range of tolerance for SRM 3252 (x-axis) and SRM 3530 (y-axis), calculated as the values above and below the consensus means that result in an acceptable Z'_{comm} score, $|Z'_{\mathrm{comm}}| \leq 2$.

Table 1-3. Data summary table for total selenium in cat food and protein drink mix. Data points highlighted in red have been flagged as potential outliers (e.g., Grubb and/or Cochran) by the NIST software package.

		Total Selenium									
		SRM 3290 Dry Cat Food (mg/kg)					SRM 3252 Protein Drink Mix (mg/kg)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target				0.524	0.046				0.566	0.035
	C002	2.25	1.32	1.29	1.620	0.546	4.36	2.34	2.49	3.063	1.125
	C005										
	C006	0.35	0.56	0.52	0.477	0.112	0.63	0.6	0.62	0.617	0.015
	C009	0.6415	0.6198		0.631	0.015	1.0022	0.8165		0.909	0.131
	C011	0.846	0.828	0.843	0.839	0.010	0.79	0.814	0.788	0.797	0.014
	C012	0.5128	0.51	0.509	0.511	0.002	0.6082	0.6438	0.647	0.633	0.022
	C013	0.499	0.517	0.452	0.489	0.034	0.378	0.495	0.472	0.448	0.062
	C014										
	C016	0.82	0.81	0.81	0.813	0.006	0.6555	0.83029	0.69925	0.728	0.091
	C017	0.59	0.61	0.63	0.610	0.020	0.61	0.63	0.6	0.613	0.015
	C019										
	C020										
	C021	0.461	0.578	0.642	0.560	0.092	0.504	0.489	0.484	0.492	0.010
	C025	0.797	0.821	0.894	0.837	0.051	0.703	0.721	0.728	0.717	0.013
	C027	0.4836	0.5217	0.5077	0.504	0.019	0.5295	0.5163	0.5188	0.522	0.007
	C030	0.5571	0.5731	0.5858	0.572	0.014	0.5739	0.5858	0.587	0.582	0.007
	C031										
	C032										
	C033										
	C035	0.477	0.495	0.499	0.490	0.012	0.504	0.53	0.502	0.512	0.016
	C039						0.589	0.632	0.61	0.610	0.022
	C043										
	C044	0.446	0.39	0.426	0.421	0.028	0.361	0.335	0.343	0.346	0.013
	C046										
	C047										
	C048	0.47	0.47	0.46	0.467	0.006	0.49	0.53	0.54	0.520	0.026
	C049										
	C050										
	C051										
	C054	0.94	1.04	1.03	1.003	0.055	1.1	1.1	1.34	1.180	0.139
	C055										
Community Results		Consensus Mean			0.605		Consensus Mean			0.623	
		Consensus Standard Deviation			0.166		Consensus Standard Deviation			0.202	
		Maximum			1.620		Maximum			3.063	
		Minimum			0.421		Minimum			0.346	
		N			16		N			17	

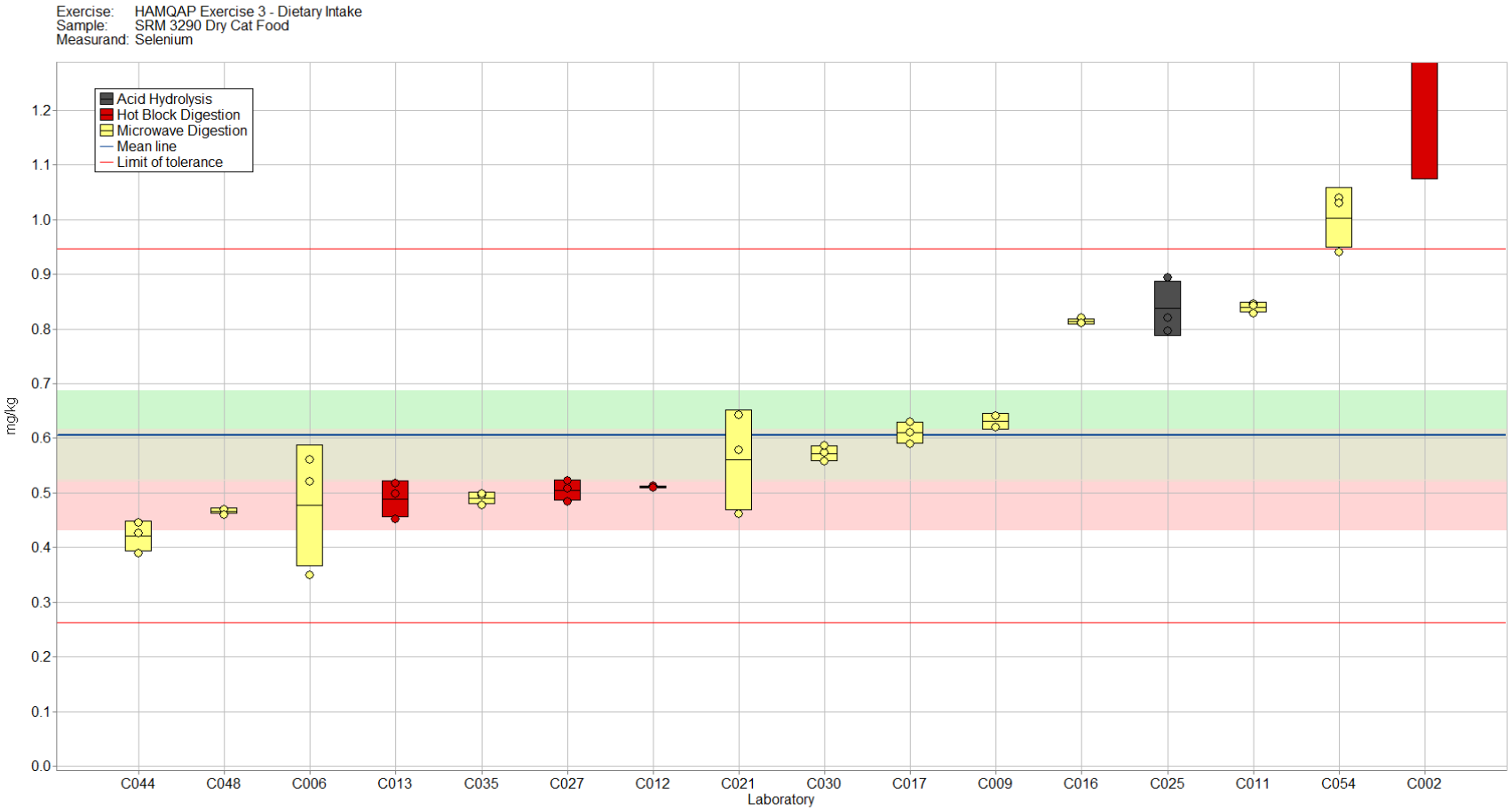

Figure 1-7. Selenium in SRM 3290 Dry Cat Food (data summary view – sample preparation method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The 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'_{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_{NIST}) and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$.

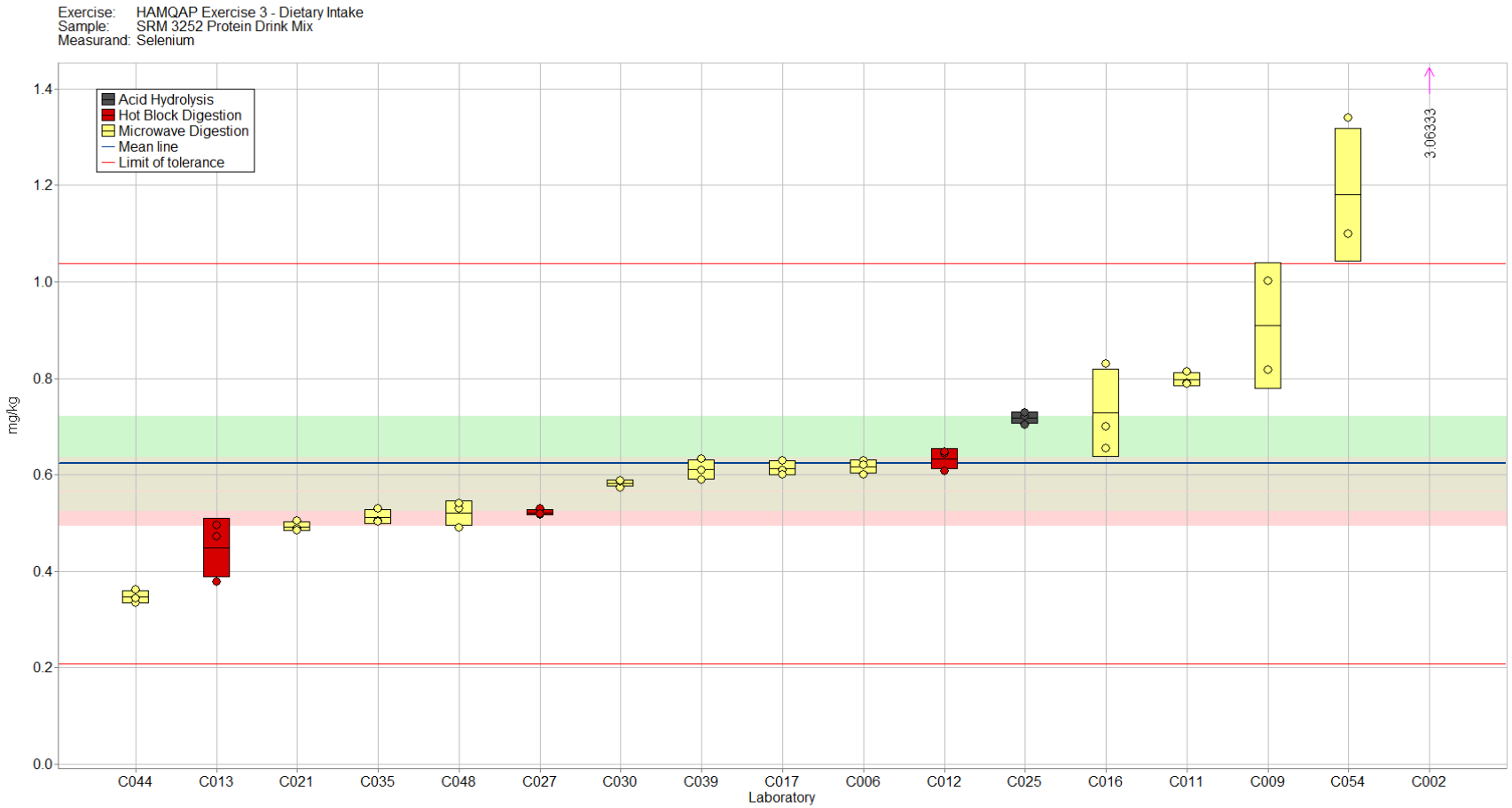

Figure 1-8. Selenium in SRM 3252 Protein Drink Mix (data summary view – sample preparation method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. 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'_{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_{NIST}) and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$.

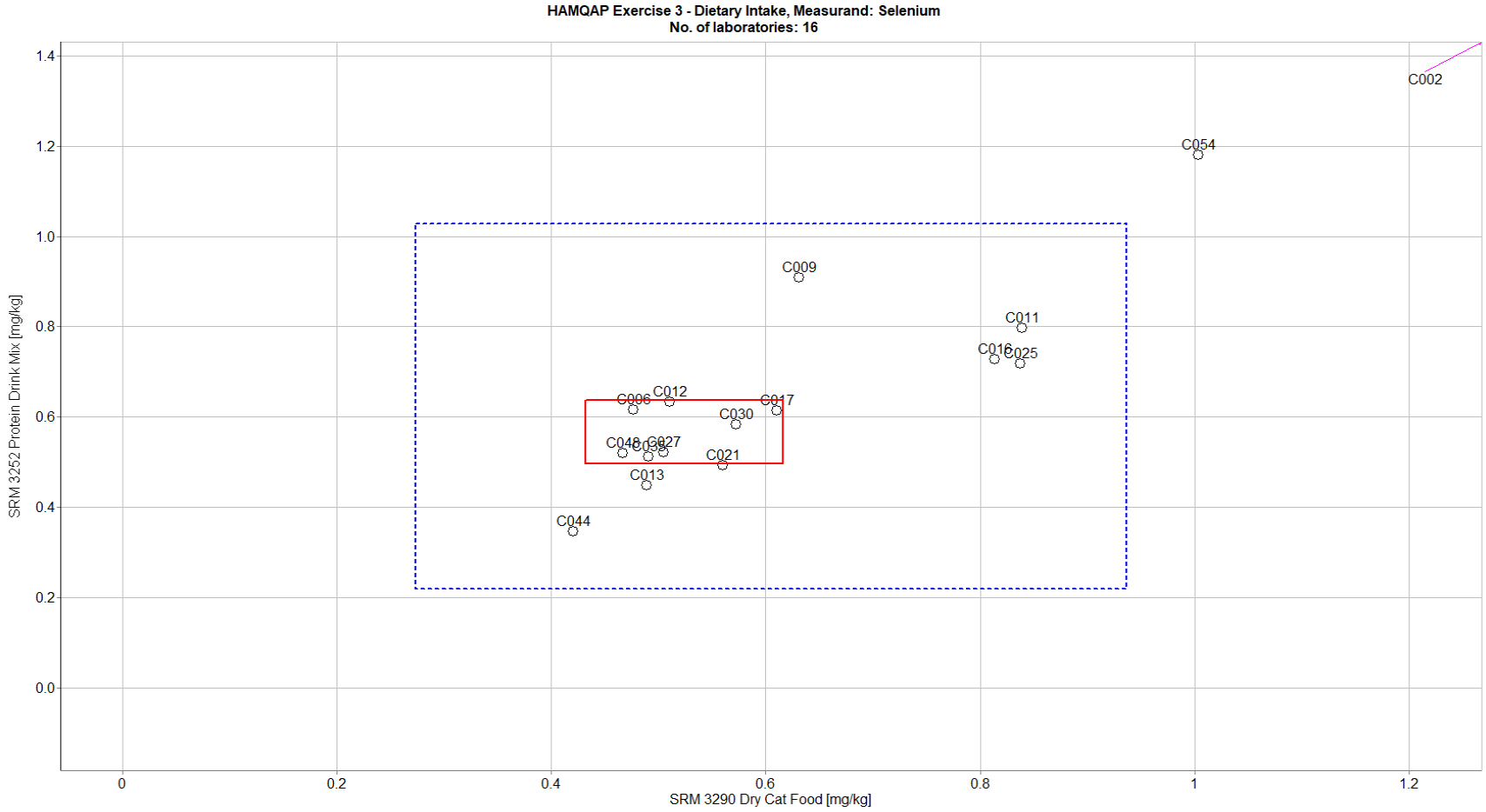

Figure 1-9. Laboratory means for selenium in SRM 3290 Dry Cat Food and SRM 3252 Protein Drink Mix (sample/sample comparison view). In this view, the individual laboratory mean for one sample (SRM 3290) is compared to the individual laboratory mean for a second sample (SRM 3252). The solid red box represents the NIST range of tolerance for the two samples, SRM 3290 (x-axis) and SRM 3252 (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, $|Z_{\mathrm{NIST}}| \leq 2$. The dotted blue box represents the consensus range of tolerance for SRM 3290 (x-axis) and SRM 3252 (y-axis), calculated as the values above and below the consensus means that result in an acceptable Z'_{comm} score, $|Z'_{\mathrm{comm}}| \leq 2$.

Human Metabolites Sample Information

Human Milk. Participants were provided with three vials, each containing 5 mL of frozen human milk. Before use, participants were instructed to allow the material to thaw at room temperature for at least 30 min prior to sampling, use the material immediately after thawing, gently mix the contents prior to removal of a test portion for analysis, and use a sample size appropriate for their usual in-house method of analysis. Participants were asked to avoid exposing the material to direct sun or UV light, to store the material at temperatures between –20 °C and –80 °C, and to prepare one sample and report one value from each vial provided. The approximate analyte levels were not reported to participants prior to the study. The NIST-determined value for iodine was assigned using results from NIST by ICP-MS. Values for selenium and the thyroid hormones in these materials were not determined by NIST prior to the study. The NIST-determined value and uncertainty for iodine in human milk is provided in the table below.

Analyte	NIST-Determined Mass Fraction in SRM 1953 (µg/kg)
Iodine (I)	193 ± 2

Human Serum A and B. Participants were provided with three vials of Human Serum A and three vials of Human Serum B, each containing 2 mL of frozen human serum. Serum A is from a pool of healthy adult males and Serum B is from a pool of healthy, premenopausal adult females. Both serums are unfortified. Before use, participants were instructed to allow the material to thaw at room temperature for at least 30 min prior to sampling, use the material immediately after thawing, gently mix the contents prior to removal of a test portion for analysis, and use a sample size appropriate for their usual in-house method of analysis. Participants were asked to avoid exposing the material to direct 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. The approximate analyte levels were not reported to participants prior to the study. Values for iodine, selenium, and the thyroid hormones in these materials were not determined by NIST prior to the study.

Human Metabolites Study Results

The enrollment and reporting statistics for the human metabolites nutritional elements study are described in the table below. Some of the reported values were non-quantitative (zero or below LOQ) but are included in the participation statistics. No laboratories reported values for T3 or T4.

		Number of Laboratories Reporting Results (Percent Participation)		
Analyte	Number of Laboratories Requesting Samples	Human Milk	Serum A	Serum B
Iodine (I)	7	0 (0 %)	1 (14 %)	1 (14 %)
Selenium (Se)	10	2 (20 %)	3 (30 %)	3 (30 %)
TSH	4	0 (0 %)	1 (25 %)	1 (25 %)

- The between-laboratory variabilities for selenium were 17 % for human milk and over 100 % for both human serum samples. Only one laboratory reported quantitative results for iodine, so between-laboratory variability could not be determined.
- All laboratories reported using ICP-MS as their analytical method for measuring selenium and the analytical method used for iodine and TSH was not reported.
- Participating laboratories reported using hot block or microwave digestion for selenium preparation. No sample preparation method was reported for iodine or TSH preparation.

Human Metabolites Technical Recommendations

In this study, too few data were reported to allow for meaningful conclusions to be drawn.

- Selenium contamination from the environment does not normally impact analytical testing.
- The digestion procedure is critical to the accuracy of selenium determination with conditions requiring the breakdown of the organoselenium compounds.
  - Recommended digestion procedures include mixtures of nitric, perchloric, and sulfuric acids with temperatures of up to 300 °C for open beaker techniques.
  - For microwave digestion, nitric acid should be sufficient with high temperatures and high pressures.
- CRMs are available and may be used for assay validation.

Table 1-4. Individualized data summary table (NIST) for total iodine, total selenium, and thyroid hormones in human milk and serum.

National Institute of Standards and Technology

HAMQAP Exercise 3 - 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*
Total Iodine	Human Serum A		ng/g					1				
Total Iodine	Human Serum B		ng/g					1				
Total Iodine	SRM 1953 Organic Contaminants in Non-Fortified Human Milk		ng/g	190	2		0	0			193	2
Total Selenium	Human Serum A		ng/g					3	70	82		
Total Selenium	Human Serum B		ng/g					3	70	80		
Total Selenium	SRM 1953 Organic Contaminants in Non-Fortified Human Milk		ng/g					2	16.2	2.7		
Triiodothyronine (T3)	Human Serum A		ng/g					0				
Triiodothyronine (T3)	Human Serum B		ng/g					0				
Triiodothyronine (T3)	SRM 1953 Organic Contaminants in Non-Fortified Human Milk		ng/g					0				
Thyroxine (T4)	Human Serum A		ng/g					0				
Thyroxine (T4)	Human Serum B		ng/g					0				
Thyroxine (T4)	SRM 1953 Organic Contaminants in Non-Fortified Human Milk		ng/g					0				
Thyroid-stimulating Hormone (TSH)	Human Serum A		ng/g					1				
Thyroid-stimulating Hormone (TSH)	Human Serum B		ng/g					1				
Thyroid-stimulating Hormone (TSH)	SRM 1953 Organic Contaminants in Non-Fortified Human Milk		ng/g					0				

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 1-5. Data summary table for total iodine in human milk and serum.

		Total Iodine														
		SRM 1953 Organic Contaminants in Non-Fortified Human Milk (ng/g)					Human Serum A (ng/g)					Human Serum B (ng/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target				193	2										
	C005															
	C006															
	C027															
	C031															
	C032															
	C033															
	C060						54	52.8	54	53.6	0.7	55.2	54.7	54.6	54.8	0.3
Community Results		Consensus Mean					Consensus Mean					Consensus Mean				
		Consensus Standard Deviation					Consensus Standard Deviation					Consensus Standard Deviation				
		Maximum					Maximum			53.6		Maximum			54.8	
		Minimum					Minimum			53.6		Minimum			54.8	
		N			0		N			1		N			1	

Table 1-6. Data summary table for total selenium in human milk and serum.

		Total Selenium													
		SRM 1953 Organic Contaminants in Non-Fortified Human Milk (ng/g)					Human Serum A (ng/g)					Human Serum B (ng/g)			
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target															
	C005															
	C006	17	17	18	17.3	0.6	12	13	13	12.7	0.6	12	12	12	12.0	0.0
	C012	15.5	13.6	16.2	15.1	1.3	84.5	89	86.2	86.6	2.3	76.3	79.4	76.9	77.5	1.6
	C027															
	C031															
	C032															
	C033															
	C054															
	C060						118	122	123	121.0	2.6	114	109	112	111.7	2.5
	C062															
Community Results		Consensus Mean			16.2		Consensus Mean			73.4		Consensus Mean			67.1	
		Consensus Standard Deviation			2.7		Consensus Standard Deviation			82.1		Consensus Standard Deviation			80.0	
		Maximum			17.3		Maximum			121.0		Maximum			111.7	
		Minimum			15.1		Minimum			12.7		Minimum			12.0	
		N			2		N			3		N			3	

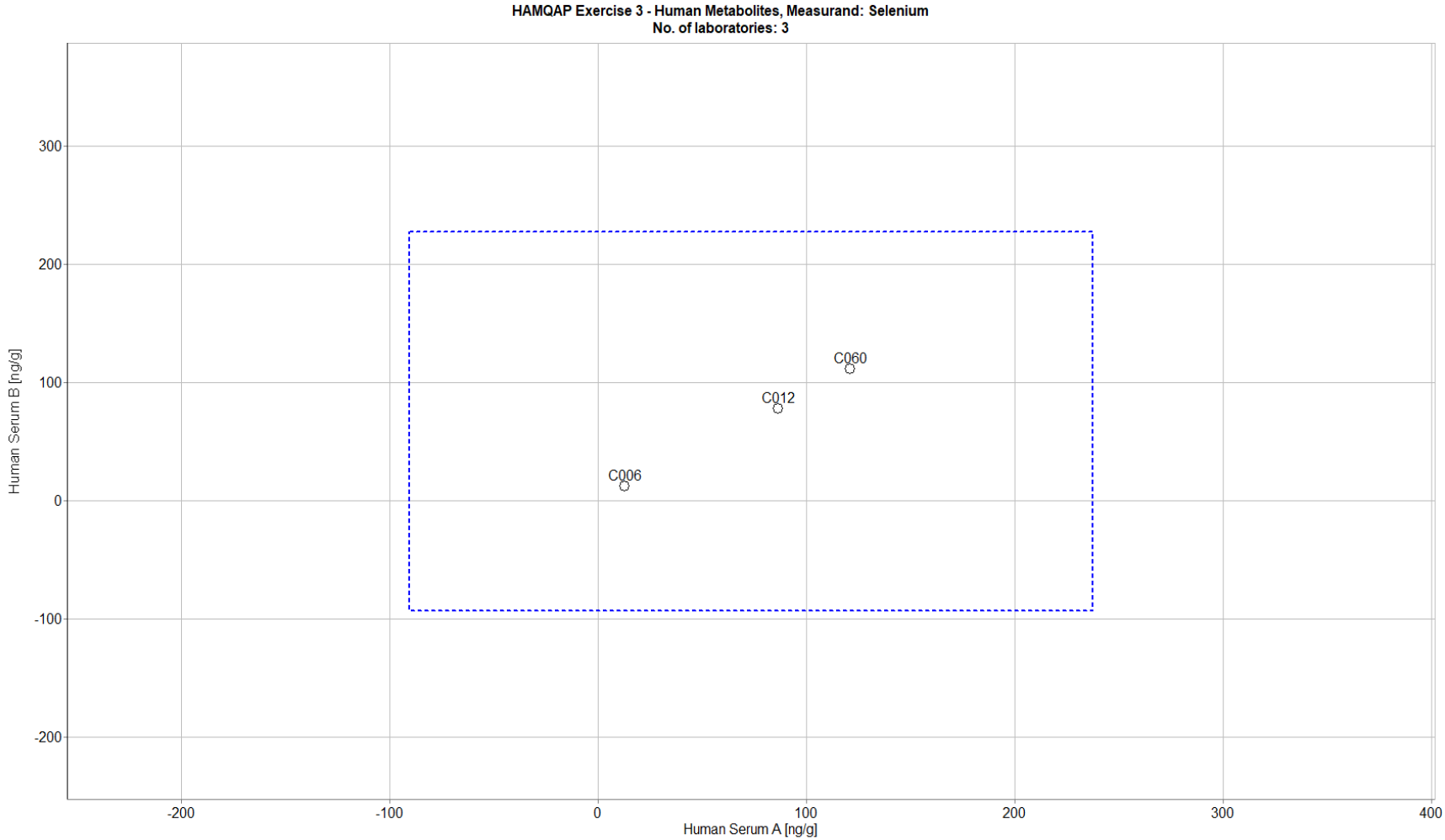

Figure 1-10. Laboratory means for selenium in Human Serum A and Human Serum B (sample/sample comparison view). In this view, the individual laboratory mean for one sample (Human Serum A) is compared to the mean for a second sample (Human Serum B). The dotted blue box represents the consensus range of tolerance for the two samples, Human Serum A (x-axis) and Human Serum B (y-axis), calculated as the values above and below the consensus means that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$.

Table 1-7. Data summary table for thyroid-stimulating hormone (TSH) in human milk and serum.

		Thyroid-stimulating Hormone (TSH)													
		SRM 1953 Organic Contaminants in Non-Fortified Human Milk (ng/g)					Human Serum A (ng/g)					Human Serum B (ng/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target															
	C005						0.0178	0.0177	0.0179	0.0178	0.0001	0.0135	0.0135	0.137	0.055	0.071
	C027															
	C032															
	C062															
Community Results		Consensus Mean					Consensus Mean					Consensus Mean				
		Consensus Standard Deviation					Consensus Standard Deviation					Consensus Standard Deviation				
		Maximum					Maximum			0.0178		Maximum			0.055	
		Minimum					Minimum			0.0178		Minimum			0.055	
		N			0		N			1		N			1	

Nutritional Elements Overall Study Comparison

The following information illustrates the importance for both the food community and the clinical community to improve their methodologies for iodine and selenium measurements.

- Selenium and iodine are nonmetal elements that are a class of more challenging analytes to measure, as demonstrated by the results of this study.
  - Iodine appears in various chemical forms during sample digestion and sample introduction, which can challenge the accurate measurement of the element.
  - Organic selenium is more likely to be found in food sources (cat food) where inorganic selenium is more likely to be found in dietary supplements (protein drink mix), leading to different measurement challenges depending on the matrix.
  - The challenges associated with the measurement of selenium and iodine can be identified and remedied through participation in future HAMQAP studies.
- Iodine is a necessary mineral for human health and accurate methods of analysis for both foods and biomarkers of status must be established.
- Because selenium is a necessary dietary mineral for human health, but can be toxic if overconsumed, biomarkers must be accurately measured.
- Human milk is often the sole source of nutrition for infants and contains iodine and selenium, but levels vary depending on the mother's diet. As a result, accurate methods for determination of these nutrients in human milk are critical to understand intake in this important population.

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

Study Overview

In this study, participants were provided with samples of black cohosh rhizome and hemp protein powder for dietary intake and Human Blood A and SRM 1401 Trace Metals in Frozen Human Blood (Level 1) for human metabolism. Participants in the dietary intake study were 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 dietary supplement matrix. Participants in the human metabolites study were asked to use in-house analytical methods to determine the mass fractions (µg/L) of arsenic (As), cadmium (Cd), lead (Pb), and mercury (Hg) in each human blood sample. Potential uptake of toxic elements from the soil may lead to contamination of plant-based foods and supplements and negative health outcomes for consumers.[6,7,8,9] In the United States, cGMPs require dietary supplement manufacturers to establish limits on contaminants, therefore laboratories must establish scientifically valid methods for the determination of toxic elements to demonstrate the products meet the specifications in the U.S. FDA Code of Federal Regulations (21 CFR 111.70(b)(3)). Testing of these foods and supplements can ensure safety for consumers and testing of human blood can identify such consumer exposure to toxic elements.

Dietary Intake Sample Information

Black Cohosh Rhizome. Participants were provided with three packets, each containing approximately 3 g of powdered black cohosh rhizome. Before use, participants were instructed to thoroughly mix the contents of each packet and to use a sample size of at least 0.25 g. Participants were asked to store the material at controlled room temperature, 20 °C to 25 °C, and to prepare a single sample and to report a single value from each packet provided. Approximate analyte levels were not reported to participants prior to the study. The target values were assigned for As, Cd, and Pb using results from NIST by ICP-MS, and for Hg using results from NIST by CV ID ICP-MS. The NIST-determined values and uncertainties are provided in the table below on an as-received basis.

Analyte	NIST-Determined Mass Fractions in Black Cohosh Rhizome (mg/kg)
Arsenic (As)	0.30 ± 0.02
Cadmium (Cd)	0.2433 ± 0.0084
Lead (Pb)	2.236 ± 0.046
Mercury (Hg)	0.01312 ± 0.00031

Hemp Protein Powder. Participants were provided with three packets, each containing approximately 3 g of powdered hemp protein. Before use, participants were instructed to

[6] Arsenic Factsheet. National Biomonitoring Program, Centers for Disease Control and Prevention. https://www.cdc.gov/biomonitoring/Arsenic_FactSheet.html (accessed November 2019).
[7] Cadmium Factsheet. National Biomonitoring Program, Centers for Disease Control and Prevention. https://www.cdc.gov/biomonitoring/Cadmium_FactSheet.html (accessed November 2019).
[8] Mercury Factsheet. National Biomonitoring Program, Centers for Disease Control and Prevention. https://www.cdc.gov/biomonitoring/Mercury_FactSheet.html (accessed November 2019).
[9] Lead Factsheet. National Biomonitoring Program, Centers for Disease Control and Prevention. https://www.cdc.gov/biomonitoring/Lead_factsheet.html (accessed November 2019).

thoroughly mix the contents of each packet and to use a sample size of at least 0.5 g for As, Cd, and Pb analysis. A sample size of at least 0.1 mg was recommended for Hg analysis. Participants were asked to store the material at controlled room temperature, 20 °C to 25 °C, and to prepare a single sample and to report a single value from each packet provided. Approximate analyte levels were not reported to participants prior to the study. Target values for As, Cd, and Pb in the hemp protein powder were assigned using results from NIST by ICP-MS, and a target value for Hg in using results from NIST by direct combustion AAS. The NIST-determined values and uncertainty for As, Cd, Pb, and Hg in hemp protein powder are provided in the table below on an as-received basis.

Analyte	NIST-Determined Mass Fractions in Hemp Protein Powder (mg/kg)
Arsenic (As)	0.0087 ± 0.0011
Cadmium (Cd)	0.02591 ± 0.00070
Lead (Pb)	0.0050 ± 0.0018
Mercury (Hg)	0.00460 ± 0.00030

Dietary Intake Study Results

- The enrollment and reporting statistics for the toxic elements 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)	
		Black Cohosh Rhizome	Hemp Protein Powder
As	42	30 (71 %)	30 (71 %)
Cd	42	30 (71 %)	30 (71 %)
Pb	42	30 (71 %)	29 (69 %)
Hg	40	30 (75 %)	29 (72 %)

- The consensus range was within the target range or overlapped the target range for all analytes except Pb in black cohosh, where the consensus range was below the target range.
- The between-laboratory variabilities for each sample-analyte pair are summarized below, showing that performance was best for black cohosh.

Analyte	Between-Laboratory Variability (% RSD)	
	Black Cohosh Rhizome	Hemp Protein Powder
As	21 %	69 %
Cd	13 %	16 %
Pb	11 %	88 %
Hg	31 %	76 %

- The sample preparation methods reported by participating laboratories are summarized in the table below. Most laboratories reported using either microwave digestion or hot block digestion for all four analytes.

	Percent Reporting	
Reported Method	As, Cd, Pb	Hg
Microwave Digestion	77 %	68 %
Hot Block Digestion	17 %	18 %
Acid Hydrolysis	3 %	4 %
Open Beaker Digestion	3 %	4 %
Thermal Decomposition	0 %	8 %

- A majority of laboratories reported using ICP-MS for determination of toxic elements.

Technical Recommendations

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

- For all analytes, no pattern was observed between reported result and analytical method.
- Sample preparation methods should be well established before analyzing unknown samples. Established quality control materials (SRMs, CRMs, RMs, and in-house materials) and accepted methods of analysis can assist in this process.
- Between-laboratory variability was much lower for the black cohosh samples than the hemp powder samples for all analytes except Cd, where the between-laboratory variability was approximately the same for both materials. The low concentrations of these elements in the hemp powder likely resulted in higher between-laboratory variability for these determinations.
- Because of the very low concentrations of all analytes in both materials, detection of the analyte in the sample may be improved by limiting the number of dilutions performed, however matrix effects may become more significant. A matrix-matched calibration curve may reduce some of the matrix interferences. A better alternative may be to perform standard additions; however, this option is more time consuming.
- The determination of the LOQ and the MDL is important when concentrations are low. Analysis of an appropriate number of procedural blanks can be critical in the determination of LOQ and MDL 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.
- For arsenic, less than half of the laboratories reporting data were within the 95 % confidence interval of the consensus mean for black cohosh and less than half of the laboratories reported data within the NIST target range, as shown in **Figures 2-1 and 2-3**. Of the laboratories

reporting quantitative values for arsenic in hemp powder, most were within both the 95 % confidence interval for the consensus mean and the NIST target range **(Figures 2-2 and 2-4)**.

- Results produced by microwave digestion with ICP-MS were most consistent with the target ranges (**Figures 2-1 through 2-4**).
- **Figure 2-5** shows that some laboratories achieved results within the target range for both samples while some laboratories reported results that were closer to the target for one sample than for the other. The differences in the two matrices or the concentration levels of As may have resulted in these difficulties.
  - Calibration curves must be linear and include the lowest and highest values expected to be measured in the sample solutions.
  - Difficulty in the digestion of samples can cause bias and/or increased variability between samples.
    - Plant materials may be easier to digest if a small amount of HF is used along with HNO_3 due to the higher content of silica.
    - The high temperatures of a microwave digestion system should ensure complete digestion of the materials prior to analysis.
    - 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 which point subsequent heating will not result in loss of arsenic.
      - Open-beaker digestion may not be the best choice for As sample preparation and may lead to low results.
      - Following closed digestion, vessels should be opened with care to ensure that no As is lost as a result of inadvertent venting.
    - Incomplete sample digestion may produce interferences that cause signal enhancement or suppression, 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 As in these two materials.
  - Some laboratories reported using ID ICP-MS as the analytical method used. ID ICP-MS is not a practicable method for As because As is monoisotopic. Measurement methods should be reported correctly and completely.
- For cadmium, approximately half of the laboratories reporting data were within both the 95 % confidence interval of the consensus mean and the NIST target range for both materials (**Figures 2-6 through 2-9**).
  - **Figure 2-10** shows that many laboratories were able to measure both samples well. Those laboratories that reported low results may have had errors with calibration. Calibration curves must be linear and include the lowest and highest values expected to be measured in the sample solutions.
  - The boiling point of Cd is high and volatile loss of Cd should not be a concern.
  - Spectral 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.

- For lead in black cohosh, **Figures 2-11 and 2-13** show that less than half of the laboratories reported data within the 95 % confidence interval of the consensus mean and only two of the laboratories reported data within the NIST target range. For hemp powder, **Figures 2-12 and 2-14** show that less than half of the laboratories reported data within the 95 % confidence interval of the consensus mean and approximately half of the laboratories reported quantitative data within the NIST target range.
  - Lead is easily digested and volatile loss of Pb 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 black cohosh is nearly 100 times greater than that in the hemp powder, $PbCl_2$ precipitation may have resulted in fewer laboratories reporting accurate results in the black cohosh material compared to the hemp powder.
  - Calibration curves must be linear and include the lowest and highest values expected to be measured in the sample solutions. Solutions falling outside of the linear curve may give erroneous answers, either values that are too high or too low.
  - No linear trend was observed in **Figure 2-15** between the reported results for Pb in the two materials. However, many of the reported values for black cohosh were lower than expected, indicating that the material itself may have caused a greater difficulty in either the sample preparation or the analytical methodology.
  - Some laboratories reported high sample-to-sample variability (47 % to 93 %) in the hemp powder, which may be caused by the low sample concentrations, difficulties in sample preparation, incomplete sample digestion, or calibration curves which do not encompass all sample solutions measured.
  - Analysis of an appropriate number of procedural blanks is always important and can be critical when sample concentrations are near the LOQs or MDLs or, as in this case, 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. The suggested minimum number of blanks to prepare is usually equal to the number of samples being prepared.
- For Hg, **Figures 2-16 through 2-19** show that approximately half of the laboratories reported quantitative data within the 95 % confidence interval of the consensus mean for both materials. Several laboratories reported data with very large within-laboratory variability for both materials.
  - No linear trend was observed between the results for Hg in these materials **(Figure 2-20)**.
- Hg is volatile, so care must be taken to not lose Hg during sample preparation. Microwave digestion is the best method for sample preparation for Hg analysis.
  - The low levels of Hg in the hemp powder may be close to the MDL for some techniques.
    - A sufficient number of procedural blanks must be used to determine an accurate MDL and LOQ. Blanks and backgrounds for Hg measurements may be large, leading to high detection limits and making determination of low-level samples difficult.
    - Low concentrations of Hg 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.
  - Low level measurements are often complicated by contamination from sources such as poorly cleaned labware or other laboratory materials.
  - Hg carryover between samples is common on many instruments, which can lead to erratic results if an adequate washout time is not used after each measurement. Use of dilute HCl in the rinse solution may decrease the length of necessary washout time.
- 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. Calibration curves must be linear and include the lowest and highest values expected to be measured in the sample solutions.

Table 2-1. Individualized data summary table (NIST) for toxic elements in black cohosh rhizome and hemp protein powder.

National Institute of Standards and Technology

HAMQAP Exercise 3 - 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*
Total Arsenic	Black Cohosh Rhizome	mg/kg		0.3	0.02		0	29	0.257	0.055	0.3	0.02
Total Arsenic	Hemp Protein Powder	mg/kg		0.0087	0.0011		0	19	0.0097	0.0067	0.00866	0.0011
Cadmium	Black Cohosh Rhizome	mg/kg		0.2433	0.0084		0	30	0.23	0.03	0.243	0.0084
Cadmium	Hemp Protein Powder	mg/kg		0.026	0.0007		0	27	0.0262	0.0041	0.0259	0.0007
Lead	Black Cohosh Rhizome	mg/kg		2.236	0.046		0	30	1.92	0.22	2.24	0.046
Lead	Hemp Protein Powder	mg/kg		0.005	0.0018		0	17	0.012	0.011	0.005	0.0018
Mercury	Black Cohosh Rhizome	mg/kg		0.01312	0.00031		0	24	0.0144	0.0044	0.0131	0.00031
Mercury	Hemp Protein Powder	mg/kg		0.005	0.0003		0	17	0.0055	0.0042	0.0046	0.0003

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 total arsenic in black cohosh rhizome and hemp protein powder. Data points highlighted in red have been flagged as potential outliers (e.g., Grubb and/or Cochran) by the NIST software package.

		Total Arsenic									
		Black Cohosh Rhizome (mg/kg)					Hemp Protein Powder (mg/kg)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target				0.30	0.02				0.0087	0.0011
	C001	0.185	0.221	0.365	0.257	0.095	0	0	0		
	C002	0.3	0.3	0.31	0.303	0.006	0.01	0.01	0.02	0.0133	0.0058
	C004	0.22	0.229	0.229	0.226	0.005	0.0097	0.0106	0.01	0.0101	0.0005
	C005										
	C006	0.29	0.29	0.27	0.283	0.012	0.01	0.01	0.012	0.0107	0.0012
	C008										
	C009	0.2227	0.2218		0.222	0.001	< 0.1000	< 0.1000			
	C011	0.281	0.307	0.293	0.294	0.013	0.012	0.013	0.012	0.0123	0.0006
	C012	0.2973	0.2773	0.2663	0.280	0.016	0.0196	0.0207	0.0249	0.0217	0.0028
	C013	0.219	0.218	0.21	0.216	0.005	< 0.0444	< 0.0444	< 0.0444		
	C014	0.23	0.22	0.206	0.219	0.012	0	0	0		
	C015	0.289	0.292	0.279	0.287	0.007	0.0118	0.0094	0.0114	0.0109	0.0013
	C016	0.16899	0.16024	0.16802	0.166	0.005	< 0.0100	< 0.0100	< 0.0100		
	C017	0.29	0.31	0.29	0.297	0.012	< 0.0500	< 0.0500	< 0.0500		
	C018	0	0	0			0	0	0		
	C019										
	C020										
	C021	0.249	0.267	0.262	0.259	0.009	0.0135	0.0121	0.0123	0.0126	0.0008
	C024										
	C025	0.233	0.23	0.237	0.233	0.004	< 0.0300	< 0.0300	< 0.0300		
	C027	0.20123	0.20195	0.20187	0.202	0.000	0.01284	0.01304	0.01297	0.0130	0.0001
	C028	0.217	0.211	0.222	0.217	0.006	0.063	0.063	0.06	0.0620	0.0017
	C030	0.2547	0.2598	0.2743	0.263	0.010	0.0084	0.0087	0.0085	0.0085	0.0002
	C031										
	C032										
	C034	0.2682	0.259	0.2768	0.268	0.009	0.0189	0.018	0.0146	0.0172	0.0023
	C035	0.285	0.273	0.287	0.282	0.008	< 0.0100	< 0.0100	< 0.0100		
	C037	0.2	0.183	0.193	0.192	0.009	0.00803	0.00812	0.011	0.0091	0.0017
	C038	0.267	0.263	0.247	0.259	0.011	< 0.0500	< 0.0500	< 0.0500		
	C039	0.255	0.244	0.243	0.247	0.007	0	0	0		
	C043										
	C044	0.21	0.22	0.22	0.217	0.006	0.01	0.01	0.01	0.0100	0.0000
	C045										
	C046	0.188	0.191	0.193	0.191	0.003	0.01	0.01	0.011	0.0103	0.0006
	C047	0.279	0.283	0.298	0.287	0.010	< 0.0160	< 0.0160	< 0.0160		
	C048	0.26	0.31	0.25	0.273	0.032	0.018	0.016	0.016	0.0167	0.0012
	C049										
	C050										
	C052	0.313	0.303	0.327	0.314	0.012	0.011	0.01	0.011	0.0107	0.0006
	C053	0.39	0.38	0.35	0.373	0.021	0.05	< 0.0500	< 0.0500	0.0500	
	C054	0.4	0.29	0.39	0.360	0.061	0.1	0.1	0.08	0.0933	0.0115
	C055										
Community Results		Consensus Mean			0.257		Consensus Mean			0.0097	
		Consensus Standard Deviation			0.055		Consensus Standard Deviation			0.0067	
		Maximum			0.373		Maximum			0.0933	
		Minimum			0.166		Minimum			0.0085	
		N			29		N			17	

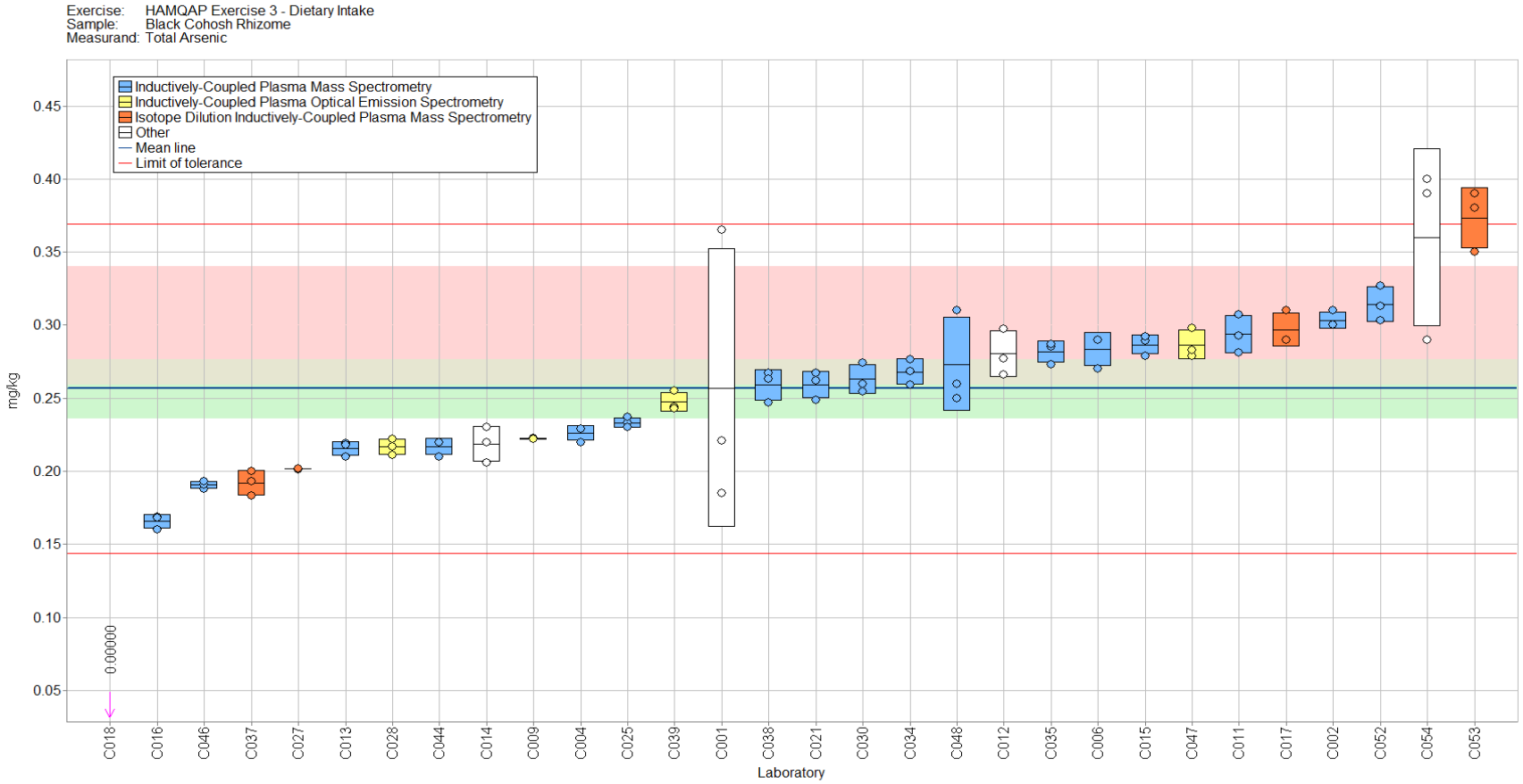

Figure 2-1. Total arsenic in Black Cohosh Rhizome (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue 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'_{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_{NIST}) and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$.

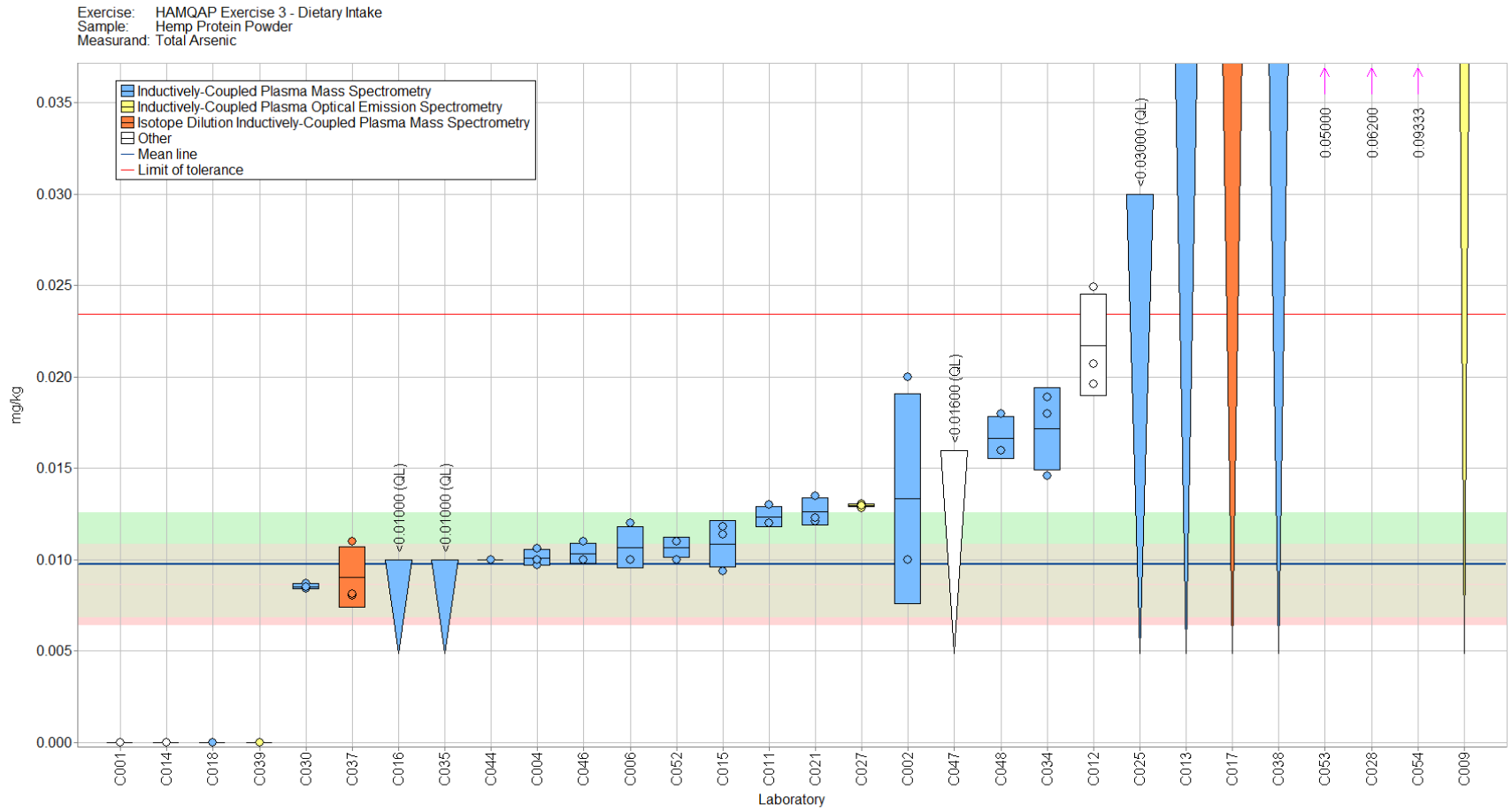

Figure 2-2. Total arsenic in Hemp Protein Powder (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue 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'_{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_{NIST}) and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$.

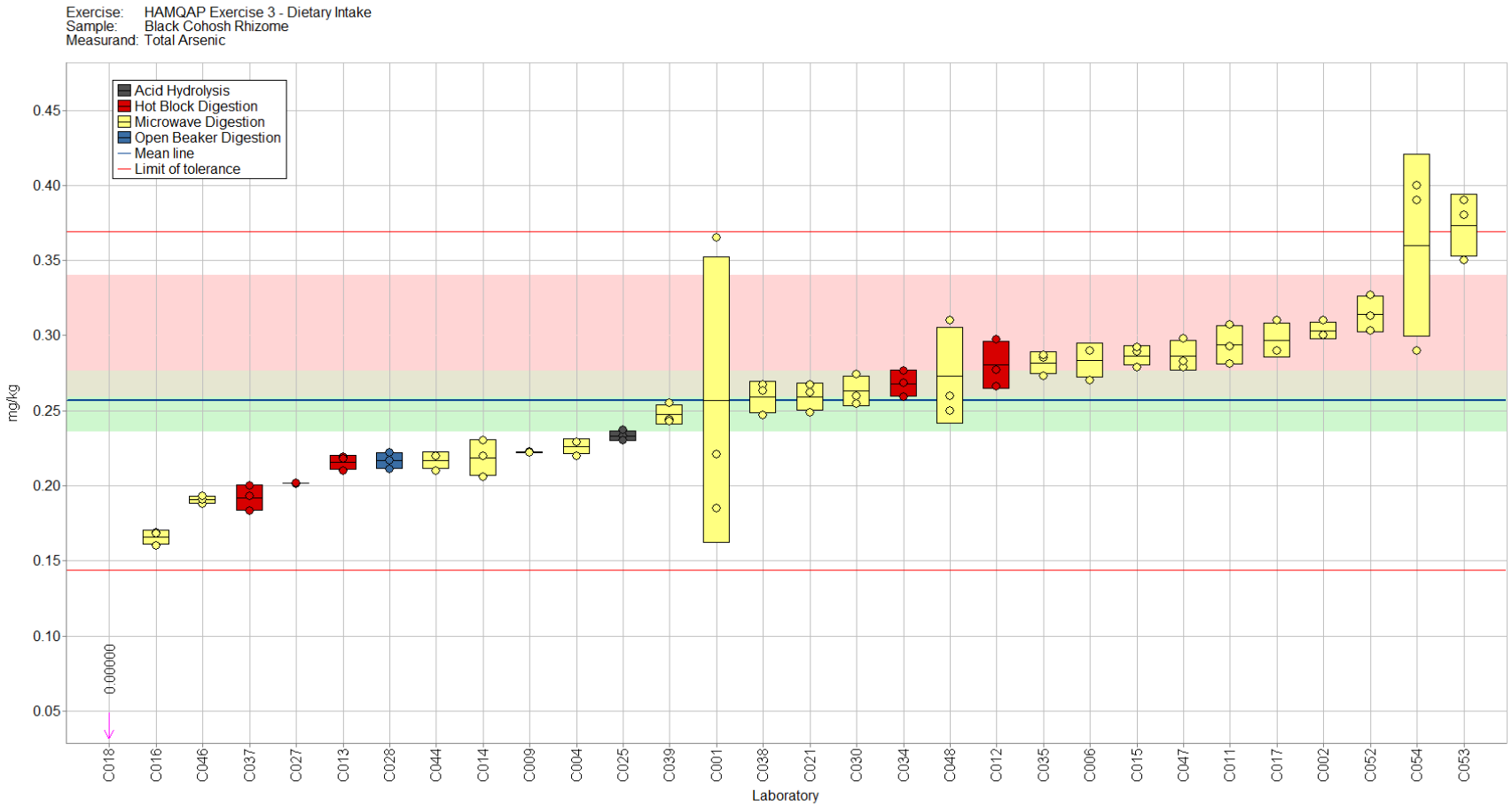

Figure 2-3. Total arsenic in Black Cohosh Rhizome (data summary view – sample preparation method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. 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'_{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_{NIST}) and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$.

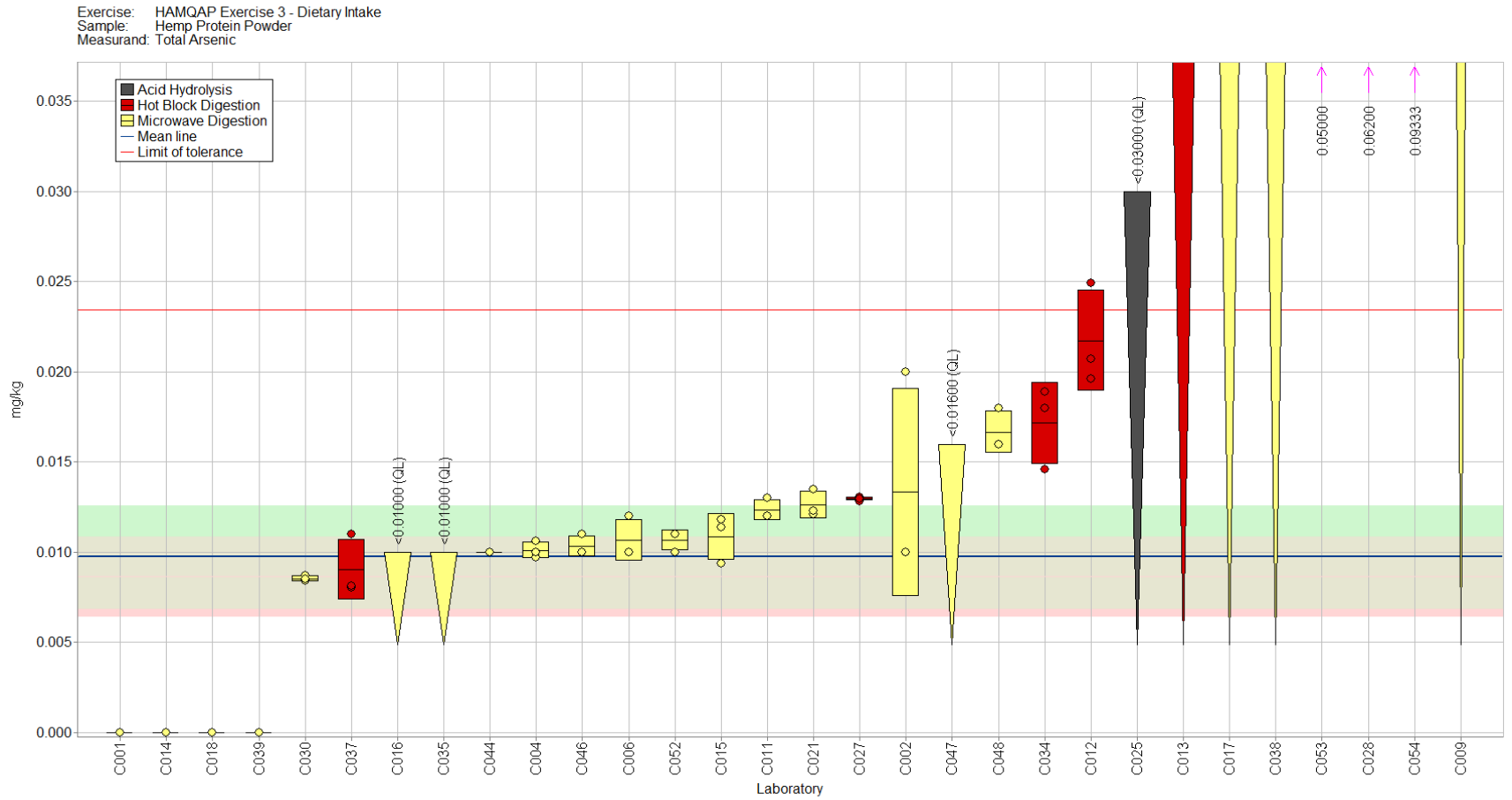

Figure 2-4. Total arsenic in Hemp Protein Powder (data summary view – sample preparation method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. 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'_{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_{NIST}) and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$.

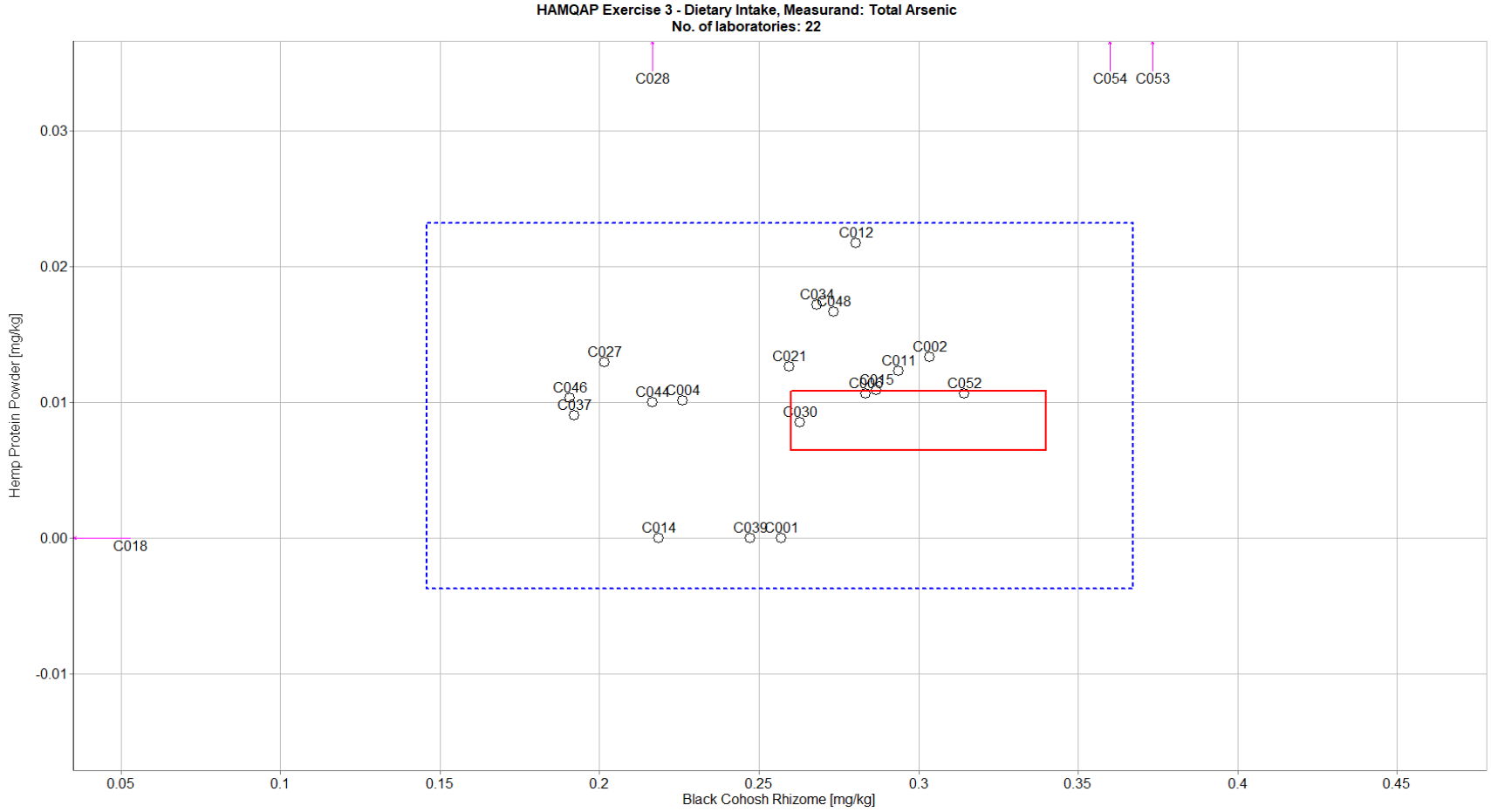

Figure 2-5. Laboratory means for total arsenic in Black Cohosh Rhizome and Hemp Protein Powder (sample/sample comparison view). In this view, the individual laboratory mean for one sample (black cohosh rhizome) is compared to the mean for a second sample (hemp protein powder). The solid red box represents the NIST range of tolerance for the two samples, black cohosh rhizome (x-axis) and hemp protein powder (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, $|Z_{\mathrm{NIST}}| \leq 2$. The dotted blue box represents the consensus range of tolerance for black cohosh rhizome (x-axis) and hemp protein powder (y-axis), calculated as the values above and below the consensus means that result in an acceptable Z'_{comm} score, $|Z'_{\mathrm{comm}}| \leq 2$.

Table 2-3. Data summary table for cadmium in black cohosh rhizome and hemp protein powder. Data points highlighted in red have been flagged as potential outliers (e.g., Grubb and/or Cochran) by the NIST software package.

		Cadmium									
		Black Cohosh Rhizome (mg/kg)					Hemp Protein Powder (mg/kg)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target				0.2433	0.0084				0.02591	0.00070
	C001	0.263	0.264	0.274	0.2670	0.0061	0.03	0.031	0.03	0.03033	0.00058
	C002	0.23	0.24	0.24	0.2367	0.0058	0.03	0.03	0.02	0.02667	0.00577
	C004	0.206	0.207	0.207	0.2067	0.0006	0.0208	0.021	0.0211	0.02097	0.00015
	C005										
	C006	0.22	0.22	0.22	0.2200	0.0000	0.022	0.025	0.026	0.02433	0.00208
	C008										
	C009	0.2381	0.2375		0.2378	0.0004	< 0.1000	< 0.1000			
	C011	0.233	0.239	0.236	0.2360	0.0030	0.026	0.025	0.025	0.02533	0.00058
	C012	0.2593	0.2633	0.2673	0.2633	0.0040	0.0227	0.0223	0.0216	0.02220	0.00056
	C013	0.226	0.221	0.226	0.2243	0.0029	0.0253	0.0257	0.028	0.02633	0.00146
	C014	0.215	0.219	0.215	0.2163	0.0023	0.032	0.024	0.026	0.02733	0.00416
	C015	0.254	0.261	0.261	0.2587	0.0040	0.0396	0.0394	0.0348	0.03793	0.00272
	C016	0.17255	0.18113	0.18406	0.1792	0.0060	0.02	0.02	0.02	0.02000	0.00000
	C017	0.26	0.24	0.24	0.2467	0.0115	< 0.0500	< 0.0500	< 0.0500		
	C018	0.214	0.222	0.22	0.2187	0.0042	0.025	0.033	0.027	0.02833	0.00416
	C019										
	C020										
	C021	0.205	0.197	0.207	0.2030	0.0053	0.0244	0.0248	0.0264	0.02520	0.00106
	C024										
	C025	0.512	0.24	0.232	0.3280	0.1594	0.028	0.025	0.026	0.02633	0.00153
	C027	0.14788	0.14158	0.14205	0.1438	0.0035	0.02971	0.02971	0.02746	0.02896	0.00130
	C028	0.226	0.224	0.218	0.2227	0.0042	0.037	0.028	0.029	0.03133	0.00493
	C030	0.248	0.2508	0.2467	0.2485	0.0021	0.0253	0.0264	0.0253	0.02567	0.00064
	C031										
	C032										
	C034	0.2362	0.2312	0.2412	0.2362	0.0050	0.278	0.0262	0.025	0.10973	0.14572
	C035	0.237	0.246	0.242	0.2417	0.0045	0.026	0.027	0.024	0.02567	0.00153
	C037	0.193	0.197	0.193	0.1943	0.0023	0.0204	0.0209	0.0215	0.02093	0.00055
	C038	0.242	0.23	0.232	0.2347	0.0064	0.026	0.026	0.027	0.02633	0.00058
	C039	0.217	0.208	0.205	0.2100	0.0062	0.028	0.027	0.027	0.02733	0.00058
	C043										
	C044	0.24	0.24	0.22	0.2333	0.0115	0.03	0.02	0.03	0.02667	0.00577
	C045										
	C046	0.19	0.195	0.192	0.1923	0.0025	0.024	0.024	0.024	0.02400	0.00000
	C047	0.256	0.252	0.259	0.2557	0.0035	0.0285	0.0277	0.0246	0.02693	0.00206
	C048	0.23	0.23	0.23	0.2300	0.0000	0.0249	0.0247	0.0247	0.02477	0.00012
	C049										
	C050										
	C052	0.303	0.294	0.335	0.3107	0.0215	0.031	0.031	0.033	0.03167	0.00115
	C053	0.28	0.26	0.25	0.2633	0.0153	< 0.0500	< 0.0500	< 0.0500		
	C054	0.37	0.23	0.3	0.3000	0.0700	0.04	0.08	0.16	0.09333	0.06110
	C055										
Community Results		Consensus Mean			0.2336		Consensus Mean			0.02624	
		Consensus Standard Deviation			0.0301		Consensus Standard Deviation			0.00412	
		Maximum			0.3280		Maximum			0.10973	
		Minimum			0.1438		Minimum			0.02000	
		N			30		N			27	

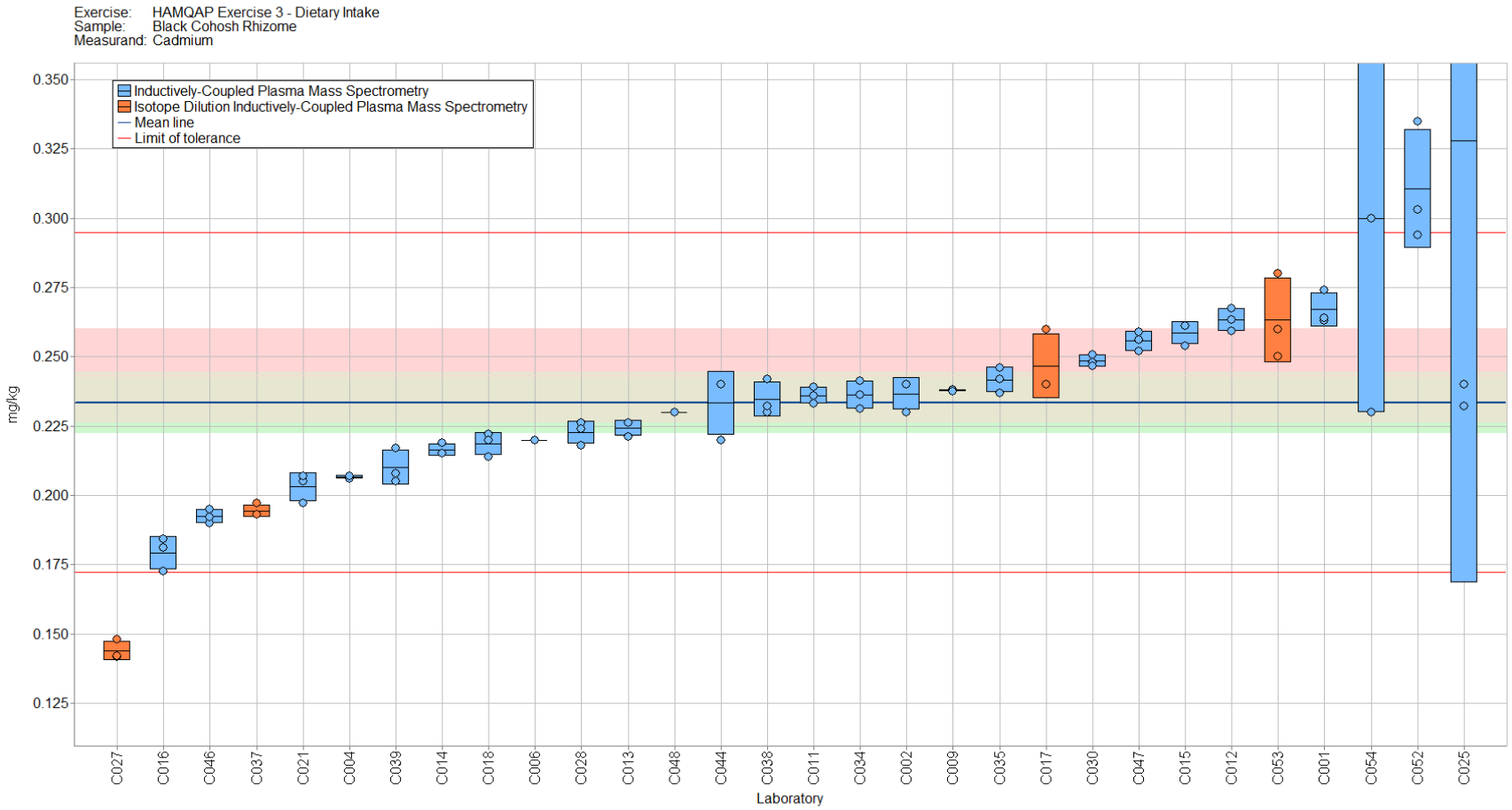

Figure 2-6. Cadmium in Black Cohosh Rhizome (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line 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'_{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_{NIST}) and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$.

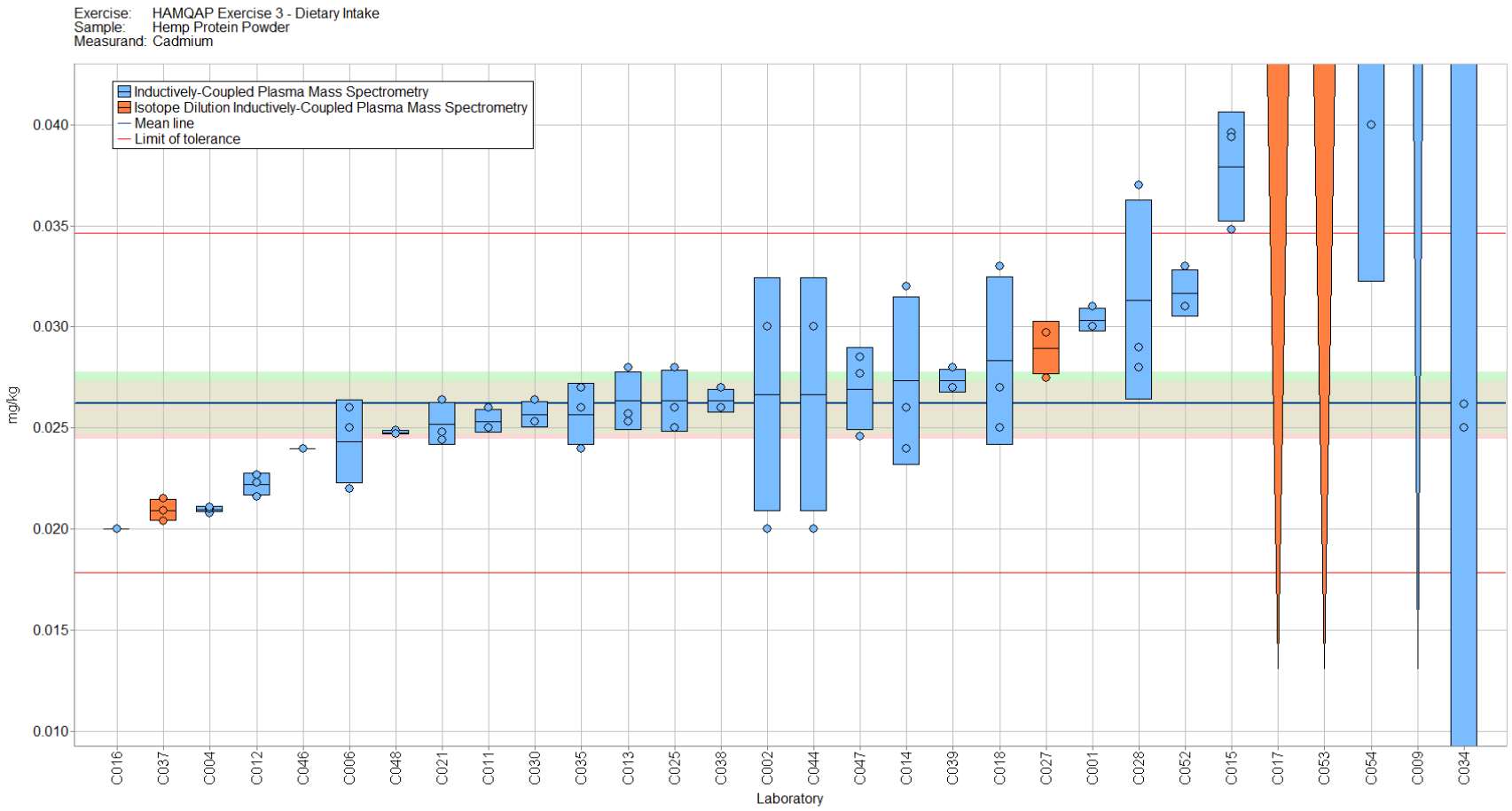

Figure 2-7. Cadmium in Hemp Protein Powder (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line 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'_{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_{NIST}) and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$.

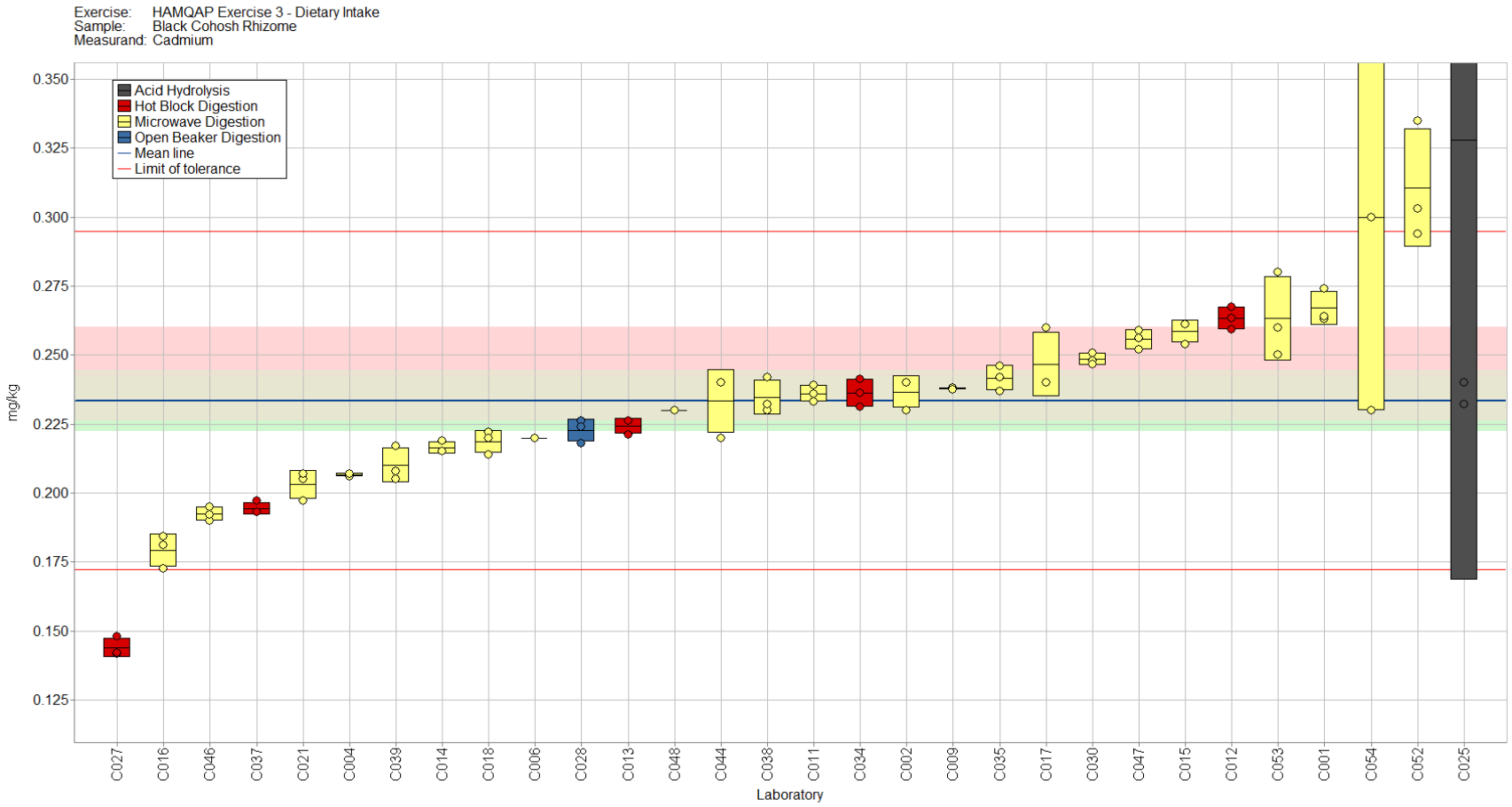

Figure 2-8. Cadmium in Black Cohosh Rhizome (data summary view – sample preparation method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The 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'_{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_{NIST}) and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$.

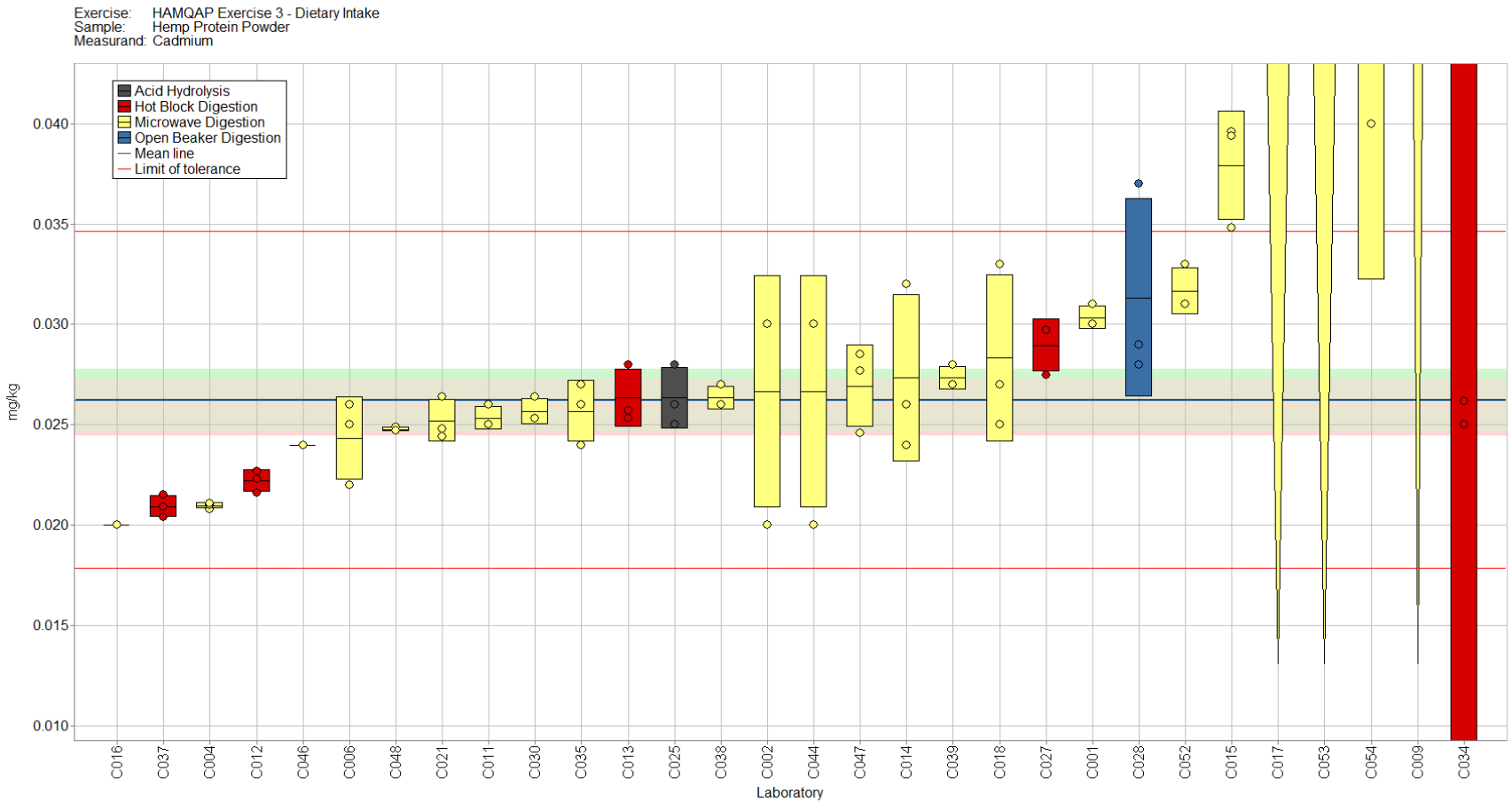

Figure 2-9. Cadmium in Hemp Protein Powder (data summary view – sample preparation method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The 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'_{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_{NIST}) and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$.

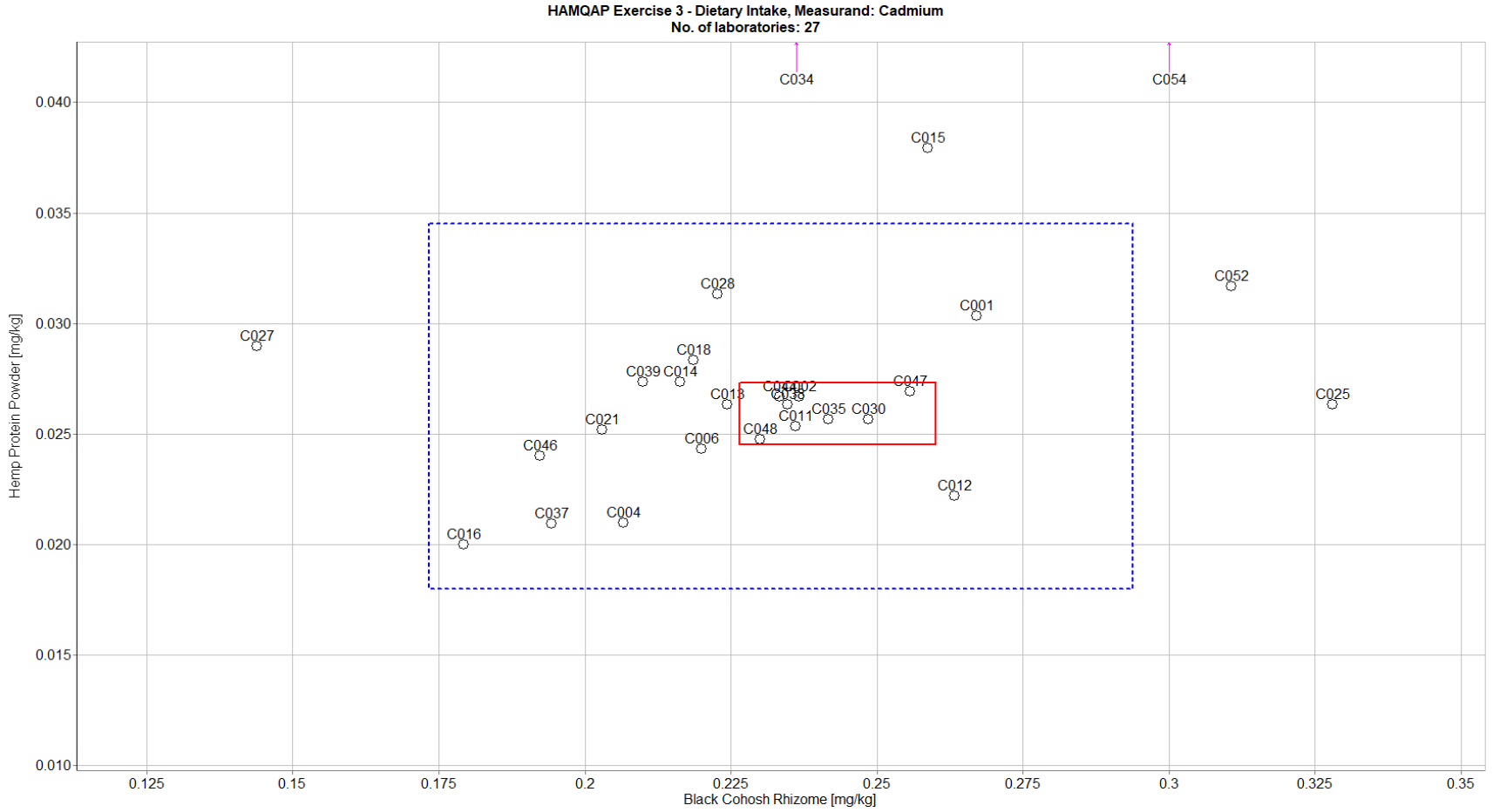

Figure 2-10. Laboratory means for cadmium in Black Cohosh Rhizome and Hemp Protein Powder (sample/sample comparison view). In this view, the individual laboratory mean for one sample (black cohosh rhizome) is compared to the mean for a second sample (hemp protein powder). The solid red box represents the NIST range of tolerance for the two samples, black cohosh rhizome (x-axis) and hemp protein powder (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, $|Z_{\mathrm{NIST}}| \leq 2$. The dotted blue box represents the consensus range of tolerance for black cohosh rhizome (x-axis) and hemp protein powder (y-axis), calculated as the values above and below the consensus means that result in an acceptable Z'_{comm} score, $|Z'_{\mathrm{comm}}| \leq 2$.

Table 2-4. Data summary table for lead in black cohosh rhizome and hemp protein powder. Data points highlighted in red have been flagged as potential outliers (e.g., Grubb and/or Cochran) by the NIST software package.

		Lead									
		Black Cohosh Rhizome (mg/kg)					Hemp Protein Powder (mg/kg)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target				2.236	0.046				0.0050	0.0018
	C001	2.557	2.452	2.526	2.512	0.054	0.052	0.031	0.043	0.0420	0.0105
	C002	2.01	1.99	2.01	2.003	0.012	0	0	0		
	C004	2.02	2.04	2.05	2.037	0.015	0.0028	0.004	0.0026	0.0031	0.0008
	C005										
	C006	2	1.9	1.9	1.933	0.058	0.0021	0.0033	0.0031	0.0028	0.0006
	C008										
	C009	1.881	1.986		1.934	0.074					
	C011	2.074	2.067	2.098	2.080	0.016	< 0.0050	< 0.0050	< 0.0050		
	C012	1.9878	2.0621	2.0196	2.023	0.037	0.0144	0.0108	0.0374	0.0209	0.0144
	C013	1.93	1.88	1.9	1.903	0.025	< 0.0222	< 0.0222	< 0.0222		
	C014	1.694	1.7	1.754	1.716	0.033	0.01	0.009	0.013	0.0107	0.0021
	C015	1.95	2.01	2.02	1.993	0.038	0.00978	0.0149	0.0094	0.0114	0.0031
	C016	1.99761	2.04039	2.05929	2.032	0.032	0.002	0.003	0.002	0.0023	0.0006
	C017	1.86	2	1.89	1.917	0.074	< 0.0500	< 0.0500	< 0.0500		
	C018	5.91	5.536	5.438	5.628	0.249	0	0.372	0.239	0.2037	0.1885
	C019										
	C020										
	C021	4.15	4.18	4.15	4.160	0.017	0.0022	0.0028	0.0033	0.0028	0.0006
	C024										
	C025	2.384	2.083	2.186	2.218	0.153	< 0.0100	< 0.0100	< 0.0100		
	C027	1.64008	1.62575	1.5922	1.619	0.025	0.06355	0.03653	0.02527	0.0418	0.0197
	C028	1.843	1.832	1.84	1.838	0.006	0.032	0.034	0.021	0.0290	0.0070
	C030	1.9577	1.9986	2.0128	1.990	0.029	0.0023	0.0027	0.0022	0.0024	0.0003
	C031										
	C032										
	C034	1.812	1.805	1.779	1.799	0.017	0.006	0.0043	0.0044	0.0049	0.0010
	C035	2.13	1.99	2.04	2.053	0.071	< 0.0100	< 0.0100	< 0.0100		
	C037	1.64	1.66	1.67	1.657	0.015	< 0.0050	< 0.0050	< 0.0050		
	C038	2.006	1.919	1.866	1.930	0.071	0.046	0.019	0.018	0.0277	0.0159
	C039	1.734	1.709	1.66	1.701	0.038	0	0	0		
	C043										
	C044	1.81	1.81	1.77	1.797	0.023	< 0.0100	< 0.0100	< 0.0100		
	C045										
	C046	1.645	1.695	1.653	1.664	0.027	0.005	0.002	0.005	0.0040	0.0017
	C047	2.05	2.01	2.02	2.027	0.021	< 0.0090	< 0.0090	< 0.0090		
	C048	1.9	2	1.9	1.933	0.058	< 0.0030	< 0.0030	< 0.0030		
	C049										
	C050										
	C052	2.34	2.28	2.52	2.380	0.125	< 0.0090	< 0.0090	< 0.0090		
	C053	2	1.9	1.9	1.933	0.058	0.04	< 0.0100	< 0.0100	0.0400	
	C054	1.91	1.35	1.5	1.587	0.290	0.08	0.08	0.08	0.0800	0.0000
	C055										
Community Results		Consensus Mean			1.922		Consensus Mean			0.0121	
		Consensus Standard Deviation			0.216		Consensus Standard Deviation			0.0107	
		Maximum			5.628		Maximum			0.2037	
		Minimum			1.587		Minimum			0.0023	
		N			30		N			16	

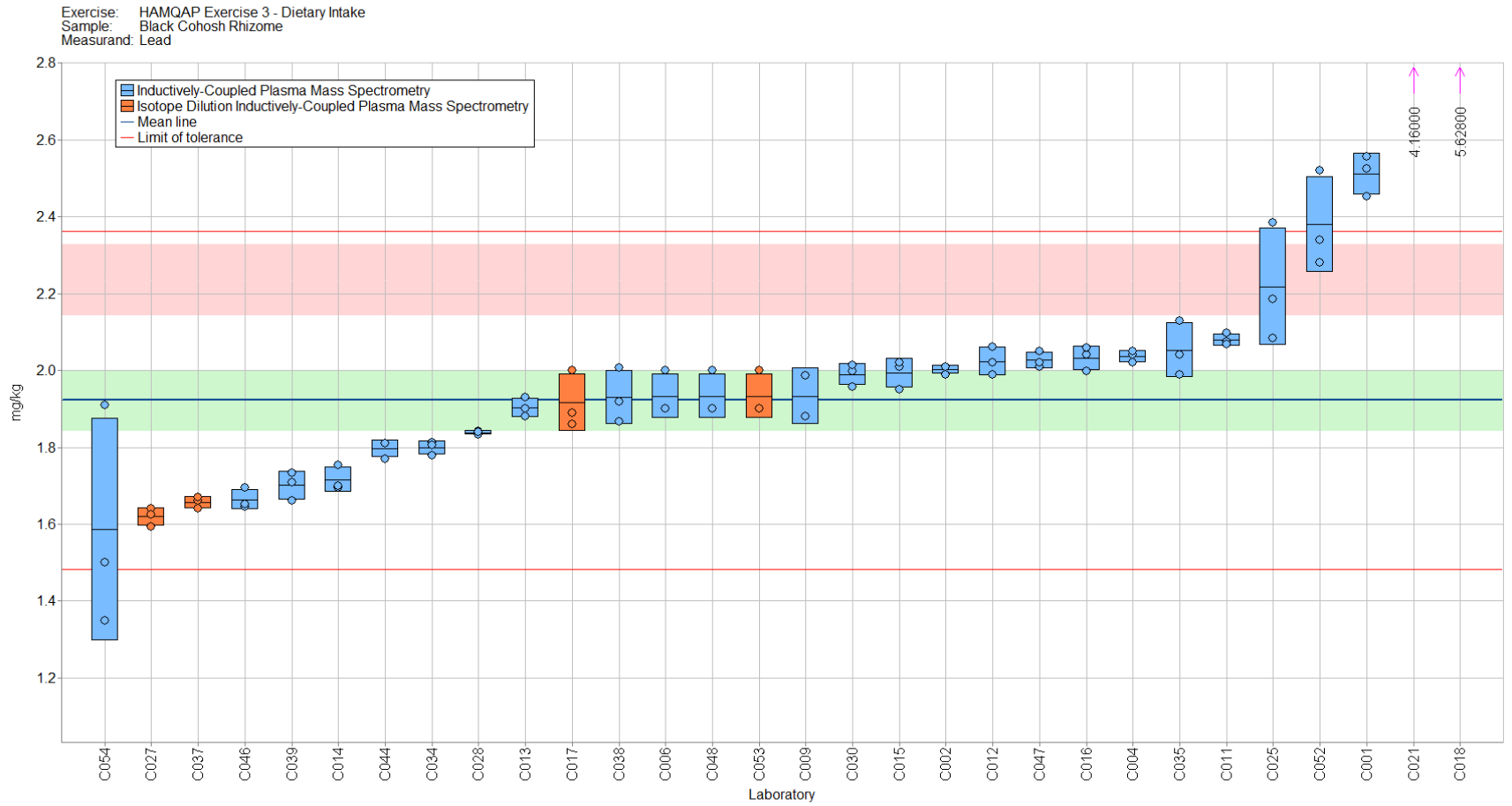

Figure 2-11. Lead in Black Cohosh Rhizome (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line represents 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'_{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_{NIST}) and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$.

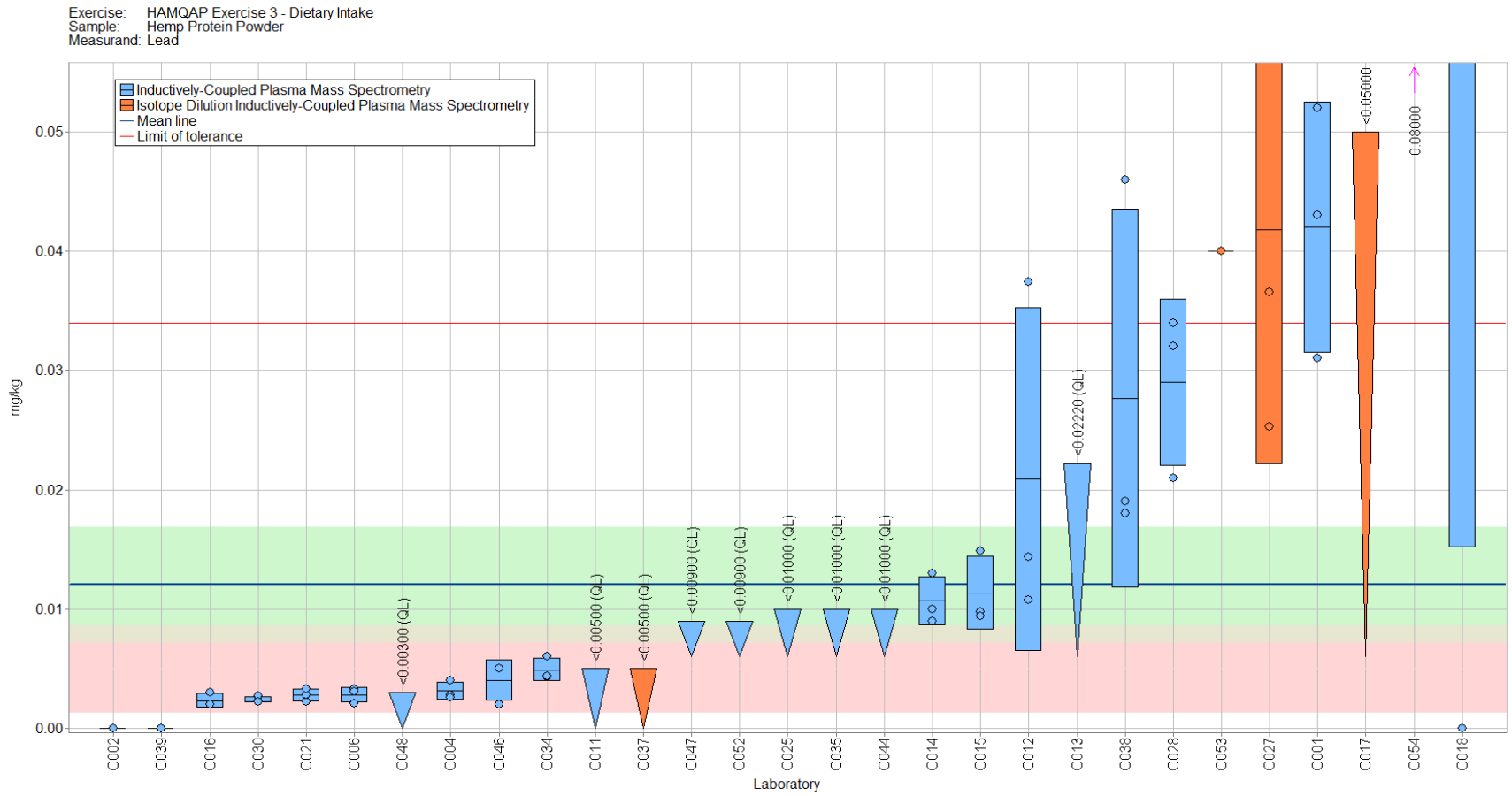

Figure 2-12. Lead in Hemp Protein Powder (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line represents 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'_{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_{NIST}) and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$.

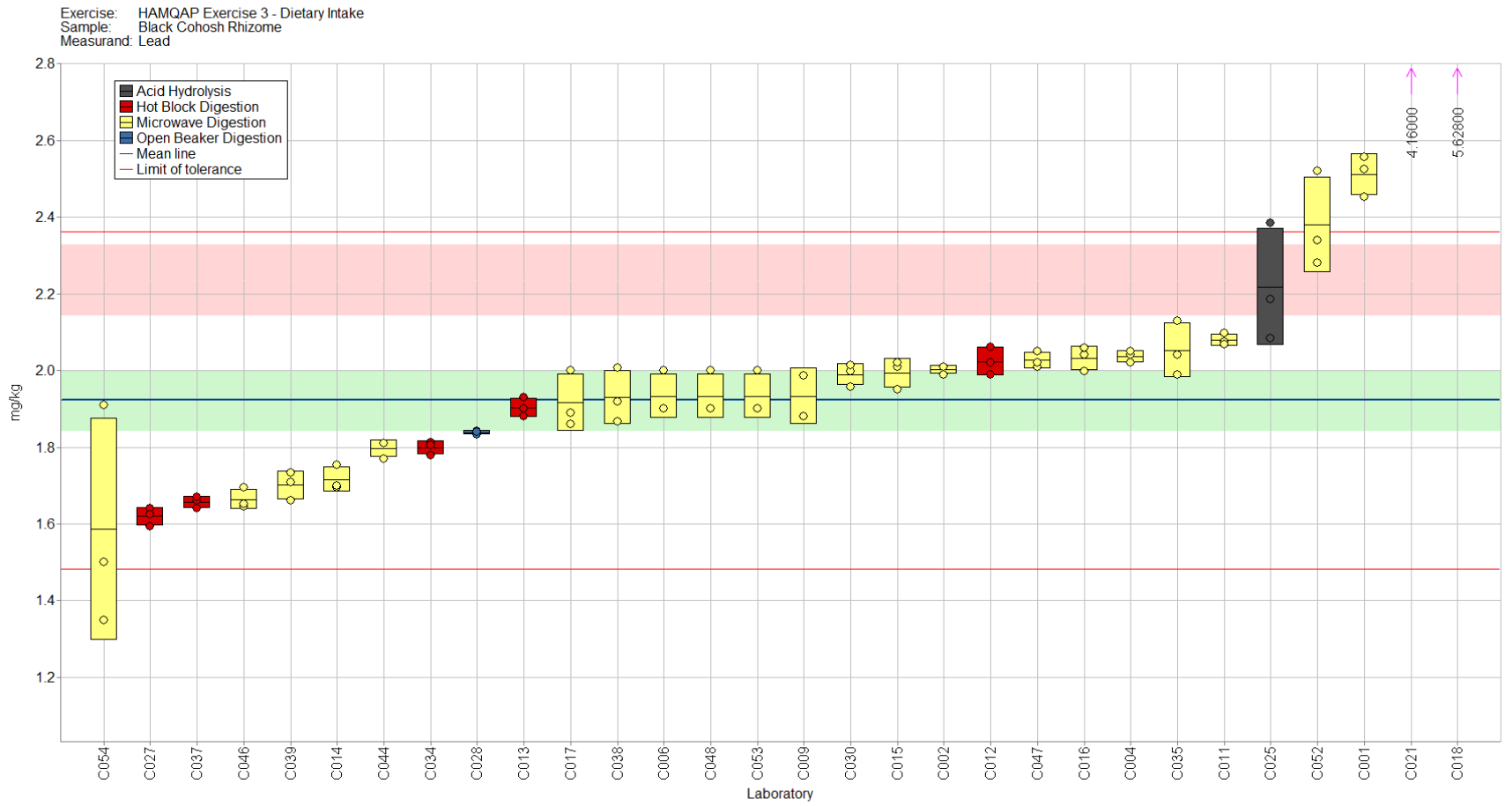

Figure 2-13. Lead in Black Cohosh Rhizome (data summary view – sample preparation method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The solid 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'_{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_{NIST}) and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$.

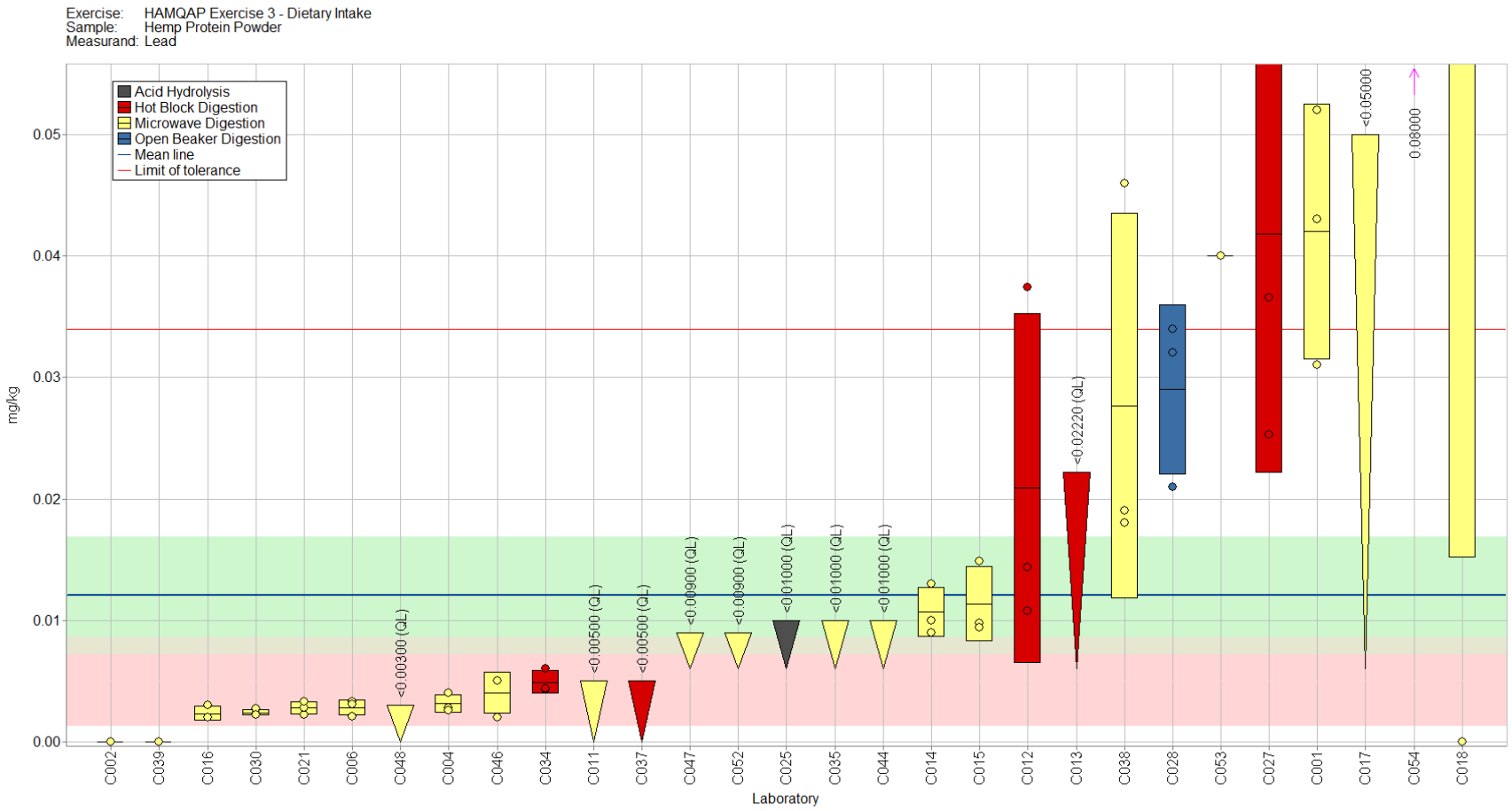

Figure 2-14. Lead in Hemp Protein Powder (data summary view – sample preparation method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The solid 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'_{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_{NIST}) and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$.

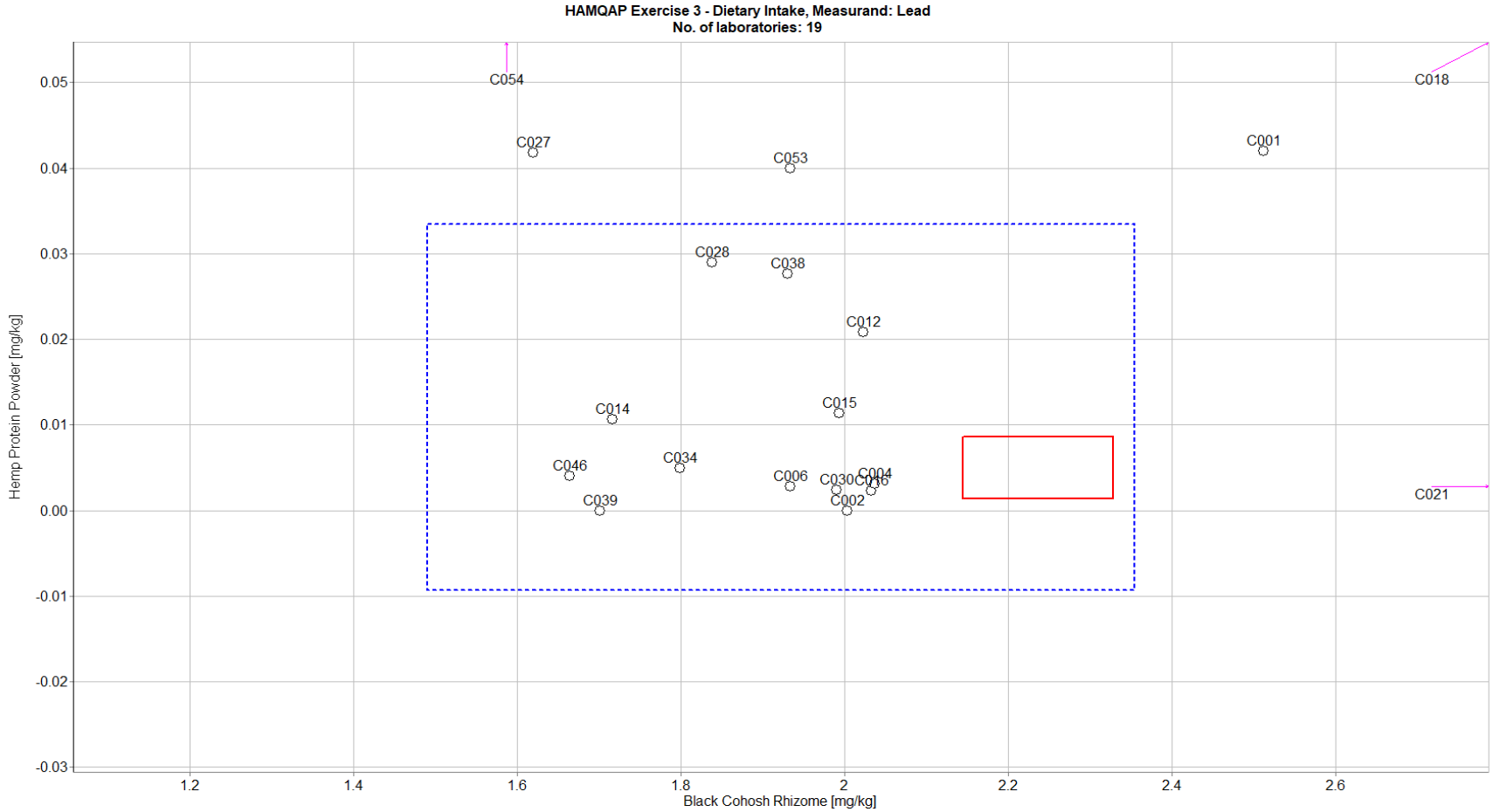

Figure 2-15. Laboratory means for lead in Black Cohosh Rhizome and Hemp Protein Powder (sample/sample comparison view). In this view, the individual laboratory mean for one sample (black cohosh rhizome) is compared to the mean for a second sample (hemp protein powder). The solid red box represents the NIST range of tolerance for the two samples, black cohosh rhizome (x-axis) and hemp protein powder (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, $|Z_{\mathrm{NIST}}| \leq 2$. The dotted blue box represents the consensus range of tolerance for black cohosh rhizome (x-axis) and hemp protein powder (y-axis), calculated as the values above and below the consensus means that result in an acceptable Z'_{comm} score, $|Z'_{\mathrm{comm}}| \leq 2$.

Table 2-5. Data summary table for mercury in black cohosh rhizome and hemp protein powder. Data points highlighted in red have been flagged as potential outliers (e.g., Grubb and/or Cochran) by the NIST software package.

		Mercury									
		Black Cohosh Rhizome (mg/kg)					Hemp Protein Powder (mg/kg)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target				0.01312	0.00031				0.00460	0.00030
	C001	0	0	0			0.002	0	0	0.00067	0.00115
	C002	0.03	0.03	0.02	0.02667	0.00577	0.01	0.01	0.01	0.01000	0.00000
	C004	0.0123	0.0126	0.0122	0.01237	0.00021	0.00351	0.00354	0.00347	0.00351	0.00004
	C006	0.011	0.011	0.01	0.01067	0.00058	0.0027	0.0027	0.0035	0.00297	0.00046
	C008										
	C009	< 0.0300	< 0.0300								
	C011	0.018	0.017	0.018	0.01767	0.00058	< 0.0050	< 0.0050	< 0.0050		
	C012	0.0329	0.0268	0.0312	0.03030	0.00315	0.0143	0.0625	0.0114	0.02940	0.02870
	C013	< 0.0190	< 0.0190	< 0.0190			< 0.0173	< 0.0173	< 0.0173		
	C014	0.014	0.012	0.012	0.01267	0.00115	0.003	0.002	0.014	0.00633	0.00666
	C015	0.013	0.012	0.0115	0.01217	0.00076	0.00442	0.00357	0.00415	0.00405	0.00043
	C016	0.0164	0.0104	0.0107	0.01250	0.00338	0.002	0.002	0.002	0.00200	0.00000
	C017	< 0.0500	< 0.0500	< 0.0500			< 0.0500	< 0.0500	< 0.0500		
	C018	0.019	0.018	0.015	0.01733	0.00208	0.007	0.007	0.005	0.00633	0.00115
	C019										
	C020										
	C021	0.028	0.024	0.025	0.02567	0.00208	0.0085	0.0091	0.0095	0.00903	0.00050
	C024										
	C025	0.019	0.011	0.016	0.01533	0.00404	< 0.0100	< 0.0100	< 0.0100		
	C027	0.02039	0.01672	0.0151	0.01740	0.00271	0.00705	0.0067	0.00518	0.00631	0.00099
	C028	0.016	0.012	0.014	0.01400	0.00200	0.013	0.01	0.014	0.01233	0.00208
	C030	0.0131	0.0111	0.0129	0.01237	0.00110	0.0037	0.0033	0.0032	0.00340	0.00026
	C031										
	C032										
	C034	0.0223	0.0183	0.0174	0.01933	0.00261	0.0038	0.0041	0.0049	0.00427	0.00057
	C035	0.014	0.014	0.014	0.01400	0.00000	< 0.0100	< 0.0100	< 0.0100		
	C037	0.0113	0.012	0.00982	0.01104	0.00111	< 0.00250	< 0.00250	< 0.00250		
	C038	< 0.0500	< 0.0500	< 0.0500			< 0.0500	< 0.0500	< 0.0500		
	C039	0.012	0.011	0.01	0.01100	0.00100	0.011	0.008	0.008	0.00900	0.00173
	C043										
	C044	0.01	0.01	0.01	0.01000	0.00000	< 0.0100	< 0.0100	< 0.0100		
	C046	0	0	0			0	0	0		
	C047	0.013	0.013	0.013	0.01300	0.00000	0.004	0.004	0.004	0.00400	0.00000
	C048	0.012	0.012	0.013	0.01233	0.00058	< 0.0100	< 0.0100	< 0.0100		
	C049										
	C050										
	C052	0.014	0.013	0.016	0.01433	0.00153	< 0.0090	< 0.0090	< 0.0090		
	C053	0.0199	0.0172	0.0141	0.01707	0.00290	< 0.0100	< 0.0100	< 0.0100		
	C054	0.03	0.01	0.02	0.02000	0.01000	0.01	0.01	0.01	0.01000	0.00000
	C055										
Community Results		Consensus Mean			0.01443		Consensus Mean			0.00551	
		Consensus Standard Deviation			0.00441		Consensus Standard Deviation			0.00417	
		Maximum			0.03030		Maximum			0.02940	
		Minimum			0.01000		Minimum			0.00067	
		N			24		N			17	

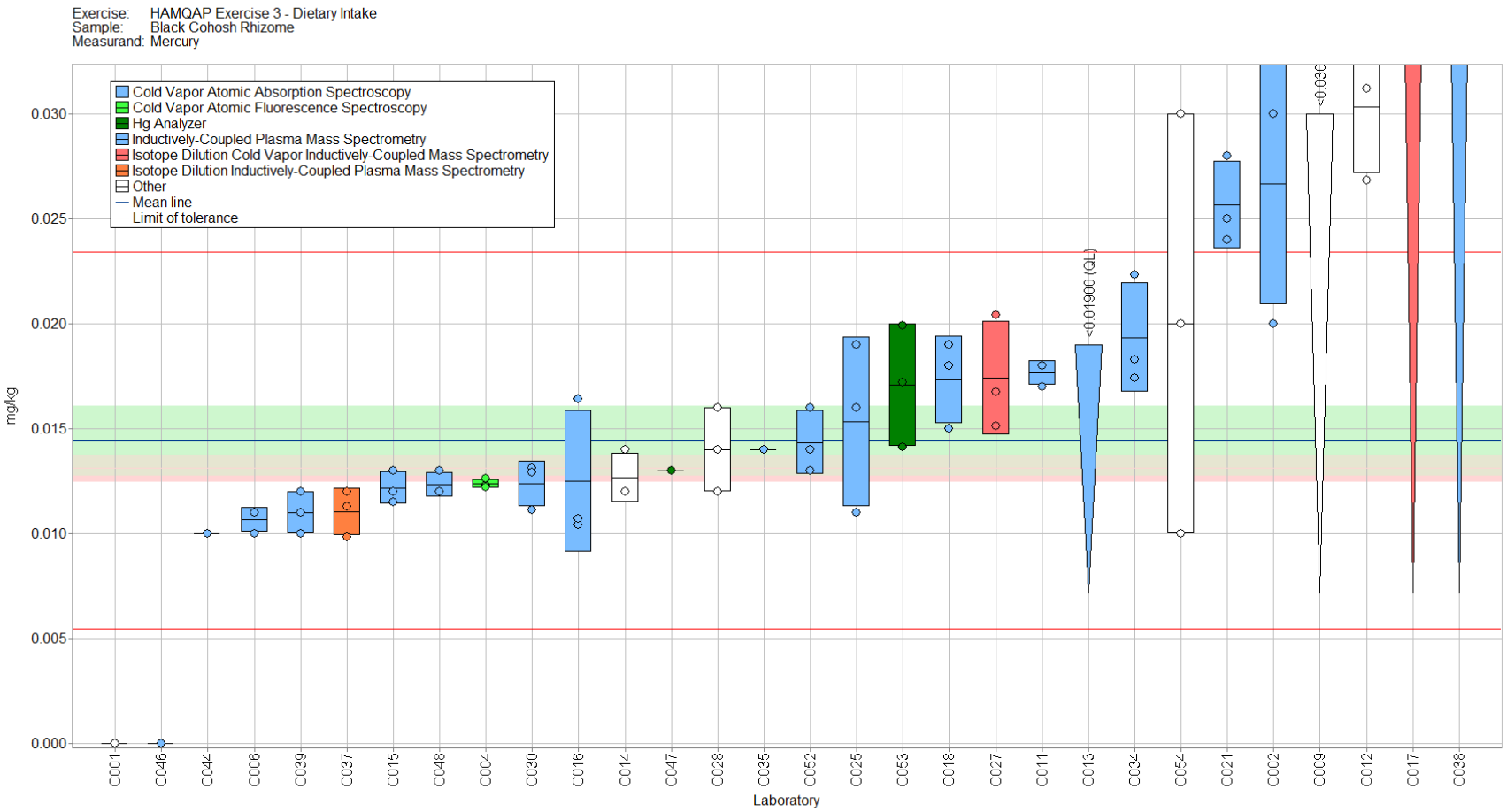

Figure 2-16. Mercury in Black Cohosh Rhizome (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line 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'_{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_{NIST}) and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$.

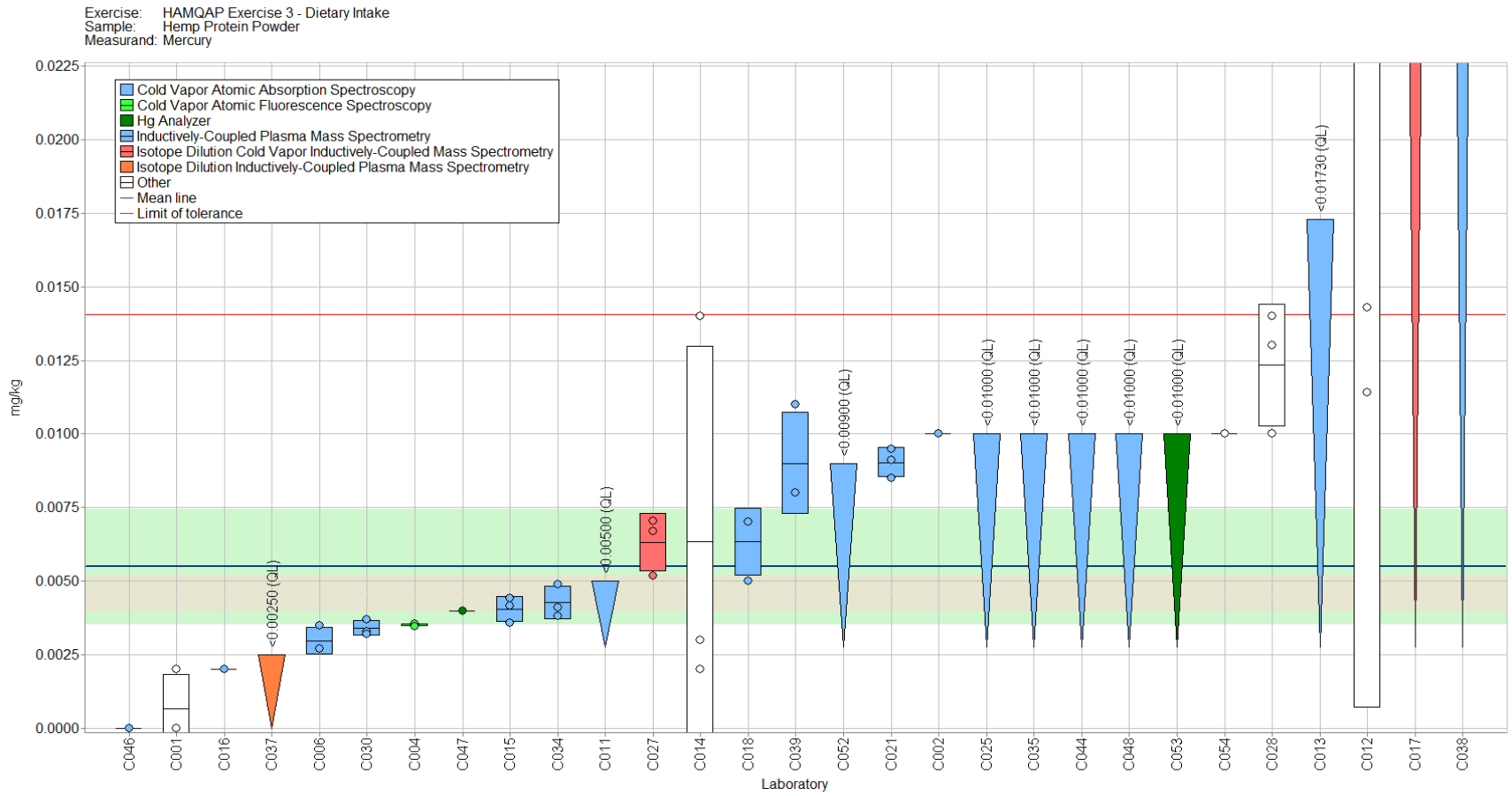

Figure 2-17. Mercury in Hemp Protein Powder (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line 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'_{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_{NIST}) and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$.

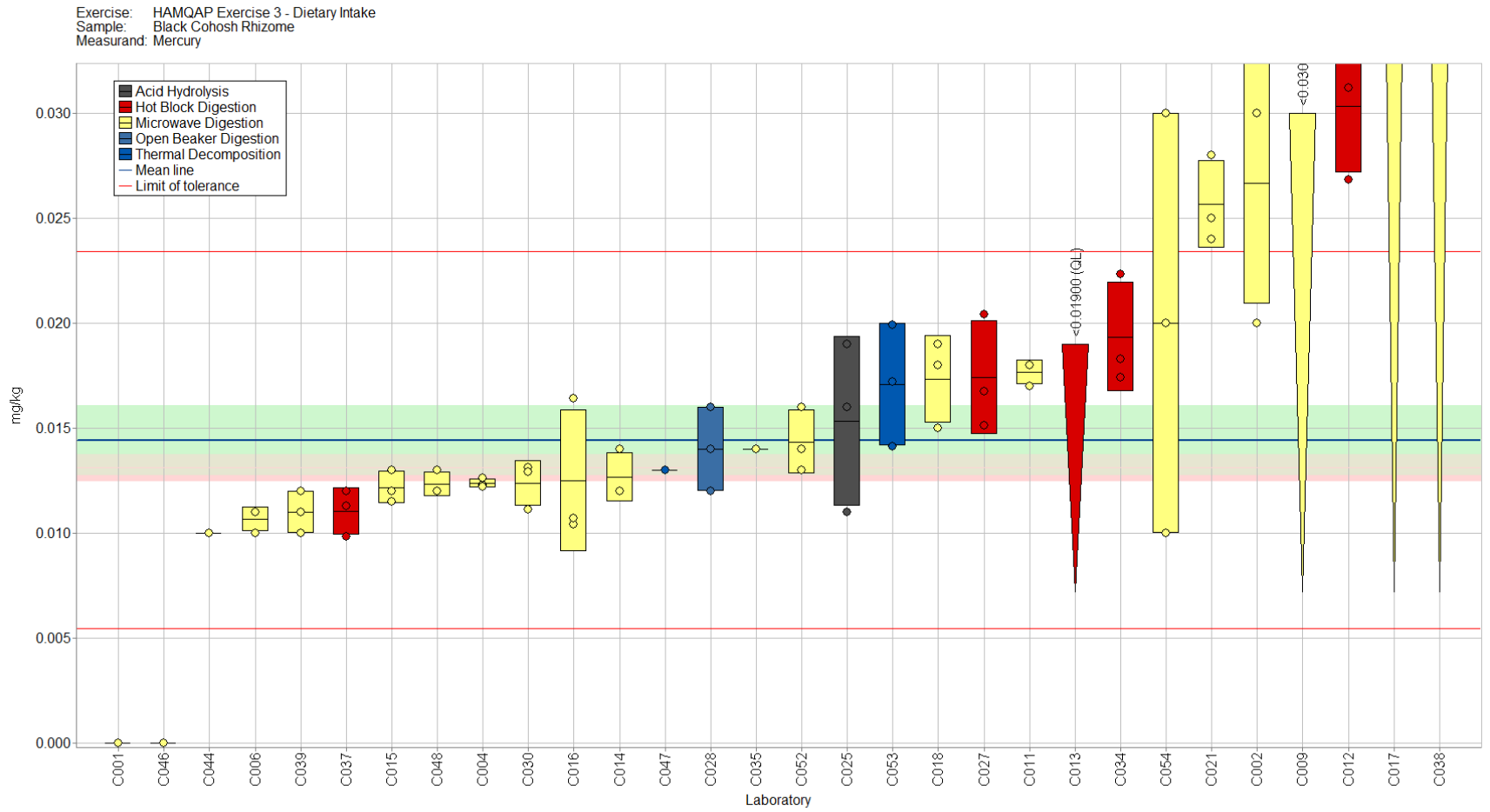

Figure 2-18. Mercury in Black Cohosh Rhizome (data summary view – sample preparation method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The 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'_{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_{NIST}) and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$.

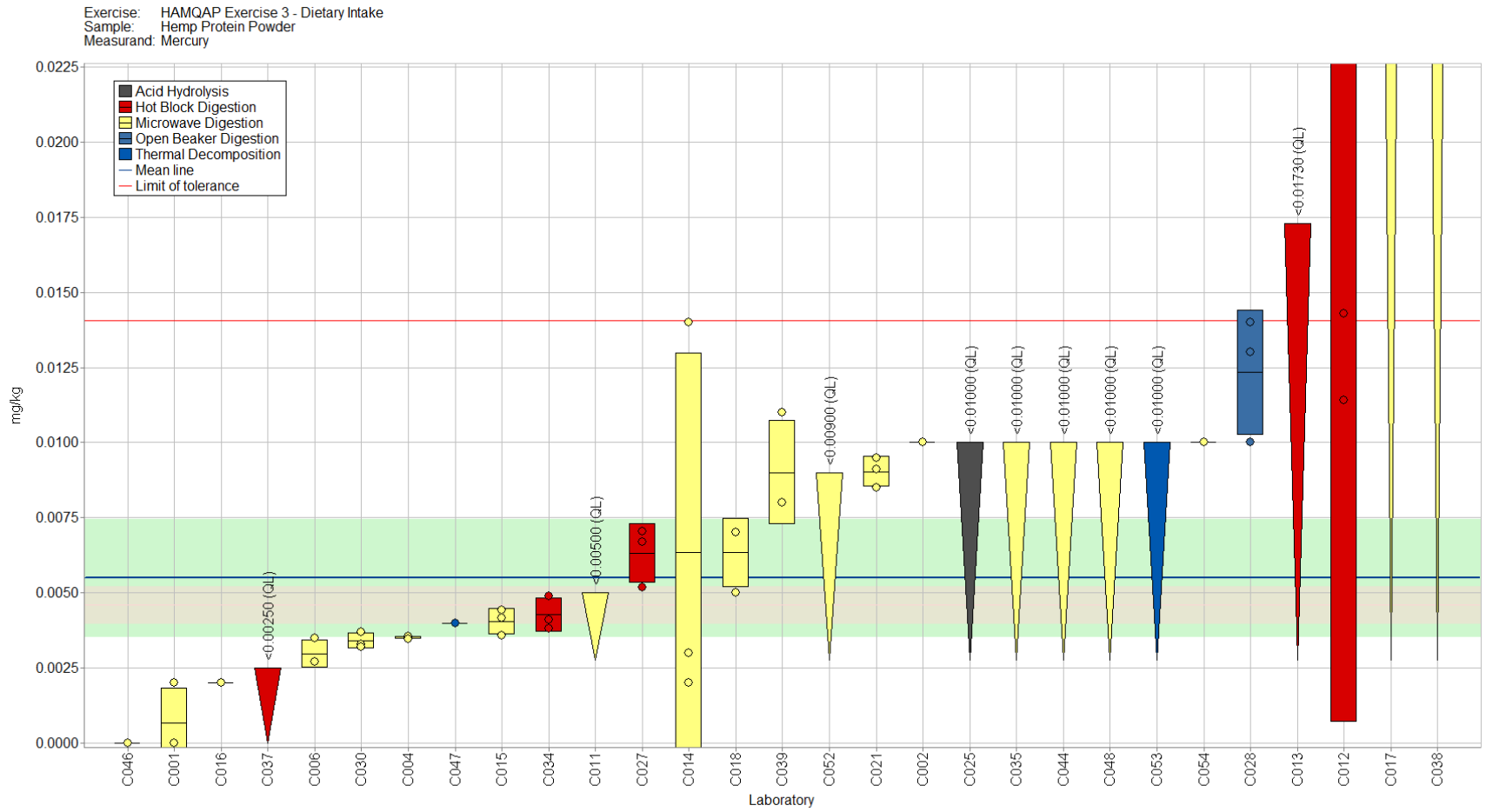

Figure 2-19. Mercury in Hemp Protein Powder (data summary view – sample preparation method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The 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'_{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_{NIST}) and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$.

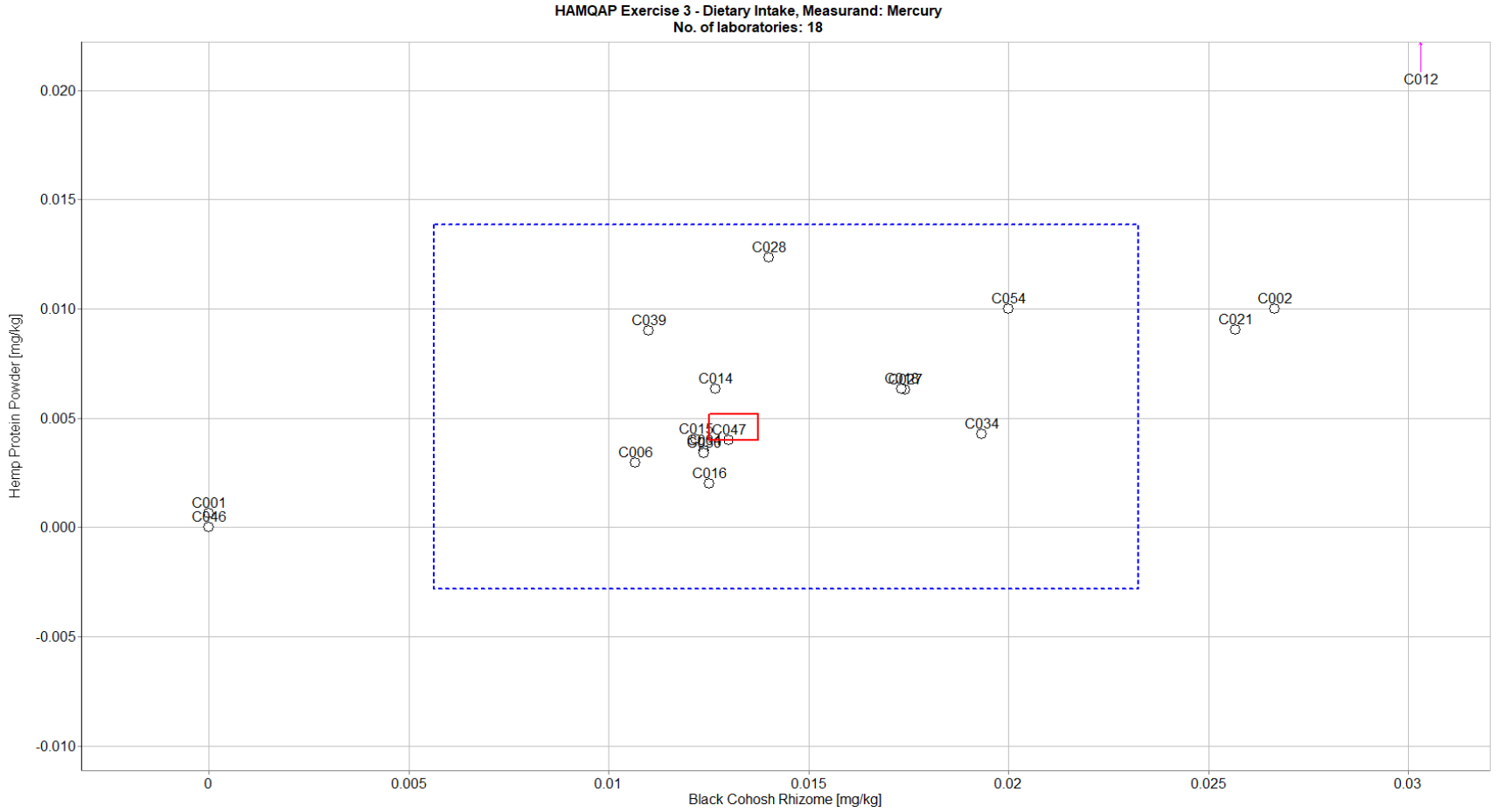

Figure 2-20. Laboratory means for mercury in Black Cohosh Rhizome and Hemp Protein Powder (sample/sample comparison view). In this view, the individual laboratory mean for one sample (black cohosh rhizome) is compared to the mean for a second sample (hemp protein powder). The solid red box represents the NIST range of tolerance for the two samples, black cohosh rhizome (x-axis) and hemp protein powder (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, $|Z_{\mathrm{NIST}}| \leq 2$. The dotted blue box represents the consensus range of tolerance for black cohosh rhizome (x-axis) and hemp protein powder (y-axis), calculated as the values above and below the consensus means that result in an acceptable Z'_{comm} score, $|Z'_{\mathrm{comm}}| \leq 2$.

Human Metabolites Sample Information

Human Blood A. Participants were provided with three vials, each containing 2 mL of frozen human blood. Before use, participants were instructed to allow the material to thaw at room temperature for at least 30 min prior to sampling, use the material immediately after thawing, gently mix the contents prior to removal of a test portion for analysis, and use a sample size appropriate for their usual in-house method of analysis. Participants were asked to avoid exposing the material to direct UV light, to store the material at or below –80 °C, and to prepare one sample and report one value from each vial provided. The approximate analyte levels were not reported to participants prior to the study. The NIST-determined values for Human Blood A were assigned using results from NIST by ICP-MS for As, ID-ICP-MS for Cd and Pb, and ID-CV-ICP-MS for Hg. The NIST-determined values and uncertainties for As, Cd, Pb, and Hg in Human Blood A are provided in the table below.

Analyte	NIST-Determined Mass Concentration in Human Blood A (µg/L)
Arsenic (As)	5.82 ± 0.26
Cadmium (Cd)	0.328 ± 0.019
Lead (Pb)	14.59 ± 0.17
Mercury (Hg)	1.352 ± 0.032

Human Blood B. Participants were provided with three vials, each containing 1.6 mL of frozen human blood. Before use, participants were instructed to allow the material to thaw at room temperature for at least 30 min prior to sampling, use the material immediately after thawing, gently mix the contents prior to removal of a test portion for analysis, and use a sample size appropriate for their usual in-house method of analysis. Participants were asked to avoid exposing the material to direct UV light, to store the material at or below –80 °C, and to prepare one sample and report one value from each vial provided. The approximate analyte levels were not reported to participants prior to the study, and target values for As, Cd, Pb, and Hg in SRM 1401 Level 1 have not been determined at NIST.

Human Metabolites Study Results

- The enrollment and reporting statistics for the toxic elements 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)	
		Human Blood A	SRM 1401 (Level 1)
As	10	5 (50 %)	3 (30 %)
Cd	9	3 (33 %)	4 (44 %)
Pb	10	5 (50 %)	5 (50 %)
Hg	9	4 (44 %)	4 (44 %)

- The consensus mean was within the target range for Hg in Human Blood A. No other consensus means were within the target ranges for Human Blood A.
- The between-laboratory variabilities for each sample-analyte pair are summarized below, showing that performance was best for lead measurements.

	Between-Laboratory Variability (% RSD)	
Analyte	Human Blood A	Human Blood B
As	88 %	31 %
Cd	>100 %	83 %
Pb	19 %	35 %
Hg	54 %	39 %

- The sample preparation methods reported by participating laboratories were either microwave digestion or hot block digestion for all four analytes.
- Most laboratories reported using ICP-MS for determination of these analytes in the human blood samples. One laboratory reported using AAS for all analytes. One laboratory reported using ICP-OES for As determination, and one laboratory reported using CV atomic fluorescence spectroscopy for Hg determination n.

Human Metabolites Technical Recommendations

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

- Overall, the largest between-laboratory variability was observed for Cd. The smallest between-laboratory variability was observed for Pb.
- The low participation rate in this study may indicate that determination of these analytes in the blood matrices was particularly challenging. The complexity of the matrix can enhance or suppress analyte signals, notably Cd and Pb, and will be extremely challenging for laboratories using ICP-MS, even in energy discrimination or collision cell mode.
- The low participation rate may also indicate that laboratories do not have established methods or protocols that they use routinely for these analytes or matrices.
  - Interested laboratories should participate in workshops or training available in the measurement of toxic metals in blood.
  - Laboratories with existing measurement procedures should publish in the peer-reviewed literature to promote knowledge exchange with other laboratories.
- Using a known quality assurance sample (CRM, SRM, RM, or in-house control) may assist with method development and method validation.

Table 2-6. Individualized data summary table (NIST) for toxic elements in human blood.

National Institute of Standards and Technology

HAMQAP Exercise 3 - 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*
Total Arsenic	Human Blood A	µg/L		5.82	0.52		0	5	9	7.9	5.82	0.52
Total Arsenic	SRM 1401 Trace Metals in Frozen Human Blood (Level 1)	µg/L						3	8.1	2.5		
Cadmium	Human Blood A	µg/L		0.328	0.0038		0	3	0.31	0.63	0.328	0.0038
Cadmium	SRM 1401 Trace Metals in Frozen Human Blood (Level 1)	µg/L						4	0.5	0.41		
Lead	Human Blood A	µg/L		14.59	0.34		0	5	16	3.1	14.6	0.34
Lead	SRM 1401 Trace Metals in Frozen Human Blood (Level 1)	µg/L						5	18.6	6.6		
Mercury	Human Blood A	µg/L		1.352	0.064		0	4	1.22	0.65	1.35	0.064
Mercury	SRM 1401 Trace Metals in Frozen Human Blood (Level 1)	µg/L						4	0.96	0.38		

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 2-7. Data summary table for total arsenic in human blood. Data points highlighted in red have been flagged as potential outliers (e.g., Grubb and/or Cochran) by the NIST software package.

		Total Arsenic									
		Human Blood A (µg/L)					SRM 1401 Trace Metals in Frozen Human Blood (Level 1) (µg/L)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target				5.82	0.52					
	C004	6.69	5.91	6.51	6.37	0.41					
	C005	18.91	16.61	18.15	17.89	1.17	9.05	10.11	10.75	9.97	0.86
	C006	3.7	1.7	2.3	2.57	1.03	-2.2	-4	5.9	-0.10	5.27
	C012	12.7	13	13.6	13.10	0.46	9.75	9.22	9.12	9.36	0.34
	C027										
	C031										
	C032										
	C054										
	C060	5	5	5	5.00	0.00					
	C062										
Community Results		Consensus Mean			8.99		Consensus Mean			8.12	
		Consensus Standard Deviation			7.87		Consensus Standard Deviation			2.51	
		Maximum			17.89		Maximum			9.97	
		Minimum			2.57		Minimum			-0.10	
		N			5		N			3	

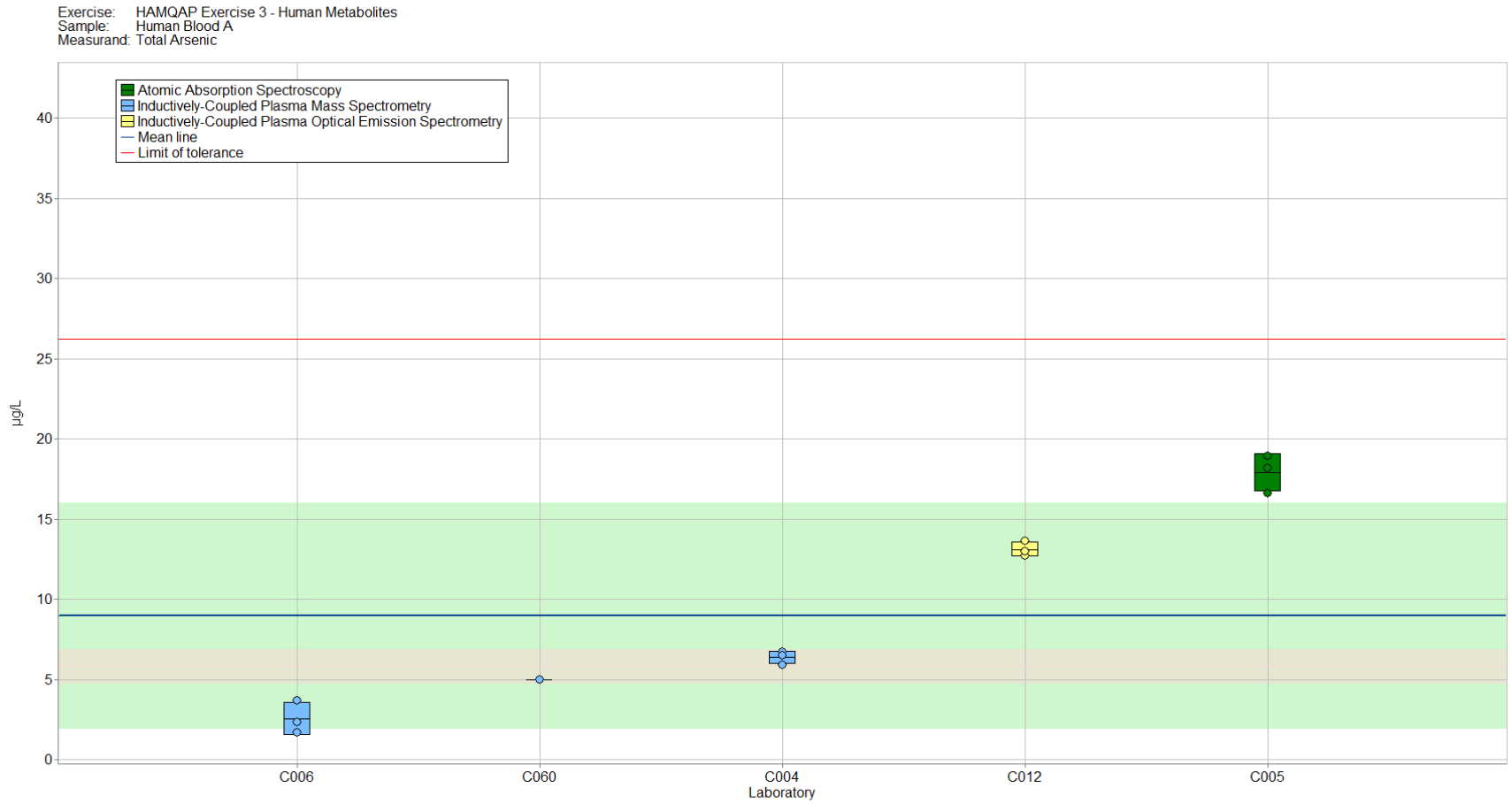

Figure 2-21. Total arsenic in Human Blood A (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line 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'_{comm} score, $|Z'_{\mathrm{comm}}| \leq 2$, 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_{NIST}) and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\mathrm{NIST}}| \leq 2$.

Table 2-8. Data summary table for cadmium in human blood. Data points highlighted in red have been flagged as potential outliers (e.g., Grubb and/or Cochran) by the NIST software package.

		Cadmium									
		Human Blood A (µg/L)					SRM 1401 Trace Metals in Frozen Human Blood (Level 1) (µg/L)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target				0.3280	0.0038					
	C004										
	C005	0.41	0.42	0.41	0.4133	0.0058	0.55	0.51	0.56	0.5400	0.0265
	C006	0.1	0.4	0.7	0.4000	0.3000	9.2	0.8	1.6	3.8667	4.6361
	C012	0.106	0.106		0.1060	0.0000	0.424	0.106	0.318	0.2827	0.1619
	C027										
	C031										
	C032										
	C054										
	C060						0.7	0.6	0.7	0.6667	0.0577
Community Results		Consensus Mean			0.3064		Consensus Mean			0.4964	
		Consensus Standard Deviation			0.6251		Consensus Standard Deviation			0.4113	
		Maximum			0.4133		Maximum			3.8667	
		Minimum			0.1060		Minimum			0.2827	
		N			3		N			4	

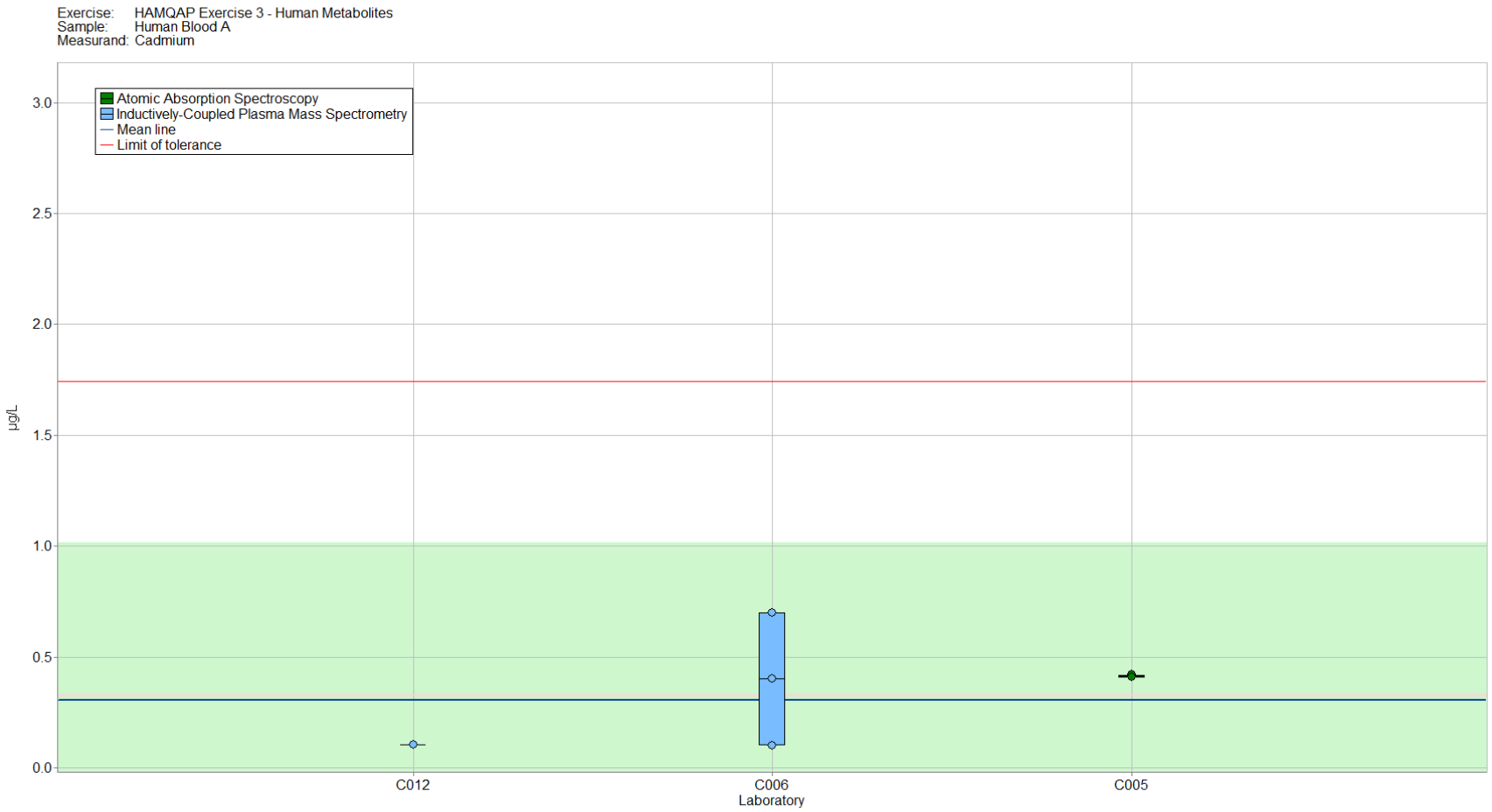

Figure 2-22. Cadmium in Human Blood A (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line represents 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'_{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_{NIST}) and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$.

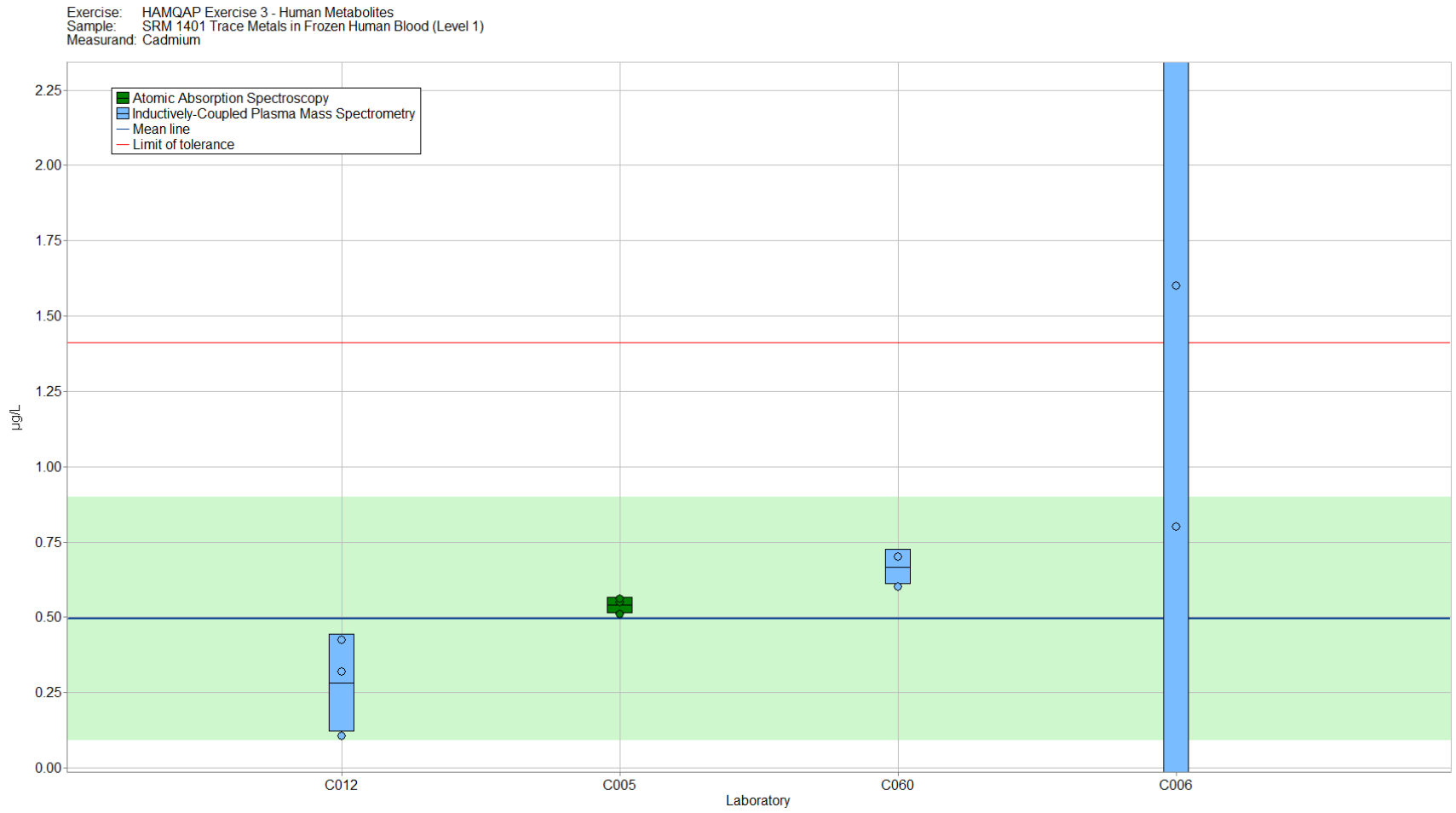

Figure 2-23. Cadmium in SRM 1401 Trace Metals in Frozen Human Blood (Level 1) (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method 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'_{comm} score, $|Z'_{\text{comm}}| \leq 2$, with the lower range set at zero. A NIST value has not been determined in this material.

Table 2-9. Data summary table for lead in human blood. Data points highlighted in red have been flagged as potential outliers (e.g., Grubb and/or Cochran) by the NIST software package.

		Lead									
		Human Blood A (µg/L)					SRM 1401 Trace Metals in Frozen Human Blood (Level 1) (µg/L)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target				14.59	0.34					
	C004	15.4	13.8	13.9	14.37	0.90	19.7	20.6	17.8	19.37	1.43
	C005	20.59	19.95	21.22	20.59	0.64	25.89	24.62	24.6	25.04	0.74
	C006	14	14	14	14.00	0.00	18	21	18	19.00	1.73
	C012	15.1	15.3	15.3	15.23	0.12	20.9	20.7	20.9	20.83	0.12
	C027										
	C031										
	C032										
	C054										
	C060	2	1	2	1.67	0.58	2	2	2	2.00	0.00
	C062										
Community Results		Consensus Mean			16.05		Consensus Mean			18.60	
		Consensus Standard Deviation			3.11		Consensus Standard Deviation			6.56	
		Maximum			20.59		Maximum			25.04	
		Minimum			1.67		Minimum			2.00	
		N			5		N			5	

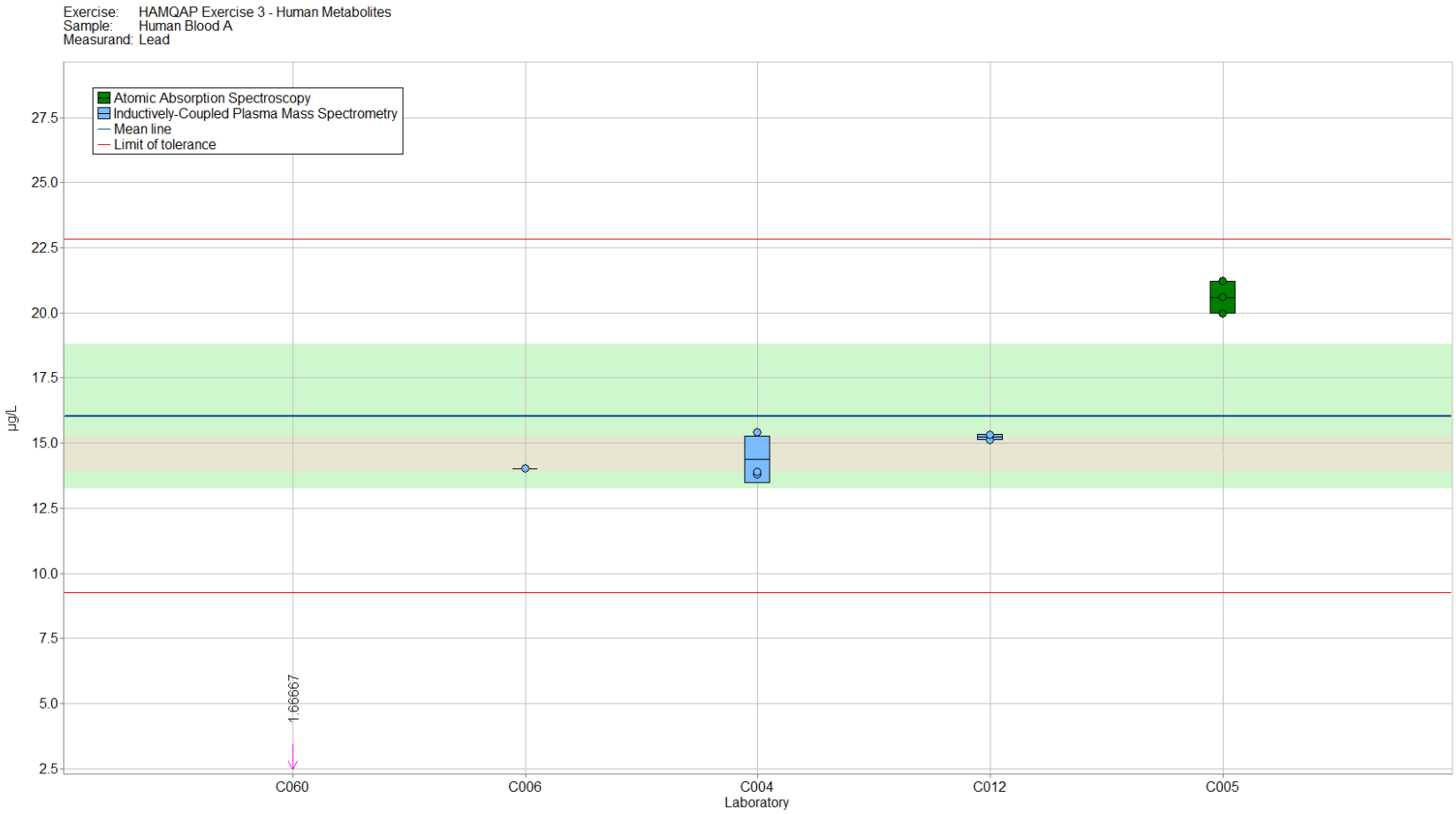

Figure 2-24. Lead in Human Blood A (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line represents 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'_{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_{NIST}) and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$.

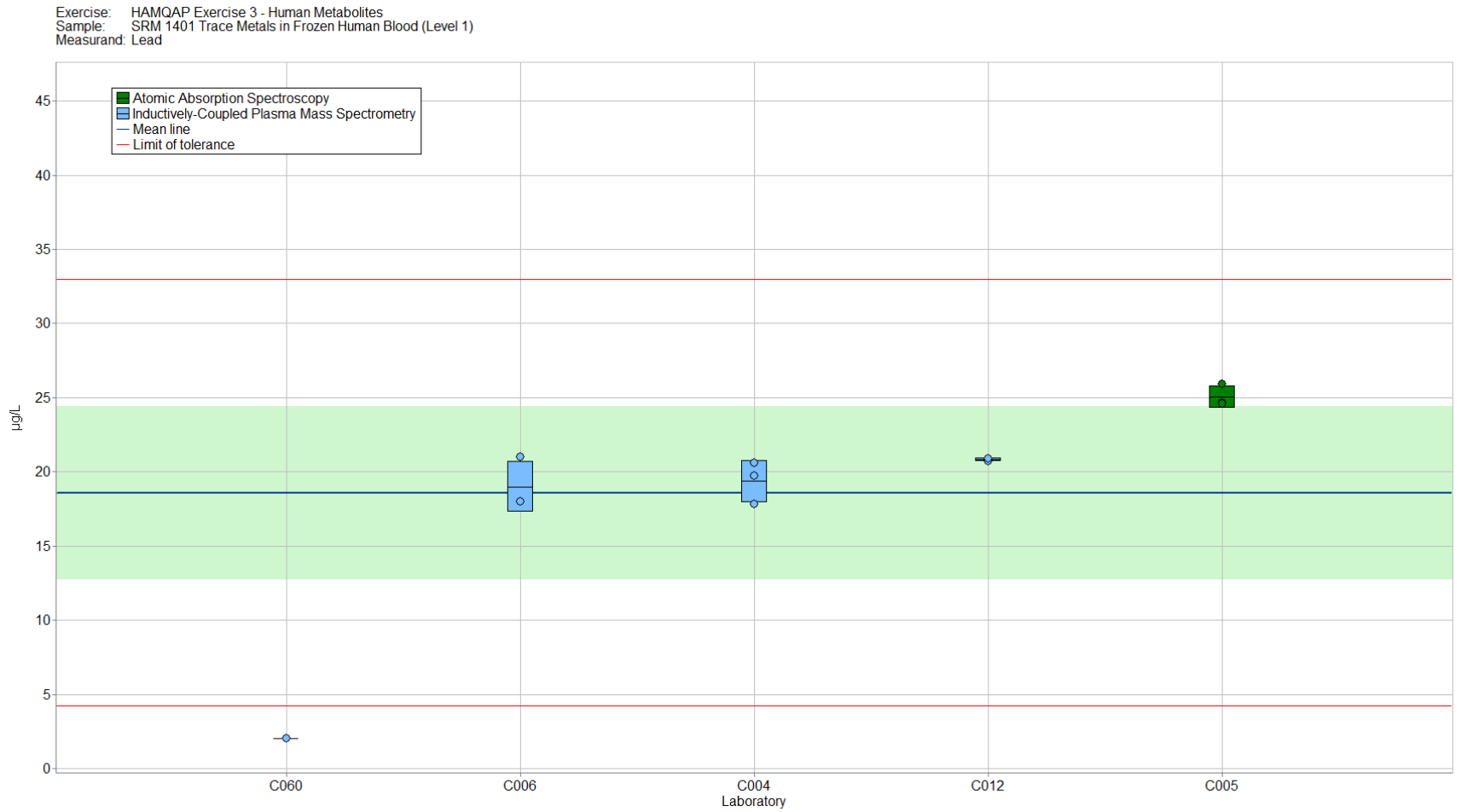

Figure 2-25. Lead in SRM 1401 Trace Metals in Frozen Human Blood (Level 1) (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method 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'_{comm} score, $|Z'_{\text{comm}}| \leq 2$. A NIST value has not been determined in this material.

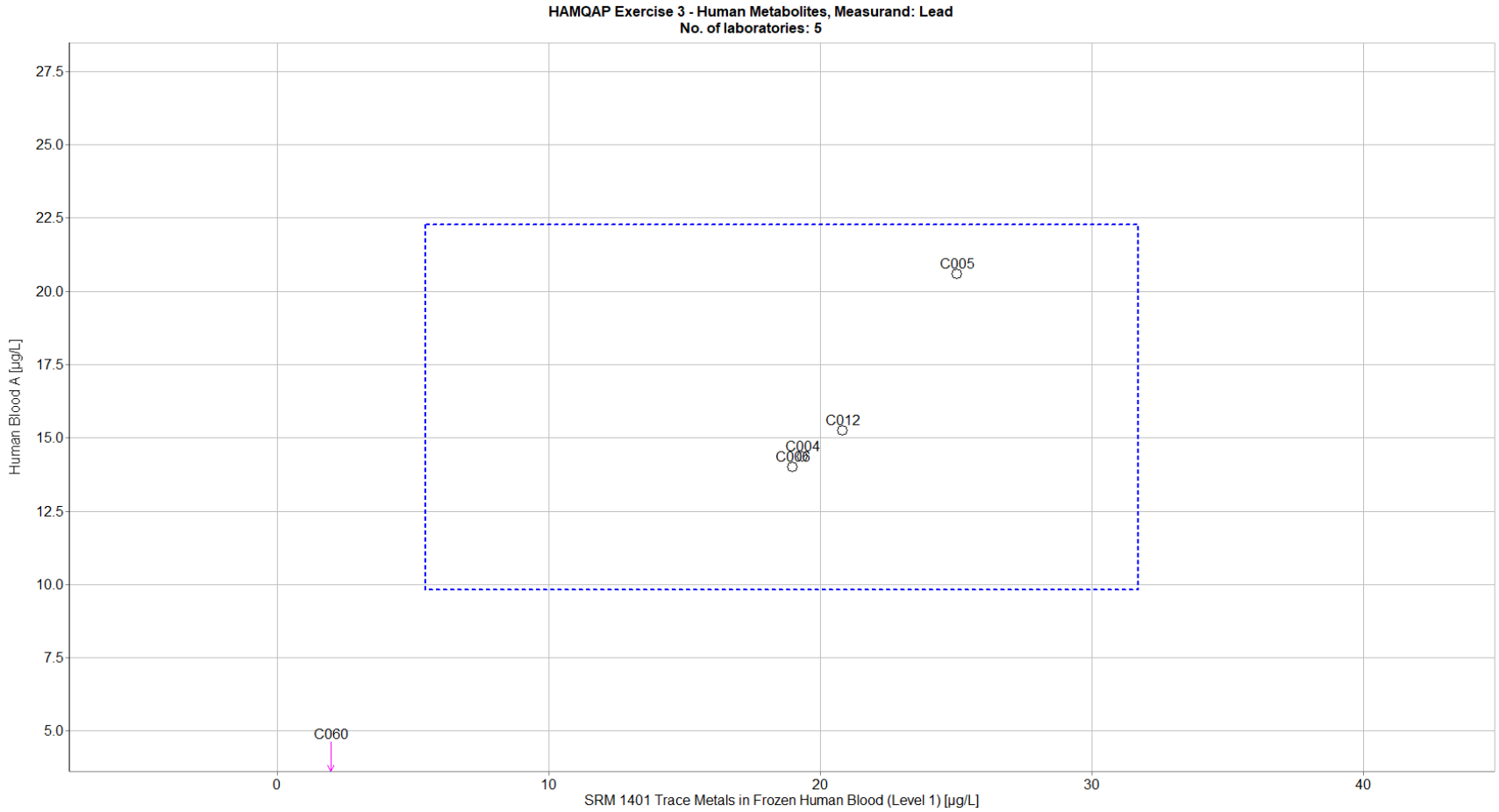

Figure 2-26. Laboratory means for lead in SRM 1401 Trace Metals in Frozen Human Blood (Level 1) and Human Blood A (sample/sample comparison view). In this view, the individual laboratory mean for one sample (SRM 1401) is compared to the mean for a second sample (human blood A). The dotted blue box represents the consensus range of tolerance for SRM 1401 (x-axis) and human blood A (y-axis), calculated as the values above and below the consensus means that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$.

Table 2-10. Data summary table for mercury in human blood.

		Mercury									
		Human Blood A (µg/L)					SRM 1401 Trace Metals in Frozen Human Blood (Level 1) (µg/L)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target				1.352	0.064					
	C004	1.17	1.21	1.15	1.177	0.031	0.917	0.922	0.961	0.933	0.024
	C006	0.7	0.3	0.7	0.567	0.231	-0.3	0.7	0.9	0.433	0.643
	C012	1.01	2.54	1.91	1.820	0.769	1.696	1.166	1.17	1.344	0.305
	C027										
	C031										
	C032										
	C054										
	C060	1.4	1.3	1.3	1.333	0.058	1	1.1	1.3	1.133	0.153
	C062										
Community Results		Consensus Mean			1.224		Consensus Mean			0.961	
		Consensus Standard Deviation			0.655		Consensus Standard Deviation			0.377	
		Maximum			1.820		Maximum			1.344	
		Minimum			0.567		Minimum			0.433	
		N			4		N			4	

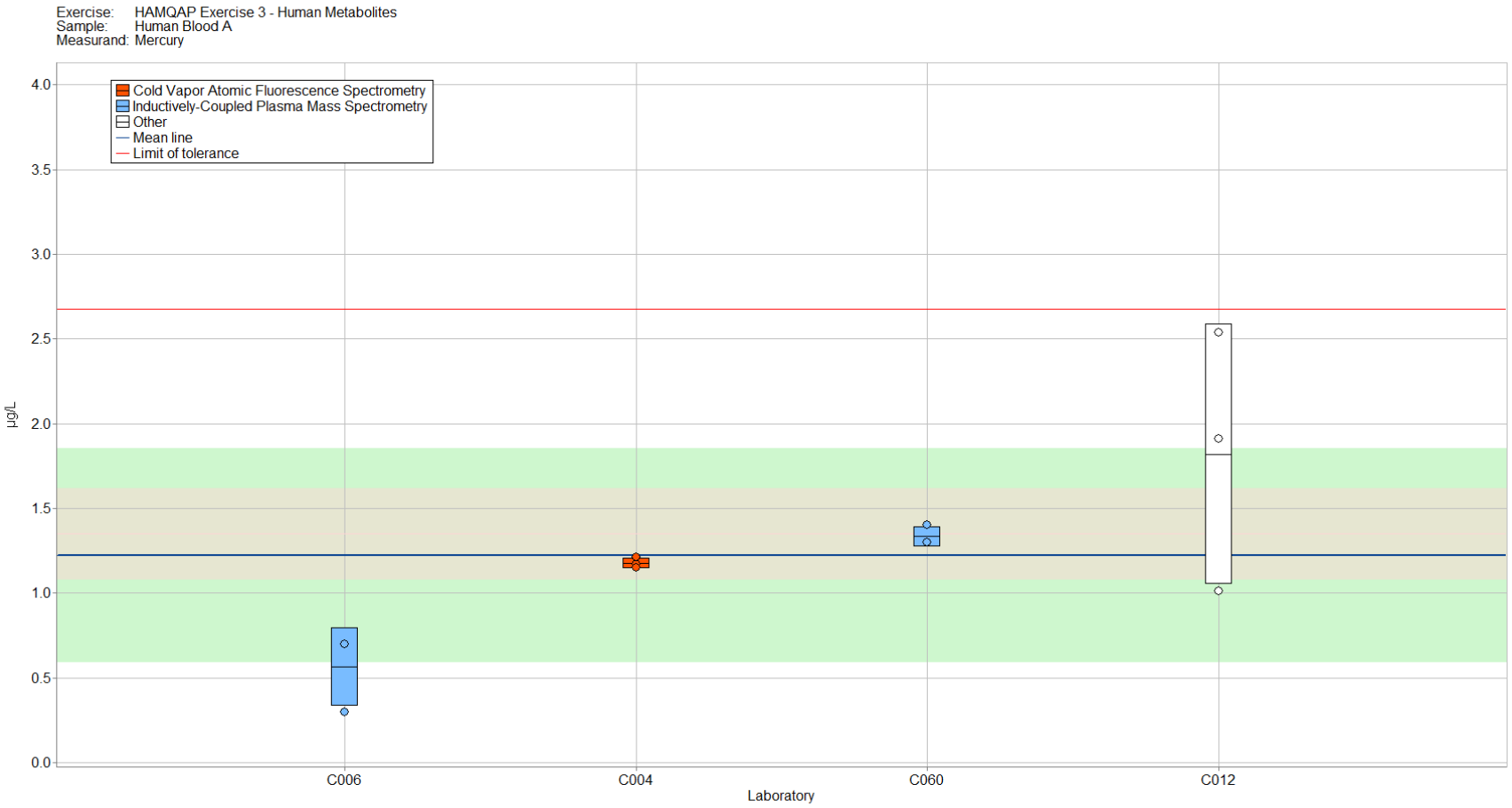

Figure 2-27. Mercury in Human Blood A (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line represents 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'_{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_{NIST}) and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$.

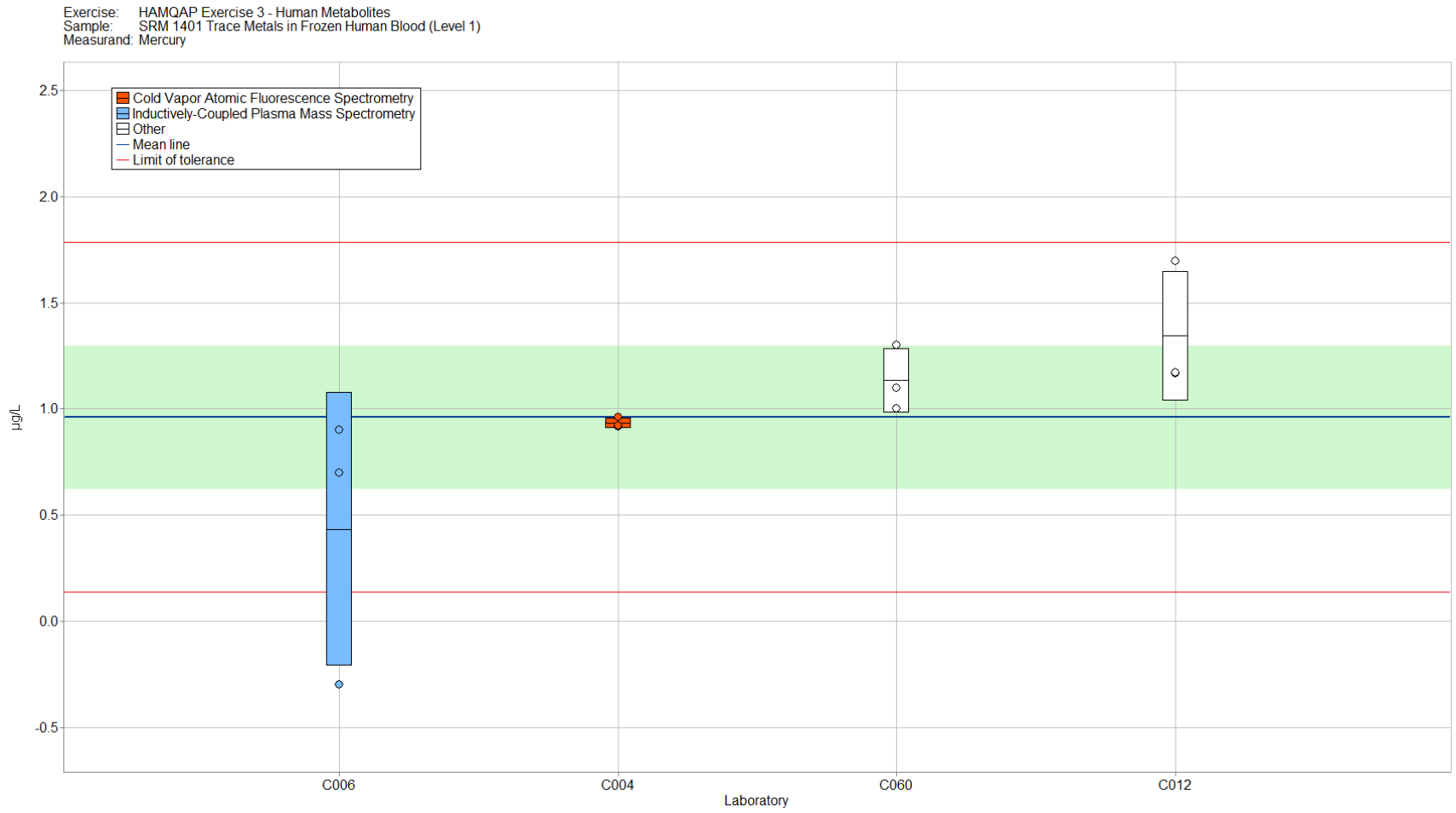

Figure 2-28. Mercury in SRM 1401 Trace Metals in Frozen Human Blood (Level 1) (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method 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'_{comm} score, $|Z'_{\text{comm}}| \leq 2$. A NIST value has not been determined in this material.

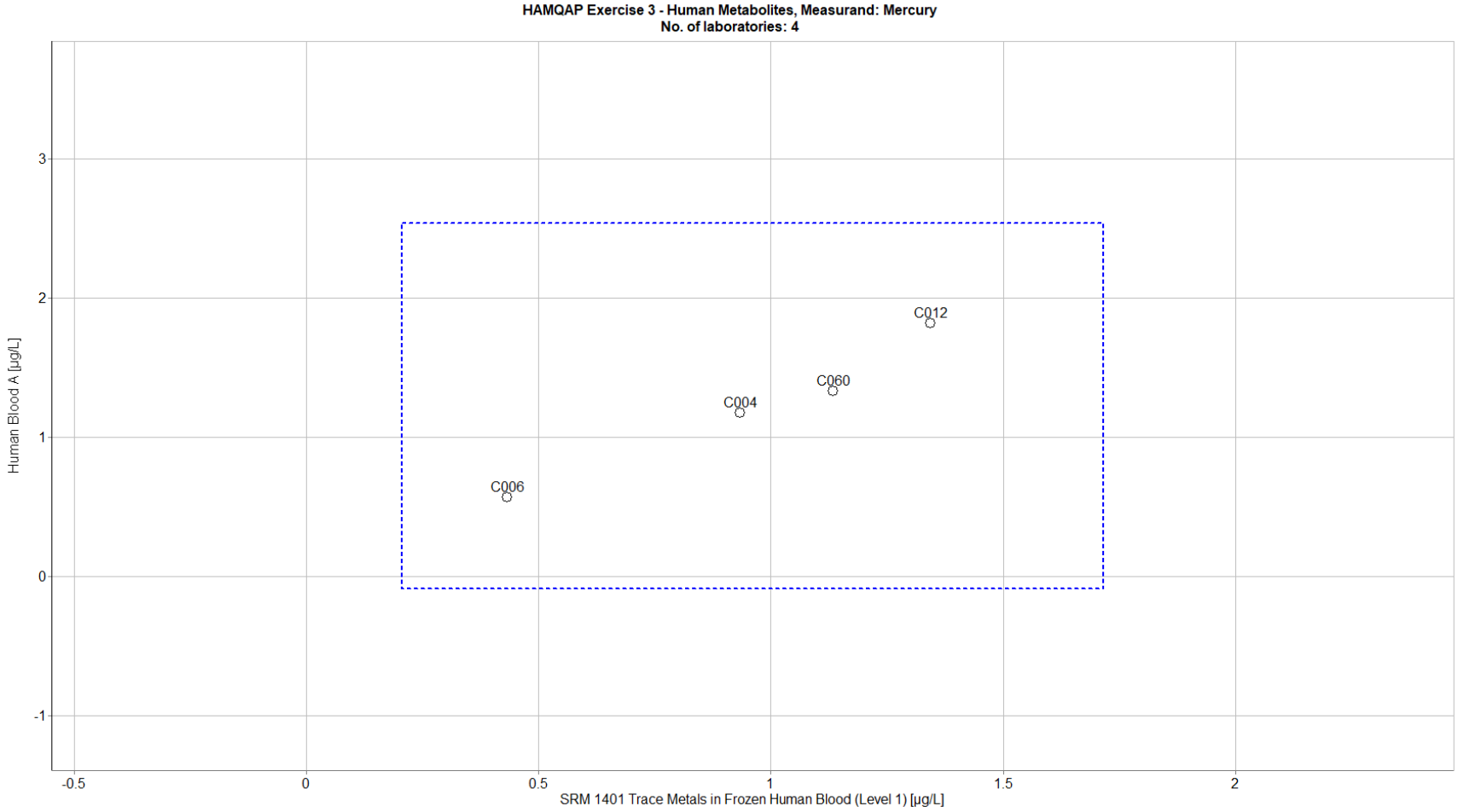

Figure 2-29. Laboratory means for mercury in SRM 1401 Trace Metals in Frozen Human Blood (Level 1) and Human Blood A (sample/sample comparison view). In this view, the individual laboratory mean for one sample (SRM 1401) is compared to the mean for a second sample (human blood A). The dotted blue box represents the consensus range of tolerance for SRM 1401 (x-axis) and human blood A (y-axis), calculated as the values above and below the consensus means that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$.

Toxic Elements Overall Study Comparison

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

- The low signup and participation rate for the human metabolism study suggests laboratories may not have an interest in participating in a toxic elements study or these studies may be too challenging.
- In general, some suggestions for participants to improve their measurements are below:
  - Sample preparation methods should be well established before analyzing unknown samples. Use established quality control materials (SRM, CRM, RM and in-house materials) and validated methods of analyses.
  - Calibration curves must be linear and include the lowest and highest values expected to be measured in the sample solutions.
  - Use of an organic solvent in the mobile phase of LC-ICP-MS may enhance sensitivity, improving of the detection limit for As in low-level sample solutions.
  - To improve the accuracy of the detection of the analyte in the sample, matrix-matched standards can be used for calibration curves. Reducing the number of sample dilutions may also improve results for unknown samples.
  - Calculation errors are often a cause for incorrect results. Use a quality assurance material (CRM, SRM, RM), or in-house prepared material, to establish that a method is in control and help find calculation errors. Once a method and quality assurance material appear to be in control, be sure results are reported in the correct units.
  - Zero and negative values are not a quantity that can be measured. If 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 MDL, LOQ, or QL.
  - Measurement methods must be correctly reported.

SECTION 3: WATER-SOLUBLE VITAMINS (Folates)

Study Overview

In this study, participants were provided with two samples for dietary intake, SRM 1869 Infant/Adult Nutritional Formula II and multivitamin tablets, and two samples for human metabolism, SRM 3949 Folate Vitamers in Frozen Human Serum (Levels 1 and 3). Participants were asked to use in-house analytical methods to determine the mass fraction (mg/kg) for dietary intake samples or molar concentration (nmol/L) for human metabolites samples of total folate and individual folate vitamers (folic acid, 5-methyltetrahydrofolate, 5-formyltetrahydrofolate, tetrahydrofolate, 5,10-methenyltetrahydrofolate, and MeFox) in each matrix. Folate is an essential vitamin, critical for the production and maintenance of new cells as well as synthesis of DNA and RNA, and adequate folate intake during pregnancy is important for the prevention of neural tube defects.[10] Naturally occurring folates in food are in the tetrahydrofolate forms, and humans obtain folic acid via fortified foods and supplements. Folate health status is evaluated through determination of folate metabolites in serum [11,12].

Dietary Intake Sample Information

Infant Formula. Participants were provided with three packets, each containing approximately 10 g of powdered material. 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 1 g. 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. The approximate analyte levels were not reported to participants prior to the study. A reference value for folic acid in SRM 1869 was assigned using results from collaborating laboratories and the manufacturer of the material. The reference value and uncertainty for folic acid in SRM 1869 are provided in the table below on an as-received basis.

Analyte	Reference Mass Fraction in SRM 1869 (mg/kg)
Folic Acid	2.239 ± 0.086

Multivitamin. Participants were provided with three bottles, each containing 30 multivitamin tablets. Before use, participants were instructed to grind all 30 tablets and mix the resulting powder thoroughly prior to removal of a test portion for analysis, and to use a sample size of at least 0.3 g. 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. Approximate analyte levels were not reported to participants prior to the study. The NIST-target value for folic acid in the multivitamin sample was assigned using results from the manufacturer of the material. The NIST-target value and uncertainty for folic acid are provided in the table below on an as-received basis.

[10] Folate Fact Sheet for Health Professionals. National Institutes of Health Office of Dietary Supplements. https://ods.od.nih.gov/factsheets/folate-healthprofessional/ (accessed February 2019).

[11] Biomarkers of Folate Status in NHANES: A Roundtable Summary. *Am J Clin Nutr*, 94 (1), 303S-312S Jul 2011. PMID 21593502.

[12] Demographic, physiologic, and lifestyle characteristics observed with serum total folate differ among folate forms. *J Nutr*, 2019. PMID 31875475.

Analyte	NIST-Target Mass Fraction in Multivitamin (mg/kg)
Folic Acid	465 ± 16

Dietary Intake Study Results

- Thirty-three laboratories enrolled in this exercise and received samples, electing to measure folic acid. In addition to measuring folic acid, thirteen labs elected to include measurements for both 5-methyltetrahydrofolate and 5-formyltetrahydrofolate, while six laboratories only elected 5-methyltetrahydrofolate.
- Eleven laboratories reported results for folic acid in the infant formula (33 % participation), and seventeen laboratories reported results for folic acid in the multivitamin (52 % participation).
- One laboratory reported results for 5-methyltetrahydrofolate in the infant formula (5 %).
- No results were reported for 5-formyltetrahydrofolate.
- For infant formula, the consensus mean for folic acid was slightly below the target range and the between-laboratory variability was high (37 % RSD) (**Table 3-2, Figure 3-1**).
- For multivitamin, the consensus mean for folic acid was below the target range and the between-laboratory variability was good (15 % RSD) (**Table 3-2, Figure 3-2**).
- The analytical methods reported by participating laboratories are summarized in the table below.

	Number of Laboratories Reporting Results (Percent Participation)	
Method	Infant Formula	Multivitamin
LC-Absorbance	6 (55 %)	12 (71 %)
LC-MS	4 (36 %)	2 (12 %)
LC-MS/MS	2 (18 %)	1 (6 %)
USP	-	1 (6 %)
Not Specified	1 (9 %)	1 (6 %)

- All values reported using LC-MS/MS methods were outside the acceptable range of twice the upper limit of tolerance.

Dietary Intake Technical Recommendations

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

- Both materials used in this exercise, infant formula and multivitamin, are fortified matrices, and are expected to contain high levels of synthetic folic acid. Endogenous folates (i.e., 5-mTHF) may be present in the infant formula sample from other ingredients (e.g., skimmed milk).
- Only one laboratory reported results for minor folates in infant formula, so no conclusions about comparability can be made. Laboratories may not be equipped to determine low levels of endogenous folates, or methods may not be capable of determining low levels in fortified samples.

- Two laboratories reported use of LC-MS/MS approaches, and reported values in both samples were significantly outside the acceptable range of twice the upper limit of tolerance. Additional information is needed to make specific recommendations, including an understanding of the extraction procedure and calibration approach, but these laboratories should review their approaches carefully for potential biases.
- Folate calibration solutions that are value assigned based on UV absorbance spectrophotometry may contain significant impurities that impact quantification. Additional purity correction by LC-absorbance analysis of calibration solutions may resolve some biases.
- The various folates have different stabilities in solution. Solution pH or the addition of antioxidants, such as ascorbic acid, should be considered to ensure all folate calibration solutions are stable throughout the duration of the sample analysis.
- Use of matrix-matched CRMs for method validation and quality assurance of the measurement process is recommended.

Table 3-1. Individualized data summary table (NIST) for folates in infant formula and multivitamin.

National Institute of Standards and Technology

HAMQAP Exercise 3 - 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*
Folic Acid	SRM 1869 Infant/Adult Nutritional Formula II	mg/kg		2.239	0.086		0	11	2.4	0.9	2.24	0.086
5-methyltetrahydrofolate	SRM 1869 Infant/Adult Nutritional Formula II	mg/kg						1	1.8	7.2		
5-formyltetrahydrofolate	SRM 1869 Infant/Adult Nutritional Formula II	mg/kg						0				
Folic Acid	Multivitamin	mg/kg		465	16		0	17	410	60	465	16

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 folic acid in infant formula and multivitamin. Data points highlighted in red have been flagged as potential outliers (e.g., Grubb and/or Cochran) by the NIST software package.

		Folic Acid									
		SRM 1869 Infant/Adult Nutritional Formula II (mg/kg)					Multivitamin (mg/kg)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target				2.239	0.086				465	16
	C002										
	C008	4	4.3	4.4	4.233	0.208	387.5	393	395.7	392	4
	C010	2.2	2.1	2.1	2.133	0.058	406	443	435	428	19
	C011										
	C012										
	C013	7.65	7.76	8.03	7.813	0.196	432	483	487	467	31
	C014	< 97.00	< 97.00	< 97.00			352	350	362	355	6
	C016	11.7	10.3	12	11.333	0.907	1180	1239	1149	1189	46
	C017										
	C019										
	C020	2.37	2.1889	2.4238	2.328	0.123	385.675	384.755	390.192	387	3
	C021						400	425	406	410	13
	C022						447.9	443.8	432.5	441	8
	C023	2.67	2.47	2.4	2.513	0.140	311.23	316.88	300.75	310	8
	C026										
	C027	1.99	2.43	2.79	2.403	0.401	461	512	481	485	26
	C028	1.8	1.7	1.7	1.733	0.058	417	401	395	404	11
	C029						417.5	418.6	417.9	418	1
	C031										
	C032										
	C033						414.97	412.94	417.25	415	2
	C035	2.34	2.33	2.38	2.350	0.026	394	383	400	392	9
	C039						464	427	442	444	19
	C043										
	C044						353	359	353	355	3
	C045										
	C046										
	C047	2.24	2.25	2.25	2.247	0.006					
	C050										
	C051										
	C053	27.8	14	15.8	19.200	7.502	476	501	452	476	25
	C054										
	C055										
Community Results		Consensus Mean			2.437		Consensus Mean			412	
		Consensus Standard Deviation			0.902		Consensus Standard Deviation			60	
		Maximum			19.200		Maximum			1189	
		Minimum			1.733		Minimum			310	
		N			11		N			17	

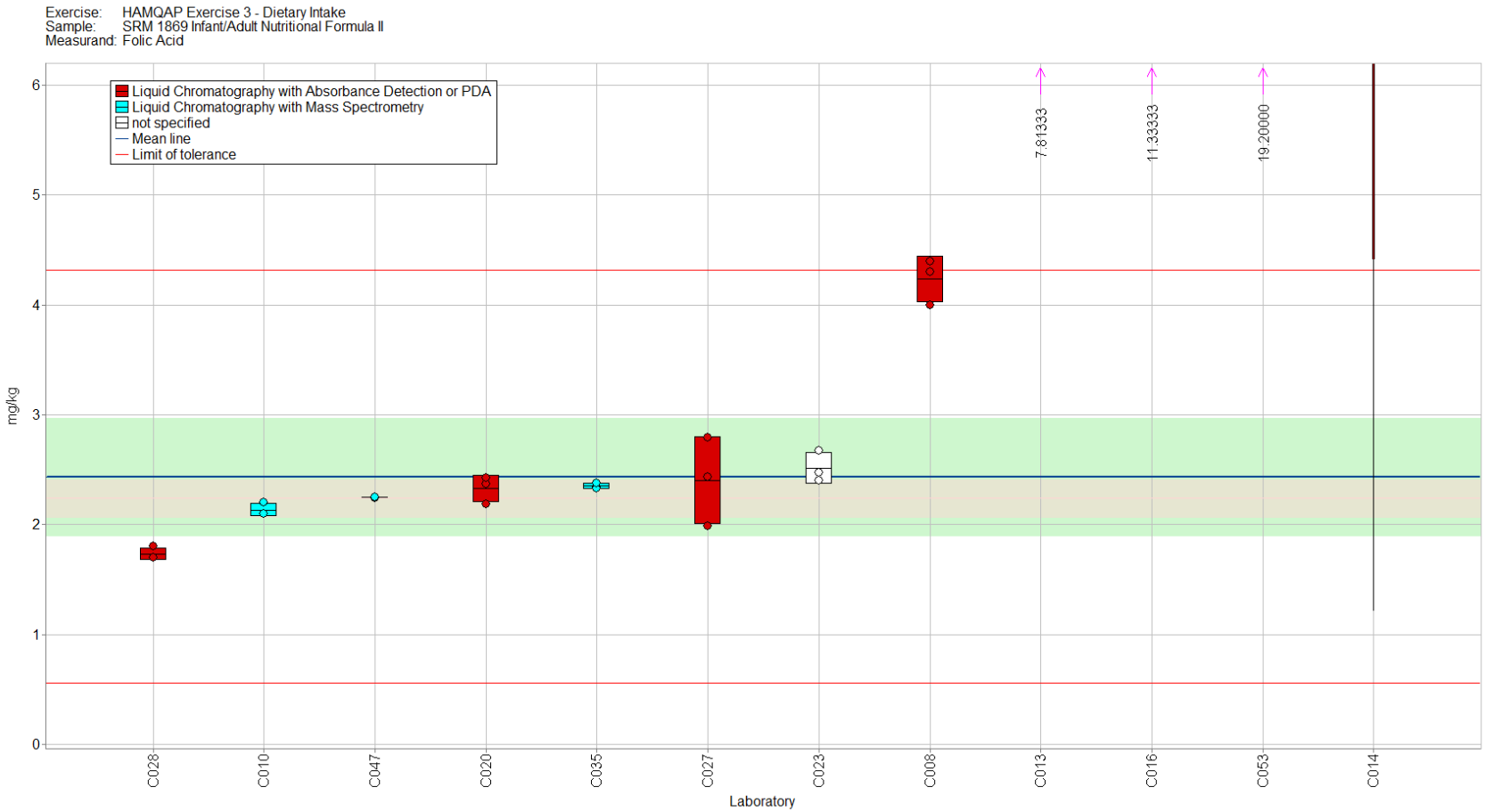

Figure 3-1. Folic acid in SRM 1869 Infant/Adult Nutritional Formula II (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line represents the consensus mean, and the green shaded region represents the consensus mean bounded by twice the consensus standard error. The solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty (U_{NIST}) and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$.

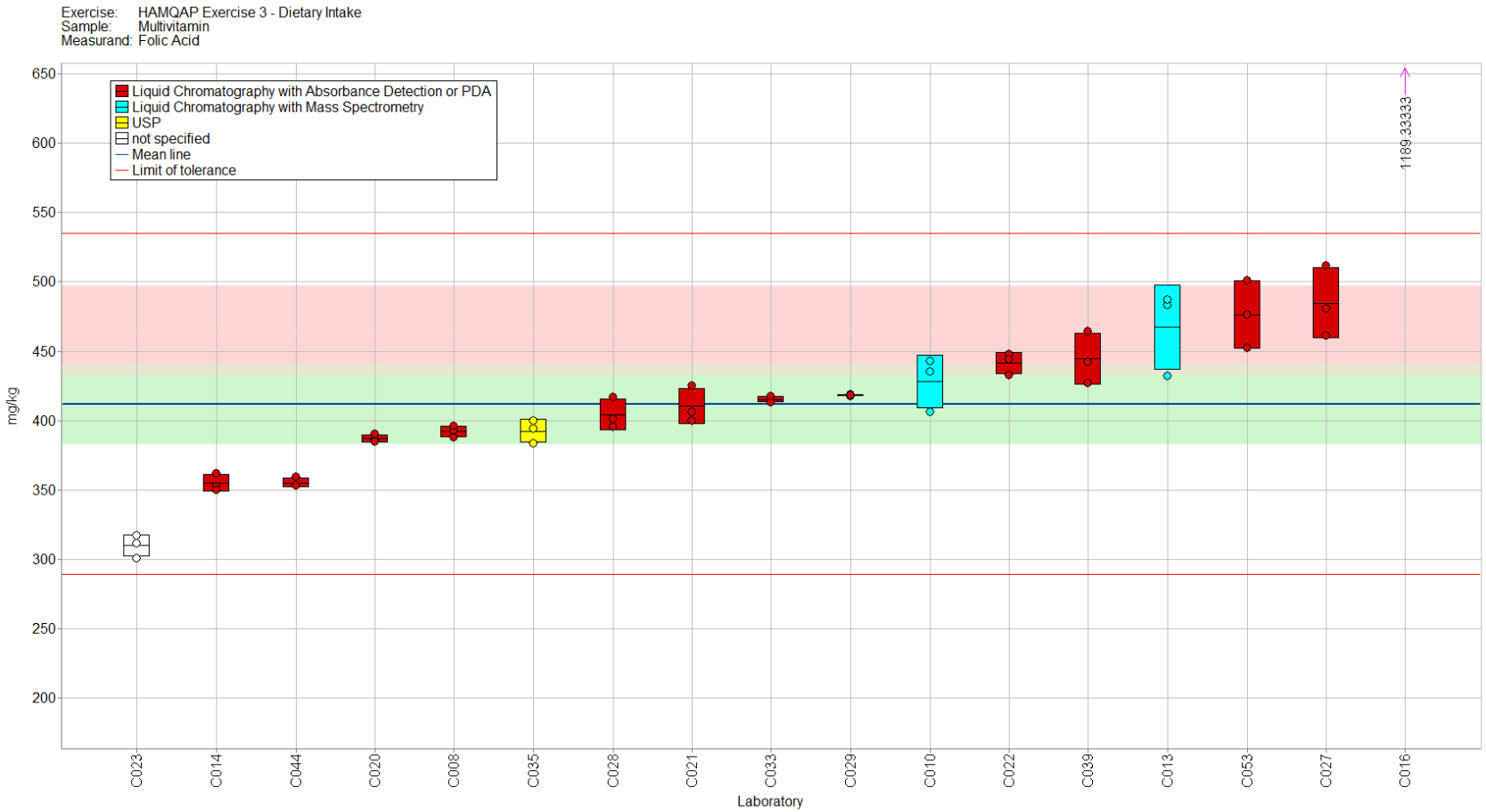

Figure 3-2. Folic acid in Multivitamin (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line represents the consensus mean, and the green shaded region represents the consensus mean bounded by twice the consensus standard error. The solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty (U_{NIST}) and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$. The NIST-target value assigned using results from the manufacturer of the material.

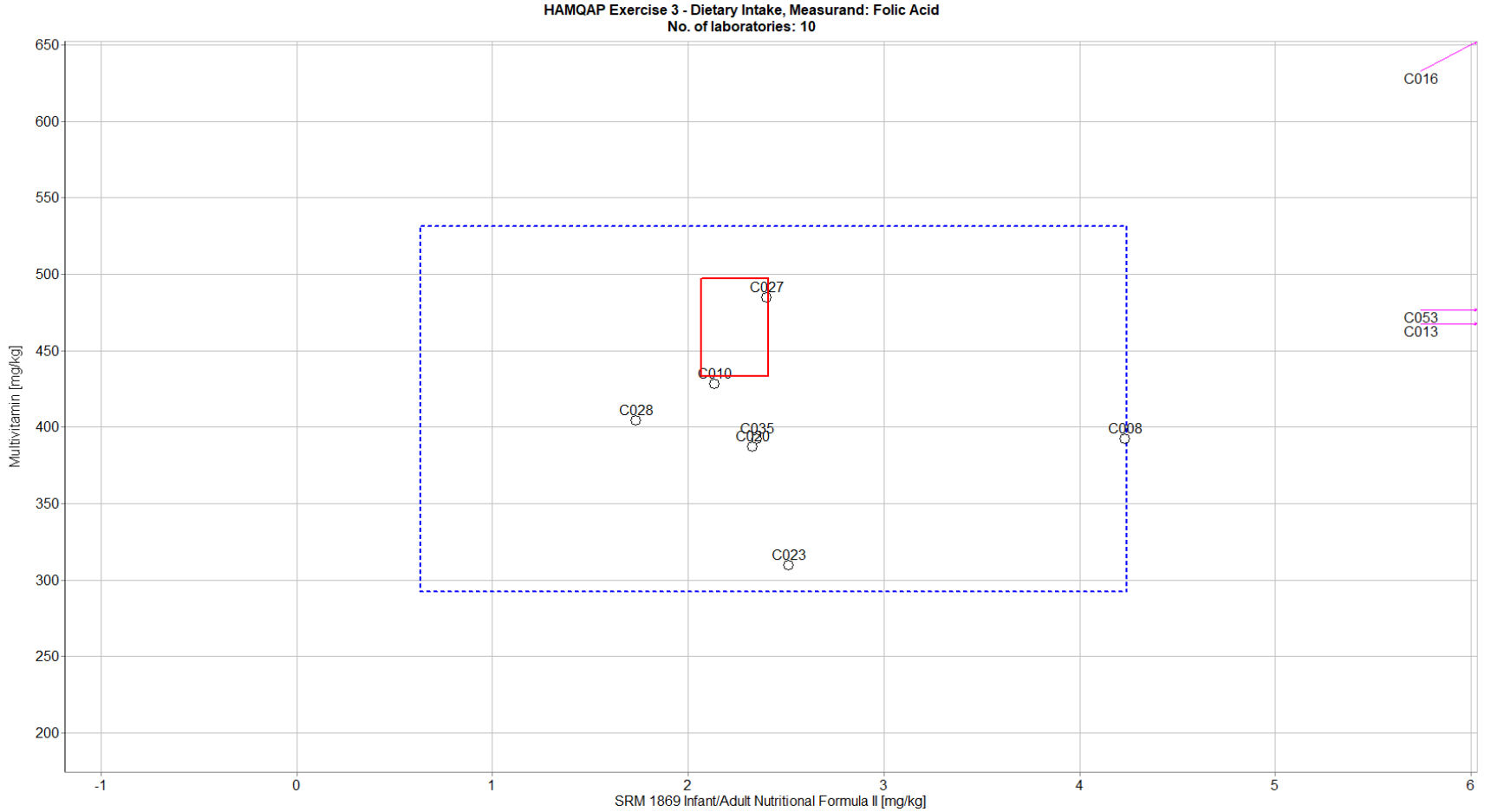

Figure 3-3. Laboratory means for folic acid in SRM 1869 Infant/Adult Nutritional Formula II and Multivitamin (sample/sample comparison view). In this view, the individual laboratory mean for one sample (SRM 1869) is compared to the individual laboratory mean for a second sample (multivitamin). The solid red box represents the NIST range of tolerance for the two samples, SRM 1869 (x-axis) and multivitamin (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, $|Z_{\mathrm{NIST}}| \leq 2$. The dotted blue box represents the consensus range of tolerance for SRM 1869 (x-axis) and multivitamin (y-axis), calculated as the values above and below the consensus means that result in an acceptable Z'_{comm} score, $|Z'_{\mathrm{comm}}| \leq 2$.

Table 3-3. Data summary table for 5-methyltetrahydrofolate in infant formula and multivitamin.

		5-methyltetrahydrofolate									
		SRM 1869 Infant/Adult Nutritional Formula II (mg/kg)					Multivitamin (mg/kg)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target										
	C002										
	C013										
	C014	< 130	< 130	< 130							
	C017										
	C020										
	C022										
	C023										
	C027	0	0	0							
	C028	3.6	3.1	3.8	3.50	0.36					
	C029										
	C031										
	C032										
	C039										
	C043										
	C046										
	C047										
	C050										
	C054										
	C055										
Community Results		Consensus Mean					Consensus Mean				
		Consensus Standard Deviation					Consensus Standard Deviation				
		Maximum			3.50		Maximum				
		Minimum			3.50		Minimum				
		N			1		N			0	

Human Metabolites Sample Information

Human Serum C. Participants were provided with three vials, each containing 1 mL of frozen human serum. Ascorbic acid was added to the pooled, off-the-clot serum to stabilize folates, and the serum was blended, bottled in 1 mL aliquots, and stored at –80 °C. Before use, participants were instructed to allow the material to thaw at room temperature for at least 30 min prior to sampling, use the material immediately after thawing, gently mix the contents prior to removal of a test portion for analysis, and use a sample size appropriate for their usual in-house method of analysis. Participants were asked to avoid exposing the material to direct UV light, to store the material at or below –80 °C, and to prepare one sample and report one value from each vial provided. The approximate analyte levels were not reported to participants prior to the study. The NIST-determined values for total folates, folic acid, 5-methyltetrahydrofolate, tetrahydrofolate, and MeFox in SRM 3949 Folate Vitamers in Frozen Human Serum (Level 1) were assigned using ID-LC-MS/MS results from NIST and CDC. The NIST-determined values and uncertainties for total folates, folic acid, 5-methyltetrahydrofolate, tetrahydrofolate, and MeFox in SRM 3949 (Level 1) are provided in the table below.

Analyte	NIST-Determined Mass Concentration in SRM 3949 (Level 1) (nmol/L)
Total Folates	17.0 ± 0.4
Folic Acid	1.00 ± 0.32
5-Methyltetrahydrofolate	14.69 ± 2.18
Tetrahydrofolate	1.14 ± 0.18
MeFox	1.58 ± 0.78

Human Serum D. Participants were provided with three vials, each containing 1 mL of frozen human serum. To obtain a measurable level of 5-formyltetrahydrofolate, the pooled, off-the-clot serum was spiked with exogenous 5-formyltetrahydrofolate. Ascorbic acid was also added to the pooled serum to stabilize folates, and the serum was blended, bottled in 1 mL aliquots, and stored at –80 °C. Before use, participants were instructed to allow the material to thaw at room temperature for at least 30 min prior to sampling, use the material immediately after thawing, gently mix the contents prior to removal of a test portion for analysis, and use a sample size appropriate for their usual in-house method of analysis. Participants were asked to avoid exposing the material to direct UV light, to store the material at or below –80 °C, and to prepare one sample and report one value from each vial provided. The approximate analyte levels were not reported to participants prior to the study. The NIST-determined values for total folates, folic acid, 5-methyltetrahydrofolate, 5-formyltetrahydrofolate, tetrahydrofolate, and MeFox in SRM 3949 Folate Vitamers in Frozen Human Serum (Level 3) were assigned using ID-LC-MS/MS results from NIST and CDC. The NIST-determined values and uncertainties for total folates, folic acid, 5-methyltetrahydrofolate, 5-formyltetrahydrofolate, tetrahydrofolate, and MeFox in SRM 3949 (Level 3) are provided in the table below.

Analyte	NIST-Determined Mass Concentration in SRM 3949 (Level 3) (nmol/L)
Total Folates	41.8 ± 0.5
Folic Acid	4.67 ± 1.01
5-Methyltetrahydrofolate	29.27 ± 3.77
5-Formyltetrahydrofolate	3.39 ± 1.86
Tetrahydrofolate	1.39 ± 0.99
MeFox	2.22 ± 0.55

Human Metabolites Study Results

- Thirteen laboratories enrolled in this exercise and received samples. Not all laboratories measured and reported results for every analyte in the study. The enrollment and reporting statistics for the study are described in the table below.

Analyte	Number of Laboratories Requesting Samples	Number of Laboratories Reporting Results (Percent Participation) SRM 3949 (Level 1)	SRM 3949 (Level 3)
Total Folates	8	3 (38 %)	3 (38 %)
Folic Acid	11	1 (9 %)	2 (18 %)
5-Methyltetrahydrofolate	5	1 (20 %)	1 (20 %)
5-Formyltetrahydrofolate	6	1 (17 %)	2 (33 %)
5,10-Methenyltetrahydrofolate	6	1 (17 %)	1 (17 %)
Tetrahydrofolate	6	1 (33 %)	1 (33 %)
MeFox	7	1 (14 %)	1 (14 %)

- The consensus mean for folic acid was within the target range for the higher-level sample, with high between-laboratory variability (76 % RSD).
- The consensus means for total folates were above the target ranges for both samples, but the consensus ranges do overlap with the target ranges. The between-laboratory variability was acceptable for total folates in both samples (14 % and 16 % RSD).
- Most laboratories reported using LC-MS/MS methods to determine folates in both serum samples. One laboratory did not specify the method used.

Human Metabolites Technical Recommendations

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

- 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.

- Folate calibration solutions that are value assigned based on UV absorbance spectrophotometry may contain significant impurities that impact quantification. Additional purity correction by LC-absorbance analysis of calibration solutions may resolve some biases.
- The various folates have different stabilities in solution. Solution pH or the addition of antioxidants, such as ascorbic acid, should be considered to ensure all folate calibration solutions are stable throughout the duration of the sample analysis.
- 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 as the requested vitamin form.

Table 3-4. Individualized data summary table (NIST) for folates in human serum.

National Institute of Standards and Technology

HAMQAP Exercise 3 - 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*
Total Folates	SRM 3949 Folate Vitamers in Frozen Human Serum (Level 1)	nmol/L		17	0.4		0	3	18.8	2.6	17	0.4
Total Folates	SRM 3949 Folate Vitamers in Frozen Human Serum (Level 3)	nmol/L		41.8	0.5		0	3	47.8	7.4	41.8	0.5
Folic Acid	SRM 3949 Folate Vitamers in Frozen Human Serum (Level 1)	nmol/L		1	0.32		0	1			1	0.32
Folic Acid	SRM 3949 Folate Vitamers in Frozen Human Serum (Level 3)	nmol/L		0	1		0	2	4.6	3.5	4.67	1.01
5-methyltetrahydrofolate	SRM 3949 Folate Vitamers in Frozen Human Serum (Level 1)	nmol/L		14.7	2.2		0	1			14.7	2.18
5-methyltetrahydrofolate	SRM 3949 Folate Vitamers in Frozen Human Serum (Level 3)	nmol/L		29.3	3.8		0	1			29.3	3.77
5-formyltetrahydrofolate	SRM 3949 Folate Vitamers in Frozen Human Serum (Level 1)	nmol/L						1				
5-formyltetrahydrofolate	SRM 3949 Folate Vitamers in Frozen Human Serum (Level 3)	nmol/L		3.39	1.86		0	2	5.00	0.64	3.39	1.86
5,10-methenyltetrahydrofolate	SRM 3949 Folate Vitamers in Frozen Human Serum (Level 1)	nmol/L						1				
5,10-methenyltetrahydrofolate	SRM 3949 Folate Vitamers in Frozen Human Serum (Level 3)	nmol/L						1				
Tetrahydrofolate	SRM 3949 Folate Vitamers in Frozen Human Serum (Level 1)	nmol/L		1.14	0.18		0	1			1.14	0.18
Tetrahydrofolate	SRM 3949 Folate Vitamers in Frozen Human Serum (Level 3)	nmol/L		1.39	0.99		0	1			1.39	0.99
MeFox	SRM 3949 Folate Vitamers in Frozen Human Serum (Level 1)	nmol/L		1.58	0.78		0	1			1.58	0.78
MeFox	SRM 3949 Folate Vitamers in Frozen Human Serum (Level 3)	nmol/L		2.22	0.55		0	1			2.22	0.55

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 3-5. Data summary table for 5,10-methenyltetrahydrofolate in human serum.

		5,10-methenyltetrahydrofolate									
		SRM 3949 Folate Vitamers in Frozen Human Serum (Level 1) (nmol/L)					SRM 3949 Folate Vitamers in Frozen Human Serum (Level 3) (nmol/L)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target										
	C027										
	C032										
	C054										
	C056	0.14	0.14	0.14	0.14	0.00	0.38	0.4	0.48	0.42	0.05
	C057										
	C064	< 0.0000	< 0.0000	< 0.0000			< 0.0000	< 0.0000	< 0.0000		
Community Results		Consensus Mean					Consensus Mean				
		Consensus Standard Deviation					Consensus Standard Deviation				
		Maximum			0.14		Maximum			0.42	
		Minimum			0.14		Minimum			0.42	
		N			1		N			1	

Table 3-6. Data summary table for 5-formyltetrahydrofolate in human serum.

		5-formyltetrahydrofolate									
		SRM 3949 Folate Vitamers in Frozen Human Serum (Level 1) (nmol/L)					SRM 3949 Folate Vitamers in Frozen Human Serum (Level 3) (nmol/L)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target										
	C027										
	C032										
	C054										
	C056	0.14	0.14	0.14	0.14	0.00	5.26	5.67	4.89	5.27	0.39
	C057										
	C064	< 0.0000	< 0.0000	< 0.0000			4.73	4.87	4.56	4.72	0.16
Community Results		Consensus Mean					Consensus Mean			5.00	
		Consensus Standard Deviation					Consensus Standard Deviation			0.64	
		Maximum			0.14		Maximum			5.27	
		Minimum			0.14		Minimum			4.72	
		N			1		N			2	

Table 3-7. Data summary table for 5-methyltetrahydrofolate in human serum.

		5-methyltetrahydrofolate									
		SRM 3949 Folate Vitamers in Frozen Human Serum (Level 1) (nmol/L)					SRM 3949 Folate Vitamers in Frozen Human Serum (Level 3) (nmol/L)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target				14.7	2.2				29.3	3.8
	C027										
	C032										
	C054										
	C056	15.6	15.9	14.7	15.4	0.6	29.9	33.4	27.4	30.2	3.0
	C057										
Community Results		Consensus Mean					Consensus Mean				
		Consensus Standard Deviation					Consensus Standard Deviation				
		Maximum			15.4		Maximum			30.2	
		Minimum			15.4		Minimum			30.2	
		N			1		N			1	

Table 3-8. Data summary table for folic acid in human serum.

		Folic Acid									
		SRM 3949 Folate Vitamers in Frozen Human Serum (Level 1) (nmol/L)					SRM 3949 Folate Vitamers in Frozen Human Serum (Level 3) (nmol/L)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target				1.00	0.32				4.7	1.0
	C012										
	C027										
	C031										
	C032										
	C033										
	C051										
	C054										
	C056	1.22	1.21	1.05	1.16	0.10	5.71	6.09	5.09	5.6	0.5
	C057										
	C062										
	C064	< 0.0000	< 0.0000	< 0.0000			3.51	3.88	3.59	3.7	0.2
Community Results		Consensus Mean					Consensus Mean			4.6	
		Consensus Standard Deviation					Consensus Standard Deviation			3.5	
		Maximum			1.16		Maximum			5.6	
		Minimum			1.16		Minimum			3.7	
		N			1		N			2	

Table 3-9. Data summary table for MeFox in human serum.

		MeFox									
		SRM 3949 Folate Vitamers in Frozen Human Serum (Level 1) (nmol/L)					SRM 3949 Folate Vitamers in Frozen Human Serum (Level 3) (nmol/L)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target				1.58	0.78				2.22	0.55
	C005										
	C027										
	C032										
	C054										
	C056	1.23	1.24	1.22	1.23	0.01	2	2.06	1.88	1.98	0.09165
	C057										
	C064										
Community Results		Consensus Mean					Consensus Mean				
		Consensus Standard Deviation					Consensus Standard Deviation				
		Maximum			1.23		Maximum			1.98	
		Minimum			1.23		Minimum			1.98	
		N			1		N			1	

Table 3-10. Data summary table for tetrahydrofolate in human serum.

		Tetrahydrofolate									
		SRM 3949 Folate Vitamers in Frozen Human Serum (Level 1) (nmol/L)					SRM 3949 Folate Vitamers in Frozen Human Serum (Level 3) (nmol/L)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target				1.14	0.18				1.39	0.99
	C027										
	C032										
	C054										
	C056	0.31	0.18	0.18	0.22	0.08	0.62	0.65	0.49	0.59	0.09
	C057										
	C064	< 0.0000	< 0.0000	< 0.0000			< 0.0000	< 0.0000	< 0.0000		
Community Results		Consensus Mean					Consensus Mean				
		Consensus Standard Deviation					Consensus Standard Deviation				
		Maximum			0.22		Maximum			0.59	
		Minimum			0.22		Minimum			0.59	
		N			1		N			1	

Table 3-11. Data summary table for total folates in human serum.

		Total Folates									
		SRM 3949 Folate Vitamers in Frozen Human Serum (Level 1) (nmol/L)					SRM 3949 Folate Vitamers in Frozen Human Serum (Level 3) (nmol/L)				
	Lab	**A**	**B**	**C**	**Avg**	**SD**	**A**	**B**	**C**	**Avg**	**SD**
Individual Results	Target				17.00	0.40				41.80	0.50
	C005	20.18	19.02	19.82	19.67	0.59	49.49	47.33	50.17	49.00	1.48
	C027										
	C029										
	C032										
	C054										
	C056	17.4	17.6	16.2	17.07	0.76	41.9	46.2	38.4	42.17	3.91
	C057										
	C064	18.14	21.25	19.7	19.70	1.56	52.68	53.13	50.73	52.18	1.28
Community Results		Consensus Mean			18.81		Consensus Mean			47.48	
		Consensus Standard Deviation			2.57		Consensus Standard Deviation			7.36	
		Maximum			19.70		Maximum			52.18	
		Minimum			17.07		Minimum			42.17	
		N			3		N			3	

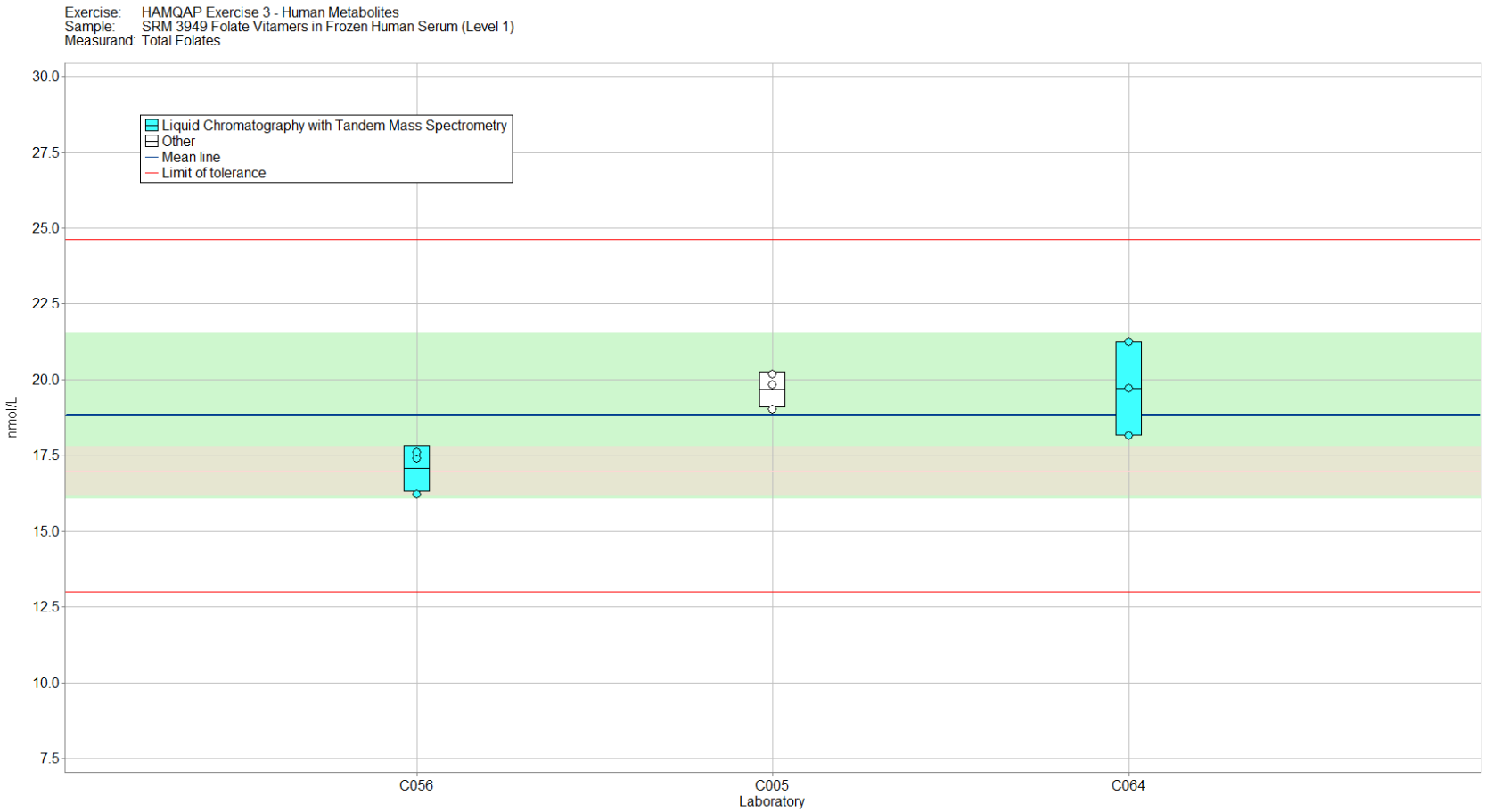

Figure 3-4. Total folates in SRM 3949 Folate Vitamers in Frozen Human Serum (Level 1) (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical 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'_{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_{NIST}) and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$.

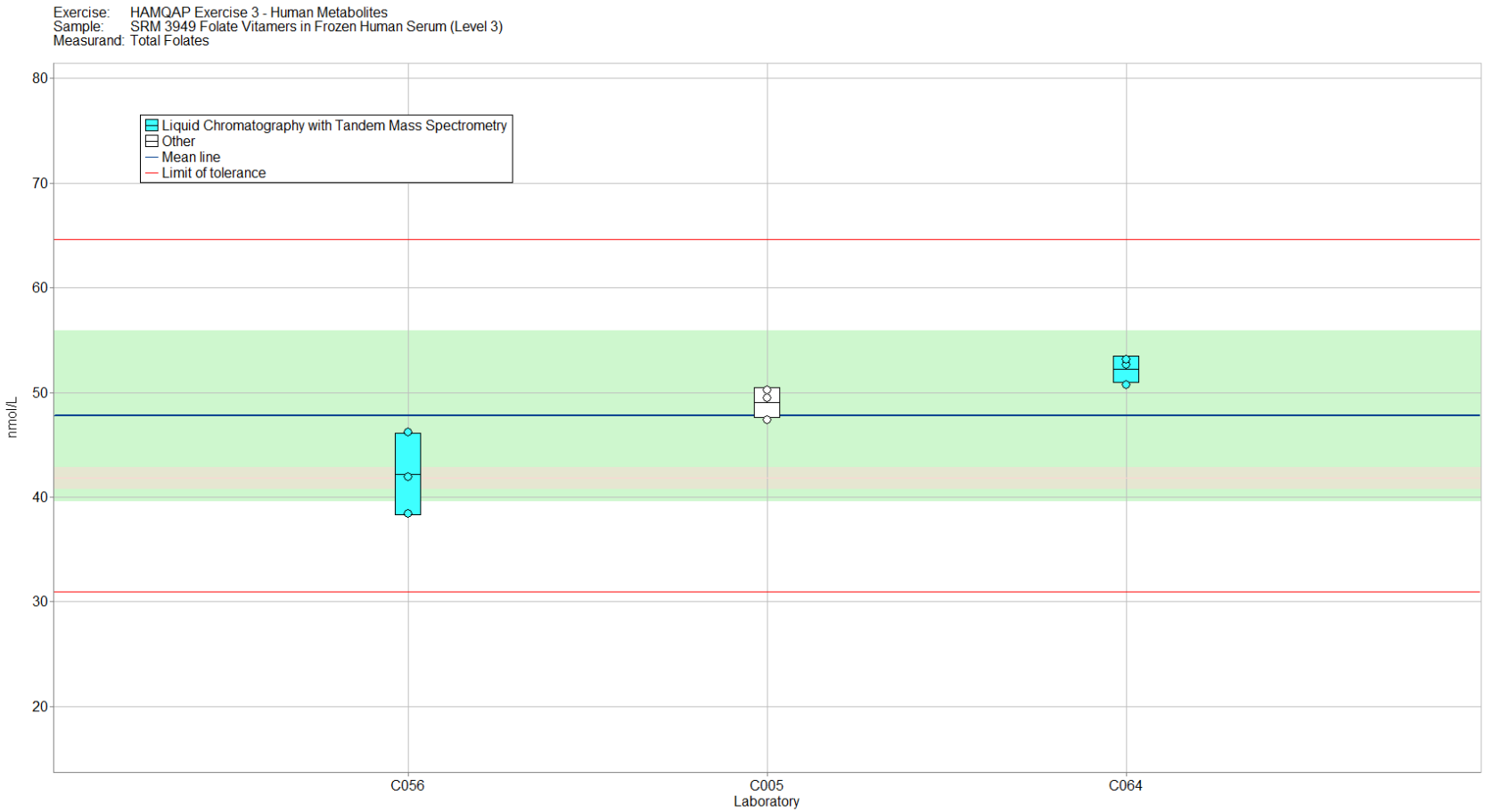

Figure 3-5. Total folates in SRM 3949 Folate Vitamers in Frozen Human Serum (Level 3) (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid 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'_{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_{NIST}) and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$.

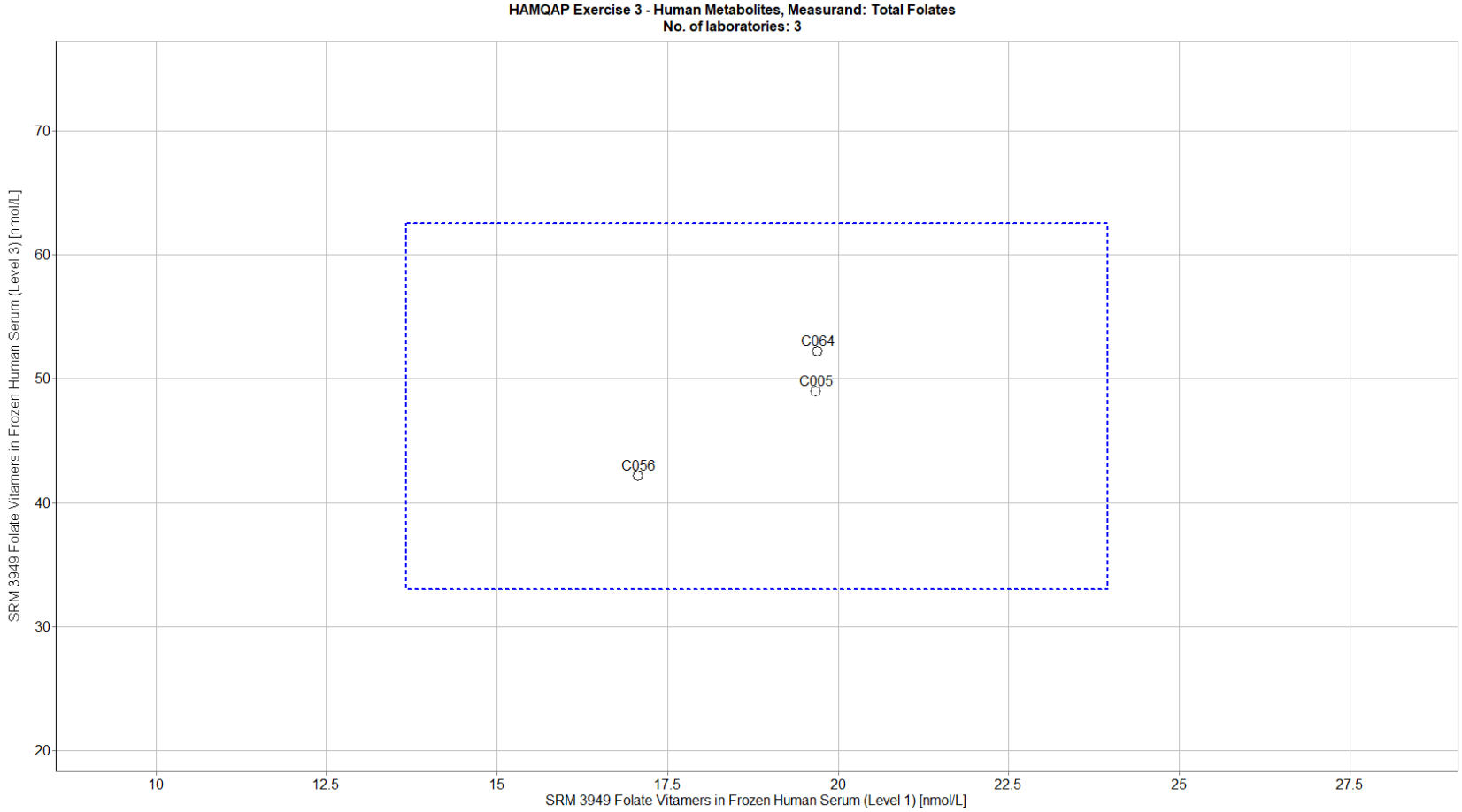

Figure 3-6. Laboratory means for total folates in SRM 3949 Folate Vitamers in Frozen Human Serum (Level 1) and SRM 3949 Folate Vitamers in Frozen Human Serum (Level 3) (sample/sample comparison view). In this view, the individual laboratory mean for one sample (Level 1) is compared to the mean for a second sample (Level 3). The dotted blue box represents the consensus range of tolerance for Level 1 (x-axis) and Level 3 (y-axis), calculated as the values above and below the consensus means that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$.

Water-Soluble Vitamins Overall Study Comparison

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

- Food laboratories have extensive experience in measuring folic acid or total folate in various food commodities, typically using a microbiological approach or LC-absorbance. With improvements in technology for sample preparation, separation, and detection, many laboratories are interested in measuring individual forms of each vitamin (fortified and endogenous, etc.) to better understand the health impact of foods and supplements. The biggest challenge for these laboratories may be in detecting low levels of endogenous forms in the presence of high levels of fortified forms.
- Clinical laboratories had lower participation for the determination of folates, but the data reported for folic acid and total folates was overall of good quality. Very little data was collected for minor folates, which may be a result of methods that lack sensitivity or specificity for these vitamers.

SECTION 4: FAT-SOLUBLE VITAMINS (Carotenoids)

Study Overview

In this study, participants were provided with two samples for dietary intake, a multivitamin and SRM 3251 Saw Palmetto (*Serenoa repens*) Extract, and two samples for human metabolism, bovine serum and SRM 968e Fat-Soluble Vitamins, Carotenoids, and Cholesterol in Human Serum. Participants were asked to use in-house analytical methods to determine and report the mass fraction (mg/kg) of as many carotenoids as possible, including lutein, zeaxanthin, and total β-carotene for intake samples, and total lutein, total zeaxanthin, total β-carotene, total lycopene, β-cryptoxanthin, total α-carotene, *trans*-lycopene, *trans*-β-carotene, total α-cryptoxanthin, and total *cis*-β-carotene for the metabolite samples. Carotenoids are responsible for the yellow and orange colors found in many fruits and vegetables. β-carotene is converted to vitamin A within the body. Vitamin A is essential to maintain normal human vision, for the function of the immune and reproductive systems, as well as the heart, lungs, kidneys, and other organs.[13] Additionally, the xanthophylls lutein and zeaxanthin have been shown to be important in slowing the progression of age related macular degeneration.[14] Carotenoids are found in a variety of foods and also in supplements and each carotenoid can be found in several forms. The bioavailability of the *cis*- and *trans*- forms of the vitamins differs, highlighting the importance of understanding the forms present in foods and supplements. Esterified forms of carotenoids are de-esterified in the body, where health status is measured by quantitative determination in serum. To maintain proper growth and function, health professionals may recommend dietary changes or supplementation to individuals with low serum levels of these vitamers.

Dietary Intake Sample Information

Multivitamin. Participants were provided with three bottles, each containing 30 multivitamin tablets. Before use, participants were instructed to grind all 30 tablets and mix the resulting powder thoroughly prior to removal of a test portion for analysis, and to use a sample size of 1 g to 1.5 g. Participants were asked to store the original, unopened bottles at controlled room temperature, 20 °C to 25 °C. After grinding, participants were asked to store the material at –20 °C. Participants were instructed to prepare one sample and report one value from each bottle provided. Approximate analyte levels were not reported to participants prior to the study. The NIST-target values for lutein and total β-carotene were assigned using results from the manufacturer of the material. The NIST-target values and uncertainties for lutein and total β-carotene are provided in the table below on an as-received basis. A target value for zeaxanthin in the multivitamin has not been determined by NIST.

Analyte	NIST-Target Mass Fraction in Multivitamin (mg/g)
Lutein	197 ± 26
Total β-carotene	682 ± 102

[13] Vitamin A Fact Sheet for Consumers. National Institutes of Health Office of Dietary Supplements. https://ods.od.nih.gov/factsheets/VitaminA-Consumer/ (accessed February 2019).

[14] Dietary Supplements for Eye Conditions. National Center for Complementary and Integrative Health. https://nccih.nih.gov/health/providers/digest/ds-eye-conditions (accessed November 2019).

Saw Palmetto (Serenoa repens) Extract. Participants were provided with three ampoules, each containing approximately 1 mL of saw palmetto extract. Before use, participants were instructed to gently mix the contents by inverting the ampoule several times and to use a minimum sample size of 100 mg. 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 ampoule provided. The approximate analyte levels were not reported to participants prior to the study. The certified value for total β-carotene was assigned using results from two LC-absorbance methods at NIST. The certified value and uncertainty for total β-carotene in the saw palmetto extract are provided in the table below on an as-received basis.

Analyte	Certified Mass Fraction in SRM 3251 (mg/g)
Total β-carotene	46.8 ± 4.6

Dietary Intake Study Results

- The table below summarizes the participation statistics for the study of carotenoids in multivitamin and saw palmetto extract.

Analyte	Number of Laboratories Requesting Samples	Number of Laboratories Reporting Results (Percent Participation)	
		Multivitamin	SRM 3251
Lutein	29	12 (41 %)	
Zeaxanthin	23	8 (35 %)	
Total β-carotene	33	17 (52 %)	16 (48 %)

- The consensus means were within the target ranges for lutein in the multivitamin and for total β-carotene in both the multivitamin and SRM 3251.
- The between-laboratory variabilities are reported below.

Analyte	Between-Laboratory Variability (% RSD)	
	Multivitamin	SRM 3251
Lutein	17 %	--
Zeaxanthin	34 %	--
Total β-carotene	28 %	22 %

- Most laboratories reported using solvent extraction followed by LC-absorbance as their sample preparation and analytical method for the determination of the carotenoids. One laboratory did not specify an analytical method.
  - For β-carotene in the multivitamin, two laboratories reported dilution as the sample preparation method used. Open beaker digestion, enzymatic hydrolysis, and saponification/base hydrolysis were each reported by one laboratory as the sample preparation method.

- For β-carotene in the saw palmetto extract, seven laboratories reported using dilution and seven laboratories reported using solvent extraction for sample preparation. Enzymatic hydrolysis, saponification/base hydrolysis, or no sample preparation method were each reported by one laboratory as the sample preparation method.
- Most laboratories reported using solvent extraction for lutein and total zeaxanthin in the multivitamin. One laboratory reported using dilution and one laboratory reported using open beaker digestion.

Dietary Intake Technical Recommendations

The following recommendations are based on results obtained from the participants in this study. In some cases, too few data were reported to allow for meaningful conclusions to be drawn. Figures were chosen to show results according to analytical method or sample preparation method depending on observed trends.

- The overall results for total β-carotene in multivitamin and saw palmetto indicate that laboratories perform well in these types of samples.
- The overall results for lutein in the multivitamin indicate that laboratories perform well in this type of sample. One laboratory was found consistently to be an outlier with values higher than the consensus mean. This laboratory also reported using dilution as the sample preparation method. Laboratories should consider using an extraction method to potentially reduce interferences that are causing positive bias.
- Reporting values for total β-carotene can be challenging for laboratories.
  - Understanding the requested information (e.g., units and chemical form) as well as correctly identifying the geometric carotenoid isomers if they are separated by the analytical method used is critical for accurate reporting.
  - Understanding and establishing calibrant purity is not straightforward. Calibrant purity and concentration assignment is best established using spectrophotometric approaches.
- Laboratories were asked to report results for total β-carotene, lutein, and zeaxanthin, but not for *cis*- or *trans*- isomers of the carotenoids. While reporting total carotenoids is acceptable for labeling purposes, labeling must be consistent with the specifications set for the product. *Trans*-isomers are reported to have higher bioactivity and bioavailability than *cis*-isomers, making this measurement capability desirable.

Table 4-1. Individualized data summary table (NIST) for carotenoids in saw palmetto extract and multivitamin.

National Institute of Standards and Technology

HAMQAP Exercise 3 - 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*
Total beta-Carotene	Multivitamin		mg/kg	680	100		0	17	530	150	682	102
Total beta-Carotene	SRM 3251 Saw Palmetto (*Serenoa repens*) Extract		mg/kg	46.8	4.6		0	16	50	11	46.8	4.6
Total Lutein	Multivitamin		mg/kg	200	26		0	12	190	33	197	26
Total Zeaxanthin	Multivitamin		mg/kg					8	18.1	6.2		

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 4-2. Data summary table for total β-carotene in saw palmetto extract and multivitamin. Data points highlighted in red have been flagged as potential outliers (e.g., Grubb and/or Cochran) by the NIST software package.

		Total β-carotene									
		SRM 3251 Saw Palmetto (*Serenoa repens*) Extract (mg/kg)					Multivitamin (mg/kg)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target				46.8	4.6				682	102
	C002										
	C005	45.5	41.7	44.1	43.8	1.9	514	613	526	551	54
	C007	56.2	56.1	54.7	55.7	0.8	574	568	579	574	6
	C008	54.3	54	53.4	53.9	0.5					
	C010						573	499	565	546	41
	C012										
	C013	38.4	42	3.78	28.1	21.1	274	281	281	279	4
	C014	0	0	0			554	562	541	552	11
	C016	131.8	116.2	159.6	135.9	22.0	812	785	816	804	17
	C019										
	C020	47.9857	48.8558	48.9618	48.6	0.5	606	639	595	613	23
	C021						512	547	514	525	20
	C026										
	C027	58.69	55.21	57.19	57.0	1.7	1054	943	811	936	122
	C028	61	65	63	63.0	2.0	592	587	589	589	3
	C029	54.6	53.98	54.21	54.3	0.3	584	646	685	638	51
	C031										
	C032										
	C033										
	C035	57	55.9	58	57.0	1.1					
	C036	32.9	40.5	39.6	37.7	4.2	481	490	468	480	11
	C038	45.5	48.7	50.5	48.2	2.5	372	299	233	301	69
	C039	59.83	52.91	52.58	55.1	4.1	640	566	648	618	45
	C040	51.33	53.98	49.42	51.6	2.3	59	102	79	80	22
	C042	25.75	28.5	26.09	26.8	1.5	178	111	175	155	38
	C044	56.6	57.3	55.9	56.6	0.7	600	653	627	627	27
	C046										
	C047										
	C050										
	C051										
	C053										
	C054										
	C055										
Community Results		Consensus Mean			49.9		Consensus Mean			532	
		Consensus Standard Deviation			11.2		Consensus Standard Deviation			153	
		Maximum			135.9		Maximum			936	
		Minimum			26.8		Minimum			80	
		N			16		N			17	

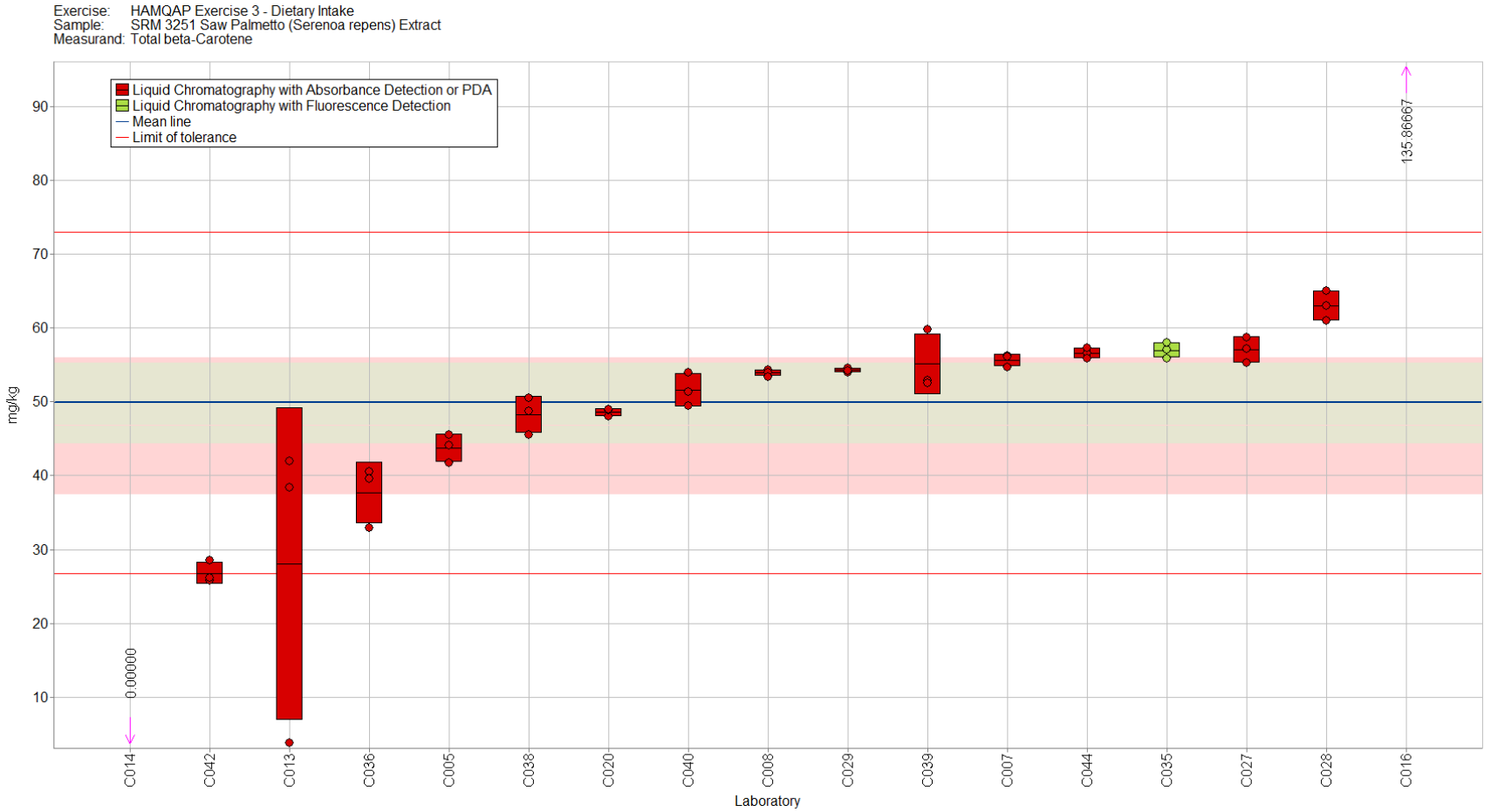

Figure 4-1. Total β-carotene in SRM 3251 Saw Palmetto Extract (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. 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'_{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_{NIST}) and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$.

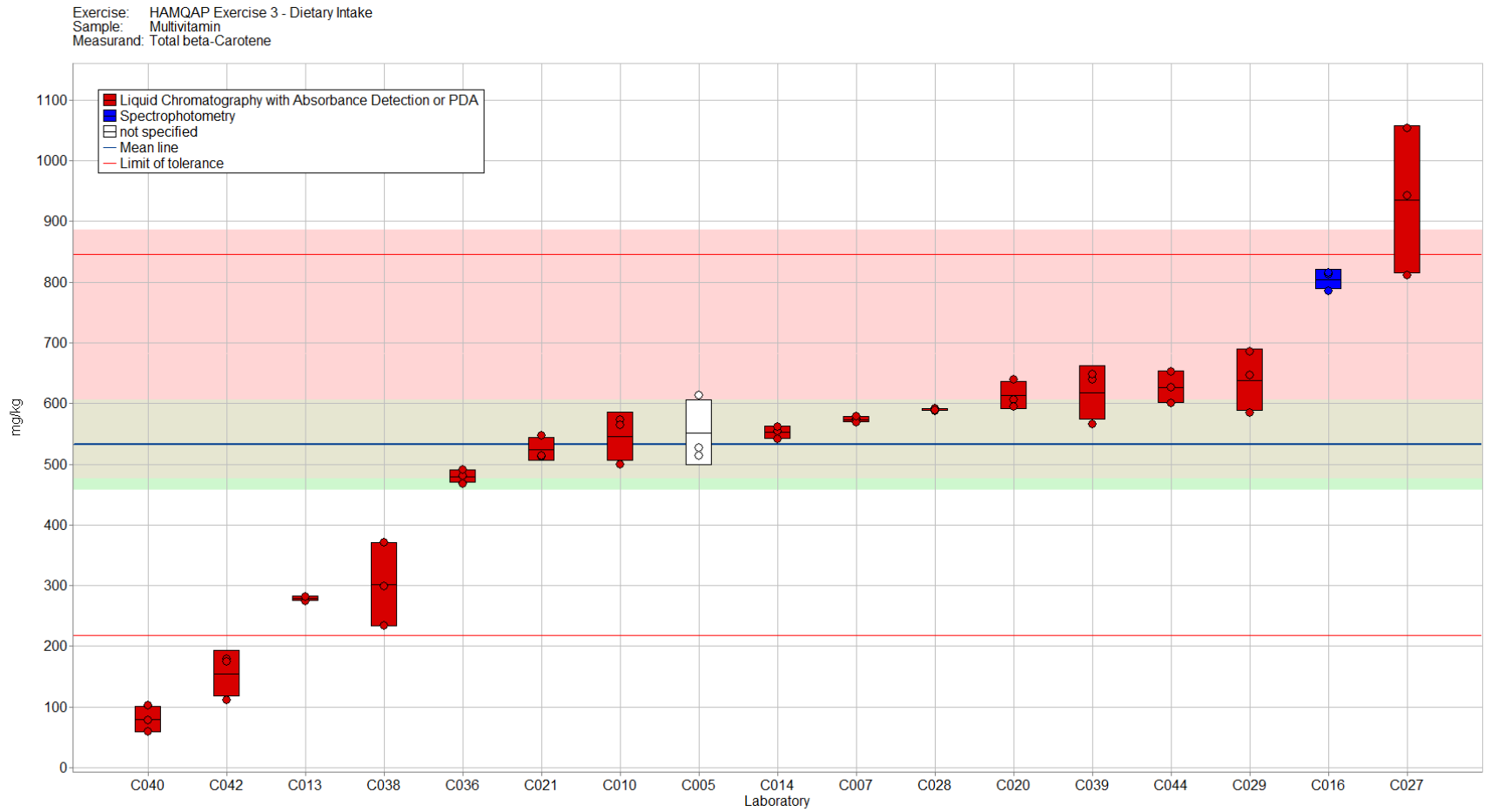

Figure 4-2. Total β-carotene in Multivitamin (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The solid 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'_{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_{NIST}) and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$. The NIST-target value assigned using results from the manufacturer of the material.

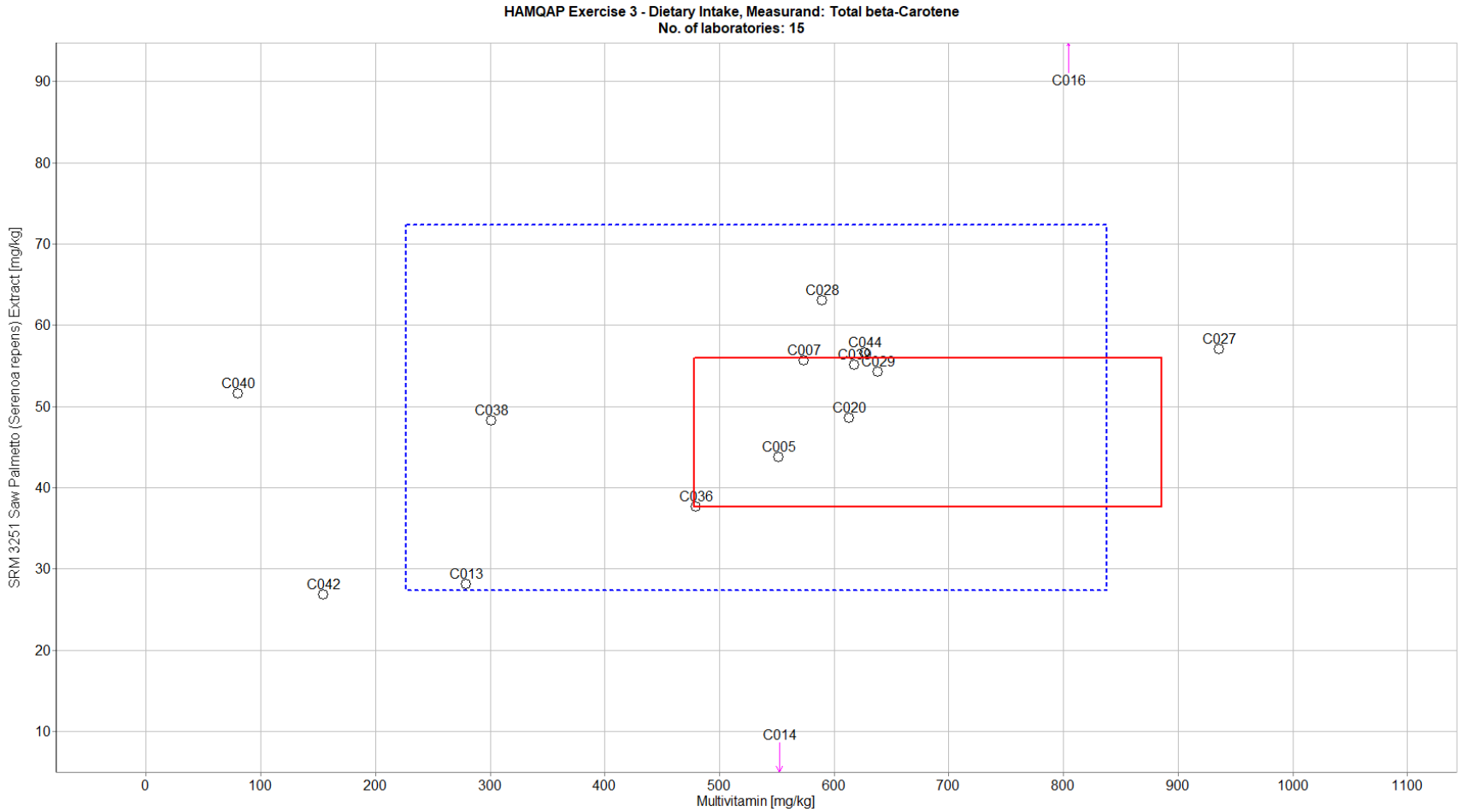

Figure 4-3. Laboratory means for total β-carotene in Multivitamin and SRM 3251 Saw Palmetto Extract (sample/sample comparison view). In this view, the individual laboratory mean for one sample (multivitamin) is compared to the mean for a second sample (SRM 3251). The solid red box represents the NIST range of tolerance for the two samples, multivitamin (x-axis) and SRM 3251 (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, $|Z_{\mathrm{NIST}}| \leq 2$. The dotted blue box represents the consensus range of tolerance for multivitamin (x-axis) and SRM 3251 (y-axis), calculated as the values above and below the consensus means that result in an acceptable Z'_{comm} score, $|Z'_{\mathrm{comm}}| \leq 2$.

Table 4-3. Data summary table for total lutein in multivitamin. Data points highlighted in red have been flagged as potential outliers (e.g., Grubb and/or Cochran) by the NIST software package.

		Total Lutein				
		Multivitamin (mg/kg)				
	Lab	A	B	C	Avg	SD
Individual Results	Target				197	26
	C002					
	C005	166.55	187.7	205.36	187	19
	C007	195	205	221	207	13
	C008					
	C012					
	C013					
	C014					
	C020					
	C021	165.2	178	153	165	13
	C025	209	200	213	207	7
	C026					
	C027	524.9	547.8	518.8	531	15
	C028	210	190	200	200	10
	C029	218	227	225	223	5
	C032					
	C033					
	C036	200.3	195.9	215.5	204	10
	C039	186.5	205.6	195.4	196	10
	C040	6.41	22.94	11.78	14	8
	C042	128.7	166	189.5	161	31
	C044	187	175	188	183	7
	C045					
	C046					
	C047					
	C050					
	C051					
	C053					
	C054					
	C055					
Community Results		Consensus Mean			193	
		Consensus Standard Deviation			33	
		Maximum			531	
		Minimum			14	
		N			12	

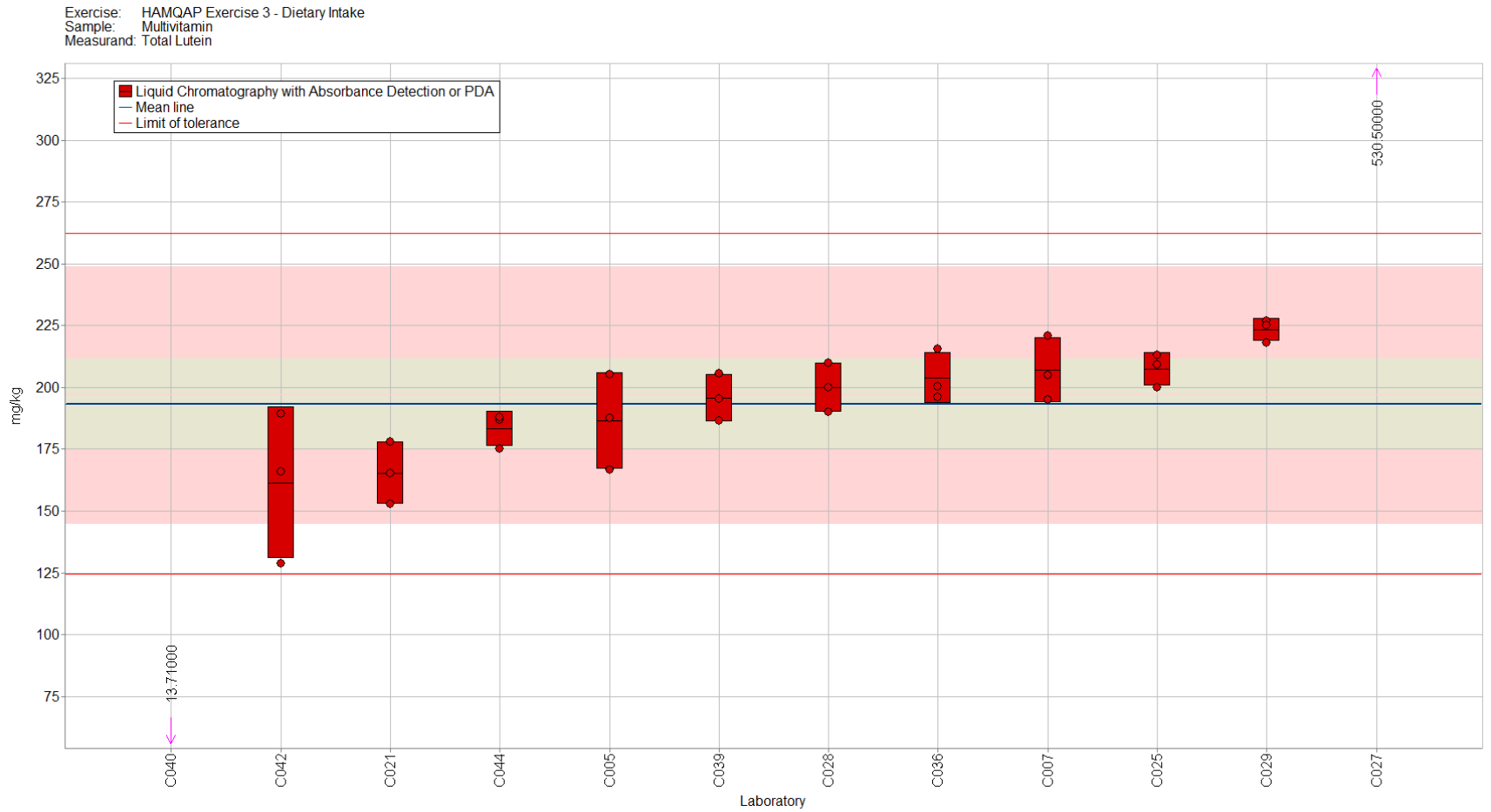

Figure 4-4. Total lutein in Multivitamin (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line represents 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'_{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_{NIST}) and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$. The NIST-target value assigned using results from the manufacturer of the material.

Table 4-4. Data summary table for total zeaxanthin in multivitamin. Data points highlighted in red have been flagged as potential outliers (e.g., Grubb and/or Cochran) by the NIST software package.

		Total Zeaxanthin				
		Multivitamin (mg/kg)				
	Lab	A	B	C	Avg	SD
Individual Results	Target					
	C002					
	C005					
	C007	16	16	16	16.0	0.0
	C008					
	C012					
	C014					
	C021	17.2	18.8	15.1	17.0	1.9
	C025	18.2	20.6	19.5	19.4	1.2
	C026					
	C027	41.6	41.9	39.6	41.0	1.3
	C028	20	20	20	20.0	0.0
	C029	20.2	22.7	20.9	21.3	1.3
	C032					
	C033					
	C039					
	C040	6.41	22.94	11.78	13.7	8.4
	C042	11.9	14.9	15.8	14.2	2.0
	C045					
	C046					
	C047					
	C050					
	C054					
	C055					
Community Results		Consensus Mean			18.1	
		Consensus Standard Deviation			6.2	
		Maximum			41.0	
		Minimum			13.7	
		N			8	

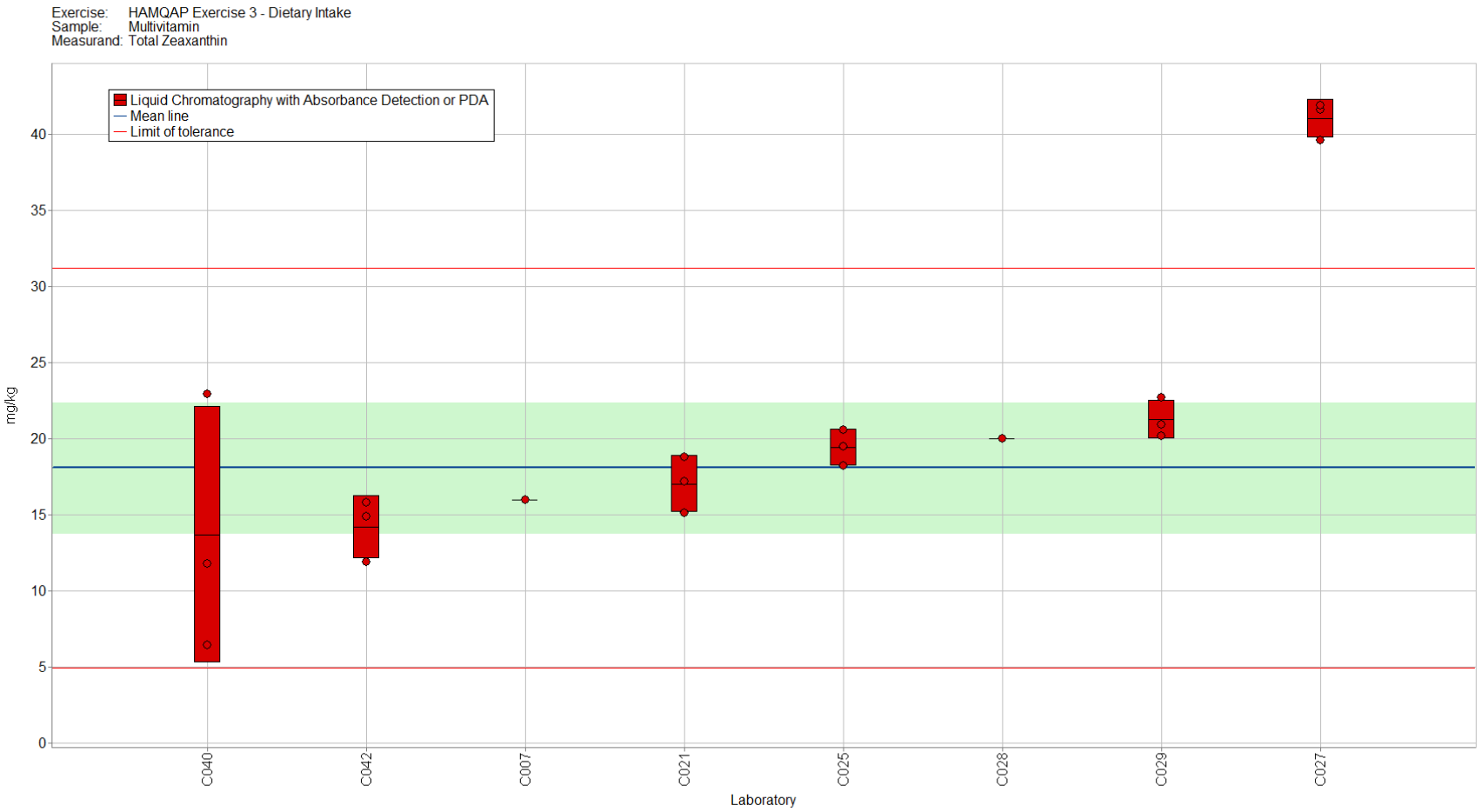

Figure 4-5. Total zeaxanthin in Multivitamin (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line 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'_{comm} score, $|Z'_{\text{comm}}| \leq 2$. A NIST value has not been determined in this material.

Human Metabolites Sample Information

Bovine Serum. Participants were provided with three vials, each containing 1 mL of frozen bovine serum. Serum was purchased from a commercial provider, describing the product as off-the-clot serum from bovine blood of grass-fed donor animals. No analytes were spiked into or stripped from the serum. The bovine serum was filtered, blended, and bottled in 1 mL aliquots and stored at –80 °C. Before use, participants were instructed to allow the material to thaw at room temperature for at least 30 min prior to sampling, use the material immediately after thawing, gently mix the contents prior to removal of a test portion for analysis, and use a sample size appropriate for their usual in-house method of analysis. Participants were asked to avoid exposing the material to direct UV light, to store the material at or below –80 °C, and to prepare one sample and report one value from each vial provided. The approximate analyte levels were not reported to participants prior to the study, and target values for carotenoids in bovine serum have not been determined at NIST.

Human Serum E. Participants were provided with three vials, each containing 1 mL of frozen human serum. This material was prepared from source plasma using blending protocols to optimize levels of various metabolites. Analyte concentrations were not adjusted by spiking. The source plasma was frozen at -80 °C, thawed, and twice filtered through Whatman 541 filter paper to convert it to serum. The serum was pooled, blended, bottled in 1 mL aliquots, and stored at -80 °C. Before use, participants were instructed to allow the material to thaw at room temperature for at least 30 min prior to sampling, use the material immediately after thawing, gently mix the contents prior to removal of a test portion for analysis, and use a sample size appropriate for their usual in-house method of analysis. Participants were asked to avoid exposing the material to direct UV light, to store the material at or below -80 °C, and to prepare one sample and report one value from each vial provided. The approximate analyte levels were not reported to participants prior to the study. The NIST-determined values for carotenoids in SRM 968e were assigned using results from two LC-absorbance methods at NIST and results from collaborating laboratories. The certified values and uncertainties for total lutein, total zeaxanthin, total β-carotene, and total β-cryptoxanthin in SRM 968e Level 1 are provided in the table below. The reference values and uncertainties for total lycopene, total α-carotene, *trans*-lycopene, and *trans*-β-carotene in SRM 968e Level 1 are also provided in the table below. The information values for total α-cryptoxanthin and total *cis*-β-carotene in SRM 968e Level 1, without associated uncertainties, are provided in the table below.

Analyte	NIST-Determined Mass Concentration in SRM 968e Level 1 (µg/mL)
Total α-carotene	0.011 ± 0.005
Trans-β-carotene	0.088 ± 0.01
Total *cis*-β-carotene	0.005
Total β-carotene	0.099 ± 0.018
Total lutein	0.067 ± 0.008
Trans-lycopene	0.135 ± 0.04
Total lycopene	0.234 ± 0.095
Total zeaxanthin	0.031 ± 0.005
Total α-cryptoxanthin	0.016
β-cryptoxanthin	0.041 ± 0.006

Human Metabolites Study Results

- Between eight and fourteen laboratories enrolled in this exercise and received samples. Not all laboratories measured and reported results for every analyte in the study. Low rates of return were observed for most analytes (all < 50%) and were especially low for *trans*-β-carotene, total *cis*-β-carotene, *trans*-lycopene, and total α-cryptoxanthin. The table below summarizes the participation statistics for the study of carotenoids in bovine serum and human serum.

Analyte	Number of Laboratories Requesting Samples	Number of Laboratories Reporting Results (Percent Participation)	
		Bovine Serum	SRM 968e
Total α-carotene	14	6 (43 %)	5 (36 %)
Trans-β-carotene	10	3 (30 %)	4 (40 %)
Total *cis*-β-carotene	9	2 (22 %)	2 (22 %)
Total β-carotene	14	5 (36 %)	5 (36 %)
Total lutein	14	6 (43 %)	6 (43 %)
Trans-lycopene	11	1 (9 %)	3 (27 %)
Total lycopene	14	3 (21 %)	6 (43 %)
Total zeaxanthin	14	4 (29 %)	5 (36 %)
Total α-cryptoxanthin	8	1 (13 %)	1 (13 %)
β-cryptoxanthin	13	5 (38 %)	6 (46 %)

- The consensus means for total β-carotene, *trans*-lycopene, and total lycopene were within the target ranges for SRM 968e, but the between-laboratory variability was high (88 %, 47 %, and 37 %, respectively). For all other analytes the consensus mean bias from the target value

ranged from 24 % to 94 % and the between-laboratory variabilities ranged from 36 % to 325 %.

- The between -laboratory variability was good for total *cis*-β-carotene and total β-carotene in the bovine serum at 13.5 % and 26 % RSD, respectively. For all other analytes, the between-laboratory variabilities ranged from 30 % to 150 %.
- Most laboratories reported using solvent extraction and LC-absorbance or PDA to determine carotenoids in both serum samples. One laboratory used an unspecified sample preparation method.

Human Metabolites Technical Recommendations

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

- For the bovine serum, the highest return rates (over 30 % participation) were for α-carotene, β-carotene, *trans*-β-carotene, β-cryptoxanthin, and lutein. For SRM 968e, the highest return rates (over 30 % participation) were for α-carotene, β-carotene, *trans*-β-carotene, β-cryptoxanthin, lycopene, zeaxanthin, and lutein. While most of the laboratories agreed reasonably well (between 25 % and 40 % RSD), the use of appropriate calibration materials and quality assurance samples to establish that a method is in control and performing correctly remains important.
- 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 as the requested vitamer.

Table 4-5. Individualized data summary table (NIST) for carotenoids in bovine serum and human serum.

National Institute of Standards and Technology

HAMQAP Exercise 3 - 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*
Total alpha-Carotene	Bovine Serum		µg/mL					6	0.023	0.013		
Total alpha-Carotene	SRM 968e Fat-Soluble Vitamins, Carotenoids, and Cholesterol in Human Serum (Level 1)		µg/mL	0.011	0.005		0	6	0.0041	0.0052	0.011	0.005
trans-beta-Carotene	Bovine Serum		µg/mL					3	2.5	0.76		
trans-beta-Carotene	SRM 968e Fat-Soluble Vitamins, Carotenoids, and Cholesterol in Human Serum (Level 1)		µg/mL	0.09	0.01		0	4	0.109	0.047	0.088	0.01
Total cis-beta-Carotene	Bovine Serum		µg/mL					2	0.37	0.05		
Total cis-beta-Carotene	SRM 968e Fat-Soluble Vitamins, Carotenoids, and Cholesterol in Human Serum (Level 1)		µg/mL	0.005				2	0.04	0.13	0.005	
Total beta-Carotene	Bovine Serum		µg/mL					5	2.5	0.65		
Total beta-Carotene	SRM 968e Fat-Soluble Vitamins, Carotenoids, and Cholesterol in Human Serum (Level 1)		µg/mL	0.099	0.018		0	5	0.107	0.094	0.099	0.018
Total Lutein	Bovine Serum		µg/mL					6	0.04	0.016		
Total Lutein	SRM 968e Fat-Soluble Vitamins, Carotenoids, and Cholesterol in Human Serum (Level 1)		µg/mL	0.067	0.008		0	6	0.11	0.04	0.067	0.008
trans-Lycopene	Bovine Serum		µg/mL					1				
trans-Lycopene	SRM 968e Fat-Soluble Vitamins, Carotenoids, and Cholesterol in Human Serum (Level 1)		µg/mL	0.14	0.04		0	3	0.15	0.07	0.135	0.04
Total Lycopene	Bovine Serum		µg/mL					4	0.018	0.019		
Total Lycopene	SRM 968e Fat-Soluble Vitamins, Carotenoids, and Cholesterol in Human Serum (Level 1)		µg/mL	0.234	0.095		0	6	0.219	0.081	0.234	0.095
Total Zeaxanthin	Bovine Serum		µg/mL					4	0.0153	0.0092		
Total Zeaxanthin	SRM 968e Fat-Soluble Vitamins, Carotenoids, and Cholesterol in Human Serum (Level 1)		µg/mL	0.031	0.005		0	5	0.06	0.04	0.031	0.005
Total alpha-Cryptoxanthin	Bovine Serum		µg/mL					1				
Total alpha-Cryptoxanthin	SRM 968e Fat-Soluble Vitamins, Carotenoids, and Cholesterol in Human Serum (Level 1)		µg/mL	0.016				1			0.016	
beta-Cryptoxanthin	Bovine Serum		µg/mL					5	0.0262	0.0089		
beta-Cryptoxanthin	SRM 968e Fat-Soluble Vitamins, Carotenoids, and Cholesterol in Human Serum (Level 1)		µg/mL	0.041	0.006		0	6	0.059	0.025	0.041	0.006

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 4-6. Data summary table for total alpha-carotene in bovine serum and human serum. Data points highlighted in red have been flagged as potential outliers (e.g., Grubb and/or Cochran) by the NIST software package.

		Total alpha-Carotene									
		Bovine Serum (µg/mL)					SRM 968e Fat-Soluble Vitamins, Carotenoids, and Cholesterol in Human Serum (Level 1) (µg/mL)				
	Lab	**A**	**B**	**C**	**Avg**	**SD**	**A**	**B**	**C**	**Avg**	**SD**
Individual Results	Target									0.0110	0.0050
	C005	0.017	0.014	0.015	0.0153	0.0015	0.004	0.004	0.003	0.0037	0.0006
	C007										
	C015										
	C026	0.021	0.021	0.021	0.0210	0.0000	0.005	0.004	0.003	0.0040	0.0010
	C027										
	C029	0.035	0.036	0.036	0.0357	0.0006	0.068	0.068	0.071	0.0690	0.0017
	C032										
	C033										
	C040	0.0235	0.0249	0.0246	0.0243	0.0007	0.00151	0.00138	0.00134	0.0014	0.0001
	C042	0.125	0.121	0.125	0.1237	0.0023		0.002	0.001	0.0015	0.0007
	C054										
	C056										
	C057										
	C065	0.016	0.017	0.019	0.0173	0.0015	0.009	0.011	0.01	0.0100	0.0010
Community Results		Consensus Mean			0.0227		Consensus Mean			0.0041	
		Consensus Standard Deviation			0.0130		Consensus Standard Deviation			0.0052	
		Maximum			0.1237		Maximum			0.0690	
		Minimum			0.0153		Minimum			0.0014	
		N			6		N			6	

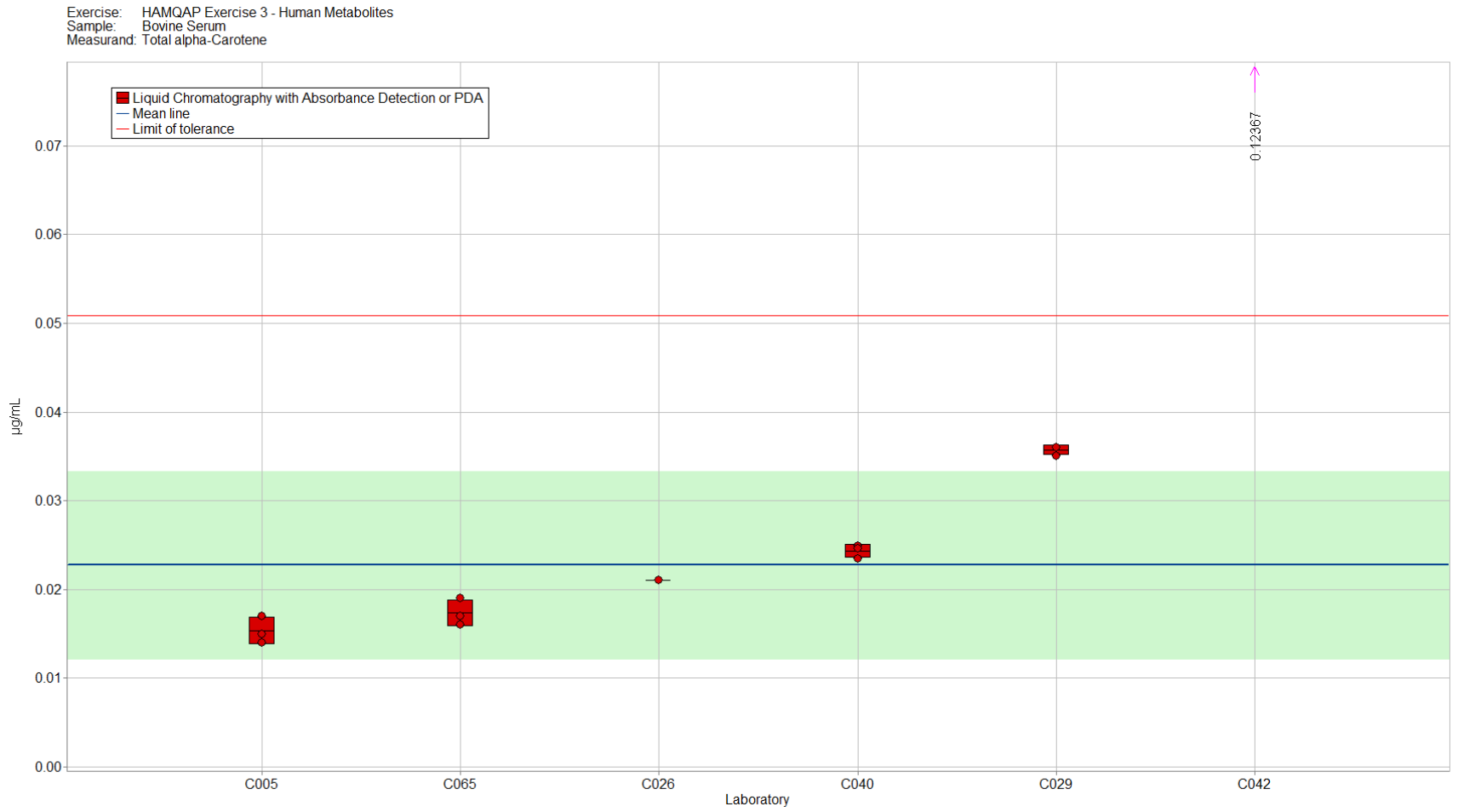

Figure 4-6. Total α-carotene in Bovine Serum (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line 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 and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$, with the lower range set at zero. A NIST value has not been determined in this material.

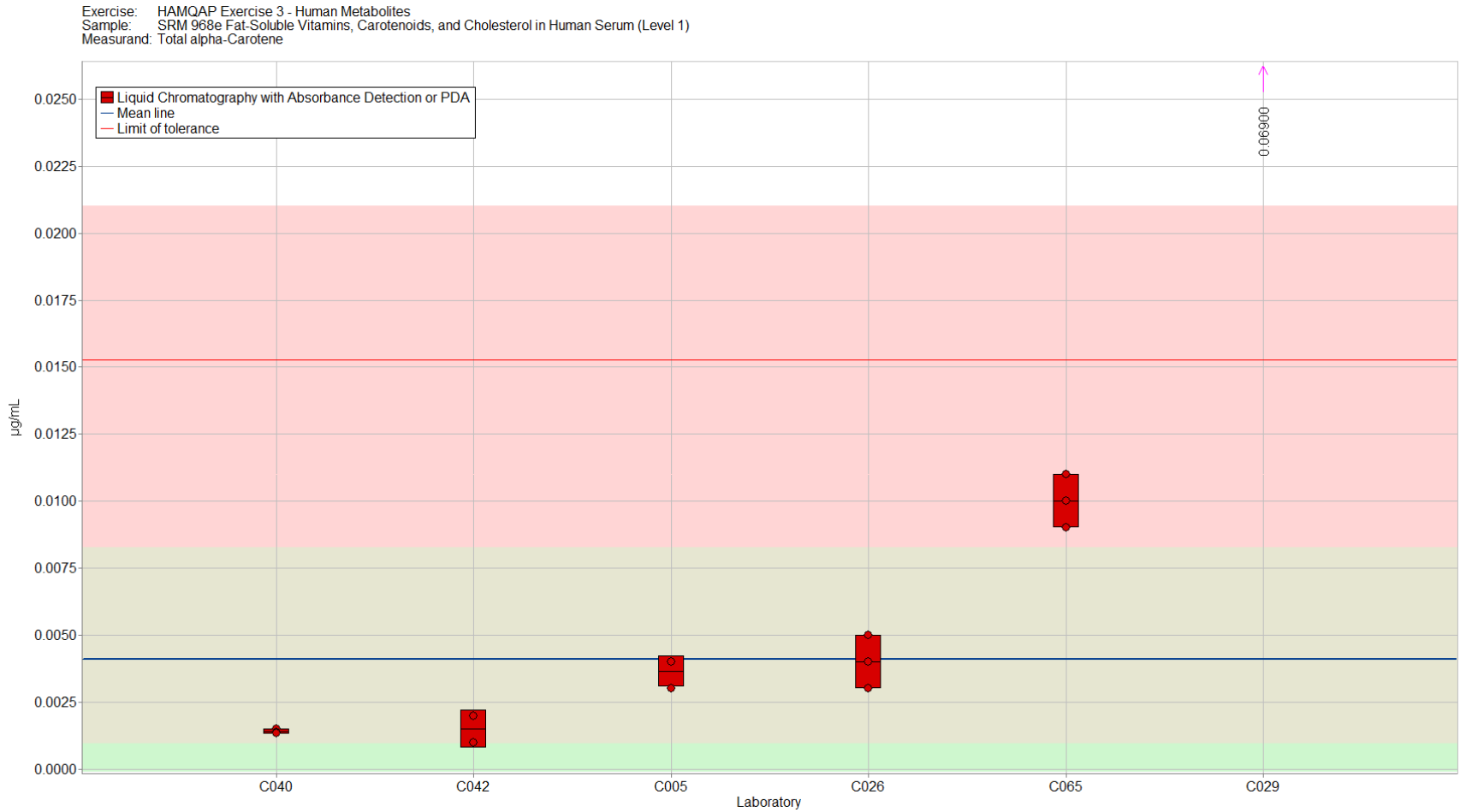

Figure 4-7. Total α-carotene in SRM 968e Fat-Soluble Vitamins, Carotenoids, and Cholesterol in Human Serum (Level 1) (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the 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 and below the consensus mean that result in an acceptable Z'_{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 its uncertainty (U_{NIST}) and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$.

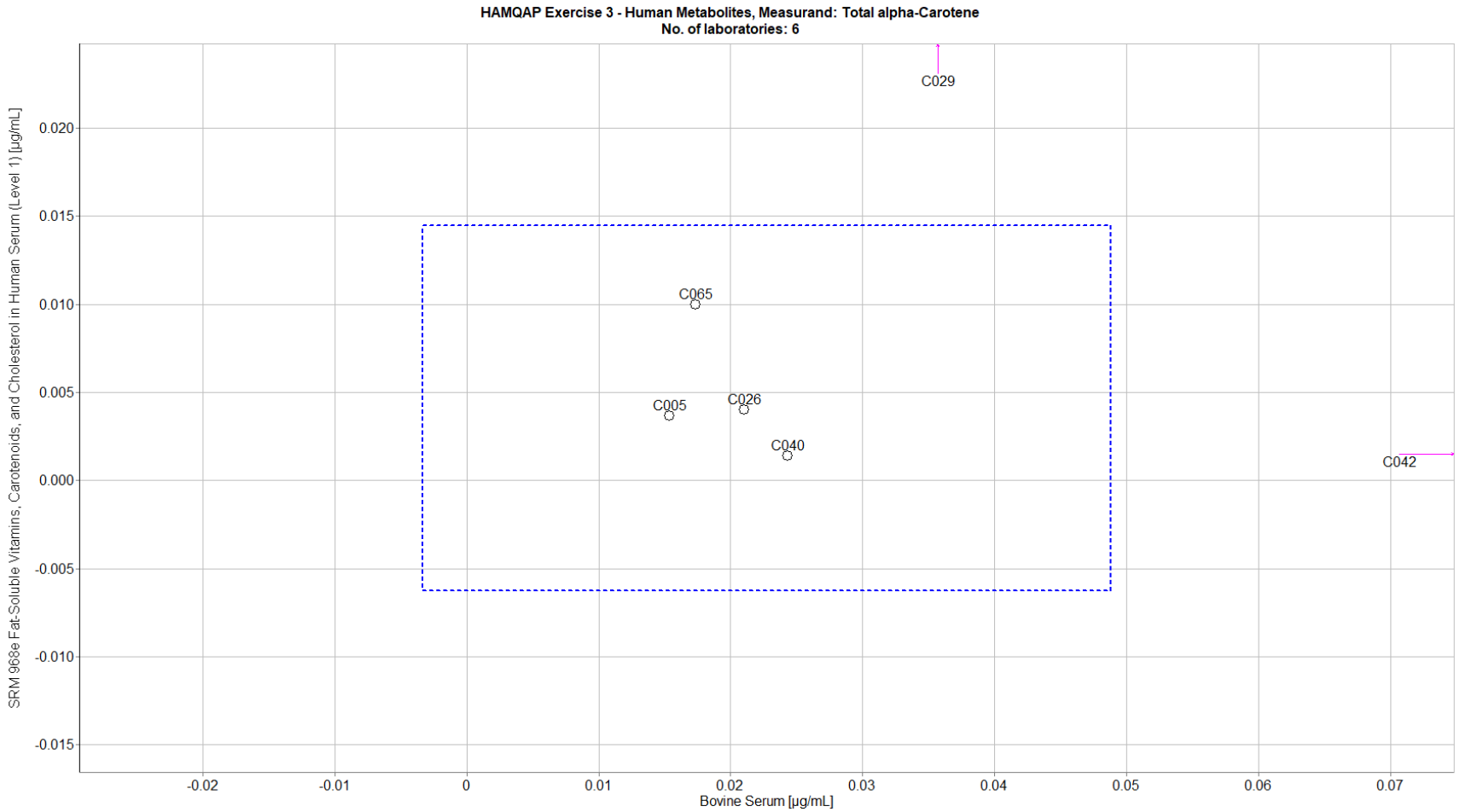

Figure 4-8. Laboratory means for total α-carotene in Bovine Serum and SRM 968e Fat-Soluble Vitamins, Carotenoids, and Cholesterol in Human Serum (Level 1) (sample/sample comparison view). In this view, the individual laboratory mean for one sample (bovine serum) is compared to the mean for a second sample (SRM 968e). The dotted blue box represents the consensus range of tolerance for bovine serum (x-axis) and SRM 968e (y-axis), calculated as the values above and below the consensus means that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$.

Table 4-7. Data summary table for *trans*-β-carotene in bovine serum and human serum.

		trans-beta-Carotene									
		Bovine Serum (µg/mL)					SRM 968e Fat-Soluble Vitamins, Carotenoids, and Cholesterol in Human Serum (Level 1) (µg/mL)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target									0.088	0.010
	C007						0.088	0.093	0.083	0.088	0.005
	C015										
	C026	2.386	2.356	2.402	2.381	0.023	0.091	0.094	0.094	0.093	0.002
	C027										
	C029	2.058	2.055	2.056	2.056	0.002	0.142	0.137	0.139	0.139	0.003
	C032										
	C033										
	C040	3.0445	3.0739	3.0462	3.055	0.017	0.1147	0.1152	0.1176	0.116	0.002
	C054										
	C056										
Community Results		Consensus Mean			2.498		Consensus Mean			0.109	
		Consensus Standard Deviation			0.765		Consensus Standard Deviation			0.047	
		Maximum			3.055		Maximum			0.139	
		Minimum			2.056		Minimum			0.088	
		N			3		N			4	

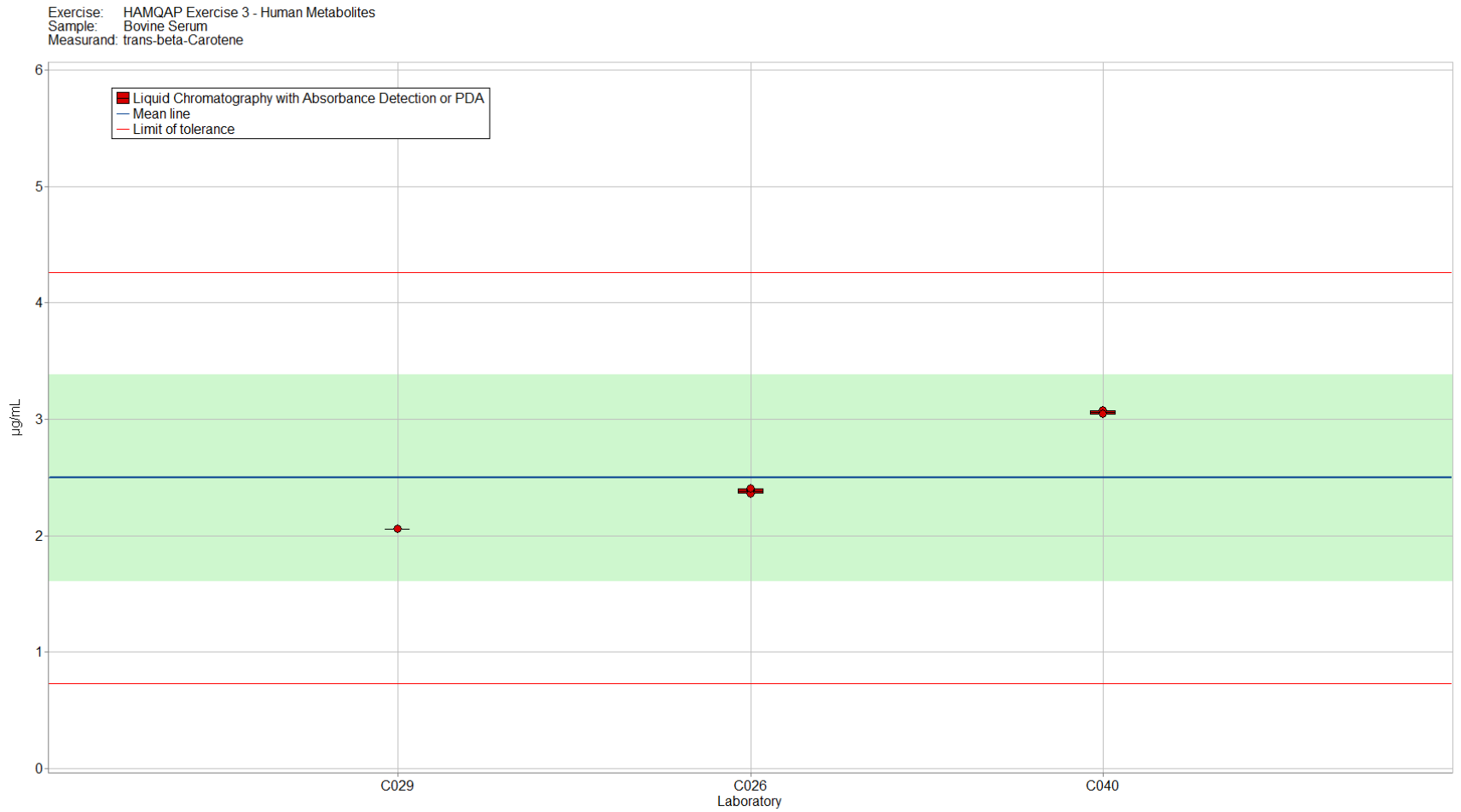

Figure 4-9. *Trans*-β-carotene in Bovine Serum (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line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

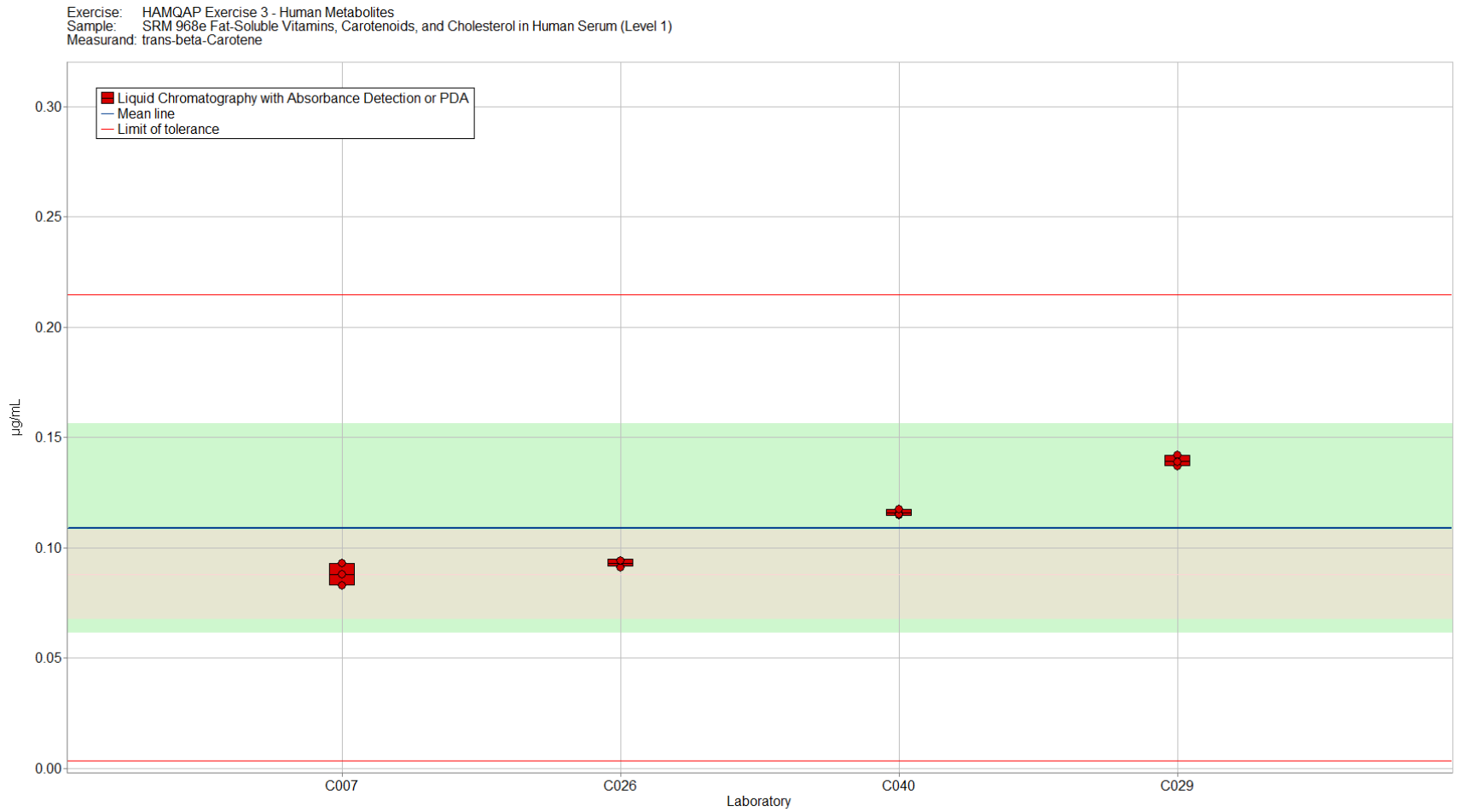

Figure 4-10. *Trans*-β-carotene in SRM 968e Fat-Soluble Vitamins, Carotenoids, and Cholesterol in Human Serum (Level 1) (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the 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'_{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_{NIST}) and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$.

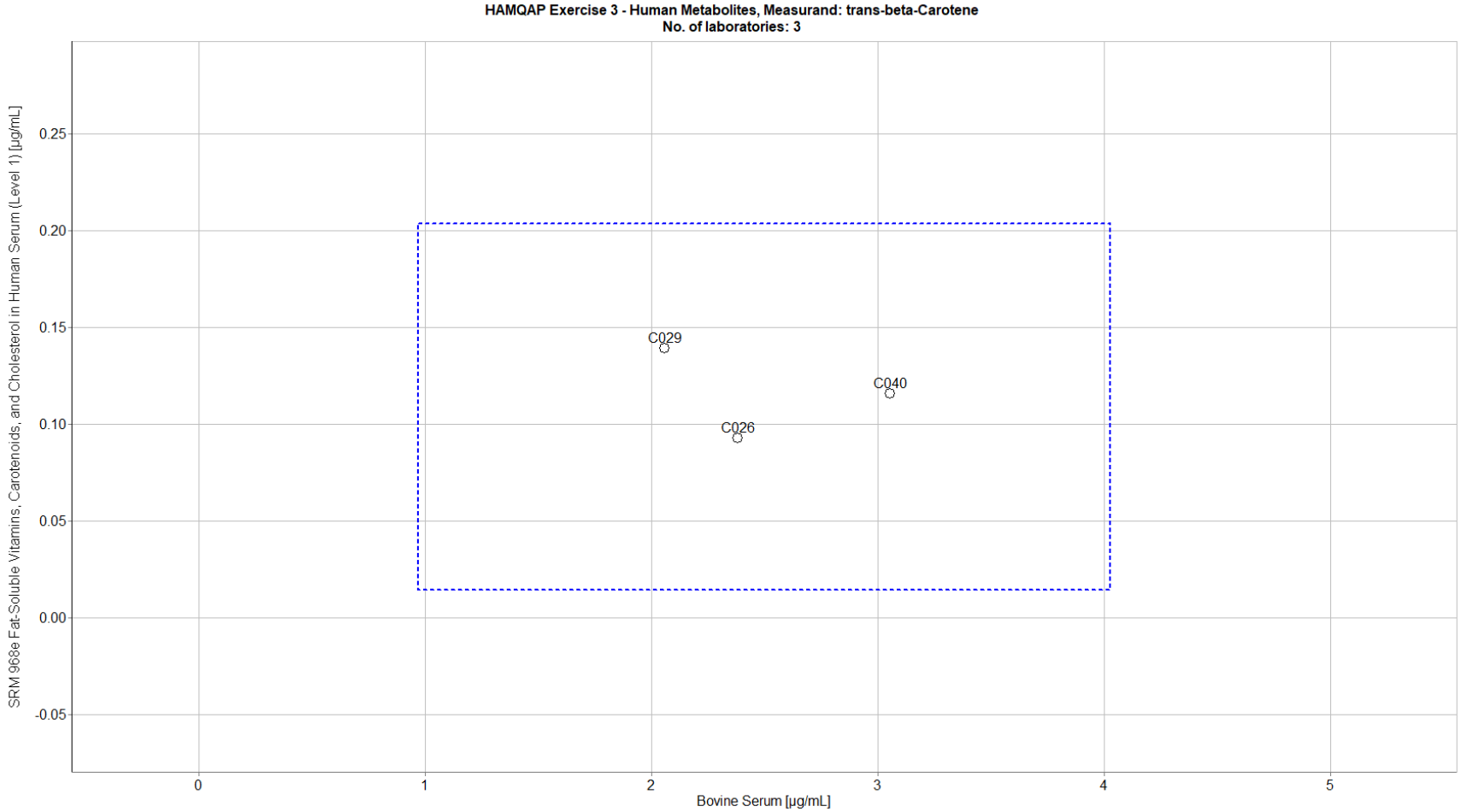

Figure 4-11. Laboratory means for *trans*-β-carotene in Bovine Serum and SRM 968e Fat-Soluble Vitamins, Carotenoids, and Cholesterol in Human Serum (Level 1) (sample/sample comparison view). In this view, the individual laboratory mean for one sample (bovine serum) is compared to the mean for a second sample (SRM 968e). The dotted blue box represents the consensus range of tolerance for bovine serum (x-axis) and SRM 968e (y-axis), calculated as the values above and below the consensus means that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$.

Table 4-8. Data summary table for total *cis*-β-carotene in bovine serum and human serum.

		Total cis-beta-Carotene									
		Bovine Serum (µg/mL)					SRM 968e Fat-Soluble Vitamins, Carotenoids, and Cholesterol in Human Serum (Level 1) (µg/mL)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target									0.01	
	C007										
	C027										
	C029	0.347	0.357	0.355	0.353	0.005	0.0680	0.0680	0.0710	0.0690	0.0017
	C032										
	C033										
	C040	0.3801	0.3754	0.3893	0.382	0.007	0.0073	0.0096	0.0084	0.0085	0.0011
	C054										
	C056										
	C065										
Community Results		Consensus Mean			0.367		Consensus Mean			0.0387	
		Consensus Standard Deviation			0.050		Consensus Standard Deviation			0.1312	
		Maximum			0.382		Maximum			0.0690	
		Minimum			0.353		Minimum			0.0085	
		N			2		N			2	

Table 4-9. Data summary table for total β-carotene in bovine serum and human serum.

		Total beta-Carotene									
		Bovine Serum (µg/mL)					SRM 968e Fat-Soluble Vitamins, Carotenoids, and Cholesterol in Human Serum (Level 1) (µg/mL)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target									0.099	0.018
	C005	2.426	2.552	2.546	2.508	0.071	0.079	0.083	0.083	0.082	0.002
	C007										
	C027										
	C029	2.405	2.412	2.411	2.409	0.004	0.21	0.205	0.21	0.208	0.003
	C031										
	C032										
	C033										
	C040	3.425	3.449	3.436	3.437	0.012	0.122	0.1248	0.126	0.124	0.002
	C042	1.325	1.433	1.439	1.399	0.064	0.023	0.02	0.022	0.022	0.002
	C054										
	C056										
	C057										
	C065	2.34	2.77	2.698	2.603	0.230	0.098	0.093	0.099	0.097	0.003
	C066										
Community Results		Consensus Mean			2.495		Consensus Mean			0.107	
		Consensus Standard Deviation			0.650		Consensus Standard Deviation			0.094	
		Maximum			3.437		Maximum			0.208	
		Minimum			1.399		Minimum			0.022	
		N			5		N			5	

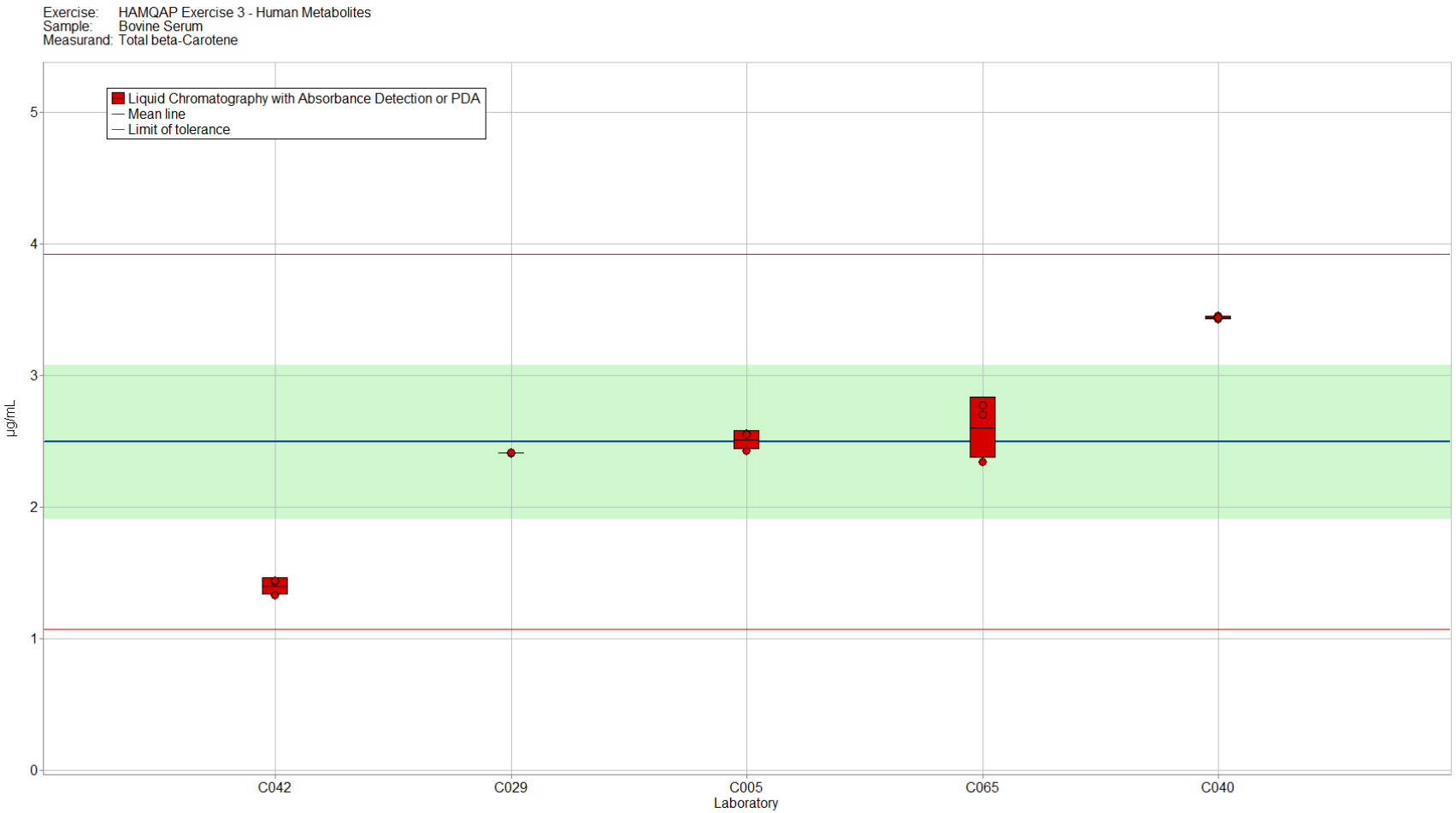

Figure 4-12. Total β-carotene in Bovine Serum (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line 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'_{comm} score, $|Z'_{\text{comm}}| \leq 2$. A NIST value has not been determined in this material.

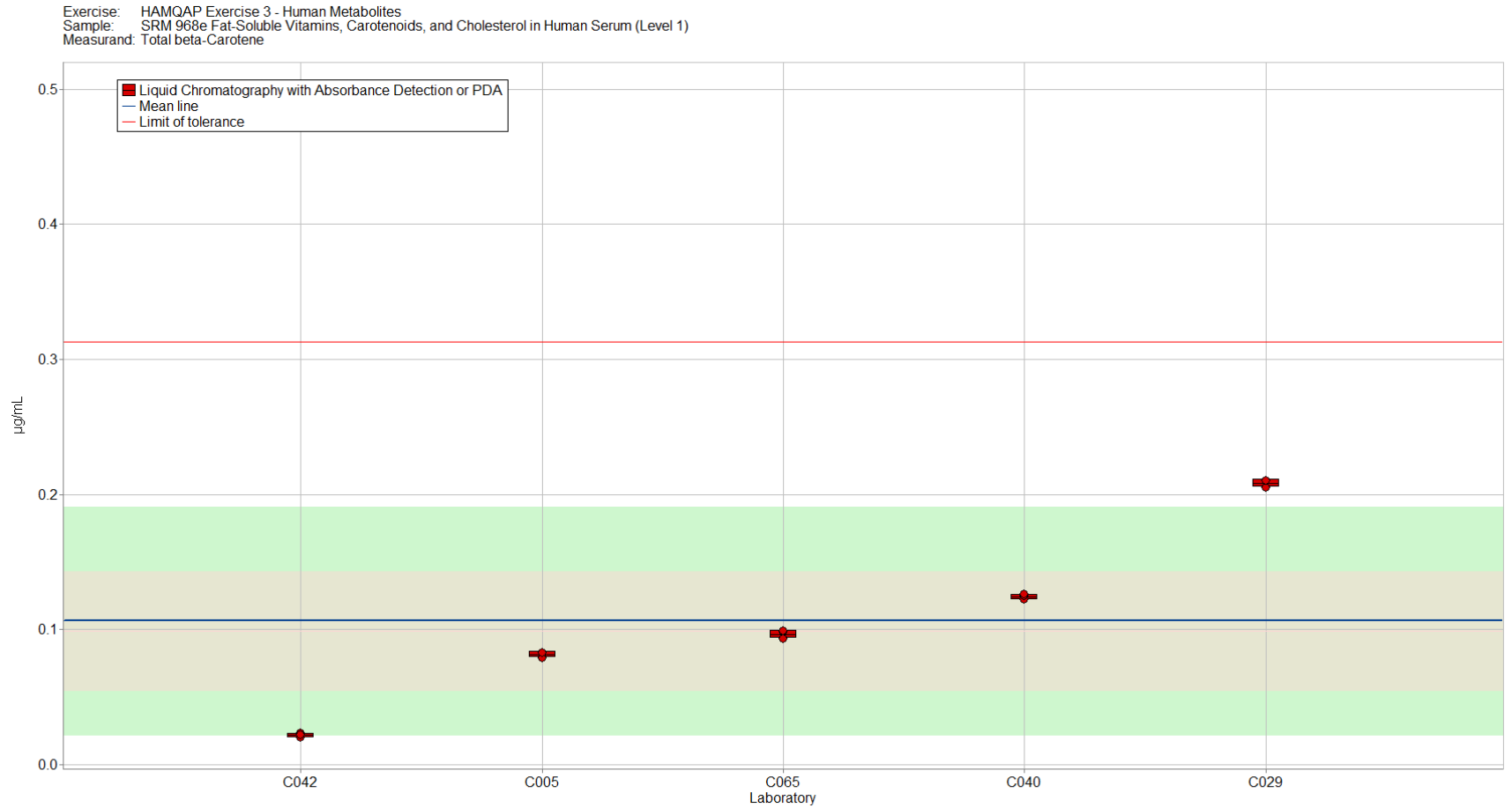

Figure 4-13. Total β-carotene in SRM 968e Fat-Soluble Vitamins, Carotenoids, and Cholesterol in Human Serum (Level 1) (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the 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 and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\mathrm{comm}}| \leq 2$, with the lower range set at zero. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by its uncertainty (U_{NIST}) and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\mathrm{NIST}}| \leq 2$.

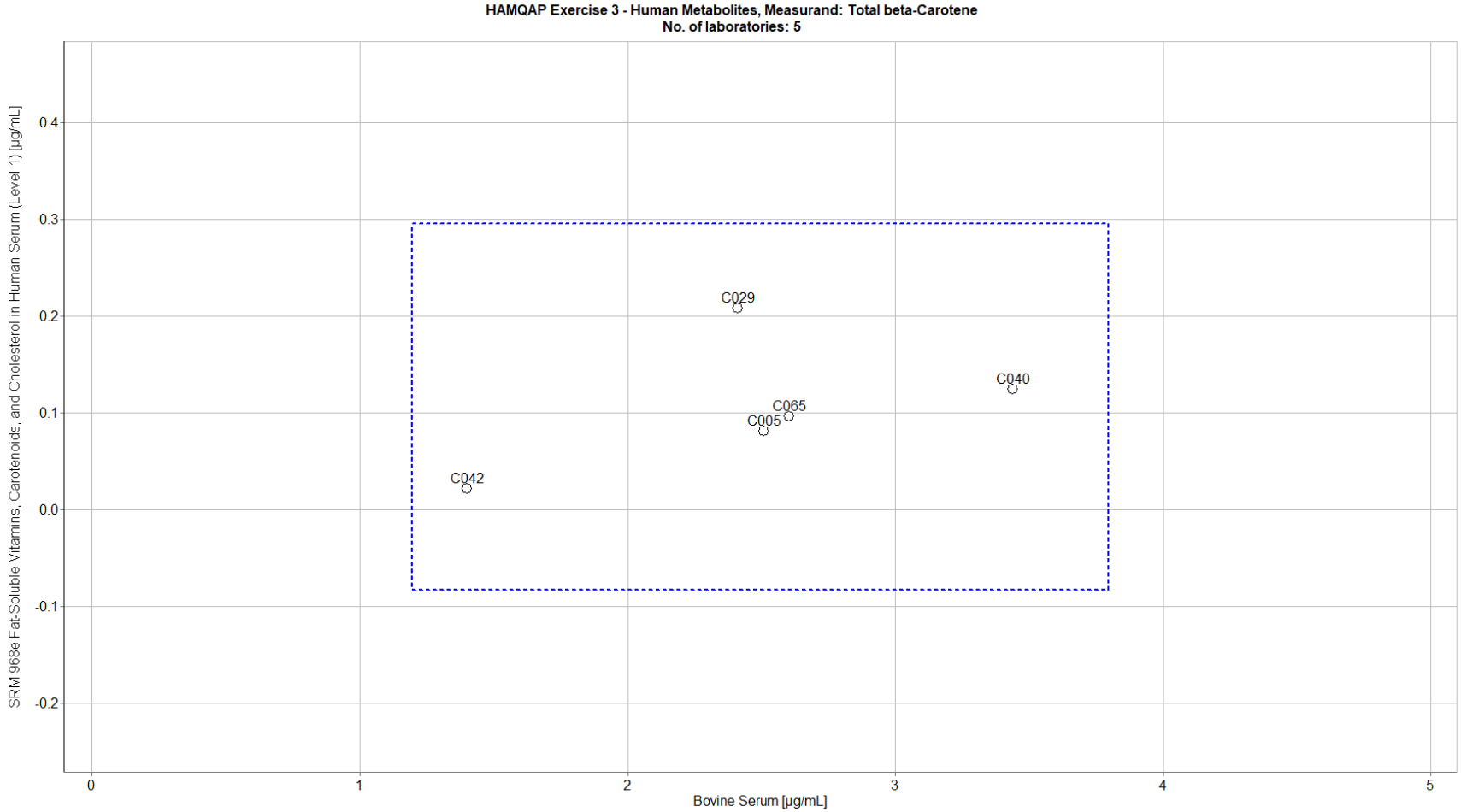

Figure 4-14. Laboratory means for total β-carotene in Bovine Serum and SRM 968e Fat-Soluble Vitamins, Carotenoids, and Cholesterol in Human Serum (Level 1) (sample/sample comparison view). In this view, the individual laboratory mean for one sample (bovine serum) is compared to the mean for a second sample (SRM 968e). The dotted blue box represents the consensus range of tolerance for bovine serum (x-axis) and SRM 968e (y-axis), calculated as the values above and below the consensus means that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$.

Table 4-10. Data summary table for total lutein in bovine serum and human serum.

		Total Lutein									
		Bovine Serum (µg/mL)					SRM 968e Fat-Soluble Vitamins, Carotenoids, and Cholesterol in Human Serum (Level 1) (µg/mL)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target									0.067	0.008
	C005	0.032	0.035	0.04	0.036	0.004	0.122	0.125	0.129	0.125	0.004
	C007										
	C015										
	C026	0.03	0.033	0.03	0.031	0.002	0.065	0.066	0.065	0.065	0.001
	C027										
	C029	0.062	0.064	0.06	0.062	0.002	0.106	0.102	0.104	0.104	0.002
	C032										
	C033										
	C040	0.0259	0.0252	0.0258	0.026	0.000	0.1098	0.1074	0.1118	0.110	0.002
	C042	0.047	0.047	0.047	0.047	0.000	0.095	0.092	0.097	0.095	0.003
	C054										
	C056										
	C057										
	C065	0.038	0.039	0.042	0.040	0.002	0.157	0.16	0.17	0.162	0.007
Community Results		Consensus Mean			0.040		Consensus Mean			0.110	
		Consensus Standard Deviation			0.016		Consensus Standard Deviation			0.040	
		Maximum			0.062		Maximum			0.162	
		Minimum			0.026		Minimum			0.065	
		N			6		N			6	

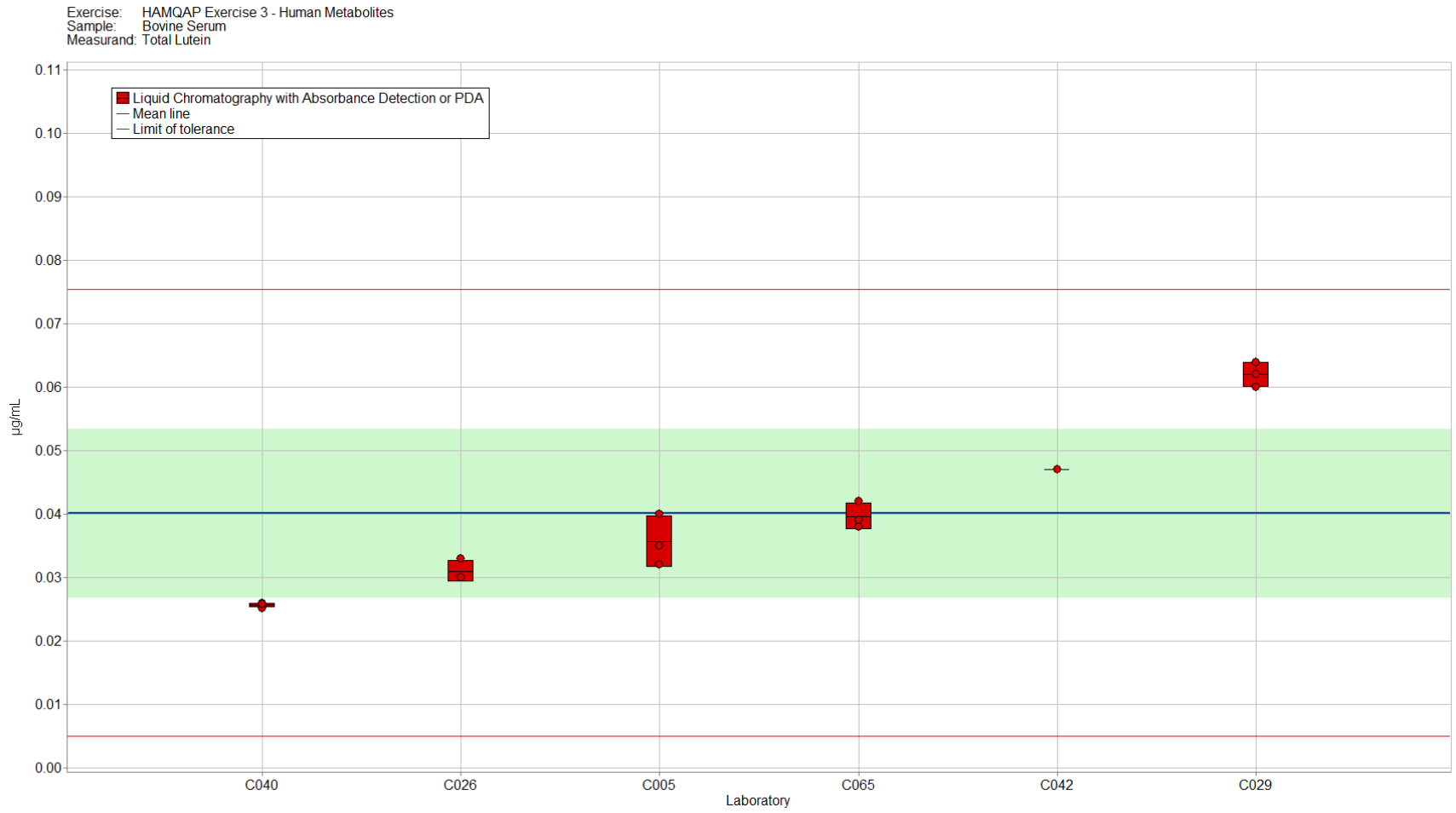

Figure 4-15. Total lutein in Bovine Serum (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line represents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

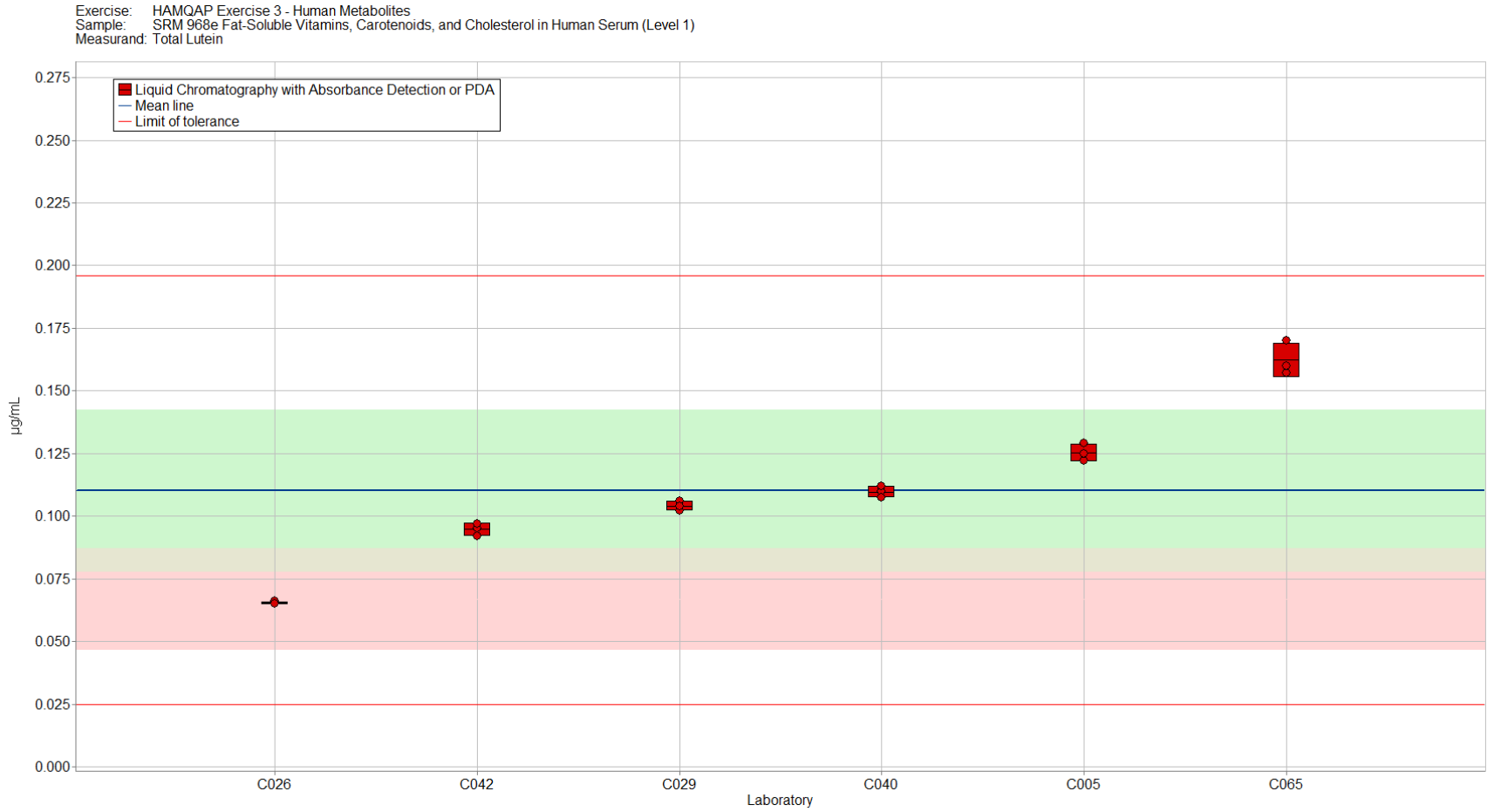

Figure 4-16. Total lutein in SRM 968e Fat-Soluble Vitamins, Carotenoids, and Cholesterol in Human Serum (Level 1) (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data 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'_{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_{NIST}) and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$.

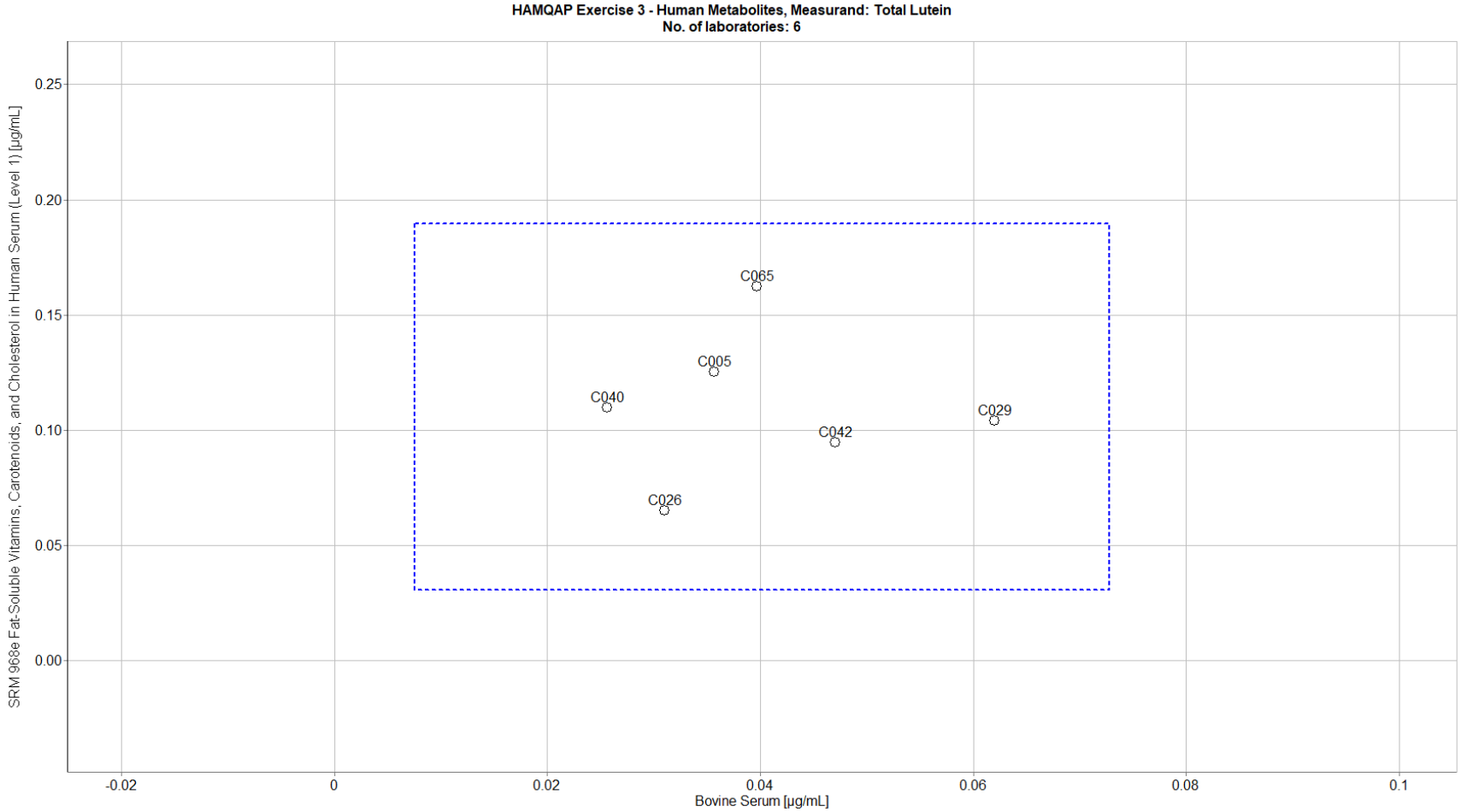

Figure 4-17. Laboratory means for total lutein in Bovine Serum and SRM 968e Fat-Soluble Vitamins, Carotenoids, and Cholesterol in Human Serum (Level 1) (sample/sample comparison view). In this view, the individual laboratory mean for one sample (bovine serum) is compared to the mean for a second sample (SRM 968e). The dotted blue box represents the consensus range of tolerance for bovine serum (x-axis) and SRM 968e (y-axis), calculated as the values above and below the consensus means that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$.

Table 4-11. Data summary table for *trans*-lycopene in bovine serum and human serum. Data points highlighted in red have been flagged as potential outliers (e.g., Grubb and/or Cochran) by the NIST software package.

		trans-Lycopene									
		Bovine Serum (µg/mL)					SRM 968e Fat-Soluble Vitamins, Carotenoids, and Cholesterol in Human Serum (Level 1) (µg/mL)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target									0.135	0.040
	C005										
	C007										
	C015										
	C026	< 0.0000	< 0.0000	< 0.0000			0.751	0.753	0.784	0.763	0.019
	C027										
	C029	< 0.0100	< 0.0100	< 0.0100			0.134	0.128	0.132	0.131	0.003
	C032										
	C033										
	C040	0.00241	0.01952	0.00399	0.0086	0.0095	0.161	0.1588	0.1627	0.161	0.002
	C054										
	C056										
Community Results		Consensus Mean					Consensus Mean			0.146	
		Consensus Standard Deviation					Consensus Standard Deviation			0.070	
		Maximum			0.0086		Maximum			0.763	
		Minimum			0.0086		Minimum			0.131	
		N			1		N			3	

Table 4-12. Data summary table for total lycopene in bovine serum and human serum. Data points highlighted in red have been flagged as potential outliers (e.g., Grubb and/or Cochran) by the NIST software package.

		Total Lycopene									
		Bovine Serum (µg/mL)					SRM 968e Fat-Soluble Vitamins, Carotenoids, and Cholesterol in Human Serum (Level 1) (µg/mL)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target									0.234	0.095
	C005	0.016	0.016	0.016	0.016	0.000	0.197	0.211	0.219	0.209	0.011
	C007						0.281	0.232	0.242	0.252	0.026
	C027										
	C029	< 0.0100	< 0.0100	< 0.0100			0.255	0.248	0.255	0.253	0.004
	C032										
	C033										
	C040	0.0426	0.0602	0.0463	0.050	0.009	0.302	0.2972	0.3028	0.301	0.003
	C042	0.001			0.001		0.001	0.001	0.001	0.001	0.000
	C051										
	C054										
	C056										
	C057										
	C065	0.007	0.01	0.009	0.009	0.002	0.206	0.201	0.208	0.205	0.004
	C066										
Community Results		Consensus Mean			0.018		Consensus Mean			0.219	
		Consensus Standard Deviation			0.019		Consensus Standard Deviation			0.081	
		Maximum			0.050		Maximum			0.301	
		Minimum			0.001		Minimum			0.001	
		N			3		N			6	

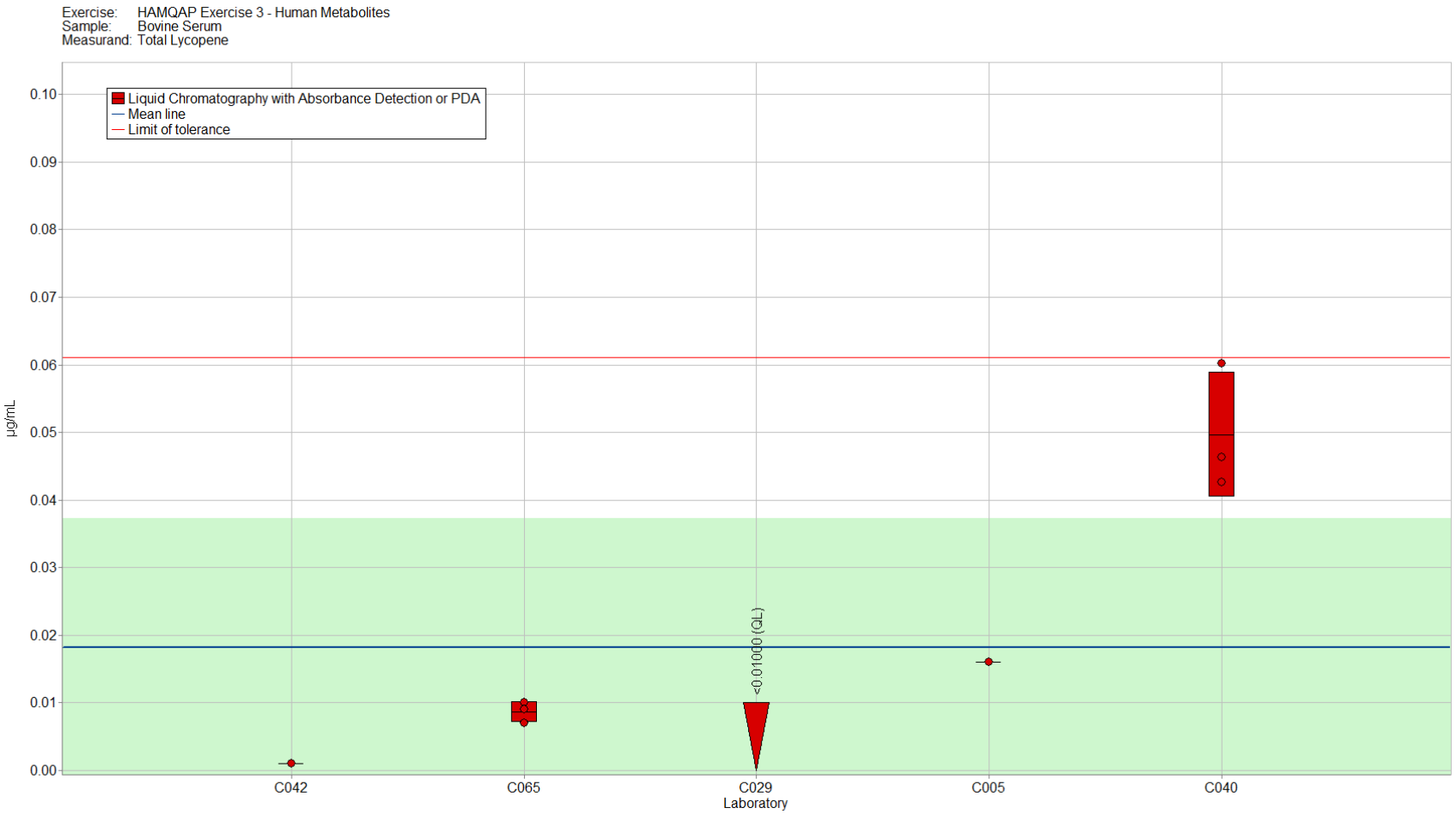

Figure 4-18. Total lycopene in Bovine Serum (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line 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 and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$, with the lower range set at zero. A NIST value has not been determined in this material.

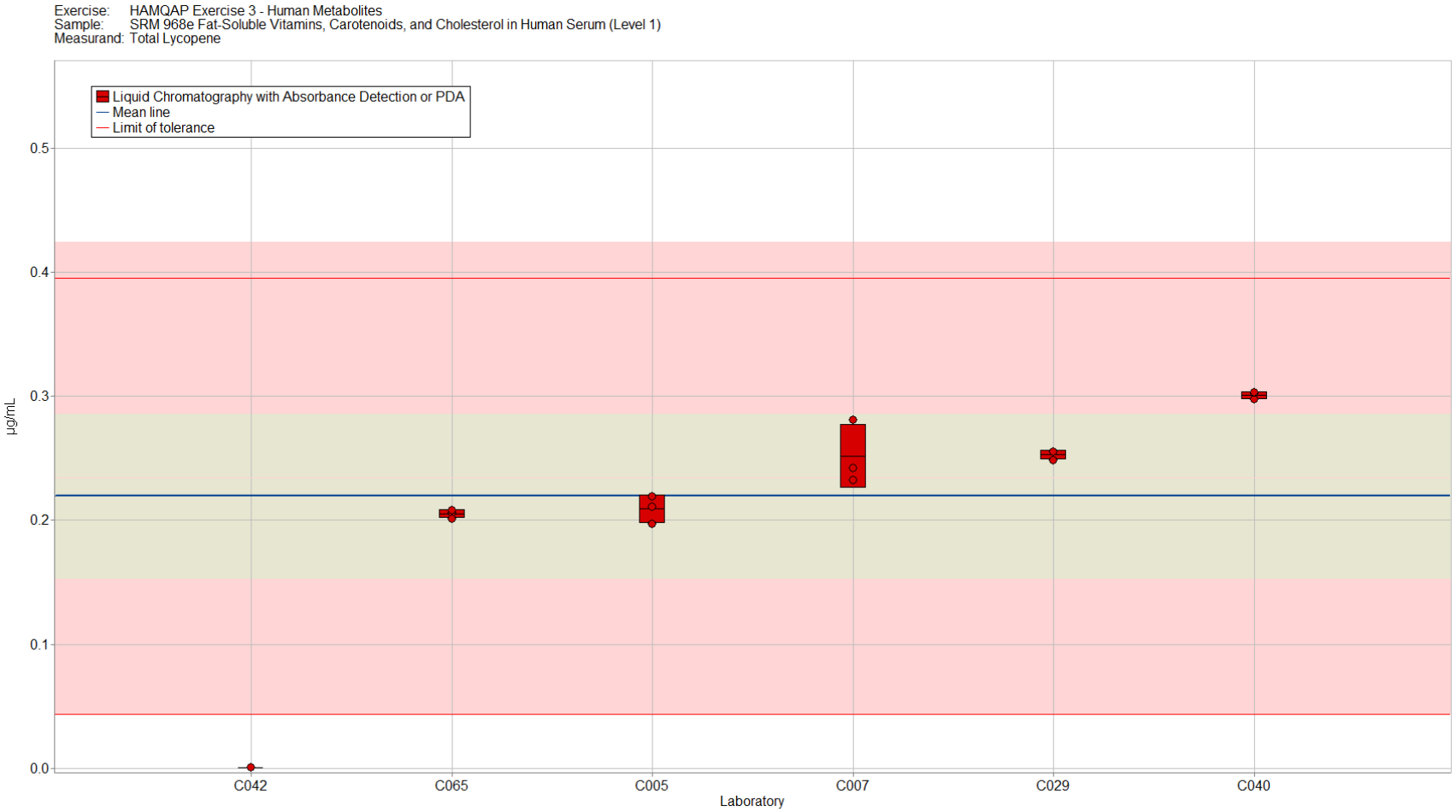

Figure 4-19. Total lycopene in SRM 968e Fat-Soluble Vitamins, Carotenoids, and Cholesterol in Human Serum (Level 1) (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data 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'_{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_{NIST}) and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$.

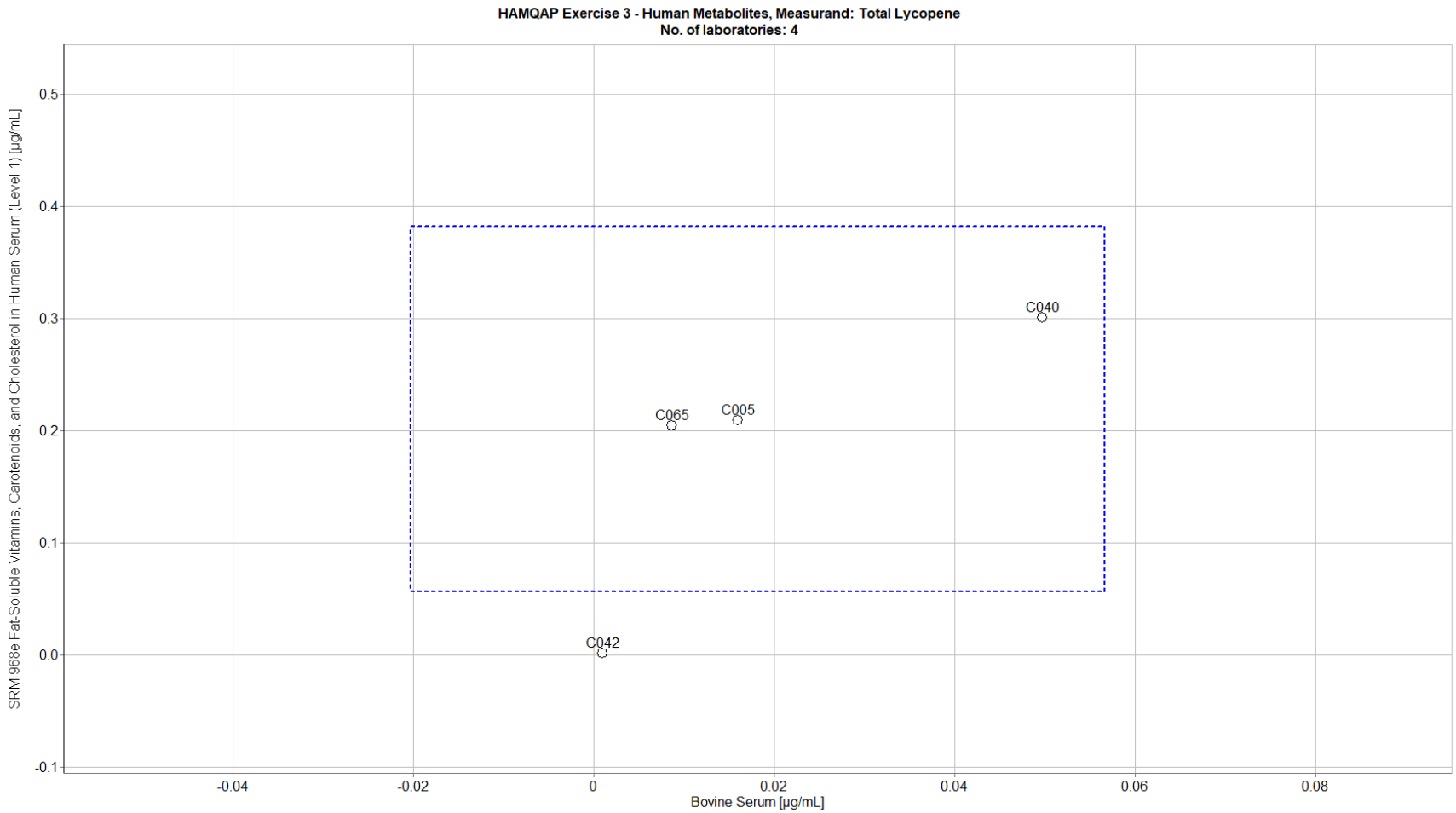

Figure 4-20. Laboratory means for total lycopene in Bovine Serum and SRM 968e Fat-Soluble Vitamins, Carotenoids, and Cholesterol in Human Serum (Level 1) (sample/sample comparison view). In this view, the individual laboratory mean for one sample (bovine serum) is compared to the mean for a second sample (SRM 968e). The dotted blue box represents the consensus range of tolerance for bovine serum (x-axis) and SRM 968e (y-axis), calculated as the values above and below the consensus means that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$.

Table 4-13. Data summary table for total zeaxanthin in bovine serum and human serum.

		Total Zeaxanthin									
		Bovine Serum (µg/mL)					SRM 968e Fat-Soluble Vitamins, Carotenoids, and Cholesterol in Human Serum (Level 1) (µg/mL)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target									0.031	0.005
	C005										
	C007						0.045	0.045	0.046	0.045	0.001
	C015										
	C026	0.003	0.004	0.003	0.0033	0.0006	0.027	0.028	0.027	0.027	0.001
	C027										
	C029	0.026	0.026	0.023	0.0250	0.0017	0.068	0.067	0.066	0.067	0.001
	C032										
	C033										
	C040	0.0259	0.0252	0.0258	0.0256	0.0004	0.1098	0.1074	0.1118	0.110	0.002
	C042	0.006	0.008	0.008	0.0073	0.0012	0.037	0.035	0.038	0.037	0.002
	C054										
	C056										
	C057										
	C065										
Community Results		Consensus Mean			0.0153		Consensus Mean			0.057	
		Consensus Standard Deviation			0.0093		Consensus Standard Deviation			0.040	
		Maximum			0.0256		Maximum			0.110	
		Minimum			0.0033		Minimum			0.027	
		N			4		N			5	

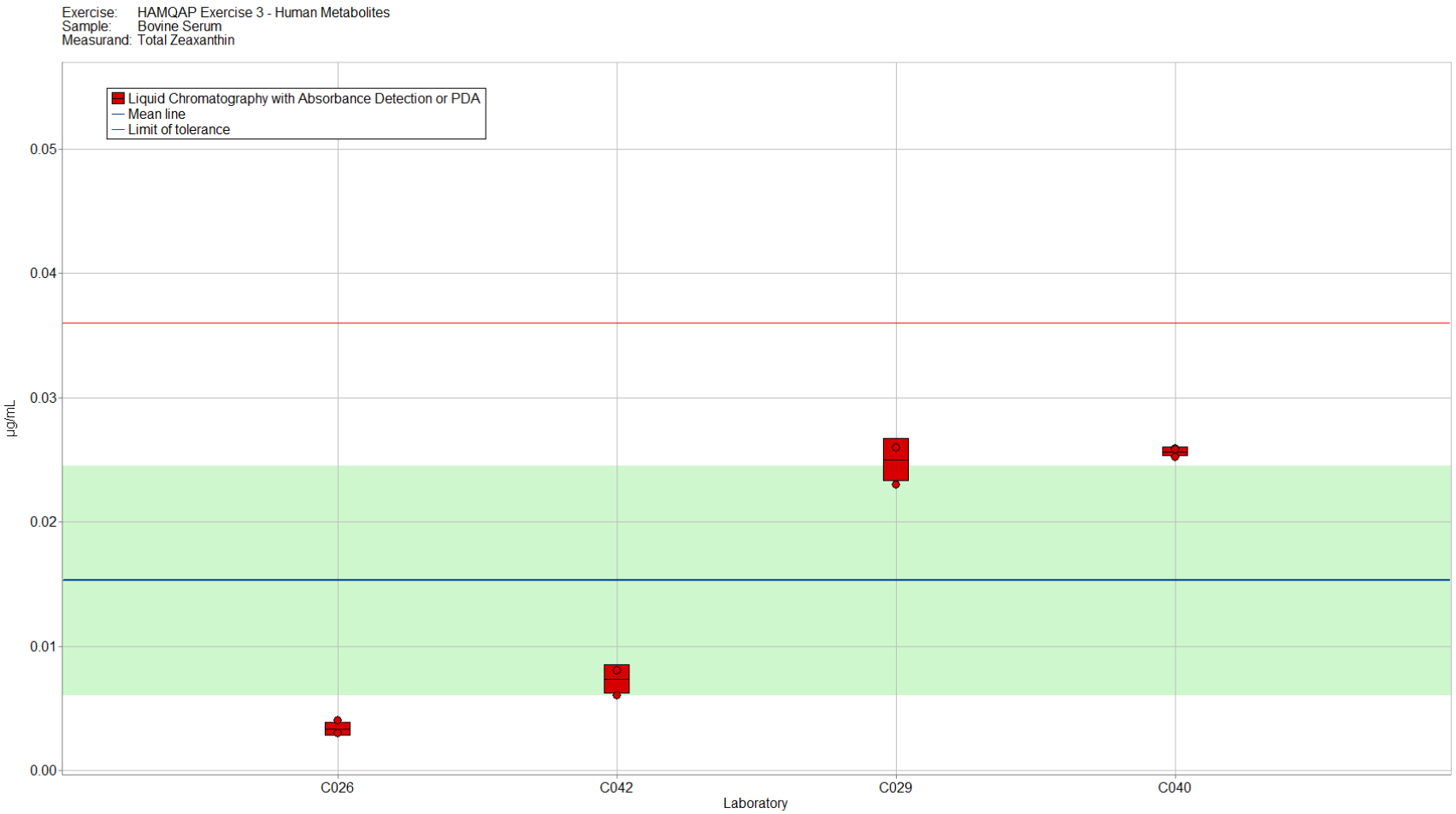

Figure 4-21. Total zeaxanthin in Bovine Serum (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line 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 and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$, with the lower range set at zero. A NIST value has not been determined in this material.

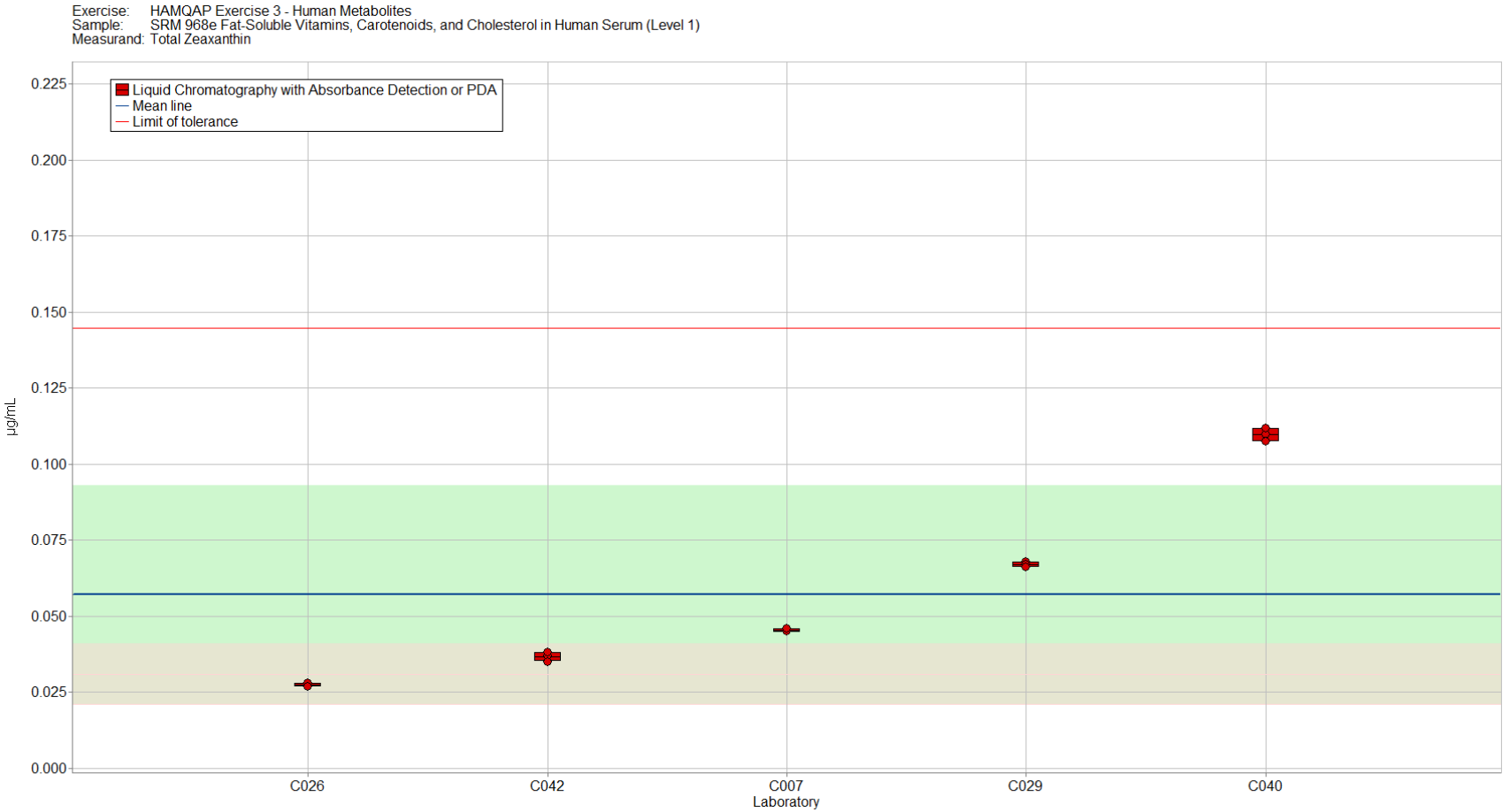

Figure 4-22. Total zeaxanthin in SRM 968e Fat-Soluble Vitamins, Carotenoids, and Cholesterol in Human Serum (Level 1) (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the 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 and below the consensus mean that result in an acceptable Z'_{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 its uncertainty (U_{NIST}) and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$.

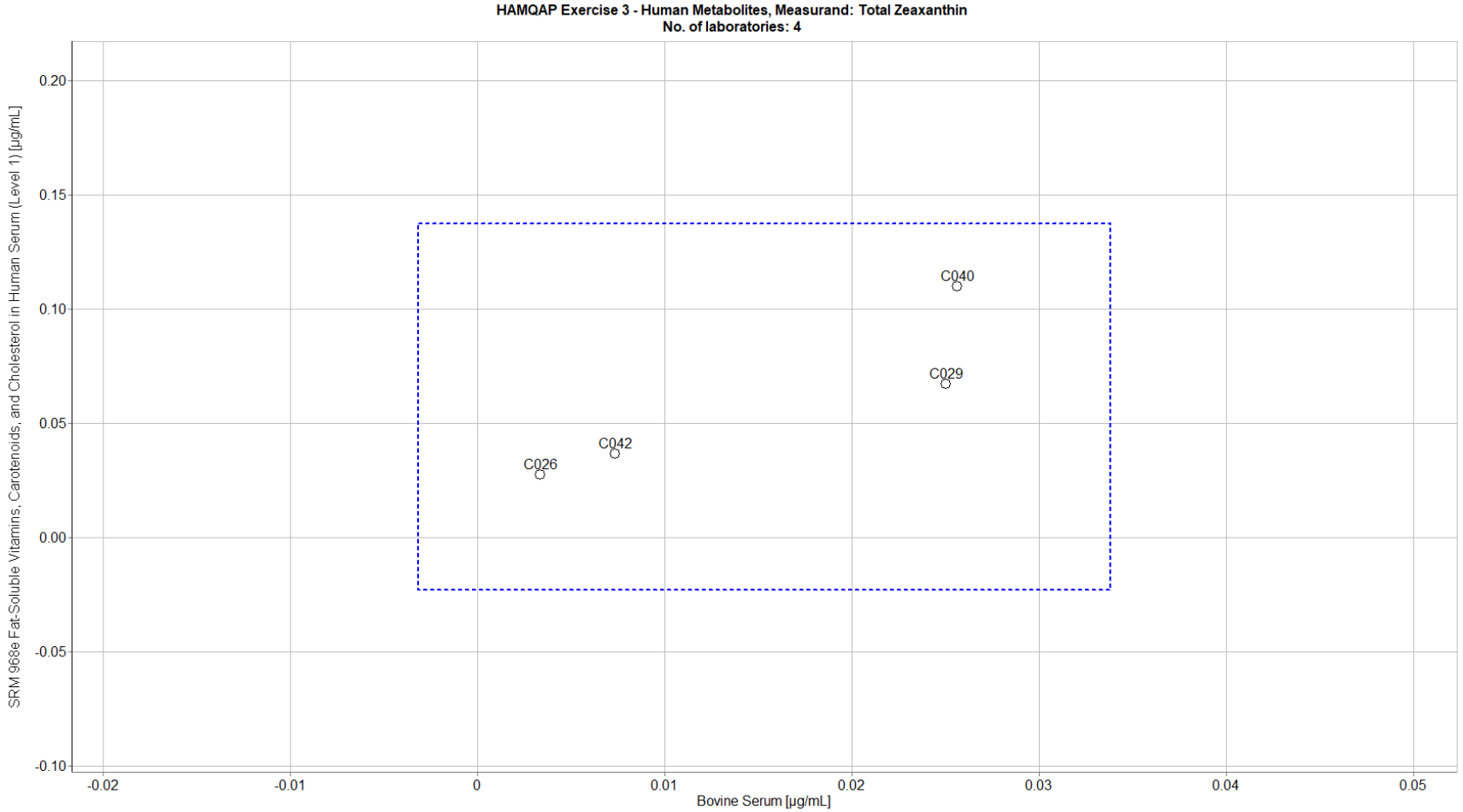

Figure 4-23. Laboratory means for total zeaxanthin in Bovine Serum and SRM 968e Fat-Soluble Vitamins, Carotenoids, and Cholesterol in Human Serum (Level 1) (sample/sample comparison view). In this view, the individual laboratory mean for one sample (bovine serum) is compared to the mean for a second sample (SRM 968e). The dotted blue box represents the consensus range of tolerance for bovine serum (x-axis) and SRM 968e (y-axis), calculated as the values above and below the consensus means that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$.

Table 4-14. Data summary table for total α-cryptoxanthin in bovine serum and human serum.

		Total alpha-Cryptoxanthin									
		Bovine Serum (µg/mL)					SRM 968e Fat-Soluble Vitamins, Carotenoids, and Cholesterol in Human Serum (Level 1) (µg/mL)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target									0.016	
	C007										
	C027										
	C029	0.041	0.036	0.033	0.0367	0.0040	0.045	0.045	0.043	0.0443	0.0012
	C032										
	C054										
	C056										
	C057										
	C065										
Community Results		Consensus Mean					Consensus Mean				
		Consensus Standard Deviation					Consensus Standard Deviation				
		Maximum			0.0367		Maximum			0.0443	
		Minimum			0.0367		Minimum			0.0443	
		N			1		N			1	

Table 4-15. Data summary table for β-cryptoxanthin in bovine serum and human serum.

		beta-Cryptoxanthin									
		Bovine Serum (µg/mL)					SRM 968e Fat-Soluble Vitamins, Carotenoids, and Cholesterol in Human Serum (Level 1) (µg/mL)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target									0.041	0.006
	C005	0.027	0.026	0.028	0.0270	0.0010	0.05	0.053	0.054	0.0523	0.0021
	C007						0.055	0.06	0.054	0.0563	0.0032
	C015										
	C026	0.011	0.01	0.008	0.0097	0.0015	0.034	0.036	0.035	0.0350	0.0010
	C027										
	C029	0.035	0.032	0.03	0.0323	0.0025	0.075	0.072	0.075	0.0740	0.0017
	C032										
	C040	0.0275	0.0271	0.0259	0.0268	0.0008	0.08923	0.08544	0.08634	0.0870	0.0020
	C042										
	C054										
	C056										
	C057										
	C065	0.036	0.024	0.036	0.0320	0.0069	0.045	0.045	0.052	0.0473	0.0040
Community Results		Consensus Mean			0.0262		Consensus Mean			0.0587	
		Consensus Standard Deviation			0.0089		Consensus Standard Deviation			0.0251	
		Maximum			0.0323		Maximum			0.0870	
		Minimum			0.0097		Minimum			0.0350	
		N			5		N			6	

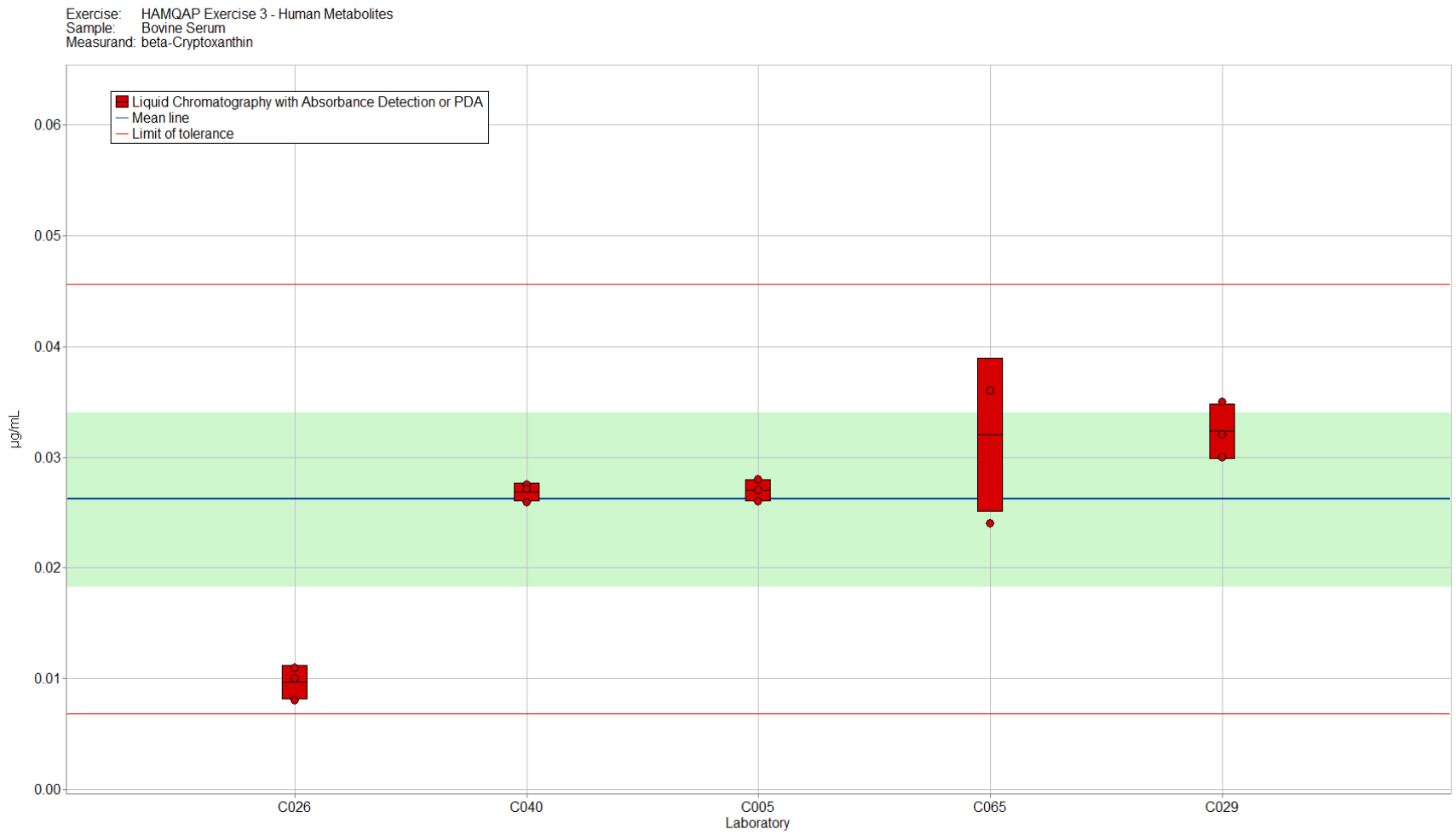

Figure 4-24. β-cryptoxanthin in Bovine Serum (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line 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'_{comm} score, $|Z'_{\text{comm}}| \leq 2$. A NIST value has not been determined in this material.

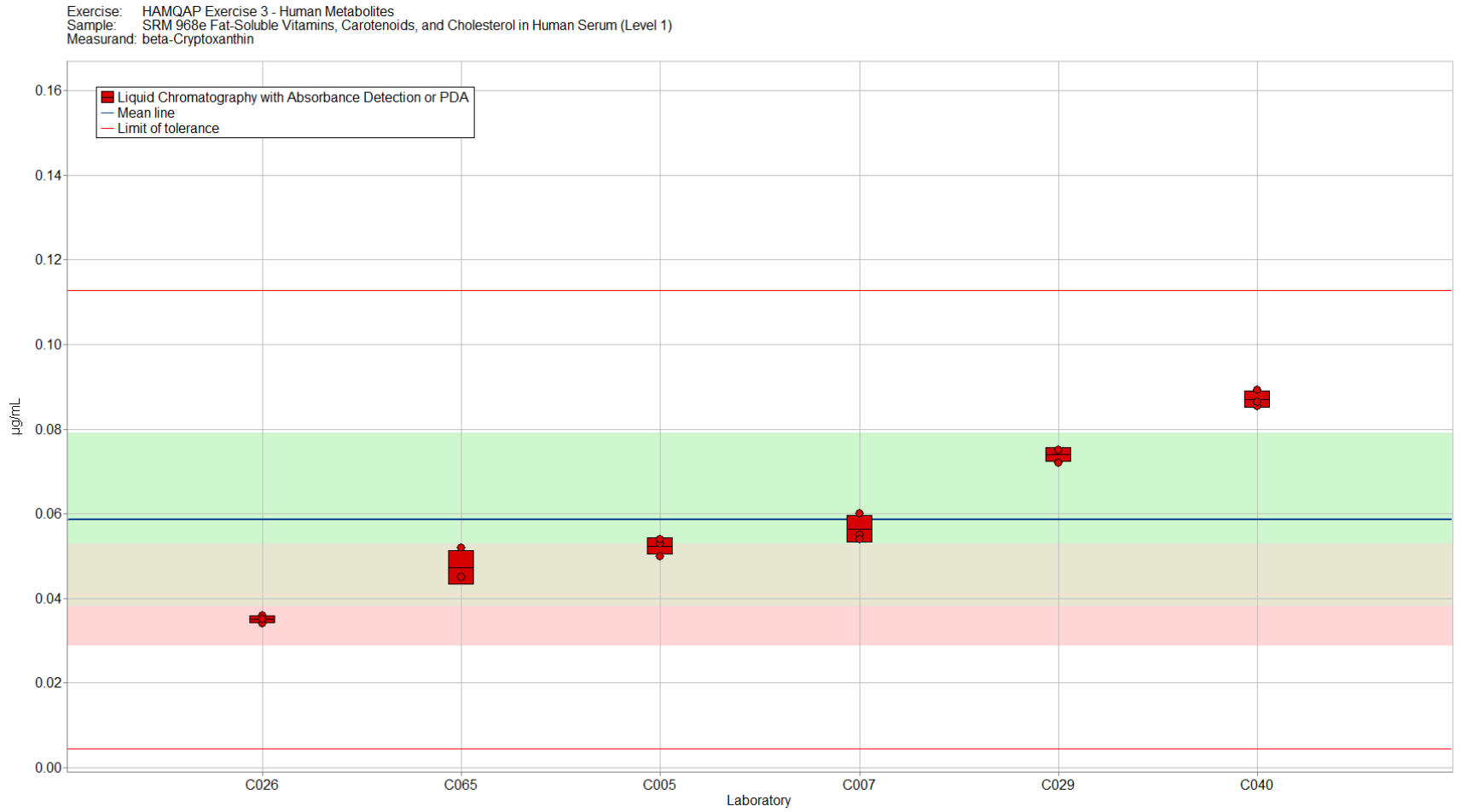

Figure 4-25. β-cryptoxanthin in SRM 968e Fat-Soluble Vitamins, Carotenoids, and Cholesterol in Human Serum (Level 1) (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the 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 represents the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{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_{NIST}) and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$.

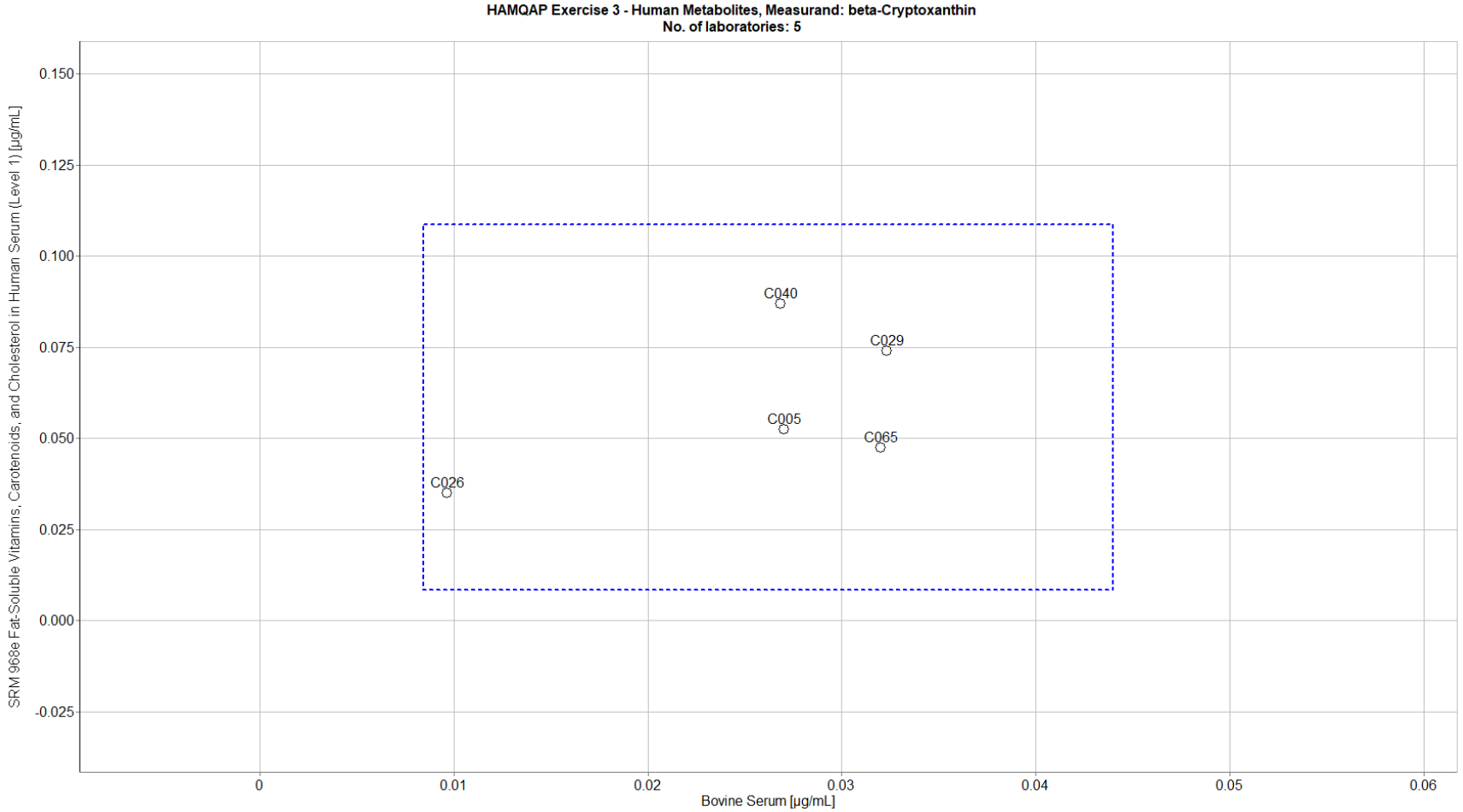

Figure 4-26. Laboratory means for β-cryptoxanthin in Bovine Serum and SRM 968e Fat-Soluble Vitamins, Carotenoids, and Cholesterol in Human Serum (Level 1) (sample/sample comparison view). In this view, the individual laboratory mean for one sample (bovine serum) is compared to the mean for a second sample (SRM 968e). The dotted blue box represents the consensus range of tolerance for bovine serum (x-axis) and SRM 968e (y-axis), calculated as the values above and below the consensus means that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$.

Fat-Soluble Vitamins Overall Study Comparison

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

- Fewer laboratories reported results for the bovine and human serum than for the multivitamin and saw palmetto extract. In addition, greater deviation from the target values was observed for the clinical samples than for the dietary intake samples. This greater deviation in the clinical measurements may be influenced by several factors.
  - The concentrations of carotenoids in the clinical samples were significantly lower than the concentration in the dietary intake samples. In most cases, the difference was at least an order of magnitude. Lower levels are generally more difficult to measure accurately.
  - Carotenoids are often found in bound forms and require release by hydrolysis or enzymatic methods, especially in clinical samples.
  - Measurement of the carotenoids may have been more straightforward for laboratories.
    - Carotenoids were fortified in the multivitamin tablets, however no special sample preparation was required to release the analytes from gelatin encapsulation.
    - The β-carotene found in the saw palmetto extract is endogenous but requires no additional sample preparation for analysis.

SECTION 5: NATURAL PRODUCT (Ubiquinone)

Study Overview

In this study, participants were provided with two commercial coenzyme Q10 (CoQ10) supplements. Commercial supplement A was labeled as containing only CoQ10 at approximately 400 mg/tablet and commercial supplement B was labeled as containing CoQ10 at approximately 30 mg/tablet, plus garlic. Participants were asked to use in-house analytical methods to determine the mass fraction (mg/g) of ubiquinone (CoQ10) in each matrix. Ubiquinone is naturally found in the body and is used in the cellular production of energy and to prevent cellular components from free radical damage.[15] Due to the critical function of ubiquinone, supplementation has been studied for use in prevention of cardiac disease and reduction of neurological disease symptoms as well as multiple other disease states. In addition, aged garlic extract has been reported to reduce cardiovascular risk factors, although the clinical study results are inconclusive.[16]

Dietary Intake Sample Information

Supplement A. Participants were provided with three packets, each containing 20 gelcaps. The gelcaps were heat-sealed inside aluminized bags. Before use, participants were instructed to composite the contents of the packet, to mix thoroughly, and to use a sample size appropriate for their usual in-house method of analysis. After compositing, participants were asked to store the material at controlled room temperature, 20 °C to 25 °C, to analyze the material within two days, and to prepare one sample and report one value from each packet provided. The approximate analyte level was not reported to participants prior to the study. The NIST-determined value was assigned based on results from a previous interlaboratory comparison. The NIST-determined value and uncertainty for ubiquinone in commercial supplement A are provided in the table below.

Analyte	NIST-Determined Mass Concentration in Supplement A (mg/g)
Ubiquinone	374 ± 55

Supplement B. Participants were provided with three packets, each containing 20 gelcaps. The gelcaps were heat-sealed inside aluminized bags. Before use, participants were instructed to composite the contents of the packet, to mix thoroughly, and to use a sample size appropriate for their usual in-house method of analysis. After compositing, participants were asked to store the material at controlled room temperature, 20 °C to 25 °C, to analyze the material within two days, and to prepare one sample and report one value from each packet provided. The approximate analyte level was not reported to participants prior to the study. The NIST-determined value was assigned based on results from a previous interlaboratory comparison. The NIST-determined value and uncertainty for ubiquinone in commercial supplement B are provided in the table below.

Analyte	NIST-Determined Mass Concentration in Supplement B (mg/g)
Ubiquinone	14.9 ± 3.1

[15] Coenzyme Q10 (PDQ®)–Health Professional Version. National Institutes of Health National Cancer Institute. https://www.cancer.gov/about-cancer/treatment/cam/hp/coenzyme-q10-pdq (accessed February 2019).
[16] Garlic. National Center for Complementary and Integrative Health. https://nccih.nih.gov/health/garlic/ataglance.htm (accessed November 2019).

Dietary Intake Study Results

- Twenty-five laboratories enrolled in this exercise and received samples to measure ubiquinone. Sixteen laboratories reported results for each sample (64 % participation).
- The between-laboratory variability was good for the determination of ubiquinone in commercial supplement A (13 % RSD) and commercial supplement B (11 % RSD).
- All laboratories that reported analytical method information indicated using LC-absorbance.
- The consensus means for ubiquinone in the supplements A and B were within the target ranges (**Figure 5-1 and 5-2**).
- As seen in **Figure 5-3**, only four laboratories (25 %) fell outside of the target range for both supplements.
- The target range in **Figure 5-3** is from a previous interlaboratory study. The consensus range for this exercise is smaller indicating that there is greater agreement among the laboratories for both of the supplements.

Dietary Intake Technical Recommendations

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

- The determination of ubiquinone, at levels of 15 mg/g to 400 mg/g, with or without the presence of garlic, does not appear to be a challenge for most laboratories (**Figures 5-1 and 5-2**).
- Laboratories reporting large within-laboratory variability should investigate the completeness of the extraction during sample preparation.
  - Any extraction procedure should be optimized to determine the most effective extraction solvent to ensure exhaustive extraction of the analyte from the matrix.
  - The optimum number of extraction cycles must be determined by sequential re-extraction of the sample matrix until no further increase in yield is observed. Sequential extractions may be needed if the extraction solvent becomes saturated during the first (or only) extraction cycle.
- Laboratories reporting results flagged as outliers should check for calculation errors. One example is to confirm that factors for all dilutions have been properly tabulated.

Table 5-1. Individualized data summary table (NIST) for ubiquinone in CoQ10 supplements.

National Institute of Standards and Technology

HAMQAP Exercise 3 - 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*
ubiquinone	Supplement A	mg/g		370	55		0	16	370	48	374	55
ubiquinone	Supplement B	mg/g		14.9	3.1		0	16	14.0	1.6	14.9	3.1

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 ubiquinone in CoQ10 dietary supplements. Data points highlighted in red have been flagged as potential outliers (e.g., Grubb and/or Cochran) by the NIST software package.

		Ubiquinone									
		Supplement A (mg/g)					Supplement B (mg/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target				374	55				14.9	3.1
	C002										
	C003	381	385	399	388	9	15.5	15.2	15.0	15.2	0.3
	C008	381	385	385	384	2	14	14	13.9	14.0	0.1
	C011	371	380	378	376	5	13.3	14.5	14.2	14.0	0.6
	C012										
	C013	336	282	259	292	40	34.9	29.5	30.1	31.5	3.0
	C014	435	434	437	435	2	14	14	14	14.0	0.0
	C016	205	204	189	199	9	12.76	12.5	12.65	12.6	0.1
	C020	375	390	393	386	10	14.4	14.5	14.4	14.4	0.1
	C021	326	332	341	333	8	13.7	13.2	14.0	13.6	0.4
	C023										
	C025	395	393	388	392	4	15.7	15.9	15.7	15.8	0.1
	C026										
	C027	407	388	397	397	9	15.0	14.7	16.7	15.5	1.0
	C028	405	379	481	422	53	12.5	12.9	11.2	12.2	0.9
	C029	397	361	408	389	25	15.2	14.3	14.3	14.6	0.5
	C032										
	C039	381	371	373	375	5	14.4	14.5	14.6	14.5	0.1
	C041	243	236	247	242	6	11.9	11.7	11.2	11.6	0.4
	C044	72	72	69	71	2	6.1	5.1	5.2	5.5	0.6
	C047										
	C050										
	C053	370	351	352	358	11	14.2	14.4	14.3	14.3	0.1
	C054										
	C055										
Community Results		Consensus Mean			371		Consensus Mean			14.0	
		Consensus Standard Deviation			48		Consensus Standard Deviation			1.6	
		Maximum			435		Maximum			31.5	
		Minimum			71		Minimum			5.5	
		N			16		N			16	

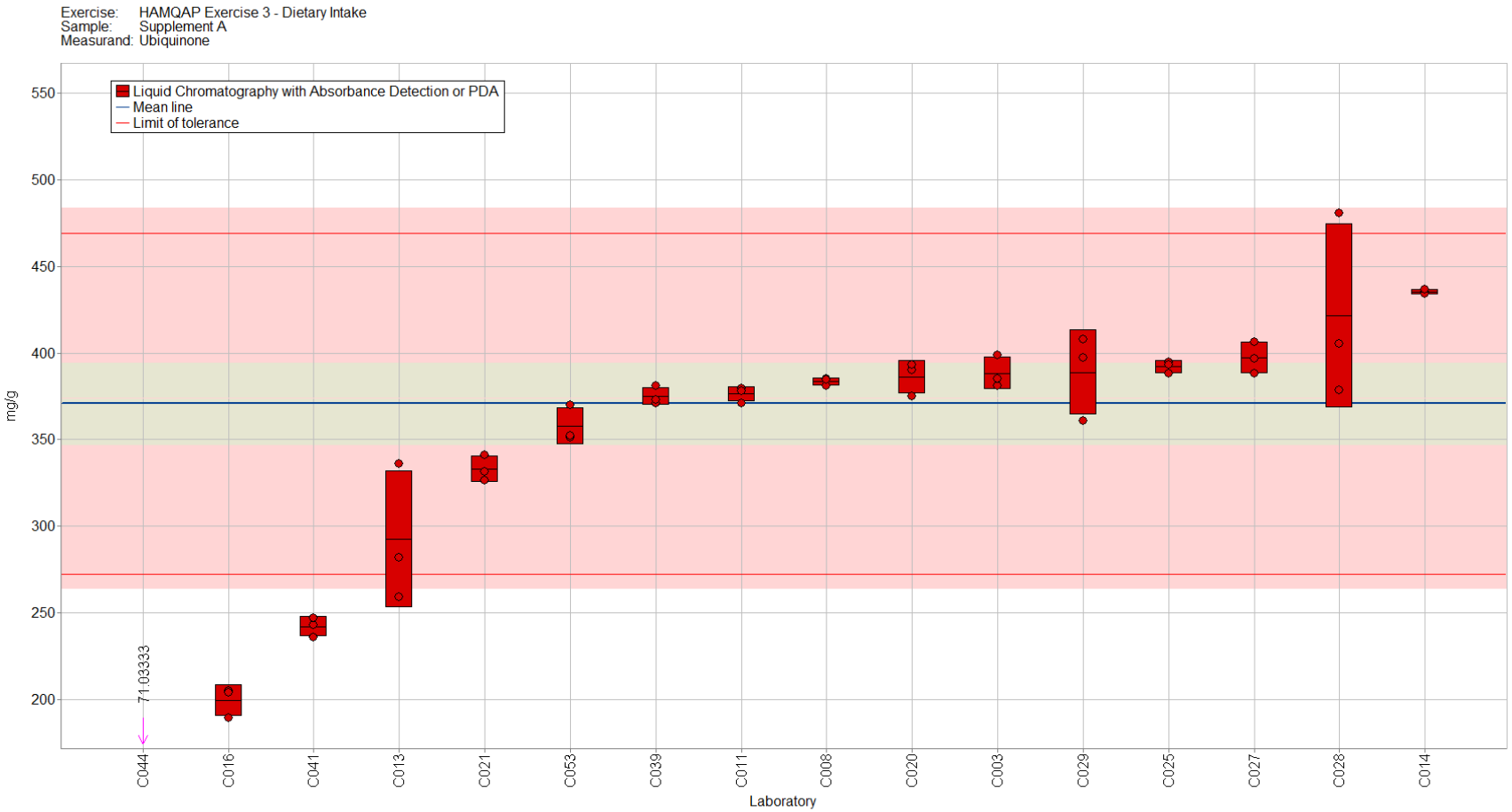

Figure 5-1. Ubiquinone in Supplement A (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line represents 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'_{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_{NIST}) and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$.

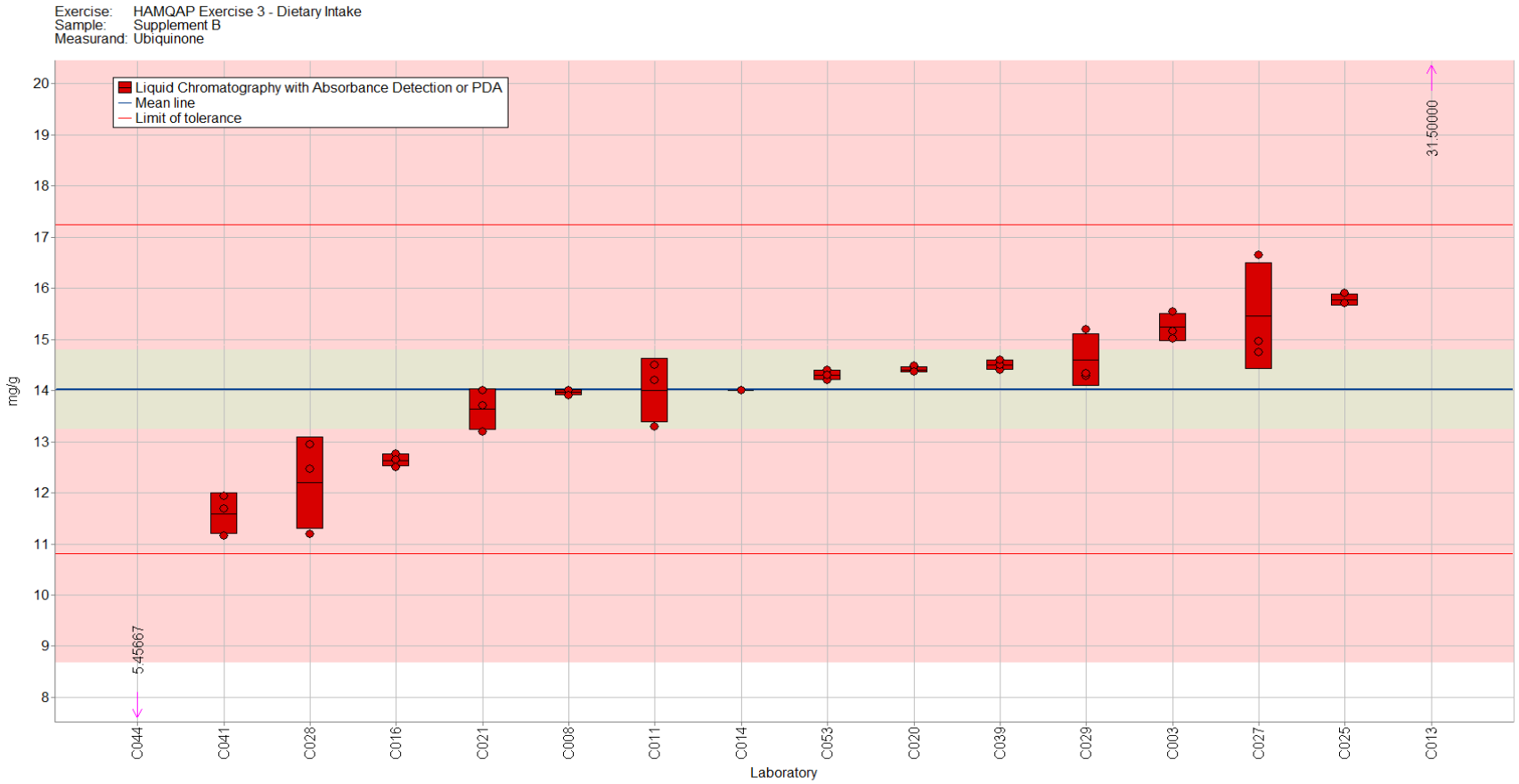

Figure 5-2. Ubiquinone in Supplement B (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line represents 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'_{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_{NIST}) and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$.

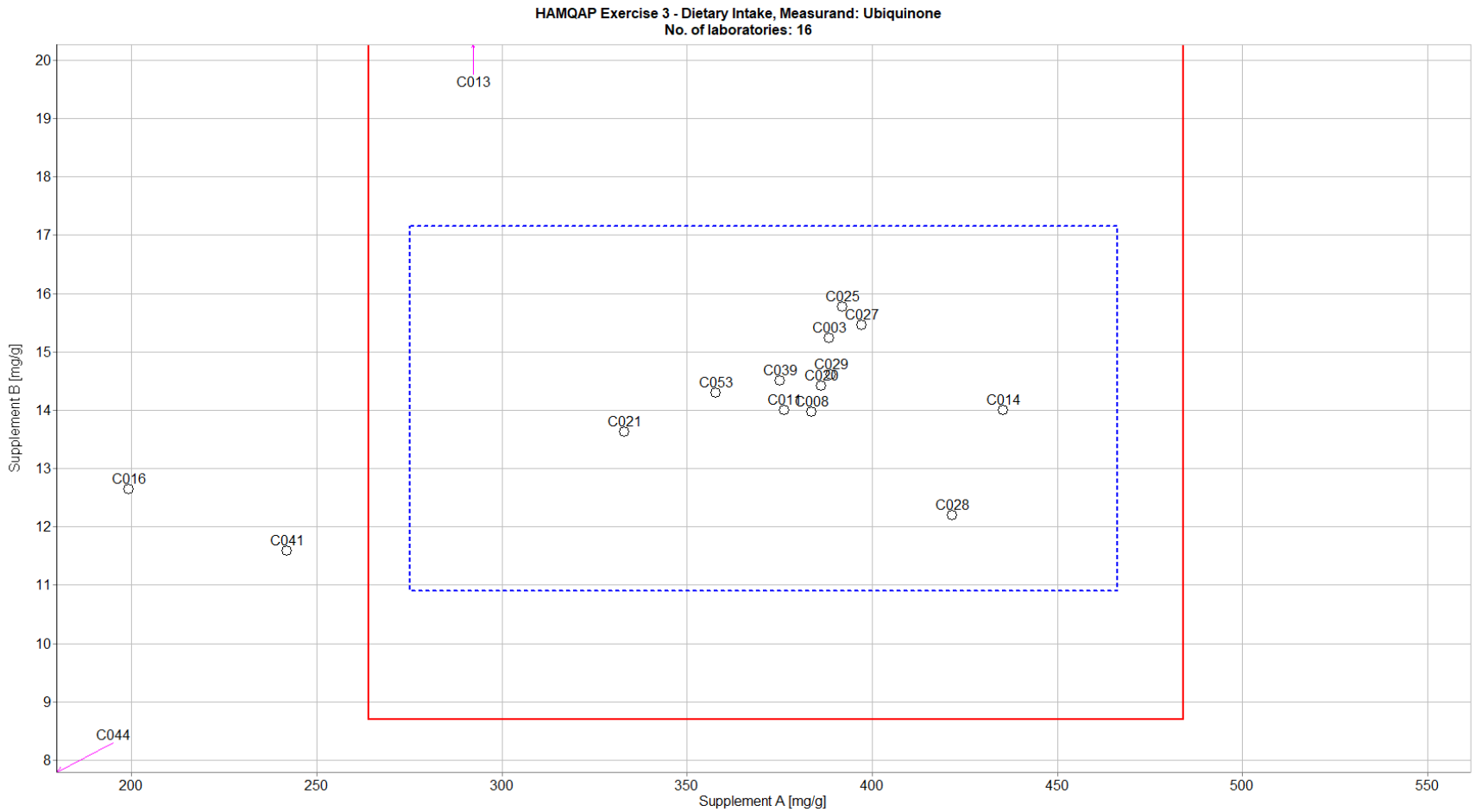

Figure 5-3. Laboratory means for ubiquinone in Supplement A and Supplement B (sample/sample comparison view). In this view, the individual laboratory mean for one sample (supplement A) is compared to the individual laboratory mean for a second sample (supplement B). The solid red box represents the NIST range of tolerance for the two samples, supplement A (x-axis) and supplement B (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, $|Z_{\mathrm{NIST}}| \leq 2$. The dotted blue box represents the consensus range of tolerance for supplement A (x-axis) and supplement B (y-axis), calculated as the values above and below the consensus means that result in an acceptable Z'_{comm} score, $|Z'_{\mathrm{comm}}| \leq 2$.

Human Metabolites Sample Information

Bovine Serum. Participants were provided with three vials, each containing 1 mL of frozen bovine serum. Serum was purchased from a commercial provider, describing the product as off-the-clot serum from bovine blood of grass-fed donor animals. No analytes were spiked into or stripped from the serum. The bovine serum was filtered, blended, and bottled in 1 mL aliquots and stored at –80 °C. Before use, participants were instructed to allow the material to thaw at room temperature for at least 30 min prior to sampling, use the material immediately after thawing, gently mix the contents prior to removal of a test portion for analysis, and use a sample size appropriate for their usual in-house method of analysis. Participants were asked to avoid exposing the material to direct UV light, to store the material at or below –80 °C, and to prepare one sample and report one value from each vial provided. The approximate analyte levels were not reported to participants prior to the study, and a target value for ubiquinone in bovine serum has not been determined at NIST.

Human Serum F. Participants were provided with three vials, each containing 1 mL of frozen human serum. Bovine thrombin and calcium chloride were added to convert the plasma to serum, which was then was dialyzed to remove bovine thrombin, calcium chloride, and anticoagulants. Salts were added back into the serum, and the material was pooled along with isotonic saline, blended, bottled in 1 mL aliquots, and stored at –80 °C. Before use, participants were instructed to allow the material to thaw at room temperature for at least 30 min prior to sampling, use the material immediately after thawing, gently mix the contents prior to removal of a test portion for analysis, and use a sample size appropriate for their usual in-house method of analysis. Participants were asked to avoid exposing the material to direct UV light, to store the material at or below –70 °C, and to prepare one sample and report one value from each vial provided. The approximate analyte level was not reported to participants prior to the study. The NIST-determined value for ubiquinone in SRM 968e (Level 3) was assigned using results from collaborating laboratories. The information value for ubiquinone in SRM 968e Level 3, without an associated uncertainty, is provided in the table below.

Analyte	NIST-Determined Mass Concentration in SRM 968e (Level 3) (µg/mL)
Ubiquinone	1.4

Human Metabolites Study Results

- Ten laboratories enrolled in this exercise and received samples to measure CoQ10 as ubiquinone. Three laboratories reported results for each sample (30 % participation).
- The between-laboratory variability was good for the determination of ubiquinone in bovine serum (15 % RSD) and human serum (21 % RSD).
- Three different analytical methods were reported, including LC-absorbance, LC with electrochemical detection, and LC-MS/MS.
- All laboratories overlapped the consensus means for ubiquinone for both serum samples (**Figure 5-4 and 5-5**), and all laboratories fell within the consensus range for both serum samples (**Figure 5-6**).

Human Metabolites Technical Recommendations

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

- 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 5-3. Individualized data summary table (NIST) for ubiquinone in bovine serum and human serum.

National Institute of Standards and Technology

HAMQAP Exercise 3 - 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*
ubiquinone	Bovine Serum	µg/mL					4	0.205	0.031		
ubiquinone	SRM 968e Fat-Soluble Vitamins, Carotenoids, and Cholesterol in Human Serum (Level 3)	µg/mL	1.4				4	1.29	0.27	1.4	

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 5-4. Data summary table for ubiquinone in bovine serum and human serum.

		ubiquinone									
		Bovine Serum (µg/mL)					SRM 968e Fat-Soluble Vitamins, Carotenoids, and Cholesterol in Human Serum (Level 3) (µg/mL)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target									1.40	
	C012										
	C015										
	C026	0.216	0.19	0.199	0.202	0.013	1.278	1.269	1.278	1.28	0.01
	C027										
	C029	0.18018	0.18606	0.17697	0.181	0.005	1.047	1.08	1.039	1.06	0.02
	C032										
	C054										
	C058	0.24	0.23	0.24	0.237	0.006	1.39	1.42	1.29	1.37	0.07
	C059	0.2	0.2	0.2	0.200	0.000	1.5	1.5	1.4	1.47	0.06
	C066										
Community Results		Consensus Mean			0.205		Consensus Mean			1.29	
		Consensus Standard Deviation			0.031		Consensus Standard Deviation			0.27	
		Maximum			0.237		Maximum			1.47	
		Minimum			0.181		Minimum			1.06	
		N			4		N			4	

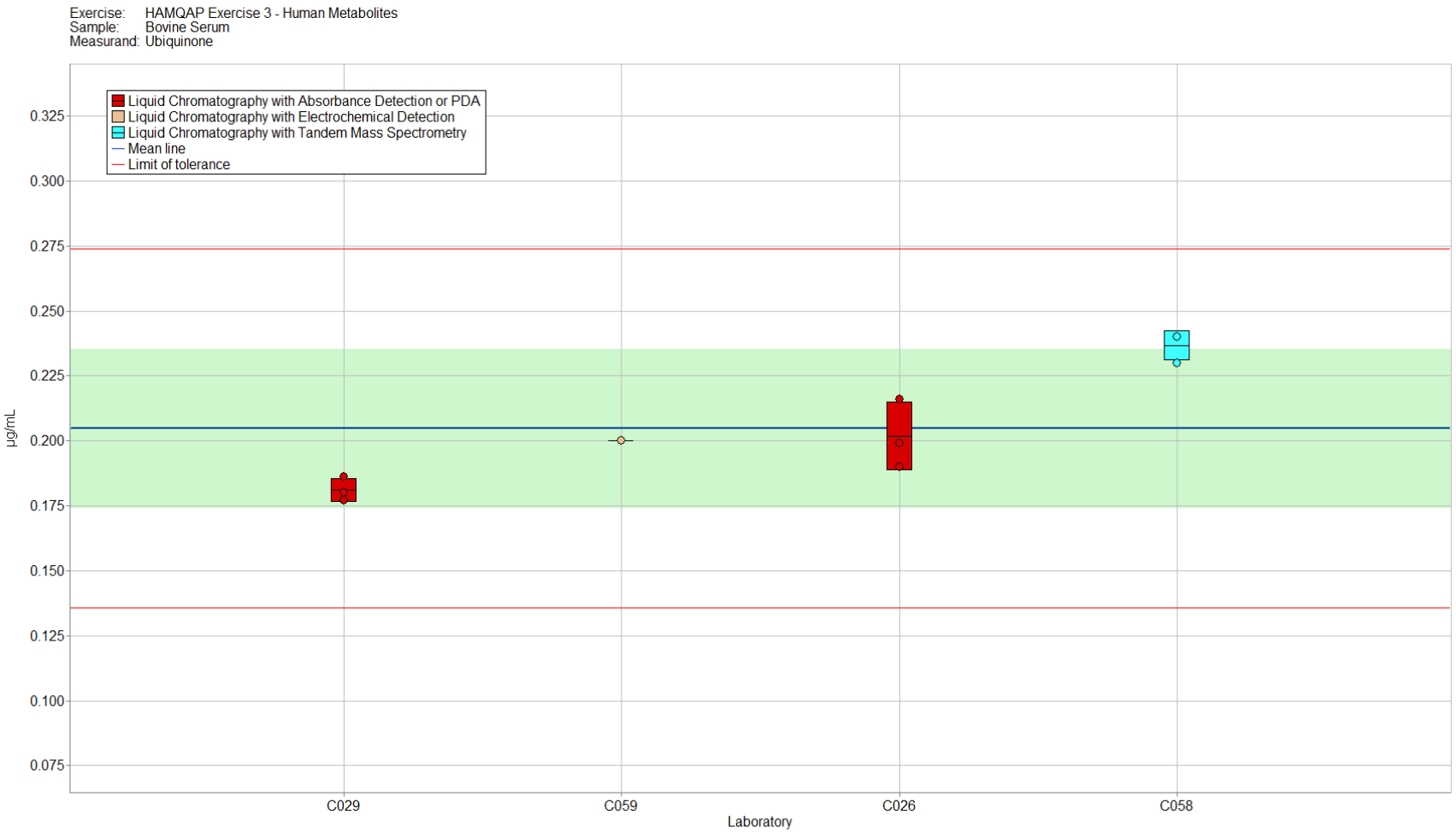

Figure 5-4. Ubiquinone in Bovine Serum (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line represents 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'_{comm} score, $|Z'_{\text{comm}}| \leq 2$. A NIST value has not been determined in this material**.**

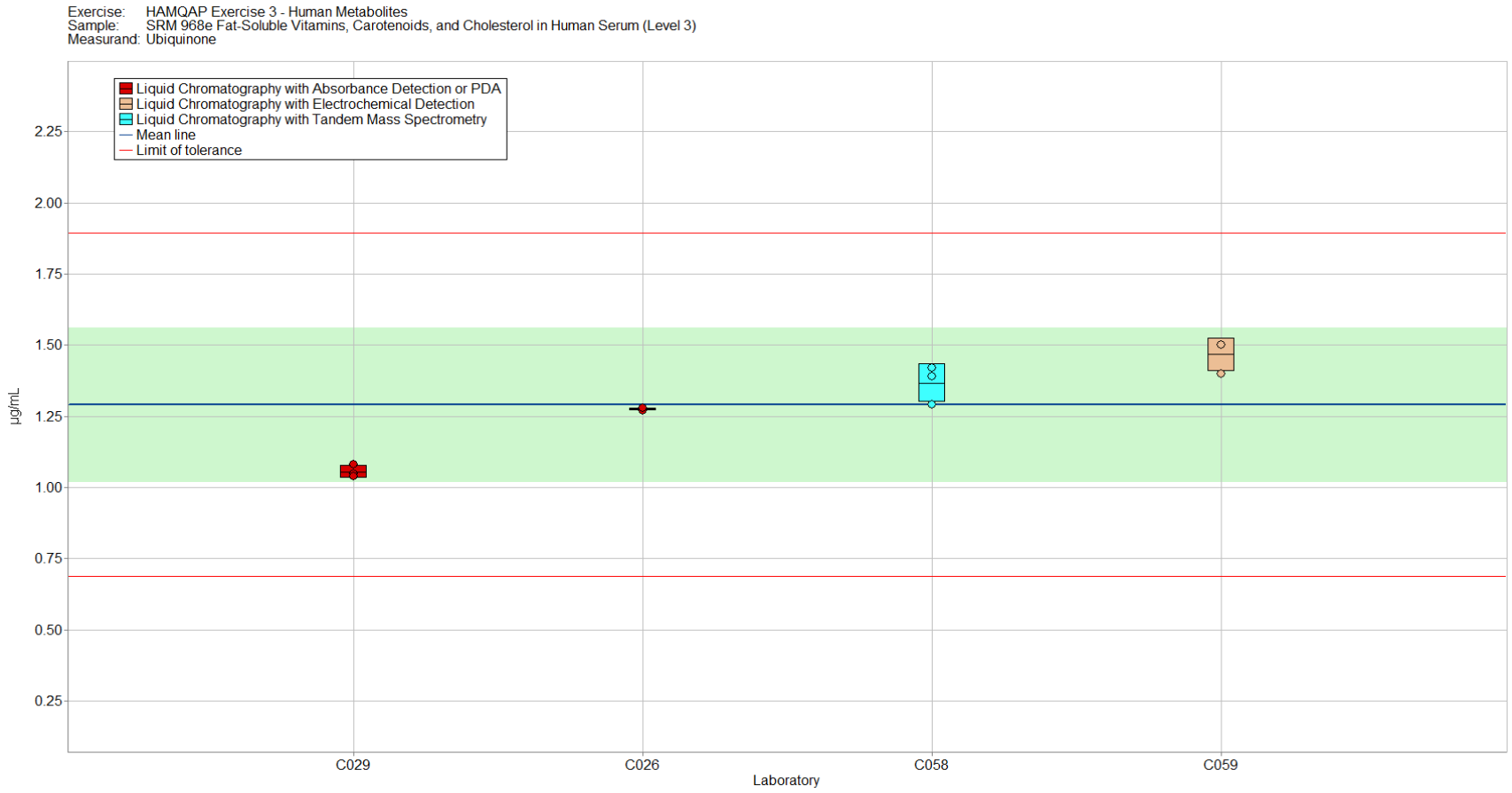

Figure 5-5. Ubiquinone in SRM 968e Fat-Soluble Vitamins, Carotenoids, and Cholesterol in Human Serum (Level 3) (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid 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'_{comm} score, $|Z'_{\text{comm}}| \leq 2$. A NIST information value (1.4 µg/mL) has been determined in this material, but without an associated uncertainty with which to produce a target range.

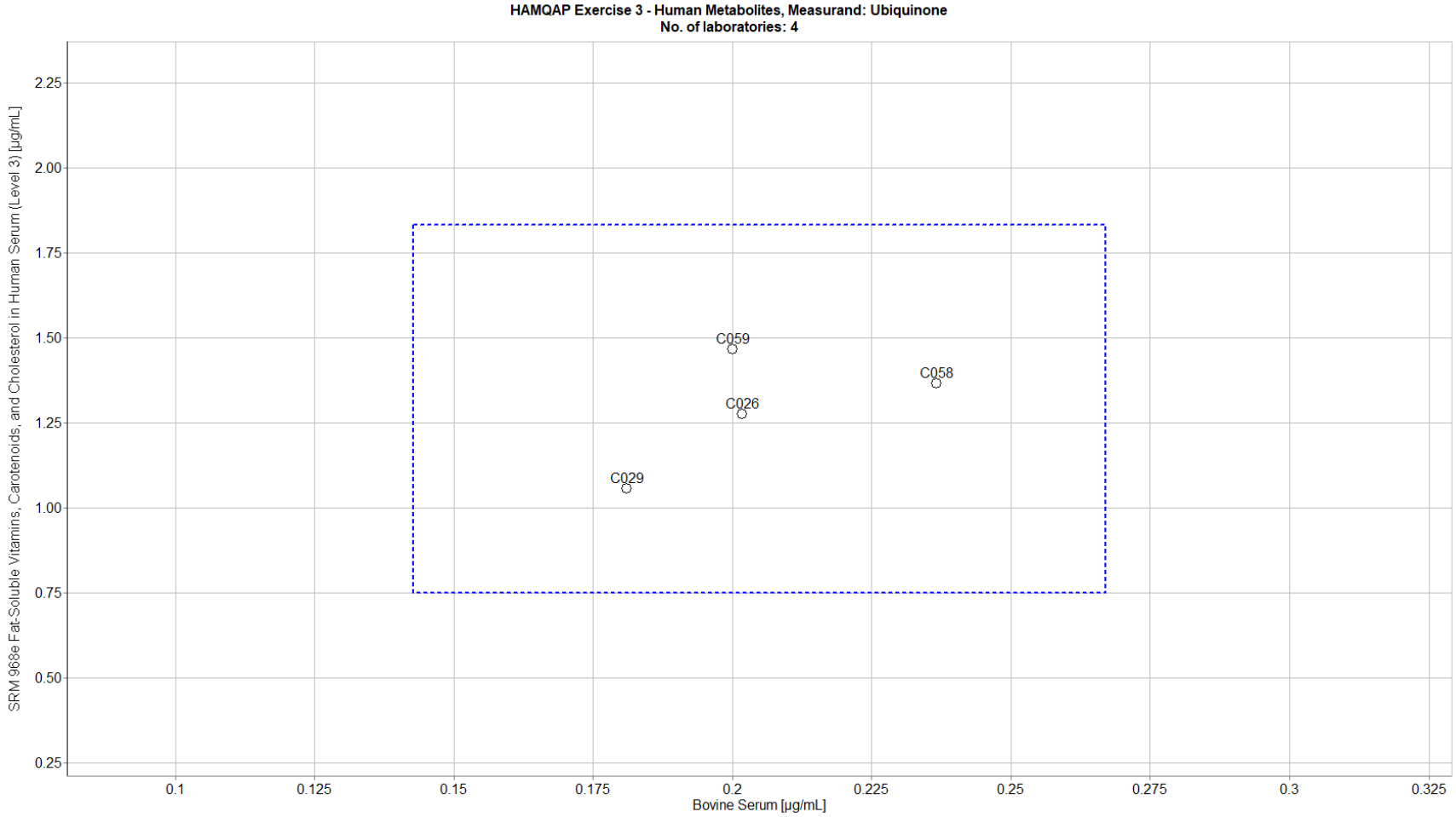

Figure 5-6. Laboratory means for ubiquinone in Bovine Serum and SRM 968e Fat-Soluble Vitamins, Carotenoids, and Cholesterol in Human Serum (Level 3) (sample/sample comparison view). In this view, the individual laboratory mean for one sample (bovine serum) is compared to the mean for a second sample (SRM 968e). The dotted blue box represents the consensus range of tolerance for Bovine Serum (x-axis) and SRM 968e (y-axis), calculated as the values above and below the consensus means that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$.

Natural Products Overall Study Comparison

Overall, laboratories measuring ubiquinone in supplements and serum were successful based on the limited results reported.

- A few laboratories reported data outside of the target range for the supplements, but overall results were excellent.
- 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 in the clinical community.

SECTION 6: BOTANICALS (Isoflavones)

Study Overview

In this study, participants were provided with two soy-based and two red clover-based dietary supplement ingredient samples; SRM 3237 Soy Protein Concentrate, SRM 3236 Soy Protein Isolate, Red Clover Flowers, and Red Clover Extract. Participants were asked to use in-house analytical methods to determine the mass fraction (mg/kg) of select isoflavones (daidzein, daidzin, genistein, genistin, glycitein, glycitin, biochanin A, formononetin) and coumestrol in each matrix. Isoflavones, a subclass of flavonoids, can elicit estrogenic activity due to their chemical structures and have been investigated for biological properties that may influence health and disease, such as reduced cholesterol, improved bone health for postmenopausal women, and reduced risk of some cancers.[17,18] Biochanin A and formononetin are related isoflavones that are reduced to genistein and daidzein by gut bacteria, and share the estrogenic/antiestrogenic, antioxidant, and antiproliferative activities of the prominent isoflavones daidzein, daidzin, genistein, genistin, glycitein, and glycitin.[19] Coumestrol, though not an isoflavone, has an estrogen-like structure and can modulate the activity of certain steroid receptors. Current research attempts to elucidate both positive and adverse effects of isoflavone consumption on several health outcomes including cancer risk, obesity, and cognitive, bone, and cardiovascular function. Accurate information on the identification and quantitation of these compounds in foods and dietary supplements is critical to the interpretation of future clinical studies.

Dietary Intake Sample Information

Soy Protein Concentrate. Participants were provided with three packets, each containing 10 g of powdered soy protein concentrate. The material was prepared by a manufacturer of food and agricultural products and packaged into single-use, nitrogen-flushed pouches. Before use, participants were instructed to mix the contents of the packet thoroughly, and to use a sample size appropriate for their usual in-house methods of analysis. 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. The approximate analyte levels were not reported to participants prior to the study. The certified mass fraction value for daidzin and reference mass fraction values for genistin and glycitin were determined at NIST from results obtained using LC-absorbance and ID-LC-MS. The NIST-determined values and uncertainties for daidzin, genistin, and glycitin in SRM 3237 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 (5.8 %). Target values for daidzein, genistein, glycitein, biochanin A, formononetin, and coumestrol in SRM 3237 have not been determined.

[17] Soy. National Center for Complimentary and Integrative Health. https://nccih.nih.gov/health/soy/ataglance.htm (accessed November 2019)

[18] Red Clover. National Center for Complimentary and Integrative Health. https://nccih.nih.gov/health/redclover/ataglance.htm (accessed November 2019)

[19] Bhagwat, S., Haytowitz, DB, and Holden, JM. 2008. USDA Database for the Isoflavone Content of Selected Foods, Release 2.0. U.S. Department of Agriculture, Agricultural Research Service, Nutrient Data Laboratory https://www.ars.usda.gov/ARSUserFiles/80400525/Data/isoflav/Isoflav_R2.pdf (Accessed July 2019).

Analyte	NIST-Determined Mass Fraction in SRM 3237 (mg/kg) (dry-mass basis)	(as-received basis)
Daidzin	7.79 ± 0.34	7.34 ± 0.32
Genistin	12.3 ± 2.1	11.6 ± 2.0
Glycitin	0.81 ± 0.14	0.76 ± 0.13

Red Clover Flowers. Participants were provided with three packets, each containing 3.3 g of ground red clover flowers. The red clover was harvested in Eolia, MO, in June 2008, and ground to a fine powder. The ground material was sieved to 80 mesh prior to packaging in nitrogen-flushed polyethylene bags, which were then sealed inside nitrogen-flushed aluminized plastic bags along with two packets of silica gel. Before use, participants were instructed to mix the contents of the packet thoroughly, and to use a sample size appropriate for their usual in-house methods of analysis. 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. The approximate analyte levels were not reported to participants prior to the study, and target values for isoflavones and coumestrol in the red clover flowers have not been determined.

Soy Protein Isolate. Participants were provided with three packets, each containing 10 g of soy protein isolate powder. The material was prepared by a manufacturer of food and agricultural products and packaged into single-use, nitrogen-flushed pouches. Before use, participants were instructed to mix the contents of the packet thoroughly, and to use a sample size appropriate for their usual in-house methods of analysis. 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. The approximate analyte levels were not reported to participants prior to the study. The certified mass fraction values for daidzein, daidzin, genistein, genistin, glycitein, and glycitin were determined at NIST from results obtained using LC-absorbance and ID-LC-MS. The certified values and uncertainties for daidzein, daidzin, genistein, genistin, glycitein, and glycitin in SRM 3236 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 (4.9 %). Target values for biochanin A, formononetin, and coumestrol in SRM 3236 have not been determined.

Analyte	Certified Mass Fraction in SRM 3236 (mg/kg) (dry-mass basis)	(as-received basis)
Daidzein	104.3 ± 0.5	99.19 ± 0.47
Daidzin	174 ± 23	165 ± 21.9
Genistin	329 ± 10	313 ± 9.51
Genistein	183 ± 14	174 ± 13.3
Glycitin	31.4 ± 0.5	29.86 ± 0.47
Glycitein	22.7 ± 0.2	21.59 ± 0.19

Red Clover Extract. Participants were provided with three tins, each containing 1.5 g of red clover extract. The red clover was harvested in Eolia, MO, in June 2008, and the extract was packaged in screw-cap tins coated with a food grade lacquer. Before use, participants were instructed to mix the contents of the tin thoroughly, and to use a sample size appropriate for their usual in-house methods of analysis. 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 tin provided. The approximate analyte levels were not reported to participants prior to the study, and target values for isoflavones and coumestrol in the red clover extract have not been determined.

Dietary Intake Study Results

- Seven to fifteen laboratories enrolled in this exercise and received samples to measure each of the isoflavones and coumestrol. The table below lists the participation statistics for each analyte.

	Number of Laboratories Requesting	Number of Laboratories Reporting Results (Percent Participation)			
Analyte	Samples	Soy Protein Concentrate	Soy Protein Isolate	Red Clover Extract	Red Clover Flowers
Daidzein	14	3 (21 %)	6 (43 %)	--	--
Daidzin	14	4 (29 %)	5 (36 %)	--	--
Genistein	15	1 (7 %)	6 (40 %)	--	--
Genistin	15	5 (33 %)	5 (33 %)	--	--
Glycitein	13	2 (15 %)	6 (46 %)	--	--
Glycitin	13	2 (15 %)	5 (38 %)	--	--
Biochanin A	10	--	--	4 (40 %)	4 (40 %)
Formononetin	9	--	--	4 (44 %)	4 (44 %)
Coumestrol	7	0 (0 %)	1 (14 %)	1 (14 %)	1 (14 %)

- The between-laboratory variabilities are reported below.

	Between-Laboratory Variability (% RSD)			
Analyte	Soy Protein Concentrate	Soy Protein Isolate	Red Clover Extract	Red Clover Flowers
Daidzein	>100 %	38 %	--	--
Daidzin	>100 %	41 %	--	--
Genistein	--	52 %	--	--
Genistin	83 %	84 %	--	--
Glycitein	>100 %	54 %	--	--
Glycitin	>100 %	74 %	--	--
Biochanin A	--	--	48 %	45 %
Formononetin	--	--	50 %	45 %
Coumestrol	--	--	--	--

- The between-laboratory variability could not be determined for coumestrol in any of the samples or for genistein in soy protein concentrate based on insufficient data.
- All participating laboratories reported the use of LC-absorbance as their analytical method.

Dietary Intake Technical Recommendations

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

- For most laboratories, daidzein, genistein, and glycitein were below the limit of detection by LC-absorbance in soy protein concentrate.
- Laboratories reporting results that were below the target value should examine sample preparation conditions. Target values for isoflavones were determined using hydrolysis to release acetyl and malonyl esters of the isoflavones. Laboratories not performing a hydrolysis may not be capturing the contribution of the acetyl and malonyl esters to the total isoflavone content, which may lead to low results.
  - **Figure 6-13** is an example of a possible bi-modal distribution caused by hydrolysis differences in which laboratories can be separated into two groups based on whether a hydrolysis step was included in the sample preparation. Additional data and information from participants are necessary to draw more solid conclusions.
  - Results may still be valid if the samples were not hydrolyzed. However, laboratories should be careful to report sample preparation conditions when reporting results to customers.
- Improper calibration may be a cause of measurement error. The upward trend in observed in **Figure 6-5**, **Figure 6-10**, **Figure 6-17**, and **Figure 6-20**, in which laboratories reported high results for both samples or low results for both samples, indicates a potential calibration 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.
  - Individual calibrants should be used for quantitation whenever possible. For example, a daidzein calibrant should not be used for the quantitation of daidzin.
- Laboratories reporting large sample-to-sample variability should investigate the completeness of the extraction during sample preparation.
  - Any extraction procedure should be optimized to determine the most effective extraction solvent to ensure exhaustive extraction of the analyte from the matrix.
  - The optimum number of extraction cycles must be determined by sequential re-extraction of the sample matrix until no further increase in yield is observed. Sequential extractions may be needed if the extraction solvent becomes saturated during the first (or only) extraction cycle.
- Laboratories reporting results flagged as outliers should check for calculation errors. One example is to confirm that factors for all dilutions have been properly tabulated.

Table 6-1. Data summary table for isoflavones and coumestrol in soy and red clover.

National Institute of Standards and Technology

HAMQAP Exercise 3 - 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*
Daidzein	SRM 3237 Soy Protein Concentrate	mg/kg						3	0.8	3.3		
Daidzein	SRM 3236 Soy Protein Isolate	mg/kg		99.19	0.48		0	6	100	37	99.2	0.476
Daidzin	SRM 3237 Soy Protein Concentrate	mg/kg		7.34	0.32		0	4	10	11	7.34	0.32
Daidzin	SRM 3236 Soy Protein Isolate	mg/kg		170	22		0	5	120	49	165	21.9
Genistein	SRM 3237 Soy Protein Concentrate	mg/kg						1	10	33		
Genistein	SRM 3236 Soy Protein Isolate	mg/kg		170	13		0	6	160	81	174	13.3
Genistin	SRM 3237 Soy Protein Concentrate	mg/kg		10	2		0	5	10	10	11.6	1.98
Genistin	SRM 3236 Soy Protein Isolate	mg/kg		312.9	9.5		0	5	220	190	313	9.51
Glycitein	SRM 3237 Soy Protein Concentrate	mg/kg						2	0.6	1.1		
Glycitein	SRM 3236 Soy Protein Isolate	mg/kg		21.59	0.19		0	6	20	13	21.6	0.19
Glycitin	SRM 3237 Soy Protein Concentrate	mg/kg		0.76	0.13		0	2	0.8	1.2	0.763	0.132
Glycitin	SRM 3236 Soy Protein Isolate	mg/kg		29.86	0.48		0	5	20	16	29.9	0.476
Coumestrol	SRM 3237 Soy Protein Concentrate	mg/kg						0				
Coumestrol	SRM 3236 Soy Protein Isolate	mg/kg						1				
Coumestrol	Red Clover Extract	mg/kg						1				
Coumestrol	Red Clover Flowers	mg/kg						1				
Biochanin A	Red Clover Extract	mg/kg						4	4310	2100		
Biochanin A	Red Clover Flowers	mg/kg						4	1550	700		
Formononetin	Red Clover Extract	mg/kg						4	11300	5700		
Formononetin	Red Clover Flowers	mg/kg						4	2080	940		

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 daidzein in soy. Data points highlighted in red have been flagged as potential outliers (e.g., Grubb and/or Cochran) by the NIST software package.

		Daidzein									
		SRM 3237 Soy Protein Concentrate (mg/kg)					SRM 3236 Soy Protein Isolate (mg/kg)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target									99.19	0.48
	C002										
	C014										
	C022	0.53	0.32	0.49	0.447	0.112	97.55	98.09	98.72	98.12	0.59
	C023										
	C027	0	0	0			83.04	83.86	82.06	82.99	0.90
	C028	1.9	2	2	1.967	0.058	79.9	79.1	73.7	77.57	3.37
	C029	< 0.0100	< 0.0100	< 0.0100			112.18	113.76	114.7	113.55	1.27
	C032										
	C033	30.9	32.2	38.9	34.000	4.293	393	393	393	393.00	0.00
	C039										
	C044						119	118	118	118.33	0.58
	C045										
	C054										
	C055										
Community Results		Consensus Mean			0.804		Consensus Mean			98.11	
		Consensus Standard Deviation			3.329		Consensus Standard Deviation			36.89	
		Maximum			34.000		Maximum			393.00	
		Minimum			0.447		Minimum			77.57	
		N			3		N			6	

Figure 6-1. Daidzein in SRM 3237 Soy Protein Concentrate (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). 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 and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$, with the lower range set at zero. A NIST value has not been determined in this material.

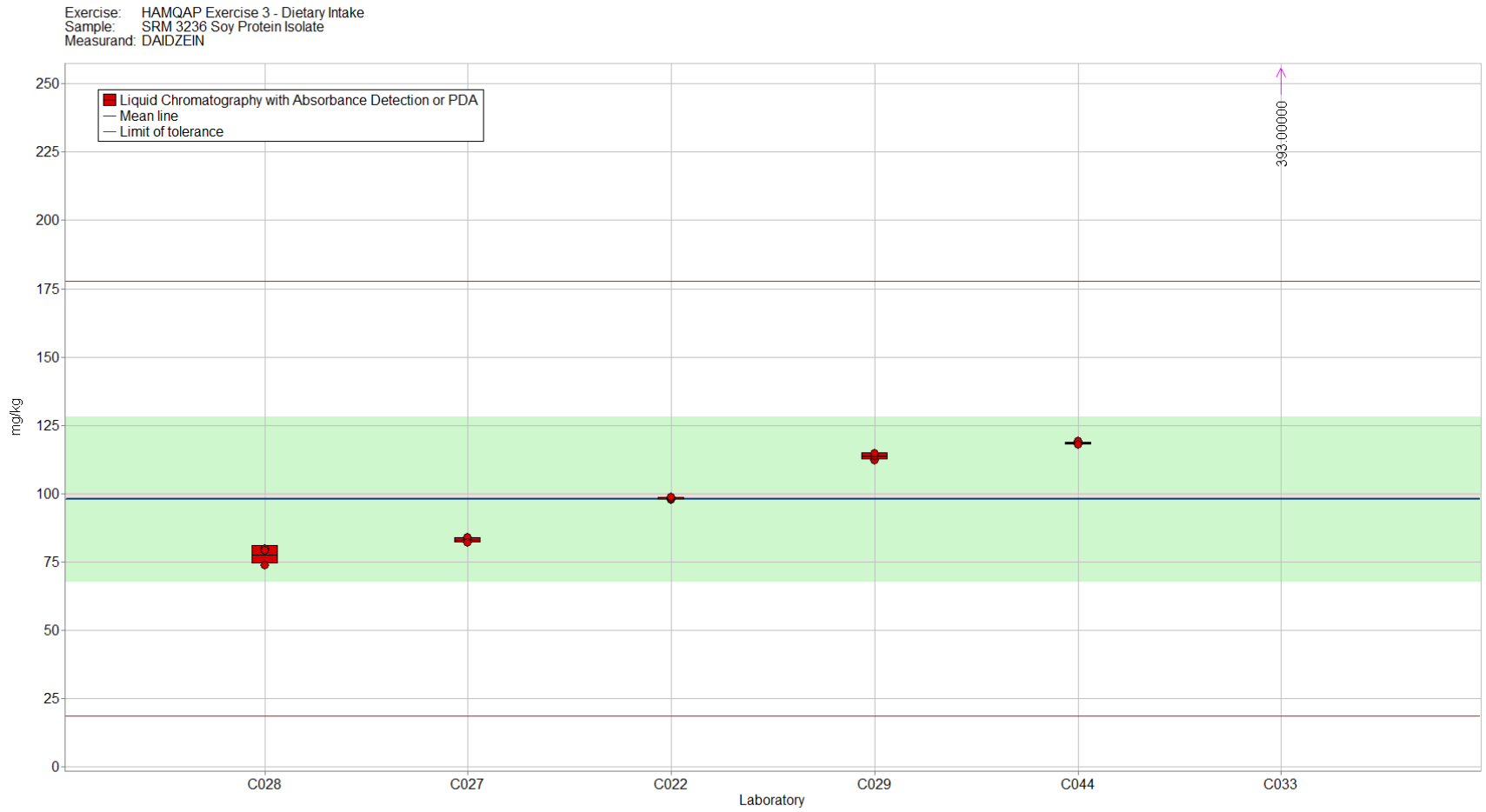

Figure 6-2. Daidzein in SRM 3236 Soy Protein Isolate (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The solid blue line represents the consensus mean, and the green shaded region represents 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'_{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_{NIST}) and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$.

Table 6-3. Data summary table for daidzin in soy.

		Daidzin									
		SRM 3237 Soy Protein Concentrate (mg/kg)					SRM 3236 Soy Protein Isolate (mg/kg)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target				7.34	0.32				165	22
	C002										
	C014										
	C022	11.81	11.7	12.28	11.93	0.31	192.99	193.95	193.46	193.5	0.5
	C023										
	C027	0	0	0			91.78	91.88	90.32	91.3	0.9
	C028	4.8	5.5	5.1	5.13	0.35	68.8	69.9	67.3	68.7	1.3
	C029	10.46	10.73	10.32	10.50	0.21	166.78	169.79	171.14	169.2	2.2
	C032										
	C033										
	C039										
	C044	5.07	4.85	4.68	4.87	0.20	76.1	75.2	75.8	75.7	0.5
	C045										
	C054										
	C055										
Community Results		Consensus Mean			6.49		Consensus Mean			119.5	
		Consensus Standard Deviation			10.65		Consensus Standard Deviation			48.8	
		Maximum			11.93		Maximum			193.5	
		Minimum			4.87		Minimum			68.7	
		N			4		N			5	

Figure 6-3. Daidzin in SRM 3237 Soy Protein Concentrate (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). 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 and below the consensus mean that result in an acceptable Z'_{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 its uncertainty (U_{NIST}) and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$.

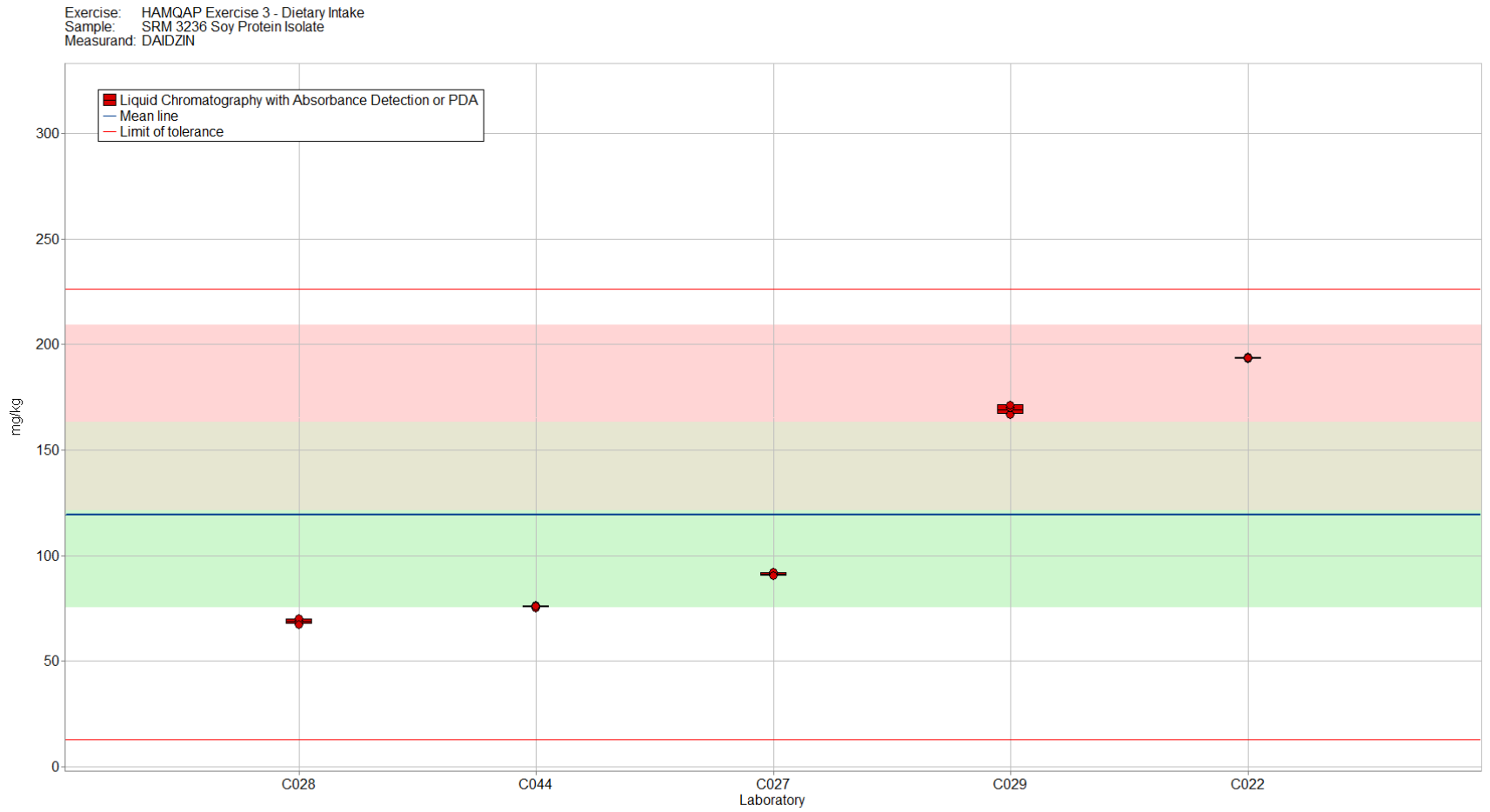

Figure 6-4. Daidzin in SRM 3236 Soy Protein Isolate (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The solid blue line represents the consensus mean, and the green shaded region represents 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'_{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_{NIST}) and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$.

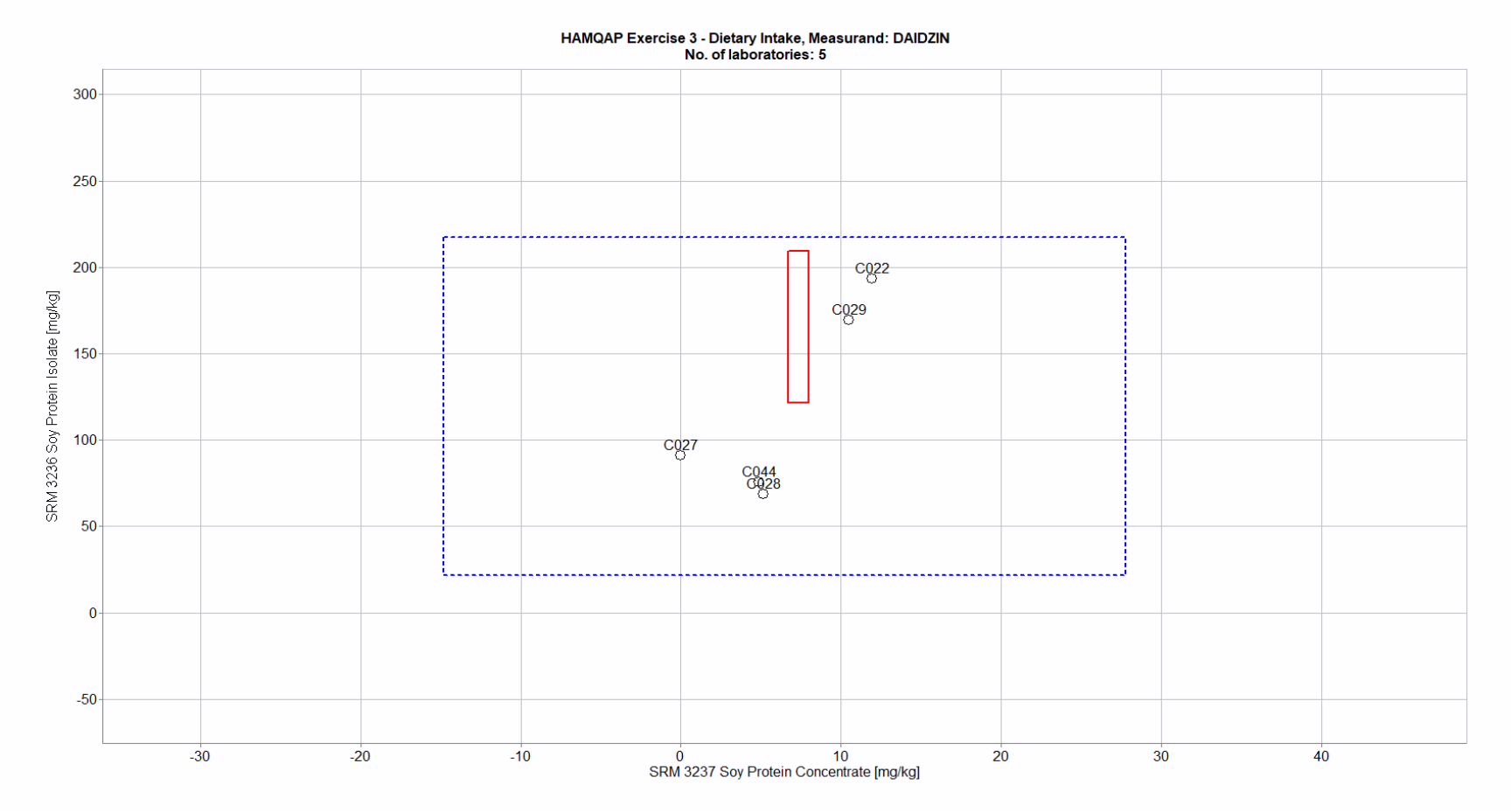

Figure 6-5. Laboratory means for daidzin in SRM 3237 Soy Protein Concentrate and SRM 3236 Soy Protein Isolate (sample/sample comparison view). In this view, the individual laboratory mean for one sample (SRM 3237) is compared to the individual laboratory mean for a second sample (SRM 3236). The solid red box represents the NIST range of tolerance for SRM 3237 (x-axis) and SRM 3236 (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, $|Z_{\mathrm{NIST}}| \leq 2$. The dotted blue box represents the consensus range of tolerance for SRM 3237 (x-axis) and SRM 3236 (y-axis), calculated as the values above and below the consensus means that result in an acceptable Z'_{comm} score, $|Z'_{\mathrm{comm}}| \leq 2$.

Table 6-3. Data summary table for genistein in soy. Data points highlighted in red have been flagged as potential outliers (e.g., Grubb and/or Cochran) by the NIST software package.

		Genistein									
		SRM 3237 Soy Protein Concentrate (mg/kg)					SRM 3236 Soy Protein Isolate (mg/kg)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target									174	13
	C002										
	C014										
	C022						158.26	158.84	160.98	159.4	1.4
	C023										
	C027	0	0	0			168.88	171.49	169.03	169.8	1.5
	C028						105.9	103.7	97.1	102.2	4.6
	C029	< 0.0100	< 0.0100	< 0.0100			139.9	143.58	143.47	142.3	2.1
	C032										
	C033	15	15.7	14.7	15.13	0.51	559	558	576	564.3	10.1
	C039										
	C044						206	206	204	205.3	1.2
	C045										
	C046										
	C054										
	C055										
Community Results		Consensus Mean			7.57		Consensus Mean			155.8	
		Consensus Standard Deviation			32.79		Consensus Standard Deviation			80.6	
		Maximum			15.13		Maximum			564.3	
		Minimum			15.13		Minimum			102.2	
		N			1		N			6	

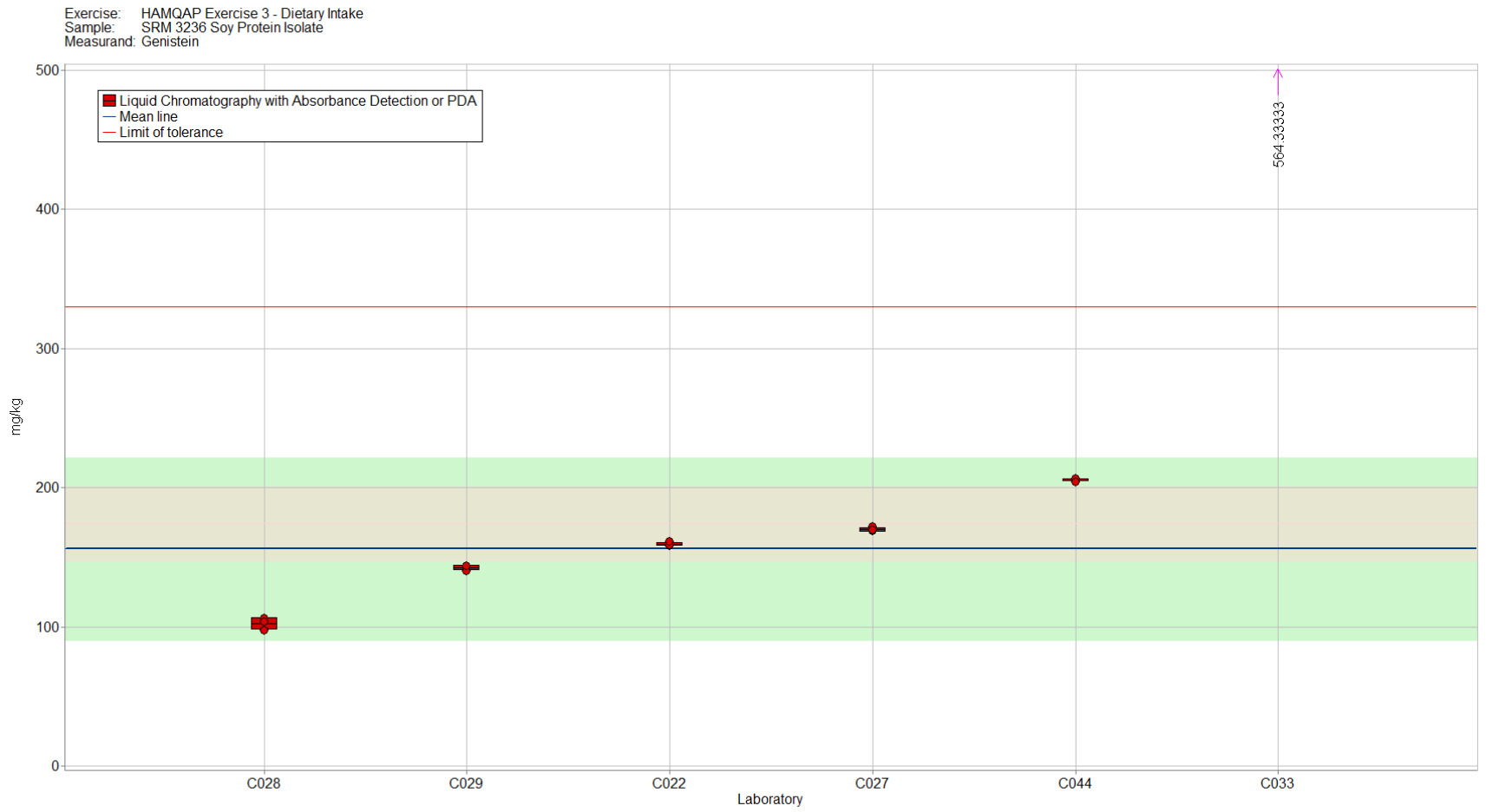

Figure 6-7. Geinstein in SRM 3236 Soy Protein Isolate (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red line represents the upper consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{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 its uncertainty (U_{NIST}) and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$.

Table 6-3. Data summary table for genistin in soy.

		Genistin									
		SRM 3237 Soy Protein Concentrate (mg/kg)					SRM 3236 Soy Protein Isolate (mg/kg)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target				11.6	2.0				312.9	9.5
	C002										
	C014										
	C022	18.74	17.7	19.31	18.58	0.82	323.41	323.67	324.79	324.0	0.7
	C023										
	C027	6.46	8.06	6.06	6.86	1.06	199.3	195.36	193.65	196.1	2.9
	C028	3.7	4.8	4.3	4.27	0.55	69.6	70.5	69.4	69.8	0.6
	C029	17.24	17.19	17.2	17.21	0.03	305.94	309.82	313.35	309.7	3.7
	C032										
	C033										
	C039										
	C044	12.3	12.4	12.7	12.47	0.21	227	224	222	224.3	2.5
	C045										
	C046										
	C054										
	C055										
Community Results		Consensus Mean			11.88		Consensus Mean			224.8	
		Consensus Standard Deviation			10.07		Consensus Standard Deviation			190.4	
		Maximum			18.58		Maximum			324.0	
		Minimum			4.27		Minimum			69.8	
		N			5		N			5	

Figure 6-8. Genistin in SRM 3237 Soy Protein Concentrate (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). 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 and below the consensus mean that result in an acceptable Z'_{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 its uncertainty (U_{NIST}) and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$.

Figure 6-9. Geinstin in SRM 3236 Soy Protein Isolate (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). 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 and below the consensus mean that result in an acceptable Z'_{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 its uncertainty (U_{NIST}) and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$.

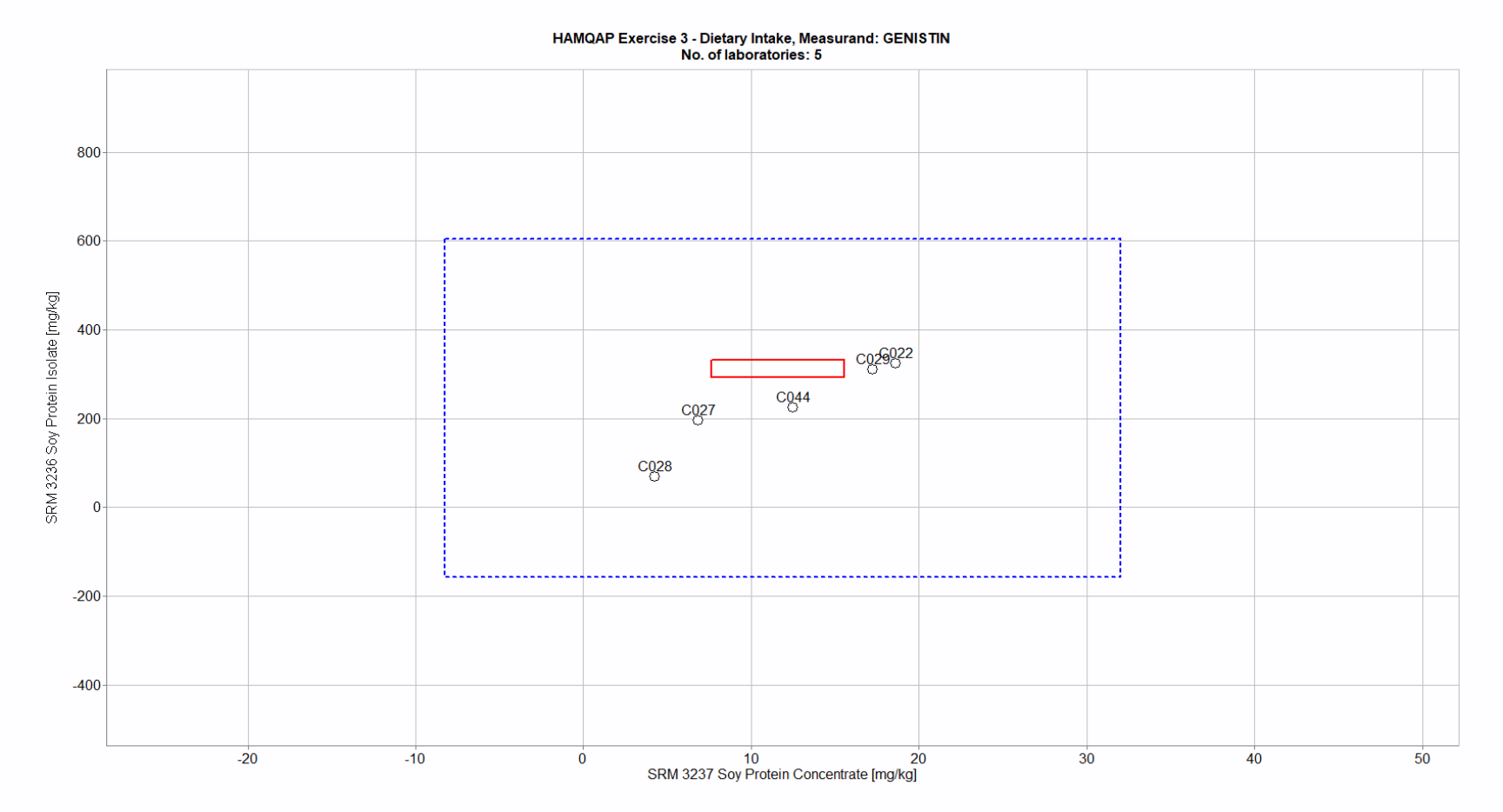

Figure 6-10. Laboratory means for genistin in SRM 3237 Soy Protein Concentrate and SRM 3236 Soy Protein Isolate (sample/sample comparison view). In this view, the individual laboratory mean for one sample (SRM 3237) is compared to the individual laboratory mean for a second sample (SRM 3236). The solid red box represents the NIST range of tolerance for SRM 3237 (x-axis) and SRM 3236 (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, $|Z_{\mathrm{NIST}}| \leq 2$. The dotted blue box represents the consensus range of tolerance for SRM 3237 (x-axis) and SRM 3236 (y-axis), calculated as the values above and below the consensus means that result in an acceptable Z'_{comm} score, $|Z'_{\mathrm{comm}}| \leq 2$.

Table 6-4. Data summary table for glycitein in soy. Data points highlighted in red have been flagged as potential outliers (e.g., Grubb and/or Cochran) by the NIST software package.

		Glycitein									
		SRM 3237 Soy Protein Concentrate (mg/kg)					SRM 3236 Soy Protein Isolate (mg/kg)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target									21.59	0.19
	C002										
	C014										
	C022	1.47	1.78	1.23	1.49	0.28	27.14	27.38	28.67	27.73	0.82
	C023										
	C027	0	0	0			19.81	20.95	20.47	20.41	0.57
	C028	0.5	0.92	1	0.81	0.27	10.9	10.9	10	10.60	0.52
	C029	< 0.0100	< 0.0100	< 0.0100			23.59	21.85	22.41	22.62	0.89
	C032										
	C033	0	0	0			68.8	77.5	70.8	72.37	4.56
	C039										
	C044						27.5	27.3	27.3	27.37	0.12
	C054										
	C055										
Community Results		Consensus Mean			0.58		Consensus Mean			23.53	
		Consensus Standard Deviation			1.11		Consensus Standard Deviation			12.84	
		Maximum			1.49		Maximum			72.37	
		Minimum			0.81		Minimum			10.60	
		N			2		N			6	

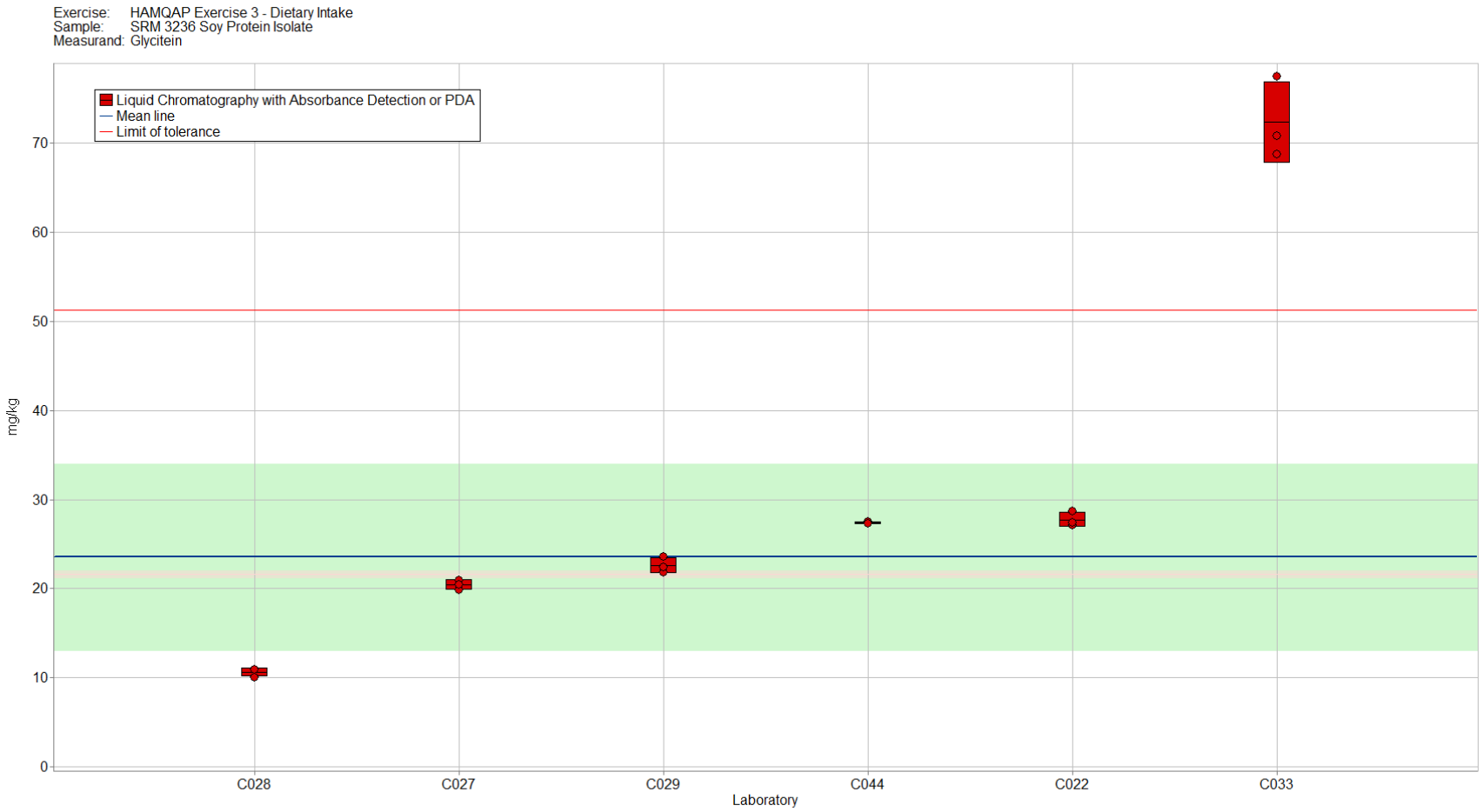

Figure 6-11. Glycitein in SRM 3236 Soy Protein Isolate (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The solid blue line represents the consensus mean, and the green shaded region 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 and below the consensus mean that result in an acceptable Z'_{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 its uncertainty (U_{NIST}) and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$.

Table 6-5. Data summary table for glycitin in soy.

		Glycitin									
		SRM 3237 Soy Protein Concentrate (mg/kg)					SRM 3236 Soy Protein Isolate (mg/kg)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target				0.76	0.13				29.86	0.48
	C002										
	C014										
	C022						34	33.88	33.87	33.92	0.07
	C023										
	C027	0	0	0			16.22	16.06	15.27	15.85	0.51
	C028	1	1	0.8	0.93	0.12	6.9	7.4	6.3	6.87	0.55
	C029	1.31	1.156	1.526	1.33	0.19	34.39	34.39	35.08	34.62	0.40
	C032										
	C033										
	C039										
	C044						14.3	13.4	14	13.90	0.46
	C054										
	C055										
Community Results		Consensus Mean			0.75		Consensus Mean			21.03	
		Consensus Standard Deviation			1.16		Consensus Standard Deviation			15.61	
		Maximum			1.33		Maximum			34.62	
		Minimum			0.93		Minimum			6.87	
		N			2		N			5	

Figure 6-12. Glycitin in SRM 3237 Soy Protein Concentrate (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). 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 and below the consensus mean that result in an acceptable Z'_{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 its uncertainty (U_{NIST}) and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$.

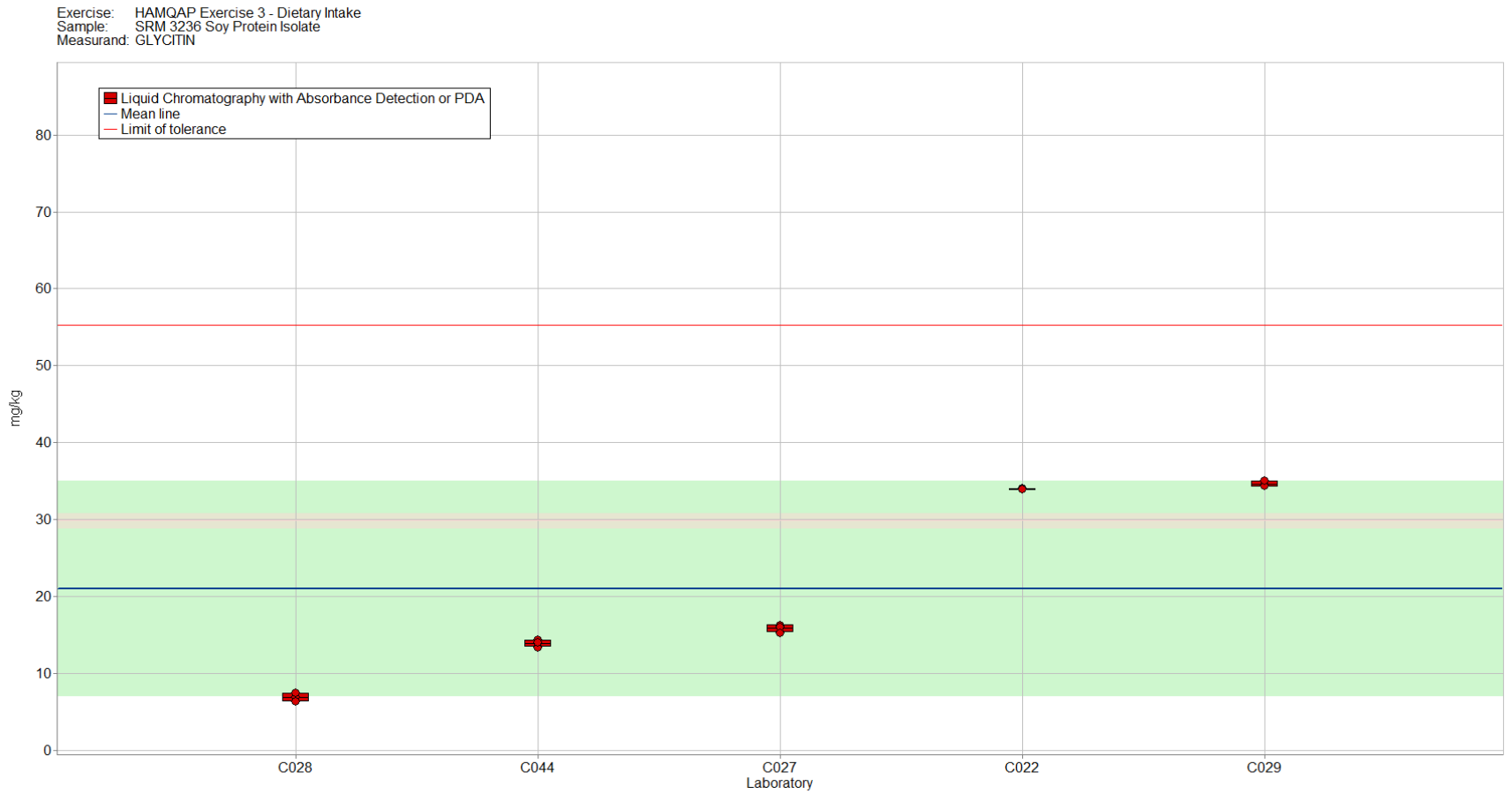

Figure 6-13. Glycitin in SRM 3236 Soy Protein Isolate (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red line represents the upper consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{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 its uncertainty (U_{NIST}) and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$.

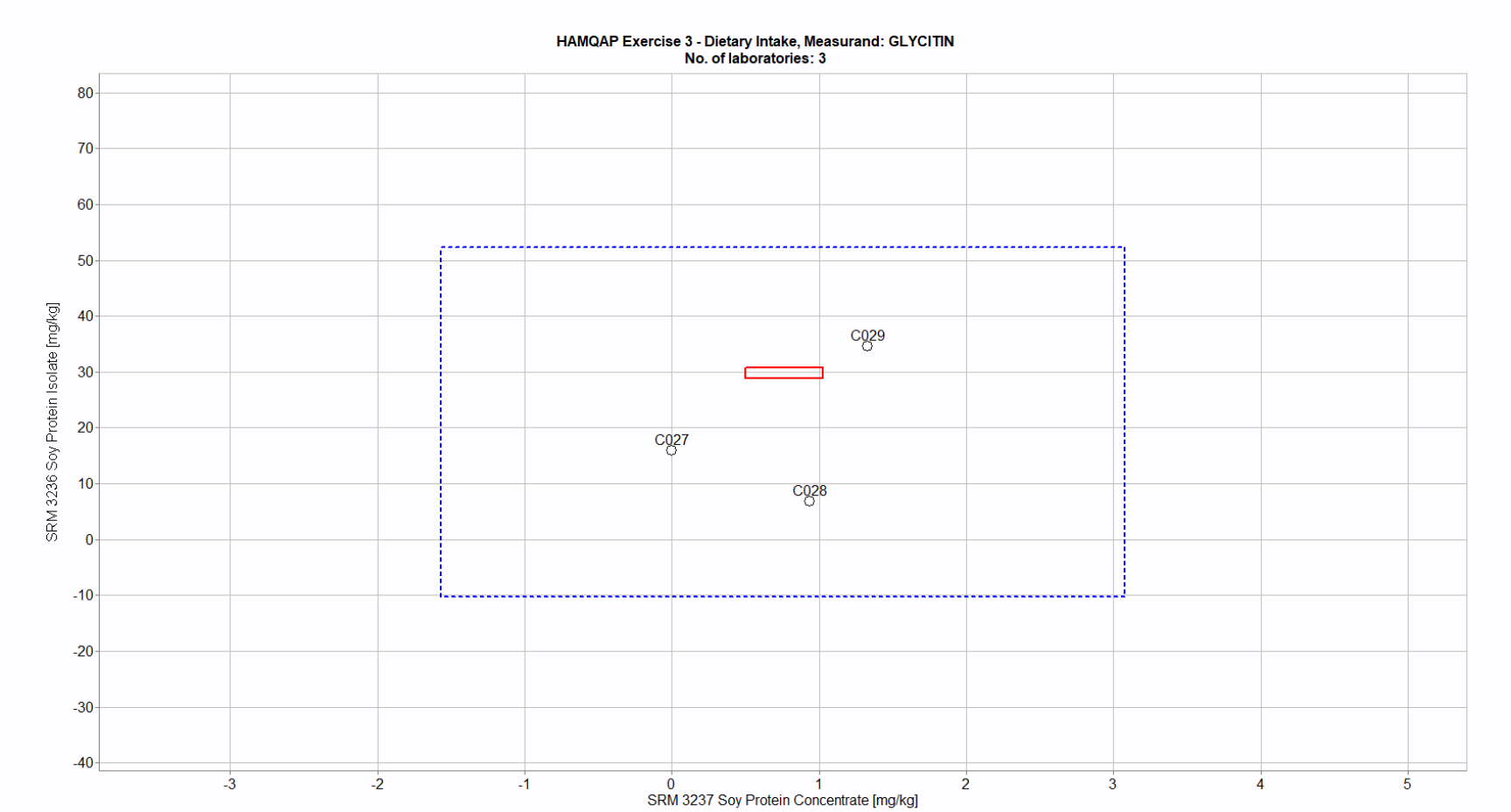

Figure 6-14. Laboratory means for glycitin in SRM 3237 Soy Protein Concentrate and SRM 3236 Soy Protein Isolate (sample/sample comparison view). In this view, the individual laboratory mean for one sample (SRM 3237) is compared to the individual laboratory mean for a second sample (SRM 3236). The solid red box represents the NIST range of tolerance for SRM 3237 (x-axis) and SRM 3236 (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, $|Z_{\mathrm{NIST}}| \leq 2$. The dotted blue box represents the consensus range of tolerance for SRM 3237 (x-axis) and SRM 3236 (y-axis), calculated as the values above and below the consensus means that result in an acceptable Z'_{comm} score, $|Z'_{\mathrm{comm}}| \leq 2$.

Table 6-6. Data summary table for coumestrol in red clover and soy.

		Coumestrol																			
		Red Clover Extract (mg/kg)					Red Clover Flowers (mg/kg)					SRM 3237 Soy Protein Concentrate (mg/kg)					SRM 3236 Soy Protein Isolate (mg/kg)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target																				
	C014																				
	C022																				
	C027	280.96	214.54	224.53	240	36	389.59	418.56	458.48	422	35	0	0	0			12.89	11.41	14.12	12.8	1.4
	C029																				
	C032																				
	C054																				
	C055																				
Community Results		Consensus Mean					Consensus Mean					Consensus Mean					Consensus Mean				
		Consensus Standard Deviation					Consensus Standard Deviation					Consensus Standard Deviation					Consensus Standard Deviation				
		Maximum			240		Maximum			422		Maximum					Maximum			12.8	
		Minimum			240		Minimum			422		Minimum					Minimum			12.8	
		N			1		N			1		N			0		N			1	

Table 6-3. Data summary table for biochanin A in red clover.

		Biochanin A									
		Red Clover Extract (mg/kg)					Red Clover Flowers (mg/kg)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target										
	C002										
	C014										
	C022	3649	3621	3769	3680	79	1518	1562	1523	1534	24
	C027	4559.18	4613.55	4772.21	4648	111	1022.63	1515.29	1082.56	1207	269
	C028	5938.3	5660.4	6304	5968	323	2650.2	1940.8	2138	2243	366
	C029	2975	2890	2928	2931	43	1236	1202	1153	1197	42
	C032										
	C045										
	C054										
	C055										
Community Results		Consensus Mean			4307		Consensus Mean			1545	
		Consensus Standard Deviation			2082		Consensus Standard Deviation			701	
		Maximum			5968		Maximum			2243	
		Minimum			2931		Minimum			1197	
		N			4		N			4	

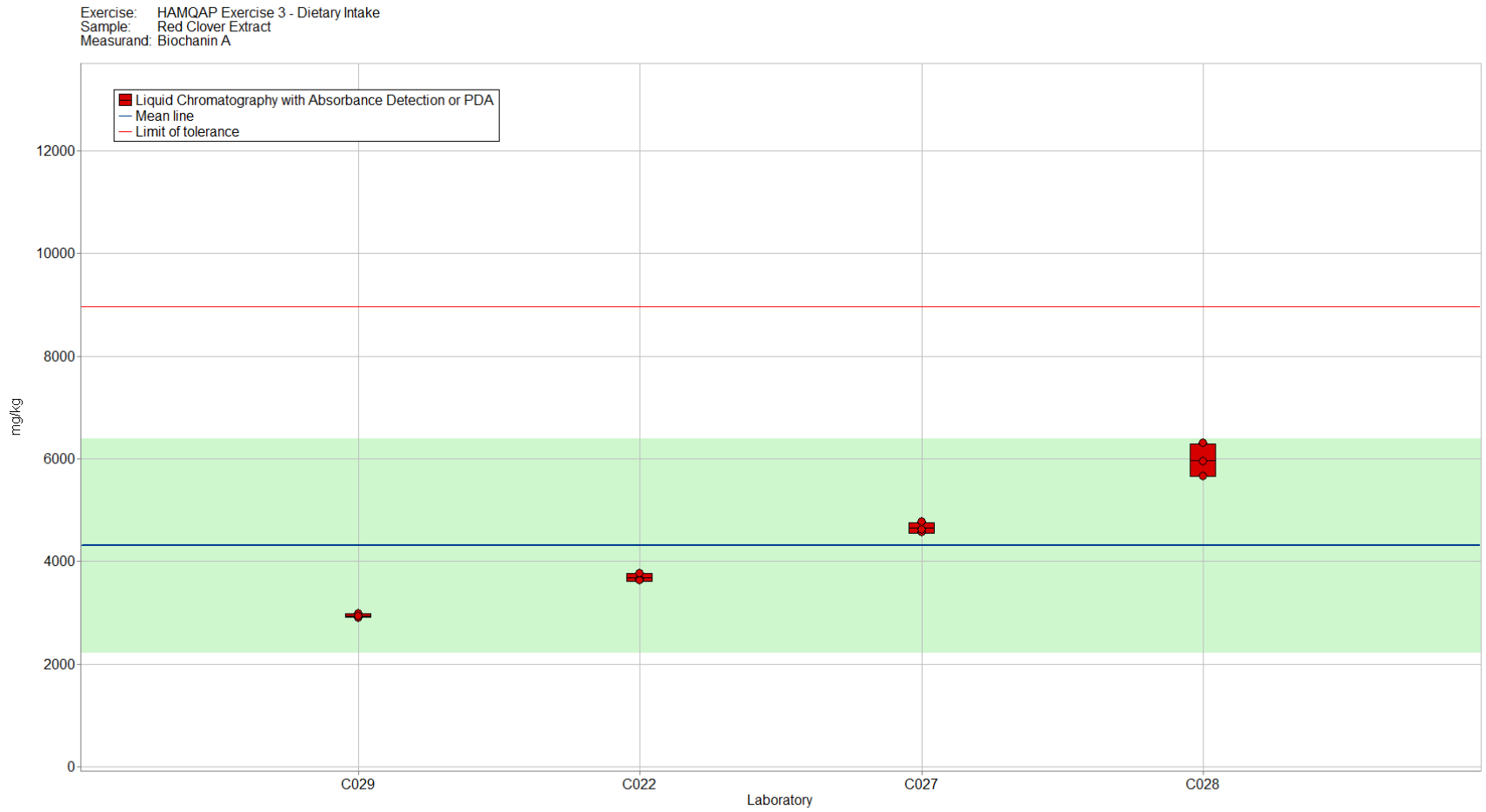

Figure 6-15. Biochanin A in Red Clover Extract (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red line represents the upper consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$, with the lower range set at zero. A NIST value has not been determined in this material.

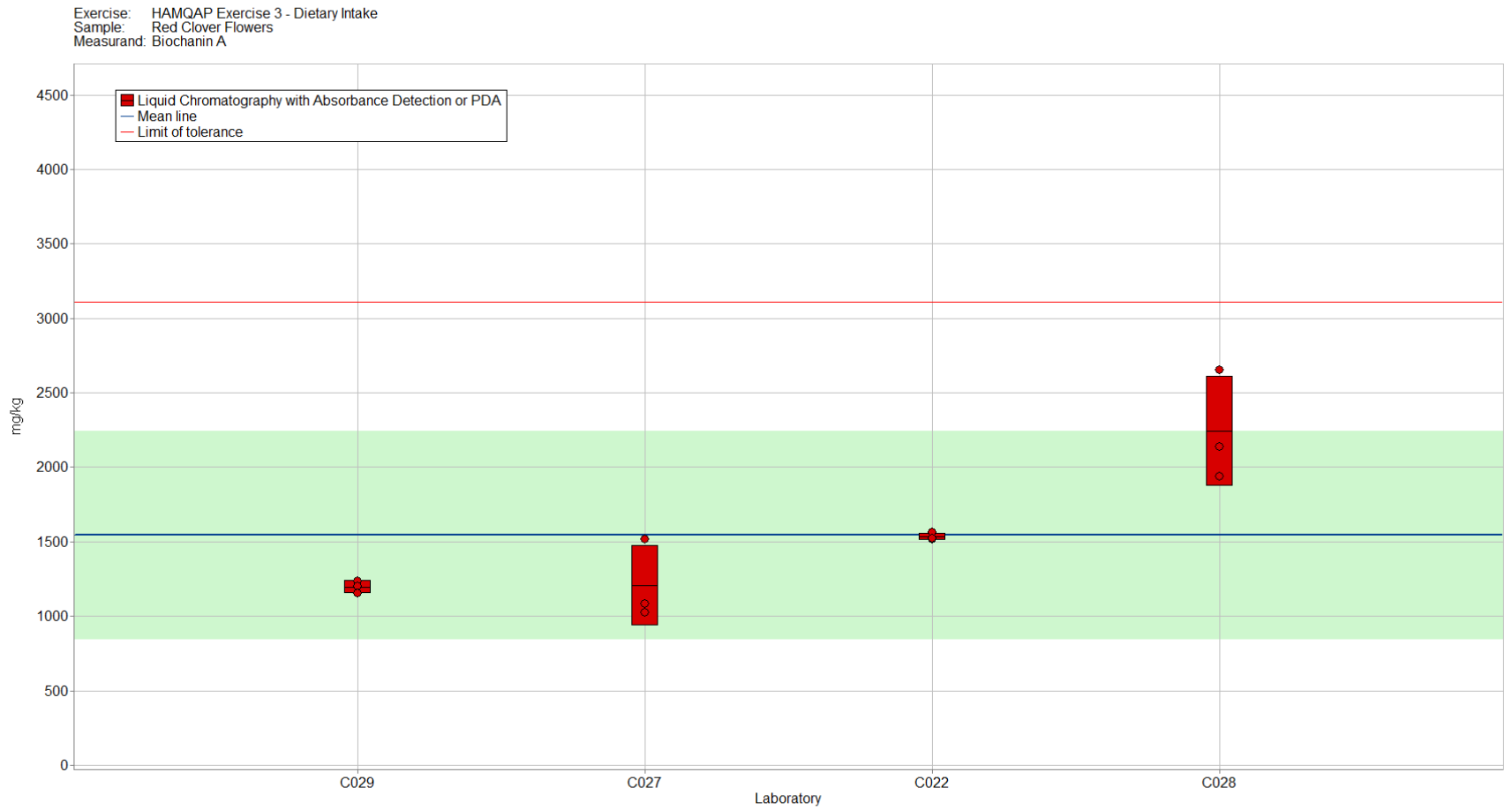

Figure 6-16. Biochanin A in Red Clover Flowers (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red line represents the upper consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$, with the lower range set at zero. A NIST value has not been determined in this material.

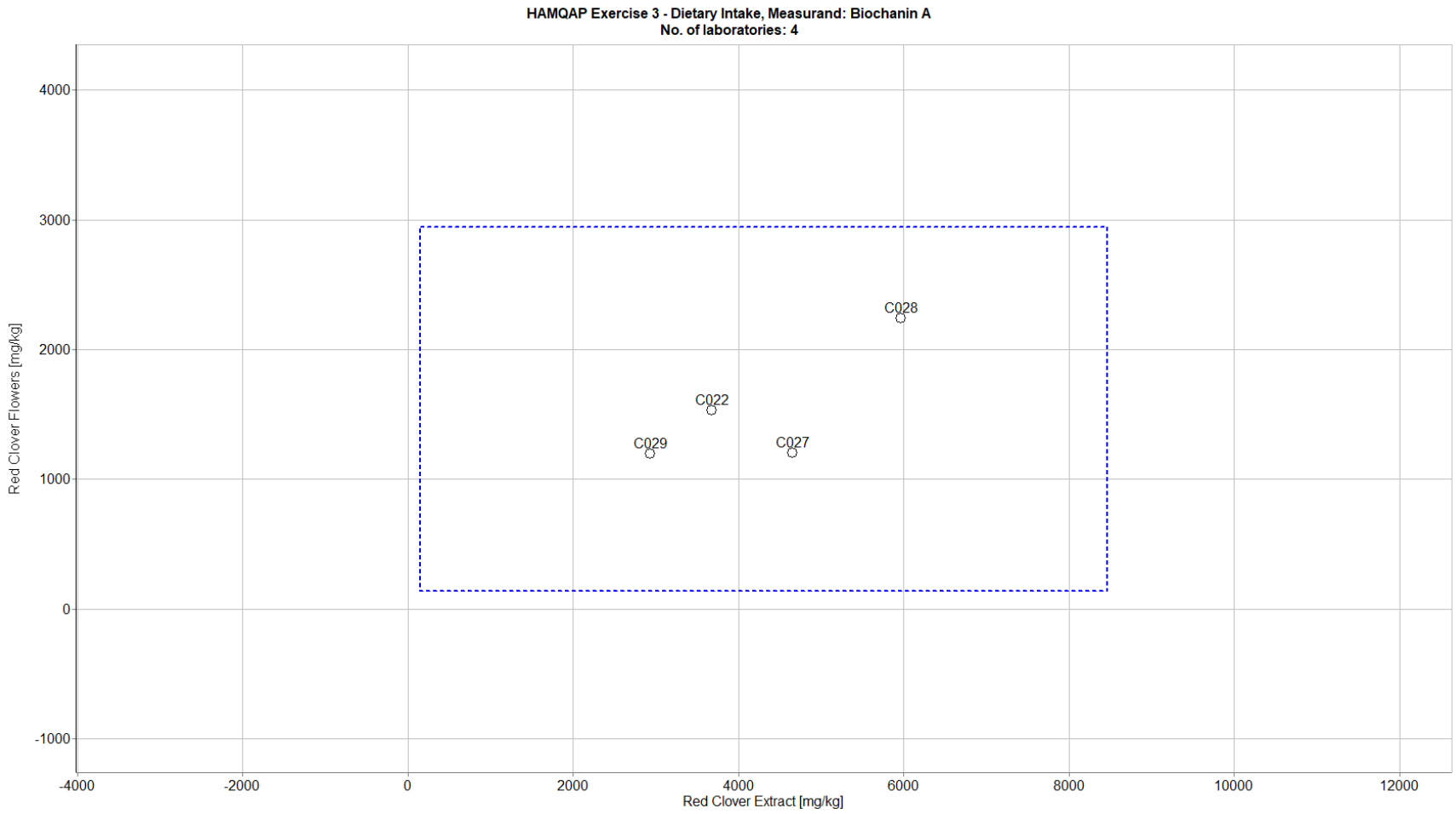

Figure 6-17. Laboratory means for biochanin A in Red Clover Extract and Red Clover Flowers (sample/sample comparison view). In this view, the individual laboratory mean for one sample (extract) is compared to the individual laboratory mean for a second sample (flowers). The dotted blue box represents the consensus range of tolerance for extract (x-axis) and flowers (y-axis), calculated as the values above and below the consensus means that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$.

Table 6-3. Data summary table for formononetin in red clover. Data points highlighted in red have been flagged as potential outliers (e.g., Grubb and/or Cochran) by the NIST software package.

		Formononetin									
		Red Clover Extract (mg/kg)					Red Clover Flowers (mg/kg)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target										
	C014										
	C022	9873	9861	10407	10047	312	1880	1928	1879	1896	28
	C027	9113.57	9850.71	10227.3	9731	567	1248.85	1217.62	1302.83	1256	43
	C028	17780.1	18477	18254.4	18171	356	4157.7	4508.3	4762.8	4476	304
	C029	7126	7165	7310	7200	97	1735	1673	1614	1674	61
	C032										
	C045										
	C054										
	C055										
Community Results		Consensus Mean			11287		Consensus Mean			2079	
		Consensus Standard Deviation			5661		Consensus Standard Deviation			941	
		Maximum			18171		Maximum			4476	
		Minimum			7200		Minimum			1256	
		N			4		N			4	

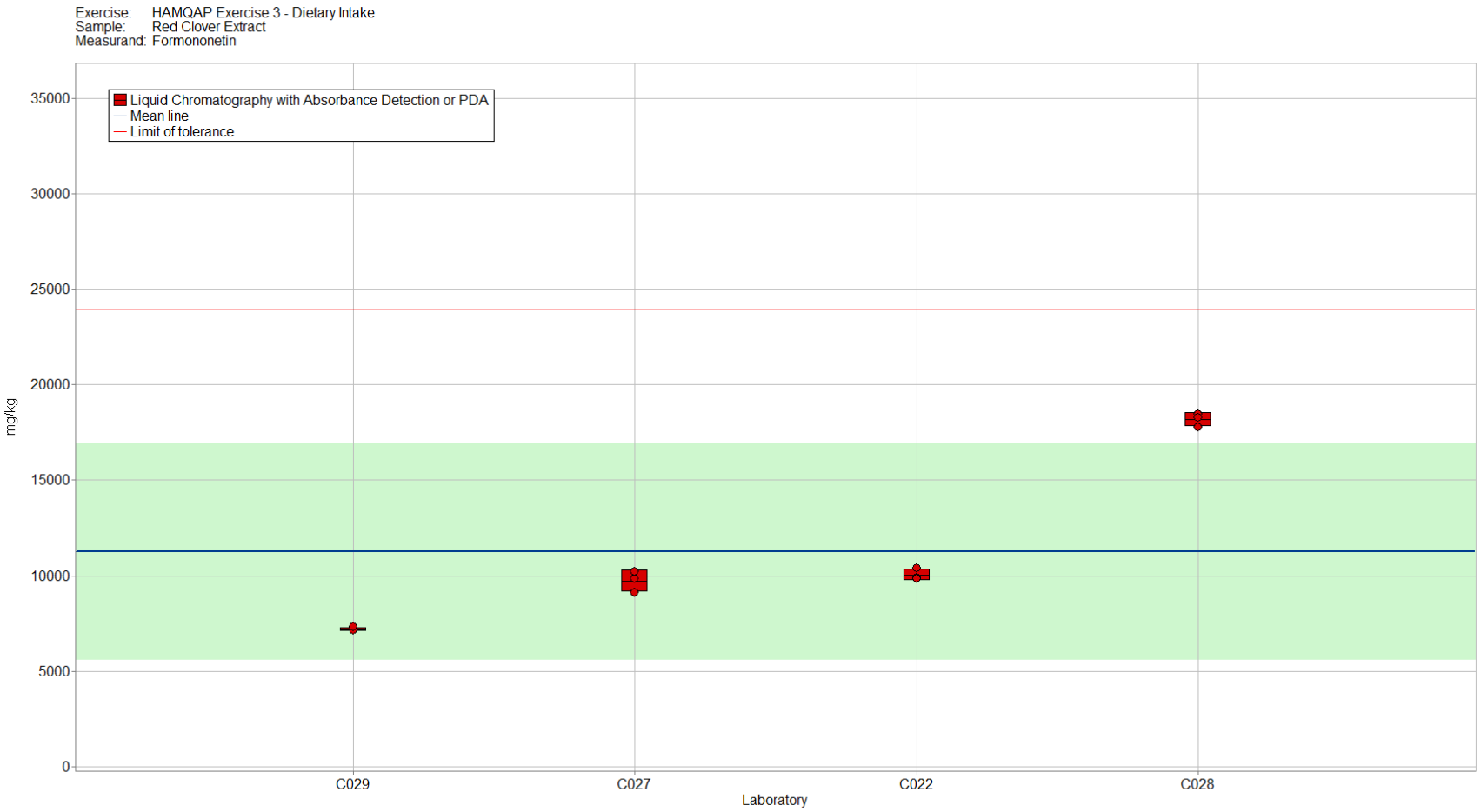

Figure 6-18. Formononetin in Red Clover Extract (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red line represents the upper consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$, with the lower range set at zero. A NIST value has not been determined in this material.

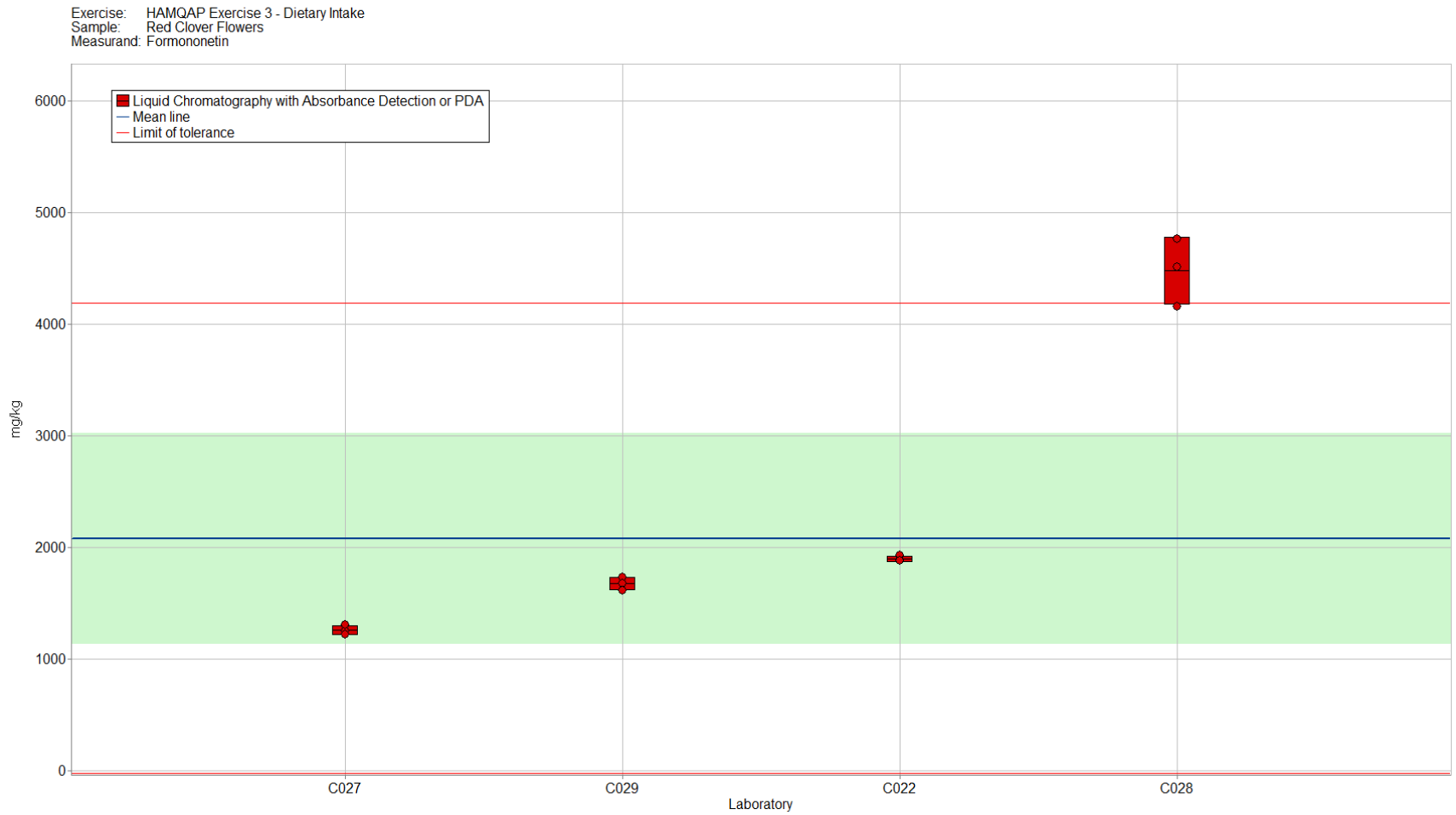

Figure 6-19. Formononetin in Red Clover Flowers (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The solid blue line represents the consensus mean, and the green shaded region represents 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'_{comm} score, $|Z'_{\text{comm}}| \leq 2$. A NIST value has not been determined in this material.

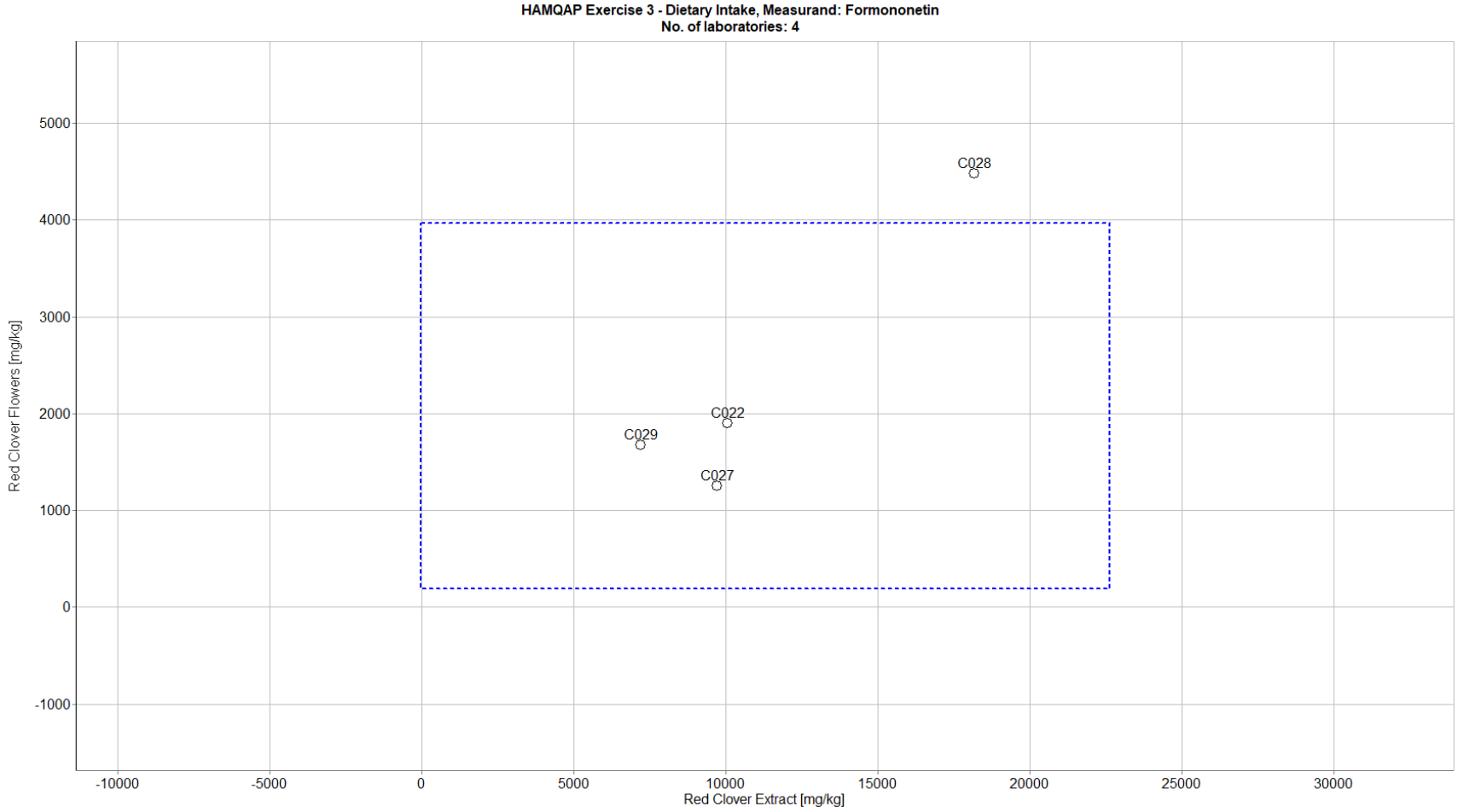

Figure 6-20. Laboratory means for formononetin in Red Clover Extract and Red Clover Flowers (sample/sample comparison view). In this view, the individual laboratory mean for one sample (extract) is compared to the individual laboratory mean for a second sample (flowers). The dotted blue box represents the consensus range of tolerance for extract (x-axis) and flowers (y-axis), calculated as the values above and below the consensus means that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$.

SECTION 7: CONTAMINANTS (Furans)

Study Overview

In this study, participants were provided with two NIST SRMs for dietary intake, SRM 2383a Baby Food Composite and SRM 3233 Fortified Breakfast Cereal, as well as roasted coffee beans. Participants were asked to use in-house analytical methods to determine the mass fraction (ng/g) of furans and alkyl furans (furan, 2-methylfuran, 2-ethylfuran, 2-propylfuran, 2-butylfuran, 2-pentylfuran, 2,5-dimethylfuran, 2-acetylfuran, 2-methoxymethylfuran, furfural, and vinyl furan) in each matrix. Furans can be formed in heat-treated foods and are known to be carcinogenic to humans and have recently been added to the California Proposition 65 list.[20,21] The metabolites of furans are typically monitored in the urine and can indicate exposure from diet, tobacco, or the environment.

Dietary Intake Sample Information

Baby Food. Participants were provided with one jar containing 70 g of slurried baby food. The baby food is a mixture of water, orange juice concentrate, corn, rice flour, papaya puree, spinach, macaroni, carrots, tomato paste, and non-fat milk powder. Before use, participants were instructed to mix the contents of the jar thoroughly and to use a sample size appropriate for their in-house method of analysis. Participants were asked to store the material under refrigeration, 2 °C to 8 °C, in the original unopened jar, and to prepare three samples and report three values from the single jar provided. The approximate analyte levels were not reported to participants prior to the study, and target values for furans and alkyl furans in SRM 2383a have not been determined at NIST.

Cereal. Participants were provided with one bottle containing 60 g of ground breakfast cereal. Before use, participants were instructed to mix the contents of the bottle thoroughly by rotating and/or rolling and to use a sample size appropriate for their in-house method of analysis. Participants were asked to store the material at controlled room temperature, 20 °C to 25 °C, and to prepare three samples and report three values from the single bottle provided. The approximate analyte levels were not reported to participants prior to the study, and target values for furans and alkyl furans in SRM 3233 have not been determined at NIST.

Coffee. Participants were provided with one bag containing 100 g of whole roasted coffee beans. Before use, participants were instructed to grind the entire bag of coffee beans and mix the resulting powder thoroughly, and to use a sample size appropriate for their in-house method of analysis. Participants were asked to store the material at controlled room temperature, 20 °C to 25 °C, and to prepare three samples and report three values from the single bag provided. The approximate analyte levels were not reported to participants prior to the study, and target values for furans and alkyl furans in the coffee have not been determined at NIST.

[20] Dioxins, Furans and Dioxin-Like Polychlorinated Biphenyls Factsheet. Centers for Disease Control and Prevention National Biomonitoring Program. https://www.cdc.gov/biomonitoring/DioxinLikeChemicals_FactSheet.html (accessed November 2019).

[21] Proposition 65. California Office of Environmental Health and Hazard Assessment. https://oehha.ca.gov/proposition-65 (accessed November 2019).

Dietary Intake Study Results

- Nine laboratories enrolled in this exercise and received samples to measure furans and/or alkyl furans. Two laboratories reported results for each sample (22 % participation).
- The variability between these two laboratories was poor in all samples (> 100 % RSD).
- Both laboratories that reported results indicated using GC-MS to measure all analytes.

Dietary Intake Technical Recommendations

The following general recommendations are offered, as too few data were reported to allow for meaningful specific conclusions to be drawn.

- Laboratories reporting large within-laboratory variability should investigate the completeness of the extraction during sample preparation.
    - Any extraction procedure should be optimized to determine the most effective extraction solvent to ensure exhaustive extraction of the analyte from the matrix.
    - The optimum number of extraction cycles must be determined by sequential re-extraction of the sample matrix until no further increase in yield is observed. Sequential extractions may be needed if the extraction solvent becomes saturated during the first (or only) extraction cycle.
    - "Zero" is not a quantity that can be measured, and therefore a more appropriate result would be to report that a value is below the MDL, LOQ, or 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 7-1. Individualized data summary table (NIST) for furans in foods.

National Institute of Standards and Technology

HAMQAP Exercise 3 - Contaminants

		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*
Furan	SRM 2383a Baby Food Composite	ng/g						1	0.01	0.038		
Furan	SRM 3233 Fortified Breakfast Cereal	ng/g						2	490	2000		
Furan	Coffee	ng/g						2	760	3100		
2-Methylfuran	SRM 2383a Baby Food Composite	ng/g						1	0	0.02		
2-Methylfuran	SRM 3233 Fortified Breakfast Cereal	ng/g						2	160	670		
2-Methylfuran	Coffee	ng/g						2	1910	7700		
2-Ethylfuran	SRM 2383a Baby Food Composite	ng/g						0				
2-Ethylfuran	SRM 3233 Fortified Breakfast Cereal	ng/g						1	0.019	0.063		
2-Ethylfuran	Coffee	ng/g						1	0.12	0.47		
2-Propylfuran	SRM 2383a Baby Food Composite	ng/g						0				
2-Propylfuran	SRM 3233 Fortified Breakfast Cereal	ng/g						1	0.03	0.13		
2-Propylfuran	Coffee	ng/g						1	0.3	1.1		
2-Butylfuran	SRM 2383a Baby Food Composite	ng/g						0				
2-Butylfuran	SRM 3233 Fortified Breakfast Cereal	ng/g						1	0.05	0.22		
2-Butylfuran	Coffee	ng/g						0				
2-Pentylfuran	SRM 2383a Baby Food Composite	ng/g						1	0.013	0.054		
2-Pentylfuran	SRM 3233 Fortified Breakfast Cereal	ng/g						2	70	310		
2-Pentylfuran	Coffee	ng/g						1	0.33	0.82		
2,5-Dimethylfuran	SRM 2383a Baby Food Composite	ng/g						1	0.008	0.027		
2,5-Dimethylfuran	SRM 3233 Fortified Breakfast Cereal	ng/g						1	0.04	0.17		
2,5-Dimethylfuran	Coffee	ng/g						2	140	580		
2-Acetylfuran	SRM 2383a Baby Food Composite	ng/g						0				
2-Acetylfuran	SRM 3233 Fortified Breakfast Cereal	ng/g						1	0	6		
2-Acetylfuran	Coffee	ng/g						2	10600	45000		
2-Methoxymethylfuran	SRM 2383a Baby Food Composite	ng/g						0				
2-Methoxymethylfuran	SRM 3233 Fortified Breakfast Cereal	ng/g						0				
2-Methoxymethylfuran	Coffee	ng/g						0				
Furfural	SRM 2383a Baby Food Composite	ng/g						1				
Furfural	SRM 3233 Fortified Breakfast Cereal	ng/g						1	0.4	1.8		
Furfural	Coffee	ng/g						0				
Vinyl furan	SRM 2383a Baby Food Composite	ng/g						0				
Vinyl furan	SRM 3233 Fortified Breakfast Cereal	ng/g						0				
Vinyl furan	Coffee	ng/g						0				

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 furan in foods.

		Furan														
		SRM 2383a Baby Food Composite (ng/g)					SRM 3233 Fortified Breakfast Cereal (ng/g)					Coffee (ng/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target															
	C005															
	C025	0.024	0.021	0.016	0.0203	0.0040	1.504	1.609	1.788	1.63	0.14	1.983	2.059	1.822	1.95	0.12
	C027	0	0	0			909	993	1011	971	54	1388	1731	1416	1512	190
	C032															
	C043															
	C045															
	C047															
	C050															
	C054															
	C055															
Community Results		Consensus Mean			0.0102		Consensus Mean			486.32		Consensus Mean			756.81	
		Consensus Standard Deviation			0.0383		Consensus Standard Deviation			2014		Consensus Standard Deviation			3076	
		Maximum			0.0203		Maximum			971		Maximum			1512	
		Minimum			0.0203		Minimum			1.63		Minimum			1.95	
		N			1		N			2		N			2	

Table 7-3. Data summary table for 2-methylfuran in foods.

		2-Methylfuran														
		SRM 2383a Baby Food Composite (ng/g)					SRM 3233 Fortified Breakfast Cereal (ng/g)					Coffee (ng/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target															
	C005															
	C025	0.01	0.01	0.009	0.00967	0.00058	0.666	0.53	0.576	0.591	0.069	5.256	5.332	4.802	5.13	0.29
	C027	0	0	0			301	312	325	313	12	3540	4393	3497	3810	505
	C032															
	C043															
	C045															
	C047															
	C050															
	C054															
	C055															
Community Results		Consensus Mean			0.00483		Consensus Mean			157		Consensus Mean			1908	
		Consensus Standard Deviation			0.02034		Consensus Standard Deviation			667		Consensus Standard Deviation			7749	
		Maximum			0.00967		Maximum			313		Maximum			3810	
		Minimum			0.00967		Minimum			0.591		Minimum			5.13	
		N			1		N			2		N			2	

Table 7-4. Data summary table for 2-ethylfuran in foods.

		2-Ethylfuran														
		SRM 2383a Baby Food Composite (ng/g)					SRM 3233 Fortified Breakfast Cereal (ng/g)					Coffee (ng/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target															
	C005															
	C025	< 0.0100	< 0.0100	< 0.0100			0.044	0.023	0.045	0.037	0.012	0.197	0.251	0.27	0.239	0.038
	C027	0	0	0			0	0	0			0	0	0		
	C032															
	C043															
	C045															
	C047															
	C050															
	C054															
	C055															
Community Results		Consensus Mean					Consensus Mean			0.019		Consensus Mean			0.120	
		Consensus Standard Deviation					Consensus Standard Deviation			0.063		Consensus Standard Deviation			0.467	
		Maximum					Maximum			0.037		Maximum			0.239	
		Minimum					Minimum			0.037		Minimum			0.239	
		N			0		N			1		N			1	

Table 7-5. Data summary table for 2-propylfuran in foods.

		2-Propylfuran														
		SRM 2383a Baby Food Composite (ng/g)					SRM 3233 Fortified Breakfast Cereal (ng/g)					Coffee (ng/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target															
	C005															
	C025	< 0.0100	< 0.0100	< 0.0100			0.063	0.056	0.062	0.0603	0.0038	0.536	0.482	0.655	0.558	0.089
	C027	0	0	0			0	0	0			0	0	0		
	C032															
	C043															
	C045															
	C047															
	C050															
	C054															
	C055															
Community Results		Consensus Mean					Consensus Mean			0.0302		Consensus Mean			0.279	
		Consensus Standard Deviation					Consensus Standard Deviation			0.1276		Consensus Standard Deviation			1.100	
		Maximum					Maximum			0.0603		Maximum			0.558	
		Minimum					Minimum			0.0603		Minimum			0.558	
		N			0		N			1		N			1	

Table 7-6. Data summary table for 2-butylfuran in foods.

		2-Butylfuran														
		SRM 2383a Baby Food Composite (ng/g)					SRM 3233 Fortified Breakfast Cereal (ng/g)					Coffee (ng/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target															
	C005															
	C025	< 0.0100	< 0.0100	< 0.0100			0.105	0.098		0.1015	0.0049	< 0.0100	< 0.0100	< 0.0100		
	C027	0	0	0			0	0	0			0	0	0		
	C032															
	C043															
	C045															
	C047															
	C050															
	C054															
	C055															
Community Results		Consensus Mean					Consensus Mean			0.0508		Consensus Mean				
		Consensus Standard Deviation					Consensus Standard Deviation			0.2175		Consensus Standard Deviation				
		Maximum					Maximum			0.1015		Maximum				
		Minimum					Minimum			0.1015		Minimum				
		N			0		N			1		N			0	

Table 7-7. Data summary table for 2-pentylfuran in foods.

		2-Pentylfuran														
		SRM 2383a Baby Food Composite (ng/g)					SRM 3233 Fortified Breakfast Cereal (ng/g)					Coffee (ng/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target															
	C005															
	C025	0.024	0.025	0.029	0.0260	0.0026	0.403	0.439	0.62	0.49	0.12	0.892	0.893	0.193	0.66	0.40
	C027	0	0	0			154	142	149	148	6	0	0	0		
	C032															
	C043															
	C045															
	C047															
	C050															
	C054															
	C055															
Community Results		Consensus Mean			0.0130		Consensus Mean			74.4		Consensus Mean			0.33	
		Consensus Standard Deviation			0.0538		Consensus Standard Deviation			314		Consensus Standard Deviation			0.82	
		Maximum			0.0260		Maximum			148		Maximum			0.66	
		Minimum			0.0260		Minimum			0.49		Minimum			0.66	
		N			1		N			2		N			1	

Table 7-8. Data summary table for 2-acetylfuran in foods.

		2-Acetylfuran														
		SRM 2383a Baby Food Composite (ng/g)					SRM 3233 Fortified Breakfast Cereal (ng/g)					Coffee (ng/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target															
	C005															
	C025	< 0.0100	< 0.0100	< 0.0100			1.926	3.249	13.077	6.084	6.092	16.495	16.984	19.45	17.6	1.6
	C027	0	0	0			0	0	0			20422	21898	21122	21147	738
	C032															
	C043															
	C045															
	C047															
	C050															
	C054															
	C055															
Community Results		Consensus Mean					Consensus Mean			3.042		Consensus Mean			10582	
		Consensus Standard Deviation					Consensus Standard Deviation			6.041		Consensus Standard Deviation			45282	
		Maximum					Maximum			6.084		Maximum			21147	
		Minimum					Minimum			6.084		Minimum			17.6	
		N			0		N			1		N			2	

Table 7-9. Data summary table for 2,5-dimethylfuran in foods.

		2,5-Dimethylfuran														
		SRM 2383a Baby Food Composite (ng/g)					SRM 3233 Fortified Breakfast Cereal (ng/g)					Coffee (ng/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target															
	C005															
	C025	0.016	0.017	0.016	0.01633	0.00058	0.087	0.075	0.08	0.0807	0.0060	1.074	1.058	0.854	1.00	0.12
	C027	0	0	0			0	0	0			272	321	261	285	32
	C032															
	C043															
	C045															
	C047															
	C050															
	C054															
	C055															
Community Results		Consensus Mean			0.00817		Consensus Mean			0.0403		Consensus Mean			143	
		Consensus Standard Deviation			0.02663		Consensus Standard Deviation			0.1692		Consensus Standard Deviation			577	
		Maximum			0.01633		Maximum			0.0807		Maximum			285	
		Minimum			0.01633		Minimum			0.0807		Minimum			1.00	
		N			1		N			1		N			2	

Table 7-10. Data summary table for 2-methylfuran in foods.

		2-Methylfuran														
		SRM 2383a Baby Food Composite (ng/g)					SRM 3233 Fortified Breakfast Cereal (ng/g)					Coffee (ng/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target															
	C005															
	C025	0.01	0.01	0.009	0.00967	0.00058	0.666	0.53	0.576	0.591	0.069	5.256	5.332	4.802	5.13	0.29
	C027	0	0	0			301	312	325	313	12	3540	4393	3497	3810	505
	C032															
	C043															
	C045															
	C047															
	C050															
	C054															
	C055															
Community Results		Consensus Mean			0.00483		Consensus Mean			157		Consensus Mean			1908	
		Consensus Standard Deviation			0.02034		Consensus Standard Deviation			667		Consensus Standard Deviation			7749	
		Maximum			0.00967		Maximum			313		Maximum			3810	
		Minimum			0.00967		Minimum			0.591		Minimum			5.13	
		N			1		N			2		N			2	

Table 7-11. Data summary table for furfural in foods.

		Furfural														
		SRM 2383a Baby Food Composite (ng/g)					SRM 3233 Fortified Breakfast Cereal (ng/g)					Coffee (ng/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target															
	C005															
	C025	< 0.0100	< 0.0100	< 0.0100			0.964	0.786	0.9	0.883	0.090	< 0.0100	< 0.0100	< 0.0100		
	C027	16.634	19.611	16.196	17.5	1.9	0	0	0			0	0	0		
	C032															
	C043															
	C045															
	C047															
	C050															
	C054															
	C055															
Community Results		Consensus Mean					Consensus Mean			0.442		Consensus Mean				
		Consensus Standard Deviation					Consensus Standard Deviation			1.807		Consensus Standard Deviation				
		Maximum			17.5		Maximum			0.883		Maximum				
		Minimum			17.5		Minimum			0.883		Minimum				
		N			1		N			1		N			0	