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Health Assessment Measurements Quality Assurance Program: Exercise 5 Final Report

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

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LIST OF ACRONYMS

AMRM	Analytical Methods and Reference Materials
CDC	US Centers for Disease Control and Prevention
cGMP	current Good Manufacturing Practice
COA	Certificate of Analysis
CRM	Certified Reference Material
DNA	Deoxyribonucleic Acid
DSQAP	Dietary Supplements Quality Assurance Program
EPA	Environmental Protection Agency
FAAS	Flame Atomic Absorption Spectrometry
FDA	US Food and Drug Administration
FES	Flame Emission Spectrometry
GC	Gas Chromatography
GC-FID	Gas Chromatography with Flame Ionization Detection
GC-MS	Gas Chromatography Mass Spectrometry
HAMQAP	Health Assessment Measurements Quality Assurance Program
IC-CD	Ion Chromatography with Conductivity Detection
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
ICP-MS w/KED	ICP-MS with kinetic energy discrimination
ICP-OES	Inductively Coupled Plasma Optical Emission Spectrometry
ID ICP-MS	Isotope Dilution Inductively Coupled Plasma 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
FAMEs	Fatty Acid Methyl Esters
LC-absorbance	Liquid Chromatography with Absorbance Detection
LC-fluorescence	Liquid Chromatography with Fluorescence Detection
LC-MS	Liquid Chromatography Mass Spectrometry
LOQ	Limit of Quantification
NHANES	National Health and Nutrition Examination Survey
NIST	National Institute of Standards and Technology
NIH	National Institutes of Health
ODS	Office of Dietary Supplements
RMP	Reference Measurement Procedure
QAP	Quality Assurance Program
QC	Quality Control
QL	Quantification Limit
RM	Reference Material
RSD	Relative Standard Deviation
SD	Standard Deviation
SF-ICP-MS	Sector Field Inductively Coupled Plasma Mass Spectrometry
SODF	Solid Oral Dosage Form
SRM	Standard Reference Material
TIMS	Thermal Ionization Mass Spectrometry
WDXRF	Wavelength Dispersive X-ray Fluorescence Spectrometry

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ABSTRACT

HAMQAP was launched in collaboration with the NIH Office of Dietary Supplements (ODS) in 2017. HAMQAP was established to enable laboratories to improve the accuracy of measurements in samples that represent human intake (e.g., foods, dietary supplements, tobacco) and samples that represent human metabolism (e.g., blood, serum, plasma, urine) for demonstration of proficiency and/or compliance with various regulations. Analytes are paired where possible to represent the full spectrum of health assessment. Exercise 5 of this program offered the opportunity for laboratories to assess their in-house measurements of nutritional elements (calcium, iron, potassium, and sodium), contaminants (arsenic and arsenic species, chlorate and perchlorate), water-soluble vitamins (several B vitamins), fat-soluble vitamins (vitamin D and metabolites), fatty acids (select omega-3 and omega-6 fatty acids), botanicals (catechins), natural products (xanthines), and proximates in foods and dietary supplements, and corresponding biomarkers/metabolites in clinical specimens (human red blood cells, sera, and urine).

INTRODUCTION

HAMQAP was established 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, natural products) are paired with samples that represent human metabolism (e.g., blood, serum, plasma, urine)[1], where possible, to represent the full spectrum of intake and metabolism for health assessment. Reports and certificates of participation are provided and may be used to demonstrate compliance with the 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. Trends observed in HAMQAP exercises could be used by ODS and NIST to identify problematic matrices and analytes for which consensus-based methods of analysis would benefit the dietary supplements and clinical communities.

NIST has decades of experience in the administration of QAPs, and HAMQAP builds on the approach taken by the former DSQAP by providing a wide range of matrices and analytes. The HAMQAP design combines activities of DSQAP, FAQAP, MMQAP, and VitDQAP, and emphasizes emerging and challenging measurements in the dietary supplement, food, and clinical matrix categories. Participating laboratories are interested in evaluating in-house methods on a

[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.

wide variety of challenging, real-world matrices to demonstrate that their performance is comparable to that of the community and that their methods provide accurate results. In areas where few standard methods have been recognized, HAMQAP offers a unique tool for assessment of the quality of measurements and provides feedback about performance that can assist participants in improving laboratory operations.

This report summarizes the results from the fifth exercise of HAMQAP. Sixty-four laboratories responded to the dietary intake portion and twenty-one laboratories responded to the human metabolites portion of the call for participants distributed in October 2019 (see table below). Four human metabolites studies were cancelled prior to shipment due to low enrollment. Samples were shipped to participants in February 2020 and results were returned to NIST by April 2020. This report contains the final data and information that was disseminated to the participants in January 2021.

Study Group	Dietary Intake Study	Human Metabolites Study
Nutritional Elements	Calcium, Iron, Potassium, Sodium Rice Flour, Wheat Flour	~~Calcium, Iron, Potassium, Sodium~~ * ~~Human Serum~~
Toxic Elements	Arsenic, Arsenic Species Aquacultured and Wild Shrimp	~~Arsenic, Arsenic Species~~ * ~~Human Urine~~
Water-Soluble Vitamins	B Vitamins Multivitamin, Infant Formula	~~Homocysteine~~* ~~Methylmalonic Acid,~~ ~~Human Serum~~
Fat-Soluble Vitamins	Vitamin D Multivitamin, Infant Formula	Vitamin D Metabolites Human Serum
Fatty Acids	Omega-3, Omega-6 Fatty Acids Fish Oil, Aquacultured & Wild Salmon	Omega-3, Omega-6 Fatty Acids Human Red Blood Cells
Botanicals	Catechins Green Tea Extract & Ground Capsules	Not Offered
Natural Products	Xanthines Yerba Mate, Green Tea Leaves, Extracts, and Ground Capsules	Not Offered
Contaminants	Chlorate, Perchlorate Nutritional Formula	~~Chlorate, Perchlorate~~ * ~~Human Urine~~
Proximates	Proximates Almond Flour, Hazelnut Flour	Not Offered

* 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.

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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.

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Statistics

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

Individualized Data Table

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

Section 1 of the data table (*Your Results*) contains the laboratory results as reported, including the mean 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, *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.

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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 encompasses the NIST target value bounded by twice its uncertainty (U_{95} or U_{NIST}).

Figures

Data Summary View (Method Comparison Data Summary View)

In this view, individual laboratory data (diamonds) 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. Major program goals include both reducing the size of the consensus zone and centering the consensus zone about the target value. Analysis of an appropriate reference material as part of a quality control scheme can help to identify sources of bias for laboratories reporting results that are significantly different from the target zone. In the case in which a method comparison is relevant, different colored data points may be used to identify laboratories that used a specific approach to sample preparation, analysis, or quantitation.

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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.

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SECTION 1: NUTRITIONAL ELEMENTS (Calcium, Iron, Potassium, Sodium)

Study Overview

In this study, participants were provided with samples of rice flour and SRM 1567b Wheat Flour for dietary intake. Participants were asked to use in-house analytical methods to determine the mass fractions (mg/kg) of calcium (Ca), iron (Fe), potassium (K), and sodium (Na) in wheat and rice flour. Consumers worldwide are being urged to limit dietary intake of Na and increase dietary intake of minerals such as Ca, Fe, and K as part of strategies to reduce chronic disease through improved nutrition.[4,5,6] Accurate measurement of Ca, Fe, K, and Na in foods is necessary for understanding daily intake of these elements and related health outcomes. Potential Na contamination from the environment when analyzing low sodium foods such as wheat and rice flours challenges methods from sample preparation to instrumental measurement.

Dietary Intake Sample Information

Rice Flour. Participants were provided with one bottle containing approximately 50 g of material. Participants were asked to store the material at controlled room temperature, 20 °C to 25 °C, in the original unopened bottles and to prepare three samples and report three values from the single bottle provided. Before use, participants were instructed to mix the contents of the bottle thoroughly and to use a sample size of at least 0.5 g. Approximate analyte levels were not reported to participants prior to the study. The NIST-determined values for Ca, Fe, and K in rice flour were assigned using results from NIST by ICP-OES and WDXRF. The NIST-determined values and expanded uncertainties are provided in the table below, both on a dry-mass basis and on an as-received basis accounting for moisture of the material (9.7 %).

	NIST-Determined Mass Fractions in Rice Flour (mg/kg)	
Analyte	(dry mass basis)	(as-received basis)
Calcium (Ca)	90.0 ± 33.0	81.3 ± 29.8
Iron (Fe)	12.98 ± 2.02	11.72 ± 1.82
Potassium (K)	3175 ± 49	2867 ± 44

Wheat Flour. Participants were provided with one bottle containing approximately 50 g of material. Participants were asked to store the material at controlled room temperature, 20 °C to 25 °C, in the original unopened bottles and to prepare three samples and report three values from the single bottle provided. Before use, participants were instructed to mix the contents of the bottle thoroughly and to use a sample size of at least 0.5 g. Approximate analyte levels were not reported to participants prior to the study. The certified value for calcium in SRM 1567b was assigned using results from NIST by FAAS, FES, WDXRF. The certified value for iron in SRM 1567b was assigned using results from NIST by FAAS, INAA, TIMS, WDXRF. The certified value for potassium in SRM 1567b was assigned using results from NIST by INAA and WDXRF. The

[4] FDA Nutrition Innovation Strategy. US Food and Drug Administration. https://www.fda.gov/food/food-labeling-nutrition/fda-nutrition-innovation-strategy (accessed March 2020).
[5] EU Salt Reduction Framework. European Commission. https://ec.europa.eu/health/sites/health/files/nutrition_physical_activity/docs/salt_report1_en.pdf (accessed March 2020).
[6] Sodium intake for adults and children: Guideline. World Health Organization. https://www.who.int/nutrition/publications/guidelines/sodium_intake/en/ (accessed March 2020).

certified value for sodium in SRM 1567b was assigned using results from NIST by ICP-OES, INAA, SF-ICP-MS. The NIST-determined values and expanded uncertainties are provided in the table below, both on a dry-mass basis as listed in the COA, and on an as-received basis accounting for moisture of the material (7.1 %) and with a further expanded uncertainty for evaluation of laboratory performance.

	NIST-Determined Mass Fractions in SRM 1567b Wheat Flour (mg/kg)	
Analyte	(dry-mass basis)	(as-received basis)[a]
Calcium (Ca)	191.4 ± 3.3	177.8 ± 6.1
Iron (Fe)	14.11 ± 0.33	13.11 ± 0.61
Potassium (K)	1325 ± 20	1231 ± 37
Sodium (Na)	6.71 ± 0.21	6.23 ± 0.39

[a] Associated expanded uncertainties for the target zone for acceptable performance are calculated as 2*(U_{95} or U_{NIST}).

Dietary Intake Study Results

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

Analyte	Number of Laboratories Requesting Samples	Number of Laboratories Reporting Results (Percent Participation)	
		Rice Flour	Wheat Flour
Calcium (Ca)	29	19 (66 %)	19 (66 %)
Iron (Fe)	30	18 (60 %)	19 (63 %)
Potassium (K)	29	18 (62 %)	18 (62 %)
Sodium (Na)	29	16 (55 %)	16 (55 %)

- For the rice flour sample, the target range for calcium completely overlaps the consensus range **(Figures 1-1** and **1-3**), the target range for iron overlaps the consensus mean and the lower half of the consensus range **(Figures 1-6** and **1-8)**, and the consensus and target ranges for potassium just overlap at the upper edge of the consensus range (**Figures 1-11** and **1-13**). No target range was available for sodium in the rice flour sample.
- For the wheat flour sample, the upper edge of the target range for calcium barely overlaps the lower edge of the consensus range (**Figures 1-2** and **1-4**), the target ranges for iron and sodium overlap the lower edges of the respective consensus ranges (**Figures 1-7** and **1-9,** and **1-17** and **1-19**), and the target range for potassium overlaps the top half of the consensus range and just touches the mean (**Figures 1-12** and **1-14**).

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- The between-laboratory variabilities were excellent or very good (see table below).

	Between-Laboratory Variability (% RSD)	
Analyte	Rice Flour	Wheat Flour
Calcium (Ca)	4 %	2 %
Iron (Fe)	8 %	5 %
Potassium (K)	3 %	2 %
Sodium (Na)	9 %	12 %

- Most laboratories reported using either microwave digestion or hot block digestion for sample preparation in the determination of all four analytes (see table below). The sample preparation methods reported by participating laboratories are shown in **Figures 1-1** and **1-2**, **1-6** and **1-7**, **1-11** and **1-12,** and **1-16** and **1-17** for Ca, Fe, K, and Na, respectively, and reported below.

Reported Sample Preparation Method	Percent Reporting			
	Ca	Fe	K	Na
Microwave Digestion	47 %	47 %	50 %	56 %
Hot Block Digestion	42 %	42 %	44 %	38 %
Solvent Extraction	5 %	5 %	6 %	6 %
Acid Hydrolysis	5 %	5 %	--	--

- Most laboratories reported using ICP-OES for determination of all four analytes (see table below). The analytical methods reported by participating laboratories are shown in **Figures 1-3** and **1-4**, **1-8** and **1-9**, **1-13** and **1-14,** and **1-18** and **1-19**, for Ca, Fe, K, and Na, respectively, and reported below.

Reported Analytical Method	Percent Reporting			
	Ca	Fe	K	Na
ICP-OES	58 %	58 %	56 %	56 %
ICP-MS	32 %	21 %	22 %	25 %
ID ICP-MS	5 %	11 %	11 %	6 %
ICP-MS w/ KED	--	5 %	6 %	6 %
Spectrophotometry	--	5 %	--	--
Other	5 %	--	6 %	6 %

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Dietary Intake Technical Recommendations

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

- Multiple trends related to sample preparation approaches were noted. The hot block sample preparation method appears to be more scattered across the upper and lower ends of the data range for the rice flour compared to the microwave digestion method.
- The results by microwave digestion are more closely clustered around the target values, which suggests that microwave digestion is a better choice than the hot block digestion for the analyte and sample combinations of this exercise. The more consistent high temperature environment of the microwave resulted in more consistent and complete sample digestion and thus reduced likelihood of low-biased results, while the closed vessel of the microwave digestion minimized the potential environmental contamination and thus reduced the likelihood of high-biased results.
- Results of some laboratories showed larger than expected within-laboratory variability which may be indicative of sample processing errors such as incomplete digestion or contamination, or the use of a smaller then recommended sample size for analysis.

- Digestion with nitric acid is sufficient for these analytes and samples.
- No trend was observed based on the analytical method used for any element.
- Multiple trends indicating potential calibration issues were identified.
  - Most laboratories reported results within the target range for calcium in rice flour (**Figures 1-1 and 1-3)**. Only four laboratories reported results within the target range for calcium in wheat flour with most laboratories reporting results above the target value (**Figures 1-2, 1-4,** and **1-5)** indicating possible calibration errors.
  - An upward trend in the sample/sample comparison plots for each element (**Figures 1-5, 1-10, 1-15,** and **1-20**), in which laboratories that reported high (or low) results for one sample also reported high (or low) results for the second sample, indicates possible calibration errors.
  - Linearity of calibration curve across the range of analyte concentrations within the extracted samples must be confirmed. Dilution or concentration of sample digests can be used to adjust the levels into the linear calibration range. The most accurate measurements can be achieved by making sure the sample concentrations fall within the middle of the calibration curve.
  - Sodium concentrations were very low in both samples and potassium concentrations were high. The best practice would be to measure these two analytes separately.
  - Analysis of a quality assurance material (such as a CRM or in-house QC material) will help to establish that the measurement procedure is in control, avoiding biased results.
- Outlying results for iron were often high, indicating a possible issue with contamination of the samples during sample preparation. Contamination from iron may occur in laboratories containing exposed metal.
  - **Figure 1-10** indicates that approximately half of the laboratories are able to measure iron in both rice and wheat flour very well.
  - Some of the laboratories are able to measure one but not both of the samples well (**Figures 1-6** through **1-10**).
- Analysis of an appropriate number of procedural blanks can be critical, especially when concentrations of analytes are low, such as sodium is in these samples.

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- Analysis of blanks can provide information about whether variability is arising from the sample preparation procedure. A suggested minimum number of blanks to prepare is often equal to the number of samples being prepared.
- Blank analysis is also critical in the determination of LOQ and MDL or when trying to reduce sample-to-sample variability.

- When using ICP-MS, make proper use of the instrumental features.
  - Many ICP-MS instruments run in pulse mode, which is more sensitive than analog mode. Instruments typically switch automatically between pulse and analog modes depending on the dynamic range in use, and therefore the instrument must be calibrated for both modes. To ensure that the calibration curve is linear in the pulse mode, consider using a narrower range of calibration points and ensure all solutions fall within this lower range.
  - Collision cell or reaction cell mode can be used to reduce or eliminate the interferences for Ca ($^{40}Ar^{+}$, $^{12}C^{16}O_2$, $^{14}N_2{}^{16}O$), Fe ($^{40}Ar^{14}N$, $^{40}Ar^{16}O$, $^{40}Ar^{16}OH$), and K ($^{38}Ar_1H^{+}$, $^{40}Ar_1H^{+}$) caused by molecular ions that have the same mass-to-charge ratio.
- When using ICP-OES, monitoring more than one wavelength for each analyte helps identify interferences or background shifts due to matrix effects at a given wavelength and helps identify and prevent bias.
- Contamination from the environment may be problematic for determination of sodium due to the low levels found in these two samples so care must be taken to follow good laboratory practices. CRMs are available and may be used for assay validation to ensure no environmental contamination.
- The use of appropriate calibration materials and quality assurance samples to establish that a method is in control and being performed correctly may reduce the likelihood of outlying data. Quality assurance samples can be commercially available reference materials (CRMs, SRMs, or RMs) or materials prepared in-house.
- Measurement results should be reported accurately.
  - Sodium is monoisotopic so isotope-dilution ICP-MS is not a practicable option.
  - Zero is 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.
  - Laboratories reporting results flagged as outliers should check for calculation errors when preliminary data tables are sent for inspection. One example is to confirm that factors for all dilutions have been properly tabulated or that results are reported in correct reporting units.

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Table 1-1. Individualized data summary table (NIST) for nutritional elements in rice flour and wheat flour.

National Institute of Standards & Technology

HAMQAP Exercise 5 - 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*
Calcium	Rice Flour	mg/kg		81.3	29.8			19	102	3.8	81.3	29.8
Calcium	SRM 1567b Wheat Flour	mg/kg		177.8	6.1			19	191	4.5	177.8	6.1
Iron	Rice Flour	mg/kg		11.72	1.82			18	13	1.1	11.72	1.82
Iron	SRM 1567b Wheat Flour	mg/kg		13.1	0.61			19	14	0.69	13.1	0.61
Potassium	Rice Flour	mg/kg		2867	44			18	2690	72	2867	44
Potassium	SRM 1567b Wheat Flour	mg/kg		1231	37			18	1200	20	1231	37
Sodium	Rice Flour	mg/kg						16	14.5	1.3		
Sodium	SRM 1567b Wheat Flour	mg/kg		6.23	0.39			16	7.55	0.87	6.23	0.39

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

		Calcium									
		Rice Flour (mg/kg)					SRM 1567b Wheat Flour (mg/kg)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target				81.3	29.8				177.8	6.1
	E001	115	111	110	112.0	2.6	184	172	170	175.3	7.6
	E002	132.411	129.69	111.78	124.6	11.2	219.54	216.218	218.71	218.2	1.7
	E003	84.7	78.2	81.5	81.5	3.3	174	173	174	173.7	0.6
	E005	114	116	92	107.3	13.3	185	205	193	194.3	10.1
	E006										
	E007	93.54	93.81	94.54	94.0	0.5	192.7	194.6	195.3	194.2	1.3
	E008										
	E011										
	E015	91.92	92.94	111.97	98.9	11.3	166	166	164	165.3	1.2
	E016	114.6	121.9	101.8	112.8	10.2	220.3	190.7	219.6	210.2	16.9
	E019										
	E020										
	E025	82.88	90.1	86.39	86.5	3.6	166	186	175	175.7	10.0
	E027	113	113	112	112.7	0.6	197	196	203	198.7	3.8
	E029	89.79	93.86	91.39	91.7	2.1	185.2	184.5	183.9	184.5	0.7
	E030	90.3	103	112	101.8	10.9	201	197	200	199.3	2.1
	E031										
	E033	99.953	96.259	100.893	99.0	2.4	182.434	185.904	189.378	185.9	3.5
	E034	93.1	97.69	94.31	95.0	2.4	192	191.8	190.1	191.3	1.0
	E035	99.7	106	104	103.2	3.2	208	202	191	200.3	8.6
	E036										
	E037										
	E040										
	E041	98.5	118.2	119.3	112.0	11.7	197.9	186	181.2	188.4	8.6
	E042	1.075	1.307	0.935	1.1	0.2	0.277	0.254	0.2	0.2	0.0
	E045	120.2	134.9	143.6	132.9	11.8	218.9	282.9		250.9	45.3
	E047	99.2	80.1	87.2	88.8	9.7	181	171	166	172.7	7.6
	E051	88.1	87.5	89	88.2	0.8	194	198	195	195.7	2.1
	E072										
Community Results		Consensus Mean			102.1		Consensus Mean			191.4	
		Consensus Standard Deviation			3.8		Consensus Standard Deviation			4.5	
		Maximum			132.9		Maximum			250.9	
		Minimum			1.1		Minimum			0.2	
		N			19		N			19	

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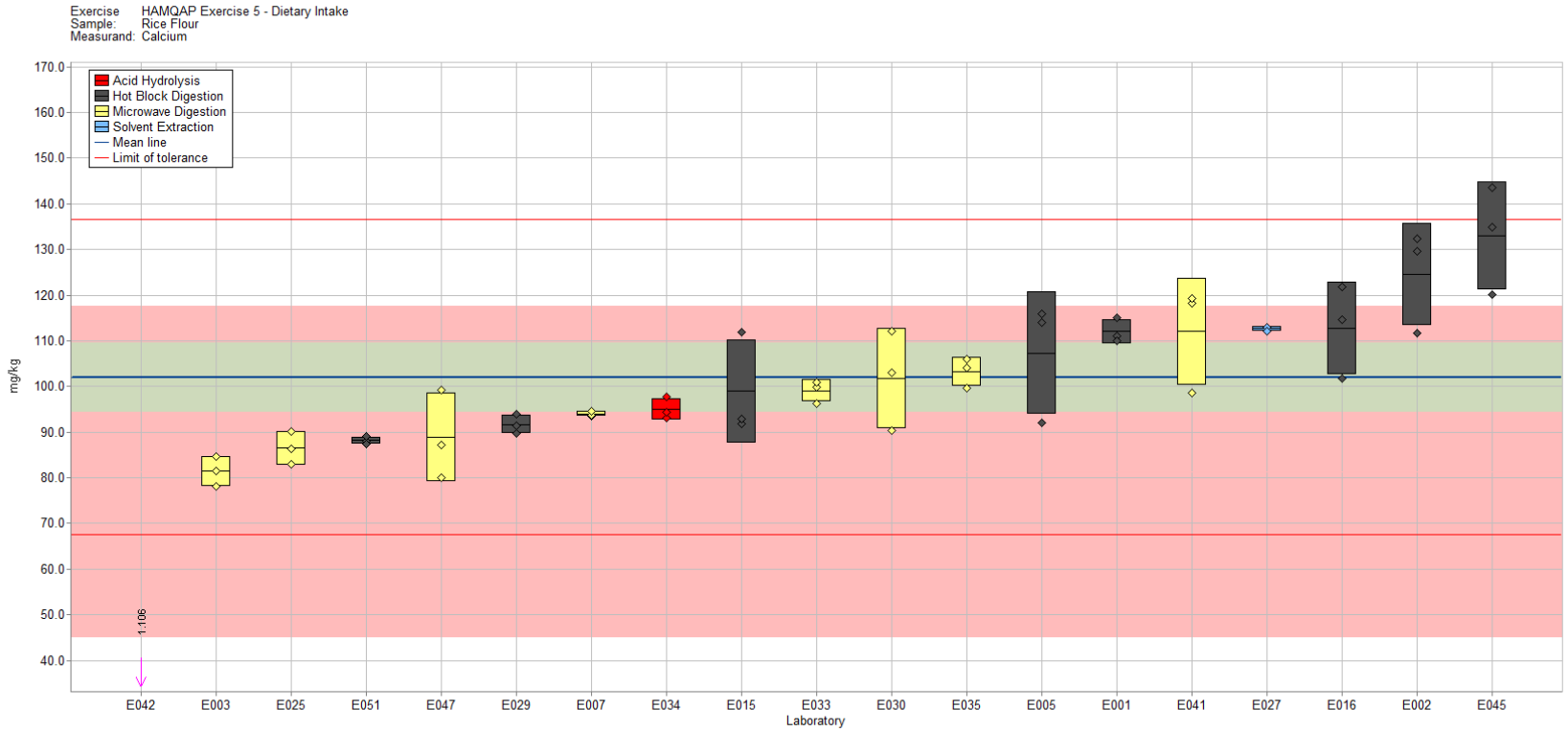

Figure 1-1. Calcium in Rice Flour (data summary view – sample preparation method). In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The solid blue 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, $|Z_{\text{NIST}}| \leq 2$.

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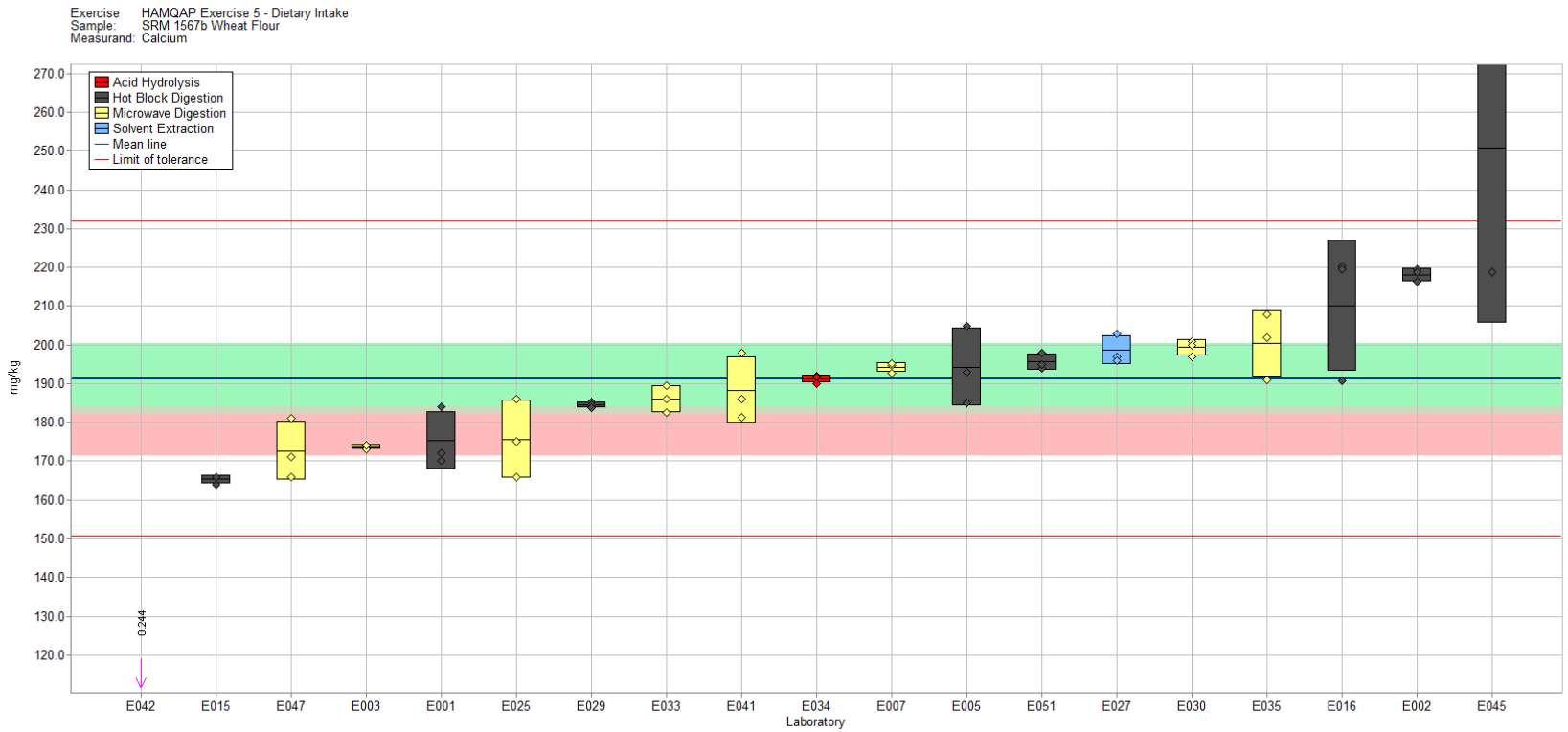

Figure 1-2. Calcium in SRM 1567b Wheat Flour (data summary view – sample preparation method). In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The 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, $|Z_{\text{NIST}}| \leq 2$.

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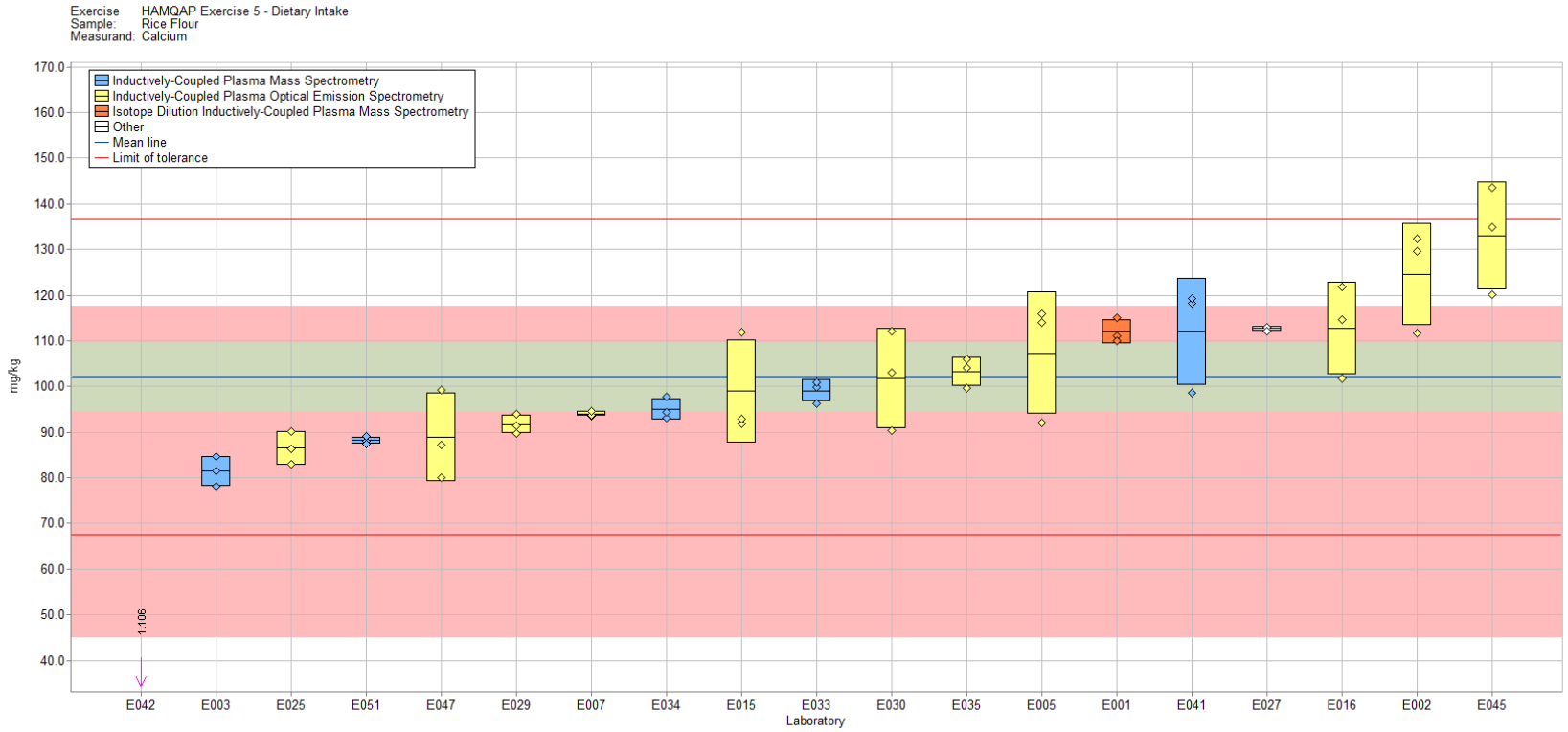

Figure 1-3. Calcium in Rice Flour (data summary view – analytical method). In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line represents 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, $|Z_{\text{NIST}}| \leq 2$.

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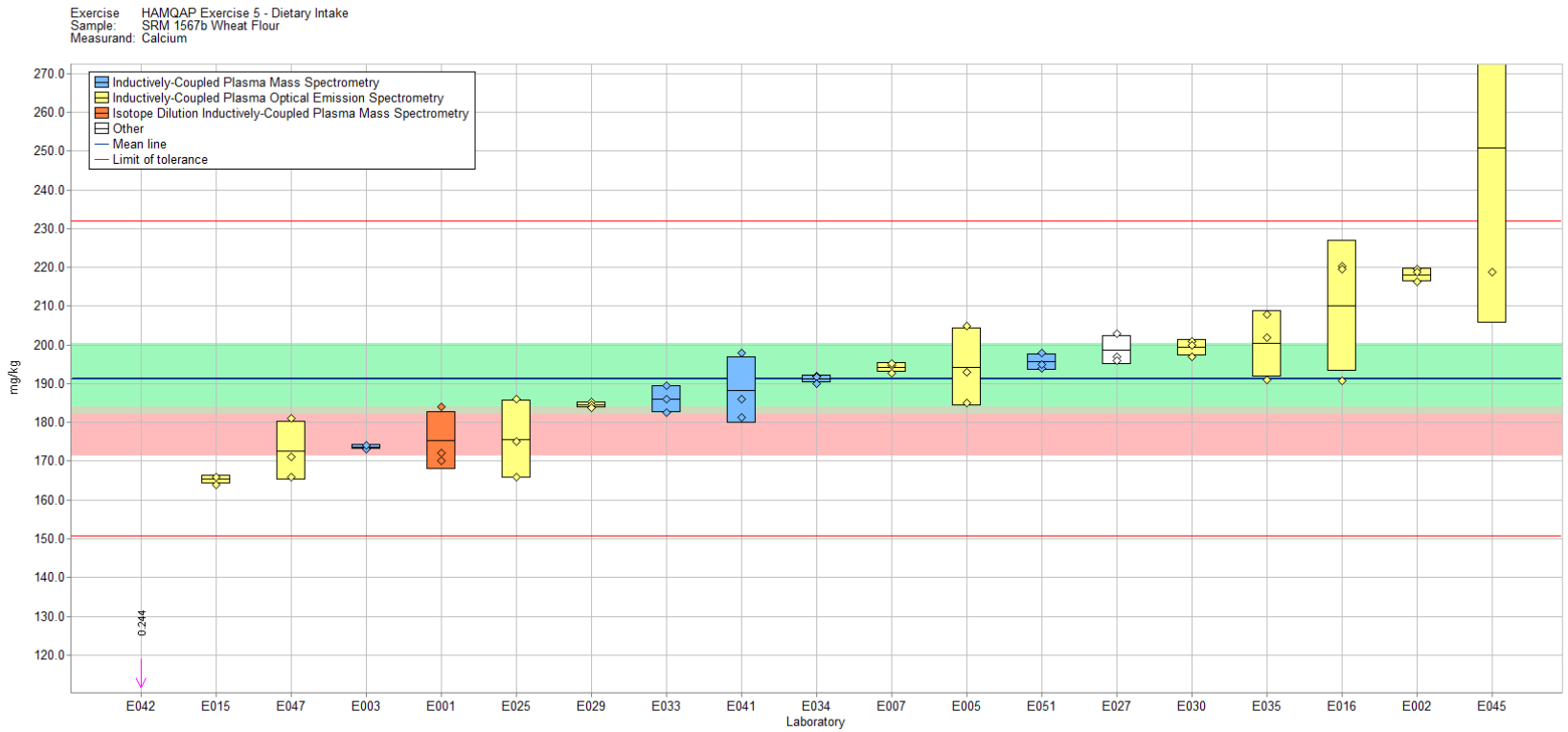

Figure 1-4. Calcium in SRM 1567b Wheat Flour (data summary view – analytical method). In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line 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, $|Z_{\text{NIST}}| \leq 2$.

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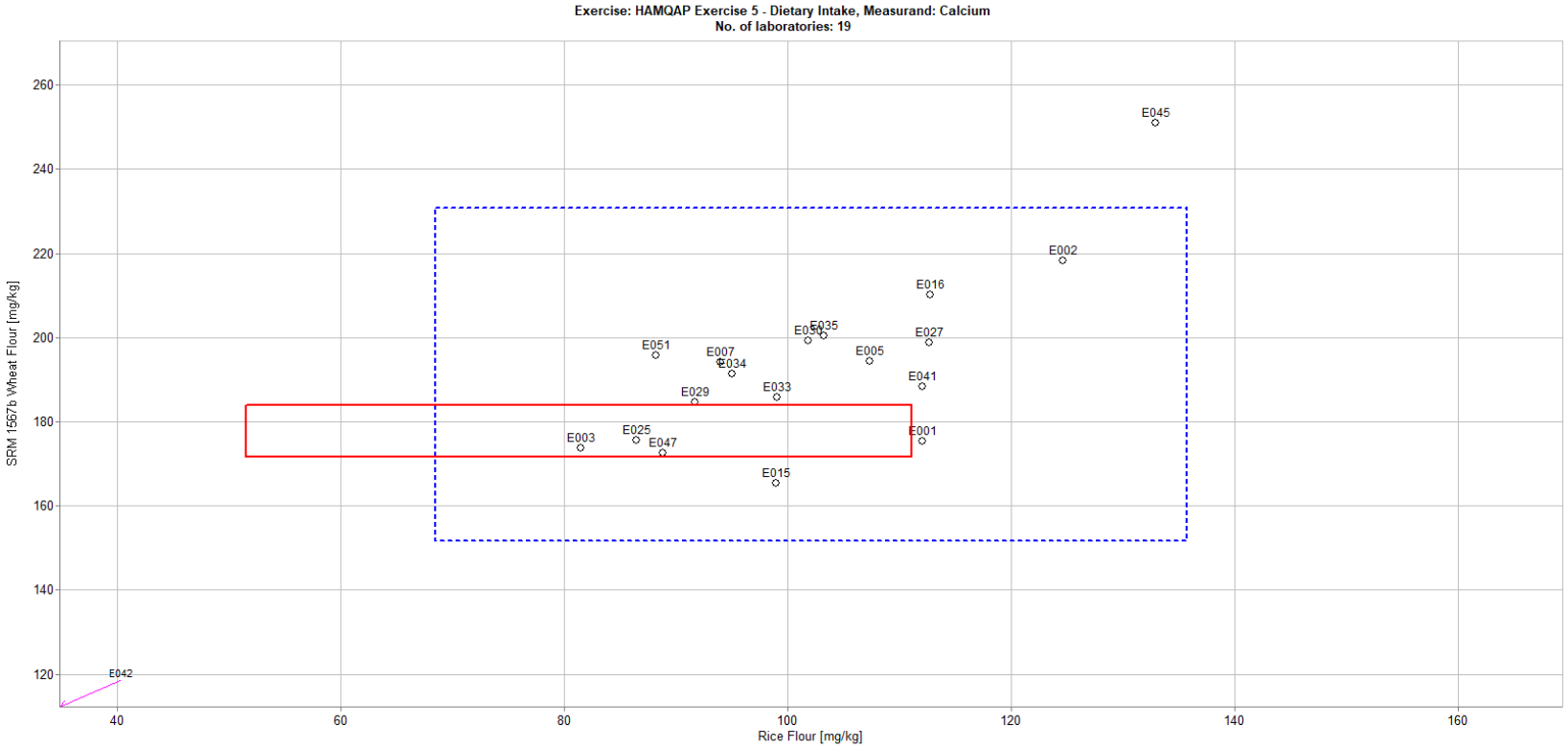

Figure 1-5. Laboratory means for calcium in Rice Flour and SRM 1567b Wheat Flour (sample/sample comparison view). In this view, the individual laboratory mean for one sample (rice flour) is compared to the individual laboratory mean for a second sample (wheat flour). The solid red box represents the NIST range of tolerance for the two samples, wheat flour (x-axis) and rice flour (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 wheat flour (x-axis) and rice flour (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 iron in rice flour and wheat flour. Data points highlighted in red have been flagged as potential outliers (e.g., Grubb and/or Cochran) by the NIST software package.

		Iron									
		Rice Flour (mg/kg)					SRM 1567b Wheat Flour (mg/kg)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target				11.72	1.82				13.11	0.61
	E001	15.1	14.3	13.8	14.40	0.66	14.6	15.1	14.3	14.67	0.40
	E002	20.746	19.156	19.847	19.92	0.80	23.441	23.409	23.645	23.50	0.13
	E003	11	11	11	11.00	0.00	12	14	13	13.00	1.00
	E005	8	7	6	7.00	1.00	9	7	7	7.67	1.15
	E006										
	E007	11.69	11.73	11.87	11.76	0.09	12.7	12.76	12.94	12.80	0.12
	E008										
	E010										
	E011										
	E015	24.66	103.76	26.17	51.53	45.24	25.4	32.3	104	53.90	43.52
	E016	11.02	9.75	8.99	9.92	1.03	15.61	14.72	14.39	14.91	0.63
	E019										
	E020										
	E025	11.7	10.97	11.13	11.27	0.38	13	12.39	12.55	12.65	0.32
	E027	22.1	20.7	20.4	21.07	0.91	16.5	15.5	16.4	16.13	0.55
	E029	19.73	17.49	17.91	18.38	1.19	17.83	16.66	16.46	16.98	0.74
	E030	16.7	17.3	17.1	17.03	0.31	12.6	12.9	13.2	12.90	0.30
	E031										
	E033	14.716	12.416	12.052	13.06	1.44	12.76	12.884	22.086	15.91	5.35
	E034	11.83	12.13	10.48	11.48	0.88	13.46	12.28	12.81	12.85	0.59
	E035	11.8	12	11.9	11.90	0.10	12.5	13.1	12.1	12.57	0.50
	E036										
	E037										
	E040										
	E041	13.2	15.9	17.9	15.67	2.36	18.4	16.2	16.8	17.13	1.14
	E042	0.023	0.026	0.02	0.02	0.00	0.0192	0.0195	0.0192	0.02	0.00
	E045						12.84	13.85		13.35	0.71
	E047	12.1	9.29	10.4	10.60	1.42	13.5	9.43	10.2	11.04	2.16
	E051	12.2	11.9	11.8	11.97	0.21	13.4	12.5	12.3	12.73	0.59
	E072										
Community Results		Consensus Mean			13.02		Consensus Mean			13.95	
		Consensus Standard Deviatio			1.08		Consensus Standard Deviatio			0.69	
		Maximum			51.53		Maximum			53.90	
		Minimum			0.02		Minimum			0.02	
		N			18		N			19	

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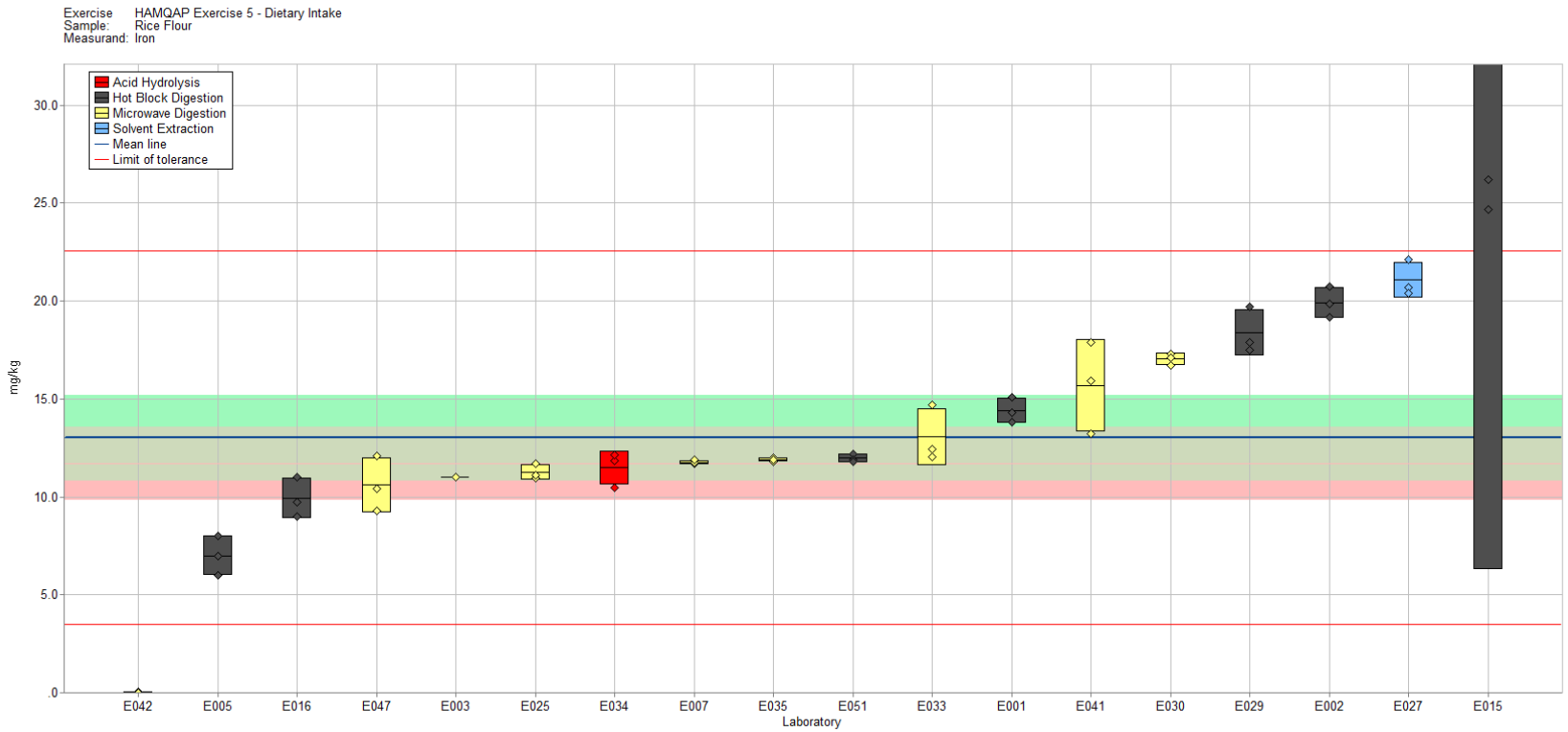

Figure 1-6. Iron in Rice Flour (data summary view – sample preparation method). In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The solid blue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

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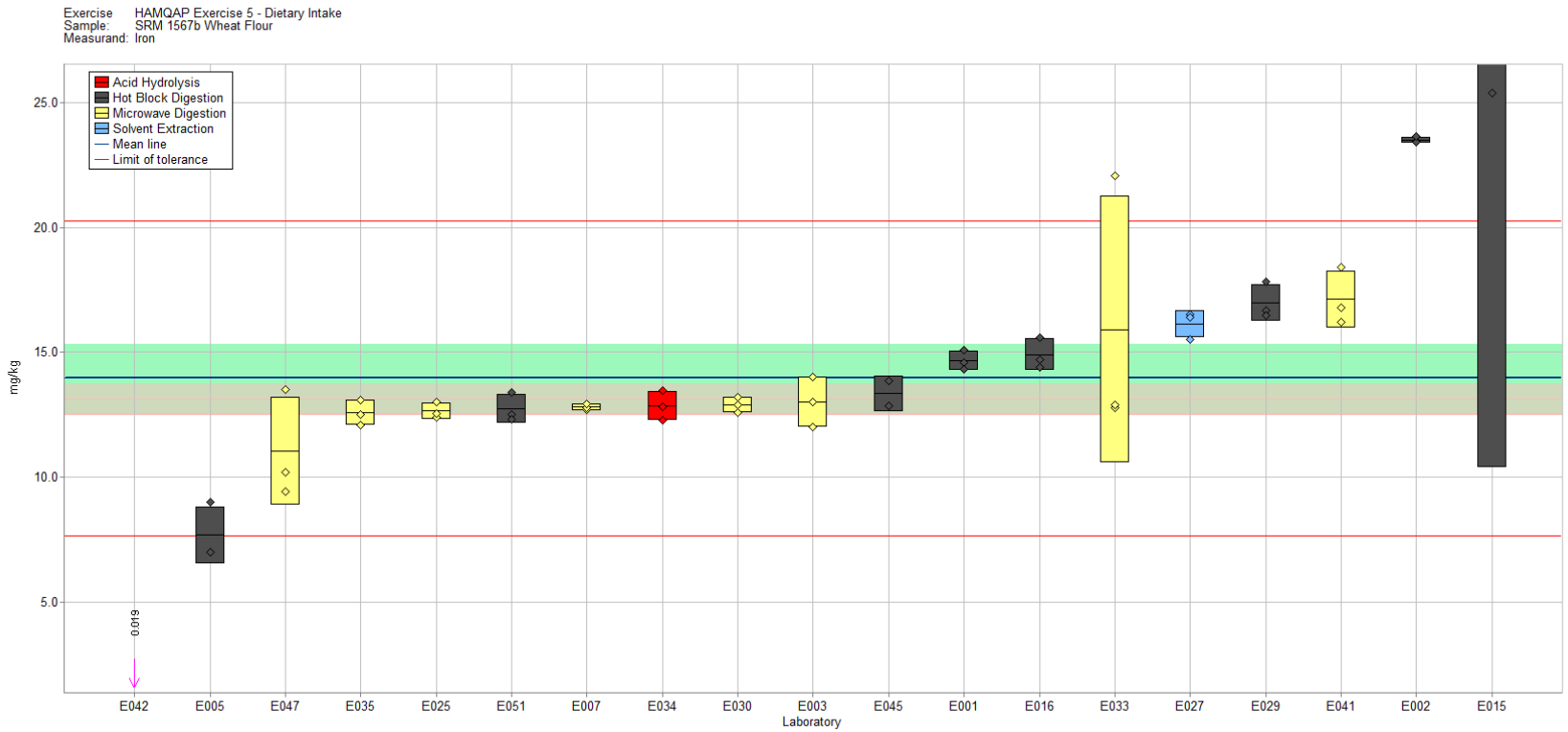

Figure 1-7. Iron in SRM 1567b Wheat Flour (data summary view – sample preparation method). In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The 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 (behind the green) 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, $|Z_{\text{NIST}}| \leq 2$.

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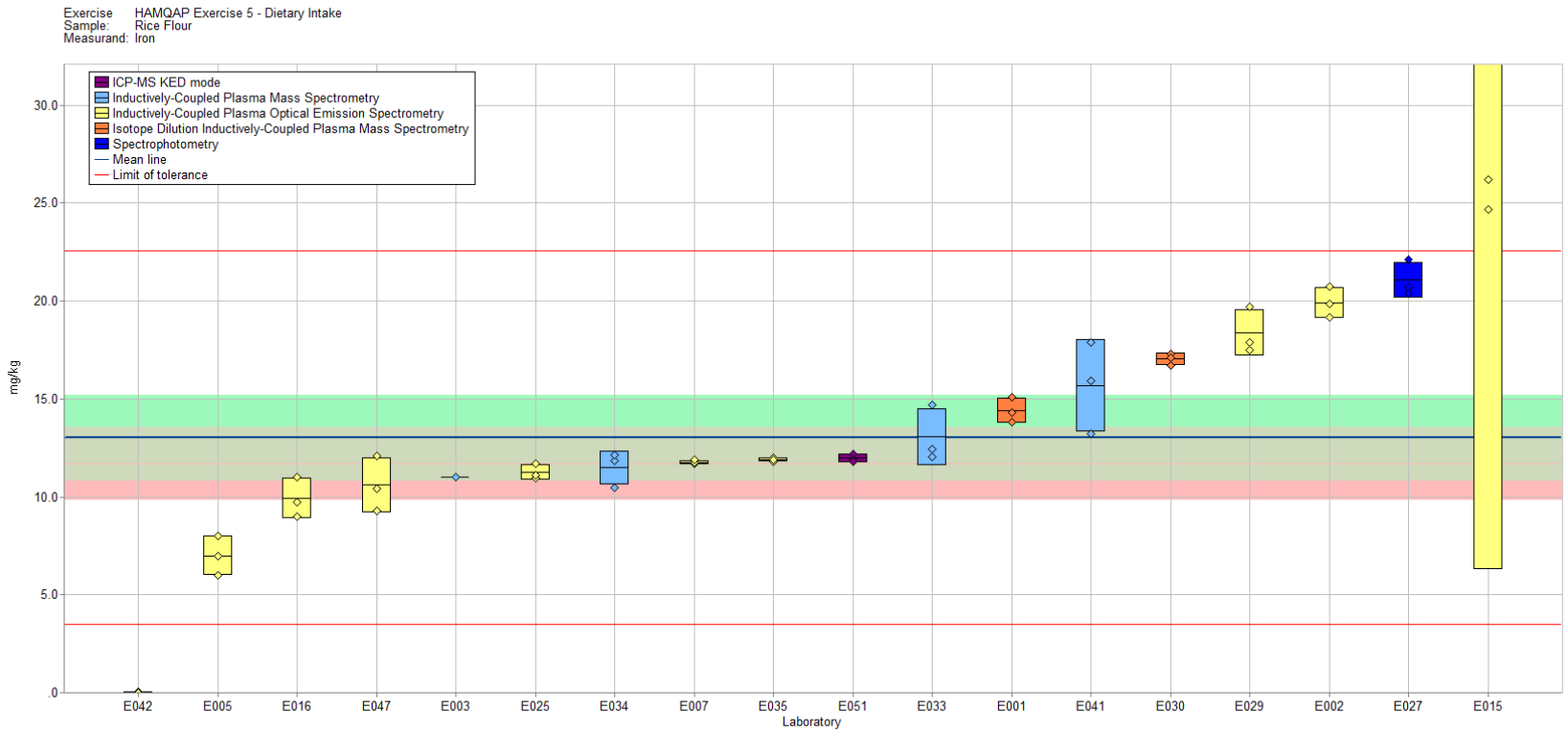

Figure 1-8. Iron in Rice flour (data summary view – analytical method). In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line represents 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, $|Z_{\text{NIST}}| \leq 2$.

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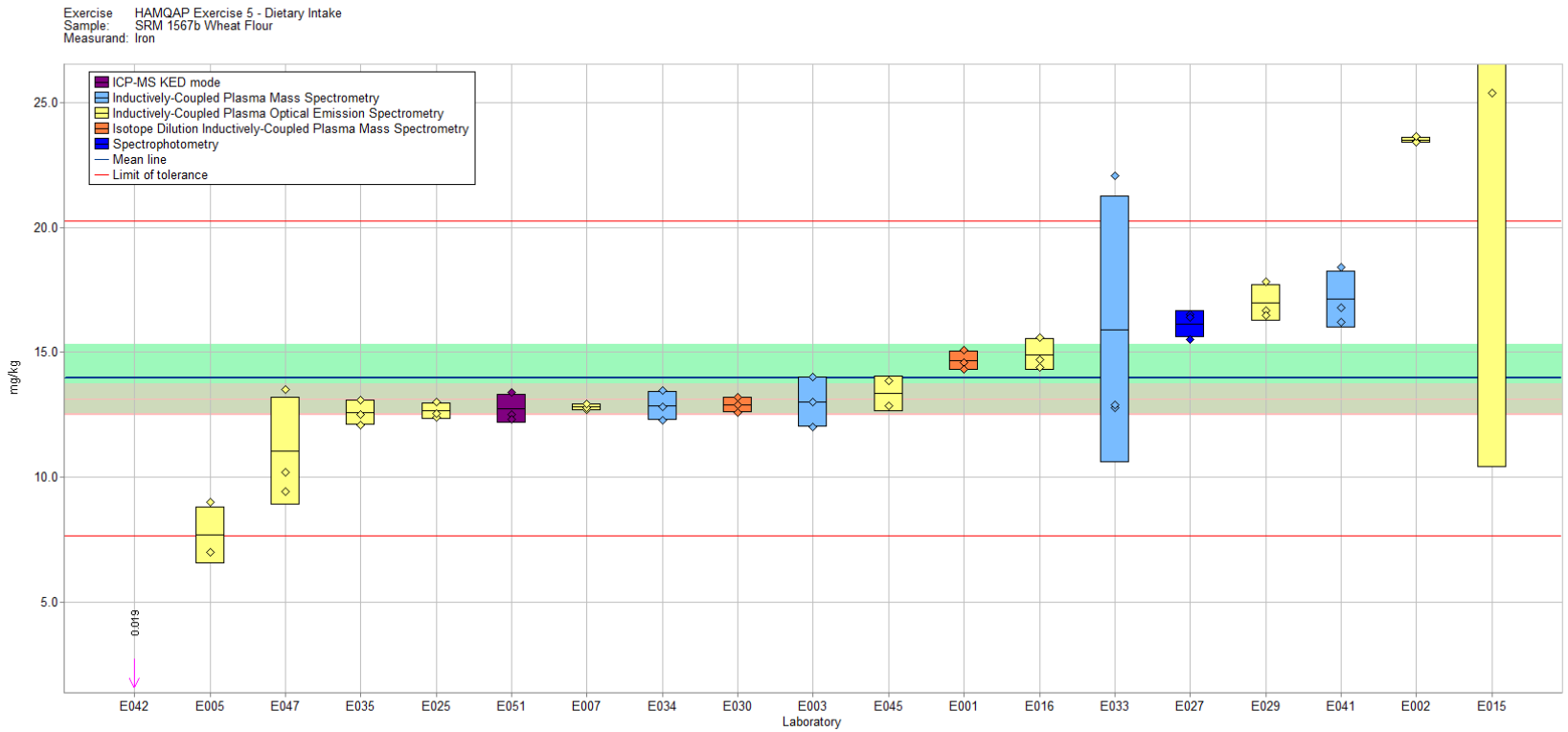

Figure 1-9. Iron in SRM 1567b Wheat Flour (data summary view – analytical method). In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line 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 (behind the green) 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, $|Z_{\text{NIST}}| \leq 2$.

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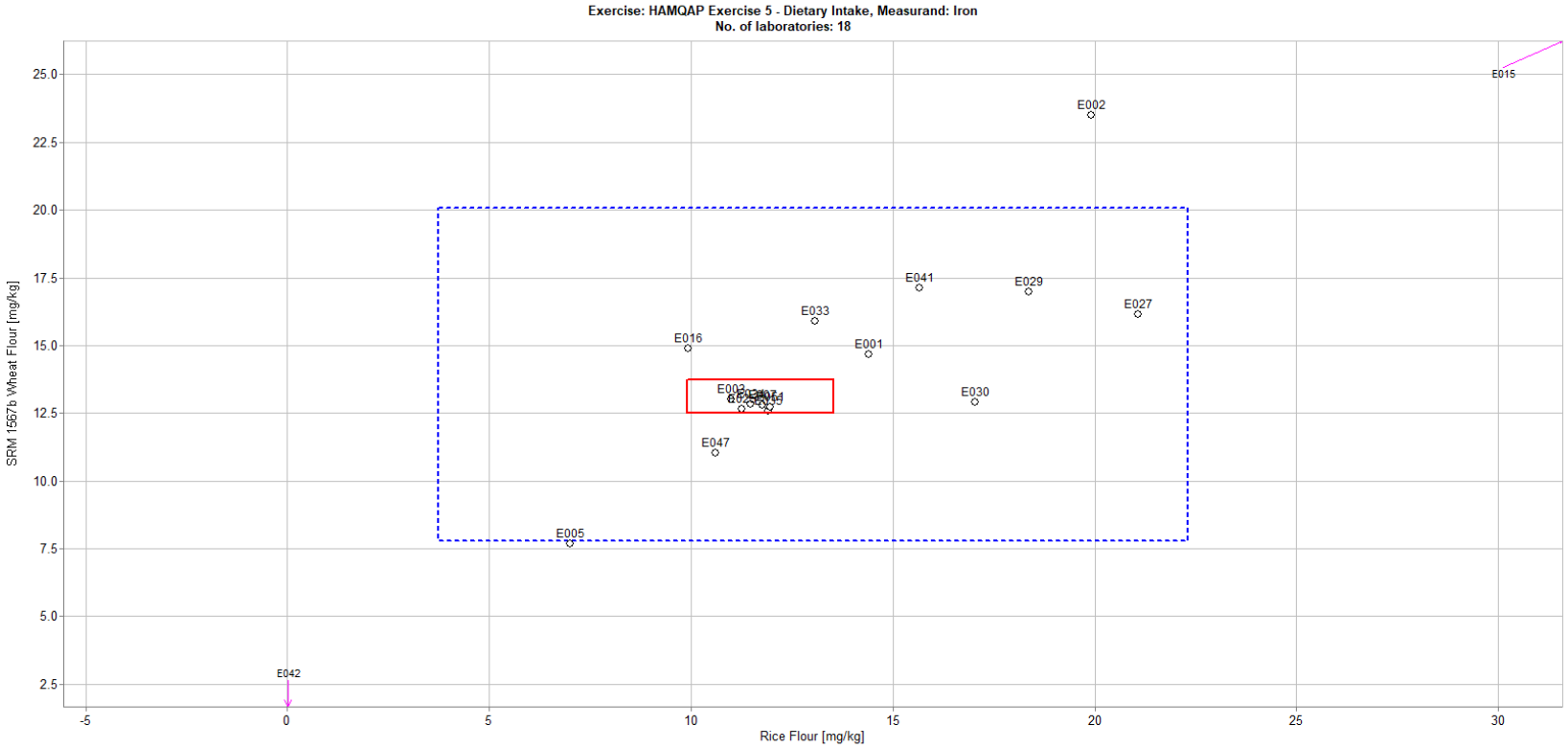

Figure 1-10. Laboratory means for iron in Rice Flour and SRM 1567b Wheat Flour (sample/sample comparison view). In this view, the individual laboratory mean for one sample (rice flour) is compared to the individual laboratory mean for a second sample (wheat flour). The solid red box represents the NIST range of tolerance for the two samples, wheat flour (x-axis) and rice flour (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 wheat flour (x-axis) and rice flour (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 1-4. Data summary table for potassium in rice flour and wheat flour. Data points highlighted in red have been flagged as potential outliers (e.g., Grubb and/or Cochran) by the NIST software package.

		Potassium									
		Rice Flour (mg/kg)					SRM 1567b Wheat Flour (mg/kg)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target				2867	44				1231	37
	E001	2770	2760	2780	2770	10	1240	1240	1240	1240	0
	E002	3176.46	3214.919	3210.54	3201	21	1957.55	1994.79	2142.73	2032	98
	E003	2465	2320	2393	2393	73	1195	1121	1158	1158	37
	E005	2887	2774	2697	2786	96	1170	1221	1204	1198	26
	E006										
	E007	2580	2550	2620	2583	35	1180	1170	1180	1177	6
	E008										
	E010										
	E011										
	E015	2439	2360	2398	2399	40	1123	1114	1133	1123	10
	E016	1667.6	1278.3	1324.1	1423	213	1824	1604	1484	1637	172
	E019										
	E020										
	E025	2467	2588	2470	2508	69	1121	1192	1128	1147	39
	E027	2737	2725	2642	2701	52	1218	1195	1238	1217	22
	E029	2922	2854	2954	2910	51	1224	1194	1194	1204	17
	E030	2720	2750	2632	2701	61	1225	1159	1258	1214	50
	E031										
	E033	2764.705	2803.025	2776.37	2781	20	1244.33	1244.84	1235.409	1242	5
	E035	2480	2620	2530	2543	71	1180	1160	1140	1160	20
	E036										
	E037										
	E040										
	E041	2253	2566	2726	2515	241	1172.3	1166.2	1159	1166	7
	E042	2.208	2.346	2.349	2	0	4.412	5.393	4.472	5	1
	E045	2941	2948		2945	5	1277	1239		1258	27
	E047	2630	2730	2820	2727	95	1170	1150	1140	1153	15
	E051	2726	2766	2726	2739	23	1302	1290	1268	1287	17
	E072										
Community Results		Consensus Mean			2689		Consensus Mean			1196	
		Consensus Standard Deviation			72		Consensus Standard Deviation			20	
		Maximum			3201		Maximum			2032	
		Minimum			2		Minimum			5	
		N			18		N			18	

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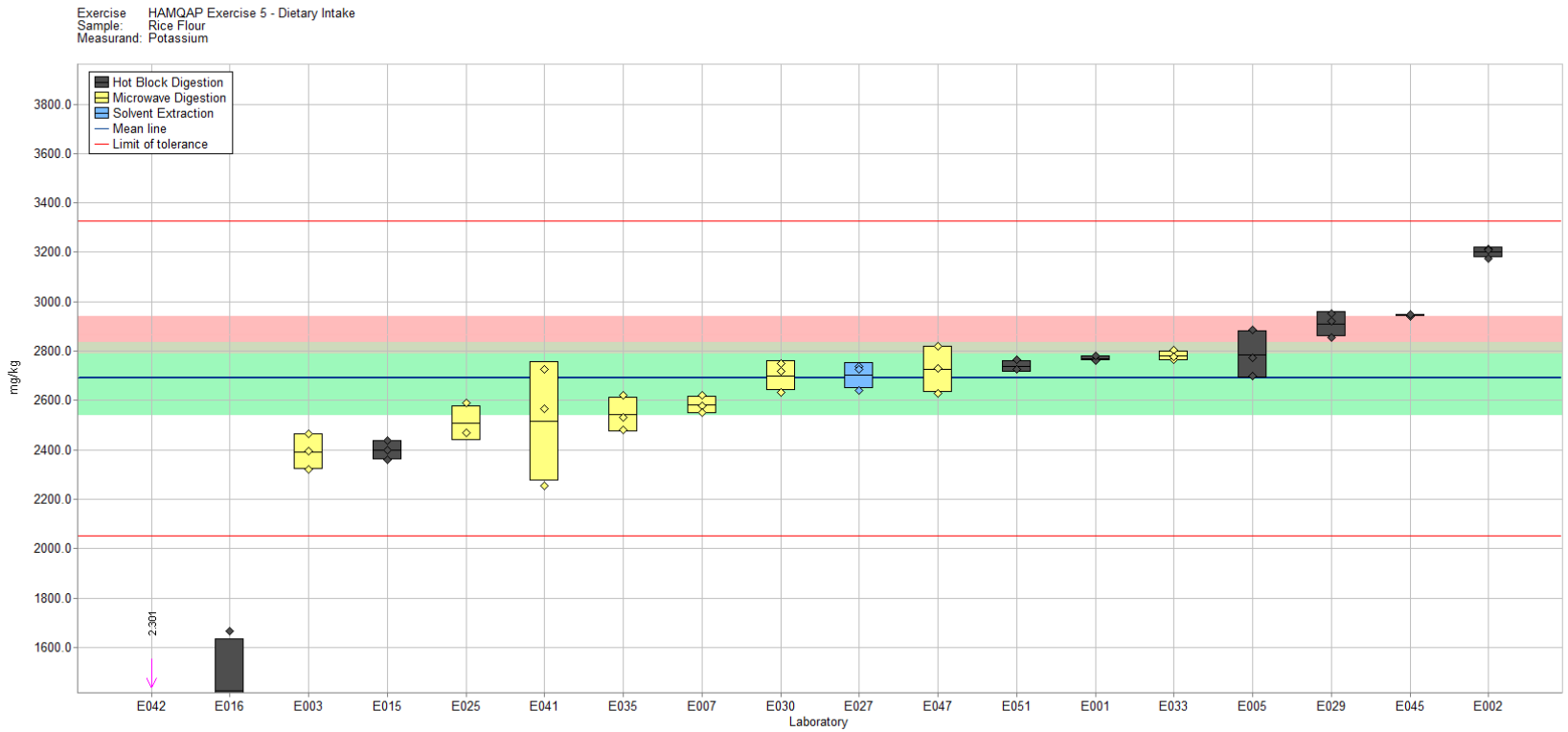

Figure 1-11. Potassium in Rice Flour (data summary view – sample preparation method). In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The solid 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, $|Z_{\text{NIST}}| \leq 2$.

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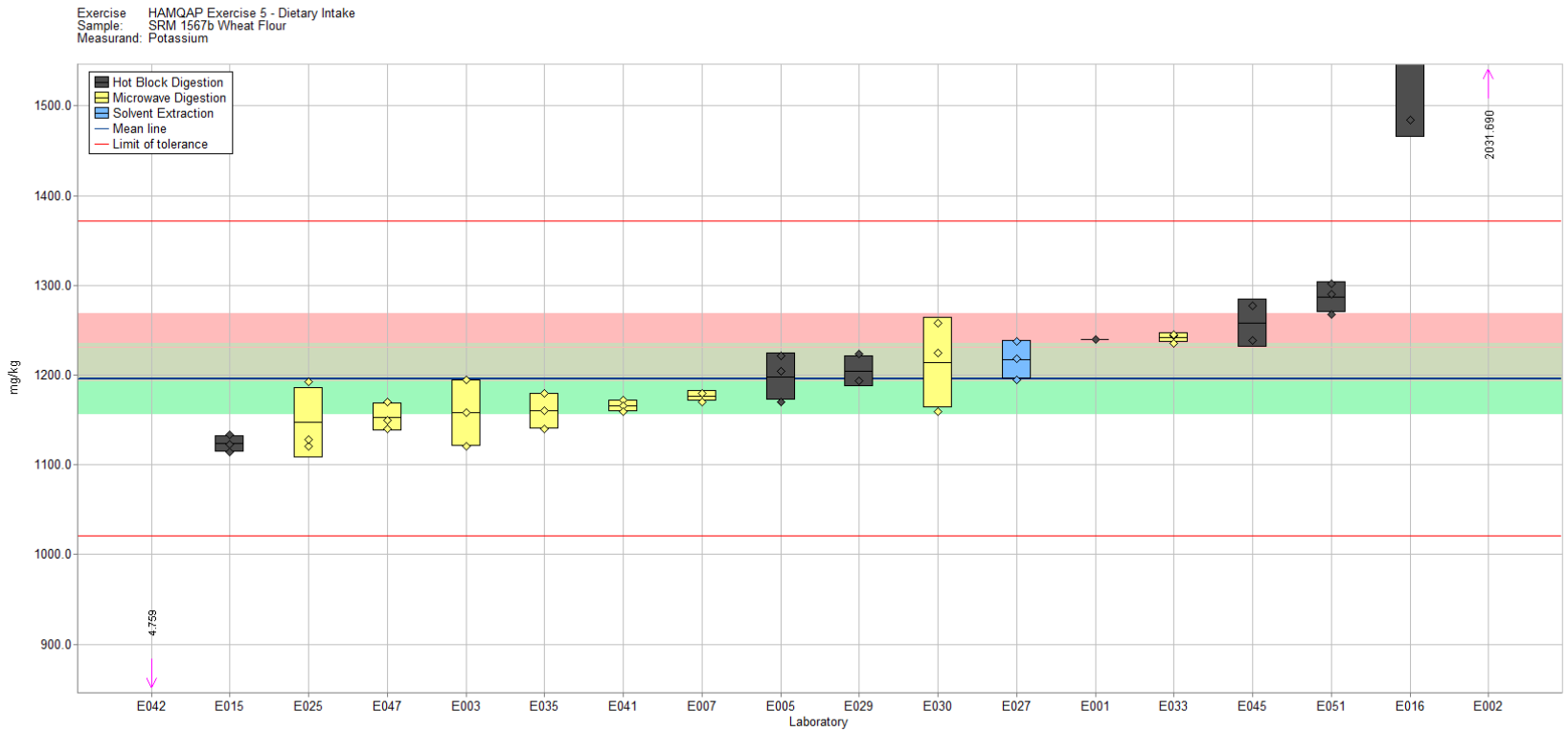

Figure 1-12. Potassium in SRM 1567b Wheat Flour (data summary view – sample preparation method). In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. 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, $|Z_{\text{NIST}}| \leq 2$.

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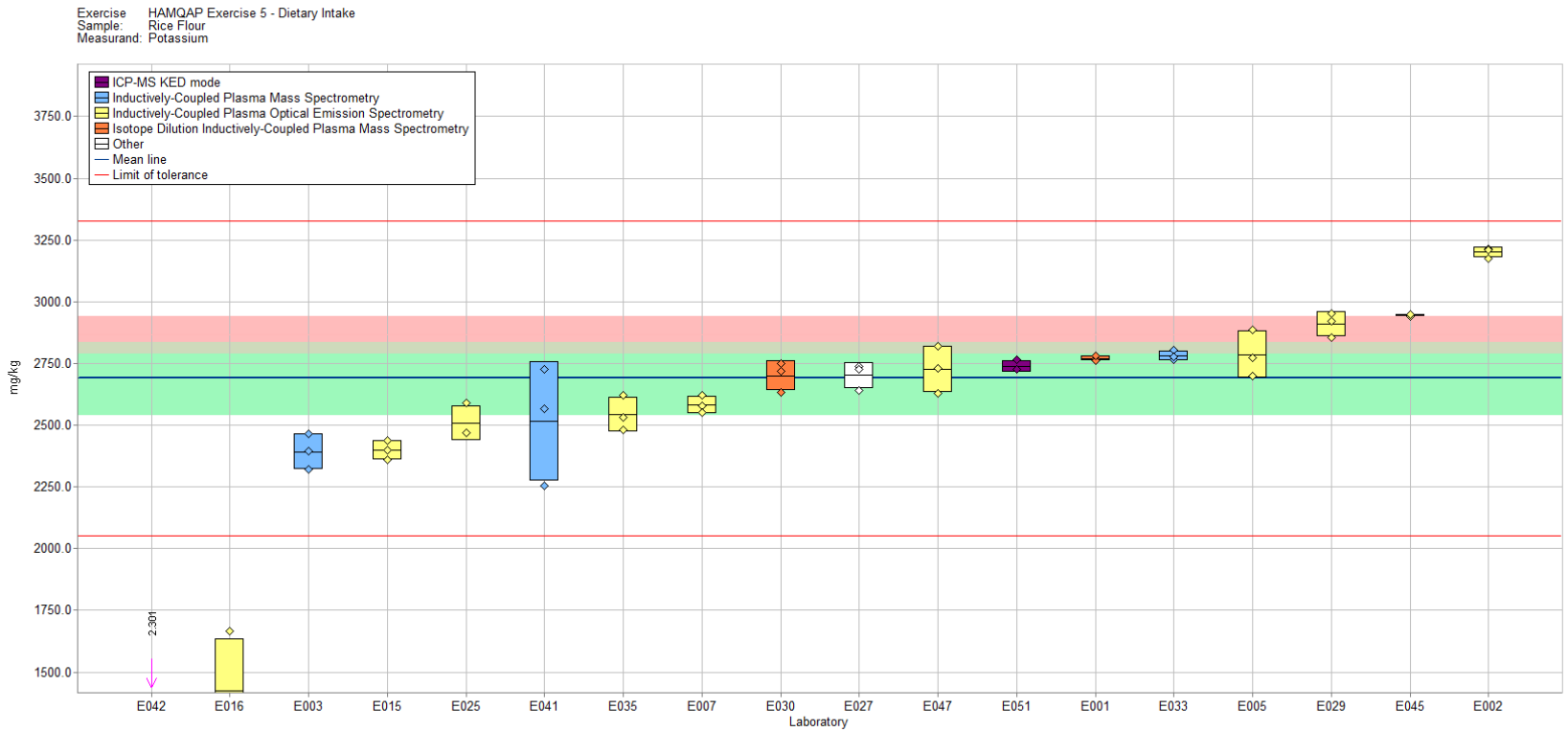

Figure 1-13. Potassium in Rice Flour (data summary view – analytical method). In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line represents 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, $|Z_{\text{NIST}}| \leq 2$.

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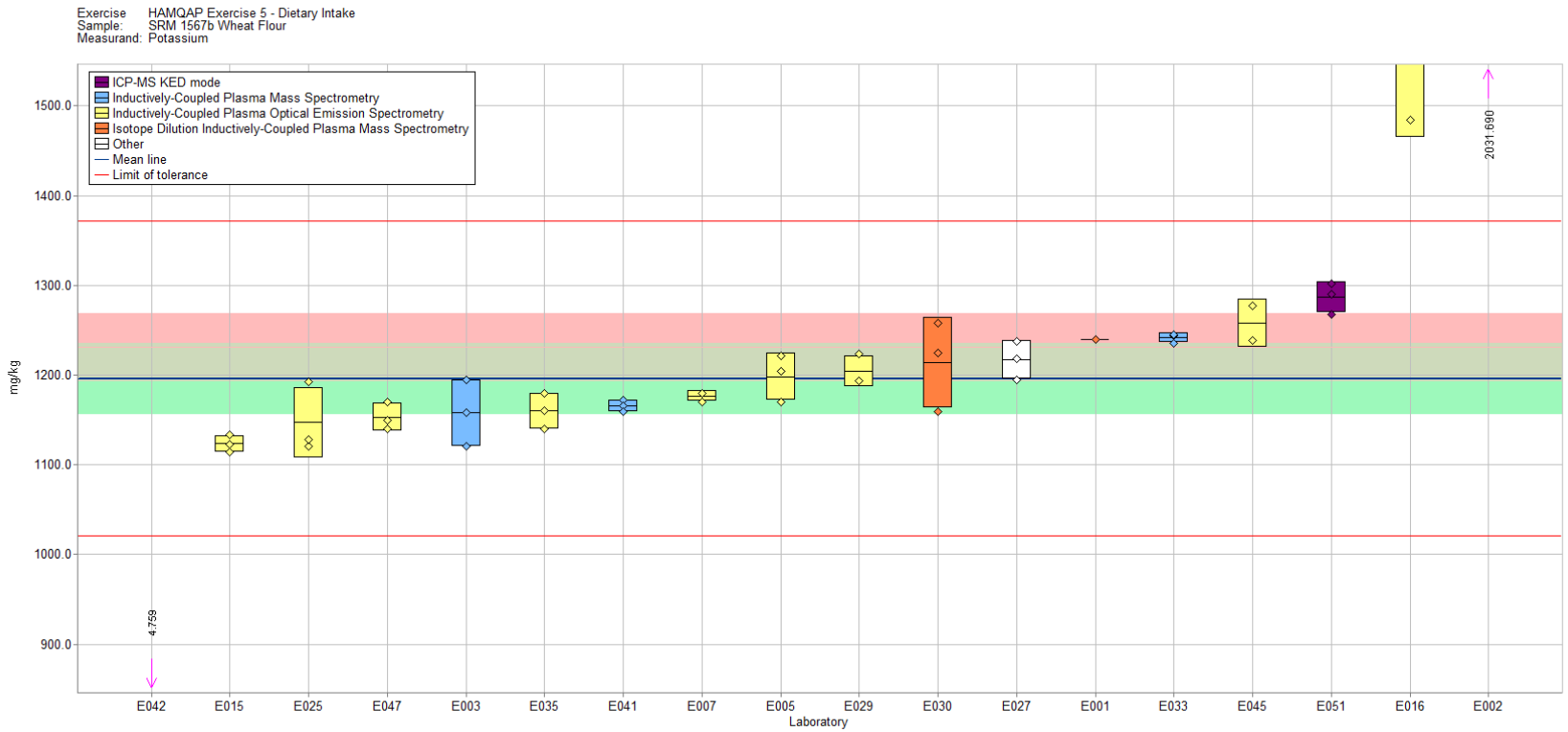

Figure 1-14. Potassium in SRM 1567b Wheat Flour (data summary view – analytical method). In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue 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, $|Z_{\text{NIST}}| \leq 2$.

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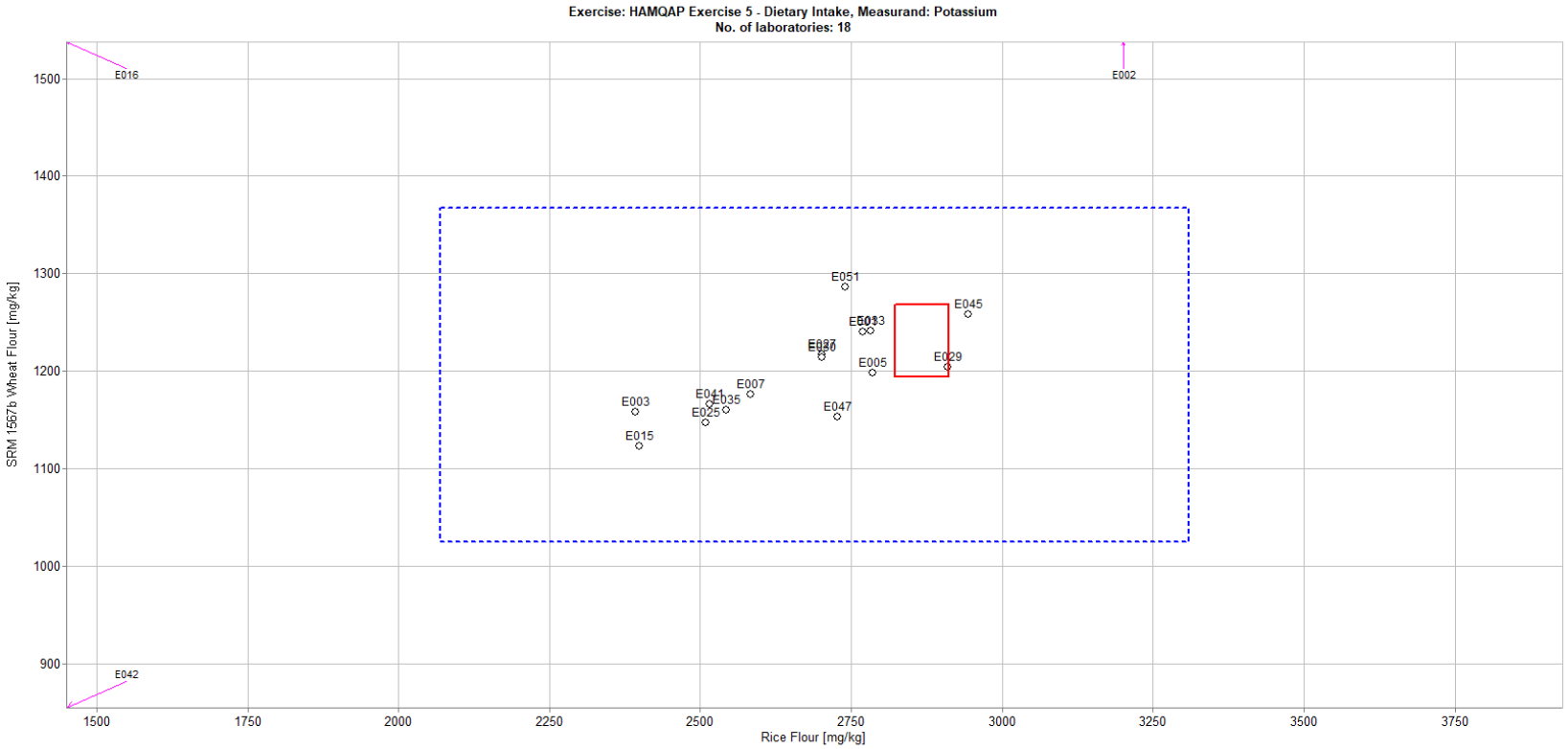

Figure 1-15. Laboratory means for potassium in Rice Flour and SRM 1567b Wheat Flour (sample/sample comparison view). In this view, the individual laboratory mean for one sample (rice flour) is compared to the individual laboratory mean for a second sample (wheat flour). The solid red box represents the NIST range of tolerance for the two samples, wheat flour (x-axis) and rice flour (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 wheat flour (x-axis) and rice flour (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 1-5. Data summary table for sodium in rice flour and wheat flour. Data points highlighted in red have been flagged as potential outliers (e.g., Grubb and/or Cochran) by the NIST software package.

		Sodium									
		Rice Flour (mg/kg)					SRM 1567b Wheat Flour (mg/kg)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target									6.23	0.39
	E001	17	16	16	16.3	0.6	9	9	8	8.67	0.58
	E002	24.994	23.517	21.616	23.4	1.7	18.973	19.418	19.125	19.17	0.23
	E003	19	16	18	17.7	1.5	8	9	9	8.67	0.58
	E005										
	E006										
	E007	12.34	12.85	12.4	12.5	0.3	4.587	4.658	4.554	4.60	0.05
	E008										
	E010										
	E011										
	E015	0.76	0.47	0.45	0.6	0.2	0.77	0.91	0.86	0.85	0.07
	E016	6.03	8.27	7.45	7.3	1.1	7.96	8.01	6.44	7.47	0.89
	E019										
	E020										
	E025	14.27	14.92	14.47	14.6	0.3	7.83	8.44	7.86	8.04	0.34
	E027	15.9	16	15.6	15.8	0.2	8.97	9.19	9.38	9.18	0.21
	E029	17.76	17.16	17.7	17.5	0.3	11.89	10.63	10.03	10.85	0.95
	E030	14.8	13.8	13.5	14.0	0.7	6.38	7.57	6.83	6.93	0.60
	E031										
	E033	26.3	15.288	23.825	21.8	5.8	9.663	9.289	16.871	11.94	4.27
	E035	19.4	18.4	18.8	18.9	0.5	9.07	9.43	11.7	10.07	1.43
	E036										
	E037										
	E040										
	E041	11.7	13.2	15	13.3	1.7	6.68	6.32	6.46	6.49	0.18
	E042	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	E045										
	E047	14.1	10.8	12.2	12.4	1.7	6.26	4.13	3.14	4.51	1.59
	E051	14.8	14.3	14.3	14.5	0.3	7.3	7.2	7.1	7.20	0.10
	E072										
Community Results		Consensus Mean			14.5		Consensus Mean			7.55	
		Consensus Standard Deviation			1.3		Consensus Standard Deviation			0.87	
		Maximum			23.4		Maximum			19.17	
		Minimum			0.0		Minimum			0.00	
		N			16		N			16	

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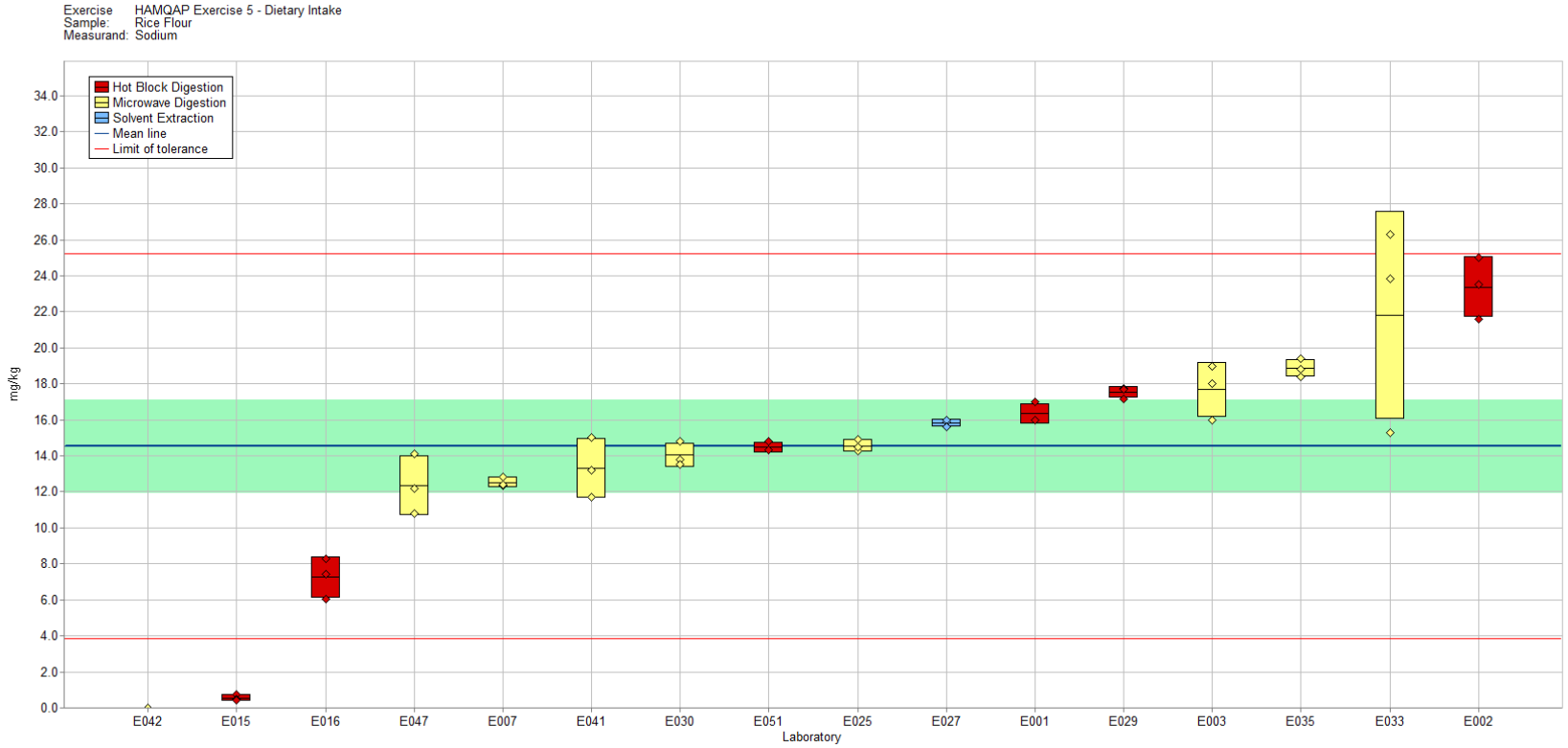

Figure 1-16. Sodium in Rice Flour (data summary view – sample preparation method). In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The solid blue 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, $|Z'_{\text{comm}}| \leq 2$. A NIST value has not been determined in this material.

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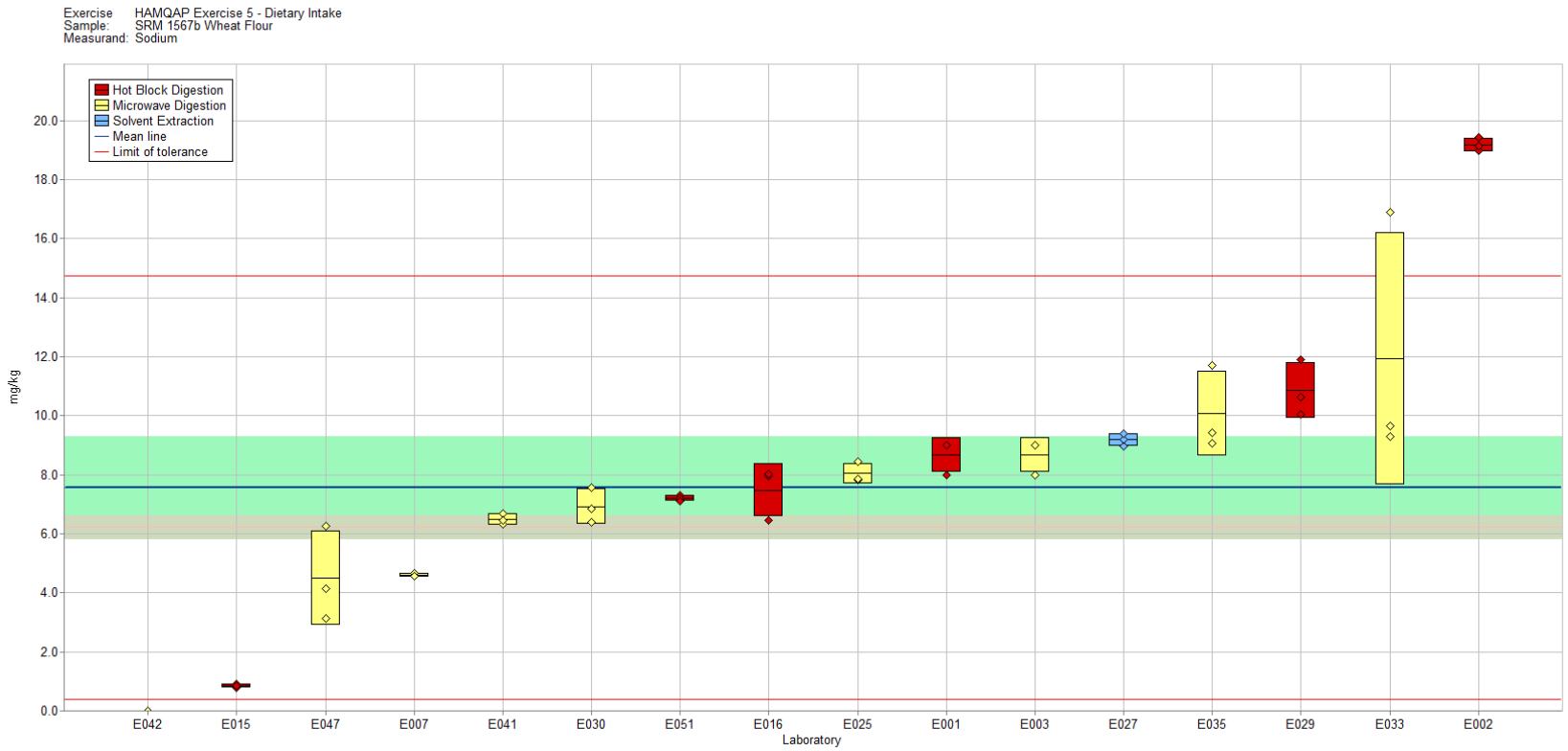

Figure 1-17. Sodium in SRM 1567b Wheat Flour (data summary view – sample preparation method). In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The 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, $|Z_{\text{NIST}}| \leq 2$.

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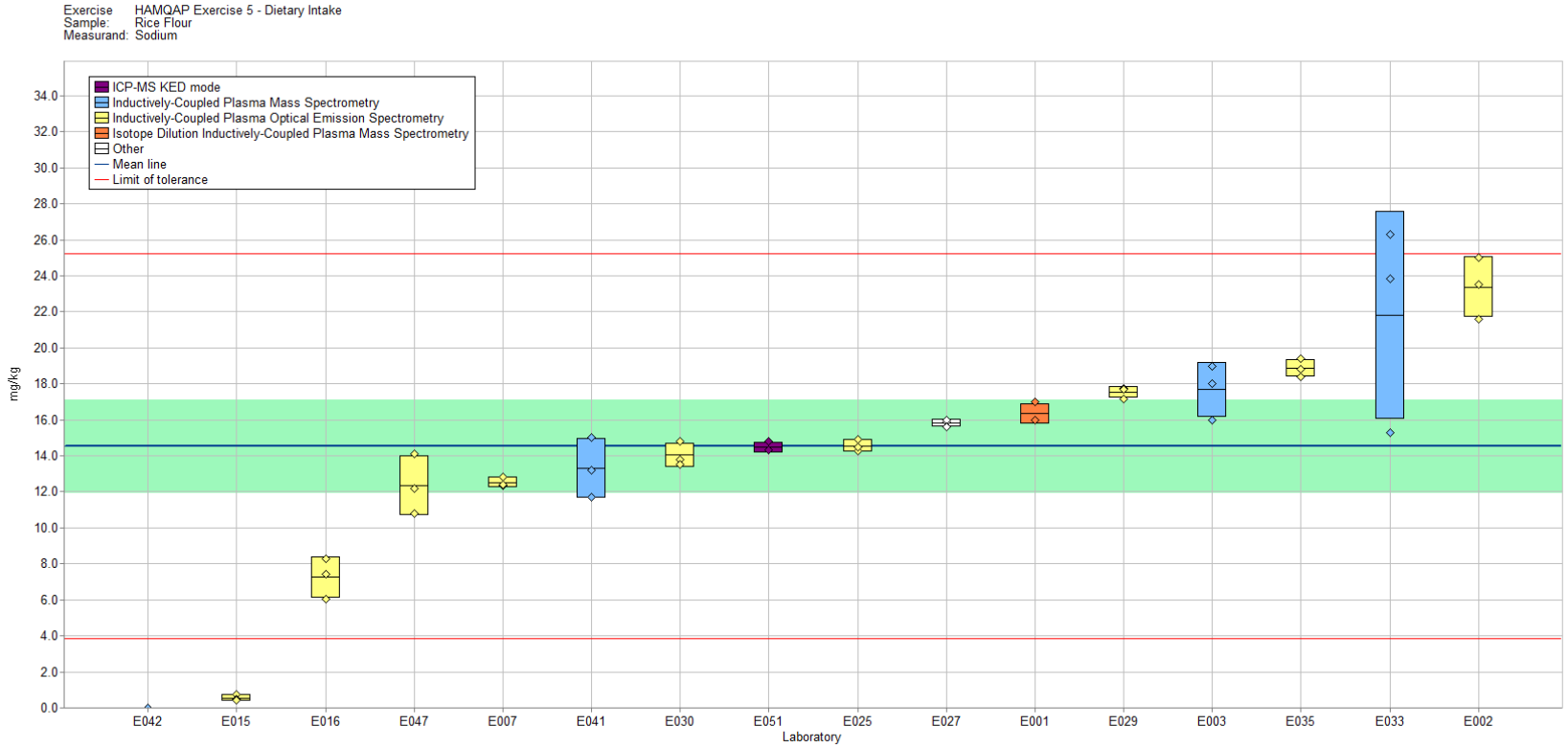

Figure 1-18. Sodium in Rice Flour (data summary view – analytical method). In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line represents 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.

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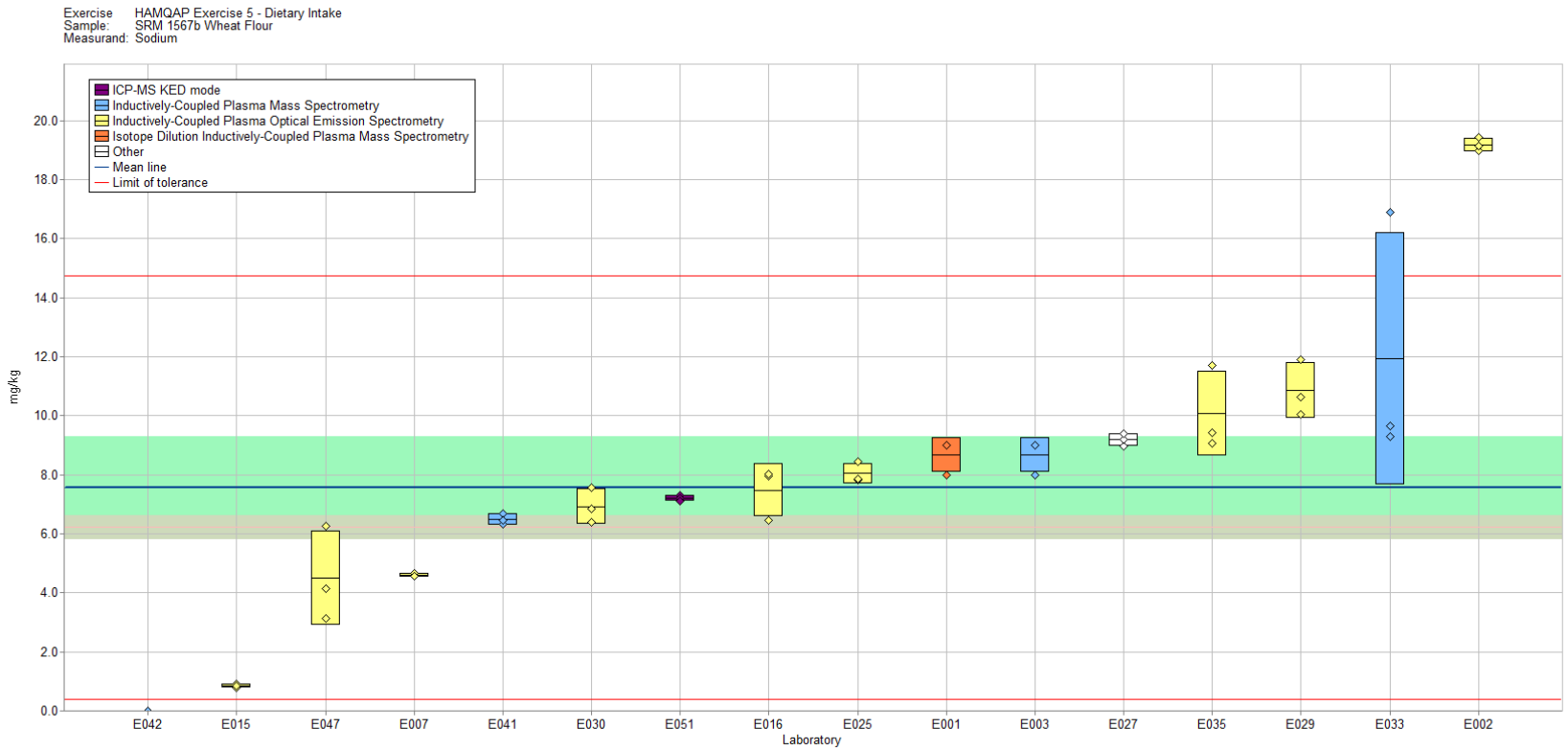

Figure 1-19. Sodium in SRM 1567b Wheat Flour (data summary view – analytical method). In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line 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, $|Z_{\text{NIST}}| \leq 2$.

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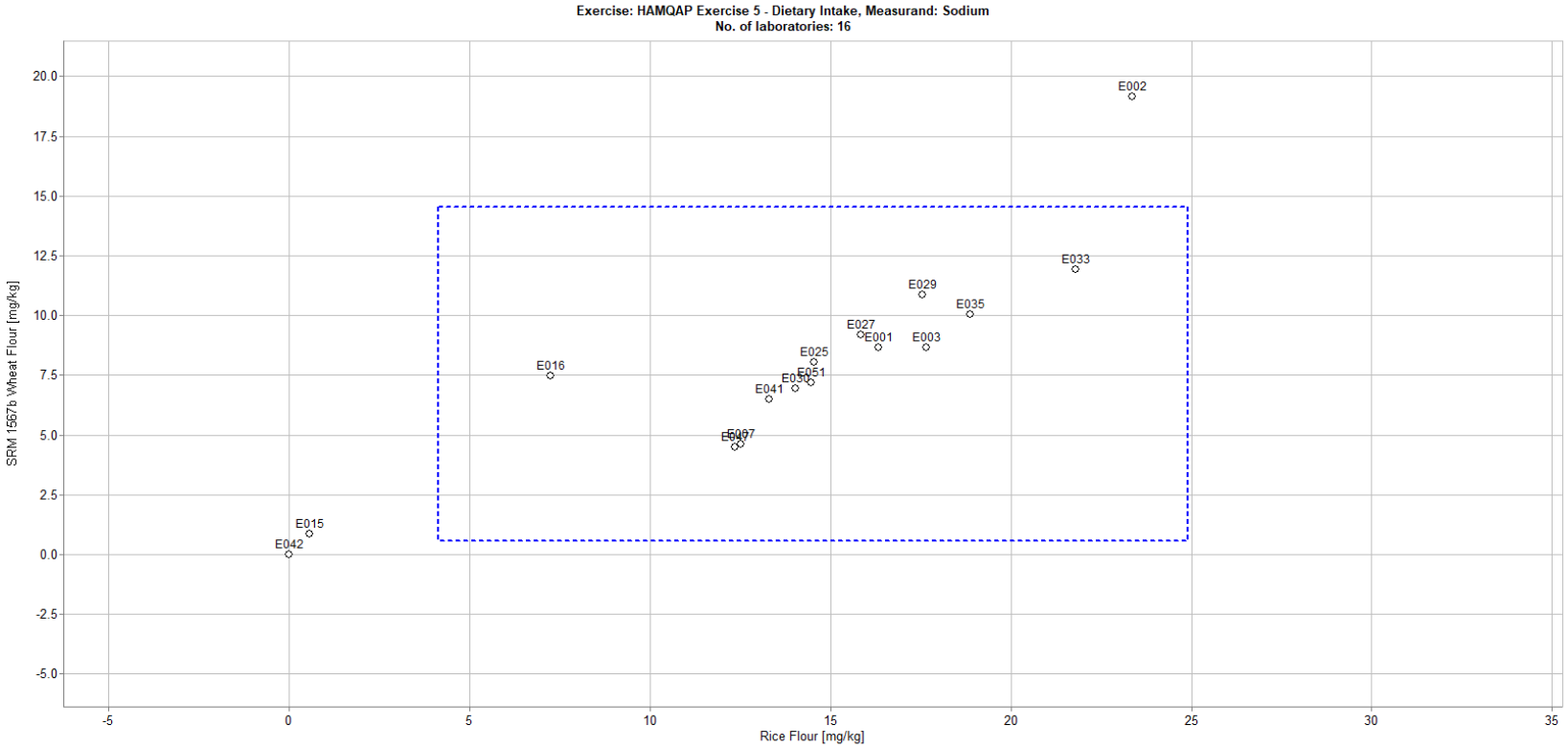

Figure 1-20. Laboratory means for sodium in Rice Flour and SRM 1567b Wheat Flour (sample/sample comparison view). In this view, the individual laboratory mean for one sample (rice flour) is compared to the individual laboratory mean for a second sample (wheat flour). The dotted blue box represents the consensus range of tolerance for wheat flour (x-axis) and rice flour (y-axis), calculated as the values above and below the consensus means that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$.

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SECTION 2: TOXIC ELEMENTS (Arsenic and Arsenic Species)

Study Overview

In this study, participants were provided with samples of aquacultured shrimp and wild-caught shrimp for dietary intake. Participants were asked to use in-house analytical methods to determine the mass fractions (mg/kg or ng/g) of total arsenic (tAs) and several arsenic species including inorganic arsenic (iAs), monomethylarsonic acid (MMA), dimethylarsinic acid (DMA), and arsenobetaine (AB) in each matrix. Arsenic occurs naturally in many foods, but inorganic arsenic specifically is highly toxic and the focus of regulation in food sources. The EPA established the Safe Drinking Water Act (SDWA) which limits the amount of inorganic arsenic in drinking water to 10 µg/L (10 ppb).[7] The FDA has proposed action levels for inorganic arsenic in apple juice at 10 ppb and recently issued Inorganic Arsenic in Rice Cereals for Infants: Action Level Guidance for Industry which limits inorganic arsenic in those commodities to 100 ppb.[8] Most arsenic in seafood is in the form of non-toxic arsenobetaine, however aquacultured seafood may contain other arsenic species from the water source, use of antibiotics, or exposure to pollutants. In this study, measurement of total arsenic and arsenic species will expose variability between laboratories and determine if significant differences exist between the types and levels of arsenic found in aquacultured and wild-caught shrimp. For the identification of farmed shrimp fraudulently labeled as wild-caught, the ratios of the arsenic species may be more important than the actual concentrations of the arsenic species.

Dietary Intake Sample Information

Aquacultured Shrimp. Participants were provided with a single jar containing approximately 6 g of aquacultured whiteleg shrimp (*Litopenaeus vannamei*) obtained from Boligee, AL. Edible portions of the shrimp were cryomilled and the fresh frozen powder bottled in glass jars and stored at –80 °C. Participants were asked to store the material at –70 °C or colder in the original unopened jar until use to ensure the material retains its powdered form. Participants were asked to prepare three samples and to report three values from the single jar provided. Before use, participants were instructed to keep the jar on dry ice during sampling, to mix the contents of the jar thoroughly, and take samples immediately upon removal from the freezer. If the sample in the jar lost the powdered form, participants were instructed to refreeze at –70 °C or colder for several hours to allow the material to return to the powdered form. If the material was stored at –20 °C, participants were instructed to allow the material to thaw completely then blend the contents of the entire jar, preferably with a handheld homogenizer or immersion blender, prior to sampling. A sample size of at least 0.5 g was recommended. The approximate analyte levels were not reported to participants prior to the study. The NIST-determined values for tAs and DMA were assigned using results from NIST by LC-ICP-MS. The NIST-determined values and uncertainties for tAs and DMA are provided in the table below on an as-received basis.

[7] US Code of Federal Regulations. Safe Drinking Water Act (21 CFR 165.110(b)(4)(iii)(A)). https://www.epa.gov/sdwa (accessed August 2020).

[8] FDA CFSAN Risk & Safety Assessments. US Food and Drug Administration; Arsenic in Rice and Rice Products Risk Assessment (FDA-2016-D-1099). https://www.fda.gov/food/cfsan-risk-safety-assessments/arsenic-rice-and-rice-products-risk-assessment (accessed August 2020).

Analyte	NIST-Determined Mass Fractions in Aquacultured Shrimp (mg/kg)
tAs	0.135 ± 0.039
DMA	0.0026 ± 0.00168

Wild-Caught Shrimp. Participants were provided with a single jar containing approximately 6 g of wild-caught brown shrimp (*Farfantepenaeus aztecus)* caught off the coast of Charleston, SC. Edible portions of the shrimp were cryomilled and the fresh frozen powder bottled in glass jars and stored at –80 °C. Participants were asked to store the material at –70 °C or colder in the original unopened jar until use to ensure the material retains its powdered form. Participants were asked to prepare three samples and to report three values from the single jar provided. Before use, participants were instructed to keep the jar on dry ice during sampling, to mix the contents of the jar thoroughly, and take samples immediately upon removal from the freezer. If the sample in the jar lost the powdered form, participants were instructed to refreeze at –70 °C or colder for several hours to allow the material to return to the powdered form. If the material was stored at –20 °C, participants were instructed to allow the material to thaw completely then blend the contents of the entire jar, preferably with a handheld homogenizer or immersion blender, prior to sampling. A sample size of at least 0.5 g was recommended. The approximate analyte levels were not reported to participants prior to the study. The NIST-determined values for tAs and AB were assigned using results from NIST by LC-ICP-MS. The NIST-determined values and uncertainties for tAs and AB are provided in the table below on an as-received basis.

Analyte	NIST-Determined Mass Fractions in Wild-Caught Shrimp (mg/kg)
tAs	10.16 ± 1.50
AB	9.85 ± 0.45

Dietary Intake Study Results

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

Analyte	Number of Laboratories Requesting Samples	Number of Laboratories Reporting Results (Percent Participation)	
		Aquacultured Shrimp	Wild-Caught Shrimp
tAs	27	18 (67 %)	18 (67 %)
iAs	10	5 (50%)	6 (60%)
MMA	5	3 (60 %)	3 (60 %)
DMA	6	2 (33 %)	4 (67 %)
AB	5	4 (80 %)	5 (100 %)

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- For the aquacultured shrimp, the consensus range for total arsenic was completely within the target range with only a few outliers (**Figures 2-1** and **2-3**).
- For the wild-caught shrimp, the consensus range for total arsenic was completely within the target range with only a few outliers (**Figures 2-2** and **2-4**), while the target range for arsenobetaine fell in the middle of the consensus range (**Figure 2-9**) and centered over the consensus mean.
- The between-laboratory variabilities were excellent for total arsenic in both aquacultured shrimp and wild-caught shrimp (4 % and 3 %, respectively), and the within-laboratory variabilities were good (< 25 %). The between-laboratory variabilities for inorganic arsenic and arsenobetaine were both good in the wild-caught shrimp (16 % and 12 %, respectively) but were not good in the aquacultured shrimp where analyte mass fractions were lower (see table below).

	Between-Laboratory Variability (% RSD)	
Analyte	Aquacultured Shrimp	Wild-Caught Shrimp
tAs	4 %	3 %
iAs	80 %	16 %
MMA	>100 %	89 %
DMA	--	>100 %
AB	57 %	12 %

- Most laboratories reported using microwave digestion as their sample preparation method for determination of total arsenic (78 % and 72 % for aquacultured and wild-caught respectively). However, when analyzing the arsenic species, less harsh sample preparation methods were used. The sample preparation methods used are listed below by number of laboratories reporting for each matrix and analyte pair.

	Number of Laboratories Reporting for Aquacultured/Wild-Caught Shrimp				
Sample Preparation Method	tAs	iAs	MMA	DMA	AB
Microwave Digestion	14 / 13	1 / 1	--	--	--
Hot Block	3 / 3	2 / 2	2 / 2	1 / 1	2 / 2
Acid Hydrolysis	1 / 2	0 / 1	--	--	--
Solvent Extraction	--	--	--	0 /1	0 /1
Other/None	--	2 / 2	1 / 1	1 / 2	2 / 2

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- Most laboratories (89 %) reported using some variation of ICP-MS for the determination of arsenic species. The analytical methods used are listed below by number of laboratories reporting for each matrix and analyte pair.

Sample Preparation Method	Number of Laboratories Reporting for Aquacultured/Wild-Caught Shrimp				
	tAs	iAs	MMA	DMA	AB
ICP-MS	9 / 9	2 / 3	1 / 1	0 / 1	2 / 2
ICP-MS w/KED	3 / 3	1 / 1	--	0 / 1	0 / 1
ID ICP-MS	4 / 3	1 / 1	1 / 1	1 / 1	1 / 1
ICP-OES	2 / 3	--	--	--	--
Spectrophotometry	--	1 / 1	1 / 1	1 / 1	1 / 1

Dietary Intake Technical Recommendations

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

- No significant bias was observed between the results obtained by different sample preparation techniques or instrumental techniques in either sample for total arsenic.
- As shown in the data tables (**Tables 2-1, 2-2,** and **2-6**), most of the arsenic in the wild-caught shrimp is in the form of arsenobetaine. Conversely, the majority of the arsenic in the aquacultured shrimp is not extracted in the hydrophilic fraction and hence not determined in the measurements.
  - Arsenic levels as well as the distribution of arsenic species may be useful for chemically distinguishing aquacultured and wild-caught seafood products.
  - Most laboratories performed well on the determination of total arsenic and arsenobetaine, indicating that their measurement procedures are in control for those analytes (**Figures 2-1** through **2-5** and **Figure 2-9**).
  - Most of the laboratories that determined inorganic arsenic in wild-caught shrimp (**Figure 2-7**) appeared to agree. Unfortunately, too few participants reported data for either sample to make statistical analyses and meaningful recommendations.
- Shrimp and similar matrices can be difficult to digest for determination of total arsenic, requiring high temperatures or the use of a small amount of HF in addition to oxidizing reagents to ensure complete digestion of the sample prior to instrumental analysis.
  - Sample preparation methods should be well established before analyzing unknown samples. Established quality control materials (SRM, CRM, RM and in-house materials when not commercially available) and methods of analyses should be used whenever possible.
  - Arsenic is volatile and can be lost during sample preparation; therefore, open-beaker digestion should not be used.
    - Closed-vessel digestions should be opened with care ensuring that no arsenic is lost as a result of inadvertent venting.
    - The high temperatures of a vigorous microwave digestion should convert all volatile organoarsenic species to arsenic acid (AsV), at which point subsequent heating will not result in loss of arsenic.

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- The level of total arsenic in the aquacultured shrimp was 100 times lower than in the wild-caught shrimp. Determination of low analyte levels requires careful laboratory preparation and practice.
  - Determination and understanding of LOQ and MDL will prevent reports of "zero" concentration and allow acceptable performance even when a result is below LOQ or MDL.
  - 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 observed variability is arising from the sample preparation procedure. The suggested minimum number of blanks to prepare is equal to the number of samples being prepared.
  - Laboratories should consider minimizing sample dilution to increase the analyte contents in measurement samples. With minimum sample dilution, however, matrix effects may become significant.
    - Matrix matched standards used for calibration curves may improve the detection of the analyte in the sample.
    - When concentrations in sample solutions are low, use of an organic solvent in the mobile phase may enhance sensitivity and improve the detection limit for arsenic.
    - When using ICP-MS, collision cell technology can be employed to minimize many of the molecular interferences that may be found when determining arsenic in these two materials.
  - Calibration curves must be linear and include the lowest and highest values expected to be measured in the sample solutions for best results. Extrapolation of the curve may cause incorrect results.
- Measurement results should be reported accurately.
  - ID-ICP-MS is not likely a useful method for total arsenic or arsenic speciation since there is only one stable isotope or arsenic.
  - Zero is not an appropriate quantity to be reported as discussed above. A more appropriate result would be to report that a value is below the MDL, LOQ, or QL.
  - Laboratories reporting results flagged as outliers should check for calculation errors when preliminary data tables are sent for inspection. One example is to confirm that factors for all dilutions have been properly tabulated and that results are reported in correct reporting units.

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Table 2-1. Individualized data summary table (NIST) for arsenic and arsenic species in aquacultured and wild-caught shrimp.

National Institute of Standards & Technology

HAMQAP Exercise 5 - 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	Aquacultured Shrimp	mg/kg	0.135	0.039			18	0.131	0.005	0.135	0.039
Total Arsenic	Wild Shrimp	mg/kg	10.16	1.50			18	10.6	0.28	10.16	1.50
Inorganic Arsenic (iAs)	Aquacultured Shrimp	ng/g					3	7.9	6.3		
Inorganic Arsenic (iAs)	Wild Shrimp	ng/g					6	90	14		
Monomethylarsonic acid (MMA)	Aquacultured Shrimp	ng/g					2	0.2	0.4		
Monomethylarsonic acid (MMA)	Wild Shrimp	ng/g					3	2.8	2.5		
Dimethylarsinic acid (DMA)	Aquacultured Shrimp	ng/g	2.62	1.68			1			2.62	1.68
Dimethylarsinic acid (DMA)	Wild Shrimp	ng/g					3	4.7	5.5		
Arsenobetaine (AB)	Aquacultured Shrimp	mg/kg					4	0.0161	0.0091		
Arsenobetaine (AB)	Wild Shrimp	mg/kg	9.85	0.45			5	9.9	1.2	9.85	0.45

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 (tAs) in aquacultured and wild-caught shrimp. Data points highlighted in red have been flagged as potential outliers (e.g., Grubb and/or Cochran) by the NIST software package.

		Total Arsenic									
		Aquacultured Shrimp (mg/kg)					Wild Shrimp (mg/kg)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target				0.135	0.039				10.16	1.50
	E001	0.137	0.131	0.133	0.134	0.003	11.6	11.6	11.7	11.63	0.06
	E002	0.1	0.101	0.096	0.099	0.003	8.829	8.916	8.821	8.86	0.05
	E003	0.12	0.11	0.12	0.117	0.006	6.33	6.86	6.6	6.60	0.27
	E005	0.15	0.15	0.14	0.147	0.006	11.25	11.19	11.29	11.24	0.05
	E007	0.152	0.163	0.162	0.159	0.006	12.2	12.2	12.1	12.17	0.06
	E008										
	E009	0.12	0.17	0.15	0.147	0.025	9.58	12.2	13.47	11.75	1.98
	E010										
	E018										
	E020										
	E021										
	E026	0.131	0.127	0.139	0.132	0.006	9.86	9.63	9.65	9.71	0.13
	E029	0.126	0.116	0.118	0.120	0.005	10.026	10.705	10.551	10.43	0.36
	E030										
	E033										
	E034	0.133	0.131	0.122	0.129	0.006	9.825	9.709	10.12	9.88	0.21
	E035	0.12	0.13	0.19	0.147	0.038	10.9	11.33	11.43	11.22	0.28
	E036										
	E037										
	E040	0.125	0.129	0.127	0.127	0.002	9.59	9.73	9.71	9.68	0.08
	E041	0.13	0.19	0.13	0.150	0.035	10.4	11.8	10.6	10.93	0.76
	E042	0.092		0.085	0.089	0.005	11.467	11.555	11.28	11.43	0.14
	E045	0.115	0.118		0.117	0.002	10.827	10.371	10.889	10.70	0.28
	E047	0.124	0.118	0.12	0.121	0.003	10.95	10.15	9.71	10.27	0.63
	E048	0.1557	0.1483	0.1581	0.154	0.005	11.01	11.01	11.25	11.09	0.14
	E049	0.146	0.156	0.161	0.154	0.008	11.7	11.1	11.4	11.40	0.30
	E051	0.11	0.13	0.11	0.117	0.012	10	9.94	9.96	9.97	0.03
Community Results		Consensus Mean			0.131		Consensus Mean			10.65	
		Consensus Standard Deviation			0.005		Consensus Standard Deviation			0.28	
		Maximum			0.159		Maximum			12.17	
		Minimum			0.089		Minimum			6.60	
		N			18		N			18	

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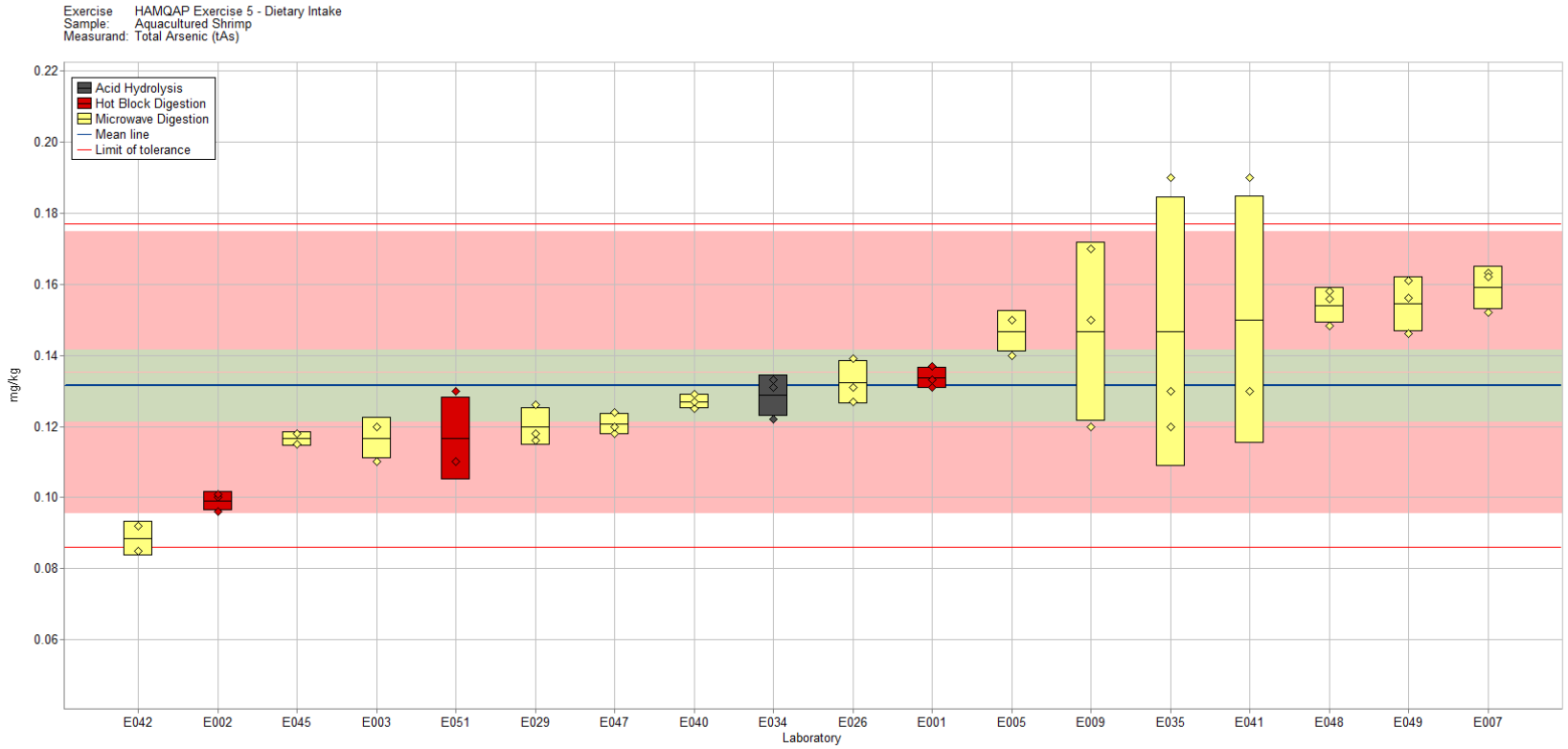

Figure 2-1. Total arsenic (tAs) in Aquacultured Shrimp (data summary view –sample preparation method). In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed.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

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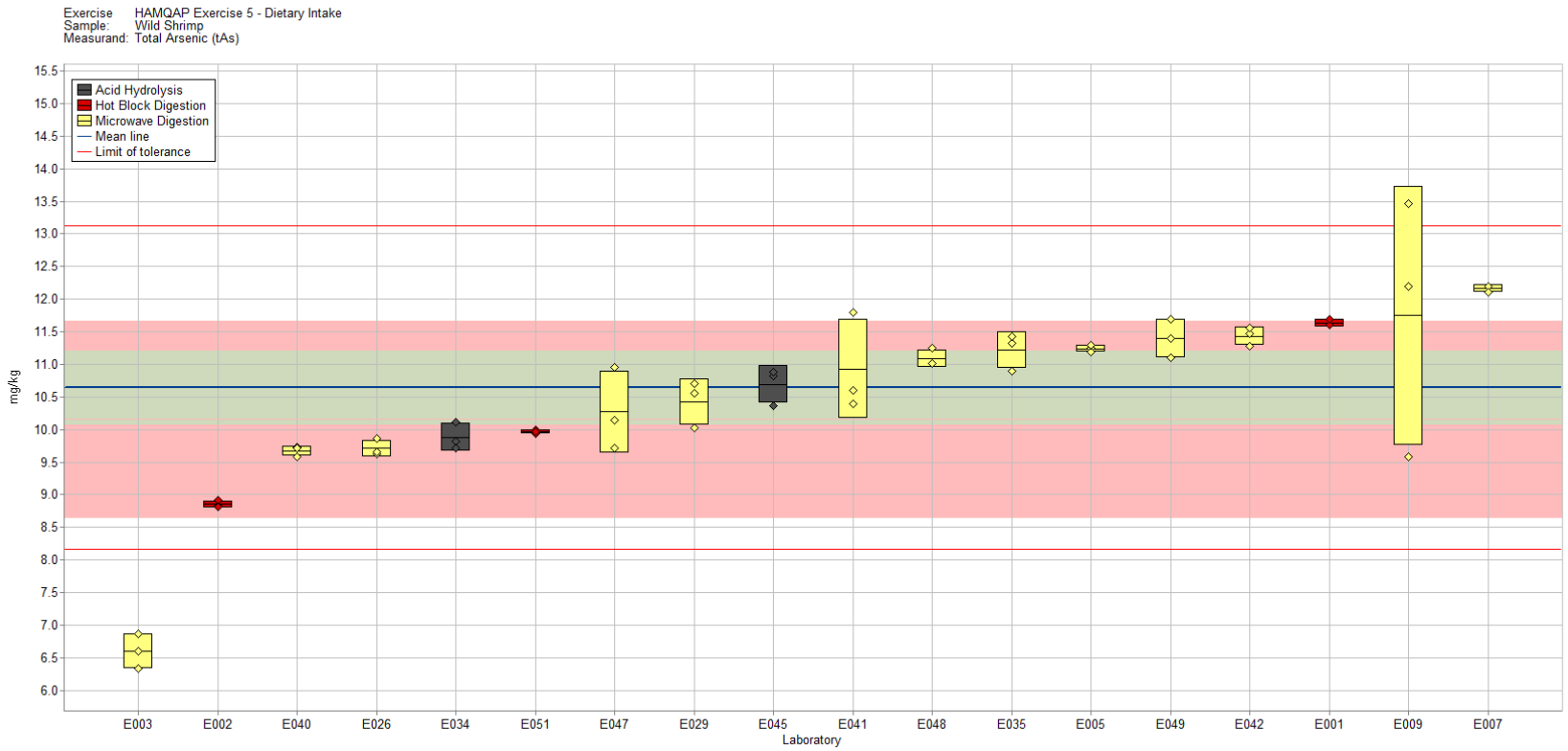

Figure 2-2. Total arsenic (tAs) in Wild-Caught Shrimp (data summary view –sample preparation method). In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. 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, $|Z_{\text{NIST}}| \leq 2$.

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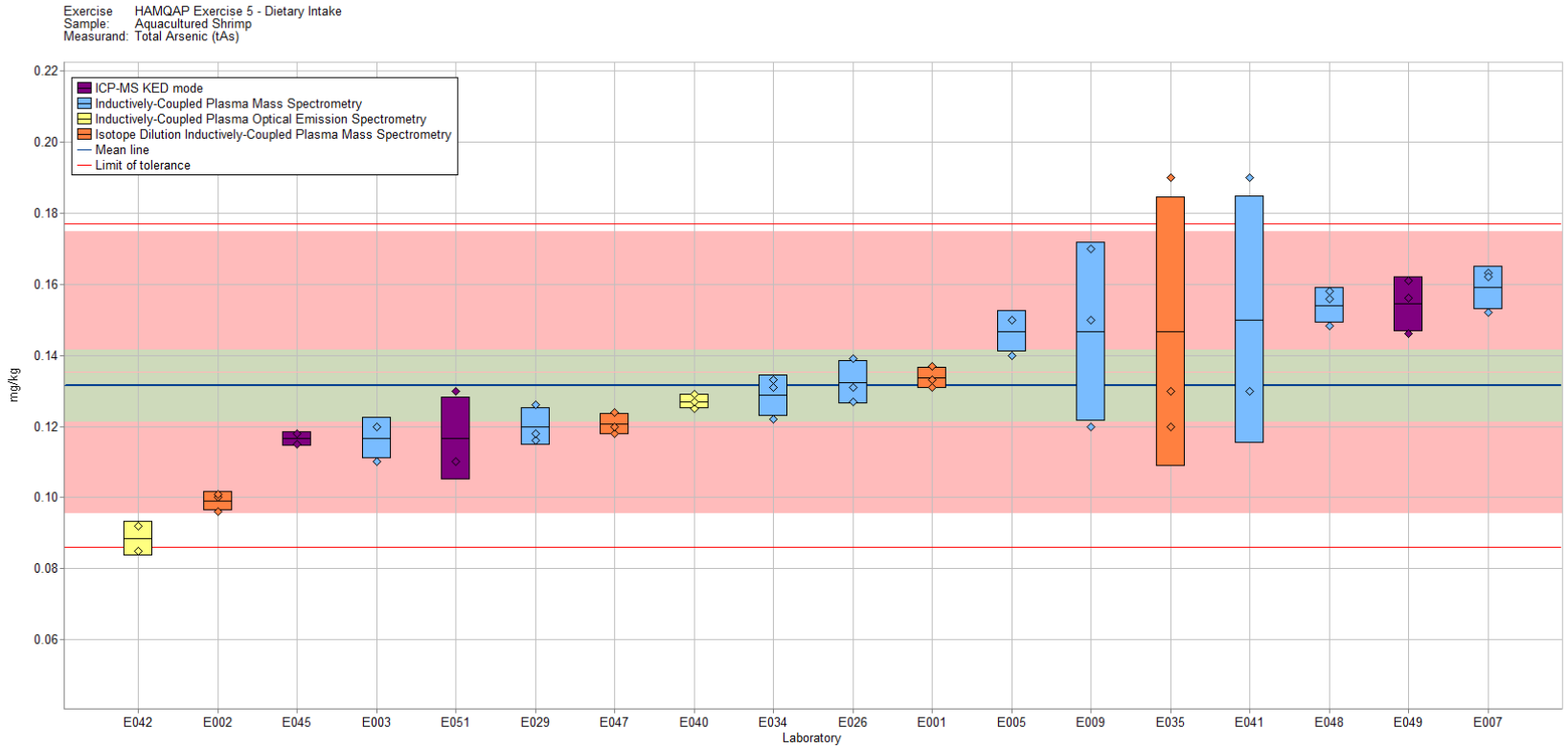

Figure 2-3. Total arsenic (tAs) in Aquacultured Shrimp (data summary view – analytical method). In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid 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, $|Z_{\text{NIST}}| \leq 2$.

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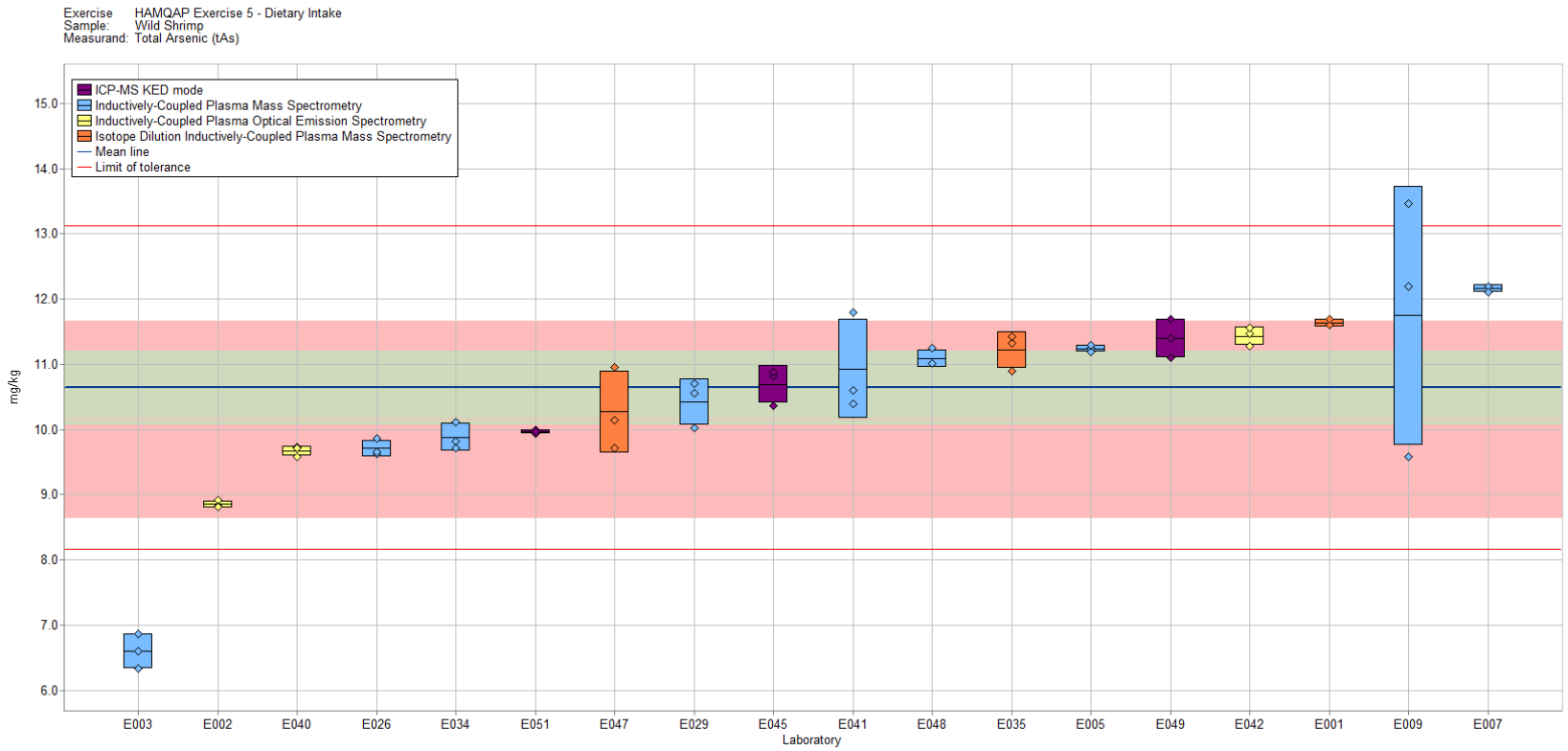

Figure 2-4. Total arsenic (tAs) in Wild-Caught Shrimp (data summary view – analytical method). In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid 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, $|Z_{\text{NIST}}| \leq 2$.

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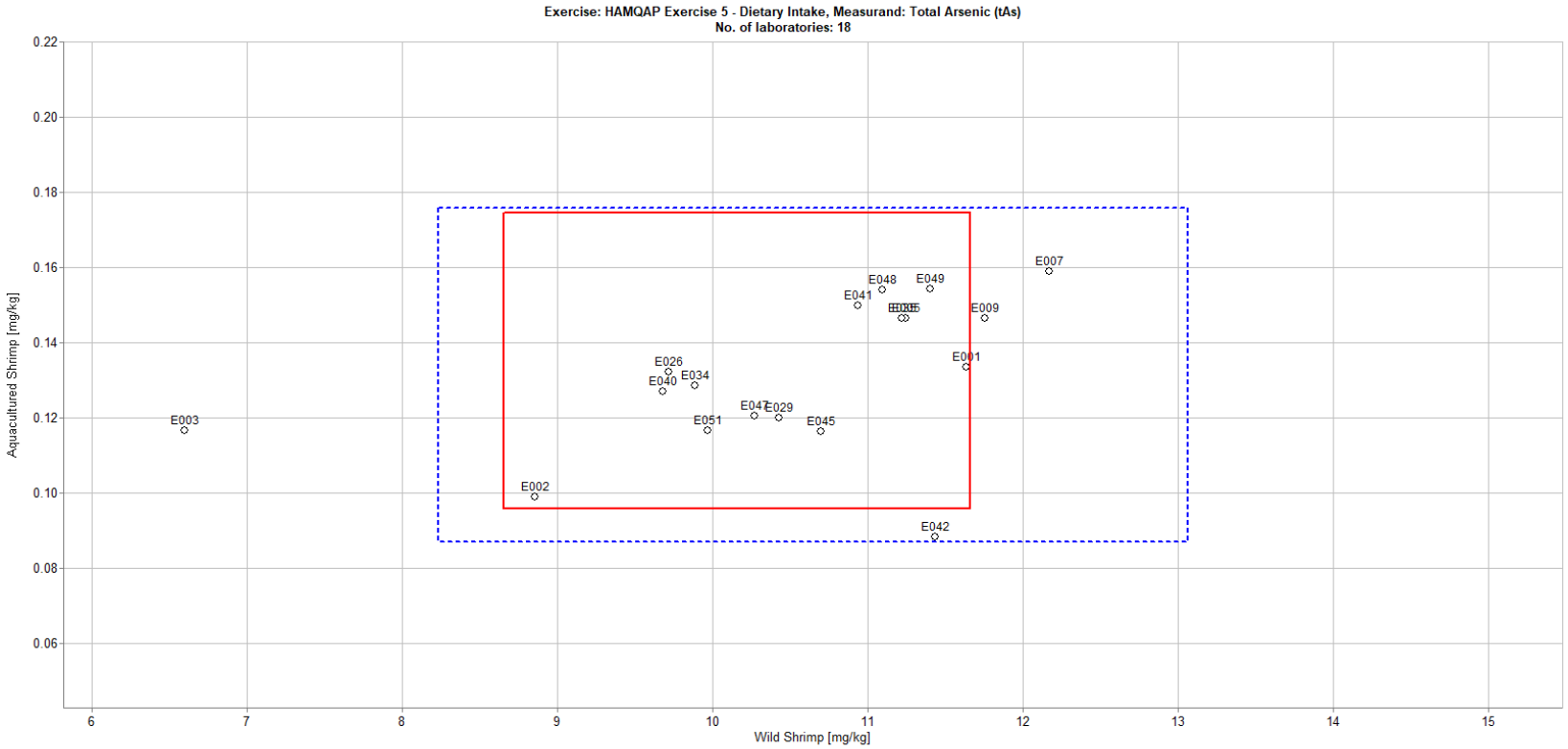

Figure 2-5. Laboratory means for total arsenic (tAs) in Aquacultured Shrimp and Wild-Caught Shrimp (sample/sample comparison view). In this view, the individual laboratory mean for one sample (aquacultured shrimp) is compared to the mean for a second sample (wild-caught shrimp). The solid red box represents the NIST range of tolerance for the two samples, wild-caught shrimp (x-axis) and aquacultured shrimp (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 wild-caught shrimp (x-axis) and aquacultured shrimp (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 inorganic arsenic (iAs) in aquacultured and wild-caught shrimp. Data points highlighted in red have been flagged as potential outliers (e.g., Grubb and/or Cochran) by the NIST software package.

		Inorganic Arsenic (iAs)									
		Aquacultured Shrimp (ng/g)					Wild Shrimp (ng/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target										
	E001	< 7.000	< 7.000	< 7.000			88.9	95.8	89.3	91.3	3.9
	E002	13	13	13	13.00	0.00	110	110	110	110.0	0.0
	E003	7.7	7.7	7.7	7.70	0.00	37	37	37	37.0	0.0
	E026	3.66	2.73	2.87	3.09	0.50	105	88	89	94.0	9.5
	E031										
	E033										
	E045						93.8	95.1	91.5	93.5	1.8
	E048										
	E049										
	E051	< 60.000	< 60.000	< 60.000			320	270	290	293.3	25.2
Community Results		Consensus Mean			7.93		Consensus Mean			85.2	
		Consensus Standard Deviatio			6.32		Consensus Standard Deviatio			13.9	
		Maximum			13.00		Maximum			293.3	
		Minimum			3.09		Minimum			37.0	
		N			3		N			6	

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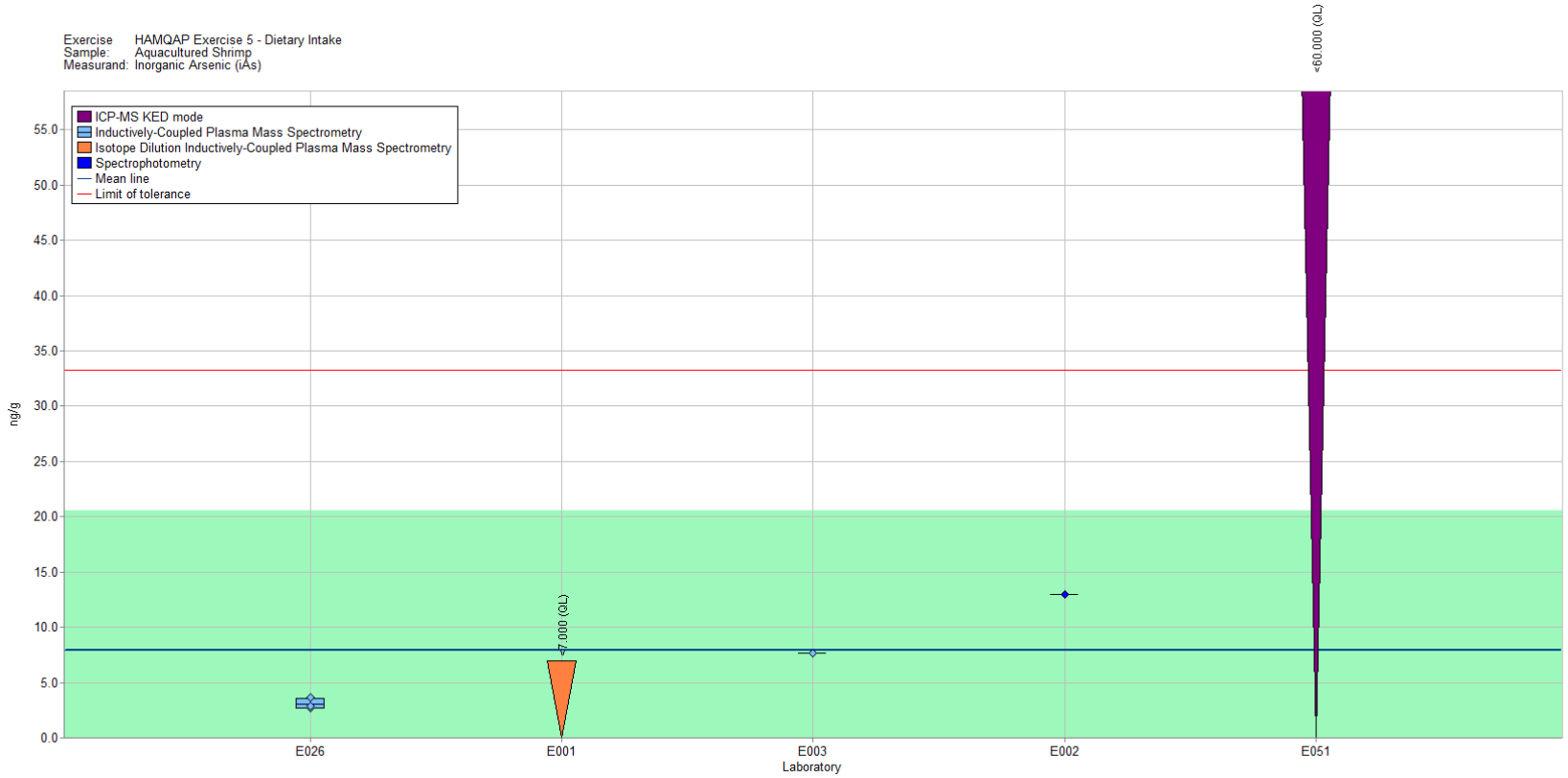

Figure 2-6. Inorganic arsenic (iAs) in Aquacultured Shrimp (data summary view – analytical method). In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The 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 consensus range of tolerance, calculated as the values above 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.

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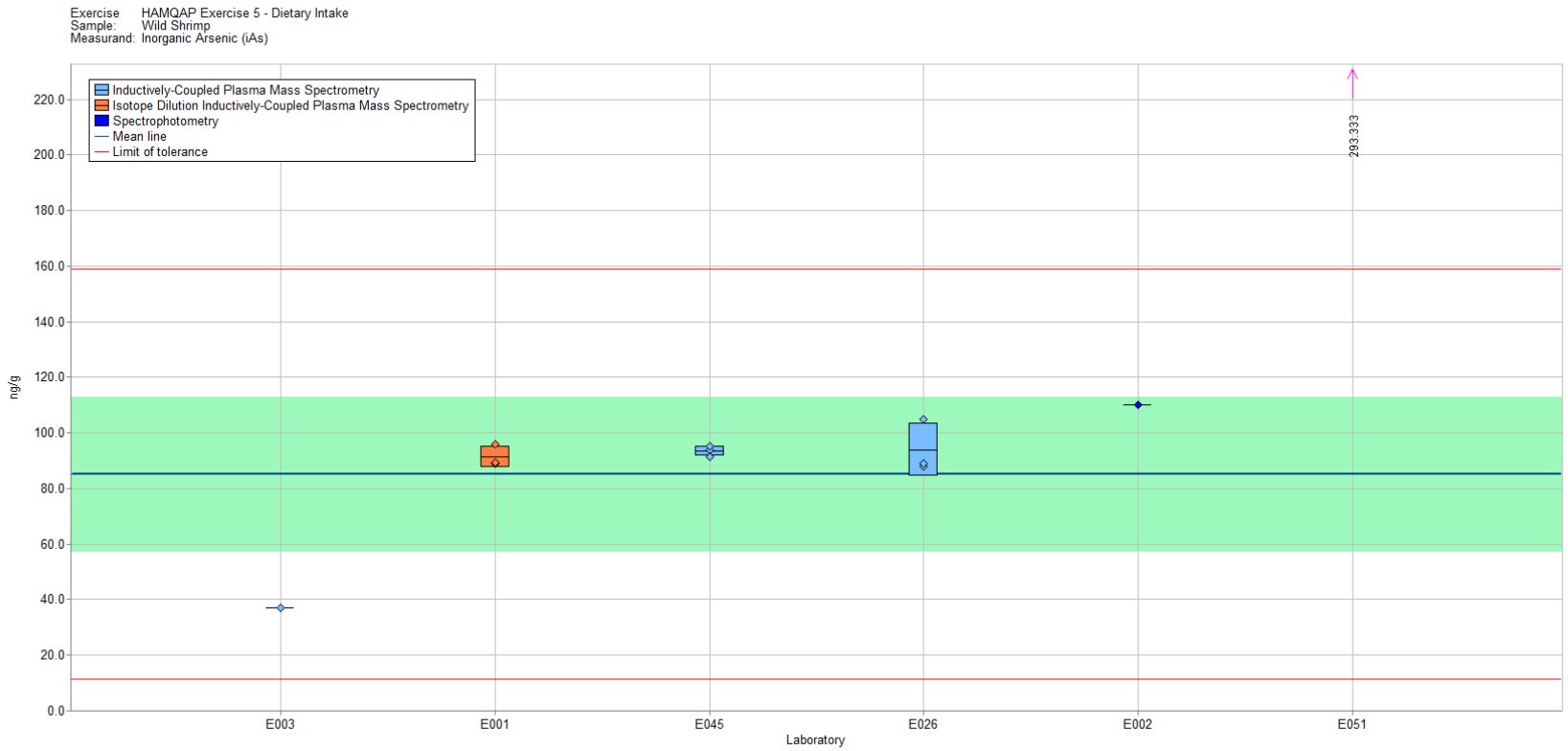

Figure 2-7. Inorganic arsenic (iAs) in Wild-Caught Shrimp (data summary view – analytical method). In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The 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.

Table 2-4. Data summary table for monomethylarsonic acid (MMA) in aquacultured and wild-caught shrimp.

		Monomethylarsonic acid (MMA)									
		Aquacultured Shrimp (ng/g)					Wild Shrimp (ng/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Result	Target										
	E001	< 3.000	< 3.000	< 3.000			5	5	5	5.00	0.00
	E002	0.00	0.00	0.00	0.000	0.000	0.00	0.00	0.00	0.00	0.00
	E026	0.244	0.453	0.295	0.331	0.109	3.55	3.07	3.1	3.24	0.27
	E048										
	E049										
Community Results		Consensus Mean			0.165		Consensus Mean			2.75	
		Consensus Standard Deviatio			0.399		Consensus Standard Deviatio			2.46	
		Maximum			0.331		Maximum			5.00	
		Minimum			0.000		Minimum			0.00	
		N			2		N			3	

Table 2-5. Data summary table for dimethylarsinic acid (DMA) in aquacultured and wild-caught shrimp.

		Dimethylarsinic acid (DMA)									
		Aquacultured Shrimp (ng/g)					Wild Shrimp (ng/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target				2.62	1.68					
	E001	< 3.000	< 3.000	< 3.000			< 3.000	< 3.000	< 3.000		
	E002	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0
	E026										
	E042										
	E048						5.2421	5.3413	4.2488	4.94	0.60
	E049						10.6	8.64	7.96	9.07	1.37
Community Results		Consensus Mean					Consensus Mean			4.67	
		Consensus Standard Deviation					Consensus Standard Deviation			5.45	
		Maximum			0.00		Maximum			9.07	
		Minimum			0.00		Minimum			0.00	
		N			1		N			3	

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Table 2-6. Data summary table for arsenobetaine (AB) in aquacultured and wild-caught shrimp. Data points highlighted in red have been flagged as potential outliers (e.g., Grubb and/or Cochran) by the NIST software package.

		Arsenobetaine (AB)									
		Aquacultured Shrimp (mg/kg)					Wild Shrimp (mg/kg)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target									9.85	0.45
	E001	0.054	0.052	0.051	0.0523	0.0015	12.5	13	13.3	12.93	0.40
	E002	0.00	0.00	0.00	0.0000	0.0000	0.00	0.00	0.00	0.00	0.00
	E026	0.014	0.013	0.012	0.0130	0.0010	9.31	8.76	8.81	8.96	0.30
	E048	0.0078	0.0084	0.0082	0.0081	0.0003	9.5019	9.7163	9.5413	9.59	0.11
	E049						10.5	9.97	10.2	10.22	0.27
Community Results		Consensus Mean			0.0161		Consensus Mean			9.87	
		Consensus Standard Deviation			0.0091		Consensus Standard Deviation			1.20	
		Maximum			0.0523		Maximum			12.93	
		Minimum			0.0000		Minimum			0.00	
		N			4		N			5	

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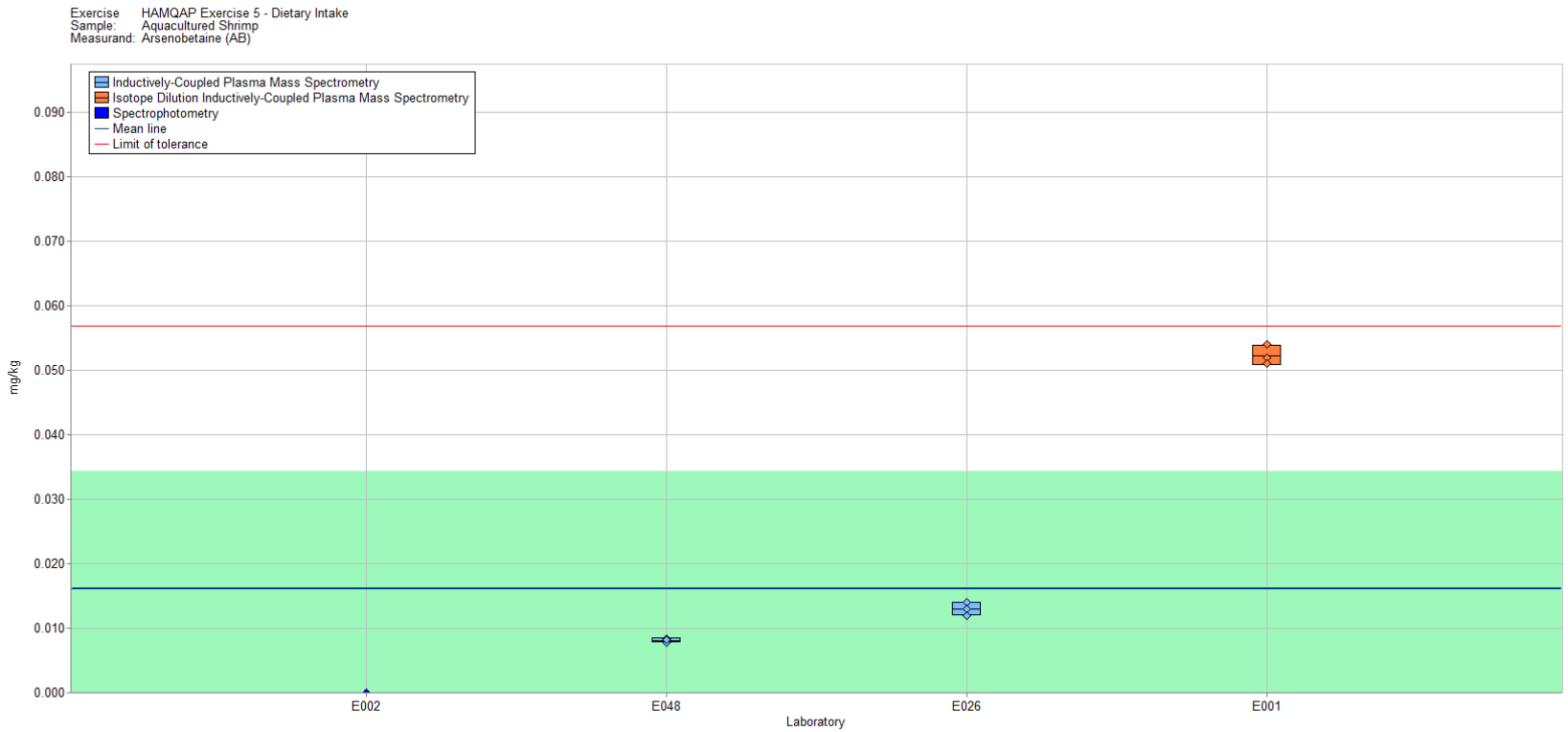

Figure 2-8. Arsenobetaine (AB) in Aquacultured Shrimp (data summary view – analytical method). In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid 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 consensus range of tolerance, calculated as the values above 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.

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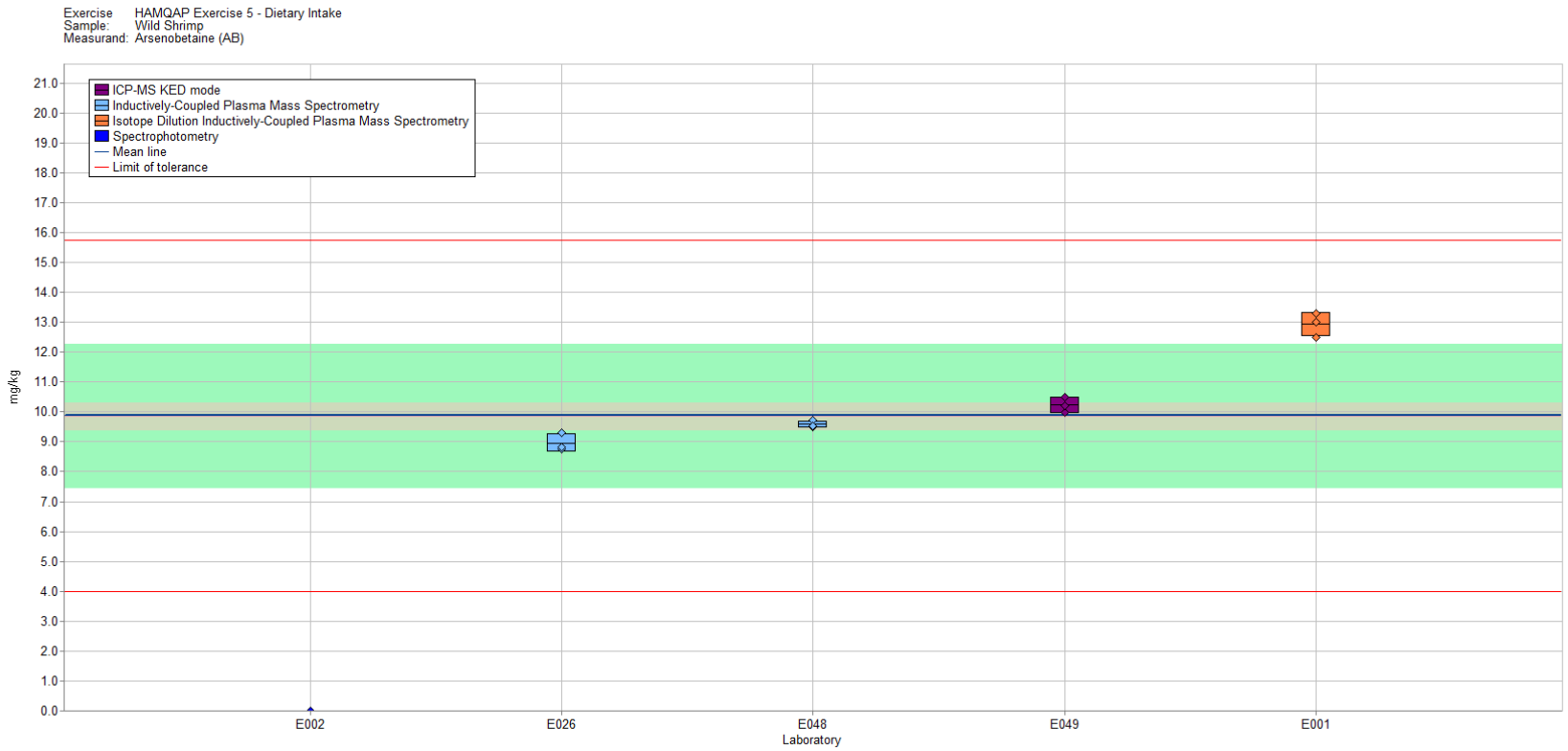

Figure 2-9. Arsenobetaine (AB) in Wild-Caught Shrimp (data summary view – analytical method). In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid 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, $|Z_{\text{NIST}}| \leq 2$.

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SECTION 3: WATER-SOLUBLE VITAMINS (B Vitamins)

Study Overview

In this study, participants were provided with samples of infant formula and multivitamin tablets for dietary intake. Participants were asked to use in-house analytical methods to determine the mass fraction (mg/kg) of thiamine, riboflavin, niacin, niacinamide, pantothenic acid, and pyridoxine in each matrix. Vitamins are nutrients required for specific and vital functions in the body and are crucial for maintaining optimal health. The various water-soluble B vitamins, for example, function as coenzymes in the metabolism of fatty acids, glucose, and proteins.[9,10,11,12,13] Specific B vitamins are critical for immune and nervous system function, DNA synthesis, and red blood cell formation. Most water-soluble vitamins are not substantially stored by the body, so humans require a continuous daily supply in the diet. Accurate measurement of water-soluble vitamins in foods provides confidence for both food labeling and dietary intake studies.

Dietary Intake Sample Information

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

Analyte	NIST-Determined Mass Fraction in Infant Formula A (mg/kg)
Thiamine (Vitamin B_1)	14.48 ± 0.17
Riboflavin (Vitamin B_2)	16.93 ± 0.36
Niacinamide (Vitamin B_3)	105.1 ± 49.0
Pantothenic Acid (Vitamin B_5)	72.6 ± 14.5
Pyridoxine (Vitamin B_6)	14.6 ± 1.1

[9] Thiamin Fact Sheet for Health Professionals. National Institutes of Health Office of Dietary Supplements. https://ods.od.nih.gov/factsheets/Thiamin-HealthProfessional/ (accessed July 15, 2020).
[10] Riboflavin Fact Sheet for Health Professionals. National Institutes of Health Office of Dietary Supplements. https://ods.od.nih.gov/factsheets/Riboflavin-HealthProfessional/ (accessed July 15, 2020).
[11] Niacin Fact Sheet for Health Professionals. National Institutes of Health Office of Dietary Supplements. https://ods.od.nih.gov/factsheets/Niacin-HealthProfessional/ (accessed July 15, 2020).
[12] Pantothenic Acid Fact Sheet for Health Professionals. National Institutes of Health Office of Dietary Supplements. https://ods.od.nih.gov/factsheets/PantothenicAcid-HealthProfessional/ (accessed July 15, 2020).
[13] Vitamin B6 Fact Sheet for Health Professionals. National Institutes of Health Office of Dietary Supplements. https://ods.od.nih.gov/factsheets/VitaminB6-HealthProfessional/ (accessed July 15, 2020).

Multivitamin. Participants were provided with three bottles, each containing 30 multivitamin tablets. Participants were asked to store the material at controlled room temperature, 20 °C to 25 °C, in the original unopened bottles and to prepare one sample and report one value from each bottle provided. Before use, participants were instructed to grind all 30 tablets 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. Approximate analyte levels were not reported to participants prior to the study. The NIST-determined values for thiamine, riboflavin, niacin, pantothenic acid, and pyridoxine in the multivitamin sample were assigned using results from the manufacturer of the material. The NIST-determined values and uncertainties for the B vitamins are provided in the table below on an as-received basis.

Analyte	NIST-Determined Mass Fraction in Multivitamin (mg/kg)		
Thiamine (Vitamin B_1)	1080	±	160
Riboflavin (Vitamin B_2)	1280	±	150
Niacin (Vitamin B_3)	12320	±	320
Pantothenic Acid (Vitamin B_5)	6910	±	1100
Pyridoxine (Vitamin B_6)	1334	±	54

Dietary Intake Study Results

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

Analyte	Number of Laboratories Requesting Samples	Number of Laboratories Reporting Results (Percent Participation)	
		Infant Formula A	Multivitamin
Thiamine (Vitamin B_1)	30	10 (33 %)	19 (63 %)
Riboflavin (Vitamin B_2)	29	11 (38 %)	18 (62 %)
Niacinamide (Vitamin B_3)	30	10 (33 %)	10 (33 %)
Niacin (Vitamin B_3)	30	5 (17 %)	15 (50 %)
Vitamin B_3	30	15 (50 %)	25 (83 %)
Pantothenic Acid (Vitamin B_5)	28	11 (39 %)	16 (57 %)
Pyridoxine (Vitamin B_6)	20	9 (45 %)	15 (75 %)

- In the infant formula sample, the consensus means for thiamine and riboflavin were slightly below the target ranges, while the consensus means for niacinamide and pantothenic acid overlapped the target range, and the consensus mean for pyridoxine was slightly below the target range.
- In the multivitamin sample, the consensus means for thiamine and pyridoxine were slightly above the target range while the consensus means for riboflavin, niacin, and pantothenic acid overlapped the target ranges.

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- The between-laboratory variabilities were excellent or very good for all vitamins expected to be in the samples (see table below). High between-laboratory variability was only observed for vitamins not expected to be present in the samples, including niacinamide in the multivitamin (100 % RSD) and niacin in the infant formula (63 % RSD).

	Between-Laboratory Variability (% RSD)	
Analyte	Infant Formula A	Multivitamin
Thiamine (Vitamin B_1)	9 %	5 %
Riboflavin (Vitamin B_2)	9 %	4 %
Niacinamide (Vitamin B_3)	7 %	100 %
Niacin (Vitamin B_3)	63 %	2 %
Pantothenic Acid (Vitamin B_5)	3 %	2 %
Pyridoxine (Vitamin B_6)	8 %	2 %

- Most laboratories reported using solvent extraction or dilution for sample preparation in the determination of all analytes (see table below).

	Reported Analytical Method Number (Percent) Reporting					
	Infant Formula A			Multivitamin		
Analyte	Solvent Extraction	Dilution	Acid Hydrolysis	Solvent Extraction	Dilution	Open Beaker Digestion
Thiamine (Vitamin B_1)	7 (70 %)	2 (20 %)	1 (10 %)	12 (63 %)	6 (32 %)	1 (5 %)
Riboflavin (Vitamin B_2)	7 (64 %)	3 (27 %)	1 (9 %)	12 (67 %)	5 (28 %)	1 (6 %)
Niacinamide (Vitamin B_3)	7 (70 %)	2 (20 %)	1 (10 %)	7 (70 %)	2 (20 %)	1 (10 %)
Niacin (Vitamin B_3)	3 (60 %)	1 (20 %)	1 (20 %)	10 (67 %)	5 (33 %)	--
Pantothenic Acid (Vitamin B_5)	7 (64 %)	3 (27 %)	1 (9 %)*	12 (75 %)	3 (19 %)	1 (6 %)
Pyridoxine (Vitamin B_6)	6 (67 %)	2 (22 %)	1 (11 %)	11 (73 %)	4 (27 %)	--

*This laboratory reported using enzymatic hydrolysis.

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- Most laboratories reported using LC-Abs for determination of all analytes (see table below).

	Reported Analytical Method Number (Percent) Reporting			
	Infant Formula A		Multivitamin	
Analyte	LC-Abs	LC-MS or LC-MS/MS	LC-Abs	LC-MS or LC-MS/MS
Thiamine (Vitamin B_1)	6 (33 %)	4 (63 %)	18 (95 %)	1 (5 %)
Riboflavin (Vitamin B_2)	7 (63 %)	4 (36 %)	17 (94 %)	1 (6 %)
Niacinamide (Vitamin B_3)	6 (60 %)	4 (40 %)	9 (90 %)	1 (10 %)
Niacin (Vitamin B_3)	4 (80 %)	1 (20 %)	14 (93 %)	1 (7 %)
Pantothenic Acid (Vitamin B_5)	6 (55 %)	4 (36 %)	13 (81%)	3 (19 %)
Pyridoxine (Vitamin B_6)	6 (67 %)	3 (33 %)	14 (93 %)	1 (7 %)

Dietary Intake Technical Recommendations

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

- For thiamine (vitamin B_1), the consensus mean was very close to the target range in the infant formula, but the consensus range was significantly larger than the target range (**Figure 3-1** and **Figure 3-3**). In the multivitamin sample, the consensus range was more comparable in size to the target range but also somewhat higher (**Figure 3-2** and **Figure 3-4**).
  - In the infant formula sample, laboratories using dilution as a sample preparation approach for thiamine analysis reported results that were lower than the consensus and target values (**Figure 3-1**).
  - In the multivitamin sample, laboratories using dilution as a sample preparation approach for thiamine analysis reported results that were more likely to be higher or lower than the consensus and target ranges than within those ranges (**Figure 3-2**).
  - These trends indicate that a more robust sample preparation approach may be required for accurate determination of thiamine in these matrices.
  - No additional trends were noted for other sample preparation techniques or analytical methods.
- For riboflavin (vitamin B_2), the consensus mean was below the target range in the infant formula, but the target range did overlap the higher end of the consensus range (**Figure 3-6** and **Figure 3-8**). In the multivitamin sample, the consensus mean overlapped the higher end of the target range (**Figure 3-7** and **Figure 3-9**). No trends were noted based on the reported sample preparation techniques or analytical methods.
- Each sample contained a different form of vitamin B_3, with infant formula containing only niacinamide and the multivitamin containing only niacin. The consensus mean and range for niacinamide in the infant formula overlapped the target range (**Figure 3-11** and **Figure 3-13**). In the multivitamin sample, the consensus range for niacin overlapped the target range (**Figure 3-16** and **Figure 3-18**).
  - In the infant formula sample, laboratories using dilution as a sample preparation approach for niacinamide analysis reported results that were higher than the consensus and target values (**Figure 3-11**). No trends were noted for other sample preparation techniques or analytical methods.

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- In the multivitamin sample, no trends were noted based on sample preparation techniques or analytical methods.
- Several laboratories reported results for niacin in the infant formula sample, a compound that was not included in the sample formulation (**Figure 3-15** and **Figure 3-17**). One laboratory reported values comparable to the level of niacinamide in the sample, indicating that the analyte may have been misidentified.
- Several laboratories reported results for niacinamide in the multivitamin sample, a compound that was not included in the sample formulation (**Figure 3-12** and **Figure 3-14**). Two of these laboratories reported values comparable to the level of niacin in the sample, indicating that the analyte may have been misidentified.
- Chromatographic peak identity should always be confirmed using appropriate reference standards, especially for niacin and niacinamide which are very similar in structure and molecular mass (differing by only 1 mass unit). Analytical methods should be able to clearly distinguish between these two analytes to be effective in nutrient determination.

- For pantothenic acid (vitamin B_5), the consensus means overlapped the target ranges for both samples (**Figure 3-19** through **Figure 3-22**).
  - In both samples, laboratories using dilution as a sample preparation approach for pantothenic acid analysis reported results that were more likely to be higher or lower than the consensus and target ranges than within those ranges. This trend indicates that a more robust sample preparation approach may be required for accurate determination of pantothenic acid in these matrices.
  - No additional trends were noted based on the reported sample preparation techniques or analytical methods.
- For pyridoxine (vitamin B_6), the consensus means overlapped the target ranges but were above the target ranges for both samples (**Figure 3-24** through **Figure 3-27**).
  - In the infant formula sample, laboratories using dilution as a sample preparation approach for pyridoxine analysis reported results that were more likely to be higher than the consensus and target ranges than within those ranges. This trend indicates that a more robust sample preparation approach may be required for accurate determination of pyridoxine in infant formula.
  - No additional trends were noted based on the reported sample preparation techniques or analytical methods.
- Prior to subsampling for analysis, the entire bottle of multivitamin tablets must be properly ground and homogenized. This practice helps reduce variability due to between-tablet differences and improves repeatability.
- Several of the vitamins (e.g., thiamine, riboflavin, pantothenic acid) may decompose in light, therefore samples and standards should be prepared under amber or attenuated lighting.
- Calculations and reporting units must be verified prior to submission of results. Laboratories often report results in the wrong units or forget a dilution factor during the calculation of the final results, resulting in poor performance on the study.
- The use of appropriate calibration materials and quality assurance samples to establish that a method is in control and being performed correctly may reduce the likelihood of outlying data. Quality assurance samples can be commercially available reference materials (CRMs, SRMs, or RMs) or materials prepared in-house.

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

National Institute of Standards & Technology

HAMQAP Exercise 5 - 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*
Thiamine (Vitamin B_1)	Infant Formula A	mg/kg		14.48	0.17			10	14.1	1.3	14.48	0.17
Thiamine (Vitamin B_1)	Multivitamin	mg/kg		1080	160			19	1240	64	1080	160
Riboflavin (Vitamin B_2)	Infant Formula A	mg/kg		16.93	0.36			11	14.8	1.4	16.93	0.36
Riboflavin (Vitamin B_2)	Multivitamin	mg/kg		1280	150			18	1330	58	1280	150
Niacinamide (Vitamin B_3)	Infant Formula A	mg/kg		105.1	49			10	116	7.9	105.1	49
Niacinamide (Vitamin B_3)	Multivitamin	mg/kg						10	130	130		
Niacin (Vitamin B_3)	Infant Formula A	mg/kg						5	240	150		
Niacin (Vitamin B_3)	Multivitamin	mg/kg		12320	320			15	12200	220	12320	320
Pantothenic Acid (Vitamin B_5)	Infant Formula A	mg/kg		72.6	14.5			11	72.8	2.3	72.6	14.5
Pantothenic Acid (Vitamin B_5)	Multivitamin	mg/kg		6910	1100			16	6990	130	6910	1100
Pyridoxine (Vitamin B_6)	Infant Formula A	mg/kg		14.6	1.1			9	15.8	1.2	14.6	1.1
Pyridoxine (Vitamin B_6)	Multivitamin	mg/kg		1334	54			15	1370	24	1334	54

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 thiamine (vitamin B_1) 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.

		Thiamine (Vitamin B_1)									
		Infant Formula A (mg/kg)					Multivitamin (mg/kg)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target				14.48	0.17				1080	160
	E001						1540	1550	1640	1577	55
	E002	16.221	16.19	16.214	16.21	0.02	1374.38	1485.95	1457.12	1439	58
	E003										
	E004						1171	1148	1153	1157	12
	E005	14	14	14	14.00	0.00	1280	1240	1250	1257	21
	E006						1440	1340	1420	1400	53
	E007	15.09	14.43	14.41	14.64	0.39	1440	1450	1421	1437	15
	E008										
	E010	18.6618	18.2415	18.4394	18.45	0.21	1366.2	1327.8	1358	1351	20
	E011										
	E012	3.75	3.68	3.4	3.61	0.19	2.41	2.4	2.5	2	0
	E013						740	765	739	748	15
	E015										
	E016						872.3	905.3	821.4	866	42
	E020										
	E021										
	E023	13.3	13.6	13.9	13.60	0.30	1090	1010	1060	1053	40
	E025						1179	1118	1158	1152	31
	E030	16.8	18.7	22.8	19.43	3.07	1236	1298	1291	1275	34
	E031										
	E033						730	740		735	7
	E035										
	E037										
	E040	9.9	10.4	7.7	9.33	1.44	1219.9	1204.7	1207.8	1211	8
	E041	14.3	15.2	14.5	14.67	0.47	1315	1330	1340	1328	13
	E042	12.53	12.726	12.503	12.59	0.12					
	E043						1294.8	1252.8	1295.7	1281	25
	E044						1167.2	1297	1308.7	1258	79
	E046						1885	1906	1885	1892	12
	E047										
	E072										
Community Results		Consensus Mean			14.10		Consensus Mean			1240	
		Consensus Standard Deviation			1.27		Consensus Standard Deviation			64	
		Maximum			19.43		Maximum			1892	
		Minimum			3.61		Minimum			2	
		N			10		N			19	

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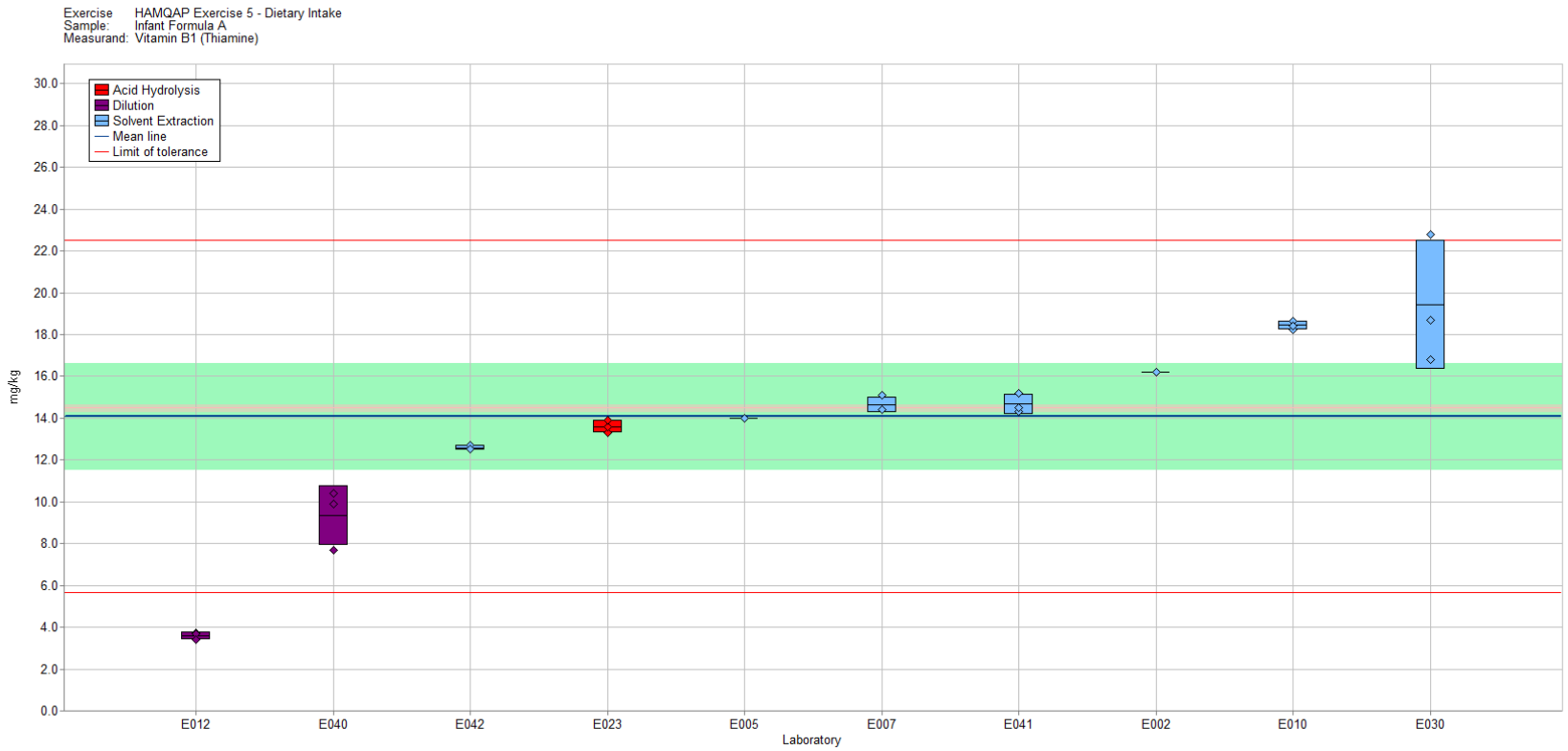

Figure 3-1. Thiamine (vitamin B_1) in Infant Formula A (data summary view – sample preparation method). In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. 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, $|Z_{\text{NIST}}| \leq 2$.

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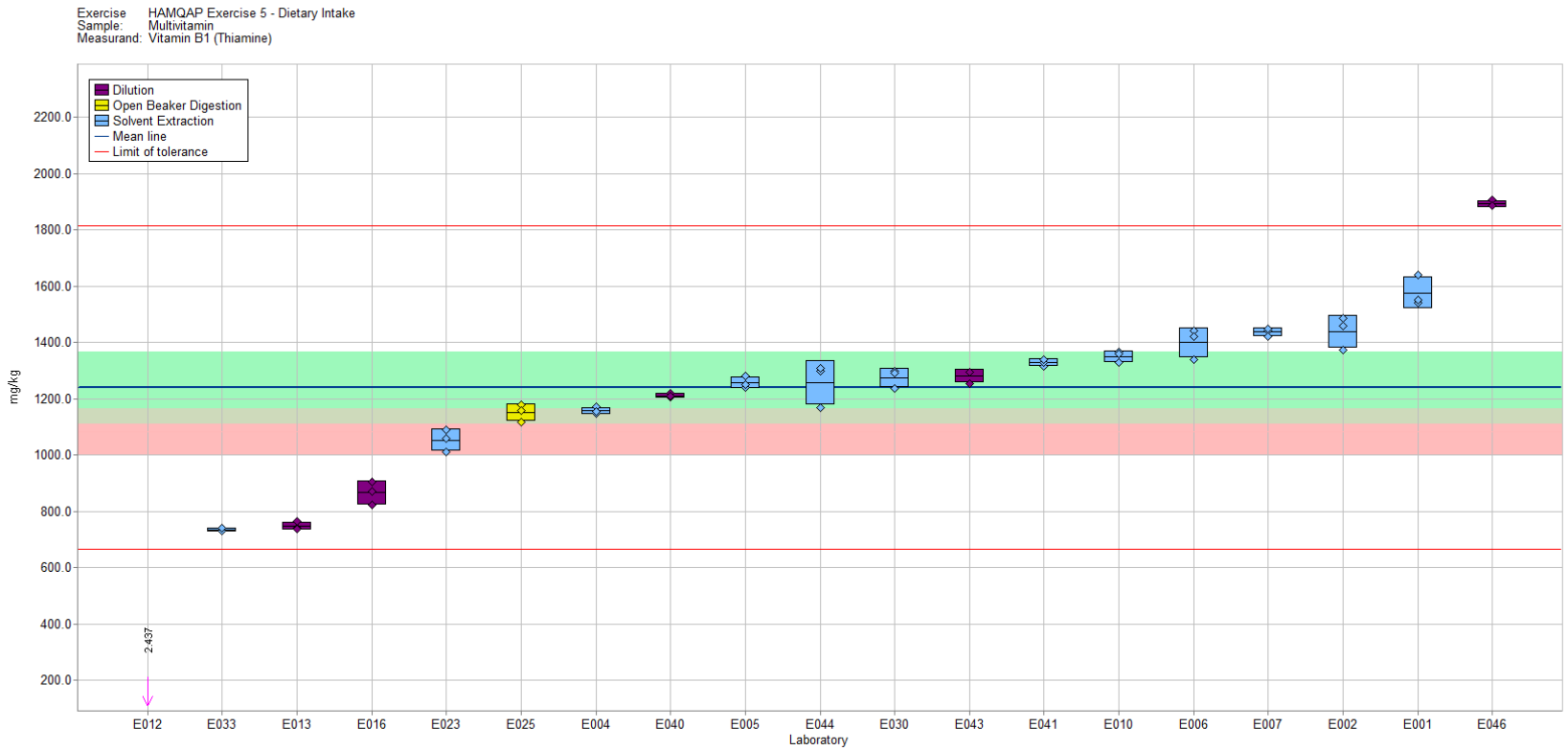

Figure 3-2. Thiamine (vitamin B_1) in Multivitamin (data summary view – sample preparation method). In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. 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, $|Z_{\text{NIST}}| \leq 2$.

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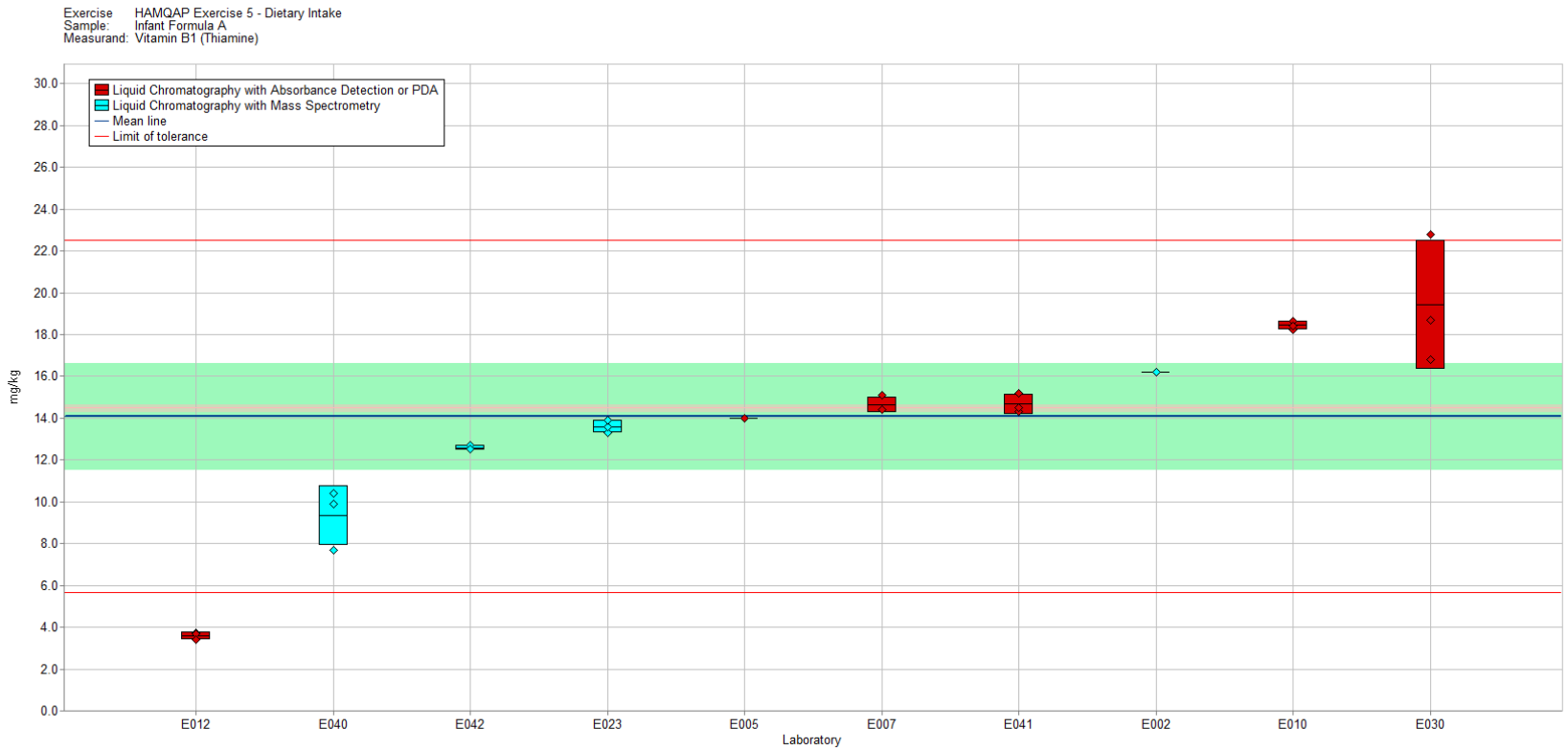

Figure 3-3. Thiamine (vitamin B1) in Infant Formula A (data summary view – analytical method). In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid 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, $|Z_{\text{NIST}}| \leq 2$.

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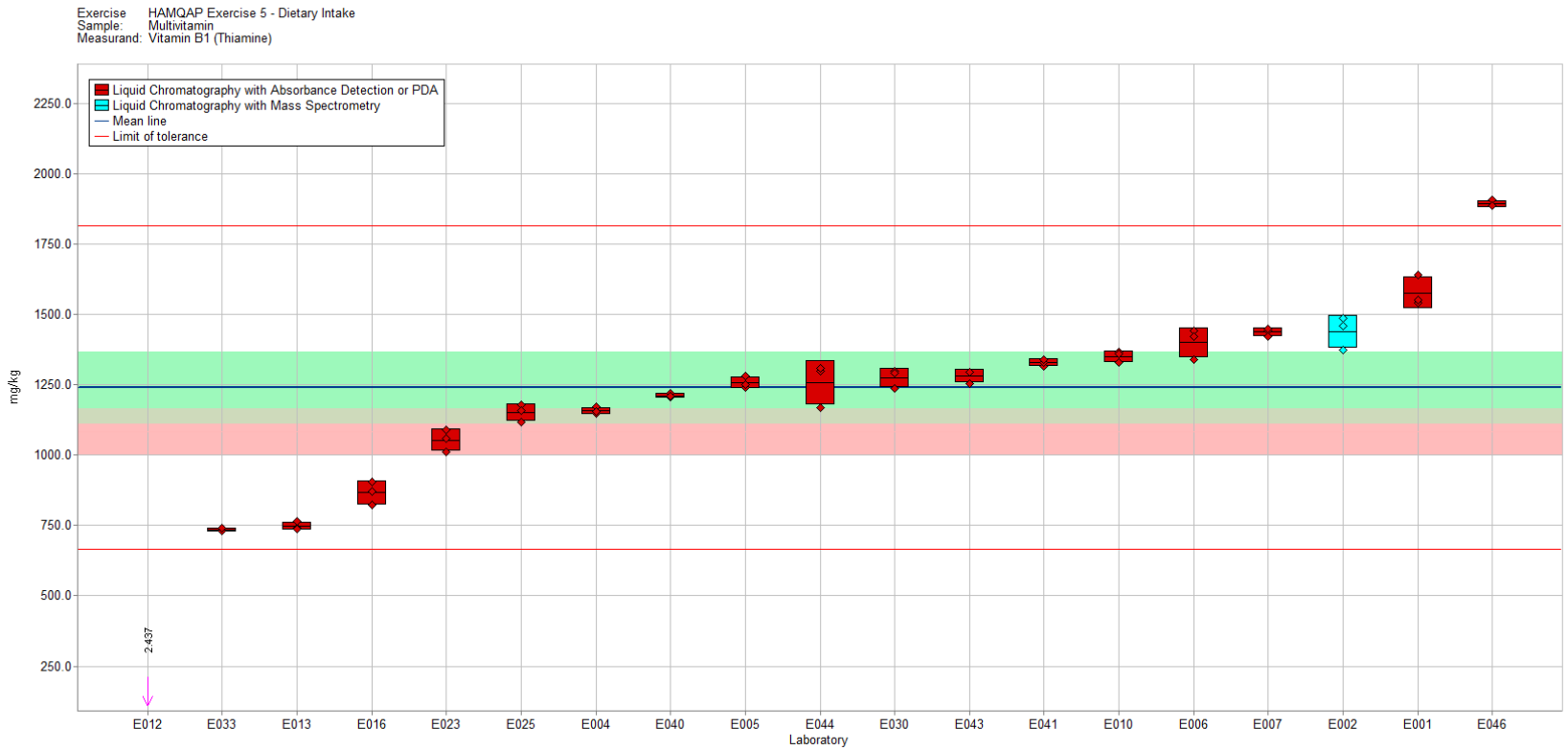

Figure 3-4. Thiamine (vitamin B_1) in Multivitamin (data summary view – analytical method). In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue 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, $|Z_{\text{NIST}}| \leq 2$.

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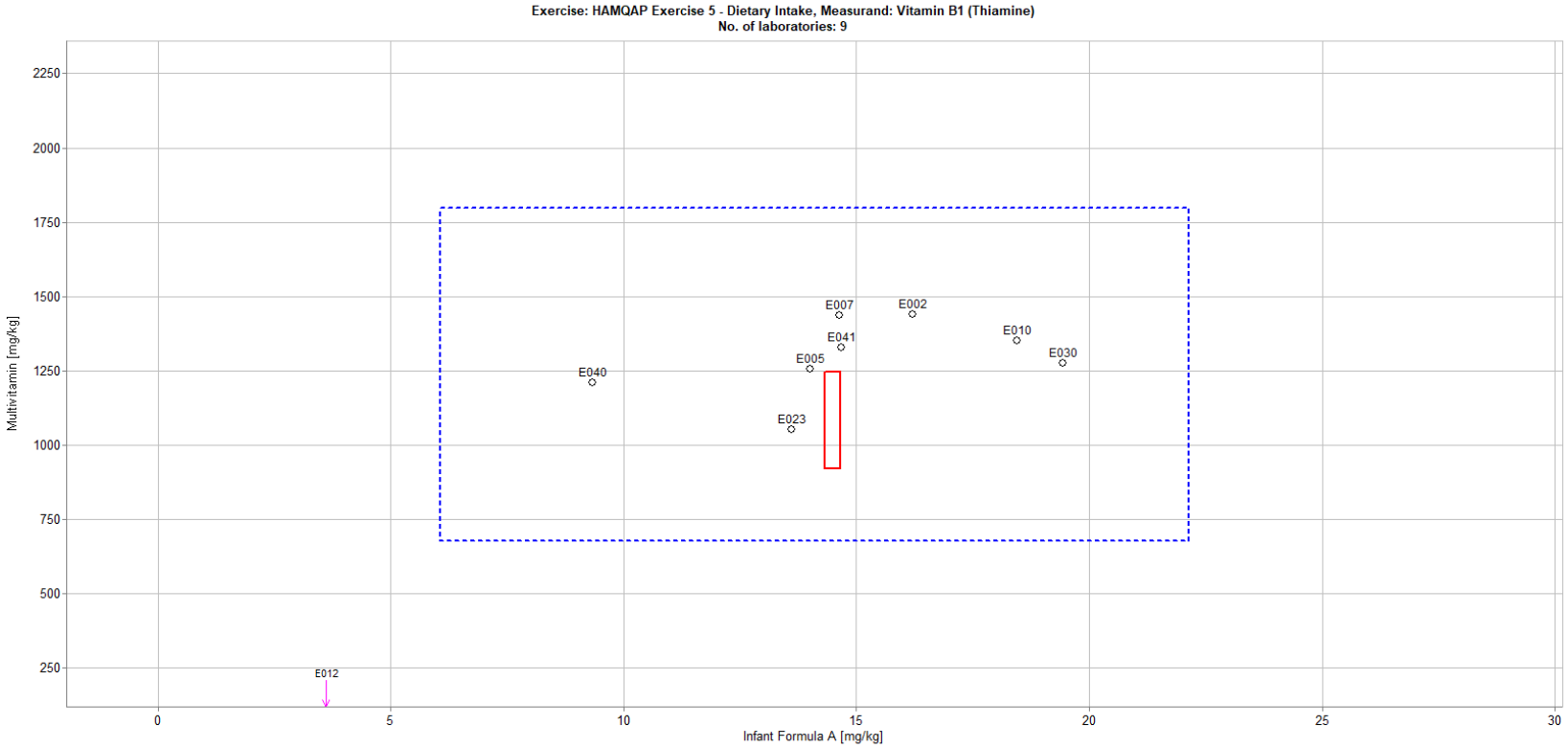

Figure 3-5. Laboratory means for thiamine (vitamin B_1) in Infant Formula A and Multivitamin (sample/sample comparison view). In this view, the individual laboratory mean for one sample (infant formula) is compared to the individual laboratory mean for a second sample (multivitamin). The solid red box represents the NIST range of tolerance for the two samples, infant formula (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 infant formula (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 riboflavin (vitamin B_2) 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.

		Riboflavin (Vitamin B_2)									
		Infant Formula A (mg/kg)					Multivitamin (mg/kg)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target				16.93	0.36				1280	150
	E001						1430	1490	1530	1483	50
	E002	16.374	16.96	16.39	16.57	0.33	1383.05	1359.2	1328.52	1357	27
	E003										
	E004						1390	1388	1381	1386	5
	E005	20	20	20	20.00	0.00	1650	1620	1620	1630	17
	E006										
	E007						1565	1594	1589	1583	16
	E008										
	E010	17.8556	17.7909	17.9923	17.88	0.10	1567.4	1494.1	1503.6	1522	40
	E011										
	E012	3.66	3.45	3.39	3.50	0.14	4.12	4.19	4.3	4	0
	E013	< 20.0	< 20.0	< 20.0	< 20.0		1350	1390	1370	1370	20
	E015										
	E016						622	619.6	605.9	616	9
	E020										
	E021										
	E023	15.2	15.3	15.4	15.30	0.10	1240	1280	1240	1253	23
	E025						930.5	933.5	875.8	913	32
	E030	16.3	18.7	16.9	17.30	1.25	1271	1243	1262	1259	14
	E031										
	E033						970	1050		1010	57
	E035										
	E037										
	E040	19.7	15.5	18	17.73	2.11	1433.4	1433.3	1325.4	1397	62
	E041	12.6	12	10.8	11.80	0.92	1350	1348	1362	1353	8
	E042	5.345	5.117	5.245	5.24	0.11					
	E043						1482.2	1515.8	1486.3	1495	18
	E044						1323.4	1331.6	1394.8	1350	39
	E047	15	15	15	15.00	0.00	1200	1210	1180	1197	15
	E072										
Community Results		Consensus Mean			14.82		Consensus Mean			1327	
		Consensus Standard Deviation			1.37		Consensus Standard Deviation			58	
		Maximum			20.00		Maximum			1630	
		Minimum			3.50		Minimum			4	
		N			11		N			18	

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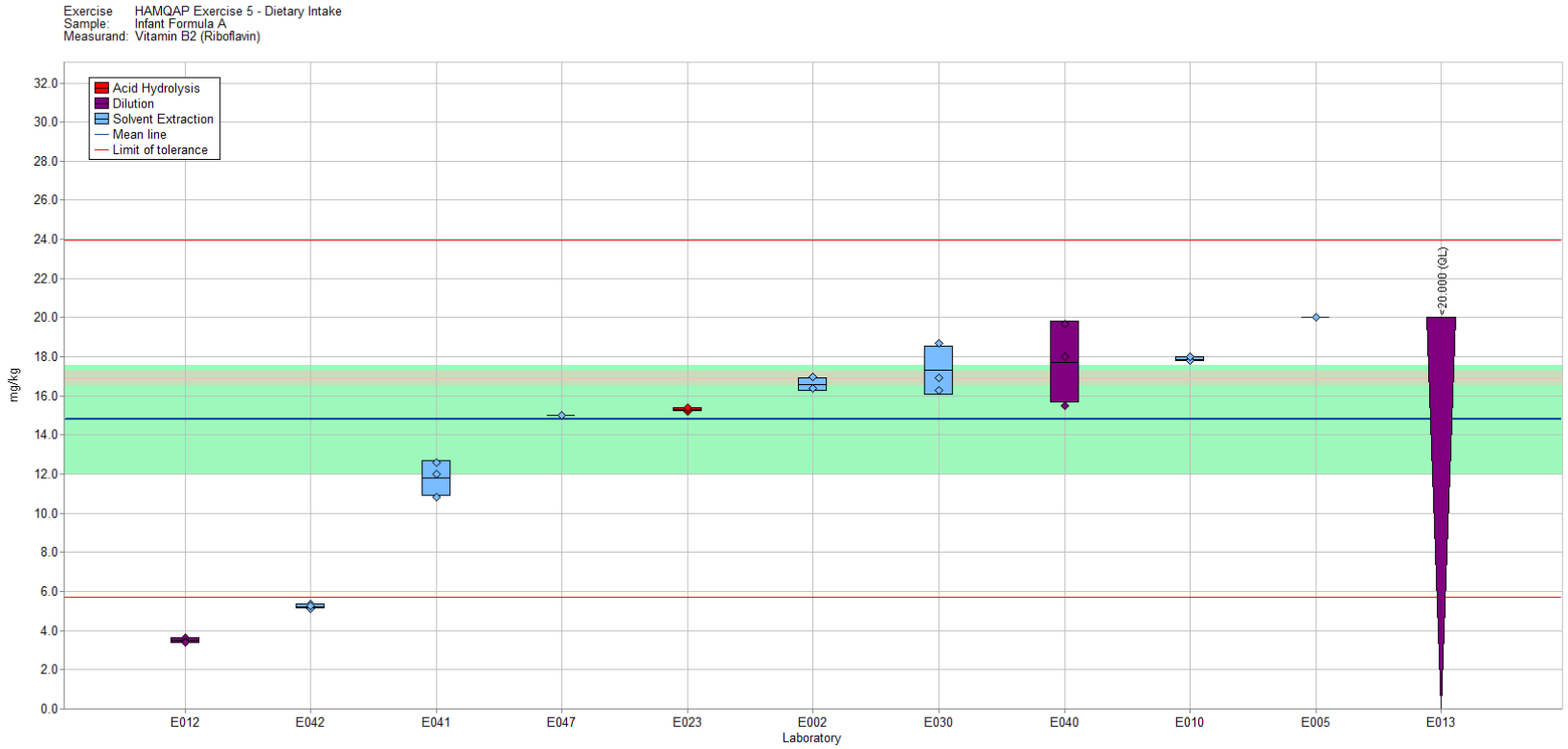

Figure 3-6. Riboflavin (vitamin B_2) in Infant Formula A (data summary view – sample preparation method). In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method 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, $|Z_{\text{NIST}}| \leq 2$.

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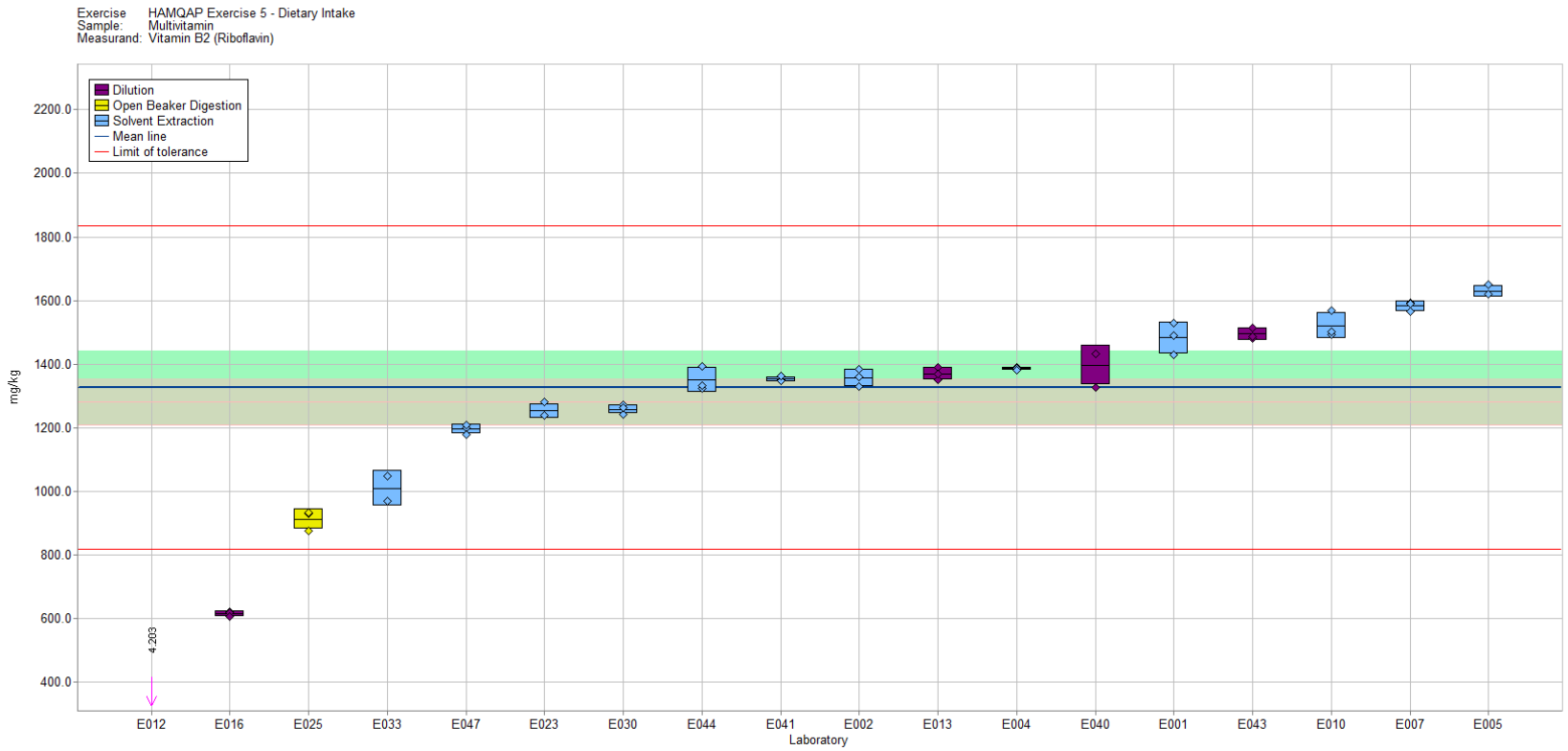

Figure 3-7. Riboflavin (vitamin B_2) in Multivitamin (data summary view – sample preparation method). In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. 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, $|Z_{\text{NIST}}| \leq 2$.

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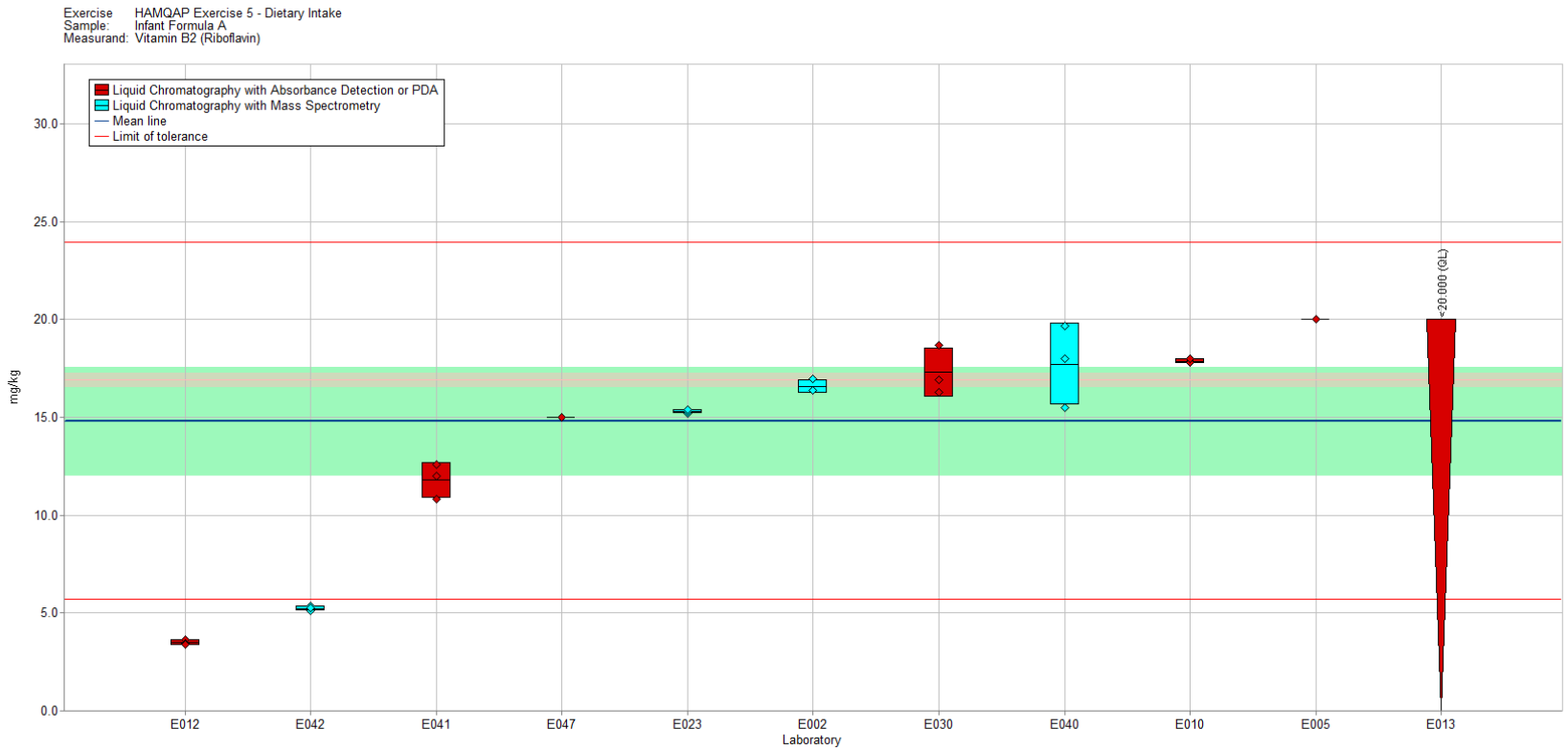

Figure 3-8. Riboflavin (vitamin B2) in Infant Formula A (data summary view – analytical method). In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid 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, $|Z_{\text{NIST}}| \leq 2$.

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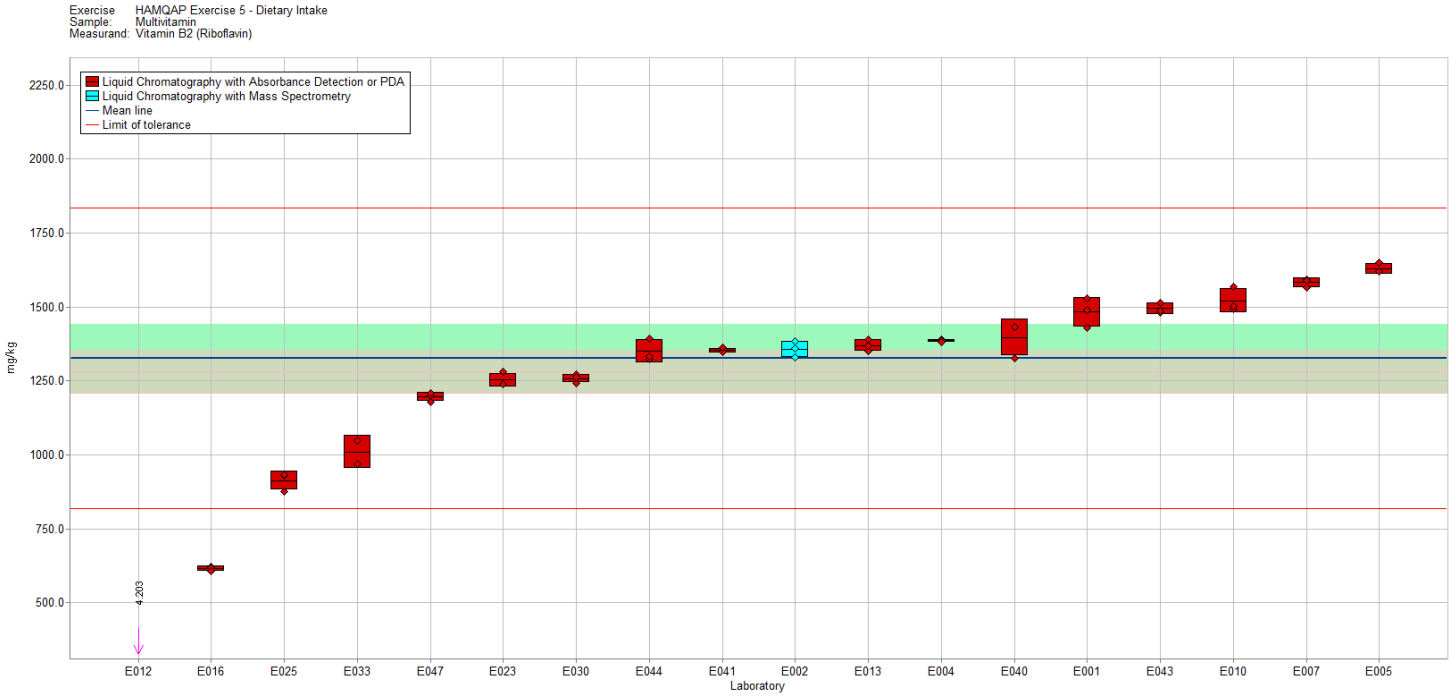

Figure 3-9. Riboflavin (vitamin B_2) in Multivitamin (data summary view – analytical method). In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid 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, $|Z_{\text{NIST}}| \leq 2$.

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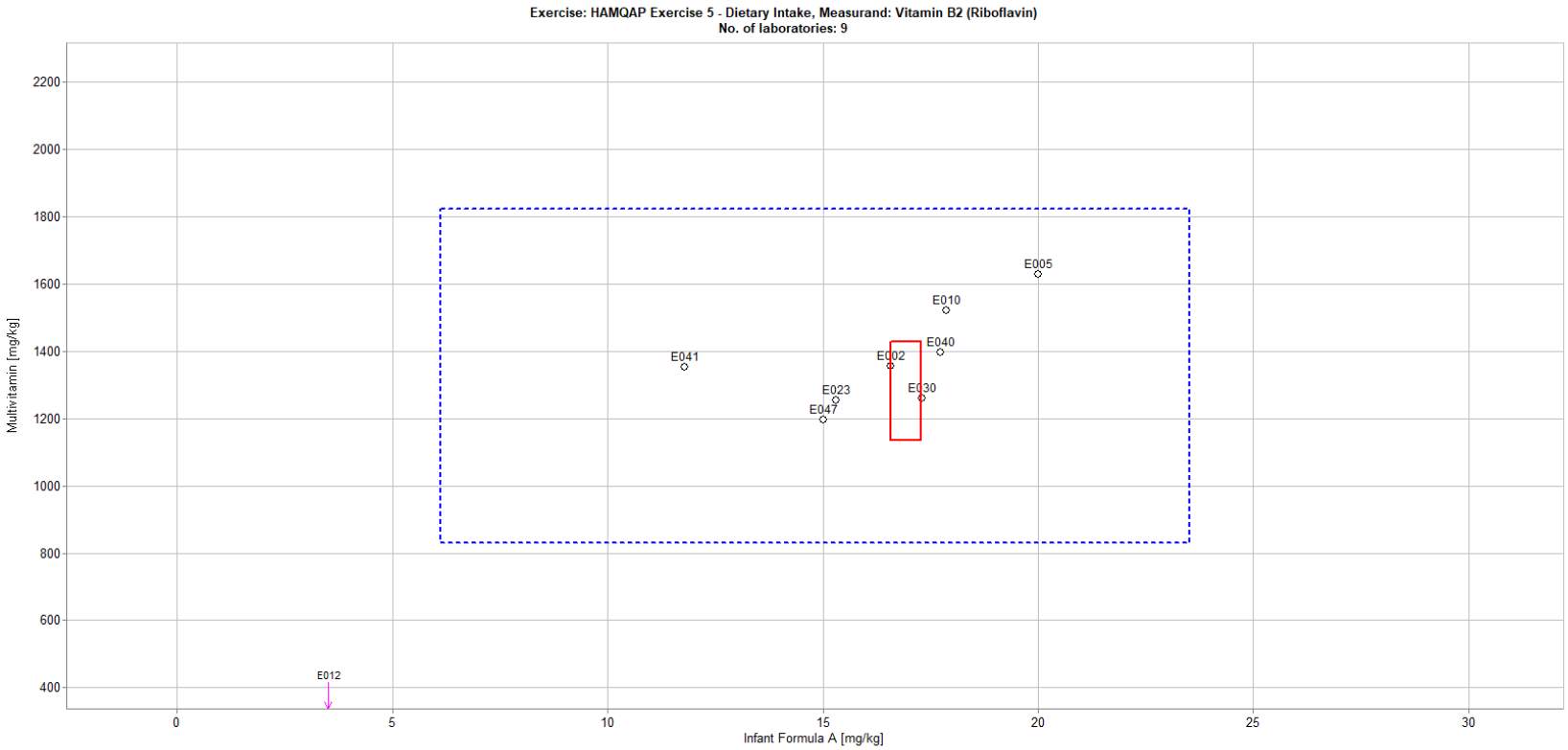

Figure 3-10. Laboratory means for riboflavin (vitamin B_2) in Infant Formula A and Multivitamin (sample/sample comparison view). In this view, the individual laboratory mean for one sample (infant formula) is compared to the individual laboratory mean for a second sample (multivitamin). The solid red box represents the NIST range of tolerance for the two samples, infant formula (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 infant formula (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 3-4. Data summary table for niacinamide (vitamin B_3) 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.

		Niacinamide (Vitamin B_3)									
		Infant Formula A (mg/kg)					Multivitamin (mg/kg)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target				105.1	49.0					
	E001										
	E002	112.03	110.65	111.99	111.6	0.8	269	220	235	241	25
	E003										
	E004										
	E005	100	101	101	100.7	0.6	12190	12150	12420	12253	146
	E006										
	E007	99.95	97.79	97.89	98.5	1.2	122	126	115	121	6
	E008										
	E010	111.3156	111.0948	110.8816	111.1	0.2					
	E011										
	E012										
	E013	< 200	< 200	< 200	< 200		< 200	< 200	< 200	< 200	
	E015										
	E016						2336	2141	2543	2340	201
	E020										
	E021										
	E023	112	112	110	111.3	1.2	< 500	< 500	< 500	< 500	
	E025						75.12	75.87	80.54	77	3
	E030	126	139	127	130.7	7.2	< 10.0	< 10.0	< 10.0	< 10.0	
	E031										
	E033						90	90		90	0
	E035										
	E037										
	E040	165	140.4	168.3	157.9	15.2					
	E041										
	E042	110.2	114.8	114.6	113.2	2.6					
	E043										
	E044										
	E046										
	E047	340	340	350	343.3	5.8	11550	11360	11370	11427	107
	E072										
Community Results		Consensus Mean			116.1		Consensus Mean			132	
		Consensus Standard Deviation			7.9		Consensus Standard Deviation			134	
		Maximum			343.3		Maximum			12253	
		Minimum			98.5		Minimum			77	
		N			10		N			10	

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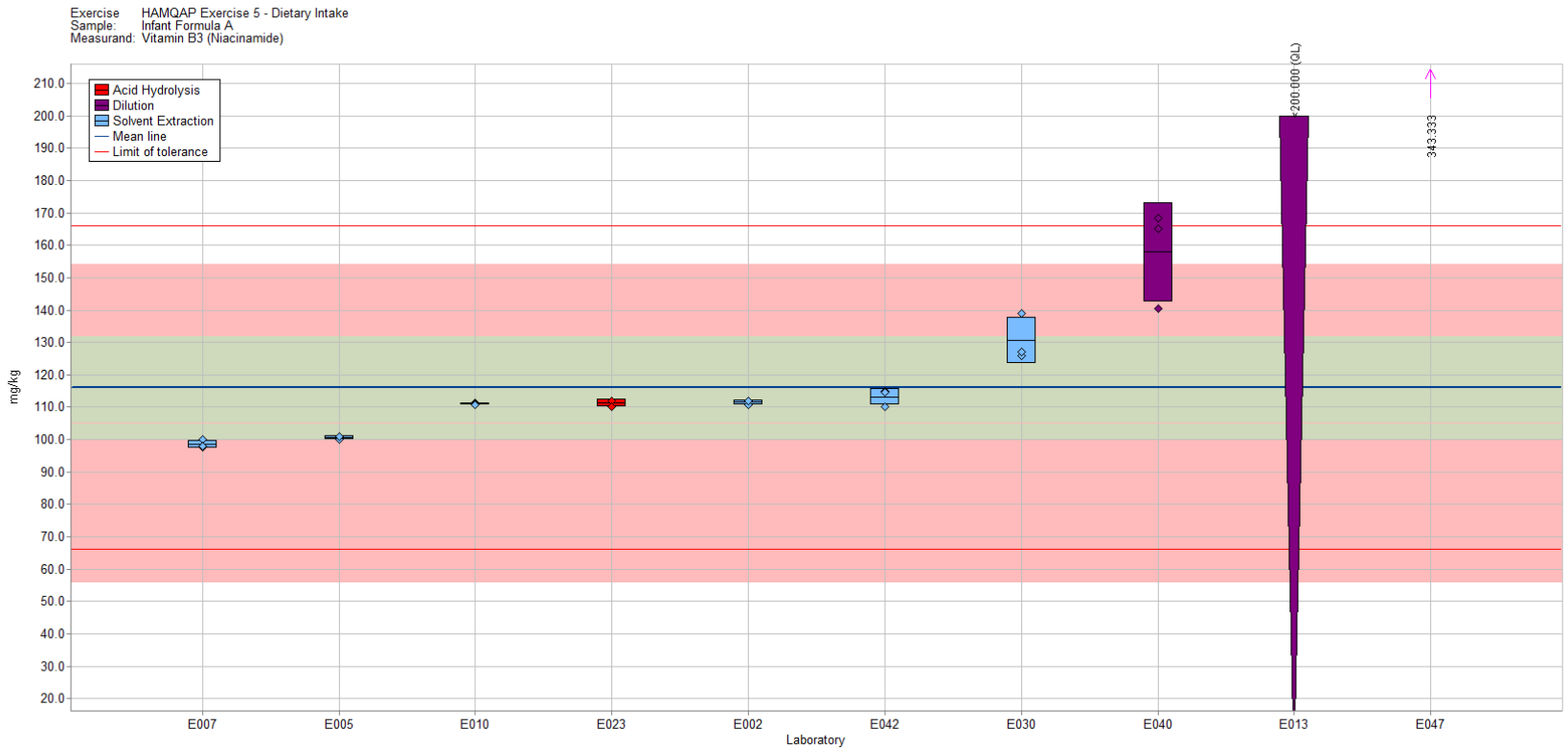

Figure 3-11. Niacinamide (vitamin B_3) in Infant Formula A (data summary view – sample preparation method). In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method 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, $|Z_{\text{NIST}}| \leq 2$.

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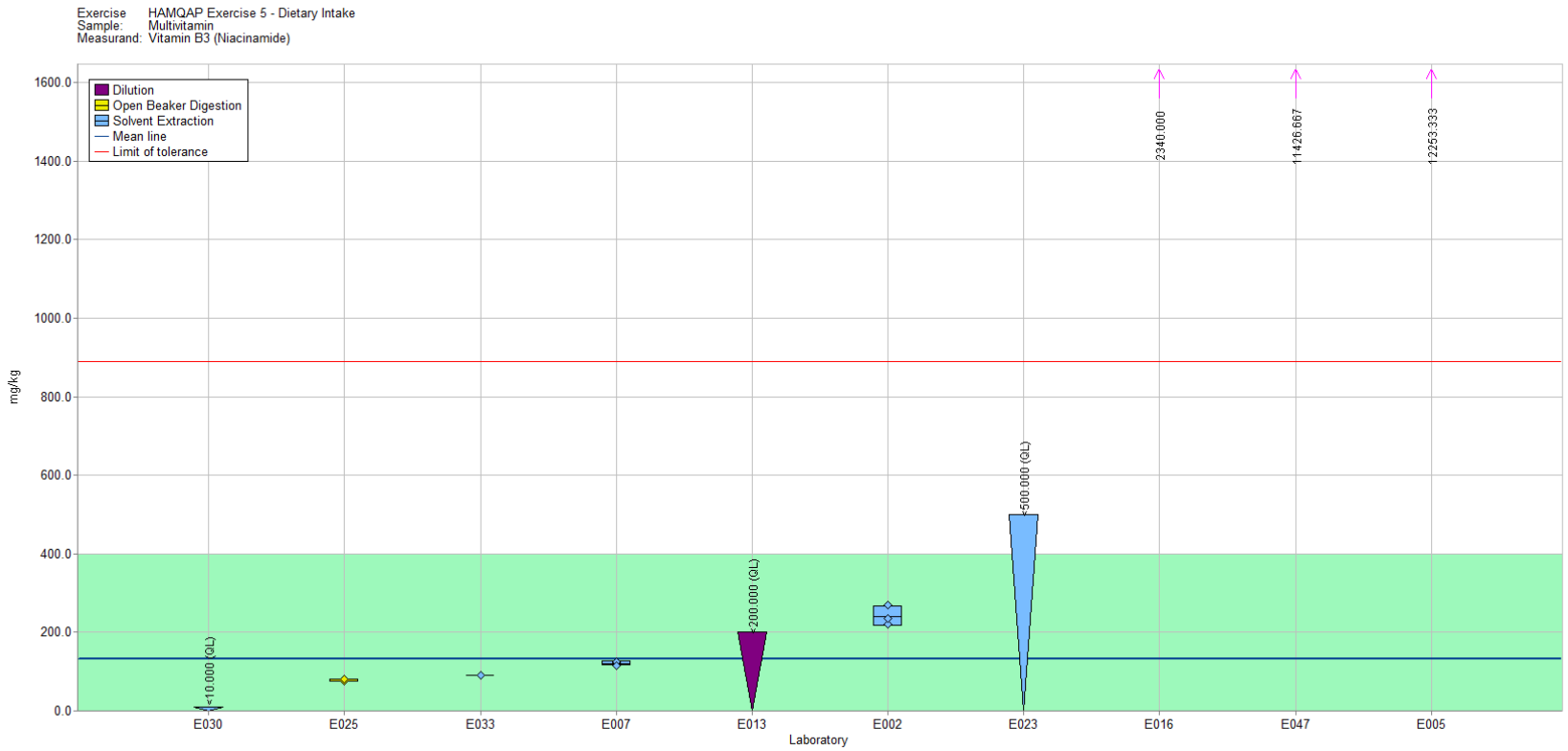

Figure 3-12. Niacinamide (vitamin B_3) in Multivitamin (data summary view – sample preparation method). In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red line represents the upper consensus range of tolerance, calculated as the values above the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$ with the lower limit set at zero. A target value for niacinamide has not been determined in this material.

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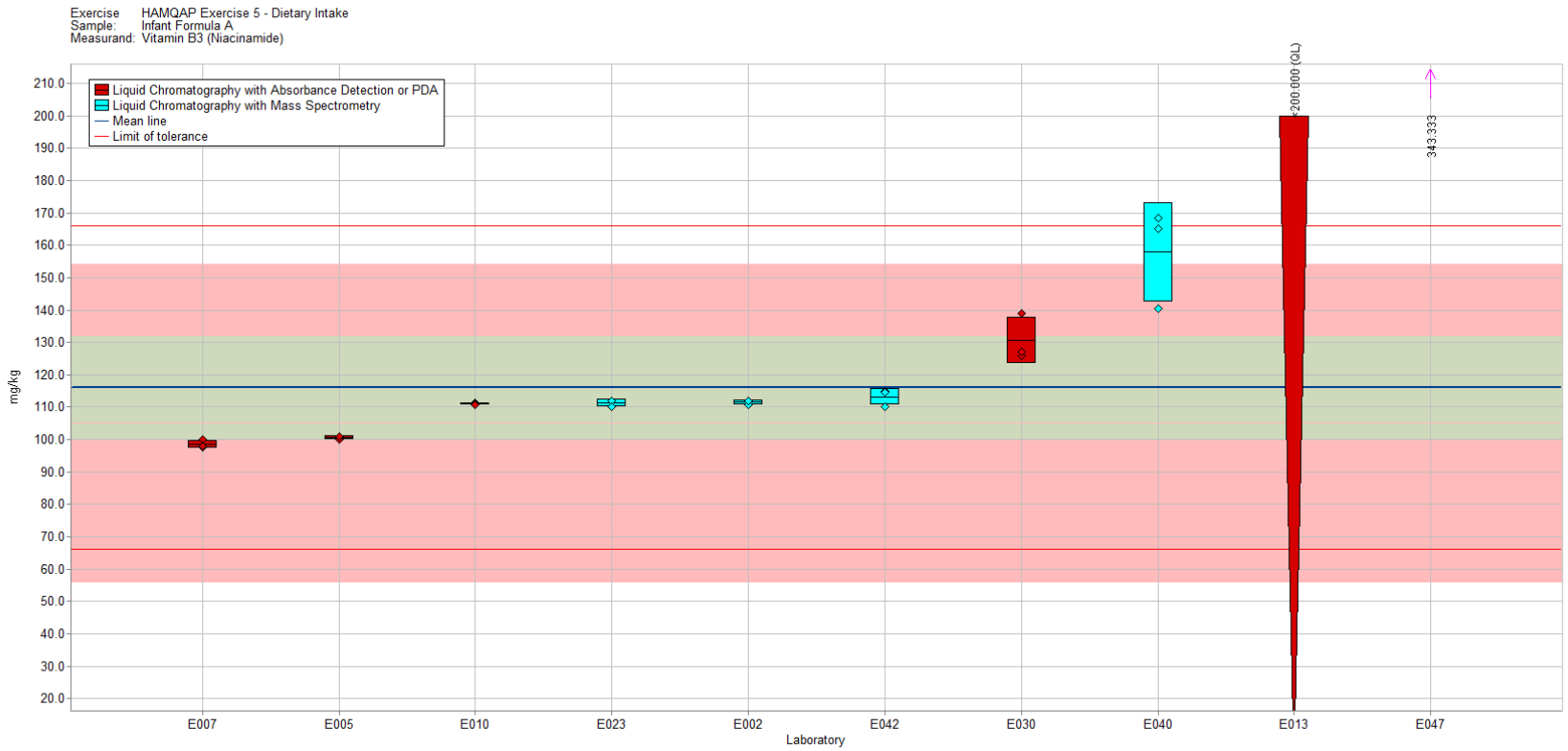

Figure 3-13. Niacinamide (vitamin B_3) in Infant Formula A (data summary view – analytical method). In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The 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, $|Z_{\text{NIST}}| \leq 2$.

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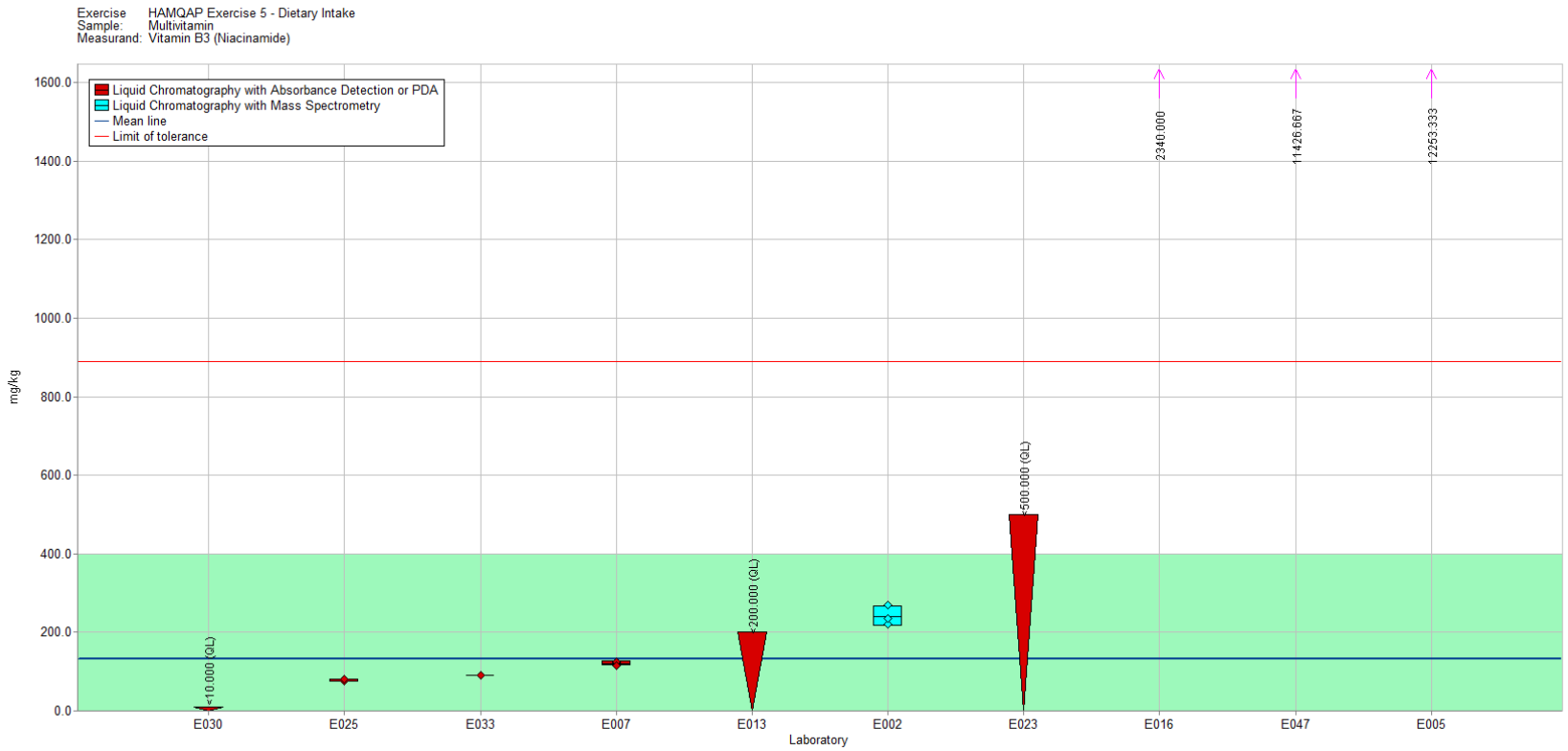

Figure 3-14. Niacinamide (vitamin B_3) in Multivitamin (data summary view – analytical method). In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red line represents the upper consensus range of tolerance, calculated as the values above the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$ with the lower limit set at zero. A target value for niacinamide has not been determined in this material.

Table 3-5. Data summary table for niacin (vitamin B_3) 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.

		Niacin (Vitamin B_3)									
		Infant Formula A (mg/kg)					Multivitamin (mg/kg)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target									12320	320
	E001						11200	11300	11000	11167	153
	E002	265.48	267.22	270.16	268	2	12358.03	12369.52	12360.94	12363	6
	E003										
	E004						12752	12709	12693	12718	31
	E005										
	E006						12500	13100	12400	12667	379
	E007	509	497	482	496	14	12309	12143	12385	12279	124
	E008										
	E010						13320	13009.6	13194.1	13175	156
	E011										
	E012	4.88	4.5	4.45	5	0	6.45	6.5	6.71	7	0
	E013										
	E014										
	E015										
	E016						877.4	808.3	843.9	843	35
	E019										
	E020										
	E021										
	E023	112	112	110	111	1					
	E025										
	E027										
	E030						12186	12370	12673	12410	246
	E031										
	E032										
	E033						11140	11670		11405	375
	E035										
	E037										
	E038										
	E040						12460	12984.3	12350.2	12598	339
	E041										
	E042										
	E043						12195.5	11945.3	12146.5	12096	133
	E044						11637.3	11978.1	11793.6	11803	171
	E046						12367	12156	12267	12263	106
	E047	340	340	350	343	6	11550	11360	11370	11427	107
	E057										
	E072										
Community Results		Consensus Mean			245		Consensus Mean			12182	
		Consensus Standard Deviation			150		Consensus Standard Deviation			221	
		Maximum			496		Maximum			13175	
		Minimum			5		Minimum			7	
		N			5		N			15	

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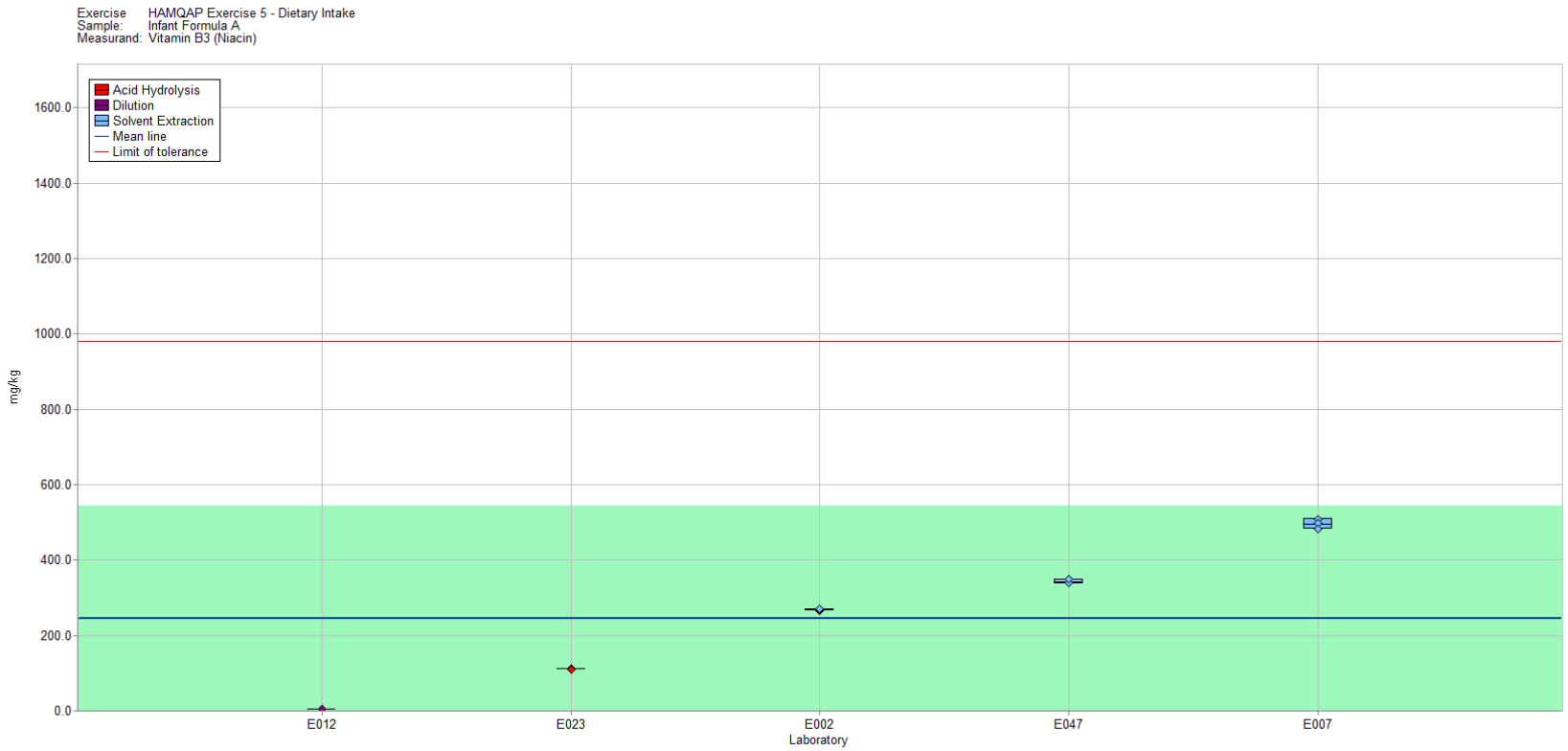

Figure 3-15. Niacin (vitamin B_3) in Infant Formula A (data summary view – sample preparation method). In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red line represents the upper consensus range of tolerance, calculated as the values above the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$ with the lower limit set at zero. A target value for niacin has not been determined in this material.

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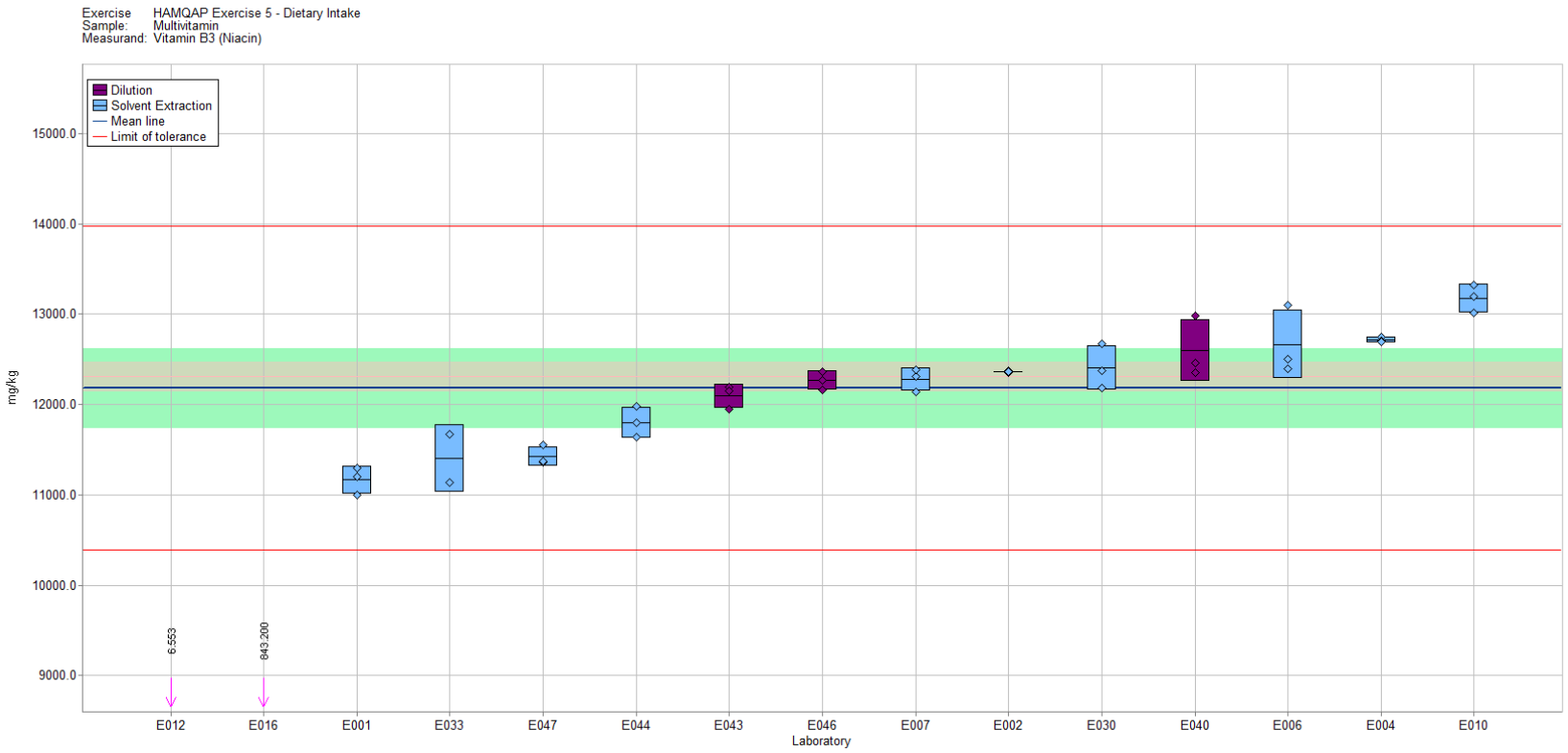

Figure 3-16. Niacin (vitamin B_3) in Multivitamin (data summary view – sample preparation method). In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. 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, $|Z_{\text{NIST}}| \leq 2$.

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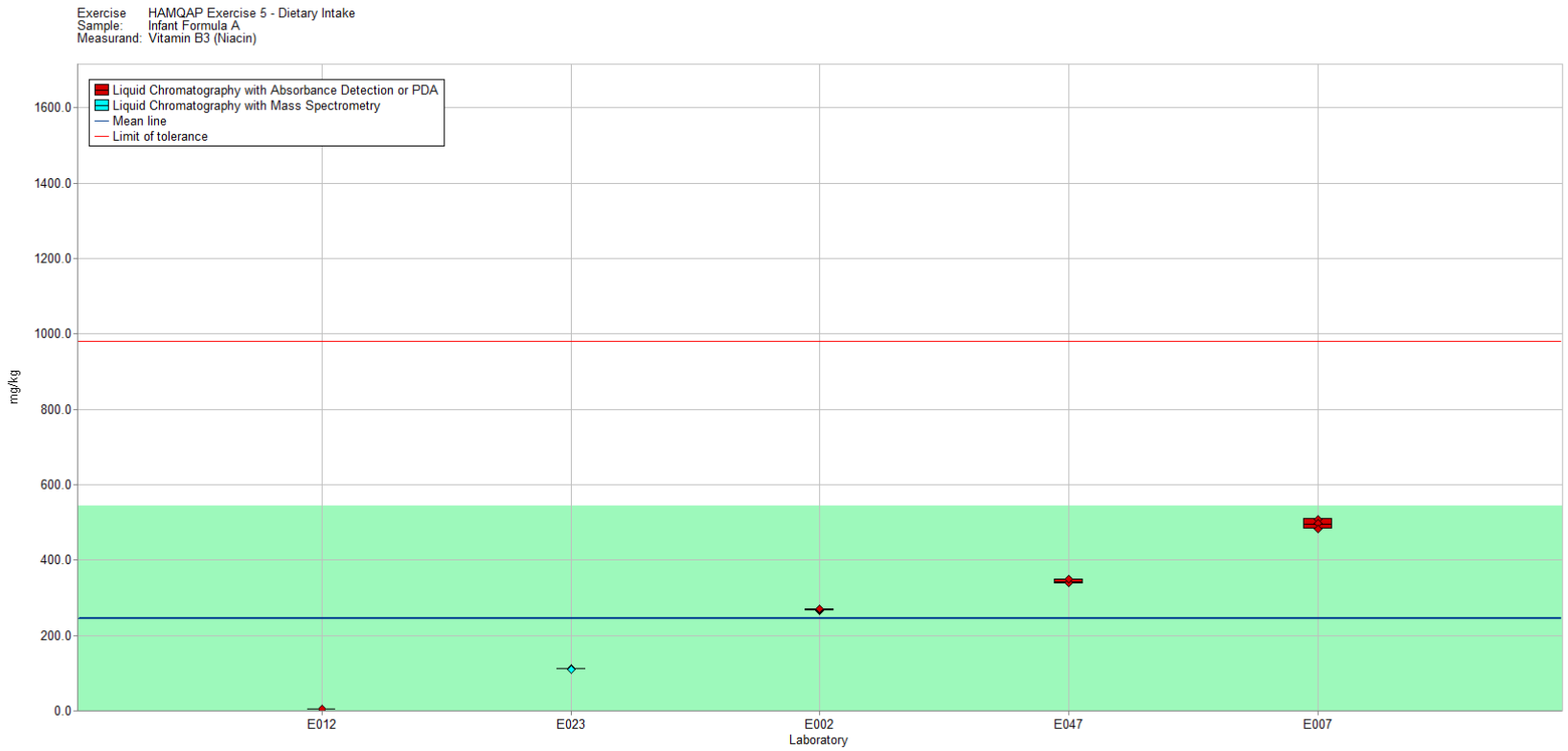

Figure 3-17. Niacin (vitamin B_3) in Infant Formula A (data summary view – analytical method). In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red line represents the upper consensus range of tolerance, calculated as the values above the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$ with the lower limit set at zero. A target value for niacin has not been determined in this material.

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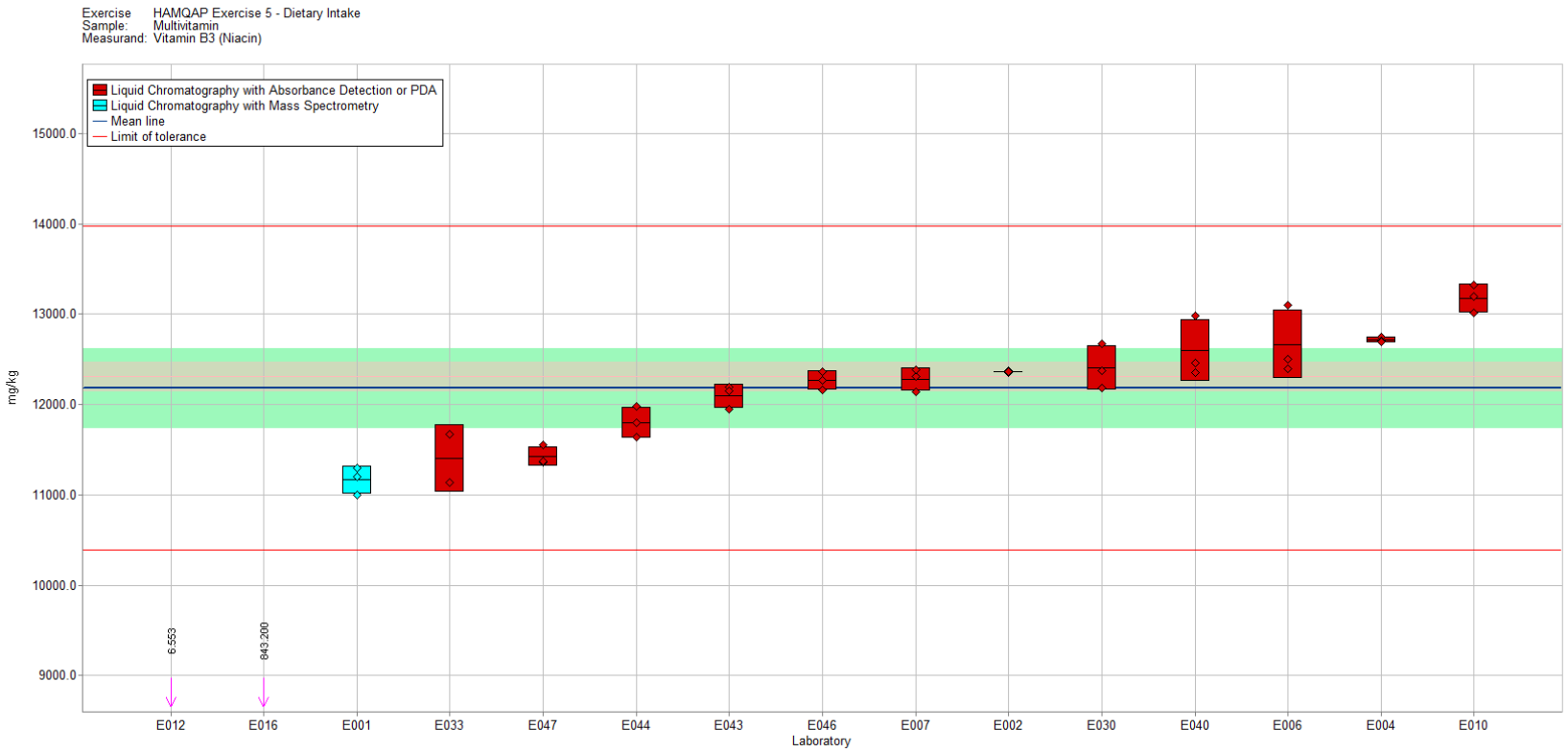

Figure 3-18. Niacin (vitamin B_3) in Multivitamin (data summary view – analytical method). In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue 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, $|Z_{\text{NIST}}| \leq 2$.

Table 3-6. Data summary table for pantothenic acid (vitamin B_5) 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.

		Pantothenic Acid (Vitamin B_5)									
		Infant Formula A (mg/kg)					Multivitamin (mg/kg)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target				72.6	14.5				6910	1100
	E001						7360	7440	7250	7350	95
	E002	75.62	74.12	75.3	75.0	0.8	7011.52	7089.05	7042.97	7048	39
	E003										
	E004						7108	7002	7117	7076	64
	E005	61	60	60	60.3	0.6	7510	7570	7280	7453	153
	E006						6640	6720	6480	6613	122
	E007	75.6	72.9	73.9	74.1	1.4	7187	7280	7102	7190	89
	E008										
	E010	71.0723	75.9825	78.0959	75.1	3.6	6439.1	6623.6	6848.5	6637	205
	E011										
	E012	3.34	3.5	3.2	3.3	0.2	2.4	2.45	2.55	2	0
	E013	< 200	< 200	< 200	< 200						
	E015										
	E016						1219	1232	1087	1179	80
	E020										
	E021										
	E023	69	69.1	68.7	68.9	0.2	6460	6200	4730	5797	933
	E025						6922	6902	6845	6890	40
	E030	78.1	73.2	75.3	75.5	2.5	7339	7259	7363	7320	54
	E031										
	E033						6900	6700		6800	141
	E035										
	E037										
	E040	100	69.1	103.7	90.9	19.0					
	E041						7416	7757	7029	7401	364
	E042	72.727	69.577	70.624	71.0	1.6					
	E043						6924	6702.9	6916.1	6848	125
	E047	70	70	70	70.0	0.0	7100	7160	6950	7070	108
	E072										
Community Results		Consensus Mean			72.8		Consensus Mean			6993	
		Consensus Standard Deviation			2.3		Consensus Standard Deviation			128	
		Maximum			90.9		Maximum			7453	
		Minimum			3.3		Minimum			2	
		N			11		N			16	

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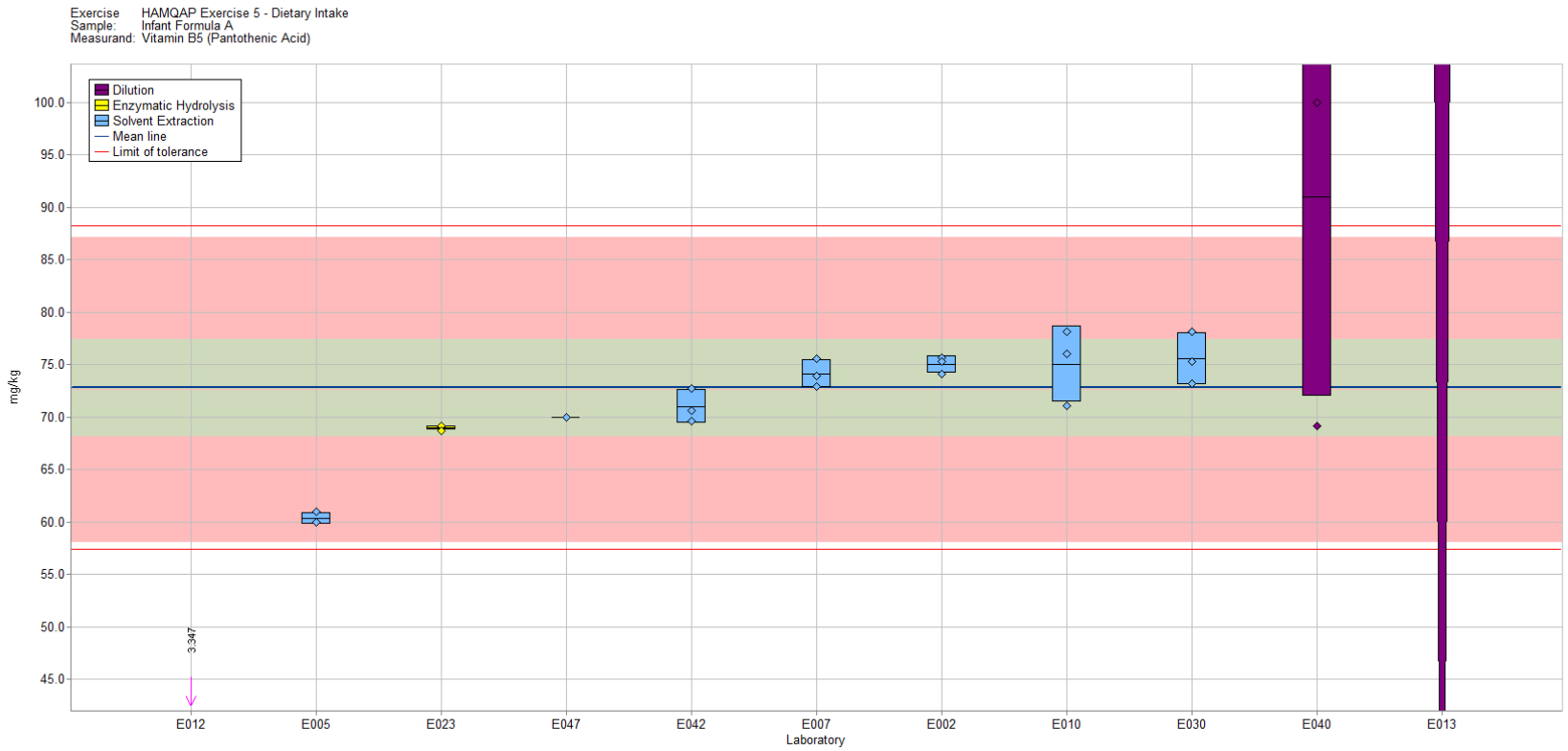

Figure 3-19. Pantothenic acid (vitamin B_5) in Infant Formula A (data summary view – sample preparation method). In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation 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, $|Z_{\text{NIST}}| \leq 2$.

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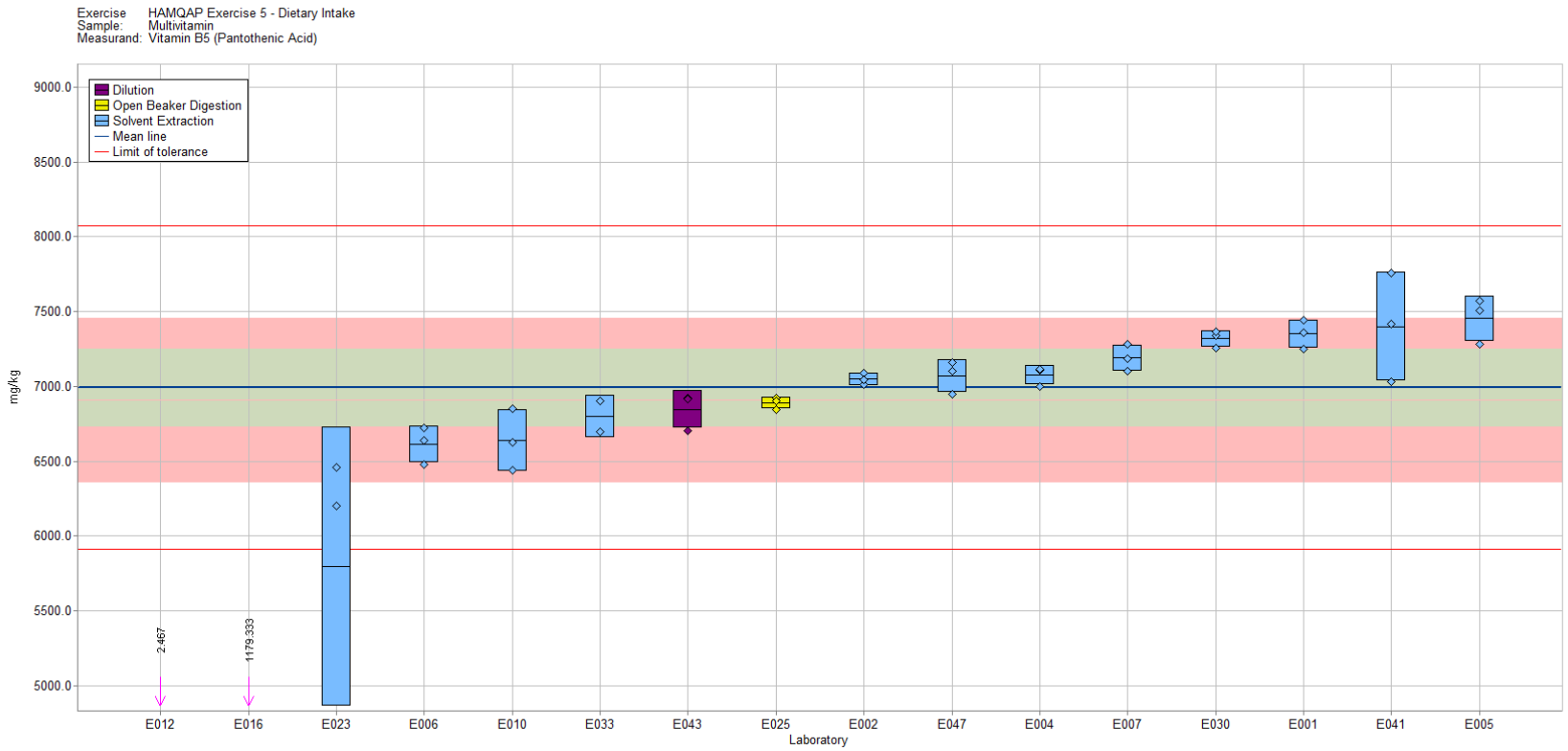

Figure 3-20. Pantothenic acid (vitamin B_5) in Multivitamin (data summary view – sample preparation method). In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method 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, $|Z_{\text{NIST}}| \leq 2$.

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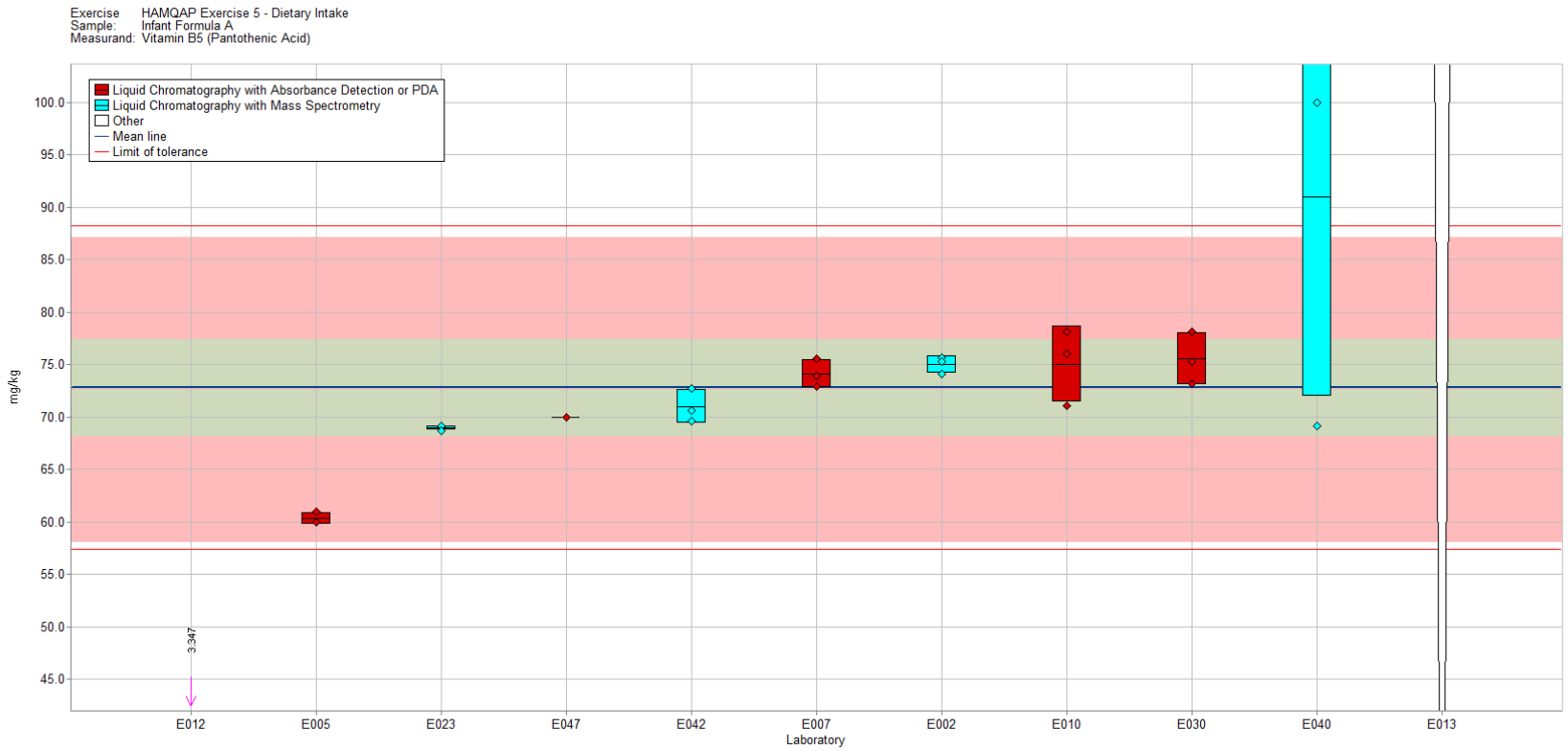

Figure 3-21. Pantothenic acid (vitamin B_5) in Infant Formula A (data summary view – analytical method). In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. 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, $|Z_{\text{NIST}}| \leq 2$.

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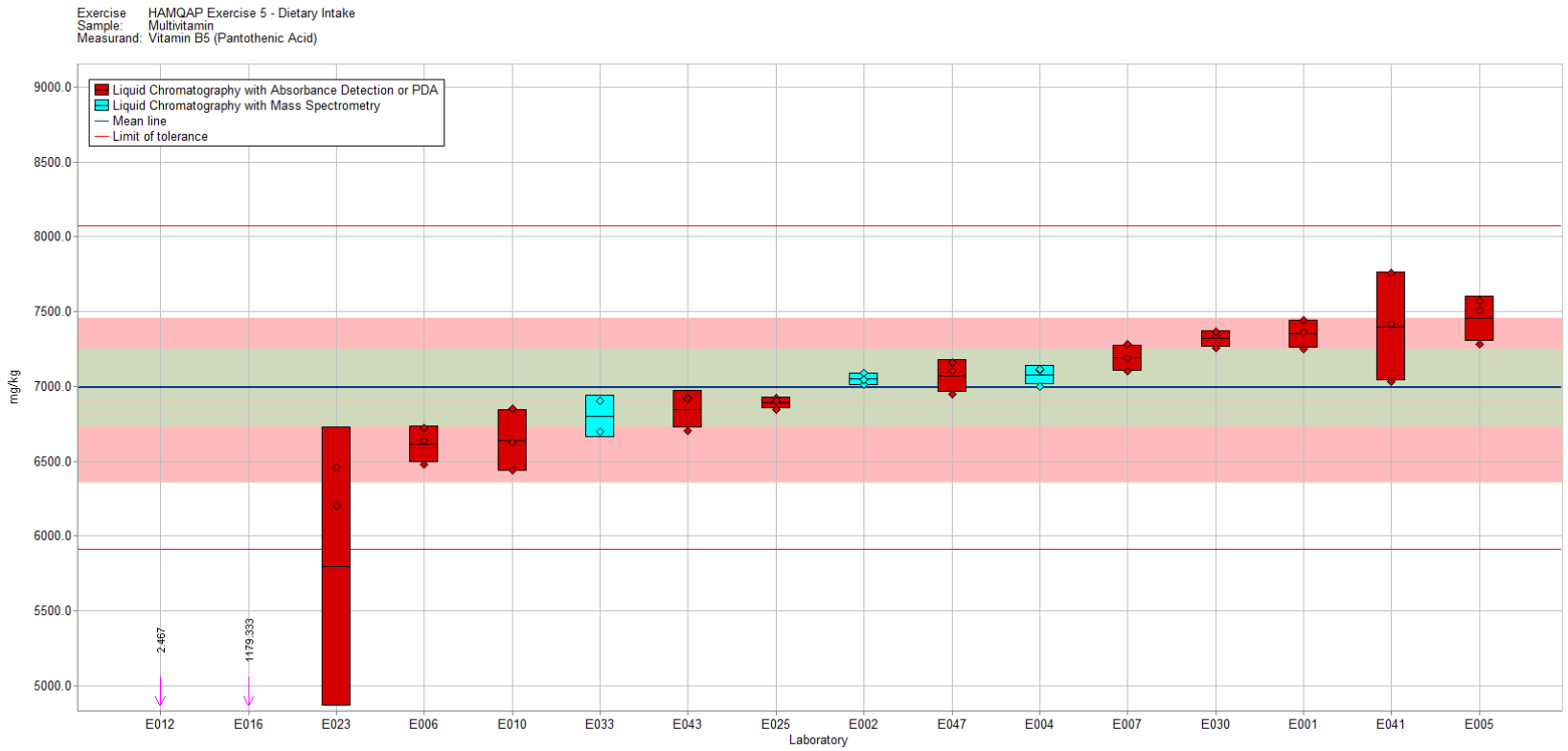

Figure 3-22. Pantothenic acid (vitamin B_5) in Multivitamin (data summary view – analytical method). In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The 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, $|Z_{\text{NIST}}| \leq 2$.

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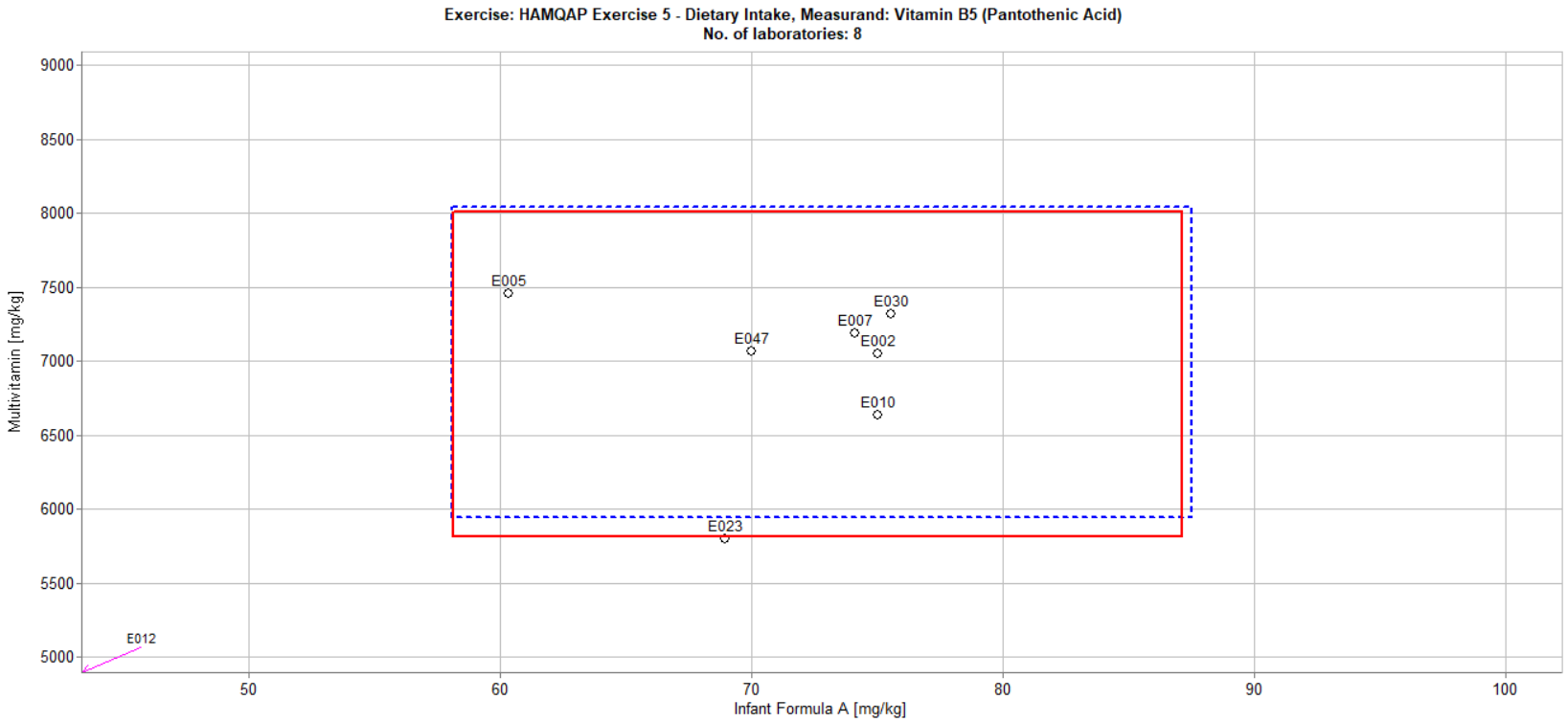

Figure 3-23. Laboratory means for pantothenic acid (vitamin B_5) in Infant Formula A and Multivitamin (sample/sample comparison view). In this view, the individual laboratory mean for one sample (infant formula) is compared to the individual laboratory mean for a second sample (multivitamin). The solid red box represents the NIST range of tolerance for the two samples, infant formula (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 infant formula (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 3-7. Data summary table for pyridoxine (vitamin B_6) 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.

		Pyridoxine (Vitamin B_6)									
		Infant Formula A (mg/kg)					Multivitamin (mg/kg)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target				14.6	1.1				1334	54
	E001						1630	1700	1730	1687	51
	E002	15.206	15.215	14.735	15.1	0.3	1483.54	1488.19	1452.74	1475	19
	E004						1308	1296	1353	1319	30
	E005	13	12	12	12.3	0.6	1270	1310	1360	1313	45
	E006						1340	1440	1300	1360	72
	E007	18.68	17.74	17.6	18.0	0.6	2058	1945	2059	2021	66
	E010	13.3423	13.4625	13.5445	13.4	0.1	1495.6	1348.8	1364.8	1403	81
	E013	< 30.0	< 30.0	< 30.0	< 30.0		1960	2050	1960	1990	52
	E016						392.8	331.4	320.7	348	39
	E020										
	E021										
	E023	14.3	14	14.1	14.1	0.2	1350	1320	1280	1317	35
	E030	18.4	16.4	17.9	17.6	1.0	1304	1367	1382	1351	41
	E031										
	E035										
	E037										
	E040	22.9	20.9	20.5	21.4	1.3	1366	1472.4	1326.3	1388	76
	E041	15.1	15.1	15.2	15.1	0.1	1321	1307	1354	1327	24
	E043						1344.2	1341.6	1302.5	1329	23
	E044						1362.5	1357.2	1334.5	1351	15
	E046										
	E072										
Community Results		Consensus Mean			15.8		Consensus Mean			1370	
		Consensus Standard Deviatio			1.2		Consensus Standard Deviatio			24	
		Maximum			21.4		Maximum			2021	
		Minimum			12.3		Minimum			348	
		N			9		N			15	

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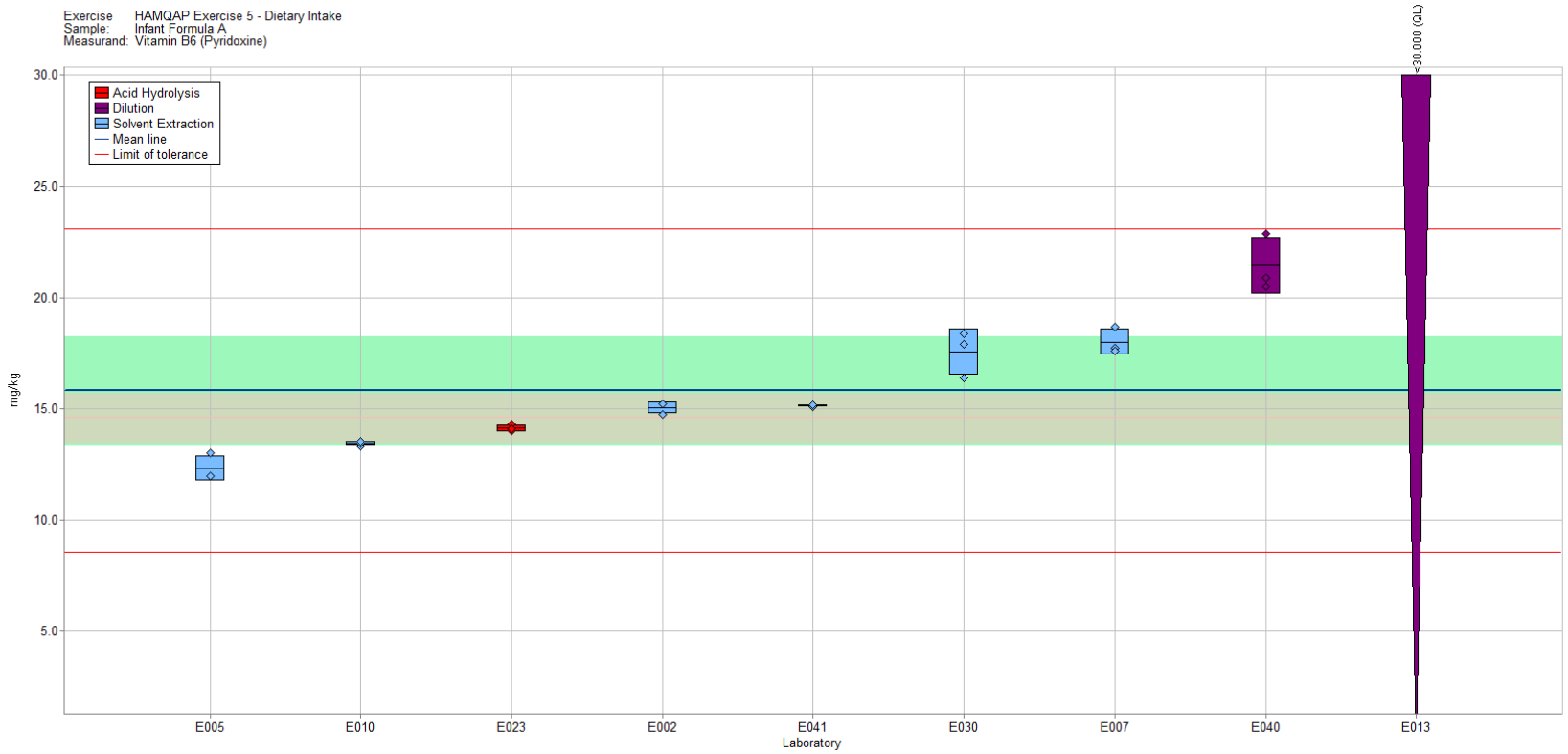

Figure 3-24. Pyridoxine (vitamin B_6) in Infant Formula A (data summary view – sample preparation method). In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. 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, $|Z_{\text{NIST}}| \leq 2$.

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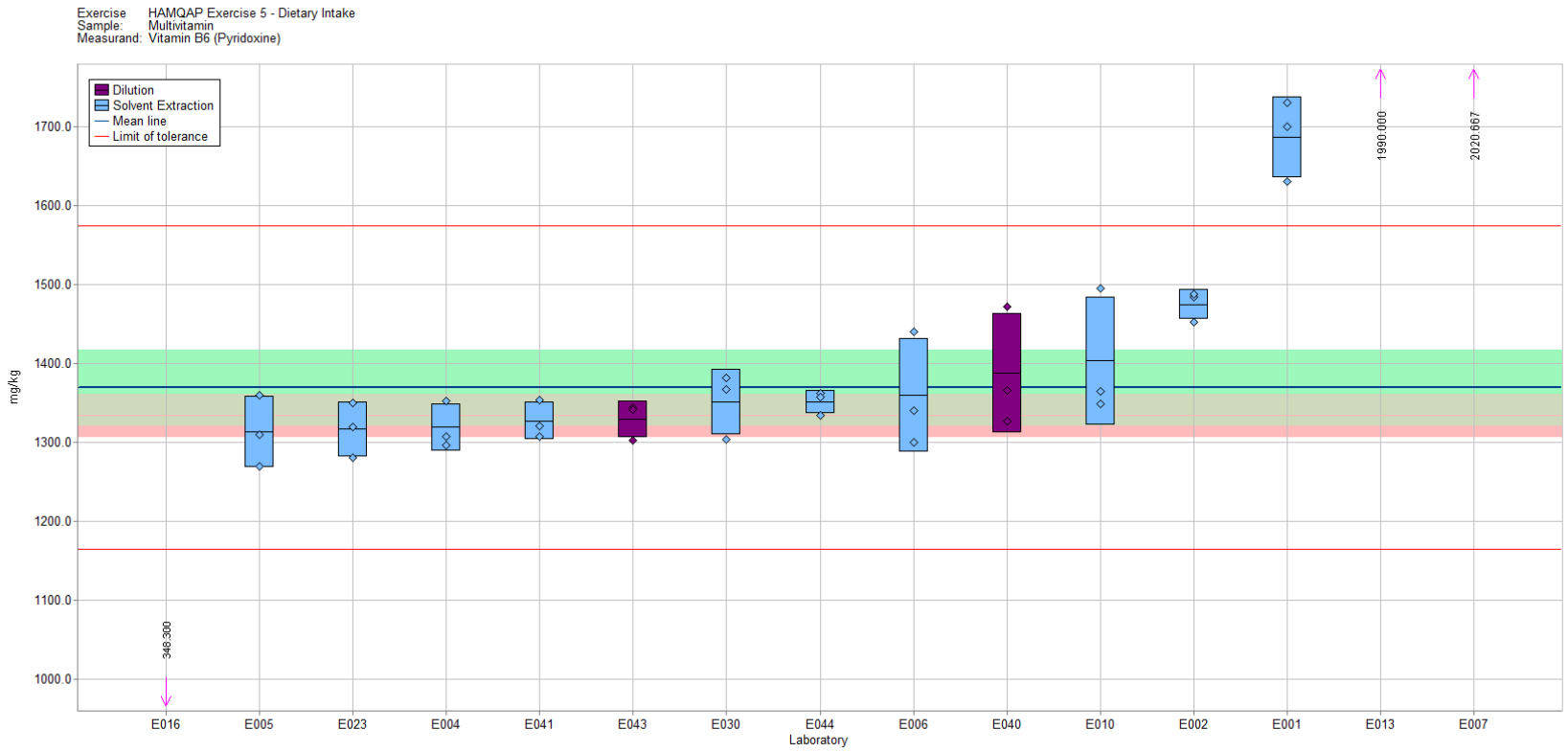

Figure 3-25. Pyridoxine (vitamin B_6) in Multivitamin (data summary view – sample preparation method). In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. 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, $|Z_{\text{NIST}}| \leq 2$.

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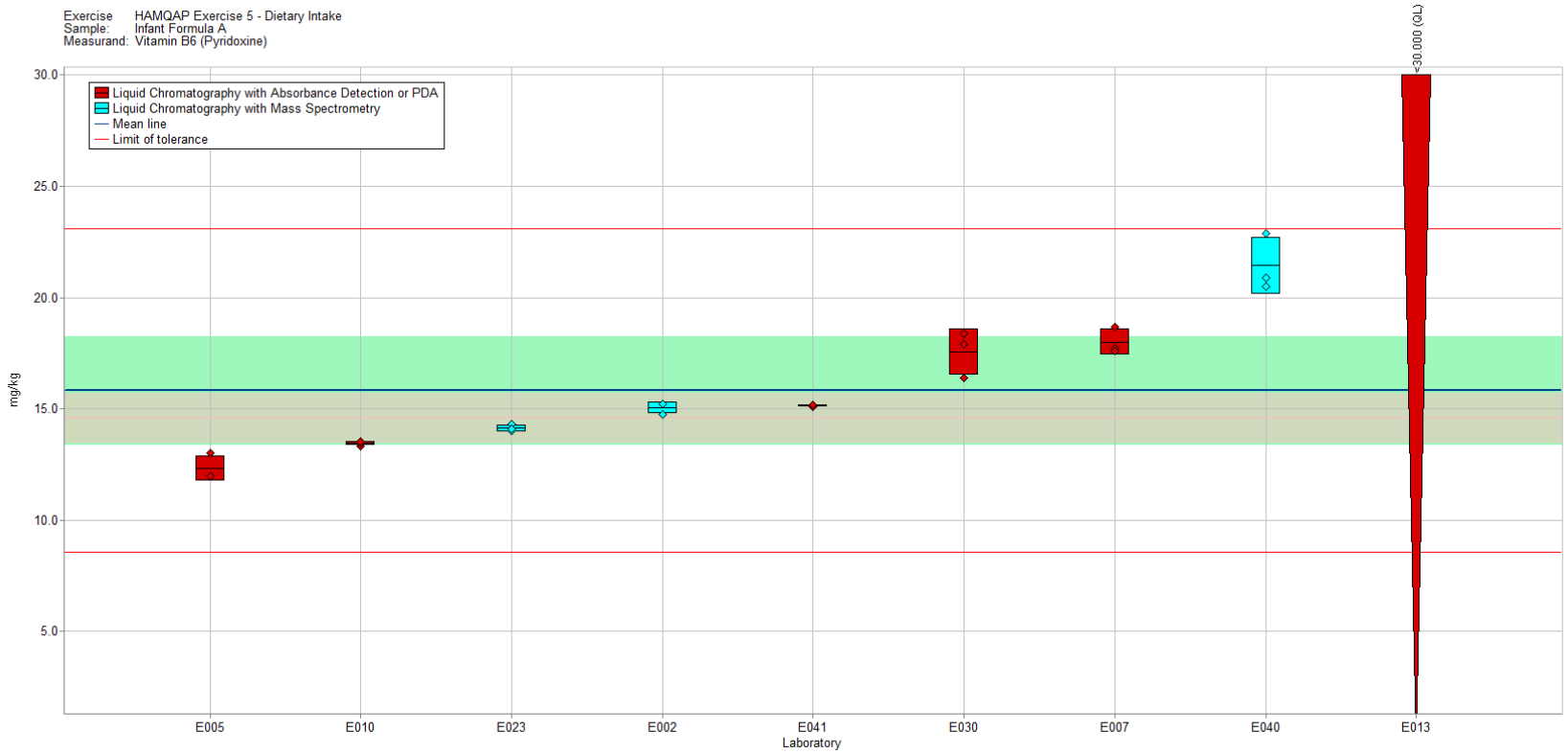

Figure 3-26. Pyridoxine (vitamin B_6) in Infant Formula A (data summary view – analytical method). In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The 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, $|Z_{\text{NIST}}| \leq 2$.

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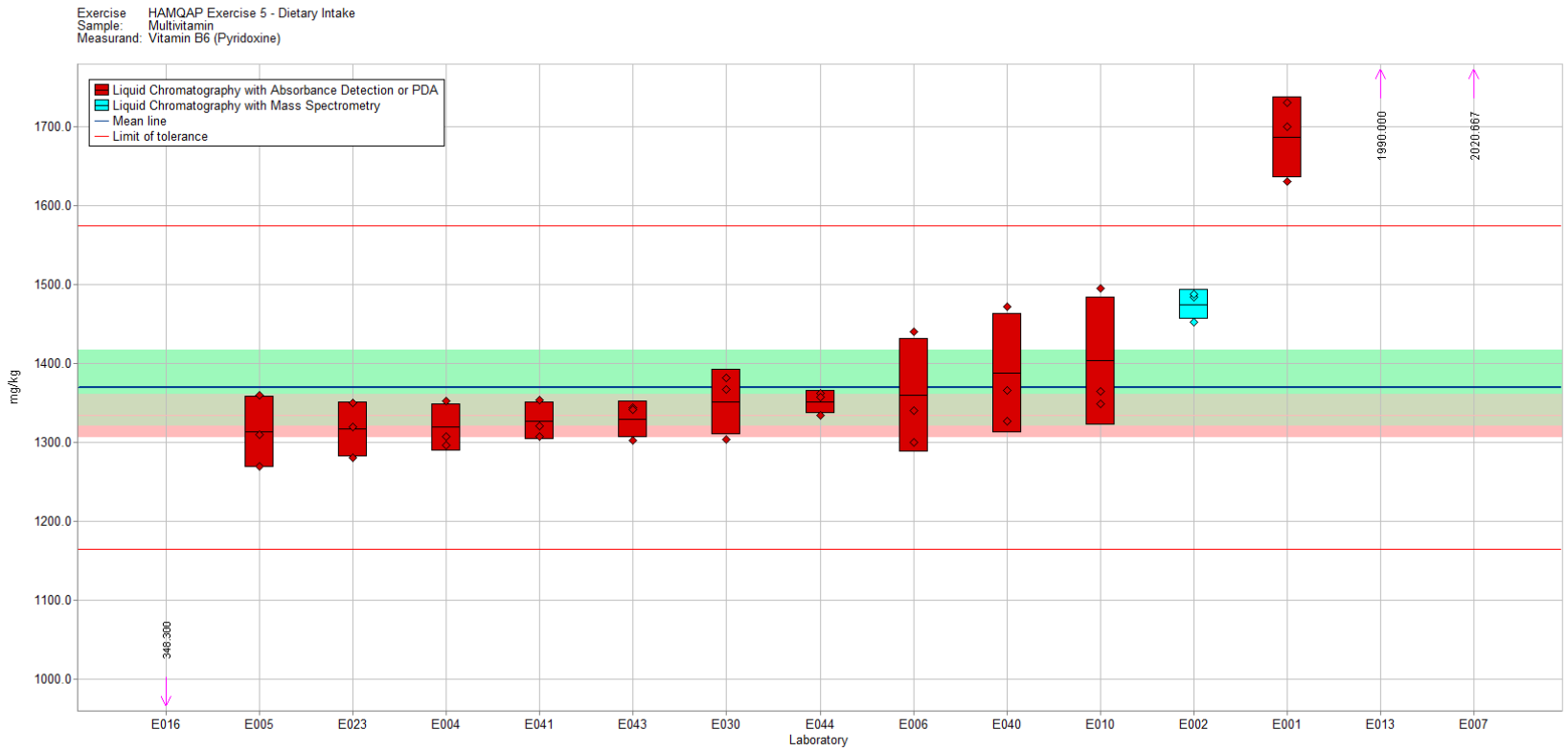

Figure 3-27. Pyridoxine (vitamin B_6) in Multivitamin (data summary view – analytical method). In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid 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, $|Z_{\text{NIST}}| \leq 2$.

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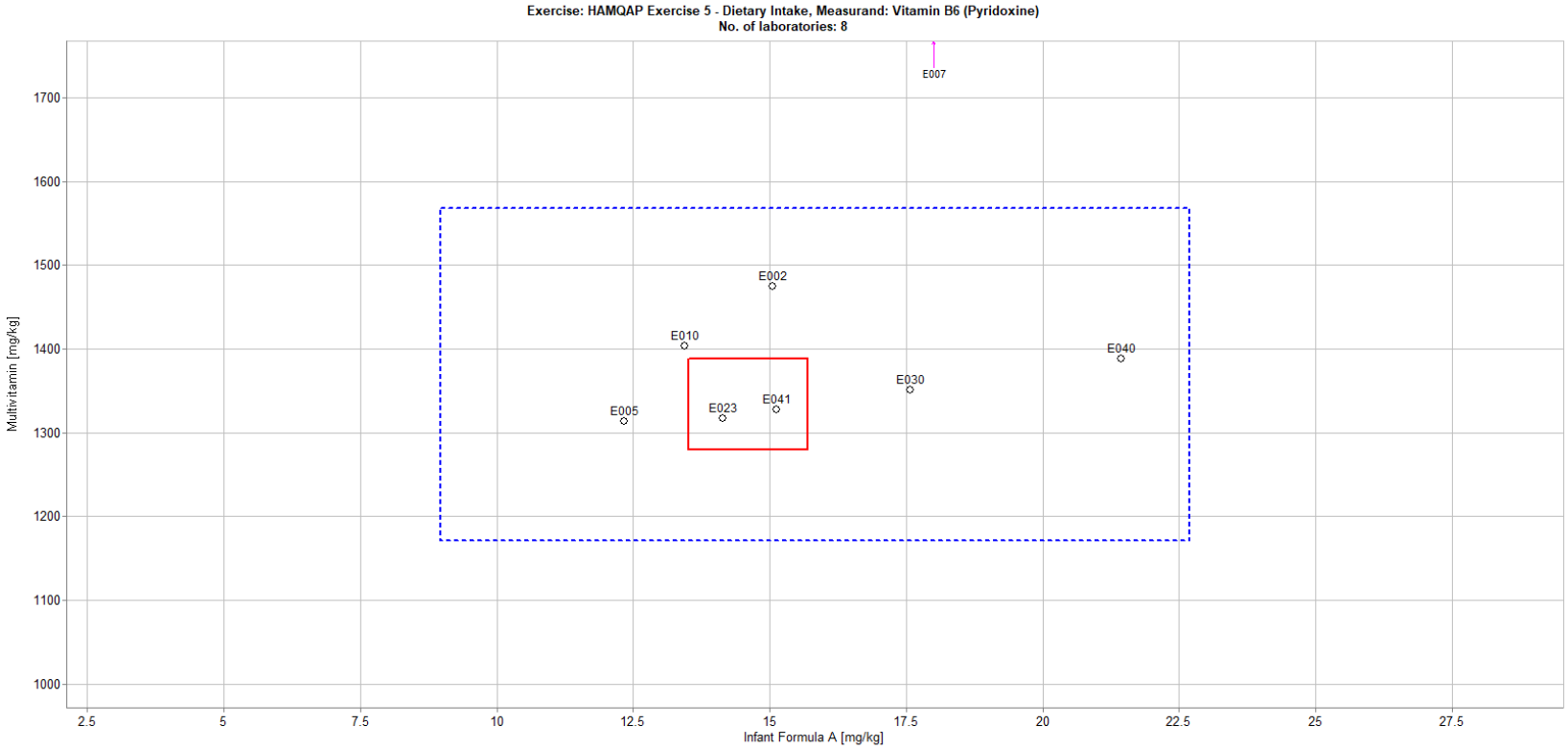

Figure 3-28. Laboratory means for pyridoxine (vitamin B_6) in Infant Formula A and Multivitamin (sample/sample comparison view). In this view, the individual laboratory mean for one sample (infant formula) is compared to the individual laboratory mean for a second sample (multivitamin). The solid red box represents the NIST range of tolerance for the two samples, infant formula (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 infant formula (x-axis) and multivitamin (y-axis), calculated as the values above and below the consensus means that result in an acceptable Z'_{comm} score, $|Z'_{\mathrm{comm}}| \leq 2$.

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SECTION 4: FAT-SOLUBLE VITAMINS (Vitamin D)

Study Overview

In this study, participants were provided with samples of infant formula and multivitamin for dietary intake, and with samples of human serum F, human serum G, and a set of calibration solutions (SRM 2972a 25-Hydroxyvitamin D Calibration Solutions) for human metabolism. Participants were asked to use in-house analytical methods to determine and report the mass fraction (mg/kg) of vitamin D and vitamin D metabolites in the various materials. Vitamin D is a fat-soluble nutrient produced in the body upon exposure to UV rays (e.g., sunlight) and obtained through dietary intake. Not many foods naturally contain vitamin D, and numerous fortified foods and dietary supplements are available.[14] Vitamin D has two isomer forms, vitamin D_2 and vitamin D_3, which both undergo two hydroxylation steps in the body to produce the active form of the vitamin, 1,25-dihydroxyvitamin D [1,25(OH)D]. The intermediate form, 25-hydroxyvitamin D [25(OH)D], is the primary marker used to assess vitamin D status. Vitamin D is an essential nutrient for promoting calcium absorption and maintaining serum calcium and phosphate levels required for proper bone growth. Vitamin D has also been associated with many other health related roles, including modulation of cell growth, neuromuscular and immune functions, and reduction of inflammation. Studies are ongoing to determine the effects of vitamin D supplementation and serum levels on conditions such as cancer, diabetes, and pregnancy outcomes. Even so, the relationships between vitamin D intake, supplementation, and status continue to be areas of debate. For example, some analyses of NHANES datasets have raised concerns about vitamin D intake estimates and status measurements, in that the observed occurrence of vitamin D deficiency in adults, measured through 25(OH)D serum concentrations does not match the expected deficiencies suggested by the intake survey results. Improved information on the levels of amount of vitamin D metabolites present in foods could help in understanding the discrepancies. Additionally, considerable variability in measurement of 25(OH)D is known to potentially confound the interpretation of serum concentration assessments. The accurate and reliable measurement of vitamin D and its various metabolites in both intake and metabolite matrices is key towards understanding intake requirements and health benefits.

Dietary Intake Sample Information

Infant Formula A. Participants were provided with three packets, each containing approximately 10 g of powdered infant formula. Participants were asked to store the material at –20 °C in the original unopened packet and to prepare one sample and report one value from each packet provided. Before use, participants were instructed to thoroughly mix the contents of the packet prior to removal of a test portion for analysis, and to use a sample size of at least 1 g. The approximate analyte levels were not reported to participants prior to the study. The NIST-determined values for vitamin D_2, vitamin D_3, and total vitamin D in the infant formula sample were assigned using results from the manufacturer of the material. The NIST-determined values and uncertainties for vitamin D are provided in the table below on an as-received basis.

[14] Vitamin D Fact Sheet for Health Professionals. National Institutes of Health Office of Dietary Supplements. https://ods.od.nih.gov/factsheets/VitaminD-HealthProfessional/ (accessed June 2020).

Analyte	NIST-Determined Mass Fraction in Infant Formula A (mg/kg)
Vitamin D_2	0.116 ± 0.011
Vitamin D_3	0.106 ± 0.007
Total Vitamin D	0.222 ± 0.012

Multivitamin. Participants were provided with three bottles, each containing 30 multivitamin tablets. Participants were asked to store the material at controlled room temperature, 20 °C to 25 °C, in the original unopened bottles and to prepare one sample and report one value from each bottle provided. Before use, participants were instructed to grind all 30 tablets and mix the resulting powder thoroughly prior to removal of a test portion for analysis, and to use a sample size of at least 1 g. Approximate analyte levels were not reported to participants prior to the study. The NIST-determined values for vitamin D_2, vitamin D_3, and total vitamin D in the infant formula sample were assigned using results from the manufacturer of the material. The NIST-determined values and uncertainties for vitamin D are provided in the table below on an as-received basis.

Analyte	NIST-Determined Mass Fraction in Multivitamin (mg/kg)
Vitamin D_2	10.73 ± 0.70
Vitamin D_3	10.8 ± 1.5
Total Vitamin D	21.5 ± 1.6

Dietary Intake Study Results

- Thirty-six laboratories enrolled in this exercise and received samples to measure vitamin D. The table below summarizes the participation statistics. Some of the reported values were non-quantitative (zero or below LOQ) but are included here in the participation and reporting statistics. Total vitamin D was determined by the sum of vitamin D_2 and vitamin D_3 reported by each individual laboratory, with one laboratory reporting total vitamin D as a single result.

Analyte	Number of Laboratories Requesting Samples	Number of Laboratories Reporting Results (Percent Participation)	
		Infant Formula	Multivitamin
Vitamin D_2	36	10 (28 %)	14 (39 %)
Vitamin D_3	36	13 (36 %)	18 (50 %)
Total Vitamin D	36	11 (36 %)	19 (53 %)

- Consensus means for all analytes and matrices were below the target range. The consensus range overlapped the target range for vitamin D_3 in both the infant formula and the multivitamin, and for vitamin D_2 in the infant formula.
- The between-laboratory variabilities for vitamin D_2 and vitamin D_3 were acceptable in the multivitamin and high for the infant formula (see table below). The between-laboratory variability for total vitamin D in both materials was high, though is harder to interpret since

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the value is the sum of the individual analytes and not a single measurement, except for one laboratory.

	Between-Laboratory Variability (% RSD)	
Analyte	Infant Formula	Multivitamin
Vitamin D_2	61 %	13 %
Vitamin D_3	50 %	11 %
Total Vitamin D	38 %	20 %

- For the determination of vitamin D in the infant formula and multivitamin, a majority of laboratories reported preparing samples using solvent extraction (see table below). Other reported sample preparation approaches included derivatization, saponification, and QuEChERs.

	Reported Analytical Method Number (Percent) Reporting			
	Infant Formula A		Multivitamin	
Analyte	Vitamin D_2	Vitamin D_3	Vitamin D_2	Vitamin D_3
Solvent Extraction	5 (50 %)	8 (62 %)	10 (71 %)	12 (67 %)
Derivatization	1 (10 %)	1 (8 %)	1 (7 %)	1 (6 %)
Saponification	1 (10 %)	1 (8 %)	1 (7 %)	2 (11 %)
QuEChERs	1 (10 %)	1 (8 %)	1 (7 %)	1 (6 %)
None/Other	2 (20 %)	2 (15 %)	2 (14 %)	2 (11 %)

- For the determination of vitamin D in the infant formula and multivitamin, a majority of laboratories reported using LC-Abs or LC with MS as the analytical method (see table below).

	Reported Analytical Method Number (Percent) Reporting			
	Infant Formula A		Multivitamin	
Analyte	Vitamin D_2	Vitamin D_3	Vitamin D_2	Vitamin D_3
LC-Abs	4 (40 %)	6 (46 %)	9 (60 %)	11 (61 %)
LC-MS or LC-MS/MS	6 (60 %)	6 (46 %)	6 (40 %)	7 (39 %)
LC-FL	--	1 (8 %)	--	--

Dietary Intake Technical Recommendations

The following recommendations are based on results obtained from the participants in this study. Figures were chosen to show results according to analytical method.

- Many of the results reported for both vitamin D_2 and vitamin D_3 in the infant formula were within the 95 % consensus range of tolerance and several of these were near the target value.

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    - Four laboratories reported results significantly higher than the target range, which may be due to improper reporting units or miscalculation of dilution factors. Calculations and reporting units must be verified prior to data submission.
    - When using absorbance as a detection method, compounds that absorb at the same wavelength used for detection of vitamin D (e.g., other vitamin D species such as pre-vitamin D or vitamin D metabolites, matrix components) may cause chromatographic interference and overestimation of the mass fraction of vitamin D in an unknown sample. All LC separations should be thoroughly evaluated for suitable resolution of known or suspected potential interferences.
    - For vitamin D compounds, calibrant purity and concentration assignment is best established using spectrophotometric approaches. Improper calibration characterization may lead to biased results.
- Many of the results reported for vitamin D_2 and vitamin D_3 in the multivitamin were within the 95 % consensus range of tolerance, though the consensus mean was lower than the target value especially for vitamin D_2 (**Figures 4-2** and **4-5**).
    - Vitamin D is susceptible to oxidation and degradation, and so is often encapsulated within a material formulation to protect the vitamin while the product is stored longer term before use. Sample preparation techniques must be able to fully extract the analytes from the sample matrix and also reduce the chance of analyte degradation. It is critical to use reduced lighting/yellow lighting when conducting preparation techniques, and store samples in the dark or in amber colored vials.
    - For vitamin D compounds, calibrant purity and concentration assignment is best established using spectrophotometric approaches. Improper calibration characterization may lead to biased results. Also, calculations and reporting units must be verified prior to data submission.
- The use of appropriate calibration materials and quality assurance samples to establish that a method is in control and being performed correctly may reduce the likelihood of outlying data. Quality assurance samples can be commercially available reference materials (CRMs, SRMs, or RMs) or materials prepared in-house.

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Table 4-1. Individualized data summary table (NIST) for vitamin D in infant formula and multivitamin.

National Institute of Standards & Technology

HAMQAP Exercise 5 - 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*
Vitamin D_2 (Ergocalciferol)	Infant Formula A	mg/kg	0.116	0.011			7	0.084	0.052	0.116	0.011
Vitamin D_2 (Ergocalciferol)	Multivitamin	mg/kg	10.73	0.70			14	7.07	0.94	10.73	0.70
Vitamin D_3 (Cholecalciferol)	Infant Formula A	mg/kg	0.106	0.007			11	0.068	0.034	0.106	0.007
Vitamin D_3 (Cholecalciferol)	Multivitamin	mg/kg	10.8	1.5			18	10	1	10.8	1.5
Total Vitamin D (Vitamin D_2 + Vitamin D_3)	Infant Formula A	mg/kg	0.222	0.012			11	0.33	0.12	0.222	0.012
Total Vitamin D (Vitamin D_2 + Vitamin D_3)	Multivitamin	mg/kg	21.5	1.6			19	6	1.2	21.53	1.6

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 Vitamin D_2 (ergocalciferol) 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.

		Vitamin D_2 (Ergocalciferol)									
		Infant Formula A (mg/kg)					Multivitamin (mg/kg)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target				0.116	0.011				10.73	0.70
	E001						8.21	8.31	7.8	8.11	0.27
	E002	0.95	1.1	1.29	1.113	0.170	16.51	16.59	18.14	17.08	0.92
	E003						3.685	3.472	3.697	3.62	0.13
	E004										
	E005	0.0525	0.0516	0.0533	0.052	0.001	5.73	5.74	6.11	5.86	0.22
	E006										
	E007										
	E008										
	E010						6.1857	7.9077	8.497	7.53	1.20
	E011										
	E012	3	2.9	2.6	2.833	0.208	15	15	15.3	15.10	0.17
	E014	0.066	0.056	0.058	0.060	0.005	11.13	8.53	9.34	9.67	1.33
	E015	2.28	1.39	2.19	1.953	0.490	1.23	1.32	1.29	1.28	0.05
	E016						0.117	0.129	0.108	0.12	0.01
	E019										
	E020										
	E021										
	E023	0.109	0.107	0.108	0.108	0.001	7.55	7.82	7.8	7.72	0.15
	E024										
	E025										
	E027										
	E030						6.77	6.7	7.05	6.84	0.19
	E031										
	E032										
	E033						6.1	5.5		5.80	0.42
	E035										
	E037										
	E038										
	E040						7.98	7.17	7.35	7.50	0.43
	E041										
	E042										
	E043						8.15	8.03	8	8.06	0.08
	E044										
	E047										
	E057	0.114	0.114	0.122	0.117	0.005					
	E072										
Community Results		Consensus Mean			0.084		Consensus Mean			7.07	
		Consensus Standard Deviation			0.052		Consensus Standard Deviation			0.94	
		Maximum			2.833		Maximum			17.08	
		Minimum			0.052		Minimum			0.12	
		N			7		N			14	

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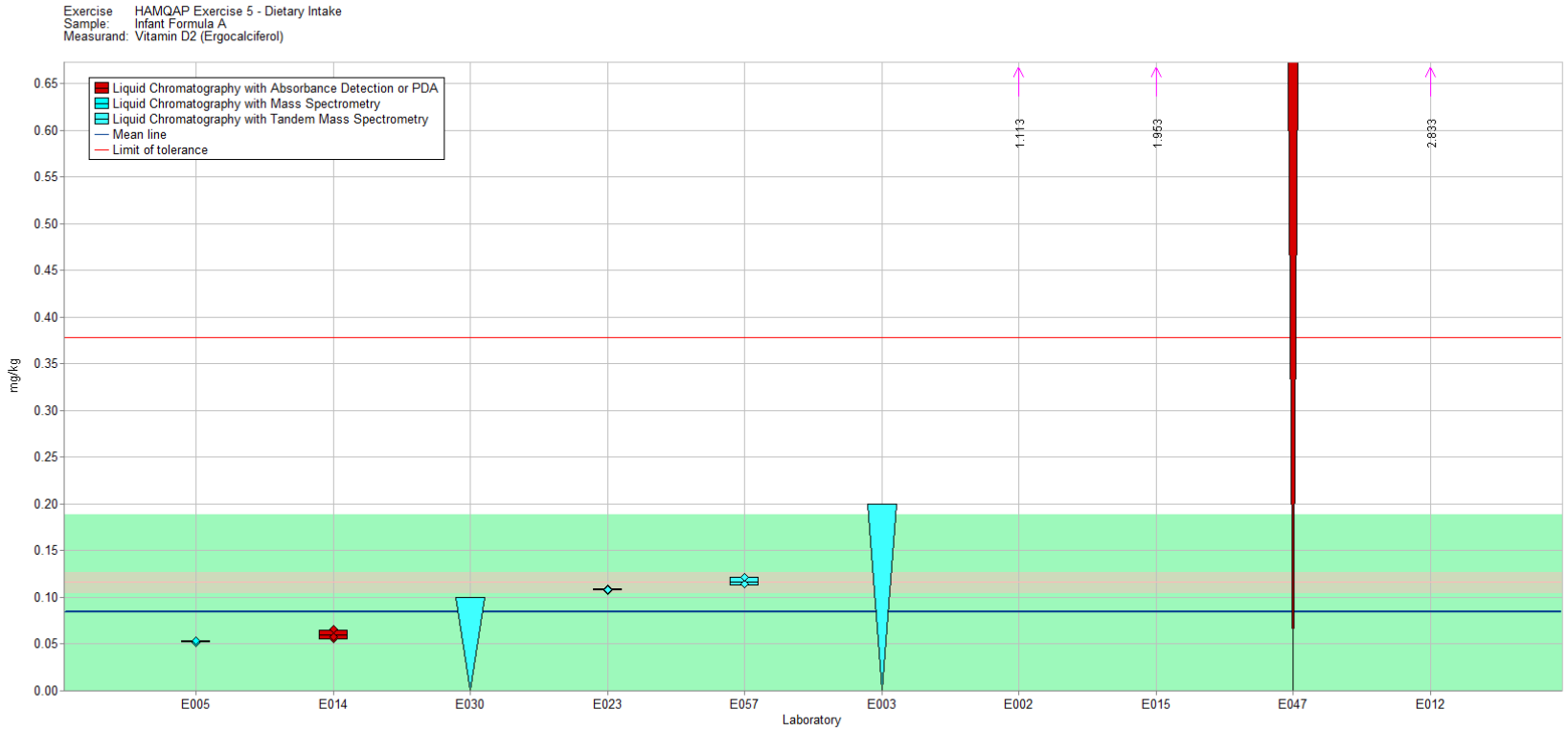

Figure 4-1. Vitamin D_2 (ergocalciferol) in Infant Formula A (data summary view – analytical method). In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The 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 consensus range of tolerance, calculated as the value above the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$, with the lower limit set to zero. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by its uncertainty (U_{NIST}) and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$.

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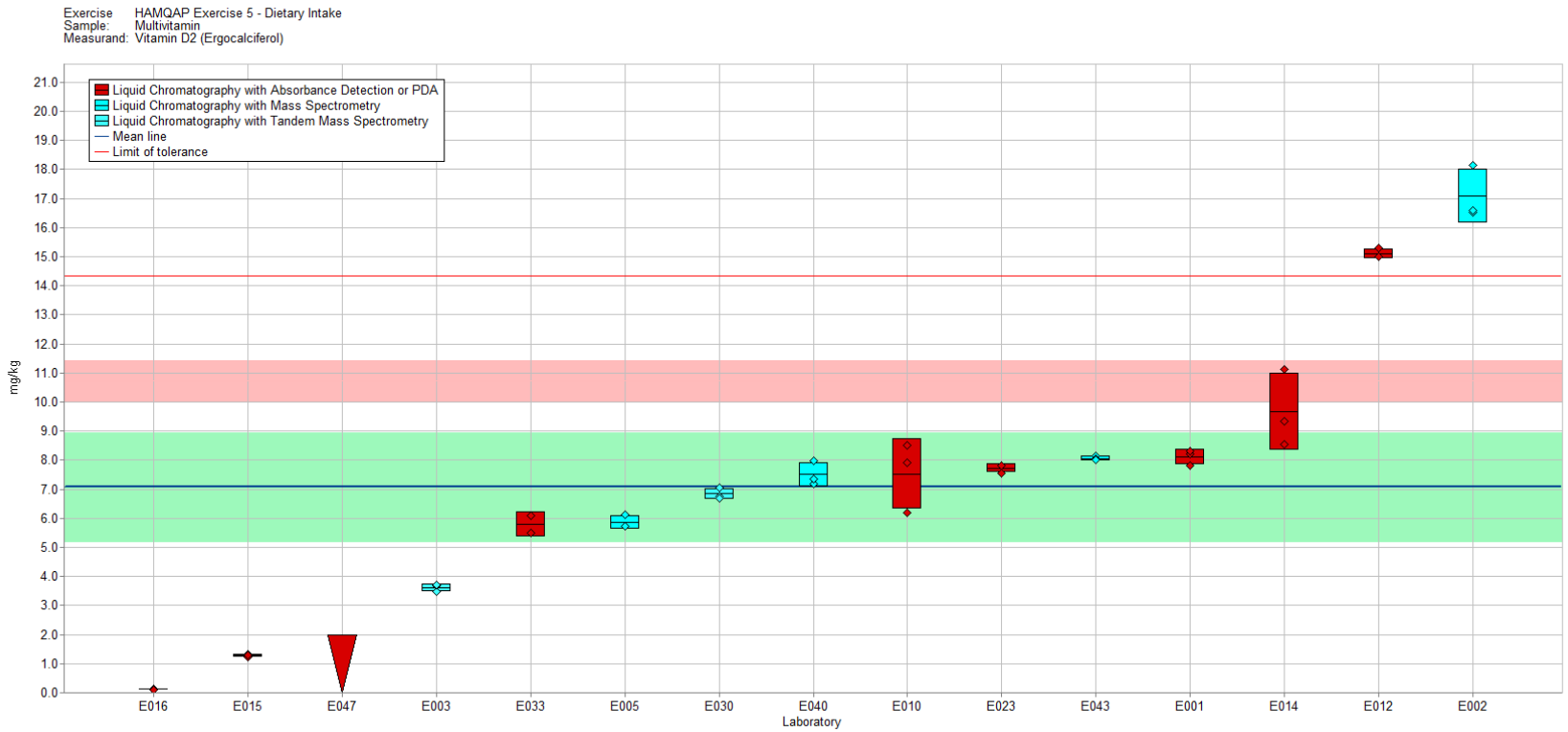

Figure 4-2. Vitamin D_2 (ergocalciferol) in Multivitamin (data summary view – analytical method). In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The red solid line represents the consensus range of tolerance, calculated as the value above the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$, with the lower limit set to zero. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by its uncertainty (U_{NIST}) and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$.

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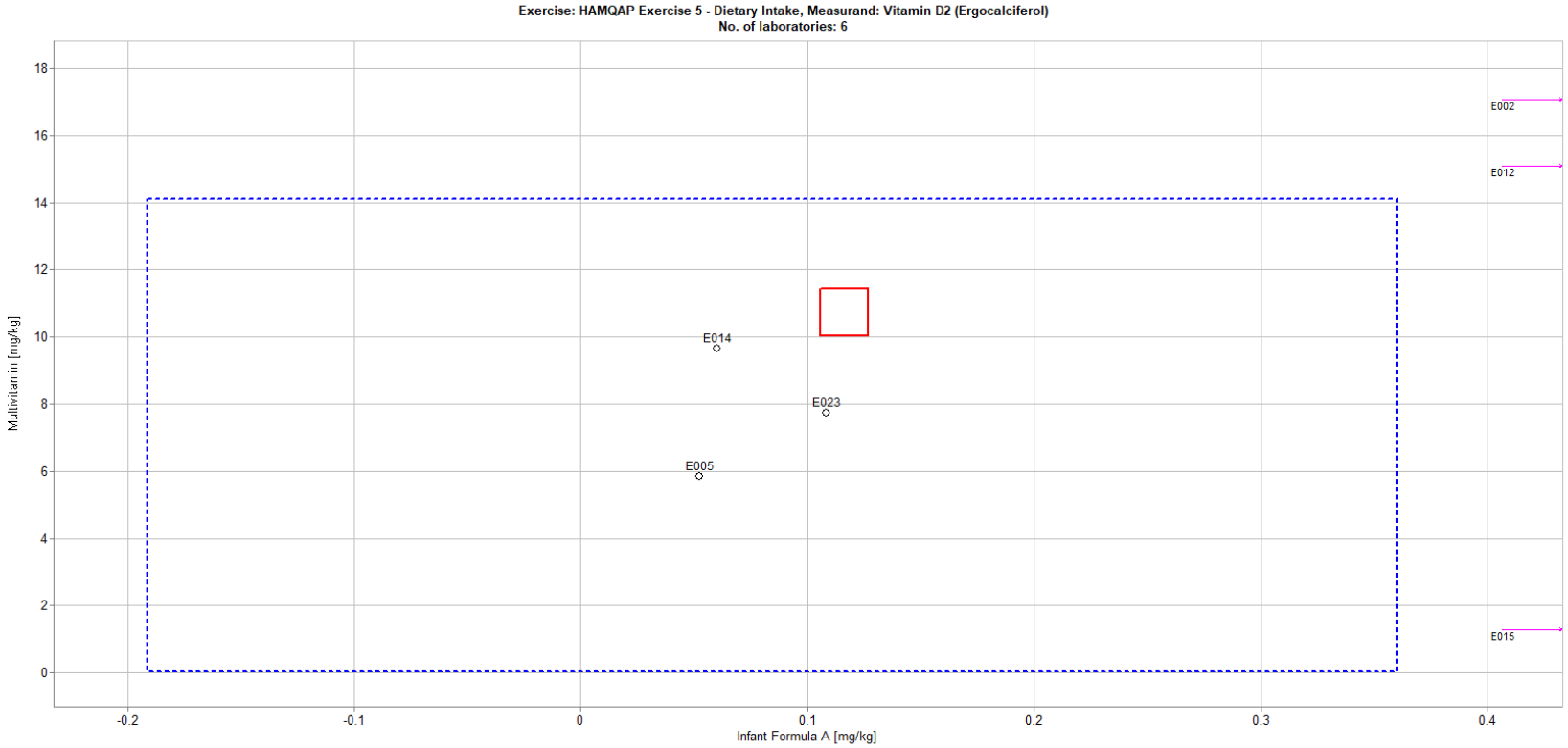

Figure 4-3. Laboratory means for vitamin D2 (ergocalciferol) in Infant Formula A and Multivitamin (sample/sample comparison view). In this view, the individual laboratory mean for one sample (infant formula) is compared to the individual laboratory mean for a second sample (multivitamin). The solid red box represents the NIST range of tolerance for the two samples, infant formula (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 infant formula (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 4-3. Data summary table for Vitamin D_3 (cholecalciferol) 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.

		Vitamin D_3 (Cholecalciferol)									
		Infant Formula A (mg/kg)					Multivitamin (mg/kg)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target				0.106	0.007				10.8	1.5
	E001						7.69	8.05	7.24	7.7	0.4
	E002	0.06	0.07	0.08	0.070	0.010	11.07	9.98	9.9	10.3	0.7
	E003						8.99	8.586	9.109	8.9	0.3
	E004										
	E005	0.0523	0.0511	0.0522	0.052	0.001	5.59	5.53	5.49	5.5	0.1
	E006										
	E007	1.15	1.19	1.1	1.147	0.045	12.7	12.8	13	12.8	0.2
	E008										
	E010										
	E011										
	E012	2	2	2.2	2.067	0.115	16.1	16	16.24	16.1	0.1
	E014	0.047	0.047	0.052	0.049	0.003	7.72	7.49	7.65	7.6	0.1
	E015	1.5	2.12	1.48	1.700	0.364	0.31	0.38	0.34	0.3	0.0
	E016						0.134	0.147	0.121	0.1	0.0
	E019										
	E020										
	E021										
	E023	0.102	0.0998	0.103	0.102	0.002	14.05	14.97	13.97	14.3	0.6
	E024										
	E025										
	E027										
	E030						8.54	8.39	7.96	8.3	0.3
	E031										
	E032						9.3	9.3	9.3	9.3	0.0
	E033						15.9	14.6		15.3	0.9
	E035										
	E037										
	E038										
	E040						8.82	8.12	7.55	8.2	0.6
	E041	10.9	12.1	12.5	11.833	0.833	9.95	8.73	5.98	8.2	2.0
	E042	0.00	0.00	0.00	0.000	0.000					
	E043						9.13	9	9.21	9.1	0.1
	E044						12.04	13.09	12.15	12.4	0.6
	E047	0.1	0.1	0.1	0.100	0.000	3.7	3.1	3.1	3.3	0.3
	E057	0.102	0.109	0.1	0.104	0.005					
	E072										
Community Results		Consensus Mean			0.068		Consensus Mean			9.0	
		Consensus Standard Deviation			0.034		Consensus Standard Deviation			1.0	
		Maximum			11.833		Maximum			16.1	
		Minimum			0.000		Minimum			0.1	
		N			11		N			18	

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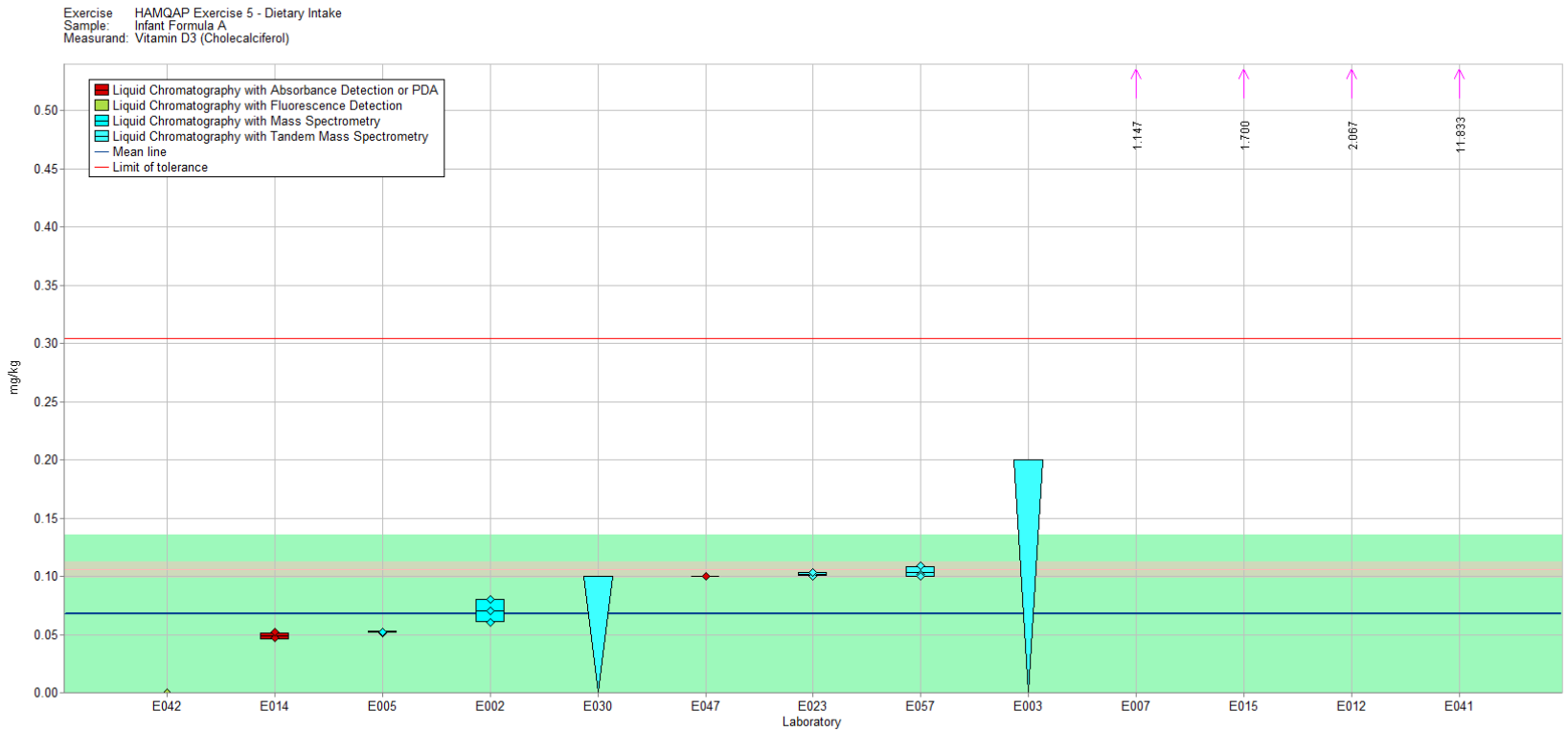

Figure 4-4. Vitamin D_3 (cholecalciferol) in Infant Formula A (data summary view – analytical method). In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. 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 consensus range of tolerance, calculated as the value above the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$, with the lower limit set to zero. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by its uncertainty (U_{NIST}) and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$.

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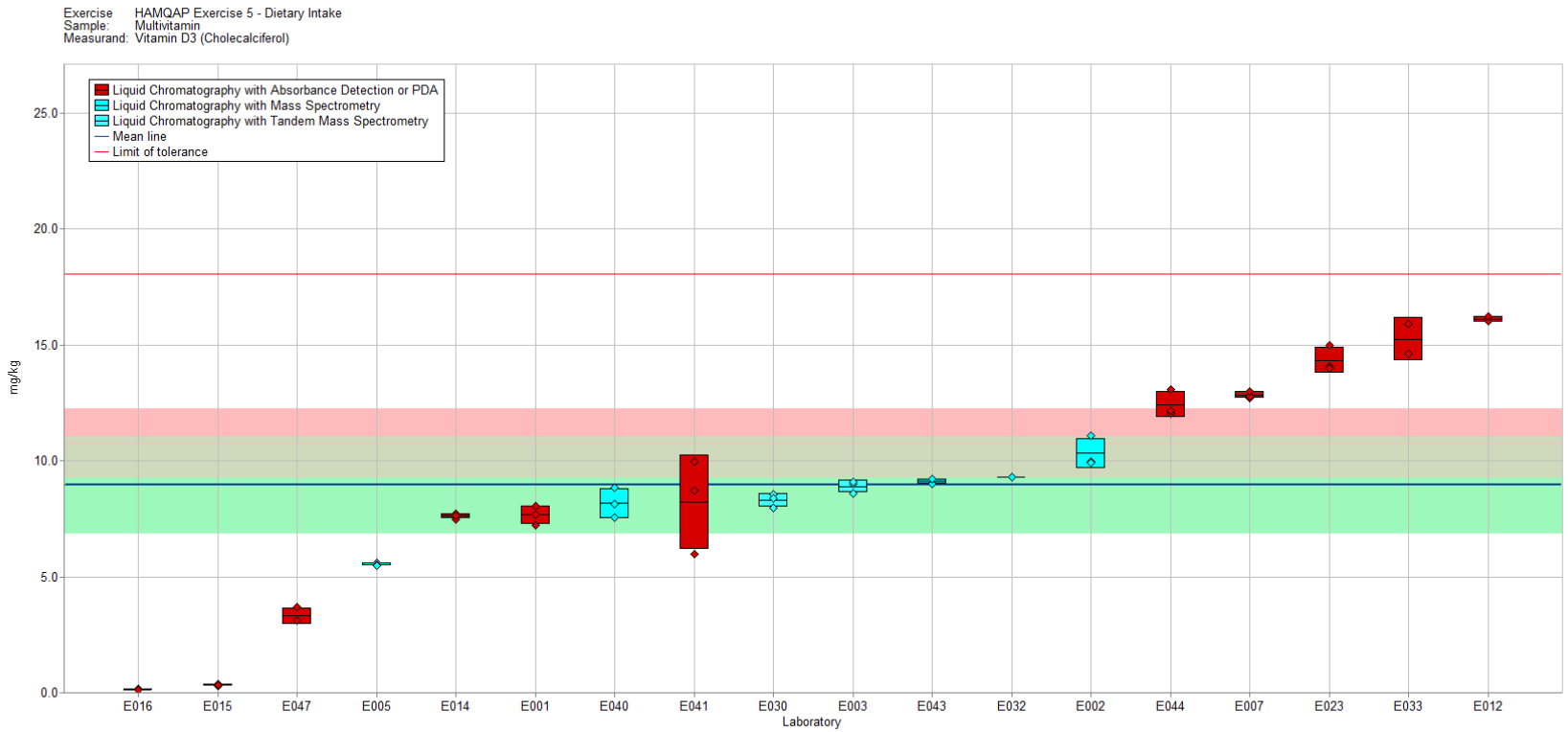

Figure 4-5. Vitamin D_3 (cholecalciferol) in Multivitamin (data summary view – analytical method). In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The 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 consensus range of tolerance, calculated as the value above the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$, with the lower limit set to zero. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by its uncertainty (U_{NIST}) and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$.

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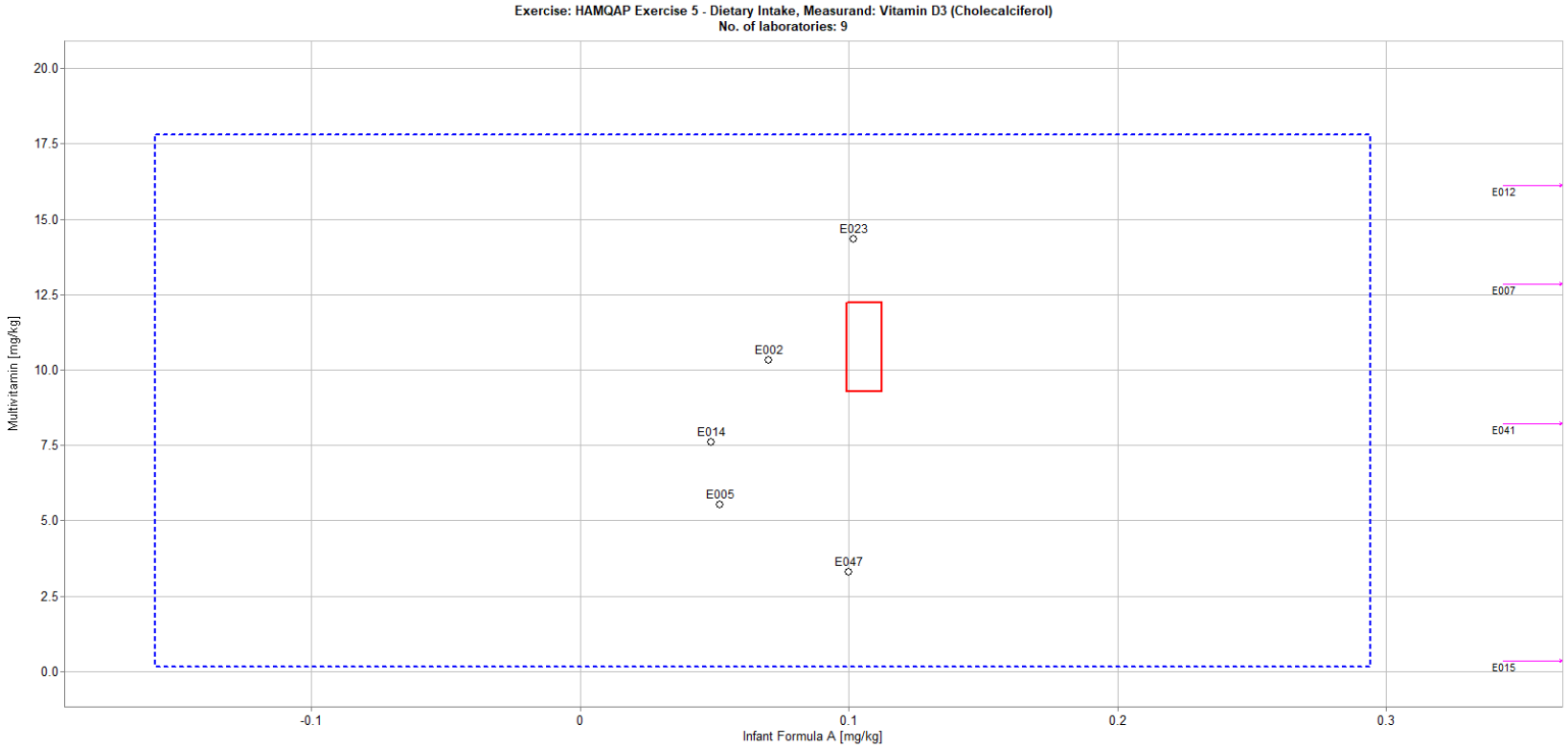

Figure 4-6. Laboratory means for Vitamin D3 (cholecalciferol) in Infant Formula A and Multivitamin (sample/sample comparison view). In this view, the individual laboratory mean for one sample (infant formula) is compared to the individual laboratory mean for a second sample (multivitamin). The solid red box represents the NIST range of tolerance for the two samples, infant formula (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 infant formula (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 4-4. Data summary table for total vitamin D 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.

		Total Vitamin D (Vitamin D2 + Vitamin D3)									
		Infant Formula A (mg/kg)					Multivitamin (mg/kg)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target				0.222	0.012				21.5	1.6
	E001						8.21	8.31	7.8	8.1	0.3
	E002	1.01	1.17	1.37	1.183	0.180	16.57	16.66	18.22	17.2	0.9
	E003						3.685	3.472	3.697	3.6	0.1
	E004										
	E005	0.1048	0.1027	0.1055	0.104	0.001	5.7823	5.7911	6.1622	5.9	0.2
	E006										
	E007	1.15	1.19	1.1	1.147	0.045	1.15	1.19	1.1	1.1	0.0
	E008										
	E010						6.1857	7.9077	8.497	7.5	1.2
	E011										
	E012	5	4.9	4.8	4.900	0.100	17	17	17.5	17.2	0.3
	E014	0.113	0.103	0.11	0.109	0.005	11.177	8.577	9.392	9.7	1.3
	E015	3.78	3.51	3.67	3.653	0.136	2.73	3.44	2.77	3.0	0.4
	E016						0.117	0.129	0.108	0.1	0.0
	E019										
	E020										
	E021										
	E023	0.211	0.2068	0.211	0.210	0.002	7.652	7.9198	7.903	7.8	0.2
	E024										
	E025										
	E027										
	E030						6.77	6.7	7.05	6.8	0.2
	E031										
	E032										
	E033						6.1	5.5		5.8	0.4
	E035										
	E037										
	E038										
	E040						7.98	7.17	7.35	7.5	0.4
	E041	10.9	12.1	12.5	11.833	0.833	10.9	12.1	12.5	11.8	0.8
	E042	0.00	0.00	0.00	0.000	0.000	0.00	0.00	0.00	0.0	0.0
	E043						8.15	8.03	8	8.1	0.1
	E044										
	E046										
	E047	0.1	0.1	0.1	0.100	0.000	0.1	0.1	0.1	0.1	0.0
	E057	0.216	0.223	0.222	0.220	0.004	0.102	0.109	0.1	0.1	0.0
	E072										
Community Results		Consensus Mean			0.332		Consensus Mean			6.0	
		Consensus Standard Deviation			0.125		Consensus Standard Deviation			1.2	
		Maximum			11.833		Maximum			17.2	
		Minimum			0.000		Minimum			0.0	
		N			11		N			19	

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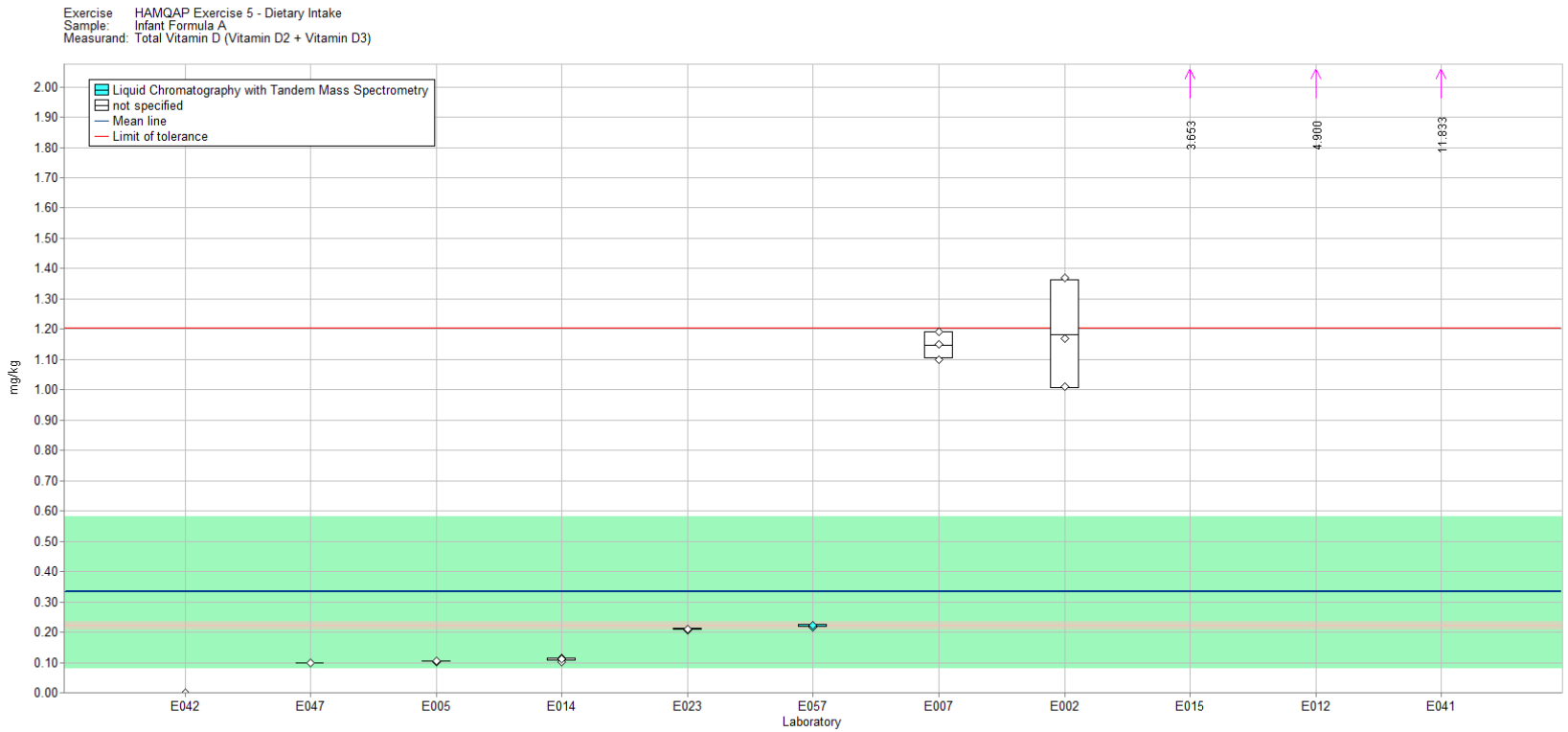

Figure 4-7. Total vitamin D in Infant Formula A (data summary view – analytical method). In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue 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 consensus range of tolerance, calculated as the value above the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\mathrm{comm}}| \leq 2$, with the lower limit set to zero. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by its uncertainty (U_{NIST}) and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\mathrm{NIST}}| \leq 2$.

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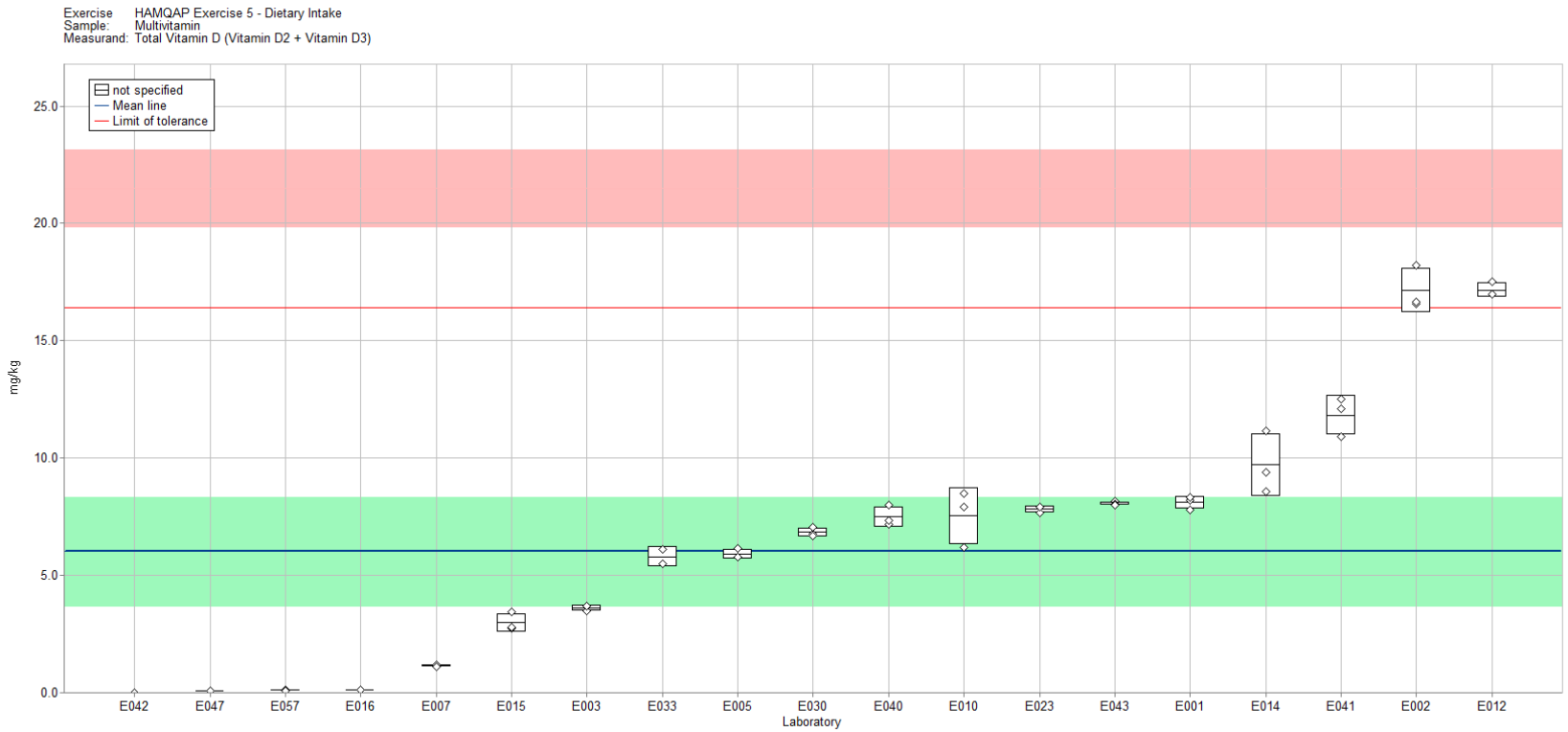

Figure 4-8. Total vitamin D in Multivitamin (data summary view – analytical method). In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red line represents the consensus range of tolerance, calculated as the value above the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$, with the lower limit set to zero. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by its uncertainty (U_{NIST}) and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$.

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Human Metabolites Sample Information

Human Serum F and G. Participants were provided with three vials of human serum F and three vials of human serum G, each containing nominally 1 mL of frozen human serum. Participants were asked to avoid exposing the material to direct sun or UV light, to store the material at or below –70 °C, and to prepare one sample and report one value from each vial provided. Before use, participants were instructed to allow the material to thaw at room temperature for at least 30 min prior to sampling, use the material immediately after thawing, and gently mix the contents prior to removal of a test portion for analysis. The approximate analyte levels were not reported to participants prior to the study. NIST assigned values were determined by isotope dilution liquid chromatography tandem mass spectrometry (ID-LC-MS/MS). The NIST-determined values and uncertainties for $25(OH)D_2$, $25(OH)D_3$, 3-epi-$25(OH)D_3$, and $24,25(OH)_2D_3$ are listed in the table below. Total 25(OH)D was calculated by the sum of $25(OH)D_2$ and $25(OH)D_3$. Target values for vitamin D_2, vitamin D_3, 3-epi-$25(OH)D_2$, and $1,25(OH)_2D_3$ in the human serum materials have not been determined by NIST.

	NIST-Determined Mass Fractions (µg/g)	
Analyte	Human Serum F	Human Serum G
$25(OH)D_2$	2.00 ± 0.10	23.49 ± 0.60
$25(OH)D_3$	11.9 ± 0.05	9.6 ± 0.48
Total 25(OH)D	13.9 ± 0.12	33.1 ± 0.8
3-epi-$25(OH)D_3$	0.49 ± 0.12	0.46 ± 0.16
$24,25(OH)_2D_3$	0.57 ± 0.04	0.73 ± 0.04

SRM 2972a 25-Hydroxyvitamin D Calibration Solutions. To increase participation and improve measurements of vitamin D metabolites in human serum matrices, participants were provided with one box consisting of four ampoules, each containing approximately 1.2 mL of ethanolic vitamin D solution. These solutions were intended to be used for calibrant preparation. Participants were asked to store the ampoules in -20 °C or colder prior to use. The analyte concentration information can be found on the certificate for SRM 2972a 25-Hydroxyvitamin D Calibration Solutions.[15]

Human Metabolites Study Results

- Between seven and fourteen laboratories enrolled in this exercise and received samples to measure vitamin D and vitamin D metabolites in human serum (see table below). For many of the measurands, participation was < 25 %. This could be due to difficulty in measuring the analytes or due to the lack of interest in quantifying certain measurands in these matrices.

[15] SRM 2972a 25-Hydroxyvitamin D Calibration Solutions Certificate of Analysis. National Institute of Standards and Technology (NIST). https://www-s.nist.gov/srmors/certificates/2972A.pdf (accessed August 2020).

Analyte	Number of Laboratories Requesting Samples	Number of Laboratories Reporting Results (Percent Participation)	
		Human Serum F	Human Serum G
$25(OH)D_2$	12	5 (42 %)	6 (50 %)
$25(OH)D_3$	14	8 (57 %)	8 (57 %)
Total 25(OH)D	12	2 (17 %)	2 (17 %)
3-epi-$25(OH)D_3$	9	3 (33 %)	3 (33 %)
3-epi-$25(OH)D_2$	7	0	0
$1,25(OH)_2D_3$	8	2 (25 %)	2 (25 %)
$24,25(OH)_2D_3$	8	2 (25 %)	2 (25 %)
Vitamin D2	9	0	0
Vitamin D3	10	0	1 (10 %)

- The between-laboratory variabilities were acceptable for total 25(OH)D, $25(OH)D_3$ and $25(OH)D_2$, but high for 3-epi-$25(OH)D_3$ considering that calibration solutions were provided for all three of these measurands. The RSDs are difficult to interpret for $1,25(OH)_2D_3$ and $24,25(OH)D_2D_3$ due to the low participation, but the two laboratories that reported results were both within or close to the target ranges. Variabilities for each analyte/sample pair are reported in the table below.

Analyte	Between-Laboratory Variability (% RSD)	
	Human Serum F	Human Serum G
$25(OH)D_2$	15%	9%
$25(OH)D_3$	2%	5%
Total 25(OH)D	6%	15%
3-epi-$25(OH)D_3$	31%	21%
$1,25(OH)_2D_3$	> 100%	>100%
$24,25(OH)_2D_3$	3%	7%

- All laboratories reported using LC-MS or LC-MS/MS for the detection of vitamin D metabolites in human serum.
- For $25(OH)D_3$, the consensus mean was only slightly higher than the target range in Serum F (**Figure 4-9**) and within the target range for Serum G (**Figure 4-10**), with most individual laboratory results within 10% of the target value. One laboratory reported a value much higher than the target range.
- For $25(OH)D_2$, the consensus means were within the target ranges for Serum F (**Figure 4-12**) and Serum G (**Figure 4-13**), but with individual laboratory results around 20% from the target value
- For 3-epi-$25(OH)D_3$, the consensus means were higher than the ranges for Serum F (**Figure 4-15**) and Serum G (**Figure 4-16**).

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- For 24,25$(OH)_2D_3$, even though only two laboratories reported results, the consensus means were within the target ranges for Serum F (**Figure 4-17**) and Serum G (**Figure 4-18**), with individual laboratory results within 10% of the target value.

Human Metabolites Technical Recommendations

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

- Optimization of chromatography conditions and MS parameters is important for ensuring accurate and reproducible results. For accurate determination with LC-MS techniques, complete resolution of interfering compounds (i.e., 25$(OH)D_3$ and 3-epi-25$(OH)D_3$) prior to MS detection is critical. Coelution is a common cause of biased or erroneous results.
- Sample preparation should always be optimized to aim for total extraction and reduce analyte degradation or loss.
- Laboratories should ensure that calibration solutions are prepared accurately. Obtaining accurate purity assessment, using mass instead of volume for solution preparation, and reduced lighting are all examples of things that could improve calibration.
- For 24,25$(OH)D_3$, the two participating laboratories show great promise in the ability to measure 24,25$(OH)D_3$ in human serum. However, overall community capabilities cannot be assessed due to the low participation rate.
- 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.
- 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. For this particular study, participants were provided with a box of SRM 2972a 25-Hydroxyvitamin D Calibration Solutions to assist in the calibration aspect of these measurements.

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Table 4-5. Individualized data summary table (NIST) for vitamin D and vitamin D metabolites in human serum.

National Institute of Standards & Technology

HAMQAP Exercise 5 - 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*
Vitamin D_3 (Cholecalciferol)	Human Serum F	ng/mL					0				
Vitamin D_3 (Cholecalciferol)	Human Serum G	ng/mL					1				
Vitamin D_2 (Ergocalciferol)	Human Serum F	ng/mL					0				
Vitamin D_2 (Ergocalciferol)	Human Serum G	ng/mL					0				
25-Hydroxyvitamin D_3	Human Serum F	ng/mL	11.9	0.05			8	12.6	0.28	11.9	0.05
25-Hydroxyvitamin D_3	Human Serum G	ng/mL	9.6	0.48			8	10.3	0.52	9.6	0.48
25-Hydroxyvitamin D_2	Human Serum F	ng/mL	2.00	0.10			5	2.45	0.37	2.00	0.10
25-Hydroxyvitamin D_2	Human Serum G	ng/mL	23.5	0.6			6	27.4	2.3	23.5	0.6
Total 25-Hydroxyvitamin D	Human Serum F	ng/mL	13.9	0.12			2	14.8	0.84	13.9	0.12
Total 25-Hydroxyvitamin D	Human Serum G	ng/mL	33.1	0.8			2	37.9	5.6	33.1	0.8
3-epi-25-Hydroxvitamin D_3	Human Serum F	ng/mL	0.49	0.12			3	1.25	0.39	0.49	0.12
3-epi-25-Hydroxvitamin D_3	Human Serum G	ng/mL	0.46	0.16			3	1.11	0.23	0.46	0.16
3-epi-25-Hydroxvitamin D_2	Human Serum F	ng/mL					0				
3-epi-25-Hydroxvitamin D_2	Human Serum G	ng/mL					0				
1,25-Dihydroxyvitamin D_3	Human Serum F	ng/mL					2	0.2	0.4		
1,25-Dihydroxyvitamin D_3	Human Serum G	ng/mL					2	0.2	0.54		
24,25-Dihydroxyvitamin D_3	Human Serum F	ng/mL	0.57	0.04			2	0.551	0.016	0.57	0.04
24,25-Dihydroxyvitamin D_3	Human Serum G	ng/mL	0.73	0.04			2	0.674	0.049	0.73	0.04

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 25-hydroxyvitamin D_3 in human serum. Data points highlighted in red have been flagged as potential outliers (e.g., Grubb and/or Cochran) by the NIST software package.

		25-Hydroxyvitamin D_3									
		Human Serum F (ng/mL)					Human Serum G (ng/mL)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target				11.9	0.05				9.60	0.48
	E002										
	E014										
	E024										
	E038										
	E057										
	E063	87.36	89.856	84.864	87.360	2.496	72.384	64.896	69.888	69.06	3.81
	E064	12.2	12	12.3	12.167	0.153	9.59	9.53	9.5	9.54	0.05
	E065	13.9	12.5	12.7	13.033	0.757	10.1	9.14	11.2	10.15	1.03
	E066	12.8	13.8	37.9	21.500	14.212	11	10.5	12.5	11.33	1.04
	E067										
	E068	12.9	12.7	12.9	12.833	0.115	9.58	9.94	10.7	10.07	0.57
	E069	12.7	12.5	12.5	12.567	0.115	9.89	9.8	10	9.90	0.10
	E070	12.22	12.3	12.65	12.390	0.229	12	11.69	11.5	11.73	0.25
	E071	11.901	14.032	11.681	12.538	1.299	8.075	9.06	10.681	9.27	1.32
Community Results		Consensus Mean			12.588		Consensus Mean			10.28	
		Consensus Standard Deviation			0.281		Consensus Standard Deviation			0.52	
		Maximum			87.360		Maximum			69.06	
		Minimum			12.167		Minimum			9.27	
		N			8		N			8	

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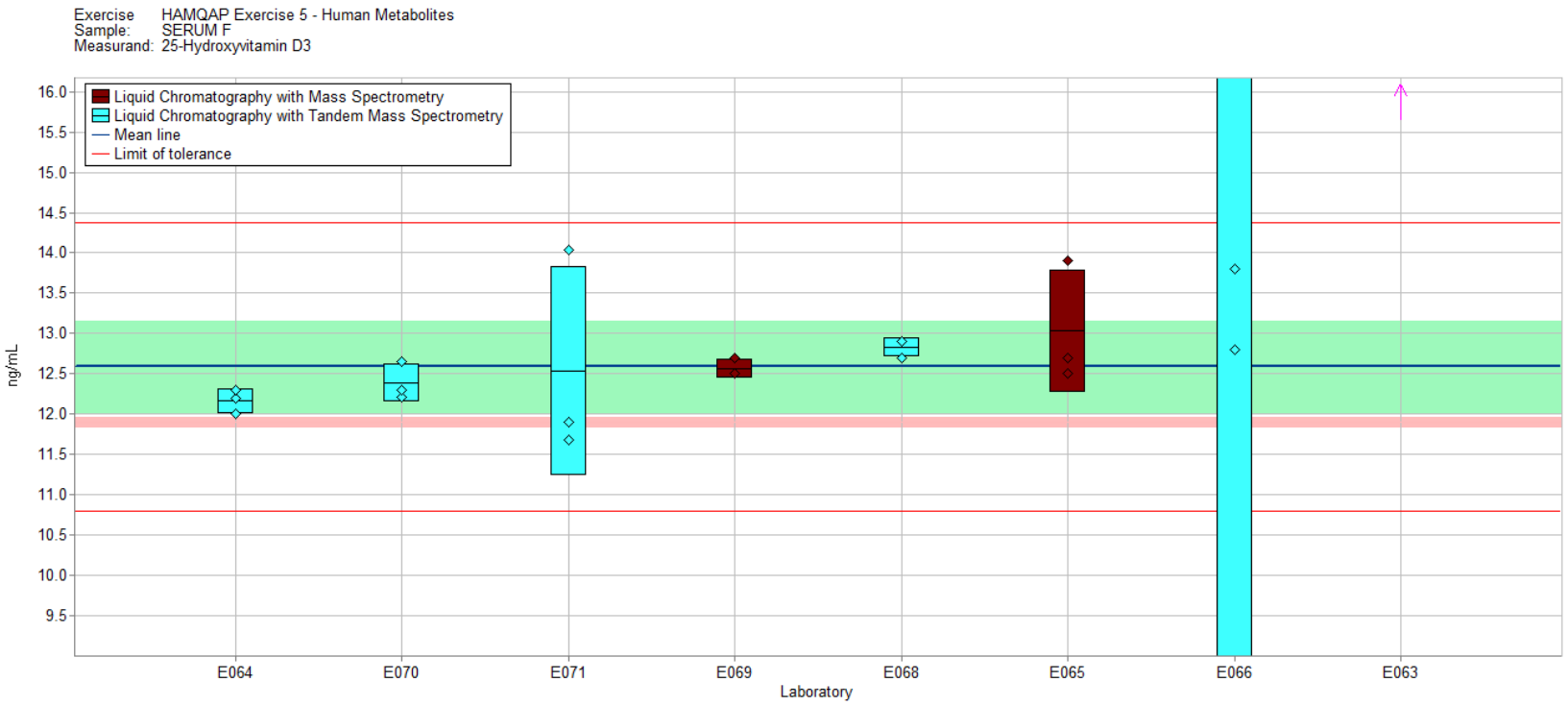

Figure 4-9. 25-Hydroxyvitamin D_3 in Human Serum F (data summary view – analytical method). In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue 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, $|Z_{\text{NIST}}| \leq 2$.

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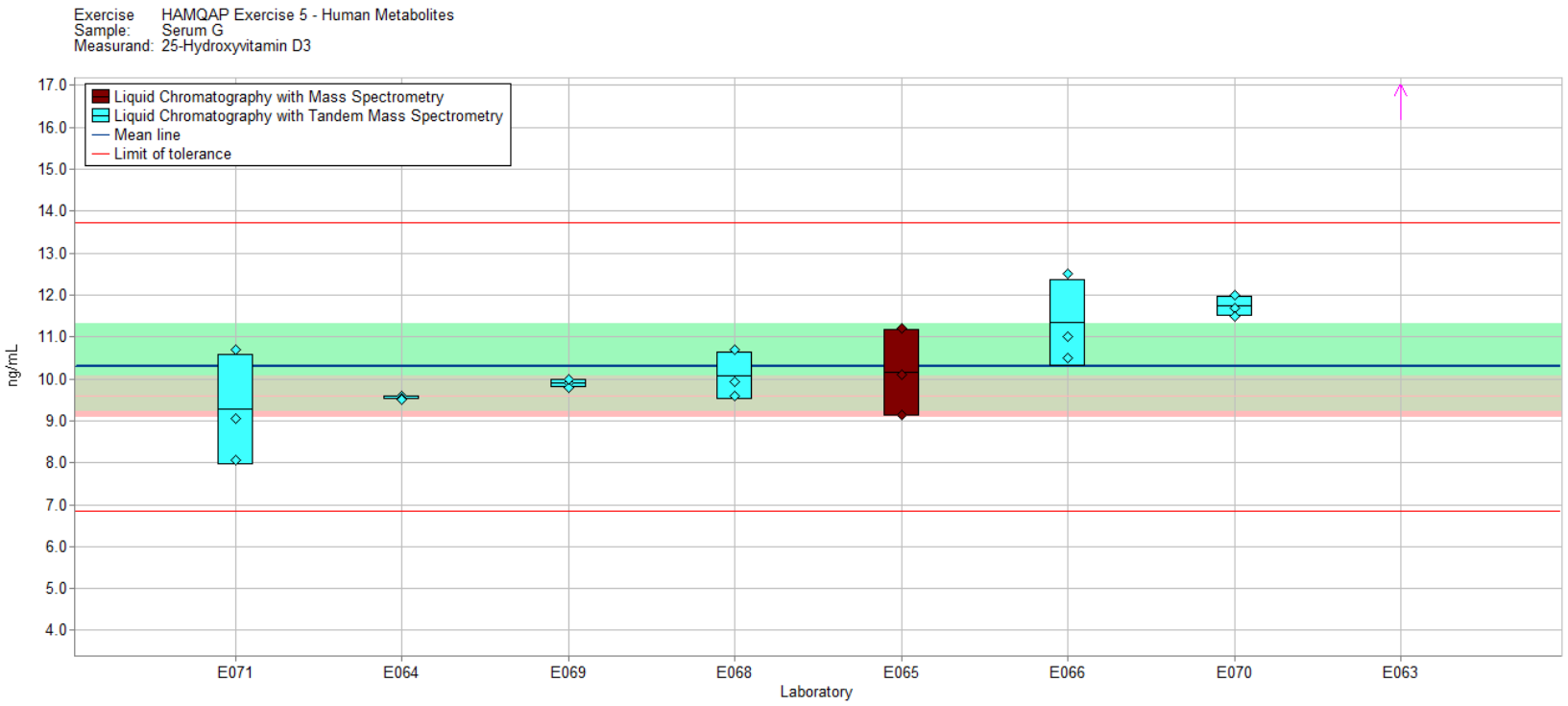

Figure 4-10. 25-Hydroxyvitamin D_3 in Human Serum G (data summary view – analytical method). In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid 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, $|Z_{\text{NIST}}| \leq 2$.

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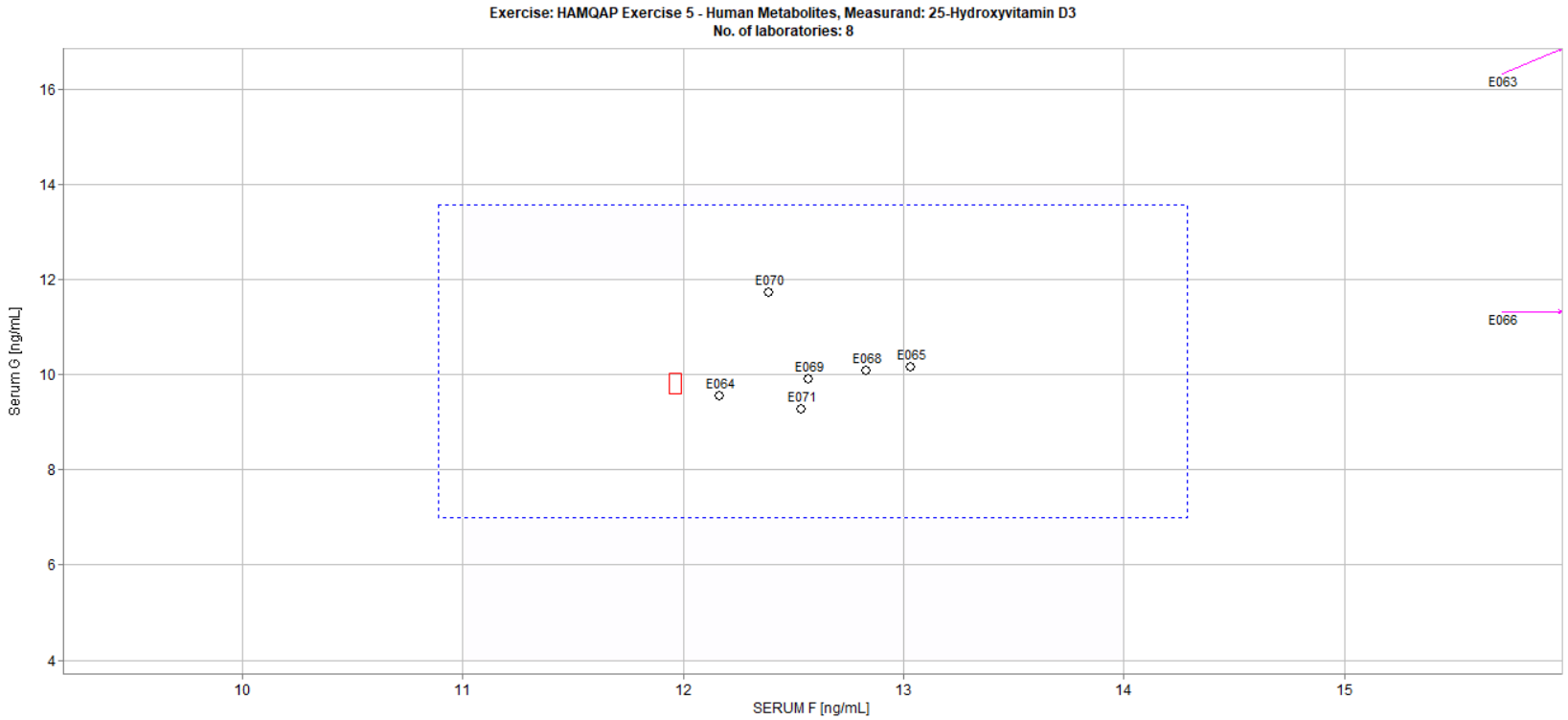

Figure 4-11. Laboratory means for 25-hydroxyvitamin D_3 in Human Serum F and Human Serum G (sample/sample comparison view). In this view, the individual laboratory mean for one sample (serum F) is compared to the individual laboratory mean for a second sample (serum G). The solid red box represents the NIST range of tolerance for the two samples, serum F (x-axis) and serum G (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 infant formula (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 4-7. Data summary table for 25-hydroxyvitamin D_2 in human serum. Data points highlighted in red have been flagged as potential outliers (e.g., Grubb and/or Cochran) by the NIST software package.

		25-Hydroxyvitamin D_2									
		Human Serum F (ng/mL)					Human Serum G (ng/mL)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target				2.00	0.10				23.49	0.60
	E002										
	E014										
	E024										
	E038										
	E057										
	E063						117.312	122.304	112.32	117.31	4.99
	E064	2.62	2.56	2.51	2.56	0.06	27.1	29.1	27.6	27.93	1.04
	E065	1.8	1.1	1.23	1.38	0.37	28.6	38	26.5	31.03	6.12
	E067										
	E068	2.53	2.43	2.35	2.44	0.09	23.8	25.3	23.4	24.17	1.00
	E069	2.23	2.15	2.15	2.18	0.05	24.9	23.8	24.2	24.30	0.56
	E071	4.493	5.092	4.49	4.69	0.35	29.582	28.196	30.383	29.39	1.11
Community Results		Consensus Mean			2.45		Consensus Mean			27.36	
		Consensus Standard Deviation			0.37		Consensus Standard Deviation			2.35	
		Maximum			4.69		Maximum			117.31	
		Minimum			1.38		Minimum			24.17	
		N			5		N			6	

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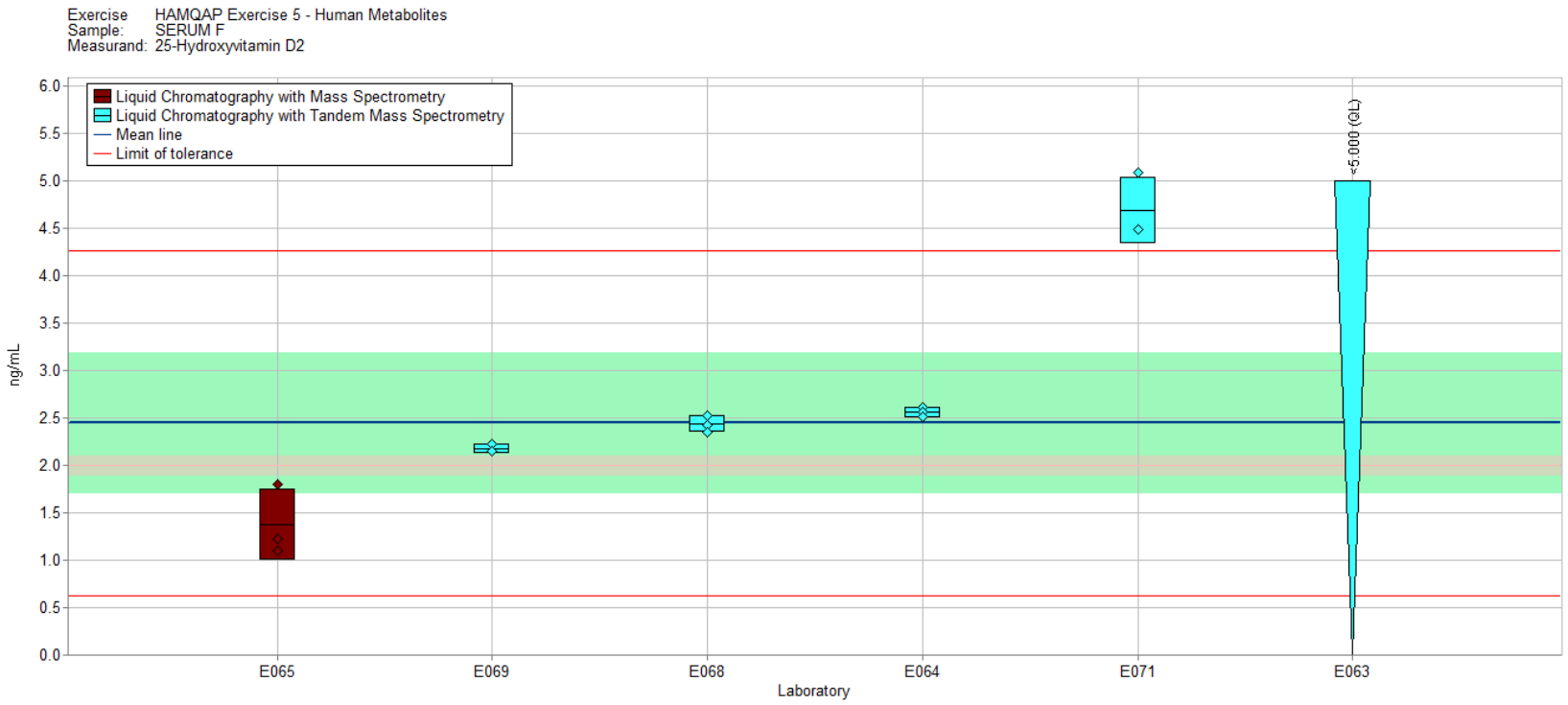

Figure 4-12. 25-Hydroxyvitamin D_2 in Human Serum F (data summary view – analytical method). In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid 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, $|Z_{\text{NIST}}| \leq 2$.

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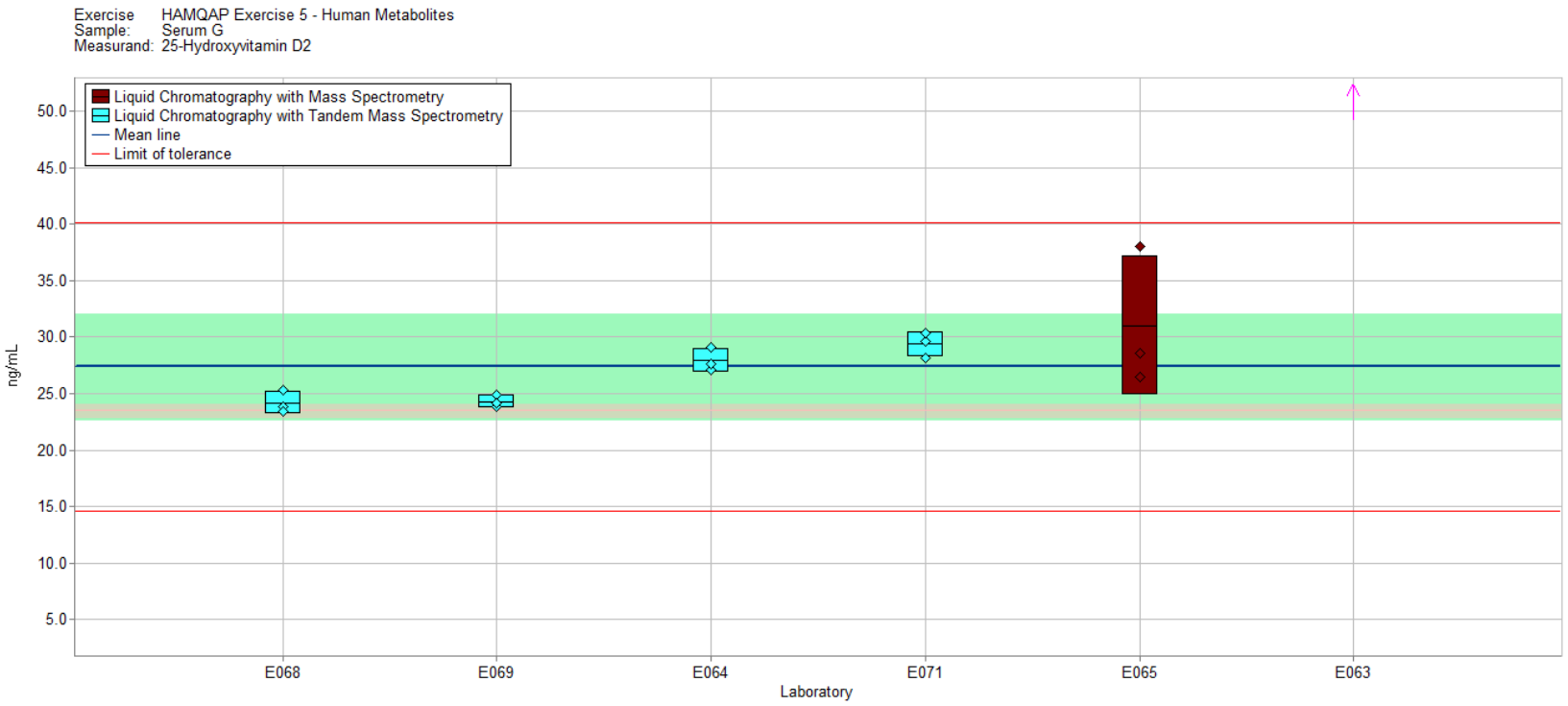

Figure 4-13. 25-Hydroxyvitamin D_2 in Human Serum G (data summary view – analytical method). In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid 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, $|Z_{\text{NIST}}| \leq 2$.

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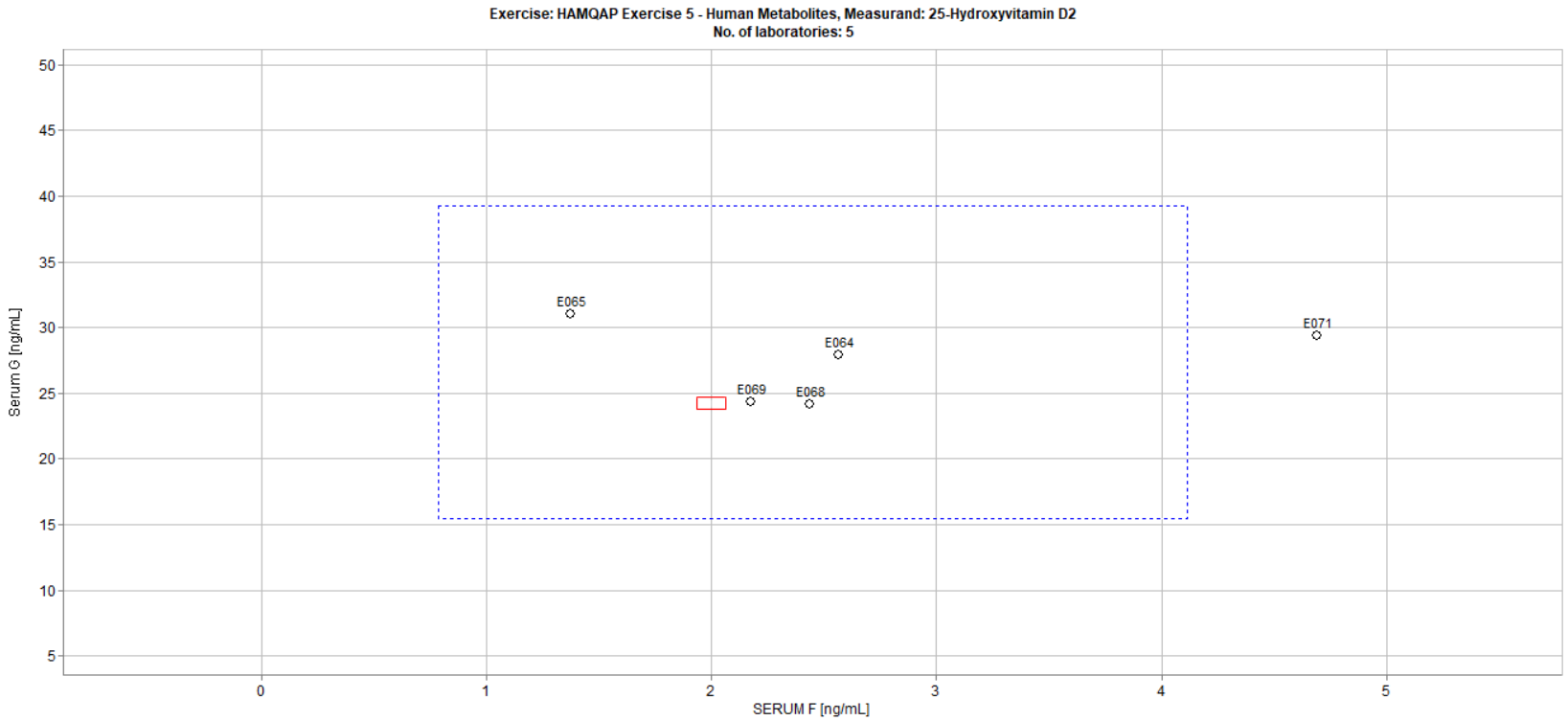

Figure 4-14. Laboratory means for 25-hydroxyvitamin D_2 in Human Serum F and Human Serum G (sample/sample comparison view). In this view, the individual laboratory mean for one sample (serum F) is compared to the individual laboratory mean for a second sample (serum G). The solid red box represents the NIST range of tolerance for the two samples, serum F (x-axis) and serum G (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 infant formula (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 4-8. Data summary table for 3-epi-25-hydroxvitamin D_3 in human serum.

		3-epi-25-Hydroxvitamin D_3									
		Human Serum F (ng/mL)					Human Serum G (ng/mL)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target				0.49	0.12				0.46	0.16
	E002										
	E014										
	E024										
	E038										
	E057										
	E064	0.845	0.795	0.722	0.79	0.06	0.919	0.729	0.818	0.82	0.10
	E065										
	E068	0.91	1.12	1.02	1.02	0.11	0.83	0.99	0.97	0.93	0.09
	E071	1.849	2.096	1.9	1.95	0.13	1.588	1.44	1.711	1.58	0.14
Community Results		Consensus Mean			1.25		Consensus Mean			1.11	
		Consensus Standard Deviation			0.39		Consensus Standard Deviation			0.23	
		Maximum			1.95		Maximum			1.58	
		Minimum			0.79		Minimum			0.82	
		N			3		N			3	

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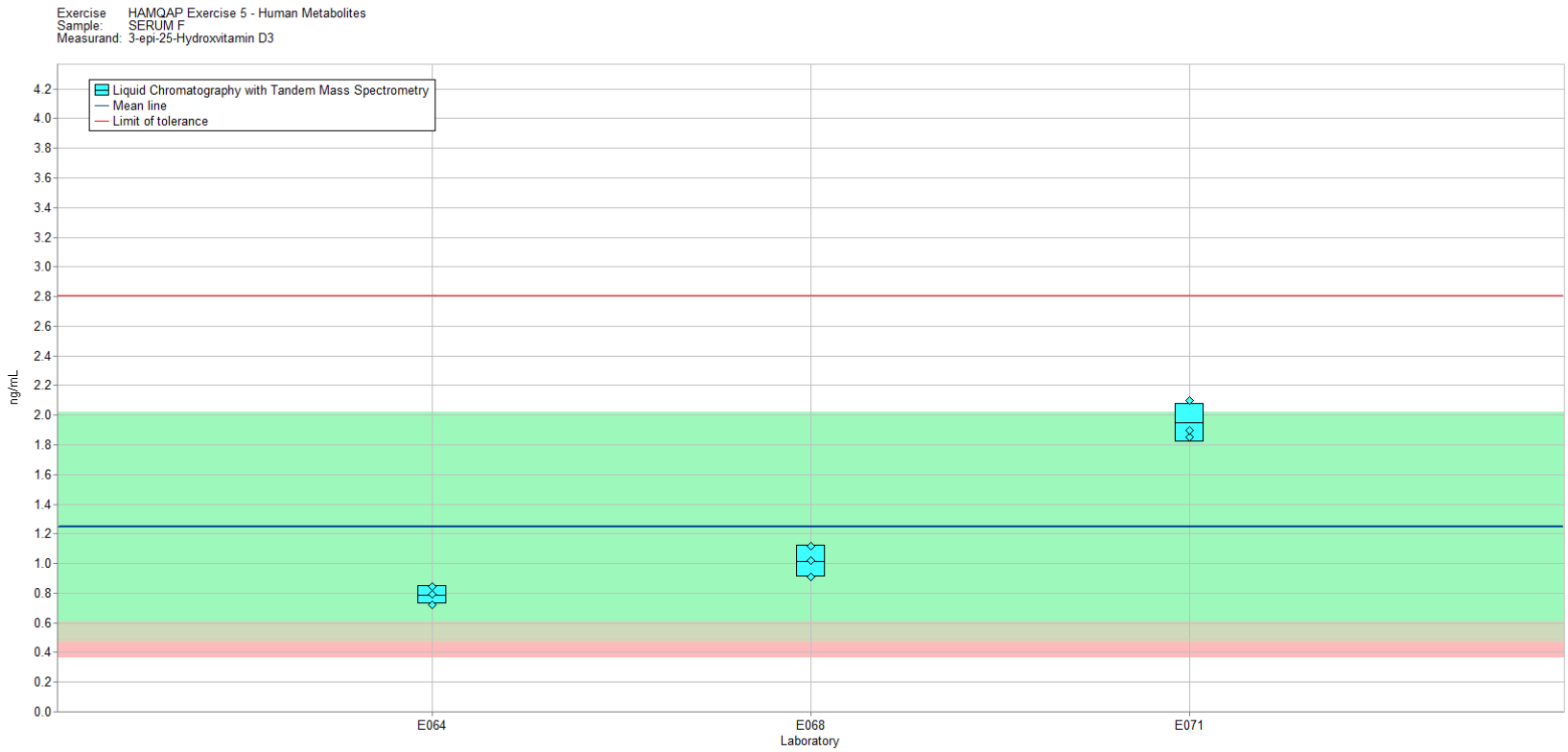

Figure 4-15. 3-epi-25-Hydroxyvitamin D_3 in Human Serum F (data summary view – analytical method). In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The 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 represent the consensus range of tolerance, calculated as the value above the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$, with the lower limit set to zero. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by its uncertainty (U_{NIST}) and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$.

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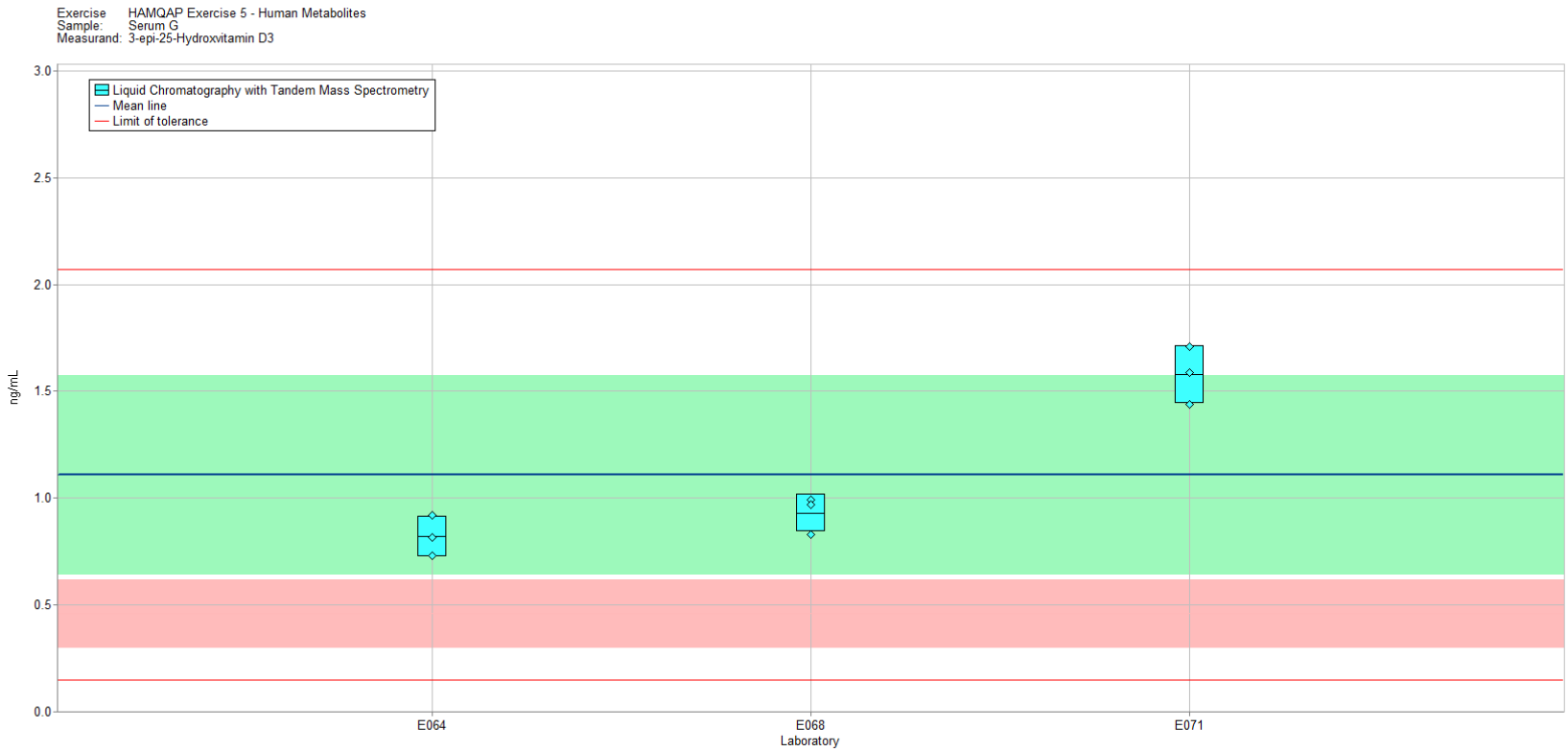

Figure 4-16. 3-epi-25-Hydroxyvitamin D_3 in Human Serum G (data summary view – analytical method). In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The 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, $|Z_{\text{NIST}}| \leq 2$.

Table 4-9. Data summary table for 1,25-dihydroxyvitamin D_3 in human serum.

		1,25-Dihydroxyvitamin D_3									
		Human Serum F (ng/mL)					Human Serum G (ng/mL)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target										
	E002										
	E014										
	E024										
	E038										
	E063	0.3024	0.312	0.3072	0.307	0.005	0.3744	0.396	0.3672	0.379	0.015
	E065										
	E066	0.0448	0.0517	0.0443	0.047	0.004	0.0231	1.00E-10	0.0311	0.018	0.016
	E071										
Community Results		Consensus Mean			0.177		Consensus Mean			0.199	
		Consensus Standard Deviation			0.403		Consensus Standard Deviation			0.539	
		Maximum			0.307		Maximum			0.379	
		Minimum			0.047		Minimum			0.018	
		N			2		N			2	

Table 4-10. Data summary table for 24,25-dihydroxyvitamin D_3 in human serum.

		24,25-Dihydroxyvitamin D_3									
		Human Serum F (ng/mL)					Human Serum G (ng/mL)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target				0.57	0.04				0.73	0.04
	E002										
	E014										
	E024										
	E038										
	E064	0.557	0.53	0.539	0.542	0.014	0.668	0.657	0.62	0.648	0.025
	E065										
	E069	0.57	0.58	0.53	0.560	0.026	0.71	0.67	0.72	0.700	0.026
	E071										
Community Results		Consensus Mean			0.551		Consensus Mean			0.674	
		Consensus Standard Deviation			0.016		Consensus Standard Deviation			0.049	
		Maximum			0.560		Maximum			0.700	
		Minimum			0.542		Minimum			0.648	
		N			2		N			2	

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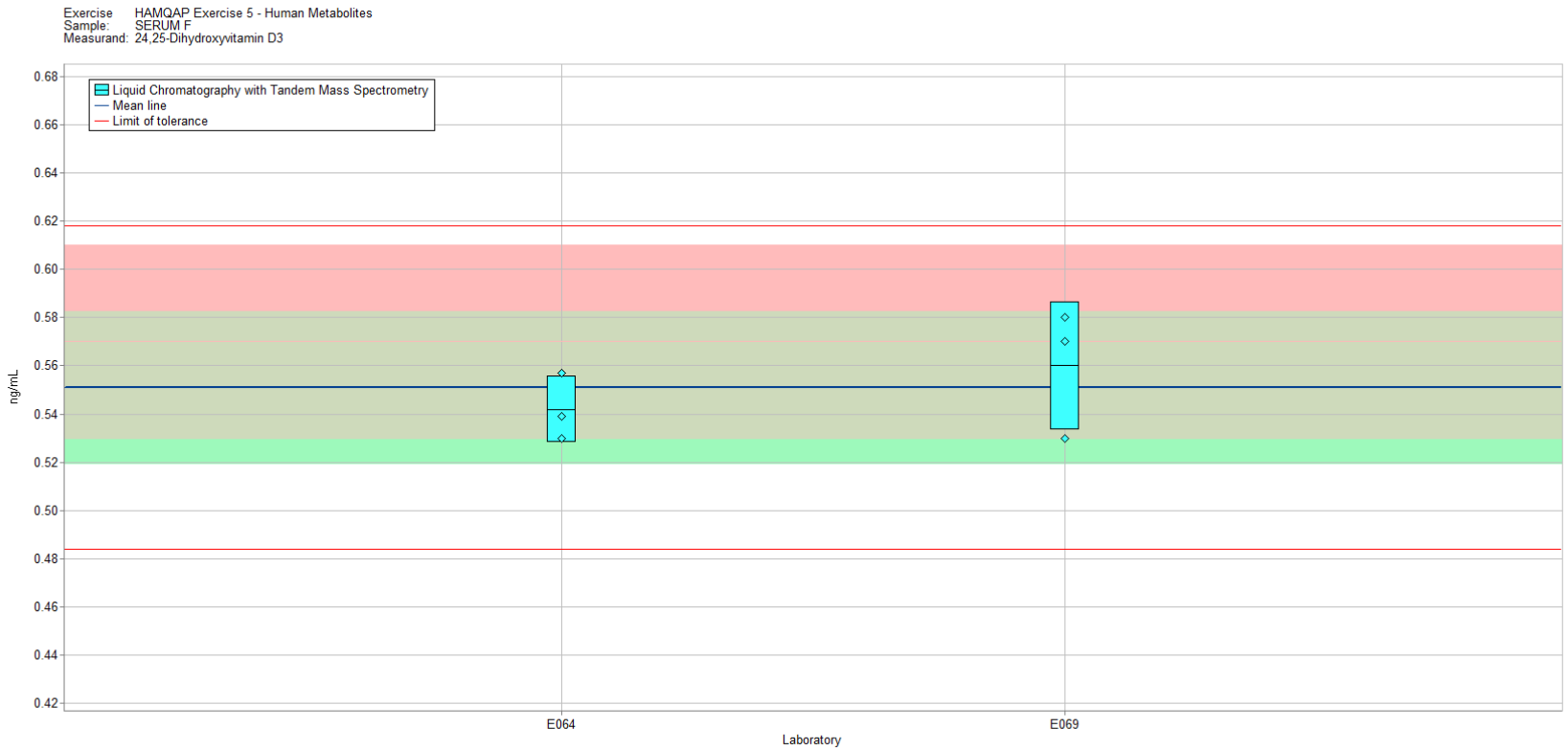

Figure 4-17. 24,25-Dihydroxyvitamin D_3 in Human Serum F (data summary view – analytical method). In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid 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, $|Z_{\text{NIST}}| \leq 2$.

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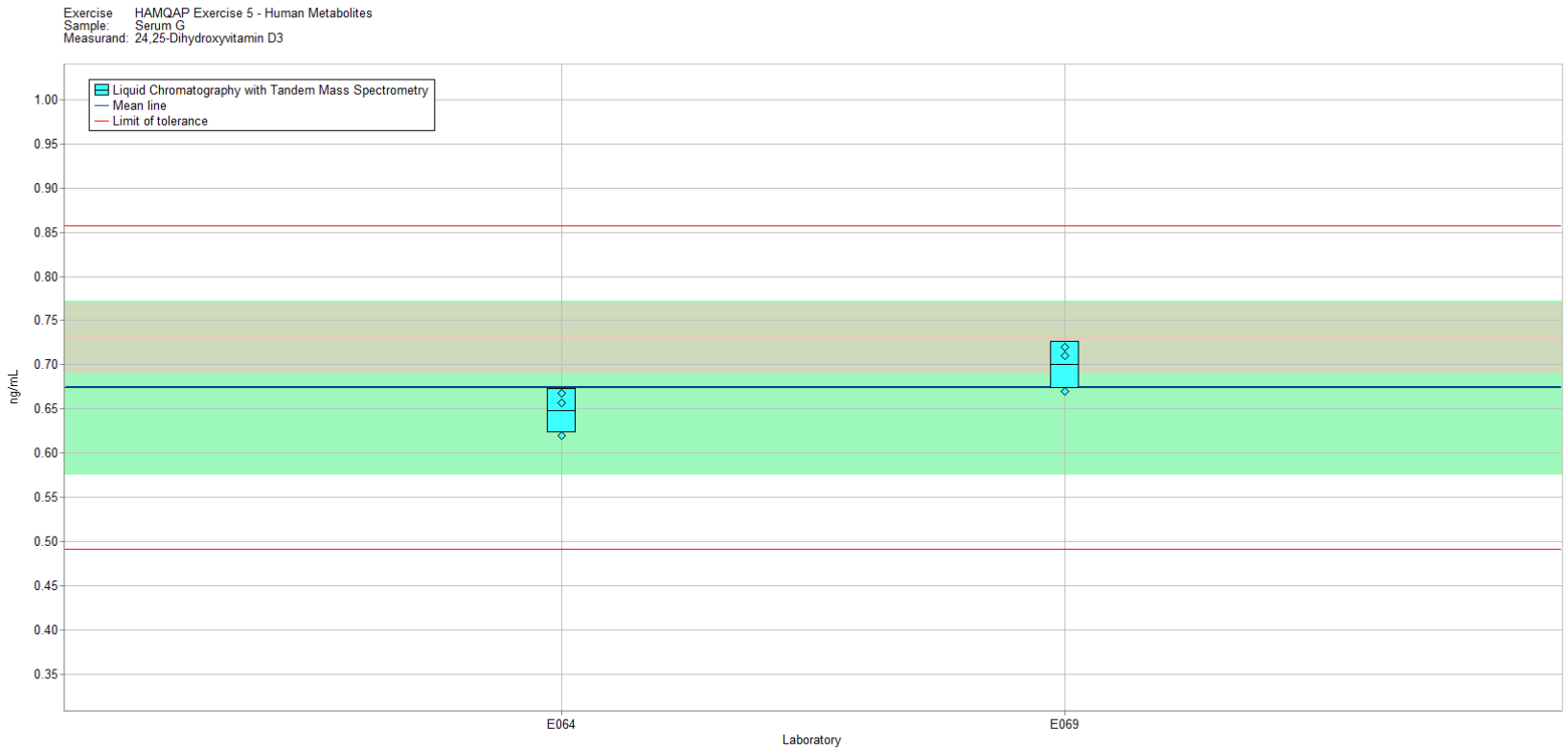

Figure 4-18. 24,25-Dihydroxyvitamin D_3 in Human Serum G (data summary view – analytical method). In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid 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, $|Z_{\text{NIST}}| \leq 2$.

Fat-Soluble Vitamins Overall Study Comparison

Overall, laboratories measuring vitamin D and vitamin D metabolites in infant formula, multivitamin, and serum matrices were successful based on the limited results reported.

- A few laboratories reported data outside of the target ranges for the intake samples, but overall results were excellent. Laboratories reporting values significantly higher than the target and consensus range for vitamin D in the infant formula should double check their calculations, and assure they are using appropriate calibration materials and quality assurance samples to establish that their method is in control and being performed correctly.
- Clinical laboratories had lower participation, but those laboratories reporting results were in good agreement. Knowing that patient vitamin D status is routinely assessed and the significance of these metabolites in clinical research, the low sign up and low participation numbers are unexpected. The limited number of participating laboratories could indicate the measurement is challenging or that clinical community interest is focused on only a few analytes, with limited interest for metabolites other than $25(OH)D_2$ and $25(OH)D_3$.
- Calibration solutions were provided for the human metabolites study and had a positive impact on the measurements made by reporting laboratories.

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

Study Overview

In this study, participants were provided with samples of SRM 3275 Omega-3 and Omega-6 Fatty Acids in Fish Oil Level 3, aquacultured salmon, and wild salmon for dietary intake, as well as two samples of human red blood cells (RBC) for human metabolism. Participants were asked to use in-house analytical methods to determine the mass fraction (mg/g) of omega-3 and omega-6 fatty acids in each intake matrix and percentage of total RBC fatty acids (%) in each metabolism sample. Omega-3 fatty acids are important components of the phospholipids that form the structures of cell membranes.[16] In addition, omega-3 and omega-6 fatty acids provide energy for the body and are used to form eicosanoids, which are mediators of inflammation, vasoconstriction, and platelet aggregation. Some researchers propose that the relative intakes of omega-3 and omega-6 fatty acids may have important implications for the pathogenesis of chronic diseases such as cardiovascular disease and cancer, but an optimal ratio has not been defined. Biomedical research has mostly focused on three omega-3 fatty acids, α-linolenic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA), and two omega-6 fatty acids, linoleic acid and arachidonic acid (AA or ARA). Dietary sources of EPA and DHA include fish and fish oils, as fatty acids originally synthesized by microalgae further down the food chain accumulate in fish tissues. ALA and other omega-6 fatty acids can be found in plant sources such as plant oils, chia seeds, and walnuts. Omega-3 and omega-6 fatty acid health status can be evaluated by measuring individual components in plasma or serum phospholipids, but values can vary substantially based on an individual's most recent intake and as such do not reflect long-term dietary consumption. Understanding intake of omega-3 and omega-6 fatty acids and their impact on inflammation and disease can advance clinical research investigating how manipulating the omega-6 to omega-3 ratio may yield positive health outcomes. In addition, the fatty acid profile of aquacultured seafood may differ from that of wild-caught seafood, based on the water and nutrient sources, use of antibiotics, or exposure to pollutants. In this study, measurement of these important fatty acids will reveal possible sources of variability between laboratories and determine if significant differences exist between the types and levels of fatty acids found in aquacultured and wild-caught salmon. For identification of farmed salmon fraudulently labeled as wild-caught, the ratios of the fatty acid species may be even more important than the actual concentrations.

Dietary Intake Sample Information

Fish Oil. Participants were provided with three ampoules of SRM 3275 Omega-3 and Omega-6 Fatty Acids in Fish Oil Level 3, each containing 1.2 mL of fish oil. Level 3 is a concentrate containing 60 % long-chain omega-3 fatty acids. Participants were asked to store the material under refrigeration, 2 °C to 8 °C, in the original unopened ampoules and to prepare one sample and report one value from each ampoule provided. Before use, participants were instructed to thoroughly mix the contents of the ampoule prior to removal of a test portion for analysis, and to use a sample size of at least 0.5 g. The approximate analyte levels were not reported to participants prior to the study. A certified value for linoleic acid in SRM 3275 Level 3 was assigned using results from NIST by GC-FID and GC-MS. Reference values for ALA, EPA, and DHA in SRM 3275 Level 3 were assigned using results from NIST by GC-FID. The NIST-determined

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

values and uncertainties for omega-3 and omega-6 fatty acids in SRM 3275 are provided in the table below, reported both as the fatty acid methyl esters (FAMEs) as listed on the Certificate of Analysis and as the free fatty acids (FFAs), using standard molecular weight conversion factors, with expanded uncertainties for the purpose of determining Z_{NIST} scores.[17] A target value for arachidonic acid was not determined.

	NIST-Determined Mass Fractions in SRM 3275-3 (mg/g)	
Analyte	(FAMEs from COA)	(FFAs with Expanded U)
ALA	6.61 ± 0.31	6.29 ± 0.59
Linoleic Acid	13.49 ± 0.45	12.85 ± 0.86
EPA	154 ± 9	150 ± 20
DHA	104 ± 5	100 ± 10

Aquacultured Salmon. Participants were provided with a single jar containing approximately 6 g of aquacultured Coho salmon (*Oncorhynchus kisutch*) obtained from Rochester, WA. Edible parts of the salmon were cryomilled and the fresh frozen powder bottled in glass jars and stored at –80 °C. Participants were asked to store the material at –70 °C or colder in the original unopened jar until use to ensure the material retains its powdered form. Participants were asked to prepare three samples and to report three values from the single jar provided. Before use, participants were instructed to keep the jar on dry ice during sampling, to mix the contents of the jar thoroughly, and take samples immediately upon removal from the freezer. If the sample in the jar lost the powdered form, participants were instructed to refreeze at –70 °C or colder for several hours to allow the material to return to the powdered form. If the material was stored at –20 °C, participants were instructed to allow the material to thaw completely then blend the contents of the entire jar, preferably with a handheld homogenizer or immersion blender, prior to sampling. A sample size of at least 0.5 g was recommended. The approximate analyte levels were not reported to participants prior to the study. Target values for linoleic acid, ARA, EPA, and DHA in the aquacultured salmon were assigned using duplicate results from NIST by GC-FID. The NIST-determined values and uncertainties for omega-3 and omega-6 fatty acids in the aquacultured salmon are provided in the table below. A target value for ALA was not determined.

Analyte	NIST-Determined Mass Fractions in Aquacultured Salmon (mg/g)
Linoleic Acid	6.59 ± 0.84
ARA	0.91 ± 0.21
EPA	5.97 ± 0.98
DHA	10.2 ± 2.3

Wild Salmon. Participants were provided with a single jar containing approximately 6 g of wild Coho salmon (*Oncorhynchus kisutch*) caught off the coast of Alaska. Edible parts of the salmon were cryomilled and the fresh frozen powder bottled in glass jars and stored at –80 °C. Participants

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

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were asked to store the material at –70 °C or colder in the original unopened jar until use to ensure the material retains its powdered form. Participants were asked to prepare three samples and to report three values from the single jar provided. Before use, participants were instructed to keep the jar on dry ice during sampling, to mix the contents of the jar thoroughly, and take samples immediately upon removal from the freezer. If the sample in the jar lost the powdered form, participants were instructed to refreeze at –70 °C or colder for several hours to allow the material to return to the powdered form. If the material was stored at –20 °C, participants were instructed to allow the material to thaw completely then blend the contents of the entire jar, preferably with a handheld homogenizer or immersion blender, prior to sampling. A sample size of at least 0.5 g was recommended. The approximate analyte levels were not reported to participants prior to the study. Target values for ALA, EPA, and DHA in the wild salmon were assigned using duplicate results from NIST by GC-FID. The NIST-determined values and uncertainties for omega-3 and omega-6 fatty acids in the wild salmon are provided in the table below. Target values for linoleic acid and ARA were not determined.

Analyte	NIST-Determined Mass Fractions in Wild Salmon (mg/g)
ALA	0.230 ± 0.034
EPA	2.32 ± 0.80
DHA	7.8 ± 3.2

Dietary Intake Study Results

- Twenty-one laboratories enrolled in this exercise and received samples to measure fatty acids in the fish oil and salmon samples. Eight to 12 laboratories reported results for each analyte, resulting in 38 % to 57 % participation. Participation statistics for each analyte are described in more detail below.

Analyte	Number of Laboratories Requesting Samples	Number of Laboratories Reporting Results (Percent Participation)		
		SRM 3275 Level 3	Aquacultured Salmon	Wild Salmon
ALA	21	12 (57 %)	8 (38 %)	8 (38 %)
Linoleic Acid	21	11 (52 %)	8 (38 %)	8 (38 %)
ARA	21	11 (52 %)	8 (38 %)	8 (38 %)
EPA	21	12 (57 %)	8 (38 %)	8 (38 %)
DHA	21	12 (57 %)	8 (38 %)	8 (38 %)

- The consensus ranges for all fatty acids overlapped the target ranges, except for linoleic acid in SRM 3275 Level 3, where the consensus range was almost completely above the target range (**Figure 5-7**).
- The between-laboratory variabilities were excellent for all analytes in all matrices, at 14 % RSD or less. Variabilities for each analyte/sample pair are reported in the table below.

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	Between-Laboratory Variability (RSD)		
Analyte	SRM 3275 Level 3	Aquacultured Salmon	Wild Salmon
ALA	4 %	10 %	11 %
Linoleic Acid	8 %	11 %	14 %
ARA	3 %	4 %	5 %
EPA	3 %	6 %	9 %
DHA	3 %	14 %	14 %

- Laboratories reported using derivatization to fatty acid methyl esters, hot block digestion, acid hydrolysis, and solvent extraction as their sample preparation methods. One laboratory did not report a sample preparation method.
- Laboratories reported using GC-FID as their analytical method for determination of fatty acids in these samples.

Dietary Intake Technical Recommendations

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

- The between-laboratory variability was lower for the fish oil samples than for the salmon samples, most likely because the sample preparation for oils is more straightforward than for the salmon. Laboratories should be aware of the level of sample preparation required and avoid sample over-processing (e.g., unneeded extraction steps) or under-processing (e.g., incomplete homogenization) that may introduce atypical errors such as losses or interferences.
- One laboratory consistently reported high results with respect to the consensus and/or target ranges, indicating a unique method challenge.
- No sample preparation approach was noted to perform better or worse than others reported.
- For ALA (**Figure 5-4** and **Figure 5-5**) and linolenic acid (**Figure 5-9** and **Figure 5-10**), the sample-sample comparison plots show an upward trend in which laboratories reporting high (or low) values on one sample are also reporting high (or low) values on the second sample (respectively). This trend often results from calibration biases that affect both samples similarly.
- Overall, the mass fraction determined for each fatty acid was higher in the aquacultured salmon than in the wild-caught salmon. The differences ranged from small (1.5 times greater for DHA) to large (16 times greater for linoleic acid). The results of this study indicate that fatty acid ratios may be useful in detecting authenticity of seafood source.
- 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.
- 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.
- 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.

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Table 5-1. Individualized data summary table (NIST) for fatty acids in fish oil and salmon.

National Institute of Standards & Technology

HAMQAP Exercise 5 - Fatty Acids

Analyte	Sample	Lab Code: NIST Units	1. Your Results x_i	s_i	Z'_{comm}	Z_{NIST}	2. Community Results N	x^*	s^*	3. Target x_{NIST}	U
Total alpha-Linolenic Acid (C18:3 n-3)	SRM 3275 Omega-3 and Omega-6 Fatty Acids in Fish Oil Level 3	mg/g	6.29	0.59			11	6.38	0.24	6.29	0.59
Total alpha-Linolenic Acid (C18:3 n-3)	Aquacultured Salmon	mg/g					8	1.15	0.11		
Total alpha-Linolenic Acid (C18:3 n-3)	Wild Salmon	mg/g	0.230	0.034			8	0.273	0.029	0.230	0.034
Total Linoleic Acid (C18:2 n-6)	SRM 3275 Omega-3 and Omega-6 Fatty Acids in Fish Oil Level 3	mg/g	12.85	0.86			11	10.2	0.84	12.85	0.86
Total Linoleic Acid (C18:2 n-6)	Aquacultured Salmon	mg/g	6.59	0.84			8	5.76	0.62	6.59	0.84
Total Linoleic Acid (C18:2 n-6)	Wild Salmon	mg/g					8	0.354	0.051		
Total Arachidonic Acid (C20:4 n-6)	SRM 3275 Omega-3 and Omega-6 Fatty Acids in Fish Oil Level 3	mg/g					11	12.5	0.39		
Total Arachidonic Acid (C20:4 n-6)	Aquacultured Salmon	mg/g	0.91	0.21			8	0.96	0.042	0.91	0.21
Total Arachidonic Acid (C20:4 n-6)	Wild Salmon	mg/g					8	0.16	0.0077		
Total EPA (C20:5 n-3)	SRM 3275 Omega-3 and Omega-6 Fatty Acids in Fish Oil Level 3	mg/g	150	20			12	148	4.3	150	20
Total EPA (C20:5 n-3)	Aquacultured Salmon	mg/g	5.97	0.98			8	5.8	0.32	5.97	0.98
Total EPA (C20:5 n-3)	Wild Salmon	mg/g	2.32	0.80			8	2.03	0.19	2.32	0.80
Total DHA (C22:6 n-3)	SRM 3275 Omega-3 and Omega-6 Fatty Acids in Fish Oil Level 3	mg/g	100	10			12	96.6	3.3	100	10
Total DHA (C22:6 n-3)	Aquacultured Salmon	mg/g	10.2	2.3			8	8.4	1.2	10.2	2.3
Total DHA (C22:6 n-3)	Wild Salmon	mg/g	7.8	3.2			8	5.59	0.77	7.8	3.2

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

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Table 5-2. Data summary table for total α-linolenic acid (ALA) in fish oil and salmon. Data points highlighted in red have been flagged as potential outliers (e.g., Grubb and/or Cochran) by the NIST software package.

		Total alpha-Linolenic Acid (C18:3 n-3)													
		SRM 3275 Omega-3 and Omega-6 Fatty Acids in Fish Oil Level 3 (mg/g)					Aquacultured Salmon (mg/g)					Wild Salmon (mg/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target				6.293	0.590									0.230	0.034
	E002	11.1	11	11	11.033	0.058	6.6	6.7	6.55	6.62	0.08	0.3	0.3	0.4	0.333	0.058
	E004	6.161	6.162	6.157	6.160	0.003										
	E005	6.38	6.37	6.38	6.377	0.006	1.2	1.19	1.16	1.18	0.02	0.25	0.22	0.27	0.247	0.025
	E006	6.47	6.42	6.45	6.447	0.025										
	E008															
	E014	6.09	6.082	5.892	6.021	0.112	1.104	1.085	1.141	1.11	0.03	0.26	0.258	0.277	0.265	0.010
	E017															
	E020															
	E021															
	E022															
	E028	5.95	5.73	5.9	5.860	0.115	0.73	0.795	0.815	0.78	0.04	0.203	0.22	0.179	0.201	0.021
	E030															
	E032															
	E033	6.1	5.8	5.8	5.900	0.173										
	E038	5.43	5.95	5.6	5.660	0.265	1.21	1.24	1.26	1.24	0.03	0.34	0.34	0.35	0.343	0.006
	E039															
	E047	7.93	7.31	7.02	7.420	0.465	1.24	1.44	1.37	1.35	0.10	0.3	0.26	0.29	0.283	0.021
	E050						7.6	6.7		7.15	0.64	4.5	5		4.750	0.354
	E051	7	6.9	7.3	7.067	0.208										
	E057	6.97	6.89	6.87	6.910	0.053	1.23	1.26	1.28	1.26	0.03	0.23	0.25	0.24	0.240	0.010
	E072															
Community Results		Consensus Mean			6.382		Consensus Mean			1.15		Consensus Mean			0.273	
		Consensus Standard Deviation			0.239		Consensus Standard Deviation			0.11		Consensus Standard Deviation			0.029	
		Maximum			11.033		Maximum			7.15		Maximum			4.750	
		Minimum			5.660		Minimum			0.78		Minimum			0.201	
		N			11		N			8		N			8	

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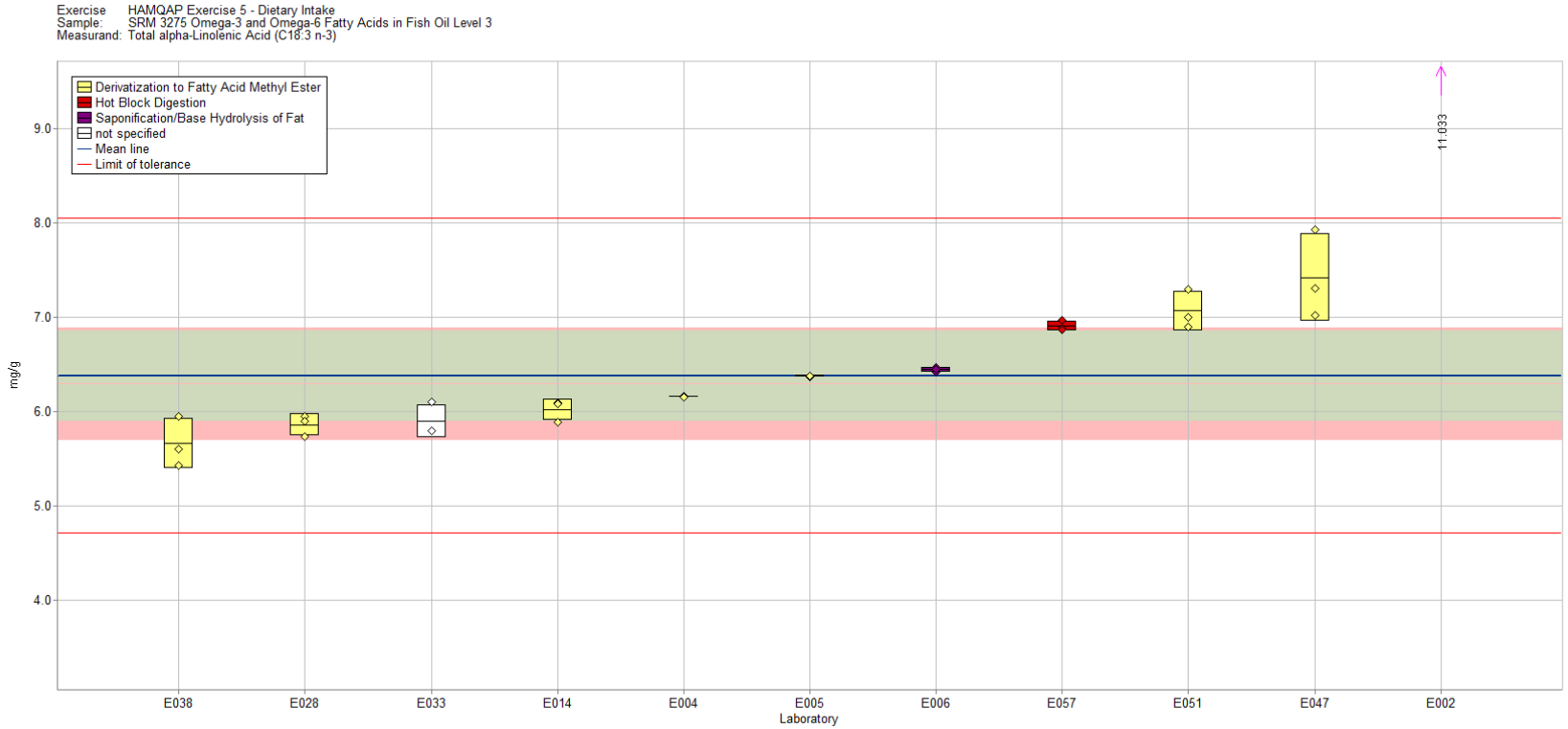

Figure 5-1. Total α-linolenic acid (ALA) in SRM 3275 Omega-3 and Omega-6 Fatty Acids in Fish Oil (Level 3) (data summary view – sample preparation method). In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation employed. The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red 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, $|Z_{\text{NIST}}| \leq 2$.

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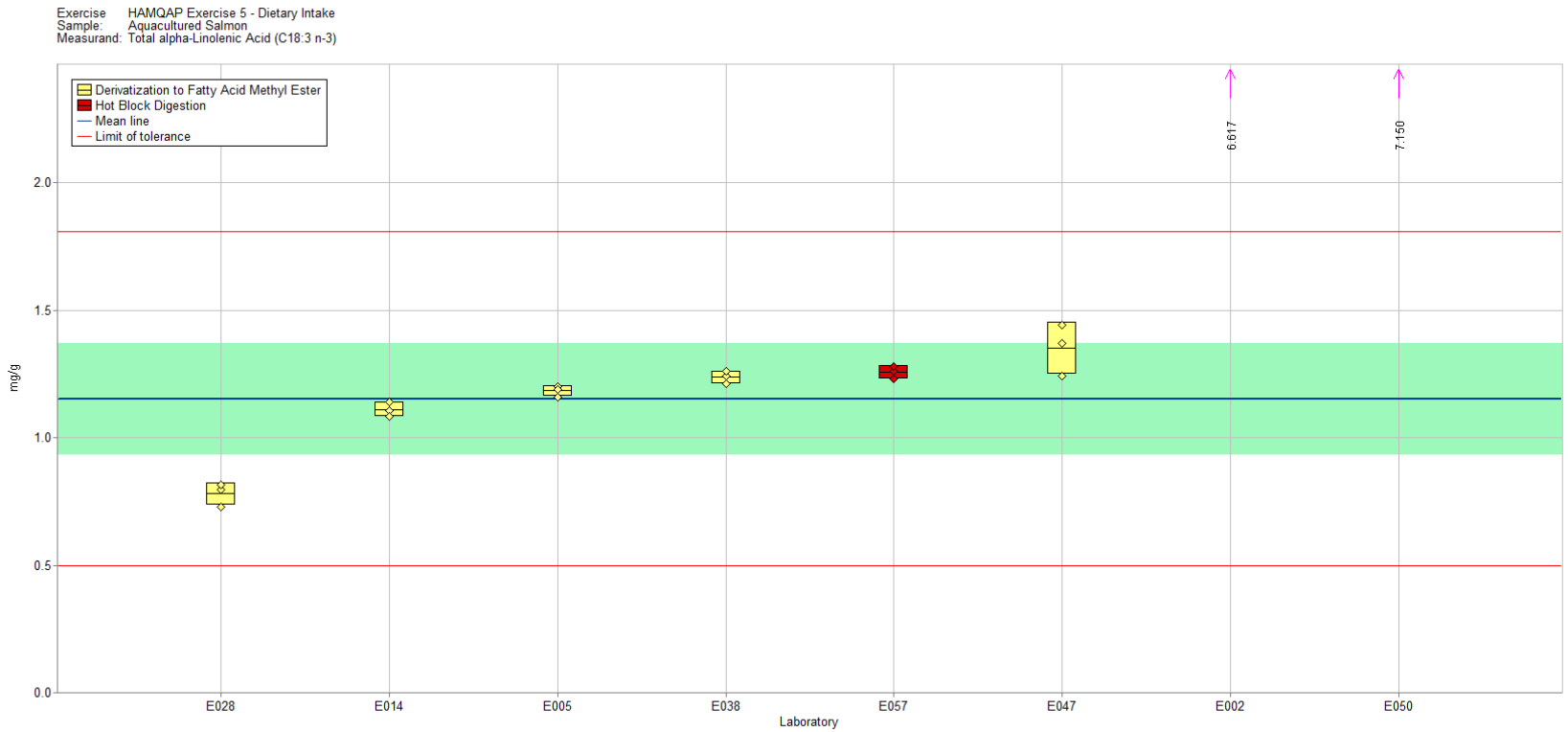

Figure 5-2. Total α-linolenic acid (ALA) in Aquacultured Salmon (data summary view – sample preparation method). In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation 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, $|Z'_{\text{comm}}| \leq 2$. A NIST value has not been determined in this material.

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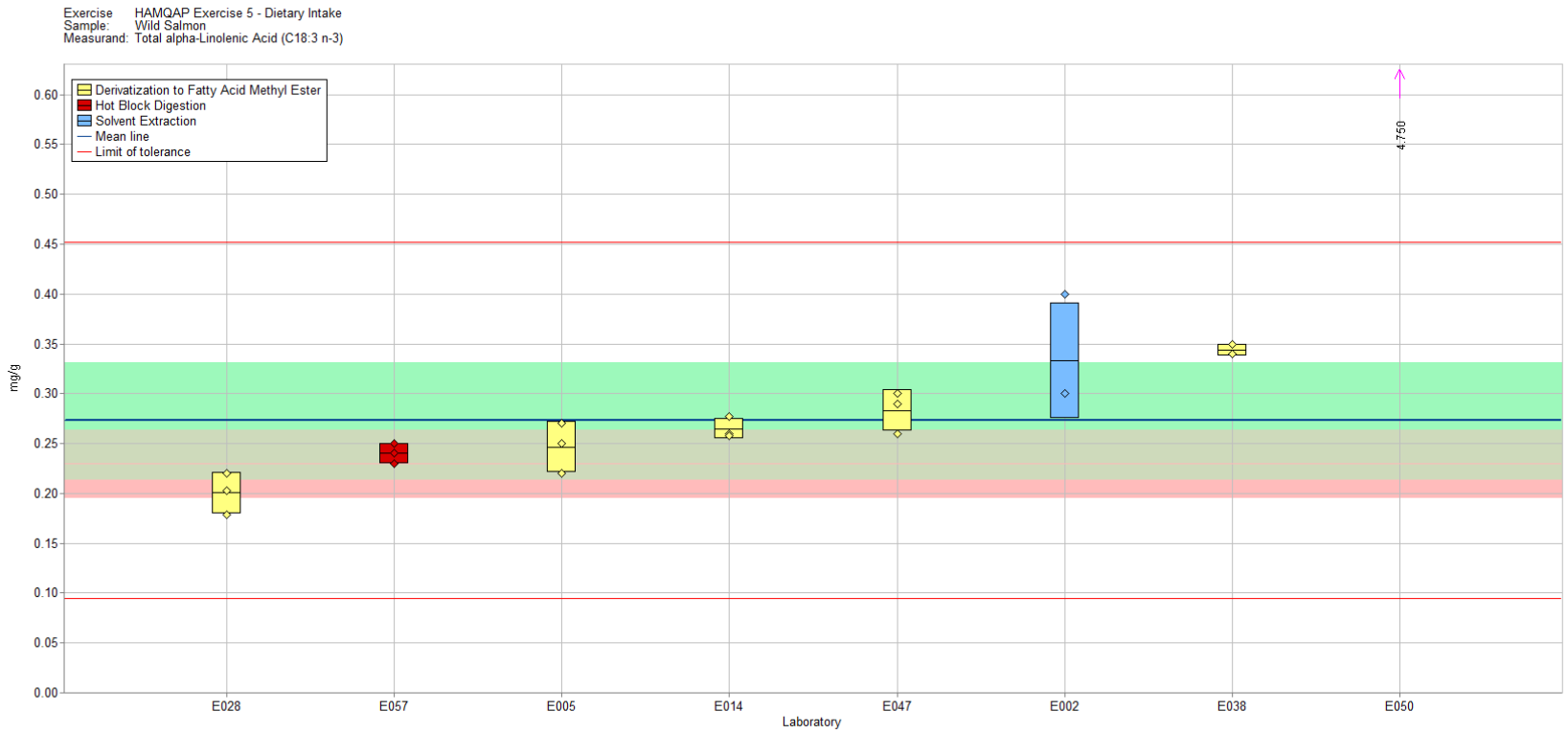

Figure 5-3. Total α-linolenic acid (ALA) in Wild Salmon (data summary view – sample preparation method). In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method 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, $|Z'_{\mathrm{comm}}| \leq 2$. 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, $|Z_{\mathrm{NIST}}| \leq 2$.

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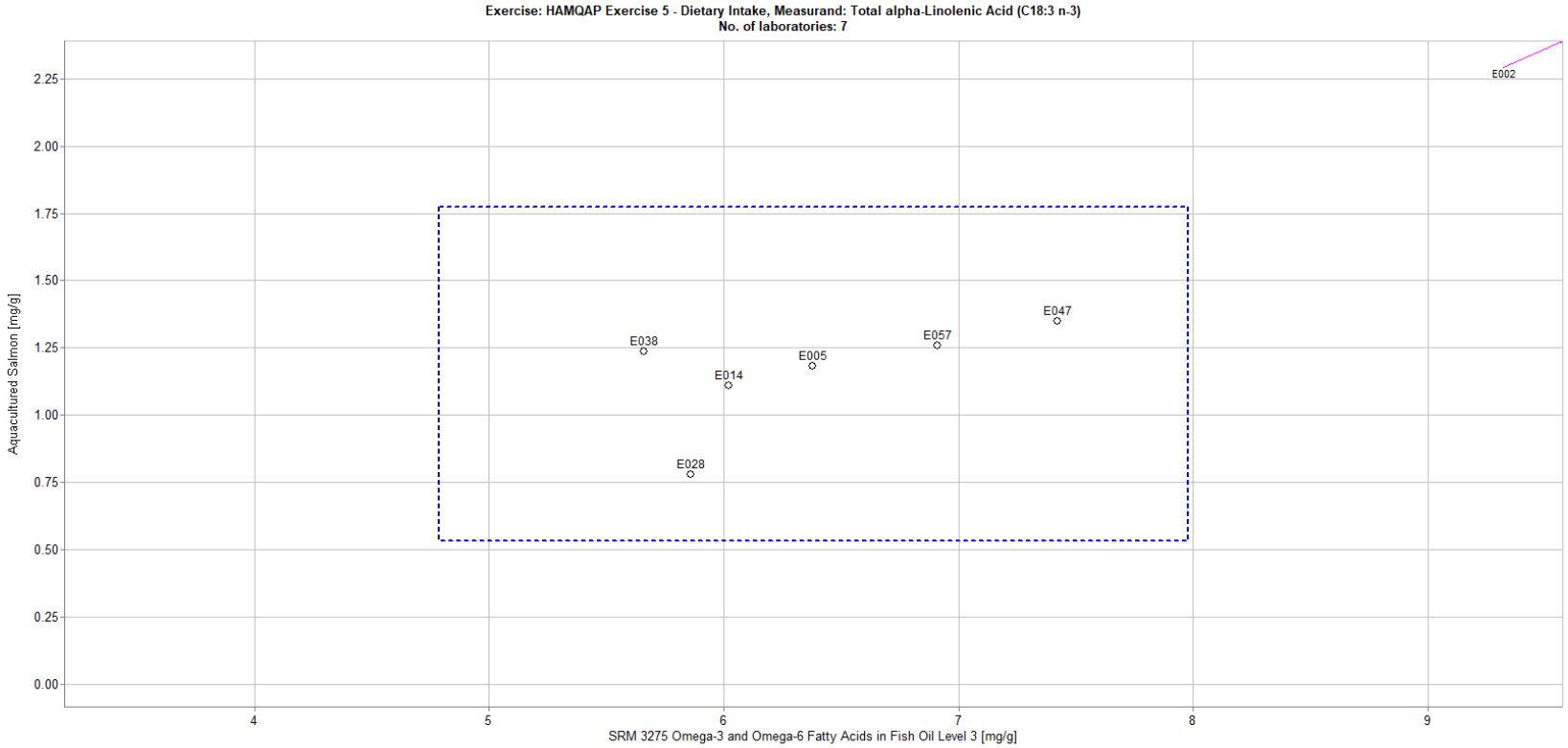

Figure 5-4. Laboratory means for total α-linolenic acid (ALA) in SRM 3275 Omega-3 and Omega-6 Fatty Acids in Fish Oil Level 3 and Aquacultured Salmon (sample/sample comparison view). In this view, the individual laboratory mean for one sample (SRM 3275 Level 3) is compared to the individual laboratory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

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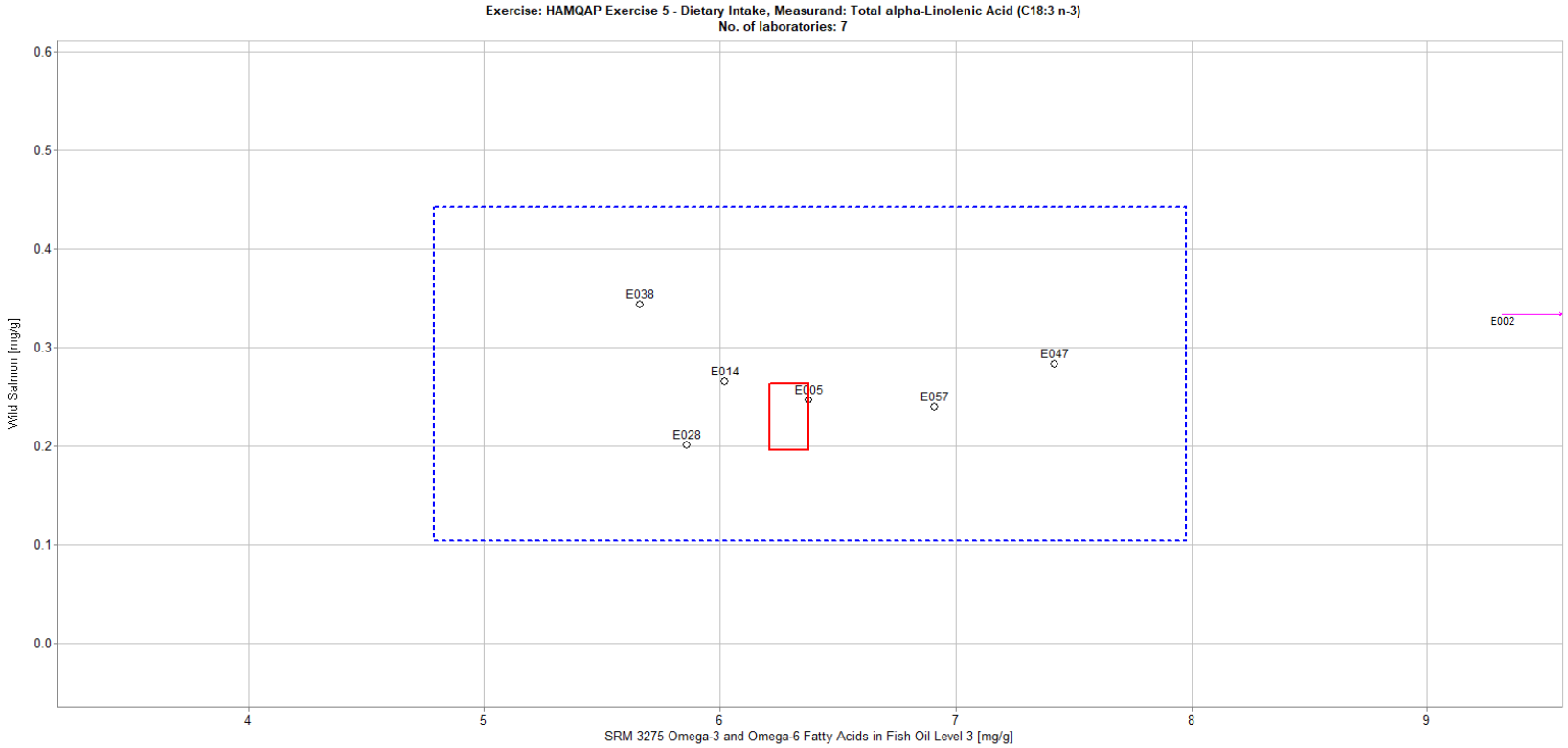

Figure 5-5. Laboratory means for total α-linolenic acid (ALA) in SRM 3275 Omega-3 and Omega-6 Fatty Acids in Fish Oil Level 3 and Wild Salmon (sample/sample comparison view). In this view, the individual laboratory mean for one sample (SRM 3275 Level 3) is compared to the individual laboratory mean for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

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Table 5-3. Data summary table for total linoleic acid in fish oil and salmon. Data points highlighted in red have been flagged as potential outliers (e.g., Grubb and/or Cochran) by the NIST software package.

		Total Linoleic Acid (C18:2 n-6)														
		SRM 3275 Omega-3 and Omega-6 Fatty Acids in Fish Oil Level 3 (mg/g)					Aquacultured Salmon (mg/g)					Wild Salmon (mg/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target				12.85	0.86				6.59	0.84					
	E002	6.1	6.1	6.05	6.08	0.03	1.25	1.2	1.2	1.22	0.03	0.2	0.2	0.25	0.217	0.029
	E004	11.85	11.8	11.81	11.82	0.03										
	E005	11.15	11.14	11.16	11.15	0.01	6.48	6.44	6.28	6.40	0.11	0.36	0.33	0.4	0.363	0.035
	E006	11.33	11.35	11.4	11.36	0.04										
	E008															
	E014	10.29	10.29	10.08	10.22	0.12	5.752	5.721	5.995	5.82	0.15	0.368	0.366	0.371	0.368	0.003
	E017															
	E020															
	E021															
	E022															
	E028	9.75	9.36	9.73	9.61	0.22	4.165	4.265	4.377	4.27	0.11	0.274	0.313	0.289	0.292	0.020
	E030															
	E032															
	E033	10.8	10.3	10.2	10.43	0.32										
	E038	4.21	4.45	4.32	4.33	0.12	6.77	6.96	7.1	6.94	0.17	0.45	0.47	0.47	0.463	0.012
	E039															
	E047	13.6	14	13.3	13.63	0.35	7.1	7.28	6.73	7.04	0.28	0.46	0.37	0.4	0.410	0.046
	E050						41.8	33.6		37.70	5.80	7.5	7.8		7.650	0.212
	E051	32.1	32.1	32	32.07	0.06										
	E057	11.96	11.42	11.8	11.73	0.28	6.57	6.77	6.86	6.73	0.15	0.37	0.38	0.35	0.367	0.015
	E072															
Community Results		Consensus Mean			10.20		Consensus Mean			5.76		Consensus Mean			0.354	
		Consensus Standard Deviation			0.84		Consensus Standard Deviation			0.62		Consensus Standard Deviation			0.051	
		Maximum			32.07		Maximum			37.70		Maximum			7.650	
		Minimum			4.33		Minimum			1.22		Minimum			0.217	
		N			11		N			8		N			8	

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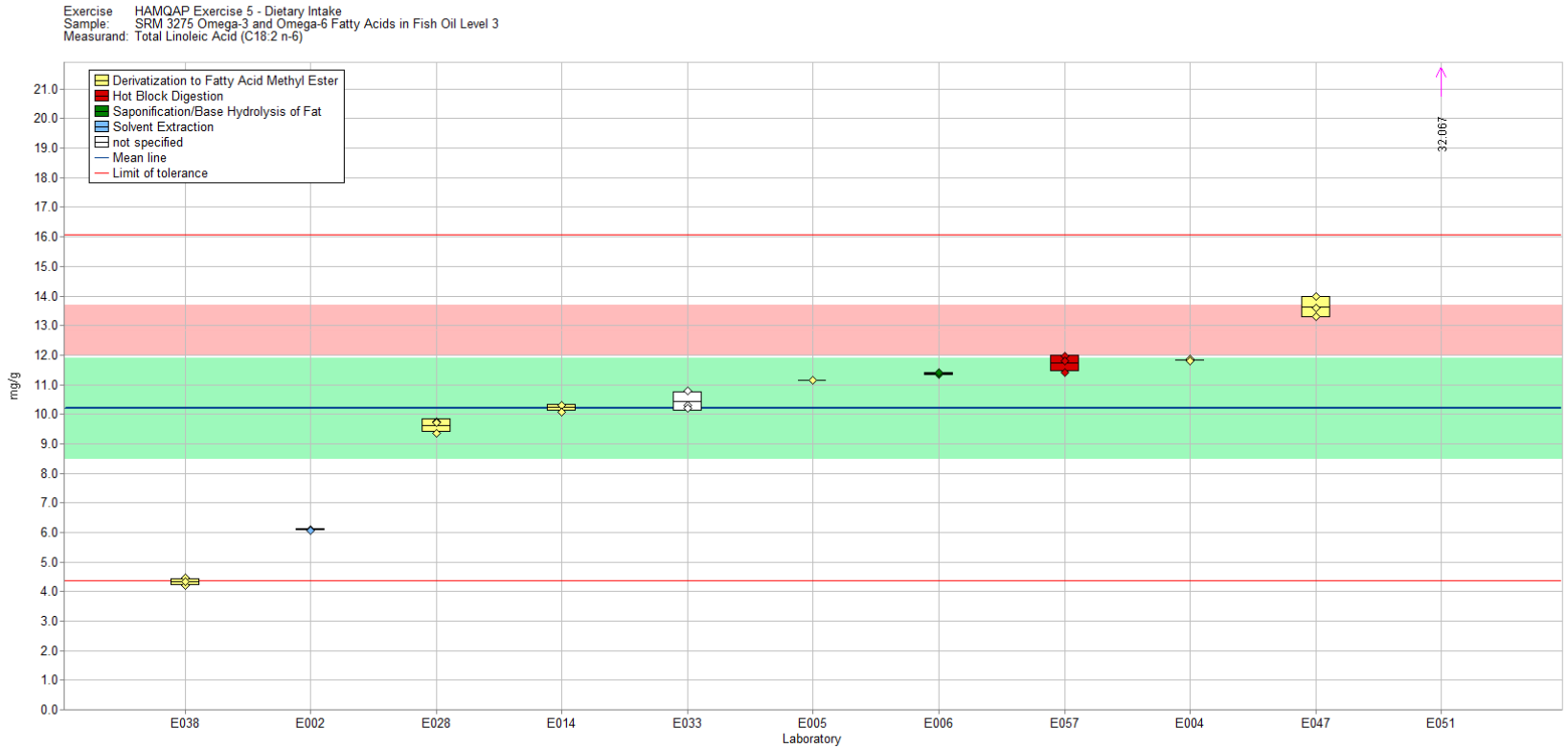

Figure 5-6. Total linolenic acid in SRM 3275 Omega-3 and Omega-6 Fatty Acids in Fish Oil (Level 3) (data summary view – sample preparation method). In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point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

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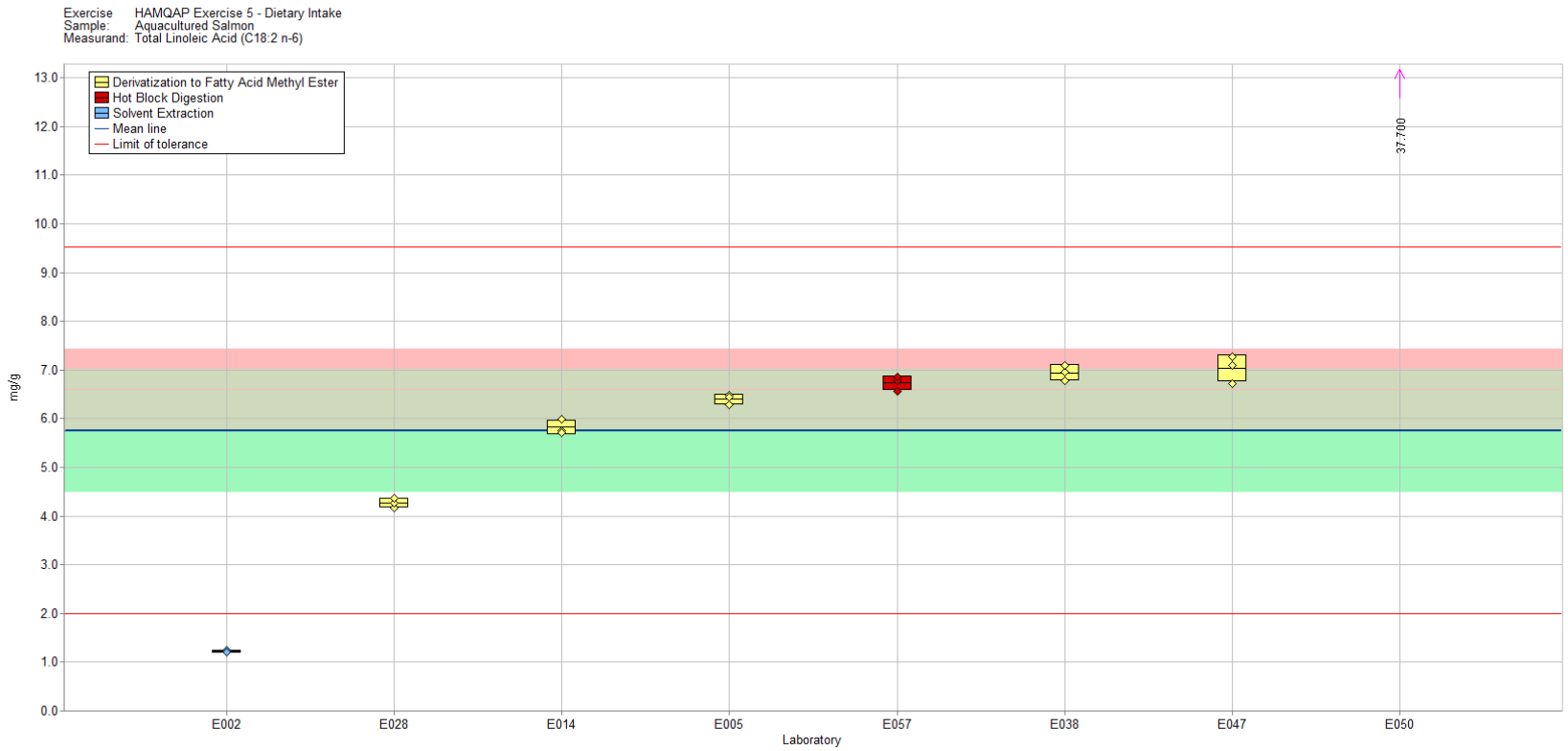

Figure 5-7. Total linolenic acid in Aquacultured Salmon (data summary view – sample preparation method). In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method 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, $|Z_{\text{NIST}}| \leq 2$.

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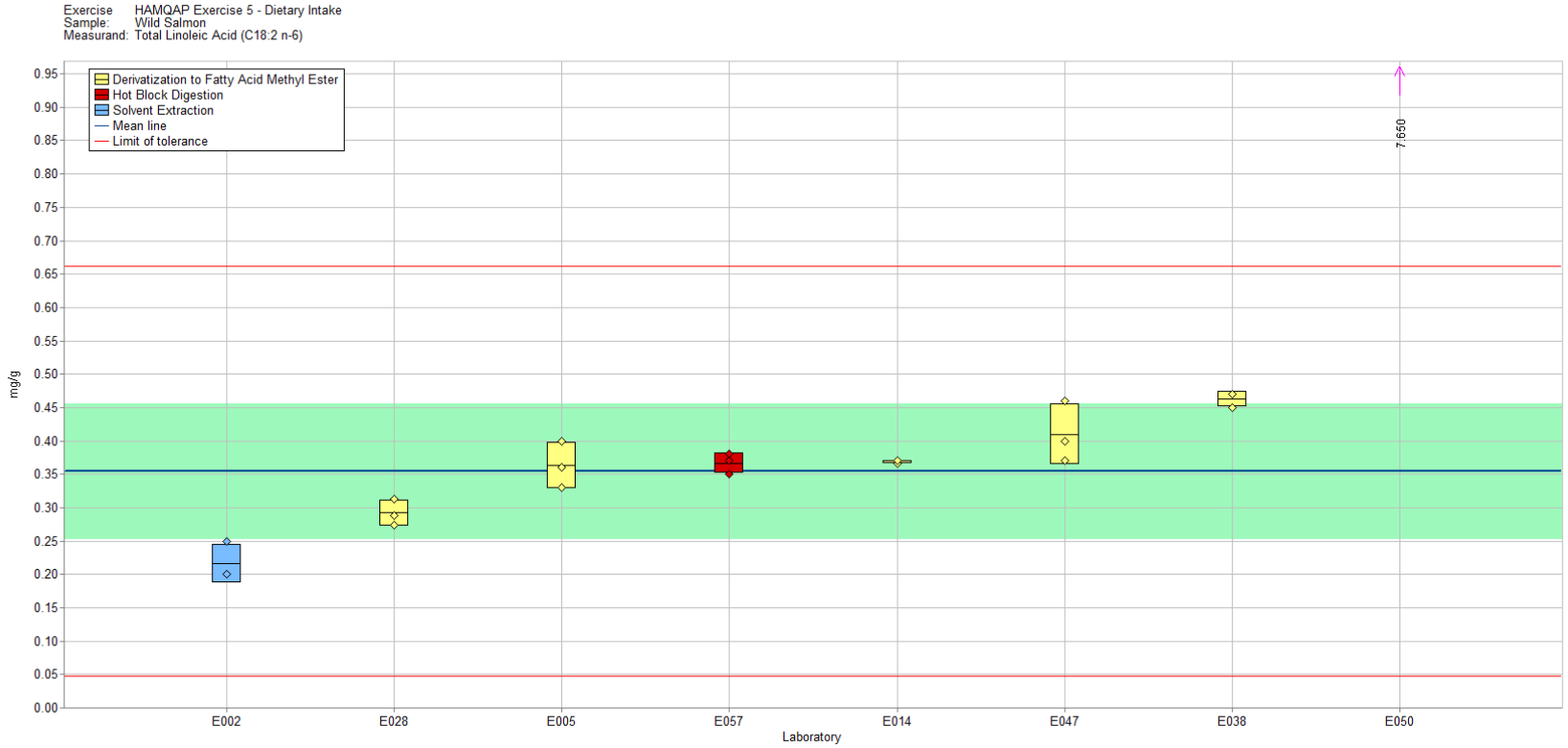

Figure 5-8. Total linolenic acid in Wild Salmon (data summary view – sample preparation method). In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. 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, $|Z'_{\text{comm}}| \leq 2$. A NIST value has not been determined in this material.

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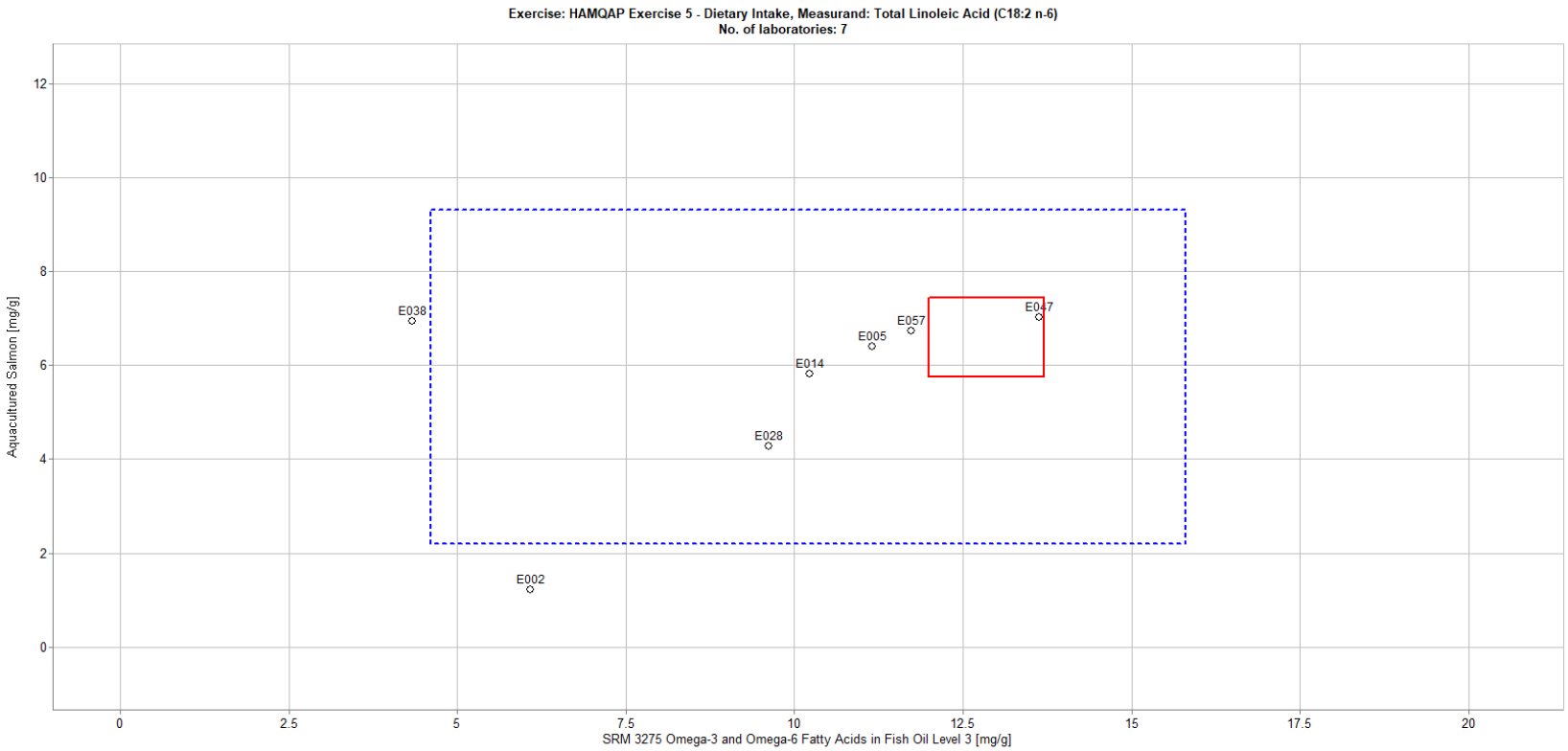

Figure 5-9. Laboratory means for total linolenic acid in SRM 3275 Omega-3 and Omega-6 Fatty Acids in Fish Oil Level 3 and Aquacultured Salmon (sample/sample comparison view). In this view, the individual laboratory mean for one sample (SRM 3275 Level 3) is compared to the individual laboratory mean for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

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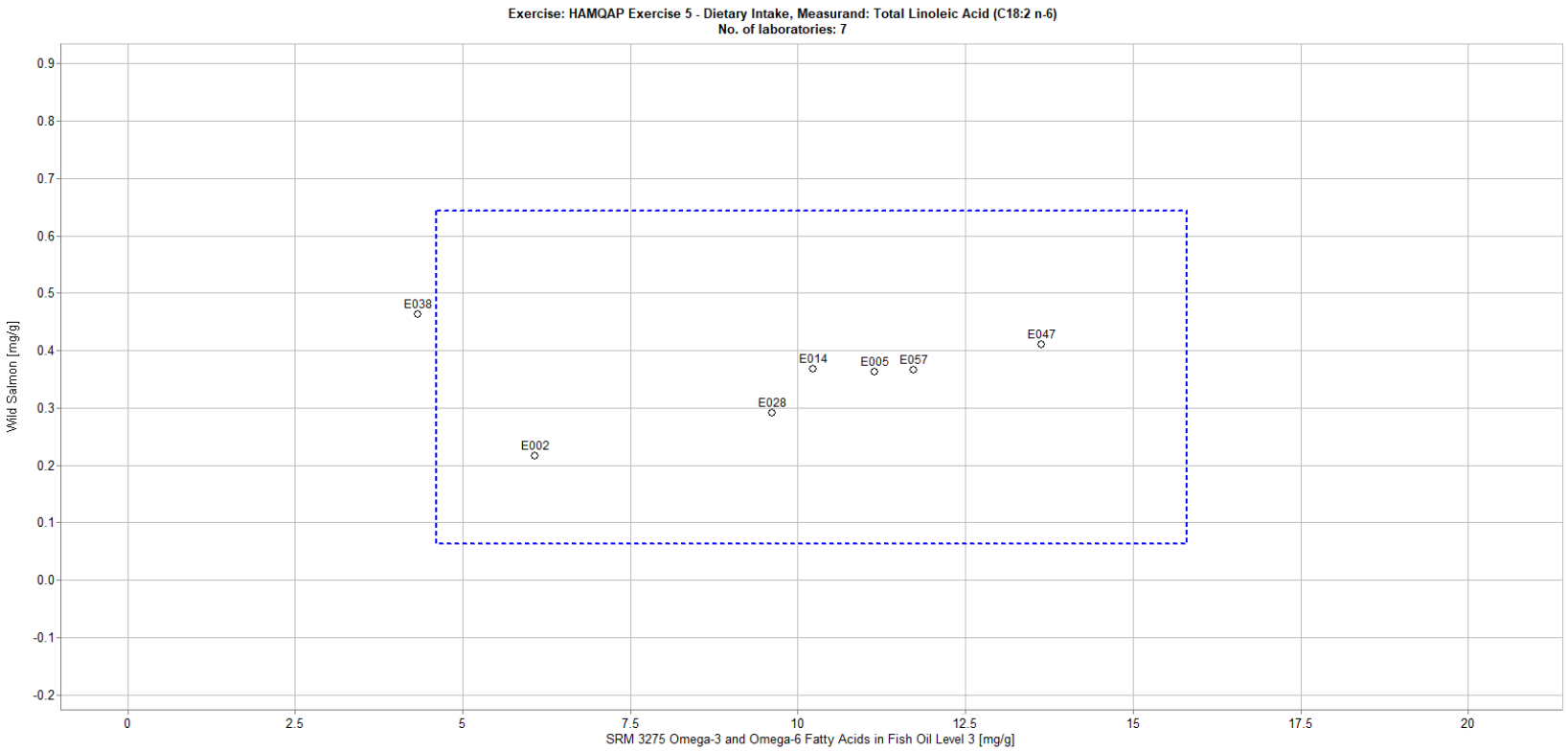

Figure 5-10. Laboratory means for total linolenic acid in SRM 3275 Omega-3 and Omega-6 Fatty Acids in Fish Oil Level 3 and Wild Salmon (sample/sample comparison view). In this view, the individual laboratory mean for one sample (SRM 3275 Level 3) is compared to the individual laboratory mean for a second sample (salmon). The dotted blue box represents the consensus range of tolerance for SRM 3275 Level 3 (x-axis) and salmon (y-axis), calculated as the values above and below the consensus means that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$.

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Table 5-4. Data summary table for total arachidonic acid (ARA) in fish oil and salmon. Data points highlighted in red have been flagged as potential outliers (e.g., Grubb and/or Cochran) by the NIST software package.

		Total Arachidonic Acid (C20:4 n-6)														
		SRM 3275 Omega-3 and Omega-6 Fatty Acids in Fish Oil Level 3 (mg/g)					Aquacultured Salmon (mg/g)					Wild Salmon (mg/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target									0.91	0.21					
	E002	12.5	12.35	12.15	12.33	0.18	1	1	1	1.000	0.000	0.1	0.15	0.15	0.133	0.029
	E004	12.96	13.11	13.11	13.06	0.09										
	E005	12.45	12.44	12.47	12.45	0.02	0.99	0.98	0.96	0.977	0.015	0.14	0.13	0.16	0.143	0.015
	E006	12.45	12.52	12.56	12.51	0.06										
	E008															
	E014	11.38	11.38	11.05	11.27	0.19	0.922	0.873	0.95	0.915	0.039	0.165	0.166	0.174	0.168	0.005
	E017															
	E020															
	E021															
	E022															
	E028	11.42	11.03	11.85	11.43	0.41	0.646	0.659	0.683	0.663	0.019	0.15	0.172	0.139	0.154	0.017
	E030															
	E032															
	E033	12.9	12.5	12.4	12.60	0.26										
	E038	10.27	11.35	10.69	10.77	0.54	0.94	0.95	0.98	0.957	0.021	0.17	0.17	0.18	0.173	0.006
	E039															
	E047	14.6	14.5	13.3	14.13	0.72	1.13	1.1	1.27	1.167	0.091	0.18	0.36	0.26	0.267	0.090
	E050						16.5	16.8		16.650	0.212	8.2	8.3		8.250	0.071
	E051	14.1	14.1	14.2	14.13	0.06										
	E057	13.2	13.11	12.83	13.05	0.19	0.94	0.94	0.98	0.953	0.023	0.16	0.16	0.16	0.160	0.000
	E072															
Community Results		Consensus Mean			12.52		Consensus Mean			0.960		Consensus Mean			0.160	
		Consensus Standard Deviation			0.39		Consensus Standard Deviation			0.042		Consensus Standard Deviation			0.008	
		Maximum			14.13		Maximum			16.650		Maximum			8.250	
		Minimum			10.77		Minimum			0.663		Minimum			0.133	
		N			11		N			8		N			8	

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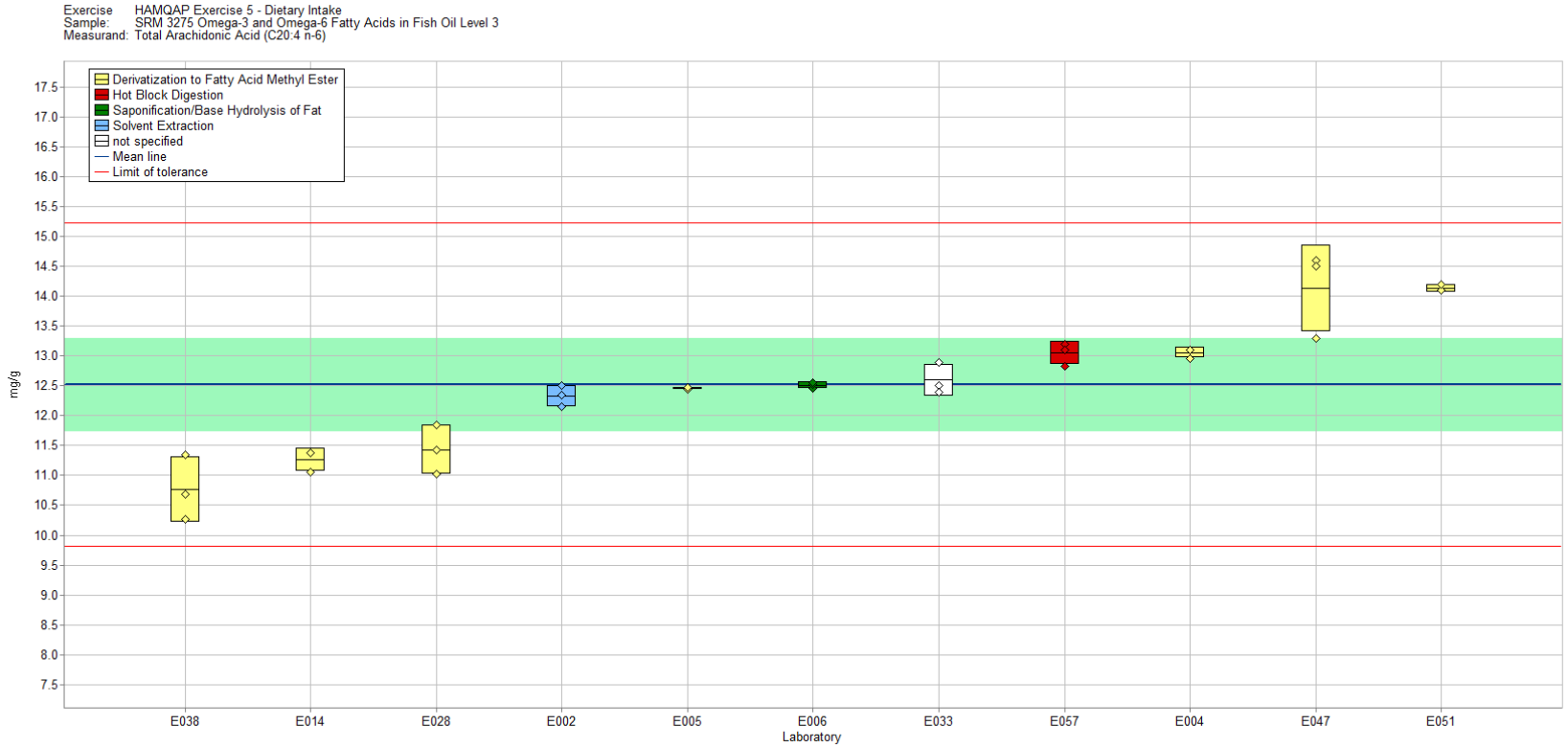

Figure 5-11. Total arachidonic acid (ARA) in SRM 3275 Omega-3 and Omega-6 Fatty Acids in Fish Oil (Level 3) (data summary view – sample preparation method). In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the 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, $|Z'_{\text{comm}}| \leq 2$. A NIST value has not been determined in this material.

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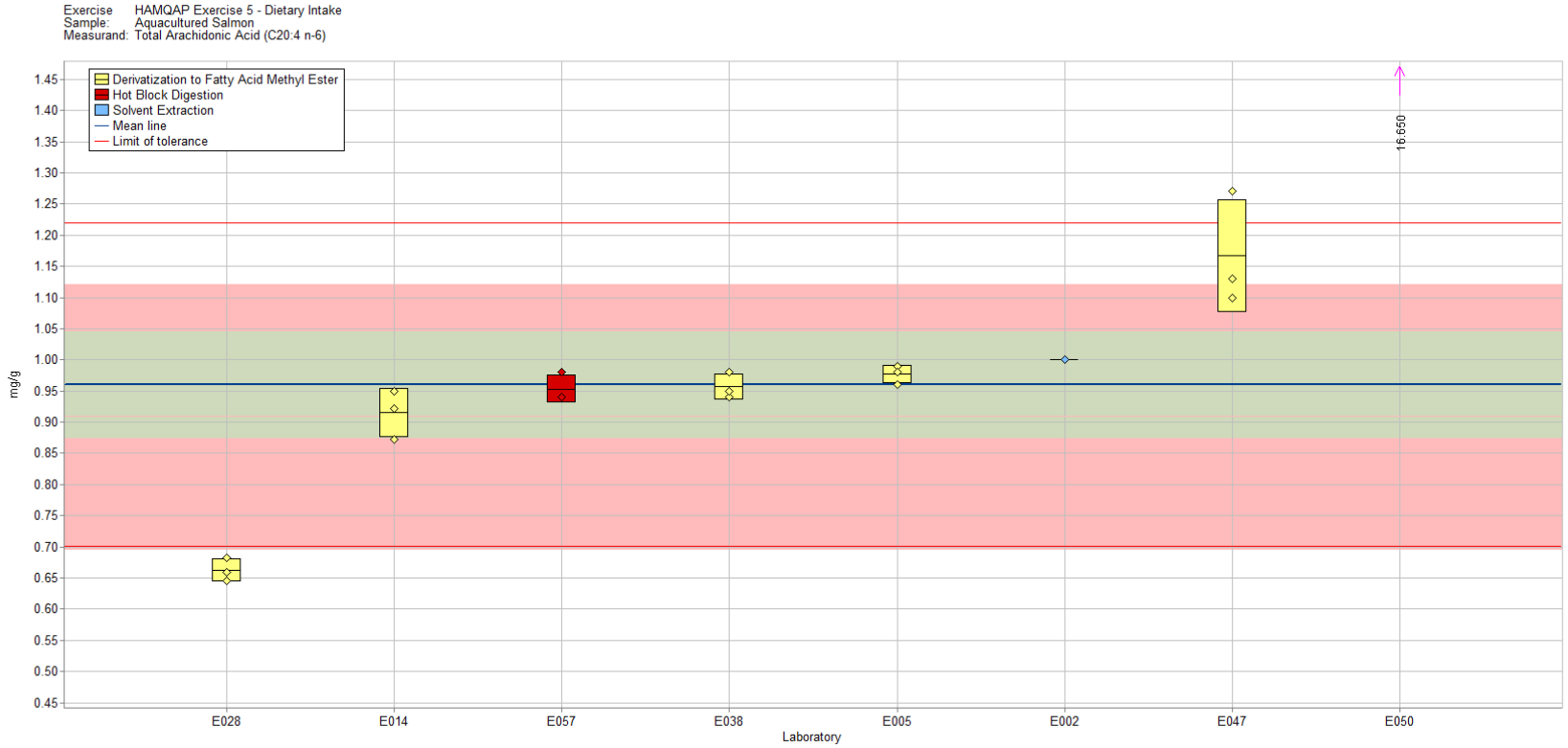

Figure 5-12. Total arachidonic acid (ARA) in Aquacultured Salmon (data summary view – sample preparation method). In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation 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, $|Z'_{\mathrm{comm}}| \leq 2$. 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, $|Z_{\mathrm{NIST}}| \leq 2$.

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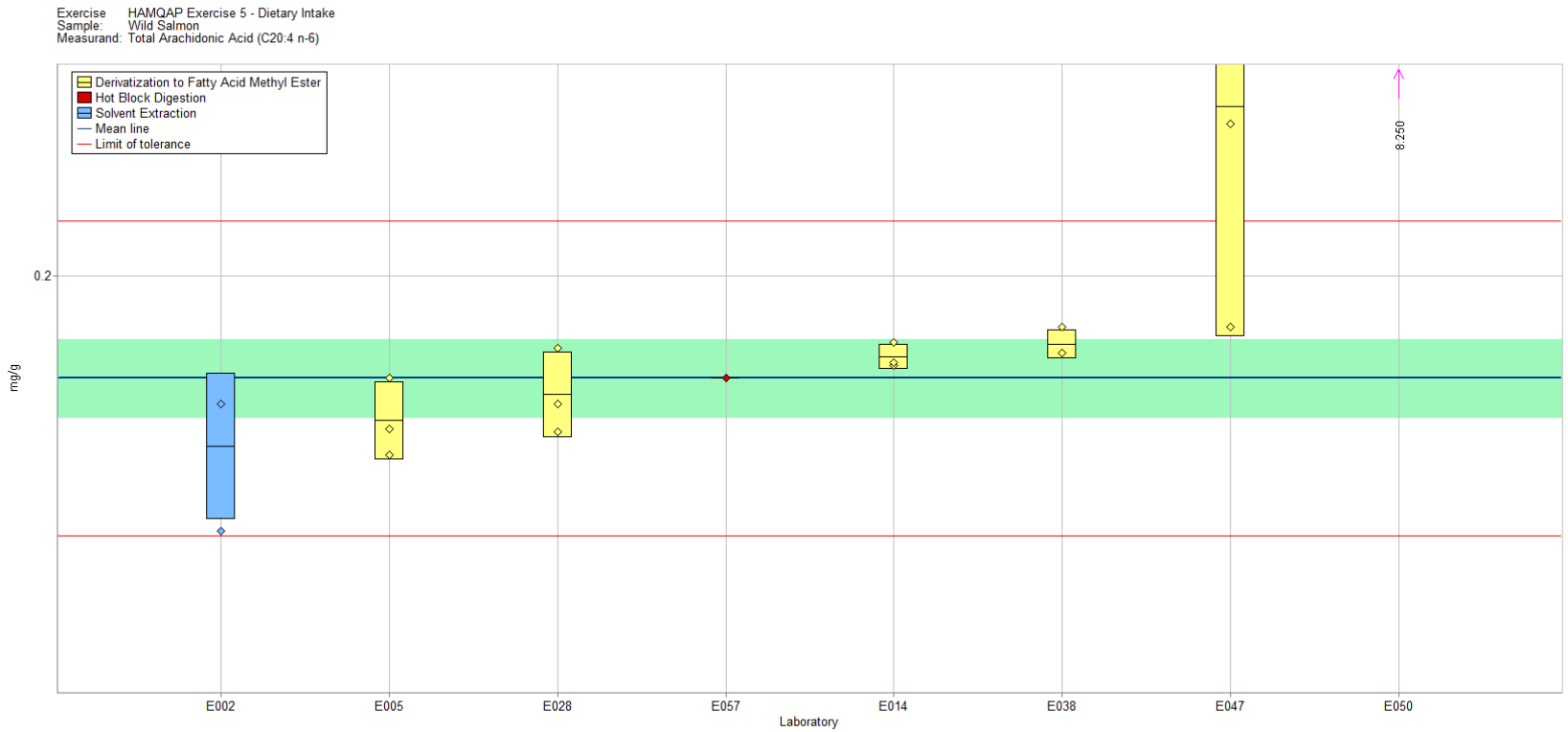

Figure 5-13. Total arachidonic acid (ARA) in Wild Salmon (data summary view – sample preparation method). In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method 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, $|Z'_{\text{comm}}| \leq 2$. A NIST value has not been determined in this material.

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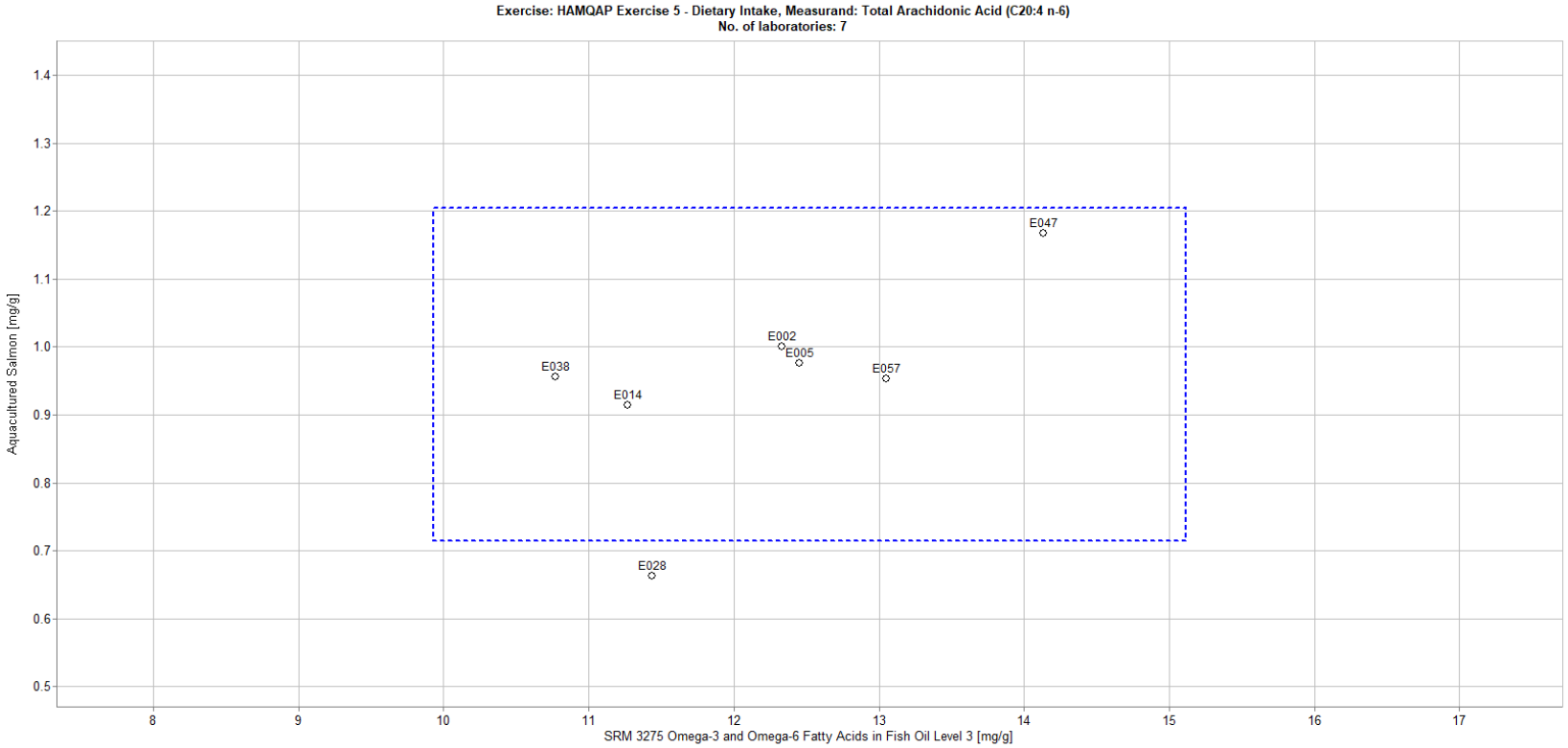

Figure 5-14. Laboratory means for total arachidonic acid (ARA) in SRM 3275 Omega-3 and Omega-6 Fatty Acids in Fish Oil Level 3 and Aquacultured Salmon (sample/sample comparison view). In this view, the individual laboratory mean for one sample (SRM 3275 Level 3) is compared to the individual laboratory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

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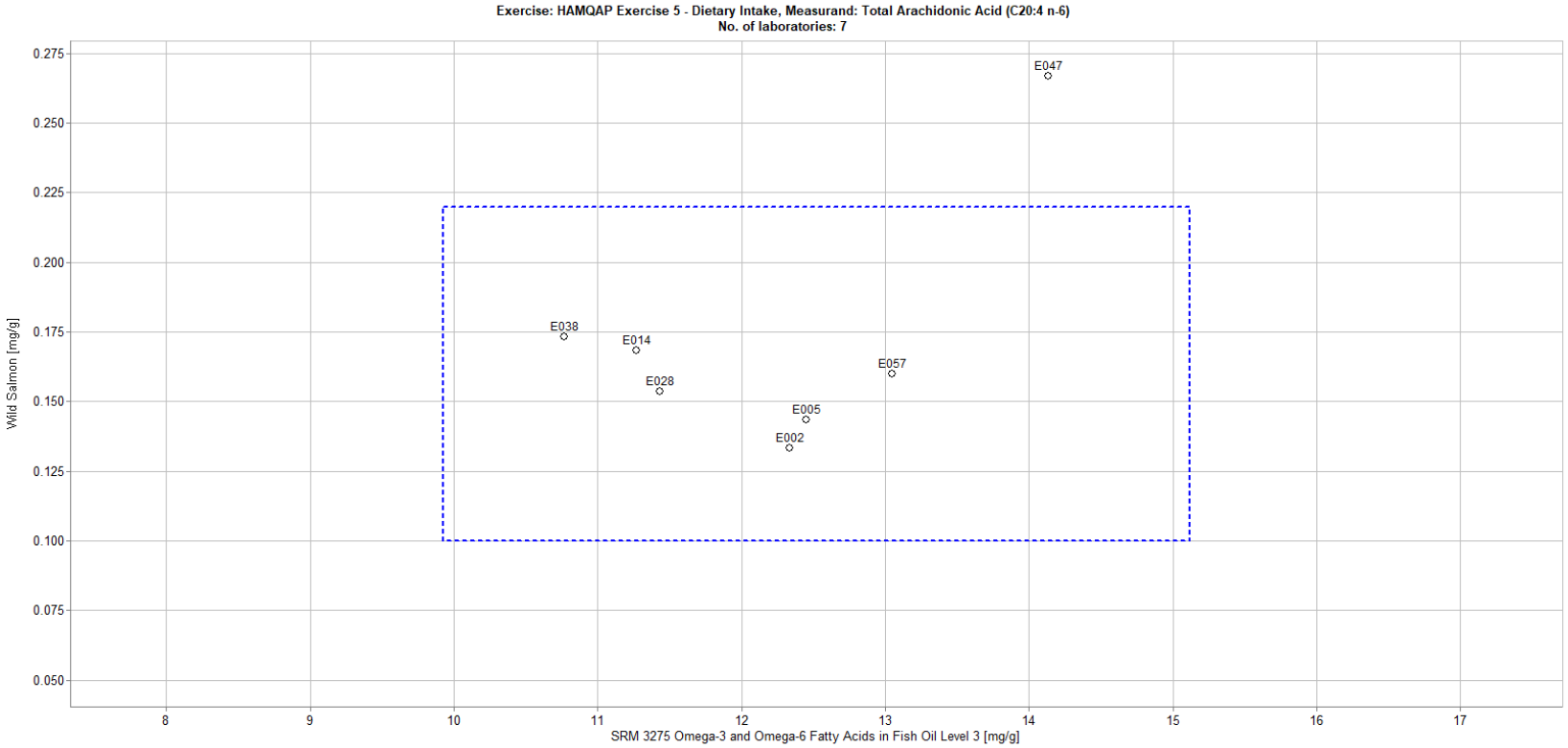

Figure 5-15. Laboratory means for total arachidonic acid (ARA) in SRM 3275 Omega-3 and Omega-6 Fatty Acids in Fish Oil Level 3 and Wild Salmon (sample/sample comparison view). In this view, the individual laboratory mean for one sample (SRM 3275 Level 3) is compared to the individual laboratory mean for a second sample (salmon). The dotted blue box represents the consensus range of tolerance for SRM 3275 Level 3 (x-axis) and salmon (y-axis), calculated as the values above and below the consensus means that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$.

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Table 5-5. Data summary table for total EPA in fish oil and salmon. Data points highlighted in red have been flagged as potential outliers (e.g., Grubb and/or Cochran) by the NIST software package.

		Total EPA (C20:5 n-3)													
		SRM 3275 Omega-3 and Omega-6 Fatty Acids in Fish Oil Level 3 (mg/g)					Aquacultured Salmon (mg/g)					Wild Salmon (mg/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target				147.2	17.2				5.97	0.98				2.32	0.80
	E002	151.45	150	150.25	150.6	0.8	5.95	6.1	6.2	6.08	0.13	2	2.05	2.1	2.05	0.05
	E004	153.22	152.88	152.8	153.0	0.2										
	E005	149.75	149.52	149.75	149.7	0.1	5.95	5.92	5.75	5.87	0.11	2.04	1.87	2.17	2.03	0.15
	E006	153.9	154.3	155	154.4	0.6										
	E008															
	E014	136.7	136.3	132.4	135.1	2.4	5.594	5.451	5.74	5.60	0.14	1.983	1.948	1.96	1.96	0.02
	E017	290.54	291.55	291.34	291.1	0.5										
	E020															
	E021															
	E022															
	E028	139.77	135.38	139.36	138.2	2.4	3.867	3.899	4.028	3.93	0.09	1.572	1.802	1.485	1.62	0.16
	E030															
	E032															
	E033	137.4	129.9	128.7	132.0	4.7										
	E038	130.06	142.3	135.95	136.1	6.1	6.23	6.3	6.5	6.34	0.14	2.53	2.54	2.61	2.56	0.04
	E039															
	E047	170	172	150	164.0	12.2	6.6	6.63	6.63	6.62	0.02	2.17	2.2	2.46	2.28	0.16
	E050						90.4	96.3		93.35	4.17	89.4	92		90.70	1.84
	E051	168.5	168.7	167.9	168.4	0.4										
	E057	150.57	152.55	149.01	150.7	1.8	5.52	5.7	5.85	5.69	0.17	1.67	1.69	1.7	1.69	0.02
	E072															
Community Results		Consensus Mean			148.4		Consensus Mean			5.80		Consensus Mean			2.03	
		Consensus Standard Deviation			4.3		Consensus Standard Deviation			0.32		Consensus Standard Deviation			0.19	
		Maximum			291.1		Maximum			93.35		Maximum			90.70	
		Minimum			132.0		Minimum			3.93		Minimum			1.62	
		N			12		N			8		N			8	

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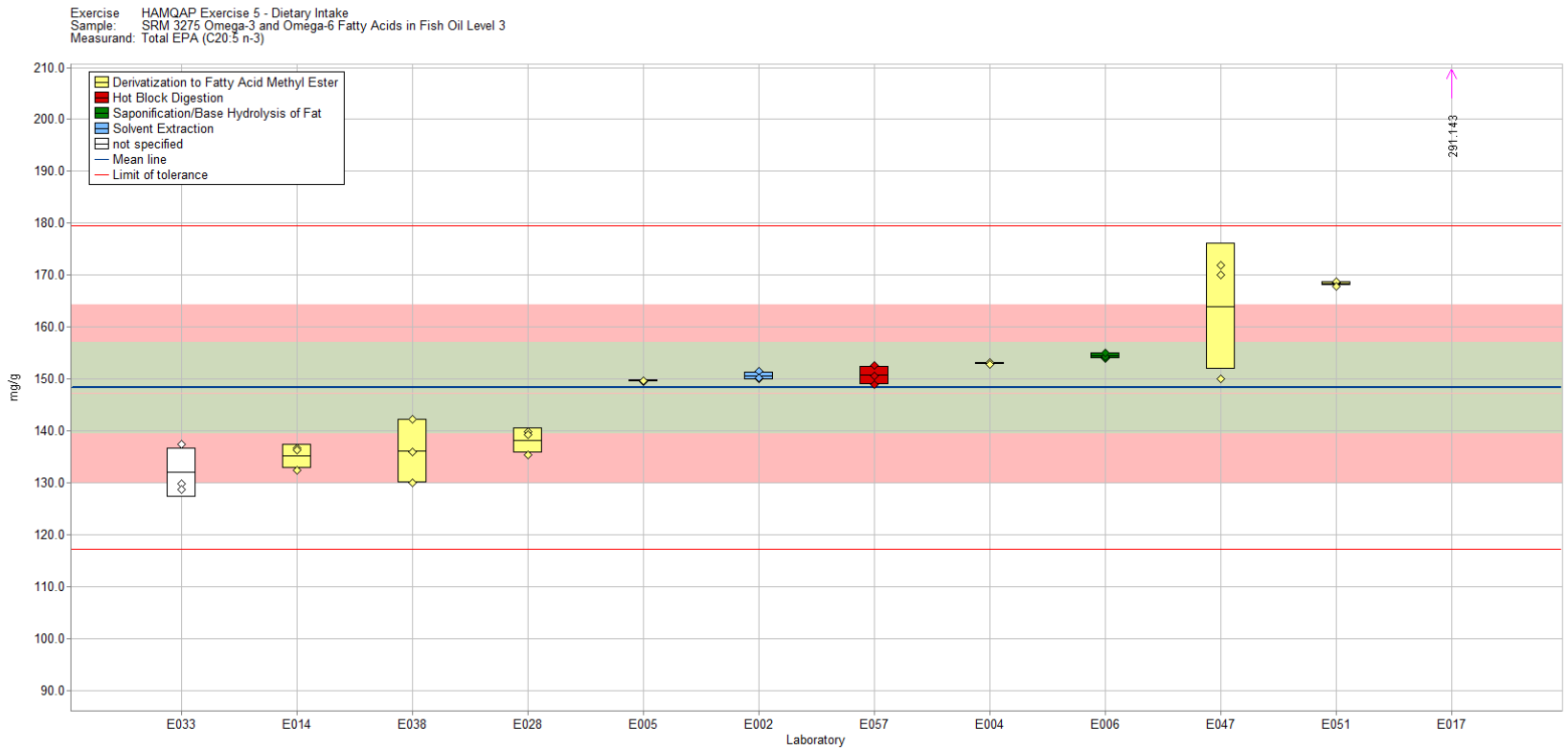

Figure 5-16. Total EPA in SRM 3275 Omega-3 and Omega-6 Fatty Acids in Fish Oil (Level 3) (data summary view – sample preparation method). In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents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

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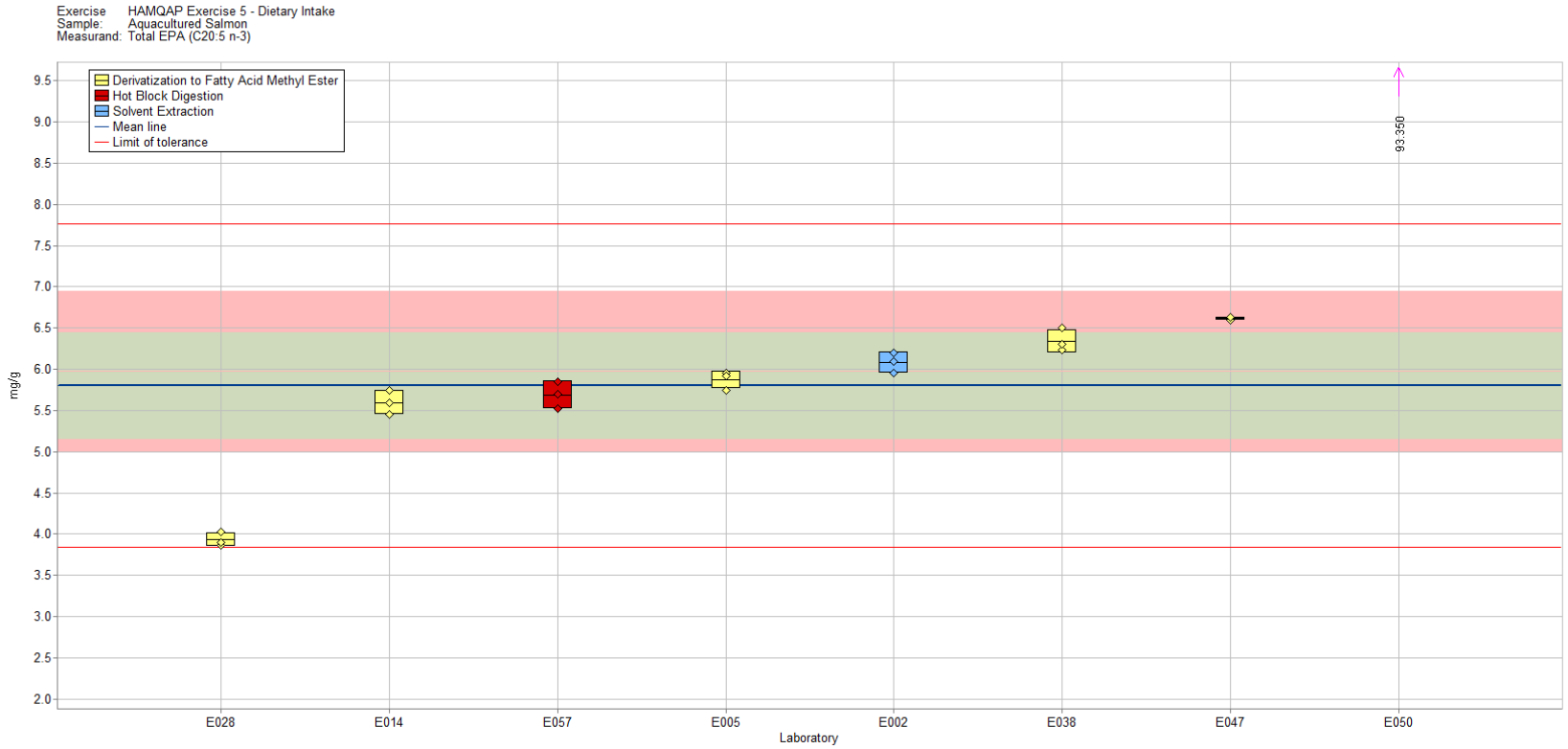

Figure 5-17. Total EPA in Aquacultured Salmon (data summary view – sample preparation method). In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. 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, $|Z_{\text{NIST}}| \leq 2$.

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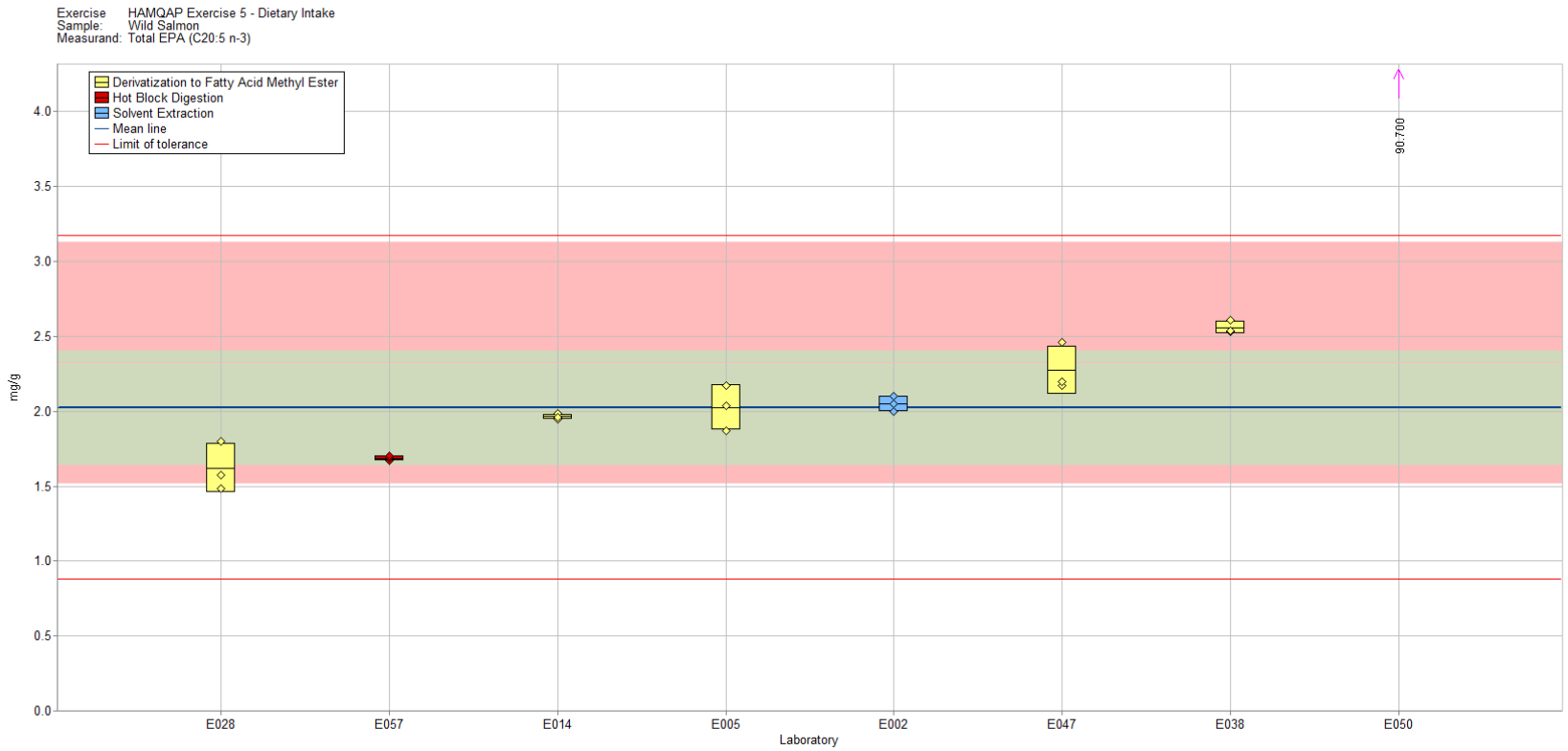

Figure 5-18. Total EPA in Wild Salmon (data summary view – sample preparation method). In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The solid 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, $|Z_{\text{NIST}}| \leq 2$.

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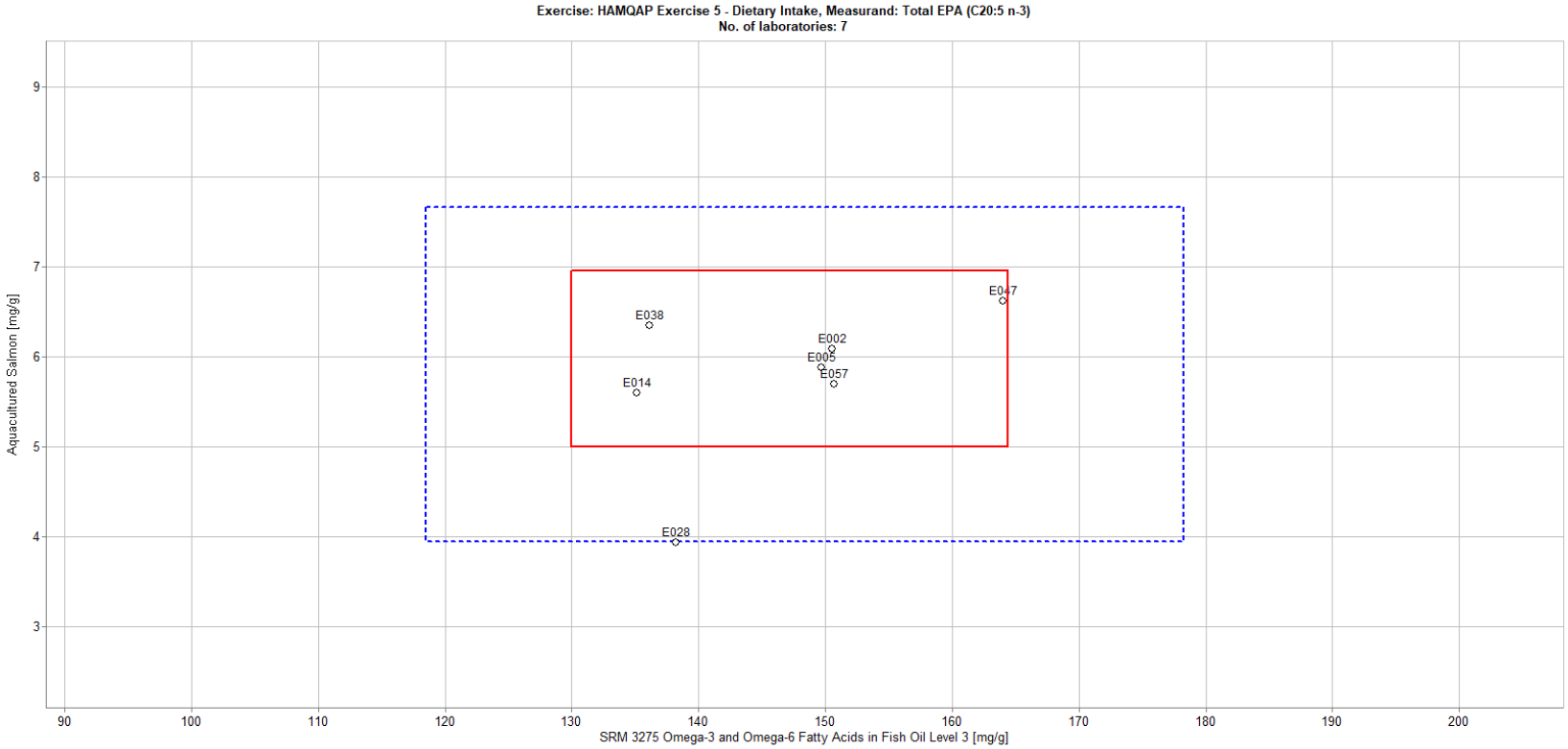

Figure 5-19. Laboratory means for total EPA in SRM 3275 Omega-3 and Omega-6 Fatty Acids in Fish Oil Level 3 and Aquacultured Salmon (sample/sample comparison view). In this view, the individual laboratory mean for one sample (SRM 3275 Level 3) is compared to the individual laboratory mean for a second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

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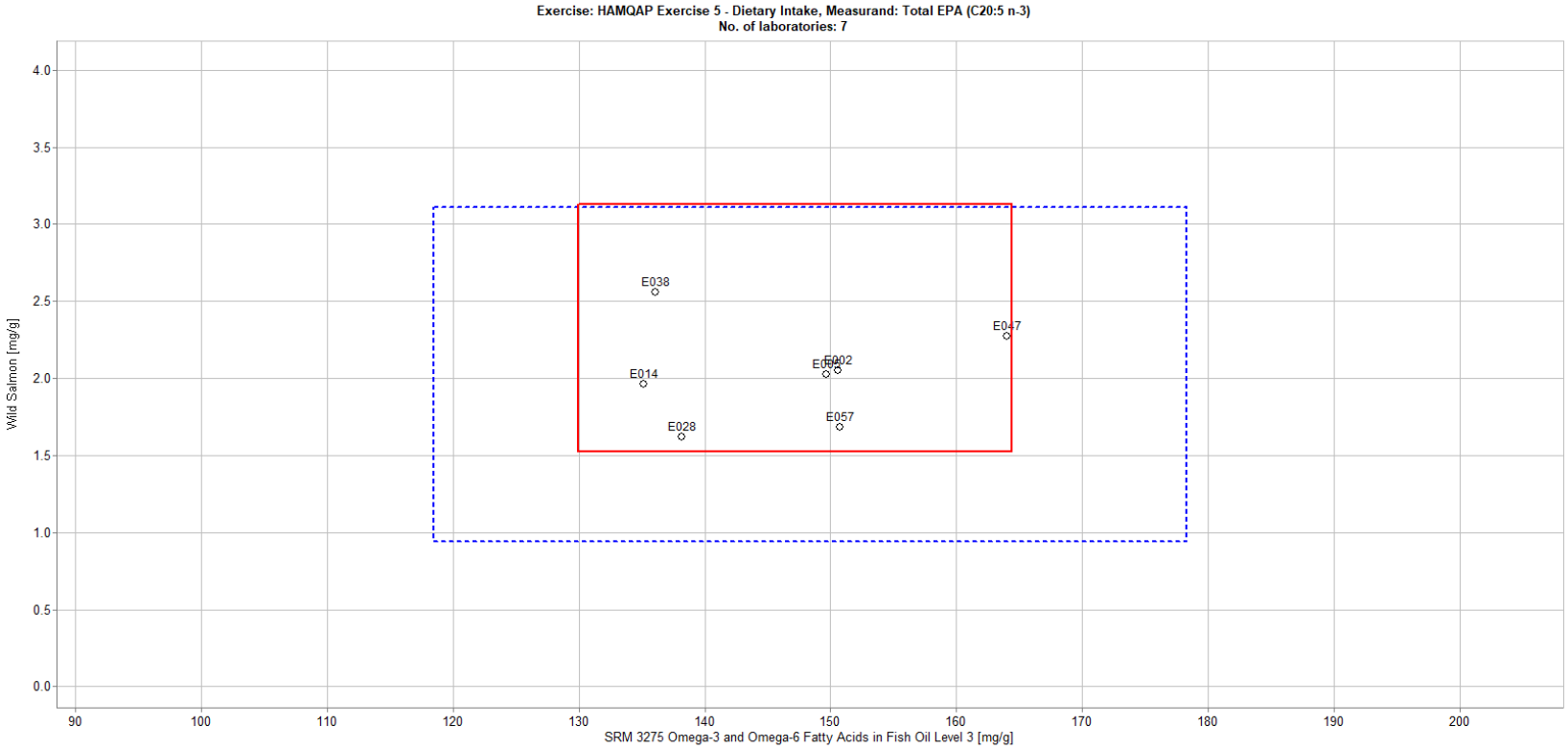

Figure 5-20. Laboratory means for total EPA in SRM 3275 Omega-3 and Omega-6 Fatty Acids in Fish Oil Level 3 and Wild Salmon (sample/sample comparison view). In this view, the individual laboratory mean for one sample (SRM 3275 Level 3) is compared to the individual laboratory mean for a second sample (salmon). The solid red box represents the NIST range of tolerance for the two samples, SRM 3275 Level 3 (x-axis) and salmon (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 3275 Level 3 (x-axis) and salmon (y-axis), calculated as the values above and below the consensus means that result in an acceptable Z'_{comm} score, $|Z'_{\mathrm{comm}}| \leq 2$.

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Table 5-6. Data summary table for total DHA in fish oil and salmon. Data points highlighted in red have been flagged as potential outliers (e.g., Grubb and/or Cochran) by the NIST software package.

		Total DHA (C22:6 n-3)														
		SRM 3275 Omega-3 and Omega-6 Fatty Acids in Fish Oil Level 3 (mg/g)					Aquacultured Salmon (mg/g)					Wild Salmon (mg/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target				99.7	9.6				10.2	2.3				7.76	3.18
	E002	96.3	95.25	94.55	95.4	0.9	9.7	9.85	9.7	9.75	0.09	6.15	6.45	6.55	6.38	0.21
	E004	99.05	98.89	98.9	98.9	0.1										
	E005	101.45	101.41	101.54	101.5	0.1	10.42	10.39	10.16	10.32	0.14	7.08	6.58	7.41	7.02	0.42
	E006	100.9	100.4	100.9	100.7	0.3										
	E008															
	E014	89.4	88.7	86.4	88.2	1.6	8.432	8.388	9.089	8.64	0.39	5.805	5.671	5.716	5.73	0.07
	E017	192.46	193.12	192.19	192.6	0.5										
	E020															
	E021															
	E022															
	E028	96.32	93.41	96.15	95.3	1.6	6.498	6.536	6.773	6.60	0.15	5.118	5.897	4.887	5.30	0.53
	E030															
	E032															
	E033	86.3	82.3	81.1	83.2	2.7										
	E038	698.67	763.17	723.8	728.5	32.5	0.01	0.01	0.01	0.01	0.00	0.01	0.01	0.01	0.01	0.00
	E039															
	E047	106	107	91.7	101.6	8.6	10.6	10.6	12.8	11.33	1.27	6.73	6.85	8.96	7.51	1.25
	E050						320.4	358.3		339.35	26.80	506.6	481.5		494.05	17.75
	E051	104.9	104.7	104.2	104.6	0.4										
	E057	96.62	98.35	95.79	96.9	1.3	9.14	8.84	9.05	9.01	0.15	4.83	4.81	4.95	4.86	0.08
	E072															
Community Results		Consensus Mean			96.6		Consensus Mean			8.42		Consensus Mean			5.59	
		Consensus Standard Deviation			3.3		Consensus Standard Deviation			1.21		Consensus Standard Deviation			0.77	
		Maximum			728.5		Maximum			339.35		Maximum			494.05	
		Minimum			83.2		Minimum			0.01		Minimum			0.01	
		N			12		N			8		N			8	

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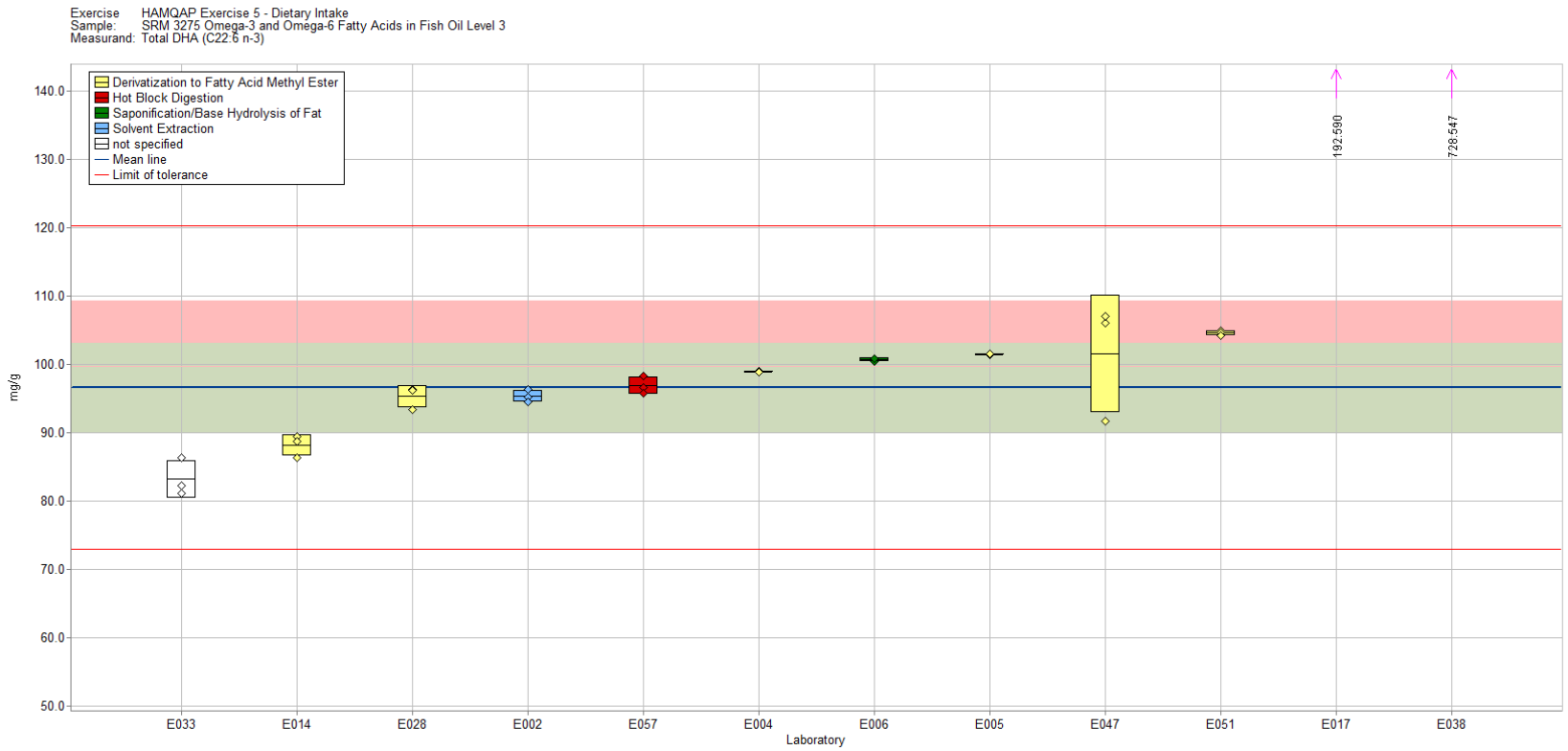

Figure 5-21. Total DHA in SRM 3275 Omega-3 and Omega-6 Fatty Acids in Fish Oil (Level 3) (data summary view – sample preparation method). In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents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

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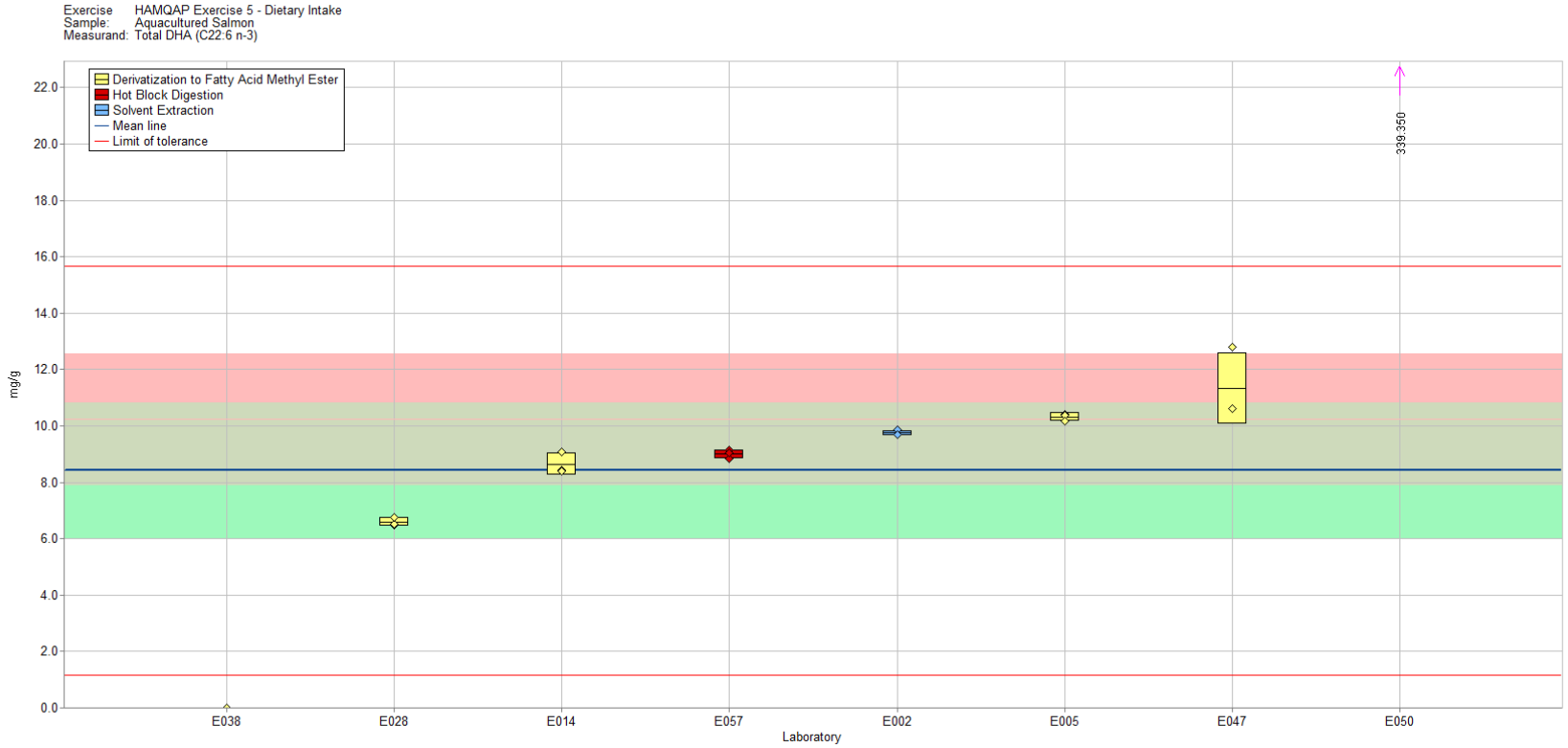

Figure 5-22. Total DHA in Aquacultured Salmon (data summary view – sample preparation method). In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. 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, $|Z_{\text{NIST}}| \leq 2$.

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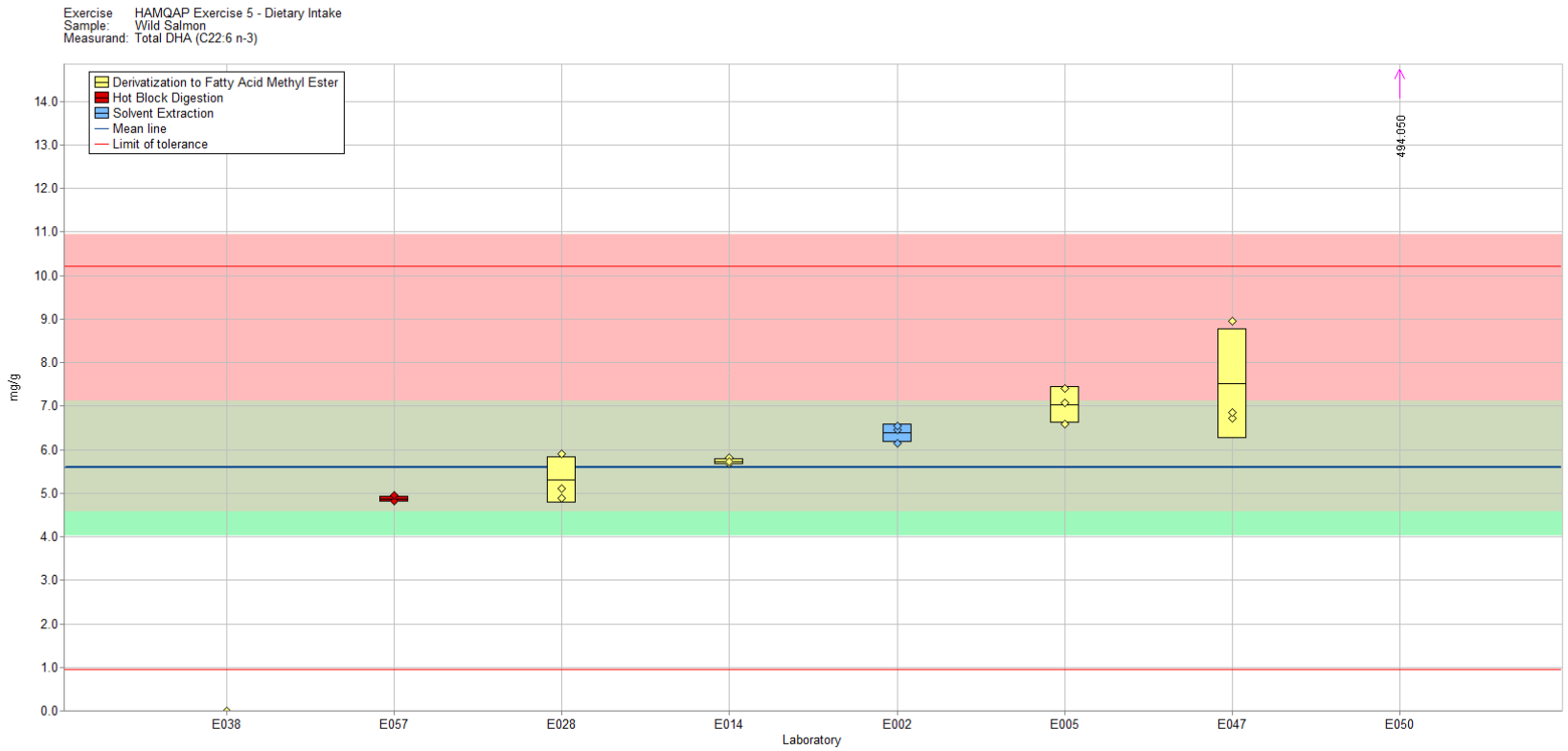

Figure 5-23. Total DHA in Wild Salmon (data summary view – sample preparation method). In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The solid 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, $|Z_{\text{NIST}}| \leq 2$.

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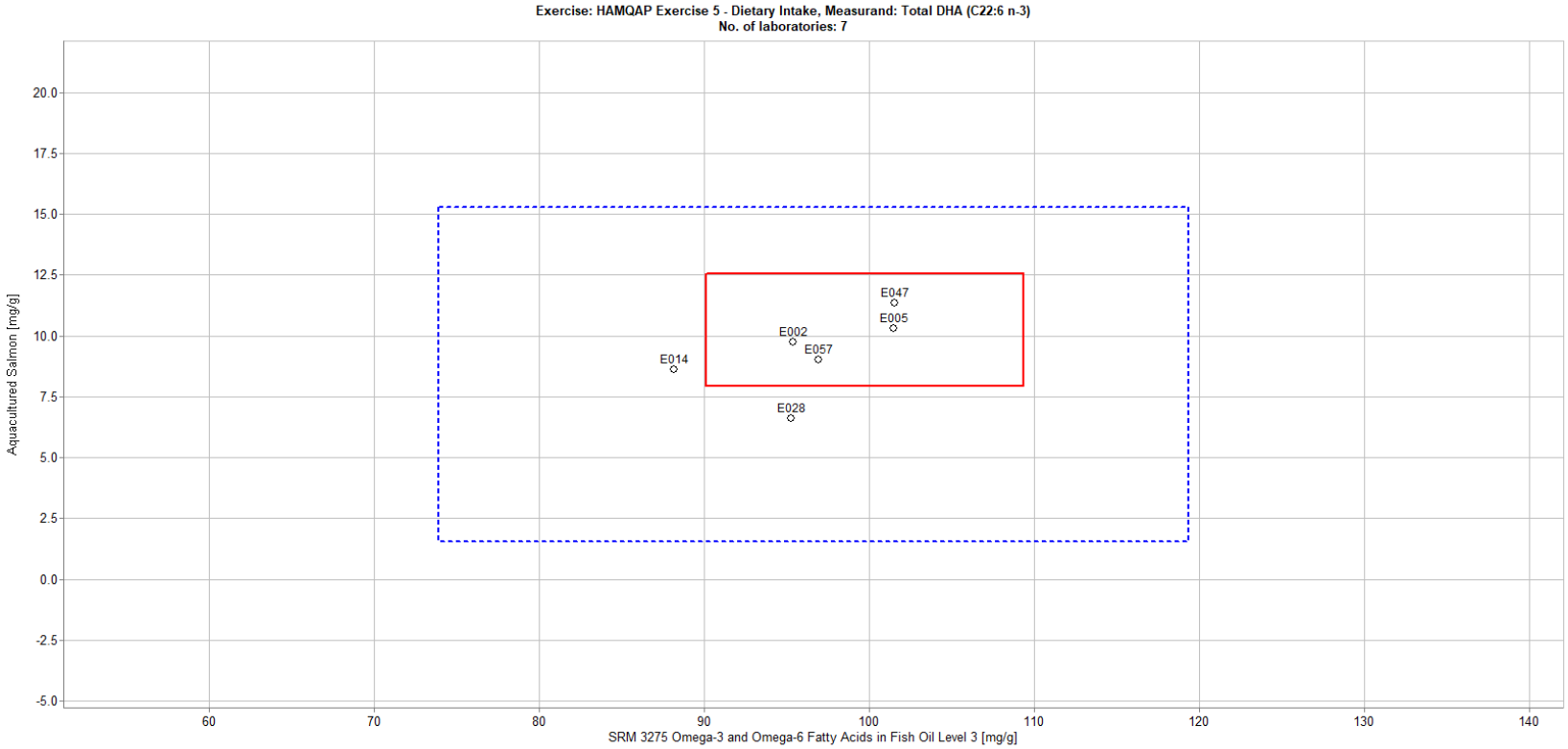

Figure 5-24. Laboratory means for total DHA in SRM 3275 Omega-3 and Omega-6 Fatty Acids in Fish Oil Level 3 and Aquacultured Salmon (sample/sample comparison view). In this view, the individual laboratory mean for one sample (SRM 3275 Level 3) is compared to the individual laboratory mean for a second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

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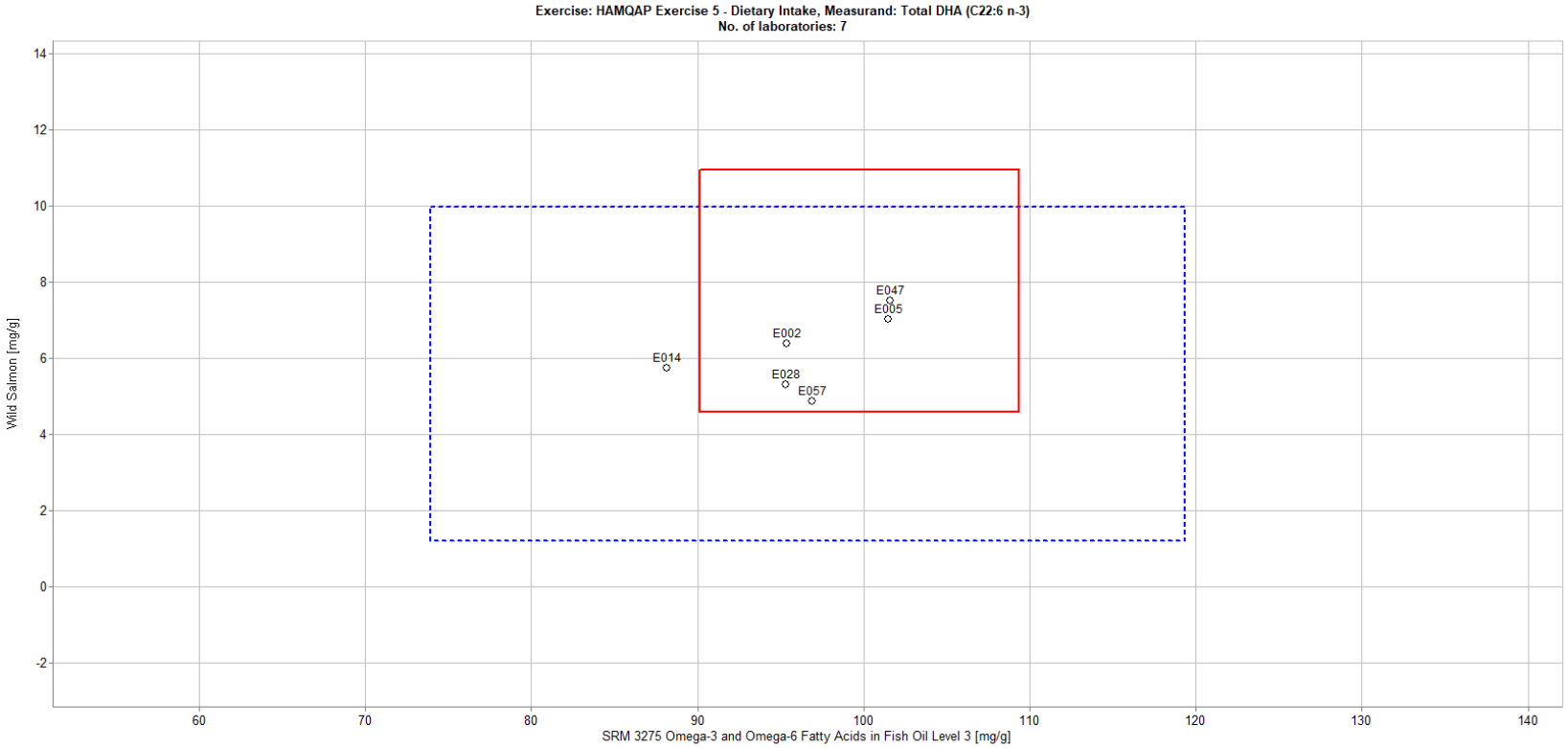

Figure 5-25. Laboratory means for total DHA in SRM 3275 Omega-3 and Omega-6 Fatty Acids in Fish Oil Level 3 and Wild Salmon (sample/sample comparison view). In this view, the individual laboratory mean for one sample (SRM 3275 Level 3) is compared to the individual laboratory mean for a second sample (salmon). The solid red box represents the NIST range of tolerance for the two samples, SRM 3275 Level 3 (x-axis) and salmon (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 3275 Level 3 (x-axis) and salmon (y-axis), calculated as the values above and below the consensus means that result in an acceptable Z'_{comm} score, $|Z'_{\mathrm{comm}}| \leq 2$.

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Human Metabolites Sample Information

Human Red Blood Cells A and B. Participants were provided with three vials each of Human RBCs Sample A and Human RBCs Sample B, each containing 0.6 mL of frozen human red blood cells. RBC A was collected from six healthy donors and RBC B was collected from two healthy donors. Participants were asked to avoid exposing the material to direct sun or UV light, to store the material at or below –70 °C, and to prepare one sample and report one value from each vial provided. Before use, participants were instructed to allow the material to thaw at room temperature for at least 30 min prior to sampling, use the material immediately after thawing, gently mix the contents prior to removal of a test portion for analysis, and use their usual in-house method of analysis. The approximate analyte levels were not reported to participants prior to the study. The NIST-determined target values for the weight percent of EPA and DHA per total fatty acids were assigned using results from ID-GC-MS analysis by CDC. The target values for EPA and DHA and their associated uncertainties are provided in the table below.

	NIST-Determined Weight Percent (based on Total Fatty Acids) in Human RBC (%)	
Analyte	RBC A	RBC B
EPA	0.340 ± 0.040	2.44 ± 0.20
DHA	2.21 ± 0.012	6.48 ± 0.52

Human Metabolites Study Results

- Thirteen laboratories enrolled in this exercise and received samples to measure each of the fatty acids in human serum. Five laboratories reported results for EPA and DHA for both samples (38 % participation).
- The consensus ranges for both fatty acids overlapped the target ranges.
  - The consensus mean for EPA was centered within the target range for RBC A (**Figure 5-26**) but was near the upper limit of the target range for RBC B (**Figure 5-27**).
  - The consensus means for DHA were near the center of the target ranges for both samples (**Figure 5-29** and **Figure 5-30**).
- The between-laboratory variabilities were excellent for both analytes in both matrices, at 3.2 % RSD or less. Variabilities for each analyte/sample pair are reported in the table below.

	Between-Laboratory Variability (% RSD)	
Analyte	RBC A	RBC B
EPA	2.4 %	2.5 %
DHA	5.0 %	3.2 %

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

Human Metabolites Technical Recommendations

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

- No trends were noted with respect to the sample preparation or analytical methods reported by the participants.
- Overall, participants performed well on this study, indicating their ability to determine the relative percentage of EPA and DHA in human red blood cell samples.
  - The analytes in this study were quantified relative to the total amount of fatty acids present in the sample. As a result, the ability to accurately extract all fats and quantify total EPA and DHA was not evaluated.
  - Future studies should include a measure of total EPA and DHA to identify method biases and ensure accuracy in this approach, which may be used to define interventions and evaluate human health status.
- 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.

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Table 5-7. Individualized data summary table (NIST) for fatty acids in human red blood cells.

National Institute of Standards & Technology

HAMQAP Exercise 5 - Fatty Acids

	Lab Code:	NIST	1. Your Results				2. Community Results			3. Target	
Analyte	Sample	Units	x_i	s_i	Z'_{comm}	Z_{NIST}	N	x*	s*	x_{NIST}	U
EPA (C20:5 n-3)	Red Blood Cells A	%	0.34	0.04			5	0.328	0.0078	0.34	0.04
EPA (C20:5 n-3)	Red Blood Cells B	%	2.44	0.2			5	2.61	0.064	2.44	0.2
DHA (C22:6 n-3)	Red Blood Cells A	%	2.21	0.12			5	2.18	0.11	2.21	0.12
DHA (C22:6 n-3)	Red Blood Cells B	%	6.48	0.52			5	6.58	0.21	6.48	0.52

x_i Mean of reported values

s_i Standard deviation of reported values

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

Z_{NIST} Z-score with respect to NIST value

N Number of quantitative values reported

x* Robust mean of reported values

s* Robust standard deviation

x_{NIST} NIST-assessed value

U expanded uncertainty about the NIST-assessed value

Table 5-8. Data summary table for EPA in human red blood cells.

		EPA (C20:5 n-3)									
		Red Blood Cells A (%)					Red Blood Cells B (%)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target				0.340	0.040				2.440	0.200
	E002										
	E014										
	E024										
	E028										
	E033										
	E038	0.31	0.36	0.32	0.330	0.026	2.71	2.75	2.76	2.740	0.026
	E039										
	E050	0.31	0.3	0.31	0.307	0.006	2.72	2.69	2.7	2.703	0.015
	E057	0.31	0.32	0.31	0.313	0.006	2.52	2.47	2.56	2.517	0.045
	E065										
	E068	0.334	0.361	0.338	0.344	0.015	2.5	2.46	2.42	2.460	0.040
	E070	0.31	0.37	0.36	0.347	0.032	2.56	2.68	2.62	2.620	0.060
	E073										
Community Results		Consensus Mean			0.328		Consensus Mean			2.608	
		Consensus Standard Deviation			0.008		Consensus Standard Deviation			0.064	
		Maximum			0.347		Maximum			2.740	
		Minimum			0.307		Minimum			2.460	
		N			5		N			5	

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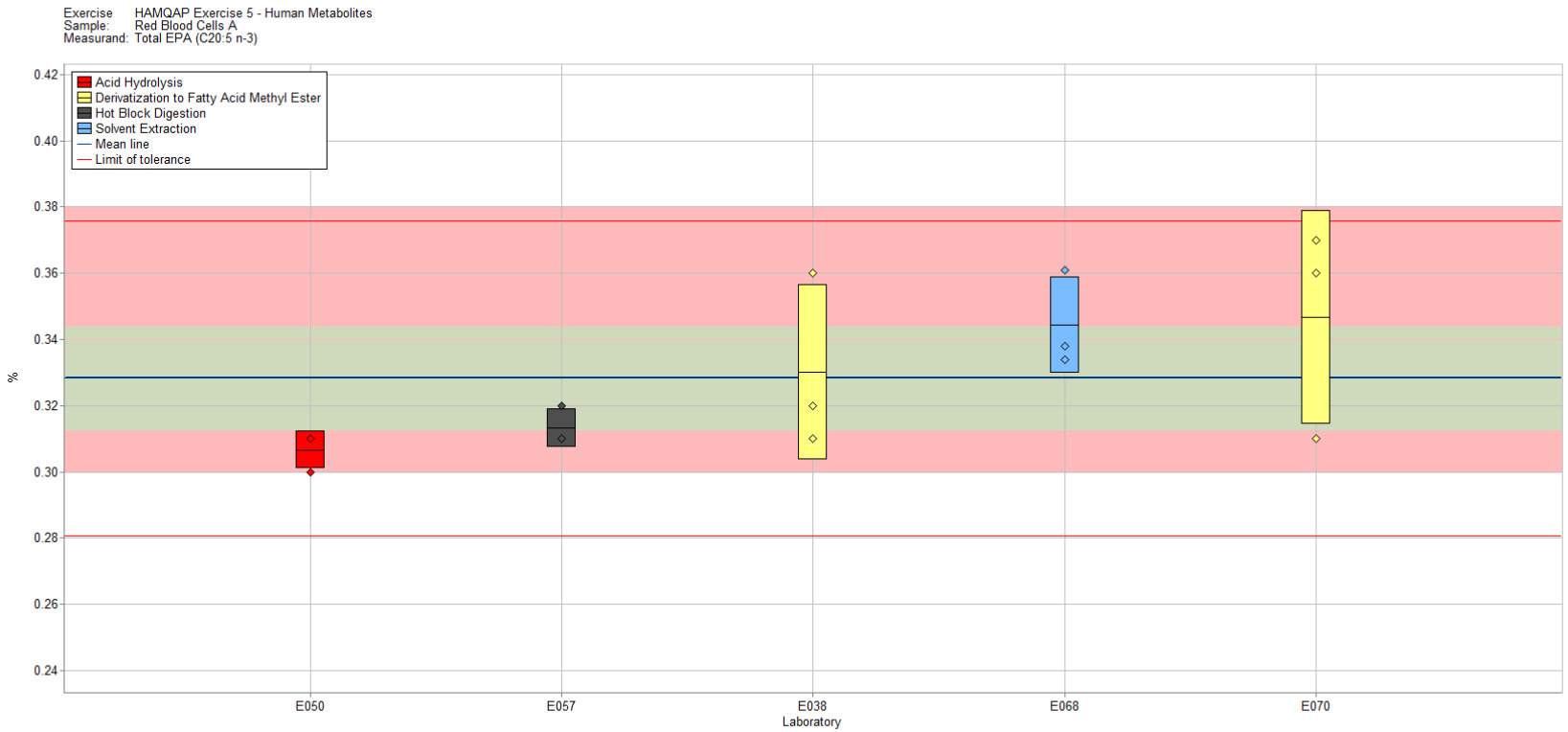

Figure 5-26. EPA in Human Red Blood Cells A (data summary view – sample preparation method). In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The 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, $|Z_{\text{NIST}}| \leq 2$.

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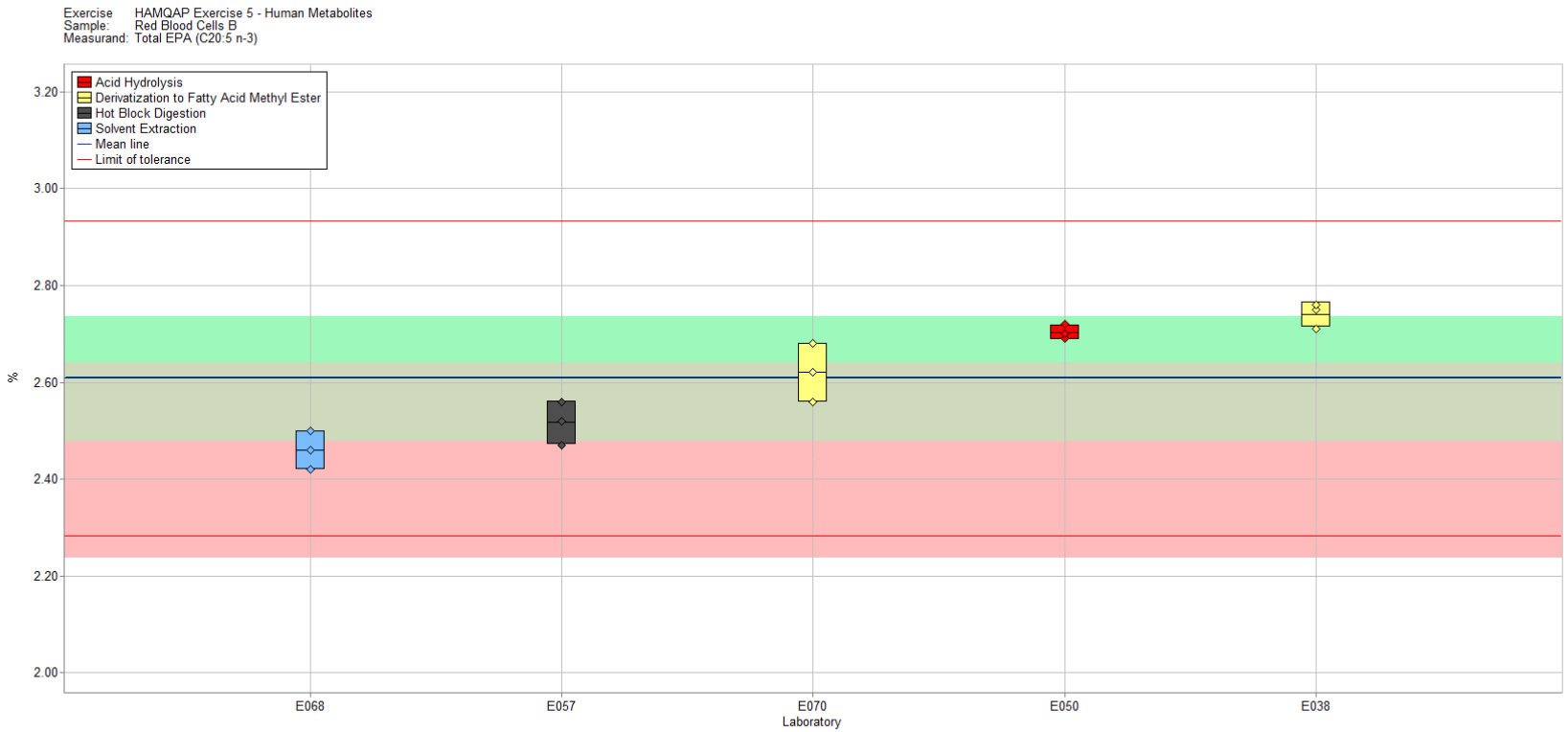

Figure 5-27. EPA in Human Red Blood Cells B (data summary view – sample preparation method). In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The 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, $|Z_{\text{NIST}}| \leq 2$.

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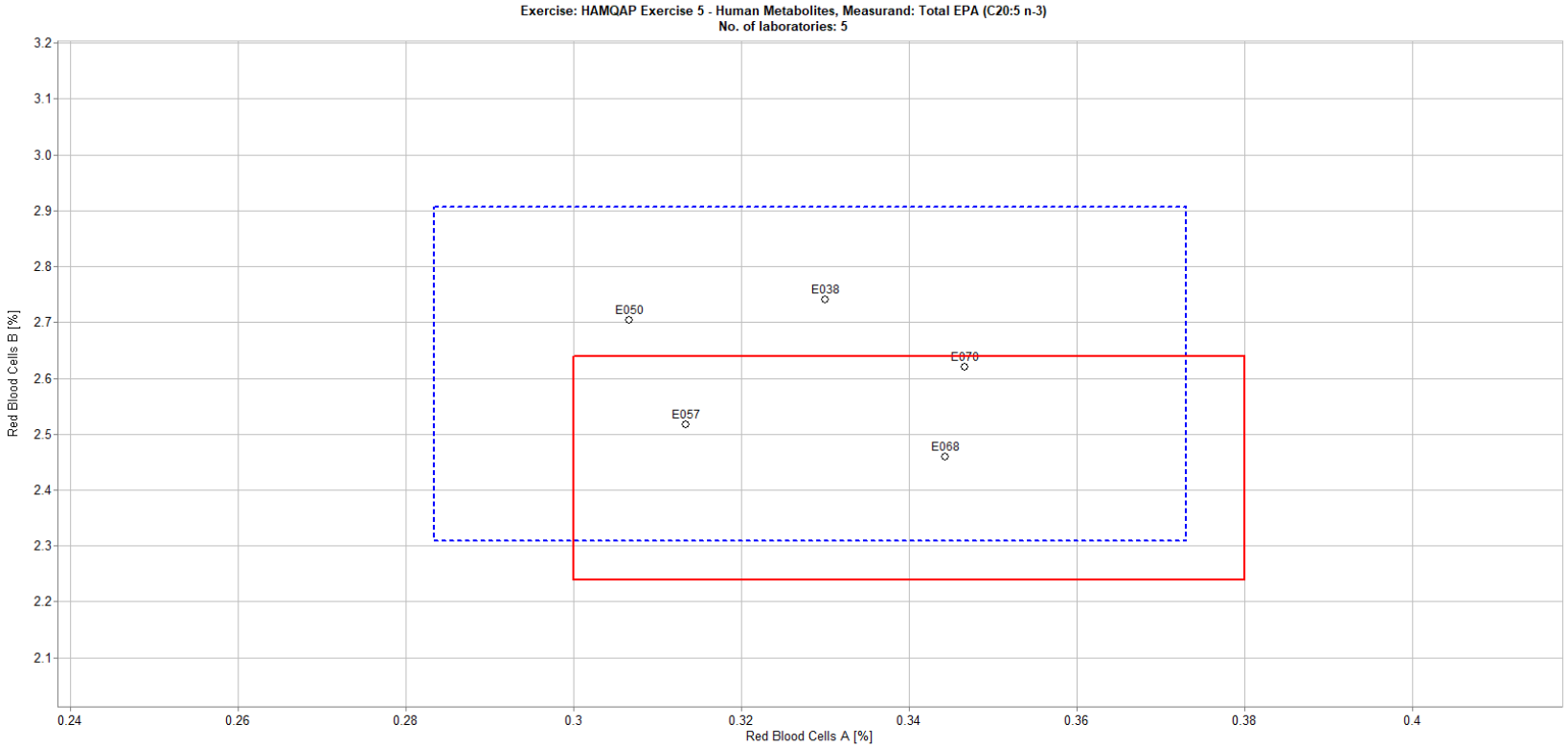

Figure 5-28. Laboratory means for EPA in Human Red Blood Cells A and Human Red Blood Cells B (sample/sample comparison view). In this view, the individual laboratory mean for one sample (RBC A) is compared to the individual laboratory mean for a second sample (RBC B). The solid red box represents the NIST range of tolerance for the two samples, RBC A (x-axis) and RBC B (y-axis), which encompasses the target values bounded by their uncertainties (U_{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 RBC A (x-axis) and RBC B (y-axis), calculated as the values above and below the consensus means that result in an acceptable Z'_{comm} score, $|Z'_{\mathrm{comm}}| \leq 2$.

Table 5-9. Data summary table for DHA in human red blood cells.

		DHA (C22:6 n-3)									
		Red Blood Cells A (%)					Red Blood Cells B (%)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target				2.21	0.12				6.48	0.52
	E002										
	E014										
	E024										
	E028										
	E033										
	E038	1.88	2.09	2.13	2.03	0.13	6.6	6.53	6.67	6.60	0.07
	E039										
	E050	2.48	2.46	2.44	2.46	0.02	7.46	7.48	7.43	7.46	0.03
	E057	2.21	2.22	2.21	2.21	0.01	6.66	6.56	6.64	6.62	0.05
	E065										
	E068	2.17	2.18	2.19	2.18	0.01	6.69	6.53	6.37	6.53	0.16
	E070	2	1.93	2.04	1.99	0.06	5.88	5.85	5.81	5.85	0.04
	E073										
Community Results		Consensus Mean			2.18		Consensus Mean			6.58	
		Consensus Standard Deviation			0.11		Consensus Standard Deviation			0.21	
		Maximum			2.46		Maximum			7.46	
		Minimum			1.99		Minimum			5.85	
		N			5		N			5	

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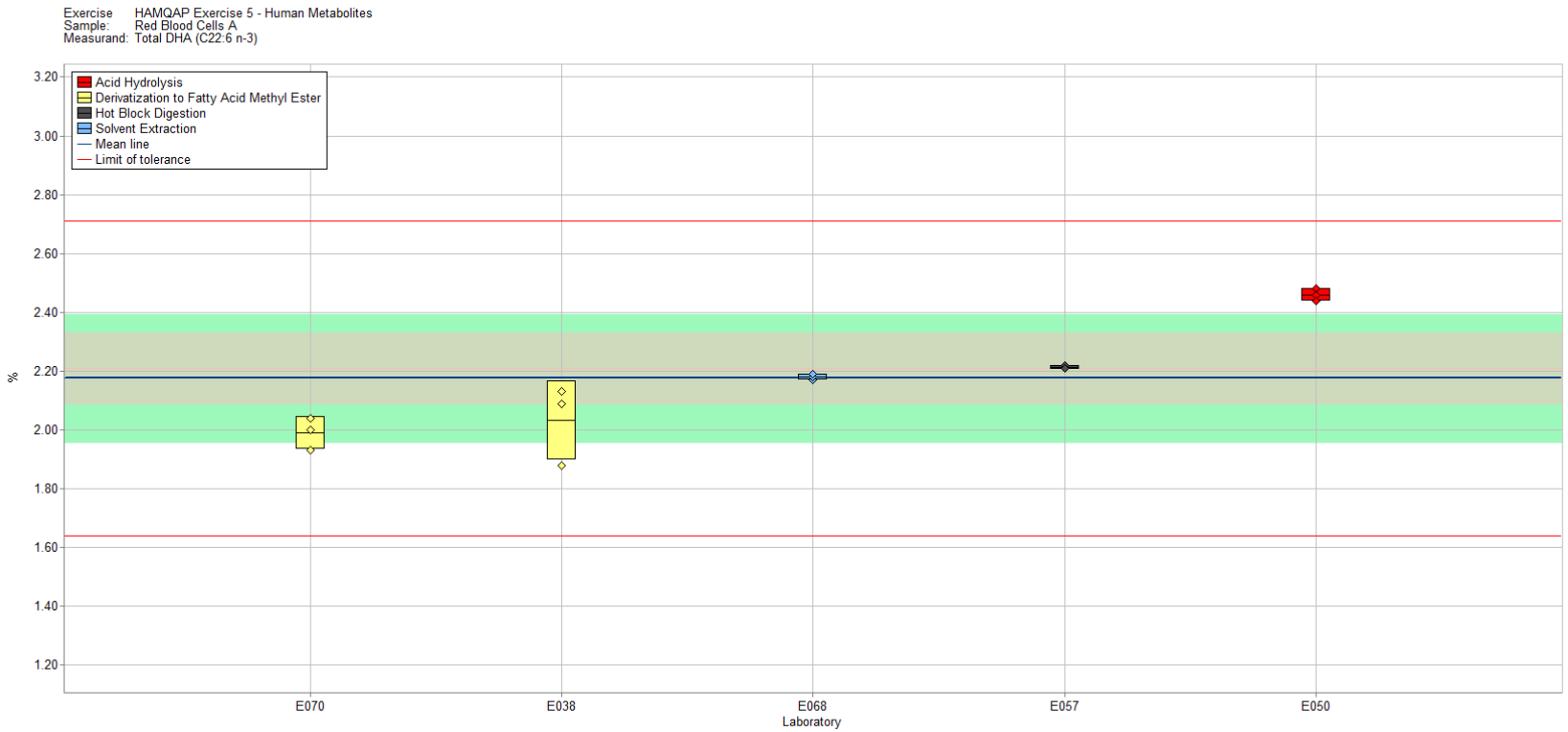

Figure 5-29. DHA in Human Red Blood Cells A (data summary view – sample preparation method). In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red line represents the upper consensus range of tolerance, calculated as the values above the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$, with the lower limit set at zero. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by its uncertainty (U_{NIST}) and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$.

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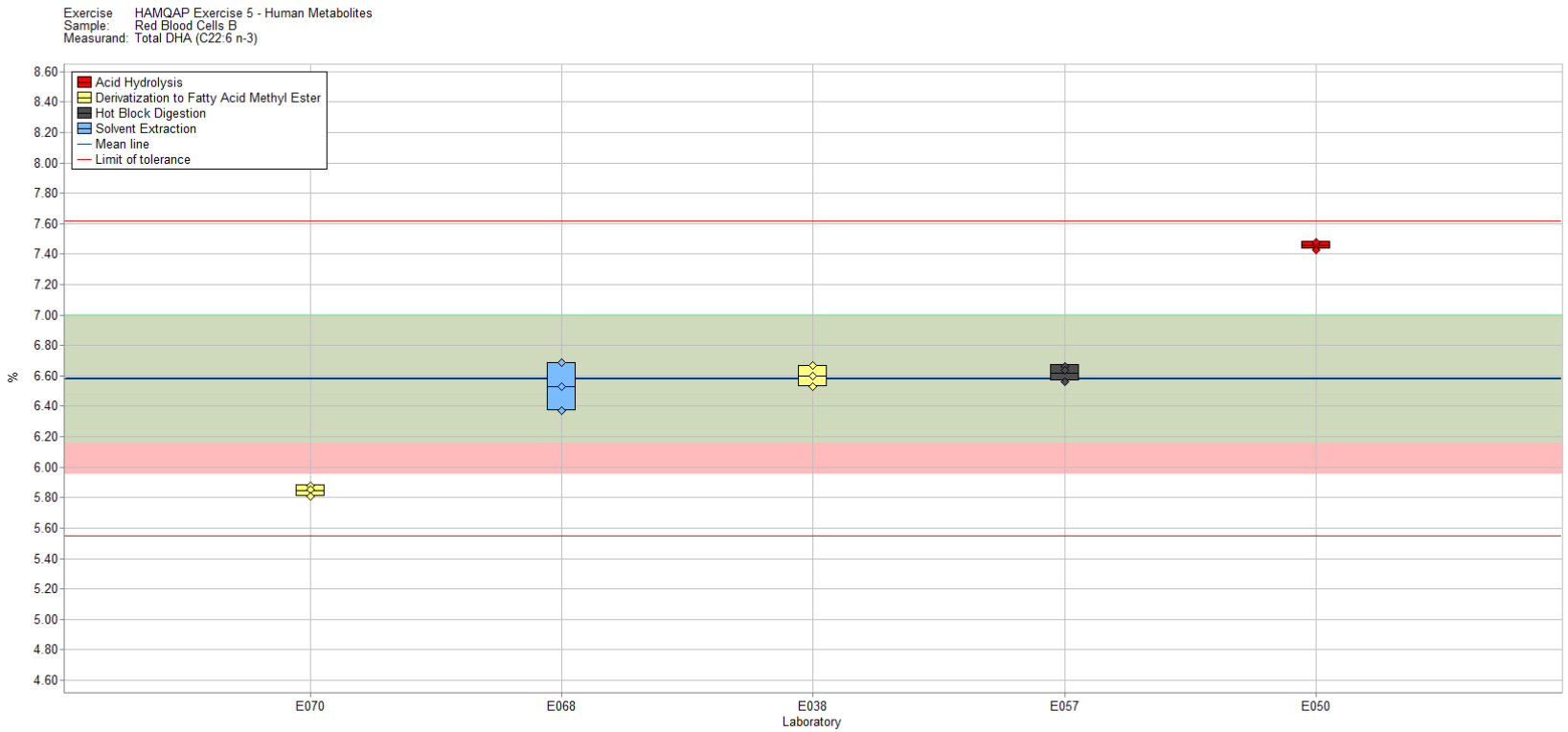

Figure 5-30. DHA in Human Red Blood Cells B (data summary view – sample preparation method). In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The 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, $|Z_{\text{NIST}}| \leq 2$.

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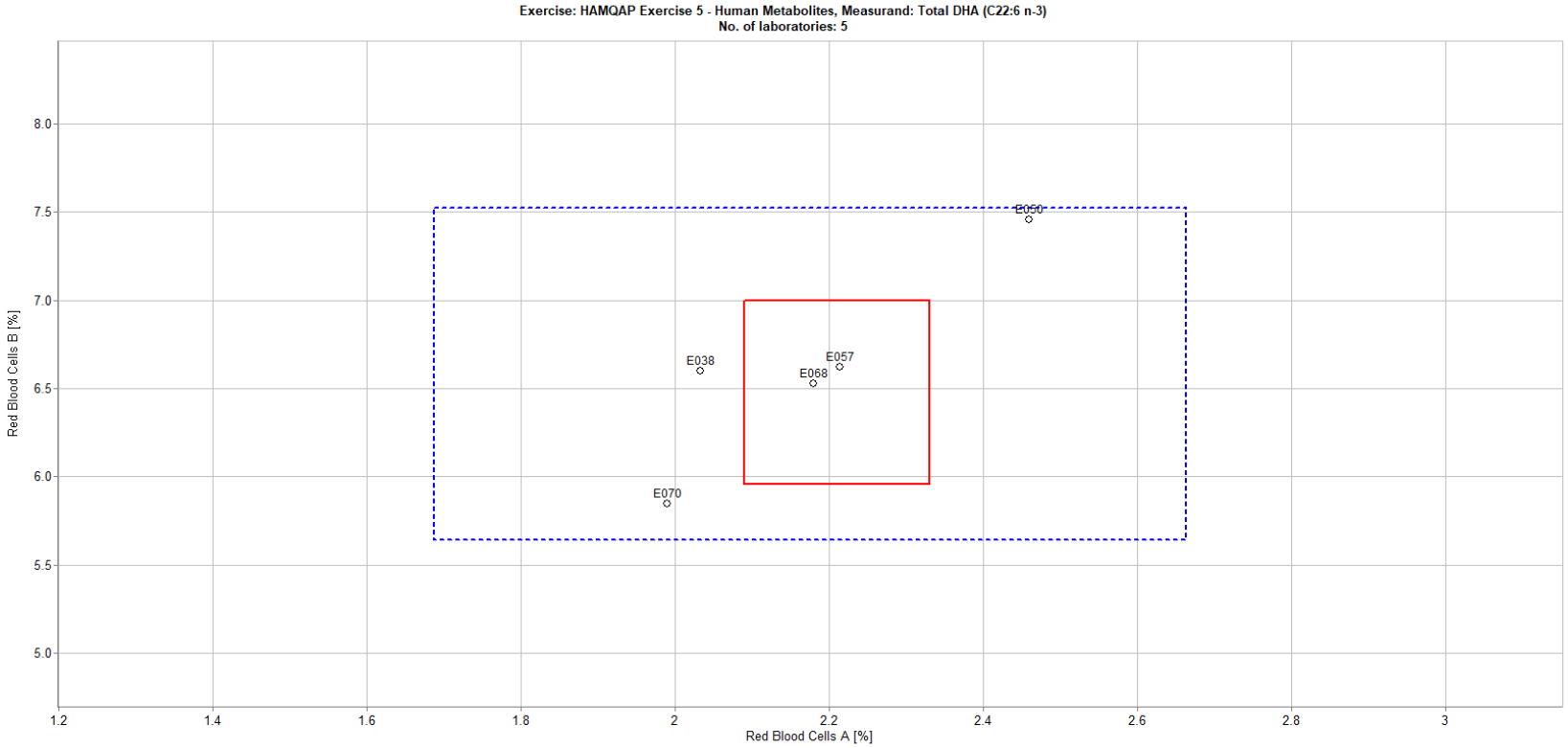

Figure 5-31. Laboratory means for DHA in Human Red Blood Cells A and Human Red Blood Cells B (sample/sample comparison view). In this view, the individual laboratory mean for one sample (RBC A) is compared to the individual laboratory mean for a second sample (RBC B). The solid red box represents the NIST range of tolerance for the two samples, RBC A (x-axis) and RBC B (y-axis), which encompasses the target values bounded by their uncertainties (U_{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 RBC A (x-axis) and RBC B (y-axis), calculated as the values above and below the consensus means that result in an acceptable Z'_{comm} score, $|Z'_{\mathrm{comm}}| \leq 2$.

Fatty Acids Overall Study Comparison

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

- The between-laboratory variability was lower for the fish oil samples than for the salmon samples. One laboratory consistently reported high results with respect to the consensus and/or target ranges, indicating a unique method challenge. Laboratories should be aware of the level of sample preparation required and avoid sample over- and under-processing.
- 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.

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SECTION 6: BOTANICALS (Catechins)

Study Overview

In this study, participants were provided with samples of SRM 3255 Green Tea (*Camellia sinensis*) Extract and SRM 3256 Green Tea-Containing Solid Oral Dosage Form (SODF). Participants were asked to use in-house analytical methods to determine the mass fraction (mg/g) of select catechins, gallic acid, and L-theanine in each matrix. Green tea (*Camellila sinensis*) is used as a food, a dietary supplement, and a traditional medicine for its purported health effects. The consumption of green tea is purported to improve mental focus and sleep quality (attributed to L-theanine) and offer possible protective effects against heart disease and cancer (attributed to catechins and gallic acid).[18] Measurement of catechins in these products is important for ensuring accuracy of product labels and also for understanding both positive and negative health outcomes related to consumption of such products.

Dietary Intake Sample Information

Green Tea Extract. Participants were provided with three packets, each containing 1 g of green tea extract. Participants were asked to store the material at controlled room temperature, 20 °C to 25 °C, and to prepare one sample and report one value from each packet provided. Before use, participants were instructed to mix the contents of the packet thoroughly, and to use a sample size of at least 100 mg. The approximate analyte levels were not reported to participants prior to the study. Certified values for catechins and reference values for gallic acid and L-theanine in SRM 3255 were assigned using results from NIST by LC-Abs and LC-MS, and from collaborating laboratories using LC-FL and/or LC-Abs. The NIST-determined values and uncertainties are provided in the table below, both on a dry-mass basis as listed on the COA, and on an as-received basis accounting for moisture of the material (3.2 %) with a further expanded uncertainty for evaluation of laboratory performance.

	NIST-Determined Mass Fractions in SRM 3255 (mg/g)	
Analyte	(dry-mass basis)	(as-received basis)[(a)]
(+)-Catechin	9.17 ± 0.93	8.88 ± 1.80
(–)-Epicatechin	47.3 ± 6.7	45.8 ± 13.0
(–)-Epicatechin gallate	100.3 ± 7.8	97.2 ± 15.1
(–)-Epigallocatechin	81.8 ± 6.5	79.2 ± 12.6
(–)-Epigallocatechin gallate	422 ± 19	408.8 ± 36.8
(–)-Gallocatechin	22.0 ± 1.7	21.3 ± 3.3
(–)-Gallocatechin gallate	39.0 ± 2.0	37.8 ± 3.9
Gallic acid	3.231 ± 0.086	3.13 ± 0.17
L-theanine	0.340 ± 0.008	0.329 ± 0.016

(a) Associated expanded uncertainties for the target zone for acceptable performance are calculated as 2*(U_{95} or U_{NIST}).

[18] Green Tea. National Institutes of Health National Center for Complementary and Integrative Health https://www.nccih.nih.gov/health/green-tea (accessed June 2020).

Green Tea-Containing SODF. Participants were provided with three packets, each containing 2.5 g of powdered oral-dosage material. Participants were asked to store the material at controlled room temperature, 20 °C to 25 °C, and to prepare one sample and report one value from each packet provided. Before use, participants were instructed to mix the contents of the packet thoroughly, allow contents to settle for one minute prior to opening to minimize the loss of fine particles, and to use a sample size of at least 100 mg. The approximate analyte levels were not reported to participants prior to the study. Certified values for catechins and reference values for (-)-gallocatechin gallate and L-theanine in SRM 3256 were assigned using results from NIST by LC-Abs and LC-MS, and from collaborating laboratories using LC-Abs and/or LC-MS. The NIST-determined values and uncertainties are provided in the table below, both on a dry-mass basis as listed on the COA, and on an as-received basis accounting for moisture of the material (2.4 %) with a further expanded uncertainty for evaluation of laboratory performance.

	NIST-Determined Mass Fractions in SRM 3256 (mg/g)	
Analyte	(dry-mass basis)	(as-received basis)[a]
(+)-Catechin	2.63 ± 0.18	2.57 ± 0.35
(–)-Epicatechin	12.0 ± 2.6	11.72 ± 5.08
(–)-Epicatechin gallate	17.1 ± 2.6	16.70 ± 5.08
(–)-Epigallocatechin	30.7 ± 5.7	30.0 ± 11.1
(–)-Epigallocatechin gallate	71.1 ± 6.6	69.4 ± 12.9
(–)-Gallocatechin	7.55 ± 0.28	7.37 ± 0.55
(–)-Gallocatechin gallate	4.6 ± 1.8	4.49 ± 3.52
Gallic acid	13.10 ± 0.49	12.79 ± 0.96
L-theanine	3.7 ± 1.2	3.613 ± 2.343

[a] Associated expanded uncertainties for the target zone for acceptable performance are calculated as 2*(U_{95} or U_{NIST}).

Dietary Intake Study Results

- A total of 30 laboratories enrolled in this exercise, with between 15 and 30 laboratories requesting samples to measure one or more of the 7 catechins, gallic acid, and L-theanine in green tea extract (SRM 3255) and SODF (SRM 3256). The enrollment and reporting statistics for the botanicals study is described in the table below. Some of the reported values were non-quantitative (zero or below LOQ) but are included in the participation and reporting statistics.

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Analyte	Number of Laboratories Requesting Samples	Number of Laboratories Reporting Results (Percent Participation) Green Tea Extract SRM 3255	Green Tea SODF SRM 3256
(+)-Catechin	19	12 (63 %)	12 (63 %)
(–)-Epicatechin	18	12 (67 %)	12 (67 %)
(–)-Epicatechin gallate	30	11 (37 %)	11 (37 %)
(–)-Epigallocatechin	18	11 (61 %)	11 (61 %)
(–)-Epigallocatechin gallate	17	12 (71 %)	12 (71 %)
(–)-Gallocatechin	15	6 (40 %)	6 (40 %)
(–)-Gallocatechin gallate	16	10 (63 %)	10 (63 %)
L-theanine	22	6 (27 %)	6 (27 %)
Gallic acid	17	4 (24 %)	4 (24 %)

- The between-laboratory variabilities were acceptable for most analytes in the green tea extract (SRM 3255) and SODF (SRM 3256) (see table below). Variabilities for L-theanine in SRM 3255 and gallic acid in SRM 3256 were very large (> 60 % RSD).

Analyte	Between-Laboratory Variability (% RSD) Green Tea Extract SRM 3255	Green Tea SODF SRM 3256
(+)-Catechin	11%	15%
(–)-Epicatechin	5%	8%
(–)-Epicatechin gallate	4%	3%
(–)-Epigallocatechin	8%	14%
(–)-Epigallocatechin gallate	1%	1%
(–)-Gallocatechin	10%	9%
(–)-Gallocatechin gallate	4%	5%
L-theanine	88%	4%
Gallic acid	15%	60%

- In both the green tea extract and the SODF, the consensus means were well within the NIST target ranges for most analytes. However, this was not observed for the following:
  - For the extract, the consensus mean for (–)-gallocatechin gallate (**Figure 6-19**) was above the NIST target range.
  - In both samples, two laboratories reported levels significantly higher than the NIST target range for (–)-gallocatechin gallate (**Figures 6-19** and **6-20**).
  - In the SODF sample, the consensus mean just touched the top edge of the NIST target range (**Figure 6-23**).

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    - For the extract, the consensus mean for L-theanine was significantly higher than the target range. (**Figures 6-25**).
- All participating laboratories reported using LC-Abs for determination of the analytes in the green tea samples.
- Most laboratories reported using solvent extraction as the sample preparation method for the green tea samples. Other reported sample preparation techniques included dilution, open beaker digestion, and derivatization. All sample preparation techniques seem to perform equally well, though greater between-laboratory variability was observed for laboratories reporting use of solvent extraction.

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Dietary Intake Technical Recommendations

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

- Higher variability was observed for a few measurands, such as (–)-gallocatechin gallate, L-theanine, and gallic acid; calibration errors, suitable LC validation, and sample preparation are likely causes.
    - Laboratories reporting results below the target value or large sample-to-sample variability should examine sample preparation conditions. Complete extraction of these analytes from the botanical matrices may require use of less common solvents or multiple extraction cycles.
        - Any extraction procedure should be optimized to determine the most effective extraction solvent and 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.
    - Definite linear trends indicating possible calibration errors were observed in **Figures 6-9** and **6-18**, though very few participants reported data for (-)-gallocatechin.
        - 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 matched calibrants should be used for quantitation whenever possible.
- Laboratories reporting appropriate data results for an analyte in one material but either high or low results for the second material may be experiencing more difficulty in sample preparation of one material over another (**Figures 6-6** and **6-12**).
- Laboratories reporting results flagged as outliers should check for errors in calculations or reporting units. Confirm that all dilution factors have been properly tabulated.

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Table 6-1. Data summary table for catechins, gallic acid, and L-theanine in green tea extract and green tea-containing SODF.

National Institute of Standards & Technology

HAMQAP Exercise 5 - 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*
(+)-catechin	SRM 3255 Green Tea (Camellia sinensis) Extract		mg/g	8.88	1.80			12	7.95	0.88	8.88	1.80
(+)-catechin	SRM 3256 Green Tea-Containing Solid Oral Dosage Form		mg/g	2.57	0.35			11	2.4	0.35	2.57	0.35
(-)-epicatechin	SRM 3255 Green Tea (Camellia sinensis) Extract		mg/g	45.8	13.0			12	38.3	2.1	45.8	13.0
(-)-epicatechin	SRM 3256 Green Tea-Containing Solid Oral Dosage Form		mg/g	11.70	5.08			12	9.69	0.75	11.70	5.08
(-)-epicatechin gallate	SRM 3255 Green Tea (Camellia sinensis) Extract		mg/g	97.2	15.1			11	94.9	3.5	97.2	15.1
(-)-epicatechin gallate	SRM 3256 Green Tea-Containing Solid Oral Dosage Form		mg/g	16.70	5.08			11	19.4	0.63	16.70	5.08
(-)-epigallocatechin	SRM 3255 Green Tea (Camellia sinensis) Extract		mg/g	79.2	12.6			11	82.4	6.6	79.2	12.6
(-)-epigallocatechin	SRM 3256 Green Tea-Containing Solid Oral Dosage Form		mg/g	30.0	11.1			11	30	4	30.0	11.1
(-)-epigallocatechin gallate	SRM 3255 Green Tea (Camellia sinensis) Extract		mg/g	408.8	36.8			12	406	2.5	408.8	36.8
(-)-epigallocatechin gallate	SRM 3256 Green Tea-Containing Solid Oral Dosage Form		mg/g	69.4	12.9			12	80	1	69.4	12.9
(-)-gallocatechin	SRM 3255 Green Tea (Camellia sinensis) Extract		mg/g	21.3	3.3			6	19.8	1.9	21.3	3.3
(-)-gallocatechin	SRM 3256 Green Tea-Containing Solid Oral Dosage Form		mg/g	7.37	0.55			5	7.74	0.71	7.37	0.55
(-)-gallocatechin gallate	SRM 3255 Green Tea (Camellia sinensis) Extract		mg/g	37.8	3.9			10	42.8	1.5	37.8	3.9
(-)-gallocatechin gallate	SRM 3256 Green Tea-Containing Solid Oral Dosage Form		mg/g	4.49	3.52			10	7.45	0.34	4.49	3.52
Gallic Acid	SRM 3255 Green Tea (Camellia sinensis) Extract		mg/g	3.13	0.17			6	3.05	0.46	3.13	0.17
Gallic Acid	SRM 3256 Green Tea-Containing Solid Oral Dosage Form		mg/g	12.79	0.96			6	13.8	8.2	12.79	0.96
L-theanine	SRM 3255 Green Tea (Camellia sinensis) Extract		mg/g	0.329	0.015			4	1.6	1.4	0.329	0.015
L-theanine	SRM 3256 Green Tea-Containing Solid Oral Dosage Form		mg/g	3.613	2.343			4	4.25	0.17	3.613	2.343

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 (+)-catechin in green tea extract and green tea-containing SODF. Data points highlighted in red have been flagged as potential outliers (e.g., Grubb and/or Cochran) by the NIST software package.

		(+)-catechin									
		SRM 3255 Green Tea (*Camellia sinensis*) Extract (mg/g)					SRM 3256 Green Tea-Containing Solid Oral Dosage Form (mg/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target				8.88	1.80				2.57	0.35
	E001	8.59	8.73	8.47	8.60	0.13	2.73	2.69	2.75	2.72	0.03
	E002	9.19	9.26	8.87	9.11	0.21	2.65	2.22	2.21	2.36	0.25
	E004	9.96	10.32	10.05	10.11	0.19	2.01	2.71	3.34	2.69	0.67
	E005	27.67	25.85	27.47	27.00	1.00	8.98	8.73	9.29	9.00	0.28
	E006										
	E007										
	E009	8.147	8.207	7.544	7.97	0.37	2.49	2.46	2.51	2.49	0.03
	E012										
	E013	8.42	8.24	8.24	8.30	0.10	2.3	2.26	2.29	2.28	0.02
	E017	4.841	4.79	4.825	4.82	0.03	1.5	1.513	1.579	1.53	0.04
	E020										
	E021										
	E022										
	E025	7.74	7.86	7.69	7.76	0.09	1.87	1.94	1.87	1.89	0.04
	E030	4.83	4.47	4.47	4.59	0.21	1.55	1.55	1.6	1.57	0.03
	E031										
	E034	7.575	7.281	7.487	7.45	0.15	< 200	< 200	< 200	< 200	
	E041	137.9	136.2	137.7	137.27	0.93	260	262	260	260.67	1.15
	E043	10.67	11.57	10.31	10.85	0.65	3.89	4.3	4.09	4.09	0.21
Community Results		Consensus Mean			7.95		Consensus Mean			2.40	
		Consensus Standard Deviation			0.88		Consensus Standard Deviation			0.35	
		Maximum			137.27		Maximum			260.67	
		Minimum			4.59		Minimum			1.53	
		N			12		N			11	

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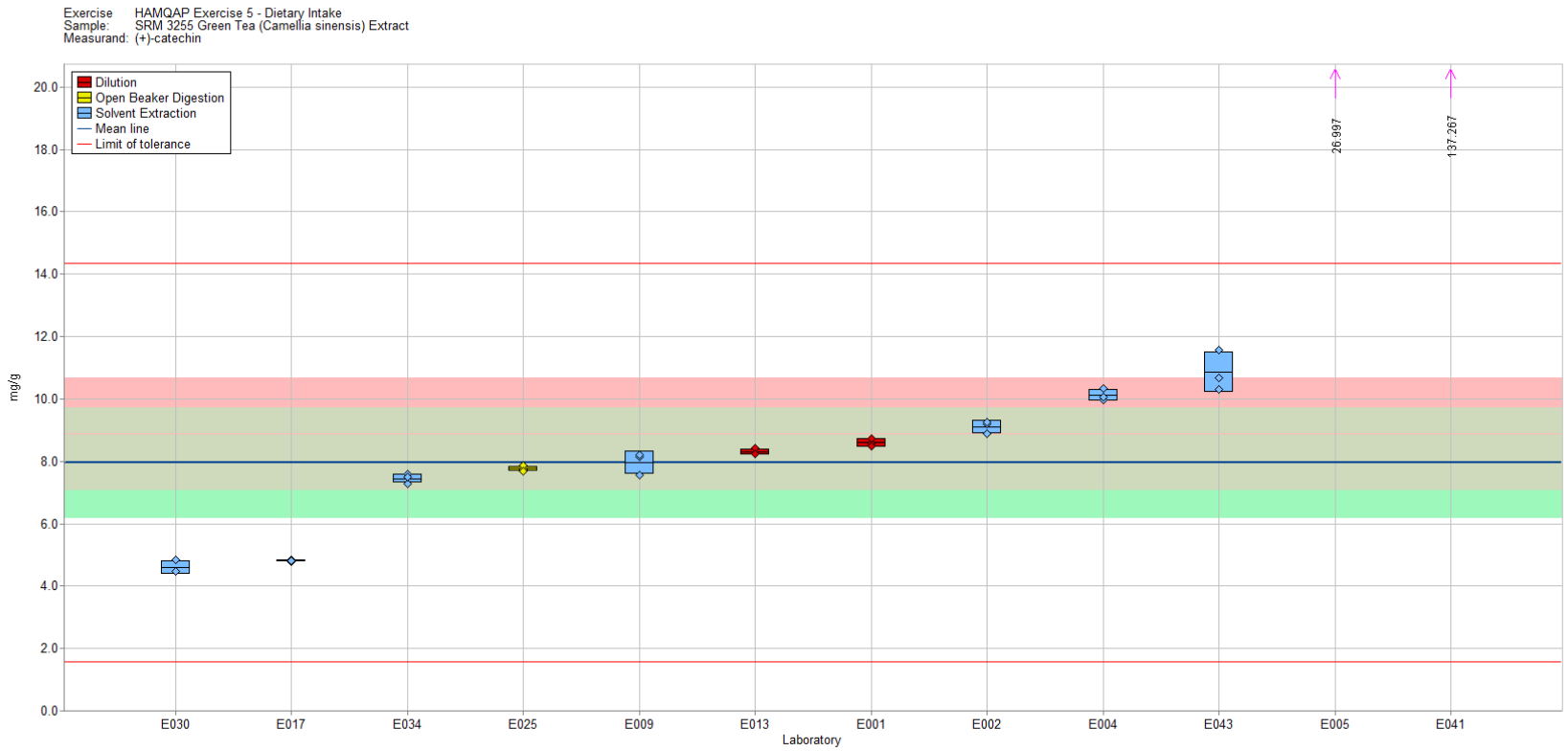

Figure 6-1. (+)-Catechin in SRM 3255 Green Tea (*Camellia sinensis*) Extract (data summary view – sample preparation method). In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample 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, $|Z_{\text{NIST}}| \leq 2$.

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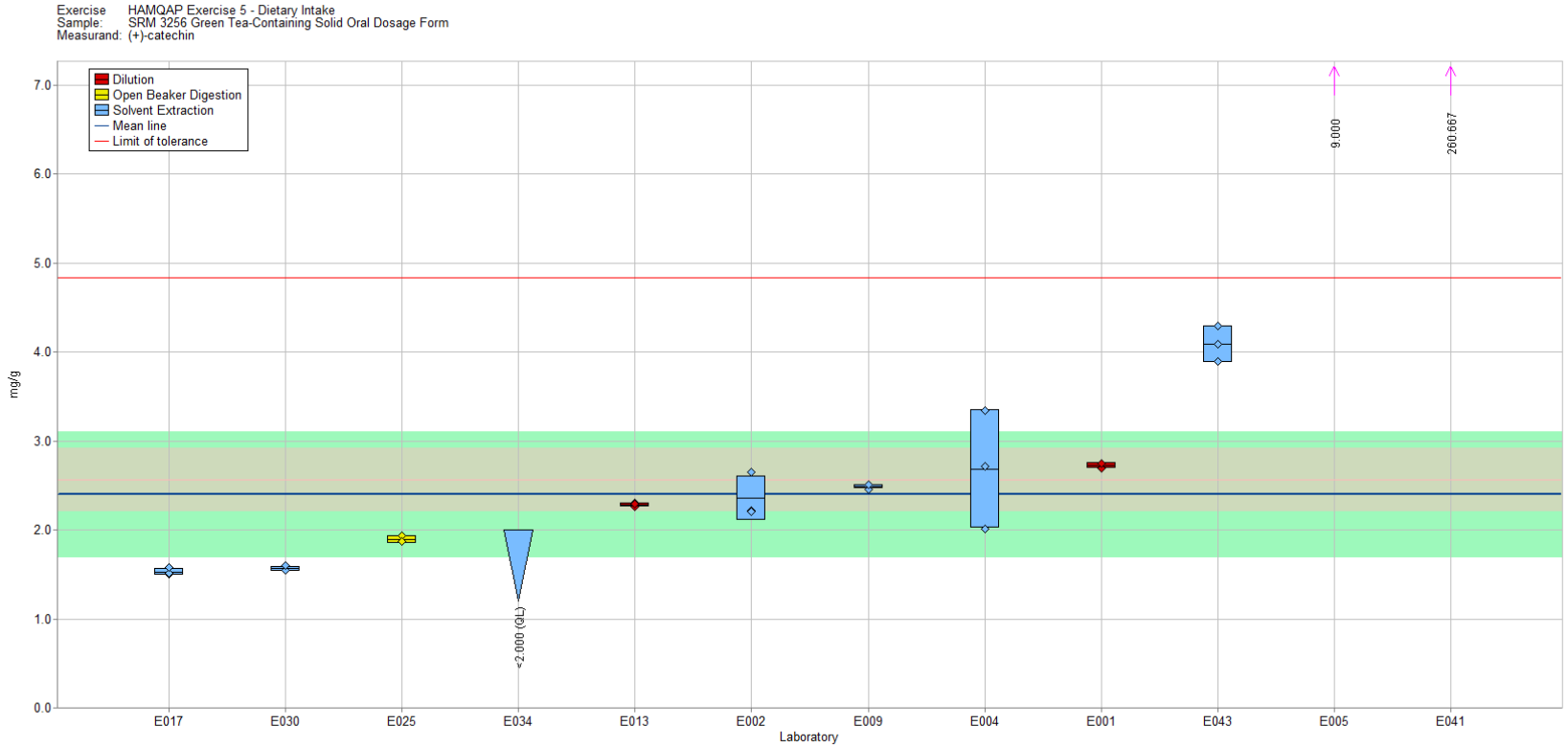

Figure 6-2. (+)-Catechin in SRM 3256 Green Tea-Containing SODF (data summary view – sample preparation method). In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red line represents the consensus range of tolerance, calculated as the value above the consensus mean that results 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, $|Z_{\text{NIST}}| \leq 2$.

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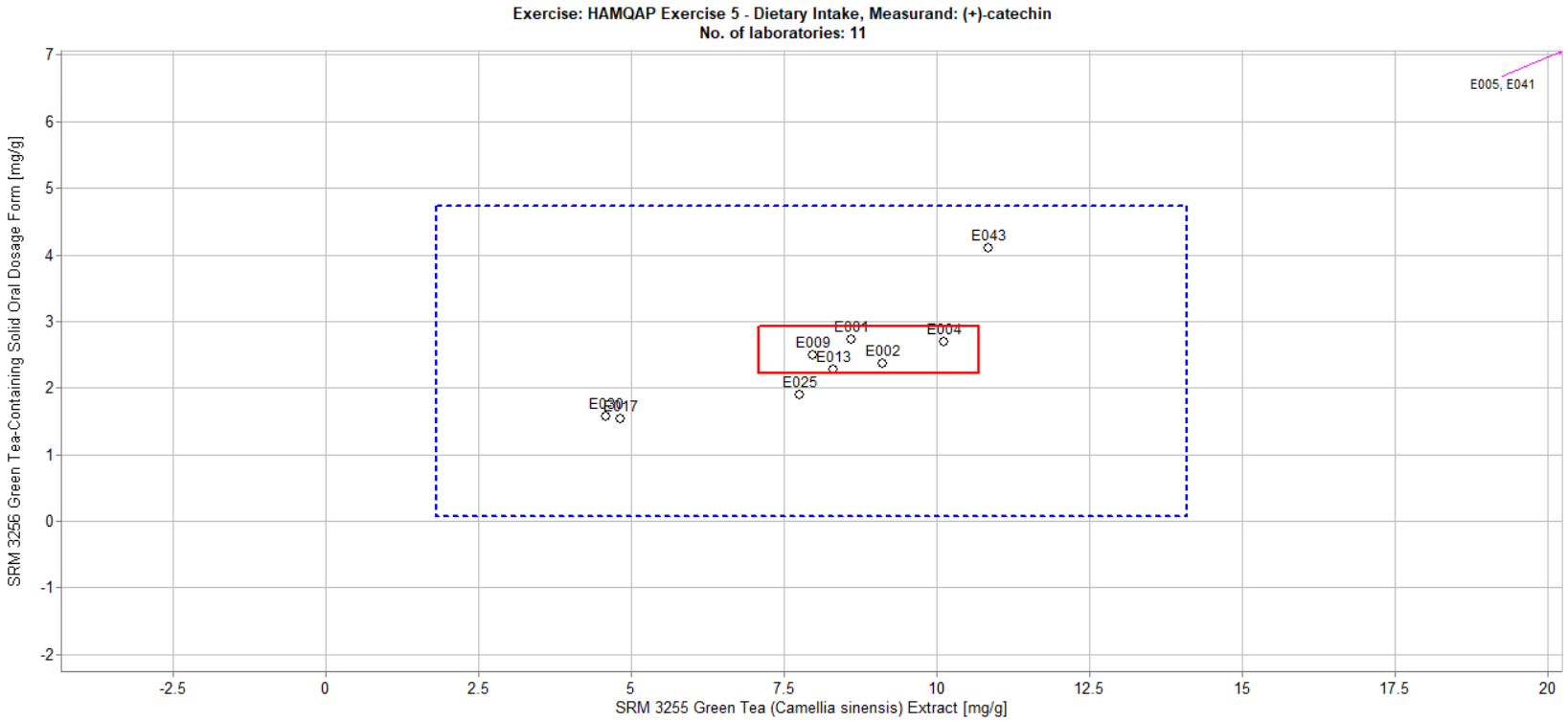

Figure 6-3. Laboratory means for (+)-catechin in SRM 3255 Green Tea (*Camellia sinensis*) Extract and SRM 3256 Green Tea-Containing SODF (sample/sample comparison view). In this view, the individual laboratory mean for one sample (SRM 3255) is compared to the individual laboratory mean for a second sample (SRM 3256). The solid red box represents the NIST range of tolerance for the two samples, SRM 3255 (x-axis) and SRM 3256 (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 3255 (x-axis) and SRM 3256 (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 (–)-epicatechin in green tea extract and green tea-containing SODF.

		(-)-epicatechin									
		SRM 3255 Green Tea (*Camellia sinensis*) Extract (mg/g)					SRM 3256 Green Tea-Containing Solid Oral Dosage Form (mg/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target				45.8	13.0				11.72	5.08
	E001	41.3	40.5	40.1	40.6	0.6	9.67	9.7	9.78	9.72	0.06
	E002	38.94	39.81	38.46	39.1	0.7	10.24	10.17	10.66	10.36	0.27
	E004	41.71	40.48	41	41.1	0.6	14.56	13.14	12.6	13.43	1.01
	E005	36.75	37.06	37.11	37.0	0.2	6.99	6.8	7.38	7.06	0.30
	E006										
	E009	38.68	39.13	35.72	37.8	1.9	8.85	8.47	8.82	8.71	0.21
	E012										
	E013	44.4	43.3	43.1	43.6	0.7	10.7	10.8	10.9	10.80	0.10
	E017	24.318	24.28	24.479	24.4	0.1	7.822	7.982	8.039	7.95	0.11
	E020										
	E021										
	E022										
	E025	44.1	45	45	44.7	0.5	12.1	12.1	12.3	12.17	0.12
	E030	25.6	25.3	25.3	25.4	0.2	6.7	6.49	6.61	6.60	0.11
	E031										
	E034	25.069	25.422	25.281	25.3	0.2	7.923	8.69	7.612	8.08	0.55
	E041	51.99	50.82	49.67	50.8	1.2	10.9	11.7	11.6	11.40	0.44
	E043	45.07	44.98	43.3	44.5	1.0	9.73	10.2	10.16	10.03	0.26
Community Results		Consensus Mean			38.3		Consensus Mean			9.69	
		Consensus Standard Deviation			2.1		Consensus Standard Deviation			0.75	
		Maximum			50.8		Maximum			13.43	
		Minimum			24.4		Minimum			6.60	
		N			12		N			12	

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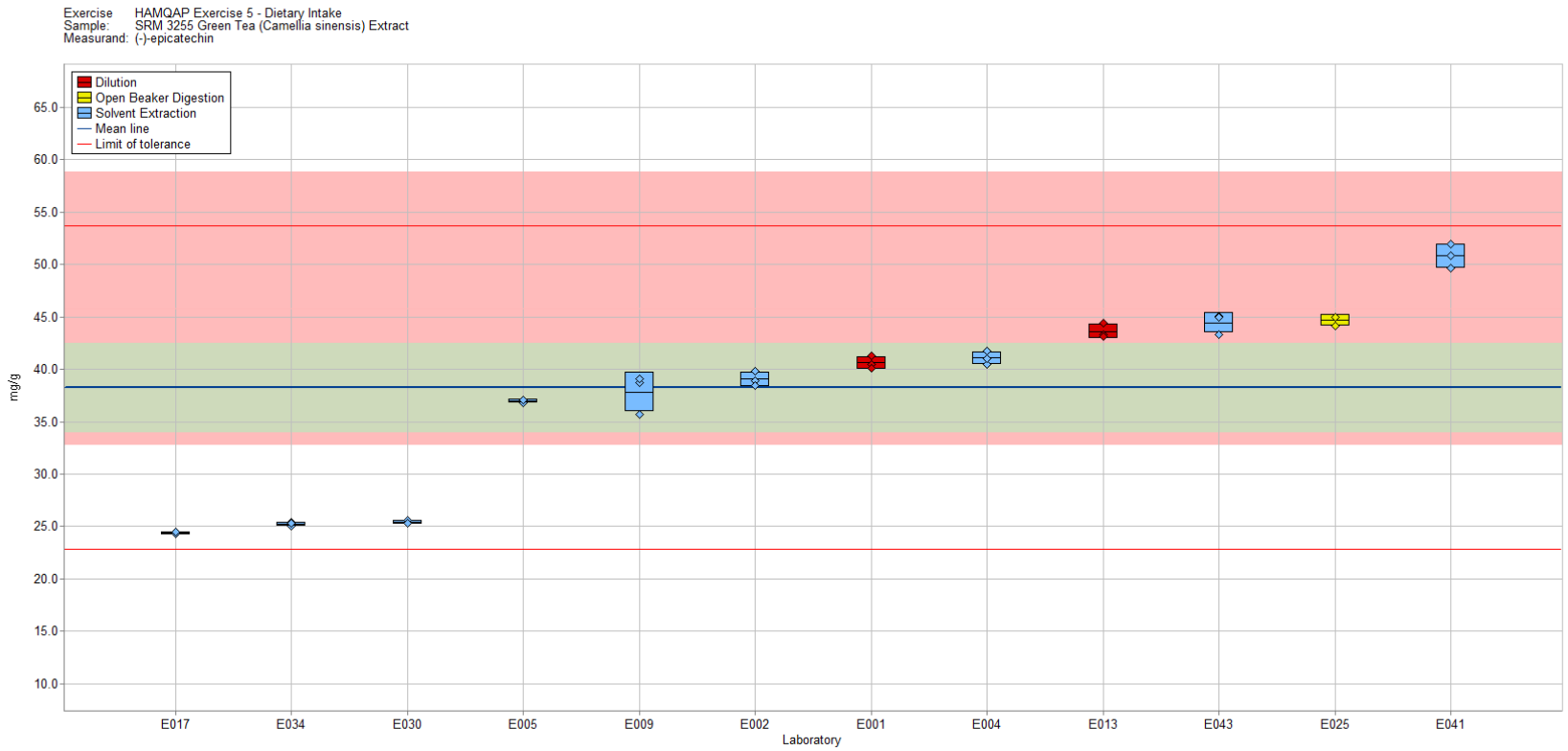

Figure 6-4. (–)-Epicatechin in SRM 3255 Green Tea (*Camellia sinensis*) Extract (data summary view – sample preparation method). In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample 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, $|Z_{\text{NIST}}| \leq 2$.

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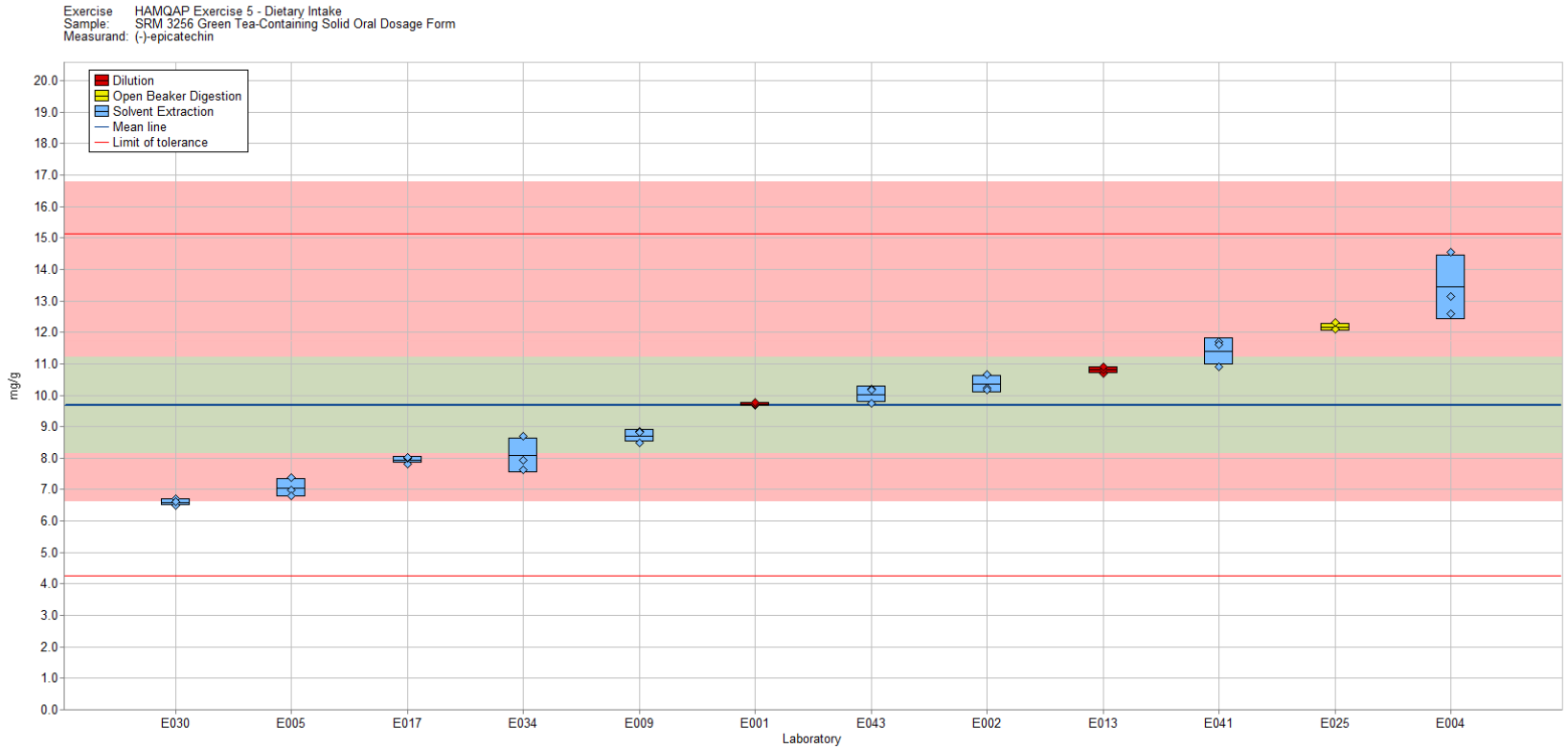

Figure 6-5. (–)-Epicatechin in SRM 3256 Green Tea-Containing SODF (data summary view – sample preparation method). In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation 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, $|Z_{\text{NIST}}| \leq 2$.

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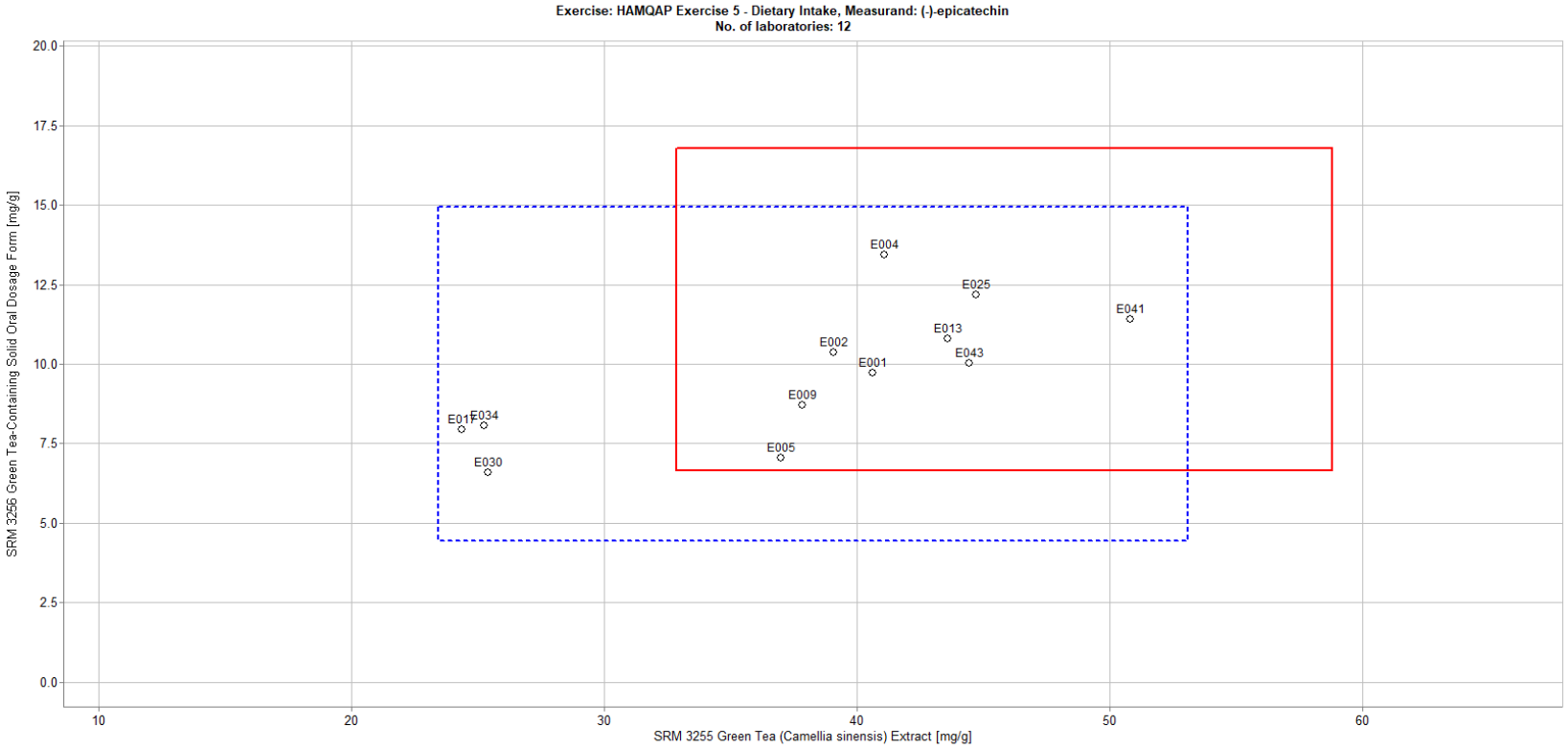

Figure 6-6. Laboratory means for (–)-epicatechin in SRM 3255 Green Tea (*Camellia sinensis*) Extract and SRM 3256 Green Tea-Containing SODF (sample/sample comparison view). In this view, the individual laboratory mean for one sample (SRM 3255) is compared to the individual laboratory mean for a second sample (SRM 3256). The solid red box represents the NIST range of tolerance for the two samples, SRM 3255 (x-axis) and SRM 3256 (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 3255 (x-axis) and SRM 3256 (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 (–)-epicatechin gallate in green tea extract and green tea-containing SODF. Data points highlighted in red have been flagged as potential outliers (e.g., Grubb and/or Cochran) by the NIST software package.

		(-)-epicatechin gallate									
		SRM 3255 Green Tea (*Camellia sinensis*) Extract (mg/g)					SRM 3256 Green Tea-Containing Solid Oral Dosage Form (mg/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target				97.2	15.1				16.70	5.08
	E001	88	87.4	86.8	87.4	0.6	18.1	17.9	17.9	17.97	0.12
	E002	80.89	81.9	81.55	81.4	0.5	17.85	16.52	16.88	17.08	0.69
	E004	99.38	99.25	101.26	100.0	1.1	21.32	20.81	20.76	20.96	0.31
	E005										
	E006										
	E007										
	E009	94.87	89.77	94.88	93.2	2.9	19.6	18.9	19.3	19.27	0.35
	E010										
	E012										
	E013	86.7	87.8	93	89.2	3.4	19	19.2	19.2	19.13	0.12
	E015										
	E017	124.738	124.174	125.428	124.8	0.6	25.562	26.048	26.659	26.09	0.55
	E018										
	E020										
	E021										
	E022										
	E024										
	E025	93.6	95.3	95.7	94.9	1.1	18.8	18.7	18.9	18.80	0.10
	E030	119	118	118	118.3	0.6	24	23.2	23.9	23.70	0.44
	E031										
	E033										
	E034	84.9	85.553	86.633	85.7	0.9	18.528	18.857	17.322	18.24	0.81
	E035										
	E037										
	E040										
	E041	96.02	94.38	95.73	95.4	0.9	19.7	19.4	19.6	19.57	0.15
	E042										
	E043	93.32	92.45	92.03	92.6	0.7	17.89	17.39	17.95	17.74	0.31
	E046										
	E047										
Community Results		Consensus Mean			94.9		Consensus Mean			19.40	
		Consensus Standard Deviation			3.5		Consensus Standard Deviation			0.63	
		Maximum			124.8		Maximum			26.09	
		Minimum			81.4		Minimum			17.08	
		N			11		N			11	

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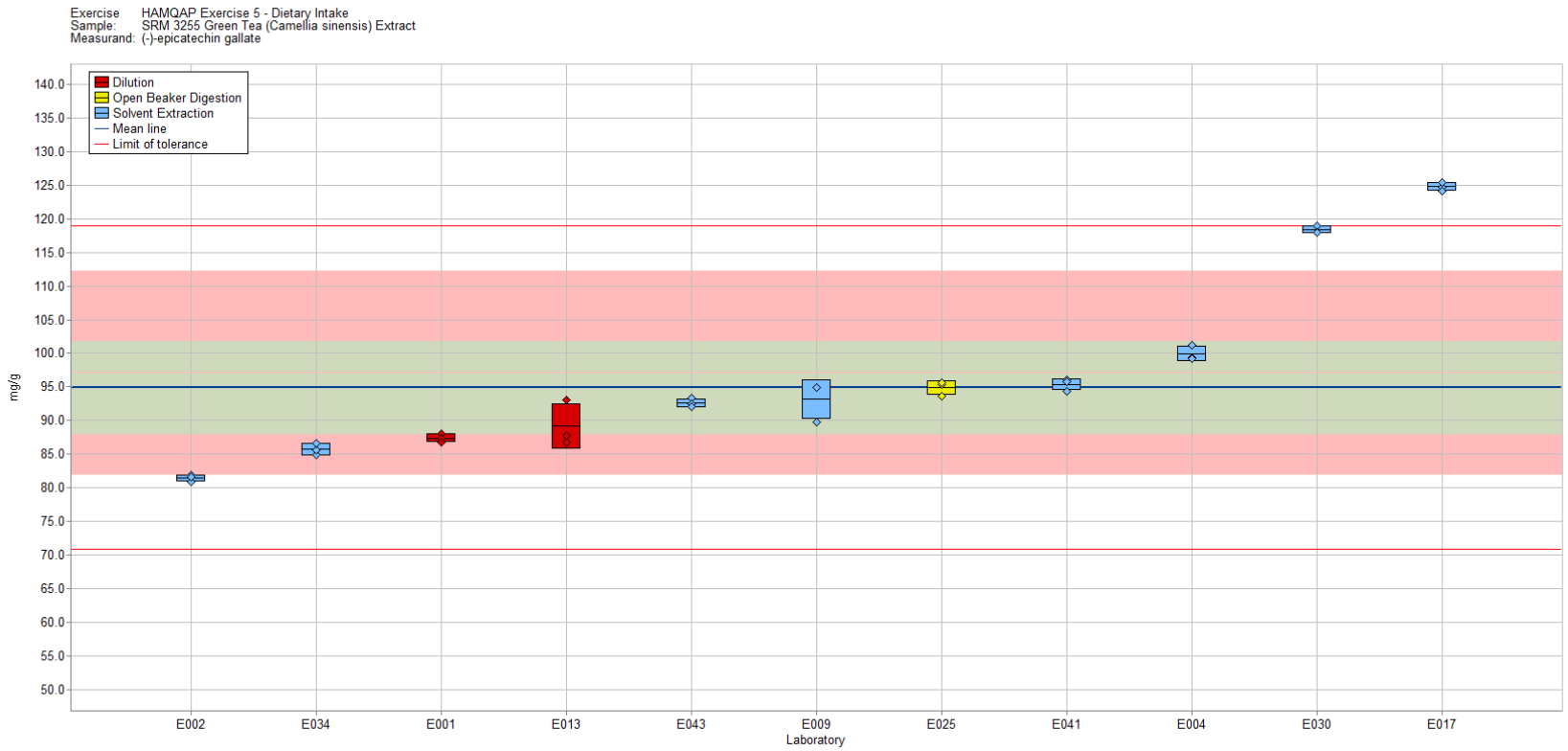

Figure 6-7. (–)-Epicatechin gallate in SRM 3255 Green Tea (*Camellia sinensis*) Extract (data summary view – sample preparation method). In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents 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, $|Z_{\text{NIST}}| \leq 2$.

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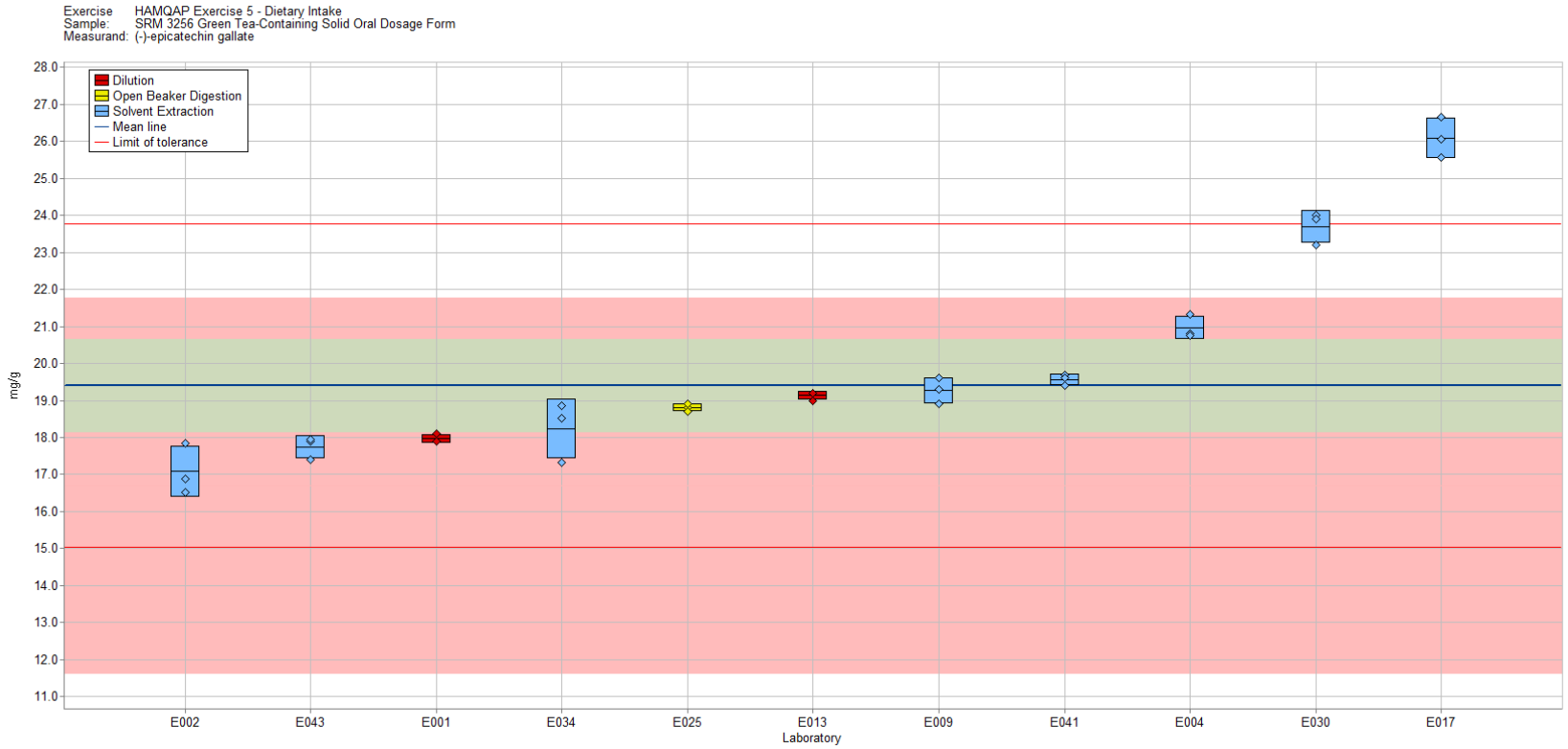

Figure 6-8. (–)-Epicatechin gallate in SRM 3256 Green Tea-Containing SODF (data summary view – sample preparation method). In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation 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, $|Z_{\text{NIST}}| \leq 2$.

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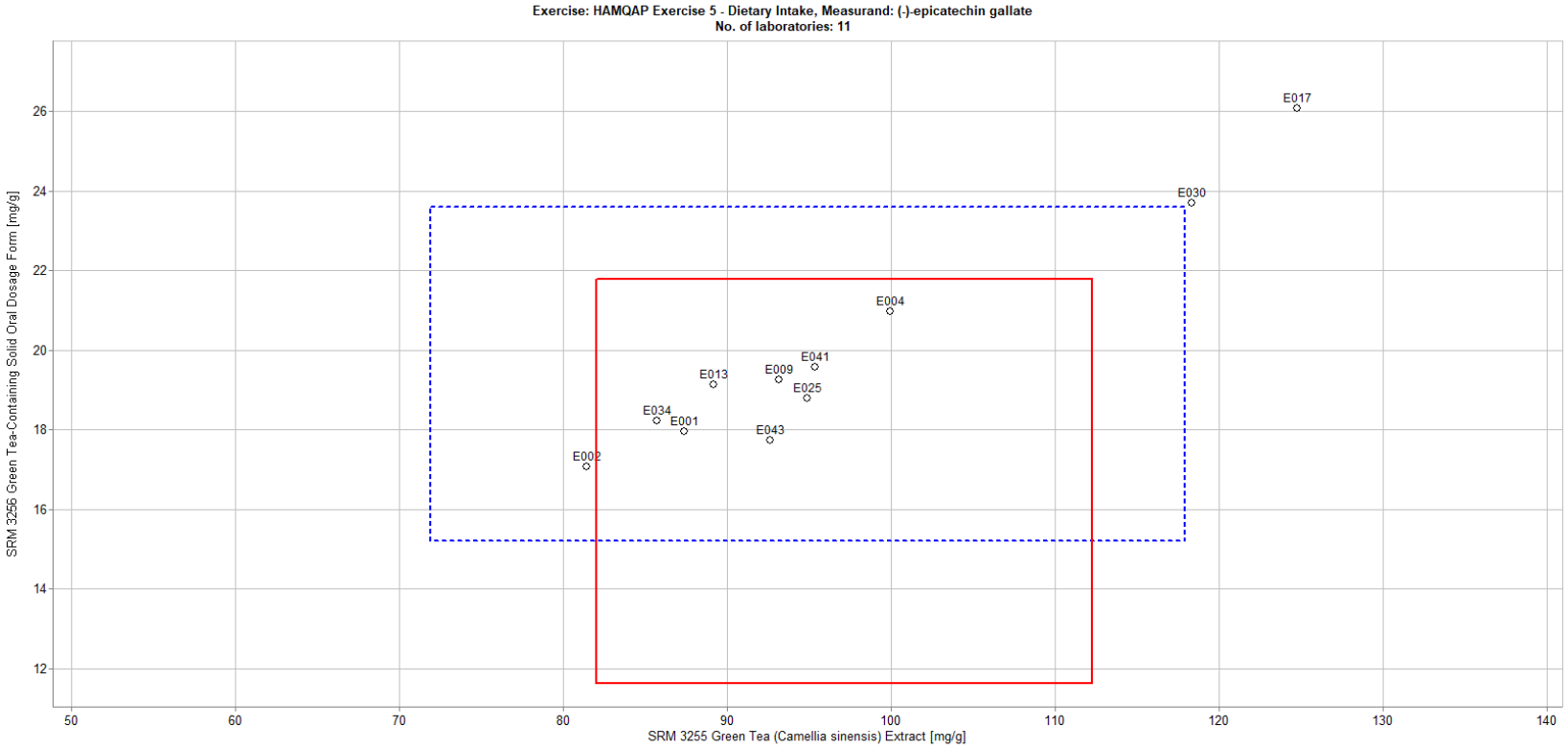

Figure 6-9. Laboratory means for (–)-epicatechin gallate in SRM 3255 Green Tea (*Camellia sinensis*) Extract and SRM 3256 Green Tea-Containing SODF (sample/sample comparison view). In this view, the individual laboratory mean for one sample (SRM 3255) is compared to the individual laboratory mean for a second sample (SRM 3256). The solid red box represents the NIST range of tolerance for the two samples, SRM 3255 (x-axis) and SRM 3256 (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 3255 (x-axis) and SRM 3256 (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 6-5. Data summary table for (–)-epigallocatechin in green tea extract and green tea-containing SODF. Data points highlighted in red have been flagged as potential outliers (e.g., Grubb and/or Cochran) by the NIST software package.

		(-)-epigallocatechin									
		SRM 3255 Green Tea (*Camellia sinensis*) Extract (mg/g)					SRM 3256 Green Tea-Containing Solid Oral Dosage Form (mg/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target				79.2	12.6				30.0	11.1
	E001	75.6	77.7	78.6	77.3	1.5	28.5	28.4	28.7	28.5	0.2
	E002	86.33	85.41	86.34	86.0	0.5	35.53	34.37	35.9	35.3	0.8
	E004	101.2	97.27	106.45	101.6	4.6	44.57	43.22	44.07	44.0	0.7
	E006										
	E009	85.33	82.38	88.19	85.3	2.9	28.3	26.8	27.8	27.6	0.8
	E012										
	E013	65.7	64.4	64.3	64.8	0.8	20.9	21.8	21.9	21.5	0.6
	E017	74.844	80.515	75.336	76.9	3.1	138.129	137.988	140.775	139.0	1.6
	E020										
	E021										
	E022										
	E025	76.3	76.5	76.9	76.6	0.3	28.7	28.4	29.1	28.7	0.4
	E030	18.4	18.4	18.4	18.4	0.0	8.83	8.54	6.87	8.1	1.1
	E031										
	E033										
	E034	139.145	140.353	142.095	140.5	1.5	37.532	38.066	35.137	36.9	1.6
	E041	92.06	98.69	100.5	97.1	4.4	31.4	31.2	34.5	32.4	1.9
	E043	76.17	76.39	75.56	76.0	0.4	27.03	26.96	27.38	27.1	0.2
Community Results		Consensus Mean			82.4		Consensus Mean			29.1	
		Consensus Standard Deviation			6.6		Consensus Standard Deviation			4.0	
		Maximum			140.5		Maximum			139.0	
		Minimum			18.4		Minimum			8.1	
		N			11		N			11	

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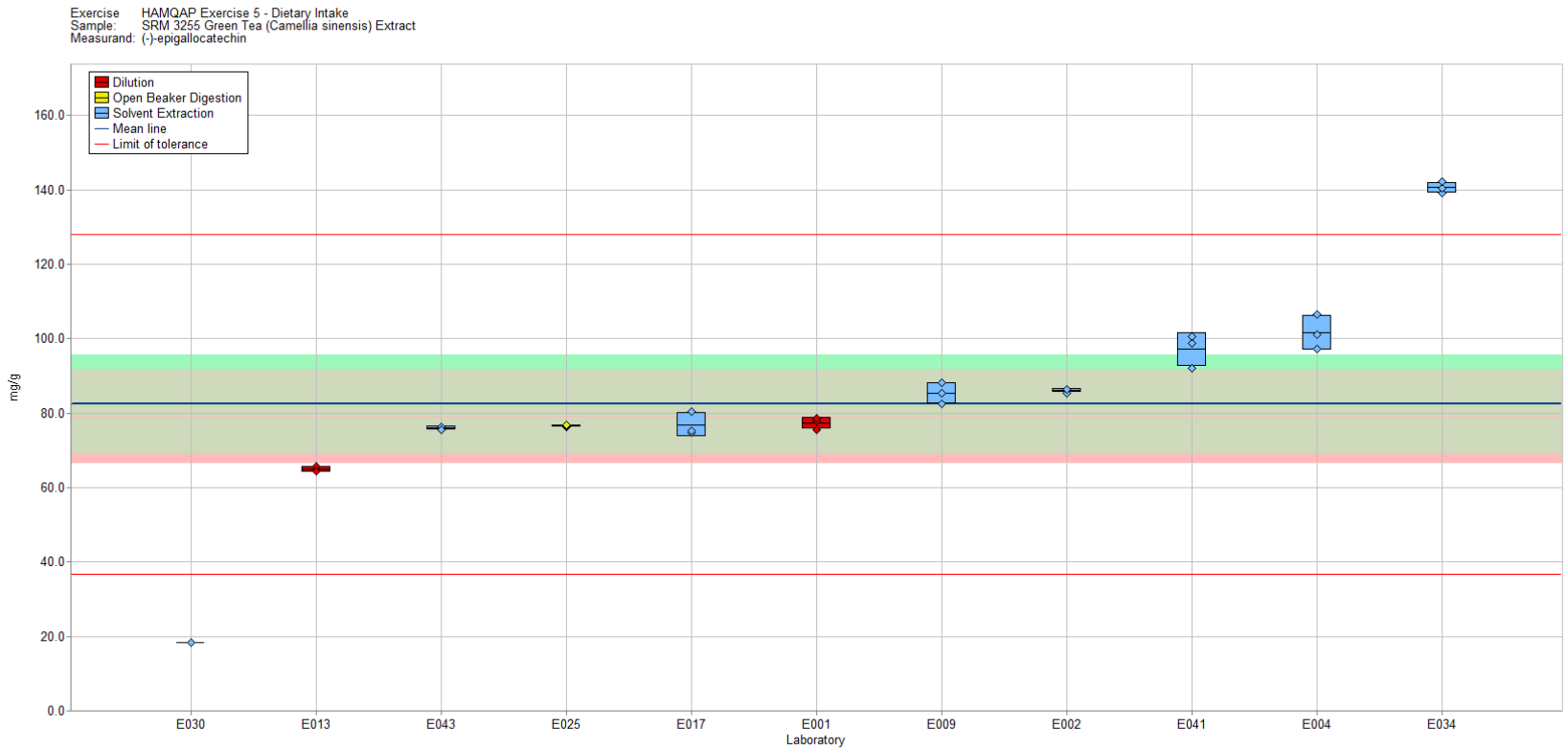

Figure 6-10. (–)-Epigallocatechin in SRM 3255 Green Tea (*Camellia sinensis*) Extract (data summary view – sample preparation method). In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents 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, $|Z_{\text{NIST}}| \leq 2$.

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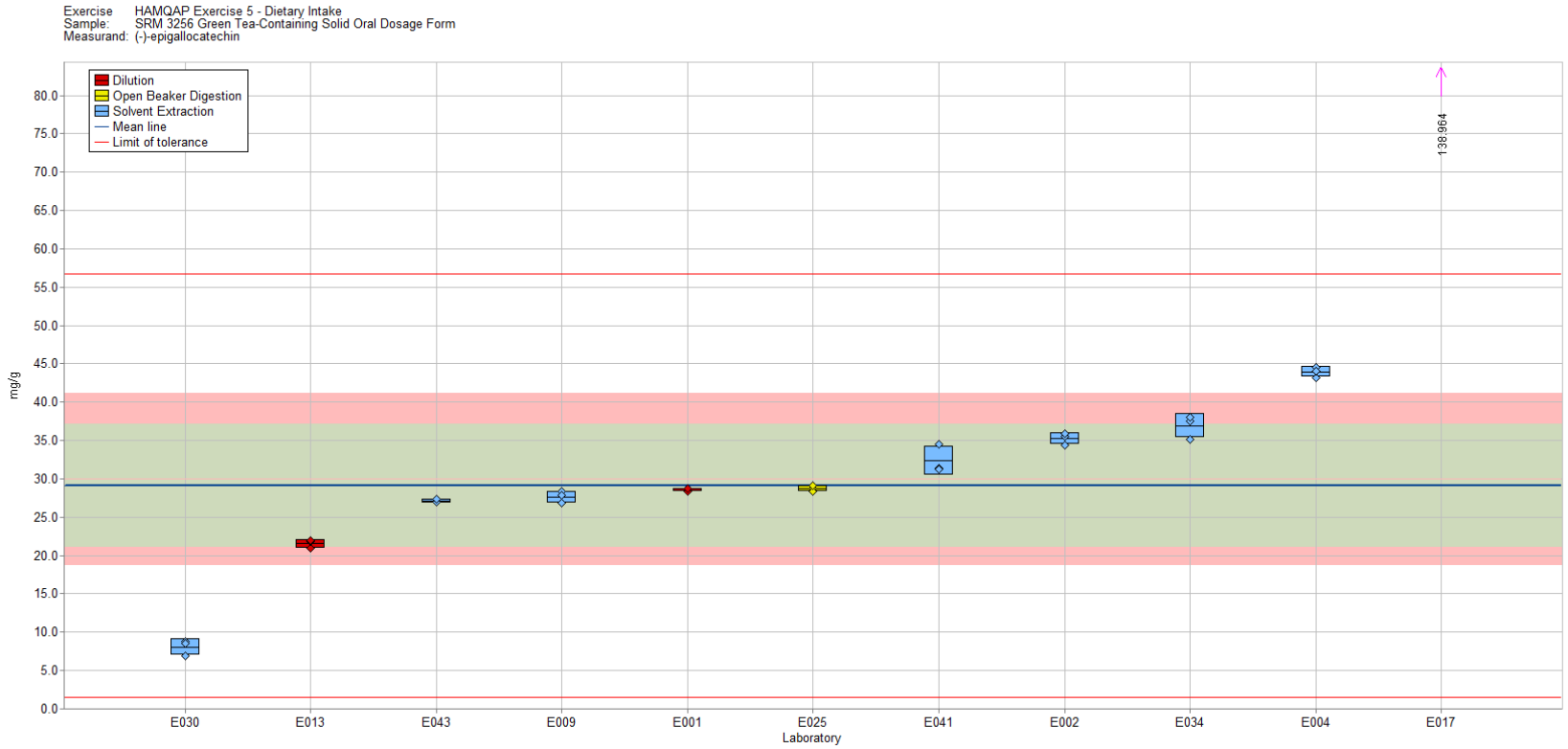

Figure 6-11. (–)-Epigallocatechin in SRM 3256 Green Tea-Containing SODF (data summary view – sample preparation method). In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation 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, $|Z_{\text{NIST}}| \leq 2$.

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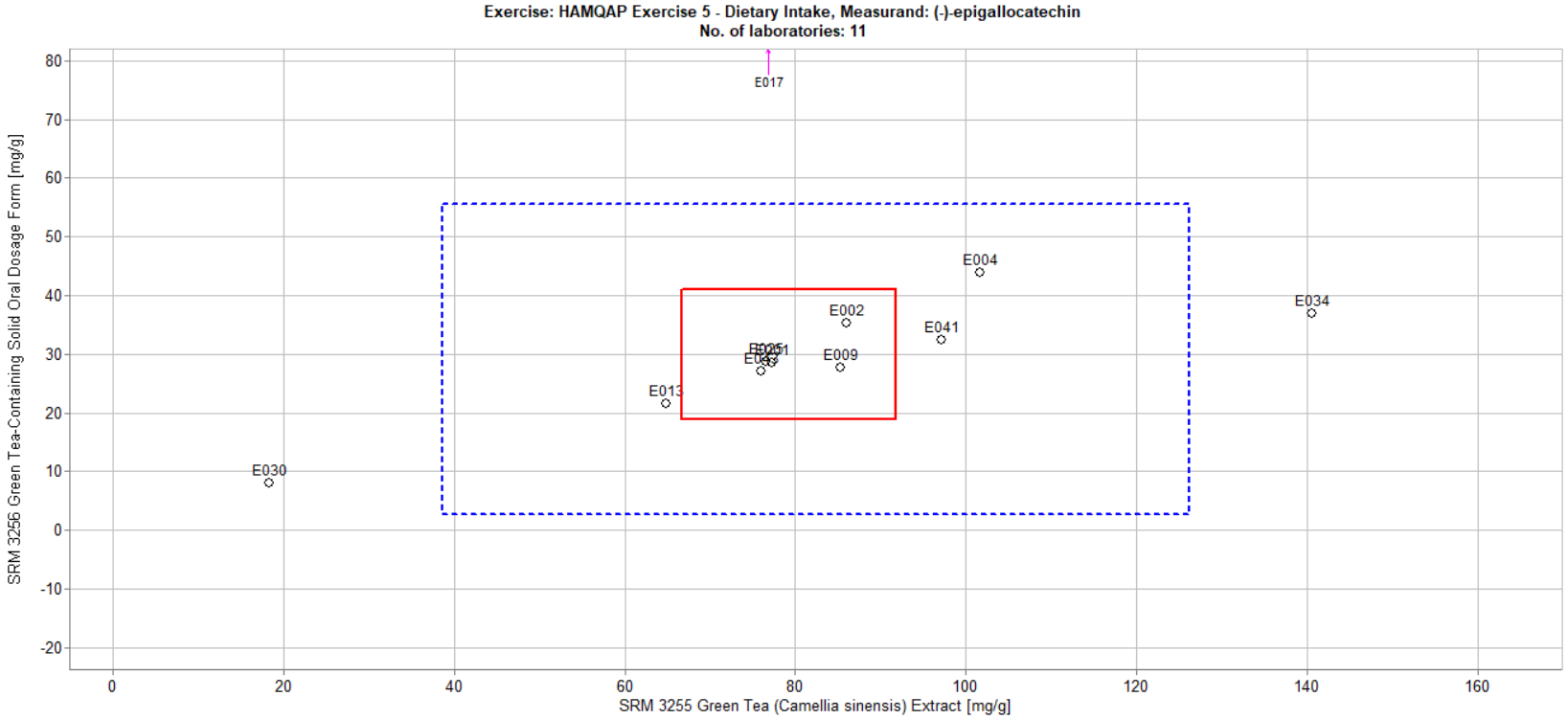

Figure 6-12. Laboratory means for (–)-epigallocatechin in SRM 3255 Green Tea (*Camellia sinensis*) Extract and SRM 3256 Green Tea-Containing SODF (sample/sample comparison view). In this view, the individual laboratory mean for one sample (SRM 3255) is compared to the individual laboratory mean for a second sample (SRM 3256). The solid red box represents the NIST range of tolerance for the two samples, SRM 3255 (x-axis) and SRM 3256 (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 3255 (x-axis) and SRM 3256 (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 (–)-epigallocatechin gallate in green tea extract and green tea-containing SODF. Data points highlighted in red have been flagged as potential outliers (e.g., Grubb and/or Cochran) by the NIST software package.

		(-)-epigallocatechin gallate									
		SRM 3255 Green Tea (*Camellia sinensis*) Extract (mg/g)					SRM 3256 Green Tea-Containing Solid Oral Dosage Form (mg/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target				408.8	36.8				69.4	12.9
	E001	400	398	396	398.0	2.0	81.4	81.4	81.7	81.5	0.2
	E002	395.61	394.75	401.17	397.2	3.5	76.99	76.29	78.03	77.1	0.9
	E004	406.51	403.51	405.38	405.1	1.5	81.15	80.15	80.08	80.5	0.6
	E006										
	E007	399.84	396.86	390.52	395.7	4.8	80.96	81.52	79.41	80.6	1.1
	E009	440.3	421.1	446.9	436.1	13.4	87.6	84.3	86.7	86.2	1.7
	E013	403	408	429	413.3	13.8	80.9	82.8	83.1	82.3	1.2
	E017	402.529	402.392	404.493	403.1	1.2	77.144	79.491	80.931	79.2	1.9
	E020										
	E021										
	E022										
	E025	398	400	401	399.7	1.5	79	78.4	79.2	78.9	0.4
	E030	407	402	403	404.0	2.6	79.8	77.3	79.6	78.9	1.4
	E031										
	E034	402.475	404.958	410.919	406.1	4.3	78.775	81.625	74.785	78.4	3.4
	E041	421.6	417.9	417.1	418.9	2.4	83.8	82.6	85.3	83.9	1.4
	E043	418.06	415.06	409.86	414.3	4.1	69.36	66.43	69.73	68.5	1.8
Community Results		Consensus Mean			406.1		Consensus Mean			80.2	
		Consensus Standard Deviation			2.5		Consensus Standard Deviation			1.0	
		Maximum			436.1		Maximum			86.2	
		Minimum			395.7		Minimum			68.5	
		N			12		N			12	

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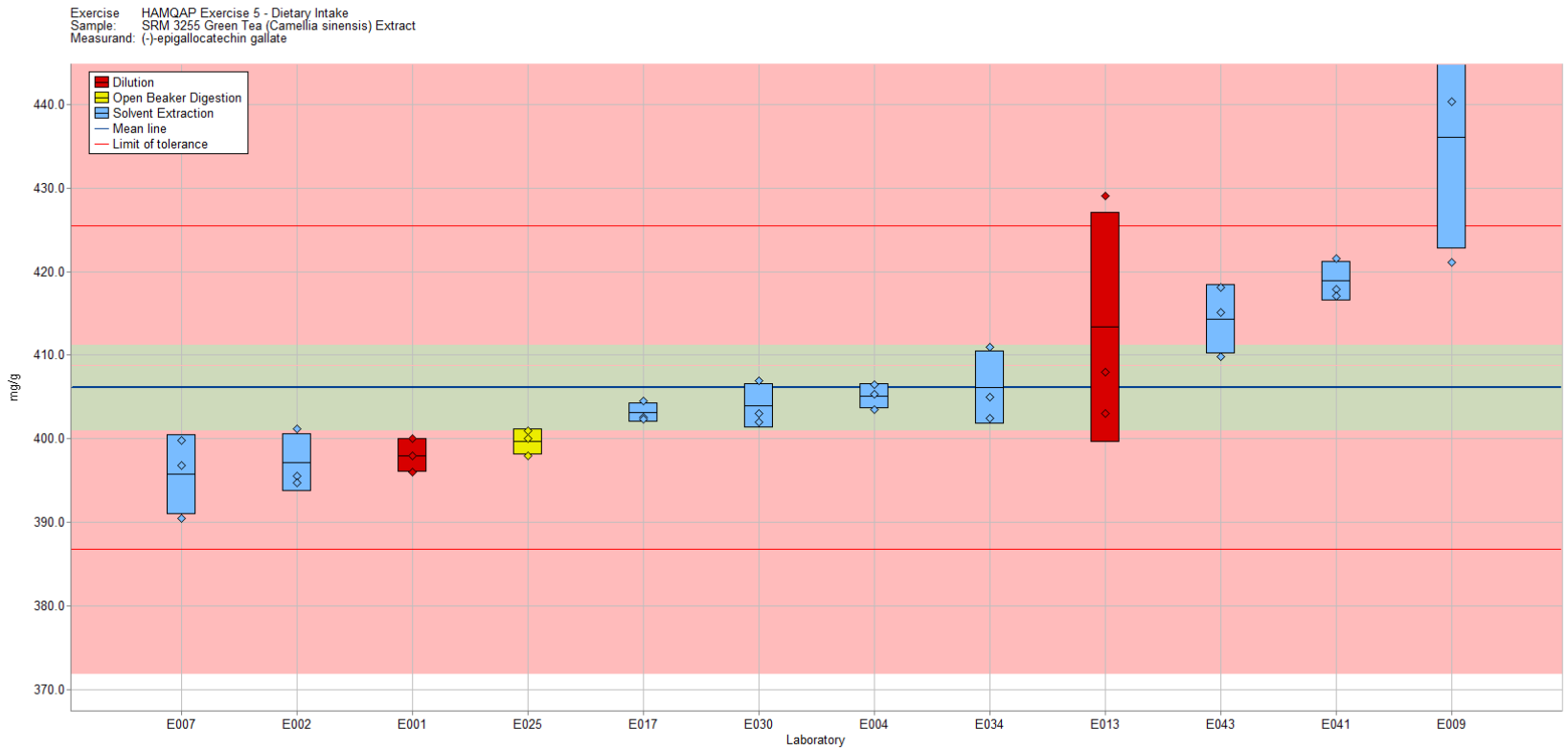

Figure 6-13. (–)-Epigallocatechin gallate in SRM 3255 Green Tea (*Camellia sinensis*) Extract (data summary view – sample preparation method). In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents 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, $|Z_{\text{NIST}}| \leq 2$.

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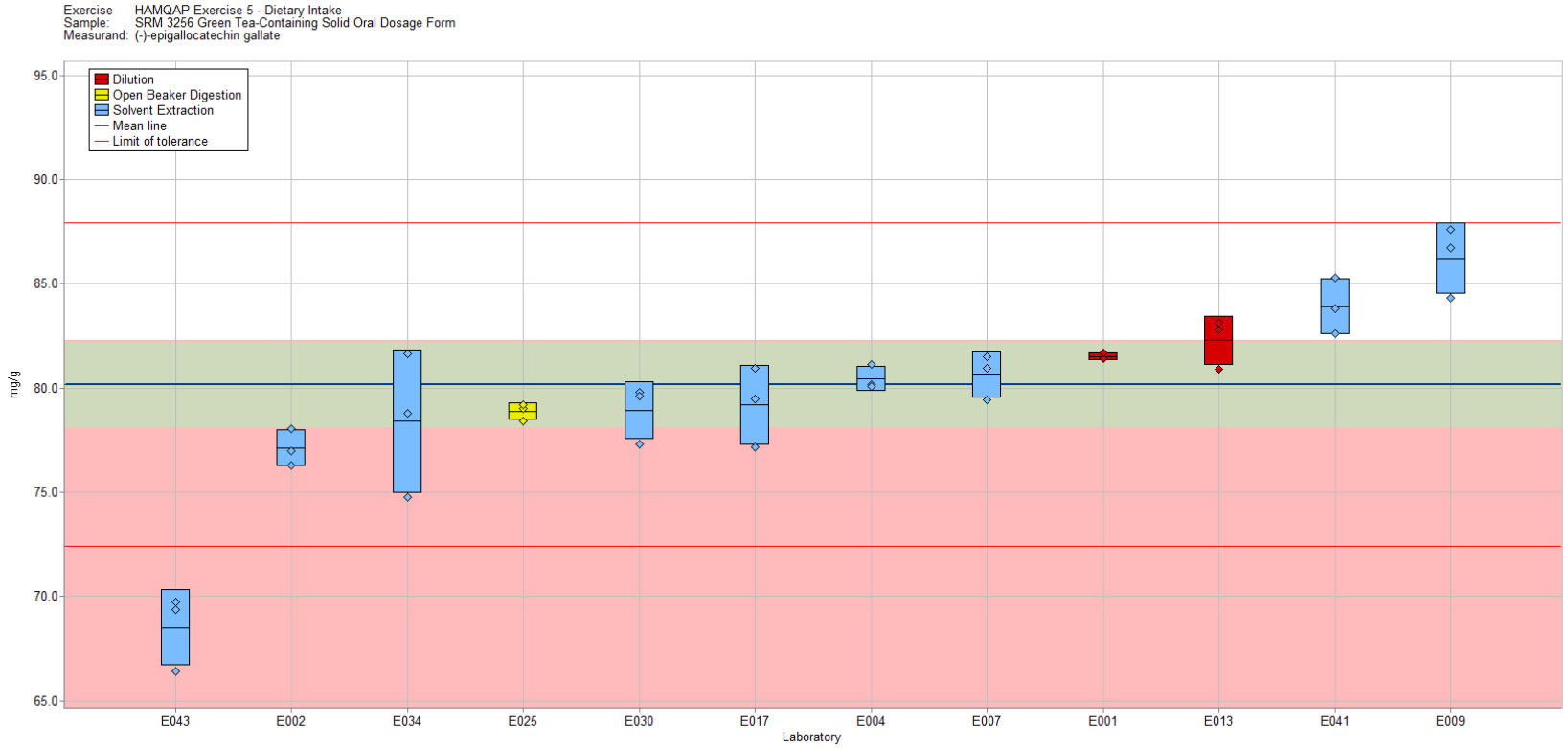

Figure 6-14. (–)-Epigallocatechin gallate in SRM 3256 Green Tea-Containing SODF (data summary view – sample preparation method). In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample 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, $|Z_{\text{NIST}}| \leq 2$.

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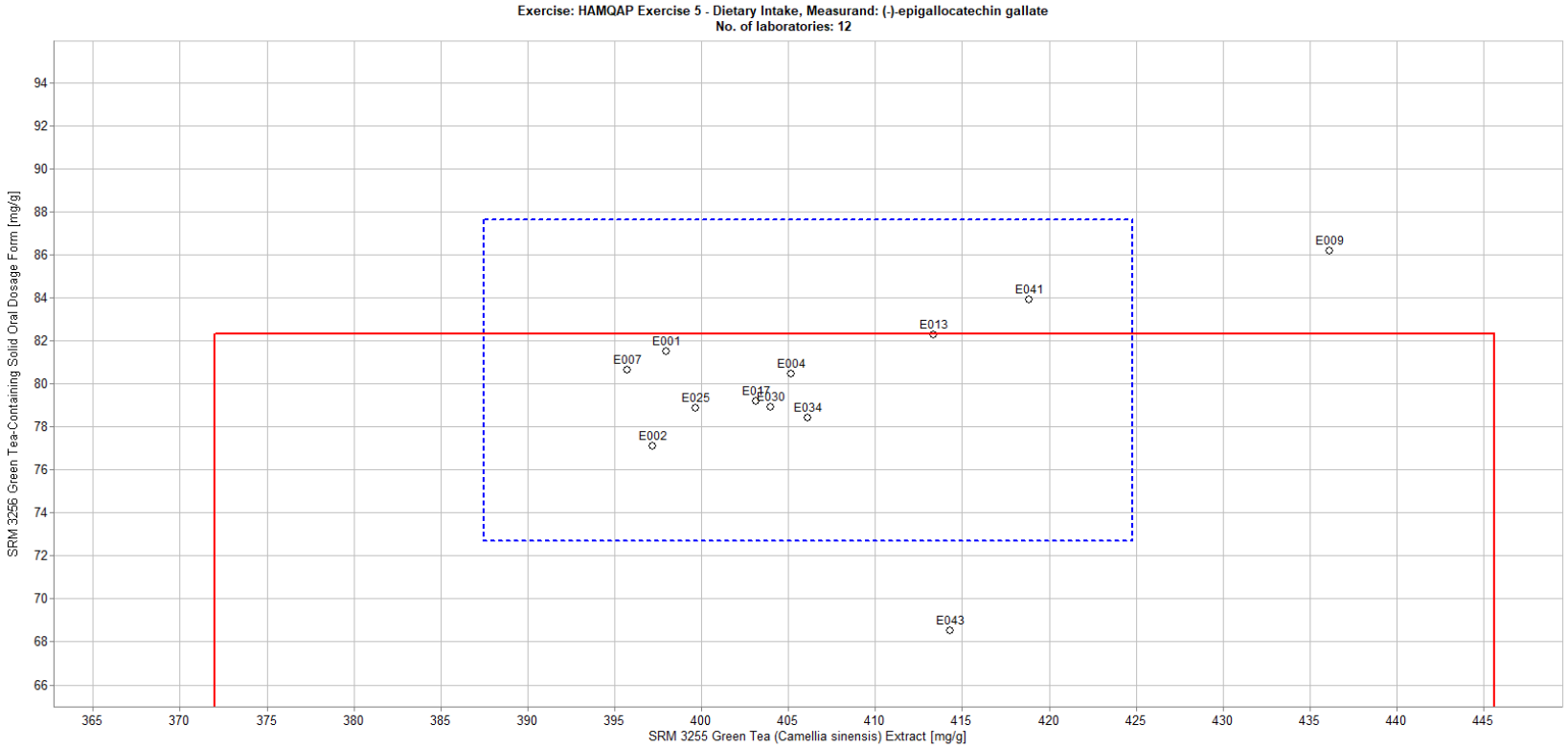

Figure 6-15. Laboratory means for (–)-epigallocatechin gallate in SRM 3255 Green Tea (*Camellia sinensis*) Extract and SRM 3256 Green Tea-Containing SODF (sample/sample comparison view). In this view, the individual laboratory mean for one sample (SRM 3255) is compared to the individual laboratory mean for a second sample (SRM 3256). The solid red box represents the NIST range of tolerance for the two samples, SRM 3255 (x-axis) and SRM 3256 (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 3255 (x-axis) and SRM 3256 (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 6-7. Data summary table for (–)-gallocatechin in green tea extract and green tea-containing SODF. Data points highlighted in red have been flagged as potential outliers (e.g., Grubb and/or Cochran) by the NIST software package.

		(-)-gallocatechin									
		SRM 3255 Green Tea (*Camellia sinensis*) Extract (mg/g)					SRM 3256 Green Tea-Containing Solid Oral Dosage Form (mg/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target				21.3	3.3				7.37	0.55
	E001										
	E002	18.99	17.03	19.05	18.4	1.1	8.46	7.35	6.9	7.57	0.80
	E004	8.1	7.49	8.06	7.9	0.3	2.15	1.89	1.7	1.91	0.23
	E006										
	E009	20.62	20.68	19.07	20.1	0.9	7.9	7.74	8.15	7.93	0.21
	E013										
	E017										
	E020										
	E021										
	E022										
	E025	19.8	20.1	19.9	19.9	0.2	7.83	7.81	7.92	7.85	0.06
	E030										
	E031										
	E034	24.069	23.715	24.508	24.1	0.4					
	E043	22.73	24.84	21.99	23.2	1.5	8.95	8.96	9	8.97	0.03
Community Results		Consensus Mean			19.8		Consensus Mean			7.74	
		Consensus Standard Deviation			1.9		Consensus Standard Deviation			0.71	
		Maximum			24.1		Maximum			8.97	
		Minimum			7.9		Minimum			1.91	
		N			6		N			5	

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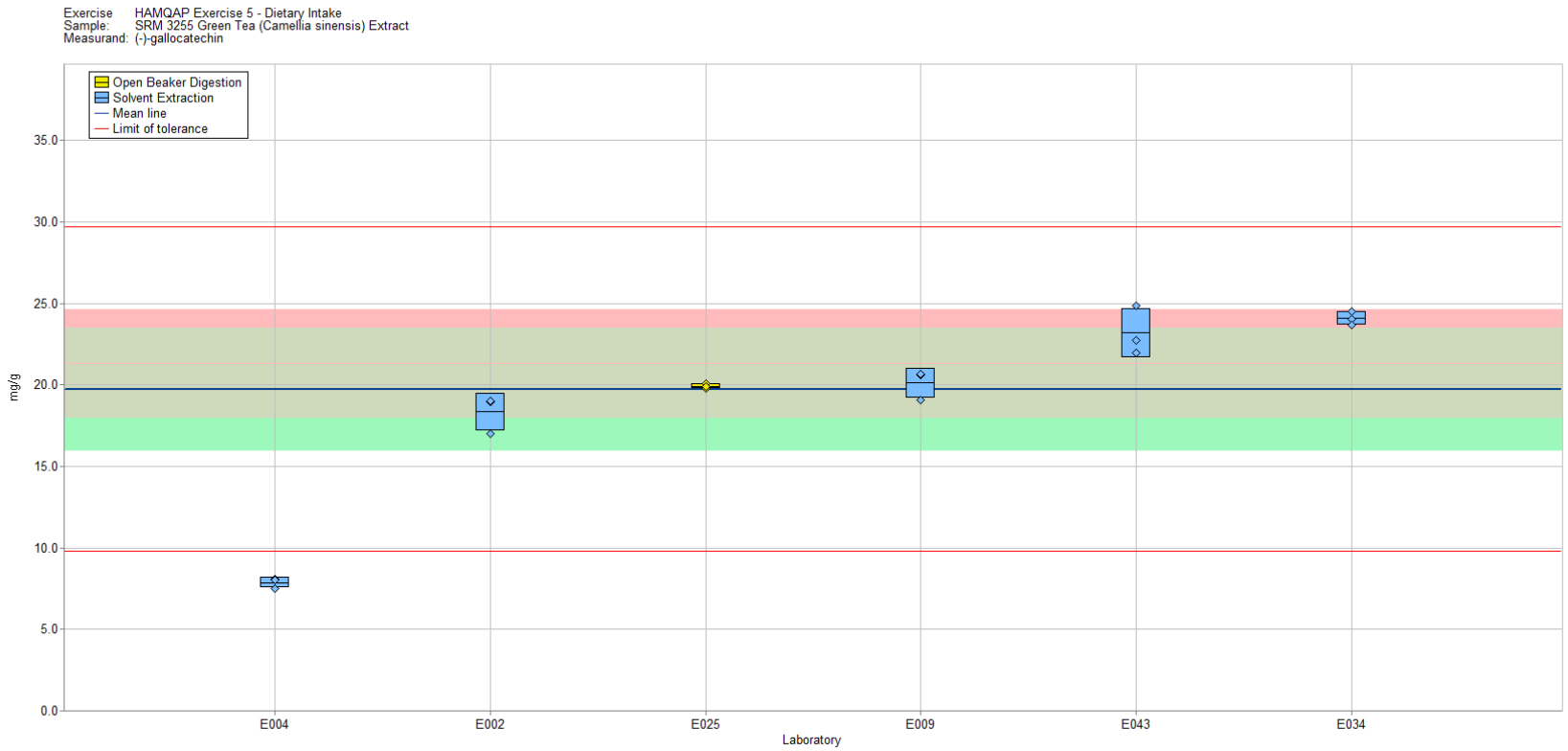

Figure 6-16. (–)-Gallocatechin in SRM 3255 Green Tea (*Camellia sinensis*) Extract (data summary view – sample preparation method). In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the 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, $|Z_{\text{NIST}}| \leq 2$.

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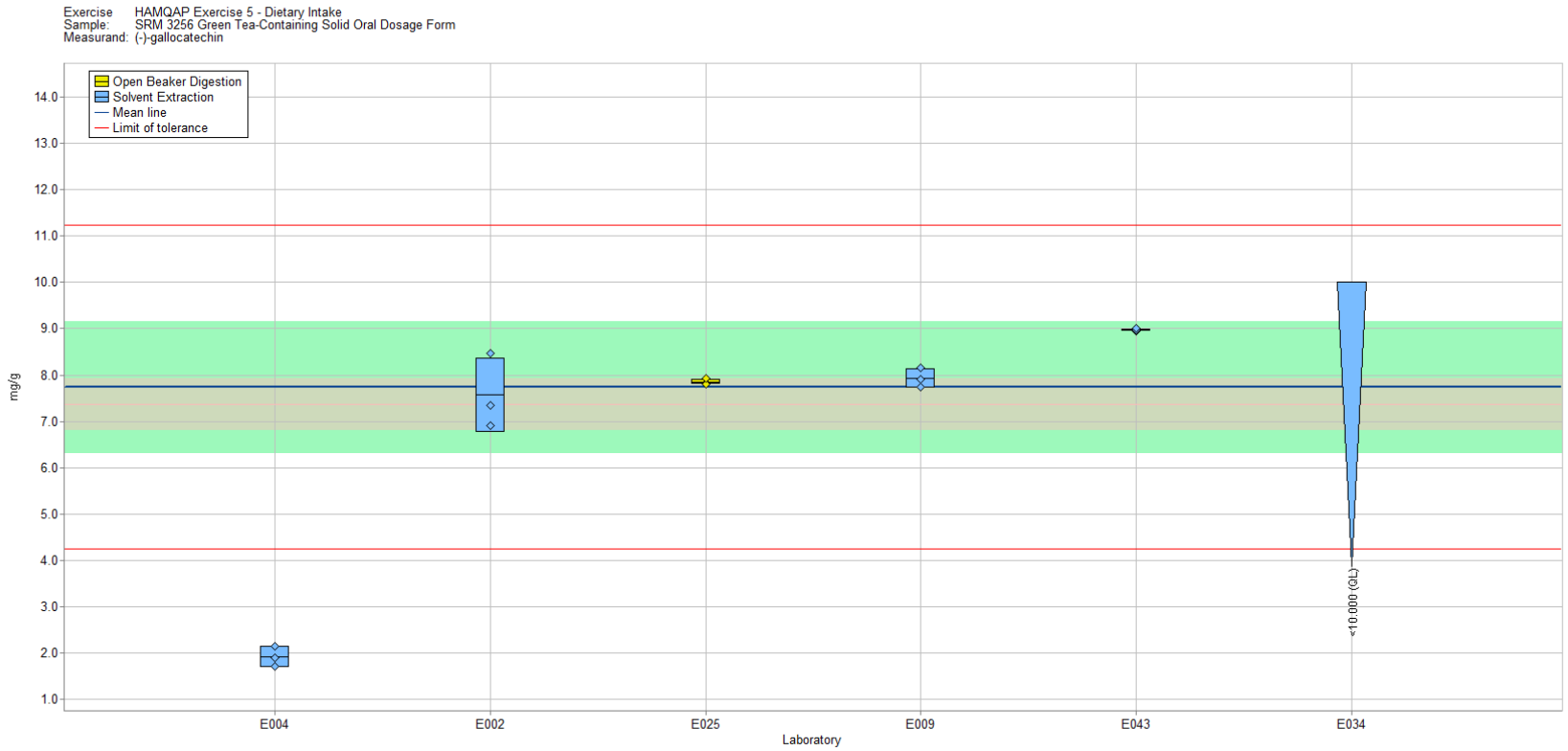

Figure 6-17. (–)-Gallocatechin in SRM 3256 Green Tea-Containing SODF (data summary view – sample preparation method). In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation 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, $|Z_{\text{NIST}}| \leq 2$.

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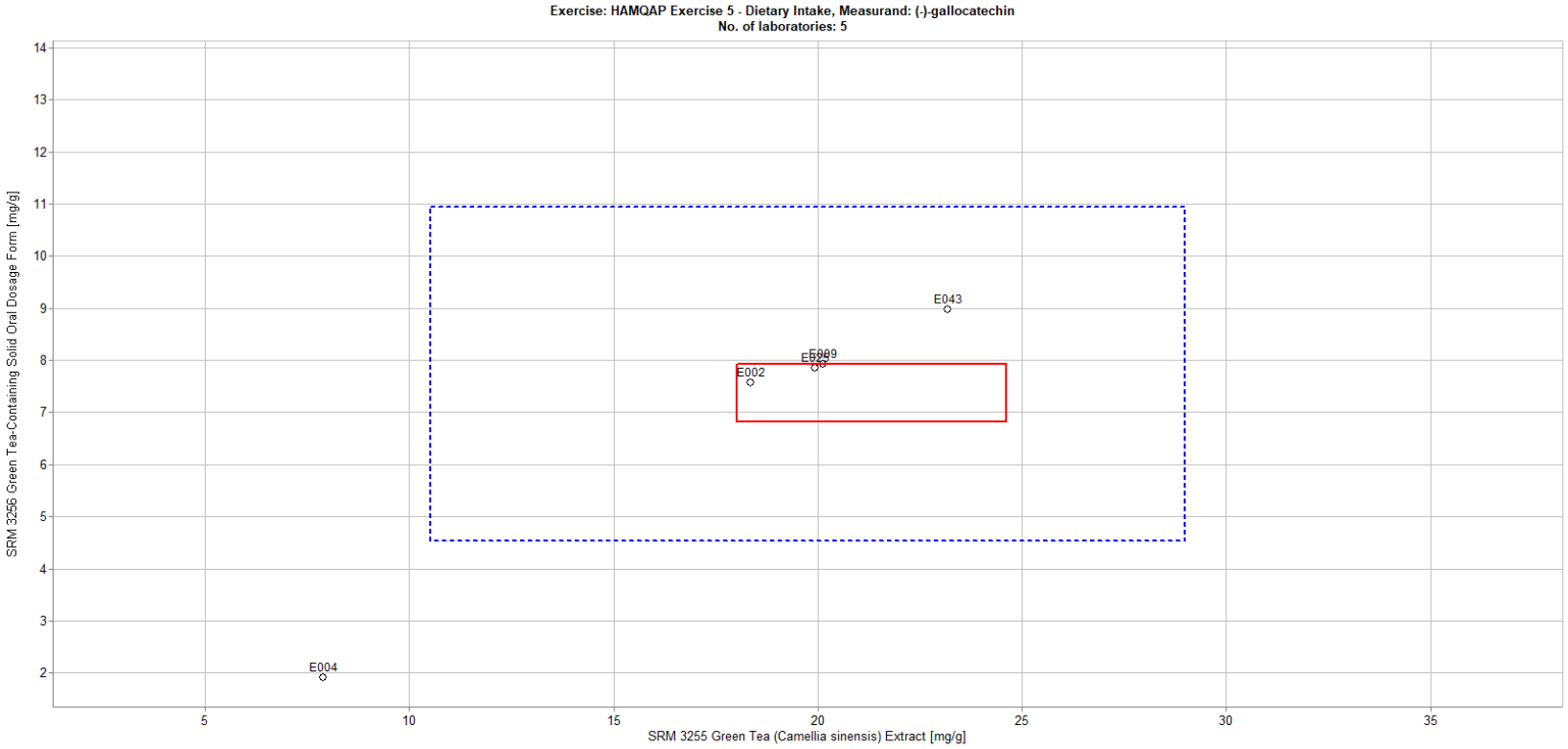

Figure 6-18. Laboratory means for (–)-gallocatechin in SRM 3255 Green Tea (*Camellia sinensis*) Extract and SRM 3256 Green Tea-Containing SODF (sample/sample comparison view). In this view, the individual laboratory mean for one sample (SRM 3255) is compared to the individual laboratory mean for a second sample (SRM 3256). The solid red box represents the NIST range of tolerance for the two samples, SRM 3255 (x-axis) and SRM 3256 (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 3255 (x-axis) and SRM 3256 (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 6-8. Data summary table for (–)-gallocatechin gallate in green tea extract and green tea-containing SODF.

		(-)-gallocatechin gallate									
		SRM 3255 Green Tea (*Camellia sinensis*) Extract (mg/g)					SRM 3256 Green Tea-Containing Solid Oral Dosage Form (mg/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target				37.8	3.9				4.49	3.52
	E001	38.5	38.3	38	38.3	0.3	6.59	6.59	6.63	6.60	0.02
	E002	48.61	41.66	50.66	47.0	4.7	7.55	7.46	7.69	7.57	0.12
	E004	46.45	46.93	47.65	47.0	0.6	9.31	8.83	8.9	9.01	0.26
	E006										
	E009	38.3	36.12	38.39	37.6	1.3	7	6.9	7.15	7.02	0.13
	E013	40	39.2	39.3	39.5	0.4	6.6	6.73	6.77	6.70	0.09
	E017	43.425	43.588	43.7	43.6	0.1	7.096	7.384	7.432	7.30	0.18
	E018										
	E020										
	E021										
	E022										
	E025	43.5	45.2	45.1	44.6	1.0	8.86	8.76	8.89	8.84	0.07
	E030	47.6	47.6	47.8	47.7	0.1	9.06	8.75	9.13	8.98	0.20
	E031										
	E034	37.706	37.535	37.992	37.7	0.2	6.365	6.478	5.983	6.28	0.26
	E043	45.54	44.45	43.85	44.6	0.9	6.12	6.04	6.54	6.23	0.27
Community Results		Consensus Mean			42.8		Consensus Mean			7.45	
		Consensus Standard Deviation			1.5		Consensus Standard Deviation			0.34	
		Maximum			47.7		Maximum			9.01	
		Minimum			37.6		Minimum			6.23	
		N			10		N			10	

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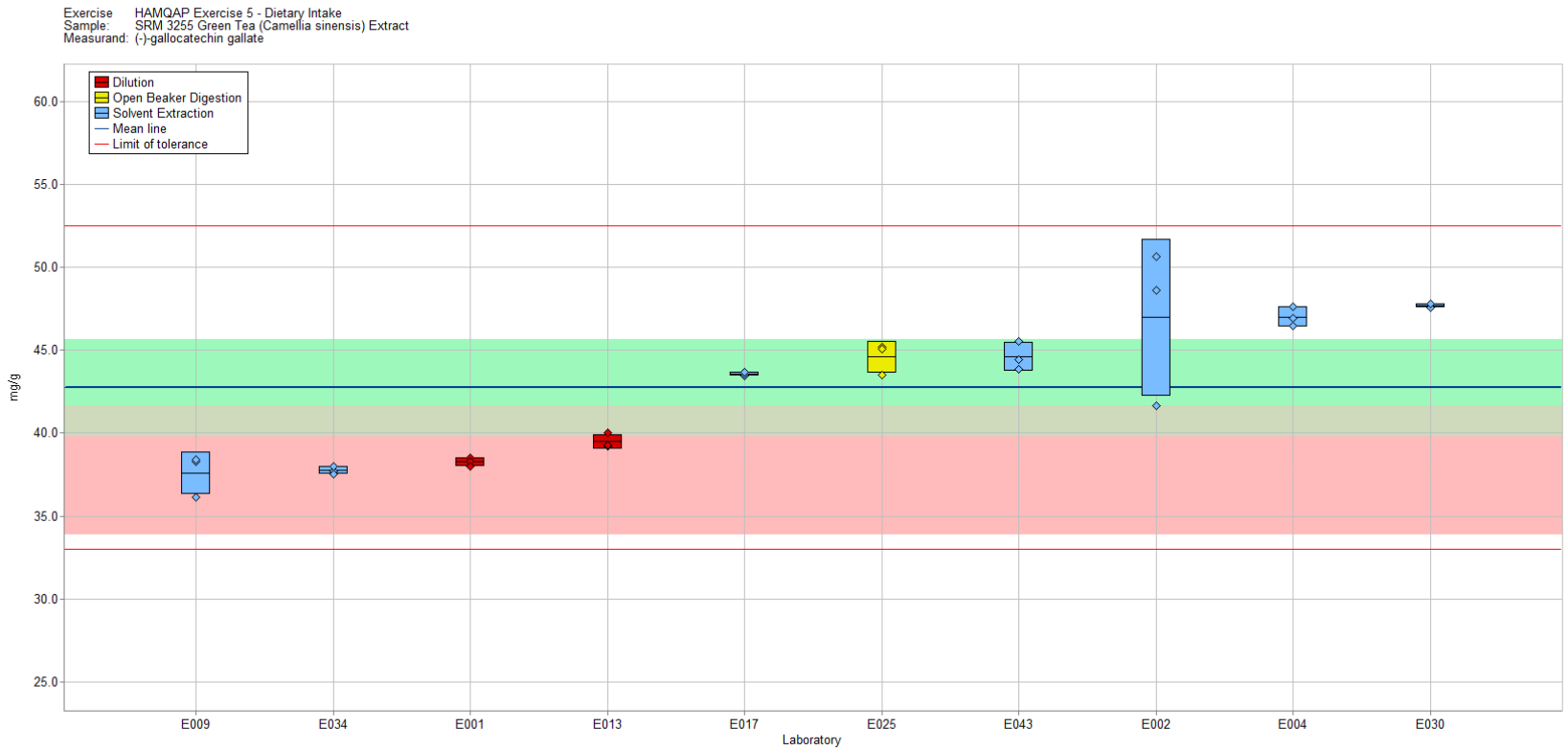

Figure 6-19. (–)-Gallocatechin gallate in SRM 3255 Green Tea (*Camellia sinensis*) Extract (data summary view – sample preparation method). In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents 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, $|Z_{\text{NIST}}| \leq 2$.

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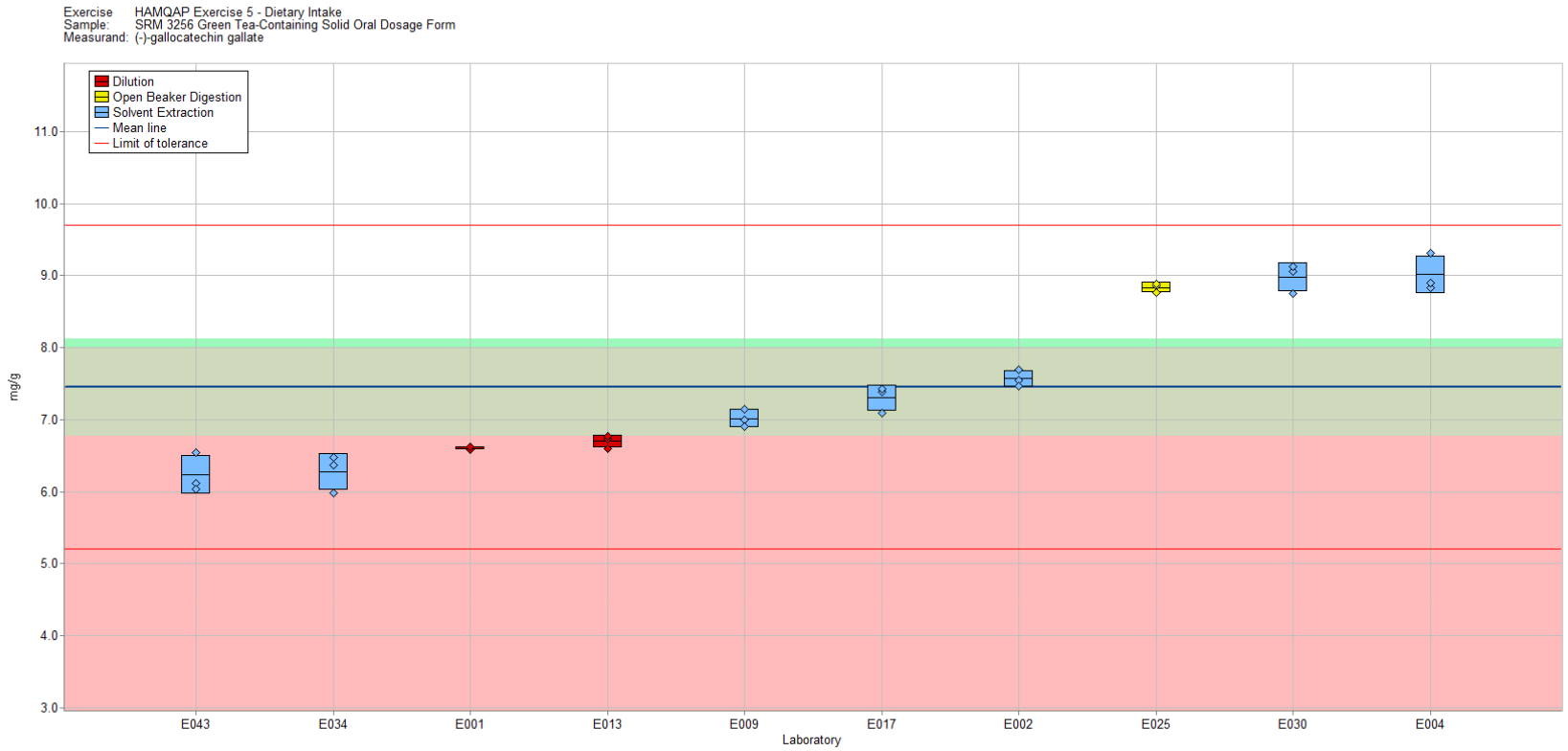

Figure 6-20. (–)-Gallocatechin gallate in SRM 3256 Green Tea-Containing SODF (data summary view – sample preparation method). In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample 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, $|Z_{\text{NIST}}| \leq 2$.

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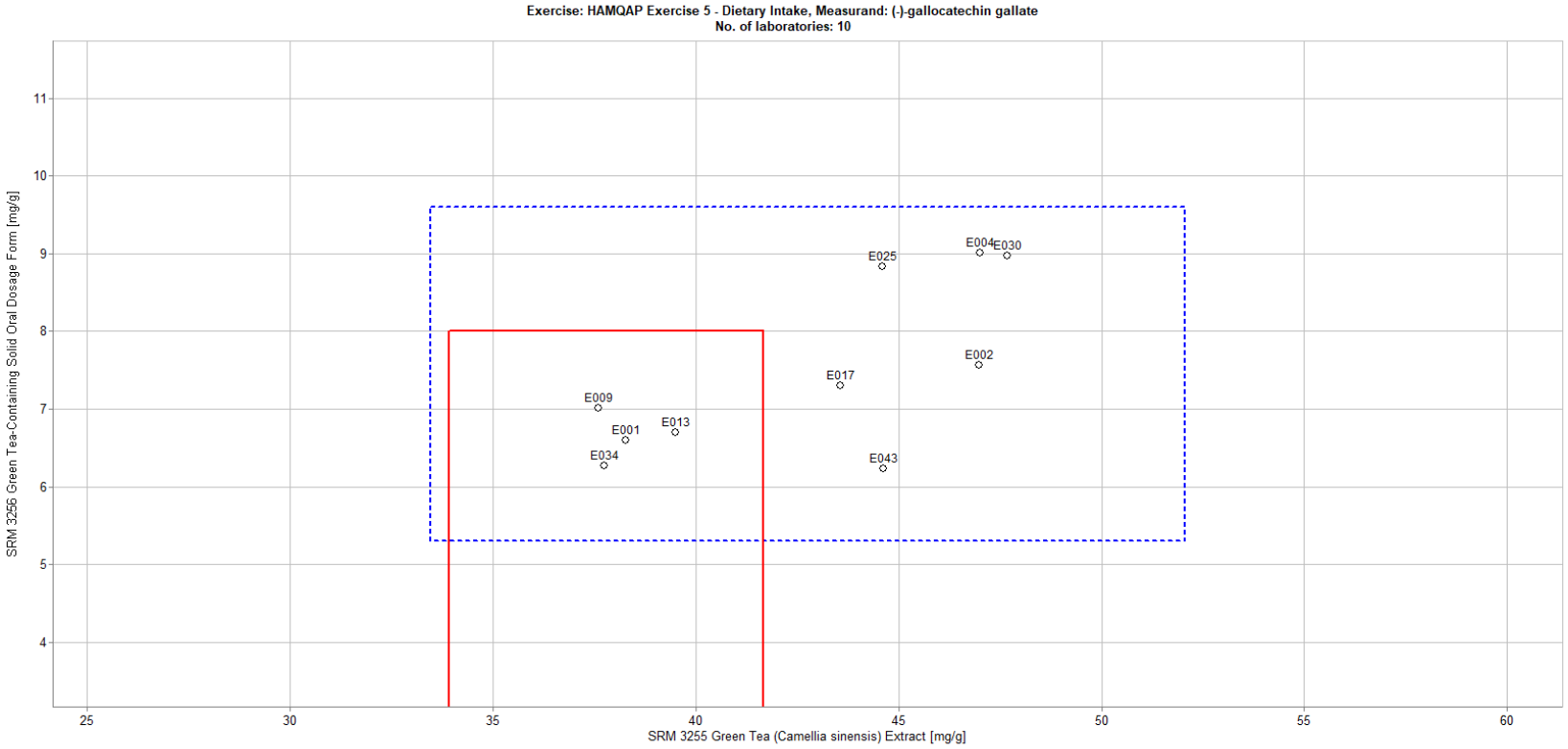

Figure 6-21. Laboratory means for (–)-gallocatechin gallate in SRM 3255 Green Tea (*Camellia sinensis*) Extract and SRM 3256 Green Tea-Containing SODF (sample/sample comparison view). In this view, the individual laboratory mean for one sample (SRM 3255) is compared to the individual laboratory mean for a second sample (SRM 3256). The solid red box represents the NIST range of tolerance for the two samples, SRM 3255 (x-axis) and SRM 3256 (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 3255 (x-axis) and SRM 3256 (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 6-9. Data summary table for gallic acid in green tea extract and green tea-containing SODF. Data points highlighted in red have been flagged as potential outliers (e.g., Grubb and/or Cochran) by the NIST software package.

		Gallic Acid									
		SRM 3255 Green Tea (*Camellia sinensis*) Extract (mg/g)					SRM 3256 Green Tea-Containing Solid Oral Dosage Form (mg/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target				3.13	0.17				12.79	0.96
	E001										
	E002	2.13	2.82	3	2.65	0.46	2.25	2.22	2.19	2.22	0.03
	E004										
	E006										
	E009										
	E013	2.96	3.01	3.07	3.01	0.06	10.9	11.3	11.3	11.17	0.23
	E017										
	E018										
	E021										
	E022										
	E025	3.02	3.08	3.03	3.04	0.03	12.3	12.3	12.9	12.50	0.35
	E030	7.83	8.36	8.33	8.17	0.30	29	28.7	27.5	28.40	0.79
	E034	29.752	29.05	27.955	28.92	0.91	103.36	114.231	104.705	107.43	5.93
	E035										
	E041										
	E042										
	E043	3.611	3.501	3.409	3.51	0.10	14.416	14.86	14.906	14.73	0.27
Community Results		Consensus Mean			3.05		Consensus Mean			13.80	
		Consensus Standard Deviation			0.46		Consensus Standard Deviation			8.24	
		Maximum			28.92		Maximum			107.43	
		Minimum			2.65		Minimum			2.22	
		N			6		N			6	

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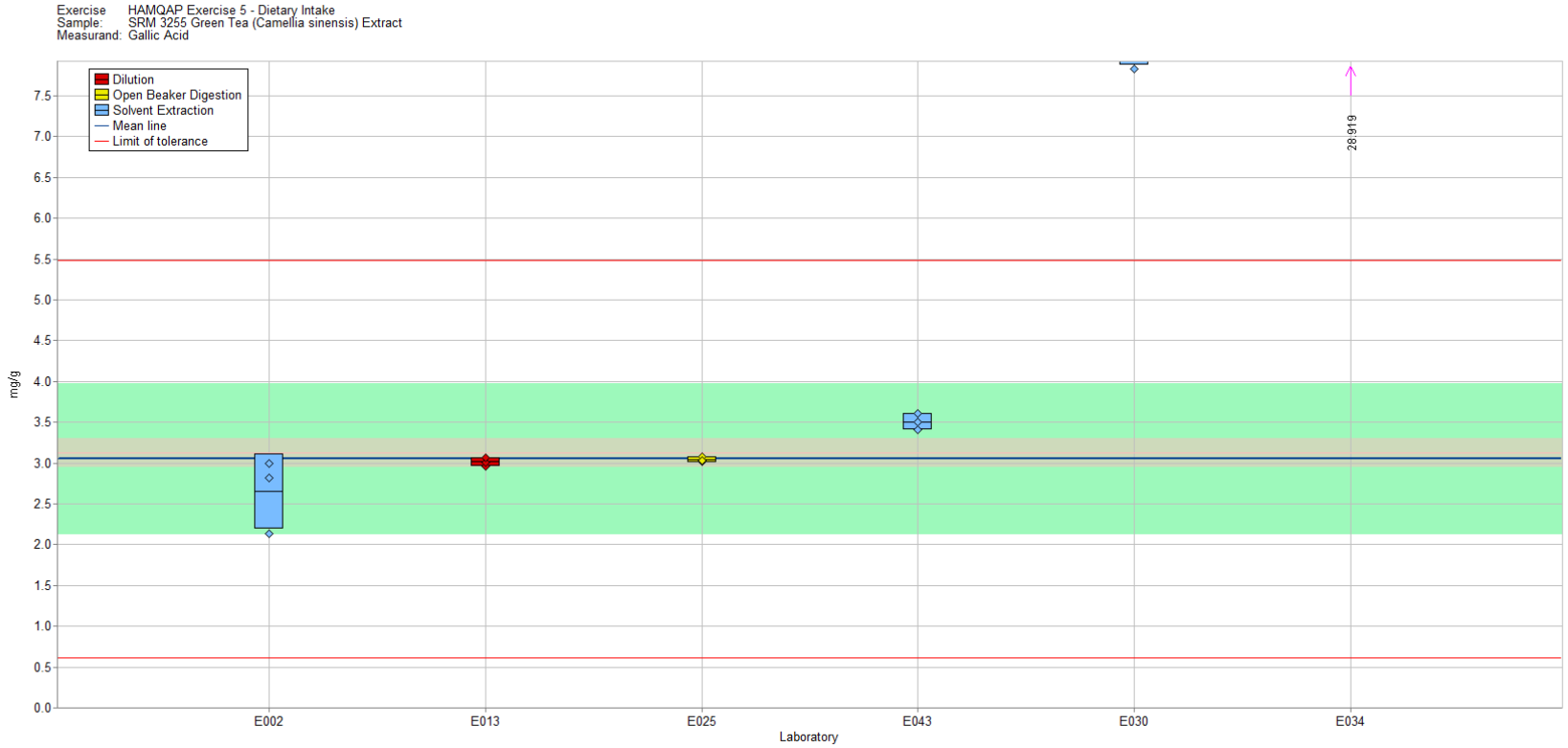

Figure 6-22. Gallic acid in SRM 3255 Green Tea (*Camellia sinensis*) Extract (data summary view – sample preparation method). In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample 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, $|Z_{\text{NIST}}| \leq 2$.

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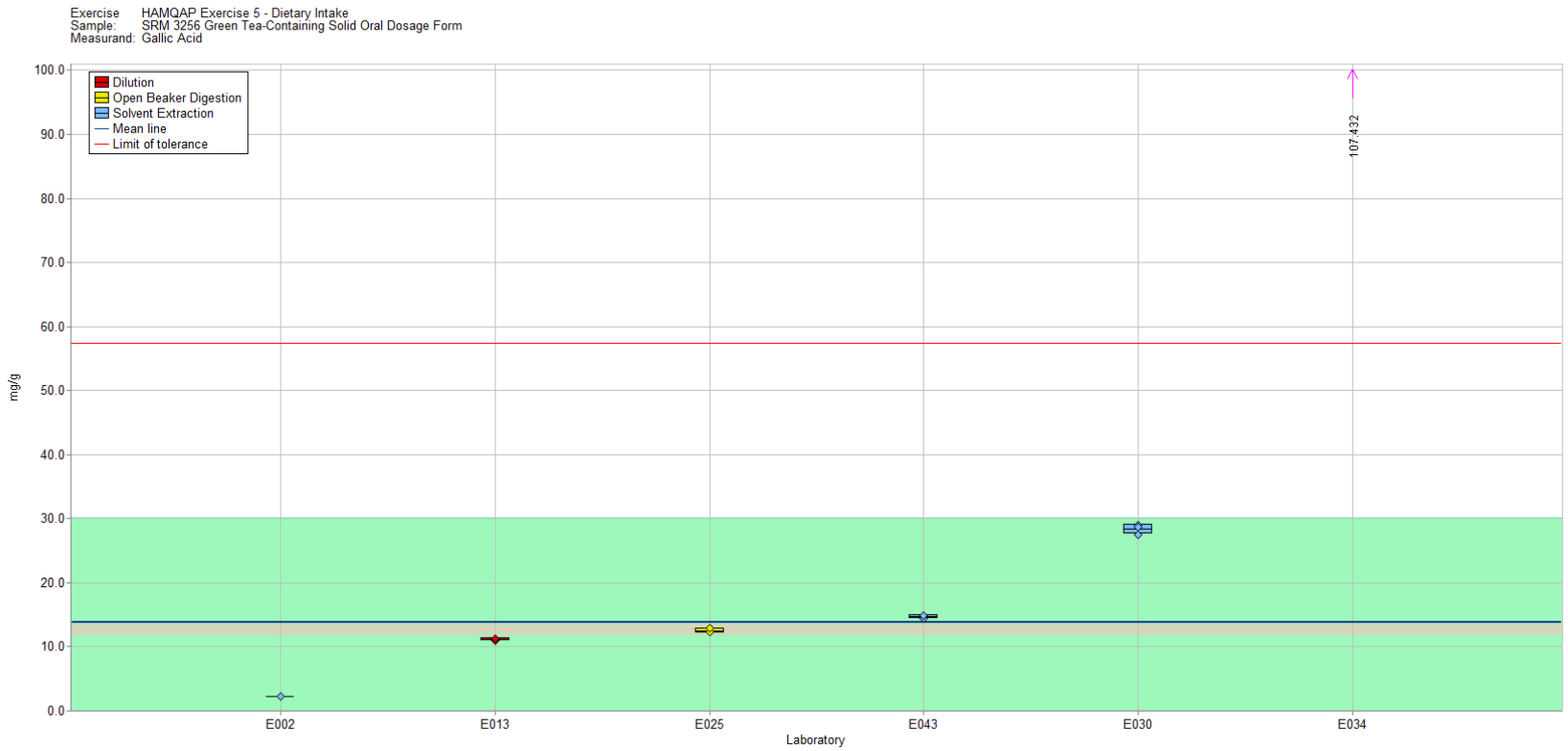

Figure 6-23. Gallic acid in SRM 3256 Green Tea-Containing SODF (data summary view – sample preparation method). In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red line represent the consensus range of tolerance, calculated as the value above the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$, with the lower limit set to zero. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty (U_{NIST}) and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$.

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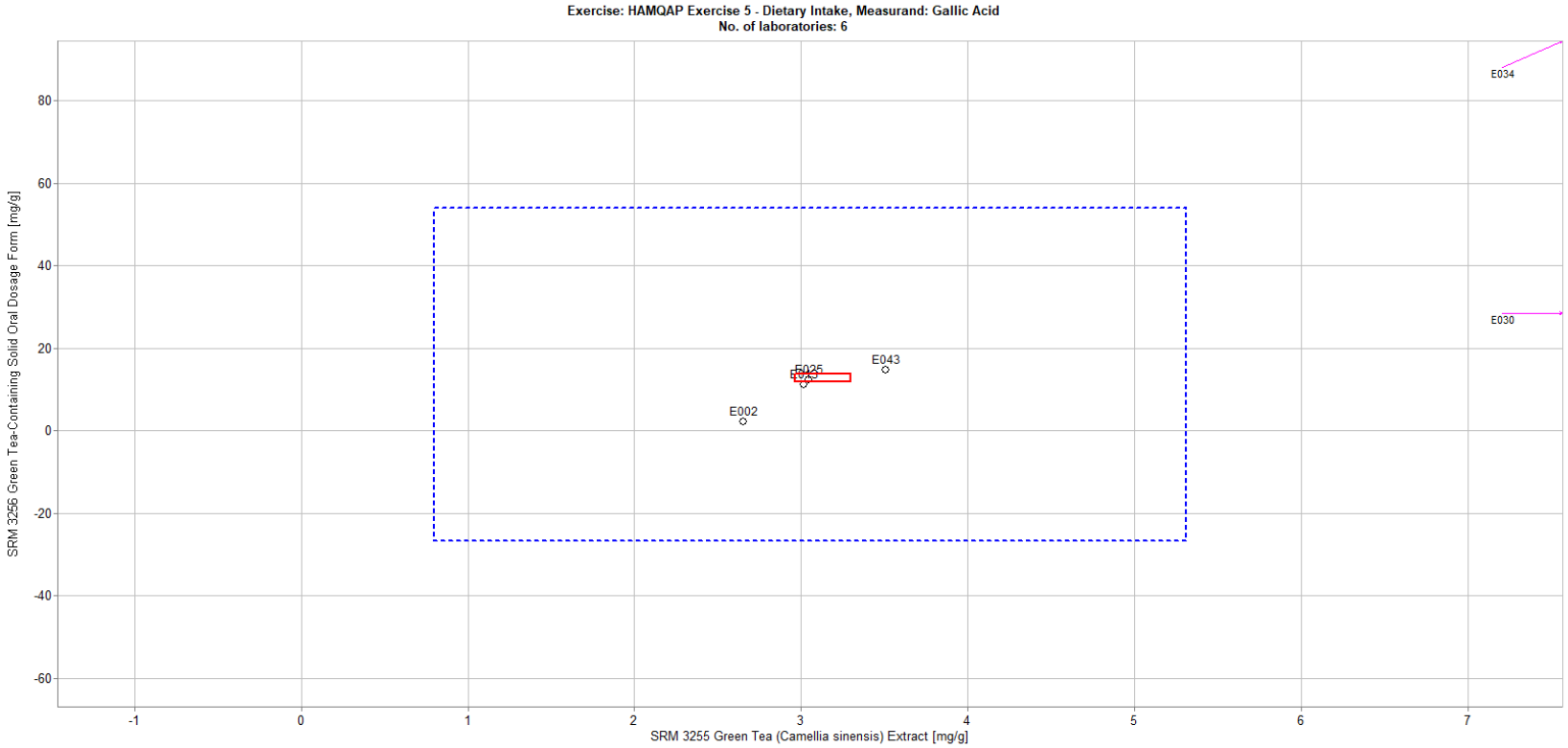

Figure 6-24. Laboratory means for gallic acid in SRM 3255 Green Tea (*Camellia sinensis*) Extract and SRM 3256 Green Tea-Containing SODF (sample/sample comparison view). In this view, the individual laboratory mean for one sample (SRM 3255) is compared to the individual laboratory mean for a second sample (SRM 3256). The solid red box represents the NIST range of tolerance for the two samples, SRM 3255 (x-axis) and SRM 3256 (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 3255 (x-axis) and SRM 3256 (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 6-10. Data summary table for L-theanine in green tea extract and green tea-containing SODF.

		L-theanine									
		SRM 3255 Green Tea (*Camellia sinensis*) Extract (mg/g)					SRM 3256 Green Tea-Containing Solid Oral Dosage Form (mg/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target				0.329	0.016				3.613	2.343
	E001										
	E002	3.783	3.711	3.19	3.561	0.324	4.63	4.839	4.667	4.712	0.112
	E004										
	E006										
	E007										
	E009										
	E010	0.4322	0.3925	0.4418	0.422	0.026	3.8185	3.8425	3.7248	3.795	0.062
	E012										
	E013	0.588	0.589	0.531	0.569	0.033	3.85	3.69	3.9	3.813	0.110
	E015										
	E017										
	E020										
	E021										
	E022										
	E025	1.79	1.861	1.914	1.855	0.062	4.421	4.789	4.777	4.662	0.209
	E030										
	E034										
	E035										
	E040										
	E041										
	E042										
	E043										
Community Results		Consensus Mean			1.602		Consensus Mean			4.246	
		Consensus Standard Deviation			1.412		Consensus Standard Deviation			0.166	
		Maximum			3.561		Maximum			4.712	
		Minimum			0.422		Minimum			3.795	
		N			4		N			4	

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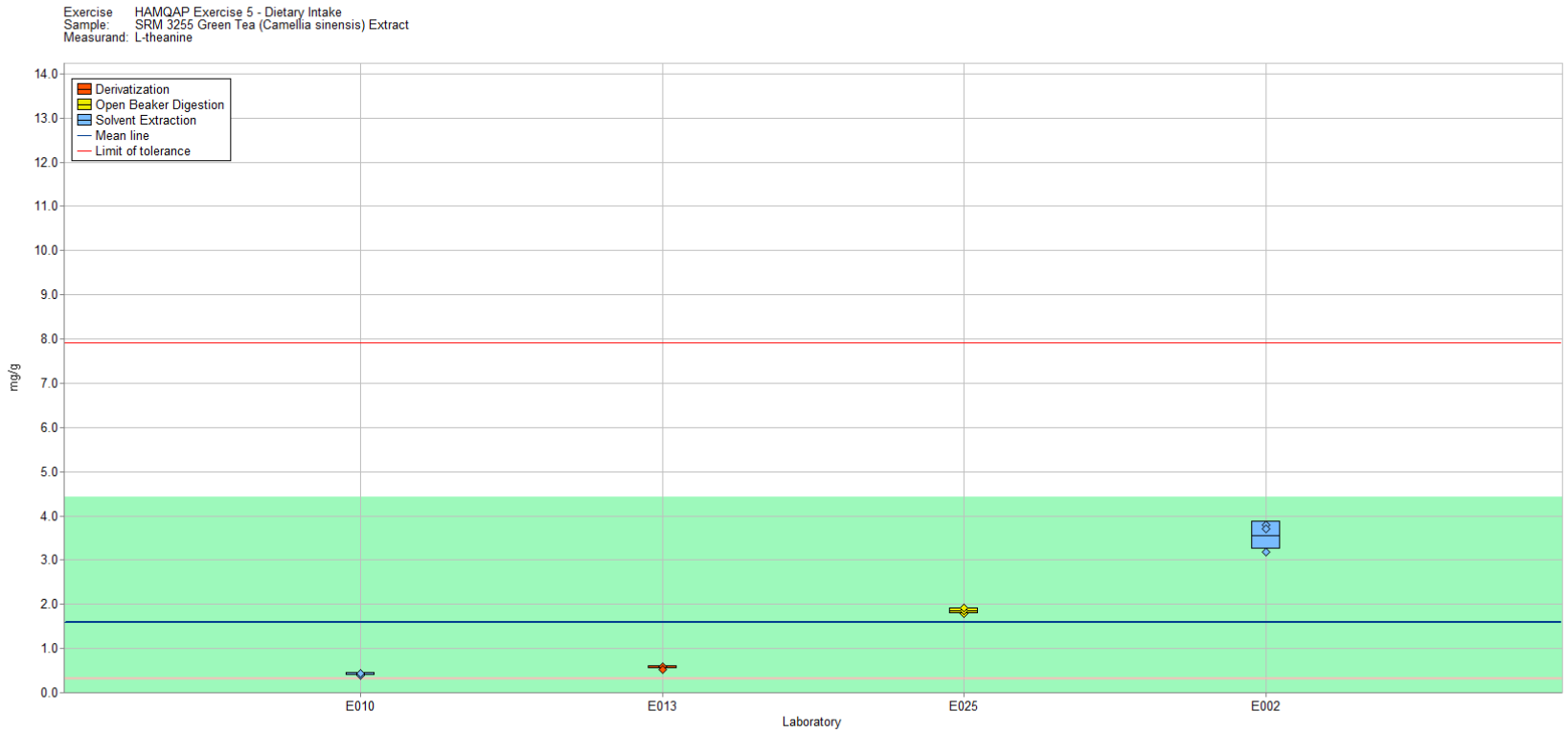

Figure 6-25. L-theanine in SRM 3255 Green Tea (*Camellia sinensis*) Extract (data summary view – sample preparation). In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red line represent the consensus range of tolerance, calculated as the value above the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$, with the lower limit set to zero. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty (U_{NIST}) and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$.

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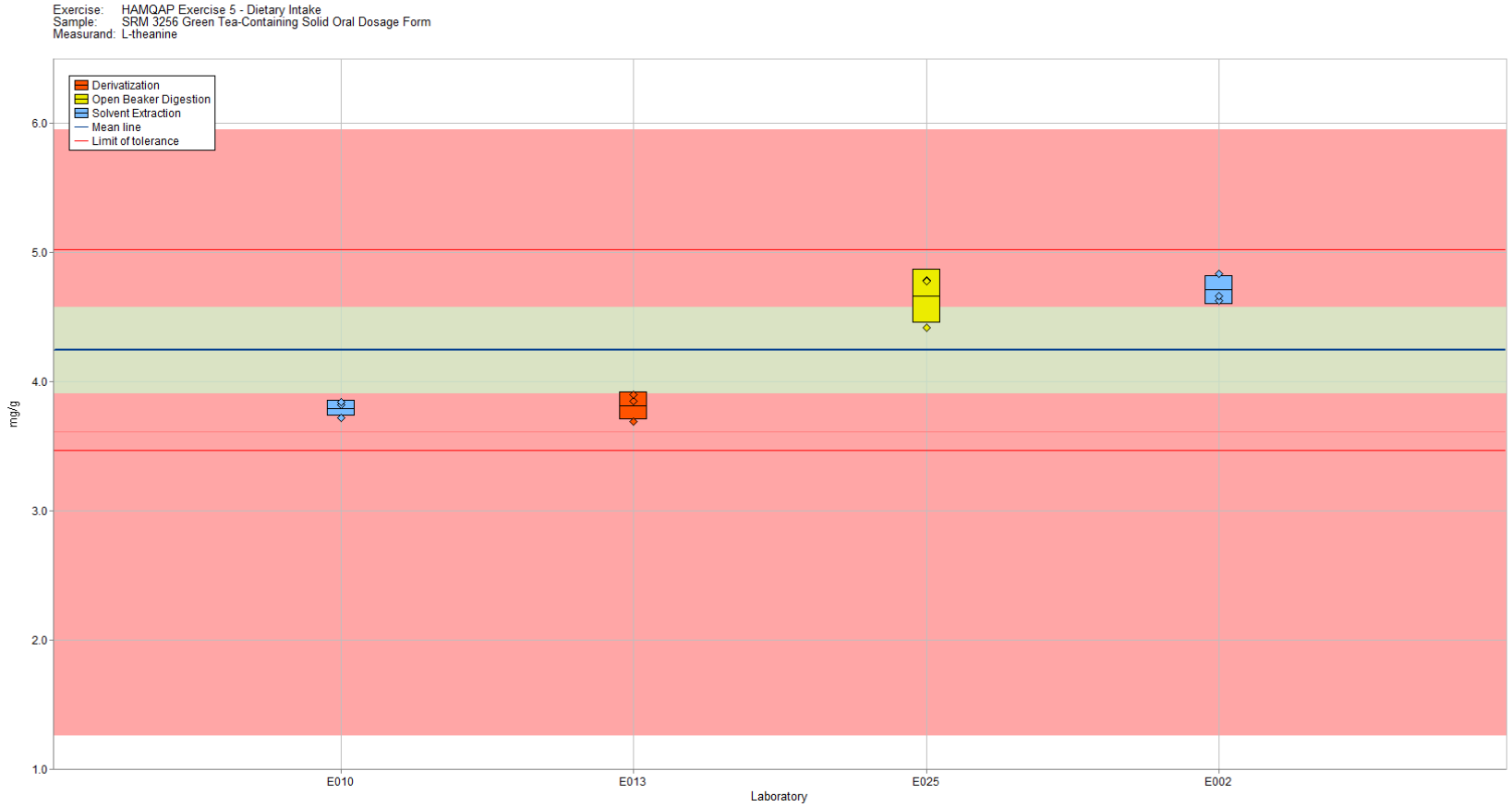

Figure 6-26. L-theanine in SRM 3256 Green Tea-Containing SODF (data summary view – sample preparation). In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. 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, $|Z_{\text{NIST}}| \leq 2$.

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SECTION 7: NATURAL PRODUCTS (Xanthines)

Study Overview

In this study, participants were provided with samples of SRM 3254 Green Tea (*Camellia sinensis*) Leaves, SRM 3255 Green Tea (*Camellia sinensis*) Extract, SRM 3256 Green Tea-Containing Solid Oral Dosage Form (SODF), and SRM 3253 Yerba Mate Leaves. Participants were asked to use in-house analytical methods to determine the mass fractions (mg/g) of caffeine, theobromine, and theophylline in each matrix. Caffeine and other xanthines such as theobromine and theophylline are included in many performance enhancing supplements.[19] Caffeine is a central nervous system stimulant that is rapidly absorbed into the bloodstream and may improve exercise performance and focus while reducing drowsiness. Side effects of caffeine consumption, however, include increased heart rate, insomnia, stomach discomfort, and anxiety. Yerba mate and green tea extract are two natural caffeine-containing products often used to enhance exercise performance. Measurement of xanthines in these products is important for ensuring accuracy of product labels and also for understanding both positive and negative health outcomes related to consumption of such products.

Dietary Intake Sample Information

Green Tea Leaves. Participants were provided with three packets, each containing 3 g of green tea powder. Participants were asked to store the material at controlled room temperature, 20 °C to 25 °C, and to prepare one sample and report one value from each packet provided. Before use, participants were instructed to mix the contents of the packet thoroughly, allow contents to settle for one minute prior to opening to minimize the loss of fine particles, and to use a sample size of at least 100 mg. The approximate analyte levels were not reported to participants prior to the study. Certified values for caffeine and theobromine in SRM 3254 were assigned using results from NIST by LC-Abs and LC-MS, and from collaborating laboratories using LC-FL and/or LC-Abs. A target level for theophylline in SRM 3254 has not been determined. The NIST-determined values and uncertainties are provided in the table below, both on a dry-mass basis as listed on the COA, and on an as-received basis accounting for moisture of the material (5.2 %) with a further expanded uncertainty for evaluation of laboratory performance.

	NIST-Determined Mass Fractions in SRM 3254 (mg/g)	
Analyte	(dry-mass basis)	(as-received basis)[(a)]
Caffeine	23.5 ± 1.8	22.3 ± 3.4
Theobromine	0.463 ± 0.052	0.439 ± 0.099

[(a)] Associated expanded uncertainties for the target zone for acceptable performance are calculated as 2*(U_{95} or U_{NIST}).

Green Tea Extract. Participants were provided with three packets, each containing 1 g of green tea extract. Participants were asked to store the material at controlled room temperature, 20 °C to 25 °C, and to prepare one sample and report one value from each packet provided. Before use, participants were instructed to mix the contents of the packet thoroughly, and to use a sample size

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

of at least 100 mg. The approximate analyte levels were not reported to participants prior to the study. Certified values for caffeine and theobromine in SRM 3255 were assigned using results from NIST by LC-Abs and LC-MS, and from collaborating laboratories by LC-FL and/or LC-Abs. A reference value for theophylline in SRM 3255 was assigned using results from NIST by LC-MS. The NIST-determined mass fraction values and uncertainties are provided in the table below, both on a dry-mass basis as shown on the COA, and on an as-received basis accounting for moisture of the material (3.2 %) with a further expanded uncertainty for evaluation of laboratory performance.

	NIST-Determined Mass Fractions in SRM 3255 (mg/g)	
Analyte	(dry-mass basis)	(as-received basis)[a]
Caffeine	36.9 ± 2.7	35.7 ± 5.2
Theobromine	0.867 ± 0.076	0.84 ± 0.15
Theophylline	0.087 ± 0.002	0.084 ± 0.004

[a] Associated expanded uncertainties for the target zone for acceptable performance are calculated as 2*(U_{95} or U_{NIST}).

Green Tea-Containing SODF. Participants were provided with three packets, each containing 2.5 g of powdered oral-dosage material. Participants were asked to store the material at controlled room temperature, 20 °C to 25 °C, and to prepare one sample and report one value from each packet provided. Before use, participants were instructed to mix the contents of the packet thoroughly, allow contents to settle for one minute prior to opening to minimize the loss of fine particles, and to use a sample size of at least 100 mg. The approximate analyte levels were not reported to participants prior to the study. Certified values for caffeine and theobromine in SRM 3256 were assigned using results from NIST and collaborating laboratories by LC-UV and LC-MS. A reference value for theophylline in SRM 3256 was assigned using results from NIST by LC-MS. The NIST-determined mass fraction values and uncertainties are provided in the table below, both on a dry-mass basis as shown on the COA, and on an as-received basis accounting for moisture of the material (2.3 %) with a further expanded uncertainty for evaluation of laboratory performance.

	NIST-Determined Mass Fractions in SRM 3256 (mg/g)	
Analyte	(dry-mass basis)	(as-received basis)[a]
Caffeine	70.0 ± 2.6	68.3 ± 5.1
Theobromine	1.04 ± 0.15	1.02 ± 0.29
Theophylline	0.060 ± 0.002	0.059 ± 0.004

[a] Associated expanded uncertainties for the target zone for acceptable performance are calculated as 2*(U_{95} or U_{NIST}).

Yerba Mate Leaves. Participants were provided with three packets, each containing 3 g of powdered yerba mate leaves. Participants were asked to store the material at controlled room temperature, 20 °C to 25 °C, and to prepare one sample and report one value from each packet provided. Before use, participants were instructed to mix the contents of the packet thoroughly, allow contents to settle for one minute prior to opening to minimize the loss of fine particles, and

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to use a sample size of at least 100 mg. Target levels for caffeine, theobromine, and theophylline have not been determined in SRM 3253.

Dietary Intake Study Results

- Thirty laboratories enrolled in this exercise and received samples to measure some or all of the select xanthines in green tea and yerba mate. The enrollment and reporting statistics for the botanicals study is described in the table below.

Analyte	Number of Laboratories Requesting Samples	Number of Laboratories Reporting Results (Percent Participation)			
		Leaves	Extract	SODF	Yerba Mate
Caffeine	30	19 (63 %)	18 (60 %)	17 (57 %)	19 (63 %)
Theobromine	20	12 (60 %)	10 (50 %)	10 (50 %)	12 (60 %)
Theophylline	13	4 (31 %)	6 (46 %)	6 (46 %)	4 (31 %)

- The between-laboratory variabilities were good for caffeine and theobromine in the green tea and yerba mate (see table below). Variabilities for theophylline were very large based on the limited number of quantitative results reported.

Analyte	Between-Laboratory Variability (% RSD)			
	Leaves	Extract	SODF	Yerba Mate
Caffeine	2.8 %	1.8 %	0.85 %	2.1 %
Theobromine	15 %	10 %	4.8 %	4.8 %
Theophylline	82 %	66 %	95 %	42 %

- For the green tea samples, the consensus means for caffeine (**Figures 7--1** through **7-3**) and theobromine (**Figure 7-8** through **7-11)** were within the NIST target range. The confidence intervals for the consensus means for theophylline for SRM 3255 (**Figure 7-16)** and SRM 3256 (**Figure 7-17)** were very large in comparison to the NIST target range.
- All participating laboratories reported using LC-absorbance for determination of the select xanthines in the green tea and yerba mate samples.
- Most laboratories reported using solvent extraction for determination of the select xanthines in the green tea and yerba mate samples. The remaining laboratories either reported using dilution or open beaker digestion.

Dietary Intake Technical Recommendations

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

- For the analysis of theophylline, overall participation was low and limits the ability to make technical recommendations.
  - Larger laboratory participation is recommended for better insight on community needs.

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    - Low participation may be the result of laboratories not having adequate in-house analytical methods for the extraction and quantification of theophylline in natural products.
- Low between-laboratory variability and within-laboratory variability for both caffeine and theobromine indicate that laboratories have these methods in control for these samples.
    - Some laboratories reported outlying values for caffeine that trended on the low end of the reported values. These laboratories reported using similar extraction techniques but optimization of in-house methods or use of a matrix CRM will ensure complete extraction of caffeine.
    - Laboratories reporting results above the targeted values should examine sample preparation and separation conditions. Extraction conditions could produce potential chromatographic interferences resulting in reported values that are biased high relative to the true value.
- Linear trends were observed for the theophylline materials, possibly caused by improper calibration, a frequent source of measurement error (**Figures 7-19** through **7-21**). However, very few participants reported data for theophylline, making it hard for statistical analyses and recommendations to be meaningful.
    - 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.
- The Youden plots (**Figures 7-12** and **7-13**) indicate that some laboratories reported appropriate data results for theobromine for one material but either a high or low result for a second material. Laboratories may experience more difficulties in sample preparation of one material over another when measuring theobromine.
- Laboratories reporting results flagged as outliers should check for errors in calculations or reporting units. Confirm that all dilution factors have been properly tabulated.

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Table 7-1. Data summary table for caffeine, theobromine, and theophylline in green tea and yerba mate.

National Institute of Standards & Technology

HAMQAP Exercise 5 - Natural Products

		Lab Code:	NIST	1. Your Results				2. Community Results			3. Target	
Analyte	Sample		Units	x_i	s_i	Z'_{comm}	Z_{NIST}	N	x*	s*	x_{NIST}	*U*
Caffeine	SRM 3254 Green Tea (Camellia sinensis) Leaves		mg/g	22.3	3.4			19	21.5	0.6	22.3	3.4
Caffeine	SRM 3255 Green Tea (Camellia sinensis) Extract		mg/g	35.7	5.2			18	38	0.7	35.7	5.2
Caffeine	SRM 3256 Green Tea-Containing Solid Oral Dosage Form		mg/g	68.3	5.1			17	70.2	0.6	68.3	5.1
Caffeine	SRM 3253 Yerba Mate Leaves		mg/g					19	10.1	0.21		
Theobromine	SRM 3254 Green Tea (Camellia sinensis) Leaves		mg/g	0.439	0.099			11	0.479	0.074	0.439	0.099
Theobromine	SRM 3255 Green Tea (Camellia sinensis) Extract		mg/g	0.840	0.15			9	0.852	0.085	0.840	0.15
Theobromine	SRM 3256 Green Tea-Containing Solid Oral Dosage Form		mg/g	1.02	0.29			9	1.05	0.051	1.02	0.29
Theobromine	SRM 3253 Yerba Mate Leaves		mg/g					11	1.47	0.071		
Theophylline	SRM 3254 Green Tea (Camellia sinensis) Leaves		mg/g					4	0.54	0.44		
Theophylline	SRM 3255 Green Tea (Camellia sinensis) Extract		mg/g	0.084	0.004			5	0.99	0.65	0.084	0.004
Theophylline	SRM 3256 Green Tea-Containing Solid Oral Dosage Form		mg/g	0.059	0.004			5	0.98	0.94	0.059	0.004
Theophylline	SRM 3253 Yerba Mate Leaves		mg/g					4	3.4	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

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Table 7-2. Data summary table for caffeine in green tea and yerba mate. Data points highlighted in red have been flagged as potential outliers (e.g., Grubb and/or Cochran) by the NIST software package.

		Caffeine																			
		SRM 3254 Green Tea (*Camellia sinensis*) Leaves (mg/g)					SRM 3255 Green Tea (*Camellia sinensis*) Extract (mg/g)					SRM 3256 Green Tea-Containing Solid Oral Dosage Form (mg/g)					SRM 3253 Yerba Mate Leaves (mg/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target				22.3	3.4				35.7	5.2				68.3	5.1					
	E001	22	22.1	22	22.03	0.06	36.9	37.2	37.1	37.07	0.15	69.6	69.8	69.6	69.67	0.12	9.76	9.74	9.67	9.72	0.05
	E002	19.69	19.84	21.67	20.40	1.10	33.55	42.35	41.25	39.05	4.79	71.73	73.23	70.85	71.94	1.20	10.64	10.68	11.14	10.82	0.28
	E004						39.9	38.22	35.86	37.99	2.03	70.05	69.92	69.56	69.84	0.25					
	E005	18.58	17.92	17.48	17.99	0.55	37.09	37.06	37.22	37.12	0.09	70.21	69.62	68.48	69.44	0.88	10.38	9.73	10.27	10.13	0.35
	E006						21.8	21.6	21.3	21.57	0.25										
	E007	20.74	21.18	21.07	21.00	0.23	37.63	37.51	37.66	37.60	0.08	70.73	70.32	70.55	70.53	0.21	11.55	11.56	11.68	11.60	0.07
	E009	21.1	22.1	22.8	22.00	0.85	38.68	39.1	37.7	38.49	0.72	71.7	70.7	70.3	70.90	0.72	9.59	9.71	9.59	9.63	0.07
	E010	22.6054	22.8571	22.9112	22.79	0.16	37.2458	37.7043	37.7199	37.56	0.27	70.4262	70.001	70.0329	70.15	0.24	9.87	9.7918	9.8483	9.84	0.04
	E012																				
	E013	18.5	18.8	18.7	18.67	0.15	37.4	37	36.9	37.10	0.26	68.6	69.1	68.6	68.77	0.29	14.5	14.2	14.1	14.27	0.21
	E015																				
	E017	19.833	19.04	19.189	19.35	0.42	38.014	36.111	38.36	37.50	1.21	60.948	61.16	62.18	61.43	0.66	9.81	9.612	9.609	9.68	0.12
	E018																				
	E020																				
	E021																				
	E022																				
	E024																				
	E025	23.6	23.4	23.2	23.40	0.20	36.3	36.6	36.5	36.47	0.15	71	70.7	71.8	71.17	0.57	10.6	10.6	10.6	10.60	0.00
	E030	45.4	43	41.4	43.27	2.01											14.5	15	13.6	14.37	0.71
	E031																				
	E033	23.3	23.6	24.1	23.67	0.40	45.9	46.6	46.5	46.33	0.38	72.9	74.7	73.2	73.60	0.96	8.8	8.4	8.1	8.43	0.35
	E034	7.501	7.463	7.388	7.45	0.06	11.767	11.658	12.063	11.83	0.21	23.199	23.016	23.32	23.18	0.15	3.221	3.181	3.198	3.20	0.02
	E035	23.79	23.88	23.5	23.72	0.20	52.62	52.12	53.25	52.66	0.57	73.31	72.98	73.99	73.43	0.52	10.26	9.83	9.83	9.97	0.25
	E037																				
	E040	22.2	22.3	22.2	22.23	0.06	35.5	34.6	35	35.03	0.45	67.7	69.6	68	68.43	1.02	10	9.9	10	9.97	0.06
	E041	24.5	24.3	24.4	24.40	0.10											10.2	10.4	10.2	10.27	0.12
	E042	22.26	22.45	22.32	22.34	0.10	34.2	36.2	35.9	35.43	1.08	344	346	350	346.67	3.06	10.03	10.13	10.07	10.08	0.05
	E043	20.81	21.02	21.32	21.05	0.26	38.09	37.92	38.77	38.26	0.45	71.2	70.8	70.24	70.75	0.48	10.25	10.02	9.85	10.04	0.20
	E046	21.47	21.37	21.68	21.51	0.16											10.749	10.826	10.799	10.79	0.04
	E047	18.9	18.7	18.93	18.84	0.13	45.3	42.45	44.8	44.18	1.52	61.9	68.2	70.5	66.87	4.45	9.33	9.39	9.55	9.42	0.11
Community Results		Consensus Mean			21.49		Consensus Mean			37.97		Consensus Mean			70.21		Consensus Mean			10.07	
		Consensus Standard Deviation			0.60		Consensus Standard Deviation			0.70		Consensus Standard Deviation			0.60		Consensus Standard Deviation			0.21	
		Maximum			43.27		Maximum			52.66		Maximum			346.67		Maximum			14.37	
		Minimum			7.45		Minimum			11.83		Minimum			23.18		Minimum			3.20	
		N			19		N			18		N			17		N			19	

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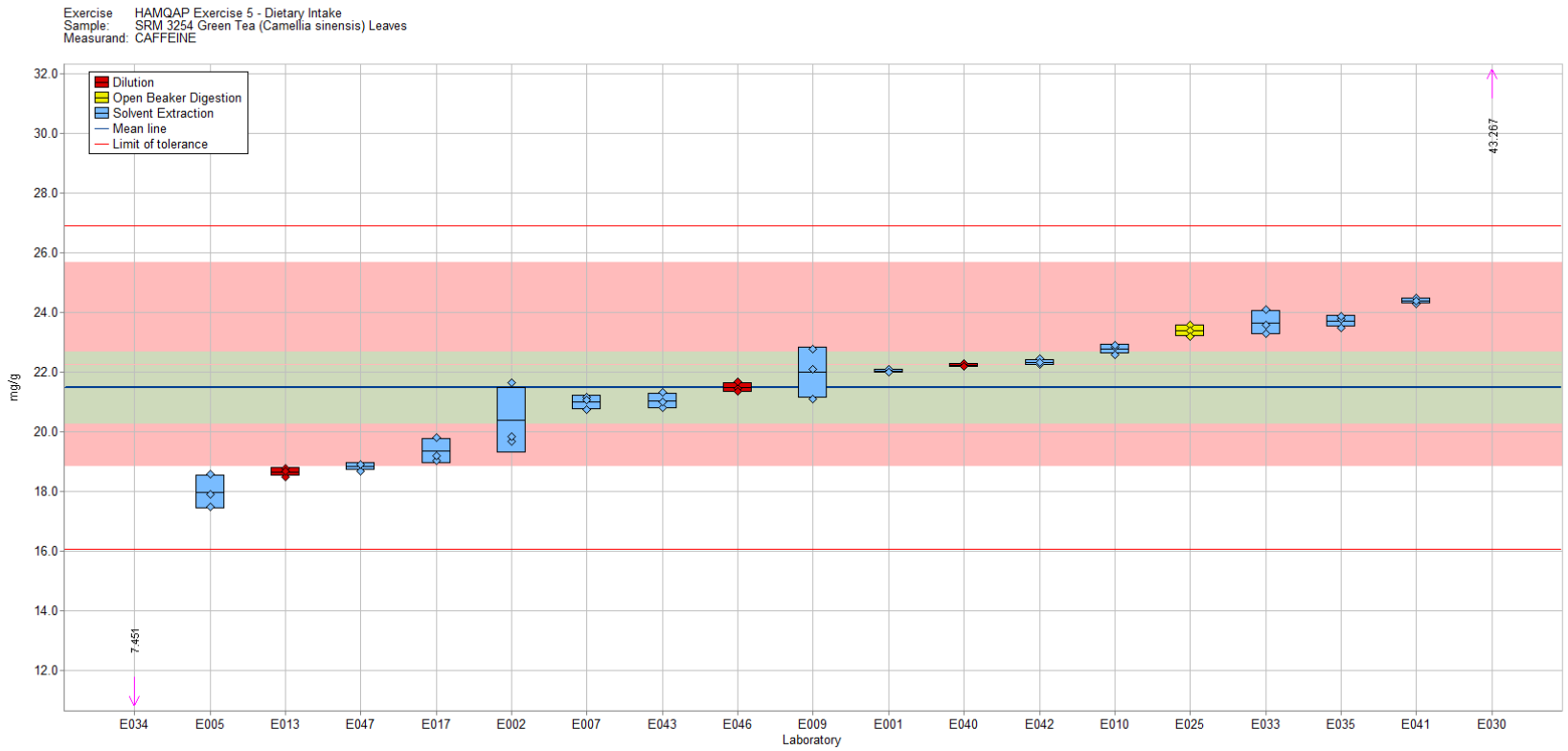

Figure 7-1. Caffeine in SRM 3254 Green Tea (*Camellia sinensis*) Leaves (data summary view – sample preparation method). In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation 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, $|Z_{\text{NIST}}| \leq 2$.

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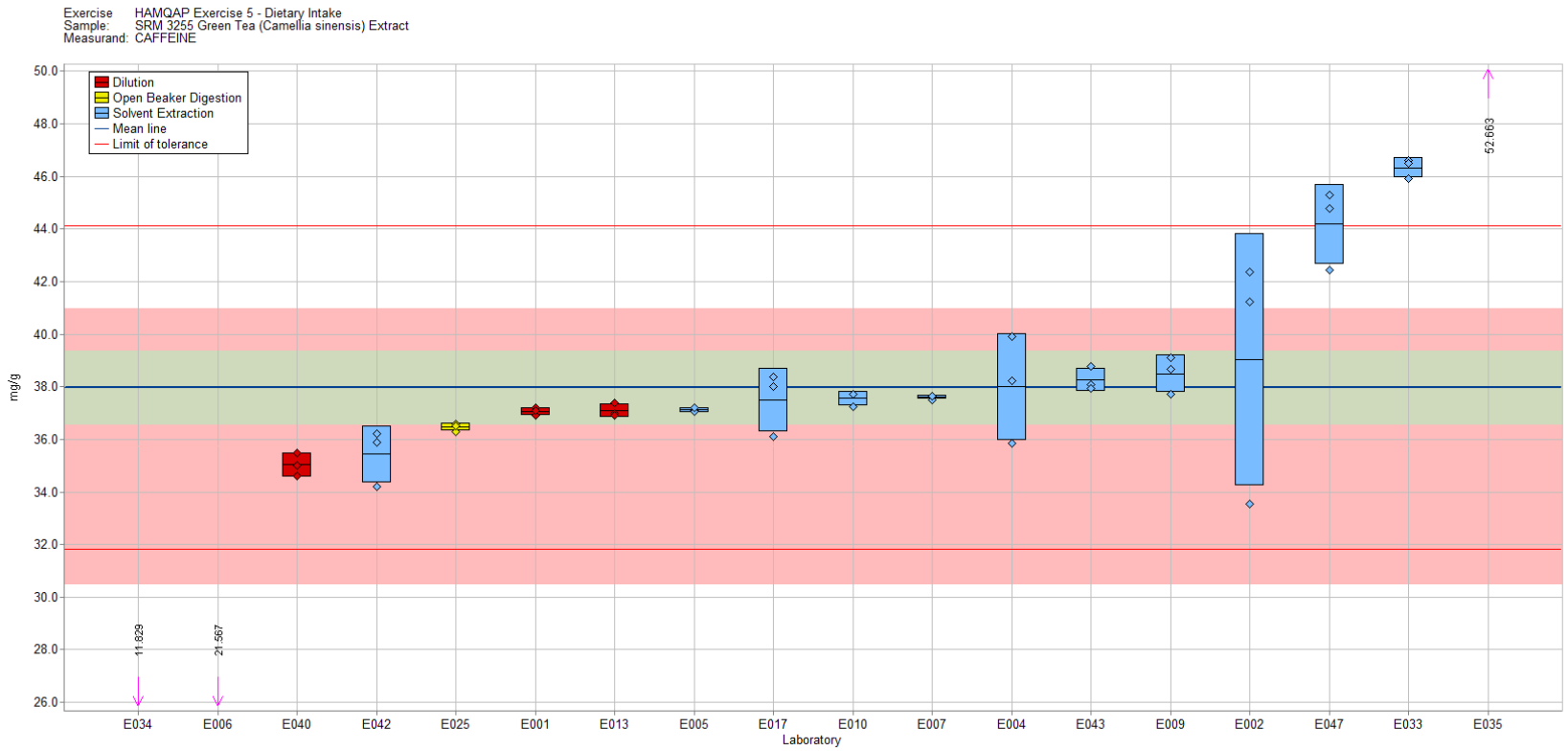

Figure 7-2. Caffeine in SRM 3255 Green Tea (*Camellia sinensis*) Extract (data summary view – sample preparation method). In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation 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, $|Z_{\text{NIST}}| \leq 2$.

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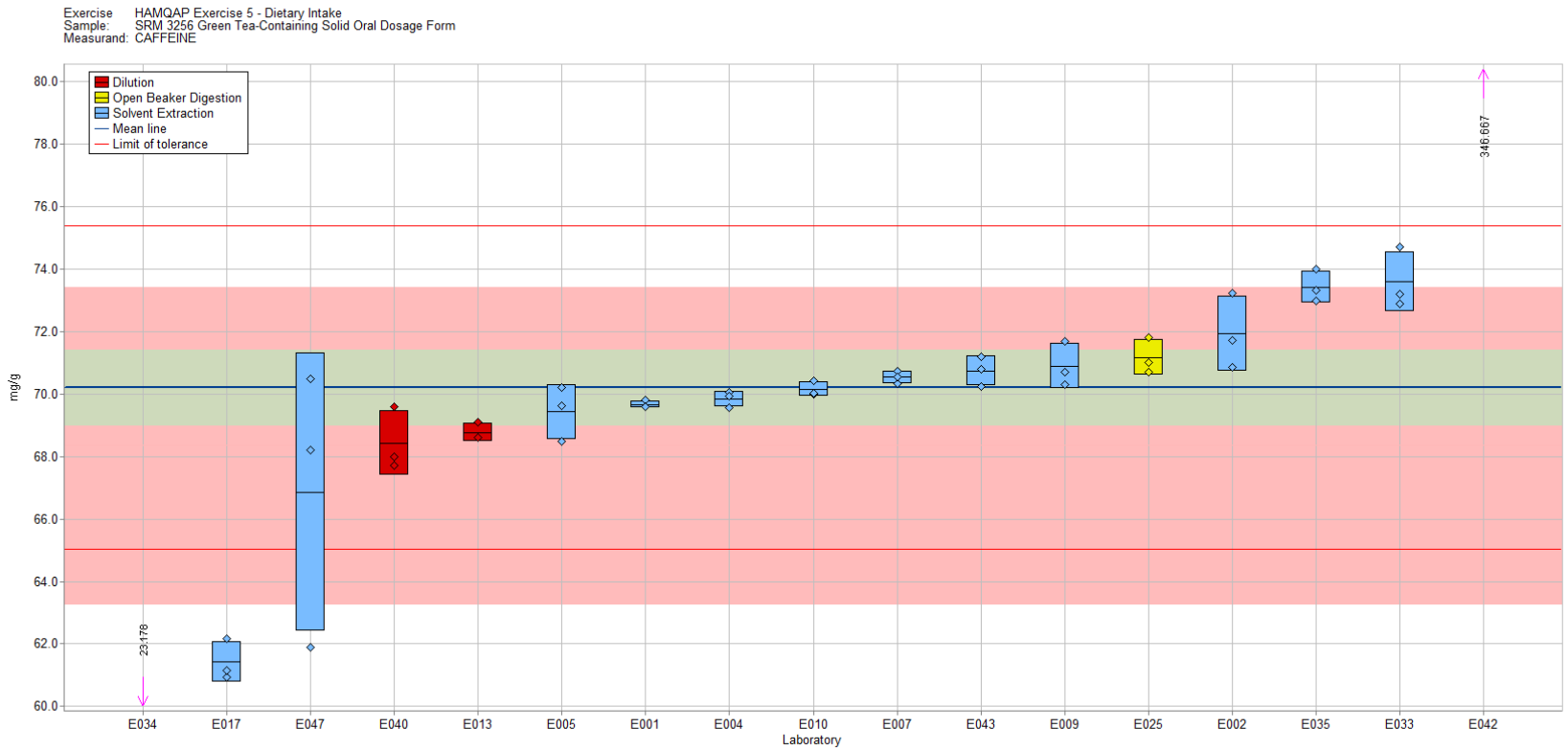

Figure 7-3. Caffeine in SRM 3256 Green Tea-Containing SODF (data summary view – sample preparation method). In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method 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, $|Z_{\text{NIST}}| \leq 2$.

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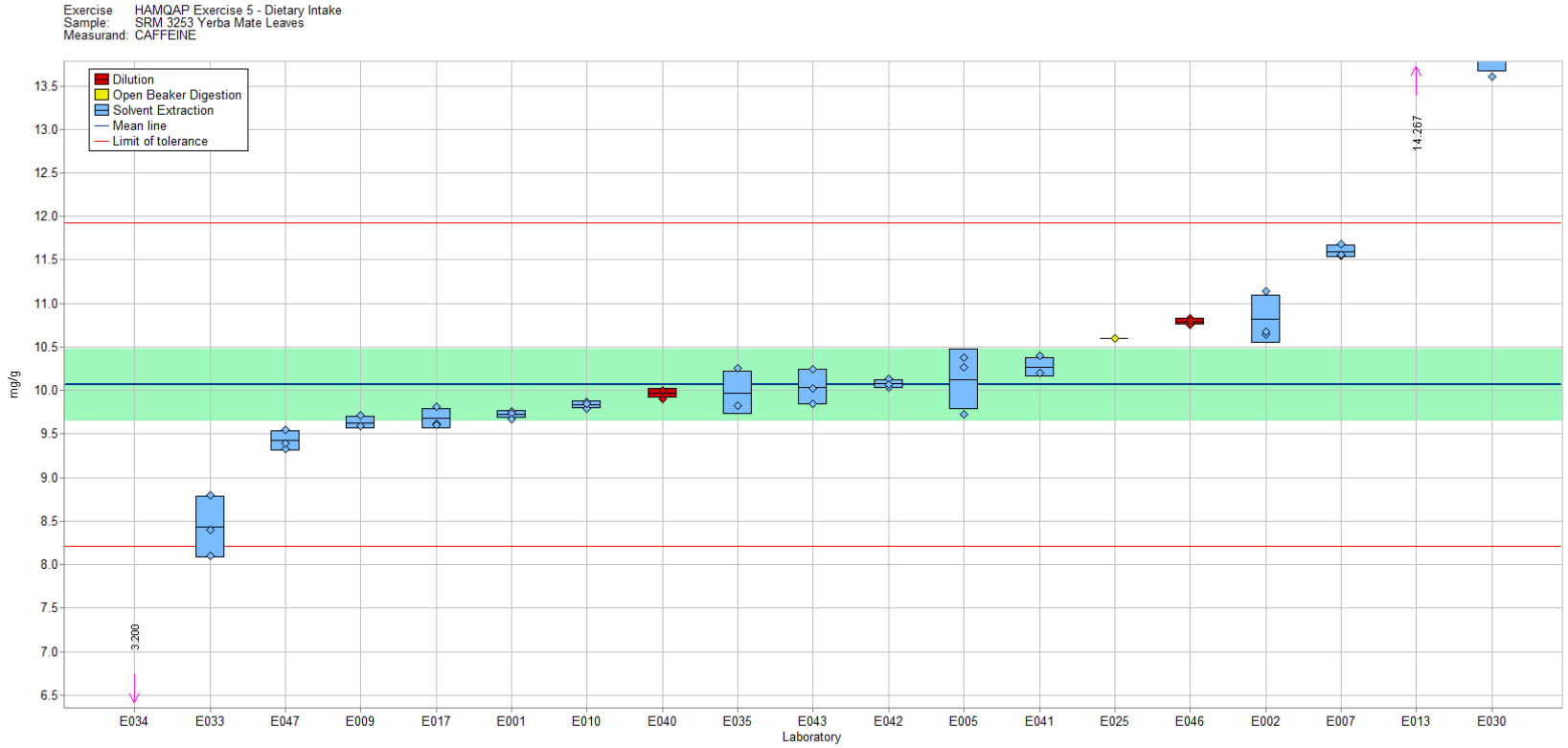

Figure 7-4. Caffeine in SRM 3253 Yerba Mate Leaves (data summary view – sample preparation method). In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The solid blue line represents the consensus mean, and the green shaded region 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 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.

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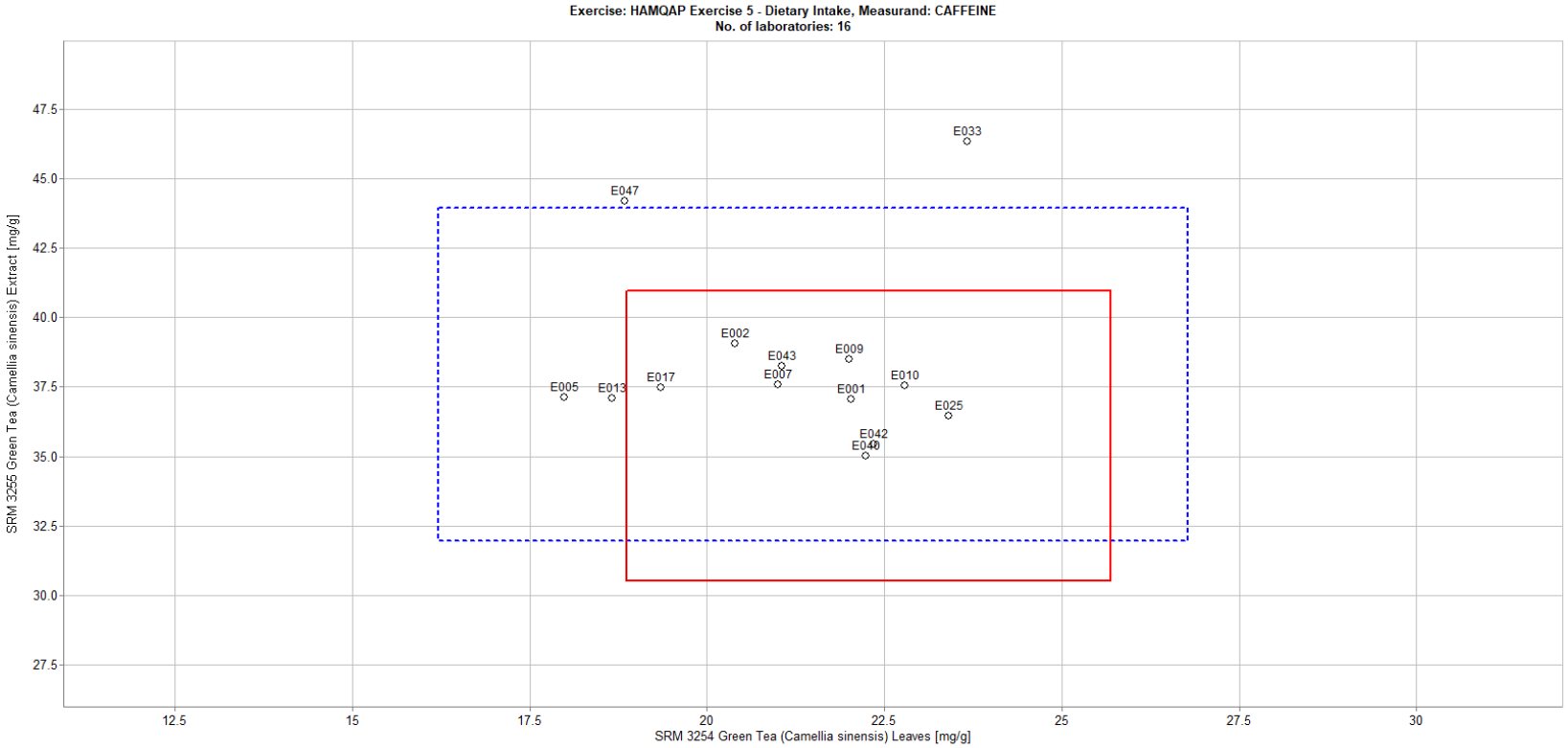

Figure 7-5. Laboratory means for caffeine in SRM 3254 Green Tea (*Camellia sinensis*) Leaves and SRM 3255 Green Tea (*Camellia sinensis*) Extract (sample/sample comparison view). In this view, the individual laboratory mean for one sample (SRM 3254) is compared to the individual laboratory mean for a second sample (SRM 3255). The solid red box represents the NIST range of tolerance for the two samples, SRM 3254 (x-axis) and SRM 3255 (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 3254 (x-axis) and SRM 3255 (y-axis), calculated as the values above and below the consensus means that result in an acceptable Z'_{comm} score, $|Z'_{\mathrm{comm}}| \leq 2$.

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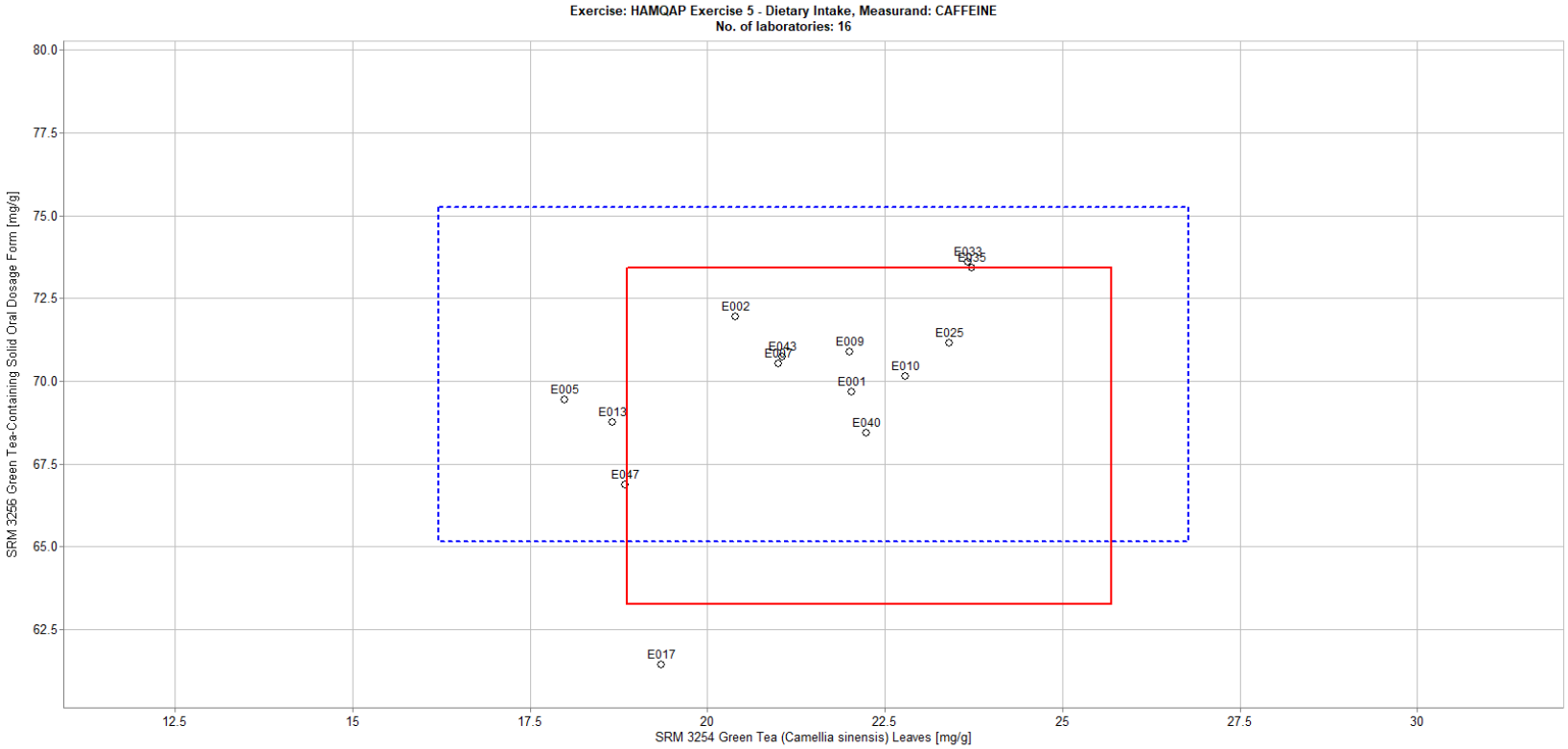

Figure 7-6. Laboratory means for caffeine in SRM 3254 Green Tea (*Camellia sinensis*) Leaves and SRM 3256 Green Tea (*Camellia sinensis*) SODF (sample/sample comparison view). In this view, the individual laboratory mean for one sample (SRM 3254) is compared to the individual laboratory mean for a second sample (SRM 3256). The solid red box represents the NIST range of tolerance for the two samples, SRM 3254 (x-axis) and SRM 3256 (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 3254 (x-axis) and SRM 3256 (y-axis), calculated as the values above and below the consensus means that result in an acceptable Z'_{comm} score, $|Z'_{\mathrm{comm}}| \leq 2$.

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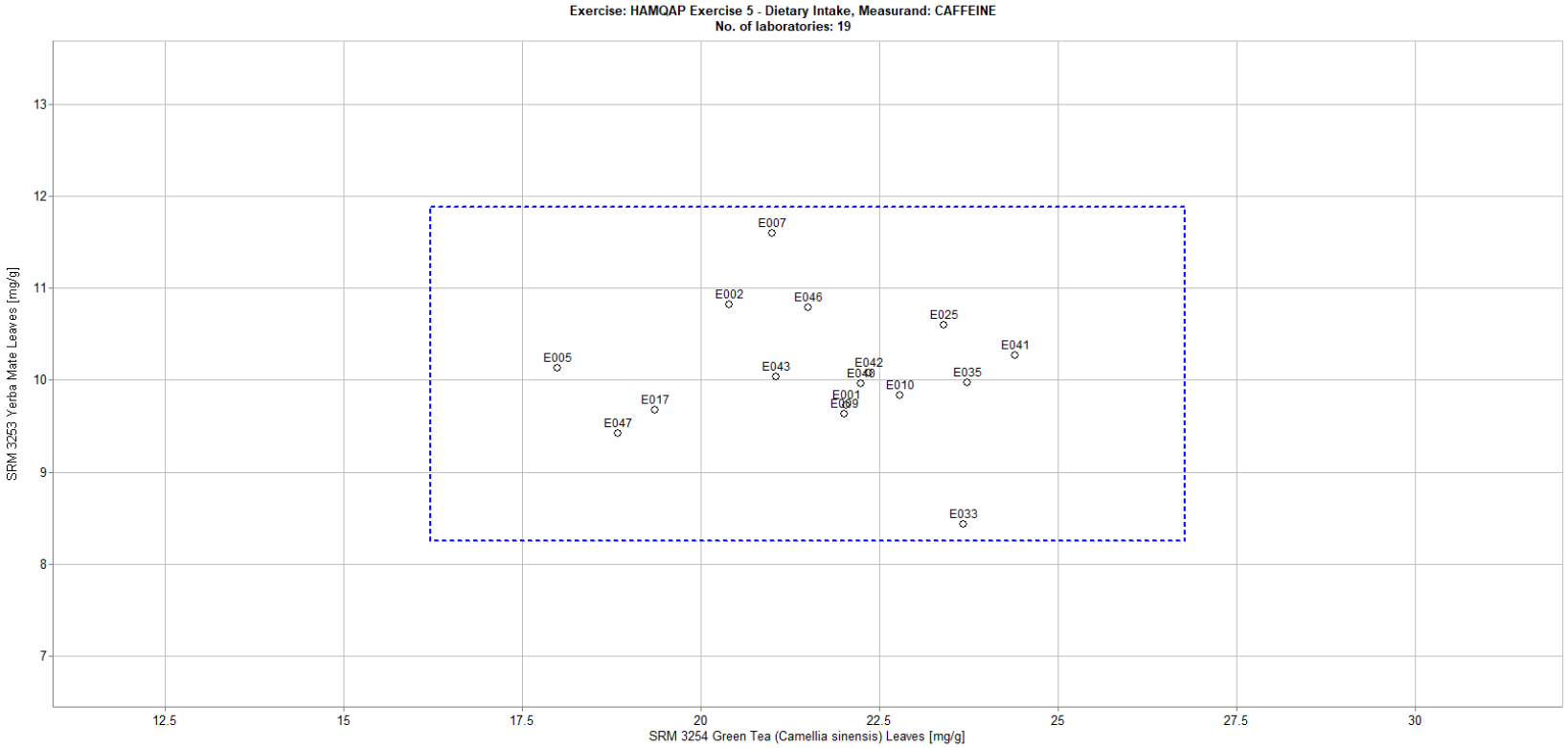

Figure 7-7. Laboratory means for caffeine in SRM 3254 Green Tea (*Camellia sinensis*) Leaves and SRM 3253 Yerba Mate Leaves (sample/sample comparison view). In this view, the individual laboratory mean for one sample (SRM 3254) is compared to the individual laboratory mean for a second sample (SRM 3253). The dotted blue box represents the consensus range of tolerance for SRM 3254 (x-axis) and SRM 3253 (y-axis), calculated as the values above and below the consensus means that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$.

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Table 7-3. Data summary table for theobromine in green tea and yerba mate. Data points highlighted in red have been flagged as potential outliers (e.g., Grubb and/or Cochran) by the NIST software package.

		Theobromine																			
		SRM 3254 Green Tea (*Camellia sinensis*) Leaves (mg/g)					SRM 3255 Green Tea (*Camellia sinensis*) Extract (mg/g)					SRM 3256 Green Tea-Containing Solid Oral Dosage Form (mg/g)					SRM 3253 Yerba Mate Leaves (mg/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target				0.439	0.099				0.840	0.15				1.02	0.29					
	E001	< 0.100	< 0.100	< 0.100	< 0.100		< 0.100	< 0.100	< 0.100	< 0.100		< 0.100	< 0.100	< 0.100	< 0.100		< 0.100	< 0.100	< 0.100	< 0.100	
	E002	0.68	0.58	0.65	0.637	0.051	0.86	0.89	0.91	0.887	0.025	0.97	0.95	0.89	0.937	0.042	1.3	1.19	1.4	1.297	0.105
	E004																				
	E005	1	1.04	1.05	1.030	0.026	2.31	2.38	2.63	2.440	0.168	1.84	1.96	2.01	1.937	0.087	1.34	1.4	1.43	1.390	0.046
	E006						0.418	0.419	0.425	0.421	0.004										
	E009	0.071	0.069	0.07	0.070	0.001						0.97	1.01	1.19	1.057	0.117	1.32	1.34	1.32	1.327	0.012
	E010	0.441	0.4394	0.4528	0.444	0.007	0.9714	0.9765	1.0061	0.985	0.019	1.0997	1.085	1.0801	1.088	0.010	1.4847	1.4776	1.4785	1.480	0.004
	E013	0.369	0.0366	0.362	0.256	0.190	0.89	0.87	0.886	0.882	0.011	0.975	0.963	0.963	0.967	0.007	1.39	1.36	1.35	1.367	0.021
	E017	0.548	0.53	0.515	0.531	0.017	1.093	1.094	1.107	1.098	0.008	1.228	0.993	1.106	1.109	0.118	1.81	1.847	1.84	1.832	0.020
	E020																				
	E021																				
	E022																				
	E025	0.492	0.48	0.488	0.487	0.006	0.929	0.92	0.931	0.927	0.006	1.26	1.25	1.28	1.263	0.015	1.44	1.44	1.44	1.440	0.000
	E030	0.297	0.3	0.331	0.309	0.019											1.14	1.1	1.08	1.107	0.031
	E040																				
	E041	0.55	0.56	0.68	0.597	0.072											1.49	1.51	1.51	1.503	0.012
	E042																				
	E043	0.66	0.64	0.67	0.657	0.015	0.978	1.054	0.966	0.999	0.048	1.067	1.028	1.056	1.050	0.020	1.82	1.78	1.75	1.783	0.035
	E046																				
	E047	0.41	0.37		0.390	0.028	0.57		0.57	0.570	0.000		0.93	1	0.965	0.049		1.62	1.64	1.630	0.014
Community Results		Consensus Mean			0.479		Consensus Mean			0.852		Consensus Mean			1.055		Consensus Mean			1.469	
		Consensus Standard Deviation			0.074		Consensus Standard Deviation			0.085		Consensus Standard Deviation			0.051		Consensus Standard Deviation			0.071	
		Maximum			1.030		Maximum			2.440		Maximum			1.937		Maximum			1.832	
		Minimum			0.070		Minimum			0.421		Minimum			0.937		Minimum			1.107	
		N			11		N			9		N			9		N			11	

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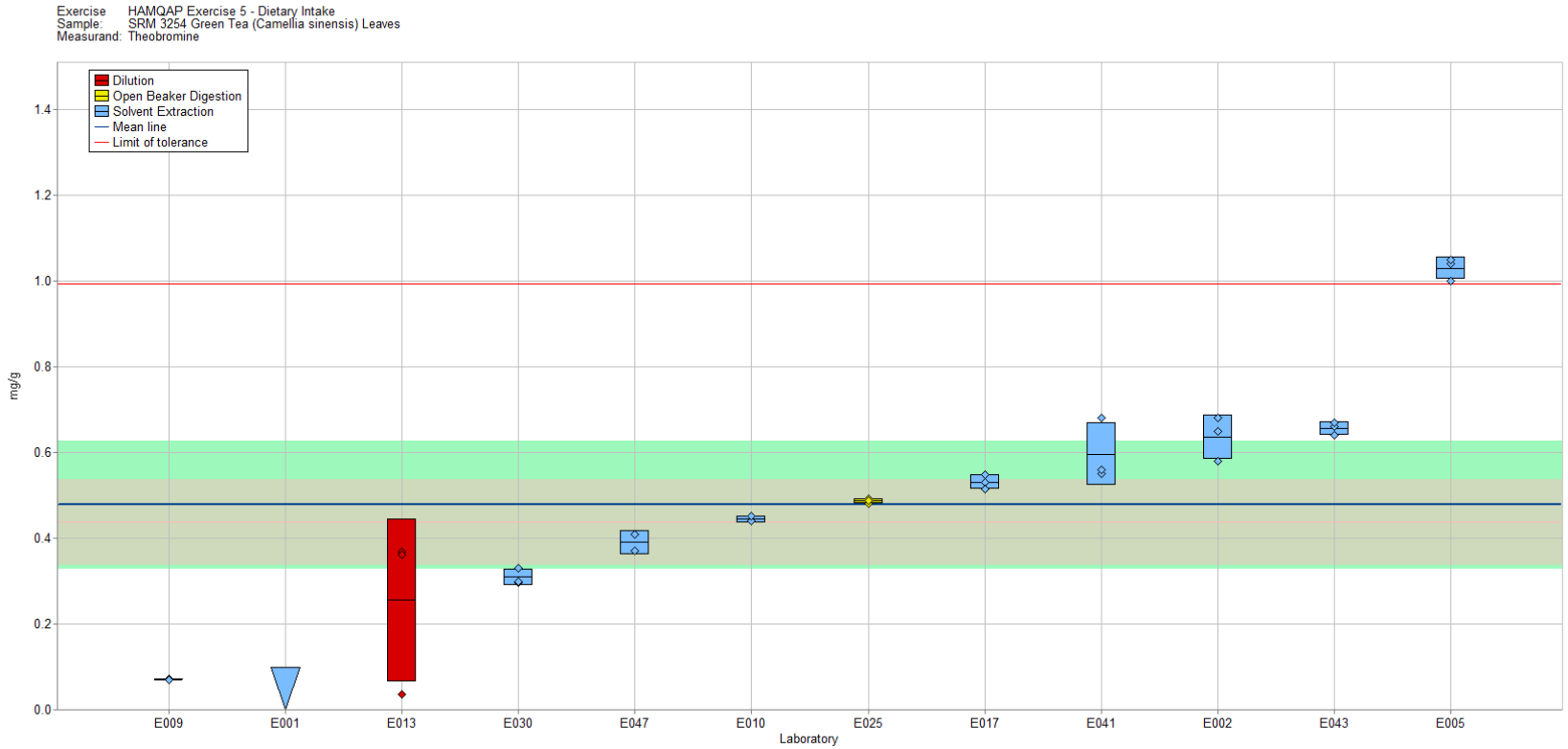

Figure 7-8. Theobromine in SRM 3254 Green Tea (*Camellia sinensis*) Leaves (data summary view – sample preparation method). In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red line represents the consensus range of tolerance, calculated as the values above the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\mathrm{comm}}| \leq 2$, with the lower limit set at zero. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty (U_{NIST}) and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\mathrm{NIST}}| \leq 2$.

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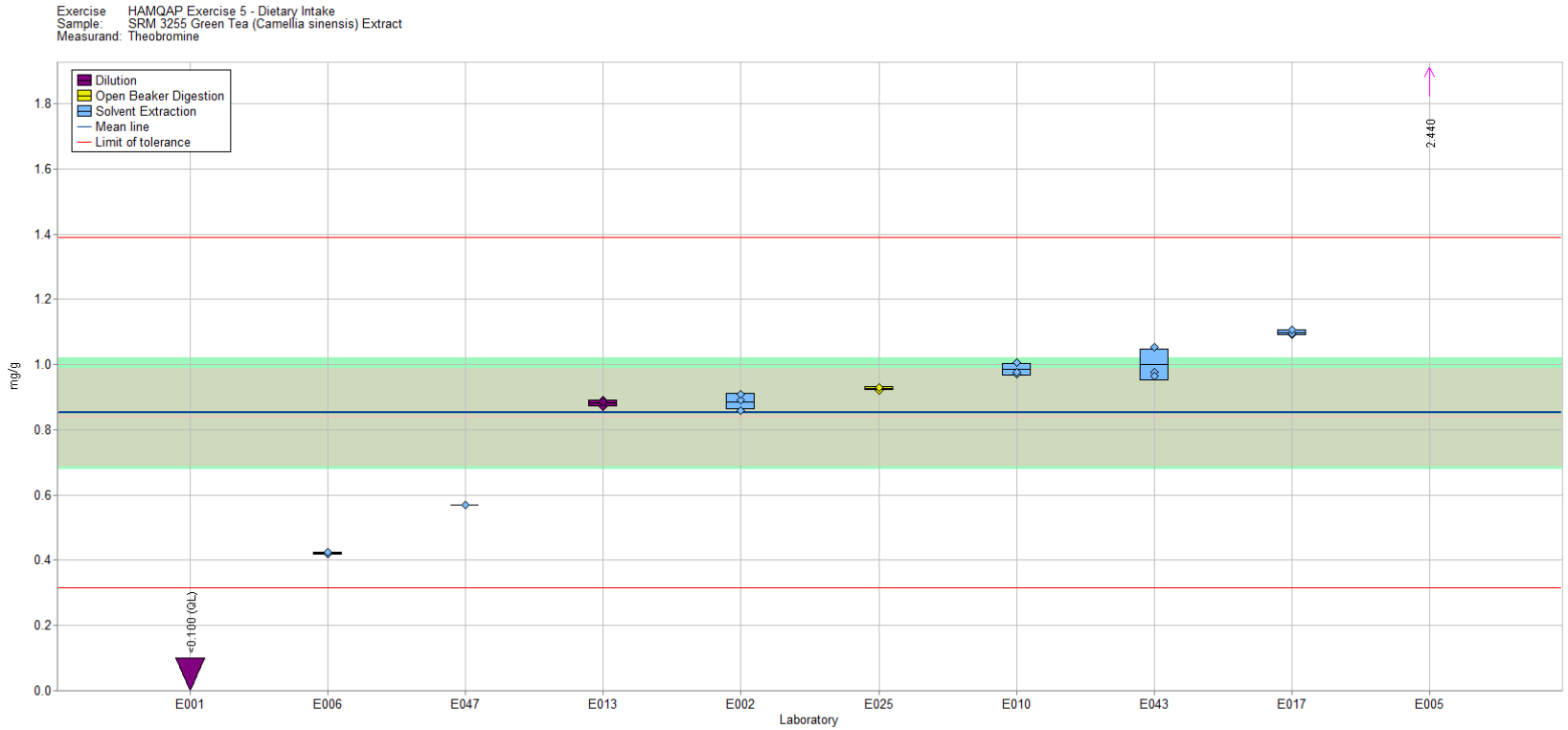

Figure 7-9. Theobromine in SRM 3255 Green Tea (*Camellia sinensis*) Extract (data summary view – sample preparation method). In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample 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, $|Z_{\text{NIST}}| \leq 2$.

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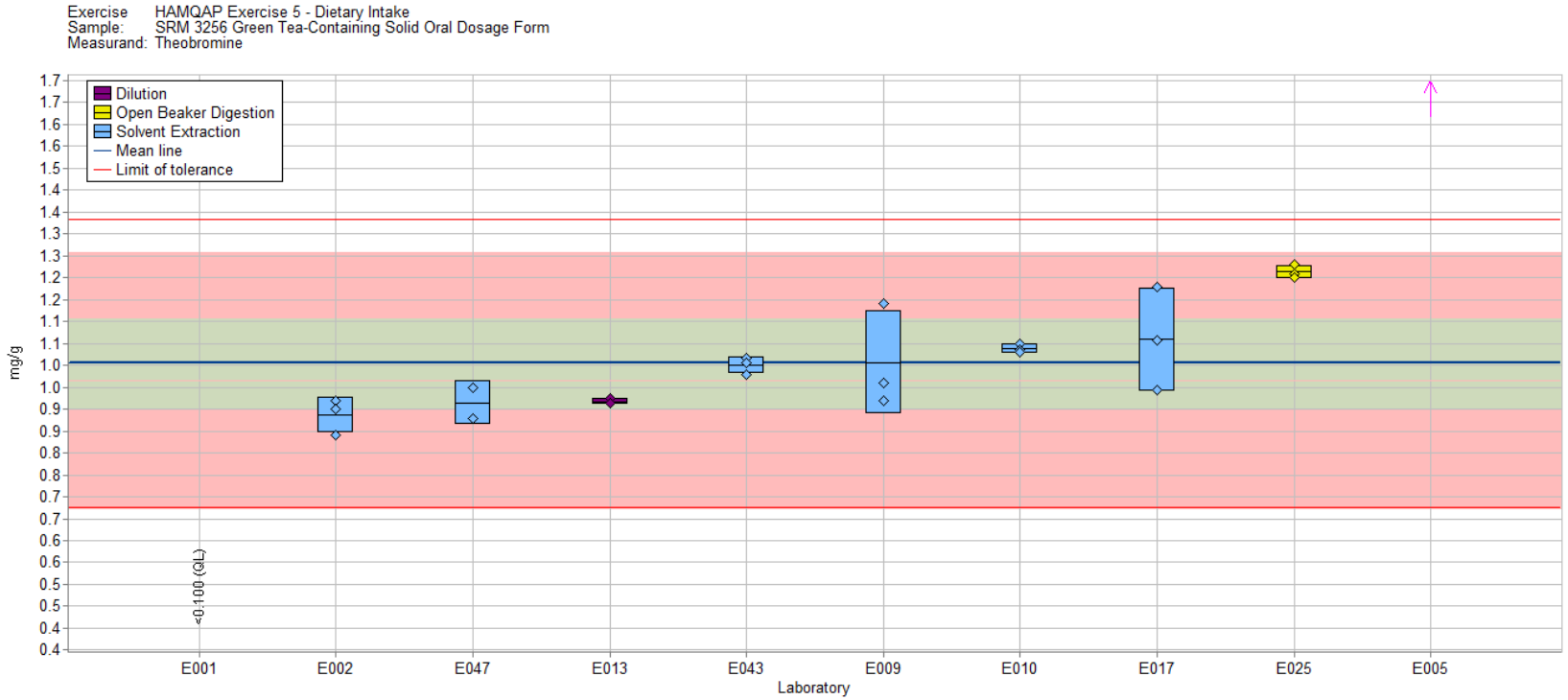

Figure 7-10. Theobromine in SRM 3256 Green Tea-Containing SODF (data summary view – sample preparation method). In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation 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, $|Z_{\text{NIST}}| \leq 2$.

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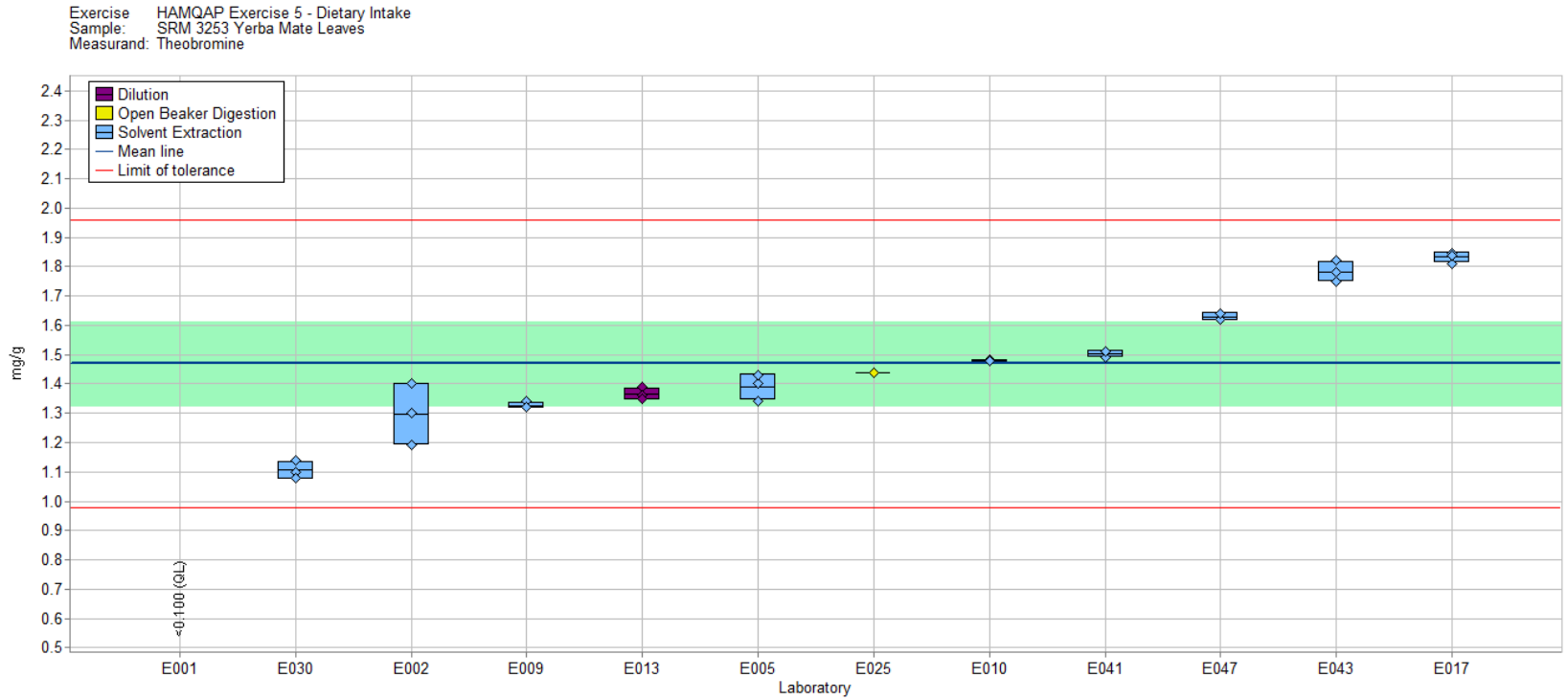

Figure 7-11. Theobromine in SRM 3253 Yerba Mate Leaves (data summary view – sample preparation method). In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The solid blue line represents the consensus mean, and the green shaded 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 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.

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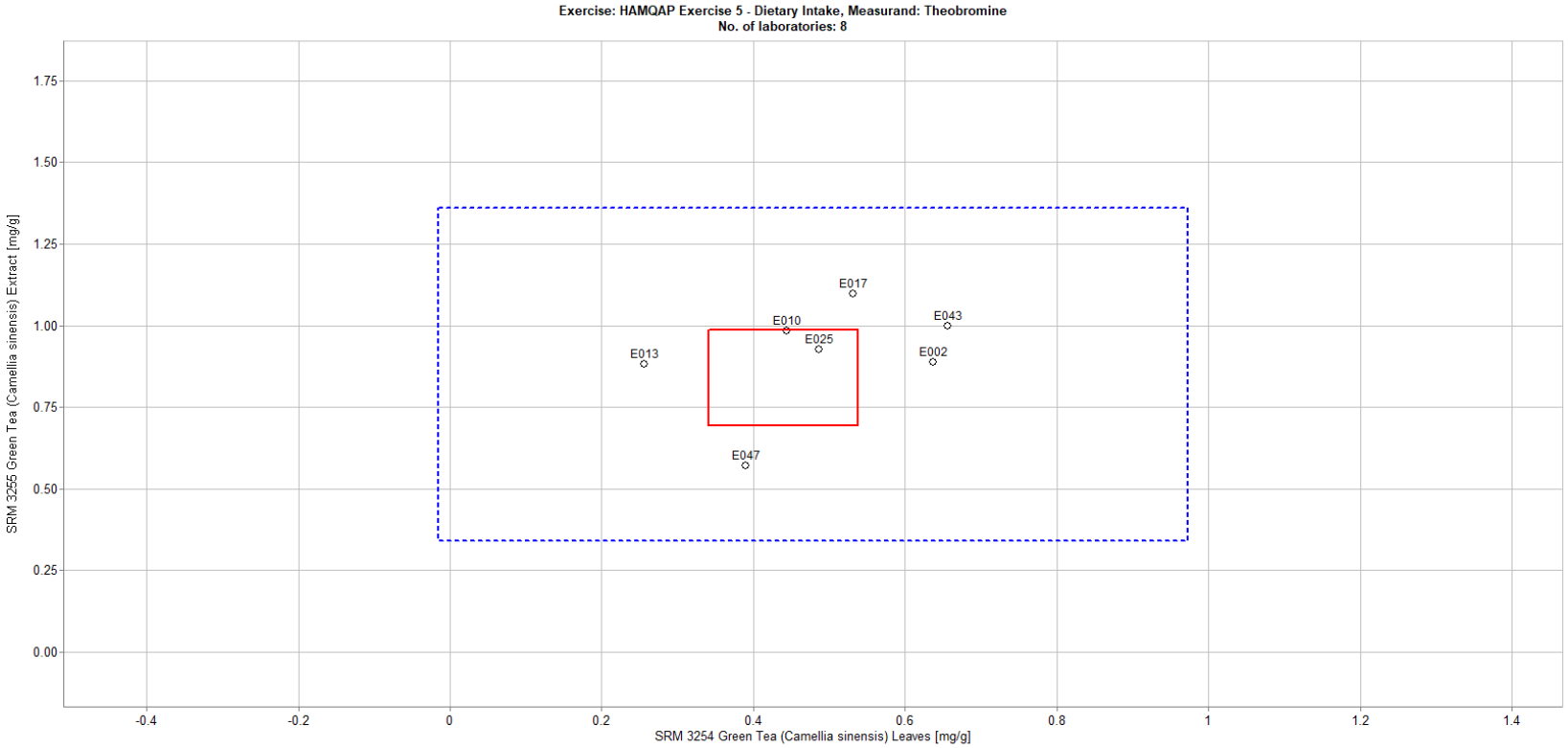

Figure 7-12. Laboratory means for theobromine in SRM 3254 Green Tea (*Camellia sinensis*) Leaves and SRM 3255 Green Tea (*Camellia sinensis*) Extract (sample/sample comparison view). In this view, the individual laboratory mean for one sample (SRM 3254) is compared to the individual laboratory mean for a second sample (SRM 3255). The solid red box represents the NIST range of tolerance for the two samples, SRM 3254 (x-axis) and SRM 3255 (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 3254 (x-axis) and SRM 3255 (y-axis), calculated as the values above and below the consensus means that result in an acceptable Z'_{comm} score, $|Z'_{\mathrm{comm}}| \leq 2$.

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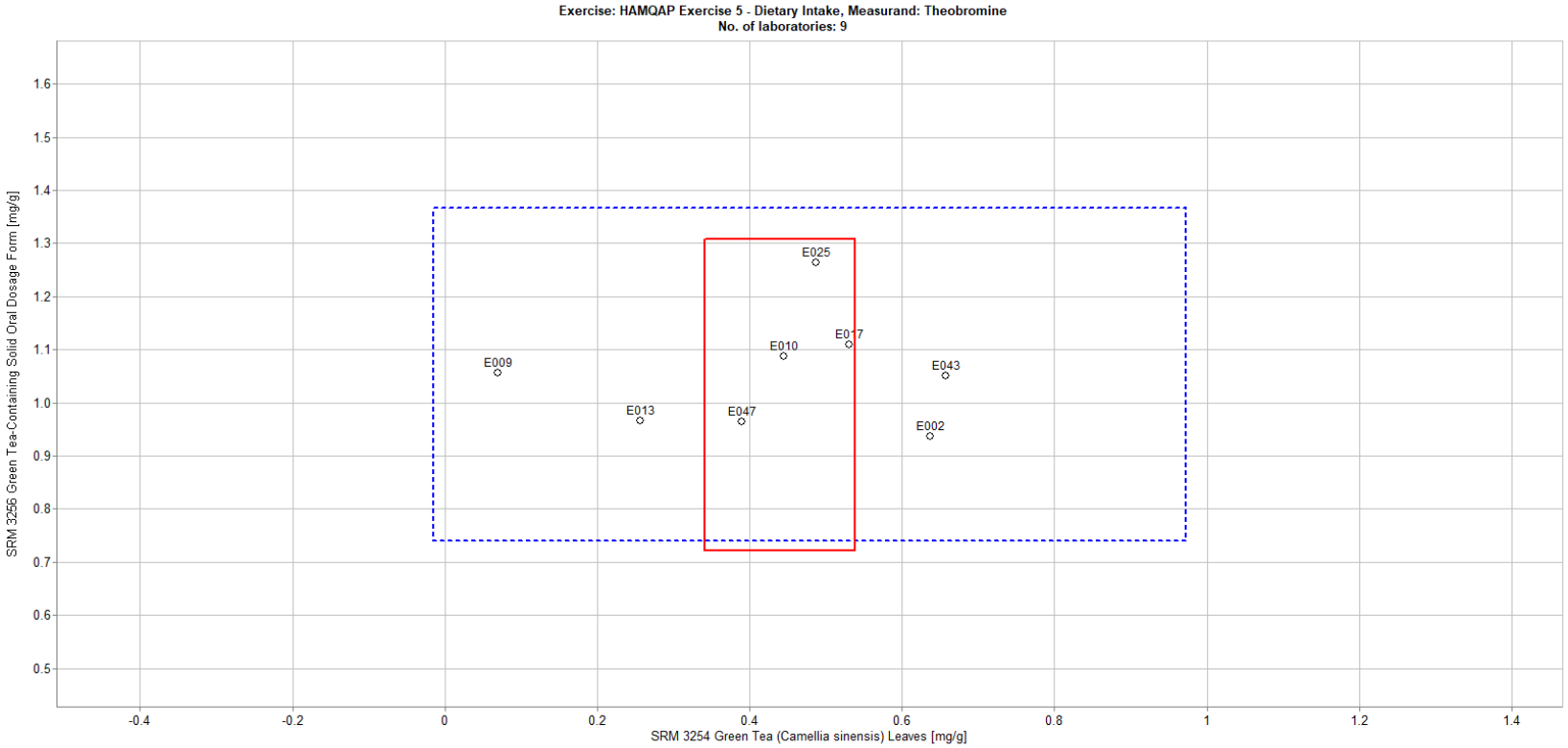

Figure 7-13. Laboratory means for theobromine in SRM 3254 Green Tea (*Camellia sinensis*) Leaves and SRM 3256 Green Tea (*Camellia sinensis*) SODF (sample/sample comparison view). In this view, the individual laboratory mean for one sample (SRM 3254) is compared to the individual laboratory mean for a second sample (SRM 3256). The solid red box represents the NIST range of tolerance for the two samples, SRM 3254 (x-axis) and SRM 3256 (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 3254 (x-axis) and SRM 3256 (y-axis), calculated as the values above and below the consensus means that result in an acceptable Z'_{comm} score, $|Z'_{\mathrm{comm}}| \leq 2$.

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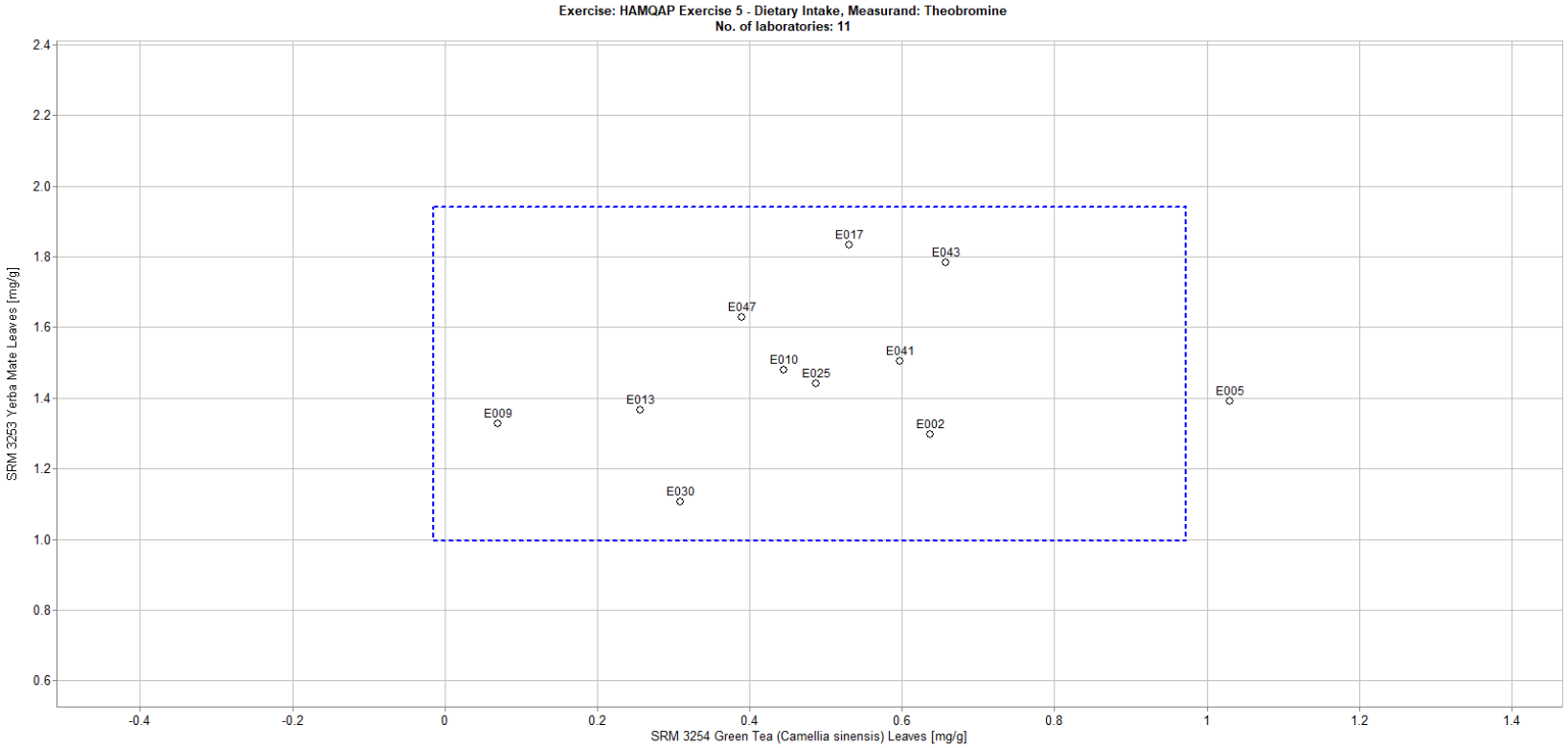

Figure 7-14. Laboratory means for theobromine in SRM 3254 Green Tea (*Camellia sinensis*) Leaves and SRM 3253 Yerba Mate Leaves (sample/sample comparison view). In this view, the individual laboratory mean for one sample (SRM 3254) is compared to the individual laboratory mean for a second sample (SRM 3253). The dotted blue box represents the consensus range of tolerance for SRM 3254 (x-axis) and SRM 3253 (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 7-4. Data summary table for theophylline in green tea and yerba mate.

		Theophylline																			
		SRM 3254 Green Tea (*Camellia sinensis*) Leaves (mg/g)					SRM 3255 Green Tea (*Camellia sinensis*) Extract (mg/g)					SRM 3256 Green Tea-Containing Solid Oral Dosage Form (mg/g)					SRM 3253 Yerba Mate Leaves (mg/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target									0.084	0.004				0.059	0.004					
	E001	0.4	0.417	0.417	0.411	0.010	0.774	0.772	0.753	0.766	0.012	1.04	1.09	1.03	1.053	0.032	1.43	1.45	1.4	1.43	0.03
	E002	1.18	1.3	1.32	1.267	0.076	2.6	3.44	3.66	3.233	0.559	2.75	2.71	2.68	2.713	0.035	2.97	2.99	2.72	2.89	0.15
	E009						< 0.09	< 0.09	< 0.09	< 0.09		< 0.09	< 0.09	< 0.09	< 0.09						
	E013																				
	E017																8.287	8.304	7.922	8.17	0.22
	E020																				
	E021																				
	E022																				
	E025	0.012	0.017	0.017	0.015	0.003	0.063	0.069	0.06	0.064	0.005	0.019	0.02	0.022	0.020	0.002					
	E030																				
	E040																				
	E043						0.116	0.112	0.117	0.115	0.003	0.078	0.078	0.072	0.076	0.003					
	E047			0.45	0.450			0.82		0.820		1.02			1.020		1.52			1.52	
Community Results		Consensus Mean			0.536		Consensus Mean			0.987		Consensus Mean			0.977		Consensus Mean			3.38	
		Consensus Standard Deviation			0.441		Consensus Standard Deviation			0.651		Consensus Standard Deviation			0.937		Consensus Standard Deviation			1.43	
		Maximum			1.267		Maximum			3.233		Maximum			2.713		Maximum			8.17	
		Minimum			0.015		Minimum			0.064		Minimum			0.020		Minimum			1.43	
		N			3		N			4		N			4		N			3	

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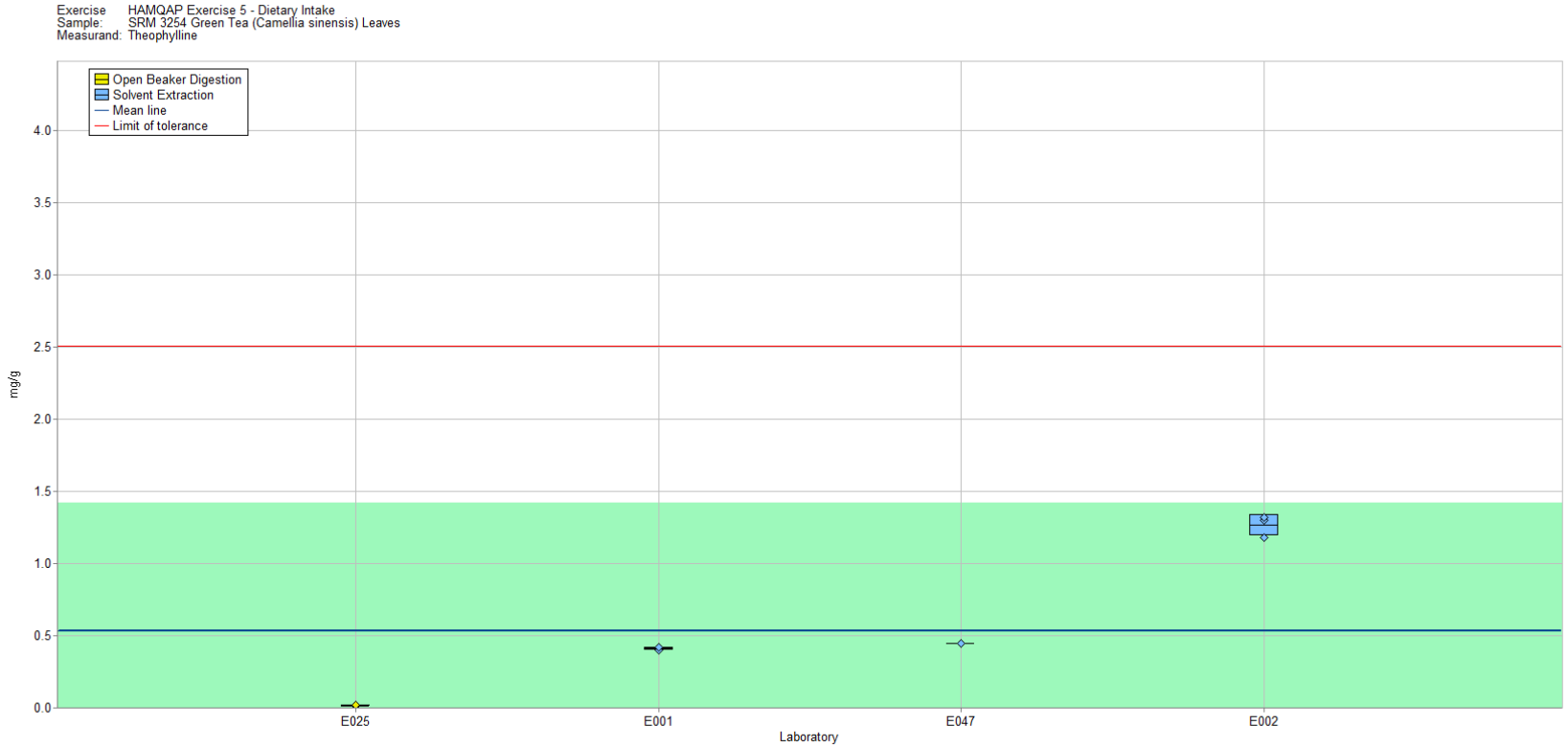

Figure 7-15. Theophylline in SRM 3254 Green Tea (*Camellia sinensis*) Leaves (data summary view – sample preparation method). In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red line represents the consensus range of tolerance, calculated as the values above the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$, with the lower limit set at zero. A NIST value has not been determined in this material.

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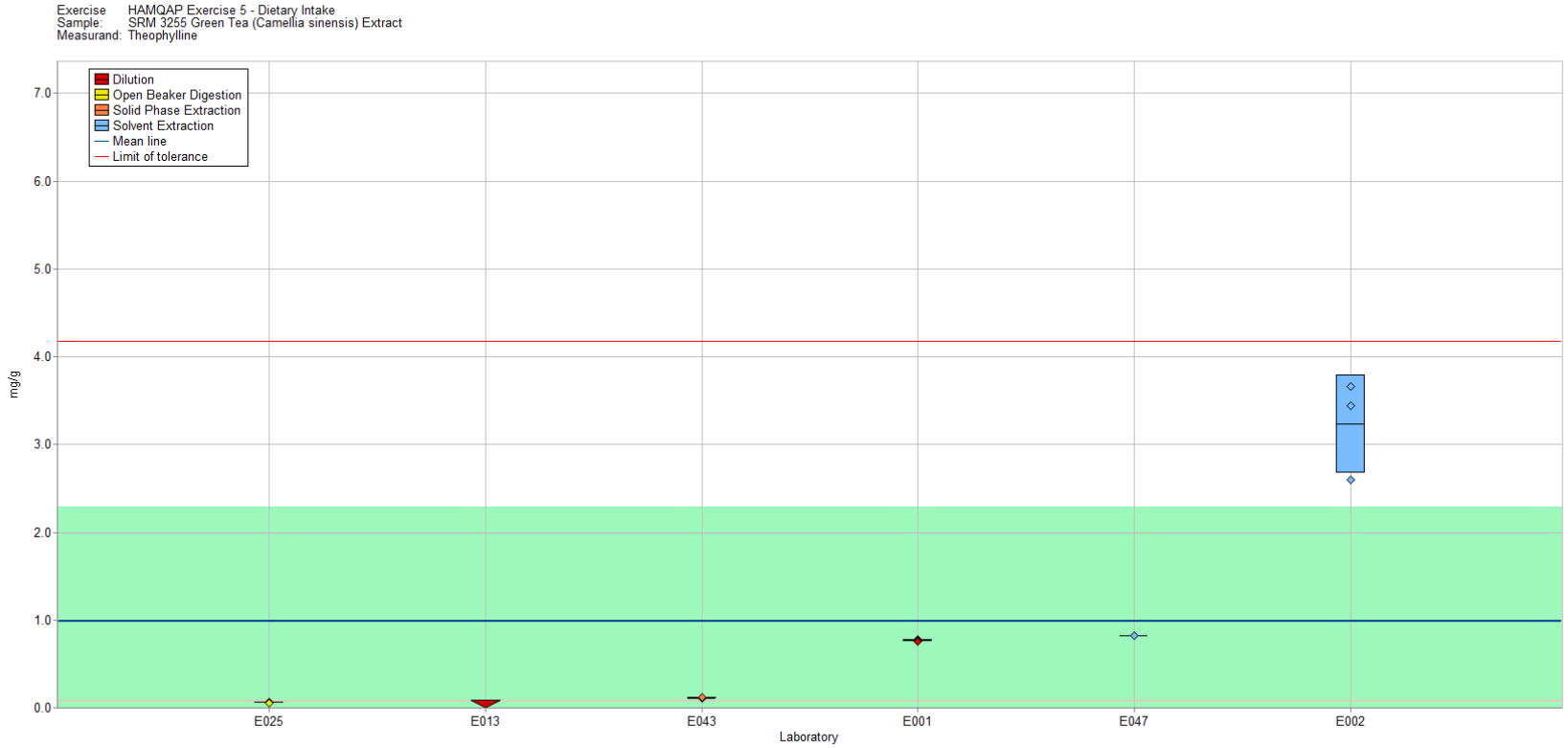

Figure 7-16. Theophylline in SRM 3255 Green Tea (*Camellia sinensis*) Extract (data summary view – sample preparation method). In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red line represents the consensus range of tolerance, calculated as the values above the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$, with the lower limit set at zero. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty (U_{NIST}) and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$.

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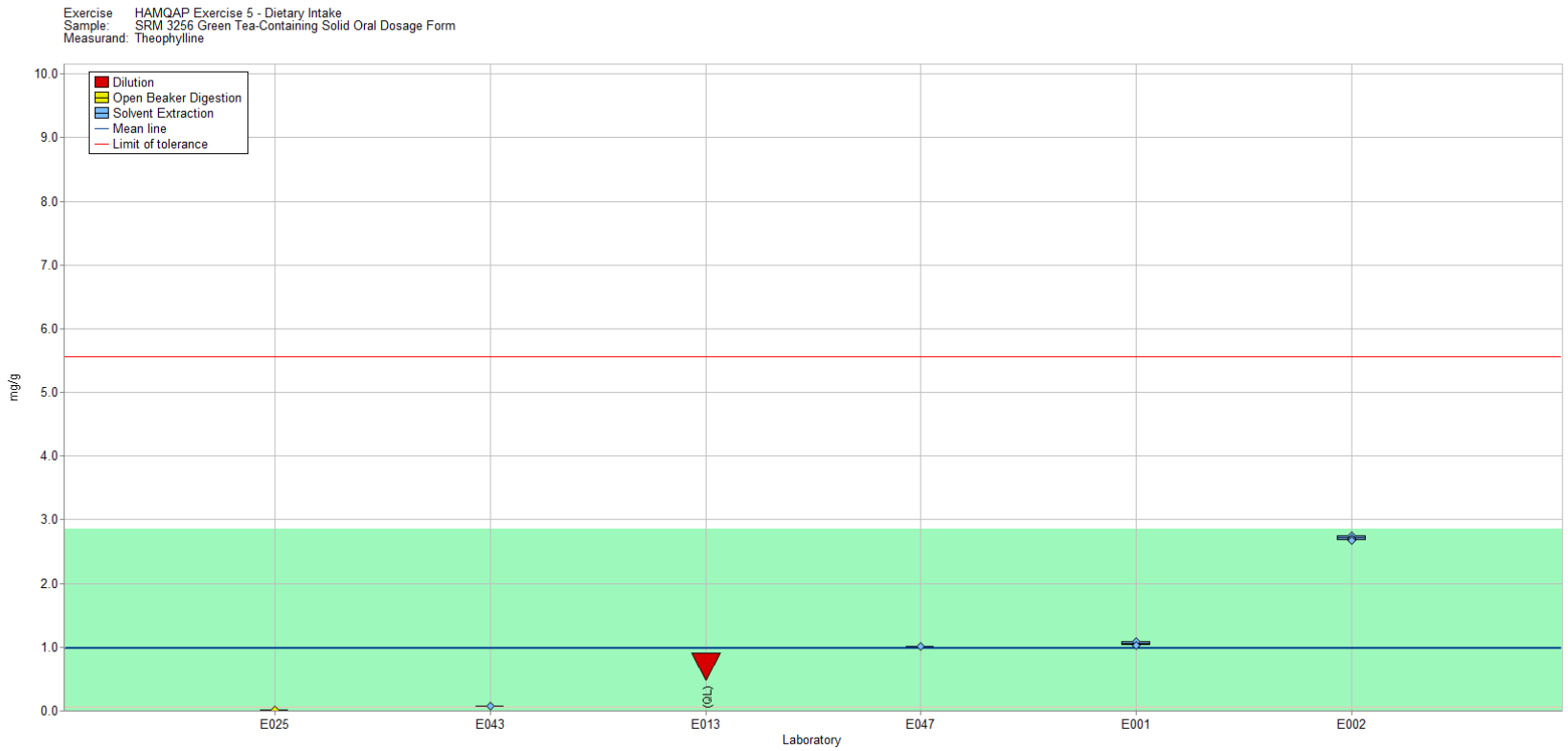

Figure 7-17. Theophylline in SRM 3256 Green Tea-Containing SODF (data summary view – sample preparation method). In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red line represents the consensus range of tolerance, calculated as the values above the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$, with the lower limit set at zero. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty (U_{NIST}) and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$.

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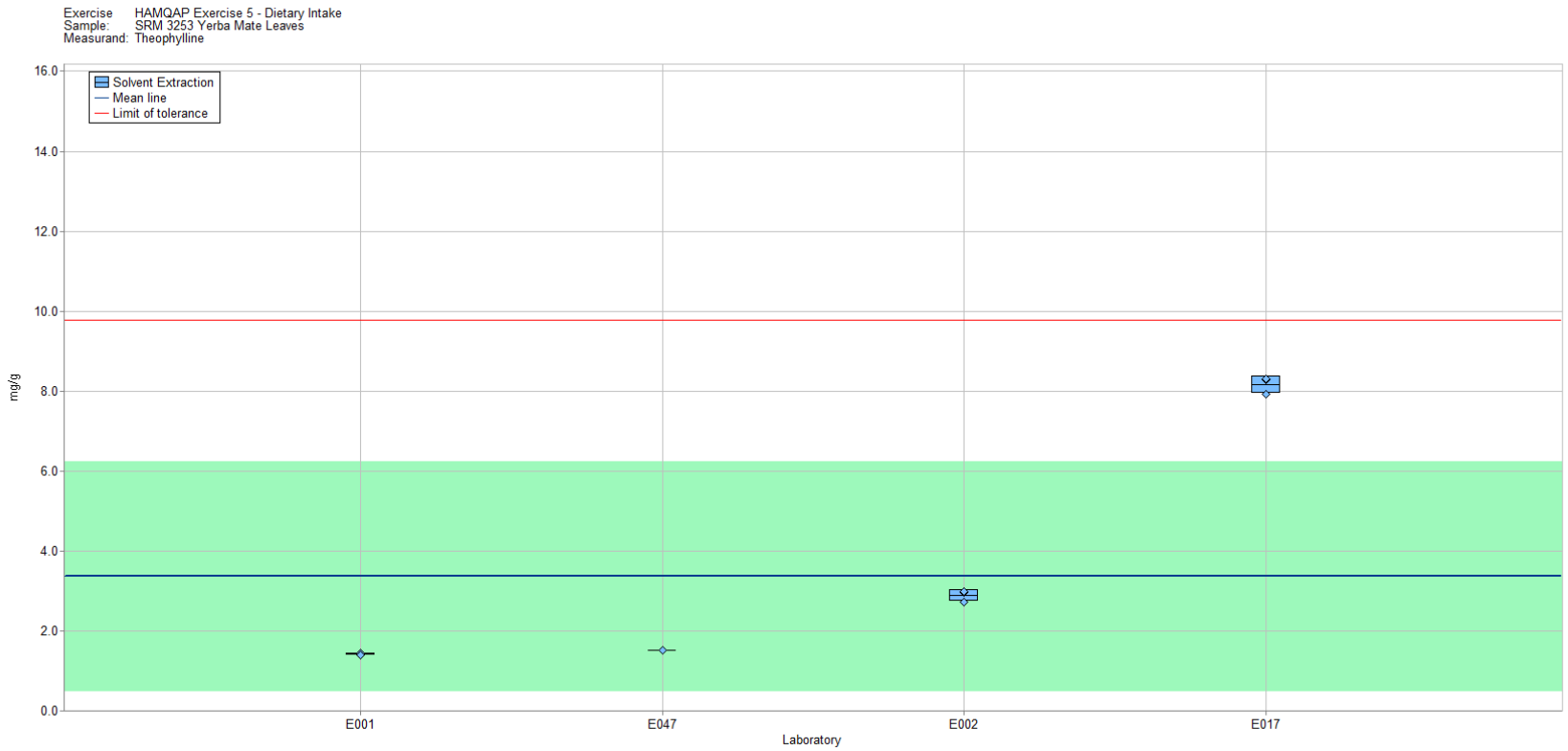

Figure 7-18. Theophylline in SRM 3253 Yerba Mate Leaves (data summary view – sample preparation method). In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The solid blue line represents the consensus mean, and the green shaded 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 the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$, with the lower limit set at zero. A NIST value has not been determined in this material.

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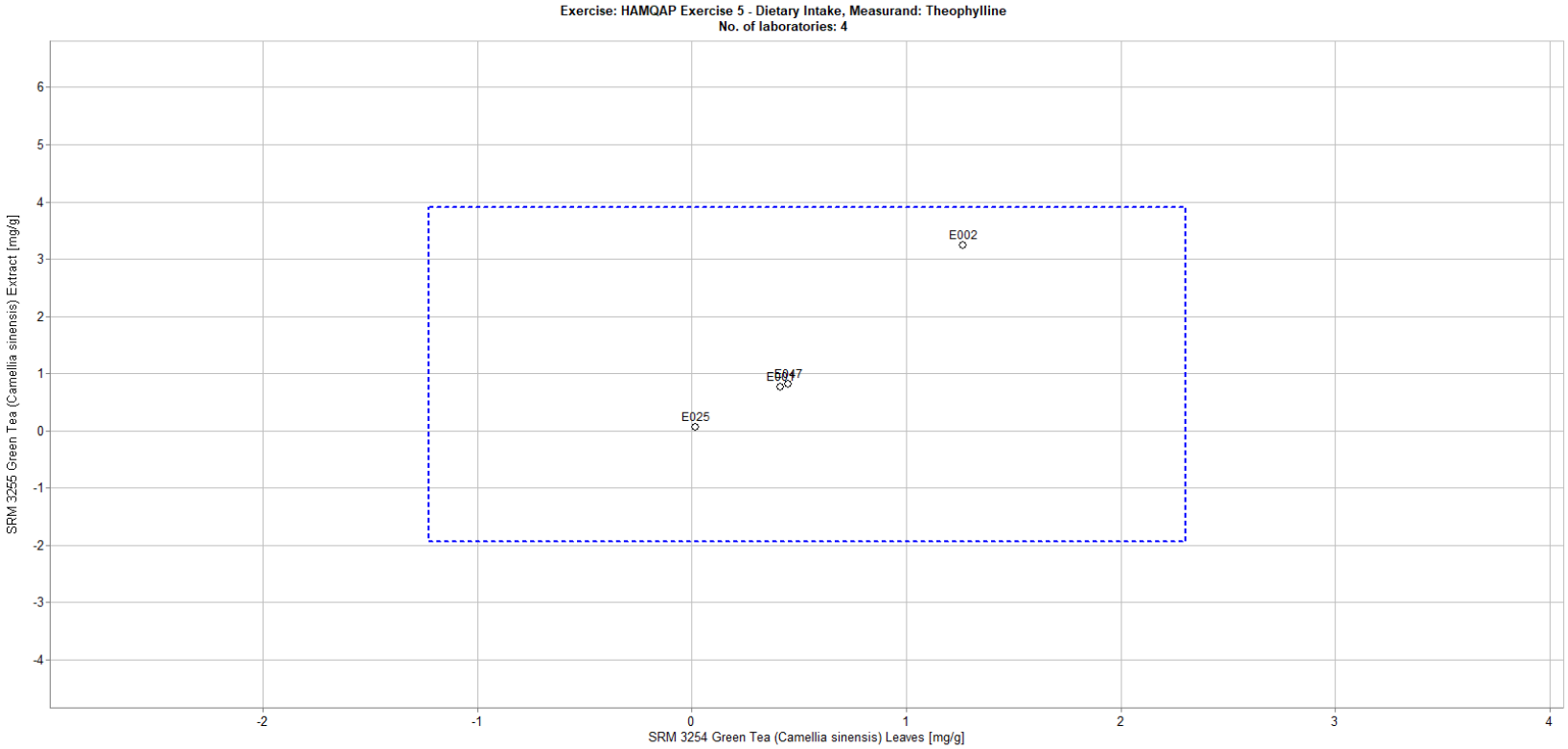

Figure 7-19. Laboratory means for theophylline in SRM 3254 Green Tea (*Camellia sinensis*) Leaves and SRM 3255 Green Tea (*Camellia sinensis*) Extract (sample/sample comparison view). In this view, the individual laboratory mean for one sample (SRM 3254) is compared to the individual laboratory mean for a second sample (SRM 3255). The dotted blue box represents the consensus range of tolerance for SRM 3254 (x-axis) and SRM 3255 (y-axis), calculated as the values above and below the consensus means that result in an acceptable Z'_{comm} score, $|Z'_{\mathrm{comm}}| \leq 2$.

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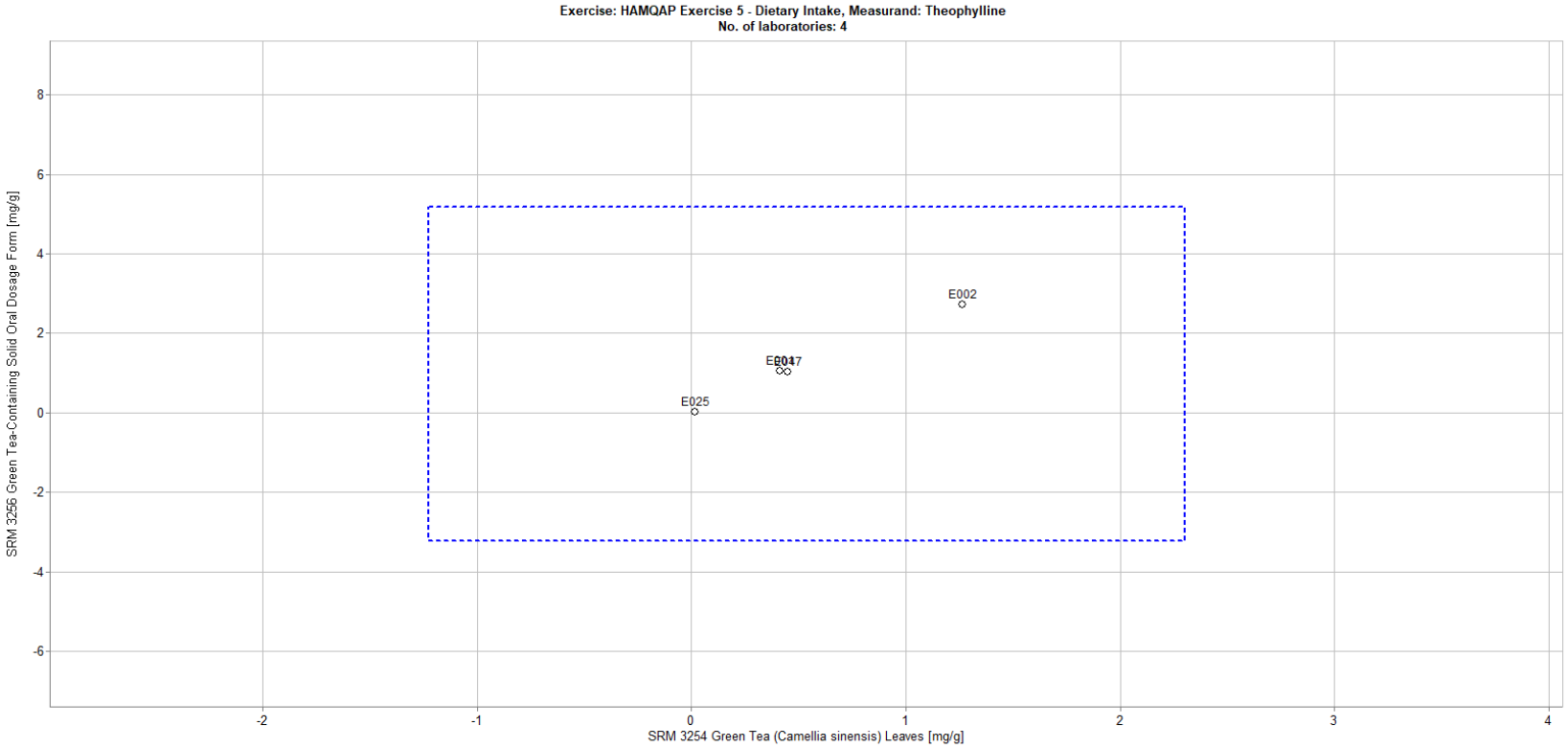

Figure 7-20. Laboratory means for theophylline in SRM 3254 Green Tea (*Camellia sinensis*) Leaves and SRM 3256 Green Tea (*Camellia sinensis*) SODF (sample/sample comparison view). In this view, the individual laboratory mean for one sample (SRM 3254) is compared to the individual laboratory mean for a second sample (SRM 3256). The dotted blue box represents the consensus range of tolerance for SRM 3254 (x-axis) and SRM 3256 (y-axis), calculated as the values above and below the consensus means that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$.

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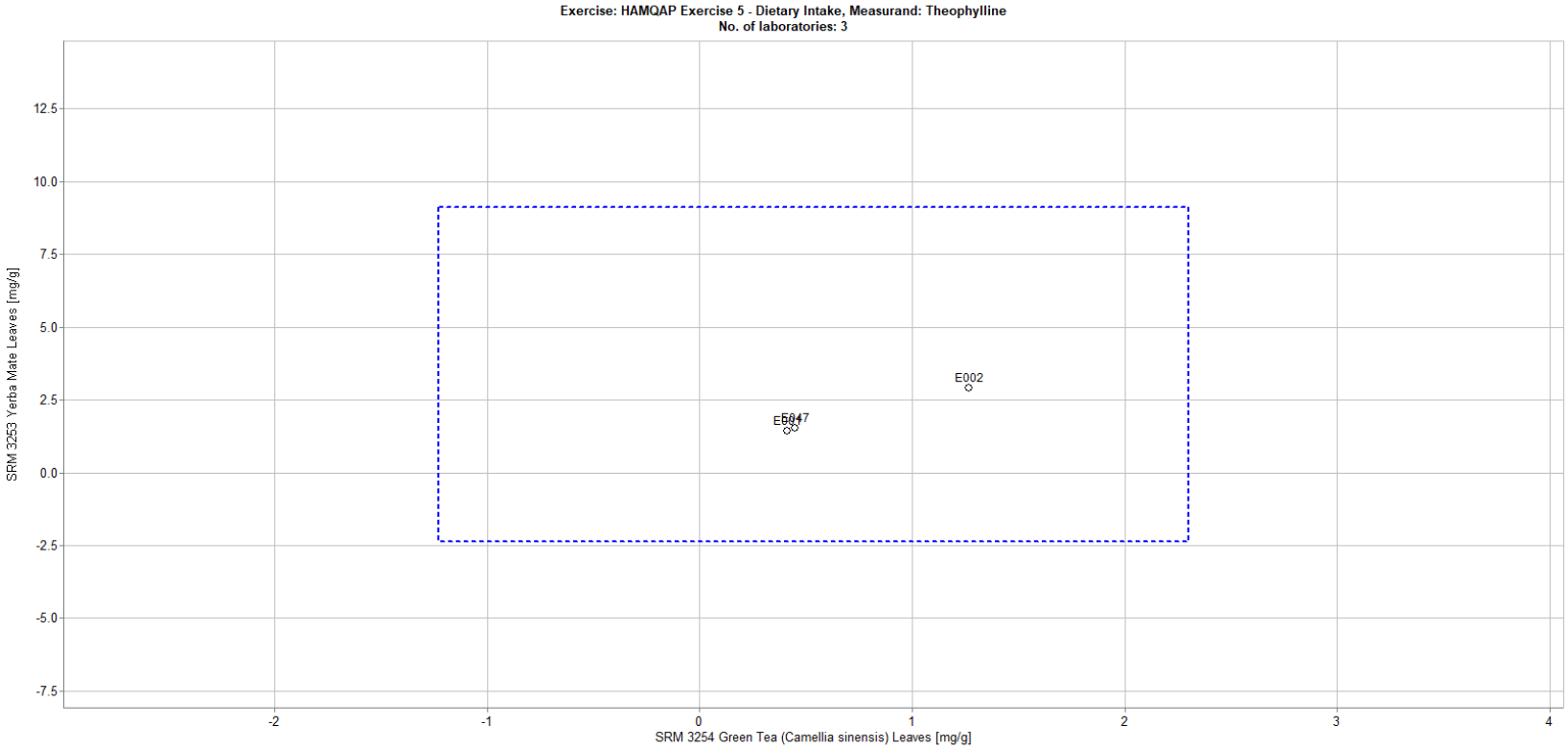

Figure 7-21. Laboratory means for theophylline in SRM 3254 Green Tea (*Camellia sinensis*) Leaves and SRM 3253 Yerba Mate Leaves (sample/sample comparison view). In this view, the individual laboratory mean for one sample (SRM 3254) is compared to the individual laboratory mean for a second sample (SRM 3253). The dotted blue box represents the consensus range of tolerance for SRM 3254 (x-axis) and SRM 3253 (y-axis), calculated as the values above and below the consensus means that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$.

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SECTION 8: CONTAMINANTS (Chlorate, Perchlorate)

Study Overview

In this study, participants were provided with SRM 1869 Infant/Adult Nutritional Formula II (milk/whey/soy-based), two other infant formulas samples, and two infant formula ingredient samples for dietary intake. Participants were asked to use in-house analytical methods to determine the mass fraction (ng/g) of chlorate and perchlorate in each matrix. Perchlorate is a chemical that occurs naturally in the environment and is also used in explosives, fireworks, road flares, and rocket propellant, resulting in the potential for widespread public exposure. Chlorine, as a sanitizing agent, plays a crucial role in food production. However, the formation of chlorate as a by-product of these chlorinated compounds has raised concerns with food regulatory bodies. Previous CDC studies have shown that nearly everyone in the United States is exposed regularly to low levels of perchlorate through eating food and drinking milk and water that contain chlorate and perchlorate, and trace levels of chlorate and perchlorate have been found in both breast milk and infant formula. High levels of perchlorate (thousands of times higher than the doses estimated from consumption of infant formula or breast milk) block the ability of the thyroid gland to use iodine, which in turn disrupts thyroid hormone production and impairs proper development of fetuses and infants. Regulations in the European Union effective 01 July 2020 place restrictions on the level of perchlorate allowable in infant formulas at 0.01 mg/kg (0.01 ng/g).[20] Measurement of chlorate and perchlorate in infant formulas is critical to understand infant exposure and reduce the risk of long-term harm to health.

Dietary Intake Sample Information

Infant Formula B. Participants were provided with three packets of SRM 1869, each containing 10 g of powdered infant formula. Participants were asked to store the material at controlled room temperature, 20 °C to 25 °C, to use a sample size appropriate for their in-house method of analysis, and to prepare one sample and report one value from each packet provided. Before use, participants were instructed to mix the contents of the packet thoroughly. The approximate analyte levels were not reported to participants prior to the study. The NIST-determined mass fraction for chlorate in SRM 1869 was assigned using data from a collaborating laboratory using LC-MS/MS. The NIST-determined mass fraction and uncertainty are reported in the table below on an as-received basis. A target value for perchlorate in SRM 1869 has not been determined at NIST.

Analyte	NIST-Determined Mass Fraction in SRM 1869 (ng/g)
Chlorate	120 ± 24

Infant Formulas C and F. Participants were provided with one can of each material, each containing approximately 1 lb (453 g) of powdered infant formula. Participants were asked to store the materials at controlled room temperature, 20 °C to 25 °C, to use a sample size appropriate for their in-house method of analysis, and to prepare three samples and report three values from each of the single cans provided. Before use, participants were instructed to mix the contents of each can thoroughly. The approximate analyte levels were not reported to participants prior to the

[20] Commission Regulation (EU) 2020/685 of 20 May 2020 amending Regulation (EC) No 1881/2006 as regards maximum levels of perchlorate in certain foods. Available at https://eur-lex.europa.eu/legal-content/EN/TXT/?qid=1591337530687&uri=CELEX:32020R0685 (accessed 30 Jun 2020).

study. The NIST-determined mass fractions for chlorate in Infant Formulas C and F were assigned using data from a collaborating laboratory using LC-MS/MS. The NIST-determined mass fractions and uncertainties are reported in the table below on an as-received basis. Target values for perchlorate in Infant Formulas C and F have not been determined at NIST.

	NIST-Determined Mass Fraction (ng/g)	
Analyte	Infant Formula C	Infant Formula F
Chlorate	300 ± 60	400 ± 80

Infant Formulas D and E. Participants were provided with one packet of each material, each containing approximately 100 g of powdered infant formula raw ingredient. Participants were asked to store the materials at controlled room temperature, 20 °C to 25 °C, to use a sample size appropriate for their in-house method of analysis, and to prepare three samples and report three values from each of the single packets provided. Before use, participants were instructed to mix the contents of each packet thoroughly. The approximate analyte levels were not reported to participants prior to the study. The NIST-determined mass fraction for chlorate in Infant Formula D was assigned using data from a collaborating laboratory using LC-MS/MS. The NIST-determined mass fraction and uncertainty are reported in the table below on an as-received basis. Target values for chlorate in Infant Formula E and perchlorate in Infant Formulas D and E have not been determined at NIST.

Analyte	NIST-Determined Mass Fraction in Infant Formula D (ng/g)
Chlorate	50 ± 10

Dietary Intake Study Results

- Thirteen laboratories enrolled in this exercise and received samples to measure chlorate and/or perchlorate.
  - Eleven or twelve laboratories reported quantitative results for chlorate in each sample (85 % to 92 % participation).
  - For the low-level perchlorate samples (B, C, and D), 1 to 2 laboratories reported quantitative results (8 % to 15 % participation).
  - For the higher-level perchlorate samples (E and F), 8 to 11 laboratories reported quantitative results (62 % to 85 % participation).
- The variability between the laboratories for chlorate was between 3 % and 11 % in the various materials. The variability between the laboratories for perchlorate was between 6 % and 7 % for the two materials containing higher perchlorate levels.
- Laboratories reported using solvent extraction (75 %), solid phase extraction (17 %), and dilution (8 %) to prepare infant formula samples for chlorate and perchlorate analysis.
- Laboratories indicated using liquid chromatography with mass spectrometry or tandem mass spectrometry (LC-MS or LC-MS/MS, 92 %) or ion chromatography with mass spectrometry (IC-MS, 8 %) for determination of chlorate and perchlorate in the infant formula samples.

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Dietary Intake Technical Recommendations

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

- Overall, laboratory performance was very good for laboratories measuring chlorate and perchlorate in these infant formula matrices.
- Analysis of chlorate and perchlorate are subject to contamination from everyday laboratory conditions.
  - Care must be taken to perform analyses in a chlorate- and perchlorate-free environment, which includes use of dedicated glassware, reagents, and other apparatuses.
  - Solvent and reagent blanks should be included with the analytical protocol to identify any potential biases that could arise from sample or instrument contamination.
- Most laboratories reported use of solvent extraction to prepare infant formula samples for analysis of chlorate and perchlorate. No trends were observed that correlated reported results with the sample preparation approach used.
- Most laboratories reported use of MS-based methodologies for determination of chlorate and perchlorate. Those that did not also utilize an isotopically labeled internal standard reported results that were outlying with respect to the consensus. Isotopically labeled internal standards, added at the beginning of the analytical procedure, often result in improved accuracy and precision of final results.
- The greatest variability for chlorate was observed for sample D, one of the ingredient materials, which had the lowest chlorate level.
  - This matrix may have been more challenging based both on the low level and the nature of the ingredient (high protein and low fat).
  - Between-laboratory variability did not decrease with increasing concentration of chlorate in other matrices.
  - Between-laboratory variability was low (4 %) for sample E, also an ingredient matrix.
- No trends were observed for within laboratory variability for chlorate or perchlorate.
- Any extraction procedure should be optimized to determine the most effective extraction solvent to ensure exhaustive extraction of the analyte from the matrix.
- Some laboratories responded to a follow-up call for additional method and laboratory information. No trends were noted between performance and laboratory level of experience or frequency of testing.
- "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.

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Table 8-1. Individualized data summary table (NIST) for chlorate and perchlorate in infant formulas.

National Institute of Standards & Technology

HAMQAP Exercise 5 - 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*
Chlorate	SRM 1869 Infant/Adult Nutritional Formula II (milk/whey/soy-based)	ng/g	120	24			11	104	2.56	120	24
Chlorate	Infant Formula C	ng/g	300	60			12	265	14.2	300	60
Chlorate	Infant Formula D	ng/g	50	10			11	66.8	6.07	50	10
Chlorate	Infant Formula E	ng/g					11	1441	59.1		
Chlorate	Infant Formula F	ng/g	400	80			12	328	15.6	400	80
Perchlorate	SRM 1869 Infant/Adult Nutritional Formula II (milk/whey/soy-based)	ng/g					2	0.617	1.764		
Perchlorate	Infant Formula C	ng/g					2	0.450	1.294		
Perchlorate	Infant Formula D	ng/g					1				
Perchlorate	Infant Formula E	ng/g					12	30.0	1.55		
Perchlorate	Infant Formula F	ng/g					8	5.75	0.394		

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

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

		Chlorate																						
		SRM 1869 Infant/Adult Nutritional Formula II (milk/whey/soy-based) (ng/g)					Infant Formula C (ng/g)					Infant Formula D (ng/g)					Infant Formula E (ng/g)					Infant Formula F (ng/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD	A	B	C	Avg	SD	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target				120	24				300	60				50	10									400	80
	E001	100	100	110	103.3	5.8	260	260	260	260.0	0.0	90	80	80	83.3	5.8	1470	1380	1850	1567	249	320	350	330	333.3	15.3
	E023	76	78	83	79.0	3.6	200	202	198	200.0	2.0	47	44	51	47.3	3.5	1270	1240	1290	1267	25	229	198	242	223.0	22.6
	E027	116	110	108	111.3	4.2	140	144	129	137.7	7.8											248	316	288	284.0	34.2
	E052	104	107	105	105.3	1.5	282	283	282	282.3	0.6	64	62	60	62.0	2.0	1382	1394	1398	1391	8	314	319	318	317.0	2.6
	E053	100	100	100	100.0	0.0	270	230	230	243.3	23.1	160	150	160	156.7	5.8	1240	1230	1200	1223	21	290	270	320	293.3	25.2
	E054	96.47	95.93	95.84	96.1	0.3	252.6	263.05	254.37	256.7	5.6	53.09	53.79	49.95	52.3	2.0	1429.81	1434.85	1478.94	1448	27	315.41	321.14	316.83	317.8	3.0
	E055	106	110	90	102.0	10.6	281			281.0		93			93.0		1401			1401		355			355.0	
	E056	101	110		105.5	6.4	299	291		295.0	5.7	71.9	67.6		69.8	3.0	1526	1604		1565	55	375	361		368.0	9.9
	E058	107.47	104.45	106.35	106.1	1.5	295.59	321.61	306.41	307.9	13.1	63.01	60.23	60.4	61.2	1.6	1525.05	1507.75	1503	1512	12	356.61	335.92	369.75	354.1	17.1
	E059	< 10.0	< 10.0	< 10.0	< 10.0		4.52	5.79	4.56	5.0	0.7	58.36	51.05	59.02	56.1	4.4	25.86	19.78	25.71	24	3	56.66	50.92	51.41	53.0	3.2
	E060	110	110	110	110.0	0.0	290	290	290	290.0	0.0	75	76	76	75.7	0.6	1500	1500	1400	1467	58	360	360	370	363.3	5.8
	E061																									
	E062	114	117	112	114.3	2.5	309	299	312	306.7	6.8	62	65.1	69.4	65.5	3.7	1565	1563	1586	1571	13	381	372	376	376.3	4.5
	E072																									
Community Results		Consensus Mean			103.8		Consensus Mean			263.7		Consensus Mean			64.7		Consensus Mean			1441		Consensus Mean			322.3	
		Consensus Standard Deviation			3.1		Consensus Standard Deviation			15.9		Consensus Standard Deviation			7.0		Consensus Standard Deviation			63		Consensus Standard Deviation			18.9	
		Maximum			114.3		Maximum			307.9		Maximum			156.7		Maximum			1571		Maximum			376.3	
		Minimum			79.0		Minimum			5.0		Minimum			47.3		Minimum			24		Minimum			53.0	
		N			11		N			12		N			11		N			11		N			12	

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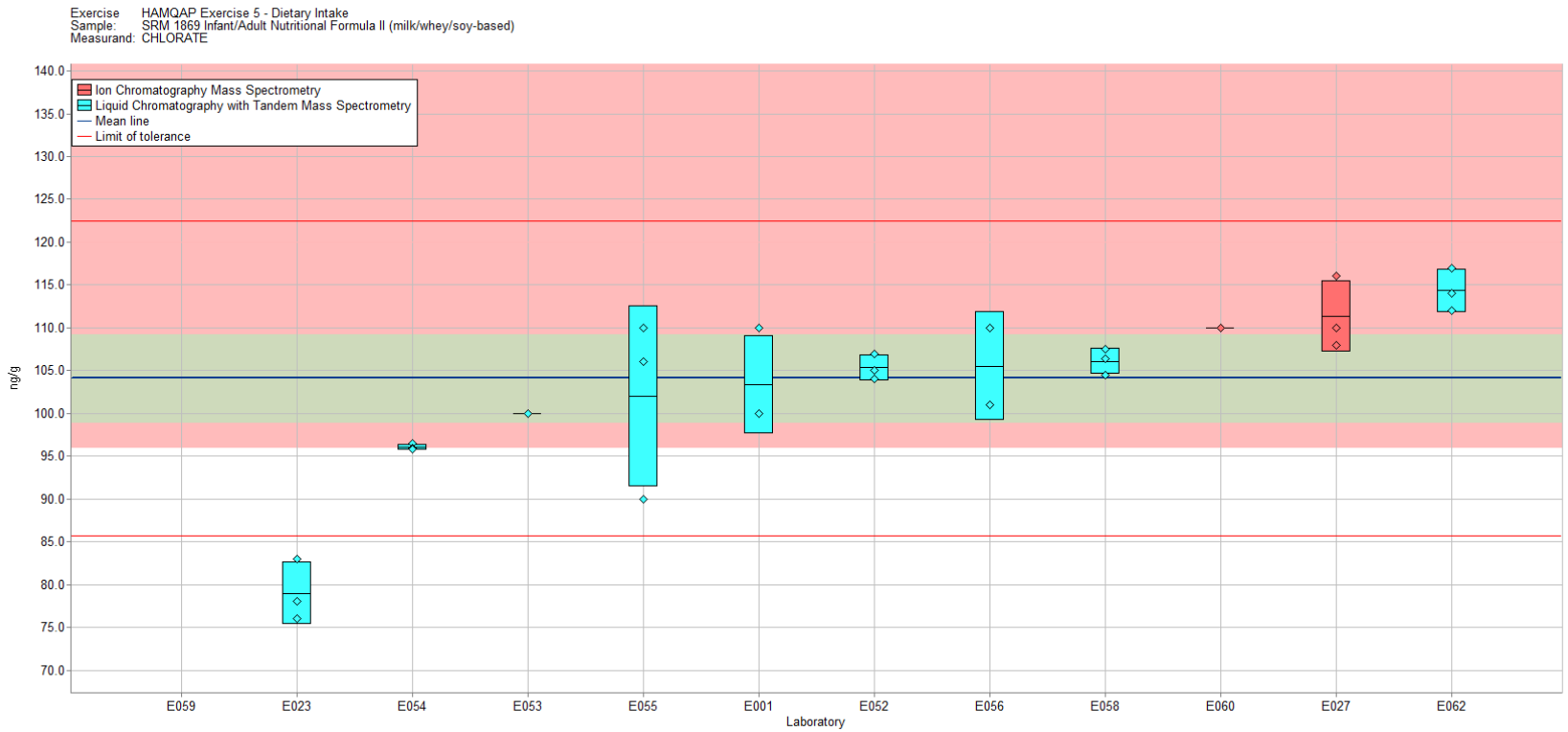

Figure 8-1. Chlorate in SRM 1869 Infant/Adult Nutritional Formula II (milk/whey/soy-based) (data summary view – analytical method). In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the 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 twice its uncertainty (U_{NIST}) and represents the range that results in an acceptable Z'_{NIST} score, $|Z'_{\text{NIST}}| \leq 2$.

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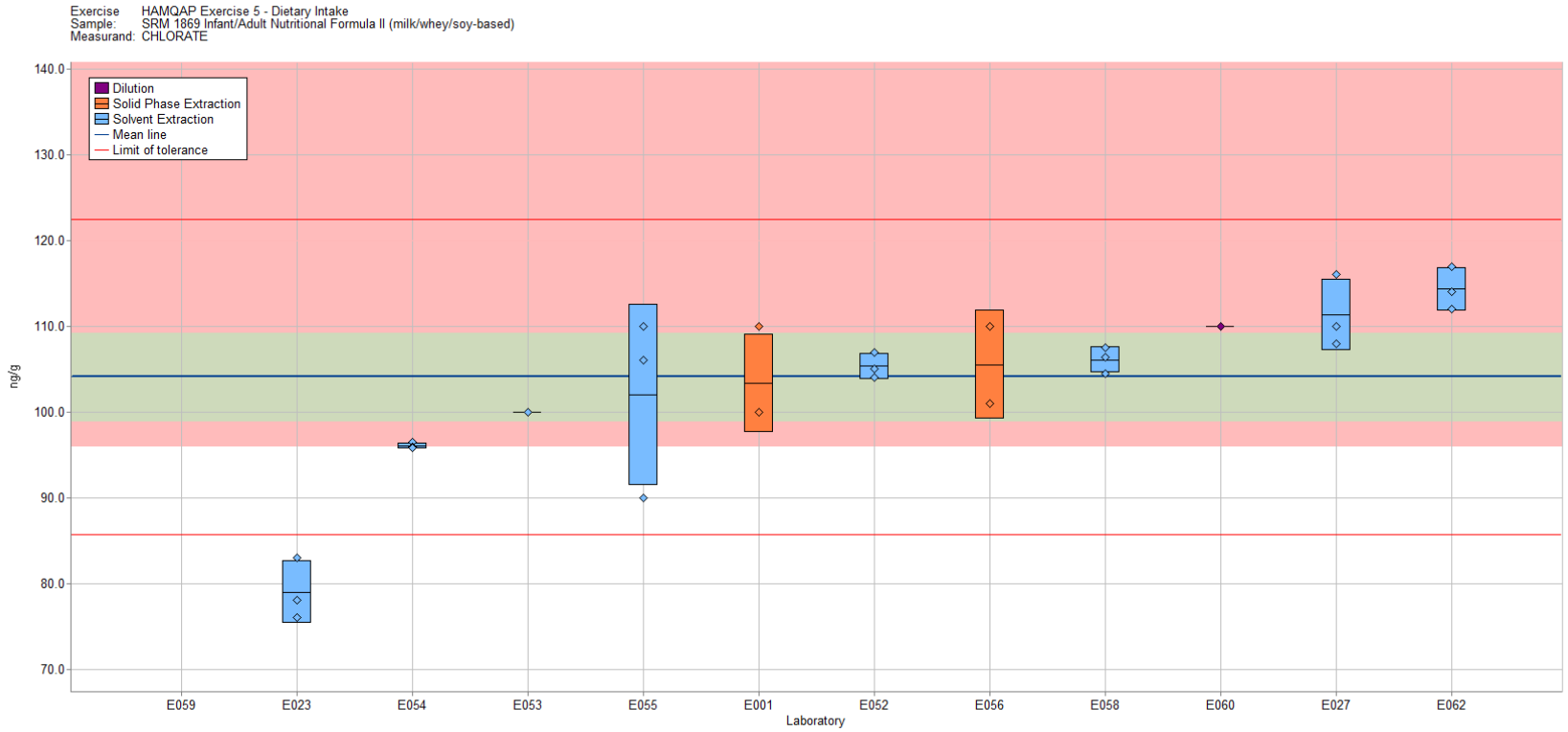

Figure 8-2. Chlorate in SRM 1869 Infant/Adult Nutritional Formula II (milk/whey/soy-based) (data summary view – sample preparation method). In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents 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, $|Z'_{\text{NIST}}| \leq 2$.

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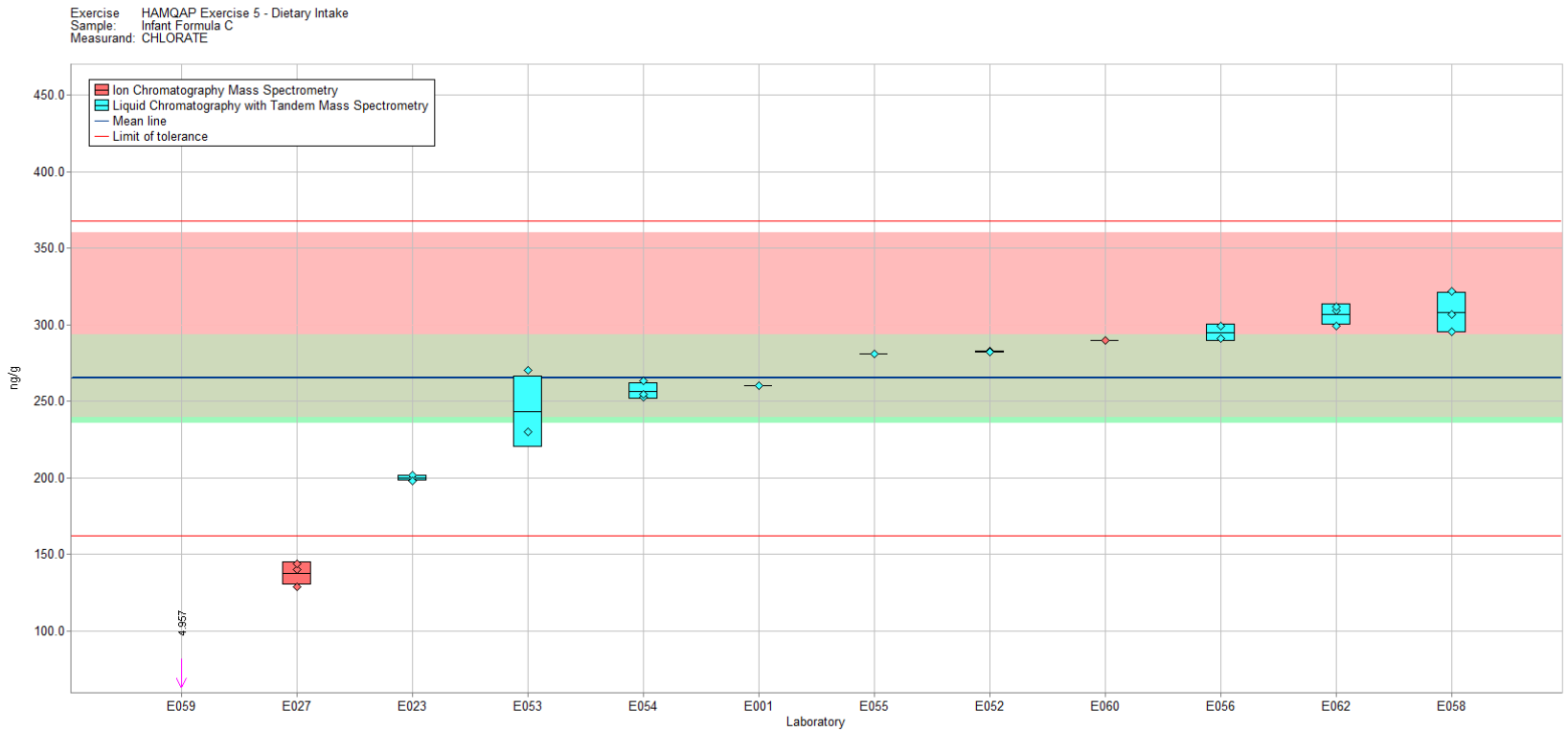

Figure 8-3. Chlorate in Infant Formula C (data summary view – analytical method). In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line represents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

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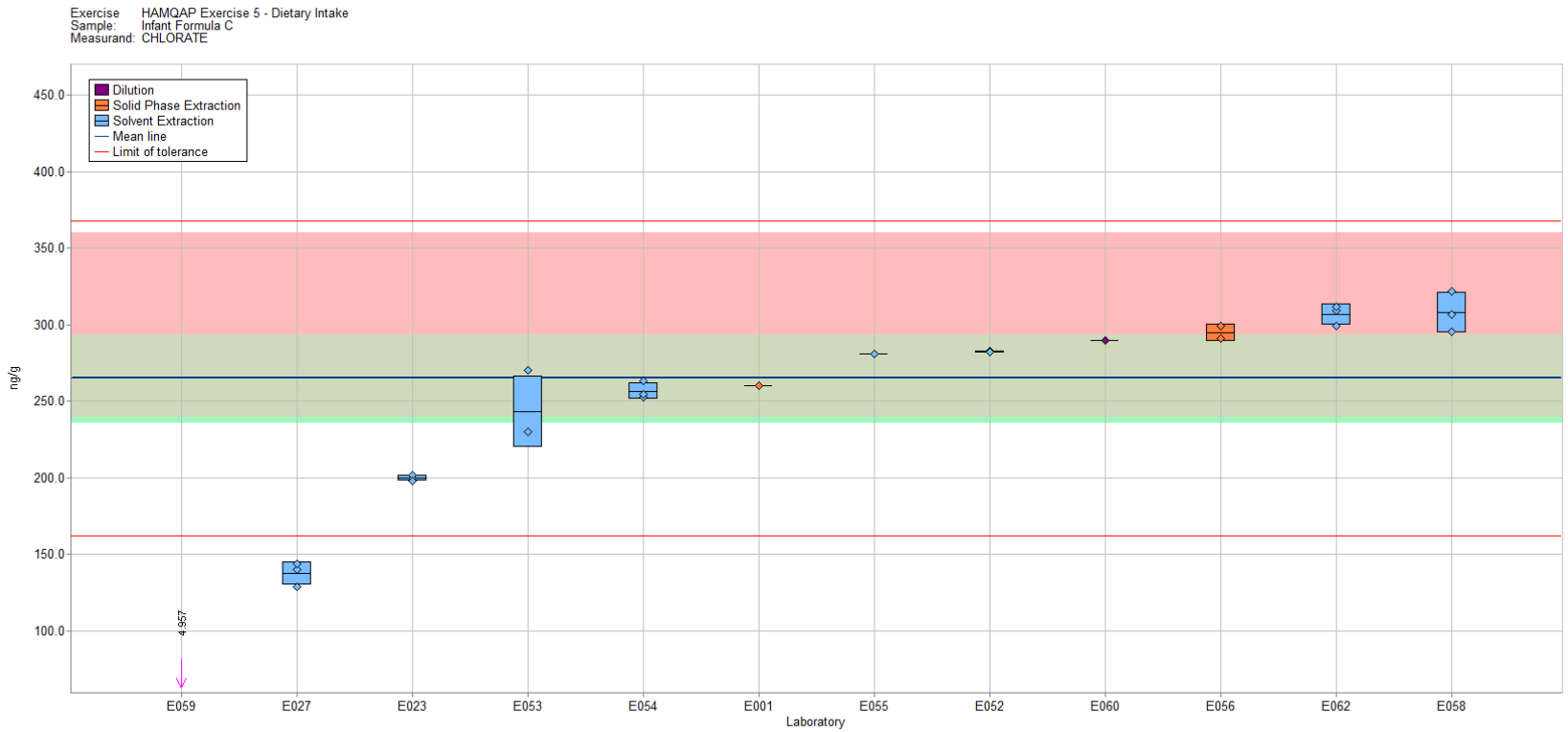

Figure 8-4. Chlorate in Infant Formula C (data summary view – sample preparation method). In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The solid 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, $|Z'_{\text{NIST}}| \leq 2$.

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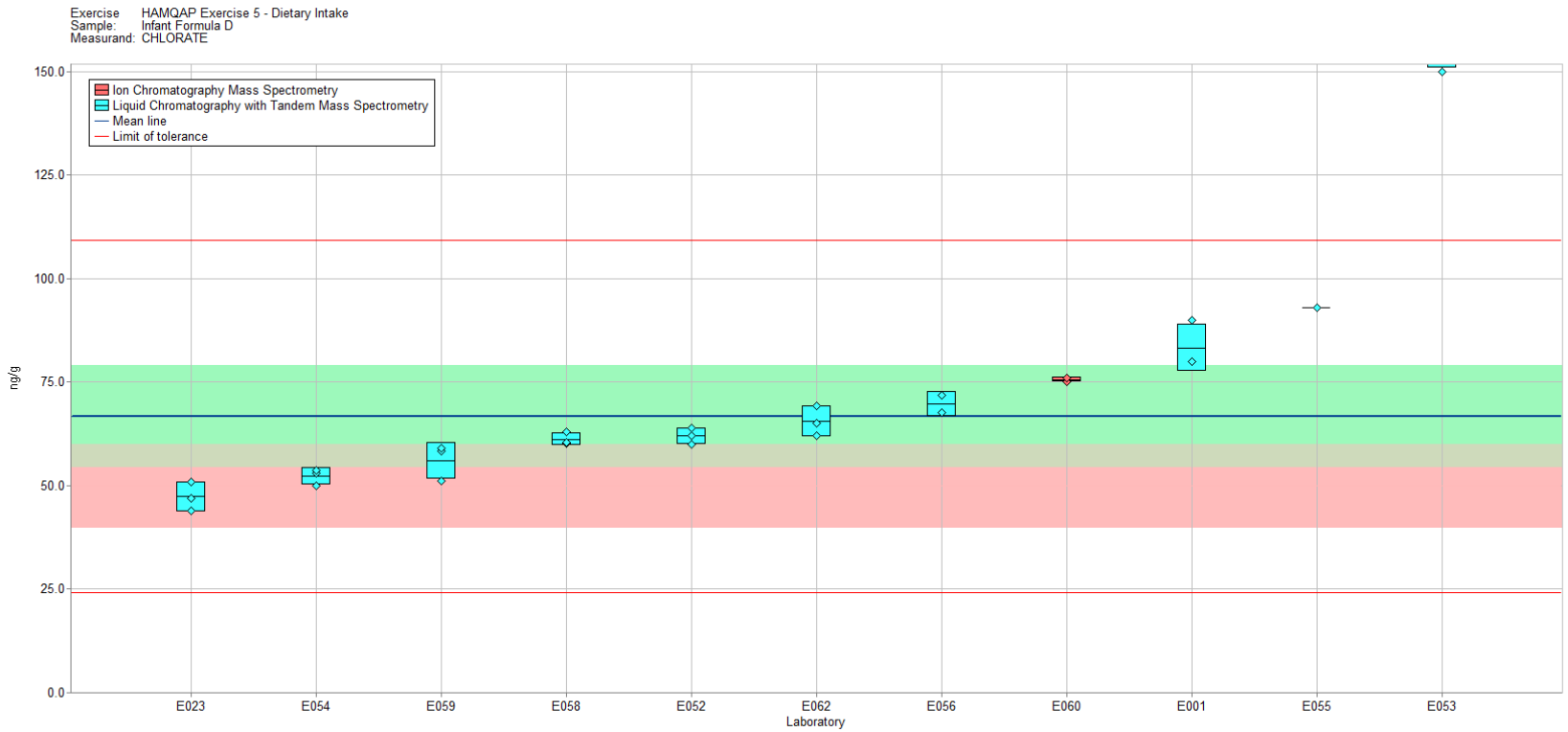

Figure 8-5. Chlorate in Infant Formula D (data summary view – analytical method). In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line represents 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 twice its uncertainty (U_{NIST}) and represents the range that results in an acceptable Z'_{NIST} score, $|Z'_{\text{NIST}}| \leq 2$.

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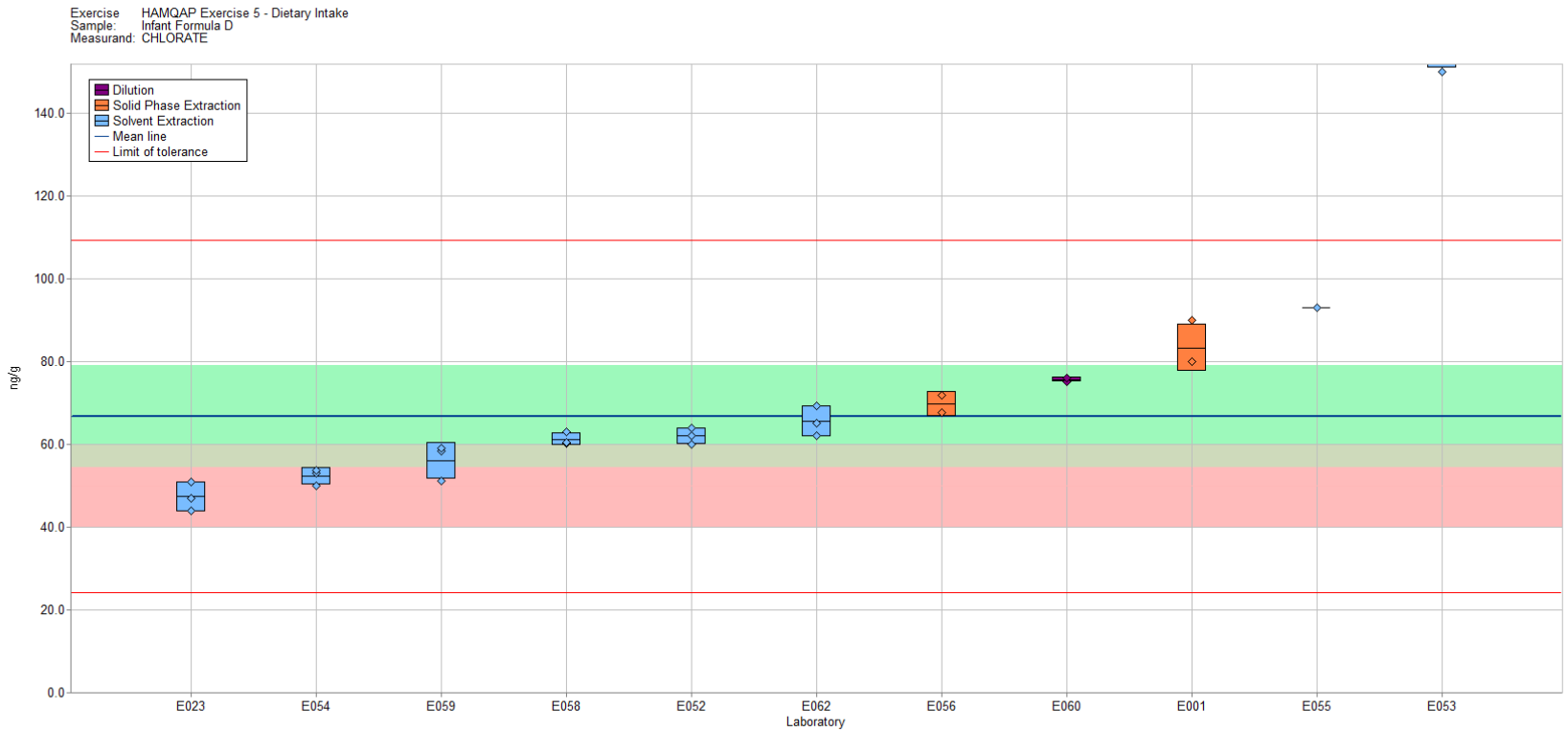

Figure 8-6. Chlorate in Infant Formula D (data summary view – sample preparation method). In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The solid 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, $|Z'_{\text{NIST}}| \leq 2$.

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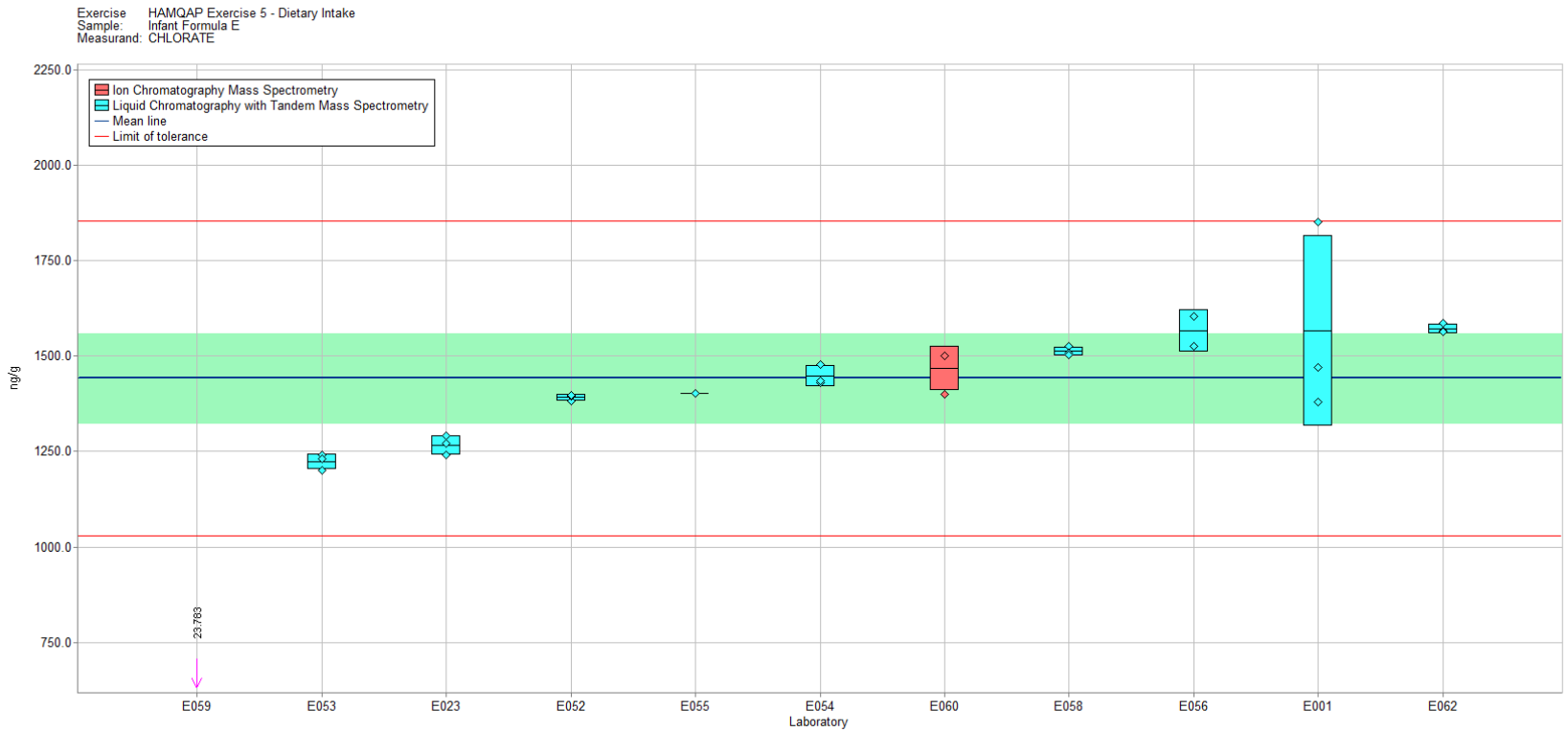

Figure 8-7. Chlorate in Infant Formula E (data summary view – analytical method). In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line represents 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.

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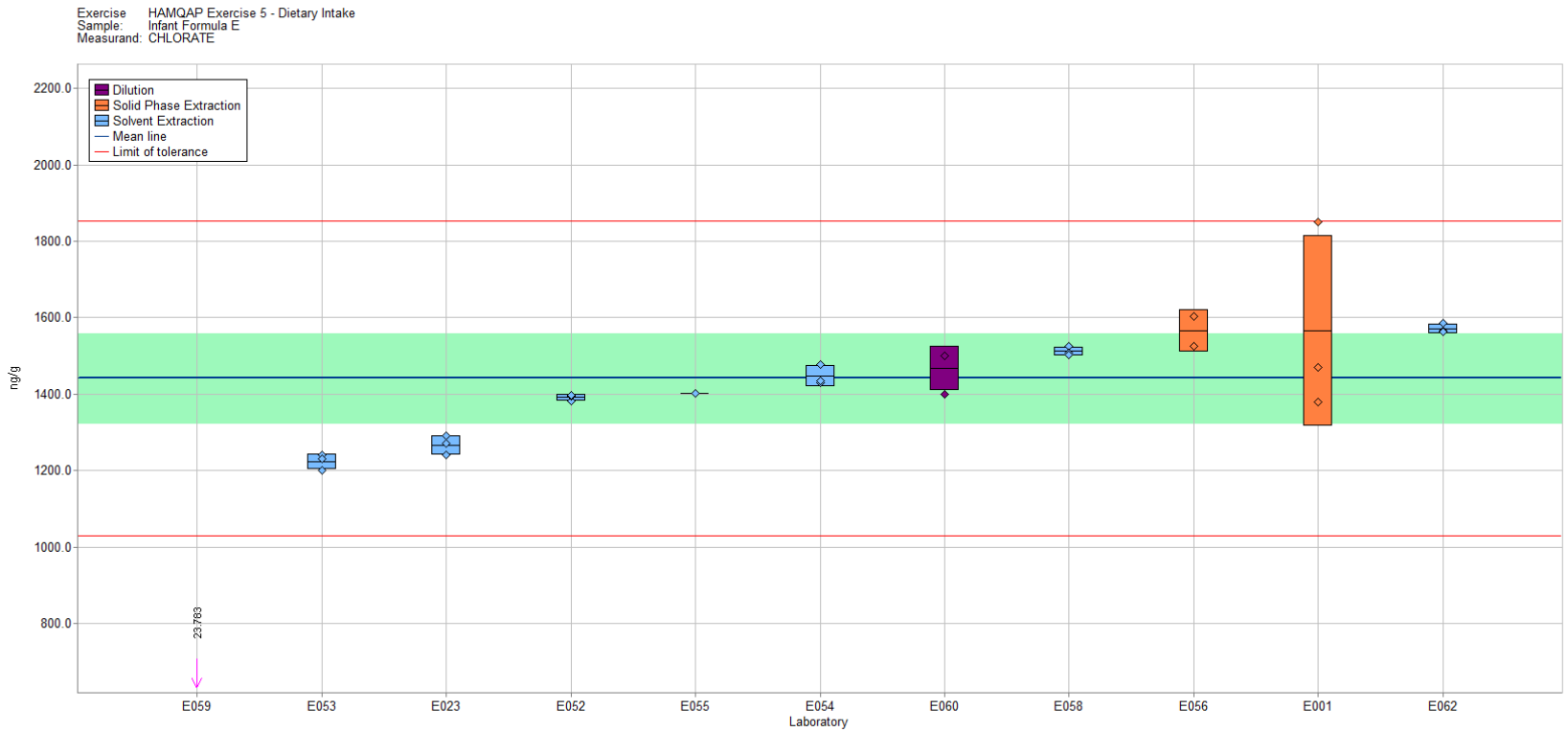

Figure 8-8. Chlorate in Infant Formula E (data summary view – sample preparation method). In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The solid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

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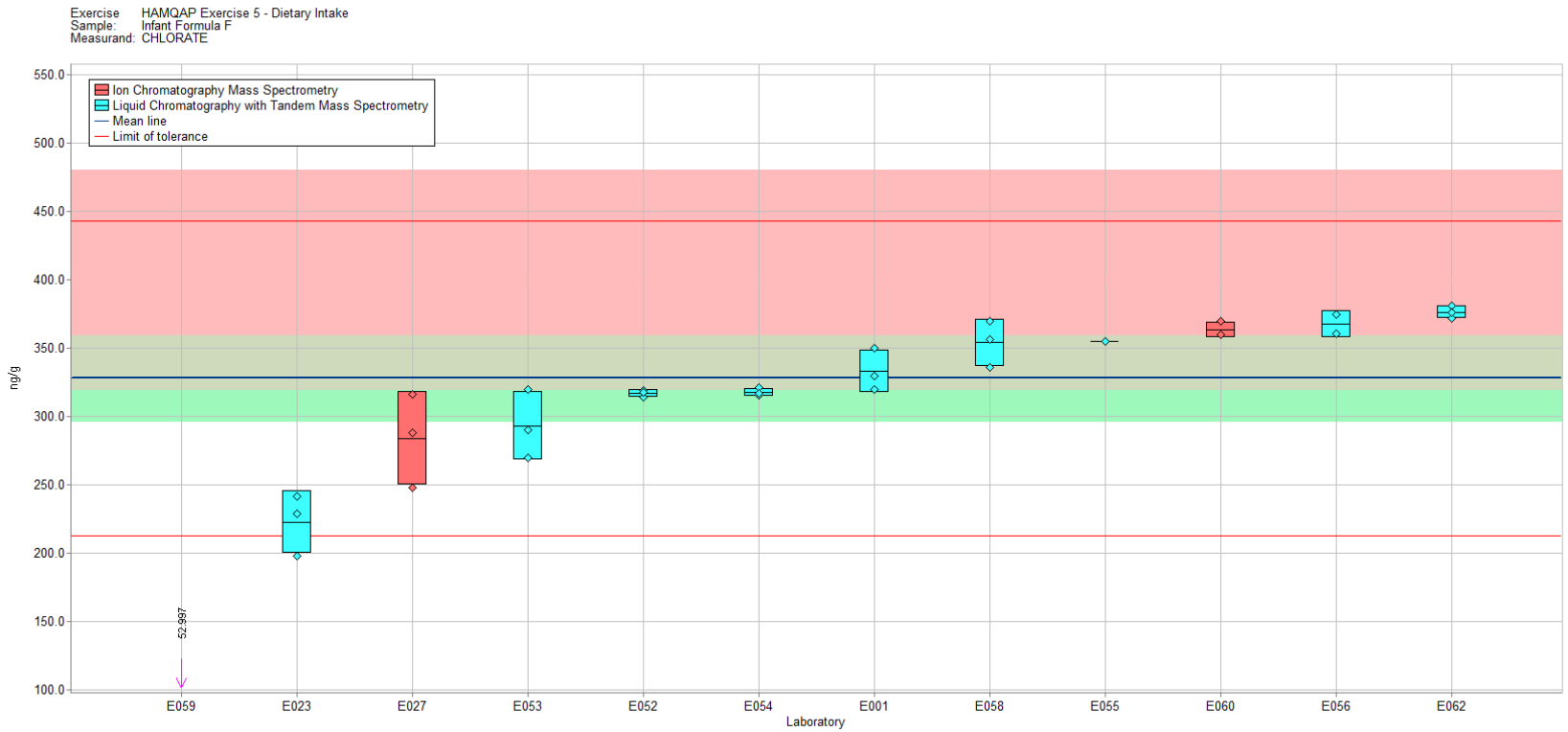

Figure 8-9. Chlorate in Infant Formula F (data summary view – analytical method). In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line represents 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 twice its uncertainty (U_{NIST}) and represents the range that results in an acceptable Z'_{NIST} score, $|Z'_{\text{NIST}}| \leq 2$.

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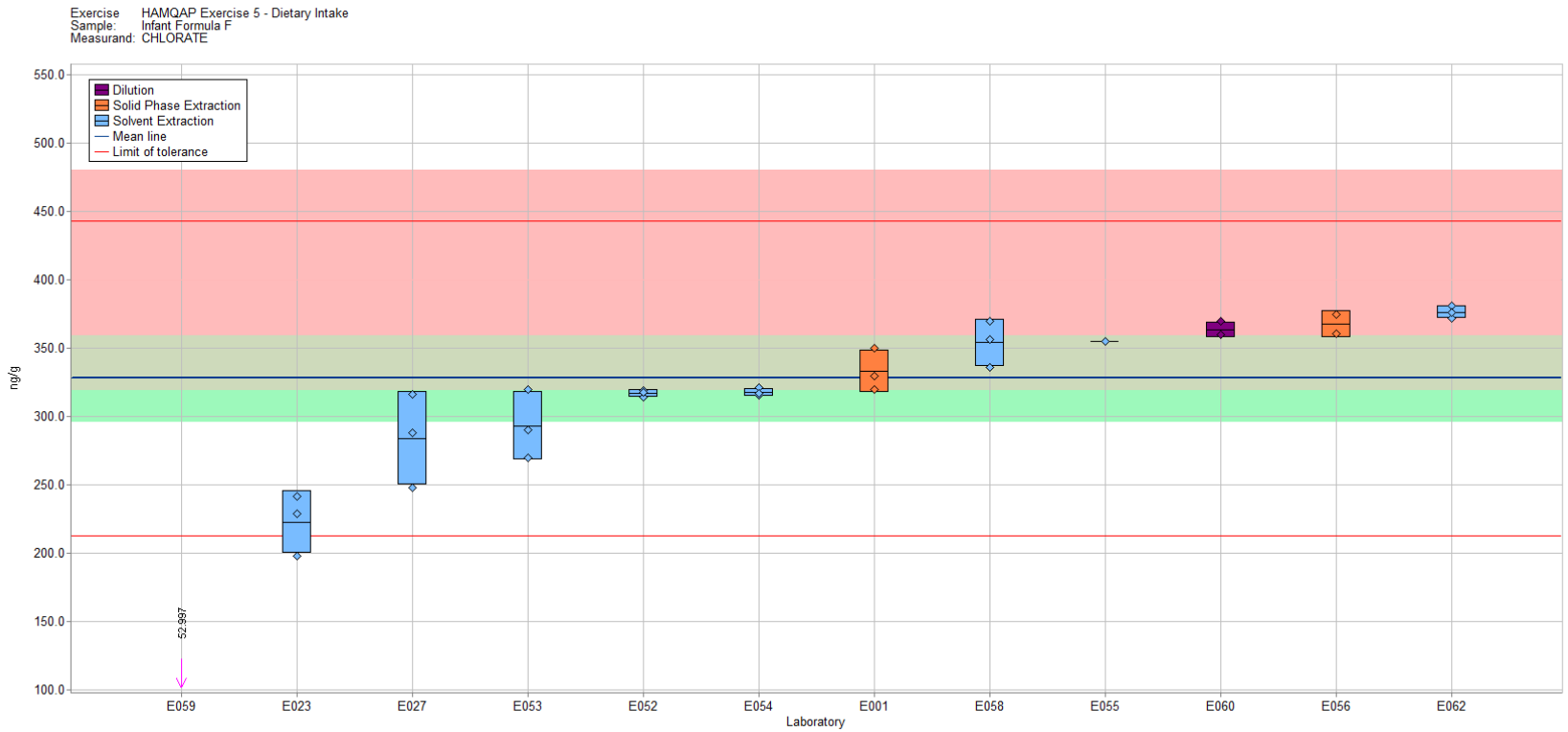

Figure 8-10. Chlorate in Infant Formula F (data summary view – sample preparation method). In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The 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, $|Z'_{\text{NIST}}| \leq 2$.

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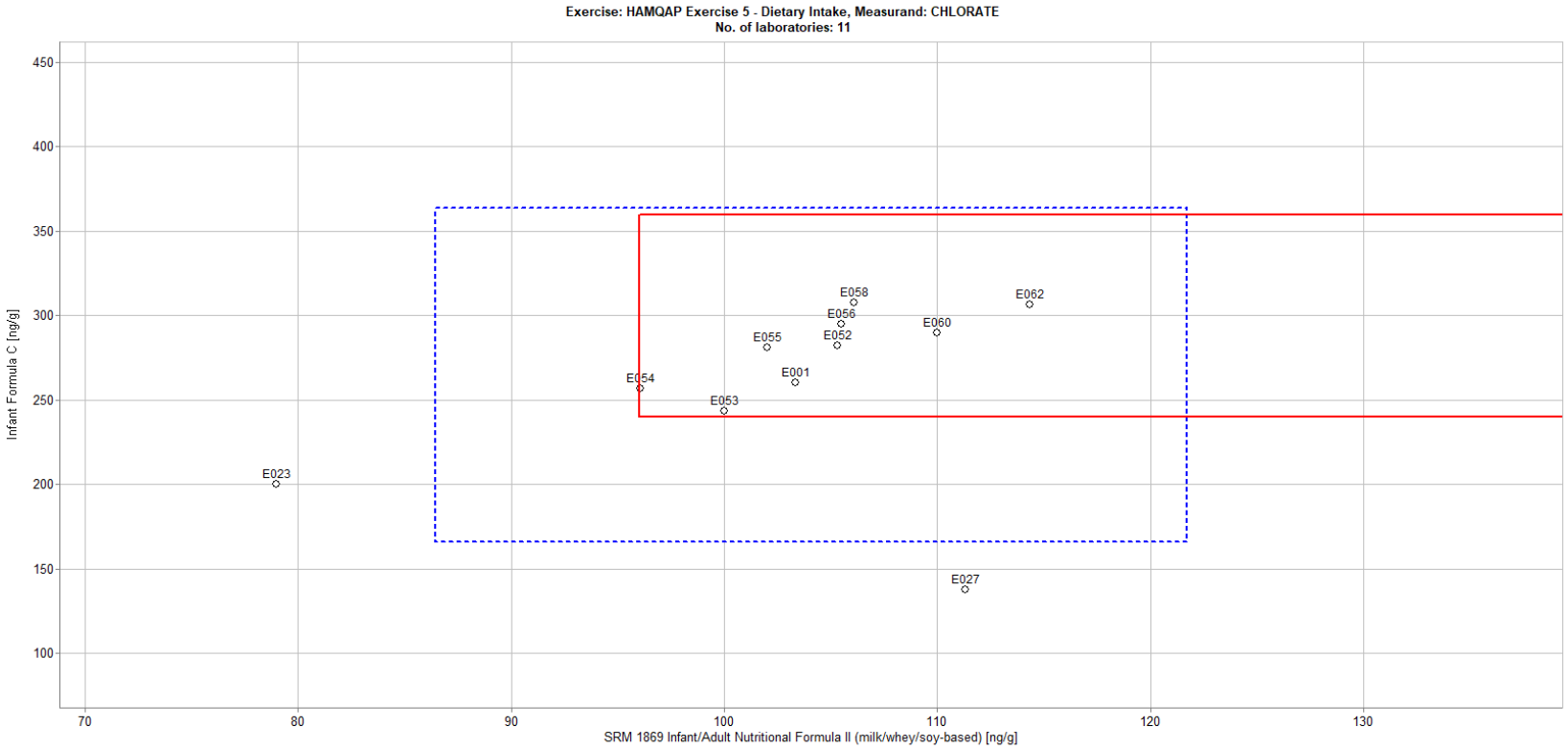

Figure 8-11. Laboratory means for chlorate in SRM 1869 Infant/Adult Nutritional Formula II (milk/whey/soy-based) and Infant Formula C (sample/sample comparison view). In this view, the individual laboratory mean for one sample (SRM 1869) is compared to the mean for a second sample (Infant Formula C). The solid red box represents the NIST range of tolerance for the two samples, SRM 1869 (x-axis) and Infant Formula C (y-axis), which encompasses the target values bounded by their uncertainties (U_{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 Infant Formula C (y-axis), calculated as the values above and below the consensus means that result in an acceptable Z'_{comm} score, $|Z'_{\mathrm{comm}}| \leq 2$.

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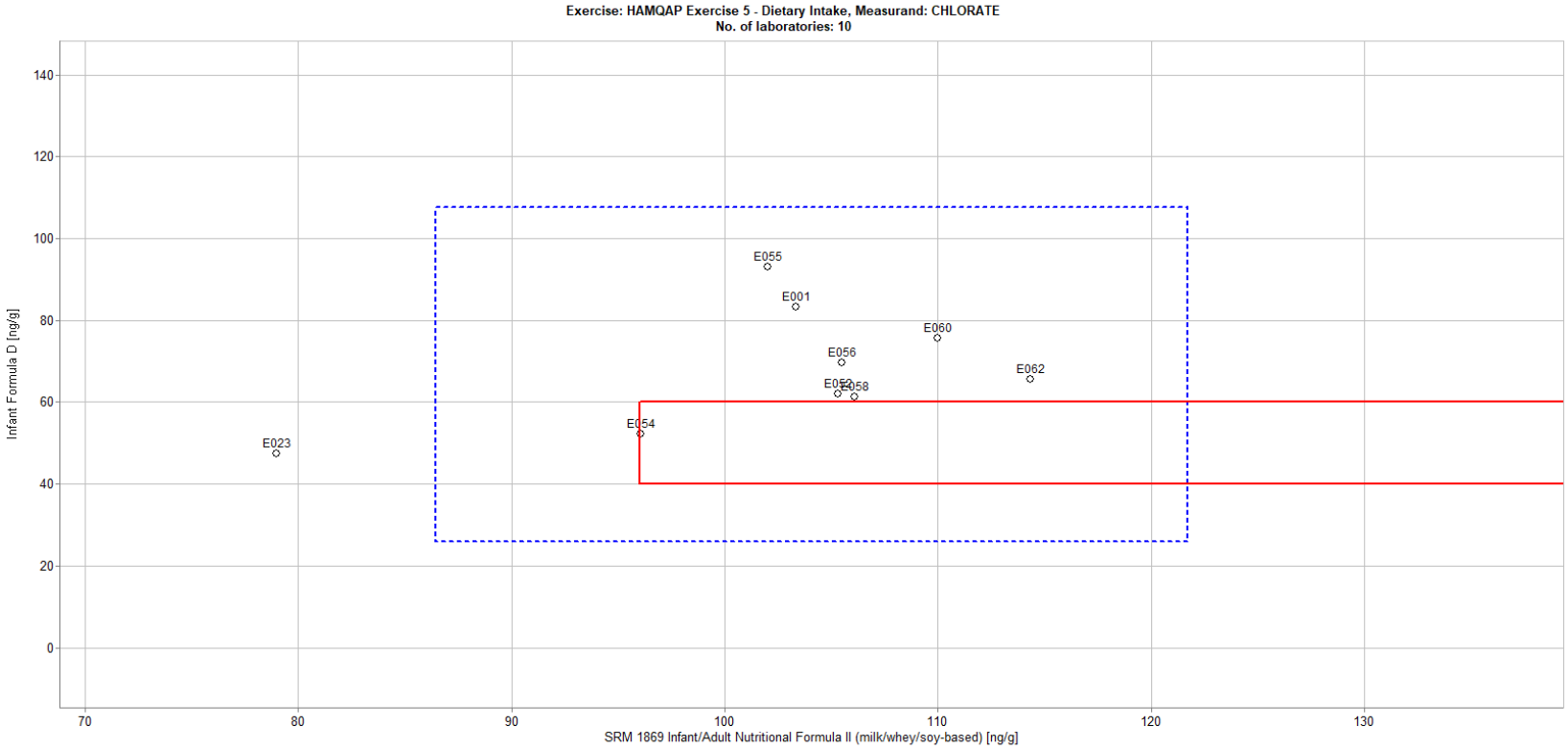

Figure 8-12. Laboratory means for chlorate in SRM 1869 Infant/Adult Nutritional Formula II (milk/whey/soy-based) and Infant Formula D (sample/sample comparison view). In this view, the individual laboratory mean for one sample (SRM 1869) is compared to the mean for a second sample (Infant Formula D). The solid red box represents the NIST range of tolerance for the two samples, SRM 1869 (x-axis) and Infant Formula D (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 Infant Formula D (y-axis), calculated as the values above and below the consensus means that result in an acceptable Z'_{comm} score, $|Z'_{\mathrm{comm}}| \leq 2$.

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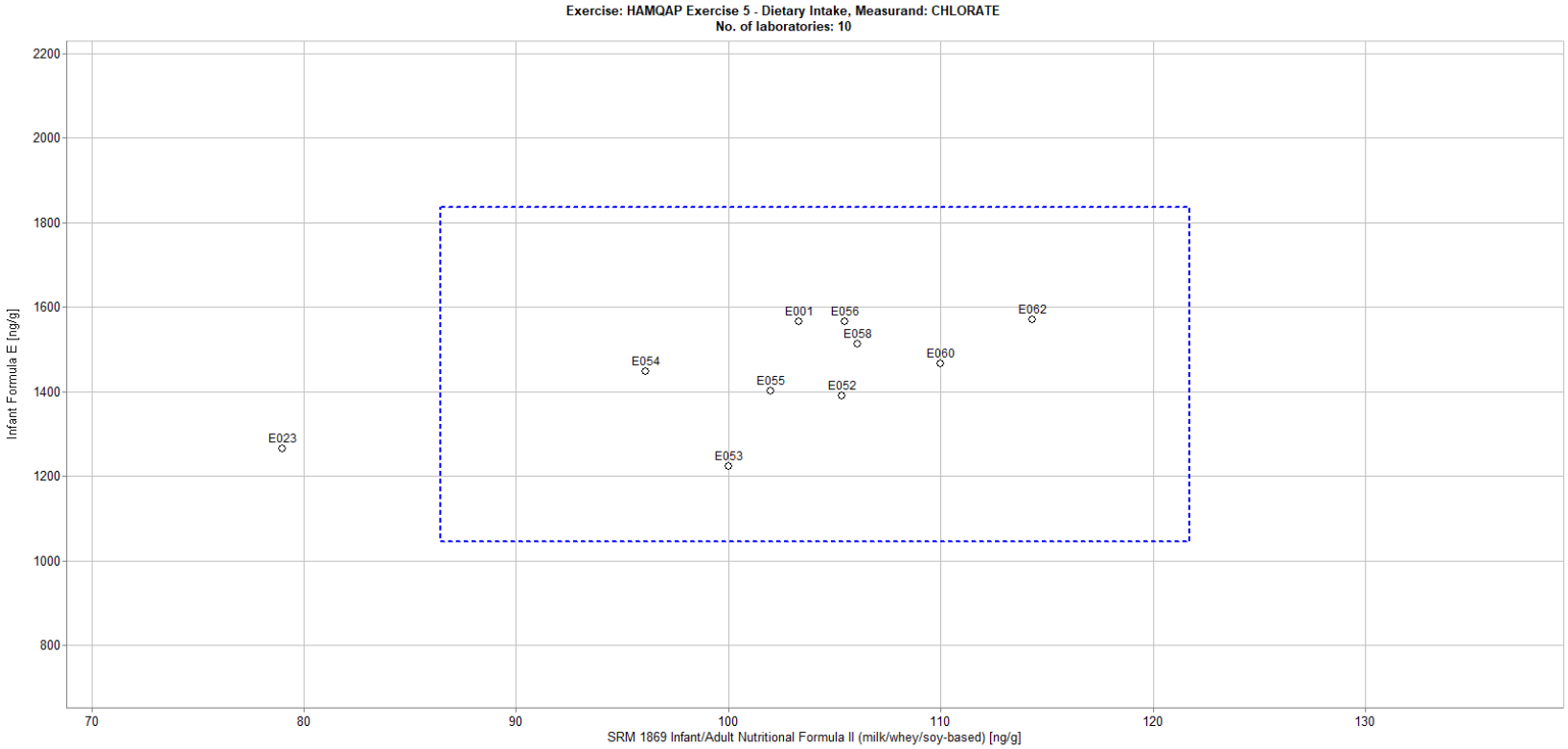

Figure 8-13. Laboratory means for chlorate in SRM 1869 Infant/Adult Nutritional Formula II (milk/whey/soy-based) and Infant Formula E (sample/sample comparison view). In this view, the individual laboratory mean for one sample (SRM 1869) is compared to the mean for a second sample (Infant Formula E).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

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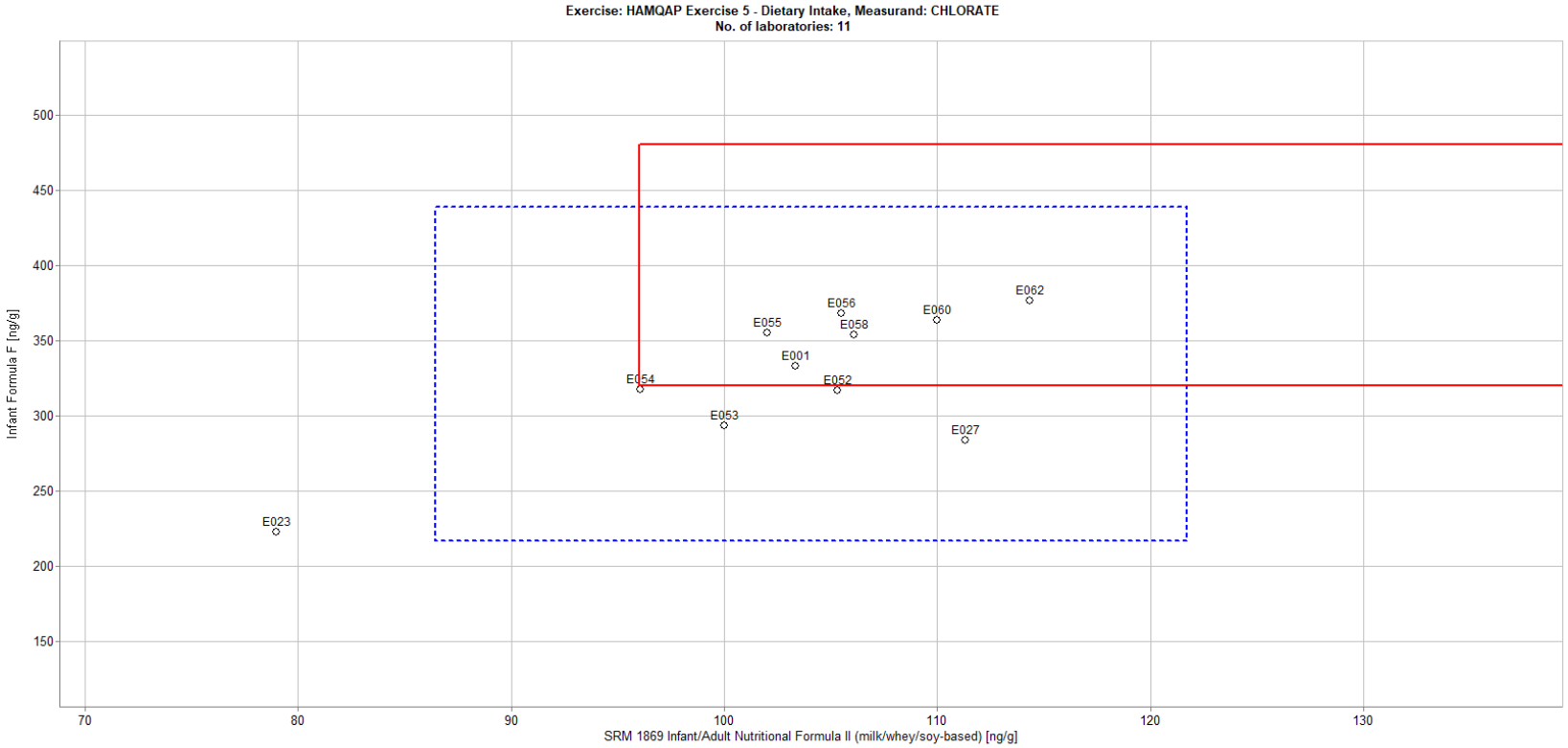

Figure 8-14. Laboratory means for chlorate in SRM 1869 Infant/Adult Nutritional Formula II (milk/whey/soy-based) and Infant Formula F (sample/sample comparison view). In this view, the individual laboratory mean for one sample (SRM 1869) is compared to the mean for a second sample (Infant Formula F). The solid red box represents the NIST range of tolerance for the two samples, SRM 1869 (x-axis) and Infant Formula F (y-axis), which encompasses the target values bounded by their uncertainties (U_{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 Infant Formula F (y-axis), calculated as the values above and below the consensus means that result in an acceptable Z'_{comm} score, $|Z'_{\mathrm{comm}}| \leq 2$.

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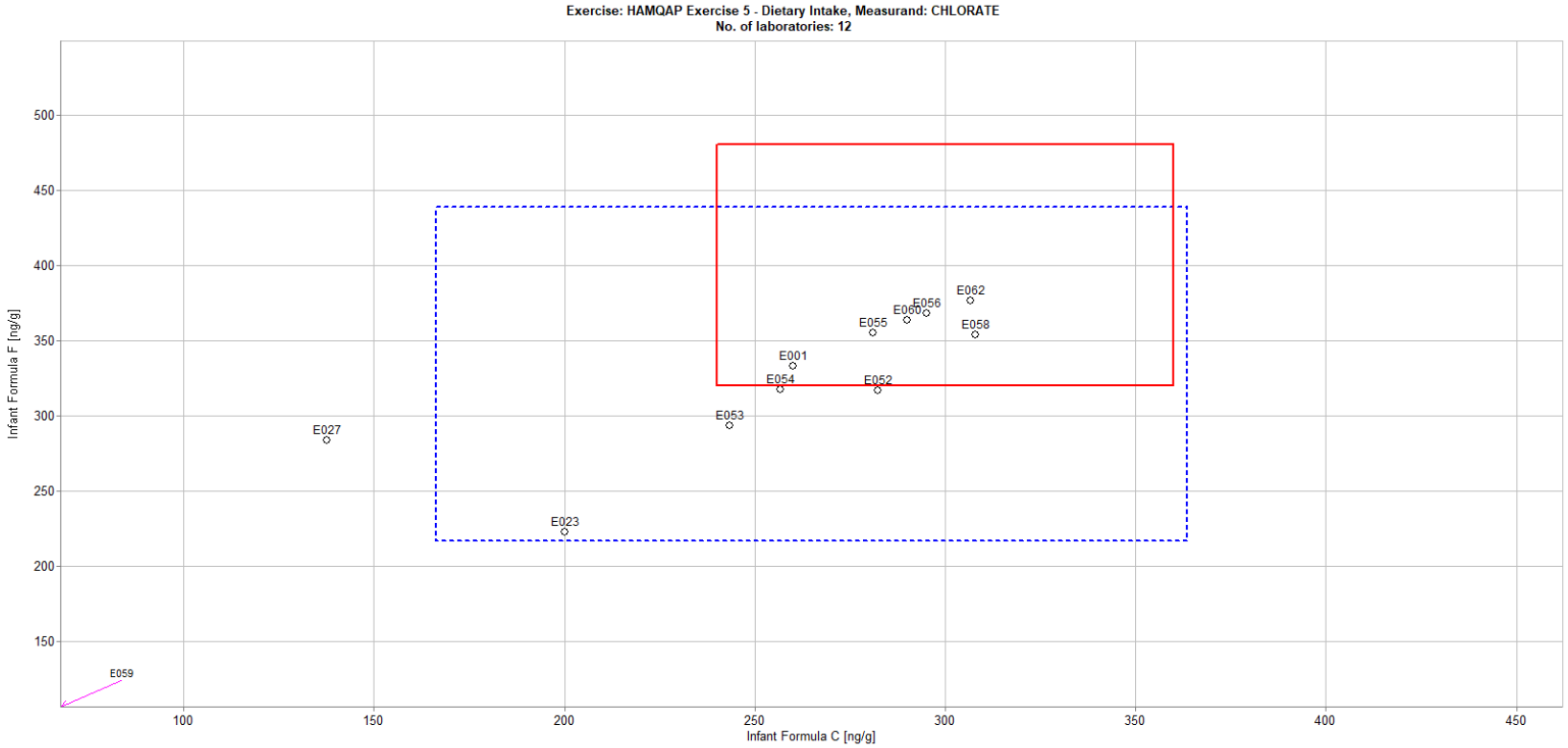

Figure 8-15. Laboratory means for chlorate in Infant Formula C and Infant Formula F (sample/sample comparison view). In this view, the individual laboratory mean for one sample (Infant Formula C) is compared to the mean for a second sample (Infant Formula F). The dotted blue box represents the consensus range of tolerance for Infant Formula C (x-axis) and Infant Formula F (y-axis), calculated as the values above and below the consensus means that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$.

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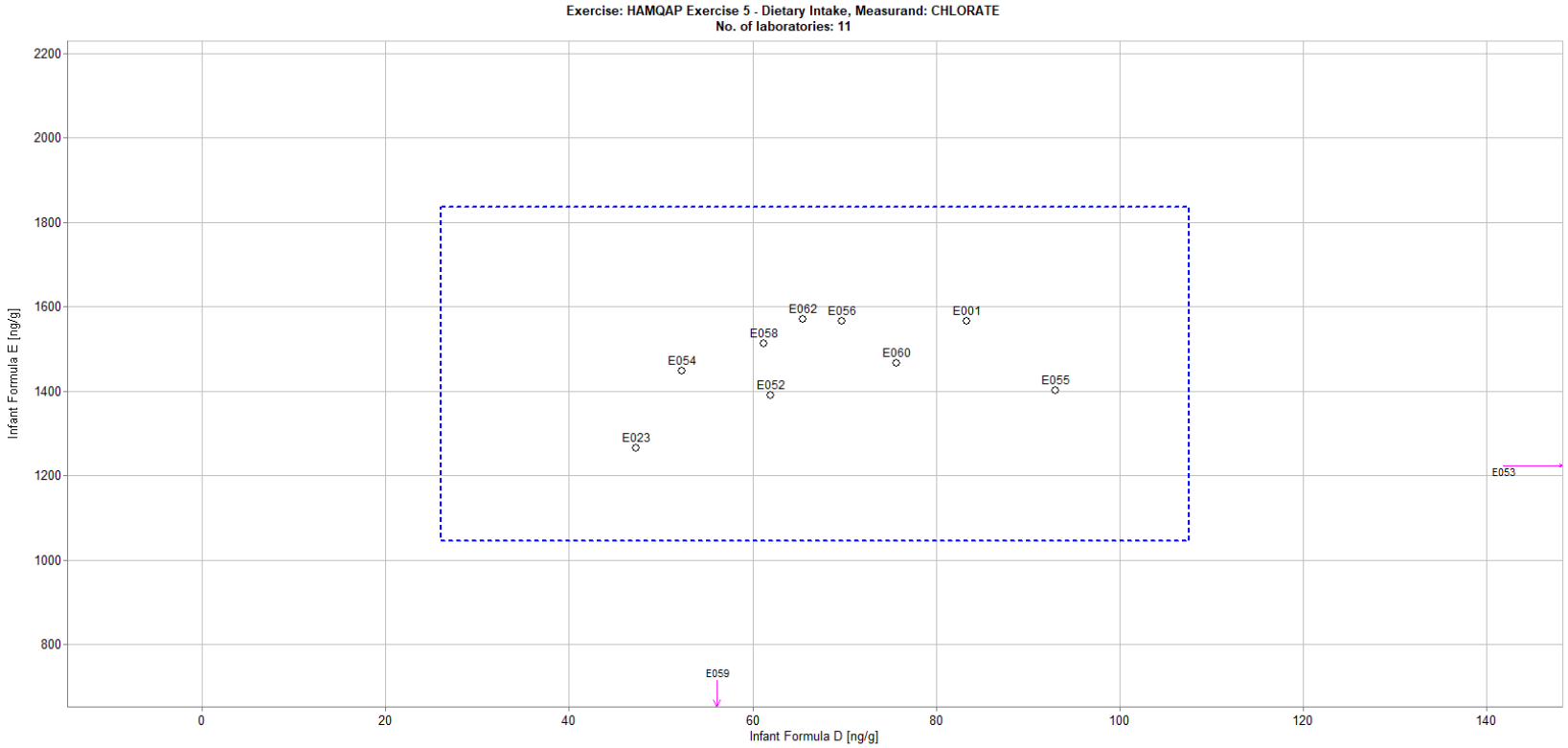

Figure 8-16. Laboratory means for chlorate in Infant Formula D and Infant Formula E (sample/sample comparison view). In this view, the individual laboratory mean for one sample (Infant Formula D) is compared to the mean for a second sample (Infant Formula E). The dotted blue box represents the consensus range of tolerance for Infant Formula D (x-axis) and Infant Formula E (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 8-3. Data summary table for perchlorate in infant formulas. Data points highlighted in red have been flagged as potential outliers (e.g., Grubb and/or Cochran) by the NIST software package.

		Perchlorate																					
		SRM 1869 Infant/Adult Nutritional Formula II (milk/whey/soy-based) (ng/g)					Infant Formula C (ng/g)					Infant Formula D (ng/g)					Infant Formula E (ng/g)					Infant Formula F (ng/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD	A	B	C	Avg	SD	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target																									
	E001	1.2	1.1	1.4	1.23	0.15	0.9	1	0.8	0.90	0.10	< 0.50	< 0.50	< 0.50	< 0.50		26.4	27	27.1	26.8	0.4	5.8	5	5.4	5.40	0.40
	E023	< 10.0	< 10.0	< 10.0	< 10.0		< 10.0	< 10.0	< 10.0	< 10.0		< 10.0	< 10.0	< 10.0	< 10.0		28	26	28	27.3	1.2	< 10.0	< 10.0	< 10.0	< 10.0	
	E027	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	27.3	31.1	25.6	28.0	2.8	0.00	0.00	0.00	0.00	0.00
	E052	< 2.0	< 2.0	< 2.0	< 2.0		< 2.0	< 2.0	< 2.0	< 2.0		< 2.0	< 2.0	< 2.0	< 2.0		28	30	29	29.0	1.0	6	6	7	6.33	0.58
	E053	< 2.5	< 2.5	< 2.5	< 2.5		< 2.5	< 2.5	< 2.5	< 2.5		< 2.5	< 2.5	< 2.5	< 2.5		41	32	40	37.7	4.9	5.5	5.1	6.4	5.67	0.67
	E054	< 2.0	< 2.0	< 2.0	< 2.0		< 2.0	< 2.0	< 2.0	< 2.0		< 2.0	< 2.0	< 2.0	< 2.0		27.26	27.41	30.4	28.4	1.8	4.46	3.5	4.83	4.26	0.69
	E055																36			36.0						
	E056	< 10.0	< 10.0	< 10.0	< 10.0		< 10.0	< 10.0	< 10.0	< 10.0		< 10.0	< 10.0	< 10.0	< 10.0		23	29		26.0	4.2	< 10.0	< 10.0	< 10.0	< 10.0	
	E058	< 2.5	< 2.5	< 2.5	< 2.5		< 2.5	< 2.5	< 2.5	< 2.5		< 2.5	< 2.5	< 2.5	< 2.5		32.95	33.22	29.51	31.9	2.1	6.34	6.21	5.46	6.00	0.48
	E059	< 10.0	< 10.0	< 10.0	< 10.0		< 10.0	< 10.0	< 10.0	< 10.0		< 10.0	< 10.0	< 10.0	< 10.0		< 10.0	< 10.0	< 10.0	< 10.0		< 10.0	< 10.0	< 10.0	< 10.0	
	E060	< 2.0	< 2.0	< 2.0	< 2.0		< 2.0	< 2.0	< 2.0	< 2.0		< 2.0	< 2.0	< 2.0	< 2.0		33	33	32	32.7	0.6	6.2	5.8	5.9	5.97	0.21
	E061	< 10.0	< 10.0	< 10.0	< 10.0		< 10.0	< 10.0	< 10.0	< 10.0		< 10.0	< 10.0	< 10.0	< 10.0		23	23	21	22.3	1.2	< 10.0	< 10.0	< 10.0	< 10.0	
	E062	< 2.0	< 2.0	< 2.0	< 2.0		< 2.0	< 2.0	< 2.0	< 2.0		< 2.0	< 2.0	< 2.0	< 2.0		32.6	33.8	33.9	33.4	0.7	6.61	6.43	6.76	6.60	0.17
	E072																									
Community Results		Consensus Mean			0.41		Consensus Mean			0.30		Consensus Mean					Consensus Mean			30.0		Consensus Mean			5.75	
		Consensus Standard Deviation			0.68		Consensus Standard Deviation			0.50		Consensus Standard Deviation					Consensus Standard Deviation			1.8		Consensus Standard Deviation			0.41	
		Maximum			1.23		Maximum			0.90		Maximum			0.00		Maximum			37.7		Maximum			6.60	
		Minimum			0.00		Minimum			0.00		Minimum			0.00		Minimum			22.3		Minimum			0.00	
		N			2		N			2		N			1		N			11		N			8	

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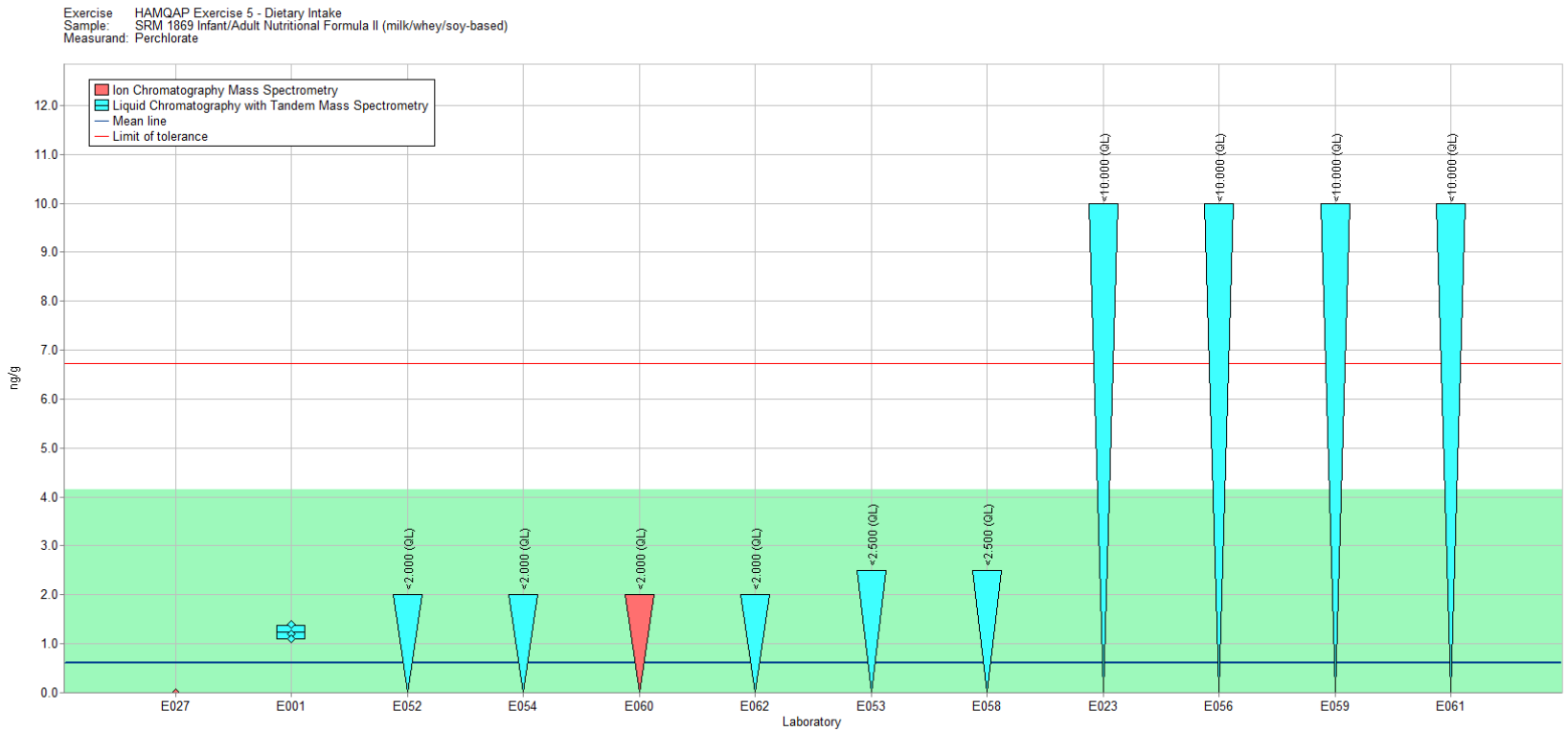

Figure 8-17. Perchlorate in SRM 1869 Infant/Adult Nutritional Formula II (milk/whey/soy-based) (data summary view – analytical method). In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red line represents the upper consensus range of tolerance, calculated as the values above the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$, with the lower limit set at zero. A NIST value has not been determined in this material.

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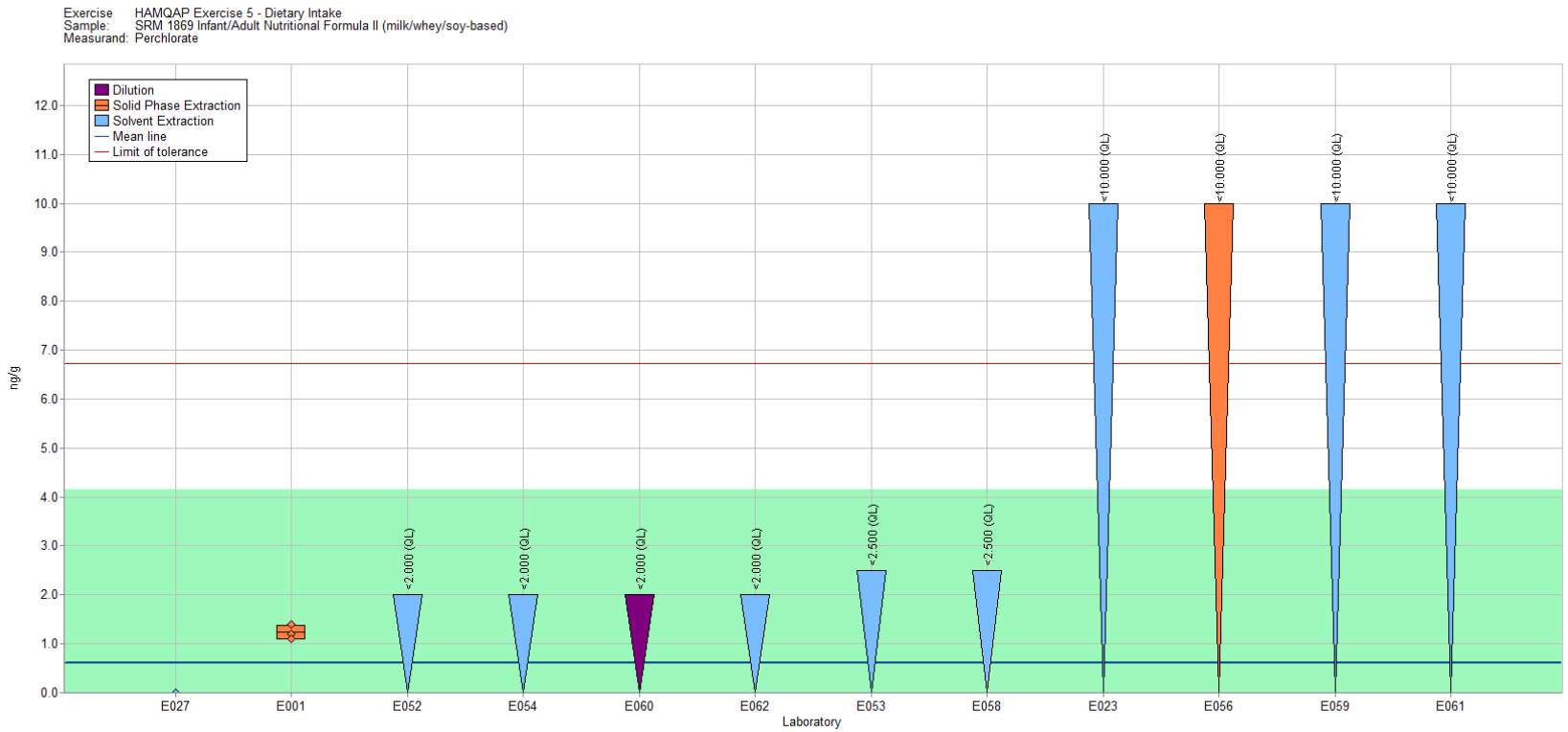

Figure 8-18. Perchlorate in SRM 1869 Infant/Adult Nutritional Formula II (milk/whey/soy-based) (data summary view – sample preparation method). In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red line represents the upper consensus range of tolerance, calculated as the values above the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$, with the lower limit set at zero. A NIST value has not been determined in this material.

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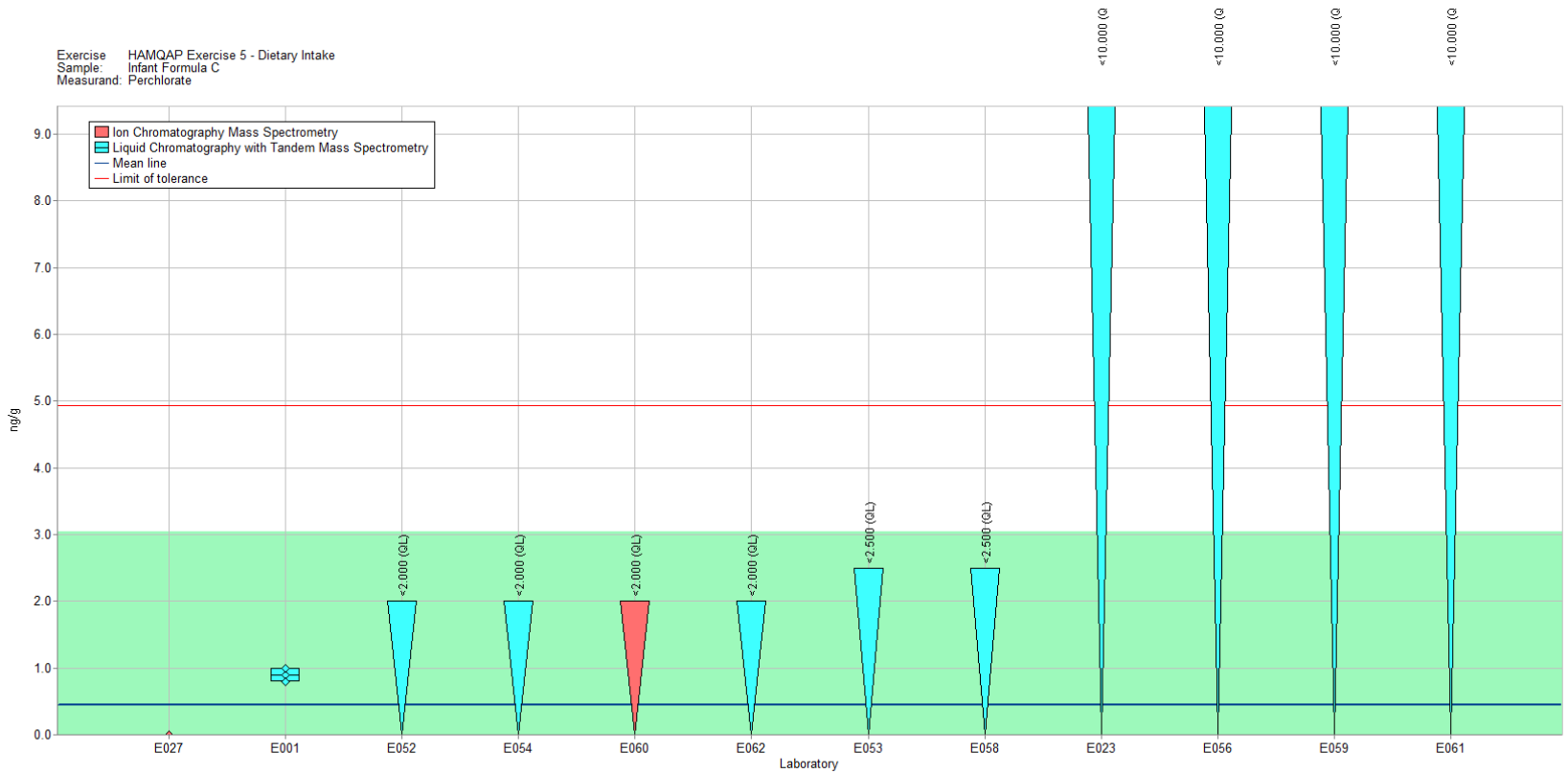

Figure 8-19. Perchlorate in Infant Formula C (data summary view – analytical method). In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red line represents the upper consensus range of tolerance, calculated as the values above the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$, with the lower limit set at zero. A NIST value has not been determined in this material.

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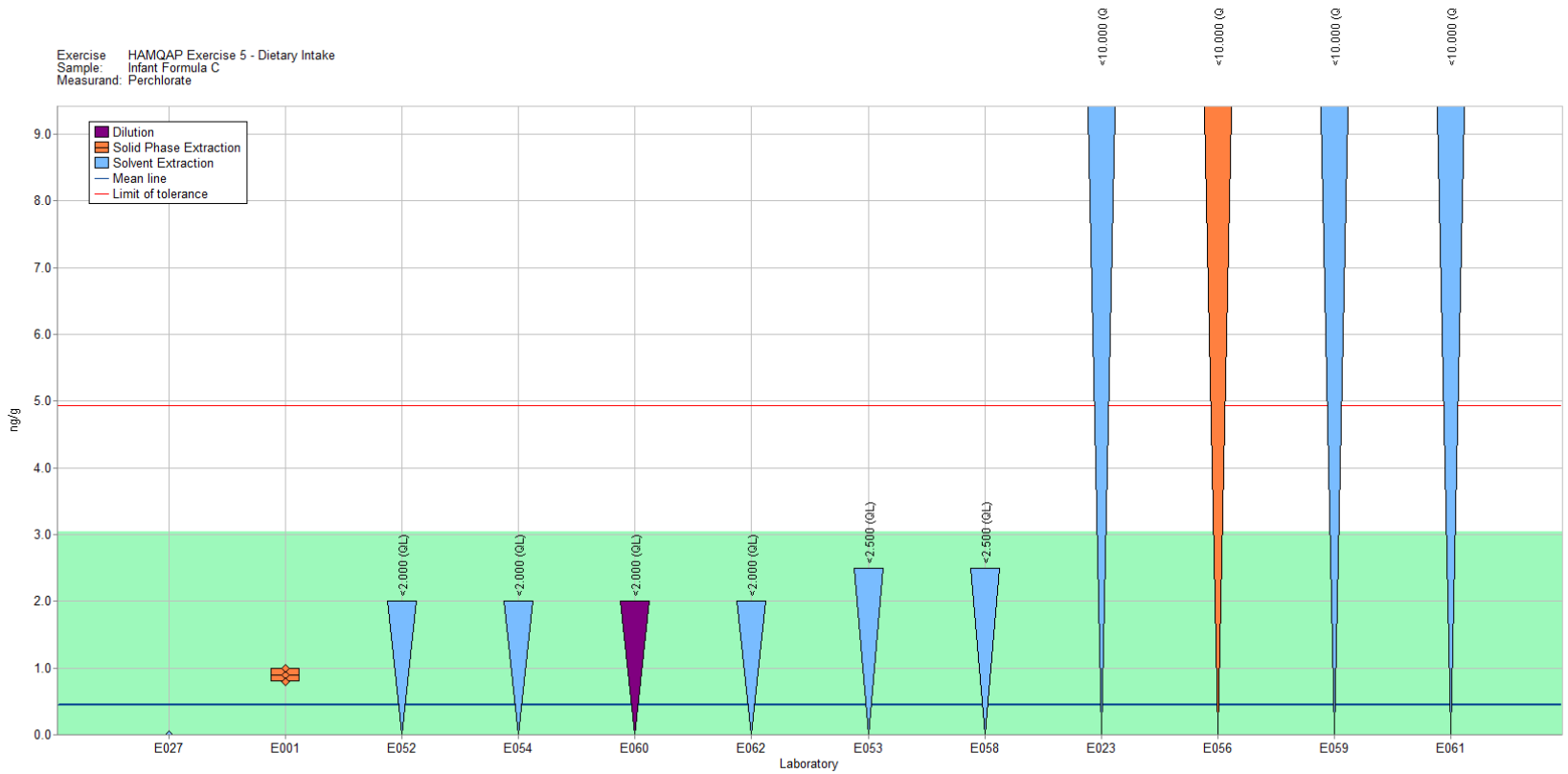

Figure 8-20. Perchlorate in Infant Formula C (data summary view – sample preparation method). In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red line represents the upper consensus range of tolerance, calculated as the values above the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$, with the lower limit set at zero. A NIST value has not been determined in this material.

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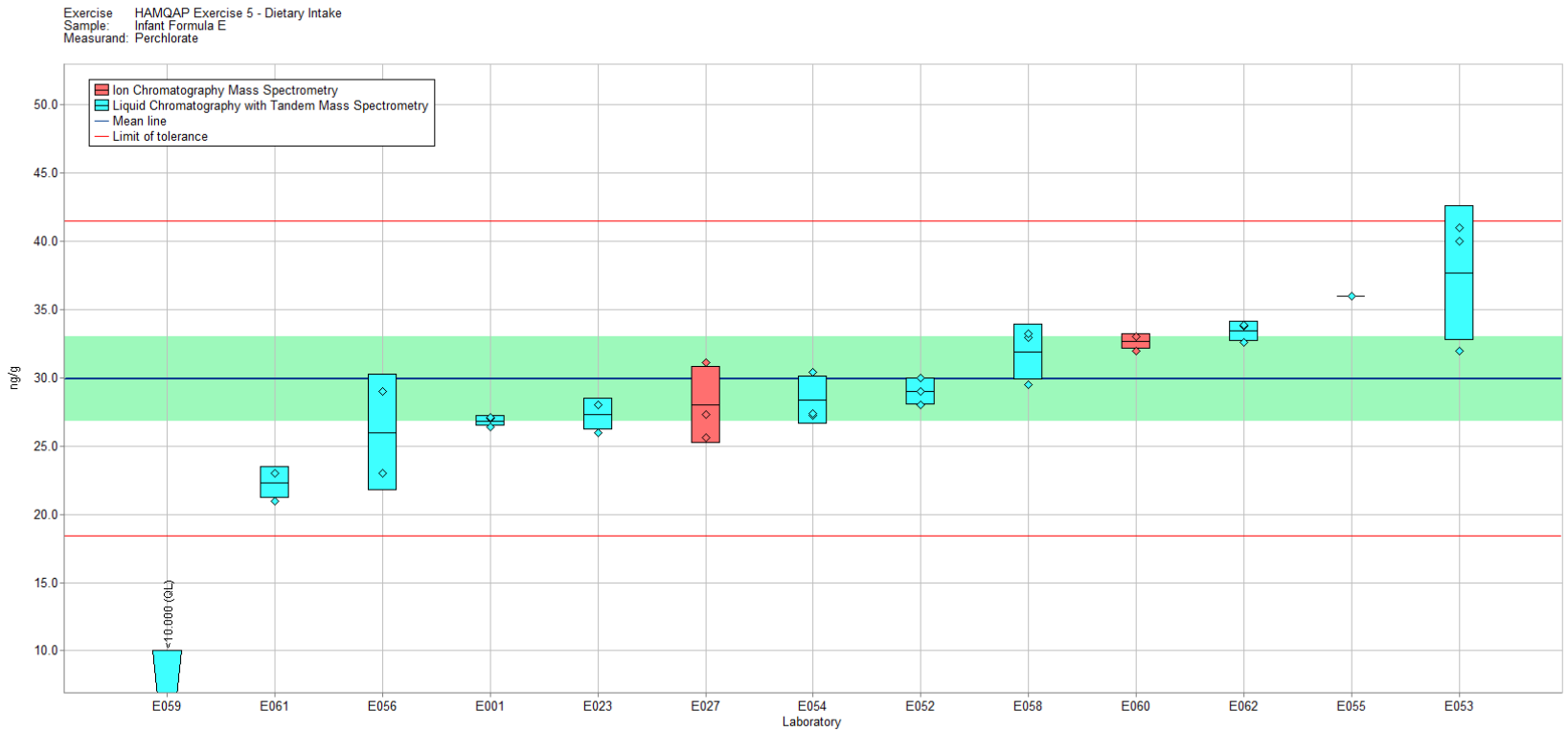

Figure 8-21. Perchlorate in Infant Formula E (data summary view – analytical method). In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line 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.

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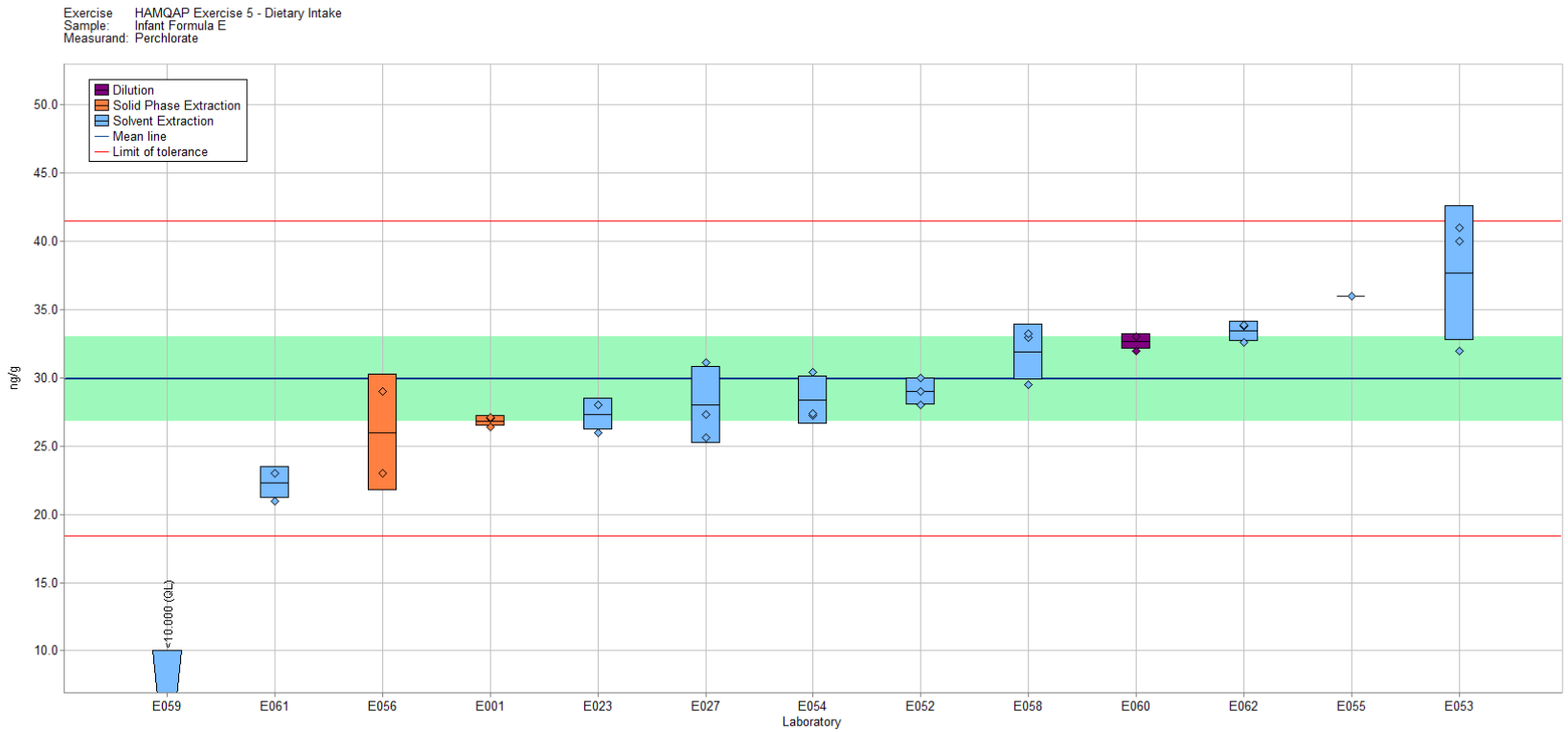

Figure 8-22. Perchlorate in Infant Formula E (data summary view – sample preparation method). In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The 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, $|Z'_{\text{comm}}| \leq 2$. A NIST value has not been determined in this material.

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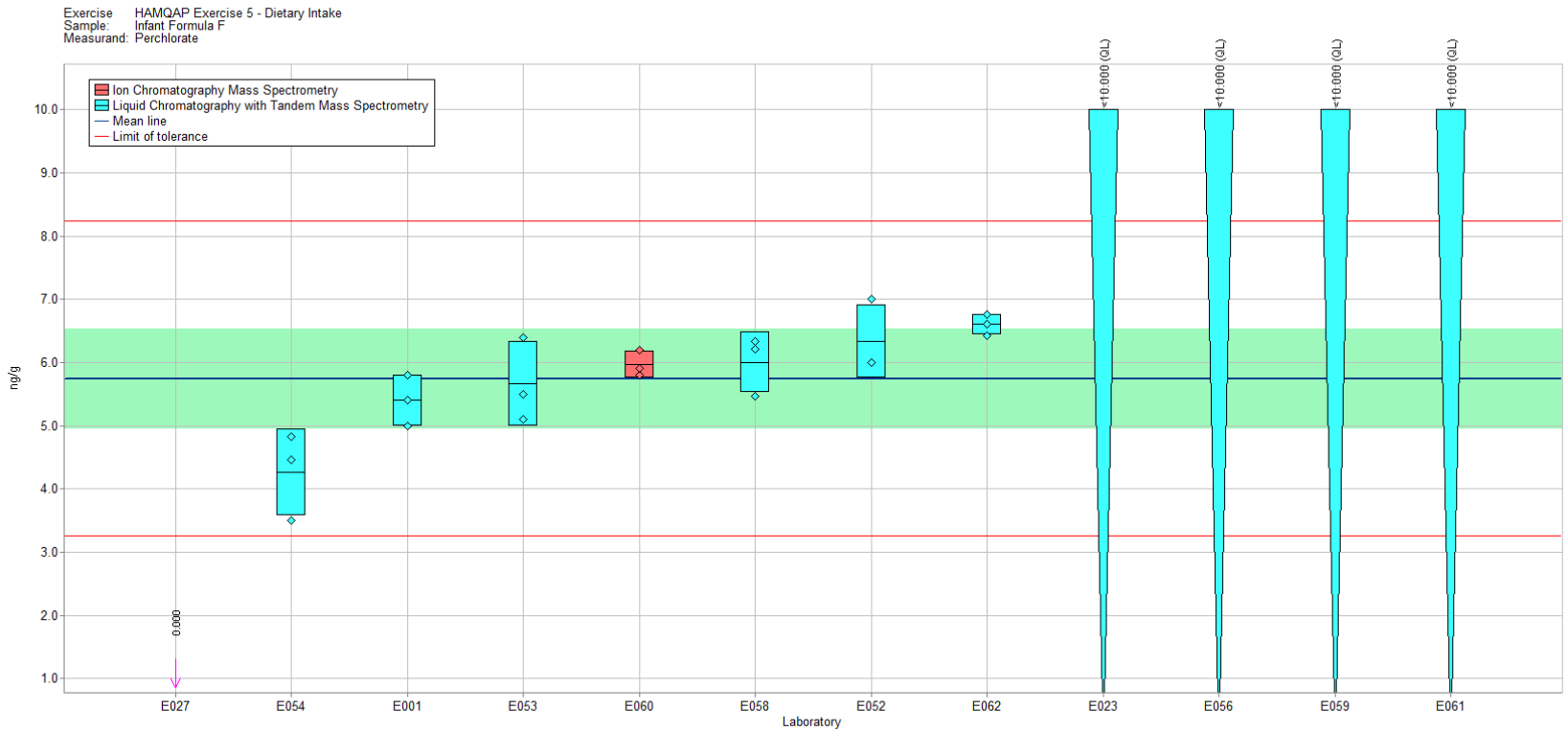

Figure 8-23. Perchlorate in Infant Formula F (data summary view – analytical method). In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line 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.

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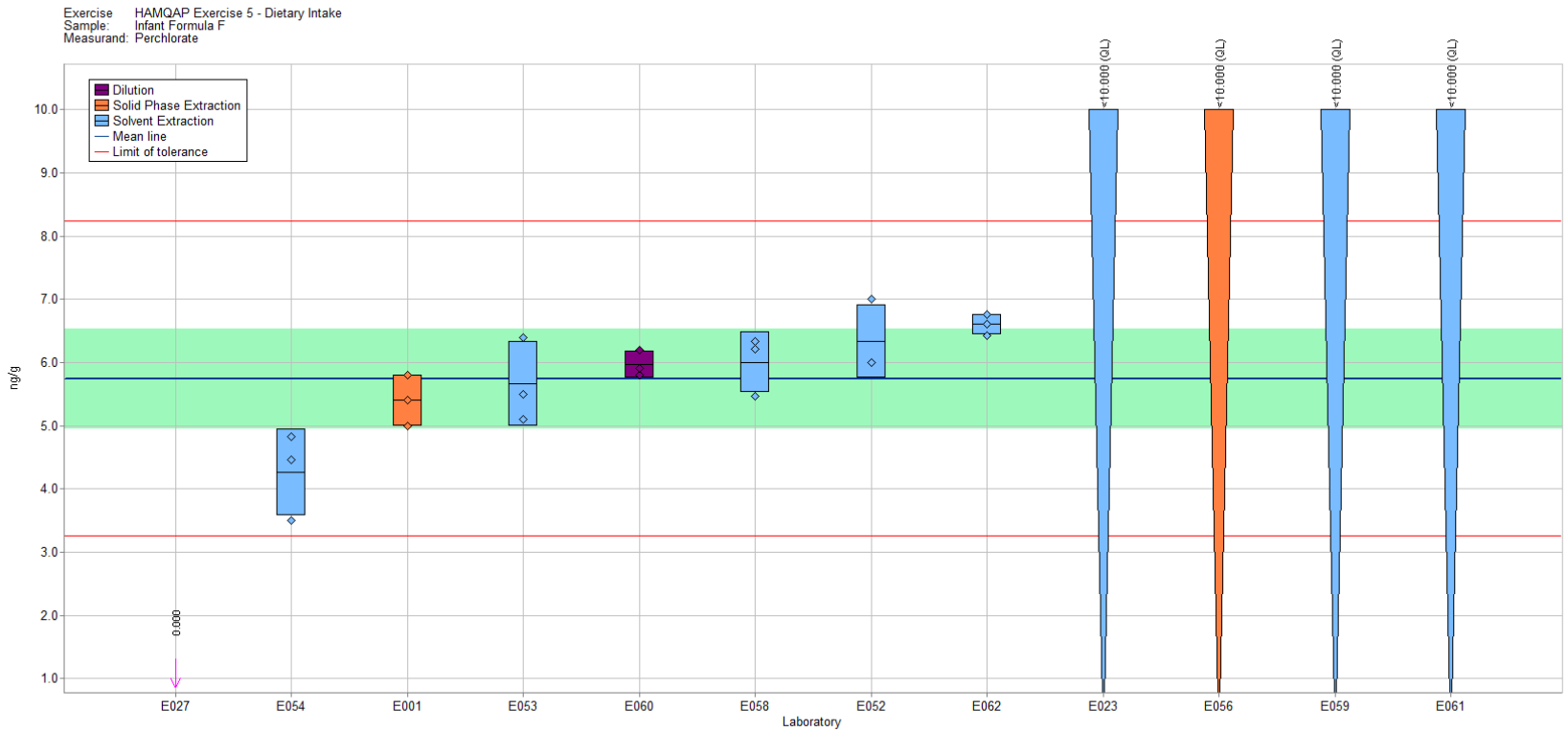

Figure 8-24. Perchlorate in Infant Formula F (data summary view – sample preparation method). In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The 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, $|Z'_{\text{comm}}| \leq 2$. A NIST value has not been determined in this material.

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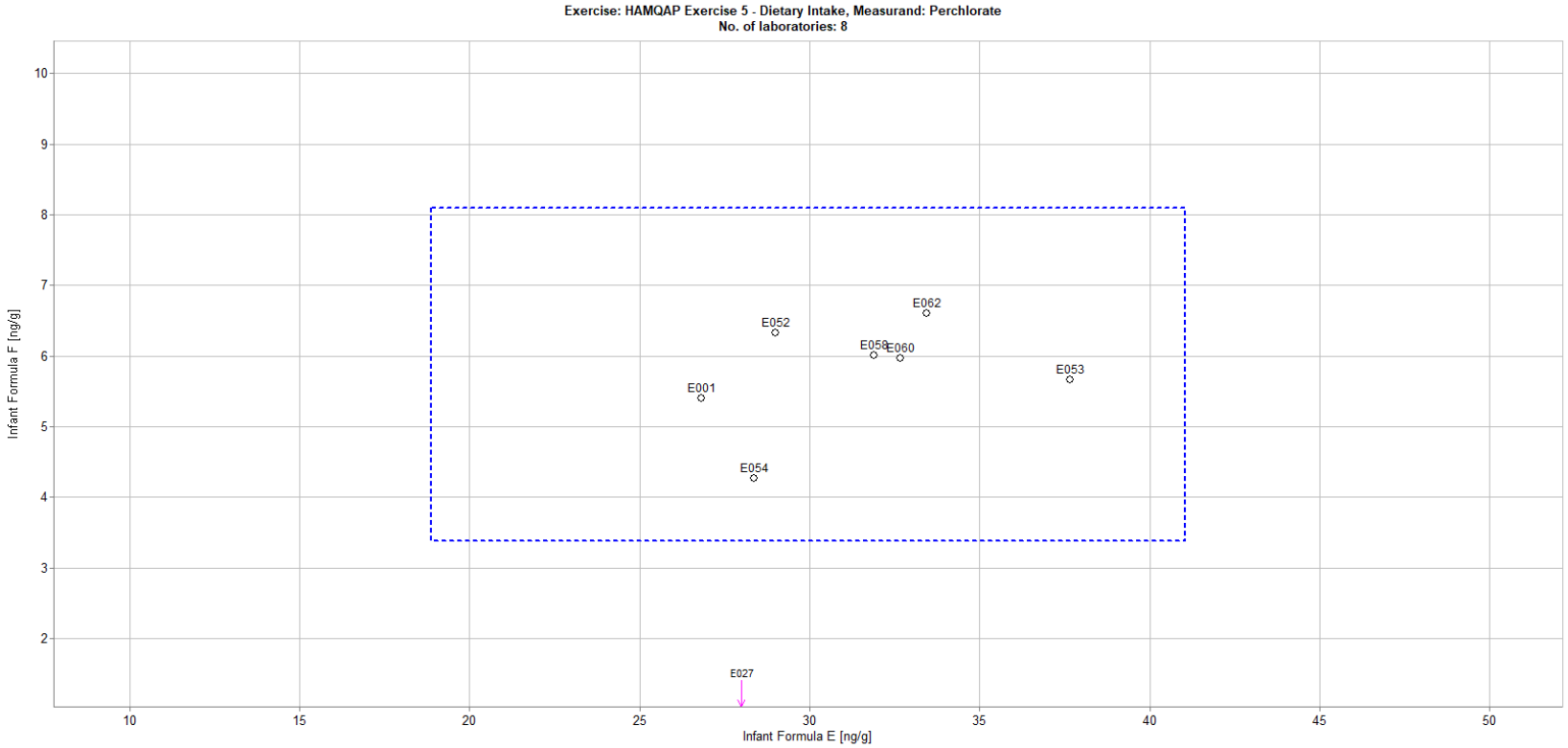

Figure 8-6. Laboratory means for perchlorate in Infant Formula E and Infant Formula F (sample/sample comparison view). In this view, the individual laboratory mean for one sample (Infant Formula E) is compared to the mean for a second sample (Infant Formula F). The dotted blue box represents the consensus range of tolerance for Infant Formula E (x-axis) and Infant Formula F (y-axis), calculated as the values above and below the consensus means that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$.

SECTION 9: PROXIMATES

Study Overview

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

Dietary Intake Sample Information

Almond Flour. Participants were provided with three packets each containing 5 g of blanched ground almond flour. Participants were asked to store the material under refrigeration, 2 °C to 8 °C, to use a sample size appropriate for their in-house method of analysis, and to prepare one sample and report one value from each packet provided. Before use, participants were instructed to mix the contents of the packet thoroughly and use a nitrogen conversion factor of 5.18 for calculation of total protein, as recommended in AOAC Official Method 950.48. The approximate analyte levels were not reported to participants prior to the study, and target values for proximates and calories in the almond flour have not been determined at NIST.

Hazelnut Flour. Participants were provided with three packets each containing 5 g of ground hazelnut flour. Participants were asked to store the material under refrigeration, 2 °C to 8 °C, to use a sample size appropriate for their in-house method of analysis, and to prepare one sample and report one value from each packet provided. Before use, participants were instructed to mix the contents of the packet thoroughly and use a nitrogen conversion factor of 5.30 for calculation of total protein, as recommended in AOAC Official Method 950.48. The approximate analyte levels were not reported to participants prior to the study, and target values for proximates and calories in the hazelnut flour have not been determined at NIST.

Dietary Intake Study Results

- Sixteen laboratories enrolled in this exercise and received samples to measure one or more analyte in the nut flours.
  - Six laboratories reported results for ash in each sample (38 % participation).
  - Four laboratories reported results for each of the other proximates in each sample (25 % participation).
- The between-laboratory variability for fat was 7 % in the almond flour and 15 % in the hazelnut flour. Laboratories reported determination of fat through summation of total fatty acids as triglycerides (75 %) or Rose-Gottlieb/Mojonnier acid extraction (25 %).
- The between-laboratory variability for protein was 10 % in the almond flour and 8 % in the hazelnut flour. Laboratories reported determination of nitrogen by combustion (50 %) or Kjeldahl (50 %), and conversion to protein using the recommended factors of 5.18 for almond flour and 5.30 for hazelnut flour.

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- The between-laboratory variability for carbohydrates was 55 % in the almond flour and 11 % in the hazelnut flour. Most laboratories reported determination of carbohydrates through calculation (75 %). One laboratory (25 %) did not report the method used.
- The between-laboratory variability for calories was 3 % in the almond flour and 10 % in the hazelnut flour. All laboratories reported determination of calories through calculation (100 %).
- The between-laboratory variability for ash was 8 % in the almond flour and 7 % in the hazelnut flour. Most laboratories reported determination of ash by weight loss after ignition in a muffle furnace (67 %). One laboratory (17 %) reported using thermogravimetric analysis, and one laboratory did not report the method used.
- The between-laboratory variability for solids was less than 1 % in both flours. Laboratories reported determination of solids by drying in a forced-air oven (50 %), drying in a vacuum oven (25 %), or by thermogravimetric analysis (25 %).

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.

- In general, all results should be checked closely to avoid calculation errors and to be sure that results are reported in the requested units.
  - One laboratory reported extremely high, outlying results for ash in both materials. These outlying results were likely due to a miscalculation or misinterpretation of the requested data.
  - Two laboratories reported extremely low, outlying results for calories in both materials. These outlying results were likely due to a misinterpretation of the requested units.
- The use of appropriate calibration materials and quality assurance samples to establish that a method is in control and performing correctly may reduce the likelihood of outlying data. Quality assurance samples can be commercially available reference materials (CRMs, SRMs, or RMs) or materials prepared in-house. Numerous food matrix CRMs are available with assigned values for proximates and calories.
- A linear calibration curve which surrounds the expected sample concentration values should be used for calculations. This curve should include both the lowest and highest expected concentration values of the sample solutions. Extrapolation of results beyond calibration curves may result in incorrect values.

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Table 9-1. Individualized data summary table (NIST) for proximates in almond and hazelnut flour.

National Institute of Standards & Technology

HAMQAP Exercise 5 - Proximates

	Lab Code:	**NIST**	**1. Your Results**				**2. Community Results**			**3. Target**	
Analyte	Sample	Units	x_i	s_i	Z'_{comm}	Z_{NIST}	N	x*	s*	x_{NIST}	*U*
Fat	Almond Flour	%					4	55.7	4.1		
Fat	Hazelnut Flour	%					4	11.7	1.7		
Protein	Almond Flour	%					4	26.1	2.7		
Protein	Hazelnut Flour	%					4	35.2	2.9		
Carbohydrates	Almond Flour	%					4	10.6	5.8		
Carbohydrates	Hazelnut Flour	%					4	37.2	4.1		
Calories	Almond Flour	kcal/100 g					4	665	18		
Calories	Hazelnut Flour	kcal/100 g					4	386	38		
Ash	Almond Flour	%					6	2.89	0.23		
Ash	Hazelnut Flour	%					6	5.63	0.42		
Solids	Almond Flour	%					4	96.3	0.77		
Solids	Hazelnut Flour	%					4	94.6	0.7		

x_i Mean of reported values

s_i Standard deviation of reported values

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

Z_{NIST} Z-score with respect to NIST value

N Number of quantitative values reported

x* Robust mean of reported values

s* Robust standard deviation

x_{NIST} NIST-assessed value

U expanded uncertainty about the NIST-assessed value

Table 9-2. Data summary table for fat in almond and hazelnut flour.

		Fat									
		Almond Flour (%)					Hazelnut Flour (%)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target										
	E001										
	E002	58.64	58.26	59.01	58.6	0.4	12.41	12.78	12.58	12.6	0.2
	E006										
	E021										
	E029										
	E030	51.5	50	49.7	50.4	1.0	10.8	10.8	10.5	10.7	0.2
	E031										
	E033	62.34	59.6	59.2	60.4	1.7	9.5	9.4	9.4	9.4	0.1
	E037										
	E047	53.71	53.09		53.4	0.4	13.06	14.73		13.9	1.2
Community Results		Consensus Mean			55.7		Consensus Mean			11.7	
		Consensus Standard Deviation			4.1		Consensus Standard Deviation			1.7	
		Maximum			60.4		Maximum			13.9	
		Minimum			50.4		Minimum			9.4	
		N			4		N			4	

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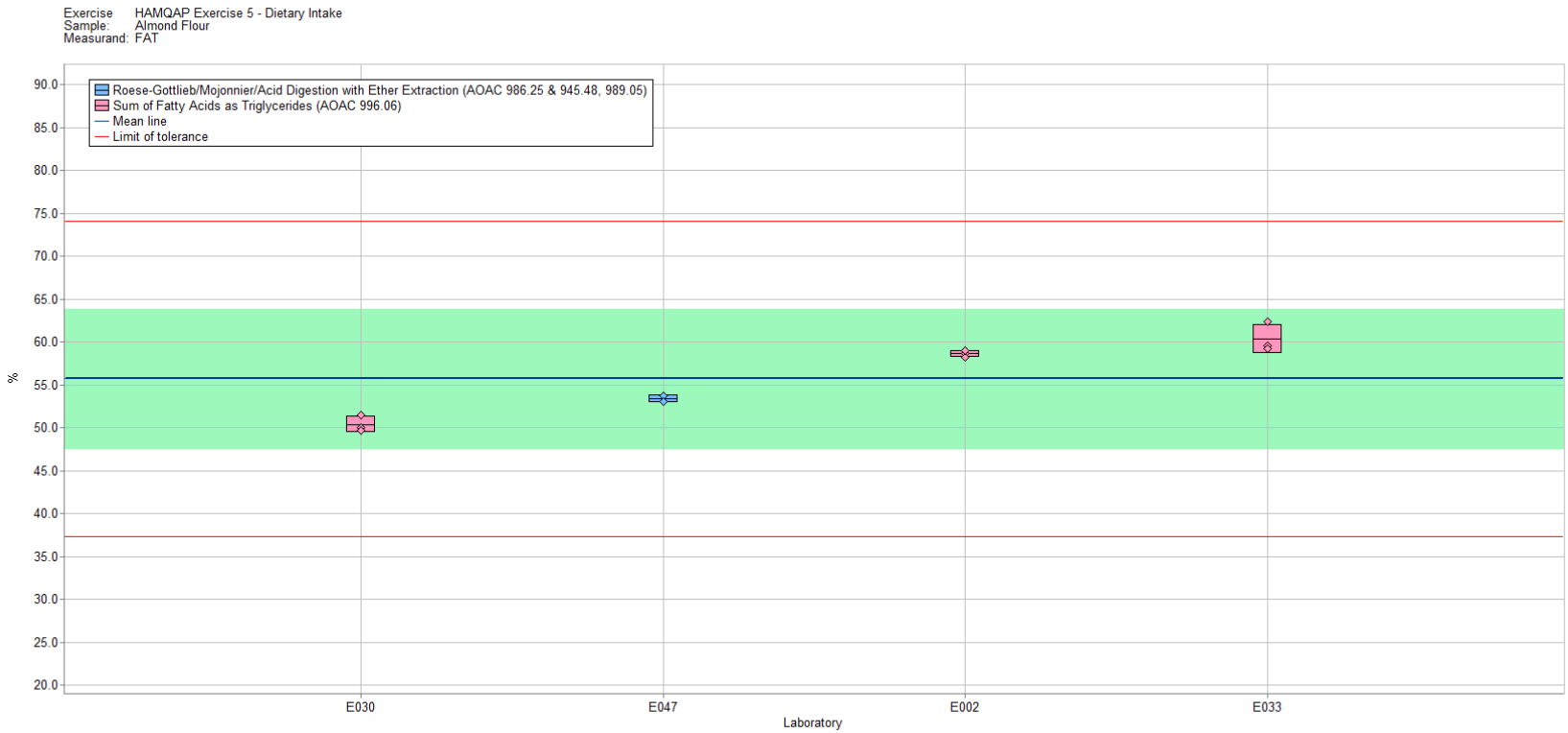

Figure 9-1. Fat in Almond Flour (data summary view – analytical method). In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line represents 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.

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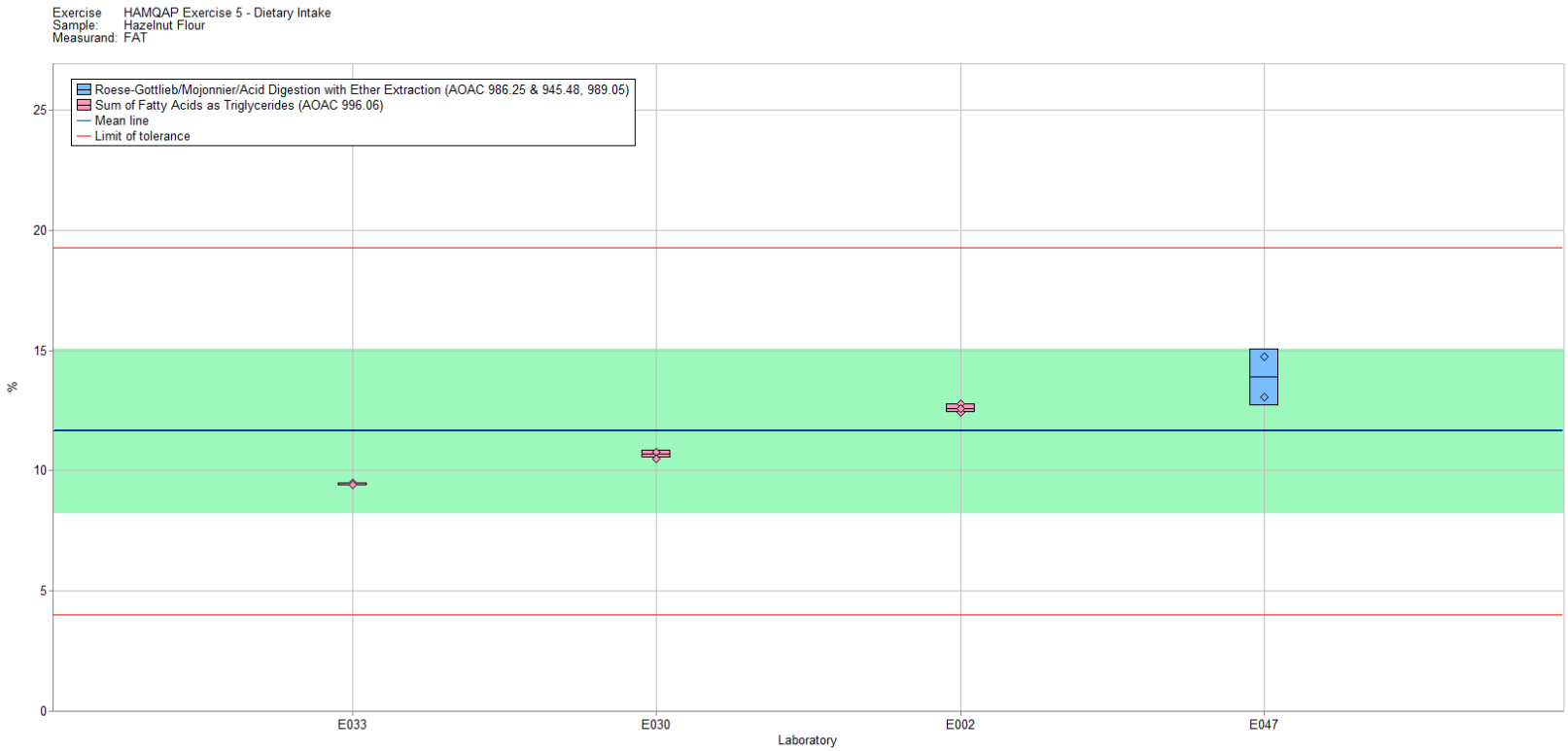

Figure 9-2. Fat in Hazelnut Flour (data summary view – analytical method). In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line represents 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.

Table 9-3. Data summary table for protein in almond and hazelnut flour.

		Protein									
		Almond Flour (%)					Hazelnut Flour (%)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target										
	E001										
	E002	24.9	25.28	24.8	25.0	0.3	35.38	35.58	35.86	35.6	0.2
	E006										
	E019										
	E020										
	E021										
	E030	22.4	22.7	22.7	22.6	0.2	33.7	33	31.9	32.9	0.9
	E031										
	E033	28.8	28.3	28.8	28.6	0.3	32.8	30.9	31.4	31.7	1.0
	E036										
	E037										
	E042										
	E047	28	28.1		28.1	0.1	40.6	40.4		40.5	0.1
Community Results		Consensus Mean			26.1		Consensus Mean			35.2	
		Consensus Standard Deviation			2.7		Consensus Standard Deviation			2.9	
		Maximum			28.6		Maximum			40.5	
		Minimum			22.6		Minimum			31.7	
		N			4		N			4	

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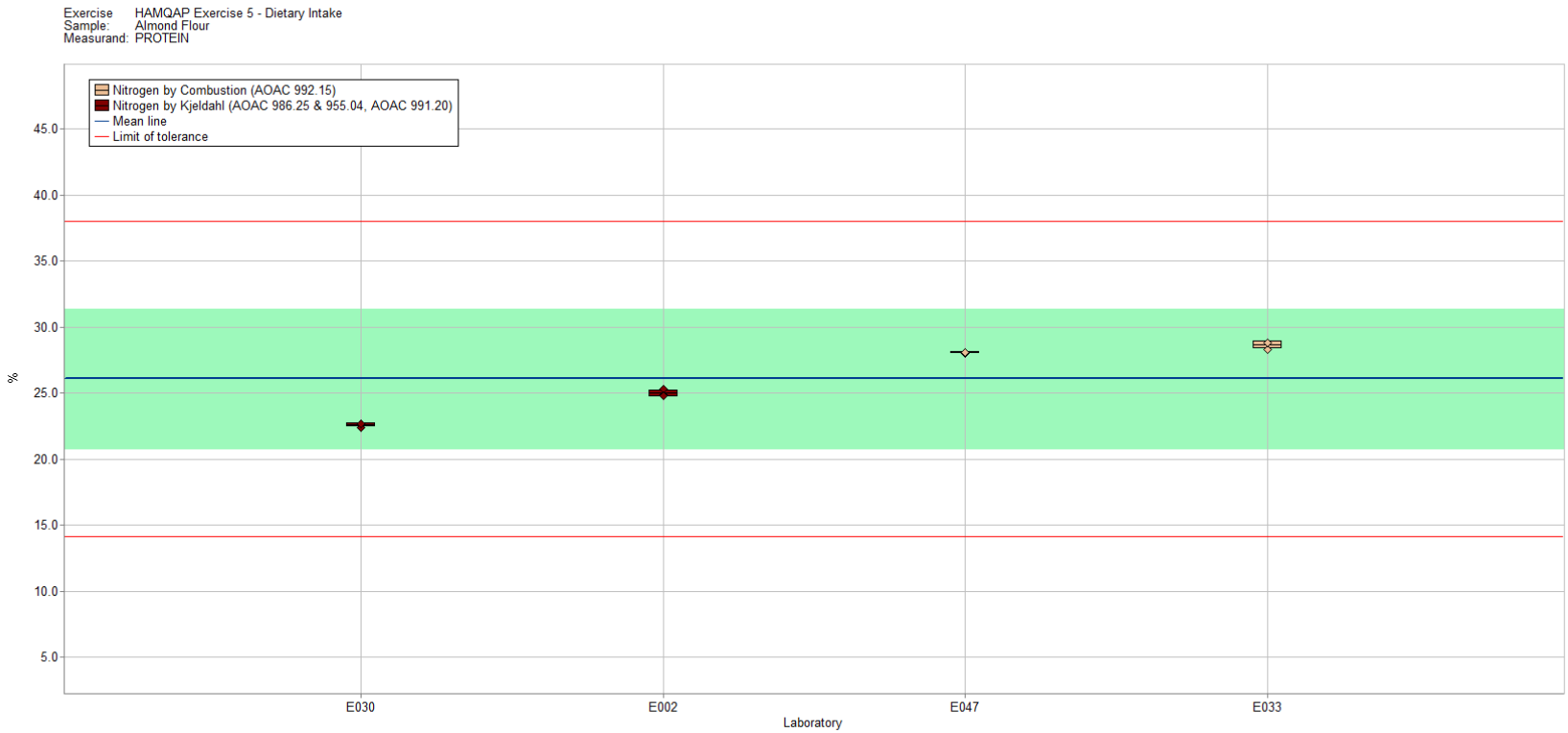

Figure 9-3. Protein in Almond Flour (data summary view – analytical method). In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line represents 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.

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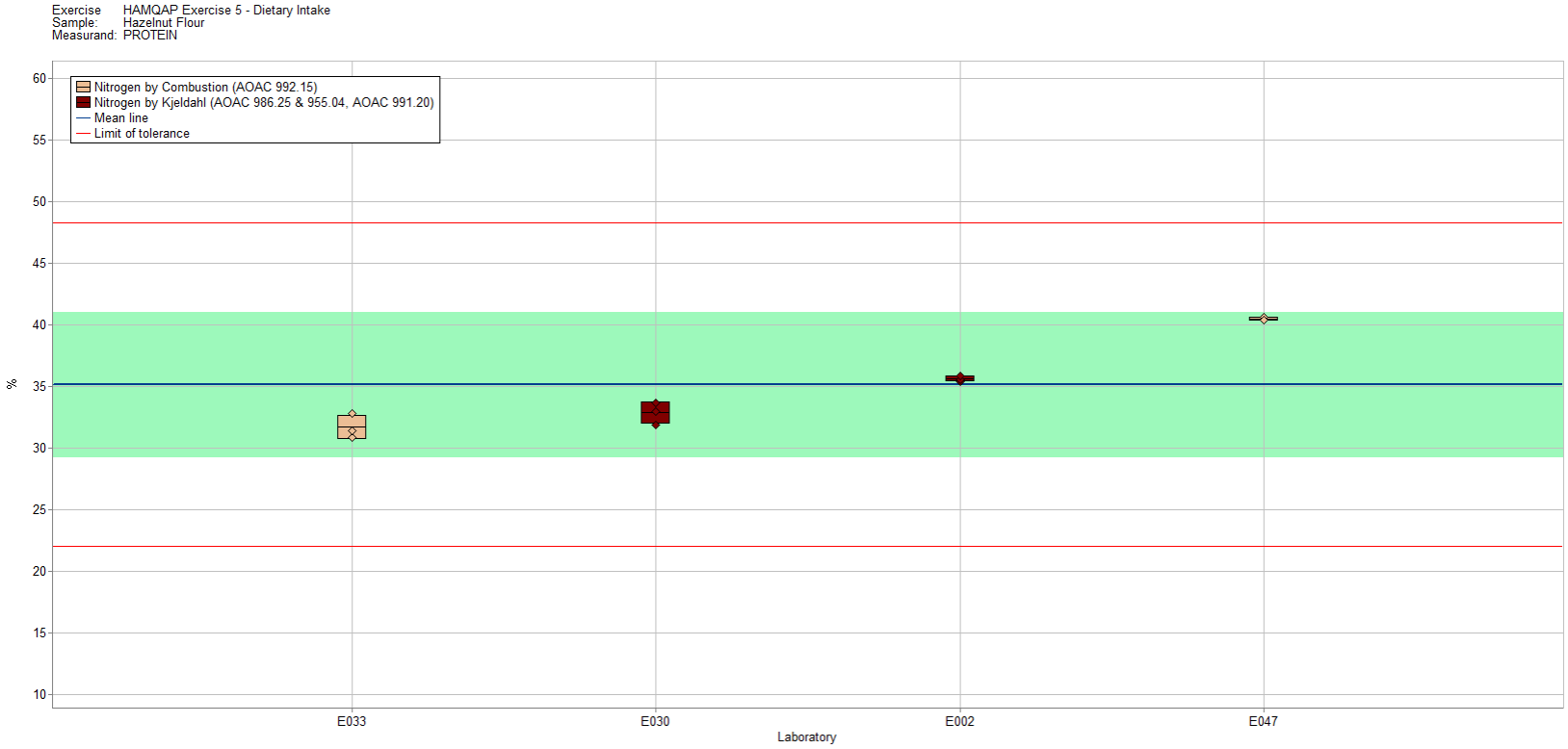

Figure 9-4. Protein in Hazelnut Flour (data summary view – analytical method). In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line represents 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.

Table 9-4. Data summary table for carbohydrates in almond and hazelnut flour.

		Carbohydrates									
		Almond Flour (%)					Hazelnut Flour (%)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target										
	E001										
	E002	7.02	6.95	7.16	7.0	0.1	29.43	29.4	29.46	29.4	0.0
	E006										
	E009										
	E019										
	E020										
	E021										
	E029										
	E030	18.9	19.8	20.2	19.6	0.7	43.2	43.5	45.6	44.1	1.3
	E031										
	E033	1.5	5.2	4.8	3.8	2.0	45.2	47.8	31.4	41.5	8.8
	E035										
	E036										
	E037										
	E042										
	E047	11.7	12.21		12.0	0.4	34.41	33.17		33.8	0.9
Community Results		Consensus Mean			10.6		Consensus Mean			37.2	
		Consensus Standard Deviation			5.8		Consensus Standard Deviation			4.1	
		Maximum			19.6		Maximum			44.1	
		Minimum			3.8		Minimum			29.4	
		N			4		N			4	

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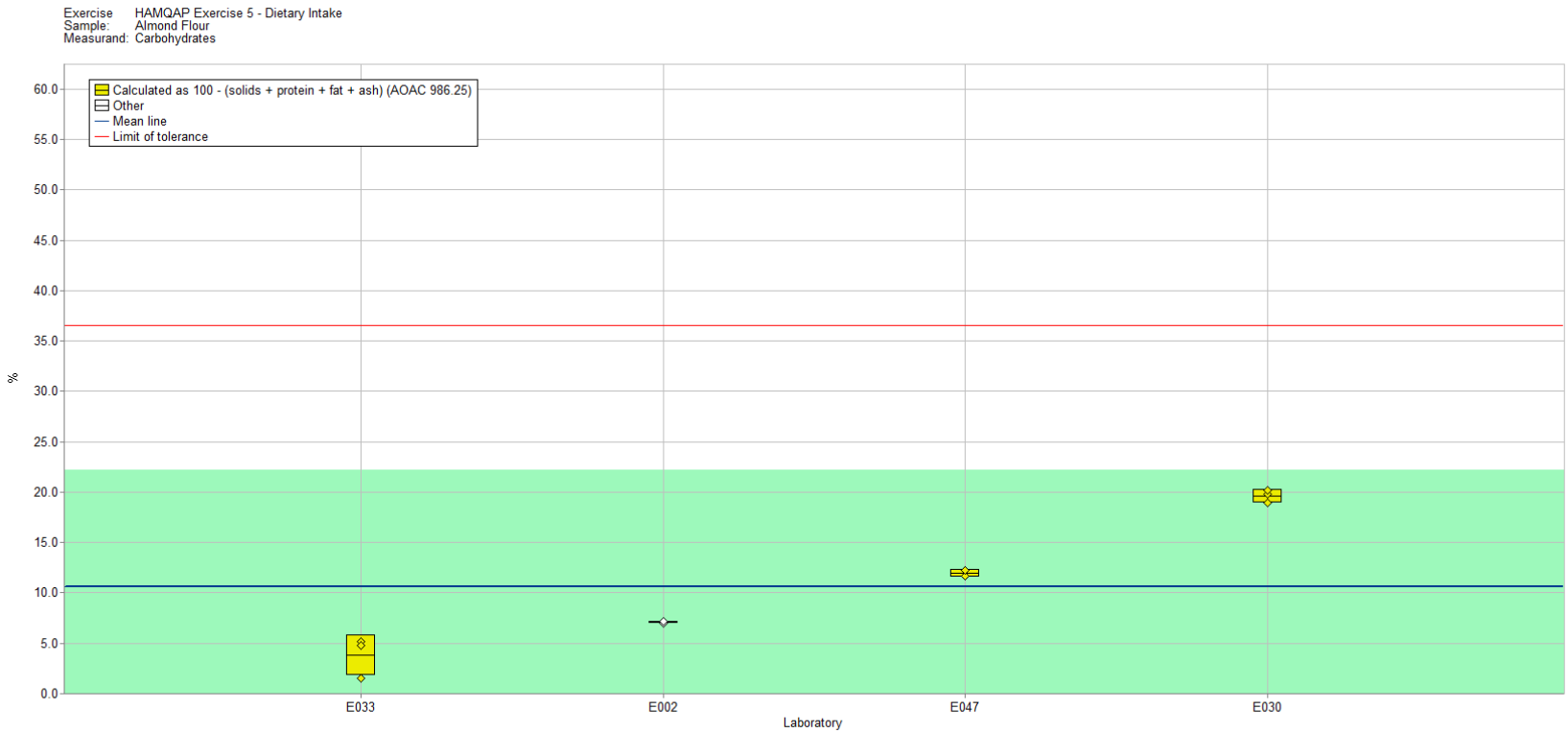

Figure 9-5. Carbohydrates in Almond Flour (data summary view – analytical method). In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red line represents the upper consensus range of tolerance, calculated as the values above the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$, with the lower limit set at zero. A NIST value has not been determined in this material.

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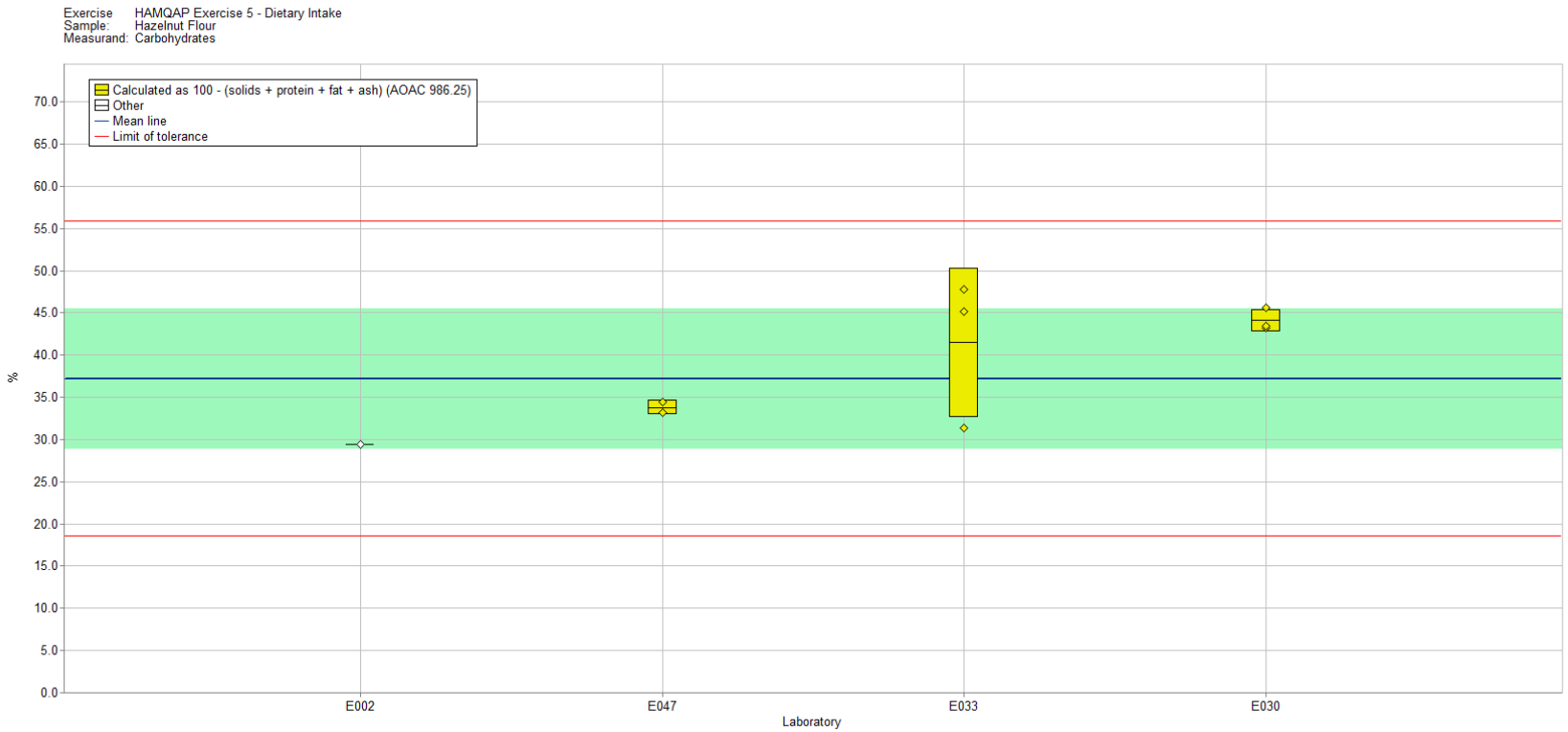

Figure 9-6. Carbohydrates in Hazelnut Flour (data summary view – analytical method). In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line 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.

Table 9-5. Data summary table for calories in almond and hazelnut flour. Data points highlighted in red have been flagged as potential outliers (e.g., Grubb and/or Cochran) by the NIST software package.

		Calories									
		Almond Flour (kcal/100 g)					Hazelnut Flour (kcal/100 g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target										
	E001										
	E002	655.44	653.26	658.93	656	3	370.93	374.94	374.5	373	2
	E006										
	E021										
	E030	0.629	0.62	0.619	0.623	0.006	0.405	0.403	0.404	0.404	0.001
	E033	682.4	670.2	667.3	673	8	397.5	399.3	400.8	399	2
	E047	0.642	0.639		0.641	0.002	0.418	0.454		0.436	0.025
Community Results		Consensus Mean			665		Consensus Mean			386	
		Consensus Standard Deviation			18		Consensus Standard Deviation			38	
		Maximum			673		Maximum			399	
		Minimum			0.623		Minimum			0.404	
		N			4		N			4	

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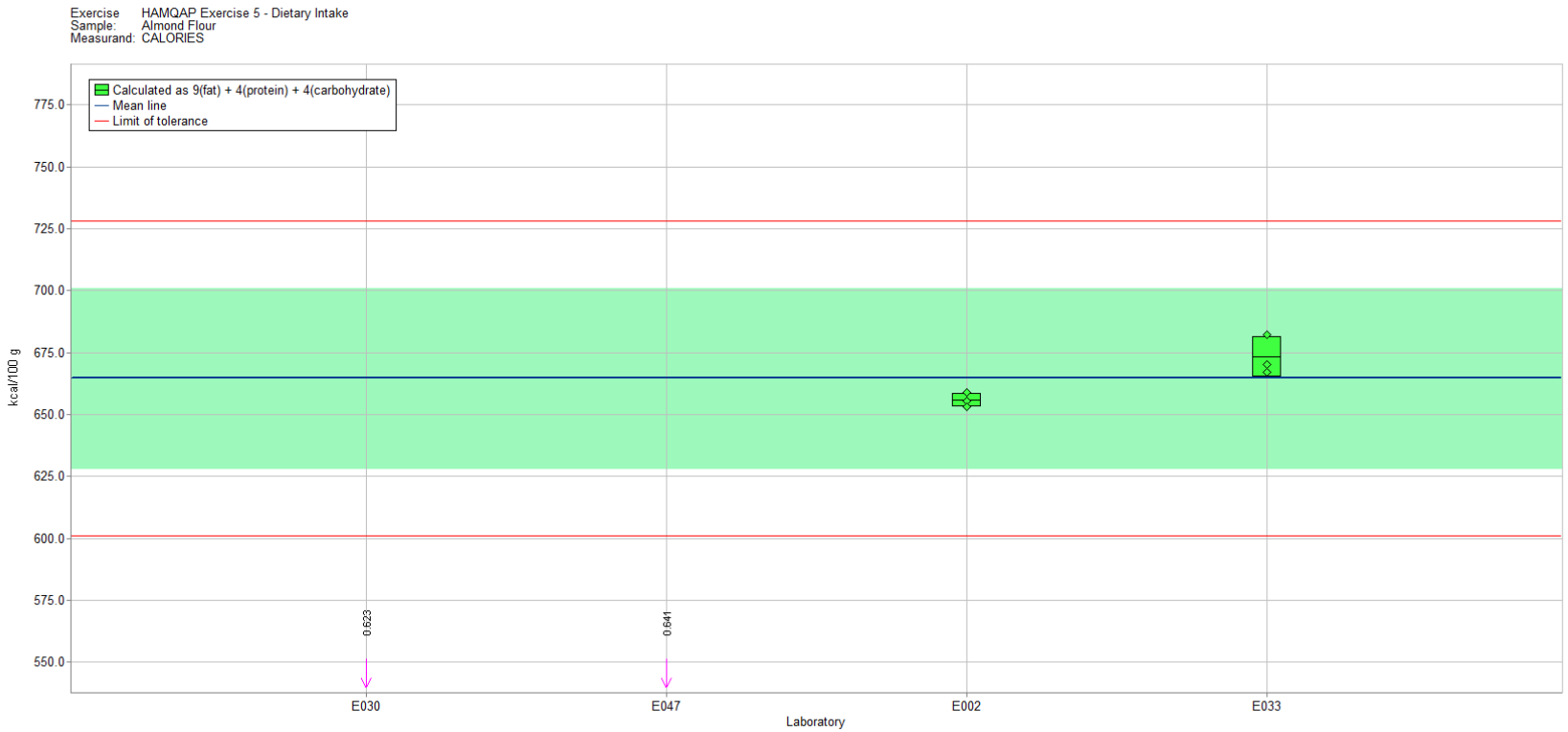

Figure 9-7. Calories in Almond Flour (data summary view – analytical method). In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line represents 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.

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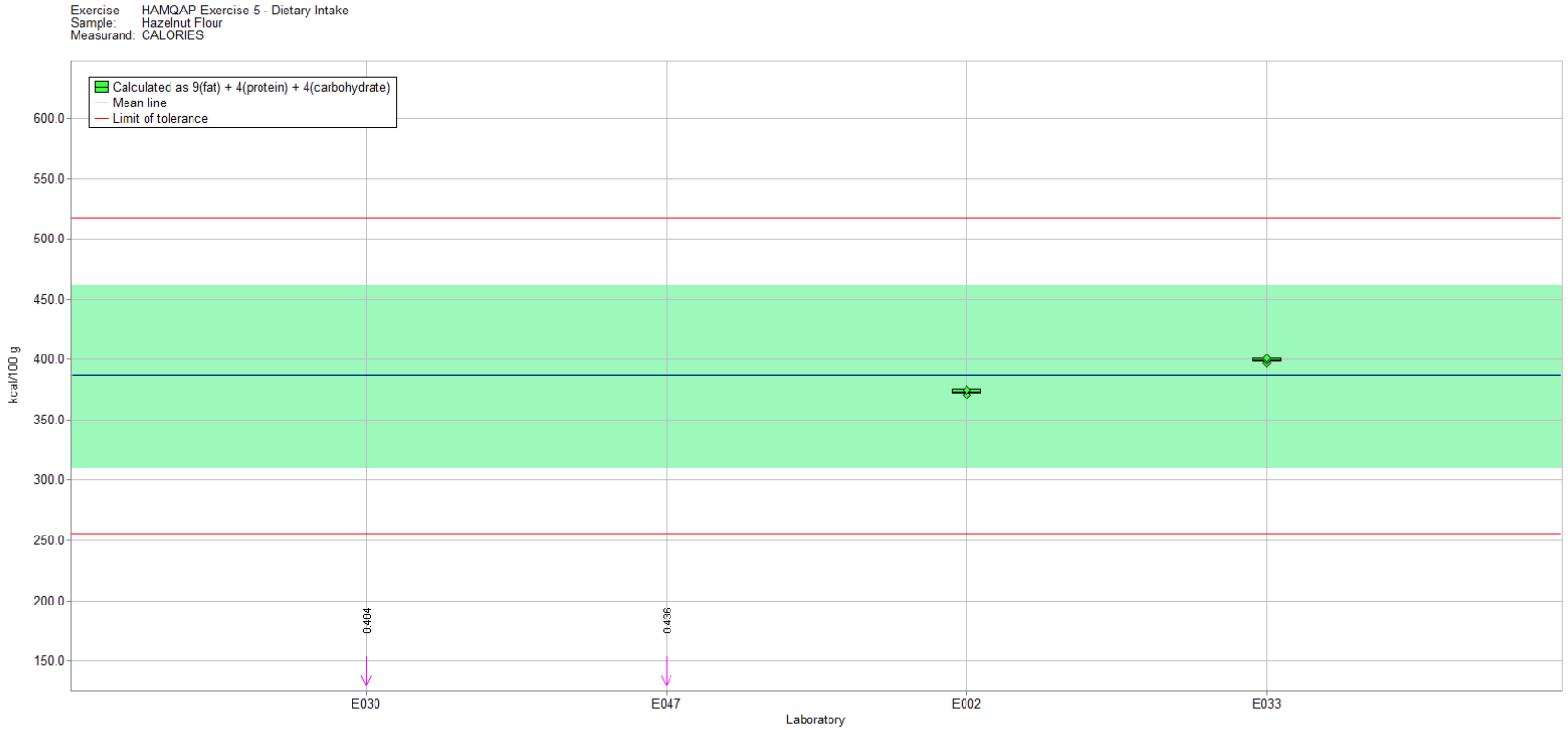

Figure 9-8. Calories in Hazelnut Flour (data summary view – analytical method). In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line represents 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.

Table 9-6. Data summary table for ash in almond and hazelnut flour. Data points highlighted in red have been flagged as potential outliers (e.g., Grubb and/or Cochran) by the NIST software package.

		Ash									
		Almond Flour (%)					Hazelnut Flour (%)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target										
	E001										
	E002	3.21	3.17	3.1	3.16	0.06	6.2	6.18	6.25	6.21	0.04
	E006										
	E009	2.61	2.81	2.37	2.60	0.22	3.57	3.43	3.3	3.43	0.14
	E019										
	E020										
	E021										
	E029										
	E030	2.69	2.67	2.67	2.68	0.01	5.68	6.13	5.73	5.85	0.25
	E031										
	E033	2.83	2.85	2.87	2.85	0.02	5.91	5.83	5.45	5.73	0.25
	E035	84.6	78.4	69.9	77.63	7.38	93.01	92.66	92.35	92.67	0.33
	E036										
	E037										
	E047	3.19	3.1		3.15	0.06	6.41	6.24		6.33	0.12
Community Results		Consensus Mean			2.89		Consensus Mean			5.63	
		Consensus Standard Deviation			0.23		Consensus Standard Deviation			0.42	
		Maximum			77.63		Maximum			92.67	
		Minimum			2.60		Minimum			3.43	
		N			6		N			6	

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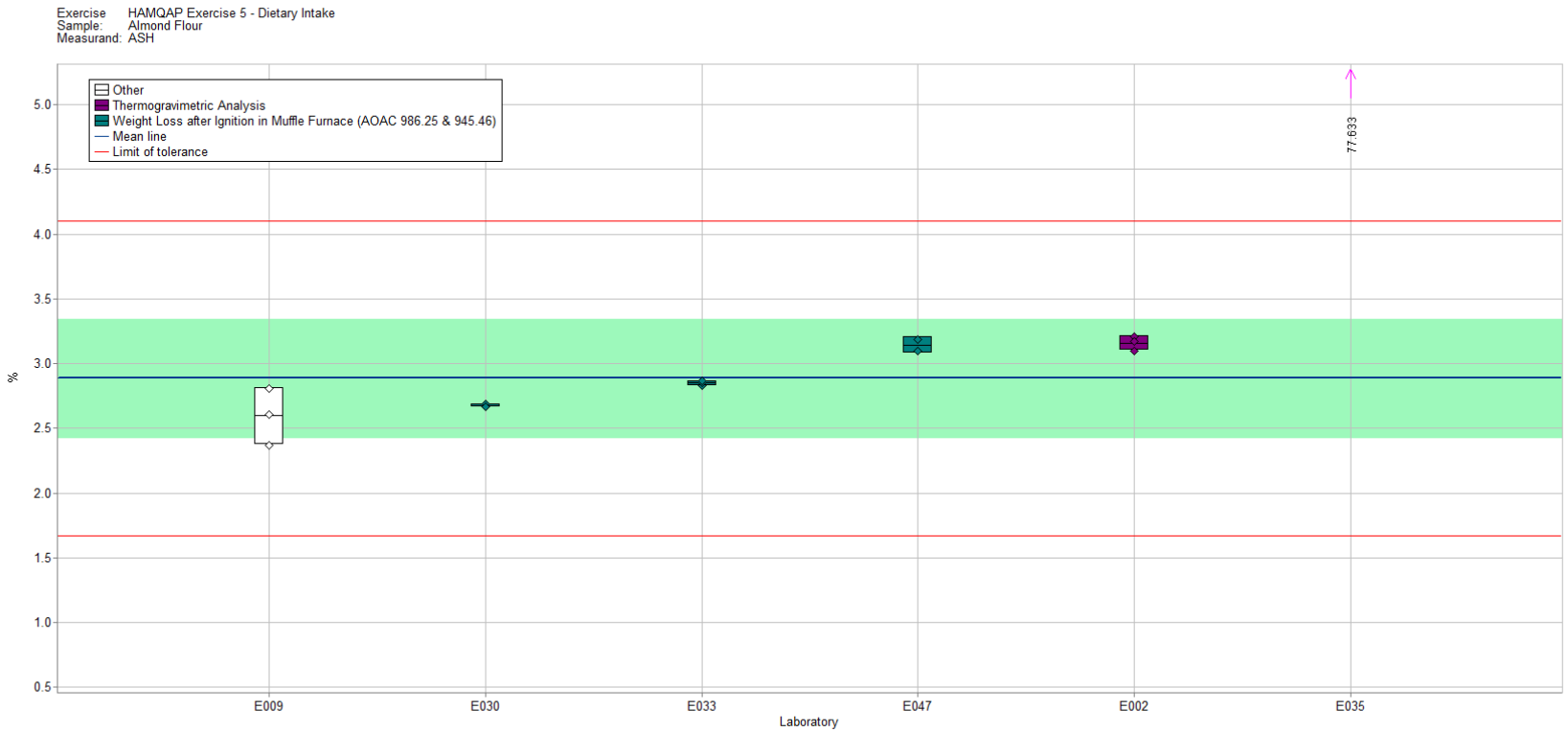

Figure 9-9. Ash in Almond Flour (data summary view – analytical method). In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line represents 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.

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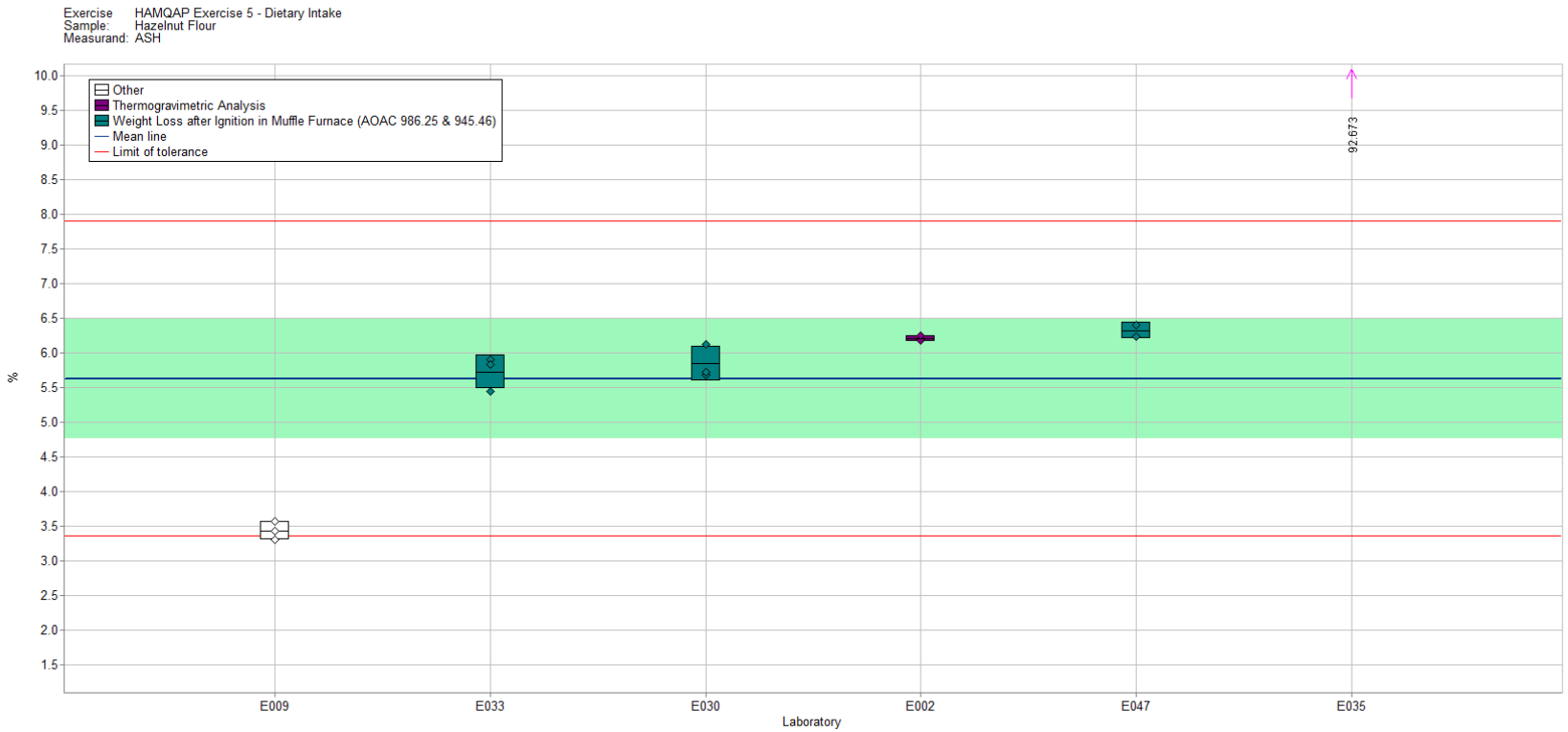

Figure 9-10. Ash in Hazelnut Flour (data summary view – analytical method). In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line represents 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.

Table 9-7. Data summary table for solids in almond and hazelnut flour.

		Solids									
		Almond Flour (%)					Hazelnut Flour (%)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target										
	E001										
	E002	97.67	97.7	97.73	97.70	0.03	96.65	96.96	96.43	96.68	0.27
	E006										
	E009										
	E019										
	E020										
	E021										
	E029										
	E030	95.5	95.22	95.3	95.34	0.14	93.38	93.48	93.7	93.52	0.16
	E031										
	E033	95.5	95.9	95.7	95.70	0.20	93.4	93.9	93.9	93.73	0.29
	E035										
	E036										
	E037										
	E042										
	E047	96.3	96.5		96.40	0.14	94.48	94.54		94.51	0.04
Community Results		Consensus Mean			96.29		Consensus Mean			94.61	
		Consensus Standard Deviation			0.77		Consensus Standard Deviation			0.70	
		Maximum			97.70		Maximum			96.68	
		Minimum			95.34		Minimum			93.52	
		N			4		N			4	

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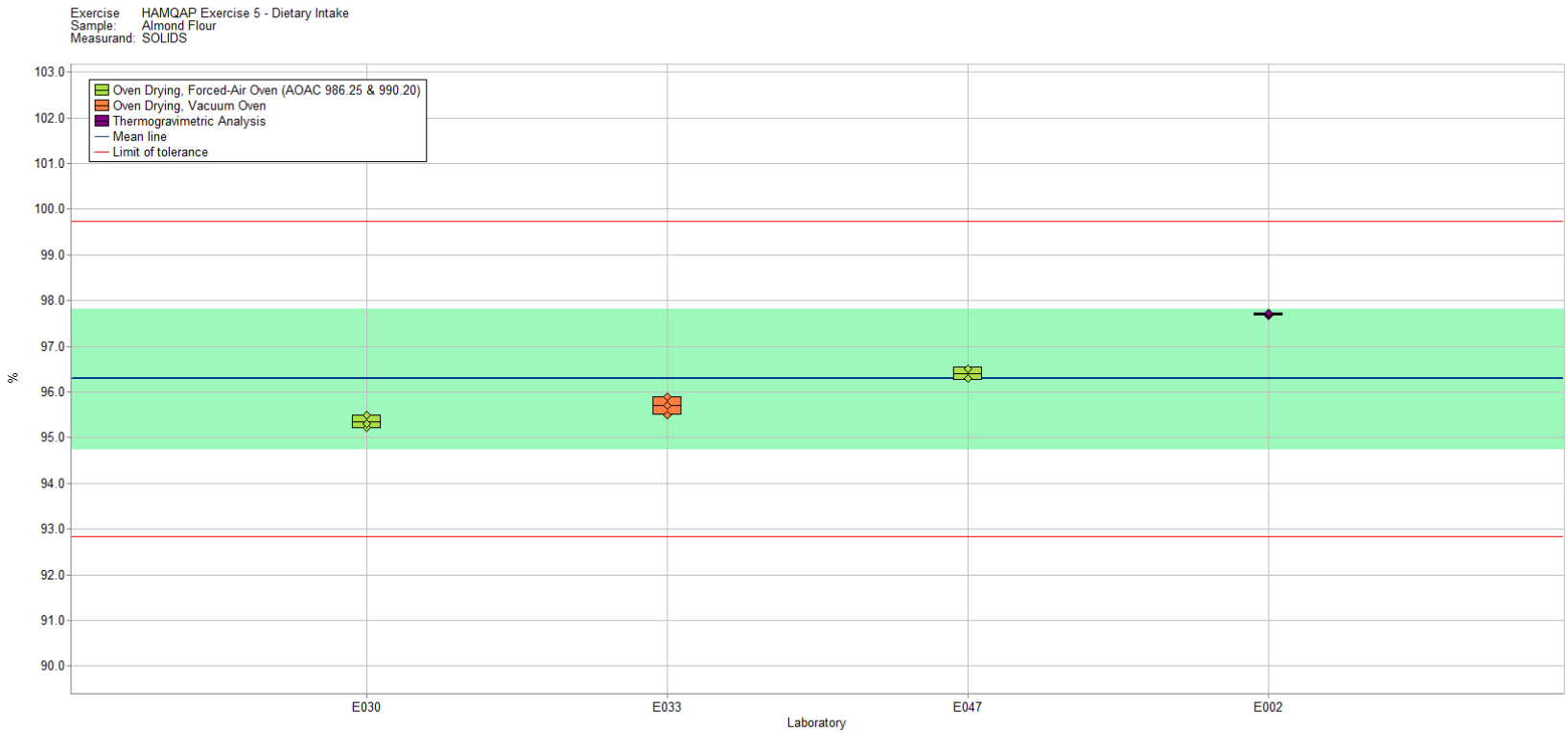

Figure 9-11. Solids in Almond Flour (data summary view – analytical method). In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line represents 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.

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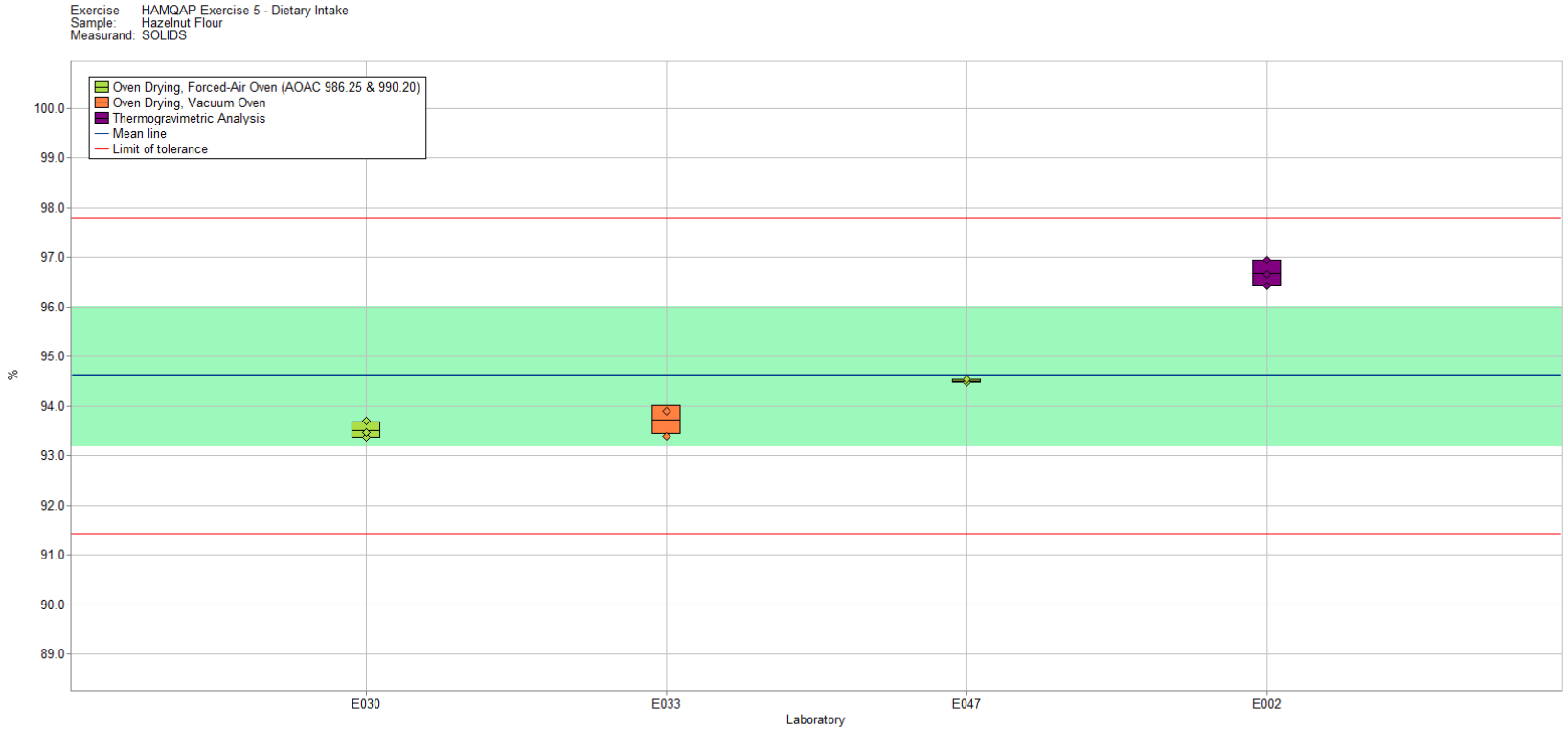

Figure 9-12. Solids in Hazelnut Flour (data summary view – analytical method). In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line represents 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.