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Health Assessment Measurements Quality Assurance Program: Exercise 6 Final Report

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LIST OF ACRONYMS

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

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

ABSTRACT

The Health Assessment Measurements Quality Assurance Program (HAMQAP) was launched in collaboration with the National Institutes of Health (NIH) Office of Dietary Supplements (ODS) in 2017. HAMQAP was established to enable laboratories to improve the accuracy of measurements in samples that represent human intake (e.g., foods, dietary supplements, tobacco) and samples that represent human metabolism (e.g., blood, serum, plasma, urine) for demonstration of proficiency and/or compliance with various regulations. Analytes are paired where possible to represent the full spectrum of health assessment. Exercise 6 of this program offered the opportunity for laboratories to assess their in-house measurements of nutritional elements (chlorine, iodine, chromium, molybdenum, and selenium), toxic elements (arsenic, cadmium, lead, and mercury), contaminants (chlorate and perchlorate, glyphosate and aminomethylphosphonic acid (AMPA)), water-soluble vitamins (biotin and vitamin C), fat-soluble vitamins (vitamins A and E), fatty acids (select omega-3 and omega-6 fatty acids), botanicals (anthocyanidins), natural products (caffeine, theobromine, and theophylline), and proximates (fat, protein, carbohydrates, solids, ash, and calories) in foods and dietary supplements, and corresponding biomarkers/metabolites in clinical specimens (human red blood cells).

INTRODUCTION

HAMQAP was formed in 2017, in part as a collaboration with the NIH-ODS and represents ongoing efforts at NIST that were supported previously via historical quality assurance programs (QAPs), including the Dietary Supplements Laboratory QAP (DSQAP), Fatty Acids in Human Serum QAP (FAQAP), Micronutrients Measurement QAP (MMQAP), and Vitamin D Metabolites QAP (VitDQAP).

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

NIST has decades of experience in the administration of QAPs, and HAMQAP builds on the approach taken by the former DSQAP by providing a wide range of matrices and analytes. The HAMQAP design combines activities of DSQAP, FAQAP, MMQAP, and VitDQAP, and emphasizes emerging and challenging measurements in the dietary supplement, food, and clinical matrix categories. Participating laboratories are interested in evaluating in-house methods on a wide variety of challenging, real-world matrices to demonstrate that their performance is comparable to that of the community and that their methods provide accurate results. In areas where few standard methods have been recognized, HAMQAP offers a unique tool for assessment of the quality of measurements and provides feedback about performance that can assist participants in improving laboratory operations.

This report summarizes the results from the sixth exercise of HAMQAP. Eighty-eight laboratories responded to the dietary intake portion and sixteen laboratories responded to the human metabolites portion of the call for participants distributed in August 2020 (see table below). Seven human metabolites studies were cancelled prior to shipment due to low enrollment. Samples were shipped to participants in January 2021 and results were returned to NIST by March 2021. This report contains the final data and information that was disseminated to the participants in September 2021.

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

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

* Study not sponsored by the NIH ODS.

** Cancelled due to low enrollment (less than 10 laboratories registered).

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

OVERVIEW OF DATA TREATMENT AND REPRESENTATION

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

Statistics

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

Individualized Data Table

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

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

Also included in Section 1 are two Z-scores. The first Z-score, Z'_{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):

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

or

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

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 (N), the consensus mean value determined for each analyte ($\bar{x}^*$), and a consensus robust estimate of the standard deviation of the reported values (s^*).³ Consensus means and standard deviations are calculated using the laboratory means; if a laboratory reported a single value, the reported value is not included in determination of the consensus values.³ Additional information on calculation of the consensus mean and standard deviation can be found in the previous section.

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

Summary Data Table

This data table includes a summary of all reported data for a particular analyte in a particular study. Participants can compare the raw data for their laboratory to data reported by the other participating laboratories and to the consensus data. A blank indicates that the laboratory signed up and received samples for that analyte and matrix, but NIST does not have data on file for that laboratory. Data points highlighted in blue have been identified as outside the consensus tolerance limits and would be estimated to result in an unacceptable Z'_{comm} score, $|Z'_{\text{comm}}| \geq 2$. Data points highlighted in red have a zero or non-numeric data point. The standard deviation (SD) for the target value in this table is the uncertainty (U_{NIST}) around the target value.

Figures

Data Summary View (Method Comparison Data Summary View)

In this view, individual laboratory data (circles) are plotted with the individual laboratory standard deviation (rectangle). Laboratories reporting values below the LOQ are shown in this view as downward triangles beginning at the LOQ, reported as quantification limit (QL) on the figures. Laboratories reporting values as “below LOQ” can still be successful in the study if the target value is also below the laboratory LOQ. The blue solid line represents the consensus mean, and the green shaded area represents the 95 % confidence interval for the consensus mean, based on the standard error of the consensus mean. The uncertainty in the consensus mean is calculated using the equation below, based on the repeatability standard deviation (s_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.

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

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.

Sample/Sample Comparison View

In this view, the individual laboratory results for one sample (e.g., NIST Standard Reference Material (SRM) with a certified, reference, or NIST-determined value; a less challenging matrix) are compared to the results for another sample (e.g., NIST SRM with a more challenging matrix; a commercial sample). The solid red box represents the target zone for the first sample (x-axis) and the second sample (y-axis), if available. The dotted blue box represents the consensus zone for the first sample (x-axis) and the second sample (y-axis). The axes of this graph are centered about the consensus mean values for each sample or control, to a limit of twice the range of tolerance (values that result in an acceptable Z' score, $|Z'| \leq 2$). Depending on the variability in the data, the axes may be scaled proportionally to better display the individual data points for each laboratory. In some cases, when the consensus and target ranges have limited overlap, the solid red box may only appear partially on the graph. If the variability in the data is high (greater than 100 % relative standard deviation (RSD)), the dotted blue box may also only appear partially on the graph. These views emphasize trends in the data that may indicate potential calibration issues or method biases. One program goal is to identify such calibration or method biases and assist participants in improving analytical measurement capabilities. In some cases, when two equally challenging materials are provided, the same view (sample/sample comparison) can be helpful in identifying commonalities or differences in the analysis of the two materials.

SECTION 1: NUTRITIONAL ELEMENTS (Chlorine, Iodine, Chromium, Molybdenum, Selenium)

Study Overview

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

Dietary Intake Sample Information

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

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

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

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

Dietary Intake Study Results

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

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

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

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

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

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

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

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

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

Dietary Intake Technical Recommendations

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

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

- When using ICP-MS as the analytical methodology for iodine, carryover between analyses may be observed for samples prepared in an acidic solution. The addition of a surfactant to sample solutions (e.g., Triton X-100) will improve washout of iodine. The rinse solution used between sample readings should be slightly basic, above pH 7, and contain Triton X-100.
- During sample preparation, iodine can adhere to tetrafluoroethylene (TFM) vessels, so perfluoroalkoxy (PFA) vessels or quartz vessels are recommended to improve repeatability.
- For chromium, **Figures 1-12, 1-14, and 1-15** indicate that most laboratories are within the NIST target range for infant formula indicating that the sample preparation and analytical methodology for chromium in this matrix is valid.
 - For the infant formula, the few values that were outside the consensus tolerance limits may need to be confirmed they are reported in the correct units or calculations may need to be checked.
 - Incomplete digestion of either sample matrix may have resulted in results with a large variability and/or values below the target range.
 - Use of collision cell gas is recommended since polyatomic interferences can occur from the plasma, $^{35}\text{Cl}^{16}\text{O}^{1}\text{H}^{+}$, $^{40}\text{Ar}^{12}\text{C}^{+}$, $^{37}\text{Cl}^{15}\text{N}^{+}$. The collision gas flow rate may need to be adjusted to reduce polyatomic interferences from the matrix itself, such as in the multivitamin/multielement samples.
 - For matrices with high total dissolved solids such as the multivitamin/multielement tablets, samples may need to be diluted appropriately in order to achieve accurate results.
 - Sample solutions should be stored in dilute nitric acid, at 1.5% or higher, to maintain stability.
 - Preparation of procedural blanks is a key step to identify sources of bias such as contamination from autosampler vials.
- For molybdenum, the sample/sample comparison view (**Figure 1-20**) shows a linear trend for both samples except for some participants reporting results outside the consensus tolerance limits.
 - Trends of this type often indicate calibration errors. Laboratories should check that all sample solution concentrations are within the linear calibration range.
 - Laboratories reporting results that are either extremely low or have high sample-to-sample variability may have incompletely digested their samples.
 - Polyatomic interferences may occur, $^{40}\text{Ar}^{39}\text{K}^{16}\text{O}^{+}$, $^{39}\text{K}^{41}\text{K}^{16}\text{O}^{+}$, $^{41}\text{K}_2^{16}\text{O}^{+}$, but the use of collision cell technology with either He or H gas should improve or eliminate these interferences.
 - Isobaric interferences could be caused by Zr or Ru but should be negligible since the concentration of these elements is low in these two matrices.
 - Preparation of procedural blanks is a key step to identify sources of bias such as reagent impurities.
- For selenium, the sample/sample comparison view (**Figure 1-25**) shows a slight linear trend for much of the sample data indicating a possible calibration error. Values that are low may indicate matrix-induced signal suppression, which may be avoided with the use of an internal standard.
 - The digestion procedure is critical to the accuracy of selenium determination. Digestion of the multivitamin/multielement tablets is difficult in comparison to the infant formula due

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

- To breakdown the organoselenium compounds, mixtures of nitric, hydrofluoric, and perchloric acids with temperatures of up to 200 °C are recommended for open beaker digestion techniques. For microwave digestion, nitric acid and a small amount of HF with high pressure and high temperature are recommended. A small amount of HF ensures complete digestion and more accurate selenium determination.
- When using ICP-MS, collision cell technology can be used to minimize polyatomic interferences caused by molecular ions that have the same mass-to-charge ratio as selenium, such as $^{40}\text{Ar}^{38}\text{Ar}^+$, $^{40}\text{Ar}^{37}\text{Cl}^+$, and $^{40}\text{Ar}_2^+$.
- Validation tools, (e.g. Certified Reference Materials (CRMs)) are available and should be used to confirm accuracy of measurement techniques. When selecting a CRM, choose matrix-matched materials that have the analytes of interest, where possible.
- All results should be reported accurately.
 - Zero is not a quantity that can be measured. If measured values are below quantitation limits, results should be reported as such. A more appropriate result would be to report that a value is below the LOQ or QL.
 - Laboratories reporting results outside the consensus tolerance levels should check for calculation errors. One example is to confirm that factors for all dilutions have been properly tabulated and that results are reported in correct reporting units.

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

HAMQAP Exercise 6 - Nutritional Elements											
Lab Code:		NIST	1. Your Results				2. Community Results			3. Target	
Analyte	Sample	Units	x_i	s_i	Z'_{comm}	Z_{NIST}	N	x^*	s^*	x_{NIST}	U
Chlorine	Infant Formula A	mg/kg	6610	10.8			5	6640	170	6610	10.8
Chlorine	SRM 3280 Multivitamin/Multielement Tablets	mg/kg	52300	2270			5	50800	3100	52300	2270
Chromium	Infant Formula A	mg/kg	1.04	0.0484			21	1.02	0.076	1.04	0.0484
Chromium	SRM 3280 Multivitamin/Multielement Tablets	mg/kg	92.4	2.7			24	100	11	92.4	2.7
Iodine	Infant Formula A	mg/kg	2.1	0.076			9	2.08	0.84	2.1	0.076
Iodine	SRM 3280 Multivitamin/Multielement Tablets	mg/kg	131	6.5			11	120	23	131	6.5
Molybdenum	Infant Formula A	mg/kg	1.8	0.0745			20	1.7	0.21	1.8	0.0745
Molybdenum	SRM 3280 Multivitamin/Multielement Tablets	mg/kg	69.7	4.4			21	70	12	69.7	4.4
Selenium	Infant Formula A	mg/kg	0.836	0.0358			20	0.79	0.12	0.836	0.0358
Selenium	SRM 3280 Multivitamin/Multielement Tablets	mg/kg	17.2	0.44			21	20	2	17.2	0.44
			x_i	Mean of reported values			N	Number of quantitative values reported		x_{NIST}	NIST-assessed value
			s_i	Standard deviation of reported values						U	expanded uncertainty
			Z'_{comm}	Z'-score with respect to community consensus			x^*	Robust mean of reported values			about the NIST-assessed value
			Z_{NIST}	Z-score with respect to NIST value			s^*	Robust standard deviation			

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

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

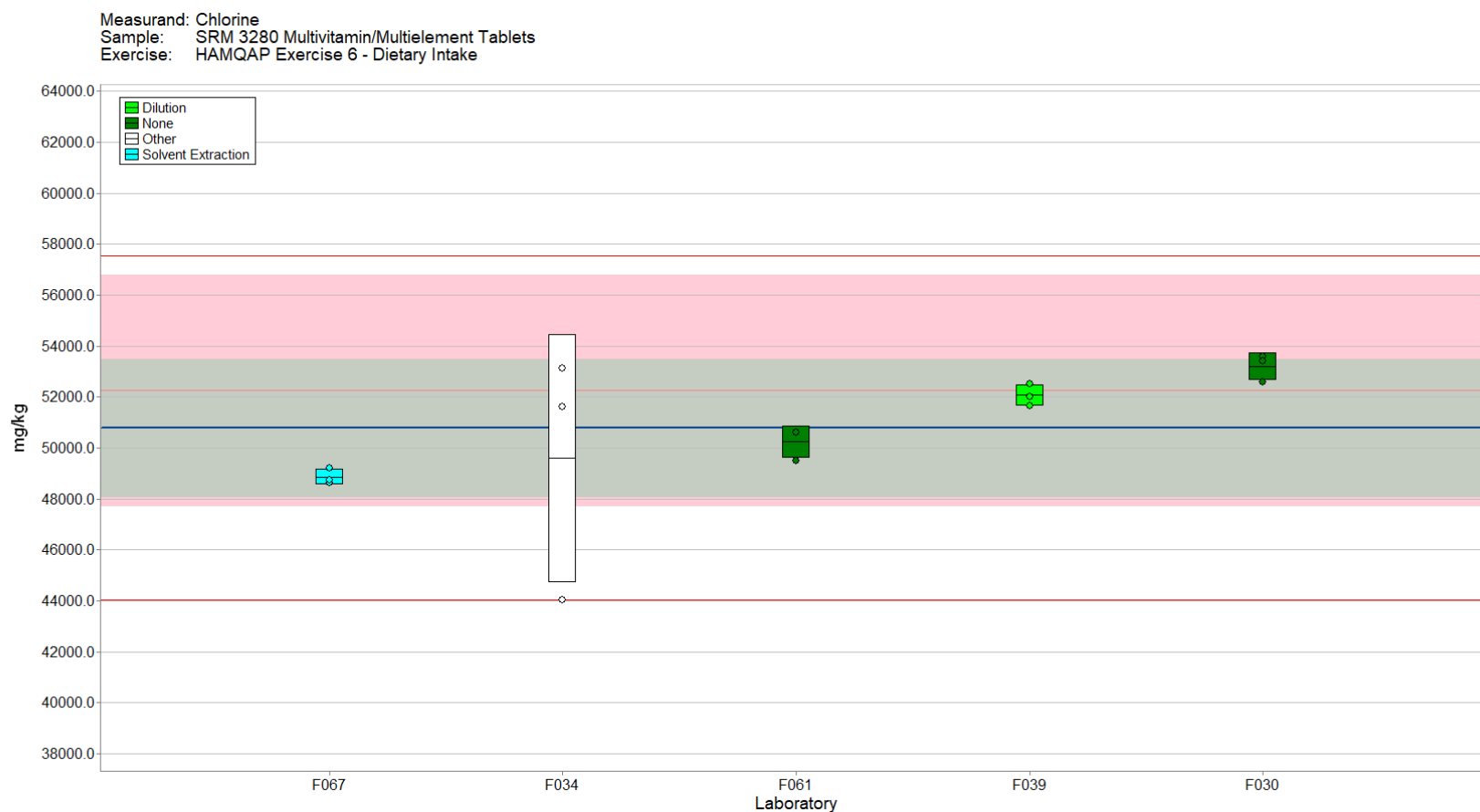


Figure 1-1. Chlorine in SRM 3280 Multivitamin/Multielement Tablets (data summary view – sample preparation method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation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

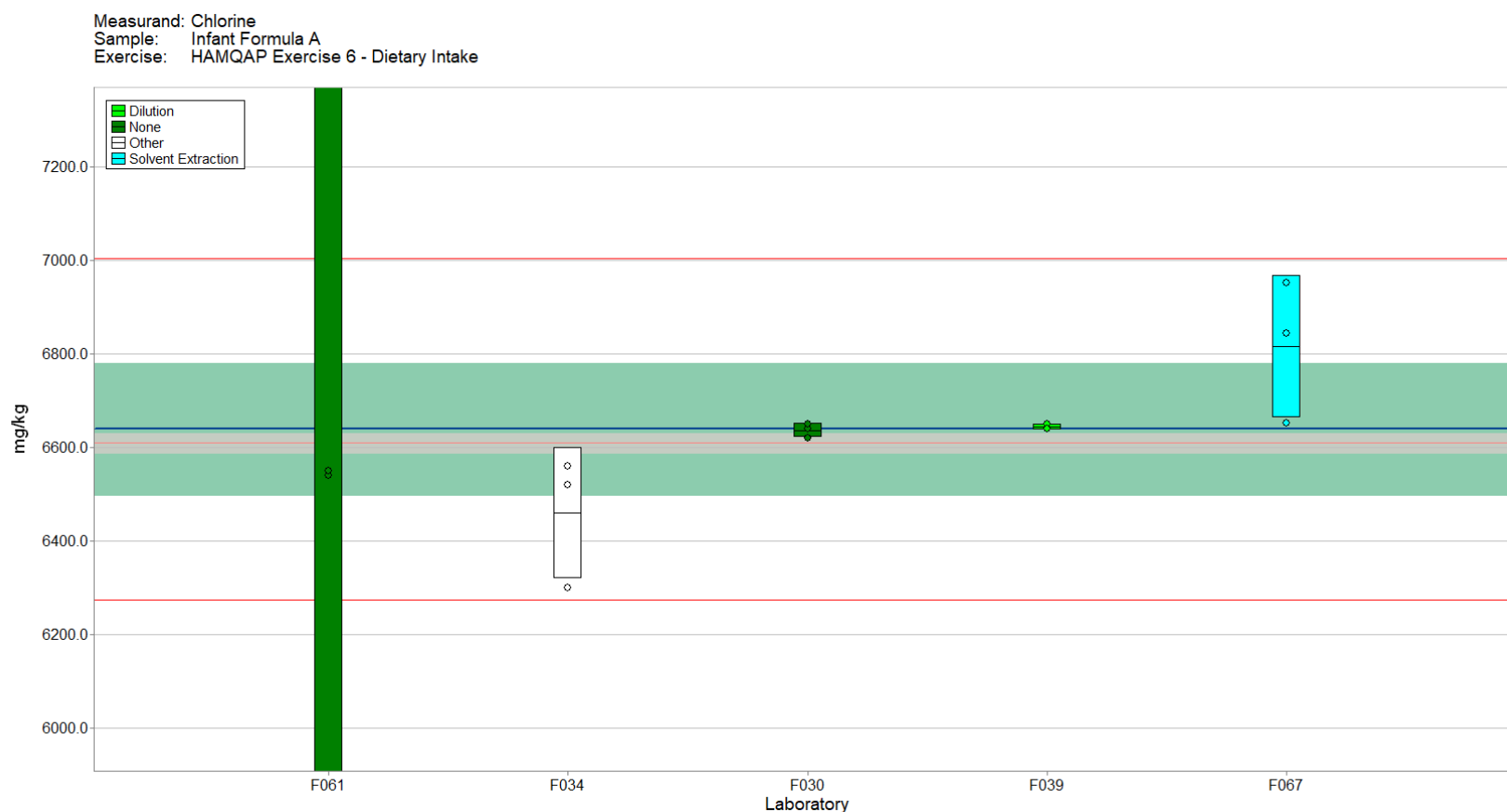


Figure 1-2. Chlorine in Infant Formula A (data summary view – sample preparation method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The solid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

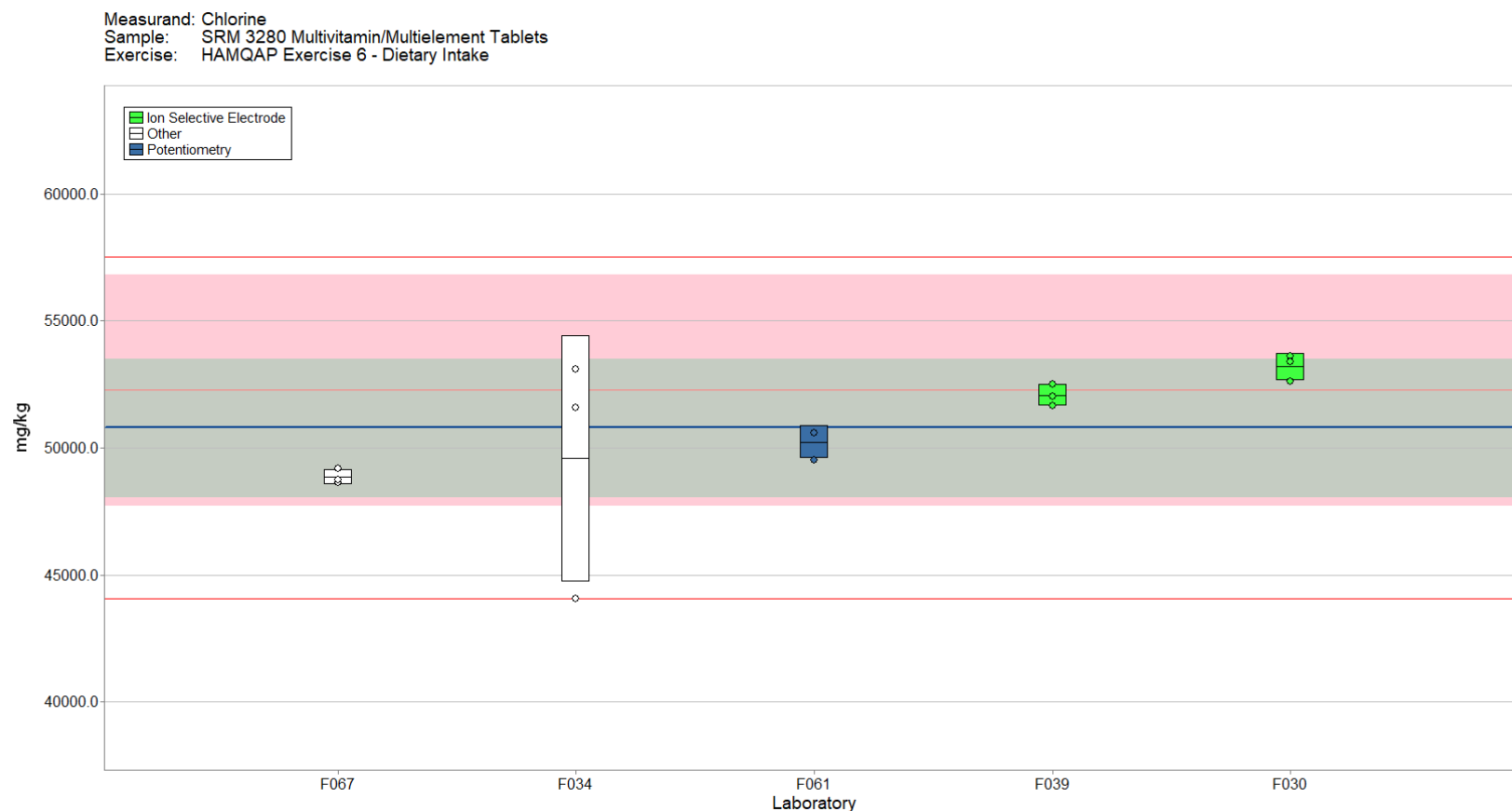


Figure 1-3. Chlorine in SRM 3280 Multivitamin/Multielement (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty (U_{NIST}) and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$.

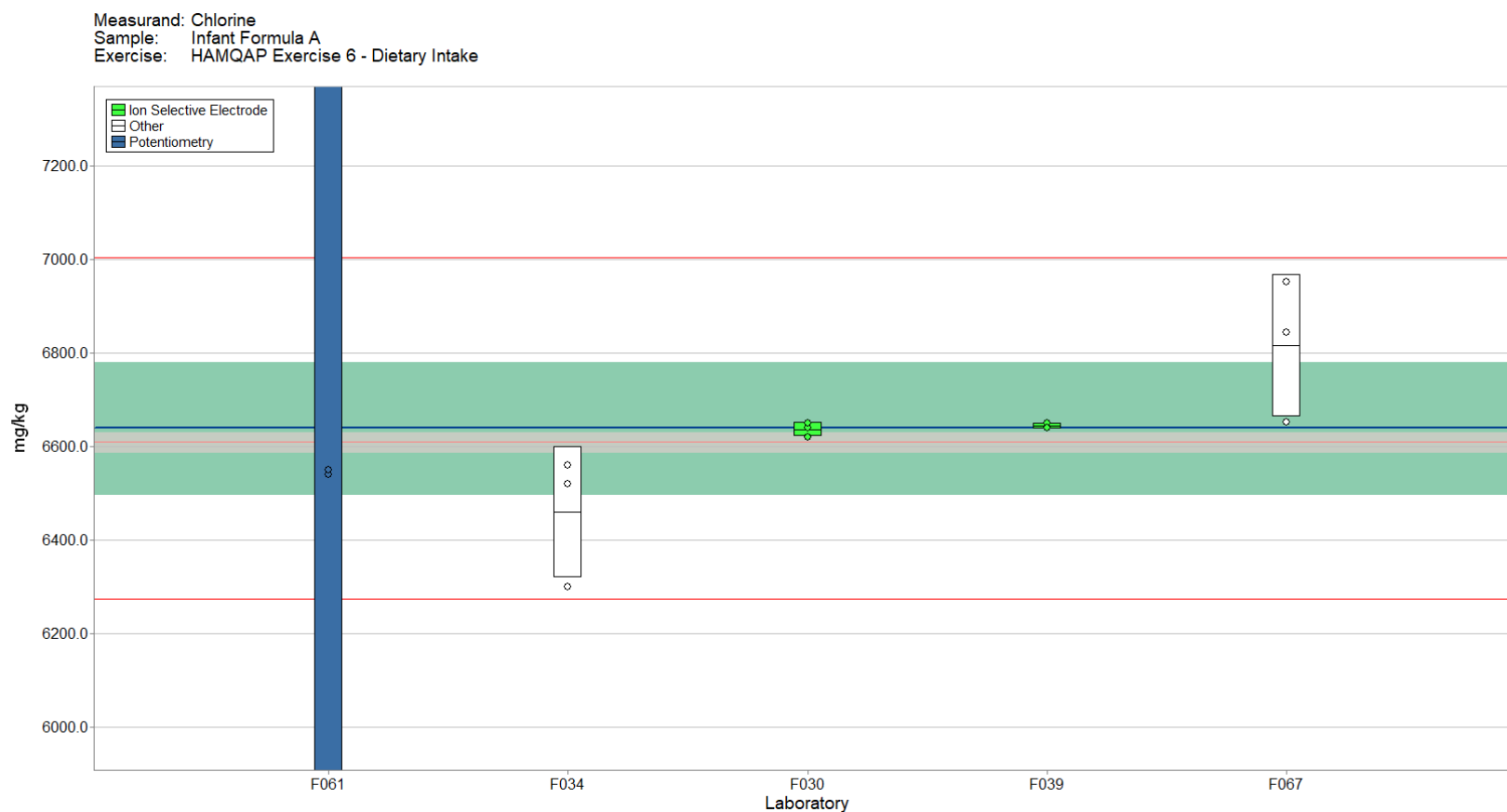


Figure 1-4. Chlorine in Infant Formula A (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line represents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

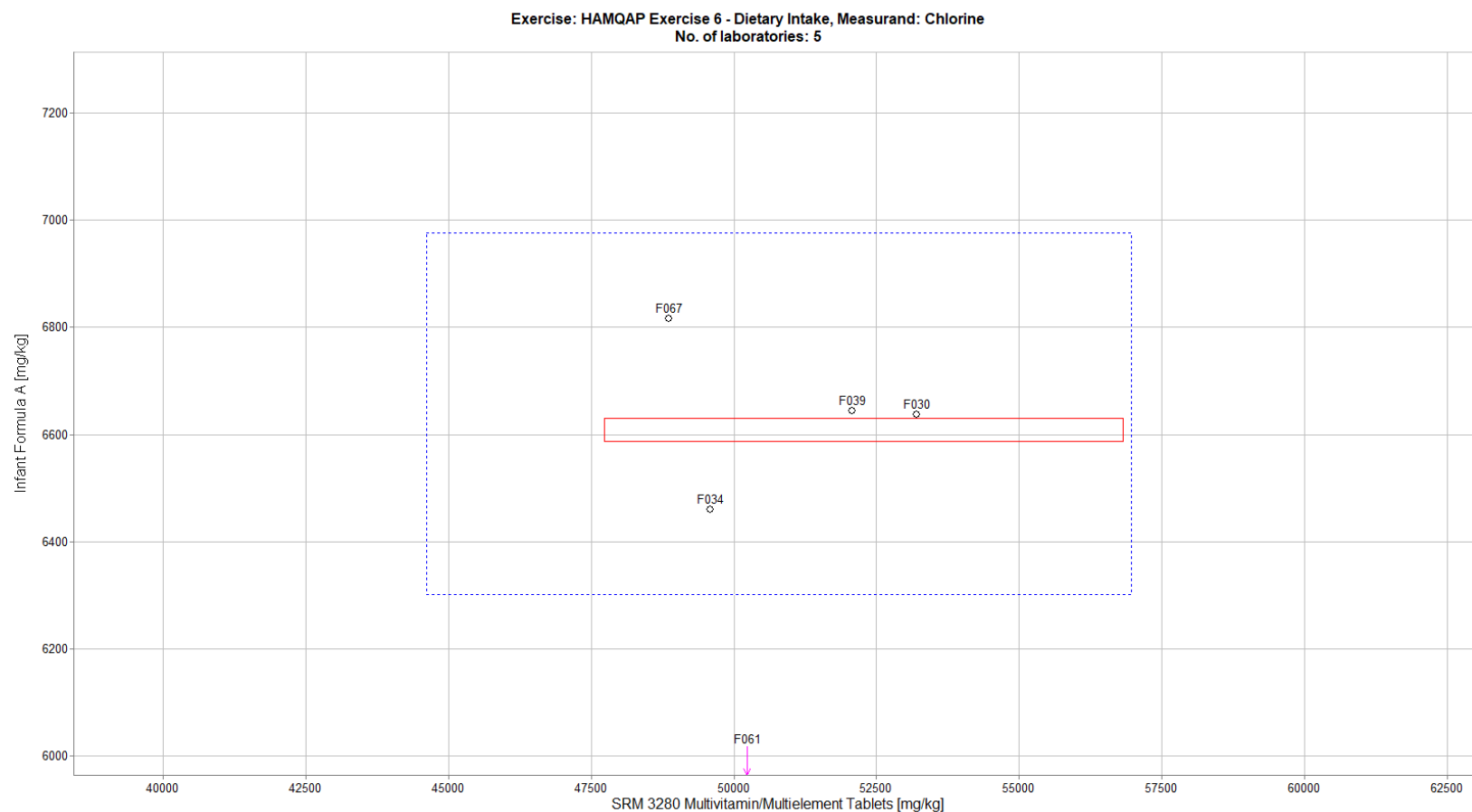


Figure 1-5. Laboratory means for chlorine in SRM 3280 Multivitamin/Multielement Tablets and Infant Formula A (sample/sample comparison view). In this view, the individual laboratory mean for one sample (SRM 3280) is compared to the individual laboratory mean for a second sample (Infant Formula A). The solid red box represents the NIST range of tolerance for the two samples, SRM 3280 (x-axis) and Infant Formula A (y-axis), which encompasses the target values bounded by their uncertainties (U_{NIST}) and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$. The dotted blue box represents the consensus range of tolerance for SRM 3280 (x-axis) and Infant Formula A (y-axis), calculated as the values above and below the consensus means that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$.

Table 1-3. Data summary table for iodine in 3280 Multivitamin/Multielement Tablets and Infant Formula A. Data points highlighted in blue have been identified as outside the consensus tolerance limits and would be estimated to result in an unacceptable Z'_{comm} score, $|Z'_{\text{comm}}| \geq 2$.

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

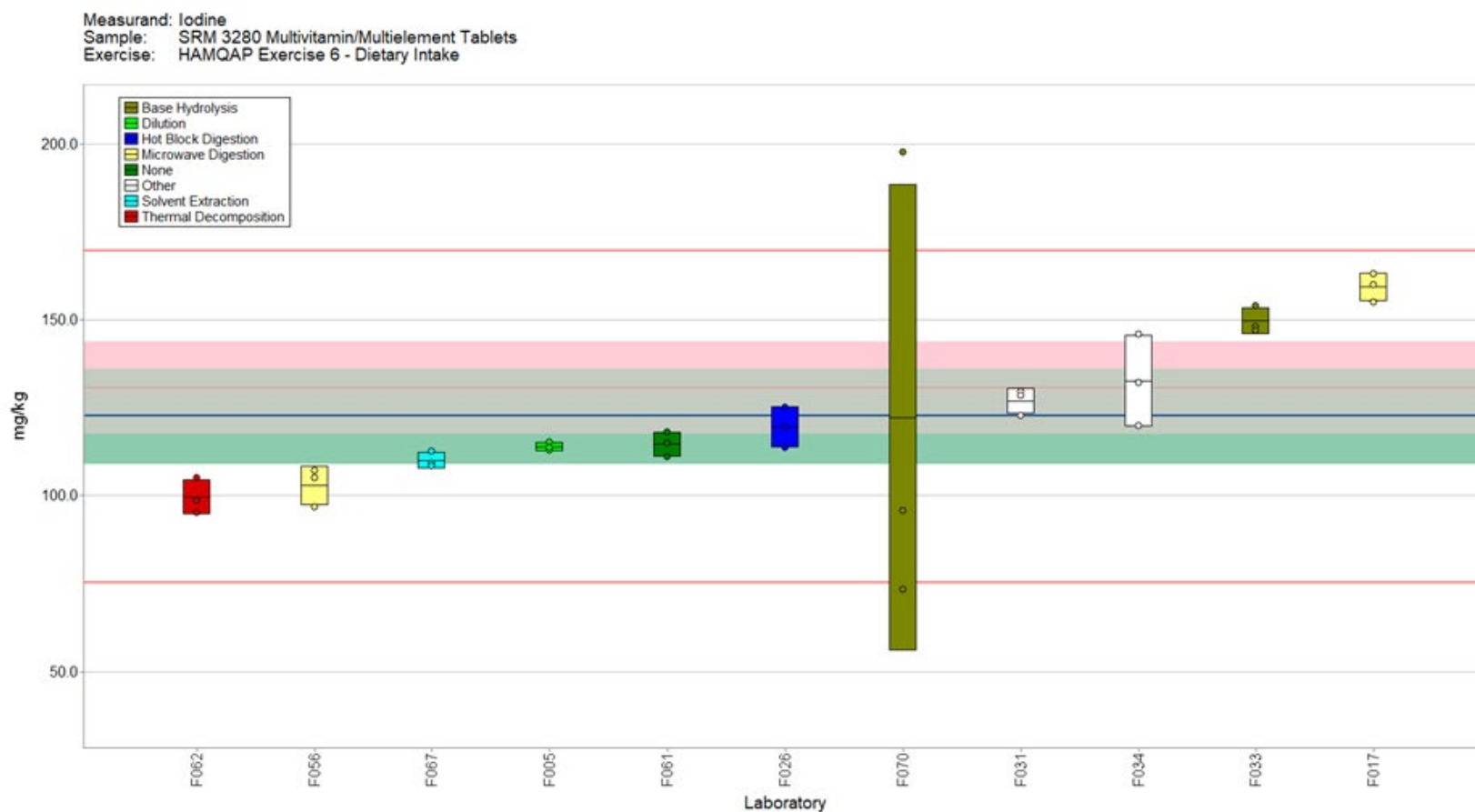


Figure 1-6. Iodine in SRM 3280 Multivitamin/Multielement Tablets (data summary view – sample preparation method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty (U_{NIST}) and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$.

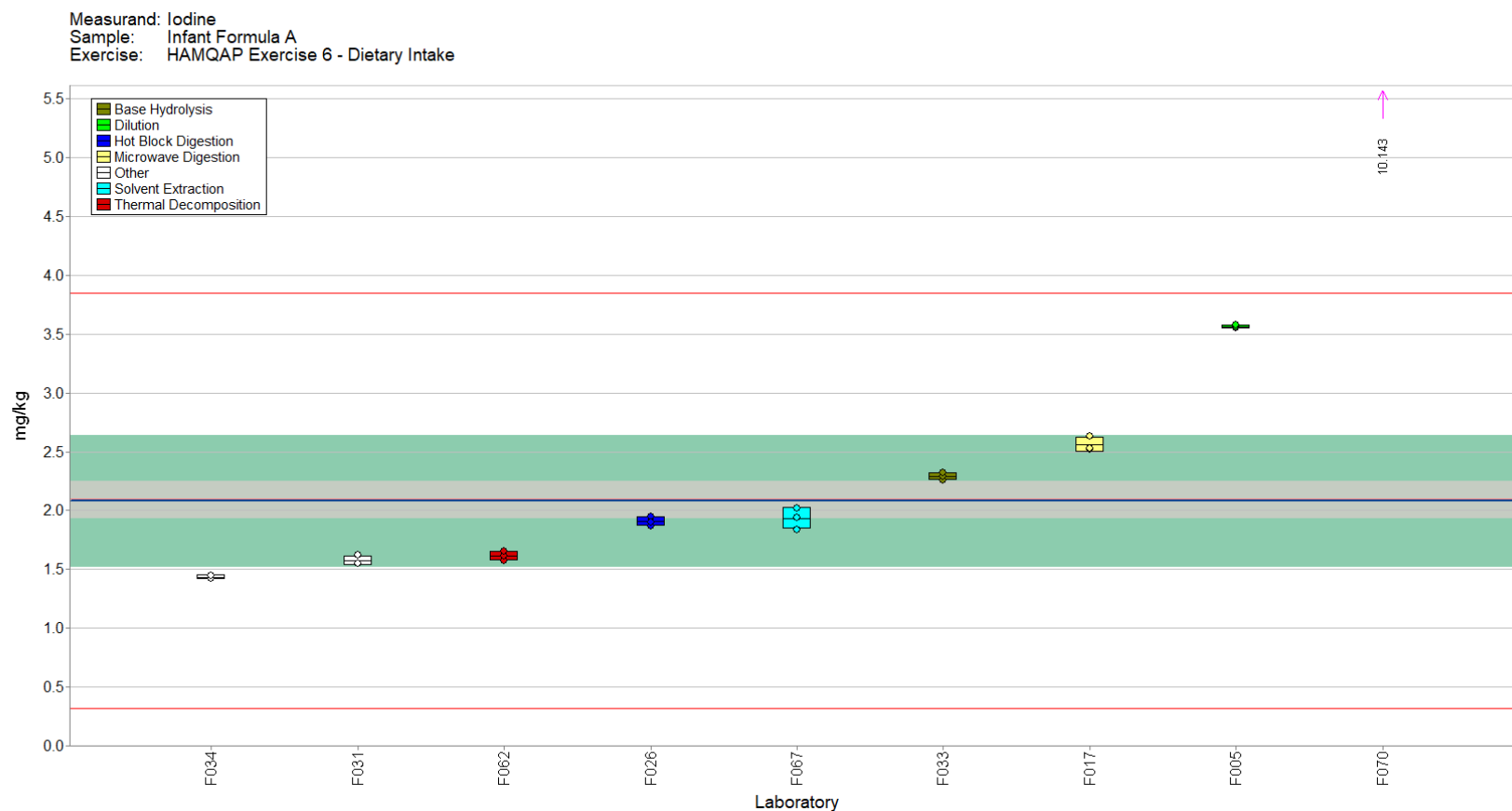


Figure 1-7. Iodine in Infant Formula A (data summary view – sample preparation method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty (U_{NIST}) and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$.

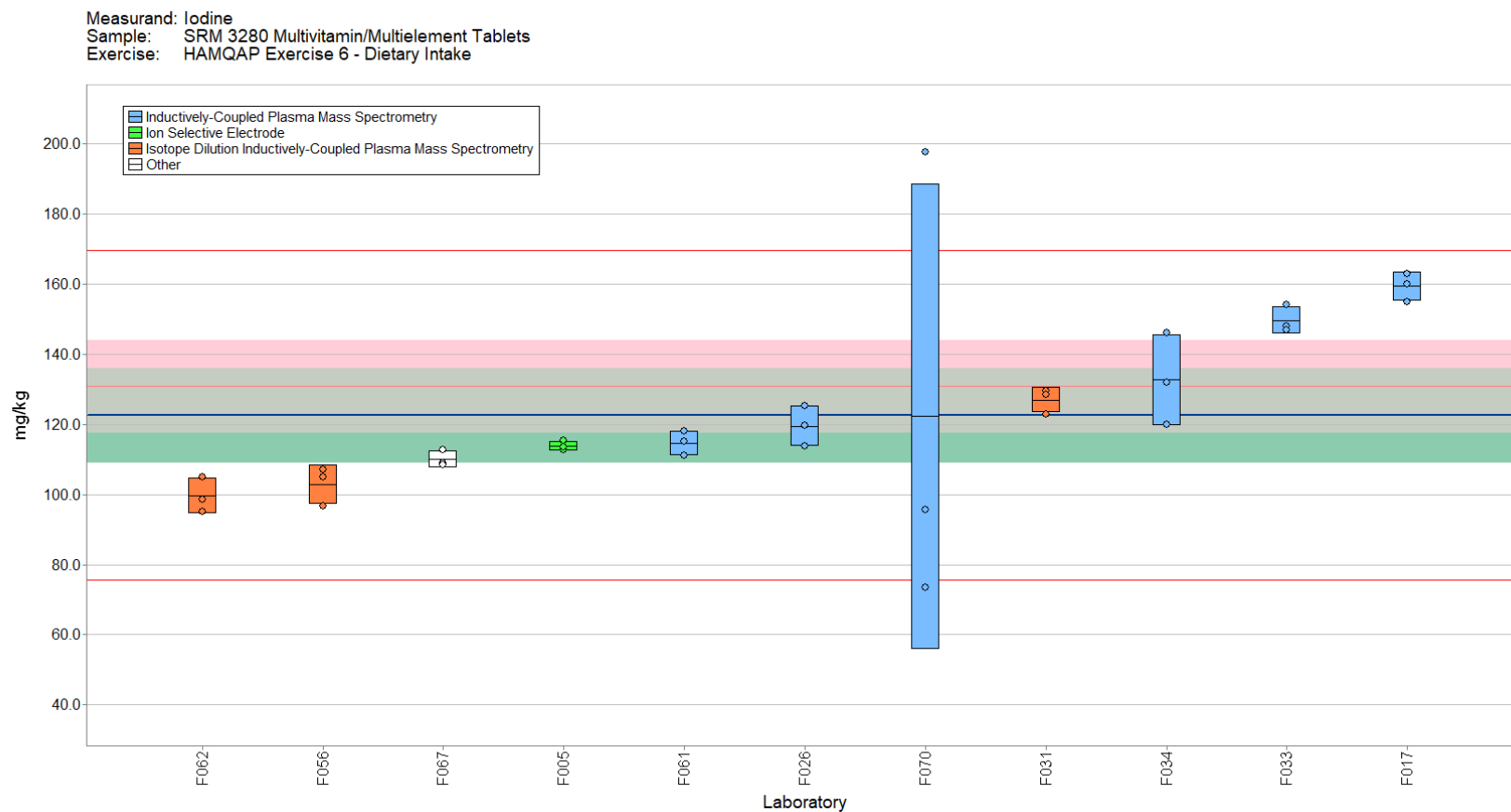


Figure 1-8. Iodine in SRM 3280 Multivitamin/Multielement Tablets (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty (U_{NIST}) and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$.

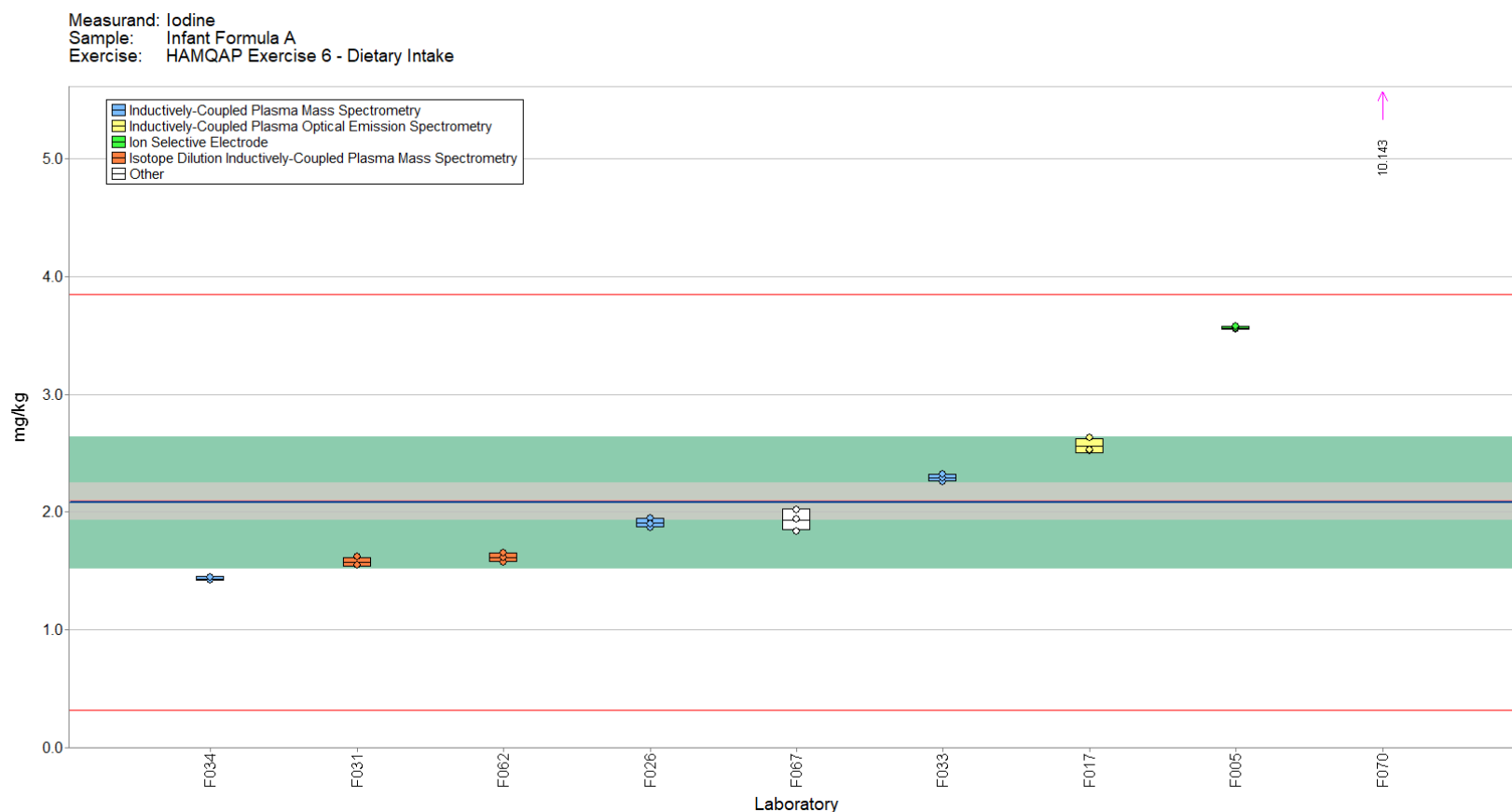


Figure 1-9. Iodine in Infant Formula A (data summary view –analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line represents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

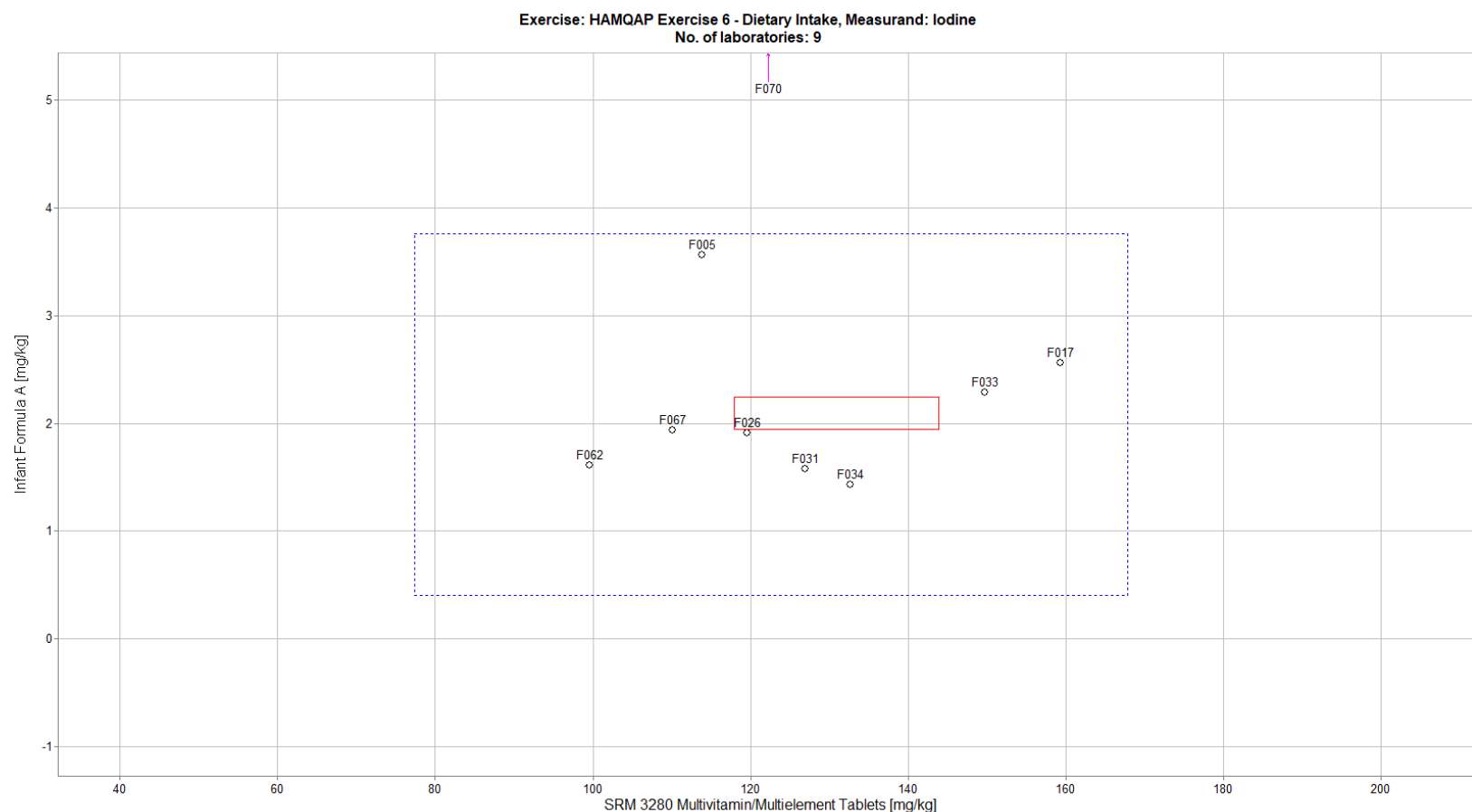


Figure 1-10. Laboratory means for iodine in SRM 3280 Multivitamin/Multielement Tablets and Infant Formula A (sample/sample comparison view). In this view, the individual laboratory mean for one sample (SRM 3280) is compared to the individual laboratory mean for a second sample (Infant Formula A). The solid red box represents the NIST range of tolerance for the two samples, SRM 3280 (x-axis) and Infant Formula A (y-axis), which encompasses the target values bounded by their uncertainties (U_{NIST}) and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$. The dotted blue box represents the consensus range of tolerance for SRM 3280 (x-axis) and Infant Formula A (y-axis), calculated as the values above and below the consensus means that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$.

Table 1-4. Data summary table for chromium in 3280 Multivitamin/Multielement Tablets and Infant Formula A. Data points highlighted in blue have been identified as outside the consensus tolerance limits and would be estimated to result in an unacceptable Z'_{comm} score, $|Z'_{\text{comm}}| \geq 2$.

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

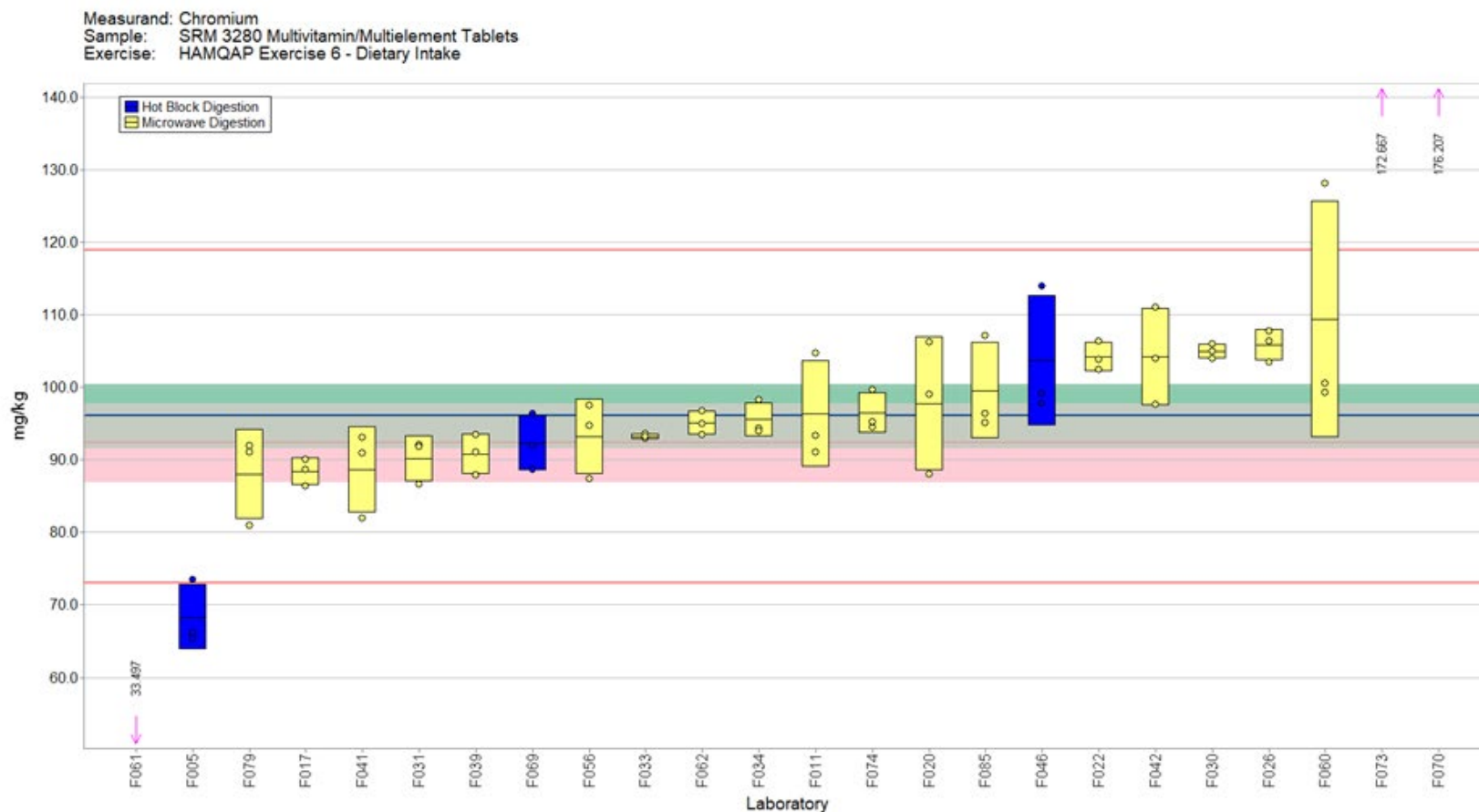


Figure 1-11. Chromium in SRM 3280 Multivitamin/Multielement Tablets (data summary view – sample preparation method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty (U_{NIST}) and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$.

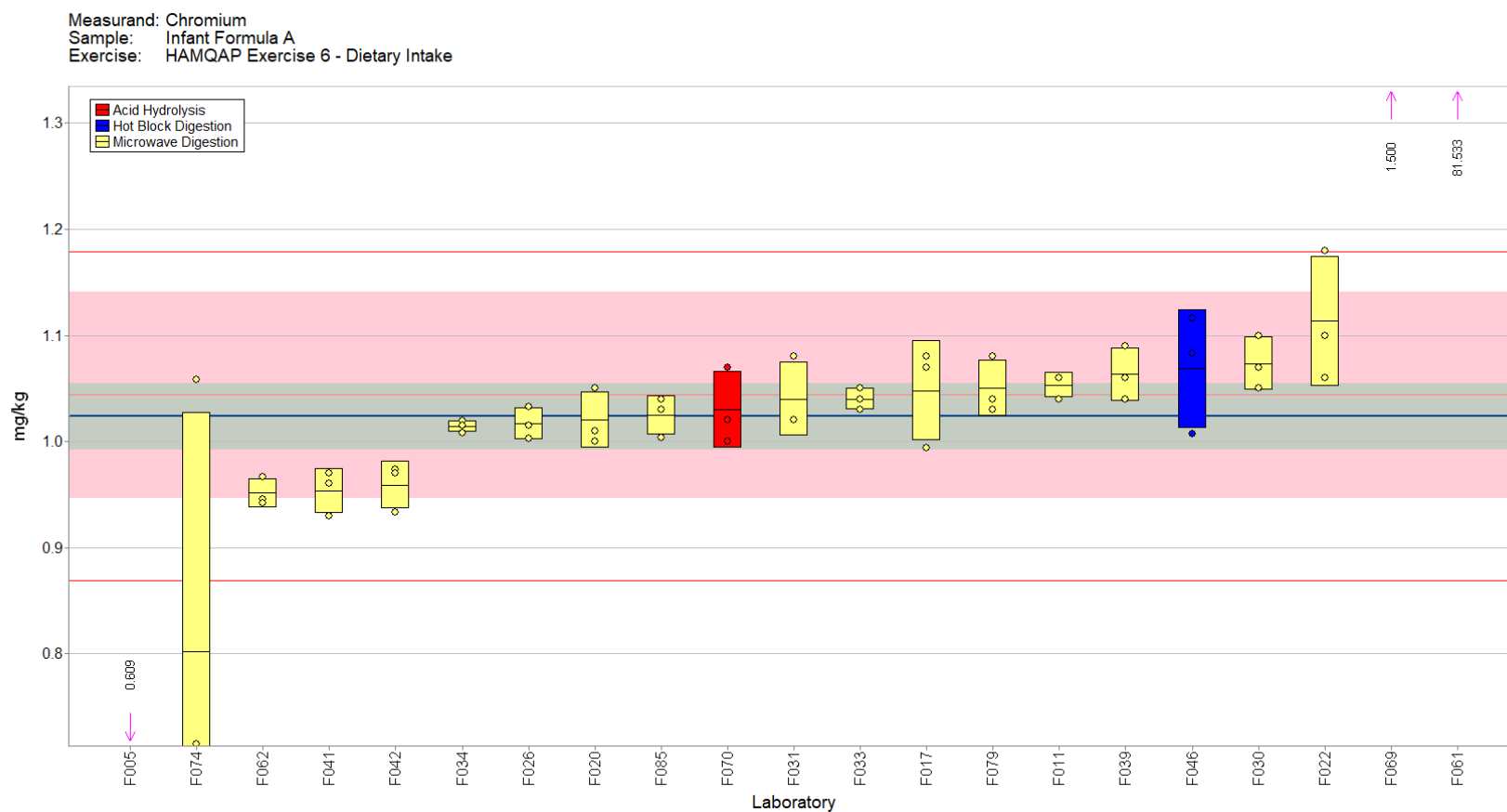


Figure 1-12. Chromium in Infant Formula A (data summary view – sample preparation method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty (U_{NIST}) and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$.

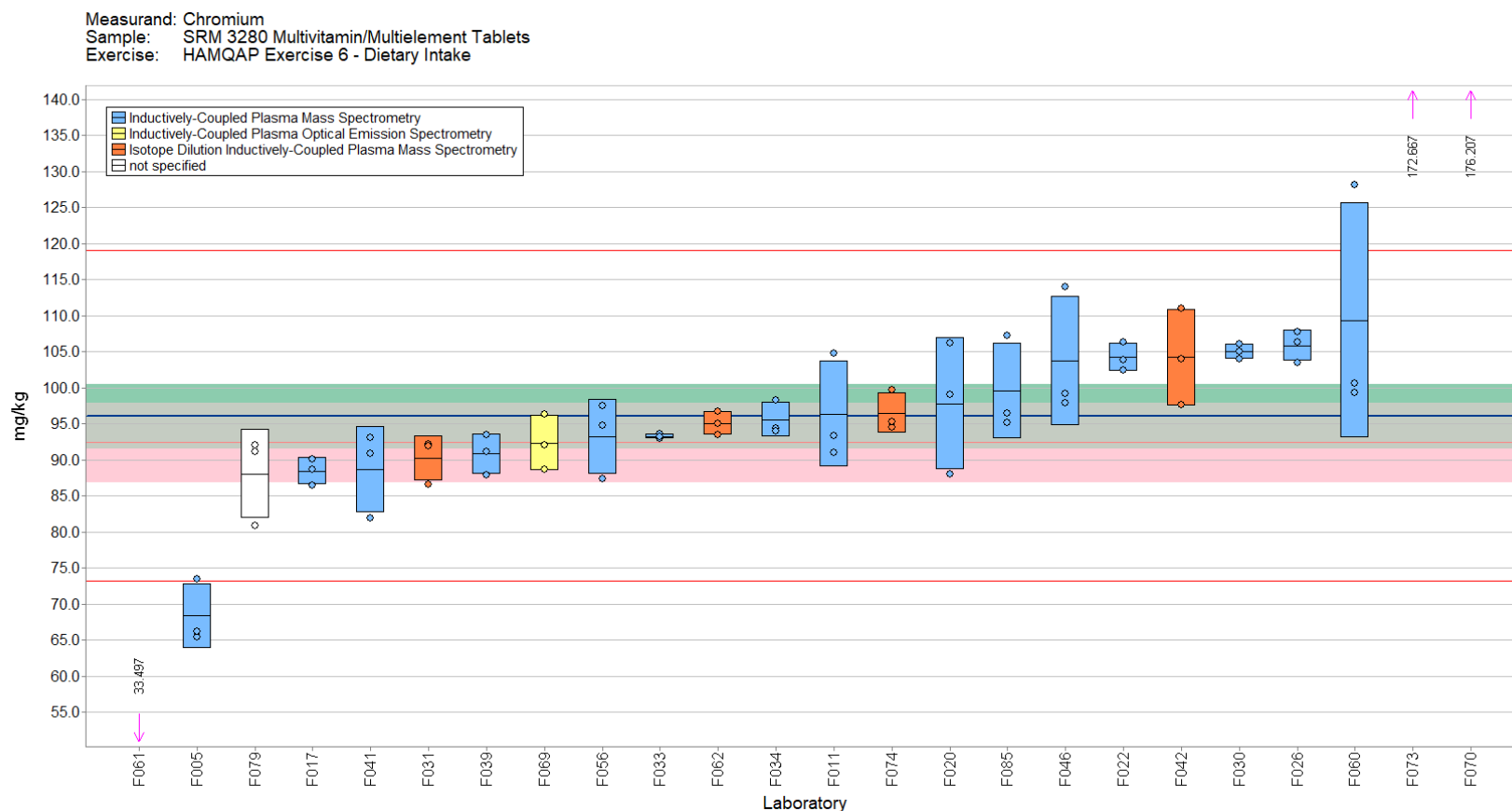


Figure 1-13. Chromium in SRM 3280 Multivitamin/Multielement Tablets (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty (U_{NIST}) and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$.

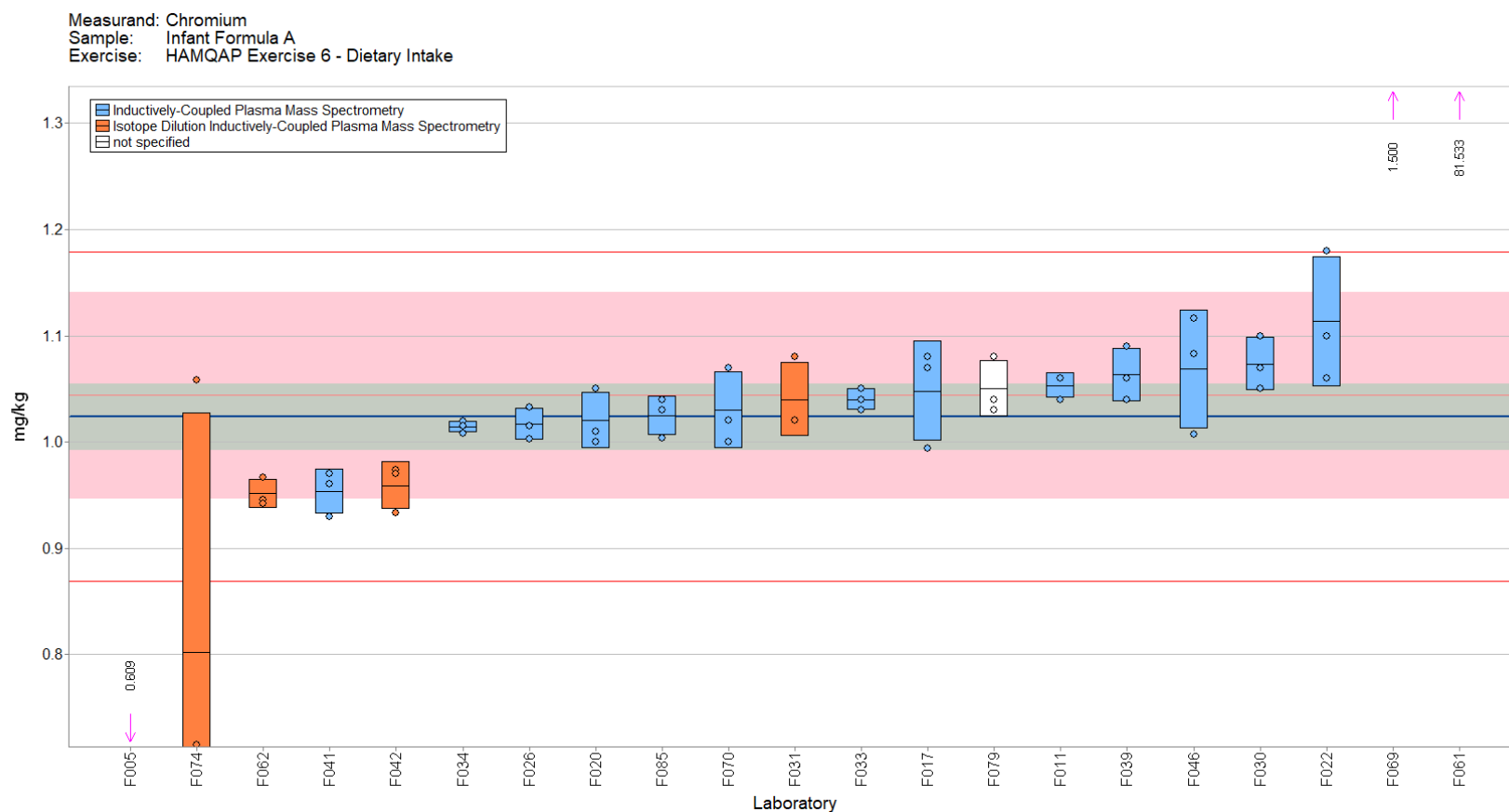


Figure 1-14. Chromium in Infant Formula A (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty (U_{NIST}) and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$.

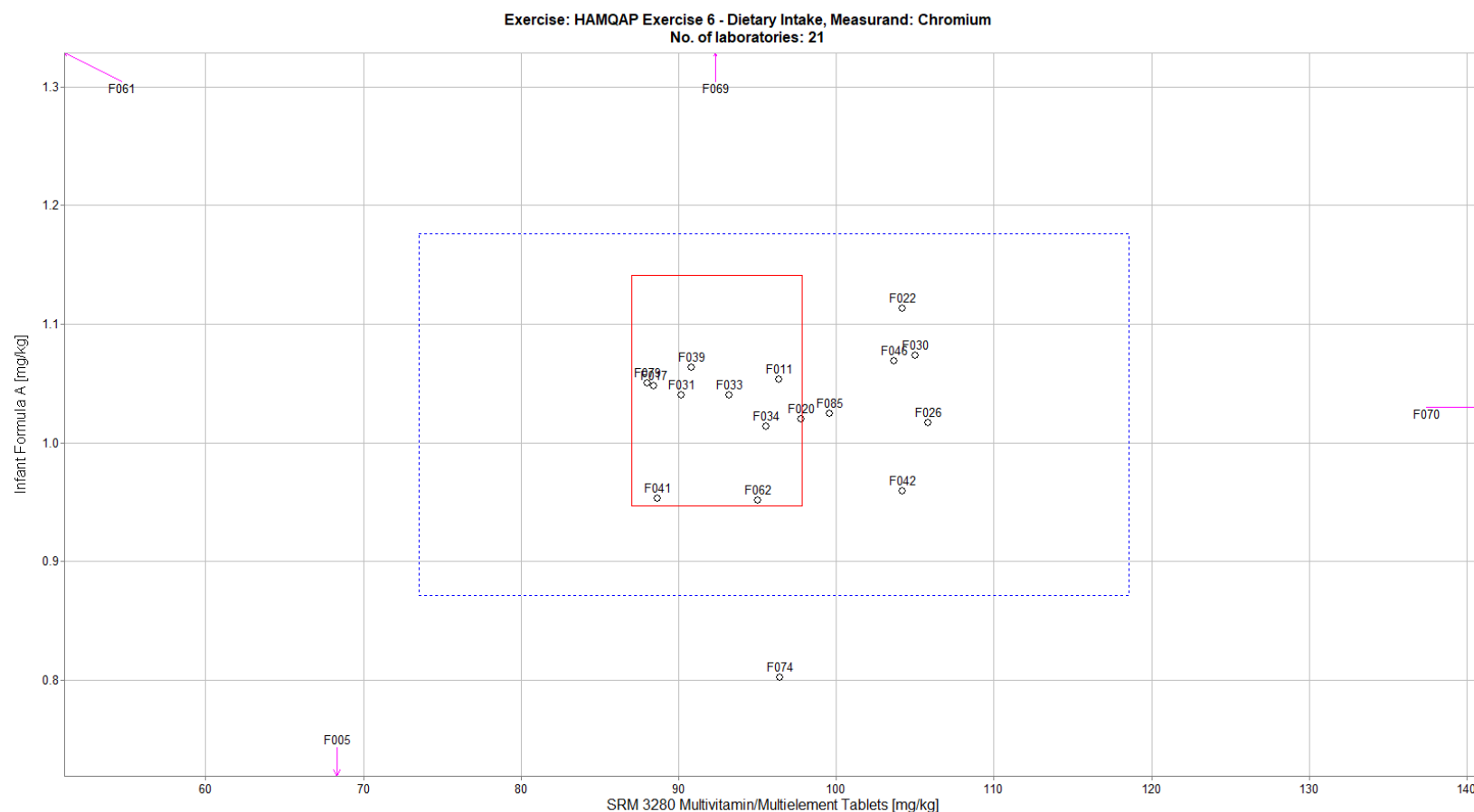


Figure 1-15. Laboratory means for chromium in SRM 3280 Multivitamin/Multielement Tablets and Infant Formula A (sample/sample comparison view). In this view, the individual laboratory mean for one sample (SRM 3280) is compared to the individual laboratory mean for a second sample (Infant Formula A). The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

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

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

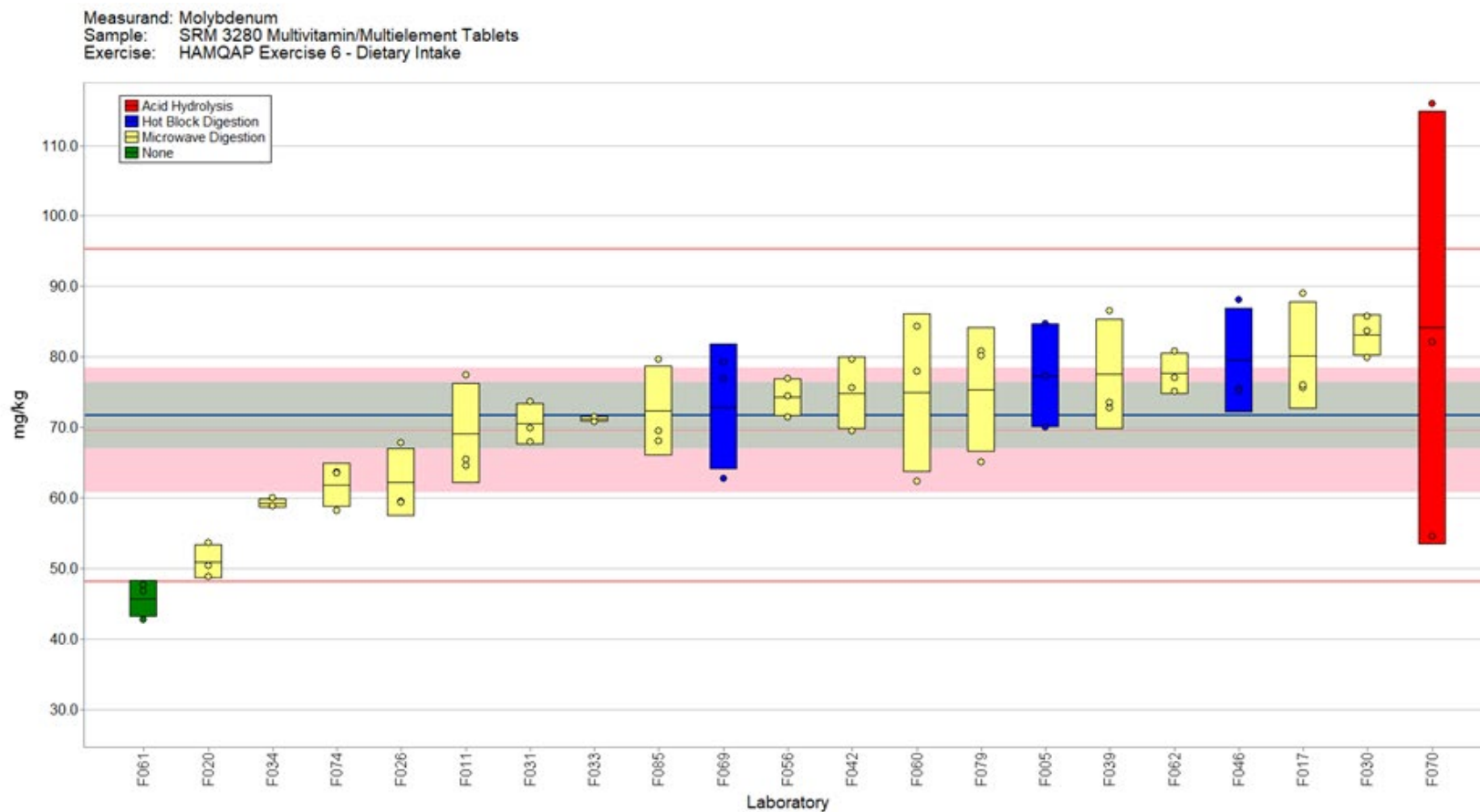


Figure 1-16. Molybdenum in SRM 3280 Multivitamin/Multielement Tablets (data summary view – sample preparation method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty (U_{NIST}) and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$.

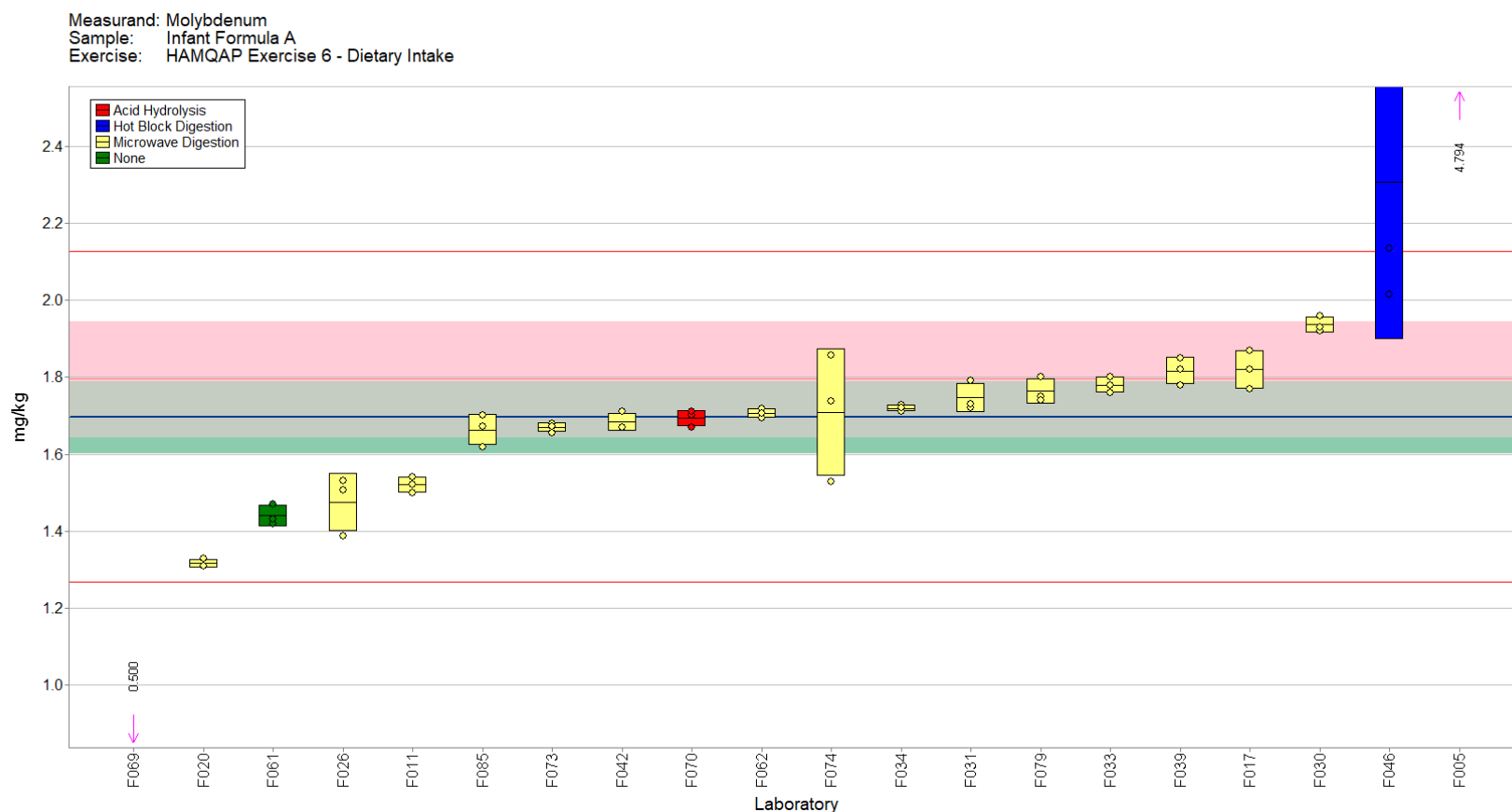


Figure 1-17. Molybdenum in Infant Formula A (data summary view – sample preparation method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty (U_{NIST}) and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$.

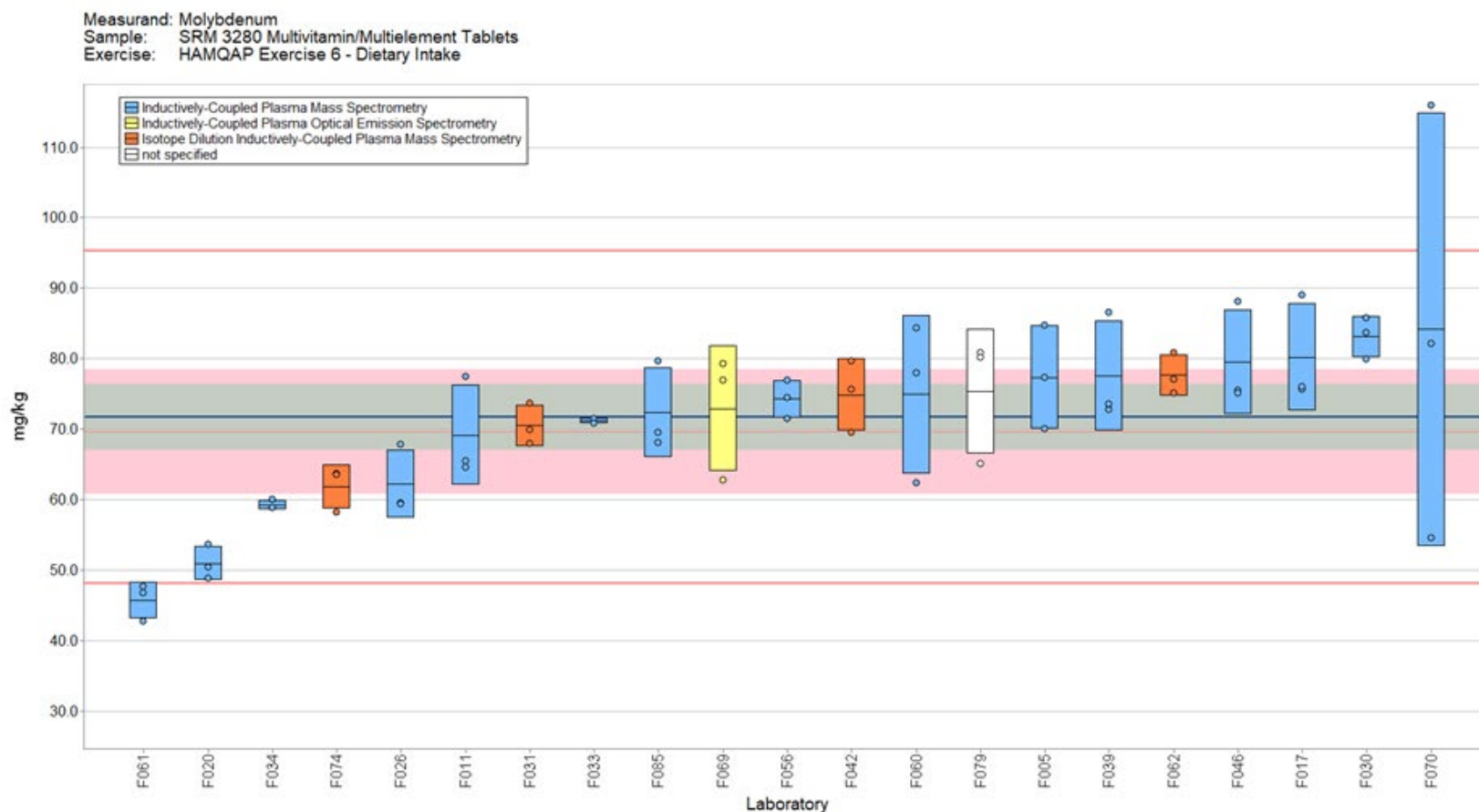


Figure 1-18. Molybdenum in SRM 3280 Multivitamin/Multielement Tablets (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$. The solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty (U_{NIST}) and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$.

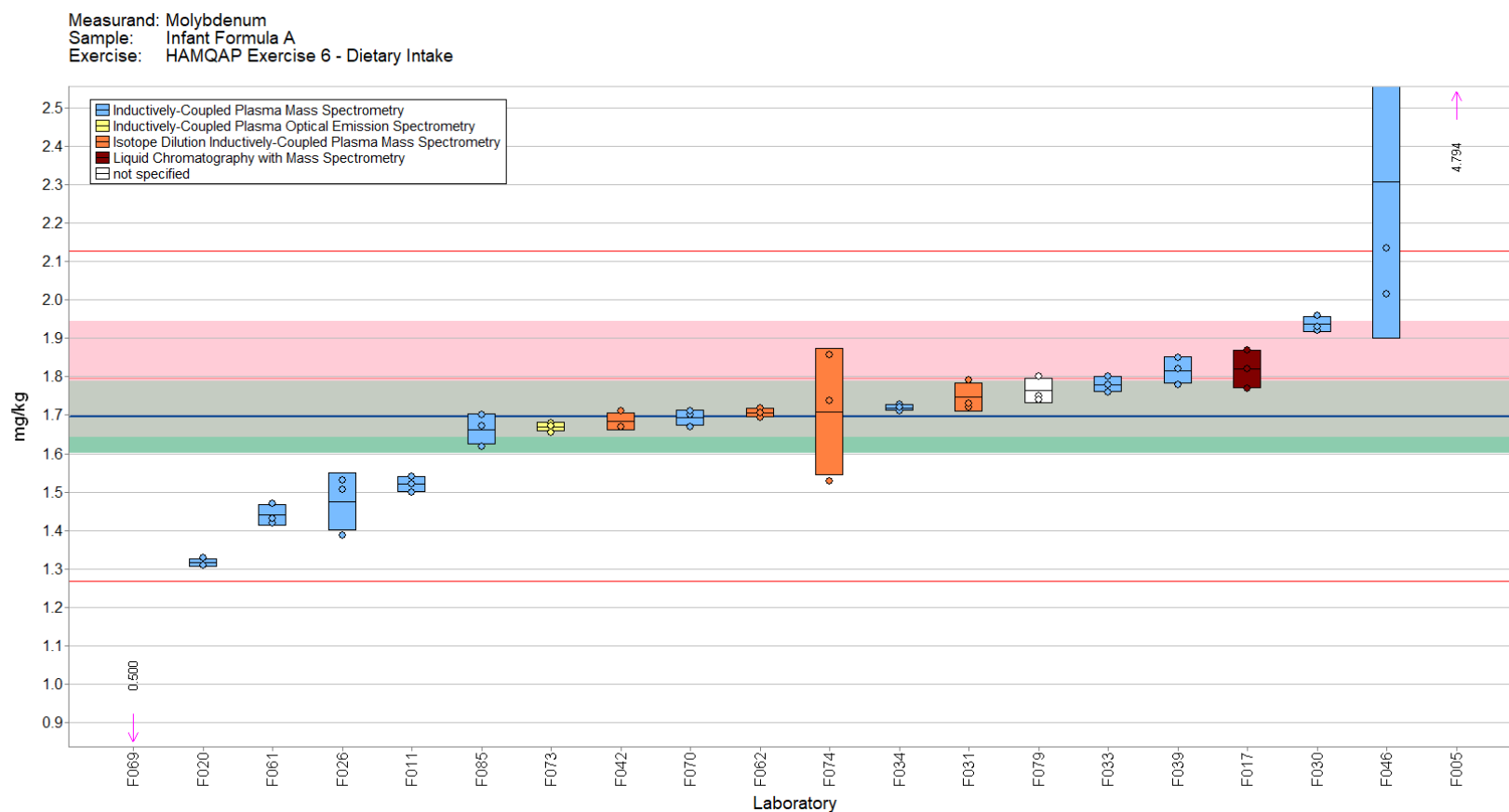


Figure 1-19. Molybdenum in Infant Formula A (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty (U_{NIST}) and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$.

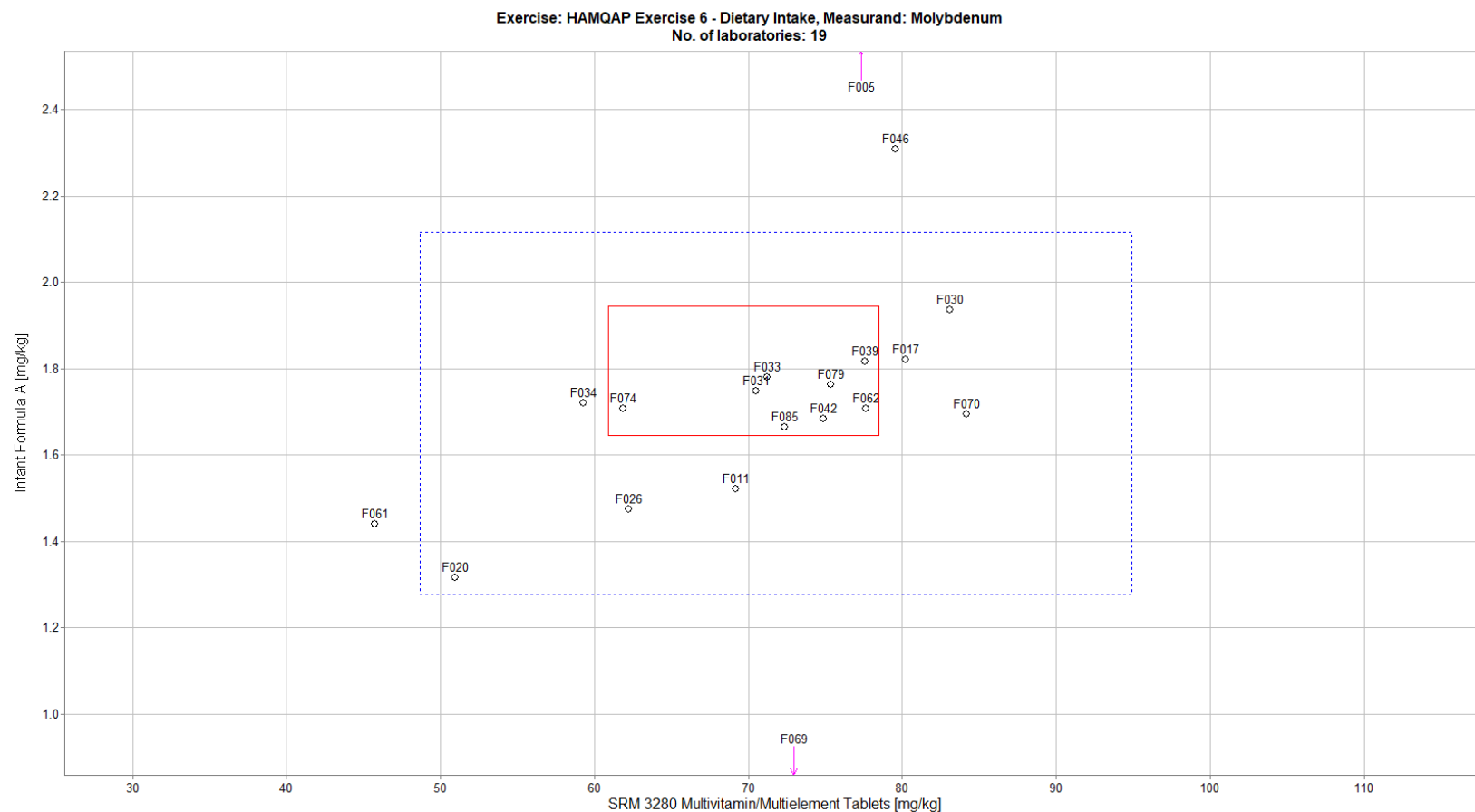


Figure 1-20. Laboratory means for molybdenum in SRM 3280 Multivitamin/Multielement Tablets and Infant Formula A (sample/sample comparison view). In this view, the individual laboratory mean for one sample (SRM 3280) is compared to the individual laboratory mean for a second sample (Infant Formula A). The solid red box represents the NIST range of tolerance for the two samples, SRM 3280 (x-axis) and Infant Formula A (y-axis), which encompasses the target values bounded by their uncertainties (U_{NIST}) and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$. The dotted blue box represents the consensus range of tolerance for SRM 3280 (x-axis) and Infant Formula A (y-axis), calculated as the values above and below the consensus means that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$.

Table 1-6. Data summary table for selenium in 3280 Multivitamin/Multielement Tablets and Infant Formula A. Data points highlighted in blue have been identified as outside the consensus tolerance limits and would be estimated to result in an unacceptable Z'_{comm} score, $|Z'_{\text{comm}}| \geq 2$.

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

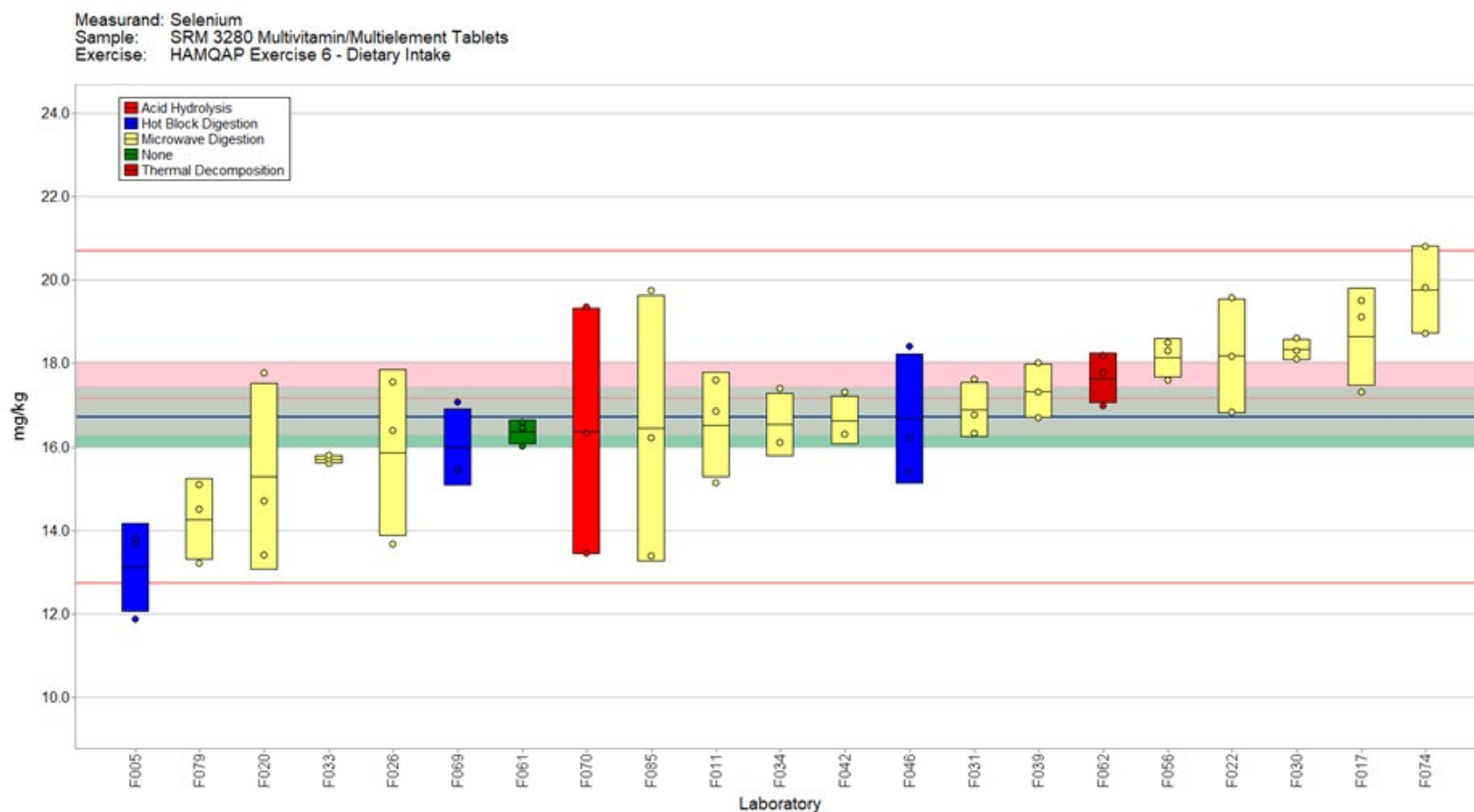


Figure 1-21. Selenium in SRM 3280 Multivitamin/Multielement Tablets (data summary view – sample preparation method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty (U_{NIST}) and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$.

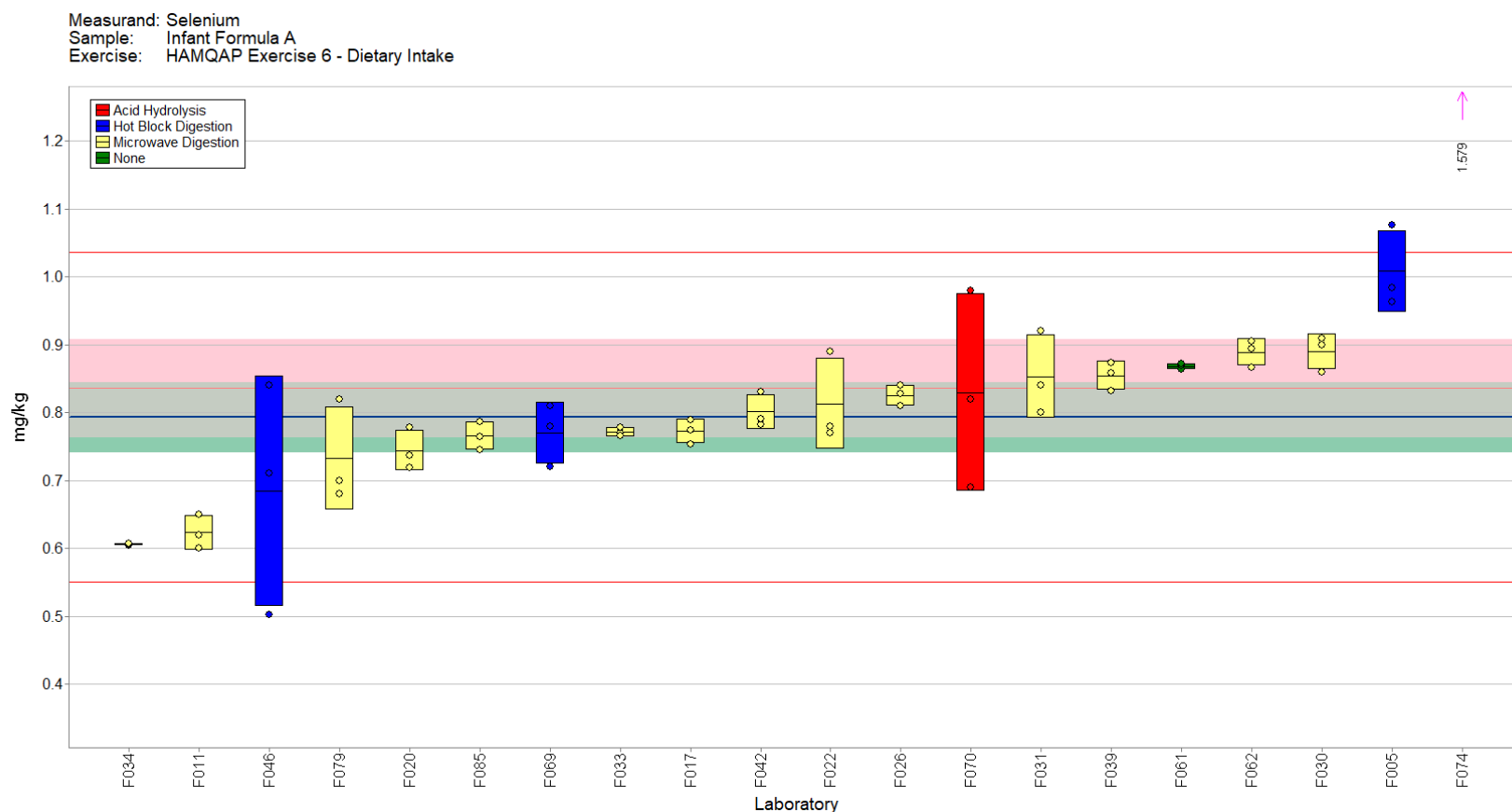


Figure 1-22. Selenium in Infant Formula A (data summary view – sample preparation method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty (U_{NIST}) and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$.

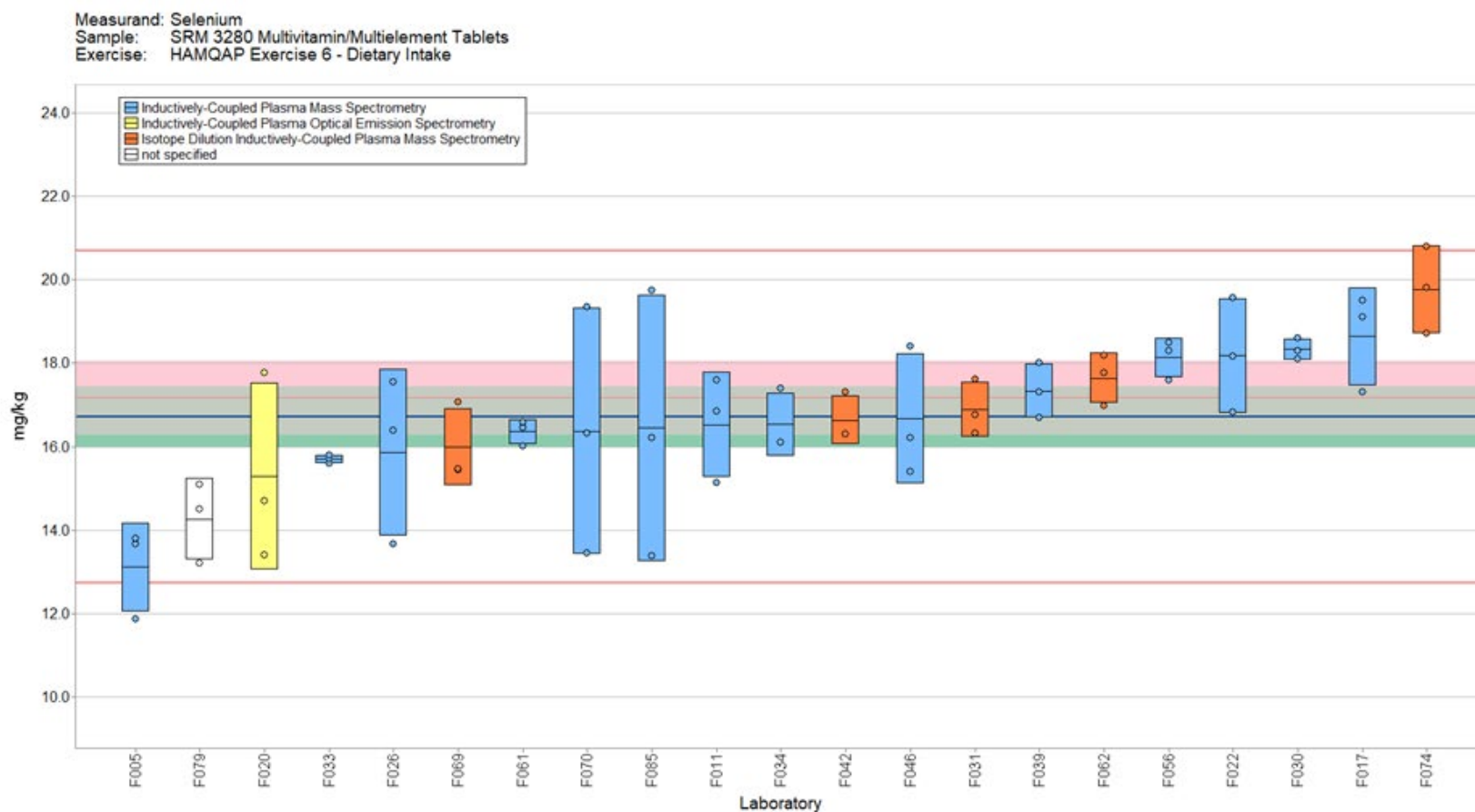


Figure 1-23. Selenium in SRM 3280 Multivitamin/Multielement Tablets (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The red solid lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty (U_{NIST}) and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$.

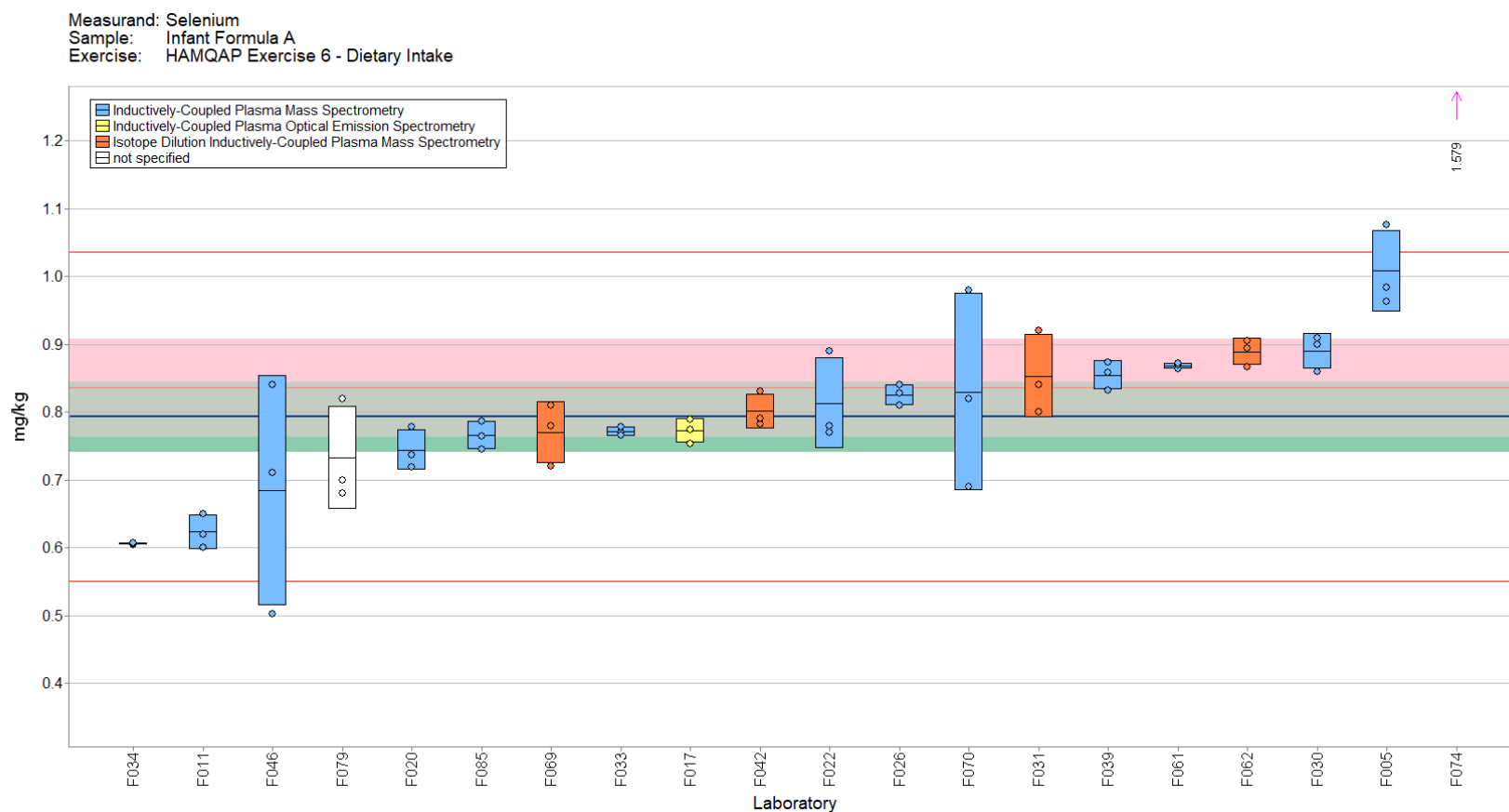


Figure 1-24. Selenium in Infant Formula A (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The red solid lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty (U_{NIST}) and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$.

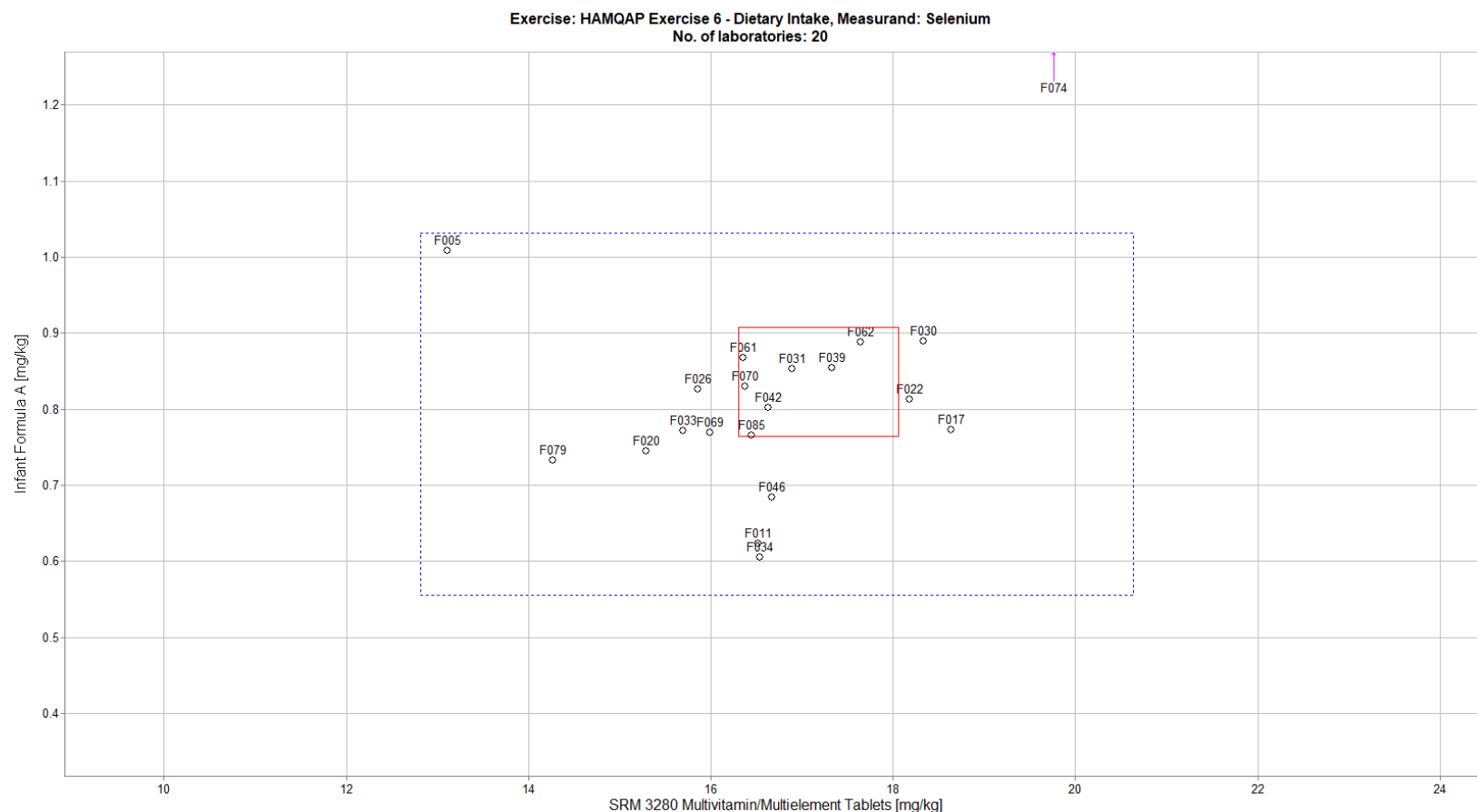


Figure 1-25. Laboratory means for selenium in SRM 3280 Multivitamin/Multielement Tablets and Infant Formula A (sample/sample comparison view). In this view, the individual laboratory mean for one sample (SRM 3280) is compared to the individual laboratory mean for a second sample (Infant Formula A). The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

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

Study Overview

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

Dietary Intake Sample Information

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

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

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

NIST-Determined Mass Fractions
in SRM 3256 Green Tea-Containing SODF (mg/kg)

<u>Analyte</u>	<u>(dry-mass basis)</u>	<u>(as-received basis)</u>
Arsenic (As)	0.269 ± 0.019	0.263 ± 0.019
Cadmium (Cd)	0.025 ± 0.002	0.024 ± 0.002
Lead (Pb)	0.316 ± 0.030	0.309 ± 0.029
Mercury (Hg)	0.014 ± 0.002	0.014 ± 0.002

Dietary Intake Study Results

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

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

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

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

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

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

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

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

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

Dietary Intake Technical Recommendations

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

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

the molecular ion interferences that may be found when analyzing arsenic in these materials.

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

- Mercury is volatile so care must be taken to not lose mercury during sample preparation. Microwave digestion is the best sample preparation method for mercury analysis. Laboratories that reported using hot block or open beaker digestion had a greater sample-to-sample variability.
- The low levels of mercury in the Rice Flour may be close to the LOQ for some techniques.
 - Since levels in blanks and backgrounds for mercury measurements may be large, leading to high detection limits and making determination of low-level samples difficult, a sufficient number of procedural blanks should be used to determine an accurate LOQ.
 - Low concentrations of mercury are not stable in solution over time. Samples should be prepared as near as possible to the time of analysis. Samples containing low concentrations of Hg may be more stable in dilute HCl than in dilute HNO₃.
 - Acidification of sample solutions will help prevent loss of Hg by adsorption. The addition of dichromate will help prevent loss of Hg through volatilization.
- The sensitivity of ICP-MS is low for Hg. Using cold vapor Hg generation increases sensitivity of ICP-MS and allows lower levels of Hg to be measured.
- Mercury carryover between samples is common and can lead to erratic results. Adequate washout time is needed after each measurement. The use of dilute HCl in the rinse solution may decrease the length of the washout time needed.
- Laboratories reporting measured values at or above the upper limit of the range of tolerance also reported larger within-laboratory variability indicating a potential calibration issue.
- All results should be reported accurately.
 - Zero is not a quantity that can be measured. If measured values are below detection limits, results should be reported as such. A more appropriate result would be to report that a value is below the LOQ or QL.
 - Laboratories reporting results outside the consensus tolerance levels should check for calculation errors. One example is to confirm that factors for all dilutions have been properly tabulated and that results are reported in correct reporting units.

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

HAMQAP Exercise 6 - Toxic Elements										
Lab Code:		NIST	1. Your Results				2. Community Results			3. Target
Analyte	Sample	Units	x_i	s_i	Z'_{comm}	Z_{NIST}	N	x^*	s^*	x_{NIST} U
Arsenic	SRM 3256 Green Tea-Containing SODF	mg/kg	0.263	0.019			34	0.266	0.038	0.263 0.019
Arsenic	Rice Flour	mg/kg	0.32	0.033			31	0.341	0.053	0.32 0.033
Cadmium	SRM 3256 Green Tea-Containing SODF	mg/kg	0.024	0.002			32	0.022	0.004	0.024 0.002
Cadmium	Rice Flour	mg/kg	0.009	0.00074			21	0.009	0.002	0.009 0.00074
Mercury	SRM 3256 Green Tea-Containing SODF	mg/kg	0.014	0.002			28	0.013	0.004	0.014 0.002
Mercury	Rice Flour	mg/kg	0.002	0.00005			14	0.004	0.004	0.002 0.00005
Lead	SRM 3256 Green Tea-Containing SODF	mg/kg	0.309	0.029			36	0.281	0.043	0.309 0.029
Lead	Rice Flour	mg/kg	0.017	0.0045			26	0.018	0.009	0.017 0.0045
			x_i	Mean of reported values			N	Number of quantitative values reported		x_{NIST} NIST-assessed value
			s_i	Standard deviation of reported values						U expanded uncertainty
			Z'_{comm}	Z'-score with respect to community consensus			x^*	Robust mean of reported values		about the NIST-assessed value
			Z_{NIST}	Z-score with respect to NIST value			s^*	Robust standard deviation		

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

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

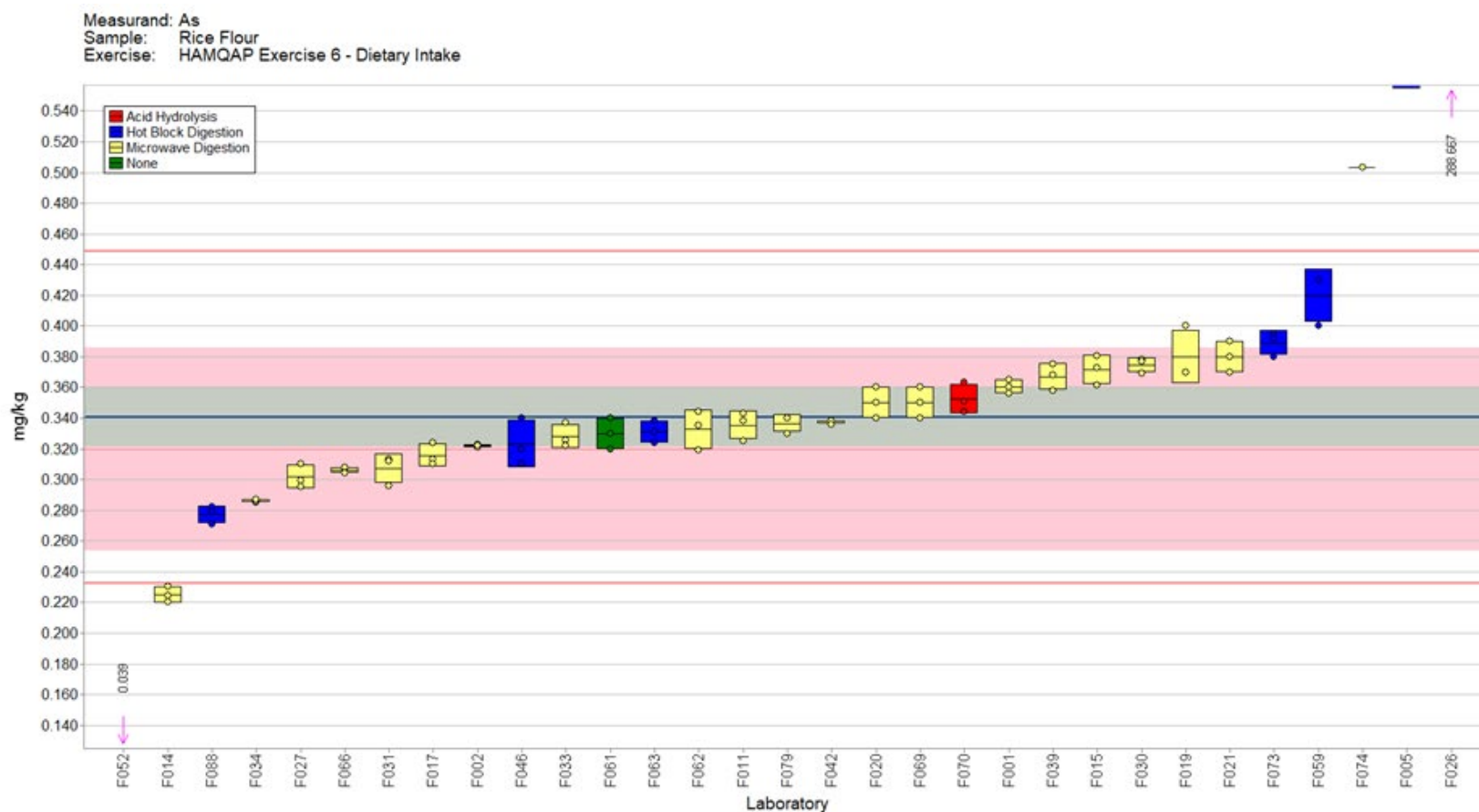


Figure 2-1. Arsenic in Rice Flour (data summary view –sample preparation method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The solid blue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

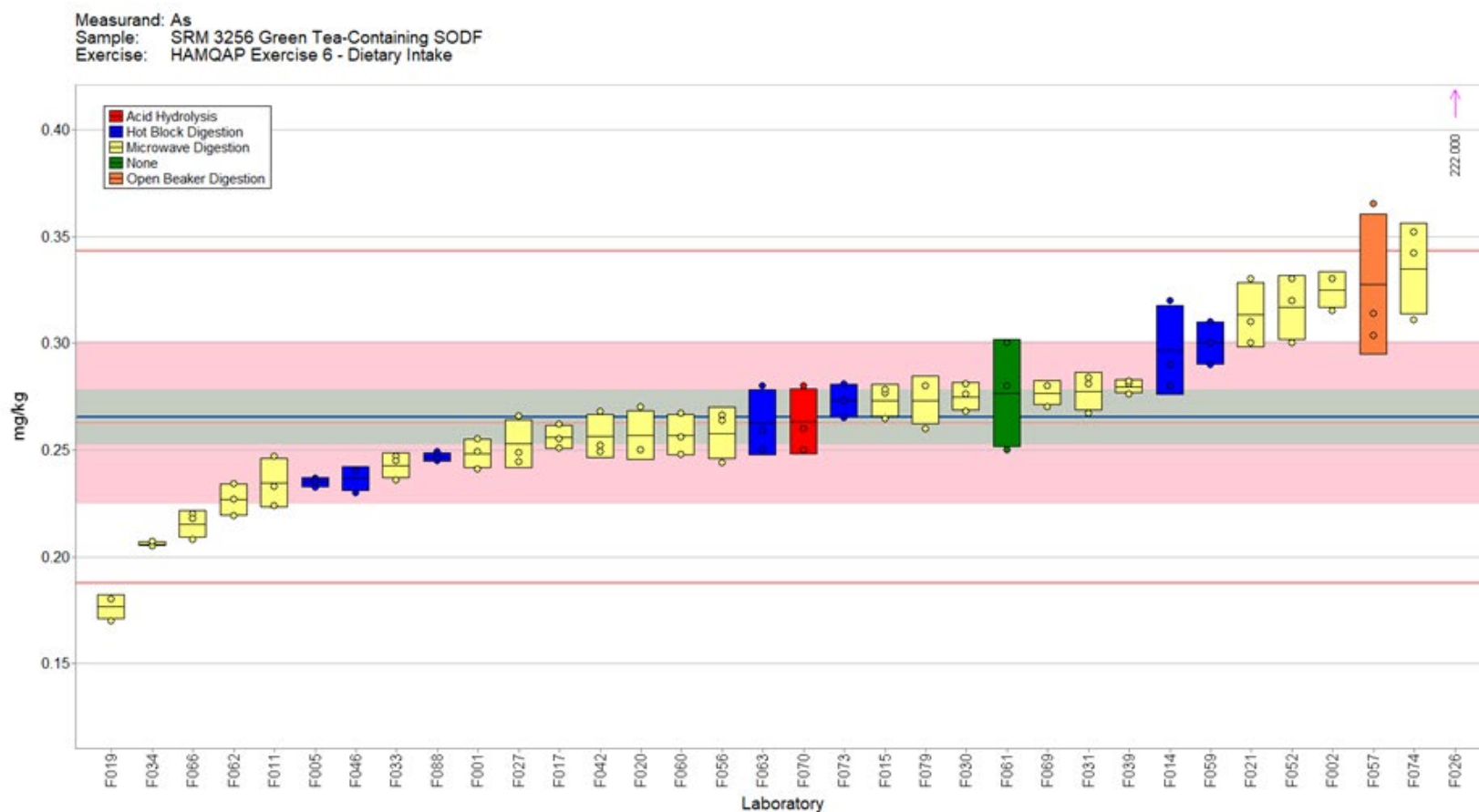


Figure 2-2. Arsenic in SRM 3256 Green Tea-Containing SODF (data summary view –sample preparation method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method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

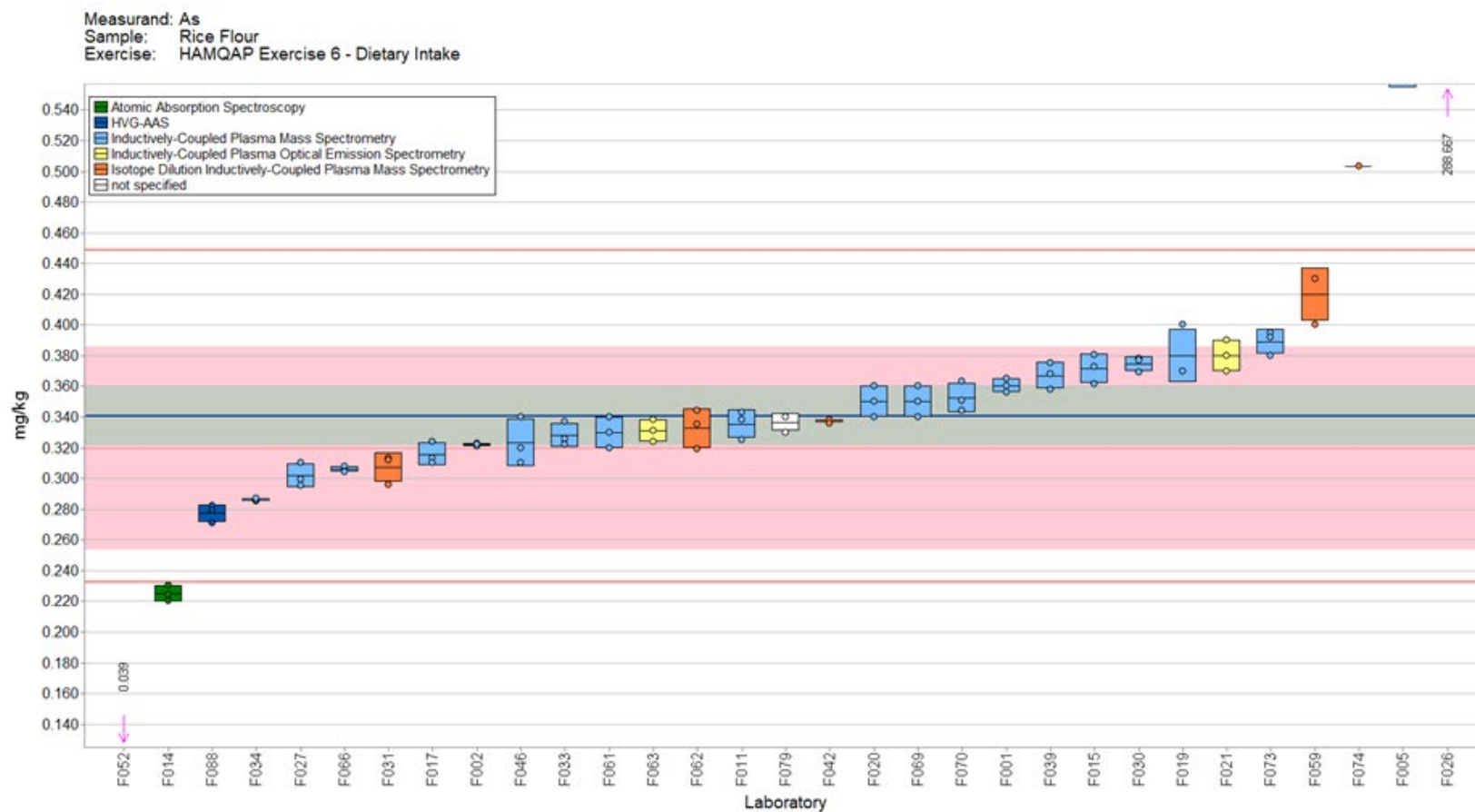


Figure 2-3. Arsenic in Rice Flour (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line represents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

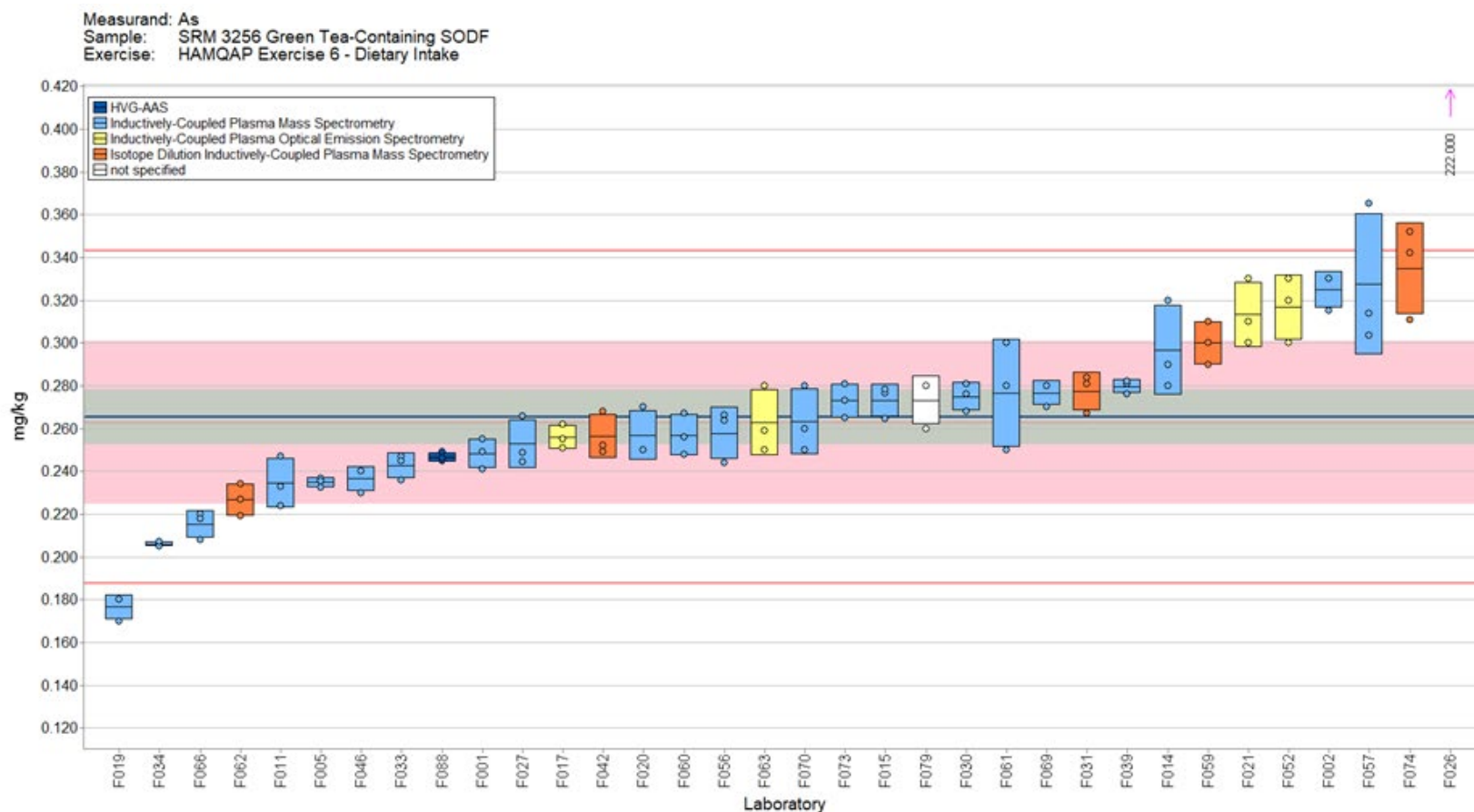


Figure 2-4. Arsenic in SRM 3256 Green Tea-Containing SODF (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty (U_{NIST}) and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$.

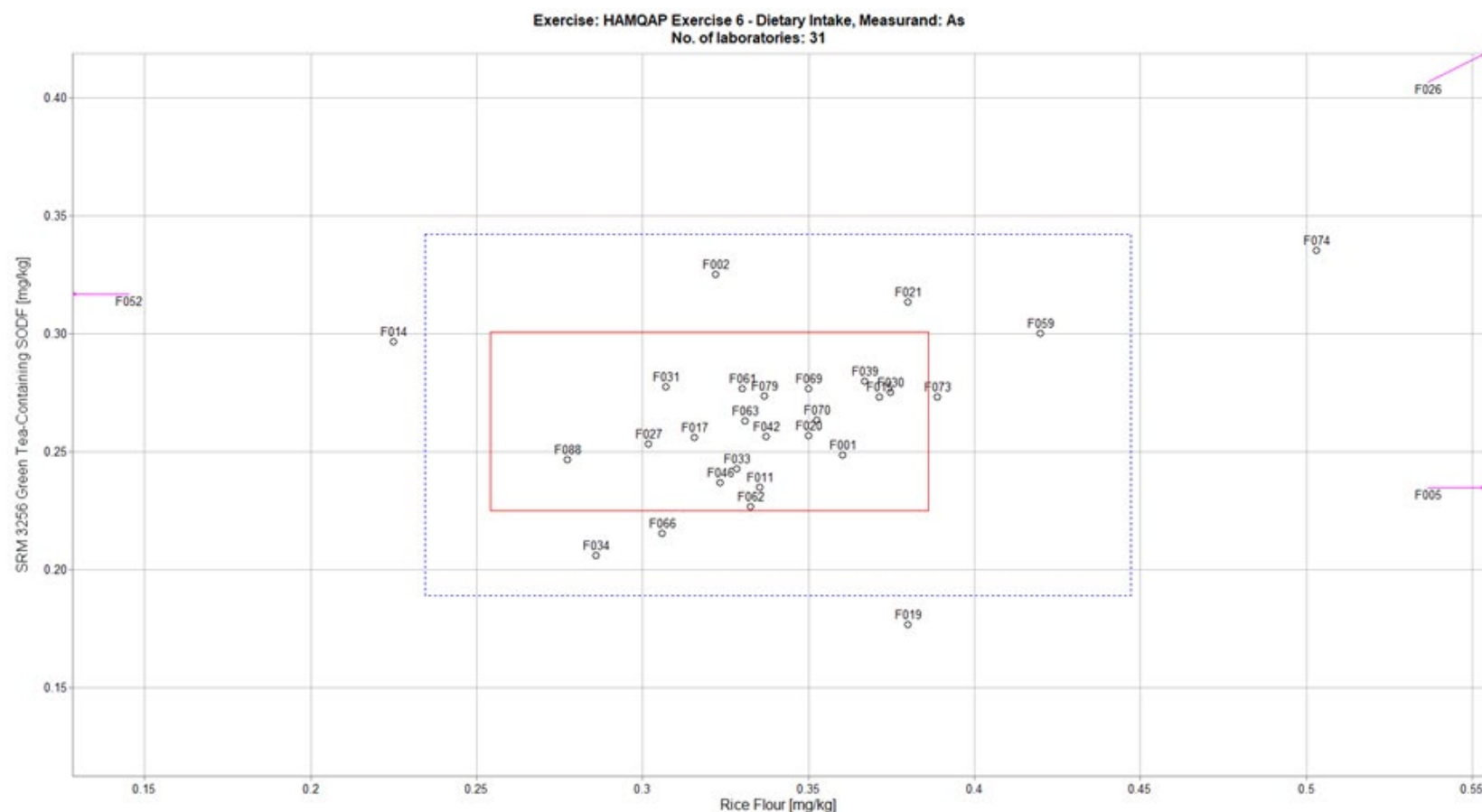


Figure 2-5. Laboratory means for arsenic in Rice Flour and SRM 3256 Green Tea-Containing SODF (sample/sample comparison view). In this view, the individual laboratory mean for one sample (Rice Flour) is compared to the mean for a second sample (SRM 3256). The solid red box represents the NIST range of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

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

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

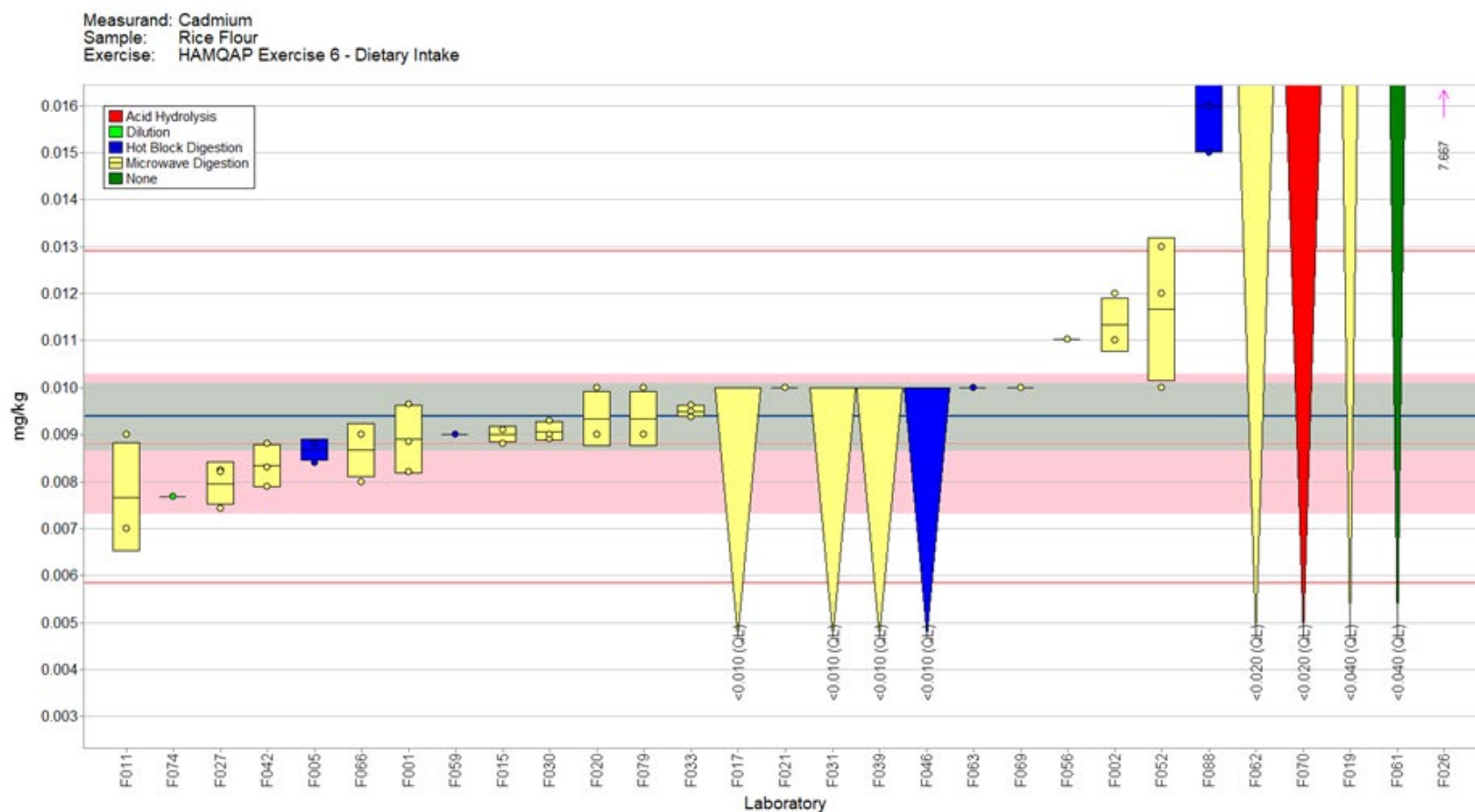


Figure 2-6. Cadmium in Rice Flour (data summary view –sample preparation method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The solid blue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

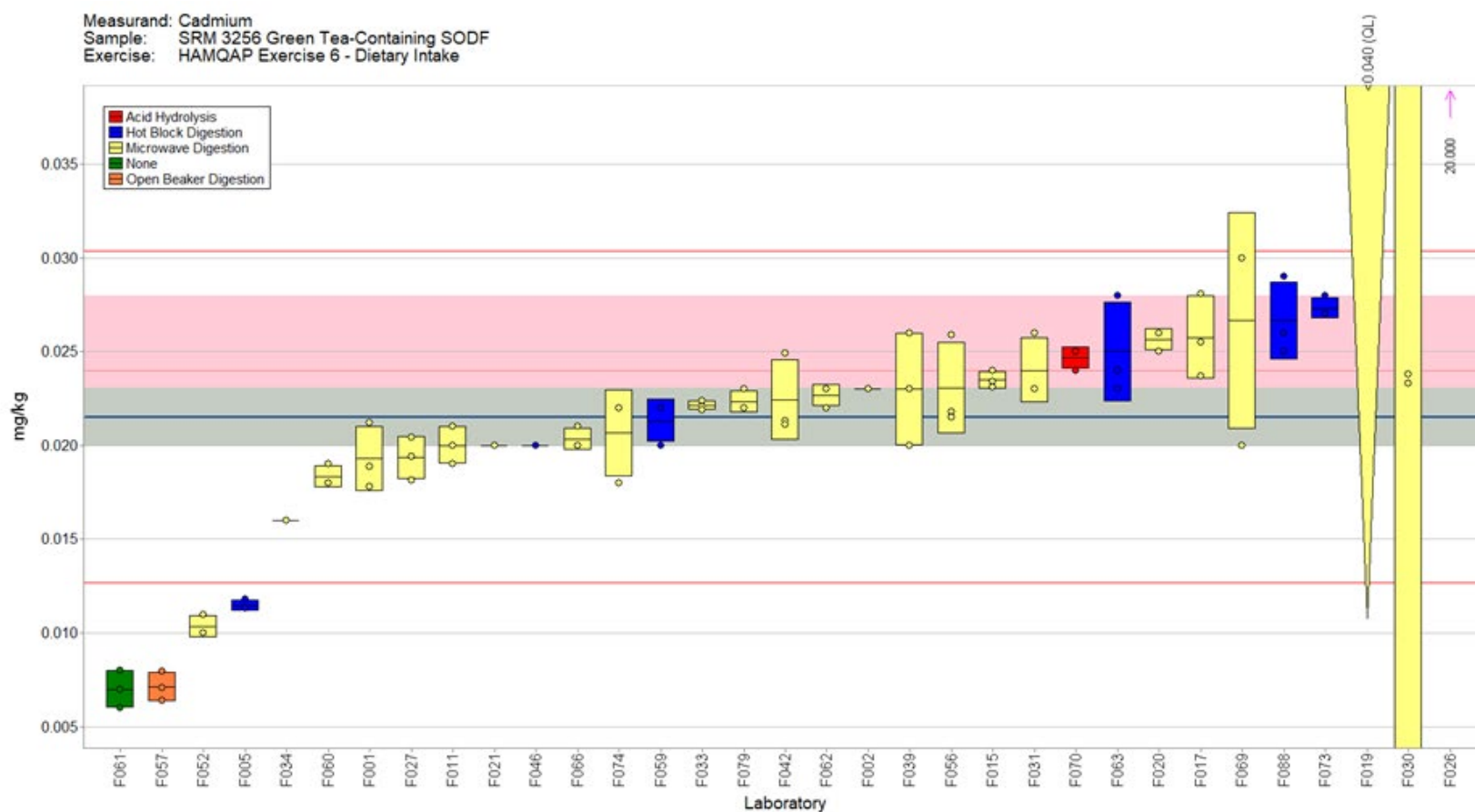


Figure 2-7. Cadmium in SRM 3256 Green Tea-Containing SODF (data summary view –sample preparation method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method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

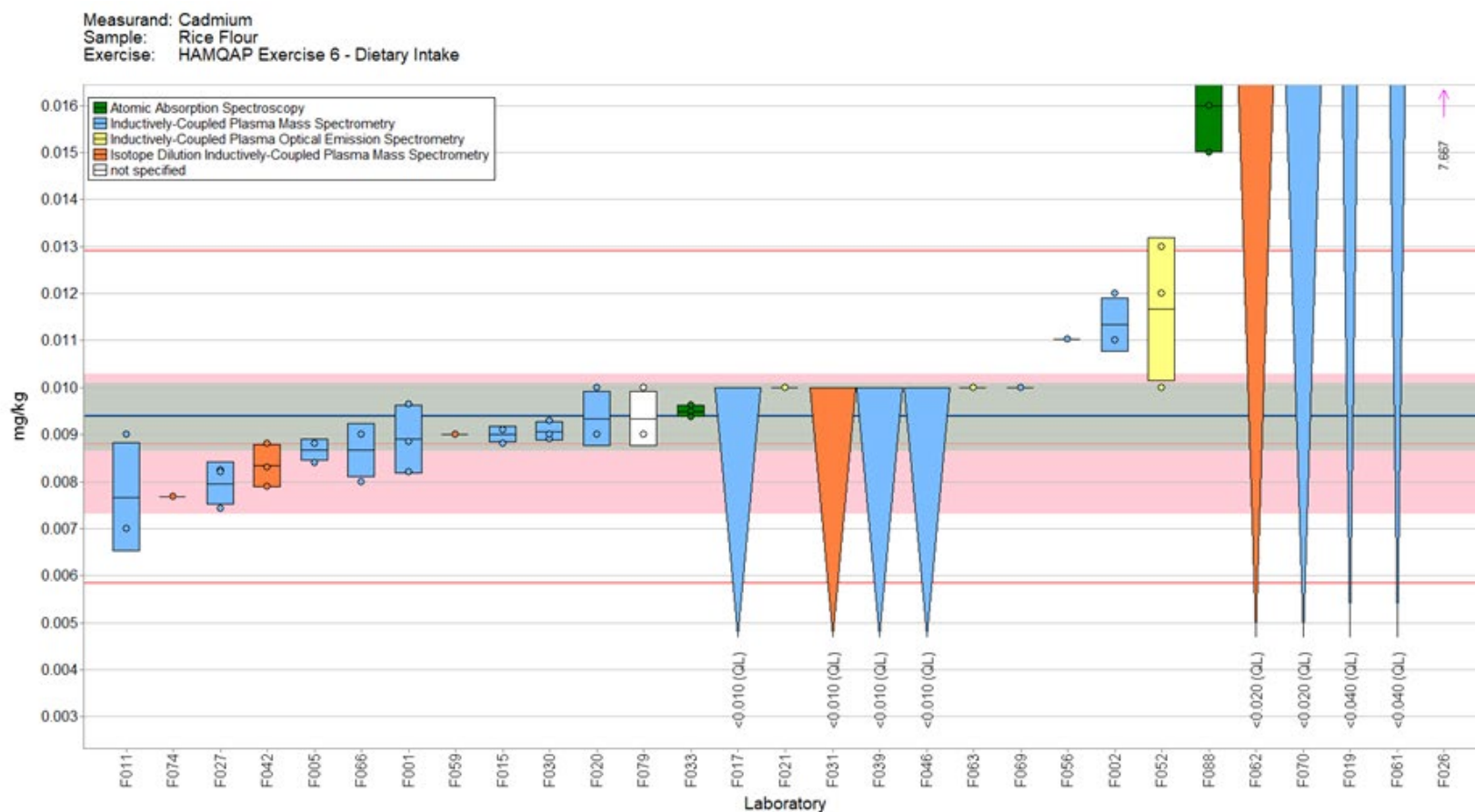


Figure 2-8. Cadmium in Rice Flour (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line represents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

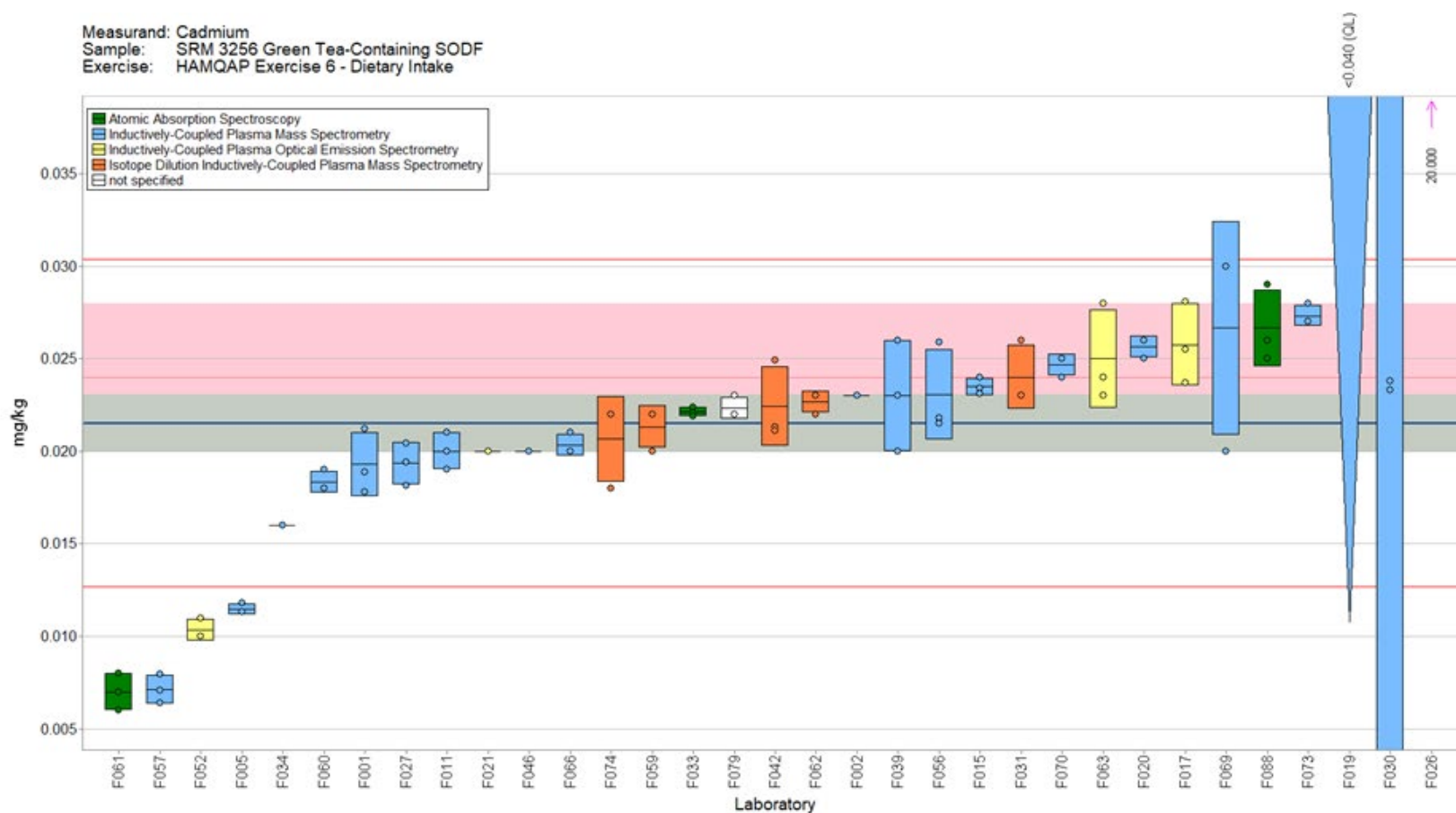


Figure 2-9. Cadmium in SRM 3256 Green Tea-Containing SODF (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty (U_{NIST}) and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$.

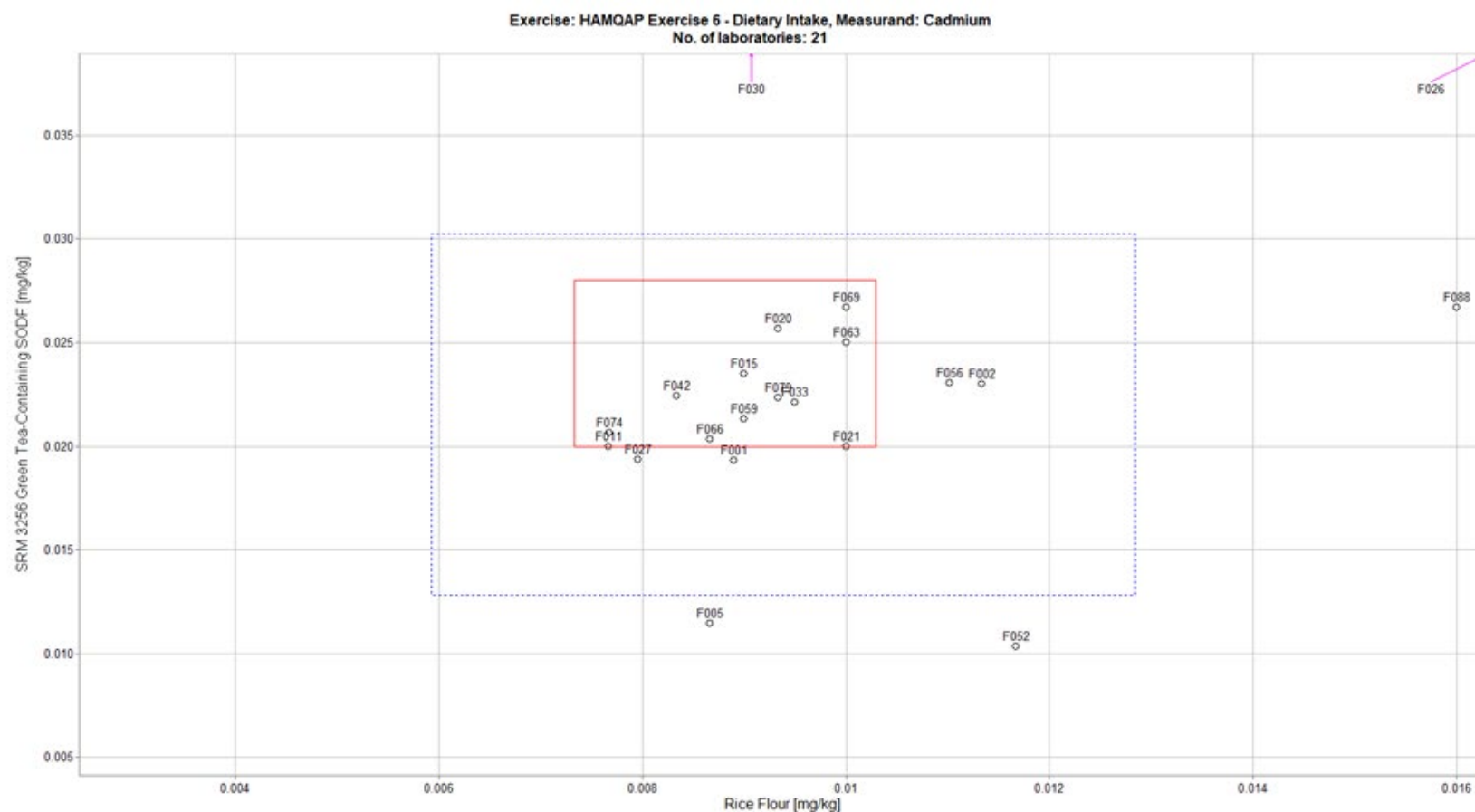


Figure 2-10. Laboratory means for cadmium in Rice Flour and SRM 3256 Green Tea-Containing SODF (sample/sample comparison view). In this view, the individual laboratory mean for one sample (Rice Flour) is compared to the mean for a second sample (SRM 3256). The solid red box represents the NIST range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

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

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

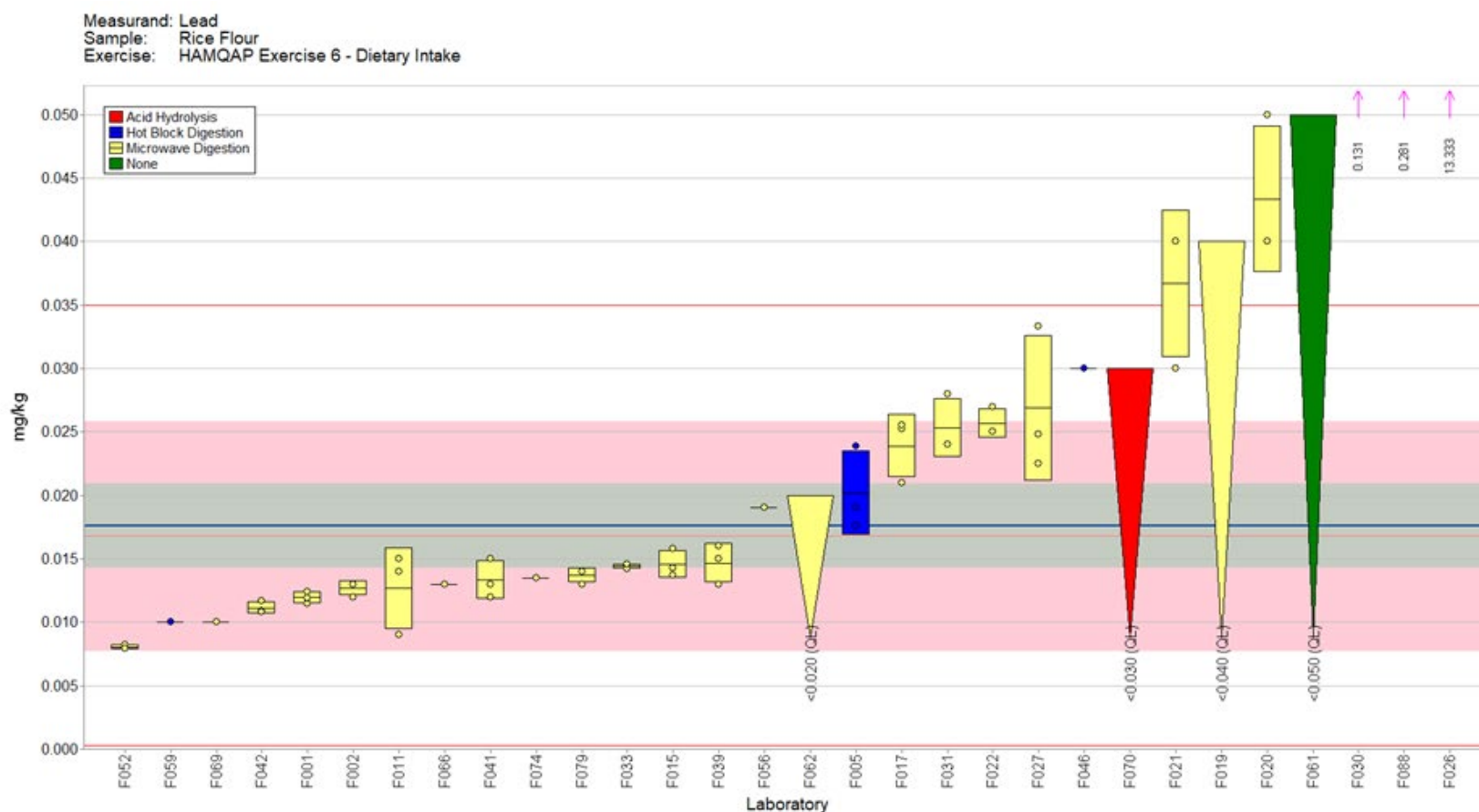


Figure 2-11. Lead in Rice Flour (data summary view –sample preparation method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$, with the lower range set at zero. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty (U_{NIST}) and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$.

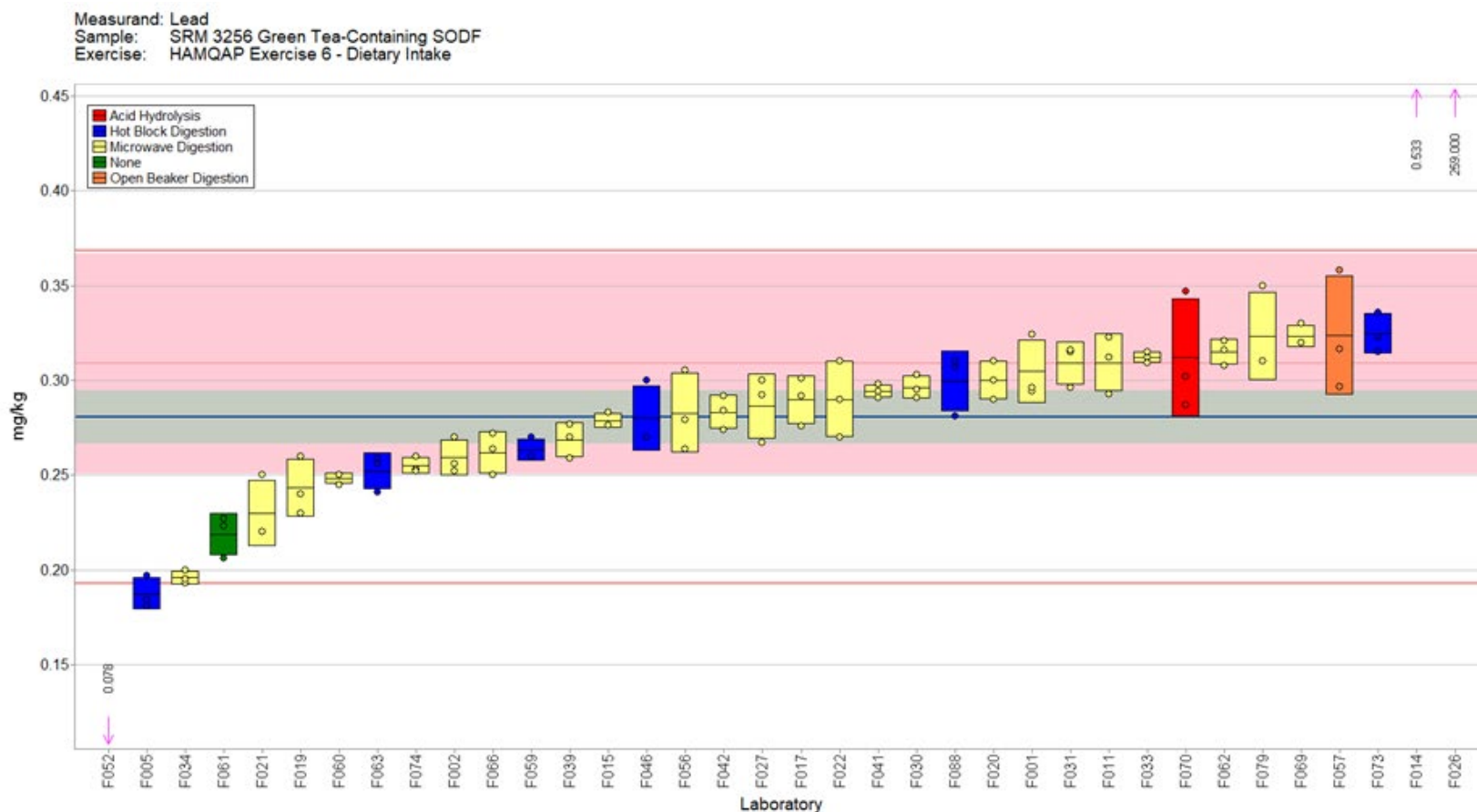


Figure 2-12. Lead in SRM 3256 Green Tea-Containing SODF (data summary view –sample preparation method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty (U_{NIST}) and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$.

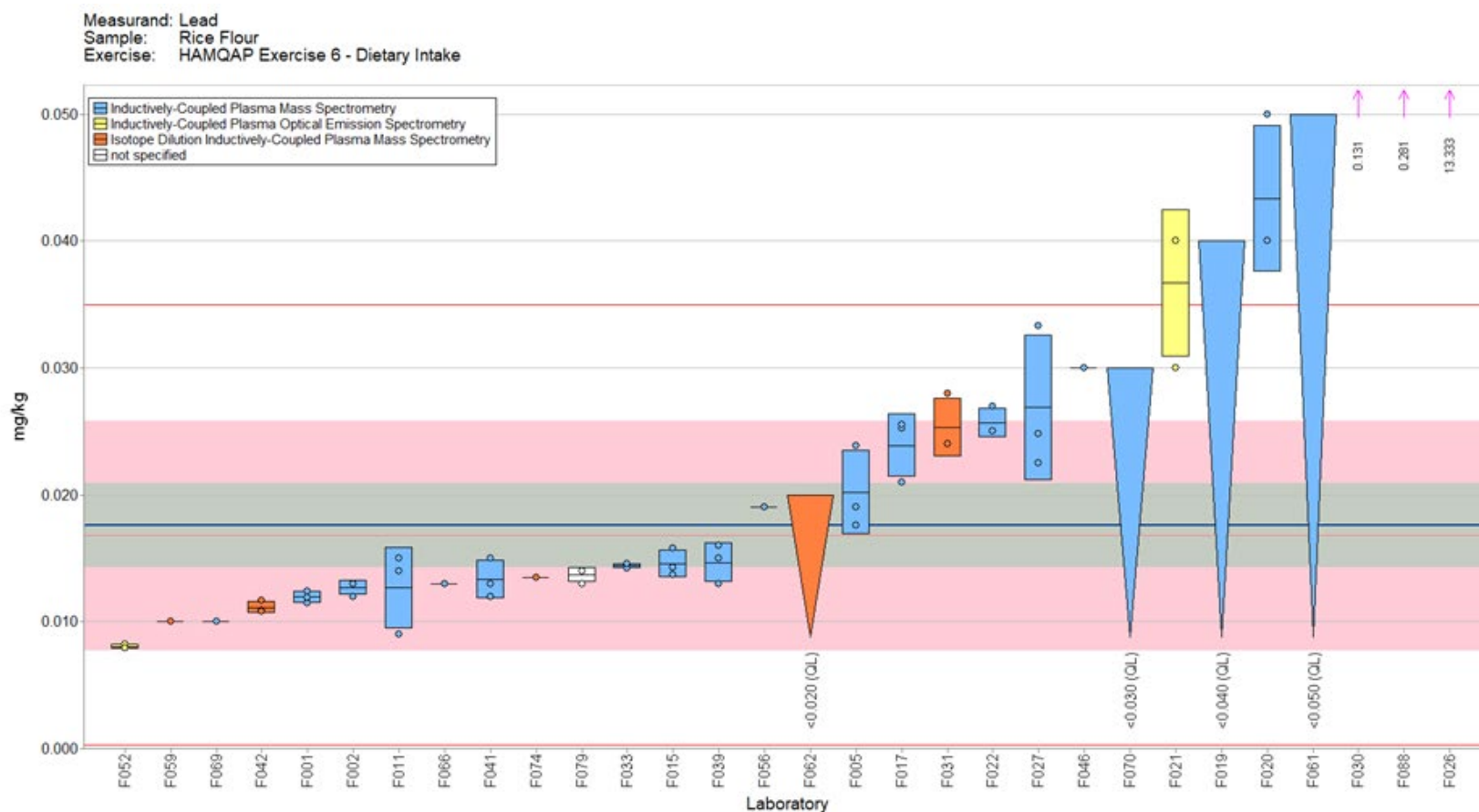


Figure 2-13. Lead in Rice Flour (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$, with the lower range set to zero. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty (U_{NIST}) and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$.

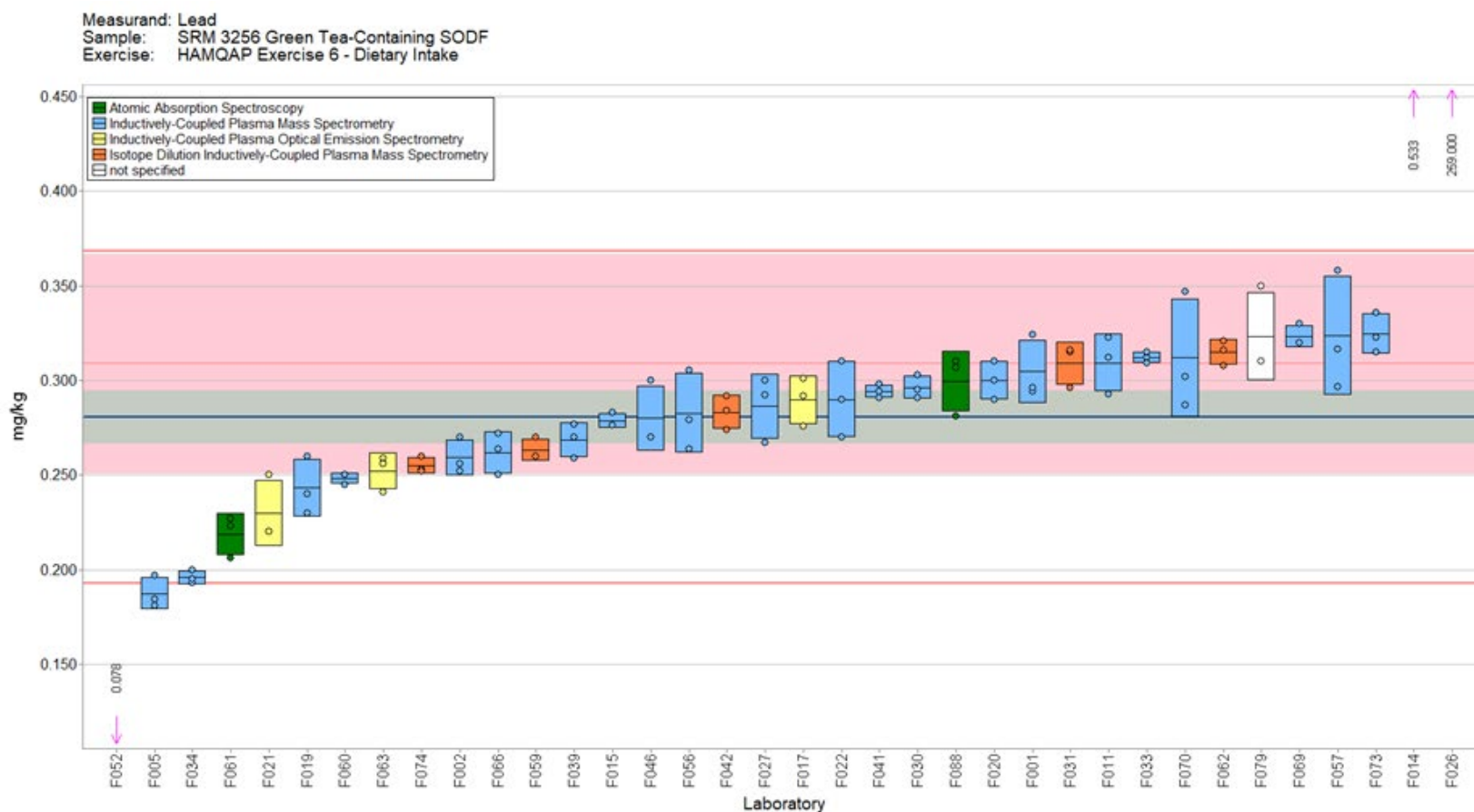


Figure 2-14. Lead in SRM 3256 Green Tea-Containing SODF (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty (U_{NIST}) and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$.

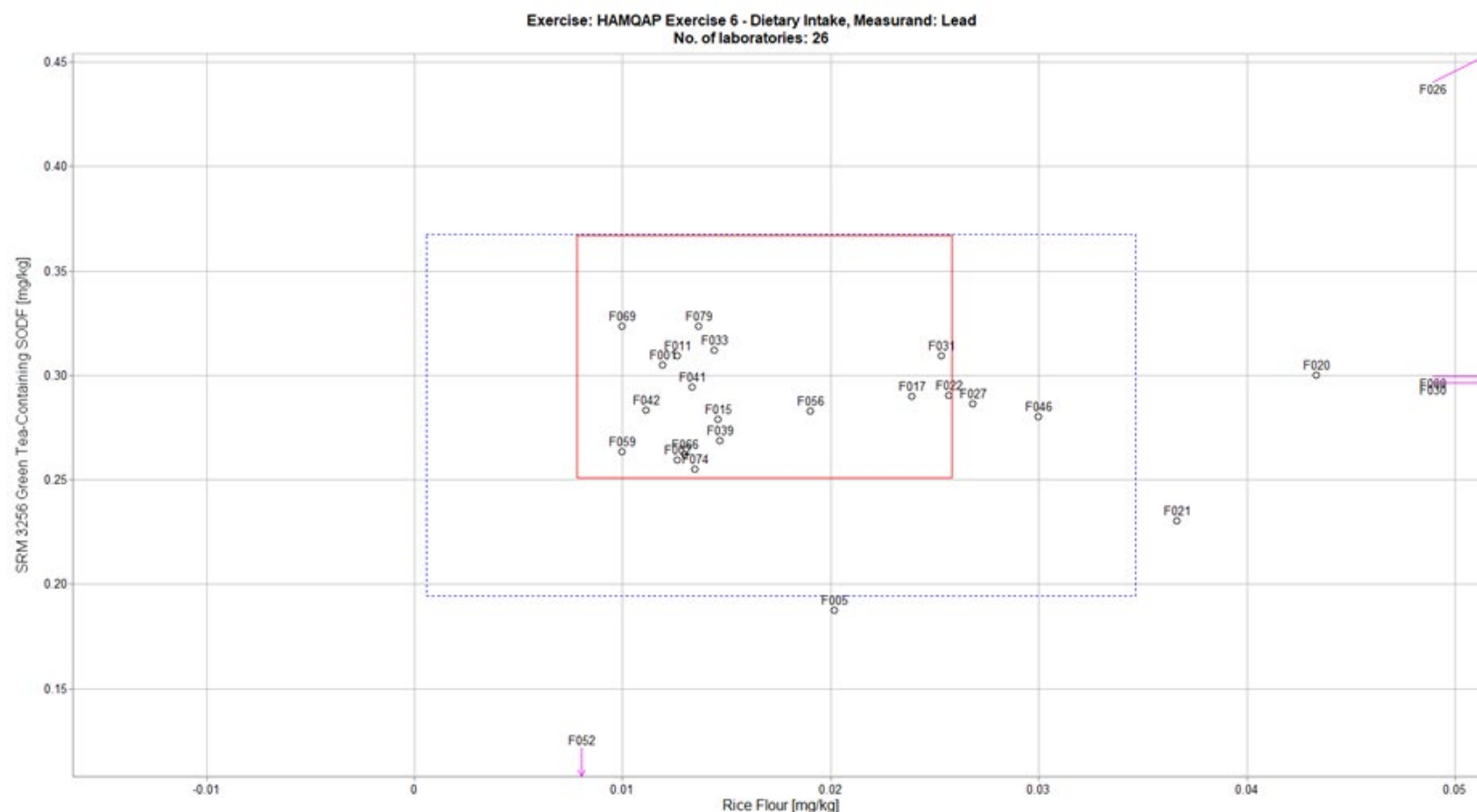


Figure 2-15. Laboratory means for lead in Rice Flour and SRM 3256 Green Tea-Containing SODF (sample/sample comparison view). In this view, the individual laboratory mean for one sample (Rice Flour) is compared to the mean for a second sample (SRM 3256). The solid red box represents the NIST range of tolerance for the two samples, Rice Flour (x-axis) and SRM 3256 (y-axis), which encompasses the target values bounded by their uncertainties (U_{NIST}) and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$. The dotted blue box represents the consensus range of tolerance for Rice Flour (x-axis) and SRM 3256 (y-axis), calculated as the values above and below the consensus means that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$.

Table 2-5. Data summary table for mercury in Rice Flour and SRM 3256 Green Tea-Containing SODF. Data points highlighted in blue have been identified as outside the consensus tolerance limits and would be estimated to result in an unacceptable Z'_{comm} score, $|Z'_{\text{comm}}| \geq 2$. Data points highlighted in red have a zero or non-numeric data point.

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

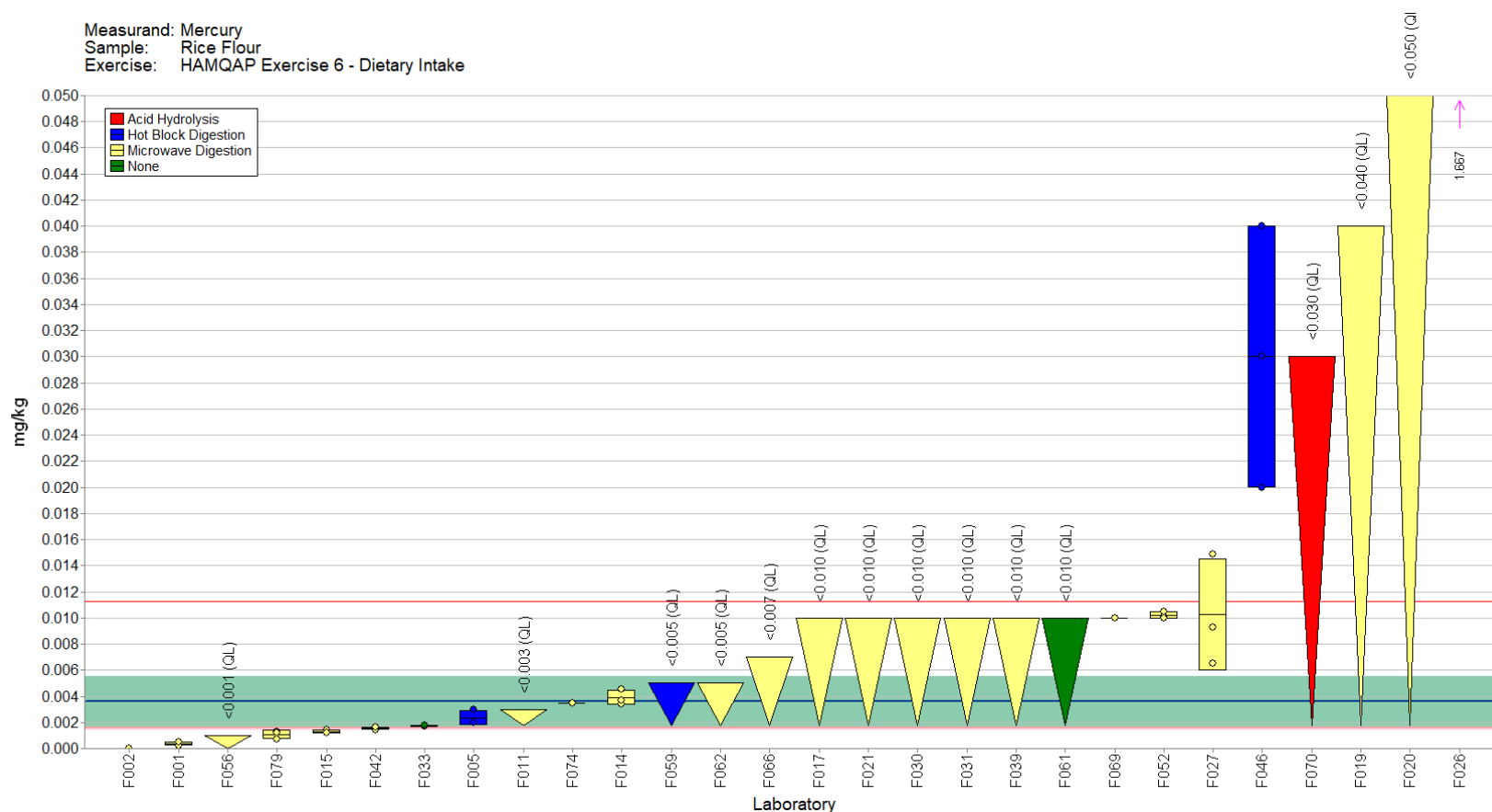


Figure 2-16. Mercury in Rice Flour (data summary view –sample preparation method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The solid blue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

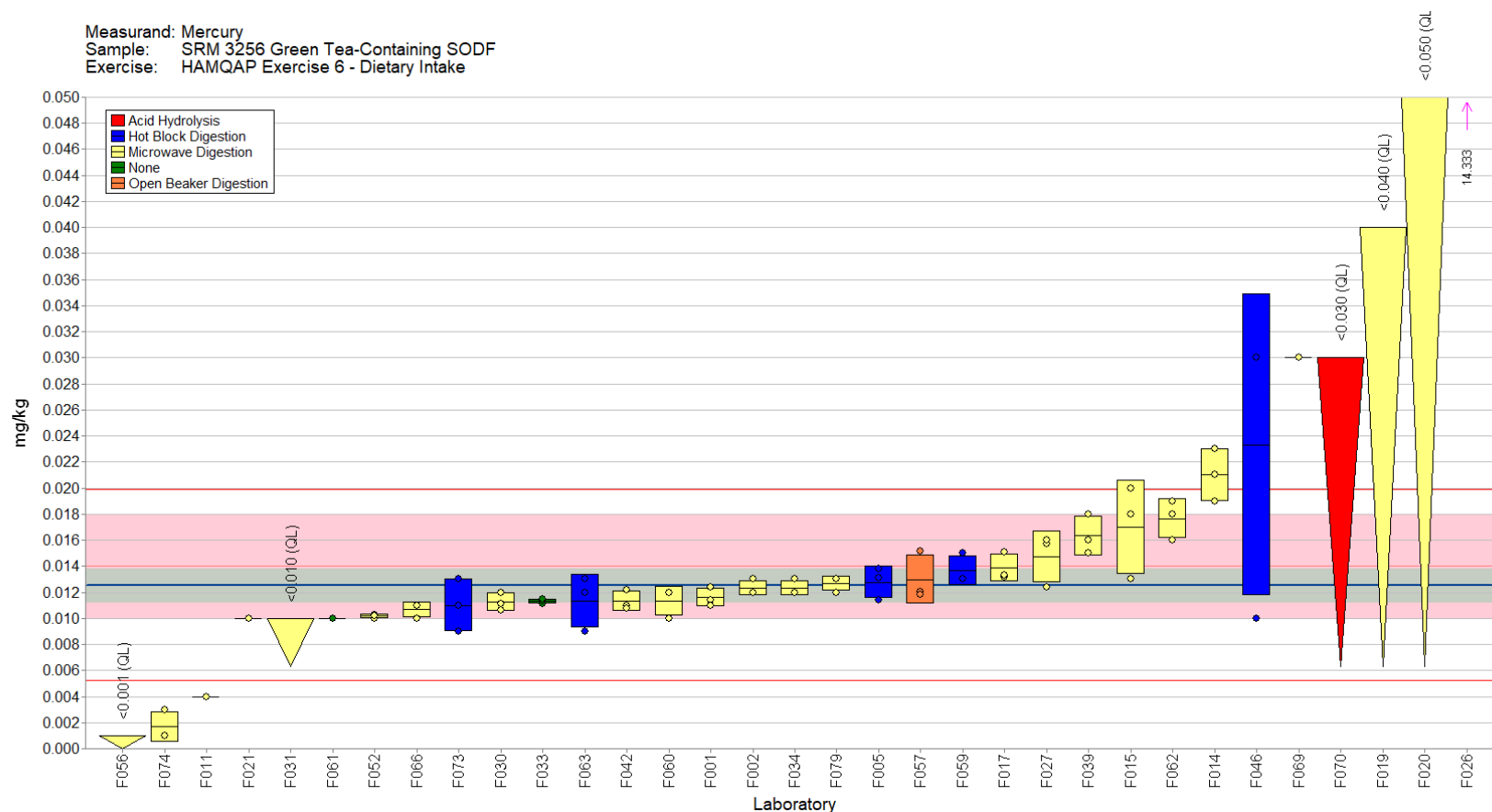


Figure 2-17. Mercury in SRM 3256 Green Tea-Containing SODF (data summary view –sample preparation method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method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

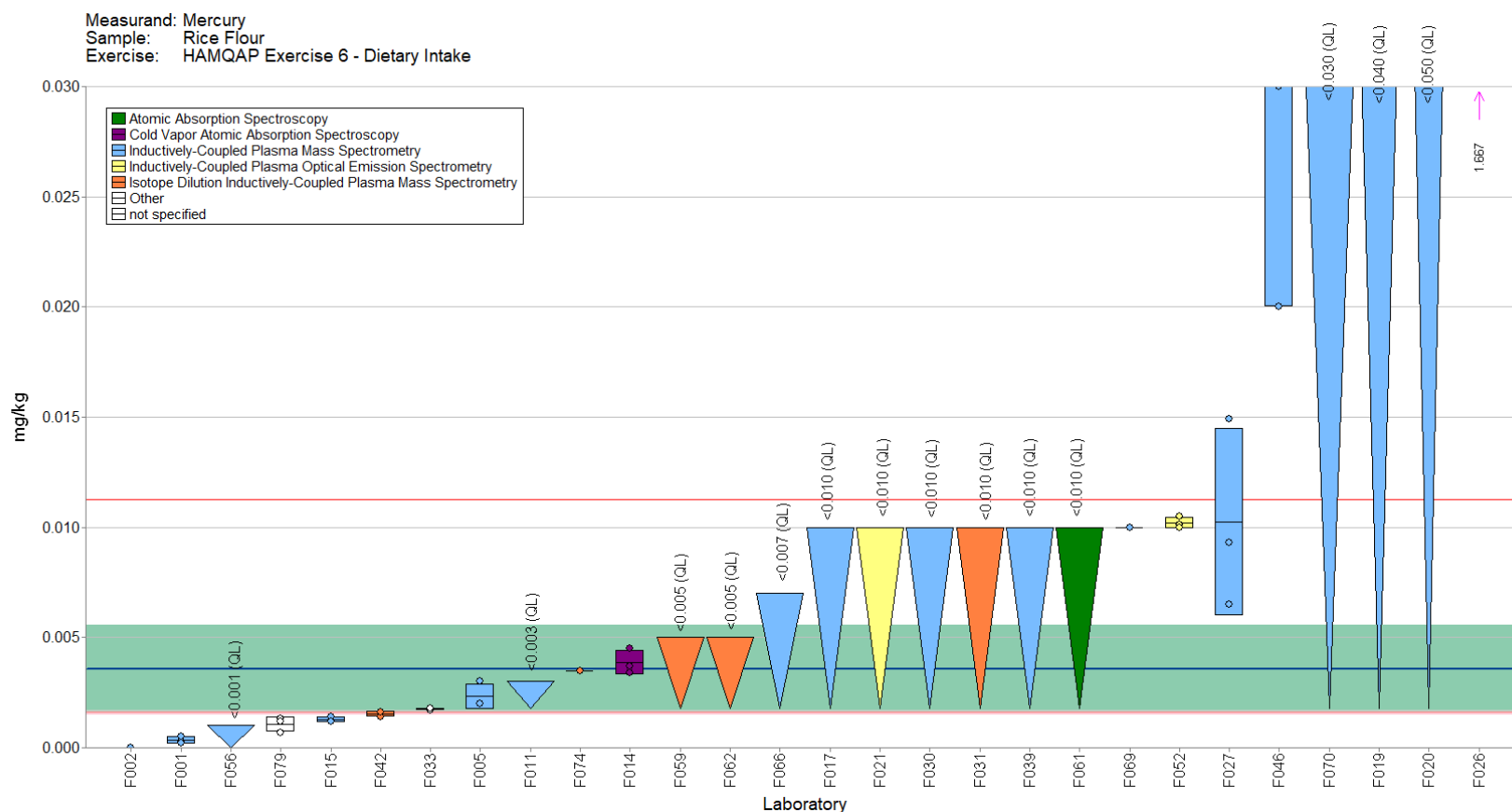


Figure 2-18. Mercury in Rice Flour (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line represents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

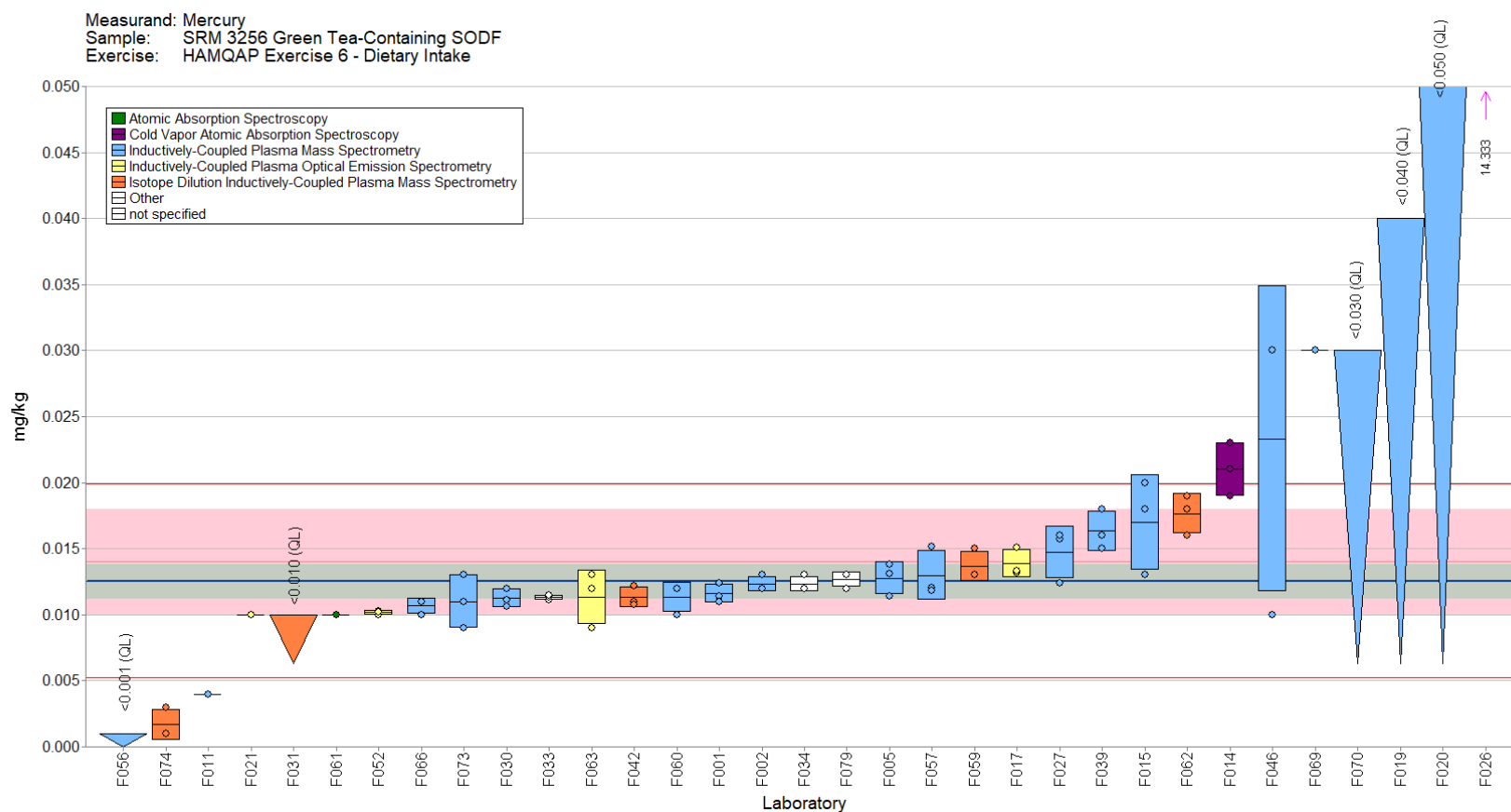


Figure 2-19. Mercury in SRM 3256 Green Tea-Containing SODF (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty (U_{NIST}) and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$.

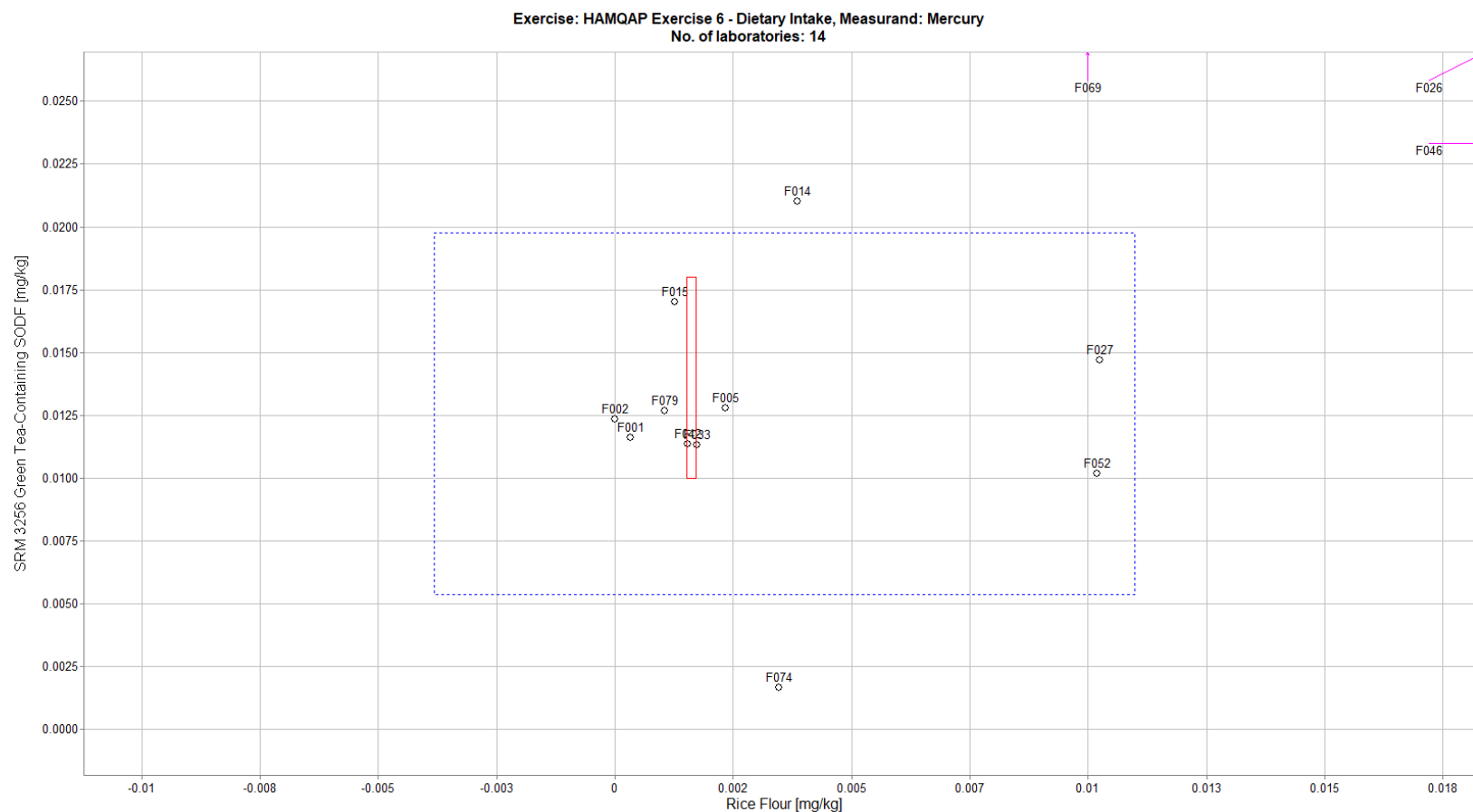


Figure 2-20. Laboratory means for mercury in Rice Flour and SRM 3256 Green Tea-Containing SODF (sample/sample comparison view). In this view, the individual laboratory mean for one sample (Rice Flour) is compared to the mean for a second sample (SRM 3256). The solid red box represents the NIST range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

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

Study Overview

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

Dietary Intake Sample Information

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

<u>Analyte</u>	<u>NIST-Determined Mass Fraction in Infant Formula A (mg/kg)</u>		
Biotin	2.14	$\pm$	0.14
Vitamin C (Ascorbic Acid)	22.04	$\pm$	1.66

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

<u>Analyte</u>	<u>NIST-Determined Mass Fraction in Multivitamin B (mg/kg)</u>		
Biotin	946	$\pm$	36
Vitamin C (Ascorbic Acid)	46700	$\pm$	2600

Dietary Intake Study Results

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

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

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

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

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

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

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

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

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

Dietary Intake Technical Recommendations

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

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

- Calculations and reporting units must be verified prior to submission of results. Laboratories often report results in the wrong units or forget a dilution factor during the calculation of the final results, resulting in poor performance for the study.
- The use of appropriate calibration materials and quality assurance samples to establish that a method is in control and being performed correctly may reduce the likelihood of outlying data. Quality assurance samples can be commercially available reference materials (CRMs, SRMs, or RMs) or materials prepared in-house.

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

HAMQAP Exercise 6 - Water-Soluble Vitamins									
Lab Code:		NIST	1. Your Results				2. Community Results		
Analyte	Sample	Units	x_i	s_i	Z'_{comm}	Z_{NIST}	N	x^*	s^*
Biotin	Infant Formula A	mg/kg	2.13	0.142			14	2.06	0.28
Biotin	Multivitamin B	mg/kg	22	1.66			16	23.6	9.2
Vitamin C (Ascorbic Acid)	Infant Formula A	mg/kg	946	36			21	970	170
Vitamin C (Ascorbic Acid)	Multivitamin B	mg/kg	46700	2600			25	42800	5100
			x_i	Mean of reported values			N	Number of quantitative values reported	
			s_i	Standard deviation of reported values				x_{NIST}	NIST-assessed value
			Z'_{comm}	Z'-score with respect to community consensus			x^*	Robust mean of reported values	
			Z_{NIST}	Z-score with respect to NIST value			s^*	Robust standard deviation	
								U	expanded uncertainty about the NIST-assessed value

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

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

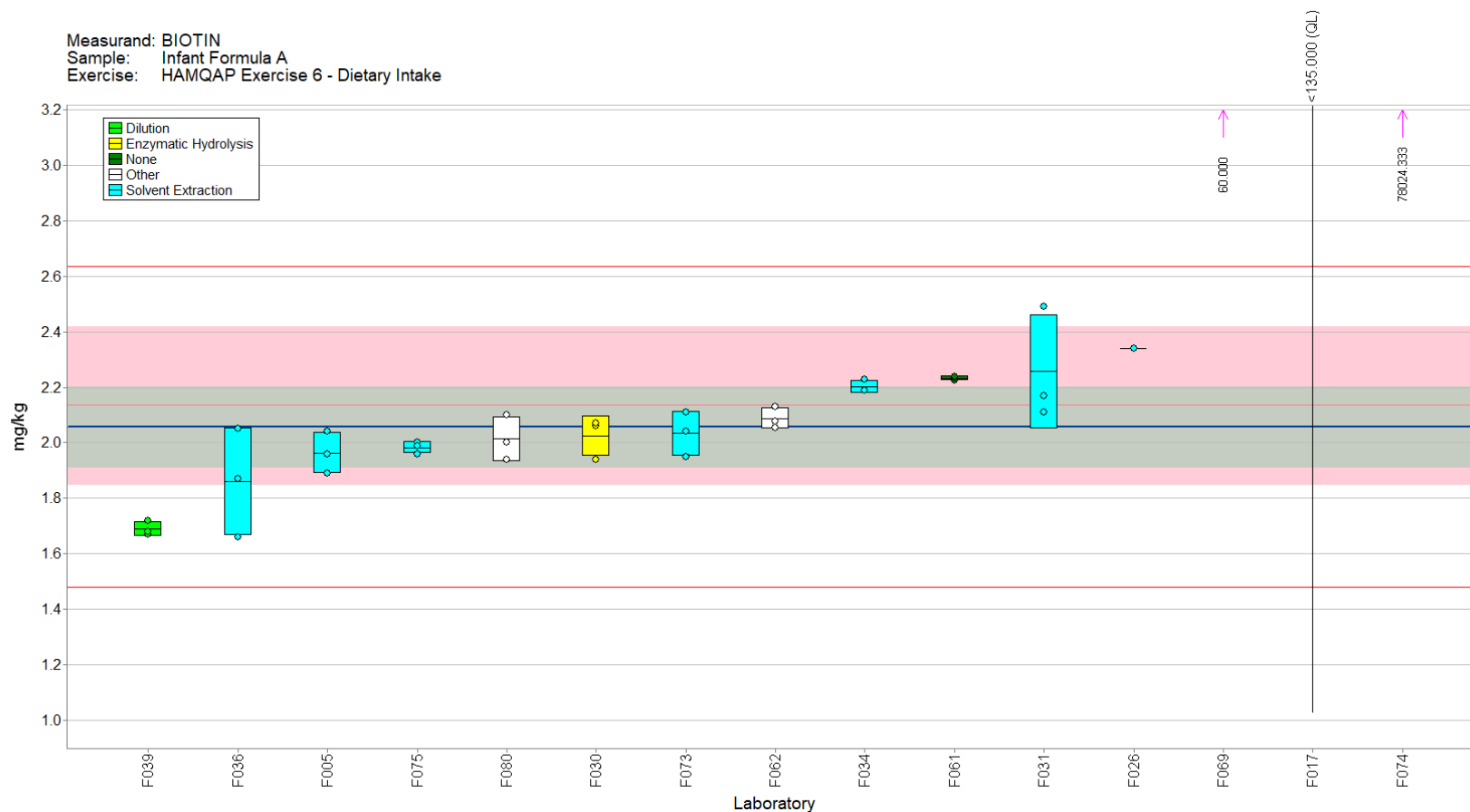


Figure 3-1. Biotin in Infant Formula A (data summary view – sample preparation). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty (U_{NIST}) and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$.

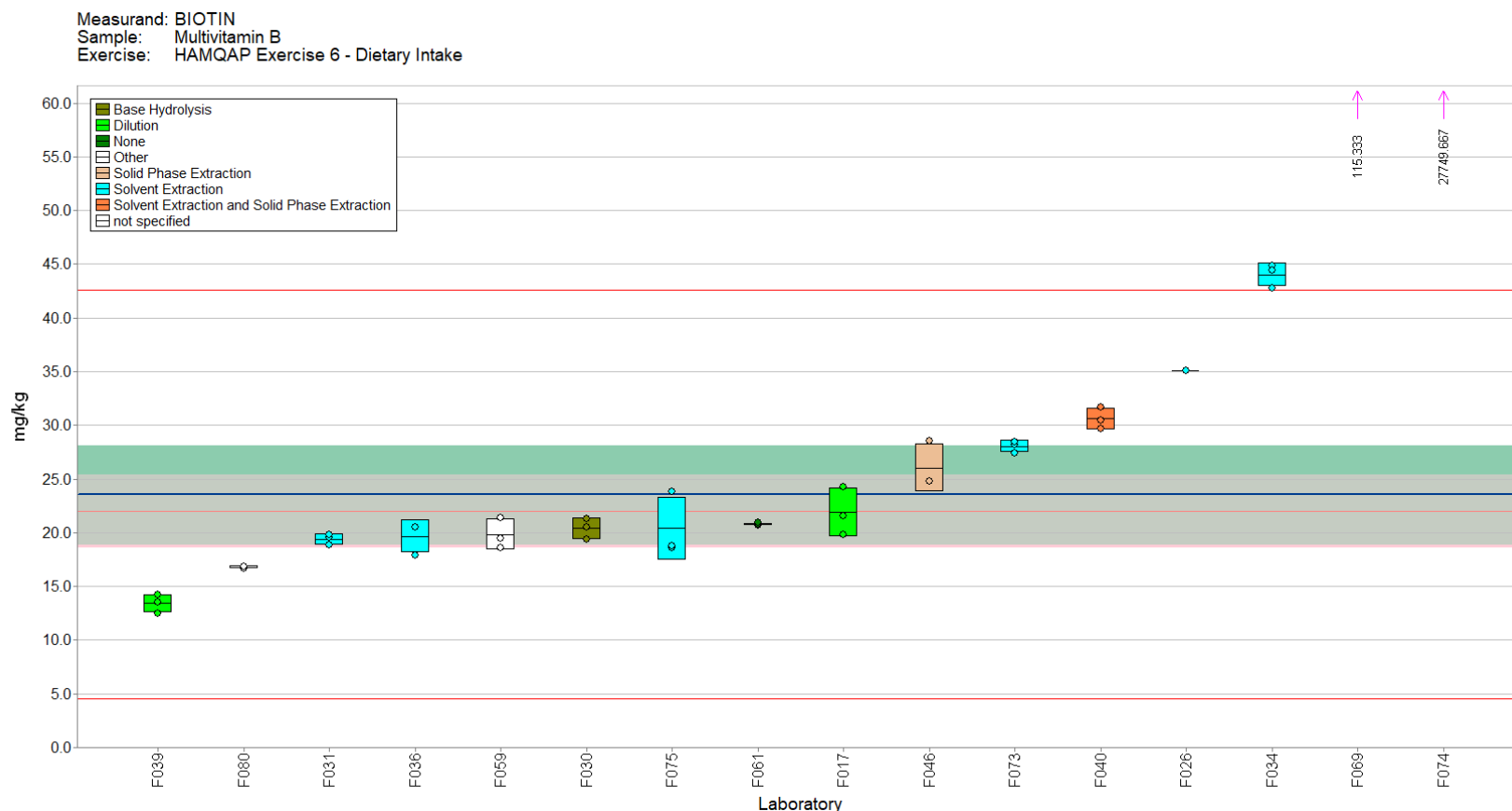


Figure 3-2. Biotin in Multivitamin B (data summary view – sample preparation). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The solid blue line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

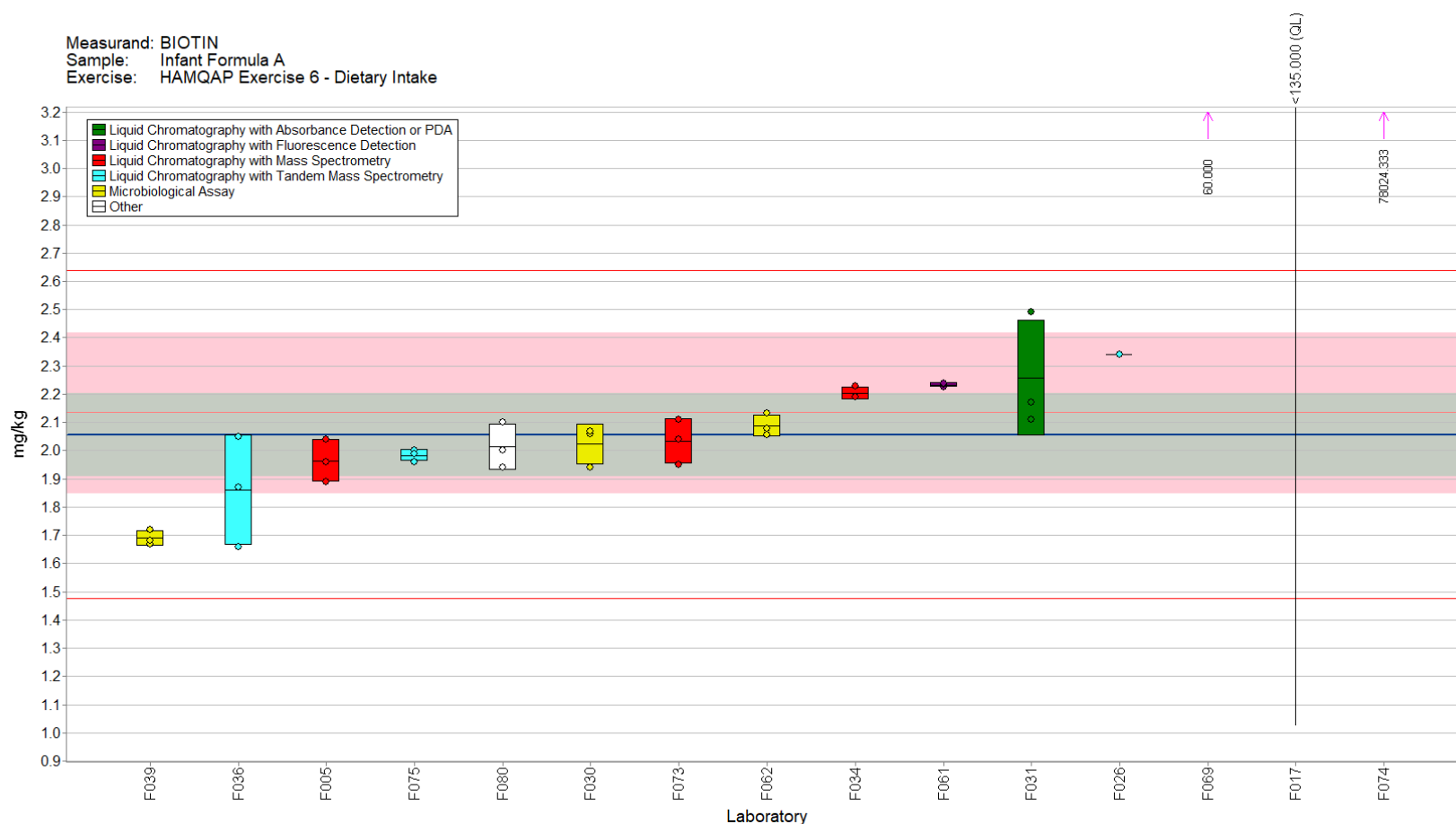


Figure 3-3. Biotin in Infant Formula A (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line represents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

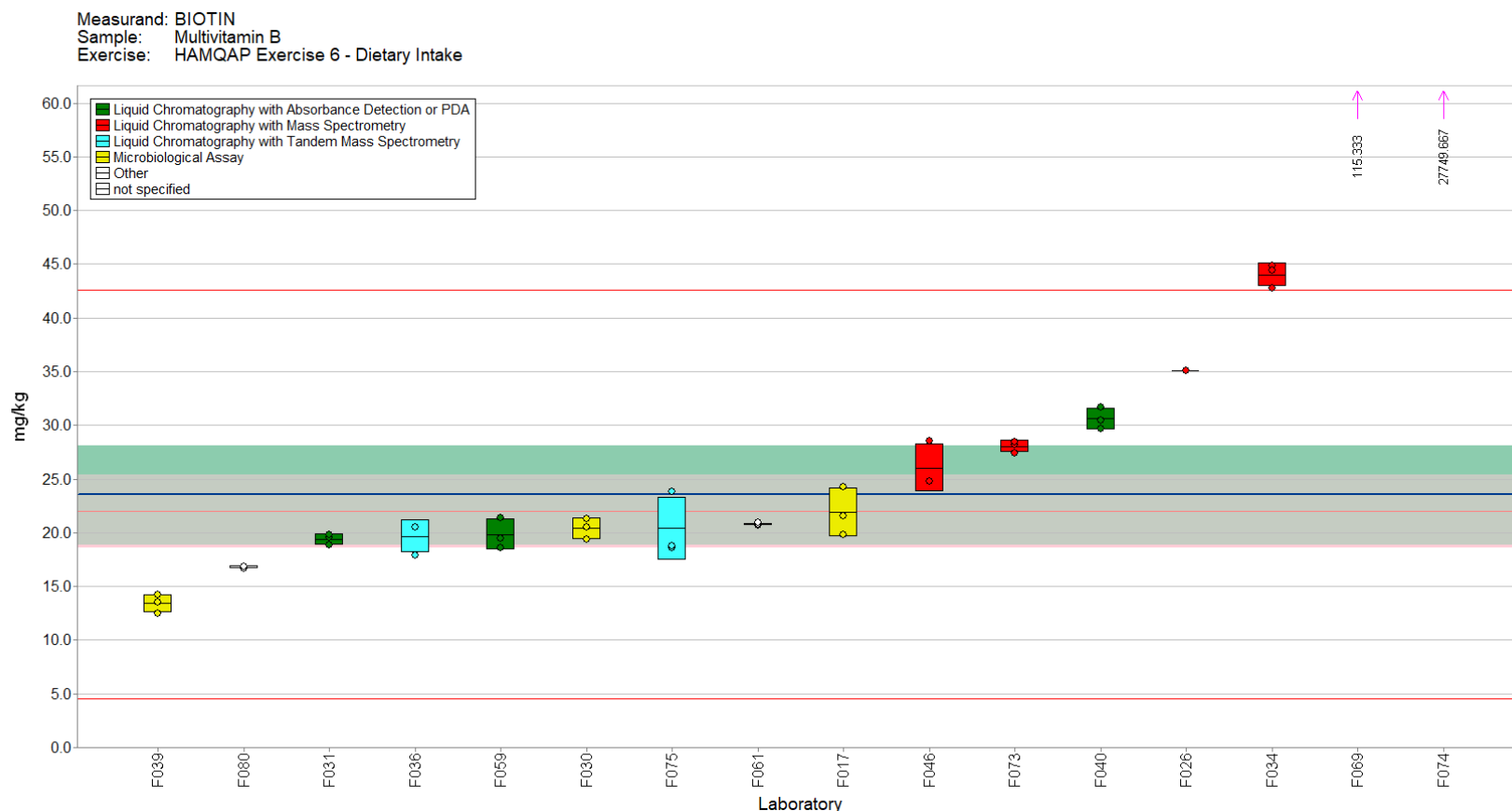


Figure 3-4. Biotin in Multivitamin B (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty (U_{NIST}) and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$.

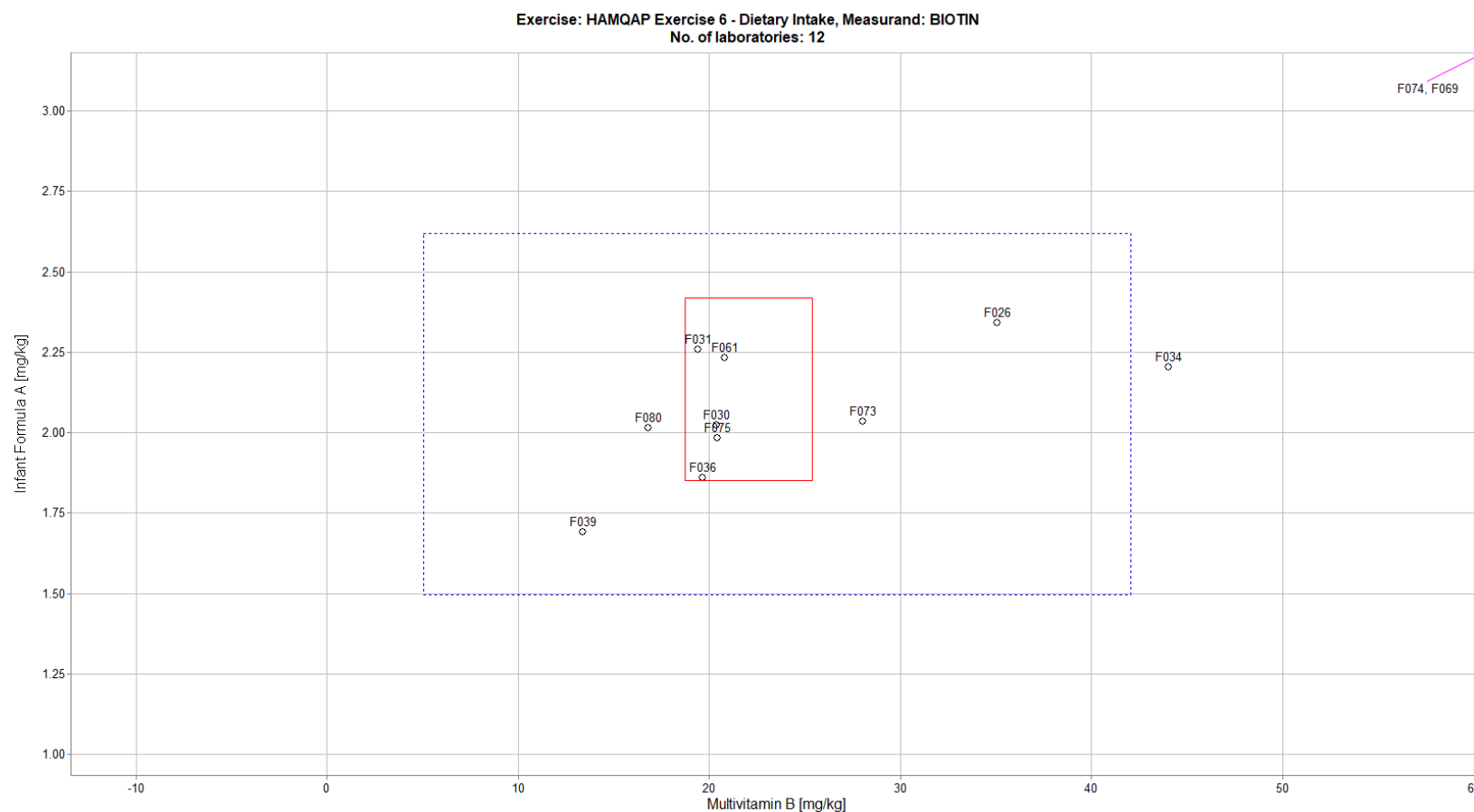


Figure 3-5. Laboratory means for biotin in Infant Formula A and Multivitamin B (sample/sample comparison view). In this view, the individual laboratory mean for one sample (Infant Formula A) is compared to the mean for a second sample (multivitamin). The solid red box represents the NIST range of tolerance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

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

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

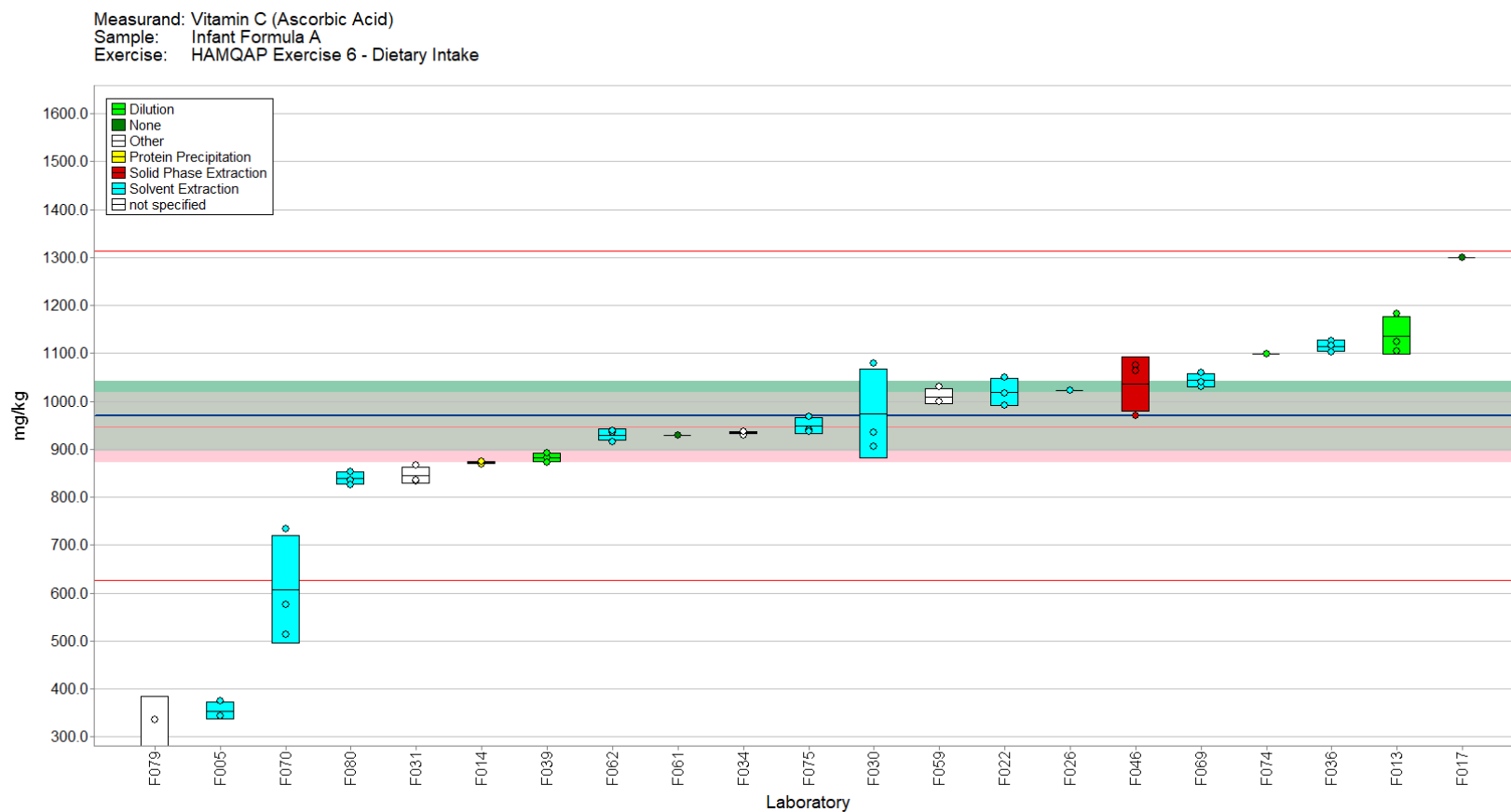


Figure 3-6. Vitamin C (ascorbic acid) in Infant Formula A (data summary view – sample preparation). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty (U_{NIST}) and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$.

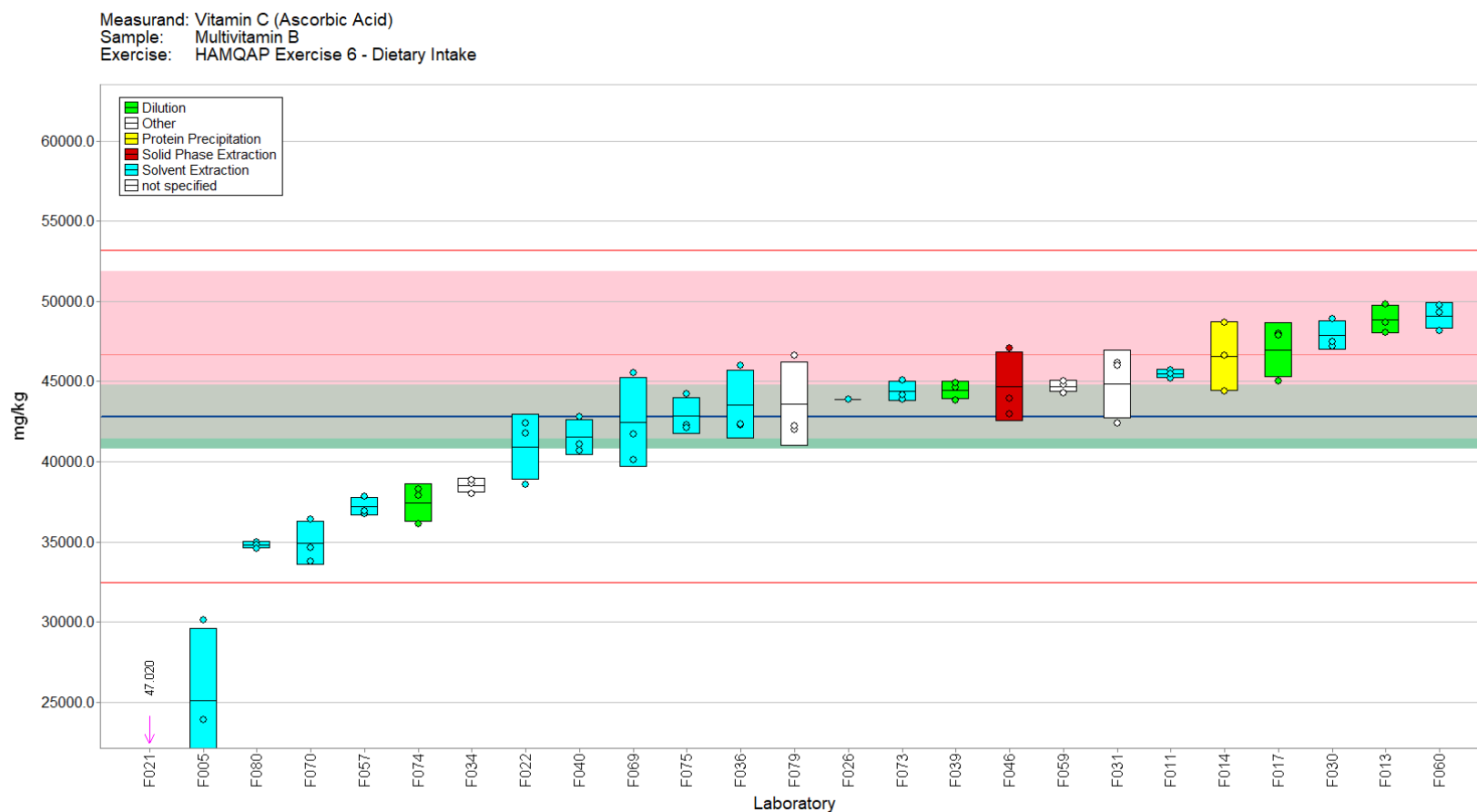


Figure 3-7. Vitamin C (ascorbic acid) in Multivitamin B (data summary view – sample preparation). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed.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

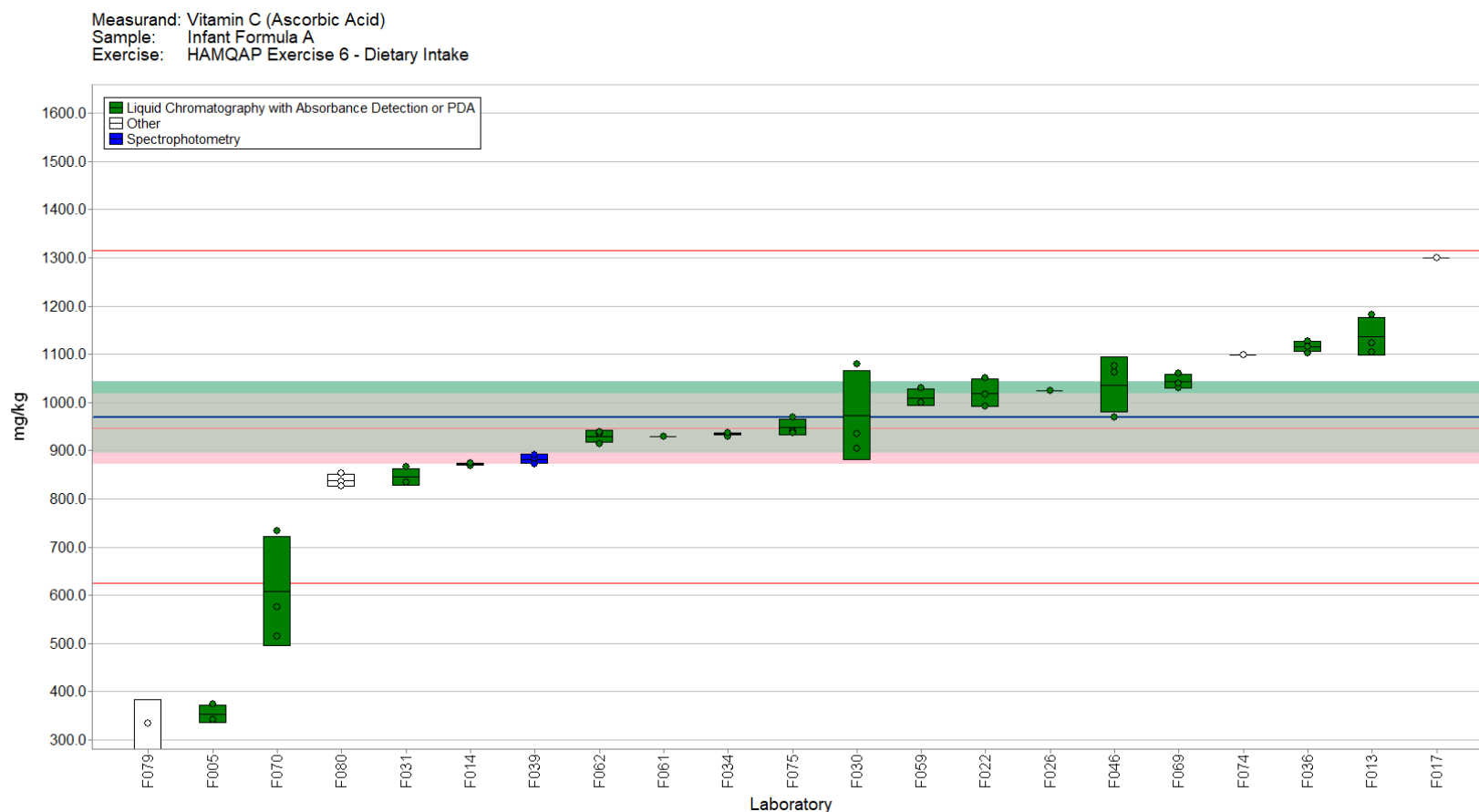


Figure 3-8. Vitamin C (ascorbic acid) in Infant Formula A (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty (U_{NIST}) and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$.

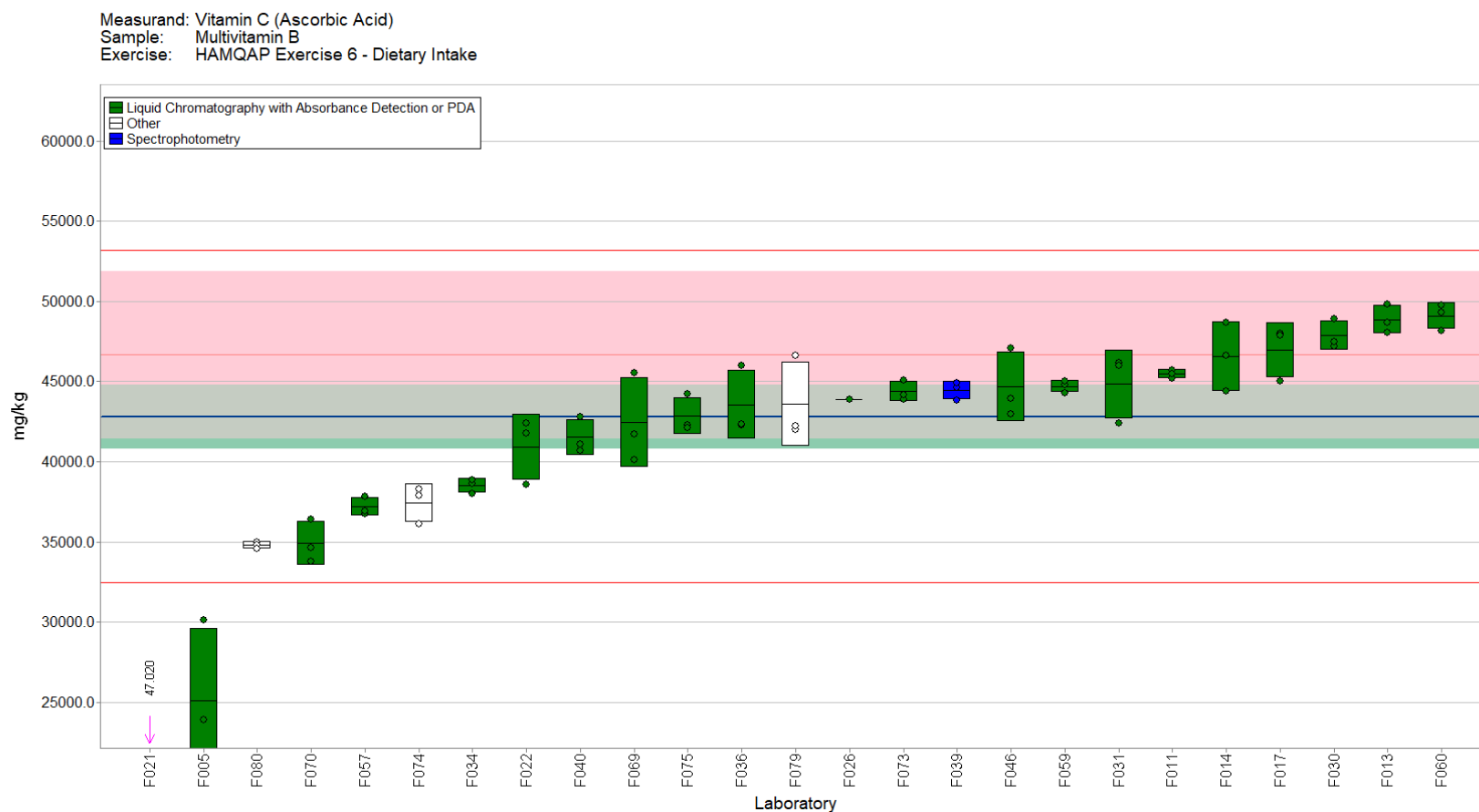


Figure 3-9. Vitamin C (ascorbic acid) in Multivitamin B (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty (U_{NIST}) and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$.

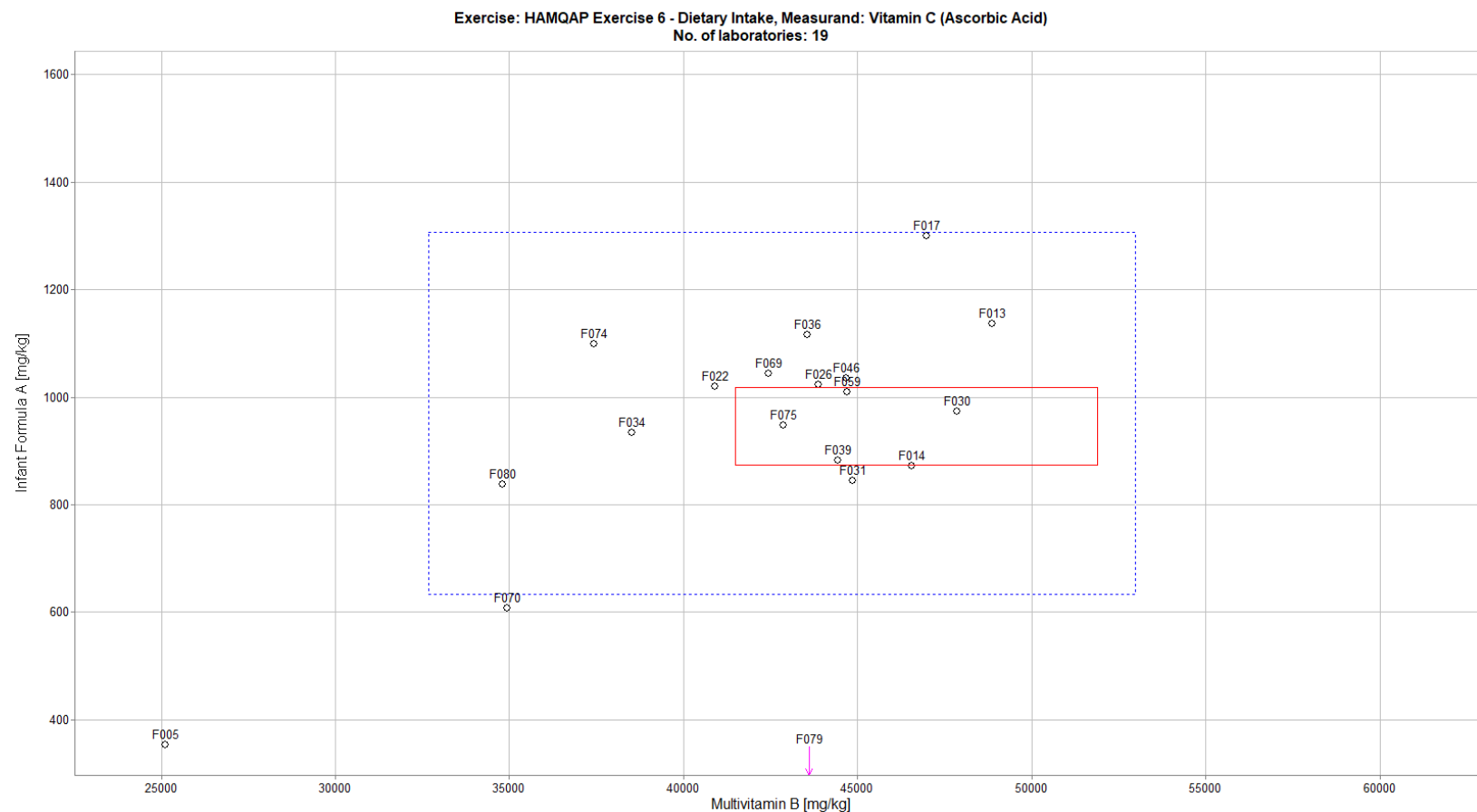


Figure 3-10. Laboratory means for vitamin C (ascorbic acid) in Infant Formula A and Multivitamin B (sample/sample comparison view). In this view, the individual laboratory mean for one sample (Infant Formula A) is compared to the mean for a second sample (multivitamin). The solid red box represents the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

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

Study Overview

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

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 packets and to prepare one sample and report one value from each packet provided. Before use, participants were instructed to thoroughly mix the contents of the packet prior to removal of a test portion for analysis, and to use a sample size of at least 3 g for the determination of vitamin A related compounds and 2 g for the determination of vitamin E related compounds. Approximate analyte levels were not reported to participants prior to the study. The NIST-determined values for vitamins A and E related compounds in the infant formula sample were assigned using results from the manufacturer of the material and are provided in the table below on an as-received basis.

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

Multivitamin B. Participants were provided with three bottles, each containing 30 multivitamin tablets. Participants were asked to store the material at controlled room temperature (20°C to 25°C) in the original unopened bottles and to prepare one sample and report one value from each bottle provided. Before use, participants were instructed to grind all 30 tablets, mix the resulting powder thoroughly prior to removal of a test portion for analysis, and to use a sample size of at least 2 g for the determination of both vitamin A and vitamin E compounds. After grinding,

participants were instructed to store the resulting powder at –20 °C or colder and analyze the material within two days for analytes in this study. Approximate analyte levels were not reported to participants prior to the study. The NIST-determined values for vitamins A and E compounds in the multivitamin sample were assigned using results from the manufacturer of the material and are provided in the table below on an as-received basis.

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

Dietary Intake Study Results

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

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

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

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

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

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

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

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

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

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

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

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 for all measurands, plus additional figures to show sample preparation for alpha-tocopherol.

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

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

- For alpha-tocopherol, two significantly different groups of reported values were identified based on sample preparation.
 - Laboratories that reported using saponification/base hydrolysis reported higher values than laboratories reporting other preparation techniques. Laboratories using hydrolysis techniques likely converted other forms present (i.e., alpha-tocopherol acetate) to alpha-tocopherol which resulted in a high bias for this analyte.
 - A few laboratories that reported using solvent extraction reported values very close to the target (**Figures 4-13 and 4-15**).
- For alpha-tocopheryl acetate, the 95 % confidence interval for the consensus mean was just overlapped with the upper portion of the target range of tolerance. Three reported that values significantly lower than the consensus range (**Figures 4-18**) should double check calibrant preparations, final calculations, and reporting units.
- Zero is not a measurable value and should not be reported.
- Overall, for fat-soluble vitamins, especially those with different chemical forms, it is important to understand what analytes are being measured and reported, and to use appropriate, high quality, and well characterized calibrants.
- Vitamin A or vitamin E compounds can be reported as totals, or as equivalents. Laboratories should choose appropriate techniques; measuring a total (by chemically converting prior to analysis) or reporting a total (by mathematically converting and combining the separately measured forms).
- Sample preparation techniques must be able to fully extract the analytes from the sample matrix, while also being mindful of analyte degradation and/or conversion. The use of reduced lighting/yellow lighting when conducting preparation techniques and storing samples in the dark or in amber colored vials can significantly reduce UV induced analyte degradation.
- In the case of tablets, sample preparation and storage are also important, being mindful of the grinding and homogenizing methods and as well as storage of ground material. Grinding shortly before sample extract and the use of cold storage when necessary can reduce the potential of analyte degradation due to the change in encapsulation.
- The use of appropriate calibration materials and quality assurance samples to establish that a method is in control and being performed correctly may reduce the likelihood of outlying data. Quality assurance samples can be commercially available reference materials (CRMs, SRMs, or RMs) or materials prepared in-house.

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

HAMQAP Exercise 6 - Fat-Soluble Vitamins											
Lab Code: NIST		1. Your Results					2. Community Results			3. Target	
Analyte	Sample	Units	x_i	s_i	Z'_{comm}	Z_{NIST}	N	x^*	s^*	x_{NIST}	U
alpha-Tocopherol	Infant Formula A	mg/kg	58.4	3.88			12	120	58	58.4	3.88
alpha-Tocopherol	Multivitamin B	mg/kg					9	160	200		
alpha-Tocopheryl Acetate	Infant Formula A	mg/kg	162	4.92			9	120	89	162	4.92
alpha-Tocopheryl Acetate	Multivitamin B	mg/kg	17900	430			15	19500	1600	17900	430
beta-Tocopherol	Infant Formula A	mg/kg	4.94	0.102			5	3.7	1.8	4.94	0.102
beta-Tocopherol	Multivitamin B	mg/kg					2	30	140		
delta-Tocopherol	Infant Formula A	mg/kg	37.9	1.02			10	30	16	37.9	1.02
delta-Tocopherol	Multivitamin B	mg/kg					3	200	200		
gamma-Tocopherol	Infant Formula A	mg/kg	114	2.19			8	120	18	114	2.19
gamma-Tocopherol	Multivitamin B	mg/kg					2	20	80		
Retinyl Acetate	Infant Formula A	mg/kg	6.73	0.226			7	8.7	3.1	6.73	0.226
Retinyl Acetate	Multivitamin B	mg/kg	895	42			12	700	110	895	42
Retinyl Palmitate	Infant Formula A	mg/kg	7.8	0.232			9	13	7.9	7.8	0.232
Retinyl Palmitate	Multivitamin B	mg/kg					4	40	230		
Total alpha-Tocopherol	Infant Formula A	mg/kg	206	5.93			12	180	100	206	5.93
Total alpha-Tocopherol	Multivitamin B	mg/kg					11	18700	1400		
Total Retinol	Infant Formula A	mg/kg	10.1	0.234			17	14.3	2.4	10.1	0.234
Total Retinol	Multivitamin B	mg/kg					14	620	140		
			x_i	Mean of reported values			N	Number of quantitative values reported		x_{NIST}	NIST-assessed value
			s_i	Standard deviation of reported values						U	expanded uncertainty
			Z'_{comm}	Z'-score with respect to community consensus			x^*	Robust mean of reported values		about the NIST-assessed value	
			Z_{NIST}	Z-score with respect to NIST value			s^*	Robust standard deviation			

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

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

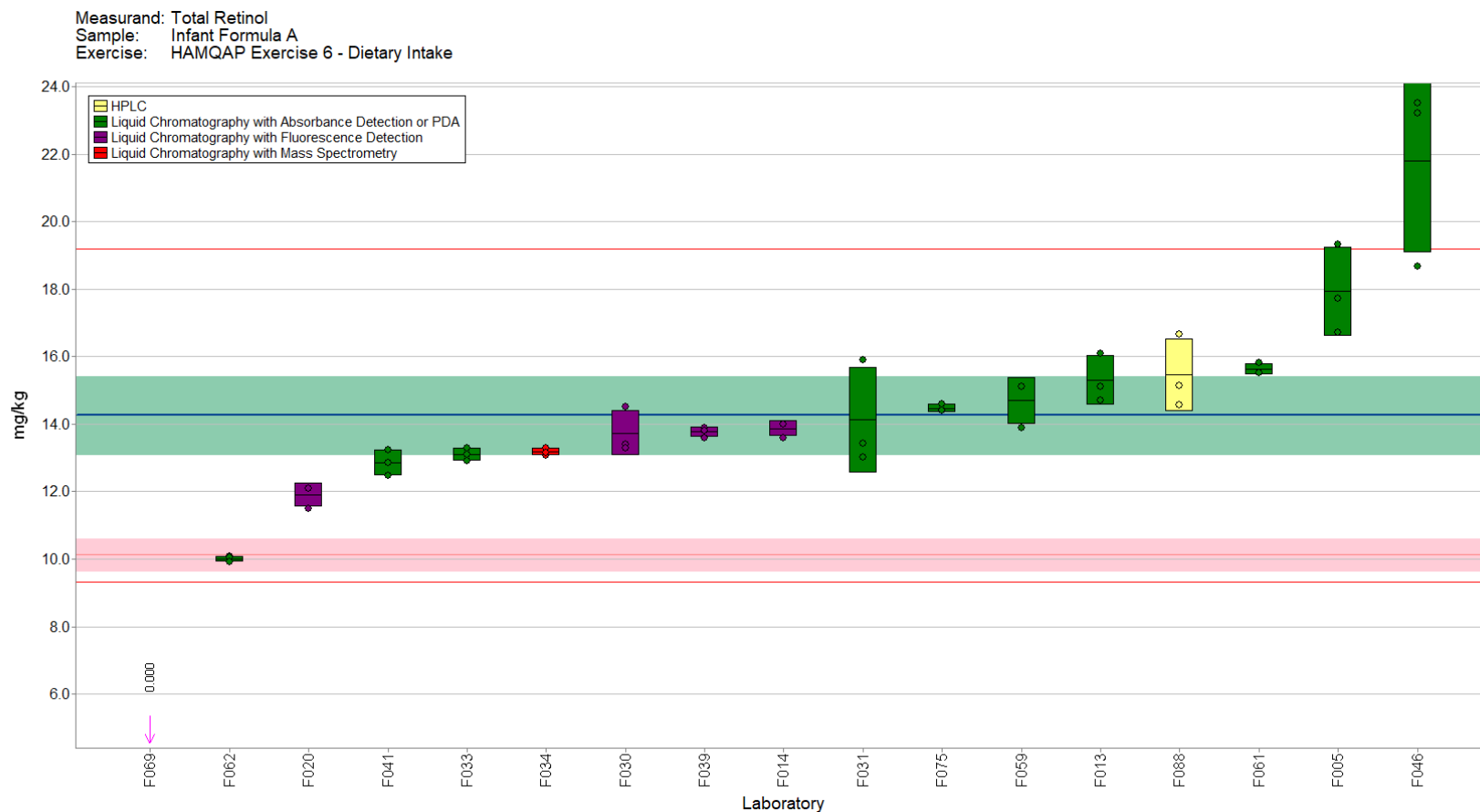


Figure 4-1. Total retinol in Infant Formula A (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line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

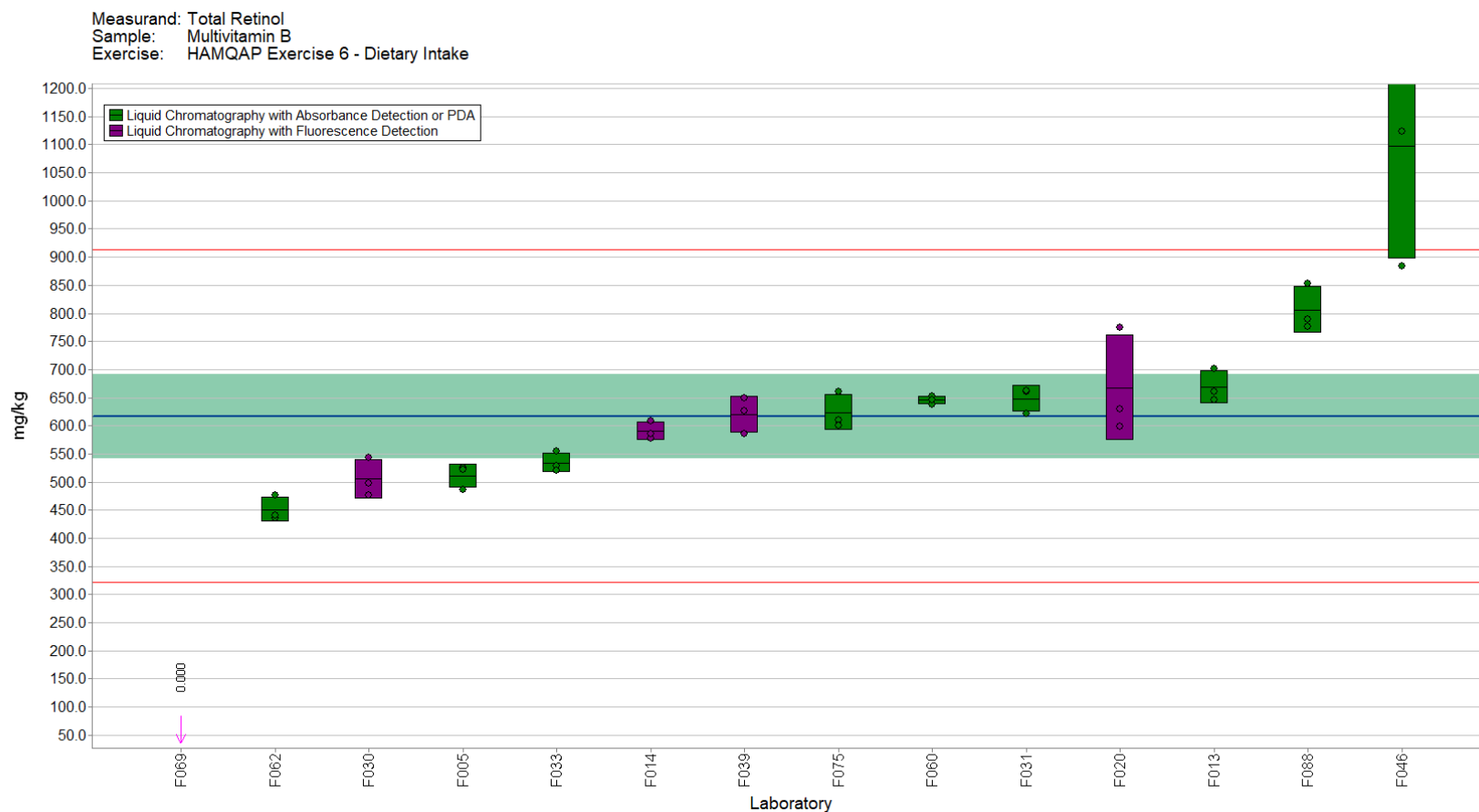


Figure 4-2. Total retinol in Multivitamin B (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line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

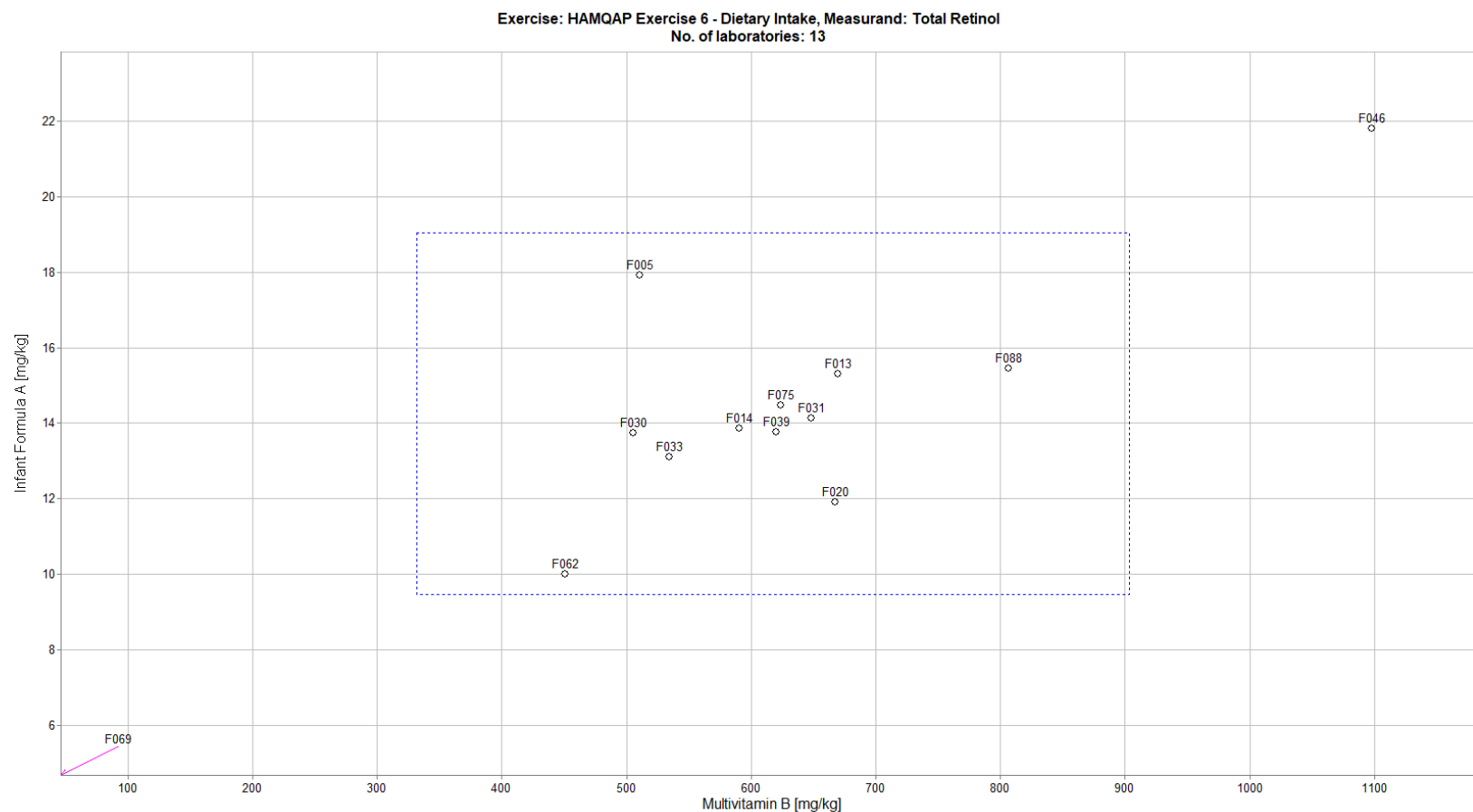


Figure 4-3. Laboratory means for total retinol in Infant Formula A and Multivitamin B (sample/sample comparison view). In this view, the individual laboratory mean for one sample (Multivitamin B) is compared to the individual laboratory mean for a second sample (Infant Formula A). The dotted blue box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

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

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

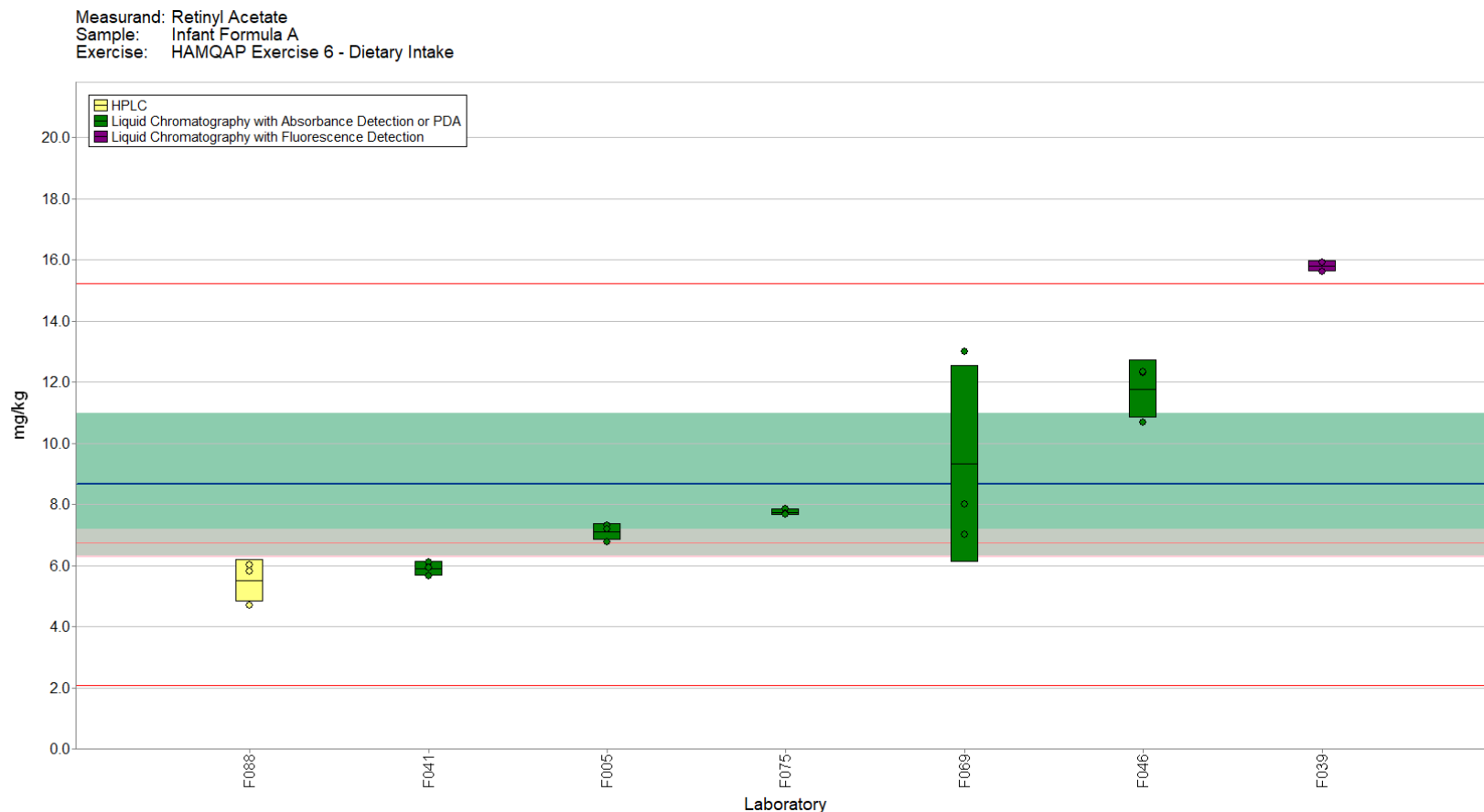


Figure 4-4. Retinyl acetate in Infant Formula A (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue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

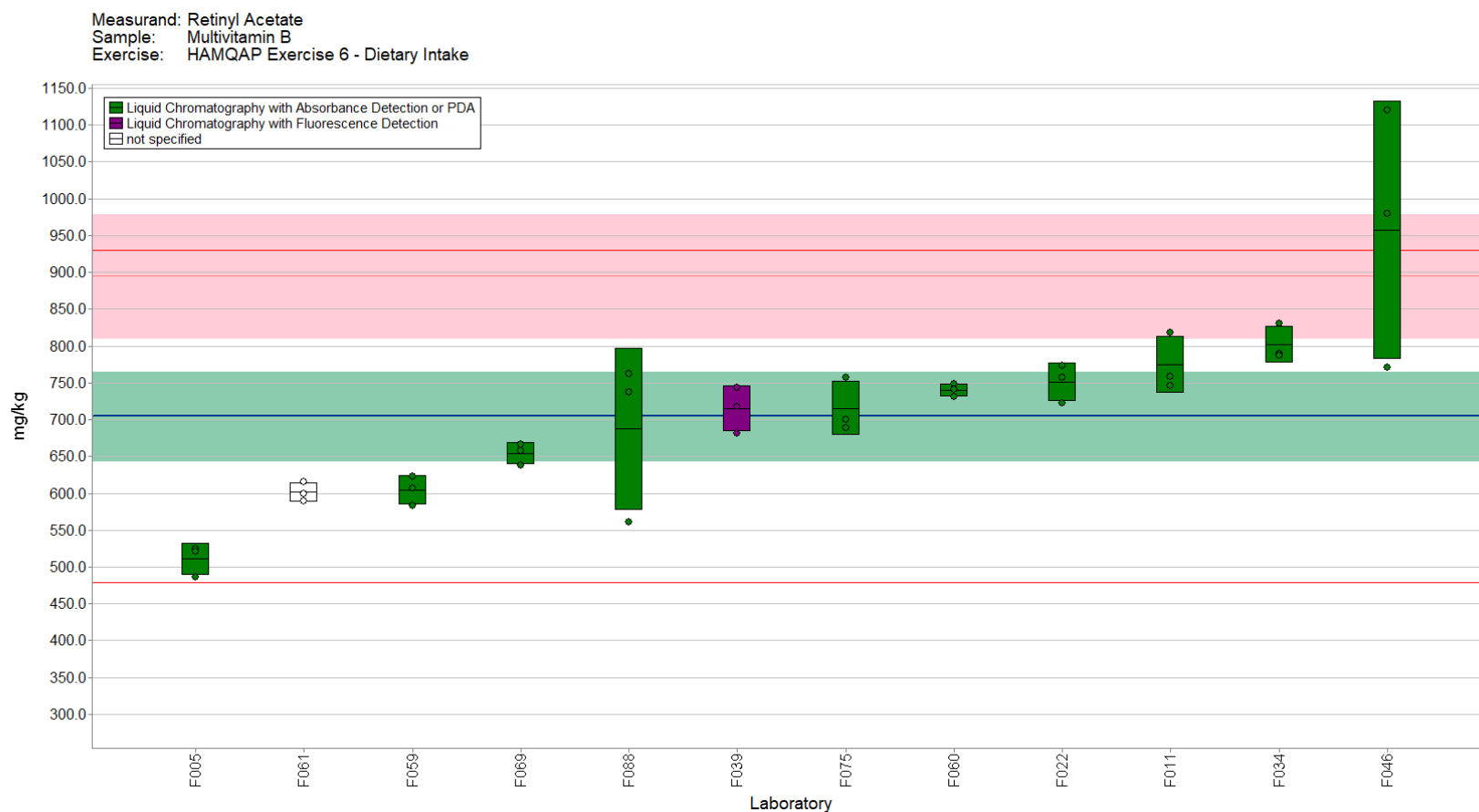


Figure 4-5. Retinyl acetate in Multivitamin B (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line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

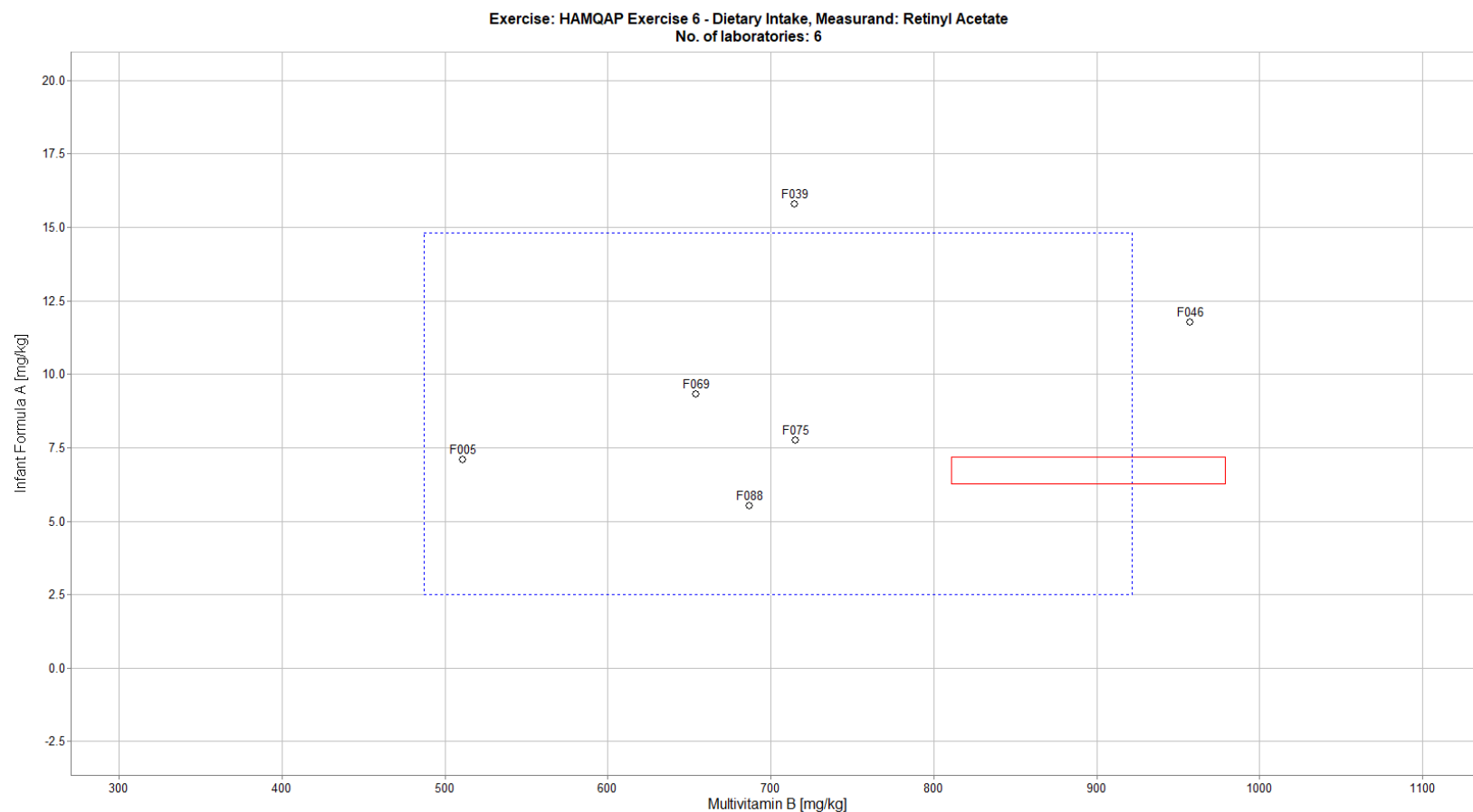


Figure 4-6. Laboratory means for retinyl acetate in Infant Formula A and Multivitamin B (sample/sample comparison view). In this view, the individual laboratory mean for one sample (multivitamin) is compared to the individual laboratory mean for a second sample (Infant Formula A). The solid red box represents the NIST range of tolerance for the two samples, Multivitamin B (x-axis) and Infant Formula A (y-axis), which encompasses the target values bounded by their uncertainties (U_{NIST}) and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$. The dotted blue box represents the consensus range of tolerance for Multivitamin B (x-axis) and Infant Formula A (y-axis), calculated as the values above and below the consensus means that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$.

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

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

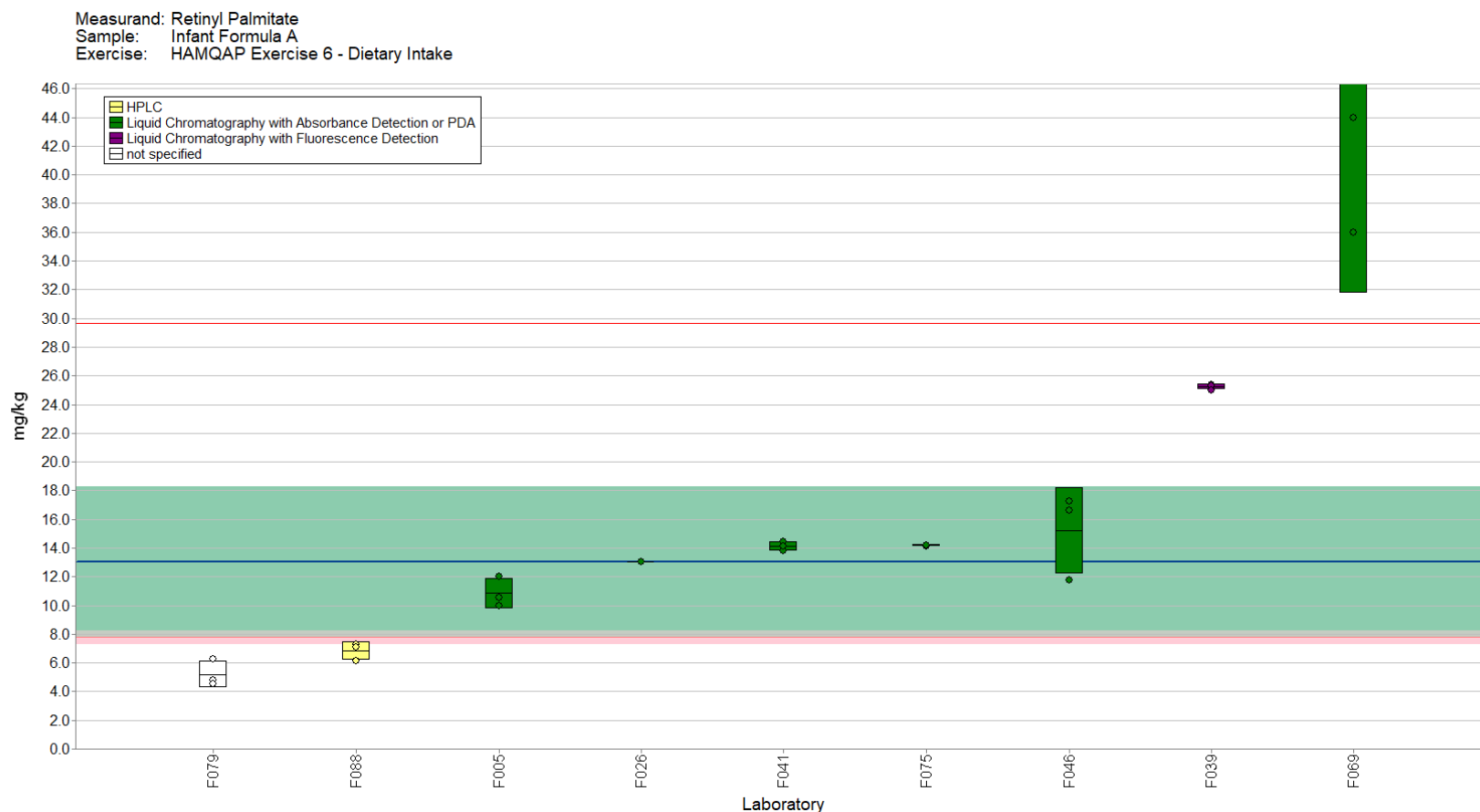


Figure 4-7. Retinyl palmitate in Infant Formula A (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The red solid lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by its uncertainty (U_{NIST}) and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$.

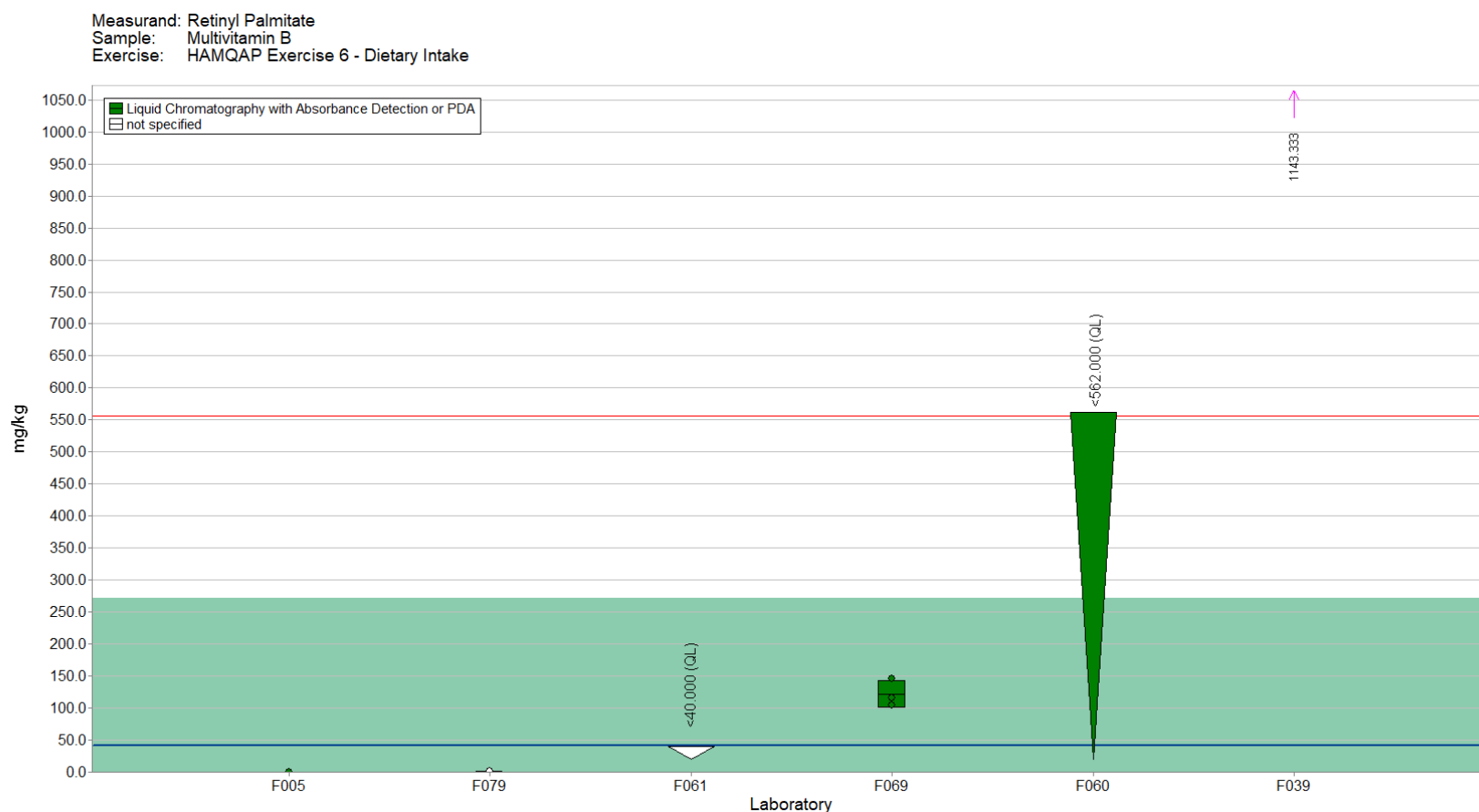


Figure 4-8. Retinyl palmitate in Multivitamin B (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The red solid lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$. A NIST value has not been determined in this material.

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

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

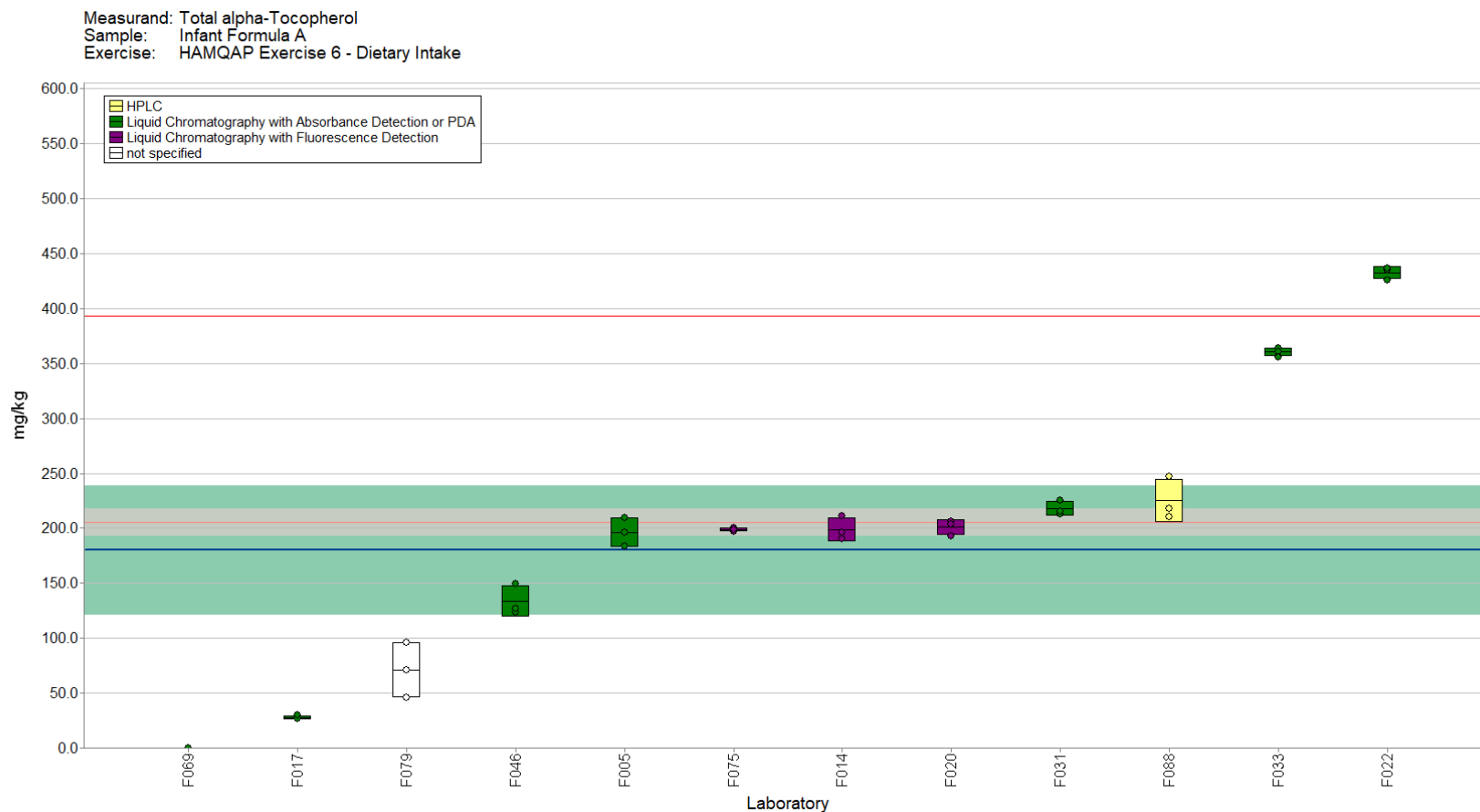


Figure 4-9. Total alpha-tocopherol in Infant Formula A (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The red solid lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by its uncertainty (U_{NIST}) and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$.

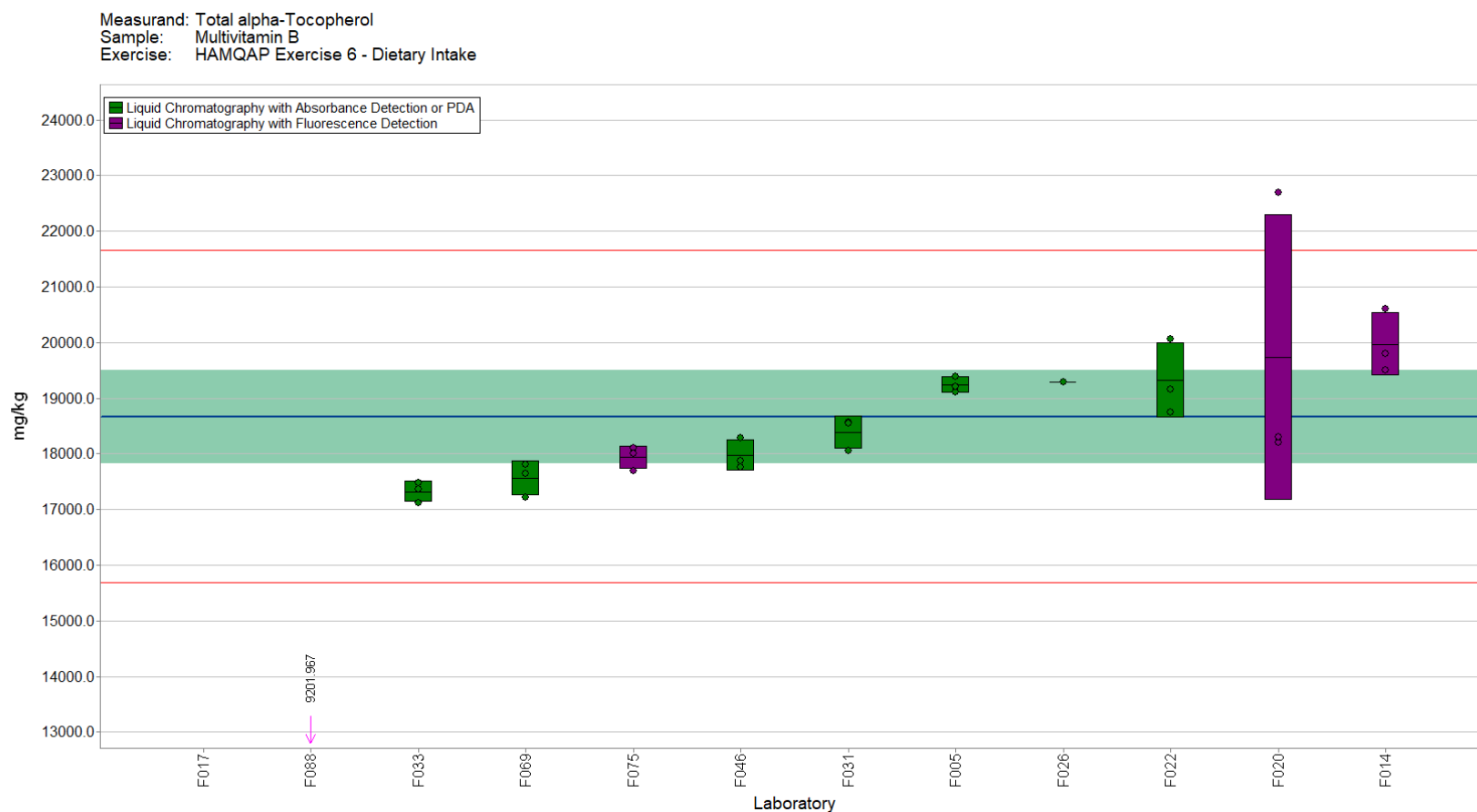


Figure 4-10. Total alpha-tocopherol in Multivitamin B (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The red solid lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$. A NIST value has not been determined in this material.

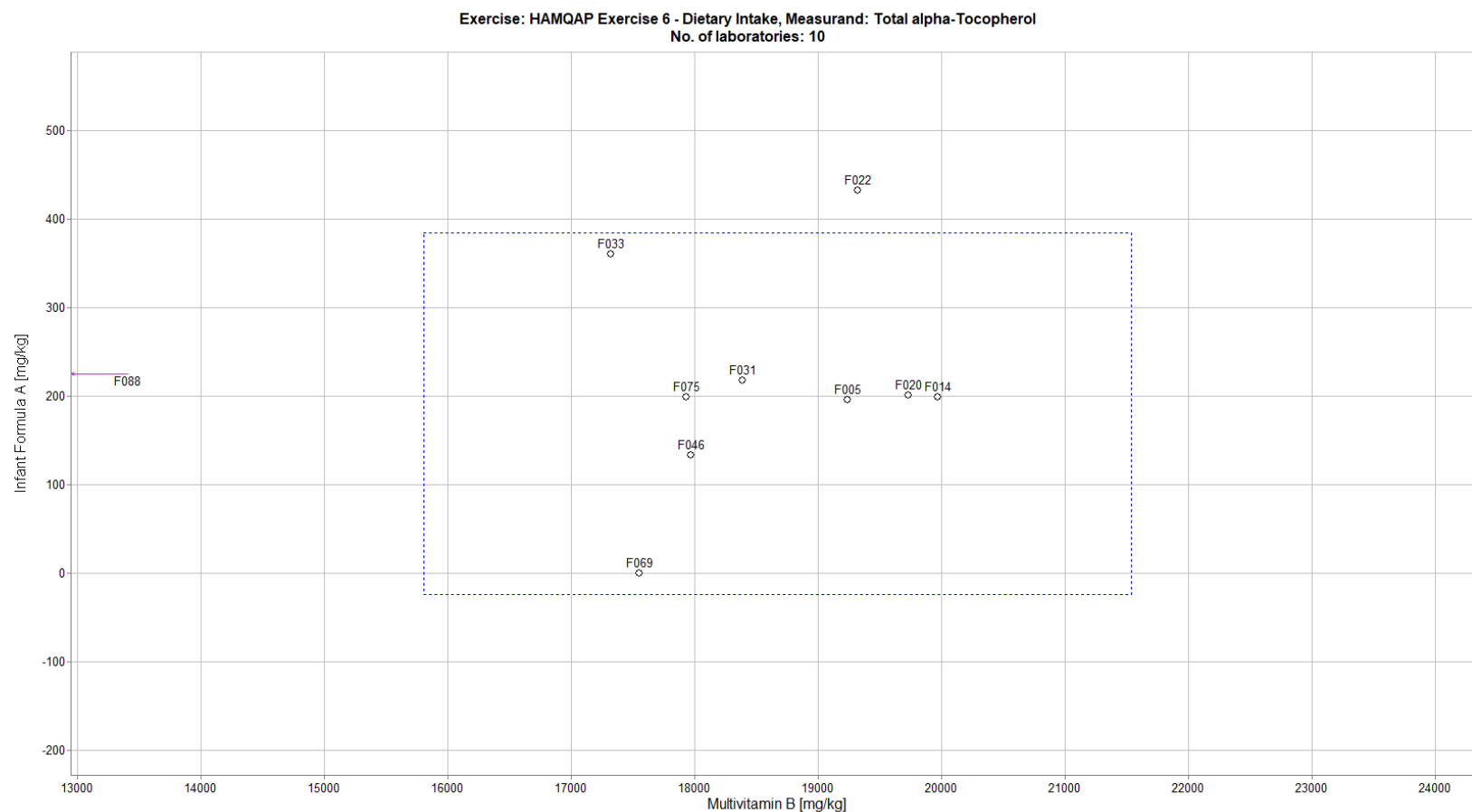


Figure 4-11. Laboratory means for total alpha-tocopherol in Infant Formula A and Multivitamin B (sample/sample comparison view). In this view, the individual laboratory mean for one sample (multivitamin) is compared to the individual laboratory mean for a second sample (Infant Formula A). The dotted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

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

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

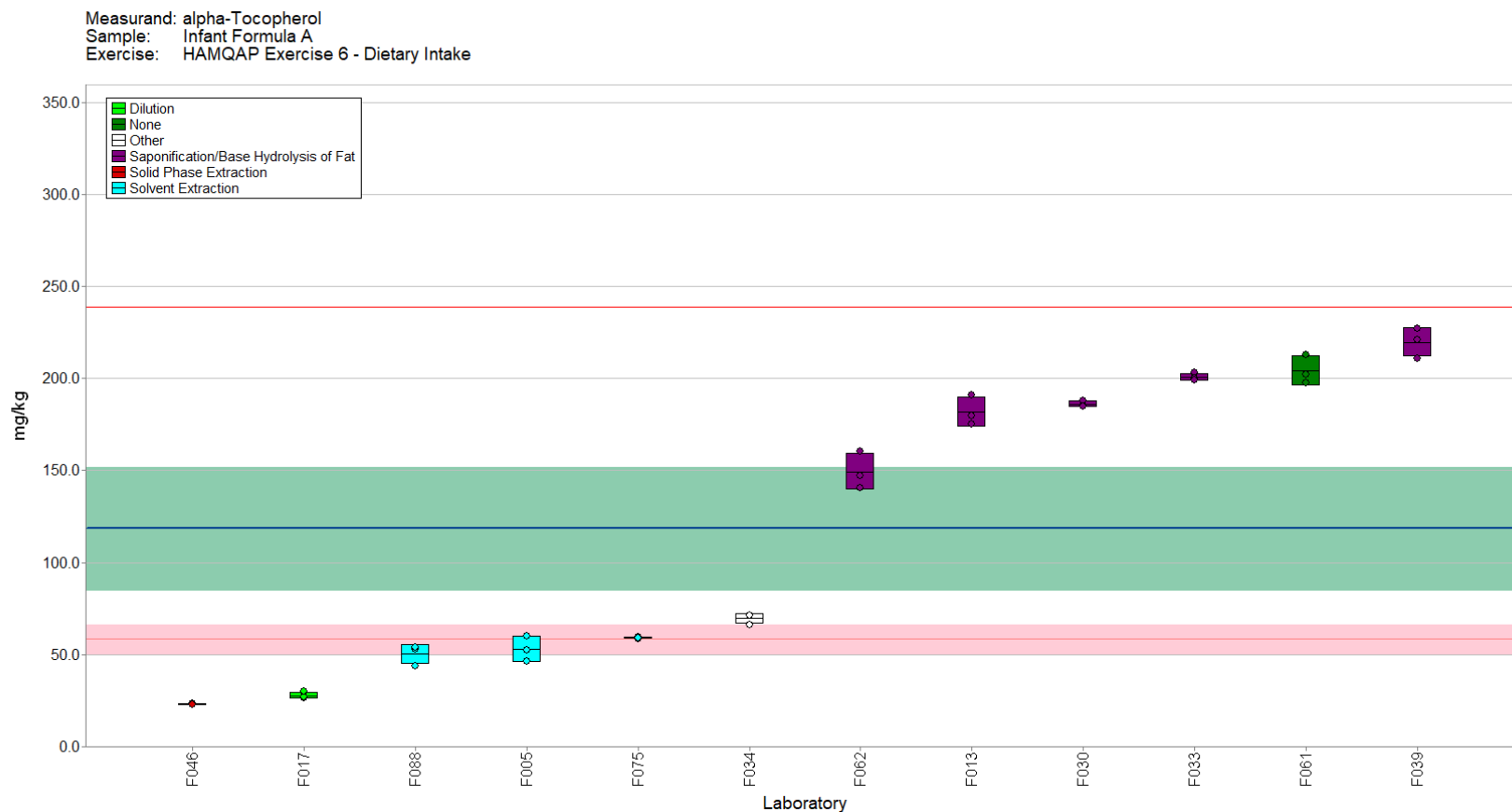


Figure 4-12. Alpha-tocopherol in Infant Formula A (data summary view – sample preparation). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation employed. The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The red solid lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by its uncertainty (U_{NIST}) and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$.

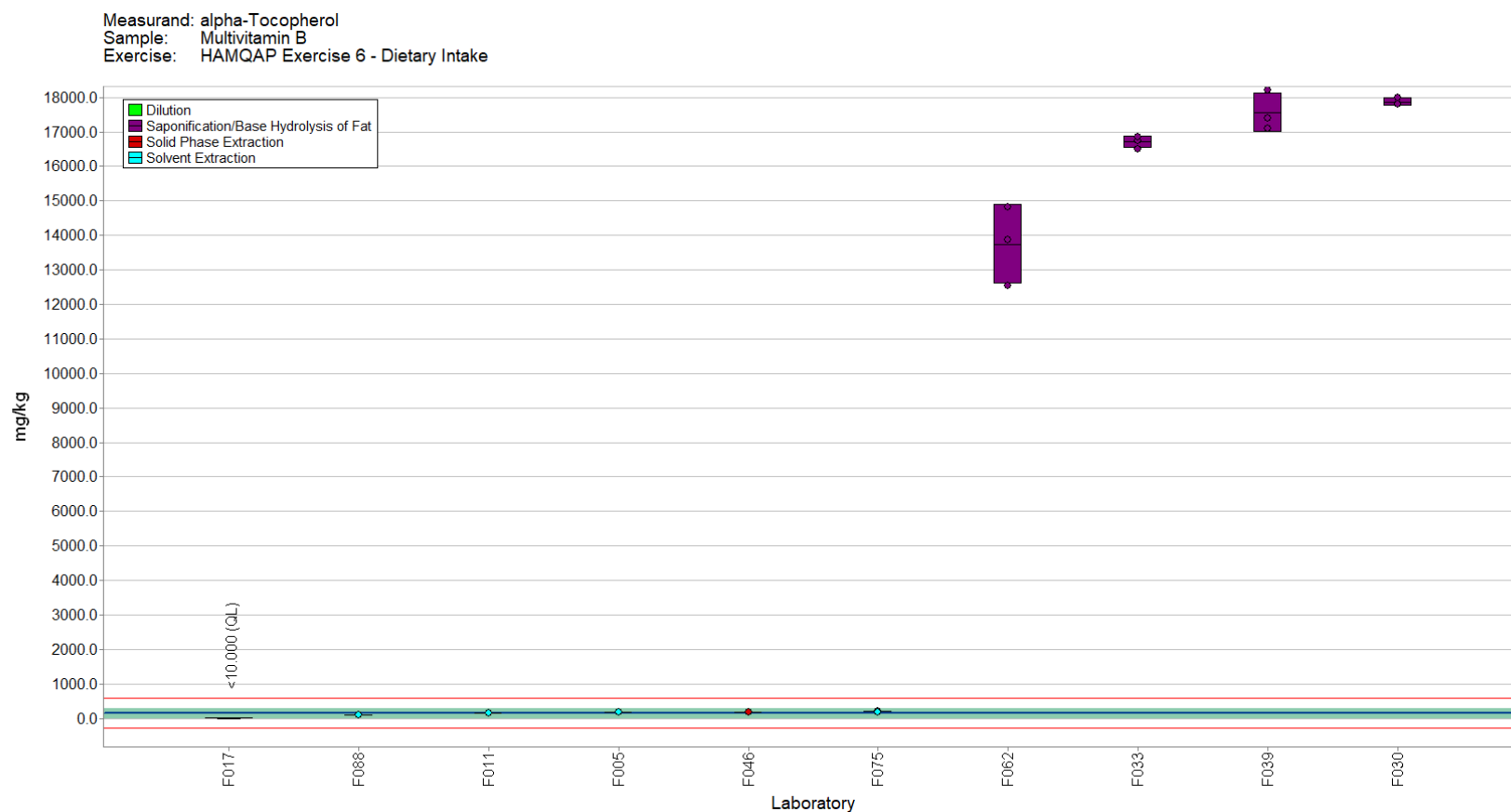


Figure 4-13. Alpha-tocopherol in Multivitamin B (data summary view – sample preparation). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation employed. The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The red solid lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$. A NIST value has not been determined in this material.

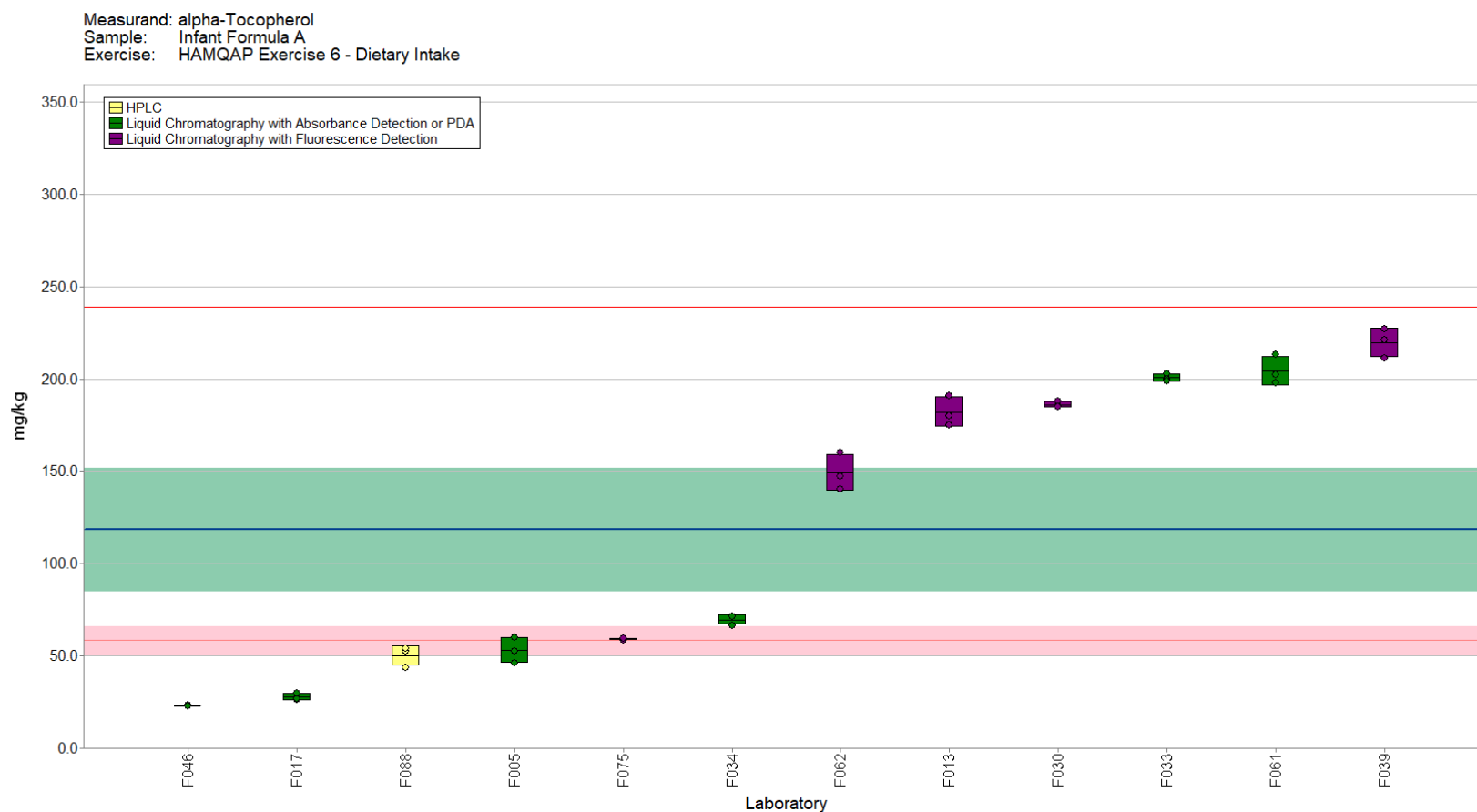


Figure 4-14. Alpha-tocopherol in Infant Formula A (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The red solid lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by its uncertainty (U_{NIST}) and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$.

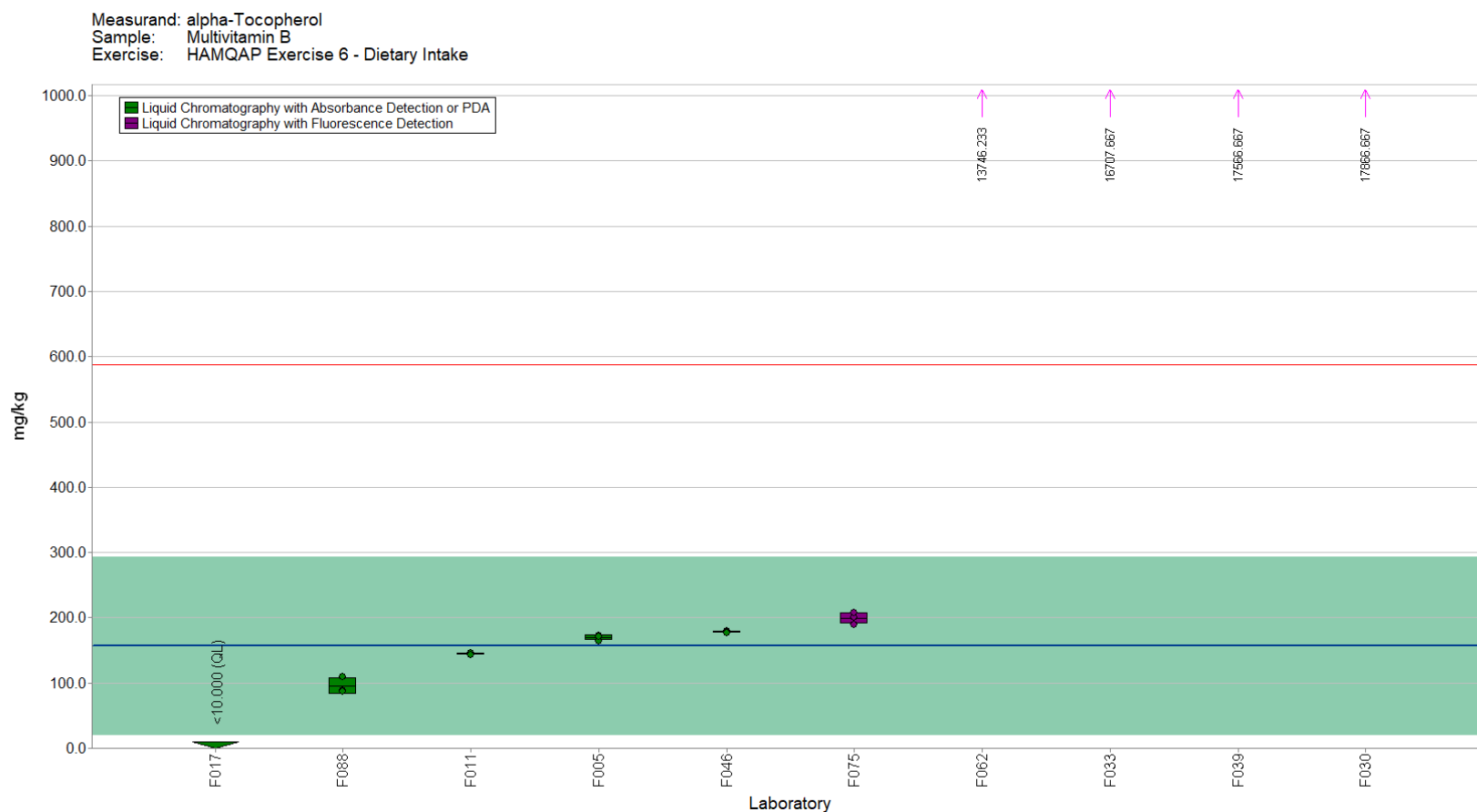


Figure 4-15. Alpha-tocopherol in Multivitamin B (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The red solid lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$. A NIST value has not been determined in this material.

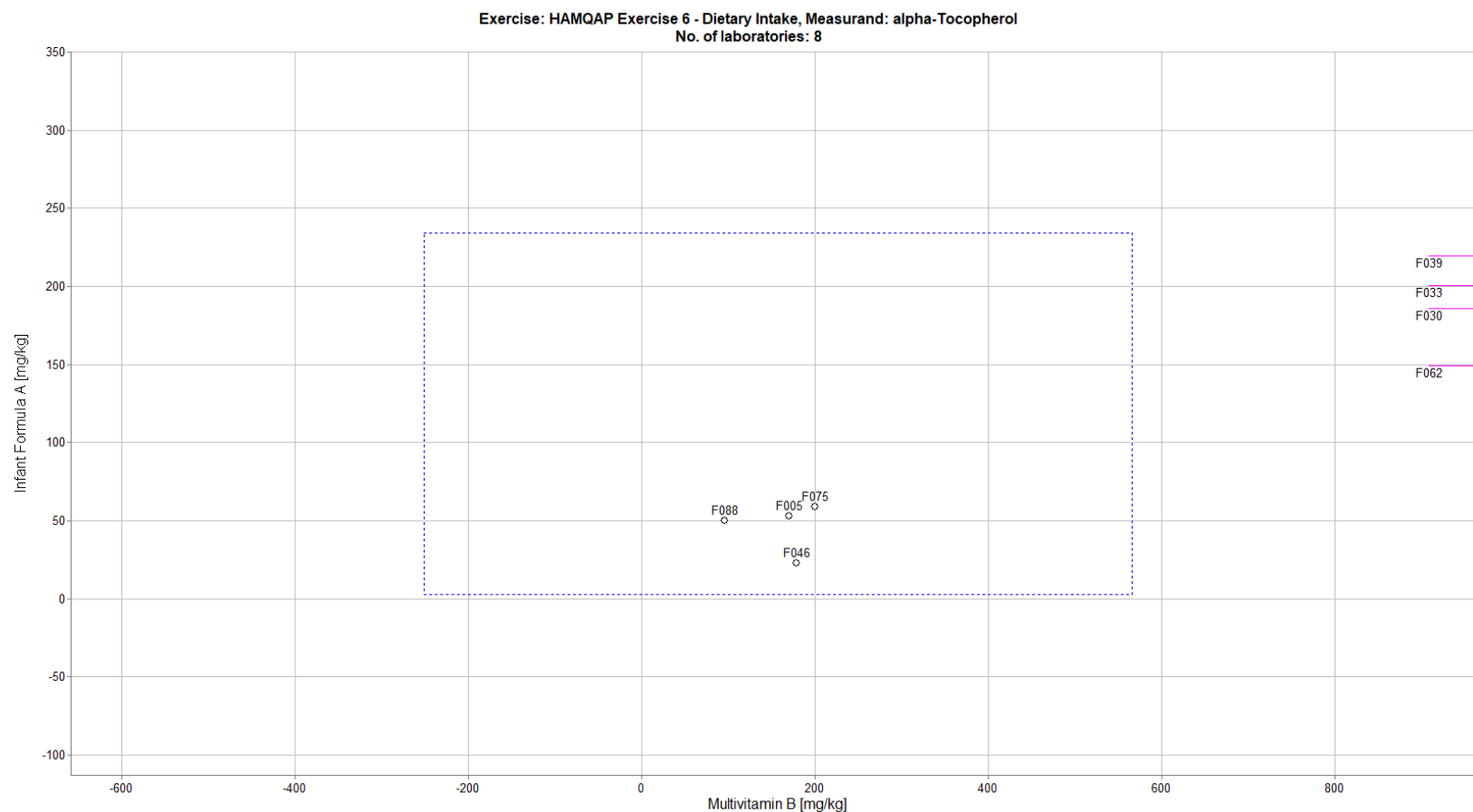


Figure 4-16. Laboratory means for alpha-tocopherol in Infant Formula A and Multivitamin B (sample/sample comparison view). In this view, the individual laboratory mean for one sample (multivitamin) is compared to the individual laboratory mean for a second sample (Infant Formula A). The dotted blue box represents the consensus range of tolerance for Multivitamin B (x-axis) and Infant Formula A (y-axis), calculated as the values above and below the consensus means that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$.

Table 4-7. Data summary table for alpha-tocopheryl acetate in Infant Formula A and Multivitamin B. Data points highlighted in blue have been identified as outside the consensus tolerance limits and would be estimated to result in an unacceptable Z'_{comm} score, $|Z'_{\text{comm}}| \geq 2$.

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

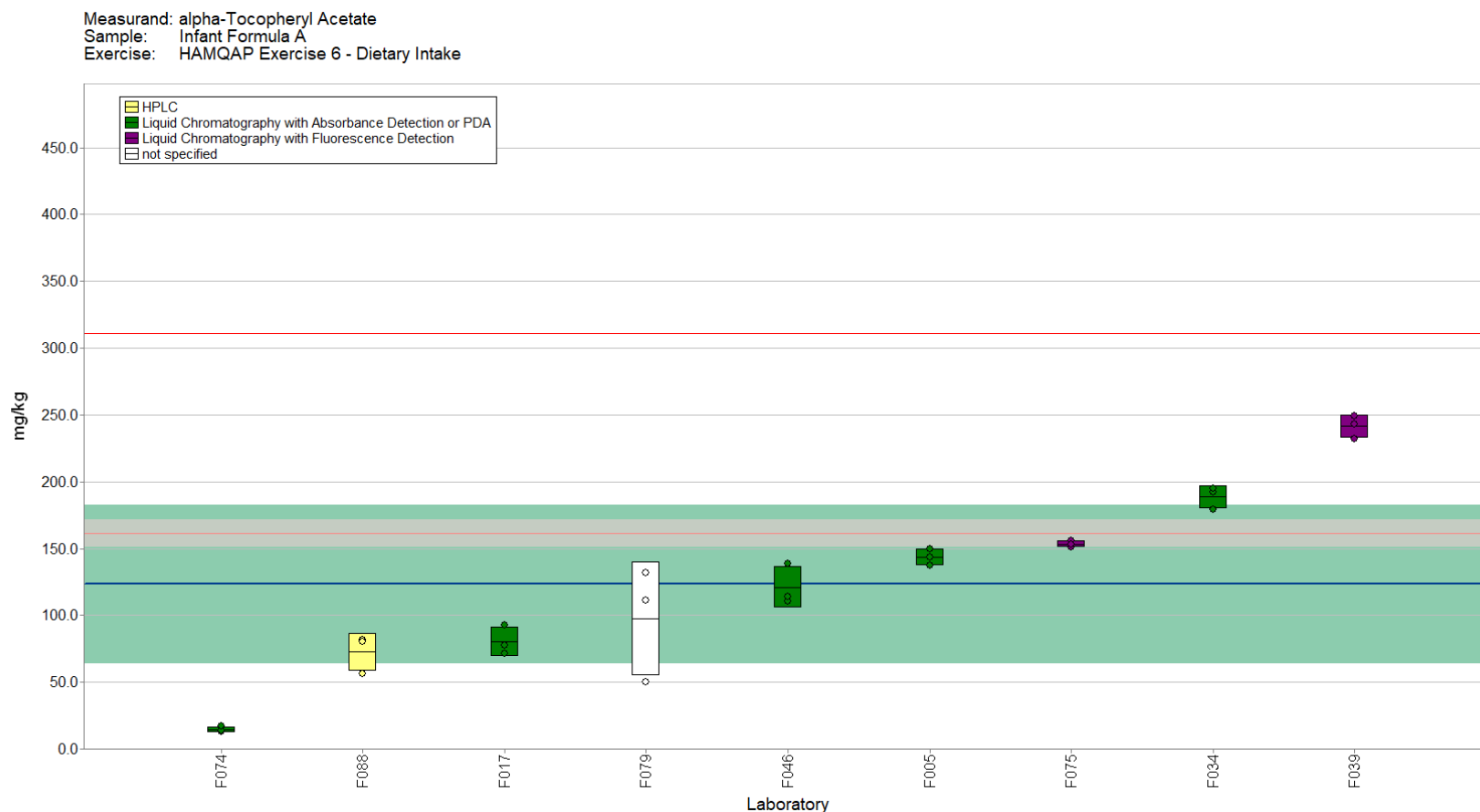


Figure 4-17. Alpha-tocopherol acetate in Infant Formula A (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The red solid lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by its uncertainty (U_{NIST}) and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$.

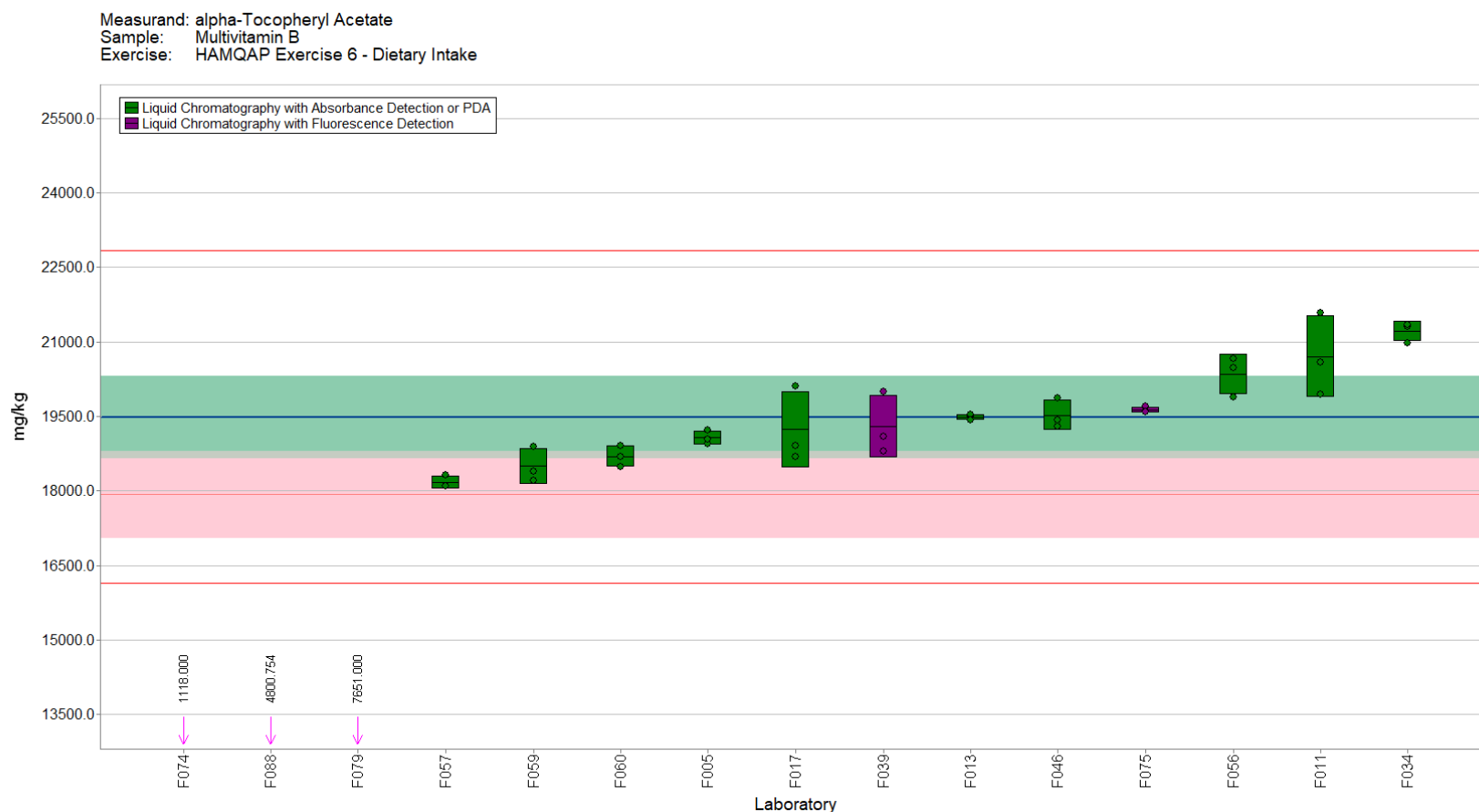


Figure 4-18. Alpha-tocopherol acetate in Multivitamin B (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The red solid lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by its uncertainty (U_{NIST}) and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$.

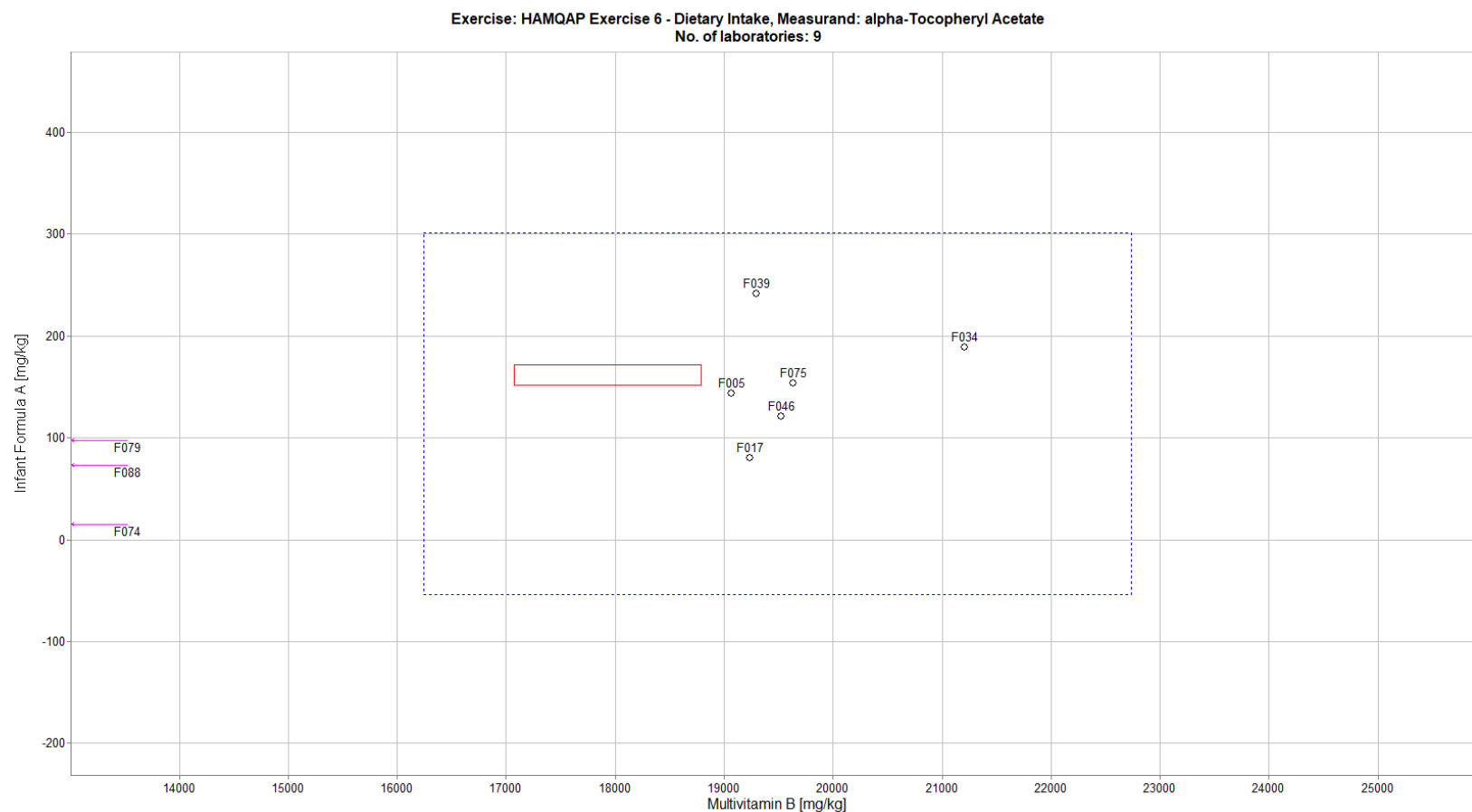


Figure 4-19. Laboratory means for alpha-tocopheryl acetate in Infant Formula A and Multivitamin B (sample/sample comparison view). In this view, the individual laboratory mean for one sample (multivitamin) is compared to the individual laboratory mean for a second sample (Infant Formula A). The solid red box represents the NIST range of tolerance for the two samples, Multivitamin B (x-axis) and Infant Formula A (y-axis), which encompasses the target values bounded by their uncertainties (U_{NIST}) and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$. The dotted blue box represents the consensus range of tolerance for Multivitamin B (x-axis) and Infant Formula A (y-axis), calculated as the values above and below the consensus means that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$.

Table 4-8. Data summary table for beta-tocopherol in Infant Formula A and Multivitamin B. Data points highlighted in red have a zero or non-numeric data point.

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

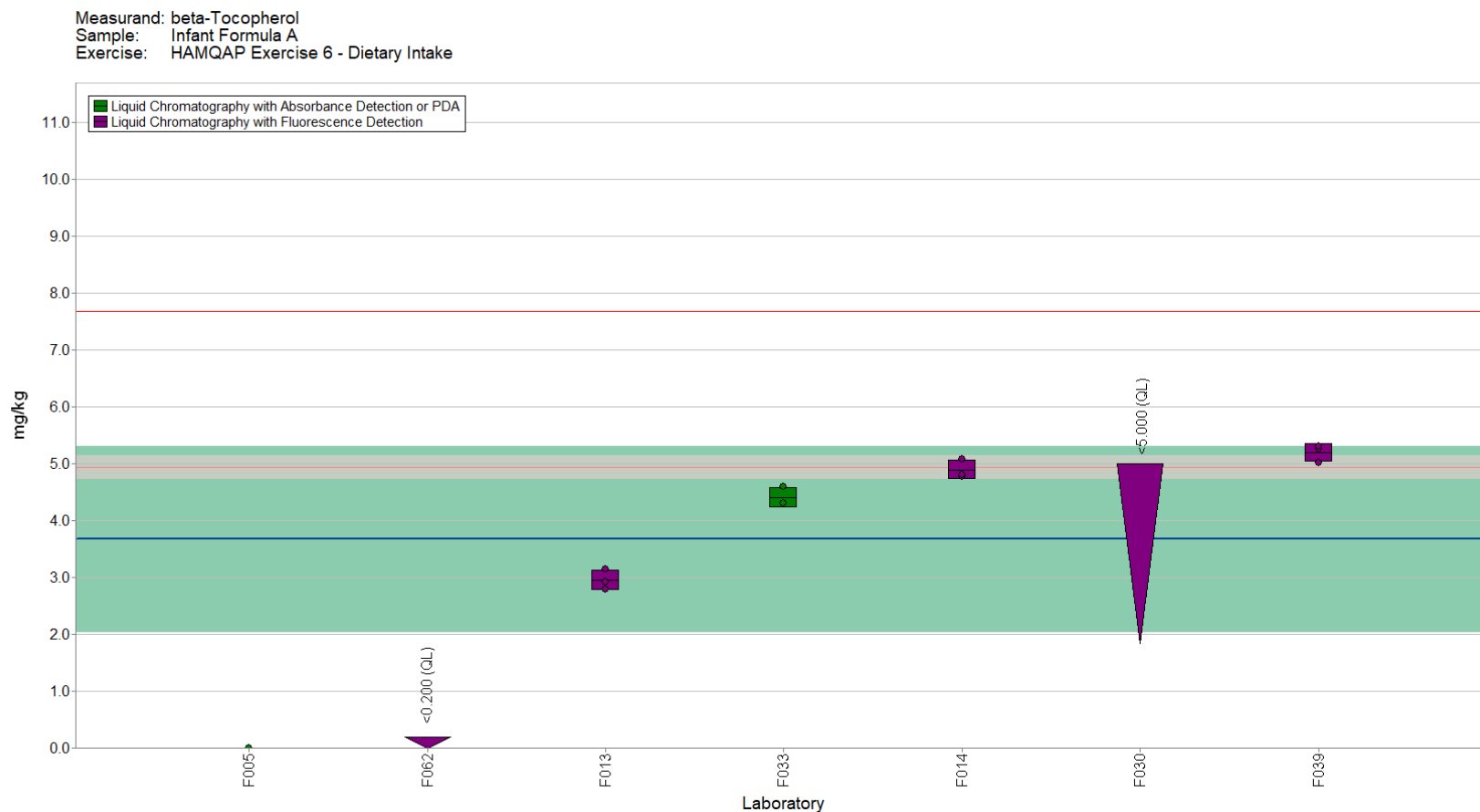


Figure 4-20. Beta-tocopherol in Infant Formula A (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The red solid lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by its uncertainty (U_{NIST}) and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$.

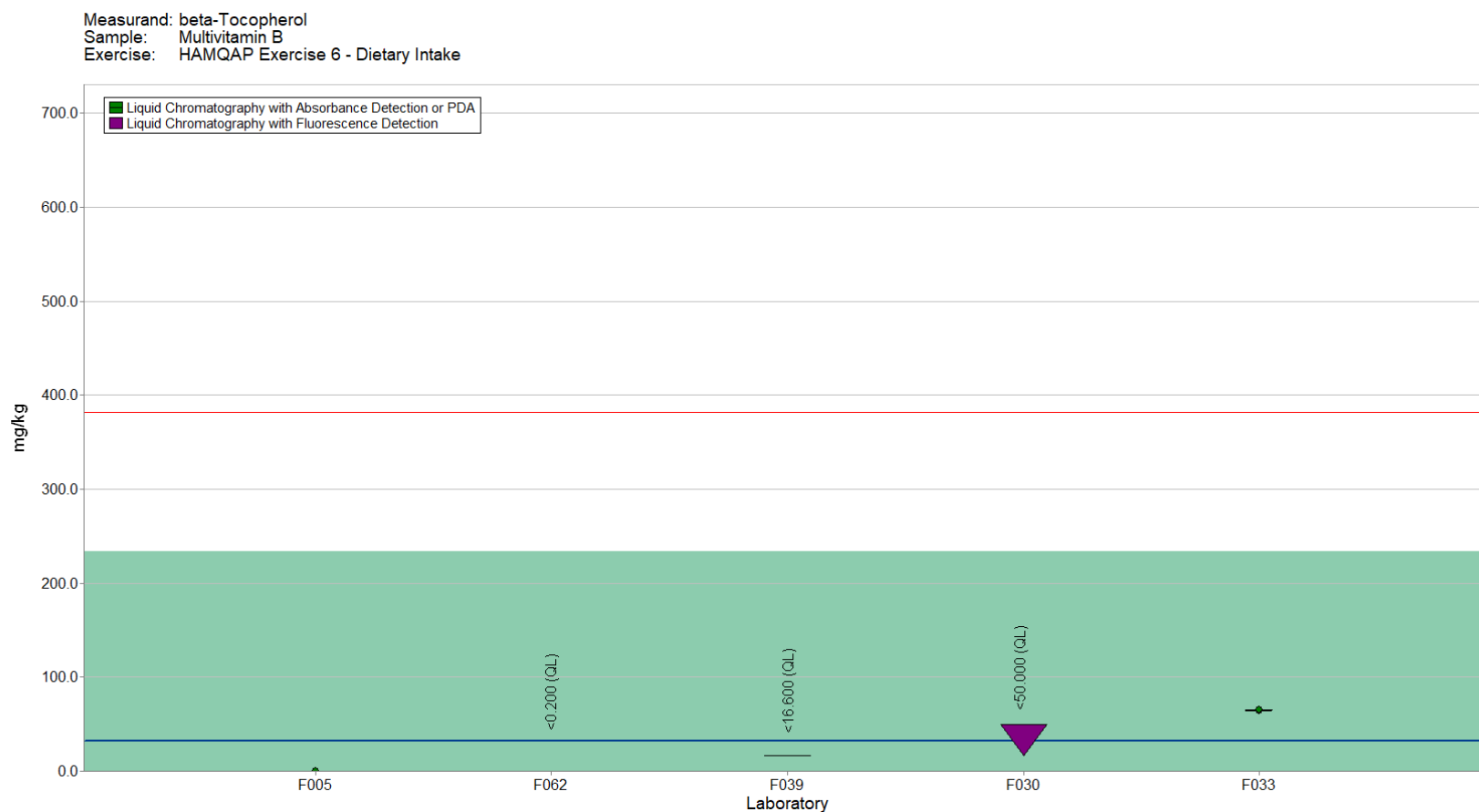


Figure 4-21. Beta-tocopherol in Multivitamin B (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The red solid lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$. A NIST value has not been determined in this material.

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

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

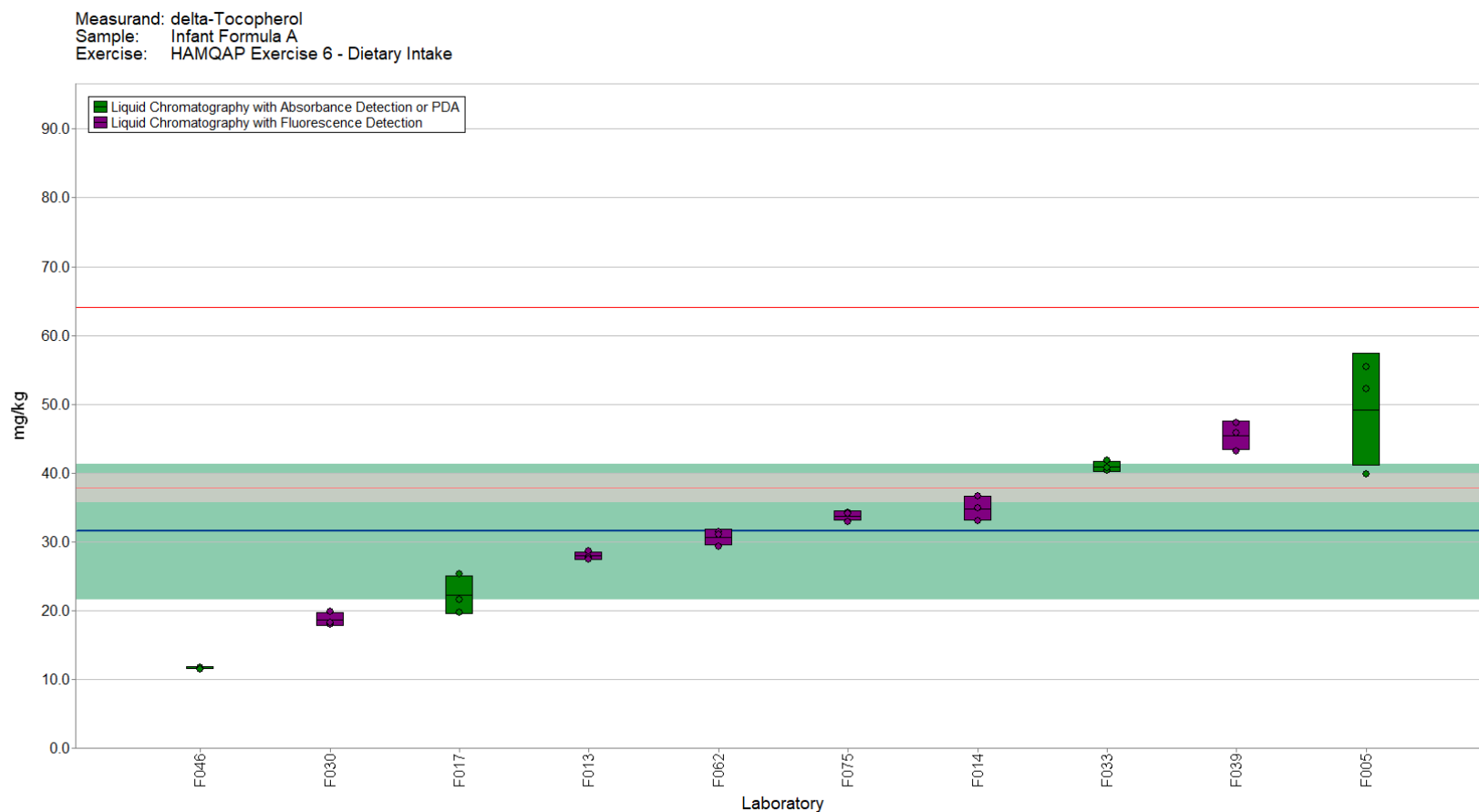


Figure 4-22. Delta-tocopherol in Infant Formula A (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The red solid lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by its uncertainty (U_{NIST}) and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$.

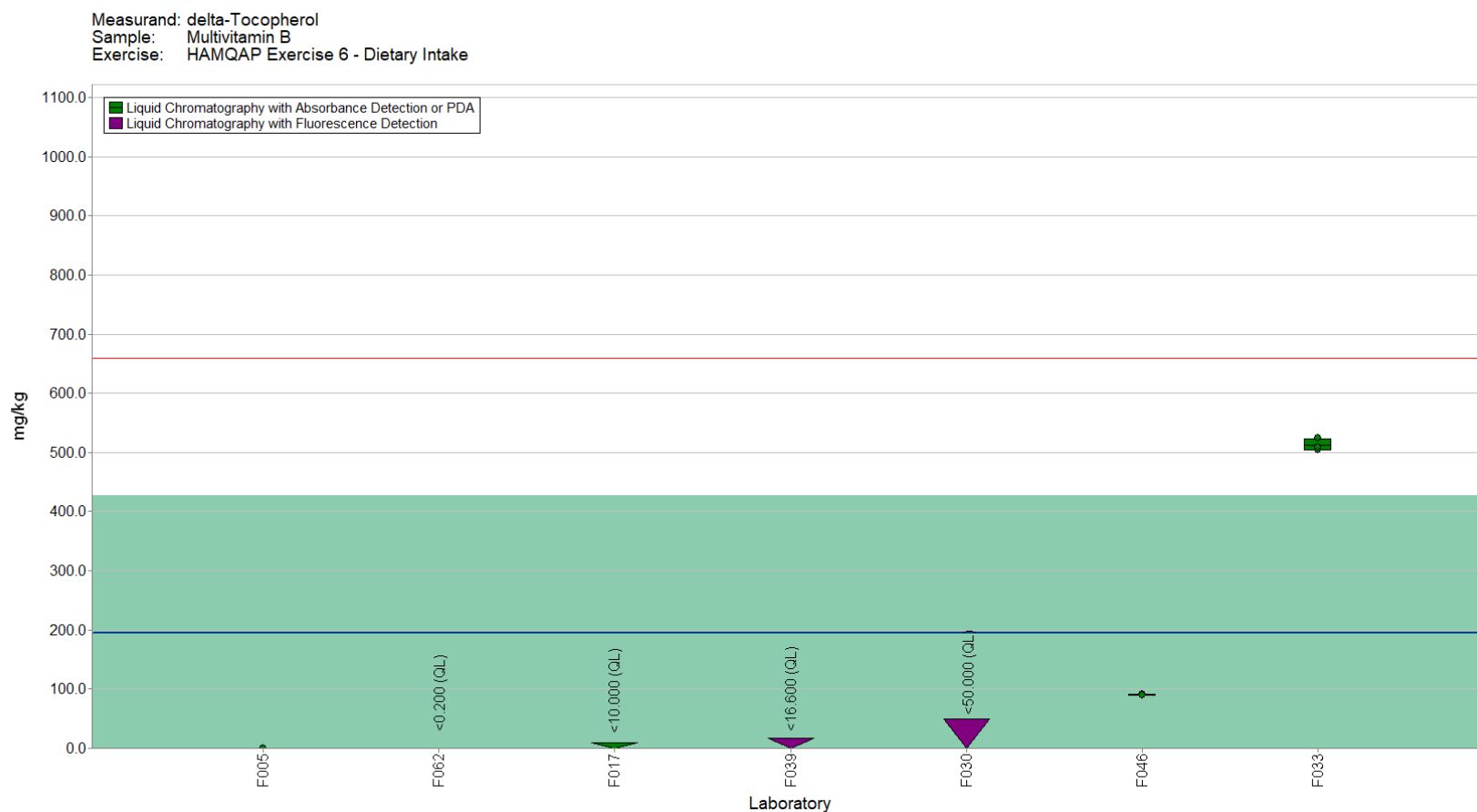


Figure 4-23. Delta-tocopherol in Multivitamin B (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The red solid lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$. A NIST value has not been determined in this material.

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

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

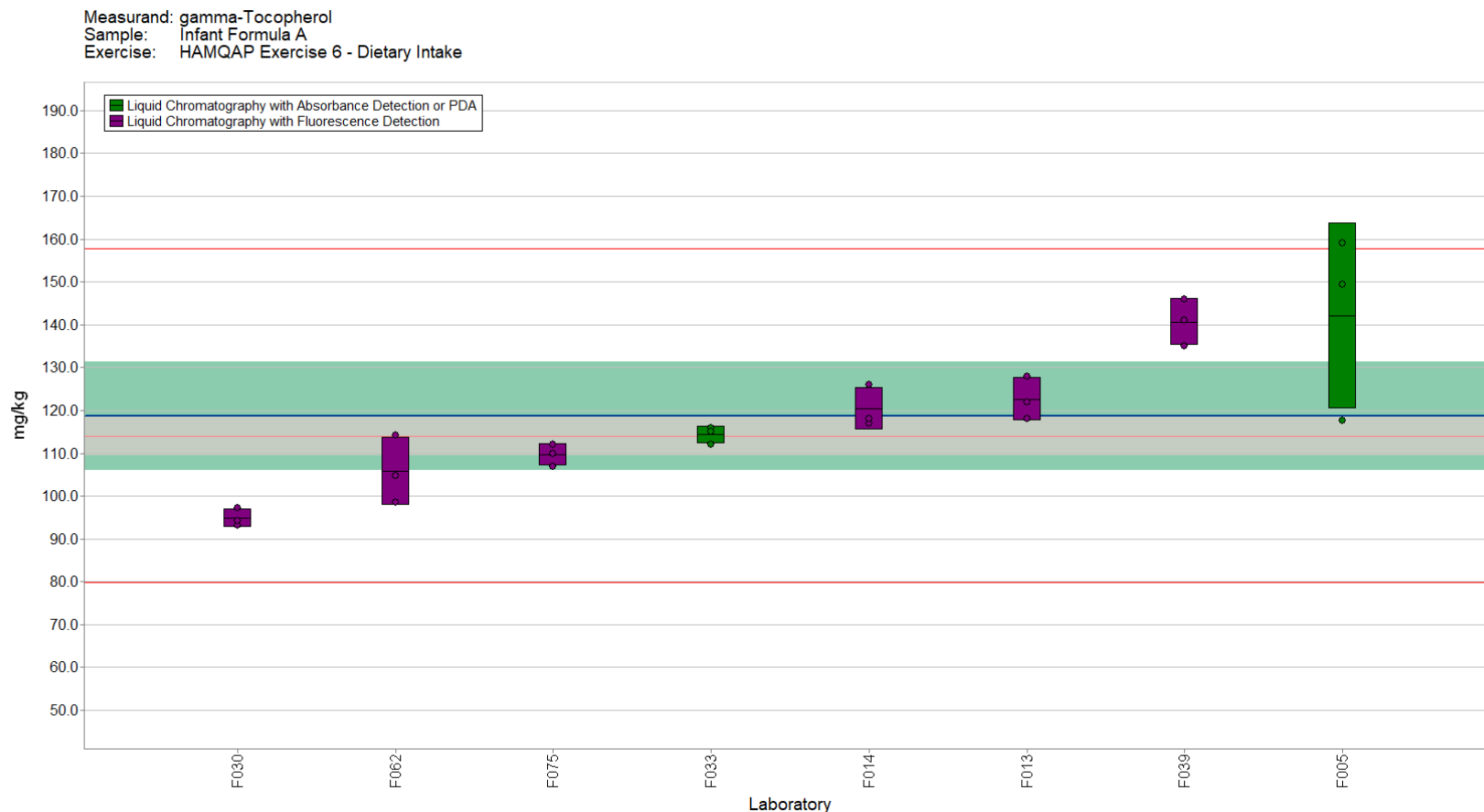


Figure 4-24. Gamma-tocopherol in Infant Formula A (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The red solid lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by its uncertainty (U_{NIST}) and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$.

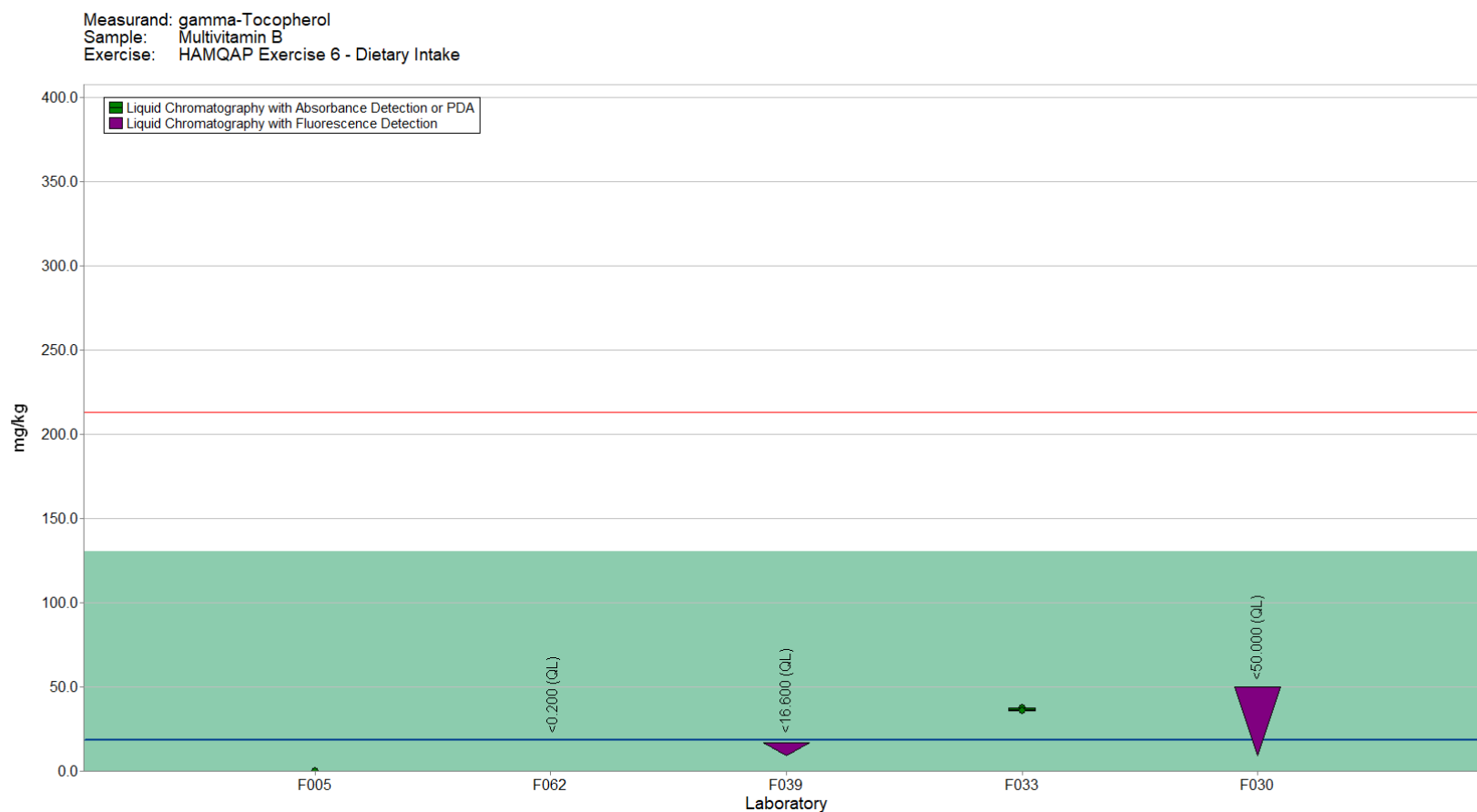


Figure 4-25. Gamma-tocopherol in Multivitamin B (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The red solid lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$. A NIST value has not been determined in this material.

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

Study Overview

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

Dietary Intake Sample Information

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

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

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

NIST-Determined Mass Fractions in SRM 3275-2 (mg/g)

<u>Analyte</u>	<u>(as FAMES)</u>	<u>(as FFAs)</u>
ALA	1.42 ± 0.12	1.35 ± 0.11
Linoleic Acid	3.00 ± 0.42	2.86 ± 0.40
ARA	22.9 ± 1.0	21.89 ± 0.96
EPA	394 ± 17	377 ± 16
DHA	187 ± 8	179 ± 8

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

Dietary Intake Study Results

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

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

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

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

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

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

Dietary Intake Technical Recommendations

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

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

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

- Laboratories reporting results flagged as outside the consensus tolerance limits should check for calculation errors. One example is to confirm that factors for all dilutions have been properly tabulated.
- The use of appropriate calibration materials and quality assurance samples to establish that a method is in control and performing correctly may reduce the likelihood of outlying data. Quality assurance samples can be commercially available reference materials (CRMs, SRMs, or RMs) or materials prepared in-house.

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

HAMQAP Exercise 6 - Fatty Acids												
		Lab Code:	NIST	1. Your Results				2. Community Results			3. Target	
Analyte	Sample		Units	x_i	s_i	Z'_{comm}	Z_{NIST}	N	x^*	s^*	x_{NIST}	U
Total Linoleic Acid (C18:2 n-6)	Commercial Anchovies		mg/g					19	40	17		
Total Linoleic Acid (C18:2 n-6)	Commercial Sardines		mg/g					18	0.44	0.57		
Total Linoleic Acid (C18:2 n-6)	SRM 3275 Omega-3 and Omega-6 Fatty Acids in Fish Oil L 2		mg/g	2.86	0.4			20	3.8	1.8	2.86	0.4
Total alpha-Linolenic Acid (C18:3 n-3)	Commercial Anchovies		mg/g					19	2.7	1.2		
Total alpha-Linolenic Acid (C18:3 n-3)	Commercial Sardines		mg/g					18	0.111	0.096		
Total alpha-Linolenic Acid (C18:3 n-3)	SRM 3275 Omega-3 and Omega-6 Fatty Acids in Fish Oil L 2		mg/g	1.35	0.114			21	1.72	0.94	1.35	0.114
Total Arachidonic Acid (C20:4 n-6)	Commercial Anchovies		mg/g					14	0.163	0.097		
Total Arachidonic Acid (C20:4 n-6)	Commercial Sardines		mg/g					17	0.32	0.22		
Total Arachidonic Acid (C20:4 n-6)	SRM 3275 Omega-3 and Omega-6 Fatty Acids in Fish Oil L 2		mg/g	21.9	0.956			21	24.9	2.7	21.9	0.956
Total EPA (C20:5 n-3)	Commercial Anchovies		mg/g					20	1.05	0.39		
Total EPA (C20:5 n-3)	Commercial Sardines		mg/g					20	3.1	2.3		
Total EPA (C20:5 n-3)	SRM 3275 Omega-3 and Omega-6 Fatty Acids in Fish Oil L 2		mg/g	377	16.2			23	390	53	377	16.2
Total DHA (C22:6 n-3)	Commercial Anchovies		mg/g					20	3.6	1.6		
Total DHA (C22:6 n-3)	Commercial Sardines		mg/g					20	3.6	2.2		
Total DHA (C22:6 n-3)	SRM 3275 Omega-3 and Omega-6 Fatty Acids in Fish Oil L 2		mg/g	179	7.67			23	190	24	179	7.67
				x_i	Mean of reported values			N	Number of quantitative values reported		x_{NIST}	NIST-assessed value
				s_i	Standard deviation of reported values						U	expanded uncertainty
				Z'_{comm}	Z'-score with respect to community consensus			x^*	Robust mean of reported values		about the NIST-assessed value	
				Z_{NIST}	Z-score with respect to NIST value			s^*	Robust standard deviation			

 $\bar{x}_i$ 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

 $\bar{x}^*$ Robust mean of reported values s^* Robust standard deviation $\bar{x}_{\text{NIST}}$ NIST-assessed value U expanded uncertainty about the NIST-assessed value

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

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

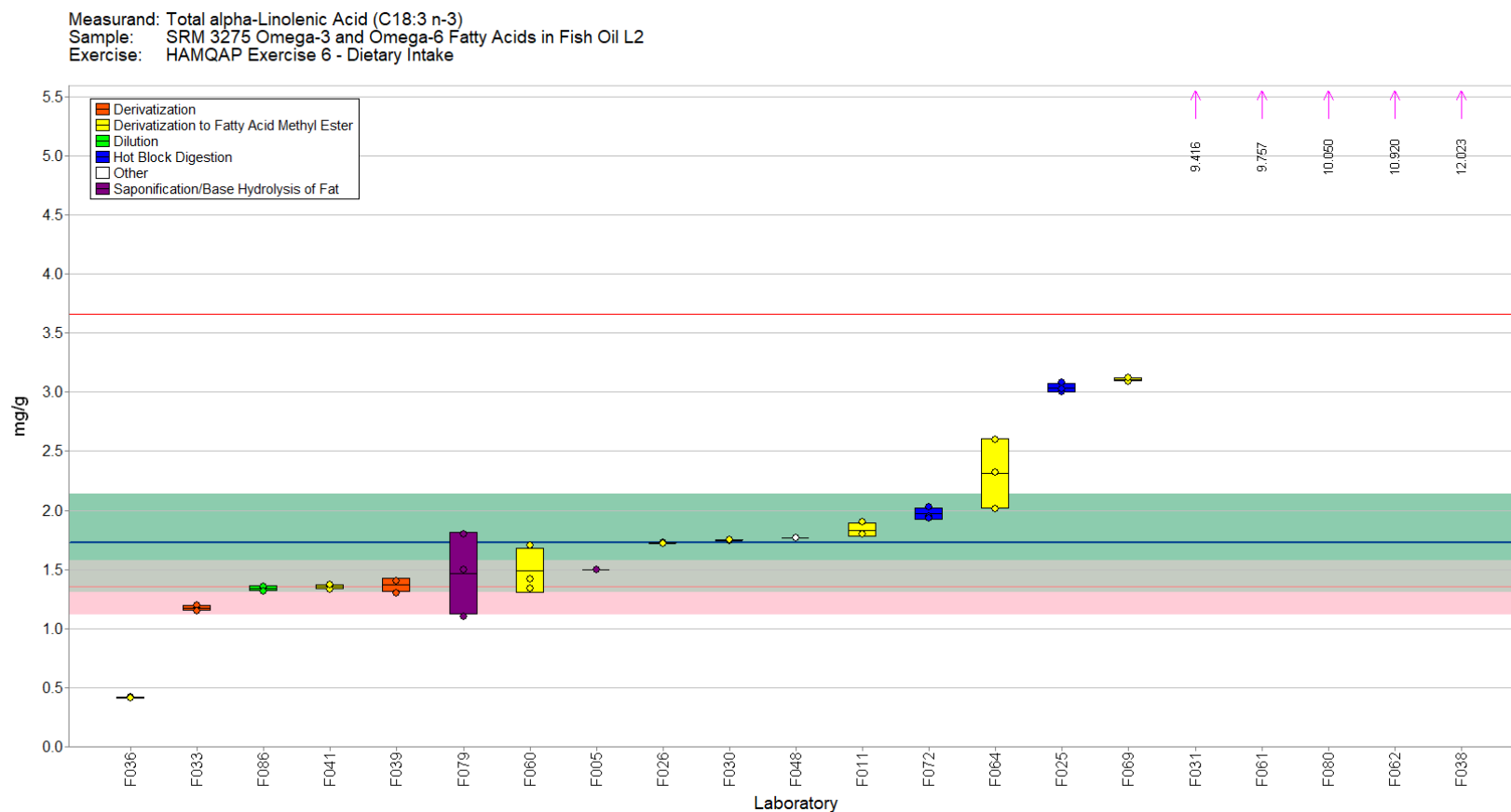


Figure 5-1. Total α -linolenic acid in SRM 3275 Omega-3 and Omega-6 Fatty Acids in Fish Oil (Level 2) (data summary view – sample preparation method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point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

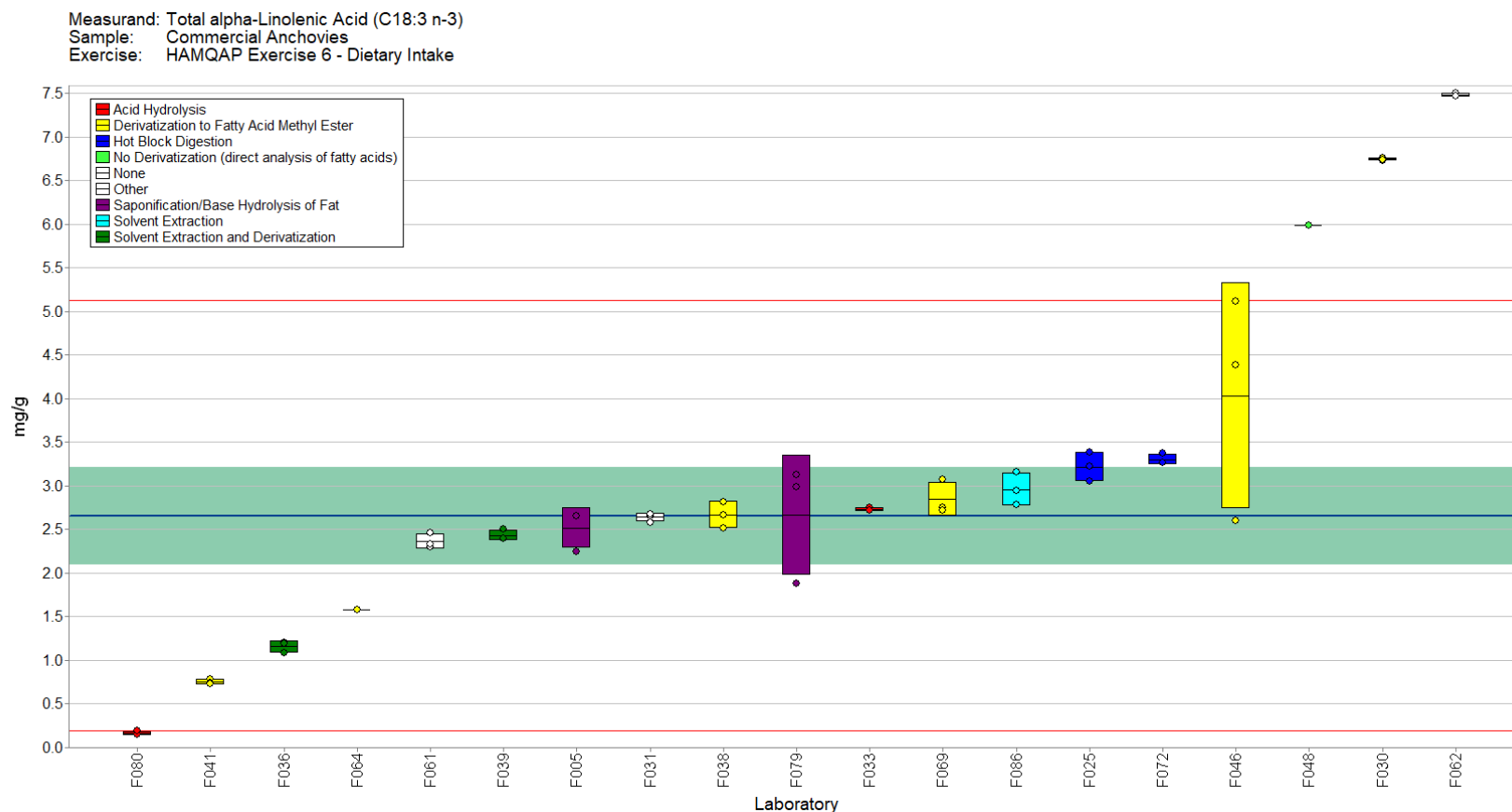


Figure 5-2. Total α -linolenic acid in commercial anchovies (data summary view – sample preparation method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method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

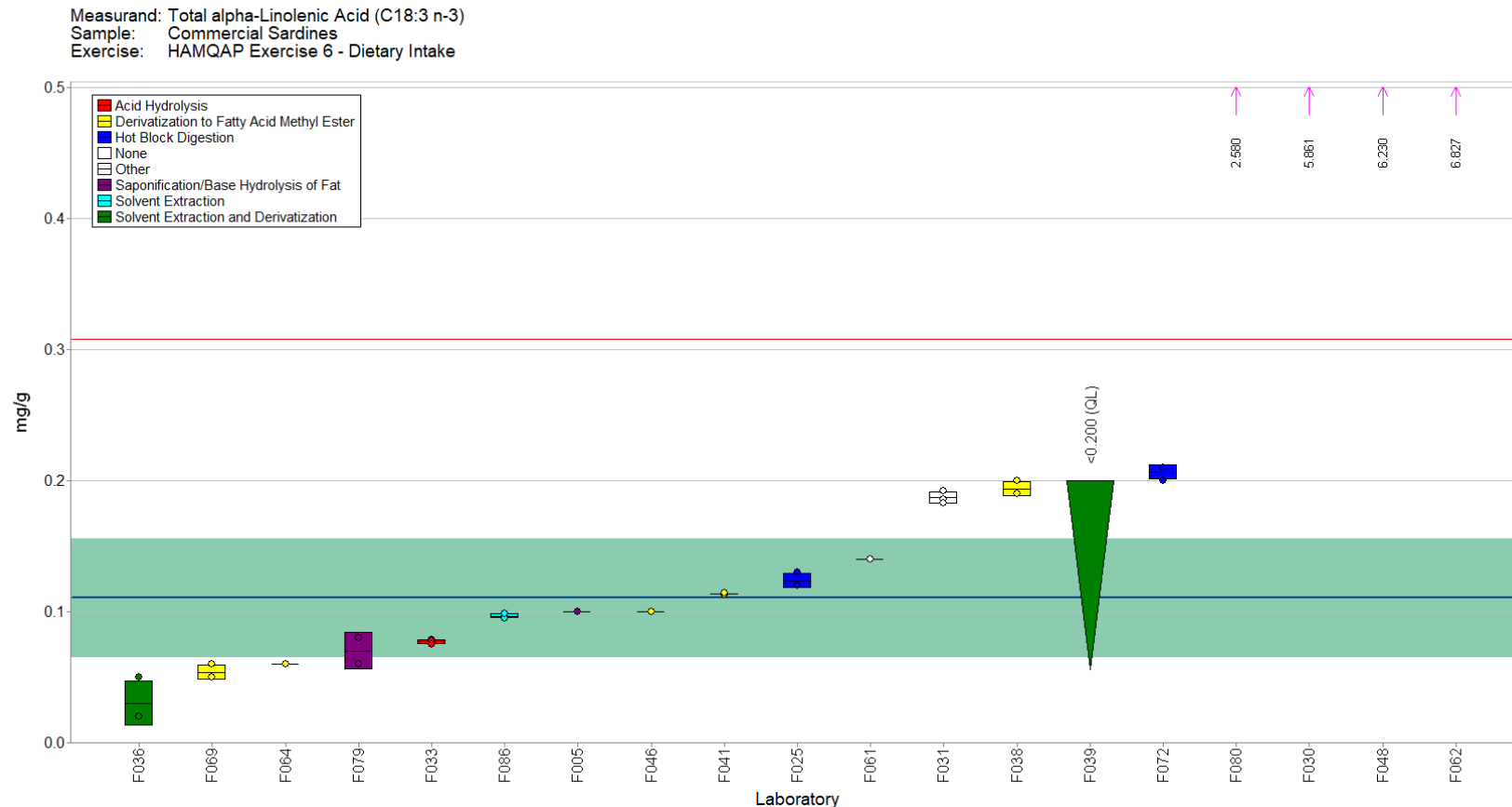


Figure 5-3. Total α -linolenic acid in commercial sardines (data summary view – sample preparation method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method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

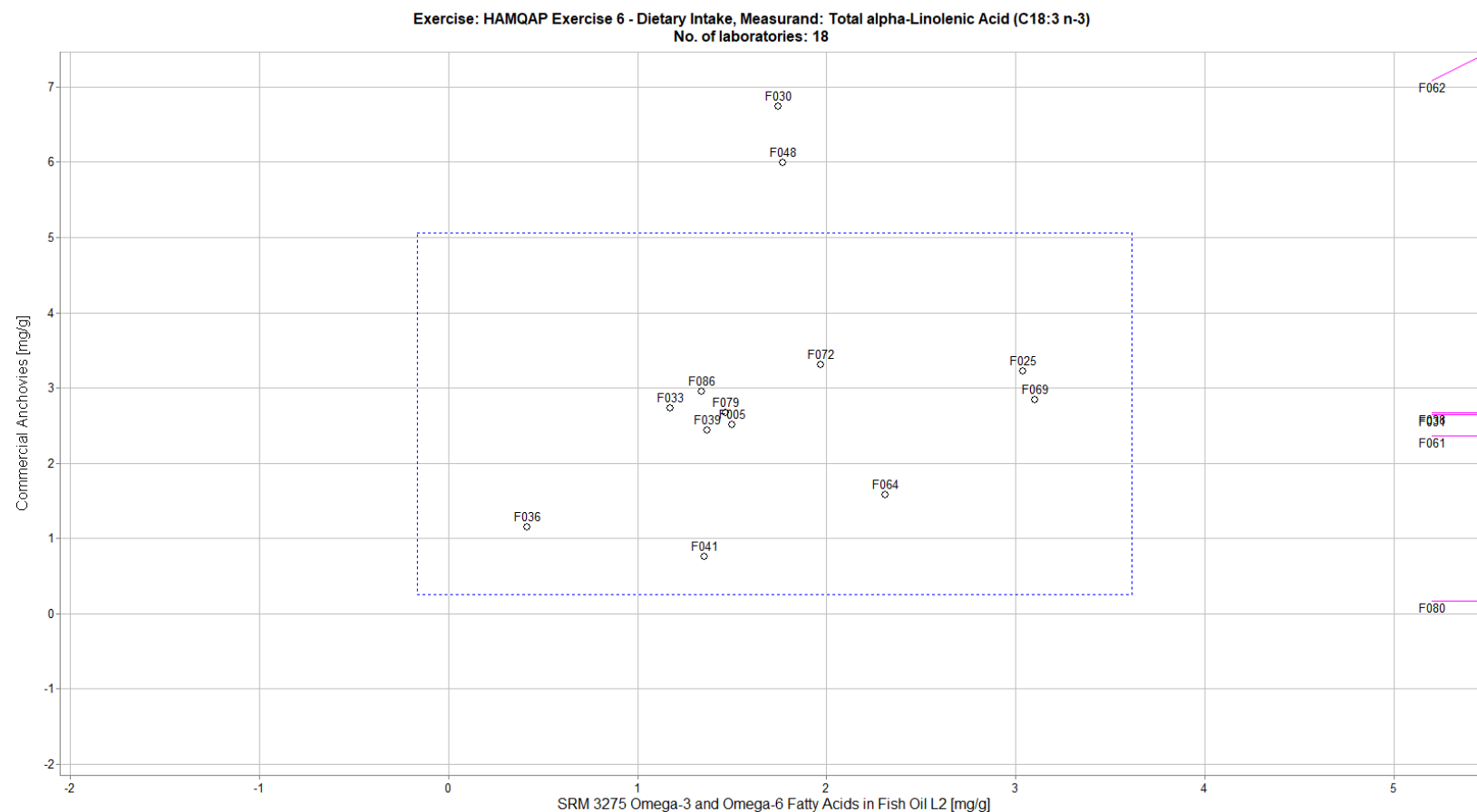


Figure 5-4. Laboratory means for total α -linolenic acid in SRM 3275 Omega-3 and Omega-6 Fatty Acids in Fish Oil Level 2 and commercial anchovies (sample/sample comparison view). In this view, the individual laboratory mean for one sample (SRM 3275 Level 2) is compared to the individual laboratory mean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

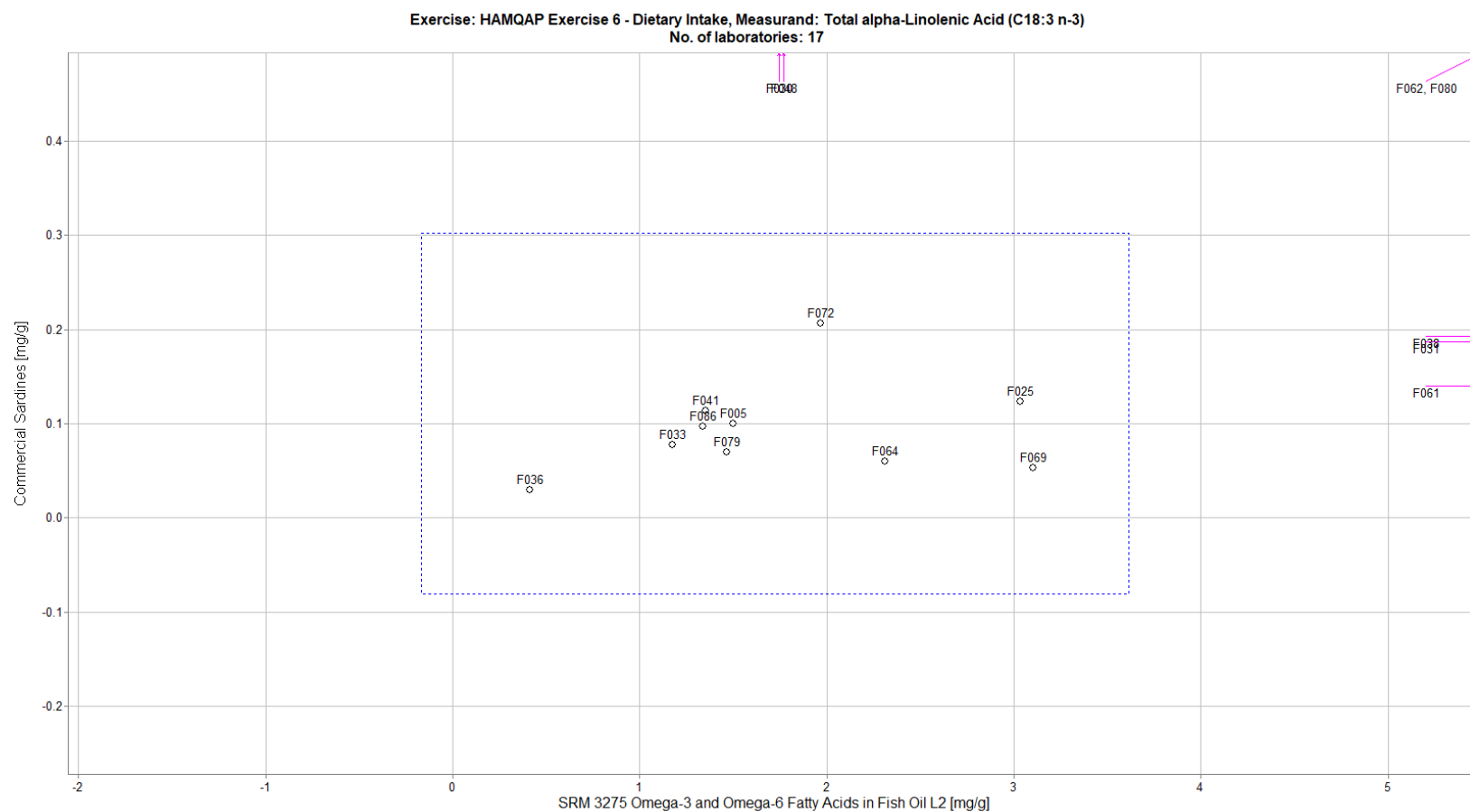


Figure 5-5. Laboratory means for total α -linolenic acid in SRM 3275 Omega-3 and Omega-6 Fatty Acids in Fish Oil Level 2 and commercial sardines (sample/sample comparison view). In this view, the individual laboratory mean for one sample (SRM 3275 Level 2) is compared to the individual laboratory mean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

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

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

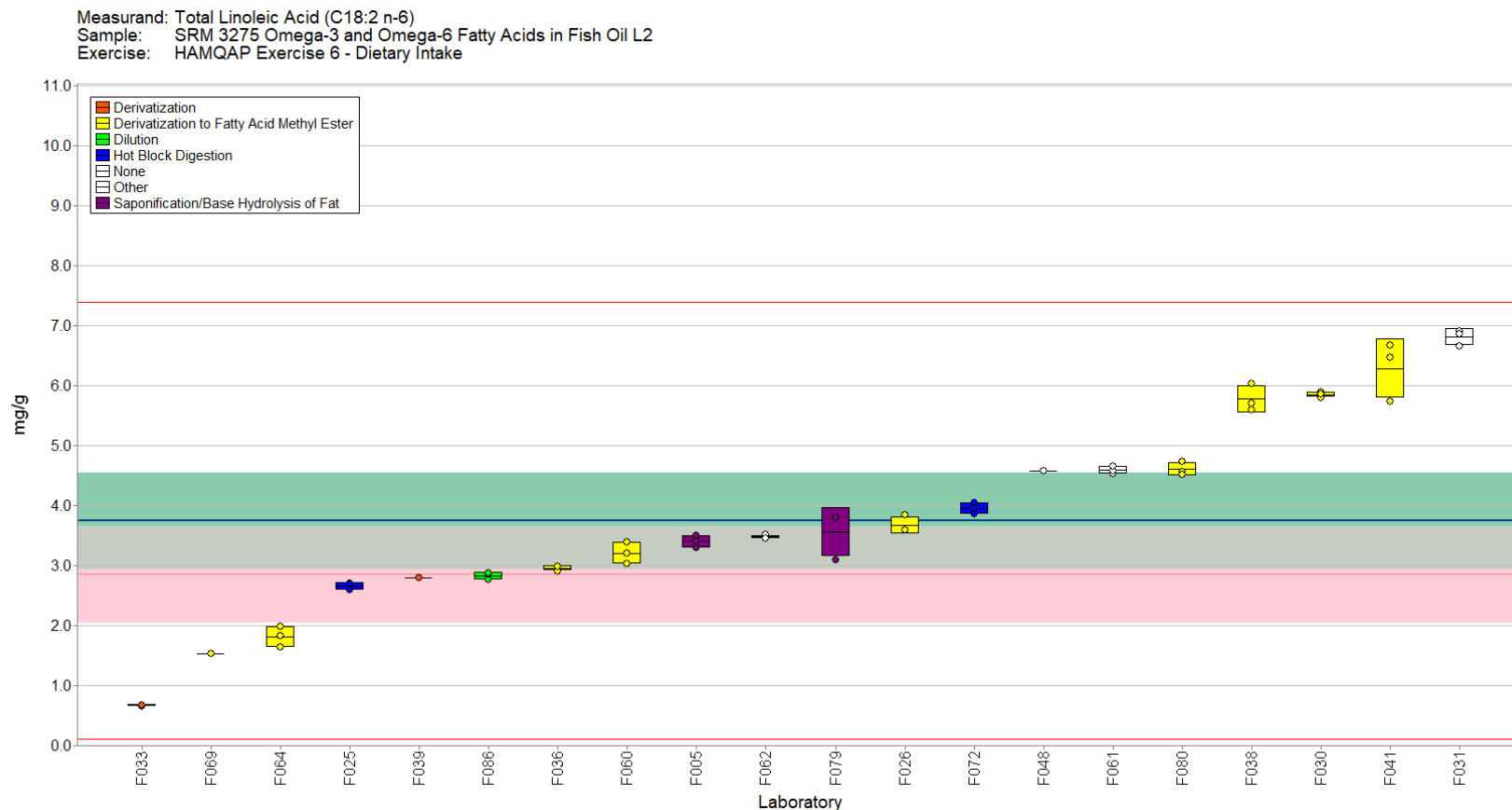


Figure 5-6. Total linolenic acid in SRM 3275 Omega-3 and Omega-6 Fatty Acids in Fish Oil (Level 2) (data summary view – sample preparation method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point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

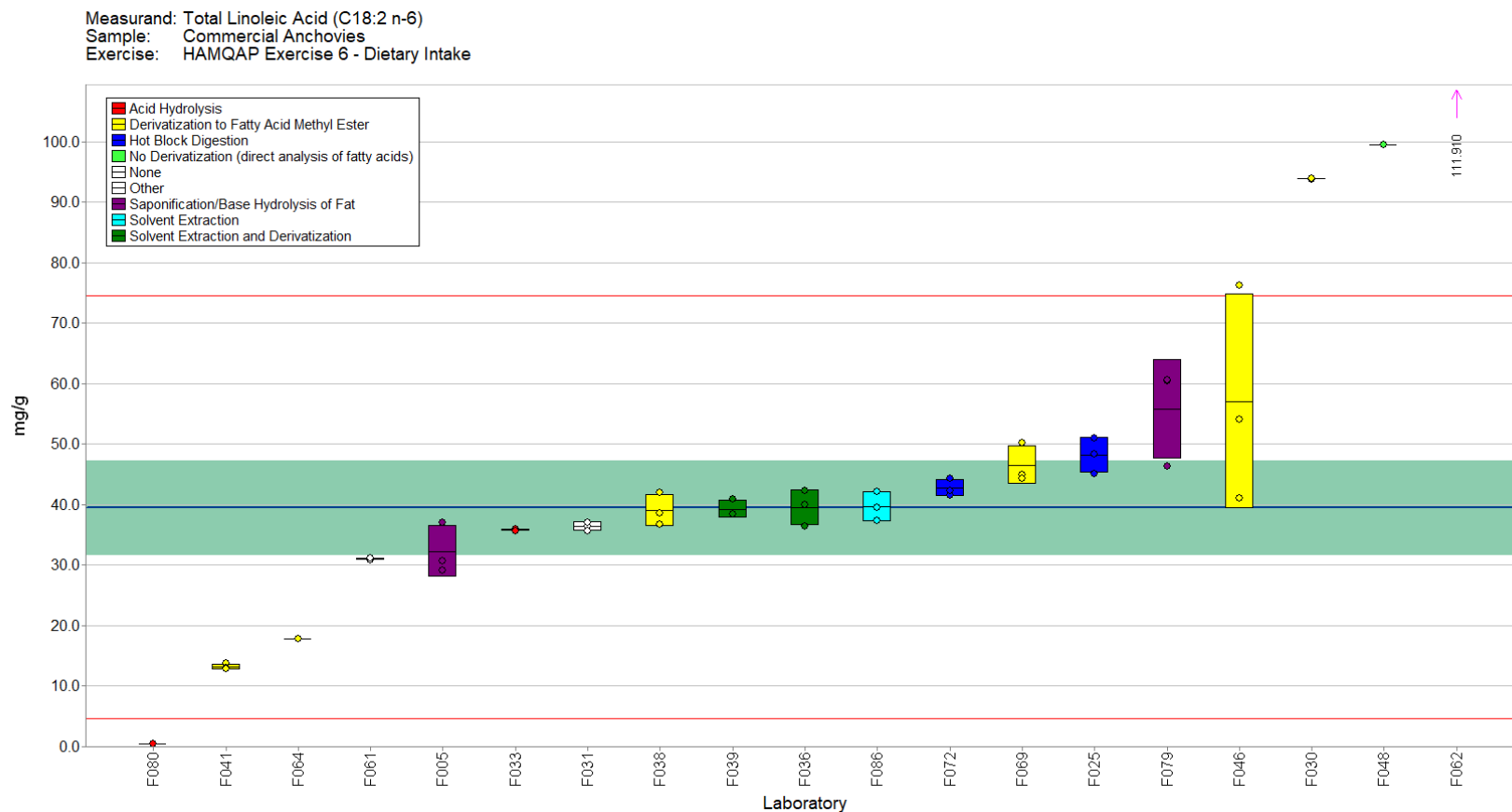


Figure 5-7. Total linolenic acid in commercial anchovies (data summary view – sample preparation method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method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

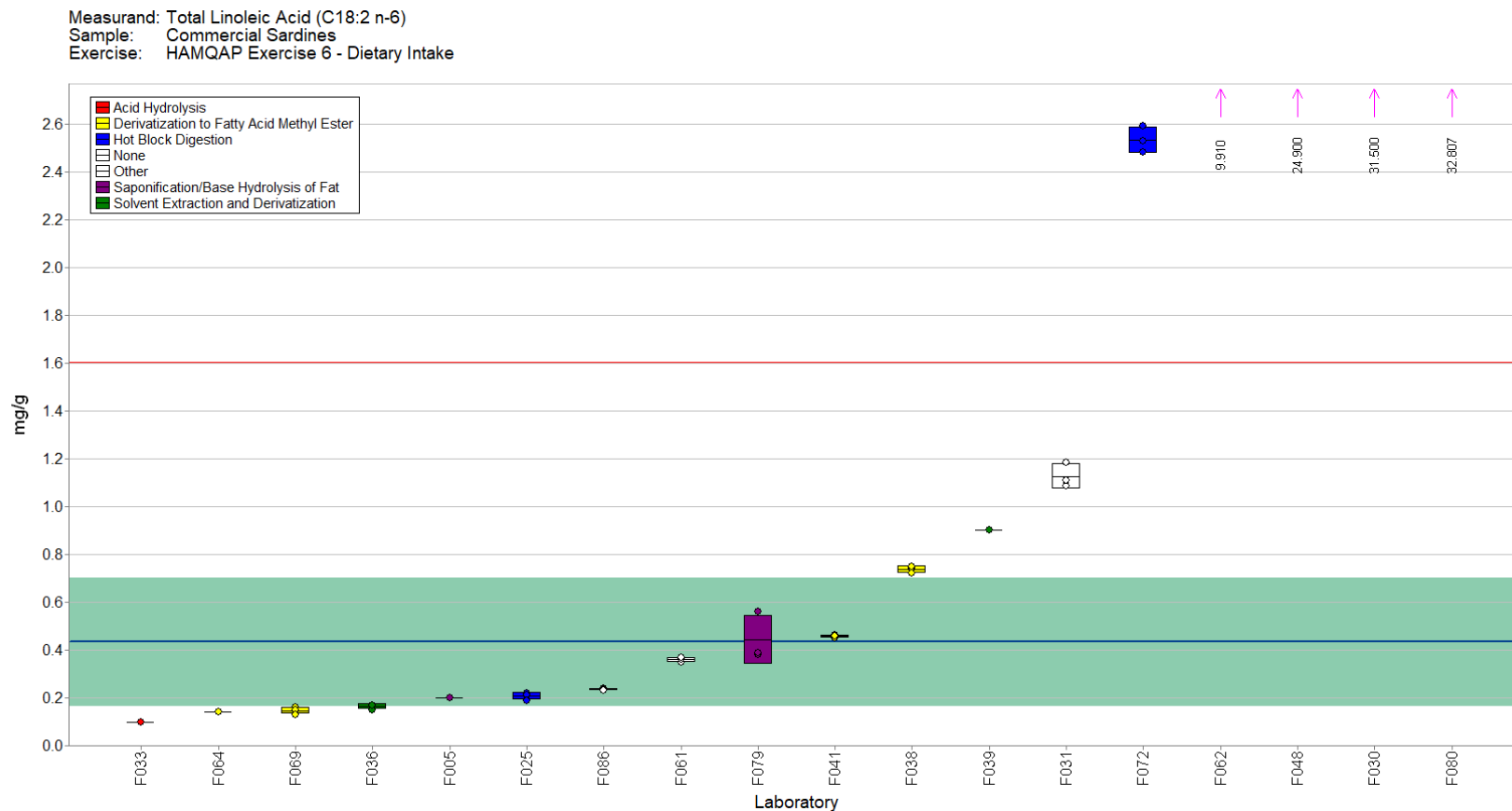


Figure 5-8. Total linolenic acid in commercial sardines (data summary view – sample preparation method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed.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

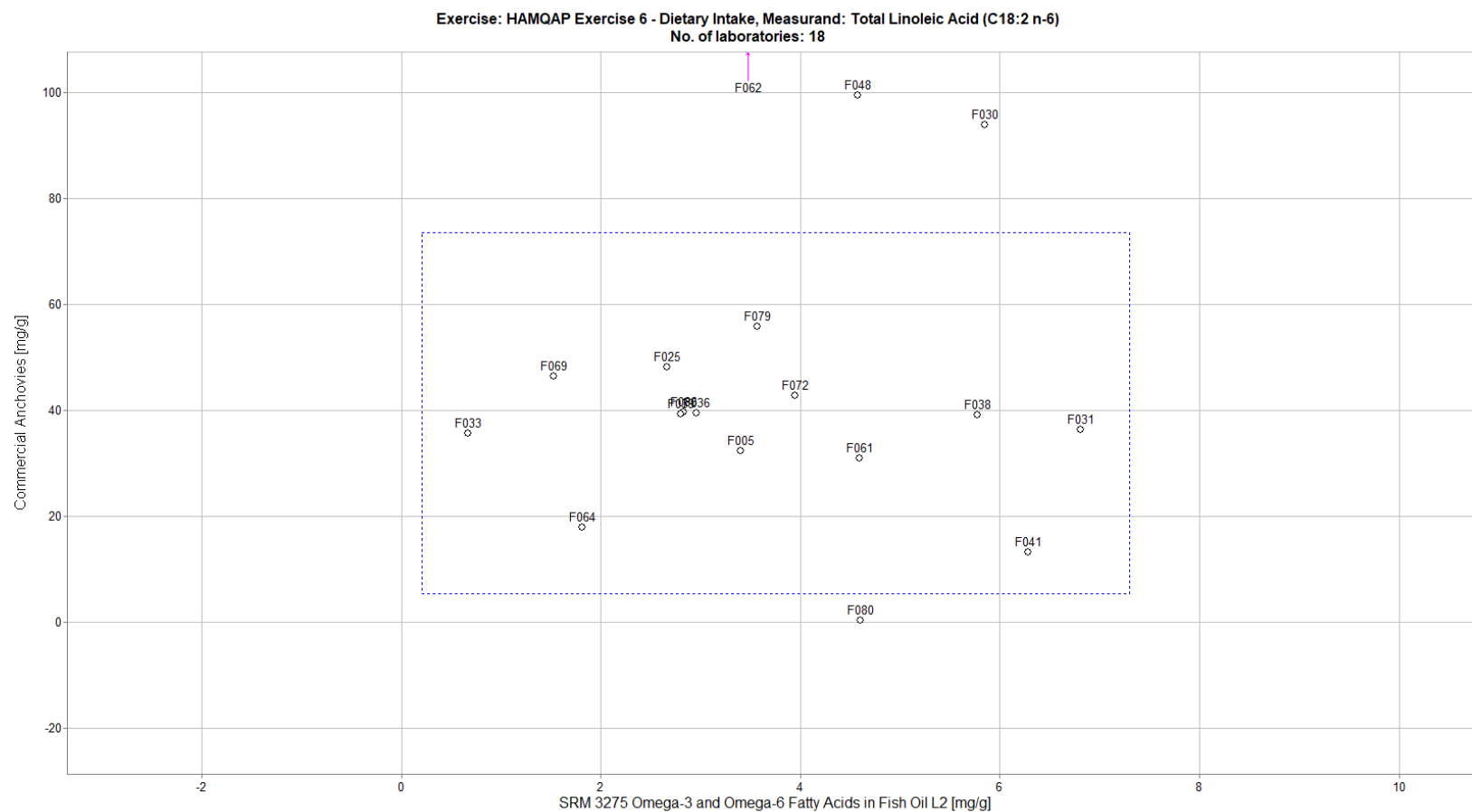


Figure 5-9. Laboratory means for total linolenic acid in SRM 3275 Omega-3 and Omega-6 Fatty Acids in Fish Oil Level 2 and commercial anchovies (sample/sample comparison view). In this view, the individual laboratory mean for one sample (SRM 3275 Level 2) is compared to the individual laboratory mean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

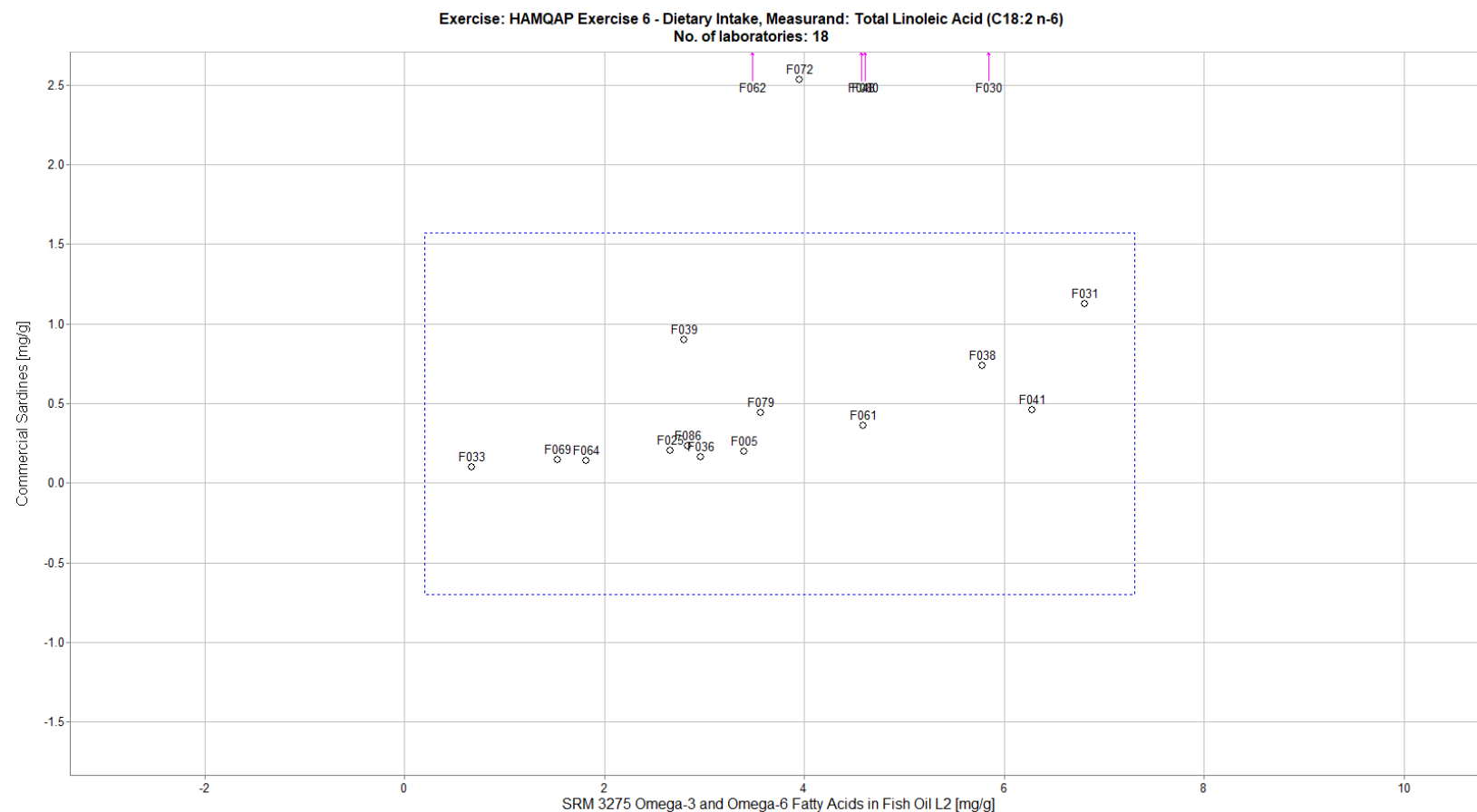


Figure 5-10. Laboratory means for total linolenic acid in SRM 3275 Omega-3 and Omega-6 Fatty Acids in Fish Oil Level 2 and commercial sardines (sample/sample comparison view). In this view, the individual laboratory mean for one sample (SRM 3275 Level 2) is compared to the individual laboratory mean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

Table 5-4. Data summary table for total arachidonic acid in SRM 3275 Omega-3 and Omega-6 Fatty Acids in Fish Oil (Level 2), commercial anchovies, and commercial sardines. Data points highlighted in blue have been identified as outside the consensus tolerance limits and would be estimated to result in an unacceptable Z'_{comm} score, $|Z'_{\text{comm}}| \geq 2$.

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

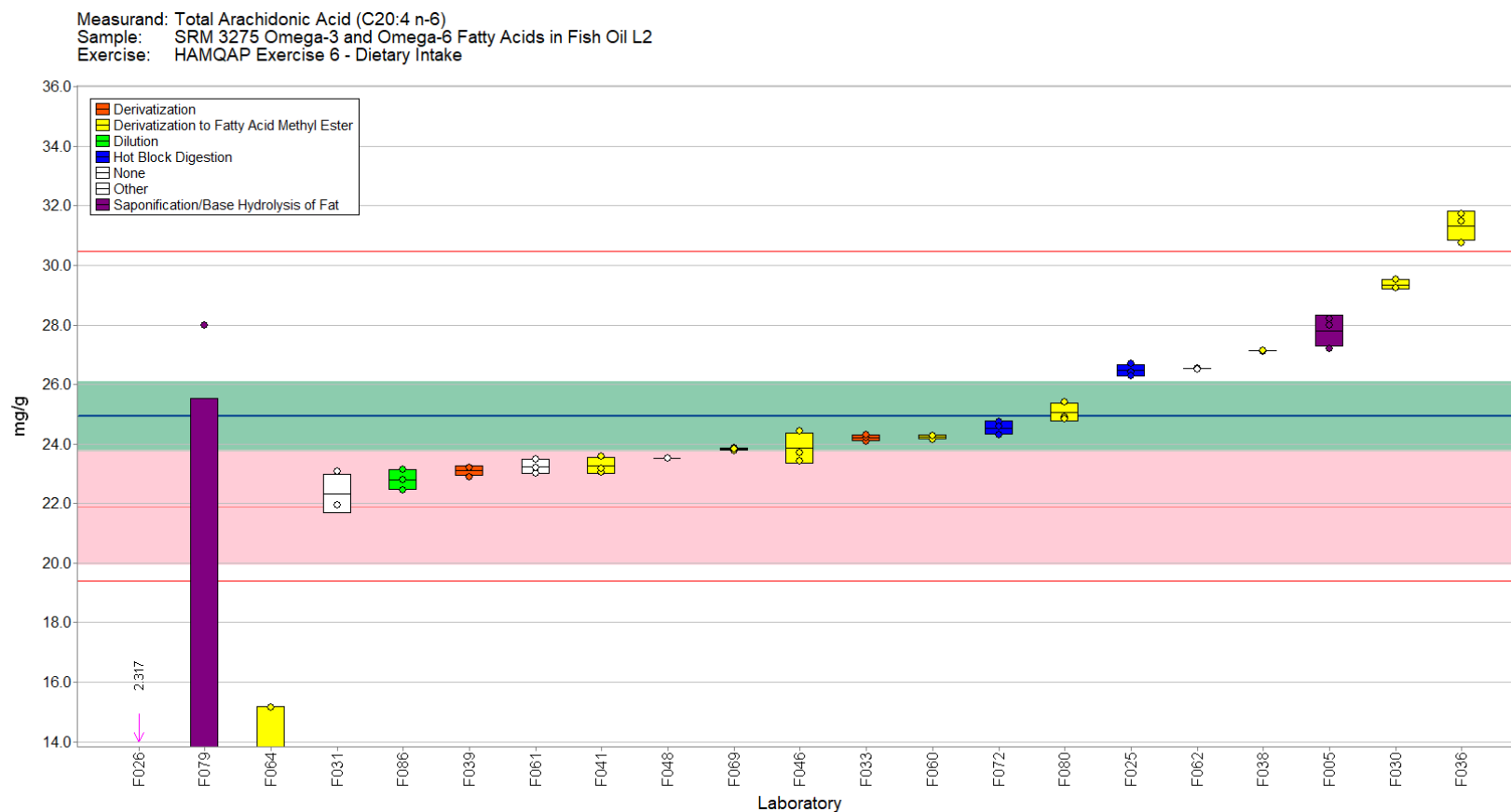


Figure 5-11. Total arachidonic acid in SRM 3275 Omega-3 and Omega-6 Fatty Acids in Fish Oil (Level 2) (data summary view – sample preparation method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The red solid lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by its uncertainty (U_{NIST}) and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$.

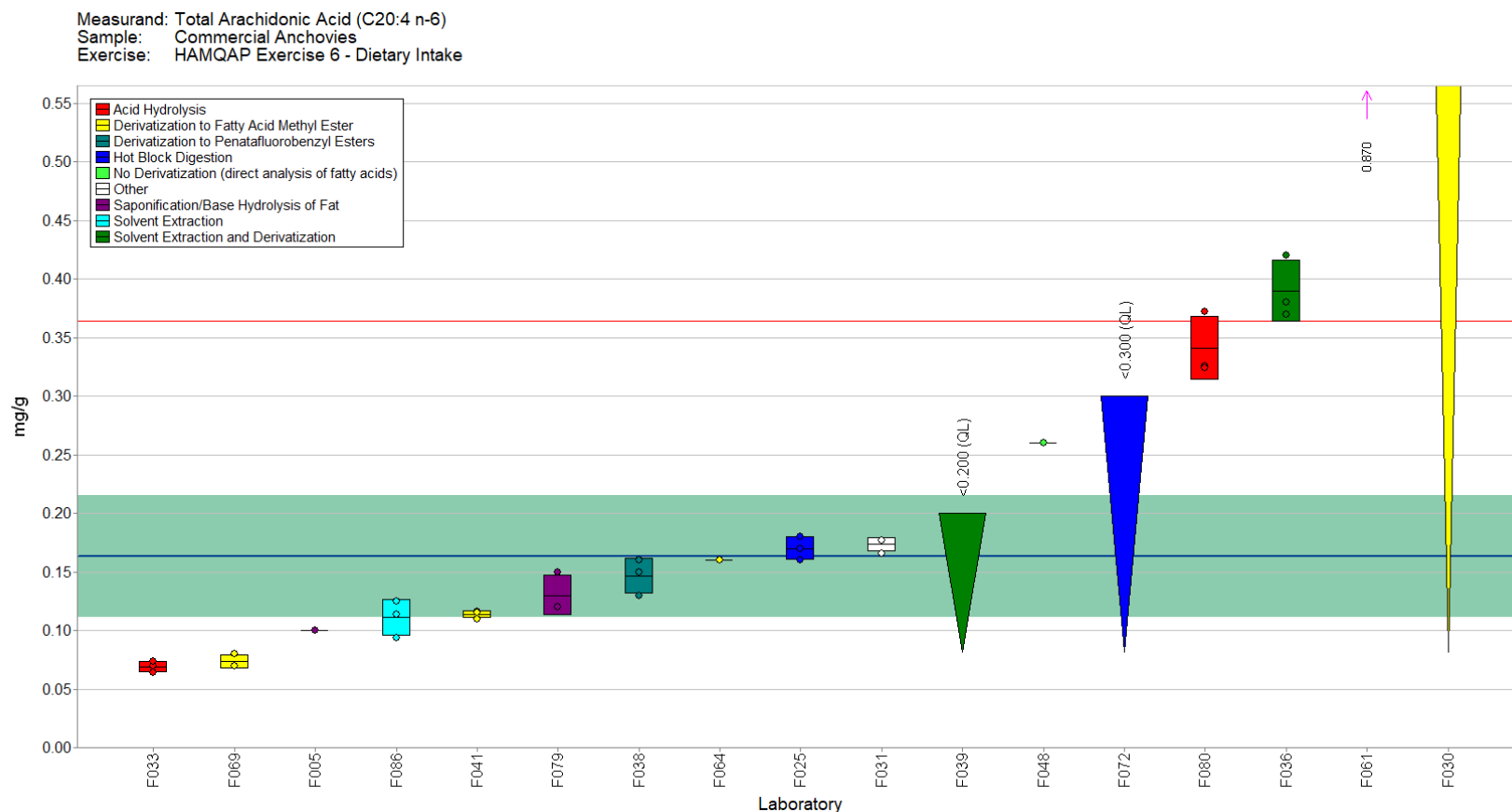


Figure 5-12. Total arachidonic acid in commercial anchovies (data summary view – sample preparation method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method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

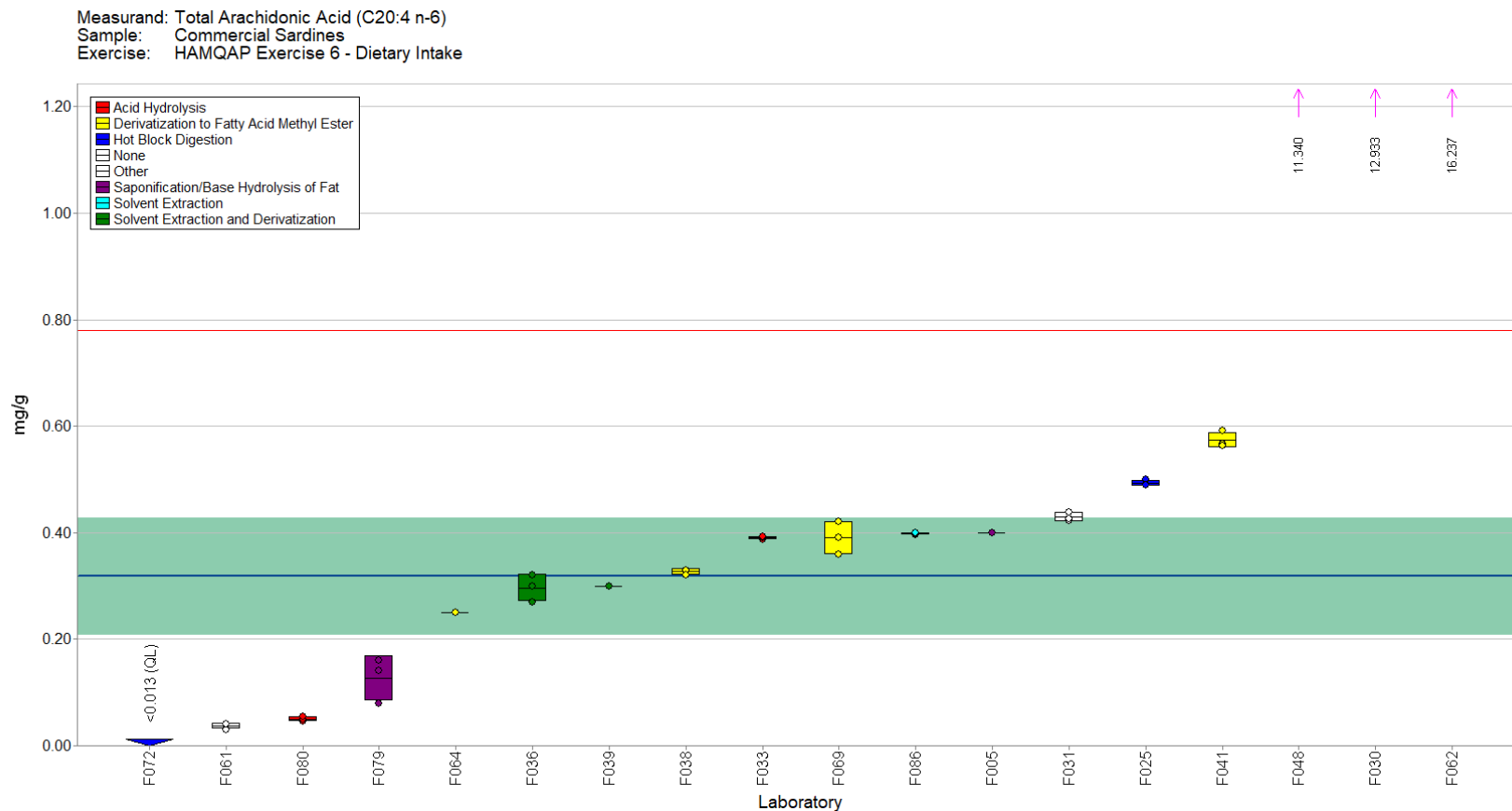


Figure 5-13. Total arachidonic acid in commercial sardines (data summary view – sample preparation method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method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

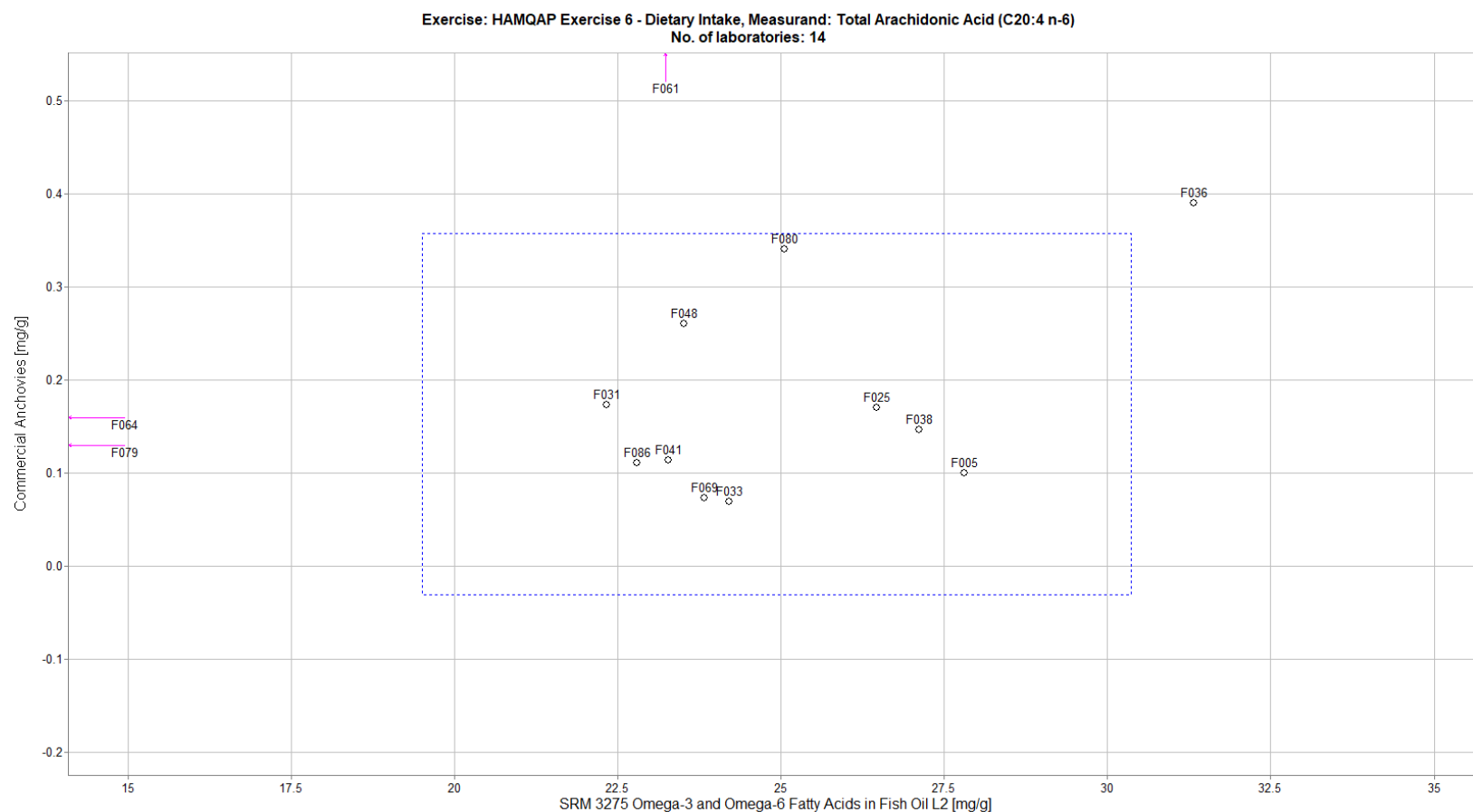


Figure 5-14. Laboratory means for total arachidonic acid in SRM 3275 Omega-3 and Omega-6 Fatty Acids in Fish Oil Level 2 and commercial anchovies (sample/sample comparison view). In this view, the individual laboratory mean for one sample (SRM 3275 Level 2) is compared to the individual laboratory mean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

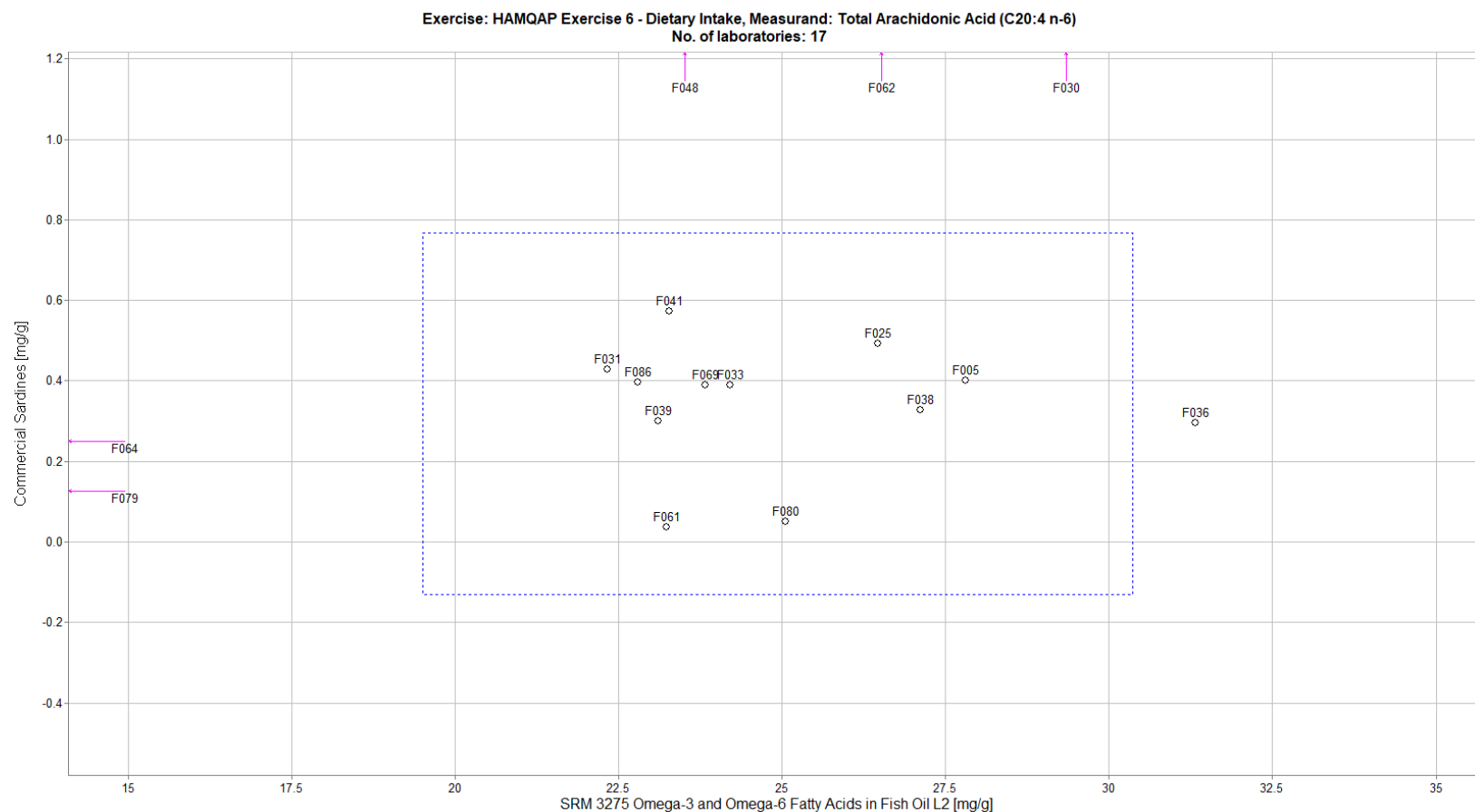


Figure 5-15. Laboratory means for total arachidonic acid in SRM 3275 Omega-3 and Omega-6 Fatty Acids in Fish Oil Level 2 and commercial sardines (sample/sample comparison view). In this view, the individual laboratory mean for one sample (SRM 3275 Level 2) is compared to the individual laboratory mean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

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

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

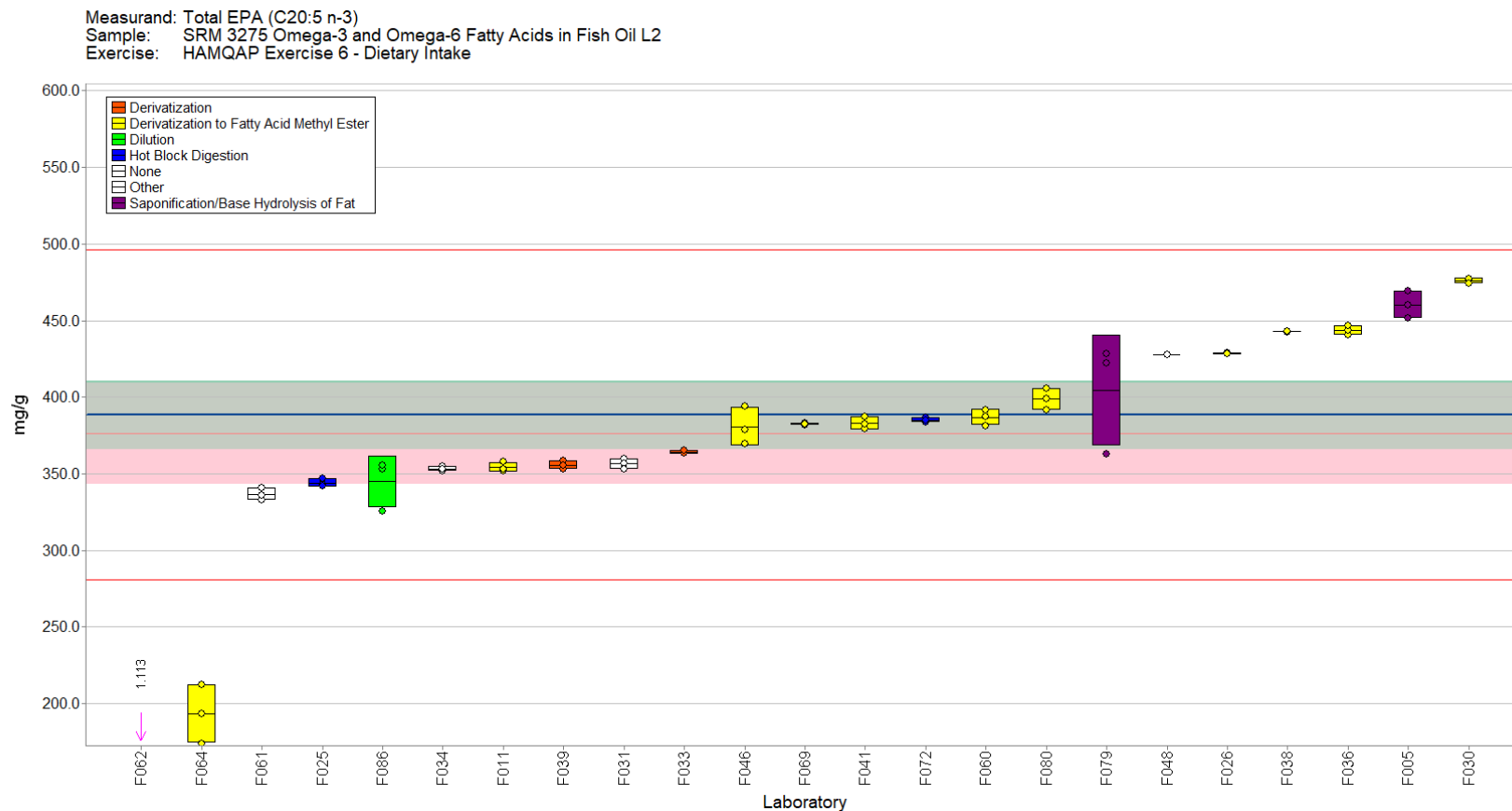


Figure 5-16. Total EPA in SRM 3275 Omega-3 and Omega-6 Fatty Acids in Fish Oil (Level 2) (data summary view – sample preparation method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The red solid lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by its uncertainty (U_{NIST}) and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$.

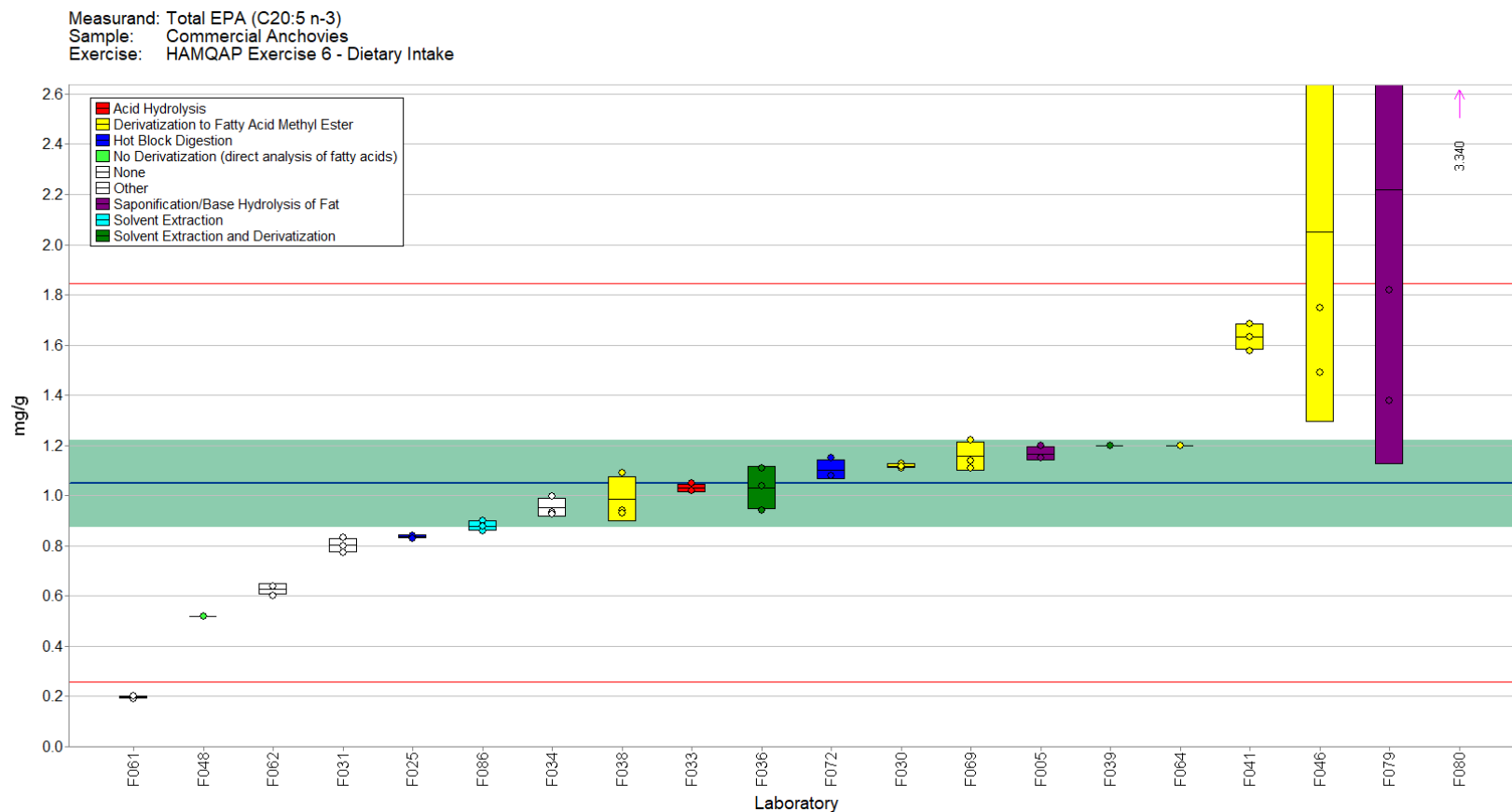


Figure 5-17. Total EPA in commercial anchovies (data summary view – sample preparation method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The red solid lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$. A NIST value has not been determined in this material.

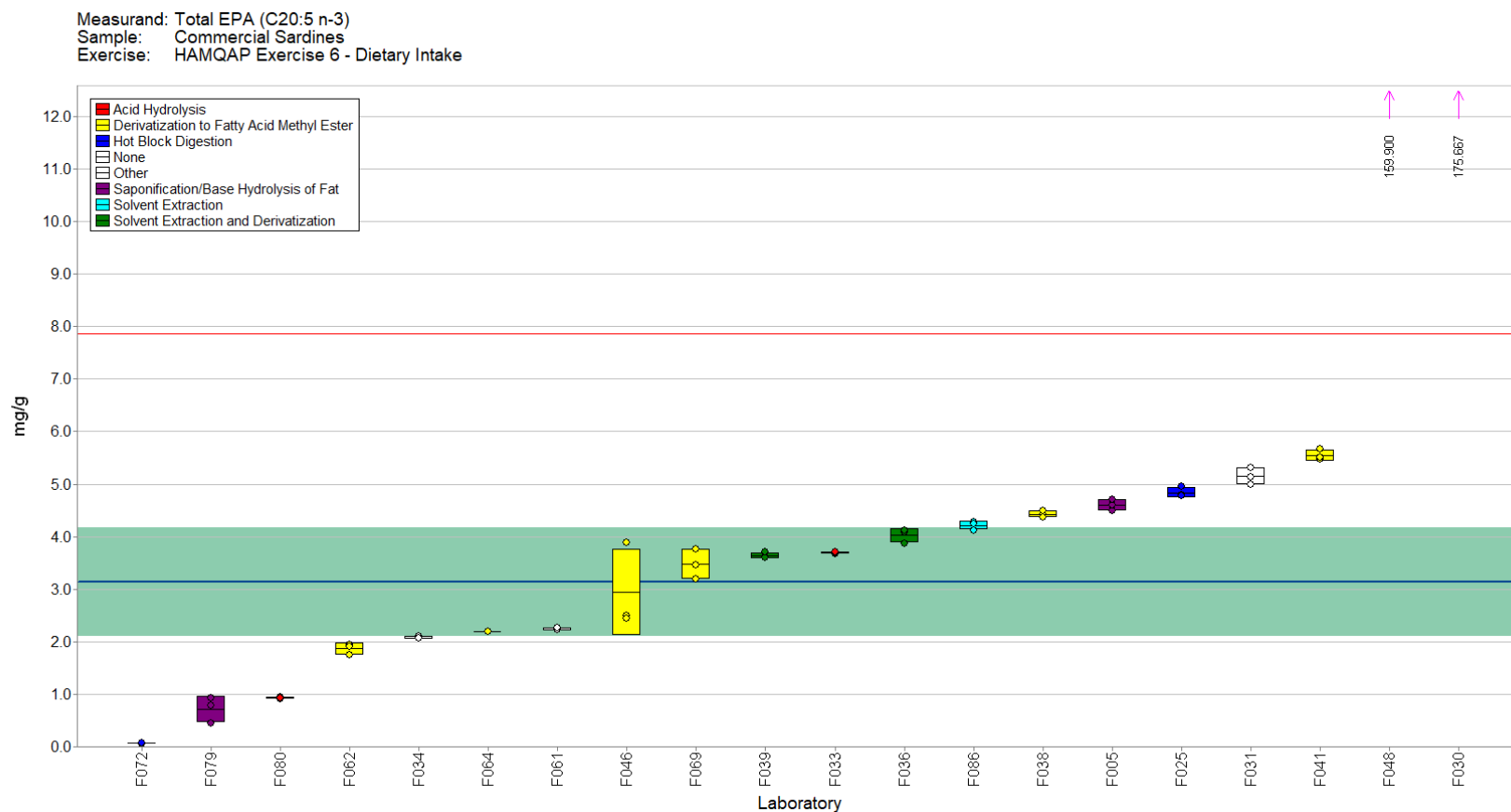


Figure 5-18. Total EPA in commercial sardines (data summary view – sample preparation method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The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

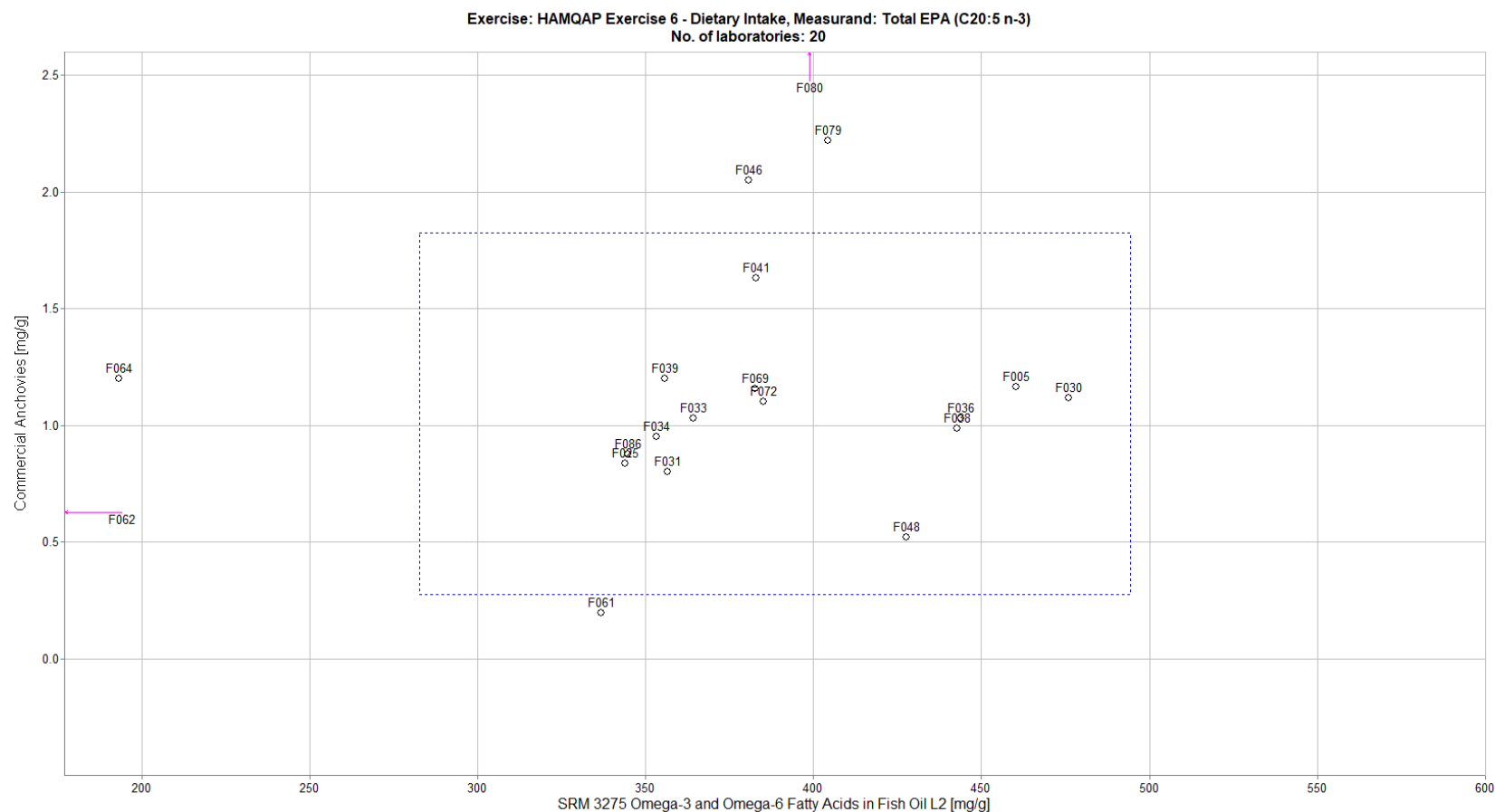


Figure 5-19. Laboratory means for total EPA in SRM 3275 Omega-3 and Omega-6 Fatty Acids in Fish Oil Level 2 and commercial anchovies (sample/sample comparison view). In this view, the individual laboratory mean for one sample (SRM 3275 Level 2) is compared to the individual laboratory mean for a second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

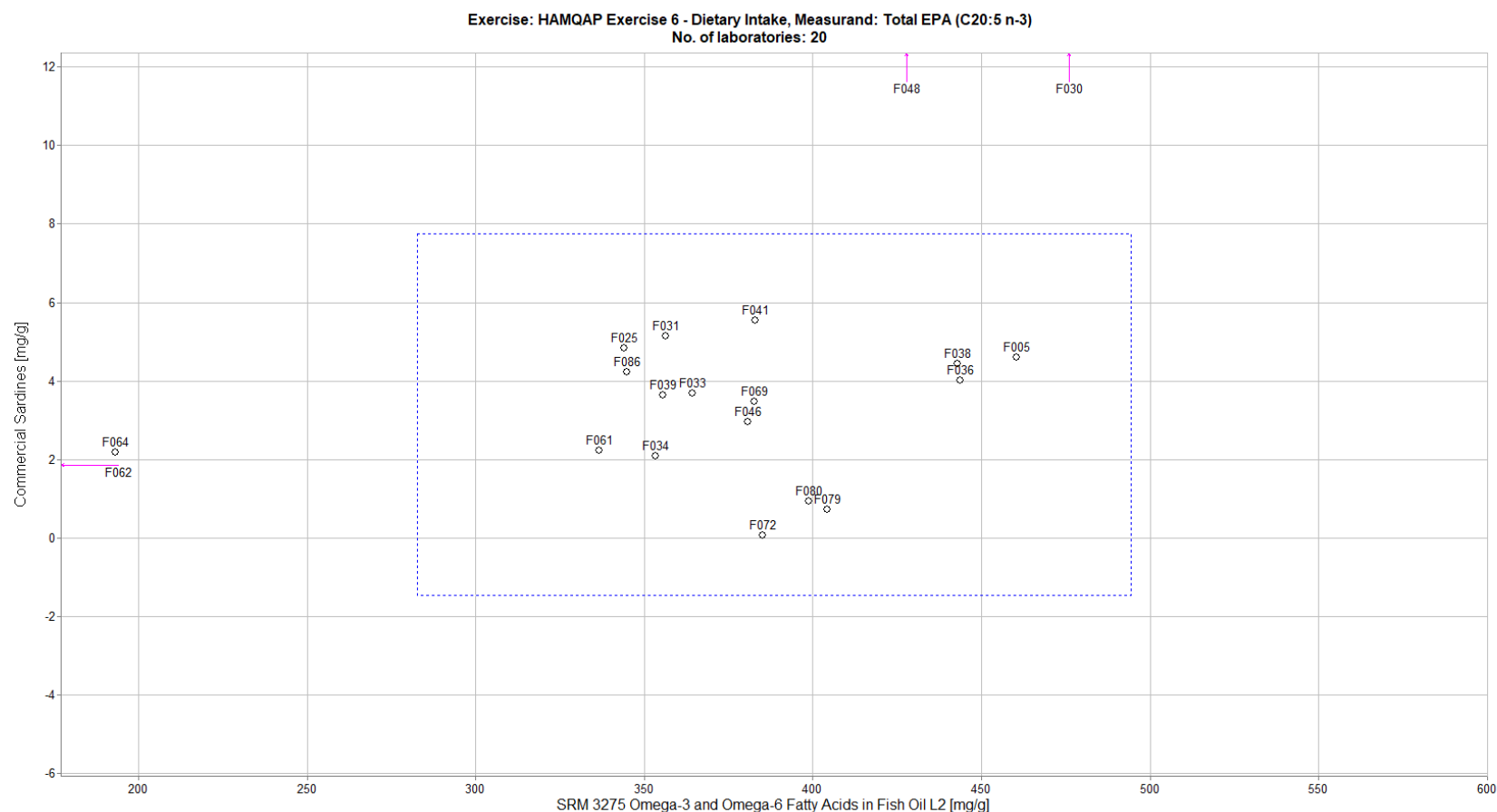


Figure 5-20. Laboratory means for total EPA in SRM 3275 Omega-3 and Omega-6 Fatty Acids in Fish Oil Level 2 and commercial sardines (sample/sample comparison view). In this view, the individual laboratory mean for one sample (SRM 3275 Level 2) is compared to the individual laboratory mean for a second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

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

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

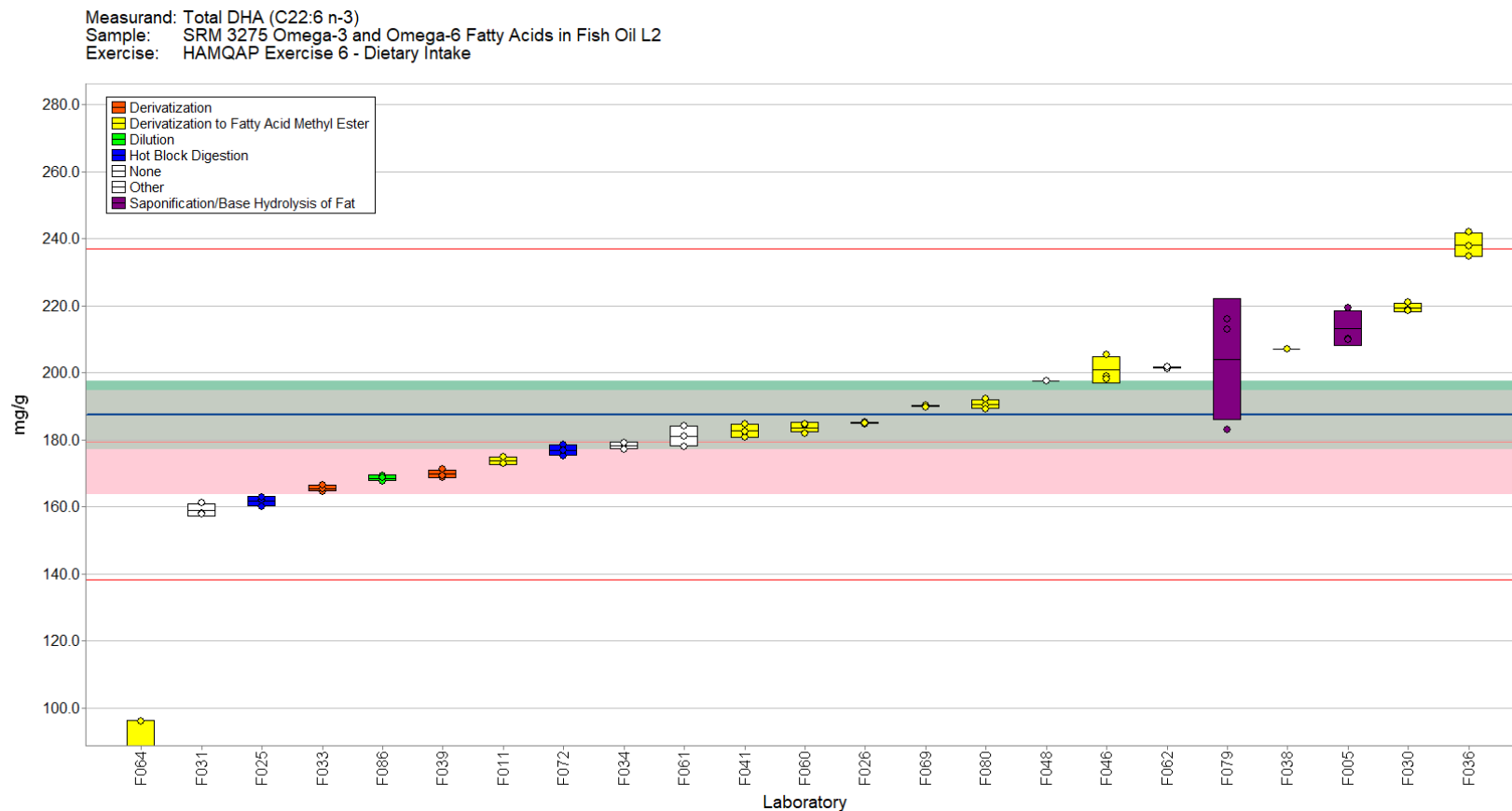


Figure 5-21. Total DHA in SRM 3275 Omega-3 and Omega-6 Fatty Acids in Fish Oil (Level 2) (data summary view – sample preparation method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The red solid lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by its uncertainty (U_{NIST}) and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$.

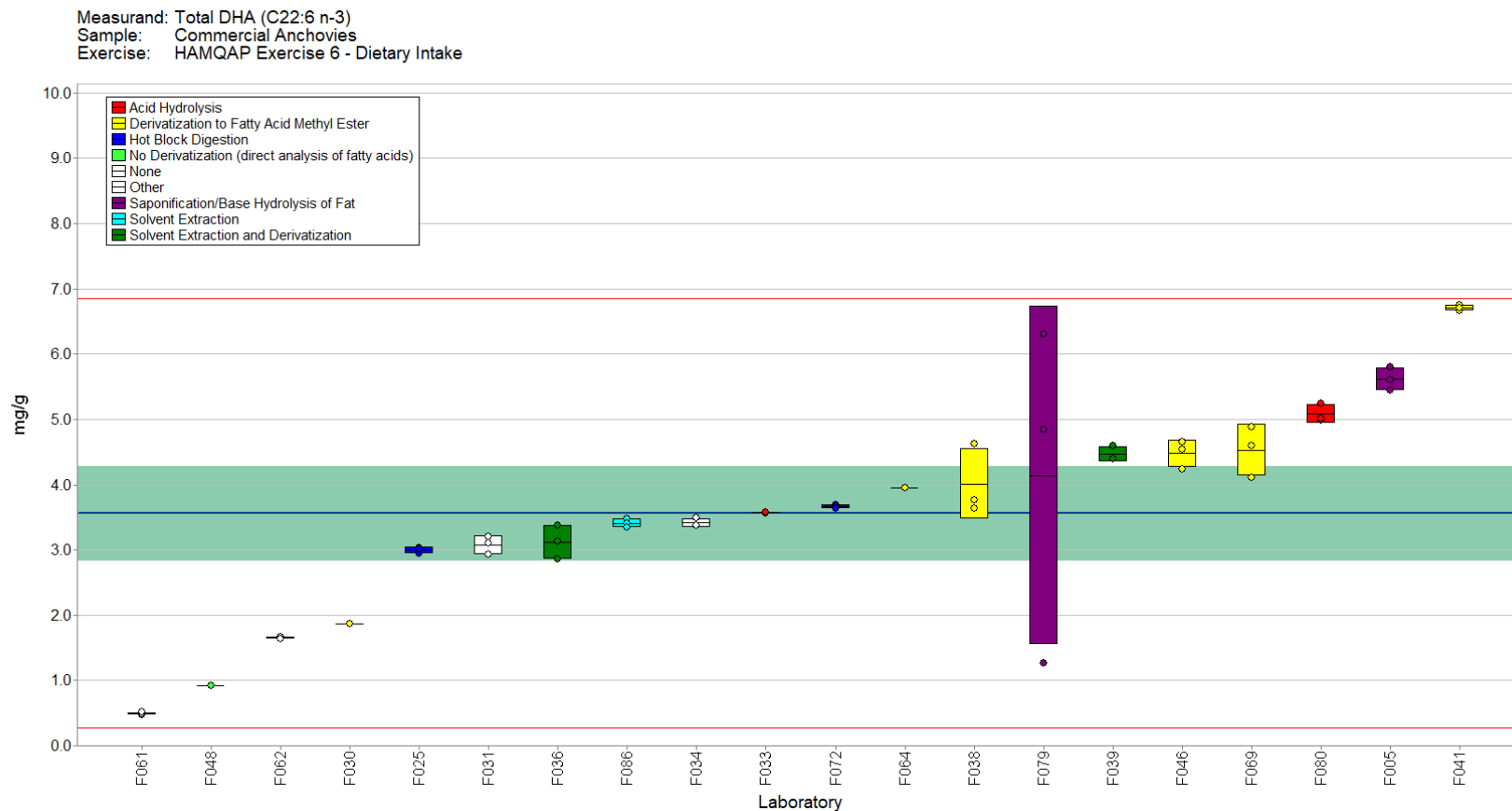


Figure 5-22. Total DHA in commercial anchovies (data summary view – sample preparation method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The red solid lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$. A NIST value has not been determined in this material.

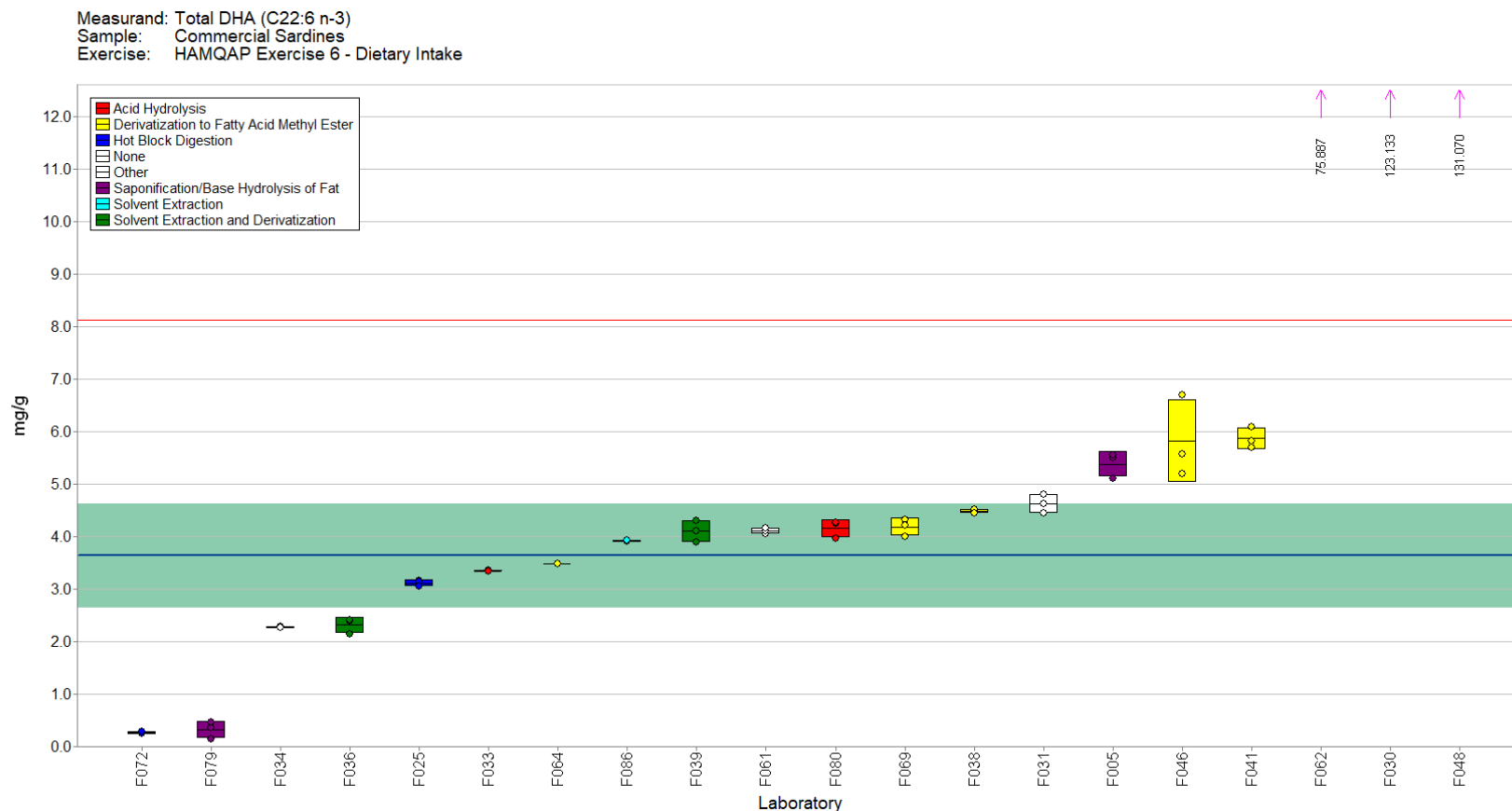


Figure 5-23. Total DHA in commercial sardines (data summary view – sample preparation method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The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

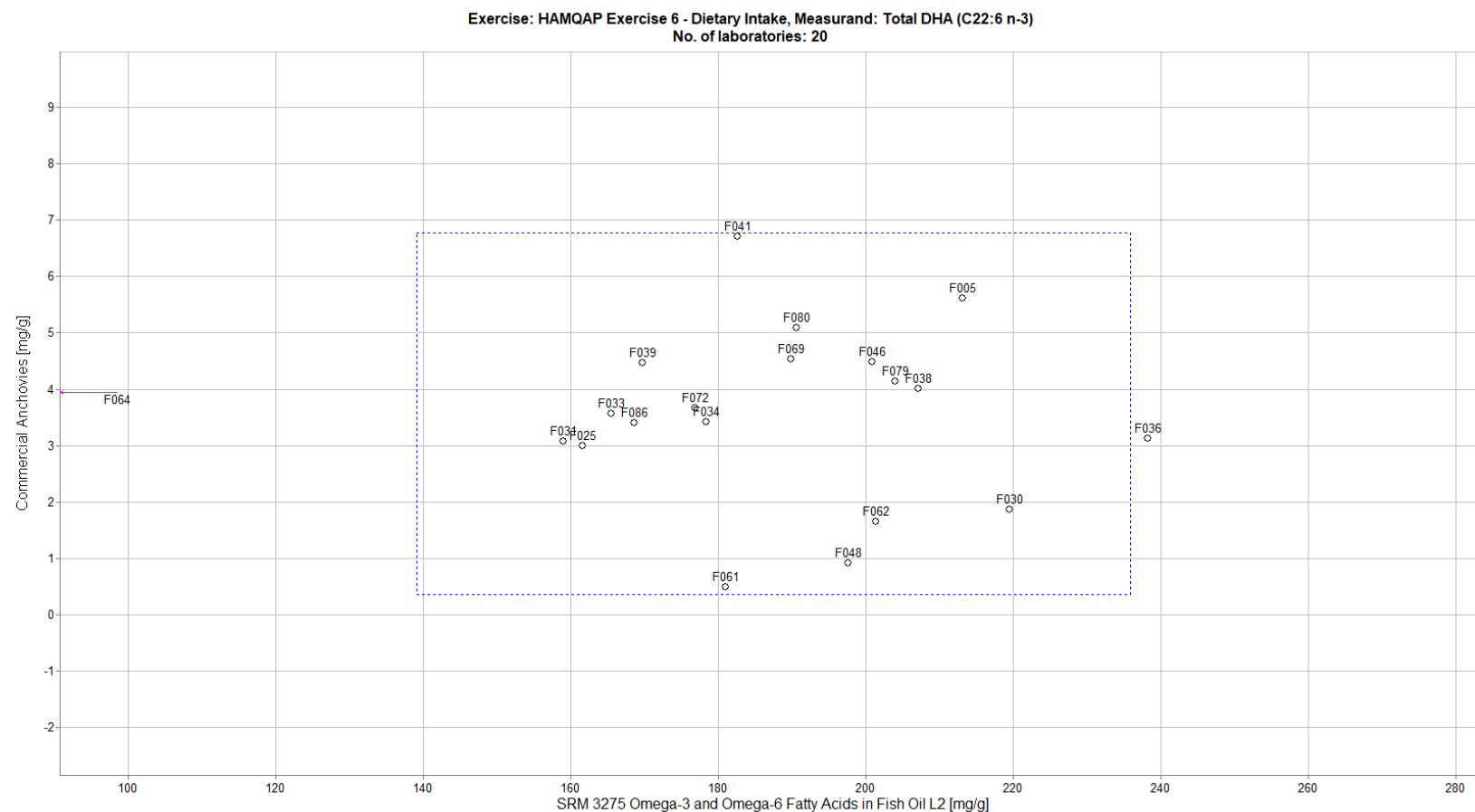


Figure 5-24. Laboratory means for total DHA in SRM 3275 Omega-3 and Omega-6 Fatty Acids in Fish Oil Level 2 and commercial anchovies (sample/sample comparison view). In this view, the individual laboratory mean for one sample (SRM 3275 Level 2) is compared to the individual laboratory mean for a second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

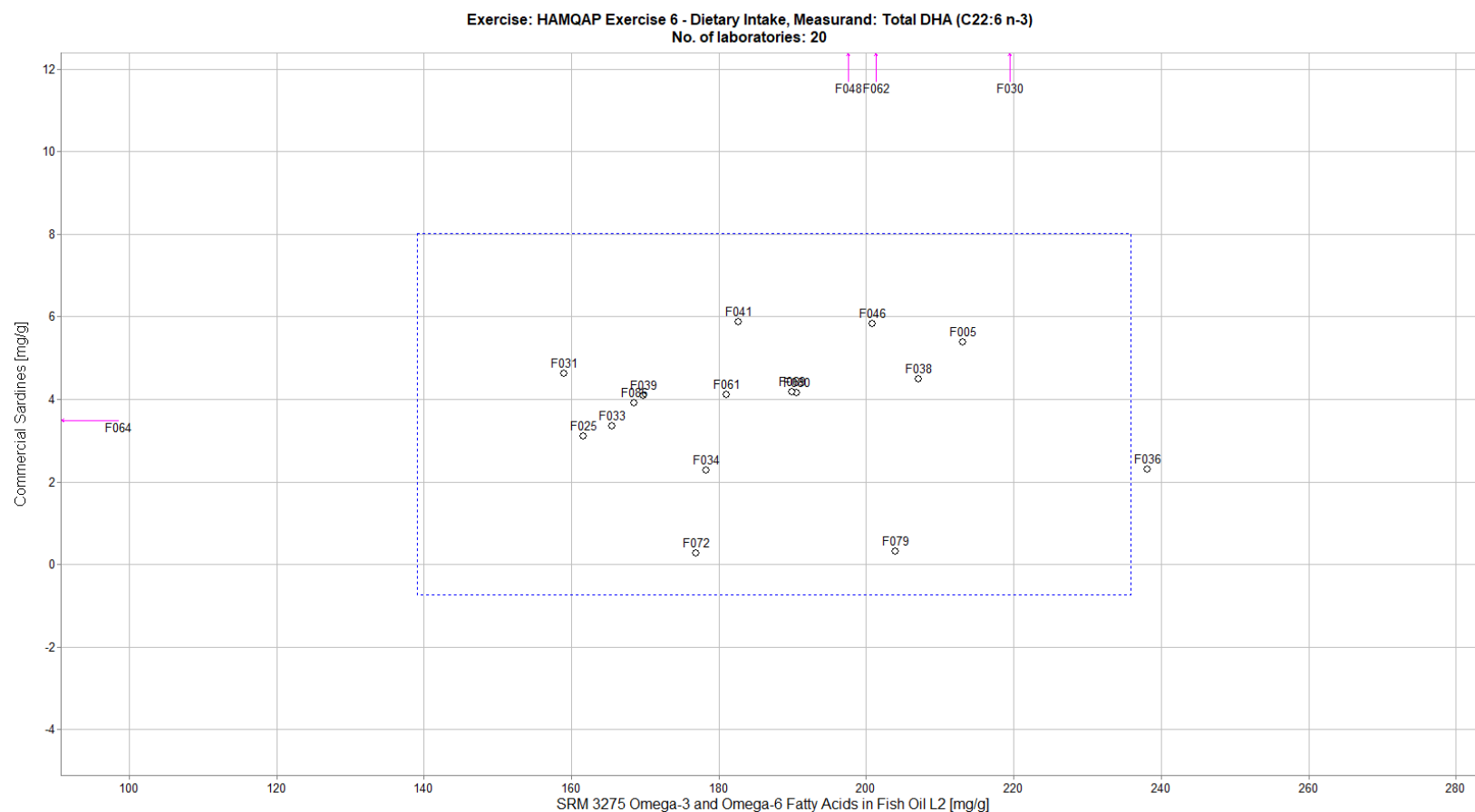


Figure 5-25. Laboratory means for total DHA in SRM 3275 Omega-3 and Omega-6 Fatty Acids in Fish Oil Level 2 and commercial sardines (sample/sample comparison view). In this view, the individual laboratory mean for one sample (SRM 3275 Level 2) is compared to the individual laboratory mean for a second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

Human Metabolites Sample Information

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

NIST-Determined Weight Percent (based on Total Fatty Acids) in Human RBC (%)

<u>Analyte</u>	<u>RBC A</u>			<u>RBC B</u>		
EPA	0.46	$\pm$	0.02	0.36	$\pm$	0.02
DHA	2.65	$\pm$	0.08	3.27	$\pm$	0.12

Human Metabolites Study Results

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

Between-Laboratory Variability for laboratories reporting in units of $\mu\text{g/mL}$ (% RSD)

<u>Analyte</u>	<u>RBC A</u>	<u>RBC B</u>
EPA	10 %	70 %
DHA	34 %	42 %

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

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

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

Human Metabolites Technical Recommendations

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

- Sufficient data points were not available to identify trends with respect to the sample preparation or analytical methods reported by the participants. However, the laboratory that tended to report higher values compared to other laboratories reported using base hydrolysis as the sample preparation method (See **Figures 5-26** through **Figure 5-29** and **Figures 5-31** through **Figures 5-34**, laboratory data points beyond chart limits). Sample preparation methods should be checked for method biases.
- Overall, the results of all of the participants agreed well for results reported as individual fatty acids in units of $\mu\text{g/mL}$ and reported as weight percent of total fatty acids with the exception of one or two laboratories reporting higher results compared to the other laboratories. These higher results could be due to a bias in calibration or a bias in the method.
- The use of appropriate calibration materials and quality assurance samples to establish that a method is in control and performing correctly may reduce the likelihood of outlying data. Quality assurance samples can be commercially available reference materials (CRMs, SRMs, or RMs) or prepared in-house.
- A linear calibration curve which surrounds the expected sample concentration values should be used for calculations. This curve should include both the lowest and highest expected concentration values of the sample solutions. Extrapolation of results beyond calibration curves may result in incorrect values.
- In general, all results should be checked closely to avoid calculation errors and to be sure that results are reported in the requested units and in the requested form.

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

HAMQAP Exercise 6 - Fatty Acids										
Lab Code:		NIST	1. Your Results				2. Community Results			3. Target
Analyte	Sample	Units	x_i	s_i	Z'_{comm}	Z_{NIST}	N	x^*	s^*	x_{NIST} U
Total EPA (C20:5 n-3)	Red Blood Cells B	ug/mL					4	10	6	
Total EPA (C20:5 n-3)	Red Blood Cells A	ug/mL					4	5.89	0.61	
Total EPA (C20:5 n-3) Weight % from total FAs	Red Blood Cells B	%	0.36	0.02			7	0.41	0.095	0.36 0.02
Total EPA (C20:5 n-3) Weight % from total FAs	Red Blood Cells A	%	0.46	0.02			7	0.481	0.085	0.46 0.02
Total DHA (C22:6 n-3)	Red Blood Cells A	ug/mL					4	30	12	
Total DHA (C22:6 n-3)	Red Blood Cells B	ug/mL					4	40	19	
Total DHA (C22:6 n-3) Weight % from total FAs	Red Blood Cells B	%	3.27	0.12			7	3.4	0.46	3.27 0.12
Total DHA (C22:6 n-3) Weight % from total FAs	Red Blood Cells A	%	2.65	0.08			7	2.8	0.2	2.65 0.08
x_i Mean of reported values							N	Number of quantitative values reported		x_{NIST} NIST-assessed value
s_i Standard deviation of reported values										U expanded uncertainty
Z'_{comm} Z'-score with respect to community consensus							x^*	Robust mean of reported values		about the NIST-assessed value
Z_{NIST} Z-score with respect to NIST value							s^*	Robust standard deviation		

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

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

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

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

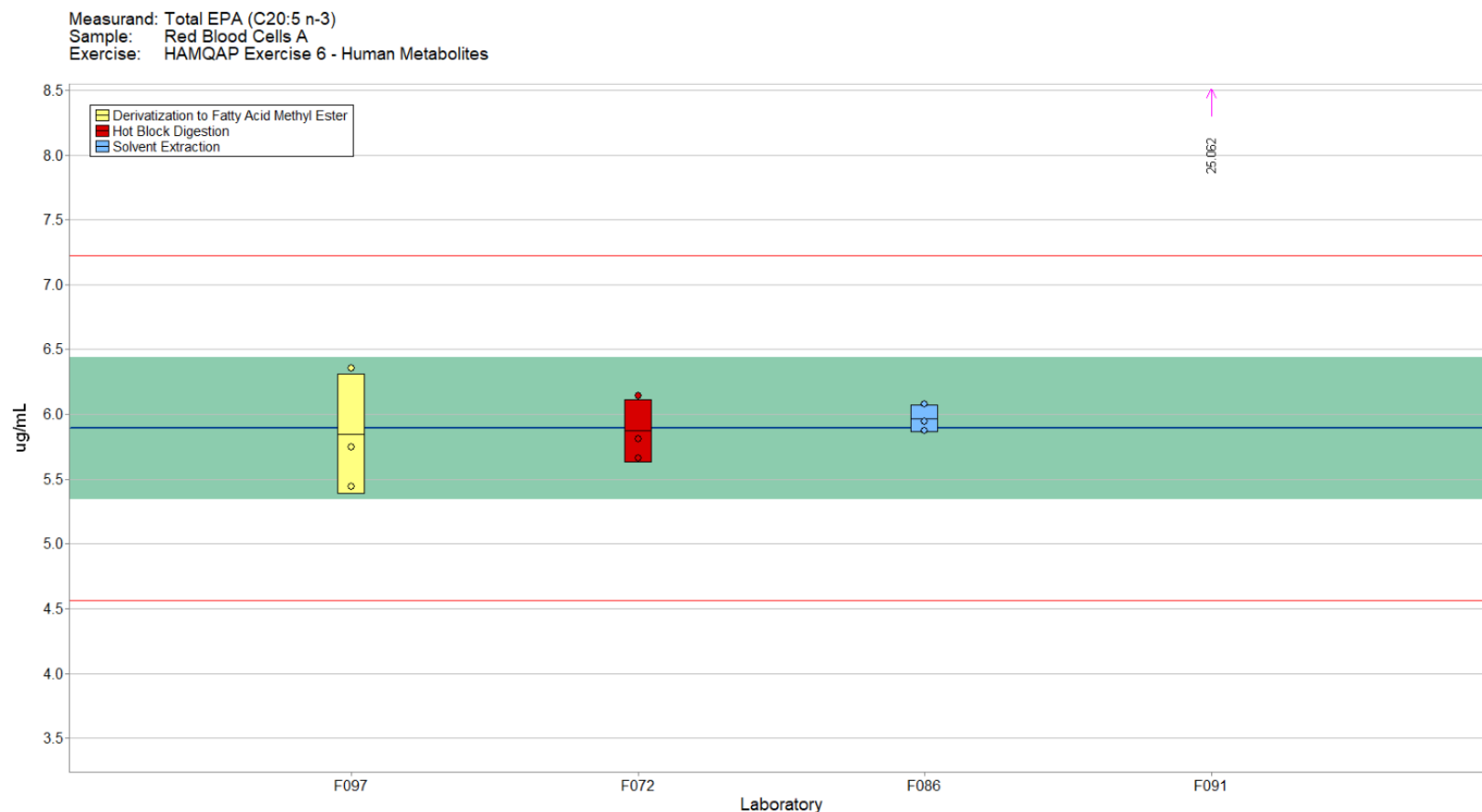


Figure 5-26. Total EPA in Human Red Blood Cells A reported in units of $\mu\text{g/mL}$ (data summary view – sample preparation method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The red solid lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$. A NIST value reported in units of $\mu\text{g/mL}$ has not been established for this material.

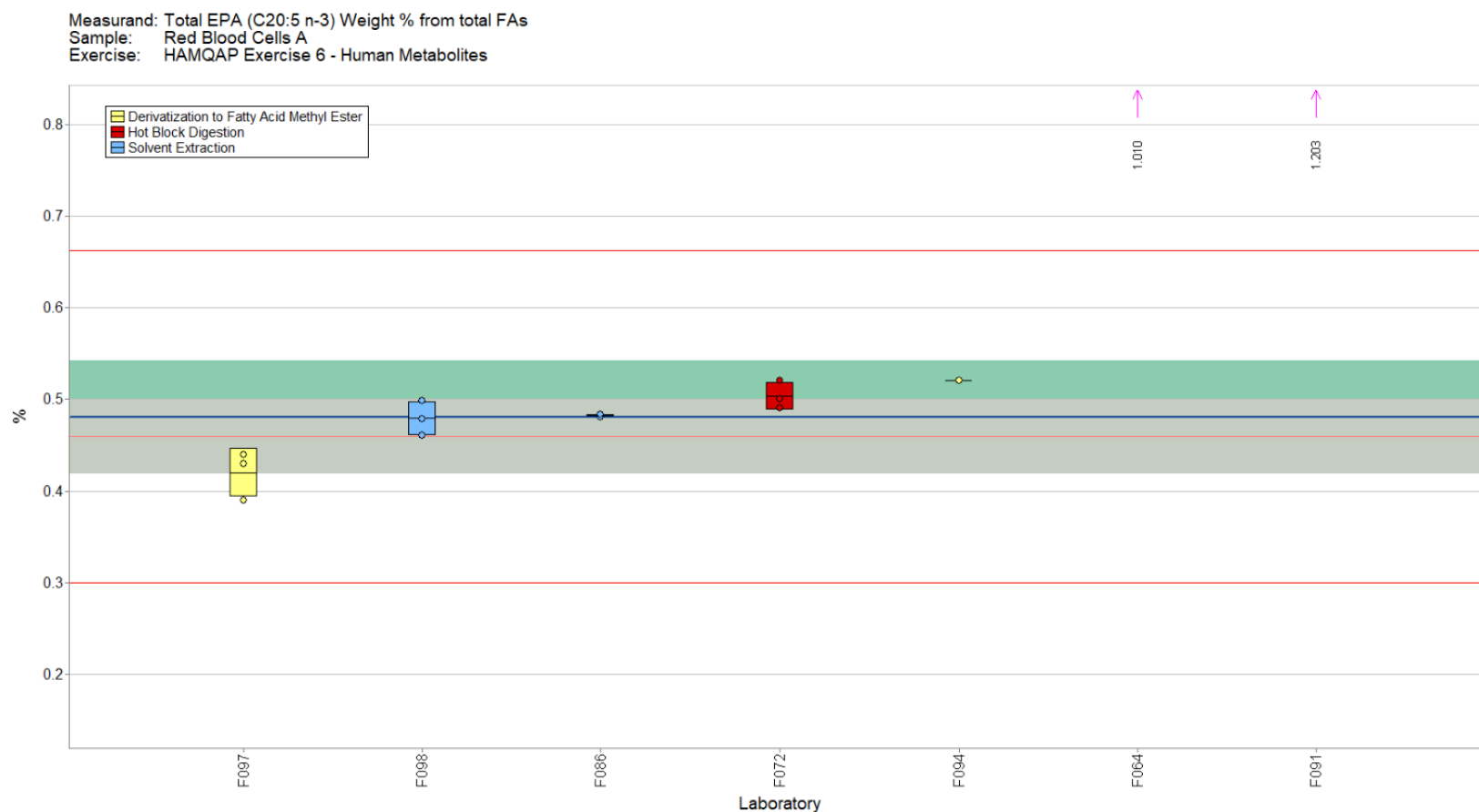


Figure 5-27. Total EPA in Human Red Blood Cells A reported as weight % from total fatty acids (data summary view – sample preparation method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The red solid lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by its uncertainty (U_{NIST}) and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$.

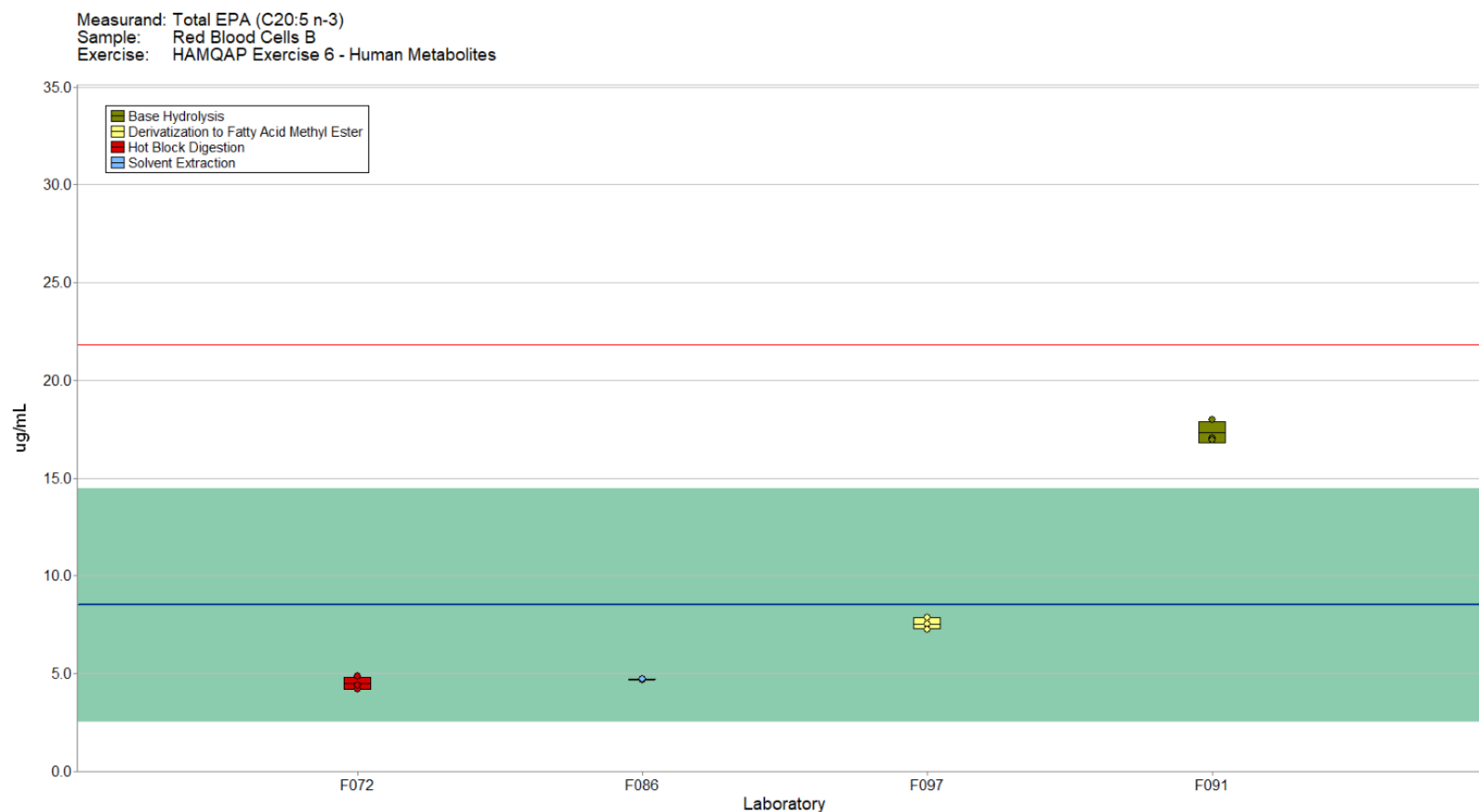


Figure 5-28. Total EPA in Human Red Blood Cells B reported in units of $\mu\text{g/mL}$ (data summary view – sample preparation method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents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

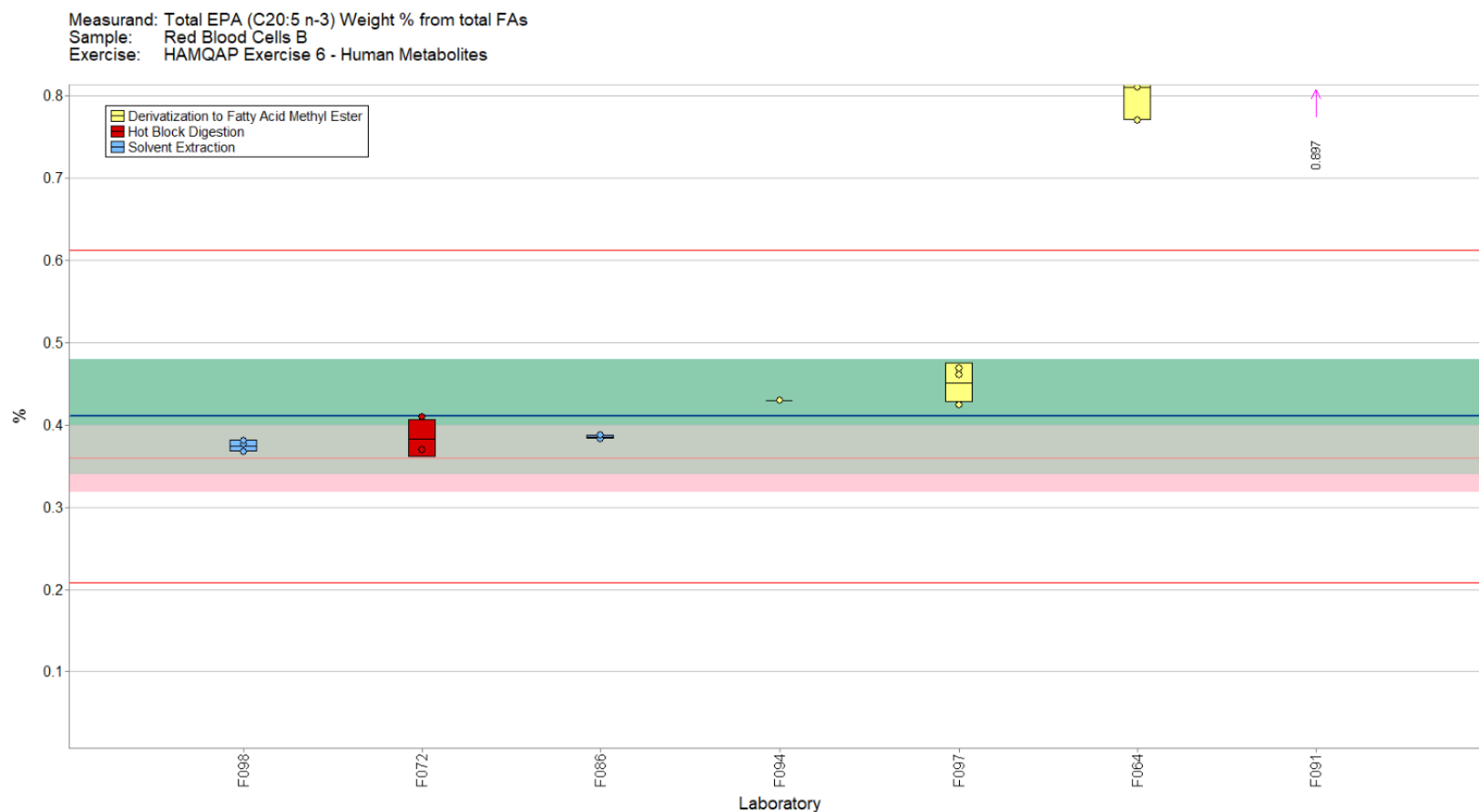


Figure 5-29. Total EPA in Human Red Blood Cells B reported as weight % from total fatty acids (data summary view – sample preparation method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The red solid lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{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, $|Z_{\text{NIST}}| \leq 2$.

Exercise: HAMQAP Exercise 6 - Human Metabolites, Measurand: Total EPA (C20:5 n-3) Weight % from total FAs
No. of laboratories: 7

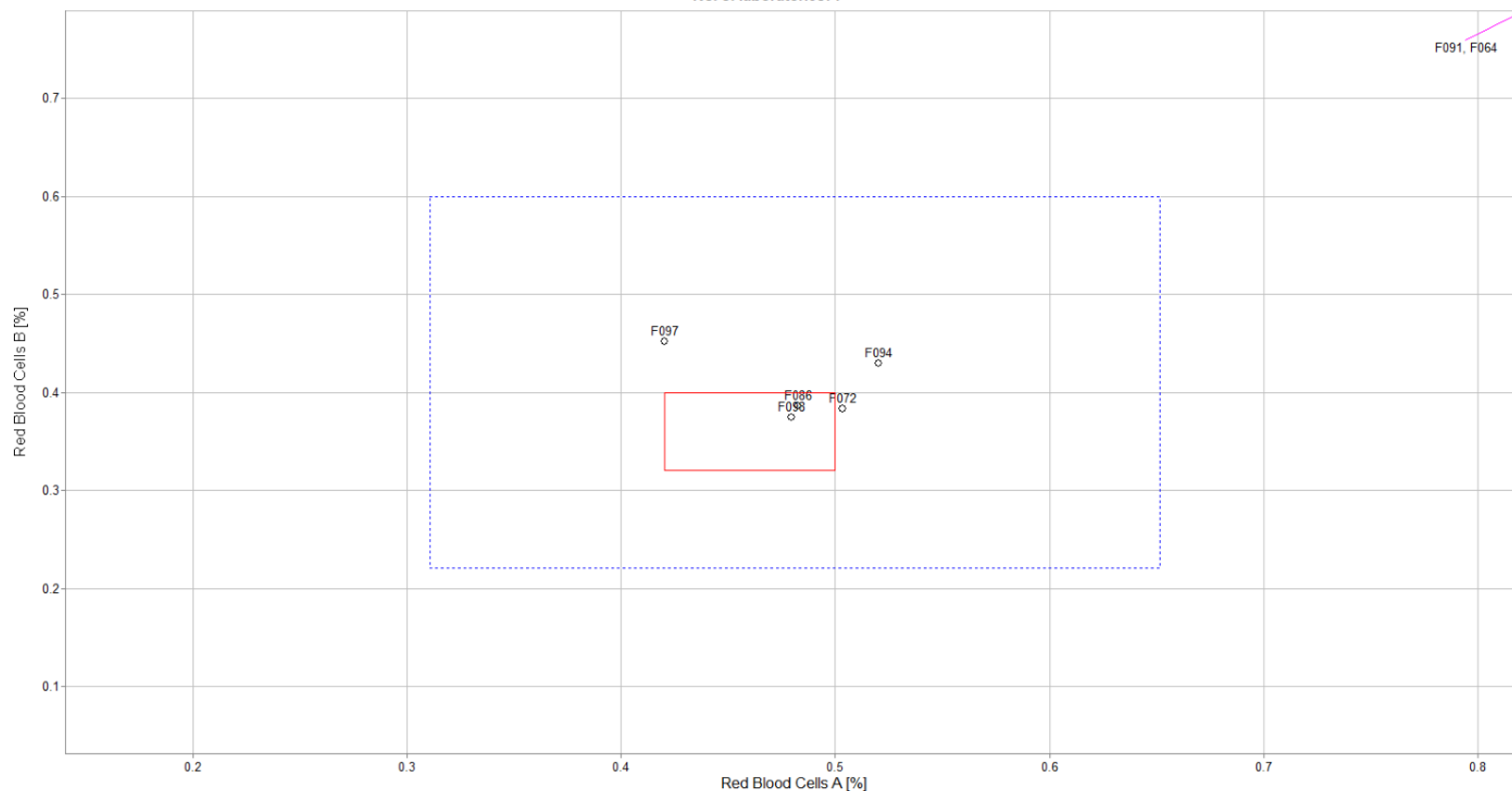


Figure 5-30. Laboratory means for total EPA in Human Red Blood Cells A and Human Red Blood Cells B (sample/sample comparison view) reported as weight % from total fatty acids. In this view, the individual laboratory mean for one sample (RBC A) is compared to the individual laboratory mean for a second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

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

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

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

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

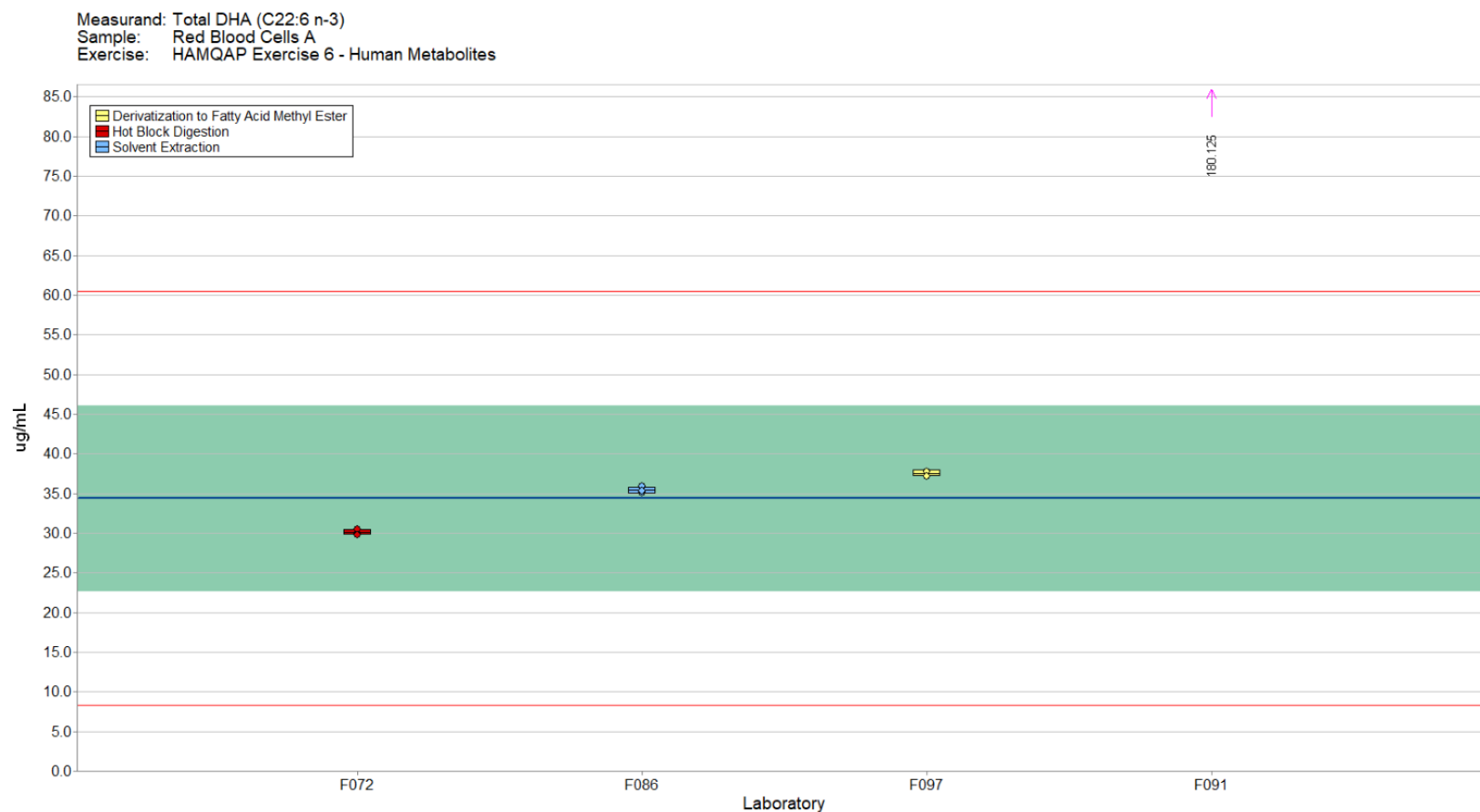


Figure 5-31. Total DHA in Human Red Blood Cells A reported in units of $\mu\text{g/mL}$ (data summary view – sample preparation method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red line represents the upper consensus range of tolerance, calculated as the values above the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$. A NIST value reported in units of $\mu\text{g/mL}$ has not been established for this material.

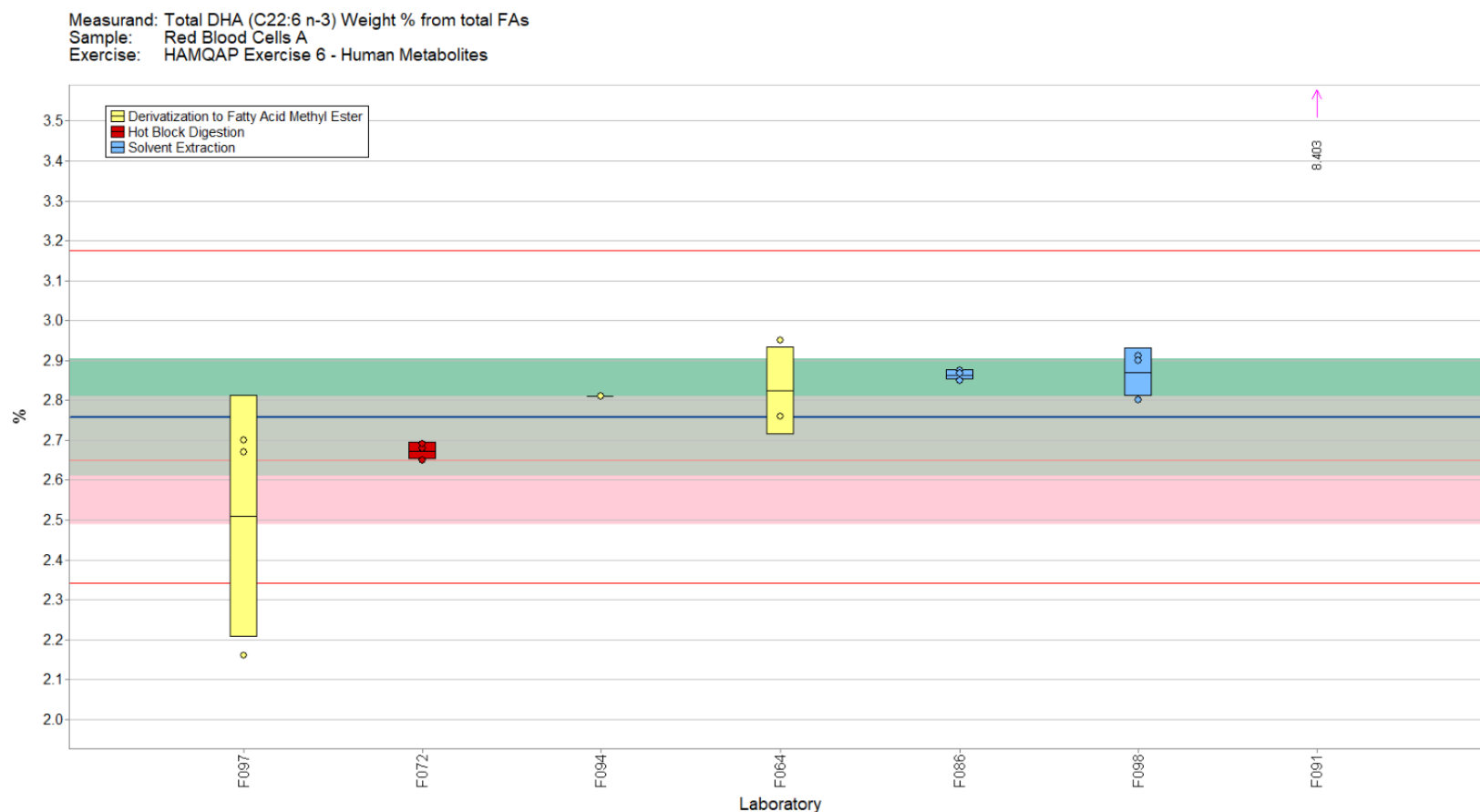


Figure 5-32. Total DHA in Human Red Blood Cells A reported as weight % from total fatty acids (data summary view – sample preparation method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The red solid lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by its uncertainty (U_{NIST}) and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$.

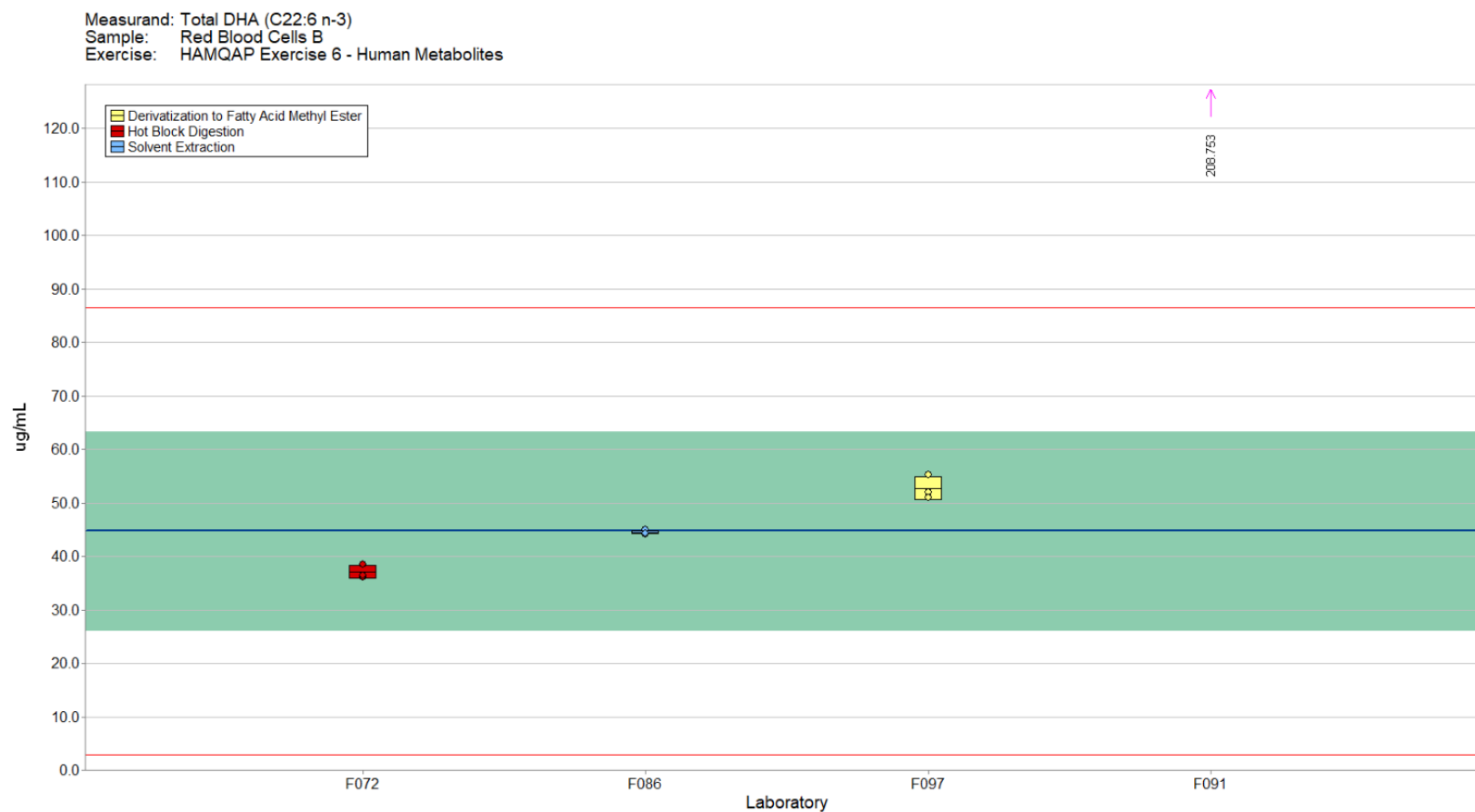


Figure 5-33. Total DHA in Human Red Blood Cells B reported in units of $\mu\text{g/mL}$ (data summary view – sample preparation method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red line represents the upper consensus range of tolerance, calculated as the values above the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$. A NIST value reported in units of $\mu\text{g/mL}$ has not been established for this material.

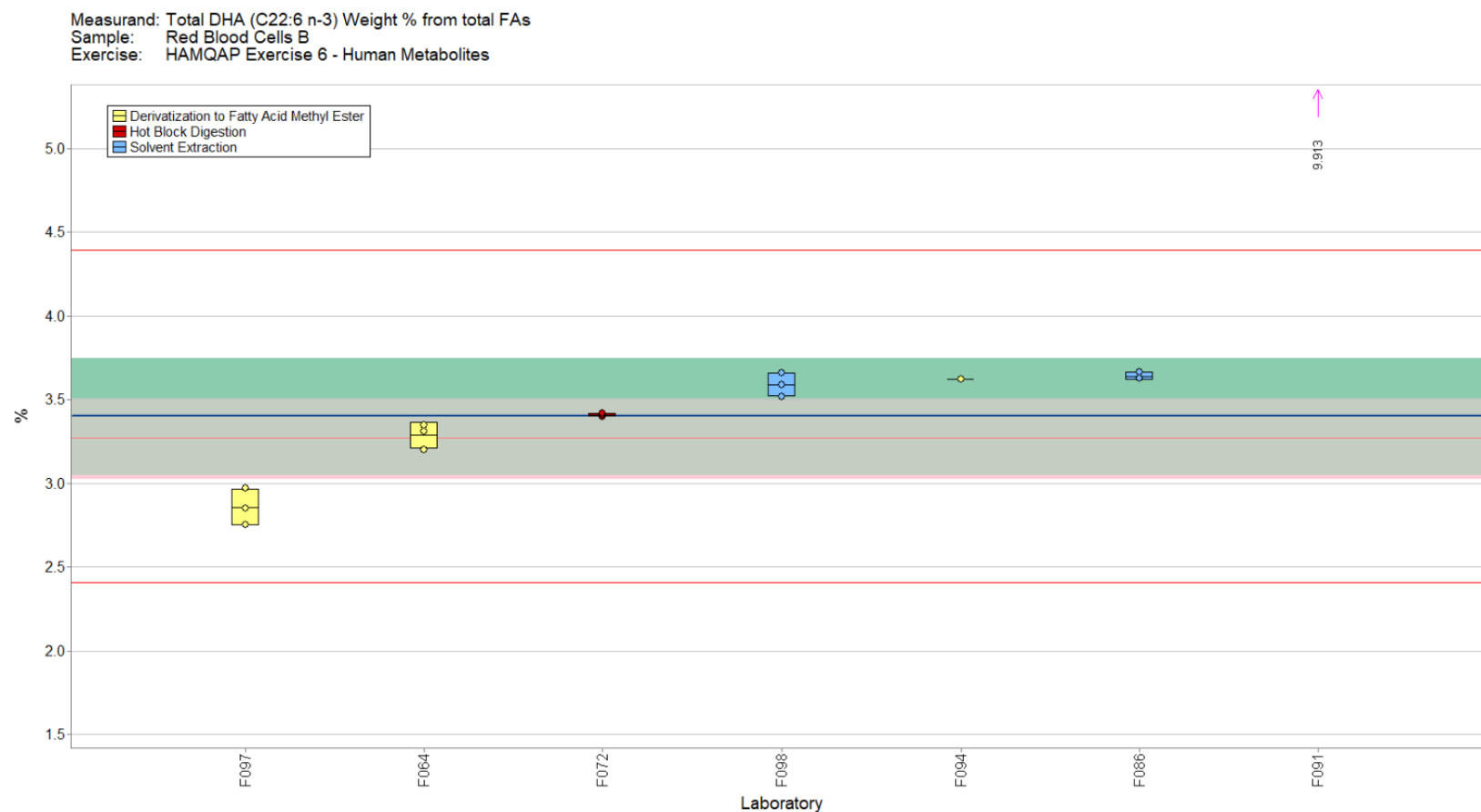


Figure 5-34. Total DHA in Human Red Blood Cells B reported as weight % from total fatty acids (data summary view – sample preparation method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The red solid lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{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, $|Z_{\text{NIST}}| \leq 2$.

Exercise: HAMQAP Exercise 6 - Human Metabolites, Measurand: Total DHA (C22:6 n-3) Weight % from total FAs
No. of laboratories: 7

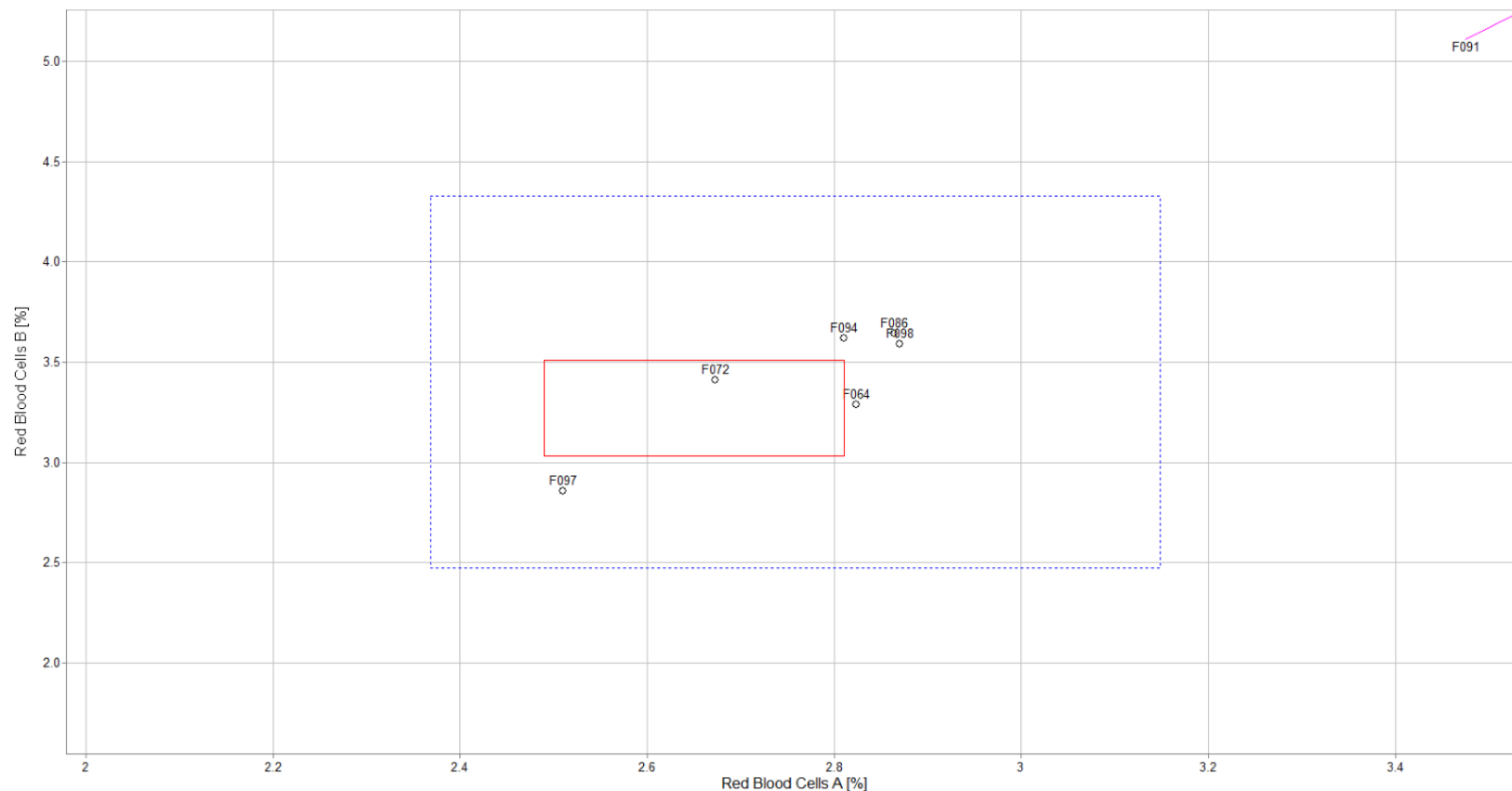


Figure 5-35. Laboratory means for total DHA in Human Red Blood Cells A and Human Red Blood Cells B reported as weight % from total fatty acids (sample/sample comparison view). In this view, the individual laboratory mean for one sample (RBC A) is compared to the individual laboratory mean for a second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

Fatty Acids Overall Study Comparison

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

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

SECTION 6: BOTANICALS (Anthocyanidins)

Study Overview

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

Dietary Intake Sample Information

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

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

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

accounting for moisture of the material (2.4 %). NIST values were not assigned for malvidin and petunidin.

NIST-Determined Mass Fraction in SRM 3281 (mg/kg)

<u>Analyte</u>	<u>(dry-mass basis)</u>	<u>(as-received basis)</u>
Cyanidin	119 ± 31	116 ± 30
Delphinidin	5.18 ± 0.68	5.06 ± 0.66
Peonidin	121 ± 22	118 ± 21

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

NIST-Determined Mass Fraction in SRM 3291 (mg/kg)

<u>Analyte</u>	<u>(as-received basis)</u>
Cyanidin	58.4 ± 5.8
Delphinidin	102.4 ± 37.6
Malvidin	42.8 ± 1.2
Petunidin	35.8 ± 0.8
Peonidin	29 ± 0.6

Dietary Intake Study Results

- Seventeen laboratories enrolled to measure the individual forms of the anthocyanidins, and 19 laboratories enrolled to measure the sum of all anthocyanidins. The enrollment and reporting statistics for the botanicals study is described in the table below. Some of the reported values were non-quantitative (zero or below LOQ) but are included in the participation and reporting statistics.

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

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

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

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

Dietary Intake Technical Recommendations

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

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

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

HAMQAP Exercise 6 - Botanicals											
Lab Code: NIST		1. Your Results					2. Community Results			3. Target	
Analyte	Sample	Units	x_i	s_i	Z'_{comm}	Z_{NIST}	N	x^*	s^*	x_{NIST}	U
Total Anthocyanidins (C3G)	SRM 3291 Bilberry Extract	mg/kg					10	282000	29000		
Total Anthocyanidins (C3G)	SRM 3287 Blueberry (Fruit)	mg/kg					9	1180	510		
Total Anthocyanidins (C3G)	SRM 3281 Cranberry (Fruit)	mg/kg					8	50	61		
Cyanidin	SRM 3291 Bilberry Extract	mg/kg	58.4	5.8			4	1730	740	58.4	5.8
Cyanidin	SRM 3287 Blueberry (Fruit)	mg/kg	290	23.7			3	3.9	7.2	290	23.7
Cyanidin	SRM 3281 Cranberry (Fruit)	mg/kg	116	30.3			3	3.5	2.9	116	30.3
Delphinidin	SRM 3291 Bilberry Extract	mg/kg	102	7.6			4	1680	1200	102	7.6
Delphinidin	SRM 3287 Blueberry (Fruit)	mg/kg	1160	227			3	10.8	5.8	1160	227
Delphinidin	SRM 3281 Cranberry (Fruit)	mg/kg	5.06	0.664			3	0	10	5.06	0.664
Malvidin	SRM 3291 Bilberry Extract	mg/kg	42.8	1.2			4	760	360	42.8	1.2
Malvidin	SRM 3287 Blueberry (Fruit)	mg/kg	1370	276			3	10	11	1370	276
Malvidin	SRM 3281 Cranberry (Fruit)	mg/kg					3	0	0		
Peonidin	SRM 3291 Bilberry Extract	mg/kg	29	0.6			4	360	77	29	0.6
Peonidin	SRM 3287 Blueberry (Fruit)	mg/kg	282	50.3			3	1.5	3.2	282	50.3
Peonidin	SRM 3281 Cranberry (Fruit)	mg/kg	118	21.5			3	0.6	1.8	118	21.5
Petunidin	SRM 3291 Bilberry Extract	mg/kg	35.8	0.8			4	880	140	35.8	0.8
Petunidin	SRM 3287 Blueberry (Fruit)	mg/kg	641	118			3	4.5	3.7	641	118
Petunidin	SRM 3281 Cranberry (Fruit)	mg/kg					3	0.18	0.34		
			x_i	Mean of reported values			N	Number of quantitative values reported		x_{NIST}	NIST-assessed value
			s_i	Standard deviation of reported values						U	expanded uncertainty
			Z'_{comm}	Z'-score with respect to community consensus			x^*	Robust mean of reported values		about the NIST-assessed value	
			Z_{NIST}	Z-score with respect to NIST value			s^*	Robust standard deviation			

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

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

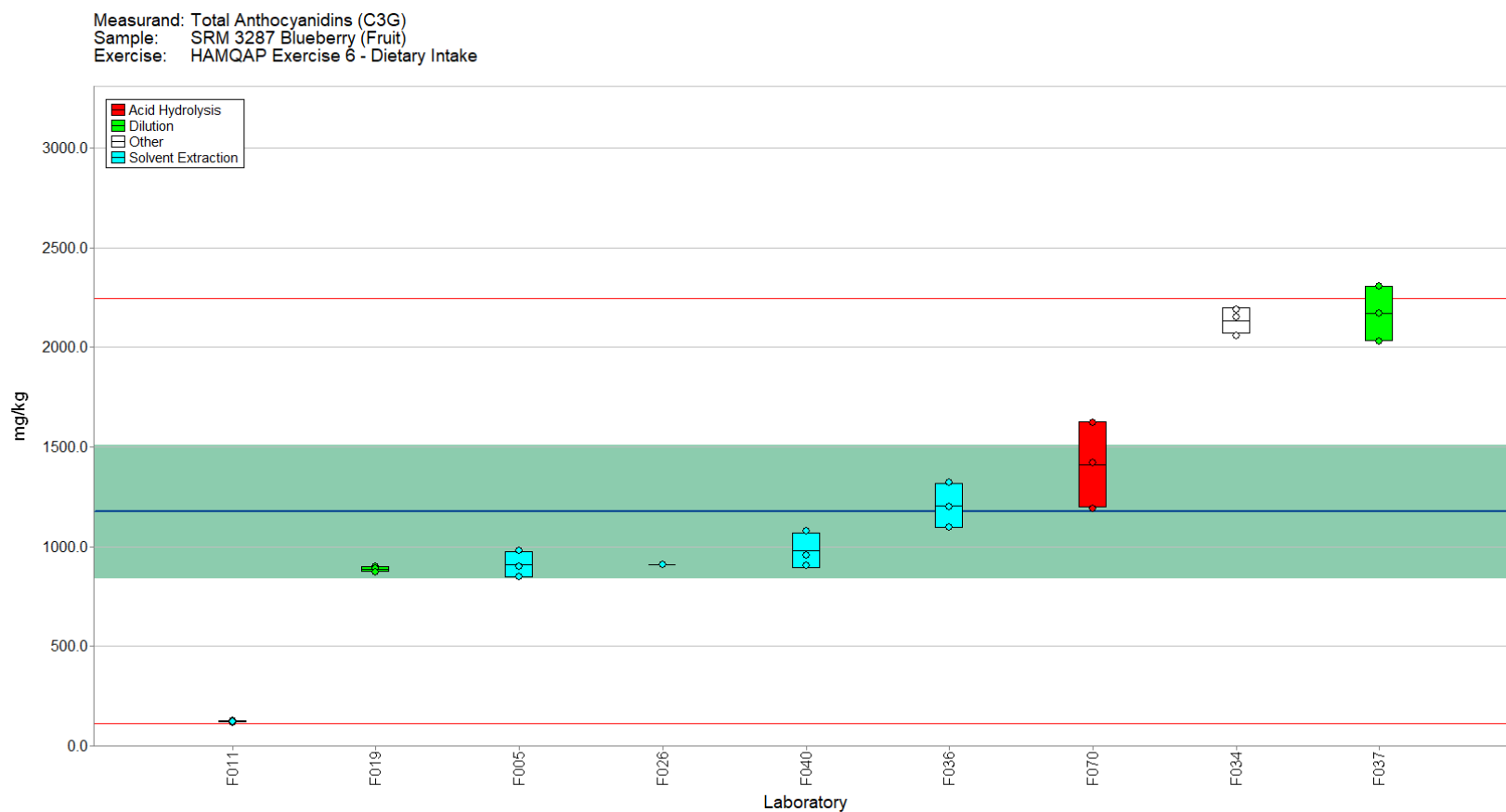


Figure 6-1. Total anthocyanidins (C3G) in SRM 3287 Blueberry (Fruit) (data summary view – sample preparation). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation employed. The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The red solid lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$. A NIST value has not been determined in this material.

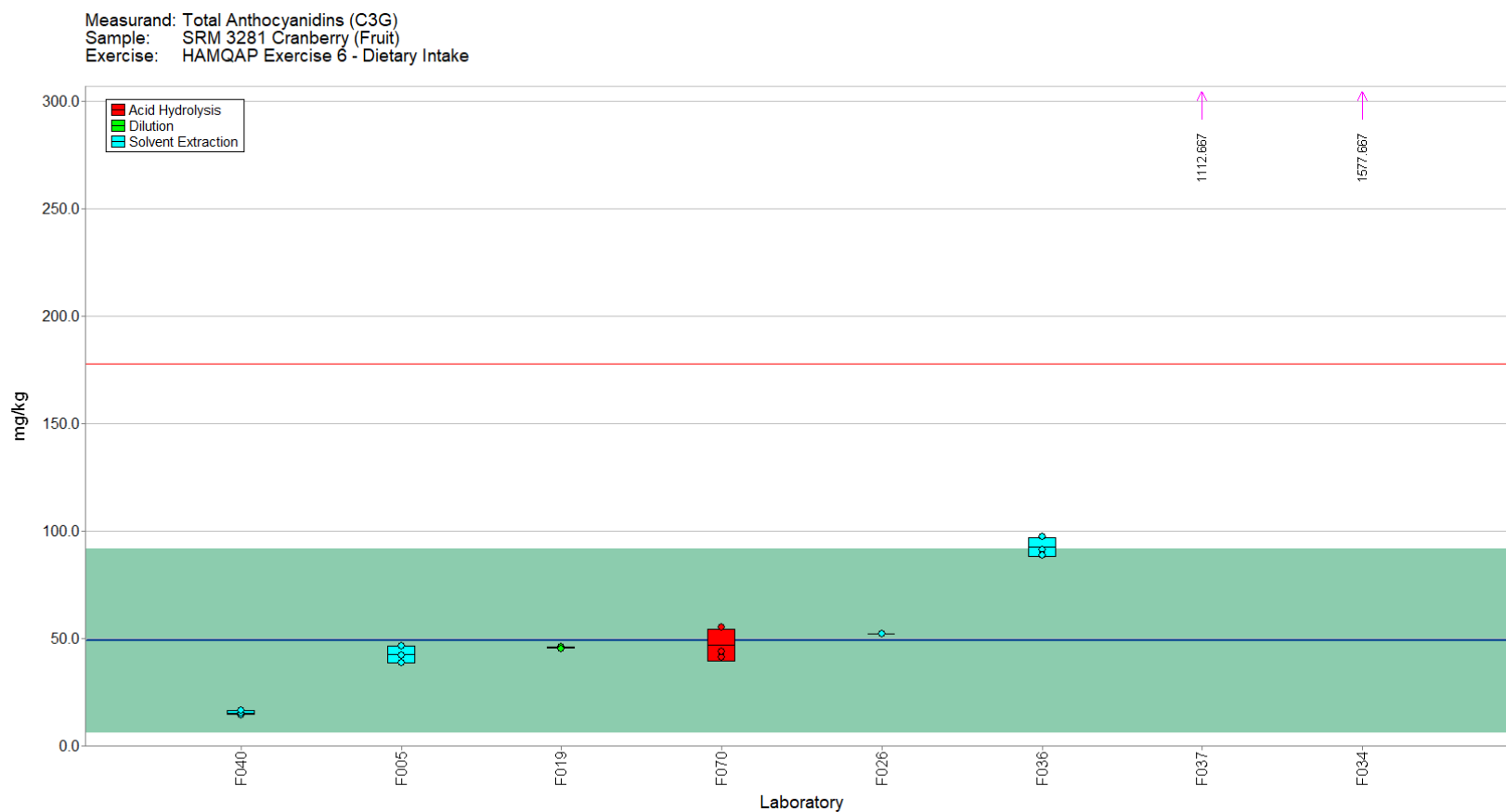


Figure 6-2. Total anthocyanidins (C3G) in SRM 3281 Cranberry (Fruit) (data summary view – sample preparation). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation employed.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

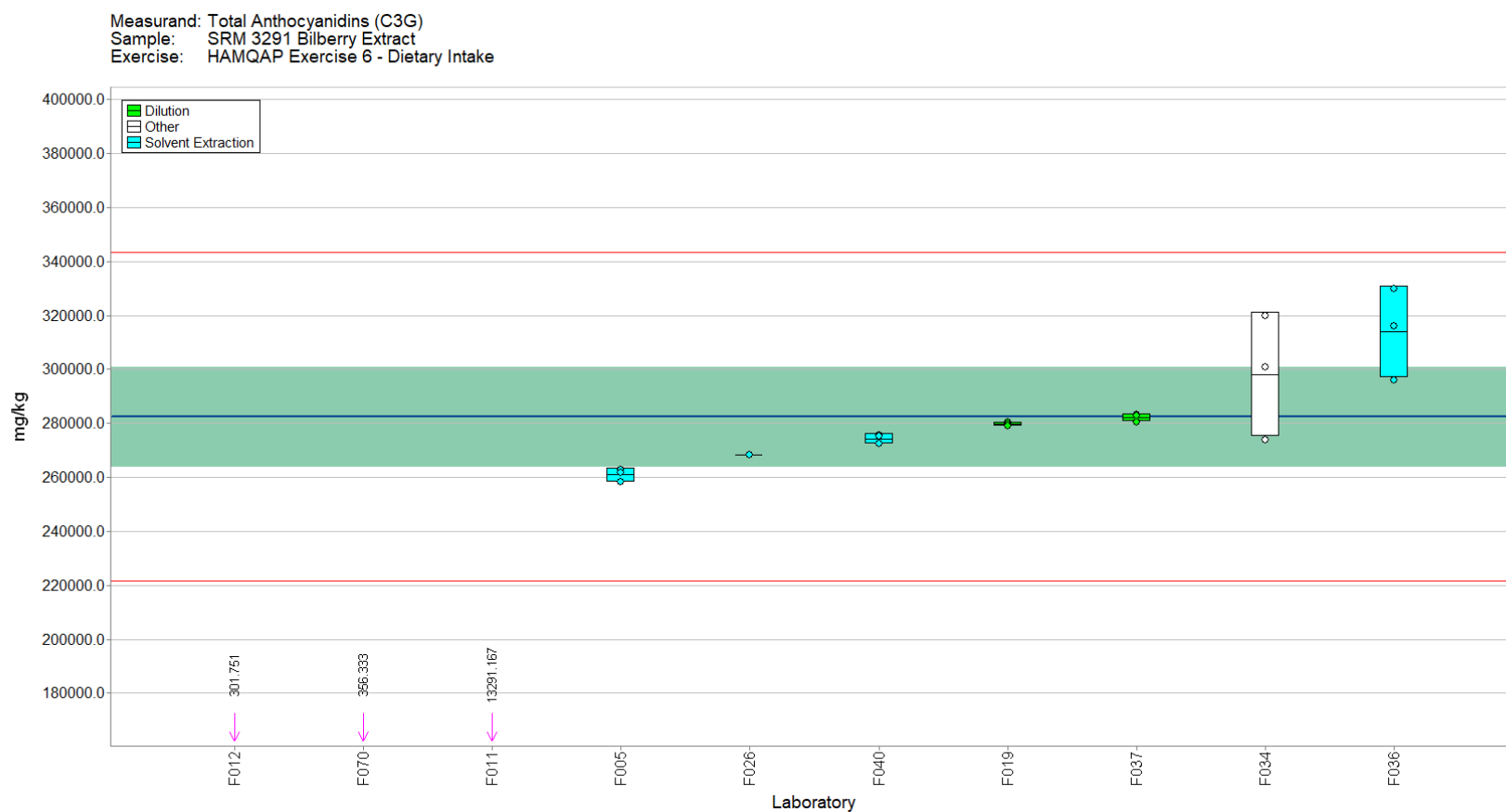


Figure 6-3. Total anthocyanidins (C3G) in SRM 3291 Bilberry Extract (data summary view – sample preparation). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation employed. The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The red solid lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$. A NIST value has not been determined in this material.

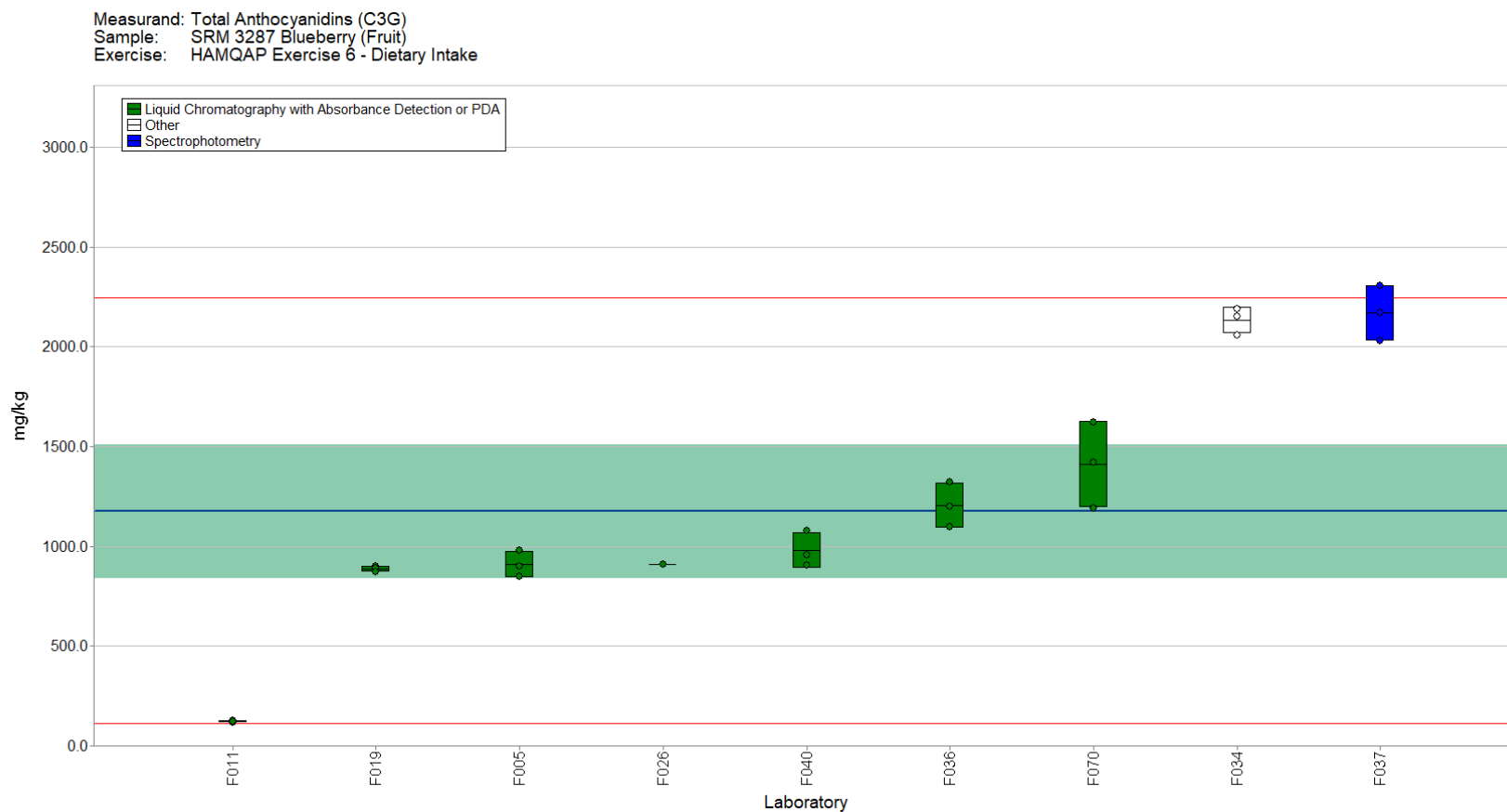


Figure 6-4. Total anthocyanidins (C3G) in SRM 3287 Blueberry (Fruit) (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The red solid lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$. A NIST value has not been determined in this material.

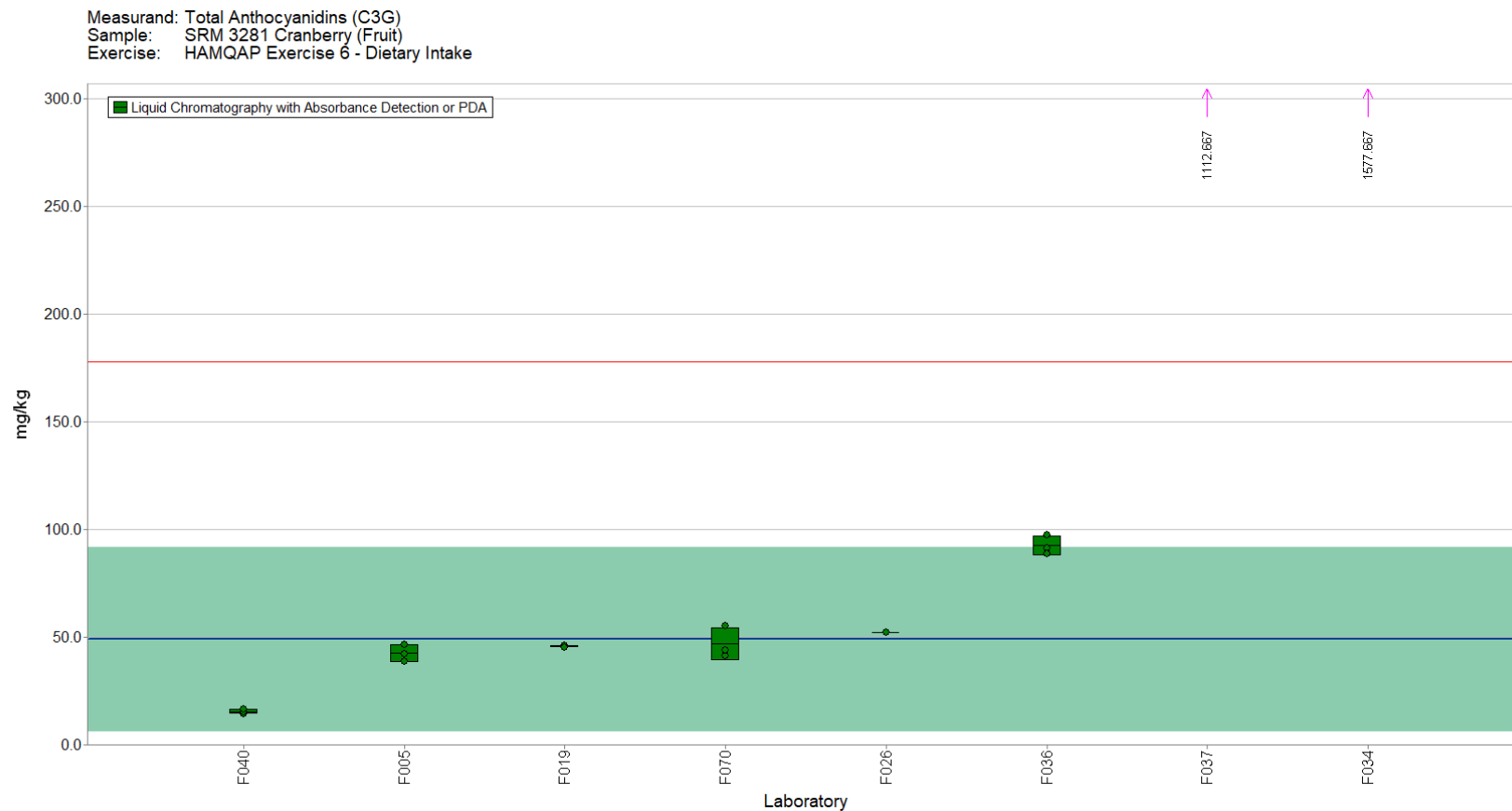


Figure 6-5. Total anthocyanidins (C3G) in SRM 3281 Cranberry (Fruit) (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed.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

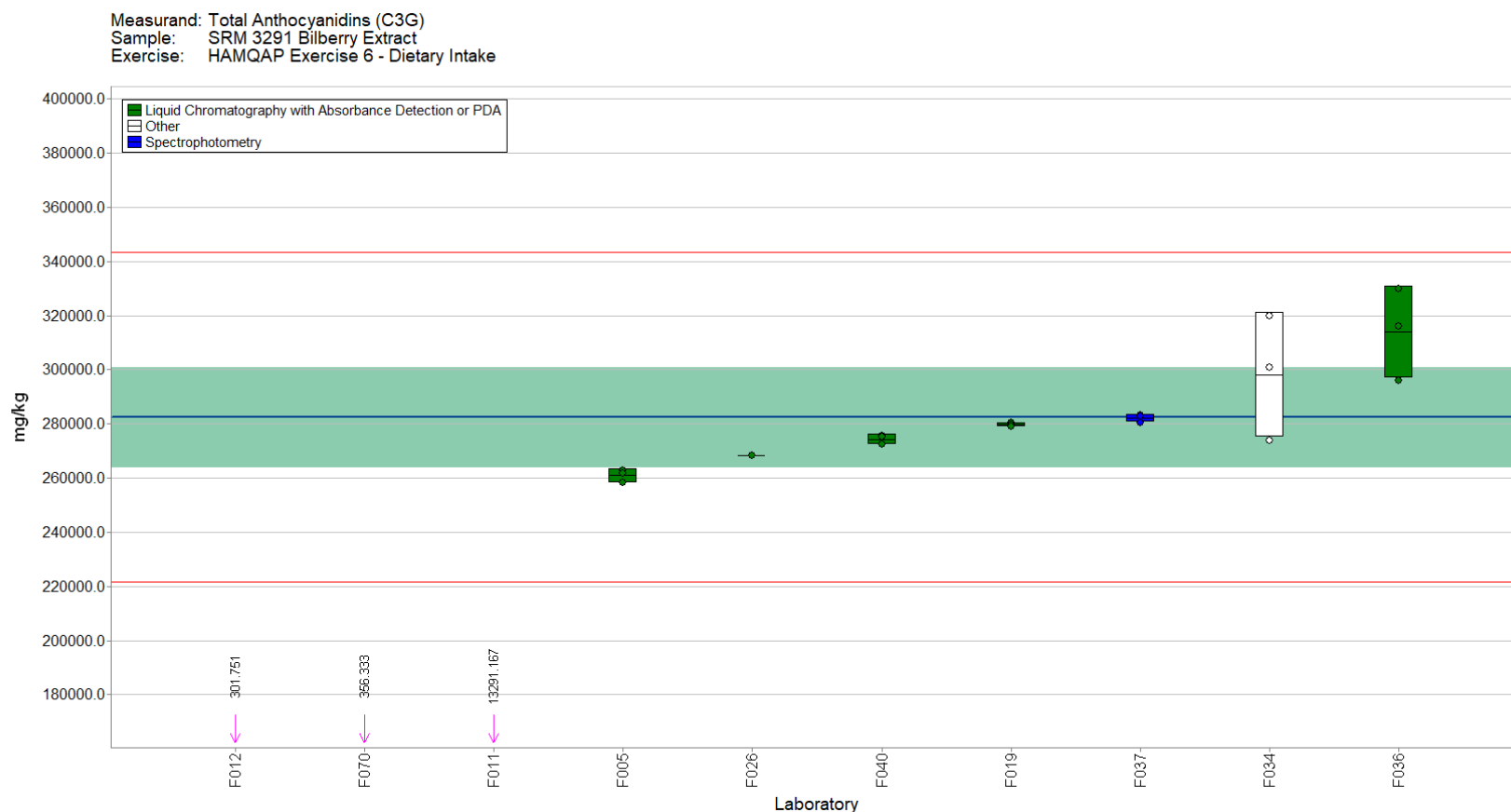


Figure 6-6. Total anthocyanidins (C3G) in SRM 3291 Bilberry Extract (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The red solid lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$. A NIST value has not been determined in this material.

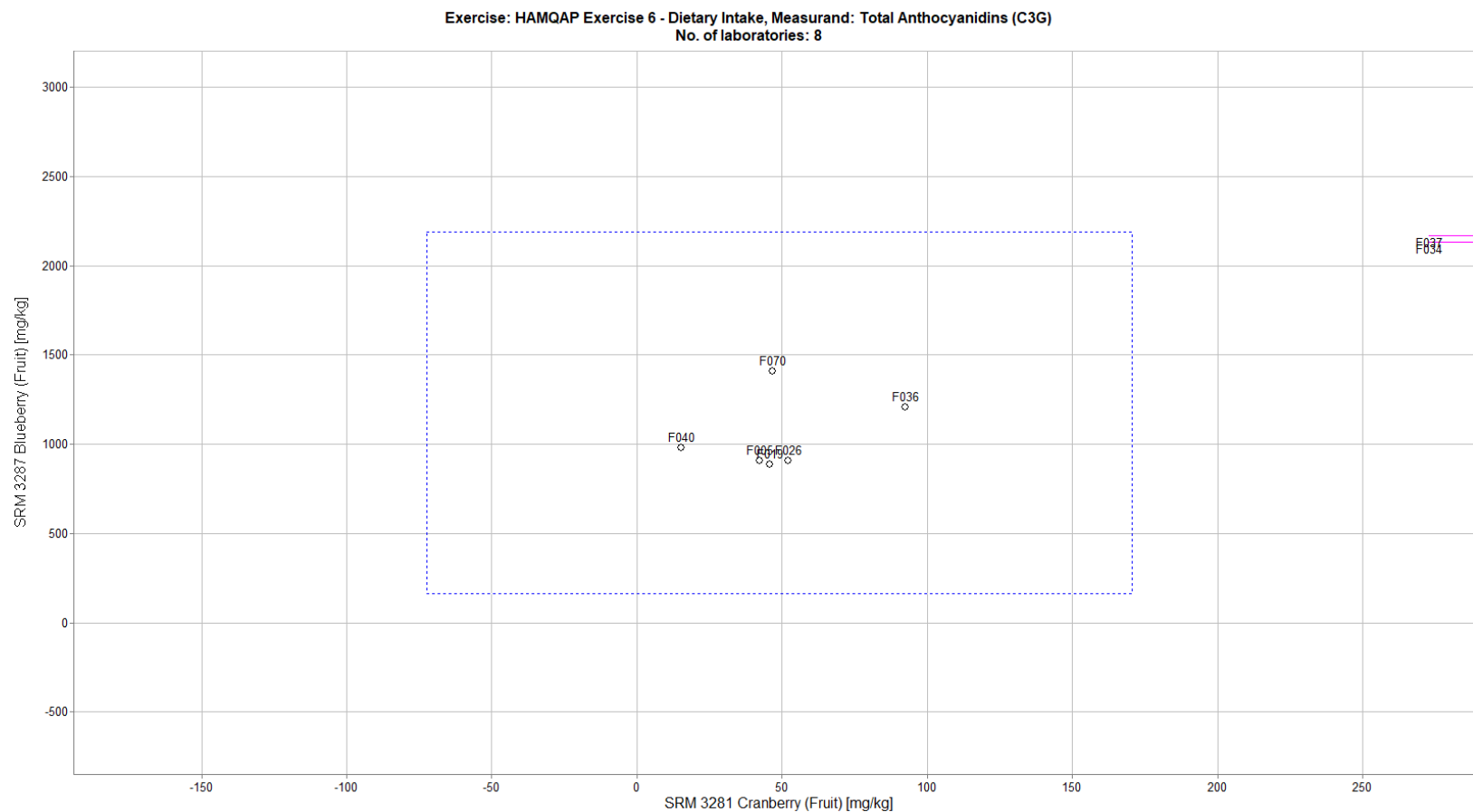


Figure 6-7. Laboratory means for total anthocyanidins (C3G) in SRM 3287 Blueberry (Fruit) and SRM 3281 Cranberry (Fruit) (sample/sample comparison view). In this view, the individual laboratory mean for one sample (SRM 3281) is compared to the individual laboratory mean for a second sample (SRM 3287).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

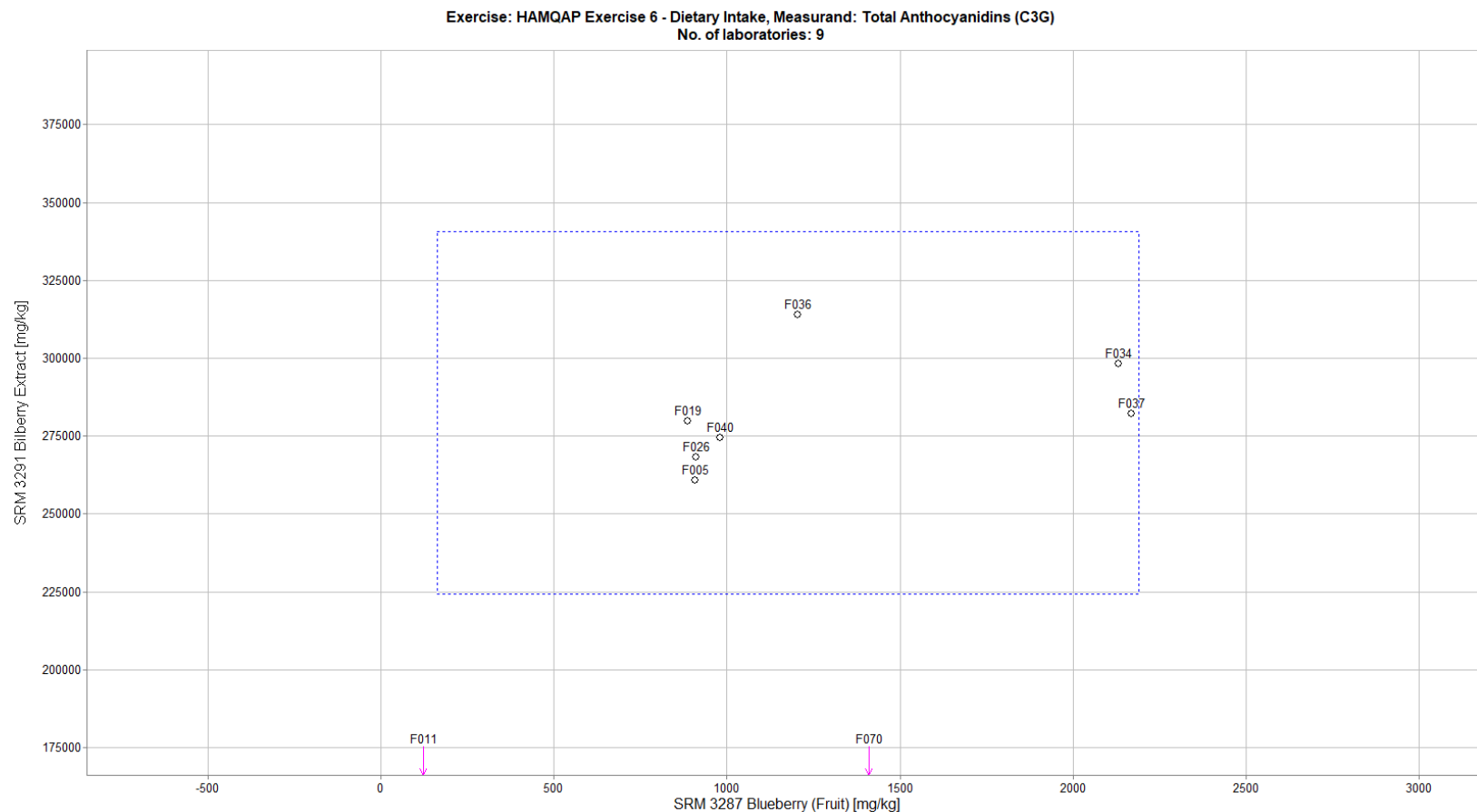


Figure 6-8. Laboratory means for total anthocyanidins (C3G) in SRM 3287 Blueberry (Fruit) and SRM 3291 Bilberry Extract (sample/sample comparison view). In this view, the individual laboratory mean for one sample (SRM 3287) is compared to the individual laboratory mean for a second sample (SRM 3291).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

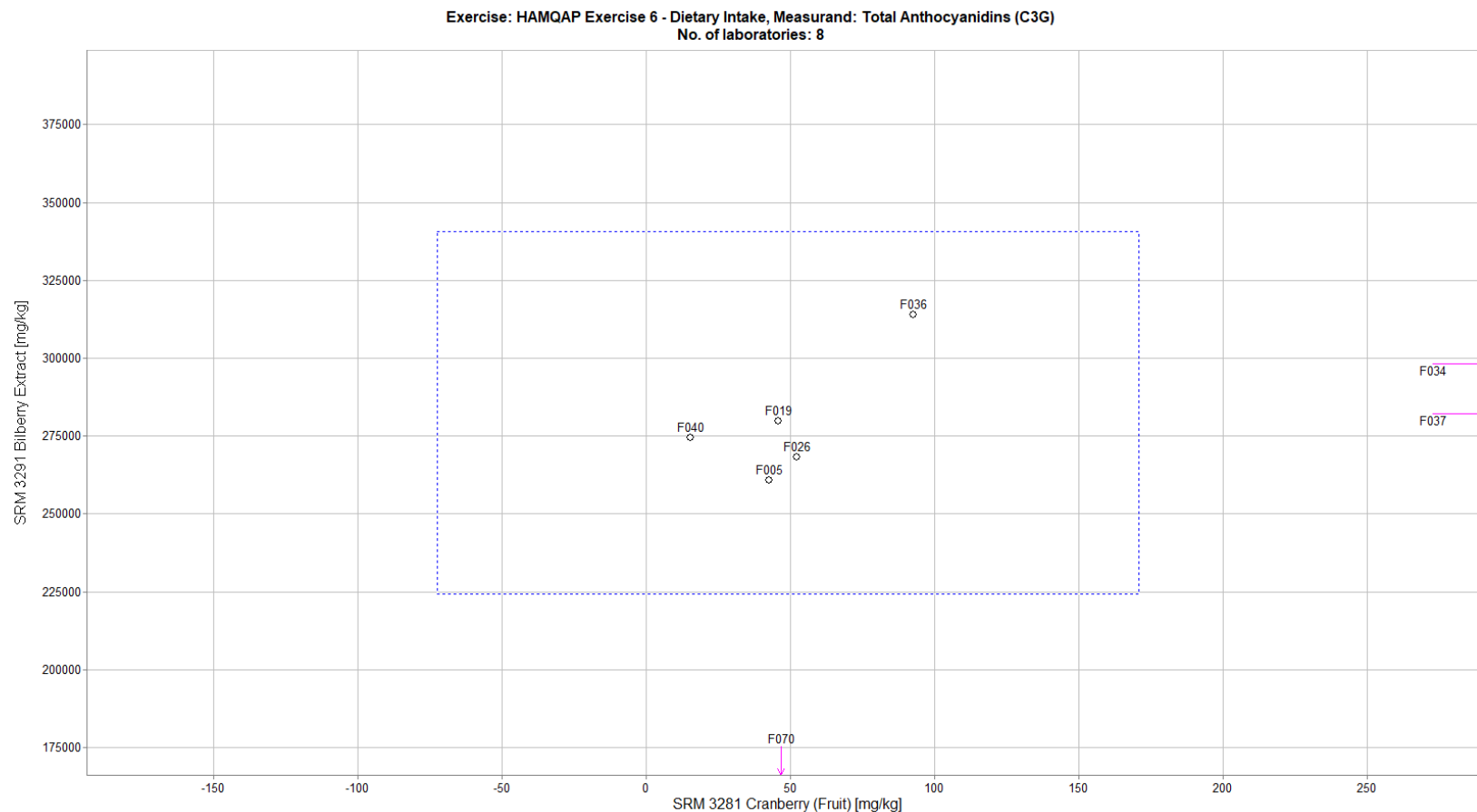


Figure 6-9. Laboratory means for total anthocyanidins (C3G) in SRM 3281 Cranberry (Fruit) and SRM 3291 Bilberry Extract (sample/sample comparison view). In this view, the individual laboratory mean for one sample (SRM 3281) is compared to the individual laboratory mean for a second sample (SRM 3291).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

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

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

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

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

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

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

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

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

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

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

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

Study Overview

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

Dietary Intake Sample Information

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

Dietary Intake Study Results

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

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

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

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

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

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

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

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

Dietary Intake Technical Recommendations

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

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

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

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

HAMQAP Exercise 6 - Natural Products											
Lab Code:		NIST	1. Your Results				2. Community Results			3. Target	
Analyte	Sample	Units	x_i	s_i	Z'_{comm}	Z_{NIST}	N	x^*	s^*	x_{NIST}	U
Caffeine	Protein Sample A	mg/g					16	0.092	0.037		
Caffeine	Protein Sample B	mg/g					18	0.403	0.092		
Theobromine	Protein Sample A	mg/g					10	1.13	0.098		
Theobromine	Protein Sample B	mg/g					11	0.98	0.15		
Theophylline	Protein Sample A	mg/g					4	0.38	0.51		
Theophylline	Protein Sample B	mg/g					4	0.024	0.042		
		x_i	Mean of reported values				N	Number of quantitative values reported		x_{NIST}	NIST-assessed value
		s_i	Standard deviation of reported values							U	expanded uncertainty
		Z'_{comm}	Z'-score with respect to community consensus				x^*	Robust mean of reported values			about the NIST-assessed value
		Z_{NIST}	Z-score with respect to NIST value				s^*	Robust standard deviation			

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

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

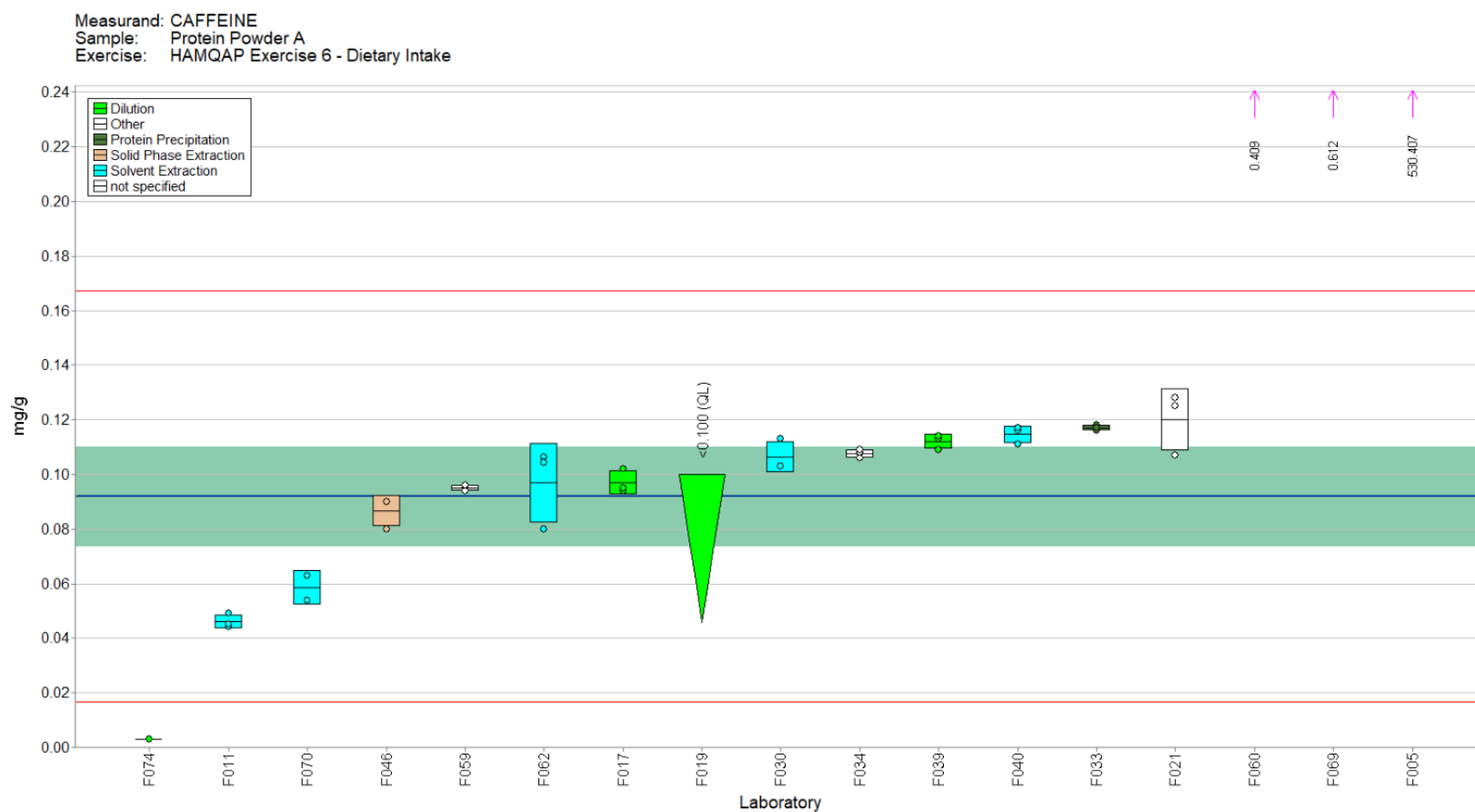


Figure 7-1. Caffeine in Protein Powder A (data summary view – sample preparation method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The red solid lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$. A NIST value has not been determined in this material.

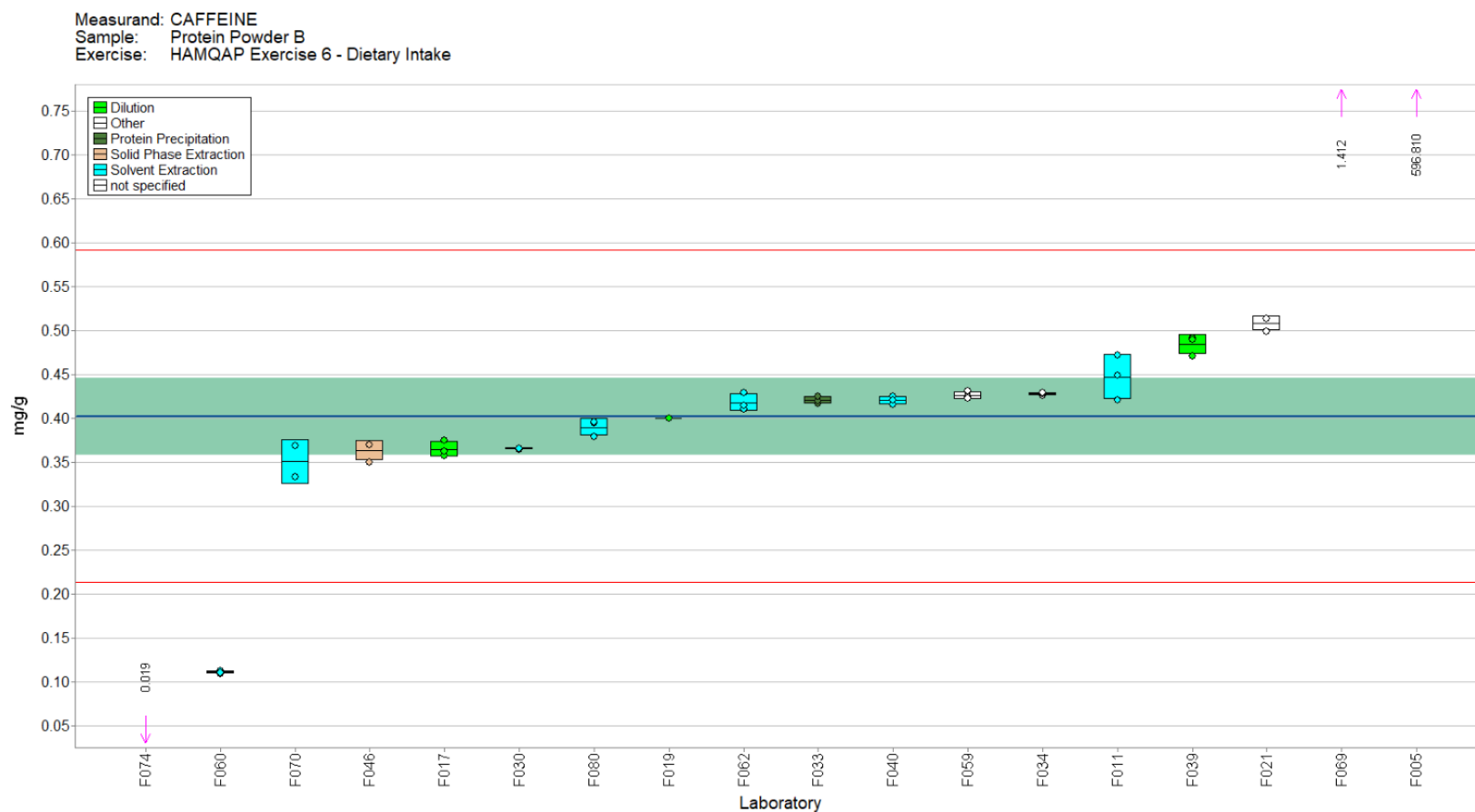


Figure 7-2. Caffeine in Protein Powder B (data summary view – sample preparation method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The solid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

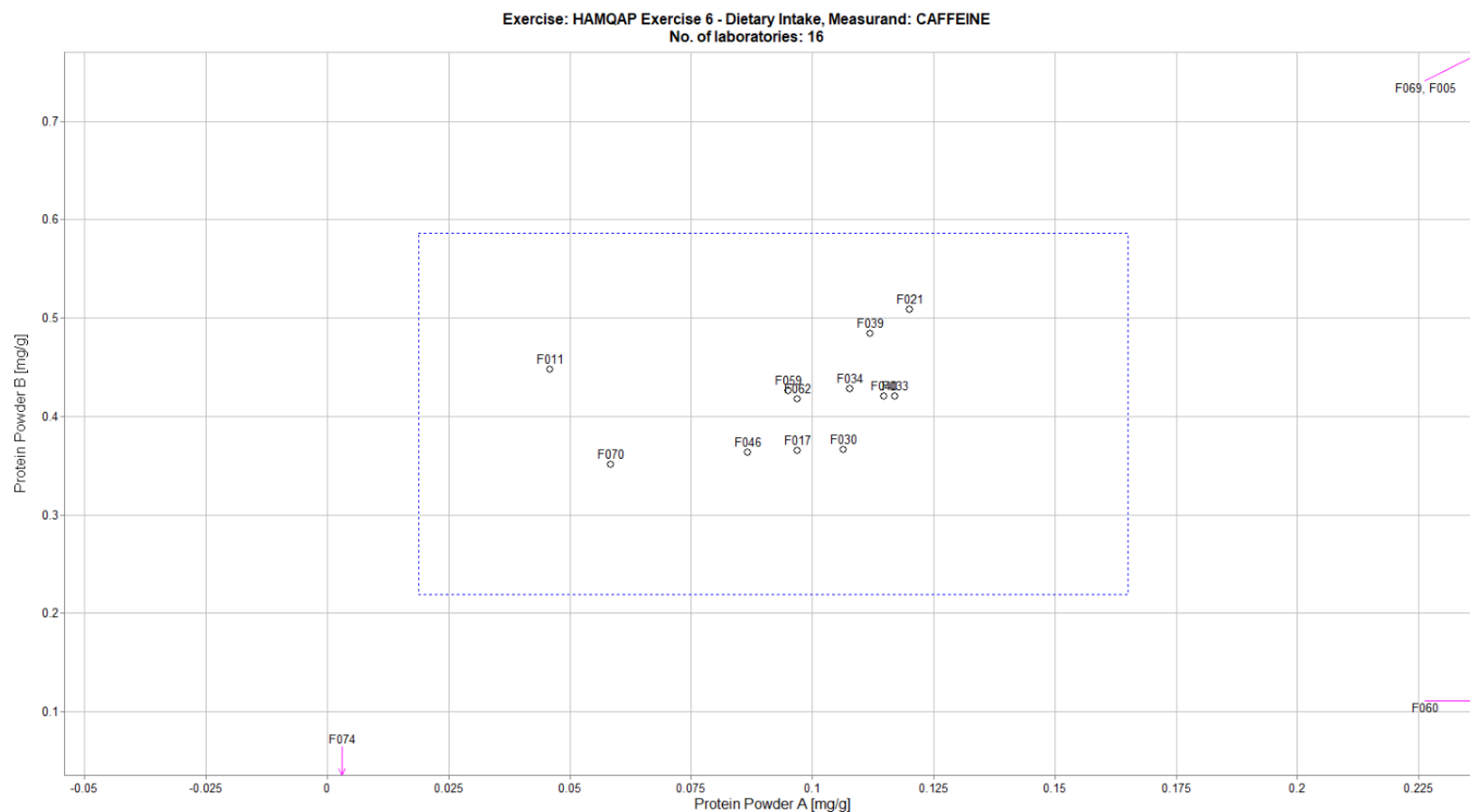


Figure 7-3. Laboratory means for caffeine in Protein Powder A and Protein Powder B (sample/sample comparison view). In this view, the individual laboratory mean for one sample (Protein Powder A) is compared to the mean for a second sample (Protein Powder B). The dotted blue box represents the consensus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

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

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



Figure 7-4. Theobromine in Protein Powder A (data summary view – sample preparation method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The red solid lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$. A NIST value has not been determined in this material.

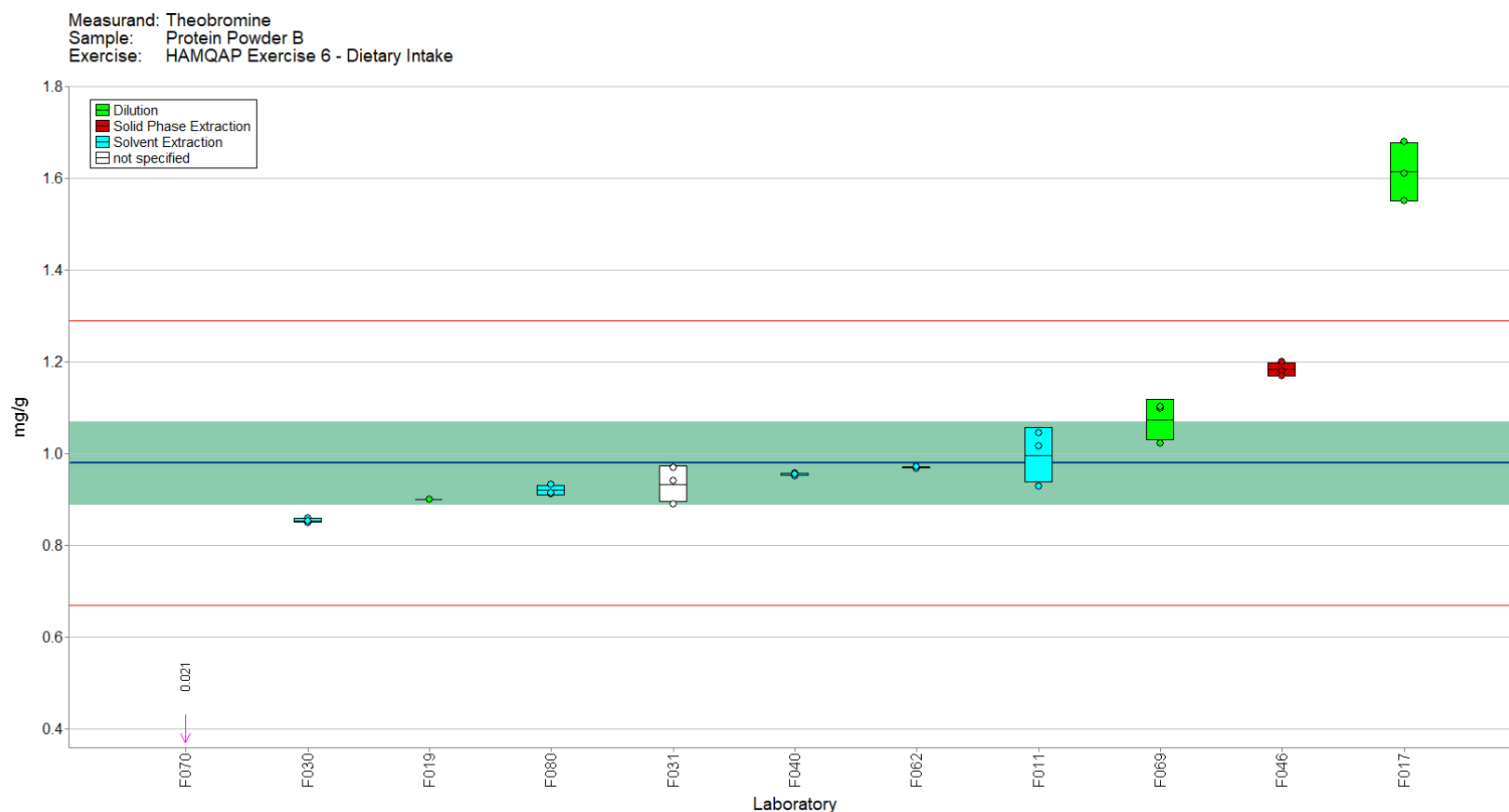


Figure 7-5. Theobromine in Protein Powder B (data summary view – sample preparation method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red line represents the upper consensus range of tolerance, calculated as the values above the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$. A NIST value has not been determined in this material.

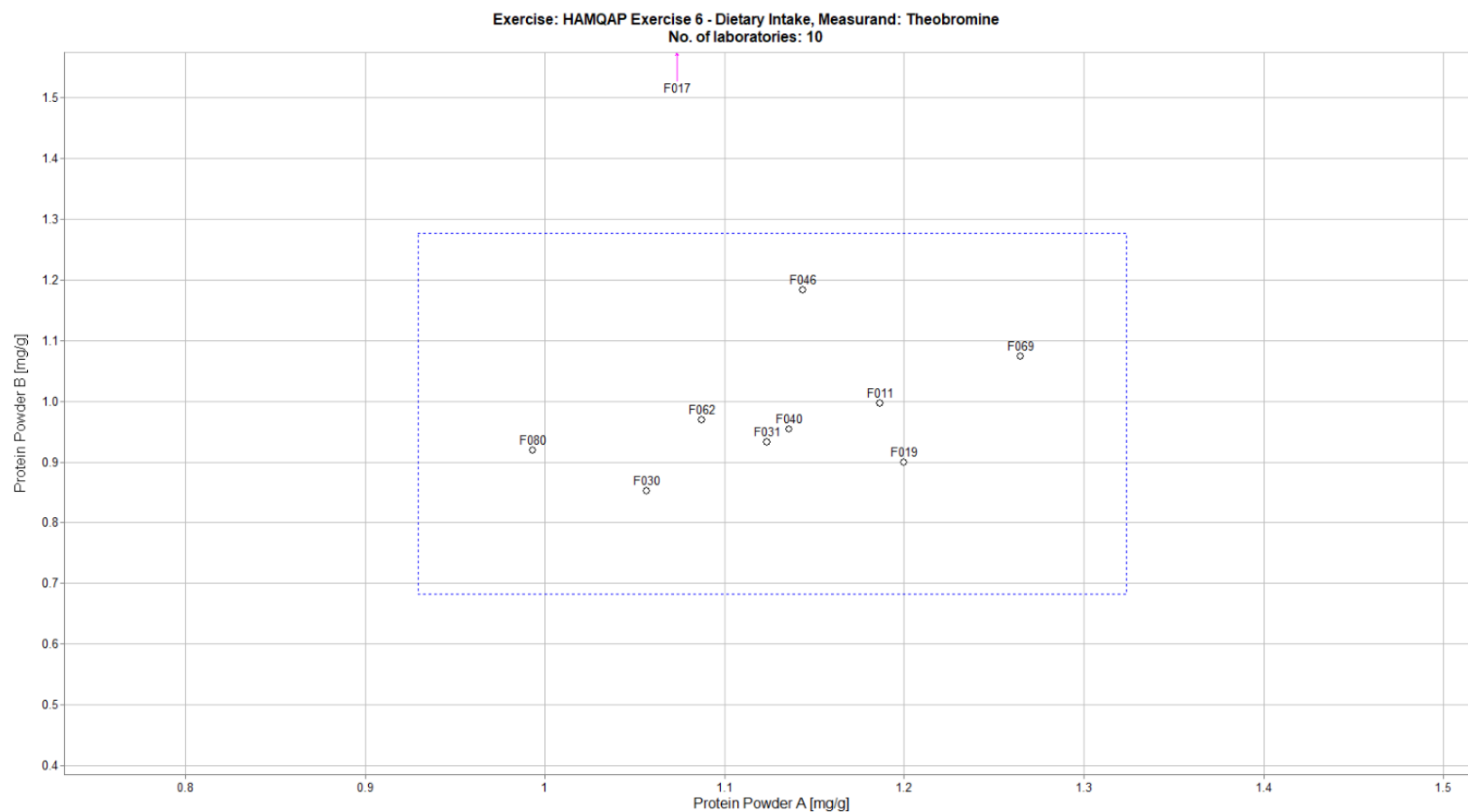


Figure 7-6. Laboratory means for theobromine in Protein Powder A and Protein Powder B (sample/sample comparison view). In this view, the individual laboratory mean for one sample (Protein Powder A) is compared to the mean for a second sample (Protein Powder B). The dotted blue box represents the consensus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

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

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

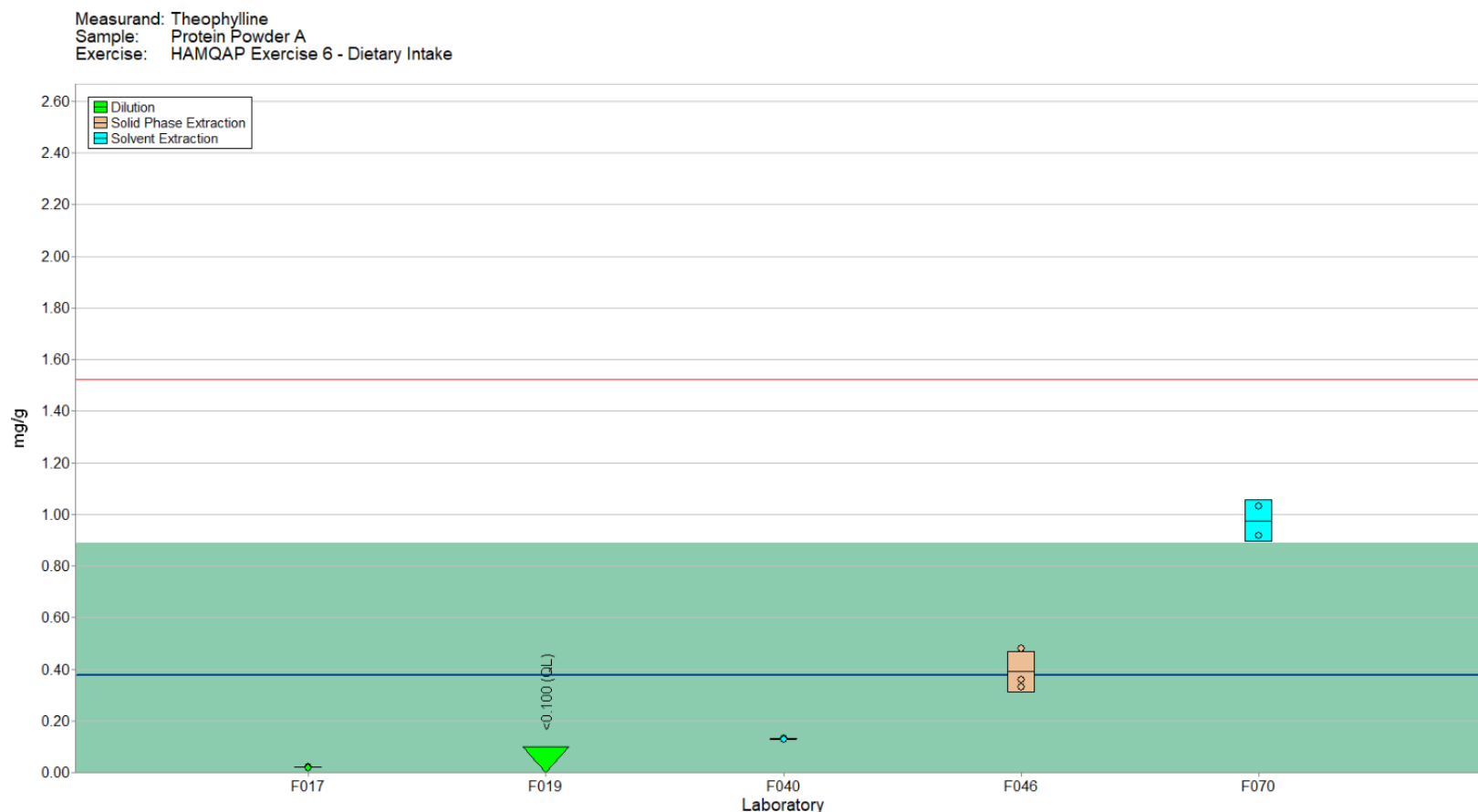


Figure 7-7. Theophylline in Protein Powder A (data summary view – sample preparation method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The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

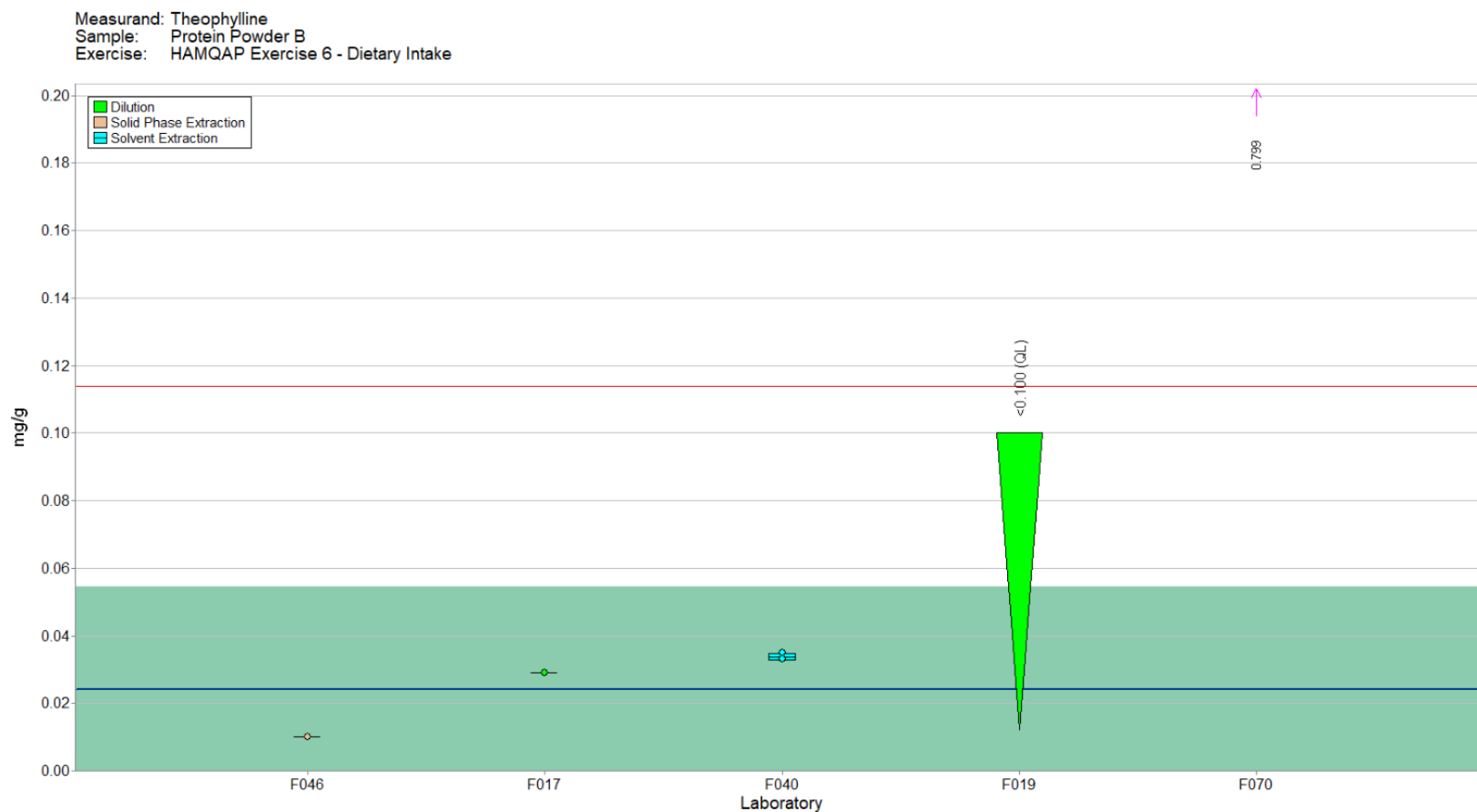


Figure 7-8. Theophylline in Protein Powder B (data summary view – sample preparation method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red line represents the upper consensus range of tolerance, calculated as the values above the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$. A NIST value has not been determined in this material.

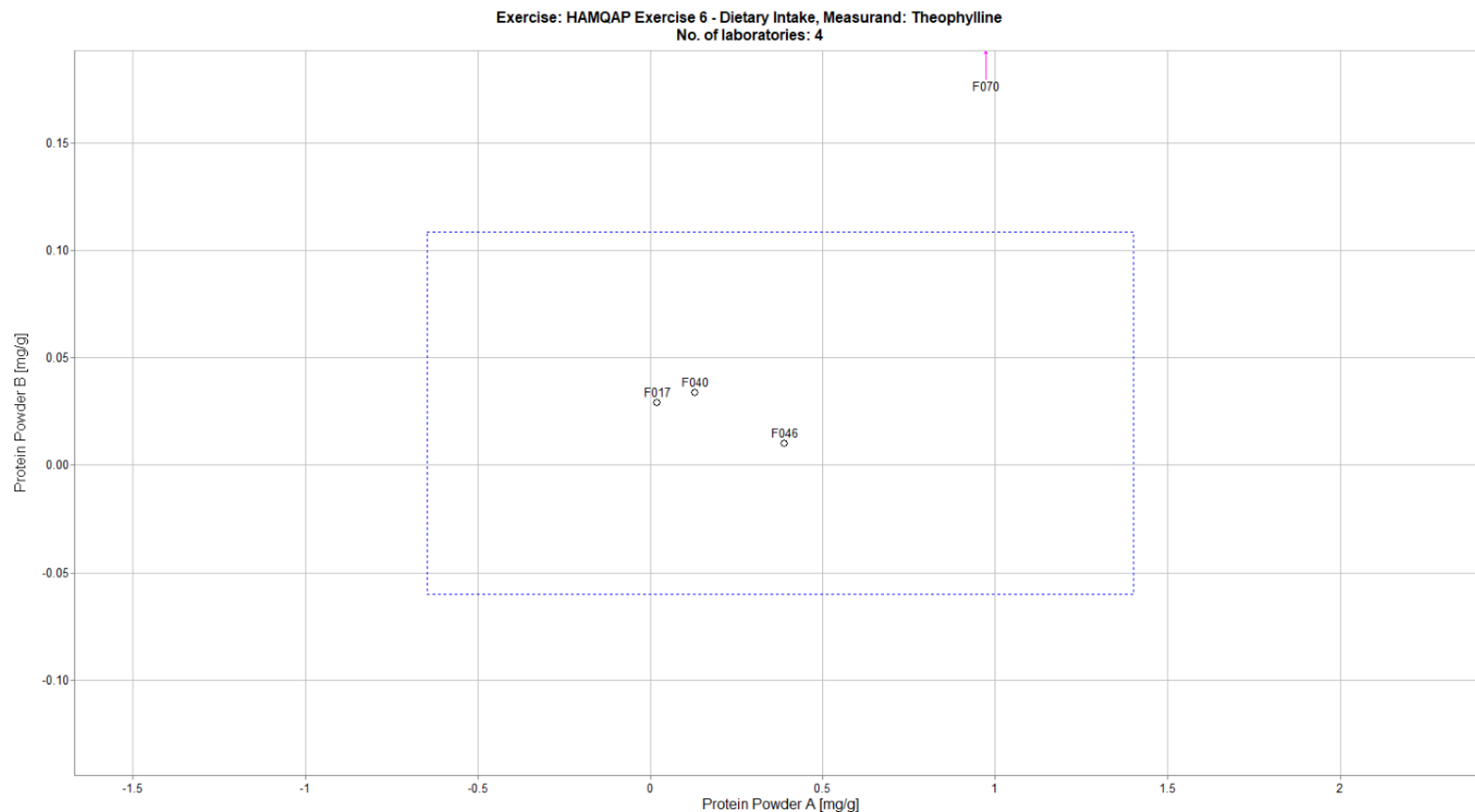


Figure 7-9. Laboratory means for theophylline in Protein Powder A and Protein Powder B (sample/sample comparison view). In this view, the individual laboratory mean for one sample (Protein Powder A) is compared to the mean for a second sample (Protein Powder B). The dotted blue box represents the consensus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

SECTION 8: CONTAMINANTS I (Chlorate, Perchlorate)

Study Overview

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

Dietary Intake Sample Information

Infant Formula B. Participants were provided three packets of SRM 1869 Infant/Adult Nutritional Formula II, a milk, whey, and soy-based infant/adult nutritional formula, each containing 10 g of material. Participants were asked to store the material at -20 °C, to thoroughly mix the contents of each packet before use, and to use a sample size appropriate for their in-house method of analysis. Participants were asked to prepare one sample and report one value from each packet provided. The approximate analyte levels were not reported to participants prior to the study. The NIST-determined values for chlorate and perchlorate were assigned using results from a previous interlaboratory comparison. The NIST-determined values and uncertainties are provided in the table below on an as-received basis.

Infant Formula C. Participants were provided with one packet of commercial whey protein concentrate containing 100 g of material. Participants were asked to store the material at controlled room temperature (20 °C to 25 °C), thoroughly mix the contents of the packet before use, and to use a sample size appropriate for their in-house method of analysis. Participants were asked to prepare three samples and report three values from the single packet provided. The approximate analyte levels were not reported to participants prior to the study. The NIST-determined values for chlorate and perchlorate were assigned using results from a previous interlaboratory comparison. The NIST-determined values and uncertainties are provided in the table below on an as-received basis.

Infant Formula D. Participants were provided with one packet of soy protein concentrate containing 20 g of material. Participants were asked to store the material at controlled room temperature (20 °C to 25 °C), to thoroughly mix the contents of the packet before use, and to use a sample size appropriate for their in-house method of analysis. Participants were asked to prepare three samples and report three values from the single packet provided. The approximate analyte

levels were not reported to participants prior to the study, and no target values have been established for chlorate and perchlorate in this sample.

Infant Formula E. Participants were provided with one packet of commercial whey protein concentrate containing 100 g of material. Participants were asked to store the material at controlled room temperature (20 °C to 25 °C), to thoroughly mix the contents of the packet before use, and to use a sample size appropriate for their in-house method of analysis. Participants were asked to prepare three samples and report three values from the single packet provided. The approximate analyte levels were not reported to participants prior to the study. The NIST-determined values for chlorate and perchlorate were assigned using results from a previous interlaboratory comparison. The NIST-determined values and uncertainties are provided in the table below on an as-received basis.

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

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

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

Dietary Intake Study Results

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

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

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

Dietary Intake Technical Recommendations

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

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

- Most laboratories reported use of MS-based methodologies for determination of chlorate and perchlorate. Isotopically labeled internal standards, added at the beginning of the analytical procedure, often result in improved accuracy and precision of results.
- No trends were observed for within laboratory variability for chlorate or perchlorate.
- Any extraction procedure should be optimized to determine the most effective extraction solvent to ensure exhaustive extraction of the analyte from the matrix.
- “Zero” is not a quantity that can be measured, and therefore a more appropriate result would be to report that a value is below the LOQ or QL.
- The use of appropriate calibration materials and quality assurance samples to establish that a method is in control and performing correctly may reduce the likelihood of outlying data. Quality assurance samples can be commercially available reference materials (CRMs, SRMs, or RMs) or materials prepared in-house.
- A linear calibration curve which surrounds the expected sample concentration values should be used for calculations. This curve should include both the lowest and highest expected concentration values of the sample solutions. Extrapolation of results beyond calibration curves may result in incorrect values.
- In general, all results should be checked closely to avoid calculation errors and to be sure that results are reported in the requested units.

Table 8-1. Individualized data summary table (NIST) for chlorate and perchlorate in infant formulas.*National Institute of Standards and Technology*

HAMQAP Exercise 6 - Contaminants I										
Lab Code:		NIST	1. Your Results				2. Community Results			3. Target
Analyte	Sample	Units	$\bar{x}_i$	s_i	Z'_{comm}	Z_{NIST}	N	$\bar{x}^*$	s^*	$\bar{x}_{\text{NIST}}$ U
Chlorate	SRM 1869 Infant/Adult Nutritional Formula II	ng/g	104	5.12			29	110	16	104 5.12
Chlorate	Infant Formula C	ng/g	1440	118			29	1780	310	1440 118
Chlorate	Infant Formula D	ng/g					24	21.8	4.8	
Chlorate	Infant Formula E	ng/g	66.8	12.1			28	50	11	66.8 12.1
Chlorate	Infant Formula F	ng/g	328	31.2			29	350	60	328 31.2
Chlorate	RM 8260 Infant Nutritional Formula	ng/g	265	28.4			29	300	53	265 28.4
Perchlorate	SRM 1869 Infant/Adult Nutritional Formula II	ng/g					7	0	2	
Perchlorate	Infant Formula C	ng/g	30	3.1			27	37.4	7.9	30 3.1
Perchlorate	Infant Formula D	ng/g					20	5.9	1.9	
Perchlorate	Infant Formula E	ng/g					5	1.3	2.4	
Perchlorate	Infant Formula F	ng/g	5.75	0.788			20	6.7	1.7	5.75 0.788
Perchlorate	RM 8260 Infant Nutritional Formula	ng/g					9	0	2	
			$\bar{x}_i$	Mean of reported values			N	Number of quantitative values reported		$\bar{x}_{\text{NIST}}$ NIST-assessed value
			s_i	Standard deviation of reported values						U expanded uncertainty
			Z'_{comm}	Z'-score with respect to community consensus			$\bar{x}^*$	Robust mean of reported values		about the NIST-assessed value
			Z_{NIST}	Z-score with respect to NIST value			s^*	Robust standard deviation		

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

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

		Chlorate														
		Infant Formula E (ng/g)					Infant Formula F (ng/g)					RM 8260 Infant Nutritional Formula (ng/g)				
Individual Results	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD	A	B	C	Avg	SD
	Target				66.8	12.14				328	31.2				265	28.4
	F003	40.8	41.71	39.12	40.5	1.3	281.04	262.28	252.03	265.1	14.7	210.71	228.89	237.52	225.7	13.7
	F004															
	F005	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	F006	54.78	56.34	54.46	55.19	1.01	430.93	417.35	431.01	426.4	7.9	371.04	370.68	372.43	371.4	0.92
	F008															
	F009	56.6	50.8	50.3	52.57	3.50	408.7	447.7	377.8	411.4	35.0	366.6	323.4	307.2	332.4	30.7
	F010	42.8	42.9	45.2	43.63	1.36	338.7	322.9	346.1	335.9	11.9	268	264	260	264.0	4.00
	F016	41	43	40.2	41.40	1.44	344.8	356.4	344.8	348.7	6.70	290	305.3	290.6	295.3	8.67
	F021															
	F023	68	57		62.50	7.78	383	400		391.5	12.0	339	330		334.5	6.36
	F024	50.3	50.1	43.9	48.10	3.64	360.8	364.4	360	361.7	2.34	289.6	289.4	294.6	291.2	2.95
	F028	49.8	49.4	49.3	49.50	0.26	391	396	418	401.7	14.4	354	349	345	349.3	4.51
	F029	330	370	430	377	50.3	290	320	310	306.7	15.3	220	260	220	233.3	23.1
	F030	36.6	36.8	36.7	36.70	0.10	381.6	294.7	278.2	318.2	55.6	264.4	303.6	289	285.7	19.8
	F031	52	40.31	40.57	44.29	6.68	334.12	323.69	297.98	318.6	18.6	288.3	262.79	245.63	265.6	21.5
	F033	66.1	64.7	68.2	66.33	1.76	402	389	399	396.7	6.81	354	365	346	355.0	9.54
	F034															
	F040	43		47	45.00	2.83	334	337	339	336.7	2.52	307	284	282	291.0	13.9
	F043	36	24	30	30.00	6.00	360	300	240	300.0	60.0	290	240	340	290.0	50.0
	F044	41	43	41	41.67	1.15	349	343	360	350.7	8.62	310	295	293	299.3	9.29
	F050						337	327	327	330.3	5.77	307	333	317	319.0	13.1
	F053		40	41	40.50	0.71	326	298	322	315.3	15.1	271	268	260	266.3	5.69
	F058	64.4	63.2	70.6	66.07	3.97	245.7	230.6	233.1	236.5	8.09	174.9	177.8	171.9	174.9	2.95
	F061	480	480	490	483.33	5.77	370	390	410	390.0	20.0	310	320	320	316.7	5.77
	F062	39	50	45	44.67	5.51	285	268	253	268.7	16.0	292	268	314	291.3	23.0
	F063	40	41	41	40.67	0.58	390	390	403	394.3	7.51	330	339	327	332.0	6.24
	F066	39	38	37	38.00	1.00	380	379	359	372.7	11.8	311	303	302	305.3	4.93
	F067															
	F068	38	31	35	34.67	3.51	279	281	290	283.3	5.86	260	252	264	258.7	6.11
	F071	37	36	32	35.00	2.65	323	333	330	328.7	5.13	281	277	278	278.7	2.08
	F078															
	F080	42	42	42	42.00	0.00	360	330	340	343.3	15.3	272	273	275	273.3	1.53
	F082	42	55	54	50.33	7.23	338	393	414	381.7	39.2	380	332	395	369.0	32.9
	F083	43.8	43.6	34.9	40.77	5.08	367	373	392	377.3	13.1	310	339	328	325.7	14.6
	F087	63.2	63.8	61.8	62.93	1.03	421	352	376	383.0	35.0	367	351	340	352.7	13.6
F088																
Community Results		Consensus Mean				45.6	Consensus Mean				346.3	Consensus Mean				299.8
		Consensus Standard Deviation				11.4	Consensus Standard Deviation				59.5	Consensus Standard Deviation				52.7
		Maximum				483.3	Maximum				426.4	Maximum				371.4
		Minimum				0	Minimum				0	Minimum				0
		N				28	N				29	N				29

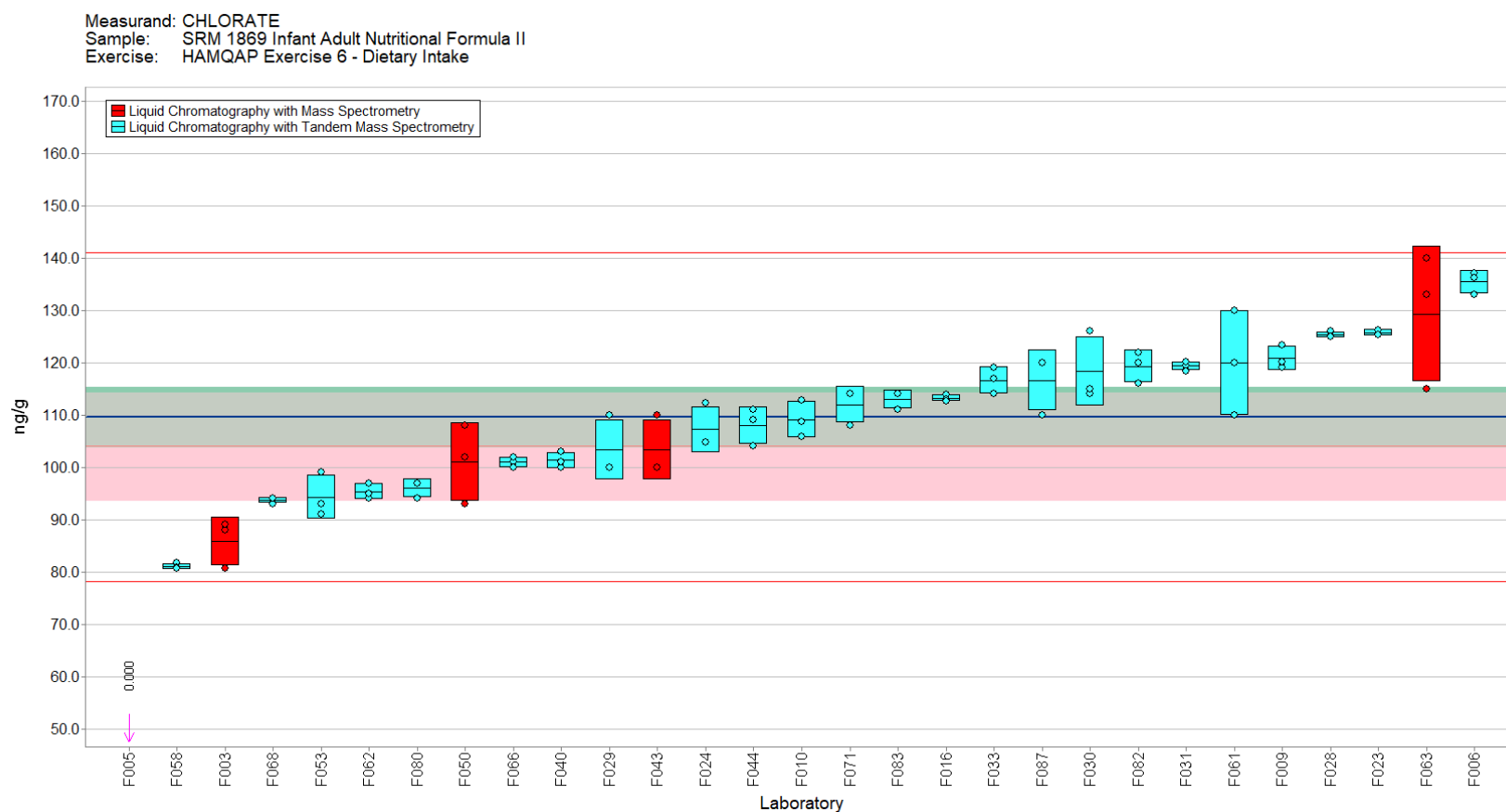


Figure 8-1. Chlorate in SRM 1869 Infant/Adult Nutritional Formula II (milk/whey/soy-based) (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The red solid lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty (U_{NIST}) and represents the range that results in an acceptable Z'_{NIST} score, $|Z'_{\text{NIST}}| \leq 2$.

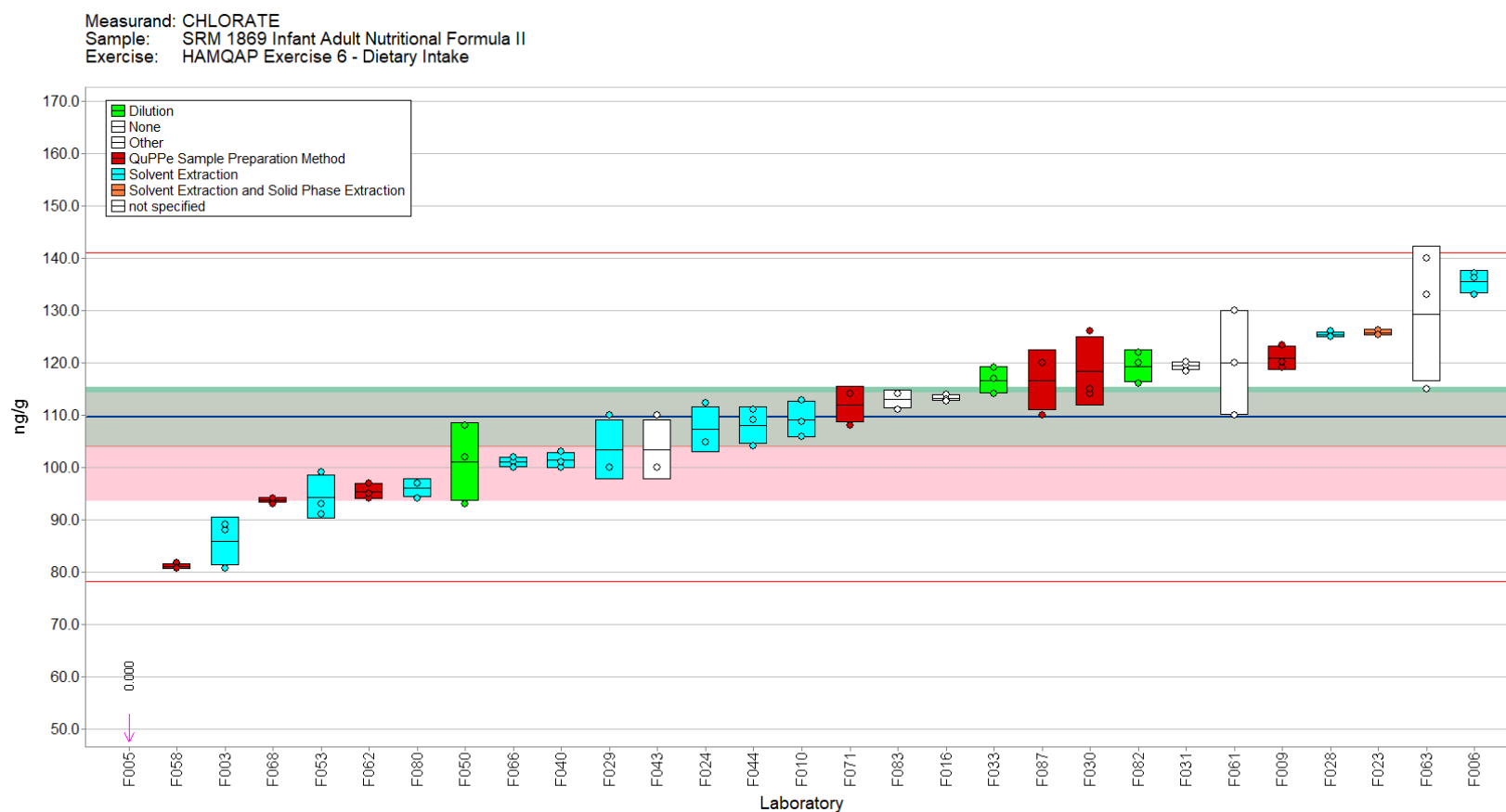


Figure 8-2. Chlorate in SRM 1869 Infant/Adult Nutritional Formula II (milk/whey/soy-based) (data summary view – sample preparation method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The red solid lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty (U_{NIST}) and represents the range that results in an acceptable Z'_{NIST} score, $|Z'_{\text{NIST}}| \leq 2$.

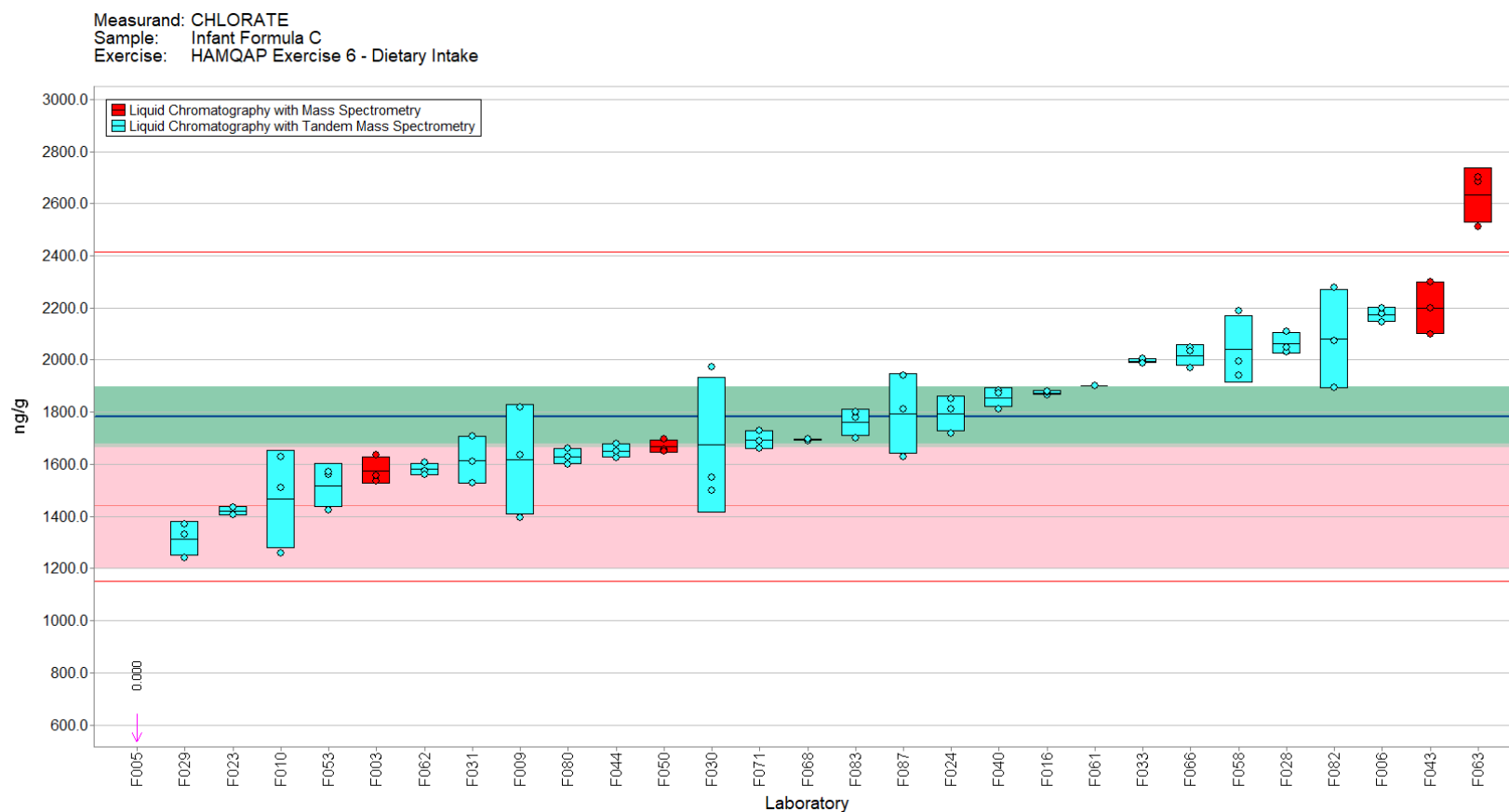


Figure 8-3. Chlorate in Infant Formula C (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The red solid lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty (U_{NIST}) and represents the range that results in an acceptable Z'_{NIST} score, $|Z'_{\text{NIST}}| \leq 2$.

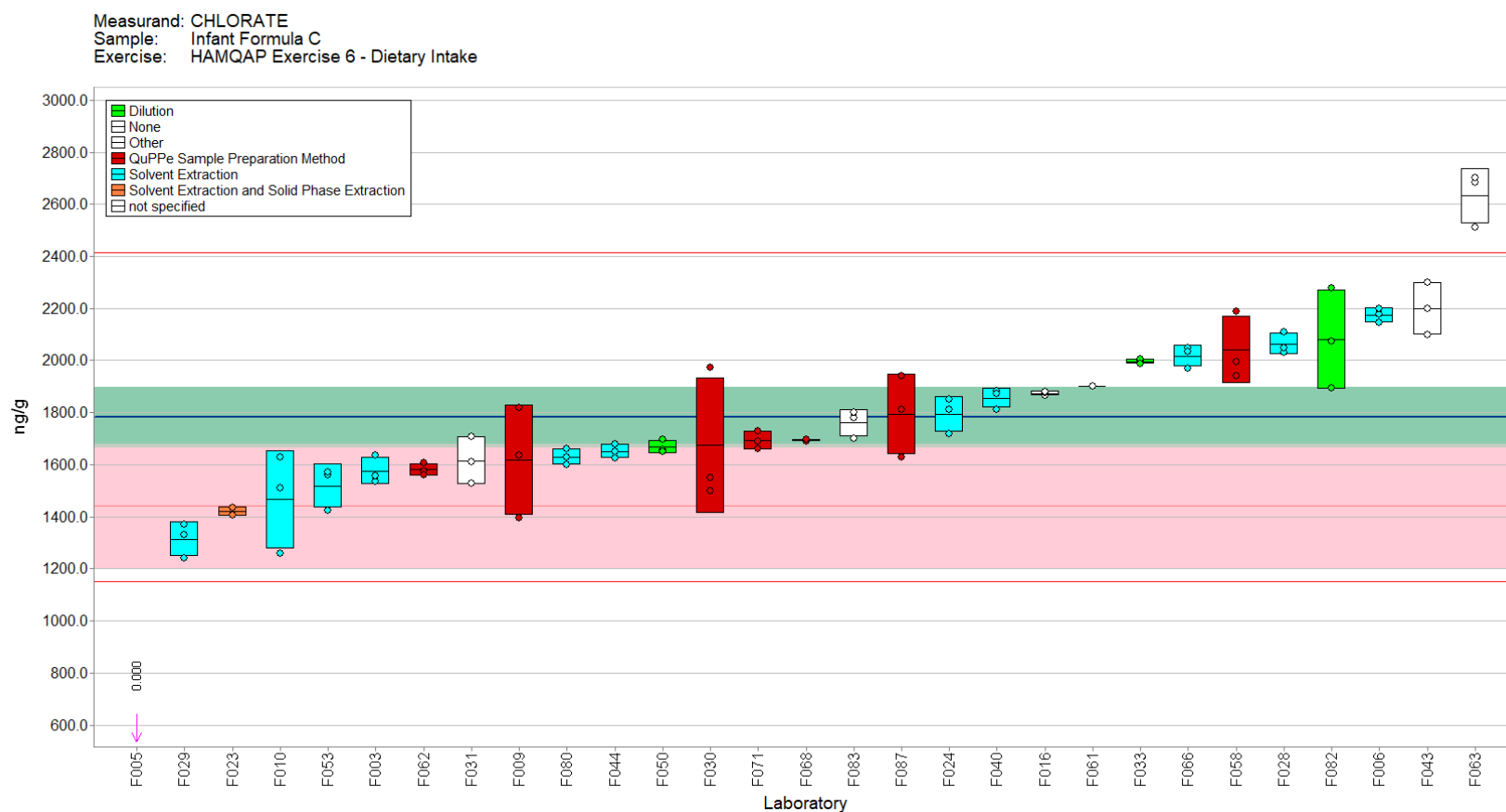


Figure 8-4. Chlorate in Infant Formula C (data summary view – sample preparation method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The red solid lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty (U_{NIST}) and represents the range that results in an acceptable Z'_{NIST} score, $|Z'_{\text{NIST}}| \leq 2$.

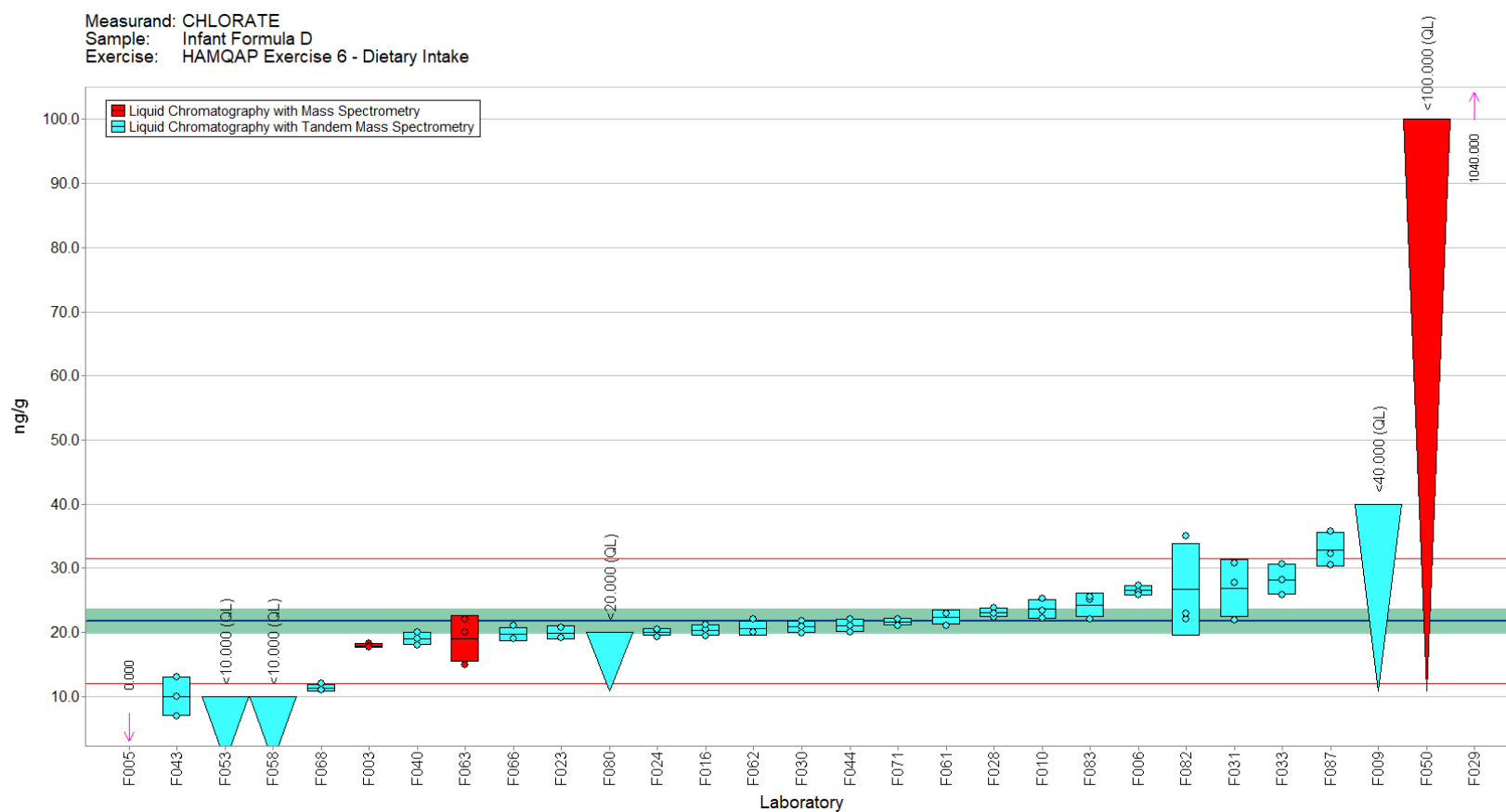


Figure 8-5. Chlorate in Infant Formula D (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The red solid lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$. A NIST value has not been determined in this material.

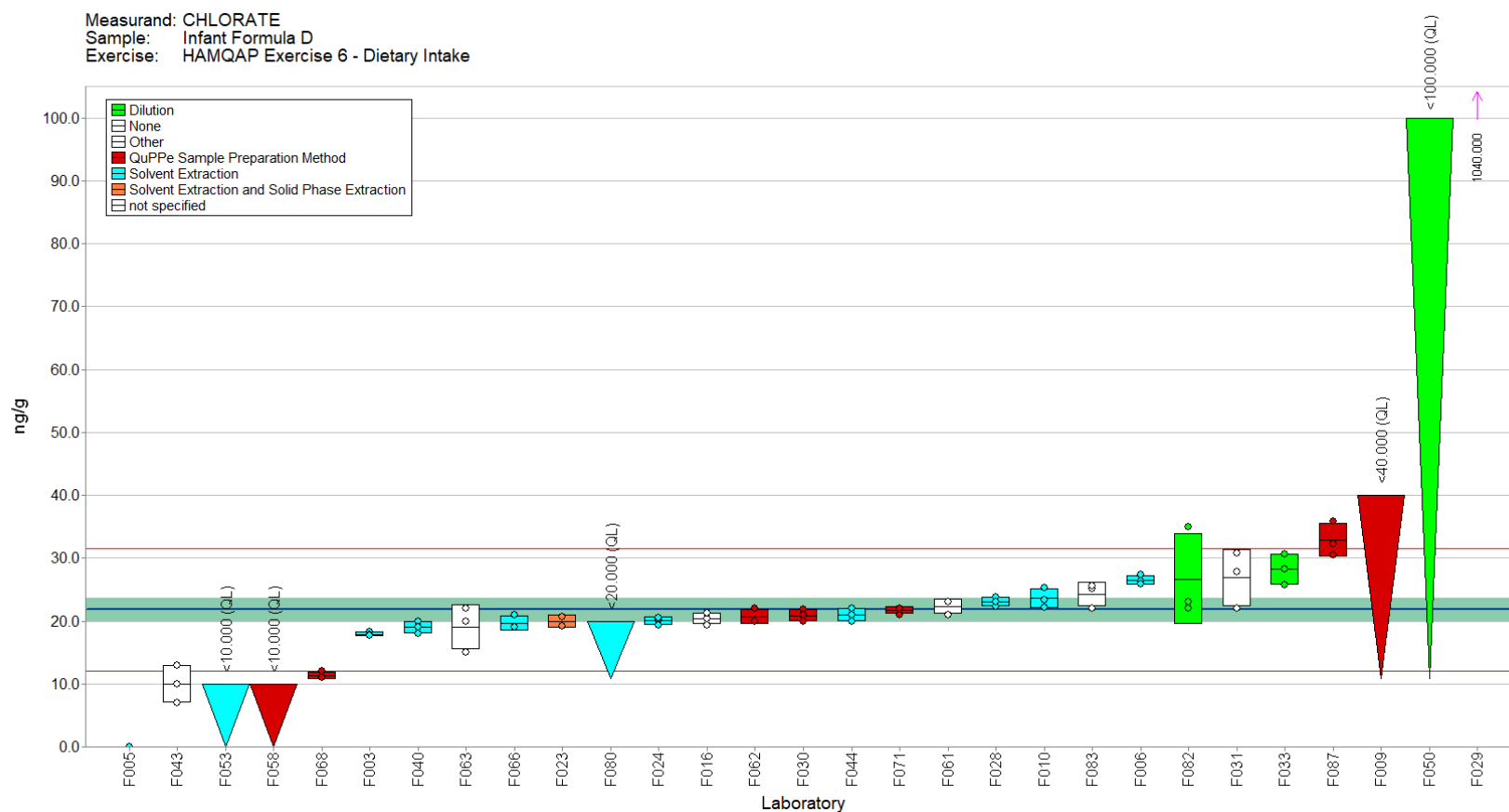


Figure 8-6. Chlorate in Infant Formula D (data summary view – sample preparation method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The red solid lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$. A NIST value has not been determined in this material.

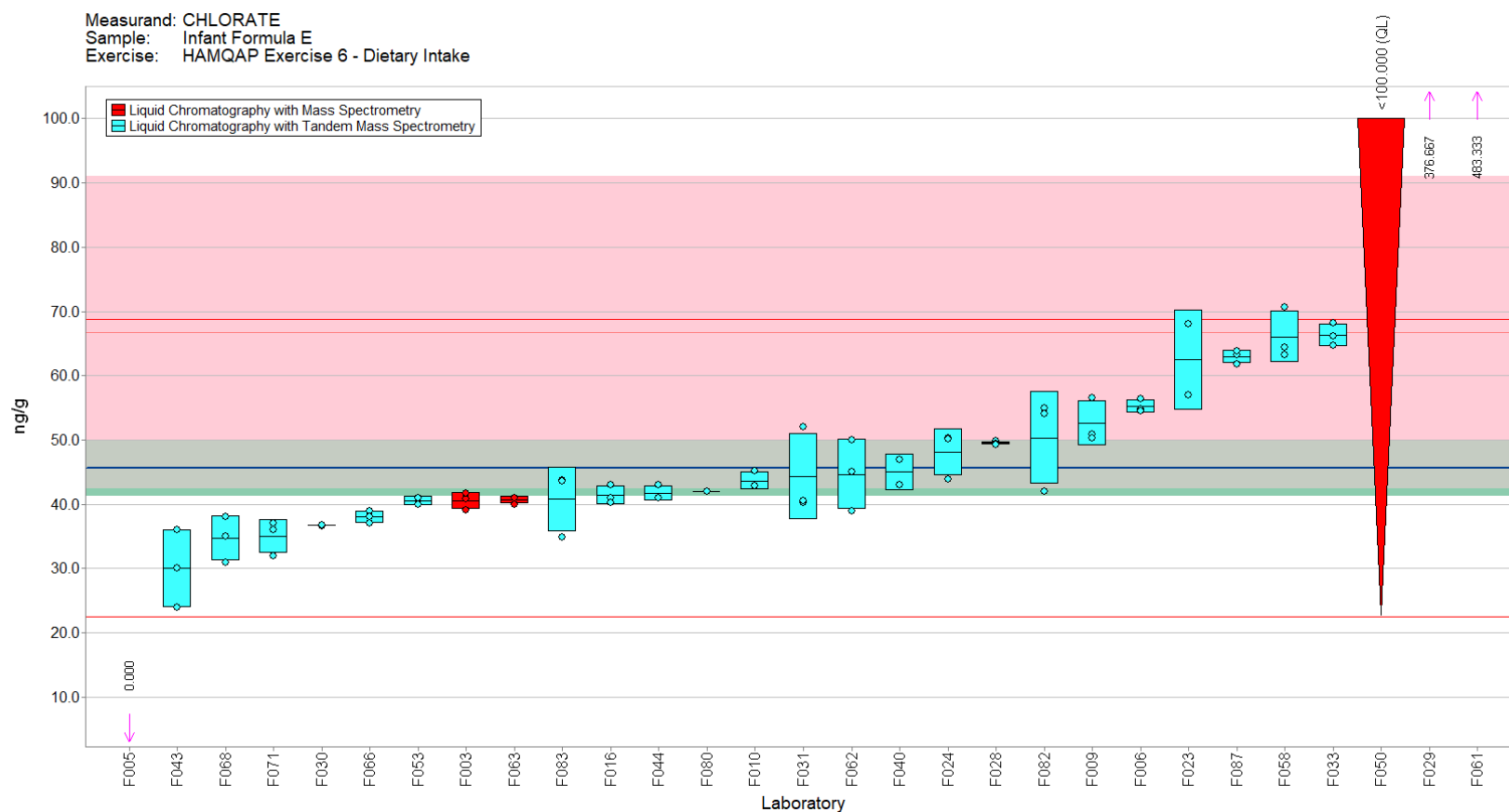


Figure 8-7. Chlorate in Infant Formula E (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The red solid lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty (U_{NIST}) and represents the range that results in an acceptable Z'_{NIST} score, $|Z'_{\text{NIST}}| \leq 2$.

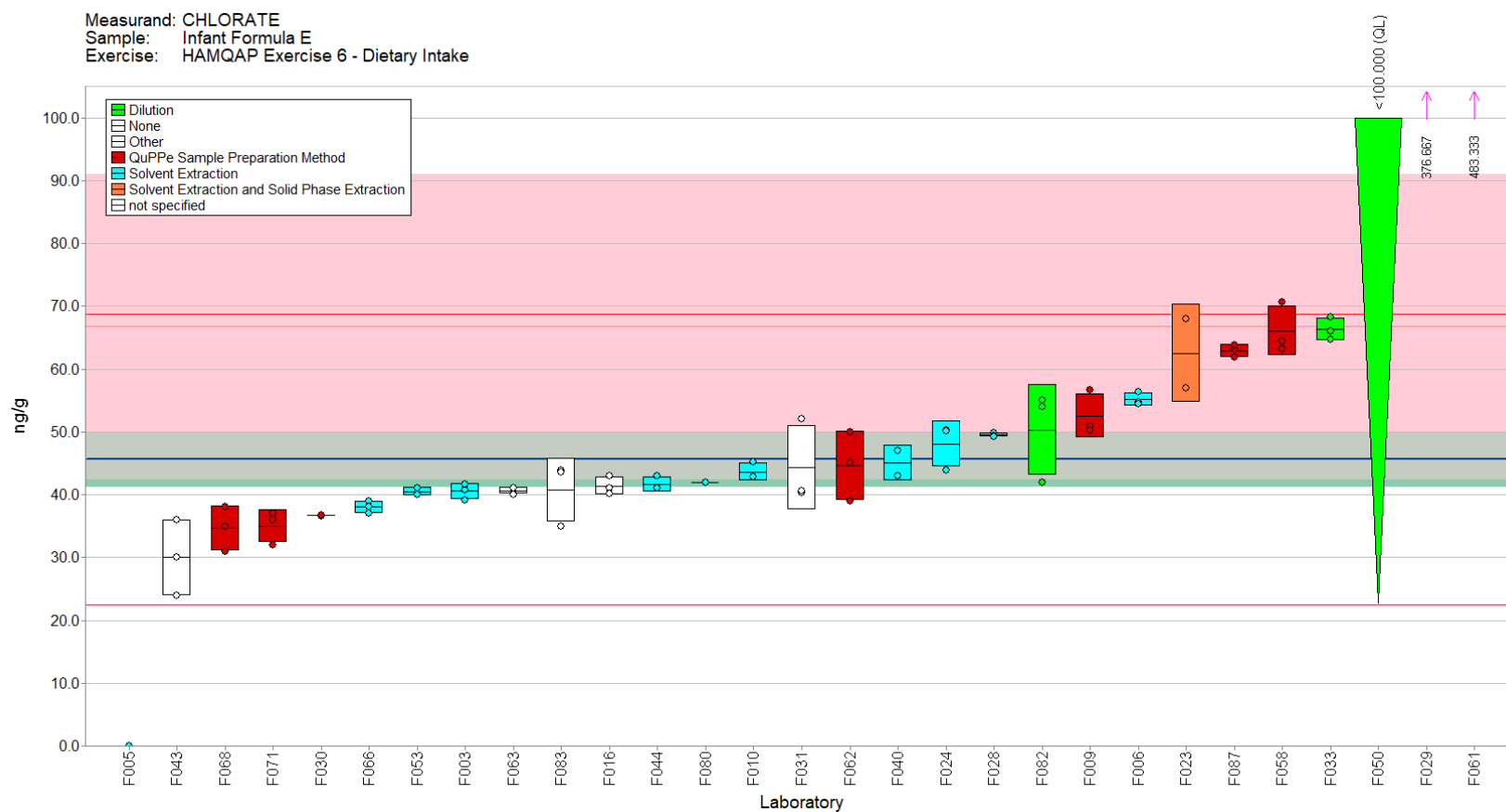


Figure 8-8. Chlorate in Infant Formula E (data summary view – sample preparation method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The solid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

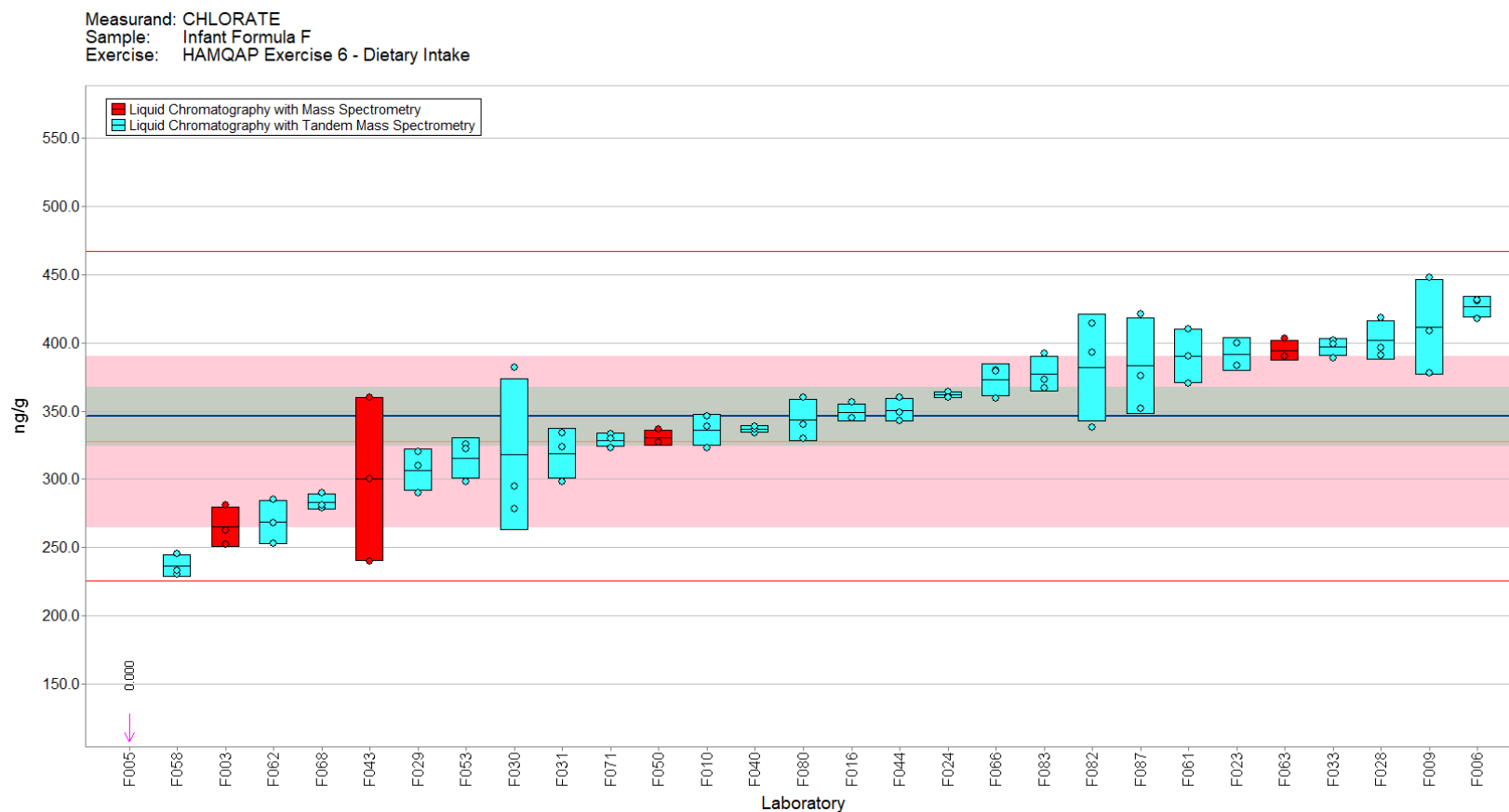


Figure 8-9. Chlorate in Infant Formula F (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The red solid lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty (U_{NIST}) and represents the range that results in an acceptable Z'_{NIST} score, $|Z'_{\text{NIST}}| \leq 2$.

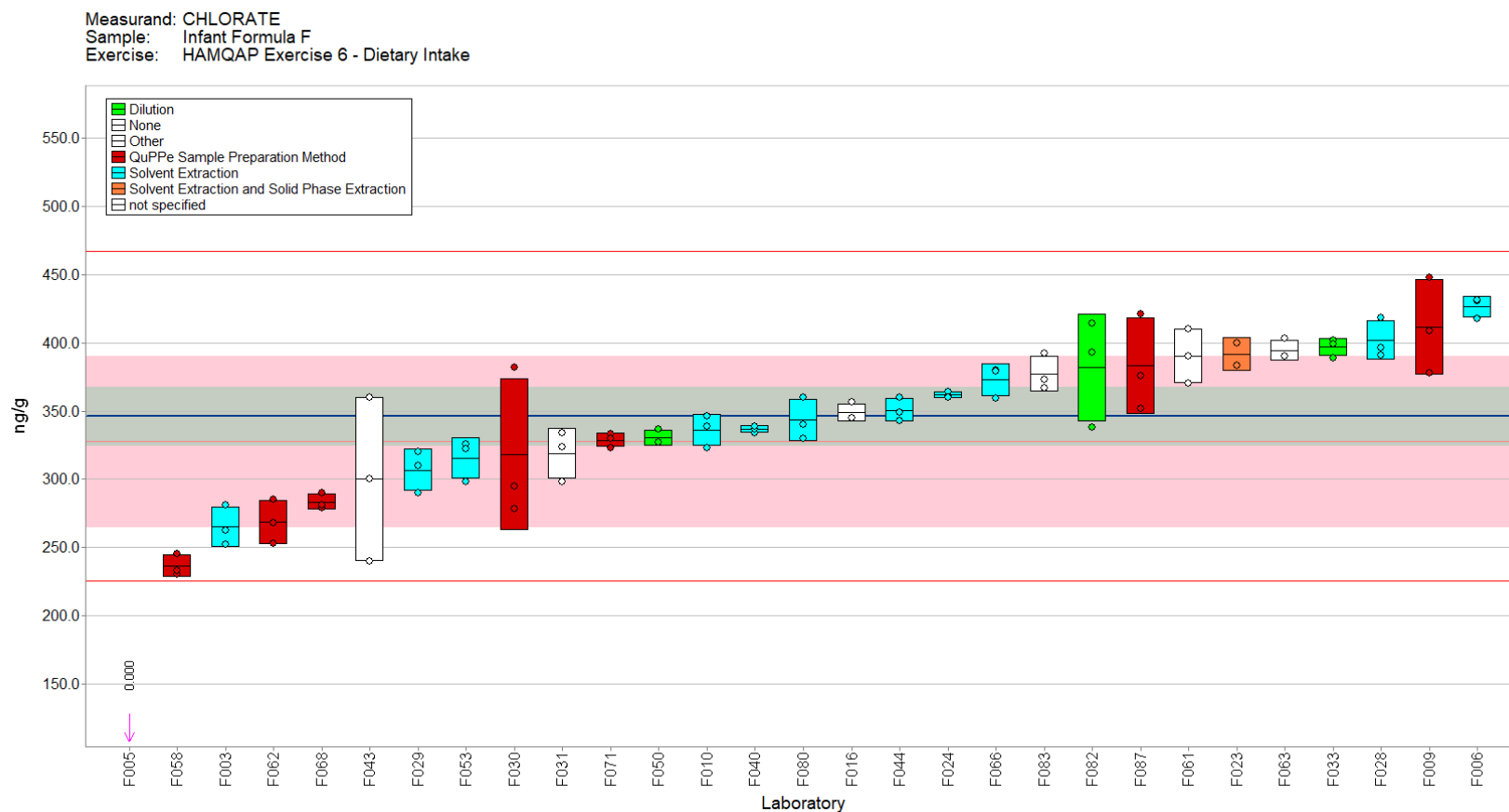


Figure 8-10. Chlorate in Infant Formula F (data summary view – sample preparation method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The red solid lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty (U_{NIST}) and represents the range that results in an acceptable Z'_{NIST} score, $|Z'_{\text{NIST}}| \leq 2$.

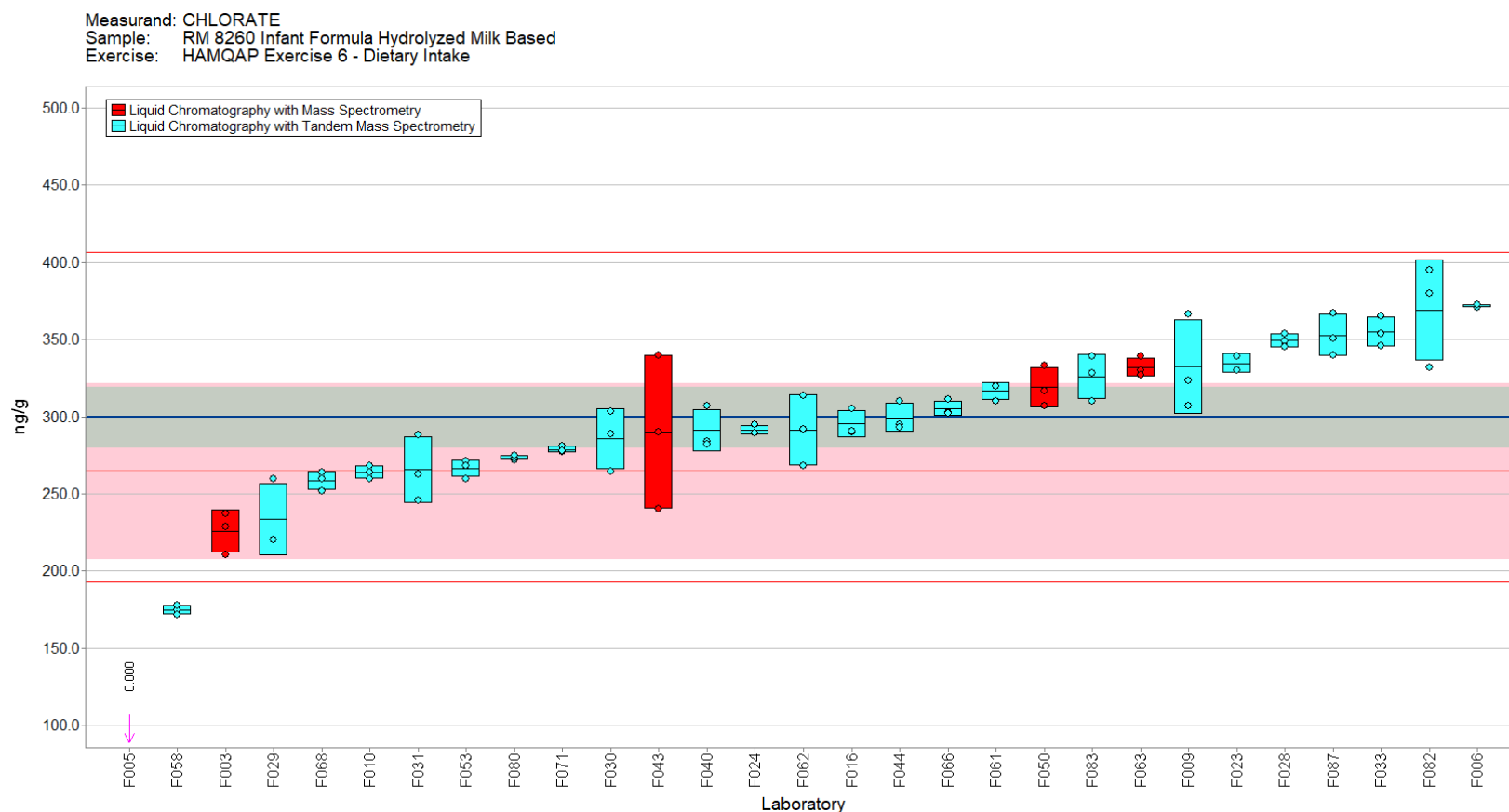


Figure 8-11. Chlorate in RM 8260 Infant Formula Hydrolyzed Milk Based (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The red solid lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty (U_{NIST}) and represents the range that results in an acceptable Z'_{NIST} score, $|Z'_{\text{NIST}}| \leq 2$.

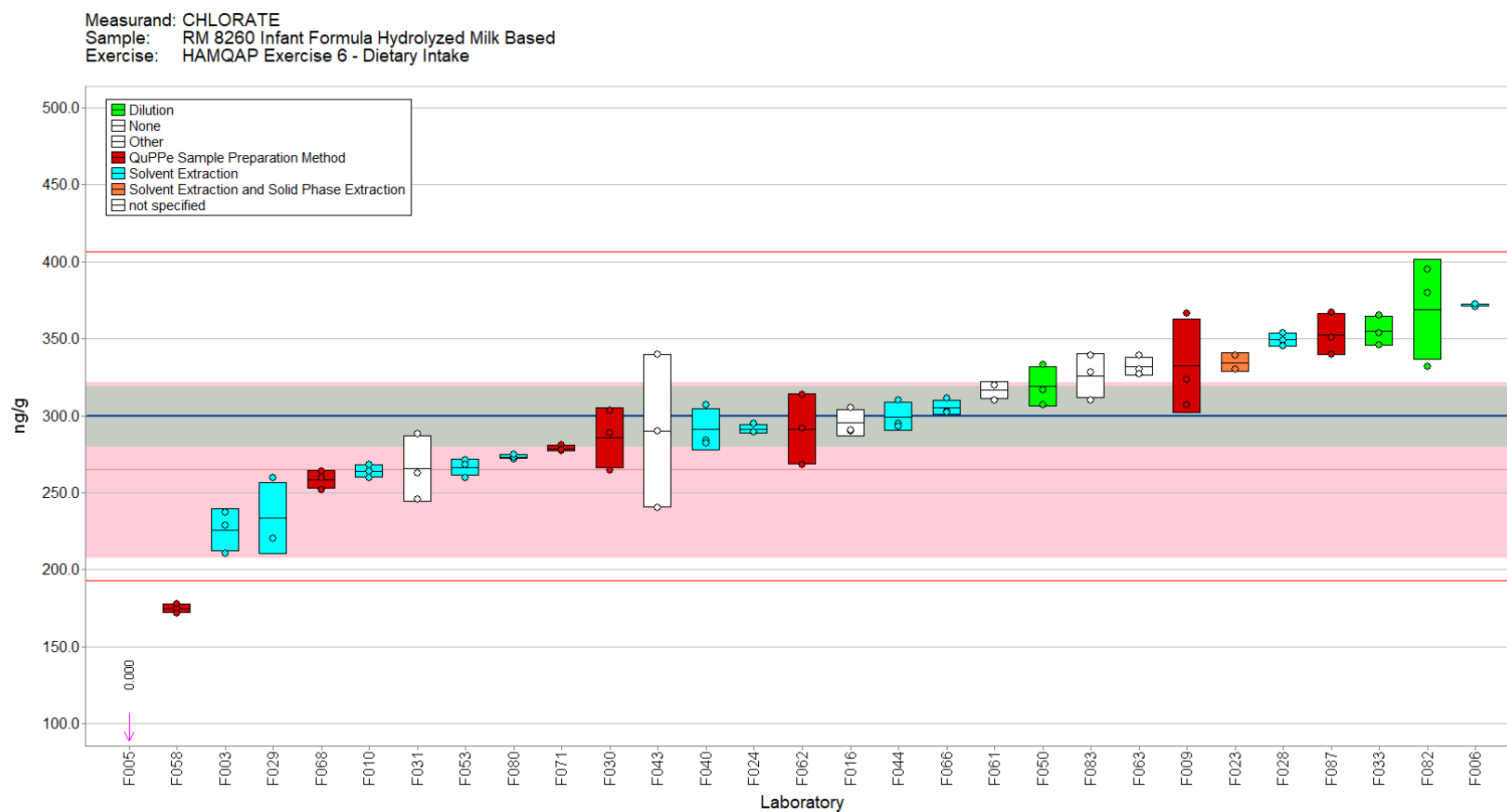


Figure 8-12. Chlorate in RM 8260 Infant Formula Hydrolyzed Milk Based (data summary view – sample preparation method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The red solid lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty (U_{NIST}) and represents the range that results in an acceptable Z'_{NIST} score, $|Z'_{\text{NIST}}| \leq 2$.

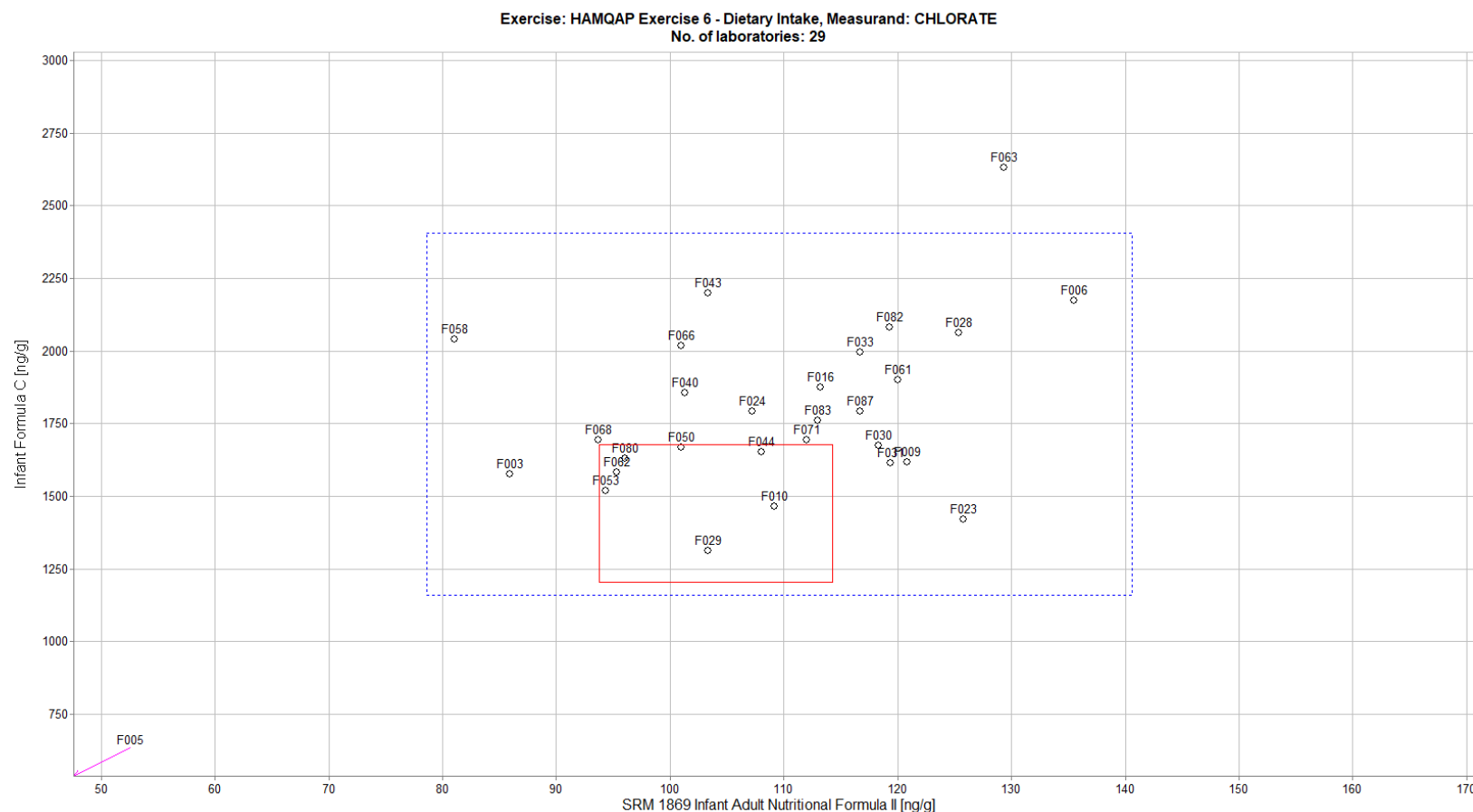


Figure 8-13. Laboratory means for chlorate in SRM 1869 Infant/Adult Nutritional Formula II (milk/whey/soy-based) and Infant Formula C (sample/sample comparison view). In this view, the individual laboratory mean for one sample (SRM 1869) is compared to the mean for a second sample (Infant Formula C).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

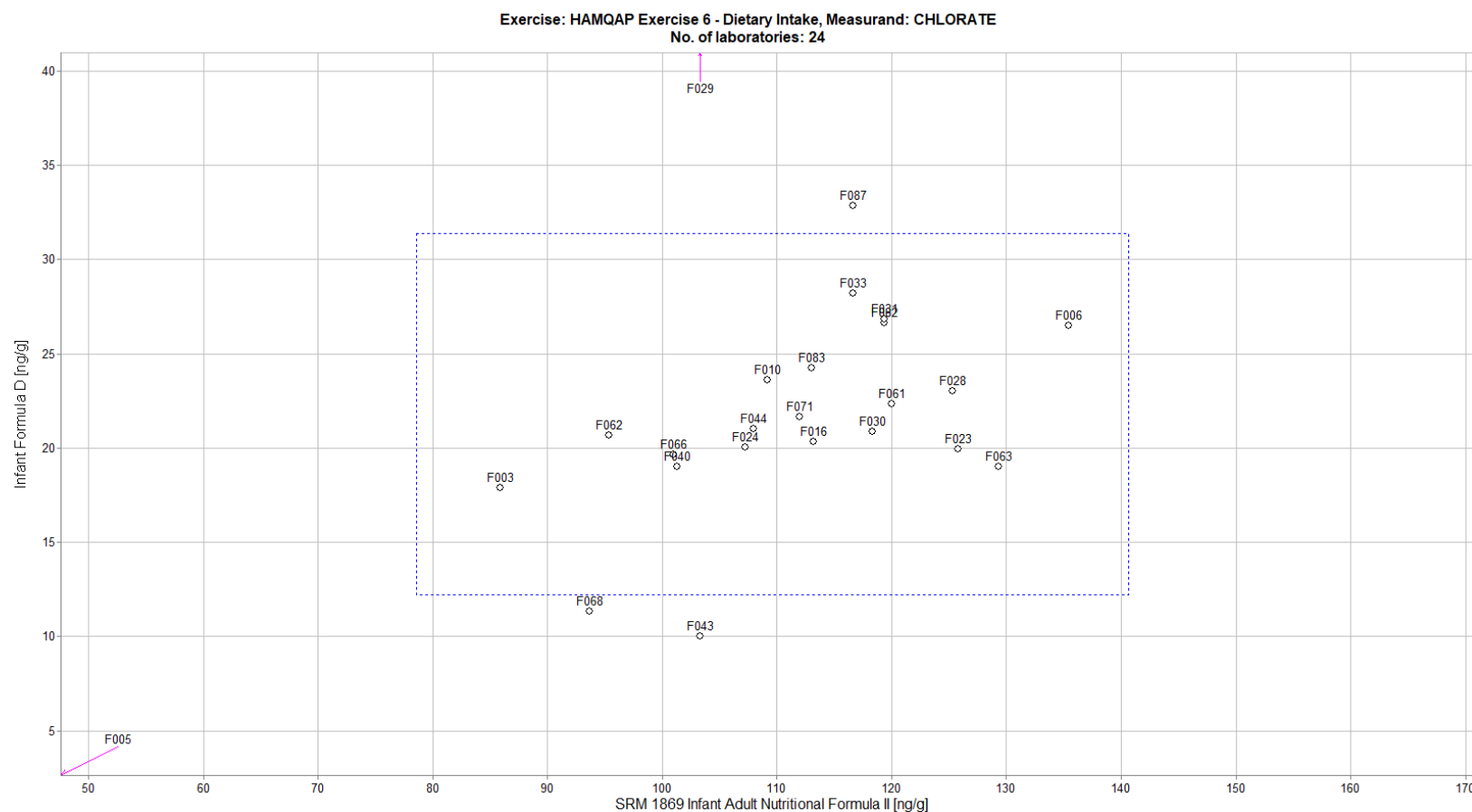


Figure 8-14. Laboratory means for chlorate in SRM 1869 Infant/Adult Nutritional Formula II (milk/whey/soy-based) and Infant Formula D (sample/sample comparison view). In this view, the individual laboratory mean for one sample (SRM 1869) is compared to the mean for a second sample (Infant Formula D).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

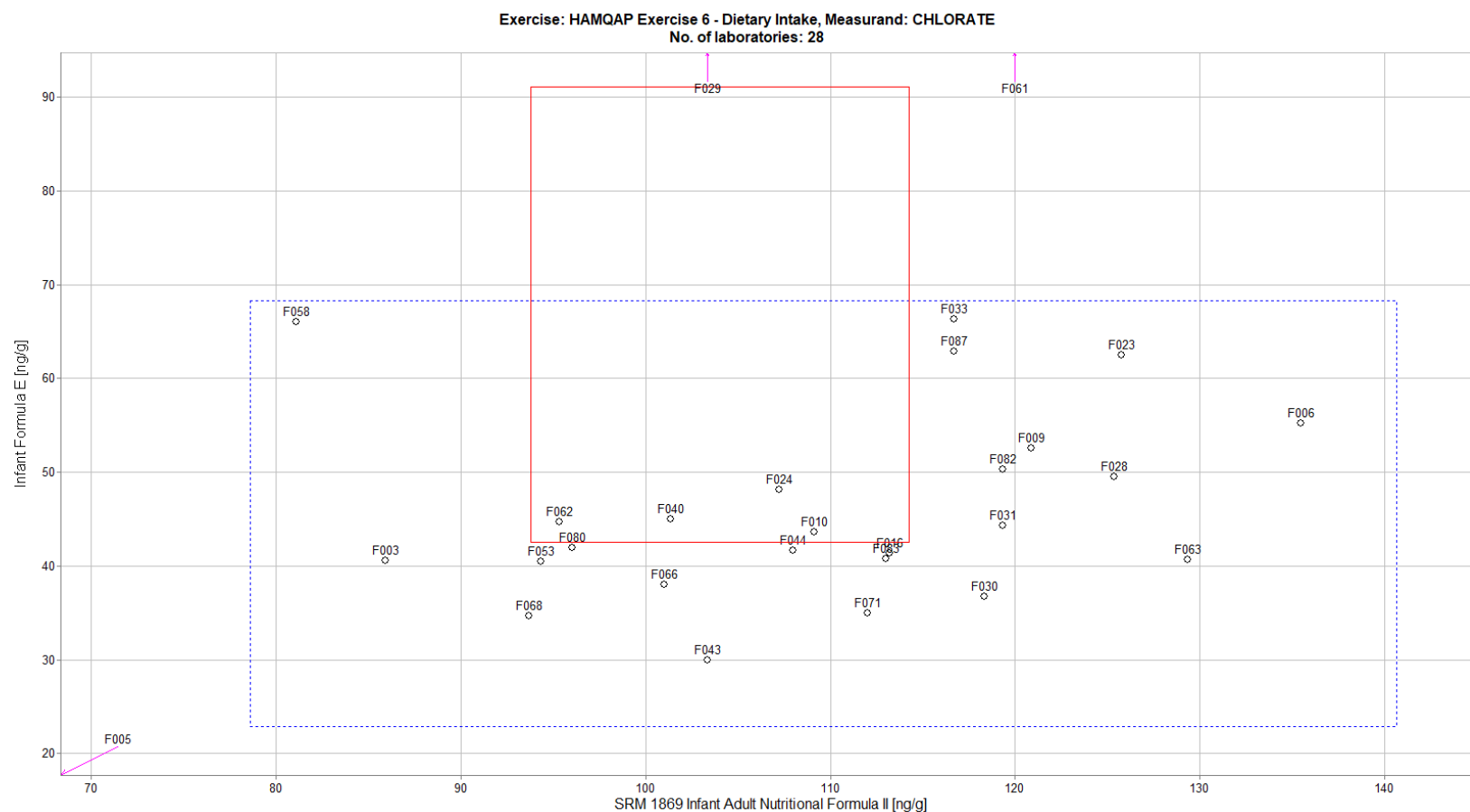


Figure 8-15. Laboratory means for chlorate in SRM 1869 Infant/Adult Nutritional Formula II (milk/whey/soy-based) and Infant Formula E (sample/sample comparison view). In this view, the individual laboratory mean for one sample (SRM 1869) is compared to the mean for a second sample (Infant Formula E).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

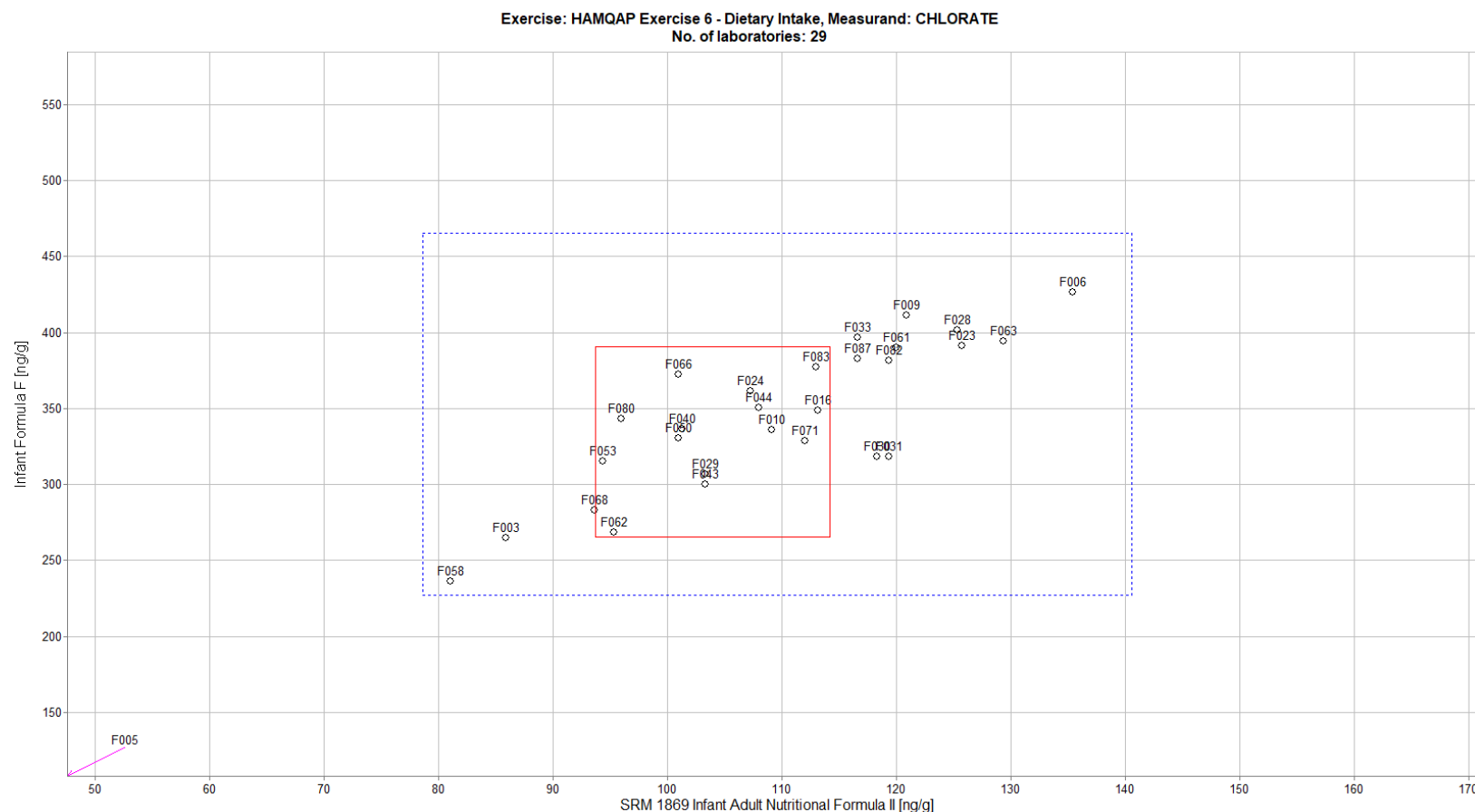


Figure 8-16. Laboratory means for chlorate in SRM 1869 Infant/Adult Nutritional Formula II (milk/whey/soy-based) and Infant Formula F (sample/sample comparison view). In this view, the individual laboratory mean for one sample (SRM 1869) is compared to the mean for a second sample (Infant Formula F).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

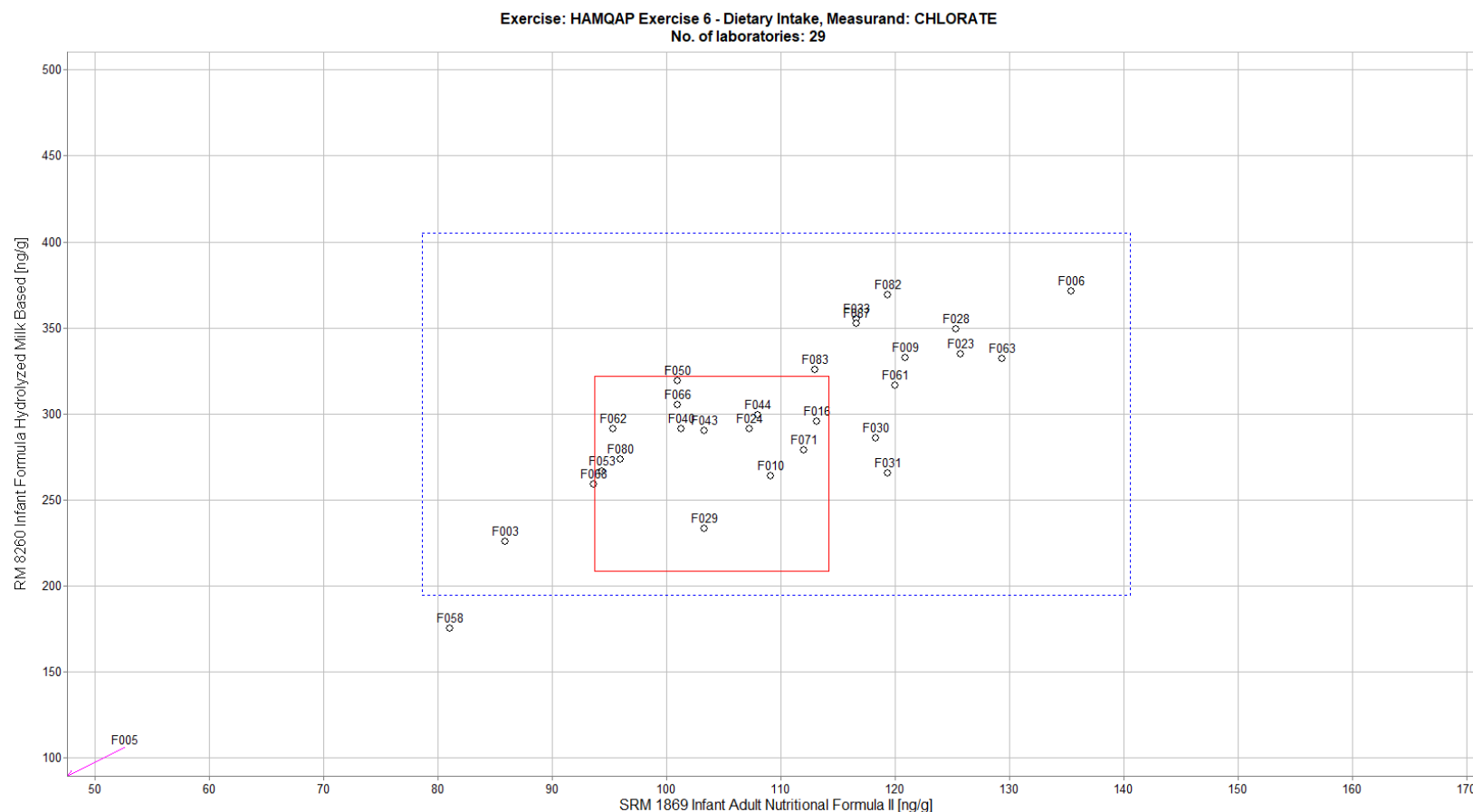


Figure 8-17. Laboratory means for chlorate in SRM 1869 Infant/Adult Nutritional Formula II (milk/whey/soy-based) and RM 8260 Infant Formula Hydrolyzed Milk Based (sample/sample comparison view). In this view, the individual laboratory mean for one sample (SRM 1869) is compared to the mean for a second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

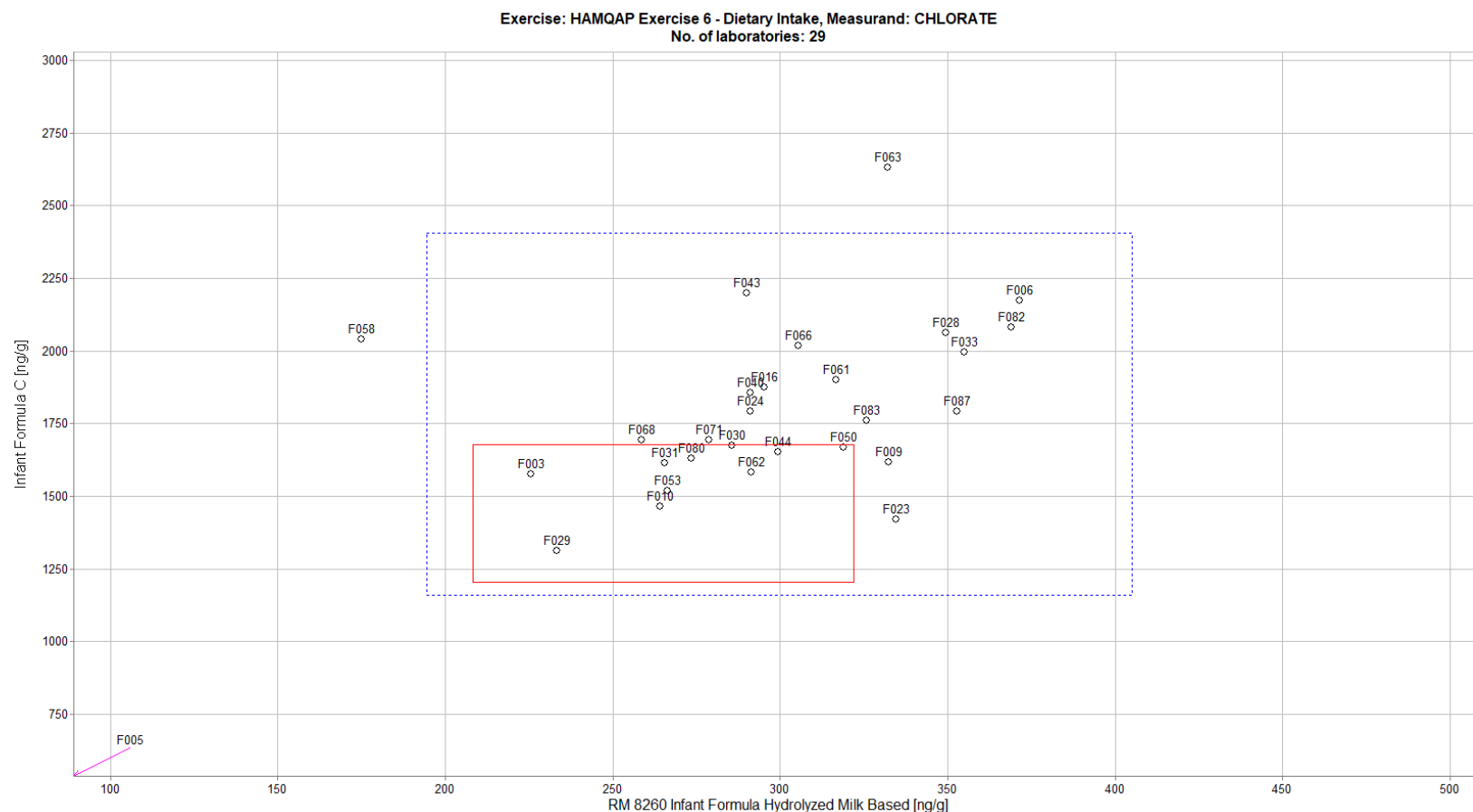


Figure 8-18. Laboratory means for chlorate in RM 8260 Infant Formula Hydrolyzed Milk Based and Infant Formula C (sample/sample comparison view). In this view, the individual laboratory mean for one sample (RM 8260) is compared to the mean for a second sample (Infant Formula C). The solid red box represents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

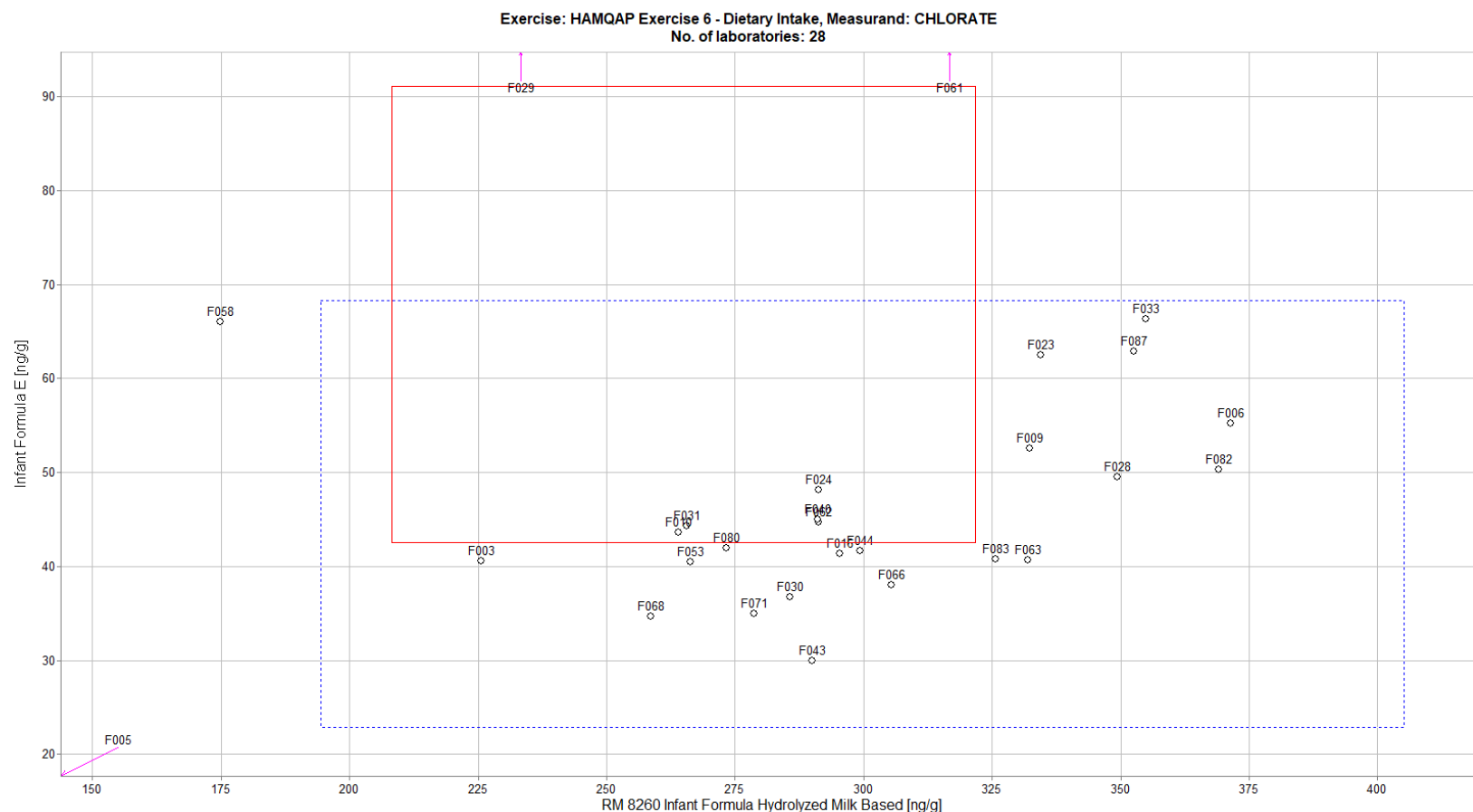


Figure 8-19. Laboratory means for chlorate in RM 8260 Infant Formula Hydrolyzed Milk Based and Infant Formula E (sample/sample comparison view). In this view, the individual laboratory mean for one sample (RM 8260) is compared to the mean for a second sample (Infant Formula E). The solid red box represents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

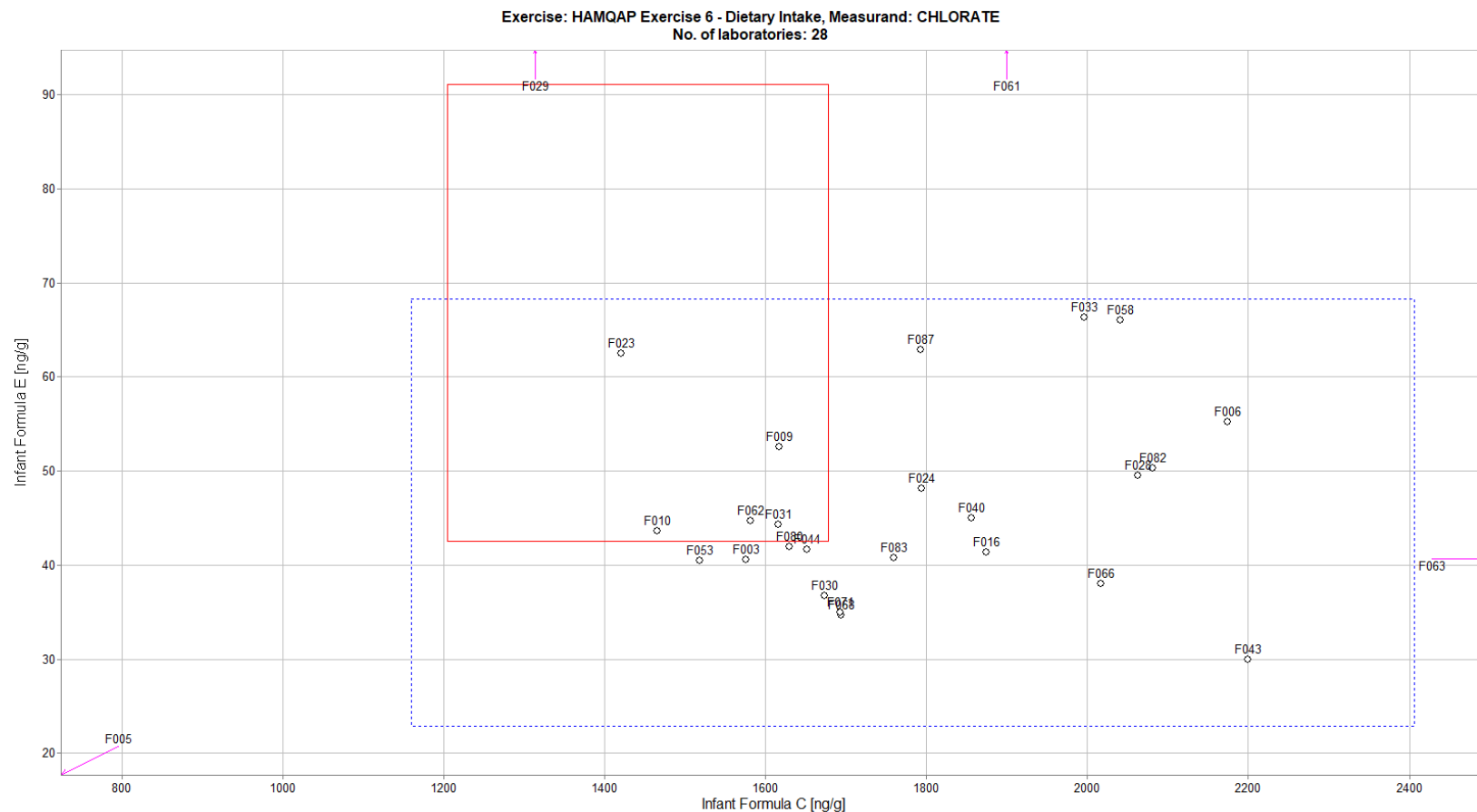


Figure 8-21. Laboratory means for chlorate in Infant Formula C and Infant Formula E (sample/sample comparison view). In this view, the individual laboratory mean for one sample (Infant Formula C) is compared to the mean for a second sample (Infant Formula E). The solid red box represents the NIST range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

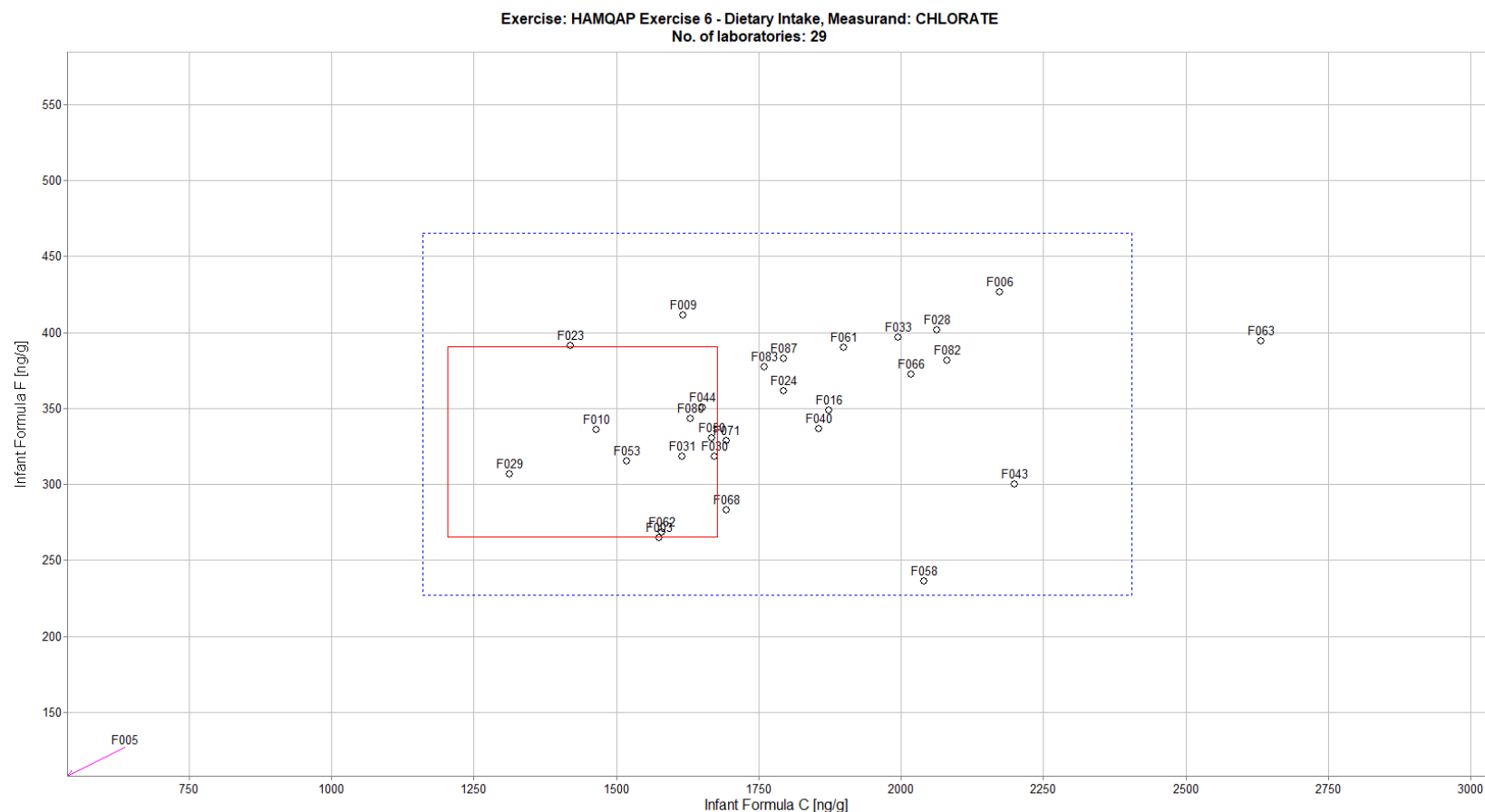


Figure 8-22. Laboratory means for chlorate in Infant Formula C and Infant Formula F (sample/sample comparison view). In this view, the individual laboratory mean for one sample (Infant Formula C) is compared to the mean for a second sample (Infant Formula F). The solid red box represents the NIST range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

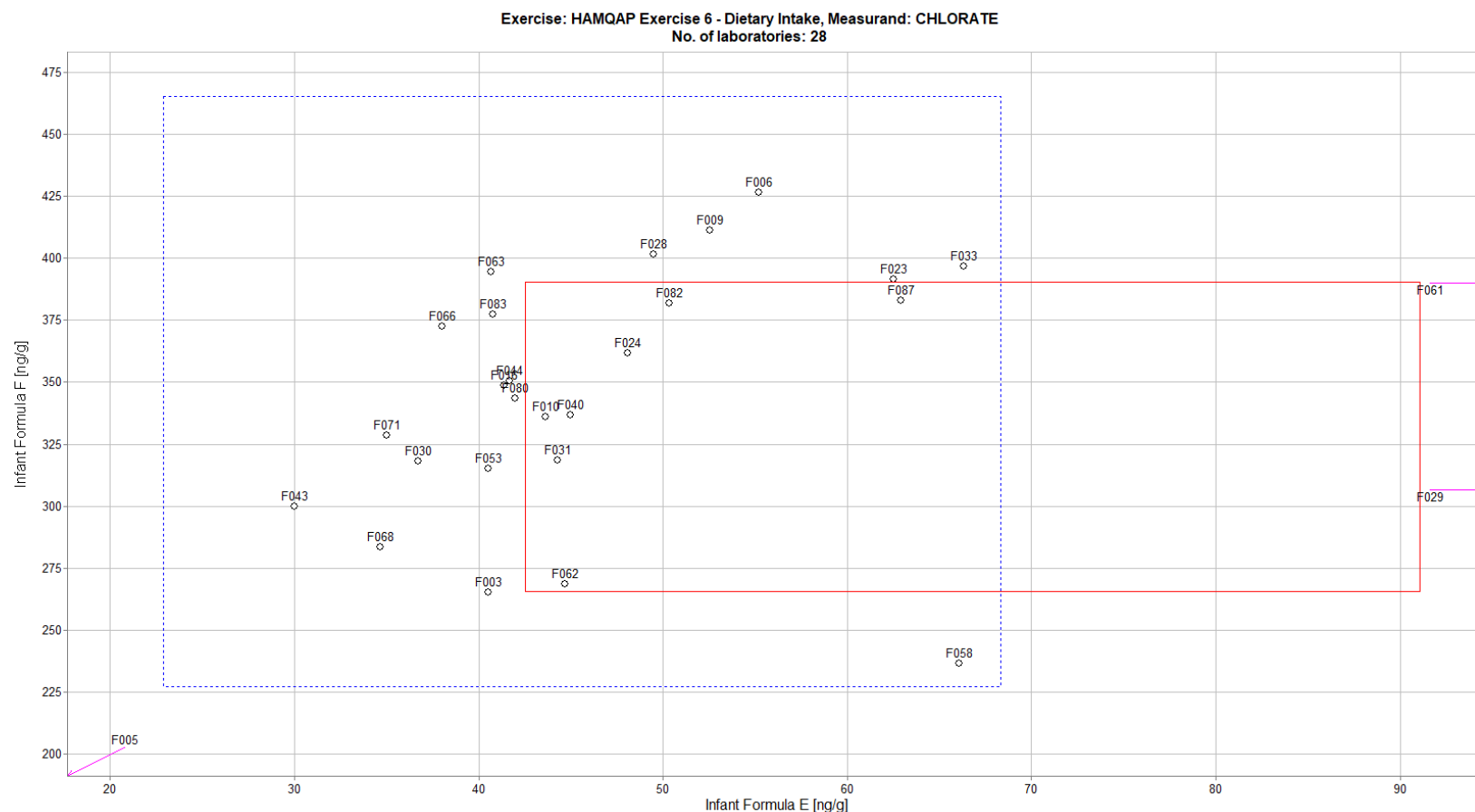


Figure 8-23. Laboratory means for chlorate in Infant Formula E and Infant Formula F (sample/sample comparison view). In this view, the individual laboratory mean for one sample (Infant Formula E) is compared to the mean for a second sample (Infant Formula F). The solid red box represents the NIST range of tolerance for the two samples Infant Formula E (x-axis) and Infant Formula F (y-axis), which encompasses the target values bounded by their uncertainties (U_{NIST}) and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$. The dotted blue box represents the consensus range of tolerance for Infant Formula E (x-axis) and Infant Formula F (y-axis), calculated as the values above and below the consensus means that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$.

Table 8-3. Data summary table for perchlorate in infant formulas. Data points highlighted in blue have been identified as outside the consensus tolerance limits and would be estimated to result in an unacceptable Z'_{comm} score, $|Z'_{\text{comm}}| \geq 2$. Data points highlighted in red have a zero or non-numeric data point. *Note that this table spans two pages.*

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

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

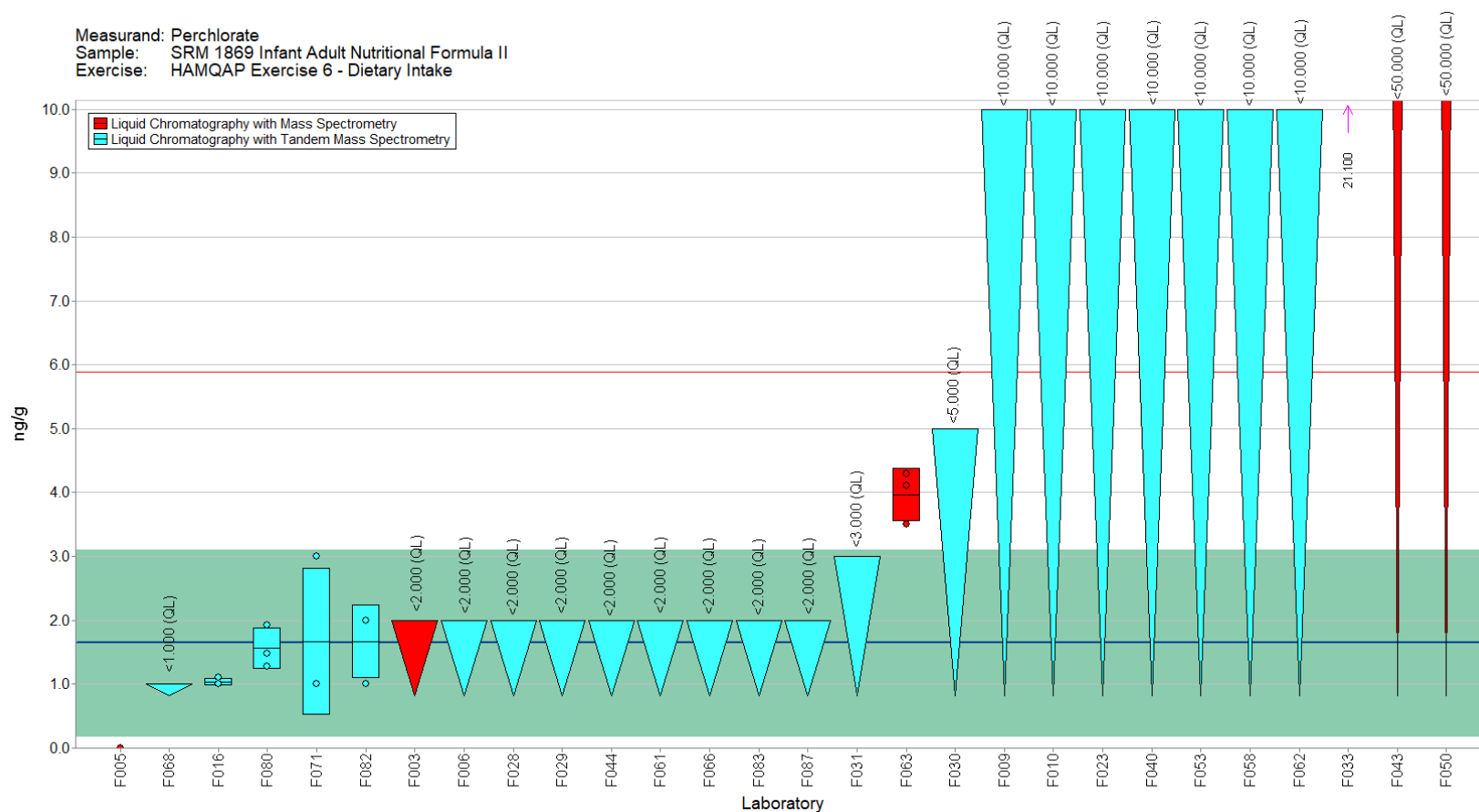


Figure 8-24. Perchlorate in SRM 1869 Infant/Adult Nutritional Formula II (milk/whey/soy-based) (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red line represents the upper consensus range of tolerance, calculated as the values above the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$, with the lower limit set at zero. A NIST value has not been determined in this material.

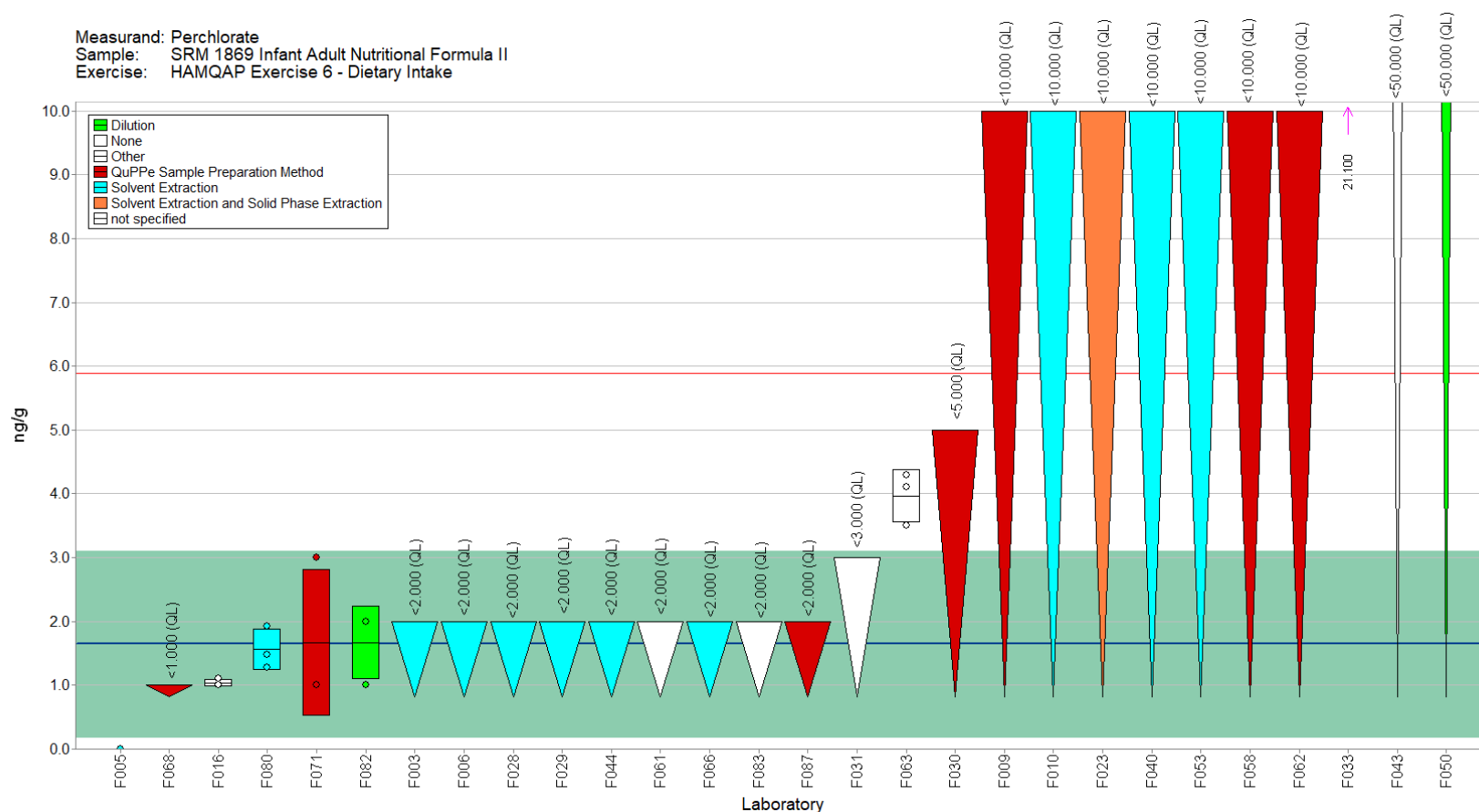


Figure 8-25. Perchlorate in SRM 1869 Infant/Adult Nutritional Formula II (milk/whey/soy-based) (data summary view – sample preparation method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red line represents the upper consensus range of tolerance, calculated as the values above the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$, with the lower limit set at zero. A NIST value has not been determined in this material.

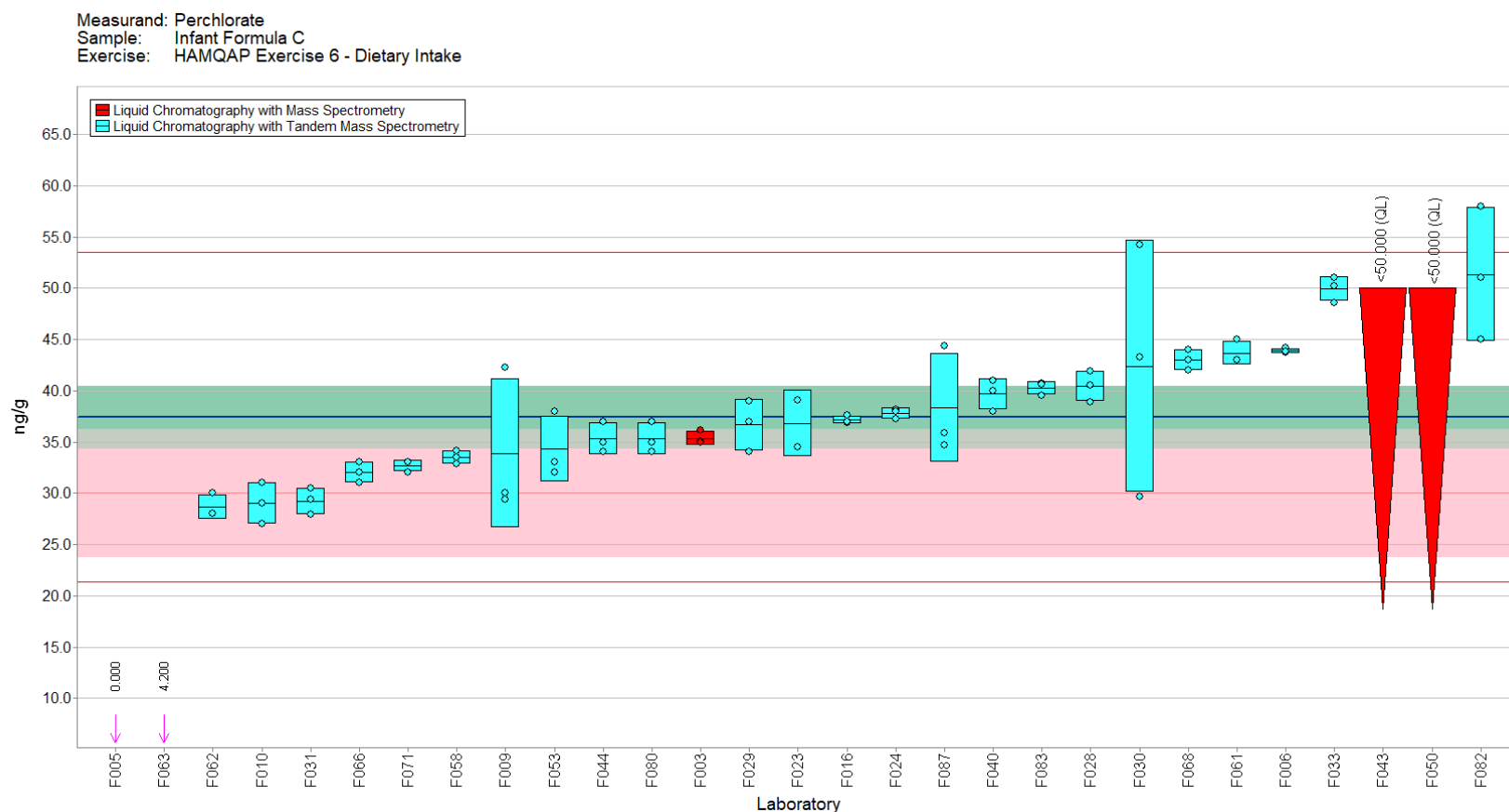


Figure 8-26. Perchlorate in Infant Formula C (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red line represents the upper consensus range of tolerance, calculated as the values above the consensus mean that result in an acceptable Z'_{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, $|Z'_{\text{NIST}}| \leq 2$.

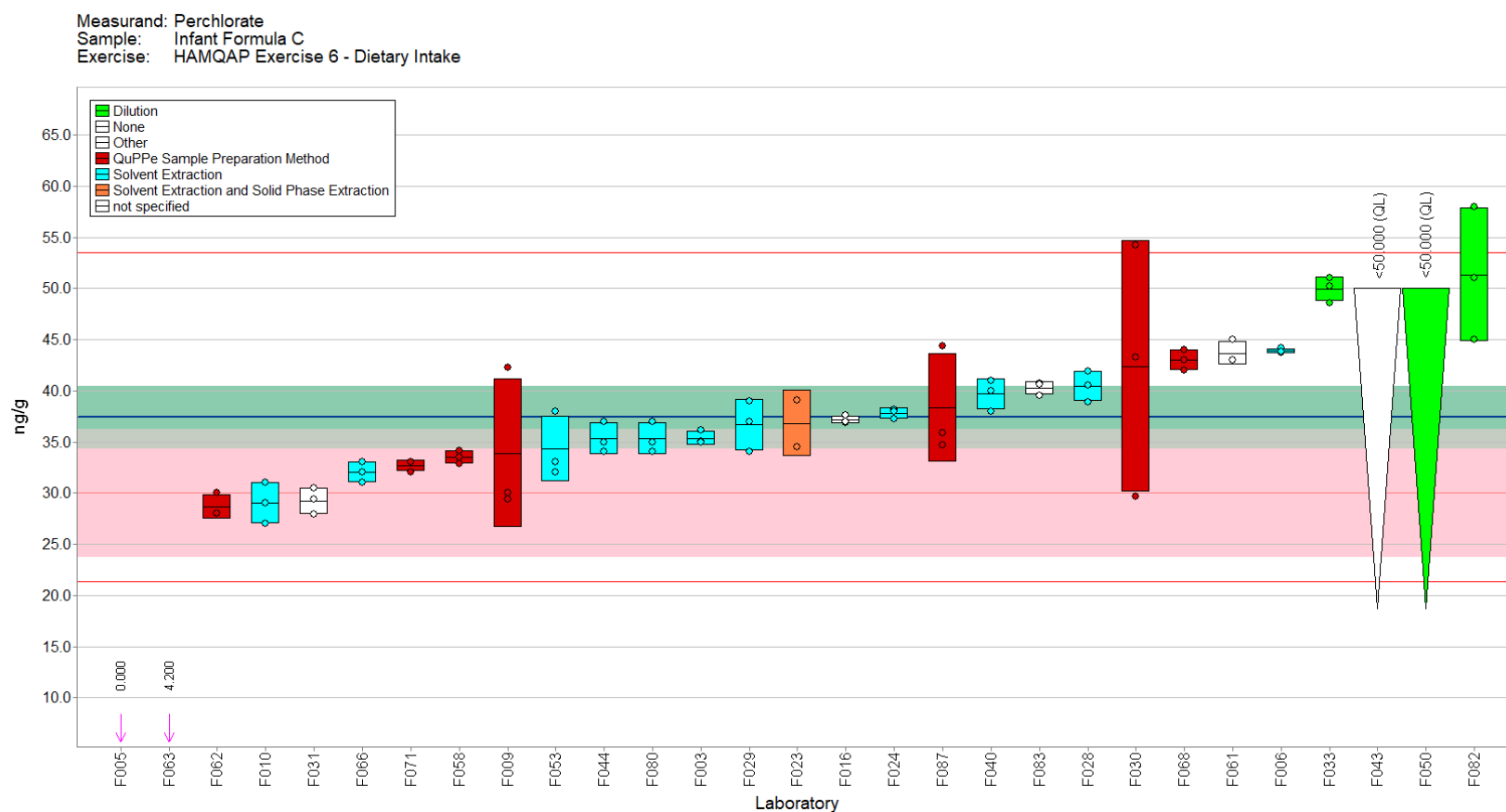


Figure 8-27. Perchlorate in Infant Formula C (data summary view – sample preparation method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red line represents the upper consensus range of tolerance, calculated as the values above the consensus mean that result in an acceptable Z'_{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, $|Z'_{\text{NIST}}| \leq 2$.

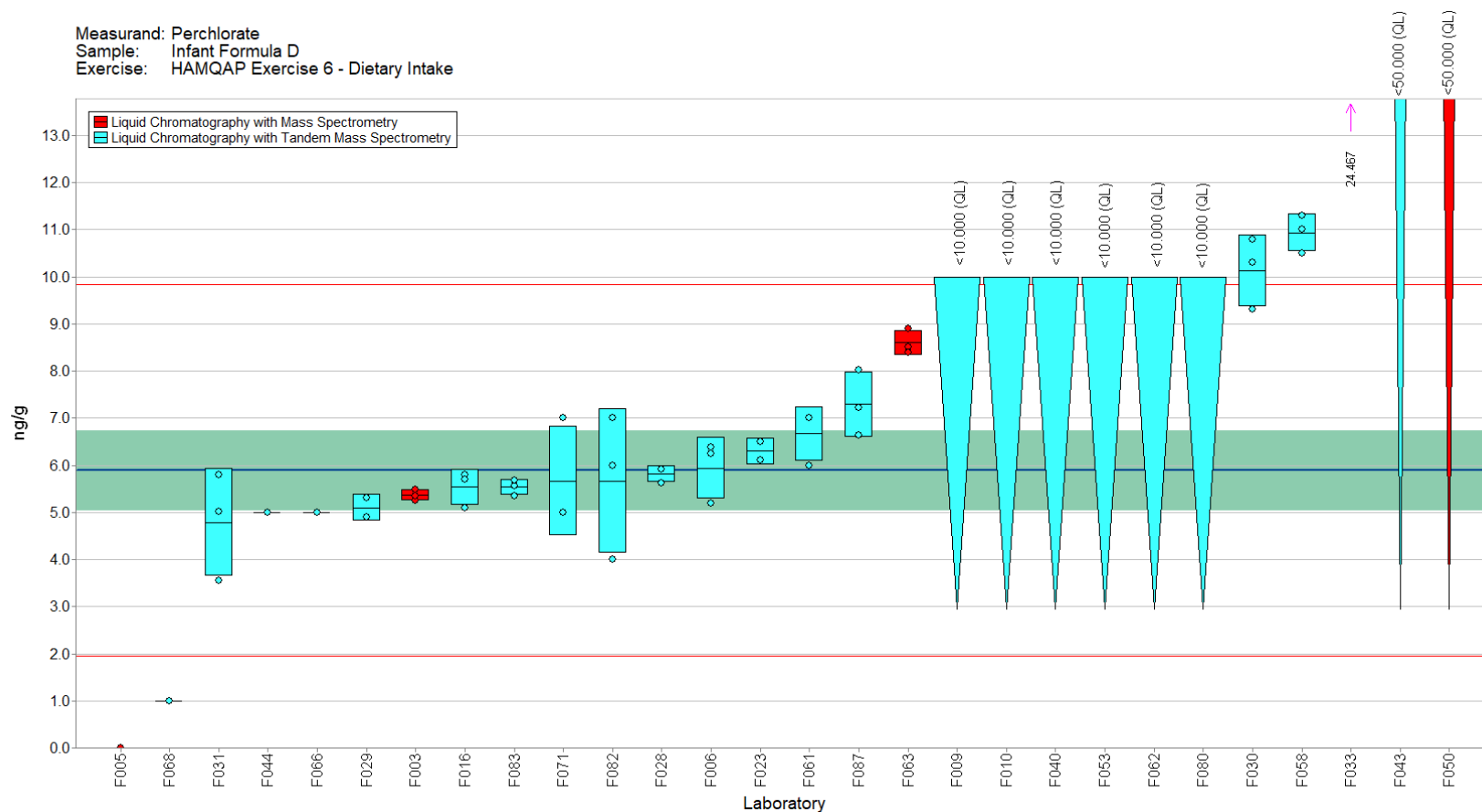


Figure 8-28. Perchlorate in Infant Formula D (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red line represents the upper consensus range of tolerance, calculated as the values above the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$, with the lower limit set at zero. A NIST value has not been determined in this material.

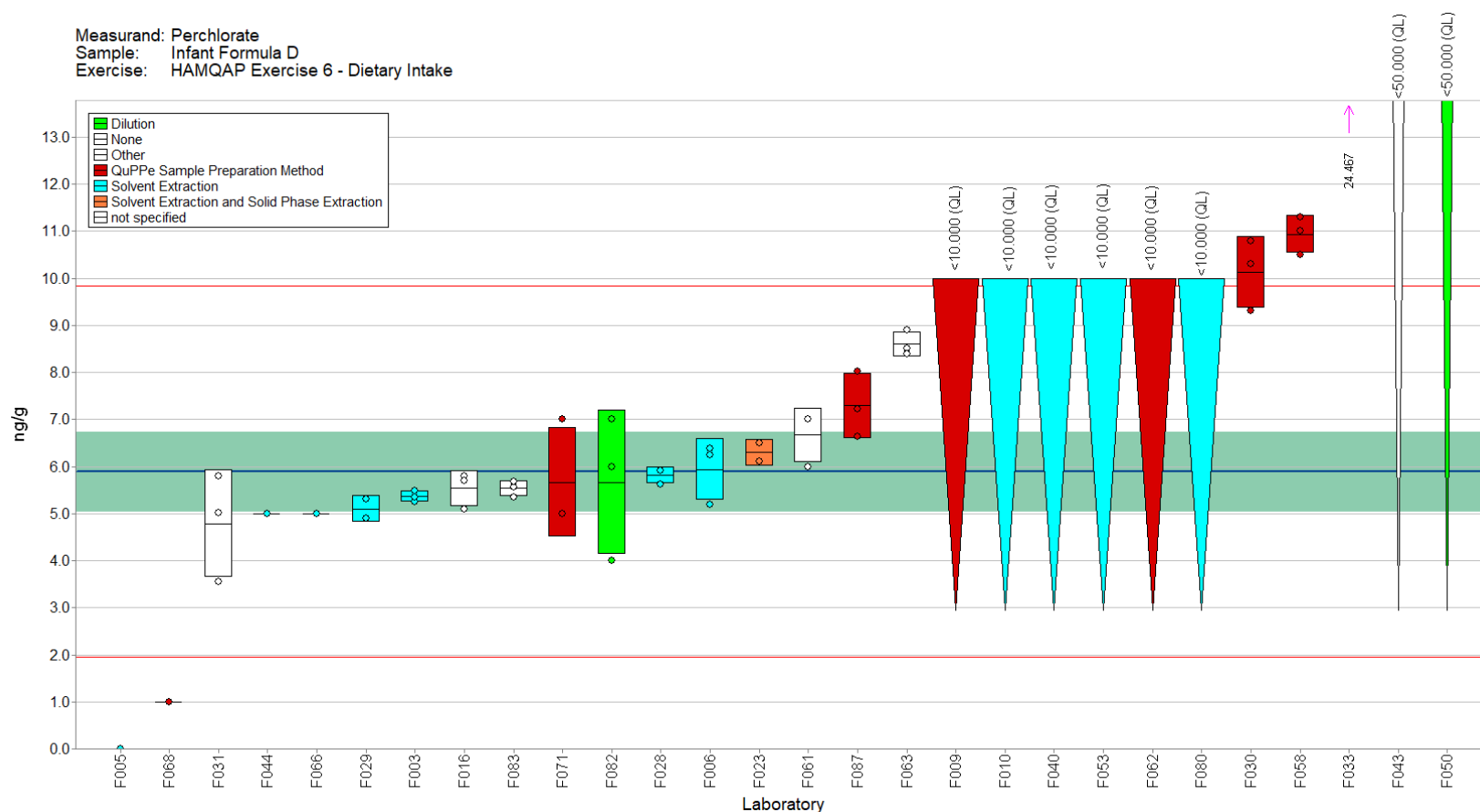


Figure 8-29. Perchlorate in Infant Formula D (data summary view – sample preparation method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red line represents the upper consensus range of tolerance, calculated as the values above the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$, with the lower limit set at zero. A NIST value has not been determined in this material.

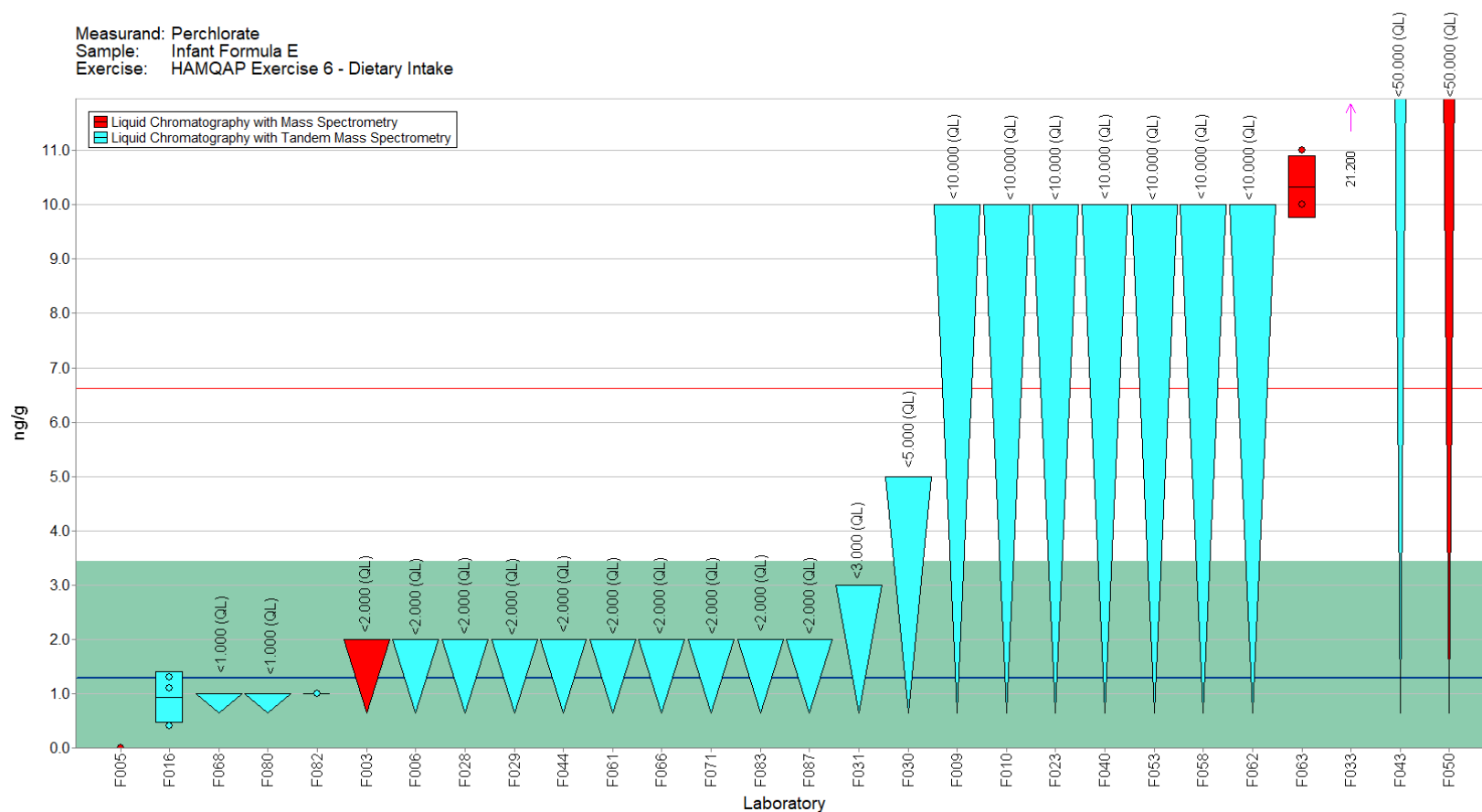


Figure 8-30. Perchlorate in Infant Formula E (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The red solid lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$. A NIST value has not been determined in this material.

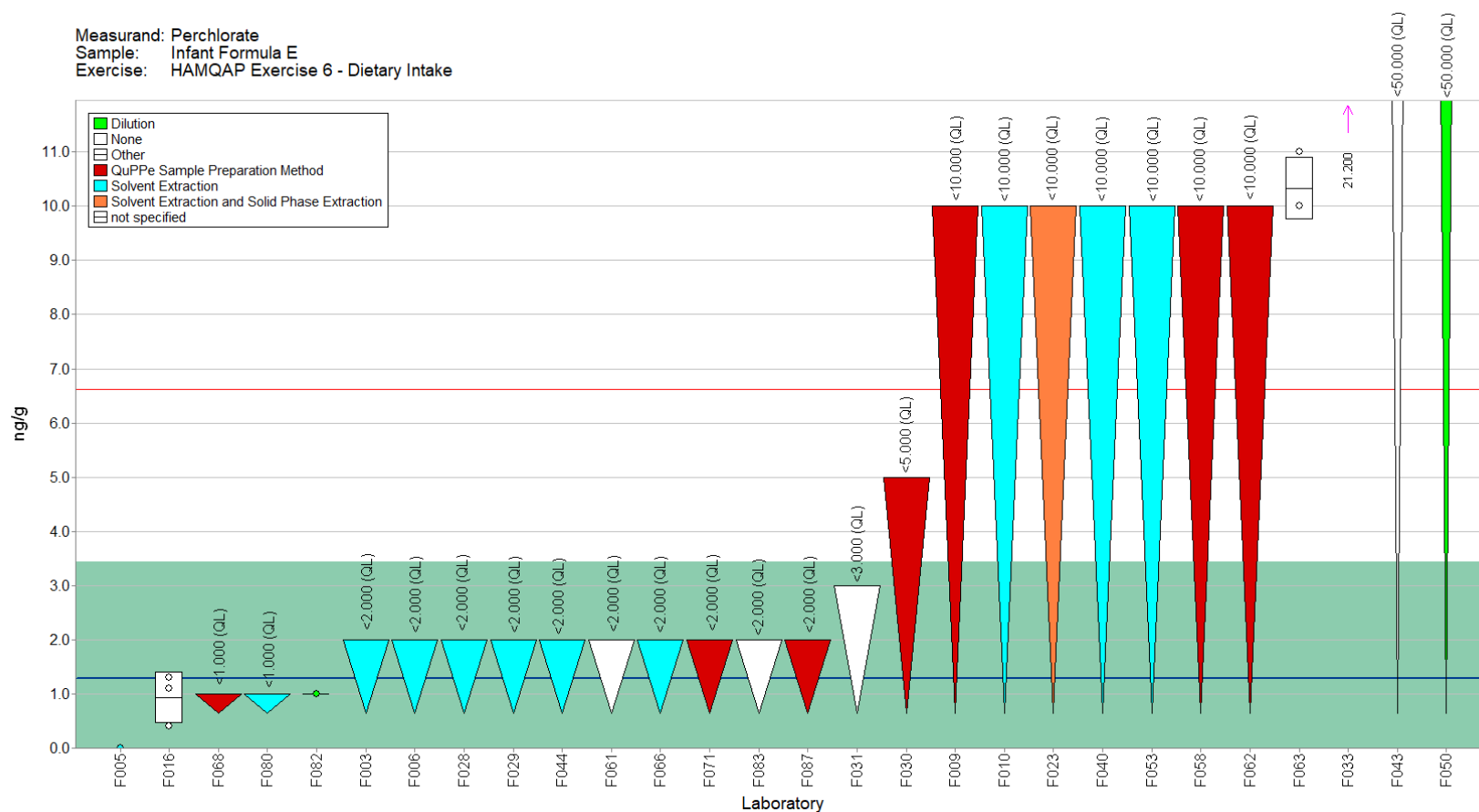


Figure 8-31. Perchlorate in Infant Formula E (data summary view – sample preparation method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The red solid lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$. A NIST value has not been determined in this material.

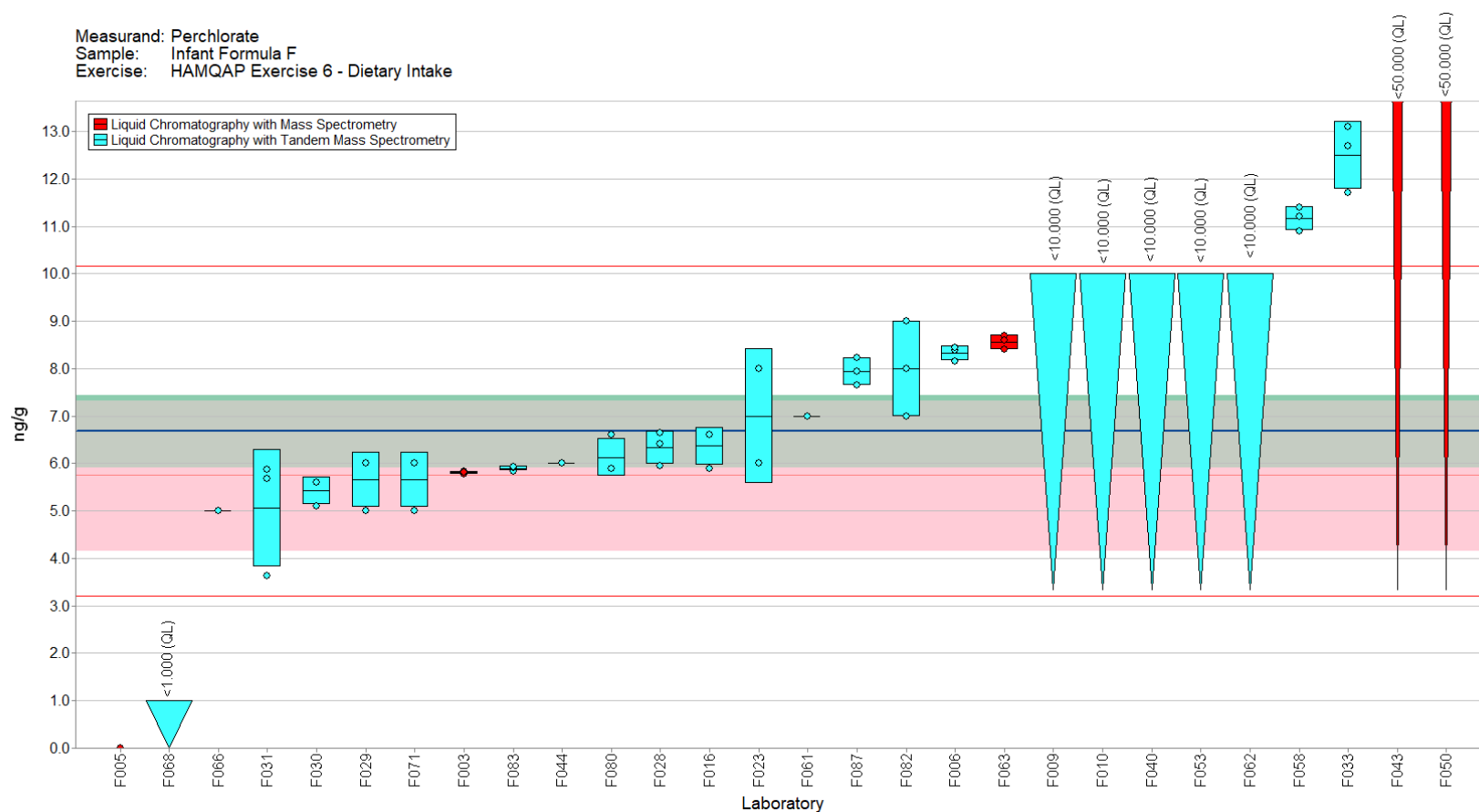


Figure 8-32. Perchlorate in Infant Formula F (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The red solid lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty (U_{NIST}) and represents the range that results in an acceptable Z'_{NIST} score, $|Z'_{\text{NIST}}| \leq 2$.

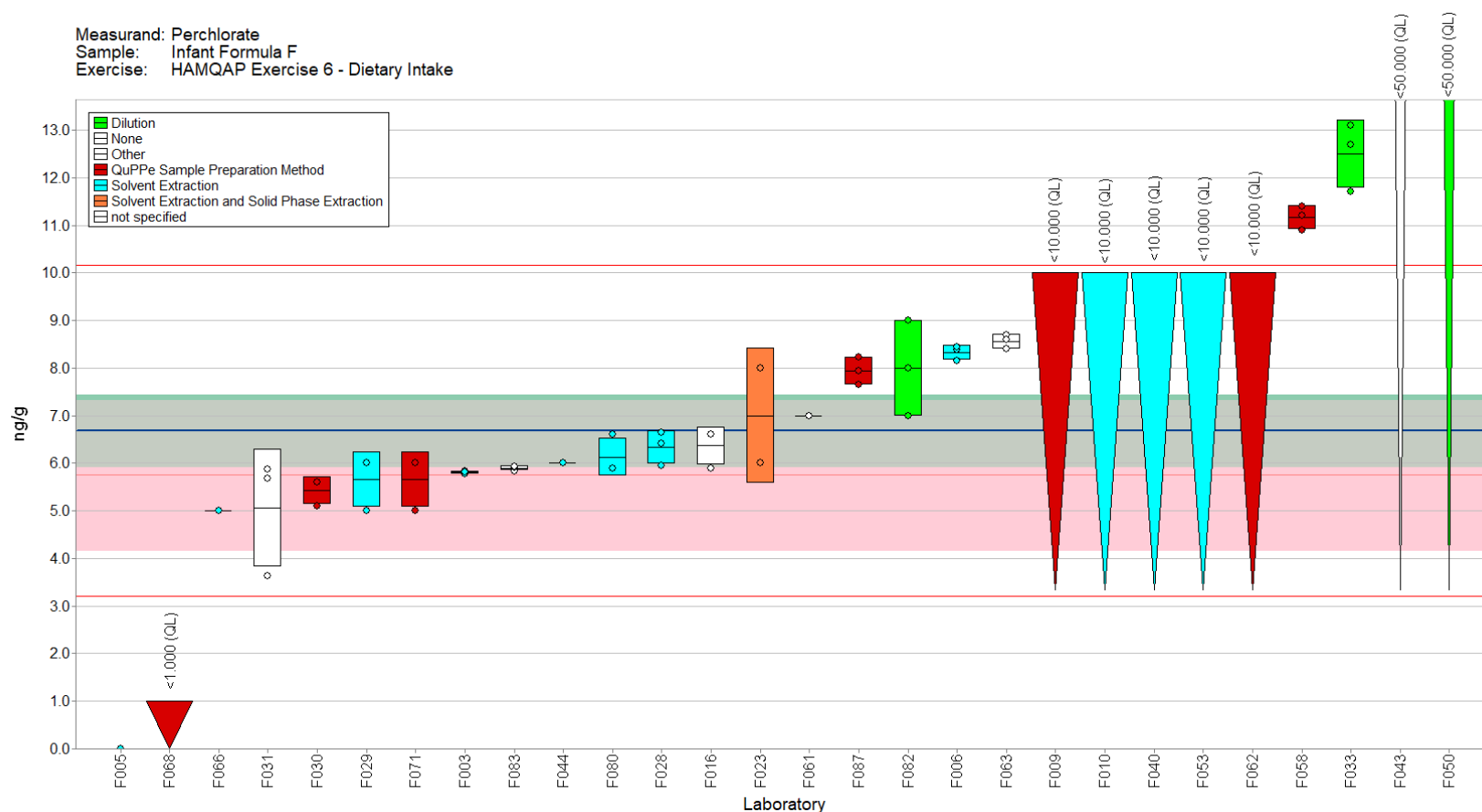


Figure 8-33. Perchlorate in Infant Formula F (data summary view – sample preparation method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed. The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The red solid lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty (U_{NIST}) and represents the range that results in an acceptable Z'_{NIST} score, $|Z'_{\text{NIST}}| \leq 2$.

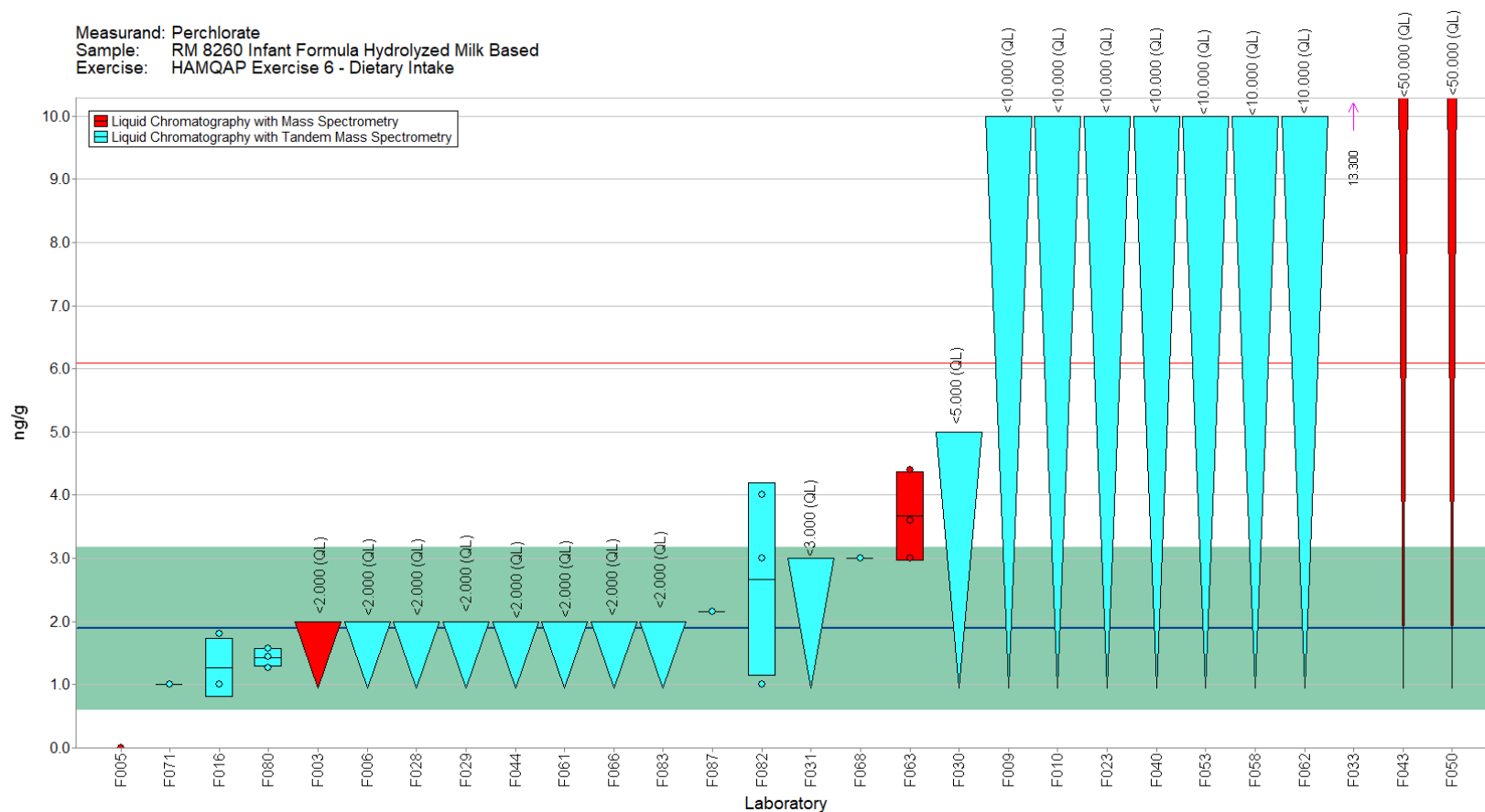


Figure 8-34. Perchlorate in RM 8260 Infant Formula Hydrolyzed Milk Based (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The red solid lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$. A NIST value has not been determined in this material.

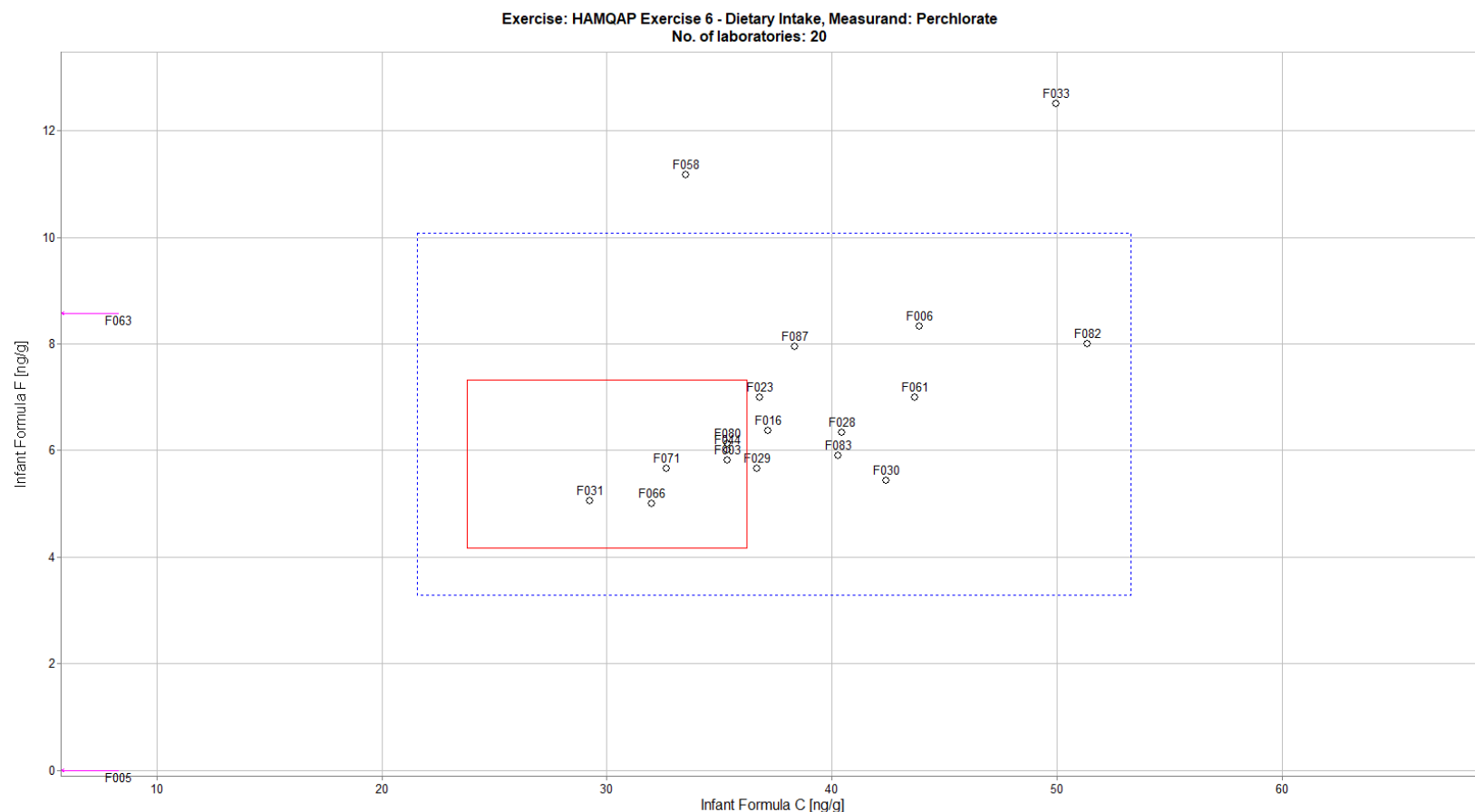


Figure 8-36. Laboratory means for perchlorate in Infant Formula C and Infant Formula F (sample/sample comparison view). In this view, the individual laboratory mean for one sample (Infant Formula C) is compared to the mean for a second sample (Infant Formula F). The solid red box represents the NIST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

SECTION 9: CONTAMINANTS II (Glyphosate, AMPA)

Study Overview

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

Dietary Intake Sample Information

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

Dietary Intake Study Results

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

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

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

<u>Analyte</u>	<u>Between-Laboratory Variability (RSD)</u>
----------------	---

	<u>Oat Flour A</u>	<u>Oat Flour B</u>
Glyphosate	21 %	30 %
AMPA	55 %	98 %

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

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

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

Dietary Intake Technical Recommendations

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

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

- No trends were observed that correlated reported results with the analytical method approach used.
- No trends were observed between direct and derivatized analysis.
- Most laboratories reported use of MS-based methodologies for determination of glyphosate and AMPA. Isotopically labeled internal standards, added at the beginning of the analytical procedure, often result in improved accuracy and precision of results.
- “Zero” is not a quantity that can be measured, and therefore a more appropriate result would be to report that a value is below the LOQ or QL.
- The use of appropriate calibration materials and quality assurance samples to establish that a method is in control and performing correctly may reduce the likelihood of outlying data. Quality assurance samples can be commercially available reference materials (CRMs, SRMs, or RMs) or materials prepared in-house.
- A linear calibration curve which surrounds the expected sample concentration values should be used for calculations. This curve should include both the lowest and highest expected concentration values of the sample solutions. Extrapolation of results beyond calibration curves may result in incorrect values.
- In general, all results should be checked closely to avoid calculation errors and to be sure that results are reported in the requested units.

Table 9-1. Individualized data summary table (NIST) for glyphosate and AMPA in Oat Flour A and Oat Flour B.*National Institute of Standards and Technology*

			HAMQAP Exercise 6 - Contaminants II						
Lab Code:		NIST	1. Your Results				2. Community Results		
Analyte	Sample	Units	x_i	s_i	Z'_{comm}	Z_{NIST}	N	x^*	s^*
AMPA	Oat Flour A	ng/g					12	20	11
AMPA	Oat Flour B	ng/g					6	8	7.9
Glyphosate	Oat Flour A	ng/g					24	420	90
Glyphosate	Oat Flour B	ng/g					24	70	20
			x_i	Mean of reported values			N	Number of quantitative values reported	
			s_i	Standard deviation of reported values					x_{NIST} NIST-assessed value
			Z'_{comm}	Z'-score with respect to community consensus			x^*	Robust mean of reported values	
			Z_{NIST}	Z-score with respect to NIST value			s^*	Robust standard deviation	
									U expanded uncertainty about the NIST-assessed value

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

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

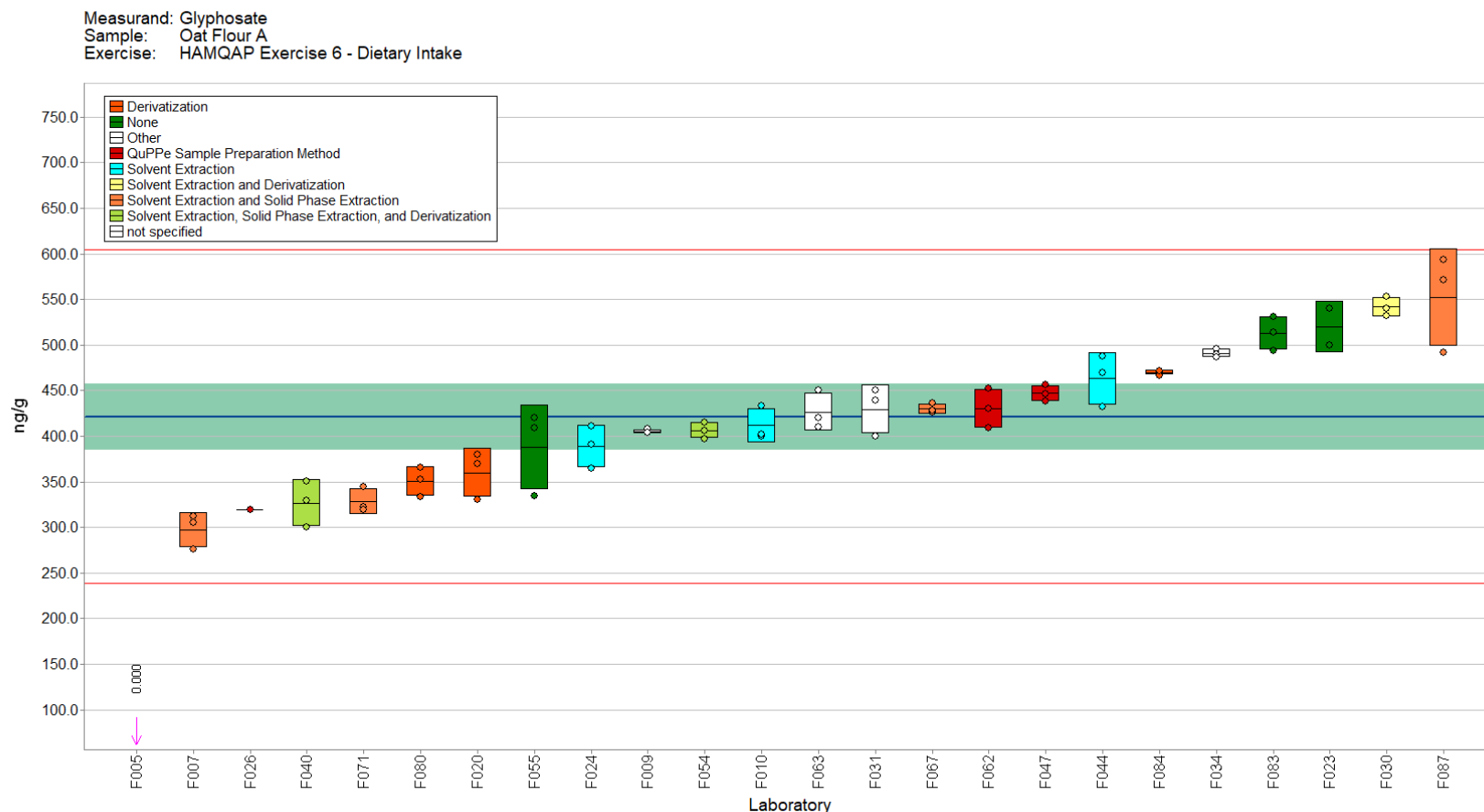


Figure 9-1. Glyphosate in Oat Flour A (data summary view –sample preparation). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation employed. The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The red solid lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$. A NIST value has not been determined in this material.

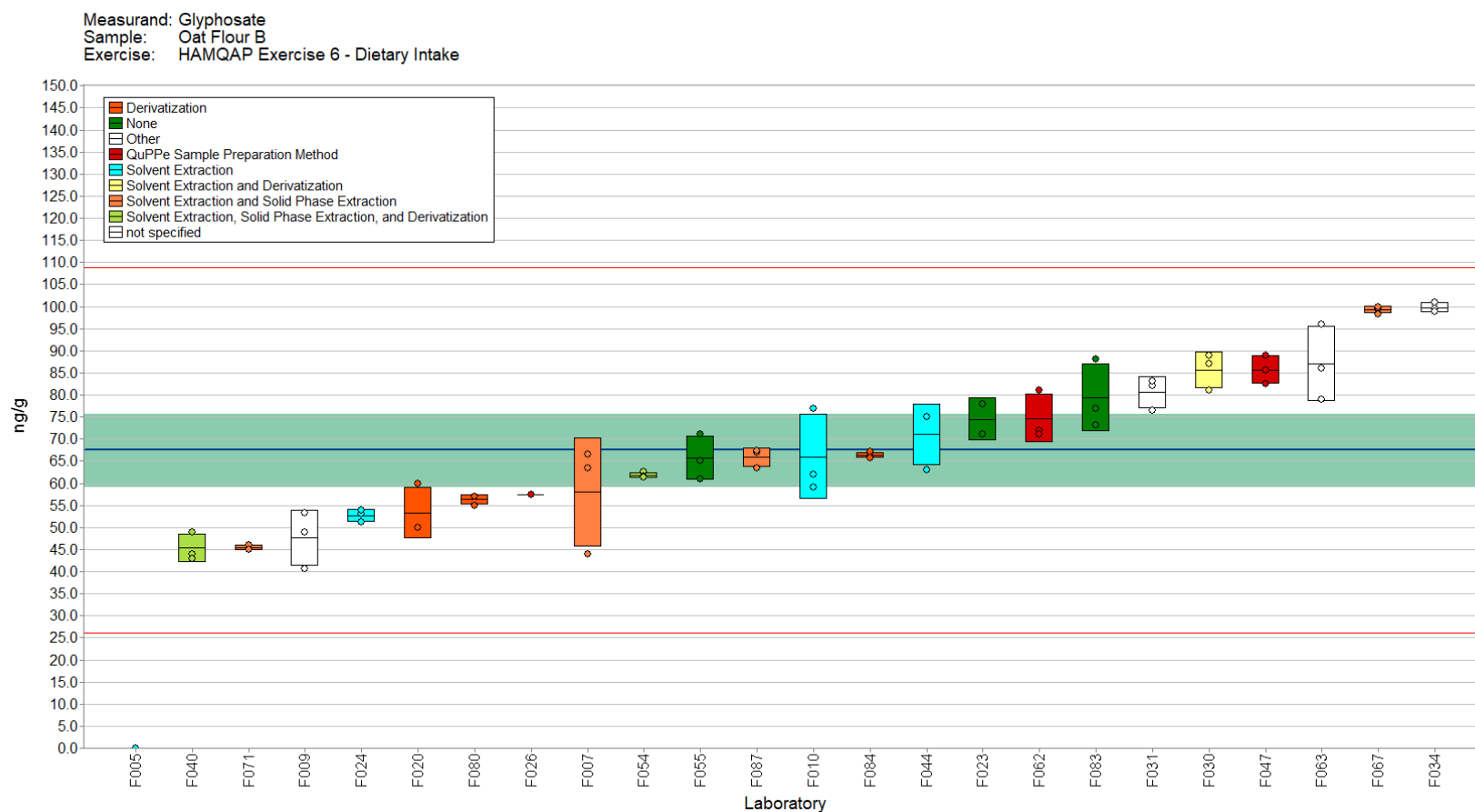


Figure 9-2. Glyphosate in Oat Flour B (data summary view – sample preparation). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation employed. The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The red solid lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$. A NIST value has not been determined in this material.

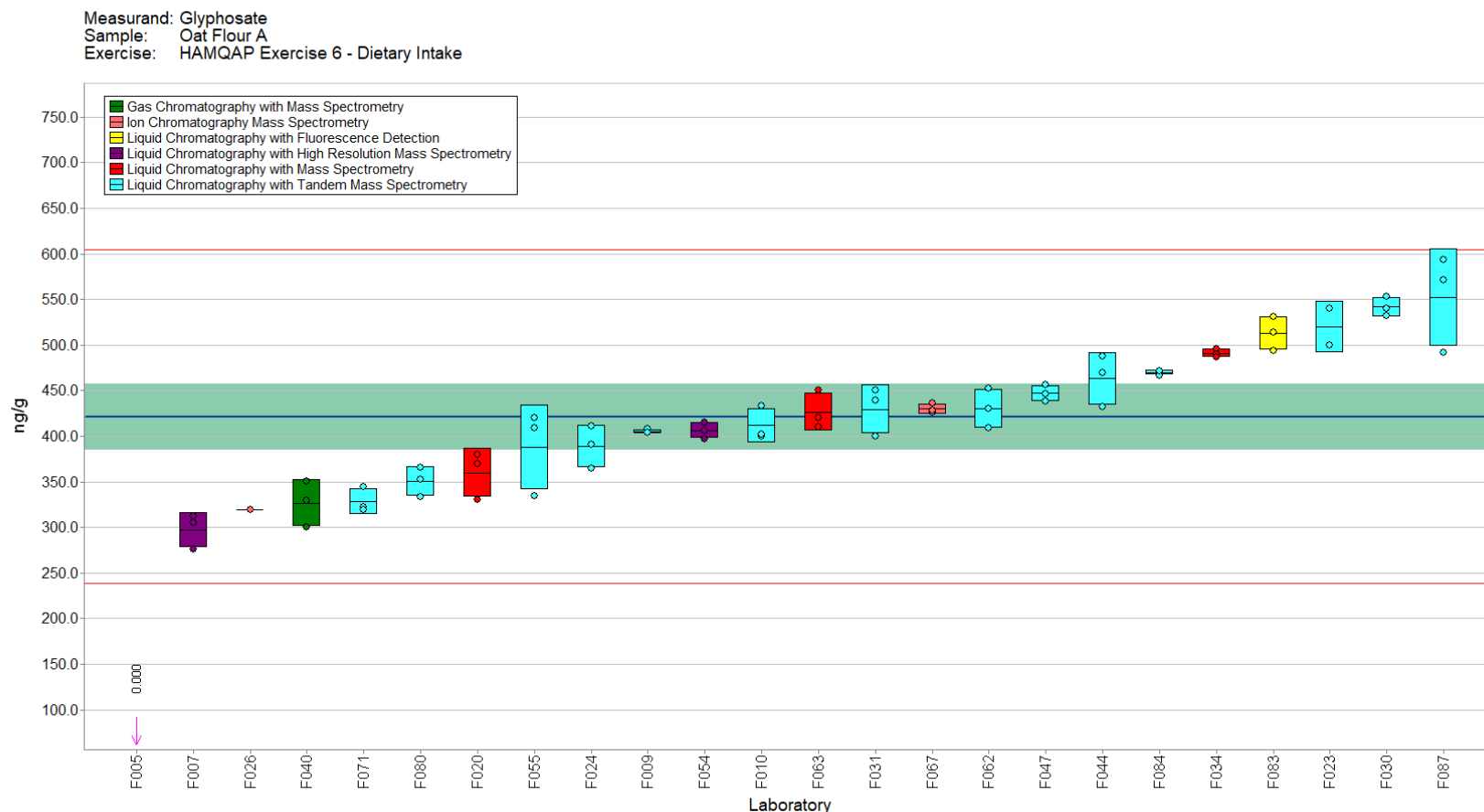


Figure 9-3. Glyphosate in Oat Flour A (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The red solid lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$. A NIST value has not been determined in this material.

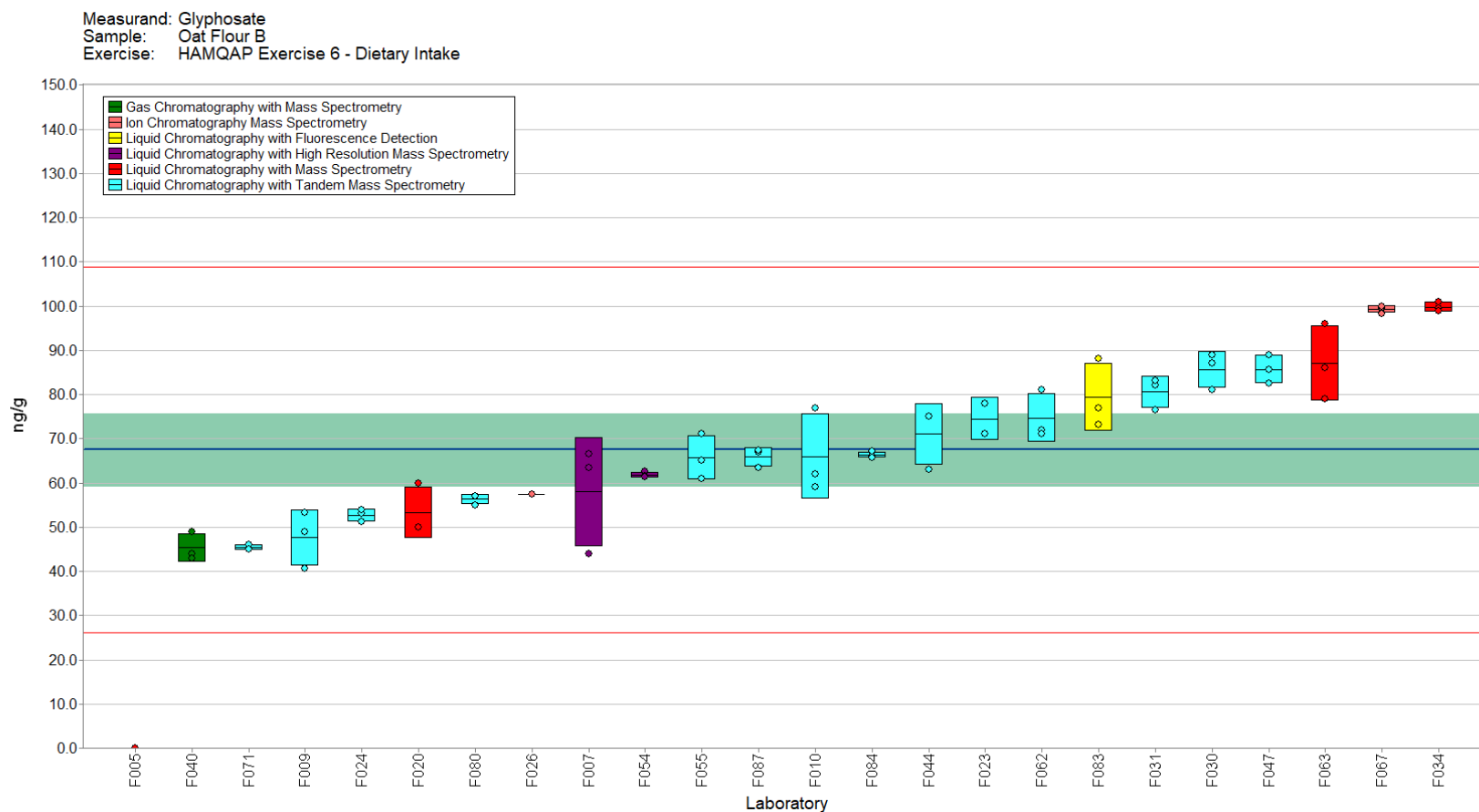


Figure 9-4. Glyphosate in Oat Flour B (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The red solid lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$. A NIST value has not been determined in this material.

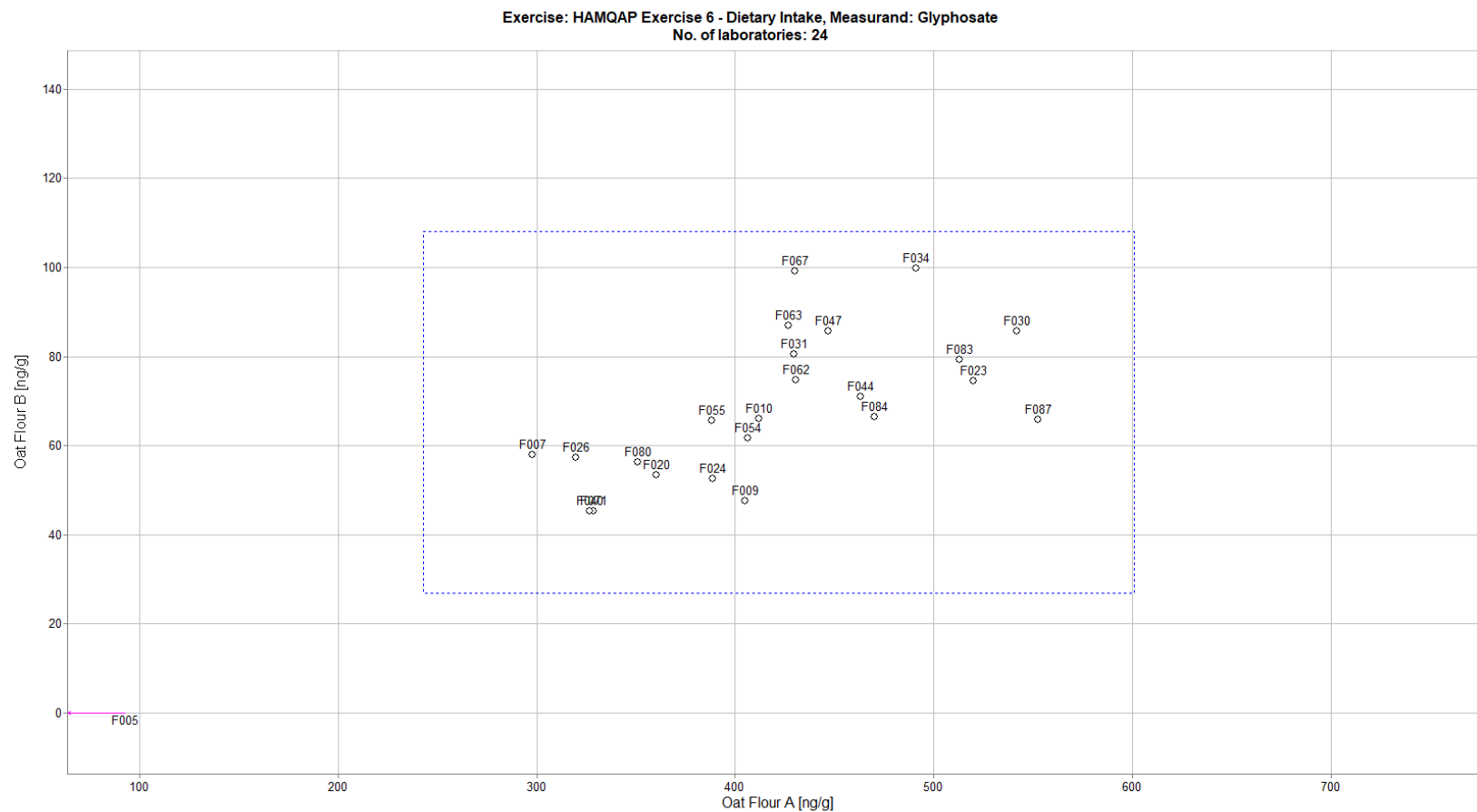


Figure 9-5. Laboratory means for glyphosate in Oat Flour A and Oat Flour B (sample/sample comparison view). In this view, the individual laboratory mean for one sample (Oat Flour A) is compared to the individual laboratory mean for a second sample (Oat Flour B). The dotted blue box represents the consensus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

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

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

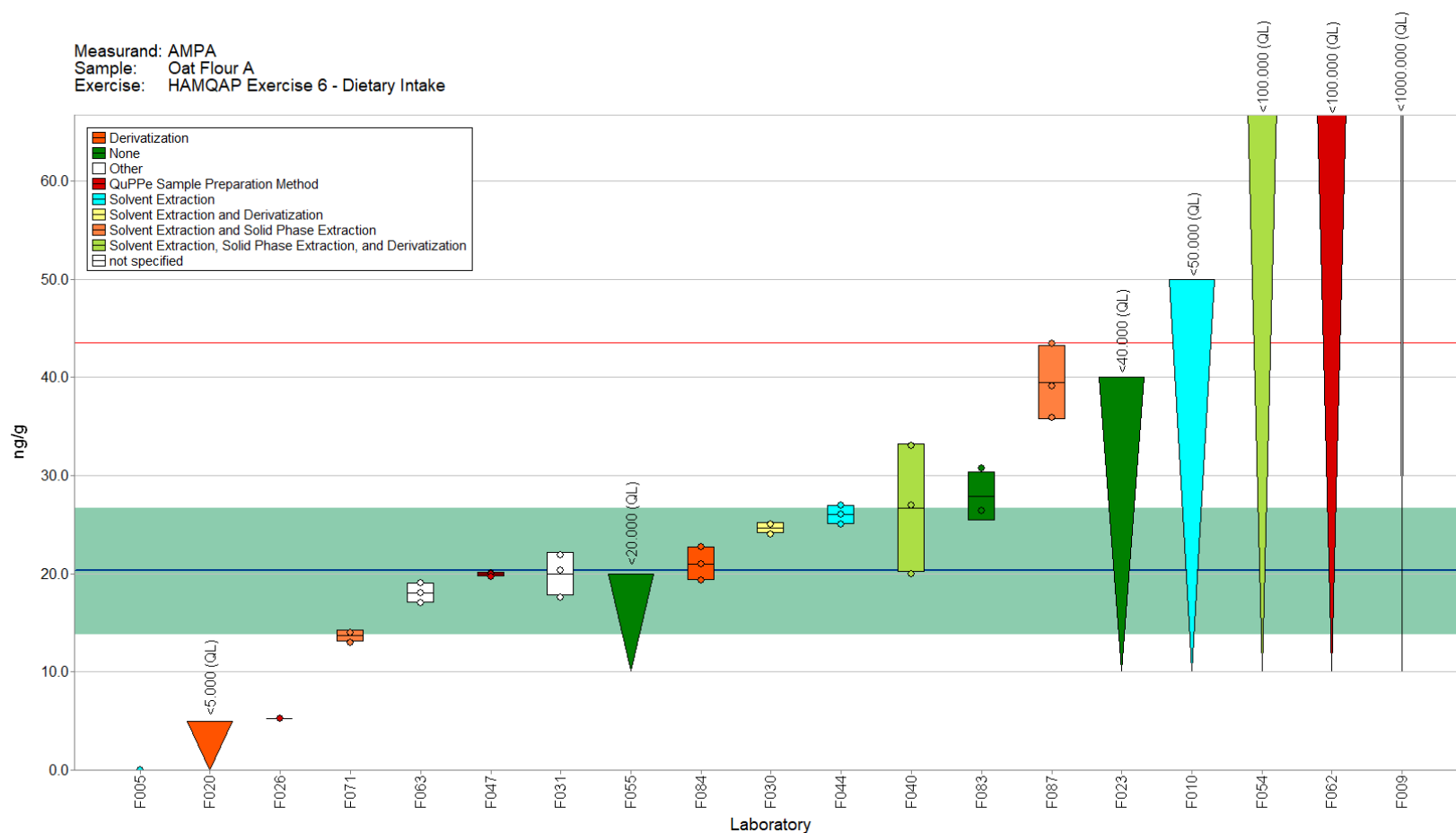


Figure 9-6. AMPA in Oat Flour A (data summary view – sample preparation). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation employed. The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red line represents the upper consensus range of tolerance, calculated as the values above the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$, with the lower limit set at zero. A NIST value has not been determined in this material.

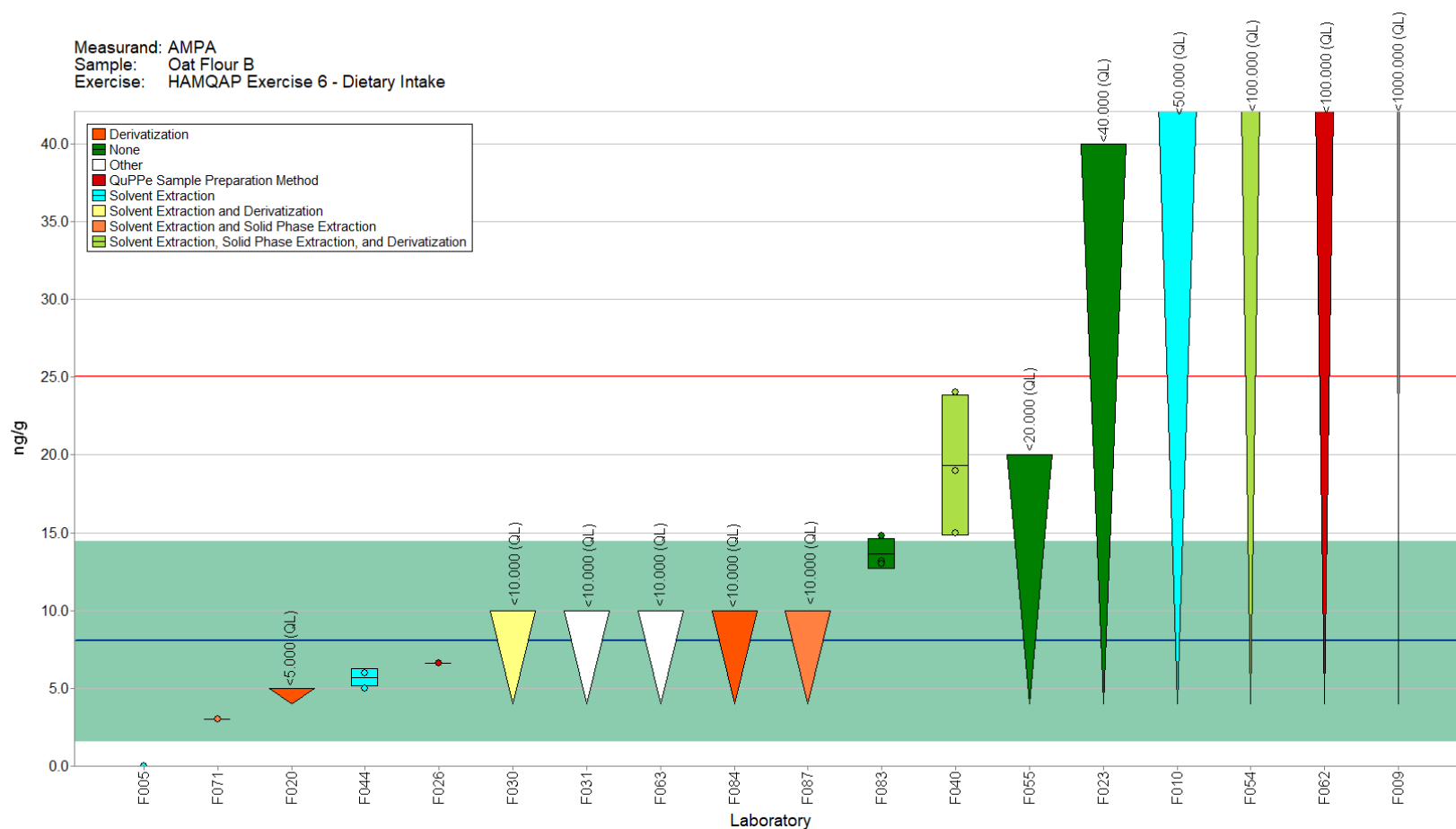


Figure 9-7. AMPA in Oat Flour B (data summary view – sample preparation). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation employed. The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red line represents the upper consensus range of tolerance, calculated as the values above the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$, with the lower limit set at zero. A NIST value has not been determined in this material.

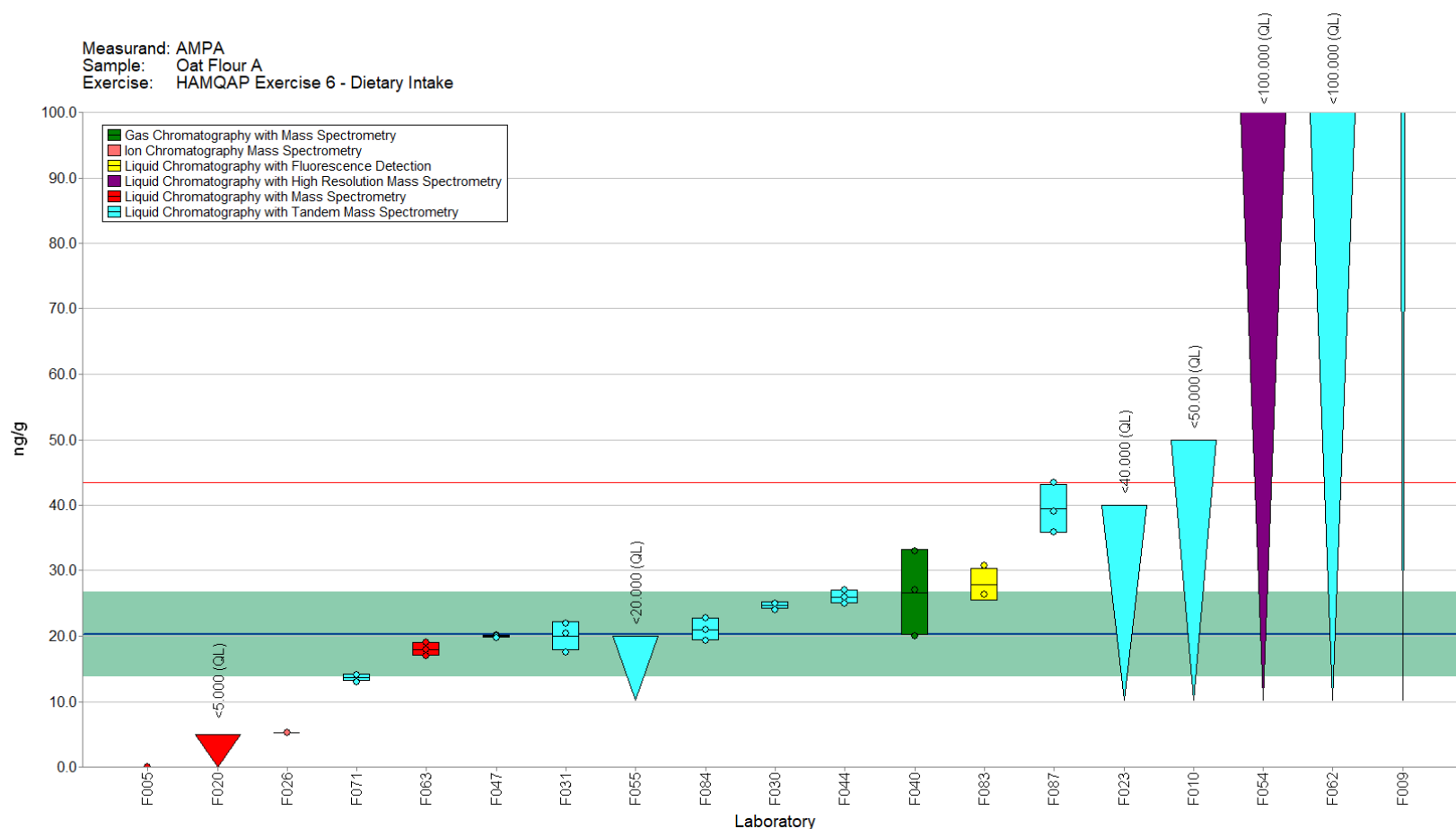


Figure 9-8. AMPA in Oat Flour A (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red line represents the upper consensus range of tolerance, calculated as the values above the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$, with the lower limit set at zero. A NIST value has not been determined in this material.

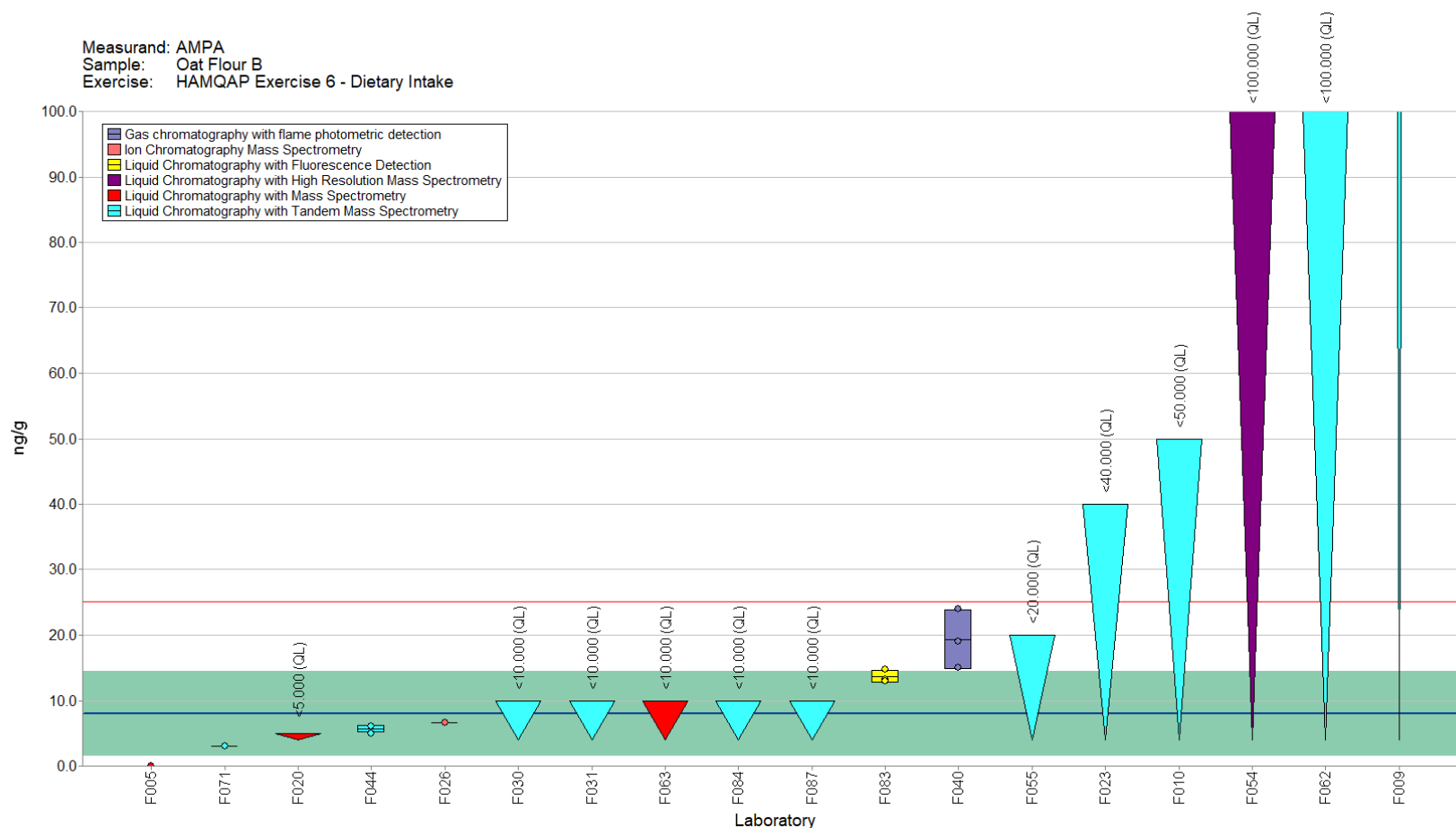


Figure 9-9. AMPA in Oat Flour B (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line represents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

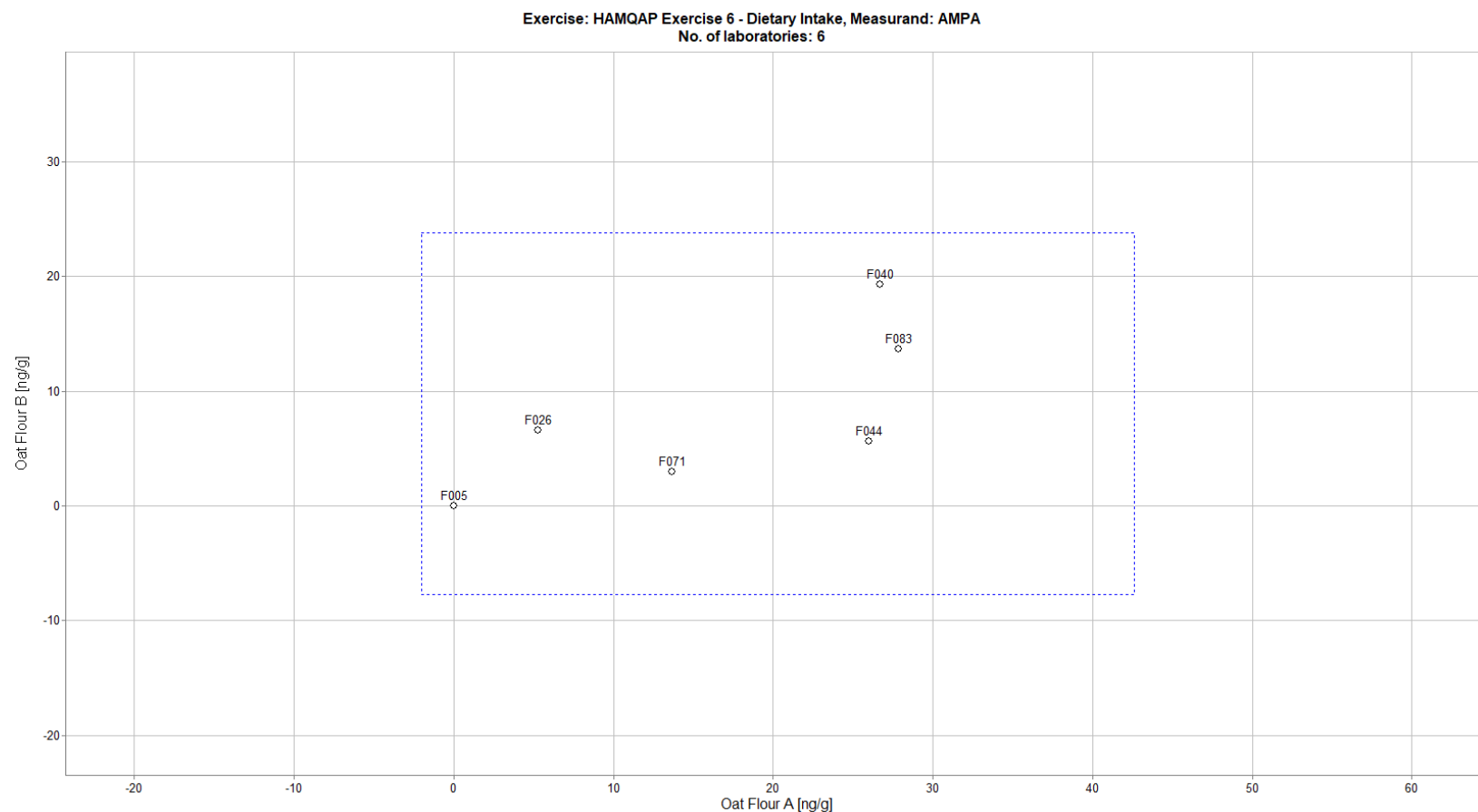


Figure 9-10. Laboratory means for AMPA in Oat Flour A and Oat Flour B (sample/sample comparison view). In this view, the individual laboratory mean for one sample (Oat Flour A) is compared to the individual laboratory mean for a second sample (Oat Flour B). The dotted blue box represents the consensus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

SECTION 10: PROXIMATES

Study Overview

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

Dietary Intake Sample Information

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

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

Rice Flour. Participants were provided with one bottle containing approximately 50 g of Rice Flour. Participants were asked to store the material at controlled room temperature (20 °C to 25 °C) in the original unopened bottle, to use a sample size appropriate for their in-house method of analysis, and to prepare three samples and report three values from the single bottle provided. Before use, participants were instructed to mix the contents of the packet thoroughly. The approximate analyte levels were not reported to participants prior to the study. The NIST-determined values for fat, protein, carbohydrates, and calories were assigned using

information from the manufacturers of the material. The NIST-determined value for solids were assigned based on NIST results for moisture of the material after desiccator drying over magnesium perchlorate ($\text{Mg}(\text{ClO}_4)_2$) for 28 d and drying in a forced air oven for 4 h at 90 °C. The NIST-determined values are provided in the table below on an as-received basis.

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

Dietary Intake Study Results

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

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

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

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

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

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

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

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

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

Dietary Intake Technical Recommendations

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

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

- In general, all results should be checked closely to avoid calculation or other errors and to be sure that results are reported in the requested units.
 - One laboratory reported extremely high, outlying results for protein in both materials. Another laboratory reported extremely low, outlying results for protein in both materials. A third laboratory reported high, outlying results for ash in both materials. These outlying results were likely due to a miscalculation or misinterpretation of the requested data.
 - One laboratory reported using AOAC Official Methods 986.25 and 990.20 for determination of fat in the samples. This method is not appropriate for determination of fat but is instead a method used for determination of solids.
- The use of appropriate calibration materials and quality assurance samples to establish that a method is in control and performing correctly may reduce the likelihood of outlying data. Quality assurance samples can be commercially available reference materials (CRMs, SRMs, or RMs) or materials prepared in-house. Numerous food matrix CRMs are available with assigned values for proximates and calories.
- A linear calibration curve which surrounds the expected sample concentration values should be used for calculations. This curve should include both the lowest and highest expected concentration values of the sample solutions. Extrapolation of results beyond calibration curves may result in incorrect values.

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

HAMQAP Exercise 6 - Proximates									
Lab Code:		NIST	1. Your Results				2. Community Results		
Analyte	Sample	Units	$\bar{x}_i$	s_i	Z'_{comm}	Z_{NIST}	N	$\bar{x}^*$	s^*
Ash	Infant Formula A	%	4.46	0.0539			15	4.5	0.14
Ash	Rice Flour	%					16	1.39	0.12
Calories	Infant Formula A	(kcal/100 g)	523	0.616			6	513	6.4
Calories	Rice Flour	(kcal/100 g)	364	9.11			6	370	15
Carbohydrates	Infant Formula A	%	54.5	0.441			9	52.2	0.89
Carbohydrates	Rice Flour	%	79.4	1.99			9	76.5	1.6
Fat	Infant Formula A	%	28.1	0.289			10	27.8	0.36
Fat	Rice Flour	%	1.6	0.04			12	2.93	0.37
Protein	Infant Formula A	%	12.9	0.318			11	12.9	0.54
Protein	Rice Flour	%	8.1	0.202			13	8.04	0.36
Solids	Infant Formula A	%	97.8	0.101			10	97.7	0.35
Solids	Rice Flour	%	90.4	0.719			11	89	0.7
			$\bar{x}_i$	Mean of reported values			N	Number of quantitative values reported	
			s_i	Standard deviation of reported values				$\bar{x}_{\text{NIST}}$	U
			Z'_{comm}	Z'-score with respect to community consensus			$\bar{x}^*$	Robust mean of reported values	
			Z_{NIST}	Z-score with respect to NIST value			s^*	Robust standard deviation	
								NIST-assessed value	
								expanded uncertainty	
								about the NIST-assessed value	

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

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

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

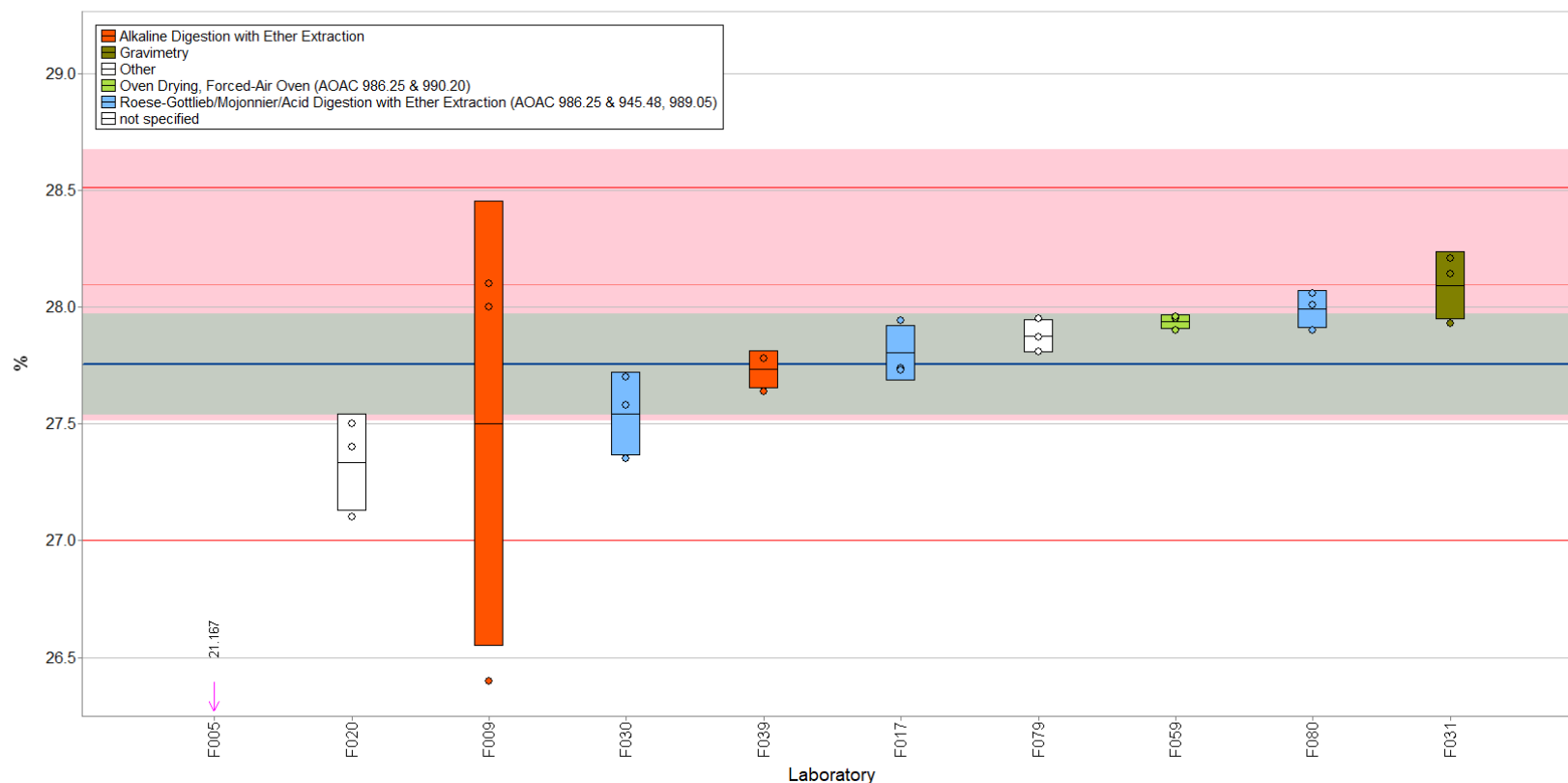


Figure 10-1. Fat in Infant Formula A (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The red solid lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty (U_{NIST}) and represents the range that results in an acceptable Z'_{NIST} score, $|Z'_{\text{NIST}}| \leq 2$.

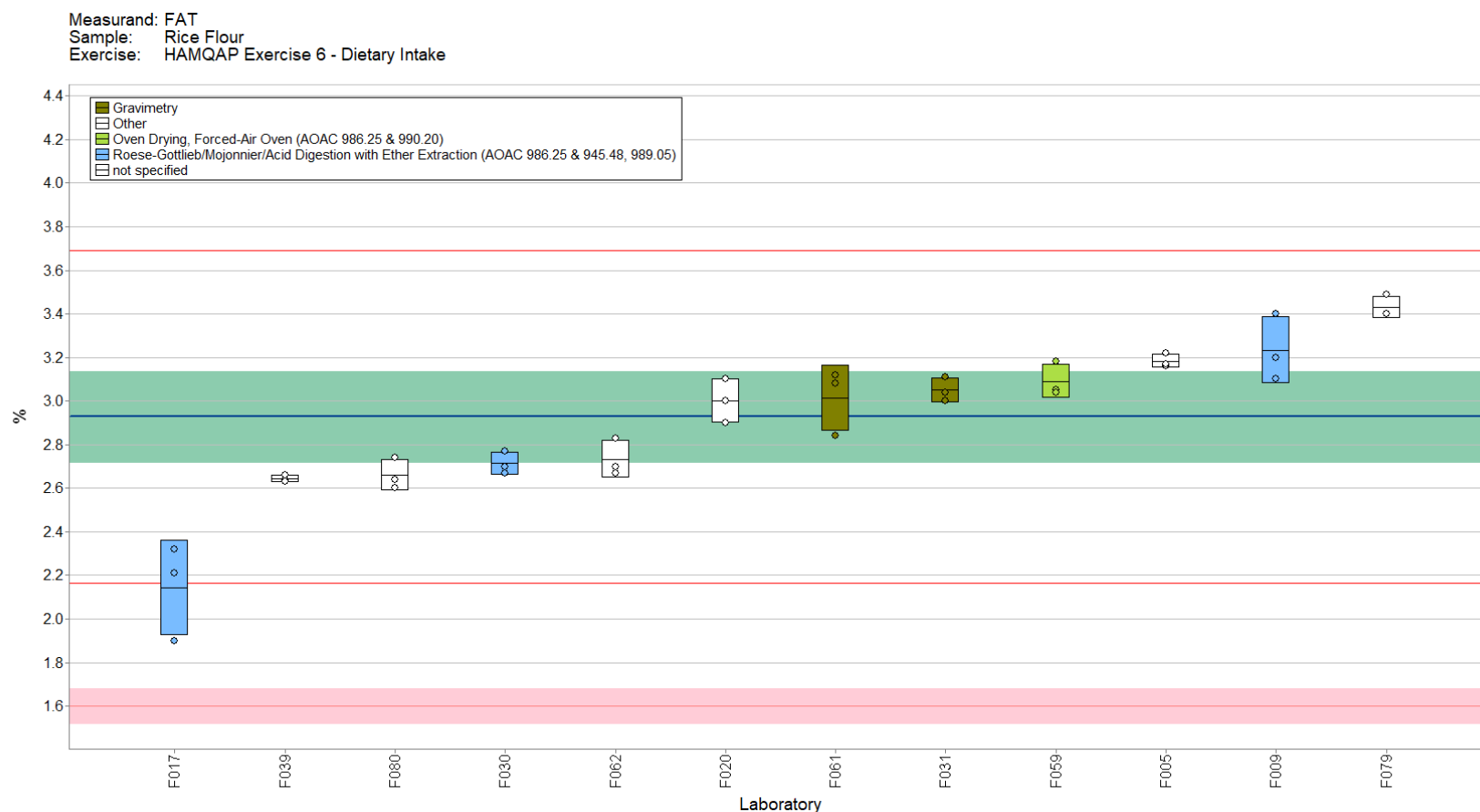


Figure 10-2. Fat in Rice Flour (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line represents the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

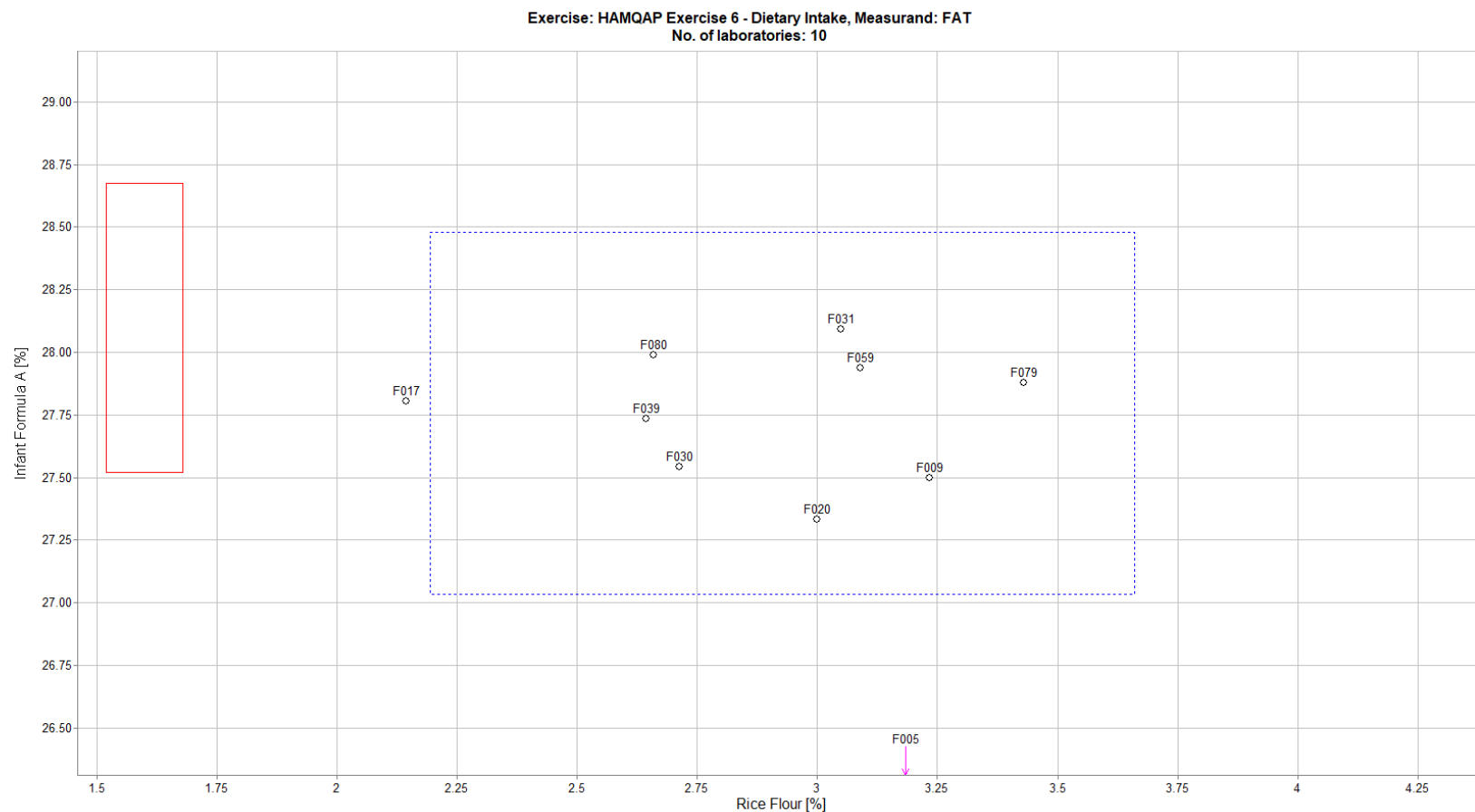


Figure 10-3. Laboratory means for fat in Infant Formula A and Rice Flour (sample/sample comparison view). In this view, the individual laboratory mean for one sample (Infant Formula A) is compared to the mean for a second sample (Rice Flour). The solid red box represents the NIST range of tolerance for the two samples, Rice Flour (x-axis) and Infant Formula A (y-axis), which encompasses the target values bounded by their uncertainties (U_{NIST}) and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$. The dotted blue box represents the consensus range of tolerance for Rice Flour (x-axis) and Infant Formula A (y-axis), calculated as the values above and below the consensus means that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$.

Table 10-3. Data summary table for protein in Infant Formula A and Rice Flour. Data points highlighted in blue have been identified as outside the consensus tolerance limits and would be estimated to result in an unacceptable Z'_{comm} score, $|Z'_{comm}| \geq 2$.

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

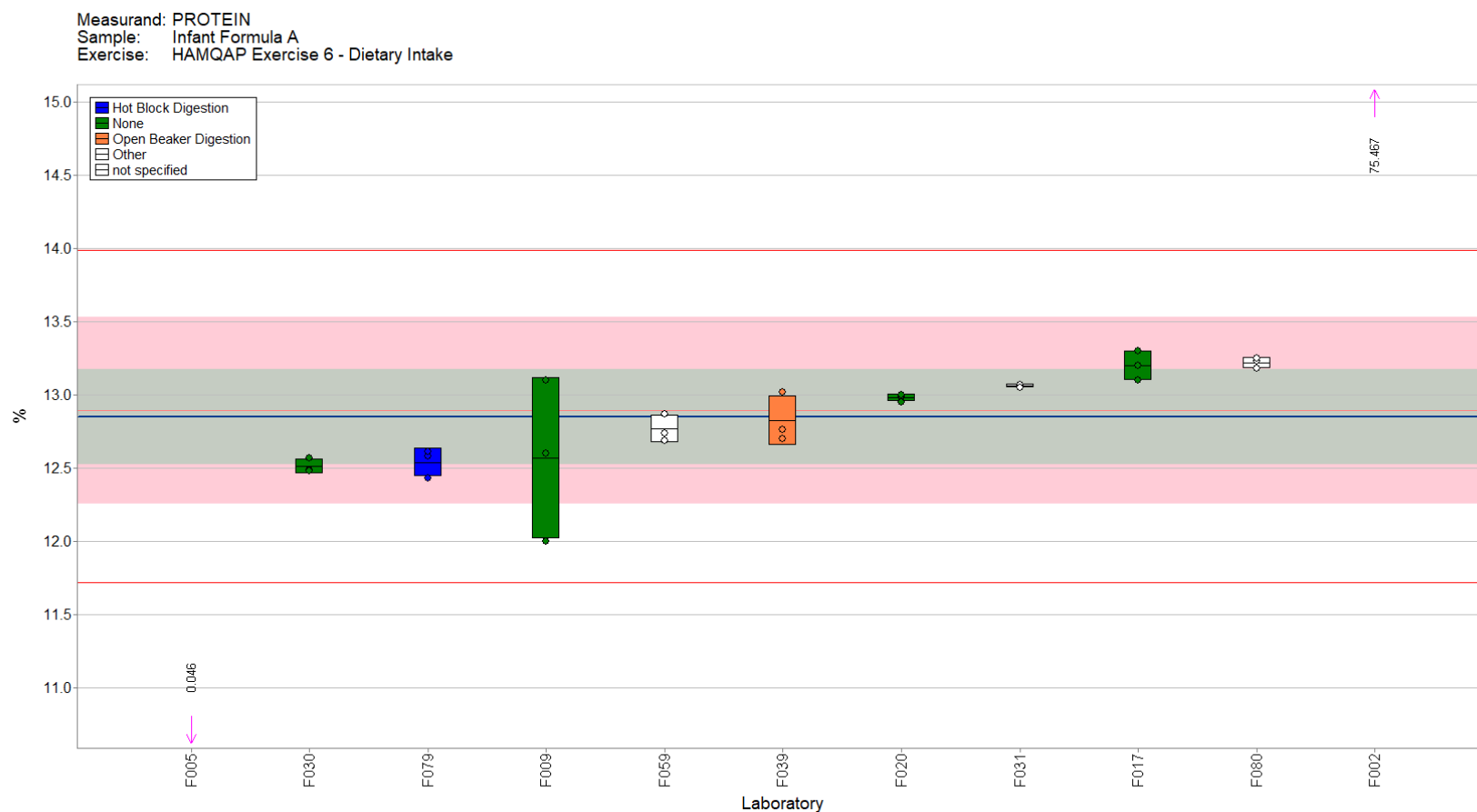


Figure 10-4. Protein in Infant Formula A (data summary view – sample preparation). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation employed. The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The red solid lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty (U_{NIST}) and represents the range that results in an acceptable Z'_{NIST} score, $|Z'_{\text{NIST}}| \leq 2$.

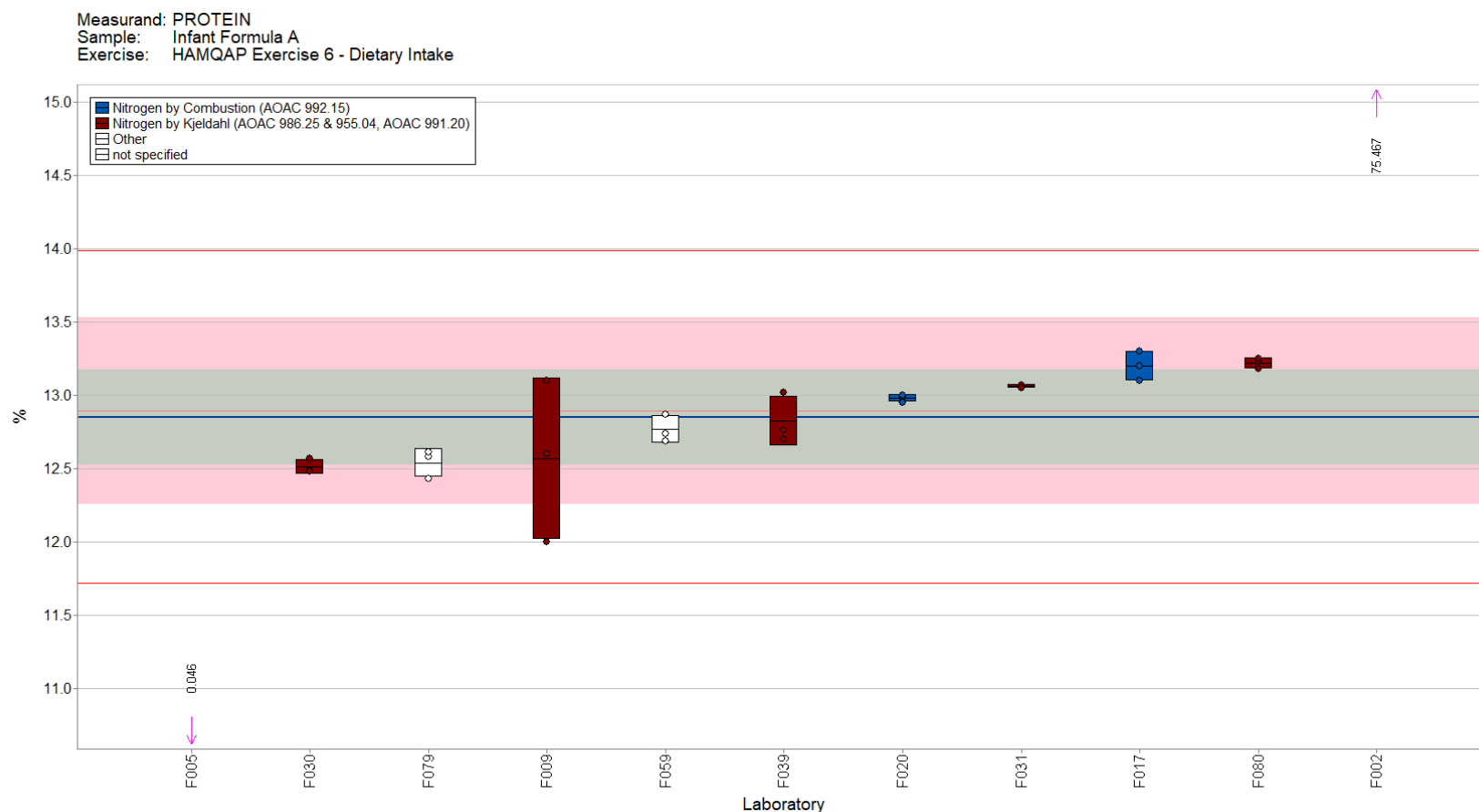


Figure 10-5. Protein in Infant Formula A (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The red solid lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty (U_{NIST}) and represents the range that results in an acceptable Z'_{NIST} score, $|Z'_{\text{NIST}}| \leq 2$.

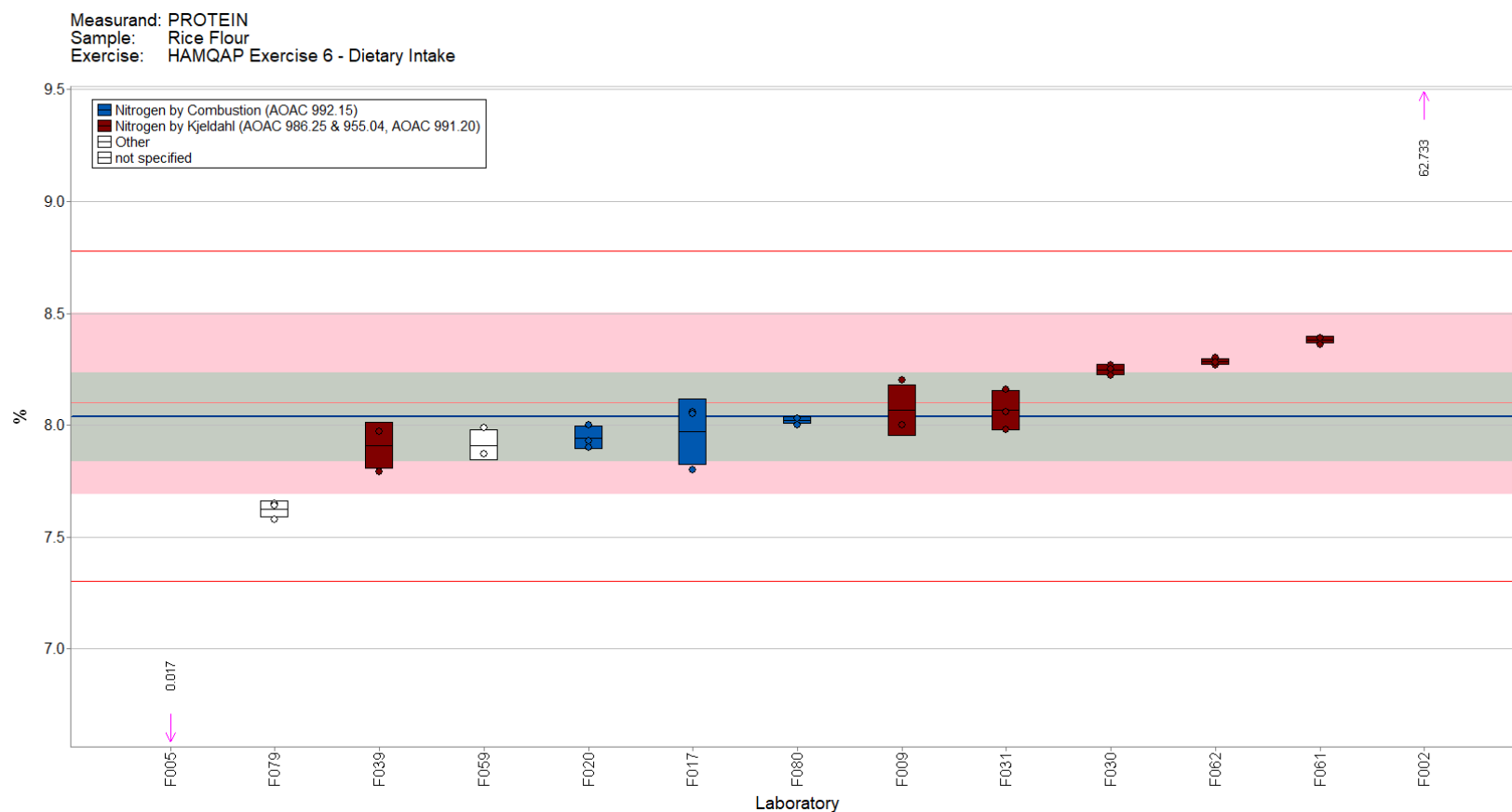


Figure 10-6. Protein in Rice Flour (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The red solid lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty (U_{NIST}) and represents the range that results in an acceptable Z'_{NIST} score, $|Z'_{\text{NIST}}| \leq 2$.

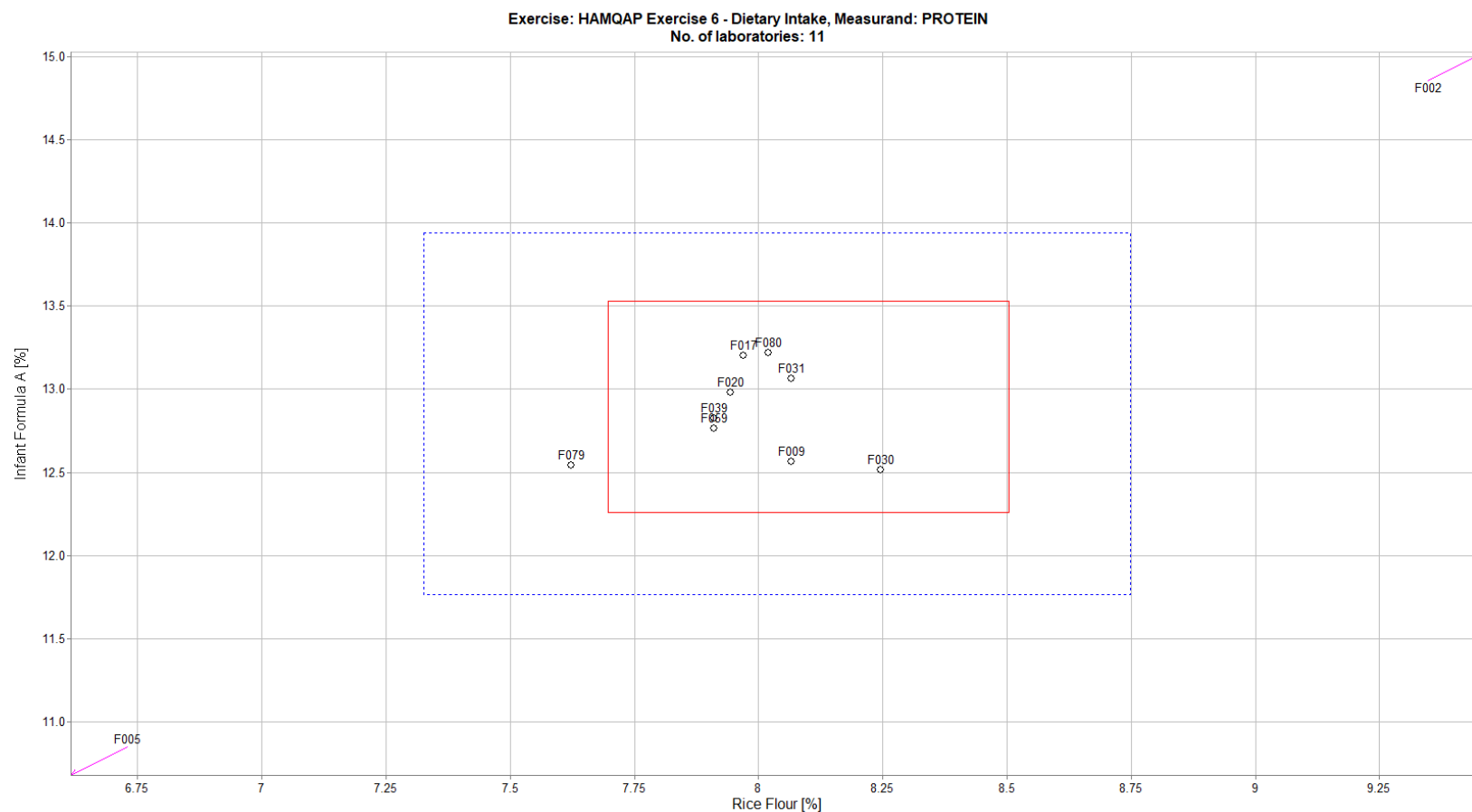


Figure 10-7. Laboratory means for protein in Infant Formula A and Rice Flour (sample/sample comparison view). In this view, the individual laboratory mean for one sample (Infant Formula A) is compared to the mean for a second sample (Rice Flour). The solid red box represents the NIST range of tolerance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

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

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

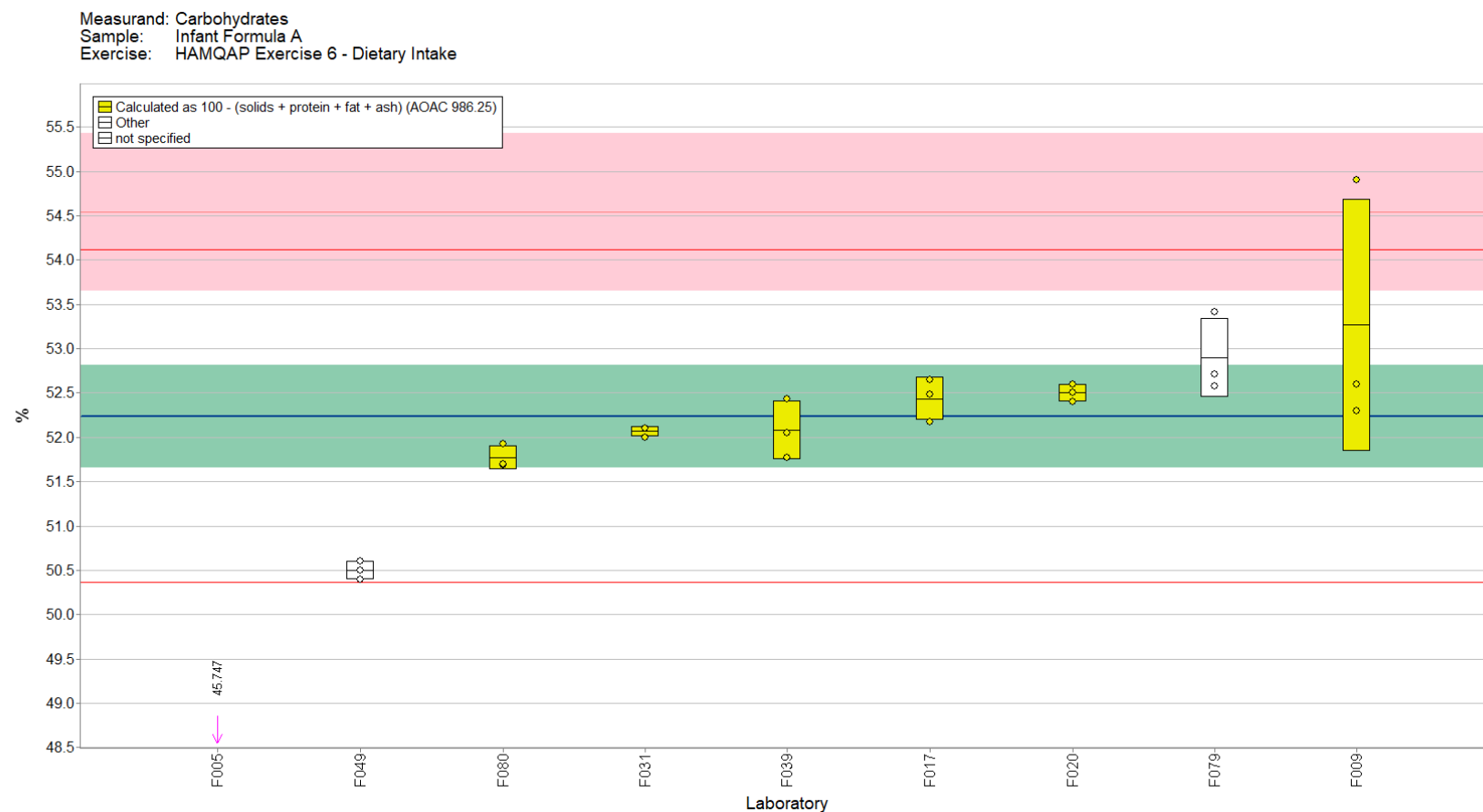


Figure 10-8. Carbohydrates in Infant Formula A (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red line represents the upper consensus range of tolerance, calculated as the values above the consensus mean that result in an acceptable Z'_{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, $|Z'_{\text{NIST}}| \leq 2$.

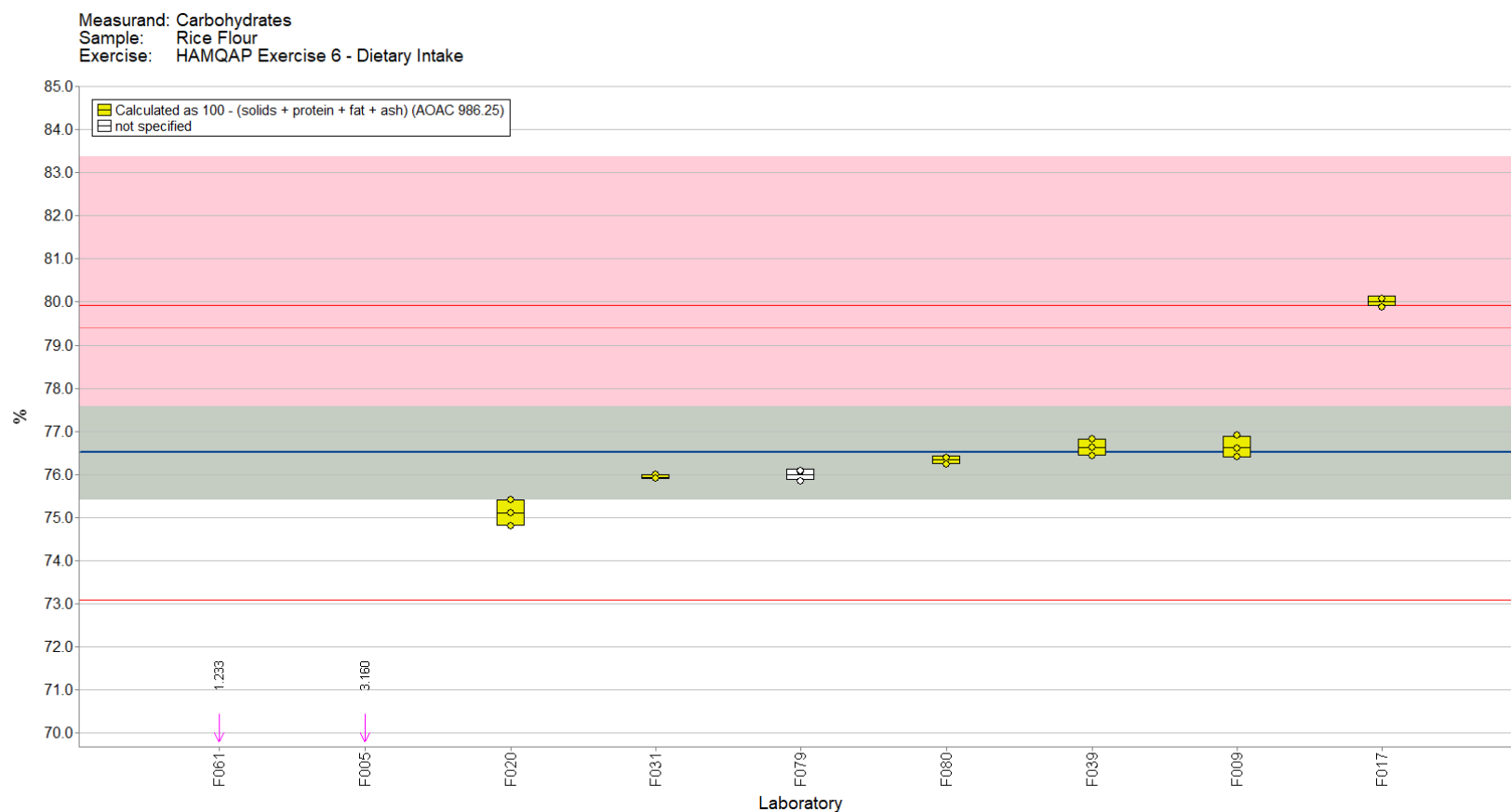


Figure 10-9. Carbohydrates in Rice Flour (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The red solid lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty (U_{NIST}) and represents the range that results in an acceptable Z'_{NIST} score, $|Z'_{\text{NIST}}| \leq 2$.

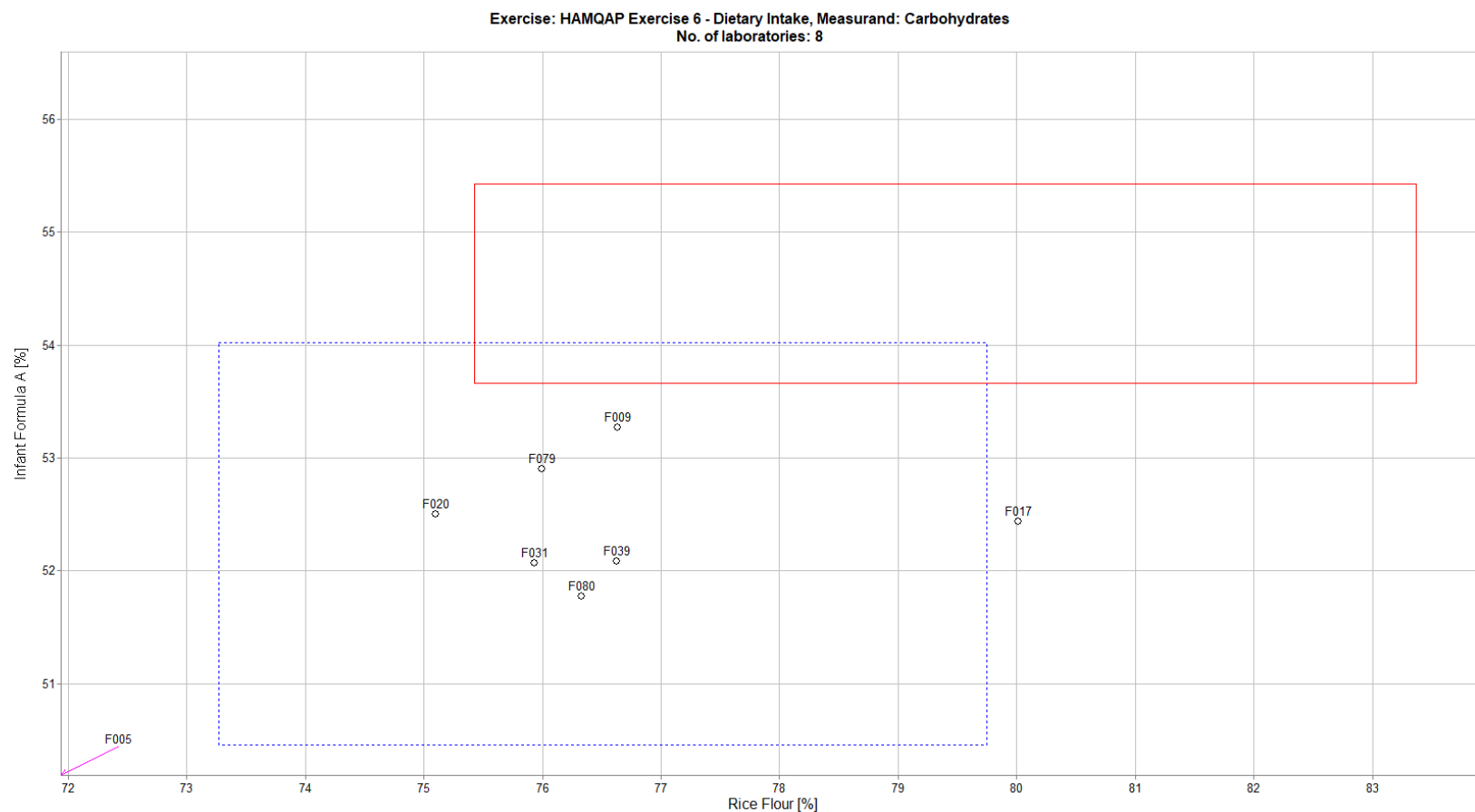


Figure 10-10. Laboratory means for carbohydrates in Infant Formula A and Rice Flour (sample/sample comparison view). In this view, the individual laboratory mean for one sample (Infant Formula A) is compared to the mean for a second sample (Rice Flour). The solid red box represents the NIST range of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

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

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

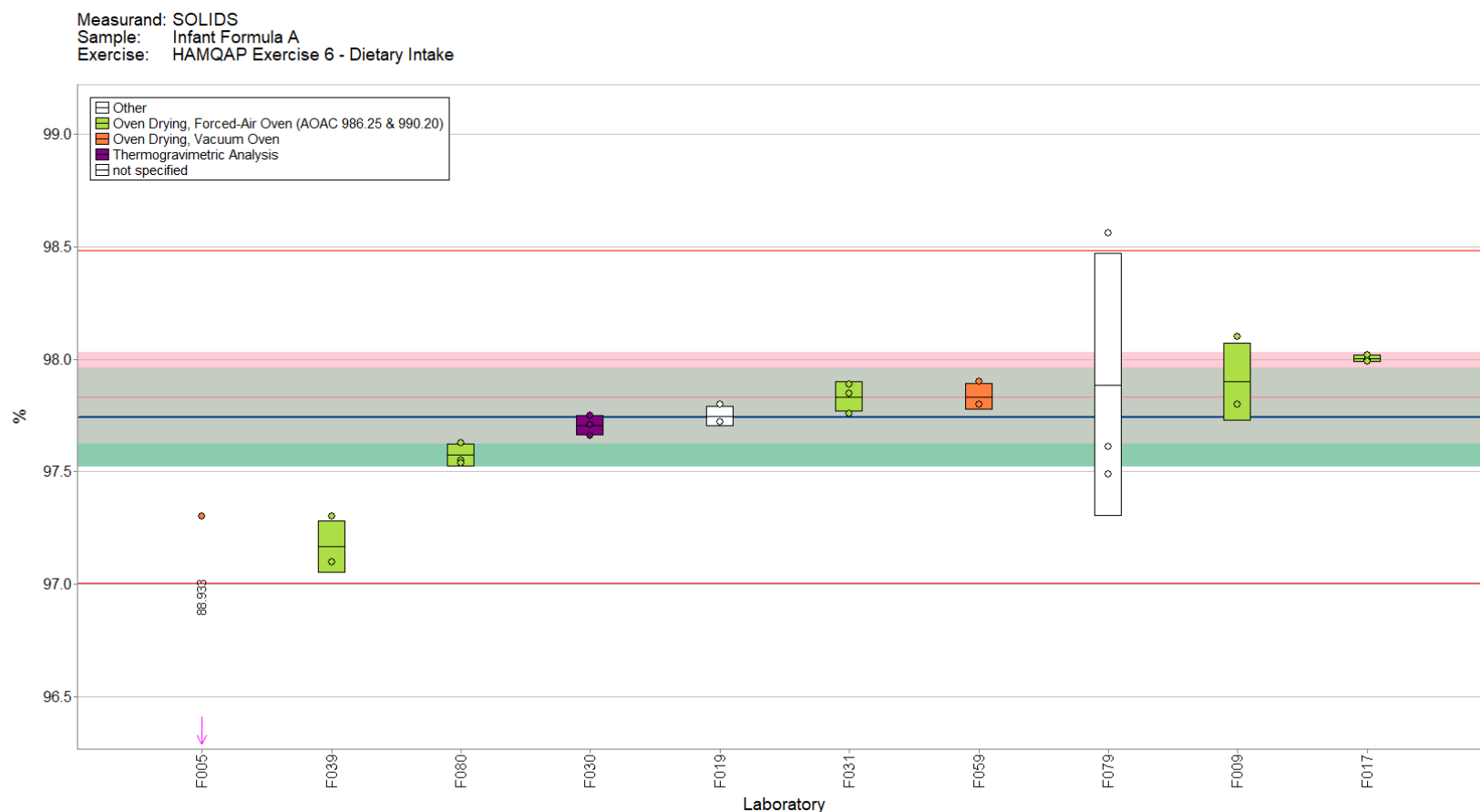


Figure 10-11. Solids in Infant Formula A (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The red solid lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty (U_{NIST}) and represents the range that results in an acceptable Z'_{NIST} score, $|Z'_{\text{NIST}}| \leq 2$.

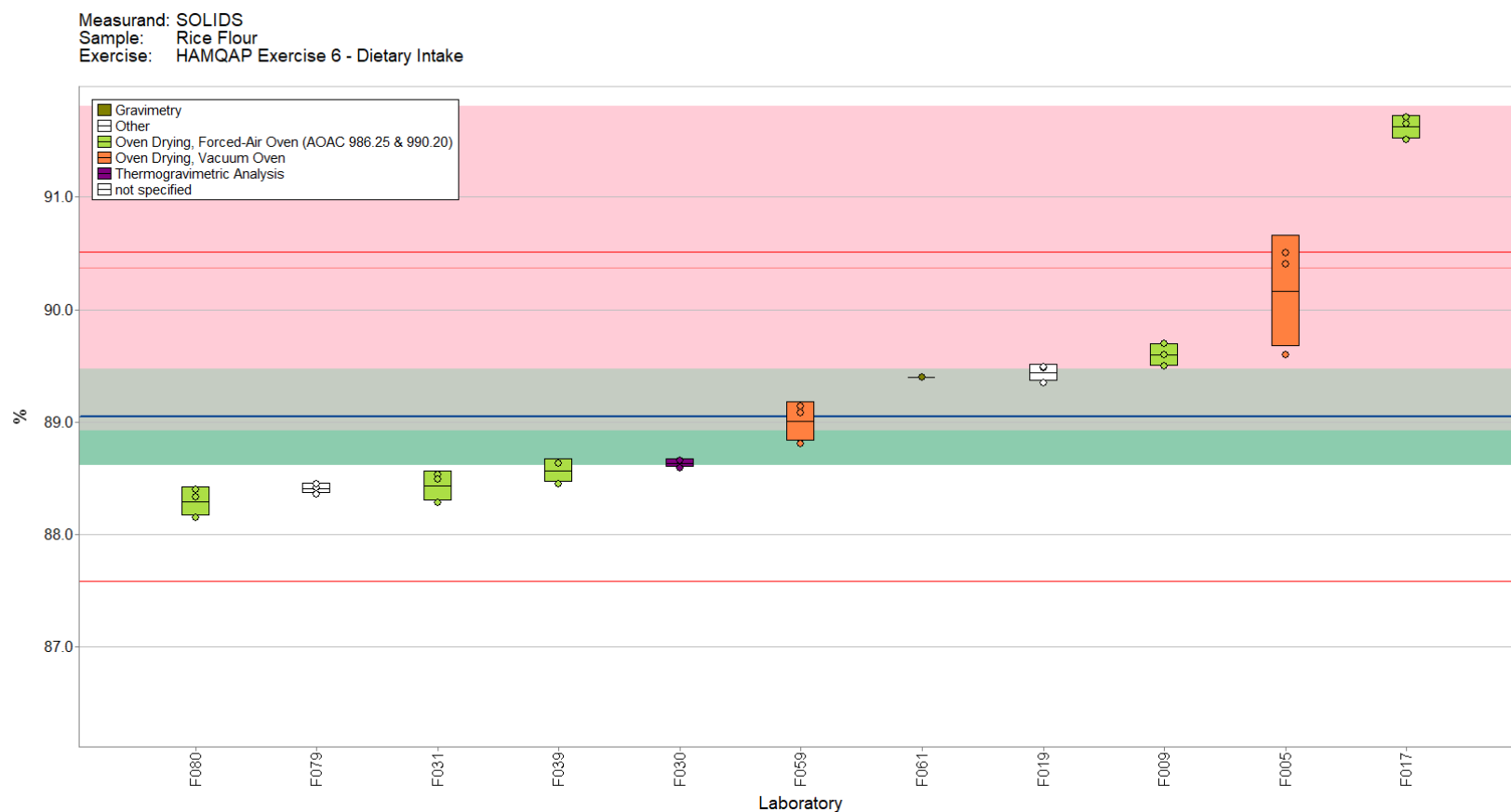


Figure 10-12. Solids in Rice Flour (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The red solid lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty (U_{NIST}) and represents the range that results in an acceptable Z'_{NIST} score, $|Z'_{\text{NIST}}| \leq 2$.

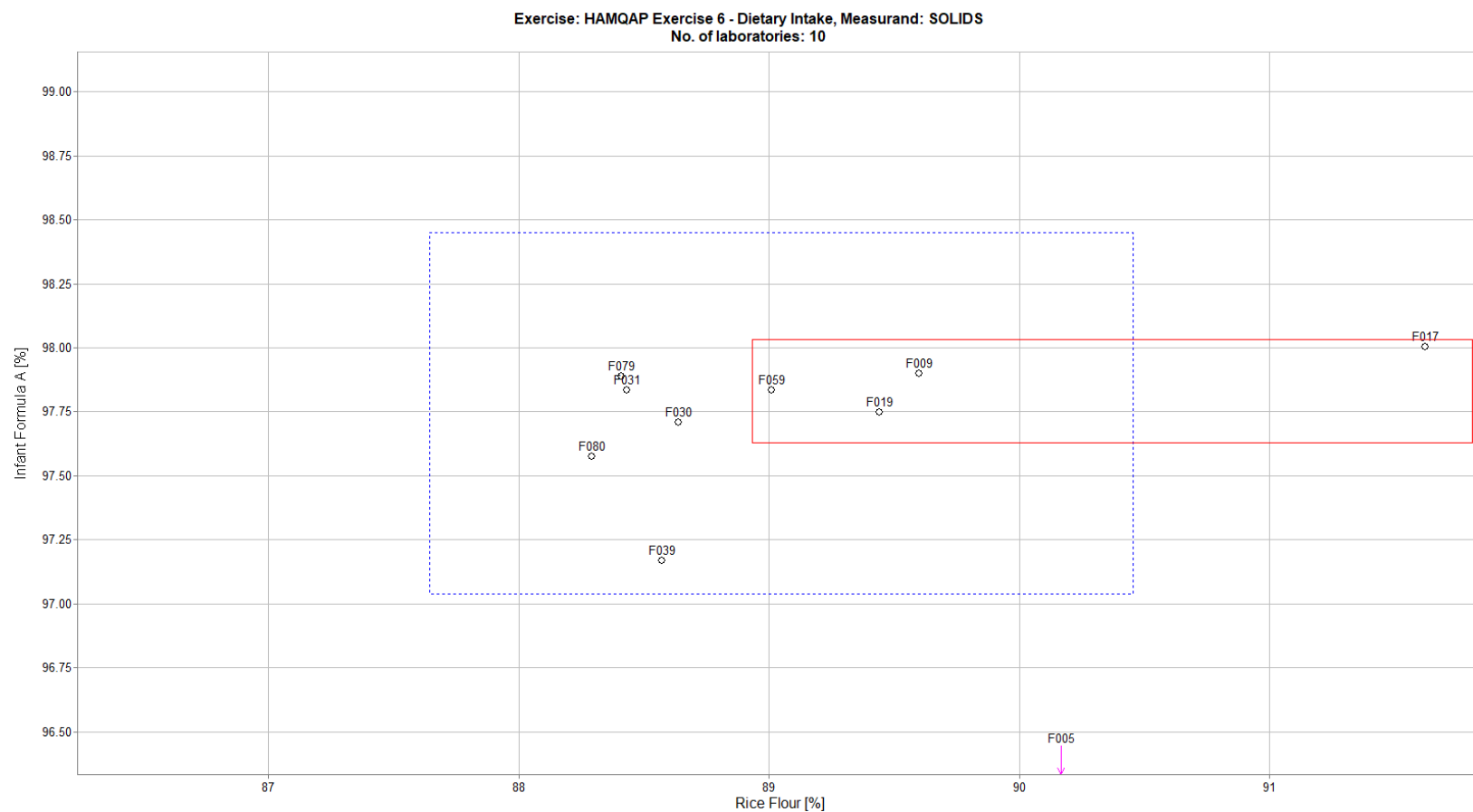


Figure 10-13. Laboratory means for solids in Infant Formula A and Rice Flour (sample/sample comparison view). In this view, the individual laboratory mean for one sample (Infant Formula A) is compared to the mean for a second sample (Rice Flour). The solid red box represents the NIST range of tolerance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

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

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

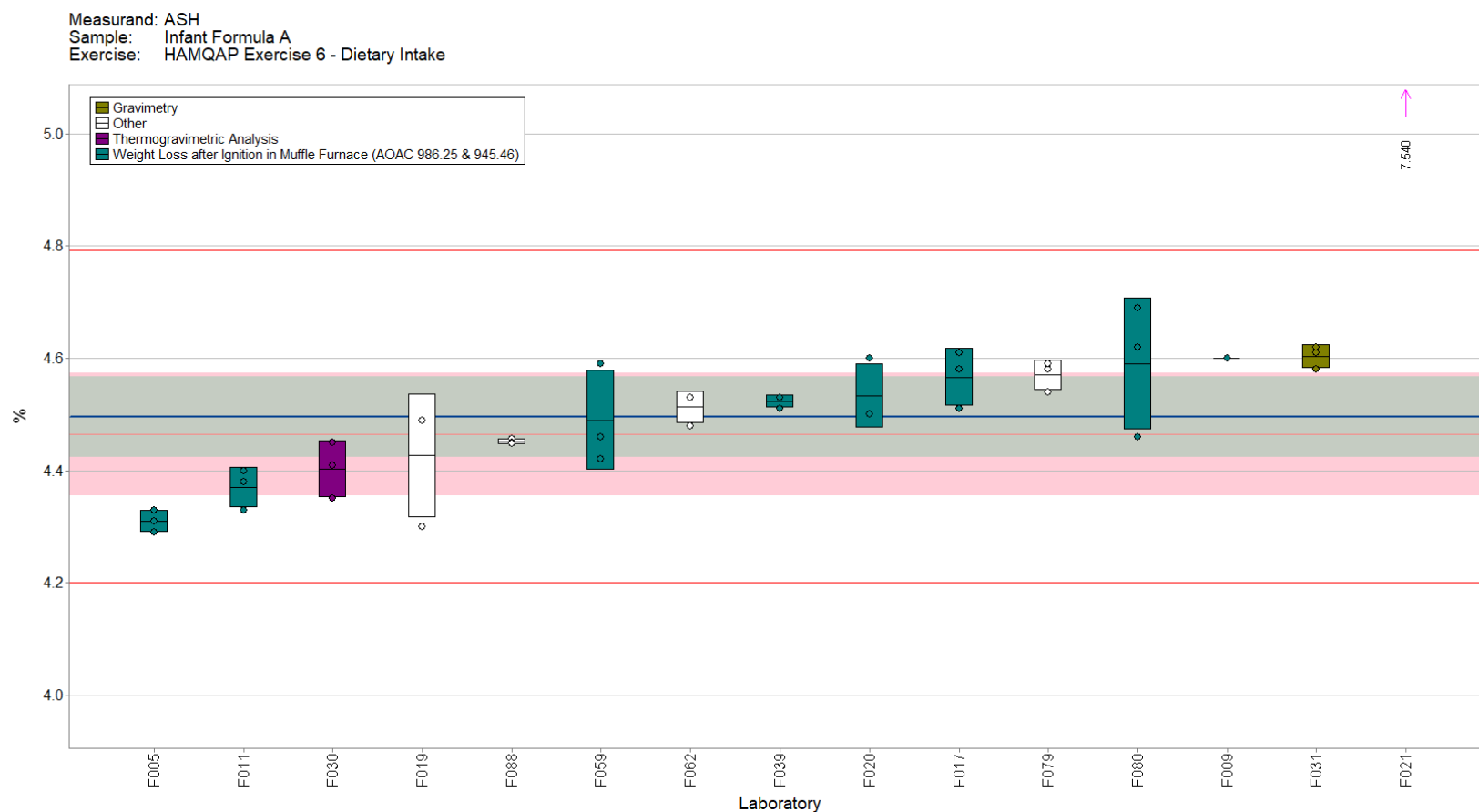


Figure 10-14. Ash in Infant Formula A (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The red solid lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty (U_{NIST}) and represents the range that results in an acceptable Z'_{NIST} score, $|Z'_{\text{NIST}}| \leq 2$.

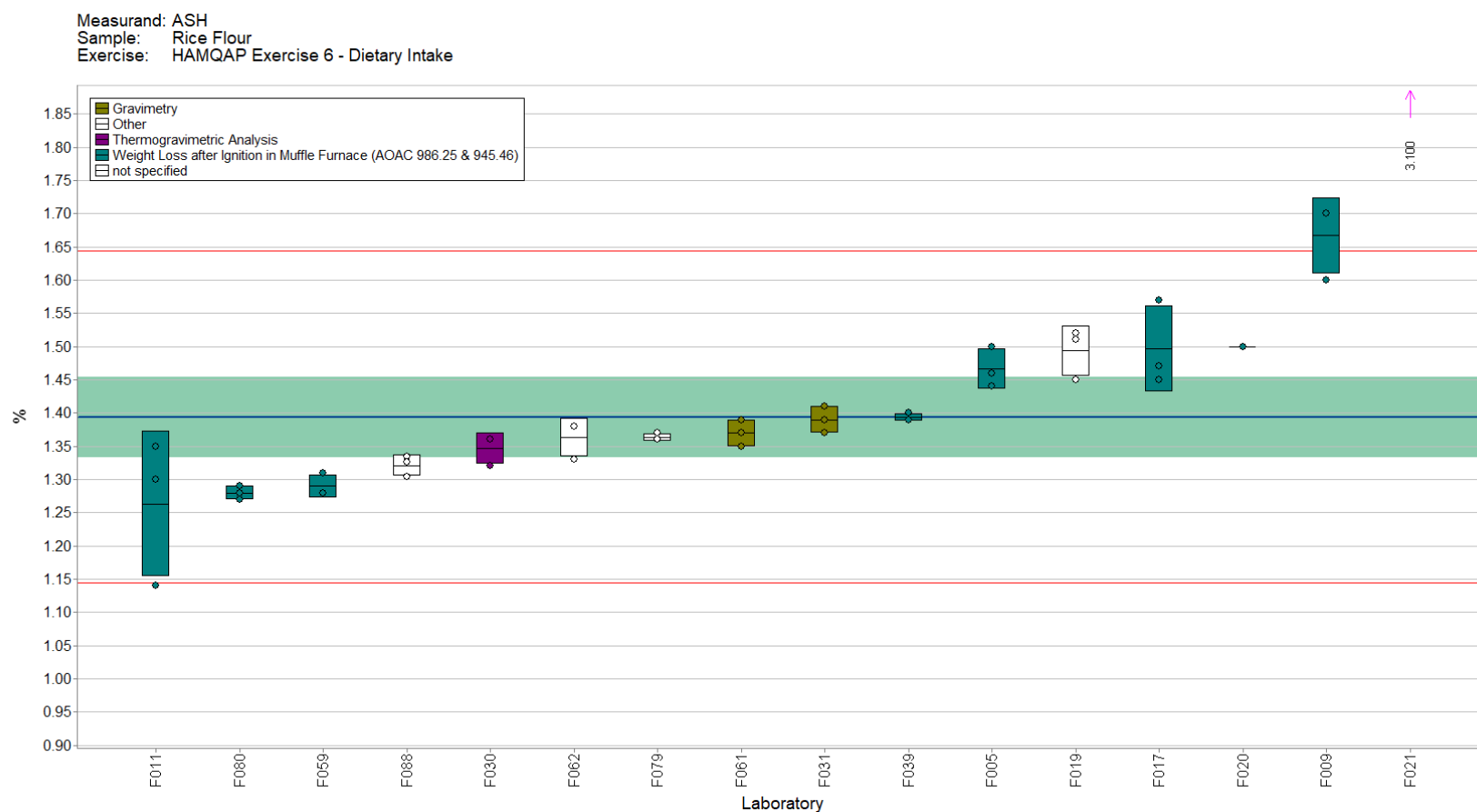


Figure 10-15. Ash in Rice Flour (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The red solid lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$. A NIST value has not been determined in this material.

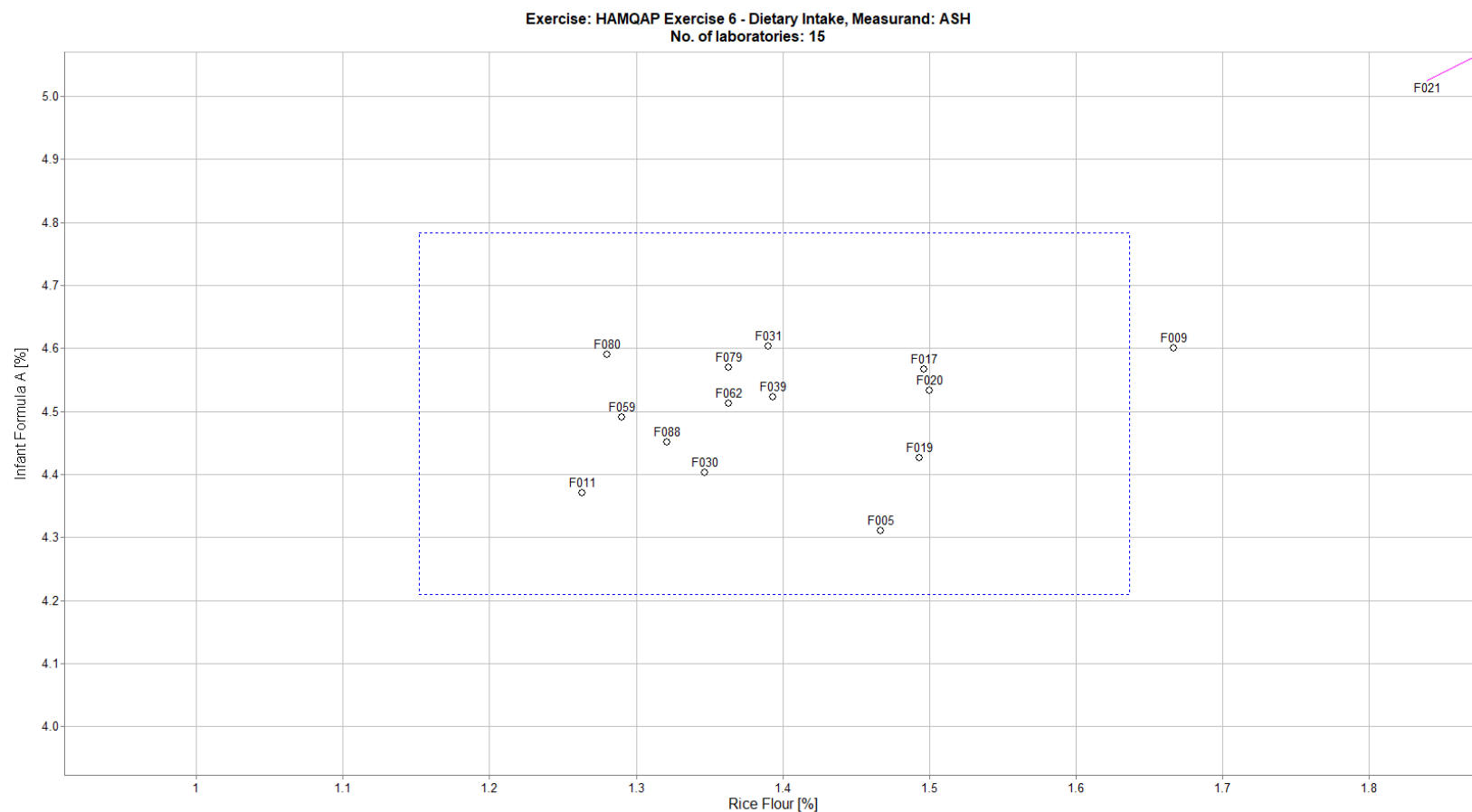


Figure 10-16. Laboratory means for ash in Infant Formula A and Rice Flour (sample/sample comparison view). In this view, the individual laboratory mean for one sample (Infant Formula A) is compared to the mean for a second sample (Rice Flour). The dotted blue box represents the consensus range of tolerance for Rice Flour (x-axis) and Infant Formula A (y-axis), calculated as the values above and below the consensus means that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$.

Table 10-7. Data summary table for calories in Infant Formula A and Rice Flour. Data points highlighted in blue have been identified as outside the consensus tolerance limits and would be estimated to result in an unacceptable Z'_{comm} score, $|Z'_{comm}| \geq 2$.

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

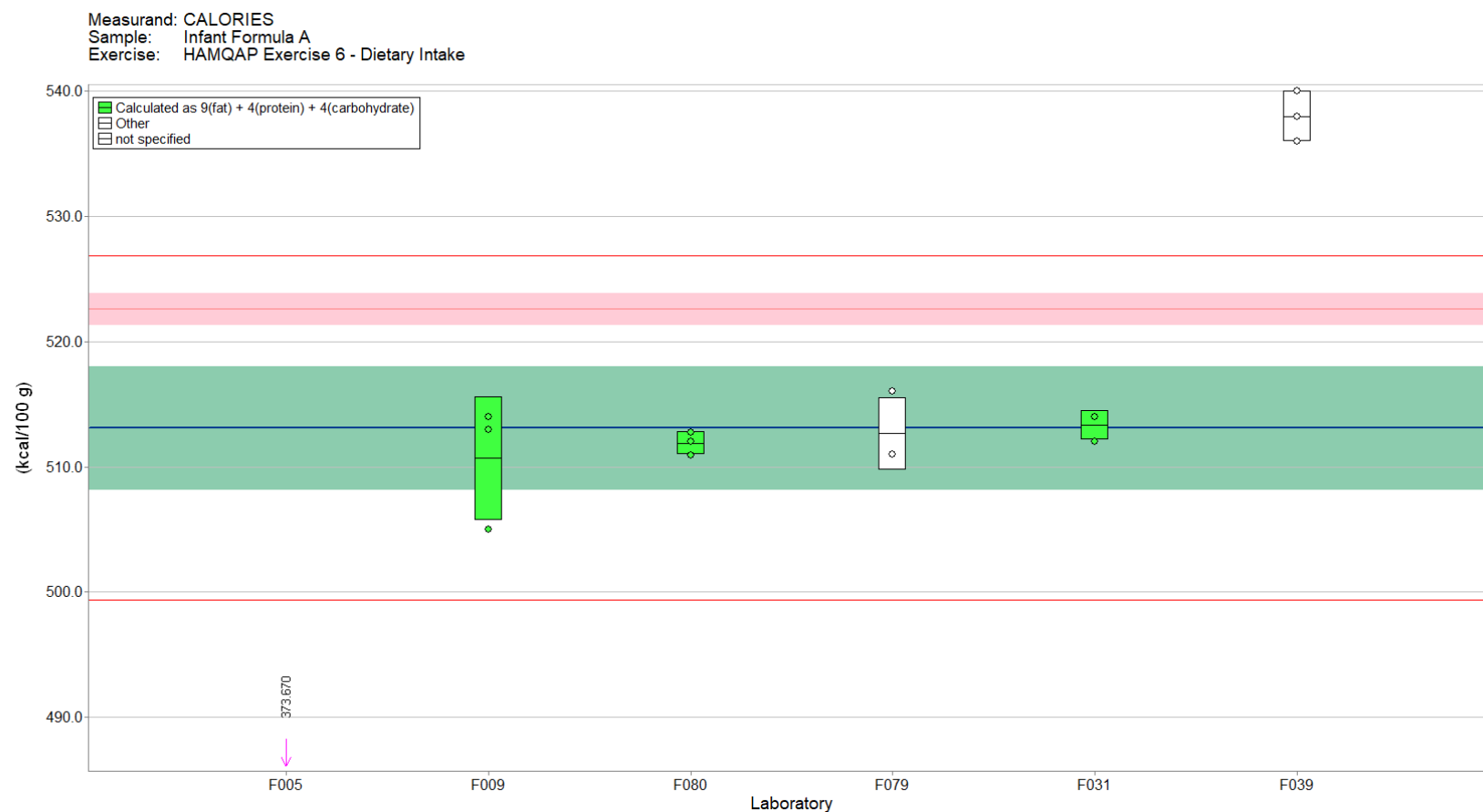


Figure 10-17. Calories in Infant Formula A (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The red solid lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty (U_{NIST}) and represents the range that results in an acceptable Z'_{NIST} score, $|Z'_{\text{NIST}}| \leq 2$.

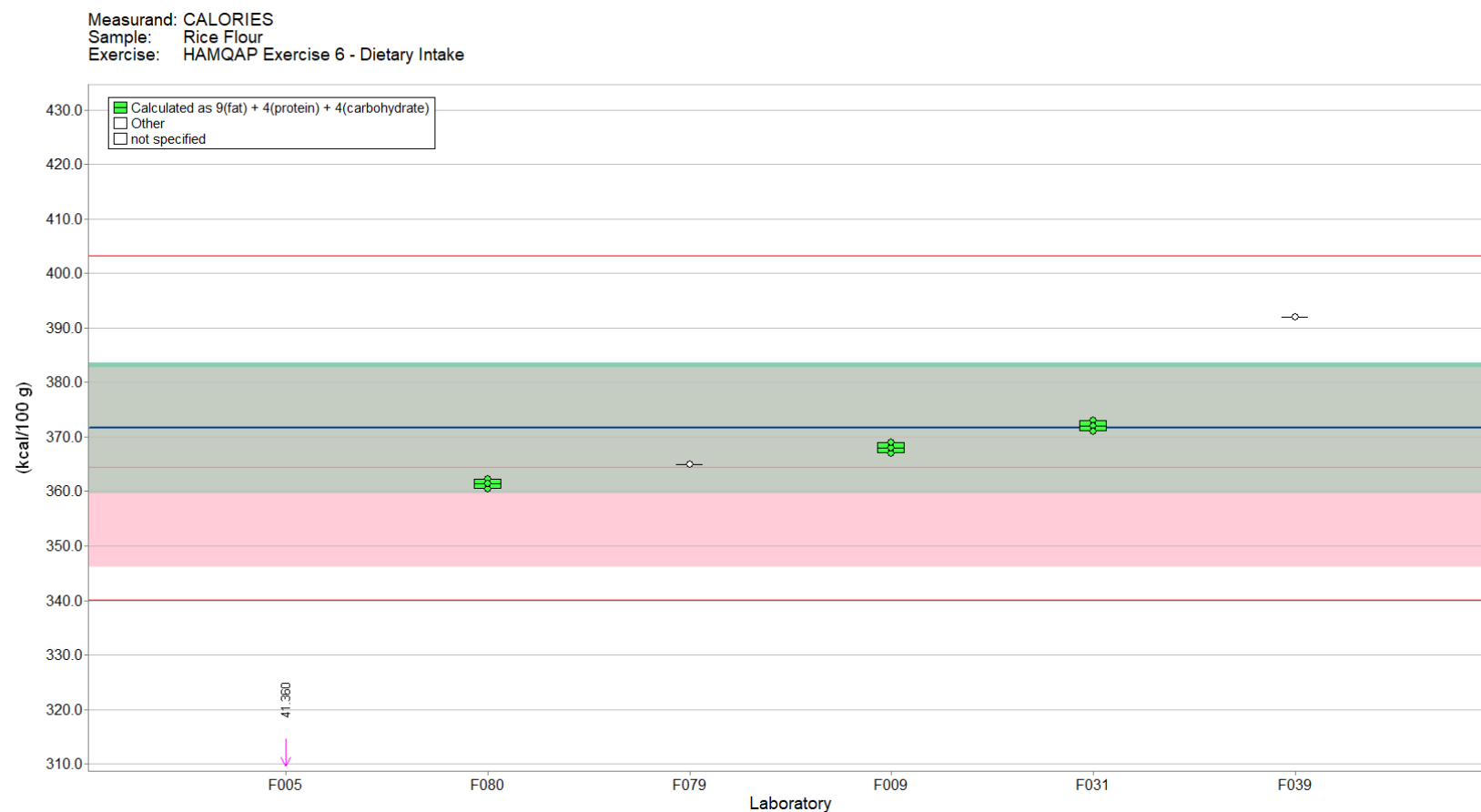


Figure 10-18. Calories in Rice Flour (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The red solid lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty (U_{NIST}) and represents the range that results in an acceptable Z'_{NIST} score, $|Z'_{\text{NIST}}| \leq 2$.

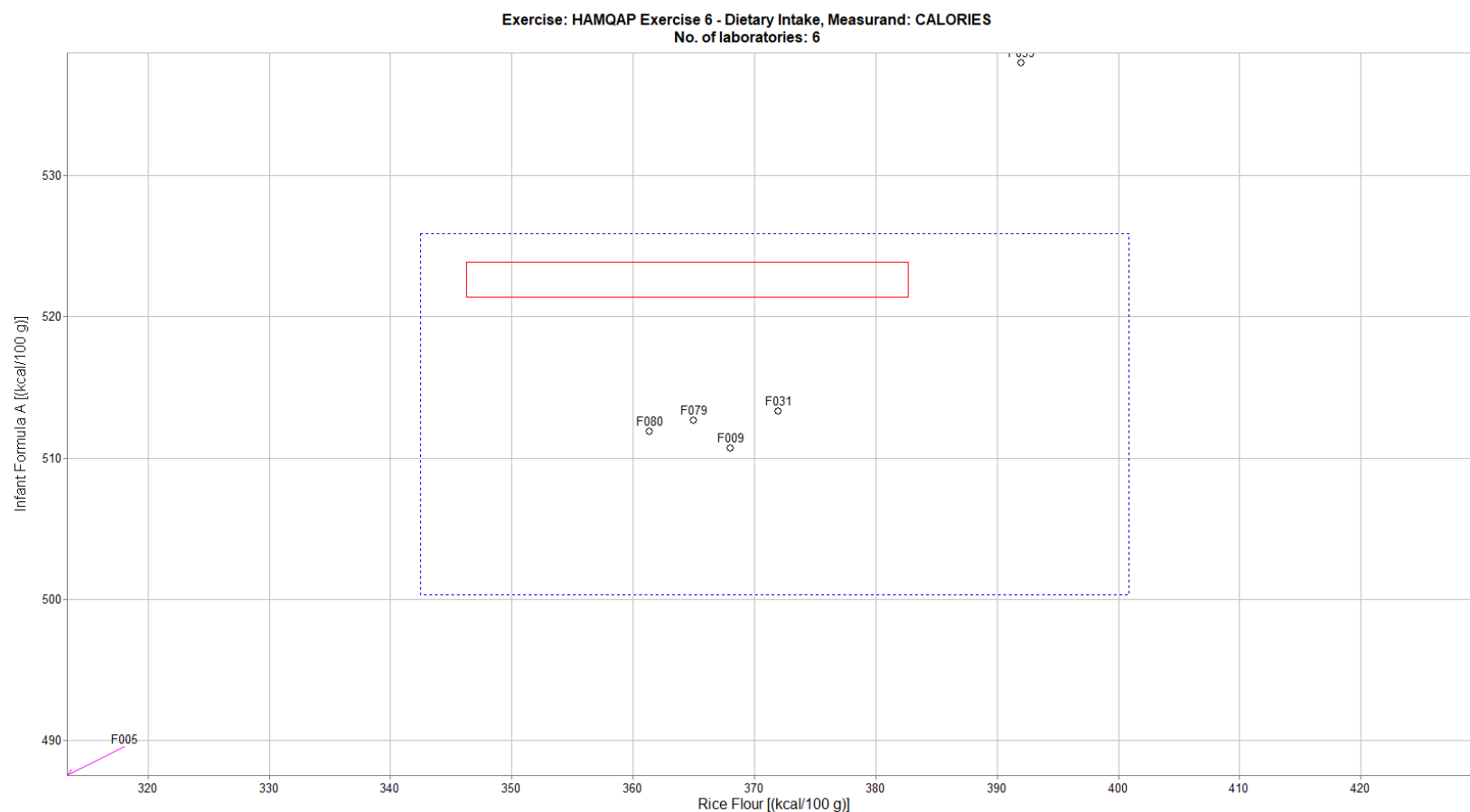


Figure 10-19. Laboratory means for calories in Infant Formula A and Rice Flour (sample/sample comparison view). In this view, the individual laboratory mean for one sample (Infant Formula A) is compared to the mean for a second sample (Rice Flour). The solid red box represents the NIST range of tolerance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