

**NIST Internal Report
NIST IR 8492**

Food Nutrition and Safety Measurements Quality Assurance Program: Exercise 2 Final Report

Colleen E. Bryan Sallee
Melissa M. Phillips
Carolyn Q. Burdette
Steven J. Christopher

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<https://doi.org/10.6028/NIST.IR.8492>

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*Chemical Sciences Division
Material Measurement Laboratory*

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July 2024



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National Institute of Standards and Technology
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NIST IR 8492
July 2024

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Publication History

Approved by the NIST Editorial Review Board on 2024-07-18

How to Cite this NIST Technical Series Publication

Bryan Sallee CE, Phillips MM, Burdette CQ, Christopher SJ (2024) Food Nutrition and Safety Measurements Quality Assurance Program: Exercise 2 Final Report. (National Institute of Standards and Technology, Gaithersburg, MD), NIST Internal Report (IR) NIST IR 8492. <https://doi.org/10.6028/NIST.IR.8492>

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Abstract

The Food Nutrition and Safety Measurements Quality Assurance Program (FNSQAP) was launched in 2021. FNSQAP was established to assist laboratories in the development and validation of new analytical methods, in improving the quality of their analytical measurements, and in supporting compliance with regulations enforced by the FDA, USDA, and international bodies. Exercise 2 of this program offered the opportunity for laboratories to assess their in-house measurements of nutritional elements (chromium, molybdenum, selenium), toxic elements (cadmium, lead), water-soluble vitamins (choline, carnitine), fat-soluble vitamins (carotenoids), fatty acids (DHA, ARA), and contaminants (glyphosate and its metabolites; phthalates) in food and infant formula samples.

Keywords

Contaminants; fat-soluble vitamins; fatty acids; Food Nutrition and Safety Measurements Quality Assurance Program (FNSQAP); glyphosate; infant formula; nutritional elements; phthalates; toxic elements; water-soluble vitamins.

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Acknowledgments

The authors thank the NIST Office of Reference Materials for providing support for sample labeling and shipment; Bruce Benner, Jr. and Jacolin Murray of the NIST Chemical Sciences Division for providing subject matter expertise; and Catherine Rimmer and John Molloy of the NIST Chemical Sciences Division for careful review of this report.

1. INTRODUCTION

The Food Nutrition and Safety Measurements Quality Assurance Program (FNSQAP) was formed in 2021 and represents ongoing efforts at the National Institute of Standards and Technology (NIST) that offer the opportunity for laboratories to assess their in-house measurements of nutritional and toxic elements, fat- and water-soluble vitamins, fatty acids, contaminants, and macronutrients in samples distributed by NIST. Reports and certificates of participation are provided and may be used to demonstrate compliance with the US Food and Drug Administration (FDA) current Good Manufacturing Practice regulations (cGMPs) or to fulfill proficiency requirements established by accreditation bodies. In the future, results from FNSQAP exercises could be used by NIST to identify problematic matrices and analytes for which consensus-based methods of analysis would benefit the food testing community.

NIST has decades of experience in the administration of Quality Assurance Programs (QAPs), and FNSQAP builds on the approach taken by the Dietary Supplement Laboratory QAP (DSQAP) and former Health Assessment Measurements QAP (HAMQAP) by providing a wide range of matrices and analytes, emphasizing critical, emerging, and/or challenging measurements in food matrices. Participating laboratories are interested in evaluating in-house methods on a wide variety of challenging, real-world matrices to demonstrate accuracy and comparability with respect to the measurement community. FNSQAP offers a unique tool for assessment of measurement quality and provides feedback about performance that can assist participants in improving laboratory operations.

This report summarizes the results from the second exercise of FNSQAP. Fifty laboratories responded to the call for participants in January 2022 to the studies available in FNSQAP Exercise 2 (Table 1-1). Samples were shipped to participants in May 2022 and results were returned to NIST in June 2022. Participants received a summary of the preliminary data in July 2022 and were given an opportunity to correct any errors by August 2022.

Table 1-1. Studies conducted as part of Exercise 2 of the FNSQAP.

Study Group	Analytes	Samples
Nutritional Elements	Cr, Mo, Se	Infant Formulas
Toxic Elements	Cd, Pb	Powdered Cacao, Chocolate Drink Mix
Water-Soluble Vitamins	Choline, Carnitine	Infant Formulas
Fat-Soluble Vitamins	β -Carotene, Lutein, Lycopene	Infant Formulas
Fatty Acids	DHA, ARA	Infant Formulas
Contaminants	Glyphosate, Aminomethylphosphonic acid (AMPA), N-acetyl-glyphosate, N-acetyl-AMPA	Turmeric, Cat Food
Contaminants	Phthalates	Infant Formula, Powdered Cheese

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

1.1. Overview of Data Treatment and Representation

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

1.1.1. 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 the NIST target value, if available. All calculations are performed in PROLab Plus (QuoData GmbH, Dresden, Germany). The consensus means and standard deviations are calculated according to the robust Q/Hampel method outlined in International Organization for Standardization (ISO) standard 13528:2022, Annex C [1].

1.1.2. Individualized Data Table

The data in this table are individualized to each participating laboratory and are provided to allow participants to directly compare their data to the summary statistics (consensus or community data as well as NIST target values, when available). The upper left of the data table includes the randomized laboratory code. Example individualized data tables are included in each section of this report using NIST as the participant; participating laboratories received uniquely coded individualized data tables in a separate distribution to protect the identity and performance of participants. The individualized data tables are presented in the format shown in Table 1-2.

Table 1-2. Exemplar individualized data summary table.

(Lab Name)

Exercise 2 – Study Name

Lab Code: (Code)			1. Your Results				2. Community Results			3. Target				
Sample ^a	Units ^b		x_i	s_i	Z'_{comm}	Z_{NIST}	N	x^*	s^*	x_{NIST}	U_{NIST}			
c_1	a_1	b_1	<i>Individual laboratory results will appear in this section; laboratory-specific results were provided to each participant separately from this report.</i>				N_1	x^*_{1}	s^*_{1}	$x_{\text{NIST}1}$	$U_{\text{NIST}1}$			
...
...
c_n	a_n	b_n					N_n	x^*_{n}	s^*_{n}	$x_{\text{NIST}n}$	$U_{\text{NIST}n}$			
			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_{NIST}	standard uncertainty about the 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								

- ^a Samples used in the study.
- ^b Units used to describe the measured values.
- ^c Analytes measured in the study.

Section 1 of the data table (*Your Results*) contains the laboratory results as reported, including the mean and standard deviation when multiple values were reported. A blank section indicates that NIST does not have data on file for that laboratory for the corresponding analyte or sample. When no value is listed for standard deviation, the participant reported a single value or a value below the limit of quantitation (LOQ).

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:

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

The second Z-score, Z_{NIST} , is calculated with respect to the NIST target value (see definition of NIST target values under Section 3 of the data table description below), using x_{NIST} and U_{NIST} , where U_{NIST} is the estimated expanded uncertainty of NIST and/or other measurements:

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

The significance of the Z-score and Z'-score is as follows [1]:

- $|Z| \leq 2$ 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}).

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

Section 2 of the data table (*Community Results*) contains the consensus results, including the number of laboratories reporting more than a single quantitative value for each analyte, the mean value determined for each analyte, and a robust estimate of the standard deviation of the reported values [1]. Consensus means and standard deviations are calculated using the laboratory means; if a laboratory reported a single value, the reported value is used as the laboratory mean [1]. 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 NIST target values for each analyte, when available. When possible, the target value is a NIST certified value, a NIST non-certified value, or a value determined at NIST that does not meet the criteria of a certified or non-certified value. A NIST certified value is a value for which NIST has the highest confidence in its accuracy in that all known or suspected sources of bias and variability have been considered [2]. NIST non-certified values are best estimates based on currently available information and may not provide metrological traceability to a higher-order reference system [2]. When a NIST certified or non-certified value has been assigned, that value is used as the NIST target value and the 95 % expanded uncertainty on the assigned value is used as u_{NIST} . For samples in which a NIST certified or non-certified value is not available, a target value may be determined at NIST using an established method or data from a collaborating laboratory. The target value represents the mean of at least three replicates, and u_{NIST} is estimated as twice the standard deviation of those replicate measurements. The standard deviations are inflated by a factor of two to protect against underestimation of uncertainties and subsequent potential implications of poor participant performance. For materials acquired from and/or evaluated as a part of 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. A unique feature of NIST QAPs is the accuracy-based component provided by comparison of participant results to a NIST value.

1.1.3. Summary Data Table

This data table includes a summary of all reported data for a specific 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 section 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. The standard deviation (SD) for the target value in this table is the uncertainty (U_{NIST}) around the target value. Data highlighted in red have been flagged as a data entry of zero or results that include text (e.g., “< LOQ” or “present”). Data highlighted in blue have been identified as outside the consensus tolerance limits and would be estimated to yield $|Z'_{comm}| > 2$. The summary data tables are presented in the format shown in Table 1-3.

Table 1-3. Exemplar data summary table.

		Analyte									
		Sample 1 (units)					Sample 2 (units)				
		A	B	C	Avg ^a	SD ^b	A	B	C	Avg	SD
Individual Results	Target				c_1	d_1				c_2	d_2
	e_1	X_{A1-1}	X_{B1-1}	X_{C1-1}	\bar{x}_{1-1}	s_{1-1}	X_{A2-1}	X_{B2-1}	X_{C2-1}	\bar{x}_{1-2}	s_{1-2}

	e_n	X_{A1-n}	X_{B1-n}	X_{C1-n}	\bar{x}_{n-1}	s_{n-1}	X_{A2-n}	X_{B2-n}	X_{C2-n}	\bar{x}_{n-2}	s_{n-2}
Community Results	Consensus Mean				f_1				f_2		
	Consensus Standard Deviation				g_1				g_2		
	Maximum				h_1				h_2		
	Minimum				i_1				i_2		
	N				j_1				j_2		

- ^a The arithmetic average of the sample replicates.
- ^b The standard deviation of the sample replicates.
- ^c The target value for the sample.
- ^d The standard deviation of the target value for the sample.
- ^e The laboratory identifier for the participant.
- ^f The robust mean of reported results.
- ^g The robust standard deviation of reported results.
- ^h The maximum of reported average results.
- ⁱ The minimum of reported average results.
- ^j The number of quantitative values reported.

1.1.4. Figures

1.1.4.1. Data Summary View (Method Comparison Data Summary View)

In this view (Fig. 1-1), individual laboratory data (diamonds) are plotted with the individual laboratory SD (rectangle). Laboratories reporting values below their method LOQ are shown in this view as downward triangles beginning at the LOQ, reported as quantification limit (QL) on the figures. Laboratories reporting values below LOQ can still be successful in the study if the target value is also below the method 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 uncertainty 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 ($n_{\text{participants}}$), and the average number of replicates reported by each participant ($n_{\text{average number of repliates per participant}}$). 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 NIST target range (values that result in an acceptable Z score, $|Z| \leq 2$). 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 for sample preparation, analysis, or quantitation.

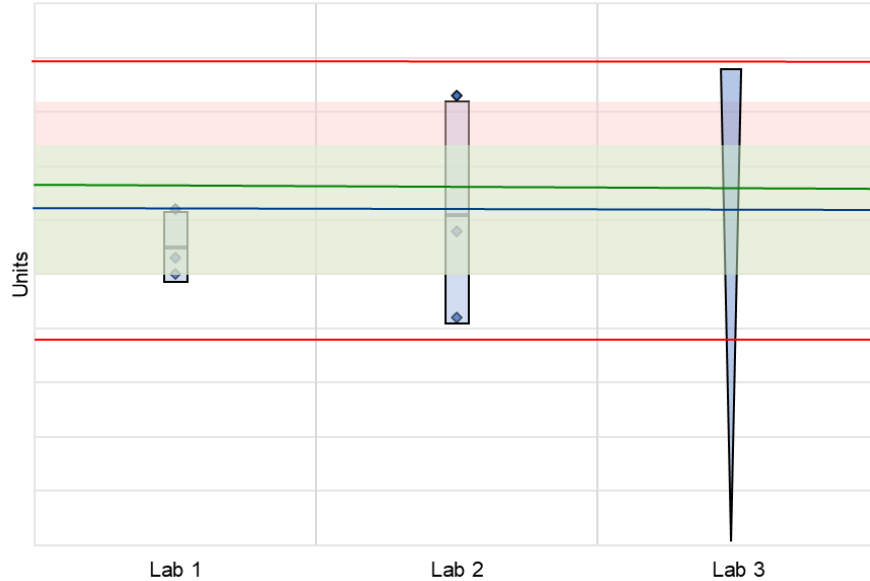


Fig. 1-1. Example data summary view.

1.1.4.2. Sample/Sample Comparison View

In this view (Fig. 1-2), the individual laboratory results for one sample are compared to the results for another sample in the study examining the same analyte. 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. Primary program goals are 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.

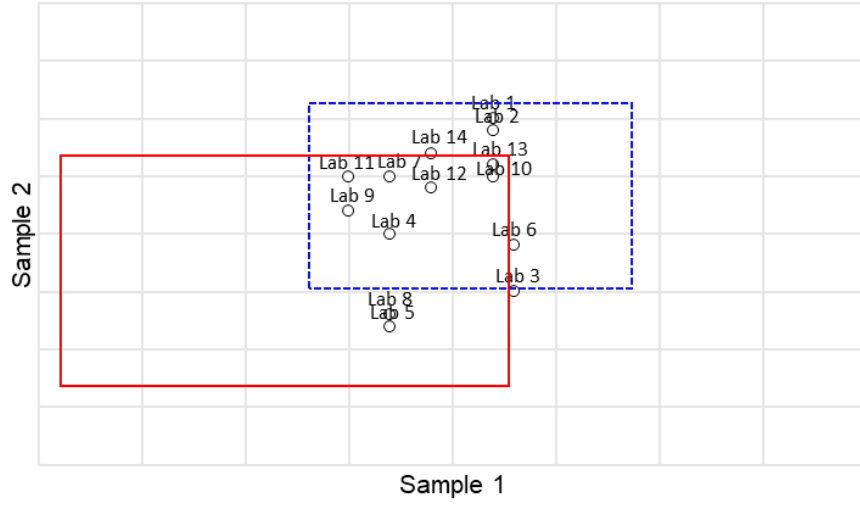


Fig. 1-2. Example sample/sample comparison view.

2. NUTRITIONAL ELEMENTS (Chromium, Molybdenum, Selenium)

2.1. Executive Summary

Nutritional elements are an important part of dietary uptake and human health, therefore accurate measurements in foods are needed to meet requirements for nutritional labelling especially for infant formula regulations. Participants in this study performed well in determination of nutritional elements regarding within-laboratory and among-laboratory measurement reproducibility except for chromium (Cr) in RM 8260. The significantly lower Cr mass fraction in RM 8260 than RM 8261 posed measurement challenges for many participating laboratories. The consensus mean ranges overlapped with the top of the target ranges for the nutritional elements in this study. Most participants reported using microwave digestion methods for sample preparation and inductively coupled plasma mass spectrometry (ICP-MS) methods for analysis. No trends were identified in the results based on these sample preparation and analysis methods. The correlation of bias in reported values between the two similar samples indicated a potential measurement issue related to method calibration.

2.2. Study Overview

Chromium, molybdenum (Mo), and selenium (Se) are essential nutritional elements required for the human body to function properly [3]. To reduce the burden of chronic diseases caused by a deficiency or excess intake, accurate assessments of these elements in foods such as infant formula are necessary to better understand the connections between dietary intake, nutritional status, and health outcomes both at individual and population levels. In this study, participants were provided with two nutritional formula samples, reference material (RM) 8260 Infant Nutritional Formula (hydrolyzed milk-based) and RM 8261 Adult Nutritional Formula (high protein). Participants were asked to use in-house analytical methods to determine the mass fractions (mg/kg) of Cr, Mo, and Se in infant formula samples. Through participation in this study, laboratories can better understand the performance of their in-house methods relative to those being used by others in the community. Participant results may be used in the value assignment of NIST reference materials included as samples in this study.

2.3. Sample Information

Participants were provided with three packets each of RM 8260 Infant Nutritional Formula (hydrolyzed milk-based) (labeled Infant Formula B) and RM 8261 Adult Nutritional Formula (high protein) (labeled Infant Formula C). Each packet contained approximately 10 g of material. Participants were asked to store the materials 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 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 nutritional elements. The approximate analyte levels were not reported to participants prior to the study. The target values for nutritional elements in RM 8260 and RM 8261 were determined using data from NIST measurements. The target values and uncertainty for nutritional elements in RM 8260 and RM 8261 are provided in Table 2-1 on an as-

received basis. The uncertainties for RM 8260 and RM 8261 were calculated and combined according to guidelines of ISO and the Joint Committee for Guides in Metrology (JCGM) [4], and the expanded uncertainty expressed as an approximately 95 % level of confidence for each nutritional element. The expanded uncertainties for each material were used as the standard uncertainties for participant data assessment as described in Section 1.1.2.

Table 2-1. Individualized data summary table for nutritional elements in infant formulas.

Laboratory-specific results and Z-scores were provided to each participant separately from this report to protect laboratory identities.

(Lab Name)

Exercise 2 – Nutritional Elements in Infant Formula

Lab Code: (Code)		1. Your Results				2. Community Results			3. Target		
Sample	Units	x_i	s_i	Z'_{comm}	Z_{NIST}	N	x^*	s^*	x_{NIST}	u_{NIST}	
Cr	RM 8260	mg/kg	<i>Individual laboratory results will appear in this section; laboratory-specific results were provided to each participant separately from this report.</i>				17	0.046	0.040	0.0243	0.0076
Cr	RM 8261	mg/kg					23	0.391	0.056	0.378	0.013
Mo	RM 8260	mg/kg					20	0.213	0.033	0.2058	0.0072
Mo	RM 8261	mg/kg					21	0.509	0.053	0.501	0.012
Se	RM 8260	mg/kg					20	0.274	0.053	0.265	0.018
Se	RM 8261	mg/kg					20	0.328	0.057	0.300	0.015
		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_{NIST}	standard uncertainty about the 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				

2.4. Study Results and Discussion

Table 2-1 summarizes and Table 2-2, Table 2-3, and Table 2-4 detail the measured mass fraction results reported by each participating laboratory for nutritional elements. The participation level was fair for nutritional elements, with 62 % to 67 % of laboratories requesting samples returning results (on average 22 of 35 laboratories). Table 2-2 reveals that of the 24 participants that submitted results for Cr, seven laboratories reported data as below LOQ for RM 8260, while only one laboratory reported as below LOQ for RM 8261. The mass fraction of Cr in RM 8260 was over ten times lower than RM 8261 and this posed a measurement challenge. Laboratories reporting LOQs above 0.16 mg/kg should consider reevaluating their method performance characteristics, as the nutritional formula community has indicated that analytical methods must be able to quantify Cr at 0.16 mg/kg [5].

Table 2-2. Data summary table for chromium in RM 8260 and RM 8261.

Data highlighted in blue have been identified as outside the consensus range of tolerance and resulted in an unacceptable Z'_{comm} score, $|Z'_{comm}| > 2$. Note: This table spans two pages; the target values and consensus values are included on both pages.

		Chromium									
		RM 8260 Infant Nutritional Formula (hydrolyzed milk-based) (mg/kg)					RM 8261 Adult Nutritional Formula (high protein) (mg/kg)				
		Lab	A	B	C	Avg	SD	A	B	C	Avg
Individual Results	Target				0.024	0.008				0.378	0.013
	B002	< 0.400	< 0.400				< 0.400	< 0.400			
	B004	0.038	0.035	0.031	0.035	0.004	0.389	0.355	0.417	0.387	0.031
	B005	2.6435	2.6757	2.6796	2.666	0.020	3.6968	3.7545	3.7628	3.738	0.036
	B007	< 0.200	< 0.200	< 0.200			0.37	0.39	0.39	0.383	0.012
	B008	0.08	0.1	0.09	0.090	0.010	0.52	0.44	0.43	0.463	0.049
	B009	0.13	0.11	0.13	0.123	0.012	0.5	0.44	0.48	0.473	0.031
	B010	0.0124	0.0028	0.0077	0.008	0.005	0.3331	0.3393	0.345	0.339	0.006
	B011	0.064	0.071	0.092	0.076	0.015	0.43	0.409	0.422	0.420	0.011
	B012										
	B013	< 0.020	< 0.020	< 0.020			0.329	0.331	0.358	0.339	0.016
	B015	0.0448	0.0609	0.0558	0.054	0.008	0.3214	0.3439	0.3533	0.340	0.016
	B018										
	B019	0.046	0.04	0.044	0.043	0.003	0.42	0.46	0.41	0.430	0.026
	B021	< 0.060	< 0.060	< 0.060			0.359	0.358	0.35	0.356	0.005
	B022	0.0124	0.0174	0.0111	0.014	0.003	0.339	0.3485	0.3543	0.347	0.008
	B023	0.024	0.028	0.022	0.025	0.003	0.389	0.381	0.357	0.376	0.017
	B024										
	B027	0.021	0.0166	0.0219	0.020	0.003	0.379	0.382	0.389	0.383	0.005
	B028	< 0.035	< 0.035	< 0.035			0.364	0.345	0.351	0.353	0.010
	B030										
	B031	0.035	0.033	0.031	0.033	0.002	0.323	0.305	0.337	0.322	0.016
	B032	0.0168	0.0258	0.0231	0.022	0.005	0.3574	0.3584	0.426	0.381	0.039
	B033										
B034	< 0.100	< 0.100	< 0.100			0.41	0.42	0.39	0.407	0.015	
B037											
B038											
B043	0.99	0.99	0.98	0.987	0.006	0.36	0.41	0.37	0.380	0.026	
B044	< 0.050	< 0.050	< 0.050			0.454	0.455	0.459	0.456	0.003	
B046											
Community Results	Consensus Mean				0.046		Consensus Mean			0.391	
	Consensus Standard Deviation				0.040		Consensus Standard Deviation			0.056	
	Maximum				2.666		Maximum			3.738	
	Minimum				0.008		Minimum			0.322	
	N				17		N			23	

Table 2-2 continued. Data summary table for chromium in RM 8260 and RM 8261.

		Chromium									
		RM 8260 Infant Nutritional Formula (hydrolyzed milk-based) (mg/kg)					RM 8261 Adult Nutritional Formula (high protein) (mg/kg)				
		Lab	A	B	C	Avg	SD	A	B	C	Avg
Individual Results	Target				0.024	0.008				0.378	0.013
	B047										
	B048										
	B050	0.092	0.081	0.074	0.082	0.009	0.422	0.468	0.443	0.444	0.023
	B051										
	B052	0.025	0.041	0.022	0.029	0.010	0.344	0.329	0.343	0.339	0.008
	B053										
	B055	0.05	0.05		0.050	0.000	0.56	0.45	0.43	0.480	0.070
Community Results		Consensus Mean			0.046		Consensus Mean			0.391	
		Consensus Standard Deviation			0.040		Consensus Standard Deviation			0.056	
		Maximum			2.666		Maximum			3.738	
		Minimum			0.008		Minimum			0.322	
		<i>N</i>			17		<i>N</i>			23	

Table 2-3. Data summary table for molybdenum in RM 8260 and RM 8261.

Data highlighted in blue have been identified as outside the consensus tolerance limits and resulted in an unacceptable Z'_{comm} score, $|Z'_{comm}| > 2$. Note: This table spans two pages; the target values and consensus values are included on both pages.

		Molybdenum									
		RM 8260 Infant Nutritional Formula (hydrolyzed milk-based) (mg/kg)					RM 8261 Adult Nutritional Formula (high protein) (mg/kg)				
		Lab	A	B	C	Avg	SD	A	B	C	Avg
Individual Results	Target				0.206	0.007				0.501	0.012
	B004	0.253	0.218	0.232	0.234	0.018	0.458	0.455	0.476	0.463	0.011
	B005	0.439	0.4618	0.4771	0.459	0.019	0.5813	0.6134	0.6718	0.622	0.046
	B007	< 0.500	< 0.500	< 0.500			0.64	0.83	0.7	0.723	0.097
	B008	0.17	0.18	0.18	0.177	0.006	0.41	0.4	0.38	0.397	0.015
	B009										
	B010	0.223	0.218	0.214	0.218	0.005	0.509	0.504	0.51	0.508	0.003
	B011	0.201	0.181	0.205	0.196	0.013	0.491	0.459	0.48	0.477	0.016
	B012										
	B013	0.199	0.202	0.199	0.200	0.002	0.496	0.484	0.503	0.494	0.010
	B015	0.2013	0.2105	0.1998	0.204	0.006	0.4816	0.4982	0.4817	0.487	0.010
	B018										
	B019	0.23	0.24	0.24	0.237	0.006	0.59	0.59	0.61	0.597	0.012
	B021	0.191	0.178	0.172	0.180	0.010	0.451	0.442	0.453	0.449	0.006
	B022	0.2096	0.2117	0.207	0.209	0.002	0.4828	0.4902	0.4797	0.484	0.005
	B023	0.23	0.235	0.227	0.231	0.004	0.582	0.545	0.488	0.538	0.047
	B027	0.21	0.205	0.205	0.207	0.003	0.498	0.506	0.494	0.499	0.006
	B028	0.239	0.242	0.246	0.242	0.004	0.588	0.572	0.584	0.581	0.008
	B030										
	B031	0.185	0.174	0.187	0.182	0.007	0.457	0.436	0.477	0.457	0.021
	B032										
	B033										
	B034	0.264	0.251	0.256	0.257	0.007	0.582	0.573	0.592	0.582	0.010
	B037										
	B038										
	B043	1.67	1.7	1.74	1.703	0.035	0.51	0.51	0.49	0.503	0.012
	B044	0.195	0.205	0.199	0.200	0.005	0.484	0.479	0.477	0.480	0.004
	B046										
B047											
B048											
Community Results	Consensus Mean				0.213		Consensus Mean			0.509	
	Consensus Standard Deviation				0.033		Consensus Standard Deviation			0.053	
	Maximum				1.703		Maximum			0.723	
	Minimum				0.177		Minimum			0.397	
	N				20		N			21	

Table 2-3 continued. Data summary table for chromium in RM 8260 and RM 8261.

		Molybdenum											
		RM 8260 Infant Nutritional Formula (hydrolyzed milk-based) (mg/kg)					RM 8261 Adult Nutritional Formula (high protein) (mg/kg)						
		Lab	A	B	C	Avg	SD	A	B	C	Avg	SD	
Individual Results	Target					167	17				91.0	9.1	
	B050	0.229	0.239	0.225	0.231	0.007	0.518	0.525	0.532	0.525	0.007		
	B051												
	B052	0.214	0.209	0.209	0.211	0.003	0.514	0.52	0.497	0.510	0.012		
	B053												
	B055	0.22	0.22	0.22	0.220	0.000	0.51	0.53	0.5	0.513	0.015		
Community Results		Consensus Mean				0.213		Consensus Mean				0.509	
		Consensus Standard Deviation				0.033		Consensus Standard Deviation				0.053	
		Maximum				1.703		Maximum				0.723	
		Minimum				0.177		Minimum				0.397	
		N				20		N				21	

Table 2-4. Data summary table for selenium in RM 8260 and RM 8261.

Data highlighted in blue have been identified as outside the consensus tolerance limits and resulted in an unacceptable Z'_{comm} score, $|Z'_{comm}| > 2$. Note: This table spans two pages; the target values and consensus values are included on both pages.

		Selenium									
		RM 8260 Infant Nutritional Formula (hydrolyzed milk-based) (mg/kg)					RM 8261 Adult Nutritional Formula (high protein) (mg/kg)				
		Lab	A	B	C	Avg	SD	A	B	C	Avg
Individual Results	Target				0.265	0.018				0.300	0.015
	B004	0.407	0.458	0.402	0.422	0.031	0.407	0.448	0.378	0.411	0.035
	B005	0.487	0.4809	0.4833	0.484	0.003	0.6821	0.6882	0.7072	0.693	0.013
	B007	< 0.500	< 0.500	< 0.500			< 0.500	< 0.500	< 0.500		
	B008	0.21	0.22	0.23	0.220	0.010	0.27	0.26	0.24	0.257	0.015
	B009	0.31	0.33	0.28	0.307	0.025	0.34	0.36	0.36	0.353	0.012
	B010	0.284	0.271	0.28	0.278	0.007	0.308	0.31	0.325	0.314	0.009
	B011	0.254	0.235	0.265	0.251	0.015	0.331	0.304	0.322	0.319	0.014
	B012										
	B013	0.242	0.248	0.246	0.245	0.003	0.291	0.282	0.28	0.284	0.006
	B015	0.2586	0.275	0.2585	0.264	0.009	0.3082	0.3157	0.3128	0.312	0.004
	B018										
	B019										
	B021	0.242	0.231	0.215	0.229	0.014	0.275	0.268	0.217	0.253	0.032
	B022	0.263	0.2868	0.3182	0.289	0.028	0.3276	0.2837	0.3252	0.312	0.025
	B023	0.223	0.247	0.247	0.239	0.014	0.311	0.295	0.284	0.297	0.014
	B027	0.279	0.262	0.268	0.270	0.009	0.321	0.328	0.327	0.325	0.004
	B028	0.282	0.282	0.267	0.277	0.009	0.343	0.344	0.345	0.344	0.001
	B030										
	B031	0.276	0.266	0.27	0.271	0.005	0.344	0.325	0.329	0.333	0.010
	B032										
	B033										
	B034	0.255	0.184	0.266	0.235	0.045	0.296	0.328	0.317	0.314	0.016
	B037										
	B038										
	B043	1.18	0.83	0.81	0.940	0.208	0.37	0.35	0.36	0.360	0.010
	B044	0.268	0.26	0.259	0.262	0.005	0.304	0.317	0.306	0.309	0.007
	B046										
B047											
B048											
Community Results	Consensus Mean				0.274		Consensus Mean			0.328	
	Consensus Standard Deviation				0.053		Consensus Standard Deviation			0.057	
	Maximum				0.940		Maximum			0.693	
	Minimum				0.220		Minimum			0.253	
	N				20		N			20	

Table 2-4 continued. Data summary table for selenium in RM 8260 and RM 8261.

Data highlighted in blue have been identified as outside the consensus tolerance limits and resulted in an unacceptable Z'_{comm} score, $|Z'_{comm}| > 2$.

		Selenium									
		RM 8260 Infant Nutritional Formula (hydrolyzed milk-based) (mg/kg)					RM 8261 Adult Nutritional Formula (high protein) (mg/kg)				
		Lab	A	B	C	Avg	SD	A	B	C	Avg
Individual Results	Target				0.265	0.018				0.300	0.015
	B050	0.56	0.529	0.509	0.533	0.026	0.558	0.616	0.614	0.596	0.033
	B051										
	B052	0.327	0.344	0.359	0.343	0.016	0.401	0.412	0.38	0.398	0.016
	B053										
	B055	0.3	0.3	0.3	0.300	0.000	0.4	0.4	0.4	0.400	0.000
Community Results		Consensus Mean			0.274		Consensus Mean			0.328	
		Consensus Standard Deviation			0.053		Consensus Standard Deviation			0.057	
		Maximum			0.940		Maximum			0.693	
		Minimum			0.220		Minimum			0.253	
		<i>N</i>			20		<i>N</i>			20	

To assess performance of methods run by individual participants and the community as a whole, repeatability and reproducibility were compared to the relevant AOAC Standard Method Performance Requirements® (SMPR®). AOAC SMPR® 2011.009 Standard Method Performance Requirements for Cr, Mo, and Se in Infant Formula and Adult/Pediatric Nutritional Formula [5] was used to evaluate nutritional element performance results for RM 8260 and RM 8261. Repeatability, demonstrated by within-laboratory variability (mean % RSD), and reproducibility, demonstrated by among-laboratory variability (% RSD), are shown in Table 2-5. Within-laboratory variabilities were mostly acceptable for all nutritional elements in both formula materials with the exception of Cr in RM 8260. Approximately 82 % of laboratories for RM 8260 had within-laboratory measured Cr mass fraction variabilities greater than 5 % RSD. The among-laboratory variabilities for nutritional elements in formula were around the published expectations of the measurement community of ≤ 15 % RSD [5]. Once again, Cr in RM 8260 was an exception with 87 % among-laboratory variability.

Table 2-5. Laboratory variabilities for nutritional elements in FNSQAP Exercise 2 formula materials relative to AOAC SMPR 2011.009 method performance requirements.

Element	Nutritional Elements					
	Within-Laboratory Variability			Among-Laboratory Variability		
	FNSQAP Ex. 2		SMPR 2011.009	FNSQAP Ex. 2		SMPR 2011.009
	RM 8260	RM 8261		RM 8260	RM 8261	
Cr	15.3 %	4.8 %	≤ 5 %	87.0 %	14.3 %	≤ 15 %
Mo	2.8 %	3.2 %	≤ 5 %	15.5 %	10.4 %	≤ 15 %
Se	5.8 %	4.0 %	≤ 5 %	19.3 %	17.4 %	≤ 15 %

As shown in Fig. 2-1, Fig. 2-2, Fig. 2-3, Fig. 2-4, Fig. 2-5, and Fig. 2-6 laboratories reported using a few different sample preparation methods for the determination of nutritional elements in the two formula samples. Numbers and percentages of laboratories described as reporting specific approaches are averages across all results for three elements and two samples. The most common sample preparation approach was a microwave digestion method (16 laboratories, 73 %); two laboratories reported using hot block digestion (9 %) and one laboratory reported using digestion without specification (4 %). Three laboratories did not report the sample preparation approach used (15 %). Notably, the laboratories indicating use of hot block digestion as the preparation method prior to analysis for determination of nutritional elements reported values biased above the 95 % confidence interval for the consensus mean in both samples for most elements. Although this preparation method is only represented by two laboratories, perhaps open beaker digestions such as hot block digestion are not ideal for nutritional element sample preparation of formula matrices. The sample preparation procedure is critical for unbiased measurements, and those that used microwave digestion methods should review protocols for future analyses to ensure complete digestion to release the analytes from the samples into solution. Greater than desired within-laboratory variability may also be due to the use of less than the recommended sample size for analysis (0.5 g) since the sample may not be homogenous below this mass.

As shown in Fig. 2-7, Fig. 2-8, Fig. 2-9, Fig. 2-10, Fig. 2-11, and Fig. 2-12, ICP-MS analytical methods (20 laboratories; 91 %) were the primary methods employed by laboratories for the determination of nutritional elements in the two formula samples. One laboratory reported using ICP-OES (5 %) and one laboratory did not report the analytical method used (5 %). Notably, the laboratory indicating use of ICP-OES as the analysis method for determination of nutritional elements reported values biased above the 95 % confidence interval for the consensus mean in both samples for all elements. Although this analysis method is only represented by one laboratory, perhaps ICP-OES is not ideal for nutritional element analysis for the mass fractions present in the formula samples. Sensitivity of the analytical method is key when determining whether the method is suitable for the analyte abundance in the sample and appropriate sample dilution for the dynamic range of the analytical method. Since ICP-MS was the most reported analytical method, some technical recommendations are provided for this analytical method. Collision cell gases or reaction cell mode can be used with ICP-MS to reduce or eliminate the interferences caused by molecular ions that have the same mass-to-charge ratio as the element of interest. Utilizing ICP-MS in kinetic energy discrimination (KED) mode can control cell-formed interferences and reduce polyatomic ion interferences created by the plasma or vacuum interface. For example, most Se isotopes suffer isobaric overlap or polyatomic interferences mainly from argon dimers (Ar_2^+) causing signal suppression or enhancement leading to bias of the measurements. Helium collision gas reduces Ar_2^+ interferences on Se. If the ICP-MS is a tandem mass spectrometer, oxygen reaction gas can be used to mass shift Se isotopes by adding an oxide, +16 m/z units higher than their native m/z state, to measure that atomic mass.

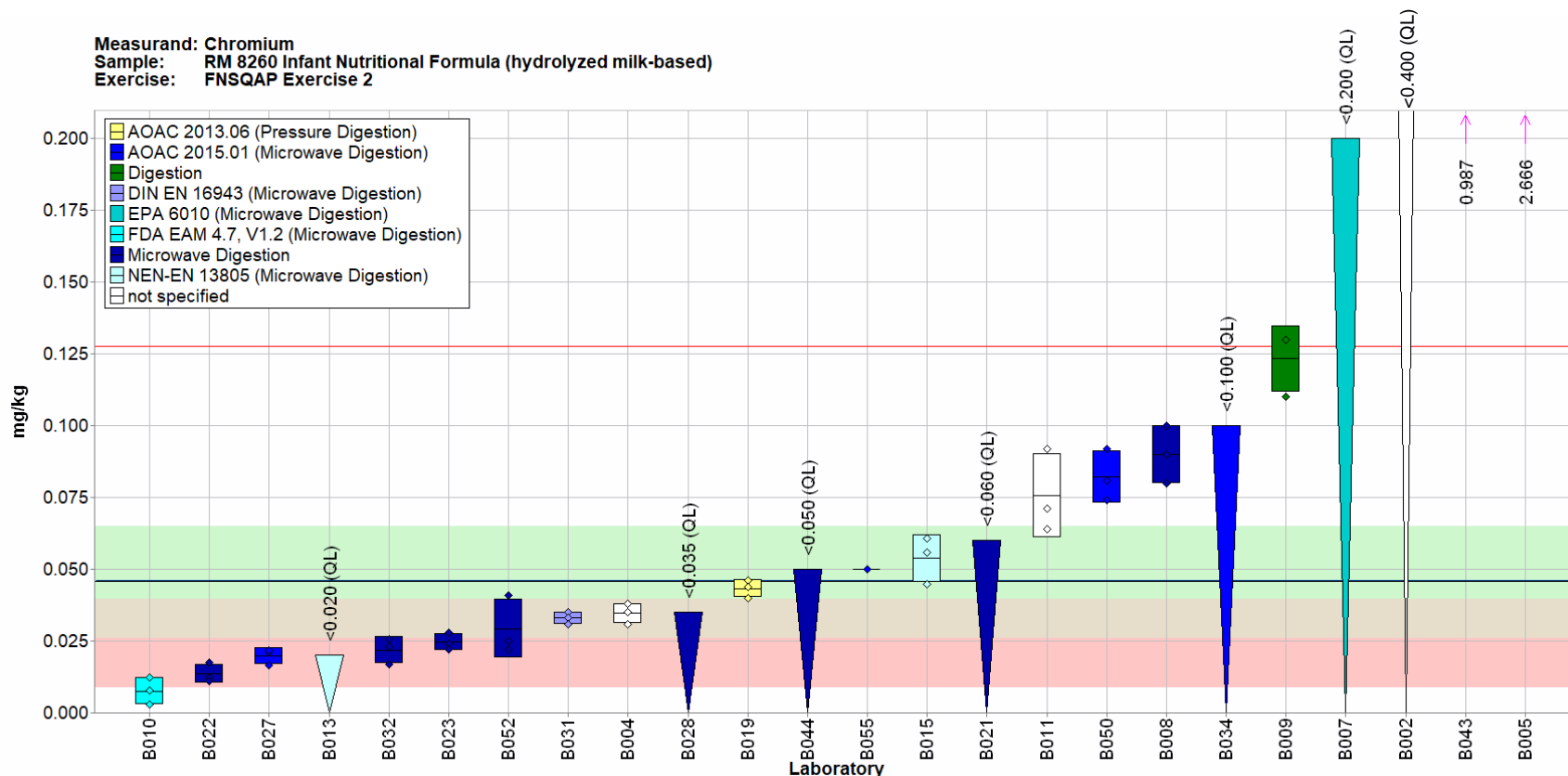


Fig. 2-1. Chromium in RM 8260 (data summary view – sample preparation method).

In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed as indicated in the figure key. Data points outside the graphical range are represented as upward arrows (the preparation methods reported by laboratories B043 and B005 were AOAC 2015.01 (microwave digestion) and hot block digestion, respectively). The 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 target range, which encompasses the target value bounded by twice its uncertainty (U_{NIST}), and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$. The beige shaded region represents the overlapping of the 95 % confidence interval for the consensus mean (green region) and the NIST target range (red region).

Measurand: Chromium
 Sample: RM 8261 Adult Nutritional Formula (high-protein)
 Exercise: FNSQAP Exercise 2

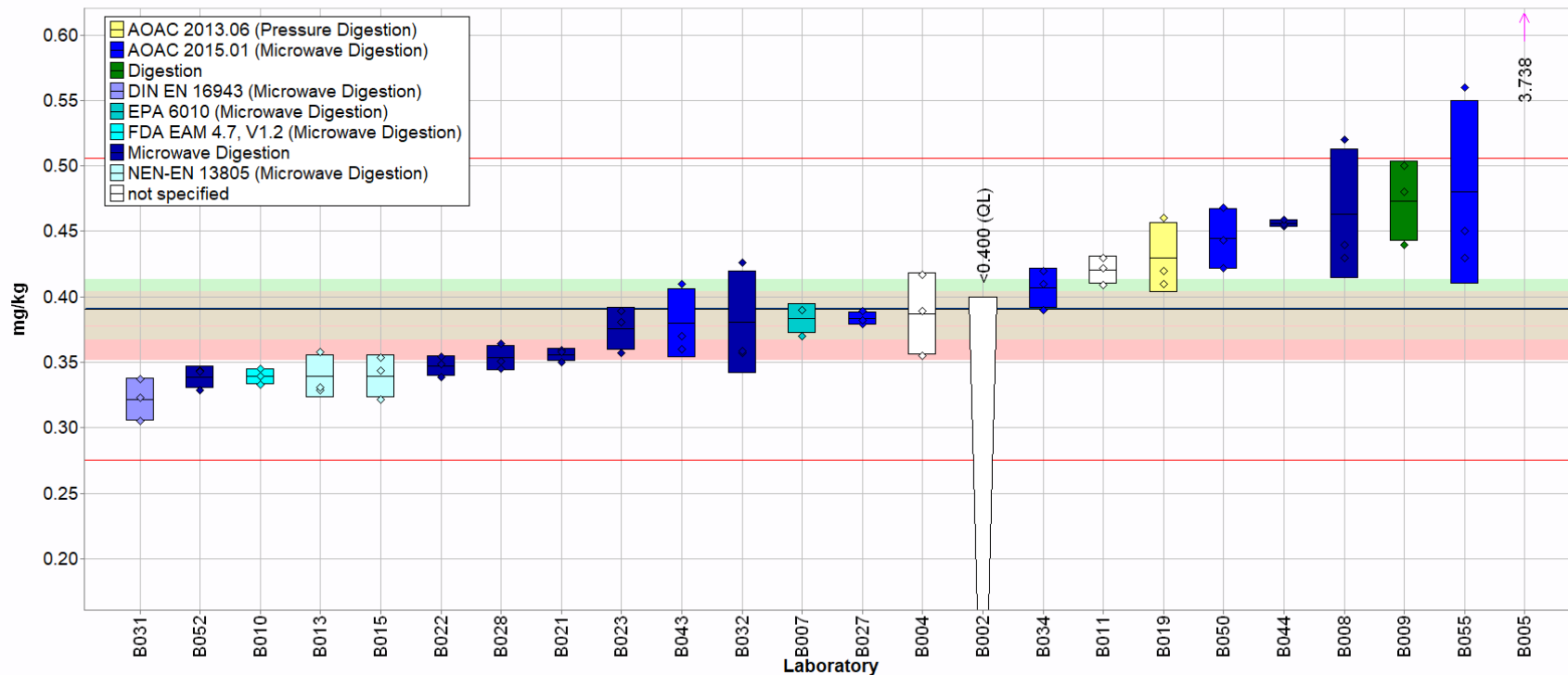


Fig. 2-2. Chromium in RM 8261 (data summary view – sample preparation method).

In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed as indicated in the figure key. Data points outside the graphical range are represented as upward arrows (the preparation method reported by laboratory B005 was hot block digestion). The 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 target range, which encompasses the target value bounded by twice its uncertainty (U_{NIST}), and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$. The beige shaded region represents the overlapping of the 95 % confidence interval for the consensus mean (green region) and the NIST target range (red region).

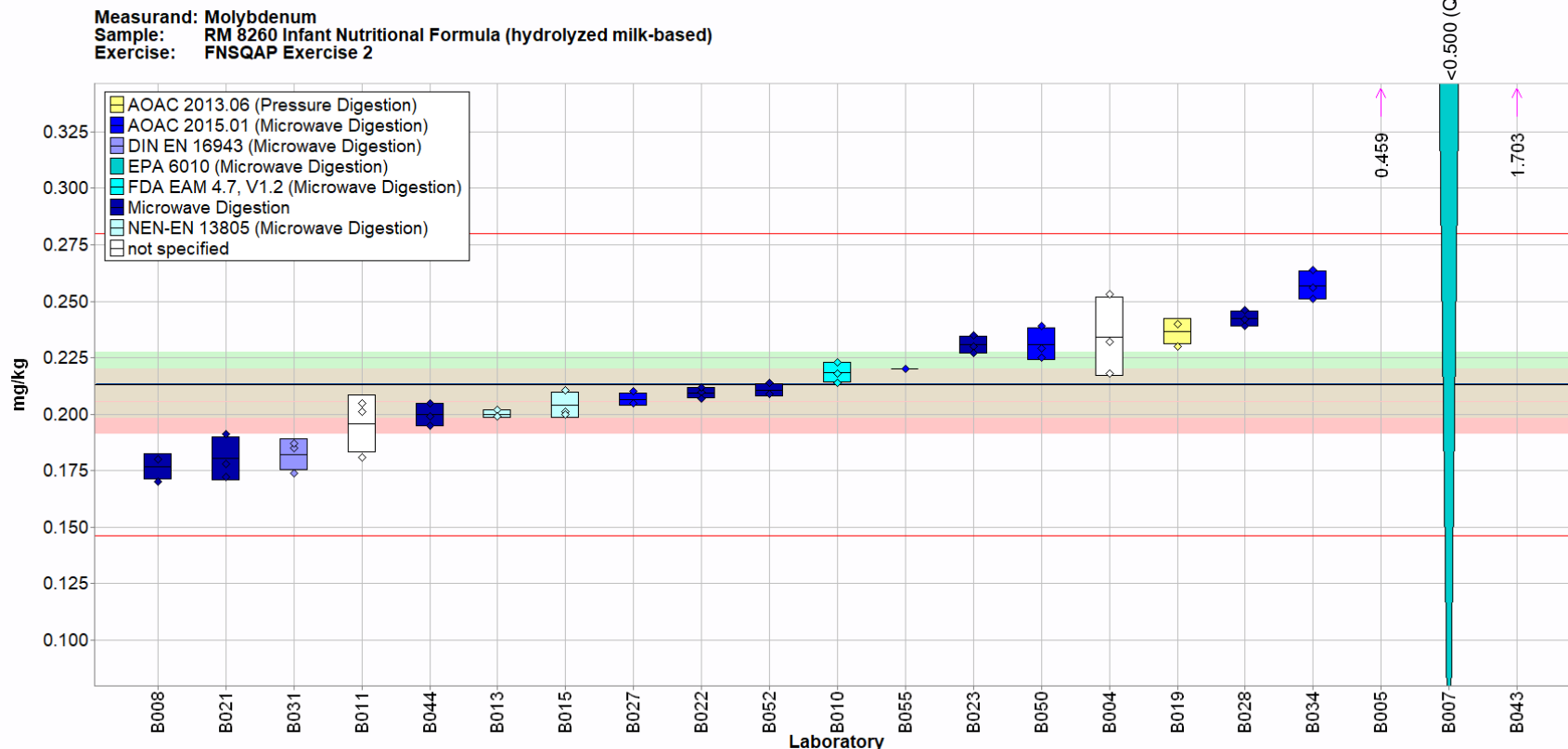


Fig. 2-3. Molybdenum in RM 8260 (data summary view – sample preparation method).

In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed as indicated in the figure key. Data points outside the graphical range are represented as upward arrows (the preparation methods reported by laboratories B005 and B043 were hot block digestion and AOAC 2015.01 (microwave digestion), respectively). The 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 target range, which encompasses the target value bounded by twice its uncertainty (U_{NIST}), and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$. The beige shaded region represents the overlapping of the 95 % confidence interval for the consensus mean (green region) and the NIST target range (red region).

Measurand: Molybdenum
 Sample: RM 8261 Adult Nutritional Formula (high-protein)
 Exercise: FNSQAP Exercise 2

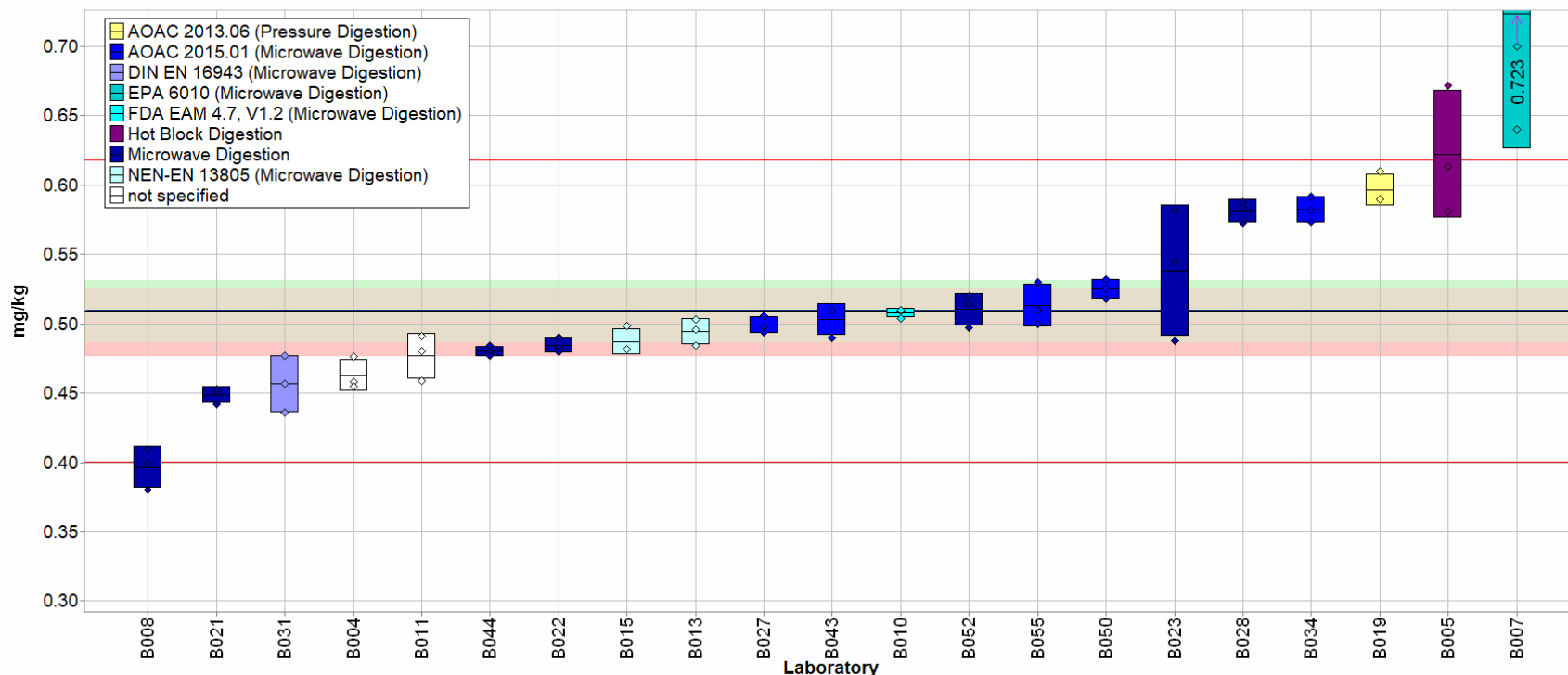


Fig. 2-4. Molybdenum in RM 8261 (data summary view – sample preparation method).

In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed as indicated in the figure key. The 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 target range, which encompasses the target value bounded by twice its uncertainty (U_{NIST}), and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$. The beige shaded region represents the overlapping of the 95 % confidence interval for the consensus mean (green region) and the NIST target range (red region).

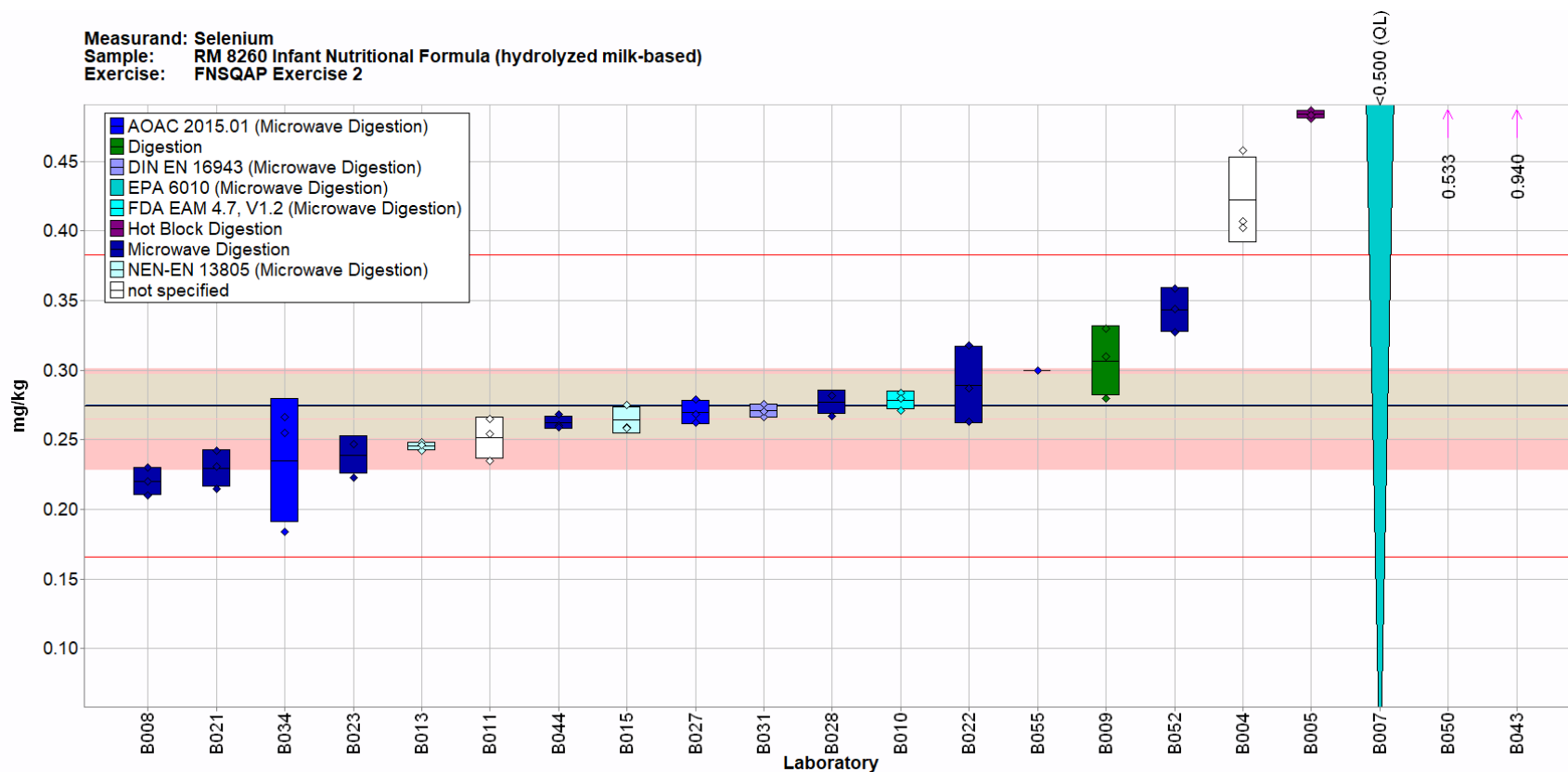


Fig. 2-5. Selenium in RM 8260 (data summary view – sample preparation method).

In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed as indicated in the figure key. Data points outside the graphical range are represented as upward arrows (the preparation method reported by laboratories B050 and B043 was AOAC 2015.01 (microwave digestion)). The 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, $|Z'_{comm}| \leq 2$. The red shaded region represents the NIST target range, which encompasses the target value bounded by twice its uncertainty (U_{NIST}), and represents the range that results in an acceptable Z_{NIST} score, $|Z_{NIST}| \leq 2$. The beige shaded region represents the overlapping of the 95 % confidence interval for the consensus mean (green region) and the NIST target range (red region).

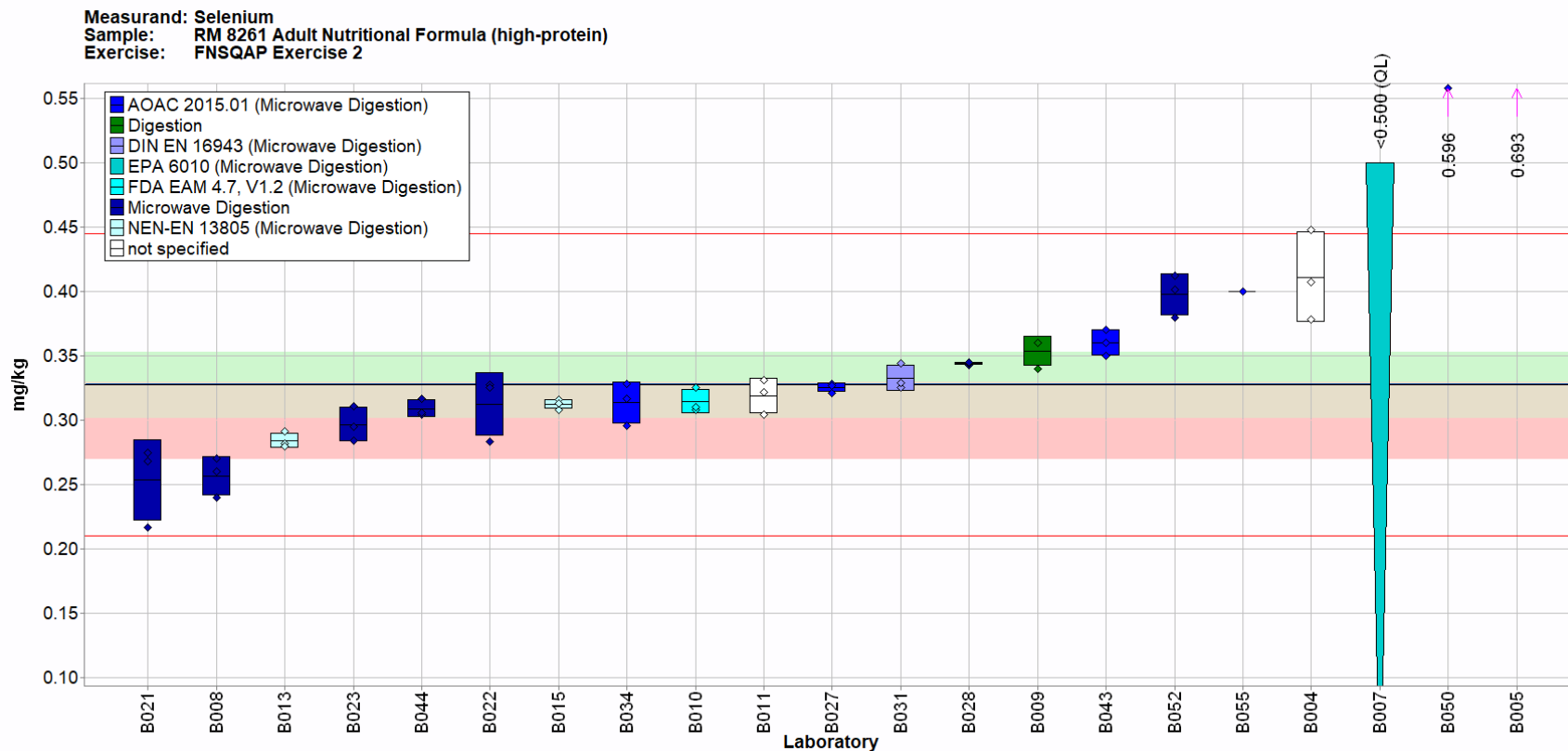


Fig. 2-6. Selenium in RM 8261 (data summary view – sample preparation method).

In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed as indicated in the figure key. Data points outside the graphical range are represented as upward arrows (the preparation methods reported by laboratories B050 and B005 were AOAC 2015.01 (microwave digestion) and hot block digestion, respectively). The 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 target range, which encompasses the target value bounded by twice its uncertainty (U_{NIST}), and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$. The beige shaded region represents the overlapping of the 95 % confidence interval for the consensus mean (green region) and the NIST target range (red region).

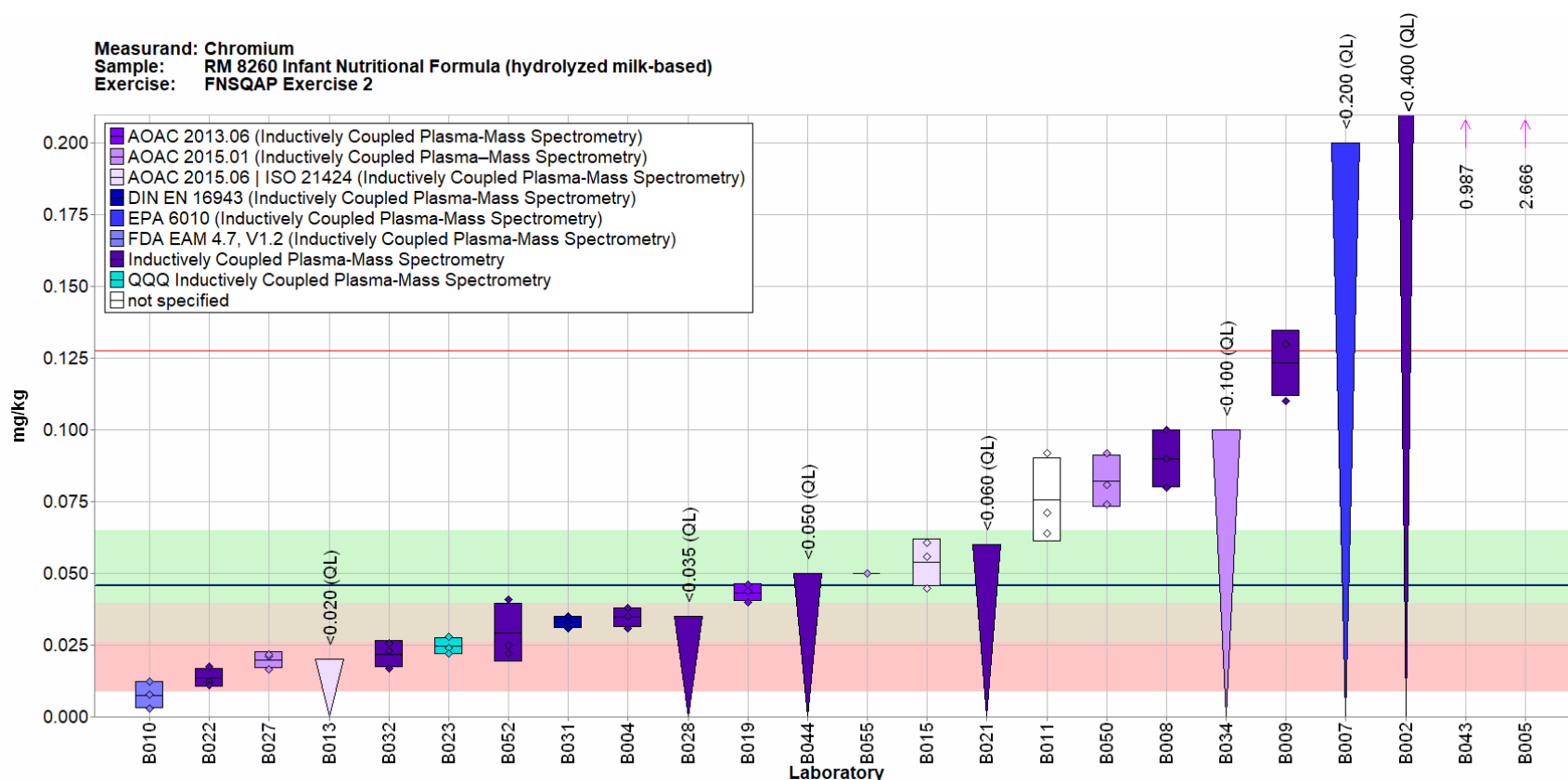


Fig. 2-7. Chromium in RM 8260 (data summary view – analytical method).

In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed as indicated in the figure key. Data points outside the graphical range are represented as upward arrows (the analytical methods reported by laboratories B043 and B005 were AOAC 2015.01 (ICP-MS) and AOAC 2011.14 (ICP-OES), respectively). The 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 target range, which encompasses the target value bounded by twice its uncertainty (U_{NIST}), and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$. The beige shaded region represents the overlapping of the 95 % confidence interval for the consensus mean (green region) and the NIST target range (red region).

Measurand: Chromium
 Sample: RM 8261 Adult Nutritional Formula (high-protein)
 Exercise: FNSQAP Exercise 2

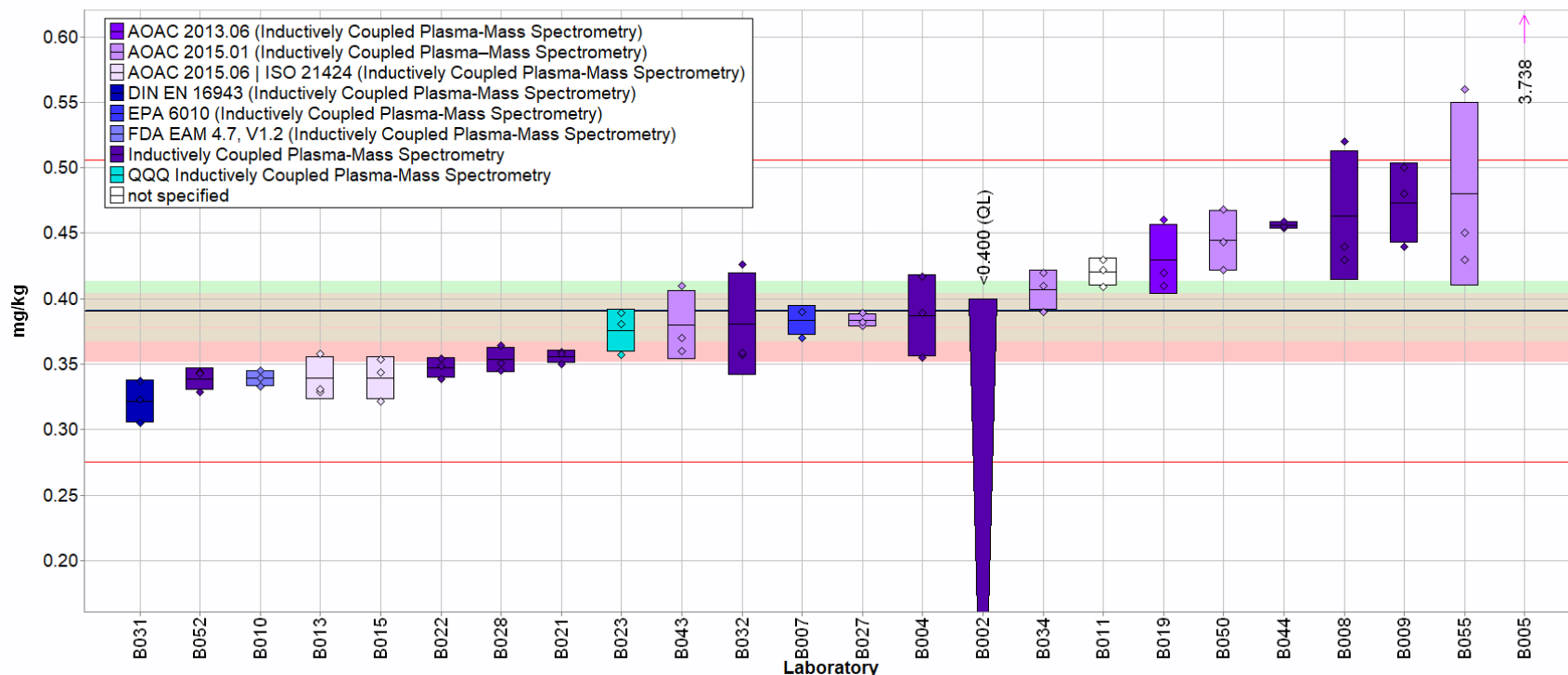


Fig. 2-8. Chromium in RM 8261 (data summary view –analytical method).

In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed as indicated in the figure key. Data points outside the graphical range are represented as upward arrows (the analytical method reported by laboratory B005 was AOAC 2011.14 (ICP-OES)). The 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 target range, which encompasses the target value bounded by twice its uncertainty (U_{NIST}), and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$. The beige shaded region represents the overlapping of the 95 % confidence interval for the consensus mean (green region) and the NIST target range (red region).

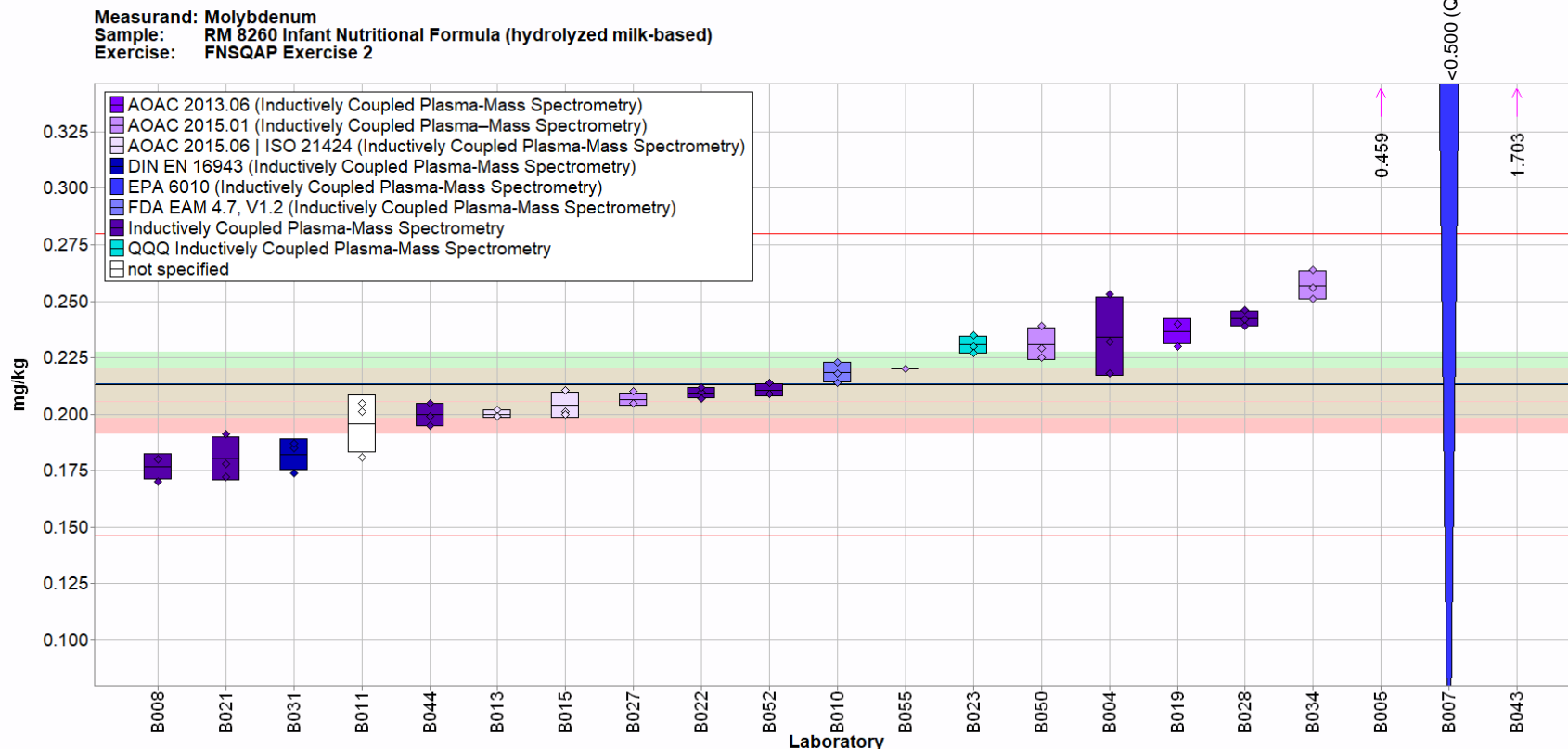


Fig. 2-9. Molybdenum in RM 8260 (data summary view – analytical method).

In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed as indicated in the figure key. Data points outside the graphical range are represented as upward arrows (the analytical methods reported by laboratories B005 and B043 were AOAC 2011.14 (ICP-OES) and AOAC 2015.01 (ICP-MS), respectively). The 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 target range, which encompasses the target value bounded by twice its uncertainty (U_{NIST}), and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$. The beige shaded region represents the overlapping of the 95 % confidence interval for the consensus mean (green region) and the NIST target range (red region).

Measurand: Molybdenum
 Sample: RM 8261 Adult Nutritional Formula (high-protein)
 Exercise: FNSQAP Exercise 2

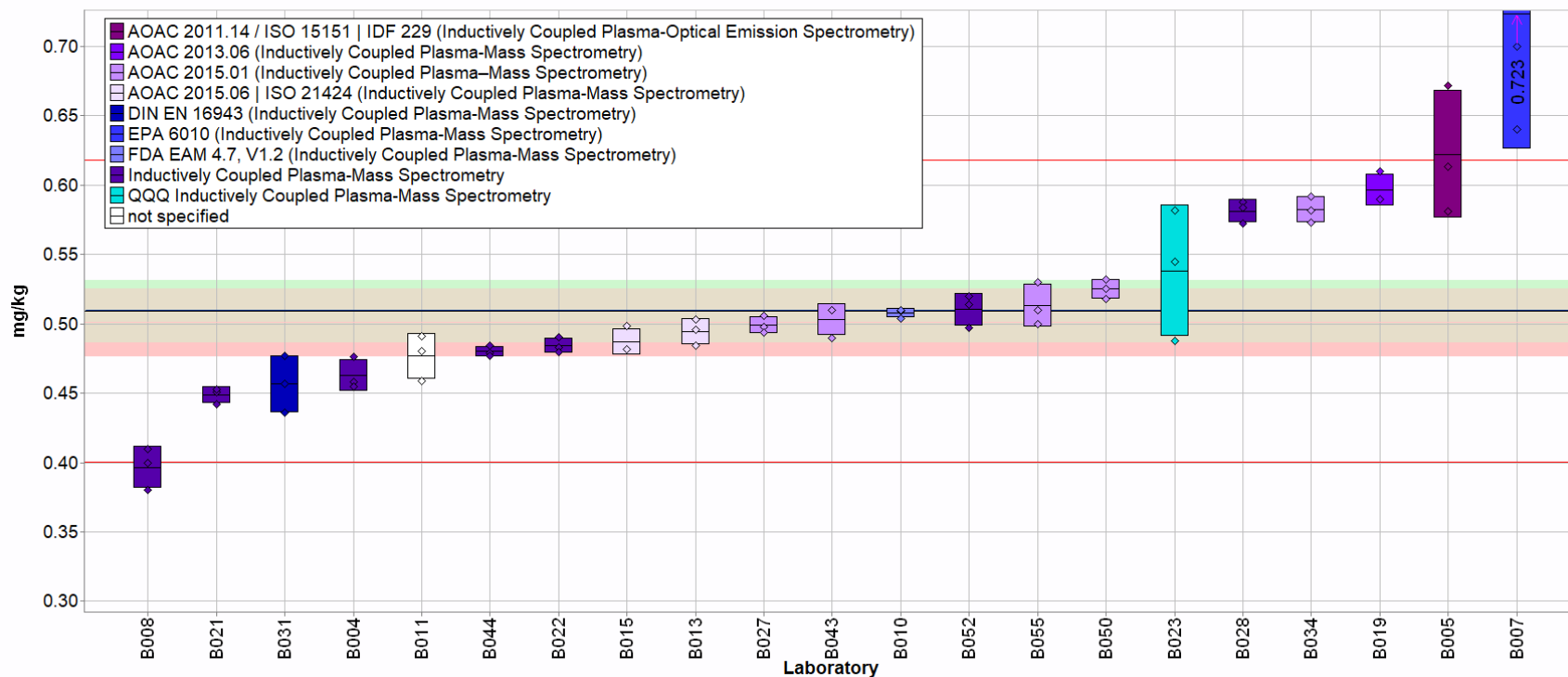


Fig. 2-10. Molybdenum in RM 8261 (data summary view – analytical method).

In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed as indicated in the figure key. The 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 target range, which encompasses the target value bounded by twice its uncertainty (U_{NIST}), and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$. The beige shaded region represents the overlapping of the 95 % confidence interval for the consensus mean (green region) and the NIST target range (red region).

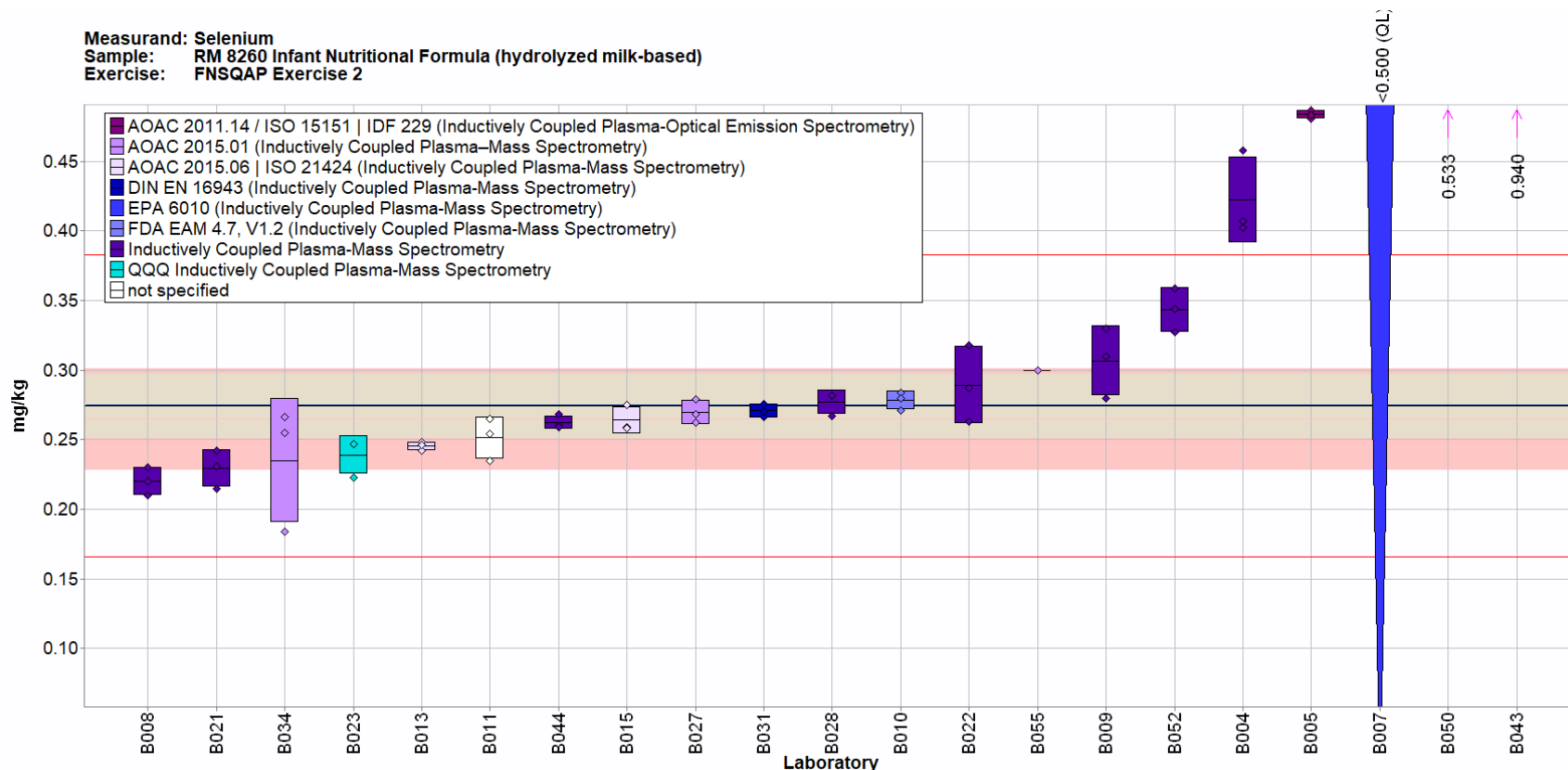


Fig. 2-11. Selenium in RM 8260 (data summary view – analytical method).

In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed as indicated in the figure key. Data points outside the graphical range are represented as upward arrows (the analytical method reported by laboratories B050 and B043 was AOAC 2015.01 (ICP-MS)). The 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 target range, which encompasses the target value bounded by twice its uncertainty (U_{NIST}), and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$. The beige shaded region represents the overlapping of the 95 % confidence interval for the consensus mean (green region) and the NIST target range (red region).

Measurand: Selenium
 Sample: RM 8261 Adult Nutritional Formula (high-protein)
 Exercise: FNSQAP Exercise 2

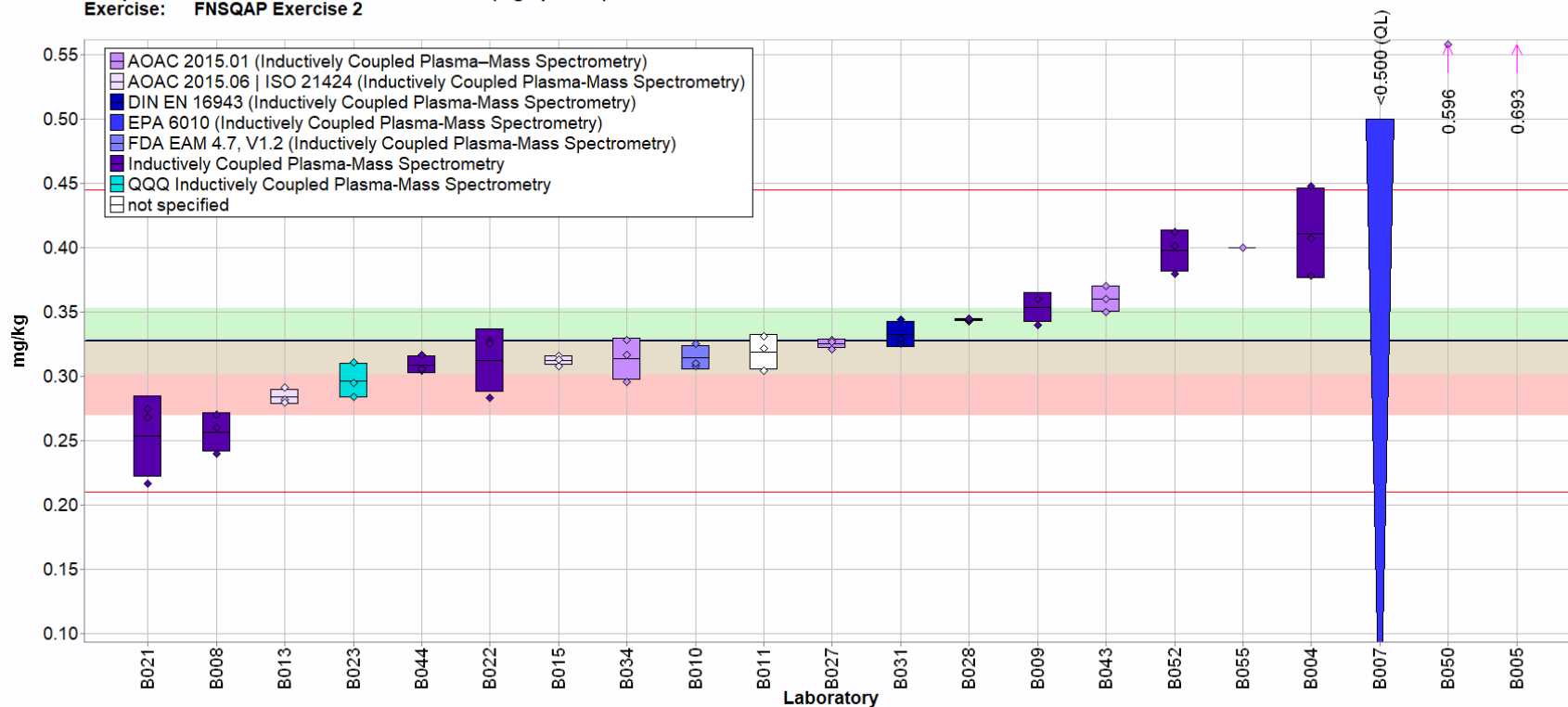


Fig. 2-12. Selenium in RM 8261 (data summary view – analytical method).

In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed as indicated in the figure key. Data points outside the graphical range are represented as upward arrows (the analytical methods reported by laboratories B050 and B005 were AOAC 2015.01 (ICP-MS) and ICP-OES, respectively). The 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 target range, which encompasses the target value bounded by twice its uncertainty (U_{NIST}), and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$. The beige shaded region represents the overlapping of the 95 % confidence interval for the consensus mean (green region) and the NIST target range (red region).

The consensus confidence interval was compared to the NIST target range for each nutritional element to assess the performance of the participants and is summarized in Table 2-6. A consensus mean within the target range is an indication that the community is performing well.

Table 2-6. Description of the consensus confidence interval in relation to the NIST target range for nutritional elements in formula samples.

Element	Consensus Confidence Interval in relation to NIST Target Range	
	RM 8260	RM 8261
Chromium (Cr)	Overlapping Above (mean above top of range)	Overlapping Above (mean at top of range)
Molybdenum (Mo)	Overlapping Above (mean at top of range)	Overlapping Above (mean at top of range)
Selenium (Se)	Within (mean above target)	Overlapping Above (mean at top of range)

Overall, laboratories performed fair in the measurement of nutritional elements in infant formula samples. Two to four participating laboratories reported Cr, Mo, and Se measured mass fraction averages outside of the consensus tolerance limits for both samples as shown in Fig. 2-13, Fig. 2-14, and Fig. 2-15. A slight positive linear trend is observed in Fig. 2-13, Fig. 2-14, and Fig. 2-15, which may indicate a global issue with calibration. Laboratories that reported measured values below the target did so consistently in these two very similar samples, and, likewise, laboratories that reported measured values above the target did so consistently between the samples. This trend was consistent between samples, but varied among nutritional elements (i.e., a laboratory was not always above the target value for all elements) with the exception of two laboratories that reported remarkably high results for all nutritional elements. Laboratories should ensure that all calibration standards have traceability to the International System of Units (SI) and meet ISO standards (such as those from NIST, another national metrology institute, or an accredited manufacturer). Calibration curves should be linear and sufficiently narrow to prevent over extension of a linear fit, which can be achieved by screening the samples to determine along which portion of the calibration curve the sample will lie. Prior to subsequent measurements, additional calibrant dilutions may be prepared to extend the calibration range; other dilutions can be excluded from the calibration curve to prevent bias.

In any laboratory exercise, calculations and reporting units must be verified prior to submission of results. Laboratories often report results in the wrong units or omit a dilution factor during the calculation of the final results, resulting in poor performance on the study. As always, consistent 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 (Certified Reference Materials (CRMs) like NIST’s SRMs or reference materials with non-certified values such as NIST’s RMs) or materials prepared in-house. Preparation and analysis of procedural blanks at the same time as samples is important to measure analyte background from the methods, which can be subtracted from the samples and used to calculate the method detection limit (MDL).

Exercise: FNSQAP Exercise 2, Measurand: Chromium
No. of laboratories: 17, Correlation coefficient: 0.7

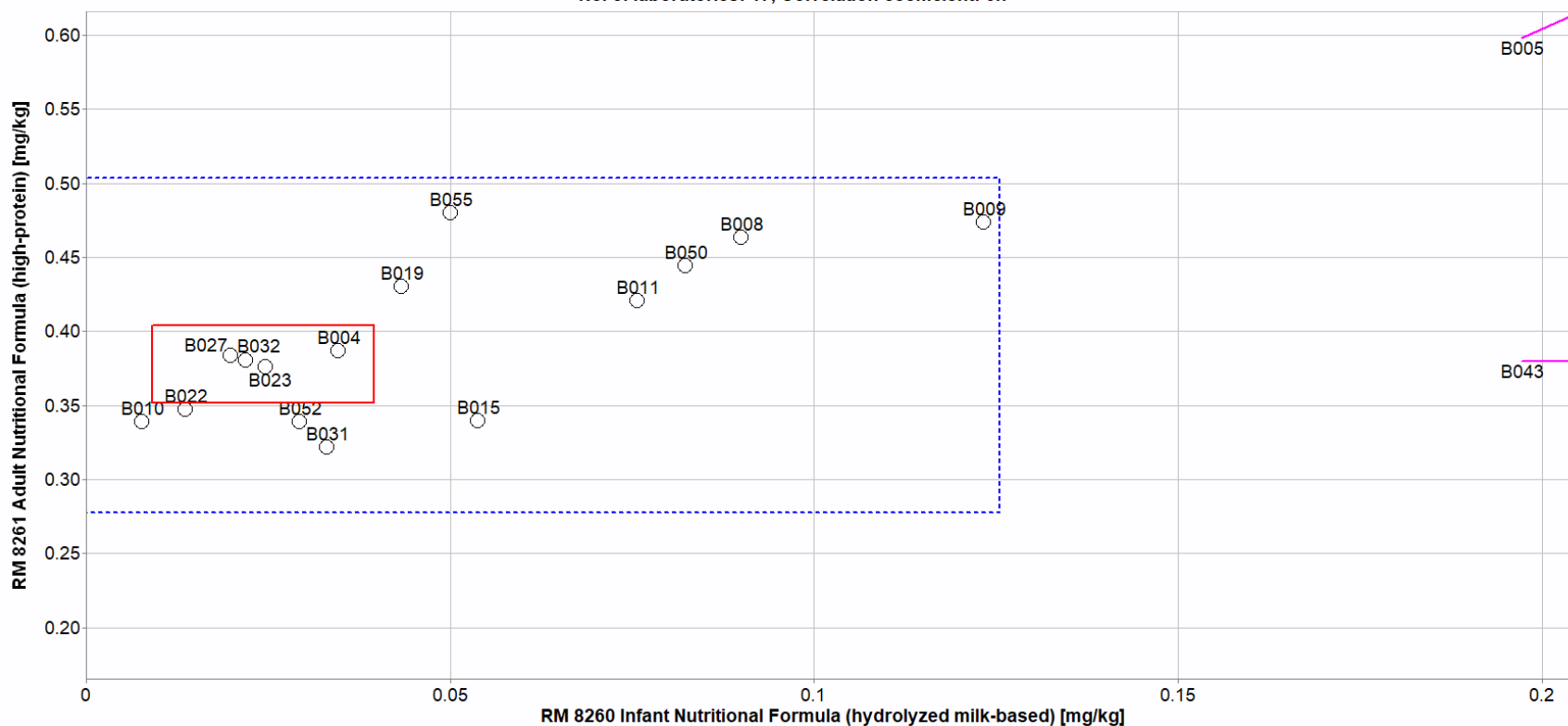


Fig. 2-13. Laboratory means for chromium in RM 8260 and RM 8261 (sample/sample comparison view).

In this view, the individual laboratory mean for one sample (RM 8260) is compared to the individual laboratory mean for a second sample (RM 8261). The solid red box represents the NIST target range for the two samples, RM 8260 (x-axis) and RM 8261 (y-axis), which encompasses the target values bounded by their uncertainties (U_{NIST}), and represents the range that results in an acceptable Z_{NIST} score, $|Z_{NIST}| \leq 2$. The dotted blue box represents the consensus range of tolerance for RM 8260 (x-axis) and RM 8261 (y-axis), calculated as the values above and below the consensus means that result in an acceptable Z'_{comm} score, $|Z'_{comm}| \leq 2$.

Exercise: FNSQAP Exercise 2, Measurand: Molybdenum
 No. of laboratories: 20, Correlation coefficient: 0.7

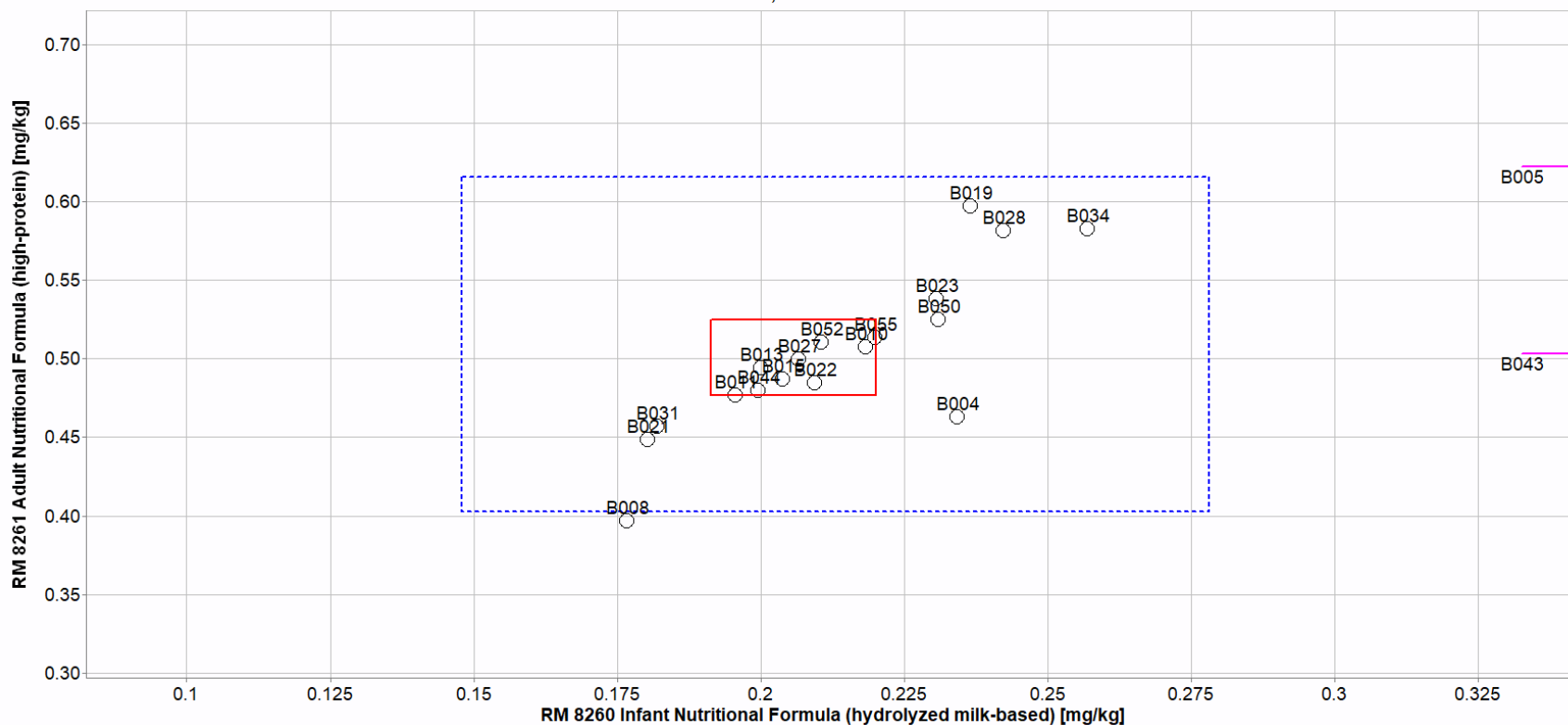


Fig. 2-14. Laboratory means for molybdenum in RM 8260 and RM 8261 (sample/sample comparison view).

In this view, the individual laboratory mean for one sample (RM 8260) is compared to the individual laboratory mean for a second sample (RM 8261). The solid red box represents the NIST target range for the two samples, RM 8260 (x-axis) and RM 8261 (y-axis), which encompasses the target values bounded by their uncertainties (U_{NIST}), and represents the range that results in an acceptable Z_{NIST} score, $|Z_{NIST}| \leq 2$. The dotted blue box represents the consensus range of tolerance for RM 8260 (x-axis) and RM 8261 (y-axis), calculated as the values above and below the consensus means that result in an acceptable Z'_{comm} score, $|Z'_{comm}| \leq 2$.

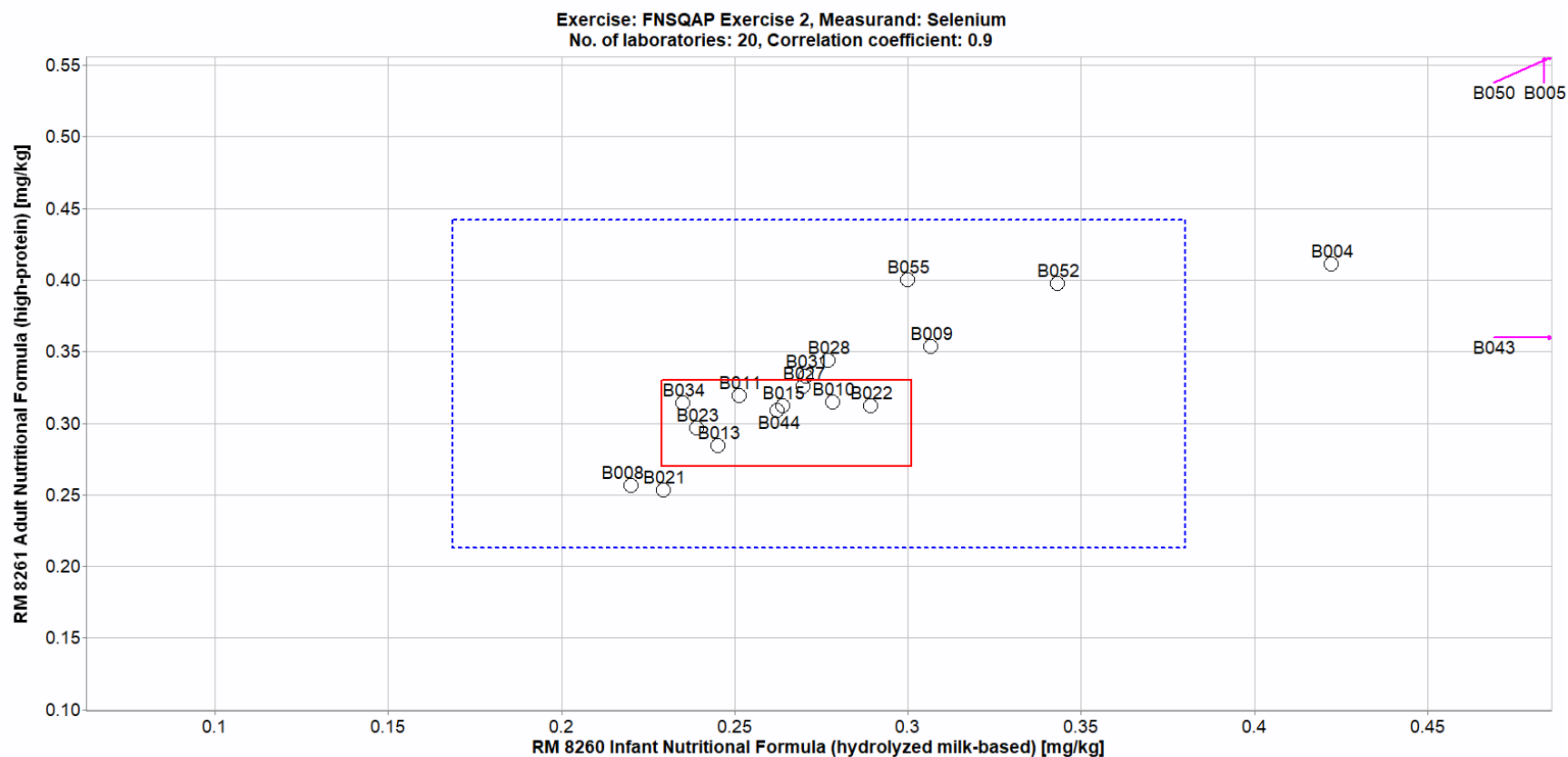


Fig. 2-15. Laboratory means for selenium in RM 8260 and RM 8261 (sample/sample comparison view).

In this view, the individual laboratory mean for one sample (RM 8260) is compared to the individual laboratory mean for a second sample (RM 8261). The solid red box represents the NIST target range for the two samples, RM 8260 (x-axis) and RM 8261 (y-axis), which encompasses the target values bounded by their uncertainties (U_{NIST}), and represents the range that results in an acceptable Z_{NIST} score, $|Z_{NIST}| \leq 2$. The dotted blue box represents the consensus range of tolerance for RM 8260 (x-axis) and RM 8261 (y-axis), calculated as the values above and below the consensus means that result in an acceptable Z'_{comm} score, $|Z'_{comm}| \leq 2$.

3. TOXIC ELEMENTS (Cadmium, Lead)

3.1. Executive Summary

To protect human health, toxic element regulatory limits have been lowered worldwide to reduce dietary exposure especially in vulnerable groups including babies and young children. This tasks laboratories to develop and use methods with greater sensitivity for accurately measuring lower levels of toxic elements in food, including cocoa, which is a high-value article in global trade. Participants in this study performed well in determination of the mass fractions of cadmium (Cd) and lead (Pb) regarding within-laboratory and among-laboratory measurement reproducibility. The significantly lower Cd mass fraction in SRM 3252 than in powdered cacao posed measurement challenges for a few participating laboratories reporting values below their LOQ. The SRM 3252 consensus mean range overlapped with the target range for Cd and was below the target range with slight overlap for Pb. Most participants reported using microwave digestion methods for sample preparation and inductively coupled plasma mass spectrometry (ICP-MS) methods for analysis. No trends were identified in the results based on these sample preparation and analysis methods. The correlation of bias in reported values between the two similar samples indicated a potential measurement issue related to method calibration.

3.2. Study Overview

Arsenic (As), Cd, Pb, and mercury (Hg) are the top four toxic elements that pose public health concerns as identified by the Agency for Toxic Substances and Disease Registry (ATSDR) and the World Health Organization (WHO) [6, 7]. Toxic elements can enter food sources from the natural environment in which they are grown and during processing. The presence of Cd and Pb in cacao and chocolate products has been reported [8]. The accuracy and precision of measurements made by food laboratories is critical for compliance with regulations from the FDA, USDA, and international bodies and to ensure product safety and customer confidence in the food supply. In this study, participants were provided with samples of powdered cacao and Standard Reference Material® (SRM®) 3252 Protein Drink Mix. Participants were asked to use in-house analytical methods to determine the mass fractions (ng/g) of Cd and Pb in each sample. Through participation in this study, laboratories can better understand the performance of their in-house methods relative to those being used by others in the community.

3.3. Sample Information

Participants were provided with three packets each of commercial powdered cacao (labeled Powdered Cacao) and SRM 3252 Protein Drink Mix (labeled Protein Powder). Each packet contained approximately 10 g of material; participants were asked to store the materials 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 thoroughly mix the contents of each packet, 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 0.5 g for the determination of the mass fractions of toxic elements. The approximate analyte levels were not reported to participants prior to the study. The target values for toxic elements in

SRM 3252 were from the Certificate of Analysis (COA) [9]. The target values and uncertainty for toxic elements in SRM 3252 are provided in Table 3-1 on an as-received basis. The uncertainties for Cd and Pb measured mass fractions in SRM 3252 were calculated and combined according to ISO/JCGM guidelines [4], and the expanded uncertainty expressed as an approximately 95 % level of confidence for each toxic element. The expanded uncertainties provided on the COA for SRM 3252 were used as the standard uncertainties for participant data assessment, as described in Section 1.1.2. Target values for Cd and Pb mass fractions in powdered cacao were not available at the time of this report.

Table 3-1. Individualized data summary table for toxic elements in cocoa.

Laboratory-specific results and Z-scores were provided to each participant separately from this report to protect laboratory identities.

(Lab Name)

Exercise 2 - Toxic Elements in Powdered Cacao and Drink Mix

Lab Code: (Code)		1. Your Results				2. Community Results			3. Target		
Sample	Units	x_i	s_i	Z'_{comm}	Z_{NIST}	N	x^*	s^*	x_{NIST}	u_{NIST}	
Cd	Cacao	ng/g	<i>Individual laboratory results will appear in this section; laboratory-specific results were provided to each participant separately from this report.</i>				29	610	55		
Cd	SRM 3252	ng/g					26	38.5	4.3	38.3	4.0
Pb	Cacao	ng/g					28	55.2	7.3		
Pb	SRM 3252	ng/g					27	35.5	6.3	38.7	0.9
		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_{NIST}	standard uncertainty about the 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						

3.4. Study Results and Discussion

Table 3-1 summarizes and Table 3-2 and Table 3-3 detail the measured mass fraction results reported by each participating laboratory for toxic elements. The participation level was good for toxic elements, with 74 % to 76 % of laboratories requesting samples returning results (on average 28 of 38 laboratories). Table 3-2 reveals that of the 29 participants that submitted results for Cd mass fraction, three laboratories reported data as below LOQ for SRM 3252, while none reported as below LOQ for powdered cacao. The mass fraction of Cd in SRM 3252 was approximately 15 times lower than powdered cacao and this posed a measurement challenge. Laboratories reporting below LOQ should implement methods with better sensitivity or evaluate their sample dilution factors.

Table 3-2. Data summary table for cadmium in powdered cacao and SRM 3252.

Data highlighted in blue have been identified as outside the consensus tolerance limits and resulted in an unacceptable Z'_{comm} score, $|Z'_{comm}| > 2$. Note: This table spans two pages; the target values and consensus values are included on both pages.

		Cadmium											
		Powdered Cacao (ng/g)					SRM 3252 Protein Drink Mix (ng/g)						
Lab		A	B	C	Avg	SD	A	B	C	Avg	SD		
Individual Results	Target											38.3	4.0
	B002	610	630		620	14	40	39		39.5	0.7		
	B004	522	565	565	551	25	40	32	31	34.3	4.9		
	B005	673.59	675.15		674	1	40.34	41.74	42.35	41.5	1.0		
	B007	640	655		648	11	43	43	39	41.7	2.3		
	B009	80	80	120	93	23	< 10.000	< 10.000	< 10.000				
	B010	607.8	646.4	618.9	624	20	38.5	40.2	37.7	38.8	1.3		
	B011	622	636	608	622	14	36	35	37	36.0	1.0		
	B012												
	B013	600	611	609	607	6	38.2	37.6	37.2	37.7	0.5		
	B014	602	621	643	622	21	37.5	35.5	37.7	36.9	1.2		
	B015	629	633		631	3	38.5	38.8	37.8	38.4	0.5		
	B016	520	480		500	28	< 40.000	< 40.000	< 40.000				
	B018	521.8	528.8	508.1	520	11	34.7	33.5	28.9	32.4	3.1		
	B019	630	630	620	627	6	28	30	28	28.7	1.2		
	B021	639	634	615	629	13	34	41	36	37.0	3.6		
	B022	629.731	601.171	582.332	604	24	37.934	36.483	38.429	37.6	1.0		
	B023	597.3	620.4	605	608	12	37.4	34.2	37.8	36.5	2.0		
	B024												
	B025	750	980	740	823	136	< 400.00	< 400.00	< 400.00				
	B027	599	598	577	591	12	35	36.2	39.4	36.9	2.3		
	B028	545	574	565	561	15	42	42	41	41.7	0.6		
	B029	592	592		592	0	44.9	42.2	42.5	43.2	1.5		
	B030												
	B031	640	700	720	687	42	45	43	43	43.7	1.2		
	B032	635	658.6		647	17	29.6	30.9	31.1	30.5	0.8		
	B033												
	B034	556	544	559	553	8	32	35	39	35.3	3.5		
	B037	65.6	65.1	63.8	65	1	41.5	41.1	40.8	41.1	0.4		
	B038												
	Community Results	Consensus Mean				610		Consensus Mean			38.5		
		Consensus Standard Deviation				55		Consensus Standard Deviation			4.3		
		Maximum				823		Maximum			45.3		
Minimum					65		Minimum			28.7			
N					29		N			26			

Table 3-2 continued. Data summary table for cadmium in powdered cacao and SRM 3252.

		Cadmium									
		Powdered Cacao (ng/g)					SRM 3252 Protein Drink Mix (ng/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target									38.3	4.0
	B039	552.9	588.6	570.8	571	18	39.56	41.65	40.13	40.4	1.1
	B043	607	620	606	611	8	39	39	39	39.0	0.0
	B044	614	612	608	611	3	48	43	43	44.7	2.9
	B046										
	B047										
	B048										
	B050	641.802	661.215	671.362	658	15	47.704	43.826	44.338	45.3	2.1
	B051										
	B054	623	627	616	622	6	36.4	40.1	36.8	37.8	2.0
Community Results		Consensus Mean				610	Consensus Mean				38.5
		Consensus Standard Deviation				55	Consensus Standard Deviation				4.3
		Maximum				823	Maximum				45.3
		Minimum				65	Minimum				28.7
		<i>N</i>				29	<i>N</i>				26

Table 3-3. Data summary table for lead in powdered cacao and SRM 3252.

Data highlighted in blue have been identified as outside the consensus tolerance limits and resulted in an unacceptable Z'_{comm} score, $|Z'_{comm}| > 2$. Note: This table spans two pages; the target values and consensus values are included on both pages.

		Lead											
		Powdered Cacao (ng/g)					SRM 3252 Protein Drink Mix (ng/g)						
Lab		A	B	C	Avg	SD	A	B	C	Avg	SD		
Individual Results	Target											38.7	0.9
	B002	55	57		56.0	1.4	39	38		38.5	0.7		
	B004	53	49	49	50.3	2.3	46	43	44	44.3	1.5		
	B005	62.3	58.34		60.3	2.8	42.21	39.28	40.01	40.5	1.5		
	B007	66	53		59.5	9.2	34	31	35	33.3	2.1		
	B009	200	130	130	153.3	40.4	280	230	200	236.7	40.4		
	B010	55.6	55.2	57.8	56.2	1.4	36.7	37	37.7	37.1	0.5		
	B011	53	50	50	51.0	1.7	26	25	26	25.7	0.6		
	B012												
	B013	50.1	51	51.7	50.9	0.8	34.2	33.6	32.6	33.5	0.8		
	B014	50.3	55	49.8	51.7	2.9	32.5	36.8	33.7	34.3	2.2		
	B015	60.6	51.2		55.9	6.6	36.4	34.7	34.7	35.3	1.0		
	B016	60	50		55.0	7.1	< 40.000	40	40	40.0	0.0		
	B018	81.7	50.7	34.8	55.7	23.9	28	17.6	23.7	23.1	5.2		
	B019	59	54	61	58.0	3.6	38	38	37	37.7	0.6		
	B021	66	51	50	55.7	9.0	35	37	34	35.3	1.5		
	B022	78.713	51.24	56.785	62.2	14.5	49.595	39.524	39.657	42.9	5.8		
	B023	50.5	49.7	50.3	50.2	0.4	36.9	32.4	34.5	34.6	2.3		
	B024												
	B025												
	B027	65.1	47.7	50.6	54.5	9.3	32.5	34.4	33.6	33.5	1.0		
	B028	51	54	83	62.7	17.7	36	37	36	36.3	0.6		
	B029	51	52.8		51.9	1.3							
	B030												
	B031	26	22	22	23.3	2.3	32	31	34	32.3	1.5		
	B032	33.7	32.1		32.9	1.1	14.6	15.8	19.2	16.5	2.4		
	B033												
	B034	70	90	70	76.7	11.5	40	50	70	53.3	15.3		
	B037	52.7	55.4	54.6	54.2	1.4	35.7	38.5	38	37.4	1.5		
	B038												
Community Results	Consensus Mean				55.2		Consensus Mean			35.5			
	Consensus Standard Deviation				7.3		Consensus Standard Deviation			6.3			
	Maximum				153.3		Maximum			236.7			
	Minimum				23.3		Minimum			16.5			
	N				28		N			27			

Table 3-3 continued. Data summary table for lead in powdered cacao and SRM 3252.

Data highlighted in blue have been identified as outside the consensus tolerance limits and resulted in an unacceptable Z'_{comm} score, $|Z'_{comm}| > 2$.

		Lead										
		Powdered Cacao (ng/g)					SRM 3252 Protein Drink Mix (ng/g)					
		Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target										38.7	0.9
	B039	51.87	47.33	46.69	48.6	2.8	27.24	32.14	41.39	33.6	7.2	
	B043	55	57	54	55.3	1.5	38	37	37	37.3	0.6	
	B044	51	65	57	57.7	7.0	37	35	37	36.3	1.2	
	B046											
	B047											
	B048											
	B050	71.455	68.918	76.181	72.2	3.7	91.993	49.585	54.748	65.4	23.1	
	B051											
B054	45.4	48	51.4	48.3	3.0	29.4	31.9	32.5	31.3	1.6		
Community Results		Consensus Mean				55.2	Consensus Mean				35.5	
		Consensus Standard Deviation				7.3	Consensus Standard Deviation				6.3	
		Maximum				153.3	Maximum				236.7	
		Minimum				23.3	Minimum				16.5	
		N				28	N				27	

To assess performance of methods run by individual participants and the community as a whole, repeatability and reproducibility were compared to the relevant AOAC SMPR®. AOAC SMPR 2012.007 Standard Method Performance Requirements for Heavy Metals in a Variety of Food and Beverages [10] was used to evaluate nutritional element performance results for SRM 3252 and powdered cacao. Repeatability, demonstrated by within-laboratory variability (mean % RSD), and reproducibility, demonstrated by among-laboratory variability (% RSD), are shown in Table 3-4. Mean within-laboratory variabilities were acceptable for all toxic elements in both cocoa materials. Only 7 % of laboratories reported within-laboratory Cd variabilities greater for powdered cacao than the method performance requirements; all laboratories reported within-laboratory Cd measured mass fraction variabilities within the requirements for SRM 3252. For each material, one laboratory reported the same measured Cd mass fraction value for all replicates so a valid within-laboratory variability could not be calculated. Twenty-nine percent of laboratories for powdered cacao and 19 % of laboratories for SRM 3252 reported within-laboratory variabilities greater than the method performance requirements for Pb. The among-laboratory variabilities for toxic elements in cocoa materials were below the published expectations of the measurement community [10].

Table 3-4. Laboratory variabilities for toxic elements in FNSQAP Exercise 2 cocoa materials relative to AOAC SMPR 2012.007 method performance requirements.

Elements	Within-Laboratory Variability			Among-Laboratory Variability		
	FNSQAP Ex. 2		SMPR	FNSQAP Ex. 2		SMPR
	Cacao	SRM 3252	2012.007	Cacao	SRM 3252	2012.007
Cd	3.5 %	4.3 %	11 % ^a ; 15 % ^b	10.1 %	12.8 %	16 % ^a ; 32 % ^b
Pb	10.1 %	8.5 %	15 %	14.9 %	18.8 %	32 %

^aMethod performance requirement for mass fraction of Cd in powdered cacao.

^bMethod performance requirement for mass fraction of Cd in SRM 3252.

The low levels of toxic elements in some cocoa samples can be challenging to measure, and laboratories must balance many factors when deciding on the most appropriate sample preparation and analysis methods to use. As shown in Fig. 3-1, Fig. 3-2, Fig. 3-3, and Fig. 3-4, laboratories reported using a few different sample preparation methods for the determination of toxic elements in the two cocoa samples. Numbers and percentages of laboratories described as reporting specific approaches are averages across all results for two elements and two samples.

The most common sample preparation approach was a microwave digestion method (20 laboratories, 70 %); one laboratory reported using hot block digestion (3.5 %) and one laboratory reported using digestion without specification (3.5 %). Six or seven laboratories did not report the sample preparation approach used (23 %). The sample preparation procedure is critical for unbiased measurements. Participants that reported use of microwave digestion methods should review protocols for future analyses to ensure complete digestion to release the analytes from the samples into solution. Failure to completely digest the organic constituents may produce matrix interferences that cause signal enhancement or suppression, introducing potential measurement bias. A high temperature and pressure closed vessel microwave digestion is suggested for these elements to fully dissolve samples in solution for liquid sample analysis methods. Since Cd and Pb have high boiling points, volatile loss of these elements is not a concern at high digestion temperatures. Samples being prepared for Pb determination should not be digested with hydrochloric acid (HCl), which can result in formation of an insoluble PbCl₂ precipitate. If HCl is used in digestion, then repeated washings of the side of the digestion vessel with dilute nitric acid (HNO₃) may redissolve the PbCl₂ into solution. Greater than desired within-laboratory variability may be due to the use of less than the recommended sample size for analysis (0.5 g) since the sample may not be homogeneous below this mass. Sample dilution in preparation greatly impacts the mass fraction of an element as-run in analysis, which can be below the sensitivity of the instrument. Multiple dilutions of a sample may need to be prepared depending on the mass fraction range of an element and analytical method sensitivity, however this must also be balanced with matrix effects that may be more significant with less sample dilution.

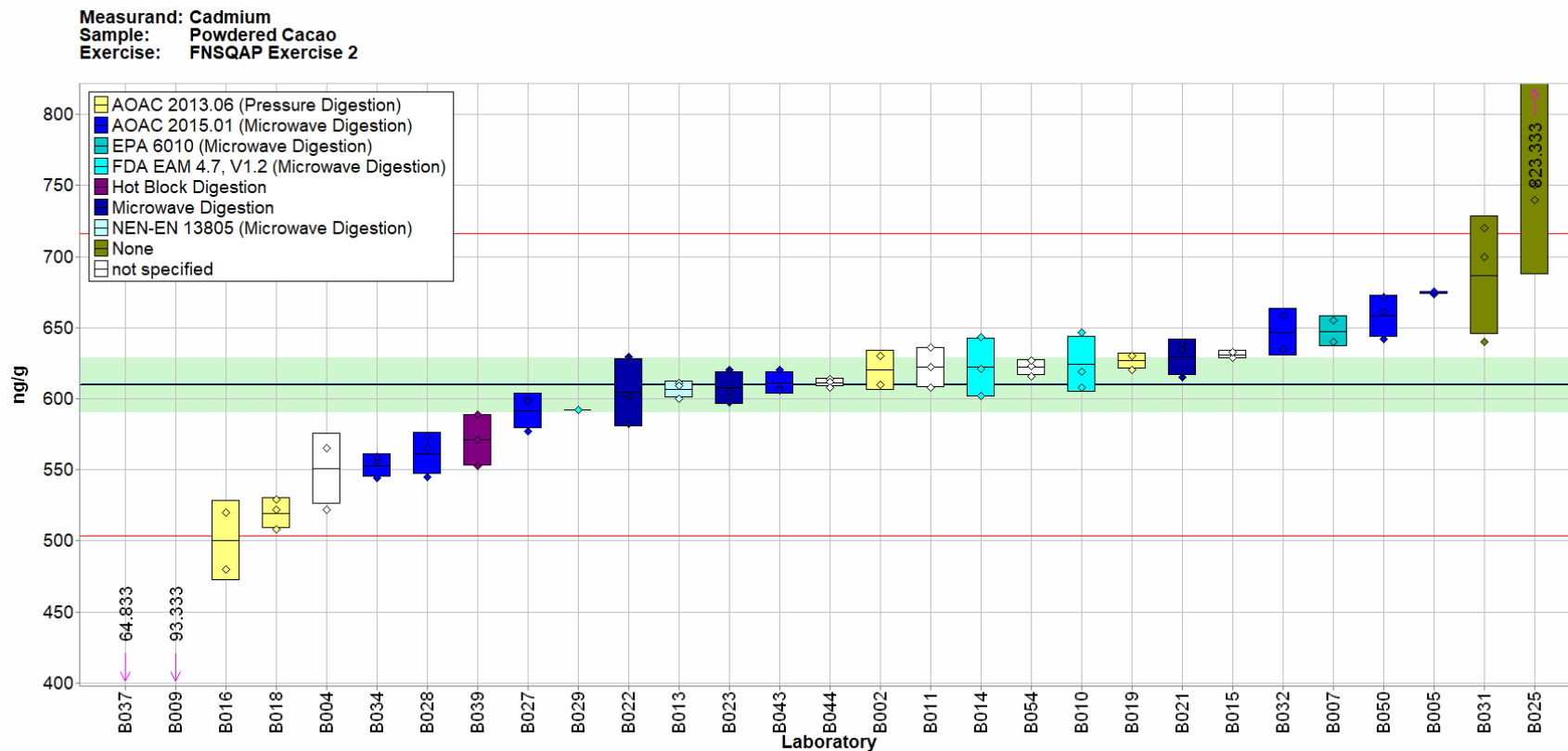


Fig. 3-1. Cadmium in Powdered Cacao (data summary view – sample preparation method).

In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed as indicated in the figure key. Data points outside the graphical range are represented as downward arrows (the preparation methods reported by laboratories B037 and B009 were FDA EAM 4.7 v1.2 (microwave digestion) and digestion, respectively). The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$. A NIST target value was not available at the time of this report.

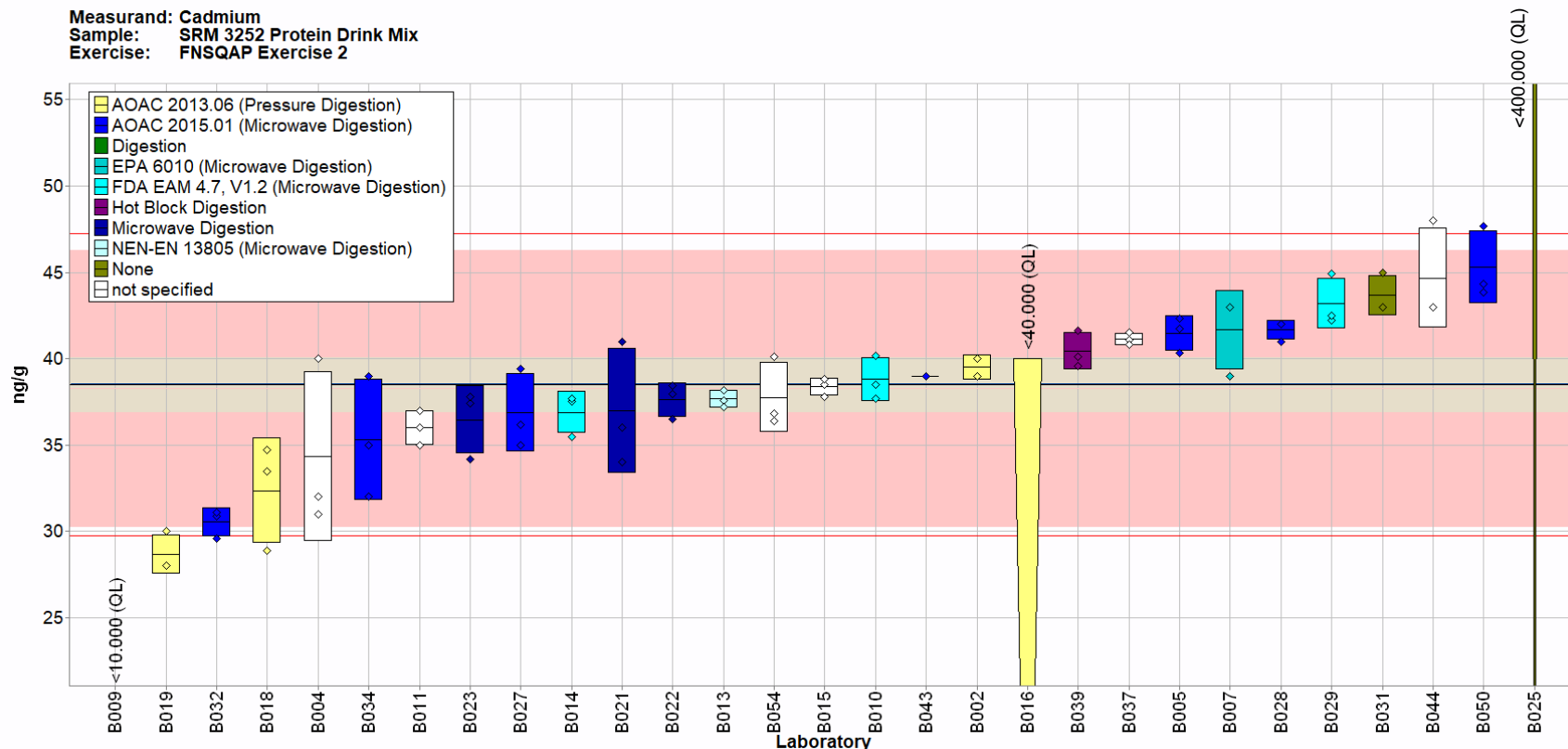


Fig. 3-2. Cadmium in SRM 3252 (data summary view – sample preparation method).

In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). A downward triangle represents data reported as an LOQ value. The color of the data point represents the sample preparation method employed as indicated in the figure key (the preparation methods reported by laboratories B009 and B025 were digestion and none, respectively). The 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 target range, which encompasses the target value bounded by twice its uncertainty (U_{NIST}), and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$. The beige shaded region represents the overlapping of the 95 % confidence interval for the consensus mean (green region) and the NIST target range (red region).

Measurand: Lead
 Sample: Powdered Cacao
 Exercise: FNSQAP Exercise 2

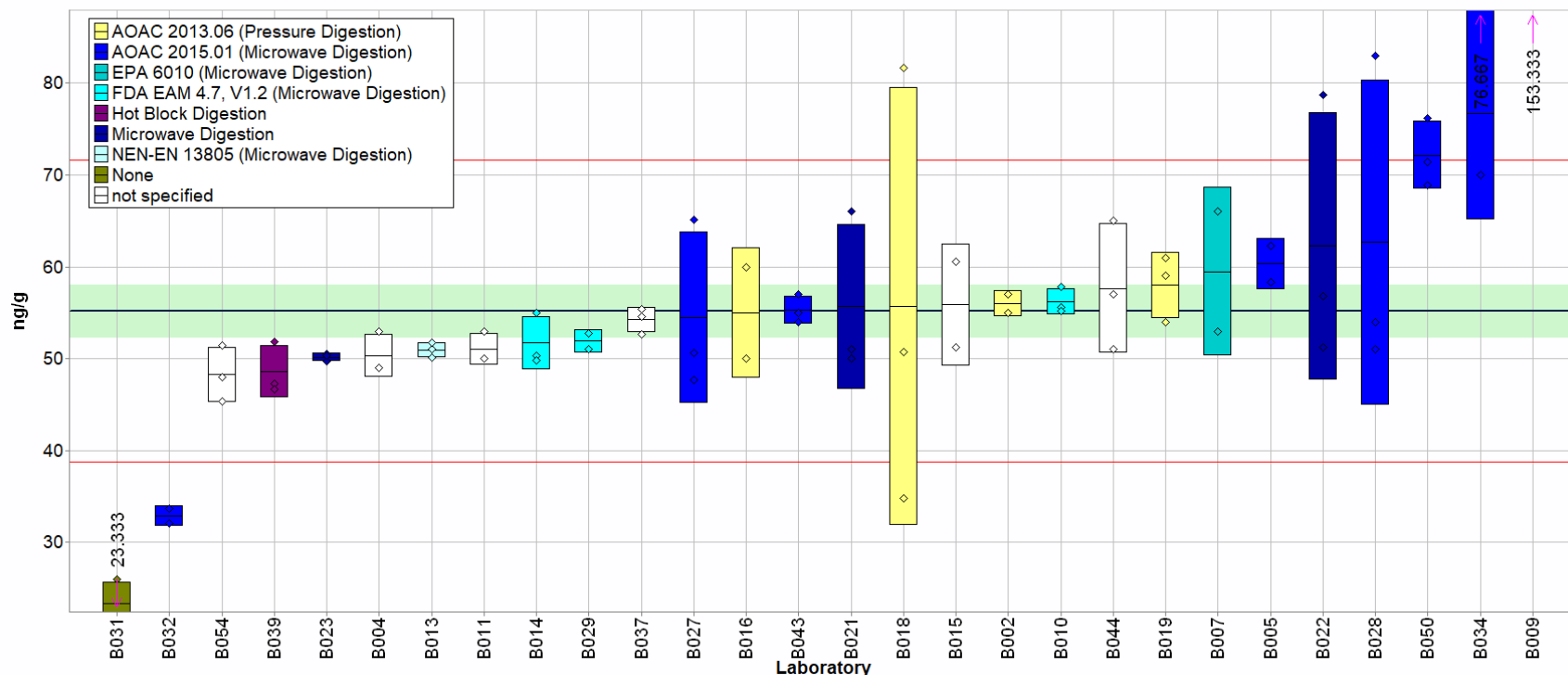


Fig. 3-3. Lead in Powdered Cacao (data summary view – sample preparation method).

In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed as indicated in the figure key. Data points outside the graphical range are represented as downward and upward arrows (the preparation method reported by laboratory B009 was digestion). The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$. A NIST target value was not available at the time of this report.

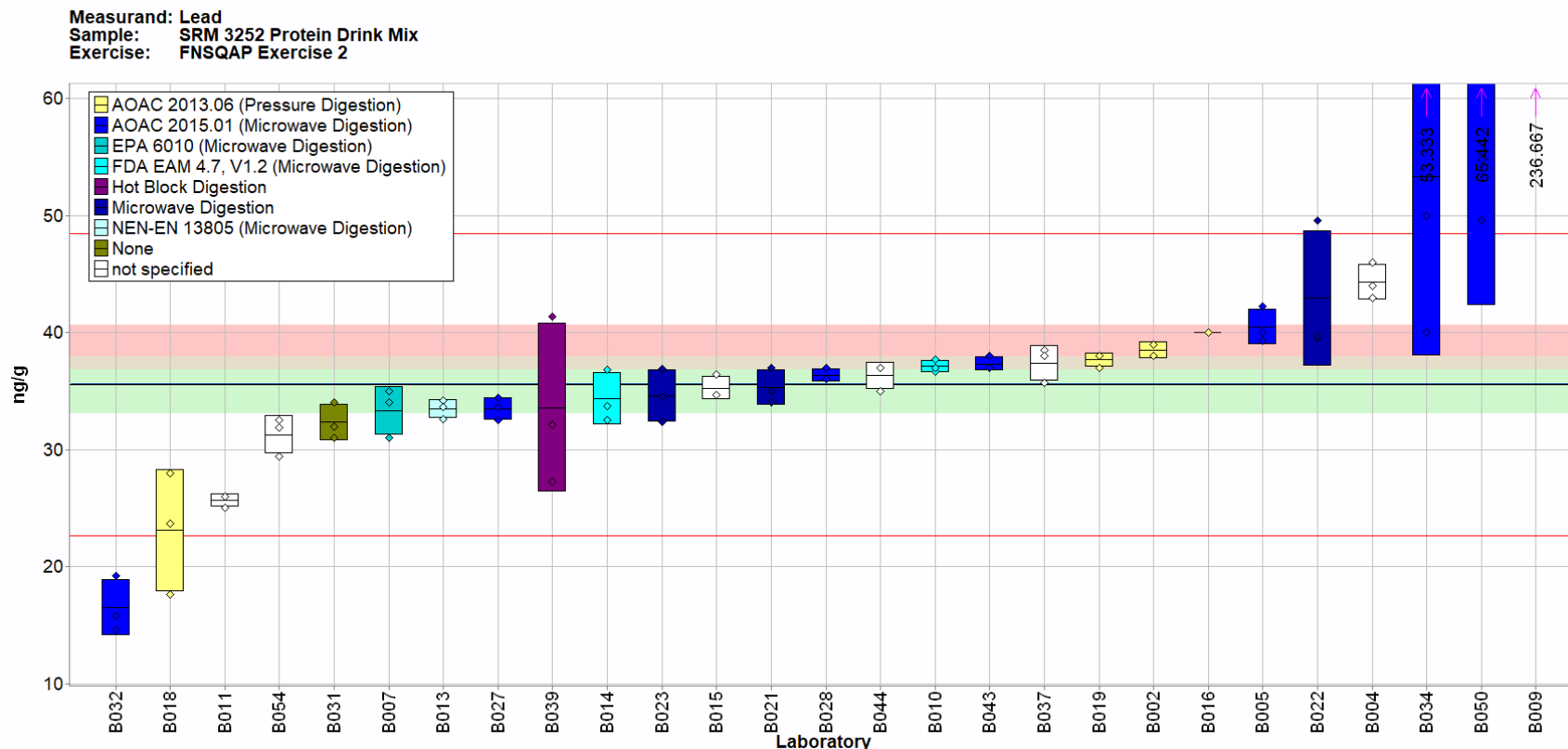


Fig. 3-4. Lead in SRM 3252 (data summary view – sample preparation method).

In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed as indicated in the figure key. Data points outside the graphical range are represented as upward arrows (the preparation method reported by laboratory B009 was digestion). The 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 target range, which encompasses the target value bounded by twice its uncertainty (U_{NIST}) and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$. The beige shaded region represents the overlapping of the 95 % confidence interval for the consensus mean (green region) and the NIST target range (red region).

As shown in Fig. 3-5, Fig. 3-6, Fig. 3-7, and Fig. 3-8, ICP-MS analytical methods (25 laboratories; 88 %) were the primary methods employed by laboratories for the determination of toxic elements in the two cocoa samples. One laboratory reported using atomic absorption spectroscopy (3.5 %), one laboratory reported using neutron activation analysis (3.4 %), and two laboratories did not report the analytical method used (7 %). As noted above, a few laboratories reported Pb mass fraction as below LOQ, which was not associated with method except for neutron activation where Cd mass fraction data were submitted as below LOQ for SRM 3252 and greatly above the 95 % confidence interval for the consensus mean in powdered cacao. Sensitivity of the analytical method is key when determining if the method is suitable for the analyte abundance in the sample.

Since ICP-MS was the most reported analytical method, some technical recommendations are provided for laboratories using this approach. Collision cell gases or reaction cell mode can be used with ICP-MS to reduce or eliminate the interferences caused by molecular ions that have the same mass-to-charge ratio as the element of interest. Utilizing ICP-MS in KED mode can control cell-formed interferences and reduce polyatomic ion interferences created by the plasma or vacuum interface. For example, cadmium can have isobaric spectral interferences such as $^{95}\text{Mo}^{16}\text{O}^+$ and $^{97}\text{Mo}^{16}\text{O}^+$ that affect the accuracy of Cd determination at 111 u and 113 u. Use of He and H₂ collision gases can effectively reduce polyatomic interferences.

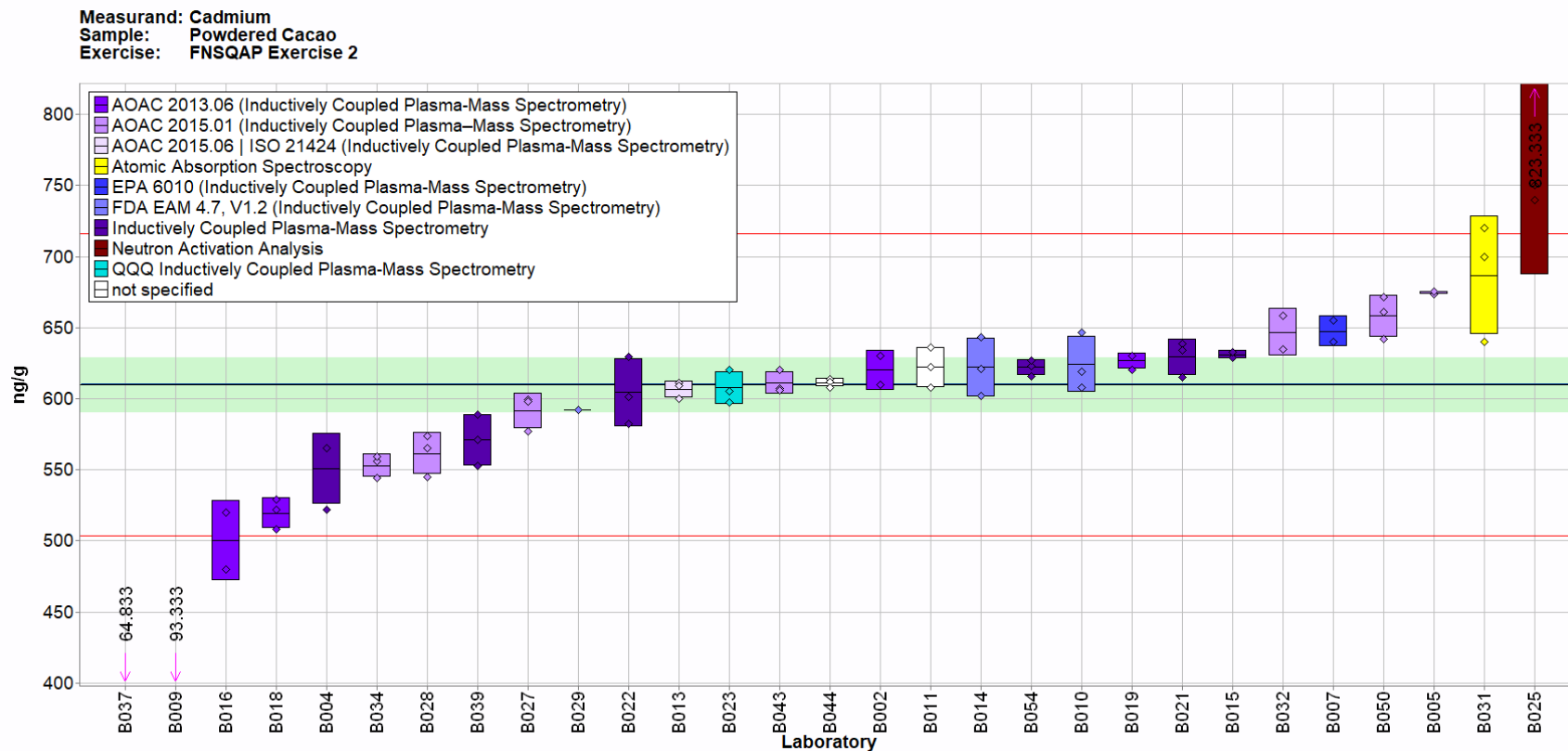


Fig. 3-5. Cadmium in Powdered Cacao (data summary view – analytical method).

In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed as indicated in the figure key. Data points outside the graphical range are represented as downward arrows (the analytical methods reported by laboratories B037 and B009 were ICP-MS and FDA EAM 4.7 V1.2 (ICP-MS), respectively). The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$. A NIST target value was not available at the time of this report.

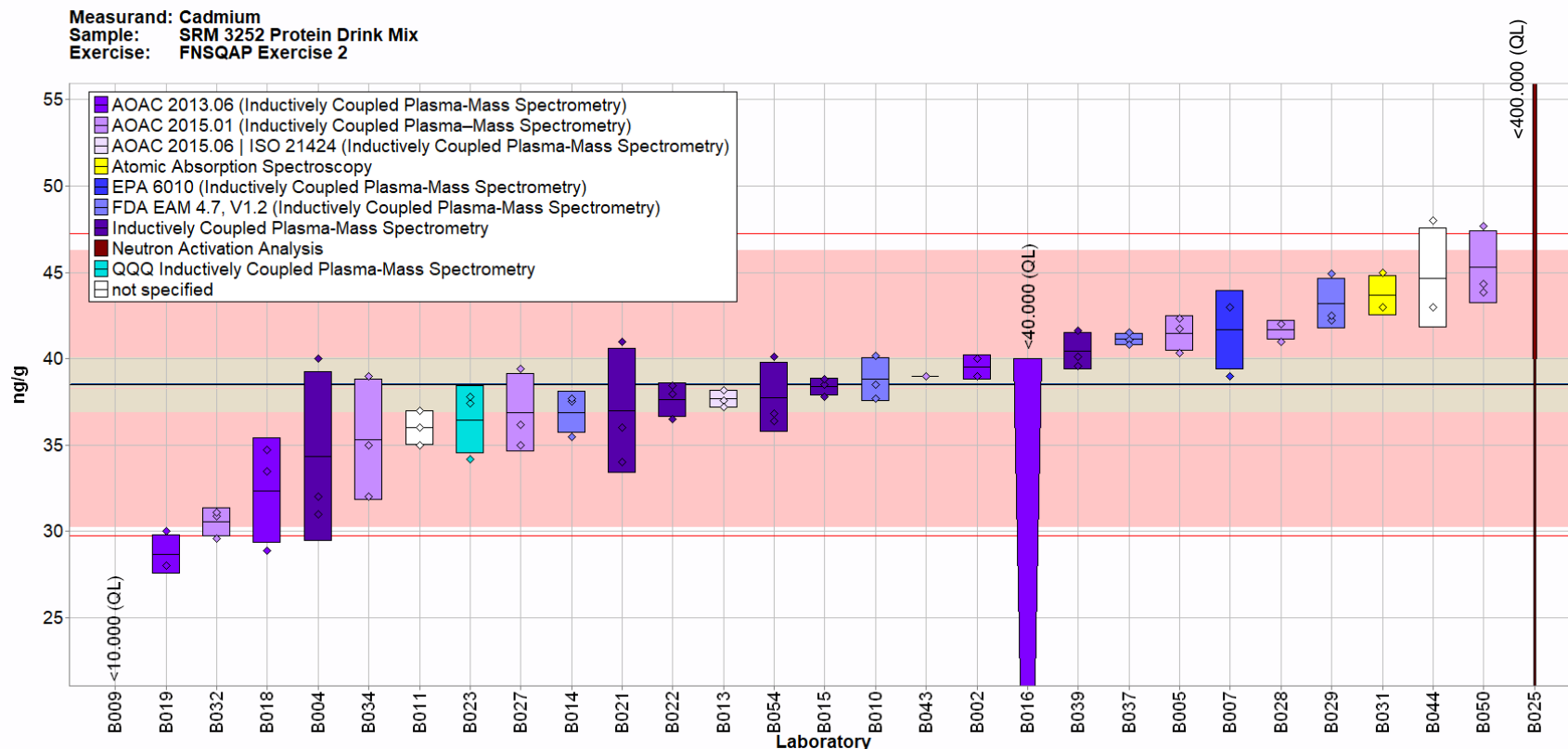


Fig. 3-6. Cadmium in SRM 3252 (data summary view – analytical method).

In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). A downward triangle represents data reported as an LOQ value. The color of the data point represents the analytical method employed as indicated in the figure key (the analytical method reported by laboratory B009 was ICP-MS). The 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 target range, which encompasses the target value bounded by twice its uncertainty (U_{NIST}), and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$. The beige shaded region represents the overlapping of the 95 % confidence interval for the consensus mean (green region) and the NIST target range (red region).

Measurand: Lead
 Sample: Powdered Cacao
 Exercise: FNSQAP Exercise 2

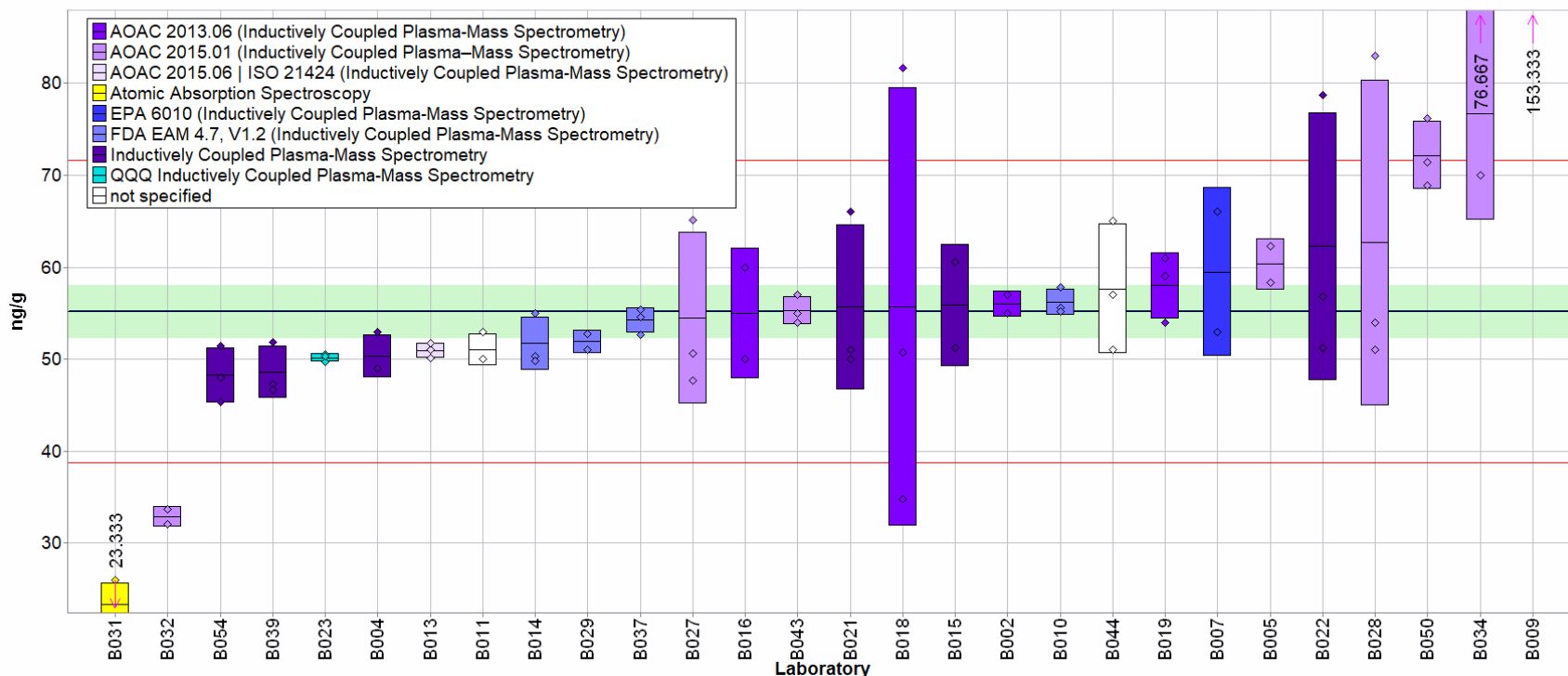


Fig. 3-7. Lead in Powdered Cacao (data summary view – analytical method).

In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed as indicated in the figure key. Data points outside the graphical range are represented as downward and upward arrows (the analytical method reported by laboratory B009 was ICP-MS). The 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, $|Z'_{comm}| \leq 2$. A NIST target value was not available at the time of this report.

Measurand: Lead
 Sample: SRM 3252 Protein Drink Mix
 Exercise: FNSQAP Exercise 2

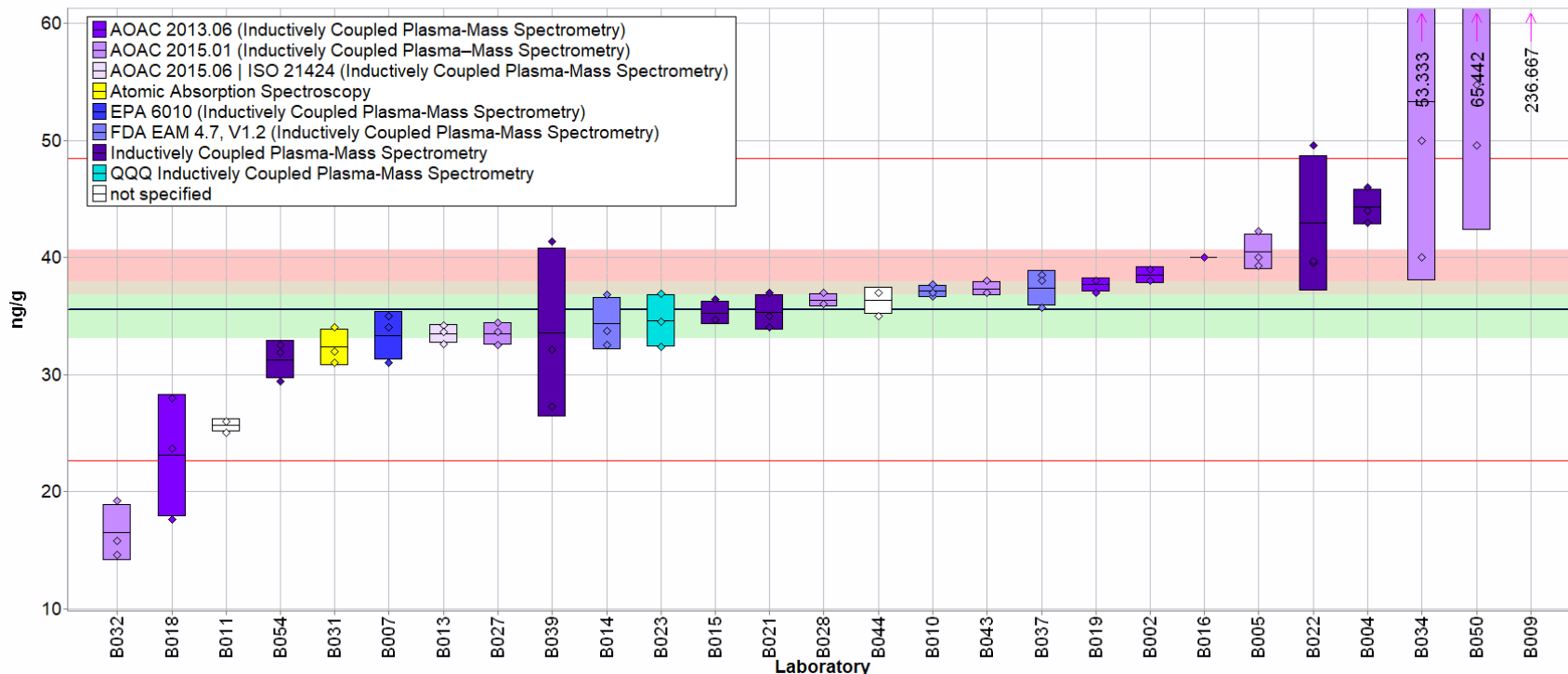


Fig. 3-8. Lead in SRM 3252 (data summary view – analytical method).

In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed as indicated in the figure key. Data points outside the graphical range are represented as upward arrows (the analytical method reported by laboratory B009 was ICP-MS). The 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 target range, which encompasses the target value bounded by twice its uncertainty (U_{NIST}), and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$. The beige shaded region represents the overlapping of the 95 % confidence interval for the consensus mean (green region) and the NIST target range (red region).

The consensus confidence interval was compared to the NIST target range for toxic elements in SRM 3252 to assess the performance of the participants. The NIST target range encompasses the consensus confidence interval for the Cd mass fraction in SRM 3252 and the consensus and target means are very close. A consensus mean within the target range is an indication that the community is performing well. For the measured mass fraction of Pb in SRM 3252, the consensus confidence interval marginally overlaps the bottom of the NIST target range, and the consensus mean is below the NIST target range.

Overall, laboratories performed well in the measurement of Cd in SRM 3252, while needing to improve measurements of Pb in the same material. At the time of this report, target values were not available for Cd and Pb mass fractions in powdered cacao to compare with the participant consensus data and the material had not been evaluated for homogeneity. Several participants reported Pb mass fraction values with greater standard deviation for the powdered cacao as shown in Fig. 3-3 and Fig. 3-7 that could indicate some possible minor heterogeneity of Pb in this material. Two to five participating laboratories had toxic element measurement averages outside of the consensus tolerance limits for both samples as shown in Fig. 3-9 and Fig. 3-10. Also, a slight positive linear trend is observed in Fig. 3-9 and Fig. 3-10, which may indicate a global issue with calibration or an equivalent level of difficulty in sample preparation/analysis between the two samples. All calibration standards should have traceability to the SI and meet ISO standards (such as those from NIST, another national metrology institute, or an accredited manufacturer). Calibration curves should be linear and sufficiently narrow to prevent over extension of a linear fit, which can be achieved by screening the samples to determine along which portion of the calibration curve the sample will lie. Prior to subsequent measurements, additional calibrant dilutions may be prepared to extend the calibration range; other dilutions can be excluded from the calibration curve to prevent bias. The method of standard additions for calibration should also be considered since this approach “matrix-matches” sample with calibrant and can improve LOQs, accuracy, and precision of measurements. For elements that are not monoisotopic, using the method of isotope dilution (ID) can result in greater accuracy, precision, and sensitivity since this approach does not rely on absolute signal intensity, but measures the signal ratios of the natural isotope of an element and the spiked isotope of an element in samples.

In any laboratory exercise, calculations and reporting units must be verified prior to submission of results. Laboratories often report results in the wrong units or omit a dilution factor during the calculation of the final results, resulting in poor performance on the study. As always, consistent 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 like NIST’s SRMs or non-certified reference materials such as NIST’s RMs) or materials prepared in-house. Additionally, preparation and analysis of procedural blanks at the same time as samples is important to measure analyte background from the methods, which can be subtracted from the samples and used to calculate the MDL. The ability for the community to measure very low mass fractions of toxic elements in food has become increasingly important as regulatory limits continue to be lowered.

Exercise: FNSQAP Exercise 2, Measurand: Cadmium
No. of laboratories: 26, Correlation coefficient: 0.1

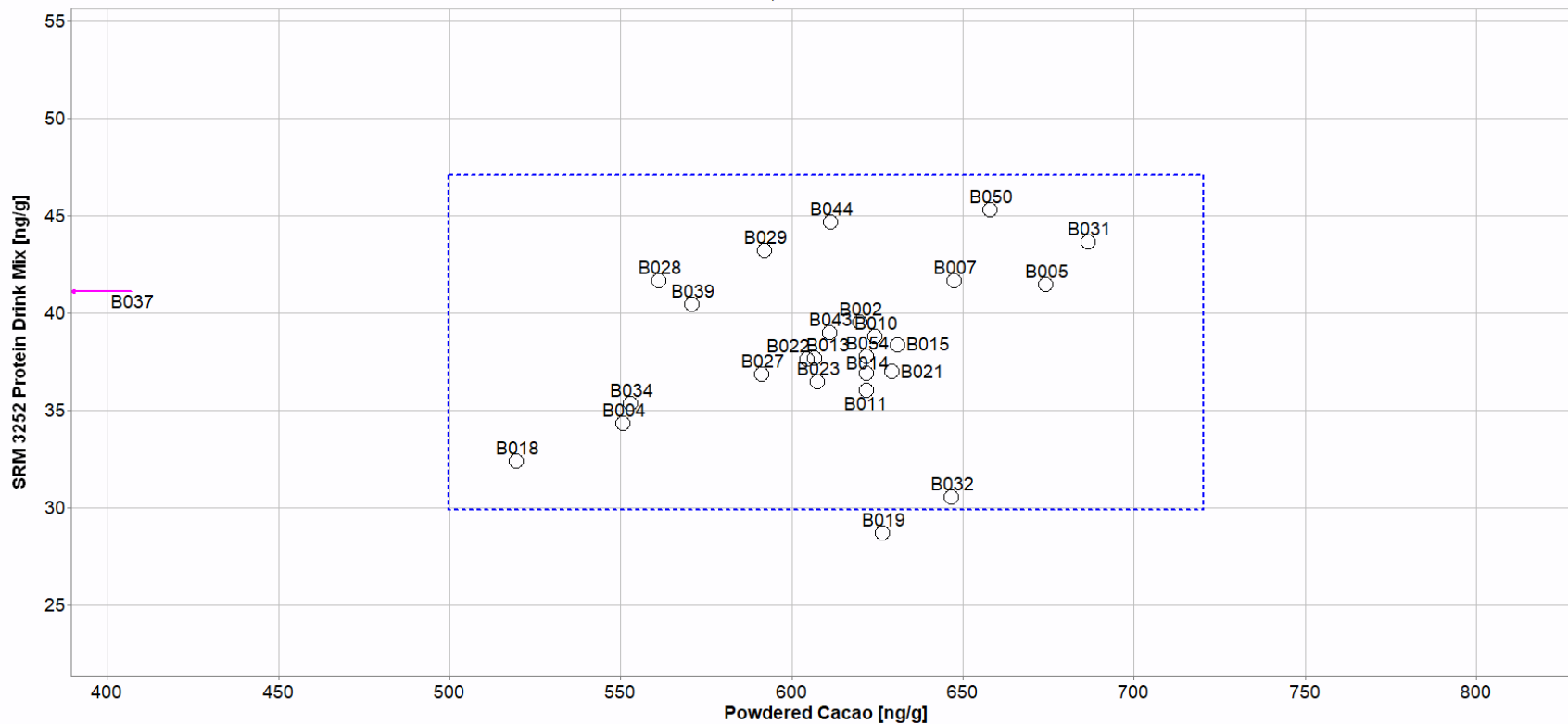


Fig. 3-9. Laboratory means for cadmium in Powdered Cacao and SRM 3252 (sample/sample comparison view).

In this view, the individual laboratory mean for one sample (Powdered Cacao) is compared to the mean for a second sample (SRM 3252). The dotted blue box represents the consensus range of tolerance for Powdered Cacao (x-axis) and SRM 3252 (y-axis), calculated as the values above and below the consensus means that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$.

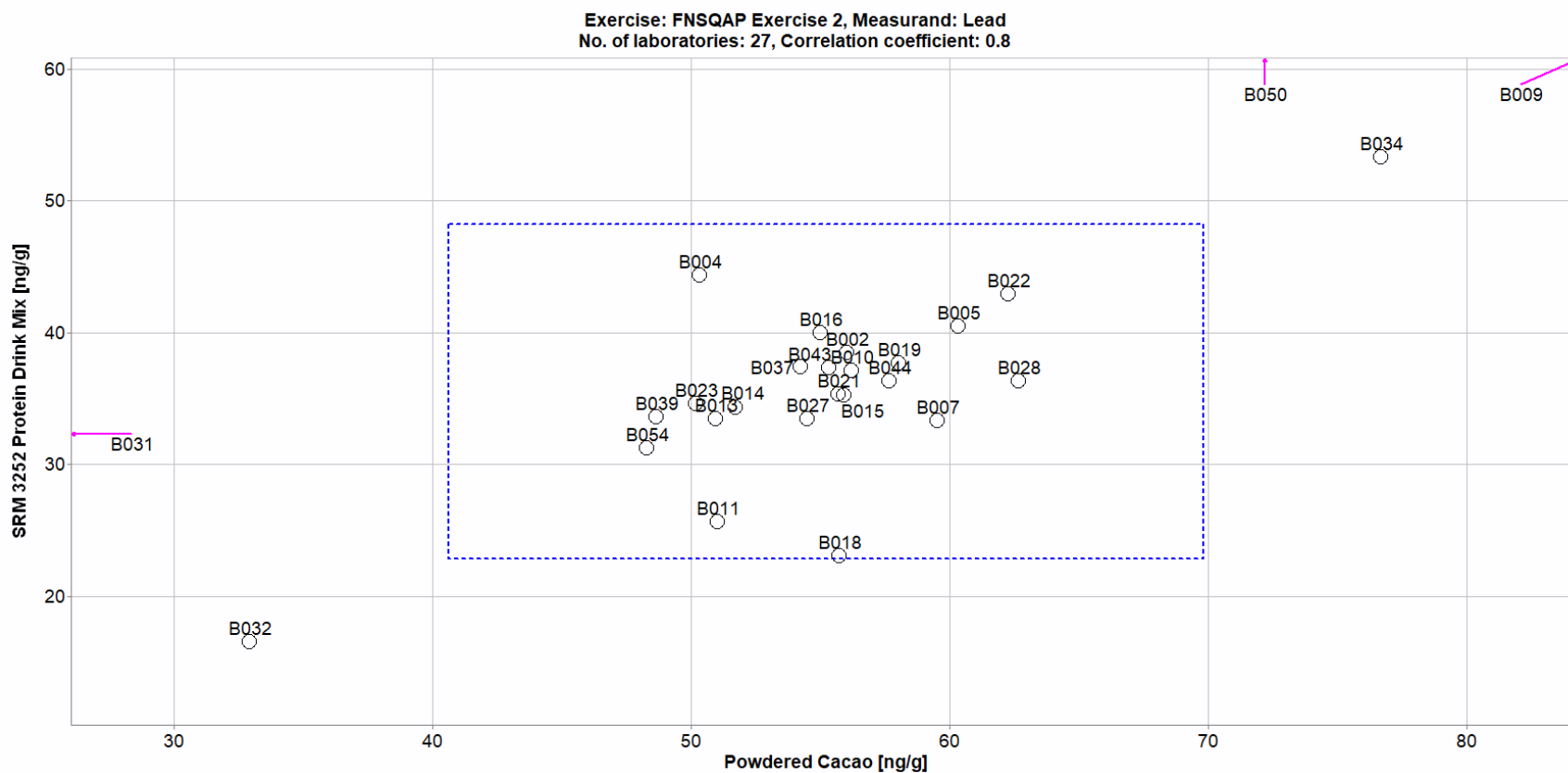


Fig. 3-10. Laboratory means for lead in Powdered Cacao and SRM 3252 (sample/sample comparison view).

In this view, the individual laboratory mean for one sample (Powdered Cacao) is compared to the mean for a second sample (SRM 3252). The dotted blue box represents the consensus range of tolerance for Powdered Cacao (x-axis) and SRM 3252 (y-axis), calculated as the values above and below the consensus means that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$.

4. WATER-SOLUBLE VITAMINS (Choline, Carnitine)

4.1. Executive Summary

Choline and carnitine are important nutrients for infant development and growth, and the fortified levels of choline and carnitine in infant foods are strictly regulated worldwide. Participants in this study performed well in determination of choline and carnitine. Laboratories relied on digestion to prepare samples for analysis, and incomplete digestion may have resulted in biased results for some participants. Laboratories utilizing this type of approach should further investigate potential bias through use of reference materials or other quality control samples. Additionally, laboratories should ensure fitness of the calibration curve at the prepared sample concentration to prevent non-linearity of detector response.

4.2. Study Overview

Choline is an essential nutrient that plays a role in liver function, normal brain development, muscle movement, nerve function, metabolism, and sleep. Carnitine, a group of compounds derived from amino acids, plays a role in energy production, and can be found in the skeletal and cardiac muscles which utilize fats for fuel. These essential nutrients provided to infants via mother's milk or infant formula are important for normal development [11, 12]. Accurately understanding the intake of choline and carnitine through measurement in fortified foods can inform future decisions about recommended dietary intakes. In this study, participants were provided with two nutritional formula samples, SRM 1849b Infant/Adult Nutritional Formula I (milk-based) and RM 8261 Adult Nutritional Formula (high protein). Participants were asked to use in-house analytical methods to determine the mass fractions (mg/kg) of choline and carnitine in the nutritional formula samples. Through participation in this study, laboratories can better understand the performance of their in-house methods relative to those being used by others in the community. Participant results may be used in the value assignment of NIST reference materials included as samples in this study.

4.3. Sample Information

Participants were provided with three packets each of SRM 1849b Infant/Adult Nutritional Formula I (milk-based) (labeled Infant Formula A) and RM 8261 Adult Nutritional Formula (high protein) (labeled Infant Formula C). Each packet contained approximately 10 g of material. Participants were asked to store the materials 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 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 1 g for the determination of the mass fractions of choline and carnitine. The approximate analyte levels were not reported to participants prior to the study. The target mass fraction values for choline and carnitine in SRM 1849b were from the COA [13, 14]. The target mass fraction values for choline and carnitine in RM 8261 were based on data provided by the material manufacturer, and the uncertainties were approximated as 10 % relative to the measured value. The target

values and uncertainties for choline and carnitine used in this study are provided in Table 4-1 on an as-received basis.

Table 4-1. Individualized data summary table for choline and carnitine in infant formulas.

Laboratory-specific results and Z-scores were provided to each participant separately from this report to protect laboratory identities.

(Lab Name)

Exercise 2 – Water-Soluble Vitamins in Infant Formula

Lab Code: (Code)		1. Your Results				2. Community Results			3. Target		
Sample	Units	x_i	s_i	Z'_{comm}	Z_{NIST}	N	x^*	s^*	x_{NIST}	u_{NIST}	
Choline	SRM 1849b	mg/kg	<i>Individual laboratory results will appear in this section; laboratory-specific results were provided to each participant separately from this report.</i>				7	924	230	1015	32
Choline	RM 8261	mg/kg					7	10400	3400	9400	940
Carnitine	SRM 1849b	mg/kg					6	150	12	160.1	2.4
Carnitine	RM 8261	mg/kg					6	76.4	7.5	67.0	6.7
		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_{NIST}	standard uncertainty about the 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						

4.4. Study Results and Discussion

4.4.1. Choline

Table 4-1 summarizes and Table 4-2 details the numerical results reported by each participating laboratory for choline. The participation level was fair for choline, with 47 % of laboratories requesting samples returning results (7 of 15 laboratories).

Table 4-2 reveals that the within-laboratory variabilities for choline measured mass fraction results were acceptable when compared to published expectations of the measurement community (Table 4-3) [16]. For among-laboratory variability, the published expectations are designed to evaluate the performance of a single method used by multiple laboratories, which is not directly applicable to the results of this study. For choline, the among-laboratory variabilities were greater than expected (25 % and 33 % for SRM 1849b and RM 8261, respectively), when considering the similarities in the analytical approaches used and the high level of choline in both samples. Additionally, the among-laboratory variability was higher for RM 8261 (33 %) compared to that for SRM 1849b (25 %), which is contrary to expectation based on the higher choline level in RM 8261 compared to SRM 1849b (Table 4-3).

Table 4-2. Data summary table for choline in SRM 1849b and RM 8261.

Data highlighted in blue have been identified as outside the consensus tolerance limits and resulted in an unacceptable Z'_{comm} score, $|Z'_{comm}| > 2$.

		Choline										
		SRM 1849b Infant/Adult Nutritional Formula I (milk-based) (mg/kg)					RM 8261 Adult Nutritional Formula (high protein) (mg/kg)					
		Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target				1014	32					9400	940
	B004	7537.91	7476.83	7507.37	7507	31	117084.8	122764.6	119924.7	119925	2840	
	B005	704	744	709	719	22	3311	3105	3203	3206	103	
	B012											
	B015	985	970	977	977	8	11595	11370	11328	11431	144	
	B017											
	B019	967	997	984	983	15	12660	12725	12743	12709	44	
	B021											
	B031	1030	1120	1130	1093	55	13030	13150	13090	13090	60	
	B043											
	B044	1030	1020	1010	1020	10	12200	12000	12200	12133	115	
	B047											
	B050											
	B051											
B053												
B055	750	750	750	750	0	7900	7500	7500	7633	231		
Community Results	Consensus Mean	920				Consensus Mean				10400		
	Consensus Standard Deviation	230				Consensus Standard Deviation				3400		
	Maximum	7507				Maximum				119925		
	Minimum	719				Minimum				3206		
	<i>N</i>	7				<i>N</i>				7		

Table 4-3. Summary of expected method performance requirements for choline and carnitine in nutritional formulas.

Standard Method Performance Requirements[®] (SMPR) ranges are expressed as the corresponding mass fraction in a reconstituted final product (reconstitution rate 25 g powder into 200 g water).

	Choline [16]		Carnitine [17]	
	SRM 1849b	RM 8261	SRM 1849b	RM 8261
Target Mass Fraction (mg/kg)	920	10400	150	76
Corresponding SMPR Range (mg/100 g)	2 – 20	20 – 200	0.16 – 20	
Expected Repeatability (RSD _r)	≤ 10 %	≤ 5 %	≤ 8 %	
Expected Reproducibility (RSD _R)	≤ 15 %	≤ 10 %	≤ 15 %	

Laboratories reported using hydrolysis (4 of 7 laboratories, 57 %) or solvent extraction (1 of 7 laboratories, 14 %) to prepare the nutritional formula samples for analysis of choline (Fig. 4-1, Fig. 4-2). Solvent extraction was conducted at room temperature for 15 min in acetic acid, which is likely insufficient to cleave choline from the numerous esterified forms. Two of the four laboratories using hydrolysis reported microwave-assisted digestion, while the other two laboratories used acid digestion without specifying use of a microwave. One laboratory indicated that acid hydrolysis was conducted at elevated temperature for 2.5 h, which yielded results more consistent with those reported following microwave digestion. The other laboratory using acid hydrolysis did not specify the hydrolysis temperature used. Two laboratories did not report the sample preparation approach employed prior to choline determination (29 %).

Fig. 4-3 and Fig. 4-4 indicate that most laboratories reported using liquid chromatography with tandem mass spectrometry detection (LC-MS/MS) for choline determination (5 of 7 laboratories, 71 %). One laboratory reported using ion chromatography with suppressed conductivity detection (14 %) and one laboratory did not report the analytical method used (14 %). No trends related to analytical method could be identified.

For both nutritional formula samples, the consensus means for measured choline mass fraction overlap the target ranges. In SRM 1849b, the widths of the consensus ranges for choline are approximately twice the widths of the target ranges (Fig. 4-1, Fig. 4-3). In RM 8261, the widths of the consensus ranges for choline and carnitine are comparable to or less than the widths of the target ranges (Fig. 4-2, Fig. 4-7).

Measurand: Choline
 Sample: SRM 1849b Infant/Adult Nutritional Formula I (milk-based)
 Exercise: FNSQAP Exercise 2

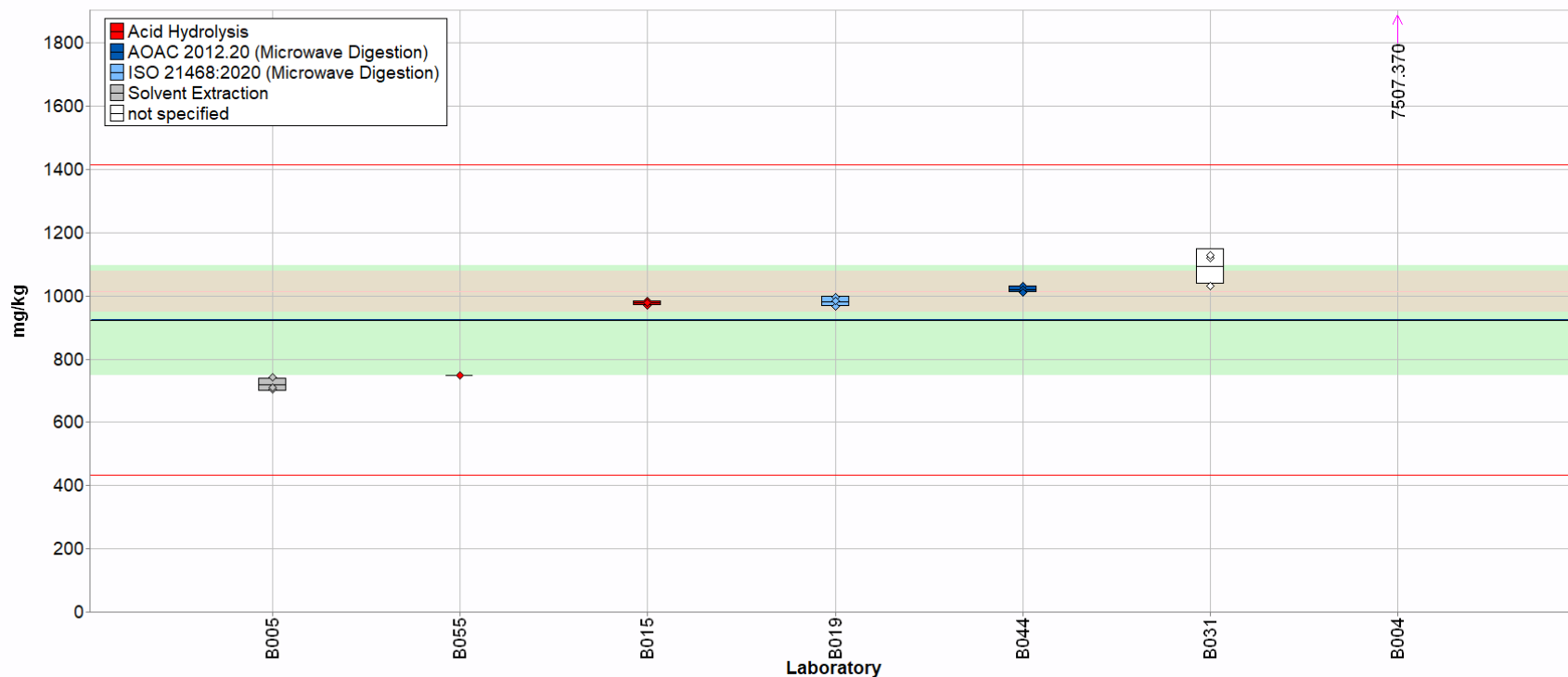


Fig. 4-1. Choline in SRM 1849b (data summary view – sample preparation method).

In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed as indicated in the figure key. Data points outside the graphical range are represented as upward arrows (laboratory B004 did not report the sample preparation approach used). The 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 target range, which encompasses the target value bounded by twice its uncertainty (U_{NIST}), and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$. The beige shaded region represents the overlapping of the 95 % confidence interval for the consensus mean (green region) and the NIST target range (red region).

Measurand: Choline
 Sample: RM 8261 Adult Nutritional Formula (high-protein)
 Exercise: FNSQAP Exercise 2

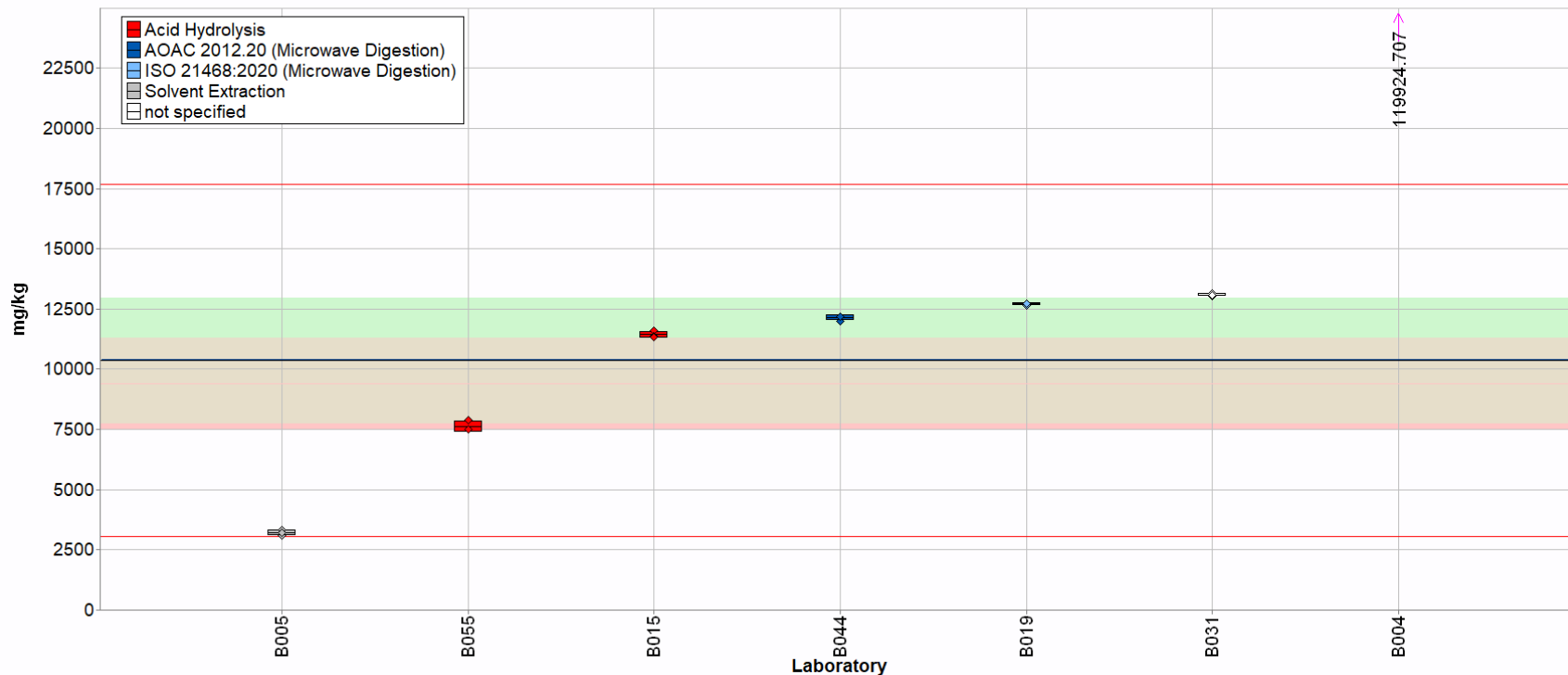


Fig. 4-2. Choline in RM 8261 (data summary view – sample preparation method).

In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed as indicated in the figure key. Data points outside the graphical range are represented as upward arrows (laboratory B004 did not report the sample preparation approach used). The 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 target range, which encompasses the target value bounded by twice its uncertainty (U_{NIST}), and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$. The beige shaded region represents the overlapping of the 95 % confidence interval for the consensus mean (green region) and the NIST target range (red region).

Measurand: Choline
 Sample: SRM 1849b Infant/Adult Nutritional Formula I (milk-based)
 Exercise: FNSQAP Exercise 2

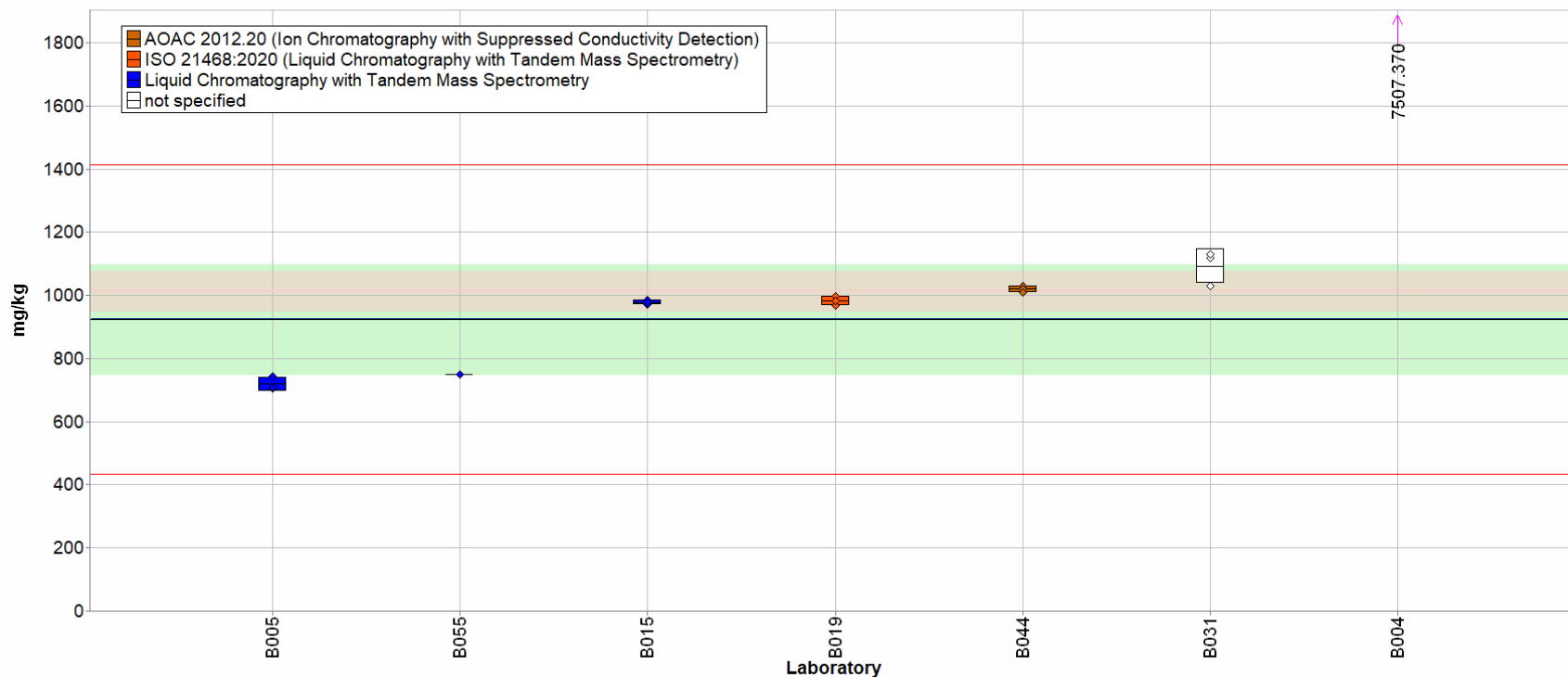


Fig. 4-3. Choline in SRM 1849b (data summary view – analytical method).

In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed as indicated in the figure key. Data points outside the graphical range are represented as upward arrows (the analytical method reported by laboratory B004 was LC-MS/MS). The 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 target range, which encompasses the target value bounded by twice its uncertainty (U_{NIST}), and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$. The beige shaded region represents the overlapping of the 95 % confidence interval for the consensus mean (green region) and the NIST target range (red region).

Measurand: Choline
 Sample: RM 8261 Adult Nutritional Formula (high-protein)
 Exercise: FNSQAP Exercise 2

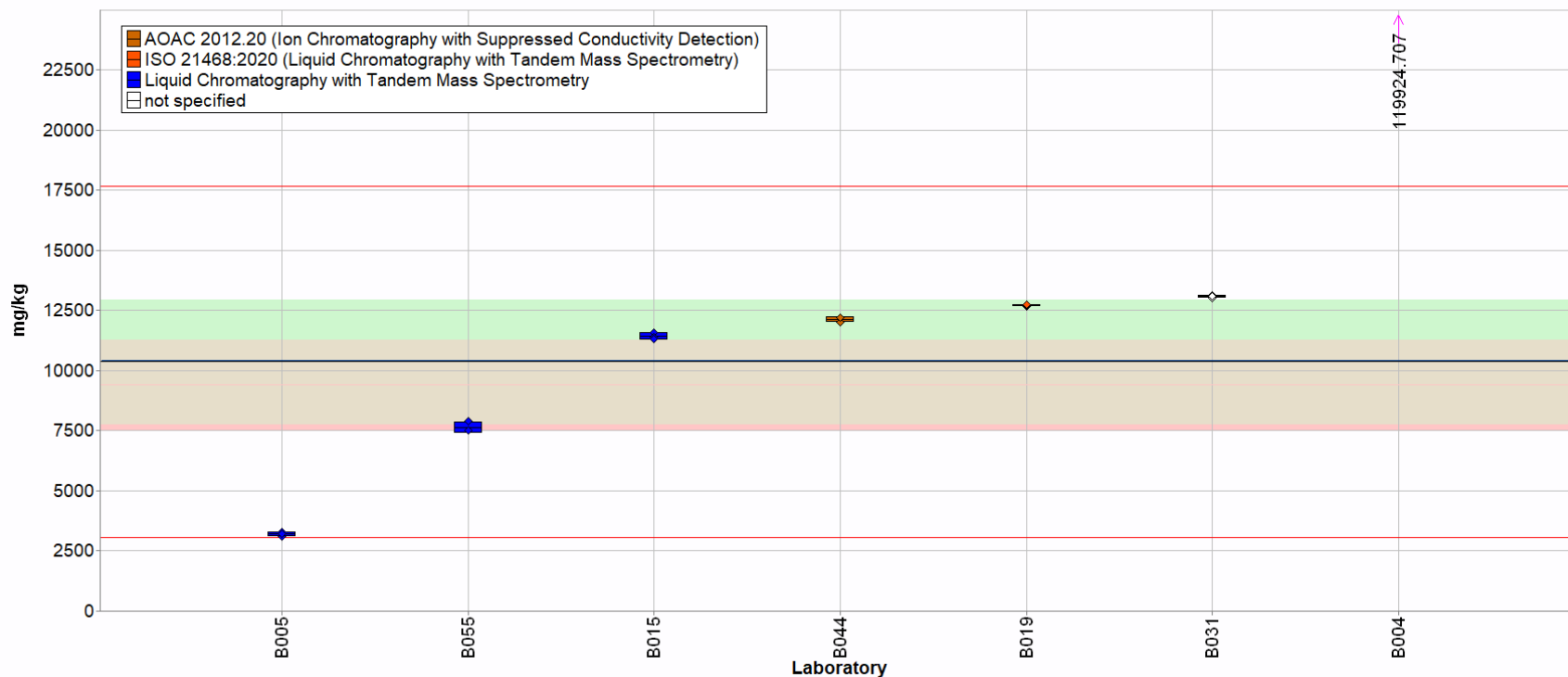


Fig. 4-4. Choline in RM 8261 (data summary view – analytical method).

In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed as indicated in the figure key. Data points outside the graphical range are represented as upward arrows (the analytical method reported by laboratory B004 was LC-MS/MS). The 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 limit of the consensus range of tolerance, calculated as the value above the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{comm}| \leq 2$. The lower limit of the community consensus range is set to zero. The red shaded region represents the NIST target range, which encompasses the target value bounded by twice its uncertainty (U_{NIST}), and represents the range that results in an acceptable Z_{NIST} score, $|Z_{NIST}| \leq 2$. The beige shaded region represents the overlapping of the 95 % confidence interval for the consensus mean (green region) and the NIST target range (red region).

The performance of laboratories in the determination of the mass fraction of choline in nutritional formula samples is highly dependent on the sample preparation technique selected. Laboratories that reported values below the target value did so consistently in these two very similar samples (Fig. 4-5), likely due to incomplete digestion of the numerous choline esters in the samples prior to separation and detection. Digestion approaches must be robust, including moderately strong acid, elevated temperature, and either use of microwave or longer digestion time to fully release choline from the matrix components.

Additionally, the level of choline in these samples was high, requiring significant sample dilution to avoid detector signal overload. With high-concentration analytes, non-linear calibration curves can impact accuracy. Calibration curves should be sufficiently narrow to prevent overextension of a linear fit. One approach is to conduct a screening experiment on the samples ahead of analysis to determine along which portion of the calibration curve the sample will lie. Prior to subsequent measurements, additional calibrant dilutions may be prepared to extend the calibration range; other dilutions can be excluded from the calibration curve to prevent bias. One laboratory reported values an order of magnitude higher than the target value, likely due to a miscalculated dilution factor. All calculations and results should be independently verified prior to submission to avoid reporting errors.

As with any laboratory exercise, consistent 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 like NIST's SRMs or non-certified reference materials such as NIST's RMs) or materials prepared in-house.

NIST has conducted one other QAP study involving measurement of choline in food samples prior to this FNSQAP study: DSQAP Exercise H in 2012 [18]. The participation rate in the previous study (54 %) was similar to this FNSQAP study (47 %), and laboratories reported results with comparable repeatability and higher reproducibility in the DSQAP study than in the current study. Higher reproducibility is expected for unfortified samples used in the DSQAP study (soy flour and egg powder), in which a greater fraction of the choline content is present in ester forms and analysis required more rigorous sample preparation than in the fortified nutritional powder samples used in this FNSQAP study. Like the current study, reporting errors were also observed in the DSQAP study in which laboratories reported values orders of magnitude higher or lower than the target values.

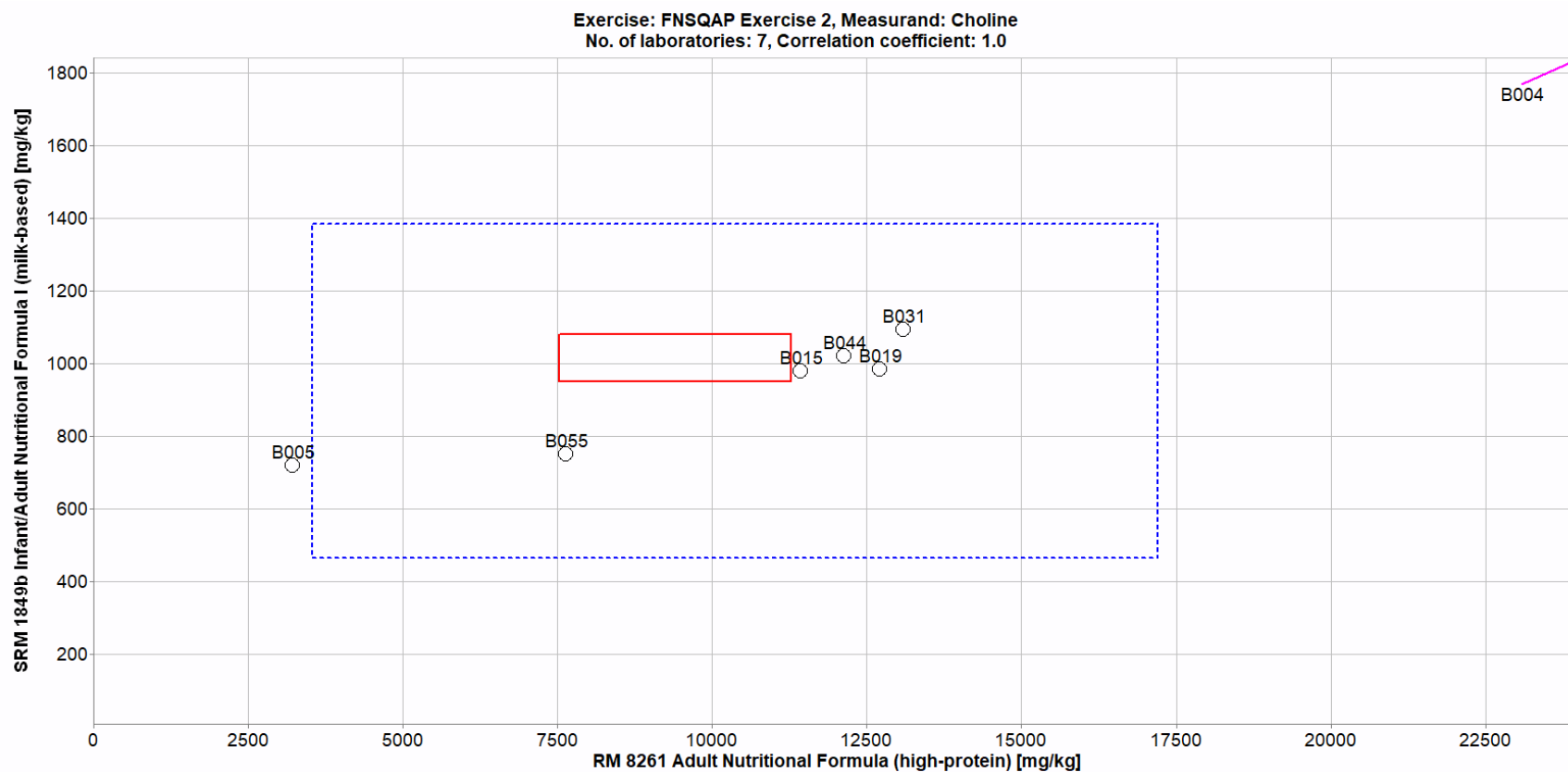


Fig. 4-5. Laboratory means for choline in RM 8261 and SRM 1849b (sample/sample comparison view).

In this view, the individual laboratory mean for one sample (RM 8261) is compared to the individual laboratory mean for a second sample (SRM 1849b). The solid red box represents the NIST target range for the two samples, RM 8261 (x-axis) and SRM 1849b (y-axis), which encompasses the target values bounded by their uncertainties (U_{NIST}), and represents the range that results in an acceptable Z_{NIST} score, $|Z_{NIST}| \leq 2$. The dotted blue box represents the consensus range of tolerance for RM 8261 (x-axis) and SRM 1849b (y-axis), calculated as the values above and below the consensus means that result in an acceptable Z'_{comm} score, $|Z'_{comm}| \leq 2$.

4.4.2. Carnitine

Table 4-1 summarizes and Table 4-4 details the numerical results reported by each participating laboratory for carnitine. The participation level was fair for carnitine, with 43 % of laboratories requesting samples returning results (6 of 14 laboratories).

Table 4-4. Data summary table for carnitine in SRM 1849b and RM 8261.

		Carnitine									
		SRM 1849b Infant/Adult Nutritional Formula I (milk-based) (mg/kg)					RM 8261 Adult Nutritional Formula (high protein) (mg/kg)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target				160.1	2.4				67.0	6.7
	B005										
	B012										
	B015	152	142	145	146.3	5.1	73	72	76	73.7	2.1
	B017										
	B019	155	156	154	155.0	1.0	76	78	76	76.7	1.2
	B021										
	B031	146	141	140	142.3	3.2	71	71	75	72.3	2.3
	B043	47.5	46.6	48.1	47.4	0.8	16.7	15.6	14.9	15.7	0.9
	B044	158.3	157.3	155.7	157.1	1.3	73.72	75.48	72.37	73.9	1.6
	B047										
	B050										
	B051										
	B053										
B055	146	159	145	150.0	7.8	86.6	87	82.2	85.3	2.7	
Community Results	Consensus Mean					150.2					76.4
	Consensus Standard Deviation					11.8					7.5
	Maximum					157.1					85.3
	Minimum					47.4					15.7
	<i>N</i>					6					6

Table 4-1 and Table 4-4 reveal that the within-laboratory variabilities and carnitine results were acceptable when compared to published expectations of the measurement community (Table 4-3) [17]. For among-laboratory variability, the published expectations are designed to evaluate the performance of a single method used by multiple laboratories, which is not directly applicable to the results of this study. However, the among-laboratory variabilities for carnitine in both nutritional formula samples were acceptable when compared to the published expectations outlined in Table 4-3.

As shown in Fig. 4-6 and Fig. 4-7, laboratories reported using microwave digestion (2 of 6 laboratories, 33 %), solvent extraction (2 of 6 laboratories, 33 %), or acid hydrolysis (1 of 6 laboratories, 17 %) to prepare the nutritional formula samples for analysis of carnitine. One laboratory did not report the sample preparation approach used (17 %). With a small number of laboratories reporting data across multiple methods, no trends were observed to correlate quality of reported results with method-specific bias.

For carnitine analysis (Fig. 4-8 and Fig. 4-9), most laboratories reported use of LC-MS/MS (5 of 6 laboratories, 83 %) while one laboratory reported use of liquid chromatography with mass spectrometry detection (LC-MS, 17 %). No trends related to analytical method could be identified.

For both nutritional formula samples, the consensus means for measured carnitine mass fraction overlap the target ranges. In SRM 1849b, the widths of the consensus ranges for carnitine are approximately twice the widths of the target ranges (Fig. 4-6, Fig. 4-8). In RM 8261, the widths of the consensus ranges for carnitine are comparable to or less than the widths of the target ranges (Fig. 4-7, Fig. 4-9). Overall, laboratories performed well in the determination of carnitine in the nutritional formula samples (Fig. 4-10). One laboratory reported values that were 20 % to 30 % of the target value, likely due to a miscalculated dilution factor.

This FNSQAP study was the first NIST study involving measurement of carnitine.

With high-concentration analytes, non-linear calibration curves can impact accuracy. Calibration curves should be sufficiently narrow to prevent overextension of a linear fit. One approach is to conduct a screening experiment on the samples ahead of analysis to determine along which portion of the calibration curve the sample will lie. Prior to subsequent measurements, additional calibrant dilutions may be prepared to extend the calibration range; other dilutions can be excluded from the calibration curve to prevent bias. Additionally, as with any laboratory exercise, consistent 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 like NIST's SRMs or non-certified reference materials such as NIST's RMs) or materials prepared in-house.

Measurand: Carnitine
 Sample: SRM 1849b Infant/Adult Nutritional Formula I (milk-based)
 Exercise: FNSQAP Exercise 2

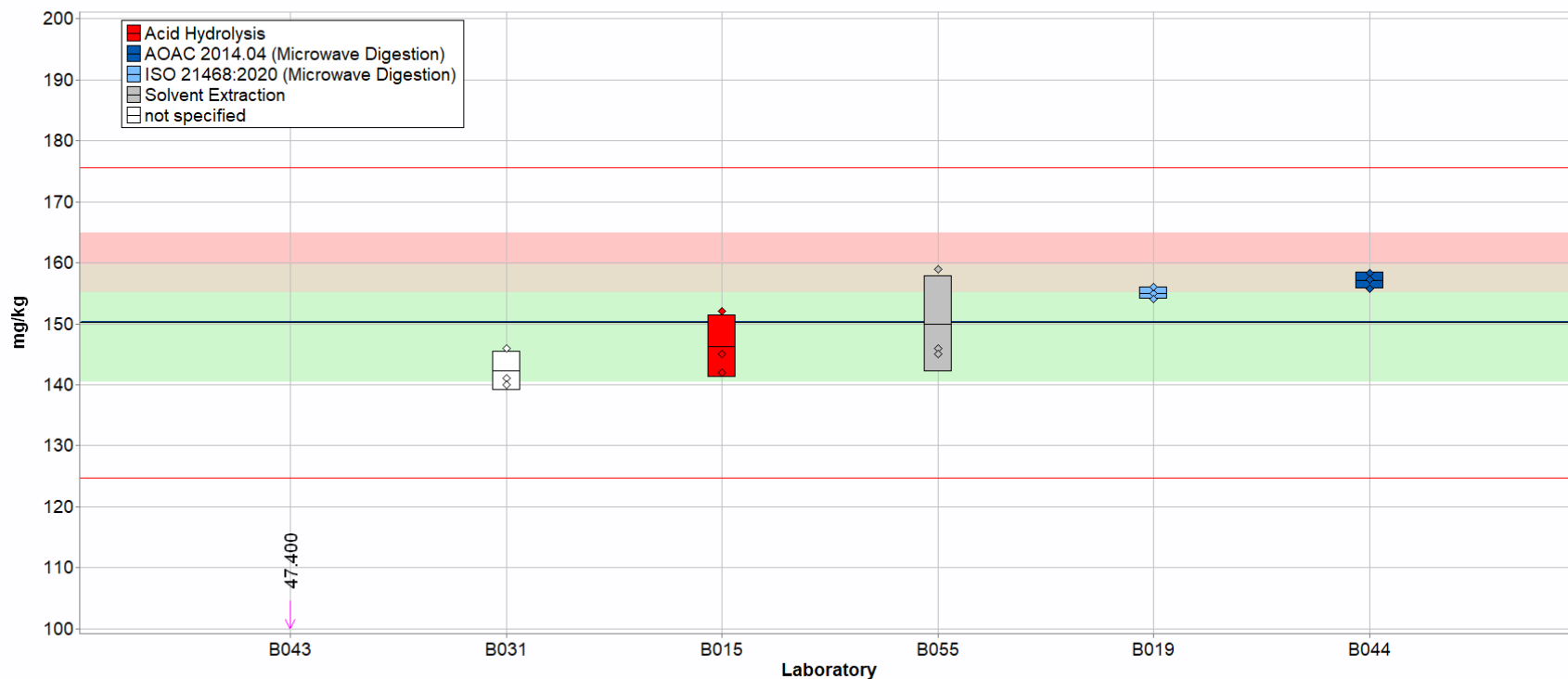


Fig. 4-6. Carnitine in SRM 1849b (data summary view – sample preparation method).

In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed as indicated in the figure key. Data points outside the graphical range are represented as downward arrows (the preparation approach reported by laboratory B043 was solvent extraction). The 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 target range, which encompasses the target value bounded by twice its uncertainty (U_{NIST}), and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$. The beige shaded region represents the overlapping of the 95 % confidence interval for the consensus mean (green region) and the NIST target range (red region).

Measurand: Carnitine
 Sample: RM 8261 Adult Nutritional Formula (high-protein)
 Exercise: FNSQAP Exercise 2

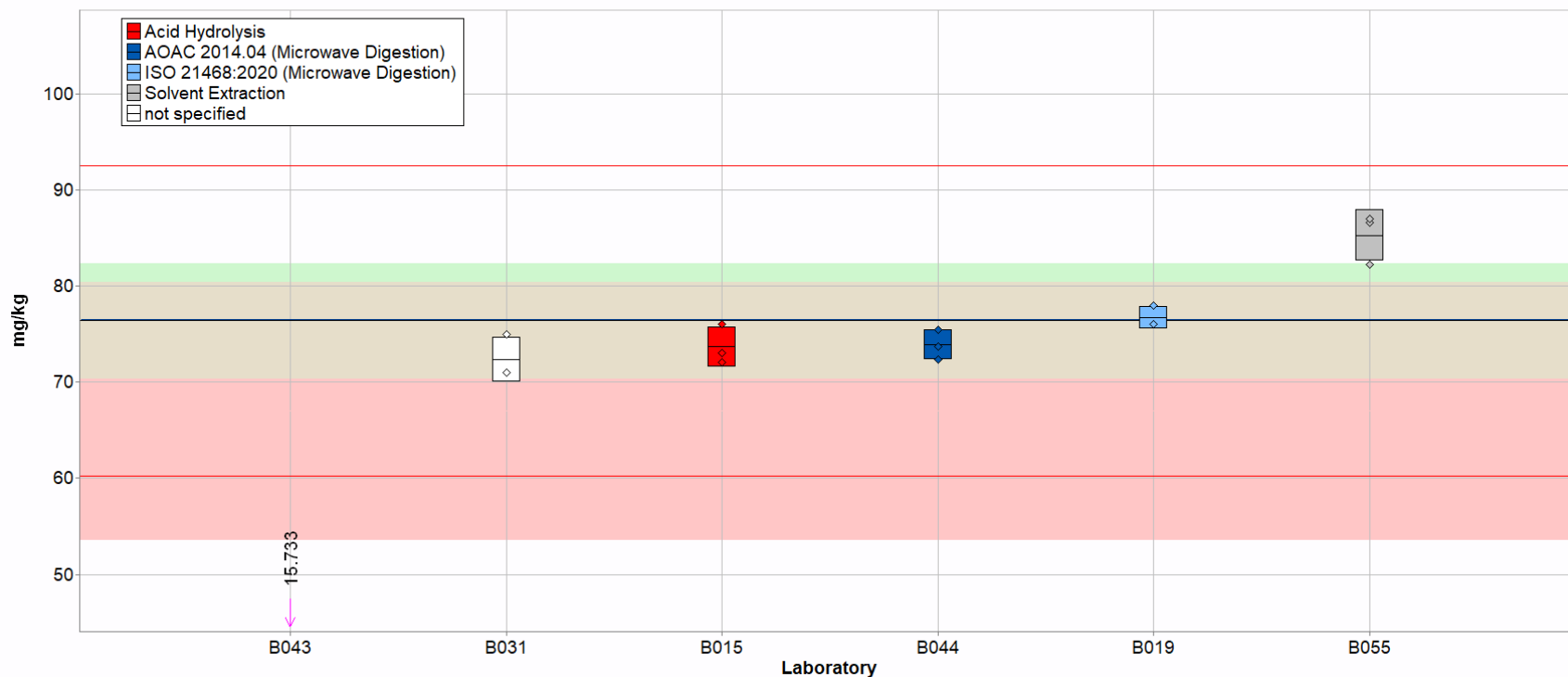


Fig. 4-7. Carnitine in RM 8261 (data summary view – sample preparation method).

In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed as indicated in the figure key. Data points outside the graphical range are represented as downward arrows (the preparation approach reported by laboratory B043 was solvent extraction). The 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 target range, which encompasses the target value bounded by twice its uncertainty (U_{NIST}), and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$. The beige shaded region represents the overlapping of the 95 % confidence interval for the consensus mean (green region) and the NIST target range (red region).

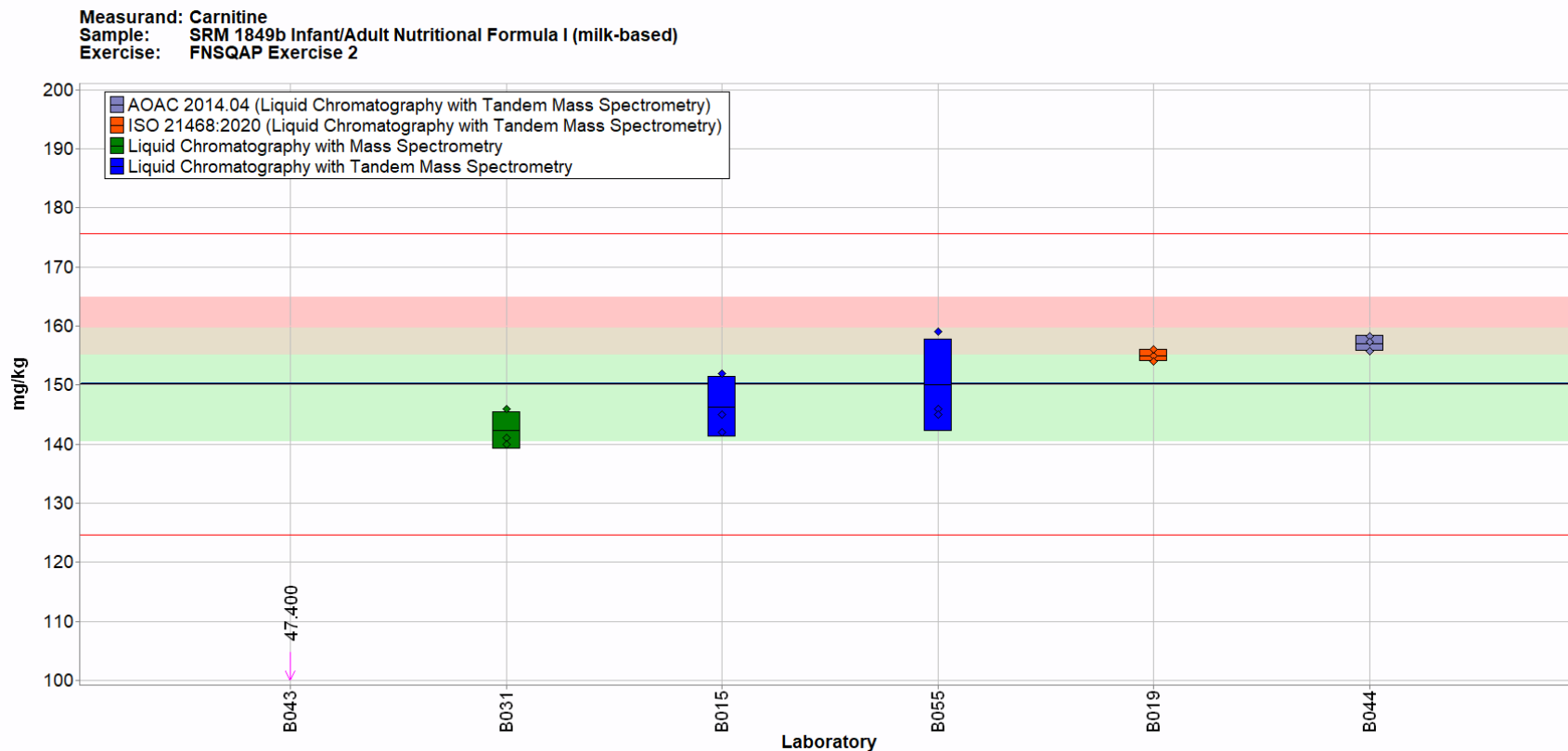


Fig. 4-8. Carnitine in SRM 1849b (data summary view – analytical method).

In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed as indicated in the figure key. Data points outside the graphical range are represented as downward arrows (the analytical method reported by laboratory B043 was LC-MS/MS). The 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 limit of the consensus range of tolerance, calculated as the value above the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$. The lower limit of the consensus range of tolerance is set to zero. The red shaded region represents the NIST target range, which encompasses the target value bounded by twice its uncertainty (U_{NIST}), and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$. The beige shaded region represents the overlapping of the 95 % confidence interval for the consensus mean (green region) and the NIST target range (red region).

Measurand: Carnitine
 Sample: RM 8261 Adult Nutritional Formula (high-protein)
 Exercise: FNSQAP Exercise 2

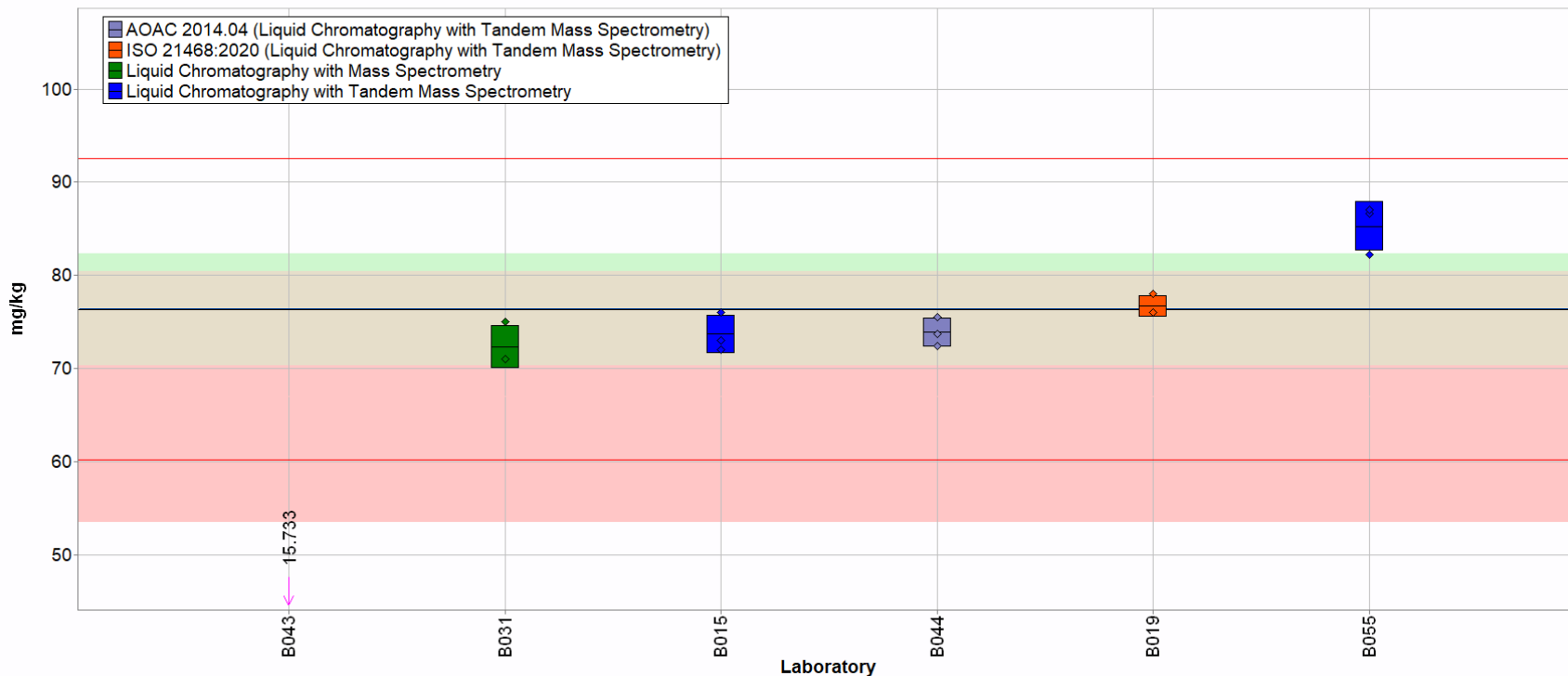


Fig. 4-9. Carnitine in RM 8261 (data summary view – analytical method).

In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed as indicated in the figure key. Data points outside the graphical range are represented as downward arrows (the analytical method reported by laboratory B043 was LC-MS/MS). The 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 limit of the consensus range of tolerance, calculated as the value above the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{comm}| \leq 2$. The lower limit of the consensus range of tolerance is set to zero. The red shaded region represents the NIST target range, which encompasses the target value bounded by twice its uncertainty (U_{NIST}), and represents the range that results in an acceptable Z_{NIST} score, $|Z_{NIST}| \leq 2$. The beige shaded region represents the overlapping of the 95 % confidence interval for the consensus mean (green region) and the NIST target range (red region).

Exercise: FNSQAP Exercise 2, Measurand: Carnitine
No. of laboratories: 6, Correlation coefficient: 0.9

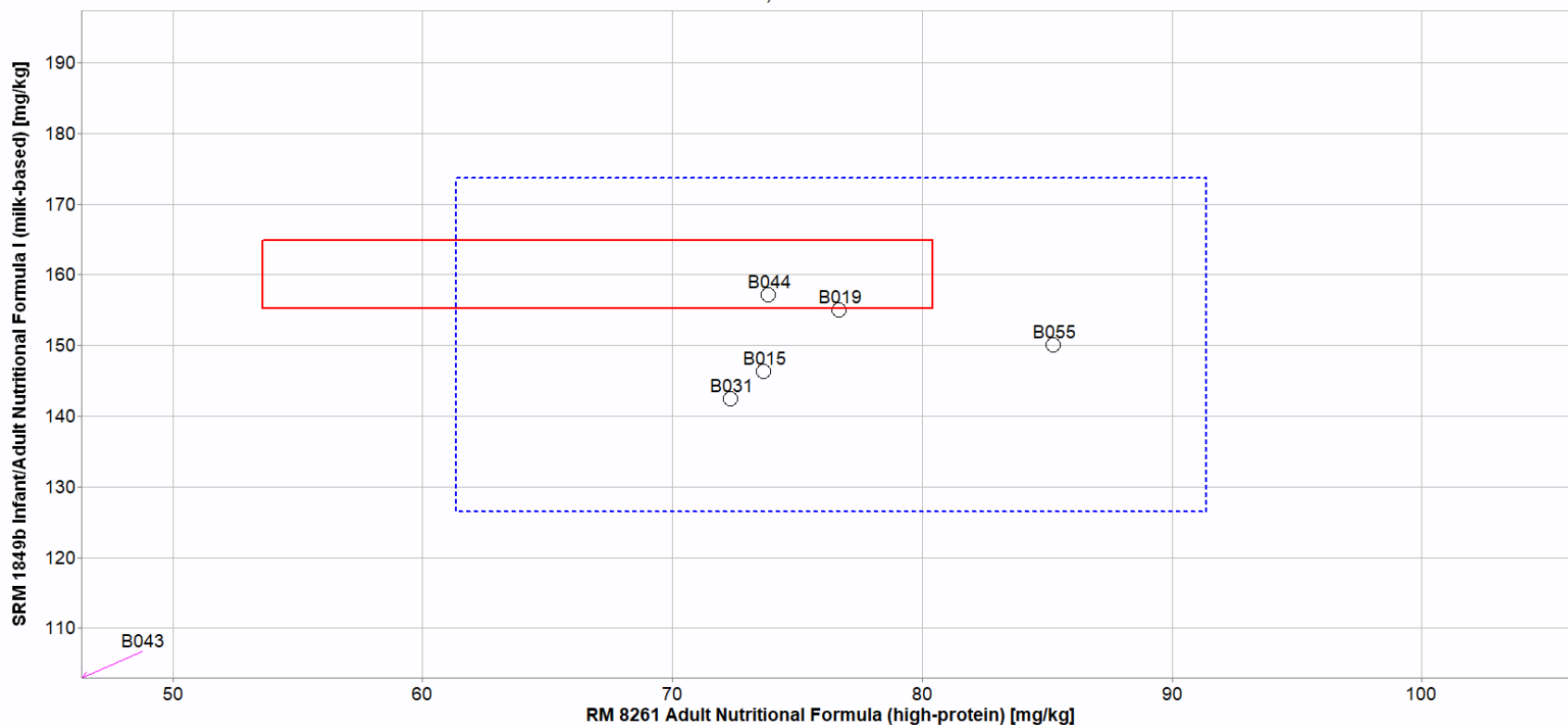


Fig. 4-10. Laboratory means for carnitine in RM 8261 and SRM 1849b (sample/sample comparison view).

In this view, the individual laboratory mean for one sample (RM 8261) is compared to the individual laboratory mean for a second sample (SRM 1849b). The solid red box represents the NIST target range for the two samples, RM 8261 (x-axis) and SRM 1849b (y-axis), which encompasses the target values bounded by their uncertainties (U_{NIST}), and represents the range that results in an acceptable Z_{NIST} score, $|Z_{NIST}| \leq 2$. The dotted blue box represents the consensus range of tolerance for RM 8261 (x-axis) and SRM 1849b (y-axis), calculated as the values above and below the consensus means that result in an acceptable Z'_{comm} score, $|Z'_{comm}| \leq 2$.

5. FAT-SOLUBLE VITAMINS (β -carotene, Lutein, Lycopene)

5.1. Executive Summary

Carotenoids are an important group of nutrients for infant development and growth, and the fortified levels of carotenoids in infant foods is strictly regulated worldwide. Participation in this study was low, and the interpretation of the small data set was confounded by presence of both major and minor outliers.

5.2. Study Overview

Carotenoids are a group of compounds essential for eye health that have also been associated with antioxidant activity and reduced risk of several different types of diseases, such as cancer and cardiovascular disease. Carotenoids such as β -carotene are considered provitamin A and are converted to retinol in the body [19]. Accurately understanding the intake and corresponding health outcomes related to carotenoid consumption through measurement in infant formulas can inform future decisions about recommended dietary intakes. In this study, participants were provided with two nutritional formula samples, SRM 1849b Infant/Adult Nutritional Formula I (milk-based) and RM 8261 Adult Nutritional Formula (high protein). Participants were asked to use in-house analytical methods to determine the mass fractions (mg/kg) of β -carotene, lutein, lycopene in the infant formula samples. Through participation in this study, laboratories can better understand the performance of their in-house methods relative to those being used by others in the community. Participant results may be used in the value assignment of NIST reference materials included as samples in this study.

5.3. Sample Information

Participants were provided with three packets each of SRM 1849b Infant/Adult Nutritional Formula I (milk-based) (labeled Infant Formula A) and RM 8261 Adult Nutritional Formula (high protein) (labeled Infant Formula C). Each packet contained approximately 10 g of material; participants were asked to store the materials 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 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 1 g for the determination of carotenoids. The approximate analyte mass fraction levels were not reported to participants prior to the study. The target values for carotenoids in SRM 1849b were from the COA [13, 14]. The target values for carotenoids in RM 8261 were based on data from the material manufacturer, and the uncertainties were approximated as 10 % relative to the measured value. The target values and uncertainties for carotenoids used in this study are provided in Table 5-1 on an as-received basis.

Table 5-1. Individualized data summary table for carotenoids in infant formulas.

Laboratory-specific results and Z-scores were provided to each participant separately from this report to protect laboratory identities.

(Lab Name)

			Exercise 2 – Fat-Soluble Vitamins in Infant Formulas								
Lab Code: (Code)		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_{NIST}
β -carotene	SRM 1849b	mg/kg	<i>Individual laboratory results will appear in this section; laboratory-specific results were provided to each participant separately from this report.</i>				4	0.43	0.65	0.545	0.007
β -carotene	RM 8261	mg/kg					3	0.27	0.14	0.050	0.005
Lutein	SRM 1849b	mg/kg					4	1.85	0.95	2.478	0.015
Lutein	RM 8261	mg/kg					4	0.17	0.14	0.230	0.023
Lycopene	SRM 1849b	mg/kg					2	1.1	4.1	1.733	0.020
Lycopene	RM 8261	mg/kg					0			0.230	0.023
			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_{NIST}	standard uncertainty about the 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			

5.4. Study Results and Discussion

5.4.1. β -carotene

Table 5-1 summarizes and Table 5-2 details the measured mass fraction results reported by each participating laboratory for β -carotene. The participation level was fair for β -carotene in this study, with 42 % of laboratories requesting samples returning results (8 of 19 laboratories). Approximately half of all reported results were qualitative, with laboratories indicating that the level of β -carotene in one or both samples were below their method limit of quantitation.

Table 5-2 reveals that the within-laboratory variabilities for three of the four participants reporting quantitative results in SRM 1849b were acceptable with respect to published expectations of the measurement community ($\leq 8\%$) (Table 5-3) [20]. For RM 8261, the three participants reporting quantitative results also indicated within-laboratory variabilities consistent with published requirements ($\leq 8\%$) despite the level of β -carotene being below the analytical range for which the requirements were established. The among-laboratory variabilities for SRM 1849b and RM 8261 were high (150 % and 52 %, respectively) compared to the published expectations of the measurement community for multiple laboratories using the same method ($\leq 15\%$) [20], even when considering the variety of methods used by the participants. Additionally, the limited number of laboratories reporting quantitative results (3 to 4 laboratories) combined with the observation of one major high outlier (Fig. 5-1 and Fig. 5-2) may inflate the observed among-laboratory variability beyond what would routinely be observed in this community for measurement of β -carotene in nutritional formulas.

Table 5-2. Data summary table for total β -carotene in SRM 1849b and RM 8261.

Data points highlighted in blue have been identified as outside the consensus tolerance limits and resulted in an unacceptable Z'_{comm} score, $|Z'_{\text{comm}}| \geq 2$.

		Total β -Carotene									
		SRM 1849b Infant/Adult Nutritional Formula I (milk-based) (mg/kg)					RM 8261 Adult Nutritional Formula (high protein) (mg/kg)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target				0.545	0.007				0.050	0.005
	B001	0.206556	0.228166	0.180402	0.205	0.024	< 0.010	< 0.010	< 0.010		
	B005										
	B009	4.99	5.31	5.33	5.210	0.191	0.93	1.01	0.88	0.940	0.066
	B012										
	B015										
	B017										
	B019										
	B021	< 0.630	< 0.630	< 0.630			< 0.630	< 0.630	< 0.630		
	B030										
	B031	< 0.600	< 0.600	< 0.600			< 0.600	< 0.600	< 0.600		
	B035	0.58	0.62	0.59	0.597	0.021	0.25	0.24	0.25	0.247	0.006
	B043										
	B044	0.5	0.5	0.5	0.500	0.000	< 0.300	< 0.300	< 0.300		
	B047										
	B050										
B051											
B052	< 0.280	< 0.280	< 0.280			< 0.280	0.291	0.311	0.301	0.014	
B053											
B055	< 10.000	< 10.000	< 10.000			< 10.000	< 10.000	< 10.000			
Community Results		Consensus Mean			0.43		Consensus Mean			0.27	
		Consensus Standard Deviation			0.65		Consensus Standard Deviation			0.14	
		Maximum			5.21		Maximum			0.94	
		Minimum			0.21		Minimum			0.25	
		N			4		N			3	

Table 5-3. Summary of expected method performance requirements for carotenoids in nutritional formulas [20].

Standard Method Performance Requirements[®] (SMPR) ranges are expressed as the corresponding mass fraction in a reconstituted final product (reconstitution rate 25 g powder into 200 g water).

	β-Carotene		Lutein		Lycopene	
	SRM 1849b	RM 8261	SRM 1849b	RM 8261	SRM 1849b	RM 8261
Target Mass Fraction (mg/kg)	0.55	0.05	2.5	0.2	1.7	0.2
Corresponding SMPR Range (μg/100 g)	1 – 100	NA	1 – 100		1 – 100	
Expected Repeatability (RSD _r)	≤ 8 %	NA	≤ 8 %		≤ 8 %	
Expected Reproducibility (RSD _R)	≤ 15 %	NA	≤ 15 %		≤ 15 %	

As shown in Fig. 5-1 and Fig. 5-2, 3 laboratories reported using saponification (38 %), two laboratories reported using enzymatic digestion (25 %), and one laboratory each reported use of base plus enzymatic digestion, extraction, and solvent extraction (13 % each). All laboratories reported use of liquid chromatography with absorbance or photodiode array detection (LC-Abs) for determination of β-carotene in the nutritional formula samples. No trends in the reported results were identified based on method information or additional details about digestion/extraction solvents, times, and temperatures and detection wavelengths. One laboratory reported values an order of magnitude higher than the target value in both samples, likely due to a miscalculated dilution factor. All calculations and results should be independently verified prior to submission to avoid reporting errors.

NIST has conducted four QAP studies involving measurement of β-carotene in food samples prior to this FNSQAP study: DSQAP Exercise D in 2009 [21], Exercise E in 2010 [21], and Exercise G in 2011 [22], and HAMQAP Exercise 3 in 2019 [23]. A review of these exercises indicated lower than previous enrollment (17 compared to 30 for past exercises) and participation rate (24 % compared to 50 % for past exercises). The sample types offered in this study (nutritional formulas) may have been of interest to fewer potential participants than historical samples (carotenoid-rich foods and supplements). The repeatability reported by participants in this study (≤ 10 % RSD) is consistent with average repeatability from previous studies, and the range of reported repeatabilities was much narrower in this study than previously reported. The low number of reported results for this study, however, prevents comparison of among-laboratory variability and bias with previous studies.

Measurand: Total beta-Carotene
 Sample: SRM 1849b Infant/Adult Nutritional Formula I (milk-based)
 Exercise: FNSQAP Exercise 2

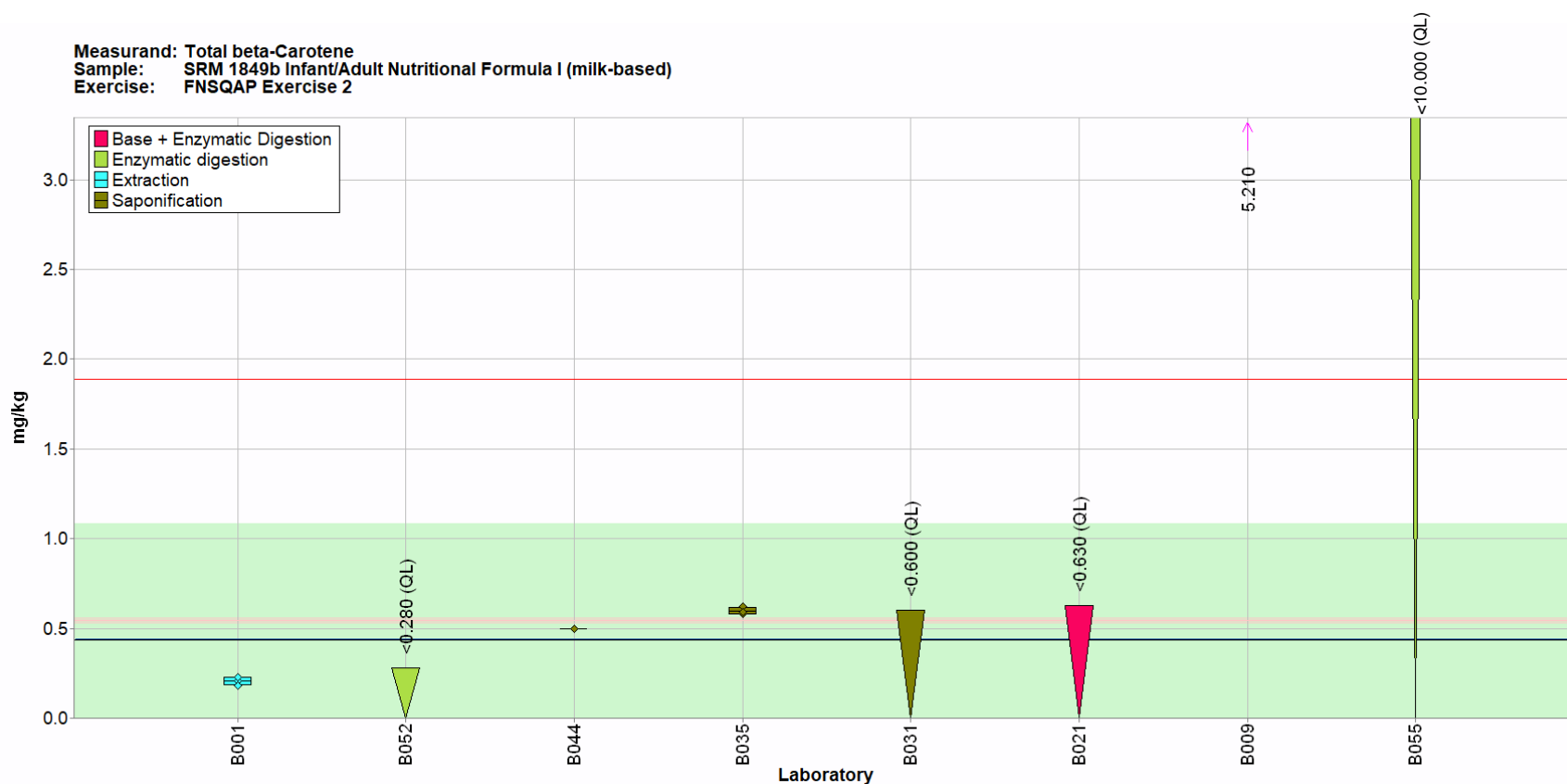


Fig. 5-1. Total β -carotene in SRM 1849b (data summary view – sample preparation method).

In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). A downward triangle represents data reported as an LOQ value. The color of the data point represents the sample preparation method employed as indicated in the figure key. Data points outside the graphical range are represented as upward arrows (the sample preparation method reported by laboratory B009 was solvent extraction). The 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 limit of the consensus range of tolerance, calculated as the value above the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{comm}| \leq 2$. The lower limit of the consensus range of tolerance is set to zero. The red shaded region represents the NIST target range, which encompasses the target value bounded by twice its uncertainty (U_{NIST}), and represents the range that results in an acceptable Z_{NIST} score, $|Z_{NIST}| \leq 2$. The beige shaded region represents the overlapping of the 95 % confidence interval for the consensus mean (green region) and the NIST target range (red region).

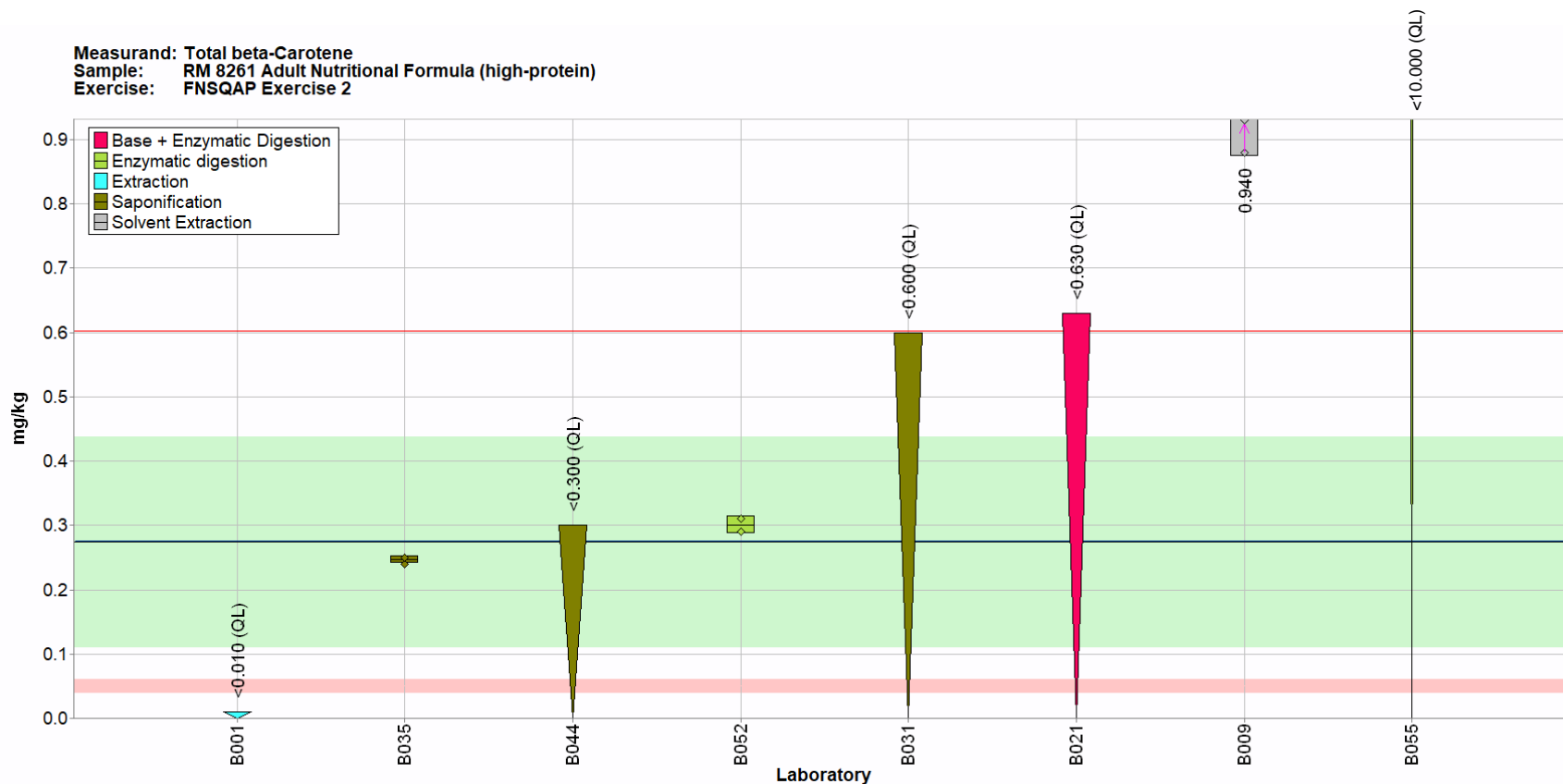


Fig. 5-2. Total β -carotene in RM 8261 (data summary view – sample preparation method).

In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). A downward triangle represents data reported as an LOQ value. The color of the data point represents the sample preparation method employed as indicated in the figure key. The 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 bound of the consensus range of tolerance, calculated as the value above the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$, with the bottom bound set to zero. The beige shaded region represents the overlapping of the 95 % confidence interval for the consensus mean (green region) and the NIST target range (red region). The NIST target range encompasses the target value bounded by twice its uncertainty (U_{NIST}), and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$.

5.4.2. Lutein

Table 5-1 summarizes and Table 5-4 details the measured mass fraction results reported by each participating laboratory for lutein. The participation level was fair for lutein in this study, with 39 % of laboratories requesting samples returning results (7 of 18 laboratories). Approximately half of all reported results were qualitative, with laboratories indicating that the level of lutein in one or both samples were below their method limit of quantitation.

Table 5-4 reveals that the within-laboratory variabilities for three of the four participants reporting quantitative results in SRM 1849b were acceptable with respect to published expectations of the measurement community ($\leq 8\%$) (Table 5-3) [20]. One laboratory reported a within-laboratory variability of 8.7 %, just outside the acceptable range. For RM 8261, two of the four participants reporting quantitative results indicated within-laboratory variabilities more than twice published requirements (17 % and 20 %). The among-laboratory variabilities for SRM 1849b and RM 8261 were also high (52 % and 83 %, respectively) compared to the published expectations of the measurement community for multiple laboratories using the same method ($\leq 15\%$) [20], even when considering the variety of methods used by the participants.

Table 5-4. Data summary table for total lutein in SRM 1849b and RM 8261.

Data points highlighted in blue have been identified as outside the consensus tolerance limits and resulted in an unacceptable Z'_{comm} score, $|Z'_{\text{comm}}| \geq 2$.

		Total Lutein									
		SRM 1849b Infant/Adult Nutritional Formula I (milk-based) (mg/kg)					RM 8261 Adult Nutritional Formula (high protein) (mg/kg)				
		Lab	A	B	C	Avg	SD	A	B	C	Avg
Individual Results	Target				2.478	0.015				0.230	0.023
	B001	1.06968	1.12269	0.946143	1.046	0.091	0.0527654	0.0684203	0.0499846	0.057	0.010
	B005	< 0.010	< 0.010	< 0.010			< 0.010	< 0.010	< 0.010		
	B009	2.53	2.46	2.5	2.497	0.035	0.14	0.13	0.14	0.137	0.006
	B012										
	B015										
	B017										
	B019										
	B021	< 1.650	< 1.650	< 1.650			< 1.650	< 1.650	< 1.650		
	B030										
	B031										
	B035	1.87	1.83	1.81	1.837	0.031	0.13	0.13	0.13	0.130	0.000
	B043										
	B047										
	B050										
	B051										
	B052	< 0.040	< 0.040	< 0.040			< 0.040	0.704	0.533	0.619	0.121
B053											
B055	1.9	2.09	2.1	2.030	0.113	< 1.000	< 1.000	< 1.000			
Community Results	Consensus Mean				1.85		Consensus Mean			0.17	
	Consensus Standard Deviation				0.95		Consensus Standard Deviation			0.14	
	Maximum				2.50		Maximum			0.62	
	Minimum				1.05		Minimum			0.06	
	<i>N</i>				4		<i>N</i>			4	

As shown in Fig. 5-3 and Fig. 5-4, one laboratory each per sample reported using saponification, enzymatic digestion, extraction, liquid-liquid extraction, and solvent extraction (25 % each). All laboratories reported use of liquid chromatography with absorbance or photodiode array detection (LC-Abs) for determination of lutein in the nutritional formula samples. No trends in the reported results were identified based on method information or additional details about digestion/extraction solvents, times, and temperatures and detection wavelengths.

Measurand: Total Lutein
 Sample: SRM 1849b Infant/Adult Nutritional Formula I (milk-based)
 Exercise: FNSQAP Exercise 2

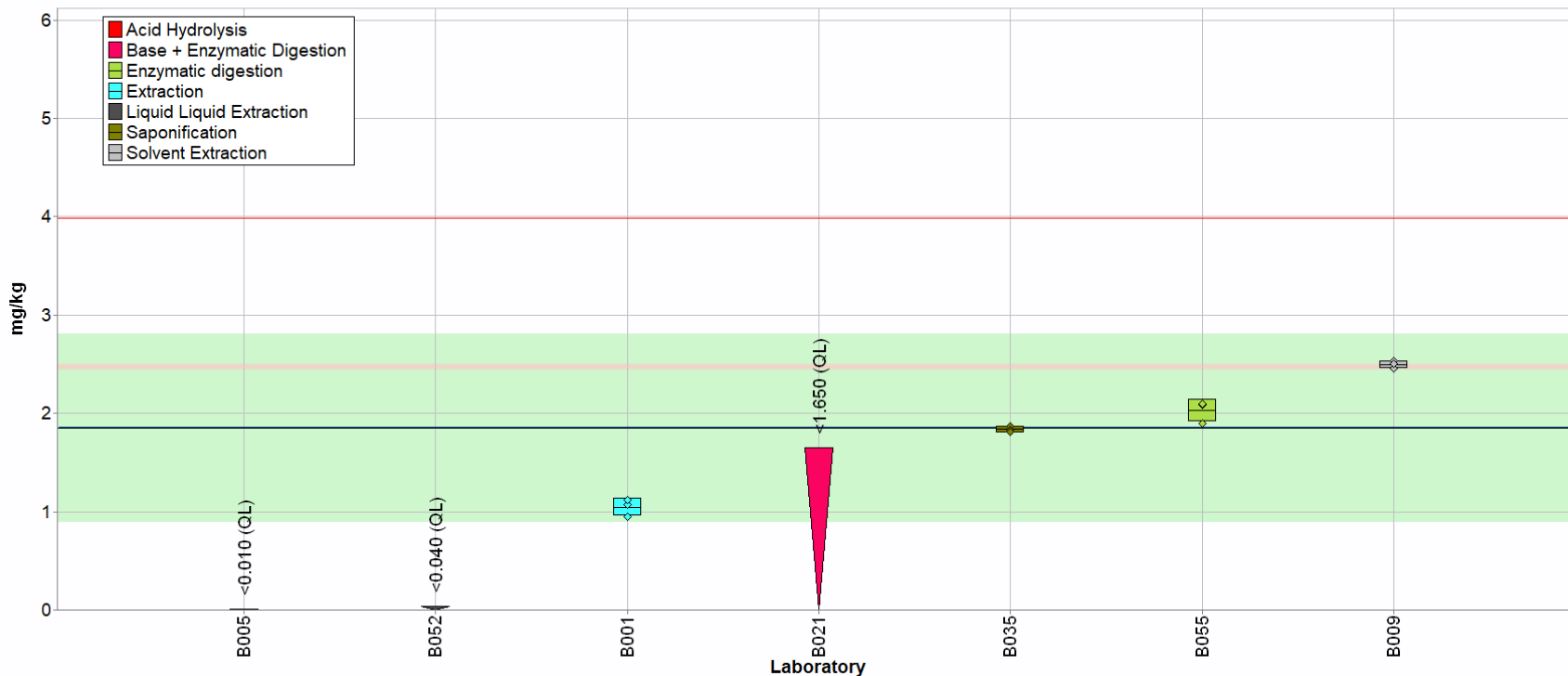


Fig. 5-3. Total lutein in SRM 1849b (data summary view – sample preparation method).

In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). A downward triangle represents data reported as an LOQ value. The color of the data point represents the sample preparation method employed as indicated in the figure key (the sample preparation methods reported by lab B005 and B052 were extraction and liquid-liquid extraction, respectively). The 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 bound of the consensus range of tolerance, calculated as the value above the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{comm}| \leq 2$, with the bottom bound set to zero. The beige shaded region represents the overlapping of the 95 % confidence interval for the consensus mean (green region) and the NIST target range (red region). The NIST target range encompasses the target value bounded by twice its uncertainty (U_{NIST}), and represents the range that results in an acceptable Z_{NIST} score, $|Z_{NIST}| \leq 2$.

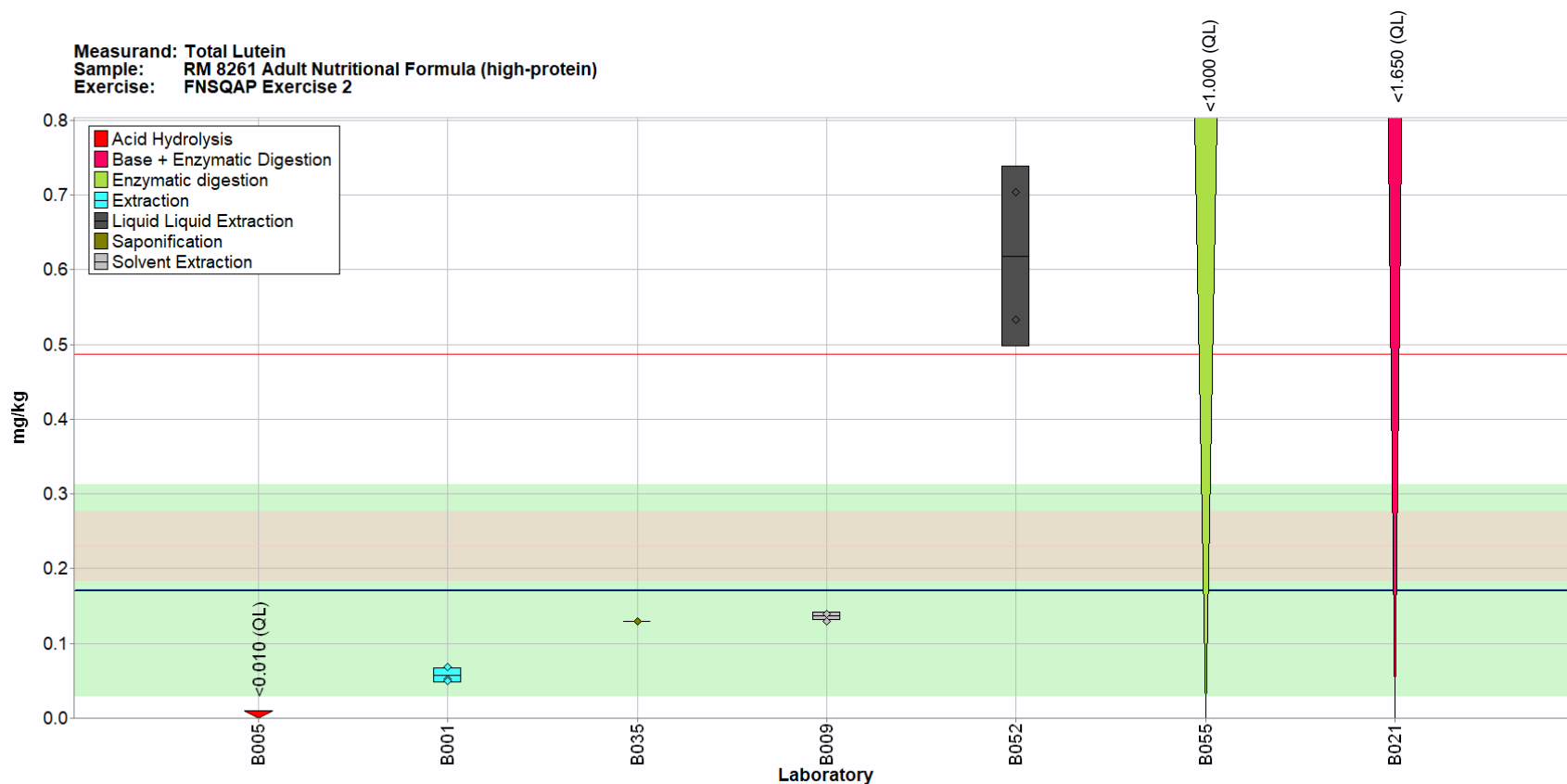


Fig. 5-4. Total lutein in RM 8261 (data summary view – sample preparation method).

In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). A downward triangle represents data reported as an LOQ value. The color of the data point represents the sample preparation method employed as indicated in the figure key. The 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 bound of the consensus range of tolerance, calculated as the value above the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{comm}| \leq 2$, with the bottom bound set to zero. The beige shaded region represents the overlapping of the 95 % confidence interval for the consensus mean (green region) and the NIST target range (red region). The NIST target range encompasses the target value bounded by twice its uncertainty (U_{NIST}), and represents the range that results in an acceptable Z_{NIST} score, $|Z_{NIST}| \leq 2$.

NIST has conducted two QAP studies involving measurement of lutein in food samples prior to this FNSQAP study: DSQAP Exercise L in 2016 [24] and HAMQAP Exercise 3 in 2019 [23]. A review of these exercises indicated lower than previous enrollment (15 compared to 33 for past exercises) and participation rate (33 % compared to 50 % for past exercises). The sample types offered in this study (nutritional formulas) may have been of interest to fewer potential participants than historical samples (carotenoid-rich foods and supplements). The repeatability reported by participants in this study is consistent with average repeatability from previous studies, however, the low number of reported results for this study prevents comparison of among-laboratory variability and bias with previous studies.

5.4.3. Lycopene

Table 5-1 summarizes and Table 5-5 details the measured mass fraction results reported by each participating laboratory for lycopene. The participation level was low for lycopene, with only 24 % of laboratories requesting samples returning results (4 of 17 laboratories). Only two laboratories reported quantitative values for lycopene in SRM 1849b, and no laboratories reported quantitative values for lycopene in RM 8261.

Table 5-5 reveals that the within-laboratory variabilities for both participants reporting quantitative results in SRM 1849b were acceptable with respect to published expectations of the measurement community ($\leq 8\%$) (Table 5-3) [20]. The limited number of laboratories reporting quantitative results (2 laboratories) combined with the observation of one major low outlier inflated the observed among-laboratory variability beyond what would routinely be observed in this community for measurement of lycopene in nutritional formulas. One laboratory reported values an order of magnitude below the target value, indicating a probable calculation error. For RM 8261, the no participants reported quantitative results.

This FNSQAP study was the first NIST study involving measurement of lycopene.

Table 5-5. Data summary table for total lycopene in SRM 1849b and RM 8261.

		Total Lycopene									
		SRM 1849b Infant/Adult Nutritional Formula I (milk-based) (mg/kg)					RM 8261 Adult Nutritional Formula (high protein) (mg/kg)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target				1.73	0.02				0.230	0.023
	B001	0.17845	0.190613	0.165869	0.18	0.01	< 0.050	< 0.050	< 0.050		
	B005										
	B012										
	B015										
	B017										
	B019										
	B021	< 2.470	< 2.470	< 2.470			< 2.470	< 2.470	< 2.470		
	B030										
	B031										
	B035	2.21	2.04	2.04	2.10	0.10	< 0.030	< 0.030	< 0.030		
	B043										
	B047										
	B050										
	B051										
	B052										
B053											
B055	< 10.000	< 10.000	< 10.000			< 10.000	< 10.000	< 10.000			
Community Results		Consensus Mean				1.1	Consensus Mean				
		Consensus Standard Deviation				4.1	Consensus Standard Deviation				
		Maximum				2.1	Maximum				
		Minimum				0.2	Minimum				
		<i>N</i>				2	<i>N</i>				0

5.4.4. Summary

For both nutritional formula samples, the number of quantitative results contributing to the carotenoid consensus means was too small for meaningful comparison to target values. Overall, conclusions cannot be drawn about community performance in the determination of carotenoids in nutritional formula samples. Laboratories reporting LOQs above 0.08 mg/kg should consider reevaluating their method performance characteristics, as the nutritional formula community has indicated that analytical methods must be able to quantify β -carotene, lutein, and lycopene at 0.08 mg/kg [20].

In any laboratory exercise, calculations and reporting units must be verified prior to submission of results. Laboratories often report results in the wrong units or omit a dilution factor during the calculation of the final results, resulting in poor performance on the study. Calibration materials for carotenoids may contain impurities, and concentrations of prepared solutions should be assigned spectrophotometrically [25]. Carotenoids are known to be unstable in matrix and solution, so laboratories should ensure that care is taken to prepare fresh solutions and to protect samples and calibrants from conditions that may accelerate degradation. As always, consistent 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 like NIST's SRMs or non-certified reference materials such as NIST's RMs) or materials prepared in-house.

6. FATTY ACIDS (DHA, ARA)

6.1. Executive Summary

Fatty acids, specifically docosahexaenoic acid (DHA) and arachidonic acid (ARA), are critical nutrients for infant brain development and growth, and the fortified levels of fatty acids in infant foods are strictly regulated worldwide. Enrollment and participation in this study was fair, but the reported data indicate comparable performance of the small number of participating laboratories. More method information is needed to understand sources of higher repeatability and reproducibility than described in published recommendations.

6.2. Study Overview

Docosahexaenoic acid (DHA) and arachidonic acid (ARA) are fatty acids found in breast milk and play important roles in early infant development. Recent European Union legislation requires addition of DHA to infant formulas [26], and some researchers encourage corresponding amounts of ARA be added as well [27, 28]. Accurate methods are needed for the detection of DHA and ARA to meet these regulatory criteria, to understand the intake of DHA and ARA through fortified foods, and to inform future decisions about recommended dietary intakes. In this study, participants were provided with two nutritional formula samples, SRM 1849b Infant/Adult Nutritional Formula I (milk-based) and RM 8260 Infant Nutritional Formula (hydrolyzed milk-based). Participants were asked to use in-house analytical methods to report the mass percent of DHA and ARA as free fatty acids (FFAs) in the infant formula samples. Through participation in this study, laboratories can better understand the performance of their in-house methods relative to those being used by others in the community. Participant results may be used in the value assignment of NIST reference materials included as samples in this study.

6.3. Sample Information

Participants were provided with three packets each of SRM 1849b Infant/Adult Nutritional Formula I (milk-based) (labeled Infant Formula A) and RM 8260 Infant Nutritional Formula (hydrolyzed milk-based) (labeled Infant Formula B). Each packet contained approximately 10 g of material; participants were asked to store the materials 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 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 0.3 g for the determination of fatty acids. The approximate analyte levels were not reported to participants prior to the study. The target values for DHA and ARA in each material were from the COA for SRM 1849b [13, 14] and the RMIS for RM 8260 [29]. The target values and uncertainties for DHA and ARA are provided in Table 6-1 on an as-received basis. The uncertainties for DHA and ARA in RM 8260 were approximated as 10 % relative to the measured value.

Table 6-1. Individualized data summary table for fatty acids in infant formulas.

Laboratory-specific results and Z-scores were provided to each participant separately from this report to protect laboratory identities.

(Lab Name)

Exercise 2 – Fatty Acids in Infant Formulas

Lab Code:		(Code)	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_{NIST}
DHA	SRM 1849b	% in FFAs	<i>Individual laboratory results will appear in this section; laboratory-specific results were provided to each participant separately from this report.</i>				7	0.052	0.011	0.056	0.001
DHA	RM 8260	% in FFAs					7	0.082	0.013	0.079	0.008
ARA	SRM 1849b	% in FFAs					7	0.159	0.021	0.163	0.001
ARA	RM 8260	% in FFAs					7	0.088	0.022	0.082	0.008
			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_{NIST}	standard uncertainty about the 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			

6.4. Study Results and Discussion

Table 6-1 summarizes and Table 6-2 and Table 6-3 detail the measured mass fraction results reported for DHA and ARA, respectively, by each participating laboratory. The participation level was fair for this study, with 42 % of laboratories requesting samples returning results (7 of 17 laboratories).

Table 6-2 reveals that four of the six non-zero within-laboratory variabilities reported for DHA were within the published expectations of the measurement community ($\leq 7\%$), with two laboratories reporting more than twice the expected level, as outlined in Table 6-4 [30]. For ARA, two of seven of the non-zero within-laboratory variabilities reported in Table 6-3 were above the published expectations of the measurement community ($\leq 7\%$). Three to four laboratories reported results for one or more sample with identical measured values, resulting in within-laboratory variabilities of zero. These laboratories should report more than one significant figure for each measured value, as appropriate to properly represent their measurement process. The among-laboratory variability for ARA in SRM 1849b (13 %) was acceptable with respect to the published expectations of the measurement community for multiple laboratories using the same method ($\leq 15\%$) [30]. The among-laboratory variabilities for ARA in RM 8260 (25 %) and for DHA in both materials (16 % to 21 %) were slightly higher than the published expectations, which is likely a result of the differences in methods used by participants.

As shown in Fig. 6-1, Fig. 6-2, Fig. 6-3, and Fig. 6-4, six laboratories reported using derivatization (86 %) and one laboratory reported using hydrolysis (14 %). For three of the four sample/analyte pairs, the laboratories using acid-catalyzed methylation reported values on average 8 % to 14 % below the consensus mean and the three laboratories using derivatization with base or an unspecified reagent reported values on average 9 % to 17 % above the consensus mean (Fig. 6-2, Fig. 6-3, and Fig. 6-4, outliers excluded). The significance of this trend is difficult to determine with this small data set but could be a potential focus for a future FNSQAP fatty acids study.

Table 6-2. Data summary table for DHA in SRM 1849b and RM 8260.

		Docosahexaenoic Acid (DHA)									
		SRM 1849b Infant/Adult Nutritional Formula I (milk-based) (% in FFAs)					RM 8260 Infant Formula (hydrolyzed milk-based) (% in FFAs)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target				0.056	0.001				0.079	0.008
	B002	0.05	0.05		0.050	0.000	0.09	0.09		0.090	0.000
	B004										
	B005	0.06	0.06	0.04	0.053	0.012	0.07	0.07	0.08	0.073	0.006
	B009	0.06	0.06	0.05	0.057	0.006	0.07	0.08	0.07	0.073	0.006
	B012										
	B015	0.043	0.059	0.058	0.053	0.009	0.093	0.093	0.093	0.093	0.000
	B019	0.045	0.042	0.041	0.043	0.002	0.07	0.064	0.063	0.066	0.004
	B021										
	B028	0.06	0.05	0.06	0.057	0.006	0.1	0.09	0.09	0.093	0.006
	B031										
	B040										
	B043										
	B044	0.05	0.05	0.05	0.050	0.000	0.09	0.09	0.08	0.087	0.006
	B046										
	B047										
	B051										
B053											
Community Results		Consensus Mean			0.052		Consensus Mean			0.082	
		Consensus Standard Deviation			0.011		Consensus Standard Deviation			0.013	
		Maximum			0.057		Maximum			0.093	
		Minimum			0.043		Minimum			0.066	
		<i>N</i>			7		<i>N</i>			7	

Table 6-3. Data summary table for ARA in SRM 1849b and RM 8260.

Data points highlighted in blue have been identified as outside the consensus tolerance limits and resulted in an unacceptable Z'_{comm} score, $|Z'_{\text{comm}}| \geq 2$.

		Arachidonic Acid (ARA)									
		SRM 1849b Infant/Adult Nutritional Formula I (milk-based) (% in FFAs)					RM 8260 Infant Formula (hydrolyzed milk-based) (% in FFAs)				
		Lab	A	B	C	Avg	SD	A	B	C	Avg
Individual Results	Target				0.163	0.001				0.082	0.008
	B002	0.16	0.16		0.160	0.000	0.2	0.2		0.200	0.000
	B004										
	B005	0.16	0.16	0.15	0.157	0.006	0.07	0.07	0.08	0.073	0.006
	B009	0.14	0.14	0.14	0.140	0.000	0.08	0.08	0.08	0.080	0.000
	B012										
	B015	0.147	0.178	0.179	0.168	0.018	0.095	0.096	0.096	0.096	0.001
	B019	0.147	0.142	0.144	0.144	0.003	0.081	0.077	0.086	0.081	0.005
	B021										
	B028	0.19	0.18	0.19	0.187	0.006	0.11	0.11	0.11	0.110	0.000
	B031										
	B040										
	B043										
	B044	0.16	0.16	0.16	0.160	0.000	0.09	0.09	0.09	0.090	0.000
	B046										
B047											
B051											
B053											
Community Results		Consensus Mean			0.159		Consensus Mean			0.088	
		Consensus Standard Deviation			0.021		Consensus Standard Deviation			0.022	
		Maximum			0.187		Maximum			0.200	
		Minimum			0.140		Minimum			0.073	
		<i>N</i>			7		<i>N</i>			7	

Table 6-4. Summary of expected method performance requirements for fatty acids in nutritional formulas [30].

Standard Method Performance Requirements[®] (SMPR) ranges are expressed as the corresponding mass fraction in a reconstituted final product (reconstitution rate 25 g powder into 200 g water).

Corresponding SMPR Range (g/100 g)	< 0.5
Expected Repeatability (RSD_r)	$\leq 7\%$
Expected Reproducibility (RSD_R)	$\leq 15\%$

Measurand: Docosahexaenoic acid (DHA)
 Sample: SRM 1849b Infant/Adult Nutritional Formula I (milk-based)
 Exercise: FNSQAP Exercise 2

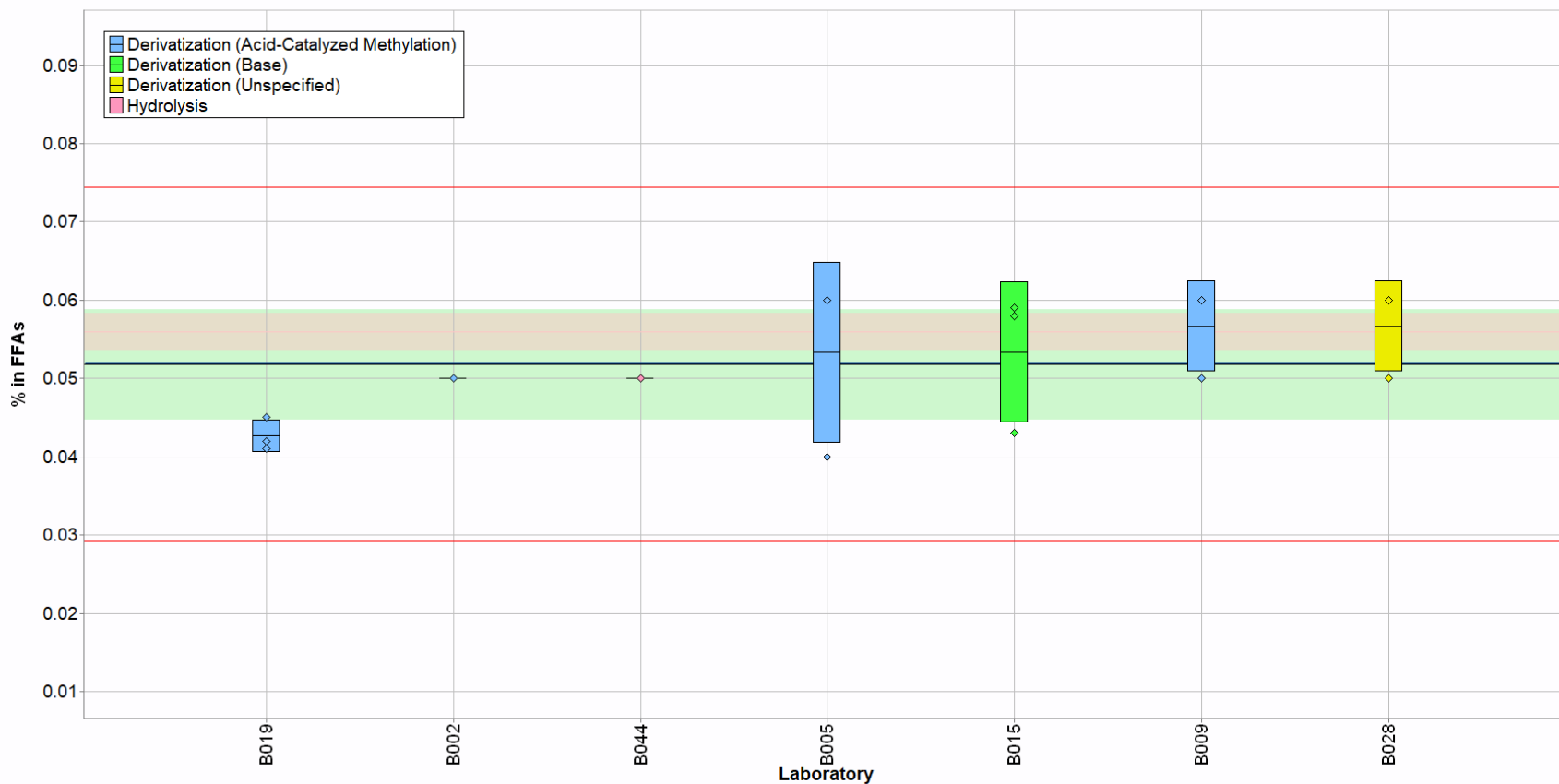


Fig. 6-1. DHA in SRM 1849b (data summary view – sample preparation method).

In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed as indicated in the figure key. The 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 beige shaded region represents the overlapping of the 95 % confidence interval for the consensus mean (green region) and the NIST target range (red region). The NIST target range encompasses the target value bounded by twice its uncertainty (U_{NIST}), and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$.

Measurand: Docosahexaenoic acid (DHA)
 Sample: RM 8260 Infant Nutritional Formula (hydrolyzed milk-based)
 Exercise: FNSQAP Exercise 2

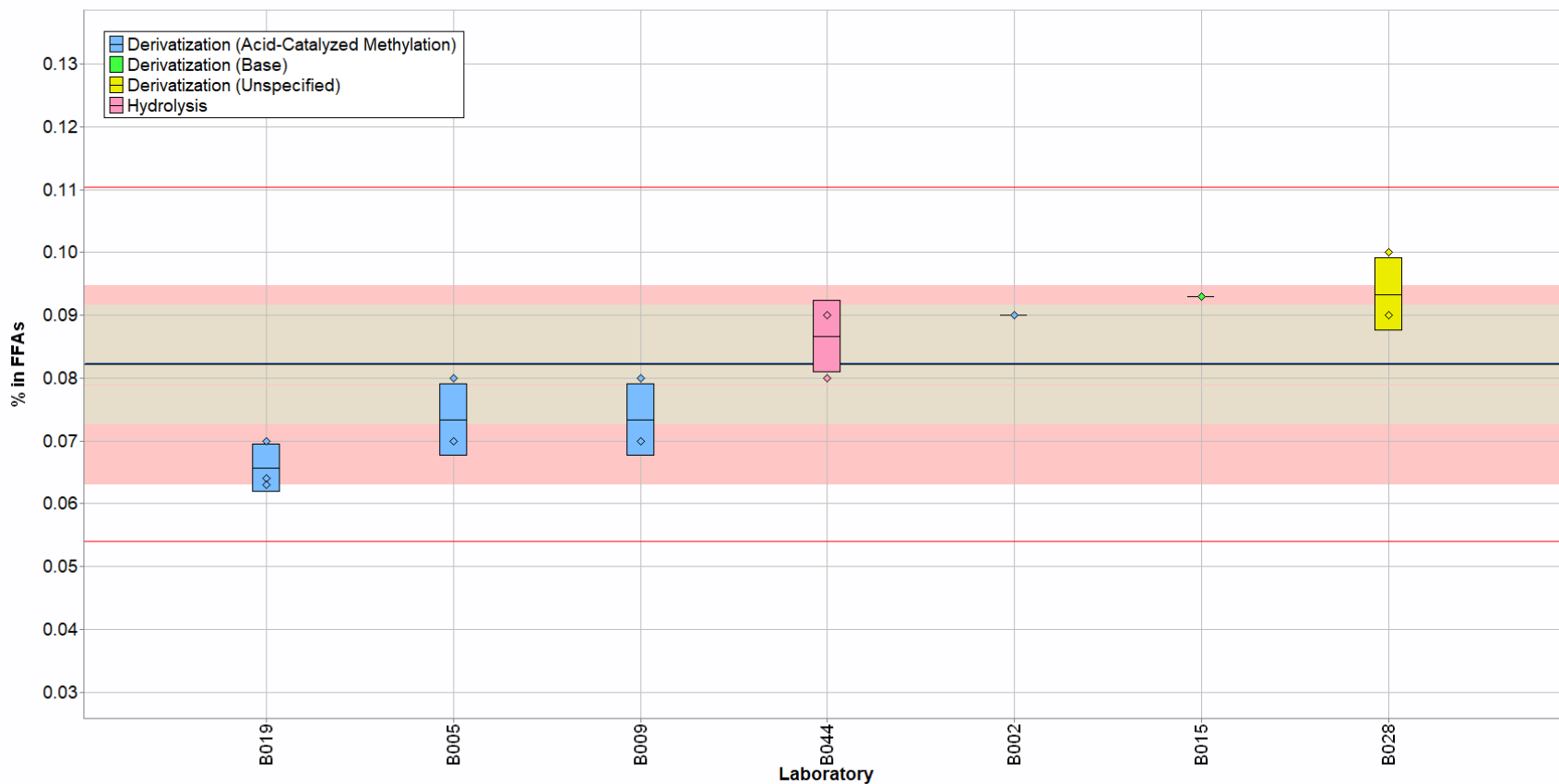


Fig. 6-2. DHA in RM 8260 (data summary view – sample preparation method).

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

Measurand: Arachidonic acid (ARA)
 Sample: SRM 1849b Infant/Adult Nutritional Formula I (milk-based)
 Exercise: FNSQAP Exercise 2

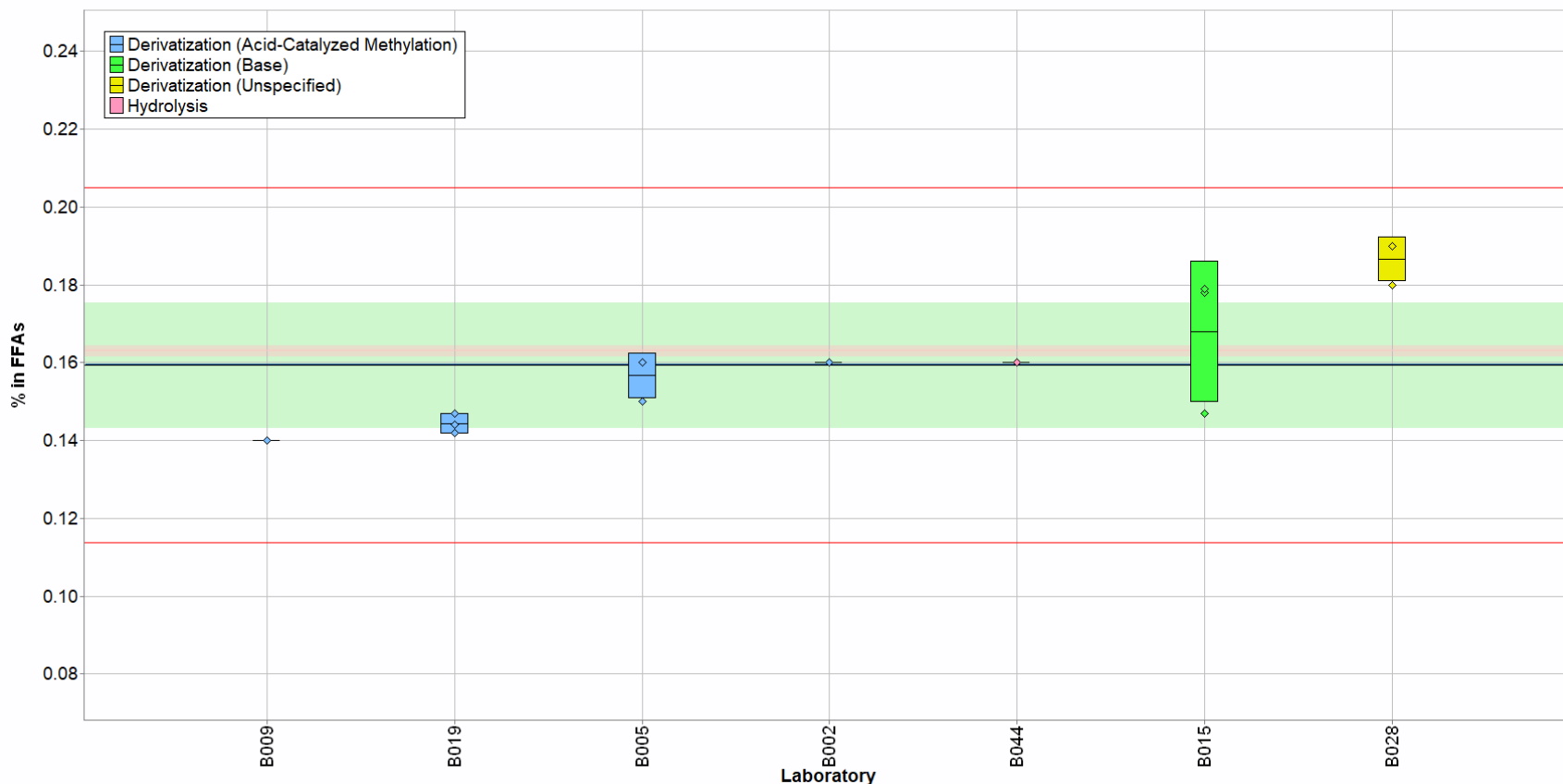


Fig. 6-3. ARA in SRM 1849b (data summary view – sample preparation method).

In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed as indicated in the figure key. The 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 beige shaded region represents the overlapping of the 95 % confidence interval for the consensus mean (green region) and the NIST target range (red region). The NIST target range encompasses the target value bounded by twice its uncertainty (U_{NIST}), and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$.

Measurand: Arachidonic acid (ARA)
 Sample: RM 8260 Infant Nutritional Formula (hydrolyzed milk-based)
 Exercise: FNSQAP Exercise 2

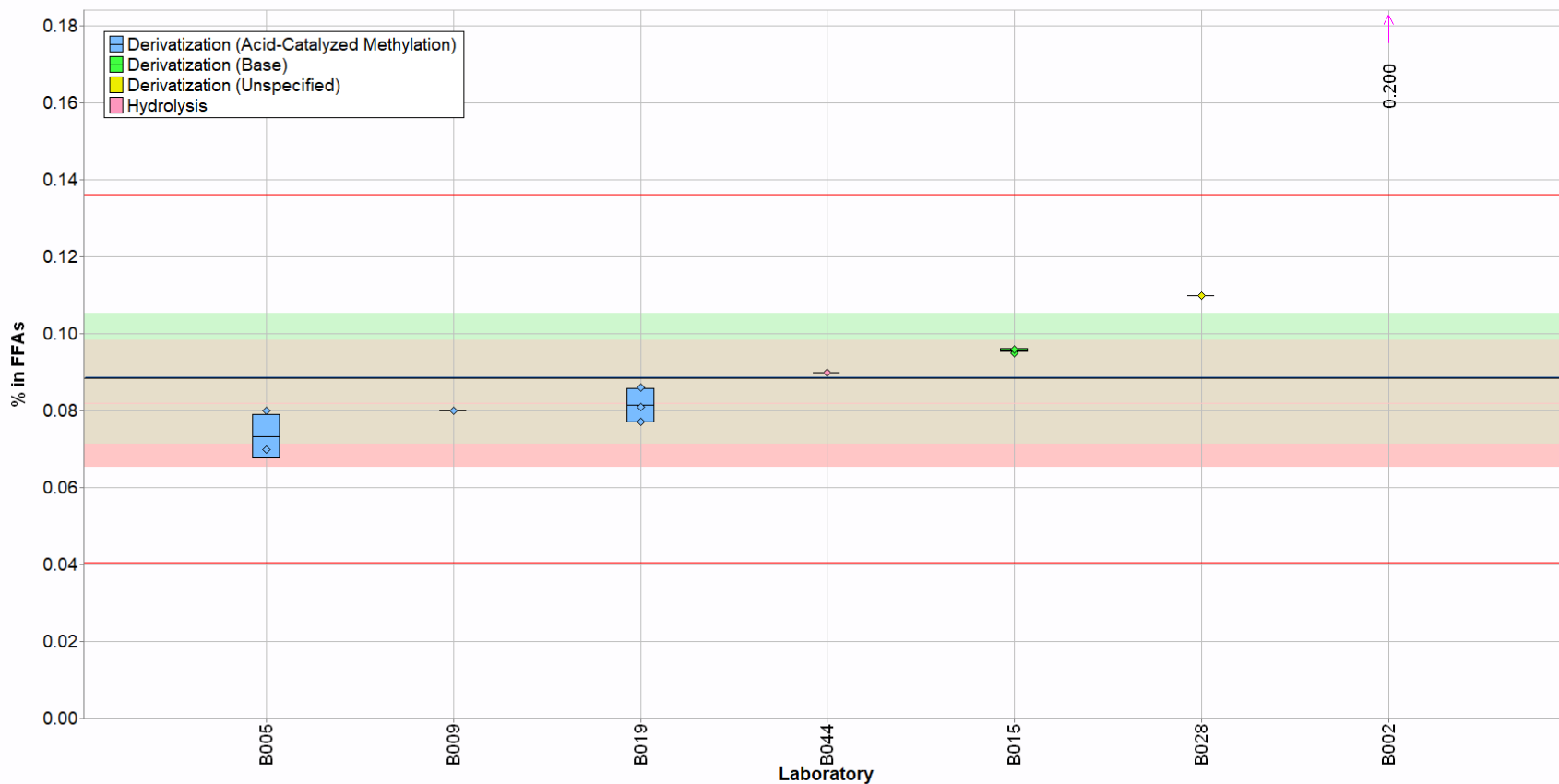


Fig. 6-4. ARA in RM 8260 (data summary view – sample preparation method).

In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed as indicated in the figure key. Data points outside the graphical range are represented as upward arrows (the sample preparation approach reported by laboratory B002 was acid-catalyzed methylation). The solid blue line represents the consensus mean, and the solid red lines represent the consensus range of 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 target range, which encompasses the target value bounded by twice its uncertainty (U_{NIST}), and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$. The beige shaded region represents the overlapping of the 95 % confidence interval for the consensus mean (green region) and the NIST target range (red region).

Laboratories reported using gas chromatography methods for determination of DHA and ARA in the nutritional formula samples, with either flame ionization detection (GC-FID, 71 %) or mass spectrometry detection (GC-MS, 29 %), as shown in Fig. 6-5, Fig. 6-6, Fig. 6-7, and Fig. 6-8. For three of the four sample/analyte pairs, the two laboratories using GC-MS reported values on average 12 % to 17 % above the consensus mean. The significance of this trend is difficult to determine with this small data set but could be a potential focus for a future FNSQAP fatty acids study.

The consensus mean measured mass fraction for both DHA and ARA were within the target ranges for RM 8260 (Fig. 6-2, Fig. 6-4, Fig. 6-6, and Fig. 6-8). In SRM 1849b, the consensus mean measured mass fractions for both DHA and ARA were slightly below the target ranges, but the entire target ranges were contained within the consensus ranges (Fig. 6-1, Fig. 6-3, Fig. 6-5, and Fig. 6-7).

NIST has conducted twelve QAP studies involving measurement of fatty acids in food samples prior to this FNSQAP study. Of these, nine have included measurement of DHA, ARA, or both.

Studies including measurement of DHA include DSQAP Exercise F in 2010 [21], Exercise J in 2013 [31], and Exercise L in 2015 [24] and HAMQAP Exercise 1 in 2017 [32], Exercise 2 in 2018 [33], Exercise 4 in 2019 [34], Exercise 5 in 2019 [35], and Exercise 6 in 2020 [36]. A review of these exercises indicated that the level of enrollment in this study (17 laboratories) was lower than past exercises (average of 26 participants per study). The participation rate was also slightly lower in this study than past exercises (41 % compared to 50 % for past exercises). The sample types offered in this study (nutritional formulas) may have been of interest to fewer potential participants than historical samples (omega-3 and -6 fatty acid-rich foods and supplements). The repeatability reported by participants in this study is consistent with average repeatability from previous studies. The among-laboratory variabilities and bias (Fig. 6-9) for the samples in this study were lower than for most previous studies involving samples requiring fatty acid extraction, which may indicate the ease with which fatty acids can be isolated from infant formula matrices compared to non-fortified foods such as fish.

ARA was measured in studies including DSQAP Exercise F in 2010 [21], Exercise H in 2012 [18], Exercise J in 2013 [31], and Exercise L in 2015 [24] and HAMQAP Exercise 1 in 2017 [32], Exercise 2 in 2018 [33], Exercise 5 in 2019 [35], and Exercise 6 in 2020 [36]. A review of these exercises indicated that the level of enrollment in this study (17 laboratories) was lower than past exercises (average of 28 participants per study). The participation rate in this study was consistent with past exercises (41 %). The sample types offered in this study (nutritional formulas) may have been of interest to fewer potential participants than historical samples (omega-3 and -6 fatty acid-rich foods and supplements). The repeatability reported by participants in this study is consistent with average repeatability from previous studies, on average less than 10 %. The among-laboratory variabilities and bias (Fig. 6-10) for the samples in this study were lower than for most previous studies involving samples requiring fatty acid extraction, which may indicate the ease with which fatty acids can be isolated from infant formula matrices compared to non-fortified foods such as fish, as also observed for DHA.

Measurand: Docosahexaenoic acid (DHA)
Sample: SRM 1849b Infant/Adult Nutritional Formula I (milk-based)
Exercise: FNSQAP Exercise 2

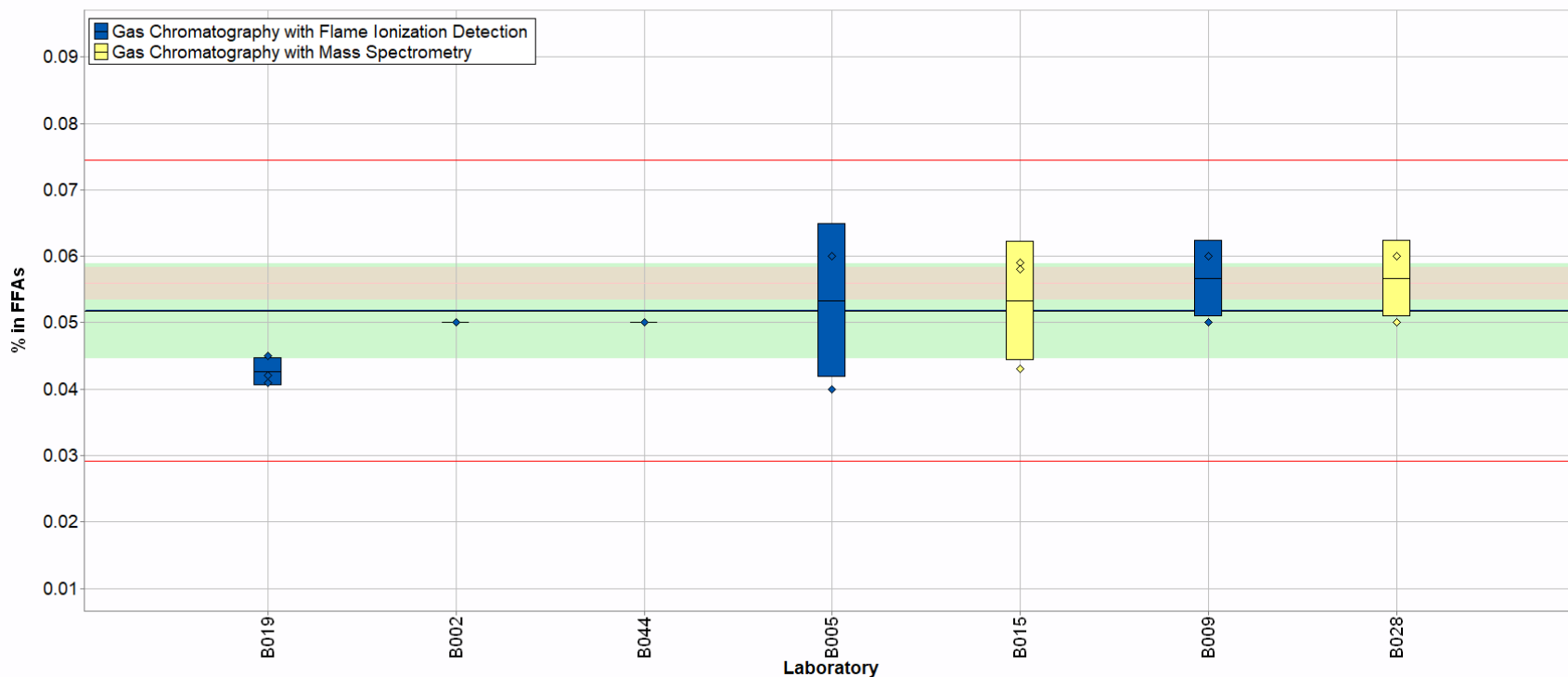


Fig. 6-5. DHA in SRM 1849b (data summary view – analytical method).

In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed as indicated in the figure key. The 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 beige shaded region represents the overlapping of the 95 % confidence interval for the consensus mean (green region) and the NIST target range (red region). The NIST target range encompasses the target value bounded by twice its uncertainty (U_{NIST}), and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$.

Measurand: Docosahexaenoic acid (DHA)
 Sample: RM 8260 Infant Nutritional Formula (hydrolyzed milk-based)
 Exercise: FNSQAP Exercise 2

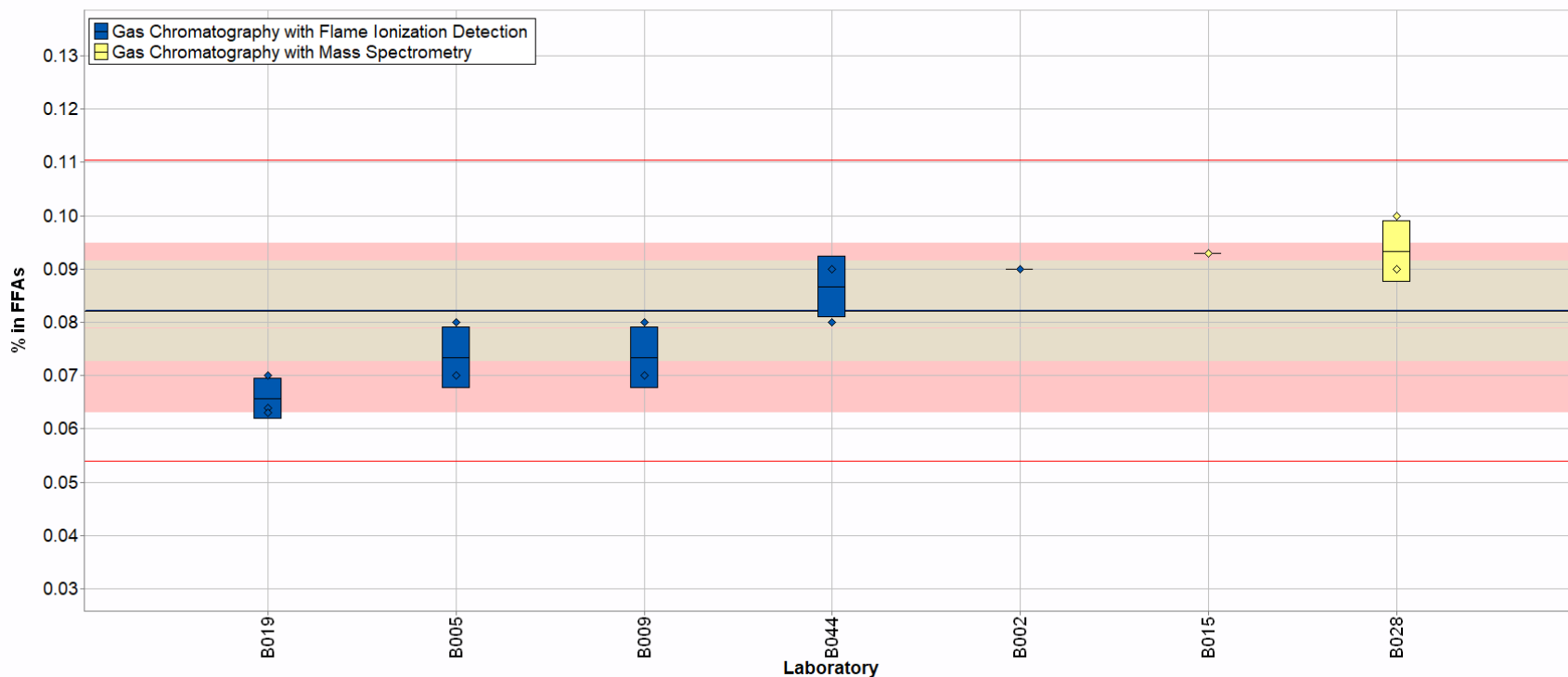


Fig. 6-6. DHA in RM 8260 (data summary view – analytical method).

In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed as indicated in the figure key. The solid blue line represents the consensus mean, and the solid red lines represent the consensus range of 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 target range, which encompasses the target value bounded by twice its uncertainty (U_{NIST}) and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$. The beige shaded region represents the overlapping of the 95 % confidence interval for the consensus mean (green region) and the NIST target range (red region).

Measurand: Arachidonic acid (ARA)
Sample: SRM 1849b Infant/Adult Nutritional Formula I (milk-based)
Exercise: FNSQAP Exercise 2

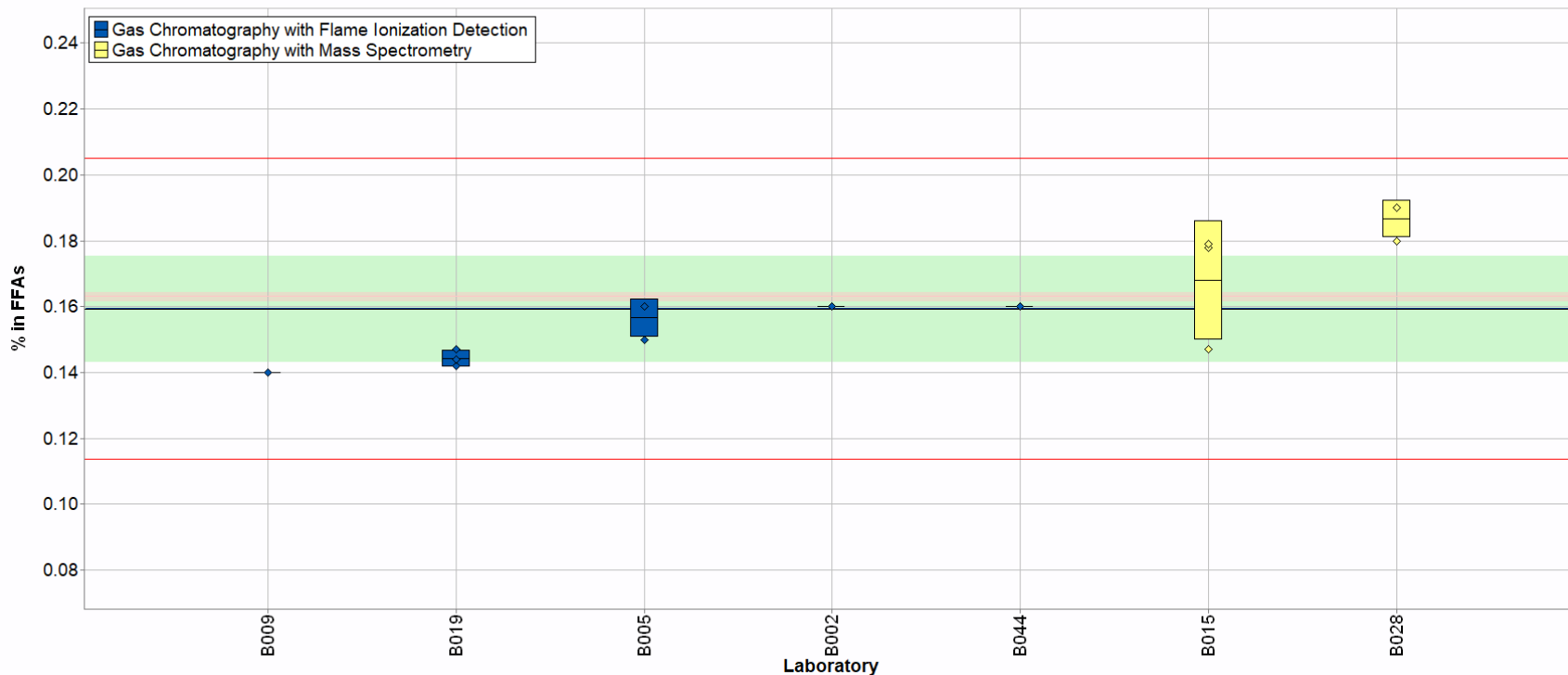


Fig. 6-7. ARA in SRM 1849b (data summary view – analytical method).

In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed as indicated in the figure key. The 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 beige shaded region represents the overlapping of the 95 % confidence interval for the consensus mean (green region) and the NIST target range (red region). The NIST target range encompasses the target value bounded by twice its uncertainty (U_{NIST}), and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$.

Measurand: Arachidonic acid (ARA)
 Sample: RM 8260 Infant Nutritional Formula (hydrolyzed milk-based)
 Exercise: FNSQAP Exercise 2

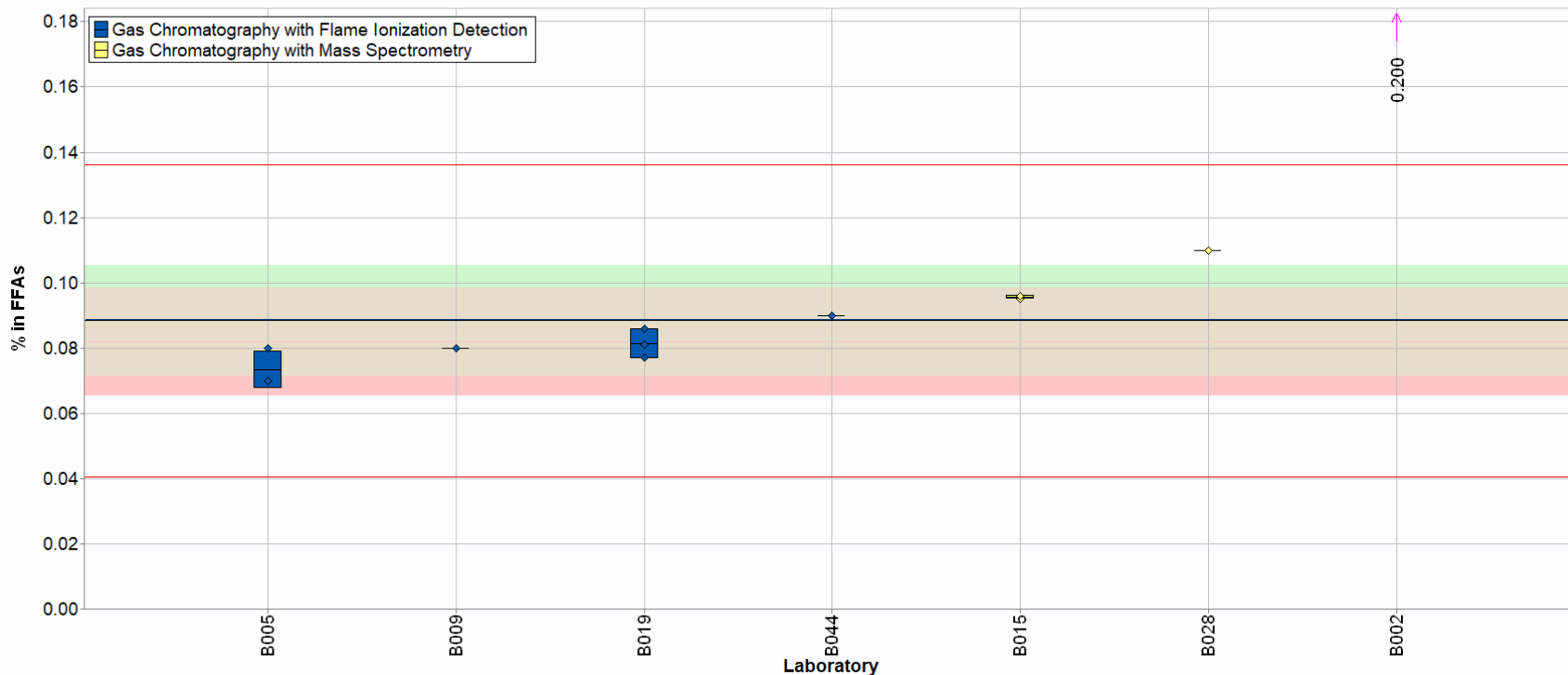


Fig. 6-8. ARA in RM 8260 (data summary view – analytical method).

In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed as indicated in the figure key. Data points outside the graphical range are represented as upward arrows (the analytical method reported by laboratory B002 was GC-FID). The solid blue line represents the consensus mean, and the solid red lines represent the consensus range of 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 target range, which encompasses the target value bounded by twice its uncertainty (U_{NIST}) and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$. The beige shaded region represents the overlapping of the 95 % confidence interval for the consensus mean (green region) and the NIST target range (red region).

Exercise: FNSQAP Exercise 2, Measurand: Docosahexaenoic acid (DHA)
No. of laboratories: 7, Correlation coefficient: 0.4

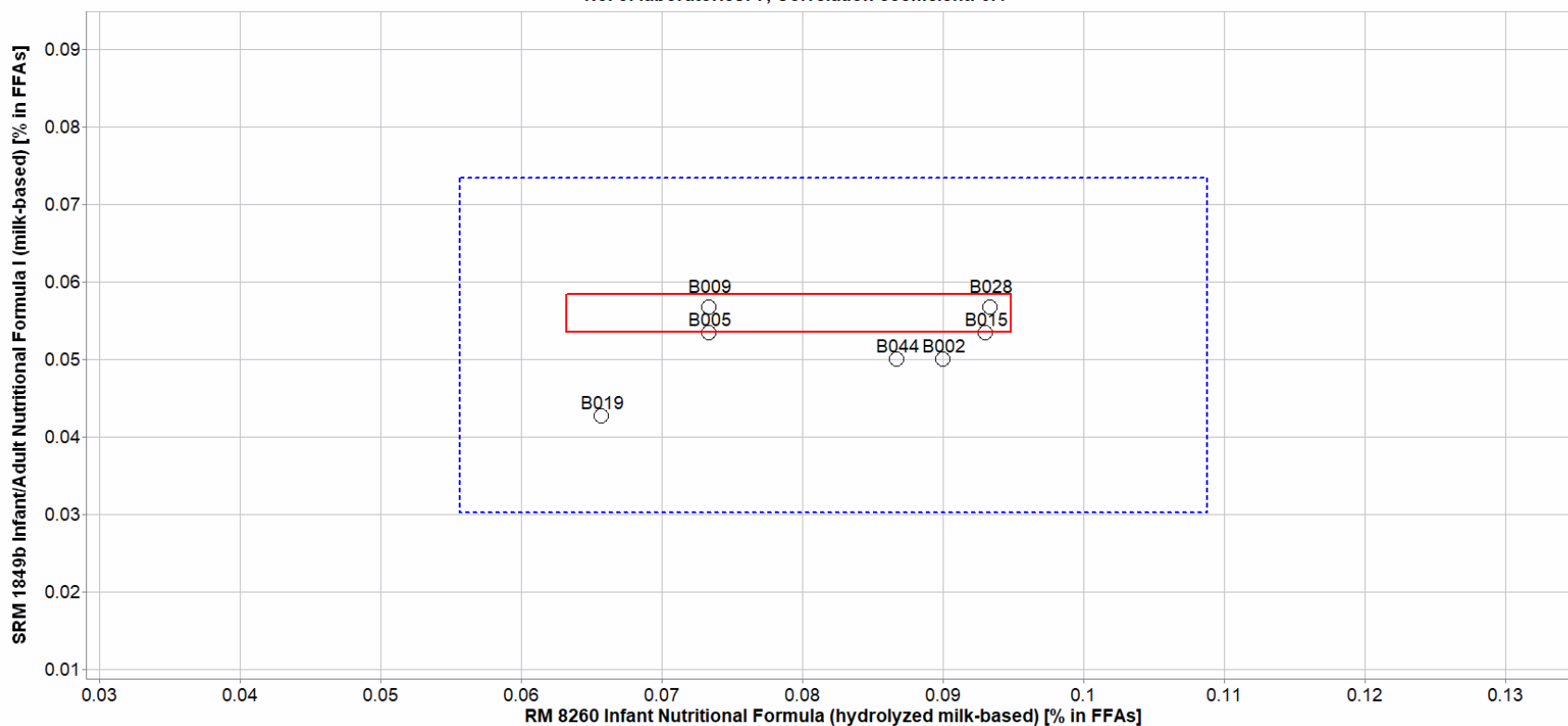


Fig. 6-9. Laboratory means for DHA in RM 8260 and SRM 1849b (sample/sample comparison view).

In this view, the individual laboratory mean for one sample (RM 8260) is compared to the individual laboratory mean for a second sample (SRM 1849b). The solid red box represents the NIST target range for the two samples, RM 8260 (x-axis) and SRM 1849b (y-axis), which encompasses the target values bounded by their uncertainties (U_{NIST}), and represents the range that results in an acceptable Z_{NIST} score, $|Z_{NIST}| \leq 2$. The dotted blue box represents the consensus range of tolerance for RM 8260 (x-axis) and SRM 1849b (y-axis), calculated as the values above and below the consensus means that result in an acceptable Z'_{comm} score, $|Z'_{comm}| \leq 2$.

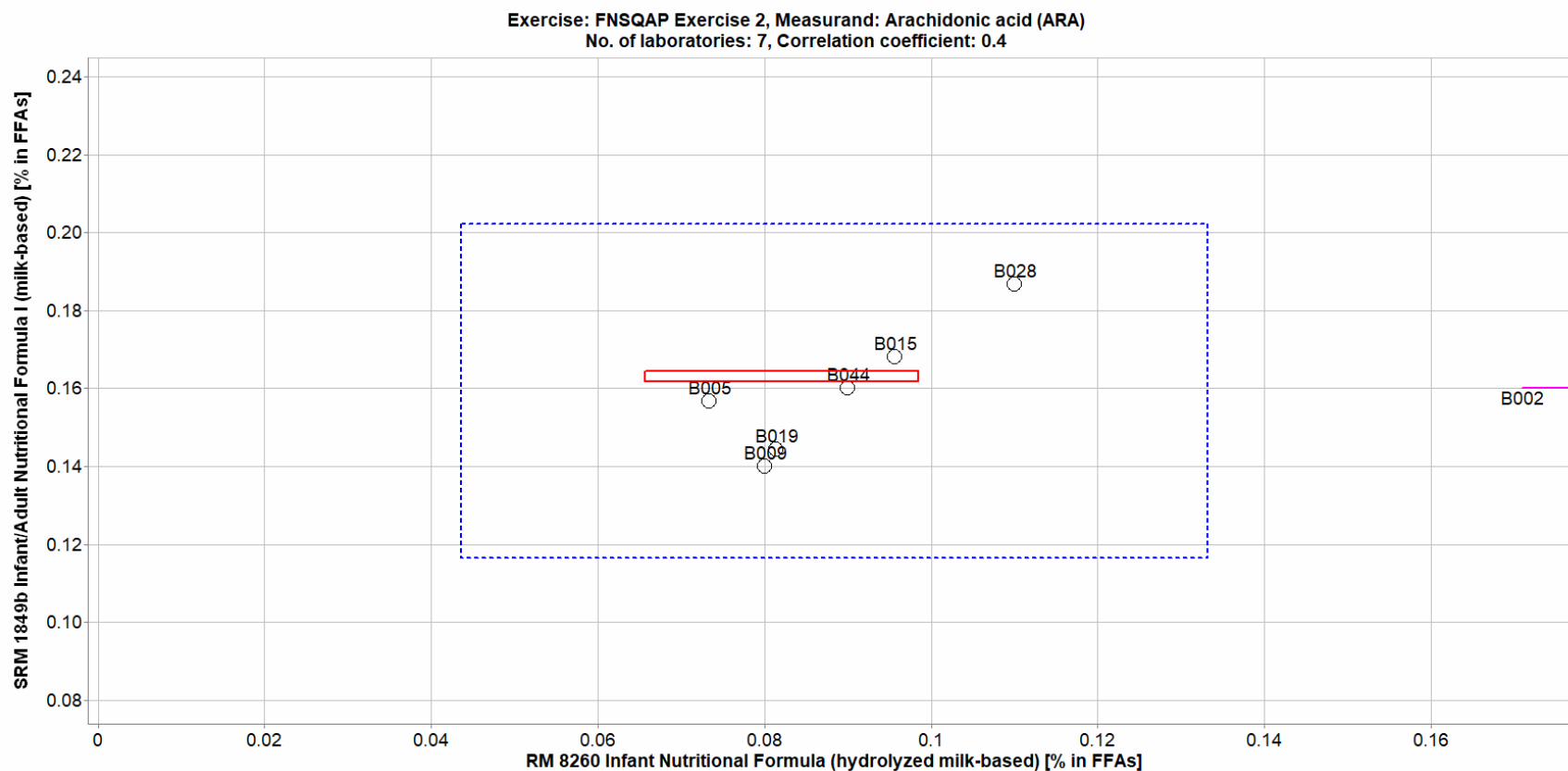


Fig. 6-10. Laboratory means for ARA in RM 8260 and SRM 1849b (sample/sample comparison view).

In this view, the individual laboratory mean for one sample (RM 8260) is compared to the individual laboratory mean for a second sample (SRM 1849b). The solid red box represents the NIST target range for the two samples, RM 8260 (x-axis) and SRM 1849b (y-axis), which encompasses the target values bounded by their uncertainties (U_{NIST}), and represents the range that results in an acceptable Z_{NIST} score, $|Z_{NIST}| \leq 2$. The dotted blue box represents the consensus range of tolerance for RM 8260 (x-axis) and SRM 1849b (y-axis), calculated as the values above and below the consensus means that result in an acceptable Z'_{comm} score, $|Z'_{comm}| \leq 2$.

Overall, the performance of participating laboratories in the determination of DHA and ARA in infant formula samples was consistent with or improved upon that observed in past studies. The small number of laboratories reporting data (7) limits the meaningfulness of any observed trends in Fig. 6-9 and Fig. 6-10. In any laboratory exercise, consistent 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 like NIST's SRMs or non-certified reference materials such as NIST's RMs) or materials prepared in-house.

7. CONTAMINANTS (Glyphosate, AMPA, N-Acetyl-Glyphosate, N-Acetyl-AMPA)

7.1. Executive Summary

To protect public health, regulators must understand human and animal dietary exposure to potentially harmful contaminants such as glyphosate, a widely used herbicide, through accurate determination of glyphosate levels in consumer products. The results of this study revealed that participating laboratories are using methods that are repeatable for determination of glyphosate and aminomethylphosphonic acid (AMPA) in the food products presented, but that further harmonization of methods across laboratories is needed.

7.2. Study Overview

Glyphosate is a widely applied broad-spectrum herbicide used to control broadleaf weeds and grasses [37]. Worldwide experts have not reached a consensus on the human toxicity of glyphosate [37, 38] and monitoring of human exposure is a critical component of understanding population health impacts. For this monitoring to be effective, methods for the detection of glyphosate mass fraction in agricultural and consumer products must be well characterized and have demonstrated accuracy. In this study, participants were provided with samples of SRM 3290 Dry Cat Food and SRM 3299 Ground Turmeric (*Curcuma longa* L.) Rhizome. Participants were asked to use in-house analytical methods to determine the mass fraction (ng/g) of glyphosate and its major metabolites aminomethylphosphonic acid (AMPA), N-acetyl-glyphosate, and N-acetyl-AMPA in each matrix. Through participation in this study, laboratories can better understand the performance of their in-house methods relative to those being used by others in the community and the related limitations of any data generated using those methods. Participant results may be used in the value assignment of NIST reference materials included as samples in this study.

7.3. Sample Information

Participants were provided three packets each of SRM 3290 Dry Cat Food (labeled Cat Food) and SRM 3299 Ground Turmeric (*Curcuma longa* L.) Rhizome (labeled Turmeric). Each packet contained approximately 10 g of material; participants were asked to store the materials 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. Participants were instructed to thoroughly mix the contents of each packet before use and to use a sample size appropriate for their in-house method of analysis. The approximate analyte levels were not reported to participants prior to the study. Target values and uncertainties for glyphosate in each material were determined using mean results and standard deviations from a collaborating laboratory; the target values and uncertainties are provided in Table 7-1 on an as-received basis. Target values for AMPA, N-acetyl-glyphosate, and N-acetyl-AMPA in both materials were not available at the time of this report.

Table 7-1. Individualized data summary table for glyphosate and its metabolites in foods.

Laboratory-specific results and Z-scores were provided to each participant separately from this report to protect laboratory identities.

(Lab Name)

Exercise 2 – Glyphosate in Foods

Lab Code: (Code)		1. Your Results				2. Community Results			3. Target		
Sample	Units	x_i	s_i	Z'_{comm}	Z_{NIST}	N	x^*	s^*	x_{NIST}	u_{NIST}	
AMPA	SRM 3290	ng/g	<i>Individual laboratory results will appear in this section; laboratory-specific results were provided to each participant separately from this report.</i>				5	24	21		
AMPA	SRM 3299	ng/g					1				
Glyphosate	SRM 3290	ng/g					7	65	80	61	27
Glyphosate	SRM 3299	ng/g					5	11.8	9.1	10.3	3.1
N-acetyl-Glyphosate	SRM 3290	ng/g					1				
N-acetyl-Glyphosate	SRM 3299	ng/g					1				
N-acetyl-AMPA	SRM 3290	ng/g					0				
N-acetyl-AMPA	SRM 3299	ng/g					1				
			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_{NIST}	standard uncertainty about the 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					

7.4. Study Results and Discussion

Table 7-1 summarizes and Table 7-2 details the measured mass fraction results for glyphosate reported by each participating laboratory. The participation level was moderate for glyphosate, with 54 % of laboratories requesting samples returning results (7 of 13 laboratories).

Table 7-2 reveals that within-laboratory variabilities were acceptable with respect to published expectations of the glyphosate measurement community ($\leq 20\%$) [39]. The among-laboratory variabilities, however, were extremely high (77 % and 122 %) with respect to the published expectations for multiple laboratories using the same method ($\leq 25\%$) [39], even when considering the variety of methods used by participants in this study. The level of glyphosate in SRM 3299 (10.3 ng/g) was close to the published LOQ requirement of 0.01 mg/kg, which may have challenged some methods used by participants.

Table 7-2. Data summary table for glyphosate in SRM 3290 and SRM 3299.

Data highlighted in blue have been identified as outside the consensus tolerance limits and resulted in an unacceptable Z'_{comm} score, $|Z'_{comm}| > 2$.

		Glyphosate									
		SRM 3290 Dry Cat Food (ng/g)					SRM 3299 Ground Turmeric (Curcuma longa L.) Rhizome (ng/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target				61	27				10.3	3.1
	B004	750.29	711.34	730.81	730.8	19.5	804.58	1035.71	920.15	920.1	115.6
	B005										
	B006	45.6	37.8	39.3	40.9	4.1	6.38	6.2	6.41	6.3	0.1
	B011										
	B012										
	B019	77	83	89	83.0	6.0	< 10.000	< 10.000	< 10.000		
	B033	75.4	82.1	70.7	76.1	5.7	10.5	10.1	11.5	10.7	0.7
	B038										
	B041	0.071	0.07	0.058	0.1	0.0	< 0.050	< 0.050	< 0.050		
	B042	118	112	115	115.0	3.0	10	10	10	10.0	0.0
	B047										
B051											
B055	75	80	76	77.0	2.6	21	20	20	20.3	0.6	
Community Results		Consensus Mean				65	Consensus Mean				11.8
		Consensus Standard Deviation				80	Consensus Standard Deviation				9.1
		Maximum				731	Maximum				920.1
		Minimum				0.1	Minimum				6.3
		N				7	N				5

As shown in Fig. 7-1 and Fig. 7-2, laboratories reported using a variety of sample preparation methods for the determination of glyphosate in the two samples. Some laboratories reported using a single-step preparation approach, while other laboratories reported using a multi-step approach. Three laboratories reported using solvent extraction with solid phase extraction (SPE) and derivatization (43 %), and one laboratory each reported use of “Quick Polar Pesticides” extraction (QuPPE), solvent extraction, and solvent extraction with solid phase extraction (14 % each). One laboratory did not report the sample preparation method used. Overall, the most accurate results were obtained using solvent extraction with an SPE clean up step, with or without derivatization.

Similarly, Fig. 7-3 and Fig. 7-4 indicate that most laboratories reported using LC-MS-based techniques for the determination of glyphosate in the two food samples. Four laboratories reported use of LC-MS/MS (57 %), one laboratory reported use of LC-MS (14 %), and one laboratory reported use of Liquid Chromatography with High Resolution Mass Spectrometry (LC-HRMS) (14 %). One laboratory reported use of GC-MS/MS (14 %).

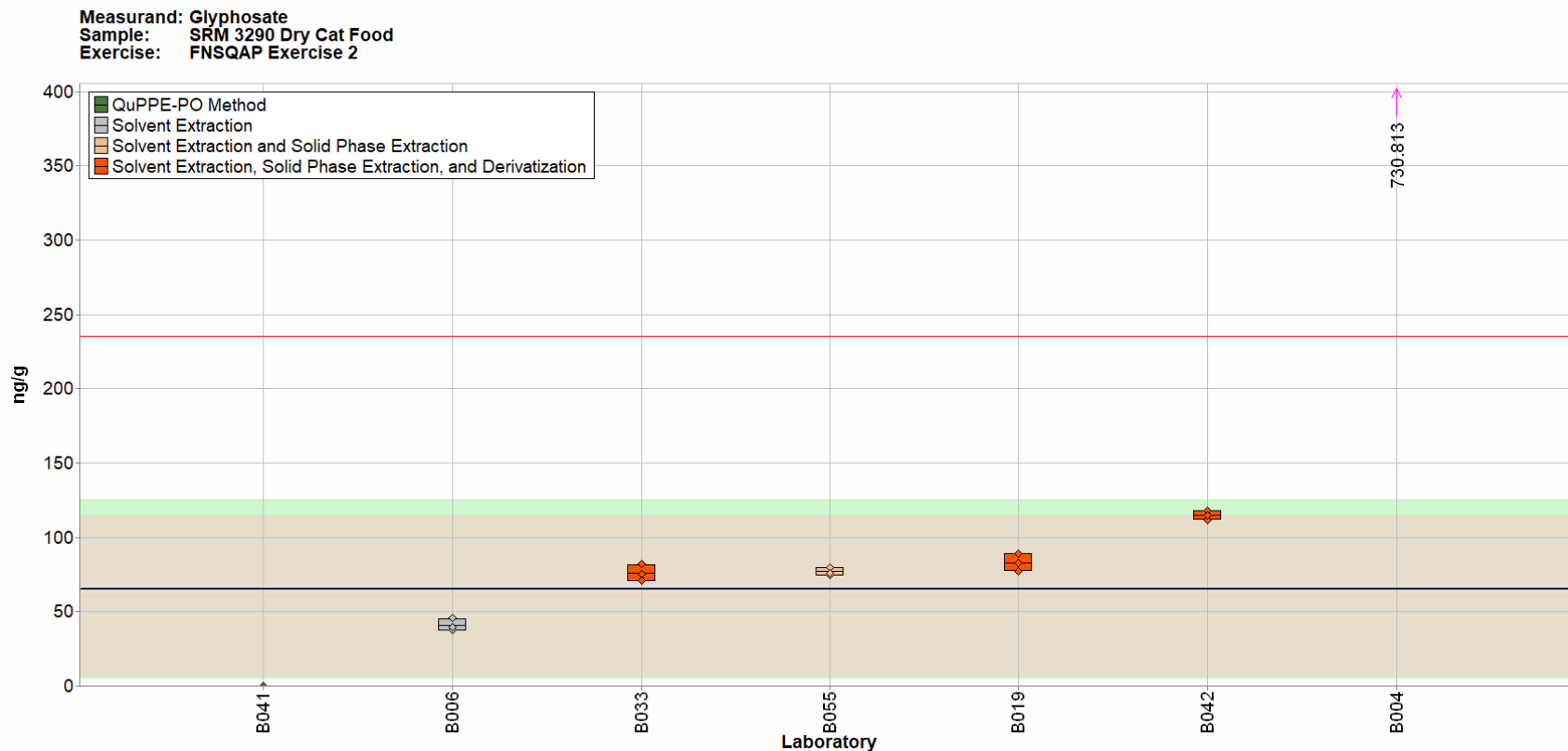


Fig. 7-1. Glyphosate in SRM 3290 (data summary view – sample preparation method).

In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed as indicated in the figure key. Data points outside the graphical range are represented as upward arrows (laboratory B004 did not specify the sample preparation approach used). The 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 bound of the consensus range of tolerance, calculated as the value above the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$, with the bottom bound set to zero. The beige shaded region represents the overlapping of the 95 % confidence interval for the consensus mean (green region) and the NIST target range (red region). The NIST target range encompasses the target value bounded by twice its uncertainty (U_{NIST}), and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$.

Measurand: Glyphosate
 Sample: SRM 3299 Ground Turmeric (*Curcuma longa* L.) Rhizome
 Exercise: FNSQAP Exercise 2

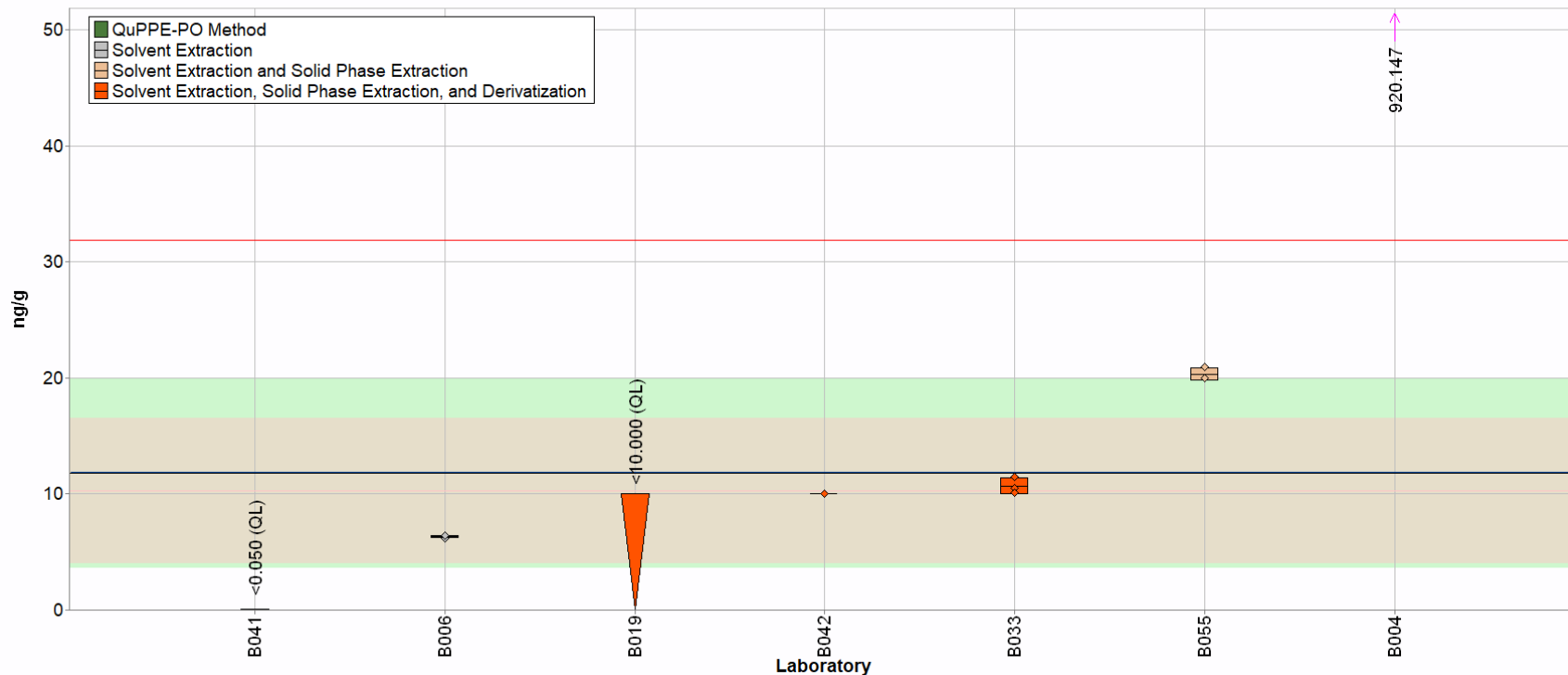


Fig. 7-2. Glyphosate in SRM 3299 (data summary view – sample preparation method).

In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). A downward triangle represents data reported as an LOQ value. The color of the data point represents the sample preparation method employed as indicated in the figure key. Data points outside the graphical range are represented as upward arrows (laboratory B004 did not specify the sample preparation approach used). The 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 bound of the consensus range of tolerance, calculated as the value above the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$, with the bottom bound set to zero. The beige shaded region represents the overlapping of the 95 % confidence interval for the consensus mean (green region) and the NIST target range (red region). The NIST target range encompasses the target value bounded by twice its uncertainty (U_{NIST}), and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$.

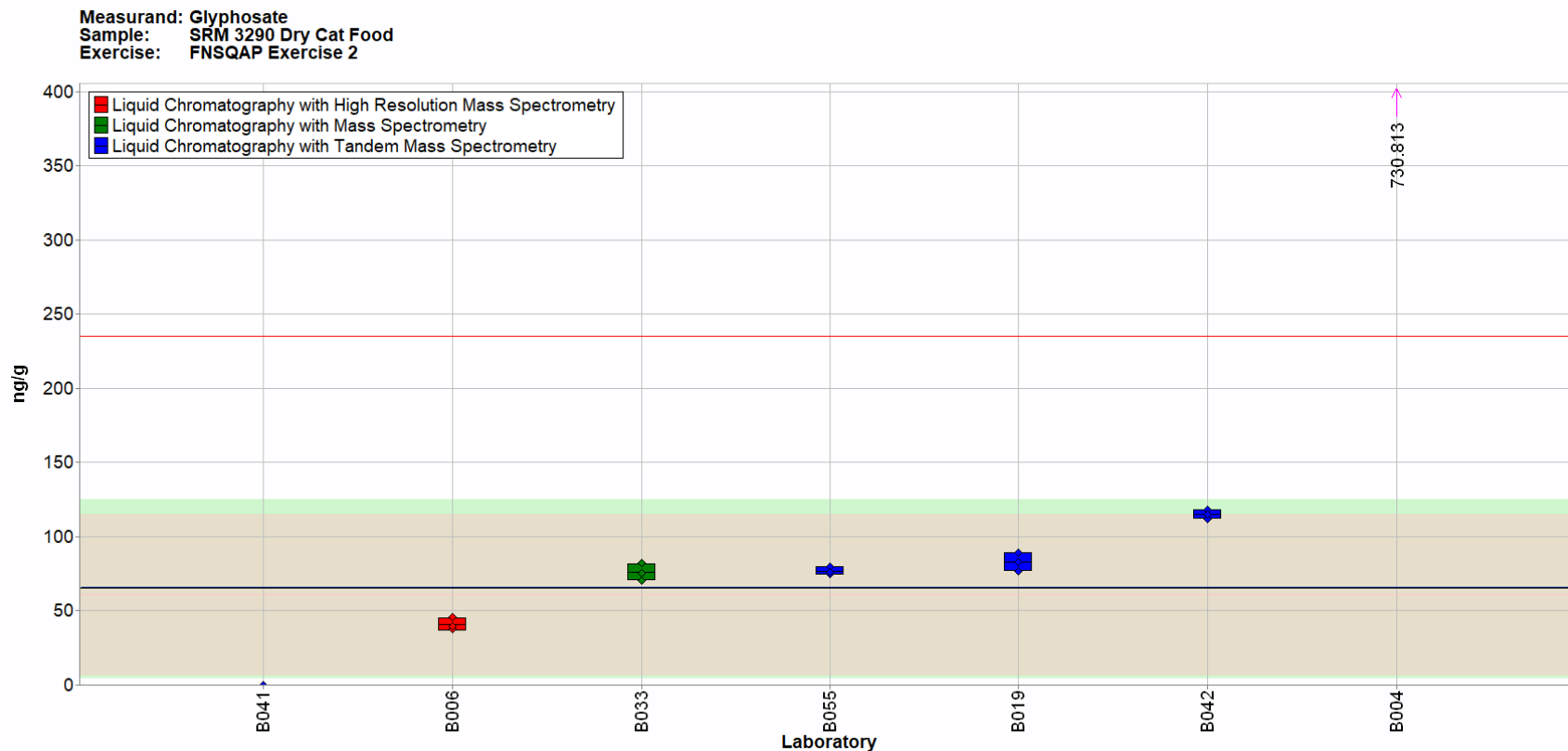


Fig. 7-3. Glyphosate in SRM 3290 (data summary view – analytical method).

In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed as indicated in the figure key. Data points outside the graphical range are represented as upward arrows (the analytical approach reported by laboratory B004 was GC-MS/MS). The 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 bound of the consensus range of tolerance, calculated as the value above the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$, with the bottom bound set to zero. The beige shaded region represents the overlapping of the 95 % confidence interval for the consensus mean (green region) and the NIST target range (red region). The NIST target range encompasses the target value bounded by twice its uncertainty (U_{NIST}), and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$.

Measurand: Glyphosate
 Sample: SRM 3299 Ground Turmeric (*Curcuma longa* L.) Rhizome
 Exercise: FNSQAP Exercise 2

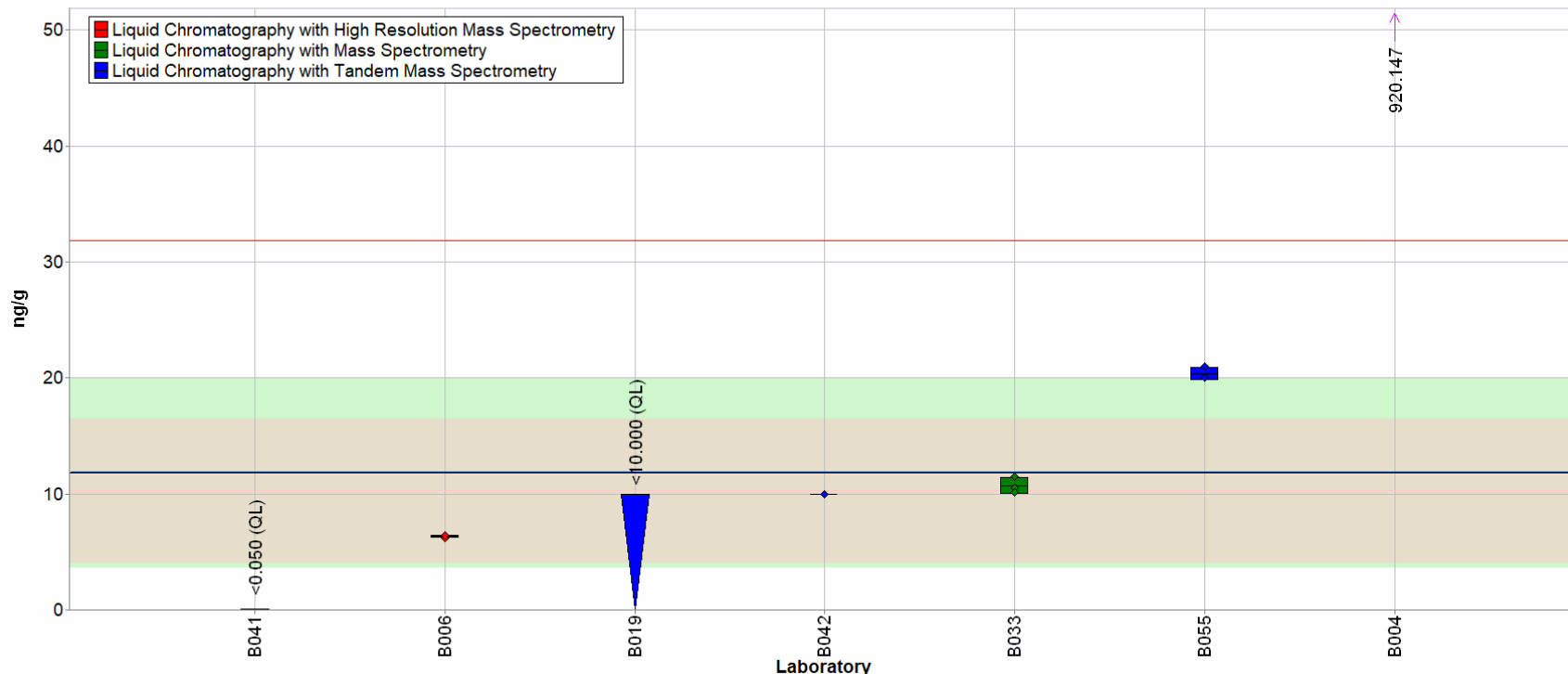


Fig. 7-4. Glyphosate in SRM 3299 (data summary view – analytical method).

In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). A downward triangle represents data reported as an LOQ value. The color of the data point represents the analytical method employed as indicated in the figure key. Data points outside the graphical range are represented as upward arrows (the analytical approach reported by laboratory B004 was GC-MS/MS). The 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 bound of the consensus range of tolerance, calculated as the value above the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{comm}| \leq 2$, with the bottom bound set to zero. The beige shaded region represents the overlapping of the 95 % confidence interval for the consensus mean (green region) and the NIST target range (red region). The NIST target range encompasses the target value bounded by twice its uncertainty (U_{NIST}), and represents the range that results in an acceptable Z_{NIST} score, $|Z_{NIST}| \leq 2$.

The glyphosate results reported by two laboratories (B004 and B041) differ from the consensus results by orders of magnitude, likely due to a calculation error. Excluding these outliers, the results obtained by a single laboratory using solvent extraction with LC-HRMS are lower than the consensus mean in both materials. While notable, additional data points are needed to determine if the trend is laboratory specific or if it is related to the sample preparation approach or analytical method. For both materials, the consensus ranges for glyphosate overlap the target ranges (Fig. 7-1, Fig. 7-2, Fig. 7-3, and Fig. 7-4).

Table 7-1 summarizes and Table 7-3 details the numerical results for AMPA reported by each participating laboratory. The participation level was slightly lower for AMPA at 50 % (6 of 12 laboratories) compared to glyphosate (54 %). Only one quantitative value was reported for AMPA in SRM 3299, thus only the reported results for AMPA in SRM 3290 will be discussed further. Table 7-3 also reveals that within-laboratory variabilities for four of five laboratories reporting quantitative results for AMPA were acceptable with respect to published expectations of this measurement community ($\leq 20\%$) [39]. The among-laboratory variability was high (88 %) with respect to the published expectations of this measurement community for multiple laboratories using the same method ($\leq 25\%$) [39].

Table 7-3. Data summary table for AMPA in SRM 3290 and SRM 3299.

		Aminomethylphosphonic Acid (AMPA)									
		SRM 3290 Dry Cat Food (ng/g)					SRM 3299 Ground Turmeric (Curcuma longa L.) Rhizome (ng/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target										
	B005										
	B006	7.49	5.09	4.1	5.6	1.7	< 4.000	< 4.000	< 4.000		
	B011										
	B012										
	B019	27	19	24	23.3	4.0	< 10.000	< 10.000	< 10.000		
	B033	21.5	20.8	19.8	20.7	0.9	< 75.000	< 75.000	< 75.000		
	B038										
	B041	< 0.050	< 0.050	< 0.050			< 0.050	< 0.050	< 0.050		
	B042	37	37	35	36.3	1.2	< 10.000	< 10.000	< 10.000		
	B047										
B051											
B055	35	35	34	34.7	0.6	16	16	16	16.0	0.0	
Community Results	Consensus Mean					24	Consensus Mean				
	Consensus Standard Deviation					21	Consensus Standard Deviation				
	Maximum					36	Maximum				
	Minimum					6	Minimum				
	N					5	N				
							1				

Fig. 7-5 depicts graphically the variety of sample preparation methods reported for the determination of AMPA in SRM 3290. As seen with glyphosate methods, some laboratories reported using a single-step preparation approach, while other laboratories reported using a multi-step approach. Three laboratories reported using solvent extraction with solid phase extraction and derivatization (50 %), and one laboratory each reported use of “Quick Polar Pesticides” extraction (QuPPE), solvent extraction, and solvent extraction with solid phase extraction (17 % each).

Similarly, Fig. 7-6 indicates that all laboratories reported using LC-MS-based techniques for the determination of AMPA in SRM 3290. Four laboratories reported use of LC-MS/MS (67 %), one laboratory reported use of LC-MS (17 %), and one laboratory reported use of LC-HRMS (17 %). As noted for glyphosate, the results for AMPA obtained using solvent extraction with LC-HRMS were also below the consensus mean for SRM 3290. Because the reported methods are so similar, no additional trends related to analytical method could be identified.

NIST has conducted two other QAP study involving measurement of glyphosate and AMPA in food samples prior to this FNSQAP study: HAMQAP Exercise 6 in 2020 [36] and FNSQAP Exercise 1 in 2021 [40]. The repeatabilities reported in this study are consistent with those from previous exercises (approximately 10 % or less for both analytes), but the reproducibilities are significantly poorer for glyphosate in this study (77 % to 120 %) compared to previous studies (21 % to 39 %). Bias of the consensus mean with respect to the target value was improved in this study (7 % to 15 %) compared to previous studies (25 % to 37 %).

Measurand: Aminomethylphosphonic Acid (AMPA)
 Sample: SRM 3290 Dry Cat Food
 Exercise: FNSQAP Exercise 2

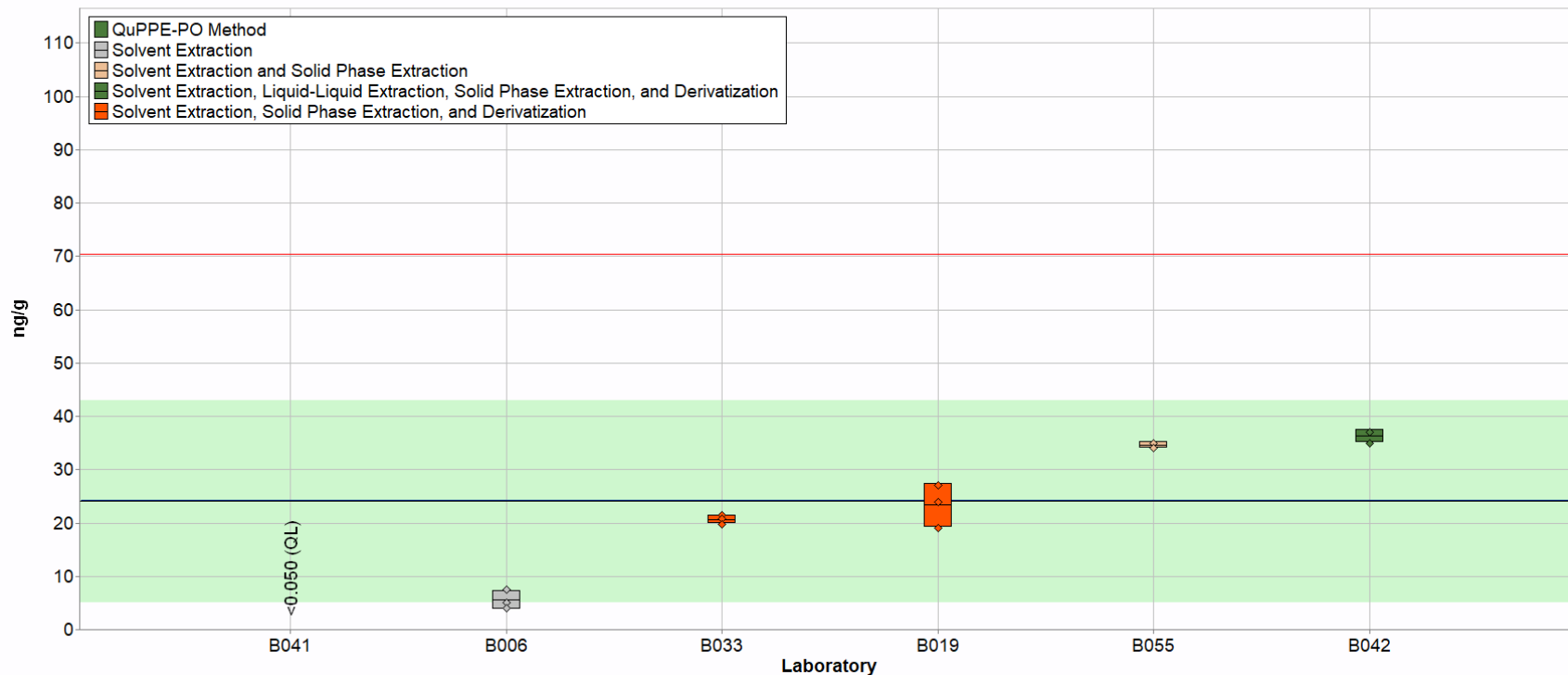


Fig. 7-5. AMPA in SRM 3290 (data summary view – sample preparation method).

In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). A downward triangle represents data reported as an LOQ value. The color of the data point represents the sample preparation method employed as indicated in the figure key (the sample preparation method reported by laboratory B041 was QUPPE-PO). The 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 bound of the consensus range of tolerance, calculated as the value above the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$, with the bottom bound set to zero. A NIST target value was not available at the time of this report.

Measurand: Aminomethylphosphonic Acid (AMPA)
Sample: SRM 3290 Dry Cat Food
Exercise: FNSQAP Exercise 2

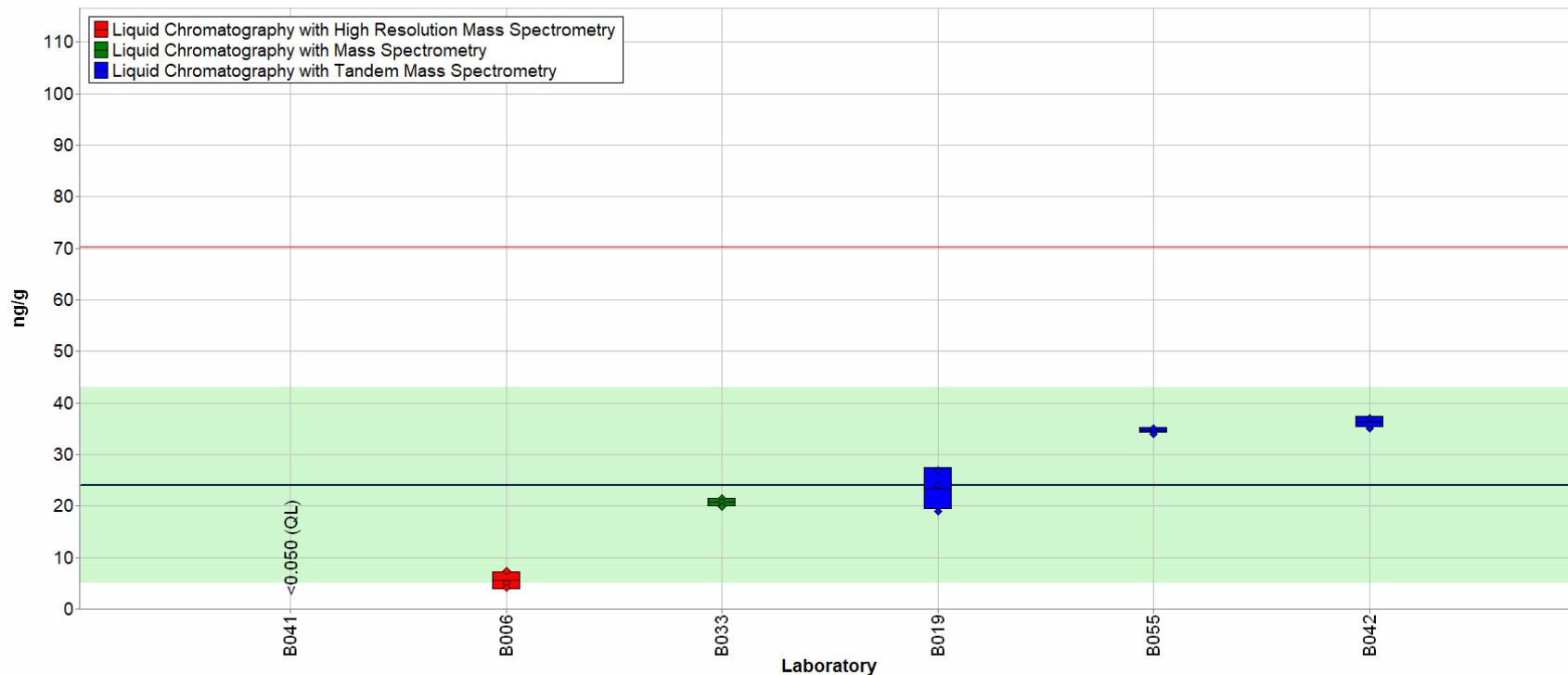


Fig. 7-6. AMPA in SRM 3290 (data summary view – analytical method).

In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). A downward triangle represents data reported as an LOQ value. The color of the data point represents the analytical method employed as indicated in the figure key (the analytical method reported by laboratory B041 was LC-MS/MS). The 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 bound of the consensus range of tolerance, calculated as the value above the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$, with the bottom bound set to zero. A NIST target value was not available at the time of this report.

Table 7-4 and Table 7-5 detail the numerical results for N-acetyl-glyphosate and N-acetyl-AMPA reported by each participating laboratory. Of the ten laboratories that indicated an intention to report results for these two analytes, only two responded and only one laboratory reported quantitative results for three of the four sample/analyte pairs. For determination of the mass fraction of N-acetyl-glyphosate and N-acetyl-AMPA, laboratories reported the use of solvent extraction or solvent extraction with solid phase extraction and derivatization for sample preparation (50 % each). One laboratory reported use of LC-MS/MS and one laboratory reported use of LC-HRMS (50 % each) The low participation and number of non-quantitative data reports indicate that these samples may not contain these minor glyphosate components or that the levels are below the quantitation limits of current methodology.

Table 7-4. Data summary table for N-acetyl-glyphosate in SRM 3290 and SRM 3299.

		N-acetyl-Glyphosate										
		SRM 3290 Dry Cat Food (ng/g)					SRM 3299 Ground Turmeric (Curcuma longa L.) Rhizome (ng/g)					
		Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target											
	B005											
	B006	< 8.000	< 8.000	< 8.000			48.6	27.6	28.5	35	12	
	B011											
	B012											
	B019											
	B041											
	B042	< 10.000	< 10.000	< 10.000			< 10.000	< 10.000	< 10.000			
	B047											
	B051											
B055												
Community Results	Consensus Mean						Consensus Mean					
	Consensus Standard Deviation						Consensus Standard Deviation					
	Maximum						Maximum					
	Minimum						Minimum					
	N	0					N					

Table 7-5. Data summary table for N-acetyl-AMPA in SRM 3290 and SRM 3299.

		N-acetyl-AMPA									
		SRM 3290 Dry Cat Food (ng/g)					SRM 3299 Ground Turmeric (Curcuma longa L.) Rhizome (ng/g)				
Lab		A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target										
	B005										
	B006	18.9	15.9	15.9	16.90	1.73	14.4	9.99	9.73	11.4	2.6
	B011										
	B012										
	B019										
	B041										
	B042	< 10.000	< 10.000	< 10.000			< 10.000	< 10.000	< 10.000		
	B047										
	B051										
B055											
Community Results	Consensus Mean						Consensus Mean				
	Consensus Standard Deviation						Consensus Standard Deviation				
	Maximum						Maximum				
	Minimum						Minimum				
	N	1					1				

Many laboratories utilize matrix-matched calibration to improve accuracy of methods for glyphosate determination. Information about calibrant preparation was not collected from participants, but future studies focused on glyphosate in foods could be designed to evaluate performance improvements related to calibration approaches. In any laboratory exercise, calculations and reporting units must be verified prior to submission of results. Laboratories often report results in the wrong units or omit a dilution factor during the calculation of the final results, resulting in poor performance on the study. As always, consistent 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 like NIST’s SRMs or non-certified reference materials such as NIST’s RMs) or materials prepared in-house.

8. CONTAMINANTS (Phthalates)

8.1. Executive Summary

To protect public health, regulators must understand human dietary exposure to potentially harmful contaminants such as phthalates, transferred from food packaging and other food contact materials, through accurate determination of phthalate levels in consumer products. Unfortunately, the participation rate in this study was extremely low and no conclusions could be drawn about laboratory or community performance.

8.2. Study Overview

Phthalates are a family of man-made chemicals used in a variety of industrial applications and are considered endocrine disruptors linked to adverse health effects. Food packaging and other food contact materials can lead to substantial phthalate concentrations in foods and increase global phthalate exposure through dietary intake [41]. Monitoring of human exposure is a critical component of understanding population health impacts, and to ensure that future studies on dangers of phthalate exposure are properly interpreted, methods for the detection and quantification of phthalates in food products must be well characterized and have demonstrated accuracy. In this study, participants were provided with samples of SRM 1869 Infant/Adult Nutritional Formula II (milk/whey/soy-based) and powdered cheese. Participants were asked to use in-house analytical methods to determine the mass fraction (ng/g) of phthalates in each matrix. Through participation in this study, laboratories can better understand the performance of their in-house methods relative to those being used by others in the community and the related limitations of any data generated using those methods. Participant results may be used in the value assignment of NIST reference materials included as samples in this study.

8.3. Sample Information

Participants were provided three packets each of SRM 1869 Infant/Adult Nutritional Formula II (milk/whey/soy-based) (labeled Infant Formula D) and powdered cheese (labeled Powdered Cheese). Packets of SRM 1869 contained 10 g of material, while packets of powdered cheese contained 3 g of material; participants were instructed to store all packets at controlled room temperature (20 °C to 25 °C). Before use, participants were instructed to mix the contents of each packet thoroughly and to prepare one sample and report one value from each packet provided using a sample size of at least 0.5 g for SRM 1869 and a mass appropriate for their in-house method of analysis for powdered cheese. The approximate analyte levels were not reported to participants prior to the study, and target values for phthalates in both materials were not available at the time of this report.

8.4. Study Results and Discussion

Nine laboratories requested samples for the phthalates in foods study, and participation rates for each analyte ranged from 13 % for di-*n*-pentyl phthalate, di-*n*-hexyl phthalate, and dicyclohexyl phthalate to 56 % for di-*n*-butyl phthalate (Table 8-1). Table 8-2 summarizes the numerical results reported by each participating laboratory. Submitted data for phthalates for which no quantitative results were submitted is summarized at the end of this section.

Table 8-1. Summary of participation rates for phthalates in foods.

Analyte	Laboratories Intending to Report Results	Laboratories Reporting Results		Laboratories Reporting Quantitative Results	
		Cheese	SRM 1869	Cheese	SRM 1869
dimethyl phthalate	9	3	3	0	0
diethyl phthalate	9	3	3	1	1
diisobutyl phthalate	9	2	2	1	1
di- <i>n</i> -butyl phthalate	9	5	5	2	4
di- <i>n</i> -pentyl phthalate	8	1	1	0	0
di- <i>n</i> -hexyl phthalate	8	1	1	0	0
bis(2-ethylhexyl) phthalate	9	4	4	2	2
benzyl butyl phthalate	9	4	4	1	0
dicyclohexyl phthalate	8	1	1	0	0
diisononyl phthalate	9	4	4	1	2

Table 8-2. Individualized data summary table for phthalates in foods.

Laboratory-specific results and Z-scores were provided to each participant separately from this report to protect laboratory identities.

(Lab Name)

Exercise 2 – Phthalates in Foods

Analyte	Lab Code: (Code)		1. Your Results				2. Community Results			3. Target	
	Sample	Units	x_i	s_i	Z'_{comm}	Z_{NIST}	N	x^*	s^*	x_{NIST}	u_{NIST}
dimethyl phthalate	Cheese	ng/g	<i>Individual laboratory results will appear in this section; laboratory-specific results were provided to each participant separately from this report.</i>				0				
dimethyl phthalate	SRM 1869	ng/g					0				
diethyl phthalate	Cheese	ng/g					1				
diethyl phthalate	SRM 1869	ng/g					1				
diisobutyl phthalate	Cheese	ng/g					1				
diisobutyl phthalate	SRM 1869	ng/g					1				
di-n-butyl phthalate	Cheese	ng/g					2	1833	3600		
di-n-butyl phthalate	SRM 1869	ng/g					4	660	600		
di-n-pentyl phthalate	Cheese	ng/g					0				
di-n-pentyl phthalate	SRM 1869	ng/g					0				
di-n-hexyl phthalate	Cheese	ng/g					0				
di-n-hexyl phthalate	SRM 1869	ng/g					0				
bis(2-ethylhexyl) phthalate	Cheese	ng/g					2	11452	47000		
bis(2-ethylhexyl) phthalate	SRM 1869	ng/g					2	11032	48000		
benzyl butyl phthalate	Cheese	ng/g					1				
benzyl butyl phthalate	SRM 1869	ng/g					0				
dicyclohexyl phthalate	Cheese	ng/g					0				
dicyclohexyl phthalate	SRM 1869	ng/g					0				
diisononyl phthalate	Cheese	ng/g					1				
diisononyl phthalate	SRM 1869	ng/g					2	13323	47000		

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_{NIST}	standard uncertainty about the 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 value		

Table 8-3, Table 8-4, Table 8-5, Table 8-6, Table 8-7, and Table 8-8 detail the measured mass fraction results for diethyl phthalate, diisobutyl phthalate, di-n-butyl phthalate, bis(2-ethylhexyl) phthalate, benzyl butyl phthalate, and diisononyl phthalate reported by each participating laboratory, respectively. Given the low number of laboratories reporting quantitative results for each compound, the performance statistics are summarized in Table 8-9 and will be discussed together.

Table 8-3. Data summary table for diethyl phthalate in foods.

		diethyl phthalate									
		Powdered Cheese (ng/g)					SRM 1869 Infant/Adult Nutritional Formula II (milk/whey/soy-based) (ng/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target										
	B004	22.8	23.3	23.05	23.05	0.25	< 0.100	< 0.100	< 0.100		
	B005	< 10.000	< 10.000	< 10.000			62	64	97	74	20
	B012										
	B019										
	B020	< 100.00	< 100.00	< 100.00			< 10.000	< 10.000	< 10.000		
	B021										
	B034										
	B038										
B051											
Community Results		Consensus Mean					Consensus Mean				
		Consensus Standard Deviation					Consensus Standard Deviation				
		Maximum					Maximum				
		Minimum					Minimum				
		N					N				

Table 8-4. Data summary table for diisobutyl phthalate in foods.

		diisobutyl phthalate									
		Powdered Cheese (ng/g)					SRM 1869 Infant/Adult Nutritional Formula II (milk/whey/soy-based) (ng/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target										
	B004	528	502	515	515	13	344.6	350.8	347.7	348	3
	B005										
	B012										
	B019										
	B020	< 100.00	< 100.00	< 100.00			< 10.000	< 10.000	< 10.000		
	B021										
	B034										
	B038										
B051											
Community Results		Consensus Mean					Consensus Mean				
		Consensus Standard Deviation					Consensus Standard Deviation				
		Maximum					Maximum				
		Minimum					Minimum				
		N					N				

Table 8-5. Data summary table for di-*n*-butyl phthalate in foods.

		di- <i>n</i> -butyl phthalate									
		Powdered Cheese (ng/g)					SRM 1869 Infant/Adult Nutritional Formula II (milk/whey/soy-based) (ng/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target										
	B004	820	726.6	773.3	773	47	200.8	203.4	202.1	202	1
	B005	< 10.000	< 10.000	< 10.000			1081	1083	445	870	368
	B012										
	B019										
	B020	< 100.00	< 100.00	< 100.00			9.7	15	14	13	3
	B021	< 20.000	< 20.000	< 20.000			< 20.000	< 20.000	< 20.000		
	B034	2340	3510	2830	2893	588	2440	2150	1350	1980	565
	B038										
B051											
Community Results		Consensus Mean				1833	Consensus Mean				660
		Consensus Standard Deviation				3554	Consensus Standard Deviation				597
		Maximum				2893	Maximum				1980
		Minimum				773	Minimum				13
		<i>N</i>				2	<i>N</i>				4

Table 8-6. Data summary table for bis(2-ethylhexyl) phthalate in foods.

		bis(2-ethylhexyl) phthalate									
		Powdered Cheese (ng/g)					SRM 1869 Infant/Adult Nutritional Formula II (milk/whey/soy-based) (ng/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target										
	B004	24089	21294	22691.5	22692	1398	21630	22318	21974	21974	344
	B005										
	B012										
	B019										
	B020	410	110	120	213	170	135	86	47	89	44
	B021	< 40.000	< 40.000	< 40.000			< 40.000	< 40.000	< 40.000		
	B034	< 500.00	< 500.00	< 500.00			< 500.00	< 500.00	< 500.00		
	B038										
B051											
Community Results		Consensus Mean				11452	Consensus Mean				11032
		Consensus Standard Deviation				47005	Consensus Standard Deviation				47874
		Maximum				22692	Maximum				21974
		Minimum				213	Minimum				89
		<i>N</i>				2	<i>N</i>				2

Table 8-7. Data summary table for benzyl butyl phthalate in foods.

		benzyl butyl phthalate									
		Powdered Cheese (ng/g)					SRM 1869 Infant/Adult Nutritional Formula II (milk/whey/soy-based) (ng/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target										
	B004	< 0.100	< 0.100	< 0.100			< 0.100	< 0.100	< 0.100		
	B005										
	B012										
	B019										
	B020	< 100.00	< 100.00	< 100.00			< 10.000	< 10.000	< 10.000		
	B021	< 20.000	< 20.000	< 20.000			< 20.000	< 20.000	< 20.000		
	B034	872	910	931	904	30	< 500.00	< 500.00	< 500.00		
	B038										
	B051										
Community Results		Consensus Mean					Consensus Mean				
		Consensus Standard Deviation					Consensus Standard Deviation				
		Maximum					Maximum				
		Minimum					Minimum				
		N					N				

Table 8-8. Data summary table for diisononyl phthalate in foods.

		diisononyl phthalate									
		Powdered Cheese (ng/g)					SRM 1869 Infant/Adult Nutritional Formula II (milk/whey/soy-based) (ng/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target										
	B004	< 1.000	< 1.000	< 1.000			< 1.000	< 1.000	< 1.000		
	B005										
	B012										
	B019										
	B020	100	120	100	107	12	240	140	58	146	91
	B021	< 100.00	< 100.00	< 100.00			< 100.00	< 100.00	< 100.00		
	B034	< 500.00	< 500.00	< 500.00			31100	27300	21100	26500	5048
	B038										
	B051										
Community Results		Consensus Mean					Consensus Mean				
		Consensus Standard Deviation					Consensus Standard Deviation				
		Maximum					Maximum				
		Minimum					Minimum				
		N					N				

Table 8-9. Summary of performance statistics for phthalates in foods.

Analyte	Laboratories Reporting Quantitative Results		Within-Laboratory Variability (%) Range (Average) ^(a)		Among-Laboratory Variability (%) ^(a)	
	Cheese	SRM 1869	Cheese	SRM 1869	Cheese	SRM 1869
diethyl phthalate	1	1	1 (NA)	26 (NA)	NA	NA
diisobutyl phthalate	1	1	3 (NA)	0.9 (NA)	NA	NA
di- <i>n</i> -butyl phthalate	2	4	6-20 (13)	0.6-42 (23)	194	90
bis(2-ethylhexyl) phthalate	2	2	6-80 (43)	2-49 (26)	410	434
benzyl butyl phthalate	1	0	3 (NA)	NA	NA	NA
diisononyl phthalate	1	2	11 (NA)	19-62 (41)	NA	350

^(a) Average within-laboratory variability and among-laboratory variability are not available when fewer than two laboratories reported quantitative results.

Overall, the results for the measurement of phthalates in foods were highly variable. One laboratory consistently reported high precision results, with RSD_r at or below 6 %. Within-laboratory variabilities observed for data from other participants, however, were consistently higher at 20 % to 80 %. Where among-laboratory variability could be calculated, laboratories did not agree (RSD_R ranging from 90 % to over 400 %). Published method performance requirements for determination of phthalates in foods are not widely available, and performance characteristics of methods developed by the US Environmental Protection Agency (EPA) for determination of phthalates in drinking and wastewater were not directly applicable.

As shown in Table 8-10, four of the five laboratories reporting results indicated use of solvent extraction in their sample preparation, while two laboratories utilized additional sample cleanup steps such as liquid-liquid extraction (LLE) and solid phase extraction (SPE). One laboratory reported use of gel permeation chromatography for sample preparation prior to analysis. All laboratories reported use of mass spectrometry-based techniques for detection of phthalates, with three laboratories using liquid chromatography for separation and two using gas chromatography.

Table 8-10. Method information reported by participants in the phthalates study.

	Sample Preparation Method	Analytical Method
B004	Gel permeation chromatography	LC-MS/MS
B005	Solvent extraction	LC-MS
B020	Solvent extraction + LLE	GC-MS/MS
B021	Solvent extraction + SPE	GC-MS/MS
B034	Extraction	LC-MS/MS

This study was the first QAP study conducted by NIST involving measurement of phthalates in food samples. Given the low participation rate in this study, few meaningful conclusions can be drawn from the data. In review of the reported results for bis(2-ethylhexyl) phthalate, di-*n*-butyl phthalate, and diisononyl phthalate, data from laboratory B020 is consistently one to two orders of magnitude lower than that reported by other laboratories. However, trends related to sample preparation or analytical method cannot be identified, and additional data would be needed to better understand any potential method biases. Methods for detecting contaminants at low levels must be well characterized, with accurately determined method detection limits and limits of quantitation. Particularly important for ubiquitous contaminants such as phthalates, laboratories should ensure these limits are defined based on detectable levels in process blanks to prevent misattribution of detected levels to sample contents.

In any laboratory exercise, calculations and reporting units must be verified prior to submission of results. Laboratories often report results in the wrong units or omit a dilution factor during the calculation of the final results, resulting in poor performance on the study. As always, consistent 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 like NIST's SRMs or non-certified reference materials such as NIST's RMs) or materials prepared in-house.

No quantitative results were reported for dimethyl phthalate (Table 8-11), di-*n*-pentyl phthalate (Table 8-12), di-*n*-hexyl phthalate (Table 8-13), or dicyclohexyl phthalate (Table 8-14) in either sample; these sample/analyte pairs will not be discussed further in this report.

Table 8-11. Data summary table for dimethyl phthalate in foods.

		dimethyl phthalate									
		Powdered Cheese (ng/g)					SRM 1869 Infant/Adult Nutritional Formula II (milk/whey/soy-based) (ng/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target										
	B004	< 0.100	< 0.100	< 0.100			< 0.100	< 0.100	< 0.100		
	B005	< 10.000	< 10.000	< 10.000			< 10.000	< 10.000	< 10.000		
	B012										
	B019										
	B020	< 100.00	< 100.00	< 100.00			< 10.000	< 10.000	< 10.000		
	B021										
	B034										
	B038										
B051											
Community Results		Consensus Mean					Consensus Mean				
		Consensus Standard Deviation					Consensus Standard Deviation				
		Maximum					Maximum				
		Minimum					Minimum				
		N					N				

Table 8-12. Data summary table for di-n-pentyl phthalate in foods.

		di-n-pentyl phthalate									
		Powdered Cheese (ng/g)					SRM 1869 Infant/Adult Nutritional Formula II (milk/whey/soy-based) (ng/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target										
	B005										
	B012										
	B019										
	B020	< 100.00	< 100.00	< 100.00			< 10.000	< 10.000	< 10.000		
	B021										
	B034										
	B038										
	B051										
	Target										
Community Results		Consensus Mean					Consensus Mean				
		Consensus Standard Deviation					Consensus Standard Deviation				
		Maximum					Maximum				
		Minimum					Minimum				
		N					N				

Table 8-13. Data summary table for di-*n*-hexyl phthalate in foods.

		di- <i>n</i> -hexyl phthalate									
		Powdered Cheese (ng/g)					SRM 1869 Infant/Adult Nutritional Formula II (milk/whey/soy-based) (ng/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target										
	B005										
	B012										
	B019										
	B020	< 100.00	< 100.00	< 100.00			< 10.000	< 10.000	< 10.000		
	B021										
	B034										
	B038										
	B051										
	Target										
Community Results		Consensus Mean					Consensus Mean				
		Consensus Standard Deviation					Consensus Standard Deviation				
		Maximum					Maximum				
		Minimum					Minimum				
		N					N				
					0					0	

Table 8-14. Data summary table for dicyclohexyl phthalate in foods.

		dicyclohexyl phthalate									
		Powdered Cheese (ng/g)					SRM 1869 Infant/Adult Nutritional Formula II (milk/whey/soy-based) (ng/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target										
	B005										
	B012										
	B019										
	B020	< 100.00	< 100.00	< 100.00			< 10.000	< 10.000	< 10.000		
	B021										
	B034										
	B038										
	B051										
	Target										
Community Results		Consensus Mean					Consensus Mean				
		Consensus Standard Deviation					Consensus Standard Deviation				
		Maximum					Maximum				
		Minimum					Minimum				
		N					N				
					0					0	

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Appendix A. List of Abbreviations and Acronyms

AMPA

Aminomethylphosphonic acid

AOAC

AOAC International, founded in 1884 as the Association of Official Agricultural Chemists. A provider of documentary standards.

ARA

Arachidonic acid

As

Arsenic

Avg

Average

Cd

Cadmium

COA

Certificate of Analysis

cGMP

current Good Manufacturing Practice

Cr

Chromium

CRM

Certified Reference Material

DHA

Docosahexaenoic acid

DSQAP

Dietary Supplement Laboratory Quality Assurance Program

EPA

US Environmental Protection Agency

FDA

US Food and Drug Administration

FNSQAP

Food Nutrition and Safety Measurements Quality Assurance Program

GC-FID

Gas Chromatography with Flame Ionization Detection

GC-MS

Gas Chromatography Mass Spectrometry

GC-MS/MS

Gas Chromatography with Tandem Mass Spectrometry Detection

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HAMQAP

Health Assessment Measurements Quality Assurance Program

HCl

Hydrochloric acid

Hg

Mercury

HNO₃

Nitric acid

ICP-MS

Inductively Coupled Plasma Mass Spectrometry

ICP-OES

Inductively Coupled Plasma Optical Emission Spectrometry

ID

Isotope Dilution

ISO

International Organization for Standardization. A provider of documentary standards.

JCGM

Joint Committee for Guides in Metrology

KED

Kinetic Energy Discrimination

LC-Abs

Liquid Chromatography with Absorbance Detection

LC-HRMS

Liquid Chromatography with High Resolution Mass Spectrometry

LC-MS

Liquid Chromatography Mass Spectrometry

LC-MS/MS

Liquid Chromatography with Tandem Mass Spectrometry

LLE

Liquid-Liquid Extraction

LOQ

Limit of Quantification

MDL

Method Detection Limit

Mo

Molybdenum

NIST

National Institute of Standards and Technology

NIST IR 8492
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Pb
Lead

PbCl₂
Lead chloride

QAP
Quality Assurance Program

QL
Quantification Limit

QuPPE
Quick Polar Pesticides extraction

RM
Reference Material

RMIS
Reference Material Information Sheet

RSD
Relative Standard Deviation, expressed as a percentage

RSD_r
Repeatability Relative Standard Deviation (Within-Laboratory Variability)

RSD_R
Reproducibility Relative Standard Deviation (Among-Laboratory Variability)

SD
Standard Deviation

SDPA
Standard Deviation for Proficiency Assessment

Se
Selenium

SI
International System of Units

SMPR
Standard Method Performance Requirements

SPE
Solid Phase Extraction

SRM
Standard Reference Material

USDA
United States Department of Agriculture

WHO
World Health Organization