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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 federal regulations enforced by the US FDA, USDA, and other international bodies. Exercise 1 of this program offered the opportunity for laboratories to assess their in-house measurements of nutritional elements (calcium, iron, potassium, and sodium), toxic elements (arsenic, cadmium, lead, and mercury), water-soluble vitamins (folic acid), fat-soluble vitamins (vitamin K), contaminants (glyphosate and acrylamide), and dietary fiber in food and infant formula samples.

Keywords

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

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1. INTRODUCTION

The Food Nutrition and Safety Measurements Quality Assurance Program (FNSQAP) was formed in 2021 and represents ongoing efforts at 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 related 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 Supplements 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 first exercise of FNSQAP. Sixty laboratories responded to the call for participants in June 2021 (Table **1-1**). Samples were shipped to participants in September 2021 and results were returned to NIST in November 2021. Participants received a summary of the preliminary data in November 2021 and were given an opportunity to correct any errors by December 2021. This report contains the final data and information that was disseminated to the participants in March 2023.

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

Study Group	Analytes	Samples
Nutritional Elements	Ca, Fe, K, Na	Infant Formula
Toxic Elements	As, Cd, Pb, Hg	Baby Food
Water-Soluble Vitamins	Folic Acid	Infant Formula
Fat-Soluble Vitamins	Vitamin K	Infant Formula
Contaminants	Glyphosate, Aminomethylphosphonic acid (AMPA), n-Acetyl-glyphosate, n-Acetyl-AMPA	Soy Protein, Mixed Diet
Contaminants	Acrylamide	Chocolate, Coffee
Dietary Fiber	Forms of soluble and insoluble dietary fiber	Breakfast Cereal, Soy Flour

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 individual laboratories. 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 13528:2015, 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 1 – 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*
c_1	a_1	b_1	*Individual laboratory results will appear in this section; laboratory-specific results were provided to each participant separately from this report.*				N_1	x^*_1	s^*_1	x_{NIST1}	U_1
…	…	…					…	…	…	…	…
…	…	…					…	…	…	…	…
c_n	a_n	b_n					N_n	x^*_n	s^*_n	x_{NISTn}	U_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*	expanded 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 indicates that NIST does not have data on file for that laboratory for the corresponding analyte or sample. An empty box for standard deviation indicates that the participant reported a single value or a value below the Limit of Quantitation (LOQ) and, therefore, that value was not included in the calculation of the consensus data [1].

Also included in Sec. 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 $2\times U_{95}$ (where U_{95} is the expanded uncertainty on an assigned value) or $2\times U_{NIST}$ (where U_{NIST} is twice the standard deviation of NIST and/or other measurements):

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

or

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

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

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

Section 2 of the data table (*Community Results*) contains the consensus results, including the number of laboratories reporting more than a single quantitative value for each analyte, the mean value determined for each analyte, and a robust estimate of the standard deviation of the reported values [1]. Consensus means and standard deviations are calculated using the laboratory means; if a laboratory reported a single value, the reported value is not included in determination of the consensus values [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 certified value, a non-certified value, or a value determined at NIST. 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]. 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. 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 particular analyte in a particular study. Participants can compare the raw data for their laboratory to data reported by the other participating laboratories and to the consensus data. A blank indicates that the laboratory signed up and received samples for that analyte and matrix, but NIST does not have data on file for that laboratory. 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 $|Z'_{\mathrm{comm}}| > 2$. 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	$x_{A1\text{-}1}$	$x_{B1\text{-}1}$	$x_{C1\text{-}1}$	$\bar{x}_{1-1}$	$s_{1\text{-}1}$	$x_{A2\text{-}1}$	$x_{B2\text{-}1}$	$x_{C2\text{-}1}$	$\bar{x}_{1-2}$	$s_{1\text{-}2}$
	…	…	…	…	…	…	…	…	…	…	…
	e_n	$x_{A1\text{-}n}$	$x_{B1\text{-}n}$	$x_{C1\text{-}n}$	$\bar{x}_{n-1}$	$s_{n\text{-}1}$	$x_{A2\text{-}n}$	$x_{B2\text{-}n}$	$x_{C2\text{-}n}$	$\bar{x}_{n-2}$	$s_{n\text{-}2}$
Community Results		Consensus Mean			f_1		Consensus Mean			f_2	
		Consensus Standard Deviation			g_1		Consensus Standard Deviation			g_2	
		Maximum			h_1		Maximum			h_2	
		Minimum			i_1		Minimum			i_2	
		N			j_1		N			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, individual laboratory data (diamonds) are plotted with the individual laboratory SD (rectangle). Laboratories reporting values below the LOQ are shown in this view as downward triangles beginning at the LOQ, reported as Quantification Limit (QL) on the figures. Laboratories reporting values below LOQ can still be successful in the study if the target value is also below the laboratory LOQ. The blue solid line represents the consensus mean, and the green shaded area represents the 95 % confidence interval for the consensus mean, based on the standard 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 ($n_{particpants}$), and the average number of replicates reported by each participant ($n_{Average\ Number\ of\ Replicates\ per\ Participant}$). 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.

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

The red shaded region represents the target zone for "acceptable" performance, which encompasses the NIST target value bounded by twice its uncertainty (U_{95} or U_{NIST}). The solid red lines represent the range of tolerance (values that result in an acceptable Z' score, $|Z'| \leq 2$). If the lower limit is below zero, the lower limit has been set to zero. In this view, the relative locations of individual laboratory data and consensus zones with respect to the target zone can be compared easily. In most cases, the target zone and the consensus zone overlap, which is the expected result. Major program goals include both reducing the size of the consensus zone and centering the consensus zone about the target value. Analysis of an appropriate reference material as part of a quality control scheme can help to identify sources of bias for laboratories reporting results that are significantly different from the target zone. In the case in which a method comparison is relevant, different colored data points may be used to identify laboratories that used a specific approach for sample preparation, analysis, or quantitation.

1.1.4.2. Sample/Sample Comparison View

In this view, the individual laboratory results for one sample (e.g., NIST Standard Reference Material® (SRM®) or Reference Material (RM) with a certified, non-certified, or NIST-determined value; a less challenging matrix) are compared to the results for another sample (e.g., NIST SRM with a more challenging matrix; a commercial sample). The solid red box represents the target zone for the first sample (x-axis) and the second sample (y-axis), if available. The dotted blue box represents the consensus zone for the first sample (x-axis) and the second sample (y-axis). The axes of this graph are centered about the consensus mean values for each sample or control, to a limit of twice the range of tolerance (values that result in an acceptable Z' score, $|Z'| \leq 2$). Depending on the variability in the data, the axes may be scaled proportionally to better display the individual data points for each laboratory. In some cases, when the consensus and target ranges

have limited overlap, the solid red box may only appear partially on the graph. If the variability in the data is high (greater than 100 % relative standard deviation (RSD)), the dotted blue box may also only appear partially on the graph. These views emphasize trends in the data that may indicate potential calibration issues or method biases. 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.

2. NUTRITIONAL ELEMENTS (Calcium, Iron, Potassium, Sodium)

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 and consensus mean ranges overlapping with target ranges. Most participants reported using microwave digestion and acid hydrolysis methods for sample preparation and Inductively Coupled Plasma Mass Spectrometry (ICP-MS) and Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) 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

Calcium (Ca), iron (Fe), potassium (K), and sodium (Na) are essential nutritional elements required for the human body to function properly. 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 infant 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 determine the mass fractions (mg/kg) of Ca, Fe, K, and Na 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 SRM 1849b Infant/Adult Nutritional Formula I (milk-based) and RM 8260 Infant Nutritional Formula (hydrolyzed milk-based). Each packet contained approximately 10 g of material; participants were asked to store the materials at –20 °C or colder 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 SRM 1849b were determined using data from the manufacturer of the material and NIST [3]. The target values for nutritional elements in RM 8260 were from the NIST Reference Material Information Sheet (RMIS). The target values and uncertainty for nutritional elements in SRM 1849b and RM 8260 are provided in Table **2-1** on an as-received basis. The uncertainty for Ca in RM 8260 was from the RMIS, while the uncertainties for Fe and K were approximated as 5 % relative to the target value. A target value for Na in RM 8260 was not available at the time of this report.

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 1 – 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*
Ca	SRM 1849b	mg/kg	*Individual laboratory results will appear in this section; laboratory-specific results were provided to each participant separately from this report.*				28	5000	310	5070	860
Ca	RM 8260	mg/kg					28	4300	280	4219	29
Fe	SRM 1849b	mg/kg					28	170	16	167	17
Fe	RM 8260	mg/kg					28	97.0	9.1	91.0	9.1
K	SRM 1849b	mg/kg					28	9100	720	8950	530
K	RM 8260	mg/kg					28	6900	610	6600	660
Na	SRM 1849b	mg/kg					26	4200	320	4130	360
Na	RM 8260	mg/kg					26	1700	170		

x_i Mean of reported values

s_i Standard deviation of reported values

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

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

N Number of quantitative values reported

x* Robust mean of reported values

s* Robust standard deviation

x_{NIST} NIST-assessed value

U expanded uncertainty about the NIST-assessed value

2.4. Study Results and Discussion

Table **2-1** summarizes and Table **2-2**, Table **2-3**, Table **2-4**, and Table **2-5** detail the numerical results reported by each participating laboratory for nutritional elements. The participation level was high for nutritional elements, with 72 % to 78 % of laboratories requesting samples returning results (on average 28 of 36 laboratories).

Table **2-2**, Table **2-3**, Table **2-4**, and Table **2-5** reveal that the within-laboratory variabilities were mostly acceptable with respect to published expectations of the measurement community of 5 % RSD for nutritional elements in formula [4]. For each nutritional element and sample type, 2 to 4 laboratories had within-laboratory variabilities greater than 5 % RSD. The between-laboratory variabilities for all nutritional elements in both SRM 1849b and RM 8260 fell below the published expectations of the measurement community of ≤ 10 % RSD [4] even with participants using a variety of methods.

Table 2-2. Data summary table for calcium in SRM 1849b and RM 8260.
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 NIST values and consensus values are included on both pages for convenience.*

		Calcium									
		SRM 1849b Infant/Adult Nutritional Formula I (milk-based) (mg/kg)					RM 8260 Infant Formula (hydrolyzed-milk based) (mg/kg)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target				5070	860				4219	29
	A001	5006.23	5033.65	5009.76	5017	15	4234.83	4276.17	4193.76	4235	41
	A002	5140	5140	5040	5107	58	4480	4380	4450	4437	51
	A004										
	A005										
	A006	5831.55	5226.13	5098.02	5385	392	4449.59	4221.99	4386.67	4353	118
	A010	4955.25	4955.25	4955.25	4955	0	4209.46	4209.46	4209.46	4209	0
	A012	4963.9	4917.59	4828.7	4903	69	4142.86	4154.32	4170.11	4156	14
	A014										
	A015	5169	5184	5186	5180	9.3	4353	4362	4385	4367	17
	A017	5167	5187	5292	5215	67	4495	4237	4379	4370	129
	A019	4440	4470	4790	4567	194	3960	4160	3920	4013	129
	A020	4760	4710	4840	4770	66	4160	4090	4050	4100	56
	A021	4685	4595	4482	4587	102	3811	3878	3954	3881	72
	A022										
	A023	4840	4840	4820	4833	12	4100	4100	3970	4057	75
	A027	5386	5570	5701	5552	158	4943	4847	4862	4884	52
	A028	5144	5228	5100	5157	65	4318	4270	4346	4311	38
	A030	4920	5070	4990	4993	75	4220	4140	4920	4427	429
	A031	5307.71	5485.24	5429.97	5408	91	4549.71	4624.66	4537.4	4571	47
	A032	4600	4750	4700	4683	76	4090	4120	4160	4123	35
	A034	5124	5100	5127	5117	15	4325	4297	4325	4316	16
	A035	4972	5124	4882	4993	122	4229	4243	4103	4192	77
	A036										
	A037										
	A038	5040	4930	4960	4977	57	4240	4210	4120	4190	62
	A039	6743	4948	4682	5458	1121	5424	5938	6301	5888	441
	A040	4620	4740	4690	4683	60	3950	3840	4060	3950	110
	A041	4610.4	4592.4	4485.5	4563	68	3964.7	3847	4326.6	4046	250
	A042	5071	4840	5233	5048	198	4625	4226	4410	4420	200
Community Results		Consensus Mean			5020		Consensus Mean			4255	
		Consensus Standard Deviation			307		Consensus Standard Deviation			281	
		Maximum			5552		Maximum			5888	
		Minimum			1065		Minimum			1005	
		N			28		N			28	

Table 2-2 continued. Data summary table for calcium in SRM 1849b and RM 8260. Data highlighted in blue have been identified as outside the consensus tolerance limits and resulted in an unacceptable Z'_{comm} score, $|Z'_{comm}| > 2$.

		Calcium									
		SRM 1849b Infant/Adult Nutritional Formula I (milk-based) (mg/kg)					RM 8260 Infant Formula (hydrolyzed-milk based) (mg/kg)				
		A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target				5070	860				4219	29
	A045	937	1144	1114	1065	112	885	1084	1045	1005	105
	A046	5160	5150	5140	5150	10	4420	4370	4420	4403	29
	A047										
	A049	5263	5431	5354	5349	84	4858	4452	4582	4631	207
	A050	5020	5065	4725	4937	185	3931	3845	4142	3973	153
	A054										
	A057	5021	5002	5035	5019	17	4317	4039	4281	4212	151
Community Results		Consensus Mean			5020		Consensus Mean			4255	
		Consensus Standard Deviation			307		Consensus Standard Deviation			281	
		Maximum			5552		Maximum			5888	
		Minimum			1065		Minimum			1005	
		N			28		N			28	

Table 2-3. Data summary table for iron in SRM 1849b and RM 8260.
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 NIST values and consensus values are included on both pages for convenience.*

		Iron									
		SRM 1849b Infant/Adult Nutritional Formula I (milk-based) (mg/kg)					RM 8260 Infant Formula (hydrolyzed-milk based) (mg/kg)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target				167	17				91.0	9.1
	A001	168.93	167.36	168.56	168	0.8	95.08	97.12	92.56	94.9	2.3
	A002	186	188	203	192	9.3	126	121	118	122	4.0
	A004										
	A005										
	A006	353.5	215.17	213.27	261	80	142.82	132.07	164.23	146	16
	A010	187.12	187.12	187.12	187	0	104.58	104.58	104.58	105	0
	A012	161.17	159.76	158	160	1.6	91.68	93.35	91.89	92.3	0.9
	A014										
	A015	161	162.2	162.7	162	0.9	92.7	93.1	93.2	93.0	0.3
	A017	174.2	158.4	176	170	10	96.3	93.7	98.6	96.2	2.5
	A019	157	161	164	161	3.5	89.1	94.6	89.9	91.2	3.0
	A020	153	154	157	155	2.1	88.2	87.4	86.5	87.4	0.9
	A021	187	181	175	181	6.0	100	97.9	101	99.6	1.6
	A022										
	A023	175	171	172	173	2.1	105	102	96.9	101	4.1
	A027	179	189	198	189	10	110	111	113	111	1.5
	A028	169	172	167	169	2.5	94.2	92.9	94.6	93.9	0.9
	A030	170	172	170	171	1.2	96	98	108	101	6.4
	A031	174.68	183.03	179.39	179	4.2	107.27	104.82	107.61	107	1.5
	A032	140.1	146	146.8	144	3.7	85.39	86.47	86.3	86.1	0.6
	A034	170.5	168.4	172.2	170	1.9	96.7	96.9	96.6	96.7	0.2
	A035	183	180	184	182	2.1	100	102	97.5	99.8	2.3
	A036										
	A037										
	A038	163	160	165	163	2.5	93	93	89	91.7	2.3
	A039	252	184	163	200	47	119	107	106	111	7.2
	A040	161	154	157	157	3.5	91.8	90.4	91.6	91.3	0.8
	A041	148.6	141.3	139.7	143	4.7	81.7	80.9	87.4	83.3	3.5
	A042	152	149.6	149.9	151	1.3	79.2	84	81.6	81.6	2.4
Community Results		Consensus Mean			169		Consensus Mean			96	
		Consensus Standard Deviation			16		Consensus Standard Deviation			8.8	
		Maximum			261		Maximum			146	
		Minimum			143		Minimum			82	
		N			29		N			29	

Table 2-3 continued. Data summary table for iron in SRM 1849b and RM 8260.
Data highlighted in blue have been identified as outside the consensus tolerance limits and resulted in an unacceptable Z'_{comm} score, $|Z'_{comm}| > 2$.

		Iron									
		SRM 1849b Infant/Adult Nutritional Formula I (milk-based) (mg/kg)					RM 8260 Infant Formula (hydrolyzed-milk based) (mg/kg)				
		A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target				167	17				91.0	9.1
	A045	174	177	196	182	12	108	124	127	120	10
	A046	156	157	157	157	0.6	90	90	90.5	90.2	0.3
	A047										
	A049	164.3	170.8	168.1	168	3.3	95.87	94.1	93.5	94.5	1.2
	A050	180	182	166	176	8.7	94	92	97.5	94.5	2.8
	A054										
	A057	164	165	165	165	0.6	92	96	95	94.3	2.1
Community Results		Consensus Mean			169		Consensus Mean			96	
		Consensus Standard Deviation			16		Consensus Standard Deviation			8.8	
		Maximum			261		Maximum			146	
		Minimum			143		Minimum			82	
		N			29		N			29	

Table 2-4. Data summary table for potassium in SRM 1849b and RM 8260.
Note: This table spans two pages; the NIST values and consensus values are included on both pages for convenience.

		Potassium									
		SRM 1849b Infant/Adult Nutritional Formula I (milk-based) (mg/kg)					RM 8260 Infant Formula (hydrolyzed-milk based) (mg/kg)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target				8950	530				6600	660
	A001	8892.91	8895.12	8935.31	8908	24	6684.59	6696.01	6551.33	6644	80
	A002	8900	9640	8850	9130	442	7320	7210	7490	7340	141
	A004										
	A005										
	A006	9827.12	10274.17	9984.84	10029	227	7414.34	7641.74	7979.81	7679	285
	A010	9757.49	9749.11	9889.08	9799	79	7020.53	6934.69	6912.11	6956	57
	A012	8650.01	8658.53	8480.02	8596	101	6246.02	6315.43	6197.18	6253	59
	A014										
	A015	9172	9295	9317	9261	78	7282	7312	7318	7304	19
	A017	9347	9550	9602	9500	135	7121	7480	7144	7248	201
	A019	8500	8630	9190	8773	367	6620	6870	6510	6667	184
	A020	9170	9240	9140	9183	51	6940	6830	6730	6833	105
	A021	9438	9027	8991	9152	248	6694	6604	6252	6517	234
	A022										
	A023	10300	9810	9830	9980	277	8360	7580	7290	7743	553
	A027	7542	7830	8045	7806	252	6476	6475	6441	6464	20
	A028	9346	9553	9265	9388	149	6961	6909	6905	6925	31
	A030	9450	9410	9390	9417	31	7010	7070	7910	7330	503
	A031	10319.34	10480.03	9982.81	10261	254	7458.45	7599.03	7508.65	7522	71
	A032	7990	8080	7990	8020	52	6060	6160	6170	6130	61
	A034	9393	9451	9523	9456	65	7020	6929	6985	6978	46
	A035	9284	9592	9397	9424	156	6994	6950	6969	6971	22
	A036										
	A037										
	A038	9080	8900	8970	8983	91	6670	6610	6720	6667	55
	A039	9864	9046	8377	9096	745	7940	7656	8392	7996	371
	A040	8610	8850	8630	8697	133	6580	6420	6760	6587	170
	A041	8053	7919.4	7678.2	7884	190	6064.7	6028.9	6616.6	6237	329
	A042	8499	8253	8679	8477	214	6433	6556	6426	6472	73
Community Results		Consensus Mean			9134		Consensus Mean			6918	
		Consensus Standard Deviation			725		Consensus Standard Deviation			610	
		Maximum			10261		Maximum			7996	
		Minimum			7806		Minimum			6130	
		N			28		N			28	

Table 2-4 continued. Data summary table for potassium in SRM 1849b and RM 8260.

		Potassium									
		SRM 1849b Infant/Adult Nutritional Formula I (milk-based) (mg/kg)					RM 8260 Infant Formula (hydrolyzed-milk based) (mg/kg)				
		A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target				8950	530				6600	660
	A045	8976	10159	10356	9830	746	7111	8023	8325	7820	632
	A046	8740	8670	8650	8687	47	6170	6190	6210	6190	20
	A047										
	A049	8673	9403	9184	9087	375	6754	7220	7300	7091	295
	A050	8865	8985	8465	8772	272	6110	5985	6375	6157	199
	A054										
	A057	9815	9694	9788	9766	64	7308	7124	7018	7150	147
Community Results		Consensus Mean			9134		Consensus Mean			6918	
		Consensus Standard Deviation			725		Consensus Standard Deviation			610	
		Maximum			10261		Maximum			7996	
		Minimum			7806		Minimum			6130	
		N			28		N			28	

Table 2-5. Data summary table for sodium in SRM 1849b and RM 8260.
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 NIST values and consensus values are included on both pages for convenience.*

		Sodium									
		SRM 1849b Infant/Adult Nutritional Formula I (milk-based) (mg/kg)					RM 8260 Infant Formula (hydrolyzed-milk based) (mg/kg)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target				4130	360					
	A001	4109.43	4122.16	4139.03	4124	15	1560.23	1567.1	1535.65	1554	17
	A002	4240	4300	4310	4283	38	1870	1840	1860	1857	15
	A004										
	A005										
	A006	4508.82	4684.35	4539.87	4578	94	1854.38	1879.5	1975.45	1903	64
	A010	4314.39	4304.4	4349.56	4323	24	1677.01	1638.32	1624.6	1647	27
	A012	4227.14	4251.57	4172.58	4217	40	1659.16	1659.07	1649.95	1656	5.3
	A014										
	A015	4373	4399	4457	4410	43	1925	1940	1951	1939	13
	A017	4170	4168	4322	4220	88	1759	1690	1665	1705	49
	A019	4060	3950	4280	4097	168	1480	1670	1480	1543	110
	A020	4060	3960	4080	4033	64	1640	1600	1590	1610	26
	A021	4259	4159	4122	4180	71	1640	1650	1551	1614	55
	A022										
	A023	4630	4470	4480	4527	90	1910	1810	1750	1823	81
	A027	3853	3999	4107	3986	127	1750	1738	1743	1744	6.0
	A028	4131	4221	4086	4146	69	1644	1626	1636	1635	9.0
	A030										
	A031	4506.69	4547.9	4438.41	4498	55	1829.04	1852.74	1829.64	1837	14
	A032	3670	3710	3700	3693	21	1510	1540	1550	1533	21
	A034	4337	4306	4321	4321	16	1785	1686	1728	1733	50
	A035	4308	4276	4347	4310	36	1741	1732	1720	1731	11
	A036										
	A037										
	A038	4240	4170	4210	4207	35	1730	1700	1750	1727	25
	A039	6104	4357	4063	4841	1103	2378	2229	2812	2473	303
	A040	3840	3940	3900	3893	50	1610	1520	1590	1573	47
	A041	3924.6	3900.9	3772.4	3866	82	1602.9	1580.6	1725.7	1636	78
	A042	3549	3968	3731	3749	210	1490	1429	1470	1463	31
Community Results		Consensus Mean			4225		Consensus Mean			1711	
		Consensus Standard Deviation			323		Consensus Standard Deviation			174	
		Maximum			5019		Maximum			2473	
		Minimum			3693		Minimum			1463	
		N			26		N			26	

Table 2-5 continued. Data summary table for sodium in SRM 1849b and RM 8260. Data highlighted in blue have been identified as outside the consensus tolerance limits and resulted in an unacceptable Z'_{comm} score, $|Z'_{comm}| > 2$.

		Sodium									
		SRM 1849b Infant/Adult Nutritional Formula I (milk-based) (mg/kg)					RM 8260 Infant Formula (hydrolyzed-milk based) (mg/kg)				
		A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target				4130	360					
	A045	5348	4794	4916	5019	291	2210	2129	2020	2120	95
	A046	4330	4360	4370	4353	21	1700	1700	1720	1707	12
	A047										
	A049	4652	4639	4548	4613	57	1781	1936	1930	1882	88
	A050										
	A054										
	A057	3712	3810	3743	3755	50	1731	1671	1766	1723	48
Community Results		Consensus Mean			4225		Consensus Mean			1711	
		Consensus Standard Deviation			323		Consensus Standard Deviation			174	
		Maximum			5019		Maximum			2473	
		Minimum			3693		Minimum			1463	
		N			26		N			26	

As shown in Fig. **2-1**, Fig. **2-2**, Fig. **2-3**, Fig. **2-4**, Fig. **2-5**, Fig. **2-6**, Fig. **2-7**, and Fig. **2-8**, laboratories reported using a variety of sample preparation methods for the determination of nutritional elements in the two infant formula samples. Numbers and percentages of laboratories described as reporting specific approaches are averages across all results for four elements and two samples. The most common sample preparation approach was microwave digestion (14 laboratories, 50 %) followed by acid hydrolysis (8 laboratories, 29 %); two laboratories reported using hot block digestion (7 %), and one laboratory each reported use of open beaker digestion, dry ashing, and no sample preparation (4 % each). One laboratory did not report the sample preparation approach used (4 %). Notably, the laboratory indicating use of open beak digestion as the preparation method prior to ICP-MS analysis for determination of nutritional elements reported values below the 95 % confidence interval for the consensus mean in both samples for all 4 elements and below the target range for Ca in RM 8260. Although this preparation method is only represented by one laboratory, perhaps open beaker digestion is not ideal for nutritional element sample preparation of infant formula matrices. The sample preparation procedure is critical for unbiased measurements, and those that used concentrated acid 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 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.

Similar to reported sample preparation approaches, Fig. **2-9**, Fig. **2-10**, Fig. **2-11**, Fig. **2-12**, Fig. **2-13**, Fig. **2-14**, Fig. **2-15**, and Fig. **2-16** indicate that a variety of analytical methods were employed for the determination of nutritional elements in the two infant formula samples. The most reported approaches were ICP-MS (12 laboratories, 42 %) and ICP-OES (12 laboratories, 44 %). Two laboratories reported using ICP-MS in Kinetic Energy Discrimination (KED) mode (7 %) and one

laboratory each reported use of Cold Vapor Atomic Absorption Spectroscopy (CV AAS), neutron activation, and Total Reflection X-ray Fluorescence (TXRF) (4 % each). No trends related to analytical method could be identified. 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 and ICP-OES were the most reported analytical methods, some technical recommendations are provided for these analytical methods. 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, Ca has common interferences such as $^{40}Ar^{+}$, $^{40}Ar^{1}H_2$, $^{12}C^{16}O_2$, and $^{14}N_2{}^{16}O$. Hydrogen collision gas removes interferences on ^{40}Ca, and He collision gas removes interferences on ^{44}Ca. When using ICP-OES, monitoring more than one wavelength for each analyte helps not only to identify interferences or background shifts due to matrix effects at a given wavelength, but also helps identify and prevent bias.

The consensus ranges for Ca, Fe, and Na in SRM 1849b (Fig. **2-1**, Fig. **2-3**, Fig. **2-7**, Fig. **2-9**, Fig. **2-11**, and Fig. **2-15**) and Fe and K in RM 8260 (Fig. **2-4**, Fig. **2-6**, Fig. **2-12**, and Fig. **2-14**) lie completely within the target ranges. The consensus range for K in SRM 1849b (Fig. **2-5** and Fig. **2-13**) and Ca in RM 8260 (Fig. **2-2** and Fig. **2-10**) extend a little above the upper edge of the target range. The widths of the consensus ranges to the target ranges were comparable for Ca in RM 8260 and K in SRM 1849b, while the target ranges were greatly wider than the consensus ranges for Ca, Fe, and Na in SRM 1849b and Fe and K in RM 8260.

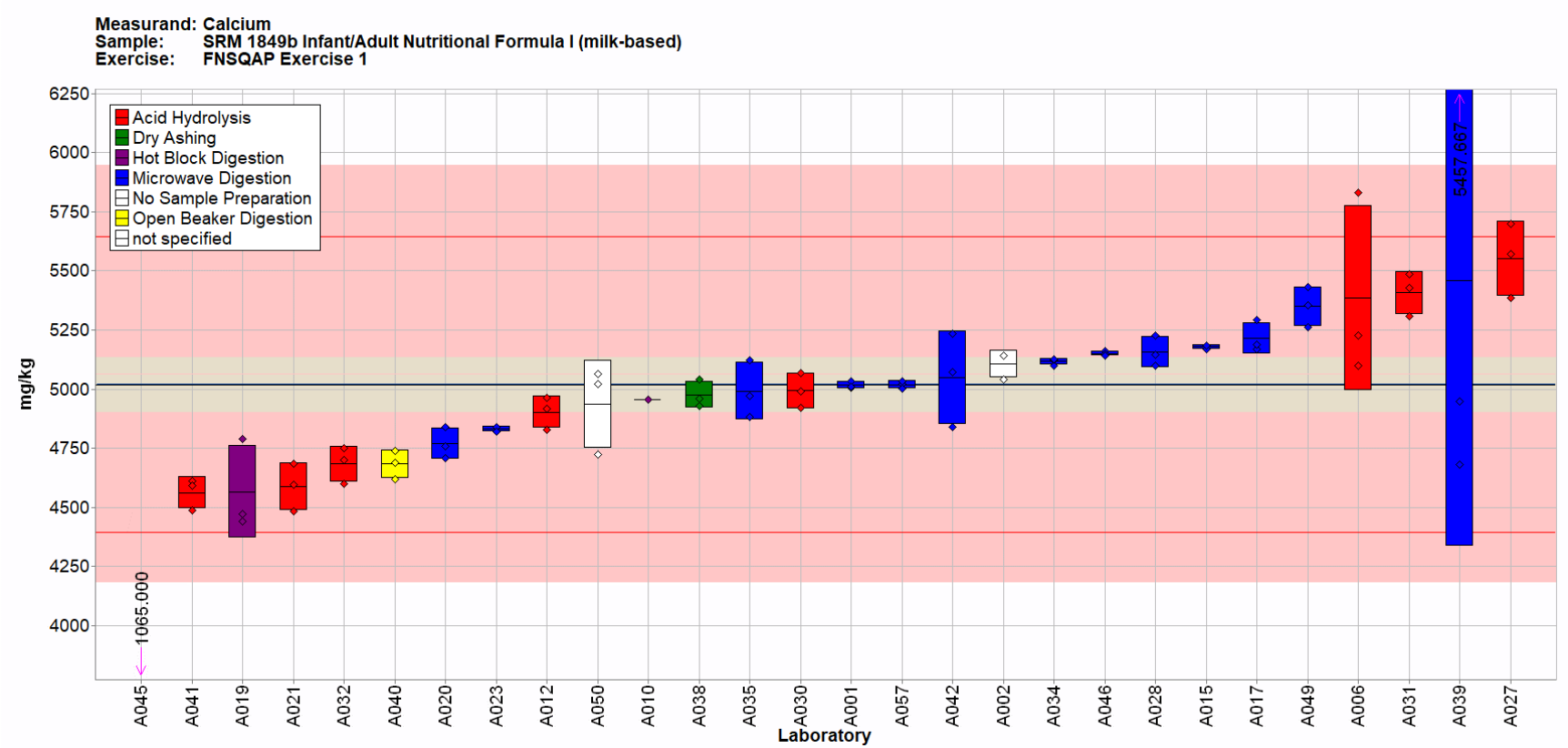

Fig. 2-1. Calcium in SRM 1849b (data summary view – sample preparation method).

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 method reported by laboratory A045 was 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, $|Z'_{\text{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty (U_{NIST}), and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$. The shaded beige region represents the overlapping of the 95 % confidence interval for the consensus mean (green region) and the NIST range of tolerance (red region).

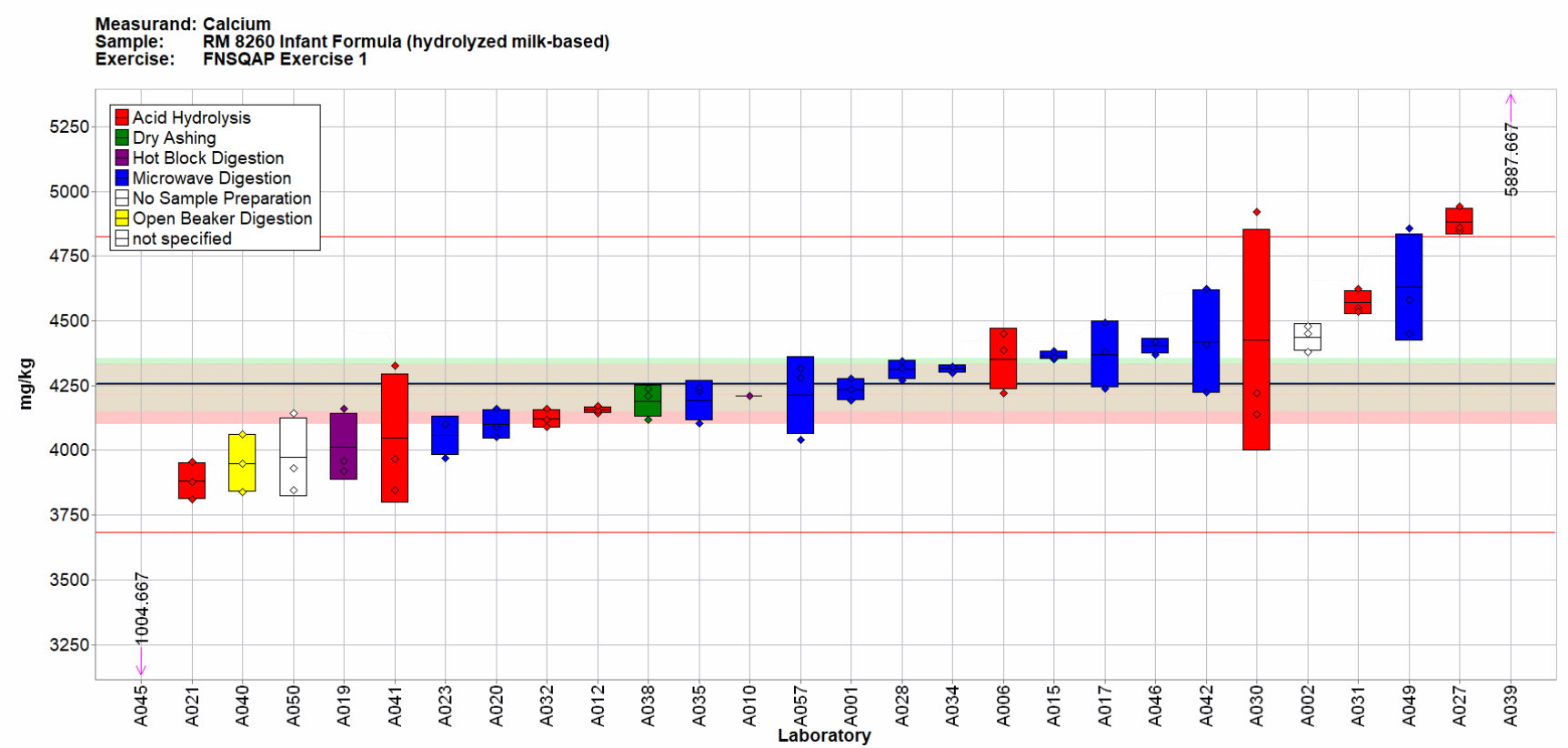

Fig. 2-2. Calcium in RM 8260 (data summary view – sample preparation method).

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 downward and upward arrows (the preparation methods reported by laboratories A039 and A045 were 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, $|Z'_{\text{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty (U_{NIST}), and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$. The shaded beige region represents the overlapping of the 95 % confidence interval for the consensus mean (green region) and the NIST range of tolerance (red region).

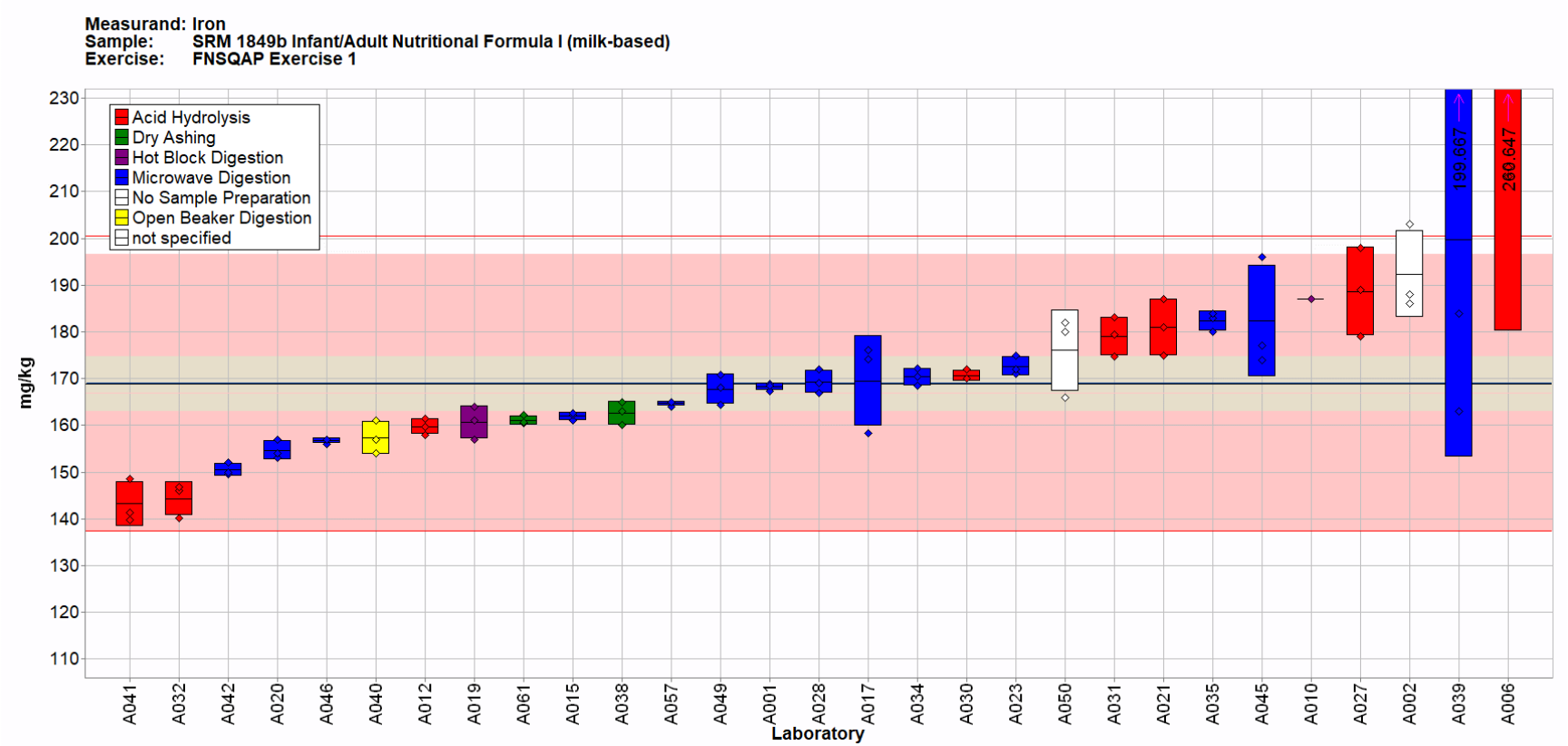

Fig. 2-3. Iron in SRM 1849b (data summary view – sample preparation method).

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 red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty (U_{NIST}), and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$. The shaded beige region represents the overlapping of the 95 % confidence interval for the consensus mean (green region) and the NIST range of tolerance (red region).

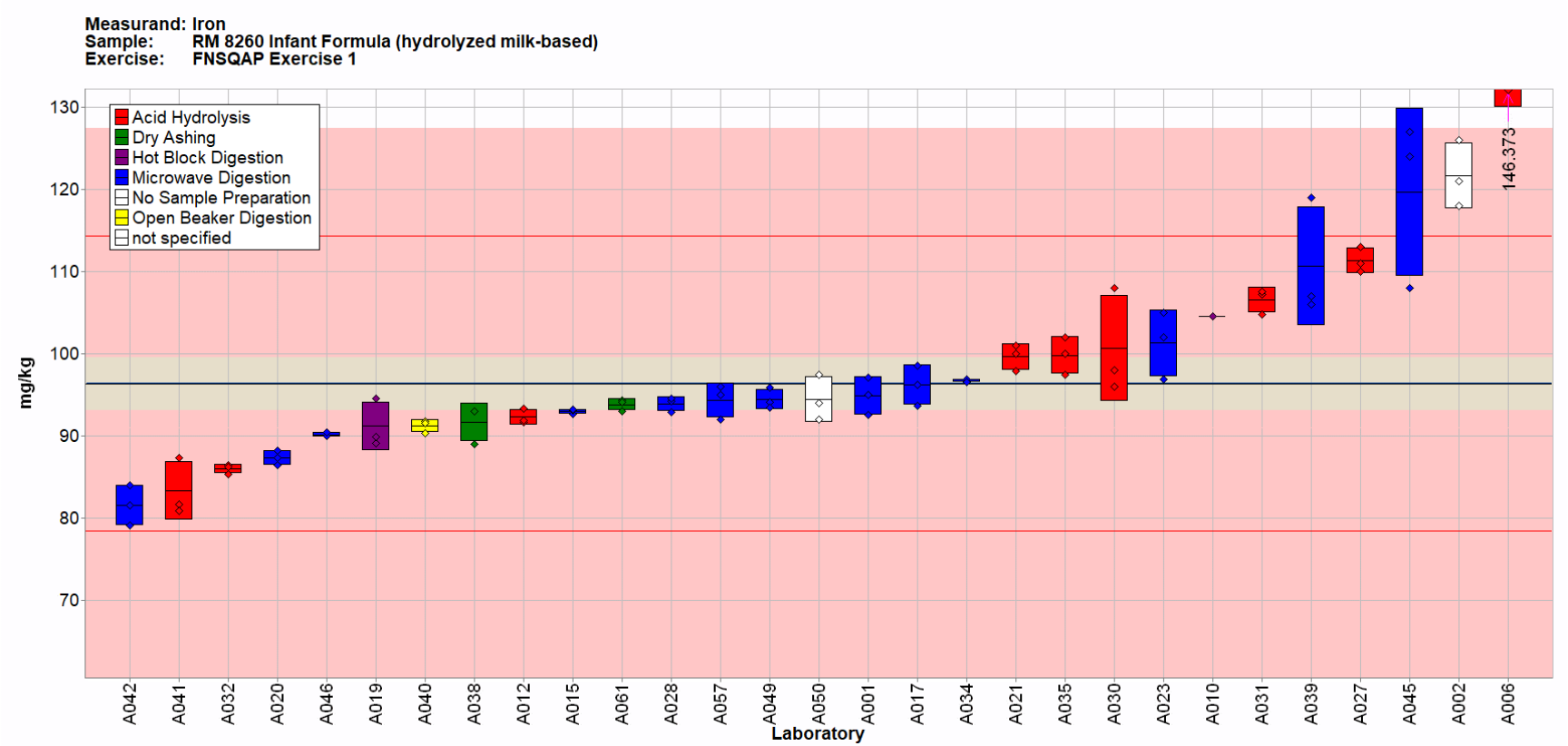

Fig. 2-4. Iron in RM 8260 (data summary view – sample preparation method).

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 green shaded region represents the 95 % confidence interval for the consensus mean. The solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty (U_{NIST}), and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$. The shaded beige region represents the overlapping of the 95 % confidence interval for the consensus mean (green region) and the NIST range of tolerance (red region).

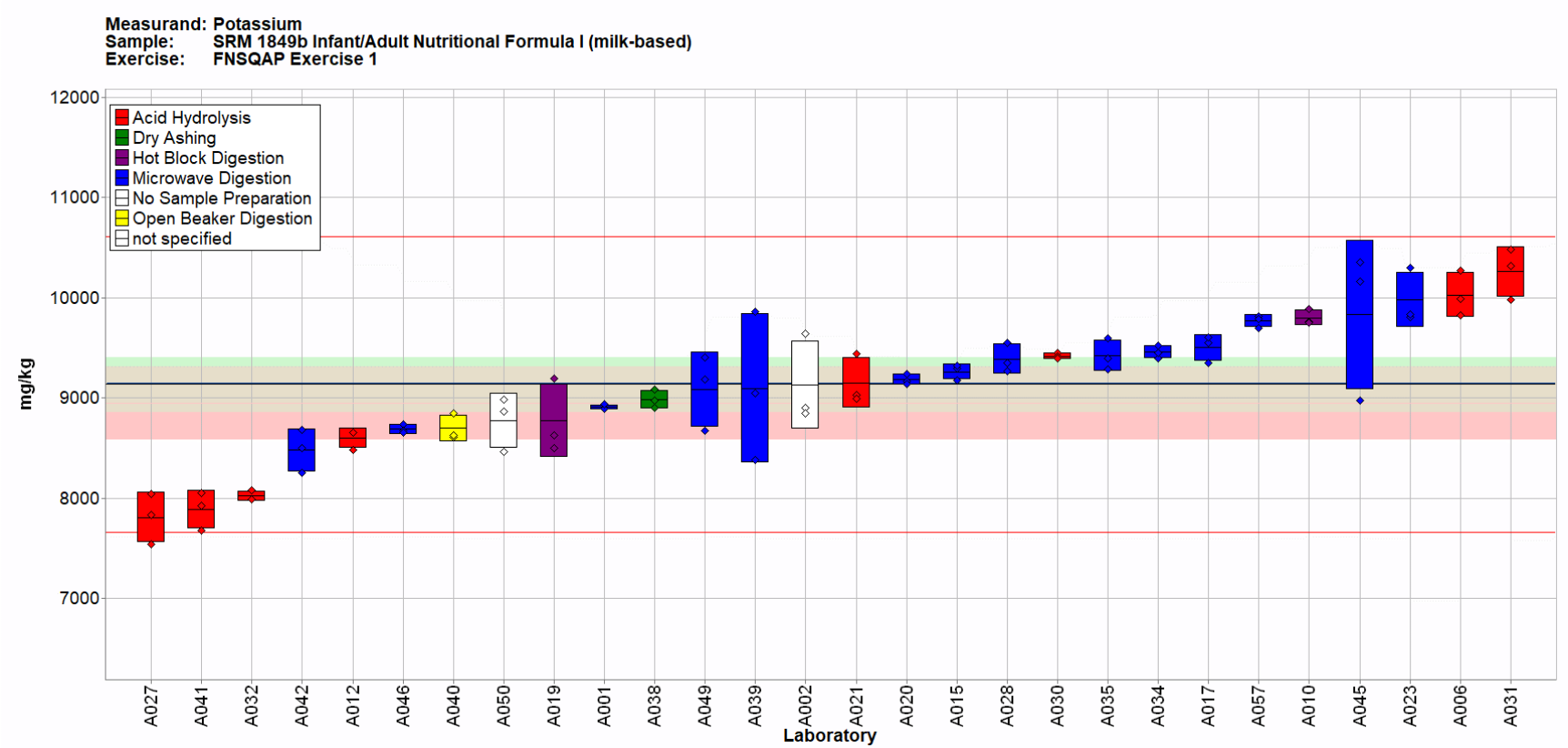

Fig. 2-5. Potassium in SRM 1849b (data summary view – sample preparation method).

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 red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty (U_{NIST}), and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$. The shaded beige region represents the overlapping of the 95 % confidence interval for the consensus mean (green region) and the NIST range of tolerance (red region).

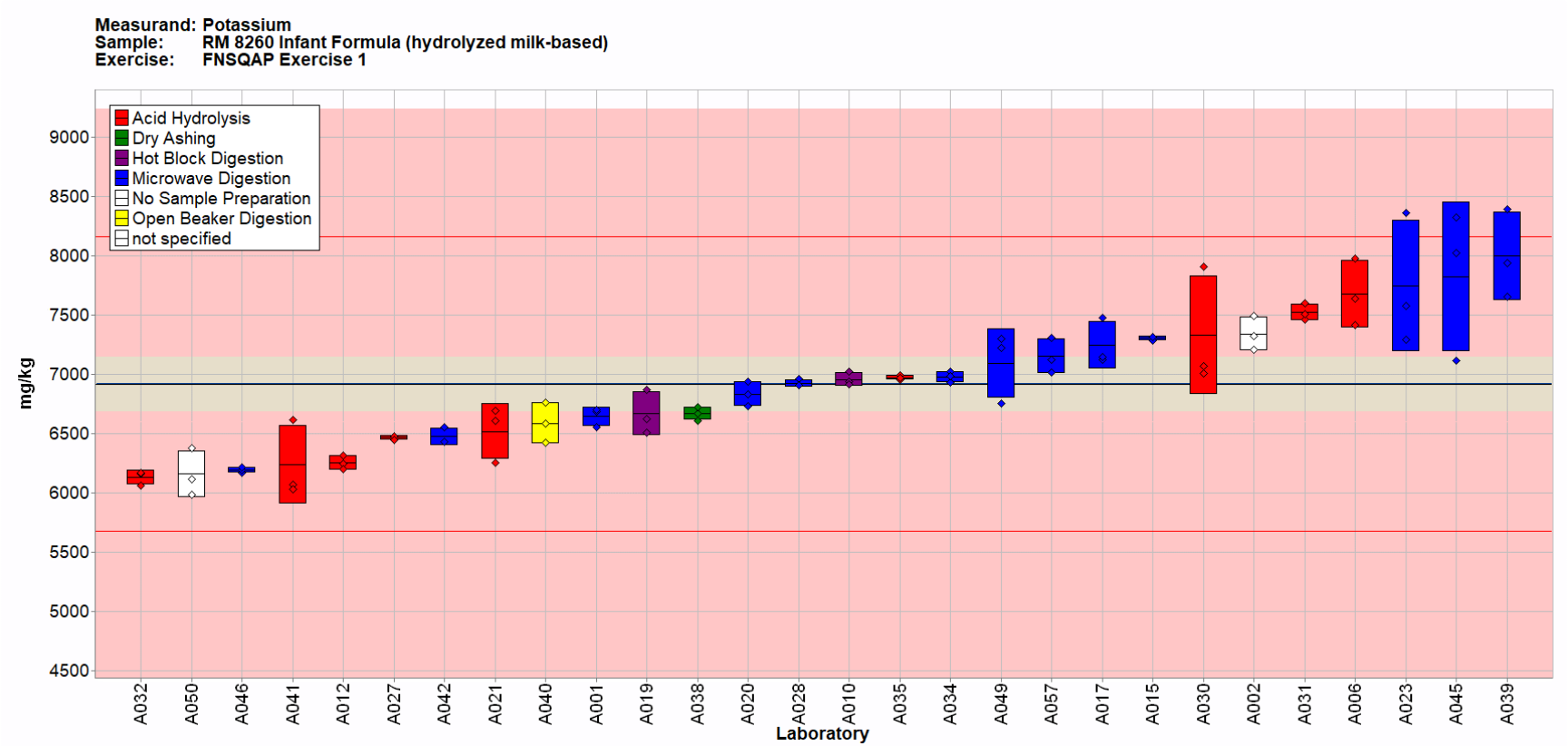

Fig. 2-6. Potassium in RM 8260 (data summary view – sample preparation method).

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 green shaded region represents the 95 % confidence interval for the consensus mean. The solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty (U_{NIST}), and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$. The shaded beige region represents the overlapping of the 95 % confidence interval for the consensus mean (green region) and the NIST range of tolerance (red region).

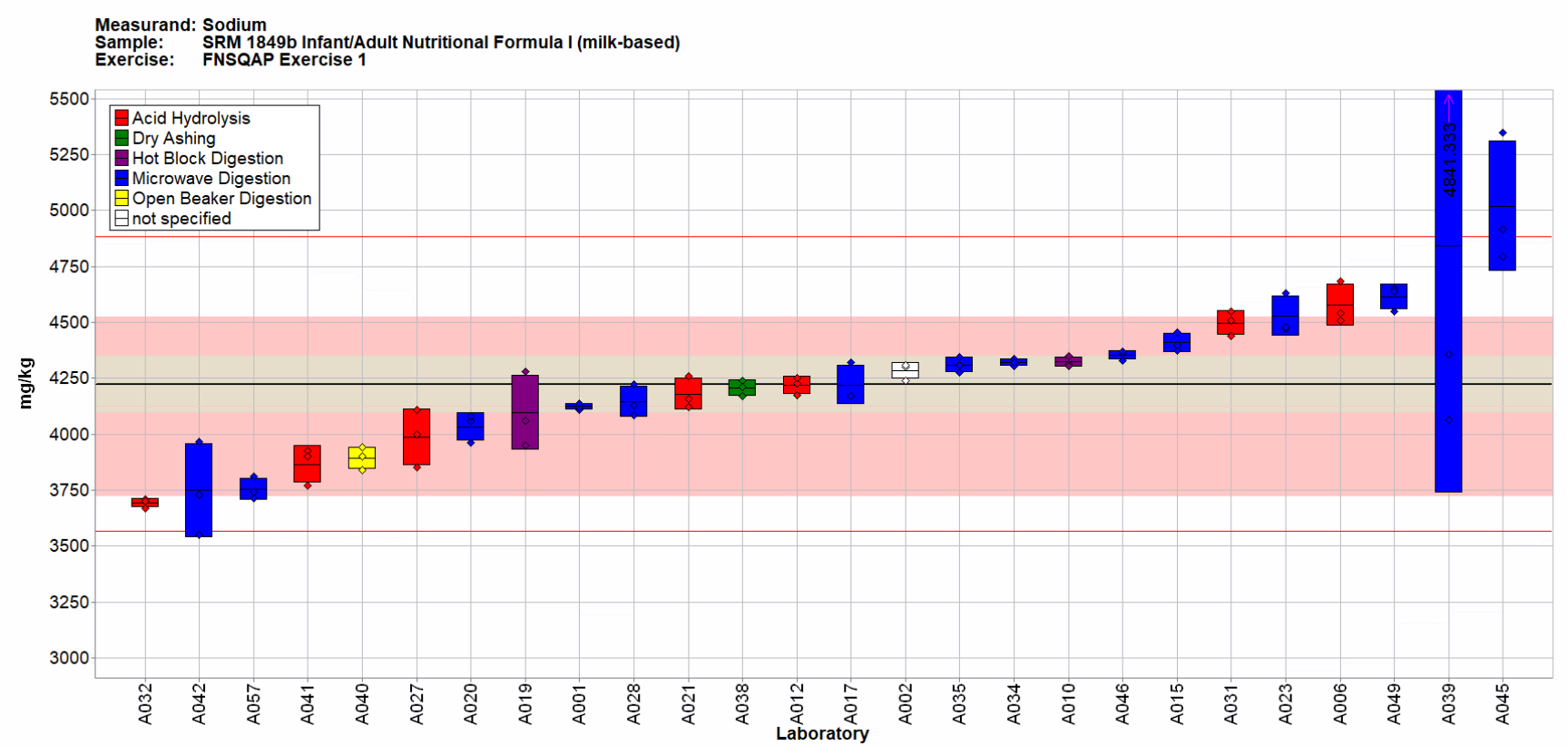

Fig. 2-7. Sodium in SRM 1849b (data summary view – sample preparation method).

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 red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty (U_{NIST}), and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$. The shaded beige region represents the overlapping of the 95 % confidence interval for the consensus mean (green region) and the NIST range of tolerance (red region).

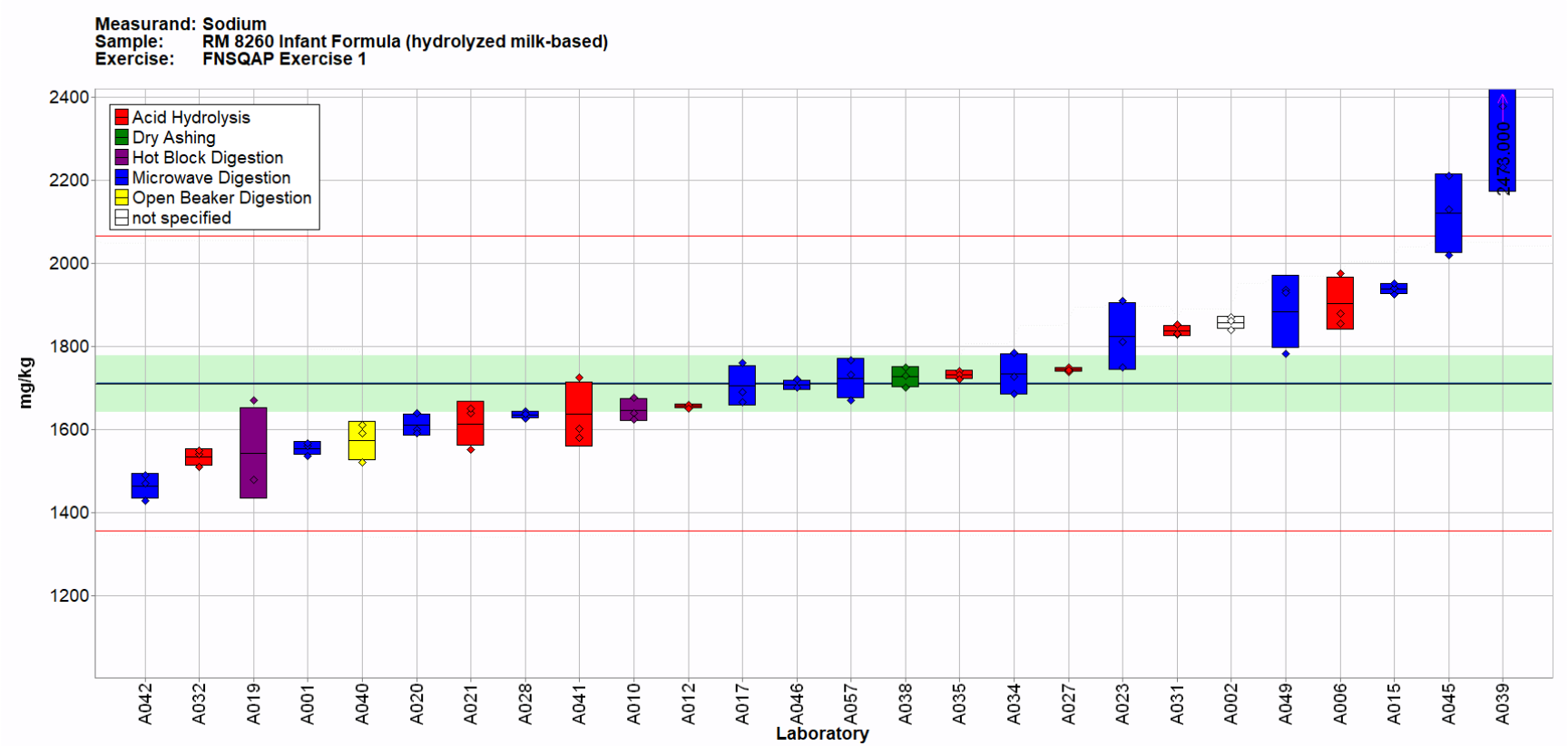

Fig. 2-8. Sodium in RM 8260 (data summary view – sample preparation method).

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 green shaded region represents the 95 % confidence interval for the consensus mean. The solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$. A NIST value has not been determined in this material.

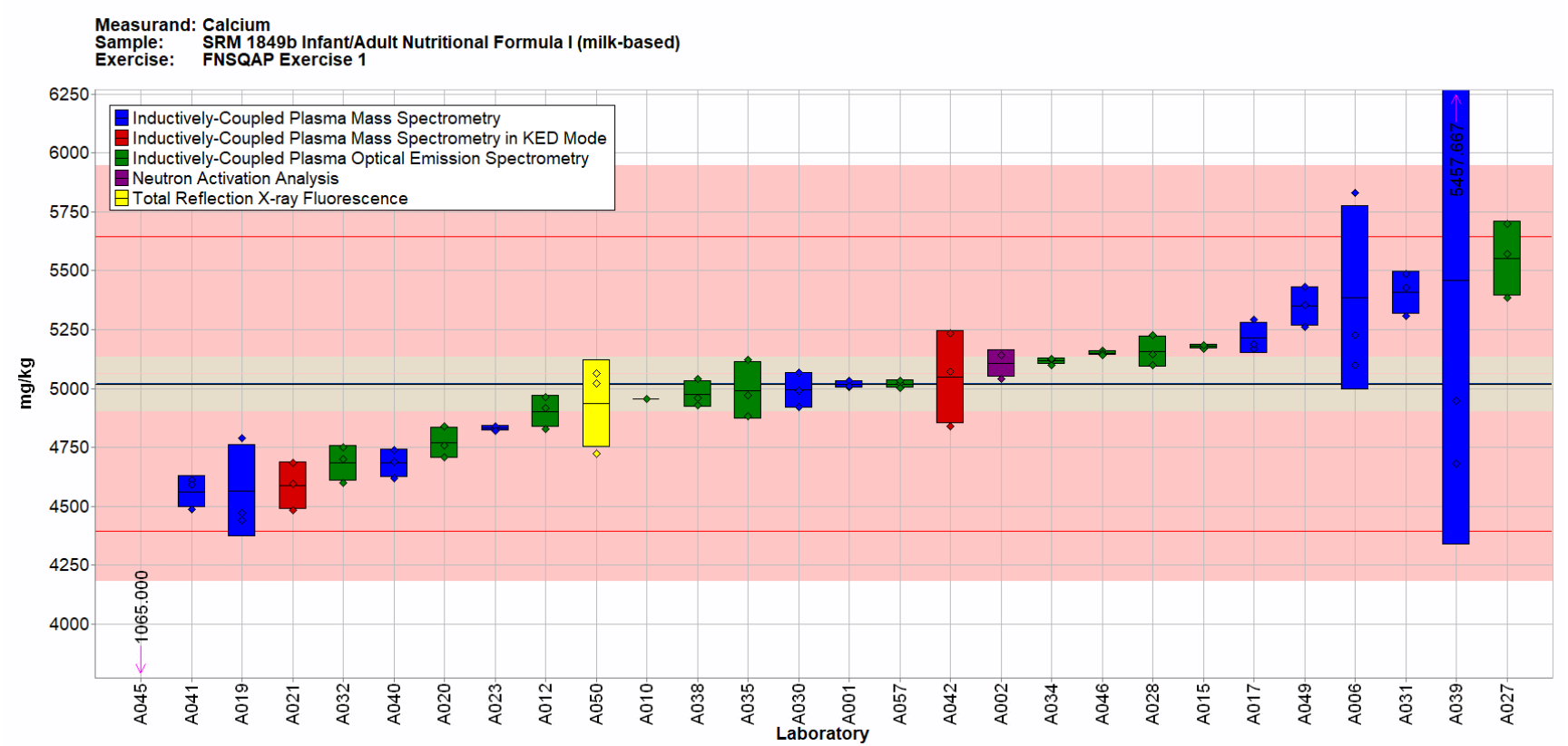

Fig. 2-9. Calcium in SRM 1849b (data summary view – analytical method).

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 A045 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 range of tolerance, which encompasses the target value bounded by twice its uncertainty (U_{NIST}), and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$. The shaded beige region represents the overlapping of the 95 % confidence interval for the consensus mean (green region) and the NIST range of tolerance (red region).

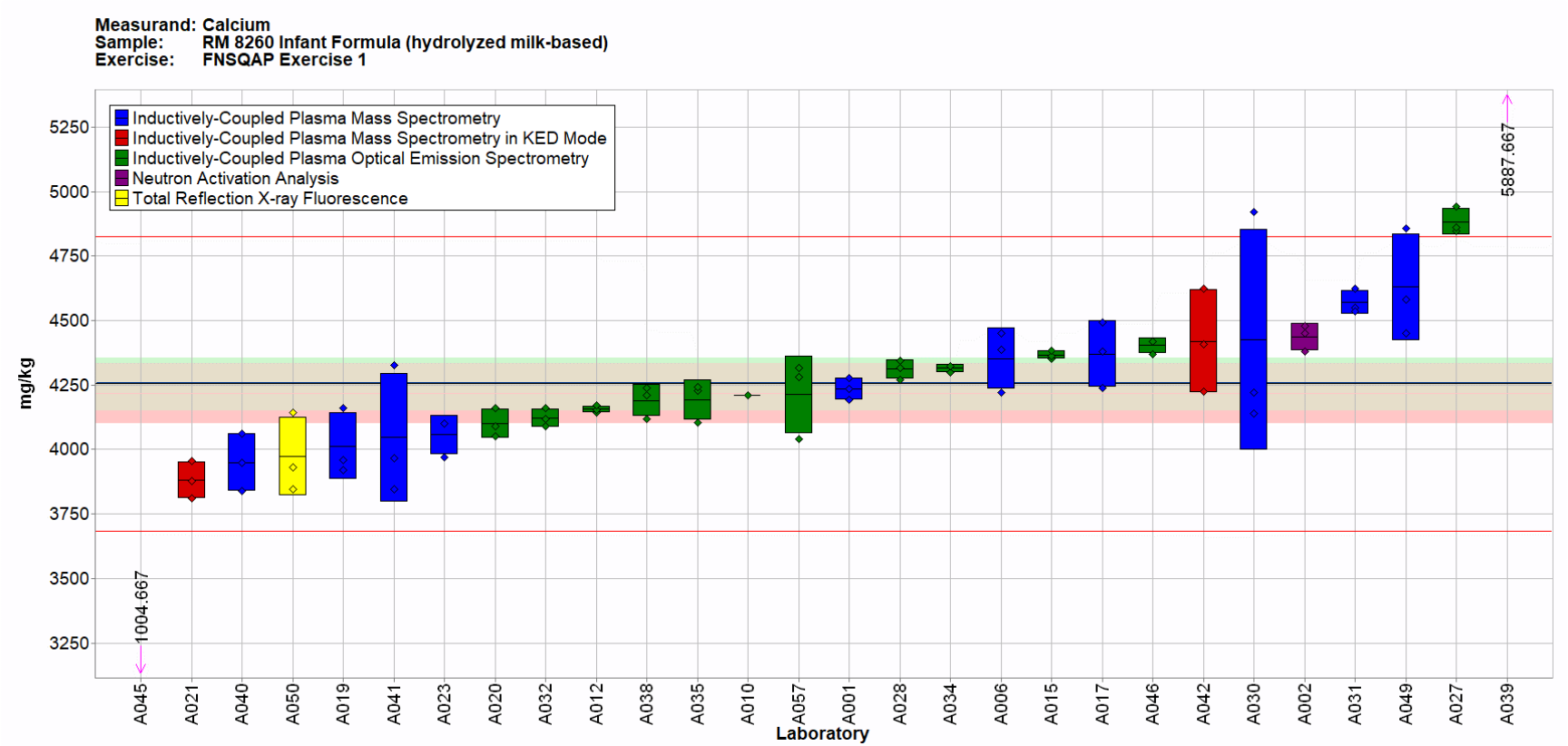

Fig. 2-10. Calcium in RM 8260 (data summary view –analytical method).

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 downward and upward arrows (the analytical methods reported by laboratories A039 and A045 were 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 range of tolerance, which encompasses the target value bounded by twice its uncertainty (U_{NIST}), and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$. The shaded beige region represents the overlapping of the 95 % confidence interval for the consensus mean (green region) and the NIST range of tolerance (red region).

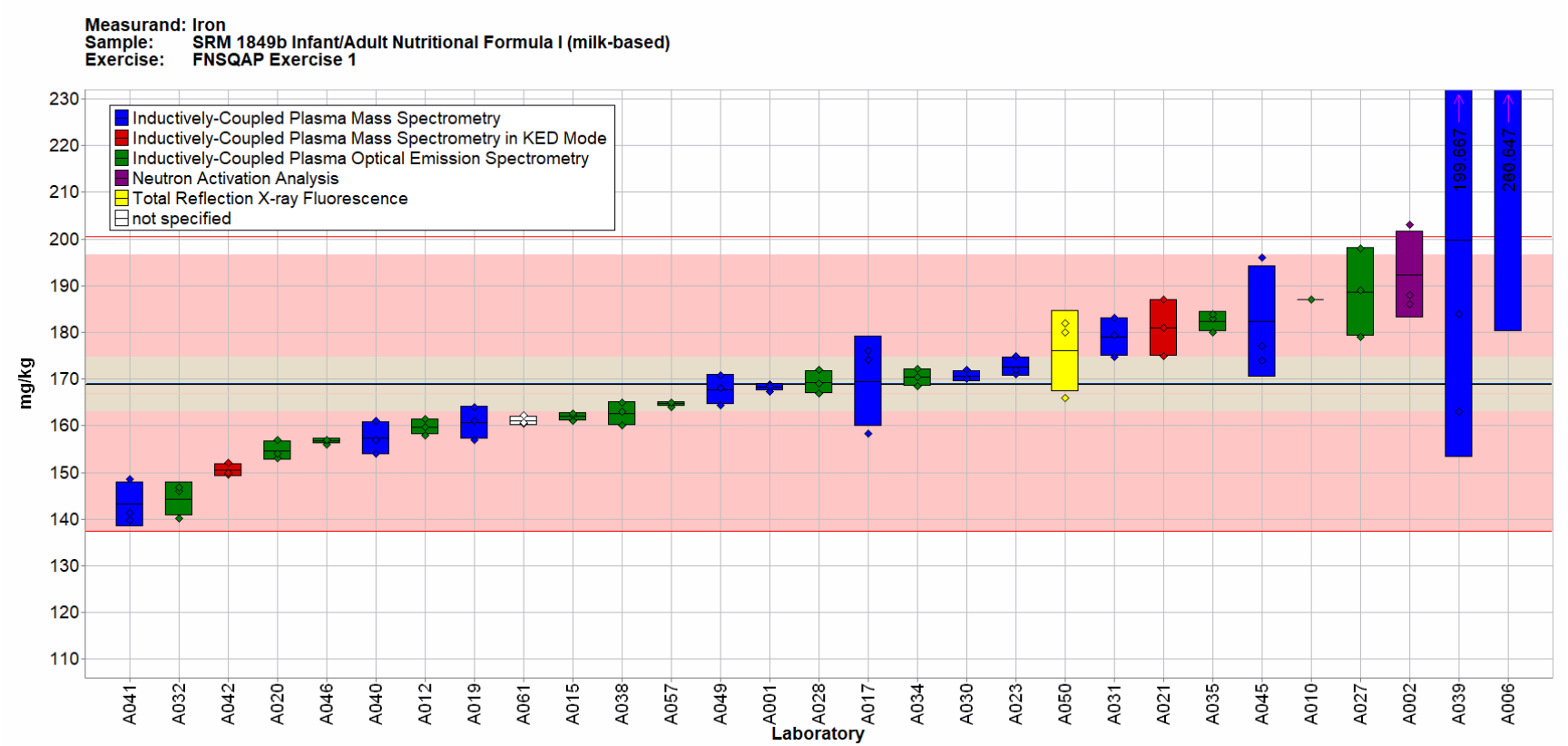

Fig. 2-11. Iron in SRM 1849b (data summary view – analytical method).

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 red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty (U_{NIST}), and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$. The shaded beige region represents the overlapping of the 95 % confidence interval for the consensus mean (green region) and the NIST range of tolerance (red region).

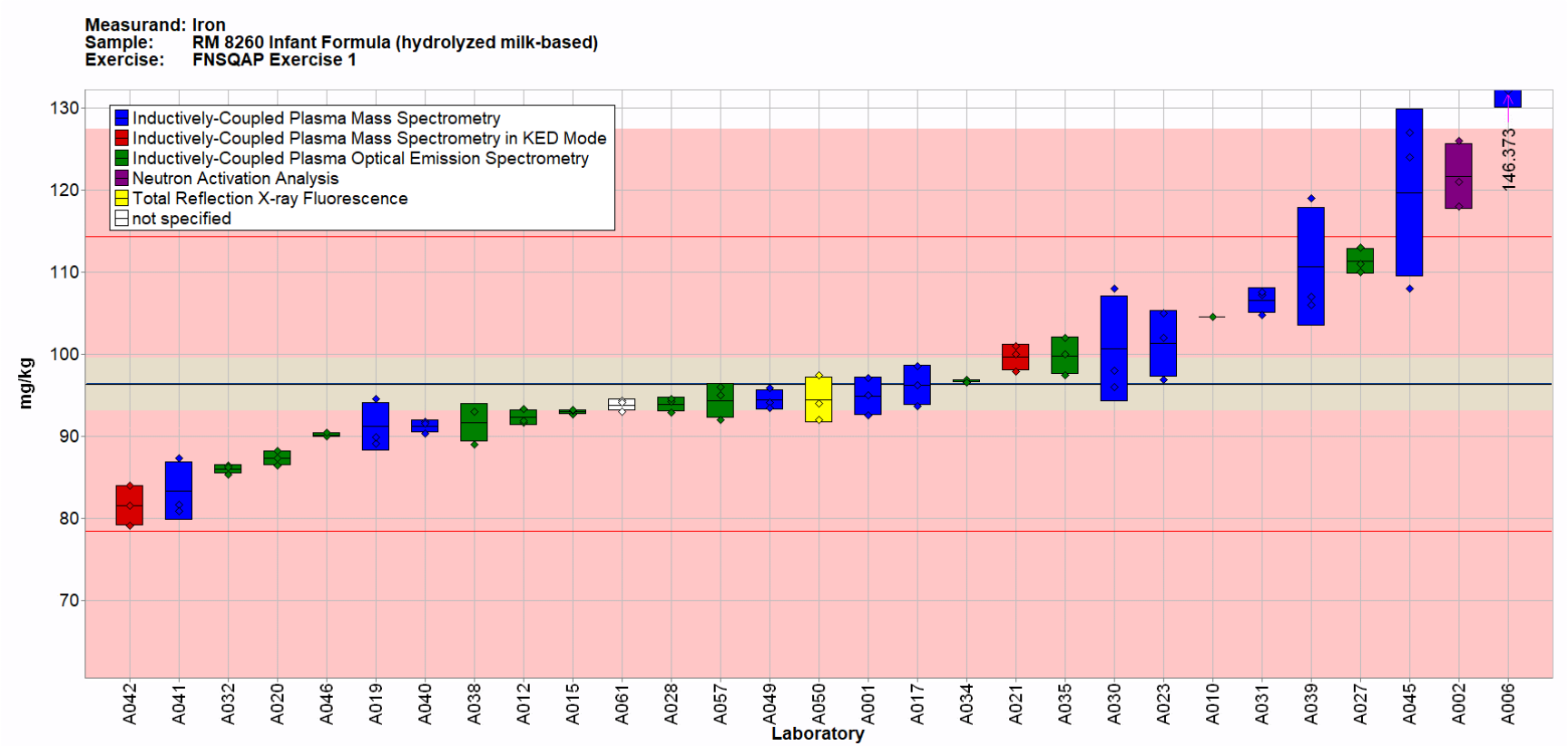

Fig. 2-12. Iron in RM 8260 (data summary view – analytical method).

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 green shaded region represents the 95 % confidence interval for the consensus mean. The solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty (U_{NIST}), and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$. The shaded beige region represents the overlapping of the 95 % confidence interval for the consensus mean (green region) and the NIST range of tolerance (red region).

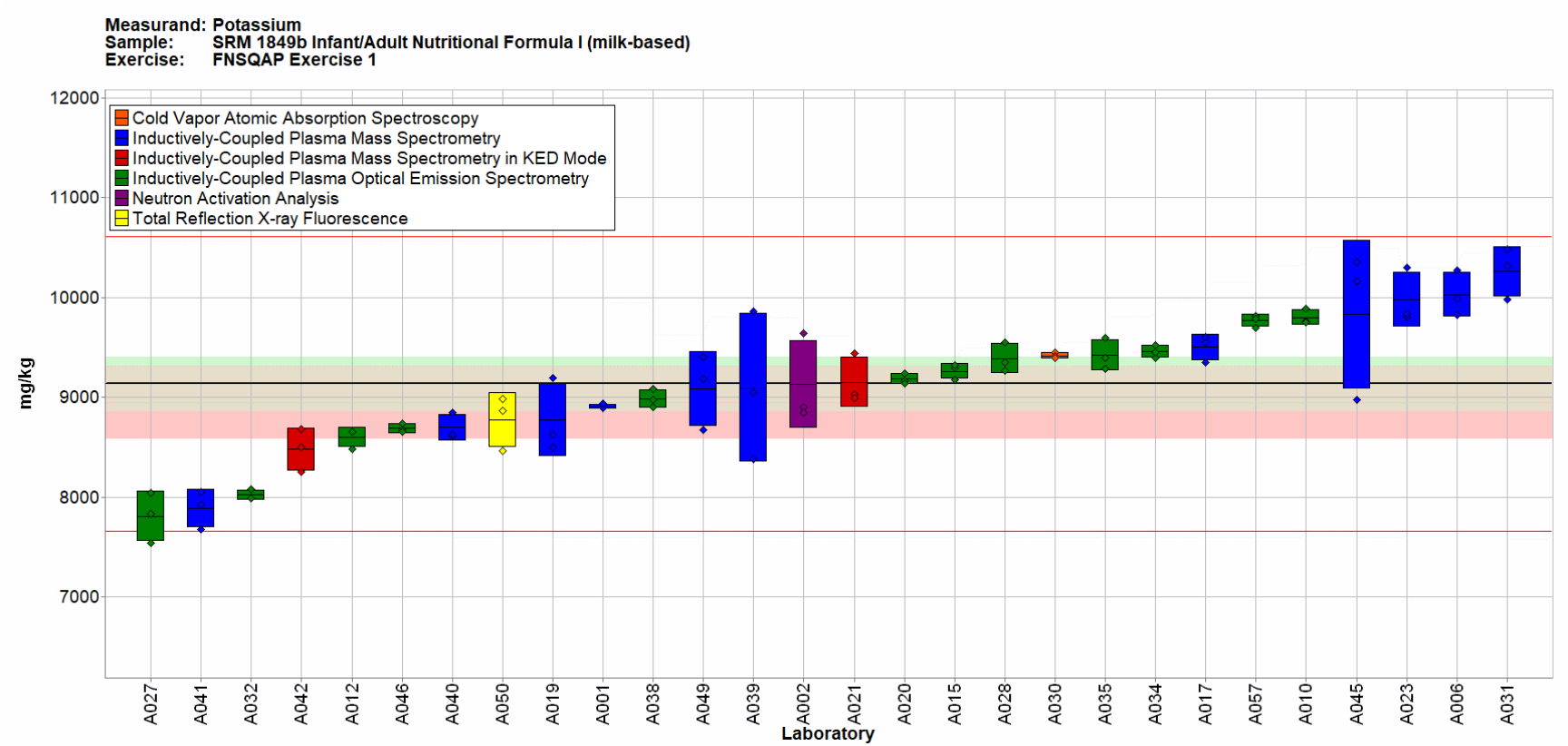

Fig. 2-13. Potassium in SRM 1849b (data summary view – analytical method).

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 red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty (U_{NIST}), and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$. The shaded beige region represents the overlapping of the 95 % confidence interval for the consensus mean (green region) and the NIST range of tolerance (red region).

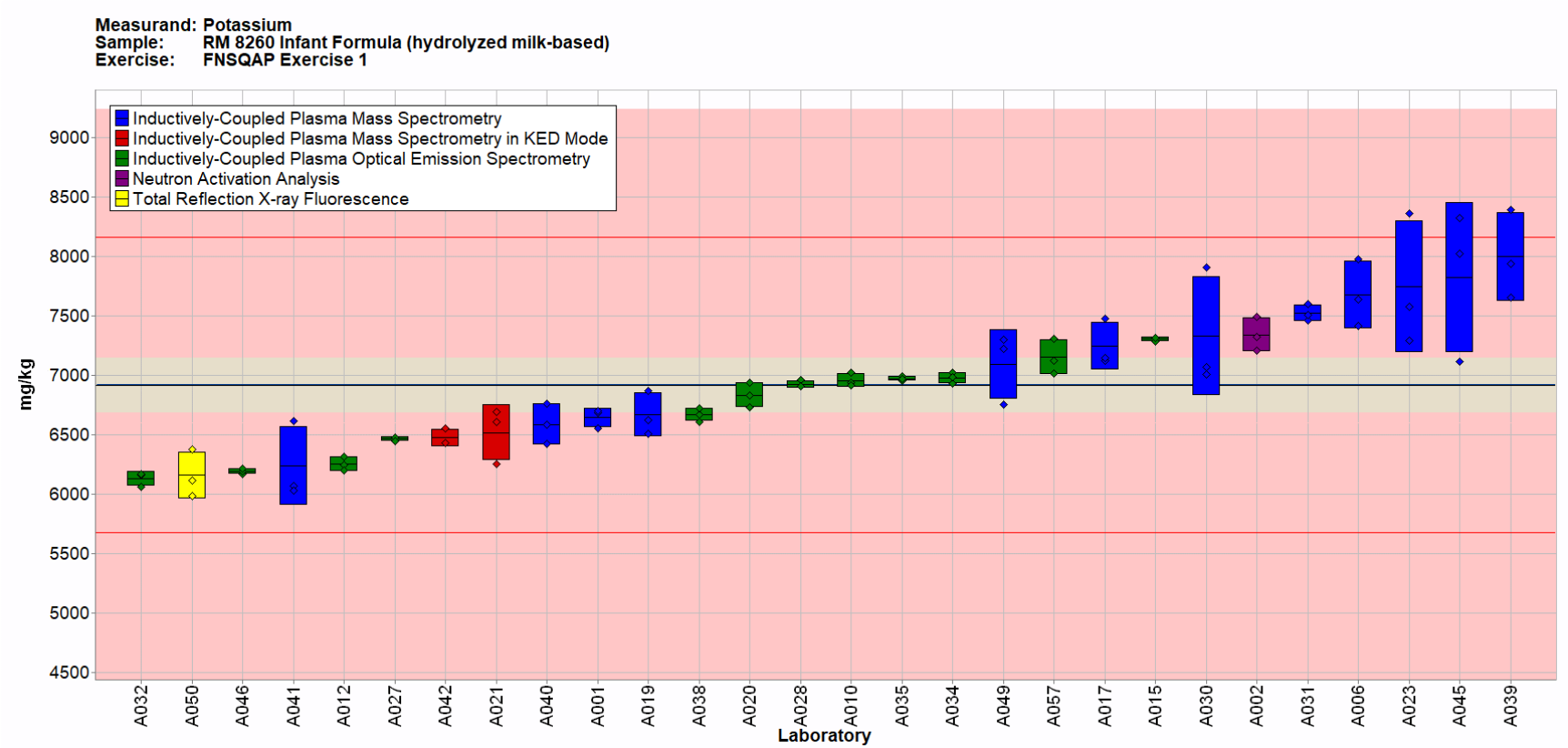

Fig. 2-14. Potassium in RM 8260 (data summary view – analytical method).

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 green shaded region represents the 95 % confidence interval for the consensus mean. The solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty (U_{NIST}), and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$. The shaded beige region represents the overlapping of the 95 % confidence interval for the consensus mean (green region) and the NIST range of tolerance (red region).

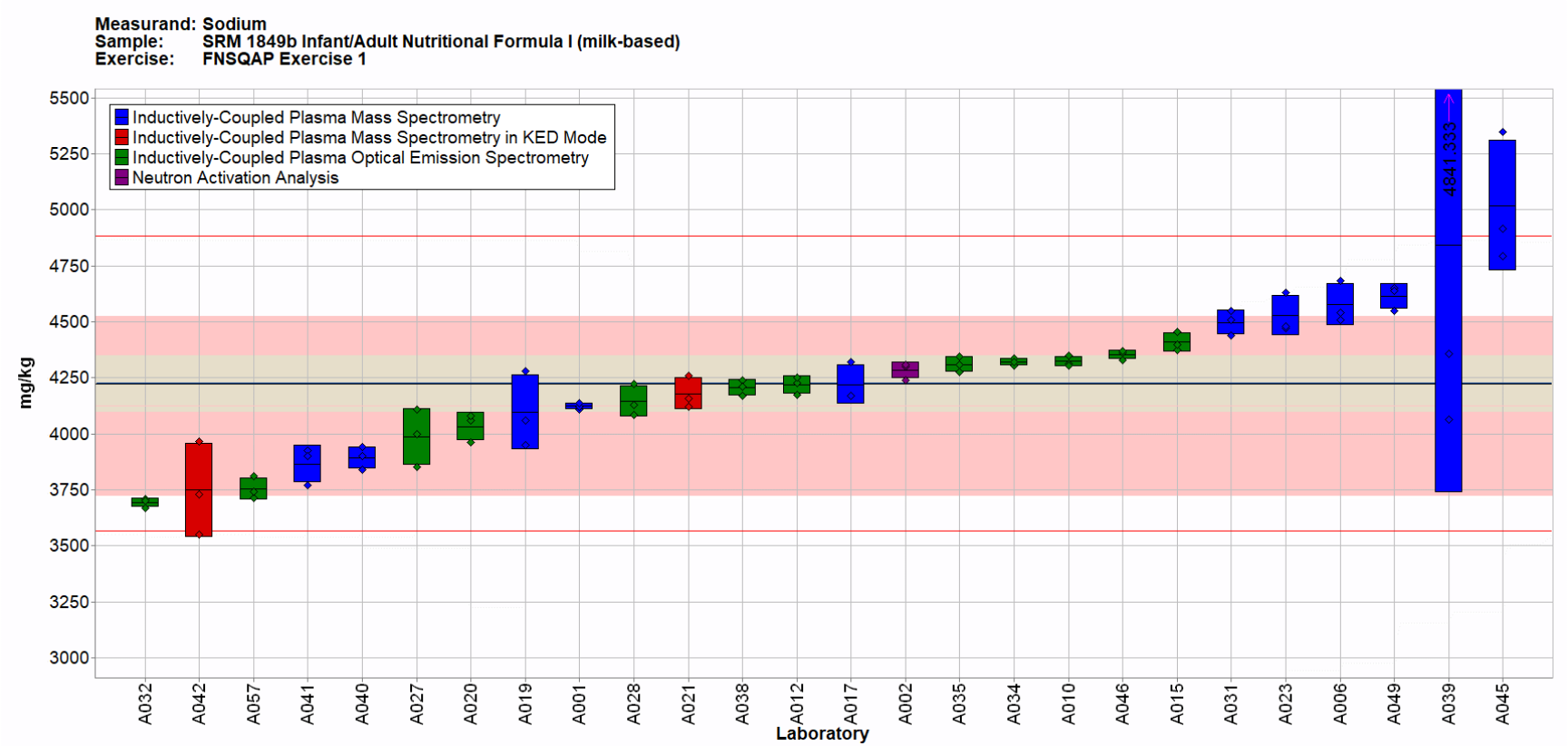

Fig. 2-15. Sodium in SRM 1849b (data summary view – analytical method).

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 red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty (U_{NIST}), and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$. The shaded beige region represents the overlapping of the 95 % confidence interval for the consensus mean (green region) and the NIST range of tolerance (red region).

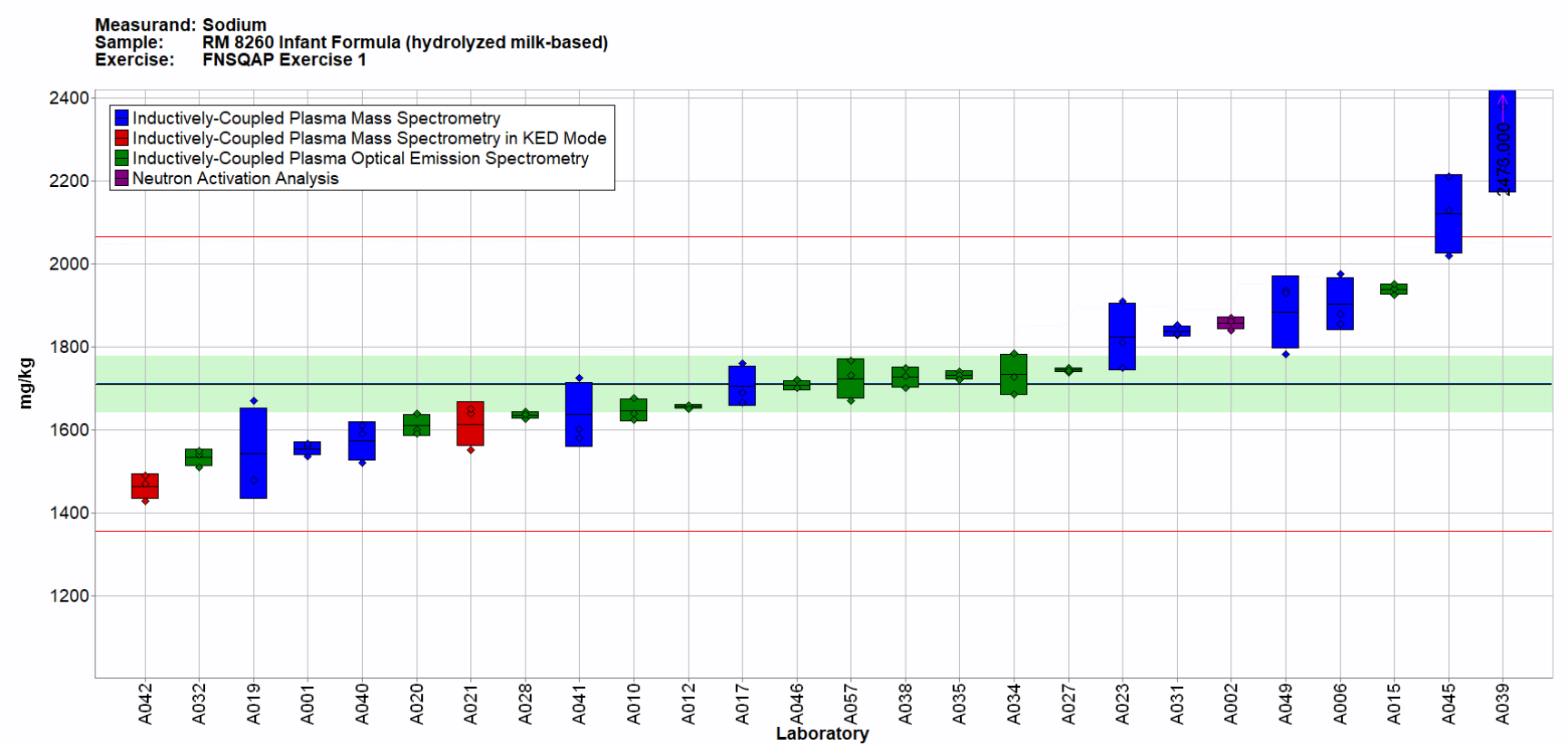

Fig. 2-16. Sodium in RM 8260 (data summary view – analytical method).

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 green shaded region represents the 95 % confidence interval for the consensus mean. The solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$. A NIST value has not been determined in this material.

Overall, laboratories performed well in the measurement of nutritional elements in infant formula samples. All participating laboratories had K measurement averages within the consensus tolerance limits for both samples. A slight positive linear trend is observed in Fig. **2-17**, Fig. **2-18**, Fig. **2-19**, and Fig. **2-20**, which may indicate a global issue with calibration. Laboratories that reported values below the target did so consistently in these two very similar samples, and, likewise, laboratories that reported values above the target did so consistently between the samples. While this trend was consistent between samples, it varied among nutritional elements (i.e., a laboratory was not always above the target value for all elements). All calibration standards should 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 that calibration range and other points can be excluded from the determination of the calibration curve to prevent bias.

NIST has conducted thirteen QAP studies involving measurement of one or more of these nutritional elements in food and supplement samples prior to this FNSQAP study, as shown in Table **2-6**.

Table 2-6. Previous NIST QAP exercises that included nutritional elements studies.

QAP Exercise	Year	Elements	Reference
DSQAP A	2007	Ca, Fe	[5]
DSQAP C	2008	Ca, Na	[5]
DSQAP E	2010	Ca, Fe	[5]
DSQAP F	2011	Na, Fe	[5]
DSQAP G	2011	Na	[6]
DSQAP H	2012	Ca	[7]
DSQAP J	2013	Ca	[8]
DSQAP K	2014	Fe	[9]
DSQAP M	2016	K	[10]
HAMQAP 1	2018	Fe	[11]
HAMQAP 4	2019	Ca, Na, K	[12]
HAMQAP 5	2020	Ca, Na, Fe, K	[13]
HAMQAP 7	2021	Ca	[14]

A review of the results from these previous exercises indicated no apparent trends in the number of laboratories reporting data, average RSD_r, RSD_R, or bias with respect to the NIST target value over time for Ca, Fe, and K. Laboratories have historically performed well measuring Ca and K with repeatability relative standard deviation (within-laboratory variability; RSD_r) below 5 % and reproducibility relative standard deviation (between laboratory variability; RSD_R) below 10 %. The between-laboratory variability of Na measurements has improved significantly over time,

from an average of 29 % in the first three DSQAP studies (2008 to 2011) to an average of 7.2 % in the more recent HAMQAP and FNSQAP studies (2019 to 2021). Interestingly, in all six studies in which Na was included, consensus means were always above the target mean. This high bias of Na measurements is likely the result of contamination or spectral interferences that laboratories are not properly addressing.

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 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 method detection limit (MDL).

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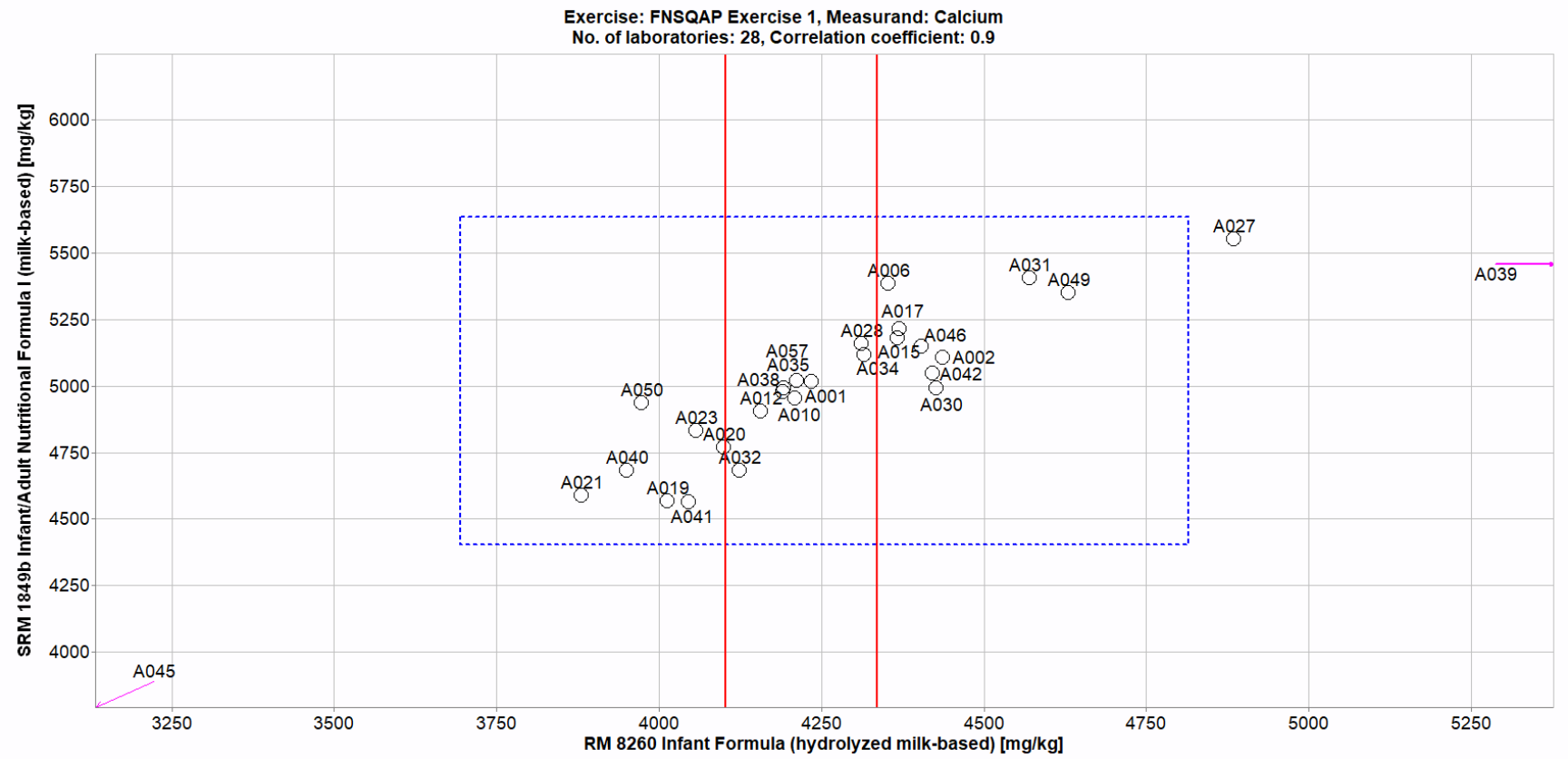

Fig. 2-17. Laboratory means for calcium in RM 8260 and SRM 1849b (sample/sample comparison view).

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 lines represents the NIST range of tolerance for RM 8260 (x-axis), which encompasses the target value bounded by its uncertainty (U_{NIST}), and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$. The NIST range of tolerance for SRM 1849b (y-axis) extends beyond the bounds of the figure. 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, $|Z'_{\text{comm}}| \leq 2$.

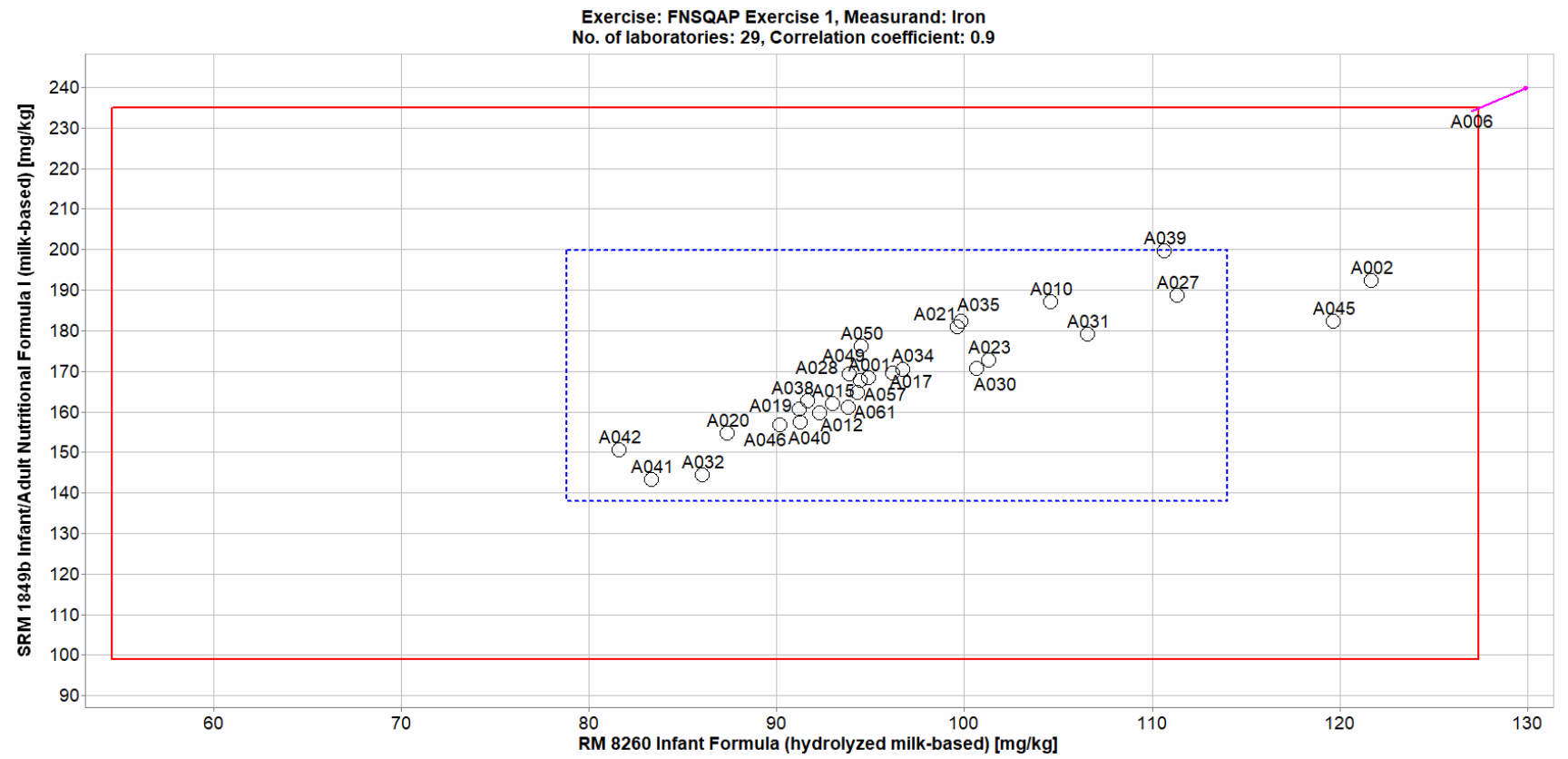

Fig. 2-18. Laboratory means for iron in RM 8260 and SRM 1849b (sample/sample comparison view).

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 range of tolerance 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, $|Z_{\text{NIST}}| \leq 2$. 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, $|Z'_{\text{comm}}| \leq 2$.

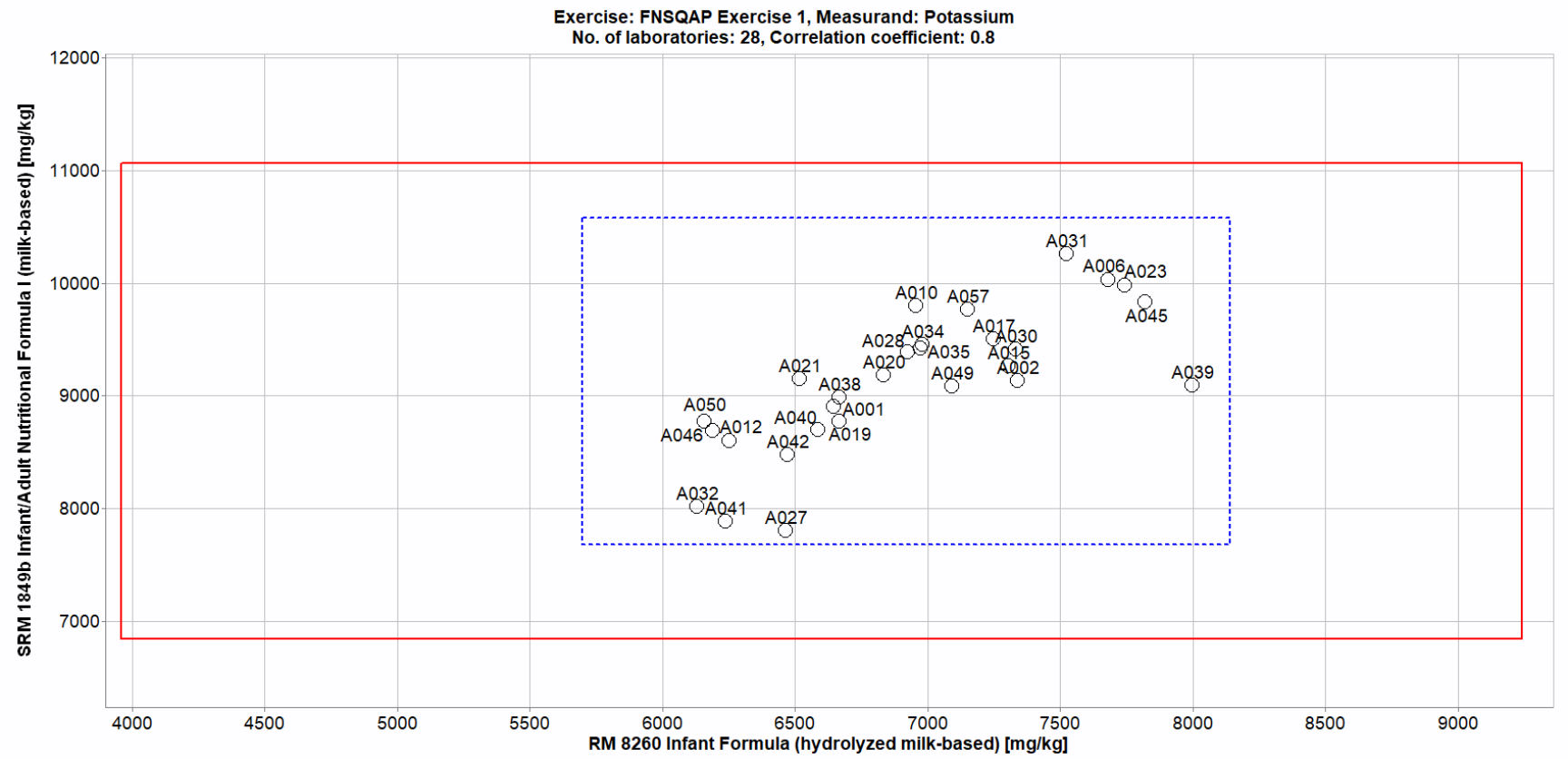

Fig. 2-19. Laboratory means for potassium in RM 8260 and SRM 1849b (sample/sample comparison view).

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 range of tolerance 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, $|Z_{\text{NIST}}| \leq 2$. 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, $|Z'_{\text{comm}}| \leq 2$.

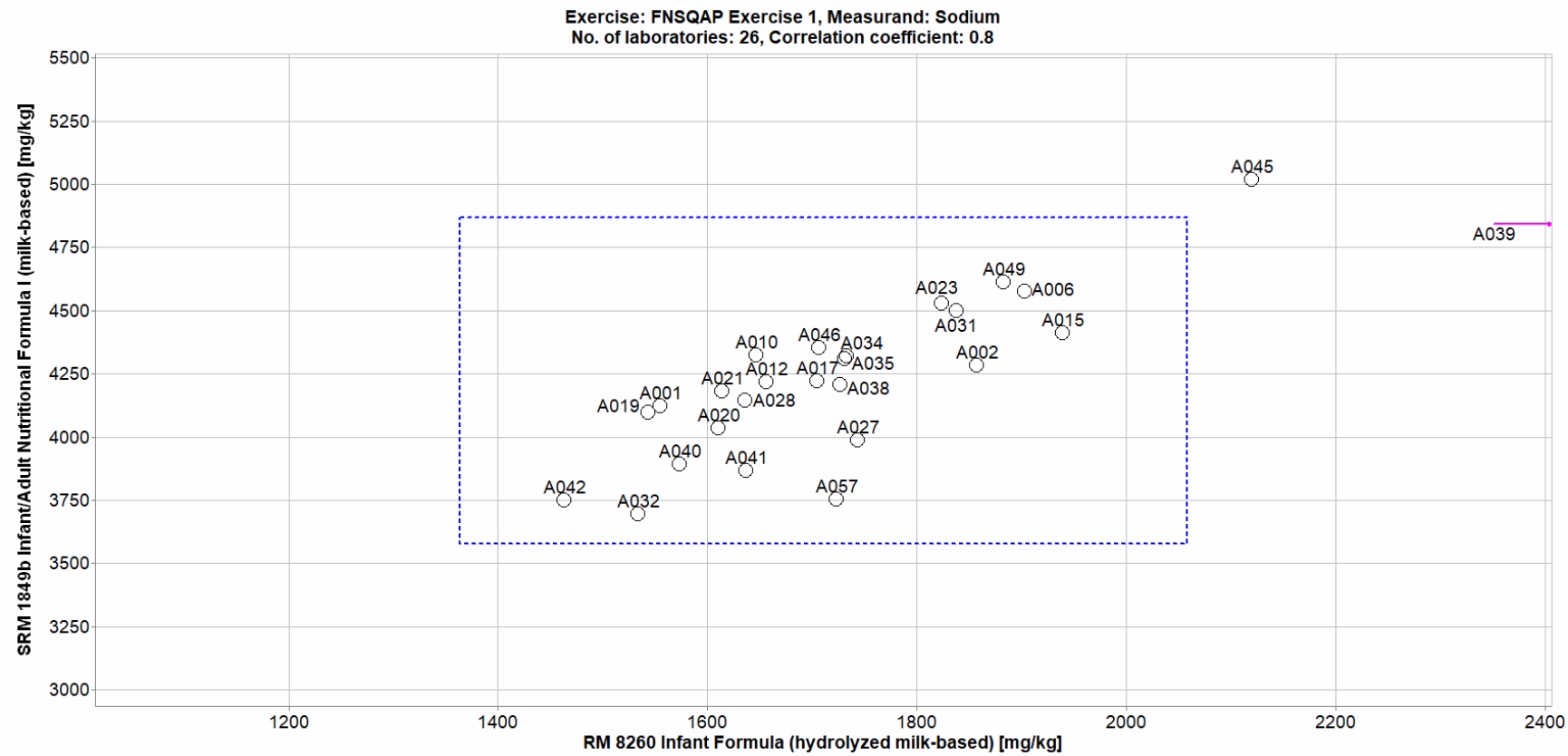

Fig. 2-20. Laboratory means for sodium in RM 8260 and SRM 1849b (sample/sample comparison view).

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 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, $|Z'_{\text{comm}}| \leq 2$. A NIST value has not been determined in this material.

3. TOXIC ELEMENTS (Arsenic, Cadmium, Lead, Mercury)

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. Participants in this study performed well in determination of arsenic and cadmium regarding within-laboratory and among-laboratory measurement reproducibility and overlap of consensus mean ranges with target ranges. Measuring the low levels of lead and mercury present in the baby food samples was a challenge for participants with about half having good within-laboratory reproducibility and overall poor among-laboratory reproducibility. Many laboratories reported qualitative data for lead and mercury providing their LOQ since these elements were present below their method reporting limits. Most participants reported using microwave digestion and acid hydrolysis methods for sample preparation and ICP-MS methods for analysis. 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), cadmium (Cd), lead (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) [15,16]. The FDA has prioritized these toxic elements in food due to the potential harm they can cause during critical times of development for babies and young children [17]. Recent news reports indicate that many common brands and types of baby food may contain higher than allowable levels of toxic elements [18]. Toxic elements can enter food sources from the natural environment in which they are grown and during processing. Because finished food products can have different element levels than the raw ingredients [18], final products were chosen as the samples for this study. The accuracy and precision of measurements made by food laboratories is critical for compliance with regulations from the FDA, United States Department of Agriculture (USDA), and international bodies and to ensure product safety and customer confidence in the food supply. In this study, participants were provided with two samples of baby food composite. Participants were asked to use in-house analytical methods to determine the mass fractions (ng/g) of As, Cd, Pb, and Hg in each baby food 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 Baby Food A and Baby Food B, which were ground materials prepared from a blend of commercially available grain-based infant snacks. Each packet contained approximately 3 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 toxic elements. The approximate analyte levels were not reported to participants prior to the study. Target values for As and Cd in Baby Food A and Baby Food B were determined at NIST using acid-assisted microwave digestion and ICP-MS. The target values and uncertainties for toxic elements in Baby Food A and Baby Food B are provided in Table **3-1** on an as-received basis. The uncertainties for As and Cd for both baby foods were approximated as 5 % relative to the target value. Target values for Pb and Hg in Baby Food A and Baby Food B were not available at the time of this report.

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

(Lab Name)

Exercise 1 – Toxic Elements in Baby Food

	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*
As	Baby Food A	ng/g	*Individual laboratory results will appear in this section; laboratory-specific results were provided to each participant separately from this report.*				33	34.0	3.9	26.7	2.7
As	Baby Food B	ng/g					33	160	15	132	13
Cd	Baby Food A	ng/g					33	62.0	5.7	52.1	5.2
Cd	Baby Food B	ng/g					30	11.0	1.4	8.89	0.89
Pb	Baby Food A	ng/g					25	9.1	5.7		
Pb	Baby Food B	ng/g					25	6.7	5.7		
Hg	Baby Food A	ng/g					17	0.7	1.1		
Hg	Baby Food B	ng/g					18	1.20	0.71		

x_i Mean of reported values
s_i Standard deviation of reported values
Z'_{comm} Z'-score with respect to community consensus
Z_{NIST} Z-score with respect to NIST value
N Number of quantitative values reported
x* Robust mean of reported values
s* Robust standard deviation
x_{NIST} NIST-assessed value
U expanded uncertainty about the NIST-assessed value

3.4. Study Results and Discussion

Table **3-1** summarizes and Table **3-2**, Table **3-3**, Table **3-4**, and Table **3-5** detail the numerical results reported by each participating laboratory for toxic elements. The participation level was high for toxic elements, with 71 % to 76 % of laboratories requesting samples returning results (on average 34 of 46 laboratories).

Table **3-2** reveals that of the 35 participants that submitted results for As, 2 laboratories reported data as below LOQ for both samples. The within-laboratory variabilities were mostly acceptable for As with respect to published expectations of the measurement community of 15 % RSD for toxic elements that range ≥ 8 ng/g (ppb) to 100 ng/g (Baby Food A) and 11 % RSD for toxic elements that range > 100 ng/g to 1 µg/g (ppm) (Baby Food B) in food [19]. Two laboratories had within-laboratory variabilities greater than 15 % RSD for Baby Food A, and 4 laboratories had within-laboratory variabilities greater than 11 % RSD for Baby Food B. One laboratory reported the same value for all three replicates for each sample so the % RSD was not calculated.

Table 3-2. Data summary table for arsenic in Baby Food A and Baby Food B.
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 NIST values and consensus values are included on both pages for convenience.*

		Arsenic									
		Baby Food A (ng/g)					Baby Food B (ng/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target				26.7	2.7				132.3	13.2
	A001	32	36	32	33.3	2.3	149	154	151	151.3	2.5
	A002	24	31	39	31.3	7.5	149	131	114	131.3	17.5
	A003	34.3	34.3	33	33.9	0.8	162.9	167.9	162.9	164.6	2.9
	A004										
	A005										
	A006	75.79	71.76	73.76	73.8	2.0	338.59	336.2	337.87	337.6	1.2
	A007	34	33.7	33	33.6	0.5	166	169	166	167.0	1.7
	A009	32	33.9	37.5	34.5	2.8	153.3	160.4	164.3	159.3	5.6
	A010	34.8	36.3	36.8	36.0	1.0	179.7	179.7	180.3	179.9	0.3
	A012	30.99	32.11	31.78	31.6	0.6	166.8	169.74	178.23	171.6	5.9
	A014	45	45	40	43.3	2.9	155	165	165	161.7	5.8
	A015	31.5	32	35	32.8	1.9	148.5	153.5	156	152.7	3.8
	A017	29.7	27.5	27.5	28.2	1.3	132.9	143.8	145	140.6	6.7
	A018										
	A019	40	40	40	40.0	0.0	170	170	170	170.0	0.0
	A020	30	31.7	30.1	30.6	1.0	150	153	154	152.3	2.1
	A021	33.07	33.83	33.74	33.5	0.4	163.76	169.51	158.15	163.8	5.7
	A022										
	A023	34.6	31.6	30.7	32.3	2.0	169	153	147	156.3	11.4
	A024	33.7	34.9	33.8	34.1	0.7	172	171	172	171.7	0.6
	A027	35	34	34	34.3	0.6	165	165	170	166.7	2.9
	A028	34.6	34.8	37.2	35.5	1.4	174.8	182.9	168.5	175.4	7.2
	A031	35.22	36.24	32.42	34.6	2.0	153.62	154.14	160.21	156.0	3.7
	A032	49.8	40.9	32.8	41.2	8.5	137.4	164.1	184	161.8	23.4
	A034	30	29	31	30.0	1.0	168	175	179	174.0	5.6
	A035	35.94	36.88	36.72	36.5	0.5	172.3	183.4	182.7	179.5	6.2
	A036										
	A037										
	A038	34	32	32	32.7	1.2	155	160	161	158.7	3.2
Community Results		Consensus Mean			33.7		Consensus Mean			161.5	
		Consensus Standard Deviation			3.9		Consensus Standard Deviation			15.1	
		Maximum			209333		Maximum			341000	
		Minimum			25.8		Minimum			131.3	
		N			33		N			33	

Table 3-2 continued. Data summary table for arsenic in Baby Food A and Baby Food B. Data highlighted in blue have been identified as outside the consensus tolerance limits and resulted in an unacceptable Z'_{comm} score, $|Z'_{comm}| > 2$.

		Arsenic									
		Baby Food A (ng/g)					Baby Food B (ng/g)				
		A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target				26.7	2.7				132.3	13.2
	A039	210000	206000	212000	209333	3055	442000	340000	241000	341000	100504
	A040	< 500	< 500	< 500			< 500	< 500	< 500		
	A041	< 0.001	< 0.001	< 0.001			< 0.001	< 0.001	< 0.001		
	A042	36.3	39.3	44.5	40.0	4.1	159.2	203.5	186.9	183.2	22.4
	A043	32	33	34	33.0	1.0	165	157	152	158.0	6.6
	A045	34.33	33.523	30.303	32.7	2.1	132.352	131.732	130.891	131.7	0.7
	A046	33.2	33.8	33.8	33.6	0.3	166.4	165	164.8	165.4	0.9
	A047										
	A049	30.479	30.847	29.705	30.3	0.6	153	149.7	158.2	153.6	4.3
	A050	28.1	26.6	22.6	25.8	2.8	156	152	156	154.7	2.3
	A052	36.7	34.5	33.2	34.8	1.8	164	161	160	161.7	2.1
	A054										
	A055										
	A056	34	34.2	33.5	33.9	0.4	170	169	170	169.7	0.6
	A057	28.5	31.6	34.7	31.6	3.1	154	145	143	147.3	5.9
	A058										
	A060										
Community Results		Consensus Mean			33.7		Consensus Mean			161.5	
		Consensus Standard Deviation			3.9		Consensus Standard Deviation			15.1	
		Maximum			209333		Maximum			341000	
		Minimum			25.8		Minimum			131.3	
		N			33		N			33	

The between-laboratory variabilities for As in both Baby Food A (12 % RSD) and Baby Food B (9 % RSD) fell well below the published expectations of the measurement community of 32 % RSD and 16 % RSD, respectively, even with participants using a variety of methods [19].

Table **3-3** reveals that of the 35 participants that submitted results for Cd, 2 laboratories reported data as below LOQ for both samples and 3 additional laboratories reported data as on LOQ value for Baby Food B. The within-laboratory variabilities for Cd were all acceptable for Baby Food B, and only 1 laboratory was greater than 15 % RSD for Baby Food A with respect to published expectations of the measurement community for toxic elements that range ≥ 8 ng/g to 100 ng/g in food [19]. One laboratory for Baby Food A and two laboratories for Baby Food B reported the same value for all three replicates of the sample so the % RSD was not calculated. The between-laboratory variabilities for Cd in both baby food samples (average 11 % RSD) fell well below the published expectations of the measurement community of 32 % RSD even with participants using a variety of methods [19].

Table 3-3. Data summary table for cadmium in Baby Food A and Baby Food B.
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 NIST values and consensus values are included on both pages for convenience.*

		Cadmium									
		Baby Food A (ng/g)					Baby Food B (ng/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target				52.1	5.2				8.9	0.9
	A001	60	61	62	61.0	1.0	11	10	11	10.7	0.6
	A002	< 210	< 210	< 210			< 380	< 380	< 380		
	A003	64.3	61.4	63.4	63.0	1.5	10.7	11.1	10.7	10.8	0.2
	A004										
	A005										
	A006	62.9	62.15	62.97	62.7	0.5	12.65	12.67	12.98	12.8	0.2
	A007	64.2	62.6	61.4	62.7	1.4	10.8	10.7	11.1	10.9	0.2
	A009	63.8	64.5	64.3	64.2	0.4	11.3	11.6	12.2	11.7	0.5
	A010	64.4	64.5	67.1	65.3	1.5	10.3	11.4	11.3	11.0	0.6
	A012	55.53	61.92	54.25	57.2	4.1	12.05	10.16	11.15	11.1	0.9
	A014	60	60	60	60.0	0.0	< 40	< 40	< 40		
	A015	67	68	69	68.0	1.0	14	14	14	14.0	0.0
	A017	52.7	50.7	55.1	52.8	2.2	9.2	10.1	9.7	9.7	0.5
	A018										
	A019	60	70	70	66.7	5.8	10	10	10	10.0	0.0
	A020	57.2	58.4	57.5	57.7	0.6	12.6	12.2	11.6	12.1	0.5
	A021	66.6	68.38	67.18	67.4	0.9	9.99	10.01	9.83	9.9	0.1
	A022										
	A023	71.9	69	67.8	69.6	2.1	13.1	12.6	13.4	13.0	0.4
	A024	65.7	65.9	65	65.5	0.5	11.4	11.3	11.2	11.3	0.1
	A027	57	58	57	57.3	0.6	10	10.5	10.5	10.3	0.3
	A028	64.2	64.5	68.5	65.7	2.4	11.2	11.9	10.9	11.3	0.5
	A031	62.3	66.02	62.42	63.6	2.1	< 25	< 25	< 25		
	A032	73.5	50.3	57	60.3	11.9	10.8	10.1	12.8	11.2	1.4
	A034	55.9	55.9	58.5	56.8	1.5	9.4	9.2	10	9.5	0.4
	A035	62.61	64.02	63.36	63.3	0.7	12.04	10.54	11.19	11.3	0.8
	A036										
	A037										
	A038	59	59	54	57.3	2.9	10	< 10	< 10	10.0	
Community Results		Consensus Mean			61.9		Consensus Mean			11.0	
		Consensus Standard Deviation			5.7		Consensus Standard Deviation			1.4	
		Maximum			67667		Maximum			17667	
		Minimum			49.8		Minimum			6.7	
		N			33		N			29	

Table 3-3 continued. Data summary table for cadmium in Baby Food A and Baby Food B. Data highlighted in blue have been identified as outside the consensus tolerance limits and resulted in an unacceptable Z'_{comm} score, $|Z'_{comm}| > 2$.

		Cadmium									
		Baby Food A (ng/g)					Baby Food B (ng/g)				
		A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target				52.1	5.2				8.9	0.9
	A039	67000	69000	67000	67667	1155	20000	16000	17000	17667	2082
	A040	< 500	< 500	< 500			< 500	< 500	< 500		
	A041	49.984	52.941	46.476	49.8	3.2	7.291	7.242	7.557	7.4	0.2
	A042	71.53	57.02	64.39	64.3	7.3	13.43	11.14	10.52	11.7	1.5
	A043	64	57	59	60.0	3.6					
	A045	57.149	55.364	53.224	55.2	2.0	10.241	12.297	11.377	11.3	1.0
	A046	62.8	63.1	63.4	63.1	0.3	11.5	11	10.9	11.1	0.3
	A047										
	A049	61.586	58.31	58.501	59.5	1.8	10.158	10.41	10.816	10.5	0.3
	A050	59.3	63.6	57.1	60.0	3.3	6.8	6.6	6.6	6.7	0.1
	A052	64.6	63.9	64.7	64.4	0.4	11.6	11.3	11.4	11.4	0.2
	A054										
	A055										
	A056	66.9	66.2	69.7	67.6	1.9	11.2	11.2	11.8	11.4	0.3
	A057	65	65.7	62.7	64.5	1.6	11.3	11.3	10.5	11.0	0.5
	A058										
	A060										
Community Results		Consensus Mean			61.9		Consensus Mean			11.0	
		Consensus Standard Deviation			5.7		Consensus Standard Deviation			1.4	
		Maximum			67667		Maximum			17667	
		Minimum			49.8		Minimum			6.7	
		N			33		N			29	

Table **3-4** reveals that of the 33 participants that submitted results for Pb, 8 laboratories reported data as below LOQ for both samples. About 60 % of participants submitting quantitative Pb results had within-laboratory variabilities acceptable in both baby foods with respect to published expectations of the measurement community of 15 % RSD for toxic elements that range ≥ 8 ng/g to 100 ng/g in food [19]. The between-laboratory variabilities for Pb in both baby food samples were greatly above (average 75 % RSD) the published expectations of the measurement community of 32 % RSD [19].

Table 3-4. Data summary table for lead in Baby Food A and Baby Food B.
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 NIST values and consensus values are included on both pages for convenience.*

		Lead									
		Baby Food A (ng/g)					Baby Food B (ng/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target										
	A001	< 10	< 10	< 10			< 10	< 10	< 10		
	A002										
	A003	6.7	7.1	7.5	7.1	0.4	3.6	4.3	3.7	3.9	0.4
	A004										
	A005										
	A006	29.23	24.95	44.21	32.8	10.1	22.31	22.05	31.65	25.3	5.5
	A007	6.93	6.57	6.61	6.7	0.2	3.74	3.64	4.03	3.8	0.2
	A009	4.3	10.3	7.2	7.3	3.0	5.4	5.6	9.3	6.8	2.2
	A010	4.3	3.3	3.2	3.6	0.6	0.2	1E-10	0.1	0.1	0.1
	A012	28.45	30.81	31.41	30.2	1.6	30.69	26.91	27.75	28.5	2.0
	A014	< 40	< 40	< 40			< 40	< 40	< 40		
	A015	14	14	15	14.3	0.6	10	10	11	10.3	0.6
	A017	6	5.5	6.2	5.9	0.4	4.1	3.3	4	3.8	0.4
	A018										
	A019	< 10	< 10	< 10			< 10	< 10	< 10		
	A020	11.1	12	13.1	12.1	1.0	8.52	9.38	7.1	8.3	1.2
	A021	6.15	5.8	6.07	6.0	0.2	2.84	2.77		2.8	0.0
	A022										
	A023	< 1.75	< 1.75	< 1.75			< 1.75	< 1.75	< 1.75		
	A024	10.1	14.6	7.07	10.6	3.8	6.41	14.1	4.5	8.3	5.1
	A027	12	12	12.5	12.2	0.3	10	9.5	9.5	9.7	0.3
	A028	6.43	6.81	6.74	6.7	0.2	3.59	3.68	3.38	3.6	0.2
	A031	< 25	< 25	< 25			< 25	< 25	< 25		
	A032	14.9	10.8	12	12.6	2.1	9.2	9.8	7.9	9.0	1.0
	A034	< 8	< 8	< 8			< 8	< 8	< 8		
	A035	11.02	10.72	11.94	11.2	0.6	9.85	8.7	9.19	9.2	0.6
	A036										
	A037										
	A038	< 10	< 10	< 10			< 10	< 10	< 10		
Community Results		Consensus Mean			9.1		Consensus Mean			6.7	
		Consensus Standard Deviation			5.7		Consensus Standard Deviation			5.7	
		Maximum			249667		Maximum			136333	
		Minimum			2.8		Minimum			0.1	
		N			25		N			25	

Table 3-4 continued. Data summary table for lead in Baby Food A and Baby Food B. 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									
		Baby Food A (ng/g)					Baby Food B (ng/g)				
		A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target										
	A039	149000	257000	343000	249666	97207	254000	50000	105000	136333	105547
	A040	< 500	< 500	< 500			< 500	< 500	< 500		
	A041	2.344	2.323	3.594	2.8	0.7	0.489	0.584	< 0.001	0.5	0.1
	A042	15.07	6.69	10.88	10.9	4.2	8.33	18.72	10.82	12.6	5.4
	A043										
	A045	86.388	62.411	68.677	72.5	12.4	47.565	49.048	45.469	47.4	1.8
	A046	7.4	6.3	6.9	6.9	0.6	3.8	3.7	4.1	3.9	0.2
	A047										
	A049	7.151	6.174	5.913	6.4	0.7	3.252	3.458	3.02	3.2	0.2
	A050	30	9	28.8	22.6	11.8	39	25.9	8.1	24.3	15.5
	A052	6.03	6.98	6.95	6.7	0.5	3.87	4.87	4.2	4.3	0.5
	A054										
	A055										
	A056	7.2	7.5	7.3	7.3	0.2	4.3	3.8	6.9	5.0	1.7
	A057	10.7	9.8	9.5	10.0	0.6	5.8	5	4.3	5.0	0.8
	A058										
	A060										
Community Results		Consensus Mean			9.1		Consensus Mean			6.7	
		Consensus Standard Deviation			5.7		Consensus Standard Deviation			5.7	
		Maximum			249667		Maximum			136333	
		Minimum			2.8		Minimum			0.1	
		N			25		N			25	

Table **3-5** reveals that of the 32 participants that submitted results for Hg, about half of the laboratories reported data as below LOQ for both samples. Additionally, a few laboratories reported the same value for sample replicates or values as 0 ng/g Hg in the baby food samples and zero is not a quantity that can be measured; results below detection limits should be reported as such. The within-laboratory variability (% RSD) was not calculated for these laboratories. About 50 % of participants submitting quantitative Hg results had within-laboratory variabilities acceptable in both baby foods with respect to published expectations of the measurement community of 15 % RSD for toxic elements that range ≥ 8 ng/g to 100 ng/g in food [19]. The between-laboratory variabilities for Hg in both baby food samples were greatly above (Baby Food A, 148 % RSD; Baby Food B, 58 % RSD) the published expectations of the measurement community of 32 % RSD [19].

Table 3-5. Data summary table for mercury in Baby Food A and Baby Food B. 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 resulted in an unacceptable Z'_{comm} score, $|Z'_{comm}| > 2$. *Note: This table spans two pages; the NIST values and consensus values are included on both pages for convenience.*

		Mercury									
		Baby Food A (ng/g)					Baby Food B (ng/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target										
	A001	6	< 5	< 5	6.00		< 5	< 5	< 5		
	A002	< 300	< 300	< 300			< 300	< 300	< 300		
	A003	0	0	0	0.00	0.00	0	0	0	0.00	0.00
	A004										
	A005										
	A006	13.82	5.51	0	6.44	6.96	1.24	0	0	0.41	0.72
	A007	< 0.8	< 0.8	< 0.8			1.09	1.14	1.18	1.14	0.05
	A009	0	1.6	2	1.20	1.06	3.1	4.1	2.8	3.33	0.68
	A010	7.1	4	3.6	4.90	1.92	5.1	4.6	3.8	4.50	0.66
	A012	< 10	< 10	< 10			< 10	< 10	< 10		
	A014	< 40	< 40	< 40			< 40	< 40	< 40		
	A015	< 1	< 1	< 1			1.2	1.2	1.2	1.20	0.00
	A017	0.41	0.39	0.42	0.41	0.02	1.09	1.17	1.07	1.11	0.05
	A018										
	A019	< 10	< 10	< 10			< 10	< 10	< 10		
	A020	1.97	1.07	0.917	1.32	0.57	1.54	1.44	1.54	1.51	0.06
	A021	0.56	0.48	0.48	0.51	0.05	1.13	1.14	1.15	1.14	0.01
	A022										
	A024	< 1.2	< 1.2	< 1.2			< 1.2	< 1.2	< 1.2		
	A027	< 4	< 4	< 4			< 4	< 4	< 4		
	A028	0.455	0.398	0.404	0.42	0.03	1.17	1.26	1.01	1.15	0.13
	A031	< 25	< 25	< 25			< 25	< 25	< 25		
	A032										
	A034	< 10	< 10	< 10			< 10	< 10	< 10		
	A035	< 9	< 9	< 9			< 9	< 9	< 9		
	A036										
	A037										
	A038	< 10	< 10	< 10			< 10	< 10	< 10		
	A039	361000	484000	452000	432333	63815	1000	1000	1000	1000	0
Community Results		Consensus Mean			0.71		Consensus Mean			1.22	
		Consensus Standard Deviation			1.05		Consensus Standard Deviation			0.71	
		Maximum			432333		Maximum			1000	
		Minimum			0.00		Minimum			0.00	
		N			16		N			18	

Table 3-5 continued. Data summary table for mercury in Baby Food A and Baby Food B. 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 resulted in an unacceptable Z'_{comm} score, $|Z'_{comm}| > 2$.

		Mercury									
		Baby Food A (ng/g)					Baby Food B (ng/g)				
		A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target										
	A040	< 500	< 500	< 500			< 500	< 500	< 500		
	A041	0.266	0.307	0.425	0.33	0.08	1.865	1.354	0.844	1.35	0.51
	A042	< 5	< 5	< 5			< 5	< 5	< 5		
	A043										
	A045	13.362	13.004	11.597	12.65	0.93	9.065	10.57	10.756	10.13	0.93
	A046	0.7	0.6	0.5	0.60	0.10	1.2	1.2	1.2	1.20	0.00
	A047										
	A049	1.389	1.341	1.116	1.28	0.15	1.713	1.291	1.782	1.60	0.27
	A050	1.8	0.6	< 0.1	1.20	0.85	2.7	0.1	< 0.1	1.40	1.84
	A052	0	0	0	0.00	0.00	1.1	0.96	1.45	1.17	0.25
	A054										
	A055										
	A056	< 2.7	< 2.7	< 2.7			< 2.7	< 2.7	< 2.7		
	A057	0.734	0.684	0.723	0.71	0.03	1.45	1.57	1.59	1.54	0.08
	A058										
	A060										
Community Results		Consensus Mean			0.71		Consensus Mean			1.22	
		Consensus Standard Deviation			1.05		Consensus Standard Deviation			0.71	
		Maximum			432333		Maximum			1000	
		Minimum			0.00		Minimum			0.00	
		N			16		N			18	

The very low levels of toxic elements in the baby food samples are 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**, Fig. **3-4**, Fig. **3-5**, Fig. **3-6**, Fig. **3-7**, and Fig. **3-8**, laboratories reported using a variety of sample preparation methods for the determination of toxic elements in the two baby food samples. Numbers and percentages of laboratories described as reporting specific approaches are averages across all results for four elements and two samples. The most common sample preparation approach was microwave digestion (20 laboratories, 59 %) followed by acid hydrolysis (10 laboratories, 30 %), and 1 laboratory each reported use of hot block digestion, open beaker digestion, and no sample preparation (3 % each). One laboratory (3 % each) for Cd and Pb and 2 laboratories (6 %) for Hg did not report the sample preparation approach used. The sample preparation procedure is critical for unbiased measurements. Participants that reported use of concentrated acid 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. Arsenic and Hg are volatile, however, and can be lost during sample preparation. Vigorous microwave digestion should convert all volatile organoarsenic species to arsenic acid (As(V)) and at this point subsequent heating will not result in loss of As. Inadvertent vessel venting and open vessel digestions can lead to loss of Hg and to loss of As species prior to conversion to As(V). 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 $PbCl_2$ precipitate. If HCl is used in digestion, then repeated washings of the side of the digestion vessel with dilute nitric acid (HNO_3) may redissolve the $PbCl_2$ 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. Additionally, low mass fractions of Hg are not stable in solution over time. Adding some HCl (3 % to 5 %) to low-level Hg solutions can help with stability, but these samples should be analyzed near to preparation.

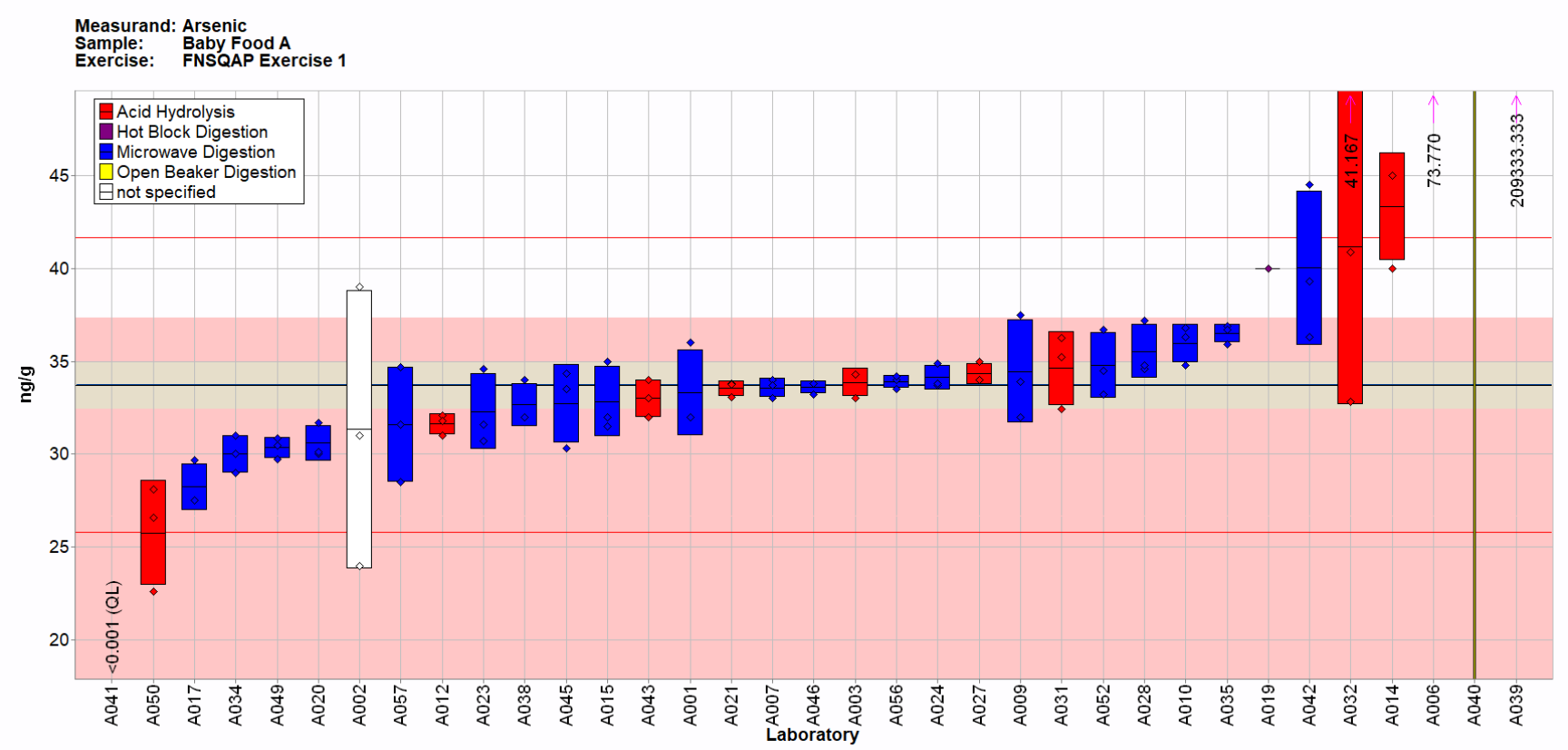

Fig. 3-1. Arsenic in Baby Food A (data summary view – sample preparation method).

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 downward and upward arrows (the preparation methods reported by laboratories A006 and A041 were acid hydrolysis and laboratory 039 was 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, $|Z'_{\text{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty (U_{NIST}), and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$. The shaded beige region represents the overlapping of the 95 % confidence interval for the consensus mean (green region) and the NIST range of tolerance (red region).

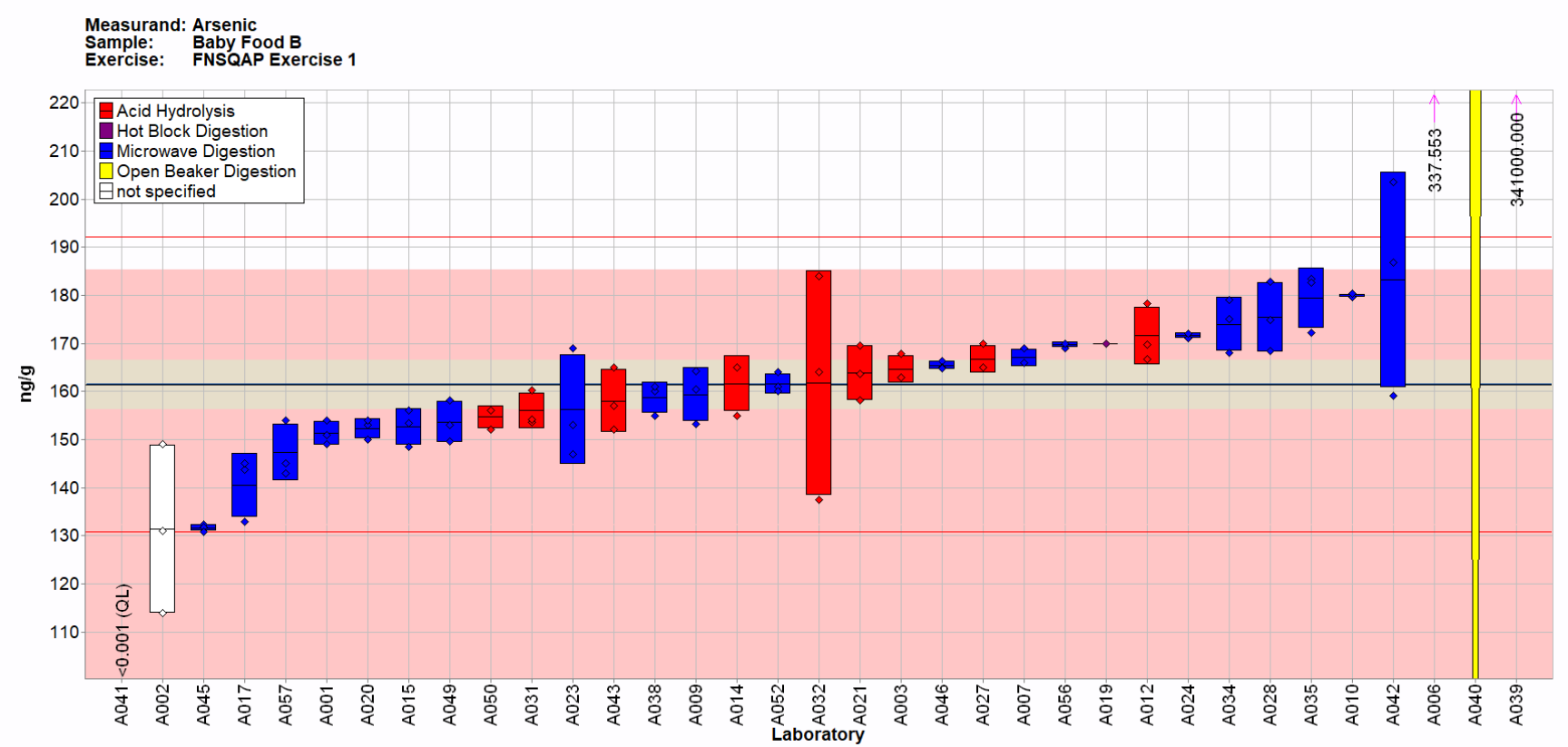

Fig. 3-2. Arsenic in Baby Food B (data summary view – sample preparation method).

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 downward and upward arrows (the preparation methods reported by laboratories A006 and A041 were acid hydrolysis and laboratory 039 was 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, $|Z'_{\text{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty (U_{NIST}), and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$. The shaded beige region represents the overlapping of the 95 % confidence interval for the consensus mean (green region) and the NIST range of tolerance (red region).

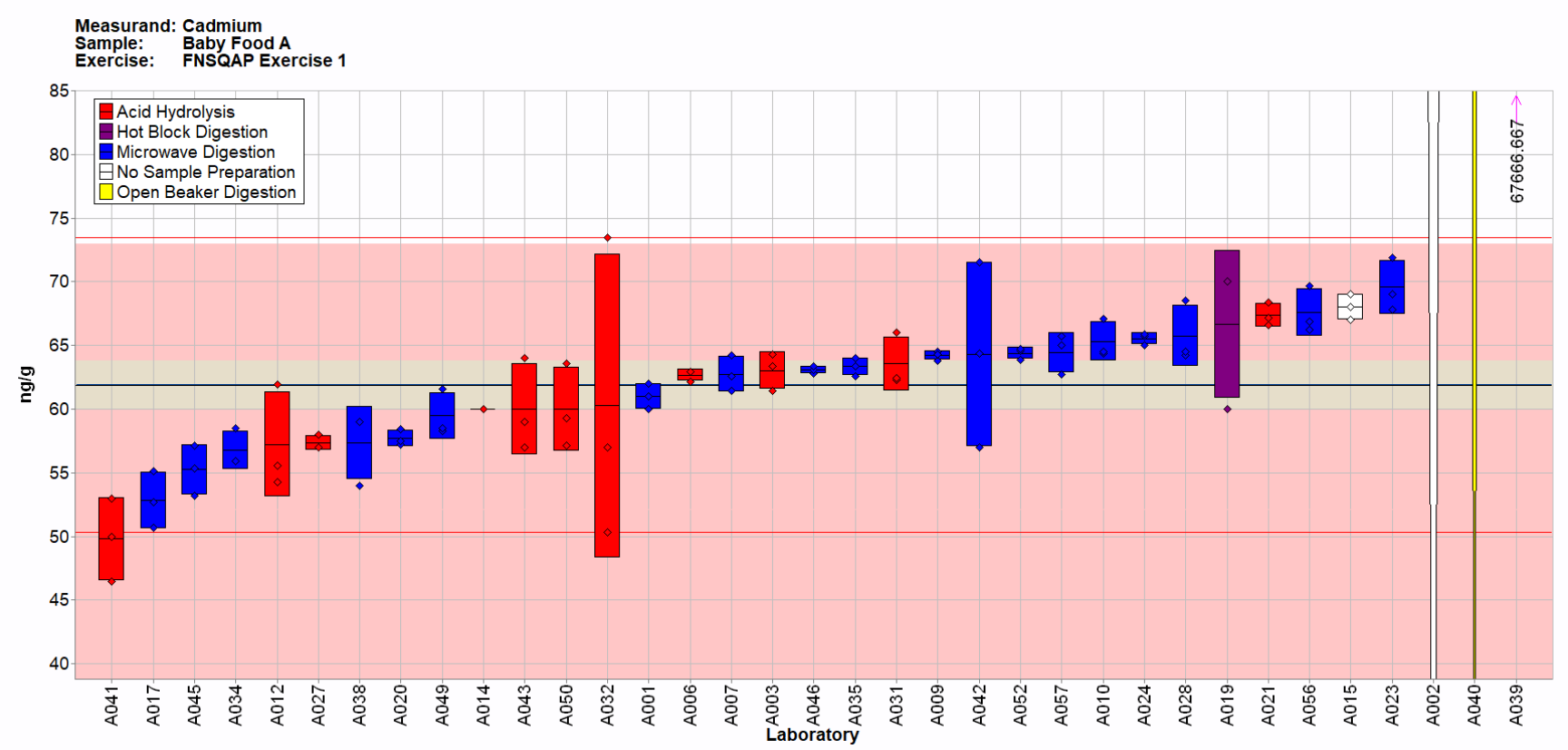

Fig. 3-3. Cadmium in Baby Food A (data summary view – sample preparation method).

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 preparation method reported by laboratory 039 was 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, $|Z'_{\text{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty (U_{NIST}), and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$. The shaded beige region represents the overlapping of the 95 % confidence interval for the consensus mean (green region) and the NIST range of tolerance (red region).

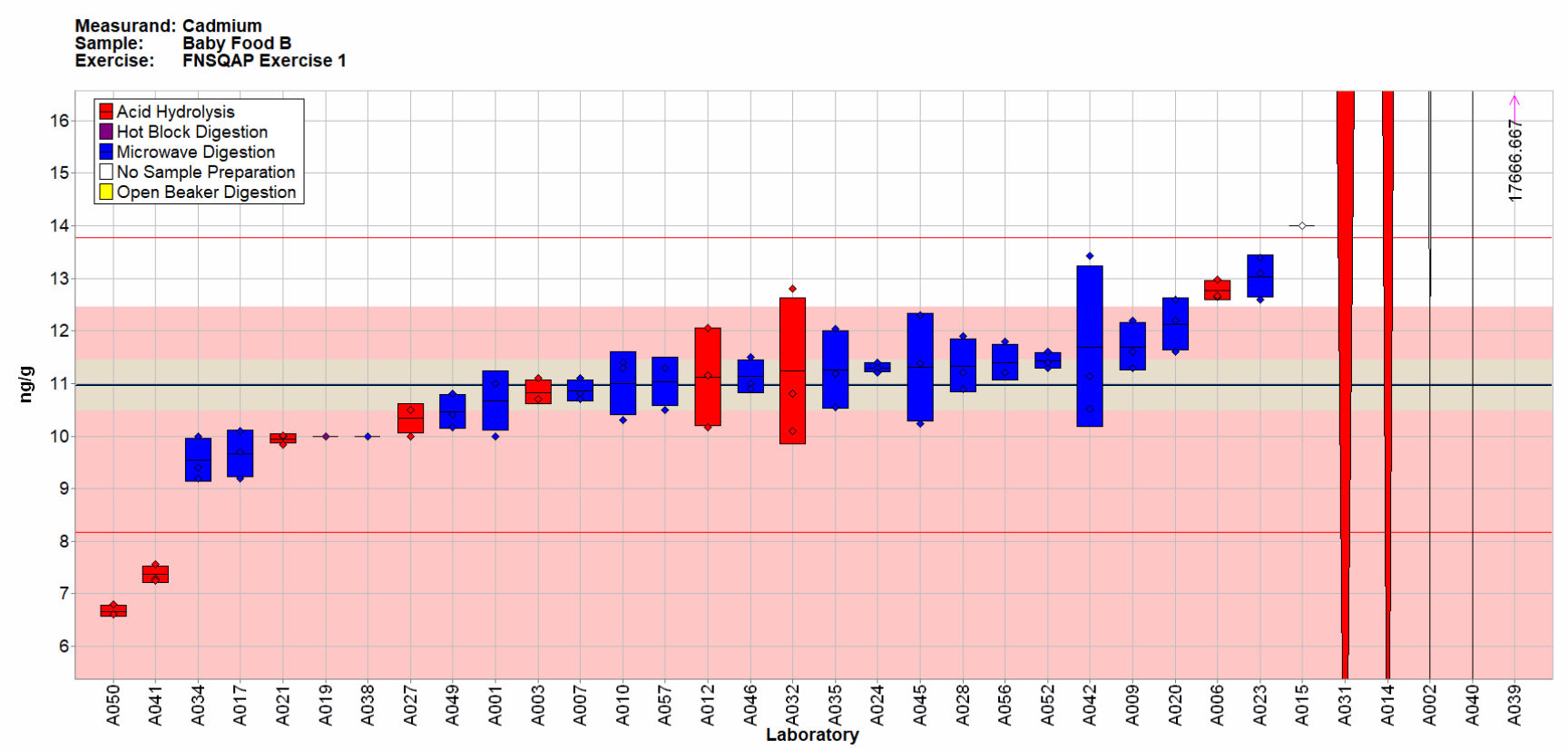

Fig. 3-4. Cadmium in Baby Food B (data summary view – sample preparation method).

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 preparation method reported by laboratory 039 was 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, $|Z'_{\text{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty (U_{NIST}) and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$. The shaded beige region represents the overlapping of the 95 % confidence interval for the consensus mean (green region) and the NIST range of tolerance (red region).

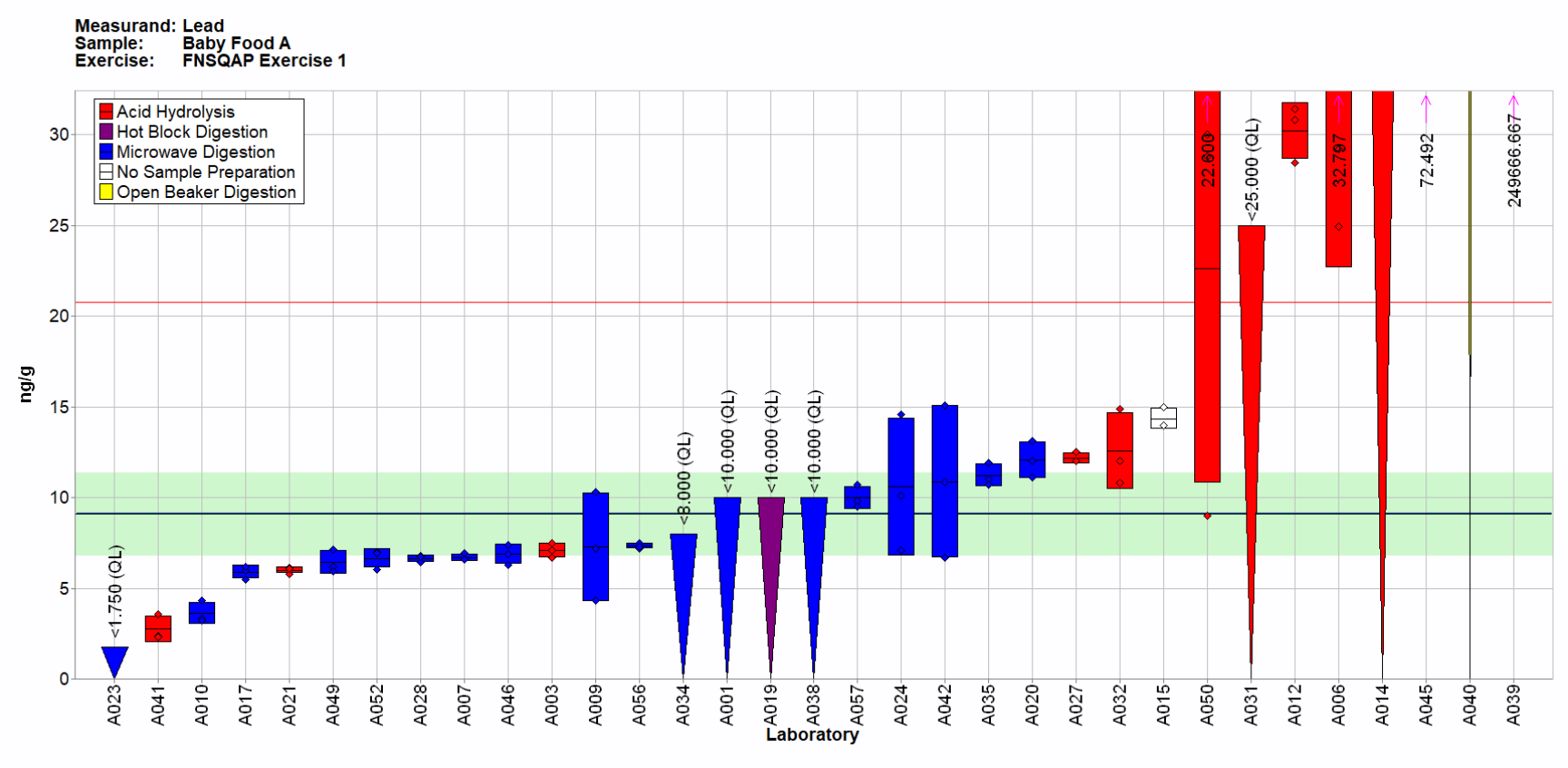

Fig. 3-5. Lead in Baby Food A (data summary view – sample preparation method).

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 preparation methods reported by laboratories A039 and A045 were 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 line represents the upper bound of the consensus range of tolerance, calculated as the value above the consensus mean that results in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$, with the lower bound set at zero. A NIST value has not been determined in this material.

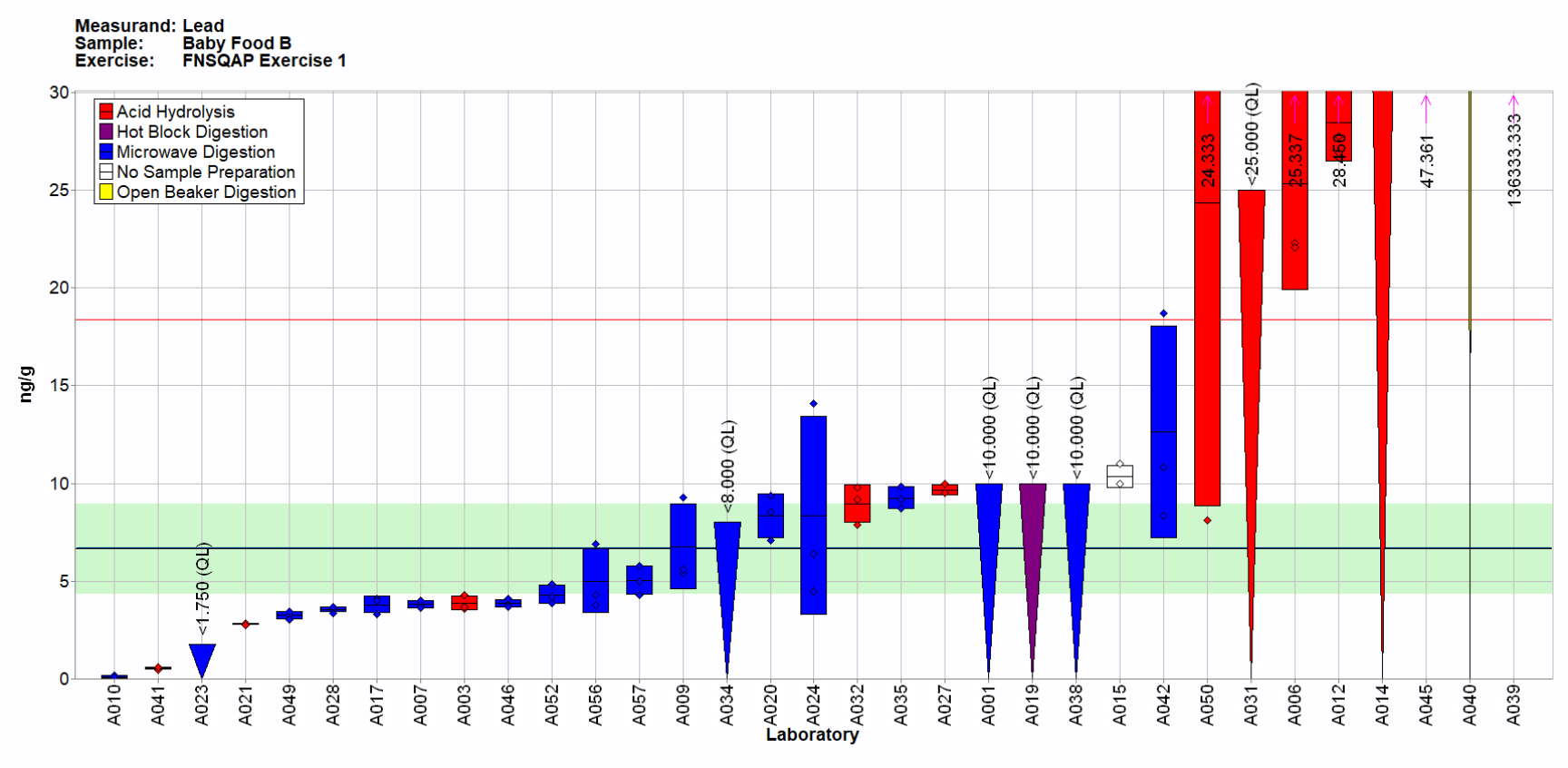

Fig. 3-6. Lead in Baby Food B (data summary view – sample preparation method).

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 preparation methods reported by laboratories A039 and A045 were 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 line represents the upper bound of the consensus range of tolerance, calculated as the value above the consensus mean that results in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$ with the lower bound set to zero. A NIST value has not been determined in this material.

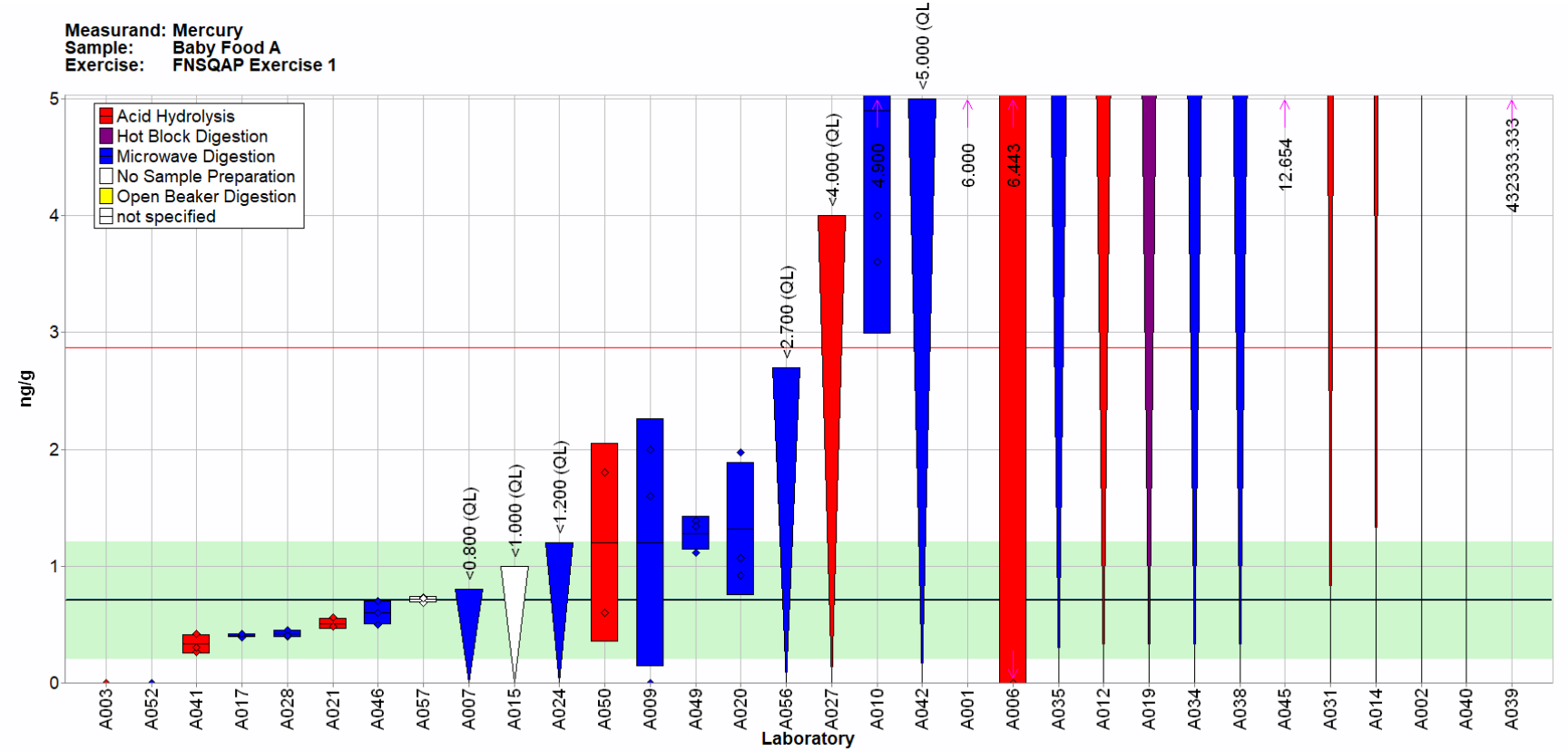

Fig. 3-7. Mercury in Baby Food A (data summary view –sample preparation method).

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 preparation methods reported by laboratories A001, A039, and A045 were 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 line represents the upper bound of consensus range of tolerance, calculated as the value above the consensus mean that results in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$, with the lower bound set at zero. A NIST value has not been determined in this material.

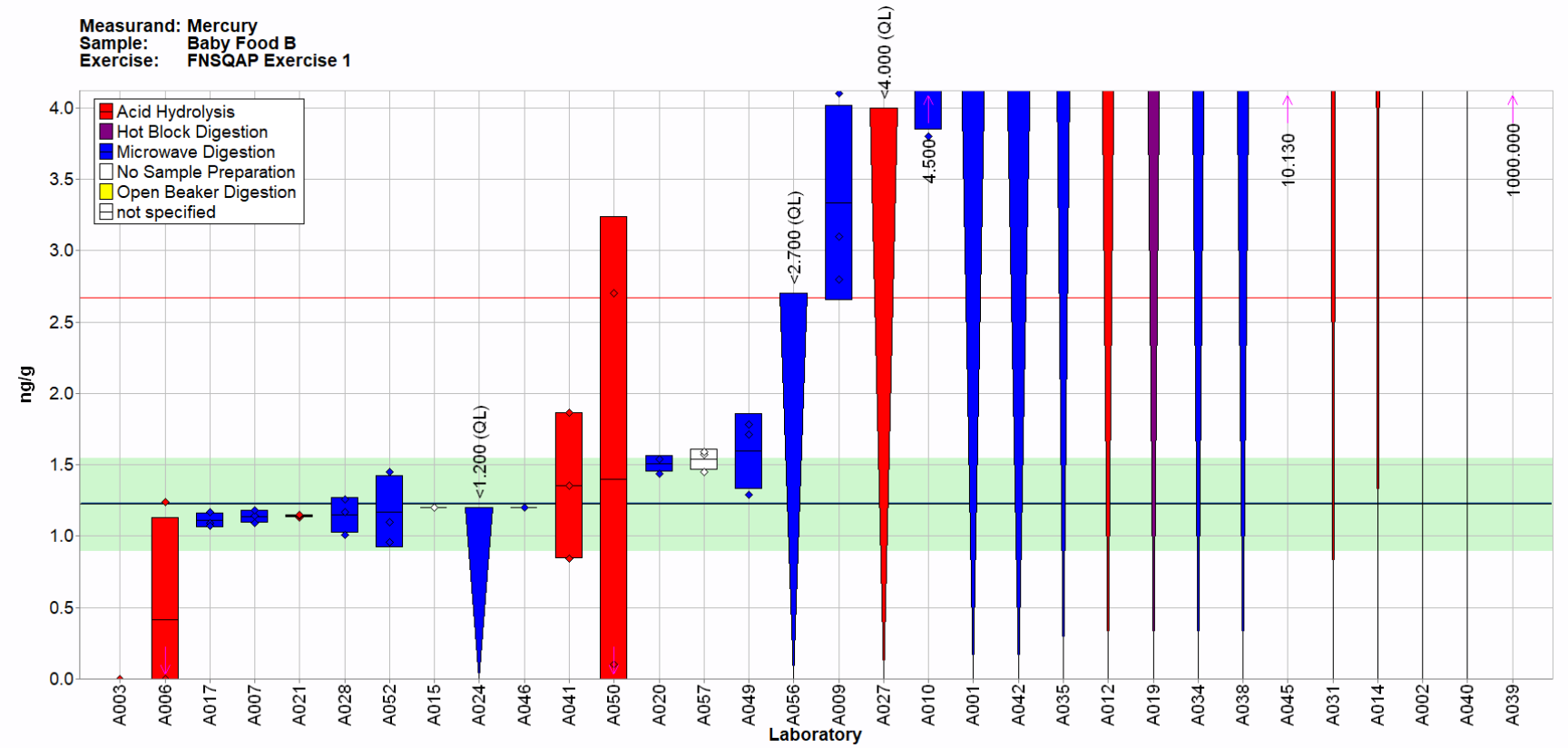

Fig. 3-8. Mercury in Baby Food B (data summary view – sample preparation method).

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 preparation methods reported by laboratories A001, A039, and A045 were 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 line represents the upper bound of the consensus range of tolerance, calculated as the value above the consensus mean that results in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$, with the lower bound set at zero. A NIST value has not been determined in this material.

Similar to reported sample preparation approaches, Fig. **3-9**, Fig. **3-10**, Fig. **3-11**, Fig. **3-12**, Fig. **3-13**, Fig. **3-14**, Fig. **3-15**, and Fig. **3-16** indicate that a variety of analytical methods were employed for the determination of toxic elements in the two baby food samples. The most reported approaches were ICP-MS (23 laboratories, 68 %) and ICP-MS in KED mode (8 laboratories, 23 %). Approximately 1 laboratory each reported use of ICP-OES, Atomic Absorption Spectroscopy (AAS), neutron activation, and Hg analyzer (Hg only) (3 % each). As noted above, several laboratories reported Pb and Hg as below LOQ, which was not associated with method except for neutron activation where all reported toxic element data were submitted as below LOQ. Sensitivity of the analytical method is key when determining if the method is suitable for the analyte abundance in the sample and the 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 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. Arsenic can have a polyatomic interference with $^{40}Ar^{35}Cl^{+}$ which forms from a combination of Ar in the plasma and Cl from the sample or diluent. Cadmium can have isobaric spectral interferences such as $^{95}Mo^{16}O^{+}$ and $^{97}Mo^{16}O^{+}$ that affect the accuracy of Cd determination at 111 u and 113 u. Use of He and H_2 collision gases can effectively reduce polyatomic interferences. Oxygen reaction cell gas can be used for elements that can form oxides to mass-shift the element for measurement to an atomic mass without overlapping inferences. The sensitivity of ICP-MS for Hg is low, and Hg carryover between samples requires long washout times between samples. Cold vapor generation for Hg measurement by ICP-MS can increase method sensitivity to accurately measure low levels of Hg and greatly reduce washout times between samples to bring Hg background down to baseline quickly for more stable results. Only one laboratory reported using an Hg analyzer for this exercise. Alternatively, use of direct combustion AAS or direct Hg analyzers for Hg analytical methods allows low detection limits and does not require sample preparation, which reduces sample throughput time.

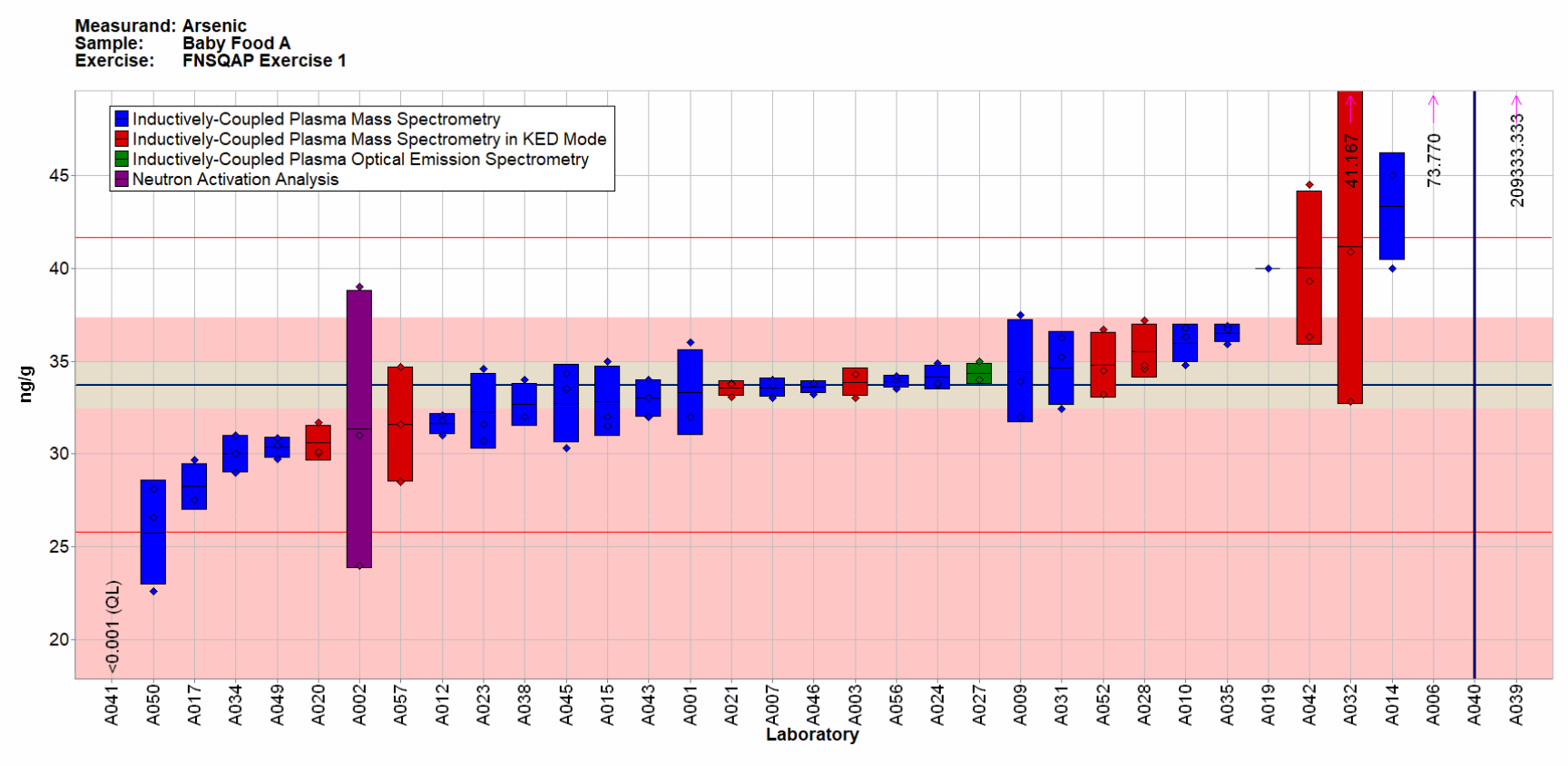

Fig. 3-9. Arsenic in Baby Food A (data summary view – analytical method).

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 downward and upward arrows (the analytical methods reported by laboratories A006, A039, and A041 were 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, $|Z'_{\mathrm{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty (U_{NIST}), and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\mathrm{NIST}}| \leq 2$. The shaded beige region represents the overlapping of the 95 % confidence interval for the consensus mean (green region) and the NIST range of tolerance (red region).

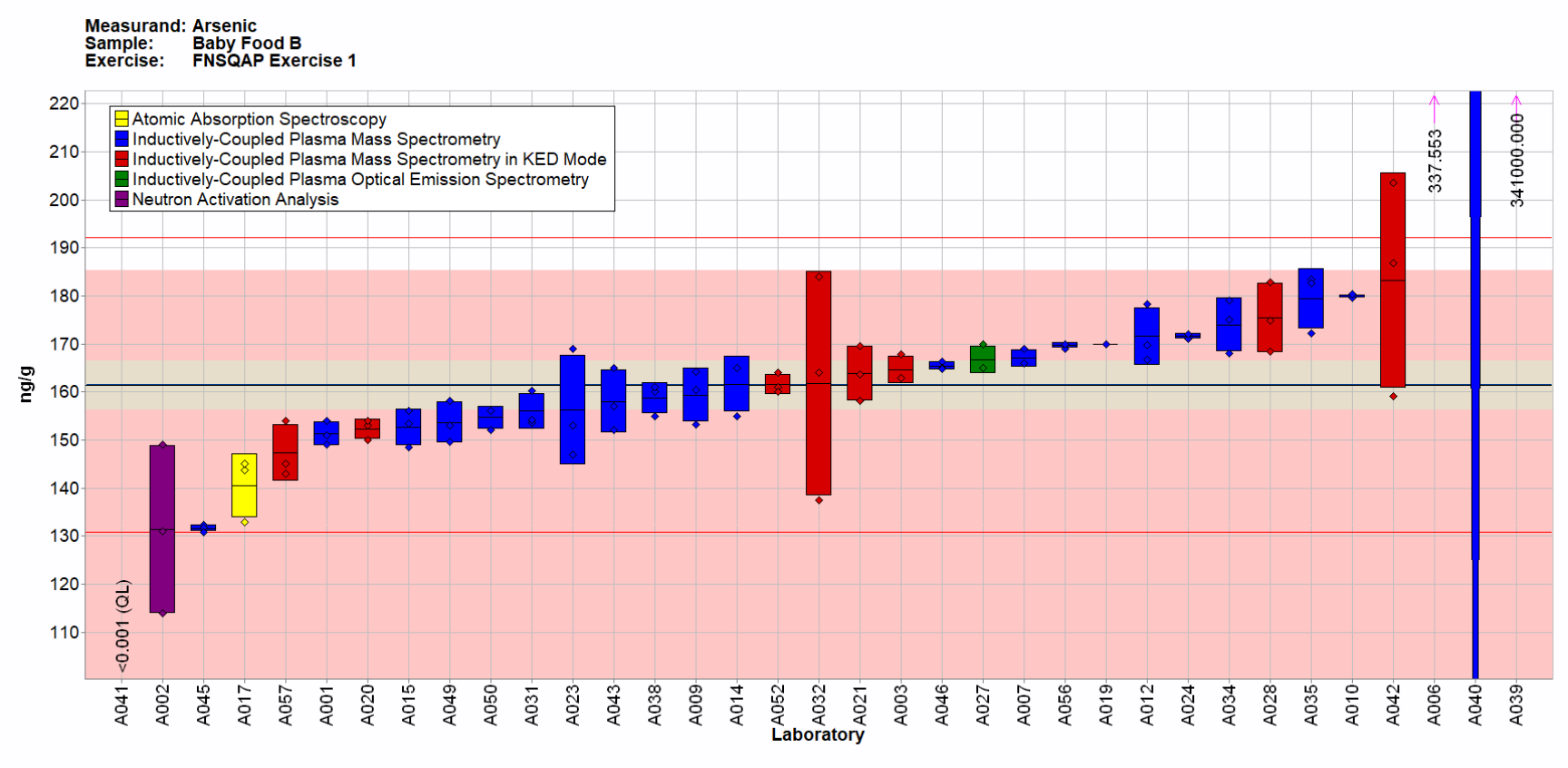

Fig. 3-10. Arsenic in Baby Food B (data summary view – analytical method).

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 downward and upward arrows (the analytical methods reported by laboratories A006, A039, and A041 were 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 range of tolerance, which encompasses the target value bounded by twice its uncertainty (U_{NIST}), and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$. The shaded beige region represents the overlapping of the 95 % confidence interval for the consensus mean (green region) and the NIST range of tolerance (red region).

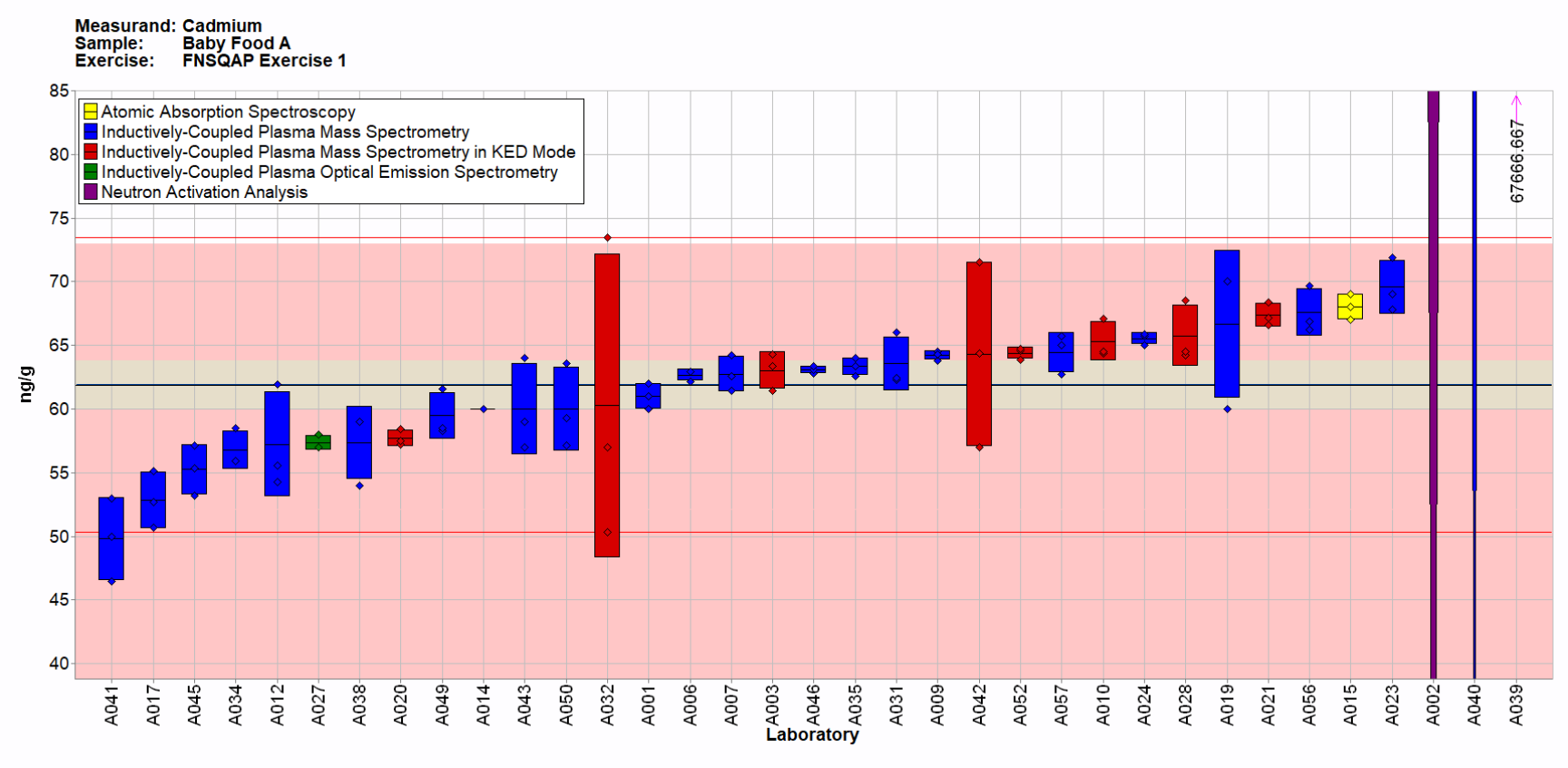

Fig. 3-11. Cadmium in Baby Food A (data summary view – analytical method).

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 method reported by laboratory A039 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 range of tolerance, which encompasses the target value bounded by twice its uncertainty (U_{NIST}), and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$. The shaded beige region represents the overlapping of the 95 % confidence interval for the consensus mean (green region) and the NIST range of tolerance (red region).

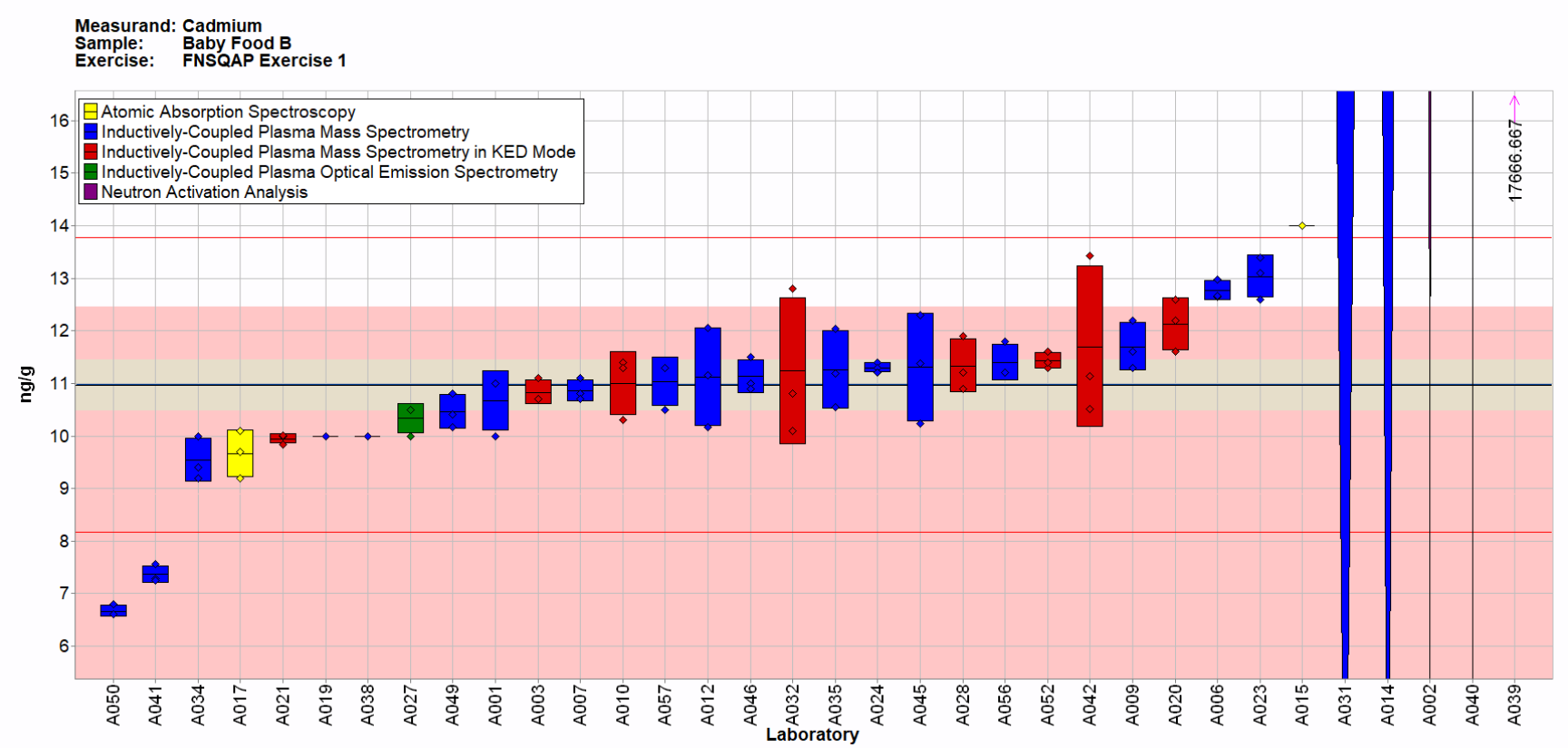

Fig. 3-12. Cadmium in Baby Food B (data summary view – analytical method).

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 method reported by laboratory A039 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 range of tolerance, which encompasses the target value bounded by twice its uncertainty (U_{NIST}), and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$. The shaded beige region represents the overlapping of the 95 % confidence interval for the consensus mean (green region) and the NIST range of tolerance (red region).

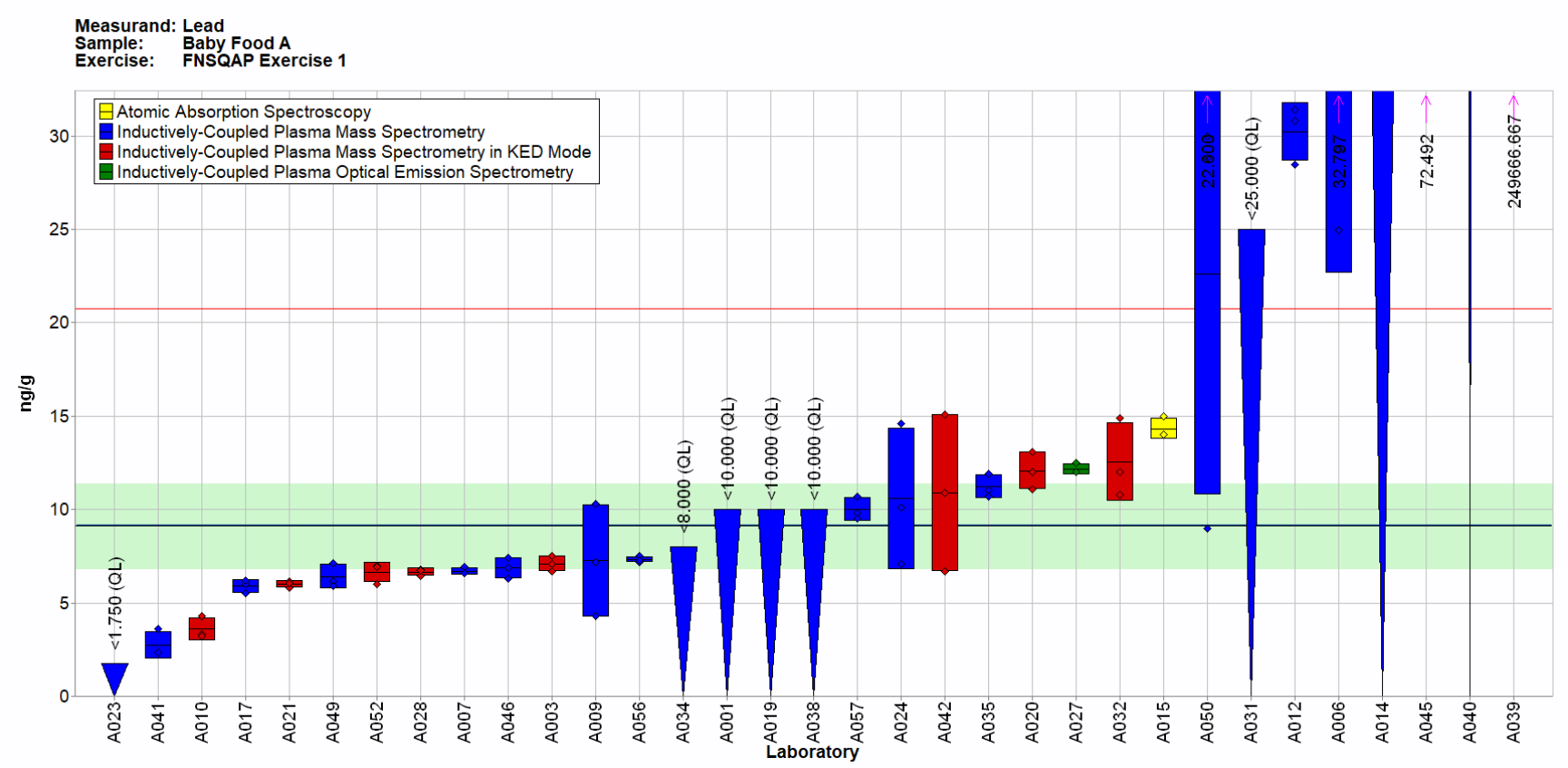

Fig. 3-13. Lead in Baby Food A (data summary view – analytical method).

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 methods reported by laboratories A039 and A045 were 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 line represents the upper bound of the consensus range of tolerance, calculated as the value above the consensus mean that results in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$, with the lower bound set at zero. A NIST value has not been determined in this material.

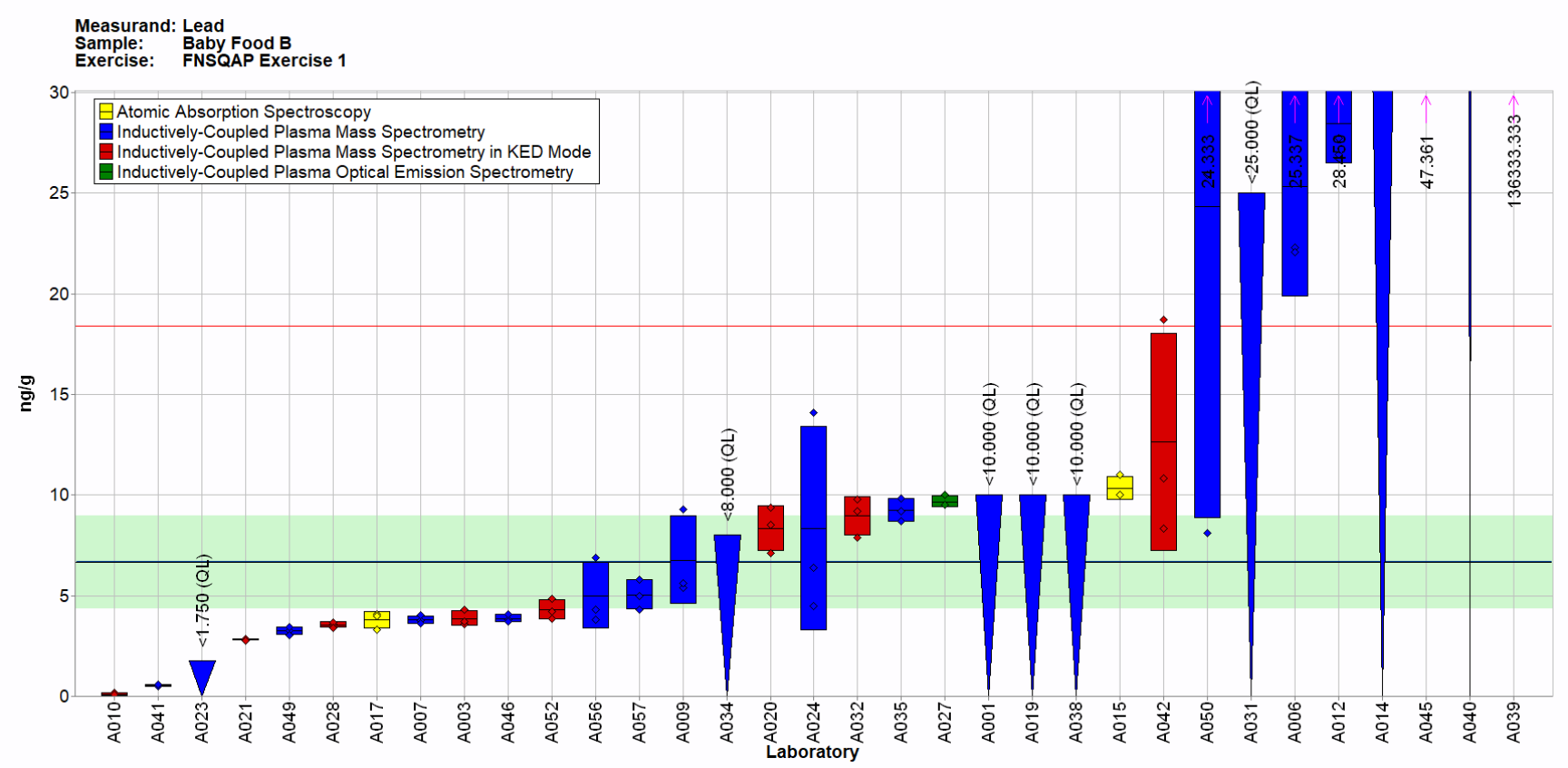

Fig. 3-14. Lead in Baby Food B (data summary view – analytical method).

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 methods reported by laboratories A039 and A045 were 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, $|Z'_{\text{comm}}| \leq 2$. A NIST value has not been determined in this material.

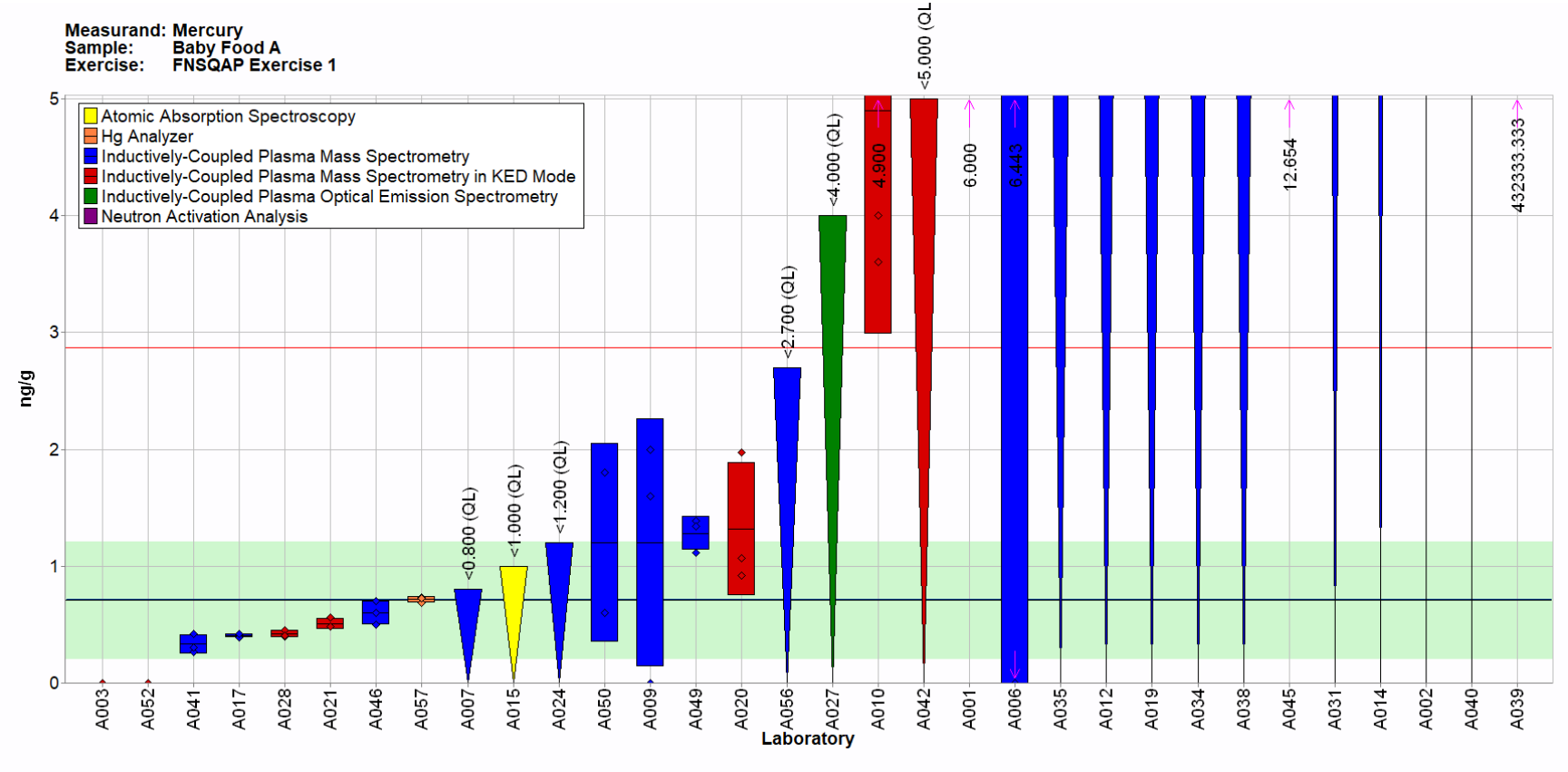

Fig. 3-15. Mercury in Baby Food A (data summary view – analytical method).

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 methods reported by laboratories A001, A039, and A045 were 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 line represents the upper bound of consensus range of tolerance, calculated as the value above the consensus mean that results in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$, with the lower bound set at zero. A NIST value has not been determined in this material.

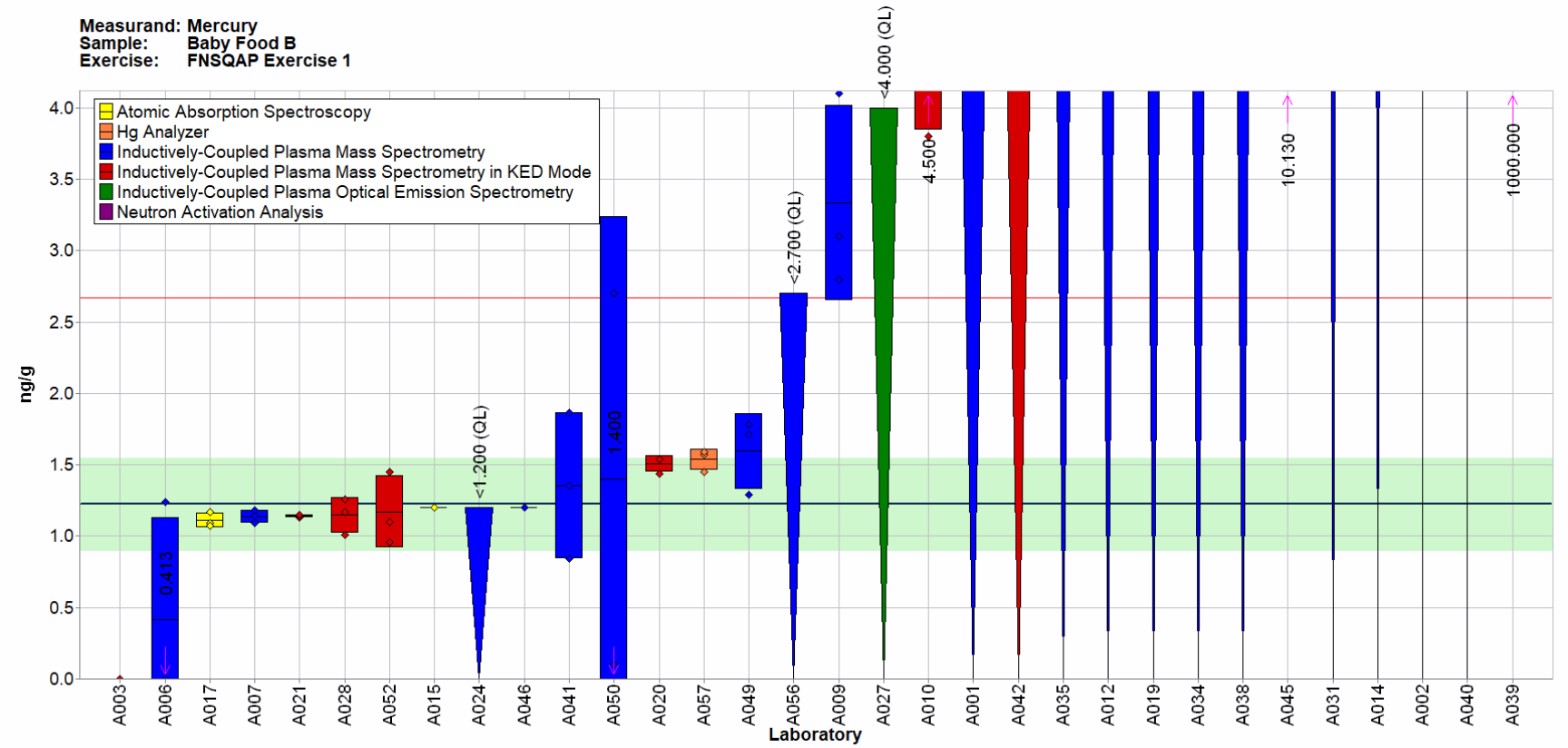

Fig. 3-16. Mercury in Baby Food B (data summary view – analytical method).

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 methods reported by laboratories A039 and A045 were 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 line represents the upper bound of the consensus range of tolerance, calculated as the value above the consensus mean that results in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$, with the lower bound set at zero. A NIST value has not been determined in this material.

Overall, laboratories performed well in the measurement of As and Cd in baby food samples. While the consensus ranges for As and Cd in both baby foods overlap with target ranges (Fig. **3-1**, Fig. **3-2**, Fig. **3-3**, Fig. **3-4**, Fig. **3-9**, Fig. **3-10**, Fig. **3-11**, and Fig. **3-12**), the consensus ranges for these elements are in the upper portion and extend above the target range as shown in Fig. **3-17** and Fig. **3-18**. The target ranges for As and Cd were significantly wider than the consensus ranges in both baby food samples. At the time of this report, target values were not available for Pb and Hg to compare with the participant consensus data.

A slight positive linear trend is observed in Fig. **3-17**, Fig. **3-18**, Fig. **3-19**, and Fig. **3-20**, which may indicate a global issue with calibration or an equivalent level of difficulty in sample digestion between the two very similar samples. Laboratories that reported values above the target or above the consensus range did so consistently across toxic elements in the study 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 that calibration range and other points can be excluded from the determination of 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. Additionally, 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 signal intensity, but measures the signal ratios of the natural isotope of an element and the spiked isotope of an element in samples.

NIST has conducted twenty QAP studies involving measurement of one or more of these toxic elements in food and supplement samples prior to this FNSQAP study, as summarized in Table **3-6**.

Table 3-6. Previous NIST QAP exercises that included toxic elements studies.

QAP Exercise	Year	Elements	Reference
DSQAP A	2007	Pb	[5]
DSQAP B	2008	As	[5]
DSQAP C	2008	As, Cd	[5]
DSQAP D	2009	Pb	[5]
DSQAP F	2011	As, Cd, Pb, Hg	[5]
DSQAP G	2011	Pb	[6]
DSQAP I	2013	Cd	[20]
DSQAP J	2013	As	[8]
DSQAP K	2014	Hg	[9]
DSQAP L	2016	As, Pb	[21]
DSQAP M	2016	As, Pb	[10]
DSQAP N	2017	As, Cd, Pb	[22]
DSQAP O	2018	As, Cd, Pb, Hg	[23]
HAMQAP 1	2018	As	[11]
HAMQAP 2	2018	As, Cd, Pb, Hg	[24]
HAMQAP 3	2019	As, Cd, Pb, Hg	[25]
HAMQAP 4	2019	Cd, Pb	[12]
HAMQAP 5	2020	As	[13]
HAMQAP 6	2021	As, Cd, Pb, Hg	[26]
HAMQAP 7	2021	As, Cd, Pb, Hg	[14]

A review of the results from these previous exercises indicated no apparent trends in the number of laboratories reporting data, average RSD_r, or bias with respect to the NIST target value for any of the toxic elements over time, or trends related to RSD_R for As and Cd. Exercises with materials containing less than 10 ng/g Pb and Hg had greatly increased RSD_R, indicating that low mass fractions of these elements are difficult for laboratories to measure. Laboratory methods for Pb and Hg will need to improve to accurately make measurements as US regulations move limits closer to zero for these and other toxic elements.

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.

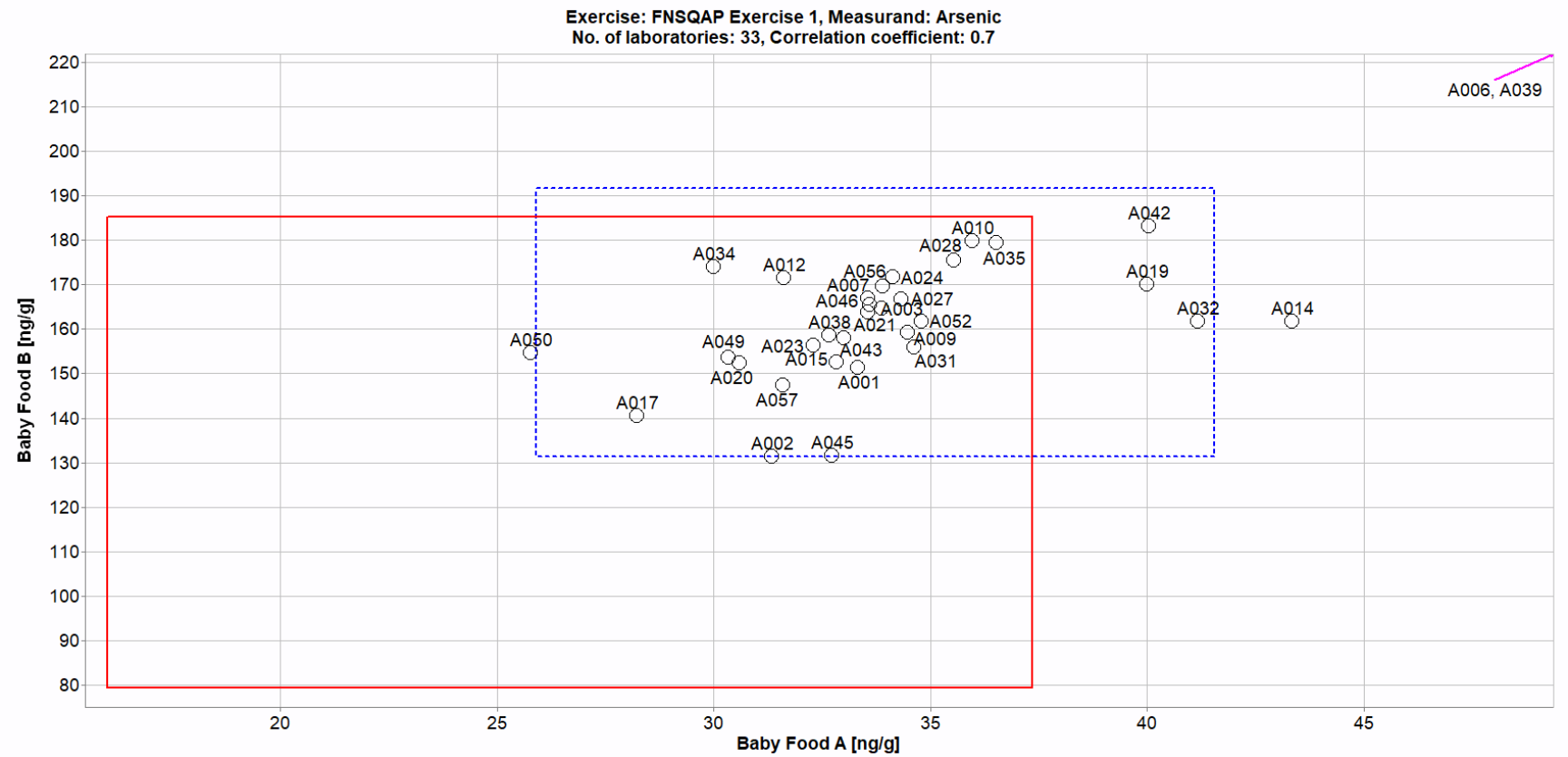

Fig. 3-17. Laboratory means for arsenic in Baby Food A and Baby Food B (sample/sample comparison view).

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

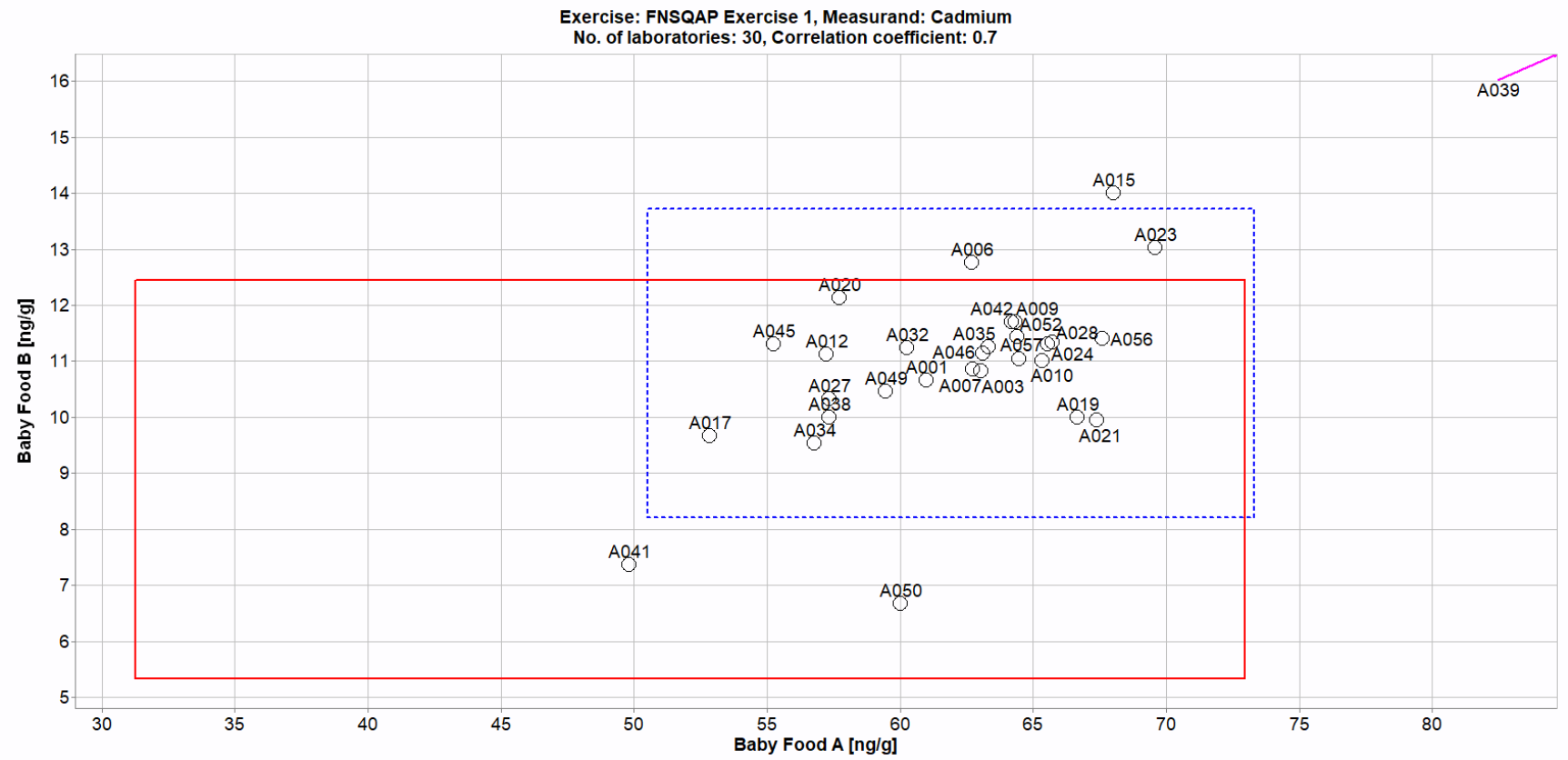

Fig. 3-18. Laboratory means for cadmium in Baby Food A and Baby Food B (sample/sample comparison view).

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

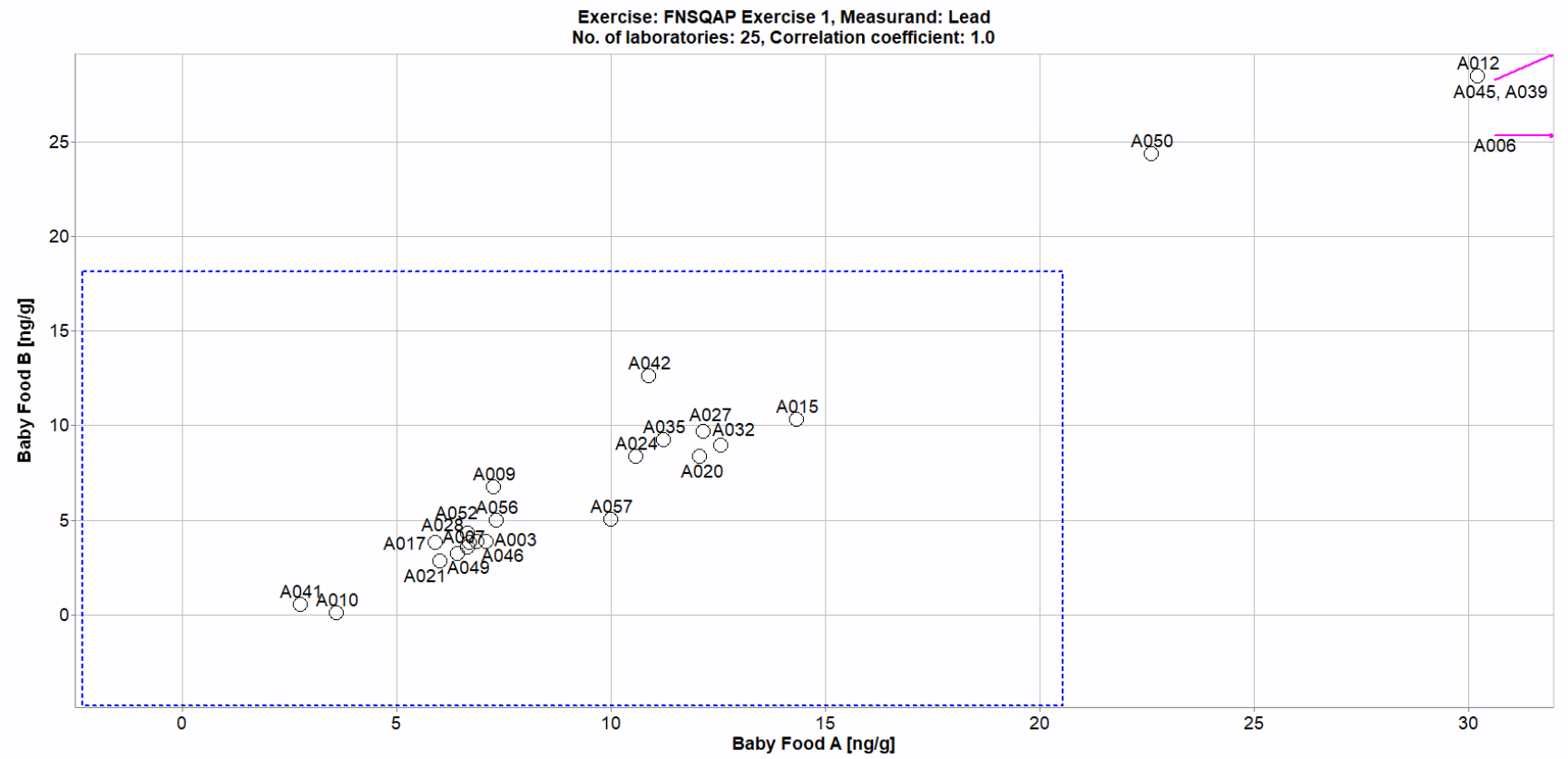

Fig. 3-19. Laboratory means for lead in Baby Food A and Baby Food B (sample/sample comparison view).

In this view, the individual laboratory mean for one sample (Baby Food A) is compared to the mean for a second sample (Baby Food B). The dotted blue box represents the consensus range of tolerance for Baby Food A (x-axis) and Baby Food B (y-axis), calculated as the values above and below the consensus means that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$. A NIST value has not been determined in these materials.

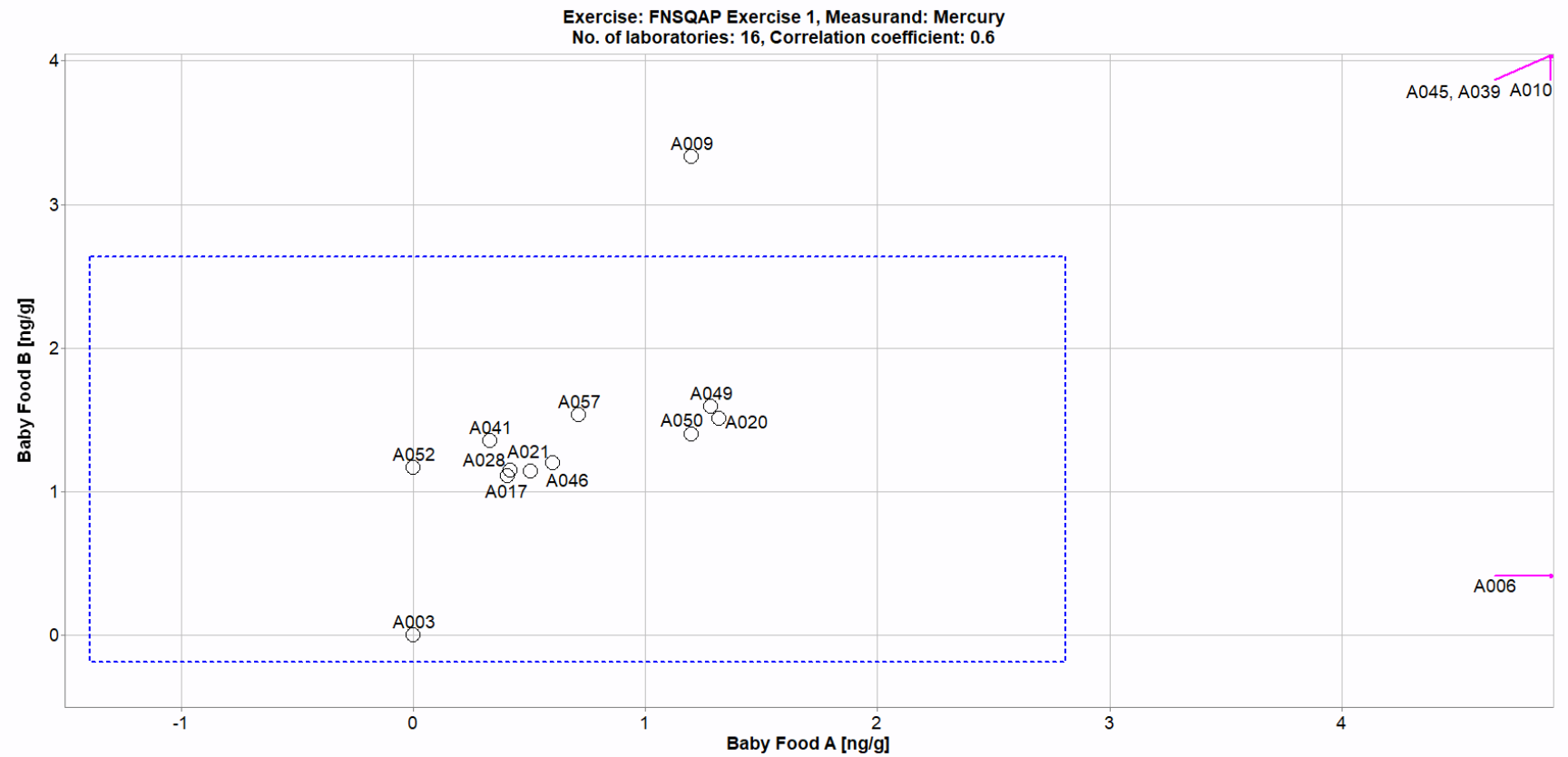

Fig. 3-20. Laboratory means for mercury in Baby Food A and Baby Food B (sample/sample comparison view).

In this view, the individual laboratory mean for one sample (Baby Food A) is compared to the mean for a second sample (Baby Food B). The dotted blue box represents the consensus range of tolerance for Baby Food A (x-axis) and Baby Food B (y-axis), calculated as the values above and below the consensus means that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$. NIST values have not been determined in these materials.

4. WATER-SOLUBLE VITAMINS (Folic Acid)

4.1. Executive Summary

Folic acid is an important nutrient for infant development and growth, and the fortified levels of folic acid in infant foods is strictly regulated worldwide. Participants in this study performed well in determination of folic acid, although the selected samples did not allow for evaluation of methods for minor folates. One approach, the use of acid or base hydrolysis for sample digestion, was identified as potentially leading to biased results and laboratories utilizing this type of approach should further investigate potential bias through use of reference materials. Additionally, the correlation of bias in reported values between the two similar samples indicated a potential measurement issue related to calibrant purity.

4.2. Study Overview

Folate is an essential vitamin, critical for the production and maintenance of new cells as well as synthesis of DNA and RNA [27]. Adequate folate intake during pregnancy is important for the prevention of neural tube defects. Naturally occurring folates in food are in the tetrahydrofolate forms, and humans obtain folic acid *via* fortified foods and supplements. For infants fed using infant formulas, folate supplementation is critical for proper growth and development. Accurately understanding the intake of folates through measurement in fortified foods can inform future decisions about recommended dietary intakes. In this study, participants were provided with two infant 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 determine the mass fractions (mg/kg) of folic acid, 5-formyltetrahydrofolate (5-FTHF), and 5-methyltetrahydrofolate (5-MTHF) 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.

4.3. Sample Information

Participants were provided with three packets each of SRM 1849b Infant/Adult Nutritional Formula I (milk-based) and RM 8260 Infant Nutritional Formula (hydrolyzed milk-based). Each packet contained approximately 10 g of material; participants were asked to store the materials at –20 °C or colder 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 folates. The approximate analyte levels were not reported to participants prior to the study. The target values for folic acid in each material were determined using data provided by the material manufacturers [3,28]; the target values and uncertainties for folic acid are provided in Table **4-1** on an as-received basis. The uncertainty for folic acid in RM 8260 was approximated as 20 % relative to the measured value. Target values for 5-FTHF and 5-MTHF in SRM 1849b and RM 8260 were not available at the time of this report.

Table 4-1. Individualized data summary table for folates 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 1 – 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*
Folic Acid	SRM 1849b	mg/kg	*Individual laboratory results will appear in this section; laboratory-specific results were provided to each participant separately from this report.*				13	2.70	0.75	2.42	0.34
Folic Acid	RM 8260	mg/kg					13	1.40	0.67	1.10	0.11
5-FTHF	SRM 1849b	mg/kg					0				
5-FTHF	RM 8260	mg/kg					0				
5-MTHF	SRM 1849b	mg/kg					1				
5-MTHF	RM 8260	mg/kg					1				

x_i Mean of reported values
s_i Standard deviation of reported values
Z'_{comm} Z'-score with respect to community consensus
Z_{NIST} Z-score with respect to NIST value
N Number of quantitative values reported
x* Robust mean of reported values
s* Robust standard deviation
x_{NIST} NIST-assessed value
U expanded uncertainty about the NIST-assessed value

4.4. Study Results and Discussion

Table **4-1** summarizes and Table **4-2** details the numerical results reported by each participating laboratory for folic acid. The participation level was high for folic acid, with 62 % of laboratories requesting samples returning results (13 of 21 laboratories). The participation rate was very low for 5-FTHF and 5-MTHF at 7 % (1 of 14 laboratories).

Only one laboratory reported results for 5-FTHF and 5-MTHF, as shown in Table **4-1**. The levels of 5-FTHF and 5-MTHF in these two milk-based samples are likely below the detection limits of most modern analytical methods. Because no consensus conclusions can be drawn, no additional tables or graphs are provided for these analytes. This single laboratory reported using enzymatic hydrolysis followed by LC-MS for determination of 5-FTHF and 5-MTHF in the two infant formula samples. The reported level of 5-FTHF was below the laboratory's method LOQ for the two samples.

Table **4-2** reveals that the within-laboratory variabilities were mostly acceptable with respect to published expectations of the measurement community as summarized in Table **4-3** [29]. For SRM 1849b, 2 of the 13 laboratories reported folate results with variability greater than expected (8 % RSD and 21 % RSD). For RM 8260, 3 of the 13 laboratories reported folate results with variability greater than expected (13 % RSD, 15 % RSD, and 60 % RSD). The between-laboratory variabilities were reasonable (28 % and 49 %) with respect to the published expectations of the measurement community for multiple laboratories using the same method, when considering the variety of methods used by the participants. The between-laboratory variabilities were nearly twice as high for RM 8260 (49 %) compared to those for SRM 1849b (28 %), which is consistent with expectations at the lower folate mass fraction in RM 8260 (Table **4-3**).

Table 4-2. Data summary table for folic acid in SRM 1849b and RM 8260.
Data highlighted in blue have been identified as outside the consensus tolerance limits and resulted in an unacceptable Z'_{comm} score, $|Z'_{comm}| > 2$.

		Folic Acid									
		SRM 1849b Infant/Adult Nutritional Formula I (milk-based) (mg/kg)					RM 8260 Infant Formula (hydrolyzed-milk based) (mg/kg)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target				2.42	0.34				1.10	0.11
	A001	2.03	1.73	1.32	1.69	0.36	0.472	0.542	0.559	0.52	0.05
	A005										
	A006	2.44	2.73	2.86	2.68	0.22	2.03	1.54	1.61	1.73	0.27
	A010	2.29	2.53	2.47	2.43	0.12	1.54	1.25	1.39	1.39	0.15
	A012	2.49	2.46	2.21	2.39	0.15	1.38	1.38	1.27	1.34	0.06
	A015	2.382	2.431	2.623	2.48	0.13	1.384	1.498	1.586	1.49	0.10
	A020	2.8	2.98	2.78	2.85	0.11	1.46	1.54	1.4	1.47	0.07
	A026										
	A027	3.06	3.05	3.08	3.06	0.02	1.88	1.86	1.85	1.86	0.02
	A031										
	A034	2.87	2.94	2.71	2.84	0.12	0.81	1.01	1.02	0.95	0.12
	A035										
	A037										
	A038	2.3	2.27	2.37	2.31	0.05	1.39	1.38	1.4	1.39	0.01
	A039										
	A040	704	708	692	701	8	4215	17569	18662	13482	8044
	A041	3.87	3.64	3.778	3.76	0.12	5.33	5.35	5	5.23	0.20
	A044	2.81	2.85	2.98	2.88	0.09	1.71	1.64	1.66	1.67	0.04
	A045	278	301	285	288	12	138	164	152	151	13
	A048										
	A054										
Community Results		Consensus Mean			2.67		Consensus Mean			1.38	
		Consensus Standard Deviation			0.75		Consensus Standard Deviation			0.67	
		Maximum			701		Maximum			13482	
		Minimum			1.69		Minimum			0.52	
		N			13		N			13	

Table 4-3. Summary of expected method performance requirements for folate in infant formula [29]. 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).

	SRM 1849b	**RM 8260**
Target Folic Acid Mass Fraction (mg/kg)	2.4	1.1
Corresponding SMPR Range (μg/100 g) [29]	> 21.5	< 21.5
Expected Repeatability (RSD_r) [29]	≤ 7 %	≤ 11 %
Expected Reproducibility (RSD_R) [29]	≤ 16 %	≤ 32 %

As shown in Fig. **4-1** and Fig. **4-2**, laboratories reported using a variety of sample preparation methods for the determination of folic acid in the two infant formula samples. The most common sample preparation approach was dilution (4 of 13 laboratories, 31 %); two laboratories reported using enzymatic hydrolysis (15 %), and 1 laboratory each reported use of acid hydrolysis, base hydrolysis, hot block digestion, solid phase extraction, and solvent extraction (8 % each). Two laboratories did not report the sample preparation approach used (15 %). Notably, the laboratory indicating use of base hydrolysis for determination of folic acid reported values below the consensus and target range in both samples. Additionally, the laboratories indicating use of acid hydrolysis and solvent extraction reported values above the consensus and target ranges in each sample. Although each of these techniques is only represented by one laboratory, perhaps these approaches are not ideal for isolation of folic acid from infant formula matrices.

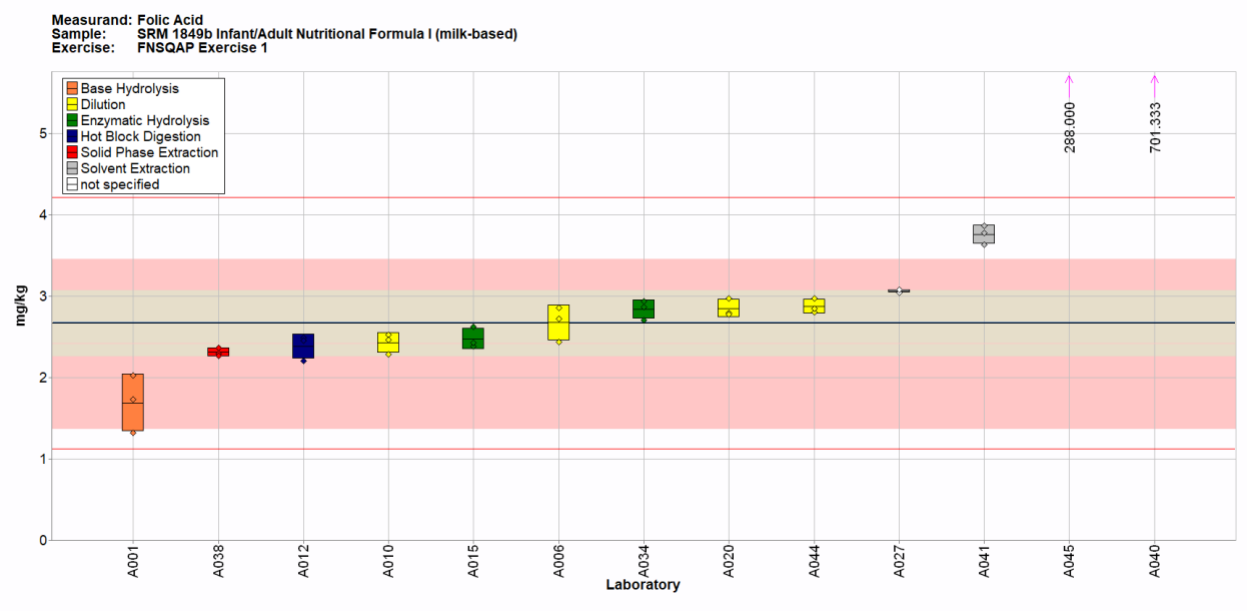

Fig. 4-1. Folic Acid in SRM 1849b (data summary view – sample preparation method).

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 (the sample preparation approach reported by laboratory A045 was acid hydrolysis; laboratory A040 did not specify the 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, $|Z'_{\mathrm{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty (U_{NIST}), and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\mathrm{NIST}}| \leq 2$. The shaded beige region represents the overlapping of the 95 % confidence interval for the consensus mean (green region) and the NIST range of tolerance (red region).

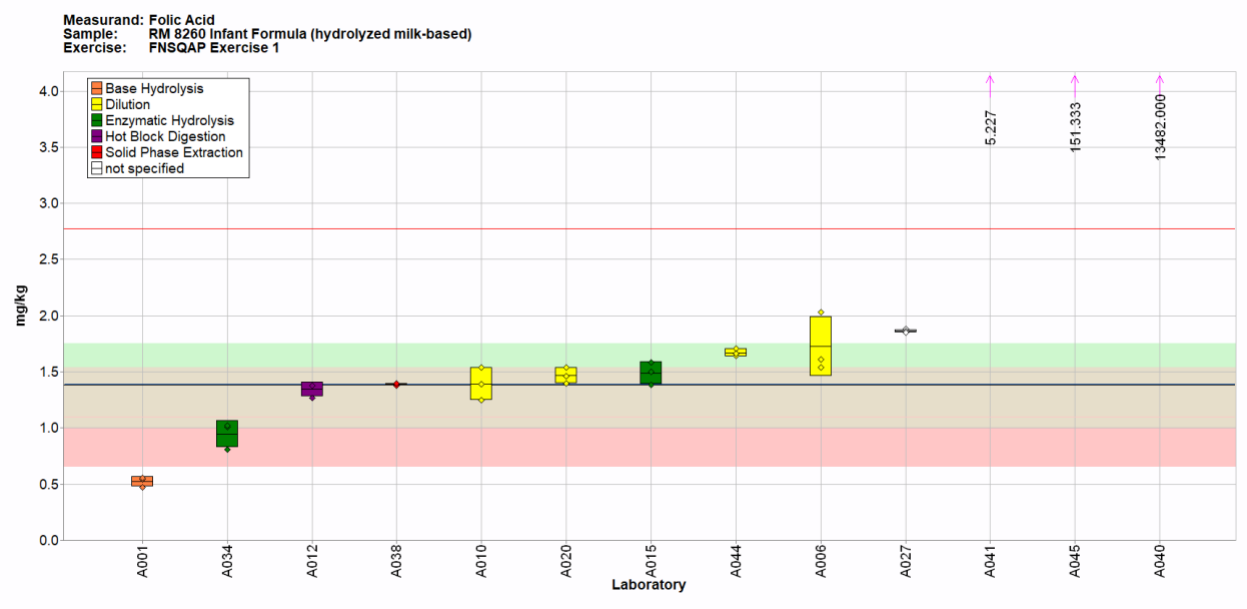

Fig. 4-2. Folic Acid in RM 8260 (data summary view – sample preparation method).

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 approaches reported by laboratories A041 and A045 were solvent extraction and acid hydrolysis, respectively; laboratory A040 did not specify the 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 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 range of tolerance, which encompasses the target value bounded by twice its uncertainty (U_{NIST}), and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$. The shaded beige region represents the overlapping of the 95 % confidence interval for the consensus mean (green region) and the NIST range of tolerance (red region).

Similarly, Fig. **4-3** and Fig. **4-4** indicate that multiple analytical methods were employed for the determination of folic acid in the two infant formula samples. The most reported approaches were Liquid Chromatography with Absorbance or Photodiode Array (PDA) detection (LC-Abs) and Liquid Chromatography with Mass Spectrometry detection (LC-MS) (3 of 13 laboratories each, 23 %). Two laboratories each reported using Liquid Chromatography with Tandem Mass Spectrometry detection (LC-MC/MS) and microbiological assay (15 % each), and 1 laboratory reported use of Enzyme-Linked Immunosorbent Assay (ELISA) (8 %). Two laboratories did not report the analytical approach used (15 %). No trends related to analytical method could be identified.

For both infant formula samples, the consensus means for folic acid lie on the upper edge of the target range (Fig. **4-1**, Fig. **4-2**, Fig. **4-3**, and Fig. **4-4**). The widths of the consensus ranges for folic acid in each sample are comparable to the widths of the target ranges.

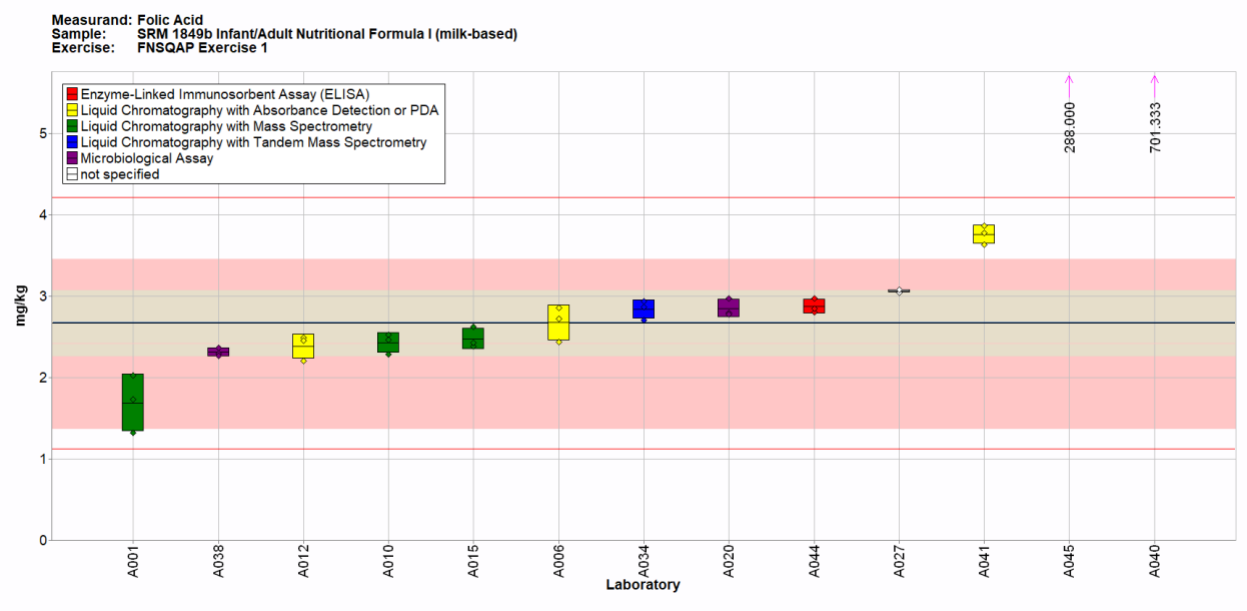

Fig. 4-3. Folic acid in SRM 1849b (data summary view – analytical method).

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 A045 was LC-MS/MS; laboratory A040 did not specify the 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, $|Z'_{\mathrm{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty (U_{NIST}), and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\mathrm{NIST}}| \leq 2$. The shaded beige region represents the overlapping of the 95 % confidence interval for the consensus mean (green region) and the NIST range of tolerance (red region).

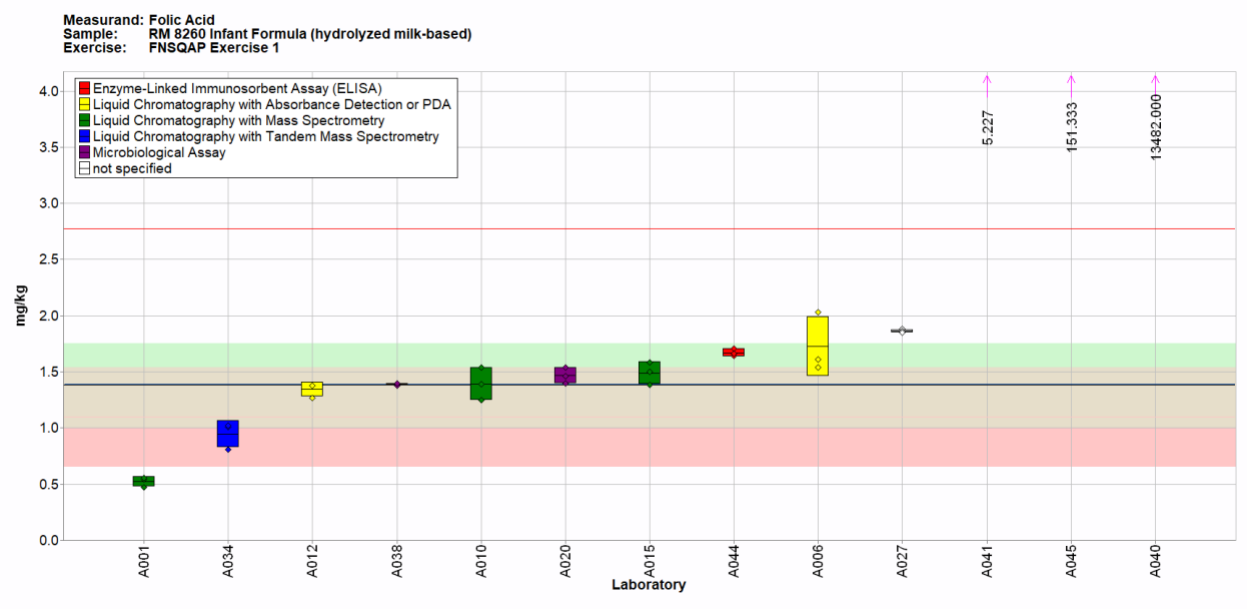

Fig. 4-4. Folic acid in RM 8260 (data summary view – analytical method).

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 A041 and A045 were LC-Abs or PDA and LC-MS/MS, respectively; laboratory A040 did not specify the 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 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 range of tolerance, which encompasses the target value bounded by twice its uncertainty (U_{NIST}), and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$. The shaded beige region represents the overlapping of the 95 % confidence interval for the consensus mean (green region) and the NIST range of tolerance (red region).

Overall, laboratories performed well in the determination of folic acid in infant formula samples. A slight linear trend is observed in Fig. **4-5**, which may indicate a global issue with calibration. Laboratories that reported values below the target did so consistently in these two very similar samples, and likewise laboratories that reported values above the target did so consistently between the samples. Potential sources of this type of calibration issue may be in the inaccurate assignment of calibrant purity (e.g., not considering potential impurities or moisture in the calibrant material) or extension of the calibration curve beyond the linear range. All calibrant materials should be of known purity, either through a statement of traceability to the SI (such as those from NIST or another national metrology institute) or through independent verification within the user's laboratory. Many standards manufacturers provide detailed documentation about purity testing; the purity should be verified in-house prior to use, and all concentrations should be adjusted to reflect known impurities. For some analytes, such as folic acid, the concentration of calibration solutions can be most accurately determined through spectrophotometry prior to analytical measurements, as described in Camara et al. [30]. Once the calibrant material has been well characterized, the calibration curve 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 that calibration range and other points can be excluded from the determination of the calibration curve to prevent bias. Another

potential contributor to the trend observed in Fig. **4-5** may be decomposition of the analytes in the samples and/or standards during analysis. Folates are known to be light-sensitive and therefore samples and standards should be prepared under amber or attenuated lighting to reduce potential degradation.

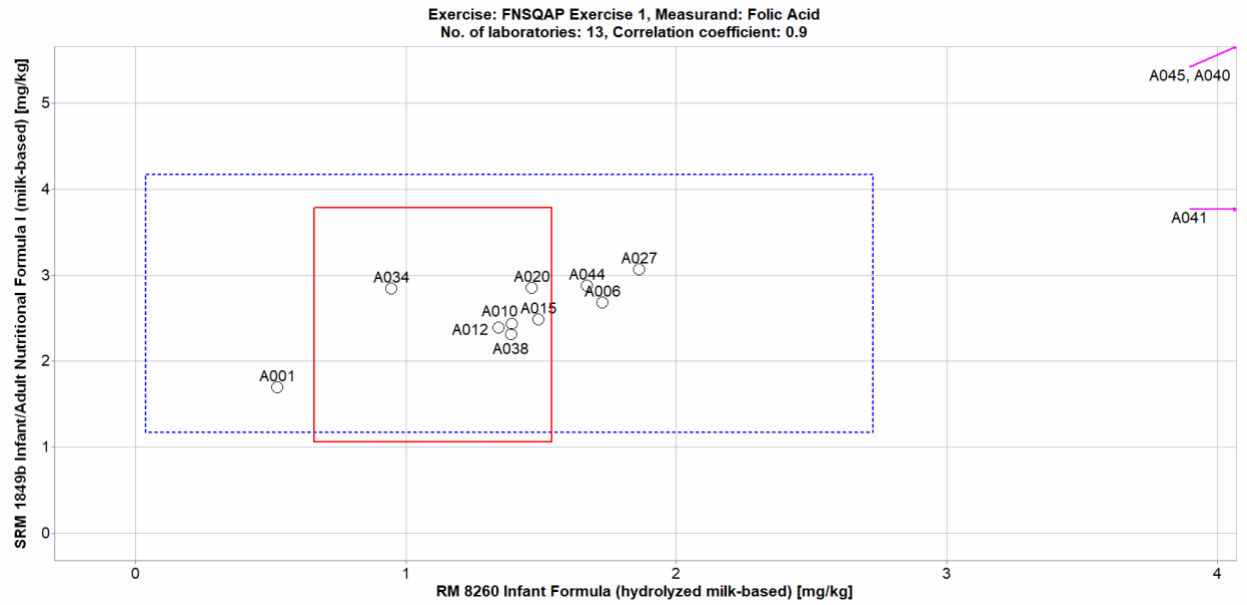

Fig. 4-5. Laboratory means for folic acid in RM 8260 and SRM 1849b (sample/sample comparison view).

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 range of tolerance 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, $|Z_{\mathrm{NIST}}| \leq 2$. 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, $|Z'_{\mathrm{comm}}| \leq 2$.

NIST has conducted four QAP studies involving measurement of folic acid in food samples prior to this FNSQAP study: DSQAP Exercise A in 2007 [5], Exercise G in 2011 [6], Exercise N in 2017 [22], and HAMQAP Exercise 3 in 2019 [25]. A review of the results from these previous exercises indicated no apparent trends in the number of laboratories reporting data, average RSD_r, RSD_R, or bias with respect to the NIST target value over time.

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.

5. FAT-SOLUBLE VITAMINS (Vitamin K_1)

5.1. Executive Summary

Vitamin K is an important nutrient for infant development and growth, and the fortified levels of vitamin K 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. Despite the limited number of reported values, the correlation of bias in reported values between the two similar samples indicated a potential measurement issue related to calibrant purity.

5.2. Study Overview

Vitamin K is a family of fat-soluble vitamins that functions as coenzymes for synthesis of proteins involved in blood clotting and bone metabolism [31]. In addition, some vitamin K-dependent proteins affect calcium uptake and regulation in the body. The average adult reaches an adequate intake of vitamin K through natural occurrence in dietary food and supplementation with multivitamins [31]. Because vitamin K transports poorly across the placenta and breast milk content of vitamin K is low, newborns are one of the highest risk groups for vitamin K deficiency that may result in bleeding, hemorrhage, and reduced bone mineralization [31]. The combination of an intramuscular dose of vitamin K at birth and fortification of infant formulas has greatly reduced the risk of vitamin K deficiency. Accurately understanding the intake and corresponding health outcomes related to vitamin K through measurement in infant formulas can inform future decisions about recommended dietary intakes. In this study, participants were provided with two infant 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 determine the mass fractions (mg/kg) of total vitamin K_1 (phylloquinone) 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) and RM 8260 Infant Nutritional Formula (hydrolyzed milk-based). Each packet contained approximately 10 g of material; participants were asked to store the materials at –20 °C or colder 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 total vitamin K_1. The approximate analyte levels were not reported to participants prior to the study. The target values for vitamin K_1 in each material were determined using data provided by the material manufacturers [3,28]; the target values and uncertainties for vitamin K_1 are provided in Table **5-1** on an as-received basis. The uncertainty for vitamin K_1 in RM 8260 was approximated as 20 % relative to the measured value.

Table 5-1. Individualized data summary table for vitamin K_1 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 1 – Fat-Soluble Vitamins in Infant Formulas

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*
SRM 1849b	mg/kg	*Individual laboratory results will appear in this section; laboratory-specific results were provided to each participant separately from this report.*				5	1.30	0.75	1.032	0.016
RM 8260	mg/kg					5	0.90	0.55	0.88	0.09

x_i	Mean of reported values	N	Number of quantitative values reported	x_{NIST}	NIST-assessed value
s_i	Standard deviation of reported values			*U*	expanded uncertainty 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

Table **5-1** summarizes and Table **5-2** details the numerical results reported by each participating laboratory. The participation level was low for this vitamin K study, with only 35 % of laboratories requesting samples returning results (6 of 17 laboratories).

Table **5-2** reveals that the within-laboratory variabilities were mostly acceptable with respect to published expectations of the measurement community (≤ 5 %) [32]. Two of the 5 laboratories reporting quantitative vitamin K results indicated variabilities greater than expected (16 % RSD to 73 % RSD); however, both laboratories were designated as outside the consensus tolerance limits. Of the remaining data sets, one laboratory reported a within-laboratory variability of 12 % for 1 sample. The between-laboratory variabilities were high (57 % and 61 %) with respect to the published expectations of the measurement community for multiple laboratories using the same method (≤ 10 %), even when considering the variety of methods used by the participants [32]. Additionally, the limited number of laboratories reporting quantitative results (5 laboratories) combined with the observation of one major and one minor high outlier (Fig. **5-1** and Fig. **5-2**) may inflate the observed between-laboratory variability beyond what would routinely be observed in this community for measurement of vitamin K_1 in infant formulas.

Table 5-2. Data summary table for total vitamin K_1 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, $|Z'_{comm}| \geq 2$.

		Vitamin K_1 (Phylloquinone)									
		SRM 1849b Infant/Adult Nutritional Formula I (milk-based) (mg/kg)					RM 8260 Infant Formula (hydrolyzed-milk based) (mg/kg)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target				1.032	0.016				0.88	0.09
	A001	< 0.372	< 0.372	< 0.372			< 0.372	< 0.372	< 0.372		
	A004										
	A005										
	A006	15.58	4.34	13.33	11.08	5.95	5.74	2.85	12.96	7.18	5.21
	A010	3	4	4	3.67	0.58	3	2	2	2.33	0.58
	A012										
	A015	1.179	1.189	1.191	1.19	0.01	0.745	0.749	0.777	0.76	0.02
	A016	0.966	0.916	0.757	0.88	0.11	0.51	0.498	0.496	0.50	0.01
	A026										
	A027	0.879	0.878	0.846	0.87	0.02	0.604	0.628	0.645	0.63	0.02
	A031										
	A035										
	A037										
	A040										
	A045										
	A048										
	A054										
Community Results		Consensus Mean			1.32		Consensus Mean			0.90	
		Consensus Standard Deviation			0.75		Consensus Standard Deviation			0.55	
		Maximum			11.08		Maximum			7.18	
		Minimum			0.87		Minimum			0.50	
		N			5		N			5	

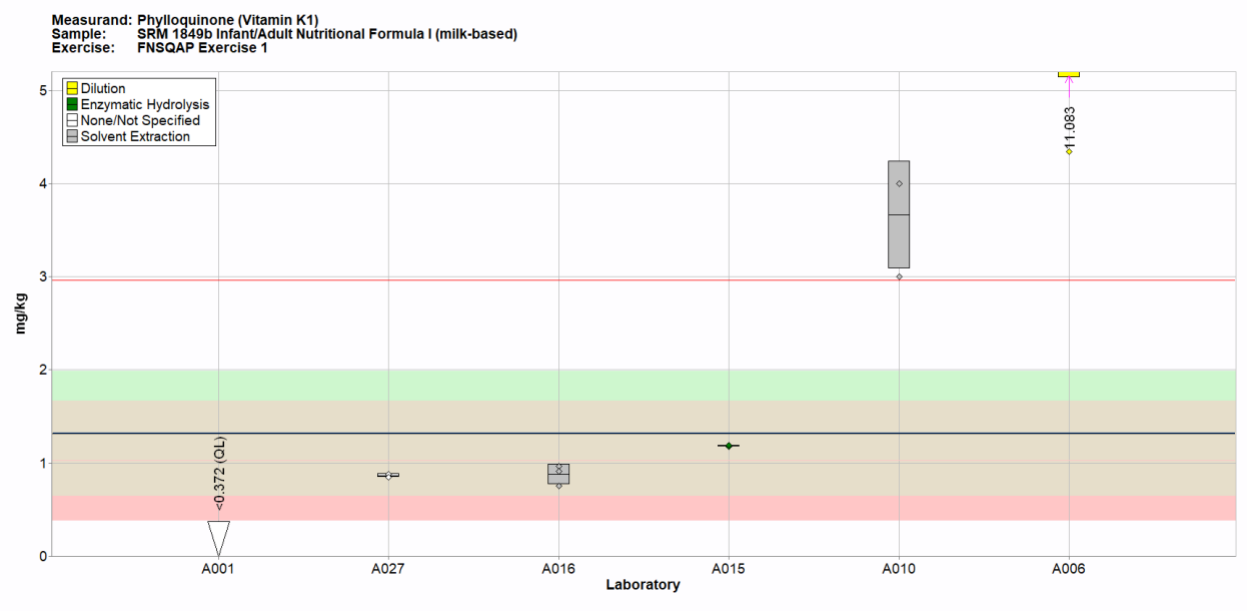

Fig. 5-1. Total vitamin K_1 in SRM 1849b (data summary view – sample preparation method).

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 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, $|Z'_{\mathrm{comm}}| \leq 2$, with the bottom bound set to zero. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty (U_{NIST}) and represents the range that results in an acceptable Z_{NIST} score,$|Z_{\mathrm{NIST}}| \leq 2$. The shaded beige region represents the overlapping of the 95 % confidence interval for the consensus mean (green region) and the NIST range of tolerance (red region).

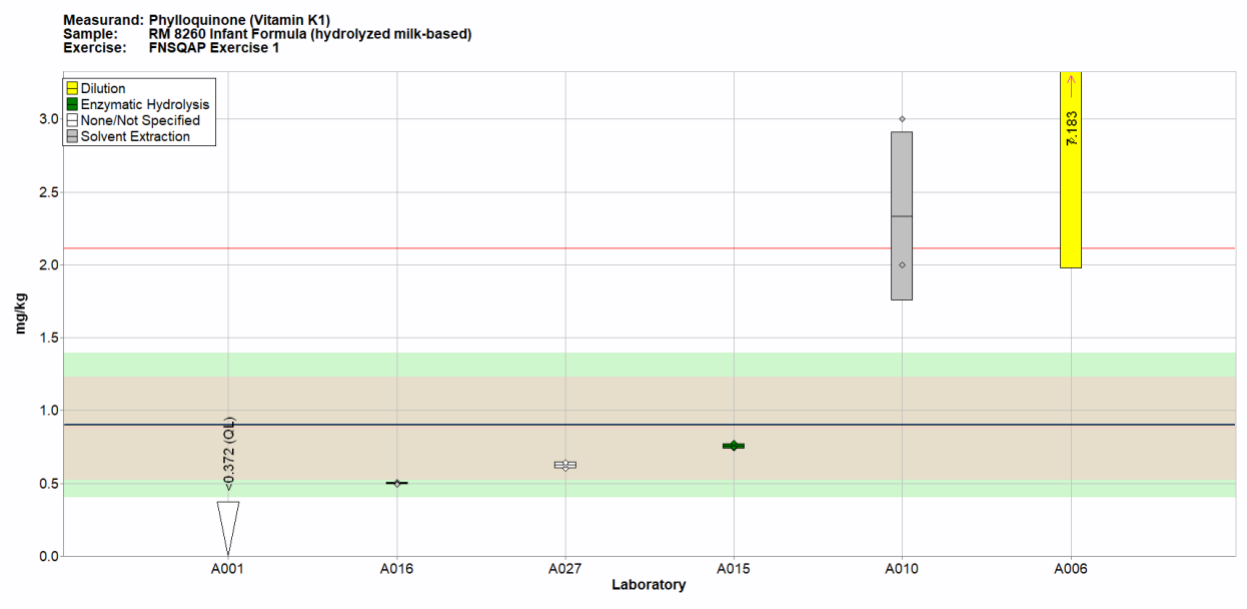

Fig. 5-2. Total vitamin K_1 in RM 8260 (data summary view – sample preparation method).

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

As shown in Fig. **5-1** and Fig. **5-2**, 2 laboratories reported using solvent extraction (33 %) and one laboratory each reported use of enzymatic hydrolysis and dilution (17 % each). Two laboratories did not report the sample preparation approach used (33 %). The laboratory indicating use of dilution for determination of vitamin K_1 reported values significantly above the consensus and target ranges in both samples. The significance of this trend is difficult to determine with only one laboratory's results, however, and is further muddled by a potential calculation error by this laboratory that reported values approximately ten-fold higher than other reported values and the consensus and target means.

Similarly, Fig. **5-3** and Fig. **5-4** indicate that all laboratories reported using liquid chromatography (LC)-based techniques for the determination of vitamin K_1 in the two infant formula samples. Two laboratories each reported use of LC-Abs and Liquid Chromatography with Fluorescence Detection (LC-FLD) (33 % each), and 1 laboratory reported using LC-MS (17 %). One laboratory did not report the analytical approach used (17 %). Notably, the laboratory reporting use of LC-MS was not able to quantify the level of vitamin K_1 in these samples, and the two laboratories reporting use of LC-Abs techniques reported values above the consensus and target ranges in both samples. As described previously, the significance of these observations is difficult to determine with such a small data set, however, and is further muddled by a potential calculation error by one laboratory that reported values approximately ten-fold higher than other reported values and the consensus and target means.

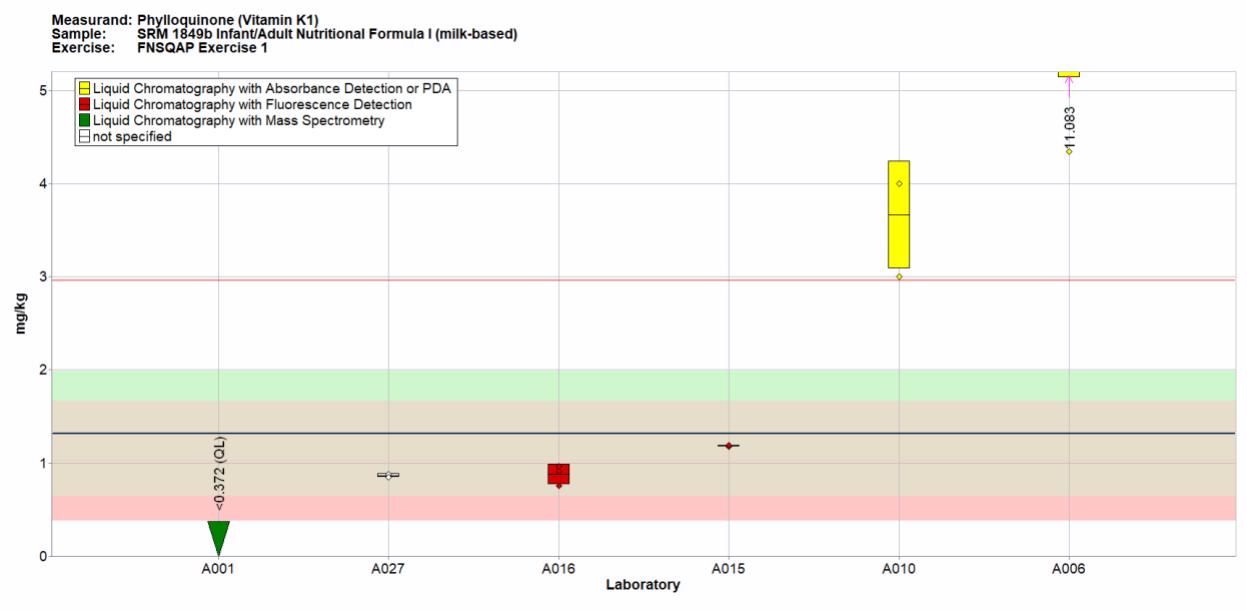

Fig. 5-3. Total vitamin K_1 in SRM 1849b (data summary view – analytical method).

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 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, $|Z'_{\mathrm{comm}}| \leq 2$, with the bottom bound set to zero. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty (U_{NIST}), and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\mathrm{NIST}}| \leq 2$. The shaded beige region represents the overlapping of the 95 % confidence interval for the consensus mean (green region) and the NIST range of tolerance (red region).

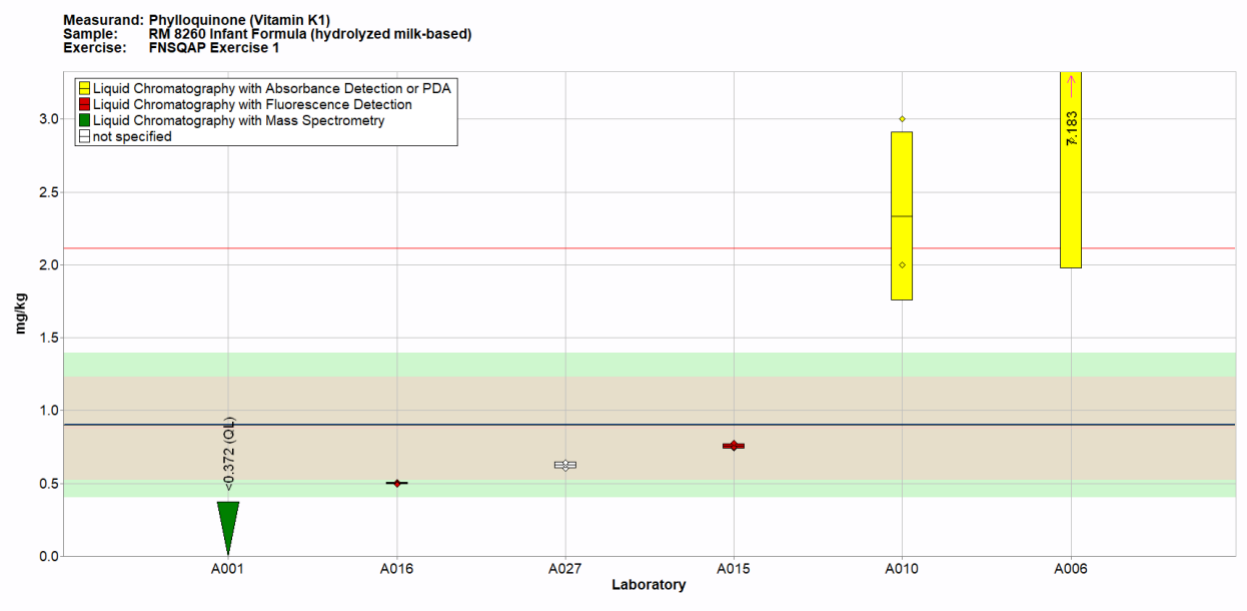

Fig. 5-4. Total vitamin K_1 in RM 8260 (data summary view – analytical method).

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 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 red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty (U_{NIST}), and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$. The shaded beige region represents the overlapping of the 95 % confidence interval for the consensus mean (green region) and the NIST range of tolerance (red region).

For both infant formula samples, the consensus means for total vitamin K_1 (phylloquinone) are within the target range (Fig. **5-1**, Fig. **5-2**, Fig. **5-3**, Fig. **5-4**). In SRM 1849b, the consensus mean (1.3 mg/kg) is slightly above the target mean (1.0 mg/kg) (Fig. **5-1**, Fig. **5-3**) while the consensus and target means in RM 8260 are statistically indistinguishable at a 99 % confidence level (0.9 mg/kg, Fig. **5-2**, Fig. **5-4**).

Overall, conclusions cannot be drawn about community performance in the determination of vitamin K_1 in infant formula samples. A slight linear trend is observed in Fig. **5-5**, which may indicate a global issue with calibration. Laboratories that reported values above the target did so consistently in these two very similar samples. Potential sources of this type of calibration issue may be in the inaccurate assignment of calibrant purity (e.g., not considering potential impurities or moisture in the calibrant material) or extension of the calibration curve beyond the linear range. All calibrant materials should be of known purity, either through a statement of traceability to the SI (such as those from NIST or another national metrology institute) or through independent verification within the user's laboratory. Many standards manufacturers provide detailed documentation about purity testing; the purity should be verified in-house prior to use and all concentrations adjusted to reflect known impurities. For vitamin K_1, the most accurate assignment of the concentration of calibration materials requires spectrophotometric evaluation using an appropriate molar extinction coefficient. Once the calibrant material has been well characterized, the calibration curve 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 that calibration range and other points can be excluded from the determination of the calibration curve to prevent bias. Another potential contributor to the trend observed in Fig. **5-5** may be decomposition of vitamin K_1 in the samples and/or standards during analysis. Vitamin K_1 is known to be light-sensitive and therefore samples and standards should be prepared under amber or attenuated lighting to reduce potential degradation.

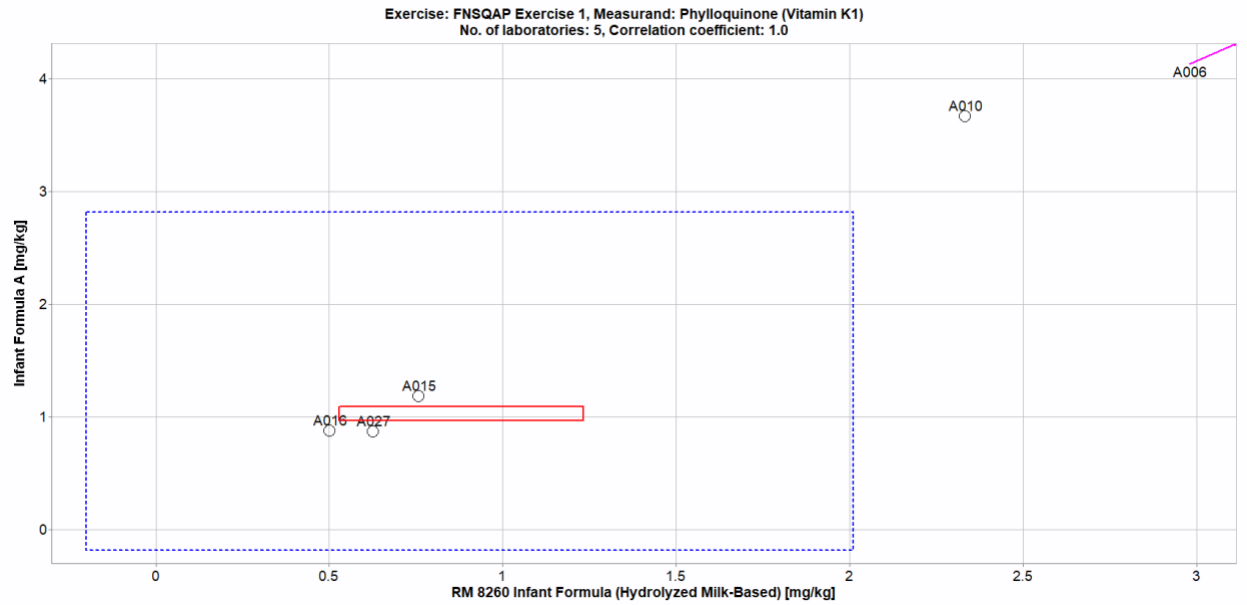

Fig. 5-5. Laboratory means for total vitamin K_1 in RM 8260 and SRM 1849b (sample/sample comparison view).

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 range of tolerance 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, $|Z_{\mathrm{NIST}}| \leq 2$. 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, $|Z'_{\mathrm{comm}}| \leq 2$.

NIST has conducted four QAP studies involving measurement of vitamin K in food samples prior to this FNSQAP study: DSQAP Exercise K in 2014 [9] and Exercise M in 2015 [10], and HAMQAP Exercise 4 in 2019 [12] and Exercise 7 in 2021 [14]. A review of the results from these previous exercises indicated no apparent trends in the number of laboratories reporting data, average RSD_r, RSD_R, or bias with respect to the NIST target value over time.

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 vitamin K typically contain approximately 10 % of the *cis*- isomer. Coupled with isomerization between the *cis*- and *trans*- forms that may occur during sample preparation, bias in reporting of *trans*-vitamin K_1 may occur. 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. CONTAMINANTS (Glyphosate, AMPA, N-Acetyl-Glyphosate, N-Acetyl-AMPA)

6.1. 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 performed well with respect to consensus-based standards in the determination of glyphosate and AMPA in the food products presented and were challenged as the levels of these contaminants approached the detection limits of modern instrumentation.

6.2. Study Overview

Glyphosate is a widely applied broad-spectrum herbicide used to control broadleaf weeds and grasses [33]. Worldwide experts have not reached a consensus on the human toxicity of glyphosate [33, 34] 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 in agricultural and consumer products must be well characterized and have demonstrated accuracy. In this study, participants were provided with samples of SRM 1548b Typical Diet and RM 8186 Soy Protein Isolate. 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.

6.3. Sample Information

Participants were provided three packets each of SRM 1548b Typical Diet and RM 8186 Soy Protein Isolate. Packets of SRM 1548b contained 5 g of material and were to be stored under refrigeration, 2 °C to 8 °C; packets of RM 8186 contained 10 g of material and were to be stored at controlled room temperature, 20 °C to 25 °C. Participants were instructed to thoroughly mix the contents of each packet before use and to prepare one sample and report one value from each packet provided using 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 and for AMPA in RM 8186 were determined using mean results and standard deviations from a collaborating laboratory; the target values and uncertainties are provided in Table **6-1** on an as-received basis. Target values for AMPA in SRM 1548b and for n-acetyl-glyphosate and n-acetyl-AMPA in both materials were not available at the time of this report.

Table 6-1. Individualized data summary table for glyphosate and AMPA in foods. Laboratory-specific results and *Z*-scores were provided to each participant separately from this report to protect laboratory identities.

(Lab Name)

Exercise 1 – 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*
AMPA	SRM 1548b	ng/g	*Individual laboratory results will appear in this section; laboratory-specific results were provided to each participant separately from this report.*				1				
AMPA	RM 8186	ng/g					7	140	49	89.6	1.0
Glyphosate	SRM 1548b	ng/g					10	90	25	68.3	7.5
Glyphosate	RM 8186	ng/g					10	50	18	33.8	1.3
n-acetyl-Glyphosate	SRM 1548b	ng/g					0				
n-acetyl-Glyphosate	RM 8186	ng/g					0				
n-acetyl-AMPA	SRM 1548b	ng/g					0				
n-acetyl-AMPA	RM 8186	ng/g					0				

x_i Mean of reported values
s_i Standard deviation of reported values
Z'_{comm} Z'-score with respect to community consensus
Z_{NIST} Z-score with respect to NIST value
N Number of quantitative values reported
x* Robust mean of reported values
s* Robust standard deviation
x_{NIST} NIST-assessed value
U expanded uncertainty about the NIST-assessed value

6.4. Study Results and Discussion

Table **6-1** summarizes and Table **6-2** details the numerical results for glyphosate reported by each participating laboratory. The participation level was high for glyphosate, with 55 % of laboratories requesting samples returning results (11 of 20 laboratories).

Table **6-2** reveals that within-laboratory variabilities were mostly acceptable with respect to published expectations of the glyphosate measurement community (≤ 20 %) [35], with only one data set for glyphosate reported at 33 % RSD. The between-laboratory variabilities were reasonable (29 % to 39 %) for the variety of methods with respect to the published expectations of the glyphosate measurement community for multiple laboratories using the same method (≤ 25 %) [35].

Table 6-2. Data summary table for glyphosate in SRM 1548b and RM 8186.
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 1548b Typical Diet (ng/g)					RM 8186 Soy Protein Isolate (ng/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target				68	7				34	1
	A004										
	A006										
	A008	152.63	177.7	217.94	183	33	71.6	71.59	66.02	70	3
	A011	200	181	155	179	23	55.5	69.5	34.8	53	17
	A012	76	80	80	79	2	41	39	38	39	2
	A013	91.7	73.5	82.7	83	9	50.8	54.9	39.1	48	8
	A021										
	A022										
	A025										
	A027	80	82	75	79	4	42	40	38	40	2
	A031	< 100	< 100	< 100			< 100	< 100	< 100		
	A033	78.1	78	68.5	75	6	35	35.5	30.6	34	3
	A037										
	A040										
	A042										
	A045	652.466038	781	726.733019	720	65	2544.10932	2051.13565	2397.62248	2331	253
	A046	68	67	68	68	1	39	40	41	40	1
	A047										
	A051	170	189.8	185.6	182	10	353.2	355.4	355	355	1
	A059	80	82	81	81	1	45	46	49	47	2
Community Results		Consensus Mean			86		Consensus Mean			46	
		Consensus Standard Deviation			25		Consensus Standard Deviation			18	
		Maximum			720		Maximum			2331	
		Minimum			68		Minimum			34	
		N			10		N			10	

As shown in Fig. **6-1** and Fig. **6-2**, 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. The most common sample preparation approach was derivatization (5 of 11 laboratories, 45 %); one laboratory each reported use of acid hydrolysis, "Quick Polar Pesticides" extraction (QuPPe), solid phase extraction, solvent extraction, solvent extraction with derivatization, and solvent extraction with solid phase extraction and derivatization (9 % each). In each matrix, the laboratories using only a derivatization step to prepare the samples reported values higher than values determined using additional steps or other approaches; three of the laboratories reporting quantitative values were outside of the consensus range for RM 8186 and all four laboratories reporting quantitative values were outside of the consensus range for SRM 1548b. Additionally,

the two laboratories using derivatization following solvent extraction and/or solid phase extraction reported the next highest values of the data set. Laboratories using only derivatization for determination of glyphosate in food matrices should be aware of non-specific reactions occurring within the complex sample that may lead to overreporting of glyphosate levels. Addition of a clean-up step such as solvent extraction and/or solid phase extraction may reduce contribution from potential interferences.

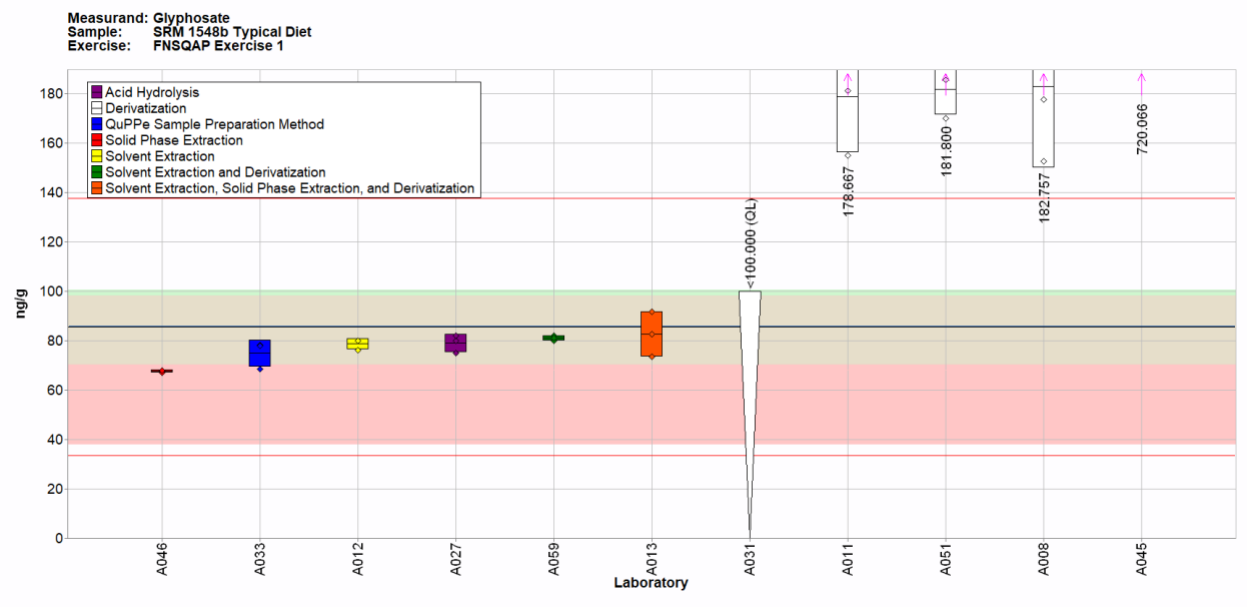

Fig. 6-1. Glyphosate in SRM 1548b (data summary view – sample preparation method).

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 approach reported by laboratory A045 was derivatization). The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\mathrm{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty (U_{NIST}), and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\mathrm{NIST}}| \leq 2$. The shaded beige region represents the overlapping of the 95 % confidence interval for the consensus mean (green region) and the NIST range of tolerance (red region).

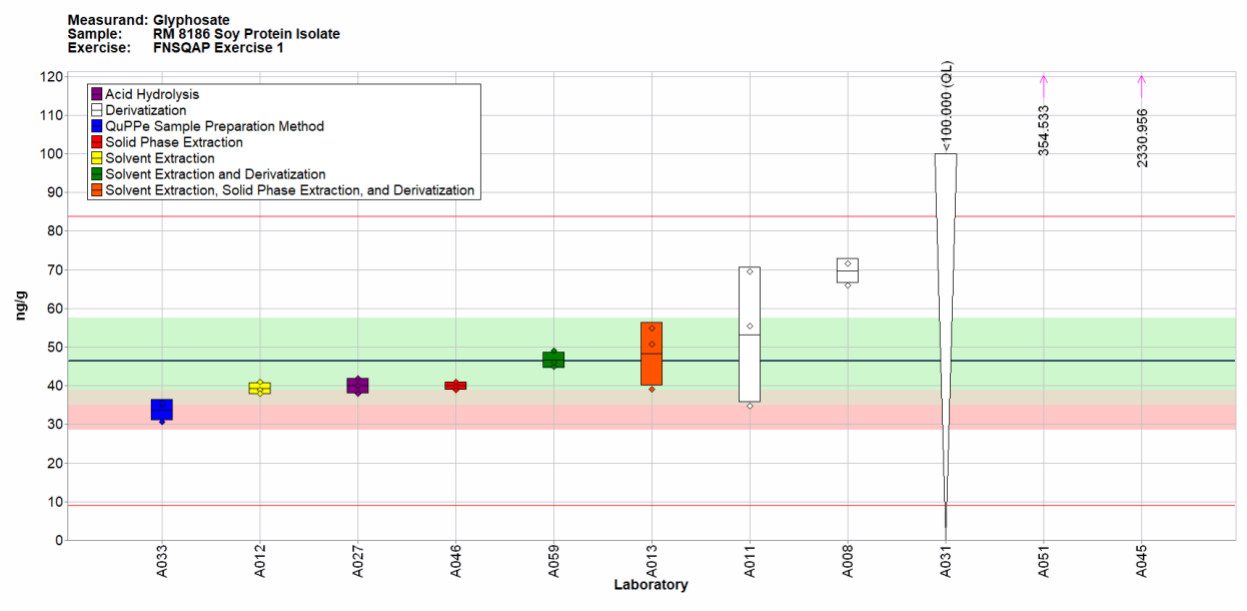

Fig. 6-2. Glyphosate in RM 8186 (data summary view – sample preparation method).

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 approaches reported by laboratories A051 and A045 were derivatization). The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty (U_{NIST}), and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$. The shaded beige region represents the overlapping of the 95 % confidence interval for the consensus mean (green region) and the NIST range of tolerance (red region).

Similarly, Fig. **6-3** and Fig. **6-4** indicate that all laboratories reported using LC-MS-based techniques for the determination of glyphosate in the two food samples. Seven laboratories reported use of LC-MS/MS (63 %), 3 laboratories reported use of LC-MS (27 %), and 1 laboratory reported us of Liquid Chromatography with High Mass Resolution Spectrometry (LC-HRMS) (9 %). No trends related to analytical method could be identified.

For SRM 1548b, the consensus range for glyphosate overlaps the upper portion of the target range (Fig. **6-1** and Fig. **6-3**). Community performance for glyphosate in RM 8186 was generally poorer, with the consensus mean falling outside of the target range and the consensus range only slightly overlapping with the target range (Fig. **6-2** and Fig. **6-4**). The expected level of glyphosate in SRM 1548b is approximately twice the level expected in RM 8186, which may contribute to the high bias in the consensus mean for the soy matrix. The between-laboratory variability for RM 8186 was also greater than that for SRM 1548b, which is consistent with difficulty in measurement of lower levels of contaminants [40]. Additionally, the protein content of soybean matrices has been demonstrated as problematic with many glyphosate extraction and analysis approaches. Soy-based samples often require additional steps for protein precipitation which result in lower reported recoveries compared to other sample types due to coprecipitation of analytes of interest and ion suppression in the mass spectrometer [36-38].

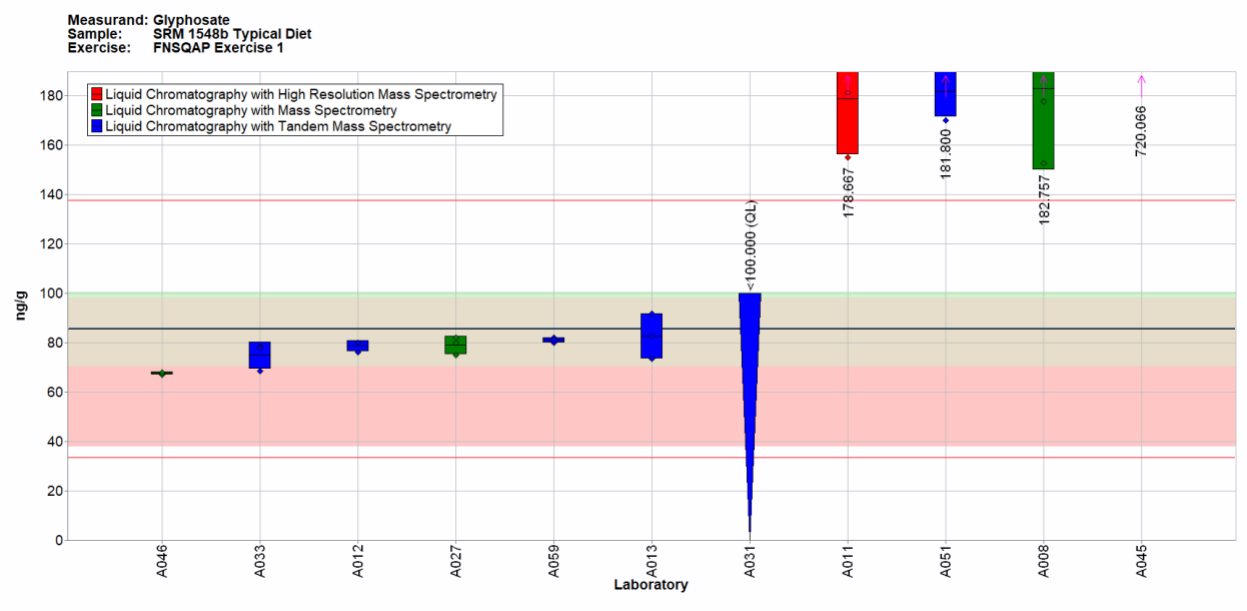

Fig. 6-3. Glyphosate in SRM 1548b (data summary view – analytical method).

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 method reported by laboratory A045 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, $|Z'_{\mathrm{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty (U_{NIST}), and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\mathrm{NIST}}| \leq 2$. The shaded beige region represents the overlapping of the 95 % confidence interval for the consensus mean (green region) and the NIST range of tolerance (red region).

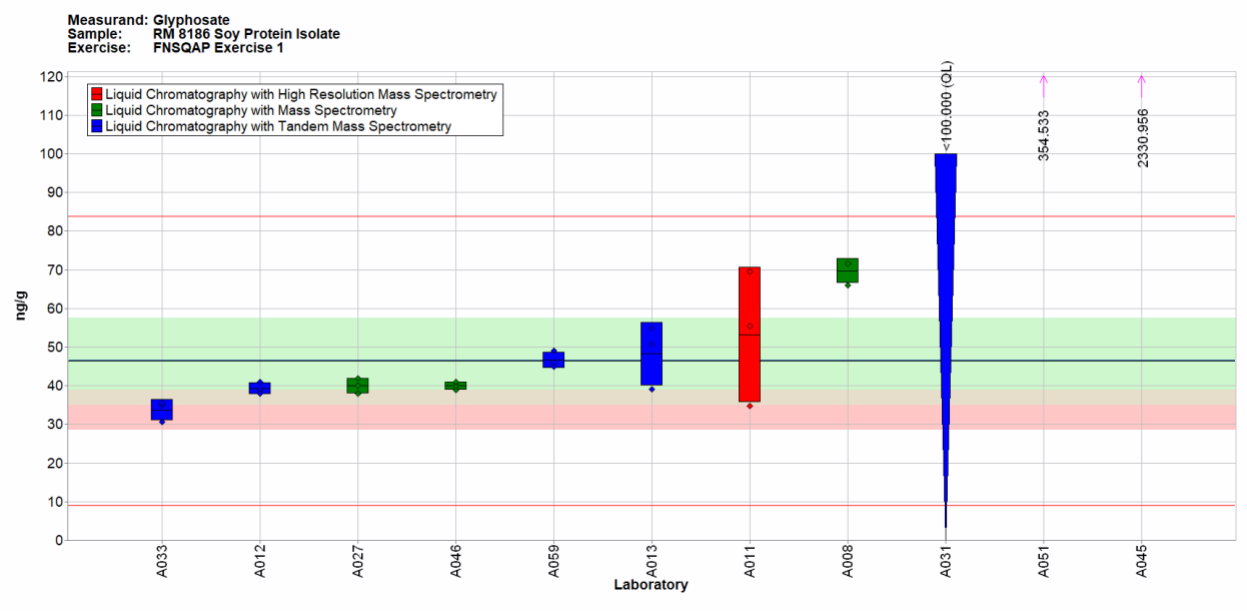

Fig. 6-4. Glyphosate in RM 8186 (data summary view – analytical method).

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 methods reported by laboratories A051 and A045 were 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 range of tolerance, which encompasses the target value bounded by twice its uncertainty (U_{NIST}) and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$. The shaded beige region represents the overlapping of the 95 % confidence interval for the consensus mean (green region) and the NIST range of tolerance (red region).

Overall, laboratories performed well in the determination of glyphosate in food samples. No clear correlation is observed in Fig. **6-5**, but in general, RM 8186 presented a greater analytical challenge to laboratories. Notably, laboratories A008 and A011 reported results that appear to be biased higher in SRM 1548b relative to the consensus and target when compared to the reported results for the soy sample, while laboratories A045 and A051 reported results that were similarly biased in the two materials relative to the consensus and target. The known challenges with determination of glyphosate from soybean materials that cause a low bias [36-38] may convolute the high-biased results reported by laboratories A008 and A011.

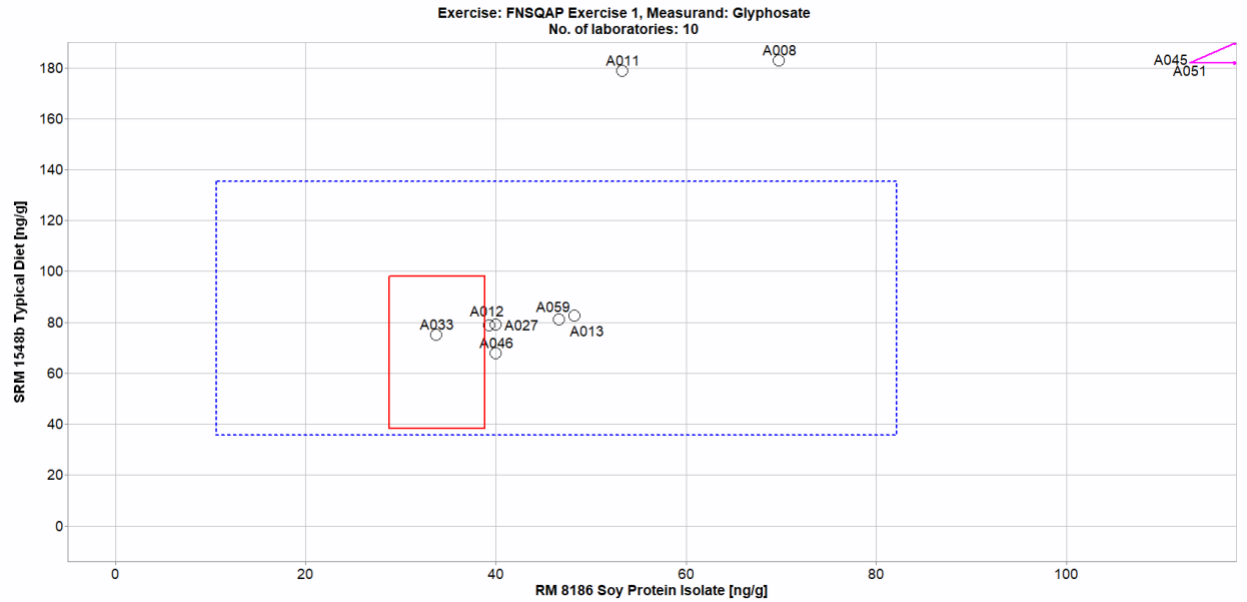

Fig. 6-5. Laboratory means for glyphosate in SRM 1548b and RM 8186 (sample/sample comparison view).

In this view, the individual laboratory mean for one sample (RM 8186) is compared to the mean for a second sample (SRM 1548b). The solid red box represents the NIST range of tolerance for the two samples, RM 8186 (x-axis) and SRM 1548b (y-axis), which encompasses the target values bounded by their uncertainties (U_{NIST}) and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\mathrm{NIST}}| \leq 2$. The dotted blue box represents the consensus range of tolerance for RM 8186 (x-axis) and SRM 1548b (y-axis), calculated as the values above and below the consensus means that result in an acceptable Z'_{comm} score, $|Z'_{\mathrm{comm}}| \leq 2$.

Table **6-1** summarizes and Table **6-3** details the numerical results for AMPA reported by each participating laboratory. The participation level was slightly lower for AMPA at 40 % (8 of 20 laboratories) compared to glyphosate (55 %). Only one quantitative value was reported for AMPA in SRM 1548b, thus only the reported results for AMPA in RM 8186 will be discussed further. Table **6-3** also reveals that within-laboratory variabilities were acceptable for AMPA with respect to published expectations of this measurement community ($\leq$ 20 %). The between-laboratory variability was reasonable (34 %) for the variety of methods with respect to the published expectations of this measurement community for multiple laboratories using the same method ($\leq$ 25 %) [35].

Table 6-3. Data summary table for AMPA in SRM 1548b and RM 8186.
Data highlighted in blue have been identified as outside the consensus tolerance limits and resulted in an unacceptable Z'_{comm} score, $|Z'_{comm}| > 2$.

		AMPA									
		SRM 1548b Typical Diet (ng/g)					RM 8186 Soy Protein Isolate (ng/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target									89.6	1.0
	A004										
	A006										
	A008	27.34	31.7	30.64	29.9	2.3	1177.1	1236.16	1306.62	1240.0	64.8
	A011	< 400	< 400	< 400			117	108	125	116.7	8.5
	A012	< 10	< 10	< 10			133	120	160	137.7	20.4
	A013	< 50	< 50	< 50			166	130	121	139.0	23.8
	A021										
	A022										
	A025										
	A027	< 100	< 100	< 100			165	164	164	164.3	0.6
	A031	< 100	< 100	< 100			< 100	< 100	< 100		
	A033	< 20	< 20	< 20			109.3	116.9	127.9	118.0	9.4
	A037										
	A040										
	A042										
	A045										
	A046										
	A047										
	A051										
	A059	< 10	< 10	< 10			188	180	192	186.7	6.1
Community Results		Consensus Mean					Consensus Mean			143.7	
		Consensus Standard Deviation					Consensus Standard Deviation			49.2	
		Maximum			29.9		Maximum			1240	
		Minimum			29.9		Minimum			116.7	
		N			1		N			7	

Fig. **6-6** depicts graphically the variety of sample preparation methods reported for the determination of AMPA in RM 8186. As seen with glyphosate methods, some laboratories reported using a single-step preparation approach, while other laboratories reported using a multi-step approach. The most common sample preparation approach was simple derivatization (3 of 8 laboratories, 38 %); one laboratory each reported use of acid hydrolysis, QuPPe, solvent extraction, solvent extraction with derivatization, and solvent extraction with solid phase extraction and derivatization (13 % each). Interestingly, the laboratories reporting use of derivatization reported both the highest and lowest quantitative values across the data set, contradictory to the data reported using derivatization for glyphosate. Notably, however, the two laboratories reporting the highest values for glyphosate did not report values for AMPA in RM 8186.

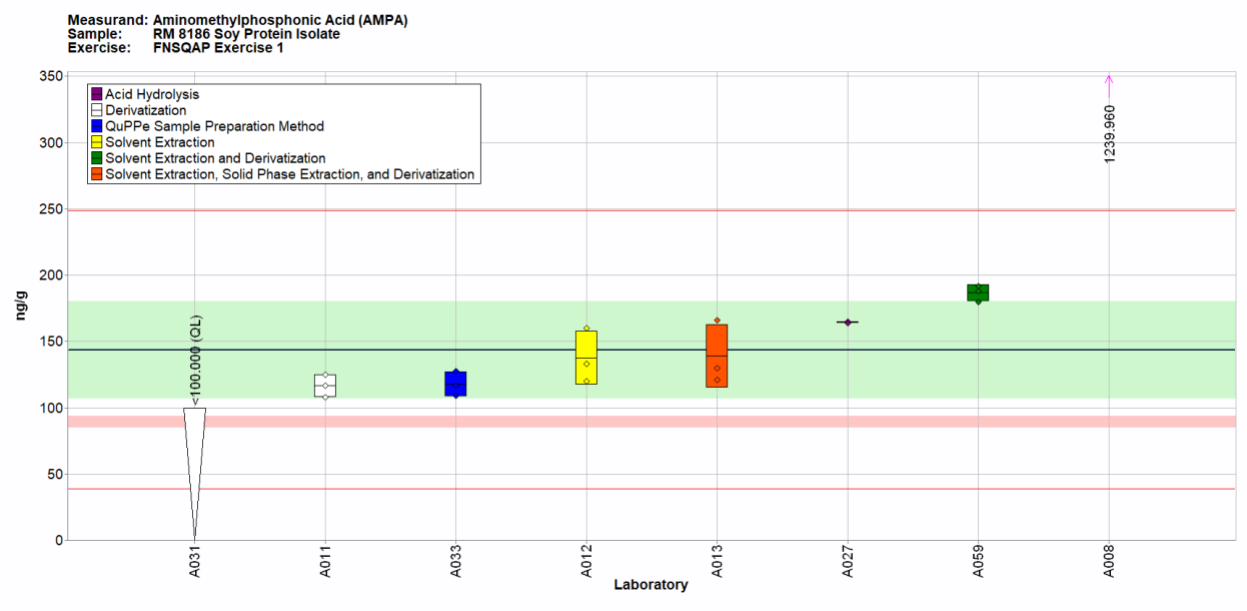

Fig. 6-6. AMPA in RM 8186 (data summary view – sample preparation method).

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 approach reported by laboratory A008 was derivatization). The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\mathrm{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty (U_{NIST}), and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\mathrm{NIST}}| \leq 2$.

The consensus range for AMPA in RM 8186 does not overlap the target range (Fig. **6-6** and Fig. **6-7**), and the consensus mean is nearly double the target mean. While the between-laboratory variability was reasonable as described previously, laboratories may have a high bias in the determination of AMPA in food products.

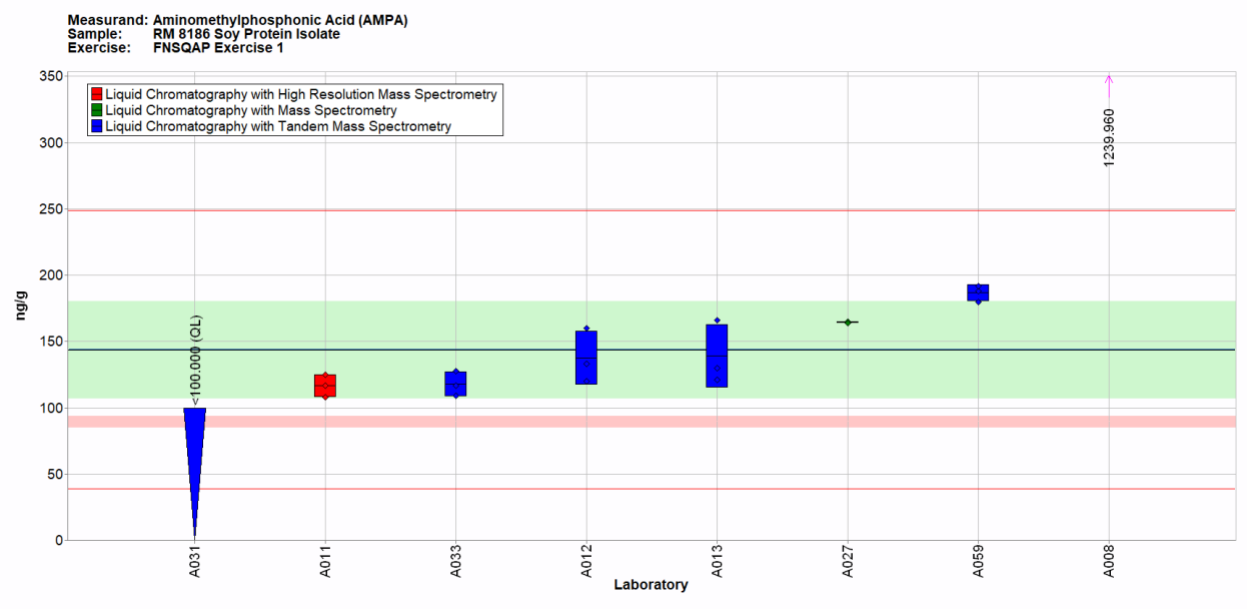

Fig. 6-7. AMPA in RM 8186 (data summary view – analytical method).

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 sample preparation approach reported by laboratory A008 was LC-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 range of tolerance, which encompasses the target value bounded by twice its uncertainty (U_{NIST}), and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$.

Similarly, Fig. **6-7** indicates that all laboratories reported using LC-MS-based techniques for the determination of AMPA in the two food samples. Five laboratories reported use of LC-MS/MS (63 %), 2 laboratories reported use of LC-MS (25 %), and 1 laboratory reported us of LC-HRMS (13 %). Because the reported methods are so similar, no trends related to analytical method could be identified.

NIST has conducted one other QAP study involving measurement of glyphosate in food samples prior to this FNSQAP study: HAMQAP Exercise 6 in 2021 [26]. A review of the results from this previous exercise indicated no apparent trends in the number of laboratories reporting data, average RSD_r, RSD_R, or bias with respect to the NIST target value over time.

Table **6-4** and Table **6-5** detail the numerical results for n-acetyl-glyphosate and n-acetyl-AMPA reported by each participating laboratory. Of the 18 laboratories that indicated an intention to report results for these 2 analytes, only 4 responded and all results were non-quantitative (below LOQ). For determination of n-acetyl-glyphosate, laboratories reported the use of solid phase extraction, solvent extraction, derivatization, and QuPPe methods for sample preparation (25 % each). Three laboratories reported use of LC-MS/MS (75 %), and 1 laboratory reported the use of LC-MS (25 %) for sample analysis. For determination of n-acetyl-AMPA, 2 laboratories reported using the QuPPe (50 %), and 1 laboratory each reported use of solvent extraction and derivatization for sample preparation (25 % each). All laboratories reported using LC-MS/MS for sample analysis. 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.

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.

Table 6-4. Data summary table for n-acetyl-glyphosate in SRM 1548b and RM 8186.

		N-acetyl-Glyphosate									
		SRM 1548b Typical Diet (ng/g)					RM 8186 Soy Protein Isolate (ng/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target										
	A004										
	A006										
	A008										
	A011										
	A012										
	A013										
	A022										
	A025										
	A031	< 100	< 100	< 100			< 100	< 100	< 100		
	A033	< 20	< 20	< 20			< 20	< 20	< 20		
	A037										
	A040										
	A042										
	A045										
	A046	< 2	< 2	< 2			< 2	< 2	< 2		
	A047										
	A051										
	A059	< 10	< 10	< 10			< 10	< 10	< 10		
Community Results		Consensus Mean					Consensus Mean				
		Consensus Standard Deviation					Consensus Standard Deviation				
		Maximum					Maximum				
		Minimum					Minimum				
		N					N				

Table 6-5. Data summary table for n-acetyl-AMPA in SRM 1548b and RM 8186.

		N-acetyl-AMPA									
		SRM 1548b Typical Diet (ng/g)					RM 8186 Soy Protein Isolate (ng/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target										
	A004										
	A006										
	A008										
	A011										
	A012	< 10	< 10	< 10			< 10	< 10	< 10		
	A013										
	A022										
	A025										
	A031	< 100	< 100	< 100			< 100	< 100	< 100		
	A033	< 20	< 20	< 20			< 20	< 20	< 20		
	A037										
	A040										
	A042										
	A045										
	A046										
	A047										
	A051										
	A059	< 10	< 10	< 10			< 10	< 10	< 10		
Community Results		Consensus Mean					Consensus Mean				
		Consensus Standard Deviation					Consensus Standard Deviation				
		Maximum					Maximum				
		Minimum					Minimum				
		N					N				

7. CONTAMINANTS (Acrylamide)

7.1. Executive Summary

To protect public health, regulators must understand human dietary exposure to potentially harmful contaminants such as acrylamide, formed during high-temperature food processing, through accurate determination of acrylamide 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.

7.2. Study Overview

Acrylamide is a chemical that is formed from naturally occurring sugars and asparagine in foods during some high-temperature cooking processes [39]. Exposure to high doses of acrylamide has been demonstrated to cause cancer in animals, and worldwide experts have declared acrylamide a human health concern. Monitoring of human exposure is a critical component of understanding population health impacts, and to ensure that future studies on dangers of acrylamide exposure are properly interpreted, methods for the detection of acrylamide in food products must be well characterized and have demonstrated accuracy. In this study, participants were provided with samples of SRM 2384 Baking Chocolate and dark roasted coffee beans. Participants were asked to use in-house analytical methods to determine the mass fraction (ng/g) of acrylamide 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 one packet each of SRM 2384 Baking Chocolate and dark roasted coffee beans. Packets of SRM 2384 contained 18 g of material and were to be stored at –20 °C or colder. Before use, participants were instructed to either melt or grate the bar. Packets of coffee beans contained 100 g of material and were to be stored at controlled room temperature, 20 °C to 25 °C. Before use, participants were instructed to grind the entire packet of coffee beans and mix the resulting powder thoroughly. Participants were instructed to prepare three samples and report three values from each packet provided using 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 acrylamide in SRM 2384 and coffee beans were determined using results from previous interlaboratory comparisons [9] the values and uncertainties are provided in Table **7-1** on an as-received basis.

Table 7-1. Individualized data summary table for acrylamide in foods.
Laboratory-specific results and *Z*-scores were provided to each participant separately from this report to protect laboratory identities.

(Lab Name)

Exercise 1 – Acrylamide 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*
SRM 2384	ng/g	*Individual laboratory results will appear in this section; laboratory-specific results were provided to each participant separately from this report.*				3	190	270	138	17
Coffee	ng/g					2	160	520	141	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*	expanded 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 numerical results reported by each participating laboratory. The participation level was low for acrylamide, with only 33 % of laboratories requesting samples returning results (3 of 9 laboratories). One laboratory did not report quantitative results for the coffee beans.

Table 7-2. Data summary table for acrylamide in SRM 2384.
Data highlighted in blue have been identified as outside the consensus tolerance limits and resulted in an unacceptable Z'_{comm} score, $|Z'_{comm}| > 2$.

		Acrylamide									
		SRM 2384 Baking Chocolate (ng/g)					**Dark Roasted Coffee (ng/g)**				
	Lab	**A**	**B**	**C**	**Avg**	**SD**	**A**	**B**	**C**	**Avg**	**SD**
Individual Results	Target				138	17				141	9
	A001										
	A004										
	A005										
	A006										
	A012	199.73	199.97		200	0.2	< 60.000	< 60.000	< 60.000		
	A019	42	43	43	42.7	0.6	45	46	42	44.3	2.1
	A031	315	320	313	316	3.6	280	285	278	281	3.6
	A037										
	A040										
Community Results		Consensus Mean			186		Consensus Mean			163	
		Consensus Standard Deviation			266		Consensus Standard Deviation			519	
		Maximum			316		Maximum			281	
		Minimum			42.7		Minimum			44.3	
		N			3		N			2	

Table **7-2** reveals that the within-laboratory variabilities were extremely low for both materials (< 3 %), and well within the published expectations for contaminants at such low levels [40]. The between-laboratory variabilities, however, were extremely high at over 100 % for both materials.

As shown in Table **7-3**, 2 laboratories reported using "Quick, Easy, Cheap, Effective, Rugged and Safe" solid phase extraction (QuEChERS) for sample preparation (67 %) while 1 laboratory reported using a combination of solvent extraction with solid phase extraction (33 %) for determination of acrylamide in these samples. All laboratories reported using LC-MS/MS as the analytical method.

Table 7-3. Method information reported by participants in the acrylamide study.

	Sample Preparation Method	Analytical Method
A012	Solvent Extraction + Solid Phase Extraction	LC-MS/MS
A019	QuEChERS	LC-MS/MS
A031	QuEChERS	LC-MS/MS

Given the low participation rate in this study, few meaningful conclusions can be drawn from the data. Two laboratories reported using very similar methods of analysis (QuEChERS with LC-MS/MS). One of these two laboratories reported consistently high results between the two samples, while the other reported consistently low results. Additional data is needed to better understand any potential method biases.

NIST has conducted one other QAP study involving measurement of acrylamide in food samples prior to this FNSQAP study: DSQAP Exercise K in 2014 [9]. A review of the results from this previous exercise indicated no apparent trends in the number of laboratories reporting data, average RSD_r, RSD_R, or bias with respect to the NIST target value over time.

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. DIETARY FIBER (IDF, HMWDF, HMW SDF, LMW SDF, SDFS, SDFP, SDF, TDF)

8.1. Executive Summary

Recently adopted standard methods have aimed to more appropriately identify and characterize various types of nutritionally relevant dietary fiber components in foods. This study demonstrated the bias of legacy methods that underestimate the contribution from various soluble fiber types. Additionally, this study revealed that assigned values for NIST SRM 3234 Soy Flour should be updated to reflect the modernized methodology for fiber determination.

8.2. Study Overview

Dietary fiber describes the parts of plant-based food that the human body cannot digest or absorb [41]. Consumption of fiber is known to promote bowel health, lower cholesterol levels, help control blood sugar, and assist in achieving and maintaining a healthy body weight [41]. Different types of fiber, namely soluble and insoluble, contribute differently to these potential health benefits. Analytically, meaningful determination of soluble and insoluble fiber in a variety of food matrices has presented a significant challenge. Understanding the fiber content of common foods is a critical component of understanding population health impacts, and to ensure that future studies on health benefits of fiber intake are properly interpreted, methods for the detection of all fiber forms in food products must be well characterized and have demonstrated accuracy. In this study, participants were provided with samples of SRM 3233 Fortified Breakfast Cereal and SRM 3234 Soy Flour. Participants were asked to use in-house analytical methods to determine the mass fraction (%) of various types of fiber in each matrix, as described in Table **8-1**.

Table 8-1. Definitions and abbreviations for target fiber types.

Fiber Type	Abbreviation	Definition
insoluble dietary fiber	IDF	--
high molecular weight soluble dietary fiber	HMW SDF	--
low molecular weight soluble dietary fiber	LMW SDF	--
soluble dietary fiber which remains soluble in 78% aqueous ethanol	SDFS	--
soluble dietary fiber that precipitates in 78% aqueous ethanol	SDFP	--
high molecular weight dietary fiber	HMWDF	IDF + SDFP
soluble dietary fiber	SDF	SDFP + SDFS
total dietary fiber	TDF	IDF + SDF

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 one bottle each of SRM 3233 Fortified Breakfast Cereal and SRM 3234 Soy Flour. Bottles of SRM 3233 contained 60 g of material and bottles of SRM 3234 contained 50 g of material. Both samples were to be stored at controlled room temperature, 20 °C to 25 °C, and participants were instructed to mix the contents of each bottle thoroughly before subsampling for analysis. Participants were instructed to prepare three samples and report three values from each bottle provided using 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 dietary fiber in SRM 3233 and SRM 3234 were from the NIST Certificate of Analysis (COA) for each material [42,43]; the values and uncertainties are provided in Table **8-2** on an as-received basis.

Table 8-2. Individualized data summary table for dietary fiber in cereals. Laboratory-specific results and *Z*-scores were provided to each participant separately from this report to protect laboratory identities.

(Lab Name)

Exercise 1 – Dietary Fiber in Cereals

	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*
IDF	SRM 3233	wt/wt %					8	7.3	1.8	6.49	0.44
IDF	SRM 3234	wt/wt %					8	14.0	2.5		
HMW SDF	SRM 3233	wt/wt %					0			2.82	0.60
HMW SDF	SRM 3234	wt/wt %					0				
LMW SDF	SRM 3233	wt/wt %					0			2.97	0.60
LMW SDF	SRM 3234	wt/wt %					0				
SDFS	SRM 3233	wt/wt %	*Individual laboratory results will appear in this section; laboratory-specific results were provided to each participant separately from this report.*				2	2.4	1.2		
SDFS	SRM 3234	wt/wt %					2	6.8	1.1		
SDFP	SRM 3233	wt/wt %					3	2.80	0.42		
SDFP	SRM 3234	wt/wt %					3	3.0	2.2		
HMWDF	SRM 3233	wt/wt %					3	14.0	3.2	2.6	1.5
HMWDF	SRM 3234	wt/wt %					3	20	16		
SDF	SRM 3233	wt/wt %					5	5.4	4.5	2.66	0.83
SDF	SRM 3234	wt/wt %					5	10	16		
TDF	SRM 3233	wt/wt %					11	10.0	3.0	12.03	0.77
TDF	SRM 3234	wt/wt %					11	20.0	8.1	17.88	0.36

x_i Mean of reported values
s_i Standard deviation of reported values
Z'_{comm} Z'-score with respect to community consensus
Z_{NIST} Z-score with respect to NIST value
N Number of quantitative values reported
x* Robust mean of reported values
s* Robust standard deviation
x_{NIST} NIST-assessed value
U expanded uncertainty about the NIST-assessed value

8.4. Study Results and Discussion

Table **8-2** summarizes and Table **8-3** details the numerical results for total dietary fiber (TDF) reported by each participating laboratory. Eighteen laboratories requested and received samples for the dietary fiber study, and one participant reported two sets of data using different methods. The participation level was high for TDF, with 58 % of laboratories requesting samples returning results (11 of 19 laboratories).

Table 8-3. Data summary table for total dietary fiber in SRM 3233 and SRM 3234. Data highlighted in blue have been identified as outside the consensus tolerance limits and resulted in an unacceptable Z'_{comm} score, $|Z'_{comm}| > 2$.

		Total Dietary Fiber (TDF = IDF + SDF)									
		SRM 3233 Fortified Breakfast Cereal (% w/w)					SRM 3234 Soy Flour (% w/w)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target				12.0	0.8				17.9	0.4
	A001	10.76	10.95	10.11	10.6	0.4	17.29	17.2	17.23	17.2	0.0
	A004										
	A006	36.84	36.21	36.71	36.6	0.3	76.49	75.42	75.6	75.8	0.6
	A012	11.69	11.59	11.45	11.6	0.1	25.27	25.25	25.47	25.3	0.1
	A015	8.8	9.3	9.6	9.2	0.4	18	19.1	19.9	19.0	1.0
	A020										
	A027	13.91	13.74	13.32	13.7	0.3	29.15	31.88	33.33	31.5	2.1
	A029	6.93	6.93	6.93	6.9	0.0	15.95	16.67	16.31	16.3	0.4
	A030										
	A031										
	A034	10.66	10.64	10.41	10.6	0.1	20.91	21.23	20.99	21.0	0.2
	A037										
	A038	12.19	13.12	12.05	12.5	0.6	26.5	26.79	27.18	26.8	0.3
	A040										
	A041	9.3	9.3	10	9.5	0.4	17.1	18.8	18.4	18.1	0.9
	A045	8.33	7.52	8.1	8.0	0.4	13	11.76	12.43	12.4	0.6
	A050										
	A053	9.69	9.56	9.57	9.6	0.1	15.82	16.08	16.43	16.1	0.3
	A061										
Community Results		Consensus Mean			10.2		Consensus Mean			20.4	
		Consensus Standard Deviation			3.0		Consensus Standard Deviation			8.1	
		Maximum			36.6		Maximum			75.8	
		Minimum			6.9		Minimum			12.4	
		N			11		N			11	

Table **8-3** reveals that within-laboratory variabilities were extremely good, with only 2 data sets reported with greater than 5 % RSD, 1 at 5.2 % RSD and 1 at 6.7 % RSD. The between-laboratory variabilities were high (29 % and 40 %) and highlight potential method differences.

As shown in Fig. **8-1** and Fig. **8-2**, laboratories reported using one of three AOAC Official Methods in the determination of total dietary fiber. The most reported method was AOAC 985.29/991.43 (7 of 11 laboratories, 64 %); 2 laboratories reported using AOAC 2017.16 (18 %), and 1 laboratory reported using AOAC 2009.01/2011.25 (9 %). In both samples, the values for total dietary fiber reported by laboratories using AOAC 985.29/991.43 were lower than all values reported by laboratories using other methods, which is further illustrated in Table **8-4** and Fig. **8-3**. This observation has been frequently reported in the literature with respect to AOAC 985.29/991.43, as common contributors to dietary fiber such as nondigestible oligosaccharides and most resistant starch are not measured by this method. Therefore, the method results in an underestimation of dietary fiber [44-47]. This method bias was addressed in the adoption of newer methods, including AOAC 2009.01/2011.25, and the approach was updated to be more biologically relevant with the adoption of AOAC 2017.16. The total dietary fiber results in this study reflect the improved accounting of the newer methods (Fig. **8-1**, Fig. **8-2**, Fig. **8-3**).

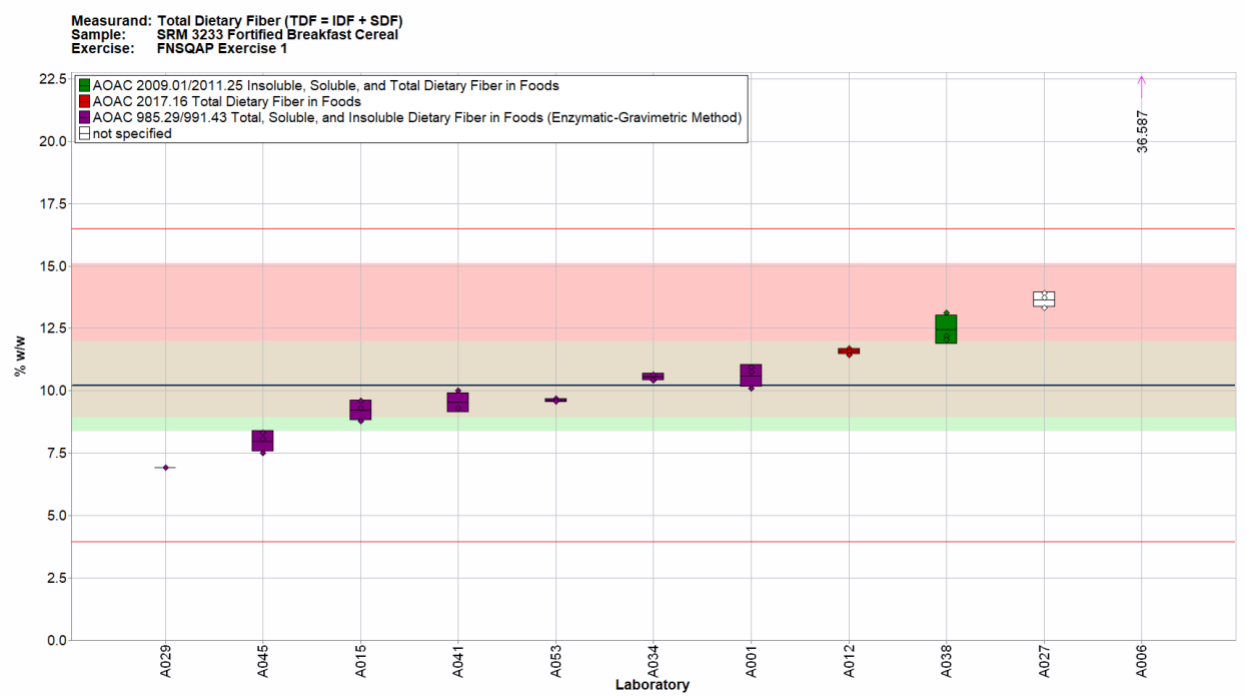

Fig. 8-1. Total dietary fiber (TDF) in SRM 3233 (data summary view – analytical method).

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 A006 was AOAC 2017.16). The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty (U_{NIST}), and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$. The shaded beige region represents the overlapping of the 95 % confidence interval for the consensus mean (green region) and the NIST range of tolerance (red region).

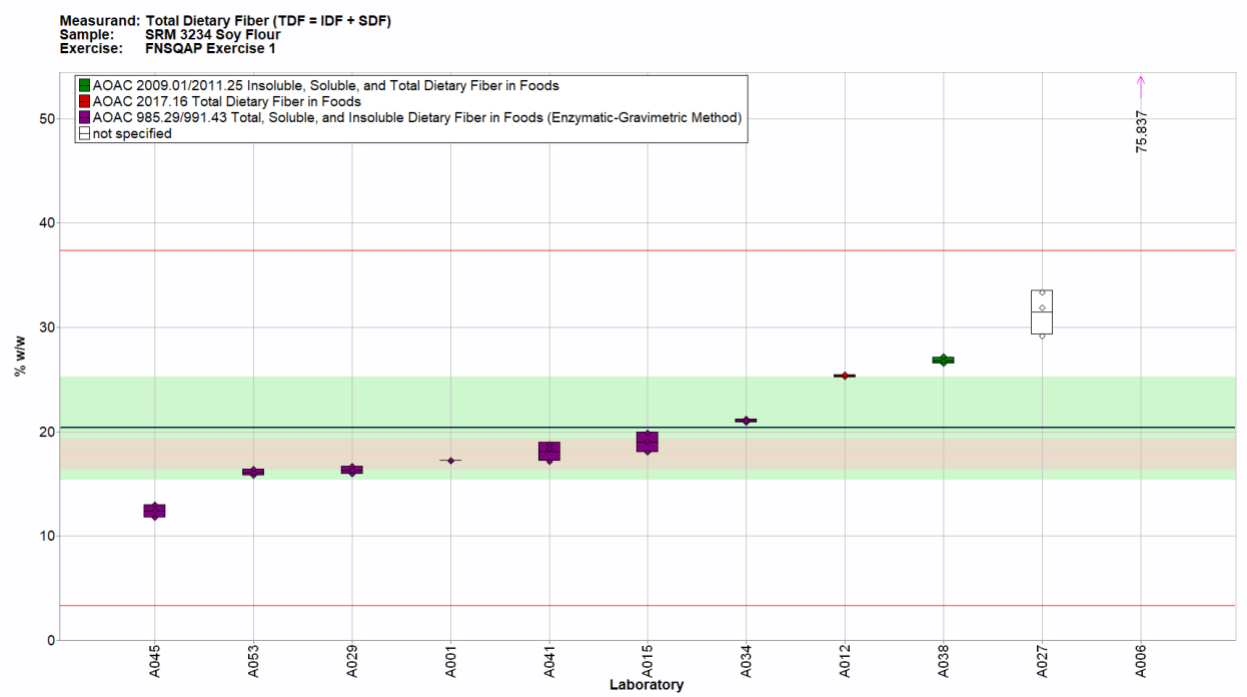

Fig. 8-2. Total dietary fiber (TDF) in SRM 3234 (data summary view – analytical method).

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 A006 was AOAC 2017.16). The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty (U_{NIST}), and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$. The shaded beige region represents the overlapping of the 95 % confidence interval for the consensus mean (green region) and the NIST range of tolerance (red region).

Table 8-4. Comparison of method means for total dietary fiber in SRM 3233 and SRM 3234. The target value describes the assigned value and expanded uncertainty from the NIST COA; the other values are the consensus means and RSD_R calculated using the data from only the specified methods.

		Total Dietary Fiber Mass Fraction (%)	
Methods Used in Calculation	**N**	**SRM 3233**	**SRM 3234**
All reported methods	11	10.2 ± 3.0	20.4 ± 8.1
AOAC 985.29/991.43	7	9.2 ± 1.6	17.2 ± 3.0
Other (not AOAC 985.29/991.43)	4	12.6 ± 3.4	27.9 ± 8.6
Target value	--	12.0 ± 1.5	17.8 ± 0.7

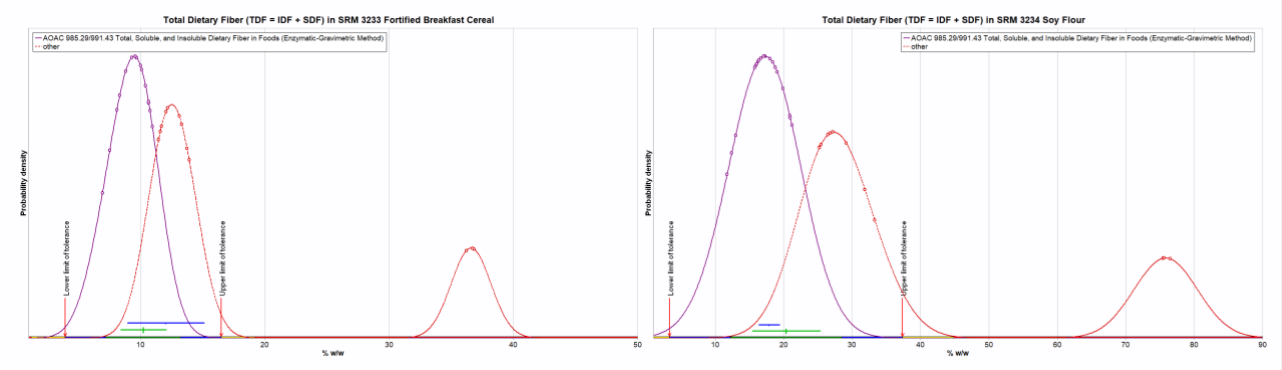

Fig. 8-3. Kernel density estimation for total dietary fiber (TDF) in SRM 3233 (left) and SRM 3234 (right).

In this view, the kernel density of the distribution is estimated as a function of a single method selection (AOAC 985.29/991.43, solid purple) compared to the estimated distribution from other reported results (dashed red). The target values are shown as the upper blue horizontal bars, and the consensus means are indicated by the lower green horizontal bars. Upper and lower limits of tolerance are indicated by red arrows.

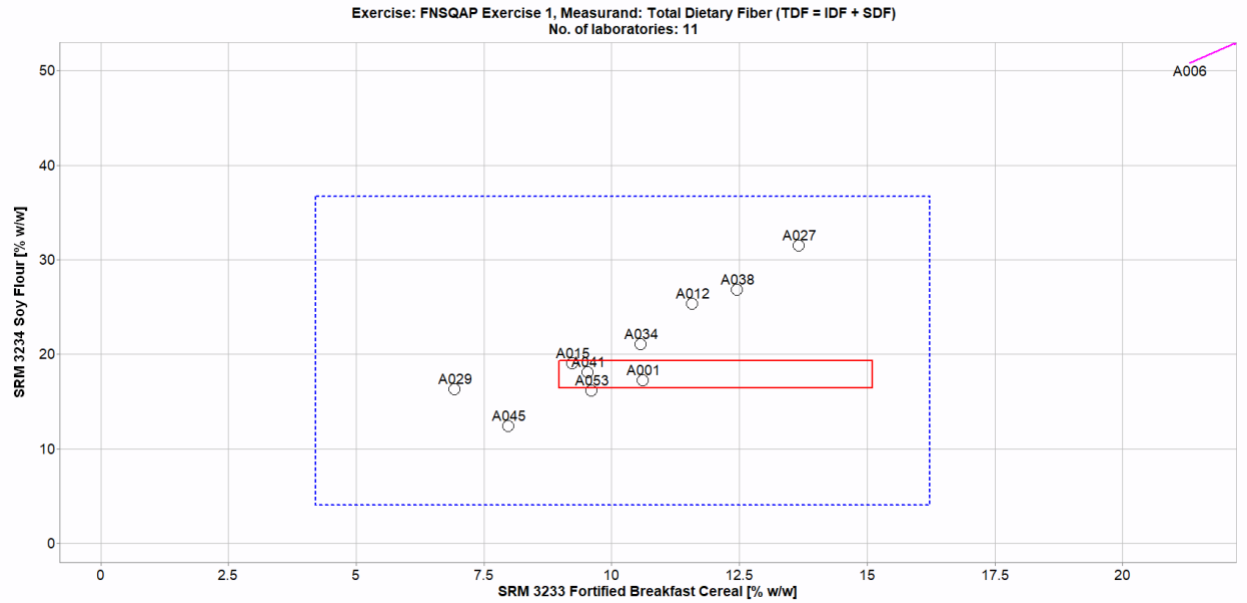

Fig. 8-4. Laboratory means for total dietary fiber (TDF) in SRM 3233 and SRM 3234 (sample/sample comparison view).

In this view, the individual laboratory mean for one sample (SRM 3233) is compared to the mean for a second sample (SRM 3234). The solid red box represents the NIST range of tolerance for the two samples, SRM 3233 (x-axis) and SRM 3234 (y-axis), which encompasses the target values bounded by their uncertainties (U_{NIST}), and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\mathrm{NIST}}| \leq 2$. The dotted blue box represents the consensus range of tolerance for SRM 3233 (x-axis) and SRM 3234 (y-axis), calculated as the values above and below the consensus means that result in an acceptable Z'_{comm} score, $|Z'_{\mathrm{comm}}| \leq 2$.

As indicated in Table **8-4**, the bias demonstrated by AOAC 985.29/991.43 for total dietary fiber in SRM 3233 is 23 % relative to the target value. In SRM 3234, however, the results reported by laboratories using AOAC 985.29/991.43 for total dietary fiber are consistent with the target value, while the mean of laboratory results based on use of other methods were biased high by 57 % relative to the target value. The data used to assign the total dietary fiber value in SRM 3234 was

collected in early 2009 ahead of publication and adoption of AOAC 2009.01 and AOAC 2011.25, which implies that AOAC 985.29/991.43 was the primary method of analysis. The data collected in this study indicate that an update of the assigned value for total dietary fiber in SRM 3234 is needed.

Table **8-2** summarizes and Table **8-5** details the numerical results for insoluble dietary fiber (IDF) reported by each participating laboratory. The participation level was good for IDF, with 42 % of laboratories requesting samples returning results (8 of 19 laboratories).

Table 8-5. Data summary table for insoluble dietary fiber in SRM 3233 and SRM 3234.

		Insoluble Dietary Fiber (IDF)									
		SRM 3233 Fortified Breakfast Cereal (% w/w)					SRM 3234 Soy Flour (% w/w)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target				6.49	0.44					
	A001										
	A004										
	A006										
	A012	7.47	7.06	7.22	7.25	0.21	15.37	15.09	15.41	15.3	0.2
	A015	6.1	6.3	6.8	6.40	0.36	14.7	15.8	16.2	15.6	0.8
	A020										
	A027	8.35	7.81	7.06	7.74	0.65	17.6	20.8	22.6	20.3	2.5
	A029										
	A030										
	A031	7.17	7.46	7.5	7.38	0.18	15.38	15.47	15.3	15.4	0.1
	A034										
	A037										
	A038	6.7	7.5	6.6	6.93	0.49	16.04	16.55	16.43	16.3	0.3
	A040										
	A041										
	A045	0.67	0.5	0.59	0.59	0.09	10.91	10.02	10.31	10.4	0.5
	A050										
	A053	6.28	5.93	6.38	6.20	0.24	15.4	15.07	14.6	15.0	0.4
	A061	7	6.85	6.75	6.87	0.13	14.69	14.75	15.34	14.9	0.4
Community Results		Consensus Mean			6.97		Consensus Mean			15.4	
		Consensus Standard Deviation			0.94		Consensus Standard Deviation			1.5	
		Maximum			7.74		Maximum			20.3	
		Minimum			0.59		Minimum			10.4	
		N			8		N			8	

Table **8-5** indicates that the within-laboratory variabilities were good, with only two data sets reported greater than 10 % RSD, one 12.5 % RSD and one 14.5 % RSD. Both high variability data sets were flagged as outliers (blue text) in Table **8-5**. The between-laboratory variabilities were also good (13 % for SRM 3233 and 9 % for SRM 3234), supporting that the method-specific

differences observed with total dietary fiber are related to the determination of soluble fiber types and not relevant to insoluble dietary fiber.

As shown in Fig. **8-5** and Fig. **8-6**, laboratories reported using one of three AOAC Official Methods in the determination of insoluble dietary fiber. The most reported method was AOAC 985.29/991.43 (5 of 8 laboratories, 63 %); one laboratory reported using AOAC 2017.16 (13 %), and one laboratory reported using AOAC 2009.01/2011.25 (13 %). No method specific trends were identified in the results, consistent with the known improvements made over time in these official methods that target proper determination of *soluble* dietary fiber components. The outlying results reported by laboratory A045 for insoluble dietary fiber in SRM 3233 indicate a potential calculation issue, as the measured values are ten-fold lower than the consensus and target values.

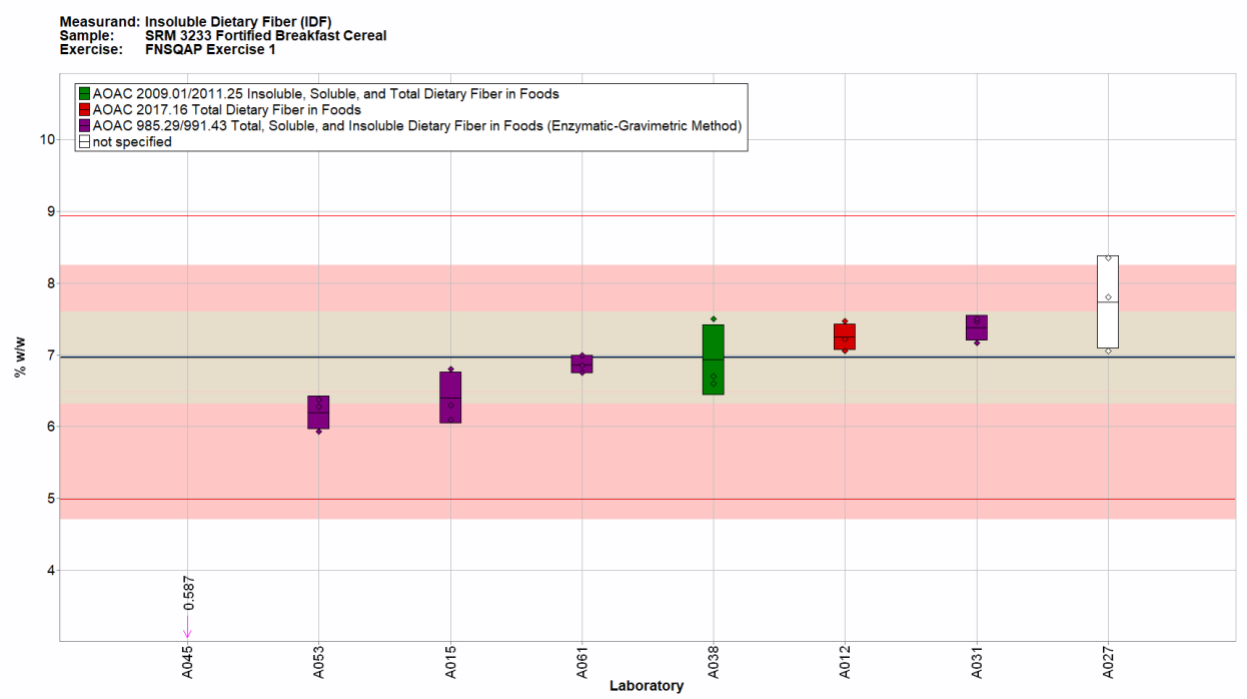

Fig. 8-5. Insoluble dietary fiber (IDF) in SRM 3233 (data summary view – analytical method).

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 A045 was AOAC 985.29/991.43). The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty (U_{NIST}), and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$. The shaded beige region represents the overlapping of the 95 % confidence interval for the consensus mean (green region) and the NIST range of tolerance (red region).

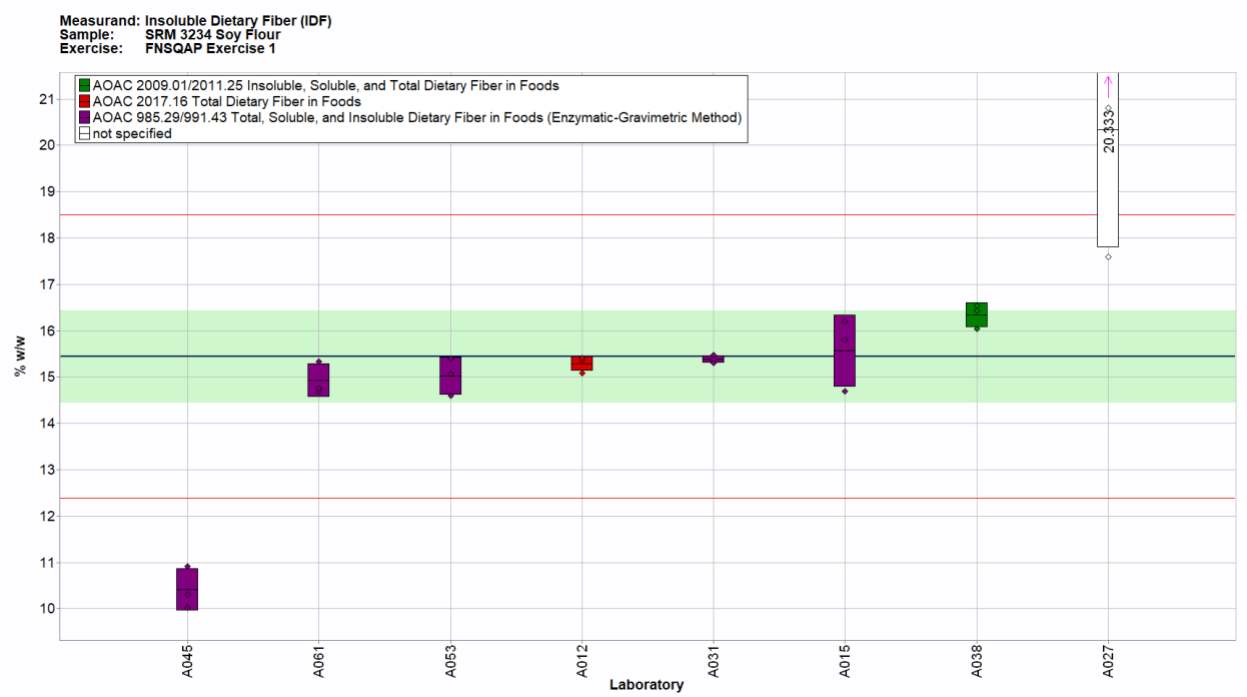

Fig. 8-6. Insoluble dietary fiber (IDF) in SRM 3234 (data summary view – analytical method).

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, $|Z'_{\mathrm{comm}}| \leq 2$. No NIST target value is available for IDF in SRM 3234.

Table **8-2** summarizes and Table **8-6** details the numerical results for soluble dietary fiber (SDF) reported by each participating laboratory. The participation level was low for SDF, with 26 % of laboratories requesting samples returning results (5 of 19 laboratories). Table **8-6** reveals that within-laboratory variabilities were good, with a median of 4.1 % RSD and a range from 0.5 % to 10.7 % RSD. The between-laboratory variabilities were extremely high (64 % for SRM 3233 and > 100 % for SRM 3234) and highlight potential method differences. Additionally, the values reported by laboratory A012 for soluble dietary fiber (Table **8-6**) are larger than the values reported for total dietary fiber (Table **8-3**), which may indicate confusion about the definition of soluble dietary fiber, as well as its determination and components.

Table 8-6. Data summary table for soluble dietary fiber in SRM 3233 and SRM 3234.

		Soluble Dietary Fiber (SDF = SDFS + SDFP)									
		SRM 3233 Fortified Breakfast Cereal (% w/w)					SRM 3234 Soy Flour (% w/w)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target				2.66	0.83					
	A001										
	A004										
	A006										
	A012	13.62	13.52	13.51	13.55	0.06	28	28.16	28.59	28.25	0.31
	A015	2.7	2.8	3	2.83	0.15	3.3	3.3	3.7	3.43	0.23
	A020										
	A027	5.56	5.93	6.26	5.92	0.35	11.55	11.08	10.73	11.12	0.41
	A029										
	A030										
	A031										
	A034										
	A037										
	A038	5.49	5.62	5.45	5.52	0.09	10.46	10.24	10.75	10.48	0.26
	A040										
	A041										
	A045	7.65	7.02	7.51	7.39	0.33	2.09	1.74	2.12	1.98	0.21
	A050										
	A053										
	A061										
Community Results		Consensus Mean			7.04		Consensus Mean			11.05	
		Consensus Standard Deviation			4.51		Consensus Standard Deviation			15.60	
		Maximum			13.55		Maximum			28.25	
		Minimum			2.83		Minimum			1.98	
		N			5		N			5	

Fig. **8-7** and Fig. **8-8** clearly demonstrate the method differences discussed previously [44-47]. Two laboratories reported using AOAC 985.29/991.43 (40 %); one laboratory reported using AOAC 2017.16 (20 %), and 1 laboratory reported using AOAC 2009.01/2011.25 (20 %). In both materials, the highly variable results for SDF are a result of the types of SDF determined by each method. Results from AOAC 985.29/991.43 do not contain all possible types of SDF and therefore this method results in an underestimation (A015 in both samples and A045 in SRM 3234). The updated methods are more inclusive of all SDF types and result in higher values (A038 and presumably A027). As described previously, the results reported by A012 using AOAC 2017.16 were likely erroneous due to a misunderstanding of the definition of the requested analyte.

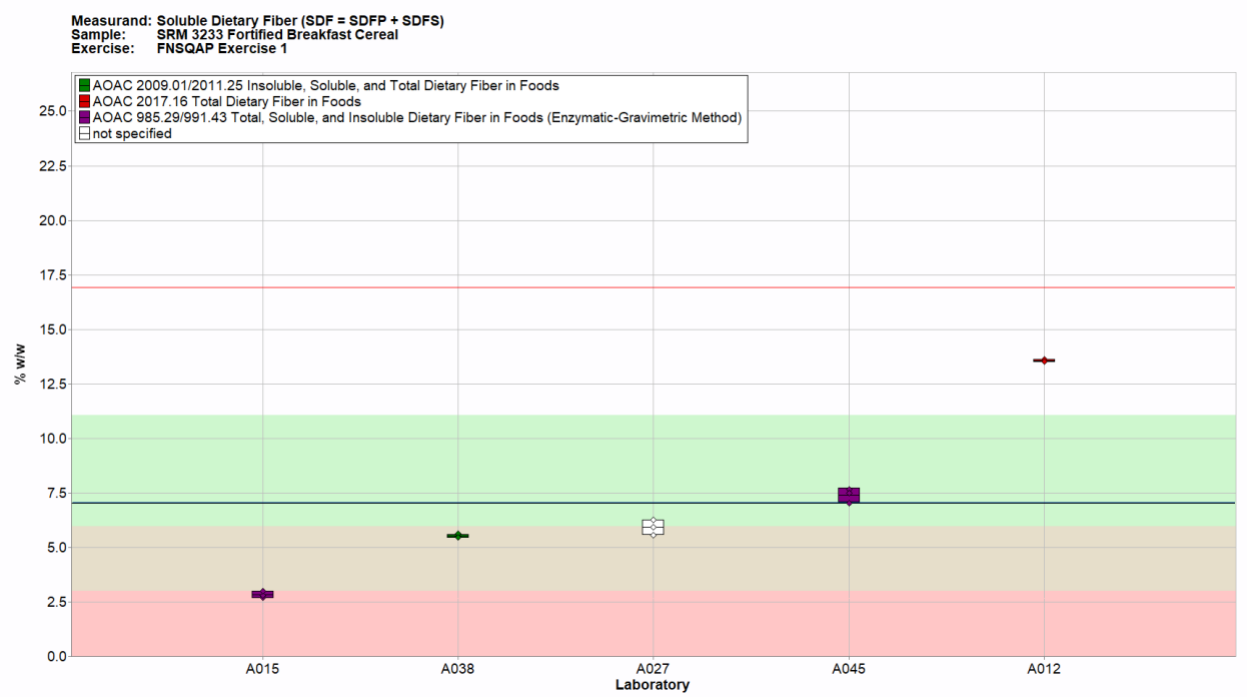

Fig. 8-7. Soluble dietary fiber (SDF) in SRM 3233 (data summary view – analytical method).

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 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, $|Z'_{\mathrm{comm}}| \leq 2$. The lower limit of the consensus range of tolerance is set to zero. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty (U_{NIST}), and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\mathrm{NIST}}| \leq 2$. The shaded beige region represents the overlapping of the 95 % confidence interval for the consensus mean (green region) and the NIST range of tolerance (red region).

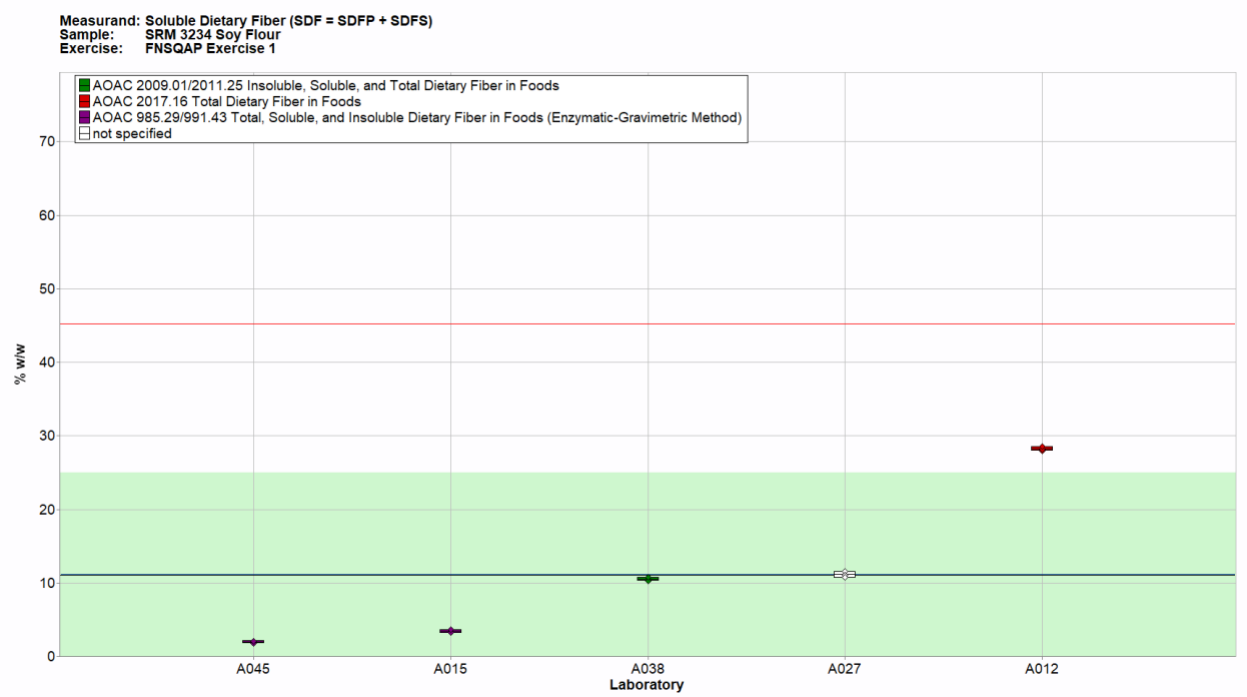

Fig. 8-8. Soluble dietary fiber (SDF) in SRM 3234 (data summary view – analytical method).

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 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, $|Z'_{\mathrm{comm}}| \leq 2$. The lower limit of the consensus range of tolerance is set to zero. No NIST target value is available for SDF in SRM 3234.

Other fiber analytes including SDFS, SDFP, and HMWDF were reported by very few laboratories (2 of 18 laboratories for SDFS and 3 of 18 laboratories for SDFP and HMWDF) as outlined in Table **8-7**, Table **8-8**, and Table **8-9**. The laboratories measuring these components reported use of AOAC 985.29/991.43 (one laboratory, SDFP and HMWDF) and AOAC 2017.16 (one laboratory, SDFS, SDFP, and HMWDF). One laboratory reporting results for SDFS, SDFP, and HMWDF did not report the method used. Given the limited number of reported results for these three analytes, no trends can be identified in the data and thus no recommendations as to method performance can be made.

Table 8-7. Data summary table for soluble dietary fiber which remains soluble in 78 % aqueous ethanol (SDFS) in SRM 3233 and SRM 3234.

		Soluble Dietary Fiber which Remains Soluble in 78% aqueous ethanol (SDFS)									
		SRM 3233 Fortified Breakfast Cereal (% w/w)					SRM 3234 Soy Flour (% w/w)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target										
	A001										
	A004										
	A006										
	A012	1.98	2.21	2.07	2.09	0.12	7.32	6.98	7.21	7.17	0.17
	A015										
	A020										
	A027	2.70	2.75	2.78	2.74	0.04	6.50	6.52	6.48	6.50	0.02
	A029										
	A030										
	A031										
	A034										
	A037										
	A038										
	A040										
	A041										
	A050										
	A053										
	A061										
Community Results		Consensus Mean			2.42		Consensus Mean			6.84	
		Consensus Standard Deviation			1.25		Consensus Standard Deviation			1.10	
		Maximum			2.74		Maximum			7.17	
		Minimum			2.09		Minimum			6.50	
		N			2		N			2	

Table 8-8. Data summary table for soluble dietary fiber that precipitates in 78 % aqueous ethanol (SDFP) in SRM 3233 and SRM 3234.

		Soluble Dietary Fiber that Precipitates in 78% aqueous ethanol (SDFP)									
		SRM 3233 Fortified Breakfast Cereal (% w/w)					SRM 3234 Soy Flour (% w/w)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target										
	A001										
	A004										
	A006										
	A012	1.91	1.95	1.76	1.87	0.10	2.89	2.65	3.32	2.95	0.34
	A015										
	A020										
	A027	2.86	3.18	3.48	3.17	0.31	5.05	4.56	4.25	4.62	0.40
	A029										
	A030										
	A031										
	A034										
	A037										
	A038										
	A040										
	A041										
	A050										
	A053										
	A061	1.93	1.93	2.06	1.97	0.08	2.73	2.91	3.12	2.92	0.20
Community Results		Consensus Mean			2.20		Consensus Mean			3.50	
		Consensus Standard Deviation			0.37		Consensus Standard Deviation			0.94	
		Maximum			3.17		Maximum			4.62	
		Minimum			1.87		Minimum			2.92	
		N			3		N			3	

Table 8-9. Data summary table for high molecular weight dietary fiber (HMWDF) in SRM 3233 and SRM 3234.

		High Molecular Weight Dietary Fiber (HMWDF = IDF + SDFP)									
		SRM 3233 Fortified Breakfast Cereal (% w/w)					SRM 3234 Soy Flour (% w/w)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target				2.56	1.47					
	A001										
	A004										
	A006										
	A012	9.72	9.37	9.38	9.49	0.20	17.95	18.27	18.26	18.16	0.18
	A015										
	A020										
	A027	11.21	10.99	10.54	10.91	0.34	22.65	25.36	26.85	24.95	2.13
	A029										
	A030										
	A031										
	A034										
	A037										
	A038										
	A040										
	A041										
	A050										
	A053										
	A061										
Community Results		Consensus Mean			9.90		Consensus Mean			18.00	
		Consensus Standard Deviation			0.82		Consensus Standard Deviation			1.48	
		Maximum			10.91		Maximum			24.95	
		Minimum			9.31		Minimum			17.85	
		N			3		N			3	

In any laboratory exercise, calculations and analyte reporting forms must be verified prior to submission of results. Laboratories often report results in the wrong units, omit a dilution factor during the calculation of the final results, or misunderstand the requested analyte form, 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.

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Appendix A. List of Acronyms

AAS	Atomic Absorption Spectroscopy
AMPA	Aminomethylphosphonic acid
AOAC	AOAC International, founded in 1884 as the Association of Official Agricultural Chemists. A provider of documentary standards.
cGMP	current Good Manufacturing Practice
CRM	Certified Reference Material
CV AAS	Cold Vapor Atomic Absorption Spectroscopy
DSQAP	Dietary Supplements Laboratory Quality Assurance Program
ELISA	Enzyme-linked Immunosorbent Assay
FDA	US Food and Drug Administration
FNSQAP	Food Nutrition and Safety Measurements Quality Assurance Program
HAMQAP	Health Assessment Measurements Quality Assurance Program
HMWDF	High Molecular Weight Dietary Fiber
HMW SDF	High Molecular Weight Soluble Dietary Fiber
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
ICP-OES	Inductively Coupled Plasma Optical Emission Spectrometry
ID	Isotope Dilution
IDF	Insoluble Dietary Fiber
ISO	International Organization for Standardization. A provider of documentary standards.
KED	Kinetic Energy Discrimination
LC-Abs	Liquid Chromatography with Absorbance detection
LC-FLD	Liquid Chromatography with Fluorescence Detection
LC-HRMS	Liquid Chromatography with High Mass Resolution Spectrometry
LC-MS	Liquid Chromatography Mass Spectrometry
LC-MS/MS	Liquid Chromatography with Tandem Mass Spectrometry
LC-PDA	Liquid Chromatography with Photodiode Array
LMW SDF	Low Molecular Weight Soluble Dietary Fiber
LOQ	Limit of Quantification
MDL	Method Detection Limit
NIST	National Institute of Standards and Technology
QAP	Quality Assurance Program
QL	Quantification Limit
QuEChERS	“Quick, Easy, Cheap, Effective, Rugged and Safe” solid phase extraction
QuPPe	Quick Polar Pesticides extraction
RM	Reference Material
RMIS	Reference Material Information Sheet
RSD	Relative Standard Deviation
RSD_r	Repeatability Relative Standard Deviation (Within-Laboratory Variability)

RSD_R	Reproducibility Relative Standard Deviation (Between-Laboratory Variability)
SD	Standard Deviation
SDF	Soluble Dietary Fiber
SDFP	Soluble Dietary Fiber that Precipitates in 78% aqueous ethanol
SDFS	Soluble Dietary Fiber which Remains Soluble in 78% aqueous ethanol
SI	International System of Units
SMPR	Standard Method Performance Requirements
SRM	Standard Reference Material
TDF	Total Dietary Fiber
TXRF	Total Reflection X-ray Fluorescence
USDA	United States Department of Agriculture