Withdrawn NIST Technical Series Publication

Warning Notice

The attached publication has been withdrawn (archived), and is provided solely for historical purposes. It may have been superseded by another publication (indicated below).

Withdrawn Publication	
Series/Number	NIST IR 8553
Title	Food Nutrition and Safety Measurements Quality Assurance Program: Exercise 3 Final Report
Publication Date(s)	December 19, 2024
Withdrawal Date	January 28, 2025
Withdrawal Note	Superseded by updated version

Superseding Publication(s) *(if applicable)*

The attached publication has been **superseded by** the following publication(s):

Series/Number	NIST IR 8553-upd1
Title	Food Nutrition and Safety Measurements Quality Assurance Program: Exercise 3 Final Report
Author(s)	Melissa M. Phillips; Colleen E. Bryan Sallee; Benjamin J. Place; Jacolin A. Murray; Alix E. Rodowa
Publication Date(s)	January 28, 2025
URL/DOI	https://doi.org/10.6028/NIST.IR.8553-upd1

Additional Information *(if applicable)*

Contact	
Latest revision of the attached publication	
Related Information	
Withdrawal Announcement Link	

Date updated: January 28, 2025

NIST NATIONAL INSTITUTE OF STANDARDS AND TECHNOLOGY
U.S. DEPARTMENT OF COMMERCE

NIST Internal Report
NIST IR 8553

Food Nutrition and Safety Measurements Quality Assurance Program: Exercise 3 Final Report

Melissa M. Phillips
Colleen E. Bryan Sallee
Benjamin J. Place
Jacolin A. Murray
Alix E. Rodowa

This publication is available free of charge from:
https://doi.org/10.6028/NIST.IR.8553

NIST NATIONAL INSTITUTE OF STANDARDS AND TECHNOLOGY
U.S. DEPARTMENT OF COMMERCE

NIST Internal Report
NIST IR 8553

Food Nutrition and Safety Measurements Quality Assurance Program: Exercise 3 Final Report

Melissa M. Phillips
Colleen E. Bryan Sallee
Jacolin A. Murray
Benjamin J. Place
Alix E. Rodowa
Chemical Sciences Division
Material Measurement Laboratory

This publication is available free of charge from:
https://doi.org/10.6028/NIST.IR.8553

December 2024

U.S. Department of Commerce
Gina M. Raimondo, Secretary

National Institute of Standards and Technology
Laurie E. Locascio, NIST Director and Under Secretary of Commerce for Standards and Technology

NIST IR 8553
December 2024

Publication History
Approved by the NIST Editorial Review Board on 2024-12-18

How to Cite this NIST Technical Series Publication
Phillips MM, Bryan Sallee CE, Murray JA, Place BJ, Rodowa AE (2024) Food Nutrition and Safety Measurements Quality Assurance Program: Exercise 3 Final Report. (National Institute of Standards and Technology, Gaithersburg, MD), NIST Internal Report (IR) NIST IR 8553. https://doi.org/10.6028/NIST.IR.8553

Author ORCID iDs
Melissa M. Phillips: 0000-0003-0477-7637
Colleen E. Bryan Sallee: 0000-0002-2334-3925
Jacolin A. Murray: 0000-0001-8063-3139
Benjamin J. Place: 0000-0003-0953-5215
Alix E. Rodowa: 0000-0002-3990-2111

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 FDA, USDA, and other international bodies. Exercise 3 of this program offered the opportunity for laboratories to assess their in-house measurements of nutritional elements (copper, magnesium, phosphorus), water-soluble vitamins (vitamin B_{12}), fat-soluble vitamins (vitamin A), and contaminants (PFAS and pesticide residues) in food and infant formula samples. Major program goals include reducing the variability in consensus results as well as centering the consensus results about the target values over time through follow-up studies and educational outreach.

Keywords

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

Table of Contents

List of Tables

List of Figures

Acknowledgments

The authors thank the NIST Office of Reference Materials for providing support for sample labeling and shipment. The authors also thank John Molloy and Catherine Rimmer of the NIST Chemical Sciences Division for careful review of this report.

1. INTRODUCTION

The Food Nutrition and Safety Measurements Quality Assurance Program (FNSQAP) was formed in 2021 and represents ongoing efforts at 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 when appropriate proficiency testing is not available. Results from FNSQAP exercises may be used by NIST to identify problematic matrices and analytes for which consensus-based methods of analysis would benefit the food testing community.

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

This report summarizes the results from the third exercise of FNSQAP. Fifty-five laboratories responded to the call for participants in May 2023 to the studies available in FNSQAP Exercise 3 (Table 1-1), and fifty laboratories completed the requirements for participation. Samples were shipped to participants in August 2023 and results were returned to NIST in September 2023. Participants received a summary of the preliminary data in September 2023 and were given an opportunity to correct any errors by October 2023.

Table 1-1. Studies conducted as part of Exercise 3 of the FNSQAP.

Study Group	Analytes	Samples
Nutritional Elements	Cu, Mg, P	Infant Formulas
Water-Soluble Vitamins	Vitamin B_{12}	Infant Formulas
Fat-Soluble Vitamins	Vitamin A	Infant Formulas
Contaminants	PFAS	Meat, Milk, Egg
Contaminants	Pesticide residues	Spinach

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

1.1.1. Statistics

Data tables and figures throughout this report contain information about the performance of each laboratory relative to that of the other participants in this study and relative to the NIST target value, if available. All calculations are performed in PROLab Plus (QuoData GmbH, Dresden, Germany). The consensus means and standard deviations are calculated according to the robust Q/Hampel method outlined in International Organization for Standardization (ISO) standard 13528:2022, Annex C [1].

1.1.2. Individualized Data Table

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

Table 1-2. Exemplar individualized data summary table.

(Lab Name)

Exercise 2 – Study Name

	Lab Code:	**(Code)**	**1. Your Results**				**2. Community Results**			**3. Target**	
	Sample[a]	Units[b]	x_i	s_i	Z'_{comm}	Z_{NIST}	N	x*	s*	x_{NIST}	u_{NIST}
c_1	a_1	b_1	*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_{NIST1}
…	…	…					…	…	…	…	…
…	…	…					…	…	…	…	…
c_n	a_n	b_n					N_n	x^*_n	s^*_n	x_{NISTn}	u_{NISTn}

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, not including single-value submissions

x* Robust mean of reported values

s* Robust standard deviation

x_{NIST} NIST-assessed value

u_{NIST} standard uncertainty about the NIST-assessed value

[a] Samples used in the study.

[b] Units used to describe the measured values.

[c] Analytes measured in the study.

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

Also included in Section 1 are two Z-scores. The first Z-score, Z'_{comm}, is calculated with respect to the community consensus value, taking into consideration bias that may result from the uncertainty in the assigned consensus value, using the consensus mean (x^*), consensus standard deviation (s^*), and standard deviation for proficiency assessment (SDPA, σ_{PT}^2) determined from the Q/Hampel estimator:

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

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

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

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

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

Section 2 of the data table (*Community Results*) contains the consensus results, including the number of laboratories reporting more than a single quantitative value for each analyte, the mean value determined for each analyte, and a robust estimate of the standard deviation of the reported values [1]. Consensus means and standard deviations are calculated using the laboratory means; if a laboratory reported a single value, the reported value is used as the laboratory mean [1]. Additional information on calculation of the consensus mean and standard deviation can be found in the previous section.

Section 3 of the data table (*Target*) contains the NIST target values for each analyte, when available. When possible, the target value is a NIST certified value, a NIST non-certified value, or a value determined at NIST that does not meet the criteria of a certified or non-certified value. A NIST certified value is a value for which NIST has the highest confidence in its accuracy in that all known or suspected sources of bias and variability have been considered [2]. NIST non-certified values are best estimates based on currently available information and may not provide metrological traceability to a higher-order reference system [2]. When a NIST certified or non-certified value has been assigned, that value is used as the NIST target value and the 95 % expanded uncertainty on the assigned value is used as u_{NIST}. For samples in which a NIST certified or non-certified value is not available, a target value may be determined at NIST using an established method or data from a collaborating laboratory. The target value represents the mean of at least three replicates, and u_{NIST} is estimated as twice the standard deviation of those replicate measurements. The standard deviations are inflated by a factor of two to protect against underestimation of uncertainties and subsequent potential implications of poor participant performance. For materials acquired from and/or evaluated as a part of another interlaboratory study or proficiency testing program, the consensus value and uncertainty from the completed round is used as the target range. Within each section of this report, the exact methods for determination of the study target values are outlined in detail. A unique feature of NIST QAPs is the accuracy-based component provided by comparison of participant results to a NIST value.

1.1.3. Summary Data Table

This data table includes a summary of all reported data for a specific analyte in a particular study. Participants can compare the raw data for their laboratory to data reported by the other participating laboratories and to the consensus data. A blank section indicates that the laboratory requested and received samples for that analyte and matrix, but NIST does not have data on file for that laboratory. Data highlighted in red have been flagged as a data entry of zero or results

that include text (e.g., "< LOQ" or "present"). Data highlighted in blue have been identified as outside the consensus tolerance limits and would be estimated to yield $|Z'_{comm}| > 2$. The summary data tables are presented in the format shown in Table 1-3.

Table 1-3. Exemplar data summary table.

		Analyte									
		Sample 1 (units)					Sample 2 (units)				
		A	**B**	**C**	**Avg**[a]	**SD**[b]	**A**	**B**	**C**	**Avg**[a]	**SD**[b]
Individual Results	Target				c_1	d_1				c_2	d_2
	e_1	x_{A1-1}	x_{B1-1}	x_{C1-1}	$\bar{x}_{1-1}$	s_{1-1}	x_{A2-1}	x_{B2-1}	x_{C2-1}	$\bar{x}_{1-2}$	s_{1-2}
	…	…	…	…	…	…	…	…	…	…	…
	e_n	x_{A1-n}	x_{B1-n}	x_{C1-n}	$\bar{x}_{n-1}$	s_{n-1}	x_{A2-n}	x_{B2-n}	x_{C2-n}	$\bar{x}_{n-2}$	s_{n-2}
Community Results		Consensus Mean			f_1		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 NIST-assigned target value for the sample.
[d] The expanded uncertainty of the NIST-assigned target value for the sample.
[e] The laboratory identifier for the participant.
[f] The robust mean of reported results.
[g] The robust standard deviation of reported results.
[h] The maximum of reported average results.
[i] The minimum of reported average results.
[j] The number of quantitative values reported.

1.1.4. Figures

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

In this view (Fig. 1-1), individual laboratory data (diamonds) are plotted with the individual laboratory SD (rectangle). Laboratories reporting values below their method LOQ are shown in this view as downward triangles beginning at the LOQ, reported as quantification limit (QL) on the figures. The blue solid line represents the consensus mean, and the green shaded area represents the 95 % confidence interval for the consensus mean, based on the standard uncertainty of the consensus mean. The uncertainty in the consensus mean is calculated using the equation below, based on the repeatability standard deviation (s_r), the reproducibility standard deviation (s_R), the number of participants reporting data ($n_{\text{participants}}$), and the average number of replicates reported by each participant ($n_{\text{average number of repliates per participant}}$). The uncertainty about the consensus mean is independent of the range of tolerance. Where appropriate, two consensus means may be calculated for the same sample if bimodality is identified in the data. In this case, two consensus means and ranges will be displayed in the data summary view.

$$u_{\text{mean}} = \sqrt{\frac{s_R^2 - s_r^2}{n_{\text{particpants}}} + \frac{s_R^2}{n_{\text{participants}} \times n_{\text{average number of replicates per participant}}}}$$

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

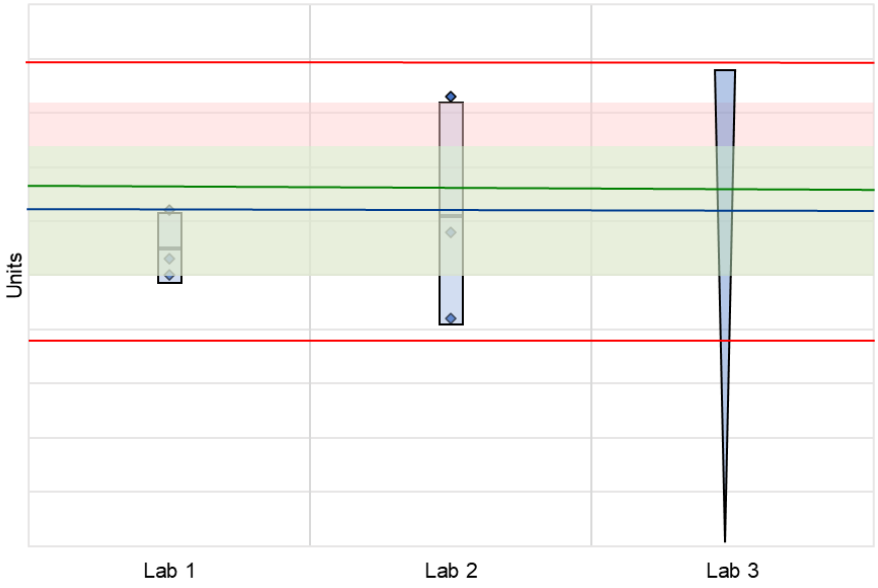

Fig. 1-1. Example data sample summary view.

1.1.4.2. Sample/Sample Comparison View

In this view (Fig. 1-2), the individual laboratory results for one sample are compared to the results for another sample in the study examining the same analyte. The solid red box represents the target zone for the first sample (x-axis) and the second sample (y-axis), if available. The dotted blue box represents the consensus zone for the first sample (x-axis) and the second sample (y-axis). The axes of this graph are centered about the consensus mean values for each sample or control, to a limit of twice the range of tolerance (values that result in an acceptable Z' score, $|Z'| \leq 2$). Depending on the variability in the data, the axes may be scaled proportionally to better display the individual data points for each laboratory. In some cases, when the consensus

and target ranges have limited overlap, the solid red box may only appear partially on the graph. If the variability in the data is high (greater than 100 % relative standard deviation (RSD)), the dotted blue box may also only appear partially on the graph. These views emphasize trends in the data that may indicate potential calibration issues or method biases. Primary program goals are to identify such calibration or method biases and assist participants in improving analytical measurement capabilities. In some cases, when two equally challenging materials are provided, the same view (sample/sample comparison) can be helpful in identifying commonalities or differences in the analysis of the two materials.

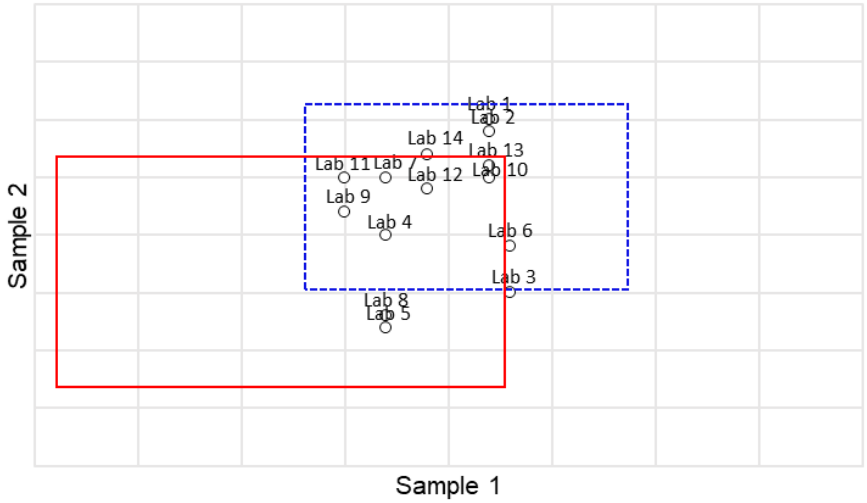

Fig. 1-2. Example sample/sample comparison view.

2. NUTRITIONAL ELEMENTS (Copper, Magnesium, Phosphorus)

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 in nutritional formula samples. Outlying data is likely attributed to analytical interferences and calibration bias.

2.2. Study Overview

Copper (Cu), magnesium (Mg), and phosphorus (P) 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, reference material (RM) 8260 Infant Nutritional Formula (hydrolyzed milk-based) and RM 8261 Adult Nutritional Formula (high protein). Participants were asked to use in-house analytical methods to determine the mass fractions (mg/kg) of Cu, Mg, and P in infant formula samples. Through participation in this study, laboratories can better understand the performance of their in-house methods relative to those being used by others in the community. Participant results may be used in the value assignment of NIST reference materials included as samples in this study.

2.3. Sample Information

Participants were provided with three packets each of RM 8260 Infant Nutritional Formula (hydrolyzed milk-based) (labeled Infant Formula A) and RM 8261 Adult Nutritional Formula (high protein) (labeled Infant Formula B). Each packet contained approximately 10 g of material. Participants were asked to store the materials at controlled room temperature (20 °C to 25 °C) in the original unopened packets and to prepare one sample and report one value per analyte 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 to ensure homogeneity of the test portion used for the determination of nutritional elements. The approximate analyte levels were not reported to participants prior to the study. The target mass fraction values for nutritional elements in each material were as described in their respective Reference Material Information Sheets (RMISs) [3],[4]. The target mass fraction values and uncertainties for nutritional elements in each material are provided in Table 2-1 on an as-received basis. The uncertainties nutritional elements in RM 8260 and RM 8261 were approximated as 10 % relative to the measured value.

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 3 – Nutritional Elements in Infant Formula

	Lab Code:	(Code)	1. Your Results				2. Community Results			3. Target	
	Sample	Units	x_i	s_i	Z'_{comm}	Z_{NIST}	N	x*	s*	x_{NIST}	u_{NIST}
Cu	RM 8260	mg/kg	*Individual laboratory results will appear in this section; laboratory-specific results were provided to each participant separately from this report.*				21	5.20	0.45	5.00	0.50
Cu	RM 8261	mg/kg					21	9.90	0.86	9.00	0.90
Mg	RM 8260	mg/kg					23	380	28	360	36
Mg	RM 8261	mg/kg					23	940	87	890	89
P	RM 8260	mg/kg					22	2300	160	2200	220
P	RM 8261	mg/kg					22	2600	210	2300	230

x_i	Mean of reported values	N	Number of quantitative values reported, not including single-value submissions	x_{NIST}	NIST-assessed value
s_i	Standard deviation of reported values			u_{NIST}	standard uncertainty about the NIST-assessed value
Z'_{comm}	Z'-score with respect to community consensus	x*	Robust mean of reported values		
Z_{NIST}	Z-score with respect to NIST value	s*	Robust standard deviation		

2.4. Study Results and Discussion

Table 2-1 summarizes and Table 2-2, Table 2-3, and Table 2-4 detail the measured mass fraction results reported by each participating laboratory for nutritional elements. The participation level was good for nutritional elements, with 76 % to 82 % of laboratories requesting samples returning results (on average 23 of 28 laboratories).

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

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

		Copper									
		RM 8260 Infant Nutritional Formula (hydrolyzed milk-based) (mg/kg)					RM 8261 Adult Nutritional Formula (high-protein) (mg/kg)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target				5.00	0.50				9.00	0.90
	C004	39.1	39.3	38.5	38.97	0.42	45.6	42.7	41	43.10	2.33
	C005	< 9.600	< 9.600	< 9.600			9.852	9.655	10.01	9.84	0.18
	C006	5.186	5.097	5.188	5.16	0.05	10.231	9.998	10.246	10.16	0.14
	C007	5.22	4.88	4.71	4.94	0.26	9.37	9.86	9.68	9.64	0.25
	C008	5.11	5.15	5.1	5.12	0.03	9.88	9.92	10	9.93	0.06
	C010	5.068	4.997	5.017	5.03	0.04	9.772	9.874	10.012	9.89	0.12
	C012	6.563	6.199	6.106	6.29	0.24	12.781	12.437	11.465	12.23	0.68
	C013	< 6.000	< 6.000	< 6.000			< 13.000	< 13.000	< 13.000		
	C014	5.13	4.97	5.29	5.13	0.16	10.6	10.5	10.2	10.43	0.21
	C015	5	6	6	5.67	0.58	10	10	7	9.00	1.73
	C016										
	C017	5.49	5.01	4.95	5.15	0.30	10.6	9.24	9.23	9.69	0.79
	C027	5.768	5.808	5.695	5.76	0.06	10.775	10.685	10.831	10.76	0.07
	C030										
	C031	4.646	4.356	5.321	4.77	0.50	8.773	8.606	8.302	8.56	0.24
	C032	3.46	3.37	3.29	3.37	0.09	8.3	8.25	7.92	8.16	0.21
	C033										
	C036	4.55	4.66	4.64	4.62	0.06	9.13	9.17	8.91	9.07	0.14
	C038	5.71	5.68	5.99	5.79	0.17					
	C039	4.99	5.16	5.22	5.12	0.12	10	10	10.2	10.07	0.12
	C040	5.314	5.378	5.384	5.36	0.04	10.631	10.736	10.895	10.75	0.13
	C042										
	C045	4.92	5.07	4.99	4.99	0.08	9.78	9.97	9.97	9.91	0.11
	C046	4.74	4.69	4.68	4.70	0.03	9.31	9.12	9.11	9.18	0.11
	C047	5.14	5.11	5.17	5.14	0.03	9.77	10.14	9.92	9.94	0.19
	C049	5.091	5.045	5.017	5.05	0.04	10.214	10.197	10.68	10.36	0.27
	C052	5.12	5.11	5.4	5.21	0.16	10	10.2	10.4	10.20	0.20
	C053										
Community Results		Consensus Mean			5.18		Consensus Mean			9.86	
		Consensus Standard Deviation			0.45		Consensus Standard Deviation			0.86	
		Maximum			38.97		Maximum			43.10	
		Minimum			3.37		Minimum			8.16	
		N			21		N			21	

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

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

		Magnesium									
		RM 8260 Infant Nutritional Formula (hydrolyzed milk-based) (mg/kg)					RM 8261 Adult Nutritional Formula (high-protein) (mg/kg)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target				360	36				890	89
	C004	595.1	560.6	522.2	559	36	1113.3	1066.5	1045.1	1075	35
	C005	363.1	362.9	361.4	362	1	868.6	867.4	864.6	867	2
	C006	382.842	374.301	375.272	377	5	938.1	926.509	931.507	932	6
	C007	422	423	417	421	3	957	1069	1022	1016	56
	C008	417	422	414	418	4	1042	1050	1057	1050	8
	C010	379.26	377.499	375.671	377	2	905.326	905.091	910.781	907	3
	C012	435.047	426.783	455.286	439	15	998.853	1043.34	1022.06	1021	22
	C013	360	340	350	350	10	800	770	810	793	21
	C014	398	390	410	399	10	1010	1000	981	997	15
	C015	340	360	360	353	12	720	810	840	790	62
	C016										
	C017	414	420	428	421	7	1013	1023	1034	1023	11
	C027	379.298	369.379	374.152	374	5	918.456	886.627	949.226	918	31
	C030										
	C031	368.74	377.05	435.99	394	37	871.35	885.11	861.05	873	12
	C032	367.5	382.14	360.15	370	11	894.88	858.02	876.66	877	18
	C033										
	C036	407.89	388.33	393.03	396	10	971.82	981.28	952.52	969	15
	C038	391	376	416	394	20	1017	1017	1046	1027	17
	C039	371	369	369	370	1	914	924	919	919	5
	C040	413.571	391.579	414.447	407	13	980.603	970.874	1020.08	991	26
	C042										
	C045	381	386	378	382	4	908	912	922	914	7
	C046	358.813	356.92	358.956	358	1	928.764	907.433	914.15	917	11
	C047	344.9	349.2	352.1	349	4	837.3	827	841	835	7
	C049	377.168	383.275	376.148	379	4	888.426	896.101	916.344	900	14
	C052	376	370	378	375	4	934	929	927	930	4
	C053										
Community Results		Consensus Mean			384		Consensus Mean			938	
		Consensus Standard Deviation			28		Consensus Standard Deviation			87	
		Maximum			559		Maximum			1075	
		Minimum			349		Minimum			790	
		N			23		N			23	

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

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

		Phosphorus									
		RM 8260 Infant Nutritional Formula (hydrolyzed milk-based) (mg/kg)					RM 8261 Adult Nutritional Formula (high-protein) (mg/kg)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target				2200	220				2300	230
	C001	2260	1980	1950	2063	171	2500	2460	2470	2477	21
	C004	5267.8	5019.5	4612	4966	331	4893.9	4485.7	4259.5	4546	322
	C005	2159	2164	2160	2161	3	2468	2451	2446	2455	12
	C006	2332.77	2283.61	2284.77	2300	28	2725.5	2673.31	2689.46	2696	27
	C007	1962	2265	2212	2146	162	2520	2345	2634	2500	146
	C008	2671	2742	2706	2706	36	2969	3014	3074	3019	53
	C010	2304.93	2261.34	2269.56	2279	23	2580.8	2620.4	2591.05	2597	21
	C012	2137.9	2142.77	2215.13	2165	43	2484.36	2496.46	2363.34	2448	74
	C013										
	C014	2360	2300	2380	2347	42	2750	2780	2710	2747	35
	C015	2290	2220	2210	2240	44	2110	2545	2495	2383	238
	C016										
	C017	2162	2185	2606	2318	250	2606	2542	2671	2606	65
	C027	2272.98	2154.21	2209.11	2212	59	2538.91	2480.78	2634.16	2551	77
	C030										
	C031	2345.69	2262.91	2763.46	2457	268	2653.61	2624.77	2586	2621	34
	C032	2551.68	2740.16	2429.5	2574	157	3021.35	2939.9	3249.05	3070	160
	C033										
	C036	2266.01	2252.08	2314.31	2277	33	2696.73	2714.26	2654.95	2689	30
	C038	2693	2600	2765	2686	83	3164	3167	3219	3183	31
	C039	2300	2260	2260	2273	23	2590	2640	2600	2610	26
	C040	236.651	231.138	232.728	234	3	252.591	256.048	260.843	256	4
	C042										
	C045	2214	2251	2210	2225	23	2530	2476	2560	2522	43
	C046	2126.15	2083.35	2089.31	2100	23	2425.28	2394.1	2409.43	2410	16
	C047	2173	2162	2174	2170	7	2379	2461	2531	2457	76
	C049										
	C052	2220	2200	2230	2217	15	2560	2630	2690	2627	65
Community Results		Consensus Mean			2275		Consensus Mean			2609	
		Consensus Standard Deviation			160		Consensus Standard Deviation			214	
		Maximum			4966		Maximum			4546	
		Minimum			234		Minimum			256	
		N			22		N			22	

Repeatability and reproducibility of methods used by individual participants and the community were compared to AOAC 2014.004 Standard Method Performance Requirements (SMPR) for Minerals and Trace Elements in Infant Formula and Adult/Pediatric Nutritional Formula [5]. Repeatability, demonstrated by within-laboratory variability (mean % RSD), and reproducibility, demonstrated by among-laboratory variability (% RSD), from this study are compared to the published expectations in Table 2-5. The mean within-laboratory variabilities were acceptable for all nutritional elements in both formula materials. For Cu and Mg in both materials and P in RM 8261, 2 to 3 laboratories per analyte/sample pair reported within-laboratory variabilities greater than 5 %. For P in RM 8260, however, 6 of 22 laboratories reported data with greater than 5 % within-laboratory variability. Higher within-laboratory variability for P in RM 8260 is not expected since RM 8260 and SRM 8261 are similar matrices with comparable P mass fractions. The among-laboratory variabilities for nutritional elements in formula were below the published expectations of the measurement community of ≤ 16 % RSD for Cu and ≤ 10 % RSD for Mg and P.

Table 2-5. Laboratory variabilities for nutritional elements in FNSQAP Exercise 3 formula materials relative to AOAC SMPR 2014.004.

	Within-Laboratory Variability			Among-Laboratory Variability		
	FNSQAP Ex. 3		SMPR	FNSQAP Ex. 3		SMPR
Elements	RM 8260	RM 8261	2014.004 [5]	RM 8260	RM 8261	2014.004 [5]
Cu	2.8 %	3.2 %	5 %	8.7 %	8.7 %	≤ 16 %
Mg	2.3 %	1.9 %	5 %	7.3 %	9.2 %	≤ 10 %
P	3.3 %	2.6 %	5 %	7.0 %	8.2 %	≤ 10 %

As shown in Fig. 2-1, Fig. 2-2, Fig. 2-3, Fig. 2-4, Fig. 2-5, and Fig. 2-6, laboratories reported using a few different sample preparation methods for the determination of nutritional elements in the two formula samples. Numbers and percentages of laboratories described as reporting specific approaches are averages across Cu and Mg results for both samples. The most common sample preparation approach was microwave digestion (16 laboratories, 70 %); five laboratories reported using hot block digestion (22 %) and one laboratory reported using hot plate digestion (4 %). One laboratory did not report the sample preparation approach used (4 %). Microwave digestion was also the most common sample preparation approach for P in both samples (15 laboratories, 68 %) followed by hot block digestion (4 laboratories, 18 %), dry ashing (2 laboratories, 9 %), and hot plate digestion (1 laboratories, 5 %). Notably, the laboratories indicating use of dry ashing as the preparation method prior to determination of P reported values biased below the 95 % confidence interval for the consensus mean in both samples. Although this preparation method is only represented by two laboratories, dry ashing may not be ideal for determination of P in formula matrices. Additionally, the values reported by the five laboratories using hot block digestion were biased high for Cu and Mg, and biased low for P. Laboratories using hot block digestion should investigate possible sources of bias through use of a CRM or other quality control sample.

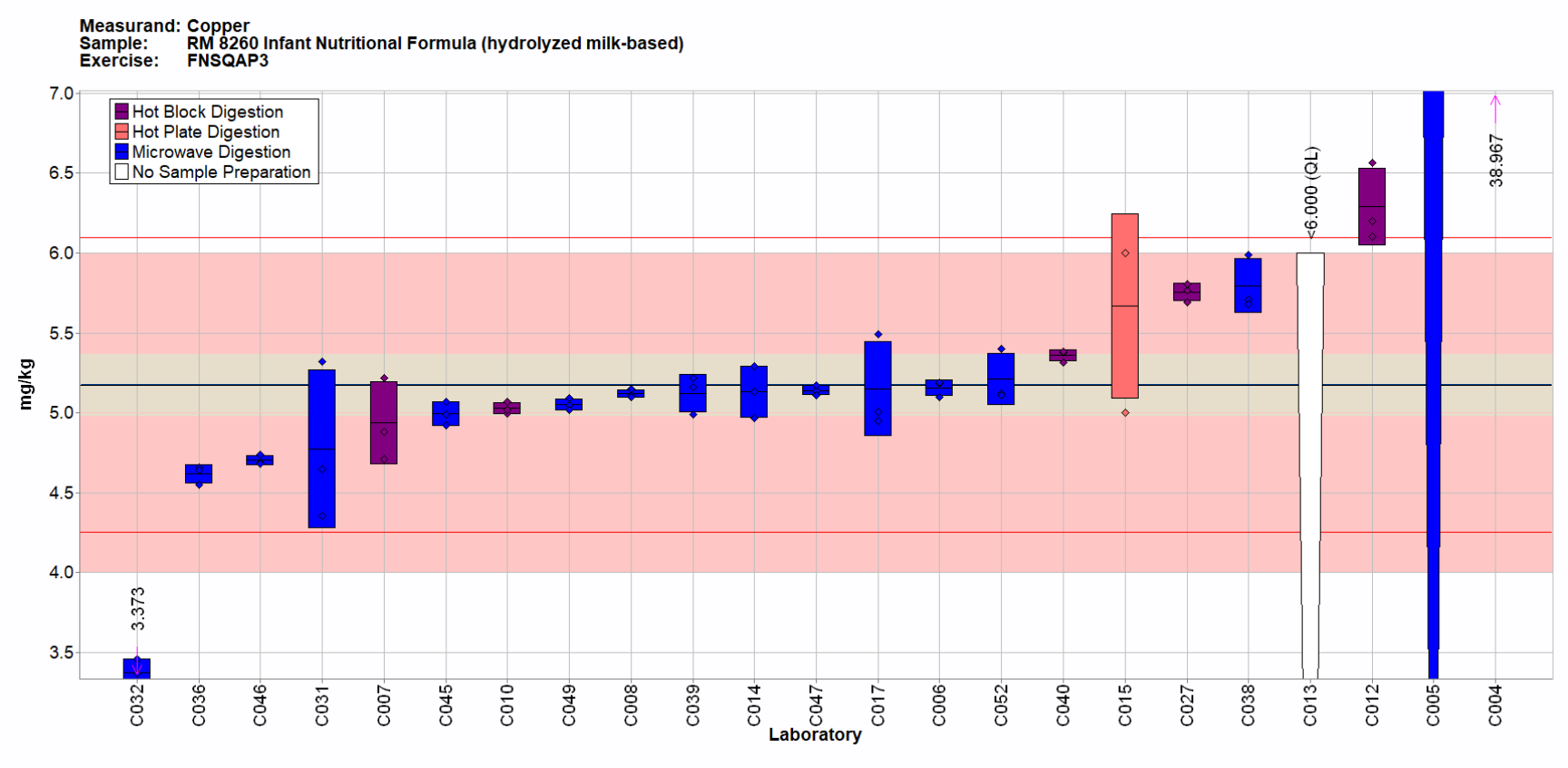

Fig. 2-1. Copper in RM 8260 (data summary view – sample preparation method).

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. Data points outside the graphical range are represented as upward arrows (the preparation method reported by laboratory C004 was microwave digestion). The solid black line represents the consensus mean, and the solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{comm}| \leq 2$. The beige shaded region represents the overlapping of the 95 % confidence interval for the consensus mean (green region) and the NIST range of tolerance (red region). The NIST range of tolerance encompasses the target value bounded by twice its uncertainty (U_{NIST}), and represents the range that results in an acceptable Z_{NIST} score, $|Z_{NIST}| \leq 2$.

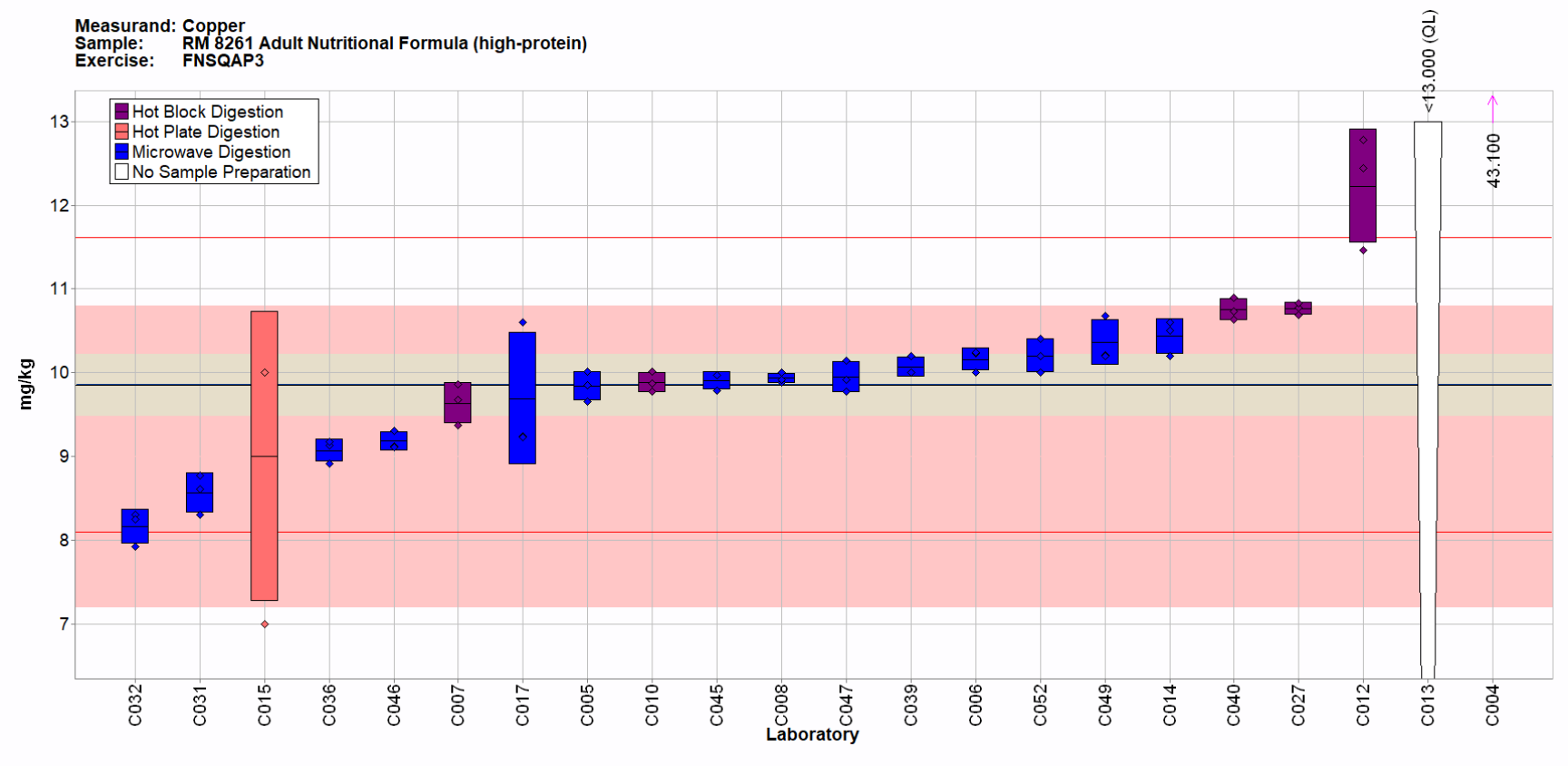

Fig. 2-2. Copper in RM 8261 (data summary view – sample preparation method).

In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). A downward triangle represents data reported as an LOQ value. The color of the data point represents the sample preparation method employed as indicated in the figure key. Data points outside the graphical range are represented as upward arrows (the preparation method reported by laboratory C004 was microwave digestion). The solid black line represents the consensus mean, and the solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{comm}| \leq 2$. The beige shaded region represents the overlapping of the 95 % confidence interval for the consensus mean (green region) and the NIST range of tolerance (red region). The NIST range of tolerance encompasses the target value bounded by twice its uncertainty (U_{NIST}), and represents the range that results in an acceptable Z_{NIST} score, $|Z_{NIST}| \leq 2$.

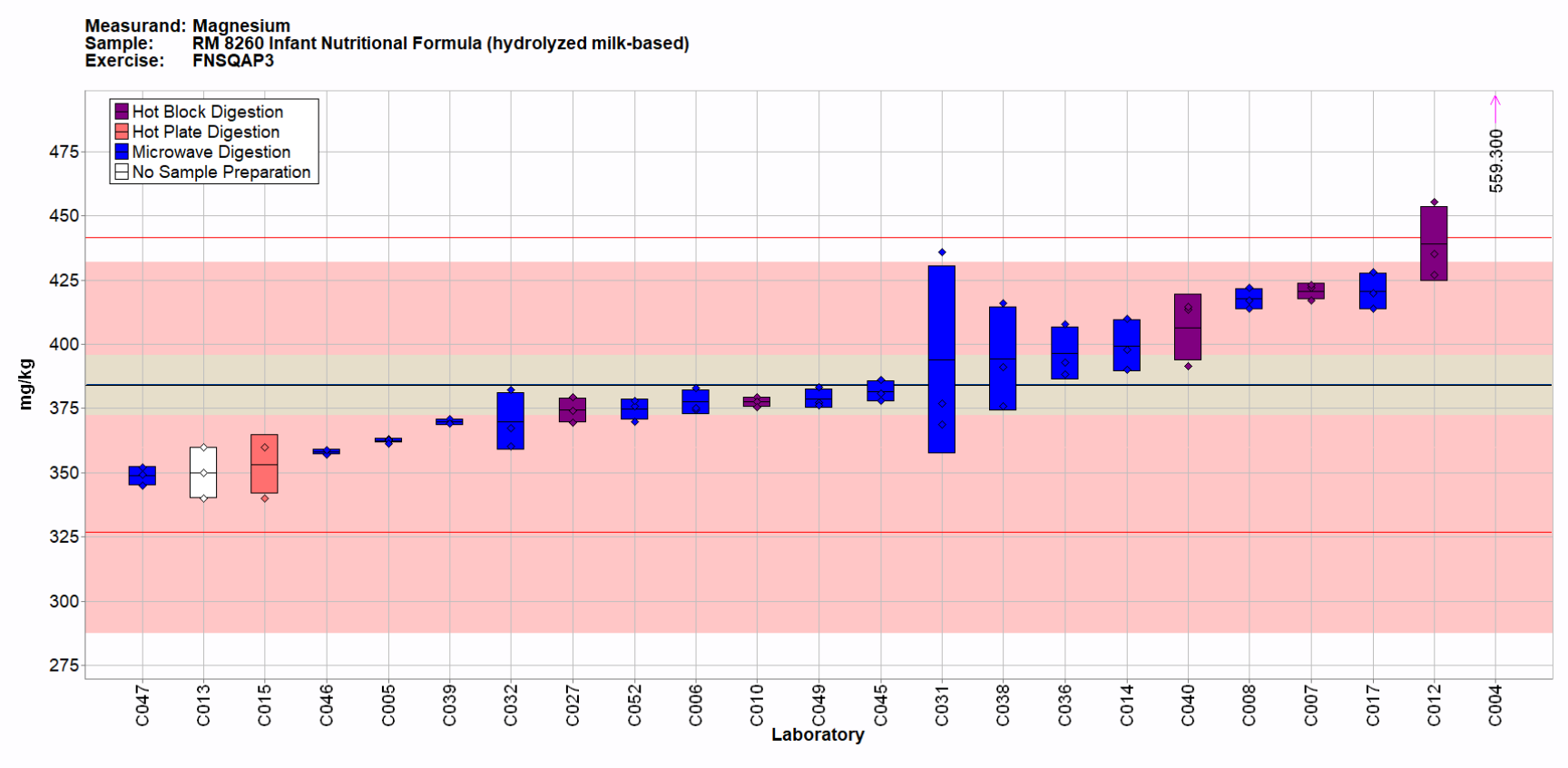

Fig. 2-3. Magnesium in RM 8260 (data summary view – sample preparation method).

In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed as indicated in the figure key. Data points outside the graphical range are represented as upward arrows (the preparation method reported by laboratory C004 was microwave digestion). The solid black line represents the consensus mean, and the solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{comm}| \leq 2$. The beige shaded region represents the overlapping of the 95 % confidence interval for the consensus mean (green region) and the NIST range of tolerance (red region). The NIST range of tolerance encompasses the target value bounded by twice its uncertainty (U_{NIST}), and represents the range that results in an acceptable Z_{NIST} score, $|Z_{NIST}| \leq 2$.

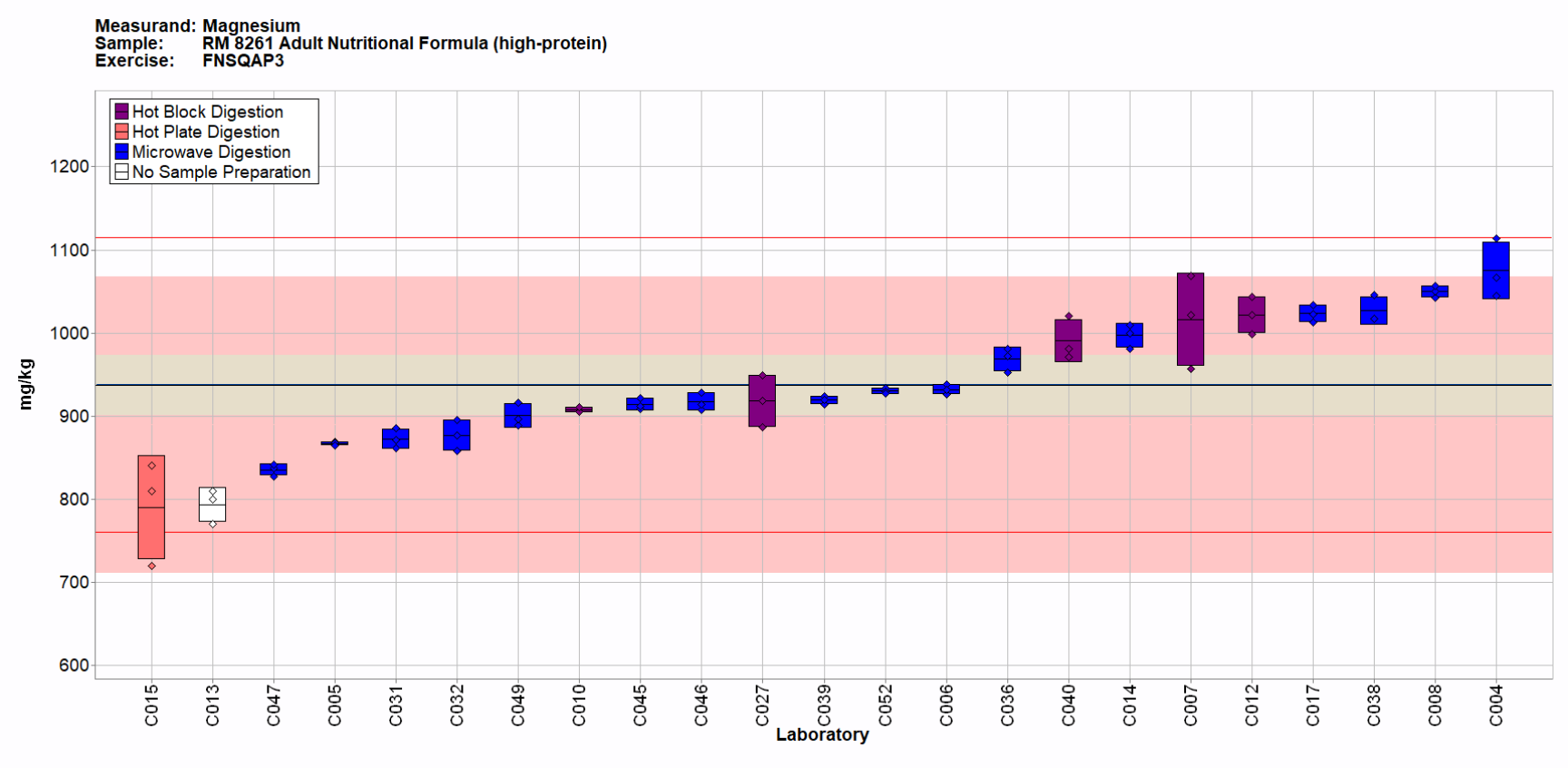

Fig. 2-4. Magnesium in RM 8261 (data summary view – sample preparation method).

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

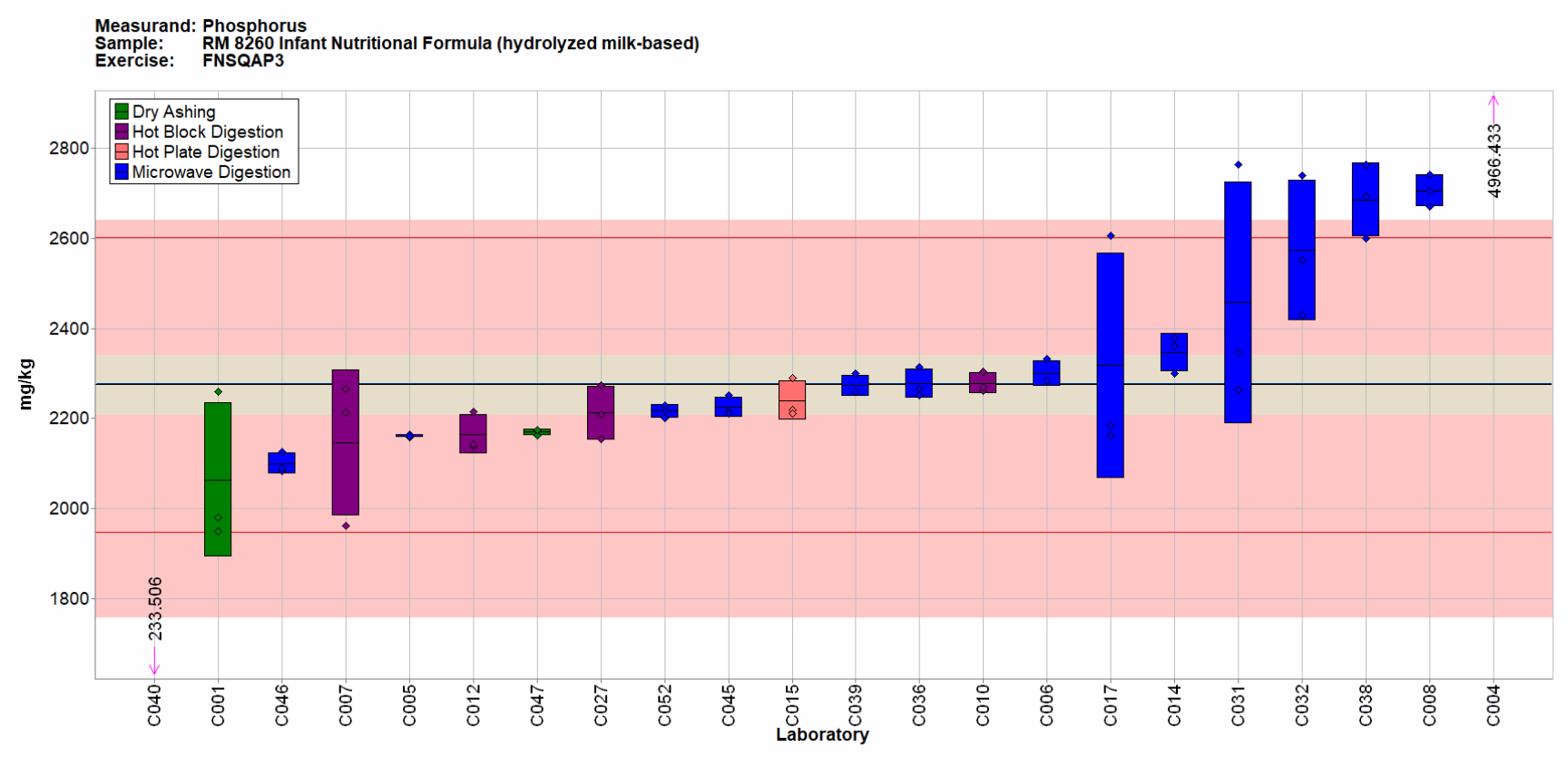

Fig. 2-5. Phosphorus in RM 8260 (data summary view – sample preparation method).

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 or upward arrows (the preparation methods reported by laboratories C040 and C004 were hot block digestion and microwave digestion, respectively). The solid black line represents the consensus mean, and the solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{comm}| \leq 2$. The beige shaded region represents the overlapping of the 95 % confidence interval for the consensus mean (green region) and the NIST range of tolerance (red region). The NIST range of tolerance encompasses the target value bounded by twice its uncertainty (U_{NIST}), and represents the range that results in an acceptable Z_{NIST} score, $|Z_{NIST}| \leq 2$.

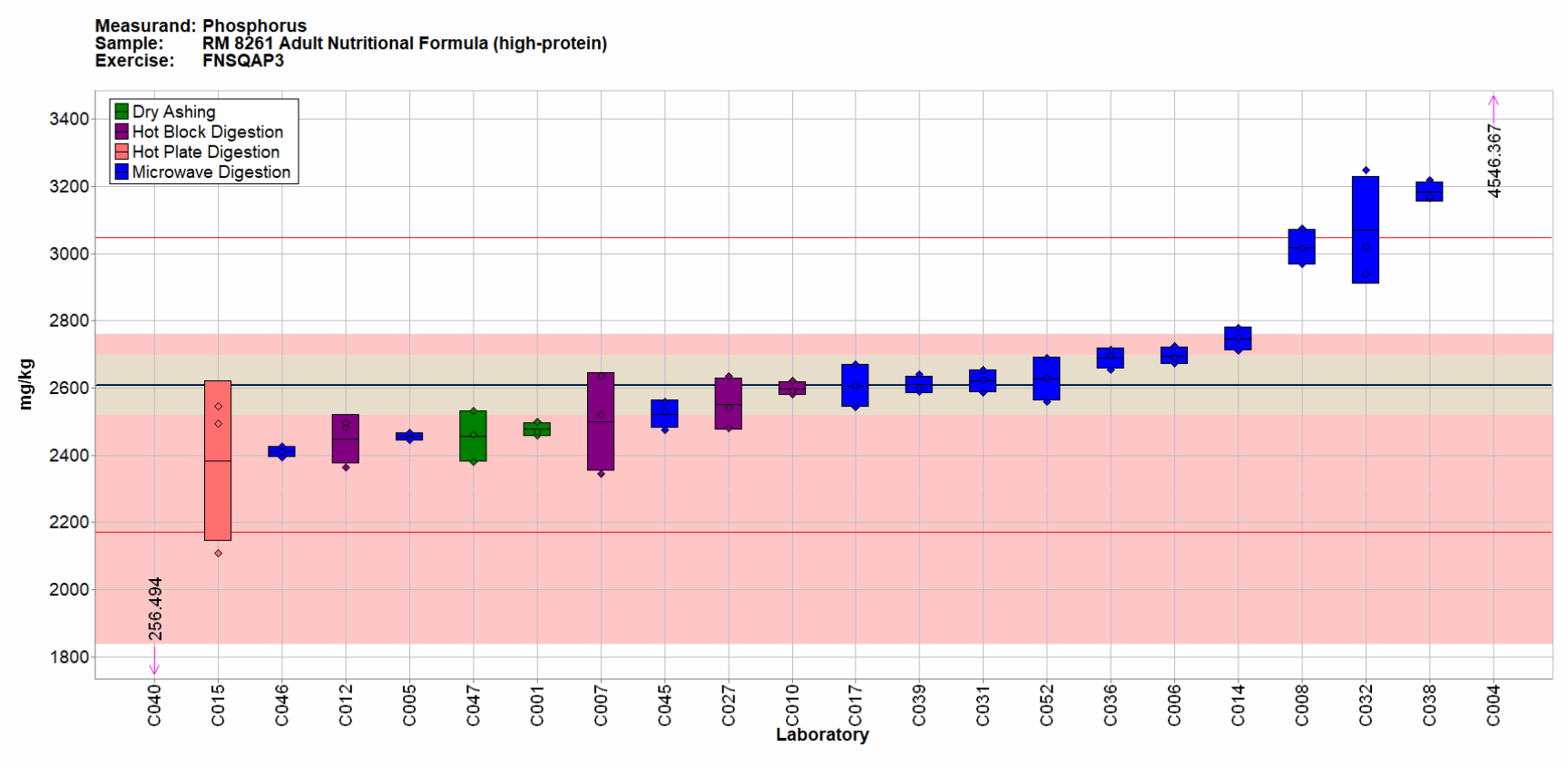

Fig. 2-6. Phosphorus in RM 8261 (data summary view – sample preparation method).

In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed as indicated in the figure key. Data points outside the graphical range are represented as downward or upward arrows (the preparation methods reported by laboratories C040 and C004 were hot block digestion and microwave digestion, respectively). The solid black line represents the consensus mean, and the solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{comm}| \leq 2$. The beige shaded region represents the overlapping of the 95 % confidence interval for the consensus mean (green region) and the NIST range of tolerance (red region). The NIST range of tolerance encompasses the target value bounded by twice its uncertainty (U_{NIST}), and represents the range that results in an acceptable Z_{NIST} score, $|Z_{NIST}| \leq 2$.

Laboratories provided additional information through a questionnaire about their sample preparation parameters including the reagents used, highest temperature during digestion, and the total digestion time. Specific digestion parameters did not point to biases for nutritional element measurements in formula samples. Greater than desired within-laboratory variability may also be due to the use of less than the recommended sample size for analysis (0.5 g) since the sample may not be homogenous below this mass.

As shown in Fig. 2-7, Fig. 2-8, Fig. 2-9, Fig. 2-10, Fig. 2-11, and Fig. 2-12, analytical methods employed by laboratories for the determination of nutritional elements in the two formula samples were about evenly split between inductively coupled plasma optical emission spectrometry (ICP-OES) (10 laboratories; 43 %) and inductively coupled plasma mass spectrometry (ICP-MS) (12 laboratories; 52 %). One laboratory (4 %) reported using instrumental neutron activation analysis (INAA) for Cu and Mg analysis, and one laboratory (4 %) reported using absorption spectrophotometry for P analysis. Notably, the laboratory indicating use of INAA as the analysis method for determination of Cu and Mg reported LOQs at the top or above the 95 % NIST range of tolerance for the target value in both formula samples. Although this analysis method is only represented by one laboratory, INAA may not be ideal for Cu and Mg analysis at the mass fractions present in the formula samples.

Sensitivity of the analytical method is key when determining whether the method is suitable for the analyte abundance in the sample and appropriate sample dilution for the dynamic range of the analytical method. Since ICP-OES and ICP-MS were the most reported analytical methods, some technical recommendations are provided for these analytical methods. No measurement bias was noted for laboratories using ICP-OES or ICP-MS for Cu and Mg determination. However, measurement bias was observed for P in RM 8260 by laboratories using ICP-OES and ICP-MS with the consensus mean approximately 13 % above the NIST target value (Fig. 2-6). When measuring P by ICP-OES using 213 nm as the wavelength, major interferences with Cu and Mo can result in inflated P measurements. The mass fractions of Cu and Mo in RM 8260 are about twice the mass fraction of these elements in RM 8261, which may have contributed to the high bias in the consensus mean. Laboratories using ICP-OES should measure P at additional suitable wavelengths to reduce impact of potential interferences from the sample matrix. Likewise, collision cell gases or reaction cell mode can be used with ICP-MS to reduce or eliminate the interferences caused by molecular ions that have the same mass-to-charge ratio as the element of interest. Utilizing ICP-MS in kinetic energy discrimination (KED) mode can control cell-formed interferences and reduce polyatomic ion interferences created by the plasma or vacuum interface. For example, monoisotopic ^{31}P has relatively high ionization potential and suffers isobaric overlap or polyatomic interferences from $^{14}N^{16}O^{1}H^{+}$ and $^{15}N^{15}N^{1}H^{+}$ causing signal enhancement and leading to bias of the measurements [6]. Use of helium collision gas reduces these interferences for P at *m/z* 31.

A comparison of the consensus confidence interval and the NIST target range for each nutritional element is summarized in Table 2-6. A consensus mean within the target range and close to the target value is an indication that the community is performing well.

Table 2-6. Comparison of consensus confidence interval and NIST target range for nutritional elements

Element	RM 8260	RM 8261
Copper (Cu)	Within (consensus mean 4 % above target value)	Within (consensus mean 10 % above target value)
Magnesium (Mg)	Within (consensus mean 7 % above target value)	Within (consensus mean 5 % above target value)
Phosphorus (P)	Within (consensus mean 3 % above target value)	Within (consensus mean 13 % above target value)

Overall, laboratories performed well in the measurement of nutritional elements in infant formula samples. Two to three participating laboratories reported Cu, Mg, and P measurement averages outside of the consensus tolerance limits for both samples as shown in Fig. 2-13, Fig. 2-14, and Fig. 2-15. A slight positive linear trend is observed in Fig. 2-13, Fig. 2-14, and Fig. 2-15, which may indicate a global issue with calibration or common measurement biases between the two samples. 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) with exception of two laboratories that were remarkably high for all nutritional elements, and one laboratory that was only low for P. 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.

In any laboratory exercise, calculations and reporting units must be verified prior to submission of results. Laboratories sometimes 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 or for an element. One laboratory reported values about ten times below the target value for P in both matrices, indicating a calculation or unit conversion error. 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 SRMs or non-certified reference materials such as NIST 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).

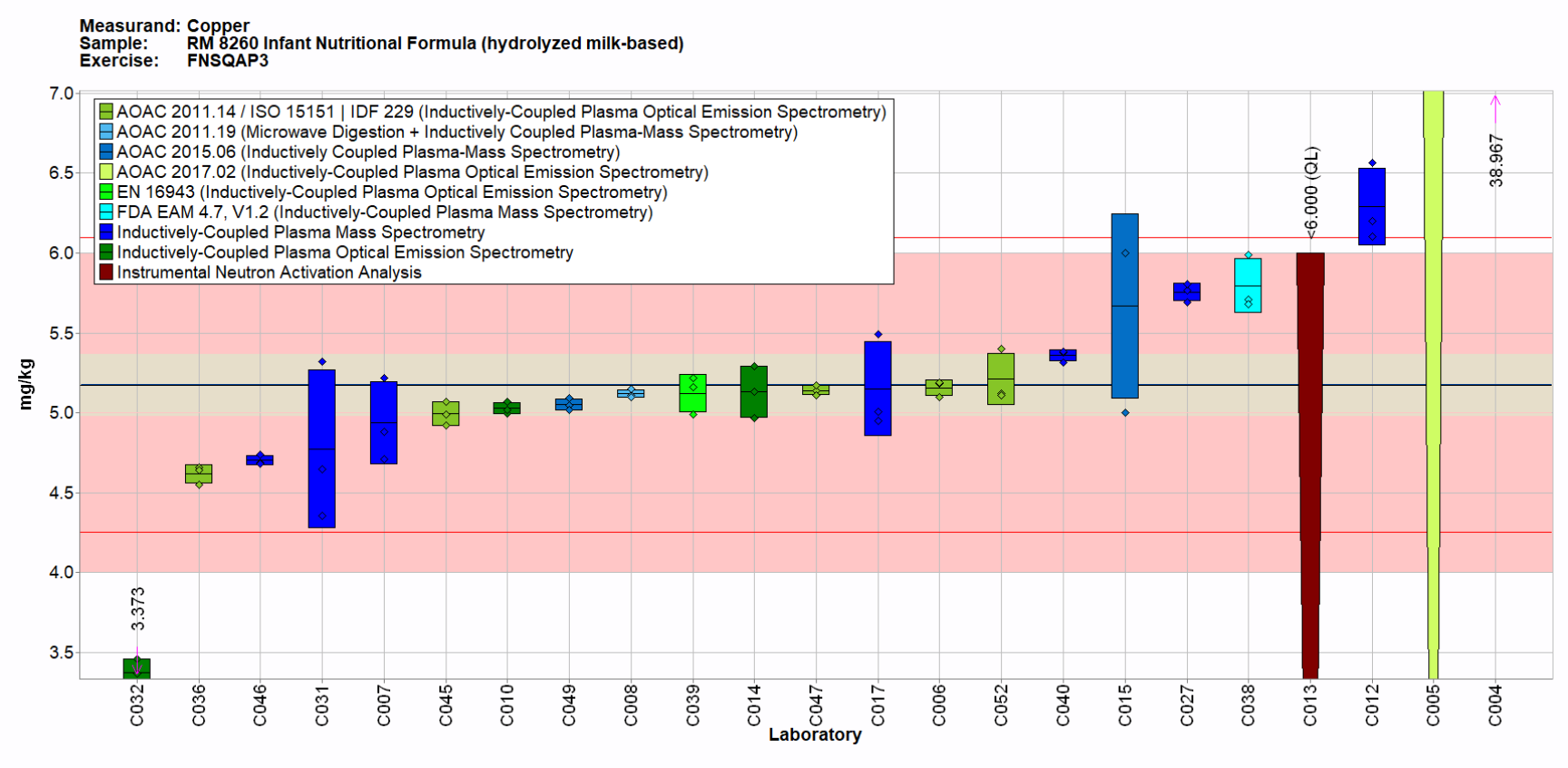

Fig. 2-7. Copper in RM 8260 (data summary view – analytical method).

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. Data points outside the graphical range are represented as upward arrows (the analytical method reported by laboratory C004 was ICP-MS). The solid black line represents the consensus mean, and the solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{comm}| \leq 2$. The beige shaded region represents the overlapping of the 95 % confidence interval for the consensus mean (green region) and the NIST range of tolerance (red region). The NIST range of tolerance encompasses the target value bounded by twice its uncertainty (U_{NIST}), and represents the range that results in an acceptable Z_{NIST} score, $|Z_{NIST}| \leq 2$.

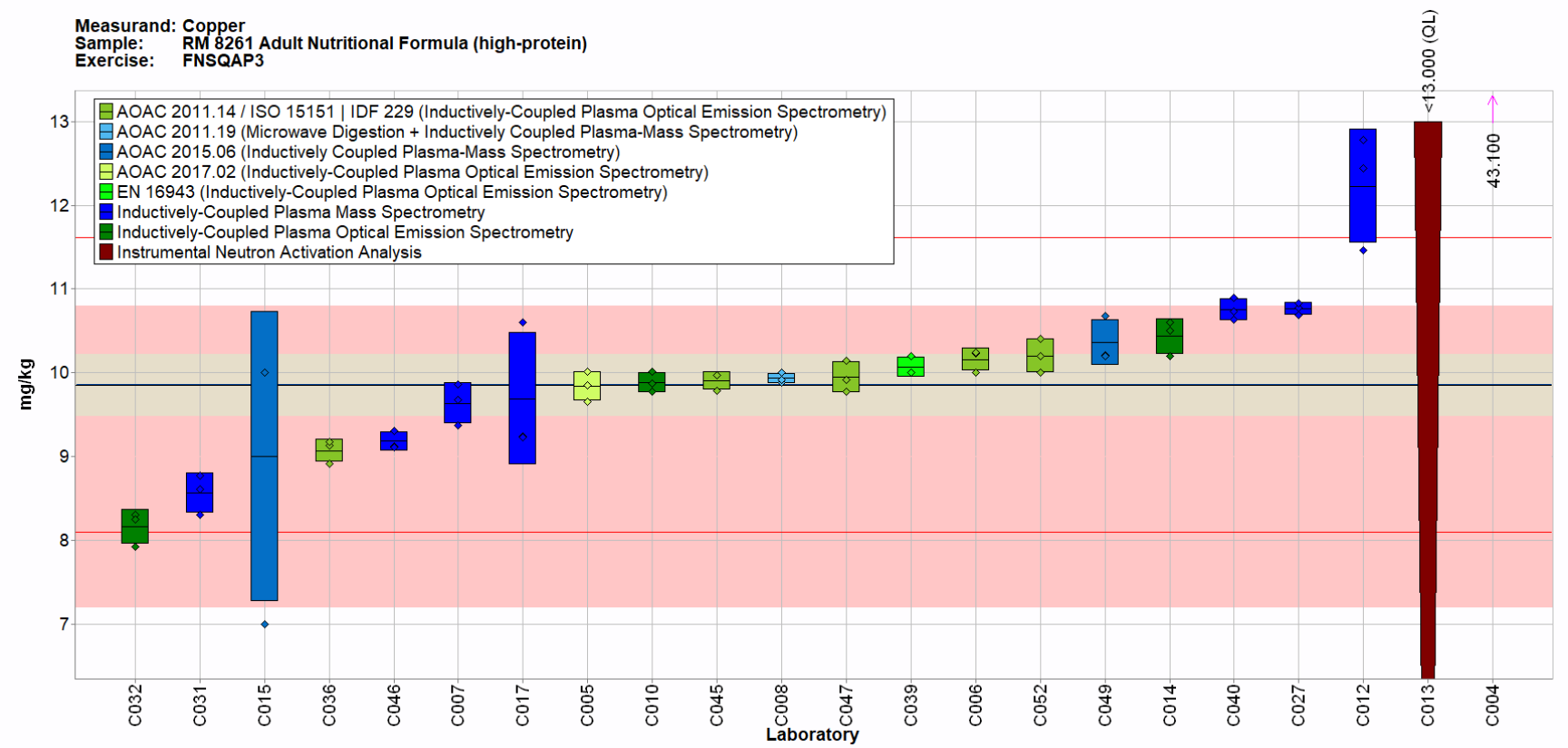

Fig. 2-8. Copper in RM 8261 (data summary view – analytical method).

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 C004 was ICP-MS). The solid black line represents the consensus mean, and the solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{comm}| \leq 2$. The beige shaded region represents the overlapping of the 95 % confidence interval for the consensus mean (green region) and the NIST range of tolerance (red region). The NIST range of tolerance encompasses the target value bounded by twice its uncertainty (U_{NIST}), and represents the range that results in an acceptable Z_{NIST} score, $|Z_{NIST}| \leq 2$.

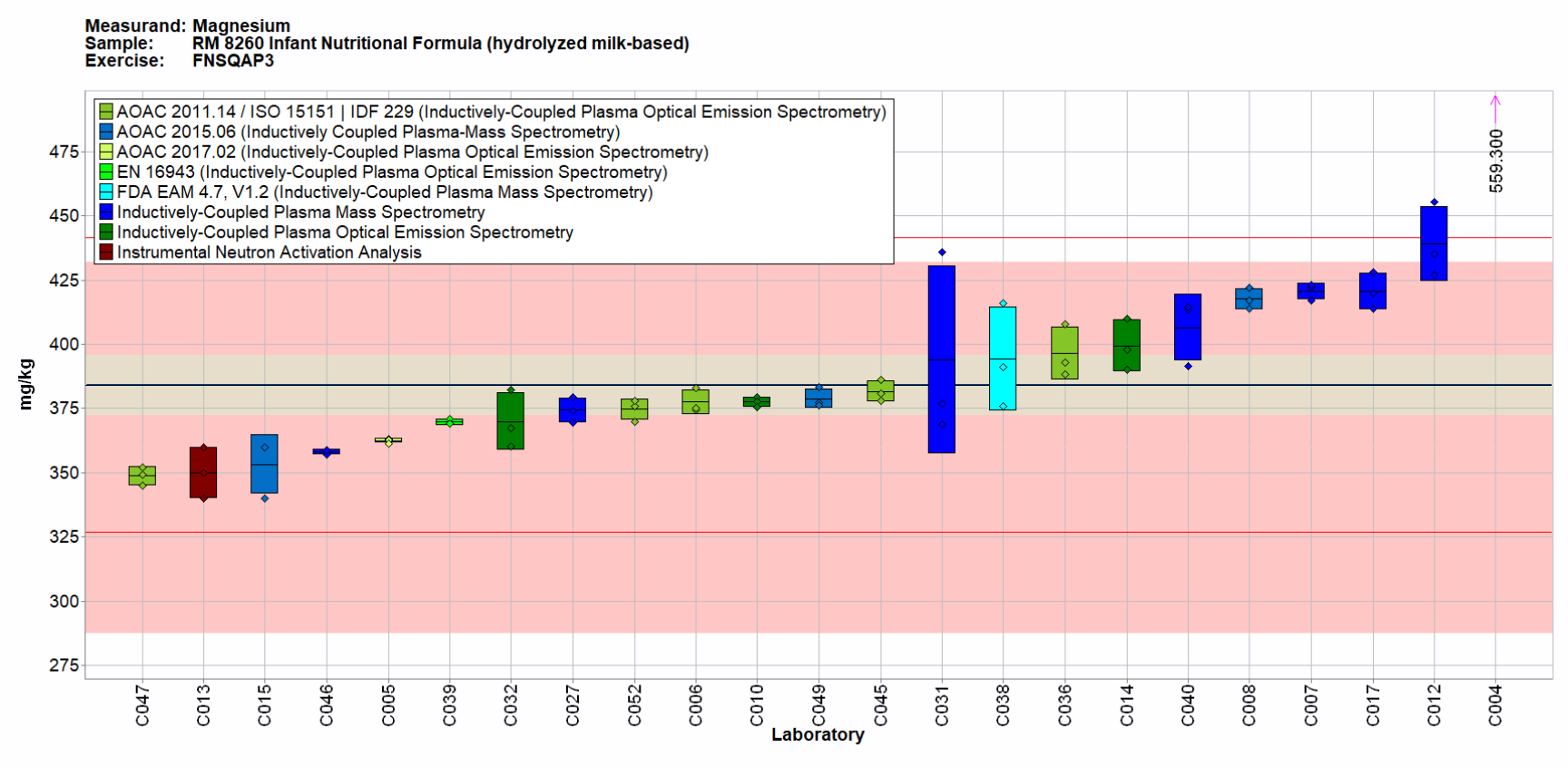

Fig. 2-9. Magnesium in RM 8260 (data summary view – analytical method).

In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed as indicated in the figure key. Data points outside the graphical range are represented as upward arrows (the analytical method reported by laboratory C004 was ICP-MS). The solid black line represents the consensus mean, and the solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{comm}| \leq 2$. The beige shaded region represents the overlapping of the 95 % confidence interval for the consensus mean (green region) and the NIST range of tolerance (red region). The NIST range of tolerance encompasses the target value bounded by twice its uncertainty (U_{NIST}), and represents the range that results in an acceptable Z_{NIST} score, $|Z_{NIST}| \leq 2$.

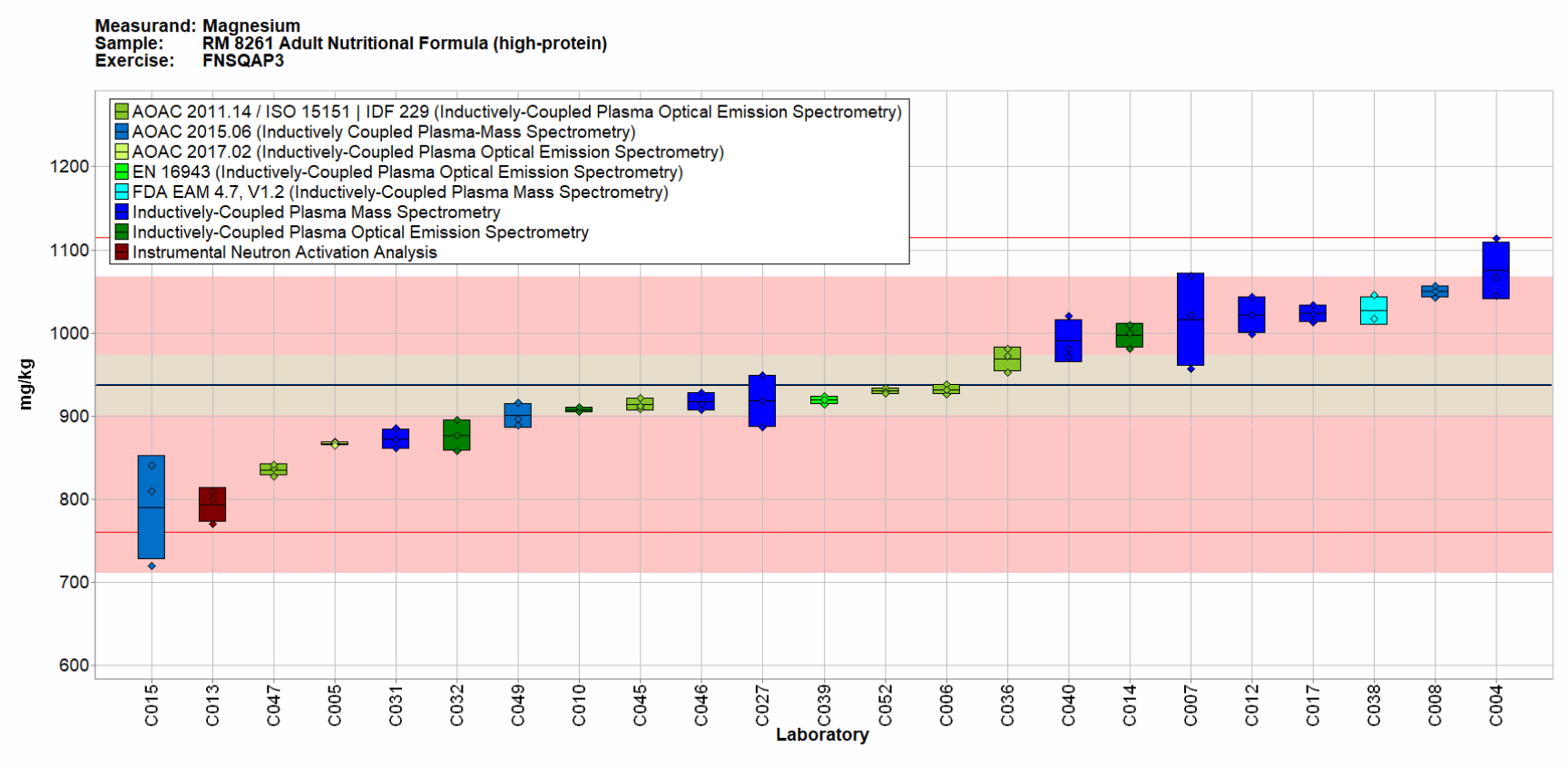

Fig. 2-10. Magnesium in RM 8261 (data summary view – analytical method).

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

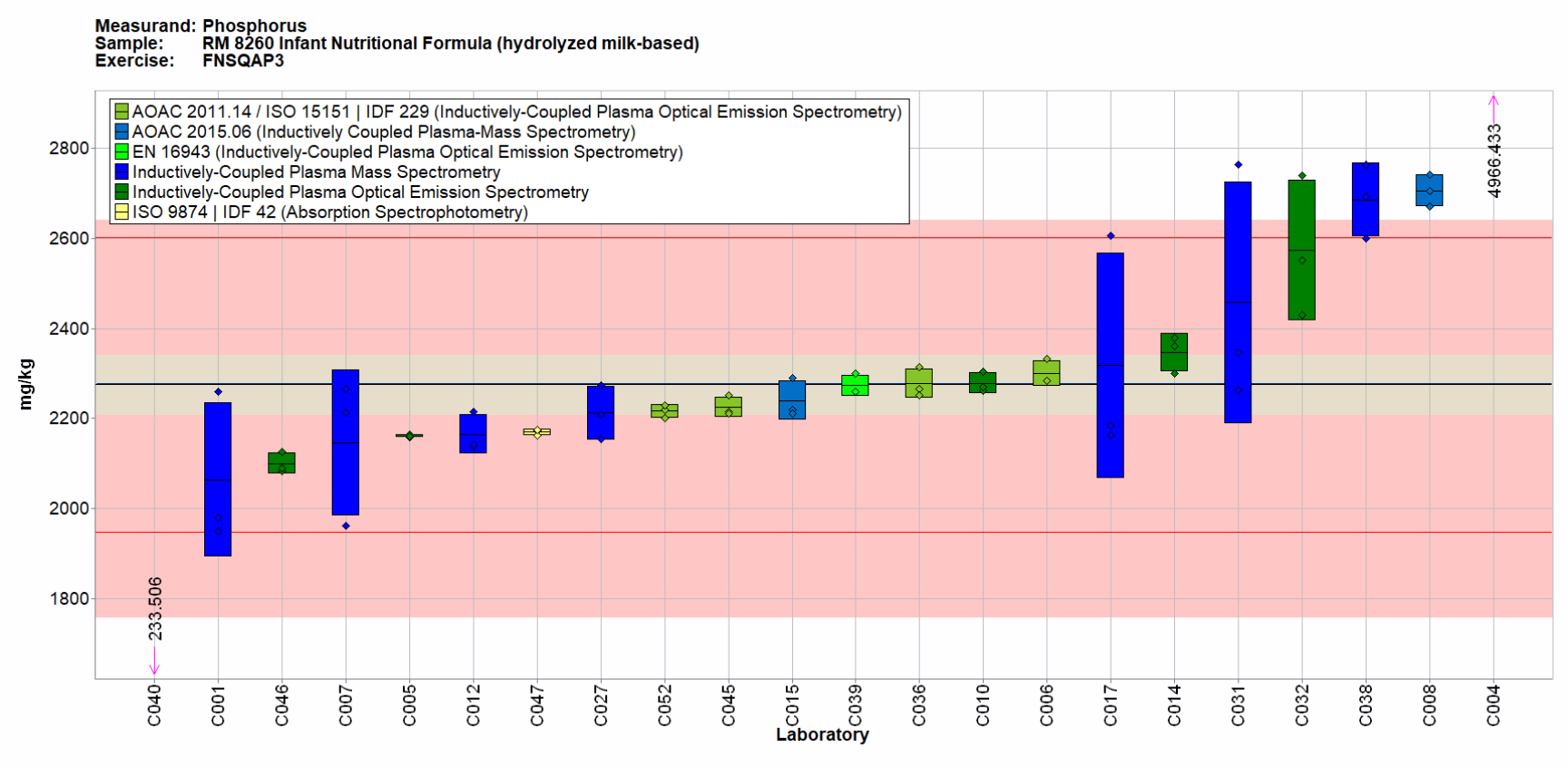

Fig. 2-11. Phosphorus in RM 8260 (data summary view – analytical method).

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 C040 and C004 were ICP-MS). The solid black line represents the consensus mean, and the solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{comm}| \leq 2$. The beige shaded region represents the overlapping of the 95 % confidence interval for the consensus mean (green region) and the NIST range of tolerance (red region). The NIST range of tolerance encompasses the target value bounded by twice its uncertainty (U_{NIST}), and represents the range that results in an acceptable Z_{NIST} score, $|Z_{NIST}| \leq 2$.

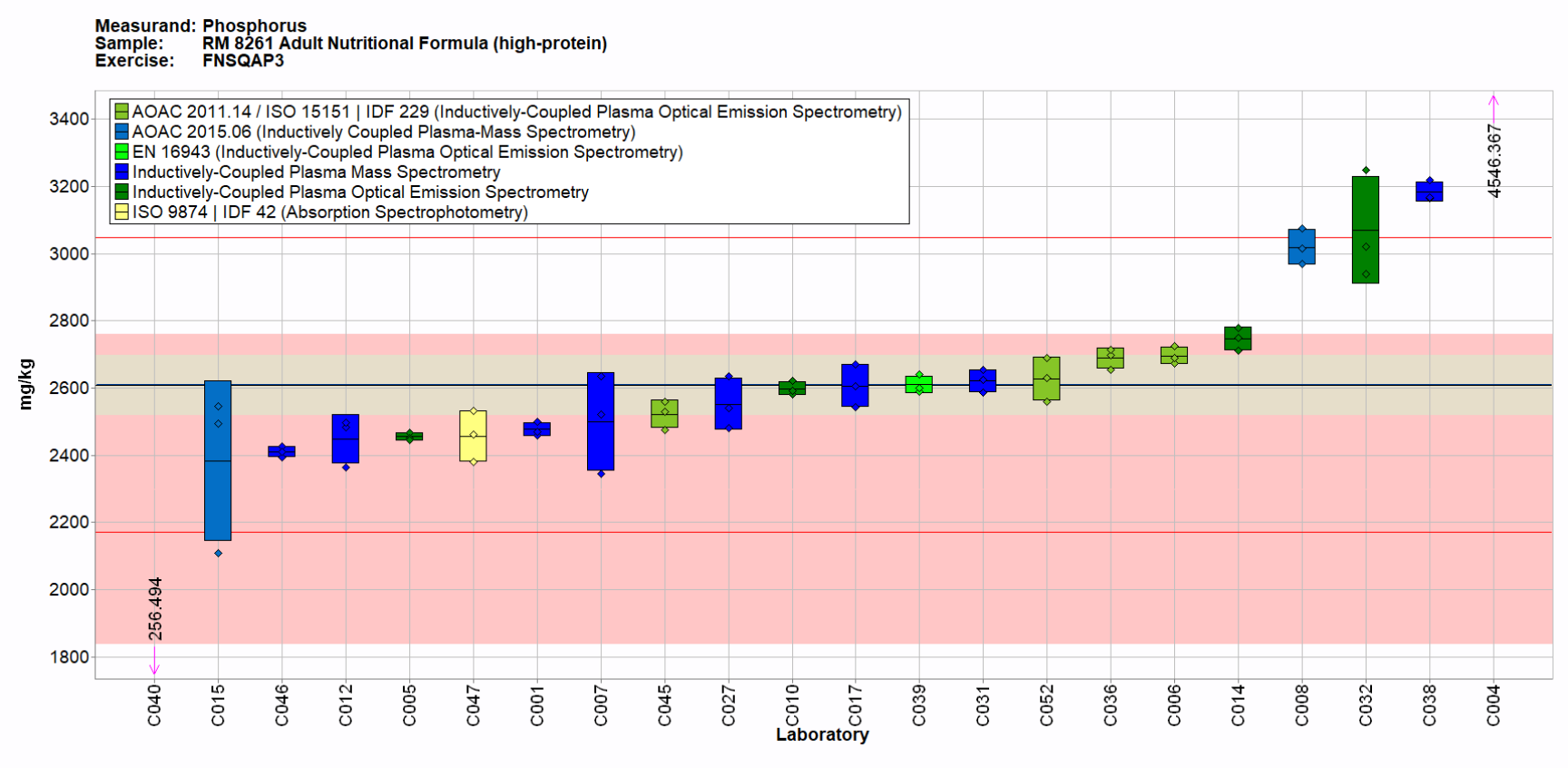

Fig. 2-12. Phosphorus in RM 8261 (data summary view – analytical method).

In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed as indicated in the figure key. Data points outside the graphical range are represented as downward and upward arrows (the analytical methods reported by laboratories C040 and C004 were ICP-MS). The solid black line represents the consensus mean, and the solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{comm}| \leq 2$. The beige shaded region represents the overlapping of the 95 % confidence interval for the consensus mean (green region) and the NIST range of tolerance (red region). The NIST range of tolerance encompasses the target value bounded by twice its uncertainty (U_{NIST}), and represents the range that results in an acceptable Z_{NIST} score, $|Z_{NIST}| \leq 2$.

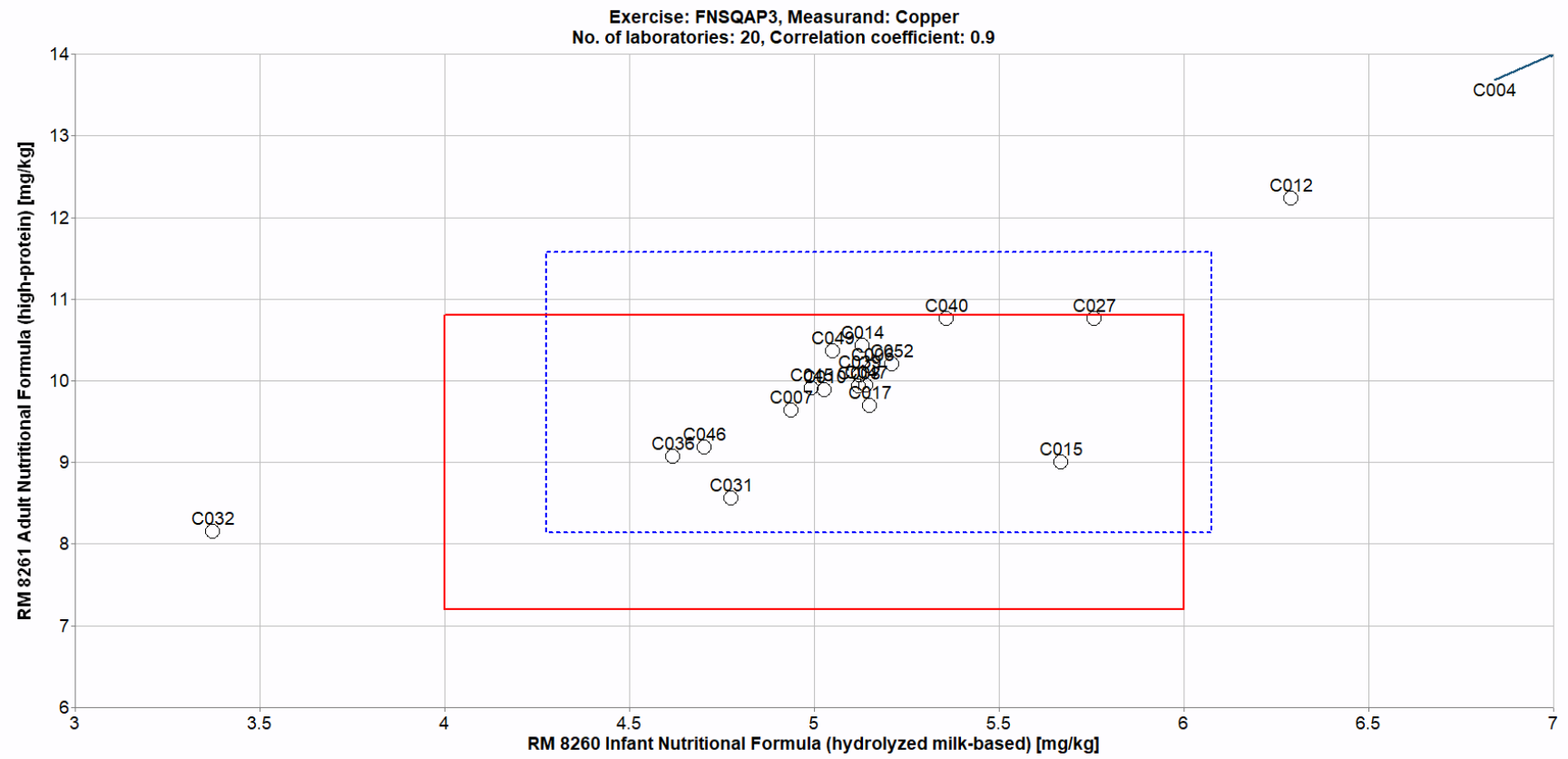

Fig. 2-13. Laboratory means for copper in RM 8260 and RM 8261 (sample/sample comparison view).

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

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

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

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

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

3. WATER-SOLUBLE VITAMINS (Vitamin B_{12})

3.1. Executive Summary

Vitamin B_{12} is an important nutrient for infant development and growth, and the fortified levels of vitamin B_{12} in infant foods are strictly regulated worldwide. Participants in this study performed well in determination of vitamin B_{12}. Laboratories that utilized cyanidation followed by sample cleanup based on solid phase extraction exhibited higher accuracy than laboratories foregoing one or both steps. Laboratories not utilizing this multistep approach should further investigate potential bias through use of reference materials.

3.2. Study Overview

Vitamin B_{12} is a group of cobalt containing compounds that assist in the development and function of the central nervous system, formation of red blood cells, and synthesis of DNA [7]. Vitamin B_{12}, provided to infants via infant formula, is important for proper development. Accurately understanding the intake of vitamin B_{12} through measurement in fortified foods such as infant formula can inform future decisions about recommended dietary intakes. In this study, participants were provided with two nutritional formula samples, RM 8260 Infant Nutritional Formula (hydrolyzed milk-based) and RM 8261 Adult Nutritional Formula (high protein). Participants were asked to use in-house analytical methods to determine the mass fraction (mg/kg) of vitamin B_{12} in the nutritional formula samples. Through participation in this study, laboratories can better understand the performance of their in-house methods relative to those being used by others in the community. Participant results may be used in the value assignment of NIST reference materials included as samples in this study.

3.3. Sample Information

Participants were provided with three packets each of RM 8260 Infant Nutritional Formula (hydrolyzed milk-based) (labeled Infant Formula A) and RM 8261 Adult Nutritional Formula (high protein) (labeled Infant Formula B). Each packet contained approximately 10 g of material. Participants were asked to store the materials at controlled room temperature (20 °C to 25 °C) in the original unopened packets and to prepare one sample and report one value from each packet provided. Before use, participants were instructed to thoroughly mix the contents of the packets prior to removal of a test portion for analysis, and to use a sample size of at least 0.5 g to ensure homogeneity of the test portion used for the determination of the mass fraction of vitamin B_{12}. The approximate analyte levels were not reported to participants prior to the study. The target mass fraction values for vitamin B_{12} in each material were as described in their respective RMISs [3, 4]. The target mass fraction values and uncertainties for vitamin B_{12} in each material are provided in Table 3-1 on an as-received basis. The uncertainties for vitamin B_{12} in RM 8260 and RM 8261 were approximated as 10 % relative to the measured value.

Table 3-1. Individualized data summary table for vitamin B_{12} in nutritional formulas.

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

(Lab Name)

Exercise 3 – Water-Soluble Vitamins in Infant Formula

	Lab Code:	**(Code)**	**1. Your Results**				**2. Community Results**			**3. Target**	
	Sample	Units	x_i	s_i	Z'_{comm}	Z_{NIST}	N	x*	s*	x_{NIST}	u_{NIST}
B_{12}	RM 8260	mg/kg	*Individual laboratory results will appear in this section; laboratory-specific results were provided to each participant separately from this report.*				13	0.0370	0.0180	0.0290	0.0029
B_{12}	RM 8261	mg/kg					13	0.066	0.023	0.059	0.006

x_i Mean of reported values

s_i Standard deviation of reported values

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

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

N Number of quantitative values reported, not including single-value submissions

x* Robust mean of reported values

s* Robust standard deviation

x_{NIST} NIST-assessed value

u_{NIST} standard uncertainty about the NIST-assessed value

3.4. Study Results and Discussion

Table 3-1 summarizes and Table 3-2 details the measured mass fraction results reported by each participating laboratory for vitamin B_{12}. The participation level was high for water-soluble vitamins, with 13 to 14 of 19 laboratories requesting samples returning results (68 % to 74 %).

Table 3-2 reveals that the within-laboratory variabilities for vitamin B_{12} measured mass fractions were acceptable for data reported by 8 of 13 laboratories for RM 8260 and for 11 of 13 laboratories for RM 8261, when compared to published expectations of the measurement community (≤ 7 %) [8]. Published expectations for among-laboratory variability are designed to evaluate the performance of a single method used by multiple laboratories. The among-laboratory variabilities in mass fractions reported for vitamin B_{12} were higher than expected (49 % and 35 % for RM 8260 and RM 8261, respectively) compared to the published expectations (≤ 11 %).

Table 3-2. Data summary table for vitamin B_{12} in RM 8260 and RM 8261.

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

		Vitamin B_{12} (Cyanocobalamin)									
		RM 8260 Infant Nutritional Formula (hydrolyzed milk-based) (mg/kg)					RM 8261 Adult Nutritional Formula (high-protein) (mg/kg)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target				0.029	0.003				0.059	0.006
	C001	0.0415	0.0423	0.0455	0.043	0.002	0.0793	0.0752	0.0704	0.075	0.004
	C006	0.0369	0.03484	0.03341	0.035	0.002	0.0722	0.06544	0.07328	0.070	0.004
	C007	18.5	19.2	19.5	19.067	0.513	12.3	12.3	12.3	12.300	0.000
	C010	0.0294	0.0288	0.029	0.029	0.000	0.0567	0.0575	0.0574	0.057	0.000
	C011	0.237	0.259	0.255	0.250	0.012	0.273	0.271	0.288	0.277	0.009
	C012	< 0.040	< 0.040	< 0.040			0.0541	0.0583	0.0594	0.057	0.003
	C014	0.0391	0.0338	0.0386	0.037	0.003	0.074	0.0766	0.0723	0.074	0.002
	C015	0.06	0.07	0.07	0.067	0.006	0.1	0.06	0.07	0.077	0.021
	C016										
	C017										
	C030										
	C033										
	C036	0.026	0.028	0.024	0.026	0.002	0.058	0.06	0.055	0.058	0.003
	C038	1.14	1.09	1.21	1.147	0.060	4.12	3.91	4.14	4.057	0.127
	C039	0.0326	0.0315	0.0324	0.032	0.001	0.0608	0.0593	0.0631	0.061	0.002
	C040	18.276	14.174	19.744	17.398	2.887	14.355	5.017	9.302	9.558	4.674
	C047	0.0342	0.0285	0.0337	0.032	0.003					
	C049										
	C052	0.0317	0.0318	0.0333	0.032	0.001	0.0642	0.0612	0.066	0.064	0.002
Community Results		Consensus Mean			0.037		Consensus Mean			0.066	
		Consensus Standard Deviation			0.018		Consensus Standard Deviation			0.023	
		Maximum			19.067		Maximum			12.300	
		Minimum			0.026		Minimum			0.057	
		N			13		N			13	

Laboratories completed a method questionnaire prior to sample distribution and provided method information upon submission of results. Combined information from these two sources is used to categorize results in the legends of Fig. 3-1 and Fig. 3-2. The key differences described by laboratories were 1) type of solution used to solubilize vitamin B_{12} from the nutritional formula samples (e.g., acetate solution, water, water with organic modifier); 2) use of a cyanidation (CN conversion) step; 3) use and type of sample cleanup step (e.g., immunoaffinity column, other solid-phase extraction (SPE) column); and 4) temperature at which samples were extracted (e.g., room temperature (RT), 25 °C to 40 °C, > 40 °C). Most laboratories reported using an acetate solution combined with CN conversion at > 40 °C (8 of 14 laboratories, 57 %), parameters that correlate with accurate determination of vitamin B_{12} in these samples. Seven of the 8 laboratories utilizing this approach reported mass fraction values for vitamin B_{12} that were within the

consensus range; 6 of the 8 were also within the NIST range of tolerance. The values reported outside the consensus mean mass fraction range using this method were high by a factor of 10, indicating a potential calculation error. The mass fraction values reported within the consensus range but outside the NIST range of tolerance were determined without a sample cleanup step, which may have resulted in presence of interfering compounds that caused a high bias. Inclusion of a specific type of cleanup step (e.g., immunoaffinity column or other) does not correlate with greater accuracy in result, but omission of a cleanup step generally resulted in mass fraction results that were biased high with respect to the consensus and target mean mass fractions.

As shown in Fig. 3-3 and Fig. 3-4, most laboratories reported using liquid chromatography with absorbance detection (LC-Abs) or photodiode array (PDA) detection for vitamin B_{12} mass fraction determination (11 of 14 laboratories, 79 %). One laboratory each reported using microbiological assay, liquid chromatography with ICP-MS, and LC-MS (7 % each). No trends related to analytical method could be identified.

For both nutritional formula samples, the consensus ranges for measured mass fraction values overlap the target mass fraction ranges and the widths of the consensus ranges are comparable in width to the target ranges.

The quality of vitamin B_{12} results in nutritional formula samples was highly dependent on the selection of specific parameters during sample preparation. Laboratories that reported measured mass fraction values above the target value did so consistently in these two very similar samples (Fig. 3-5). In most cases, laboratories reporting mass fraction values above the target range did not incorporate a sample cleanup step which may have resulted in presence of chromatographic interferences. Four laboratories reported mass fraction values an order of magnitude or more above the target values, which may indicate calculation errors as well. All calculations and results should be independently verified prior to submission to avoid reporting errors.

As with any laboratory exercise, consistent use of appropriate calibration materials and quality assurance samples to establish that a method is in control and being performed correctly may reduce the likelihood of outlying data. Quality assurance samples can be commercially available reference materials (CRMs like NIST SRMs or non-certified reference materials such as NIST RMs) or materials prepared in-house.

NIST has conducted four QAP studies involving measurement of vitamin B_{12} in food samples prior to this FNSQAP study: DSQAP Exercise F in 2010 [9] and Exercise 1 in 2021 [10], and HAMQAP Exercise 1 in 2017 [11] and Exercise 4 in 2019 [12]. Other than DSQAP Exercise 1, the average participation rate in this FNSQAP study (71 %) was increased compared to previous studies (46 % to 61 %). The average mass fraction repeatability reported by laboratories in this study (6.1 % to 8.8 %) was comparable to that in previous studies (4.8 % to 7.4 %), apart from one sample in DSQAP Exercise 1. Average mass fraction reproducibility in this study (42 %) was significantly higher than in almost all previous studies (15 %, 98 %, 12 %, and 28 % for DSQAP Exercise F, HAMQAP Exercise 1, HAMQAP Exercise 4, and DSQAP Exercise 1, respectively). All samples used in previous studies were also fortified with vitamin B_{12}, but the mass fraction of vitamin B_{12} present in the samples used in the current study were significantly lower than used in previous studies, which may have contributed to the higher among-laboratory variability.

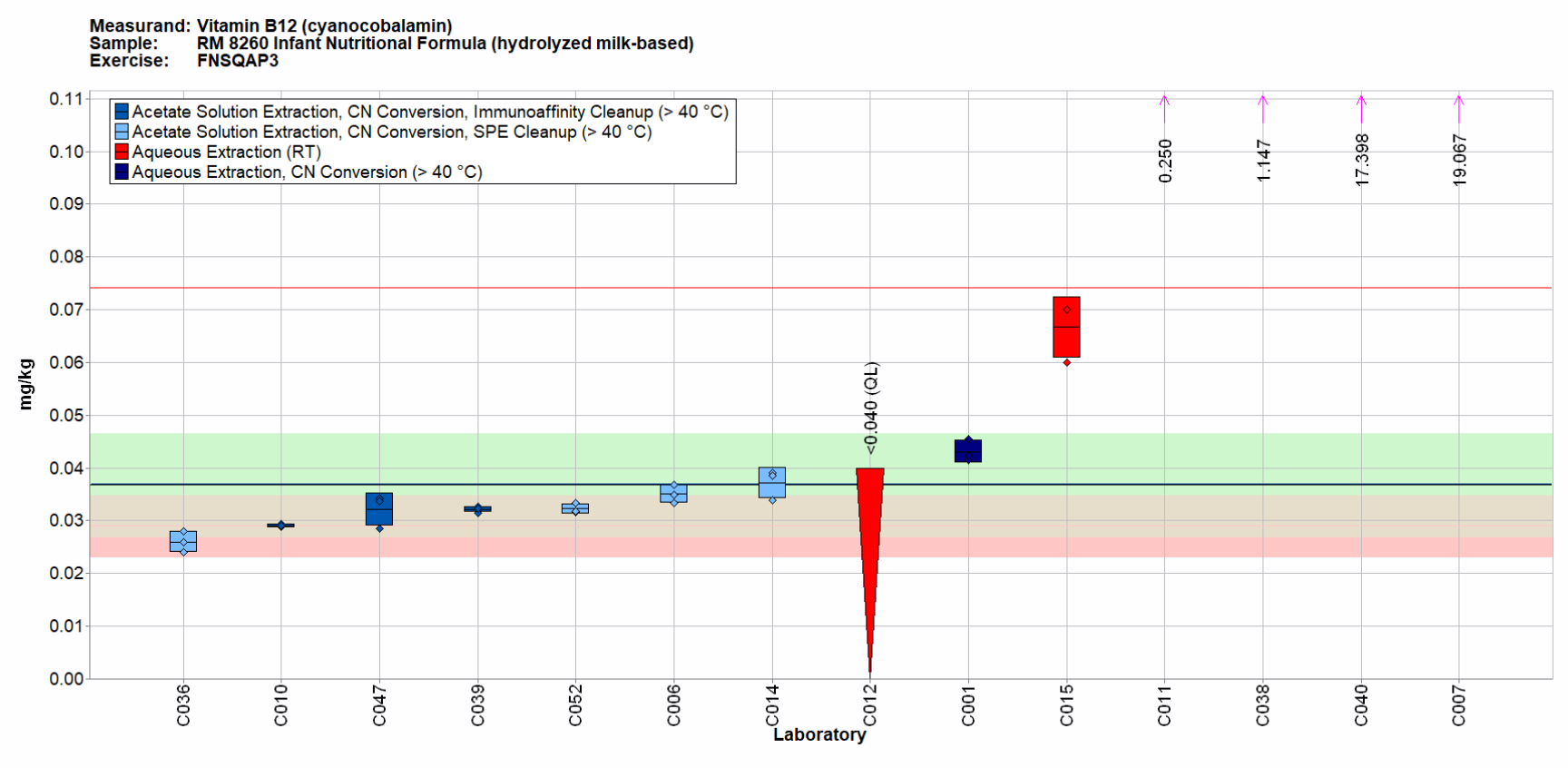

Fig. 3-1. Vitamin B_{12} in RM 8260 (data summary view – sample preparation method).

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. Data points outside the graphical range are represented as upward arrows (sample preparation approaches reported by laboratories C011, C038, C040, and C007 were Acetate Solution Extraction, CN Conversion, Immunoaffinity Cleanup (> 40 °C); Aqueous Extraction (RT); Aqueous Extraction (25 °C to 40 °C); and Solvent Extraction (RT), respectively). The solid black 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 values above the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$, with the bottom of the range set to zero. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty (U_{NIST}), and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$. The beige shaded region represents the overlapping of the 95 % confidence interval for the consensus mean (green region) and the NIST range of tolerance (red region).

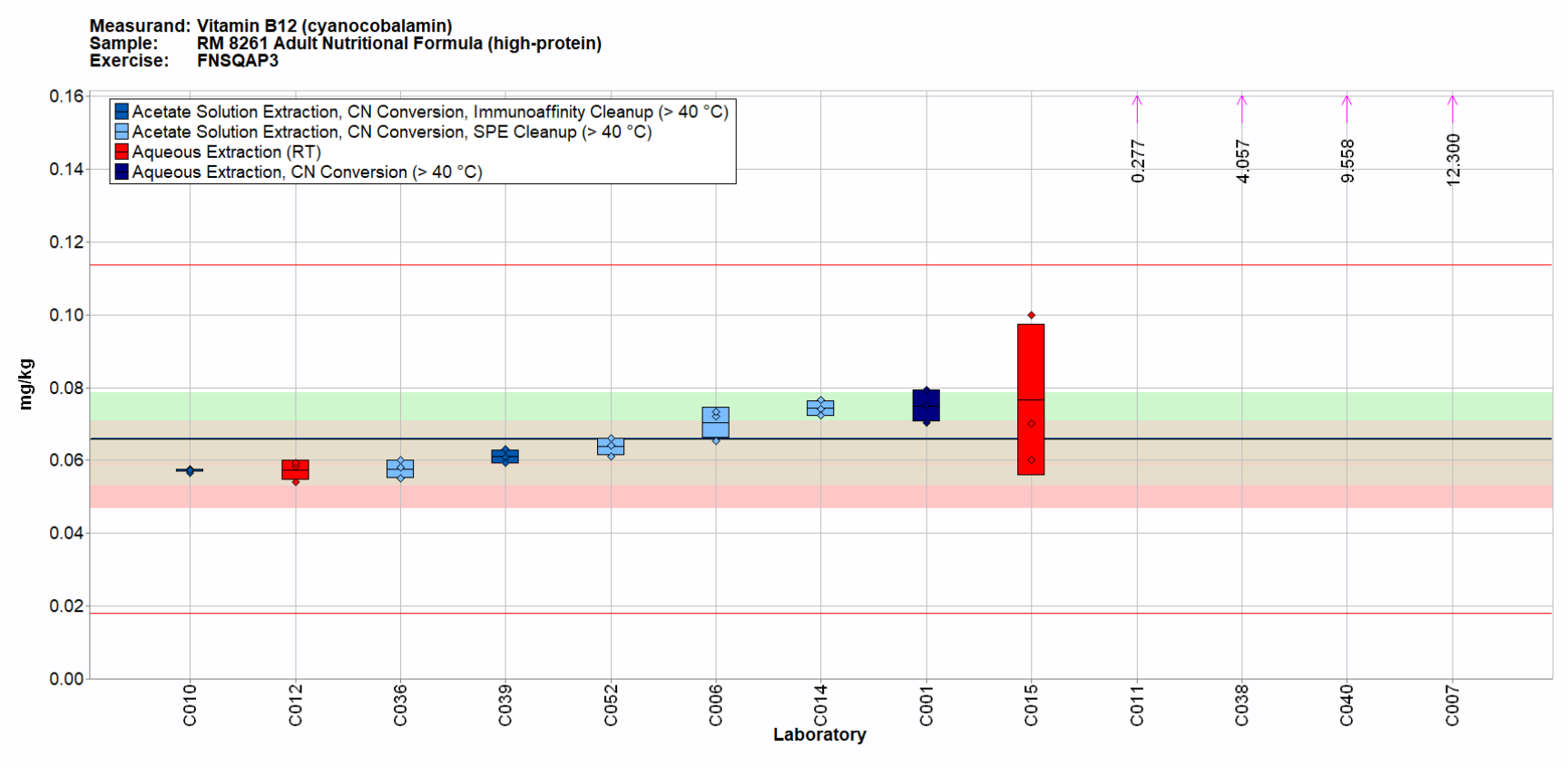

Fig. 3-2. Vitamin B_{12} in RM 8261 (data summary view – sample preparation method).

In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed as indicated in the figure key. Data points outside the graphical range are represented as upward arrows (sample preparation approaches reported by laboratories C011, C038, C040, and C007 were Acetate Solution Extraction, CN Conversion, Immunoaffinity Cleanup (> 40 °C); Aqueous Extraction (RT); Aqueous Extraction (25 °C to 40 °C); and Solvent Extraction (RT), respectively). The solid black 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 beige shaded region represents the overlapping of the 95 % confidence interval for the consensus mean (green region) and the NIST range of tolerance (red region).

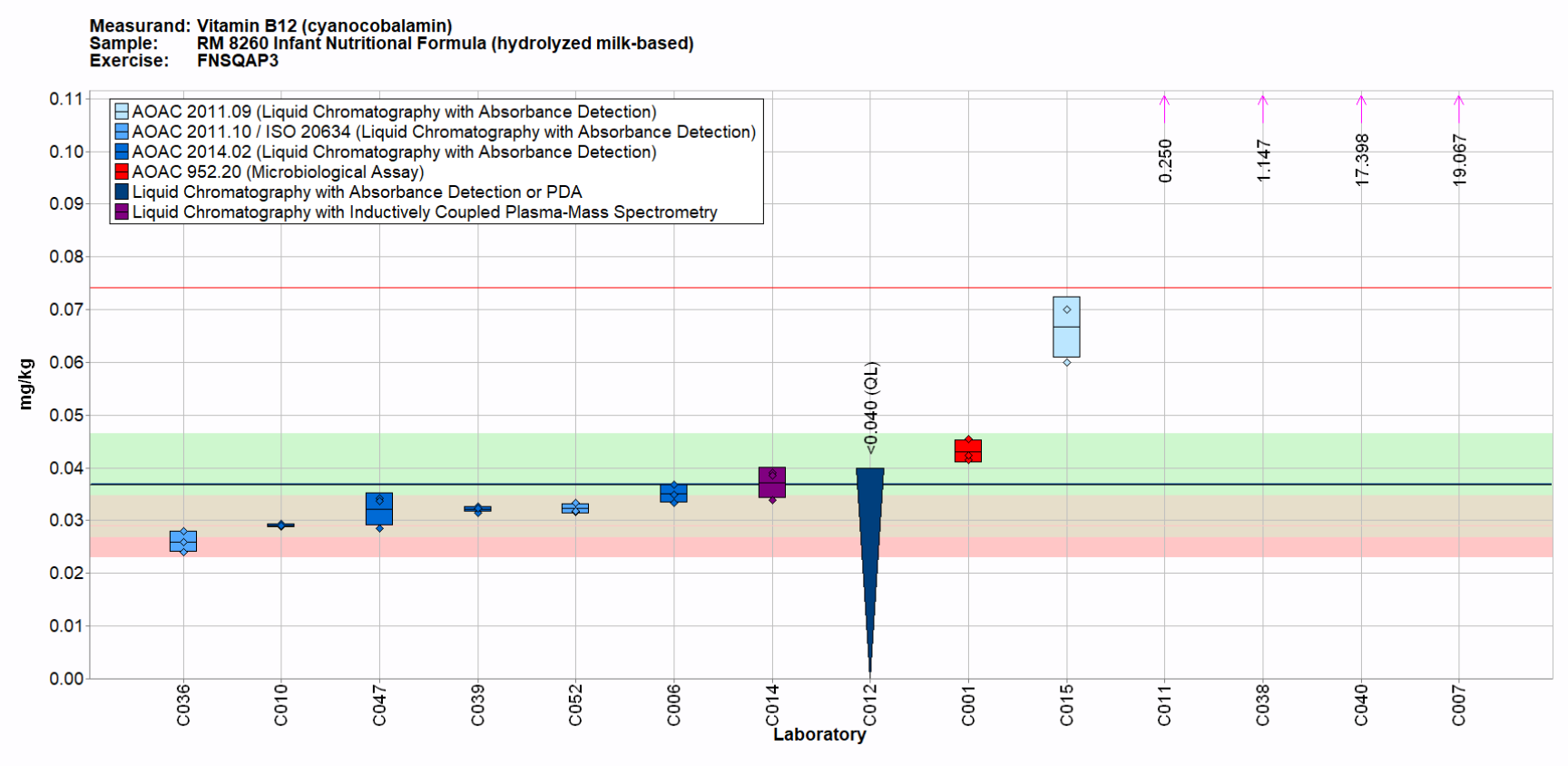

Fig. 3-3. Vitamin B_{12} in RM 8260 (data summary view – analytical method).

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. Data points outside the graphical range are represented as upward arrows (the analytical method reported by laboratory C011 was AOAC 2014.02 (LC-Abs); laboratory C038 reported using LC-MS; and laboratories C040 and C007 reported using LC-Abs or PDA). The solid black 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 values above the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$, with the bottom of the range set to zero. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty (U_{NIST}), and represents the range that results in an acceptable Z_{NIST} score, $|Z_{\text{NIST}}| \leq 2$. The beige shaded region represents the overlapping of the 95 % confidence interval for the consensus mean (green region) and the NIST range of tolerance (red region).

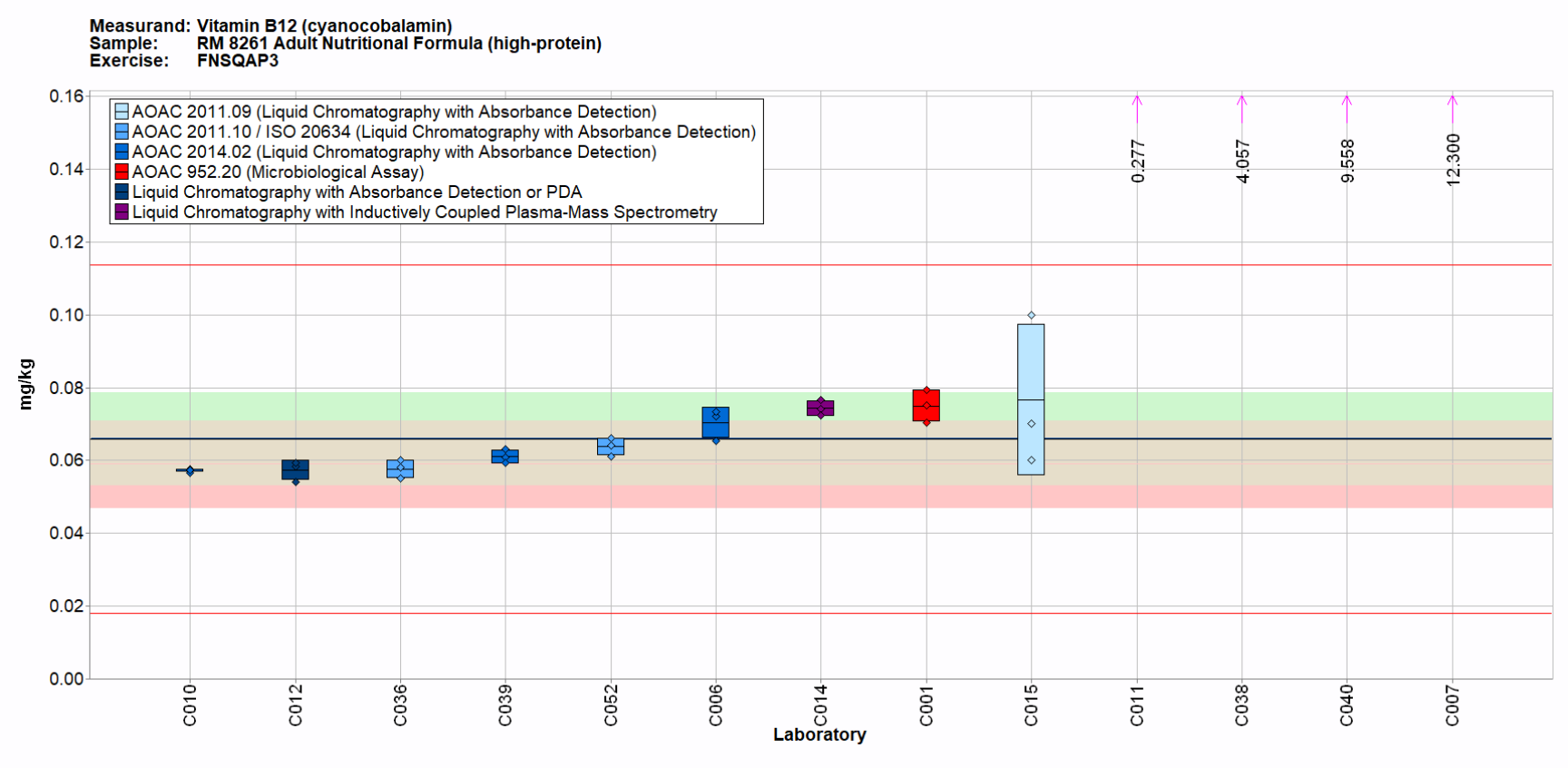

Fig. 3-4. Vitamin B_{12} in RM 8261 (data summary view – analytical method).

In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed as indicated in the figure key. Data points outside the graphical range are represented as upward arrows (the analytical method reported by laboratory C011 was AOAC 2014.02 (LC-Abs); laboratory C038 reported using LC-MS; and laboratories C040 and C007 reported using LC-Abs or PDA). The solid black 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 value above 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 beige shaded region represents the overlapping of the 95 % confidence interval for the consensus mean (green region) and the NIST range of tolerance (red region).

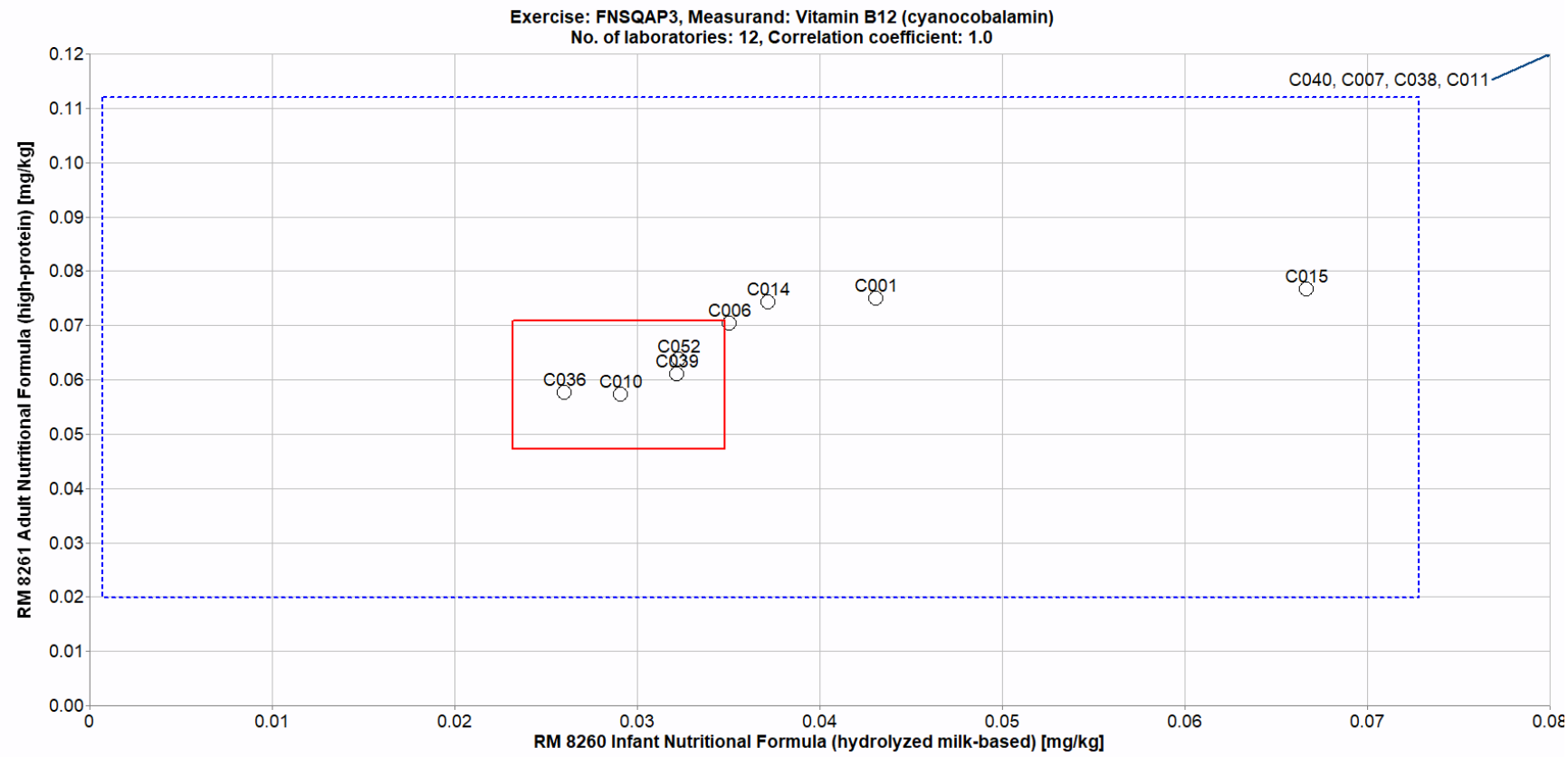

Fig. 3-5. Laboratory means for vitamin B_{12} in RM 8260 and RM 8261 (sample/sample comparison view).

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

4. FAT-SOLUBLE VITAMINS (Vitamin A)

4.1. Executive Summary

Vitamin A compounds are important for infant development and growth, and the fortified levels of vitamin A components in infant foods are strictly regulated worldwide. Overall participation in this study was good, and participating laboratories reported acceptable within-laboratory variability and improved among-laboratory variability compared to previous NIST QAP studies. However, several laboratories were challenged by nuances of analytical methods for vitamin A determination and the subsequent calculations required prior to reporting results. Additionally, the overall accuracy of current methodology may be insufficient, as the consensus means were biased low compared to target values for all analyte/sample pairs for which target values were available.

4.2. Study Overview

Vitamin A is a group of compounds that support immune function as well as cellular communication, growth, and development including formation of numerous organs [13]. Sufficient consumption of vitamin A has been connected to reduced mortality rates from measles, while high oral and topical doses of vitamin A have been linked to the incidence of birth defects [13]. Accurately understanding the intake and corresponding health outcomes related to vitamin A through measurement in infant and other nutritional formulas can inform future decisions about recommended dietary intakes. In this study, participants were provided with two nutritional formula samples, RM 8260 Infant Nutritional Formula (hydrolyzed milk-based) and RM 8261 Adult Nutritional Formula (high protein). Participants were asked to use in-house analytical methods to determine the mass fractions (mg/kg) of retinol, retinyl acetate, retinyl palmitate, and total vitamin A in the nutritional formula samples. Through participation in this study, laboratories can better understand the performance of their in-house methods relative to those being used by others in the community. Participant results may be used in the value assignment of NIST reference materials included as samples in this study.

4.3. Sample Information

Participants were provided with three packets each of RM 8260 Infant Nutritional Formula (hydrolyzed milk-based) (labeled Infant Formula A) and RM 8261 Adult Nutritional Formula (high protein) (labeled Infant Formula B). Each packet contained approximately 10 g of material; participants were asked to store the materials at controlled room temperature (20 °C to 25 °C) in the original unopened packets and to prepare one sample and report one value per analyte 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 to ensure homogeneity of the test portion used for the determination of vitamin A. The approximate analyte mass fraction levels were not reported to participants prior to the study. The target mass fraction values for vitamin A compounds in each material were as described in

their respective RMISs [3, 4]. The target mass fraction values and uncertainties for total vitamin A in RM 8260 and for retinyl acetate, retinyl palmitate, and total vitamin A in RM 8261 are provided in Table 4-1 on an as-received basis. The uncertainties for vitamin A in RM 8260 and RM 8261 were approximated as 10 % relative to the measured value. While RM 8261 is known to contain both retinyl acetate and retinyl palmitate, the forms of vitamin A used in the preparation of RM 8260 were not known prior to this study.

Table 4-1. Individualized data summary table for vitamin A compounds 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 3 – Fat-Soluble Vitamins in Infant Formulas

	Lab Code:	**(Code)**	**1. Your Results**				**2. Community Results**			**3. Target**	
Analyte	Sample	Units	x_i	s_i	Z'_{comm}	Z_{NIST}	N	x*	s*	x_{NIST}	u_{NIST}
Retinol	RM 8260	mg/kg	*Individual laboratory results will appear in this section; laboratory-specific results were provided to each participant separately from this report.*				7	5.9	2.0		
Retinol	RM 8261	mg/kg					7	4.1	1.0		
Retinyl Acetate	RM 8260	mg/kg					4	6.6	1.8		
Retinyl Acetate	RM 8261	mg/kg					4	0.72	0.16	1.20	0.12
Retinyl Palmitate	RM 8260	mg/kg					1				
Retinyl Palmitate	RM 8261	mg/kg					4	6.80	1.90	9.30	0.93
Total Vitamin A	RM 8260	mg/kg					6	6.10	0.81	7.40	0.74
Total Vitamin A	RM 8261	mg/kg					7	4.40	0.84	6.00	0.60

x_i	Mean of reported values	N	Number of quantitative values reported, not including single-value submissions	x_{NIST}	NIST-assessed value
s_i	Standard deviation of reported values			u_{NIST}	standard uncertainty about the NIST-assessed value
Z'_{comm}	Z'-score with respect to community consensus	x*	Robust mean of reported values		
Z_{NIST}	Z-score with respect to NIST value	s*	Robust standard deviation		

4.4. Study Results and Discussion

Overall participation in this study was high, with 11 of 18 laboratories requesting samples returning results for at least one form of vitamin A (61 %).

The results for each analyte in this study will be discussed in the subsequent sections. For all analytes, the performance of laboratories is compared to published expectations in AOAC SMPR 2011.003 [14]. In this SMPR, characteristics of suitable methods for determination of vitamin A compound mass fractions in infant formula and adult/pediatric nutritional formulas are described, including repeatability of ≤ 8 % and reproducibility of ≤ 16 %. Reproducibility in this context describes the among-laboratory variability for several laboratories using the same method but can be useful to understand overall community performance.

To correlate trends between reported results and particular sample preparation approaches or analytical methods, information from method questionnaires completed prior to sample distribution and from method information provided upon submission of results were used.

Detailed review of this information was used to provide insight into important method parameters that correlated to laboratory performance, when possible.

4.4.1. Retinol

Table 4-1 summarizes and Table 4-2 details the measured mass fraction results reported by each participating laboratory for retinol. The participation level was good for retinol in this study, with 47 % of laboratories requesting samples returning results (8 of 17 laboratories). Seven of the eight laboratories reported quantitative mass fraction results for retinol in both samples.

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

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

		Retinol									
		RM 8260 Infant Nutritional Formula (hydrolyzed milk-based) (mg/kg)					RM 8261 Adult Nutritional Formula (high-protein) (mg/kg)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target										
	C005	7.359	7.378	7.418	7.4	0.0	4.8	4.687	4.769	4.8	0.1
	C006										
	C010	6.794	6.806	6.807	6.8	0.0	4.843	4.739	4.864	4.8	0.1
	C012	< 10.00	< 10.00	< 10.00			< 10.00	< 10.00	< 10.00		
	C015										
	C016										
	C017	58.3	74.1	103	78.5	22.7	31.1	21	23.6	25.2	5.2
	C026	5.64	5.78	5.81	5.7	0.1	4.09	4.14	4.08	4.1	0.0
	C027	3.52	3.59	3.66	3.6	0.1	2.52	2.62	2.35	2.5	0.1
	C030										
	C033										
	C036										
	C038										
	C039	5.72	5.74	5.92	5.8	0.1	4.16	4.23	4.46	4.3	0.2
	C040										
	C047	6.23	6.24	6.1	6.2	0.1	4.31	4.19	4.29	4.3	0.1
	C049										
Community Results		Consensus Mean			5.9		Consensus Mean			4.1	
		Consensus Standard Deviation			2.0		Consensus Standard Deviation			1.0	
		Maximum			78.5		Maximum			25.2	
		Minimum			3.6		Minimum			2.5	
		N			7		N			7	

Table 4-2 reveals that for 6 of the 7 participants reporting quantitative mass fraction results, the within-laboratory variabilities were acceptable (≤ 8 %) [14]. One laboratory reported within-laboratory variability of over 20 % for each sample, and one laboratory reported that the mass

fraction of retinol in these samples was below their method limit of quantitation of 10 mg/kg. Both laboratories should review their procedures to ensure that retinol can be determined with sufficient precision (≤ 8 %) in nutritional formula samples at the level established by the community (0.56 mg/kg) [14]. The among-laboratory mass fraction variabilities reported for RM 8260 and RM 8261 were high (33 % and 25 %, respectively) compared to the published expectations (≤ 16 %) [14], but the variability is likely the result of the diversity of methods used by the participants.

Submitted method information was used to categorize results in the legends of Fig. 4-1 and Fig. 4-2. The key differences described by laboratories were 1) use of base during extraction to hydrolyze retinyl esters and 2) the extraction temperature (e.g., room temperature, 25 °C to 40 °C, > 40 °C). Most laboratories reported using base hydrolysis with liquid-liquid extraction (LLE) (75 %) and one laboratory each reported use of enzymatic hydrolysis with LLE and LLE without hydrolysis (13 % each). The three laboratories reporting retinol mass fraction values closest to the mean reported high temperature extraction (> 40 °C) while lower temperatures were used by all laboratories reporting mass fraction values in the upper half of the data set. These observations indicate the importance of high-temperature hydrolysis for the accurate determination of retinol mass fractions in nutritional formulas. One laboratory reported mass fraction values an order of magnitude higher than the consensus values in both samples, likely due to a miscalculated dilution factor. All calculations and results should be independently verified prior to submission to avoid reporting errors.

As shown in Fig. 4-3 and Fig. 4-4, most laboratories reported using LC-Abs or PDA for determination of retinol mass fractions (6 of 8 laboratories, 75 %). No specific information about monitored wavelengths or wavelength ranges was available. Two laboratories reported using LC-MS (25 %). Because the two laboratories using LC-MS reported use of an external standard calibration approach and results that were either qualitative or outlying, no trends related to analytical method could be identified.

The performance of laboratories in the determination of retinol mass fractions in nutritional formula samples was dependent on the selection of specific parameters during sample preparation. Laboratories that reported mass fraction values above or below the consensus mean did so consistently in these two very similar samples (Fig. 4-5). In most cases, laboratories reporting mass fraction values above the consensus mean utilized a lower temperature extraction procedure or did not include a base hydrolysis step. One laboratory reported mass fraction values an order of magnitude above the consensus values, which may indicate calculation errors. All calculations should be independently verified prior to submission to avoid reporting errors. Laboratories reporting method LOQs above 0.56 mg/kg should consider reevaluating their method performance characteristics, as the nutritional formula community has indicated that analytical methods must be able to quantify retinol at 0.56 mg/kg [14].

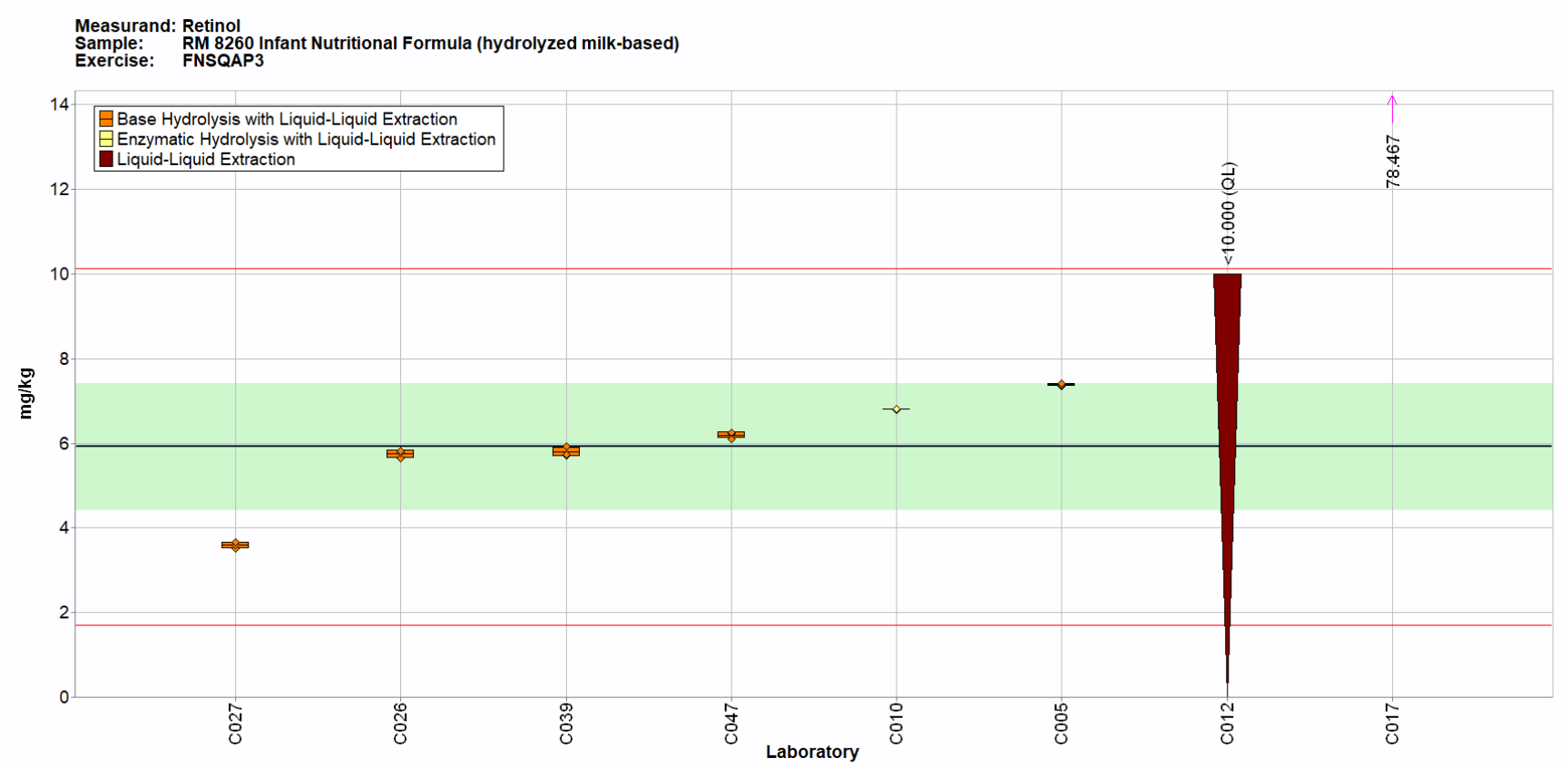

Fig. 4-1. Retinol in RM 8260 (data summary view – sample preparation method).

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. Data points outside the graphical range are represented as upward arrows (the sample preparation method reported by laboratory C017 was base hydrolysis with LLE). The solid black 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, $|Z'_{\text{comm}}| \leq 2$.

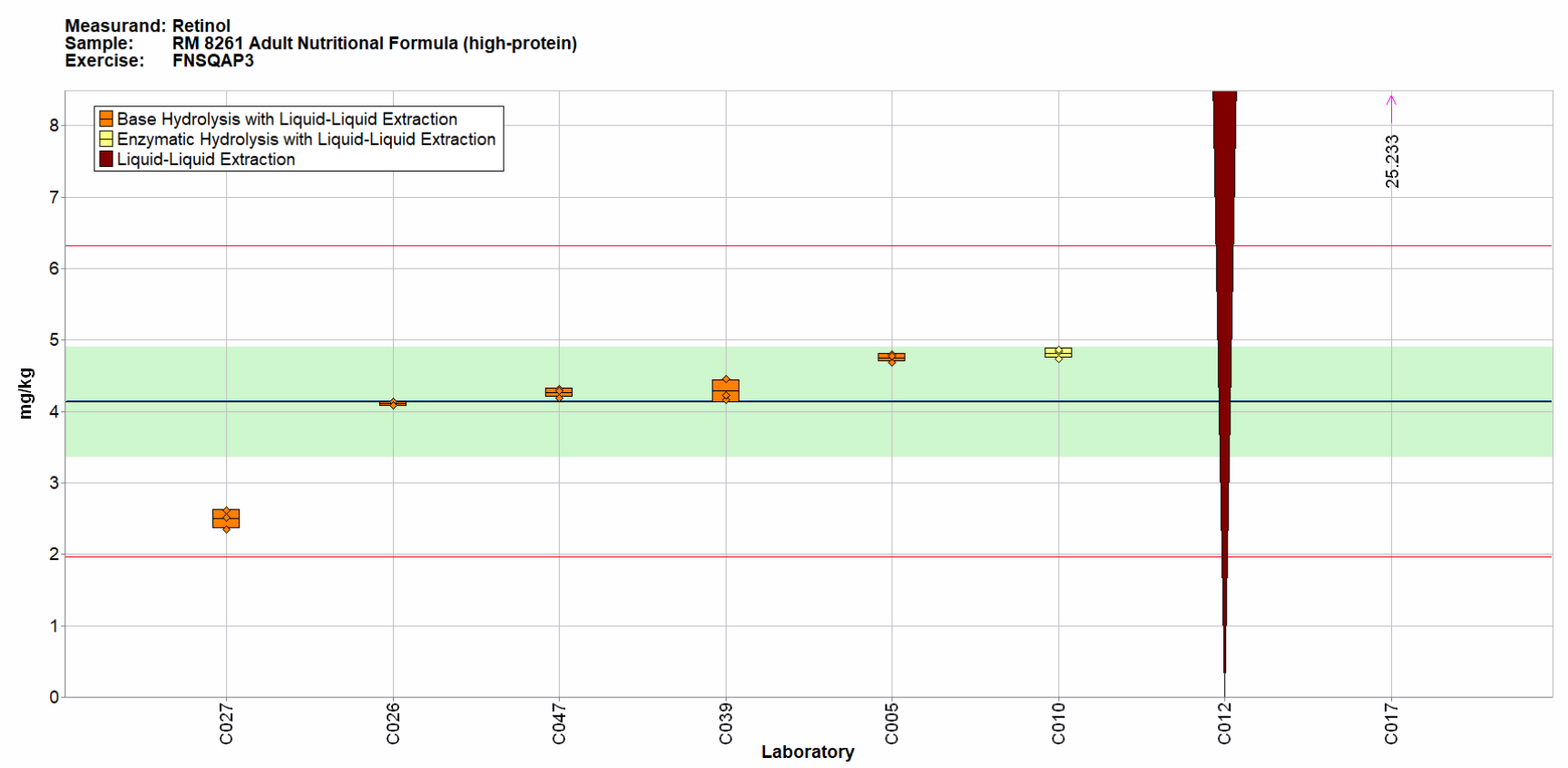

Fig. 4-2. Retinol in RM 8261 (data summary view – sample preparation method).

In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). A downward triangle represents data reported as an LOQ value. The color of the data point represents the sample preparation method employed as indicated in the figure key. Data points outside the graphical range are represented as upward arrows (the sample preparation method reported by laboratory C017 was base hydrolysis with LLE). The solid black 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, $|Z'_{\text{comm}}| \leq 2$.

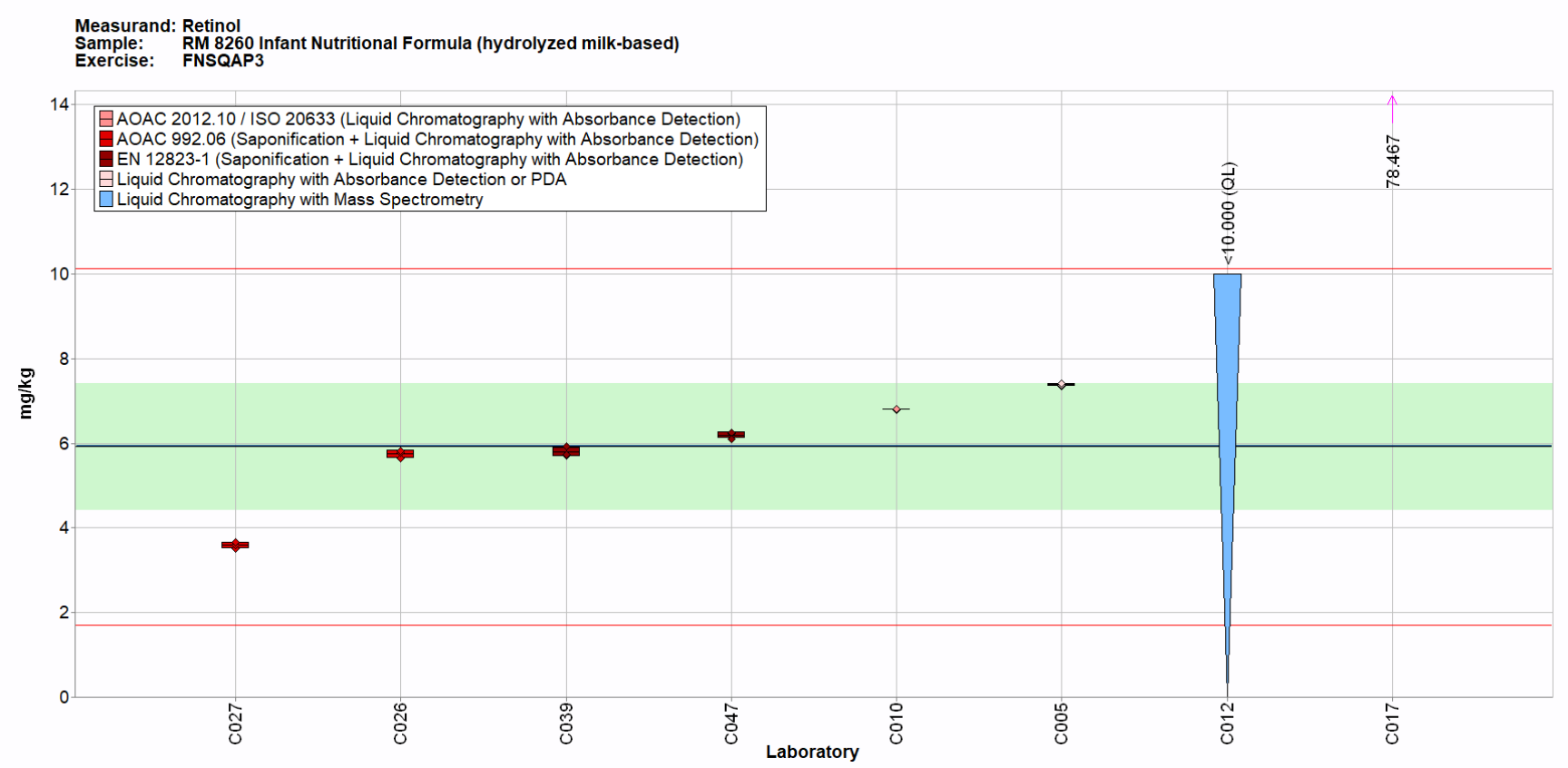

Fig. 4-3. Retinol in RM 8260 (data summary view – analytical method).

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. Data points outside the graphical range are represented as upward arrows (the analytical method reported by laboratory C017 was LC-MS). The solid black 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, $|Z'_{\text{comm}}| \leq 2$.

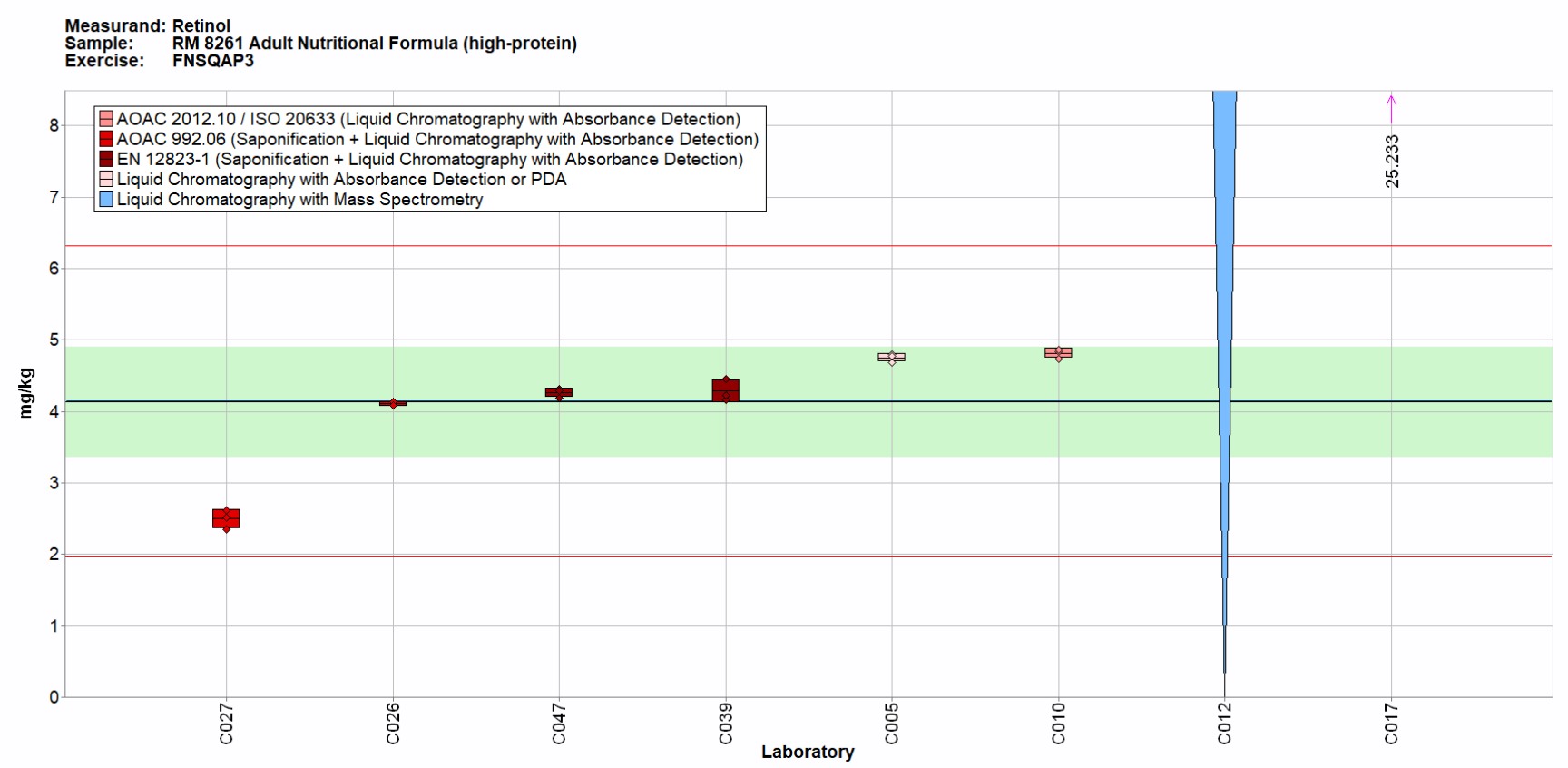

Fig. 4-4. Retinol in RM 8261 (data summary view – analytical method).

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 C017 was LC-MS). The solid black 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, $|Z'_{\text{comm}}| \leq 2$.

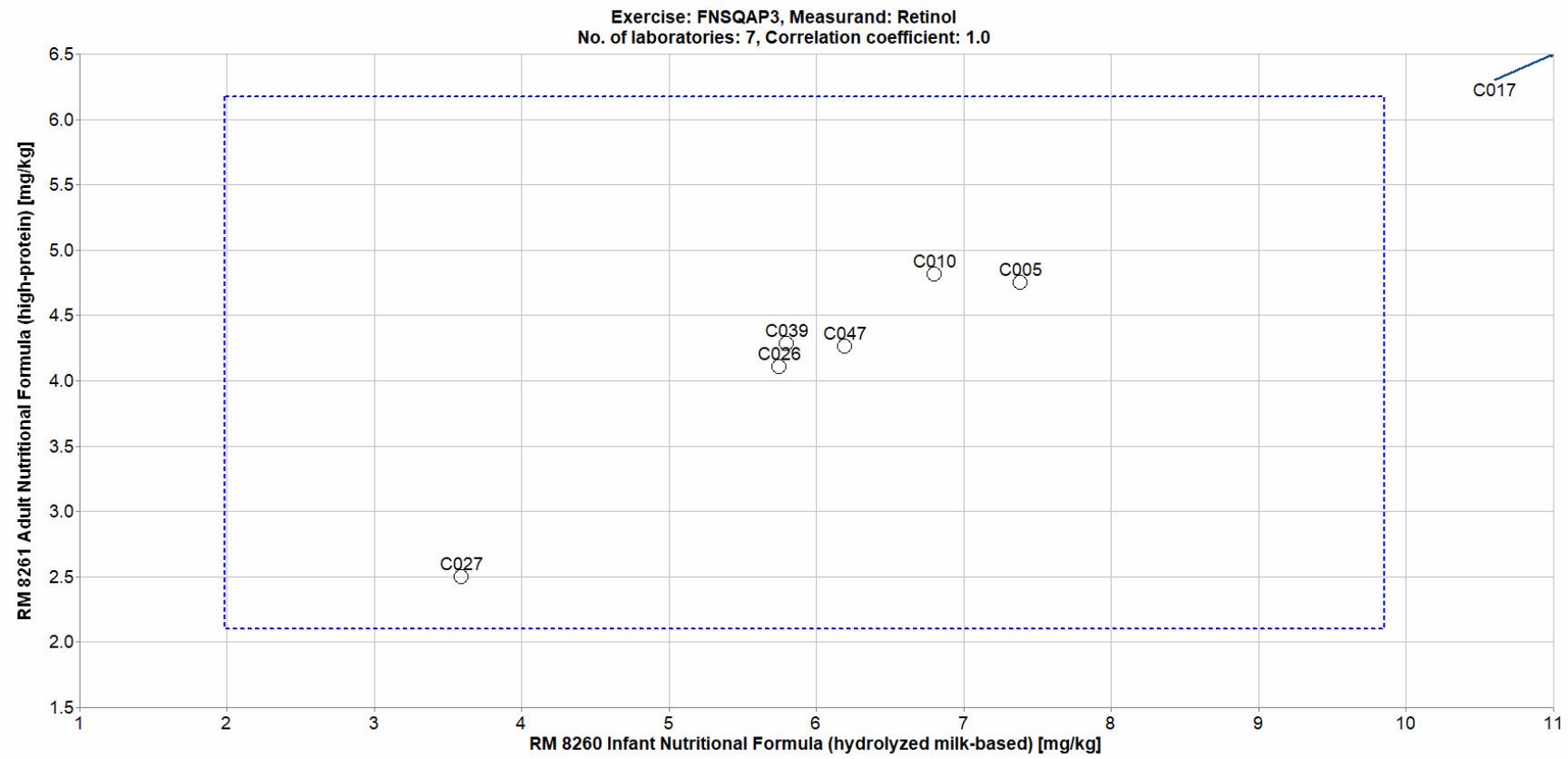

Fig. 4-5. Laboratory means for retinol in RM 8260 and RM 8261 (sample/sample comparison view).

In this view, the individual laboratory mean for one sample (RM 8260) is compared to the individual laboratory mean for a second sample (RM 8261). The dotted blue box represents the consensus range of tolerance for RM 8260 (x-axis) and RM 8261 (y-axis), calculated as the values above and below the consensus means that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$.

4.4.2. Retinyl Acetate

Table 4-1 summarizes and Table 4-3 details the measured mass fraction results reported by each participating laboratory for retinyl acetate. The participation level was poor for retinyl acetate in this study, with only 25 % of laboratories requesting samples returning results (4 of 16 laboratories).

Table 4-3. Data summary table for retinyl acetate in RM 8260 and RM 8261.

		Retinyl Acetate									
		RM 8260 Infant Nutritional Formula (hydrolyzed milk-based) (mg/kg)					RM 8261 Adult Nutritional Formula (high-protein) (mg/kg)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target									1.20	0.12
	C006	7.3165	6.9689	7.1479	7.1	0.2	0.8674	0.8806	0.8554	0.87	0.01
	C010	7.809	7.823	7.824	7.8	0.0	0.903	0.881	0.901	0.90	0.01
	C012	5.34	5.58	5.31	5.4	0.1	0.52	0.5	0.57	0.53	0.04
	C015										
	C016										
	C017										
	C026										
	C027	6.14	6.22	6.3	6.2	0.1	0.61	0.59	0.58	0.59	0.02
	C030										
	C033										
	C036										
	C038										
	C039										
	C040										
	C047										
	C049										
Community Results		Consensus Mean			6.6		Consensus Mean			0.72	
		Consensus Standard Deviation			1.8		Consensus Standard Deviation			0.16	
		Maximum			7.8		Maximum			0.90	
		Minimum			5.4		Minimum			0.53	
		N			4		N			4	

Table 4-3 reveals that the within-laboratory mass fraction variabilities were acceptable with respect to published expectations (≤ 8 %) [14]. The among-laboratory mass fraction variabilities reported for RM 8260 and RM 8261 were high (28 % and 21 %, respectively) compared to the published expectations (≤ 16 %) [14], but the high variability is likely the result of the diversity of methods used by the participants.

Submitted method information was used to categorize results in the legends of Fig. 4-6 and Fig. 4-7. The key differences described by laboratories were 1) use of enzyme during extraction

to release retinyl acetate from hydrophilic protein coated fat micelles that form in the reconstituted nutritional formula prior to extraction and 2) the extraction temperature (e.g., room temperature, 25 °C to 40 °C, > 40 °C). Most laboratories reported using enzymatic hydrolysis with LLE (75 %) and one laboratory reported use of LLE without hydrolysis (25 %). The three laboratories using enzymatic hydrolysis did so at elevated temperature (25 °C to 40 °C), which facilitates release of retinyl acetate from the matrix. For this reason, the laboratory using LLE at room temperature reported lower retinyl acetate mass fraction results.

As shown in Fig. 4-8 and Fig. 4-9, most laboratories reported using LC-Abs for retinyl acetate mass fraction determination (75 %). One laboratory reported using LC-MS (25 %). With a limited number of data points, no trends related to analytical method performance could be identified.

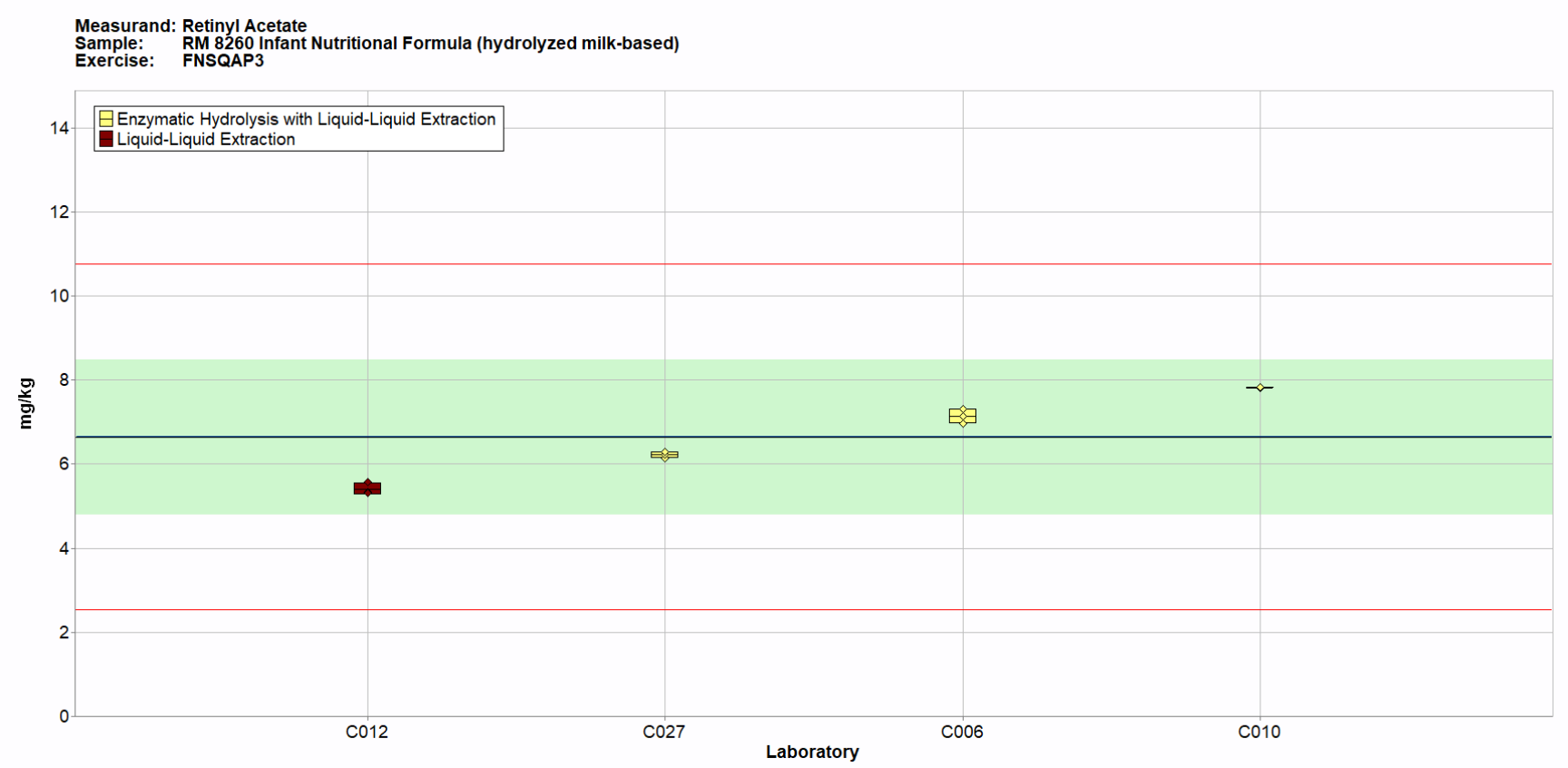

Fig. 4-6. Retinyl acetate in RM 8260 (data summary view – sample preparation method).

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 black 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, $|Z'_{\mathrm{comm}}| \leq 2$.

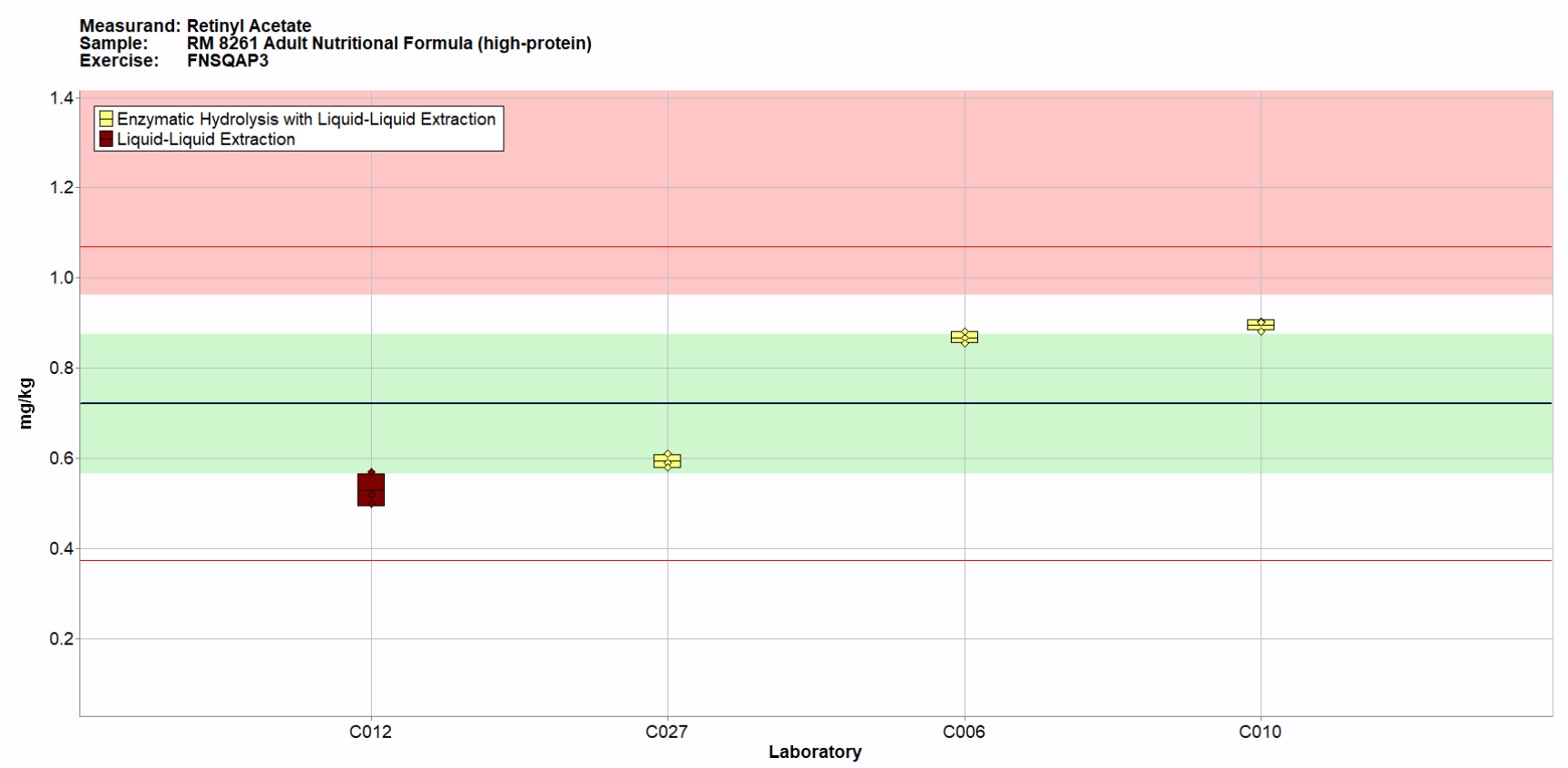

Fig. 4-7. Retinyl acetate in RM 8261 (data summary view – sample preparation method).

In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed as indicated in the figure key. The solid black 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, $|Z_{\mathrm{NIST}}| \leq 2$.

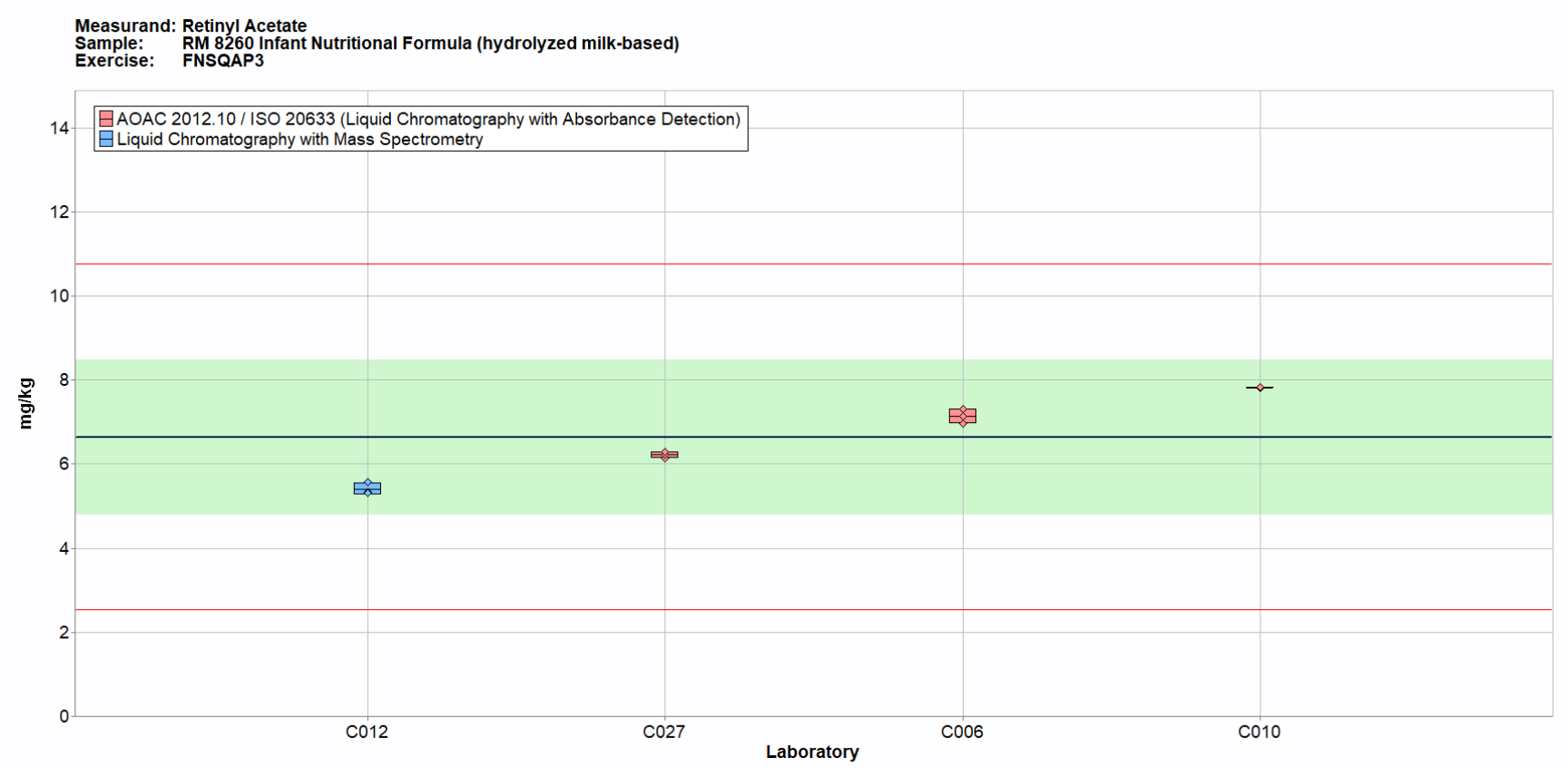

Fig. 4-8. Retinyl acetate in RM 8260 (data summary view – analytical method).

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 black 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, $|Z'_{\text{comm}}| \leq 2$.

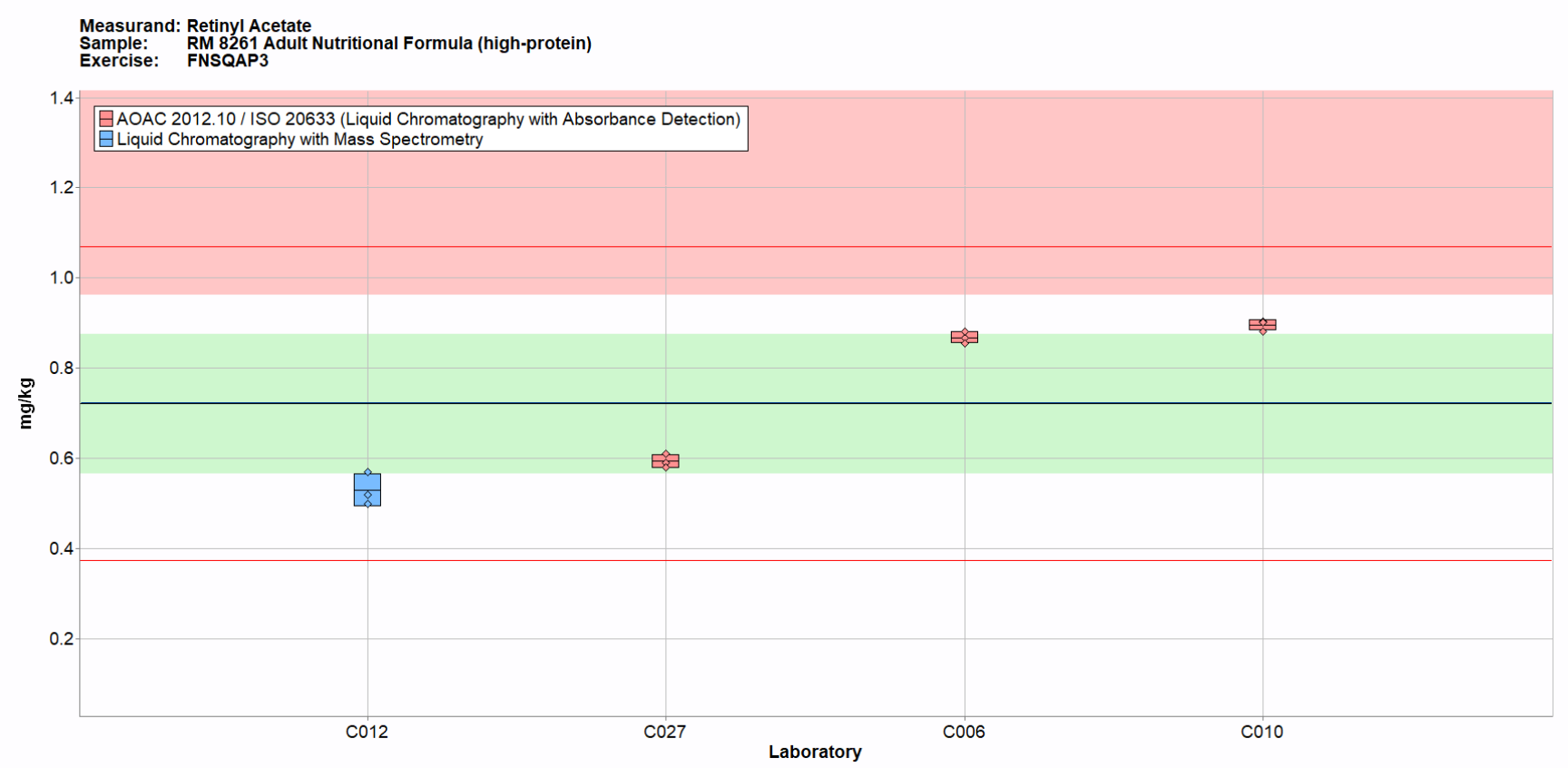

Fig. 4-9. Retinyl acetate in RM 8261 (data summary view – analytical method).

In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed as indicated in the figure key. The solid black 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, $|Z_{\mathrm{NIST}}| \leq 2$.

4.4.3. Retinyl Palmitate

Table 4-1 summarizes and Table 4-4 details the measured mass fraction results reported by each participating laboratory for retinyl palmitate. The participation level was low for retinyl palmitate in this study, with only 31 % of laboratories requesting samples returning results (5 of 16 laboratories). For RM 8260, only one laboratory reported quantitative results, indicating that the mass fraction of retinyl palmitate in this material was below the limit of quantitation for the methods of most participants. For RM 8261, 4 of 5 laboratories reported quantitative mass fraction results.

Table 4-4. Data summary table for retinyl palmitate in RM 8260 and RM 8261.

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

		Retinyl Palmitate									
		RM 8260 Infant Nutritional Formula (hydrolyzed milk-based) (mg/kg)					RM 8261 Adult Nutritional Formula (high-protein) (mg/kg)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target									9.3	0.9
	C006	< 0.160	< 0.160	< 0.160			7.0063	6.9791	6.9705	7.0	0.0
	C010	< 0.020	< 0.020	< 0.020			7.377	7.224	7.42	7.3	0.1
	C012	< 10.000	< 10.000	< 10.000			< 10.000	< 10.000	< 10.000		
	C015	13.4	12.3	13.7	13.13	0.74	56.4	53.1	54.3	54.6	1.7
	C016										
	C017										
	C026										
	C027	< 0.069	< 0.069	< 0.069			6.11	6.28	6.02	6.1	0.1
	C030										
	C033										
	C036										
	C038										
	C039										
	C040										
	C047										
	C049										
Community Results		Consensus Mean					Consensus Mean			6.8	
		Consensus Standard Deviation					Consensus Standard Deviation			1.9	
		Maximum			13.13		Maximum			54.6	
		Minimum			13.13		Minimum			6.1	
		N			1		N			4	

Table 4-4 reveals that within-laboratory mass fraction variabilities for all results were acceptable with respect to published expectations (≤ 8 %) [14]. For RM 8261, the only sample with multiple laboratories reporting quantitative mass fraction results, among-laboratory variability was high

(28 %) compared to the published expectations (≤ 16 %) [14], but the high variability is likely the result of the diversity of methods used by the participants. Three of the four laboratories reporting mass fractions were below their method LOQ for retinyl palmitate have sufficiently low quantitation limits to meet the needs of the nutritional formula community (0.56 mg/kg) [14]. The laboratory reporting an LOQ of 10 mg/kg should review their procedures to ensure that retinol can be determined in nutritional formula samples at the level established by the community.

Submitted method information was used to categorize results in the legend of Fig. 4-10. The key difference described by laboratories was use of enzyme to release retinyl palmitate from hydrophilic protein coated fat micelles in the reconstituted nutritional formula prior to or during extraction. Two laboratories reported using enzymatic hydrolysis with LLE (40 %), one laboratory reported using base hydrolysis with LLE (20 %), and two laboratories reported using LLE without hydrolysis (40 %). One laboratory using LLE without hydrolysis reported mass fraction values an order of magnitude higher than the consensus value, likely due to a miscalculated dilution factor. All calculations and results should be independently verified prior to submission to avoid reporting errors. With the small number of reported results and methods used, in addition to laboratories reporting outlying values and values below LOQ, no conclusions can be drawn related to method performance.

As shown in Fig. 4-11, most laboratories reported using LC-Abs for retinyl palmitate mass fraction determination (4 of 5 laboratories, 80 %). One laboratory reported using LC-MS (20 %). No trends related to analytical method could be identified.

The performance of laboratories in the determination of retinyl palmitate mass fractions in nutritional formula samples was dependent on the selection of specific parameters during sample preparation. One laboratory reported mass fraction values an order of magnitude above the consensus values, which may indicate calculation errors. All calculations should be independently verified prior to submission to avoid reporting errors.

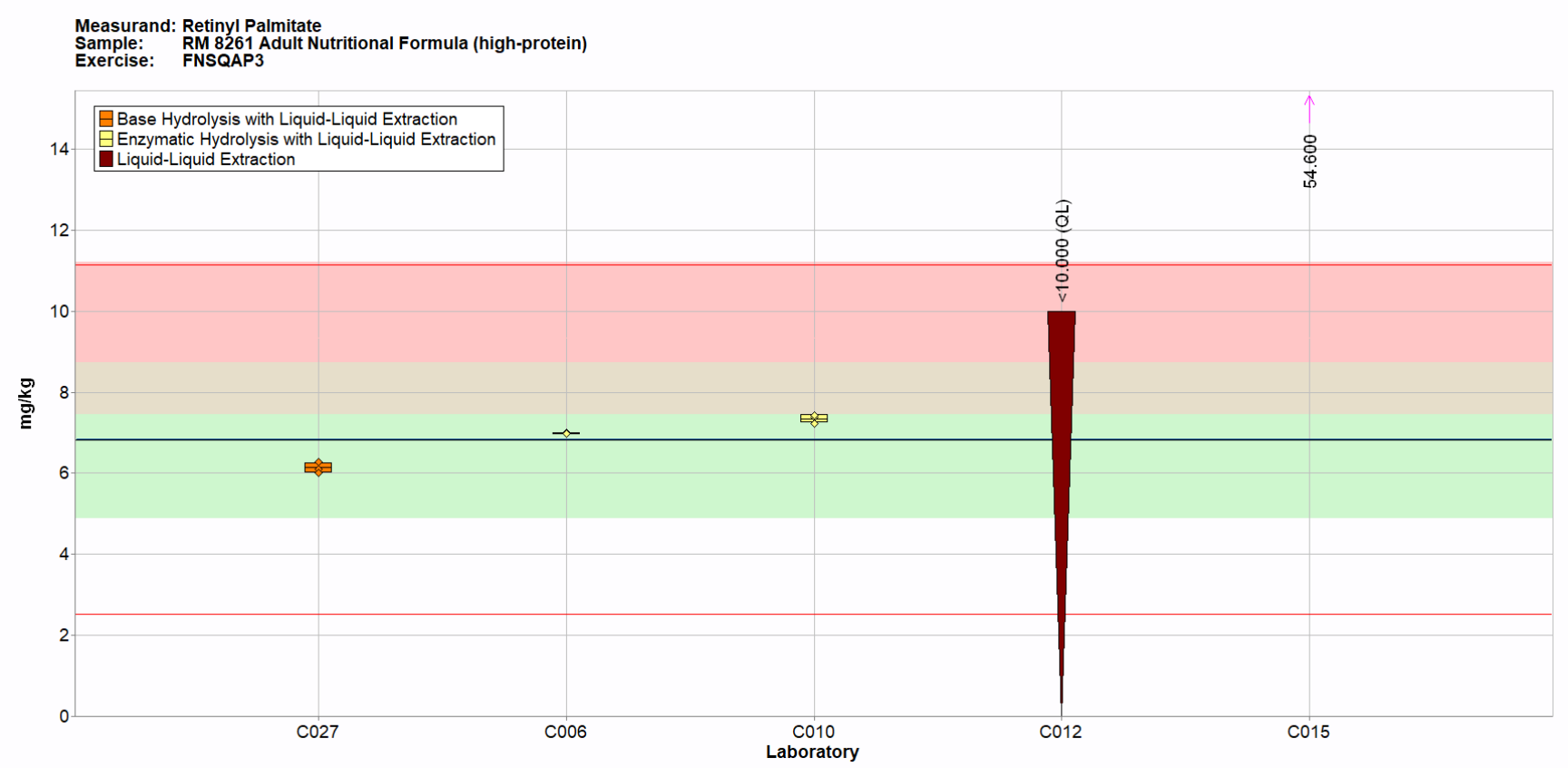

Fig. 4-10. Retinyl palmitate in RM 8261 (data summary view – sample preparation method).

In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). A downward triangle represents data reported as an LOQ value. The color of the data point represents the sample preparation method employed as indicated in the figure key. Data points outside the graphical range are represented as upward arrows (the sample preparation method reported by laboratory C015 was LLE). The solid black 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 beige shaded region represents the overlapping of the 95 % confidence interval for the consensus mean (green region) and the NIST range of tolerance (red region).

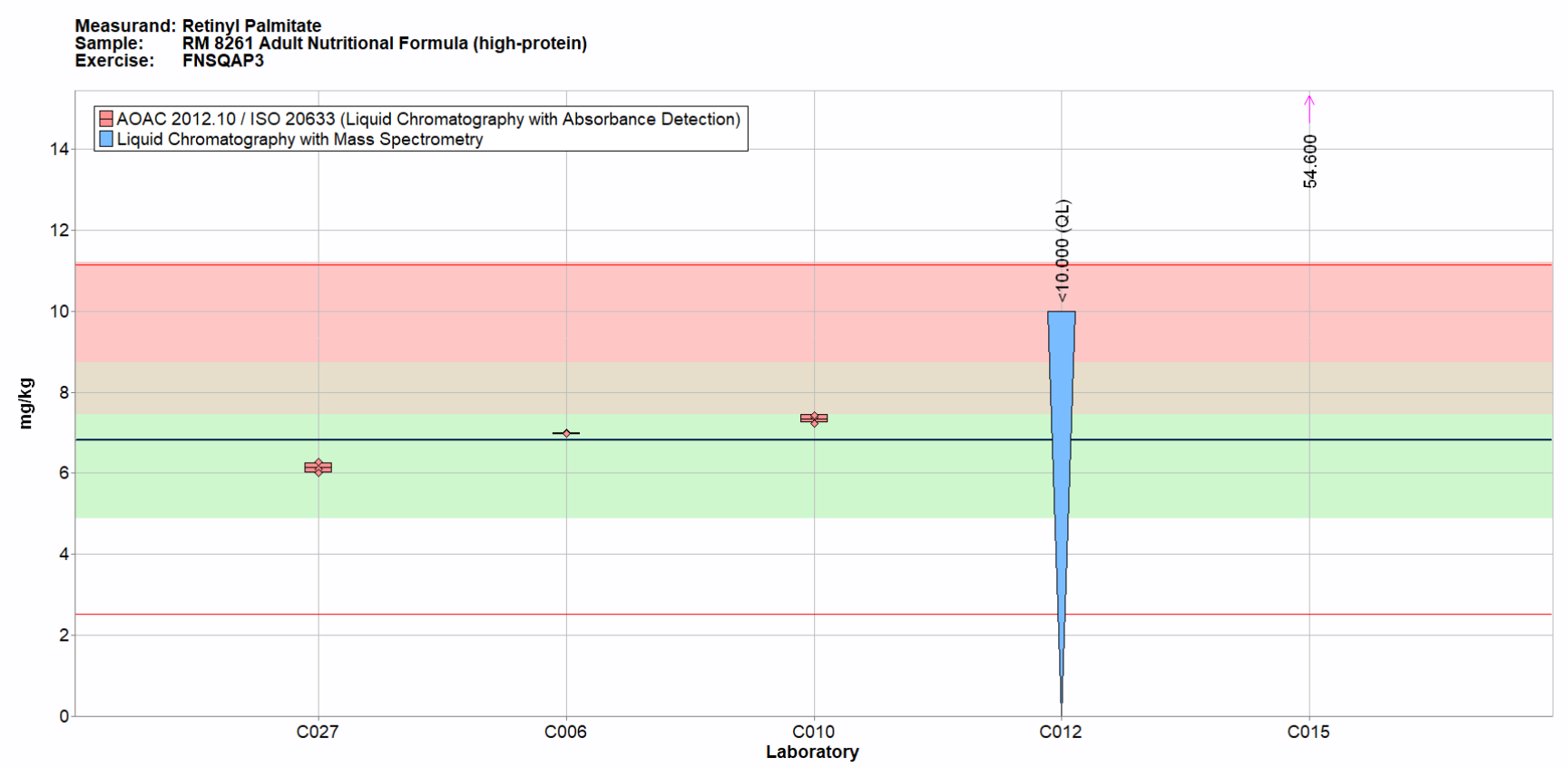

Fig. 4-11. Retinyl palmitate in RM 8261 (data summary view – analytical method).

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 C015 was AOAC 2012.09). The solid black 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 beige shaded region represents the overlapping of the 95 % confidence interval for the consensus mean (green region) and the NIST range of tolerance (red region).

4.4.4. Total Vitamin A

Table 4-1 summarizes and Table 4-5 details the measured mass fraction results reported by each participating laboratory for total vitamin A. The participation level was reasonable for total vitamin A in this study, with 41 % of laboratories requesting samples returning results (7 of 17 laboratories). Six of the 7 laboratories reported quantitative results for total vitamin A in both samples.

Table 4-5. Data summary table for total vitamin A in RM 8260 and RM 8261.

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

		Total Vitamin A									
		RM 8260 Infant Nutritional Formula (hydrolyzed milk-based) (mg/kg)					RM 8261 Adult Nutritional Formula (high-protein) (mg/kg)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target				7.40	0.74				6.00	0.60
	C006	6.3653	6.063	6.2186	6.22	0.15	4.6081	4.6047	4.578	4.60	0.02
	C010	6.794	6.806	6.807	6.80	0.01	4.843	4.739	4.864	4.82	0.07
	C012	5.34	5.58	5.31	5.41	0.15	0.52	0.5	0.57	0.53	0.04
	C015										
	C016										
	C017										
	C026	5.64	5.78	5.81	5.74	0.09	4.09	4.14	4.08	4.10	0.03
	C027										
	C030										
	C033										
	C036	6.73	6.53	6.59	6.62	0.10	4.39	4.63	4.58	4.53	0.13
	C038	< 1.000	< 1.000	< 1.000			< 1.000	< 1.000	1	1.00	
	C039										
	C040										
	C046	5.256	6.13	5.654	5.68	0.44	4.201	4.439	4.134	4.26	0.16
	C047										
	C049										
Community Results		Consensus Mean			6.08		Consensus Mean			4.38	
		Consensus Standard Deviation			0.81		Consensus Standard Deviation			0.84	
		Maximum			6.80		Maximum			4.82	
		Minimum			5.41		Minimum			0.53	
		N			6		N			7	

Table 4-5 reveals that the within-laboratory mass fraction variabilities for all submitted results were acceptable with respect to published expectations (≤ 8 %) [14]. The among-laboratory mass fraction variabilities reported for RM 8260 and RM 8261 were excellent (13 % and 19 %,

respectively) compared to the published expectations (≤ 16 %) [14]; any deviations are likely the result of the variety of methods used by the participants.

Submitted method information was used to categorize results in the legends of Fig. 4-12 and Fig. 4-13. The key differences described by laboratories were 1) use of base during extraction to hydrolyze retinyl esters; 2) use of enzymes during extraction to release vitamin A from the matrix; and 3) the extraction temperature (e.g., room temperature, 25 °C to 40 °C, > 40 °C). Three laboratories reported using base hydrolysis with LLE (43 %), and two laboratories each reported use of enzymatic hydrolysis with LLE and LLE without hydrolysis (29 % each). While the specific approach is not indicative of any trends in performance, the laboratory reporting a mass fraction of vitamin A below their method LOQ and the two laboratories reporting the lowest quantitative mass fraction values all conducted their sample preparation steps at room temperature. This may indicate the importance of elevated temperature for the accurate determination of vitamin A mass fractions in nutritional formulas.

As shown in Fig. 4-14 and Fig. 4-15, most laboratories reported using LC-Abs or PDA for total vitamin A mass fraction determination (6 of 7 laboratories, 86 %). One laboratory reported using LC-MS (14 %). No trends related to analytical method could be identified.

The performance of laboratories in the determination of vitamin A mass fractions in nutritional formula samples may have been dependent on the temperature used for extraction and/or hydrolysis. Most laboratories reported mass fraction results below the target value in both samples, indicating incomplete extraction of the vitamins from the matrix.

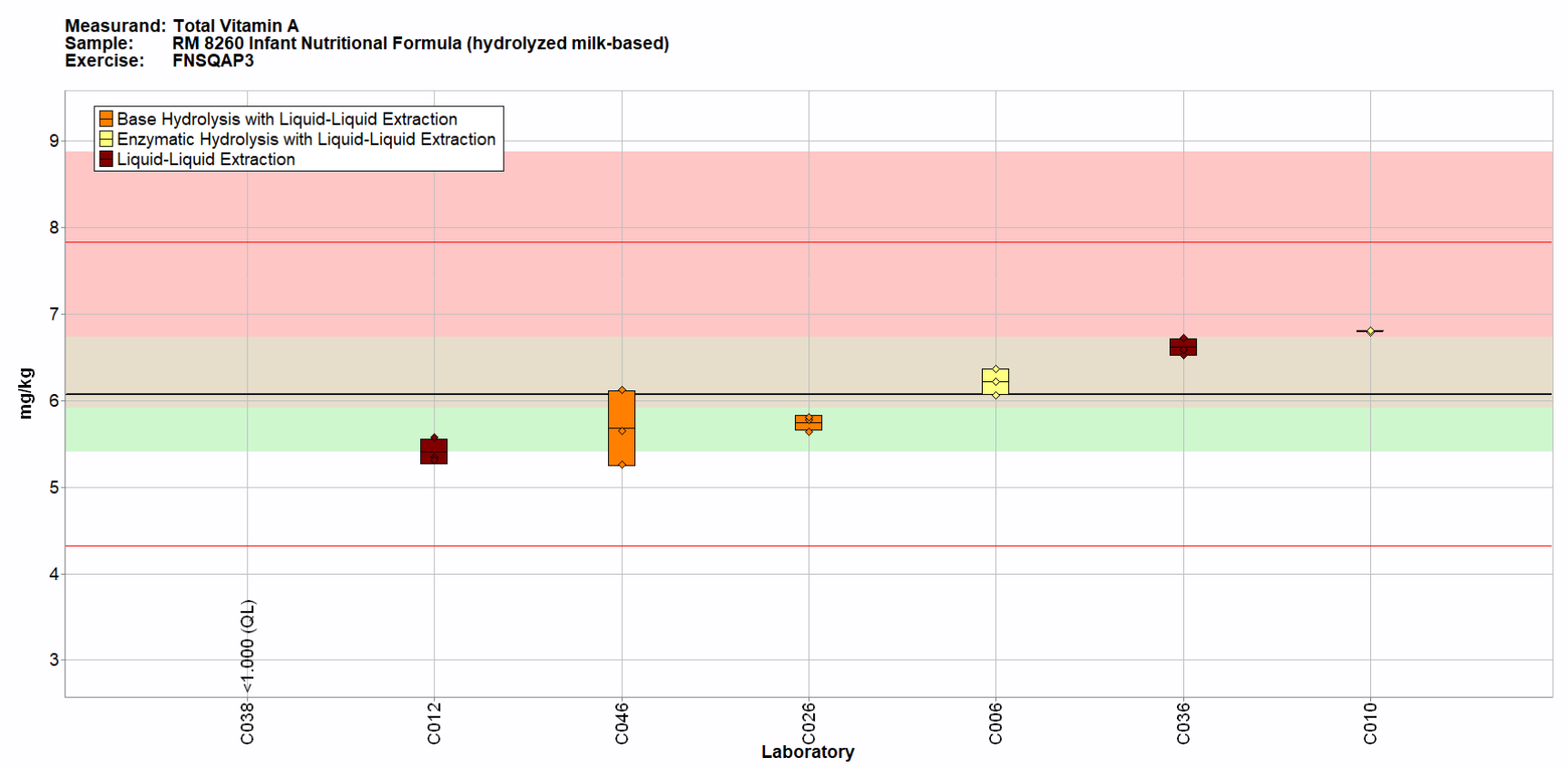

Fig. 4-12. Total vitamin A in RM 8260 (data summary view – sample preparation method).

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 sample preparation method reported by laboratory C038 was base hydrolysis with LLE). The solid black 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 beige shaded region represents the overlapping of the 95 % confidence interval for the consensus mean (green region) and the NIST range of tolerance (red region).

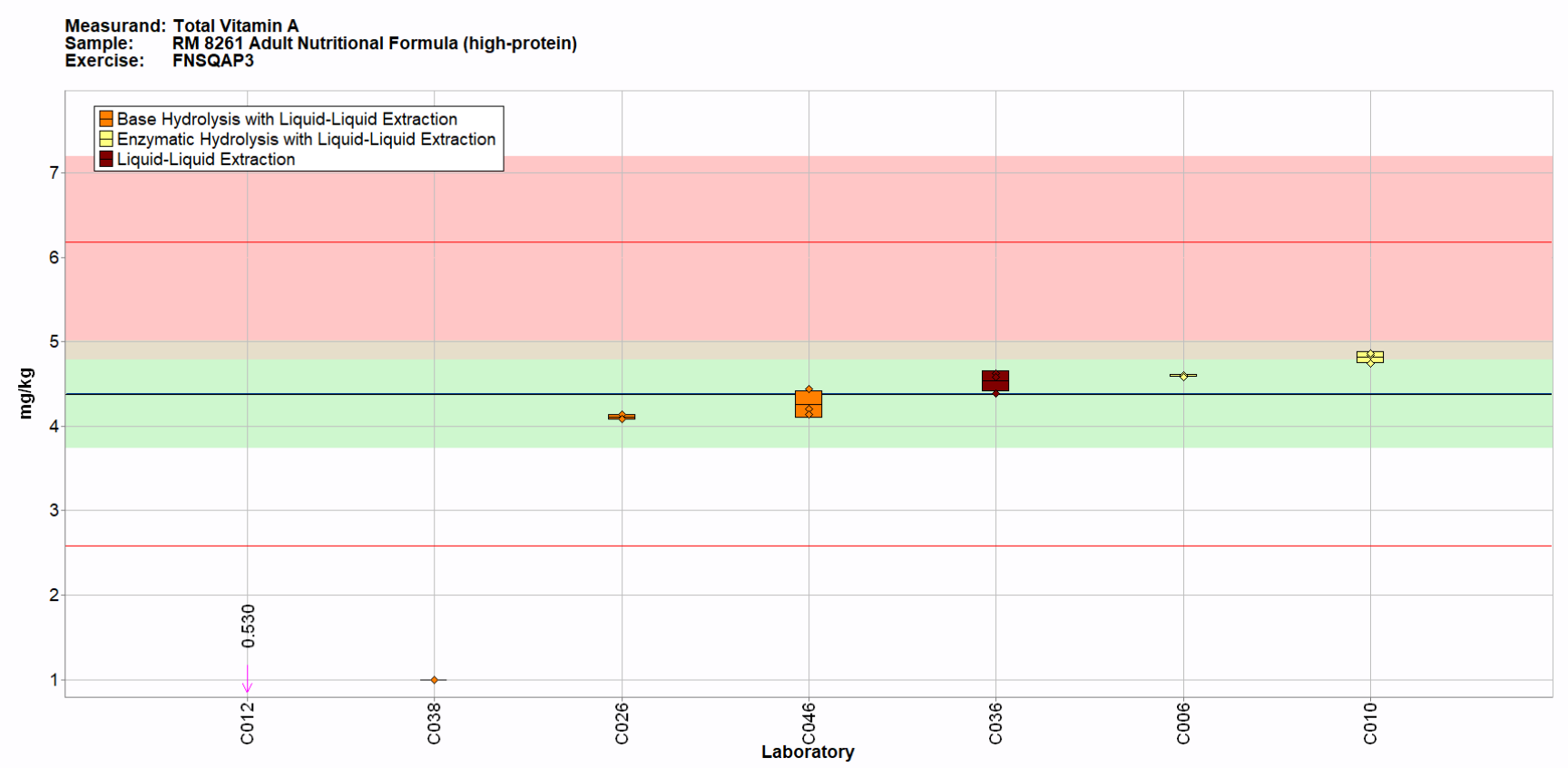

Fig. 4-13. Total vitamin A in RM 8261 (data summary view – sample preparation method).

In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation method employed as indicated in the figure key. Data points outside the graphical range are represented as downward arrows (the sample preparation method reported by laboratory C012 was LLE). The solid black 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 beige shaded region represents the overlapping of the 95 % confidence interval for the consensus mean (green region) and the NIST range of tolerance (red region).

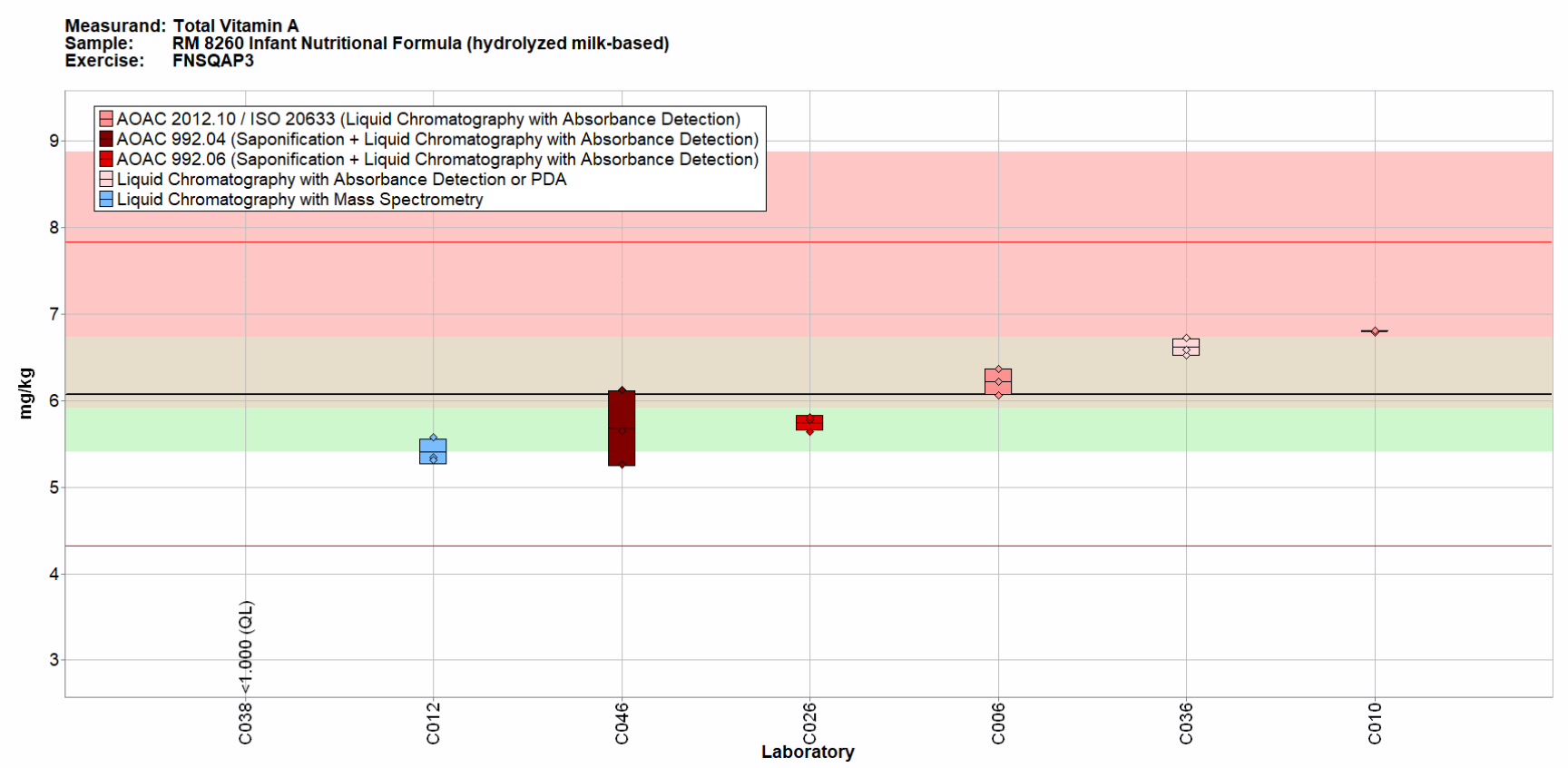

Fig. 4-14. Total vitamin A in RM 8260 (data summary view – analytical method).

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 analytical method reported by laboratory C038 was LC-Abs). The solid black 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 beige shaded region represents the overlapping of the 95 % confidence interval for the consensus mean (green region) and the NIST range of tolerance (red region).

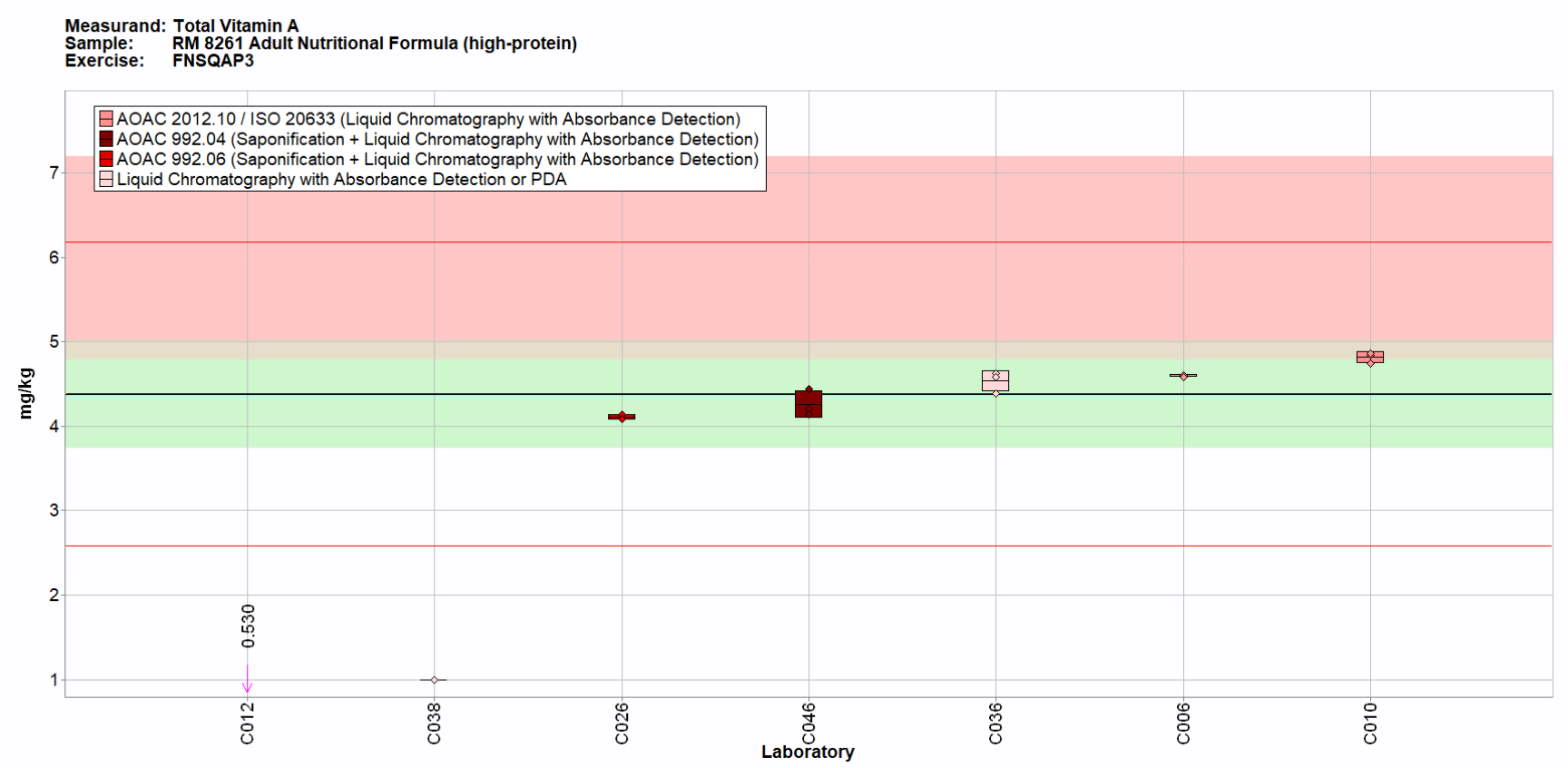

Fig. 4-15. Total vitamin A in RM 8261 (data summary view – analytical method).

In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed as indicated in the figure key. Data points outside the graphical range are represented as downward arrows (the analytical method reported by laboratory C012 was LC-MS). The solid black 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 beige shaded region represents the overlapping of the 95 % confidence interval for the consensus mean (green region) and the NIST range of tolerance (red region).

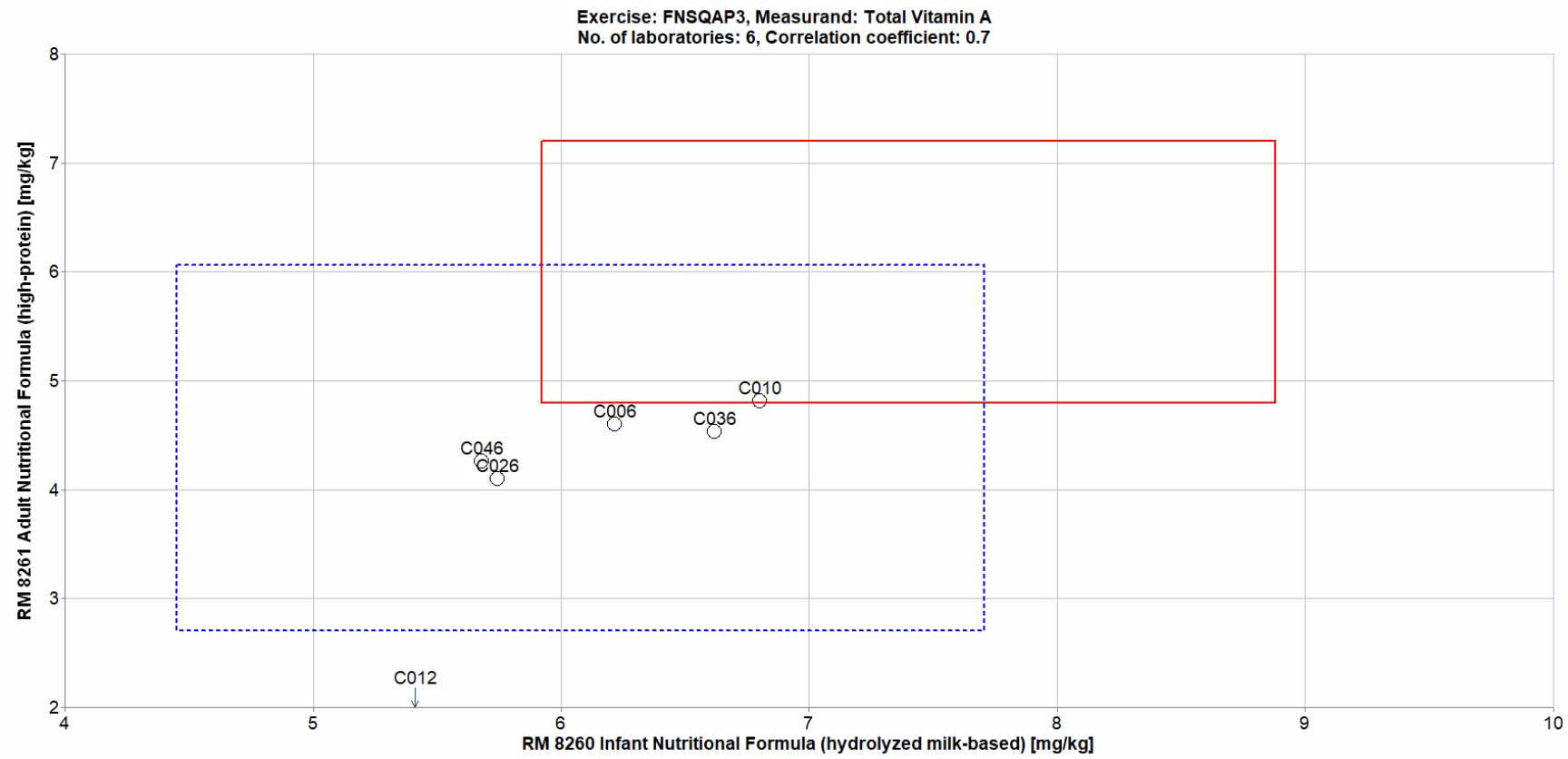

Fig. 4-16. Laboratory means for total vitamin A in RM 8260 and RM 8261 (sample/sample comparison view).

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

4.4.5. Summary and Overall Conclusions

Generally, determination of the vitamin A mass fraction in nutritional formula is accomplished following one of two general approaches. One approach is to expose the sample to somewhat harsh conditions, often with elevated temperature and strong base, to completely hydrolyze retinyl esters, followed by determination of total retinol mass fraction as total vitamin A. A second approach is to extract free retinol and retinyl esters more gently from the sample, sometimes using enzymes to release vitamers, followed by individual determination of free retinol and retinyl ester mass fractions. The total mass fraction of vitamin A is then expressed by summation of the mass fractions of each of component, converting the ester mass fractions to retinol mass fractions using the respective molecular weights.

Table 4-6 summarizes the mass fraction values reported by all participants for all vitamers in this study to facilitate comparison of reported forms with method information. In several cases, laboratories reported mass fraction values for retinol, retinyl acetate, and/or retinyl palmitate but did not report a mass fraction value for total vitamin A. When one or more vitamers are measured, a reported mass fraction value for total vitamin A, converted from the measured mass fractions of individual forms, can be useful to directly compare different methods. Even more confounding, some laboratories reported a value for total vitamin A mass fraction that is inconsistent with a summation of the mass fraction values reported for individual forms. In other cases, laboratories reported results inconsistent with the forms that would be determined using the reported method. Analysts should understand the methods being used, the forms of vitamin A being measured, and the calculations and conversions required to report mass fraction values for various forms appropriately. An example of calculation of total vitamin A from measured forms can be found in AOAC *Official Method of* Analysis 2012.10 [15] and supporting documentation.

Table 4-6. Summary of results and methods reported for all vitamin A forms.

	Value Reported in RM 8260				Value Reported in RM 8261				
Lab	Retinol	Retinyl Acetate	Retinyl Palmitate	Total Vitamin A	Retinol	Retinyl Acetate	Retinyl Palmitate	Total Vitamin A	Method Type[a]
C005	7.4	--	--	--[b]	4.8	--	--	--[b]	1
C006	--	7.1	<LOQ	6.2	--	0.87	7	4.6	2
C010	6.8	7.8	<LOQ	6.8	4.8	0.9	7.3	4.8	2
C012	<LOQ	5.4	<LOQ	5.4[c]	<LOQ	0.53	<LOQ	0.5[c]	2
C015	--	--	13.1	--[b]	--	--	54.6	--[b]	2
C017	78.5	--	--	--[b]	25.2	--	--	--[b]	1
C026	5.7	--	--	5.7	4.1	--	--	4.1	1
C027	3.6	6.2[d]	<LOQ	--[b]	2.5	0.59[d]	6.1[d]	--[b]	1
C036	--	--	--	6.6[d]	--	--	--	4.5[d]	2
C038	--	--	--	<LOQ[b]	--	--	--	1	1
C039	5.8	--	--	<LOQ[b]	4.3	--	--	<LOQ[b]	1
C047	6.2	--	--	5.7[c]	4.3	--	--	4.3	1

[a] 1 = strong hydrolysis to retinol; only retinol values expected to be reported. 2 = gentle extraction for measuring individual forms; any detected individual vitamers expected to be reported.

[b] If a value is reported for retinol, retinyl acetate, and/or retinyl palmitate, total vitamin A can be reported.

[c] Reported value for total vitamin A does not correlate to a molecular weight conversion of other reported values.

[d] Reported values are inconsistent with what would theoretically be determined based on reported method information.

NIST has conducted five QAP studies involving measurement of vitamin A in food samples prior to this FNSQAP study: DSQAP Exercise A in 2007 [16], Exercise C in 2008 [17], and Exercise I in 2012 [18], and HAMQAP Exercise 2 in 2018 [19] and Exercise 6 in 2020 [20]. Early studies focused on only retinol (DSQAP Exercise A) or retinyl palmitate (DSQAP Exercise C). Later studies focused on the entire suite of vitamin A compounds (DSQAP Exercise I and HAMQAP Exercises 2 and 6). This FNSQAP study was the first to include separate data entry fields for retinol and total vitamin A.

Table 4-7 summarizes a review of past exercises and indicates lower enrollment in this study than previous average enrollment for retinol (17), retinyl acetate (16), and retinyl palmitate (17). Participation rates for this study, however, were higher than previous averages for retinol at 47 %, similar for retinyl palmitate at 31 %, and lower for retinyl acetate at 25 %. The nutritional formulas in this study were similar to the samples from past studies, which included other nutritional formulas, multivitamins, and egg powder. The average within-laboratory mass fraction variabilities (RSD_r) reported in this study were comparable to (retinol, 5 %) or better than (retinyl acetate, 3 % and retinyl palmitate, 2 %) those reported in past studies; among-laboratory mass fraction variabilities were improved for all three vitamers (25 %, 21 %, and 28 % for retinol, retinyl acetate, and retinyl palmitate, respectively). These reduced variabilities indicate that many laboratories have improved their in-house procedures for precise and reproducible determination of vitamin A compounds in nutritional formulas over time.

Table 4-7. Summary of past QAP exercises involving measurement of vitamin A compounds in foods.

	Average				Bias (Range)
	Enrollment	Participation	RSD_r	RSD_R	
Retinol	26	42 %	6 %	46 %	+ 2 % to + 9 %
Retinyl Acetate	29	34 %	7 %	56 %	- 4 % to + 8 %
Retinyl Palmitate	26	30 %	12 %	155 %	- 4 % to + 21 %

Accuracy of current methods, however, may require further investigation. For the analytes in which target mass fraction values were available, the consensus means were lower than the target values. For retinyl acetate in RM 8261, the mass fraction bias was significant at –40 %, and the consensus range did not overlap the target range. For retinyl palmitate in RM 8261 and total vitamin A in RM 8260 and RM 8261, the consensus mass fraction means were also biased low (27 %, 18 %, and 27 %, respectively) and were outside the target ranges, although the consensus ranges did overlap the target ranges. Because this trend is not consistent with historical studies, perhaps the nutritional formulas used in this study presented unique challenges to current methodologies.

5. CONTAMINANTS (PFAS)

5.1. Executive Summary

To protect public health, regulators must understand human and animal dietary exposure to potentially harmful contaminants such as per- and polyfluorofluoroalkyl substances (PFAS) through accurate determination of their concentrations in foods. The results of this study revealed that participating laboratories are using repeatable and comparable methods for determination of perfluorohexanesulfonic acid (PFHxS), perfluornonanoic acid (PFNA), perfluorooctanoic acid (PFOA), and perfluorooctanesulfonic acid (PFOS) in the food products presented. Some laboratories should consider method modifications to increase sensitivity to relevant PFAS mass fractions in foods.

5.2. Study Overview

PFAS are a group of anthropogenic chemicals that have been widely used for decades to resist oil, water, and heat in hundreds of products including fabrics, carpeting, cleaning products, and fire-fighting foams [21]. Current scientific research indicates that human exposure to some PFAS may lead to adverse health outcomes, but more research is needed to understand the impact of each PFAS and exposure route. One potential exposure route for humans is from consumption of PFAS-contaminated foods, which can result from animals that have consumed contaminated feed or water. To better understand this exposure route, methods for the detection of PFAS in animal products must be well characterized and have demonstrated accuracy [22]. In this study, participants were provided with samples of RM 8695 PFAS in Bovine Tissue (Beef Bull) (Frozen), Research Grade Test Material (RGTM) 10225 PFAS in Bovine Milk (freeze-dried), and RGTM 10226 PFAS in Chicken Egg. Participants were asked to use in-house analytical methods to determine the mass fractions (ng/g) of six PFAS including perfluorohexanoic acid (PFHxA), PFHxS, PFNA, PFOA, PFOS, and perfluorooctanesulfonamide (PFOSA) 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.

5.3. Sample Information

Participants were provided one jar each of RM 8695 PFAS in Bovine Tissue (Beef Bull) (Frozen),[1] RGTM 10225 PFAS in Bovine Milk (freeze-dried), and RGTM 10226 PFAS in Chicken Egg (freeze-dried). Each jar of RM 8695 and RGTM 10225 contained approximately 15 g of material, and each jar of RGTM 10226 contained approximately 7 g of material. Participants were asked to store RM 8695 at or below –70 °C in the original unopened jar and to store RGTM 10225 and RGTM 10226 under refrigeration (2 °C to 5 °C) in the original unopened jars. Participants were

[1] At the time of publication of this report, RM 8695 is still in development and is not yet available for purchase from NIST. Once available, RM 8695 can be purchased at https://shop.nist.gov.

instructed to thoroughly mix the contents of each jar before use, to prepare three samples and report three values per analyte from each jar provided, and to use a sample size of at least 5 g to ensure homogeneity of the test portion used for analysis. The approximate analyte mass fractions were not reported to participants prior to the study. Target mass fraction values for PFAS in these materials were determined in single or double samples from three to four bottles. Samples were prepared as described in FDA Method C-010.02 [23] with dispersive solid-phase extraction (dSPE) but without subsequent SPE clean-up. The extracted samples containing isotopically labeled internal standards were analyzed by LC-MS/MS. Target values and uncertainties were calculated from mean mass fractions and are provided in Table 5-1 on an as-received basis.

Table 5-1. Individualized data summary table for PFAS in foods.

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

(Lab Name)

Exercise 3 – PFAS in Foods

	Lab Code:	**(Code)**	**1. Your Results**				**2. Community Results**			**3. Target**	
	Sample	Units	x_i	s_i	Z'_{comm}	Z_{NIST}	N	x*	s*	x_{NIST}	u_{NIST}
PFHxA	RGTM 10225	ng/g					0				
PFHxA	RGTM 10226	ng/g					1				
PFHxA	RM 8695	ng/g					0				
PFHxS	RGTM 10225	ng/g					0				
PFHxS	RGTM 10226	ng/g					7	0.210	0.059	0.174	0.044
PFHxS	RM 8695	ng/g					6	0.063	0.014	0.046	0.027
PFNA	RGTM 10225	ng/g					7	0.190	0.031	0.36	0.15
PFNA	RGTM 10226	ng/g	*Individual laboratory results will appear in this section; laboratory-specific results were provided to each participant separately from this report.*				9	15.0	1.8	12.7	2.6
PFNA	RM 8695	ng/g					8	1.60	0.23	1.48	0.36
PFOA	RGTM 10225	ng/g					1				
PFOA	RGTM 10226	ng/g					9	8.20	0.89	7.97	0.58
PFOA	RM 8695	ng/g					8	0.720	0.081	0.84	0.13
PFOS	RGTM 10225	ng/g					8	0.40	0.15	0.371	0.080
PFOS	RGTM 10226	ng/g					9	30.0	7.8	26.2	3.2
PFOS	RM 8695	ng/g					8	23.0	5.5	20.0	2.2
PFOSA	RGTM 10225	ng/g					0				
PFOSA	RGTM 10226	ng/g					0				
PFOSA	RM 8695	ng/g					0				

x_i	Mean of reported values	N	Number of quantitative values reported, not including single-value submissions	x_{NIST}	NIST-assessed value
s_i	Standard deviation of reported values			u_{NIST}	standard uncertainty about the NIST-assessed value
Z'_{comm}	Z'-score with respect to community consensus	x*	Robust mean of reported values		
Z_{NIST}	Z-score with respect to NIST value	s*	Robust standard deviation		

5.4. Study Results and Discussion

Seventeen laboratories requested samples for this study. For PFHxA, PFHxS, PFNA, PFOA, and PFOS, 9 to 10 reported results (53 % to 59 % participation). Of the 16 laboratories that indicated intent to measure PFOSA, 6 to 7 laboratories reported results for at least one sample (38 % to 44 % participation). As shown in Table 5-1, quantitative results were limited for PFOA and PFHxS in RGTM 10225 and for PFHxA and PFOSA in all 3 samples, indicating that the levels of these PFAS in some samples were below the LOQs of most methods.

The results for each PFAS in this study will be discussed in the subsequent sections. For all analytes, the performance of laboratories is compared to published expectations in AOAC SMPR 2023.003 [24]. In this SMPR, characteristics of suitable methods for determination of select PFAS mass fractions in foods are described, including repeatability of ≤ 20 % or ≤ 25 % and reproducibility of ≤ 40 %. Reproducibility in this context describes the among-laboratory variability for several laboratories using the same method but can be useful to understand overall community performance.

5.4.1. PFHxA

Table 5-1 summarizes and Table 5-2 details the measured mass fraction results for PFHxA reported by each participating laboratory. The limited number of quantitative results reported for PFHxA indicates that the levels of PFHxA are below the LOQs of most participant methods. Laboratories reporting LOQs higher than published expectations outlined in Table 5-3 should review practices to ensure that detection of PFHxA is achievable at minimum levels agreed upon by the PFAS measurement community.

Table 5-2. Data summary table for PFHxA in foods.

		PFHxA														
		RGTM 10225 PFAS in Bovine Milk (freeze-dried) (ng/g)					RGTM 10226 PFAS in Chicken Egg (freeze-dried) (ng/g)					RM 8695 PFAS in Bovine Tissue (Beef Bull) (ng/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target															
	C002	< 0.062	< 0.062	< 0.062			< 0.062	< 0.062	< 0.062			< 0.062	< 0.062	< 0.062		
	C019															
	C022															
	C029															
	C030															
	C034						< 0.100	< 0.100	< 0.100			< 0.100	< 0.100	< 0.100		
	C035	< 0.022	< 0.022	< 0.022			< 0.015	< 0.015	< 0.015			< 0.034	< 0.034	< 0.034		
	C036	< 0.003	< 0.003	< 0.003			< 0.003	< 0.003	< 0.003			< 0.003	< 0.003	< 0.003		
	C038	< 156.0	< 156.0	< 156.0			< 156.0	< 156.0	< 156.0			< 156.0	< 156.0	< 156.0		
	C040															
	C041	< 0.013	< 0.013	< 0.013			0.0321	0.0498	0.0346	0.04	0.01	< 0.013	< 0.013	< 0.013		
	C043	< 0.030	< 0.030	< 0.030			< 0.030	< 0.030	< 0.030			< 0.030	< 0.030	< 0.030		
	C048	< 0.500	< 0.500	< 0.500			< 0.500	< 0.500	< 0.500			< 0.500	< 0.500	< 0.500		
	C051	< 0.170	< 0.170	< 0.170			< 1.000	< 1.000	< 1.000							
	C052	< 0.150					< 0.150					< 0.150				
	C053															
	C054															
Community Results		Consensus Mean					Consensus Mean					Consensus Mean				
		Consensus Standard Deviation					Consensus Standard Deviation					Consensus Standard Deviation				
		Maximum					Maximum			0.04		Maximum				
		Minimum					Minimum			0.04		Minimum				
		N			0		N			1		N			0	

Table 5-3. Published method performance requirements for PFAS in foods [24].

	PFOS, PFOA, PFNA, PFHxS			PFHxA, PFOSA		
	LOQ (ng/g)	RSD_r (%)	RSD_R (%)	LOQ (ng/g)	RSD_r (%)	RSD_R (%)
Milk	≤ 0.08	≤ 25	≤ 40	≤ 1	≤ 25	≤ 40
Eggs	≤ 0.3	≤ 20	≤ 40	≤ 3	≤ 25	≤ 40
Beef	≤ 0.1	≤ 20	≤ 40	≤ 1	≤ 25	≤ 40

5.4.2. PFHxS

Table 5-1 summarizes and Table 5-4 details the measured mass fraction results for PFHxS reported by each participating laboratory. A limited number of quantitative results were reported for PFHxS in RGTM 10225, indicating that the level of PFHxS in this milk sample is below the LOQs of most participant methods. Laboratories reporting LOQs higher than published expectations outlined in Table 5-3 should review practices to ensure that detection of PFHxS aligns closely with levels agreed upon by the PFAS measurement community for food matrix samples.

Table 5-4 reveals that for the 6 to 7 laboratories reporting multiple quantitative mass fraction values for PFHxS in RGTM 10226 and RM 8695, respectively, the within-laboratory variabilities were acceptable with respect to published expectations of the PFAS measurement community (≤ 20 %). The among-laboratory variabilities (28 % and 22 % for RGTM 10226 and RM 8695, respectively) were also well within the published expectations for multiple laboratories using the same method (≤ 40 %). The consensus mass fraction ranges were within the target ranges for both RGTM 10226 and RM 8695.

As shown in Fig. 5-1 and Fig. 5-2, laboratories reported using a variety of sample preparation methods for the determination of PFHxS in the egg and beef samples. Two laboratories reported using only solvent extraction for isolation of PFHxS (20 %) while most laboratories reported using an additional cleanup step such as solid phase extraction to reduce potential interferences (80 %). Five laboratories indicated that freeze-dried samples were rehydrated prior to extraction (50 %); one laboratory did not rehydrate freeze-dried samples (10 %). A majority of laboratories used acidic acetonitrile for PFHxS extraction (60 %). One laboratory each reported use of unmodified methanol, unmodified acetonitrile, and a combination of basic methanol and unmodified acetonitrile for extraction (10 % each); one laboratory did not report the type of solvent used. No trends were identified correlating the sample preparation conditions with concordance of PFHxS results.

Similarly, Fig. 5-3 and Fig. 5-4 indicate that all laboratories reported using LC-MS-based techniques for the determination of PFHxS in the two food samples. Two laboratories reported use of LC-MS (20 %), 7 laboratories reported use of LC-MS/MS (70 %), and 1 laboratory reported use of LC-HRMS (10 %).

Table 5-4. Data summary table for PFHxS in foods.

		PFHxS														
		RGTM 10225 PFAS in Bovine Milk (freeze-dried) (ng/g)					RGTM 10226 PFAS in Chicken Egg (freeze-dried) (ng/g)					RM 8695 PFAS in Bovine Tissue (Beef Bull) (ng/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target									0.174	0.044				0.046	0.027
	C002	< 0.055	< 0.055	< 0.055			0.181	0.157	0.157	0.165	0.014	0.065	0.064	0.063	0.064	0.001
	C019															
	C022															
	C029															
	C030															
	C034						< 0.070	< 0.070	< 0.070			< 0.070	< 0.070	< 0.070		
	C035	< 0.023	< 0.023	< 0.023			0.26	0.267	0.25	0.259	0.009	0.0708	0.0666	0.0732	0.070	0.003
	C036	< 0.003	< 0.003	< 0.003			0.23	0.25	0.25	0.243	0.012	0.082	0.082	0.08	0.081	0.001
	C038	< 156.0	< 156.0	< 156.0			< 156.0	< 156.0	< 156.0			< 156.0	< 156.0	< 156.0		
	C040															
	C041	< 0.015	< 0.015	< 0.015			0.1529	0.176	0.1652	0.165	0.012	0.061	0.066	0.0616	0.063	0.003
	C043	< 0.007	< 0.007	< 0.007			0.2	0.22	0.23	0.217	0.015	0.038	0.035	0.041	0.038	0.003
	C048	< 0.500	< 0.500	< 0.500			0.276	0.207	0.282	0.255	0.042	< 0.500	< 0.500	< 0.500		
	C051	< 0.760	< 0.760	< 0.760			< 1.000	< 1.000	< 1.000							
	C052	< 0.500					0.187			0.187		0.0598			0.060	
	C053															
	C054															
Community Results		Consensus Mean					Consensus Mean			0.213		Consensus Mean			0.063	
		Consensus Standard Deviation					Consensus Standard Deviation			0.059		Consensus Standard Deviation			0.014	
		Maximum					Maximum			0.259		Maximum			0.081	
		Minimum					Minimum			0.165		Minimum			0.038	
		N			0		N			7		N			6	

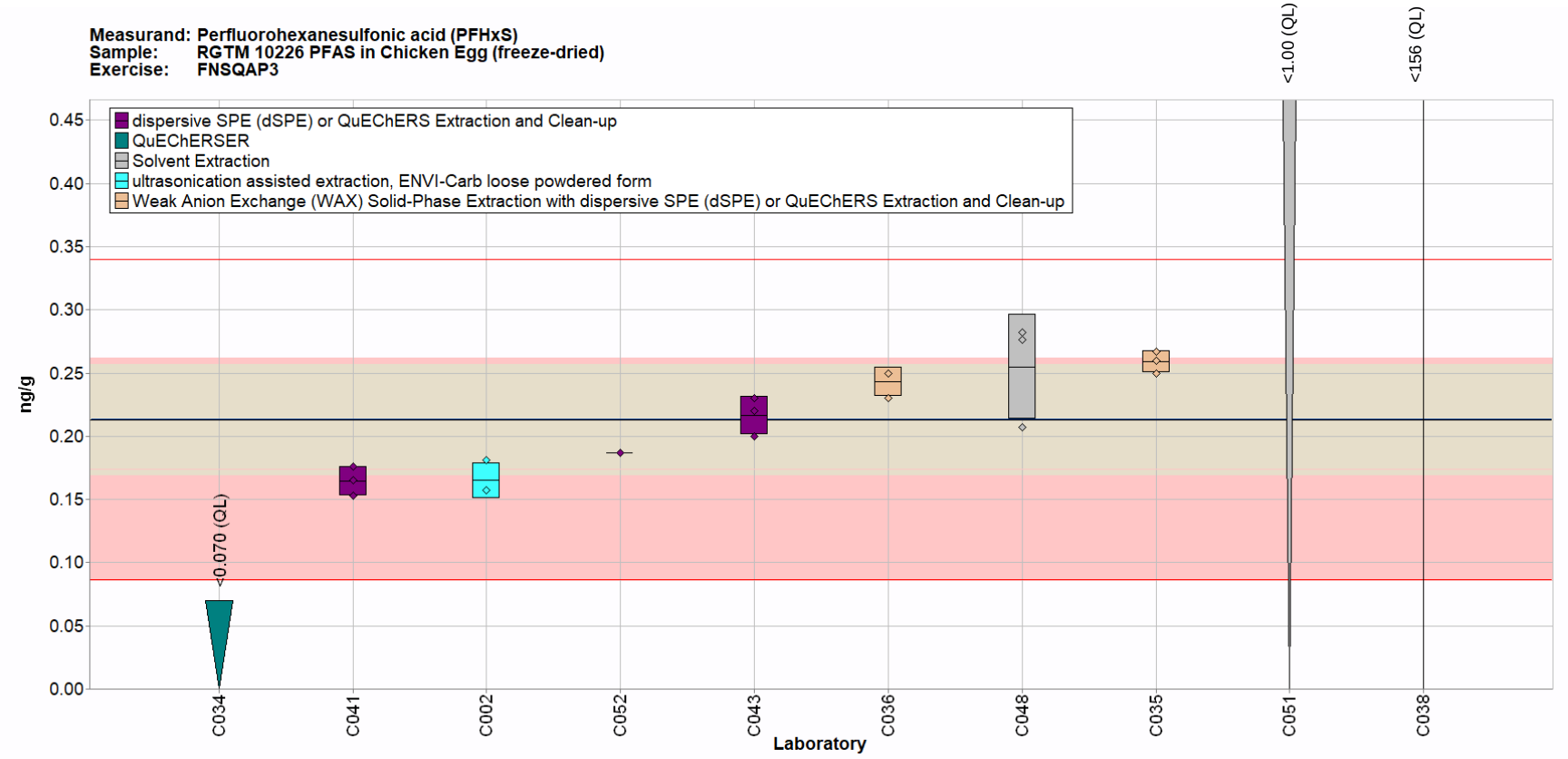

Fig. 5-1. PFHxS in RGTM 10226 (data summary view – sample preparation method).

In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). A downward triangle represents data reported as an LOQ value. The color of the data point represents the sample preparation method employed as indicated in the figure key (the sample preparation approach reported by laboratory C038 was solvent extraction). The solid blue line represents the consensus mean, and the solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{comm}| \leq 2$. The beige shaded region represents the overlapping of the 95 % confidence interval for the consensus mean (green region) and the NIST range of tolerance (red region). The NIST range of tolerance encompasses the target value bounded by twice its uncertainty (U_{NIST}), and represents the range that results in an acceptable Z_{NIST} score, $|Z_{NIST}| \leq 2$.

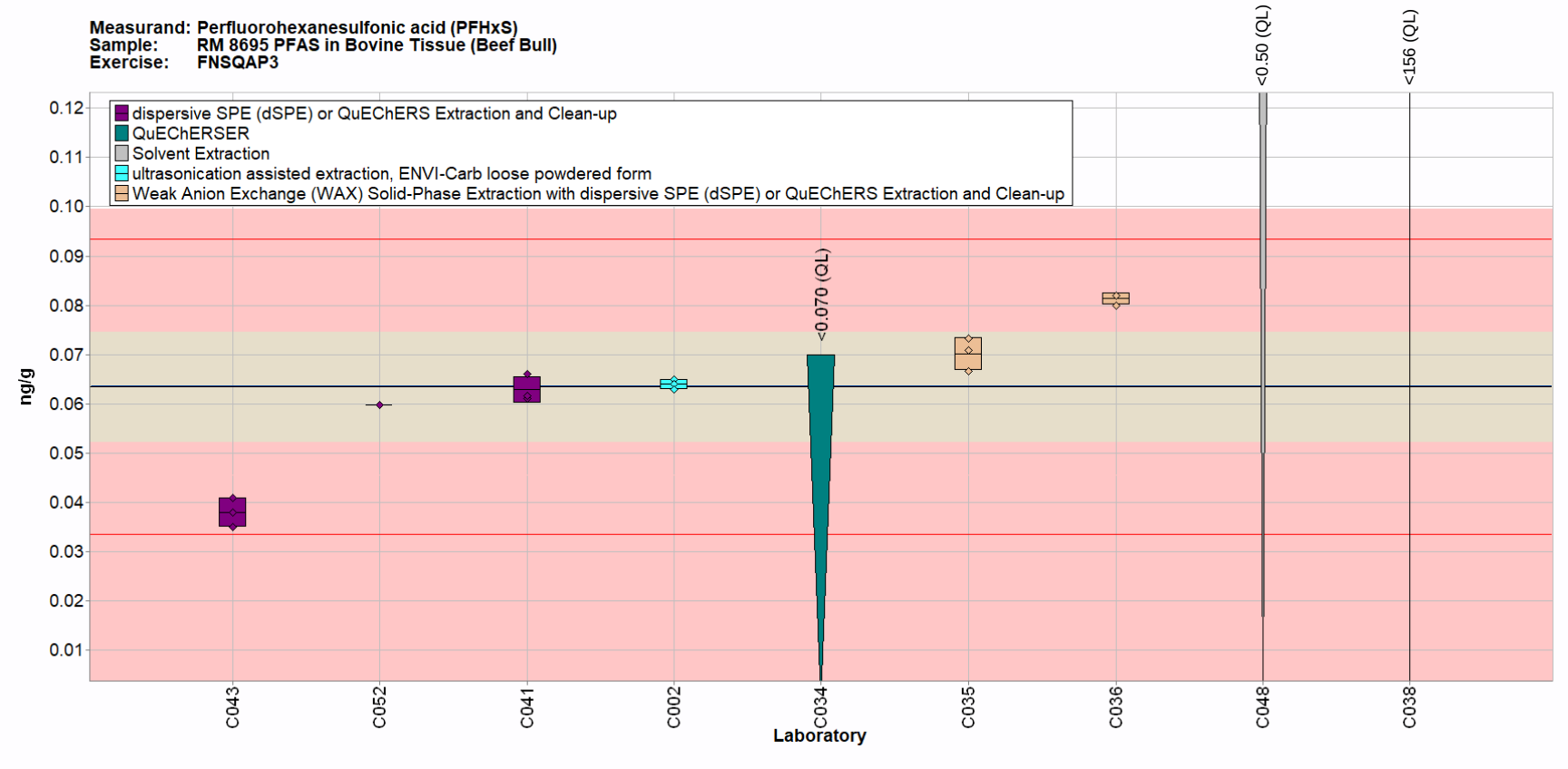

Fig. 5-2. PFHxS in RM 8695 (data summary view – sample preparation method).

In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). A downward triangle represents data reported as an LOQ value. The color of the data point represents the sample preparation method employed as indicated in the figure key (the sample preparation approach reported by laboratory C038 was solvent extraction). The solid blue line represents the consensus mean, and the solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{comm}| \leq 2$. The beige shaded region represents the overlapping of the 95 % confidence interval for the consensus mean (green region) and the NIST range of tolerance (red region). The NIST range of tolerance encompasses the target value bounded by twice its uncertainty (U_{NIST}), and represents the range that results in an acceptable Z_{NIST} score, $|Z_{NIST}| \leq 2$.

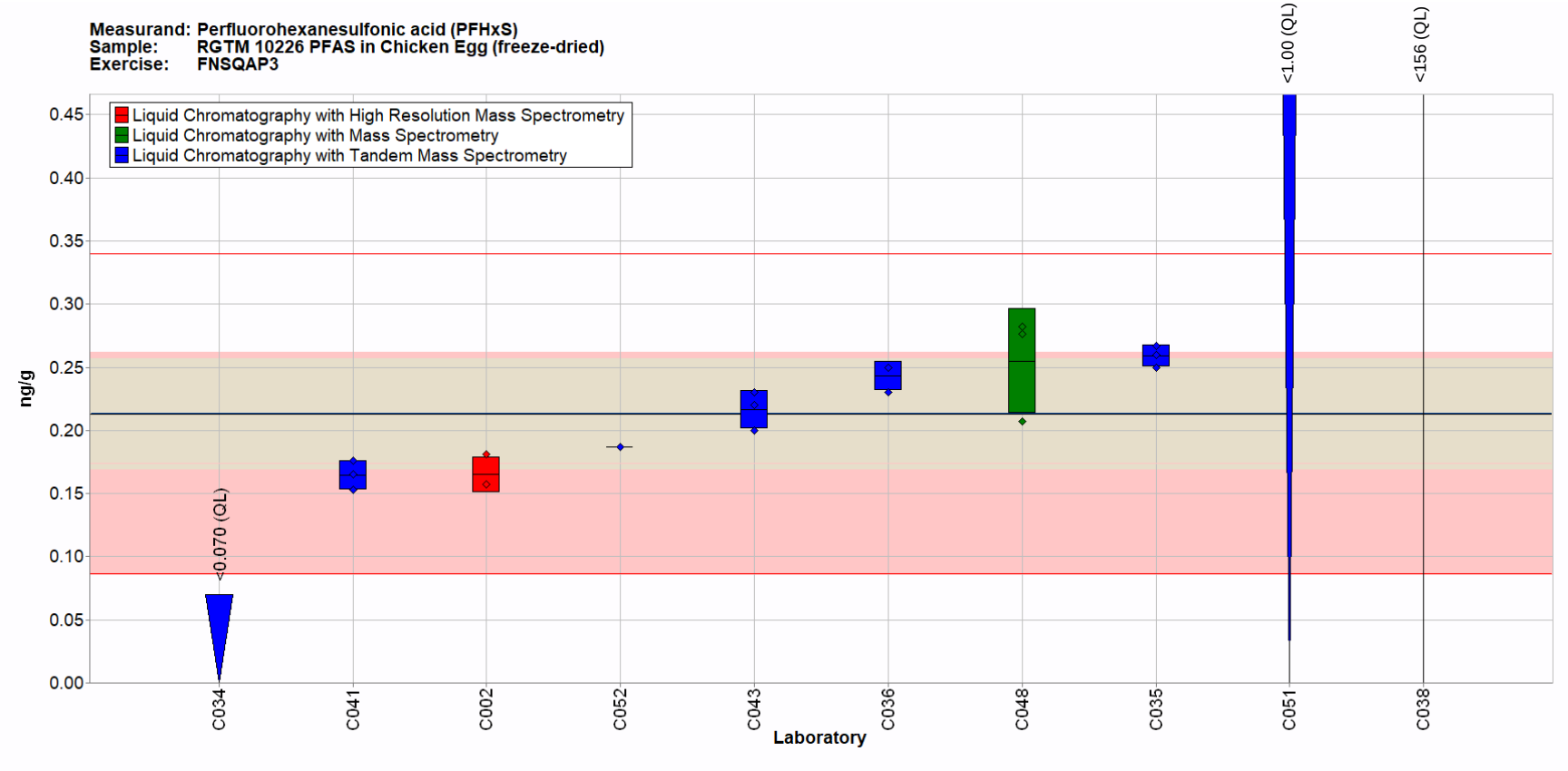

Fig. 5-3. PFHxS in RGTM 10226 (data summary view – analytical method).

In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). A downward triangle represents data reported as an LOQ value. The color of the data point represents the analytical method employed as indicated in the figure key (the analytical approach reported by laboratory C038 was LC-MS). The solid blue line represents the consensus mean, and the solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{comm}| \leq 2$. The beige shaded region represents the overlapping of the 95 % confidence interval for the consensus mean (green region) and the NIST range of tolerance (red region). The NIST range of tolerance encompasses the target value bounded by twice its uncertainty (U_{NIST}), and represents the range that results in an acceptable Z_{NIST} score, $|Z_{NIST}| \leq 2$.

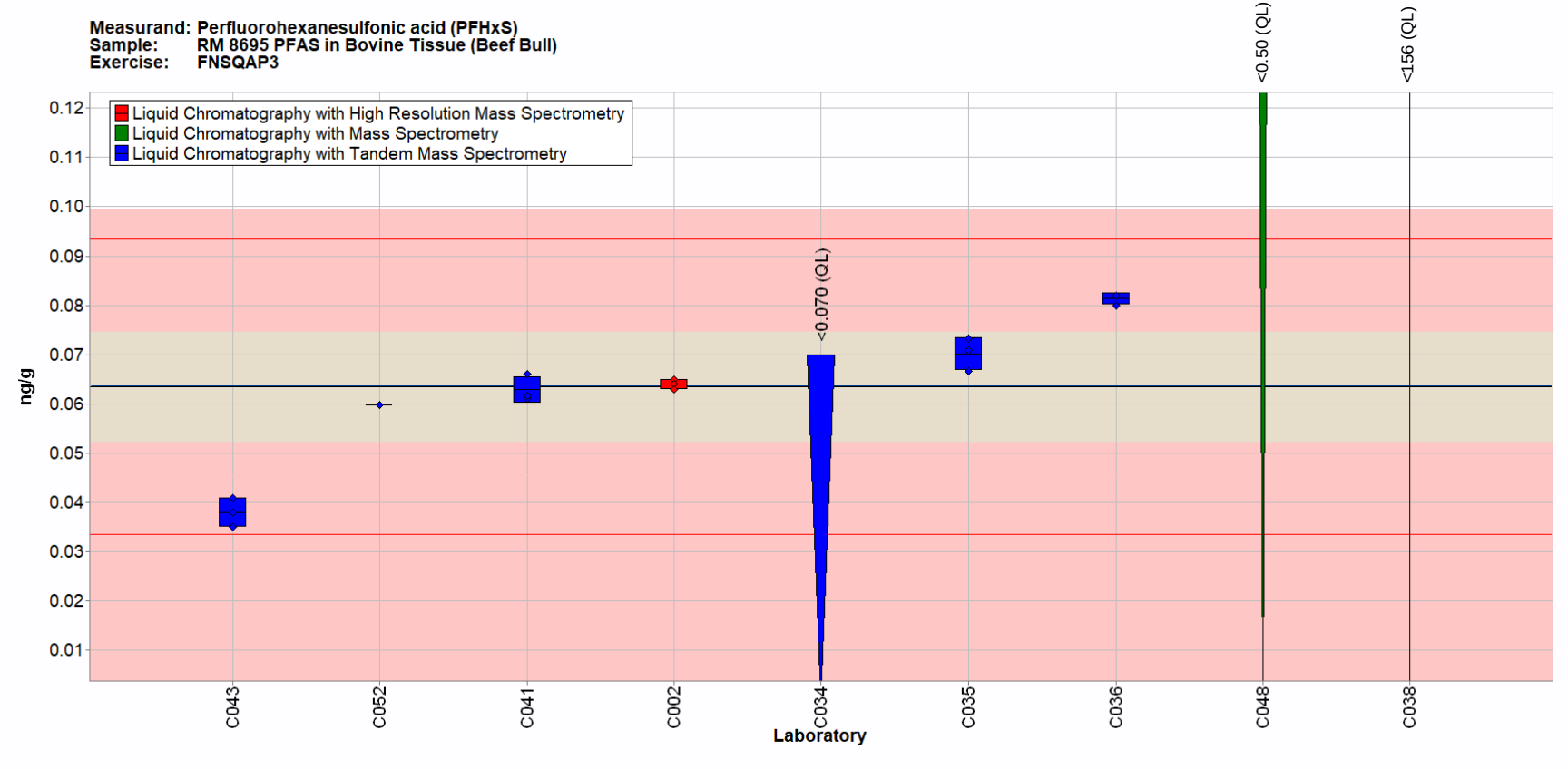

Fig. 5-4. PFHxS in RM 8695 (data summary view – analytical method).

In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). A downward triangle represents data reported as an LOQ value. The color of the data point represents the analytical method employed as indicated in the figure key (the analytical approach reported by laboratory C038 was LC-MS). The solid blue line represents the consensus mean, and the solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{comm}| \leq 2$. The beige shaded region represents the overlapping of the 95 % confidence interval for the consensus mean (green region) and the NIST range of tolerance (red region). The NIST range of tolerance encompasses the target value bounded by twice its uncertainty (U_{NIST}), and represents the range that results in an acceptable Z_{NIST} score, $|Z_{NIST}| \leq 2$.

The mass fraction results from laboratories reporting use of solvent extraction with LC-MS were either below method LOQ (for 3 of 4 sample/analyte pairs) or above the consensus mean with high repeatability (for 1 of 4 sample/analyte pairs). One of these laboratories reporting using solvent extraction with unmodified methanol, which was different than the solvent selected by all other laboratories; one additional laboratory did not report the solvent used. The method LOQs reported by laboratories using solvent extraction with LC-MS were significantly higher than the published recommendations; laboratories using solvent extraction and LC-MS for determination of PFHxS should consider method adjustments to improve sensitivity, such as modification of extraction solvent or including additional sample cleanup steps, or changing to more sensitive instrumentation such as LC-MS/MS.

The acceptable repeatability and reproducibility observed for measurements of PFHxS in RGTM 10226 and RM 8695, combined with the lack of trends observed in Fig. 5-5, indicate that laboratories are generally performing comparably in determination of PFHxS mass fractions in the represented food matrices, and any recommendations for improvement would require additional probing of method details.

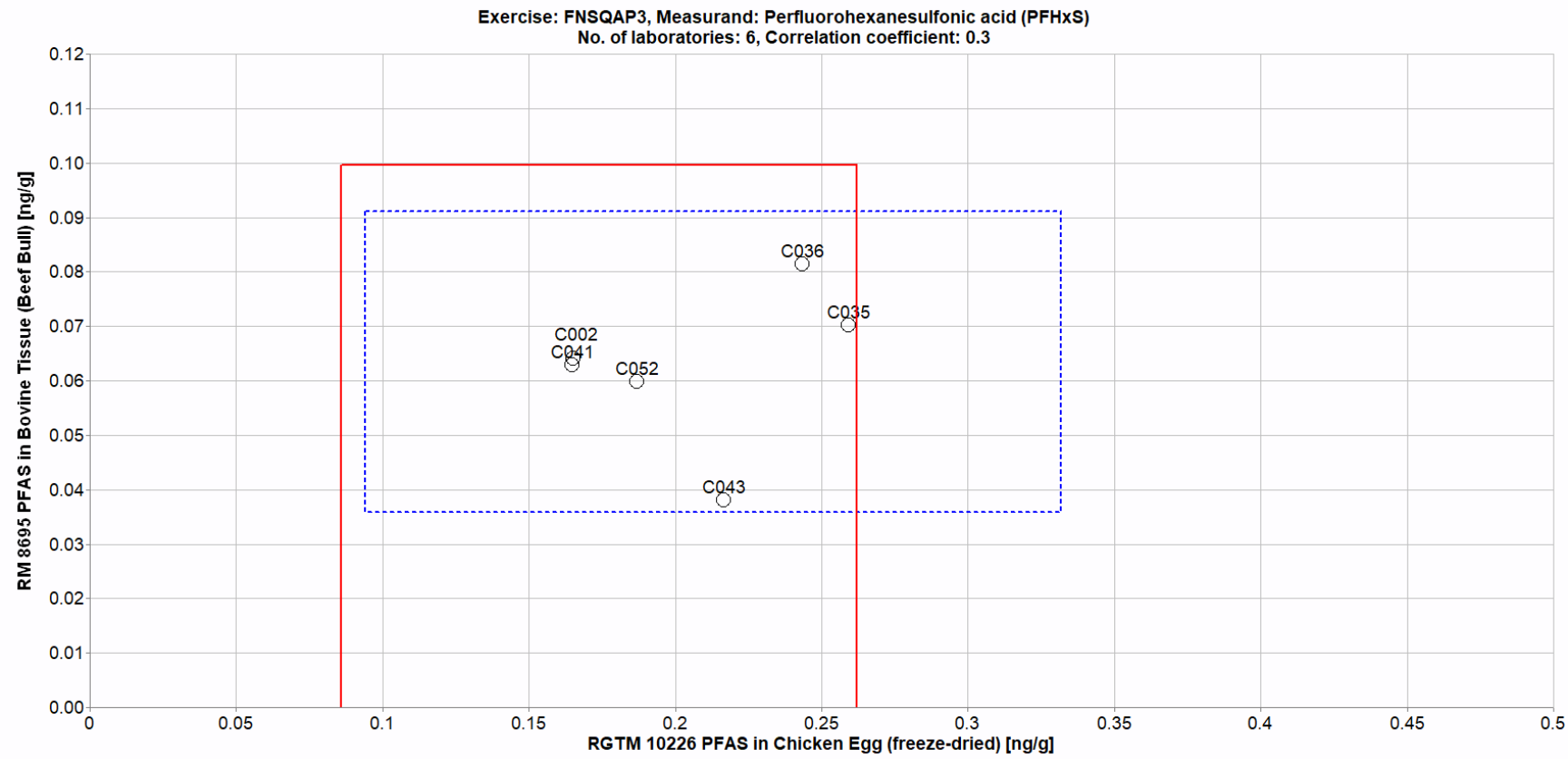

Fig. 5-5. Laboratory means for PFHxS in RGTM 10226 and RM 8695 (sample/sample comparison view).

In this view, the individual laboratory mean for one sample (RGTM 10226) is compared to the individual laboratory mean for a second sample (RM 8695). The solid red box represents the NIST range of tolerance for the two samples, RGTM 10226 (x-axis) and RM 8695 (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 RGTM 10226 (x-axis) and RM 8695 (y-axis), calculated as the values above and below the consensus means that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$.

5.4.3. PFNA

Table 5-1 summarizes and Table 5-5 details the measured mass fraction results for PFNA reported by each participating laboratory. For the 7 to 9 laboratories reporting multiple quantitative mass fraction values for PFNA in the 3 samples, the within-laboratory variabilities were acceptable with respect to published expectations of the PFAS measurement community (≤ 20 %). The among-laboratory variabilities of 16 %, 12 %, and 14 % for RGTM 10225, RGTM 10226, and RM 8695, respectively, were also well within the published expectations for multiple laboratories using the same method (≤ 40 %). The consensus mass fraction ranges were within the target ranges for all three samples.

As described for PFHxS in Section 5.4.2 and as shown in Fig. 5-8, Fig. 5-9, and Fig. 5-10, laboratories reported using a variety of sample preparation methods for the determination of PFNA in the three samples. Similarly, Fig. 5-9, Fig. 5-10, and Fig. 5-11 indicate that all laboratories reported using LC-MS-based techniques for the determination of PFNA in the three samples.

For the milk sample, two potential correlations between methodology and reported mass fraction results were noted. First, the laboratory reporting the lowest mass fraction for PFNA in the milk sample reported not rehydrating the sample with water prior to or during analysis, which was unique compared to other reported methods. The same laboratory reported use of only solvent extraction, without additional cleanup steps, using unique solvents compared to other participants (basic methanol and unmodified acetonitrile). Additionally, this laboratory was the only to report use of LC-HRMS. Unfortunately, given the multiple unique aspects of the method reported by this laboratory, no specific reason for the lower measured mass fraction can be identified. Second, as described for PFHxS, the two laboratories reporting results below their method LOQs both reported use of solvent extraction (one using unmodified methanol, one did not report the solvent used) and LC-MS to determine PFNA. Laboratories using solvent extraction and LC-MS for determination of PFNA should consider method adjustments to improve sensitivity, such as modification of extraction solvent or including additional sample cleanup steps, or changing to more sensitive instrumentation such as LC-MS/MS. These observations were specific to the results reported for the milk sample and were not apparent in the results for egg and beef samples.

For the egg sample, the laboratory using QuEChERSER [25] for sample preparation reported the lowest mass fraction for PFNA. This potential sample-specific effect was not observed for the beef sample and the laboratory did not report results for the milk sample.

Two unique observations were noted for the results reported for the beef sample, specifically with laboratories reporting use of solvent extraction and LC-MS. The lowest reported mass fractions were obtained using solvent extraction with unmodified methanol followed by LC-MS, while the other laboratory reporting use of solvent extraction and LC-MS reported results below LOQ. As described previously, laboratories using solvent extraction and LC-MS for determination of PFNA should consider method adjustments to improve sensitivity.

Table 5-5. Data summary table for PFNA in foods.

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

		PFNA													
		RGTM 10225 PFAS in Bovine Milk (freeze-dried) (ng/g)					RGTM 10226 PFAS in Chicken Egg (freeze-dried) (ng/g)					RM 8695 PFAS in Bovine Tissue (Beef Bull) (ng/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target				0.36	0.15				12.7	2.6				1.48	0.36
	C002	0.127	0.123	0.107	0.119	0.011	14.287	13.44	13.809	13.8	0.4	1.636	1.624	1.616	1.63	0.01
	C019															
	C022															
	C029															
	C030															
	C034						11.9	12.341	11.42	11.9	0.5	1.618	1.689	1.796	1.70	0.09
	C035	0.21	0.22	0.214	0.215	0.005	15	15.7	15.7	15.5	0.4	1.58	1.61	1.8	1.66	0.12
	C036	0.21	0.2	0.2	0.203	0.006	15.67	15.06	15.38	15.4	0.3	1.67	1.66	1.69	1.67	0.02
	C038	< 156.0	< 156.0	< 156.0			< 156.0	< 156.0	< 156.0			< 156.0	< 156.0	< 156.0		
	C040															
	C041	0.174	0.1798	0.1849	0.180	0.005	13.324	13.095	12.497	13.0	0.4	1.4642	1.4502	1.4148	1.44	0.03
	C043	0.211	0.216	0.218	0.215	0.004	14.2	15.3	14.2	14.6	0.6	1.82	1.89	2.04	1.92	0.11
	C048	< 0.500	< 0.500	< 0.500			15.3	14.57	15.1	15.0	0.4	1.31	1.3	1.23	1.28	0.04
	C051	0.17	0.17	0.2	0.180	0.017	17	17	18	17.3	0.6					
	C052	0.196			0.196		14.5			14.5		1.58			1.58	
	C053															
	C054															
Community Results		Consensus Mean			0.190		Consensus Mean			14.5		Consensus Mean			1.61	
		Consensus Standard Deviation			0.031		Consensus Standard Deviation			1.8		Consensus Standard Deviation			0.23	
		Maximum			0.215		Maximum			17.3		Maximum			1.92	
		Minimum			0.119		Minimum			11.9		Minimum			1.28	
		N			7		N			9		N			8	

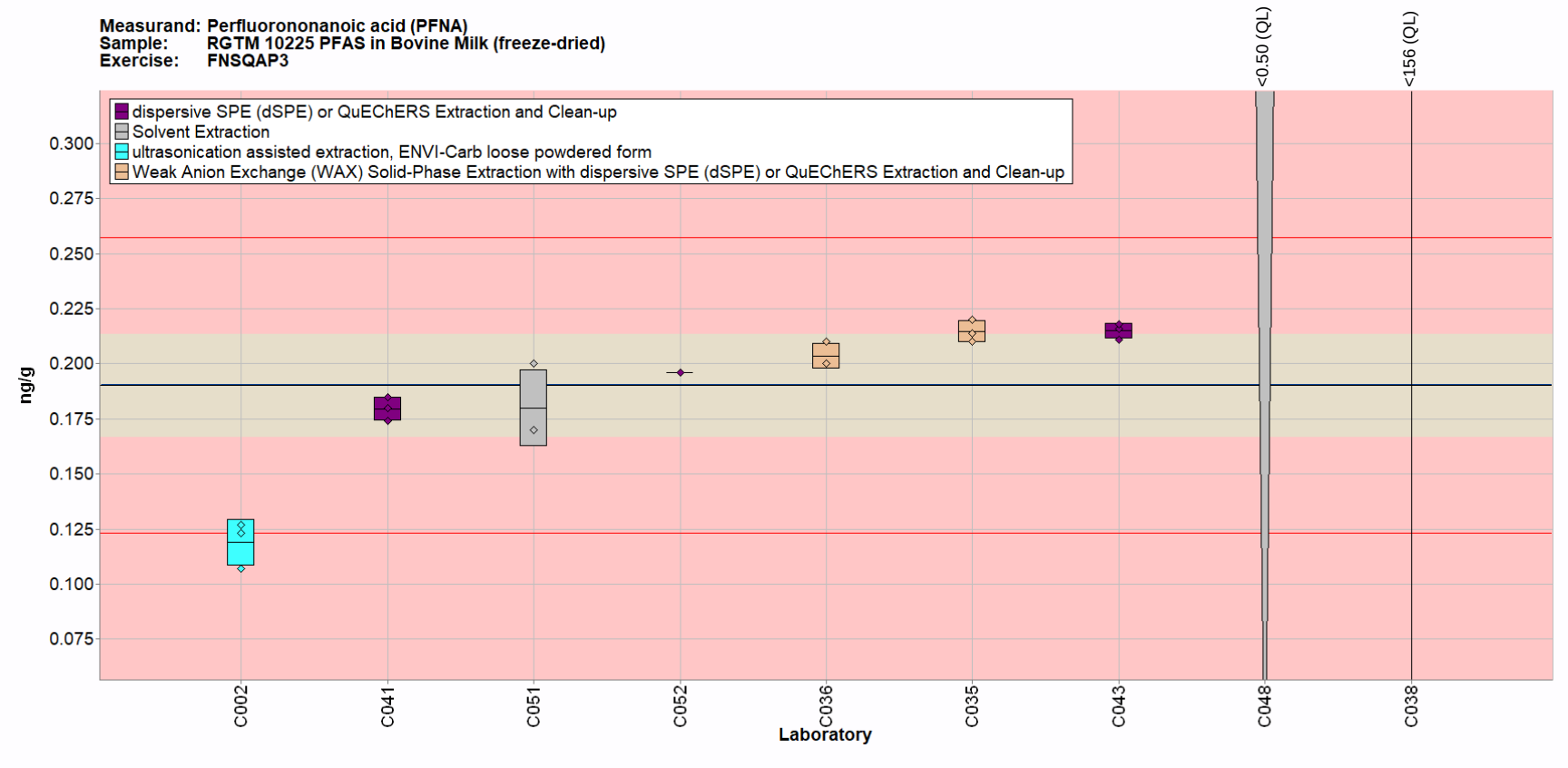

Fig. 5-6. PFNA in RGTM 10225 (data summary view – sample preparation method).

In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). A downward triangle represents data reported as an LOQ value. The color of the data point represents the sample preparation method employed as indicated in the figure key (the sample preparation approach reported by laboratory C038 was solvent extraction). The solid black line represents the consensus mean, and the solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{comm}| \leq 2$. The beige shaded region represents the overlapping of the 95 % confidence interval for the consensus mean (green region) and the NIST range of tolerance (red region). The NIST range of tolerance encompasses the target value bounded by twice its uncertainty (U_{NIST}), and represents the range that results in an acceptable Z_{NIST} score, $|Z_{NIST}| \leq 2$.

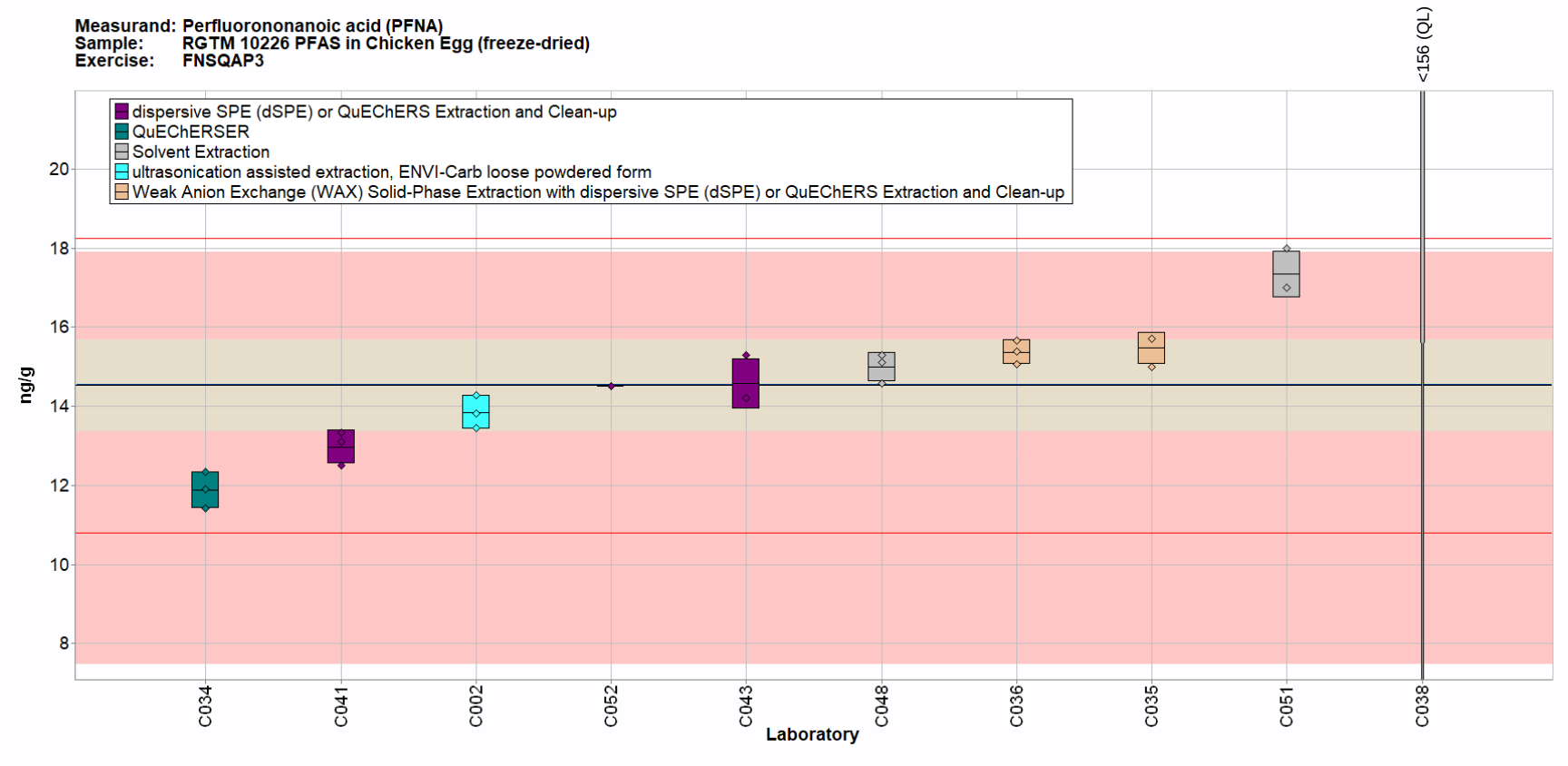

Fig. 5-7. PFNA in RGTM 10226 (data summary view – sample preparation method).

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

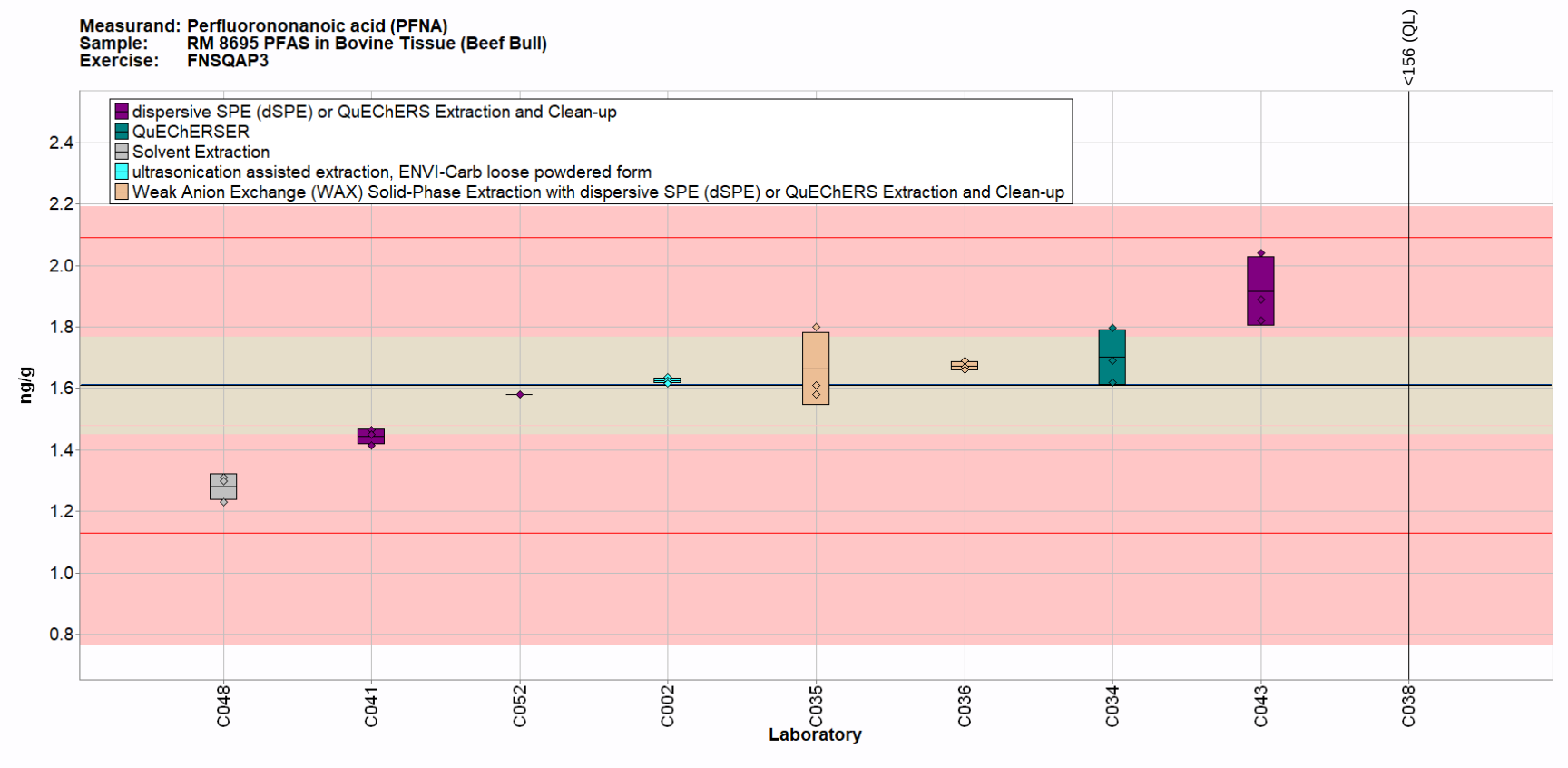

Fig. 5-8. PFNA in RM 8695 (data summary view – sample preparation method).

In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). A downward triangle represents data reported as an LOQ value. The color of the data point represents the sample preparation method employed as indicated in the figure key (the sample preparation approach reported by laboratory C038 was solvent extraction). The solid black line represents the consensus mean, and the solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{comm}| \leq 2$. The beige shaded region represents the overlapping of the 95 % confidence interval for the consensus mean (green region) and the NIST range of tolerance (red region). The NIST range of tolerance encompasses the target value bounded by twice its uncertainty (U_{NIST}), and represents the range that results in an acceptable Z_{NIST} score, $|Z_{NIST}| \leq 2$.

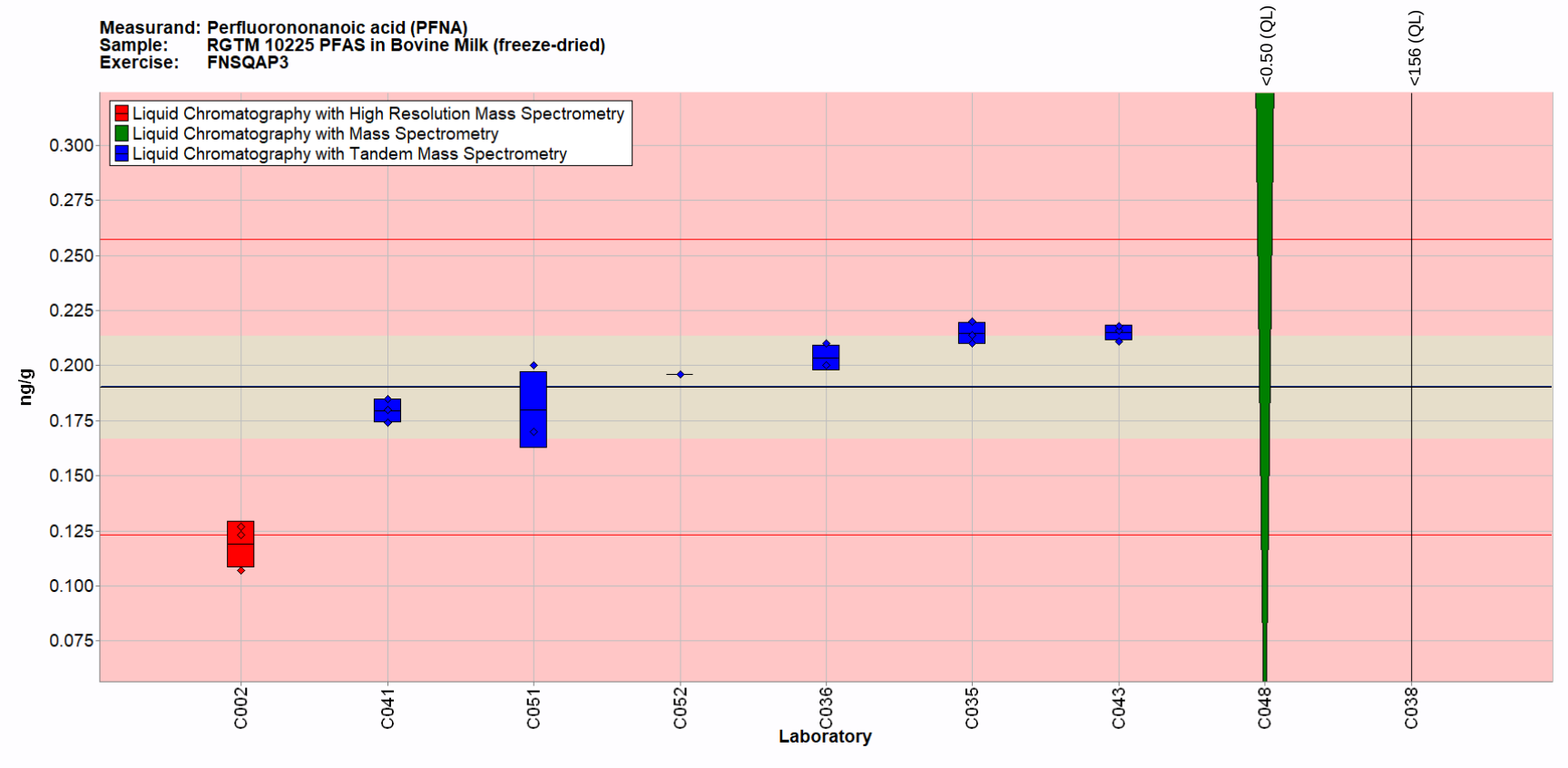

Fig. 5-9. PFNA in RGTM 10225 (data summary view – analytical method).

In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). A downward triangle represents data reported as an LOQ value. The color of the data point represents the analytical method employed as indicated in the figure key (the analytical approach reported by laboratory C038 was LC-MS). The solid black line represents the consensus mean, and the solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{comm}| \leq 2$. The beige shaded region represents the overlapping of the 95 % confidence interval for the consensus mean (green region) and the NIST range of tolerance (red region). The NIST range of tolerance encompasses the target value bounded by twice its uncertainty (U_{NIST}), and represents the range that results in an acceptable Z_{NIST} score, $|Z_{NIST}| \leq 2$.

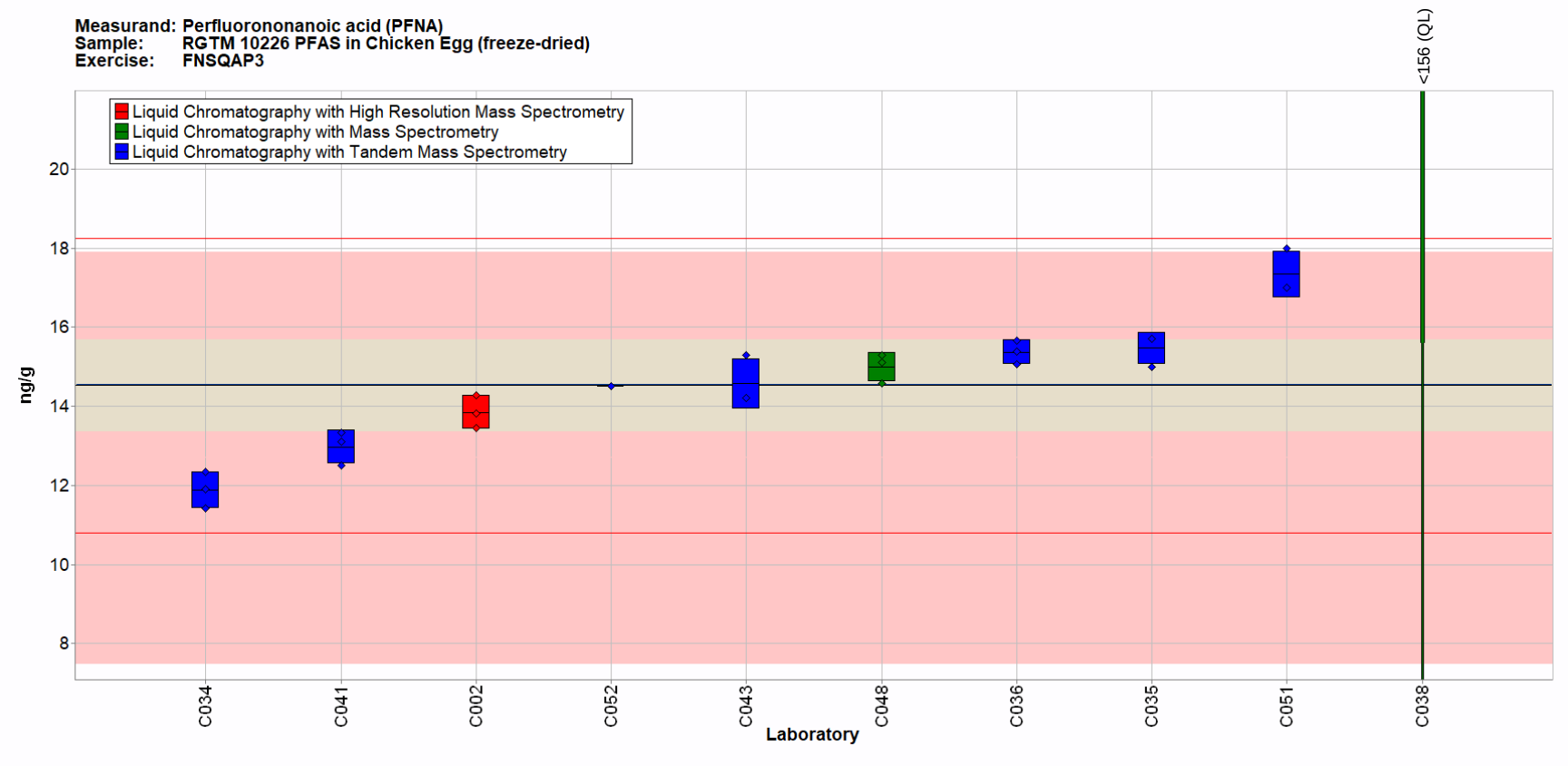

Fig. 5-10. PFNA in RGTM 10226 (data summary view – analytical method).

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

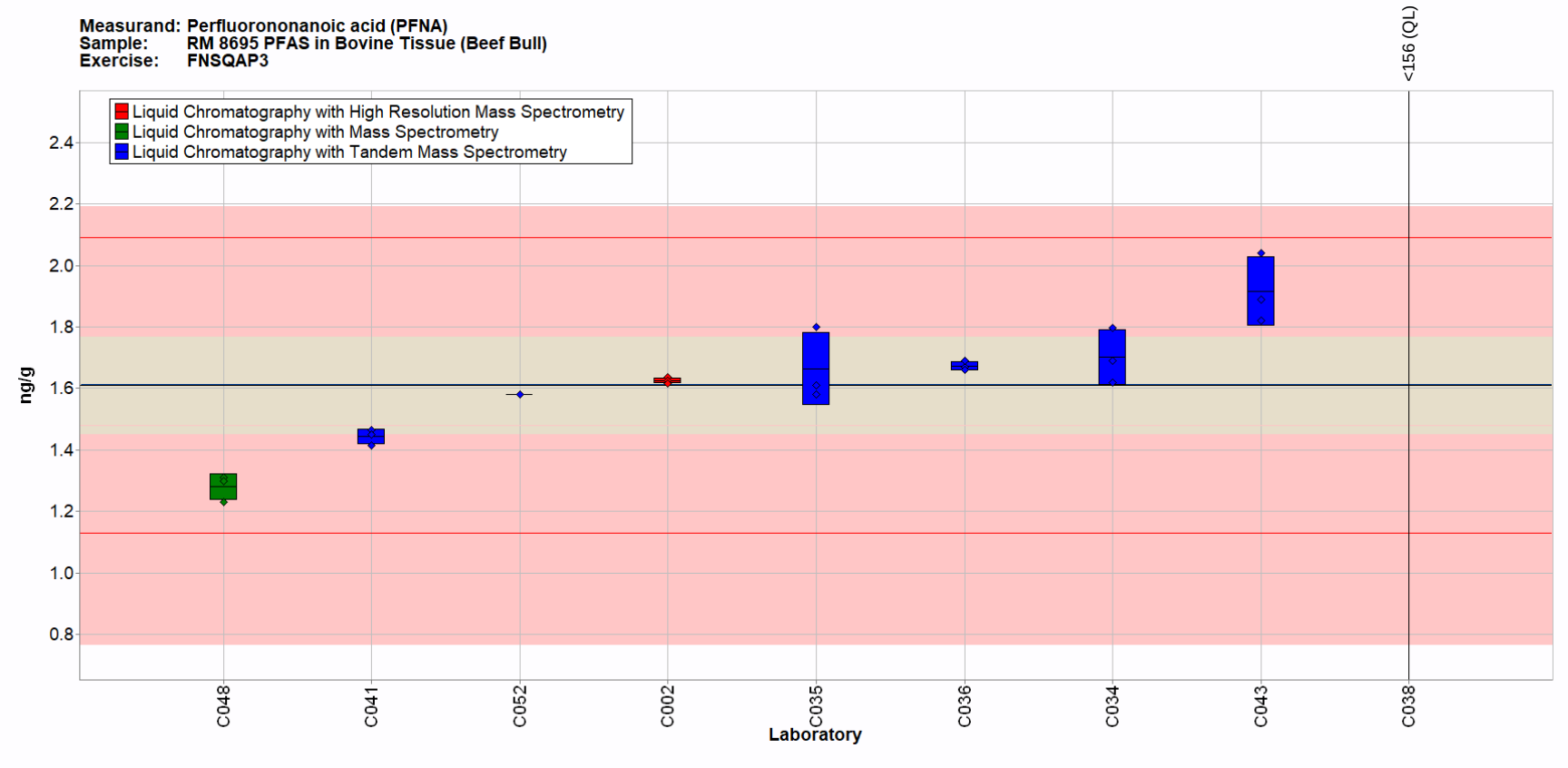

Fig. 5-11. PFNA in RM 8695 (data summary view – analytical method).

In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). A downward triangle represents data reported as an LOQ value. The color of the data point represents the analytical method employed as indicated in the figure key (the analytical approach reported by laboratory C038 was LC-MS). The solid black line represents the consensus mean, and the solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{comm}| \leq 2$. The beige shaded region represents the overlapping of the 95 % confidence interval for the consensus mean (green region) and the NIST range of tolerance (red region). The NIST range of tolerance encompasses the target value bounded by twice its uncertainty (U_{NIST}), and represents the range that results in an acceptable Z_{NIST} score, $|Z_{NIST}| \leq 2$.

The acceptable repeatability and reproducibility observed for mass fraction measurements of PFNA in RGTM 10225, RGTM 10226, and RM 8695 indicate that laboratories are performing comparably in determination of PFNA in food samples. Review of Fig. 5-12 and Fig. 5-13 reveals clustering around the 45-degree bisector in the comparison of RGTM 10225 with both samples, indicating the potential for a systematic error in the overall dataset. This clustering is less prominent in Fig. 5-14. However, given the small number of laboratories reporting results, global interpretation of trends is difficult and recommendations for improvement would require additional probing of method details.

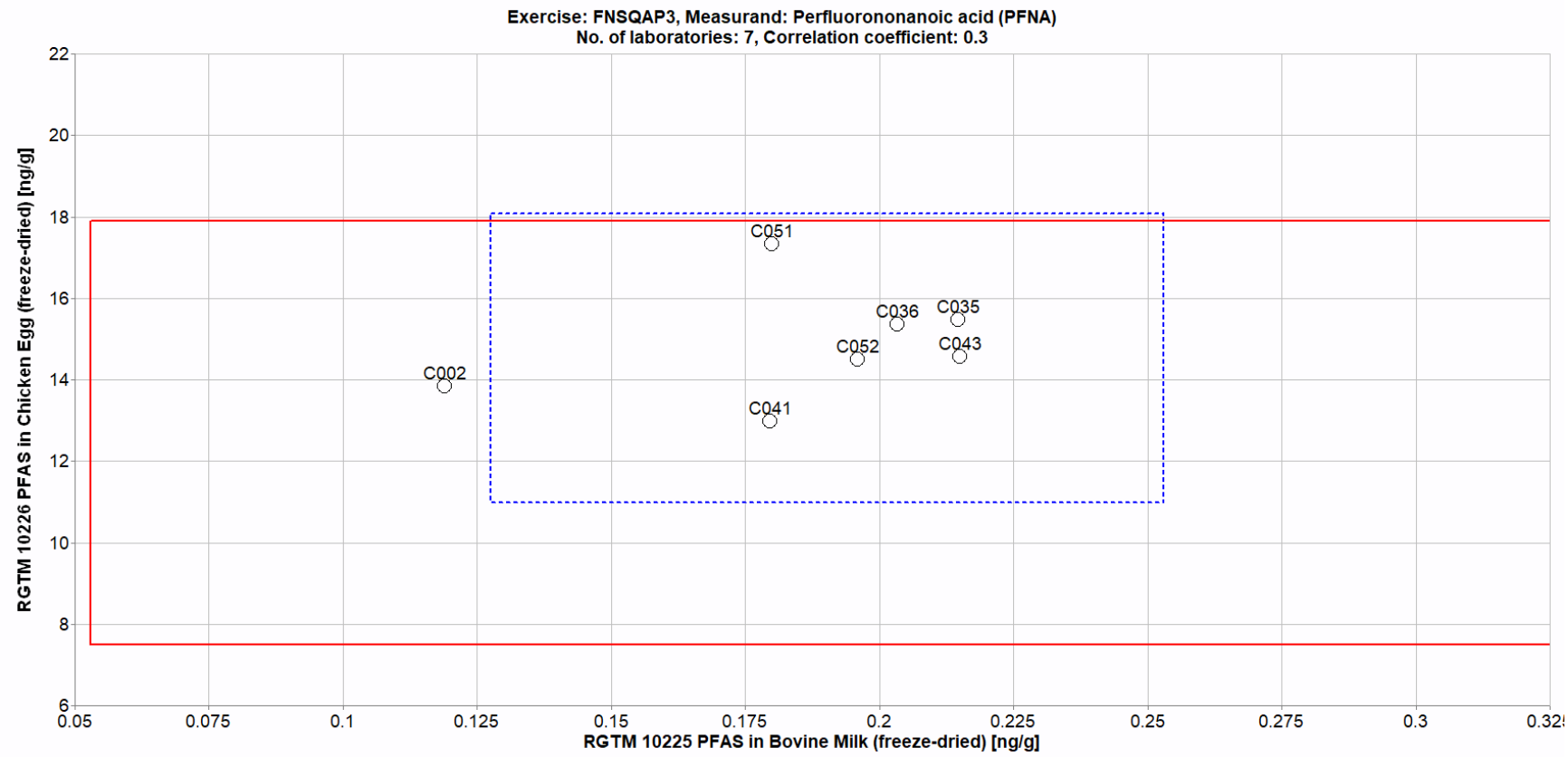

Fig. 5-12. Laboratory means for PFNA in RGTM 10225 and RGTM 10226 (sample/sample comparison view).

In this view, the individual laboratory mean for one sample (RGTM 10225) is compared to the individual laboratory mean for a second sample (RGTM 10226). The solid red box represents the NIST range of tolerance for the two samples, RGTM 10225 (x-axis) and RGTM 10226 (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 RGTM 10225 (x-axis) and RGTM 10226 (y-axis), calculated as the values above and below the consensus means that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$.

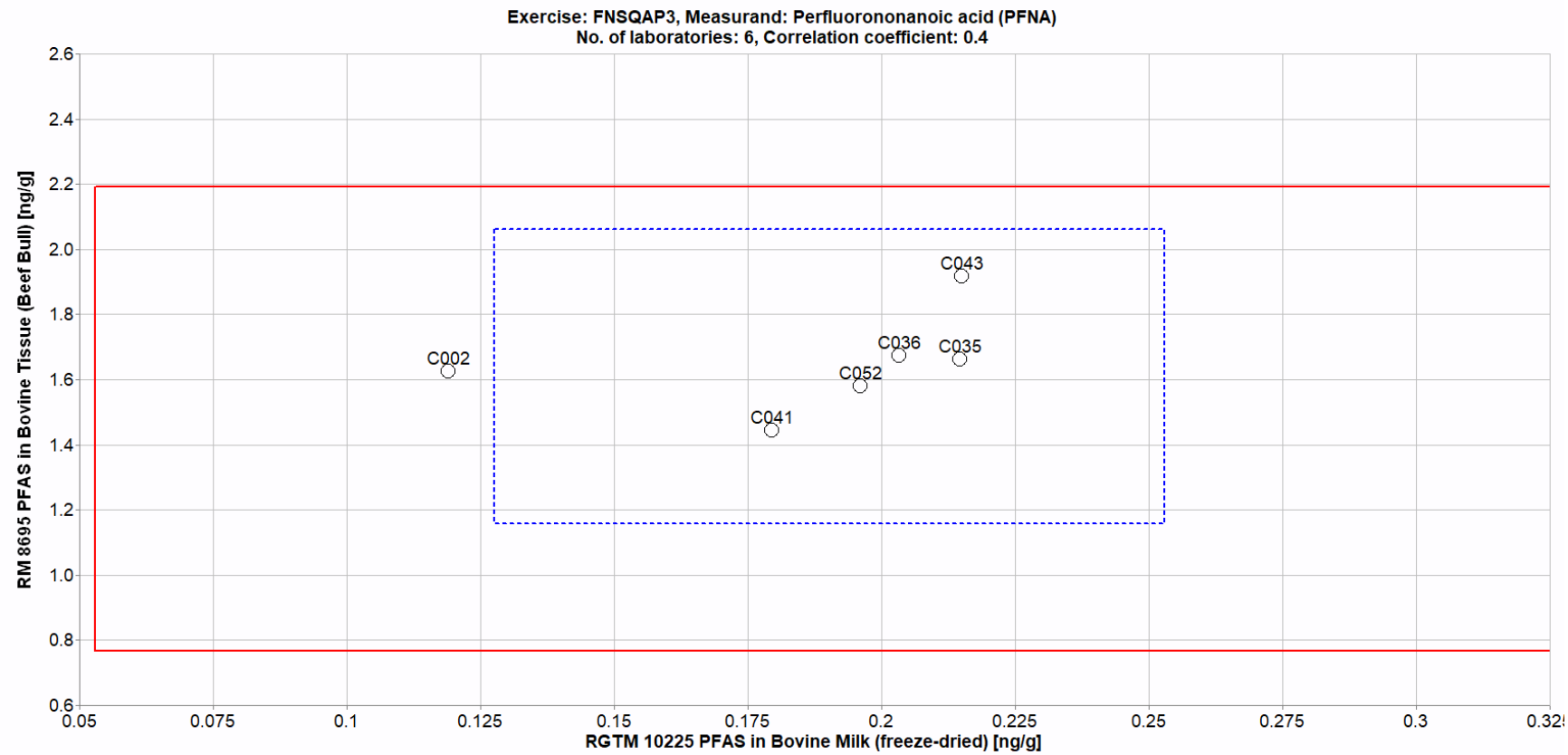

Fig. 5-13. Laboratory means for PFNA in RGTM 10225 and RM 8695 (sample/sample comparison view).

In this view, the individual laboratory mean for one sample (RGTM 10225) is compared to the individual laboratory mean for a second sample (RM 8695). The solid red box represents the NIST range of tolerance for the two samples, RGTM 10225 (x-axis) and RM 8695 (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 RGTM 10225 (x-axis) and RM 8695 (y-axis), calculated as the values above and below the consensus means that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$.

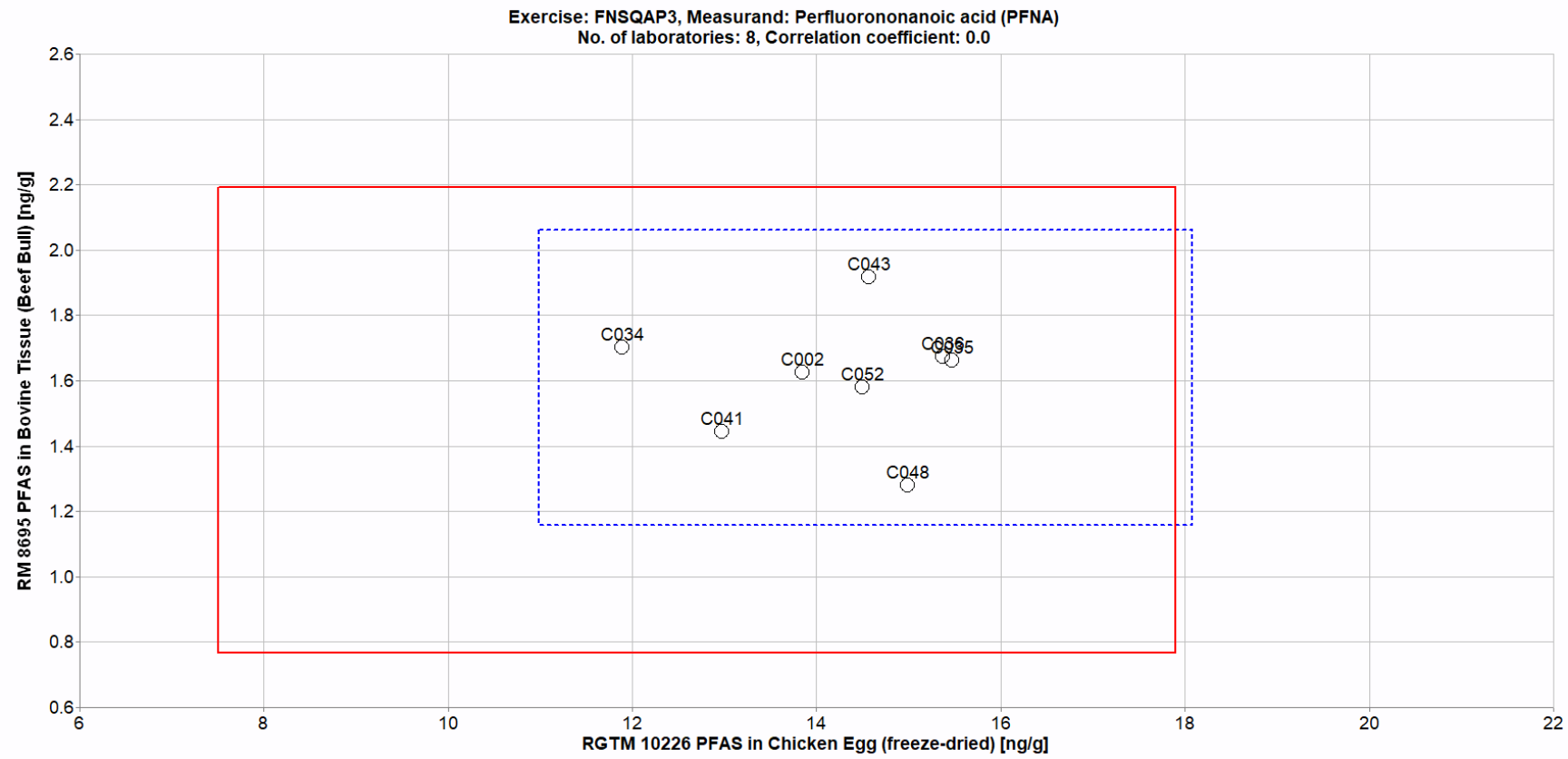

Fig. 5-14. Laboratory means for PFNA in RGTM 10226 and RM 8695 (sample/sample comparison view).

In this view, the individual laboratory mean for one sample (RGTM 10226) is compared to the individual laboratory mean for a second sample (RM 8695). The solid red box represents the NIST range of tolerance for the two samples, RGTM 10226 (x-axis) and RM 8695 (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 RGTM 10226 (x-axis) and RM 8695 (y-axis), calculated as the values above and below the consensus means that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$.

5.4.4. PFOA

Table 5-1 summarizes and Table 5-6 details the measured mass fraction results for PFOA reported by each participating laboratory. A limited number of quantitative mass fraction results were reported for PFOA in RGTM 10225, indicating that the level of PFOA in this milk sample is below the LOQs of most participant methods. Laboratories reporting LOQs higher than published expectations outlined in Table 5-3 should review practices to ensure that detection of PFOA is achievable at levels agreed upon by the PFAS measurement community.

Table 5-6 reveals that for the 8 and 9 laboratories reporting multiple quantitative mass fraction values for PFOA in RGTM 10226 and RM 8695, respectively, the within-laboratory variabilities were mostly acceptable with respect to published expectations of the PFAS measurement community (≤ 20 %). One laboratory reported mass fractions for PFOA in egg powder with 21.4 % RSD_r, slightly outside the desired range. The RSD_r of results from the one laboratory reporting quantitative mass fraction values for RGTM 10225 was 6.7 %, also within published expectations. The among-laboratory mass fraction variabilities (10.8 % and 11.2 % for RGTM 10226 and RM 8695, respectively) were also well within the published expectations for multiple laboratories using the same method (≤ 40 %). The consensus mass fraction ranges were within the target ranges for both RGTM 10226 and RM 8695.

As described in the previous sections for PFHxS and PFNA, and as shown in Fig. 5-15 and Fig. 5-16, laboratories reported using a variety of sample preparation methods for the determination of PFOA in the egg and beef samples. Similarly, Fig. 5-17 and Fig. 5-18 indicate that all laboratories reported using LC-MS-based techniques for the determination of PFOA in the two samples.

As noted for PFNA, the laboratory using QuEChERSER for sample preparation also reported the lowest mass fraction for PFOA. This potential sample-specific effect was not observed for the beef sample and the laboratory did not report results for the milk sample. Additionally, for the beef sample, the lowest reported mass fraction results were obtained using solvent extraction with unmodified methanol followed by LC-MS, while another laboratory reporting use of solvent extraction and LC-MS reported results below their method LOQ. As described previously, laboratories using solvent extraction and LC-MS for determination of PFOA should consider method adjustments to improve sensitivity. The consistency of these trends for both PFNA and PFOA may potentially indicate broader, sample-specific methodology challenges for these laboratories.

The acceptable repeatability and reproducibility observed for measurements of PFNA in RGTM 10226 and RM 8695 indicate that laboratories are generally performing comparably in determination of PFOA in food samples. Review of Fig. 5-19 does not reveal any trends, so recommendations for improvement would require additional probing of method details.

Table 5-6. Data summary table for PFOA in foods.

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

		PFOA													
		RGTM 10225 PFAS in Bovine Milk (freeze-dried) (ng/g)					RGTM 10226 PFAS in Chicken Egg (freeze-dried) (ng/g)					RM 8695 PFAS in Bovine Tissue (Beef Bull) (ng/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target									7.97	0.58				0.84	0.13
	C002	< 0.096	< 0.096	< 0.096			8.081	7.904	7.925	7.97	0.10	0.736	0.758	0.77	0.755	0.017
	C019															
	C022															
	C029															
	C030															
	C034						7.056	6.643	6.765	6.82	0.21	0.741	0.776	0.763	0.760	0.018
	C035	< 0.047	< 0.047	< 0.047			8.55	8.62	8.87	8.68	0.17	0.726	0.754	0.743	0.741	0.014
	C036	0.048	0.042	0.046	0.05	0.00	9.04	9.14	9.46	9.21	0.22	0.71	0.69	0.66	0.687	0.025
	C038	< 156.0	< 156.0	< 156.0			< 156.0	< 156.0	< 156.0			< 156.0	< 156.0	< 156.0		
	C040															
	C041	< 0.010	< 0.010	< 0.010			7.7905	8.0789	7.8114	7.89	0.16	0.6795	0.7263	0.6811	0.696	0.027
	C043	< 0.020	< 0.020	< 0.020			8.7	9.3	8.9	8.97	0.31	0.87	0.89	0.99	0.917	0.064
	C048	< 0.500	< 0.500	< 0.500			8.63	8.48	5.73	7.61	1.63	0.512	0.531	0.571	0.538	0.030
	C051	< 0.083	< 0.083	< 0.083			8.6	8.6	7.9	8.37	0.40					
	C052	< 0.050					8.47			8.47		0.692			0.692	
	C053															
	C054															
Community Results		Consensus Mean					Consensus Mean			8.23		Consensus Mean			0.722	
		Consensus Standard Deviation					Consensus Standard Deviation			0.89		Consensus Standard Deviation			0.081	
		Maximum			0.05		Maximum			9.21		Maximum			0.917	
		Minimum			0.05		Minimum			6.82		Minimum			0.538	
		N			1		N			9		N			8	

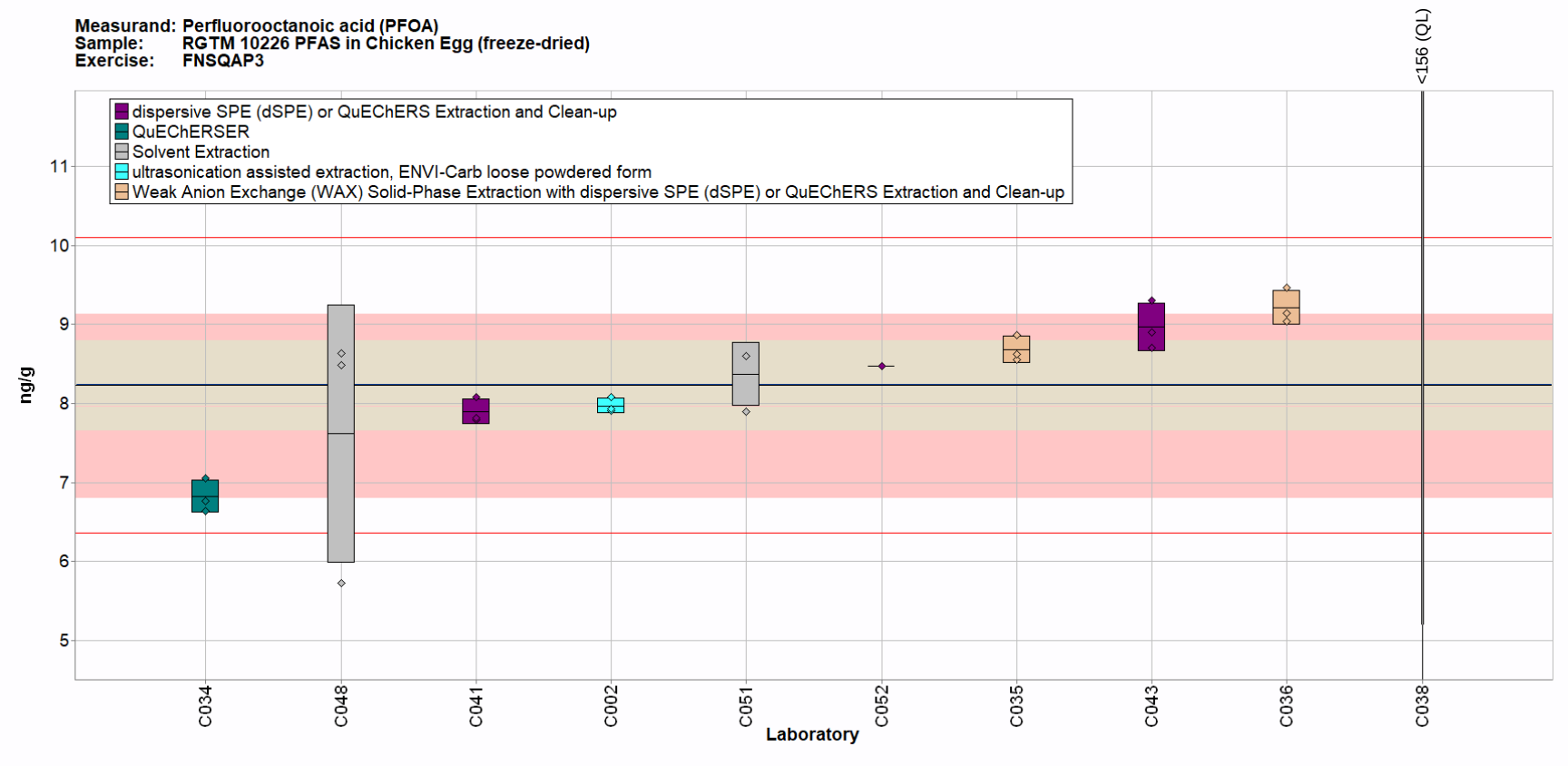

Fig. 5-15. PFOA in RGTM 10226 (data summary view – sample preparation method).

In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). A downward triangle represents data reported as an LOQ value. The color of the data point represents the sample preparation method employed as indicated in the figure key (the sample preparation approach reported by laboratory C038 was solvent extraction). The solid black line represents the consensus mean, and the solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{comm}| \leq 2$. The beige shaded region represents the overlapping of the 95 % confidence interval for the consensus mean (green region) and the NIST range of tolerance (red region). The NIST range of tolerance encompasses the target value bounded by twice its uncertainty (U_{NIST}), and represents the range that results in an acceptable Z_{NIST} score, $|Z_{NIST}| \leq 2$.

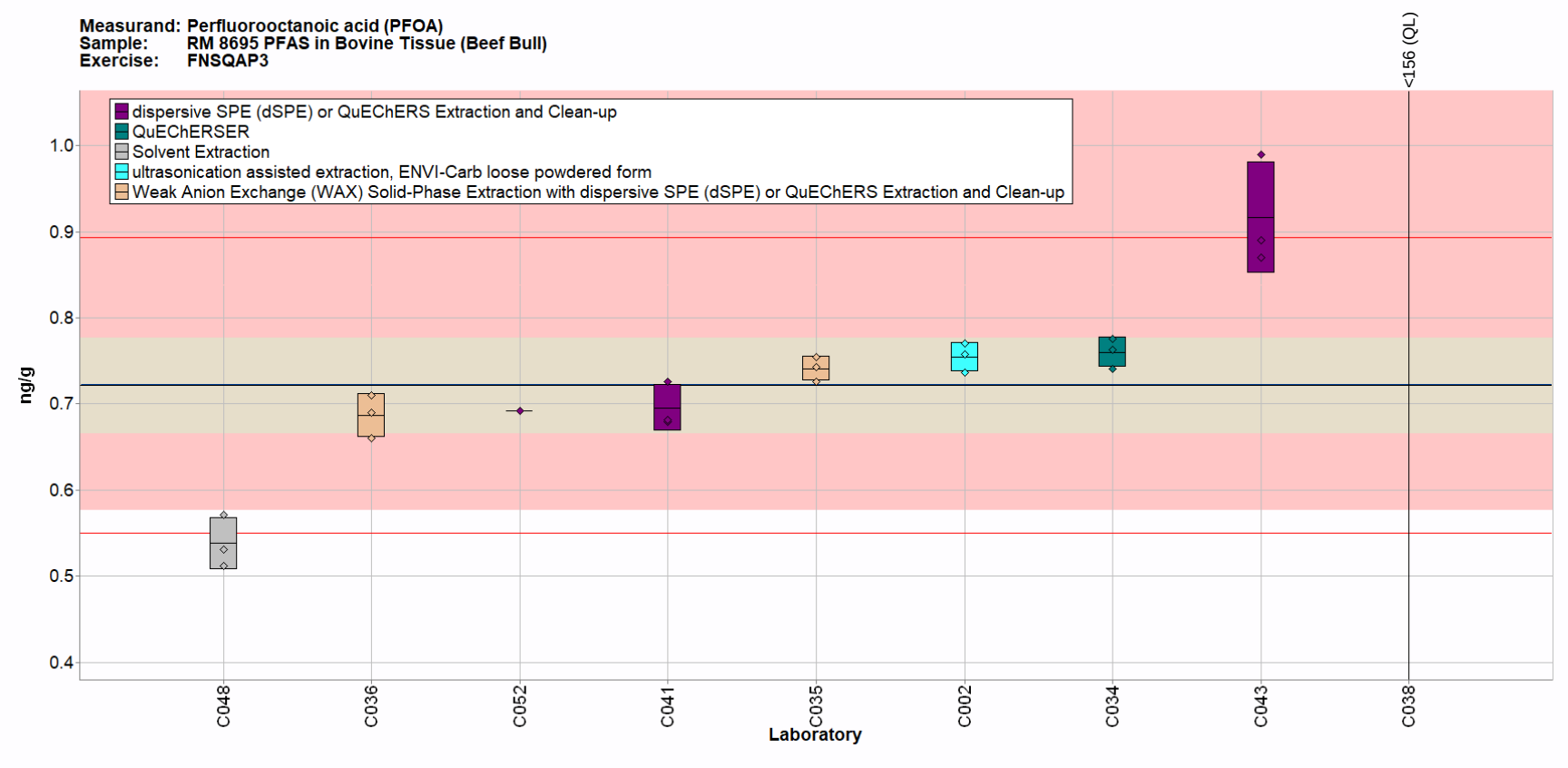

Fig. 5-16. PFOA in RM 8695 (data summary view – sample preparation method).

In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). A downward triangle represents data reported as an LOQ value. The color of the data point represents the sample preparation method employed as indicated in the figure key (the sample preparation approach reported by laboratory C038 was solvent extraction). The solid black line represents the consensus mean, and the solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{comm}| \leq 2$. The beige shaded region represents the overlapping of the 95 % confidence interval for the consensus mean (green region) and the NIST range of tolerance (red region). The NIST range of tolerance encompasses the target value bounded by twice its uncertainty (U_{NIST}), and represents the range that results in an acceptable Z_{NIST} score, $|Z_{NIST}| \leq 2$.

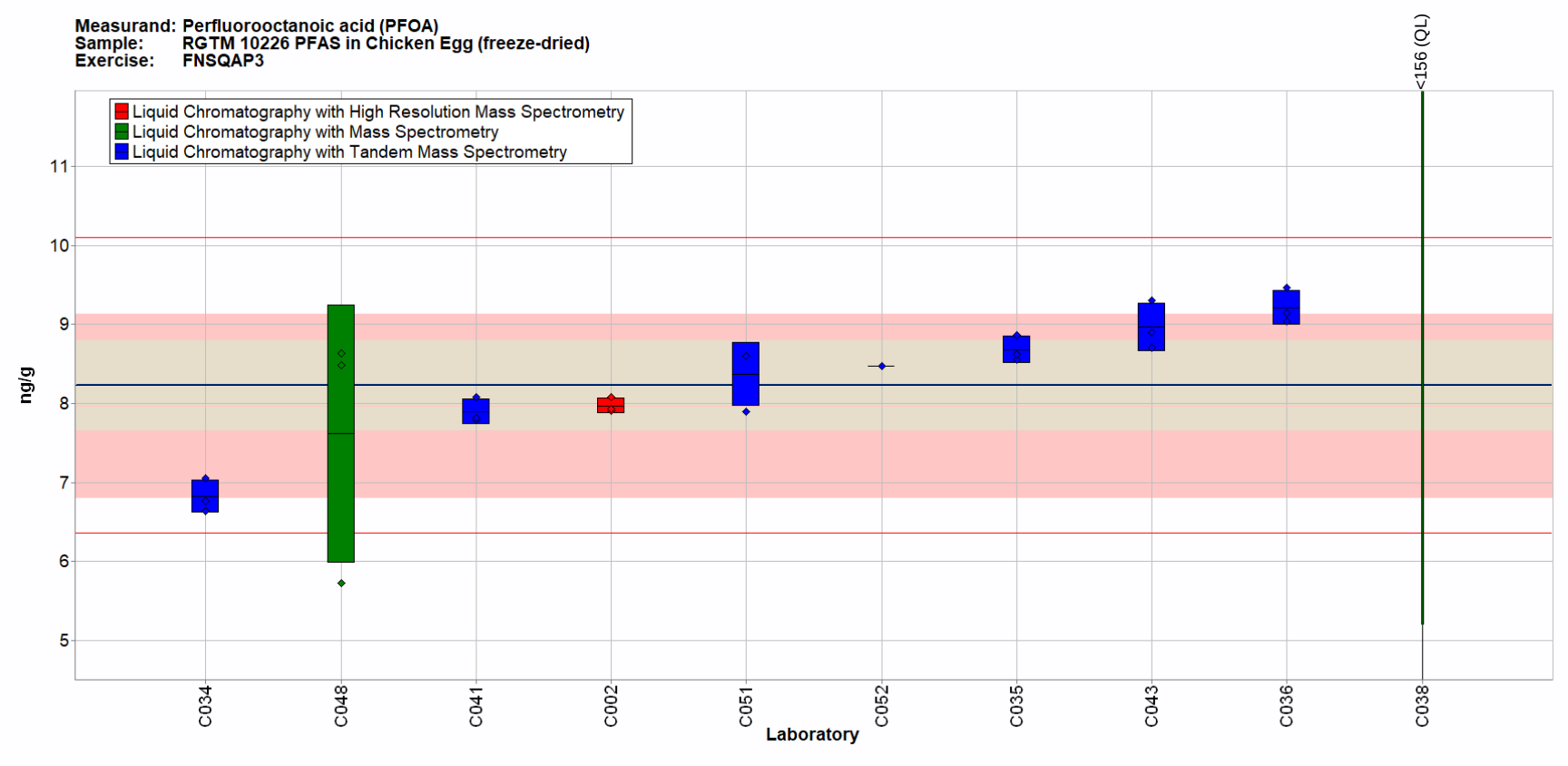

Fig. 5-17. PFOA in RGTM 10226 (data summary view – analytical method).

In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). A downward triangle represents data reported as an LOQ value. The color of the data point represents the analytical method employed as indicated in the figure key (the analytical approach reported by laboratory C038 was LC-MS). The solid black line represents the consensus mean, and the solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{comm}| \leq 2$. The beige shaded region represents the overlapping of the 95 % confidence interval for the consensus mean (green region) and the NIST range of tolerance (red region). The NIST range of tolerance encompasses the target value bounded by twice its uncertainty (U_{NIST}), and represents the range that results in an acceptable Z_{NIST} score, $|Z_{NIST}| \leq 2$.

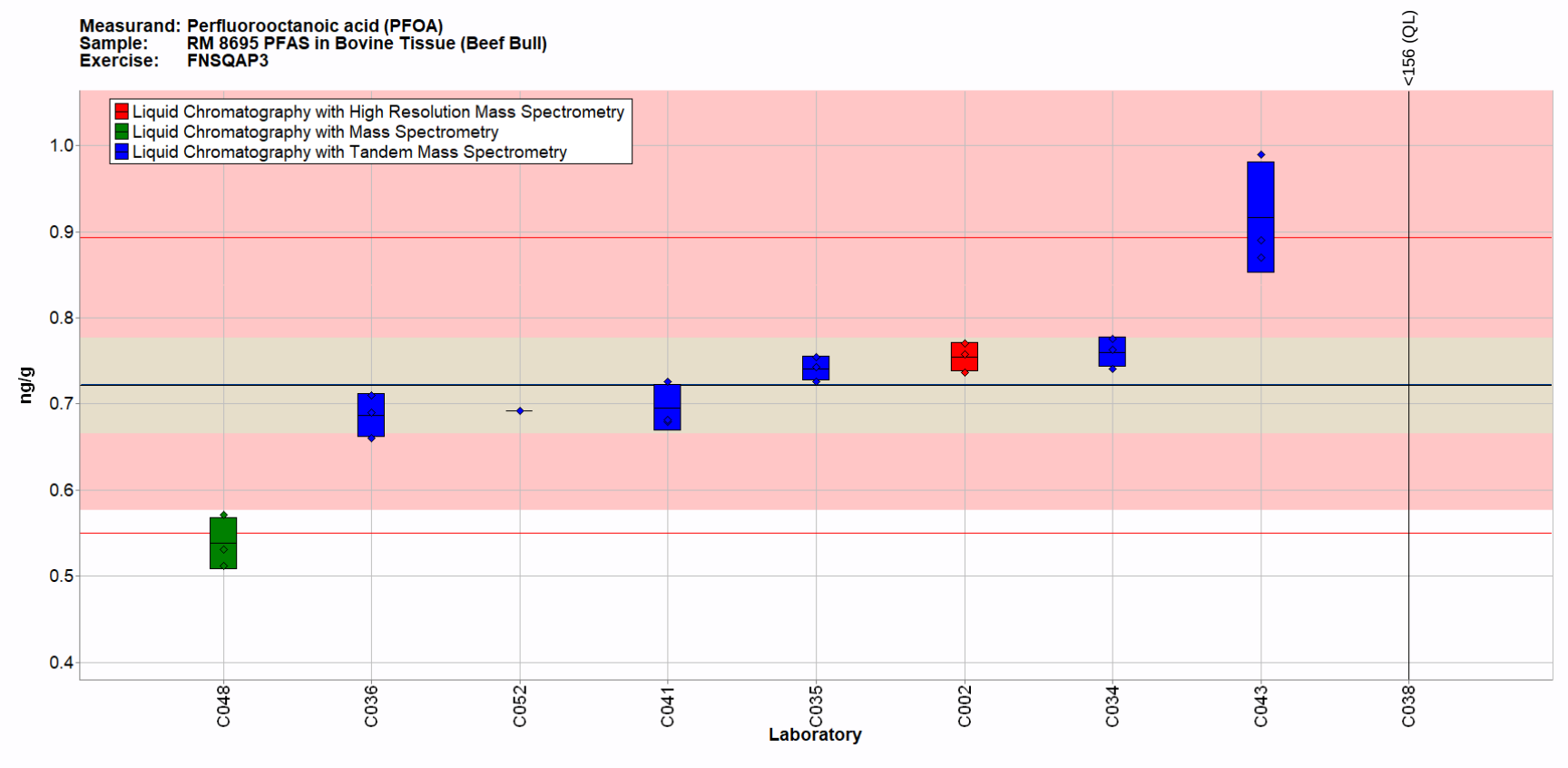

Fig. 5-18. PFOA in RM 8695 (data summary view – analytical method).

In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). A downward triangle represents data reported as an LOQ value. The color of the data point represents the analytical method employed as indicated in the figure key (the analytical approach reported by laboratory C038 was LC-MS). The solid black line represents the consensus mean, and the solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{comm}| \leq 2$. The beige shaded region represents the overlapping of the 95 % confidence interval for the consensus mean (green region) and the NIST range of tolerance (red region). The NIST range of tolerance encompasses the target value bounded by twice its uncertainty (U_{NIST}), and represents the range that results in an acceptable Z_{NIST} score, $|Z_{NIST}| \leq 2$.

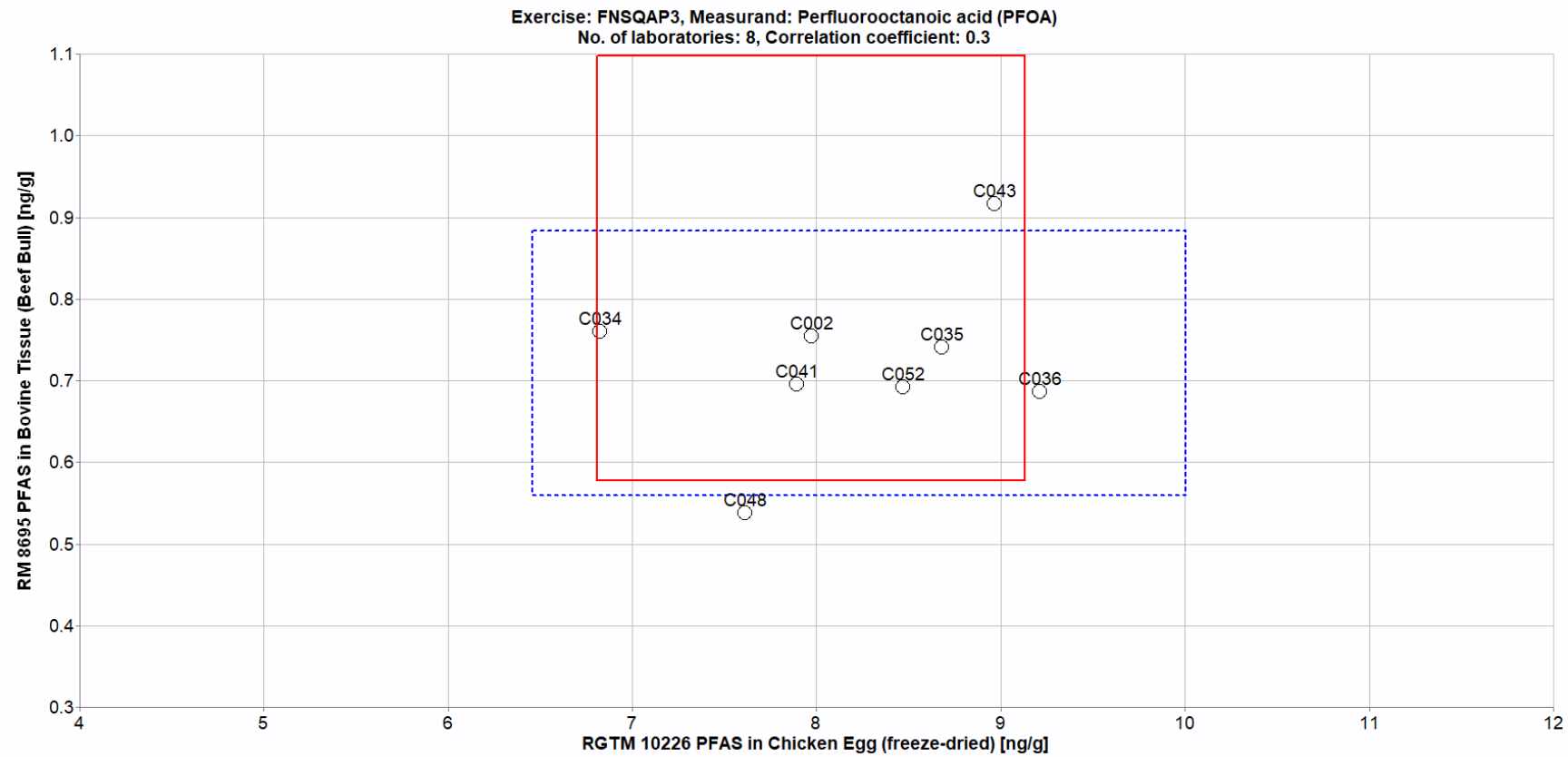

Fig. 5-19. Laboratory means for PFOA in RGTM 10226 and RM 8695 (sample/sample comparison view).

In this view, the individual laboratory mean for one sample (RGTM 10226) is compared to the individual laboratory mean for a second sample (RM 8695). The solid red box represents the NIST range of tolerance for the two samples, RGTM 10225 (x-axis) and RM 8695 (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 RGTM 10226 (x-axis) and RM 8695 (y-axis), calculated as the values above and below the consensus means that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$.

5.4.5. PFOS

Table 5-1 summarizes and Table 5-7 details the measured mass fraction results for PFOS reported by each participating laboratory. For the 8 to 9 laboratories reporting multiple quantitative mass fraction values for PFOS in the 3 samples, the within-laboratory variabilities were mostly acceptable with respect to published expectations of the PFAS measurement community (≤ 20 %). One laboratory reported mass fractions for PFOS in milk powder with 29 % RSD_r, significantly outside the desired range. The among-laboratory variabilities of 37 %, 26 %, and 24 % for RGTM 10225, RGTM 10226, and RM 8695, respectively, were also well within the published expectations for multiple laboratories using the same method (≤ 40 %). The consensus mass fraction range for PFOS in RGTM 10225 was within the target range, while the consensus mass fractions for PFOS in RGTM 10226 and RM 8695 were within the target range but the consensus range overlapped only the upper portion of the target range.

As described in the previous sections for PFHxS, PFNA, and PFOA, and as shown in Fig. 5-20, Fig. 5-21, and Fig. 5-22, laboratories reported using a variety of sample preparation methods for the determination of PFNA in the three samples. Similarly, Fig. 5-23, Fig. 5-24, and Fig. 5-25 indicate that all laboratories reported using LC-MS-based techniques for the determination of PFOS in the three samples.

As noted for PFNA and PFOA, the laboratory using QuEChERSER for sample preparation also reported the lowest mass fraction for PFOS. This potential sample-specific effect was not observed for the beef sample and the laboratory did not report results for the milk sample. Additionally, as described previously for PFNA and PFOA, the lowest reported mass fractions for PFOS in the beef sample were obtained using solvent extraction with unmodified methanol followed by LC-MS, while another laboratory reporting use of solvent extraction and LC-MS reported results below their method LOQ. Laboratories using solvent extraction and LCMS for determination of PFOS should consider method adjustments to improve sensitivity. The consistency of these trends for PFNA, PFOA, and PFOS may indicate broader, sample-specific methodology challenges for these laboratories.

The acceptable repeatability and reproducibility observed for most measurements of PFOS in RGTM 10225, RGTM 10226, and RM 8695 indicate that laboratories are generally performing comparably in determination of PFOS in food samples. Review of Fig. 5-26, Fig. 5-27, and Fig. 5-28 reveals clustering around the 45-degree bisector, indicating the potential for a systematic error in the overall dataset. However, given the small number of laboratories reporting results, global interpretation of trends is difficult and recommendations for improvement would require additional probing of method details.

Table 5-7. Data summary table for PFOS in foods.

		PFOS														
		RGTM 10225 PFAS in Bovine Milk (freeze-dried) (ng/g)					RGTM 10226 PFAS in Chicken Egg (freeze-dried) (ng/g)					RM 8695 PFAS in Bovine Tissue (Beef Bull) (ng/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target				0.371	0.080				26.2	3.2				20.0	2.2
	C002	0.377	0.322	0.362	0.35	0.03	26.49	25.835	26.212	26.2	0.3	21.207	22.353	21.725	21.8	0.6
	C019															
	C022															
	C029															
	C030															
	C034						22.972	20.584	19.663	21.1	1.7	24.302	24.174	23.371	23.9	0.5
	C035	0.558	0.503	0.523	0.53	0.03	28.5	29.8	28.1	28.8	0.9	22.4	21.6	23.1	22.4	0.8
	C036	0.48	0.49	0.49	0.49	0.01	30.04	29.66	29.2	29.6	0.4	25.19	24.27	24.44	24.6	0.5
	C038	< 156.0	< 156.0	< 156.0			< 156.0	< 156.0	< 156.0			< 156.0	< 156.0	< 156.0		
	C040															
	C041	0.4453	0.4335	0.4427	0.44	0.01	39.0855	38.8857	38.6018	38.9	0.2	27.1283	26.4193	26.2564	26.6	0.5
	C043	0.53	0.57	0.57	0.56	0.02	31.7	32.3	33.4	32.5	0.9	27.9	29.5	30.8	29.4	1.5
	C048	0.202	0.284	0.367	0.28	0.08	28.5	31.5	28.6	29.5	1.7	19	16.9	16.7	17.5	1.3
	C051	0.33	0.34	0.31	0.33	0.02	41	35	39	38.3	3.1					
	C052	0.265			0.27		22			22.0		17.7			17.7	
	C053															
	C054															
Community Results		Consensus Mean			0.40		Consensus Mean			29.7		Consensus Mean			23.0	
		Consensus Standard Deviation			0.15		Consensus Standard Deviation			7.8		Consensus Standard Deviation			5.5	
		Maximum			0.56		Maximum			38.9		Maximum			29.4	
		Minimum			0.27		Minimum			21.1		Minimum			17.5	
		N			8		N			9		N			8	

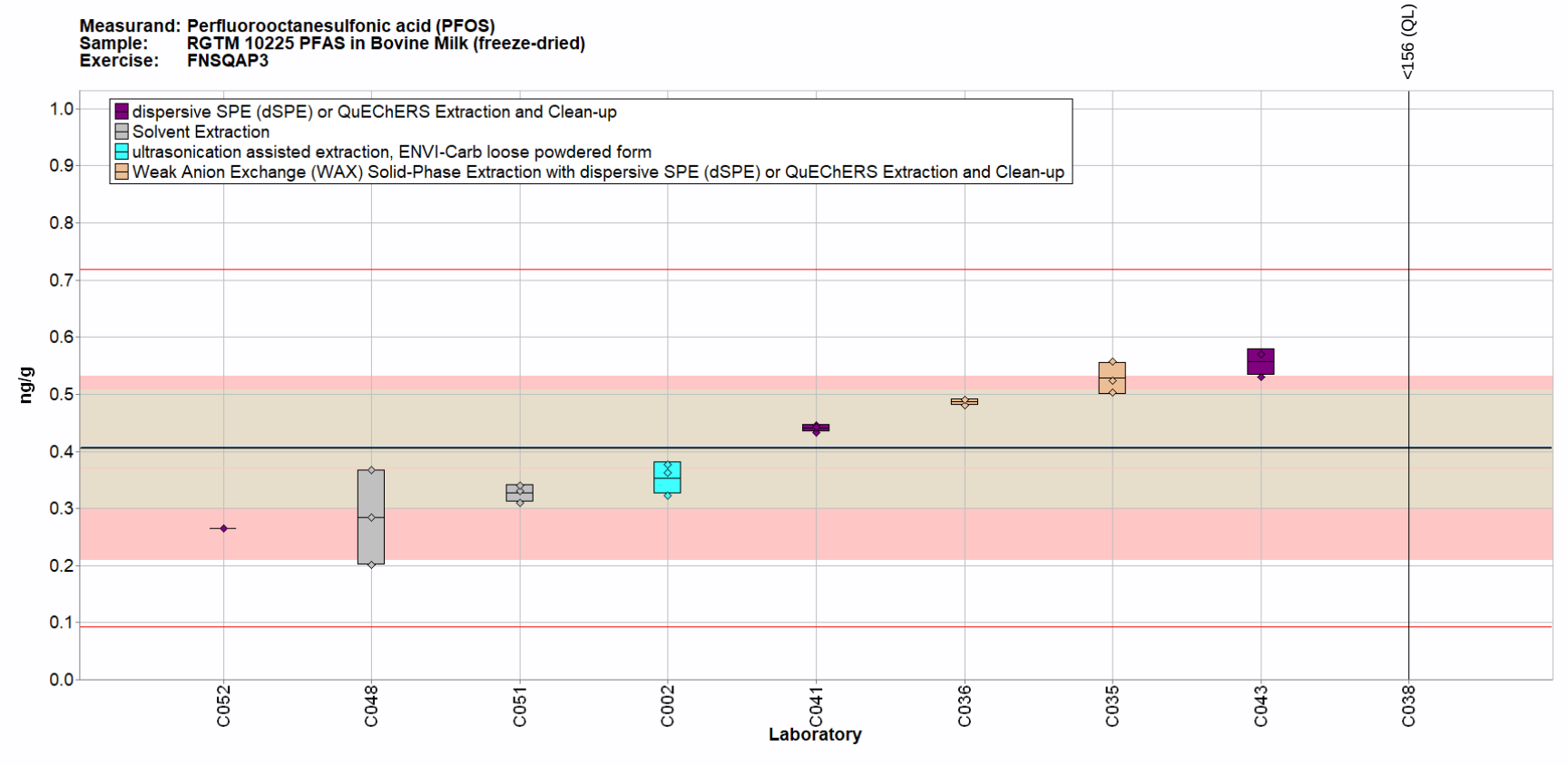

Fig. 5-20. PFOS in RGTM 10225 (data summary view – sample preparation method).

In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). A downward triangle represents data reported as an LOQ value. The color of the data point represents the sample preparation method employed as indicated in the figure key (the sample preparation approach reported by laboratory C038 was solvent extraction). The solid black line represents the consensus mean, and the solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{comm}| \leq 2$. The beige shaded region represents the overlapping of the 95 % confidence interval for the consensus mean (green region) and the NIST range of tolerance (red region). The NIST range of tolerance encompasses the target value bounded by twice its uncertainty (U_{NIST}), and represents the range that results in an acceptable Z_{NIST} score, $|Z_{NIST}| \leq 2$.

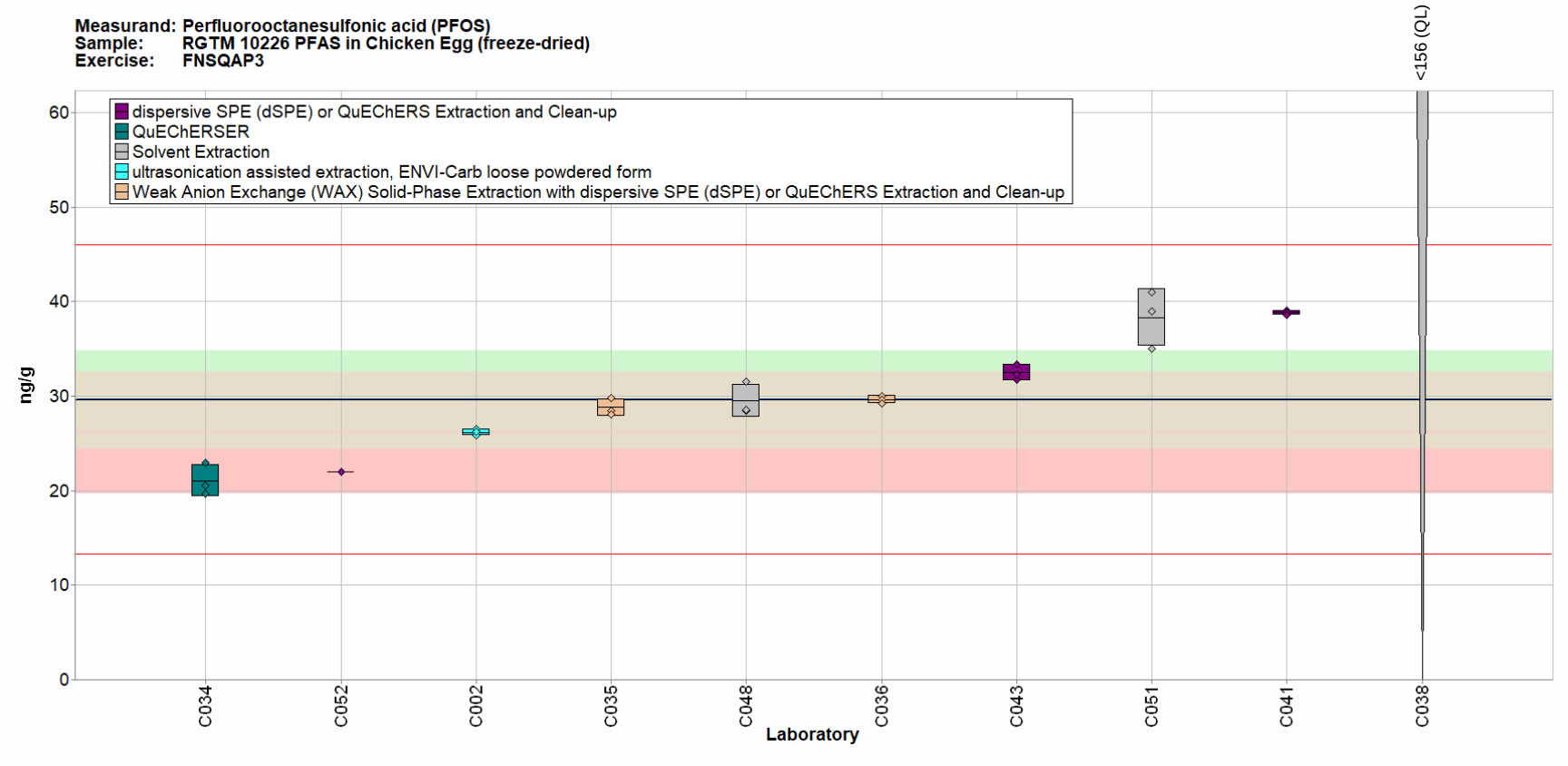

Fig. 5-21. PFOS in RGTM 10226 (data summary view – sample preparation method).

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 black 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 beige shaded region represents the overlapping of the 95 % confidence interval for the consensus mean (green region) and the NIST range of tolerance (red region).

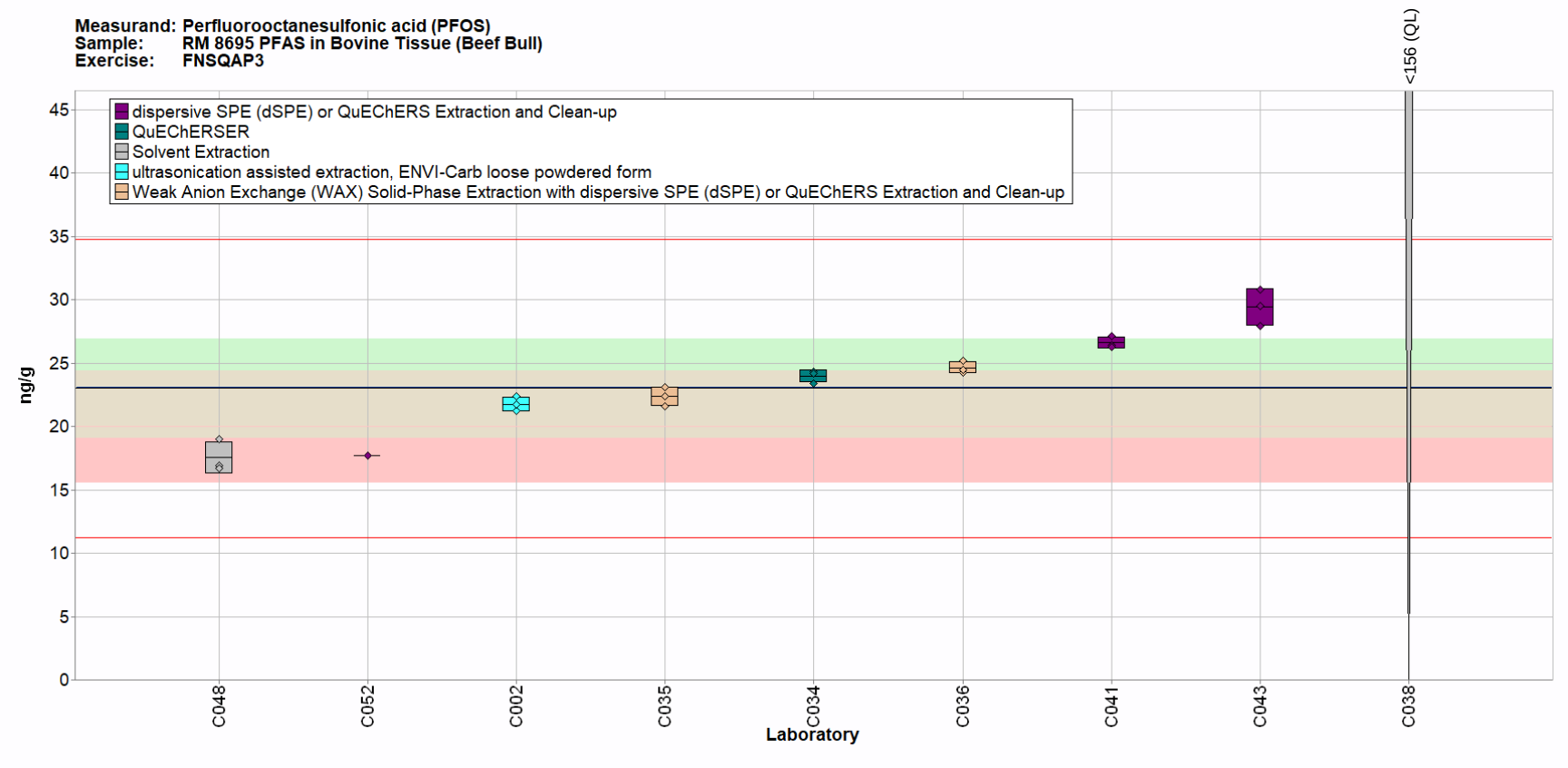

Fig. 5-22. PFOS in RM 8695 (data summary view – sample preparation method).

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 black 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 beige shaded region represents the overlapping of the 95 % confidence interval for the consensus mean (green region) and the NIST range of tolerance (red region).

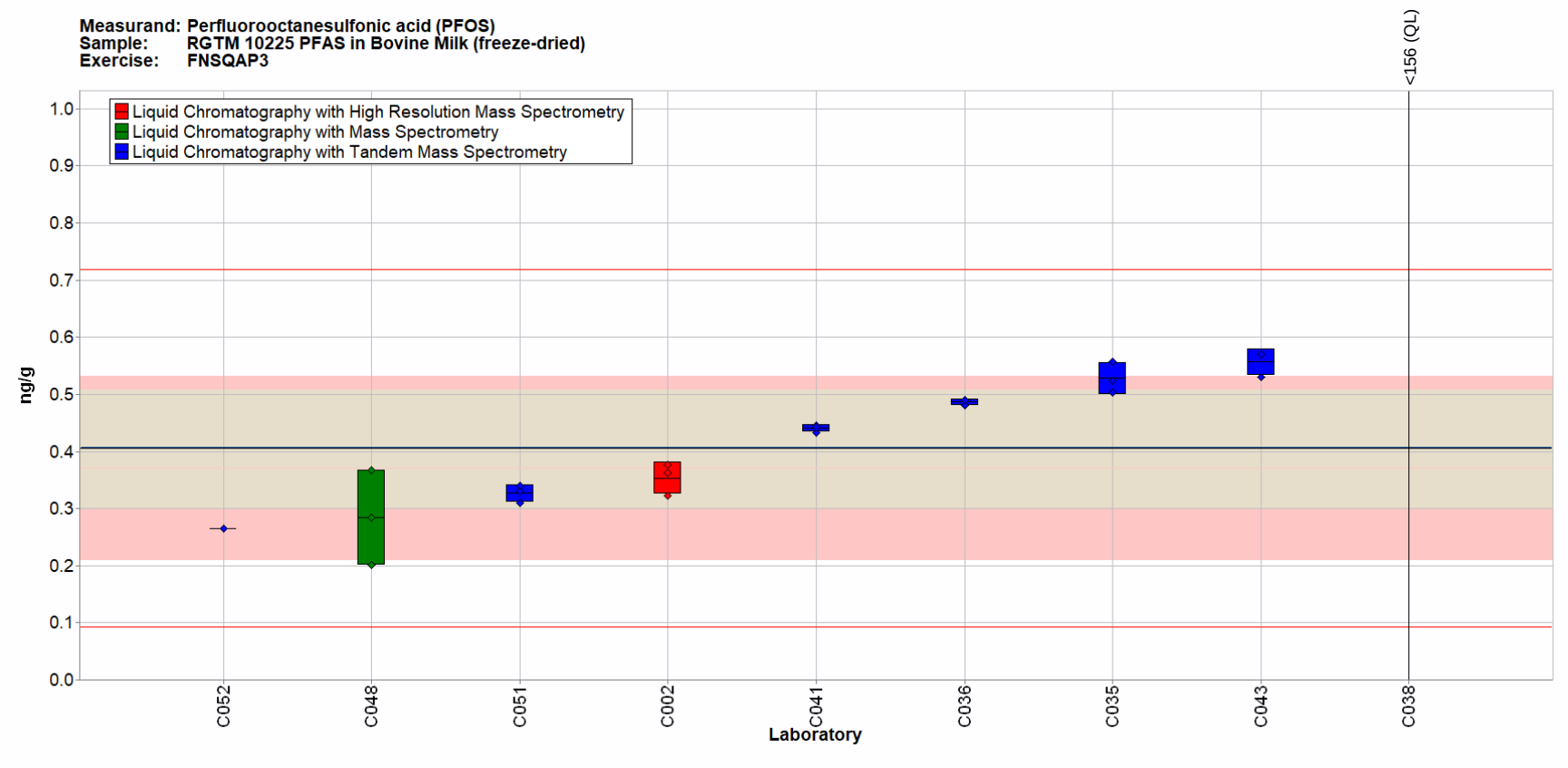

Fig. 5-23. PFOS in RGTM 10225 (data summary view – analytical method).

In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). A downward triangle represents data reported as an LOQ value. The color of the data point represents the analytical method employed as indicated in the figure key (the analytical approach reported by laboratory C038 was LC-MS). The solid black line represents the consensus mean, and the solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{comm}| \leq 2$. The beige shaded region represents the overlapping of the 95 % confidence interval for the consensus mean (green region) and the NIST range of tolerance (red region). The NIST range of tolerance encompasses the target value bounded by twice its uncertainty (U_{NIST}), and represents the range that results in an acceptable Z_{NIST} score, $|Z_{NIST}| \leq 2$.

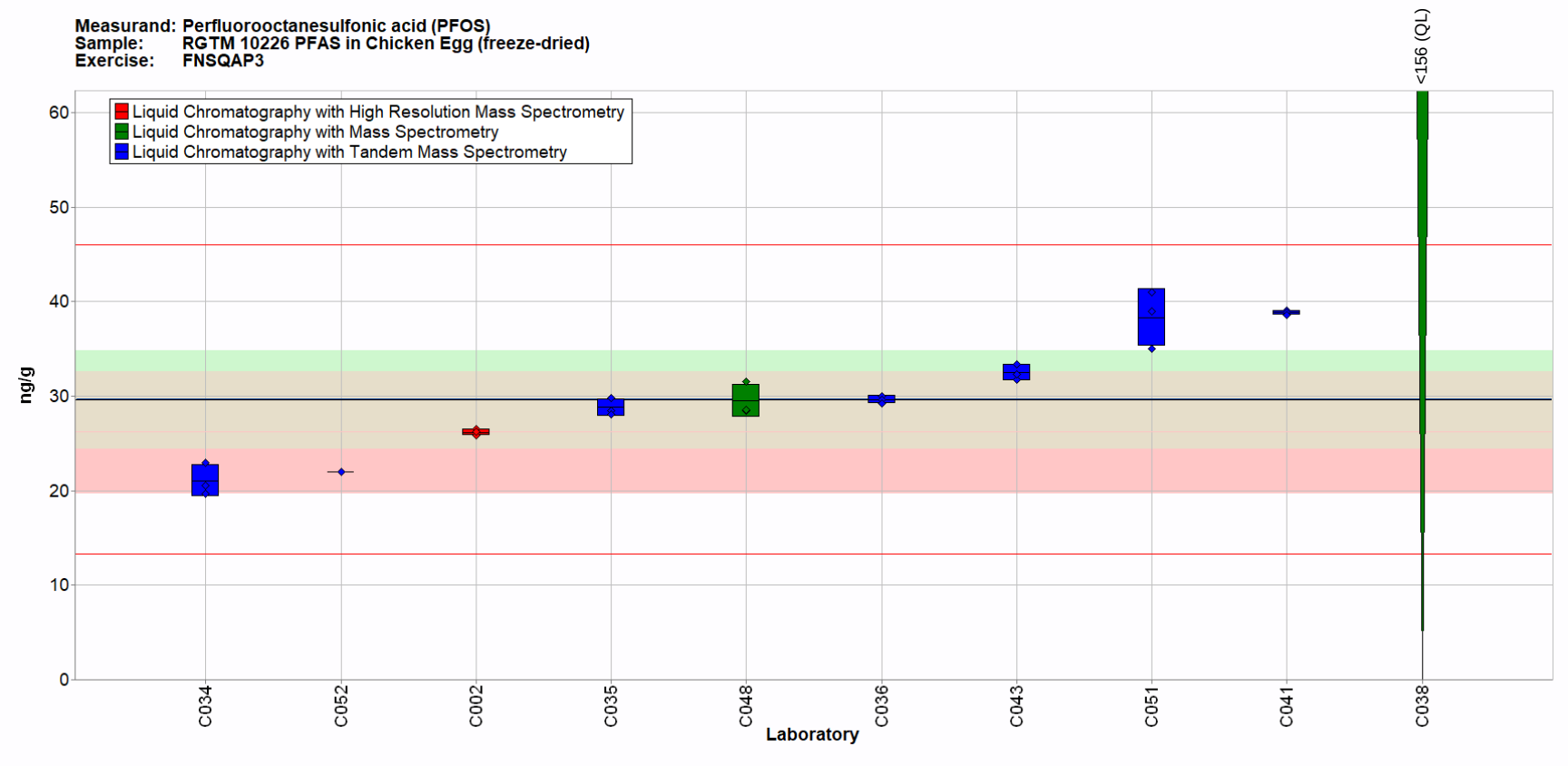

Fig. 5-24. PFOS in RGTM 10226 (data summary view – analytical method).

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 black 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 beige shaded region represents the overlapping of the 95 % confidence interval for the consensus mean (green region) and the NIST range of tolerance (red region).

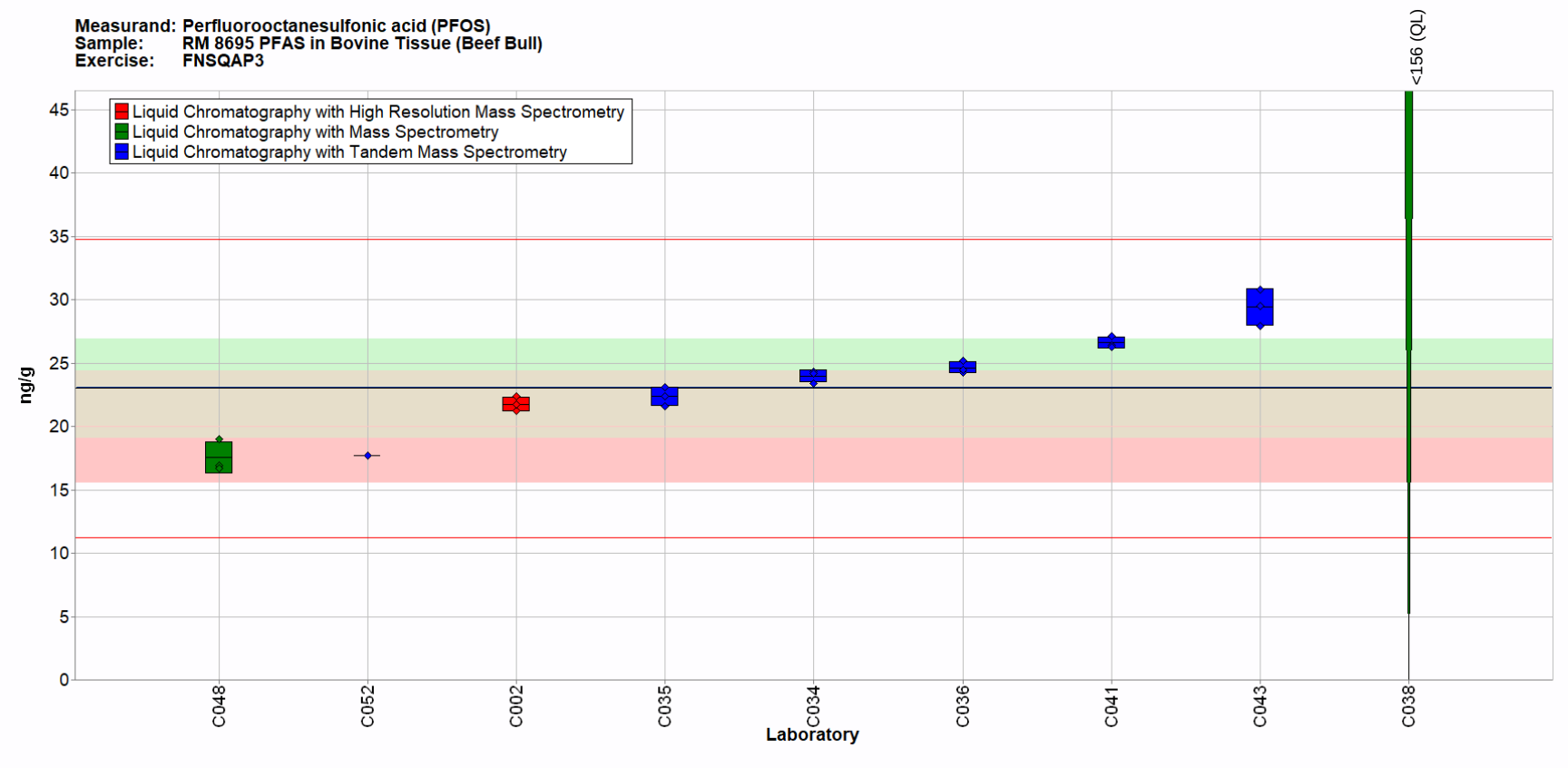

Fig. 5-25. PFOS in RM 8695 (data summary view – analytical method).

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 black 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 Z'_{comm} [OBJ] $|Z'_{\mathrm{comm}}| \leq 2$ [OBJ]. The red shaded region represents the NIST range of tolerance, which encompasses the target value bounded by twice its uncertainty (U_{NIST}), and represents Z_{NIST} [OBJ] $|Z_{\mathrm{NIST}}| \leq 2$ [OBJ]. The beige shaded region represents the overlapping of the 95 % confidence interval for the consensus mean (green region) and the NIST range of tolerance (red region).

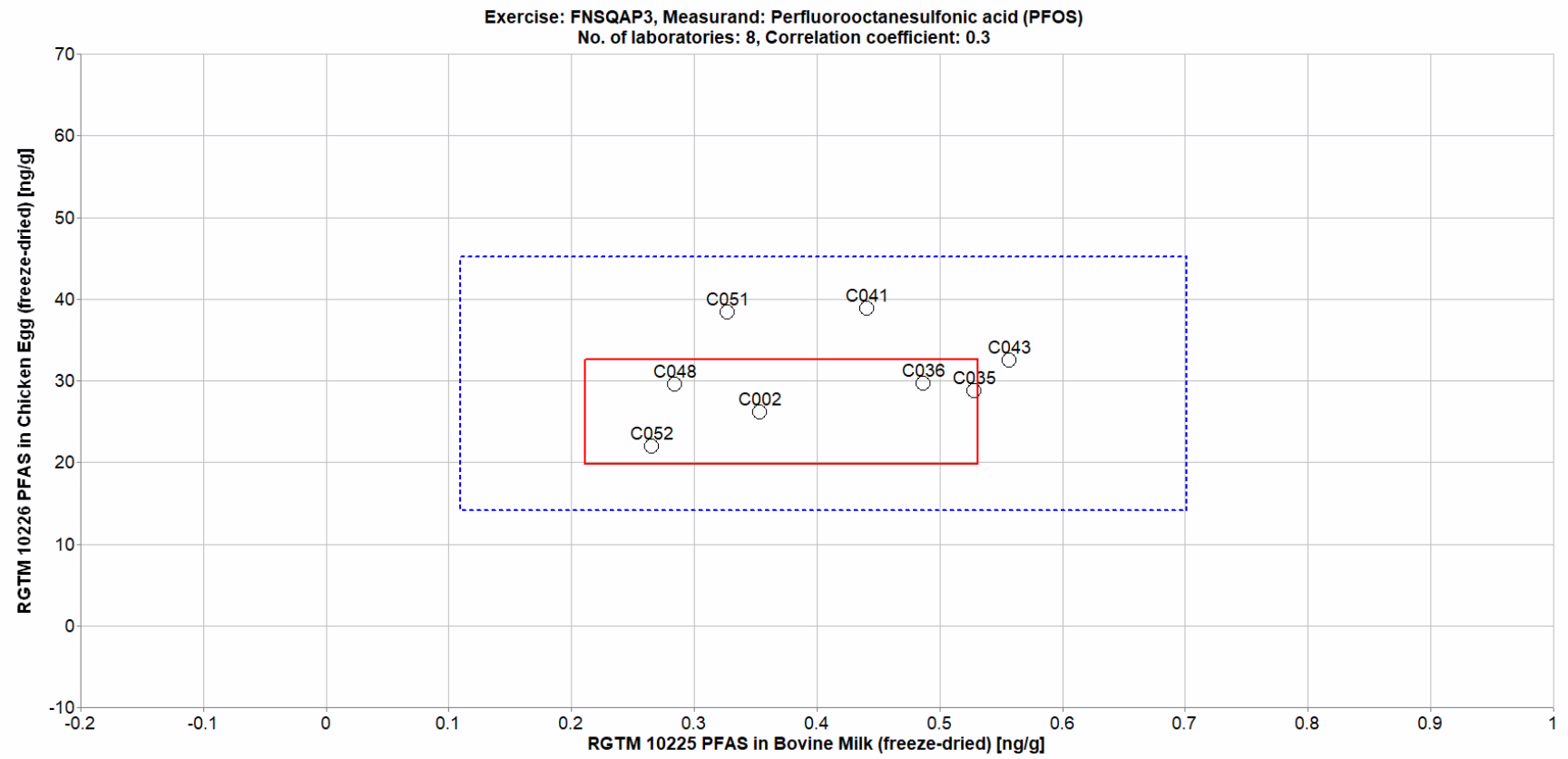

Fig. 5-26. Laboratory means for PFOS in RGTM 10225 and RGTM 10226 (sample/sample comparison view).

In this view, the individual laboratory mean for one sample (RGTM 10225) is compared to the individual laboratory mean for a second sample (RGTM 10226). The solid red box represents the NIST range of tolerance for the two samples, RGTM 10225 (x-axis) and RGTM 10226 (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 RGTM 10225 (x-axis) and RGTM 10226 (y-axis), calculated as the values above and below the consensus means that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$.

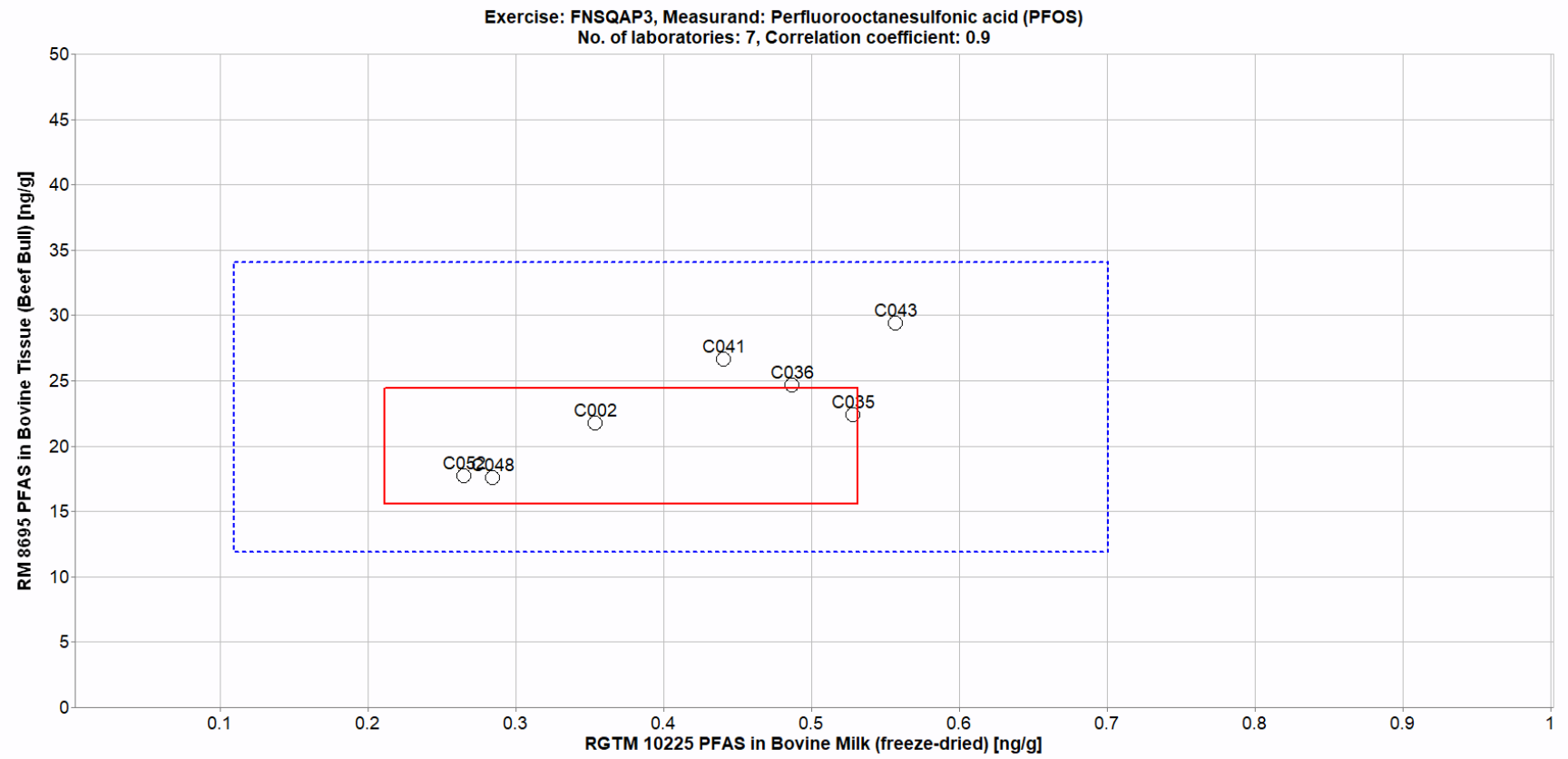

Fig. 5-27. Laboratory means for PFOS in RGTM 10225 and RM 8695 (sample/sample comparison view).

In this view, the individual laboratory mean for one sample (RGTM 10225) is compared to the individual laboratory mean for a second sample (RM 8695). The solid red box represents the NIST range of tolerance for the two samples, RGTM 10225 (x-axis) and RM 8695 (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 RGTM 10225 (x-axis) and RM 8695 (y-axis), calculated as the values above and below the consensus means that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$.

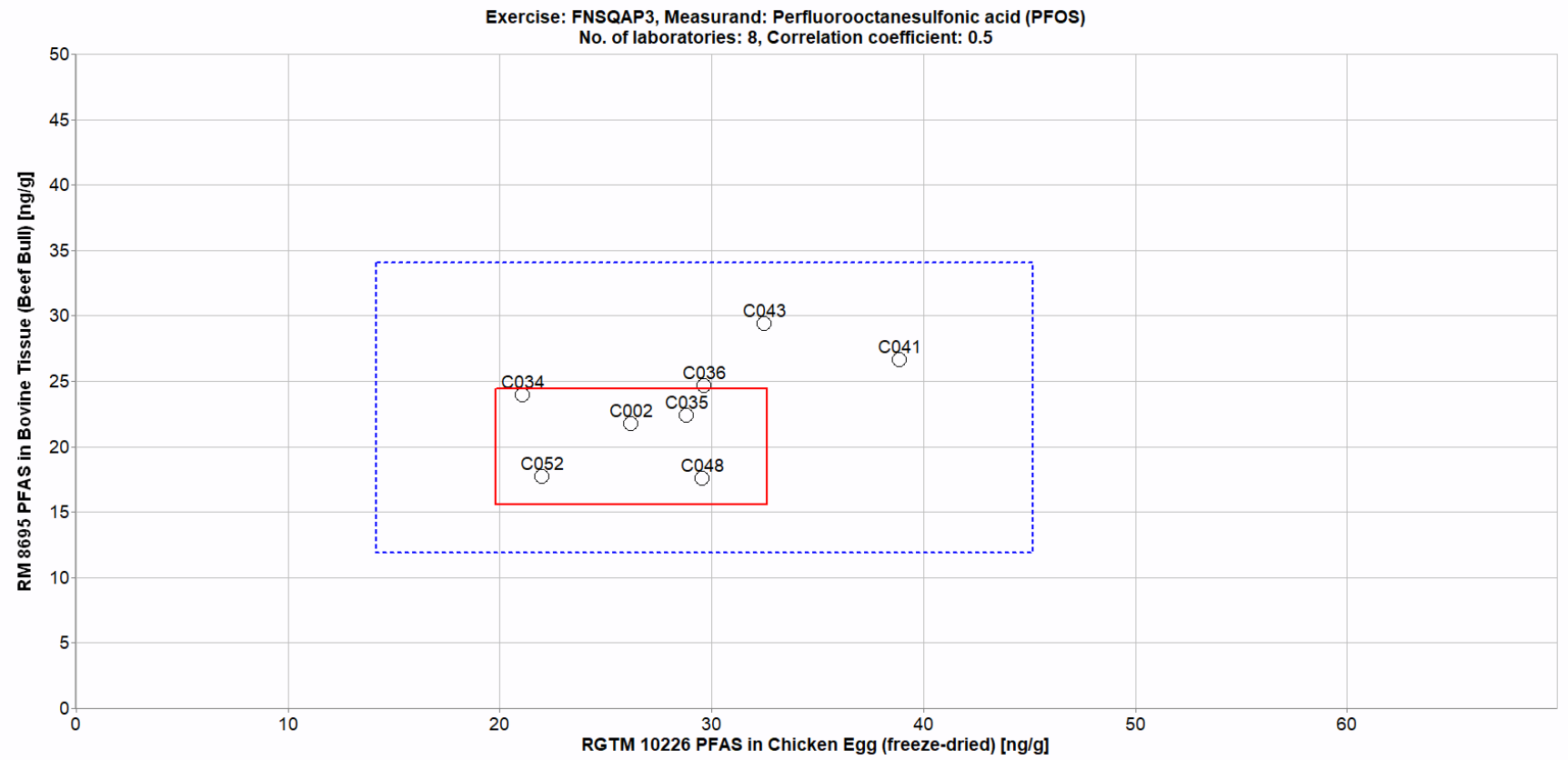

Fig. 5-28. Laboratory means for PFOS in RGTM 10226 and RM 8695 (sample/sample comparison view).

In this view, the individual laboratory mean for one sample (RGTM 10226) is compared to the individual laboratory mean for a second sample (RM 8695). The solid red box represents the NIST range of tolerance for the two samples, RGTM 10226 (x-axis) and RM 8695 (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 RGTM 10226 (x-axis) and RM 8695 (y-axis), calculated as the values above and below the consensus means that result in an acceptable Z'_{comm} score, $|Z'_{\text{comm}}| \leq 2$.

5.4.6. PFOSA

Table 5-1 summarizes and Table 5-8 details the measured mass fraction results for PFOSA reported by each participating laboratory. The limited number of quantitative mass fraction results reported for PFOSA indicates that the levels of PFOSA are below the LOQs of most participant methods. Laboratories reporting LOQs higher than published expectations outlined in Table 5-3 should review practices to ensure that detection of PFOSA is achievable at levels agreed upon by the PFAS measurement community.

Table 5-8. Data summary table for PFOSA in foods.

		PFOSA													
		RGTM 10225 PFAS in Bovine Milk (freeze-dried) (ng/g)					RGTM 10226 PFAS in Chicken Egg (freeze-dried) (ng/g)					RM 8695 PFAS in Bovine Tissue (Beef Bull) (ng/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target															
	C002	< 0.049	< 0.049	< 0.049			< 0.049	< 0.049	< 0.049			< 0.049	< 0.049	< 0.049		
	C019															
	C022															
	C029															
	C030															
	C034						< 0.100	< 0.100	< 0.100			< 0.100	< 0.100	< 0.100		
	C035	< 0.120	< 0.120	< 0.120			< 0.092	< 0.092	< 0.092			< 0.018	< 0.018	< 0.018		
	C036	< 0.300	< 0.300	< 0.300			< 0.300	< 0.300	< 0.300			< 0.300	< 0.300	< 0.300		
	C038	< 156.0	< 156.0	< 156.0			< 156.0	< 156.0	< 156.0			< 156.0	< 156.0	< 156.0		
	C040															
	C041															
	C043	< 0.010	< 0.010	< 0.010			< 0.010	< 0.010	< 0.010			< 0.010	< 0.010	< 0.010		
	C051	< 0.190	< 0.190	< 0.190			< 1.000	< 1.000	< 1.000							
	C052															
	C053															
	C054															

5.4.7. Additional PFAS Data

In addition to PFHxA, PFHxS, PFNA, PFOA, PFOS, and PFOSA, laboratories were asked to report quantitative mass fraction values or LOQs for any additional PFAS detected in the 3 samples. Data reported for perfluorobutanoic acid (PFBA), perfluoro-n-pentanoic acid (PFPeA), perfluoroheptanoic acid (PFHpA), perfluorodecanoic acid (PFDA), perfluoroheptane sulfonic acid (PFHpS), linear perfluorooctanesulfonate (L-PFOS), branched perfluorooctanesulfonate (Br-PFOS), 8:2 fluorotelomer sulfonic acid (8:2FTS), 10:2 fluorotelomer sulfonic acid (10:2FTS), perfluorodecanesulfonic acid (PFDS), perfluoroundecanoic acid (PFUnA), perfluorododecanoic acid (PFDoA), perfluorotridecanoic acid (PFTrDA), perfluorotetradecanoic acid (PFTeDA), perfluorononanesulfonic acid (PFNS), N-ethyl perfluorooctane sulfonamido acetic acid (L-NEtFOSAA), and 6:2 Fluorotelomer phosphate diester (6:2 diPAP) are summarized in Table 5-9 through Table 5-25.

A single laboratory submitted data for PFBA (Table 5-9), PFPeA (Table 5-10), PFNS (Table 5-23), N-EtFOSAA (Table 5-24), and 6:2 diPAP (Table 5-25). The limited reporting of these PFAS suggests potential analytical challenges regarding their quantification. The mass fractions of these PFAS may be below the limits of detection of commonly used methods, or may not be included as target PFAS in those methods.

Up to 5 laboratories submitted data for PFHpA (Table 5-11), PFDA (Table 5-12), PFHpS (Table 5-13), PFDS (Table 5-18), PFUnA (Table 5-19), PFDoA (Table 5-20), PFTrDA (Table 5-21) and PFTeDA (Table 5-22). Generally, the reported mass fraction results for each PFAS were of the same order of magnitude across reporting laboratories, but accuracy cannot be evaluated without NIST target values for comparison. These analytes all have commercially available standards and are common in many targeted analytical method lists. Laboratories that do not currently include these analytes in their targeted lists should consider expanding their method scope to incorporate these related PFAS.

Only two laboratories presented independent results for L-PFOS and Br-PFOS (Table 5-14 and Table 5-15, respectively). Any PFAS manufactured using ECF have the potential to produce both branched and linear isomers (e.g., all PFCAs and PFSAs), therefore laboratories can achieve greatest accuracy by integrating all isomers for which standards are commercially available. Laboratories did not specify whether calibration was based on Br-PFOS only or on a Br+L-PFOS curve. Comparing results generated using different types of calibration could lead to an increase in the observed reproducibility. Both branched and linear PFOS should be reported when possible for improved accuracy.

Two to three laboratories submitted mass fraction data for 8:2 FTS (Table 5-16), and high levels of 8:2 FTS, ranging from 65 ng/g to 1100 ng/g, were observed in the egg sample. The high variability of reported values could indicate an analytical interference, but no conclusions can be drawn from the limited amount of data. 8:2 FTS was also observed in beef bull, but at much lower mass fractions. High mass fractions of 10:2 FTS in the egg sample were also reported by two laboratories (Table 5-17).

Table 5-9. Data summary table for PFBA in foods.

		PFBA													
		RGTM 10225 PFAS in Bovine Milk (freeze-dried) (ng/g)					RGTM 10226 PFAS in Chicken Egg (freeze-dried) (ng/g)					RM 8695 PFAS in Bovine Tissue (Beef Bull) (ng/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	C034															
	C035															
	C041	< 0.42	< 0.42	< 0.42			< 0.42	< 0.42	< 0.42			< 0.42	< 0.42	< 0.42		
	C048						1.6	1.6	1.6	1.6	0.0					
	C051															
Community Results		Mean					Mean			1.6		Mean				
		Standard Deviation					Standard Deviation			0		Standard Deviation				
		N			0		N			1		N			0	

Table 5-10. Data summary table for PFPeA in foods.

		PFPeA													
		RGTM 10225 PFAS in Bovine Milk (freeze-dried) (ng/g)					RGTM 10226 PFAS in Chicken Egg (freeze-dried) (ng/g)					RM 8695 PFAS in Bovine Tissue (Beef Bull) (ng/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	C034															
	C035															
	C041	0.0270	0.0325	0.0326	0.0307	0.0032	< 0.012	< 0.012	< 0.012			< 0.012	< 0.012	< 0.012		
	C048															
	C051															
Community Results		Mean			0.0307		Mean					Mean				
		Standard Deviation			0.0032		Standard Deviation					Standard Deviation				
		N			1		N			0		N			0	

Table 5-11. Data summary table for PFHpA in foods.

		PFHpA													
		RGTM 10225 PFAS in Bovine Milk (freeze-dried) (ng/g)					RGTM 10226 PFAS in Chicken Egg (freeze-dried) (ng/g)					RM 8695 PFAS in Bovine Tissue (Beef Bull) (ng/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	C034						0.344	0.333	0.341	0.339	0.006					
	C035						0.434	0.445	0.476	0.451	0.020	0.0178	0.0183	0.0190	0.0184	0.0006
	C041	< 0.009	< 0.009	< 0.009			0.333	0.344	0.326	0.334	0.009	< 0.009	< 0.009	< 0.009		
	C048															
	C051															
Community Results		Mean					Mean			0.375		Mean			0.0184	
		Standard Deviation					Standard Deviation			0.058		Standard Deviation			0.0006	
		N			0		N			3		N			1	

Table 5-12. Data summary table for PFDA in foods.

		PFDA													
		RGTM 10225 PFAS in Bovine Milk (freeze-dried) (ng/g)					RGTM 10226 PFAS in Chicken Egg (freeze-dried) (ng/g)					RM 8695 PFAS in Bovine Tissue (Beef Bull) (ng/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	C034						33.72	31.75	29.07	31.5	2.3	2.602	2.382	2.502	2.50	0.11
	C035	0.281	0.209	0.231	0.240	0.037	44.9	40.4	40.7	42.0	2.5	2.39	2.36	2.37	2.37	0.02
	C041	0.146	0.154	0.167	0.156	0.011	30.67	26.36	25.13	27.4	2.9	1.680	1.779	1.816	1.76	0.07
	C048						46.8			46.8		1.88			1.88	
	C051	0.16	0.17	0.19	0.173	0.015										
Community Results		Mean			0.190		Mean			34.9		Mean			2.18	
		Standard Deviation			0.044		Standard Deviation			7.7		Standard Deviation			0.34	
		N			3		N			4		N			4	

Table 5-13. Data summary table for PFHpS in foods.

		PFHpS													
		RGTM 10225 PFAS in Bovine Milk (freeze-dried) (ng/g)					RGTM 10226 PFAS in Chicken Egg (freeze-dried) (ng/g)					RM 8695 PFAS in Bovine Tissue (Beef Bull) (ng/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	C034						0.18	0.145	0.082	0.136	0.050	0.106	0.16	0.137	0.134	0.027
	C035								0.108	0.108		0.155	0.152	0.178	0.162	0.014
	C041	< 0.013	< 0.013	< 0.013			0.1200	0.1208	0.1124	0.118	0.005	0.1604	0.1579	0.1651	0.161	0.004
	C048															
	C051															
Community Results		Mean					Mean			0.124		Mean			0.152	
		Standard Deviation					Standard Deviation			0.031		Standard Deviation			0.021	
		N			0		N			3		N			3	

Table 5-14. Data summary table for L-PFOS in foods.

		L-PFOS													
		RGTM 10225 PFAS in Bovine Milk (freeze-dried) (ng/g)					RGTM 10226 PFAS in Chicken Egg (freeze-dried) (ng/g)					RM 8695 PFAS in Bovine Tissue (Beef Bull) (ng/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	C034						19.42	17.153	16.145	17.6	1.7	18.314	17.873	17.578	17.92	0.37
	C035															
	C041															
	C048															
	C051	0.21	0.21	0.2	0.2067	0.0058	37	32	35	34.7	2.5					
Community Results		Mean			0.2067		Mean			26.1		Mean			17.92	
		Standard Deviation			0.0058		Standard Deviation			9.6		Standard Deviation			0.37	
		N			1		N			2		N			1	

Table 5-15. Data summary table for Br-PFOS in foods.

		Br-PFOS													
		RGTM 10225 PFAS in Bovine Milk (freeze-dried) (ng/g)					RGTM 10226 PFAS in Chicken Egg (freeze-dried) (ng/g)					RM 8695 PFAS in Bovine Tissue (Beef Bull) (ng/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	C034						3.552	3.431	3.518	3.50	0.06	5.988	6.301	5.792	6.03	0.26
	C035															
	C041															
	C048															
	C051	0.12	0.13	0.12	0.1233	0.0058	3.7	3.3	3.5	3.50	0.20					
Community Results		Mean			0.1233		Mean			3.50		Mean			6.03	
		Standard Deviation			0.0058		Standard Deviation			0.13		Standard Deviation			0.26	
		N			1		N			2		N			1	

Table 5-16. Data summary table for 8:2FTS in foods.

		8:2FTS													
		RGTM 10225 PFAS in Bovine Milk (freeze-dried) (ng/g)					RGTM 10226 PFAS in Chicken Egg (freeze-dried) (ng/g)					RM 8695 PFAS in Bovine Tissue (Beef Bull) (ng/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	C034						66.872	62.753	66.694	65.4	2.3	0.278	0.184	0.22	0.227	0.047
	C035						991	863	831	895	85	0.221	0.203	0.198	0.207	0.012
	C041															
	C048															
	C051						1100	1000	1200	1100	100					
Community Results		Mean					Mean			687		Mean			0.217	
		Standard Deviation					Standard Deviation			479		Standard Deviation			0.033	
		N			0		N			3		N			2	

Table 5-17. Data summary table for 10:2FTS in foods.

		10:2FTS													
		RGTM 10225 PFAS in Bovine Milk (freeze-dried) (ng/g)					RGTM 10226 PFAS in Chicken Egg (freeze-dried) (ng/g)					RM 8695 PFAS in Bovine Tissue (Beef Bull) (ng/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	C034															
	C035		0.1613	0.1302	0.146	0.022	2232	2439	2137	2269	154	0.303	0.327	0.31	0.313	0.012
	C041															
	C048															
	C051						2500	2200	2500	2400	173					
Community Results		Mean			0.146		Mean			1334		Mean			0.313	
		Standard Deviation			0.022		Standard Deviation			163		Standard Deviation			0.012	
		N			1		N			2		N			1	

Table 5-18. Data summary table for PFDS in foods.

		PFDS													
		RGTM 10225 PFAS in Bovine Milk (freeze-dried) (ng/g)					RGTM 10226 PFAS in Chicken Egg (freeze-dried) (ng/g)					RM 8695 PFAS in Bovine Tissue (Beef Bull) (ng/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	C034						0.904	0.663	0.533	0.700	0.188					
	C035						1.4	1.51	1.42	1.443	0.059					
	C041	< 0.010	< 0.010	< 0.010			1.235	1.226	1.233	1.231	0.005	< 0.010	< 0.010	< 0.010		
	C048						1.67			1.67						
	C051						1.2	1.3	1.3	1.267	0.058					
Community Results		Mean					Mean			1.20		Mean				
		Standard Deviation					Standard Deviation			0.32		Standard Deviation				
		N			0		N			5		N			0	

Table 5-19. Data summary table for PFUnA in foods.

		PFUnA													
		RGTM 10225 PFAS in Bovine Milk (freeze-dried) (ng/g)					RGTM 10226 PFAS in Chicken Egg (freeze-dried) (ng/g)					RM 8695 PFAS in Bovine Tissue (Beef Bull) (ng/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	C034						25.772	21.42	24.212	23.8	2.2	0.42	0.436	0.506	0.454	0.046
	C035						32.7	31.2	31.6	31.83	0.78	0.46	0.462	0.456	0.4593	0.0031
	C041	0.0194	0.0242	0.0217	0.0218	0.0024	23.763	24.058	23.594	23.8	0.2	0.3454	0.3584	0.3316	0.345	0.013
	C048						33.3			33.3						
	C051															
Community Results		Mean			0.0218		Mean			27.2		Mean			0.419	
		Standard Deviation			0.0024		Standard Deviation			4.5		Standard Deviation			0.061	
		N			1		N			4		N			3	

Table 5-20. Data summary table for PFDoA in foods.

		PFDoA													
		RGTM 10225 PFAS in Bovine Milk (freeze-dried) (ng/g)					RGTM 10226 PFAS in Chicken Egg (freeze-dried) (ng/g)					RM 8695 PFAS in Bovine Tissue (Beef Bull) (ng/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	C034						41.19	38.55	42.33	40.7	1.9	0.151	0.184	0.171	0.169	0.017
	C035						70.3	72	68.1	70.1	2.0	0.184	0.169	0.166	0.173	0.010
	C041	< 0.012	< 0.012	< 0.012			52.49	56.64	53.71	54.3	2.1	0.1334	0.1348	0.1321	0.1334	0.0013
	C048						75.3			75.3						
	C051						74	71	70	71.1	2.1					
Community Results		Mean					Mean			60		Mean			0.158	
		Standard Deviation					Standard Deviation			14		Standard Deviation			0.021	
		N			0		N			5		N			3	

Table 5-21. Data summary table for PFTrDA in foods.

		PFTrDA													
		RGTM 10225 PFAS in Bovine Milk (freeze-dried) (ng/g)					RGTM 10226 PFAS in Chicken Egg (freeze-dried) (ng/g)					RM 8695 PFAS in Bovine Tissue (Beef Bull) (ng/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	C034						12.456	8.455	8.369	9.8	2.3					
	C035						18.9	18.5	17.8	18.4	0.6			0.0202	0.0202	
	C041															
	C048						11.5			11.5						
	C051						14	14	14	14	0					
Community Results		Mean					Mean			13.8		Mean			0.0202	
		Standard Deviation					Standard Deviation			3.8		Standard Deviation				
		N			0		N			4		N			1	

Table 5-22. Data summary table for PFTeDA in foods.

		PFTeDA													
		RGTM 10225 PFAS in Bovine Milk (freeze-dried) (ng/g)					RGTM 10226 PFAS in Chicken Egg (freeze-dried) (ng/g)					RM 8695 PFAS in Bovine Tissue (Beef Bull) (ng/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	C034						31.576	25.545	27.835	28.3	3.0					
	C035						45.6	45.3	45.8	45.6	0.3	0.0296	0.0282	0.0317	0.0298	0.0018
	C041															
	C048						32.2			32.2						
	C051						39	44	44	42.3	2.9					
Community Results		Mean					Mean			38.1		Mean			0.0298	
		Standard Deviation					Standard Deviation			8.0		Standard Deviation			0.0018	
		N			0		N			4		N			1	

Table 5-23. Data summary table for PFNS in foods.

		PFNS													
		RGTM 10225 PFAS in Bovine Milk (freeze-dried) (ng/g)					RGTM 10226 PFAS in Chicken Egg (freeze-dried) (ng/g)					RM 8695 PFAS in Bovine Tissue (Beef Bull) (ng/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	C034						0.228	0.227	0.243	0.2327	0.0090					
	C035															
	C041															
	C048															
	C051															
Community Results		Mean					Mean			0.2327		Mean				
		Standard Deviation					Standard Deviation			0.0090		Standard Deviation				
		N			0		N			1		N			0	

Table 5-24. Data summary table for L-NEtFOSAA in foods.

		L-NEtFOSAA													
		RGTM 10225 PFAS in Bovine Milk (freeze-dried) (ng/g)					RGTM 10226 PFAS in Chicken Egg (freeze-dried) (ng/g)					RM 8695 PFAS in Bovine Tissue (Beef Bull) (ng/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	C034						0.256	0.18	0.18	0.205	0.044					
	C035															
	C041															
	C048															
	C051															
Community Results		Mean					Mean			0.205		Mean				
		Standard Deviation					Standard Deviation			0.044		Standard Deviation				
		N			0		N			1		N			0	

Table 5-25. Data summary table for 6:2 diPAP in foods.

		6:2 diPAP													
		RGTM 10225 PFAS in Bovine Milk (freeze-dried) (ng/g)					RGTM 10226 PFAS in Chicken Egg (freeze-dried) (ng/g)					RM 8695 PFAS in Bovine Tissue (Beef Bull) (ng/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	C034						1.236	1.196	1.133	1.188	0.052					
	C035															
	C041															
	C048															
	C051															
Community Results		Mean					Mean			1.188		Mean				
		Standard Deviation					Standard Deviation			0.052		Standard Deviation				
		N			0		N			1		N			0	

Additionally, at least one laboratory reported non-quantitative values for each of the following PFAS: perfluorobutane sulfonate (PFBS), perfluoropentane sulfonic acid (PFPeS), sodium dodecafluoro-3H-4, 8-dioxanonanoate (NaDONA), hexafluoropropylene oxide dimer acid (HFPO-DA), 9-chlorohexadecafluoro-3-oxanone-1-sulfonic acid (9Cl-PF3ONS), and 11-chloroeicosafluoro-3-oxaundecane-1-sulfonic acid (11Cl-PF3OUdS).

5.4.8. Summary and Overall Conclusions

A majority of participating laboratories using various extraction techniques, instrumentation, and processing protocols, produced equivalent data. Participating laboratories were generally in good agreement with NIST target values and consensus values, with few exceptions. Laboratories reporting outlying results should reevaluate their method detection limits, mainly to ensure data provided for customers is consistent and accurate. Further, not all extraction methods performed equivalently in this small data set. When exploring a new extraction method, rigorous evaluations should be performed in matrix to ensure high quality data. For example, in the case of dehydrated or freeze-dried materials, comparisons should be performed between hydrated and non-hydrated subsets of the same sample matrix. Ultimately, given that PFAS are prone to interpretive complications (e.g., branched and linear isomers, interferences), laboratories using less-specific methodologies (LC-MS vs. LC-MS/MS) should be wary of reporting and interpretation without quality control and technical expertise. Potential interferences (e.g., taurodeoxycholic acid, a bile acid known to occur in matrices such as chicken eggs) should be thoroughly investigated in each new matrix using chemical standards to ensure that extraction procedures (e.g., envi-carb application) and analytical set-up (e.g., guard columns, ghost columns, analytical separation) are appropriate to offset and reduce the possibility of bias in reporting.

NIST has conducted one QAP study involving measurement of PFAS in food samples prior to this FNSQAP study. The previous study, DSQAP Exercise 1, focused on the determination of 15 PFAS in a kelp sample and only one laboratory reported results [10].

6. CONTAMINANTS (Pesticide Residues)

6.1. Executive Summary

To protect public health, regulators must understand human and animal dietary exposure to potentially harmful contaminants such as pesticide residues through accurate determination of their concentrations in consumer products. The results of this study revealed that most participating laboratories are using repeatable, reproducible, and accurate methods for determination of most pesticide residues in the food product presented. Additionally, the test sample, prepared using a custom amendment procedure, proved useful in understanding community-wide method performance.

6.2. Study Overview

The EPA sets tolerances for the maximum amount of pesticide residues that are allowed to remain in foods [26, 27], and the FDA, USDA, and state enforcement agencies test food products to ensure compliance with these tolerances. In other countries, tolerances may be called maximum residue limits (MRLs). Tolerances are set based on risk assessment, starting with residue toxicity to humans, likelihood that the residue will remain on food, and other possible sources of exposure balanced with the breadth of use of the pesticide and amounts and types of foods consumed. To ensure tolerances are properly set and foods are properly monitored, methods for the detection of pesticide residues in agricultural and consumer products must be well characterized and have demonstrated accuracy. In this study, participants were provided with samples of RGTM 10190 Pesticide Residues in Frozen Spinach Leaves. Participants were asked to use in-house analytical methods to determine the mass fraction (ng/g) of 10 diverse pesticide residues (i.e., acephate, boscalid, chlorantraniliprole, clothianidin, dichlorodiphenyldichloroethylene (p,p'-DDE), dimethomorph, fluopicolide, flutriafol, mandipropamid, and permethrin) in the spinach leaves. 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.

6.3. Sample Information

Participants were provided one to two jars of RGTM 10190 Pesticides in Frozen Spinach Leaves, based on method sample size needs. The material was prepared by cryohomogenizing organic frozen spinach leaves and amending the homogenate with a custom mixture of pesticides. Each jar contained approximately 20 g of material; participants were asked to store the material at or below -20 °C in the original unopened jars and to prepare at least two samples and report at least two values per analyte from the total material provided. Participants were instructed to thoroughly mix the contents of each jar before use and to use a sample size of at least 1 g to ensure homogeneity of the test portion used for analysis. The approximate analyte mass fractions were not reported to participants prior to the study. Target mass fraction values and uncertainties for pesticide residues in RGTM 10190 were determined at NIST from duplicate

preparations from 6 jars using LC-MS/MS (acephate, boscalid, chlorantraniliprole, clothianidin, dimethomorph, fluopicolide, flutriafol, mandipropamid), gas chromatography with mass spectrometry (GC-MS) (p,p'-DDE, permethrin), and GC with tandem mass spectrometry (GC-MS/MS) (boscalid, p,p'-DDE, dimethomorph, permethrin). Stratified random sampling ensured that the jars tested were representative of the entire production lot. The variabilities within and among packaged jars were determined using ANOVA and were incorporated into the uncertainties on each target value. Target mass fraction values and uncertainties were calculated from mean results from available methods and are provided in Table 6-1 on an as-received basis.

Table 6-1. Individualized data summary table for pesticide residues in spinach.

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

(Lab Name)

Exercise 3 – Pesticides in Spinach

	Lab Code:	**(Code)**	**1. Your Results**				**2. Community Results**			**3. Target**	
	Sample	Units	x_i	s_i	Z'_{comm}	Z_{NIST}	N	x*	s*	x_{NIST}	u_{NIST}
Acephate	RGTM 10190	ng/g	*Individual laboratory results will appear in this section; laboratory-specific results were provided to each participant separately from this report.*				12	45	16	52	9
Boscalid	RGTM 10190	ng/g					12	90	30	78	18
Chlorantraniliprole	RGTM 10190	ng/g					13	194	47	190	38
Clothianidin	RGTM 10190	ng/g					11	97	32	100	11
p,p'-DDE	RGTM 10190	ng/g					11	14.5	5.5	20	12
Dimethomorph	RGTM 10190	ng/g					11	243	63	190	49
Fluopicolide	RGTM 10190	ng/g					6	230	33	200	26
Flutriafol	RGTM 10190	ng/g					10	37	20	43	10
Mandipropamid	RGTM 10190	ng/g					8	114	40	110	34
Permethrin	RGTM 10190	ng/g					16	420	190	540	100

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, not including single-value submissions

x* Robust mean of reported values

s* Robust standard deviation

x_{NIST} NIST-assessed value

u_{NIST} standard uncertainty about the NIST-assessed value

6.4. Study Results and Discussion

6.4.1. Participation Rates

Twenty-three laboratories received sample(s) for this study; of those, 19 returned results for at least one pesticide residue (83 % participation). Some laboratories reported values obtained using multiple methods and thus were treated as independent laboratories. Some laboratories reported that values were below their method LOQ; results from these laboratories were not included in calculation of consensus means and standard deviations.

More detailed participation information for each pesticide residue is summarized in Table 6-2. Participation rates were high, with over 65 % of laboratories requesting samples reporting results, for acephate, boscalid, chlorantraniliprole, p,p'-DDE, and permethrin. For fluopicolide and mandipropamid, participation rates were lower with fewer than 50 % of laboratories reporting results.

Table 6-2. Participation information for individual pesticide residues in spinach.

	Laboratories Reporting Results	Participation Rate	Laboratories Reporting Quantitative Results
Acephate	16 of 23	70 %	12
Boscalid	17 of 24	71 %	12
Chlorantraniliprole	16 of 24	67 %	13
Clothianidin	13 of 24	54 %	11
p,p'-DDE	15 of 23	65 %	11
Dimethomorph	14 of 24	58 %	11
Fluopicolide	8 of 22	36 %	6
Flutriafol	13 of 23	57 %	10
Mandipropamid	11 of 23	48 %	8
Permethrin	19 of 24	79 %	15

6.4.2. Precision

Table 6-1 summarizes and Table 6-3 through Table 6-12 detail the measured mass fraction results for acephate, boscalid, chlorantraniliprole, clothianidin, p,p'-DDE, dimethomorph, fluopicolide, flutriafol, mandipropamid, and permethrin reported by each participating laboratory, respectively.

Table 6-3. Data summary table for acephate in RGTM 10190.

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

		Acephate				
		RGTM 10190 Pesticide Residues in Frozen Spinach Leaves (ng/g)				
	Lab	A	B	C	Avg	SD
Individual Results	Target				52	9
	C003	< 10.00	< 10.00	< 10.00		
	C004	57.55			58	
	C007	38.4	42.9		41	3
	C009	43.3	44.2		44	1
	C012	41.32	46.11	35.43	41	5
	C018					
	C019	47.9			48	
	C020					
	C024					
	C028	40.2	34.9	36.9	37	3
	C029					
	C030					
	C031	32.2	32.5	30.7	32	1
	C032	54.923	88.421		72	24
	C034	86	122	67	92	28
	C036	43.79	45.86	41.72	44	2
	C038	< 10000	< 10000	< 10000		
	C040	45.23	45.26		45	0
	C046	29.8	25.8	26.2	27	2
	C047					
	C050					
	C055	32.1	31		32	1
	C056	125	168	109	133	32
Community Results		Consensus Mean			45	
		Consensus Standard Deviation			16	
		Maximum			133	
		Minimum			27	
		N			12	

Table 6-4. Data summary table for boscalid in RGTM 10190.

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

		Boscalid				
		RGTM 10190 Pesticide Residues in Frozen Spinach Leaves (ng/g)				
	Lab	A	B	C	Avg	SD
Individual Results	Target				78	18
	C003					
	C004	113.58			114	
	C005	95.1	81.1		88	10
	C007	47.4	47		47	0
	C009	74	75.8		75	1
	C012					
	C018					
	C019	84.3			84	
	C020					
	C024	101			101	
	C028	62.2	59.8	67.5	63	4
	C029					
	C030					
	C031	< 10.000	< 10.000	< 10.000		
	C032	99.647	120.85		110	15
	C034	140	272	109	174	87
	C036	70.5	74.16	66.84	71	4
	C038	< 10000	< 10000	< 10000		
	C040	76.35	92.05		84	11
	C046	62.1	62.9	62.4	62	0
	C047	74	87		81	9
	C050					
	C055	105	98.6		102	5
	C056	144	254	119	172	72
Community Results		Consensus Mean			90	
		Consensus Standard Deviation			30	
		Maximum			174	
		Minimum			47	
		N			12	

Table 6-5. Data summary table for chlorantraniliprole in RGTM 10190.

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

		Chlorantraniliprole				
		RGTM 10190 Pesticide Residues in Frozen Spinach Leaves (ng/g)				
	Lab	A	B	C	Avg	SD
Individual Results	Target				188	38
	C003					
	C004	191.75			192	
	C005	223	170		197	37
	C007	177.9	165.1		172	9
	C009	184.4	195.3		190	8
	C012					
	C018					
	C019	143.7			144	
	C020					
	C024	205			205	
	C028	205	166	172	181	21
	C029					
	C030					
	C031	270.2	217.4	199.1	229	37
	C032	218.793	262.506		241	31
	C034					
	C036	168.49	183.6	153.39	168	15
	C038	464.68	448.13	456.4	456	8
	C040	162.03	168.02		165	4
	C046	188.5	203.5	130	174	39
	C047	248	279		264	22
	C050					
	C055	199	198		199	1
	C056	833	1441	673	982	405
Community Results		Consensus Mean			195	
		Consensus Standard Deviation			47	
		Maximum			982	
		Minimum			144	
		N			13	

Table 6-6. Data summary table for clothianidin in RGTM 10190.

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

		Clothianidin				
		RGTM 10190 Pesticide Residues in Frozen Spinach Leaves (ng/g)				
	Lab	A	B	C	Avg	SD
Individual Results	Target				100	11
	C003					
	C004	104.57			105	
	C005	119	77.4		98	29
	C007	77.1	73.6		75	2
	C009	80	82.6		81	2
	C012					
	C018					
	C019	116.6			117	
	C020					
	C024					
	C028	107	93.1	97.4	99	7
	C029					
	C030					
	C031	90.4	81.7	79.2	84	6
	C032	137.146	155.404		146	13
	C034					
	C036	91.43	91.12	91.74	91	0
	C038	75.92	72.05	73.99	74	2
	C040					
	C046	97.1	116.6	118.5	111	12
	C047					
	C050					
	C055	51.7	47.9		50	3
	C056	190	267	149	202	60
Community Results		Consensus Mean			97	
		Consensus Standard Deviation			32	
		Maximum			50	
		Minimum			202	
		N			11	

Table 6-7. Data summary table for p,p'-dichlorodiphenyldichloroethylene (DDE) in RGTM 10190.

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

		p,p'-Dichlorodiphenyldichloroethylene (DDE)				
		RGTM 10190 Pesticide Residues in Frozen Spinach Leaves (ng/g)				
	Lab	A	B	C	Avg	SD
Individual Results	Target				20	12
	C003	< 10.000	< 10.000	< 10.000		
	C004					
	C005	24.8	17.7		21.3	5.0
	C007	12.5	9.6		11.1	2.1
	C009	13.2	13.7		13.5	0.4
	C012	10.61	9.41	14.85	11.6	2.9
	C018					
	C020					
	C024					
	C028	14	12.3	12.4	12.9	1.0
	C029					
	C030					
	C031	< 10.000	< 10.000	< 10.000		
	C032	15.454	23.089		19.3	5.4
	C034					
	C036	18.07	18.32	18.19	18.2	0.1
	C038	< 10000	< 10000	< 10000		
	C040					
	C046	< 10.000	< 10.000	< 10.000		
	C047	12	12		12.0	0.0
	C050	5	4	5	4.7	0.6
	C055	12.1	11.7		11.9	0.3
	C056	25	52	20	32.3	17.2
Community Results		Consensus Mean			14.5	
		Consensus Standard Deviation			5.5	
		Maximum			32.3	
		Minimum			4.7	
		N			11	

Table 6-8. Data summary table for dimethomorph in RGTM 10190.

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

		Dimethomorph				
		RGTM 10190 Pesticide Residues in Frozen Spinach Leaves (ng/g)				
	Lab	A	B	C	Avg	SD
Individual Results	Target				185	49
	C003					
	C004	379.21			379	
	C005	268	183		226	60
	C007	197.8	185.8		192	8
	C009	174.9	188.7		182	10
	C012					
	C018					
	C019	387.2			387	
	C020					
	C024	170			170	
	C028	199	184	194	192	8
	C029					
	C030					
	C031	360.6	359	551.6	424	111
	C032	212.445	305.146		259	66
	C034	329	615	252	391	157
	C036	168.58	180.35	156.8	169	12
	C038	144.06	167.18	155.62	156	12
	C040					
	C046	137	198.5	183.5	173	32
	C047					
	C050					
	C055					
	C056	334	560	256	383	158
Community Results		Consensus Mean			243	
		Consensus Standard Deviation			63	
		Maximum			424	
		Minimum			156	
		N			11	

Table 6-9. Data summary table for fluopicolide in RGTM 10190.

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

		Fluopicolide				
		RGTM 10190 Pesticide Residues in Frozen Spinach Leaves (ng/g)				
	Lab	A	B	C	Avg	SD
Individual Results	Target				197	26
	C003					
	C004					
	C007	212.2	204.3		208	6
	C009	233.1	249.9		242	12
	C012					
	C018					
	C020					
	C024	188			188	
	C028	223	226	236	228	7
	C029					
	C030					
	C031					
	C032	236.293	294.246		265	41
	C034					
	C036	220.14	226.91	223.52	224	3
	C038	< 1000	< 1000	< 1000		
	C040					
	C046					
	C047					
	C050					
	C055					
	C056	310	524	237	357	149
Community Results		Consensus Mean			230	
		Consensus Standard Deviation			33	
		Maximum			357	
		Minimum			188	
		N			6	

Table 6-10. Data summary table for flutriafol in RGTM 10190.

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

		Flutriafol				
		RGTM 10190 Pesticide Residues in Frozen Spinach Leaves (ng/g)				
	Lab	A	B	C	Avg	SD
Individual Results	Target				43	10
	C003	< 50	< 50	< 50		
	C004					
	C007	29.2	25.6		27	3
	C009	42.9	43.2		43	0
	C012					
	C018					
	C019	33.9			34	
	C020					
	C024					
	C028	43.4	39	40.7	41	2
	C029					
	C030					
	C031	33.2	34.8	37.5	35	2
	C032	46.817	61.818		54	11
	C034	187	294	142	208	78
	C036	45.01	46.84	45.92	46	1
	C038	< 10000	< 10000	< 10000		
	C040					
	C046	25.7	32.3	42.5	34	8
	C047					
	C050	14	15	14	14	1
	C055					
	C056	233	372	183	263	98
Community Results		Consensus Mean			37	
		Consensus Standard Deviation			20	
		Maximum			263	
		Minimum			14	
		N			10	

Table 6-11. Data summary table for mandipropamid in RGTM 10190.

		Mandipropamid				
		RGTM 10190 Pesticide Residues in Frozen Spinach Leaves (ng/g)				
	Lab	A	B	C	Avg	SD
Individual Results	Target				110	34
	C003					
	C004					
	C007	78.6	82.3		80	3
	C009	80.3	83.4		82	2
	C012					
	C018					
	C019	121.9			122	
	C020					
	C024	65			65	
	C028	115	120	126	120	6
	C029					
	C030					
	C031	194	178.6	162.6	178	16
	C032	114.373	150.265		132	25
	C034					
	C036	102.68	110.84	94.52	103	8
	C038	< 10000	< 10000	< 10000		
	C040					
	C046	78	110	100.5	96	16
	C047					
	C050					
	C055					
	C056	142	246	110	166	71
Community		Consensus Mean			114	
		Consensus Standard Deviation			40	
		Maximum			178	
		Minimum			65	
		N			8	

Table 6-12. Data summary table for permethrin in RGTM 10190.

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

		Permethrin				
		RGTM 10190 Pesticide Residues in Frozen Spinach Leaves (ng/g)				
	Lab	A	B	C	Avg	SD
Individual Results	Target				538	100
	C003	220	250	250	240	17
	C004	426.54			427	
	C005	563	452		508	78
	C007	348.9	373.3		361	17
	C009	384.8	396.4		391	8
	C012	750.87	836.1	882.59	823	67
	C018					
	C019	148			148	
	C020					
	C024	488			488	
	C028	394	331	348	358	33
	C029					
	C030					
	C031	295	230	269.7	265	33
	C032	659.689	933.307		796	193
	C034	713	1402	542	886	455
	C036	522.79	520.99	521.89	522	1
	C038	< 10000	< 10000	< 10000		
	C040	449.4	393.59		421	39
	C046	332.4	336.1	363.7	344	17
	C047	427	480		454	37
	C050	60	50	50	53	6
	C055	398	371		385	19
	C056					
Community		Consensus Mean			420	
		Consensus Standard Deviation			191	
		Maximum			886	
		Minimum			53	
		N			15	

Results from laboratories participating in this study are summarized in Table 6-13 and are compared to published method performance expectations as summarized in Table 6-14. The average within-laboratory mass fraction variability for all pesticide residues was within the published expectations; however, at least one laboratory reported mass fraction values with an RSD_r greater than 20 % for each residue. One participant reported the appearance of inhomogeneity of the thawed sample, which could result in large variability if the sampling size is small or if the material is not adequately mixed prior to sampling. Participants reported using sample sizes ranging from 1 g to 15 g, and no trend correlating within-laboratory variability to sample size was identified. Some laboratories reporting high within-laboratory variability used a smaller sample size (2 g or 5 g), while one laboratory using a sample size of 1 g reported low within-laboratory variability. Laboratories that reported high within-laboratory variability should review their protocols to identify potential sources of variability and attempt to minimize such errors.

The among-laboratory mass fraction variabilities ranged from 15 % to 55 % for the various pesticide residues. For chlorantraniliprole, dimethomorph, and fluocpicolide, the RSD_Rs were within the published expectations for multiple laboratories using the same method (≤ 30 %). Because the RSD_Rs were established to evaluate reproducibility of a single method used by multiple laboratories, performance for acephate, boscalid, clothianidin, p,p'-DDE, and mandipropamid (33 % to 38 %) are also acceptable considering the variety of methods used by participants in this study. For flutriafol and permethrin, laboratory results were less comparable with RSD_Rs of 55 % and 45 %, respectively. A mixture of *cis* and *trans* isomers of permethrin were present in the sample, but no specific form (e.g., *cis*, *trans*, or total) was indicated on data entry. The target value for permethrin represents a sum of *cis* and *trans* isomers, but laboratories reporting a single isomer may have contributed to the large among-laboratory variability. Future studies will more clearly instruct participants with respect to reporting residues containing isomers.

Table 6-13. Performance information for individual pesticide residues in spinach.

	Within-Laboratory Variability (RSD_r)			Among-Laboratory Variability (RSD_R)
	Range	Average	Laboratories ≥ 20 %	
Acephate	0.05 % to 33 %	11 %	3	36 %
Boscalid	0.6 % to 50 %	13 %	2	33 %
Chlorantraniliprole	0.4 % to 41 %	12 %	2	24 %
Clothianidin	0.3 % to 30 %	10 %	2	33 %
p,p'-DDE	0 % to 53 %	16 %	4	38 %
Dimethomorph	4 % to 41 %	19 %	5	26 %
Fluopicolide	1.5 % to 42 %	12 %	1	15 %
Flutriafol	0.5 % to 38 %	15 %	3	55 %
Mandipropamid	3 % to 43 %	13 %	1	35 %
Permethrin	0.2 % to 51 %	12 %	2	45 %

Table 6-14. Published method performance requirements for pesticide residues in dried cannabis flower (AOAC SMPR 2018.011) and plant-based color additives (AOAC SMPR 2024.001).

Note: p,p'-DDE was not included in the list of selected pesticides for either SMPR.

	AOAC SMPR 2018.011 [28]			AOAC SMPR 2024.001 [29]		
	LOQ (ng/g)	RSD_r (%)	RSD_R (%)	LOQ (ng/g)	RSD_r (%)	RSD_R (%)
Acephate	50	≤ 20	≤ 30	10-100	≤ 20	≤ 30
Boscalid	50					
Chlorantraniliprole	100					
Clothianidin	5					
Dimethomorph	1000					
Fluopicolide	N/A					
Flutriafol	N/A					
Mandipropamid	N/A					
Permethrin	20	≤ 20	≤ 30			

6.4.3. Sample Preparation Approaches

As shown in Fig. 6-1 through Fig. 6-10, laboratories reported using a variety of sample preparation methods for the determination of the pesticide residues in the spinach sample. Most laboratories reported using some form of sample cleanup, including forms of dispersive SPE or QuEChERS (63 % to 88 %) or traditional SPE (8 % to 13 %). Depending on the analyte, up to four laboratories reported using solvent extraction without an additional sample cleanup step (7 % to 25 %). Most laboratories that provided additional method information indicated use of acetonitrile with or without acid modification; one laboratory used methanol for sample extraction and one laboratory used a mixture of hexane and toluene with a GC method (only reporting mass fraction values for acephate and p,p'-DDE). Four laboratories did not provide information about the solvent(s) used in extraction.

The laboratories reporting use of QuEChERSER for sample preparation generally reported mass fraction values with large variability and high bias relative to the consensus and target values. These laboratories should evaluate potential sources of error in this approach that could be causing highly variable results. Otherwise, no trends correlating laboratory performance with the reported sample preparation approach were apparent.

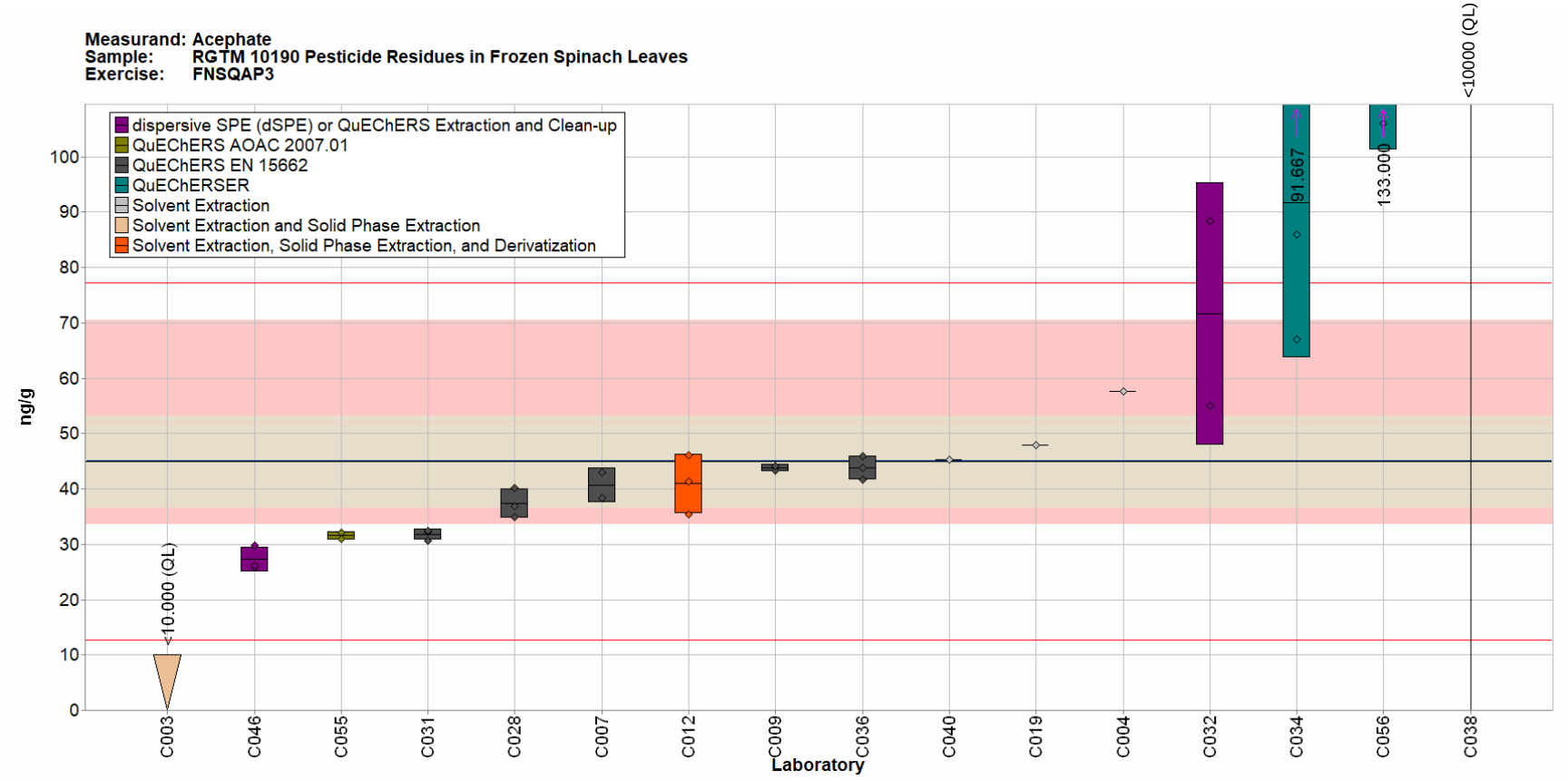

Fig. 6-1. Acephate in RGTM 10190 (data summary view – sample preparation method).

In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). A downward triangle represents data reported as an LOQ value. The color of the data point represents the sample preparation method employed as indicated in the figure key. Data points outside the graphical range are represented as upward arrows (the sample preparation method reported by laboratory C038 was solvent extraction). The solid black line represents the consensus mean, and the solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{comm}| \leq 2$. The beige shaded region represents the overlapping of the 95 % confidence interval for the consensus mean (green region) and the NIST range of tolerance (red region). The NIST range of tolerance encompasses the target value bounded by twice its uncertainty (U_{NIST}), and represents the range that results in an acceptable Z_{NIST} score, $|Z_{NIST}| \leq 2$.

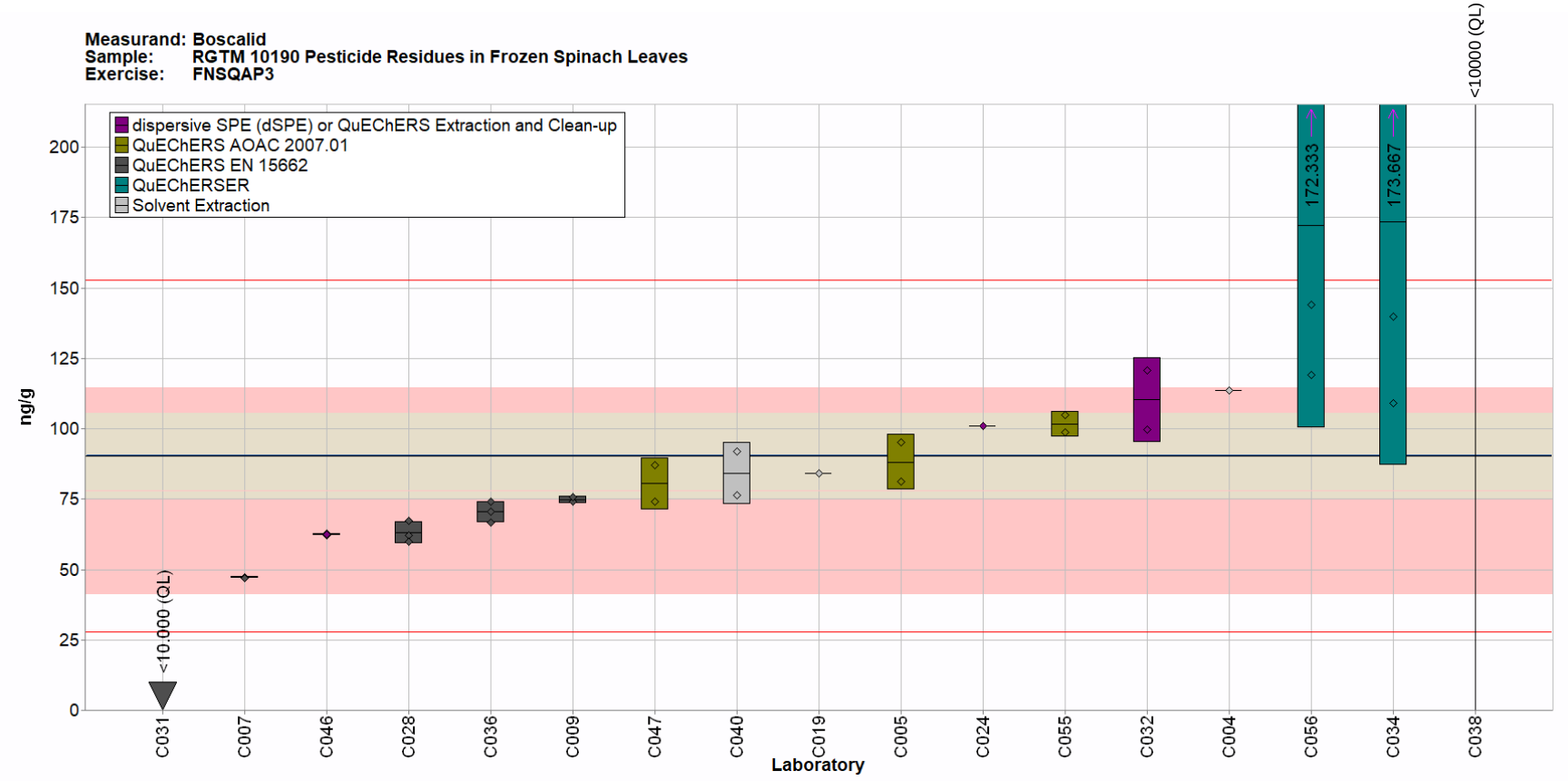

Fig. 6-2. Boscalid in RGTM 10190 (data summary view – sample preparation method).

In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). A downward triangle represents data reported as an LOQ value. The color of the data point represents the sample preparation method employed as indicated in the figure key. Data points outside the graphical range are represented as upward arrows (the sample preparation method reported by laboratory C038 was solvent extraction). The solid black line represents the consensus mean, and the solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{comm}| \leq 2$. The beige shaded region represents the overlapping of the 95 % confidence interval for the consensus mean (green region) and the NIST range of tolerance (red region). The NIST range of tolerance encompasses the target value bounded by twice its uncertainty (U_{NIST}), and represents the range that results in an acceptable Z_{NIST} score, $|Z_{NIST}| \leq 2$.

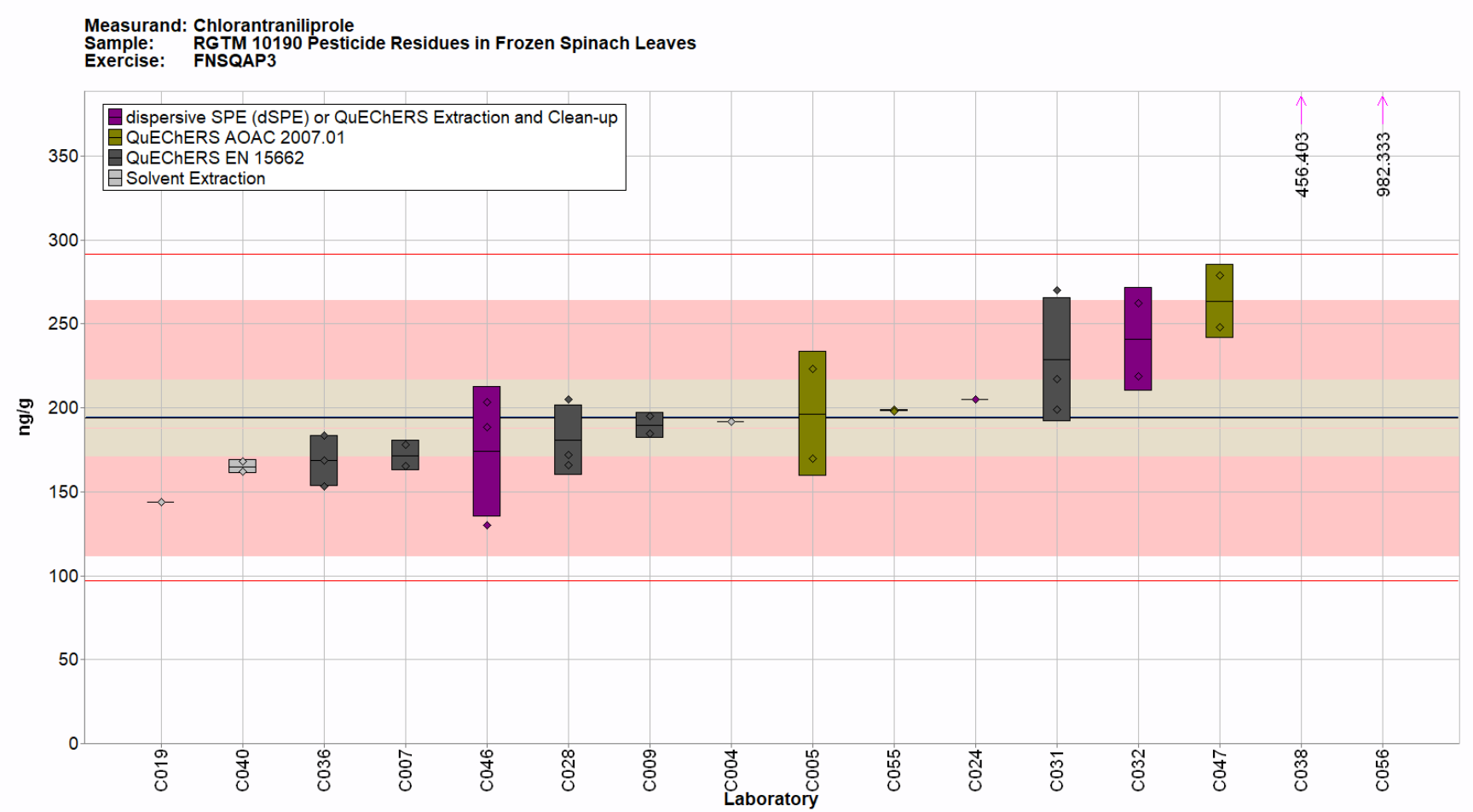

Fig. 6-3. Chlorantraniliprole in RGTM 10190 (data summary view – sample preparation method).

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 methods reported by laboratories C038 and C056 were solvent extraction and QuEChERSER, respectively). The solid black line represents the consensus mean, and the red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{comm}| \leq 2$. The beige shaded region represents the overlapping of the 95 % confidence interval for the consensus mean (green region) and the NIST range of tolerance (red region). The NIST range of tolerance encompasses the target value bounded by twice its uncertainty (U_{NIST}), and represents the range that results in an acceptable Z_{NIST} score, $|Z_{NIST}| \leq 2$.

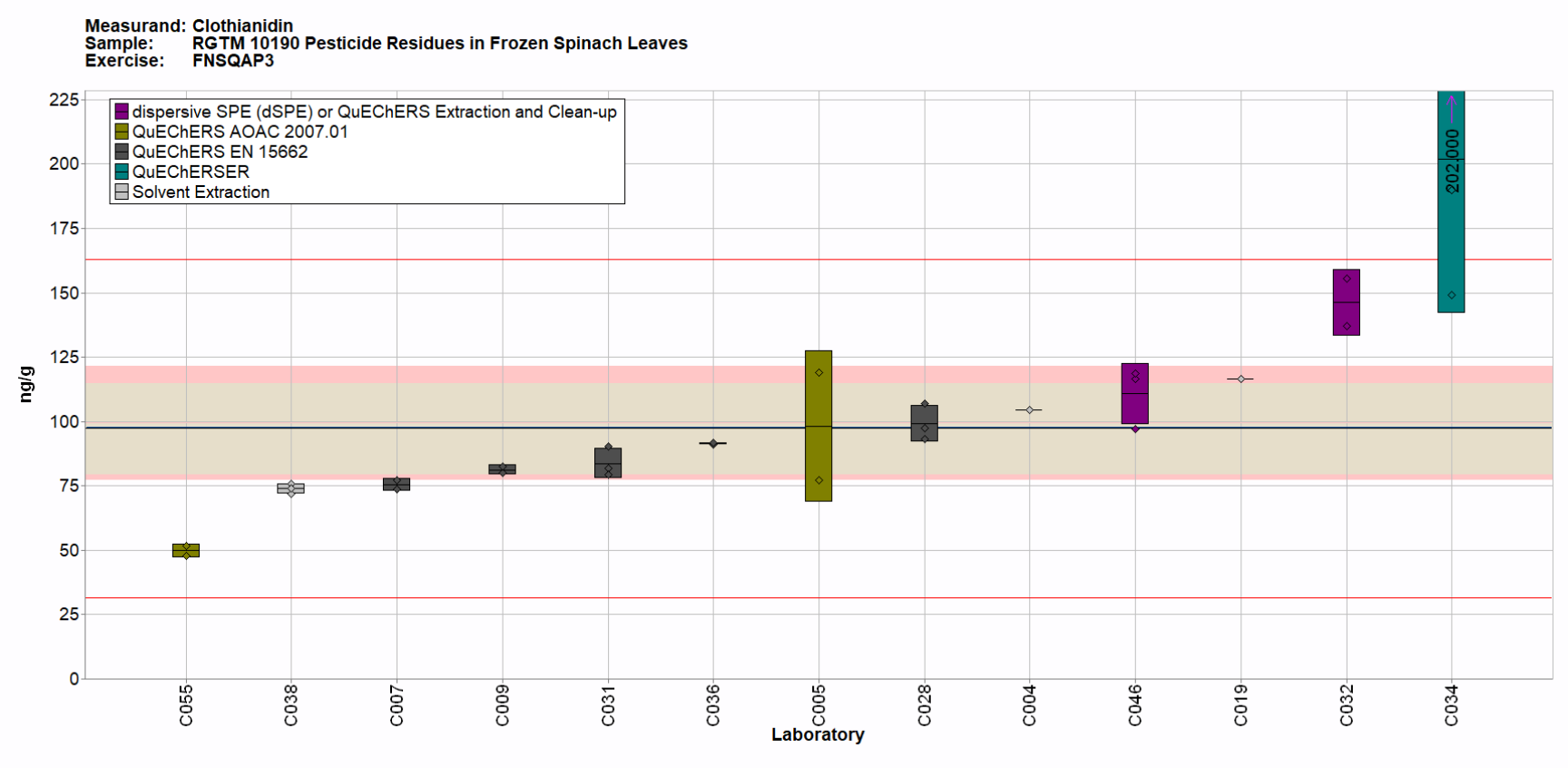

Fig. 6-4. Clothianidin in RGTM 10190 (data summary view – sample preparation method).

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

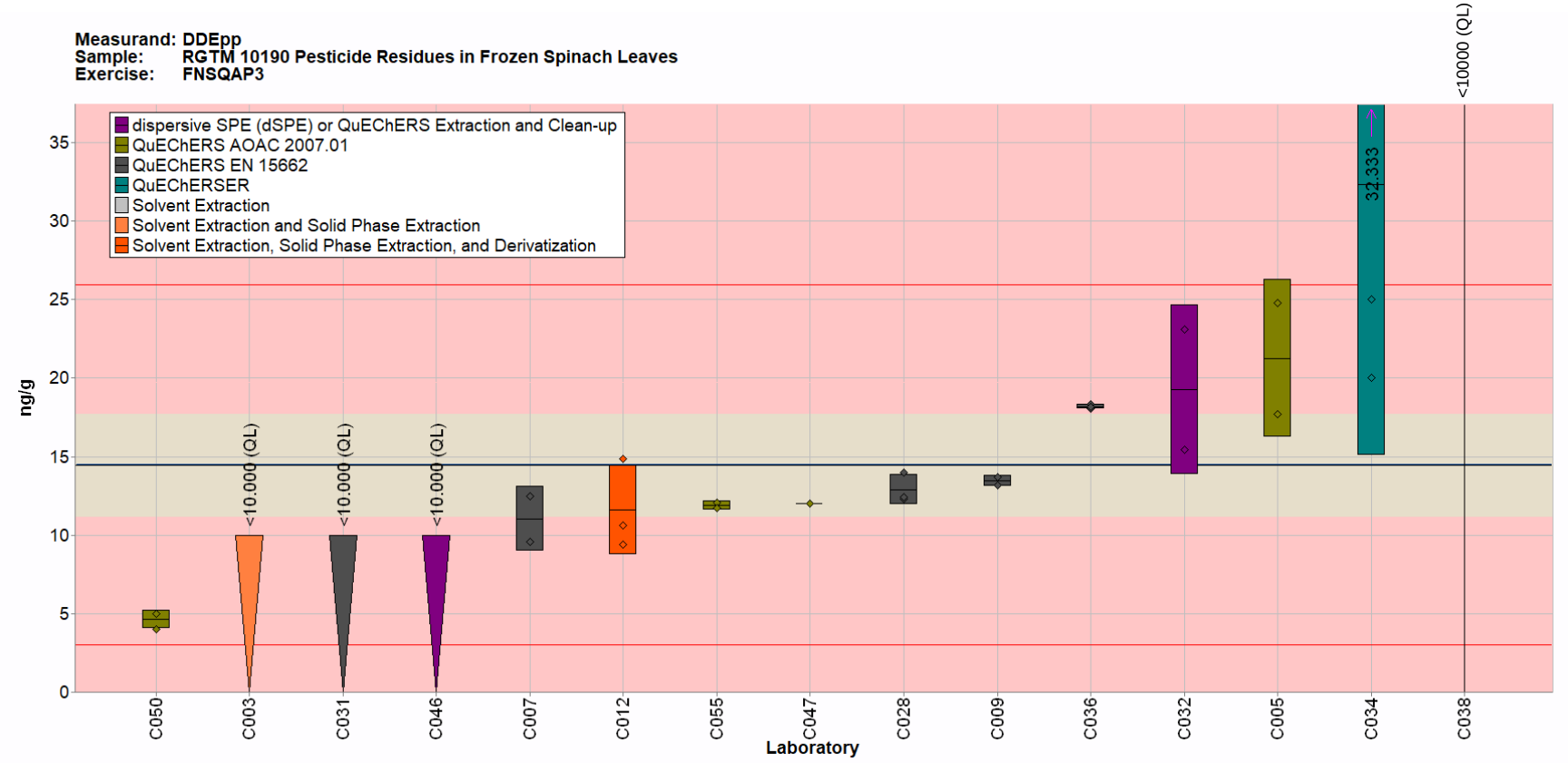

Fig. 6-5. p,p'-Dichlorodiphenyldichloroethylene in RGTM 10190 (data summary view – sample preparation method).

In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). A downward triangle represents data reported as an LOQ value. The color of the data point represents the sample preparation method employed as indicated in the figure key (the sample preparation approach reported by laboratory C038 was solvent extraction). The solid black line represents the consensus mean, and the solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{comm}| \leq 2$. The beige shaded region represents the overlapping of the 95 % confidence interval for the consensus mean (green region) and the NIST range of tolerance (red region). The NIST range of tolerance encompasses the target value bounded by twice its uncertainty (U_{NIST}), and represents the range that results in an acceptable Z_{NIST} score, $|Z_{NIST}| \leq 2$.

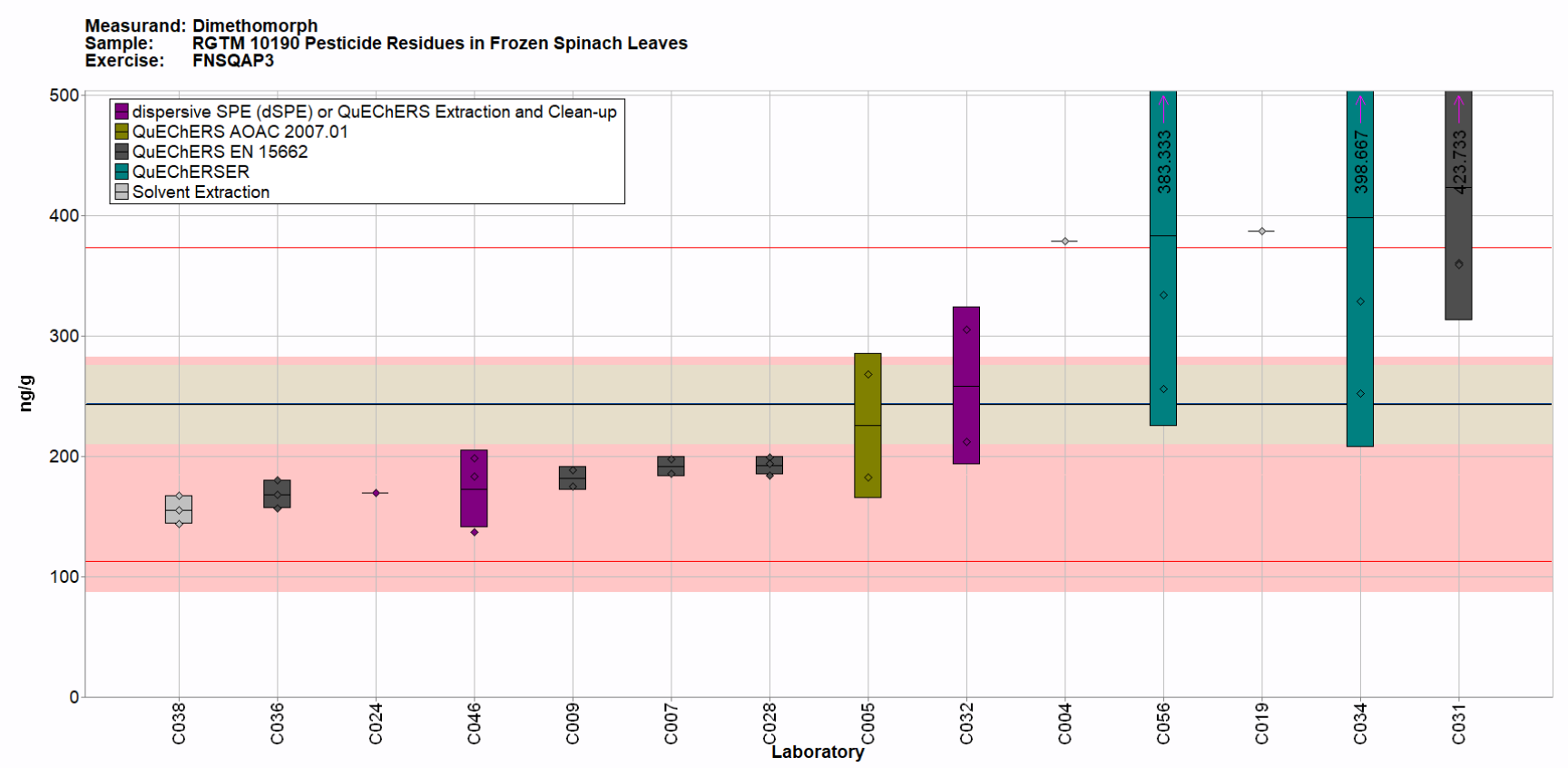

Fig. 6-6. Dimethomorph in RGTM 10190 (data summary view – sample preparation method).

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

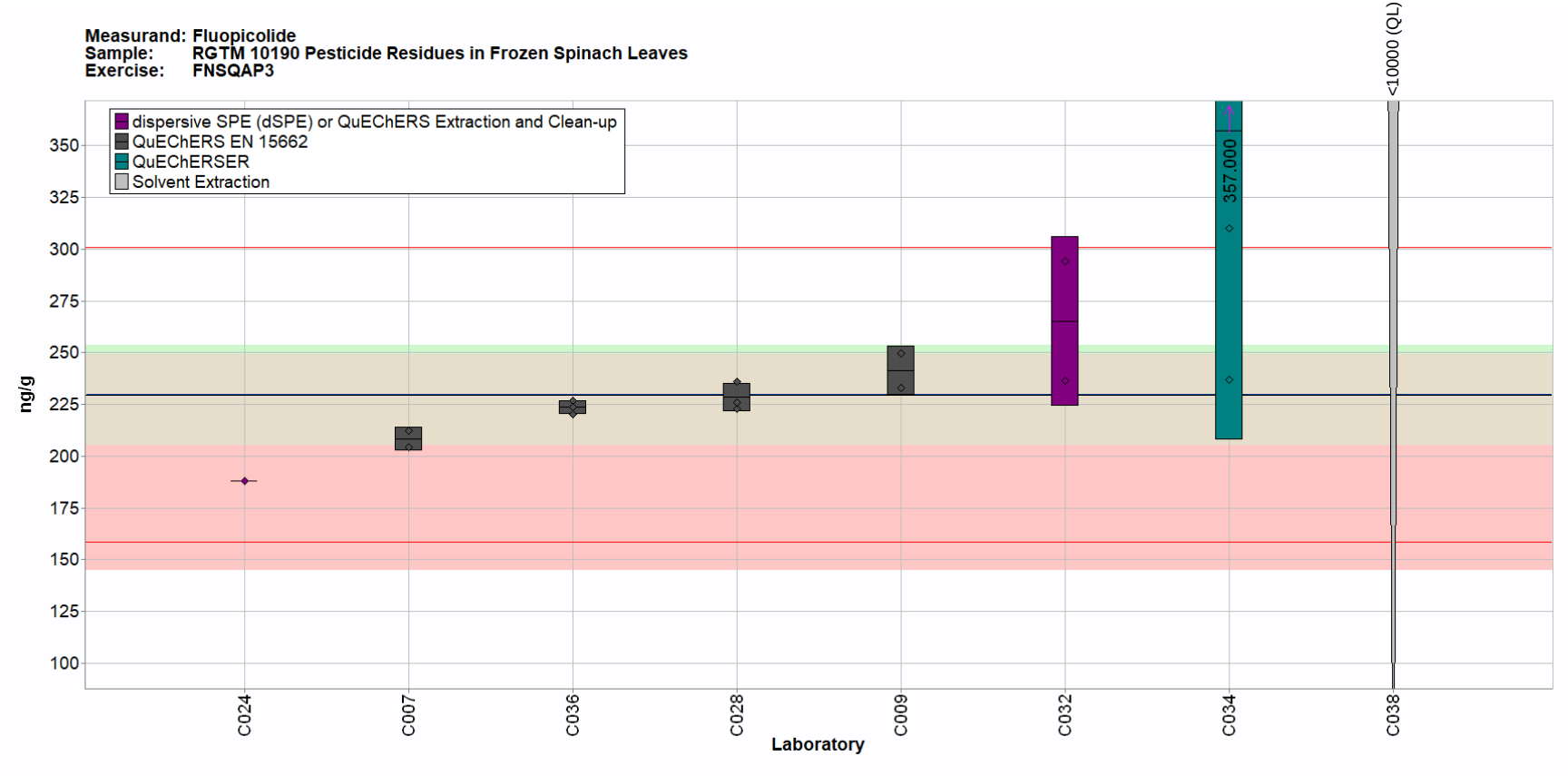

Fig. 6-7. Fluopicolide in RGTM 10190 (data summary view – sample preparation method).

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

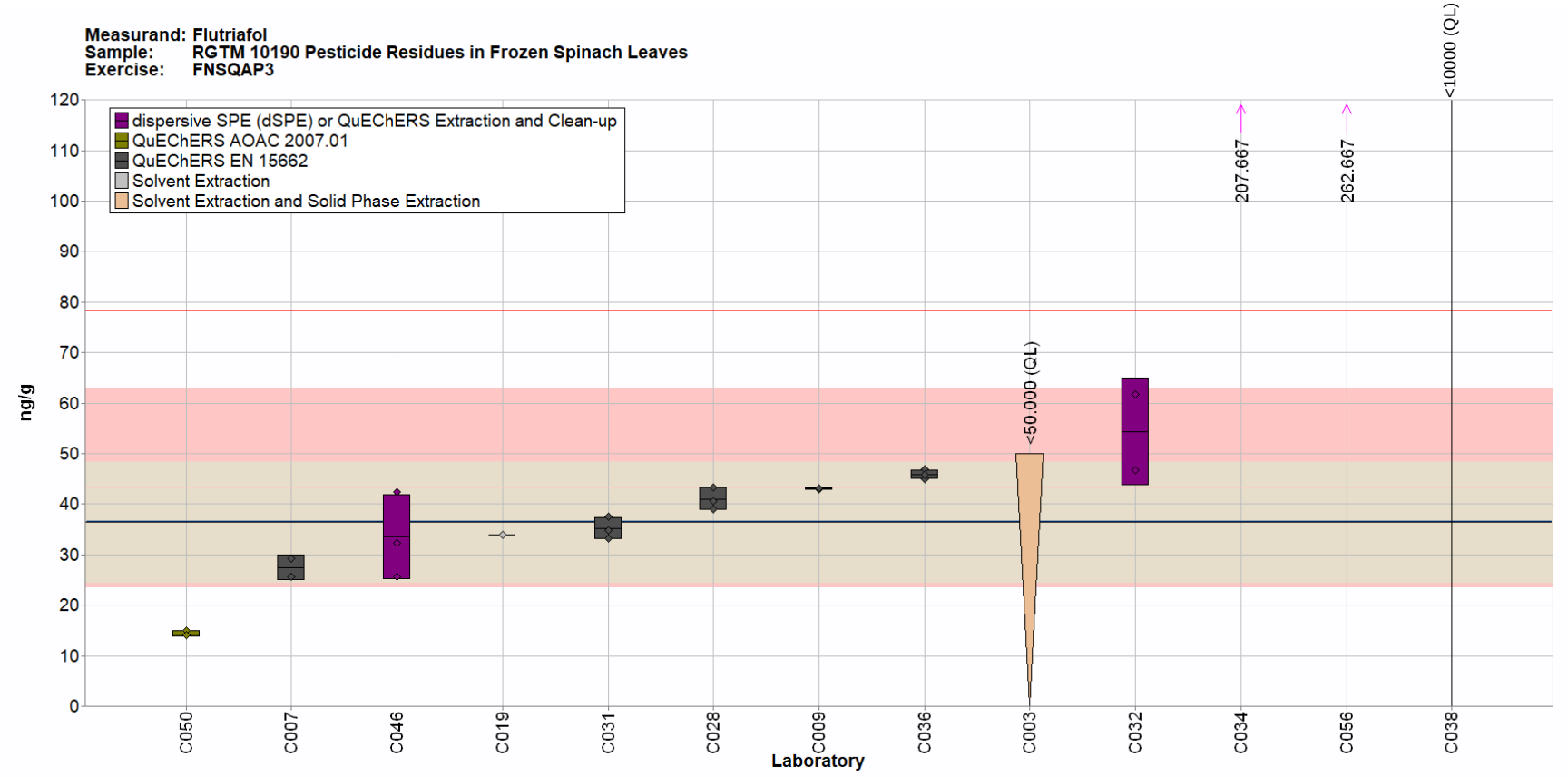

Fig. 6-8. Flutriafol in RGTM 10190 (data summary view – sample preparation method).

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, and data points outside the graphical range are represented as upward arrows. The color of the data point represents the sample preparation method employed as indicated in the figure key (the sample preparation approaches reported by laboratories C034 and C056 were QuEChERSER, and by laboratory C038 was solvent extraction). The solid black line represents the consensus mean, and the solid red line represents the upper bound of the consensus range of tolerance, calculated as the values above the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{comm}| \leq 2$. The lower bound for the consensus range of tolerance is set to zero. The beige shaded region represents the overlapping of the 95 % confidence interval for the consensus mean (green region) and the NIST range of tolerance (red region). The NIST range of tolerance encompasses the target value bounded by twice its uncertainty (U_{NIST}), and represents the range that results in an acceptable Z_{NIST} score, $|Z_{NIST}| \leq 2$.

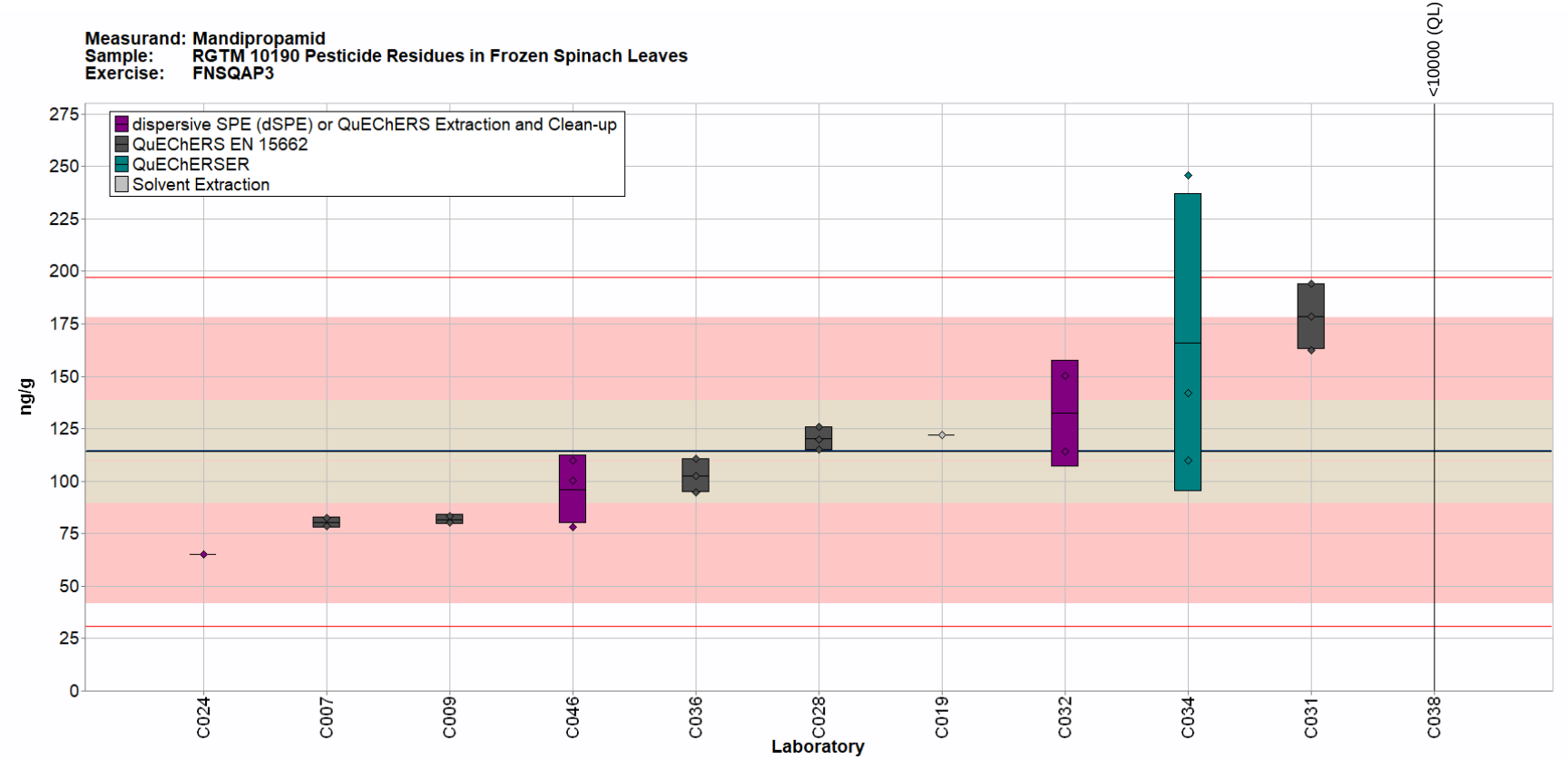

Fig. 6-9. Mandipropamid in RGTM 10190 (data summary view – sample preparation method).

In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). A downward triangle represents data reported as an LOQ value. The color of the data point represents the sample preparation method employed as indicated in the figure key (the sample preparation approach reported by laboratory C038 was solvent extraction). The solid black line represents the consensus mean, and the solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{comm}| \leq 2$. The beige shaded region represents the overlapping of the 95 % confidence interval for the consensus mean (green region) and the NIST range of tolerance (red region). The NIST range of tolerance encompasses the target value bounded by twice its uncertainty (U_{NIST}), and represents the range that results in an acceptable Z_{NIST} score, $|Z_{NIST}| \leq 2$.

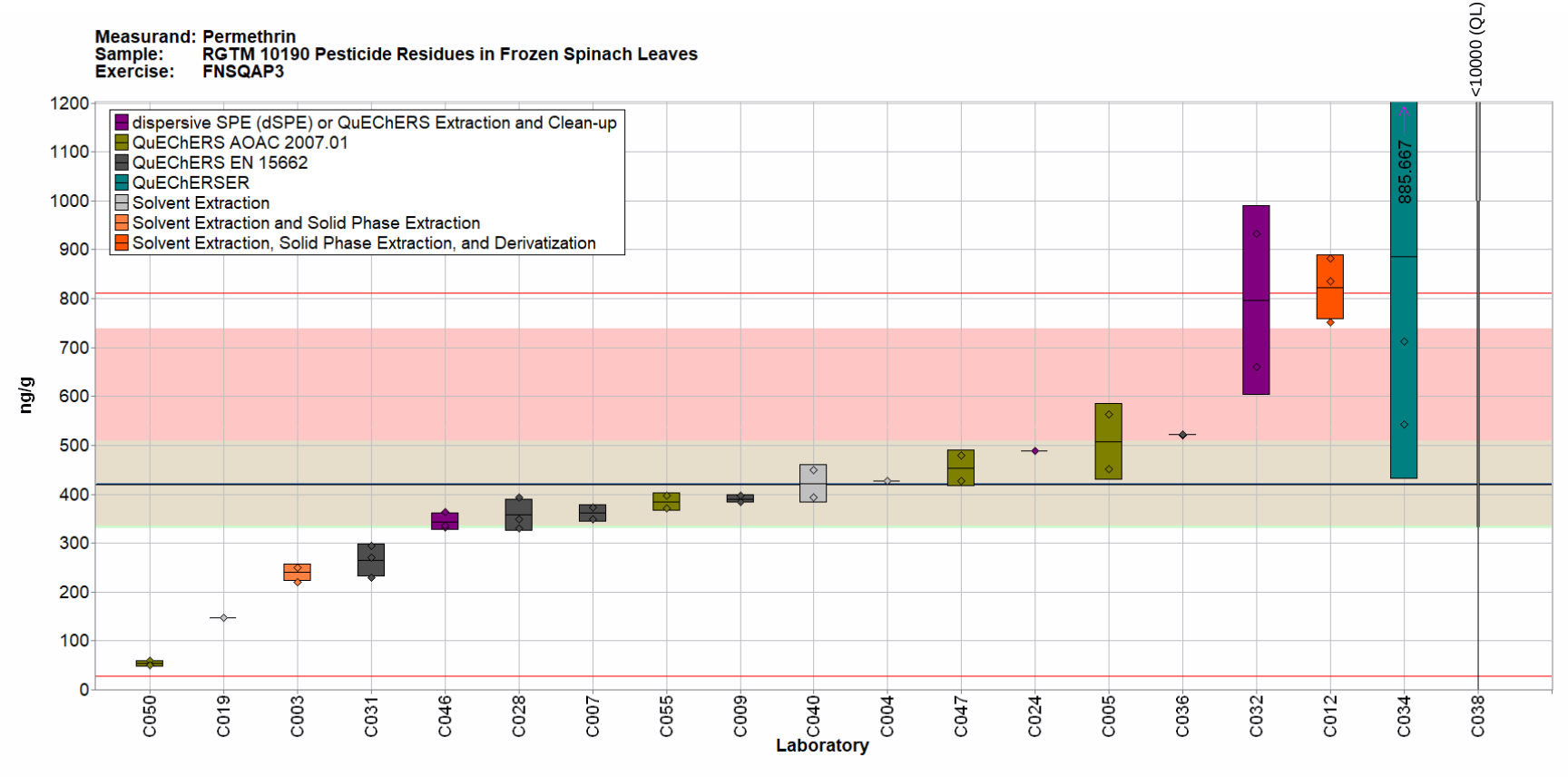

Fig. 6-10. Permethrin in RGTM 10190 (data summary view – sample preparation method).

In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). A downward triangle represents data reported as an LOQ value. The color of the data point represents the sample preparation method employed as indicated in the figure key (the sample preparation approach reported by laboratory C038 was solvent extraction). The solid black line represents the consensus mean, and the solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{comm}| \leq 2$. The beige shaded region represents the overlapping of the 95 % confidence interval for the consensus mean (green region) and the NIST range of tolerance (red region). The NIST range of tolerance encompasses the target value bounded by twice its uncertainty (U_{NIST}), and represents the range that results in an acceptable Z_{NIST} score, $|Z_{NIST}| \leq 2$.

6.4.4. Analytical Methods and Calibration

For the determination of acephate, boscalid, chlorantraniliprole, clothianidin, dimethomorph, fluopicolide, flutriafol, and mandipropamid in the spinach sample (Fig. 6-11 through Fig. 6-18), 62 % to 100 % of laboratories reported use of LC-MS, LC-MS/MS, or LC-HRMS. For determination of p,p'-DDE and permethrin in the spinach sample, as shown in Fig. 6-19 and Fig. 6-20, 79 % to 100 % of laboratories reported using GC-MS-based techniques. Two to three laboratories for each residue reported at data entry the use of an official method that includes both LC-MS and GC-MS based options, specifying the module that was used in the preliminary method survey (i.e., EN 15662:2018, AOAC 2007.001). No trends related to analytical method were identified.

In a preliminary method survey, most laboratories indicated intention to use matrix-matched calibration (60 % to 75 %), standard additions (up to 10 %), or a combination of the two (13 % to 25 %) to compensate for possible matrix effects when determining residues in the spinach sample. Up to four laboratories did not use matrix-matched calibration or standard additions to compensate for matrix effects. For calibration, 5 laboratories used an internal standard approach (26 %), 10 laboratories used an external standard approach (53 %), and two laboratories each used standard additions and a combination of internal and external standard approaches (11 % each). One laboratory did not report the type of calibration approach used. None of these specific method details correlate to performance in this study.

One laboratory reported a method LOQ of 10 000 ng/g for several pesticide residues, which is several orders of magnitude above the published expectations described in Table 6-14. Laboratories should ensure that method LOQs are accurately determined, including that calculations are free from errors and consider method modifications to ensure accurate detection of pesticide residues at accepted levels.

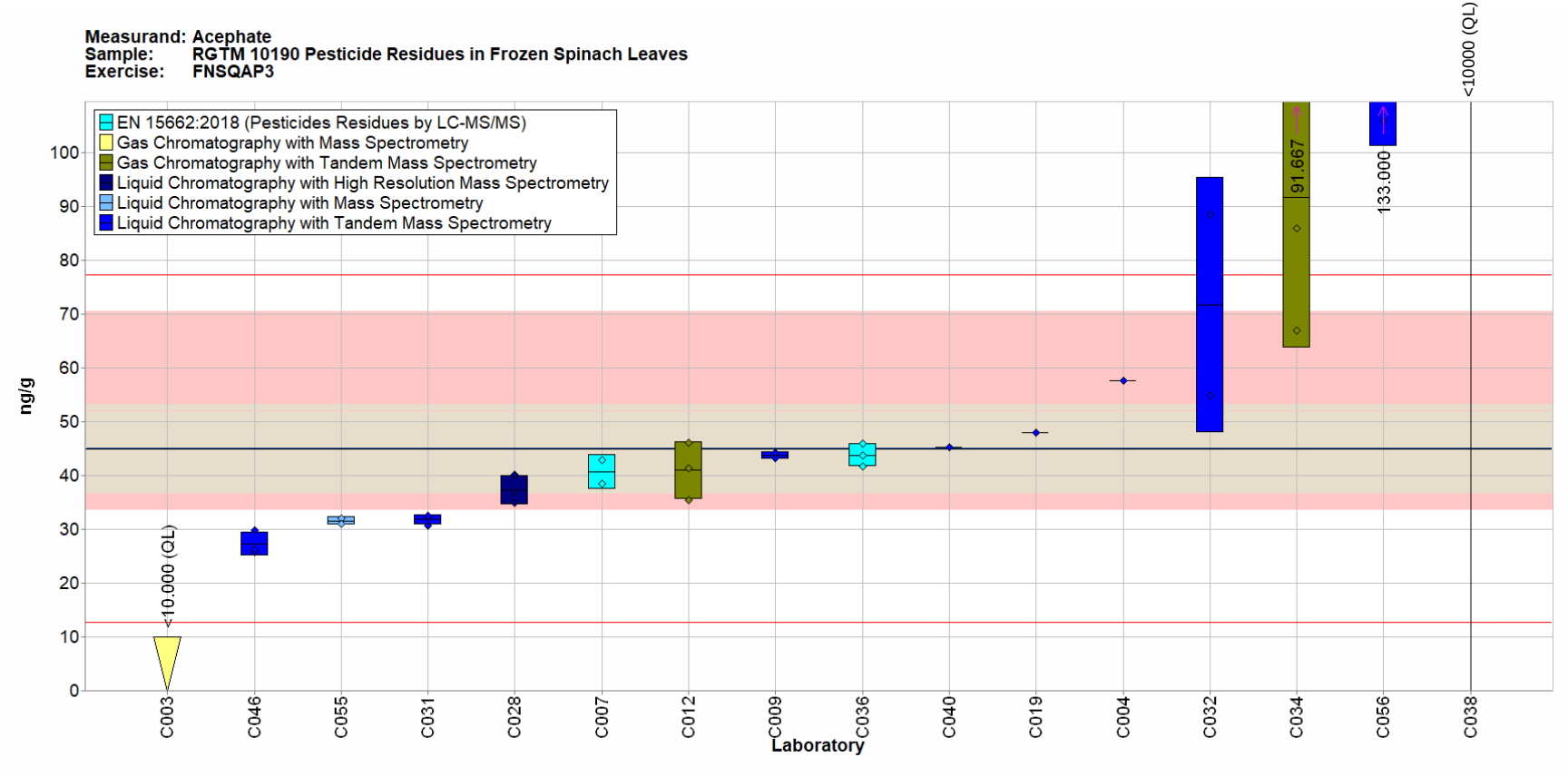

Fig. 6-11. Acephate in RGTM 10190 (data summary view – analytical method).

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 C038 was GC-MS). The solid black line represents the consensus mean, and the solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{comm}| \leq 2$. The beige shaded region represents the overlapping of the 95 % confidence interval for the consensus mean (green region) and the NIST range of tolerance (red region). The NIST range of tolerance encompasses the target value bounded by twice its uncertainty (U_{NIST}), and represents the range that results in an acceptable Z_{NIST} score, $|Z_{NIST}| \leq 2$.

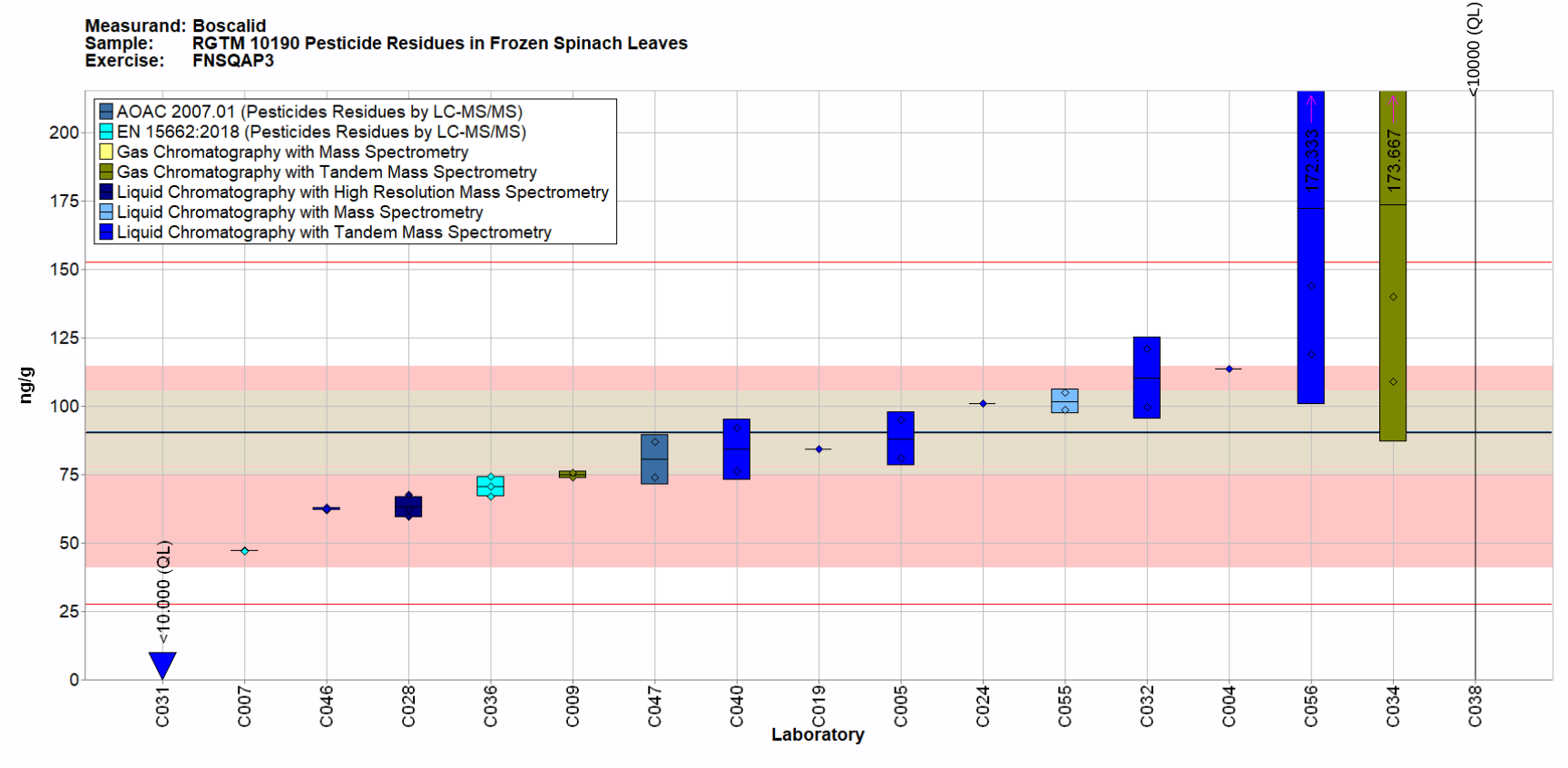

Fig. 6-12. Boscalid in RGTM 10190 (data summary view – analytical method).

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 C038 was GC-MS). The solid black line represents the consensus mean, and the solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{comm}| \leq 2$. The beige shaded region represents the overlapping of the 95 % confidence interval for the consensus mean (green region) and the NIST range of tolerance (red region). The NIST range of tolerance encompasses the target value bounded by twice its uncertainty (U_{NIST}), and represents the range that results in an acceptable Z_{NIST} score, $|Z_{NIST}| \leq 2$.

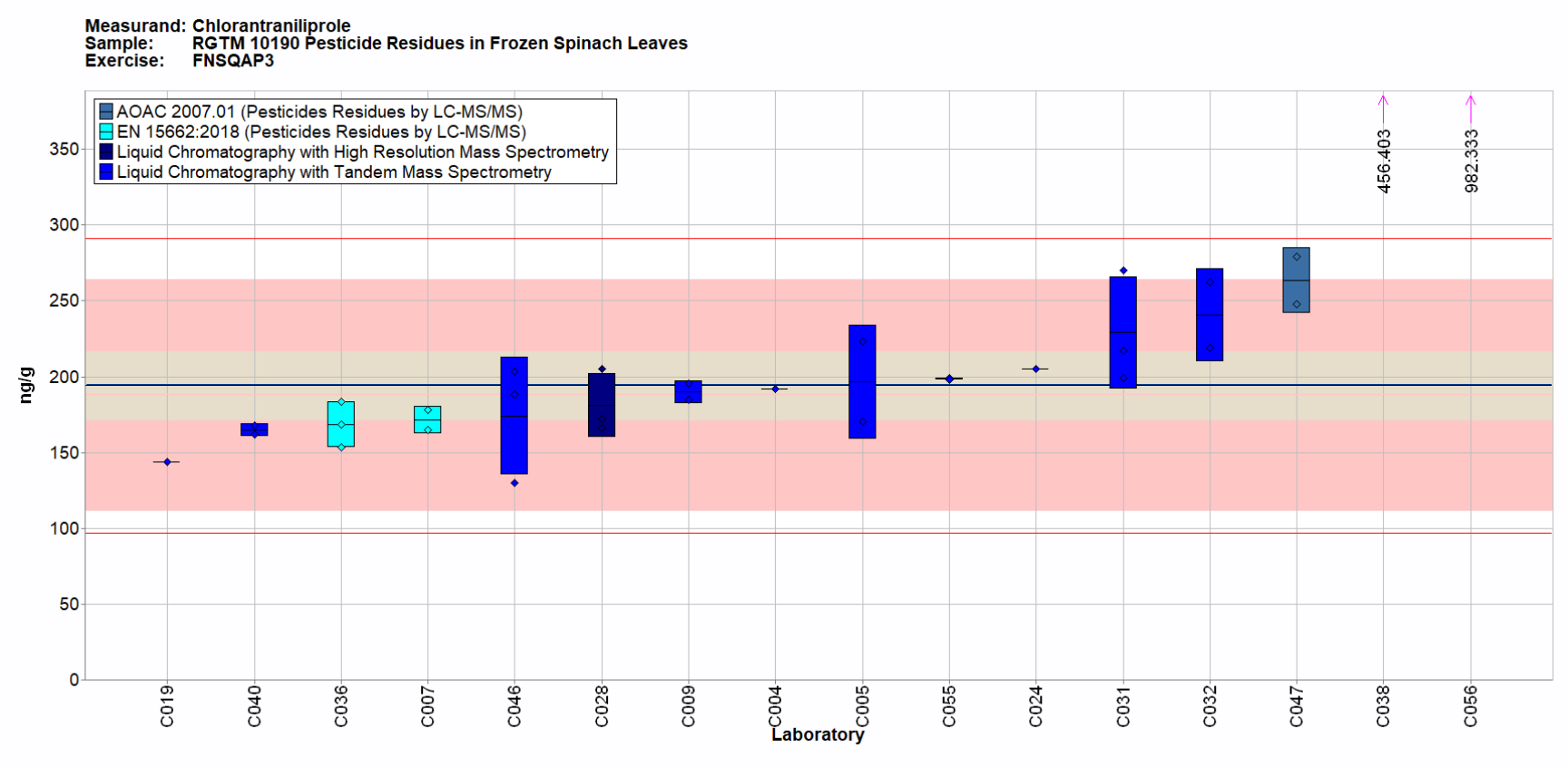

Fig. 6-13. Chlorantraniliprole in RGTM 10190 (data summary view – analytical method).

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 C038 and C056 were LC-MS and LC-MS/MS, respectively). The solid black line represents the consensus mean, and the solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{comm}| \leq 2$. The beige shaded region represents the overlapping of the 95 % confidence interval for the consensus mean (green region) and the NIST range of tolerance (red region). The NIST range of tolerance encompasses the target value bounded by twice its uncertainty (U_{NIST}), and represents the range that results in an acceptable Z_{NIST} score, $|Z_{NIST}| \leq 2$.

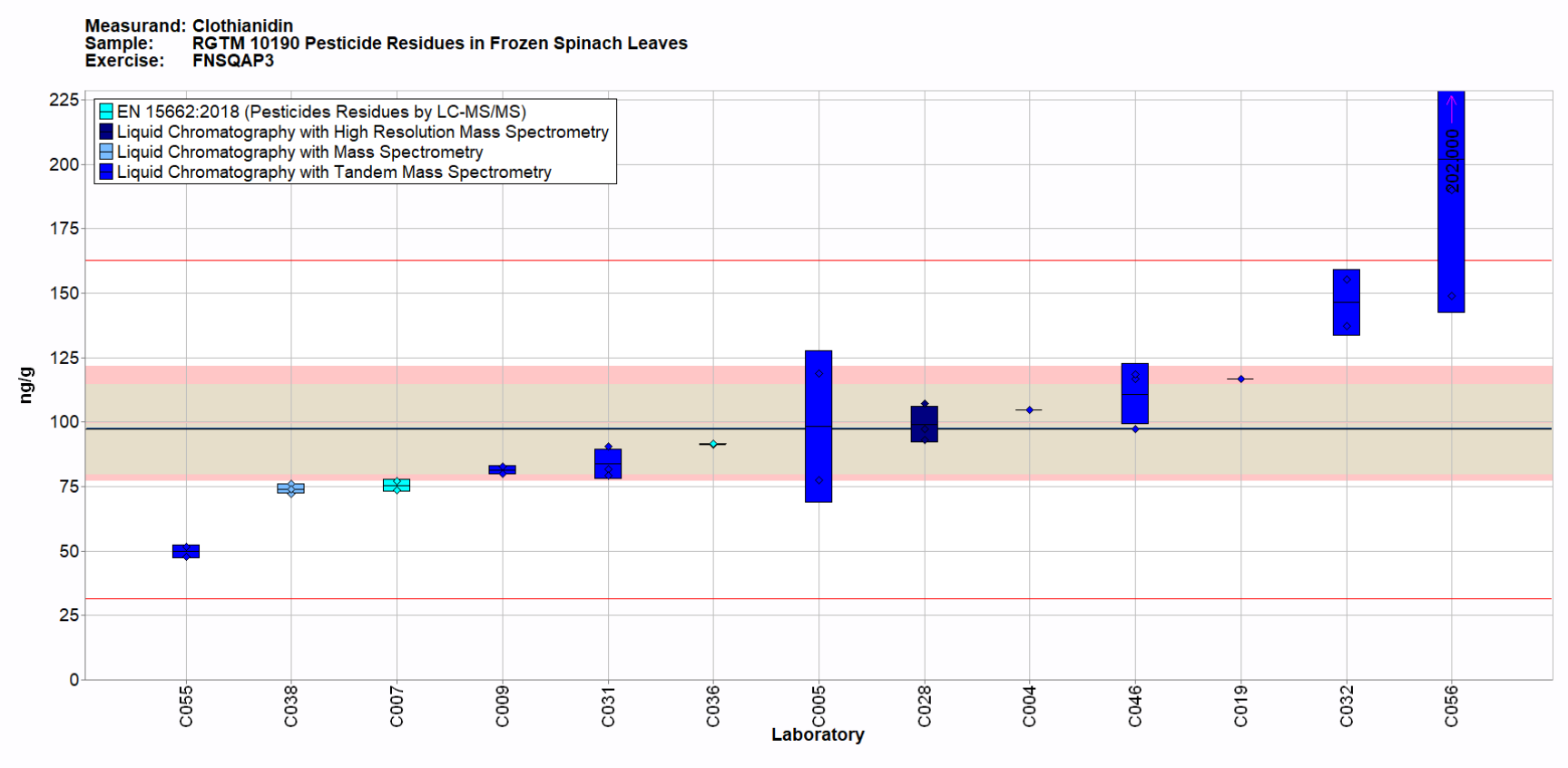

Fig. 6-14. Clothianidin in RGTM 10190 (data summary view – analytical method).

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

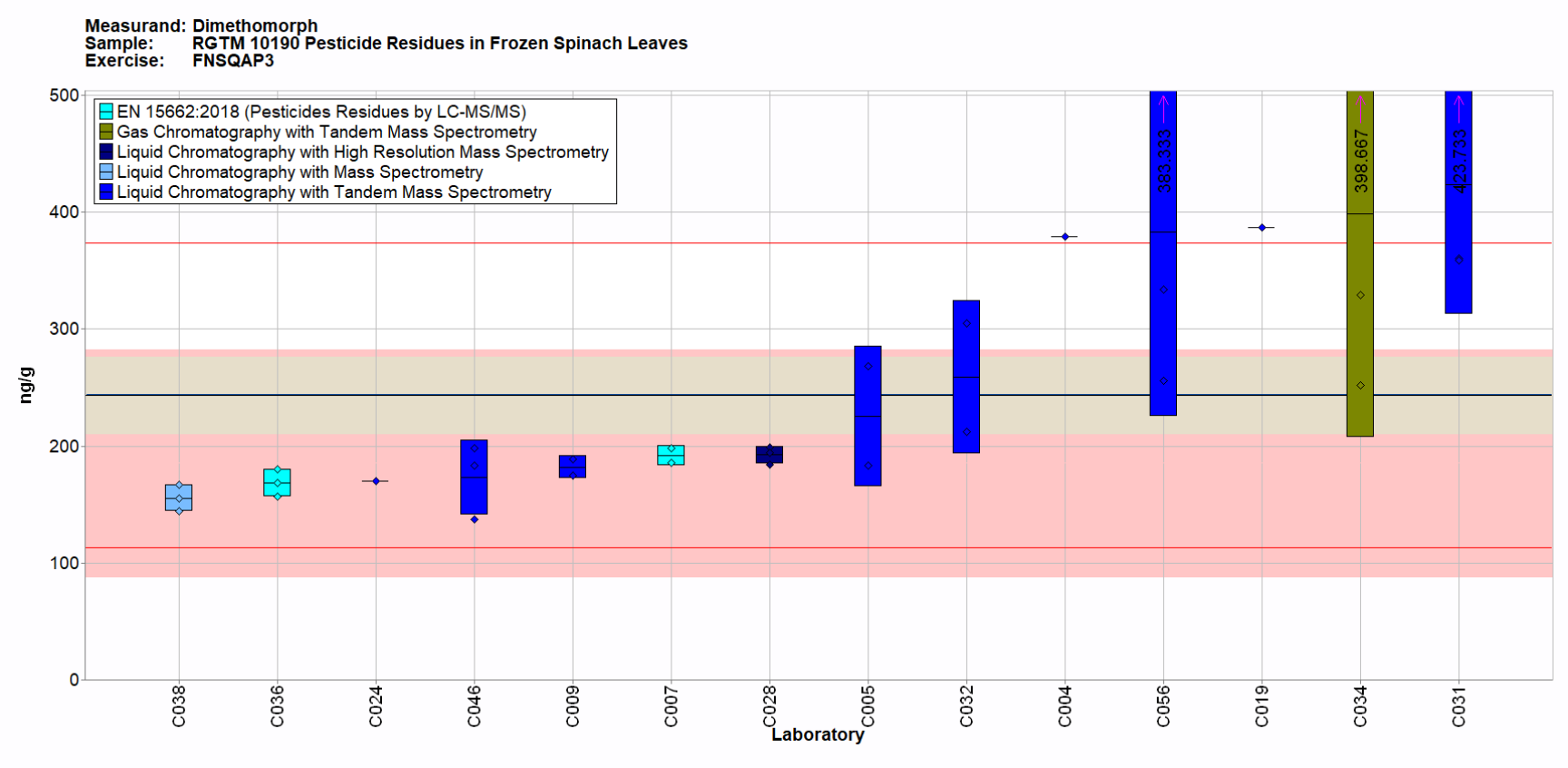

Fig. 6-15. Dimethomorph in RGTM 10190 (data summary view – analytical method).

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

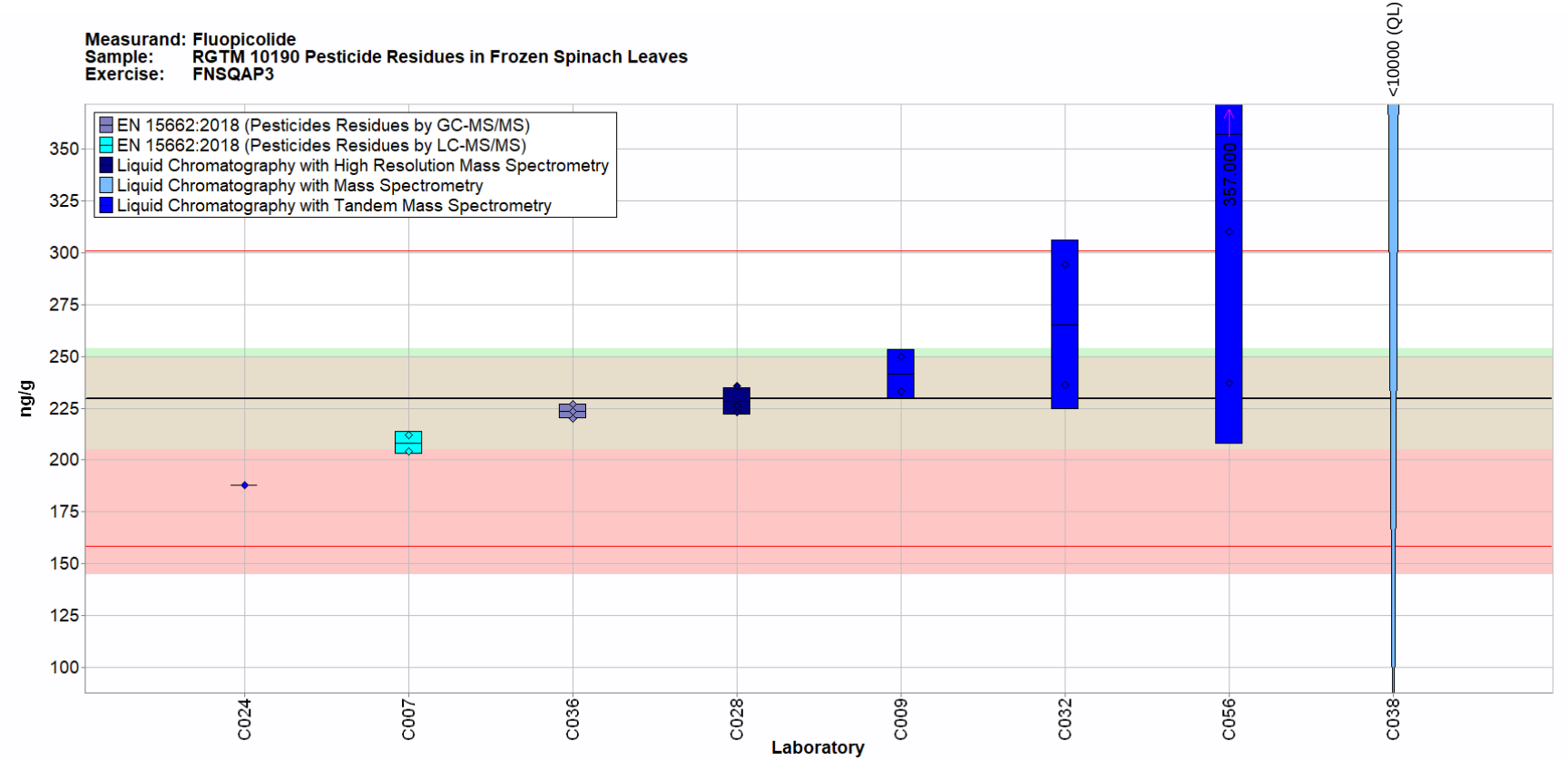

Fig. 6-16. Fluopicolide in RGTM 10190 (data summary view – analytical method).

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

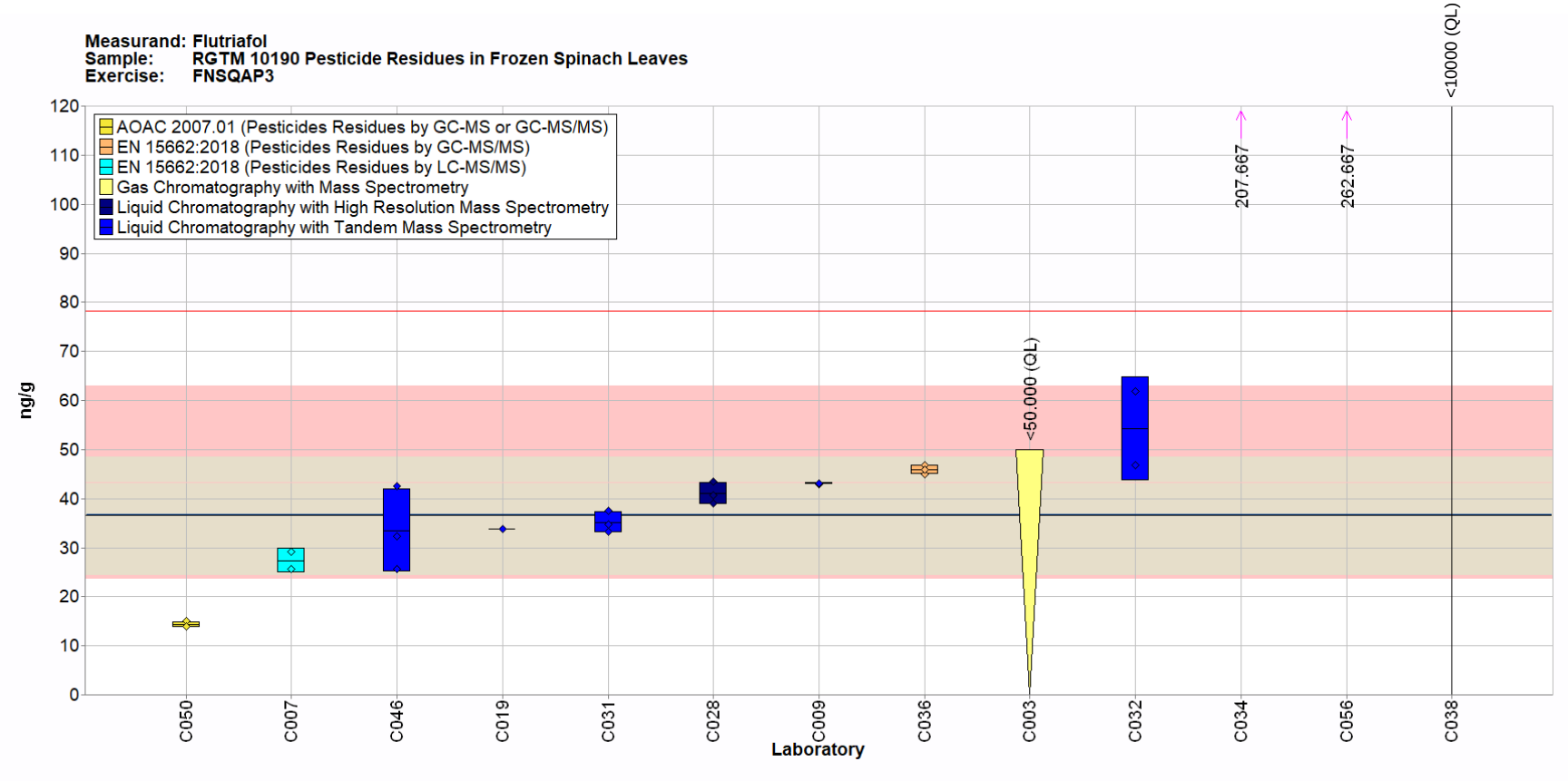

Fig. 6-17. Flutriafol in RGTM 10190 (data summary view – analytical method).

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, and data points outside the graphical range are represented as upward arrows. The color of the data point represents the analytical method employed as indicated in the figure key (the analytical methods reported by laboratories C034, C056, and C038 were GC-MS/MS, LC-MS/MS, and GC-MS, respectively). The solid black line represents the consensus mean, and the solid red line represents the upper bound of the consensus range of tolerance, calculated as the values above the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{comm}| \leq 2$. The lower bound for the consensus range of tolerance is set to zero. The beige shaded region represents the overlapping of the 95 % confidence interval for the consensus mean (green region) and the NIST range of tolerance (red region). The NIST range of tolerance encompasses the target value bounded by twice its uncertainty (U_{NIST}), and represents the range that results in an acceptable Z_{NIST} score, $|Z_{NIST}| \leq 2$.

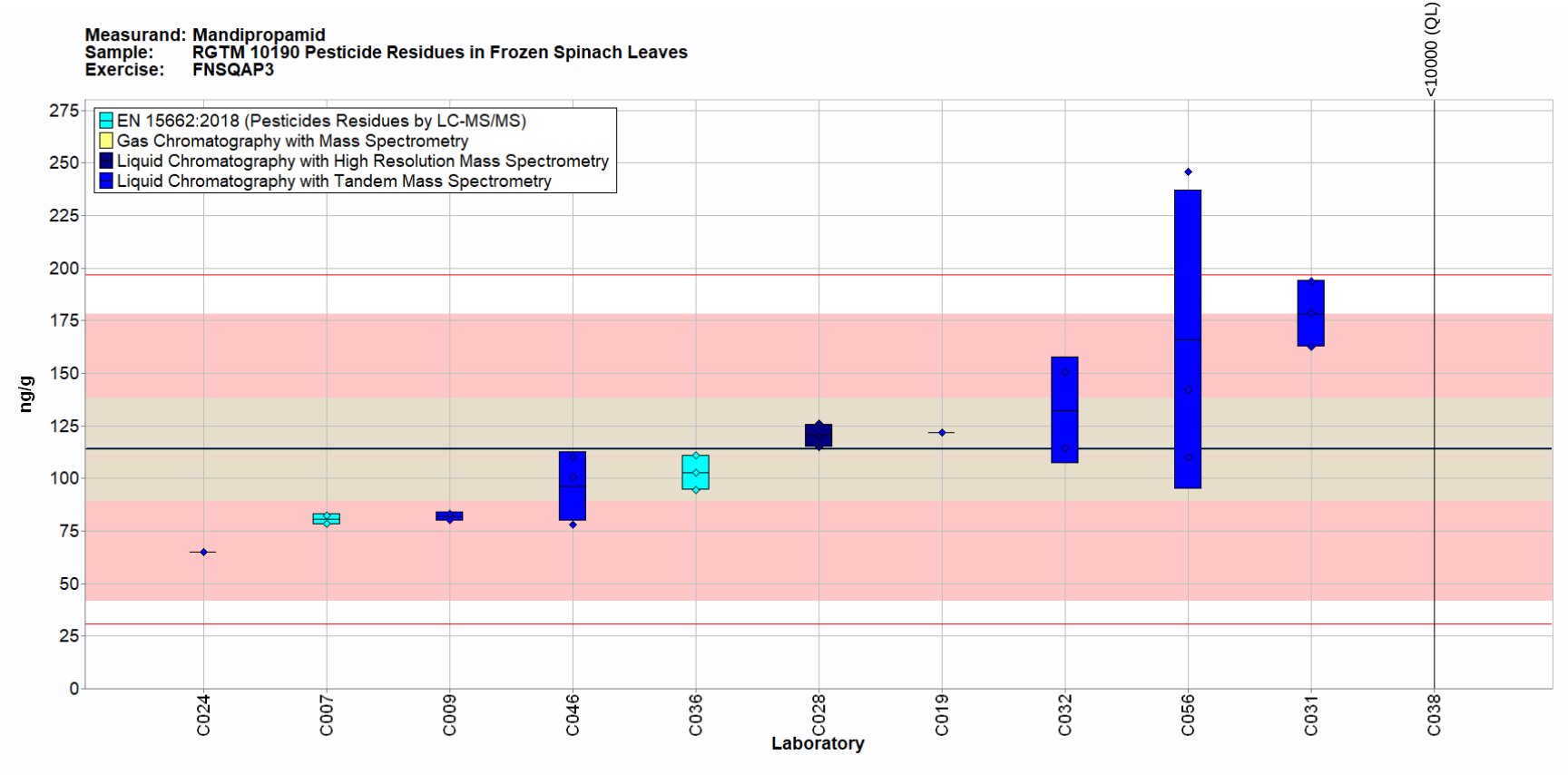

Fig. 6-18. Mandipropamid in RGTM 10190 (data summary view – analytical method).

In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). A downward triangle represents data reported as an LOQ value. The color of the data point represents the analytical method employed as indicated in the figure key (the analytical method reported by laboratory C038 was GC-MS). The solid black line represents the consensus mean, and the solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{comm}| \leq 2$. The beige shaded region represents the overlapping of the 95 % confidence interval for the consensus mean (green region) and the NIST range of tolerance (red region). The NIST range of tolerance encompasses the target value bounded by twice its uncertainty (U_{NIST}), and represents the range that results in an acceptable Z_{NIST} score, $|Z_{NIST}| \leq 2$.

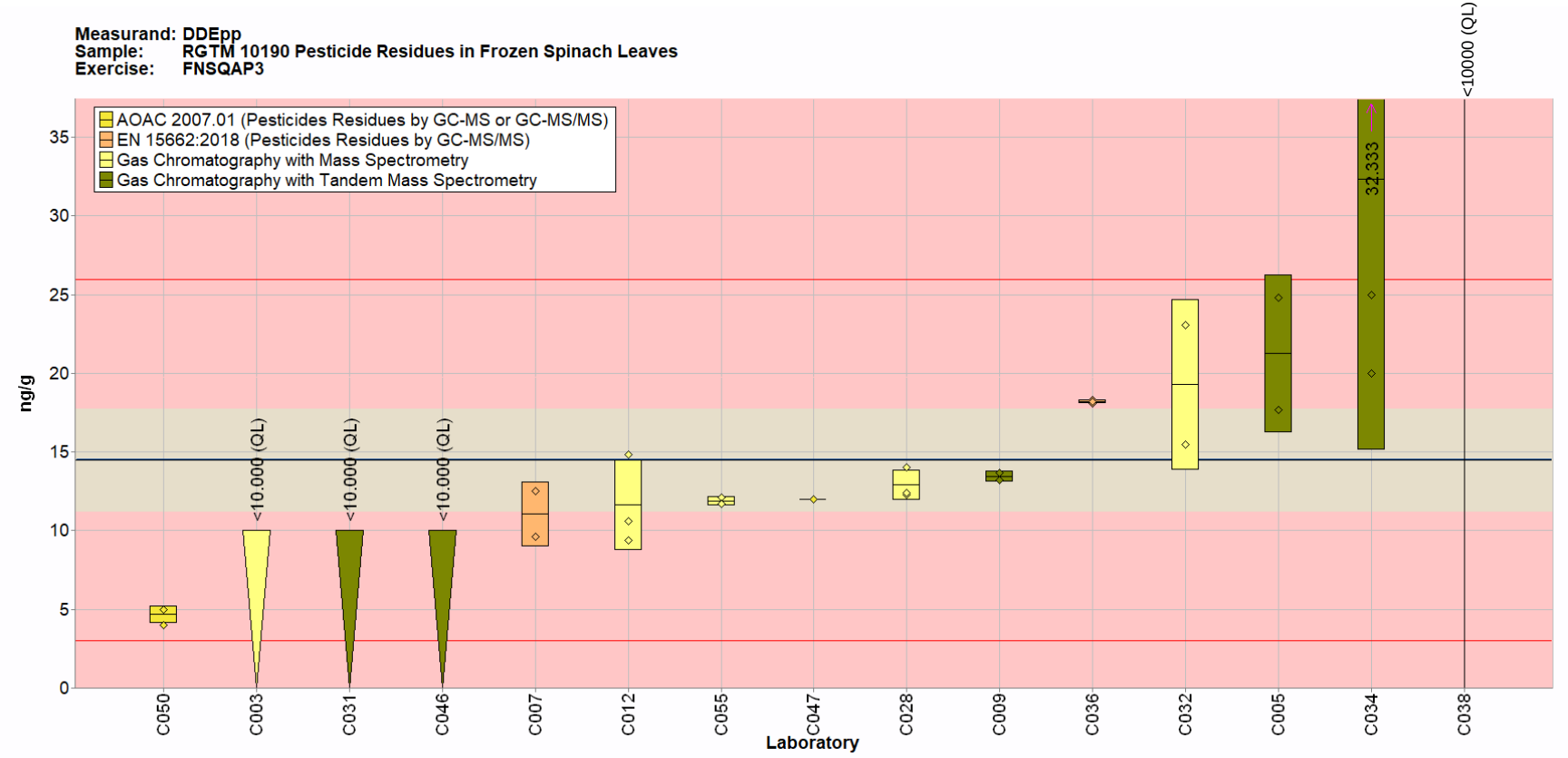

Fig. 6-19. p,p'-Dichlorodiphenyldichloroethylene in RGTM 10190 (data summary view – analytical method).

In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). A downward triangle represents data reported as an LOQ value. The color of the data point represents the analytical method employed as indicated in the figure key (the analytical method reported by laboratory C038 was GC-MS). The solid black line represents the consensus mean, and the solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{comm}| \leq 2$. The beige shaded region represents the overlapping of the 95 % confidence interval for the consensus mean (green region) and the NIST range of tolerance (red region). The NIST range of tolerance encompasses the target value bounded by twice its uncertainty (U_{NIST}), and represents the range that results in an acceptable Z_{NIST} score, $|Z_{NIST}| \leq 2$.

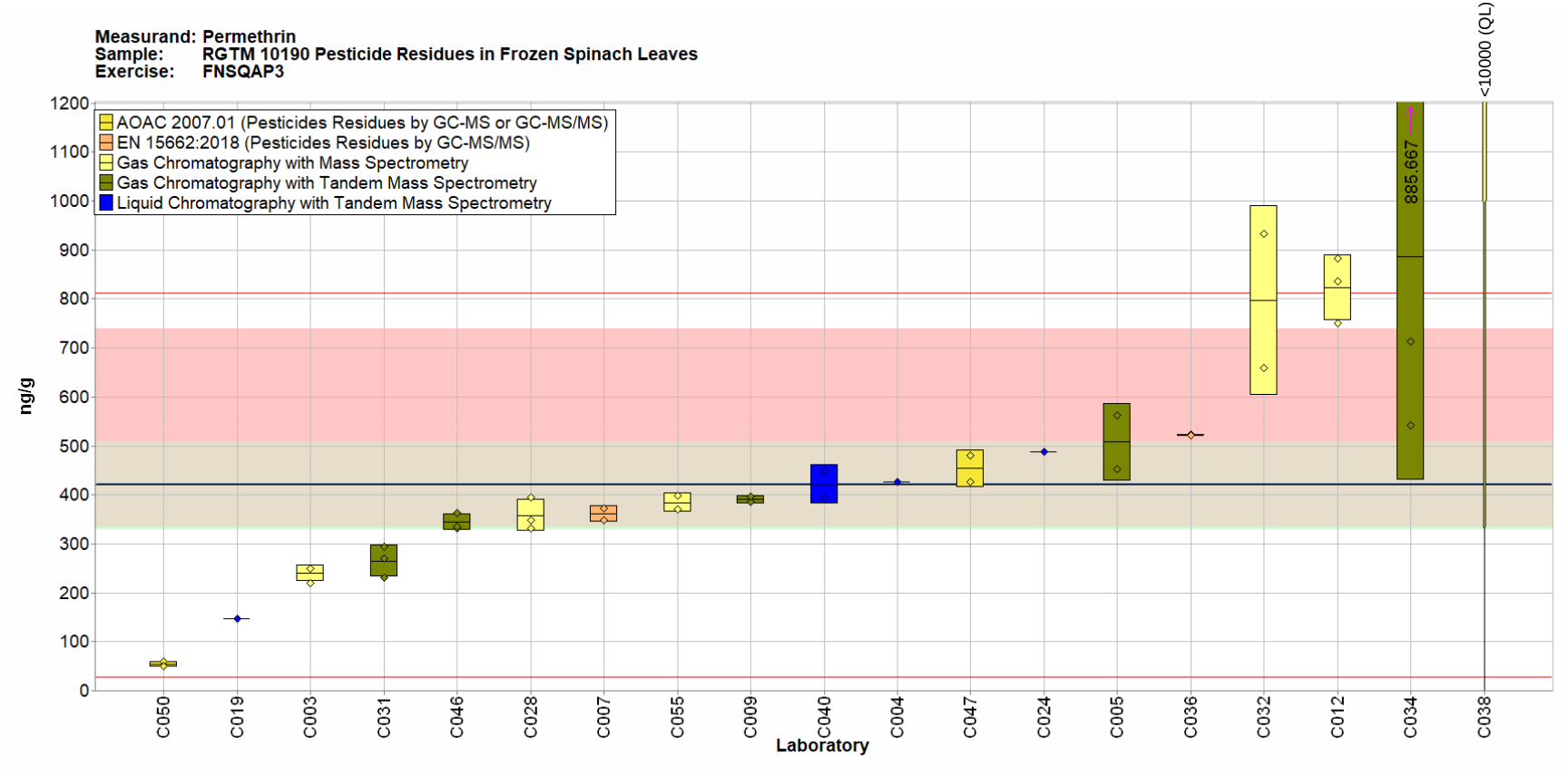

Fig. 6-20. Permethrin in RGTM 10190 (data summary view – analytical method).

In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). A downward triangle represents data reported as an LOQ value. The color of the data point represents the analytical method employed as indicated in the figure key (the analytical method reported by laboratory C038 was GC-MS). The solid black line represents the consensus mean, and the solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{comm}| \leq 2$. The beige shaded region represents the overlapping of the 95 % confidence interval for the consensus mean (green region) and the NIST range of tolerance (red region). The NIST range of tolerance encompasses the target value bounded by twice its uncertainty (U_{NIST}), and represents the range that results in an acceptable Z_{NIST} score, $|Z_{NIST}| \leq 2$.

6.4.5. Accuracy

The study material was prepared by spiking known amounts of pesticides into organic spinach. The target mass fraction values were determined by analysis using LC-MS/MS, GC-MS, and/or GC-MS/MS. While the gravimetric mass fractions from the material preparation were not used in determining the target value, the assayed values were within 11 % of the gravimetric values for all residues.

Overall, the participant consensus mass fraction values were comparable to the target values. For all residues, the consensus mass fraction ranges overlap the target ranges, and for all but fluopicolide, the consensus ranges are completely contained within the target ranges. For most residues, only one or two laboratories reported measured mass fractions outside the consensus ranges. Exceptions include mandipropamid, for which no laboratories reported mass fraction values outside the consensus range and dimethomorph, for which five laboratories reported values outside the consensus range. As stated previously, no trends were observed to correlate laboratory performance with sample size, method, matrix compensation technique, or calibration type used. Laboratories reporting mass fractions outside the consensus ranges should review their methods to evaluate potential sources of error.

6.4.6. Summary and Overall Conclusions

Laboratories participating in this study performed well with respect to accuracy and precision. Some participants reported high within-laboratory mass fraction variability, but no trend was observed correlating performance with common method parameters. The results from some laboratories were biased high, but like precision, no correlation between these results and sample size, method, matrix compensation technique, or type of calibration was identified. For measurands that can be determined by both LC and GC based methods, no trends between performance and analytical method were observed. Laboratories that reported biased or highly variable results should investigate potential sources of error such as incomplete extraction of residues from the matrix, insufficient sample size, uncompensated matrix effects, and inappropriate calibration. Routine use of reference materials or other such as quality control materials, or evaluation of recovery from spiked samples, can provide valuable information about method performance and potential sources of bias and variability.

NIST has conducted two QAP studies involving measurement of pesticide residues in food samples prior to this FNSQAP study. Both of the previous studies focused on the determination of glyphosate and glyphosate metabolites; this study was the first to include acephate, boscalid, chlorantraniliprole, clothianidin, p,p’-DDE, dimethomorph, fluopicolide, flutriafol, mandipropamid, and permethrin.

Appendix A. List of Abbreviations and Acronyms

AOAC
AOAC International, founded in 1884 as the Association of Official Agricultural Chemists. Today, the organization's legal name is AOAC INTERNATIONAL, and their long-form name is ASSOCIATION OF OFFICIAL ANALYTICAL COLLABORATION (AOAC) INTERNATIONAL. A provider of documentary standards.

Avg
Average

CN Conversion
Cyanidation

cGMP
current Good Manufacturing Practice

Cu
Copper

CRM
Certified Reference Material

DSQAP
Dietary Supplement Laboratory Quality Assurance Program

EN
European Standards, from the German name *Europäische Norm* ("European Norm")

EPA
US Environmental Protection Agency

FDA
US Food and Drug Administration

FNSQAP
Food Nutrition and Safety Measurements Quality Assurance Program

GC-MS
Gas Chromatography Mass Spectrometry

GC-MS/MS
Gas Chromatography with Tandem Mass Spectrometry Detection

HAMQAP
Health Assessment Measurements Quality Assurance Program

ICP-MS
Inductively Coupled Plasma Mass Spectrometry

ICP-OES
Inductively Coupled Plasma Optical Emission Spectrometry

INAA
Instrumental Neutron Activation Analysis

ISO
International Organization for Standardization. A provider of documentary standards.

JCGM
Joint Committee for Guides in Metrology

KED
Kinetic Energy Discrimination

LC-Abs
Liquid Chromatography with Absorbance Detection

LC-HRMS
Liquid Chromatography with High Resolution Mass Spectrometry

LC-MS
Liquid Chromatography Mass Spectrometry

LC-MS/MS
Liquid Chromatography with Tandem Mass Spectrometry

LLE
Liquid-Liquid Extraction

LOQ
Limit of Quantification

MDL
Method Detection Limit

MRL
Maximum Residue Limit

Mg
Magnesium

NIST
National Institute of Standards and Technology

P
Phosphorus

PDA
Photodiode Array

PFAS
Perfluoroalkyl Substances

PFHxA
Perfluorohexanoic Acid

PFHxS
Perfluorohexanesulfonic Acid

PFNA
Perfluornonanoic Acid

PFOA
Perfluorooctanoic Acid

PFOS

Perfluorooctanesulfonic Acid

PFOSA
Perfluorooctanesulfonamide

p,p'-DDE
dichlorodiphenyldichloroethylene

QAP
Quality Assurance Program

QL
Quantification Limit

QuEChERS
Quick, Easy, Cheap, Effective, Rugged, and Safe

QuEChERSER
Quick, Easy, Cheap, Effective, Rugged, Safe, Efficient, and Robust

RGTM
Research Grade Test Material

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 (Among-Laboratory Variability)

RT
Room Temperature

SD
Standard Deviation

SDPA
Standard Deviation for Proficiency Assessment

SI
International System of Units

SMPR
Standard Method Performance Requirements

SPE
Solid Phase Extraction

SRM
Standard Reference Material

References

[1] ISO13528:2022 (2022) Statistical methods for use in proficiency testing by interlaboratory comparison.
[2] Beauchamp CR, Camara JE, Carney J, Choquette SJ, Cole KD, DeRose PC, Duewer DL, Epstein MS, Kline MC, Lippa KA, Lucon E, Molloy J, Nelson MA, Phinney KW, Polakoski M, Possolo A, Sander LC, Schiel JE, Sharpless KE, Toman B, Winchester MR, Windover D (2021) *Metrological Tools for the Reference Materials and Reference Instruments of the NIST Material Measurement Laboratory* (Department of Commerce, Washington, D.C.), NIST Special Publication (SP) 260-136-2020. https://doi.org/10.6028/NIST.SP.260-136-2020.
[3] National Institute of Standards and Technology (2023) Reference Material Information Sheet RM 8260 Infant Nutritional Formula (hydrolyzed milk-based). https://tsapps.nist.gov/srmext/certificates/8260.pdf.
[4] National Institute of Standards and Technology (2023) Reference Material Information Sheet RM 8261 Adult Nutritional Formula (high protein). https://tsapps.nist.gov/srmext/certificates/8261.pdf.
[5] Official Methods of Analysis of AOAC INTERNATIONAL , 22nd Ed. (2014) AOAC SMPR® 2014.004 - Standard Method Performance Requirements for Minerals and Trace Elements in Infant Formula and Adult/Pediatric Nutritional Formula (AOAC INTERNATIONAL, Rockville, MD). https://www.aoac.org/wp-content/uploads/2020/11/SMPR202014_004.pdf.
[6] May TW , Wiedmeyer RH (1998) A Table of Polyatomic Interferences in ICP-MS. *Atomic Spectroscopy* 19(5):150-155. https://doi.org/10.46770/AS.1998.05.001
[7] National Institutes of Health Office of Dietary Supplements (2024) Vitamin B12 Fact Sheet for Health Professionals. https://ods.od.nih.gov/factsheets/VitaminB12-HealthProfessional/.
[8] Official Methods of Analysis of AOAC INTERNATIONAL , 22nd Ed. (2011) AOAC SMPR® 2011.005 - Standard Method Performance Requirements for Vitamin B12 in Infant Formula and Adult/Pediatric Nutritional Formula (AOAC INTERNATIONAL, Rockville, MD). https://www.aoac.org/wp-content/uploads/2020/11/SMPR202011_005.pdf.
[9] National Institute of Standards and Technology (2010) Dietary Supplements Laboratory Quality Assurance Program (DSQAP) Exercise F Final Report. https://www.nist.gov/programs-projects/dietary-supplements-laboratory-quality-assurance-program-dsqap.
[10] Burdette CQ, Hayes HV, Klingsick J, Bryan-Sallee CE, Barber CA, Christopher S, Yu L (2023) Dietary Supplement Laboratory Quality Assurance Program: Exercise 1 Final Report. (National Institute of Standards and Technology, Gaithersburg, MD), NIST Interagency/Internal Report (NISTIR) 8494. https://doi.org/10.6028/NIST.IR.8494.
[11] Barber CA, Bruce A. Benner J, Thomas JB, Burdette CQ, Camara J, Long S, Murray JA, Phillips MM, Place BJ, Rimmer CA, Wood LJ, Yu L, Chinthalapati SKR, Tai SS-C (2018) Health Assessment Measurements Quality Assurance Program: Exercise 1 Final Report. (National Institute of Standards and Technology, Gaithersburg, MD), NIST Interagency/Internal Report (NISTIR) 8237. https://doi.org/10.6028/NIST.IR.8237.
[12] Barber CA, Burdette CQ, Hayes HV, Phillips MM, Rimmer CA, Wood LJ, Yu L, Kotowski SP (2020) Health Assessment Measurements Quality Assurance Program: Exercise 4 Final Report. (National Institute of Standards and Technology, Gaithersburg, MD), NIST Interagency/Internal Report (NISTIR) 8308. https://doi.org/10.6028/NIST.IR.8308.
[13] National Institutes of Health Office of Dietary Supplements (2023) Vitamin A and Carotenoids Fact Sheet for Health Professionals. https://ods.od.nih.gov/factsheets/VitaminA-HealthProfessional/.
[14] Official Methods of Analysis of AOAC INTERNATIONAL , 22nd Ed. (2011) AOAC SMPR® 2011.003 - Standard Method Performance Requirements for Vitamin A in Infant Formula and Adult/Pediatric Nutritional Formula (AOAC INTERNATIONAL, Rockville, MD). https://www.aoac.org/wp-content/uploads/2020/11/SMPR202011_003.pdf.
[15] Official Methods of Analysis of AOAC INTERNATIONAL , 22nd Ed. (2023) AOAC Official Method 2012.10 - Simultaneous Determination of Vitamins E and A in Infant Formula and Adult Nutritionals: Normal-Phase High-Performance Liquid Chromatography. https://doi.org/10.1093/9780197610145.003.276.
[16] National Institute of Standards and Technology (2007) Dietary Supplements Laboratory Quality Assurance Program (DSQAP) Exercise A Final Report. https://www.nist.gov/document/exercise-resultspdf.
[17] National Institute of Standards and Technology (2008) Dietary Supplements Laboratory Quality Assurance Program (DSQAP) Exercise C Final Report. https://www.nist.gov/document/nist-exercise-c-linkspdf.

[18] Phillips MM, Rimmer CA, Wood LJ, Murphy KE, Vetter TW (2013) Dietary Supplement Laboratory Quality Assurance Program: Exercise I Final Report. (National Institute of Standards and Technology, Gaithersburg, MD), NIST Interagency/Internal Report (NISTIR) 7955. http://dx.doi.org/10.6028/NIST.IR.7955.
[19] Barber CA, Thomas JB, Burdette CQ, Camara J, Long S, Phillips MM, Place BJ, Rimmer CA, Wood LJ, Yu L (2019) Health Assessment Measurements Quality Assurance Program: Exercise 2 Final Report. (National Institute of Standards and Technology, Gaithersburg, MD), NIST Interagency/Internal Report (NISTIR) 8249. https://doi.org/10.6028/NIST.IR.8249.
[20] Barber CA, Burdette CQ, Hayes HV, Johnson ME, Kotoski SP, Murray JA, Phillips MM, Rimmer CA, Wood LJ, Yarberry AJ (2021) Health Assessment Measurements Quality Assurance Program: Exercise 6 Final Report. (National Institute of Standards and Technology, Gaithersburg, MD), NIST Interagency/Internal Report (NISTIR) 8394. https://doi.org/10.6028/NIST.IR.8394.
[21] Glüge J, Scheringer M, Cousins IT, DeWitt JC, Goldenman G, Herzke D, Lohmann R, Ng CA, Trier X, Wang Z (2020) An overview of the uses of per- and polyfluoroalkyl substances (PFAS). *Environmental Science: Processes & Impacts* 22(12):2345-2373. https://doi.org/10.1039/D0EM00291G.
[22] Office of Science and Technology Policy PFAS Strategy Team National Science and Technology Council (2024) Per- and Polyfluoroalkyl Substances (PFAS) Federal Research and Development Strategic Plan. https://www.whitehouse.gov/wp-content/uploads/2024/09/PFAS-STRAT-PLAN-FINAL.pdf.
[23] U.S. Food and Drug Administration. FDA Foods Program Compendium of Analytical Methods: Chemical Analytical Manual (CAM) (2024) Method C-010.02 “Determination of 16 Per- and Polyfluoroalkyl Substances (PFAS) in Processed Food using Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS). https://www.fda.gov/media/131510/download.
[24] Official Methods of Analysis of AOAC INTERNATIONAL , 22nd Ed. (2023) AOAC SMPR® 2023.003 - Standard Method Performance Requirements for Per- and Polyfluoroalkyl Substances (PFAS) in Produce, Beverages, Dairy Products, Eggs, Seafood, Meat Products, and Feed (AOAC INTERNATIONAL, Rockville, MD). https://www.aoac.org/wp-content/uploads/2023/11/SMPR-2023_003-1.pdf.
[25] Lehotay S (2022) The QuEChERSER Mega-Method. *LCGC North America* 40:13–19. https://doi.org/10.56530/lcgc.na.px2173m3.
[26] The National Archives of the U.S. (1971) Federal Register Code of Federal Regulations 40 CFR Part 180. https://www.ecfr.gov/current/title-40/chapter-I/subchapter-E/part-180.
[27] U.S. Environmental Protection Agency (2024) Regulation of Pesticide Residues on Food. https://www.epa.gov/pesticide-tolerances.
[28] Official Methods of Analysis of AOAC INTERNATIONAL , 22nd Ed. (2018) AOAC SMPR® 2018.011 - Standard Method Performance Requirements for Identification and Quantitation of Selected Pesticide Residues in Dried Cannabis Materials (AOAC INTERNATIONAL, Rockville, MD). https://www.aoac.org/wp-content/uploads/2020/11/SMPR202018_011.pdf.
[29] Official Methods of Analysis of AOAC INTERNATIONAL , 22nd Ed. (2024) AOAC SMPR® 2024.001 - Standard Method Performance Requirements for Selected Pesticides in Color Additives from Crop-Based Sources (AOAC INTERNATIONAL, Rockville, MD). https://www.aoac.org/wp-content/uploads/2024/02/SMPR-2024_001-1.pdf.