

**NIST Internal Report  
NIST IR 8559**

# **Dietary Supplement Laboratory Quality Assurance Program: Exercise 2 Final Report**

Hugh V. Hayes  
Jenna R. Klingsick  
Colleen E. Bryan Sallee  
Sanem Hosbas Coskun (Associate)

This publication is available free of charge from:  
<https://doi.org/10.6028/NIST.IR.8559>

**NIST Internal Report  
NIST IR 8559**

# **Dietary Supplement Laboratory Quality Assurance Program: Exercise 2 Final Report**

Hugh V. Hayes  
Jenna R. Klingsick  
Colleen E. Bryan Sallee  
Sanem Hosbas Coskun (Associate)  
*Chemical Sciences Division  
Material Measurement Laboratory*

This publication is available free of charge from:  
<https://doi.org/10.6028/NIST.IR.8559>

January 2025



U.S. Department of Commerce  
*Jeremy Pelter, Acting Secretary of Commerce*

National Institute of Standards and Technology  
*Craig Burkhardt, Acting Under Secretary of Commerce for Standards and Technology and Acting NIST Director*

NIST IR 8559  
January 2025

Certain commercial entities, equipment, or materials may be identified in this document in order to describe an experimental procedure or concept adequately. Such identification is not intended to imply recommendation or endorsement by the National Institute of Standards and Technology, nor is it intended to imply that the entities, materials, or equipment are necessarily the best available for the purpose.

**NIST Technical Series Policies**

[Copyright, Fair Use, and Licensing Statements](#)

[NIST Technical Series Publication Identifier Syntax](#)

**Publication History**

Approved by the NIST Editorial Review Board on 2025-01-22

**How to Cite this NIST Technical Series Publication**

Hayes HV, Klingsick JR, Bryan Sallee CE, Hosbas Coskun S (2025) Dietary Supplement Laboratory Quality Assurance Program: Exercise 2 Final Report. (National Institute of Standards and Technology, Gaithersburg, MD), NIST Internal Report (IR) NIST IR 8559. <https://doi.org/10.6028/NIST.IR.8559>

**NIST Author ORCID iDs**

Hugh V. Hayes: 0000-0002-4855-2993

Jenna R. Klingsick: 0009-0000-8793-1016

Colleen E. Bryan Sallee: 0000-0002-2334-3925

Sanem Hosbas Coskun: 0000-0002-0413-8934

## **Abstract**

The National Institute of Standards and Technology (NIST) Dietary Supplement Laboratory Quality Assurance Program (DSQAP) was launched in 2007 in part as a collaboration with the National Institutes of Health (NIH) Office of Dietary Supplements (ODS). The DSQAP enables laboratories to improve the accuracy of measurements in samples for nutrients, marker compounds, toxic elements, and/or contaminants in dietary supplement ingredients and finished products. Primary program goals include centering the consensus range about the target value and reducing measurement bias. Exercise 2 is the seventeenth DSQAP exercise (previously, they were designated “A” through “O”). Exercise 2 was designed with four studies, offering the opportunity for laboratories to assess their in-house techniques on a variety of measurements. Studies included determinations of select toxic elements in ginger and eleuthero extracts, vitamins in a multivitamin and saw palmetto extract, fatty acids in fish oils, and botanical marker compounds in dietary supplement ingredient materials and finished products. This report summarizes the results, describes observations, and provides technical recommendations for measurement improvements.

## **Keywords**

Botanicals; Consumer Safety; Dietary Supplements; Quality Assurance; Reference Materials.

## Table of Contents

<b>1. Introduction.....</b>	<b>1</b>
1.1. Background .....	1
1.2. Overview of Data Treatment and Representation .....	2
1.2.1. Statistics .....	2
1.2.2. Individualized Data Tables .....	3
1.2.3. Summary Data Tables .....	4
1.2.4. Figures .....	5
<b>2. Overall Technical Recommendations .....</b>	<b>8</b>
<b>3. Toxic Elements in Botanical Extracts .....</b>	<b>9</b>
3.1. Summary .....	9
3.2. Background .....	9
3.3. Study Information .....	9
3.4. Study Results and Technical Recommendations .....	10
3.4.1. RM 8666 Ginger ( <i>Zingiber officinale</i> ) Extract .....	12
3.4.2. Eleuthero Extract .....	19
<b>4. Fat-Soluble Vitamins in Supplements.....</b>	<b>25</b>
4.1. Summary .....	25
4.2. Background .....	25
4.3. Study Information .....	25
4.4. Study Results and Technical Recommendations .....	26
4.4.1. Total $\beta$ -carotene .....	27
4.4.2. <i>Trans</i> - $\beta$ -carotene .....	31
4.4.3. 9- <i>Cis</i> - $\beta$ -carotene .....	34
<b>5. Fatty Acids in Fish Oil .....</b>	<b>36</b>
5.1. Summary .....	36
5.2. Background .....	36
5.3. Study Information .....	36
5.4. Study Results and Technical Recommendations .....	37
5.4.1. Total Linoleic Acid.....	38
5.4.2. Total Arachidonic Acid.....	43
5.4.3. EPA .....	47
5.4.4. DPA .....	51
5.4.5. DHA .....	55

<b>6. Botanicals</b> .....	<b>59</b>
6.1. Summary .....	59
6.2. Background .....	59
6.3. Study Information .....	59
6.4. Study Results and Technical Recommendations .....	60
<b>References</b> .....	<b>61</b>
<b>Appendix A. List of Acronyms</b> .....	<b>64</b>
<b>Appendix B. Toxic Elements Supplemental Tables and Figures</b> .....	<b>66</b>
<b>Appendix C. Fat-Soluble Vitamins Supplemental Tables and Figures</b> .....	<b>79</b>
<b>Appendix D. Fatty Acids Supplemental Tables and Figures</b> .....	<b>85</b>

## List of Tables

<b>Table 1-1. Summary of DSQAP Exercise 2 Studies.</b> .....	<b>2</b>
<b>Table 1-2. Individualized Data Table Template.</b> .....	<b>3</b>
<b>Table 1-3. Summary Data Table Template.</b> .....	<b>5</b>
<b>Table 3-1. Enrollment and Participation Statistics for Elements in Botanical Extracts.</b> .....	<b>10</b>
<b>Table 3-2. Description of the consensus confidence interval in relation to the NIST target range for elements in botanical extracts.</b> .....	<b>10</b>
<b>Table 3-3. AOAC Standard Method Performance Requirements 2020.001 was used to assess participants' performance in analyzing toxic elements in botanical extracts.</b> .....	<b>11</b>
<b>Table 3-4. Summary of sample preparation methods for the determination of toxic elements in RM 8666 and Eleuthero Extract.</b> .....	<b>11</b>
<b>Table 3-5. Summary of analytical methods for the determination of toxic elements in RM 8666 and Eleuthero Extract.</b> .....	<b>11</b>
<b>Table 3-6. Summary of results and laboratory variabilities for toxic elements in RM 8666.</b> .....	<b>13</b>
<b>Table 3-7. Summary of results and laboratory variabilities for toxic elements in Eleuthero Extract.</b> ....	<b>19</b>
<b>Table 4-1. Summary of sample preparation methods for the determination of <math>\beta</math>-carotene in SRM 3251 Saw Palmetto (<i>Serenoa repens</i>) Extract and SRM 3289 Multivitamin Tablets.</b> .....	<b>26</b>
<b>Table 4-2. Summary of results and laboratory variabilities for total <math>\beta</math>-carotene in SRM 3251 Saw Palmetto (<i>Serenoa repens</i>) Extract and 3289 Multivitamin Tablets.</b> .....	<b>27</b>
<b>Table 4-3. Summary of results and laboratory variabilities for <i>trans</i>-<math>\beta</math>-carotene in SRM 3251 Saw Palmetto (<i>Serenoa repens</i>) Extract and 3289 Multivitamin Tablets.</b> .....	<b>31</b>
<b>Table 4-4. Summary of results and laboratory variabilities for 9-<i>cis</i>-<math>\beta</math>-carotene in SRM 3251 Saw Palmetto (<i>Serenoa repens</i>) Extract and 3289 Multivitamin Tablets.</b> .....	<b>34</b>
<b>Table 5-1. Summary of sample preparation methods for the determination of fatty acids in SRM 3275 Omega-3 and Omega-6 Fatty Acids in Fish Oil.</b> .....	<b>37</b>

Table 5-2. Summary of analytical methods for the determination of fatty acids in SRM 3275 Omega-3 and Omega-6 Fatty Acids in Fish Oil. ....	38
Table 5-3. Target values, consensus values, and variabilities for total linoleic acid in SRM 3275 Omega-3 and Omega-6 Fatty Acids in Fish Oil. ....	39
Table 5-4. Target values, consensus values, and variabilities for total arachidonic acid in SRM 3275 Omega-3 and Omega-6 Fatty Acids in Fish Oil. ....	43
Table 5-5. Target values, consensus values, and variabilities for EPA in SRM 3275 Omega-3 and Omega-6 Fatty Acids in Fish Oil.....	47
Table 5-6. Target values, consensus values, and variabilities for DPA in SRM 3275 Omega-3 and Omega-6 Fatty Acids in Fish Oil.....	51
Table 5-7. Target values, consensus values, and variabilities for DHA in SRM 3275 Omega-3 and Omega-6 Fatty Acids in Fish Oil.....	55
Table 6-1. Results for triterpenes in Black Cohosh Rhizome and Black Cohosh Extract. ....	60
Table B-1. Individualized data summary table (example) for toxic elements in botanical extracts.....	66
Table B-2. Data summary table for total arsenic in botanical extracts. ....	67
Table B-3. Data summary table for cadmium in botanical extracts.....	70
Table B-4. Data summary table for lead in botanical extracts. ....	73
Table B-5. Data summary table for mercury in botanical extracts. ....	76
Table C-1. Individualized data summary table (example) for fat-soluble vitamins in supplements. ....	79
Table C-2. Data summary table for total $\beta$ -carotene in supplements.....	80
Table C-3. Data summary table for <i>trans</i> - $\beta$ -carotene in supplements.....	82
Table C-4. Data summary table for 9- <i>cis</i> - $\beta$ -carotene in supplements.....	84
Table D-1. Individualized data summary table (example) for fatty acids in fish oil. ....	85
Table D-2. Data summary table for total linoleic acid in fish oil.....	86
Table D-3. Data summary table for total arachidonic acid in fish oil.....	89
Table D-4. Data summary table for total EPA in fish oil.....	92
Table D-5. Data summary table for total DPA in fish oil.....	95
Table D-6. Data summary table for total DHA in fish oil.....	98

## List of Figures

Fig. 1-1. Example data sample summary view. ....	6
Fig. 1-2. Example sample/sample comparison view.....	7
Fig. 3-1. Total arsenic in RM 8666 Ginger ( <i>Zingiber officinale</i> ) Extract (data summary view – analytical method). ....	15
Fig. 3-2. Cadmium in RM 8666 Ginger ( <i>Zingiber officinale</i> ) Extract (data summary view – analytical method). ....	16

Fig. 3-3. Lead in RM 8666 Ginger ( <i>Zingiber officinale</i> ) Extract (data summary view – analytical method). .....	17
Fig. 3-4. Mercury in RM 8666 Ginger ( <i>Zingiber officinale</i> ) Extract (data summary view – analytical method). .....	18
Fig. 3-5. Total arsenic in Eleuthero Extract (data summary view – analytical method). .....	21
Fig. 3-6. Cadmium in Eleuthero Extract (data summary view – analytical method). .....	22
Fig. 3-7. Lead in Eleuthero Extract (data summary view – analytical method). .....	23
Fig. 3-8. Mercury in Eleuthero Extract (data summary view – analytical method). .....	24
Fig. 4-1. Total $\beta$ -carotene in SRM 3251 Saw Palmetto ( <i>Serenoa repens</i> ) Extract (data summary view – sample preparation). .....	29
Fig. 4-2. Total $\beta$ -carotene in SRM 3289 Multivitamin Tablets (data summary view – sample preparation). .....	30
Fig. 4-3. <i>Trans</i> - $\beta$ -carotene in SRM 3251 Saw Palmetto ( <i>Serenoa repens</i> ) Extract (data summary view – sample preparation). .....	32
Fig. 4-4. <i>Trans</i> - $\beta$ -carotene in SRM 3289 Multivitamin Tablets (data summary view – sample preparation). .....	33
Fig. 4-5. 9- <i>Cis</i> - $\beta$ -carotene in SRM 3251 Saw Palmetto ( <i>Serenoa repens</i> ) Extract (data summary view – sample preparation). .....	35
Fig. 5-1. Total Linoleic Acid in SRM 3275-1 Omega-3 and Omega-6 Fatty Acids in Fish Oil (data summary view – analytical method). .....	40
Fig. 5-2. Total Linoleic Acid in SRM 3275-2 Omega-3 and Omega-6 Fatty Acids in Fish Oil (data summary view – analytical method). .....	41
Fig. 5-3. Total Linoleic Acid in SRM 3275-3 Omega-3 and Omega-6 Fatty Acids in Fish Oil (data summary view – analytical method). .....	42
Fig. 5-4. Total Arachidonic Acid in SRM 3275-1 Omega-3 and Omega-6 Fatty Acids in Fish Oil (data summary view – analytical method). .....	44
Fig. 5-5. Total Arachidonic Acid in SRM 3275-2 Omega-3 and Omega-6 Fatty Acids in Fish Oil (data summary view – analytical method). .....	45
Fig. 5-6. Total Arachidonic Acid in SRM 3275-3 Omega-3 and Omega-6 Fatty Acids in Fish Oil (data summary view – analytical method). .....	46
Fig. 5-7. Total EPA in SRM 3275-1 Omega-3 and Omega-6 Fatty Acids in Fish Oil (data summary view – analytical method). .....	48
Fig. 5-8. Total EPA in SRM 3275-2 Omega-3 and Omega-6 Fatty Acids in Fish Oil (data summary view – analytical method). .....	49
Fig. 5-9. Total EPA in SRM 3275-3 Omega-3 and Omega-6 Fatty Acids in Fish Oil (data summary view – analytical method). .....	50
Fig. 5-10. Total DPA in SRM 3275-1 Omega-3 and Omega-6 Fatty Acids in Fish Oil (data summary view – analytical method). .....	52
Fig. 5-11. Total DPA in SRM 3275-2 Omega-3 and Omega-6 Fatty Acids in Fish Oil (data summary view – analytical method). .....	53

Fig. 5-12. Total DPA in SRM 3275-3 Omega-3 and Omega-6 Fatty Acids in Fish Oil (data summary view – analytical method). .....	54
Fig. 5-13. Total DHA in SRM 3275-1 Omega-3 and Omega-6 Fatty Acids in Fish Oil (data summary view – analytical method). .....	56
Fig. 5-14. Total DHA in SRM 3275-2 Omega-3 and Omega-6 Fatty Acids in Fish Oil (data summary view – analytical method). .....	57
Fig. 5-15. Total DHA in SRM 3275-3 Omega-3 and Omega-6 Fatty Acids in Fish Oil (data summary view – analytical method). .....	58
Fig. B-1. Laboratory means for total arsenic in RM 8666 Ginger ( <i>Zingiber officinale</i> ) Extract and Eleuthero Extract (sample/sample comparison view).....	69
Fig. B-2. Laboratory means for cadmium in RM 8666 Ginger ( <i>Zingiber officinale</i> ) Extract and Eleuthero Extract (sample/sample comparison view).....	72
Fig. B-3. Laboratory means for lead in RM 8666 Ginger ( <i>Zingiber officinale</i> ) Extract and Eleuthero Extract (sample/sample comparison view).....	75
Fig. B-4. Laboratory means for mercury in RM 8666 Ginger ( <i>Zingiber officinale</i> ) Extract and Eleuthero Extract (sample/sample comparison view).....	78
Fig. C-1. Laboratory means for total $\beta$ -carotene in SRM 3251 Saw Palmetto ( <i>Serenoa repens</i> ) Extract and SRM 3289 Multivitamin Tablets (sample/sample comparison view).....	81
Fig. C-2. Laboratory means for <i>trans</i> - $\beta$ -carotene in SRM 3251 Saw Palmetto ( <i>Serenoa repens</i> ) Extract and SRM 3289 Multivitamin Tablets (sample/sample comparison view).....	83
Fig. D-1. Laboratory means for total linoleic acid in SRM 3275-1 and SRM 3275-2 Omega-3 and Omega-6 Fatty Acids in Fish Oil (sample/sample comparison view). .....	87
Fig. D-2. Laboratory means for total linoleic acid in SRM 3275-1 and SRM 3275-3 Omega-3 and Omega-6 Fatty Acids in Fish Oil (sample/sample comparison view). .....	88
Fig. D-3. Laboratory means for total arachidonic acid in SRM 3275-1 and SRM 3275-2 Omega-3 and Omega-6 Fatty Acids in Fish Oil (sample/sample comparison view). .....	90
Fig. D-4. Laboratory means for total arachidonic acid in SRM 3275-1 and SRM 3275-3 Omega-3 and Omega-6 Fatty Acids in Fish Oil (sample/sample comparison view). .....	91
Fig. D-5. Laboratory means for total EPA in SRM 3275-1 and SRM 3275-2 Omega-3 and Omega-6 Fatty Acids in Fish Oil (sample/sample comparison view). .....	93
Fig. D-6. Laboratory means for total EPA in SRM 3275-1 and SRM 3275-3 Omega-3 and Omega-6 Fatty Acids in Fish Oil (sample/sample comparison view). .....	94
Fig. D-7. Laboratory means for total DPA in SRM 3275-1 and SRM 3275-2 Omega-3 and Omega-6 Fatty Acids in Fish Oil (sample/sample comparison view). .....	96
Fig. D-8. Laboratory means for total DPA in SRM 3275-1 and SRM 3275-3 Omega-3 and Omega-6 Fatty Acids in Fish Oil (sample/sample comparison view). .....	97
Fig. D-9. Laboratory means for total DHA in SRM 3275-1 and SRM 3275-2 Omega-3 and Omega-6 Fatty Acids in Fish Oil (sample/sample comparison view). .....	99
Fig. D-10. Laboratory means for total DHA in SRM 3275-1 and SRM 3275-3 Omega-3 and Omega-6 Fatty Acids in Fish Oil (sample/sample comparison view).....	100

## **Acknowledgements**

We thank the following for their significant contributions through technical expertise and report reviews, comments, and edits: Catherine Rimmer (NIST), John Molloy (NIST), David Duewer (NIST), and Adam Kuszak (NIH ODS/AMRM). We thank the staff of the NIST Office of Reference Materials (ORM) for the various support aspects involved with the preparation and shipment of samples.

## 1. Introduction

### 1.1. Background

The National Institute of Standards and Technology (NIST) Dietary Supplement Laboratory Quality Assurance Program (DSQAP) was revived in 2022. DSQAP was established in 2007 in part as a collaboration with the National Institutes of Health (NIH) Office of Dietary Supplements (ODS). In 2017, the program was integrated into the Health Assessment Measurements Quality Assurance Program (HAMQAP). Now, DSQAP continues the ongoing collaborative efforts between NIST and the NIH ODS.

NIST has more than 30 years of experience in the administration of QAPs, including currently active programs [i.e., Cannabis Laboratory QAP (CannaQAP), Food Nutrition and Safety Measurements QAP (FNSQAP)] and historical programs [i.e., Micronutrients Measurement QAP (MMQAP), Vitamin D Metabolites QAP (VitDQAP), and HAMQAP]. The purpose of DSQAP is to improve the measurement capabilities of the dietary supplement and natural product measurement communities. Participating laboratories are able to demonstrate that their performance is comparable to that of the broader community and that their methods provide accurate results by evaluating in-house methods on a wide variety of challenging, real-world matrices. DSQAP is a unique tool for assessing the quality of measurements and providing feedback about performance that can assist participants in improving laboratory operations. This can be especially useful in areas where few consensus or official methods have been recognized.

Laboratories participating in DSQAP are offered opportunities to assess their in-house measurements through various studies of measurands (e.g., nutritional and toxic elements, fat- and water-soluble vitamins, marker compounds, and organic contaminants) in samples distributed by NIST. After submitting results, NIST provides laboratories with reports and certificates of participation that may be used to fulfill proficiency requirements established by accreditation bodies or to demonstrate compliance with current Good Manufacturing Practices (cGMPs). Additionally, NIST and DSQAP assist the NIH ODS Analytical Methods and Reference Materials (AMRM) program in supporting the development and dissemination of analytical tools and reference materials. ODS and NIST can use results from DSQAP exercises to identify problematic matrices and analytes for which consensus-based analysis methods would benefit the dietary supplement measurement communities.

DSQAP Exercises are leveraged to determine any community wide analytical challenges and to support measurement improvements through appropriate reference materials and educational resources as well as assisting the community with evaluating internal analytical challenges associated with measurements in botanical matrices.

While NIST QAP exercises are not proficiency tests (PT) and are not intended to pass strict evaluation of laboratory performance, they are conducted according to the International Organization for Standardization (ISO)/ International Electrotechnical Commission (IEC) 17043 and are designed to assist participants in evaluation and improvement of their measurement

capabilities. Additionally, industry stakeholders can observe measurement challenges and NIST gains knowledge to guide the production and maintenance of reference materials.

This report summarizes the results from Exercise 2 of the DSQAP. Fifty-five laboratories responded to the April 2023 call for study participation for DSQAP Exercise 2, as seen in **Table 1-1**. Samples were shipped to participants in July 2023 and results were returned to NIST by August 18, 2023. This report contains the final data and information disseminated to the participants in January 2025.

**Table 1-1.** Summary of DSQAP Exercise 2 Studies.

Study Group	Analytes	Samples
<b>Toxic Elements</b>	tAs, Cd, Pb, Hg	Ginger Extract and Eleuthero Extract
<b>Fat-Soluble Vitamins</b>	Total $\beta$ -carotene, <i>Trans</i> - $\beta$ -carotene, 9- <i>Cis</i> - $\beta$ -carotene	Multivitamin and Saw Palmetto Extract
<b>Fatty Acids</b>	LA, ARA, EPA, DPA, DHA	Fish Oil
<b>Botanicals</b>	23-epi-26-deoxyactein, 23-epi-26-deoxycimicifugoside, Actein, Cimigenol 3- $\beta$ -D-xyloside, Cimiracemoside C, Cimiracemoside D	Black Cohosh Rhizome and Extract

Each study is summarized individually with appropriate tables, figures, and text and reported by section. Additional tables and figures can be found in the Appendices. When possible, conclusions and technical recommendations are drawn for the entire exercise and reported in the **Overall Technical Recommendations** section.

## 1.2. Overview of Data Treatment and Representation

Individualized data tables and certificates are provided to the participants who have submitted data in each study in addition to this report. Examples of the data tables are also included in each section of this report. Community tables and figures are provided using randomized laboratory codes. Laboratories only know their own participation code. The statistical approaches for each type of data representation are outlined below.

### 1.2.1. Statistics

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

### 1.2.2. Individualized Data Tables

The data in **Table 1-2** is individualized to each participating laboratory and is provided to allow participants to directly compare their data to the summary statistics (consensus or community data as well as NIST-certified, non-certified, or estimated values, when available). Participating laboratories receive uniquely coded individualized data tables in a separate distribution, with the randomized laboratory code in the upper left of the data table. For example, individualized data tables included in this report are made with this section blank to protect the identity and performance of participants.

**Table 1-2.** Individualized Data Table Template.

			<b>(Laboratory Name)</b>									
			<b>Exercise 1 - (Study Name)</b>									
<b>Lab Code:</b>		<b>(code)</b>	<b>1. Your Results</b>				<b>2. Community Results</b>			<b>3. Target</b>		
Analyte	Sample	Units	$x_i$	$s_i$	$Z'_{comm}$	$Z_{NIST}$	N	$x^*$	$s^*$	$x_{NIST}$	$U_{NIST}$	
Analyte 1	Sample Name A	unit	<i>Individual laboratory results will appear in this section; Laboratory-specific results were provided to each participant separately from this report</i>				<i>Community results will appear in this section</i>			<i>Target values will appear in this section</i>		
Analyte 1	Sample Name B	unit										
Analyte 2	Sample Name A	unit										
Analyte 2	Sample Name B	unit										
Analyte 3	Sample Name A	unit										
Analyte 3	Sample Name B	unit										
			$x_i$	Mean of reported values		N	Number of quantitative values reported		$x_{NIST}$	target value		
			$s_i$	Standard deviation of reported values		$x^*$	Robust mean of reported values		$U_{NIST}$	expanded uncertainty about the target value		
			$Z'_{comm}$	Z'-score with respect to community consensus		$s^*$	Robust standard deviation					
			$Z_{NIST}$	Z-score with respect to NIST value								

Section 1 of the data table (*Your Results*) contains the laboratory results as reported, including the mean and standard deviation when multiple values were reported. A blank indicates that NIST does not have data on file from that laboratory for the corresponding analyte or matrix. An empty box for standard deviation indicates that the participant reported a single value or a value below the limit of quantification (LOQ). If the participant reported a single quantitative value, that value is still included in the calculation of the consensus data.

Also included in Section 1 of the data table 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,  $\sigma_{PT}^2$ ) determined from the Q/Hampel estimator:

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

The second Z-score,  $Z_{NIST}$ , is calculated with respect to the target value (when available), using  $x_{NIST}$  and  $U_{NIST}$ , where  $U_{NIST}$  represents the expanded uncertainty of NIST or other measurements:

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

Significance of the Z-scores:

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

Section 2 of the data table (*Community Results*) contains the consensus results, including the number of laboratories reporting at least 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 the consensus mean and standard deviation calculation can be found in the previous section.

Section 3 of the data table (*Target*) contains the target values for each analyte, when available. When a NIST Standard Reference Material (SRM) or Reference Material (RM) is used as a sample in the study, the NIST certified or non-certified values and associated uncertainties ( $U_{\text{NIST}}$ ) are used as target values. The criteria NIST uses to assign certified and non-certified values are described elsewhere [2]. Target values for other study samples may be determined at NIST or by a collaborating laboratory as the mean of at least three replicates. Target values may also be based on information provided by the material manufacturer or determined from another interlaboratory study or PT program, where the consensus value and uncertainty from the completed round is used as the target range. The exact methods for determination of the study target values are outlined in detail within each section of this report.

### 1.2.3. Summary Data Tables

This data table includes a summary of all reported data for a particular analyte in a specific study. Participants can compare the raw data from their laboratory to data reported by the other participating laboratories and to the consensus data. A blank indicates that the laboratory signed up and received samples for that analyte and matrix, but no data was returned. An empty box for standard deviation indicates that the participant reported a single value or a value below the LOQ. 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  $|Z'_{\text{comm}}| > 2$  by the PROLab software package. A summary data table example is shown in **Table 1-3** and the following are some laboratory data reporting examples. Laboratory code 4 only reported one value for one sample; therefore, no standard deviation is shown. Reported values that are outside the consensus tolerance limit are highlighted with blue text, for example as shown for Laboratory code 6. Reported values of zero, which are not appropriate results, are highlighted in red text, for example as shown for Laboratory code 10.

**Table 1-3.** Summary Data Table Template.

		Analyte 1									
		Sample Name A (unit)					Sample Name B (unit)				
Lab		A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	Target				Target	U				Target	U
	(lab code 1)	Value 1	Value 2	Value 3	Avg	SD	Value 1	Value 2	Value 3	Avg	SD
	(lab code 2)	Value 1	Value 2	Value 3	Avg	SD	Value 1	Value 2	Value 3	Avg	SD
	(lab code 3)	Value 1	Value 2	Value 3	Avg	SD	Value 1	Value 2	Value 3	Avg	SD
	(lab code 4)	Value 1			Value 1						
	(lab code 5)	Value 1	Value 2	Value 3	Avg	SD	Value 1	Value 2	Value 3	Avg	SD
	(lab code 6)	Value 1	Value 2	Value 3	Avg	SD	Value 1	Value 2	Value 3	Avg	SD
	(lab code 7)	Value 1	Value 2	Value 3	Avg	SD	Value 1	Value 2	Value 3	Avg	SD
	(lab code 8)	Value 1	Value 2	Value 3	Avg	SD	Value 1	Value 2	Value 3	Avg	SD
	(lab code 9)	Value 1	Value 2	Value 3	Avg	SD	Value 1	Value 2	Value 3	Avg	SD
(lab code 10)	0	0	0	0	0	0	0	0	0	0	
Community Results		Consensus Mean			(Avg)		Consensus Mean			(Avg)	
		Consensus Standard Deviation			(SD)		Consensus Standard Deviation			(SD)	
		Maximum			(Max)		Maximum			(Max)	
		Minimum			(Min)		Minimum			(Min)	
		N			(N)		N			(N)	

## 1.2.4. Figures

### 1.2.4.1. Data Summary View (Method Comparison Data Summary View)

In this view (**Fig. 1-1**), individual laboratory data (diamonds) are plotted with the individual laboratory standard deviation (rectangle). Laboratories reporting values below their LOQ are shown in this view as downward triangles beginning at the LOQ, reported as quantification limit (QL) on the figures. Laboratories reporting values as “below LOQ” can still be successful in the study if the target value is also below the laboratory LOQ. It is important to note that LOQs reported by participants may be artificial thresholds and not calculated instrumental quantitation limits; however, NIST uses these reported values as calculated LOQs to facilitate consensus calculations. 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, and the average number of replicates reported by each participant. The uncertainty about the consensus mean is independent of the range of tolerance.

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

The red shaded region represents the target range for “acceptable” performance, encompassing the target value bounded by twice its uncertainty ( $U_{\text{NIST}}$ ). The solid red lines

represent the range of tolerance (values that result in an acceptable  $Z'_{\text{comm}}$  score,  $|Z'_{\text{comm}}| \leq 2$ ). 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 ranges with respect to the target range can be compared easily. In most cases, the target range and the consensus range overlap in the beige shaded region, which is the desired result. Primary program goals include centering the consensus range about the target value and reducing the size of the consensus range. Analysis of an appropriate reference material as part of a quality control scheme can help identify sources of bias for laboratories reporting results significantly different from the target range. When a method comparison is relevant, different colored data points may be used to identify laboratories that used a specific approach to sample preparation, analysis, or quantitation.

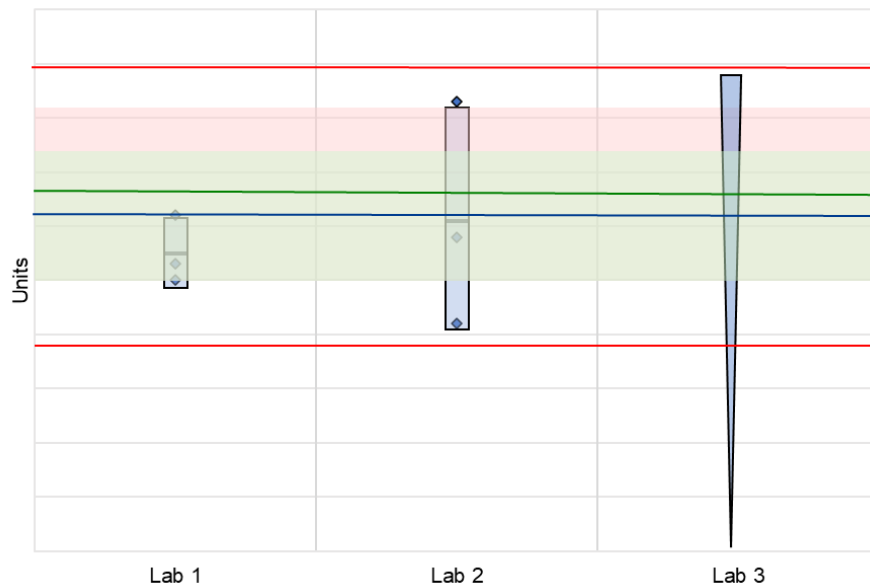
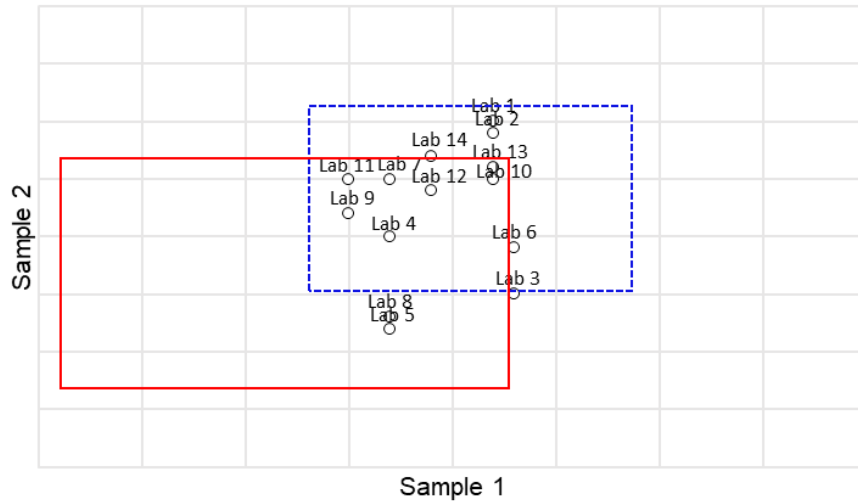


Fig. 1-1. Example data sample summary view.

#### 1.2.4.2. Sample/Sample Comparison View

In this view (**Fig. 1-2**), the individual laboratory results for one sample (e.g., NIST material with a certified target value, a less challenging matrix) are compared to the results for another sample (e.g., NIST material with a more challenging matrix, a commercial sample). The solid red box represents the target range for the first sample (x-axis) and the second sample (y-axis), if available. The dotted blue box represents the consensus range 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 to a limit of twice the range of tolerance (values that result in an acceptable  $Z'_{\text{comm}}$  score,  $|Z'_{\text{comm}}| \leq 2$ ). 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 large (greater than 100 % RSD), the dotted blue box may also only appear partially on the graph. These views emphasize

trends in the data that may indicate potential calibration issues or method biases. One program goal is to identify such calibration or method biases and assist participants in improving analytical measurement capabilities. In some cases, when two equally challenging materials are provided, the same view (sample/sample comparison) can help identify commonalities or differences in the analysis of the two materials.



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

## 2. Overall Technical Recommendations

The following general technical recommendations are essential for achieving accurate and precise measurements. Please see the individual study results and technical recommendation sections for recommendations focused on a particular sample matrix or analyte type.

Using *quality assurance or quality control materials* (commercially available reference materials or appropriately characterized in-house materials) helps establish that sample preparation methods and analytical methods are validated and performing as expected. The analysis of blanks can provide information about sources of analytical variability, such as from the sample preparation procedure or the material itself. Analysis of a statistically sufficient number of procedural blanks is important, especially when determining an LOQ or reducing sample-to-sample variability.

*Proper calibration* is critical to successful measurements. When using a calibration curve, linearity must be ensured at the mass fractions of the sample solutions being measured. The range of calibrant mass fractions should encompass the as-measured sample mass fractions. No as-measured sample mass fractions should be outside of the linear range. Materials used in calibrant preparation should be assessed for purity, and the measured purity should be used to correct the gravimetric or volumetric concentrations of the solutions used for calibration. Calibrant materials should also be assessed for the presence of residual solvents prior to use. Purity evaluation is especially critical for vitamins and botanical marker compounds. Calibrants should be prepared to match the concentration of the final sample preparation solution (i.e., similar mass fractions and similar solvent) whenever possible to avoid potential biases that may arise during sample preparation or from differences in chromatographic retention time or detector sensitivity. Adding an internal standard is recommended to help improve the precision of the instrumental measurements. Selecting the appropriate internal standard will help to correct measurement variability between the calibration standards and the samples.

*Calculations and reporting units should be verified* prior to submission of results. Laboratories often report results in the wrong units or omit a dilution factor during the calculation of the final results, resulting in poor performance on the study. The use of quality assurance or quality control materials can indicate calculation errors through comparisons to expected values. Laboratories reporting results which have been flagged as outside of consensus tolerance limits on preliminary data sheets should check for these types of errors and provide corrected results. Results should also be recorded appropriately in the online DSQAP data entry system. For example, zero is not a quantity that can be measured and should not be reported; if results are below a method LOQ, values should be reported as such (e.g., "< 0.02 %"). Blank data entry fields are only appropriate when no measurements were made.

### 3. Toxic Elements in Botanical Extracts

#### 3.1. Summary

Elemental analysis of foods and dietary supplements is critical to consumer health and safety. The goal of this study was to understand how the measurement community is performing for the determination of toxic elements in ginger and eleuthero extracts. Between 43 and 46 laboratories registered for individual elements, as seen in **Table 3-1**, with data return between 85 % and 89 %. Overall, laboratories that reported quantitative results performed well, although the mercury measurements challenged the community at the low mass fractions present in the study materials.

#### 3.2. Background

Laboratories must establish scientifically valid methods for the determination of toxic elements to demonstrate the products are safe and meet their specifications. Monitoring toxic substances in foods and dietary supplements helps prevent hazardous exposures for consumers and reduces the risk of related negative health outcomes. The U.S. Food and Drug Administration (FDA) has set goals to lower the intake of toxic elements, particularly from fish, shellfish, and foods commonly eaten by infants [3]. As regulations strive for toxic element levels as low as possible in foods and supplements, analytical methods must demonstrate appropriate LOQs. A challenge in measuring toxic elements in botanical extracts is that the low concentrations can push the necessary detection limits for laboratory methods lower.

In this study, participants were asked to use in-house analytical methods to determine the mass fractions of total arsenic (tAs), cadmium (Cd), lead (Pb), and mercury (Hg) in the botanical samples.

#### 3.3. Study Information

Participants were provided with samples of Ginger Extract (three 1 g packets, *Zingiber officinale*) and Eleuthero Extract (three 1.6 g packets, *Eleutherococcus senticosus*). RM 8666 Ginger (*Zingiber officinale*) Extract was labeled as Ginger Extract for this DSQAP exercise to conceal the identity of this material to participants and will be referred to as RM 8666 for the remainder of this report. Participants were asked to store the materials at controlled room temperature, 20 °C to 25 °C, in the original unopened packets until analysis. Before use, participants were instructed to thoroughly mix contents of the packet and to allow contents to settle for one minute before opening to minimize the loss of fine particles prior to removal of a test portion for analysis. Participants were asked to prepare one sample and report one value from each packet provided and to use a sample size of at least 0.25 g for elemental analyses. Approximate analyte levels were not reported to participants prior to the study.

Target values, associated uncertainties, and details on analytical methods are listed in the following individual sample sections.

Enrollment and participation rates, averaged for the botanical materials, for this study are detailed in **Table 3-1**. Some of the reported values were non-quantitative (zero or below LOQ) but are included in the participation and reporting statistics.

**Table 3-1.** Enrollment and Participation Statistics for Elements in Botanical Extracts.

Analyte	Number of Laboratories Requesting Samples	Number of Laboratories Reporting Results (Percent Participation) Averaged for all Samples
Total Arsenic (tAs)	46	40 (87 %)
Cadmium (Cd)	46	40 (87 %)
Lead (Pb)	45	40 (89 %)
Mercury (Hg)	44	37 (85 %)

### 3.4. Study Results and Technical Recommendations

The consensus confidence interval was compared to the NIST target range for each analyte to assess the performance of the participants and is summarized in **Table 3-2**. A consensus mean within the target range is an indication that the community is performing well.

**Table 3-2.** Description of the consensus confidence interval in relation to the NIST target range for elements in botanical extracts.

Analyte	Consensus Confidence Interval in relation to NIST Target Range	
	RM 8666	Eleuthero Extract
Total Arsenic (tAs)	Within	Overlapping
Cadmium (Cd)	Within	Within
Lead (Pb)	Within	Overlapping Below (mean below range)
Mercury (Hg)	Within	Within

In order to assess performance of methods run by individual participants and the community as a whole, repeatability and reproducibility were compared to AOAC Standard Method Performance Requirements (SMPRs). At the time of this report, no SMPRs had been published specific to these botanical extracts. One SMPR, AOAC SMPR 2020.001 Determination of Heavy Metals in a Variety of Cannabis and Cannabis-Derived Products, was identified as an acceptable proxy for the toxic elements in this study [4]. The method performance requirements for cannabis were used as the matrix was deemed the most appropriate proxy versus other SMPRs that are for foods, beverages, or infant formula. Repeatability, demonstrated by within-laboratory variability, and reproducibility, demonstrated by between-laboratory variability, are discussed in the individual sections below. The SMPR 2020.001 performance requirements are seen in **Table 3-3**.

**Table 3-3.** AOAC Standard Method Performance Requirements 2020.001 was used to assess participants' performance in analyzing toxic elements in botanical extracts.

Range	Within-Laboratory Variability (% RSD)	Between-Laboratory Variability (% RSD)
≥10 ng/g to 100 ng/g	15%	32%
> 100 ng/g to 1 µg/g	11%	16%

The majority of laboratories reported using microwave digestion as their sample preparation method to determine toxic elements in the botanical extracts as seen in **Table 3-4**. More than 10 % of laboratories reported using hot block digestion as their sample preparation method.

**Table 3-4.** Summary of sample preparation methods for the determination of toxic elements in RM 8666 and Eleuthero Extract.

Reported Sample Preparation	Percent Reporting %			
	tAs	Cd	Pb	Hg
Acid Hydrolysis	2 %	2 %	2 %	3 %
Hot Block Digestion	12 %	12 %	12 %	11 %
Microwave Digestion	73 %	73 %	76 %	73 %
No Sample Preparation	2 %	2 %	-	5 %
None Reported	10 %	10 %	10 %	8 %

Both AOAC 2013.06-2013 Arsenic, Cadmium, Mercury, and Lead in Foods [5] and AOAC 2015.01-2015 Heavy Metals in Foods [6] use inductively coupled plasma mass spectrometry (ICP-MS) as their analytical method. When combined with laboratories that reported using ICP-MS, nearly 90 % of laboratories reported using ICP-MS as their analytical method to determine toxic elements in the botanical extracts as shown in **Table 3-5**. One laboratory each reported using atomic absorption spectroscopy (AAS), inductively coupled optical emission spectrometry (ICP-OES), or instrumental neutron activation analysis (INAA) to analyze the elements in RM 8666 and Eleuthero Extract.

**Table 3-5.** Summary of analytical methods for the determination of toxic elements in RM 8666 and Eleuthero Extract.

Reported Analytical Method	Percent Reporting %			
	tAs	Cd	Pb	Hg
AOAC 2013.06	5 %	5 %	5 %	5 %
AOAC 2015.01	22 %	20 %	22 %	22 %
AAS	2 %	2 %	2 %	3 %
Cold Vapor AAS Hg Analyzer	-	-	-	3 %
High Resolution ICP-MS	-	-	2 %	-
ICP-MS	61 %	63 %	61 %	57 %
ICP-OES	2 %	2 %	2 %	3 %
INAA	2 %	2 %	-	3 %
Other/None Reported	5 %	5 %	5 %	5 %

In addition of the overall technical recommendations made in Section 2, a few key recommendations should be highlighted for determination of toxic elements in botanical extracts. Sample preparation methods should be well validated prior to analyzing unknown samples to ensure there is complete digestion. Established quality control materials (SRMs, certified reference materials, RMs, and in-house materials when not commercially available) and established methods of analysis should be used whenever possible. Larger than expected within-laboratory variability may be due to challenges in sample processing errors, the use of smaller than recommended sample sizes for analysis, or not using adequate wash-out times between samples for elements with known carryover issues, including Hg. To achieve lower LOQs, laboratories should analyze a sufficient number of blanks and use high-purity reagents along with deionized water. Laboratories should ensure they are using an appropriate sample dilution, especially when measuring samples with low mass fractions.

When using ICP-MS, laboratories should ensure proper use of the instrumental parameters and features. Many ICP-MS instruments run in pulse counting mode, which is more sensitive than analog mode. Instruments typically switch automatically between pulse counting and analog modes depending on the dynamic range and instrument sensitivity for the analyte, and therefore the instrument must either be calibrated for both modes or forced to use only pulse counting mode. To ensure that the calibration curve is linear in the pulse mode, a narrower range of calibration points should be used and all solutions should be diluted to fall within this lower range. Collision cell or reaction cell mode can be used to reduce or eliminate the interferences caused by molecular ions that have the same mass-to-charge ratio as the element of interest isotope.

Laboratories that consistently report results outside of the consensus tolerance limits should ensure they are using proper calibration techniques and are correctly calculating and reporting their results in the correct units.

#### **3.4.1. RM 8666 Ginger (*Zingiber officinale*) Extract**

Target values for tAs, Pb, and Hg in RM 8666 were taken from the Certificate of Analysis (COA) at the time of this report which is the only source for official values [7]. These values were transformed to as-measured with moisture content by using the moisture correction in the COA (0.9329 g dry mass/g as-received mass) in order for the values to be comparable to the as-received units requested for reporting by participants. These target values were determined using data collected from collaborating laboratories that used their in-house methods to analyze this material. The target value for Cd in RM 8666 was determined at NIST using nitric and hydrochloric acid assisted microwave digestion and ICP tandem mass spectrometry (ICP-MS/MS). The target values and participant consensus means for toxic elements in RM 8666 are summarized in **Table 3-6**.

**Table 3-6.** Summary of results and laboratory variabilities for toxic elements in RM 8666.

Analyte	Target Value $\pm U_{\text{NIST}}$ Mass Fraction (ng/g)	Consensus Mean $\pm$ SD Mass Fraction (ng/g)	Within-Laboratory Variability (% RSD)	Between-Laboratory Variability (% RSD)
Total Arsenic (tAs)	40.8 $\pm$ 5.0	40.3 $\pm$ 6.7	8 %	17 %
Cadmium (Cd)	6.7 $\pm$ 1.6	6.8 $\pm$ 1.5	12 %	22 %
Lead (Pb)	93.1 $\pm$ 6.5	89 $\pm$ 15	10 %	17 %
Mercury (Hg)	7.7 $\pm$ 2.4	7.4 $\pm$ 2.6	27 %	35 %

For the determination of toxic elements in RM 8666, the participation rate was above 85 % for each element, with 37 laboratories requesting samples reporting results for Hg and 40 laboratories requesting samples reporting results for tAs, Cd, and Pb. Within-laboratory variabilities were averaged for each element as shown in **Table 3-6**. The published within-laboratory variability requirement for tAs and Pb in this range is at or below 15 %, as shown in **Table 3-3** [4]. Most laboratories were below this threshold for tAs and Pb which demonstrates that the majority of participants' in-house methods achieve successful repeatability. Only two laboratories for tAs and six laboratories for Pb were above the published performance requirements. The published between-laboratory variability requirement for tAs and Pb in this range is at or below 32 %, as seen in **Table 3-3** [4]. For tAs and Pb the between-laboratory variabilities, as shown in **Table 3-6**, were below the requirements indicating that the community demonstrated acceptable agreement for the analysis of RM 8666. Since AOAC SMPR 2020.001 does not include method performance requirements for toxic elements below 10 ng/g, AOAC SMPR 2024.002 Determination of Trace Elemental Contaminants in Food and Beverages can be used as a proxy to assess the performance of laboratories measuring Cd and Hg in RM 8666 [8]. The published within-laboratory variability requirement is at or below 30 % [8]. Nearly all laboratories, except for two, were below this threshold for Cd which demonstrates that the majority of participants' in-house methods achieve successful repeatability. The average within-laboratory variability for Hg was still below the threshold but there were ten laboratories above the 30 % RSD. The published between-laboratory variability requirements for Cd and Hg in this range is at or below 44 % [8]. For Cd and Hg the between-laboratory variabilities, as shown in **Table 3-6**, were below the requirements indicating that the community demonstrated acceptable agreement for the analysis of RM 8666.

No sample preparation or analysis method bias was observed for any of the toxic elements in RM 8666.

The consensus mean was slightly below the target value for tAs, as shown in **Fig. 3-1**. The consensus mean was equal to the target value for Cd, as seen in **Fig. 3-2**. For Pb and Hg, the consensus means were below the target values, as seen in **Fig. 3-3** and **3-4**. The consensus confidence intervals were all within the target ranges for all four elements in RM 8666.

For As, Cd, and Hg, between 20 % and 40 % of laboratories reported results below LOQ. Many of the methods reported by these laboratories are capable of measuring these analytes at the levels found in RM 8666 as demonstrated in **Fig. 3-2** and **Fig. 3-4** by laboratories reporting results within the target range. Laboratories can lower their LOQs by analyzing a sufficient number of blanks and using high purity reagents and deionized water. Although laboratories

that report values below LOQ are considered successful in the study if the target value is also below the laboratory's LOQ, many of these laboratories are reporting LOQs at 10 ng/g or above, which is above the FDA regulatory limits for toxic elements in food products. AOAC SMPR 2024.002 provides LOQ requirements for toxic elements in foods and baby foods: 5 ng/g for tAs, 4 ng/g for Cd, 2 ng/g for Pb, and 1 ng/g for Hg [8]. It seems that many laboratories in this study, specifically for Cd and Hg, are setting their LOQs at the FDA regulations of 10 ng/g instead of calculating the LOQ for their laboratory's in-house methods. One of the FDA's current goals is to reduce dietary exposure to toxic elements to as low as possible, which could lead to lower regulatory limits for these elements and subsequently require analytical laboratories to lower their LOQs to be able to measure these elements in foods and related products [3].

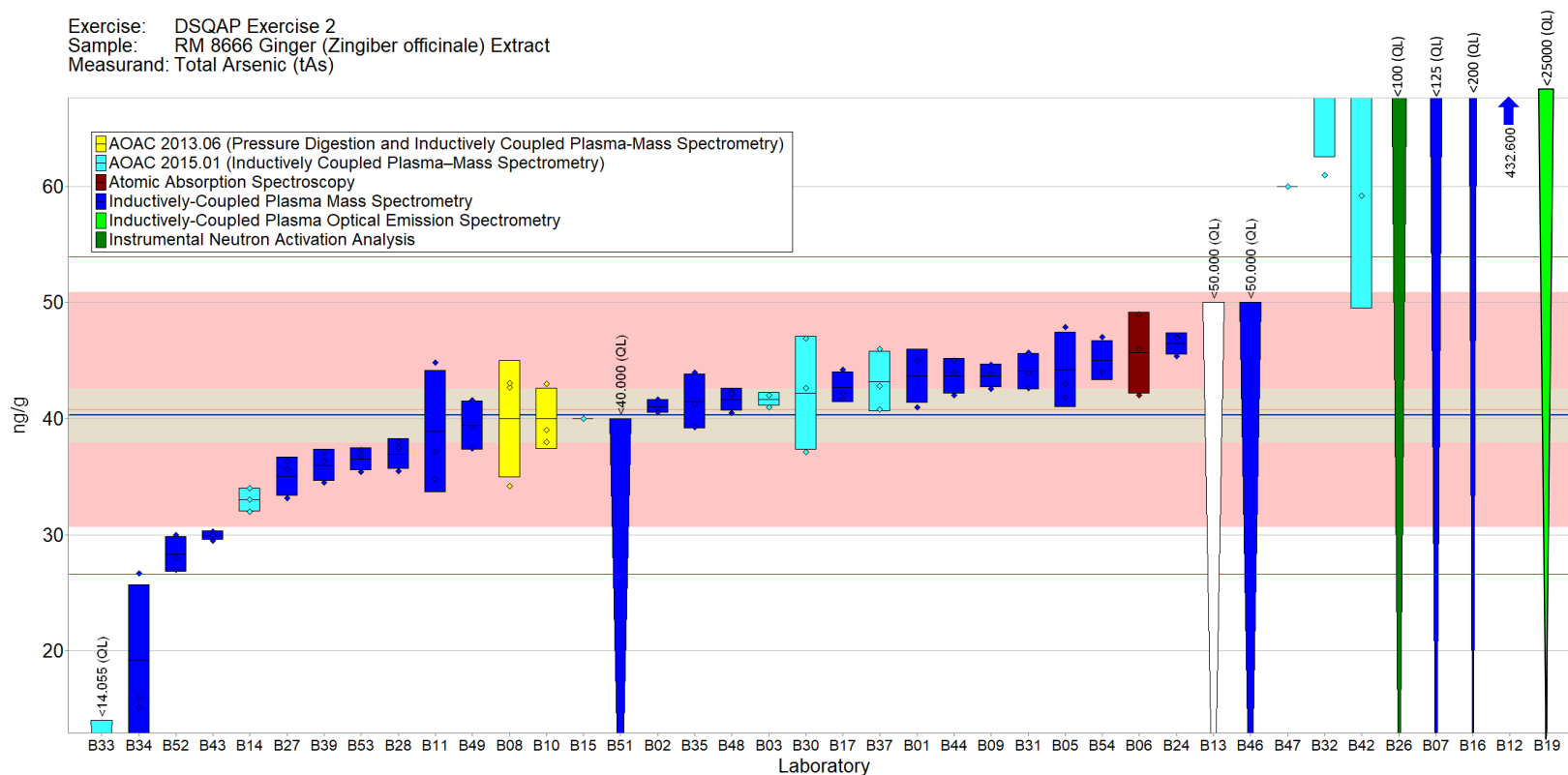
Laboratories that report values above the target ranges for As and Cd should consider potential interferences when analyzing this material. Collision cell technology can be used to minimize the molecular ion interferences that may be found when analyzing As. Residual carbon leftover after digestion of samples with high carbon content can lead to As signal enhancement when using ICP-MS. To mitigate these effects, laboratories need to digest samples at a high enough temperature to increase the oxidation potential of the acids which will decrease the amount of residual carbon. Laboratories can also mitigate these effects after digestion by adding a solvent, such as isopropanol, to samples, by carbon matrix matching the calibration solutions to the samples, or by adding carbon to the plasma, such as methane, to equally enhance both samples and standards. When using ICP-MS, spectral/isobaric interferences can make Cd difficult to measure due to the presence of certain elements (e.g., Mo, Sn, or Zr) in samples. Isobaric spectral interferences such as  $^{95}\text{Mo}^{16}\text{O}^+$  and  $^{97}\text{Mo}^{16}\text{O}^+$  can affect the accuracy of Cd determination at  $m/z$  111 and  $m/z$  113 by ICP-MS and usually result in biasing the results above the true value.

Overall laboratories performed well when analyzing Pb in RM 8666. When using ICP-MS, the three most abundant Pb isotopes ( $^{206}\text{Pb}$ ,  $^{207}\text{Pb}$ ,  $^{208}\text{Pb}$ ) should be monitored and their signals averaged to account for natural differences in Pb isotopic composition between standards and sample types.

For Hg, erratic results can occur due to carryover between samples resulting in high within-laboratory variability as seen in RM 8666. Adequate washout time is needed after each measurement and the addition of dilute HCl to the washout solution can help reduce the length of washout time needed.

Additional tables and figures for toxic elements in RM 8666 are located in **Appendix B**.

Exercise: DSQAP Exercise 2  
 Sample: RM 8666 Ginger (*Zingiber officinale*) Extract  
 Measurand: Total Arsenic (tAs)



**Fig. 3-1.** Total arsenic in RM 8666 Ginger (*Zingiber officinale*) Extract (data summary view – analytical method).

In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed (the analytical method was not specified by laboratory B13). The solid blue line represents the consensus mean, and the green shaded region (beige here due to overlap with the red NIST target range) 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  $Z'_{\text{comm}}$  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 ( $U_{\text{NIST}}$ ) and represents the range that results in an acceptable  $Z_{\text{NIST}}$  score,  $|Z_{\text{NIST}}| \leq 2$ .

Exercise: DSQAP Exercise 2  
 Sample: RM 8666 Ginger (*Zingiber officinale*) Extract  
 Measurand: Cadmium

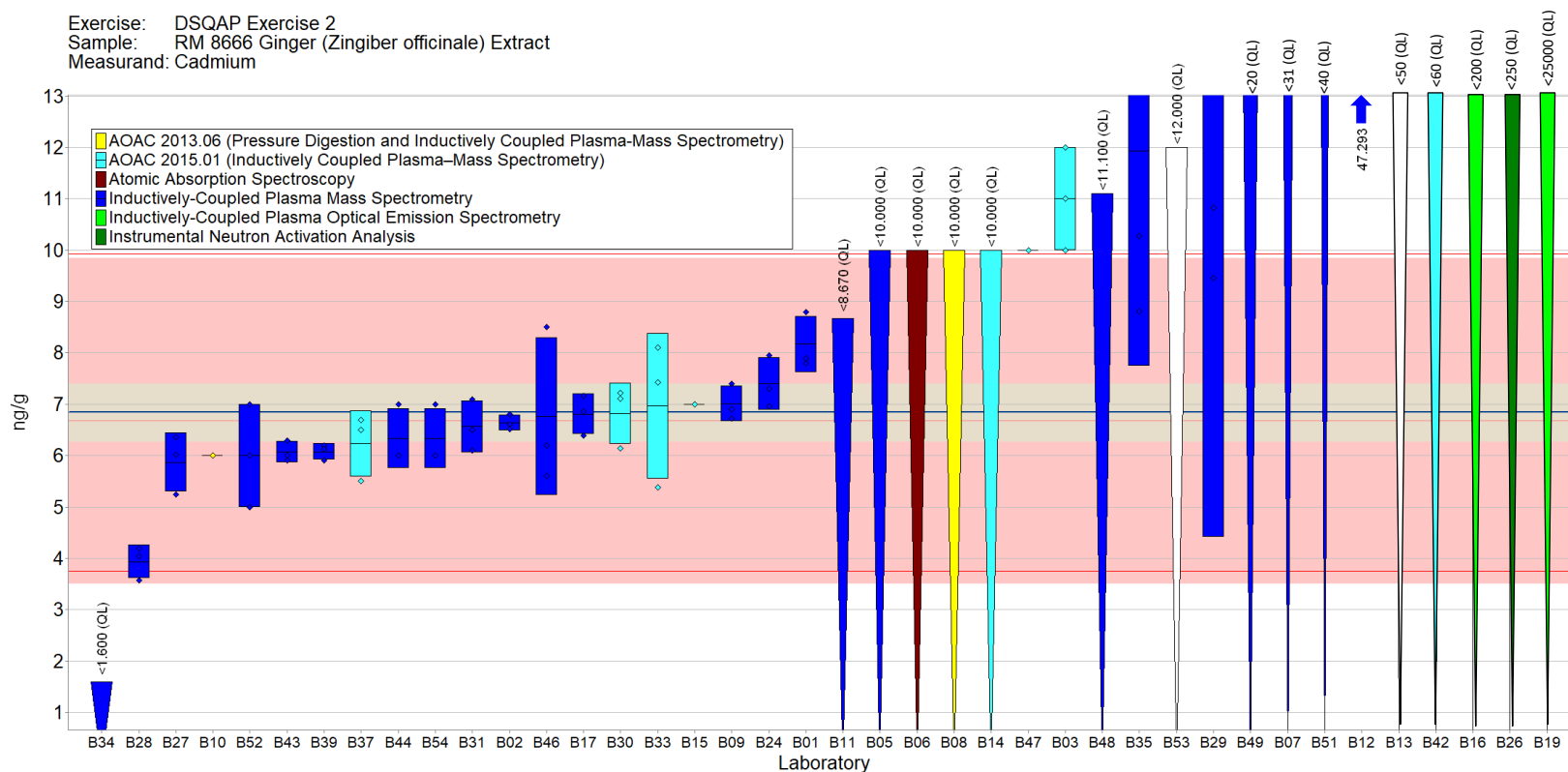
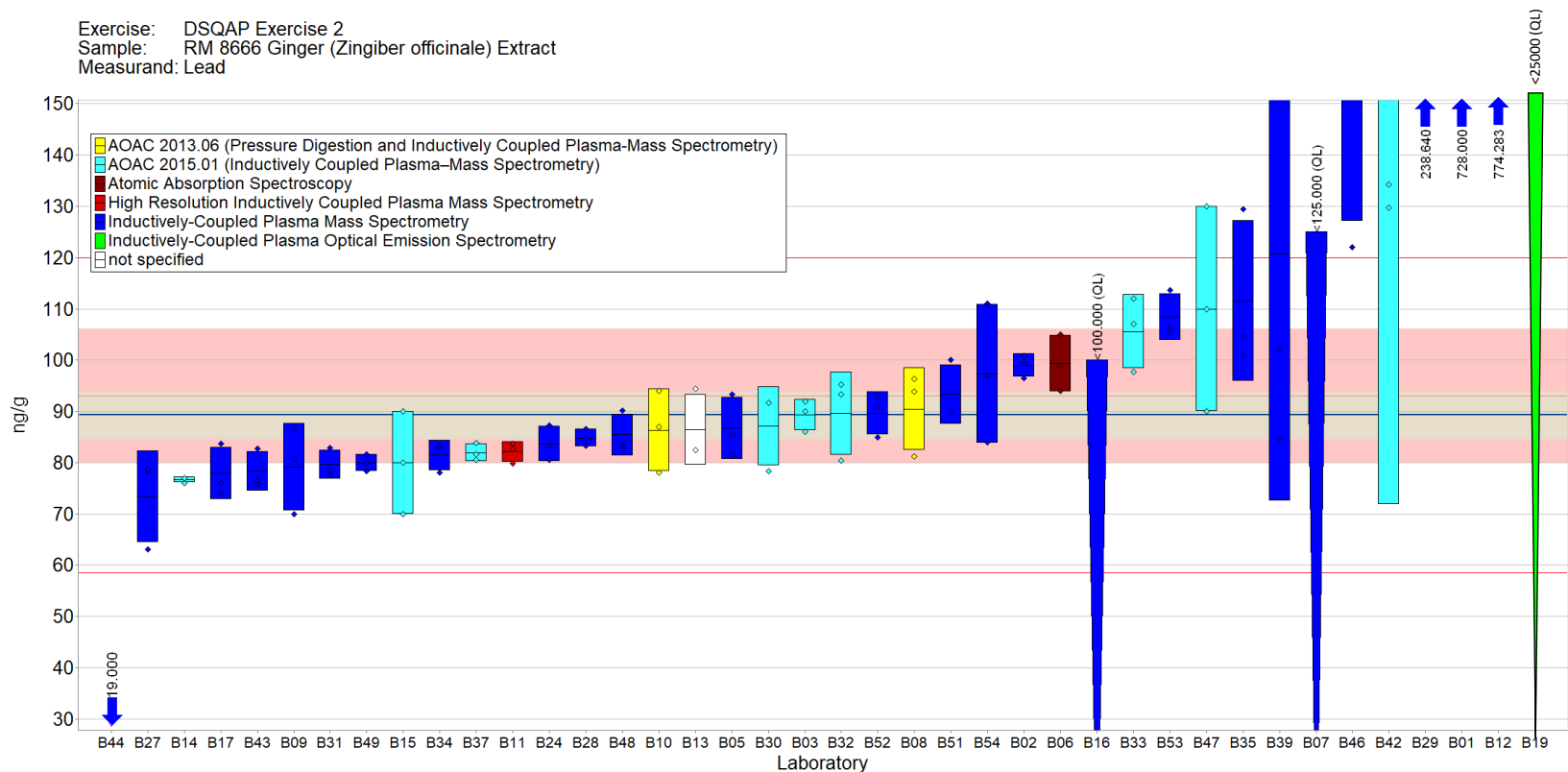


Fig. 3-2. Cadmium in RM 8666 Ginger (*Zingiber officinale*) Extract (data summary view – analytical method).

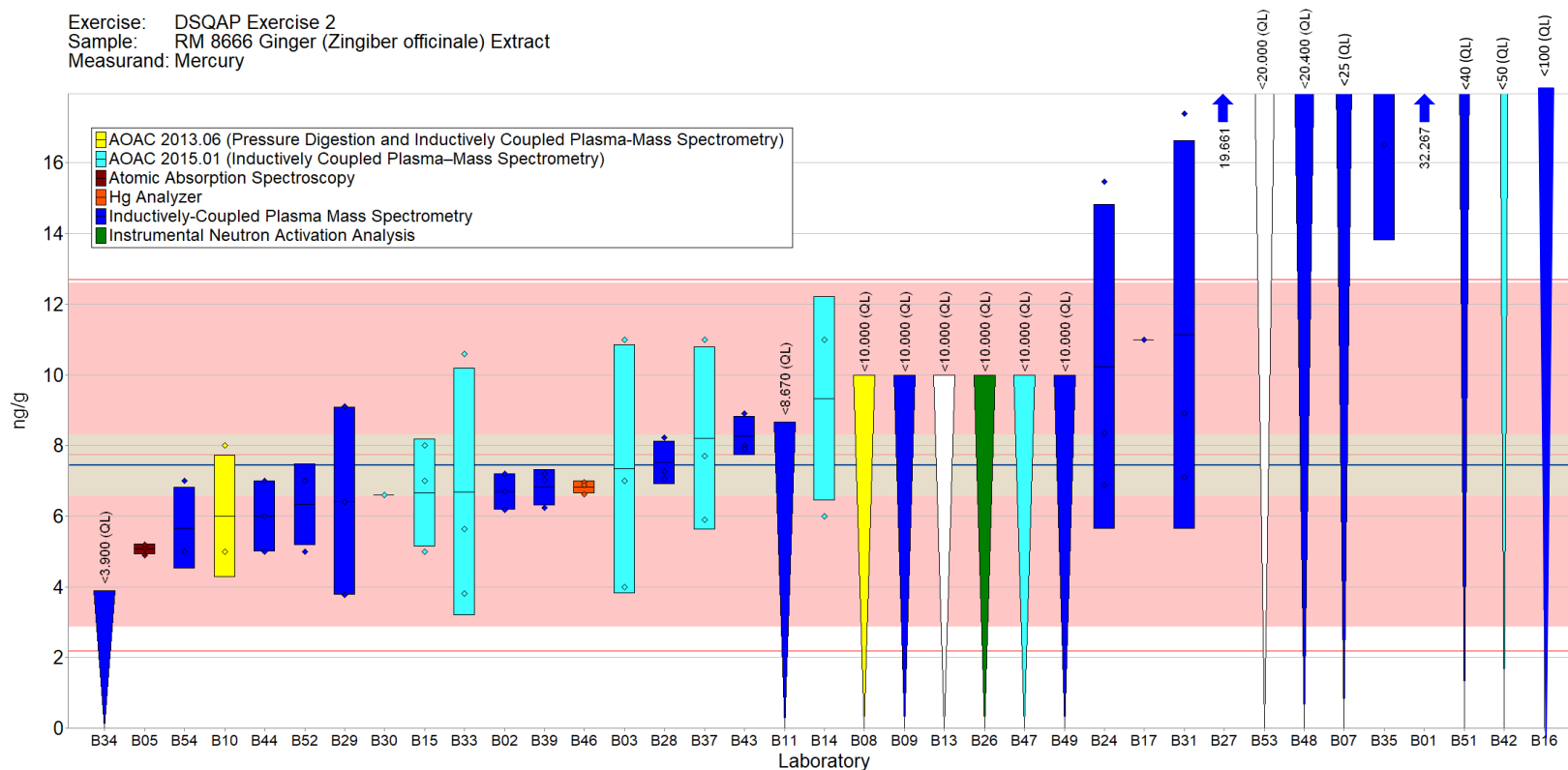
In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed (the analytical method was not specified by laboratories B10, B13, and B53). The solid blue line represents the consensus mean, and the green shaded region (beige here due to overlap with the red NIST target range) 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  $Z'_{\text{comm}}$  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 ( $U_{\text{NIST}}$ ) and represents the range that results in an acceptable  $Z_{\text{NIST}}$  score,  $|Z_{\text{NIST}}| \leq 2$ .



**Fig. 3-3.** Lead in RM 8666 Ginger (*Zingiber officinale*) Extract (data summary view – analytical method).

In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line represents the consensus mean, and the green shaded region (beige here due to overlap with the red NIST target range) 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  $Z'_{\text{comm}}$  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 ( $U_{\text{NIST}}$ ) and represents the range that results in an acceptable  $Z_{\text{NIST}}$  score,  $|Z_{\text{NIST}}| \leq 2$ .

Exercise: DSQAP Exercise 2  
 Sample: RM 8666 Ginger (*Zingiber officinale*) Extract  
 Measurand: Mercury



**Fig. 3-4.** Mercury in RM 8666 Ginger (*Zingiber officinale*) Extract (data summary view – analytical method).

In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed (the analytical method was not specified by laboratories B13 and B53). The solid blue line represents the consensus mean, and the green shaded region (beige here due to overlap with the red NIST target range) 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  $Z'_{\text{comm}}$  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 ( $U_{\text{NIST}}$ ) and represents the range that results in an acceptable  $Z_{\text{NIST}}$  score,  $|Z_{\text{NIST}}| \leq 2$ .

### 3.4.2. Eleuthero Extract

All of the target values in the Eleuthero Extract are derived from preliminary data determined at NIST. The target values for tAs and Pb in the Eleuthero Extract were determined at NIST using nitric and hydrofluoric acid assisted microwave digestion and ICP-MS. The target value for Cd in the Eleuthero Extract was determined at NIST using nitric and hydrochloric acid assisted microwave digestion and ICP-MS/MS. The target value for Hg was determined by averaging results from NIST using two methods: hydrochloric and nitric acid assisted microwave digestion and isotope dilution cold-vapor generation ICP-MS (ID-CV-ICP-MS) along with direct combustion AAS (DC AAS). Target values and participant consensus means for toxic elements in the Eleuthero Extract are summarized in **Table 3-7**.

**Table 3-7.** Summary of results and laboratory variabilities for toxic elements in Eleuthero Extract.

Analyte	Target Value $\pm U_{\text{NIST}}$ Mass Fraction (ng/g)	Consensus Mean $\pm$ SD Mass Fraction (ng/g)	Within-Laboratory Variability (% RSD)	Between-Laboratory Variability (% RSD)
Total Arsenic (tAs)	495.7 $\pm$ 8.1	498 $\pm$ 66	2.5 %	13 %
Cadmium (Cd)	63.7 $\pm$ 6.2	63.9 $\pm$ 7.9	5.6 %	12 %
Lead (Pb)	876.0 $\pm$ 8.1	843 $\pm$ 94	4.4 %	11 %
Mercury (Hg)	324 $\pm$ 79	330 $\pm$ 140	16.6 %	43 %

For the determination of toxic elements in Eleuthero Extract, the participation rate was above 85 % for each element, with 37 laboratories requesting samples reporting results for Hg and 41 laboratories requesting samples reporting results for tAs, Cd, and Pb. Within-laboratory variabilities were averaged for each element as shown in **Table 3-7**. The published within-laboratory variability requirement, as seen in **Table 3-3**, for tAs, Pb, and Hg in these concentration ranges is at or below 11 % while it is at or below 15% for Cd at this concentration [4]. Most laboratories were below this threshold for tAs, Cd, and Pb, demonstrating that most participants' in-house methods achieve successful repeatability. Only one laboratory for tAs, three laboratories for Cd, and four laboratories for Pb were above the published performance requirements. The average within-laboratory variability for Hg was much greater with 20 laboratories above the 11 % RSD recommendation. The published between-laboratory variability requirement for tAs, Pb, and Hg in these concentration ranges is at or below 16 % while it is at or below 16 % for Cd at this concentration [4]. For tAs, Cd, and Pb the between-laboratory variabilities, as shown in **Table 3-7**, were below the requirements indicating that the community demonstrated acceptable agreement for the analysis of Eleuthero Extract. The between-laboratory variability for Hg was more than twice the published recommendation. No sample preparation or analytical method bias was observed for any toxic elements in the Eleuthero Extract.

The consensus mean was slightly above the target value for tAs and the consensus confidence interval overlapped the NIST target range, as shown in **Fig. 3-5**. The consensus mean was equal to the target value for Cd and the consensus confidence interval was within the NIST target range, as seen in **Fig. 3-6**. For Pb, the consensus mean was below the NIST target range and the consensus confidence interval only slightly overlapped the lower region of the NIST target range, as shown in **Fig. 3-7**. The consensus mean was above the target value for Hg and the consensus confidence interval was within the NIST target range, as seen in **Fig. 3-8**.

Overall, laboratories performed well when measuring As and Cd in Eleuthero Extract. Laboratories that reported results below the target ranges should evaluate their sample preparation method to determine whether complete digestion of As and Cd from the extract is being achieved. A high temperature in a closed vessel system is suggested to ensure a complete digestion of the sample. At least 14 % of laboratories reported using a sample preparation method other than microwave digestion, as shown in **Table 3-4**. As previously stated in section 3.4.1, laboratories reporting values above the consensus and target ranges should consider potential interferences and utilize collision cell technology to minimize them. For As, laboratories could also be reporting results above the consensus and target ranges due to enhanced signal from residual carbon as previously mentioned in section 3.4.1.

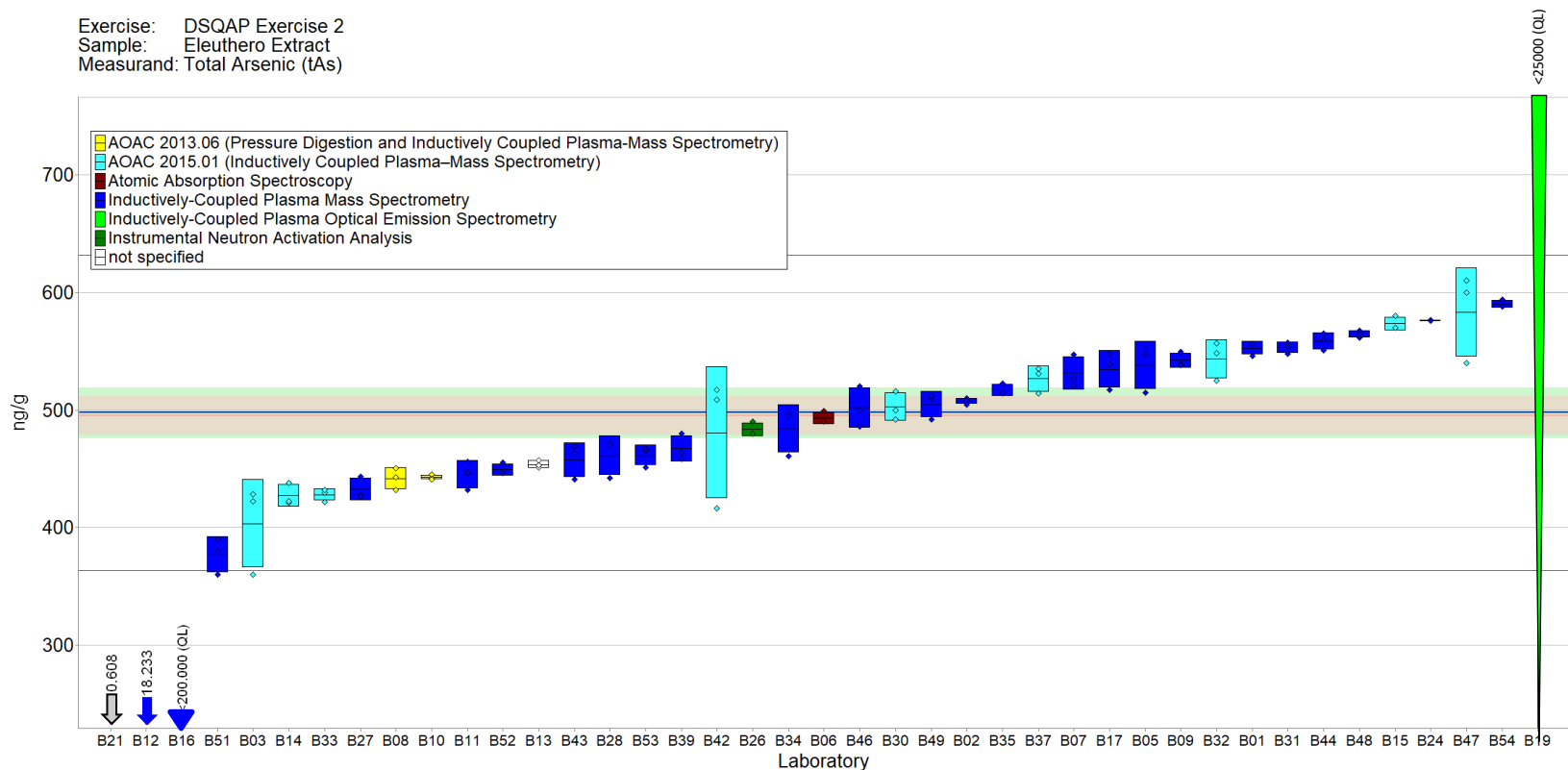
Nearly half of the laboratories reported results below the target range for Pb. If laboratories perform digestion with HCl, it can form insoluble  $\text{PbCl}_2$  precipitate and result in undissolved Pb going undetected. As Pb mass fractions increase,  $\text{PbCl}_2$  precipitation due to HCl could become more problematic and may explain why the consensus mean was below the target range for Pb in Eleuthero Extract. To prevent this bias, digestion with  $\text{HNO}_3$  is recommended for analysis of Pb. If HCl is used in digestion, dilute  $\text{HNO}_3$  should be used to repeatedly wash the side of the digestion vessels to redissolve any  $\text{PbCl}_2$  that may have formed. As previously mentioned, when using ICP-MS the three most abundant Pb isotopes ( $^{206}\text{Pb}$ ,  $^{207}\text{Pb}$ ,  $^{208}\text{Pb}$ ) should be monitored and their signals averaged.

Similar to the results in RM 8666, many laboratories reported large within-laboratory variability for the analysis of Hg in Eleuthero Extract. This could be due to Hg carryover between samples and it is recommended that adequate washout time be used after each measurement. The length of washout time can be reduced if HCl is used in the washout solution. Large within-laboratory variability could also be due to heterogeneity of the material and laboratories should ensure they are using the recommended sample size for the analysis of toxic elements.

Laboratory B21 reported values far below the consensus and target ranges for all four toxic elements and should consider evaluating if their values are reported in the correct units.

Additional tables and figures for toxic elements in Eleuthero Extract are located in **Appendix B**.

Exercise: DSQAP Exercise 2  
Sample: Eleuthero Extract  
Measurand: Total Arsenic (tAs)



**Fig. 3-5.** Total arsenic in Eleuthero Extract (data summary view – analytical method).

In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed (the analytical method reported by laboratory B21 was other). The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \leq 2$ . The red shaded region (beige here due to overlap with the green consensus confidence interval) 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,  $|Z_{NIST}| \leq 2$ .

Exercise: DSQAP Exercise 2  
 Sample: Eleuthero Extract  
 Measurand: Cadmium

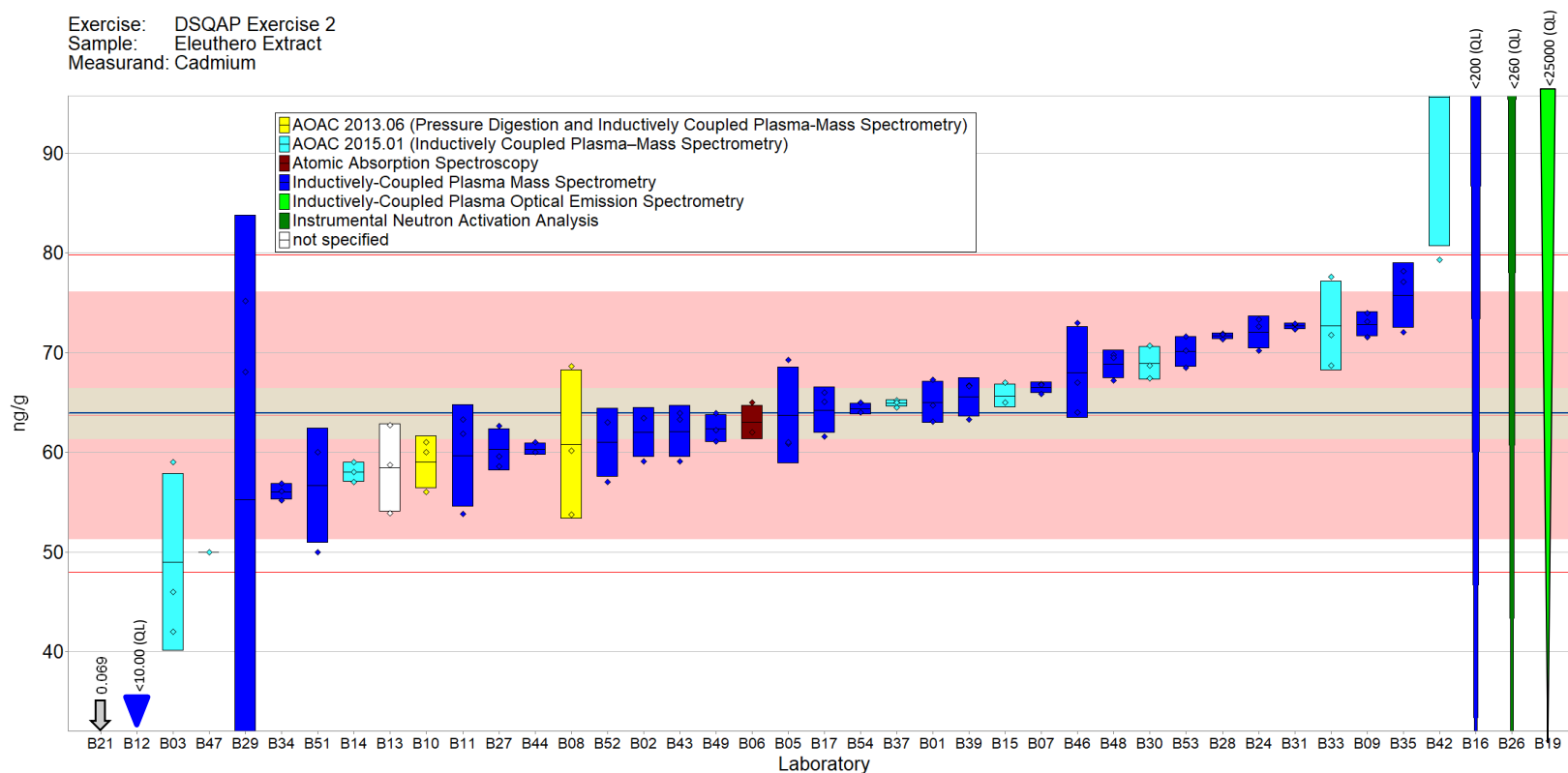


Fig. 3-6. Cadmium in Eleuthero Extract (data summary view – analytical method).

In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed (the analytical method reported by laboratory B21 was other). The solid blue line represents the consensus mean, and the green shaded region (beige here due to overlap with the red NIST target range) 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  $Z'_{\text{comm}}$  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 ( $U_{\text{NIST}}$ ) and represents the range that results in an acceptable  $Z_{\text{NIST}}$  score,  $|Z_{\text{NIST}}| \leq 2$ .

Exercise: DSQAP Exercise 2  
 Sample: Eleuthero Extract  
 Measurand: Lead

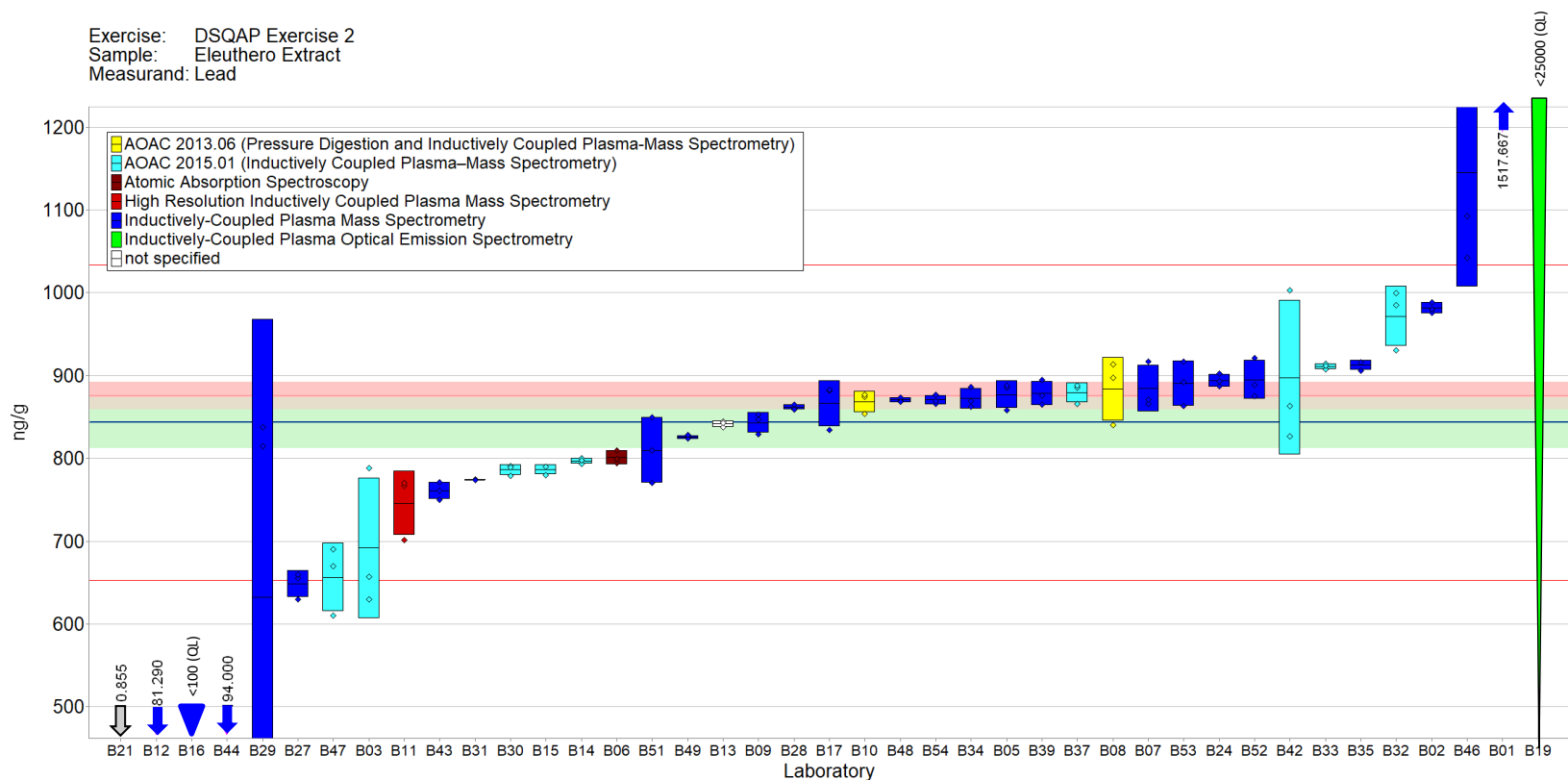
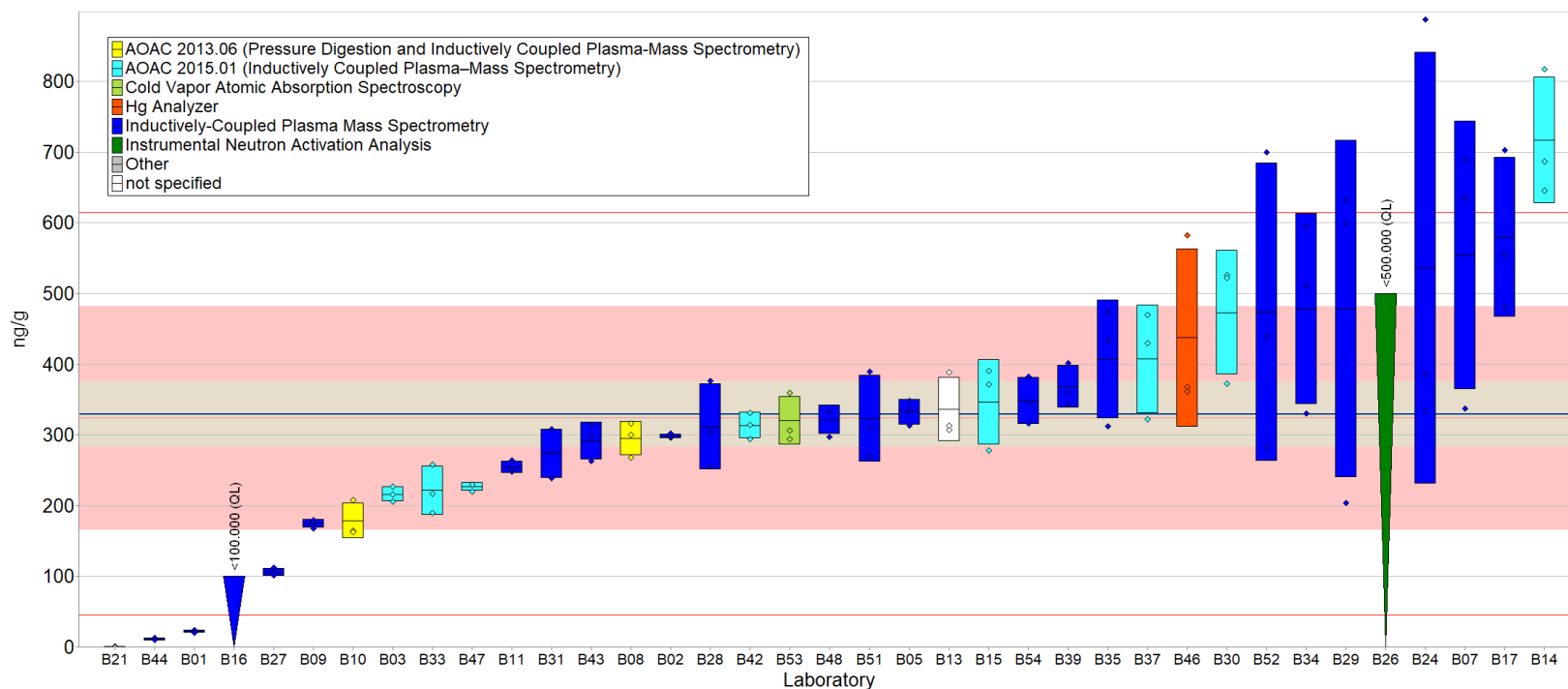


Fig. 3-7. Lead in Eleuthero Extract (data summary view – analytical method).

In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed (the analytical method reported by laboratory B21 was other). The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable  $Z'_{\text{comm}}$  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 ( $U_{\text{NIST}}$ ) and represents the range that results in an acceptable  $Z_{\text{NIST}}$  score,  $|Z_{\text{NIST}}| \leq 2$ . The shaded beige region represents the overlapping of the 95 % confidence interval for the consensus mean (green region) and the NIST range of tolerance (red region).

Exercise: DSQAP Exercise 2  
 Sample: Eleuthero Extract  
 Measurand: Mercury



**Fig. 3-8.** Mercury in Eleuthero Extract (data summary view – analytical method).

In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid blue line represents the consensus mean, and the green shaded region (beige here due to overlap with the red NIST target range) 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  $Z'_{\text{comm}}$  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 ( $U_{\text{NIST}}$ ) and represents the range that results in an acceptable  $Z_{\text{NIST}}$  score,  $|Z_{\text{NIST}}| \leq 2$ .

## 4. Fat-Soluble Vitamins in Supplements

### 4.1. Summary

This study evaluated analytical challenges associated with carotenoid measurements in dietary supplement materials. Participants were asked to measure total  $\beta$ -carotene, *trans*- $\beta$ -carotene, and 9-*cis*- $\beta$ -carotene in SRM 3289 Multivitamin Tablets and SRM 3251 Saw Palmetto (*Serenoa repens*) Extract. Fourteen and 13 laboratories reported results for total  $\beta$ -carotene in SRM 3289 and SRM 3251, respectively. Participation rates for *trans*- $\beta$ -carotene and 9-*cis*- $\beta$ -carotene were much lower with only four to six laboratories reporting results for the two  $\beta$ -carotene forms. Overall, participants did well measuring  $\beta$ -carotene in both materials with low within-laboratory variabilities. Challenges with complete extraction of  $\beta$ -carotene and calibration were observed in this study.

### 4.2. Background

Carotenoids are pigment compounds that are responsible for the yellow and orange colors found in many fruits and vegetables and certain carotenoids such as  $\beta$ -carotene,  $\alpha$ -carotene, and  $\beta$ -cryptoxanthin are a source of vitamin A. Vitamin A is essential to maintain normal human vision, for the function of the immune and reproductive systems, as well as the heart, lungs, kidneys, and other organs [9].  $\beta$ -carotene is converted to vitamin A within the body, and the *cis-trans* isomerization can affect the provitamin A activity [9]. Accurate determination of the isomers is essential for understanding intake values, bioactivity, and correlation to health benefits. In this study, participants were asked to use their in-house analytical methods for the measurement of total  $\beta$ -carotene, *trans*- $\beta$ -carotene, and 9-*cis*- $\beta$ -carotene in multivitamin tablets and saw palmetto extracts.

### 4.3. Study Information

Participants were provided samples of Saw Palmetto Extract (three ampoules containing 1 mL, *Serenoa repens*) and Multivitamin (three bottles of 30 tablets). SRM 3251 Saw Palmetto (*Serenoa repens*) Extract was labeled as Saw Palmetto Extract and SRM 3289 Multivitamin Tablets were labeled as Multivitamin for this DSQAP exercise to conceal the identities of these materials to participants and will be referred to as SRM 3251 and SRM 3289, respectively, for the remainder of the report. Participants were asked to store the materials at controlled room temperature, 20 °C to 25 °C, and to prepare one sample and report one value from each bottle and ampoule provided. Before use, participants were asked to mix each individual SRM 3251 ampoule by gently inverting at least three times. Participants were instructed to grind all 30 multivitamin tablets and mix the resulting powder thoroughly for each bottle. Participants' usual sample size for oil matrices for SRM 3251 and a sample size of at least 2 g for SRM 3289 were recommended to determine mass fractions (mg/g) of  $\beta$ -carotene. The approximate analyte levels were not reported to participants prior to the study. Target values for SRM 3251 [10] and SRM 3289 [11, 12] were obtained from their respective COAs which is the only source for official values.

#### 4.4. Study Results and Technical Recommendations

**Table 4-1** summarizes the reported sample preparation techniques used by the participants to measure total  $\beta$ -carotene in SRM 3251 and SRM 3289. Overall, participants used various sample preparation techniques with solvent extraction being the most reported. Nearly all participants, 13, reported use of liquid chromatography with absorbance/photodiode array detection (LC-Abs/PDA) for the determination of total  $\beta$ -carotene in this study. One laboratory reported use of LC with fluorescence detection (LC-FLD). No sample preparation or analytical technique trends were observed based on the limited method information received for total  $\beta$ -carotene. Due to the low reporting rates for *trans*- $\beta$ -carotene and 9-*cis*- $\beta$ -carotene, no technical recommendations on the methodology can be made for the reported values.

**Table 4-1.** Summary of sample preparation methods for the determination of  $\beta$ -carotene in SRM 3251 Saw Palmetto (*Serenoa repens*) Extract and SRM 3289 Multivitamin Tablets.

Reported Sample Preparation	Percent Reporting % (Total $\beta$ -carotene)	
	SRM 3251	SRM 3289
Dilution	23 %	14 %
Enzymatic Hydrolysis	23 %	14 %
Saponification/Base Hydrolysis	15 %	14 %
Solvent Extraction	31 %	43 %
Solvent Extraction, Solid Phase Extraction, and Derivatization	-	7 %
Other/None Reported	8 %	7 %

NIST has conducted several DSQAP studies involving the measurement of  $\beta$ -carotene over the years. Most notably Exercise D (2009) [13] and Exercise G (2017) [14]. Participants measured total  $\beta$ -carotene in SRM 3251 in Exercise D. Overall, the between-laboratory variabilities were large, and the primary recommendation was to improve calibration within the testing community [13]. Exercise G asked participants to measure total  $\beta$ -carotene, *trans*- $\beta$ -carotene, and 9-*cis*- $\beta$ -carotene in two calibration solutions along with SRM 3251. Participants performed better in this study and reported between-laboratory variabilities of 10 %, 7 %, and 17 % for total  $\beta$ -carotene, *trans*- $\beta$ -carotene, and 9-*cis*- $\beta$ -carotene, respectively [14].

The variety of reported sample preparation techniques was similar between this current study and the two previous studies, indicating no consensus methodology for  $\beta$ -carotene within the testing community. While no methodology trends were observed in these studies, calibration errors in the measurement of carotenoids are of concern. Laboratories should assign calibration concentrations spectrophotometrically since carotenoid measurements are traceable to molar absorptivity. Appropriate molar absorption coefficients should also be used based on specific solvents and temperatures within the validated method. As a starting point, the absorptivity for *trans*- $\beta$ -carotene in hexane at 29°C with a max detection wavelength of 452 nm is 2592 dL/g·cm while the absorptivity for 9-*cis*- $\beta$ -carotene at a max detection wavelength of 445 nm is 2550 dL/g·cm [15].

#### 4.4.1. Total $\beta$ -carotene

Of the 17 laboratories requesting samples for the FSV study, 13 laboratories reported results for total  $\beta$ -carotene in SRM 3251 and 14 out of 18 laboratories reported results for SRM 3289 (76 % and 78 %, respectively). The average within-laboratory variability for total  $\beta$ -carotene in SRM 3251 (3.1 %) was acceptable compared to the published standard of at or below 5 % for levels above 0.125 mg/kg (**Table 4-2**) [16]. While AOAC SMPR 2017.006 is intended to establish method requirements for determination of  $\beta$ -carotene in infant and adult/pediatric nutritional formulas, the established requirements are used here as a proxy to gauge the acceptability of the participants' performance in the provided samples. The within-laboratory variability in SRM 3289 was higher at 6.4 % and outside of the established performance requirements. The between-laboratory variabilities for SRM 3251 (24 %) and SRM 3289 (50 %) were both outside of the established performance requirements of 10 % indicating that the participants and the methods being used are not in agreement with each other [16].

**Table 4-2.** Summary of results and laboratory variabilities for total  $\beta$ -carotene in SRM 3251 Saw Palmetto (*Serenoa repens*) Extract and 3289 Multivitamin Tablets.

Material	Total $\beta$ -carotene	
	Within-Laboratory Variability (% RSD)	Between-Laboratory Variability (% RSD)
SRM 3251 Saw Palmetto ( <i>Serenoa repens</i> ) Extract	3.1 %	24 %
SRM 3289 Multivitamin Tablets	6.4 %	50 %

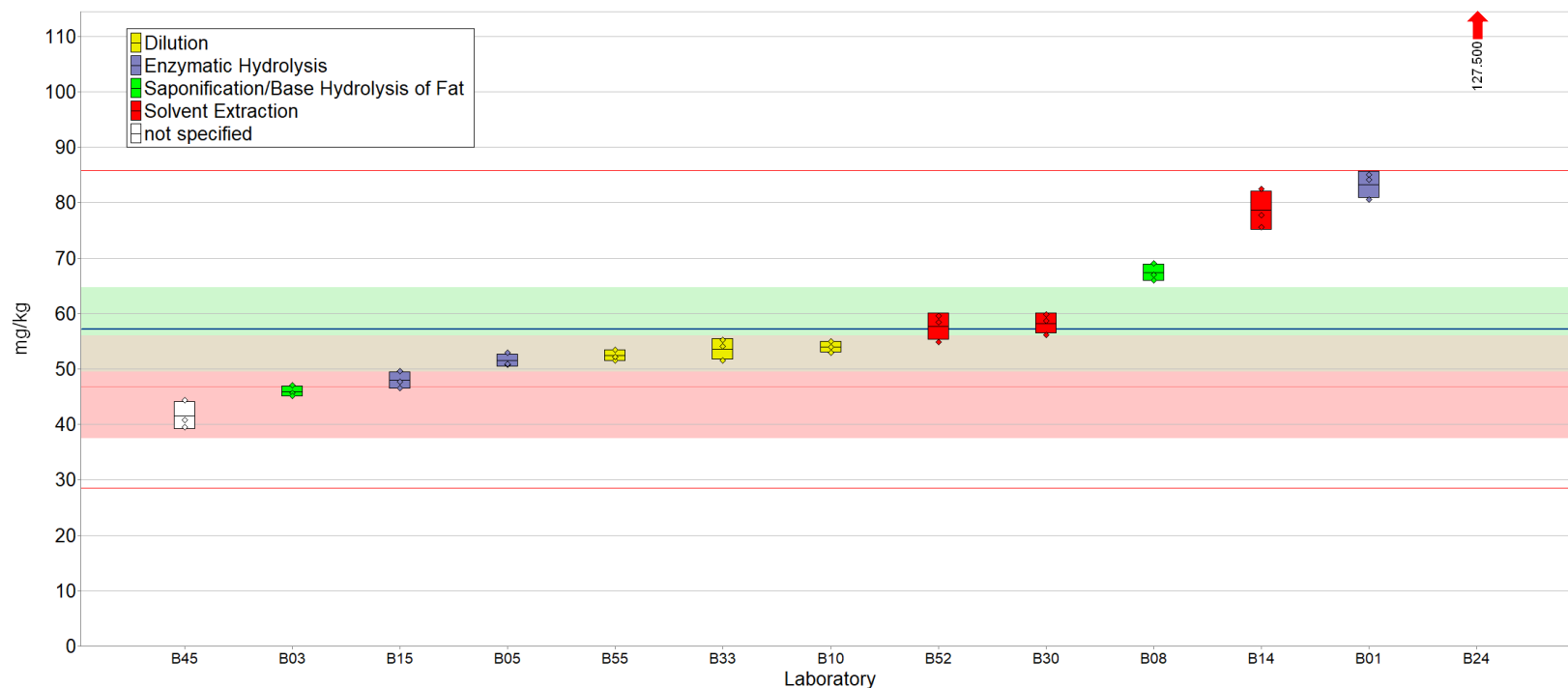
Overall, 6 of the 13 participants reporting values for total  $\beta$ -carotene in SRM 3251 were within the 95 % confidence interval for the consensus mean which overlaps with the NIST range of tolerance (**Fig. 4-1**). Three participants reported values below the 95 % confidence interval for the consensus mean; however, they were within the NIST range of tolerance. The consensus mean ( $57.16 \pm 13.81$  mg/kg) was slightly above of the NIST range of tolerance with seven participants reported values within the target range.

The results for total  $\beta$ -carotene in SRM 3289 showed the opposite trend when compared to SRM 3251 (**Fig. 4-2**). The 95 % confidence interval for the consensus mean overlapped with the lower side of the NIST range of tolerance. While 8 of the 14 participants reported results within the NIST range of tolerance, the lower consensus range indicates potential extraction issues. Fat-soluble vitamins are typically encapsulated in multivitamin products to prevent degradation and enhance bioavailability. While no sample preparation trends were observed, participants should ensure their methods are capable of breaking down the encapsulation to fully release  $\beta$ -carotene for analysis. Participant B30 indicated using solvent extraction and reported values for the oil-based material that were slightly overlapping with the upper limits of the NIST target range while their values for SRM 3289 were approximately 350 mg/kg below the lower limits of the NIST target range. This showcases the applicability of solvent extraction for total  $\beta$ -carotene in liquid and oil-based materials while highlighting the difficulty in fully releasing vitamins from encapsulation in multivitamin tablets.

Calibration of  $\beta$ -carotene isomers may also present challenges to community consensus. Concentrations of  $\beta$ -carotene calibrants are determined spectrophotometrically and appropriate molar absorption coefficients are necessary. It is important to note that  $\beta$ -carotene isomers have different absorptivity values based on solvents and temperature. The high between-laboratory variabilities for total  $\beta$ -carotene may be due, in part, to participants not agreeing on calibration approaches. Having an established methodology (solvent and temperature) with a specified molar absorption coefficient may improve community consensus for  $\beta$ -carotene measurements.

Additional tables and figures for total  $\beta$ -carotene in supplements are located in **Appendix C**.

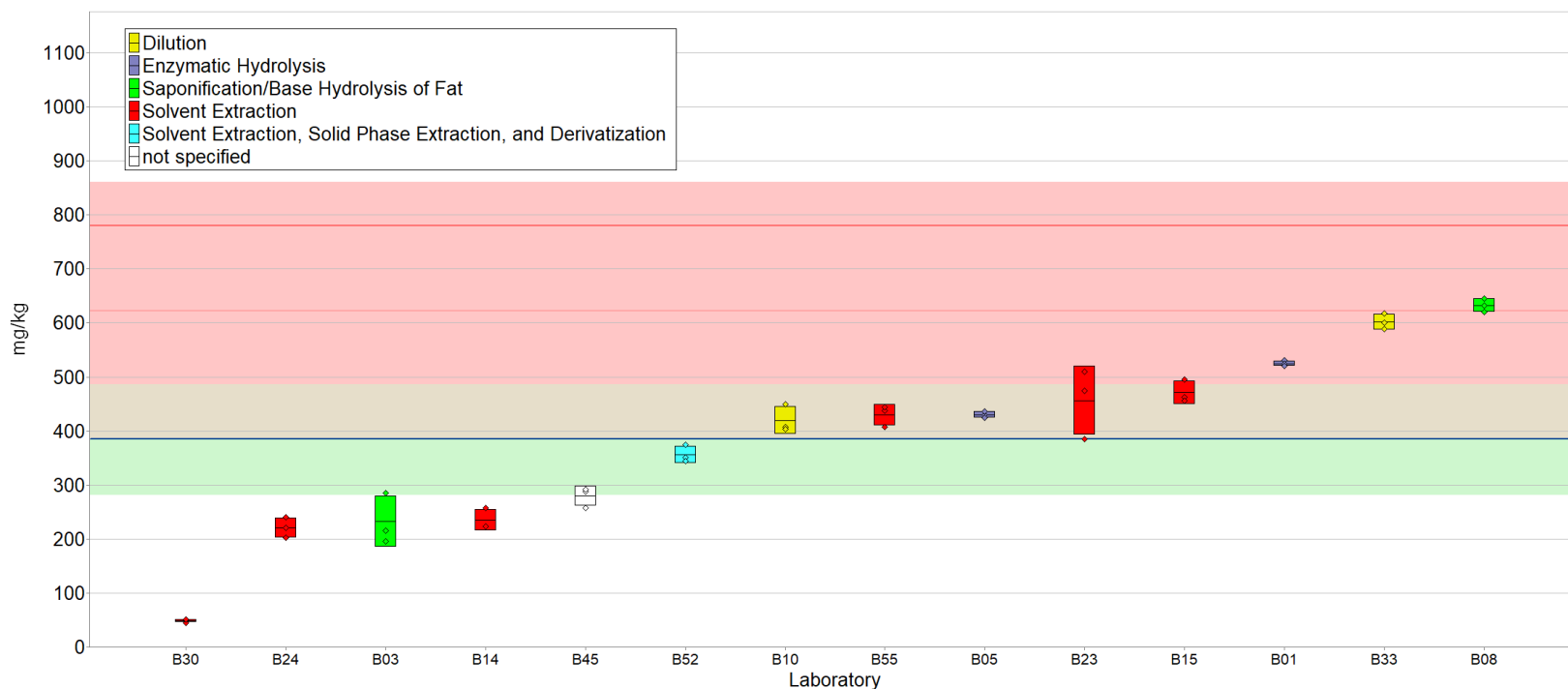
Exercise: DSQAP Exercise 2  
Sample: SRM 3251 Saw Palmetto (*Serenoa repens*) Extract  
Measurand: Total beta-Carotene



**Fig. 4-1.** Total  $\beta$ -carotene in SRM 3251 Saw Palmetto (*Serenoa repens*) Extract (data summary view – sample preparation).

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

Exercise: DSQAP Exercise 2  
Sample: SRM 3289 Multivitamin Tablets  
Measurand: Total beta-Carotene



**Fig. 4-2.** Total  $\beta$ -carotene in SRM 3289 Multivitamin Tablets (data summary view – sample preparation).

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

#### 4.4.2. *Trans*- $\beta$ -carotene

Of the 14 laboratories requesting samples for the FSV study, 6 laboratories reported results for *trans*- $\beta$ -carotene in both SRM 3251 and SRM 3289 (43 %). The average within-laboratory variability for *trans*- $\beta$ -carotene in SRM 3251 (3.4 %) was acceptable compared to the published standard of at or below 5 % for levels above 0.125 mg/kg (**Table 4-3**) [16]. The within-laboratory variability in SRM 3289 was higher at 6.5 % and outside of the referenced performance requirements. The between-laboratory variabilities for SRM 3251 (25 %) and SRM 3289 (57 %) were both outside of the established performance requirements of 10 % [16].

**Table 4-3.** Summary of results and laboratory variabilities for *trans*- $\beta$ -carotene in SRM 3251 Saw Palmetto (*Serenoa repens*) Extract and 3289 Multivitamin Tablets.

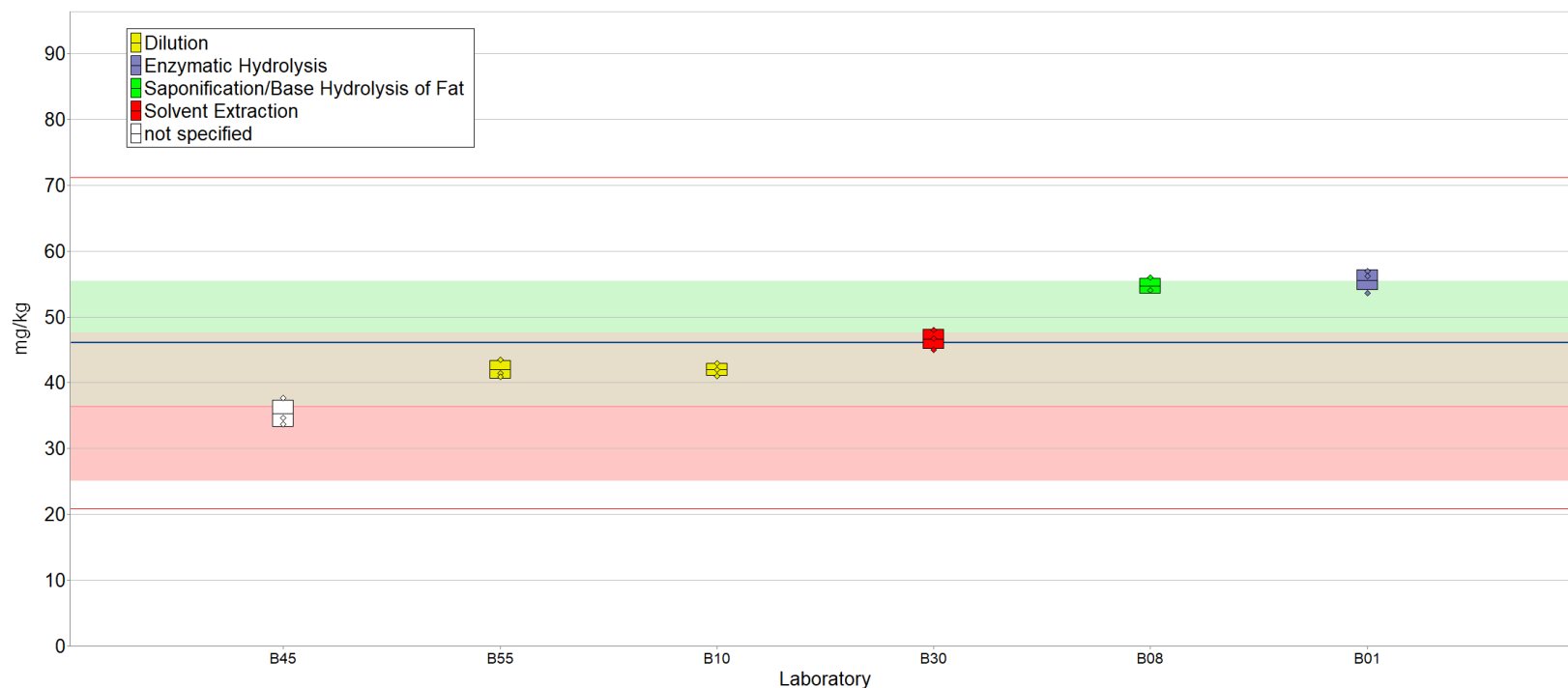
<i>Trans</i> - $\beta$ -carotene		
Material	Within-Laboratory Variability (% RSD)	Between-Laboratory Variability (% RSD)
SRM 3251 Saw Palmetto ( <i>Serenoa repens</i> ) Extract	3.4 %	25 %
SRM 3289 Multivitamin Tablets	6.5 %	57 %

While only 6 participants returned data for *trans*- $\beta$ -carotene, the results were similar to total  $\beta$ -carotene in the previous section. The consensus mean for SRM 3251 fell within the NIST target range with only two participants reporting results above the NIST range of tolerance (**Fig. 4-3**). The consensus mean for *trans*- $\beta$ -carotene and total  $\beta$ -carotene were both approximately 10 mg/kg above the respective target values which indicates potential calibration issues with the *trans*- $\beta$ -carotene isomer. It is important to note that *trans*- $\beta$ -carotene accounts for approximately 77 % of total  $\beta$ -carotene in SRM 3251. As mentioned previously, participants should use an appropriate detection wavelength and corresponding molar absorptivity to determine accurate calibrant concentrations. Participants reporting quantitative data for specific *cis/trans* isomers should use appropriate sample preparation techniques as different approaches can isomerize the  $\beta$ -carotene and affect the ratio of isomers in the sample. However, this would not be evident in the total  $\beta$ -carotene measurements.

The 95 % confidence interval for the consensus mean for *trans*- $\beta$ -carotene in SRM 3289 aligns with the reported data for total  $\beta$ -carotene (**Fig. 4-4**). While no target range is available for *trans*- $\beta$ -carotene in SRM 3289, it is likely that *trans*- $\beta$ -carotene accounts for the majority of total  $\beta$ -carotene in the multivitamin material. The between-laboratory variability was approximately twice as high for *trans*- $\beta$ -carotene in SRM 3289 than in SRM 3251. As mentioned previously, participants should ensure their methods are capable of breaking down the encapsulation to fully release  $\beta$ -carotene and isomers for analysis.

Additional tables and figures for *trans*- $\beta$ -carotene in supplements are located in **Appendix C**.

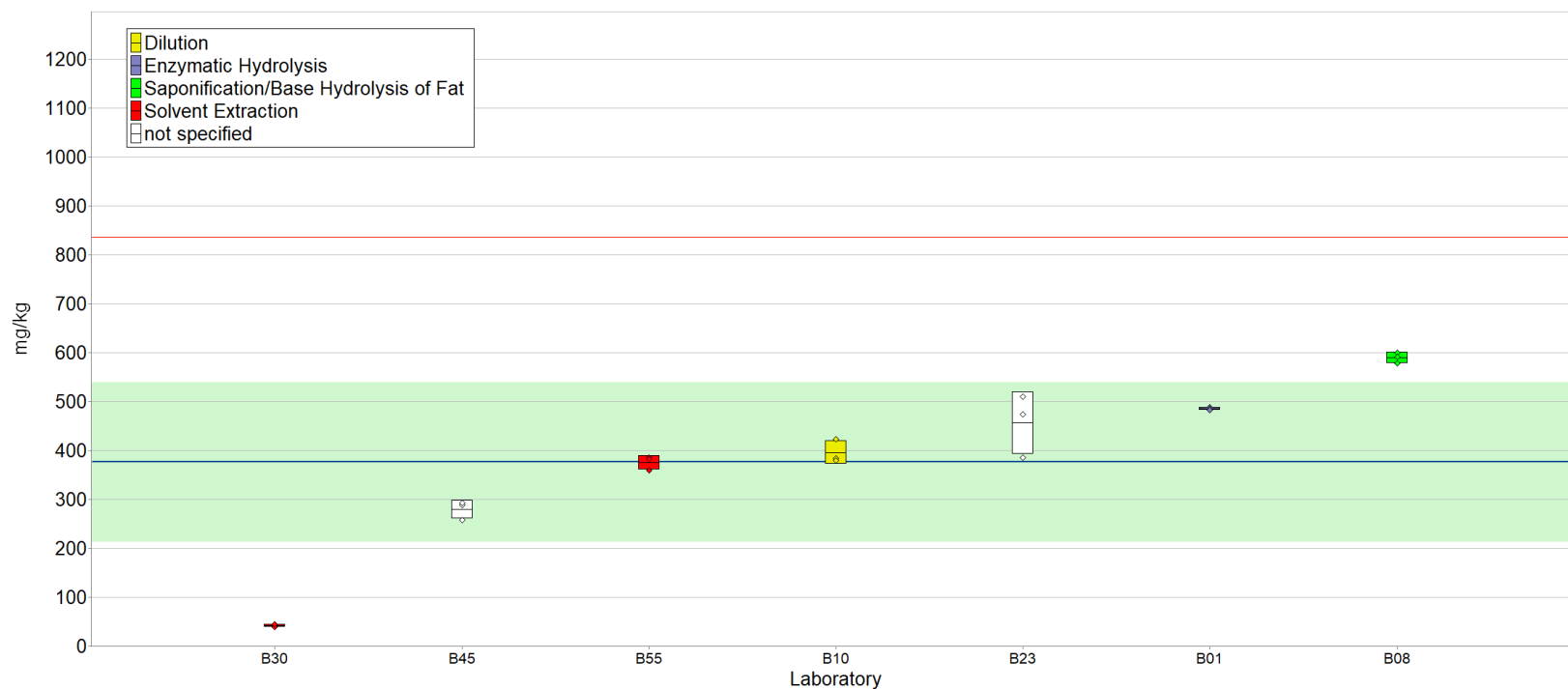
Exercise: DSQAP Exercise 2  
 Sample: SRM 3251 Saw Palmetto (*Serenoa repens*) Extract  
 Measurand: trans-beta-Carotene



**Fig. 4-3.** *Trans*-β-carotene in SRM 3251 Saw Palmetto (*Serenoa repens*) Extract (data summary view – sample preparation).

In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation employed. The solid blue line represents the consensus mean, and the green shaded region (beige here due to overlap with the red NIST target range) 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  $Z'_{\text{comm}}$  score,  $|Z'_{\text{comm}}| \leq 2$ . The red shaded region represents the NIST range of tolerance (with the target value represented as the darker red line in the center), which encompasses the target value bounded by twice its uncertainty ( $U_{\text{NIST}}$ ) and represents the range that results in an acceptable  $Z_{\text{NIST}}$  score,  $|Z_{\text{NIST}}| \leq 2$ . The shaded beige region represents the overlapping of the 95 % confidence interval for the consensus mean (green region) and the NIST range of tolerance (red region).

Exercise: DSQAP Exercise 2  
Sample: SRM 3289 Multivitamin Tablets  
Measurand: trans-beta-Carotene



**Fig. 4-4.** *Trans*- $\beta$ -carotene in SRM 3289 Multivitamin Tablets (data summary view – sample preparation).

In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation employed. The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The solid red line represents the upper bound of the consensus range of tolerance, calculated as the value above the consensus mean that results in an acceptable  $Z'_{\text{comm}}$  score,  $|Z'_{\text{comm}}| \leq 2$ , with the lower bound set to zero.

### 4.4.3. 9-*Cis*- $\beta$ -carotene

Of the 13 laboratories requesting samples for the FSV study, 5 laboratories reported results for 9-*cis*- $\beta$ -carotene in SRM 3251 (38 %). The average within-laboratory variability for 9-*cis*- $\beta$ -carotene in SRM 3251 (10 %) was outside of the published standard of at or below 5 % for levels above 0.125 mg/kg (**Table 4-4**) [16]. The between-laboratory variabilities for SRM 3251 (54 %) was outside of the referenced performance requirements of 10 %.

Table 4-4. Summary of results and laboratory variabilities for 9-*cis*- $\beta$ -carotene in SRM 3251 Saw Palmetto (*Serenoa repens*) Extract and 3289 Multivitamin Tablets.

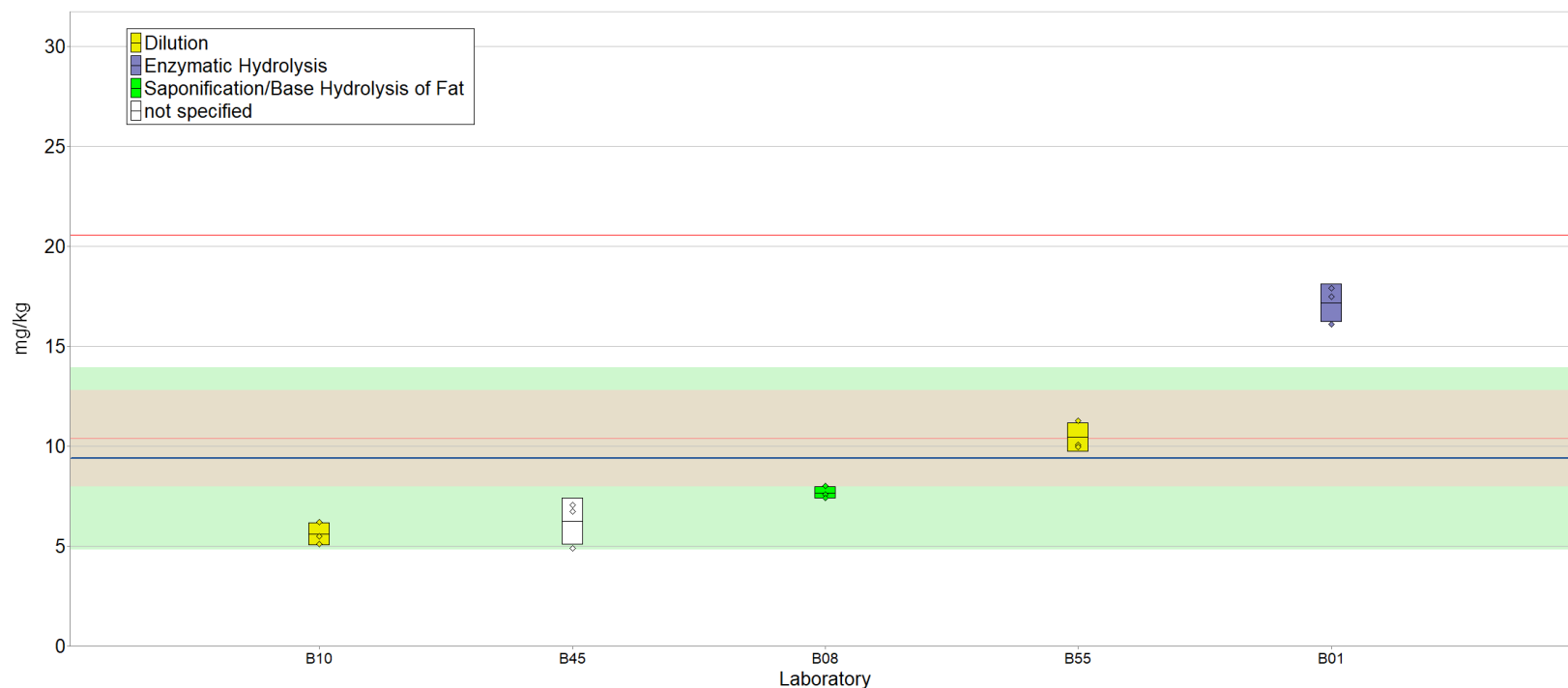
9- <i>cis</i> - $\beta$ -carotene		
Material	Within-Laboratory Variability (% RSD)	Between-Laboratory Variability (% RSD)
SRM 3251 Saw Palmetto ( <i>Serenoa repens</i> ) Extract	10 %	54 %
SRM 3289 Multivitamin Tablets	-	-

The 95 % confidence interval for the consensus mean for 9-*cis*- $\beta$ -carotene in SRM 3251 overlapped with the NIST target range (**Fig. 4-5**). While the consensus mean was within the acceptable range, the large between-laboratory variability indicates a lack of agreement within the testing community for the *cis* isomer. Due to low reporting rates for 9-*cis*- $\beta$ -carotene, interpretation of the small data set is limited. As mentioned previously, participants should be cognizant of the reported  $\beta$ -carotene form and use appropriate sample preparation techniques to limit isomerization.

SRM 3289 does not contain 9-*cis*- $\beta$ -carotene, therefore no quantitative values were reported.

Additional tables and figures for 9-*cis*- $\beta$ -carotene in supplements are located in **Appendix C**.

Exercise: DSQAP Exercise 2  
 Sample: SRM 3251 Saw Palmetto (*Serenoa repens*) Extract  
 Measurand: 9-cis-beta-Carotene



**Fig. 4-5.** 9-Cis-β-carotene in SRM 3251 Saw Palmetto (*Serenoa repens*) Extract (data summary view – sample preparation).

In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation employed. The solid blue line represents the consensus mean, and the green shaded region (beige here due to overlap with the red NIST target range) represents the 95 % confidence interval for the consensus mean. The solid red lines represent the upper bound of the consensus range of tolerance, calculated as the value above the consensus mean that result in an acceptable  $Z'_{\text{comm}}$  score,  $|Z'_{\text{comm}}| \leq 2$ , with the lower bound set to zero. The red shaded region (beige here due to overlap with the green consensus confidence interval) represents the NIST range of tolerance (with the target value represented as the darker red line in the center), which encompasses the target value bounded by twice its uncertainty ( $U_{\text{NIST}}$ ) and represents the range that results in an acceptable  $Z_{\text{NIST}}$  score,  $|Z_{\text{NIST}}| \leq 2$ .

## 5. Fatty Acids in Fish Oil

### 5.1. Summary

This study was designed to identify analytical challenges for fatty acid measurements in fish oil materials across the testing community. Participants were provided samples of SRM 3275 Omega-3 and Omega-6 Fatty Acids in Fish Oil and reported values for total linoleic acid (LA), arachidonic acid (ARA), eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA), and docosahexaenoic acid (DHA). The results from this study were also used to evaluate the stability of the SRM. Enrollment and participation in this study was fair with participation rates ranging from 66 to 86 %. The reported results highlight comparable performance across laboratories, with most participants using similar sample preparation and analytical techniques. The overall agreement between the participants' results and the NIST target ranges for the five fatty acids indicates excellent stability of SRM 3275.

### 5.2. Background

Omega-3 and omega-6 fatty acids are polyunsaturated fatty acids that play important roles in various biological functions and health benefits. Omega-3 fatty acids (such as EPA, DHA, and DPA) are crucial components of the phospholipids that form the structures of cell membranes [17]. Omega-6 fatty acids (such as LA and ARA), along with omega-3 fatty acids, are used by the body to form eicosanoids which have known functions in the cardiovascular, endocrine, immune, and pulmonary systems [17]. Due to the inability of the human body to form omega-3 fatty acids, coupled with the low conversion rate of ALA to EPA and DHA in the liver, these essential nutrients must be obtained from food and/or dietary supplementation to maintain sufficient levels for the biological functions mentioned above [18]. Common sources of intake may contain one or more forms such as free fatty acids (FFA), fatty acid esters (FAE), triglycerides, and phospholipids. Accurate determination of these important fatty acids in dietary supplements is essential for understanding intake values, bioactivity, and correlation to health benefits. In this study, participants were asked to use their in-house analytical methods for the measurement of total LA, ARA, EPA, DHA, and DPA in three fish oil materials.

### 5.3. Study Information

Participants were provided samples of Fish Oil A (three ampoules containing 1.2 mL), Fish Oil B (three ampoules containing 1.2 mL), and Fish Oil C (three ampoules containing 1.2 mL). SRM 3275 Omega-3 and Omega-6 Fatty Acids in Fish Oil were labeled as Fish Oil A, Fish Oil B, and Fish Oil C for this DSQAP exercise to conceal the identities of these materials to participants and will be referred to as SRM 3275-1, SRM 3275-2, and SRM 3275-3, respectively, for the remainder of this report. Participants were asked to store the materials in the original unopened ampoules at controlled refrigeration, 2 °C to 8 °C. Before use, participants were instructed to mix each individual ampoule by gently inverting at least three times. It was recommended to use participants' usual sample size for oil matrices and to prepare one sample

and report one value from each ampoule. The approximate analyte levels were not reported to participants prior to the study; however, target values for each fatty acid were obtained from the COA and converted from (mg / g) fatty acid methyl esters to (g / 100 g) free fatty acids and are listed in the sections below [19]. The COA is the only source for official values.

#### 5.4. Study Results and Technical Recommendations

**Table 5-1** details the reporting percentages for the various sample preparation techniques used by the participants to measure fatty acids in the three fish oil materials. Majority of the participating laboratories (72 % to 86 %) incorporated derivatization to fatty acid methyl esters (FAME) in their analysis, which is comparable to the sample preparation detailed in AOAC Official Method 2012.13 [20]. Laboratory B05 was the only participant to specify using hydrolysis to FFA and reported values within the 95 % confidence interval for the consensus means and/or NIST target ranges for ALA, EPA, DPA, and DHA. The value reported for LA was consistently high, but within the consensus range of tolerance for all three fatty acid materials. Laboratories B20 and B52 reported using saponification/base hydrolysis and reported values either at or above the consensus means for ARA, EPA, and DHA. Laboratory B52 reported values for LA below the 95 % confidence interval for the consensus mean for all three materials. The significance of these trends is difficult to determine with only one laboratory hydrolyzing to free fatty acids and two laboratories using saponification/base hydrolysis.

**Table 5-1.** Summary of sample preparation methods for the determination of fatty acids in SRM 3275 Omega-3 and Omega-6 Fatty Acids in Fish Oil.

Reported Sample Preparation	Percent Reporting %				
	LA	ARA	EPA	DPA	DHA
Derivatization to FAME	86%	81%	72%	86%	72%
Dilution	-	6%	5%	7%	5%
Hydrolysis to FFA	7%	6%	6%	7%	6%
Saponification/Base Hydrolysis	7%	6%	11%	-	11%
Other	-	-	5%	-	5%

**Table 5-2** summarizes the reporting percentages for the analytical techniques used by participants in this study. For interpretation purposes, Direct Analysis by gas chromatography with flame ionization detection (GC-FID) and GC-FID are treated as the same analytical technique due to some participants most likely choosing the first option in the method selection portion of data entry. It is important to note that AOAC 2012.13 also uses GC-FID. Therefore, approximately 87 % of participants reported using GC-FID for the analysis of the five fatty acids in the three fish oil materials. Laboratory B05 was the only participant that reported using Direct Analysis by LC-fluorescence detection (LC-FLD). Due to limited data, it cannot be determined if the fluorescence detection and/or hydrolysis to free fatty acids played a role in laboratory B05 reporting values consistently high for LA. As mentioned previously, laboratories B20 and B52 were the only two participants to use saponification/base hydrolysis; however, they both reported using GC-FID so no trends in the detection technique were observed. Laboratory B04 was the only participant who indicated using LC with high resolution mass

spectrometry (LC-HRMS) and reported values below the consensus ranges for ARA, EPA, SPA, and DHA.

**Table 5-2.** Summary of analytical methods for the determination of fatty acids in SRM 3275 Omega-3 and Omega-6 Fatty Acids in Fish Oil.

Reported Analytical Method	Percent Reporting %				
	LA	ARA	EPA	DPA	DHA
AOAC 2012.13	7%	6%	6%	7%	6%
Direct Analysis by GC-FID	36%	31%	44%	36%	44%
Direct Analysis by LC-FLD	7%	6%	6%	7%	6%
GC-FID	50%	50%	39%	36%	39%
GC-FPD	-	-	-	7%	-
LC-HRMS	-	6%	6%	7%	6%

DSQAP has conducted two previous studies involving the measurement of fatty acids in fish oil dietary supplement materials. In 2014, Exercise J [18] included the measurement of LA, ARA, EPA, and DHA in SRM 3275-2, and Exercise L (2016) [21] included the measurement of LA, ARA, EPA, and DHA in SRM 3275-1 and SRM 3275-3. Similar to this study, the majority of participants measuring fatty acids in Exercise J and Exercise L reported using derivatization to fatty acid methyl esters and GC-FID. The consensus means for EPA and DHA in all three materials and LA in SRM 3275-1 and SRM 3275-2 were within the target ranges in the two previous exercises indicating the community consistently performs well measuring select fatty acids in fish oils. The consensus mean for LA in SRM 3275-3 in Exercise L was below the target range; however, the consensus means for LA in all three materials in this current study were within the target range indicating measurement improvement within the community. Also, the consistent performance between the three DSQAP exercises ranging from 2014 to 2023 further validates material stability.

Overall, participants performed well with the consensus overlapping the NIST target ranges for LA, EPA, DPA, and DHA in all three fish oil materials (ARA does not have a target value). While no trends were observed, it is important to consistently use appropriate calibration materials and quality assurance samples to establish that a method is in control and performed correctly. Fatty acids may be reported in several different formats and laboratories should ensure all conversion factors are correct and the reported values are in the appropriate units.

#### 5.4.1. Total Linoleic Acid

Of the 21 laboratories requesting samples for total linoleic acid, 14 laboratories reported results for the three fish oil materials (67 %). The within-laboratory variabilities for total linoleic acid across the three fish oil materials ranged from 2.4 % to 8.2 % (**Table 5-3**). Generally speaking, repeatability data below 7 % are within the acceptable range [22]. The between-laboratory variabilities were much higher ranging from 66.9 to 86.2 %; however, these percentages are skewed by a few laboratories reporting values significantly outside the consensus range of tolerance.

**Table 5-3.** Target values, consensus values, and variabilities for total linoleic acid in SRM 3275 Omega-3 and Omega-6 Fatty Acids in Fish Oil.

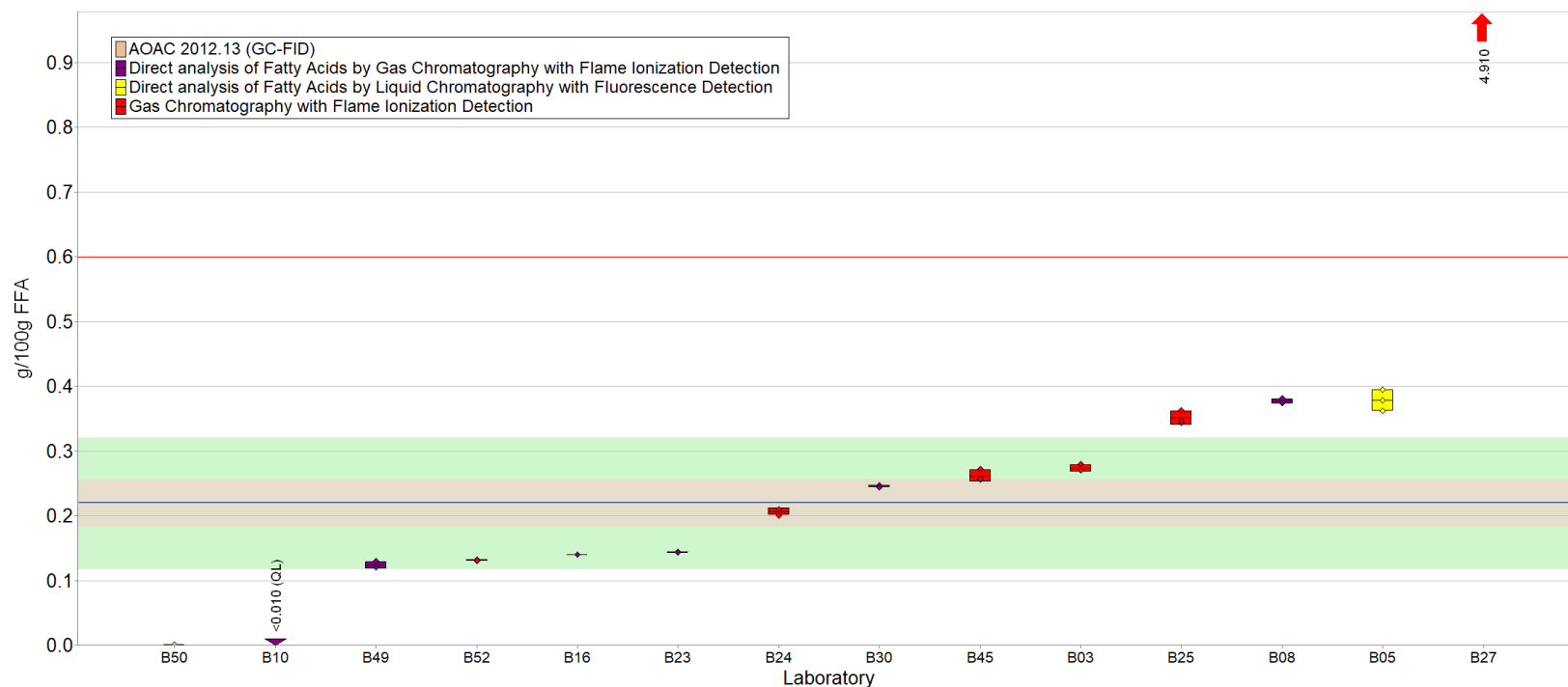
<b>Total Linoleic Acid</b>			
	SRM 3275-1	SRM 3275-2	SRM 3275-3
Target Mass Fraction $\pm U_{\text{NIST}}$ (g/100 g)	$0.220 \pm 0.018$	$0.286 \pm 0.040$	$1.285 \pm 0.043$
Consensus Mean $\pm$ SD (g/100 g)	$0.22 \pm 0.18$	$0.29 \pm 0.25$	$1.27 \pm 0.85$
Within-Laboratory Variability (Median % RSD)	3.2 %	8.2 %	2.4 %
Between-Laboratory Variability (% RSD)	81.8 %	86.2 %	66.9 %

Eight laboratories reported values for total linoleic acid in SRM 3275-1 that were within the 95% confidence interval for the consensus mean which completely overlaps with the NIST range of tolerance (**Fig. 5-1**). The consensus mean ( $(0.22 \text{ g} \pm 0.18 \text{ g})/100 \text{ g}$ ) was within the target range. Laboratories B10, B27, and B50 were outside of the consensus range of tolerance which is likely due to calculation or calibration errors.

The reported results for total linoleic acid in SRM 3275-2 (**Fig. 5-2**) and SRM 3275-3 (**Fig. 5-3**) were similar to the observed trends in SRM 3275-1. The 95 % confidence intervals for the consensus means completely encompasses the NIST range of tolerances for both materials. Laboratories B05, B08, and B25 were above the 95 % confidence interval for the consensus mean for all three materials; however, they were within the consensus range of tolerance. B05 was the only laboratory to indicate hydrolyzing to free fatty acids prior to analysis. Since most laboratories reported derivatization to fatty acid methyl esters, including B08 and B25, the higher levels reported by these three laboratories does not indicate a sample preparation bias.

Additional tables and figures for total linoleic acid in fish oils are located in **Appendix D**.

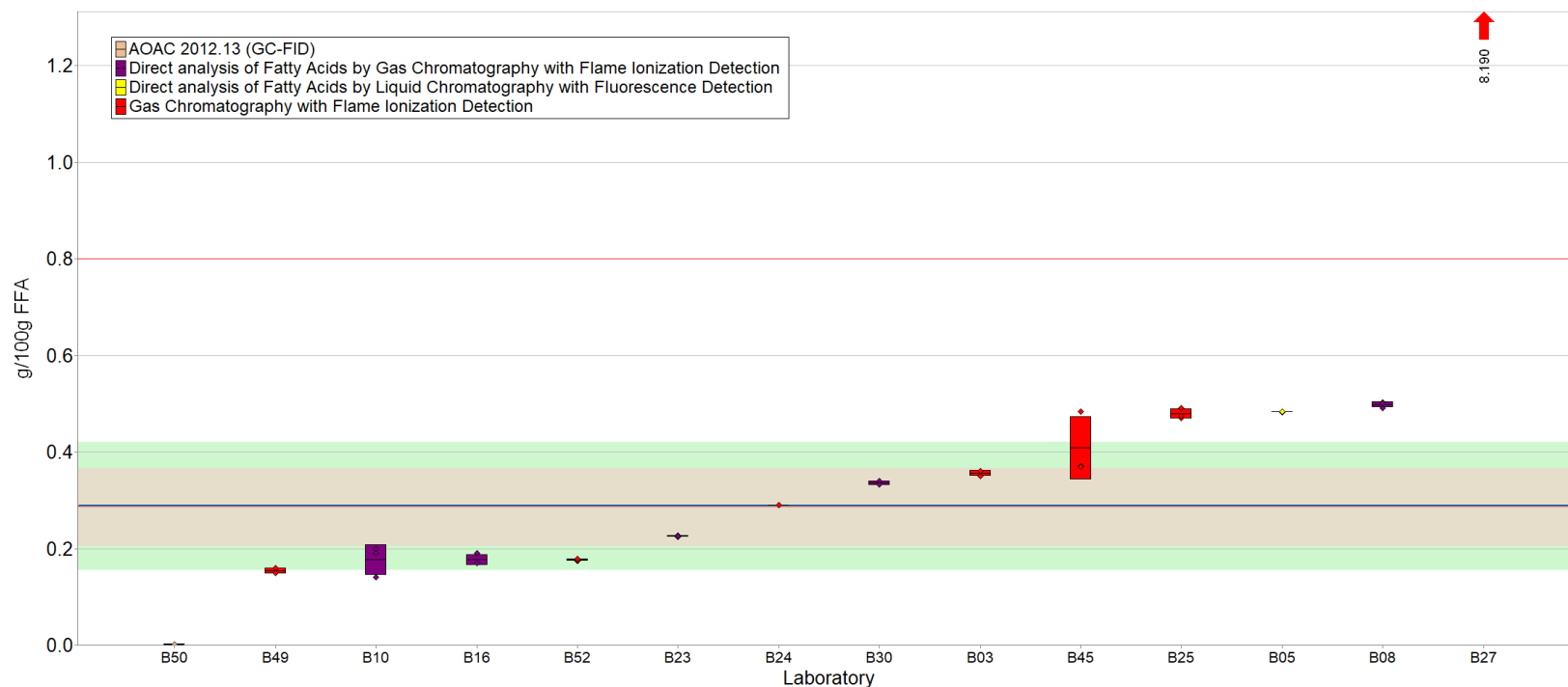
Exercise: DSQAP Exercise 2  
 Sample: SRM 3275-1 Fish Oil  
 Measurand: Total Linoleic Acid (C18:2 n-6)



**Fig. 5-1.** Total Linoleic Acid in SRM 3275-1 Omega-3 and Omega-6 Fatty Acids in Fish Oil (data summary view – analytical method).

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

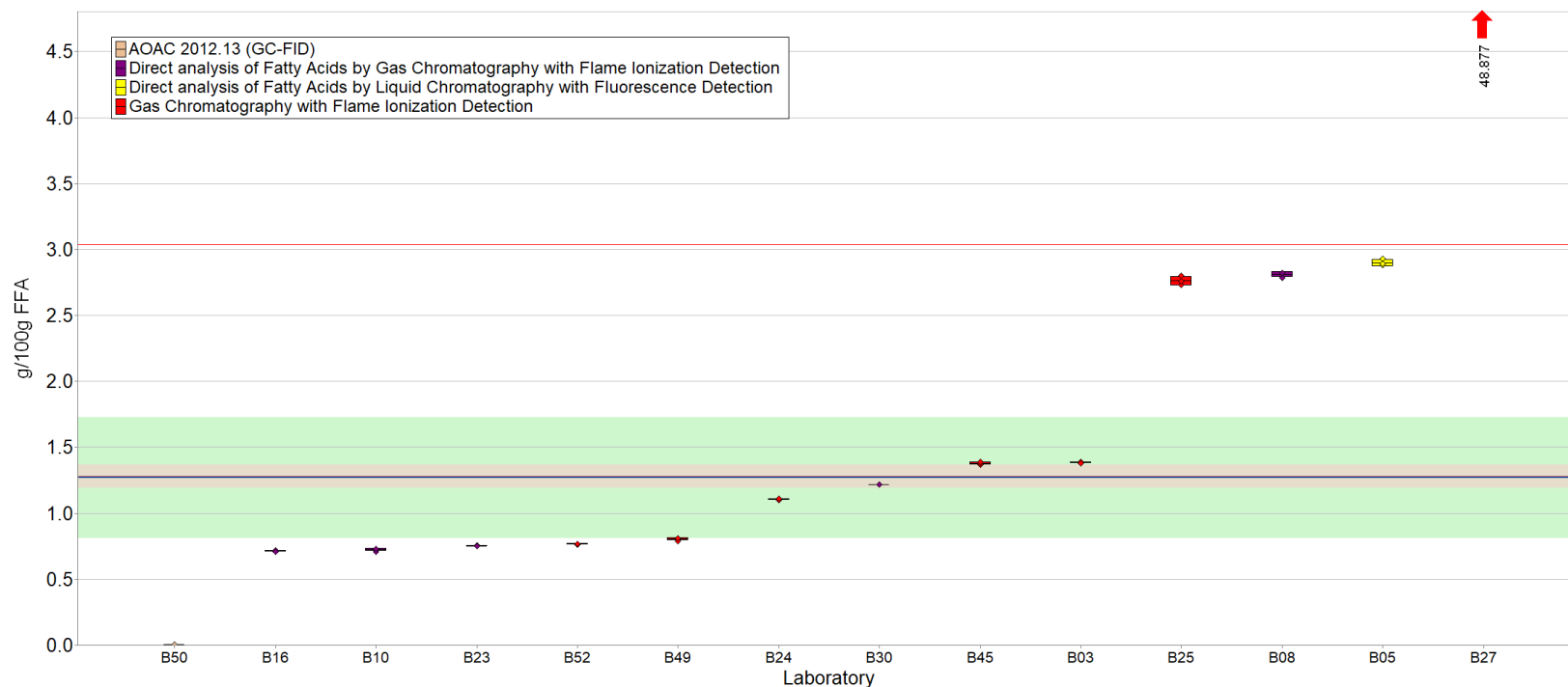
Exercise: DSQAP Exercise 2  
 Sample: SRM 3275-2 Fish Oil  
 Measurand: Total Linoleic Acid (C18:2 n-6)



**Fig. 5-2.** Total Linoleic Acid in SRM 3275-2 Omega-3 and Omega-6 Fatty Acids in Fish Oil (data summary view – analytical method).

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

Exercise: DSQAP Exercise 2  
Sample: SRM 3275-3 Fish Oil  
Measurand: Total Linoleic Acid (C18:2 n-6)



**Fig. 5-3.** Total Linoleic Acid in SRM 3275-3 Omega-3 and Omega-6 Fatty Acids in Fish Oil (data summary view – analytical method).

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

### 5.4.2. Total Arachidonic Acid

Of the 21 laboratories requesting samples for total arachidonic acid, 16 laboratories reported results for the three fish oil materials (76 %). The within-laboratory variabilities for total arachidonic acid across the three fish oil materials ranged from 4.6 % to 6.1 % (**Table 5-4**). The between-laboratory variabilities for total arachidonic acid ranged from 23.6 % to 28.8 %, indicating better community consensus for total arachidonic acid when compared to total linoleic acid.

**Table 5-4.** Target values, consensus values, and variabilities for total arachidonic acid in SRM 3275 Omega-3 and Omega-6 Fatty Acids in Fish Oil.

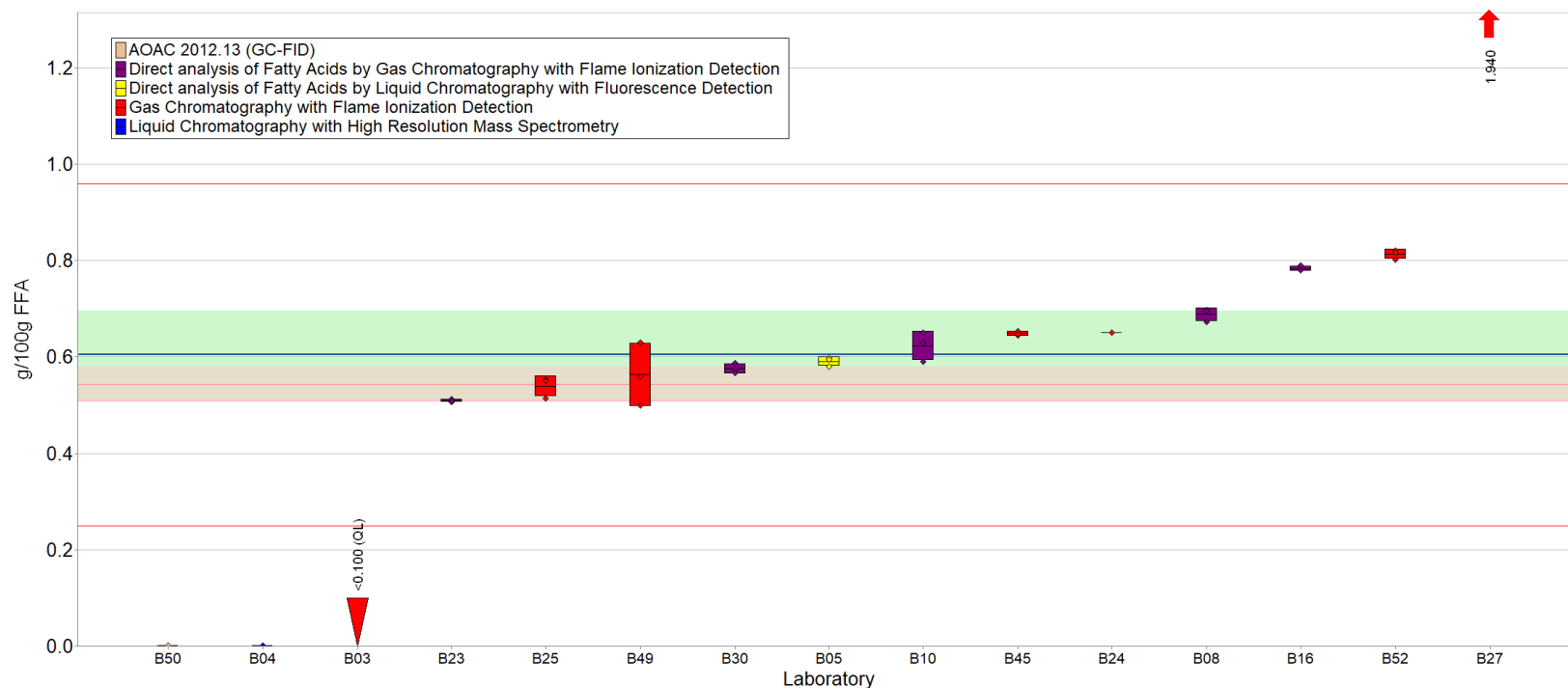
<b>Total Arachidonic Acid</b>			
	SRM 3275-1	SRM 3275-2	SRM 3275-3
Target Mass Fraction $\pm U_{\text{NIST}}$ (g/100 g)	0.544 $\pm$ 0.018	2.189 $\pm$ 0.096	-
Consensus Mean $\pm$ SD (g/100 g)	0.6 $\pm$ 0.17	2.57 $\pm$ 0.72	-
Within-Laboratory Variability (Median % RSD)	4.6 %	6.0 %	6.1 %
Between-Laboratory Variability (% RSD)	28.3 %	28.0 %	23.6 %

Overall, participants performed well when measuring total arachidonic acid in SRM 3275-1 and SRM 3275-2, as seen in **Fig. 5-4** and **Fig. 5-5**, respectively. The 95 % confidence interval for the consensus means overlap with the NIST target ranges for both fish oil materials. Since SRM 3275-3, as shown in **Fig. 5-6**, does not have a target range for total arachidonic acid, only between-laboratory comparisons are assessed.

Similar to total linoleic acid, most participants indicated derivatization to fatty acid methyl esters prior to analysis by GC-FID. Laboratories B50, B04, and B03 reported values well below the consensus range of tolerance for all three materials which is likely due to calculation and/or calibration errors. Laboratory B27 reported values for total arachidonic acid in SRM 3275-1 three times higher than the target value; however, the reported values for SRM 3275-2 and SRM 3275-3 were within the consensus range of tolerance. Participants should ensure all conversion and dilution factors are correct.

Additional tables and figures for total arachidonic acid in fish oils are located in **Appendix D**.

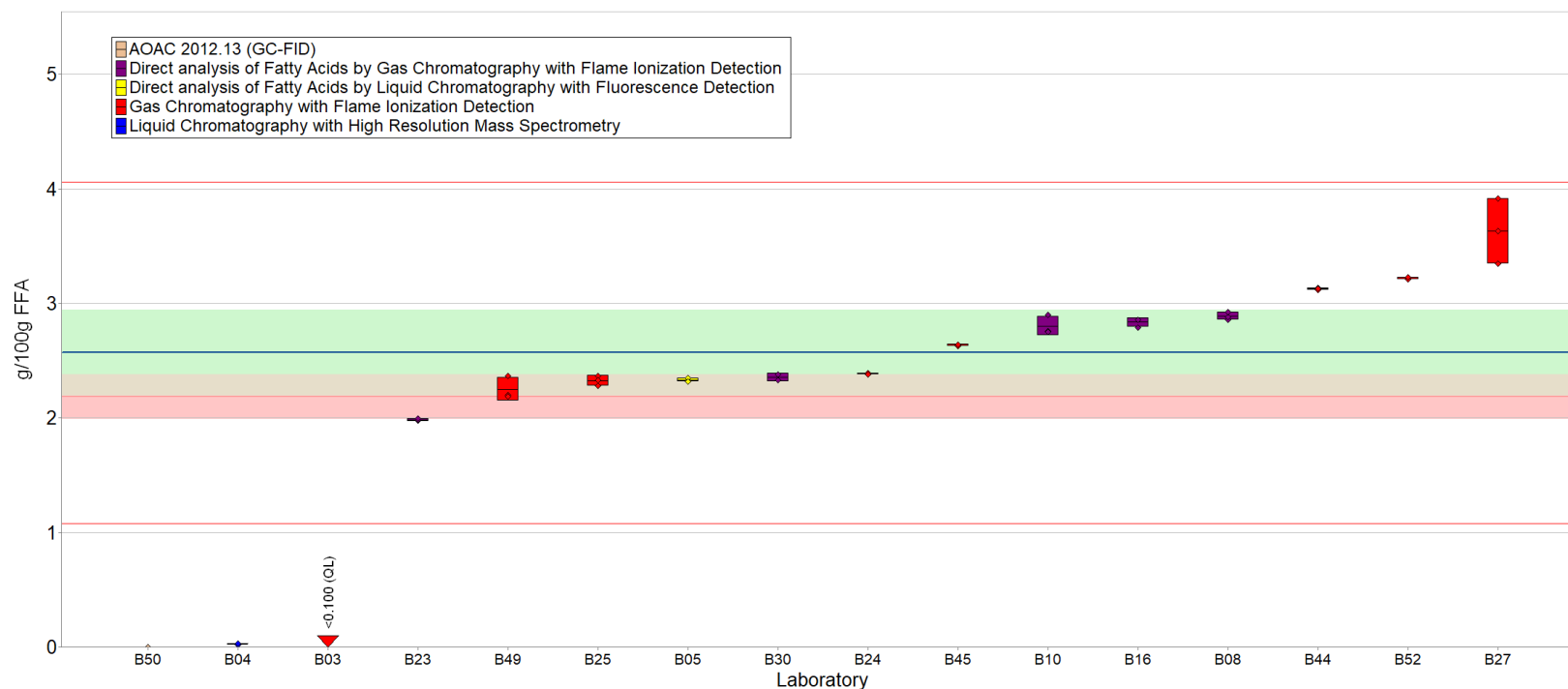
Exercise: DSQAP Exercise 2  
 Sample: SRM 3275-1 Fish Oil  
 Measurand: Total Arachidonic Acid (C20:4 n-6)



**Fig. 5-4.** Total Arachidonic Acid in SRM 3275-1 Omega-3 and Omega-6 Fatty Acids in Fish Oil (data summary view – analytical method).

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

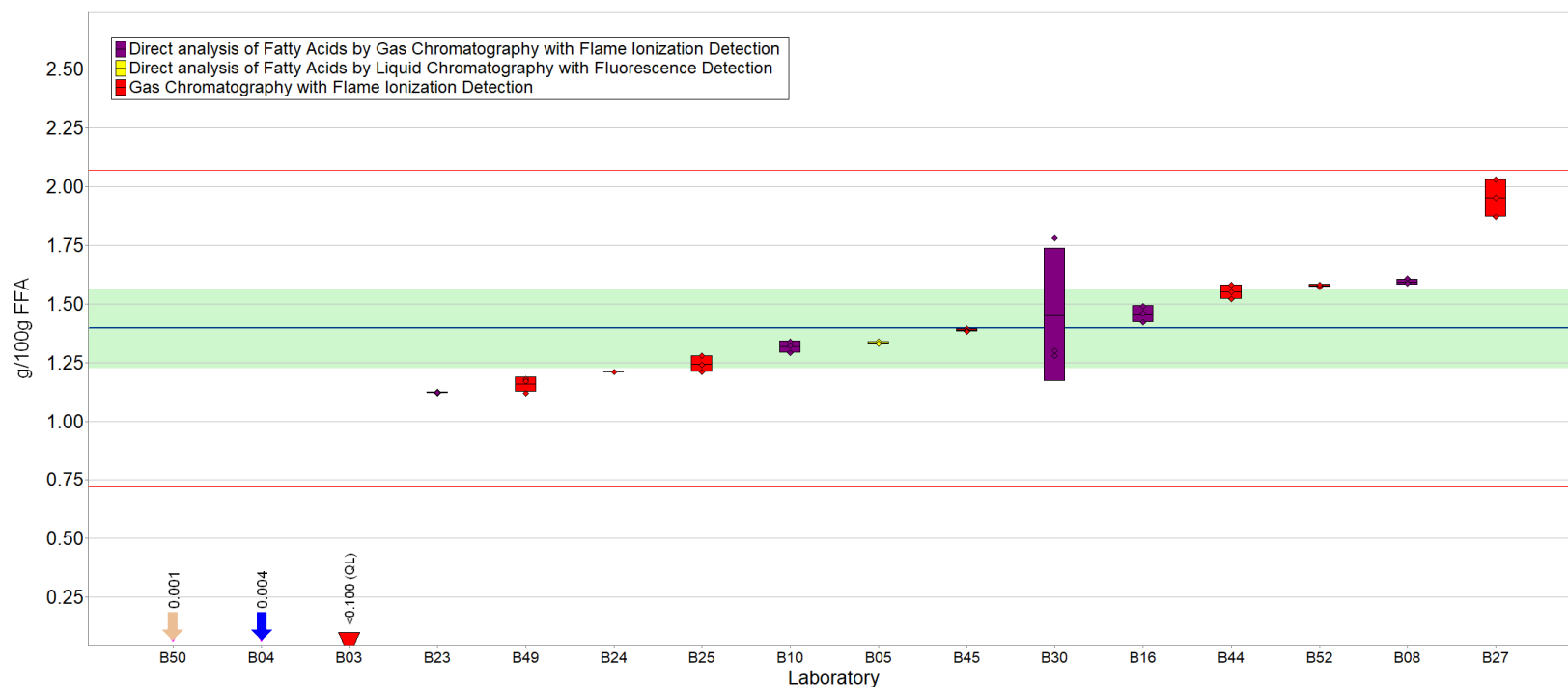
Exercise: DSQAP Exercise 2  
 Sample: SRM 3275-2 Fish Oil  
 Measurand: Total Arachidonic Acid (C20:4 n-6)



**Fig. 5-5.** Total Arachidonic Acid in SRM 3275-2 Omega-3 and Omega-6 Fatty Acids in Fish Oil (data summary view – analytical method).

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

Exercise: DSQLP Exercise 2  
 Sample: SRM 3275-3 Fish Oil  
 Measurand: Total Arachidonic Acid (C20:4 n-6)



**Fig. 5-6.** Total Arachidonic Acid in SRM 3275-3 Omega-3 and Omega-6 Fatty Acids in Fish Oil (data summary view – analytical method).

In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed (the analytical methods reported by laboratory B50 was AOAC 2012.13 (GC-FID) and laboratory B04 was LC-HRMS). The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The red solid lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that results in an acceptable  $Z'_{\text{comm}}$  score,  $|Z'_{\text{comm}}| \leq 2$ .

### 5.4.3. EPA

Of the 22 laboratories requesting samples for EPA, 18 laboratories reported results for the three fish oil materials (82 %). The within-laboratory variabilities for EPA were excellent across the three fish oil materials and ranged from 2.5 % to 4.6 % (**Table 5-5**). The between-laboratory variabilities for EPA ranged from 15.6 % to 20.9 % which indicates better community consensus for EPA when compared to total arachidonic acid and total linoleic acid.

**Table 5-5.** Target values, consensus values, and variabilities for EPA in SRM 3275 Omega-3 and Omega-6 Fatty Acids in Fish Oil.

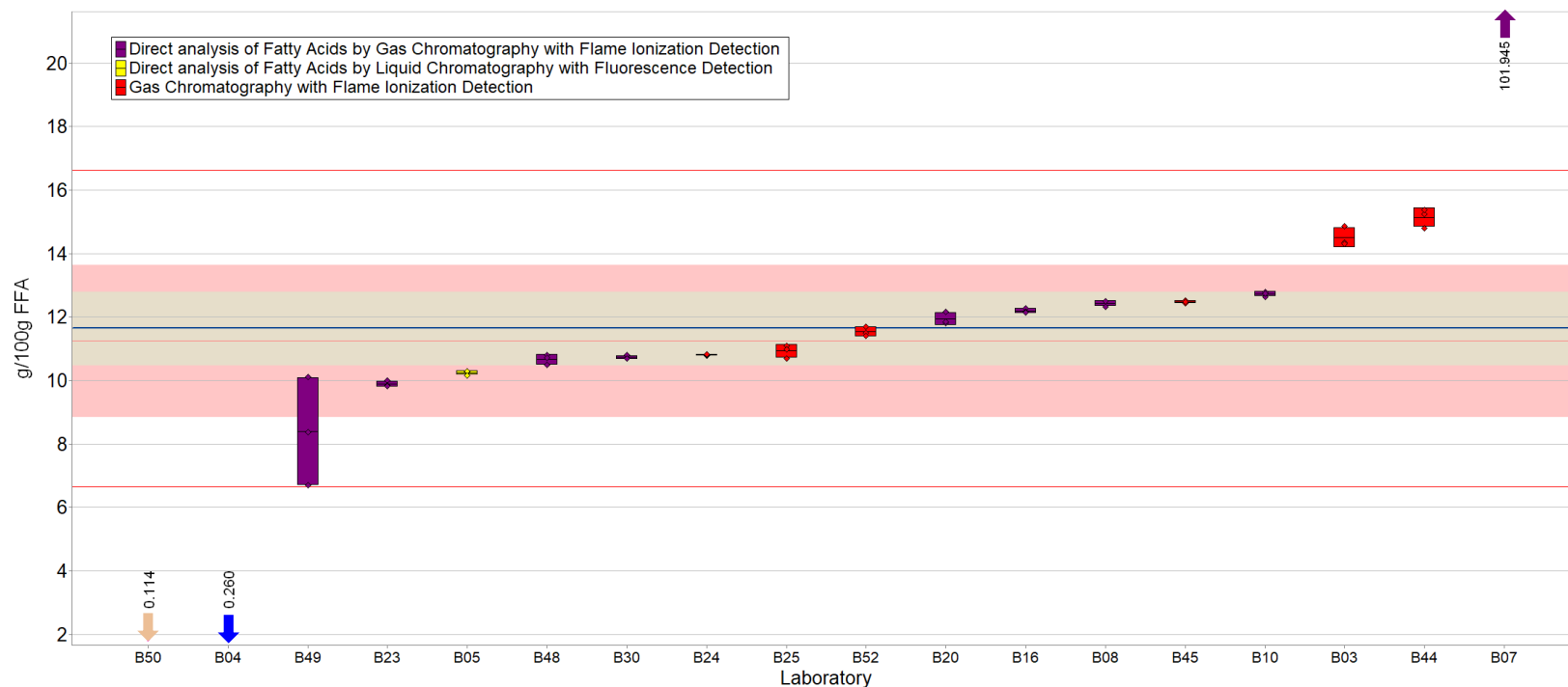
	EPA		
	SRM 3275-1	SRM 3275-2	SRM 3275-3
Target Mass Fraction $\pm U_{\text{NIST}}$ (g/100 g)	11.25 $\pm$ 1.19	39.23 $\pm$ 1.69	15.33 $\pm$ 0.89
Consensus Mean $\pm$ SD (g/100 g)	11.64 $\pm$ 2.43	41.39 $\pm$ 8.20	16.26 $\pm$ 2.53
Within-Laboratory Variability (Median % RSD)	4.6 %	2.5 %	3.3 %
Between-Laboratory Variability (% RSD)	20.9 %	19.8 %	15.6 %

Participants performed well with measuring EPA in SRM 3275-1, SRM 3275-2, and SRM 3275-3, as seen in **Fig. 5-7**, **Fig. 5-8**, and **Fig. 5-9**, respectively. The 95 % confidence interval for the consensus means overlap with the NIST target ranges for all three fish oil materials indicating participants' ability to accurately measure EPA in fish oil matrices. The within- and between-laboratory variabilities were lower for EPA when compared to other fatty acids which is likely due to the higher levels of EPA in these materials. These levels of EPA do not challenge the analytical range of methods within the community.

Laboratories B04 and B50 reported values for EPA one order of magnitude below the target values for all three materials while laboratory B07 reported values one order of magnitude above the target values. These participants should ensure all dilution factors are accounted for and that conversion calculations are correct.

Additional tables and figures for EPA in fish oils are located in **Appendix D**.

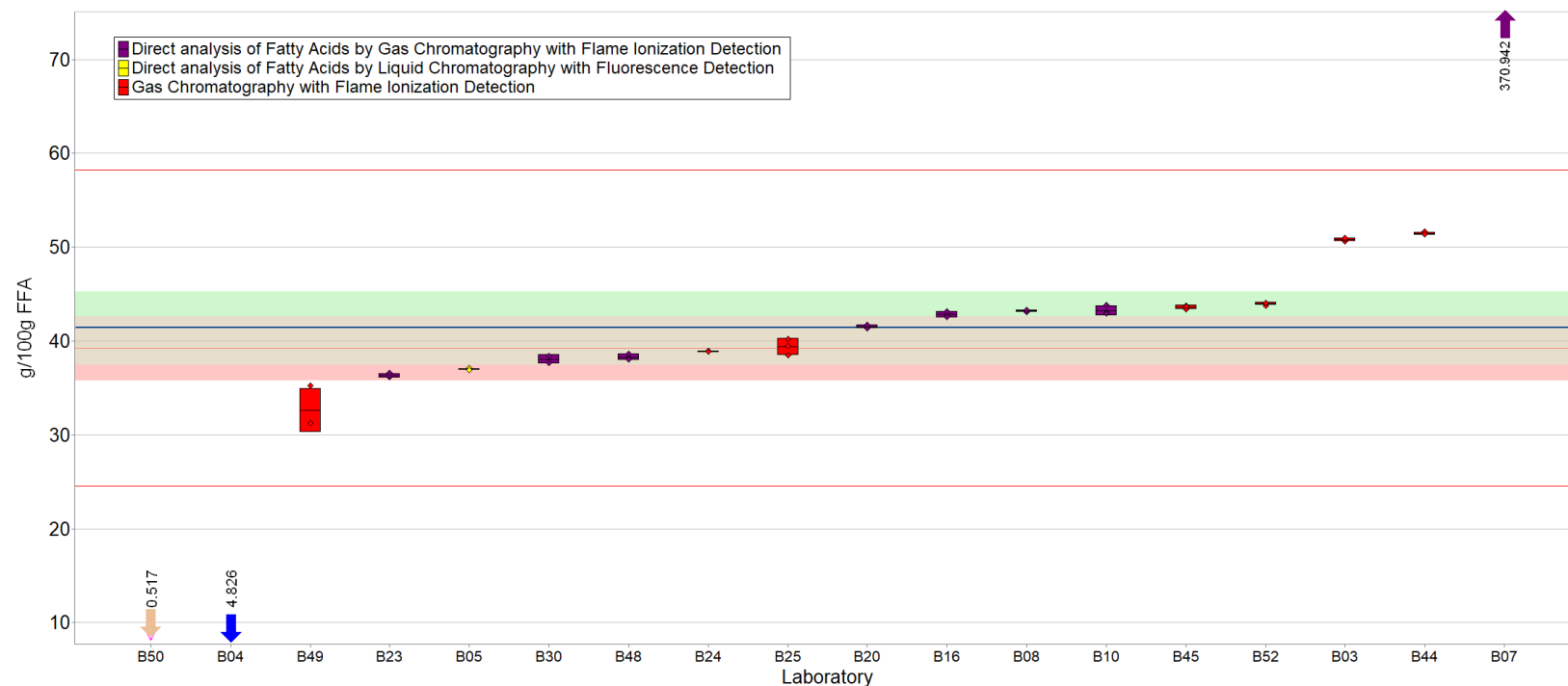
Exercise: DSQAP Exercise 2  
Sample: SRM 3275-1 Fish Oil  
Measurand: Total EPA (C20:5 n-3)



**Fig. 5-7.** Total EPA in SRM 3275-1 Omega-3 and Omega-6 Fatty Acids in Fish Oil (data summary view – analytical method).

In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed (the analytical methods reported by laboratory B50 was AOAC 2012.13 (GC-FID) and laboratory B04 was LC-HRMS). The solid blue line represents the consensus mean, and the green shaded region (beige here due to overlap with the red NIST target range) represents the 95 % confidence interval for the consensus mean. The red solid lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable  $Z'_{\text{comm}}$  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 ( $U_{\text{NIST}}$ ) and represents the range that results in an acceptable  $Z_{\text{NIST}}$  score,  $|Z_{\text{NIST}}| \leq 2$ .

Exercise: DSQAP Exercise 2  
Sample: SRM 3275-2 Fish Oil  
Measurand: Total EPA (C20:5 n-3)



**Fig. 5-8.** Total EPA in SRM 3275-2 Omega-3 and Omega-6 Fatty Acids in Fish Oil (data summary view – analytical method).

In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed (the analytical methods reported by laboratory B50 was AOAC 2012.13 (GC-FID) and laboratory B04 was LC-HRMS). The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The red solid lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable  $Z'_{\text{comm}}$  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 ( $U_{\text{NIST}}$ ) and represents the range that results in an acceptable  $Z_{\text{NIST}}$  score,  $|Z_{\text{NIST}}| \leq 2$ . The shaded beige region represents the overlapping of the 95 % confidence interval for the consensus mean (green region) and the NIST range of tolerance (red region).

Exercise: DSQAP Exercise 2  
Sample: SRM 3275-3 Fish Oil  
Measurand: Total EPA (C20:5 n-3)

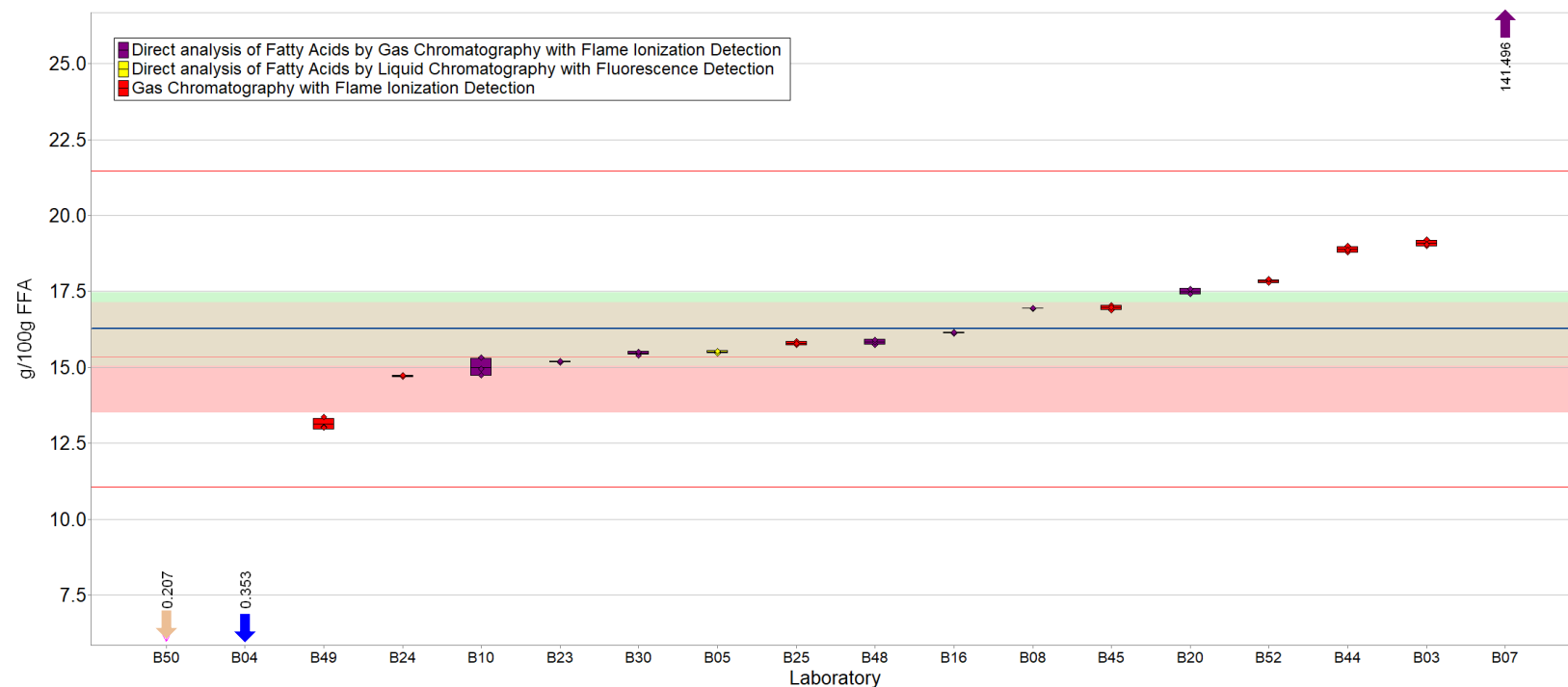


Fig. 5-9. Total EPA in SRM 3275-3 Omega-3 and Omega-6 Fatty Acids in Fish Oil (data summary view – analytical method).

In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed (the analytical methods reported by laboratory B50 was AOAC 2012.13 (GC-FID) and laboratory B04 was LC-HRMS). The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The red solid lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that results in an acceptable  $Z'_{\text{comm}}$  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 ( $U_{\text{NIST}}$ ) and represents the range that results in an acceptable  $Z_{\text{NIST}}$  score,  $|Z_{\text{NIST}}| \leq 2$ . The shaded beige region represents the overlapping of the 95 % confidence interval for the consensus mean (green region) and the NIST range of tolerance (red region).

#### 5.4.4. DPA

Of the 20 laboratories requesting samples for DPA, only 14 laboratories reported results for the three fish oil materials (70 %). The within-laboratory variabilities for DPA ranged from 2.6 % to 4.2 % (**Table 5-6**) indicating excellent method repeatability across all three fish oil materials. The between-laboratory variabilities for DPA ranged from 16.9 % to 22.5 % which is skewed high due to two laboratories reporting values outside of the consensus range of tolerance.

**Table 5-6.** Target values, consensus values, and variabilities for DPA in SRM 3275 Omega-3 and Omega-6 Fatty Acids in Fish Oil.

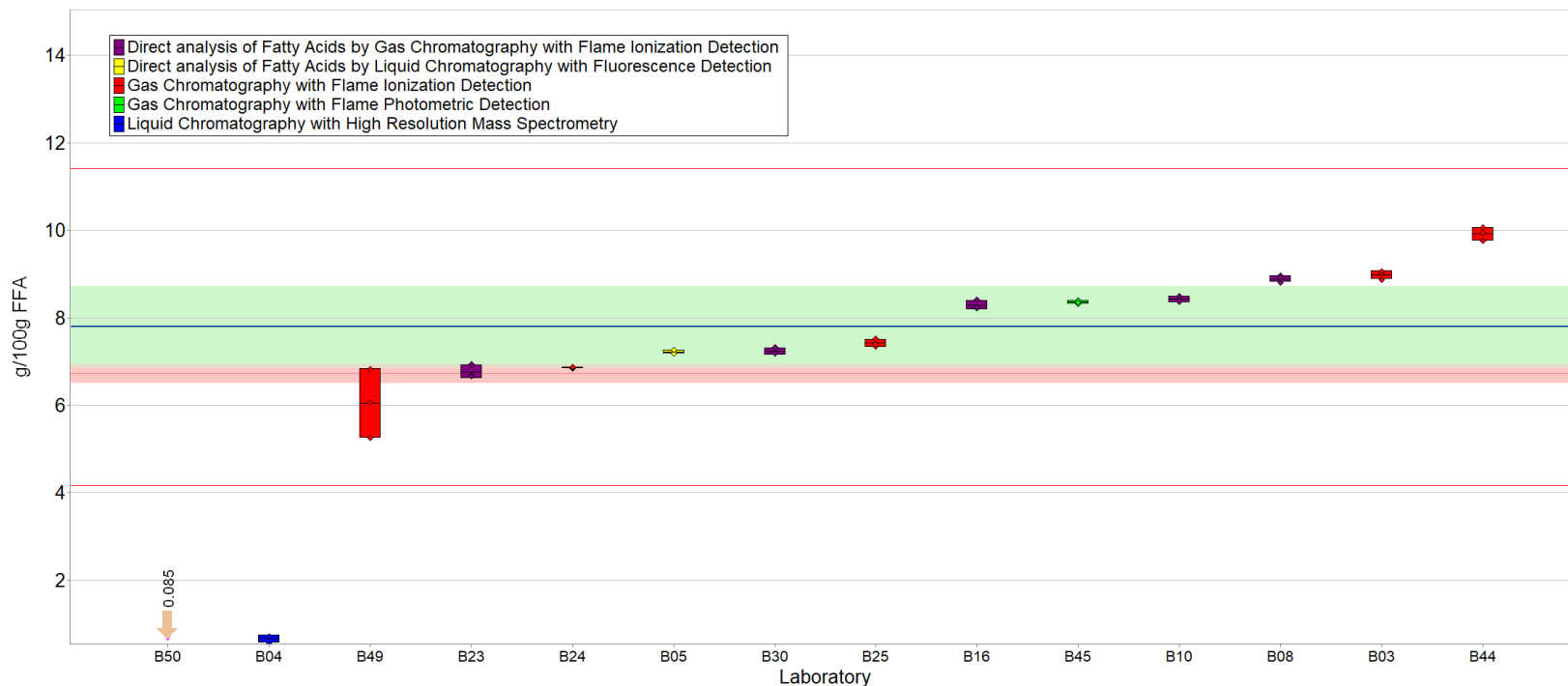
	DPA		
	SRM 3275-1	SRM 3275-2	SRM 3275-3
Target Mass Fraction $\pm U_{\text{NIST}}$ (g/100 g)	11.25 $\pm$ 1.19	39.23 $\pm$ 1.69	15.33 $\pm$ 0.89
Consensus Mean $\pm$ SD (g/100 g)	11.64 $\pm$ 2.43	41.39 $\pm$ 8.20	16.26 $\pm$ 2.53
Within-Laboratory Variability (Median % RSD)	4.2 %	4.1 %	2.6 %
Between-Laboratory Variability (% RSD)	22.5 %	17.1 %	16.9 %

Participants performed well with measuring DPA in SRM 3275-1, SRM 3275-2, and SRM 3275-3, as shown in **Fig. 5-10**, **Fig. 5-11**, and **Fig 5-12**, respectively. The 95 % confidence interval for the consensus means overlap with the NIST target ranges for all three fish oil materials indicating participants' ability to accurately measure DPA in fish oil matrices. While the 95 % confidence interval for the consensus mean for SRM 3275-1 slightly overlaps with the NIST target range, it is important to note that the NIST range of tolerance for this material is nearly half the size when compared to SRM 3275-2 at similar mass fraction levels.

Similarly to EPA, the levels of DPA in the three fish oil materials should not challenge the analytical range of methods within the community. This is highlighted by the excellent within-laboratory variabilities and overall community consensus for DPA in SRM 3275-1, SRM 3275-2, and SRM 3275-3.

Additional tables and figures for DPA in fish oils are located in **Appendix D**.

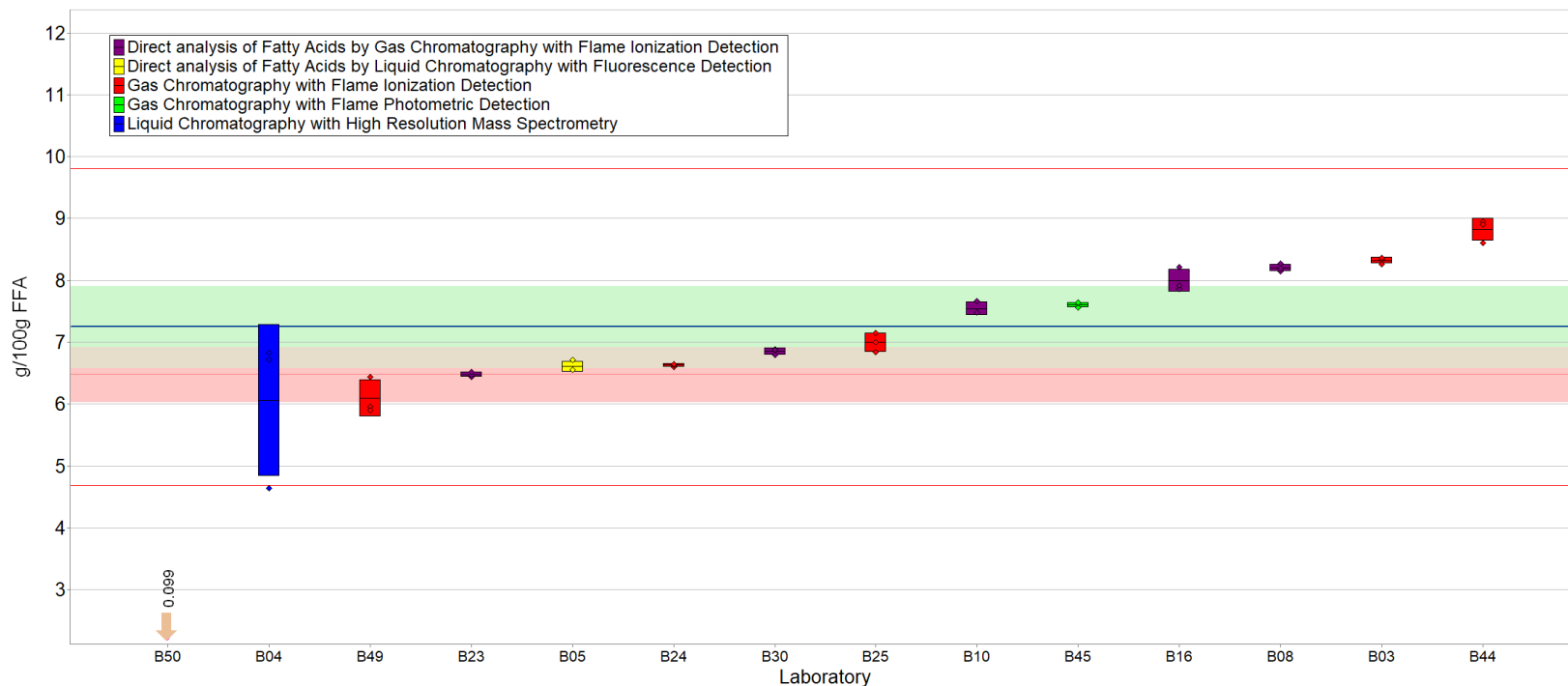
Exercise: DSQAP Exercise 2  
 Sample: SRM 3275-1 Fish Oil  
 Measurand: Total DPA (C22:5 n-3)



**Fig. 5-10.** Total DPA in SRM 3275-1 Omega-3 and Omega-6 Fatty Acids in Fish Oil (data summary view – analytical method).

In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed (the analytical method reported by laboratory B50 was AOAC 2012.13 (GC-FID)). The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The red solid lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable  $Z'_{\text{comm}}$  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 ( $U_{\text{NIST}}$ ) and represents the range that results in an acceptable  $Z_{\text{NIST}}$  score,  $|Z_{\text{NIST}}| \leq 2$ .

Exercise: DSQAP Exercise 2  
Sample: SRM 3275-2 Fish Oil  
Measurand: Total DPA (C22:5 n-3)



**Fig. 5-11.** Total DPA in SRM 3275-2 Omega-3 and Omega-6 Fatty Acids in Fish Oil (data summary view – analytical method).

In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed (the analytical method reported by laboratory B50 was AOAC 2012.13 (GC-FID)). The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The red solid lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable  $Z'_{\text{comm}}$  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 ( $U_{\text{NIST}}$ ) and represents the range that results in an acceptable  $Z_{\text{NIST}}$  score,  $|Z_{\text{NIST}}| \leq 2$ . The shaded beige region represents the overlapping of the 95 % confidence interval for the consensus mean (green region) and the NIST range of tolerance (red region).

Exercise: DSQAP Exercise 2  
Sample: SRM 3275-3 Fish Oil  
Measurand: Total DPA (C22:5 n-3)

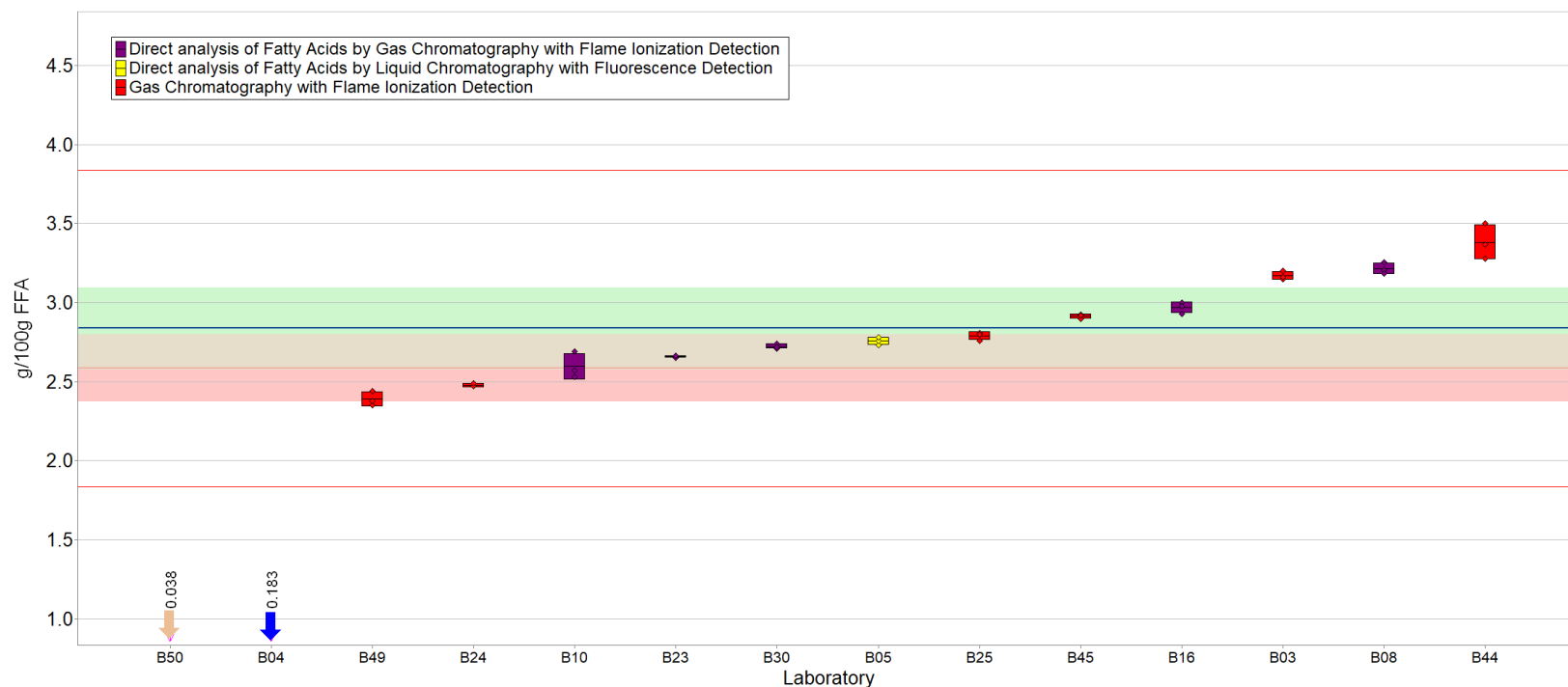


Fig. 5-12. Total DPA in SRM 3275-3 Omega-3 and Omega-6 Fatty Acids in Fish Oil (data summary view – analytical method).

In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed (the analytical methods reported by laboratory B50 was AOAC 2012.13 (GC-FID) and laboratory B04 was Liquid Chromatography with High Resolution Mass Spectrometry). The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The red solid lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that results in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \leq 2$ . 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,  $|Z_{NIST}| \leq 2$ . The shaded beige region represents the overlapping of the 95 % confidence interval for the consensus mean (green region) and the NIST range of tolerance (red region).

### 5.4.5. DHA

Of the 22 laboratories requesting samples for DHA, 17 laboratories reported results for the three fish oil materials (82 %). The within-laboratory variabilities for DHA ranged from 2.3 % to 5.0 % (**Table 5-7**) indicating excellent method repeatability across all three fish oil materials. The between-laboratory variabilities for DHA ranged from 15.3 % to 23.1 % which is skewed high due to three laboratories reporting values well outside of the consensus range of tolerance.

**Table 5-7.** Target values, consensus values, and variabilities for DHA in SRM 3275 Omega-3 and Omega-6 Fatty Acids in Fish Oil.

	DHA		
	SRM 3275-1	SRM 3275-2	SRM 3275-3
Target Mass Fraction $\pm U_{\text{NIST}}$ (g/100 g)	11.25 $\pm$ 1.19	39.23 $\pm$ 1.69	15.33 $\pm$ 0.89
Consensus Mean $\pm$ SD (g/100 g)	45.29 $\pm$ 10.44	18.78 $\pm$ 3.43	10.38 $\pm$ 1.59
Within-Laboratory Variability (Median % RSD)	5.0 %	3.6 %	2.3 %
Between-Laboratory Variability (% RSD)	23.1 %	18.3 %	15.3 %

As shown in **Fig. 5-13**, **Fig. 5-14**, and **Fig 5-15**, participants performed well measuring DHA in the three fish oil materials. The 95 % confidence interval for the consensus means overlapped with the NIST target ranges for SRM 3275-1, SRM 3275-2, and SRM 3275-3 which highlights the participants' ability to accurately measure DHA in fish oil matrices. Laboratory B07 was roughly one order of magnitude higher than the NIST target value for all three materials indicating a potential calculation error. Laboratory B50 was approximately one order of magnitude below the NIST target value for all three materials while laboratory B04 was an order of magnitude below the NIST target value for SRM 3275-1 and SRM 3275-3. Participants should ensure all calculations are correct and conversion and dilution factors are accounted for.

Additional tables and figures for DHA in fish oils are located in **Appendix D**.

Exercise: DSQAP Exercise 2  
Sample: SRM 3275-1 Fish Oil  
Measurand: Total DHA (C22:6 n-3)

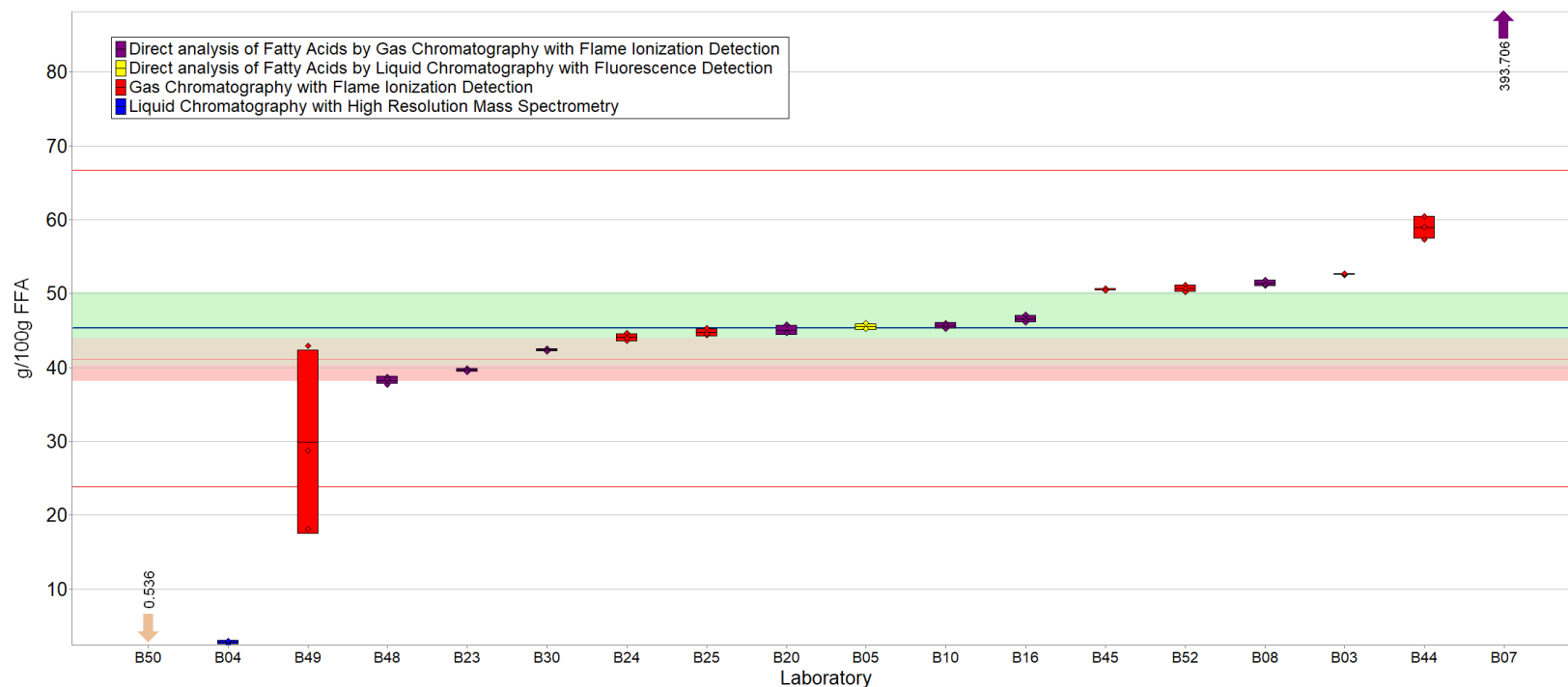


Fig. 5-13. Total DHA in SRM 3275-1 Omega-3 and Omega-6 Fatty Acids in Fish Oil (data summary view – analytical method).

In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed (the analytical method reported by laboratory B50 was AOAC 2012.13 (GC-FID)). The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The red solid lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable  $Z'_{\text{COMM}}$  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 ( $U_{\text{NIST}}$ ) and represents the range that results in an acceptable  $Z_{\text{NIST}}$  score,  $|Z_{\text{NIST}}| \leq 2$ . The shaded beige region represents the overlapping of the 95 % confidence interval for the consensus mean (green region) and the NIST range of tolerance (red region).

Exercise: DSQLP Exercise 2  
Sample: SRM 3275-2 Fish Oil  
Measurand: Total DHA (C22:6 n-3)

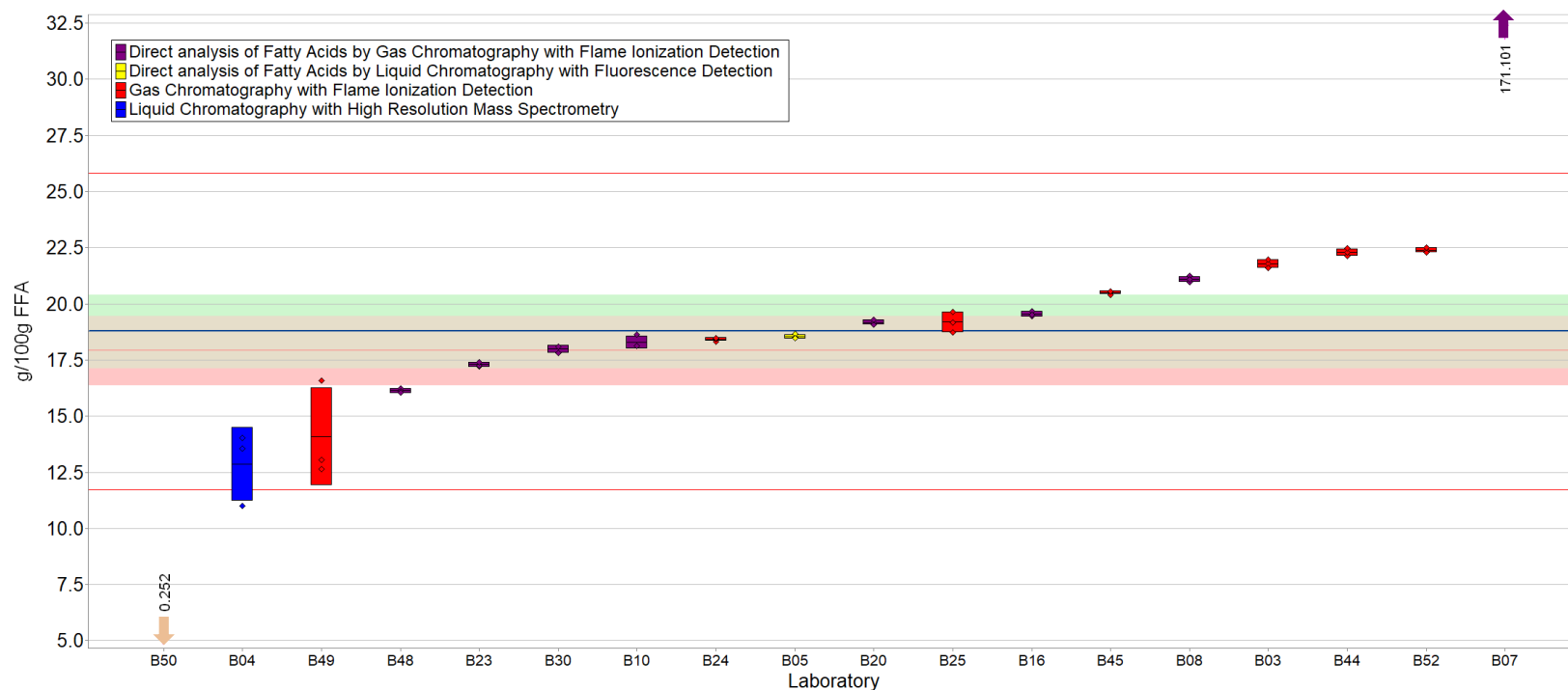


Fig. 5-14. Total DHA in SRM 3275-2 Omega-3 and Omega-6 Fatty Acids in Fish Oil (data summary view – analytical method).

In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed (the analytical methods reported by laboratory B50 was AOAC 2012.13 (GC-FID)). The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The red solid lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable  $Z'_{\text{COMM}}$  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 ( $U_{\text{NIST}}$ ) and represents the range that results in an acceptable  $Z_{\text{NIST}}$  score,  $|Z_{\text{NIST}}| \leq 2$ . The shaded beige region represents the overlapping of the 95 % confidence interval for the consensus mean (green region) and the NIST range of tolerance (red region).

Exercise: DSQAP Exercise 2  
Sample: SRM 3275-3 Fish Oil  
Measurand: Total DHA (C22:6 n-3)

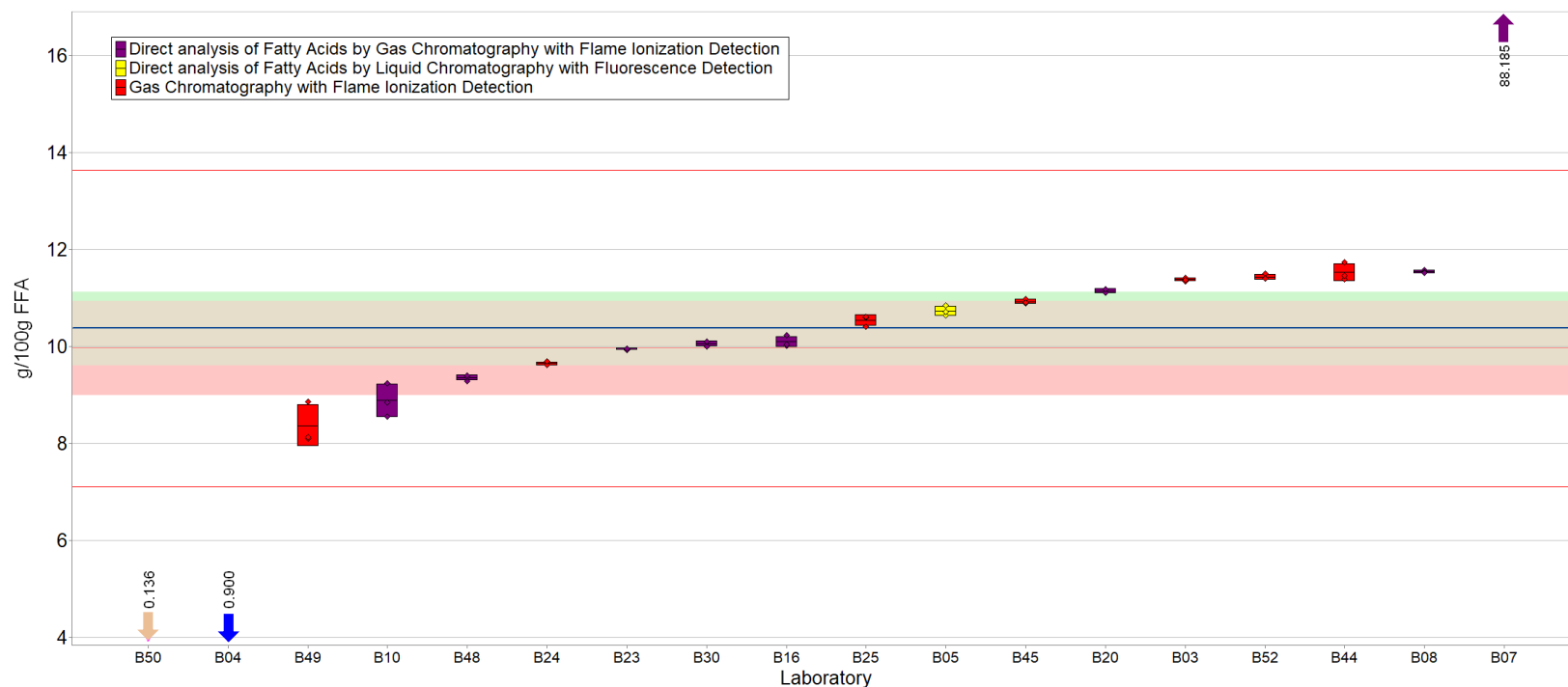


Fig. 5-15. Total DHA in SRM 3275-3 Omega-3 and Omega-6 Fatty Acids in Fish Oil (data summary view – analytical method).

In this view, individual laboratory data are plotted (diamonds) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed (the analytical methods reported by laboratory B50 was AOAC 2012.13 (GC-FID) and laboratory B04 was LC-HRMS). The solid blue line represents the consensus mean, and the green shaded region represents the 95 % confidence interval for the consensus mean. The red solid lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that results in an acceptable  $Z'_{\text{comm}}$  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 ( $U_{\text{NIST}}$ ) and represents the range that results in an acceptable  $Z_{\text{NIST}}$  score,  $|Z_{\text{NIST}}| \leq 2$ . The shaded beige region represents the overlapping of the 95 % confidence interval for the consensus mean (green region) and the NIST range of tolerance (red region).

## 6. Botanicals

### 6.1. Summary

Triterpene glycosides are a group of chemical constituents in Black cohosh (*Actaea racemosa* L.) that may contribute to its health effects and can be used as marker compounds for dietary supplement product standardization. Identification and quantitation of these phytochemicals in black cohosh materials is therefore important for dietary supplement product quality control, accurate labeling, and the overall health and safety of consumers. Participation in this study was low with one laboratory returning data. Therefore, technical recommendations are limited.

### 6.2. Background

Black cohosh is a perennial plant native to North America and has a long history of medicinal uses including the treatment of menopausal symptoms [23]. Both roots and rhizomes are sold as dietary supplements in many forms, but the chemical composition of the various supplement preparations differ considerably [24]. Part of this is due to the lack of clear evidence on which compounds in black cohosh may be responsible for the relief of menopausal symptoms. Substances in black cohosh that may account for its activity include triterpene glycosides and these marker compounds are frequently standardized to provide at least 1 mg per daily dose in dietary supplement products [24]. Standardization of analytical measurement techniques and the development of reference materials are important for the detection of these compounds as dietary supplement products continue to use black cohosh as an active ingredient. In this study participants were asked to use their in-house analytical method to detect the presence of six select triterpenes in black cohosh samples: 23-epi-26-deoxyactein, 23-epi-26-deoxycimicifugoside, actein, cimigenol 3- $\beta$ -D-xyloside, cimiracemoside C, and cimiracemoside D.

### 6.3. Study Information

Participants were provided with samples of Black Cohosh Rhizome (three packets each containing approximately 3.3 g, *A. racemosa* rhizome) and Black Cohosh Extract (three packets each containing approximately 1 g, *A. racemosa* rhizome extract). Participants were asked to store the samples at controlled room temperature, 20 °C to 25 °C, in the original unopened packets until analysis. Before use, participants were instructed to thoroughly mix the contents of the packet and to allow contents to settle for one minute prior to opening to minimize the loss of fine particles prior to removal of a test portion for analysis. For the Black Cohosh Rhizome and Black Cohosh Extract, participants were asked to use a sample size of at least 0.5 g and 0.25 g, respectively, and to prepare one sample and report one value from each packet. The approximate analyte levels were not reported to participants prior to the study.

#### 6.4. Study Results and Technical Recommendations

The enrollment for the botanicals study was low, with only five laboratories requesting samples. One laboratory returned results for all analytes and reported using dilution for sample preparation and selected “other” for analytical method. The results returned from this laboratory are presented in **Table 6-1** along with preliminary data measured at NIST using LC with mass spectrometry detection (LC-MS).

**Table 6-1.** Results for triterpenes in Black Cohosh Rhizome and Black Cohosh Extract.

Analyte	Target Values ± SD (mg/g)		Laboratory B10 Mass Fraction ± SD (mg/g)	
	Black Cohosh Rhizome	Black Cohosh Extract	Black Cohosh Rhizome	Black Cohosh Extract
23-epi-26-deoxyactein	1.67 ± 0.06	8.75 ± 0.29	1.97 ± 0.04	10.02 ± 0.07
23-epi-26-deoxycimicifugoside	0.21 ± 0.02	1.24 ± 0.11	< 0.1	< 0.1
Actein			2.43 ± 0.11	10.94 ± 0.27
Cimigenol 3-β-D-xyloside	0.15 ± 0.02	7.85 ± 0.28	1.18 ± 0.02	11.50 ± 0.33
Cimiracemoside C	0.23 ± 0.02	8.70 ± 0.35	1.26 ± 0.003	12.07 ± 0.21
Cimiracemoside D	0.22 ± 0.03	1.27 ± 0.09	1.26 ± 0.02	2.26 ± 0.09

Since only one laboratory provided results, no specific technical recommendations can be made at this time. Laboratories measuring phytochemicals in black cohosh materials should use appropriate extraction techniques and ensure complete extraction of target analytes.

## References

- [1] ISO 13528:2022 Statistical methods for use in proficiency testing by interlaboratory comparisons.
- [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. <https://doi.org/10.6028/NIST.SP.260-136-2021>.
- [3] U.S. Food and Drug Administration (2024) Closer to Zero: Reducing Childhood Exposure to Contaminants from Foods. <https://www.fda.gov/food/environmental-contaminants-food/closer-zero-reducing-childhood-exposure-contaminants-foods>. Accessed on: 13 January 2025.
- [4] Official Methods of Analysis of AOAC INTERNATIONAL 22nd Ed. (2020) AOAC SMPR 2020.001 Standard Method Performance Requirements (SMPRs) for Determination of Heavy Metals in a Variety of Cannabis and Cannabis-Derived Products. [https://www.aocac.org/wp-content/uploads/2020/07/SMPR-2020\\_001.pdf](https://www.aocac.org/wp-content/uploads/2020/07/SMPR-2020_001.pdf). Accessed on: 13 January 2025.
- [5] Julshamn K, Maage A, Norli HS, Grobecker KH, Jorhem L, Fecher P, Dowell D (2013) Determination of Arsenic, Cadmium, Mercury, and Lead in Foods by Pressure Digestion and Inductively Coupled Plasma/Mass Spectrometry: First Action 2013.06. Journal of AOAC INTERNATIONAL 96(5):1101–1102. <https://doi.org/10.5740/jaoacint.13-143>.
- [6] Briscoe M (2015) Determination of Heavy Metals in Food by Inductively Coupled Plasma–Mass Spectrometry: First Action 2015.01. Journal of AOAC INTERNATIONAL 98(4):1113–1120. <https://doi.org/10.5740/jaoac.int.2015.01>.
- [7] National Institute of Standards and Technology (2021) Certificate of Analysis RM 8666 Ginger (*Zingiber officinale*) Extract. <https://tsapps.nist.gov/srmext/certificates/8666.pdf>. Accessed on: 13 January 2025.
- [8] Official Methods of Analysis of AOAC INTERNATIONAL (2024) AOAC SMPR 2024.002 Standard Method Performance Requirements (SMPRs) for Determination of Trace Elemental Contaminants in Food and Beverages. [https://www.aocac.org/wp-content/uploads/2024/05/SMPR-2024\\_002.pdf](https://www.aocac.org/wp-content/uploads/2024/05/SMPR-2024_002.pdf). Accessed on: 13 January 2025.
- [9] 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/>. Accessed on: 13 January 2025.
- [10] National Institute of Standards and Technology (2020) Certificate of Analysis SRM 3251 Saw Palmetto (*Serenoa repens*) Extract. <https://tsapps.nist.gov/srmext/certificates/3251.pdf>. Accessed on: 13 January 2025.

- [11] National Institute of Standards and Technology (2022) Certificate of Analysis SRM 3289 Multivitamin Tablets. <https://tsapps.nist.gov/srmext/certificates/3289.pdf>. Accessed on: 13 January 2025.
- [12] Hayes, H., Mulloor, J., Nelson, M., Rimmer, K., Yu, L., and Yen, J. (2022), Certification of Standard Reference Material 3289 Multivitamin Tablets, Special Publication (NIST SP), National Institute of Standards and Technology. <https://doi.org/10.6028/NIST.SP.260-220>.
- [13] National Institute of Standards and Technology (2024) Dietary Supplements Laboratory Quality Assurance Program (DSQAP). <https://www.nist.gov/programs-projects/dietary-supplement-laboratory-quality-assurance-program-dsqap>. Accessed on: 13 January 2025.
- [14] Phillips MM, Rimmer CA, Wood LJ (2017) Dietary supplement laboratory quality assurance program: exercise G final report. <https://doi.org/10.6028/NIST.IR.8163>.
- [15] Sharpless KE, Arce-Osuna M, Thomas JB, Gill LM (1999) Value Assignment of Retinol, Retinyl Palmitate, Tocopherol, and Carotenoid Concentrations in Standard Reference Material 2383 (Baby Food Composite). *Journal of AOAC INTERNATIONAL* 82(2):288–296. <https://doi.org/10.1093/jaoac/82.2.288>.
- [16] Official Methods of Analysis of AOAC INTERNATIONAL (2017) AOAC SMPR 2017.006 Standard Method Performance Requirements (SMPRs) for Determination of  $\beta$ -Carotene in Infant and Adult/Pediatric Nutritional Formula. [https://www.aoac.org/wp-content/uploads/2020/11/SMPR202017\\_006.pdf](https://www.aoac.org/wp-content/uploads/2020/11/SMPR202017_006.pdf). Accessed on: 13 January 2025.
- [17] National Institutes of Health Office of Dietary Supplements (2023) Omega-3 Fatty Acids Fact Sheet for Health Professionals. <https://ods.od.nih.gov/factsheets/Omega3FattyAcids%20-HealthProfessional/>. Accessed on: 13 January 2025.
- [18] Harris WS (2010) Omega-3 Fatty Acids. *Encyclopedia of Dietary Supplements*, eds Coates PM, Betz JM, Blackman MR (Informa Healthcare, London and New York), 2nd Edition, pp 577–586.
- [19] National Institute of Standards and Technology (2024) Certificate of Analysis SRM 3275 Omega-3 and Omega-6 Fatty Acids in Fish Oil. <https://tsapps.nist.gov/srmext/certificates/3275.pdf>. Accessed on: 13 January 2025.
- [20] Golay P-A, Moulin J, Alewijn M, Braun U, Choo LF, Crujisen H, Delmonte P, Fontecha J, Holroyd S, Hostetler G, Lacoste F, Lehmann C, Nagelholt L, Phillips S, Ritvanen T, Rizzo A, Shimelis O, Srigley C, Sullivan D, Trossat P (2016) Determination of Labeled Fatty Acids Content in Milk Products, Infant Formula, and Adult/Pediatric Nutritional Formula by Capillary Gas Chromatography: Collaborative Study, Final Action 2012.13. *Journal of AOAC INTERNATIONAL* 99(1):210–222. <https://doi.org/10.5740/jaoacint.15-0140>.
- [21] Phillips MM, Rimmer CA, Wood LJ, Bedner M, Chieh KD, Paul RL (2014) Dietary Supplement Laboratory Quality Assurance Program : Exercise J Final Report. <https://doi.org/10.6028/NIST.IR.7997>.
- [22] Wood LJ, Phillips MM, Rimmer CA (2016) Dietary Supplement Laboratory Quality Assurance Program: Exercise L Final Report. <https://doi.org/10.6028/NIST.IR.8154>.

- [23] Official Methods of Analysis of AOAC INTERNATIONAL 22nd Ed. (2012) AOAC SMPR 2012.011 Standard Method Performance Requirements (SMPRs) for Fatty Acids, Including LCPUFAs, in Infant Formula and Adult/Pediatric Nutritional Formula. [https://www.aoac.org/wp-content/uploads/2020/11/SMPR202012\\_011.pdf](https://www.aoac.org/wp-content/uploads/2020/11/SMPR202012_011.pdf). Accessed on: 13 January 2025.
- [24] Borrelli F, Ernst E (2008) Black cohosh (*Cimicifuga racemosa*) for menopausal symptoms: A systematic review of its efficacy. *Pharmacological Research* 58(1):8–14. <https://doi.org/10.1016/j.phrs.2008.05.008>.
- [25] National Institutes of Health Office of Dietary Supplements (2020) Black Cohosh Fact Sheet for Health Professionals. <https://ods.od.nih.gov/factsheets/BlackCohosh-HealthProfessional/>. Accessed on: 13 January 2025.

## Appendix A. List of Acronyms

AAS	Atomic Absorption Spectroscopy
ARA	Arachidonic Acid
AMRM	Analytical Methods and Reference Materials
CannaQAP	Cannabis Laboratory Quality Assurance Program
cGMP	Current Good Manufacturing Practice
COA	Certificate of Analysis
DC AAS	Direct Combustion Atomic Absorption Spectrometry
DHA	Docosahexaenoic Acid
DPA	Docosapentaenoic Acid
DSQAP	Dietary Supplement Laboratory Quality Assurance Program
EPA	Eicosapentaenoic Acid
FAME	Fatty Acid Methyl Esters
FDA	US Food and Drug Administration
FFA	Free Fatty Acids
FNSQAP	Food Nutrition and Safety Measurements Quality Assurance Program
GC-FID	Gas Chromatography with Flame Ionization Detector
GC-FPD	Gas Chromatography with Flame Photometric Detector
HAMQAP	Health Assessment Measurements Quality Assurance Program
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
ICP-MS/MS	Inductively Coupled Plasma Tandem Mass Spectrometry
ICP-OES	Inductively Coupled Plasma Optical Emission Spectrometry
ID-CV-ICP-MS	Isotope Dilution Cold-Vapor Generation Inductively Coupled Plasma Mass Spectrometry
IEC	International Electrotechnical Commission
INAA	Instrumental Neutron Activation Analysis
ISO	International Organization for Standardization
LA	Linoleic Acid
LC-Abs	Liquid Chromatography with Absorbance Detection
LC-FLD	Liquid Chromatography with Fluorescence Detection
LC-MS	Liquid Chromatography Mass Spectrometry
LC-HRMS	Liquid Chromatography with High Resolution Mass Spectrometry
LOQ	Limit of Quantification
MMQAP	Micronutrients Measurement Quality Assurance Program
NIST	National Institute of Standards and Technology
NIH	National Institutes of Health
ODS	Office of Dietary Supplements
PDA	Photodiode Array Detection
PT	Proficiency Tests
QAP	Quality Assurance Program
QL	Quantification Limit
RM	Reference Material
RSD	Relative Standard Deviation

SD	Standard Deviation
SMPR	Standard Method Performance Requirements
SRM	Standard Reference Material
VitDQAP	Vitamin D Metabolites Quality Assurance Program

## Appendix B. Toxic Elements Supplemental Tables and Figures

**Table B-1.** Individualized data summary table (example) for toxic elements in botanical extracts.

### (Laboratory Name)

#### Exercise 2 - Toxic Elements in Botanical Extracts

Lab Code: (code)			1. Your Results				2. Community Results			3. Target	
Analyte	Sample	Units	$X_i$	$S_i$	$Z'_{comm}$	$Z_{NIST}$	N	$x^*$	$s^*$	$X_{NIST}$	$U_{NIST}$
Total Arsenic	RM 8666 Ginger Extract	ng/g	<i>Individual laboratory results will appear in this section; Laboratory-specific results were provided to each participant separately from this report</i>				40	40.3	6.7	40.8	5.0
Total Arsenic	Eleuthero Extract	ng/g					41	498	66	495.7	8.1
Cadmium	RM 8666 Ginger Extract	ng/g					40	6.8	1.5	6.7	1.6
Cadmium	Eleuthero Extract	ng/g					41	63.9	7.9	63.7	6.2
Lead	RM 8666 Ginger Extract	ng/g					40	89	15	93.1	6.5
Lead	Eleuthero Extract	ng/g					41	843	94	876.0	8.1
Mercury	RM 8666 Ginger Extract	ng/g					37	7.4	2.6	7.7	2.4
Mercury	Eleuthero Extract	ng/g					37	330	140	324	79
			$X_i$	Mean of reported values	N	Number of quantitative values reported	$X_{NIST}$	NIST value			
			$S_i$	Standard deviation of reported values	$x^*$	Robust mean of reported values	$U_{NIST}$	expanded uncertainty about the NIST value			
			$Z'_{comm}$	Z'-score with respect to community consensus	$s^*$	Robust standard deviation					
			$Z_{NIST}$	Z-score with respect to NIST value							

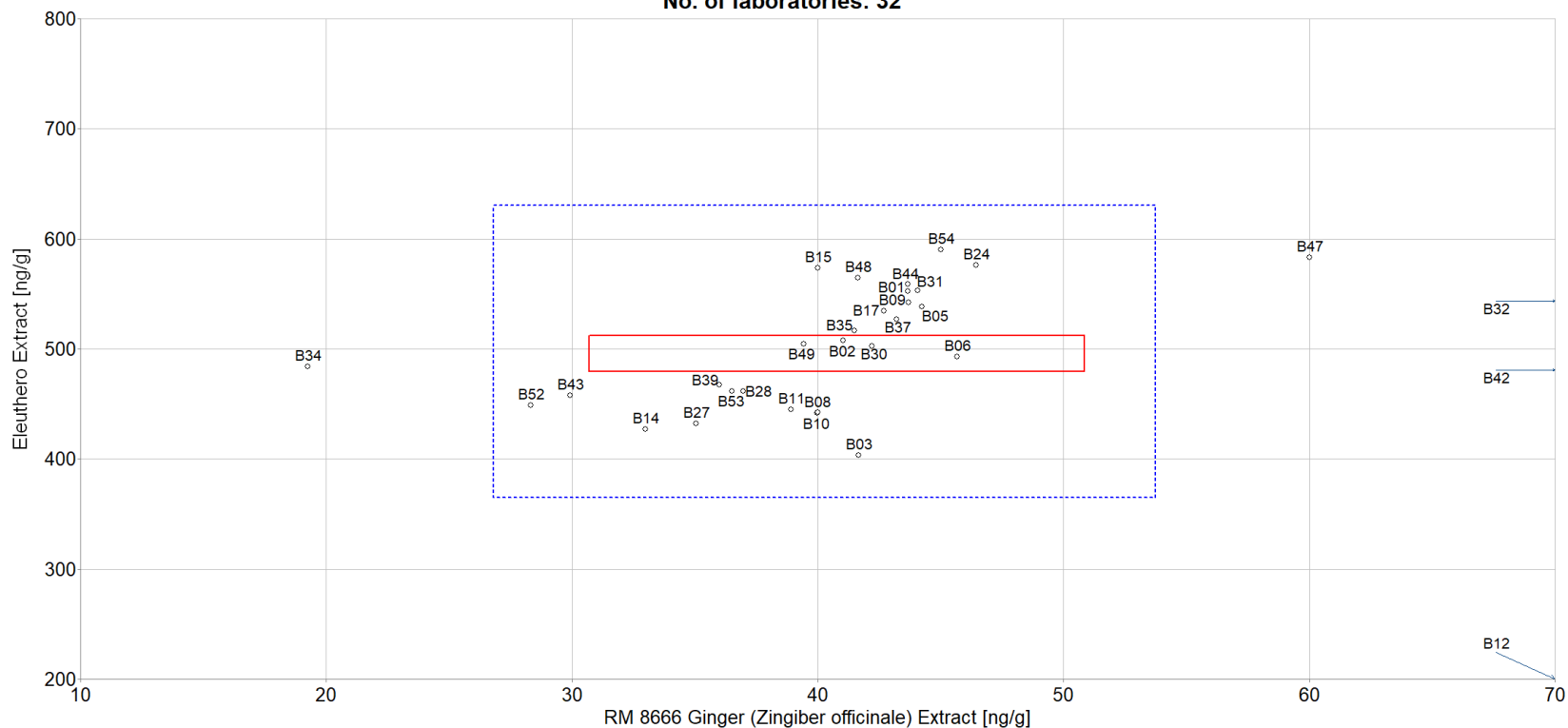
**Table B-2.** Data summary table for total arsenic in botanical extracts.

Data highlighted in blue have been identified as outside the consensus tolerance limits and would be estimated to result in an unacceptable  $Z'_{comm}$  score,  $|Z'_{comm}| > 2$ . (Table continues to next page.)

		Total Arsenic (tAs)									
		RM 8666 Ginger ( <i>Zingiber officinale</i> ) Extract (ng/g)					Eleuthero Extract (ng/g)				
Lab	Target	A	B	C	Mean	SD	A	B	C	Mean	SD
	B01	41	45	45	43.7	2.3	556	546	556	553	5.8
	B02	41.666	40.929	40.516	41.0	0.6	509.64	508.16	504.74	508	2.5
	B03	42	42	41	41.7	0.6	360	428	422	403	38
	B05	41.8	43	47.9	44.2	3.2	515	547	553	538	20
	B06	46	42	49	45.7	3.5	490	490	499	493	5.2
	B07	< 125	< 125	< 125			547.4	526.77	520.06	531	14
	B08	42.68	34.17	43.06	40.0	5.0	442.53	432.02	450.46	442	9.3
	B09	44.645	42.5924	43.8394	43.7	1.0	538.945	549.427	538.206	542	6.3
	B10	43	39	38	40.0	2.6	442	445	441	443	2.1
	B11	37.1	44.8	34.8	38.9	5.2	456	447	432	445	12
	B12	435.98	435.22	426.6	432.6	5.2	19.22	17.8	17.68	18	0.86
	B13	< 50	< 50	< 50			457.158	451.065	452.873	454	3.1
	B14	34	32	33	33.0	1.0	421	422	438	427	9.5
	B15	40	40	40	40.0	0	570	580	570	573	5.8
	B16	< 200	< 200	< 200			< 200	< 200	< 200		
	B17	41.7	44.2	42.2	42.7	1.3	548	539	517	535	16
	B19	< 25000	< 25000	< 25000			< 25000	< 25000	< 25000		
	B21						0.6055	0.621	0.598	0.61	0.012
	B23										
	B24	45.38	47.01	46.94	46.4	0.92	576.58	576.08	576.01	576	0.31
	B26	< 100	< 100	< 100			480	490	480	483	5.8
	B27	33.142	35.652	36.317	35.0	1.7	427.486	443.363	426.683	433	9.4
	B28	37.4	38	35.5	37.0	1.3	442	472	470	461	17
	B29										
	B30	42.6	46.9	37.1	42.2	4.9	516	492	500	503	12

		Total Arsenic (tAs)										
		RM 8666 Ginger ( <i>Zingiber officinale</i> ) Extract (ng/g)					Eleuthero Extract (ng/g)					
Lab		A	B	C	Mean	SD	A	B	C	Mean	SD	
B31		43.9	42.6	45.7	44.1	1.6	547.9	554.4	557.5	553	4.9	
B32		78.074	81.26	60.947	73.4	11	556.87	525.071	548.524	543	16	
B33		< 14.055	< 14.055	< 14.055			421.91	429.764	432	428	5.3	
B34		15.1	15.9	26.7	19.2	6.5	496.6	495	460.6	484	20	
B35		39.27853	41.19715	43.98863	41.5	2.4	514.0099	514.0228	522.5032	517	4.9	
B36												
B37		42.8	46	40.8	43.2	2.6	531	535	514	527	11	
B38												
B39		34.489	36.358	37.13	36.0	1.4	463.735	479.682	458.376	467	11	
B40												
B41												
B42		106	59.19	< 50	82.6	33.1	508.7	416.2	517.3	481	56	
B43		29.5	30	30.3	29.9	0.40	462.9	440.9	468.8	458	15	
B44		44	45	42	43.7	1.5	560	565	551	559	7.1	
B46		< 50	< 50	< 50			500	520	486	502	17	
B47		60	60	60	60.0	0	600	610	540	583	38	
B48		42.3	42.1	40.5	41.6	1.0	561.4	567.5	564.8	565	3.1	
B49		39.3	41.6	37.4	39.4	2.1	492	510	512	505	11	
B51		< 40	< 40	< 40			360	380	390	377	15	
B52		28	30	27	28.3	1.5	446	455	446	449	5.2	
B53		36.8	35.4	37.3	36.5	1.0	465.7	467	451.3	461	8.7	
B54		47	44	44	45.0	1.7	588	594	588	590	3.5	
Community Results		Consensus Mean				40.3		Consensus Mean				498
		Consensus Standard Deviation				6.7		Consensus Standard Deviation				66
		Maximum				432.6		Maximum				590
		Minimum				19.2		Minimum				0.61
		N				32		N				39

**Exercise: DSQAP Exercise 2, Measurand: Total Arsenic (tAs)**  
**No. of laboratories: 32**



**Fig. B-1.** Laboratory means for total arsenic in RM 8666 Ginger (*Zingiber officinale*) Extract and Eleuthero Extract (sample/sample comparison view).

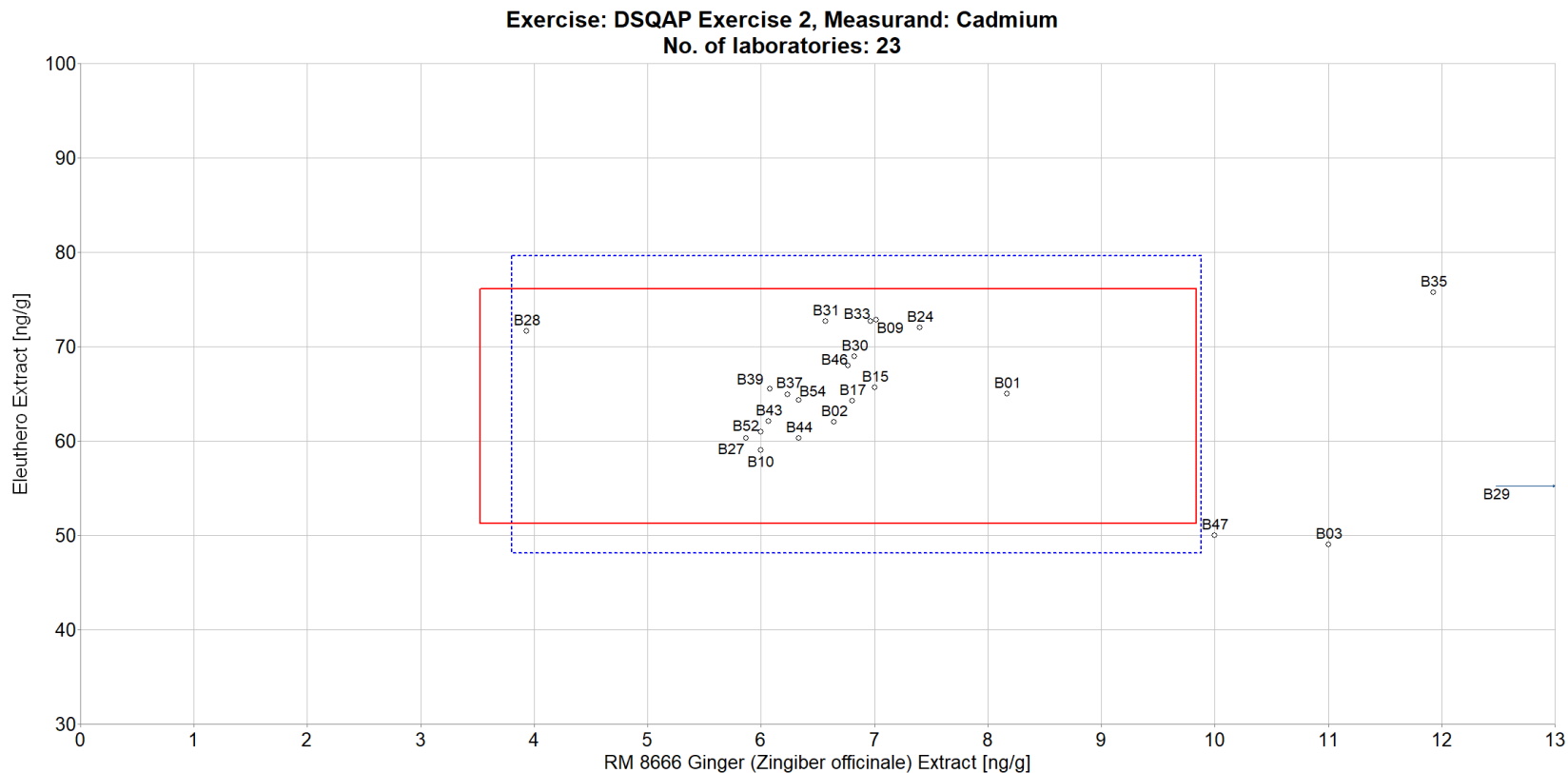
In this view, the individual laboratory mean for one sample, RM 8666, is compared to the individual laboratory mean for a second sample, Eleuthero Extract. The solid red box represents the NIST range of tolerance for the two samples, RM 8666 (x-axis) and Eleuthero Extract (y-axis), which encompasses the target values bounded by their uncertainties ( $U_{NIST}$ ) and represents the range that results in an acceptable  $Z_{NIST}$  score,  $|Z_{NIST}| \leq 2$ . The dotted blue box represents the consensus range of tolerance for RM 8666 (x-axis) and Eleuthero Extract (y-axis), calculated as the values above and below the consensus means that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \leq 2$ .

**Table B-3.** Data summary table for cadmium in botanical extracts.

Data highlighted in blue have been identified as outside the consensus range of tolerance and would be estimated to result in an unacceptable  $Z'_{\text{comm}}$  score,  $|Z'_{\text{comm}}| > 2$ . (Table continues to next page.)

		Cadmium									
		RM 8666 Ginger ( <i>Zingiber officinale</i> ) Extract (ng/g)					Eleuthero Extract (ng/g)				
		Lab	A	B	C	Mean	SD	A	B	C	Mean
<b>Individual Results</b>	Target				6.7	1.6				63.7	6.2
	B01	7.8	7.9	8.8	8.2	0.55	64.7	67.3	63.1	65.0	2.1
	B02	6.8095	6.608	6.512	6.6	0.15	63.439	63.433	59.119	62.0	2.5
	B03	12	10	11	11.0	1.0	42	46	59	49.0	8.9
	B05	< 10	< 10	< 10			60.9	61	69.3	63.7	4.8
	B06	< 10	< 10	< 10			62	62	65	63.0	1.7
	B07	< 31	< 31	< 31			66.85	65.82	66.75	66.5	0.57
	B08	< 10	< 10	< 10			68.62	60.13	53.72	60.8	7.5
	B09	6.727	6.906	7.405	7.0	0.35	73.953	73.08	71.524	72.9	1.2
	B10	6	6	6	6.0	0	61	60	56	59.0	2.6
	B11	< 8.67	< 8.67	< 8.67			61.9	63.3	53.8	59.7	5.1
	B12	46.93	48.35	46.6	47.3	0.93	< 10	< 10	< 10		
	B13	< 50	< 50	< 50			53.911	62.72	58.766	58.5	4.4
	B14	< 10	< 10	< 10			59	57	58	58.0	1.0
	B15	7	7	7	7.0	0	65	67	65	65.7	1.2
	B16	< 200	< 200	< 200			< 200	< 200	< 200		
	B17	6.39	7.17	6.86	6.8	0.39	65.1	66	61.6	64.2	2.3
	B19	< 25000	< 25000	< 25000			< 25000	< 25000	< 25000		
	B21						0.069	0.0695	0.069	0.1	0.00029
	B23										
B24	6.95	7.3	7.95	7.4	0.51	70.22	72.62	73.3	72.0	1.6	
B26	< 250	< 250	< 250			< 260	< 260	< 260			
B27	5.238	6.358	6.014	5.9	0.57	62.652	59.62	58.599	60.3	2.1	
B28	4.04	3.57	4.19	3.9	0.32	71.3	71.9	71.8	71.7	0.32	
B29	33.56	10.83	9.46	18.0	14	75.17	22.56	68.05	55.3	29	
B30	7.22	7.11	6.14	6.8	0.59	67.4	70.7	68.7	68.9	1.7	

		Cadmium										
		RM 8666 Ginger ( <i>Zingiber officinale</i> ) Extract (ng/g)					Eleuthero Extract (ng/g)					
Lab		A	B	C	Mean	SD	A	B	C	Mean	SD	
B31		6.5	6.1	7.1	6.6	0.50	72.3	72.8	72.9	72.7	0.32	
B32												
B33		5.38	7.42	8.105	7.0	1.4	71.729	77.595	68.736	72.7	4.5	
B34		< 1.6	< 1.6	< 1.6			56.9	56.1	55.2	56.1	0.85	
B35		8.81372	10.275031	16.683399	11.9	4.2	78.164772	77.083412	72.03271	75.8	3.3	
B36												
B37		6.7	6.5	5.5	6.2	0.64	65.2	64.5	65	64.9	0.36	
B38												
B39		5.905	6.201	6.128	6.1	0.15	63.295	66.716	66.626	65.5	1.9	
B40												
B41												
B42		< 60	< 60	< 60			98.84	79.28	108.6	95.6	15	
B43		6.3	6	5.9	6.1	0.21	63.9	59.1	63.3	62.1	2.6	
B44		6	7	6	6.3	0.58	61	60	60	60.3	0.58	
B46		8.5	6.2	5.6	6.8	1.5	64	73	67	68.0	4.6	
B47		10	10	10	10.0	0	50	50	50	50.0	0	
B48		< 11.1	< 11.1	< 11.1			69.8	69.5	67.2	68.8	1.4	
B49		< 20	< 20	< 20			63.9	61.1	62.2	62.4	1.4	
B51		< 40	< 40	< 40			50	60	60	56.7	5.8	
B52		7	5	6	6.0	1.0	63	63	57	61.0	3.5	
B53		< 12	< 12	< 12			70.2	71.6	68.5	70.1	1.6	
B54		6	6	7	6.3	0.58	64	64	65	64.3	0.58	
Community Results		Consensus Mean				6.8		Consensus Mean				63.9
		Consensus Standard Deviation				1.5		Consensus Standard Deviation				7.9
		Maximum				47.3		Maximum				95.6
		Minimum				3.9		Minimum				0.069
		N				24		N				37



**Fig. B-2.** Laboratory means for cadmium in RM 8666 Ginger (*Zingiber officinale*) Extract and Eleuthero Extract (sample/sample comparison view).

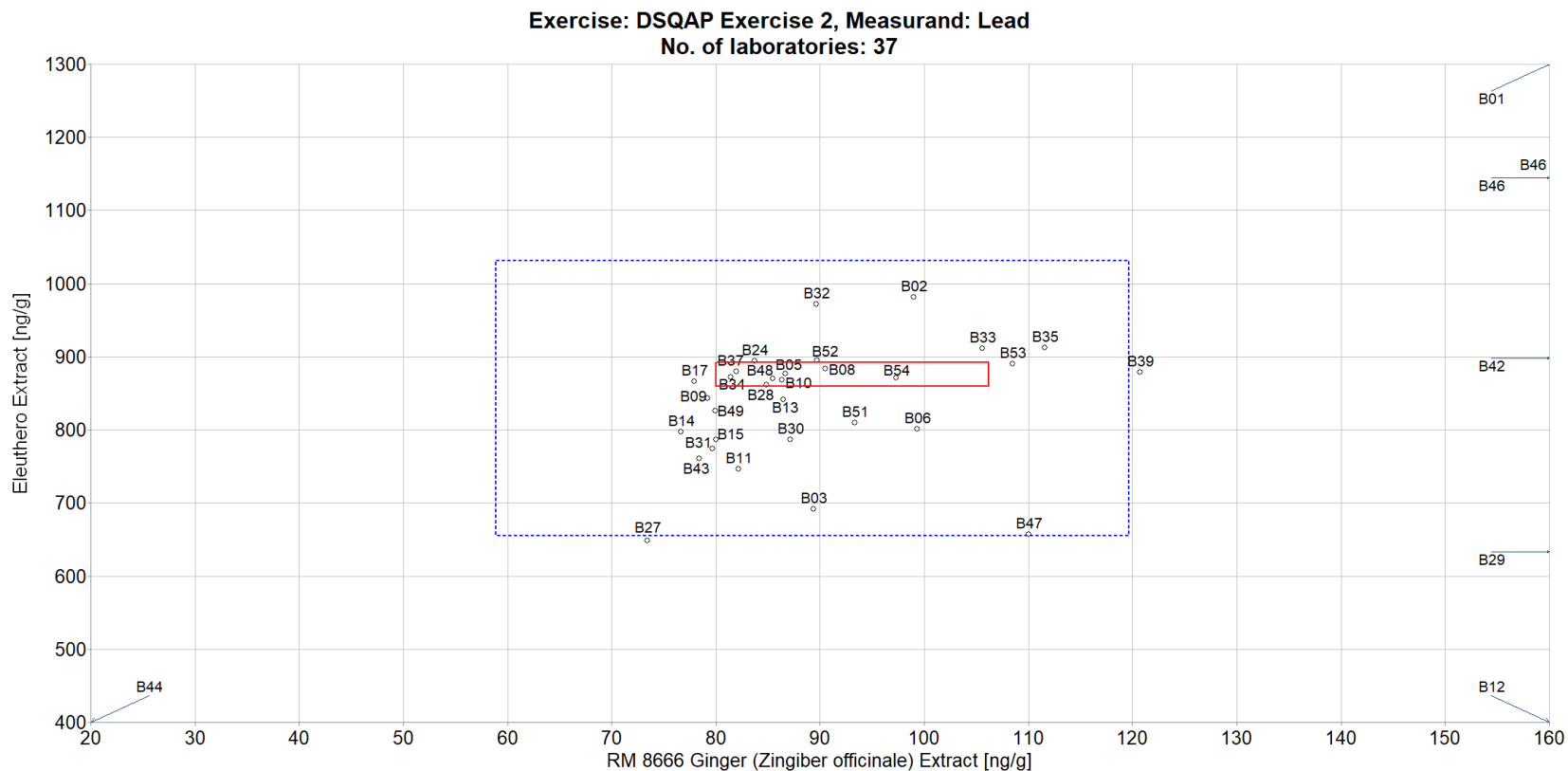
In this view, the individual laboratory mean for one sample, RM 8666, is compared to the individual laboratory mean for a second sample, Eleuthero Extract. The solid red box represents the NIST range of tolerance for the two samples, RM 8666 (x-axis) and Eleuthero Extract (y-axis), which encompasses the target values bounded by their uncertainties ( $U_{NIST}$ ) and represents the range that results in an acceptable  $Z_{NIST}$  score,  $|Z_{NIST}| \leq 2$ . The dotted blue box represents the consensus range of tolerance for RM 8666 (x-axis) and Eleuthero Extract (y-axis), calculated as the values above and below the consensus means that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \leq 2$ .

**Table B-4.** Data summary table for lead in botanical extracts.

Data highlighted in blue have been identified as outside the consensus tolerance limits and would be estimated to result in an unacceptable  $Z'_{comm}$  score,  $|Z'_{comm}| > 2$ . (Table continues to next page.)

		Lead									
		RM 8666 Ginger ( <i>Zingiber officinale</i> ) Extract (ng/g)					Eleuthero Extract (ng/g)				
Lab		A	B	C	Mean	SD	A	B	C	Mean	SD
Individual Results	Target				93	6.5				876	8.1
	B01	592	758	834	728	124	1787	1326	1440	1518	240
	B02	100.839	96.513	99.691	99	2.2	975.93	980.18	988.71	982	6.5
	B03	92	86	90	89	3.1	630	657	788	692	85
	B05	81.4	85.4	93.3	87	6.1	858	885	888	877	17
	B06	94	99	105	99	5.5	794	810	799	801	8.2
	B07	< 125	< 125	< 125			916.88	865.64	870.57	884	28
	B08	96.31	93.83	81.29	90	8.1	840.3	897.2	913.12	884	38
	B09	86.715	80.775	70.018	79	8.5	829.277	853.094	847.148	843	12
	B10	78	87	94	86	8.0	854	874	877	868	13
	B11	83.7	82.8	79.9	82	2.0	767	770	701	746	39
	B12	777.85	776.05	768.95	774	4.7	88.49	75.88	79.5	81	6.5
	B13	82.495	94.435	82.525	86	6.9	844.888	837.532	842.621	842	3.8
	B14	76	77	77	77	0.58	800	793	798	797	3.6
	B15	70	80	90	80	10	790	790	780	787	5.8
	B16	< 100	< 100	< 100			< 100	< 100	< 100		
	B17	74.1	83.7	76	78	5.1	882	883	834	866	28
	B19	< 25000	< 25000	< 25000			< 25000	< 25000	< 25000		
	B21						0.85	0.8535	0.8605	0.85	0.0053
	B23										
	B24	87.31	80.58	83.24	84	3.4	886.68	892.66	902.37	894	7.9
B27	78.395	78.731	63.025	73	9.0	655.813	659.898	629.854	649	16	
B28	83.3	86.6	84.7	85	1.7	861	865	859	862	3.1	
B29	230.84	321.03	164.05	239	79	837.69	245.42	814.93	633	336	
B30	78.3	91.6	91.6	87	7.7	791	779	789	786	6.4	
B31	82.9	77.7	78.4	80	2.8	773.8	774.3	773.8	774	0.29	

		Lead											
		RM 8666 Ginger ( <i>Zingiber officinale</i> ) Extract (ng/g)					Eleuthero Extract (ng/g)						
Lab		A	B	C	Mean	SD	A	B	C	Mean	SD		
B32		95.202	93.267	80.346	90	8.1	984.883	930.542	999.503	972	36		
B33		97.761	111.993	106.997	106	7.2	907.668	914.426	911.409	911	3.4		
B34		83.2	83	78.1	81	2.9	869	886.3	862.3	873	12		
B35		100.673	104.624	129.49	112	16	916.255	905.757	916.424	913	6.1		
B36													
B37		83.8	81.6	80.5	82	1.7	885	888	866	880	12		
B38													
B39		84.682	175.327	102.075	121	48	865.256	894.592	876.023	879	15		
B40													
B41													
B42		378.3	129.7	134.2	214	142	863.4	826.7	1003	898	93		
B43		82.8	75.8	76.5	78	3.9	761.3	750.2	771.2	761	11		
B44		19	18	20	19	1.0	191	195	196	194	2.6		
B46		178	122	181	160	33	1301	1092	1042	1145	137		
B47		90	110	130	110	20	690	610	670	657	42		
B48		83.4	82.8	90.1	85	4.1	869.4	873.7	868.4	871	2.8		
B49		78.4	81.7	79.8	80	1.7	825	824	828	826	2.1		
B51		90	90	100	93	5.8	770	810	850	810	40		
B52		85	91	93	90	4.2	889	921	875	895	24		
B53		113.7	106.1	105.6	108	4.5	916.7	891.8	862.9	890	27		
B54		84	97	111	97	14	866	869	877	871	5.7		
Community Results		Consensus Mean				89		Consensus Mean				843	
		Consensus Standard Deviation				15		Consensus Standard Deviation				94	
		Maximum				774		Maximum				1518	
		Minimum				19		Minimum				0.85	
		N				37		N				39	



**Fig. B-3.** Laboratory means for lead in RM 8666 Ginger (*Zingiber officinale*) Extract and Eleuthero Extract (sample/sample comparison view).

In this view, the individual laboratory mean for one sample, RM 8666, is compared to the individual laboratory mean for a second sample, Eleuthero Extract. The solid red box represents the NIST range of tolerance for the two samples, RM 8666 (x-axis) and Eleuthero Extract (y-axis), which encompasses the target values bounded by their uncertainties ( $U_{NIST}$ ) and represents the range that results in an acceptable  $Z_{NIST}$  score,  $|Z_{NIST}| \leq 2$ . The dotted blue box represents the consensus range of tolerance for RM 8666 (x-axis) and Eleuthero Extract (y-axis), calculated as the values above and below the consensus means that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \leq 2$ .

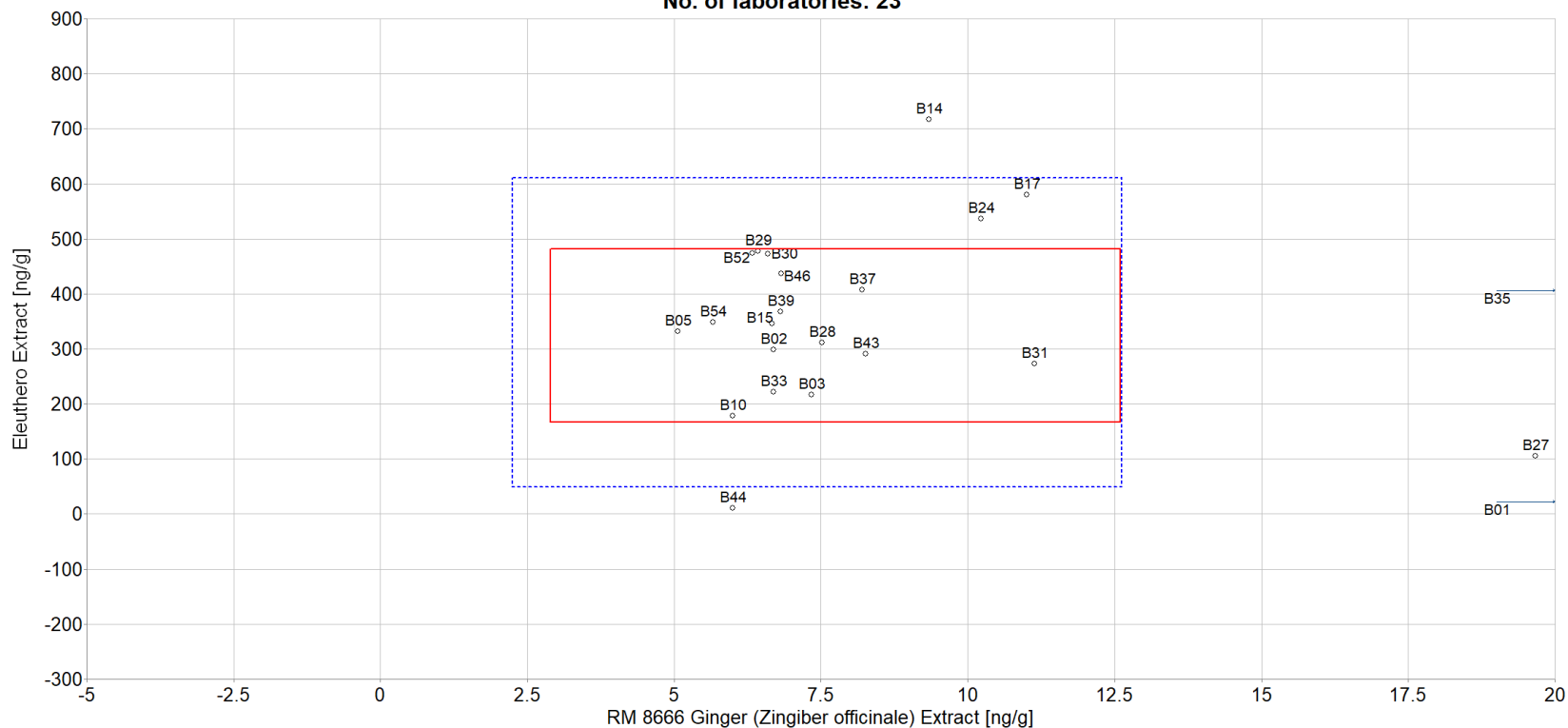
**Table B-5.** Data summary table for mercury in botanical extracts.

Data highlighted in blue have been identified as outside the consensus tolerance limits and would be estimated to result in an unacceptable  $Z'_{comm}$  score,  $|Z'_{comm}| > 2$ . (Table continues to next page.)

		Mercury									
		RM 8666 Ginger ( <i>Zingiber officinale</i> ) Extract (ng/g)					Eleuthero Extract (ng/g)				
Lab		A	B	C	Mean	SD	A	B	C	Mean	SD
Individual Results	Target				7.7	2.4				324	79
	B01	39.7	30.5	26.6	32.3	6.7	24.2	21.6	19.8	22	2.2
	B02	7.2085	6.6995	6.1705	6.7	0.52	296.09	296.771	301.84	298	3.1
	B03	11	7	4	7.3	3.5	216	227	206	216	11
	B05	5.1	5.2	4.9	5.1	0.15	348	313	336	332	18
	B06										
	B07	< 25	< 25	< 25			689.93	635.57	336.91	554	190
	B08	< 10	< 10	< 10			316.1	299.86	268.55	295	24
	B09	< 10	< 10	< 10			176.746	167.854	180.042	175	6.3
	B10	8	5	5	6.0	1.7	165	163	208	179	25
	B11	< 8.67	< 8.67	< 8.67			264	248	251	254	8.5
	B12										
	B13	< 10	< 10	< 10			388.402	306.828	313.396	336	45
	B14	11	6	11	9.3	2.9	687	817	646	717	89
	B15	7	8	5	6.7	1.5	371	278	391	347	60
	B16	< 100	< 100	< 100			< 100	< 100	< 100		
	B17	< 10	< 10	11	11.0		481	703	555	580	113
	B21						0.44325	0.6555	0.3965	0.50	0.14
	B23										
	B24	15.46	8.34	6.89	10.2	4.6	887.02	334.59	387	536	305
B26	< 10	< 10	< 10			< 500	< 500	< 500			
B27	20.582	19.968	18.432	19.7	1.1	105	101	112	106	5.6	
B28	7.07	7.26	8.22	7.5	0.62	376	303	256	312	60	
B29	9.1	6.39	3.78	6.4	2.7	631.87	204.2	599.08	478	238	
B30	6.6	< 5	< 5	6.6		526	522	372	473	88	

		Mercury											
		RM 8666 Ginger ( <i>Zingiber officinale</i> ) Extract (ng/g)					Eleuthero Extract (ng/g)						
Lab		A	B	C	Mean	SD	A	B	C	Mean	SD		
B31		17.4	7.1	8.9	11.1	5.5	238.9	273.7	308.3	274	35		
B33		10.601	5.644	3.821	6.7	3.5	258.143	216.585	189.554	221	35		
B34		< 3.9	< 3.9	< 3.9			594.5	510.5	329.9	478	135		
B35		38.2191	21.0463	16.5144	25.3	11	473.432	312.53	433.905	407	84		
B36													
B37		7.7	5.9	11	8.2	2.6	470	322	430	407	77		
B38													
B39		7.193	6.235	7.018	6.8	0.51	343.105	401.687	360.184	368	30		
B40													
B41													
B42		< 50	< 50	< 50			331.4	314.3	294.4	313	19		
B43		8	7.9	8.9	8.3	0.55	314	298.2	262.7	292	26		
B44		7	6	5	6.0	1.0	13	10	10	11	1.7		
B46		6.97	6.88	6.63	6.8	0.18	367.8	361.9	582.6	437	126		
B47		< 10	< 10	< 10			230	230	220	227	5.8		
B48		< 20.400	< 20.400	< 20.400			297.6	333.3	333.7	322	21		
B49		< 10	< 10	< 10									
B51		< 40	< 40	< 40			270	310	390	323	61		
B52		7	5	7	6.3	1.2	700	439	283	474	211		
B53		< 20	< 20	< 20			359.4	294.6	306.6	320	34		
B54		5	5	7	5.7	1.2	346	316	383	348	34		
Community Results		Consensus Mean				7.4		Consensus Mean				330	
		Consensus Standard Deviation				2.6		Consensus Standard Deviation				140	
		Maximum				32.3		Maximum				717	
		Minimum				5.1		Minimum				0.50	
		N				21		N				35	

**Exercise: DSQAP Exercise 2, Measurand: Mercury**  
**No. of laboratories: 23**



**Fig. B-4.** Laboratory means for mercury in RM 8666 Ginger (*Zingiber officinale*) Extract and Eleuthero Extract (sample/sample comparison view).

In this view, the individual laboratory mean for one sample, RM 8666, is compared to the individual laboratory mean for a second sample, Eleuthero Extract. The solid red box represents the NIST range of tolerance for the two samples, RM 8666 (x-axis) and Eleuthero Extract (y-axis), which encompasses the target values bounded by their uncertainties ( $U_{NIST}$ ) and represents the range that results in an acceptable  $Z_{NIST}$  score,  $|Z_{NIST}| \leq 2$ . The dotted blue box represents the consensus range of tolerance for RM 8666 (x-axis) and Eleuthero Extract (y-axis), calculated as the values above and below the consensus means that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \leq 2$ .

## Appendix C. Fat-Soluble Vitamins Supplemental Tables and Figures

**Table C-1.** Individualized data summary table (example) for fat-soluble vitamins in supplements.

### (Laboratory Name)

#### Exercise 2 - Fat-Soluble Vitamins in Supplements

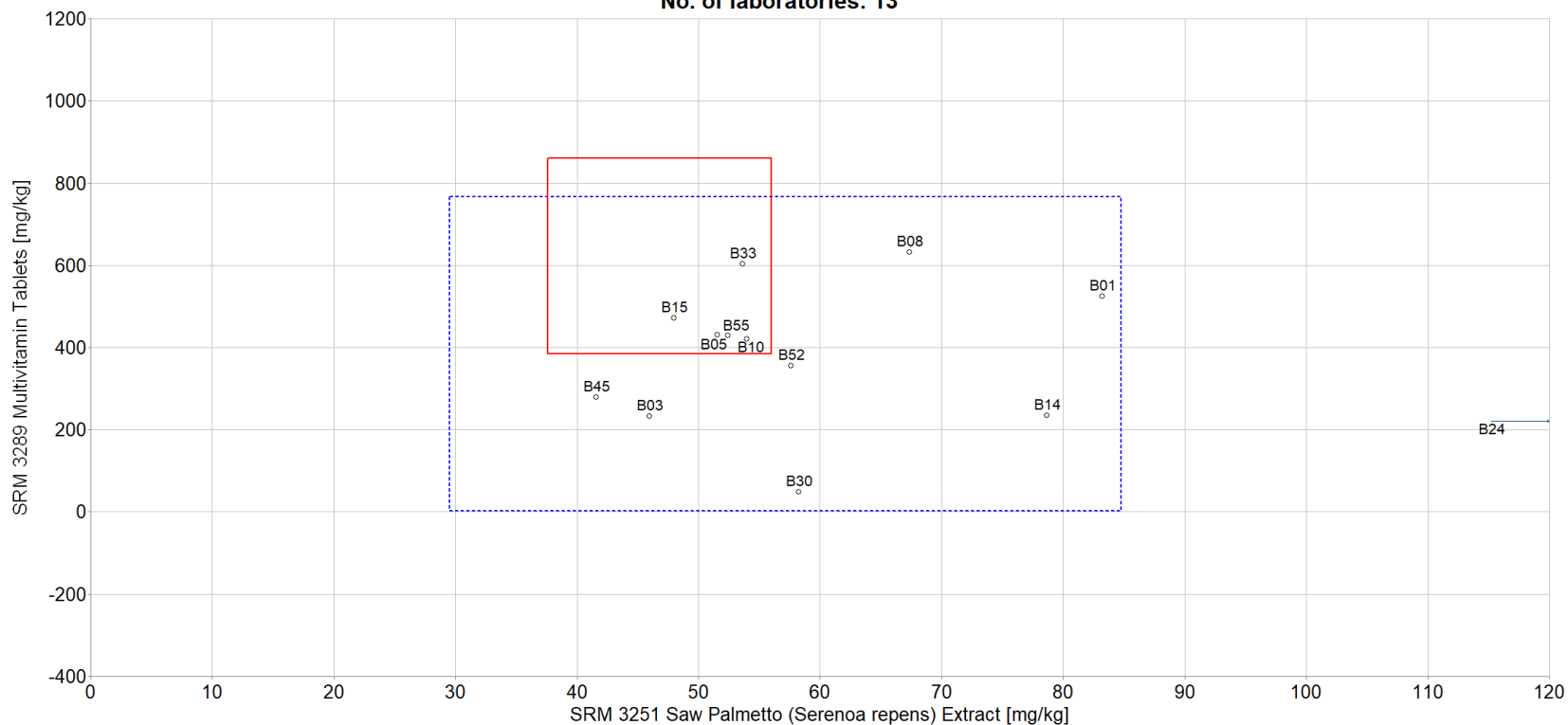
Lab Code: (code)			1. Your Results				2. Community Results			3. Target	
Analyte	Sample	Units	$X_i$	$S_i$	$Z'_{comm}$	$Z_{NIST}$	N	$x^*$	$s^*$	$X_{NIST}$	$U_{NIST}$
Total beta-Carotene	SRM 3251 Saw Palmetto Extract	mg/kg	<i>Individual laboratory results will appear in this section; Laboratory-specific results were provided to each participant separately from this report</i>				13	57.2	13.8	46.8	4.6
Total beta-Carotene	SRM 3289 Multivitamin Tablets	mg/kg					14	385	191	623	119
trans-beta-Carotene	SRM 3251 Saw Palmetto Extract	mg/kg					6	46.0	11.7	36.4	5.6
trans-beta-Carotene	SRM 3289 Multivitamin Tablets	mg/kg					7	377	215		
9-cis-beta-Carotene	SRM 3251 Saw Palmetto Extract	mg/kg					5	9.4	5.1	10.4	1.2
9-cis-beta-Carotene	SRM 3289 Multivitamin Tablets	mg/kg					4				
			$X_i$	Mean of reported values	N	Number of quantitative values reported	$X_{NIST}$	NIST value			
			$S_i$	Standard deviation of reported values			$U_{NIST}$	expanded uncertainty about the NIST 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					

**Table C-2.** Data summary table for total  $\beta$ -carotene in supplements.

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

		Total $\beta$ -Carotene										
		SRM 3251 Saw Palmetto ( <i>Serenoa repens</i> ) Extract (mg/kg)					SRM 3289 Multivitamin Tablets (mg/kg)					
Individual Results	Lab	A	B	C	Mean	SD	A	B	C	Mean	SD	
		Target				46.8	4.6				623	119
	B01	85.1	80.5	84.1	83.2	2.4	530.4	524.2	520.4	525	5.0	
	B03	45.64	45.17	47	45.9	1.0	215.4	195.4	285.8	232	47	
	B05	50.8	50.9	52.9	51.5	1.2	430.2	424.5	436.5	430	6.0	
	B08	69	66	67	67.3	1.5	645	620	632	632	13	
	B10	53	55	54	54.0	1.0	407	449	403	420	25	
	B14	82.5	75.6	77.8	78.6	3.5	258	223	224	235	20	
	B15	46.6	47.7	49.6	48.0	1.5	462	456	496	471	22	
	B18											
	B21											
	B22											
	B23						385.6	474.13	509.64	456	64	
	B24		125	130	127.5	3.5	203	240	221	221	19	
	B30	58.7	59.8	56.2	58.2	1.8	49.3	45.2	50.8	48	2.9	
	B33	55.18	54.11	51.51	53.6	1.9	600.6	588.8	617.4	602	14	
	B40											
	B45	44.3921	39.5651	40.7779	41.6	2.5	287.8693	258.1933	292.2792	279	19	
	B52	58.4	59.6	54.9	57.6	2.4	351	374	344	356	16	
	B55	51.55615	52.1765	53.4875	52.4	1.0	437.982	407.325	443.673	430	20	
Community Results		Consensus Mean				57.2		Consensus Mean				385
		Consensus Standard Deviation				13.8		Consensus Standard Deviation				191
		Maximum				127.5		Maximum				632
		Minimum				41.6		Minimum				48
		N				13		N				14

**Exercise: DSQAP Exercise 2, Measurand: Total beta-Carotene  
 No. of laboratories: 13**



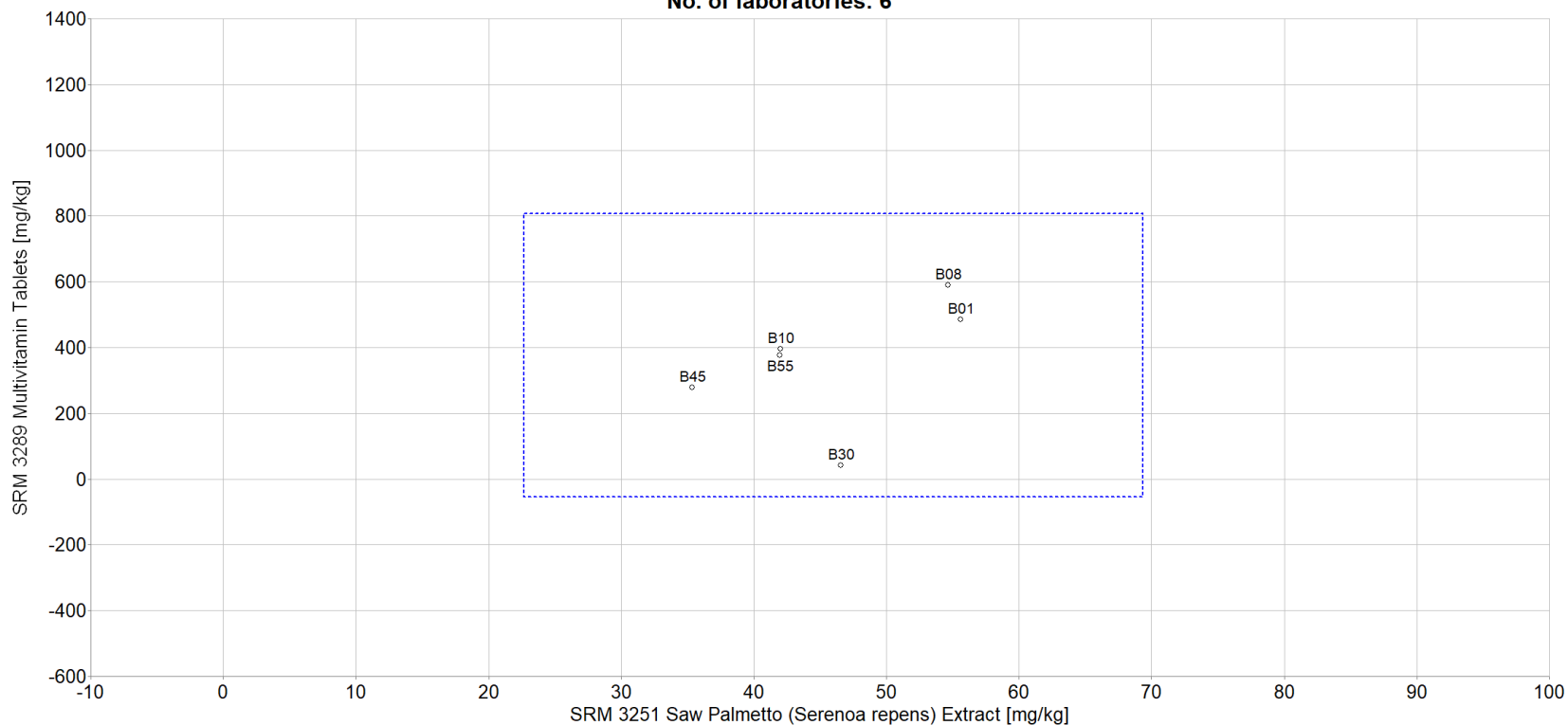
**Fig. C-1.** Laboratory means for total  $\beta$ -carotene in SRM 3251 Saw Palmetto (*Serenoa repens*) Extract and SRM 3289 Multivitamin Tablets (sample/sample comparison view).

In this view, the individual laboratory mean for one sample, SRM 3251, is compared to the individual laboratory mean for a second sample, SRM 3289. The solid red box represents the NIST range of tolerance for the two samples, SRM 3251 (x-axis) and SRM 3289 (y-axis), which encompasses the target values bounded by their uncertainties ( $U_{NIST}$ ) and represents the range that results in an acceptable  $Z_{NIST}$  score,  $|Z_{NIST}| \leq 2$ . The dotted blue box represents the consensus range of tolerance for SRM 3251 (x-axis) and SRM 3289 (y-axis), calculated as the values above and below the consensus means that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \leq 2$ .

**Table C-3.** Data summary table for *trans*- $\beta$ -carotene in supplements.

		<i>trans</i> - $\beta$ -Carotene									
		SRM 3251 Saw Palmetto ( <i>Serenoa repens</i> ) Extract (mg/kg)					SRM 3289 Multivitamin Tablets (mg/kg)				
	Lab	A	B	C	Mean	SD	A	B	C	Mean	SD
<b>Individual Results</b>	Target				36.4	5.6					
	B01	57	53.6	56.2	55.6	1.6	488.7	485.4	482.4	486	2.8
	B05										
	B08	56	54	54	54.7	1.2	600	578	591	590	11
	B10	42	43	41	42.0	1.0	384	423	380	396	24
	B14										
	B15										
	B18										
	B21										
	B22										
	B23						385.6	474.13	509.64	456	64
	B24										
	B30	46.7	48	45	46.6	1.5	43.3	39.4	44.5	42	2.7
	B40										
	B45	37.6363	34.6606	33.7086	35.3	2.0	287.869	258.193	292.279	279	19
B52											
B55	41.4795	40.8935	43.508	42.0	1.4	385.38	358.76	382.525	376	15	
<b>Community Results</b>		Consensus Mean			46.0		Consensus Mean			377	
		Consensus Standard Deviation			11.7		Consensus Standard Deviation			215	
		Maximum			55.6		Maximum			590	
		Minimum			35.3		Minimum			42	
		N			6		N			7	

**Exercise: DSQAP Exercise 2, Measurand: trans-beta-Carotene  
No. of laboratories: 6**



**Fig. C-2.** Laboratory means for *trans*- $\beta$ -carotene in SRM 3251 Saw Palmetto (*Serenoa repens*) Extract and SRM 3289 Multivitamin Tablets (sample/sample comparison view).

In this view, the individual laboratory mean for one sample, SRM 3251, is compared to the individual laboratory mean for a second sample, SRM 3289. The dotted blue box represents the consensus range of tolerance for SRM 3251 (x-axis) and SRM 3289 (y-axis), calculated as the values above and below the consensus means that result in an acceptable  $Z'_{\text{comm}}$  score,  $|Z'_{\text{comm}}| \leq 2$ .

**Table C-4.** Data summary table for 9-*cis*- $\beta$ -carotene in supplements.

		9- <i>cis</i> - $\beta$ -Carotene									
		SRM 3251 Saw Palmetto ( <i>Serenoa repens</i> ) Extract (mg/kg)					SRM 3289 Multivitamin Tablets (mg/kg)				
	Lab	A	B	C	Mean	SD	A	B	C	Mean	SD
Individual Results	Target				10.4	1.2					
	B01	16.1	17.5	17.9	17.2	0.95	< 1	< 1	< 1		
	B05										
	B08	8	7.6	7.4	7.7	0.31					
	B10	5.1	5.5	6.2	5.6	0.56	< 1	< 1	< 1		
	B14										
	B15										
	B18										
	B21										
	B22										
	B23										
	B24										
	B30										
	B40										
	B45	6.7558	4.9045	7.0693	6.2	1.2	< 0.04	< 0.04	< 0.04		
B52											
B55	10.07665	11.283	9.9795	10.4	0.73	52.602	48.565	61.148	54.1	6.4	
Community Results		Consensus Mean				9.4	Consensus Mean				
		Consensus Standard Deviation				5.1	Consensus Standard Deviation				
		Maximum				17.2	Maximum				54.1
		Minimum				5.6	Minimum				54.1
		N				5	N				1

**Appendix D. Fatty Acids Supplemental Tables and Figures**

**Table D-1.** Individualized data summary table (example) for fatty acids in fish oil.

*(Laboratory Name)*

**Exercise 2 - Fatty Acids in Fish Oil**

Lab Code: (code)			1. Your Results				2. Community Results			3. Target	
Analyte	Sample	Units	$X_i$	$S_i$	$Z'_{comm}$	$Z_{NIST}$	N	$x^*$	$s^*$	$X_{NIST}$	$U_{NIST}$
Total Linoleic Acid (C18:2 n-6)	SRM 3275-1 Fish Oil	g/100g FFA	<i>Individual laboratory results will appear in this section; Laboratory-specific results were provided to each participant separately from this report</i>				14	0.22	0.18	0.220	0.018
Total Linoleic Acid (C18:2 n-6)	SRM 3275-2 Fish Oil	g/100g FFA					14	0.29	0.25	0.286	0.040
Total Linoleic Acid (C18:2 n-6)	SRM 3275-3 Fish Oil	g/100g FFA					14	1.27	0.85	1.285	0.043
Total Arachidonic Acid (C20:4 n-6)	SRM 3275-1 Fish Oil	g/100g FFA					15	0.60	0.17	0.544	0.018
Total Arachidonic Acid (C20:4 n-6)	SRM 3275-2 Fish Oil	g/100g FFA					16	2.57	0.72	2.19	0.10
Total Arachidonic Acid (C20:4 n-6)	SRM 3275-3 Fish Oil	g/100g FFA					16	1.40	0.33		
Total EPA (C20:5 n-3)	SRM 3275-1 Fish Oil	g/100g FFA					18	11.6	2.4	11.3	1.2
Total EPA (C20:5 n-3)	SRM 3275-2 Fish Oil	g/100g FFA					18	41.4	8.2	39.2	1.7
Total EPA (C20:5 n-3)	SRM 3275-3 Fish Oil	g/100g FFA					18	16.3	2.5	15.3	0.90
Total DPA (C22:5 n-3)	SRM 3275-1 Fish Oil	g/100g FFA					14	7.8	1.8	6.73	0.11
Total DPA (C22:5 n-3)	SRM 3275-2 Fish Oil	g/100g FFA					14	7.2	1.2	6.48	0.22
Total DPA (C22:5 n-3)	SRM 3275-3 Fish Oil	g/100g FFA					14	2.84	0.48	2.59	0.11
Total DHA (C22:6 n-3)	SRM 3275-1 Fish Oil	g/100g FFA					18	45.3	10.4	41.1	1.4
Total DHA (C22:6 n-3)	SRM 3275-2 Fish Oil	g/100g FFA					18	18.8	3.4	17.93	0.77
Total DHA (C22:6 n-3)	SRM 3275-3 Fish Oil	g/100g FFA					18	10.4	1.6	9.97	0.48

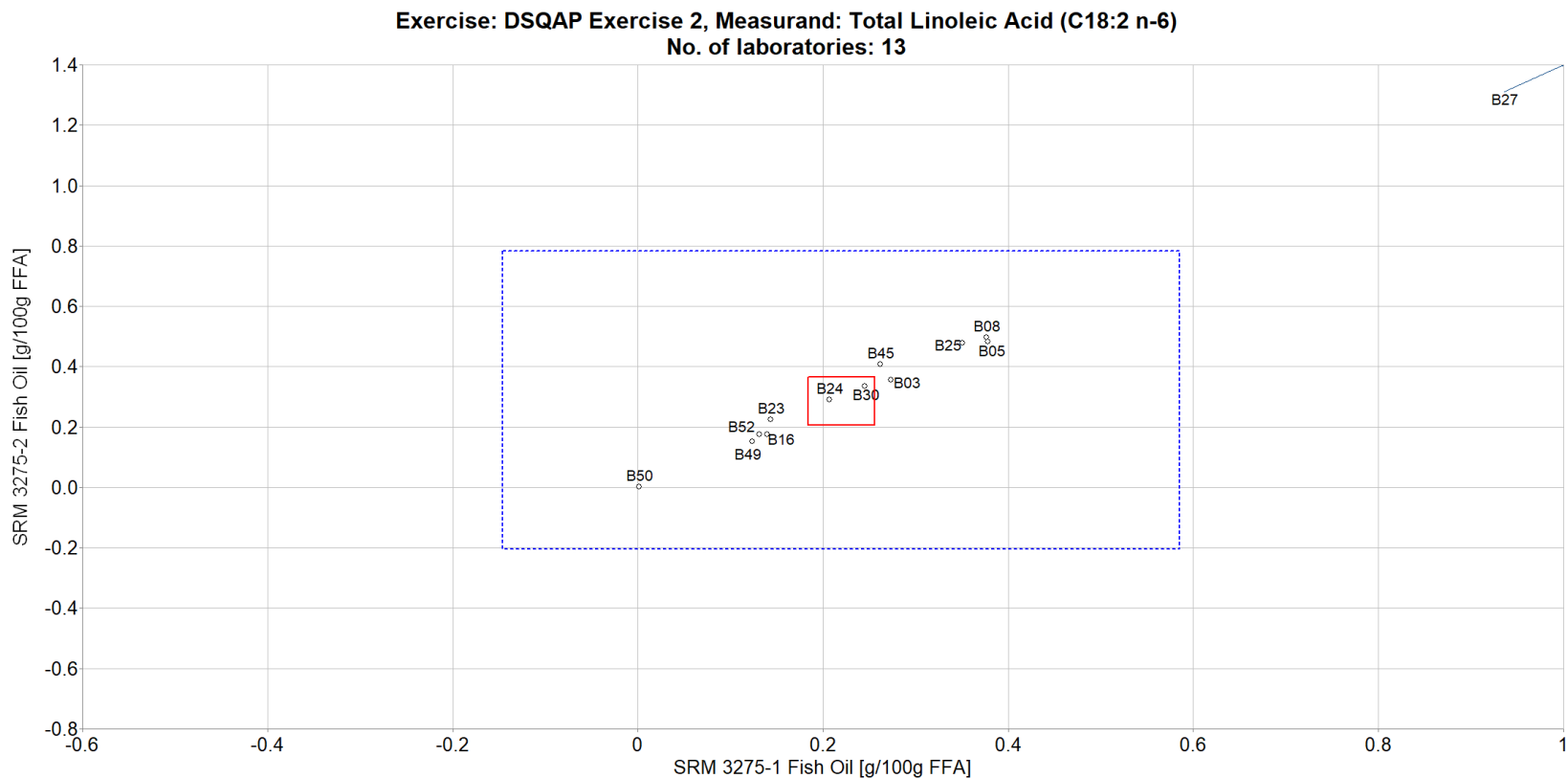
  

$X_i$	Mean of reported values	N	Number of quantitative values reported	$X_{NIST}$	NIST value
$S_i$	Standard deviation of reported values	$x^*$	Robust mean of reported values	$U_{NIST}$	expanded uncertainty about the NIST value
$Z'_{comm}$	Z'-score with respect to community consensus	$s^*$	Robust standard deviation		
$Z_{NIST}$	Z-score with respect to NIST value				

**Table D-2.** Data summary table for total linoleic acid in fish oil.

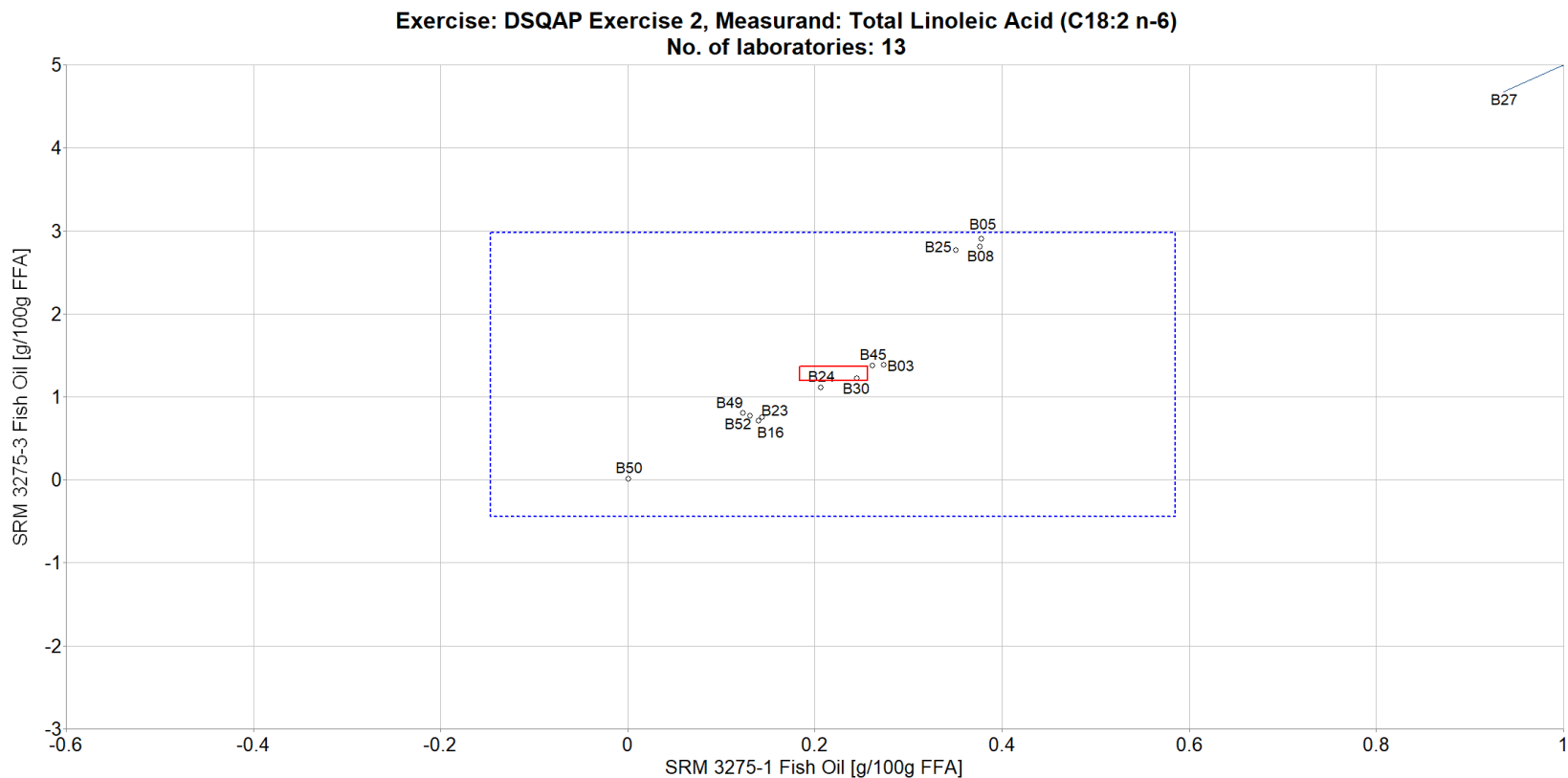
Data points highlighted in blue have been identified as outside the consensus tolerance limits and would be estimated to result in an unacceptable  $Z'_{\text{comm}}$  score,  $|Z'_{\text{comm}}| \geq 2$ .

		Total Linoleic Acid (C18:2 n-6)														
		SRM 3275-1 Omega-3 and Omega-6 Fatty Acids in Fish Oil (g/100g FFA)					SRM 3275-2 Omega-3 and Omega-6 Fatty Acids in Fish Oil (g/100g FFA)					SRM 3275-3 Omega-3 and Omega-6 Fatty Acids in Fish Oil (g/100g FFA)				
	Lab	A	B	C	Mean	SD	A	B	C	Mean	SD	A	B	C	Mean	SD
Individual Results	Target				0.220	0.018				0.286	0.040				1.285	0.043
	B03	0.27	0.27	0.28	0.27	0.0058	0.36	0.35	0.36	0.36	0.0058	1.38	1.39	1.38	1.38	0.0058
	B04															
	B05	0.362	0.378	0.395	0.38	0.017	0.482	0.483	0.484	0.48	0.0010	2.88	2.93	2.89	2.90	0.026
	B07															
	B08	0.374	0.375	0.381	0.38	0.0038	0.491	0.502	0.501	0.50	0.0061	2.787	2.824	2.824	2.81	0.021
	B10	< 0.01	< 0.01	< 0.01			0.14	0.2	0.19	0.18	0.032	0.73	0.71	0.73	0.72	0.012
	B11															
	B16	0.14	0.14	0.14	0.14	0	0.17	0.17	0.19	0.18	0.012	0.72	0.71	0.71	0.71	0.0058
	B20															
	B21															
	B22															
	B23	0.143	0.144	0.143	0.14	0.00058	0.224	0.227	0.226	0.23	0.0015	0.755	0.752	0.753	0.75	0.0015
	B24	0.2	0.21	0.21	0.21	0.0058	0.29	0.29	0.29	0.29	0	1.1	1.11	1.11	1.11	0.0058
	B25	0.3433	0.363	0.3462	0.35	0.011	0.4707	0.4902	0.4752	0.48	0.010	2.73	2.76	2.8	2.76	0.035
	B27	4.91	4.01	5.81	4.91	0.90	8.19	6.92	9.46	8.19	1.27	55.25	42.5	48.88	48.88	6.38
	B30	0.244	0.247	0.244	0.25	0.0017	0.335	0.34	0.332	0.34	0.0040	1.22	1.22	1.22	1.22	0
B40																
B44																
B45	0.256	0.272	0.257	0.26	0.0090	0.371	0.484	0.37	0.41	0.066	1.37	1.378	1.388	1.38	0.0090	
B48																
B49	0.12	0.12	0.13	0.12	0.0058	0.16	0.15	0.15	0.15	0.0058	0.81	0.79	0.81	0.80	0.012	
B50	0.001	0.001		0.001	0	0.002	0.001		0.0015	0.00071	0.007	0.006		0.0065	0.00071	
B52	0.13	0.132	0.131	0.13	0.0010	0.175	0.176	0.179	0.18	0.0021	0.768	0.761	0.77	0.77	0.0047	
Community Results		Consensus Mean				0.22	Consensus Mean				0.29	Consensus Mean				1.27
		Consensus Standard Deviation				0.18	Consensus Standard Deviation				0.25	Consensus Standard Deviation				0.85
		Maximum				4.91	Maximum				8.19	Maximum				48.88
		Minimum				0.0010	Minimum				0.0015	Minimum				0.0065
		N				13	N				14	N				14



**Fig. D-1.** Laboratory means for total linoleic acid in SRM 3275-1 and SRM 3275-2 Omega-3 and Omega-6 Fatty Acids in Fish Oil (sample/sample comparison view).

In this view, the individual laboratory mean for one sample, SRM 3275-1, is compared to the individual laboratory mean for a second sample, SRM 3275-2. The solid red box represents the NIST range of tolerance for the two samples, SRM 3275-1 (x-axis) and SRM 3275-2 (y-axis), which encompasses the target values bounded by their uncertainties ( $U_{\text{NIST}}$ ) and represents the range that results in an acceptable  $Z_{\text{NIST}}$  score,  $|Z_{\text{NIST}}| \leq 2$ . The dotted blue box represents the consensus range of tolerance for SRM 3275-1 (x-axis) and SRM 3275-2 (y-axis), calculated as the values above and below the consensus means that result in an acceptable  $Z'_{\text{comm}}$  score,  $|Z'_{\text{comm}}| \leq 2$ .



**Fig. D-2.** Laboratory means for total linoleic acid in SRM 3275-1 and SRM 3275-3 Omega-3 and Omega-6 Fatty Acids in Fish Oil (sample/sample comparison view).

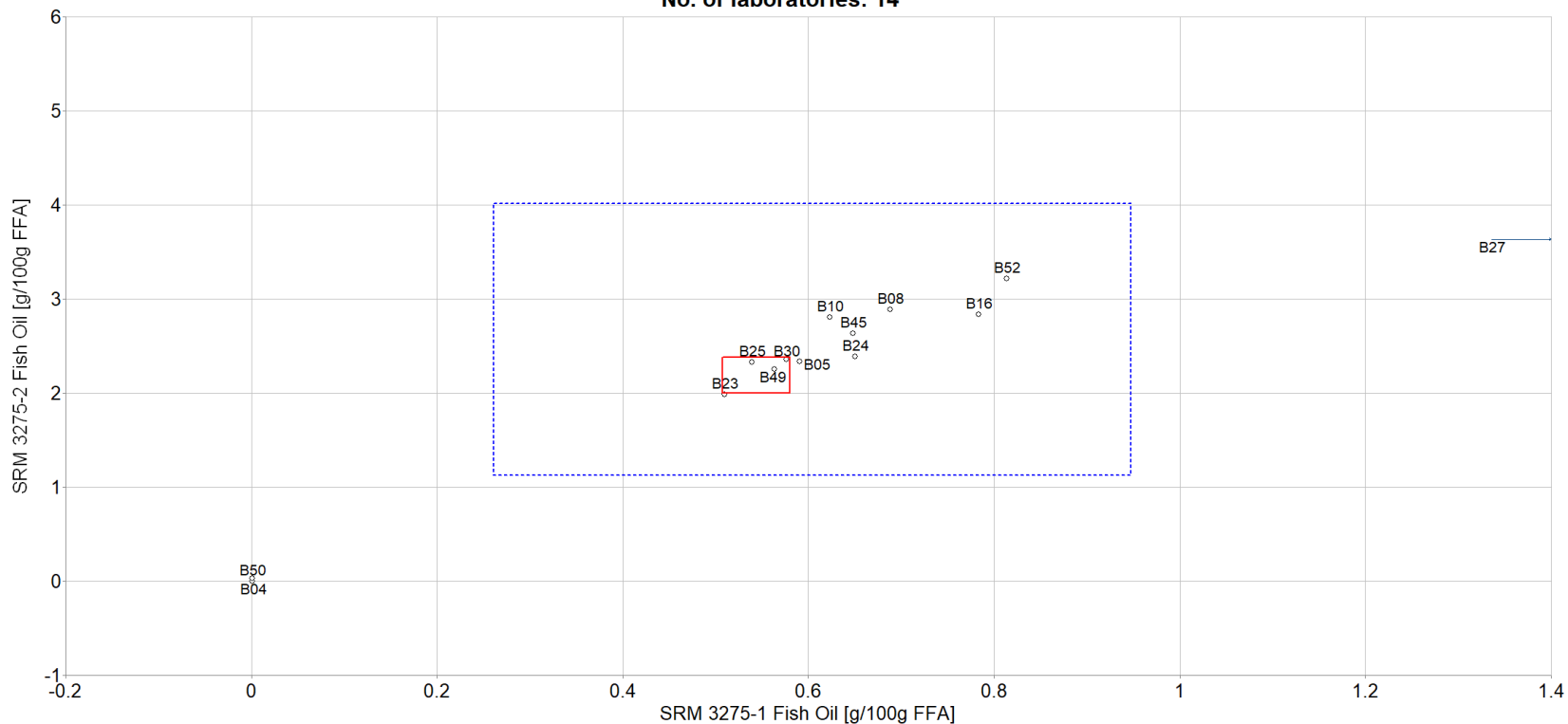
In this view, the individual laboratory mean for one sample, SRM 3275-1, is compared to the individual laboratory mean for a second sample, SRM 3275-3. The solid red box represents the NIST range of tolerance for the two samples, SRM 3275-1 (x-axis) and SRM 3275-3 (y-axis), which encompasses the target values bounded by their uncertainties ( $U_{\text{NIST}}$ ) and represents the range that results in an acceptable  $Z_{\text{NIST}}$  score,  $|Z_{\text{NIST}}| \leq 2$ . The dotted blue box represents the consensus range of tolerance for SRM 3275-1 (x-axis) and SRM 3275-3 (y-axis), calculated as the values above and below the consensus means that result in an acceptable  $Z'_{\text{comm}}$  score,  $|Z'_{\text{comm}}| \leq 2$ .

**Table D-3.** Data summary table for total arachidonic acid in fish oil.

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

		Total Arachidonic Acid (C20:4 n-6)														
		SRM 3275-1 Omega-3 and Omega-6 Fatty Acids in Fish Oil (g/100g FFA)					SRM 3275-2 Omega-3 and Omega-6 Fatty Acids in Fish Oil (g/100g FFA)					SRM 3275-3 Omega-3 and Omega-6 Fatty Acids in Fish Oil (g/100g FFA)				
	Lab	A	B	C	Mean	SD	A	B	C	Mean	SD	A	B	C	Mean	SD
Individual Results	Target				0.544	0.018				2.19	0.10					
	B03	< 0.1	< 0.1	< 0.1			< 0.1	< 0.1	< 0.1			< 0.1	< 0.1	< 0.1		
	B04	0.0014	0.0015	0.0011	0.001	0.00021	0.0316	0.0214	0.0297	0.03	0.0054	0.0031	0.004	0.0039	0.004	0.00049
	B05	0.58	0.597	0.595	0.59	0.0093	2.33	2.35	2.32	2.33	0.015	1.33	1.34	1.33	1.33	0.0058
	B07															
	B08	0.697	0.672	0.695	0.69	0.014	2.86	2.883	2.927	2.89	0.034	1.585	1.607	1.587	1.59	0.012
	B10	0.65	0.63	0.59	0.62	0.031	2.9	2.76	2.75	2.80	0.084	1.29	1.34	1.32	1.32	0.025
	B11															
	B16	0.79	0.78	0.78	0.78	0.0058	2.86	2.86	2.79	2.84	0.040	1.42	1.46	1.49	1.46	0.035
	B20															
	B21															
	B22															
	B23	0.513	0.507	0.508	0.51	0.0032	1.975	1.981	1.998	1.98	0.012	1.126	1.118	1.123	1.12	0.0040
	B24	0.65	0.65	0.65	0.65	0	2.39	2.39	2.38	2.39	0.0058	1.21	1.21	1.21	1.21	0
	B25	0.5145	0.5521	0.5506	0.54	0.021	2.28	2.37	2.33	2.33	0.045	1.21	1.24	1.28	1.24	0.035
	B27	1.94	1.56	2.32	1.94	0.38	3.63	3.35	3.92	3.63	0.29	2.03	1.87	1.95	1.95	0.080
	B30	0.574	0.567	0.587	0.58	0.010	2.38		2.33	2.36	0.035	1.28	1.3	1.78	1.45	0.28
	B40															
	B44						3.12	3.14	3.13	3.13	0.010	1.58	1.55	1.52	1.55	0.030
B45	0.654	0.644	0.647	0.65	0.0051	2.643	2.628	2.642	2.64	0.0084	1.394	1.384	1.382	1.39	0.0064	
B48																
B49	0.56	0.5	0.63	0.56	0.065	2.37	2.2	2.19	2.25	0.10	1.18	1.17	1.12	1.16	0.032	
B50	0.001	0.001		0.001	0	0.002	0.001		0.002	0.00071	0.001	0.001		0.001	0	
B52	0.802	0.821	0.817	0.81	0.010	3.22	3.21	3.23	3.22	0.010	1.58	1.57	1.58	1.58	0.0058	
Community Results		Consensus Mean				0.60	Consensus Mean				2.57	Consensus Mean				1.40
		Consensus Standard Deviation				0.17	Consensus Standard Deviation				0.72	Consensus Standard Deviation				0.33
		Maximum				1.94	Maximum				3.63	Maximum				1.95
		Minimum				0.0010	Minimum				0.0015	Minimum				0.0010
		N				14	N				15	N				15

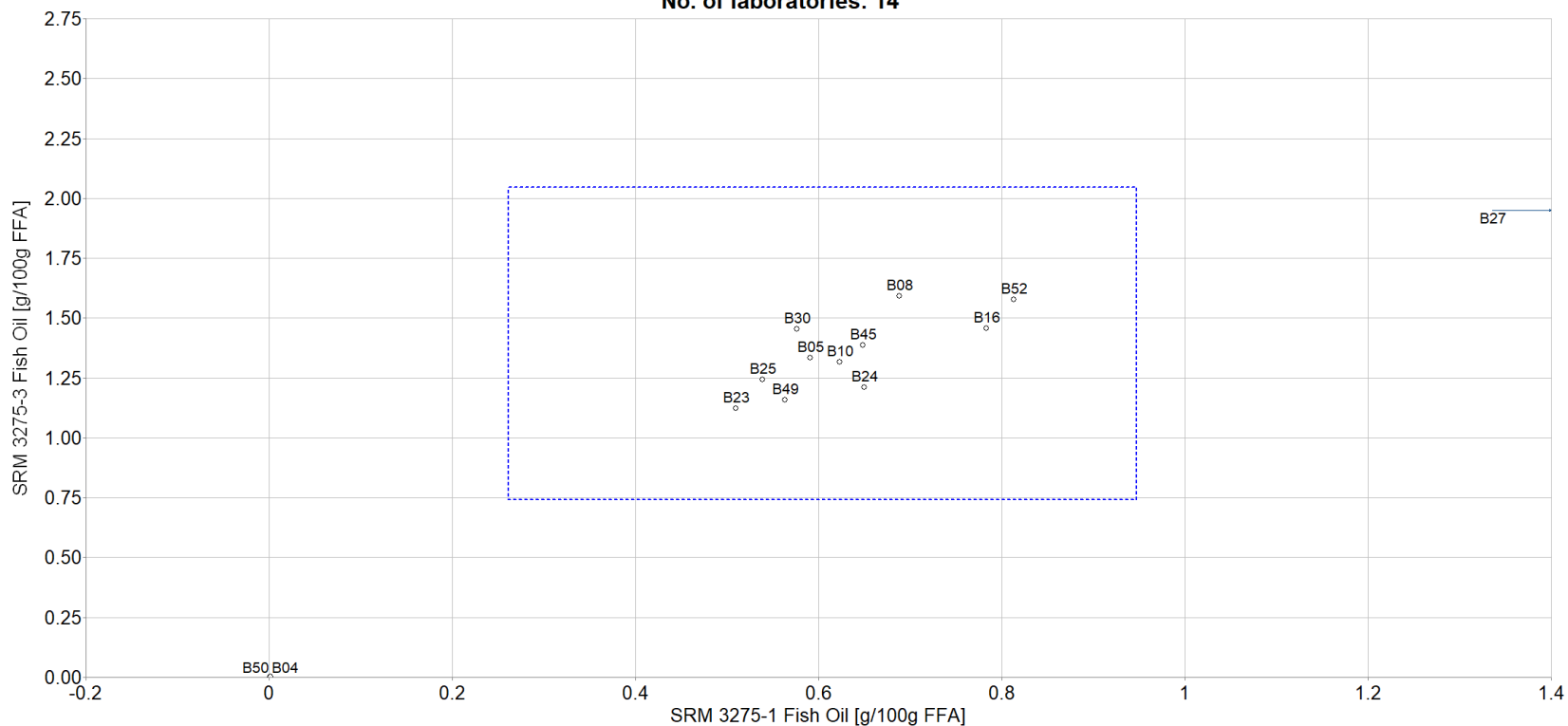
**Exercise: DSQAP Exercise 2, Measurand: Total Arachidonic Acid (C20:4 n-6)**  
**No. of laboratories: 14**



**Fig. D-3.** Laboratory means for total arachidonic acid in SRM 3275-1 and SRM 3275-2 Omega-3 and Omega-6 Fatty Acids in Fish Oil (sample/sample comparison view).

In this view, the individual laboratory mean for one sample, SRM 3275-1, is compared to the individual laboratory mean for a second sample, SRM 3275-2. The solid red box represents the NIST range of tolerance for the two samples, SRM 3275-1 (x-axis) and SRM 3275-2 (y-axis), which encompasses the target values bounded by their uncertainties ( $U_{NIST}$ ) and represents the range that results in an acceptable  $Z_{NIST}$  score,  $|Z_{NIST}| \leq 2$ . The dotted blue box represents the consensus range of tolerance for SRM 3275-1 (x-axis) and SRM 3275-2 (y-axis), calculated as the values above and below the consensus means that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \leq 2$ .

**Exercise: DSQAP Exercise 2, Measurand: Total Arachidonic Acid (C20:4 n-6)**  
**No. of laboratories: 14**



**Fig. D-4.** Laboratory means for total arachidonic acid in SRM 3275-1 and SRM 3275-3 Omega-3 and Omega-6 Fatty Acids in Fish Oil (sample/sample comparison view).

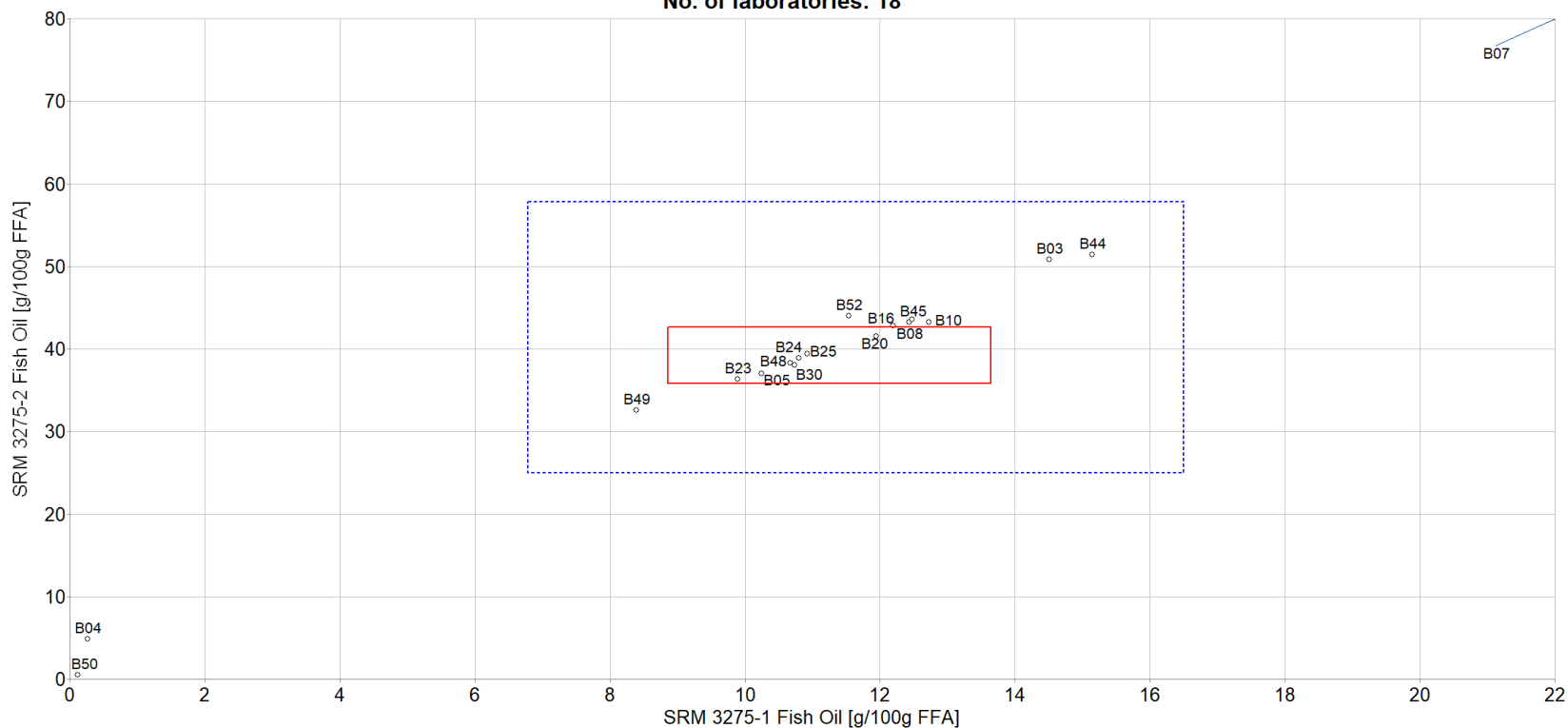
In this view, the individual laboratory mean for one sample, SRM 3275-1, is compared to the individual laboratory mean for a second sample, SRM 3275-3. The dotted blue box represents the consensus range of tolerance for SRM 3275-1 (x-axis) and SRM 3275-3 (y-axis), calculated as the values above and below the consensus means that result in an acceptable  $Z'_{\text{comm}}$  score,  $|Z'_{\text{comm}}| \leq 2$ .

**Table D-4.** Data summary table for total EPA in fish oil.

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

		Total EPA (C20:5 n-3)														
		SRM 3275-1 Omega-3 and Omega-6 Fatty Acids in Fish Oil (g/100g FFA)					SRM 3275-2 Omega-3 and Omega-6 Fatty Acids in Fish Oil (g/100g FFA)					SRM 3275-3 Omega-3 and Omega-6 Fatty Acids in Fish Oil (g/100g FFA)				
	Lab	A	B	C	Mean	SD	A	B	C	Mean	SD	A	B	C	Mean	SD
Individual Results	Target				11.3	1.2				39.2	1.7				15.3	0.90
	B01															
	B03	14.35	14.31	14.86	14.5	0.31	50.61	50.86	50.99	50.8	0.19	19.01	19.2	19.05	19.1	0.10
	B04	0.2311	0.2464	0.3024	0.3	0.038	4.7832	4.8608	4.8339	4.8	0.039	0.2482	0.4724	0.3395	0.4	0.11
	B05	10.27	10.29	10.16	10.2	0.070	36.92	37.11	36.95	37.0	0.10	15.46	15.53	15.53	15.5	0.040
	B07	97.7872	109.496	98.551	101.9	6.6	371.529	361.796	379.502	370.9	8.9	125.323	146.847	152.319	141.5	14
	B08	12.488	12.329	12.488	12.4	0.092	43.289	43.087	43.292	43.2	0.12	16.922	16.939	16.962	16.9	0.020
	B10	12.79	12.64	12.74	12.7	0.076	43.81	42.98	42.91	43.2	0.50	14.74	15.33	14.95	15.0	0.30
	B11															
	B16	12.28	12.16	12.13	12.2	0.079	42.74	43.19	42.56	42.8	0.32	16.14	16.12	16.15	16.1	0.015
	B20	12.163	11.817	11.846	11.9	0.19	41.484	41.376	41.739	41.5	0.19		17.417	17.573	17.5	0.11
	B21															
	B22															
	B23	9.998	9.848	9.821	9.9	0.10	36.149	36.293	36.584	36.3	0.22	15.188	15.152	15.202	15.2	0.026
	B24	10.77	10.8	10.82	10.8	0.025	38.85	38.95	38.84	38.9	0.061	14.69	14.69	14.74	14.7	0.029
	B25	10.7	11.1	10.98	10.9	0.21	38.49	40.25	39.47	39.4	0.88	15.73	15.85	15.78	15.8	0.06
	B30	10.8	10.7	10.7	10.7	0.058	38.4		37.7	38.1	0.49	15.4	15.5	15.5	15.5	0.06
	B40															
	B44	15.25	15.38	14.79	15.1	0.31	51.35	51.64	51.39	51.5	0.16	18.99	18.78	18.87	18.9	0.11
	B45	12.429	12.514	12.47	12.5	0.043	43.782	43.354	43.695	43.6	0.23	17.04	16.885	16.979	17.0	0.078
B48	10.81	10.71	10.48	10.7	0.17	38.68	38.15	38.04	38.3	0.34	15.73	15.84	15.91	15.8	0.091	
B49	8.37	6.7	10.11	8.4	1.7	35.28	31.26	31.26	32.6	2.3	13.05	13.02	13.36	13.1	0.19	
B50	0.134	0.093		0.1	0.029	0.606	0.428		0.5	0.13	0.221	0.192		0.2	0.021	
B52	11.7	11.5	11.4	11.5	0.15	44	43.8	44.1	44.0	0.15	17.8	17.8	17.9	17.8	0.058	
Community Results		Consensus Mean				11.6	Consensus Mean				41.4	Consensus Mean				16.3
		Consensus Standard Deviation				2.4	Consensus Standard Deviation				8.2	Consensus Standard Deviation				2.5
		Maximum				101.9	Maximum				370.9	Maximum				141.5
		Minimum				0.11	Minimum				0.52	Minimum				0.21
		N				18	N				18	N				18

**Exercise: DSQAP Exercise 2, Measurand: Total EPA (C20:5 n-3)**  
**No. of laboratories: 18**



**Fig. D-5.** Laboratory means for total EPA in SRM 3275-1 and SRM 3275-2 Omega-3 and Omega-6 Fatty Acids in Fish Oil (sample/sample comparison view).

In this view, the individual laboratory mean for one sample, SRM 3275-1, is compared to the individual laboratory mean for a second sample, SRM 3275-2. The solid red box represents the NIST range of tolerance for the two samples, SRM 3275-1 (x-axis) and SRM 3275-2 (y-axis), which encompasses the target values bounded by their uncertainties ( $U_{\text{NIST}}$ ) and represents the range that results in an acceptable  $Z_{\text{NIST}}$  score,  $|Z_{\text{NIST}}| \leq 2$ . The dotted blue box represents the consensus range of tolerance for SRM 3275-1 (x-axis) and SRM 3275-2 (y-axis), calculated as the values above and below the consensus means that result in an acceptable  $Z'_{\text{comm}}$  score,  $|Z'_{\text{comm}}| \leq 2$ .

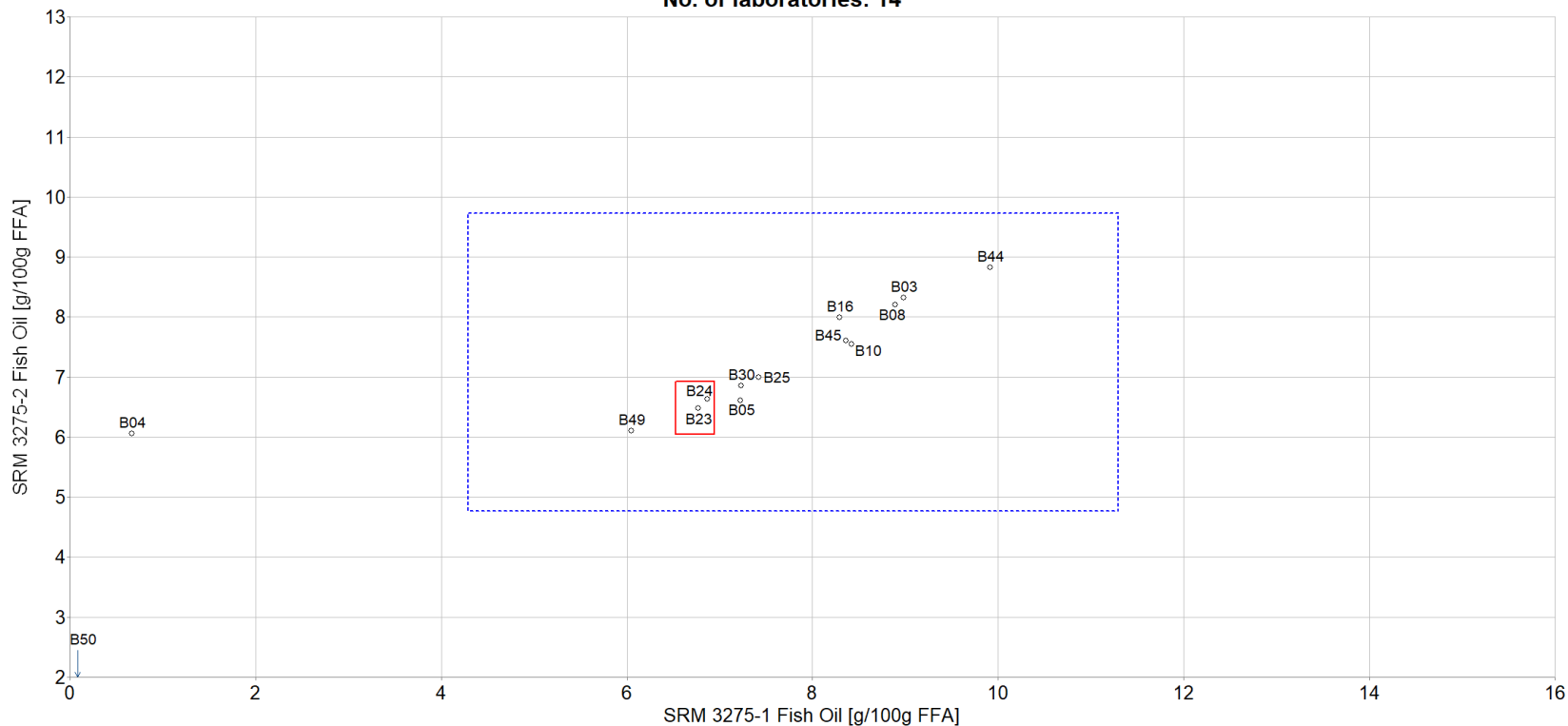


**Table D-5.** Data summary table for total DPA in fish oil.

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

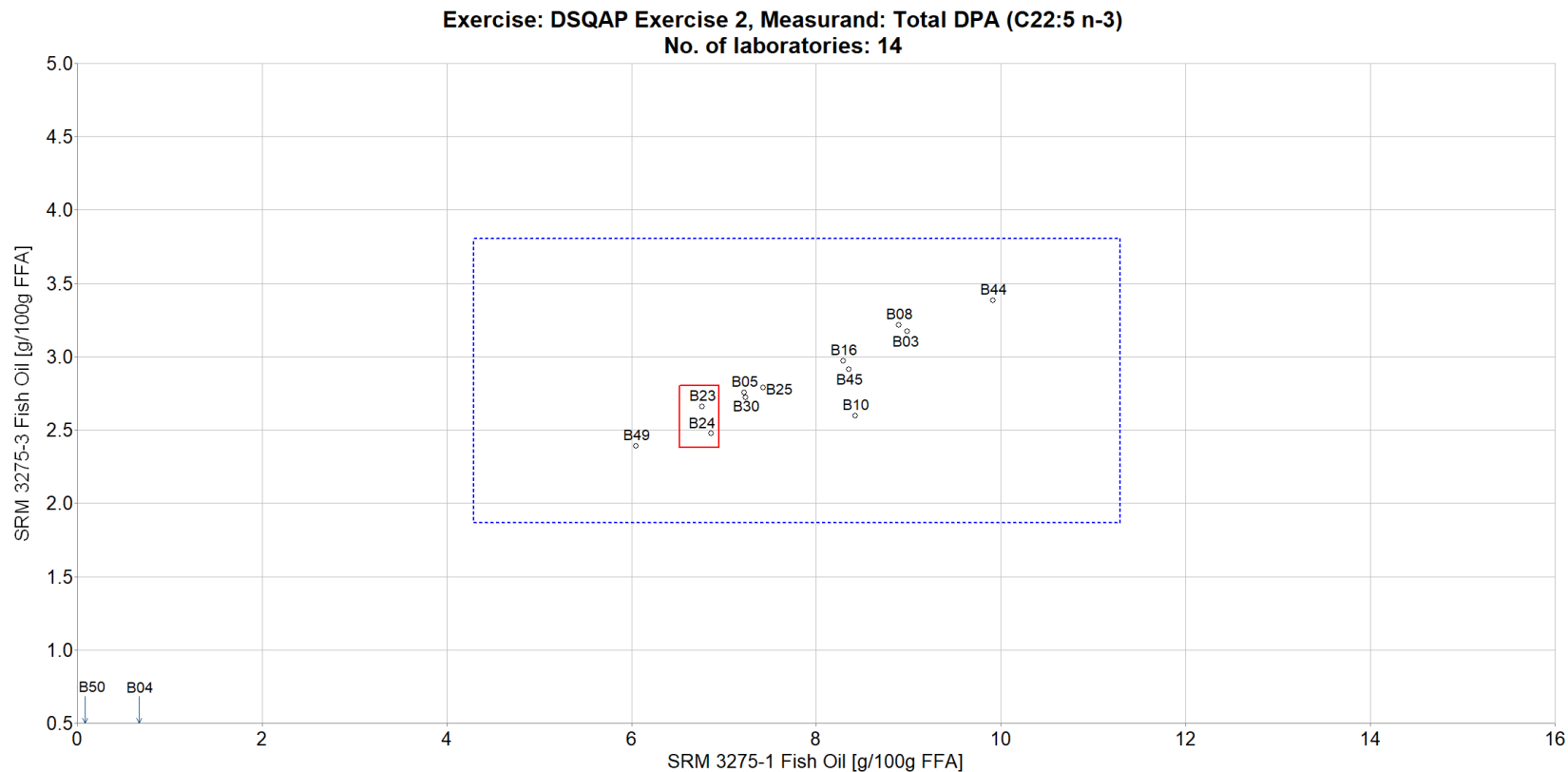
		Total DPA (C22:5 n-3)														
		SRM 3275-1 Omega-3 and Omega-6 Fatty Acids in Fish Oil (g/100g FFA)					SRM 3275-2 Omega-3 and Omega-6 Fatty Acids in Fish Oil (g/100g FFA)					SRM 3275-3 Omega-3 and Omega-6 Fatty Acids in Fish Oil (g/100g FFA)				
	Lab	A	B	C	Mean	SD	A	B	C	Mean	SD	A	B	C	Mean	SD
Individual Results	Target				6.73	0.11				6.49	0.22				2.59	0.11
	B03	9.02	9.06	8.87	9.0	0.10	8.34	8.26	8.36	8.3	0.053	3.15	3.2	3.16	3.17	0.026
	B04	0.7276	0.7117	0.5581	0.7	0.094	6.7155	4.6429	6.8249	6.1	1.2	0.1798	0.2028	0.1675	0.18	0.018
	B05	7.21	7.19	7.26	7.2	0.036	6.56	6.56	6.71	6.6	0.087	2.73	2.76	2.78	2.76	0.025
	B07															
	B08	8.957	8.805	8.912	8.9	0.078	8.268	8.149	8.189	8.2	0.061	3.206	3.256	3.183	3.22	0.037
	B10	8.5	8.41	8.36	8.4	0.071	7.67	7.48	7.48	7.5	0.11	2.53	2.69	2.57	2.60	0.083
	B11															
	B16	8.22	8.24	8.41	8.3	0.10	7.86	7.92	8.21	8.0	0.19	3	2.93	2.98	2.97	0.036
	B20															
	B21															
	B22															
	B23	6.945	6.685	6.668	6.8	0.16	6.44	6.478	6.518	6.5	0.039	2.662	2.652	2.664	2.66	0.0064
	B24	6.85	6.86	6.88	6.9	0.015	6.6	6.64	6.65	6.6	0.026	2.47	2.47	2.49	2.48	0.012
	B25	7.35	7.52	7.4	7.4	0.087	6.84	7.15	7	7.0	0.16	2.76	2.81	2.8	2.79	0.026
	B30	7.33	7.18	7.19	7.2	0.084	6.89	6.88	6.79	6.9	0.055	2.71	2.74	2.72	2.72	0.015
	B40															
	B44	9.76	10.06	9.93	9.9	0.15	8.61	8.95	8.9	8.8	0.18	3.28	3.37	3.5	3.38	0.11
	B45	8.323	8.396	8.354	8.4	0.037	7.619	7.558	7.637	7.6	0.041	2.924	2.897	2.92	2.91	0.015
	B48															
B49	6.06	5.25	6.83	6.0	0.79	6.44	5.96	5.9	6.1	0.30	2.35	2.38	2.44	2.39	0.046	
B50	0.098	0.073		0.1	0.018	0.112	0.085		0.1	0.019	0.041	0.035		0.04	0.0042	
B52																
Community Results		Consensus Mean				7.8	Consensus Mean				7.2	Consensus Mean				2.84
		Consensus Standard Deviation				1.8	Consensus Standard Deviation				1.2	Consensus Standard Deviation				0.48
		Maximum				9.9	Maximum				8.8	Maximum				3.38
		Minimum				0.086	Minimum				0.10	Minimum				0.038
		N				14	N				14	N				14

**Exercise: DSQAP Exercise 2, Measurand: Total DPA (C22:5 n-3)**  
**No. of laboratories: 14**



**Fig. D-7.** Laboratory means for total DPA in SRM 3275-1 and SRM 3275-2 Omega-3 and Omega-6 Fatty Acids in Fish Oil (sample/sample comparison view).

In this view, the individual laboratory mean for one sample, SRM 3275-1, is compared to the individual laboratory mean for a second sample, SRM 3275-2. The solid red box represents the NIST range of tolerance for the two samples, SRM 3275-1 (x-axis) and SRM 3275-2 (y-axis), which encompasses the target values bounded by their uncertainties ( $U_{NIST}$ ) and represents the range that results in an acceptable  $Z_{NIST}$  score,  $|Z_{NIST}| \leq 2$ . The dotted blue box represents the consensus range of tolerance for SRM 3275-1 (x-axis) and SRM 3275-2 (y-axis), calculated as the values above and below the consensus means that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \leq 2$ .



**Fig. D-8.** Laboratory means for total DPA in SRM 3275-1 and SRM 3275-3 Omega-3 and Omega-6 Fatty Acids in Fish Oil (sample/sample comparison view).

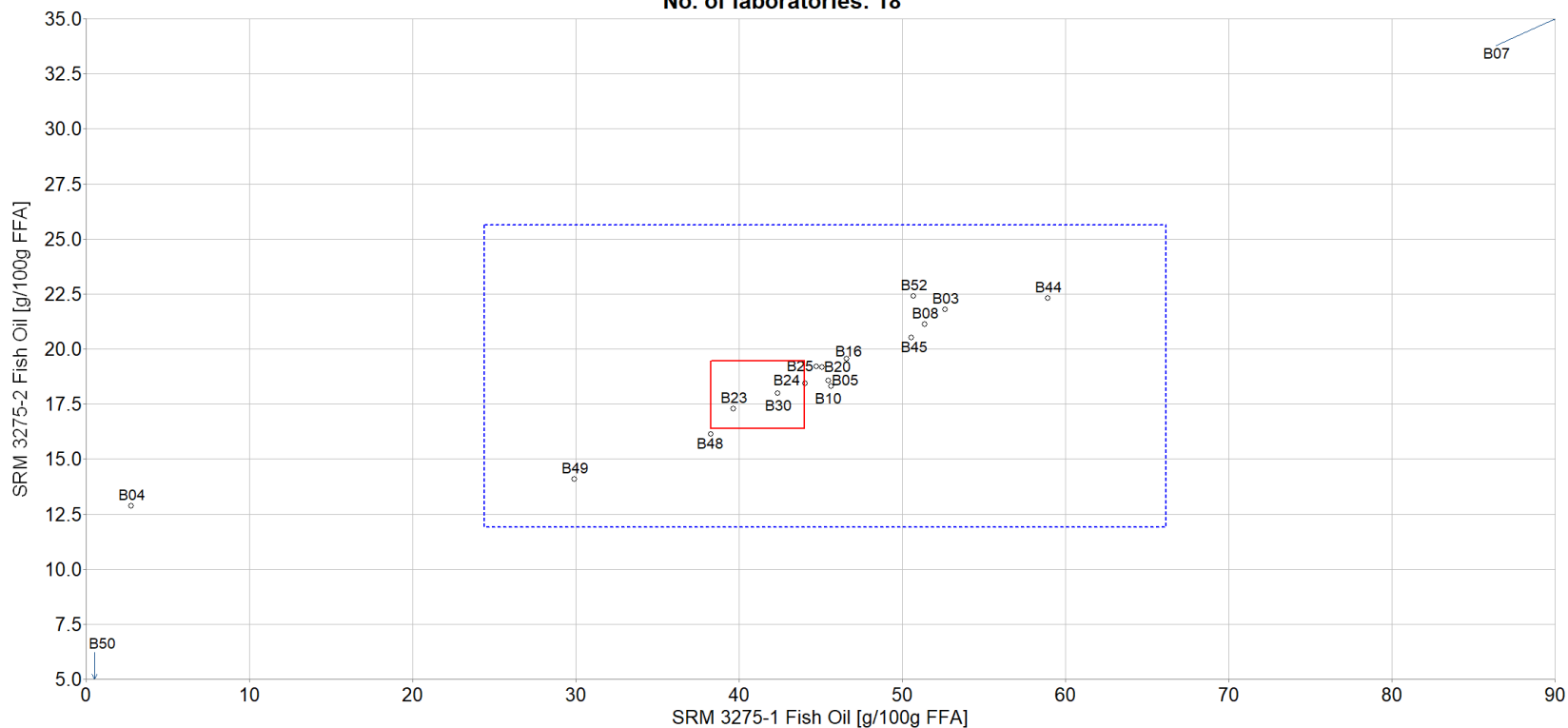
In this view, the individual laboratory mean for one sample, SRM 3275-1, is compared to the individual laboratory mean for a second sample, SRM 3275-3. The solid red box represents the NIST range of tolerance for the two samples, SRM 3275-1 (x-axis) and SRM 3275-3 (y-axis), which encompasses the target values bounded by their uncertainties ( $U_{NIST}$ ) and represents the range that results in an acceptable  $Z_{NIST}$  score,  $|Z_{NIST}| \leq 2$ . The dotted blue box represents the consensus range of tolerance for SRM 3275-1 (x-axis) and SRM 3275-3 (y-axis), calculated as the values above and below the consensus means that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \leq 2$ .

**Table D-6.** Data summary table for total DHA in fish oil.

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

		Total DHA (C22:6 n-3)														
		SRM 3275-1 Omega-3 and Omega-6 Fatty Acids in Fish Oil (g/100g FFA)					SRM 3275-2 Omega-3 and Omega-6 Fatty Acids in Fish Oil (g/100g FFA)					SRM 3275-3 Omega-3 and Omega-6 Fatty Acids in Fish Oil (g/100g FFA)				
	Lab	A	B	C	Mean	SD	A	B	C	Mean	SD	A	B	C	Mean	SD
Individual Results	Target				41.1	1.4				17.93	0.77				9.97	0.48
	B01															
	B03	52.53	52.63	52.7	52.6	0.085	21.78	21.61	21.97	21.8	0.18	11.35	11.42	11.38	11.4	0.035
	B04	2.9038	3.0079	2.4229	2.8	0.31	14.0405	10.9924	13.5609	12.9	1.6	0.8309	1.0258	0.8444	0.9	0.11
	B05	45.27	45.21	45.98	45.5	0.43	18.5	18.66	18.48	18.5	0.10	10.64	10.85	10.72	10.7	0.11
	B07	416.048	398.029	367.042	393.7	25	189.341	158.956	165.007	171.1	16	82.3544	89.5938	92.6062	88.2	5.3
	B08	51.07	51.225	51.899	51.4	0.44	21.237	20.983	21.103	21.1	0.13	11.58	11.539	11.516	11.5	0.032
	B10	46.04	45.73	45.22	45.7	0.41	18.62	18.13	18.12	18.3	0.29	8.56	9.25	8.85	8.9	0.35
	B11															
	B16	47.12	46.53	46.14	46.6	0.49	19.68	19.45	19.51	19.5	0.12	10.23	10.02	10.05	10.1	0.11
	B20	45.838	44.681	44.699	45.1	0.66	19.182	19.076	19.291	19.2	0.11		11.11	11.185	11.1	0.053
	B21															
	B22															
	B23	39.833	39.701	39.389	39.6	0.23	17.217	17.261	17.413	17.3	0.10	9.966	9.93	9.959	10.0	0.019
	B24	43.58	44	44.62	44.1	0.52	18.33	18.45	18.49	18.4	0.083	9.63	9.63	9.69	9.7	0.035
	B25	44.34	45.33	44.54	44.7	0.52	18.74	19.65	19.17	19.2	0.46	10.41	10.6	10.62	10.5	0.12
	B30	42.6	42.3	42.2	42.4	0.21	18.1	18.1	17.8	18.0	0.17	10	10.1	10.1	10.1	0.058
	B40															
	B44	59	60.46	57.34	58.9	1.6	22.14	22.28	22.47	22.3	0.17	11.74	11.39	11.47	11.5	0.18
	B45	50.546	50.707	50.391	50.5	0.16	20.572	20.403	20.554	20.5	0.093	10.981	10.89	10.913	10.9	0.047
B48	38.71	38.46	37.7	38.3	0.53	16.25	16.11	16.04	16.1	0.11	9.29	9.38	9.4	9.4	0.059	
B49	28.73	18.12	42.9	29.9	12	16.59	12.63	13.04	14.1	2.2	8.11	8.14	8.87	8.4	0.43	
B50	0.597	0.476		0.5	0.086	0.281	0.222		0.3	0.042	0.142	0.13		0.1	0.0085	
B52	51.2	50.7	50.2	50.7	0.50	22.4	22.3	22.5	22.4	0.10	11.5	11.4	11.4	11.4	0.058	
Community Results		Consensus Mean				45.3	Consensus Mean				18.8	Consensus Mean				10.4
		Consensus Standard Deviation				10.4	Consensus Standard Deviation				3.4	Consensus Standard Deviation				1.6
		Maximum				393.7	Maximum				171.1	Maximum				88.2
		Minimum				0.54	Minimum				0.25	Minimum				0.14
		N				18	N				18	N				18

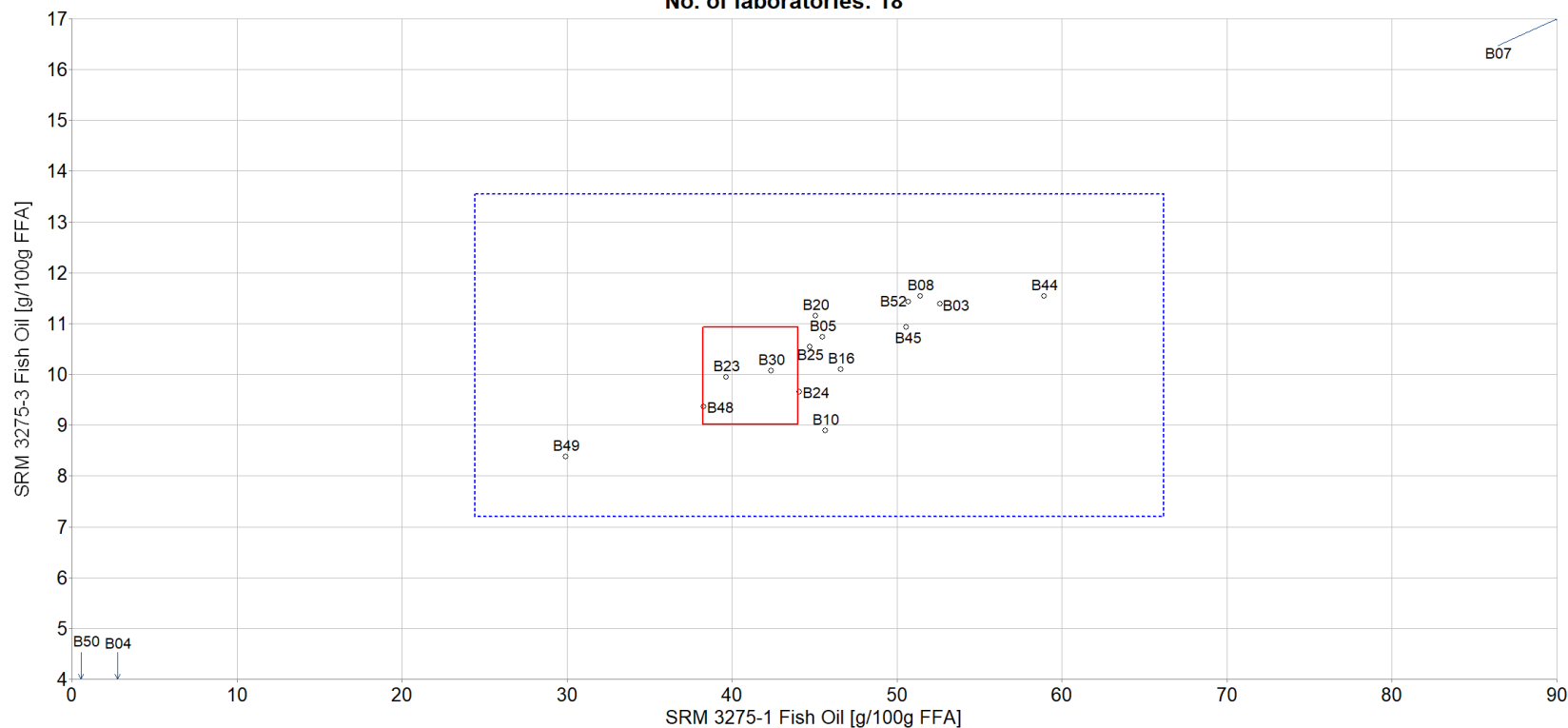
**Exercise: DSQAP Exercise 2, Measurand: Total DHA (C22:6 n-3)**  
**No. of laboratories: 18**



**Fig. D-9.** Laboratory means for total DHA in SRM 3275-1 and SRM 3275-2 Omega-3 and Omega-6 Fatty Acids in Fish Oil (sample/sample comparison view).

In this view, the individual laboratory mean for one sample, SRM 3275-1, is compared to the individual laboratory mean for a second sample, SRM 3275-2. The solid red box represents the NIST range of tolerance for the two samples, SRM 3275-1 (x-axis) and SRM 3275-2 (y-axis), which encompasses the target values bounded by their uncertainties ( $U_{NIST}$ ) and represents the range that results in an acceptable  $Z_{NIST}$  score,  $|Z_{NIST}| \leq 2$ . The dotted blue box represents the consensus range of tolerance for SRM 3275-1 (x-axis) and SRM 3275-2 (y-axis), calculated as the values above and below the consensus means that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \leq 2$ .

**Exercise: DSQAP Exercise 2, Measurand: Total DHA (C22:6 n-3)**  
**No. of laboratories: 18**



**Fig. D-10.** Laboratory means for total DHA in SRM 3275-1 and SRM 3275-3 Omega-3 and Omega-6 Fatty Acids in Fish Oil (sample/sample comparison view).

In this view, the individual laboratory mean for one sample, SRM 3275-1, is compared to the individual laboratory mean for a second sample, SRM 3275-3. The solid red box represents the NIST range of tolerance for the two samples, SRM 3275-1 (x-axis) and SRM 3275-3 (y-axis), which encompasses the target values bounded by their uncertainties ( $U_{NIST}$ ) and represents the range that results in an acceptable  $Z_{NIST}$  score,  $|Z_{NIST}| \leq 2$ . The dotted blue box represents the consensus range of tolerance for SRM 3275-1 (x-axis) and SRM 3275-3 (y-axis), calculated as the values above and below the consensus means that result in an acceptable  $Z'_{comm}$  score,  $|Z'_{comm}| \leq 2$ .