NISTIR 8203

Dietary Supplement Laboratory Quality Assurance Program: Exercise M Final Report

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https://doi.org/10.6028/NIST.IR.8203

National Institute of Standards and Technology
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February 2018

U.S. Department of Commerce
Wilbur L. Ross, Jr., Secretary

National Institute of Standards and Technology
Walter Copan, NIST Director and Under Secretary of Commerce for Standards and Technology

ABSTRACT

The NIST Dietary Supplement Laboratory Quality Assurance Program (DSQAP) was established in collaboration with the National Institutes of Health (NIH) Office of Dietary Supplements (ODS) in 2007 to enable members of the dietary supplements community to improve the accuracy of measurements for demonstration of compliance with various regulations including the dietary supplement current Good Manufacturing Practices (cGMPs). Exercise M of this program offered the opportunity for laboratories to assess their in-house measurements of nutritional elements (potassium and zinc), contaminants (arsenic and lead), water-soluble vitamins (thiamine (B_1) and riboflavin (B_2)), fat-soluble vitamins (total vitamin K_1, *cis*- and *trans*-vitamin K_1), botanical marker compounds (curcuminoids, chondroitin sulfate), and identity (chondroitin) in foods and/or botanical dietary supplement ingredients and finished products.

KEYWORDS

botanical; chondroitin; contaminants; curcuminoids; dietary supplements; foods; minerals; vitamins.

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INTRODUCTION

The dietary supplement industry in the US is booming, with two-thirds of adults considering themselves to be supplement users.[1] Consumption of dietary supplements, which includes vitamin and mineral supplements, represents an annual US expenditure of more than $40 billion. These figures represent an increasing American and worldwide trend, and as a result, it is critically important that both the quality and safety of these products are verified and maintained.

The Dietary Supplement Health and Education Act of 1994 (DSHEA) amended the Federal Food, Drug, and Cosmetic Act to create the regulatory category called dietary supplements. The DSHEA also gave the FDA authority to write current Good Manufacturing Practices (cGMPs) that require manufacturers to evaluate the identity, purity, and composition of their ingredients and finished products. In addition, the DSHEA authorized the establishment of the Office of Dietary Supplements at the National Institutes of Health (NIH ODS). To enable members of the dietary supplements community to improve the accuracy of the measurements required for compliance with these and other regulations, NIST established the Dietary Supplement Laboratory Quality Assurance Program (DSQAP) in collaboration with the NIH ODS in 2007.

The program offers the opportunity for laboratories to assess their in-house measurements of active or marker compounds, nutritional elements, contaminants (toxic elements, pesticides, mycotoxins), and fat- and water-soluble vitamins in foods as well as botanical dietary supplement ingredients and finished products. Reports and certificates of participation are provided and can be used to demonstrate compliance with the cGMPs. In addition, NIST and the DSQAP assist the ODS Analytical Methods and Reference Materials program (AMRM) at the NIH in supporting the development and dissemination of analytical tools and reference materials. In the future, results from DSQAP exercises could be used by ODS to identify problematic matrices and analytes for which an AOAC INTERNATIONAL Official Method of Analysis would benefit the dietary supplement community.

NIST has experience in the administration of quality assurance programs, but the DSQAP takes a unique approach. In other NIST quality assurance programs, a set of analytes is measured repeatedly over time in the same or similar matrices to demonstrate and improve laboratory performance. In contrast, the wide range of matrices and analytes under the "dietary supplement" umbrella means that not every laboratory is interested in every sample or analyte. The constantly changing dietary supplement market, and the enormous diversity of finished products, makes repeated determination of a few target compounds in a single matrix of little use to participants. Instead, participating laboratories are interested in testing in-house methods on a wide variety of challenging, real-world matrices to demonstrate that their performance is comparable to that of the community and that their methods provide accurate results. In an area where there are few standard methods, the DSQAP offers a unique tool for assessment of the quality of measurements, provides feedback about performance, and can assist participants in improving laboratory operations.

This report summarizes the results from the twelfth exercise of the DSQAP, Exercise M. Eighty-two laboratories responded to the call for participants distributed in October 2015. Samples

[1] Walsh, T. (2012) *Supplement Usage, Consumer Confidence Remain Steady According to New Annual Survey from CRN*. Council for Responsible Nutrition, Washington, DC.

were shipped to participants in two separate shipments, one shipment in July 2016 and one shipment in September 2016, and results were returned to NIST by December 2016. This report contains the final data and information that was disseminated to the participants in January 2018.

OVERVIEW OF DATA TREATMENT AND REPRESENTATION

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

Statistics

Data tables and graphs throughout this report contain information about the performance of each laboratory relative to that of the other participants in this study and relative to a target around the expected result, if available. All calculations are performed in PROLab Plus (QuoData GmbH, Dresden, Germany).[2] The consensus mean and standard deviation are calculated according to the robust algorithm outlined in ISO 13528:2015(E), Annex C.[3] The algorithm is summarized here in simplified form.

Initial values of the consensus mean, x^*, and consensus standard deviation, s^*, are estimated as

$$x^* = \text{median of } x_i \qquad (i = 1, 2, \ldots, n)$$
$$s^* = 1.483 \times \text{median of } |x_i - x^*| \qquad (i = 1, 2, \ldots, n).$$

These initial values for x^* and s^* are updated by first calculating the expanded standard deviation, δ, as

$$\delta = 1.5 \times s^*.$$

Then each x_i is compared to the expanded range and adjusted to x_i^* as described below to reduce the effect of outliers.

If $x_i < x^* - \delta$, then $x_i^* = x^* - \delta$.
If $x_i > x^* + \delta$, then $x_i^* = x^* + \delta$.
Otherwise, $x_i^* = x_i$.

New values of x^*, s^*, and δ are calculated iteratively until the process converges. Convergence is taken as no change from one iteration to the next in the third significant figure of s^* and in the equivalent digit in x^*:

$$x^* = \frac{\sum_{i=1}^{n} x_i^*}{n}$$

[2] Certain commercial equipment, instruments or materials are identified in this report to adequately specify the experimental procedure. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that the materials or equipment identified are necessarily the best available for the purpose.
[3] ISO 13528:2015(E), *Statistical methods for use in proficiency testing by interlaboratory comparisons*, pp. 53-54.

$$s^* = 1.134 \times \sqrt{\frac{\sum_{i=1}^{n}(x_i^* - x^*)}{n-1}}.$$

Individualized Data Table

The data in this table is individualized to each participating laboratory and is provided to allow participants to directly compare their data to the summary statistics (consensus or community data as well as NIST certified, reference, or estimated values). The upper left of the data table includes the randomized laboratory code. Tables included in this report are generated using NIST data to protect the identity and performance of participants.

Section 1 of the data table contains the laboratory results as reported, including the mean and standard deviation when multiple values were reported. A blank indicates that NIST does not have data on file for that laboratory for a particular analyte or matrix. An empty box for standard deviation indicates that only a single value was reported and therefore that value was not included in the calculation of the consensus data.[3]

Also in Section 1 are two Z-scores. The first Z-score, Z'_{comm}, is calculated with respect to the community consensus value, taking into consideration bias that may result from the uncertainty in the assigned consensus value, using x* and s*:

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

The second Z-score, Z_{NIST}, is calculated with respect to the target value (NIST certified, reference, or estimated value), using x_{NIST} and U_{95} (the expanded uncertainty) or s_{NIST} (the standard deviation of NIST measurements):

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

or

$$Z_{NIST} = \frac{x_i - x_{NIST}}{s_{NIST}}.$$

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

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

Section 2 of the data table contains the community results, including the number of laboratories reporting more than a single value for a given analyte[1], the mean value determined for each analyte, and a robust estimate of the standard deviation of the reported values.[4] Consensus means and

[4] ISO 13528:2015(E), *Statistical methods for use in proficiency testing by interlaboratory comparisons*, Annex C.

standard deviations are calculated using the laboratory means; if a laboratory reported a single value, the reported value is not included.[3] Additional information on calculation of the consensus mean and standard deviation can be found in the previous section.

Section 3 of the data table contains the target values for each analyte. When possible, the target value is a certified or reference value determined at NIST. Certified values and the associated expanded uncertainty (U_{95}) have been determined with two independent analytical methods at NIST, or by combination of a single method and NIST and results from collaborating laboratories. Reference values are assigned using NIST values obtained from the average and standard deviation of measurements made using a single analytical method at NIST or by measurements obtained from collaborating laboratories. For both certified and reference values, at least six samples have been tested and duplicate preparations from the sample package have been included, allowing the uncertainty to encompass variability due to inhomogeneity within and between packages. For samples in which a NIST certified or reference value is not available, the analytes are measured at NIST using an appropriate method. The NIST-assessed value represents the mean of at least three replicates. For materials acquired from another proficiency testing program, the consensus value and uncertainty from the completed round is used as the target range.

Summary Data Table

This data table includes a summary of all reported data for a particular analyte in a particular study. Participants can compare the raw data for their laboratory to data reported by the other participating laboratories or to the consensus data. A blank indicates that the laboratory signed up and received samples for that particular analyte and matrix, but NIST does not have data on file for that laboratory.

Graphs

Data Summary View (Method Comparison Data Summary View)

In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). Laboratories reporting values below the method quantitation limit are shown in this view as downward triangles beginning at the limit of quantitation (LOQ). Laboratories reporting values as "below LOQ" can still be successful in the study if the target value is also below the laboratory LOQ. The black solid line represents the consensus mean, and the green shaded area represents the consensus variability (standard error of the consensus mean). Where appropriate, two consensus means may be calculated for the same sample if bimodality is identified in the data. In this case, two consensus means and ranges will be displayed in the data summary view. The red shaded region represents the target zone for "acceptable" performance, which encompasses the NIST certified, reference, or estimated value bounded by its uncertainty (U_{95}) or standard deviation. The black dashed lines represent the range of tolerance (values that result in an acceptable Z' score, $|Z'| \leq 2$). The y-axis of the graph is scaled to include twice the range of tolerance; laboratory results that are above or below this range will be displayed using a red arrow pointing up or down, respectively, and the laboratory reported value. In this view, the relative locations of individual laboratory data and consensus zones with respect to the target zone can be compared easily. In most cases, the target zone and the consensus zone overlap, which is the expected result. The major program goals are to reduce the size of the consensus zone and center the consensus zone about the target value. Analysis of an appropriate reference material as part of a quality control scheme can help to identify sources of bias for laboratories reporting

results that are significantly different from the target zone. In the case in which a method comparison is relevant, different colored data points may be used to indicate laboratories that used a specific approach to sample preparation, analysis, or quantitation.

Sample/Sample Comparison View

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

NUTRITIONAL ELEMENTS (K AND ZN) IN SPINACH LEAVES AND SPIRULINA

Study Overview

In this study, participants were provided with NIST SRM 1570a Trace Elements in Spinach Leaves and one commercially prepared product, spirulina powder. Participants were asked to use in-house analytical methods to determine the mass fractions of potassium and zinc in each of the matrices and report values on an as-received basis.

Sample Information

Spinach. Participants were provided with three packets, each containing approximately 5 g of dried spinach leaves. The dried leaves were ground, homogenized, and heat-sealed inside 4 mil polyethylene bags. Before use, participants were instructed to thoroughly mix the contents of the packet and to use a sample size of at least 0.5 g. Participants were asked to store the material at controlled room temperature, 20 °C to 25 °C, and to prepare one sample and report one value from each packet provided. Approximate analyte levels were not reported to participants prior to the study. The certified value for potassium in SRM 1570a was determined at NIST using isotope dilution thermal ionization mass spectrometry (ID TIMS) and instrumental neutron activation analysis (INAA). The certified value for zinc was determined at NIST using inductively coupled plasma optical emission spectroscopy (ICP-OES) and INAA. The certified values and uncertainties are provided in the table below, both on a dry-mass basis and on an as-received basis accounting for moisture of the material (5.15 %).

	Certified Mass Fraction in SRM 1570a (mg/kg)	
Analyte	(dry-mass basis)	(as-received basis)
Potassium (K)	29000 ± 260	27500 ± 250
Zinc (Zn)	82.3 ± 3.9	78.1 ± 3.7

Spirulina. Participants were provided with three packets, each containing approximately 3 g of dried spirulina. The spirulina was ground, homogenized, and heat-sealed inside 4 mil polyethylene bags, which were then sealed inside nitrogen-flushed aluminized plastic bags along with two packets of silica gel. Before use, participants were instructed to thoroughly mix the contents of the packet and to use a sample size of at least 0.5 g. Participants were asked to store the material at controlled room temperature, 20 °C to 25 °C, and to prepare one sample and report one value from each packet provided. Approximate analyte levels were not reported to participants prior to the study. The target value for both potassium and zinc in spirulina was determined at NIST using ICP-OES. The NIST-determined values and uncertainties for potassium and zinc are provided in the table below, on an as-received basis.

	NIST-Determined Mass Fraction in Spirulina (mg/kg)
Analyte	(as-received basis)
Potassium (K)	15300 ± 370
Zinc (Zn)	7.77 ± 0.98

Study Results

- Thirty-seven laboratories enrolled in this exercise and received samples to measure potassium. Twenty-five laboratories reported results for both the spinach leaves and the spirulina powder (68 % participation).
    - The consensus mean for potassium in spirulina was within the target range, while the consensus mean for potassium in the spinach leaves was below the target range. The between-laboratory variability was acceptable for both the dried spinach leaves and the spirulina (16 % and 22 %, RSD respectively).
    - Most of the laboratories reported using inductively coupled plasma mass spectroscopy (ICP-MS) (44 %) or ICP-OES (40 %) as their analytical method for measuring potassium. The remaining laboratories reported using total reflection x-ray fluorescence spectroscopy (TXRF) (4 %) or a method is not specified (12 %).
- Forty-three laboratories enrolled in this exercise and received samples to measure zinc. Twenty-eight laboratories reported results for the spinach leaves (65 % participation) and 27 laboratories reported results for the spirulina powder (63 % participation).
    - The consensus mean for zinc in spinach leaves was within the target range, while the consensus mean for zinc in the spirulina was on the upper edge of the target range. The between-laboratory variability was acceptable for both the dried spinach leaves and the spirulina (16 % and 24 %, RSD respectively).
    - Almost half of the laboratories reported using ICP-MS (47 %) as their analytical method for measuring zinc. The remaining laboratories reported using ICP-OES, (35 %), atomic absorption spectroscopy (AAS) (7 %), TXRF (4 %), or did not report a method (7 %).

Technical Recommendations

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

- No trends were observed based on sample preparation or analytical method used.
- As shown in **Figure 5**, laboratory results follow a linear trend. Many laboratories reported data for potassium that was consistently biased for both samples, but there were also a large number of laboratories that reported the correct value for one sample but a low, or high, value for the second sample.
    - When laboratories report data biased for both samples the source is usually a calibration issue. A linear calibration curve which surrounds the expected sample concentration values should be used for calculation. This curve should include both the lowest and highest expected concentration values of the sample solutions. Extrapolation of results beyond calibration curves may result in false values.
    - For laboratories whose results do not follow this trend (i.e., reported the correct value for one sample but a low, or high, value for the second sample), the cause may be due to one sample that is more difficult to digest than the other.
- Results for zinc (**Figures 3, 4, and 6**) indicate laboratory results for the spirulina sample were almost exclusively biased high relative to the expected value, where results for the spinach sample were more likely to be biased low.
    - There may be more difficulty in the digestion of one sample material over the other.
    - A matrix interference may be present in one or both samples. The use of internal standards may reduce the impact of matrix interferences.

    - The level of zinc in the spirulina samples was approximately an order of magnitude lower than the level in the spinach samples. If proper calibration curves were not constructed, extrapolation of a higher curve to lower values may result in the observed bias.
- For both potassium and zinc, several laboratories reported data significantly outside of the target and consensus ranges. The use of appropriate quality assurance samples to establish that a method is in control and performing correctly may reduce the likelihood of outlying data. Quality assurance samples can be commercially available reference materials (CRMs, SRMs, or RMs) or prepared in-house.
- All results should be checked closely to avoid calculation errors and to be sure that results are reported in the requested units.

Table 1. Individualized data summary table (NIST) for potassium and zinc in spinach and spirulina.

National Institute of Standards & Technology

Exercise M - March 2016 - Potassium and Zinc

	Lab Code:	**NIST**	**1. Your Results**				**2. Community Results**			**3. Target**	
Analyte	Sample	Units	x_i	s_i	Z'_{comm}	Z_{NIST}	N	x*	s*	x_{NIST}	U_{95}
Potassium	Spinach	mg/kg	27500	247		0.00	25	25864	4163	27500	247
Potassium	Spirulina	mg/kg	15300	370		0.00	25	15123	3343	15300	370
Zinc	Spinach	mg/kg	78.1	3.7		0.00	28	74	12	78	4
Zinc	Spirulina	mg/kg	7.77	0.98		0.00	26	9.7	2.3	7.8	1.0

x_i Mean of reported values

s_i Standard deviation of reported values

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

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

N Number of quantitative values reported

x* Robust mean of reported values

s* Robust standard deviation

x_{NIST} NIST-assessed value

U_{95} ±95% confidence interval about the assessed value or standard deviation (s_{NIST})

Table 2. Data summary table for potassium in spinach leaves and spirulina. Data points highlighted in red have been flagged as potential outliers (e.g., difference from reference value, Grubb and/or Cochran) by the NIST software package.

		Potassium									
		SRM 1570a Trace Elements in Spinach Leaves (mg/kg)					Commercial Spirulina Powder (mg/kg)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	NIST				27500	250				15300	370
	M003										
	M005										
	M006										
	M010	30750	30400	31250	30800	427	17560	18000	17560	17707	254
	M011										
	M012	28973	32165	34144	31761	2609	25671	48514	19861	31349	15147
	M015	25	25	26	25	1	15	14	16	15	1
	M016	24621	19041	18942	20868	3251	10859	10632	10150	10547	362
	M017										
	M019	28000	27800	26300	27367	929	16100	15700	15700	15833	231
	M020	5280	5226	5265	5257	28	3082	3109	3009	3067	52
	M022	26314	27099	27253	26889	504	14816	15379	15103	15099	282
	M025										
	M026	27983	28235	27572	27930	335	16311	16186	16076	16191	118
	M028	28413	28233	28507	28384	139	15434	15617	15871	15641	219
	M032										
	M033	27175	26992	26519	26895	339	16339	16134	16054	16176	147
	M035	26200	26600	26600	26467	231	15600	15700	15700	15667	58
	M036										
	M037	25200	23600	23500	24100	954	17400	15900	10500	14600	3629
	M039										
	M041	26300	28000	28000	27433	981	15300	15300	15200	15267	58
	M042	25335	24565	24650	24850	422	14245	14990	15065	14767	453
	M046	27000	27400	24600	26333	1514	15800	15400	16400	15867	503
	M048										
	M051										
	M056	28655	27818	28347	28274	423	16562	16365	16606	16511	129
	M058	27113	28683	28761	28186	930	15667	18277	14608	16184	1888
	M061	25100	27100	27400	26533	1250	15000	15500	15400	15300	265
	M064	30100	30440	29470	30003	492	17300	16550	16630	16827	412
	M065	23565	23904	23069	23513	420	19349	19145	18944	19146	203
	M068	28	28	28	28	0	15	15	15	15	0
	M070										
	M071	28000	28000	29100	28367	635	16300	16200	16500	16333	153
	M073	23675	23185	23680	23513	284	13655	13880	13510	13682	186
	M074	3680	3754	3707	3714	37	2325	2394	2385	2368	38
	M075	30145	30275	28173	29531	1178	34142	34335	34119	34199	119
Community Results		Consensus Mean			25864		Consensus Mean			15123	
		Consensus Standard Deviation			4163		Consensus Standard Deviation			3343	
		Maximum			31761		Maximum			34199	
		Minimum			25		Minimum			15	
		N			25		N			25	

Table 3. Data summary table for zinc in spinach leaves and spirulina. Data points highlighted in red have been flagged as potential outliers (e.g., difference from reference value, Grubb and/or Cochran) by the NIST software package.

		Zinc									
		SRM 1570a Trace Elements in Spinach Leaves (mg/kg)					Commercial Spirulina Powder (mg/kg)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	NIST				78.1	3.7				7.77	0.98
	M002										
	M003										
	M005										
	M006										
	M007	86.0	102.0	80.0	89.3	11.4	10.00	11.00	10.00	10.33	0.58
	M008	89.5	90.5	89.9	90.0	0.5	9.90	10.10	10.30	10.10	0.20
	M010	83.6	81.8	83.5	83.0	1.0	10.89	10.50	10.97	10.79	0.25
	M011										
	M012	1.0	1.0	1.0	1.0	0.0	1.00	1.00	1.00	1.00	0.00
	M014										
	M015	0.1	0.1	0.1	0.1	0.0	0.01	0.01	0.01	0.01	0.00
	M016	68.7	69.2	70.8	69.6	1.1	8.65	8.95	8.61	8.74	0.19
	M017										
	M019	65.0	67.0	67.0	66.3	1.2	9.70	8.50	8.30	8.83	0.76
	M020	34.3	20.4	27.5	27.4	7.0	11.70	3.80	3.70	6.40	4.59
	M022	78.0	80.3	80.0	79.4	1.3	9.40	9.50	9.30	9.40	0.10
	M025										
	M026	86.5	86.4	85.7	86.2	0.4	12.00	12.00	12.70	12.23	0.40
	M028	194.0	204.0	210.0	202.7	8.1	59.00	51.00	58.00	56.00	4.36
	M030										
	M032										
	M033	79.9	73.0	74.2	75.7	3.7	8.60	8.40	8.70	8.57	0.15
	M035	76.5	75.2	76.1	75.9	0.7	9.50	9.10	9.10	9.23	0.23
	M036										
	M037	79.0	74.1	74.5	75.9	2.7	19.20	12.60	7.60	13.13	5.82
	M039										
	M040	65.9	66.6	65.3	65.9	0.7	7.89	7.54	7.24	7.56	0.33
	M041	74.2	79.9	80.0	78.0	3.3	9.10	8.87	10.60	9.52	0.94
	M042	70.7	69.0	66.8	68.8	2.0	12.10	10.40	11.40	11.30	0.85
	M046	81.7	83.4	84.1	83.1	1.2	< 9.70	9.30	< 9.70	9.30	
	M048										
	M051										
	M056	66.3	65.4	63.9	65.2	1.2	9.11	8.71	8.85	8.89	0.20
	M058	76.8	80.6	78.9	78.8	1.9	16.45	14.22	12.78	14.48	1.85
	M061	71.5	70.2	70.4	70.7	0.7	9.76	8.89	9.12	9.26	0.45
	M064	81.4	79.1	79.7	80.1	1.2	9.09	9.59	9.32	9.34	0.25
	M065	56.0	55.0	53.5	54.8	1.2	7.37	6.92	7.76	7.35	0.42
	M068	74.5	73.2	72.9	73.5	0.9	9.48	9.75	9.49	9.57	0.15
	M070										
	M071	80.8	78.7	81.1	80.2	1.3	10.30	10.70	10.70	10.57	0.23
	M073	61.5	62.3	64.2	62.7	1.4					
	M074	82.7	84.4	80.6	82.6	1.9	12.02	13.21	14.35	13.19	1.17
	M075	77.8	77.6	78.1	77.8	0.3	9.81	9.76	9.45	9.67	0.20
Community Results		Consensus Mean			74.1		Consensus Mean			9.71	
		Consensus Standard Deviation			12.1		Consensus Standard Deviation			2.35	
		Maximum			202.7		Maximum			56.00	
		Minimum			0.1		Minimum			0.01	
		N			28		N			26	

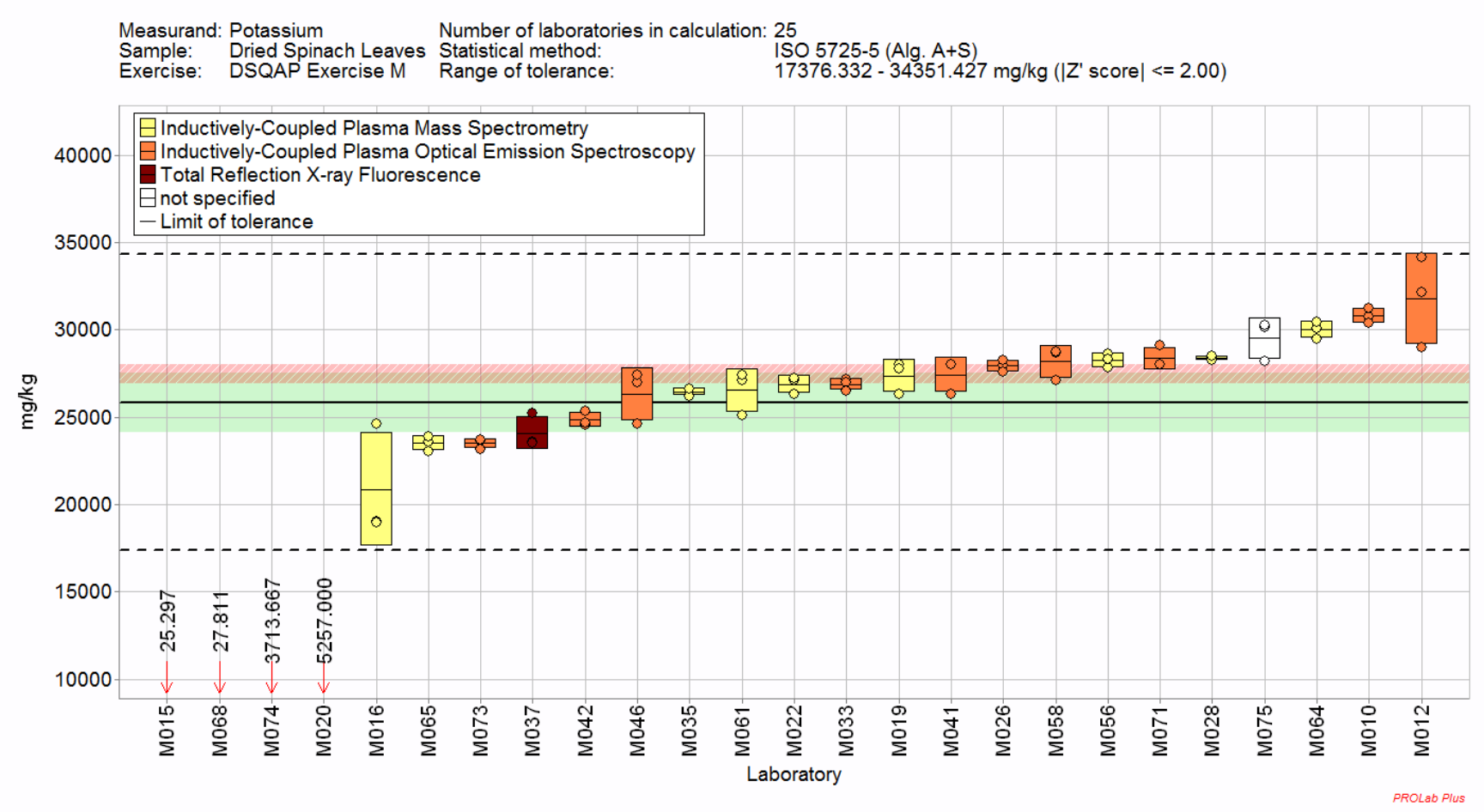

Figure 1. Potassium in SRM 1570a Trace Elements in Spinach Leaves (data summary view –analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents the consensus mean, and the green shaded region represents the consensus mean bounded by twice the consensus standard error. The black dashed lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{comm}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the NIST certified value bounded by twice its uncertainty (U_{95}), and represents the range that results in an acceptable Z'_{NIST} score, $|Z'_{NIST}| \leq 2$.

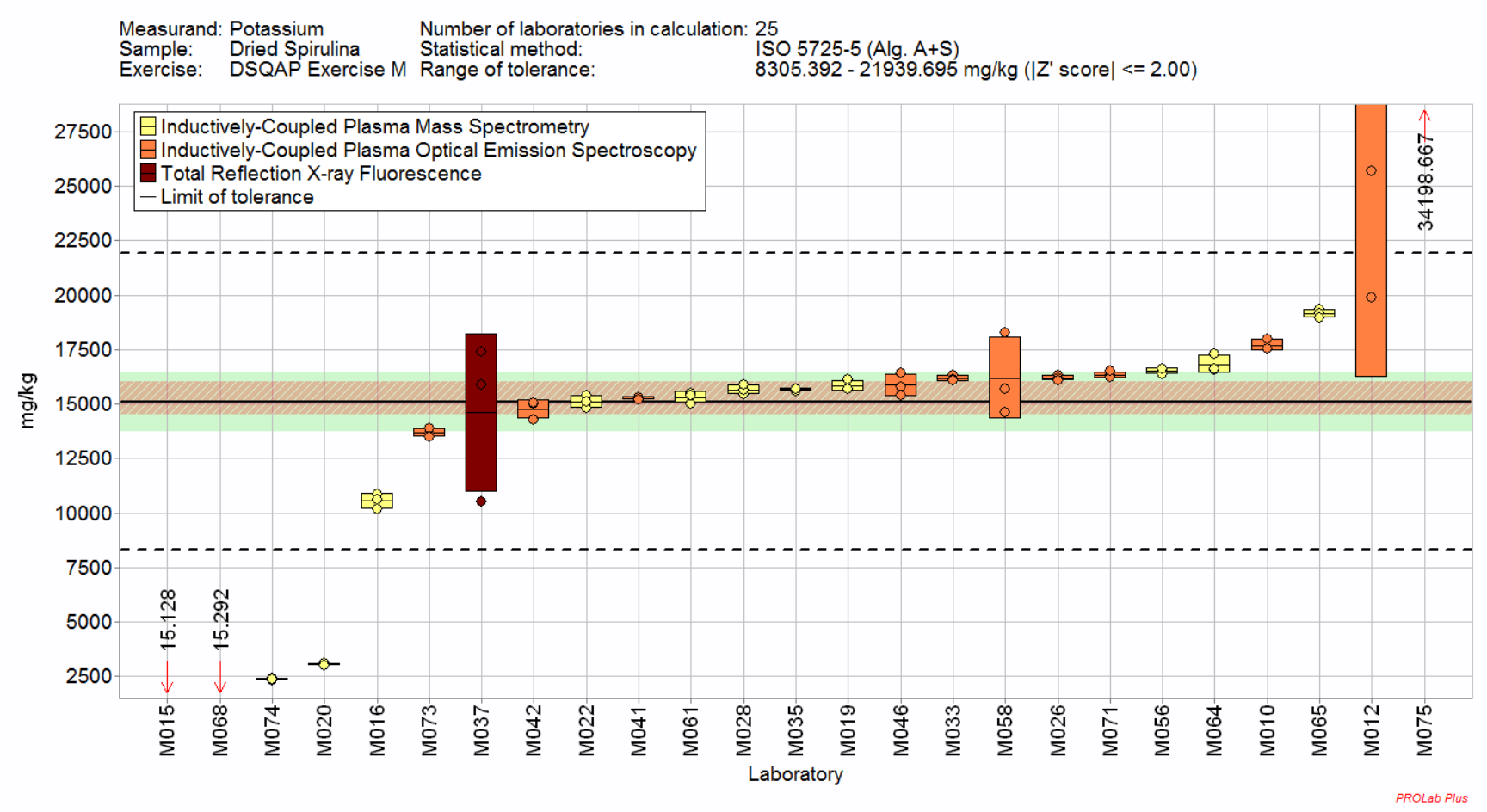

Figure 2. Potassium in spirulina (data summary view –analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents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

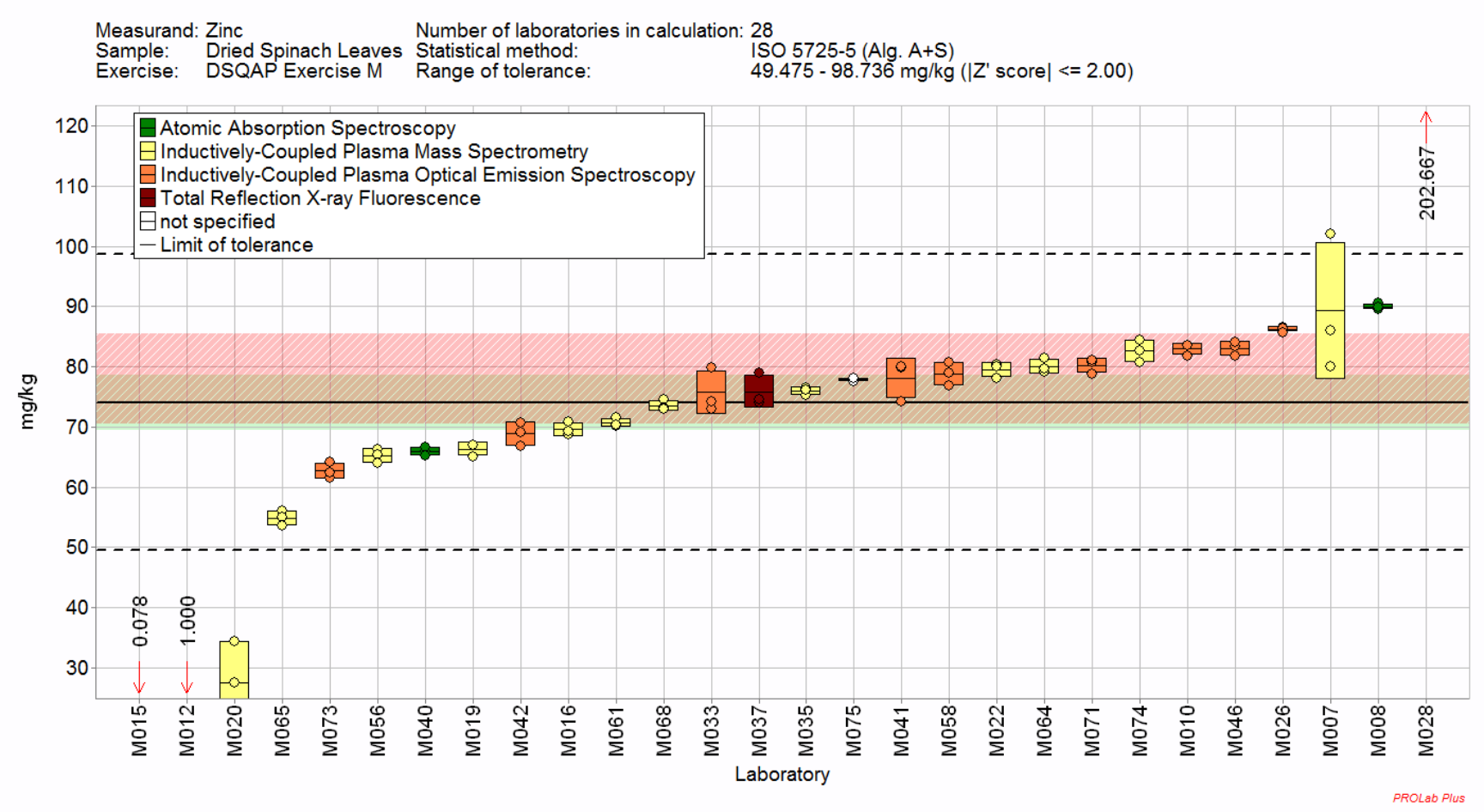

Figure 3. Zinc in SRM 1570a Trace Elements in Spinach Leaves (data summary view –analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents the consensus mean, and the green shaded region represents the consensus mean bounded by twice the consensus standard error. The black dashed lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{comm}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the NIST certified value bounded by twice its uncertainty (U_{95}), and represents the range that results in an acceptable Z'_{NIST} score, $|Z'_{NIST}| \leq 2$.

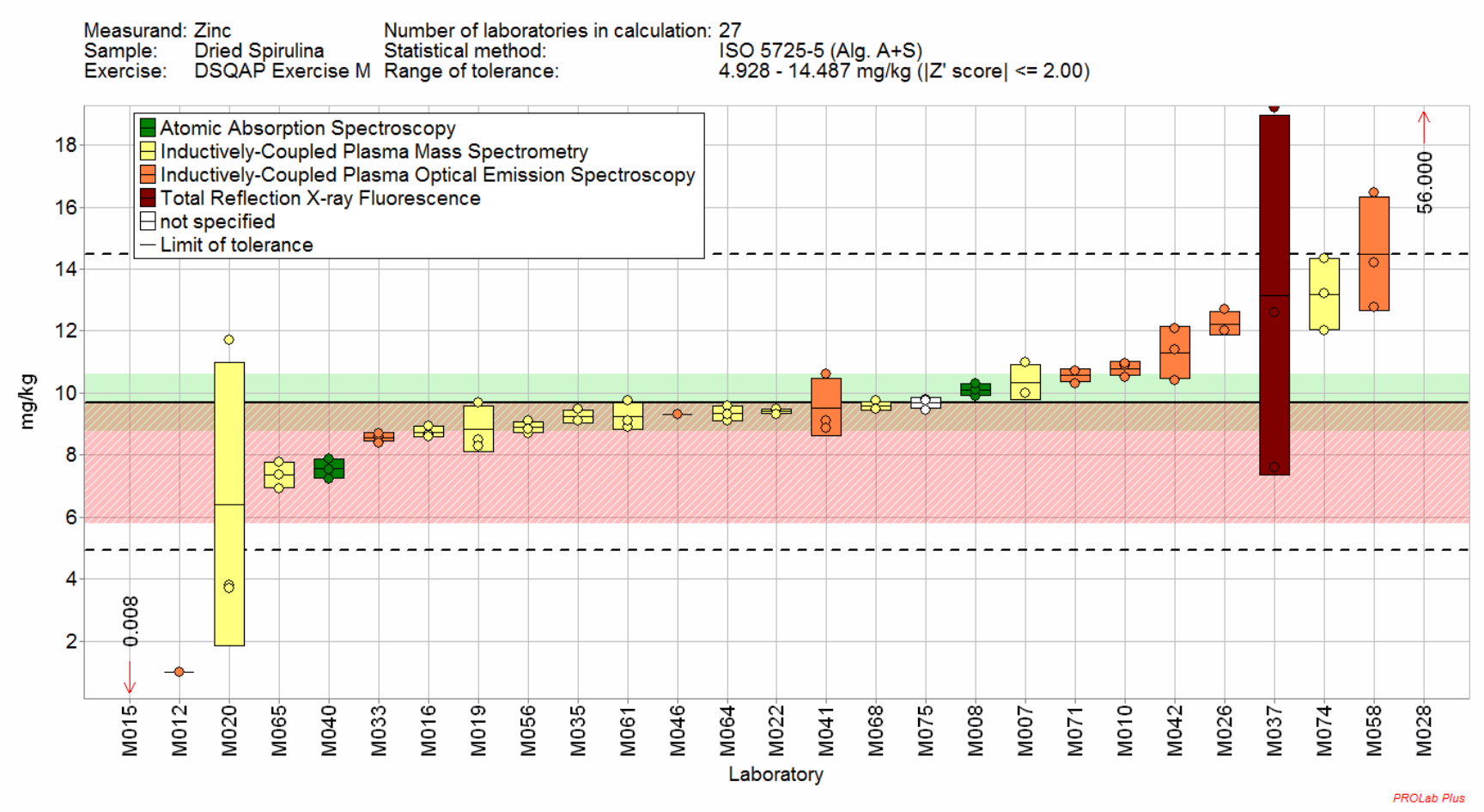

Figure 4. Zinc in spirulina (data summary view –analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents the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

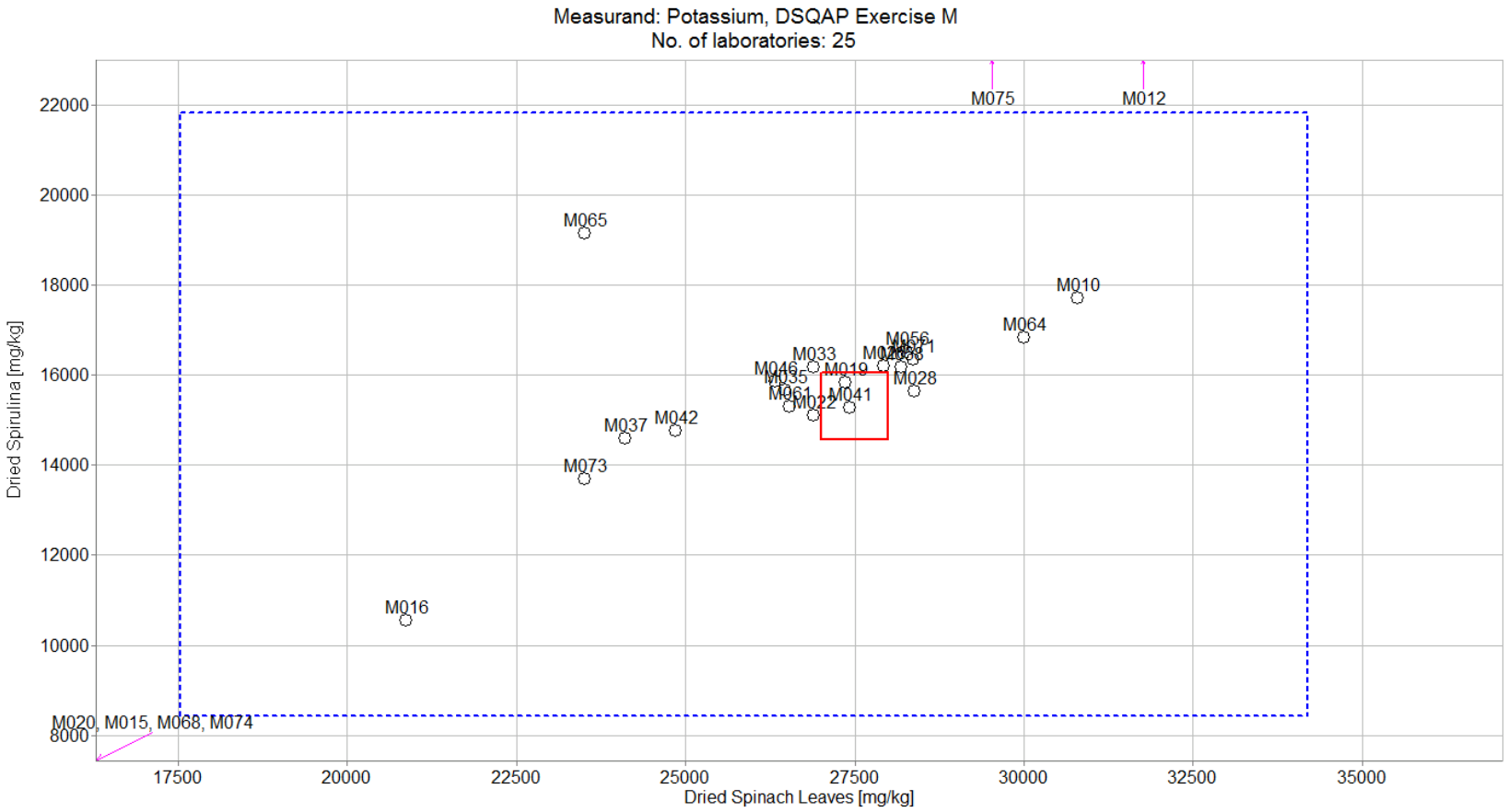

Figure 5. Laboratory means for potassium in SRM 1570a Trace Elements in Spinach Leaves and spirulina (sample/sample comparison view). In this view, the individual laboratory mean for one sample (spinach leaves) is compared to the mean for a second sample (spirulina). The solid red box represents the NIST range of tolerance for the two samples, spinach leaves (x-axis) and spirulina (y-axis), which encompasses the NIST values bounded by twice their uncertainties (U_{95}), 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 spinach leaves (x-axis) and spirulina (y-axis), calculated as the values above and below the consensus means that result in an acceptable Z'_{comm} score, $|Z'_{comm}| \leq 2$.

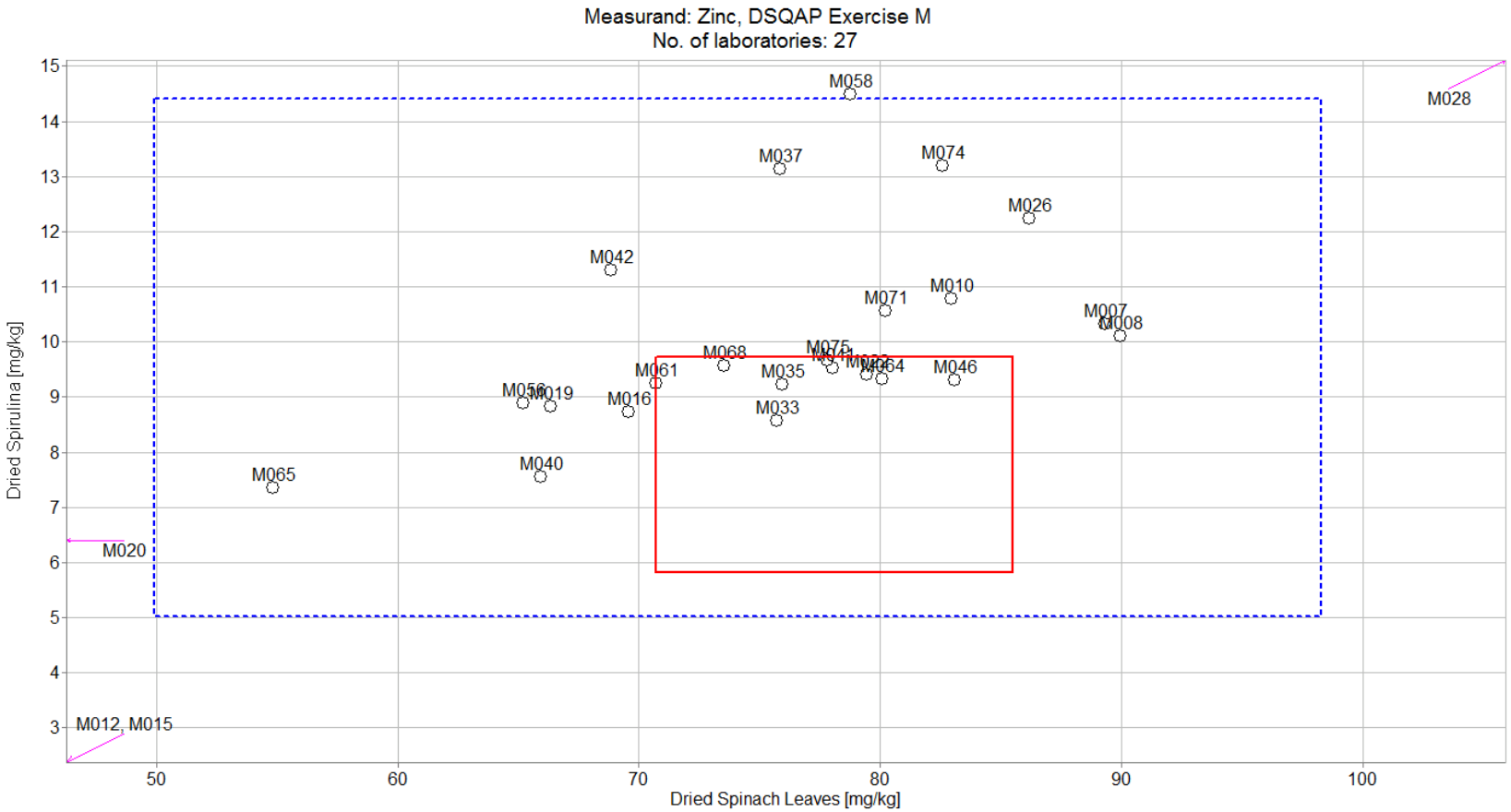

Figure 6. Laboratory means for zinc in SRM 1570a Trace Elements in Spinach Leaves and spirulina (sample/sample comparison view). In this view, the individual laboratory mean for one sample (spinach leaves) is compared to the mean for a second sample (spirulina). The solid red box represents the NIST range of tolerance for the two samples, spinach leaves (x-axis) and spirulina (y-axis), which encompasses the NIST values bounded by twice their uncertainties (U_{95}), 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 spinach leaves (x-axis) and spirulina (y-axis), calculated as the values above and below the consensus means that result in an acceptable Z'_{comm} score, $|Z'_{comm}| \leq 2$.

TOXIC ELEMENTS (PB AND AS) IN GINGER AND GINSENG DIETARY SUPPLEMENTS

Study Overview

In this study, participants were provided with two candidate NIST SRMs, SRM 3398 Ginger (*Zingiber officinale)* Rhizome and SRM 3384 Asian Ginseng (*Panax ginseng)* Rhizome. Participants were asked to use in-house analytical methods to determine the mass fractions of lead (Pb) and total arsenic (As) in each of the matrices and report values on an as-received basis. Additionally, participants were asked to determine arsenic species and to report the mass fractions of arsenic species on an as-received basis.

Sample Information

Ginger Rhizome. Participants were provided with three packets, each containing approximately 1.6 g of dried ginger rhizome. The dried rhizomes were ground, homogenized, and packaged inside 4 mil polyethylene bags, which were then sealed inside nitrogen-flushed aluminized plastic bags along with two packets of silica gel. Before use, participants were instructed to thoroughly mix the contents of each packet and use a sample size of at least 0.5 g. Participants were asked to store the material at controlled room temperature, 20 °C to 25 °C, and to report a single value from each packet provided. Approximate analyte levels were not reported to participants prior to the study. The target values for arsenic and lead in SRM 3398 Ginger (*Zingiber officinale)* Rhizome were determined at NIST using ICP-MS. The NIST-determined values and uncertainties for As and Pb are provided in the table below, on an as-received basis.

Analyte	NIST-Determined Mass Fraction in Ginger (ng/g) (as-received basis)
Lead (Pb)	1369 ± 52
Total Arsenic (As)	46900 ± 3500

A procedure-defined method was used to determined values for arsenic acid (AsV), arsenous acid (AsIII), and total inorganic arsenic (iAs) in SRM 3398 Ginger (*Zingiber officinale)* Rhizome at NIST. A 0.5 g sample was vortexed for 1 min with 10 g of sub-boiled distilled water in a centrifuge tube and allowed to sit overnight (16 h). The contents were then vortexed for 30 s and centrifuged for 30 min. The extract was t and an aliquot of arsenobetaine solution was added as an internal standard. A 1 g aliquot of the extract was centrifuged for 10 min, and aliquots of the supernatant were transferred to 15 mL centrifuge tubes to determine AsIII and AsV using the method of standard additions. Inorganic arsenic was determined as iAs = AsIII + AsV. Total arsenic in the extract was determined by a digestion of the extract and analysis using ICP-MS. The NIST-determined values and uncertainties for AsIII, AsV, iAs, and total As in the extracted sample are provided in the table below, on an as-received basis.

Analyte	NIST-Determined Mass Fraction in Ginger (ng/g) (as-received basis)
Arsenous Acid (AsIII)	3144 ± 478
Arsenic Acid (AsV)	1241 ± 301
Total Inorganic Arsenic (iAs)	4385 ± 200
Total Arsenic (As, in extract)	4425 ± 291*

* uncertainty expressed as 1 standard deviation (n=9)

Asian Ginseng Rhizome. Participants were provided with three packets, each containing approximately 3 g of dried Asian ginseng rhizome. The dried rhizomes were ground, homogenized, and packaged inside 4 mil polyethylene bags, which were then sealed inside nitrogen-flushed aluminized plastic bags along with two packets of silica gel. Before use, participants were instructed to thoroughly mix the contents of each packet and use a sample size of at least 0.5 g. Participants were asked to store the material at controlled room temperature, 20 °C to 25 °C, and to report a single value from each packet provided. Approximate analyte levels were not reported to participants prior to the study. The target values for arsenic and lead in SRM 3384 Asian Ginseng (*Panax ginseng)* Rhizome were determined at NIST using ICP-MS. The NIST-determined values and uncertainties for As and Pb are provided in the table below, on an as-received basis. Arsenic species were not determined in Asian ginseng.

Analyte	NIST-Determined Mass Fraction in Ginseng (ng/g) (as-received basis)
Lead (Pb)	6330 ± 550
Arsenic (As)	395 ± 32

Study Results

- Forty-seven laboratories enrolled in this exercise and received samples. Thirty-six laboratories reported results for lead in ginger rhizome (77 % participation). Thirty-five laboratories reported results for lead in Asian ginseng rhizome (74 % participation).
  - The consensus means for lead in both materials were within the target ranges with good between-laboratory variability (12 % and 17 % RSD) for the ginger and ginseng, respectively.
  - Most laboratories reported using ICP-MS as their analytical method for analysis for lead (94 %). Laboratories also reported using AAS (3 %) and ICP-OES (3 %).
- Thirty-five of the forty-seven enrolled laboratories reported results for total arsenic in ginger rhizome (74 % participation). Thirty-six laboratories reported results for total arsenic in Asian ginseng rhizome (77 % participation).
  - The consensus mean for total arsenic in the ginger rhizome was within the target range with high between-laboratory variability (32 % RSD).
  - The consensus mean for total arsenic in the Asian ginseng rhizome was below the target range with good between-laboratory variability (17 % RSD).
  - Most laboratories reported using ICP-MS as their analytical method for analysis of total arsenic (92 %). Laboratories also reported using AAS (3 %), ICP-OES (3 %), and TXRF (3 %).

- Twenty of the forty-seven laboratories enrolled in the arsenic speciation study (43 %). Of those, results were reported by four laboratories for total inorganic arsenic in ginger rhizome (20 % participation) and by three laboratories in Asian ginseng rhizome (15 % participation). Two laboratories reported results for AsIII and AsV in both materials (10 % participation).

Technical Recommendations

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

- Arsenic is volatile and can be lost during sample preparation, resulting in data that is biased low. The high temperatures of a vigorous microwave digestion should convert all volatile organoarsenic species to arsenic acid (AsV), at which point subsequent heating will not result in loss of arsenic.
  - Open-beaker digestion should not be used for As analysis.
  - Closed-vessel digestions should be used with care for As analysis, ensuring that no As is lost as a result of inadvertent venting.
  - Higher temperatures or the use of a small amount of HF may be needed to ensure complete digestion of plant materials for analysis of As.
- Lead is easily digested and volatile loss of Pb is not a concern. However, digestion with HCl may form a highly insoluble $PbCl_2$ precipitate so digestion with HNO_3 is recommended. Dry ashing with a small volume of acid is another recommended technique.
    - Both sample materials had high levels of lead. ICP-MS or AAS are recommend for analysis of low levels of Pb. Sensitivity of Pb is poor when using ICP-OES.
    - Some laboratories had high sample-to-sample variability for lead (20 % to > 50 %). This could be caused by incomplete sample digestion, matrix interferences, or calibration curves which do not encompass all sample solutions measured.
      - Calibration curves must be linear and include the lowest values expected to be measured and the highest values to be measured. Extrapolation of the curve may cause incorrect results.
    - An appropriate number of procedural blanks are important, and can be critical when sample concentrations are near the detection limit.
  - Calculation errors may be a cause for incorrect results. Using a quality assurance material (CRM, SRM, RM), or in-house prepared material, to establish that a method is in control will also help find calculation errors. Once a method and quality assurance material appear to be in control, be sure results are reported in the correct units.
  - An optimum extraction procedure extracts all arsenic species in a sample without causing a change in the speciation of the analytes. Mixtures of non-oxidizing neutral solvents consisting water and methanol were investigated at NIST for the extraction of arsenic species because acidic and basic solvents can hydrolyze certain arsenic species while oxidizing agents can change AsIII to AsV. Water was found to be the most effective solvent to extract the arsenic in ginger rhizome under aforementioned constraints for the measurement of AsIII and AsV in the extract of ginger rhizome; however, the extraction efficiency was low at ~ 10%.

Table 4. Individualized data summary table (NIST) for lead and arsenic in ginger and ginseng rhizome dietary supplements.

National Institute of Standards & Technology

Exercise M - March 2016 - Lead and Arsenic

	Lab Code:	**NIST**	**1. Your Results**				**2. Community Results**			**3. Target**	
Analyte	Sample	Units	x_i	s_i	Z'_{comm}	Z_{NIST}	N	x*	s*	x_{NIST}	U_{95}
Lead	Ginger	ng/g	1369	52		0.00	36	1308	160	1369	52
Lead	Ginseng	ng/g	6330	550		0.00	35	5801	995	6330	550
Total Arsenic	Ginger	ng/g	46900	3500		0.00	35	42294	13524	46900	3500
Total Arsenic	Ginseng	ng/g	395.0	32.0		0.00	36	320	55	395.0	32.0
Total Inorganic Arsenic	Ginger	ng/g	4385	200		0.00	4	24328	19856	4385	200
Total Inorganic Arsenic	Ginseng	ng/g					3	225	50		
Arsenic III	Ginger	ng/g	3144	478		0.00	2	18595	20888	3144	478
Arsenic III	Ginseng	ng/g					2	73.2	80.5		
Arsenic V	Ginger	ng/g	1241	301		0.00	1	10573	0	1241	301
Arsenic V	Ginseng	ng/g					2	134.8	33.2		

x_i Mean of reported values

s_i Standard deviation of reported values

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

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

N Number of quantitative values reported

x* Robust mean of reported values

s* Robust standard deviation

x_{NIST} NIST-assessed value

U_{95} ±95% confidence interval about the assessed value or standard deviation (s_{NIST})

Table 5. Data summary table for lead in ginger and ginseng rhizome dietary supplements. Data highlighted in red have been flagged as potential outliers (e.g., difference from reference value, Grubb and/or Cochran) by the NIST software package.

		Lead									
		Ginger (ng/g)					Ginseng (ng/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	NIST				1369	52				6330	550
	M002	12000	13100	12600	12567	551	5210	5230	5000	5147	127
	M005										
	M006										
	M007	1257	1289	1360	1302	53	4327	4359	4585	4424	141
	M010	1335	1274	1342	1317	37	13441	5746	4626	7938	4799
	M011										
	M012	1595	1545	1560	1567	26	6422	8413	7060	7298	1017
	M015	1163	1653	1219	1345	268	5919	5628	7410	6319	956
	M016	1526	1663	1465	1552	101	7025	6881	6869	6925	87
	M017	1312	1322	1321	1318	6	4478	6107	6069	5551	930
	M019	1160	1090	1145	1132	37	5005	6035	4880	5307	634
	M021	1442	1156	1318	1305	143	4380	5320	5775	5158	711
	M022	1256	1288	1263	1269	17					
	M023	1255	1250	1213	1239	23	5755	5195	5620	5523	292
	M025	1427	1282	1405	1371	78	5884	8856	5858	6866	1723
	M026	1320	1350	1370	1347	25	6870	5210	5340	5807	923
	M028	1309	1315	1294	1306	11	5868	5407	6261	5845	427
	M029	1170	1290	1260	1240	62	5870	5710	5550	5710	160
	M030	1300	1330	1280	1303	25	5170	6060	7810	6347	1343
	M031	1163	1653	1219	1345	268	5919	5628	7410	6319	956
	M032										
	M033	1350	1260	1410	1340	75	5470	5260	4990	5240	241
	M035	1170	1130	1200	1167	35	4180	4540	4220	4313	197
	M036										
	M037										
	M039	1.2	1.1	1.0	1.1	0.1	0.4	0.3	0.3	0.3	0.1
	M040	961	951	970	961	10	5530	5550	5570	5550	20
	M041	1310	1340	1340	1330	17	5740	6060	6000	5933	170
	M042	1371	1359	1385	1372	13	5717	6123	5809	5883	213
	M046	1230	1290	2920	1813	959	4830	5120	7120	5690	1247
	M047	1390	1330	1310	1343	42	5560	5580	6230	5790	381
	M048										
	M050	1279	1366	1323	1323	44	5220	7102	5063	5795	1135
	M051										
	M056	1401	1334	1378	1371	34	7800	7024	5438	6754	1204
	M059										
	M061	1180	1290	1270	1247	59	5630	5870	5470	5657	201
	M063	1117	1149	1183	1150	33	4399	15552	8481	9477	5643
	M064	1182	1131	1174	1162	27	5045	4961	5043	5016	48
	M065	1262	1285	1312	1286	25	4991	6820	6379	6063	954
	M066	1303	1355	1360	1339	31	5422	4991	6702	5705	890
	M067	1480	1320	1710	1503	196	5250	3920	8380	5850	2290
	M068	1.3	1.3	1.3	1.3	0.0	5.3	5.3	5.3	5.3	0.0
	M070										
	M071										
	M074	1052	1016	1048	1039	20	6609	3862	4898	5123	1387
	M075	2327	2199	2039	2188	144	6873	7346	6333	6851	507
Community Results		Consensus Mean			1308		Consensus Mean			5801	
		Consensus Standard Deviation			160		Consensus Standard Deviation			995	
		Maximum			12567		Maximum			9477	
		Minimum			1		Minimum			0.3	
		N			36		N			35	

Table 6. Data summary table for total arsenic in ginger and ginseng rhizome dietary supplements. Data highlighted in red have been flagged as potential outliers (e.g., difference from reference value, Grubb and/or Cochran) by the NIST software package.

		Total Arsenic									
		Ginger (ng/g)					Ginseng (ng/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	NIST				46900	3500				395	32
	M002	12205	10950	11470	11542	631	121	123	119	121	2
	M005										
	M006										
	M007						338	344	328	337	8
	M008	51328	50558	49333	50406	1006	388	380	378	382	5
	M010	29497	29931	28612	29347	672	272	234	223	243	26
	M011										
	M012	54689	59965	54732	56462	3034	422	364	442	409	41
	M015		79416	44298	61857	24832	281	301	229	270	37
	M016	34321	29060	33025	32135	2741	70	85	78	78	7
	M017	38719	52182	34096	41666	9396	287	276	271	278	8
	M019	41050	38100	49150	42767	5722	373	374	371	373	2
	M021	41950	43690	41470	42370	1168	346	329	294	323	27
	M022						340	340	343	341	2
	M023	42763	42475	40352	41863	1317	281	263	281	275	10
	M025	46286	46327	39339	43984	4023	556	470	431	486	64
	M026	4110	37280	44040	28477	21371	300	320	310	310	10
	M028	45111	53175	49218	49168	4032	356	355	358	356	2
	M029	58800	51400	55100	55100	3700	342	339	341	341	2
	M030	39500	58500	53300	50433	9819	358	353	333	348	13
	M031	44553	79416	44298	56089	20202	281	301	229	270	37
	M032										
	M033	48480	47710	56810	51000	5046	< 540	< 540	< 540	< 540	
	M035	44400	46600	46500	45833	1242	305	311	312	309	4
	M036										
	M037	12.3	13.8	18.9	15.0	3.5					
	M039	41.20	41.50	38.00	40.23	2	10.0	5.4	4.4	6.6	3.0
	M041	51500	50500	41300	47767	5623	360	391	348	366	22
	M042	40283	44869	44203	43118	2478	347	312	332	330	18
	M046	33000	31300	29200	31167	1904	331	330	283	315	27
	M047	38200	38800	38400	38467	306	295	338	351	328	29
	M048										
	M050	52429	69747	50005	57394	10767	342	320	329	330	11
	M051										
	M056	36503	32913	29639	33018	3433	255	263	276	265	11
	M059										
	M061	54200	41500	40800	45500	7543	289	294	287	290	4
	M063	65906	116721	44290	75639	37183	297	302	280	293	12
	M064	39090	44430	44930	42817	3237	369	345	344	353	14
	M065	42738	41439	50241	44806	4751	343	363	346	351	11
	M066	40277	47851	51773	46634	5844	329	321	326	325	4
	M067	60300	35100	47300	47567	12602	344	380	315	346	33
	M068	44	44	44	44	0	331	332	331	331	1
	M070						376	376	377	376	1
	M071										
	M074	7829	8134	7019	7661	576	262	231	259	251	17
	M075	45928	54742	44980	48550	5383	391	388	379	386	6
Community Results		Consensus Mean			42294		Consensus Mean			320	
		Consensus Standard Deviation			13524		Consensus Standard Deviation			55	
		Maximum			75639		Maximum			486	
		Minimum			15		Minimum			6.6	
		N			35		N			36	

Table 7. Data summary table for total inorganic arsenic in ginger and ginseng rhizome dietary supplements.

		Total Inorganic Arsenic									
		Ginger (ng/g)					Ginseng (ng/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	NIST				4385	200					
	M002										
	M006										
	M010										
	M016										
	M017	38719	37408	29779	35302	4828					
	M021	41710	43450	41270	42143	1153	190	180	160	177	15
	M022										
	M026										
	M029										
	M030										
	M036										
	M041										
	M042										
	M048										
	M051										
	M056										
	M064										
	M067	4940	4860	2790	4197	1219	217	268	233	239	26
	M068										
	M070	15435	15646	15928	15670	247	251	265	262	259	7
Community Results		Consensus Mean			24328		Consensus Mean			225	
		Consensus Standard Deviation			19856		Consensus Standard Deviation			50	
		Maximum			42143		Maximum			259	
		Minimum			4197		Minimum			177	
		N			4		N			3	

Table 8. Data summary table for arsenic III in ginger and ginseng rhizome dietary supplements.

		Arsenic III									
		Ginger (ng/g)					Ginseng (ng/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	NIST				3144	478					
	M006										
	M017										
	M021	32340	27110	35260	31570	4129	30.0	20.0	20.0	23.3	5.8
	M029										
	M030										
	M041										
	M048										
	M051										
	M056										
	M067	6650	5360	4850	5620	928	139	131	99	123	21
	M068										
	M070										
Community Results		Consensus Mean			18595		Consensus Mean			73	
		Consensus Standard Deviation			20888		Consensus Standard Deviation			80	
		Maximum			31570		Maximum			123	
		Minimum			5620		Minimum			23	
		N			2		N			2	

Table 9. Data summary table for arsenic V in ginger and ginseng rhizome dietary supplements.

		Arsenic V									
		Ginger (ng/g)					Ginseng (ng/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	NIST				1241	301					
	M006										
	M017										
	M021	9370	16340	6010	10573	5269.1	160	160	140	153	11.5
	M029										
	M030										
	M041										
	M048										
	M051										
	M056										
	M067	< 734	< 734	< 734	< 734		79	137	133	116	32
	M068										
	M070										
Community Results		Consensus Mean			10573		Consensus Mean			135	
		Consensus Standard Deviation					Consensus Standard Deviation			33	
		Maximum			10573		Maximum			153	
		Minimum			10573		Minimum			116	
		N			1		N			2	

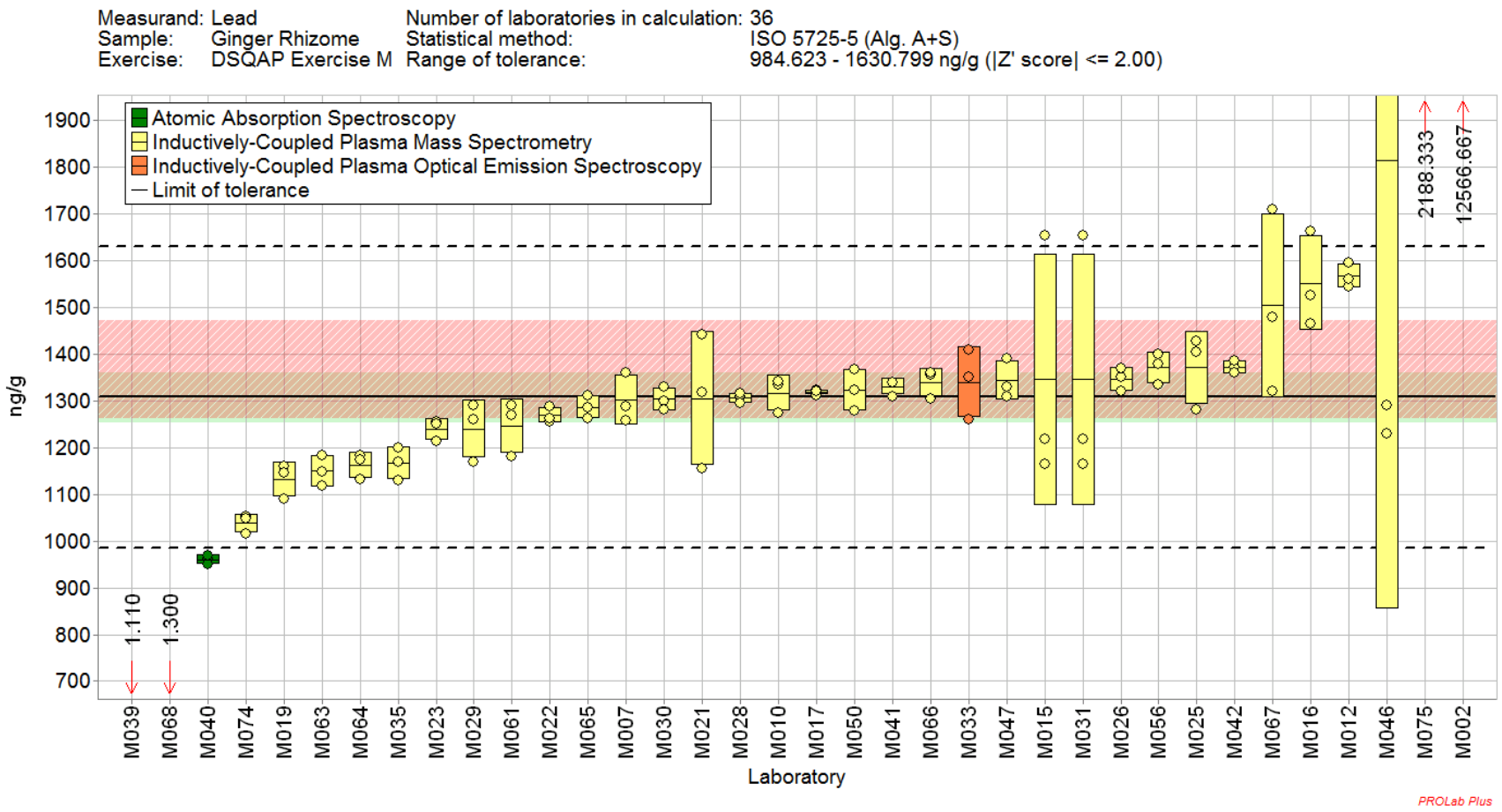

Figure 7. Lead in SRM 3398 Ginger (*Zingiber officinale*) Rhizome (data summary view –analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents the consensus mean, and the green shaded region represents the consensus mean bounded by twice the consensus standard error. The black dashed lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{comm}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the NIST-determined value bounded by twice its uncertainty (U_{95}), and represents the range that results in an acceptable Z'_{NIST} score, $|Z'_{NIST}| \leq 2$.

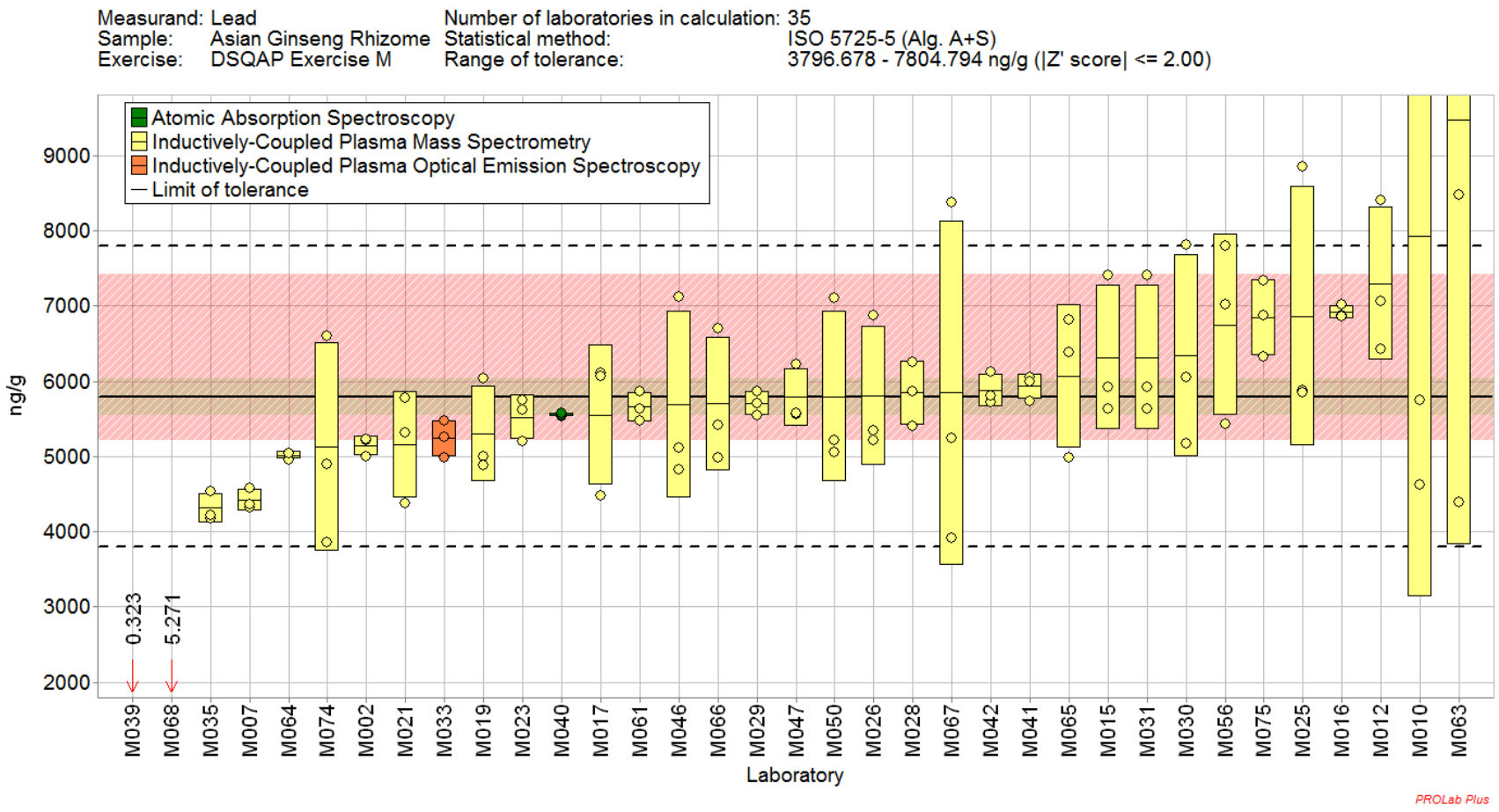

Figure 8. Lead in SRM 3384 Ground Asian Ginseng (*Panax ginseng* C.A. Meyer) Rhizome (data summary view –analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents the consensus mean, and the green shaded region represents the consensus mean bounded by twice the consensus standard error. The black dashed lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{comm}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the NIST-determined value bounded by twice its uncertainty (U_{95}), and represents the range that results in an acceptable Z'_{NIST} score, $|Z'_{NIST}| \leq 2$.

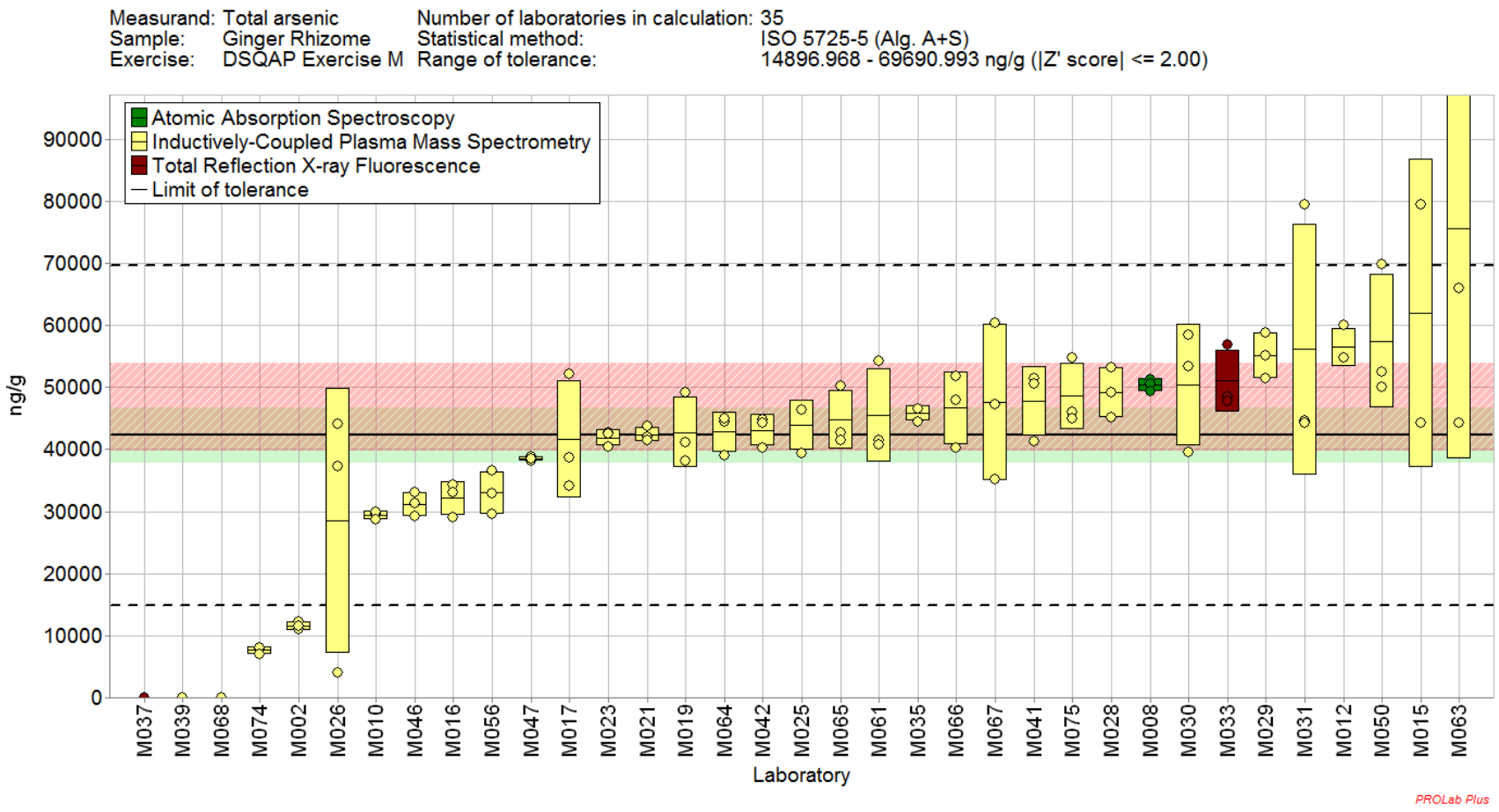

Figure 9. Total arsenic in SRM 3398 Ginger (*Zingiber officinale*) Rhizome (data summary view –analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents the consensus mean, and the green shaded region represents the consensus mean bounded by twice the consensus standard error. The black dashed lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{comm}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the NIST-determined value bounded by twice its uncertainty (U_{95}), and represents the range that results in an acceptable Z'_{NIST} score, $|Z'_{NIST}| \leq 2$.

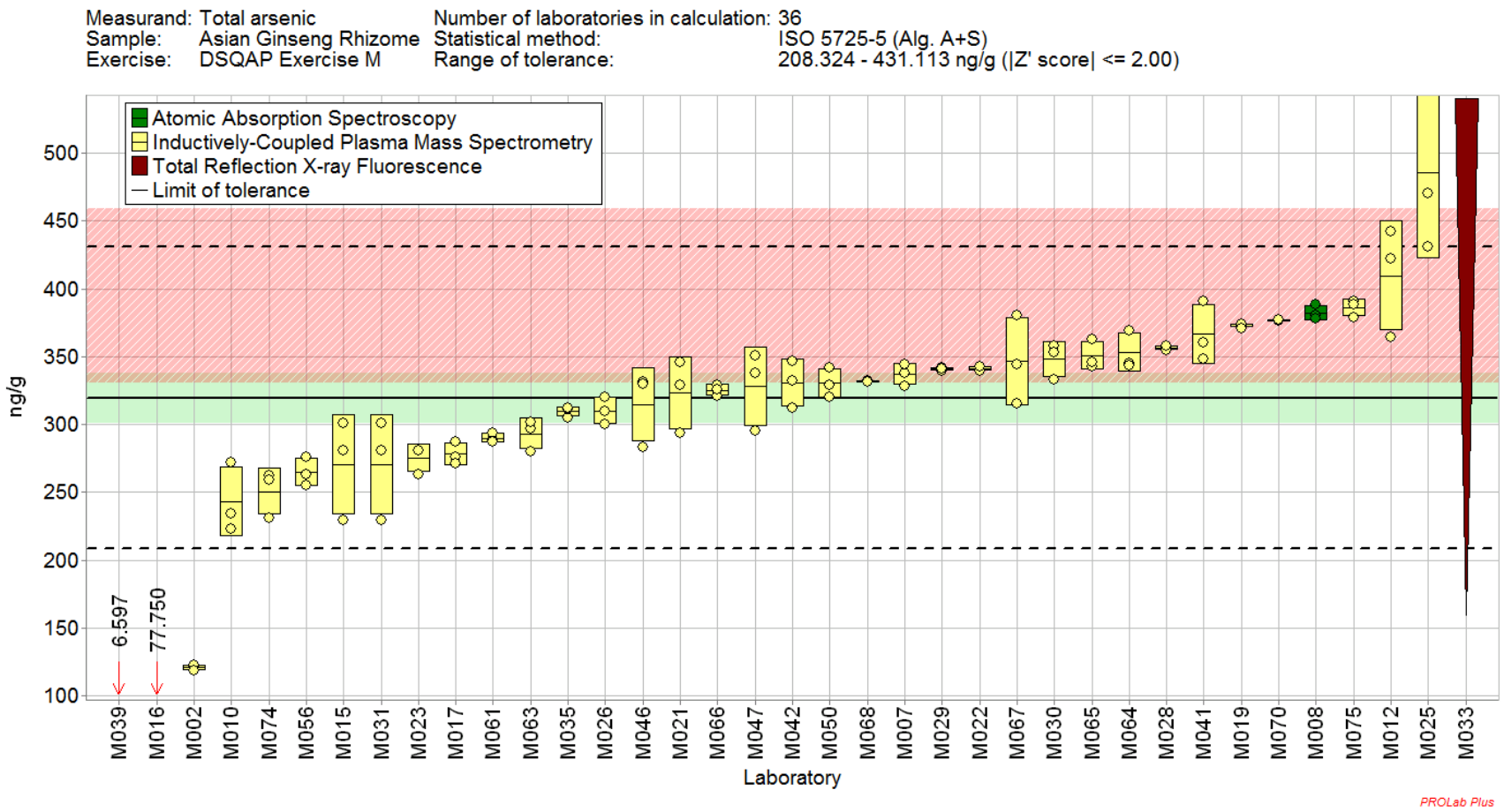

Figure 10. Total arsenic in SRM 3384 Ground Asian Ginseng (*Panax ginseng* C.A. Meyer) Rhizome (data summary view –analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. Laboratory data shown as a triangle indicates that a "less than" result was submitted, and the base of the triangle is displayed at the reported laboratory detection limit. The solid black line represents the consensus mean, and the green shaded region represents the consensus mean bounded by twice the consensus standard error. The black dashed lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{comm}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the NIST-determined value bounded by twice its uncertainty (U_{95}), and represents the range that results in an acceptable Z'_{NIST} score, $|Z'_{NIST}| \leq 2$.

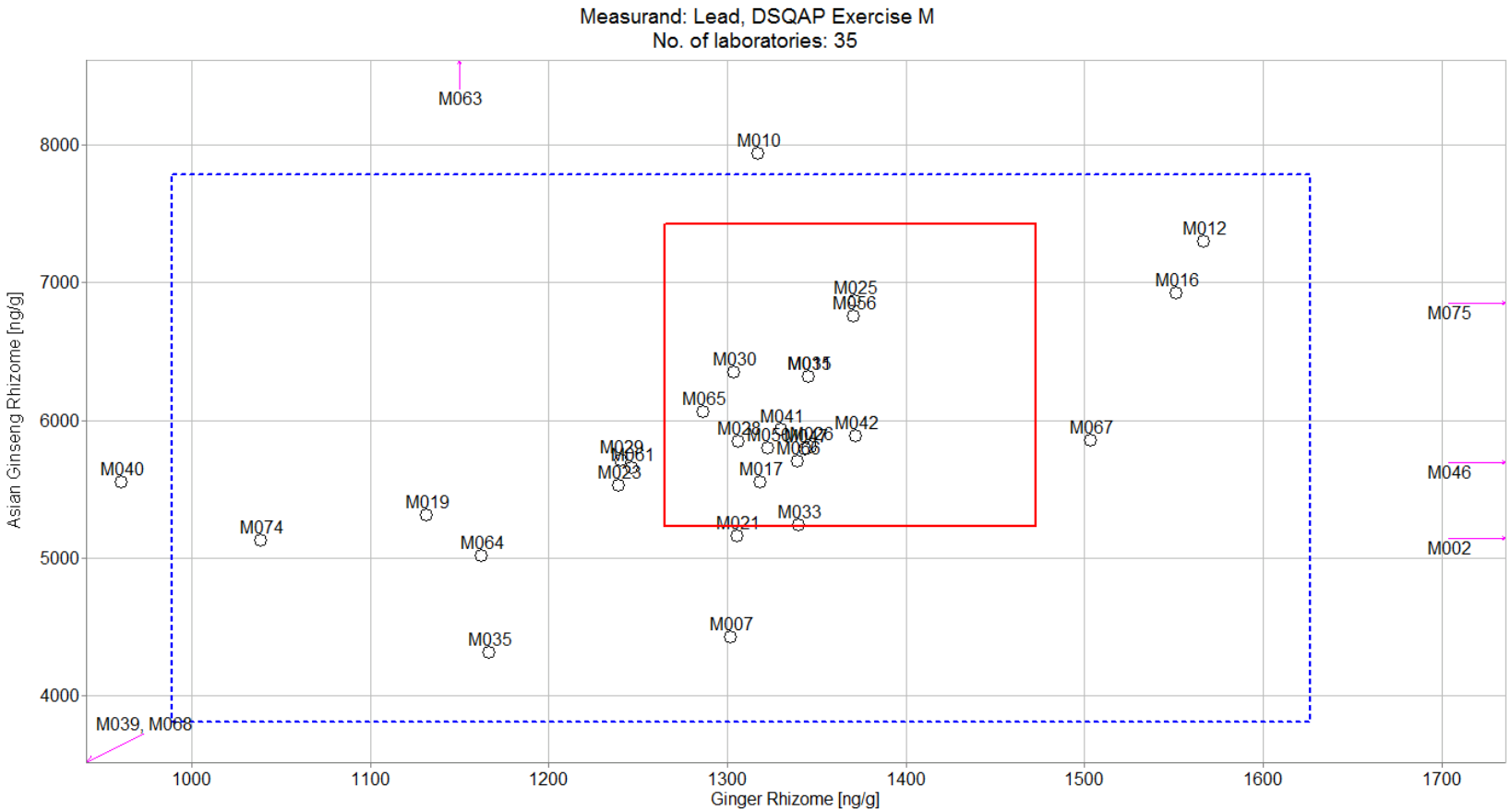

Figure 11. Laboratory means for lead in SRM 3398 Ginger (*Zingiber officinale*) Rhizome and SRM 3384 Ground Asian Ginseng (*Panax ginseng* C.A. Meyer) Rhizome (sample/sample comparison view). In this view, the individual laboratory mean for one sample (ginger rhizome) is compared to the mean for a second sample (Asian ginseng rhizome). The solid red box represents the NIST range of tolerance for the two samples, ginger rhizome (x-axis) and Asian ginseng rhizome (y-axis), which encompasses the NIST values bounded by twice their uncertainties (U_{95}), 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 ginger rhizome (x-axis) and Asian ginseng rhizome (y-axis), calculated as the values above and below the consensus means that result in an acceptable Z'_{comm} score, $|Z'_{comm}| \leq 2$.

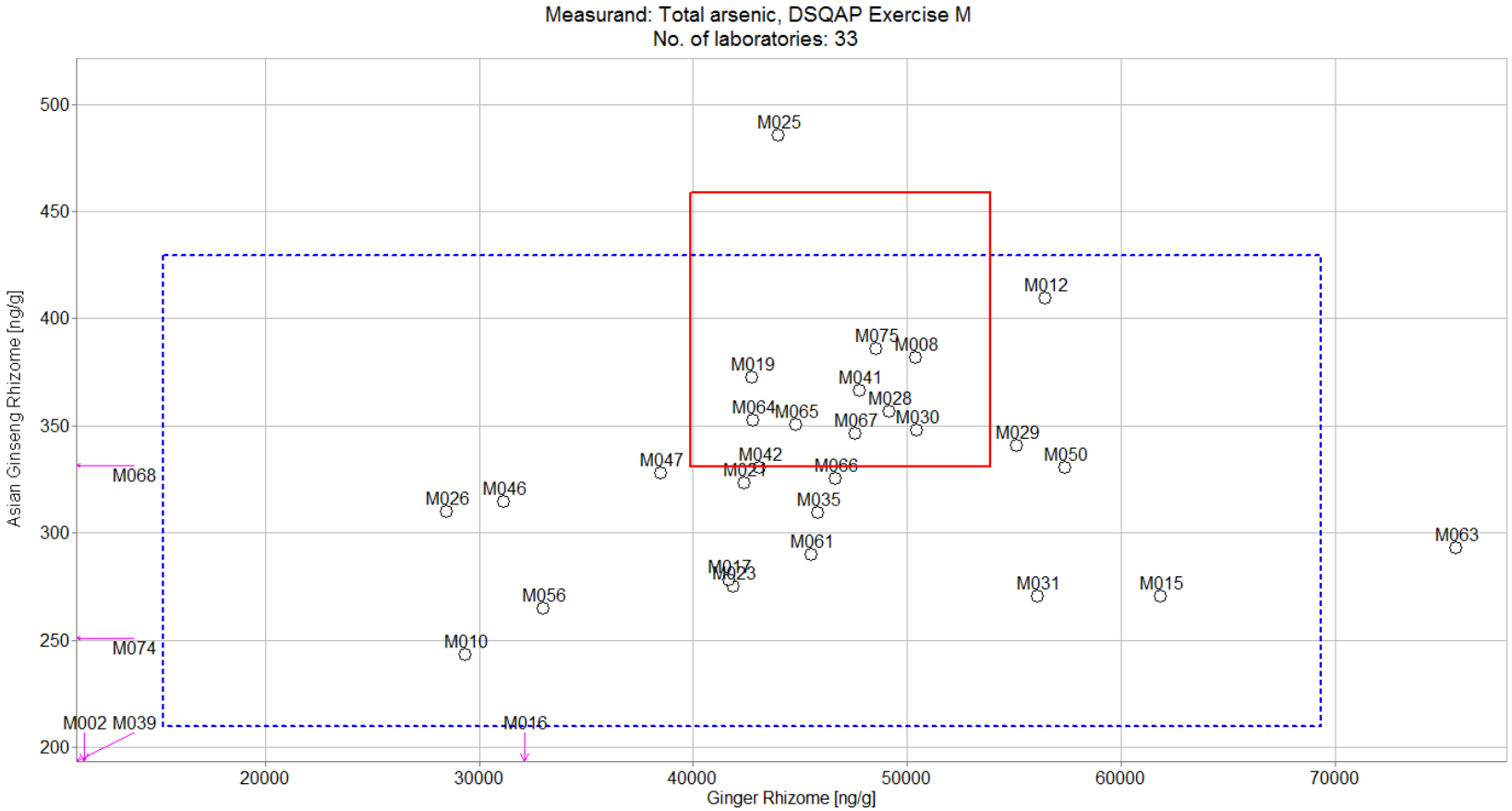

Figure 12. Laboratory means for total arsenic in SRM 3398 Ginger (*Zingiber officinale*) Rhizome and SRM 3384 Ground Asian Ginseng (*Panax ginseng* C.A. Meyer) Rhizome (sample/sample comparison view). In this view, the individual laboratory mean for one sample (ginger rhizome) is compared to the mean for a second sample (Asian ginseng rhizome). The solid red box represents the NIST range of tolerance for the two samples, ginger rhizome (x-axis) and Asian ginseng rhizome (y-axis), which encompasses the NIST values bounded by twice their uncertainties (U_{95}), 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 ginger rhizome (x-axis) and Asian ginseng rhizome (y-axis), calculated as the values above and below the consensus means that result in an acceptable Z'_{comm} score, $|Z'_{comm}| \leq 2$.

WATER-SOLUBLE VITAMINS (B_1, B_2) IN DIETARY SUPPLEMENTS

Study Overview

In this study, participants were provided with one NIST SRM, SRM 3280 Multivitamin/Multielement Tablets, and one commercially prepared product, spirulina powder. Participants were asked to use in-house analytical methods to determine the mass fraction of thiamine (B_1) and riboflavin (B_2) in each of the matrices and report values on an as-received basis.

Sample Information

Spirulina. Participants were provided with three packets containing approximately 3 g of powdered spirulina. The spirulina was blended, aliquotted, and heat-sealed inside 4 mil polyethylene bags, which were then sealed inside nitrogen-flushed aluminized plastic bags along with two packets of silica gel. Before use, participants were instructed to thoroughly mix the contents of each packet and to use a sample size of at least 0.5 g. Participants were asked to store the material at controlled room temperature, 20 °C to 25 °C, to prepare a single sample and to report a single value from each packet provided. Approximate analyte levels were not reported to participants prior to the study, and target values for these analytes have not been determined at NIST.

Multivitamin. Participants were provided with one bottle containing 30 multivitamin/multielement tablets. Before use, participants were instructed to grind a minimum of 15 tablets, mix the resulting powder thoroughly, and to use a sample size of at least 0.25 g. Participants were asked to store the material at controlled room temperature, 20 °C to 25 °C, and to prepare three samples and report three values from the single bottle provided. Approximate analyte levels were not reported to participants prior to the study. The certified values for thiamine and riboflavin in SRM 3280 Multivitamin/Multielement Tablets were determined at NIST using liquid chromatography mass spectrometry (LC-MS) and LC-absorbance, in combination with data from numerous collaborating laboratories. The certified values and uncertainties for thiamine and riboflavin are provided in the table below, both on a dry-mass basis and on an as-received basis accounting for moisture of the material (1.37 %).

	Certified Mass Fraction in SRM 3280 (mg/g)	
Analyte	(dry-mass basis)	(as-received basis)
Thiamine HCl (Vitamin B_1)	1.06 ± 0.12	1.05 ± 0.12
Riboflavin (Vitamin B_2)	1.32 ± 0.17	1.30 ± 0.17

Study Results

- Forty-six laboratories enrolled in this exercise and received samples. Thirty-two laboratories reported results for thiamine (vitamin B_1) in the multivitamin (70 % participation) and 22 laboratories reported results for thiamine in spirulina powder (48 % participation).
  - The results for thiamine in the multivitamin were divided into two subsets. One group reported values in mg/kg as requested by the shipping letter, while the other group reported values in mg/g as requested by the data entry page.

        - One subset of 17 laboratories reported results in mg/g as requested by the data entry page. These results were on the same order of magnitude as the NIST certified value as reported on the Certificate of Analysis. The consensus mean for subset 1 was within the target range for thiamine in the multivitamin with acceptable between-laboratory variability (21 % RSD).
        - Another subset of 10 laboratories reported results in mg/kg as requested by the shipping letter. After adjustment of the NIST certified value to the same units, the consensus mean for subset 2 was also within the target range for thiamine in the multivitamin with acceptable between-laboratory variability (16 % RSD).
    - The between-laboratory variability was very high for thiamine in the spirulina powder (126 % RSD).
- Of the forty-six laboratories that enrolled, thirty-one laboratories reported results for riboflavin (vitamin B_2) in the multivitamin (68 % participation) and 21 laboratories reported results for riboflavin in spirulina powder (46 % participation).
    - The results for riboflavin in the multivitamin were divided into two subsets. One group reported values in mg/kg as requested by the shipping letter, while the other group reported values in mg/g as requested by the data entry page.
        - One subset of 18 laboratories reported results in mg/g as requested by the data entry page. These results were on the same order of magnitude as the NIST certified value as reported on the Certificate of Analysis. The consensus mean for subset 1 was within the target range for riboflavin in the multivitamin with excellent between-laboratory variability (7 % RSD).
        - Another subset of 11 laboratories reported results in mg/kg as requested by the shipping letter. After adjustment of the NIST certified value to the same units, the consensus mean for subset 2 was also within the target range for riboflavin in the multivitamin with excellent between-laboratory variability (8 % RSD).
    - The between-laboratory variability was very high for riboflavin in the spirulina powder (80 % RSD).
- A majority of the laboratories reported using liquid chromatography with absorbance detection (75 %) as their instrumental method for analysis. Use of LC with fluorescence detection (10 %), microbiological assay (8 %), spectrophotometry (8 %), and LC with mass spectrometry (3 %) were also reported.

Technical Recommendations

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

- Results for the multivitamin tablet were excellent. No methods presented as significantly better or worse than any other. No systematic biases were noted.
- Inconsistent requests for reported units between the shipping letter and data entry website led to results being divided into two subsets for both thiamine and riboflavin in the multivitamin. Despite this issue, the results for both vitamins were well within the target ranges. In future studies, requests from NIST will be more consistent.
- The recommended form for reporting of thiamine data was not specified. For purposes of this report, NIST has compared all data to the form reported on the Certificate of Analysis, thiamine hydrochloride. Some laboratories, particularly those reporting values less than 1 mg/g (or 1000 mg/kg) may have reported results as thiamine ion and not as thiamine hydrochloride. Differences in the reported form for thiamine may have resulted in larger

than expected between-laboratory variability. In future studies, NIST will clearly specify the form of the vitamin requested on the shipping letter as well as on the data entry page.

- The results for both vitamins in the spirulina were highly variable, despite the excellent results for the multivitamin samples, indicating a potential challenge with the spirulina matrix.
- None of the reported analytical methods performed better than others with the spirulina matrix. Most likely the greatest challenge with the spirulina matrix is in the sample preparation, and extraction of the endogenous vitamins.

Table 10. Individualized data summary table (NIST) for vitamin B_1 and vitamin B_2 in dietary supplements.

National Institute of Standards & Technology

Exercise M - March 2016 - B Vitamins

	Lab Code:	**NIST**	**1. Your Results**				**2. Community Results**			**3. Target**	
Analyte	Sample	Units	x_i	s_i	Z'_{comm}	Z_{NIST}	N	x*	s*	x_{NIST}	U_{95}
Thiamine	Multivitamin	mg/g	1.05	0.12		0.00	29	406	611	1.05	0.12
Thiamine	Spirulina	mg/kg					20	46	58		
Thiamine (Subset 1)	Multivitamin	mg/g	1.05	0.12		0.00	17	1.10	0.23	1.05	0.12
Thiamine (Subset 2)	Multivitamin	mg/kg	1050	120		0.00	10	1078	171	1050	120
Riboflavin	Multivitamin	mg/g	1.30	0.17		0.00	28	537	787	1.30	0.17
Riboflavin	Spirulina	mg/kg					20	41	33		
Riboflavin (Subset 1)	Multivitamin	mg/g	1.30	0.17		0.00	16	1.30	0.09	1.30	0.17
Riboflavin (Subset 2)	Multivitamin	mg/kg	1302	170		0.00	10	1342	104	1302	170

x_i Mean of reported values

s_i Standard deviation of reported values

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

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

N Number of quantitative values reported

x* Robust mean of reported values

s* Robust standard deviation

x_{NIST} NIST-assessed value

U_{95} ±95% confidence interval about the assessed value or standard deviation (s_{NIST})

Table 11. Data summary table for vitamin B_1 in dietary supplements. Data highlighted in red have been flagged as potential outliers (e.g., difference from reference value, Grubb and/or Cochran) by the NIST software package.

		Thiamine									
		SRM 3280 Multivitamin/Multielement Tablets (mg/g)					Commercial Spirulina Powder (mg/kg)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	NIST				1.050	0.120					
	M001	0.876	0.893	0.885	0.885	0.009	2410	2390	2380	2393	15.28
	M002										
	M003										
	M004	1705	1710	1712	1709	4					
	M006										
	M007	1204	1213	1211	1209	5	26.1	26.5	26.4	26.3	0.2
	M010	1010	1072	908	997	83					
	M012	1.108	1.088	1.086	1.094	0.012	1.0	1.0	1.0	1.0	0.0
	M014	1.083	1.142	1.095	1.107	0.031	24.7	20.8	18.1	21.2	3.3
	M015	1032	1038	1022	1031	8	12.3	32.1	37.6	27.3	13.3
	M016	957			957		316	289	326	310	19
	M017										
	M019	10500	10600		10550	71	10.0	9.7	9.8	9.8	0.2
	M020	1.150	1.060	1.130	1.113	0.047	160	220	230	203	38
	M022										
	M023	0.900	0.888	0.893	0.894	0.006	106.7	112.2	109.8	109.6	2.8
	M024	0.796	0.818	0.797	0.804	0.012	2.45	6.59	6.10	5.05	2.26
	M025	1.060			1.060						
	M026	0.001	0.001	0.001	0.001	0.000					
	M028	1.366	1.380	1.380	1.375	0.008	16.0	16.5	17.1	16.5	0.6
	M029	960	950	978	963	14	9.37	7.26	8.53	8.39	1.06
	M032										
	M033	0.985	0.985	0.985	0.985	0.000	4.32	4.14	3.99	4.15	0.17
	M036										
	M038*	1.143	1.103	1.107	1.118	0.022					
	M039	1.104	1.097	1.156	1.119	0.032					
	M041	1120	1220	1130	1157	55	4.50	3.80	4.20	4.17	0.35
	M042	853	853	873	859	12	7.90	4.00	1.20	4.37	3.37
	M046	25.2	26.5	24.8	25.50	0.89	< 18.10	< 18.10	< 18.10	< 18.10	
	M047	0.855	0.844	0.841	0.847	0.007					
	M048										
	M051										
	M055										
	M056	1248	1187	1204	1213	31	29.2	20.6	23.5	24.4	4.4
	M058	1091	1026	1142	1087	58					
	M064										
	M065	1.160	1.100	1.140	1.133	0.031					
	M068	0.924	0.995	0.951	0.957	0.036	55.1	53.0	50.0	52.7	2.6
	M069										
	M070	839	833		836	4.24	86.6	85.1	85.3	85.7	0.8
	M071	1.380			1.380		3.30	3.40	3.30	3.33	0.06
	M073	1.129	1.133	1.128	1.130	0.003					
	M074	0.964	0.992	0.971	0.976	0.015					
	M075	0.943	0.971	0.976	0.963	0.018	115	119	122	119	4
Community Results		Consensus Mean			406		Consensus Mean			46.2	
		Consensus Standard Deviation			611		Consensus Standard Deviation			58.2	
		Maximum			10550		Maximum			2393	
		Minimum			0.00		Minimum			1.00	
		N			29		N			20	

*Data for lab M038 was reported as thiamine ion and converted by NIST to the hydrochloride form.

Table 12. Data summary table (subset 1) for vitamin B_1 in SRM 3280 Multivitamin/Multielement Tablets. Data in this group were reported on the same order of magnitude as the certified value. Data highlighted in red have been flagged as potential outliers (e.g., difference from reference value, Grubb and/or Cochran) by the NIST software package.

		Thiamine Subset 1				
		SRM 3280 Multivitamin/Multielement Tablets (mg/g)				
	Lab	A	B	C	Avg	SD
Individual Results	NIST				1.050	0.120
	M001	0.876	0.893	0.885	0.885	0.009
	M012	1.108	1.088	1.086	1.094	0.012
	M014	1.083	1.142	1.095	1.107	0.031
	M020	1.150	1.060	1.130	1.113	0.047
	M023	0.900	0.888	0.893	0.894	0.006
	M024	0.796	0.818	0.797	0.804	0.012
	M025	1.060			1.060	
	M028	1.366	1.380	1.380	1.375	0.008
	M033	0.985	0.985	0.985	0.985	0.000
	M038*	1.452	1.402	1.407	1.421	0.028
	M039	1.104	1.097	1.156	1.119	0.032
	M046	25.2	26.5	24.8	25.50	0.89
	M047	0.855	0.844	0.841	0.847	0.007
	M065	1.160	1.100	1.140	1.133	0.031
	M068	0.924	0.995	0.951	0.957	0.036
	M071	1.380			1.380	
	M073	1.129	1.133	1.128	1.130	0.003
	M074	0.964	0.992	0.971	0.976	0.015
	M075	0.943	0.971	0.976	0.963	0.018
Community Results		Consensus Mean			1.10	
		Consensus Standard Deviation			0.23	
		Maximum			25.50	
		Minimum			0.80	
		N			17	

*Data for lab M038 was reported as thiamine ion and converted by NIST to the hydrochloride form.

Table 13. Data summary table (subset 2) for vitamin B_1 in SRM 3280 Multivitamin/Multielement Tablets. Data in this group were reported roughly three orders of magnitude higher than the certified value. An error in reporting units is suspected, so this data has been modified based on this assumption in the set on the right.

		Thiamine Subset 2					Thiamine Subset 2 (modified)				
		SRM 3280 Multivitamin/Multielement Tablets (mg/kg)					SRM 3280 Multivitamin/Multielement Tablets (mg/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	NIST				1050	120				1.050	0.120
	M004	1705	1710	1712	1709	4	1.705	1.710	1.712	1.709	0.004
	M007	1204	1213	1211	1209	5	1.204	1.213	1.211	1.209	0.005
	M010	1010	1072	908	997	83	1.010	1.072	0.908	0.997	0.083
	M015	1032	1038	1022	1031	8	1.032	1.038	1.022	1.031	0.008
	M016	957			957		0.957	0.000	0.000	0.957	
	M029	960	950	978	963	14	0.960	0.950	0.978	0.963	0.014
	M041	1120	1220	1130	1157	55	1.120	1.220	1.130	1.157	0.055
	M042	853	853	873	859	12	0.853	0.853	0.873	0.859	0.012
	M056	1248	1187	1204	1213	31	1.248	1.187	1.204	1.213	0.031
	M058	1091	1026	1142	1087	58	1.091	1.026	1.142	1.087	0.058
	M070	839	833		836	4	0.839	0.833		0.836	0.004
Community Results		Consensus Mean			1078		Consensus Mean			1.08	
		Consensus Standard Deviation			171		Consensus Standard Deviation			0.17	
		Maximum			1709		Maximum			1.71	
		Minimum			836		Minimum			0.84	
		N			10		N			10	

Table 14. Data summary table for vitamin B_2 in dietary supplements. Data highlighted in red have been flagged as potential outliers (e.g., difference from reference value, Grubb and/or Cochran) by the NIST software package.

		Riboflavin									
		SRM 3280 Multivitamin/Multielement Tablets (mg/g)					Commercial Spirulina Powder (mg/kg)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	NIST				1.30	0.17					
	M001										
	M002										
	M003										
	M004	2140	2284	2389	2271	125	59.8	54.2	55.0	56.3	3.0
	M006										
	M007	1282	1296	1299	1293	9					
	M010	1490	1438	1423	1450	35					
	M012	1.30	1.40	1.40	1.40	0.06	80.0	86.0	96.0	87.3	8.1
	M014	1.40	1.30	1.30	1.40	0.06	31.8	29.1	34.9	31.9	2.9
	M015	1367	1408	1314	1363	47	4.0	3.4	3.5	3.6	0.3
	M016	1410			1410						
	M017										
	M019	9770	9830		9800	42					
	M020	1.30	1.30	1.30	1.30	0.00	5.0	6.0	2.0	4.3	2.1
	M022										
	M023	1.30	1.30	1.30	1.30	0.00	40.3	36.3	42.0	39.5	2.9
	M024	1.20	1.30	1.20	1.20	0.06	12.9	17.5	17.6	16.0	2.7
	M025	1.30			1.30		65.9	66.3	68.5	66.9	1.4
	M026	0.00	0.00	0.00	0.00	0.00					
	M028	1.40	1.40	1.40	1.40	0.00	37.0	36.0	40.0	37.7	2.1
	M029	1246	1016	1106	1123	116	30.8	34.5	36.1	33.8	2.7
	M032										
	M033	1.40	1.40	1.40	1.40	0.00	1.9	1.3	1.9	1.9	0.3
	M036										
	M038	1.10	1.00	1.10	1.10	0.06					
	M039	1.20	1.30	1.40	1.30	0.10					
	M041	1230	1350	1320	1300	62	47.7	51.1	48.9	49.2	1.7
	M042	1282	1266	1254	1268	14	70.3	65.4	72.8	69.5	3.8
	M046	1.40	1.20	1.40	1.30	0.12	142.0	101.0	155.0	132.7	28.2
	M047	1.20	1.30	1.30	1.30	0.06					
	M048										
	M051										
	M055										
	M056	1365	1464	1332	1387	69	38.9	36.8	30.4	35.4	4.4
	M058	1316	1234	1297	1282	43	141.3	119.0	137.1	132.5	11.9
	M064										
	M065	1.40	1.40	1.40	1.40	0.00					
	M068	1.40	1.30	1.40	1.30	0.06	< 0.1	< 0.1	< 0.1	< 0.1	
	M069										
	M070	1312	1339		1326	19	0.5	0.5	0.6	0.5	0.1
	M071	0.90			0.90		43.0	43.0	46.4	44.1	2.0
	M073	1.30	1.30	1.30	1.30	0.00					
	M074	1.40	1.40	1.40	1.40	0.00	30.9	32.1	32.6	31.9	0.9
	M075	1.20	1.20	1.20	1.20	0.00	28.0	27.0	27.0	27.3	0.6
Community Results		Consensus Mean			537		Consensus Mean			40.8	
		Consensus Standard Deviation			787		Consensus Standard Deviation			32.6	
		Maximum			9800		Maximum			132.7	
		Minimum			0.00		Minimum			0.5	
		N			28		N			20	

Table 15. Data summary table (subset 1) for vitamin B_2 in SRM 3280 Multivitamin/Multielement Tablets. Data in this group were reported on the same order of magnitude as the certified value. Data highlighted in red have been flagged as potential outliers (e.g., difference from reference value, Grubb and/or Cochran) by the NIST software package.

		Riboflavin Subset 1				
		SRM 3280 Multivitamin/Multielement Tablets (mg/g)				
	Lab	A	B	C	Avg	SD
Individual Results	NIST				1.302	0.168
	M012	1.343	1.399	1.359	1.367	0.029
	M014	1.372	1.346	1.335	1.351	0.019
	M020	1.260	1.290	1.320	1.290	0.030
	M023	1.268	1.288	1.281	1.279	0.010
	M024	1.210	1.260	1.210	1.227	0.029
	M025	1.320			1.320	
	M028	1.450	1.430	1.430	1.437	0.012
	M033	1.360	1.360	1.360	1.360	0.000
	M038	1.128	1.000	1.105	1.078	0.068
	M039	1.243	1.299	1.380	1.307	0.069
	M046	1.400	1.230	1.360	1.330	0.089
	M047	1.230	1.280	1.280	1.263	0.029
	M065	1.360	1.360	1.370	1.363	0.006
	M068	1.361	1.329	1.353	1.348	0.017
	M071	0.890			0.890	
	M073	1.344	1.313	1.330	1.329	0.016
	M074	1.396	1.362	1.373	1.377	0.017
	M075	1.173	1.206	1.152	1.177	0.027
Community Results		Consensus Mean			1.30	
		Consensus Standard Deviation			0.09	
		Maximum			1.44	
		Minimum			0.89	
		N			16	

Table 16. Data summary table (subset 2) for vitamin B_2 in SRM 3280 Multivitamin/Multielement Tablets. Data in this group were reported roughly three orders of magnitude higher than the certified value. An error in reporting units is suspected, so this data has been modified based on this assumption in the set on the right.

		Riboflavin Subset 2					Riboflavin Subset 2 (modified)				
		SRM 3280 Multivitamin/Multielement Tablets (mg/kg)					SRM 3280 Multivitamin/Multielement Tablets (mg/g)				
	Lab	**A**	**B**	**C**	**Avg**	**SD**	**A**	**B**	**C**	**Avg**	**SD**
Individual Results	NIST				1302	170				1.302	0.168
	M004	2140	2284	2389	2271	125	2.140	2.284	2.389	2.271	0.125
	M007	1282	1296	1299	1292	9	1.282	1.296	1.299	1.292	0.009
	M010	1490	1438	1423	1450	35	1.490	1.438	1.423	1.450	0.035
	M015	1366	1408	1314	1363	47	1.366	1.408	1.314	1.363	0.047
	M016	1410			1410		1.410			1.410	
	M029	1246	1016	1106	1123	116	1.246	1.016	1.106	1.123	0.116
	M041	1230	1350	1320	1300	62	1.230	1.350	1.320	1.300	0.062
	M042	1282	1266	1254	1268	14	1.282	1.266	1.254	1.268	0.014
	M056	1365	1464	1332	1387	68	1.365	1.464	1.332	1.387	0.068
	M058	1316	1234	1297	1282	43	1.316	1.234	1.297	1.282	0.043
	M070	1312	1339		1326	19	1.312	1.339		1.326	0.019
Community Results		Consensus Mean			1342		Consensus Mean			1.34	
		Consensus Standard Deviation			104		Consensus Standard Deviation			0.10	
		Maximum			2271		Maximum			2.27	
		Minimum			1123		Minimum			1.12	
		N			10		N			10	

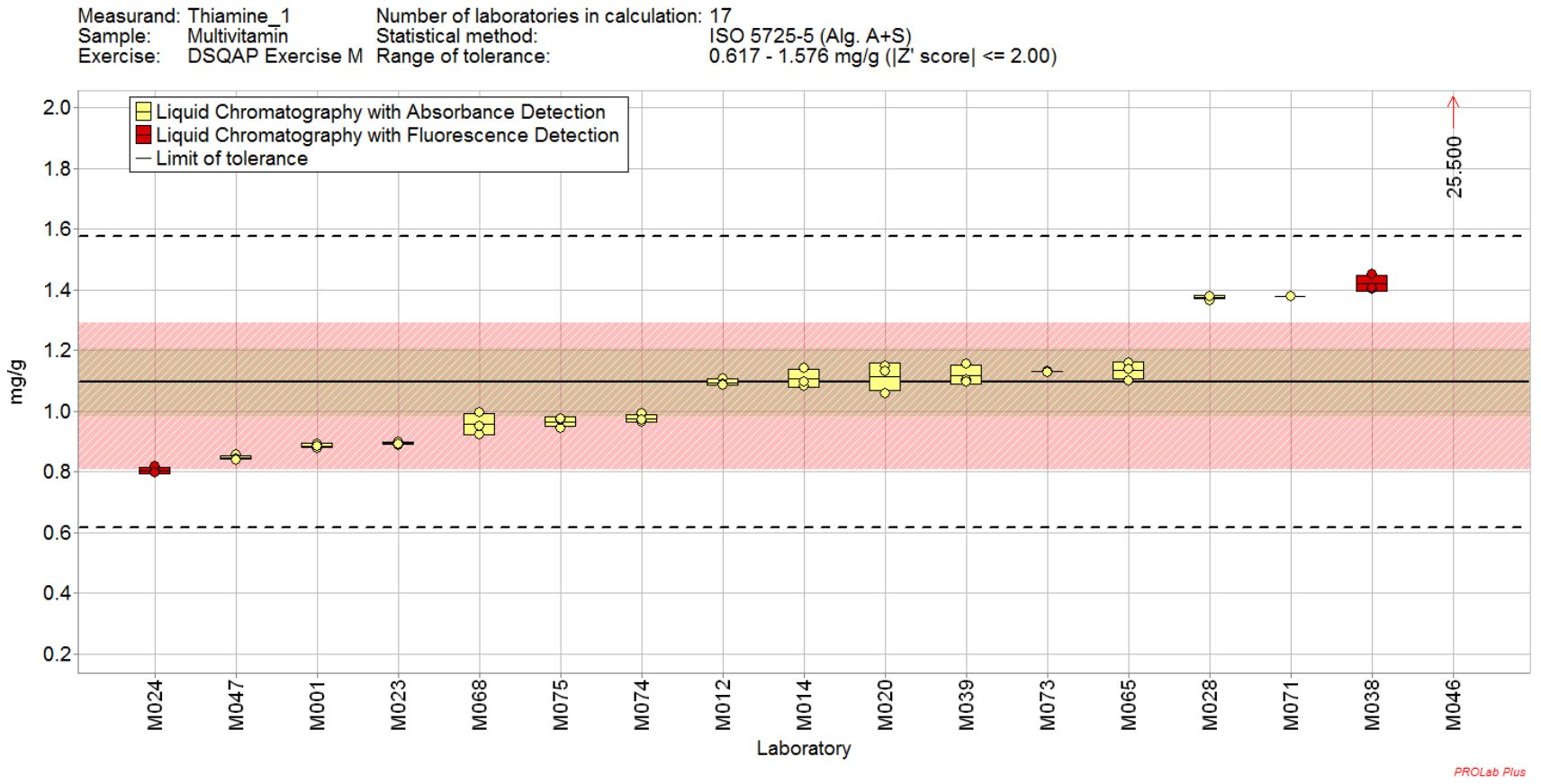

Figure 13. Vitamin B_1 (subset 1) in SRM 3280 Multivitamin/Multielement Tablets (data summary view – analytical method). Data in this group were reported on the same order of magnitude as the certified value. In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents the consensus mean, and the green shaded region represents the consensus mean bounded by twice the consensus standard error. The black dashed lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{comm}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the NIST certified value bounded by twice its uncertainty (U_{95}), and represents the range that results in an acceptable Z'_{NIST} score, $|Z'_{NIST}| \leq 2$.

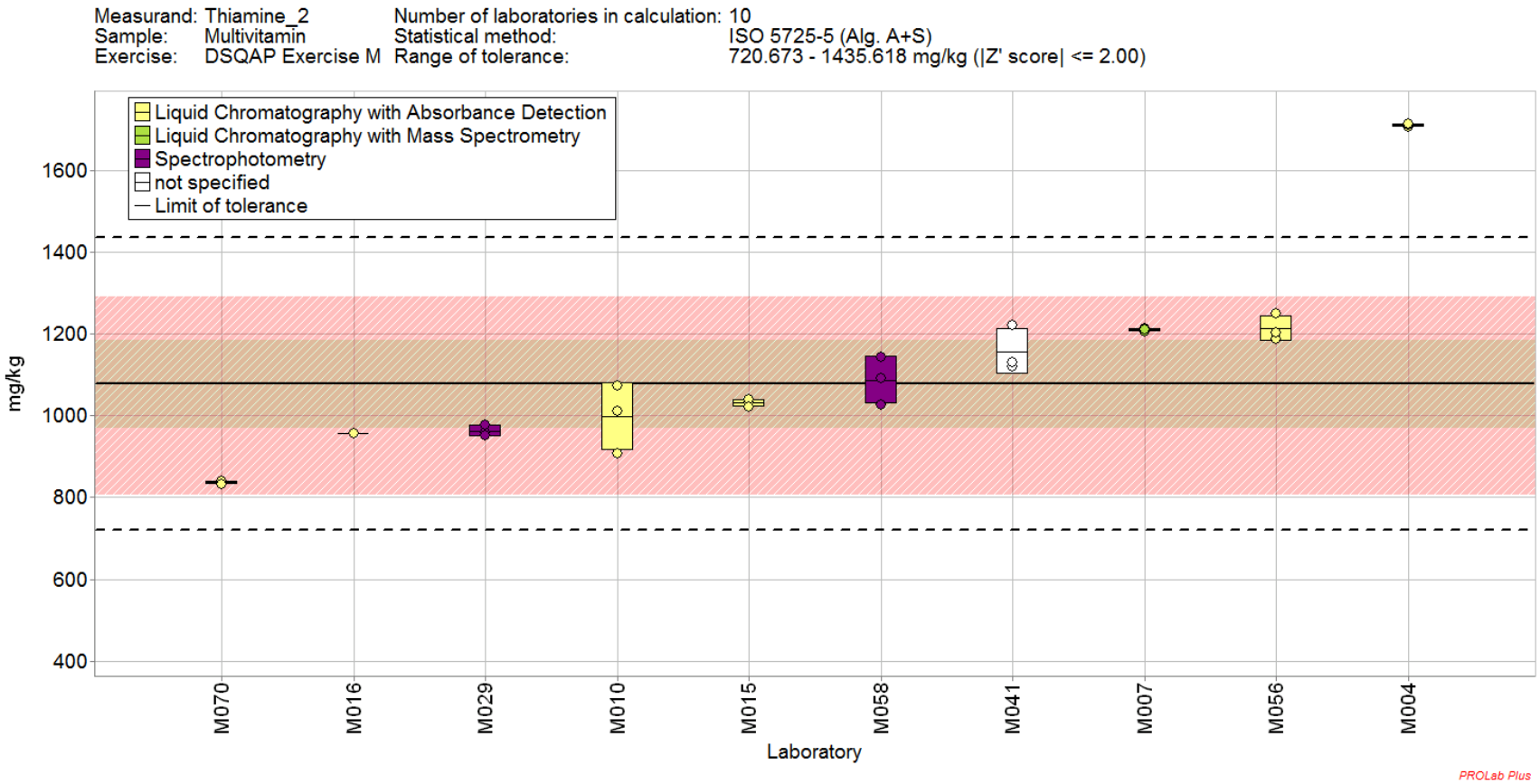

Figure 14. Vitamin B_1 (subset 2) in SRM 3280 Multivitamin/Multielement Tablets (data summary view – analytical method). Data in this group were reported roughly three orders of magnitude higher than the certified value. An error in reporting units is suspected, so this data has been modified based on this assumption. In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents the consensus mean, and the green shaded region represents the consensus mean bounded by twice the consensus standard error. The black dashed lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{comm}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the NIST certified value bounded by twice its uncertainty (U_{95}), and represents the range that results in an acceptable Z'_{NIST} score, $|Z'_{NIST}| \leq 2$.

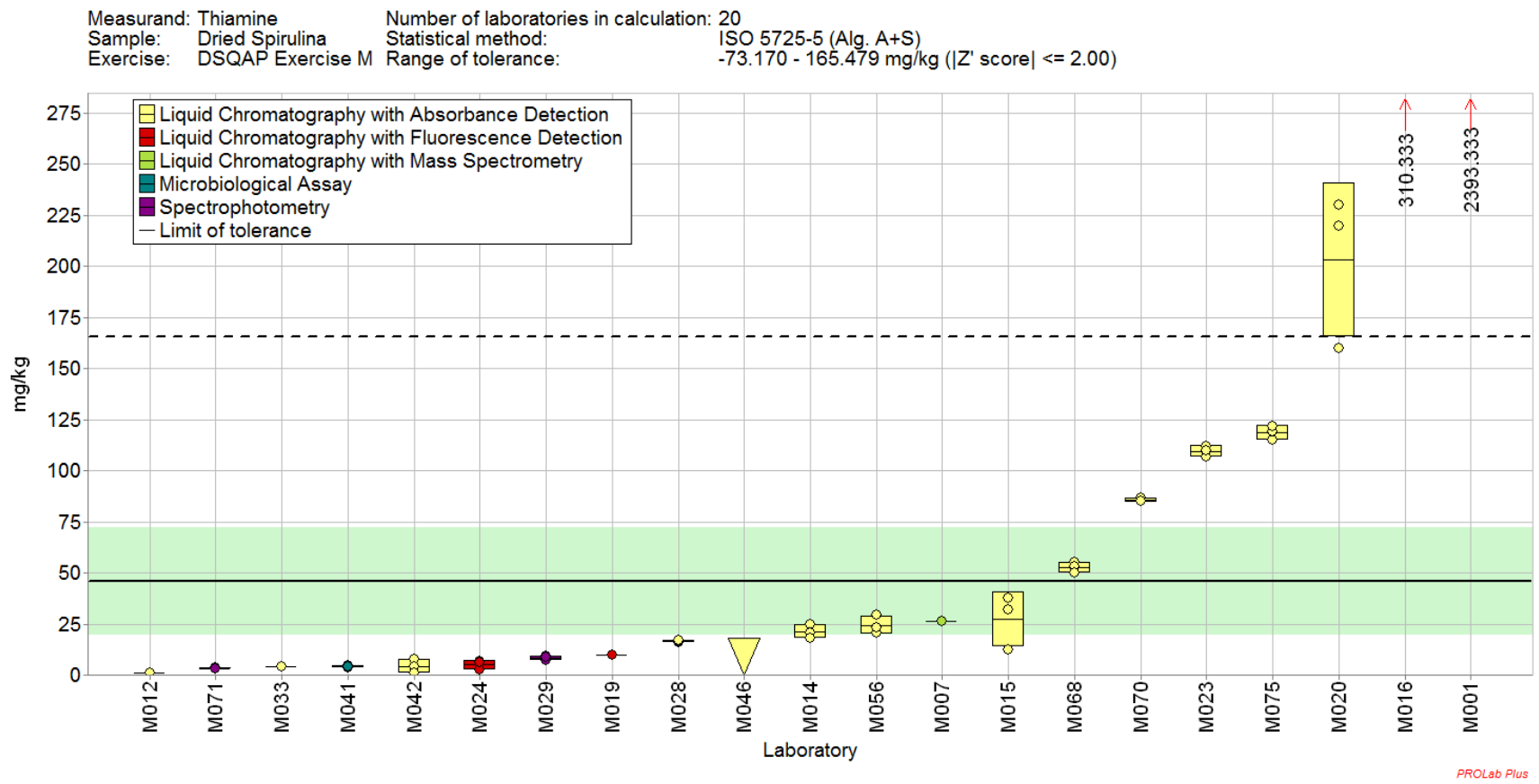

Figure 15. Vitamin B_1 in spirulina (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. Laboratory data shown as a triangle indicates that a "less than" result was submitted, and the base of the triangle is displayed at the reported laboratory detection limit. The solid black line represents the consensus mean, and the green shaded region represents the consensus mean bounded by twice the consensus standard error. The black dashed 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, $|Z'_{comm}| \leq 2$. No NIST value has been determined in this material.

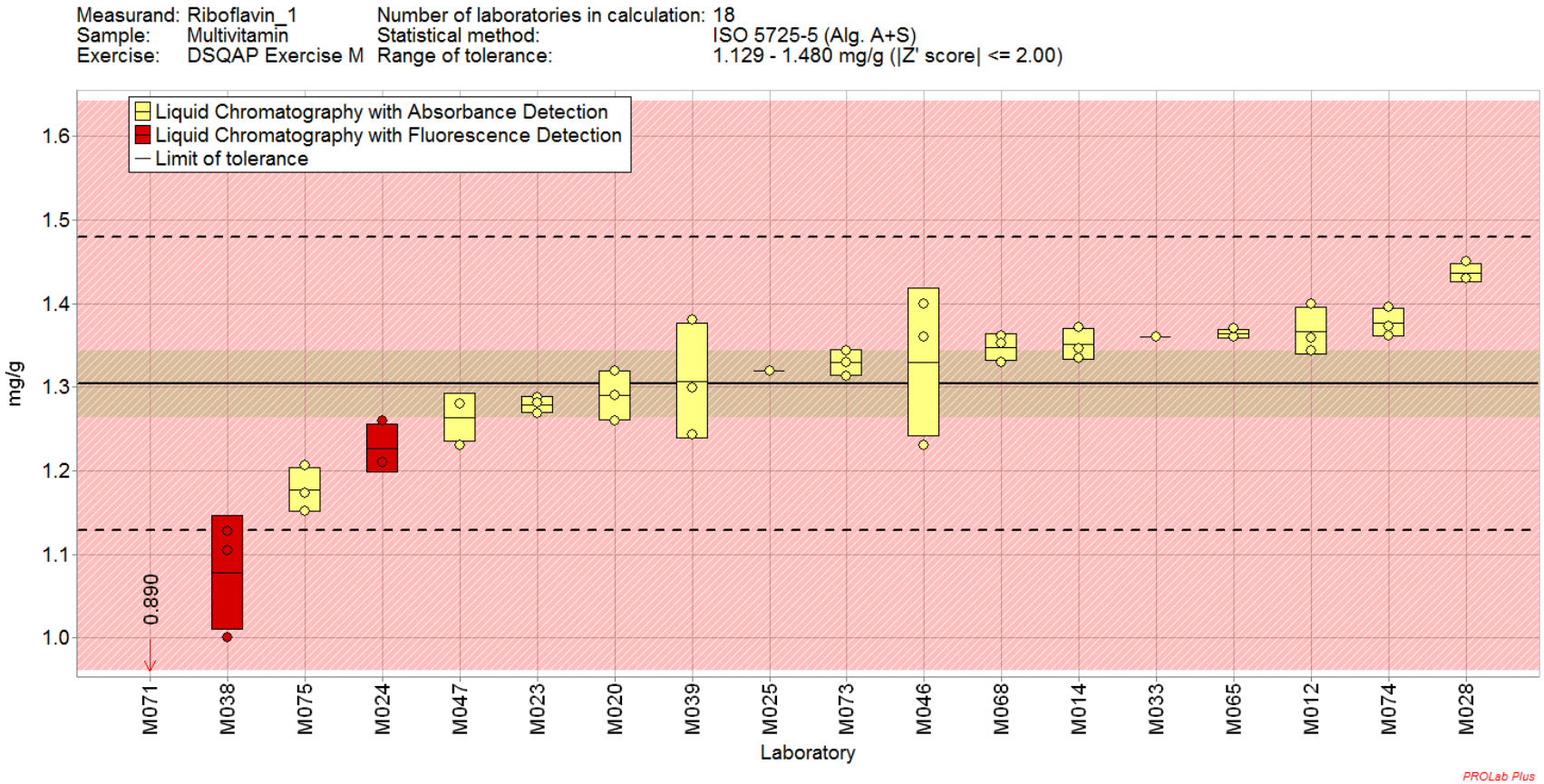

Figure 16. Vitamin B_2 (subset 1) in SRM 3280 Multivitamin/Multielement Tablets (data summary view – analytical method). Data in this group were reported on the same order of magnitude as the certified value. In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents the consensus mean, and the green shaded region represents the consensus mean bounded by twice the consensus standard error. The black dashed lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{comm}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the NIST certified value bounded by twice its uncertainty (U_{95}), and represents the range that results in an acceptable Z'_{NIST} score, $|Z'_{NIST}| \leq 2$.

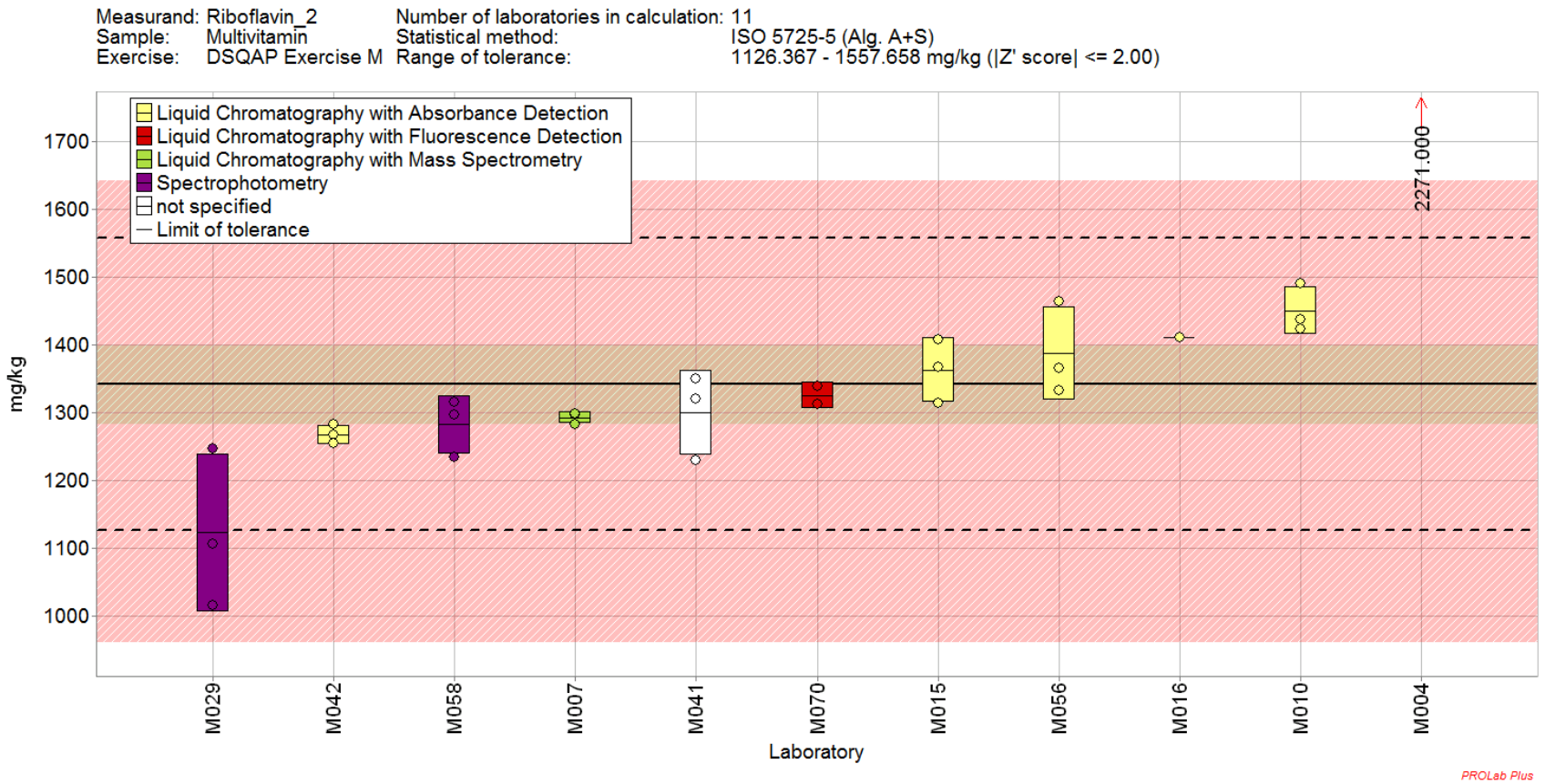

Figure 17. Vitamin B_2 (subset 2) in SRM 3280 Multivitamin/Multielement Tablets (data summary view – analytical method). Data in this group were reported roughly three orders of magnitude higher than the certified value. An error in reporting units is suspected, so this data has been modified based on this assumption. In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents the consensus mean, and the green shaded region represents the consensus mean bounded by twice the consensus standard error. The black dashed lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{comm}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the NIST certified value bounded by twice its uncertainty (U_{95}), and represents the range that results in an acceptable Z'_{NIST} score, $|Z'_{NIST}| \leq 2$.

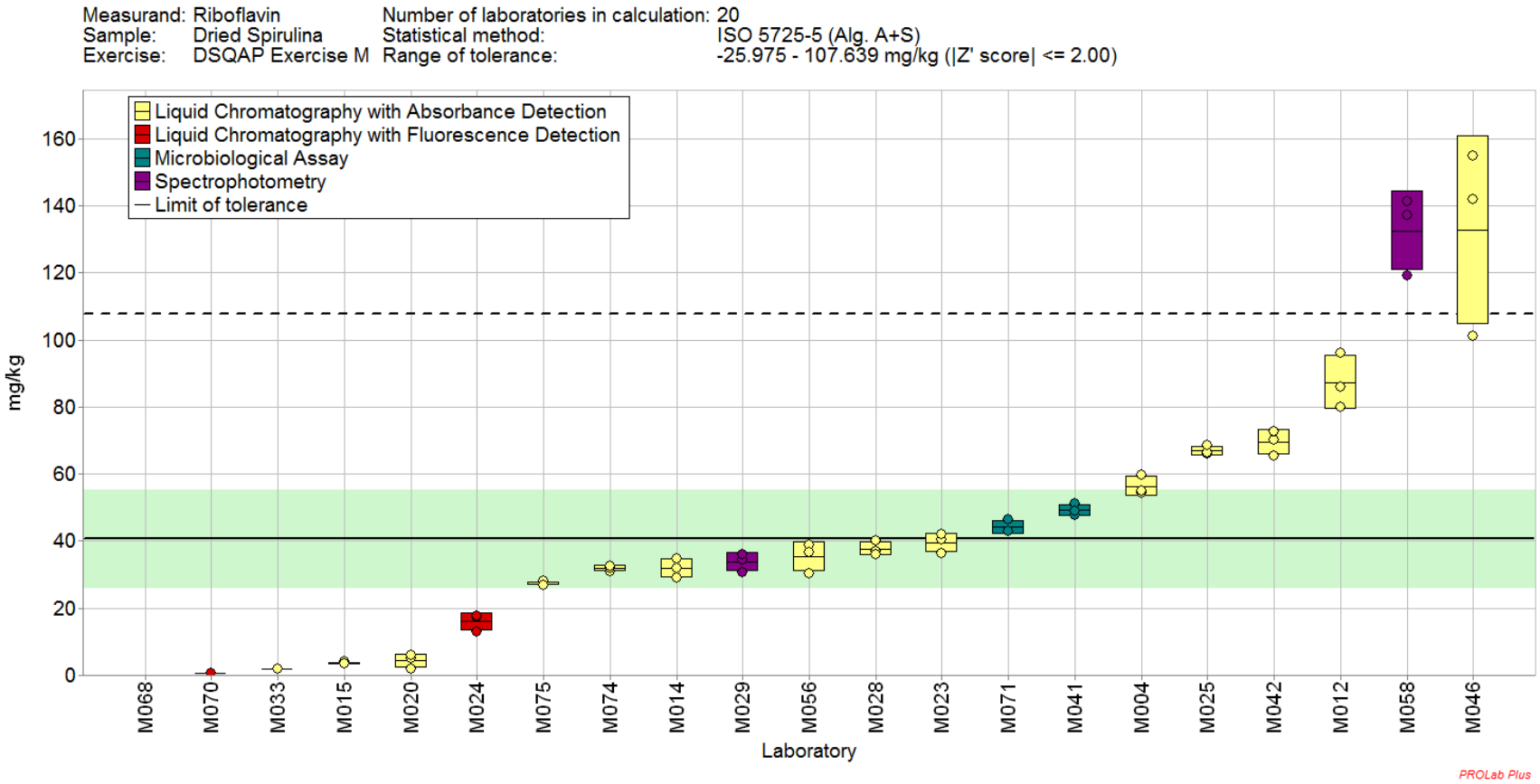

Figure 18. Vitamin B_2 in spirulina (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents the consensus mean, and the green shaded region represents the consensus mean bounded by twice the consensus standard error. The black dashed 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, $|Z'_{comm}| \leq 2$. No NIST value has been determined in this material.

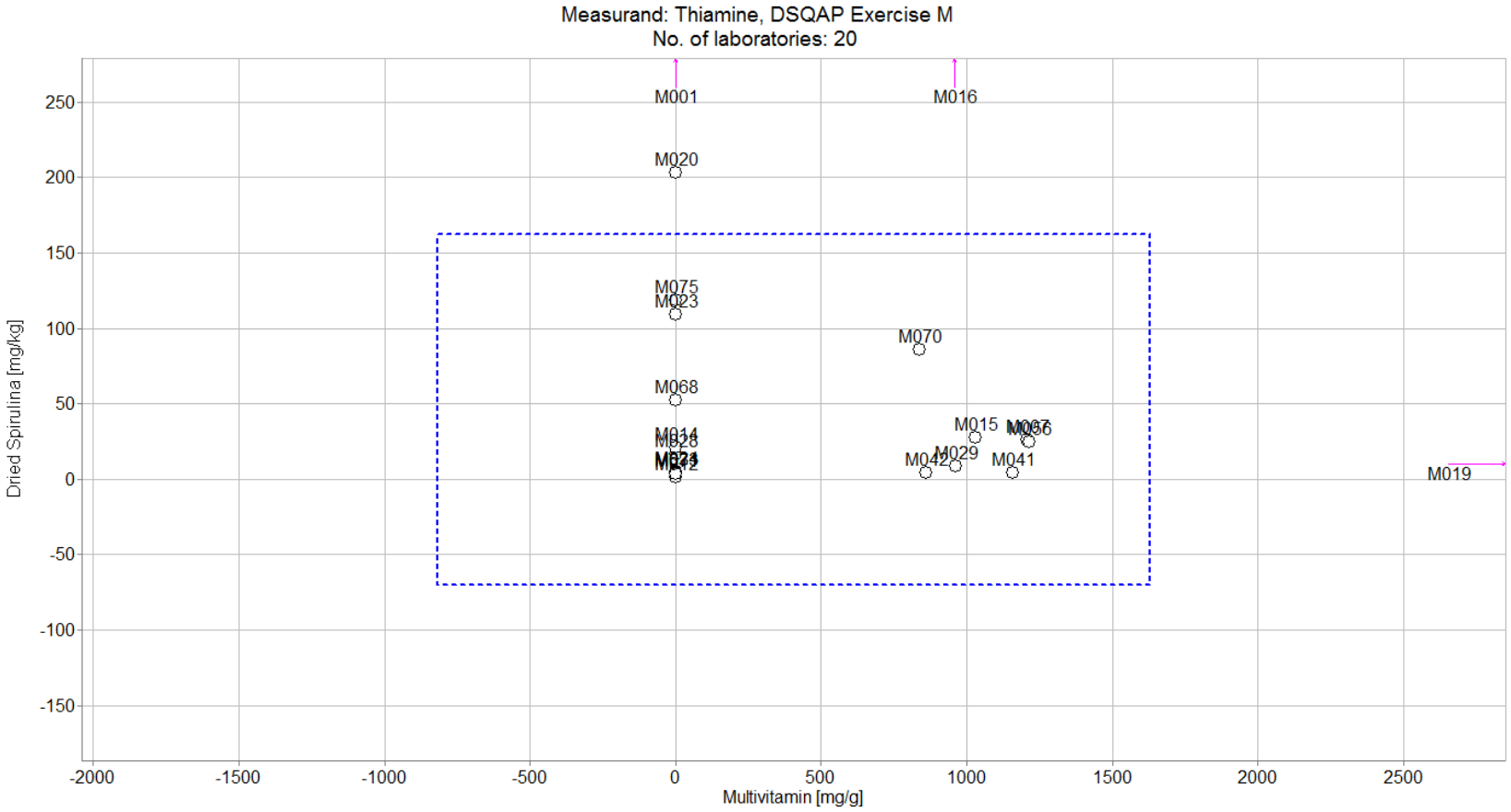

Figure 19. Laboratory means for vitamin B_1 in SRM 3280 Multivitamin/Multielement Tablets and spirulina (sample/sample comparison view). In this view, the individual laboratory mean for one sample (multivitamin) is compared to the mean for a second sample (spirulina). The dotted blue box represents the consensus range of tolerance for multivitamin (x-axis) and spirulina (y-axis), calculated as the values above and below the consensus means that result in an acceptable Z'_{comm} score, $|Z'_{comm}| \leq 2$.

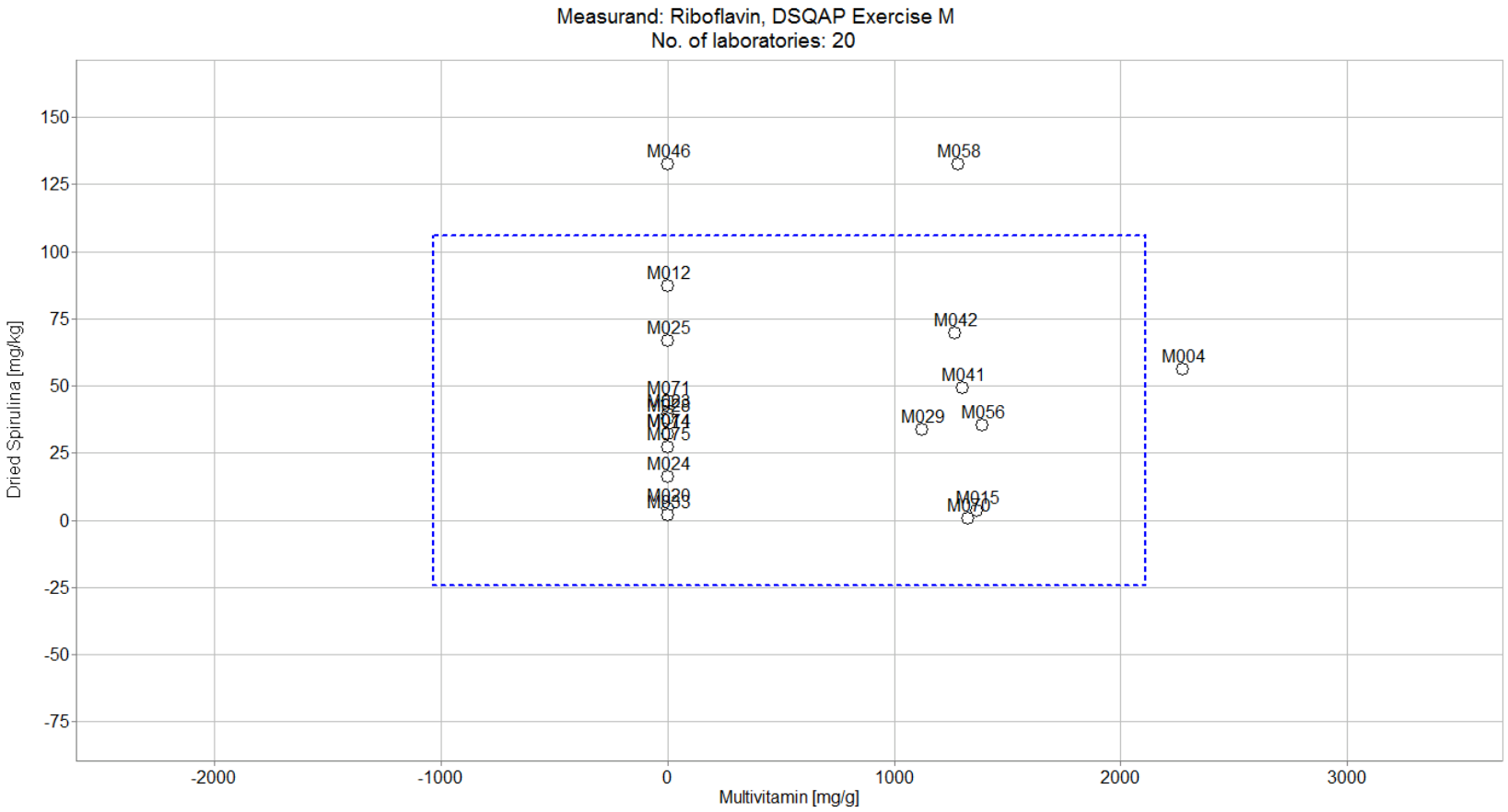

Figure 20. Laboratory means for vitamin B_2 in SRM 3280 Multivitamin/Multielement Tablets and spirulina (sample/sample comparison view). In this view, the individual laboratory mean for one sample (multivitamin) is compared to the mean for a second sample (spirulina). The dotted blue box represents the consensus range of tolerance for multivitamin (x-axis) and spirulina (y-axis), calculated as the values above and below the consensus means that result in an acceptable Z'_{comm} score, $|Z'_{comm}| \leq 2$.

FAT-SOLUBLE VITAMINS (K_1) IN DIETARY SUPPLEMENTS

Study Overview

In this study, participants were provided with two commercially prepared products, basil and kelp. Participants were asked to use in-house analytical methods to determine the mass fractions of total vitamin K_1, *cis*-vitamin K_1, and *trans*-vitamin K_1 in each of the matrices and report values on an as-received basis.

Sample Information

Basil. Participants were provided with three packets, each containing approximately 3 g of powdered basil. The basil was blended, aliquotted, and heat-sealed inside 4 mil polyethylene bags, which were then sealed inside nitrogen-flushed aluminized plastic bags along with two packets of silica gel. Before use, participants were instructed to thoroughly mix the contents of each packet and to use a sample size of at least 2 g. Participants were asked to store the material at controlled room temperature, 20 °C to 25 °C, to prepare a single sample and to report a single value from each packet provided. Approximate analyte levels were not reported to participants prior to the study, and target values for these analytes have not been determined at NIST.

Kelp. Participants were provided with three packets, each containing approximately 3 g of powdered kelp. The kelp was blended, aliquotted, and heat-sealed inside 4 mil polyethylene bags, which were then sealed inside nitrogen-flushed aluminized plastic bags along with two packets of silica gel. Before use, participants were instructed to thoroughly mix the contents of each packet and to use a sample size of at least 2 g. Participants were asked to store the material at controlled room temperature, 20 °C to 25 °C, to prepare a single sample and to report a single value from each packet provided. Approximate analyte levels were not reported to participants prior to the study, and target values for these analytes have not been determined at NIST.

Study Results

- Sixteen laboratories enrolled in this exercise and received samples. Six laboratories reported results for total vitamin K_1 in both the basil powder and the kelp powder (38 % participation). No results were reported for either *cis*-vitamin K_1 or *trans*-vitamin K_1.
  - For both basil and kelp, the between-laboratory variability was high (60 % RSD).
  - Laboratories reported using liquid chromatography (LC) with absorbance detection (33 %), LC with fluorescence detection (17 %), LC with mass spectrometry (MS) (17 %), and LC with tandem MS (17 %) as their analytical approach. One laboratory did not report the method type used.

Technical Recommendations

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

- Sample preparation steps should be carried out in the dark, or under subdued lighting, to minimize losses of vitamin K_1 due to photodecomposition. Amber autosampler vials should be used for analysis.
- Multiple extraction steps may be necessary to extract all vitamin K_1 from the sample matrix. Analysis of a reference material as a control may help determine if an extra extraction step is needed for complete recovery.

- The low participation rate in this study may indicate that the basil and kelp matrices were particularly challenging for the determination of vitamin K_1.

Table 17. Individualized data summary table (NIST) for vitamin K_1, *cis*-vitamin K_1, and *trans*-vitamin K_1 in dietary supplements.

National Institute of Standards & Technology

Exercise M - March 2016 - Vitamin K1

	Lab Code:	**NIST**	**1. Your Results**				**2. Community Results**			**3. Target**	
Analyte	Sample	Units	x_i	s_i	Z'_{comm}	Z_{NIST}	N	x*	s*	x_{NIST}	U_{95}
Total Phylloquinone	Basil	mg/kg					6	14.7	9.1		
Total Phylloquinone	Kelp	mg/kg					6	2.1	1.3		
cis-Phylloquinone	Basil	mg/kg									
cis-Phylloquinone	Kelp	mg/kg									
trans-Phylloquinone	Basil	mg/kg									
trans-Phylloquinone	Kelp	mg/kg									

x_i Mean of reported values

s_i Standard deviation of reported values

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

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

N Number of quantitative values reported

x* Robust mean of reported values

s* Robust standard deviation

x_{NIST} NIST-assessed value

U_{95} ±95% confidence interval about the assessed value or standard deviation (s_{NIST})

Table 18. Data summary table for total vitamin K_1 in dietary supplements.

		Total Vitamin K1									
		Commercial Basil Powder (mg/kg)					Commercial Kelp Powder (mg/kg)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	NIST										
	M004	14.0	14.3	17.8	15.4	2.1	4.10	4.50	3.80	4.15	0.35
	M006										
	M012										
	M017										
	M024	3.2	3.9	3.4	3.5	0.4	1.10	1.10	1.20	1.12	0.06
	M025	15.2	14.1	15.1	14.8	0.6	2.80	2.60	2.50	2.55	0.15
	M028										
	M036										
	M041	12.3	12.5	11.6	12.1	0.5	2.40	2.20	2.20	2.21	0.12
	M042										
	M046	237.0	157.0	153.0	182.3	47.4	1.70	1.60	1.80	1.65	0.10
	M055										
	M056										
	M065	15.7	12.6	14.5	14.3	1.6	1.00	1.10	1.00	1.03	0.06
	M068										
	M070										
Community Results		Consensus Mean			14.7		Consensus Mean			2.10	
		Consensus Standard Deviation			9.1		Consensus Standard Deviation			1.30	
		Maximum			182		Maximum			4.15	
		Minimum			3.5		Minimum			1.03	
		N			6		N			6	

Table 19. Data summary table for *cis*-vitamin K_1 in dietary supplements.

		***cis* -Vitamin K1**									
		Commercial Basil Powder (mg/kg)					**Commercial Kelp Powder (mg/kg)**				
	Lab	**A**	**B**	**C**	**Avg**	**SD**	**A**	**B**	**C**	**Avg**	**SD**
Individual Results	NIST										
	M004										
	M006										
	M012										
	M017										
	M024										
	M025										
	M028										
	M036										
	M041										
	M042										
	M046										
	M055										
	M056										
	M065										
	M068										
	M070										
Community Results		Consensus Mean					Consensus Mean				
		Consensus Standard Deviation					Consensus Standard Deviation				
		Maximum					Maximum				
		Minimum					Minimum				
		N			0		N			0	

Table 20. Data summary table for *trans*-vitamin K_1 in dietary supplements.

		***trans* -Vitamin K1**									
		Commercial Basil Powder (mg/kg)					**Commercial Kelp Powder (mg/kg)**				
	Lab	**A**	**B**	**C**	**Avg**	**SD**	**A**	**B**	**C**	**Avg**	**SD**
Individual Results	NIST										
	M004										
	M006										
	M012										
	M017										
	M024										
	M025										
	M028										
	M036										
	M041										
	M042										
	M046										
	M055										
	M056										
	M065										
	M068										
	M070										
Community Results		Consensus Mean					Consensus Mean				
		Consensus Standard Deviation					Consensus Standard Deviation				
		Maximum					Maximum				
		Minimum					Minimum				
		N			0		N			0	

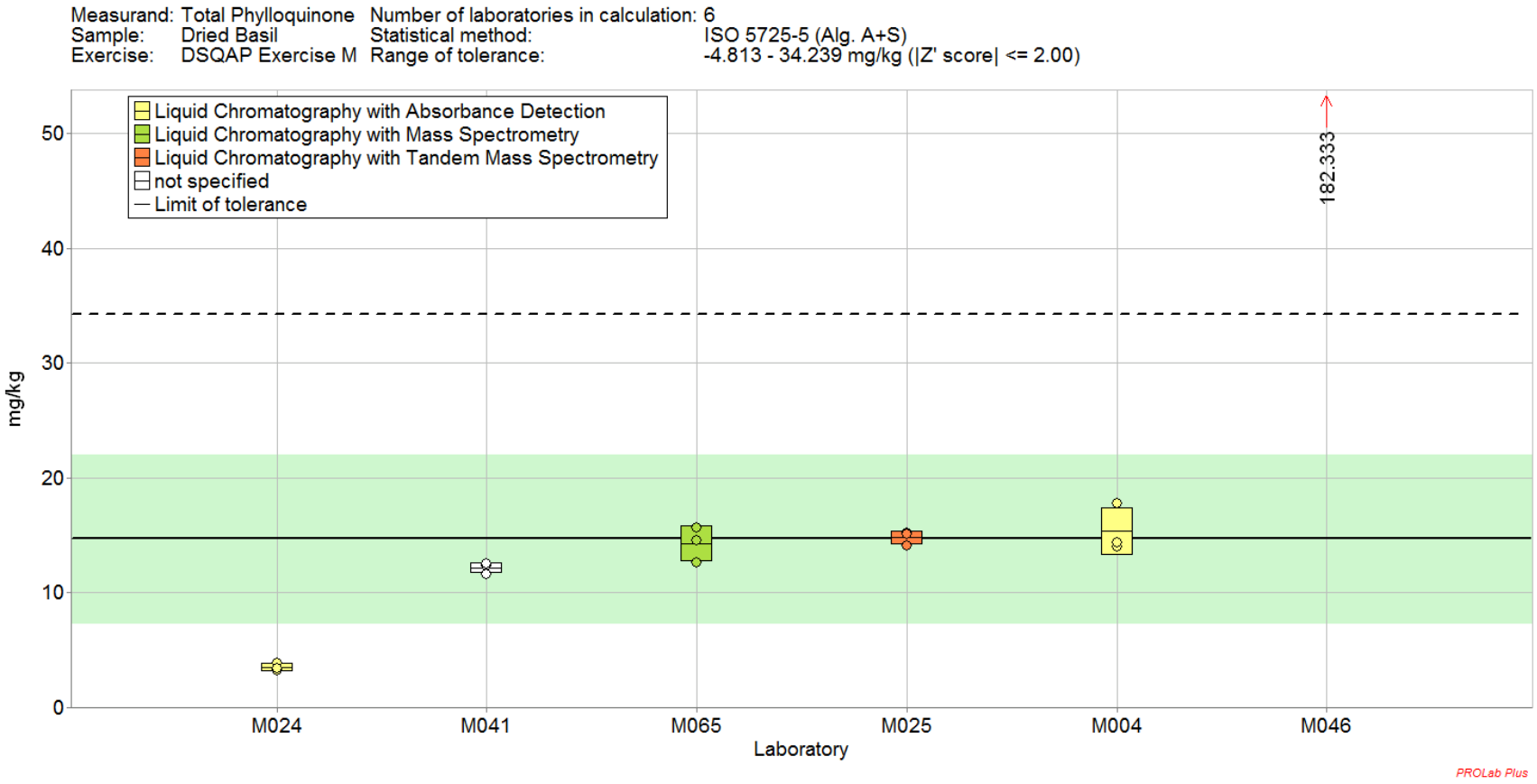

Figure 21. Total vitamin K_1 in basil (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents the consensus mean, and the green shaded region represents the consensus mean bounded by twice the consensus standard error. The black dashed 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, $|Z'_{comm}| \leq 2$. No NIST value has been determined in this material.

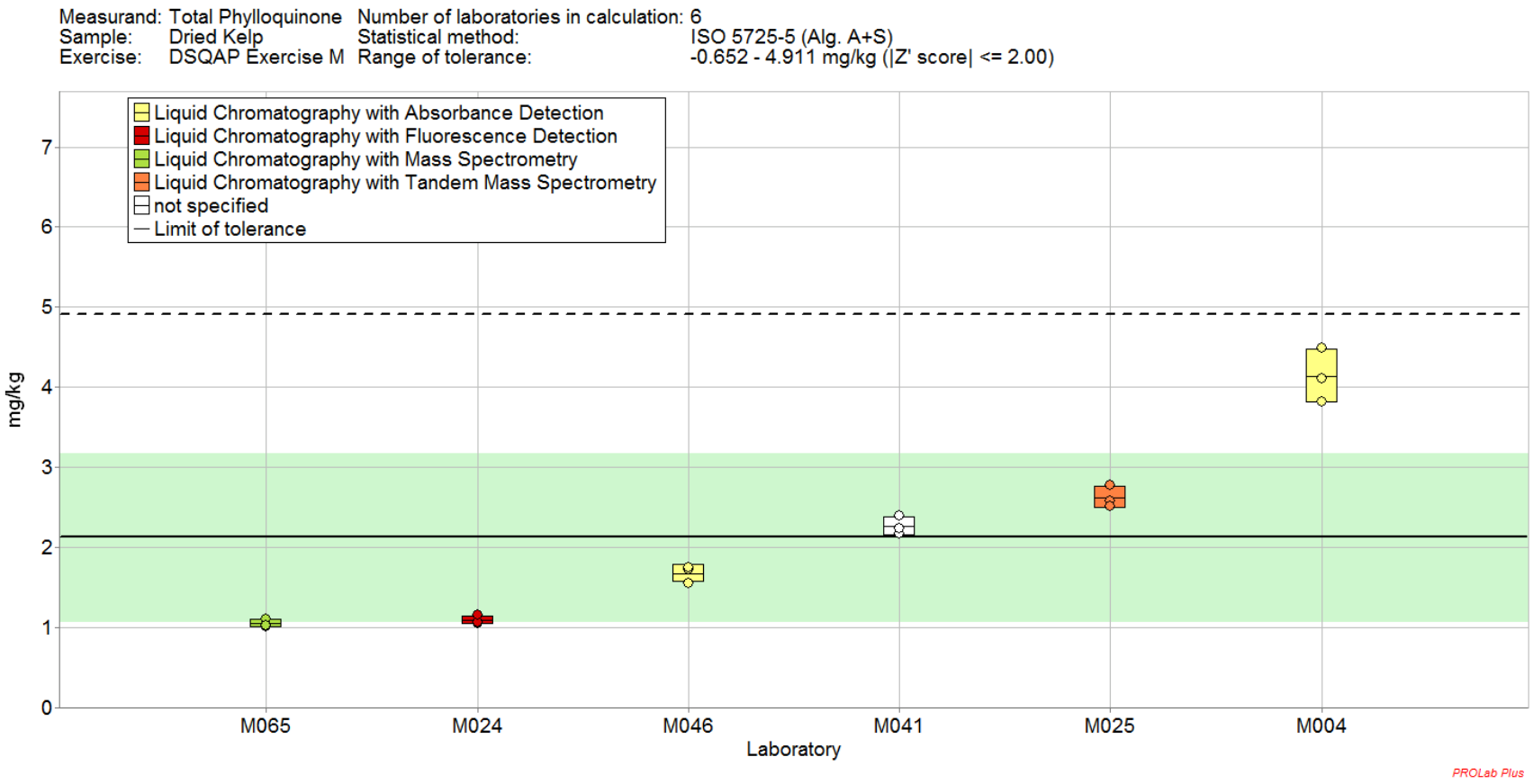

Figure 22. Total vitamin K_1 in kelp (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents the consensus mean, and the green shaded region represents the consensus mean bounded by twice the consensus standard error. The black dashed 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, $|Z'_{comm}| \leq 2$. No NIST value has been determined in this material.

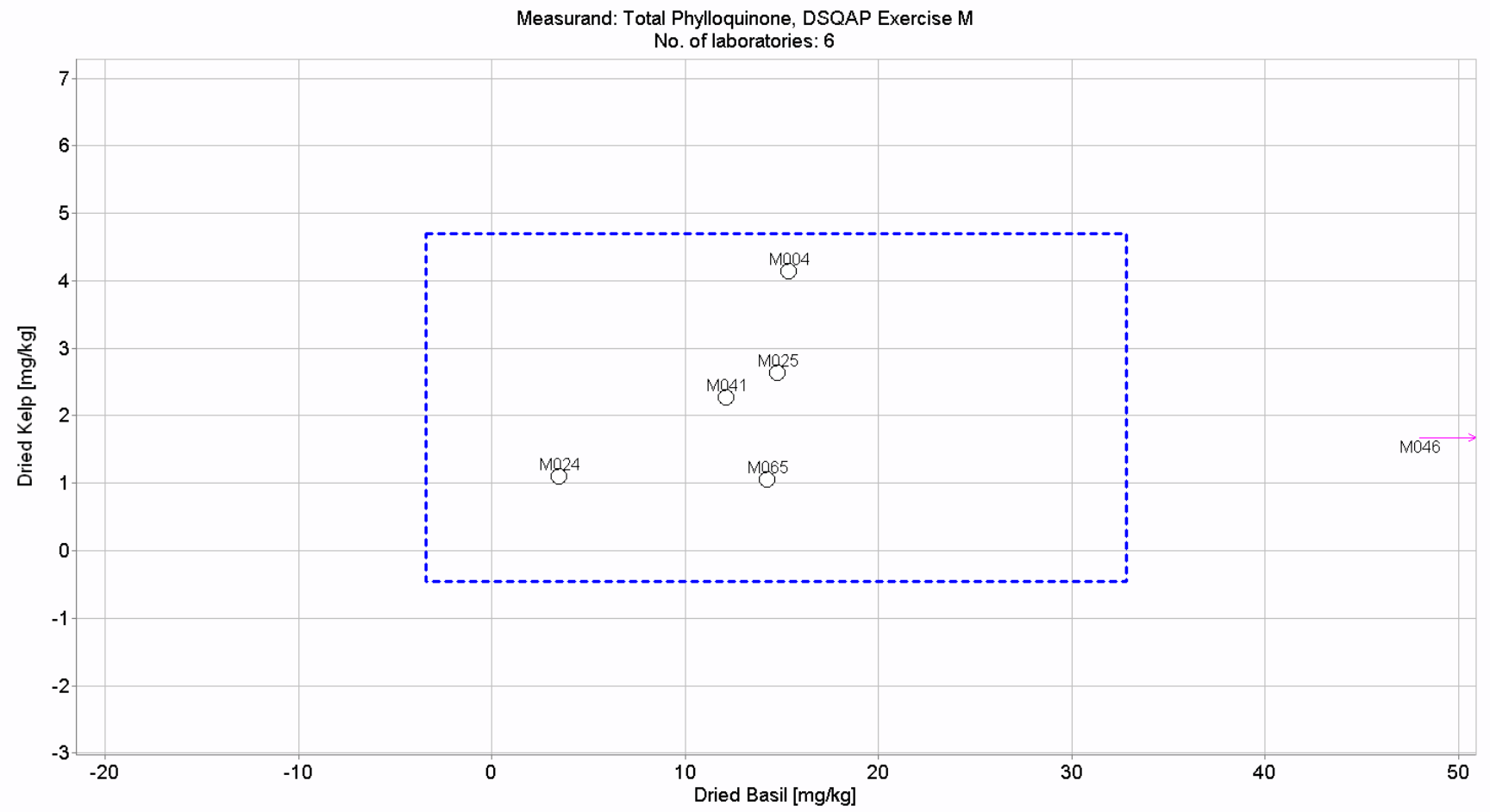

Figure 23. Laboratory means for total vitamin K_1 in dietary supplements (sample/sample comparison view). In this view, the individual laboratory mean for one sample (basil) is compared to the mean for a second sample (kelp). The dotted blue box represents the consensus range of tolerance for basil (x-axis) and kelp (y-axis), representing the consensus mean bounded by twice the reproducibility standard deviation.

CURCUMINOIDS IN TURMERIC

Study Overview

In this study, participants were provided with two candidate NIST SRMs, turmeric rhizome and curcuminoids extracted from turmeric. Participants were asked to use in-house analytical methods to determine the mass fractions of curcuminoids bisdemethoxycurcumin (BDMC), desmethoxycurcumin (DMC), and curcumin in each of the matrices and report values on an as-received basis.

Sample Information

Turmeric Rhizome. Participants were provided with three packets, each containing approximately 3 g of turmeric rhizome. The rhizome was blended, aliquotted, and heat-sealed inside 4 mil polyethylene bags, which were then sealed inside nitrogen-flushed aluminized plastic bags along with two packets of silica gel. Before use, participants were instructed to thoroughly mix the contents of each packet and to use a sample size of at least 100 mg. Participants were asked to store the material at controlled room temperature, 20 °C to 25 °C, to prepare a single sample and to report a single value from each packet provided. Approximate analyte levels were not reported to participants prior to the study. Target values and uncertainties for curcuminoids in the rhizome were determined at NIST by LC-absorbance. The NIST-determined values and standard deviations are reported in the table below on an as-received basis.

Analyte	NIST-Determined Mass Fraction (mg/g)
BDMC	3.390 ± 0.054
DMC	3.634 ± 0.064
Curcumin	11.17 ± 0.21

Curcuminoids Extracted from Turmeric. Participants were provided with three packets, each containing approximately 1 g of extract. The extract was blended, aliquotted, and heat-sealed inside 4 mil polyethylene bags, which were then sealed inside nitrogen-flushed aluminized plastic bags along with two packets of silica gel. Before use, participants were instructed to thoroughly mix the contents of each packet and to use a sample size of at least 10 mg. Participants were asked to store the material at controlled room temperature, 20 °C to 25 °C, to prepare a single sample and to report a single value from each packet provided. Approximate analyte levels were not reported to participants prior to the study. Target values and uncertainties for curcuminoids in the extract were determined at NIST by LC-absorbance. The NIST-determined values and standard deviations are reported in the table below on an as-received basis.

Analyte	NIST-Determined Mass Fraction (mg/g)
BDMC	18.25 ± 0.49
DMC	117.1 ± 1.2
Curcumin	822 ± 11

Study Results

- Thirty-one laboratories enrolled in this exercise and received samples for curcuminoids. Twenty-three laboratories reported results for curcumin (74 % participation), and 18 laboratories reported results for DMC and BDMC (69 % participation).
  - For BDMC, the consensus means were below the lower boundary of the target range in both samples. The between-laboratory variability was acceptable for BDMC (20 % RSD) for the turmeric rhizome and high (34 % RSD) for the turmeric extract.
  - For DMC, the consensus mean was below the lower boundary of the target range for the turmeric rhizome but within the target range for the extract, both with acceptable between-laboratory variability (17 % and 11 %, respectively).
  - For curcumin, the consensus means were on the upper and lower boundary of the target ranges for the turmeric rhizome and extract, respectively. The between-laboratory variability for curcumin was acceptable, at 18 % and 15 % for the turmeric rhizome and turmeric extract, respectively.
- All laboratories reported using LC with absorbance detection for determination of BDMC, DMC, and curcumin in the turmeric extract. LC-absorbance was also used for the analysis of the curcuminoids in the turmeric rhizome, with one laboratory reported using LC-MS.

Technical Recommendations

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

- The extraction procedure should be optimized for the extraction solvent and the number of extraction cycles to ensure exhaustive extraction from the matrix.
  - NIST found that the highest yield for curcuminoids was achieved using methanol as the extraction solvent.
  - Inadequate extraction from the rhizome sample may explain the low results for BDMC and DMC.
  - The optimum number of extraction cycles must be determined by sequential extraction until no further increase in yield is observed. Sequential extractions may be important in samples that contain very high concentrations of the curcuminoids, as the extraction solvent may quickly become saturated during the extraction.
- An individual calibration must be conducted for each curcuminoid for maximum accuracy.
- The purity of all calibrant materials should be rigorously determined using multiple techniques, and the final sample result corrected for any impurities. If curcuminoid impurities are identified (e.g., the standard for DMC contains curcumin), prepare separate calibration solutions for each curcuminoid to reduce potential bias.

Table 21. Individualized data summary table (NIST) for curcuminoids in turmeric.

National Institute of Standards & Technology

Exercise M - March 2016 - Curcuminoids

		Lab Code:	**NIST**	**1. Your Results**				**2. Community Results**			**3. Target**	
Analyte	Sample	Units		x_i	s_i	Z'_{comm}	Z_{NIST}	N	x*	s*	x_{NIST}	U_{95}
Bisdemethoxycurcumin	Turmeric Root	mg/g		3.390	0.054		0.00	18	3.23	0.66	3.390	0.054
Bisdemethoxycurcumin	Turmeric Extract	mg/g		18.25	0.49		0.00	18	16.2	5.5	18.25	0.49
Curcumin	Turmeric Root	mg/g		11.17	0.21		0.00	23	11.6	2.1	11.17	0.21
Curcumin	Turmeric Extract	mg/g		822	11		0.00	23	801	123	822	11
Desmethoxycurcumin	Turmeric Root	mg/g		3.634	0.064		0.00	18	3.26	0.54	3.634	0.064
Desmethoxycurcumin	Turmeric Extract	mg/g		117.1	1.2		0.00	17	116	13	117.1	1.2

x_i Mean of reported values

s_i Standard deviation of reported values

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

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

N Number of quantitative values reported

x* Robust mean of reported values

s* Robust standard deviation

x_{NIST} NIST-assessed value

U_{95} ±95% confidence interval about the assessed value or standard deviation (s_{NIST})

Table 22. Data summary table for BDMC in turmeric.

		Bisdemethoxycurcumin									
		Turmeric Rhizome (mg/g)					Tumeric Extract (mg/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	NIST				3.390	0.054				18.25	0.49
	M002	3.900	4.100	3.900	3.967	0.115	17.40	18.10	16.90	17.47	0.60
	M003										
	M004	3.774	4.024	4.107	3.968	0.173	21.33	19.45	19.06	19.95	1.22
	M006										
	M013	2.692	2.878	2.847	2.806	0.100	15.08	15.08	14.34	14.83	0.42
	M014	4.864	5.072	5.035	4.990	0.111	24.77	25.28	23.92	24.66	0.69
	M015	2.704	2.480	2.552	2.579	0.114	17.81	18.95	18.10	18.29	0.60
	M017										
	M020	2.690	2.720	3.120	2.843	0.240	17.71	18.01	17.32	17.68	0.35
	M022										
	M023	1.538	1.540	1.531	1.536	0.005	8.19	8.11	8.23	8.18	0.06
	M025	3.410	3.340	3.340	3.363	0.040	4.31	4.91	4.22	4.48	0.38
	M026	2.540	2.630	2.560	2.577	0.047	16.70	16.70	16.50	16.63	0.12
	M028										
	M032										
	M037	3.890	3.850	3.730	3.823	0.083	22.90	22.40	24.60	23.30	1.15
	M039	2.970	2.990	2.970	2.977	0.012	15.30	15.50	15.50	15.43	0.12
	M041										
	M047	3.680	3.740	3.760	3.727	0.042	18.10	20.00	18.70	18.93	0.97
	M053	2.473	2.467	2.600	2.513	0.075	14.15	14.46	14.69	14.43	0.27
	M055										
	M056	2.952	2.869	3.031	2.951	0.081	13.97	13.97	13.15	13.69	0.48
	M063	2.960	3.580	3.590	3.377	0.361	1.73	1.73	1.66	1.71	0.04
	M070	3.640	3.600	3.680	3.640	0.040	13.05	13.07	13.41	13.18	0.20
	M072	3.170	3.340	3.250	3.253	0.085	20.81	21.25	20.89	20.98	0.23
	M074	3.222	3.403	3.397	3.341	0.103	18.84	16.58	18.70	18.04	1.27
Community Results		Consensus Mean			3.232		Consensus Mean			16.19	
		Consensus Standard Deviation			0.658		Consensus Standard Deviation			5.46	
		Maximum			4.990		Maximum			24.66	
		Minimum			1.536		Minimum			1.71	
		N			18		N			18	

Table 23. Data summary table for DMC in turmeric.

		Desmethoxycurcumin									
		Turmeric Rhizome (mg/g)					Tumeric Extract (mg/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	NIST				3.634	0.064				117.1	1.2
	M002	3.500	3.700	4.100	3.767	0.306	115.7	116.7	114.9	115.8	0.9
	M003										
	M004	3.807	3.942	4.004	3.918	0.101	133.0	129.3	127.6	130.0	2.8
	M006										
	M013	2.481	2.602	2.571	2.551	0.063	105.0	107.7	99.4	104.0	4.2
	M014	4.904	5.014	4.815	4.911	0.100	134.0	135.0	132.1	133.7	1.5
	M015	2.820	2.656	2.700	2.725	0.085	114.8	115.8	107.7	112.7	4.4
	M017										
	M020	3.030	3.050	3.610	3.230	0.329	139.4	141.6	136.4	139.1	2.6
	M022										
	M023	1.608	1.606	1.587	1.600	0.012	54.0	53.8	54.9	54.2	0.6
	M025	3.630	3.560	3.570	3.587	0.038	113.0	114.0	111.0	112.7	1.5
	M026	3.040	3.140	3.050	3.077	0.055	125.0	125.0	124.0	124.7	0.6
	M028										
	M032										
	M037	3.620	3.350	3.440	3.470	0.137	112.9	122.5	122.1	119.2	5.4
	M039	3.490	3.520	3.520	3.510	0.017	116.8	117.7	118.1	117.5	0.7
	M041										
	M047	3.050	3.020	3.100	3.057	0.040	111.0	121.0	116.0	116.0	5.0
	M053	3.052	3.045	3.153	3.083	0.060	120.4	121.1	120.2	120.5	0.5
	M055										
	M056	3.392	3.290	3.317	3.333	0.053	106.1	106.5	104.9	105.8	0.8
	M063	2.950	3.390	3.410	3.250	0.260	11.8	11.7	11.5	11.7	0.1
	M070	3.540	3.470	3.590	3.533	0.060	110.5	110.7	112.1	111.1	0.9
	M072	2.690	2.420	2.340	2.483	0.183					
	M074	3.392	3.582	3.576	3.517	0.108	122.5	107.8	121.6	117.3	8.2
Community Results		Consensus Mean			3.256		Consensus Mean			115.9	
		Consensus Standard Deviation			0.544		Consensus Standard Deviation			12.8	
		Maximum			4.911		Maximum			139.1	
		Minimum			1.600		Minimum			11.7	
		N			18		N			17	

Table 24. Data summary table for curcumin in turmeric.

		Curcumin									
		Turmeric Rhizome (mg/g)					Tumeric Extract (mg/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	NIST				11.17	0.21				822	11
	M002	12.70	13.10	13.10	12.97	0.23	837	847	839	841	5
	M003										
	M004	11.69	12.05	12.31	12.02	0.31	836	846	850	844	7
	M006										
	M013	5.39	5.27	5.30	5.32	0.06	645	647	578	624	39
	M014	11.29	11.37	10.66	11.11	0.39	764	778	781	774	9
	M015	11.22	10.80	10.80	10.94	0.24	883	884	878	882	3
	M017										
	M020	10.31	10.44	12.54	11.10	1.25	968	980	945	964	18
	M022										
	M023	5.69	5.70	5.67	5.68	0.02	411	411	417	413	3
	M025	11.80	11.60	11.60	11.67	0.12	824	825	807	819	10
	M026	10.20	10.50	10.30	10.33	0.15	856	851	845	851	6
	M028	17.90	19.60	19.10	18.87	0.87	991	959	975	975	16
	M032										
	M033	974.80	953.50	961.20	963.17	10.79	17	16	16	16	0
	M037	11.20	10.70	10.40	10.77	0.40	762	752	759	758	5
	M039	10.70	10.80	10.90	10.80	0.10	780	787	789	785	5
	M040	12.80	12.60	13.00	12.80	0.20	780	783	782	781	2
	M041										
	M047	9.12	9.01	9.44	9.19	0.22	728	780	774	761	28
	M051										
	M053	10.02	9.93	10.28	10.08	0.19	778	782	776	779	3
	M054	16.00	15.00	16.00	15.67	0.58	998	1016	993	1002	12
	M055										
	M056	12.42	11.85	12.01	12.09	0.29	786	793	794	791	4
	M063	9.86	11.70	11.66	11.07	1.05	84	84	82	83	1
	M070	11.51	11.25	11.57	11.44	0.17	854	852	876	860	13
	M072	11.19	12.36	12.05	11.87	0.61	898	900	892	896	4
	M074	10.52	10.93	10.73	10.72	0.21	801	705	795	767	54
	M075	14.71	14.79	14.60	14.70	0.10	827	839	836	834	6
Community Results		Consensus Mean			11.56		Consensus Mean			801	
		Consensus Standard Deviation			2.14		Consensus Standard Deviation			123	
		Maximum			963.17		Maximum			1002	
		Minimum			5.32		Minimum			16	
		N			23		N			23	

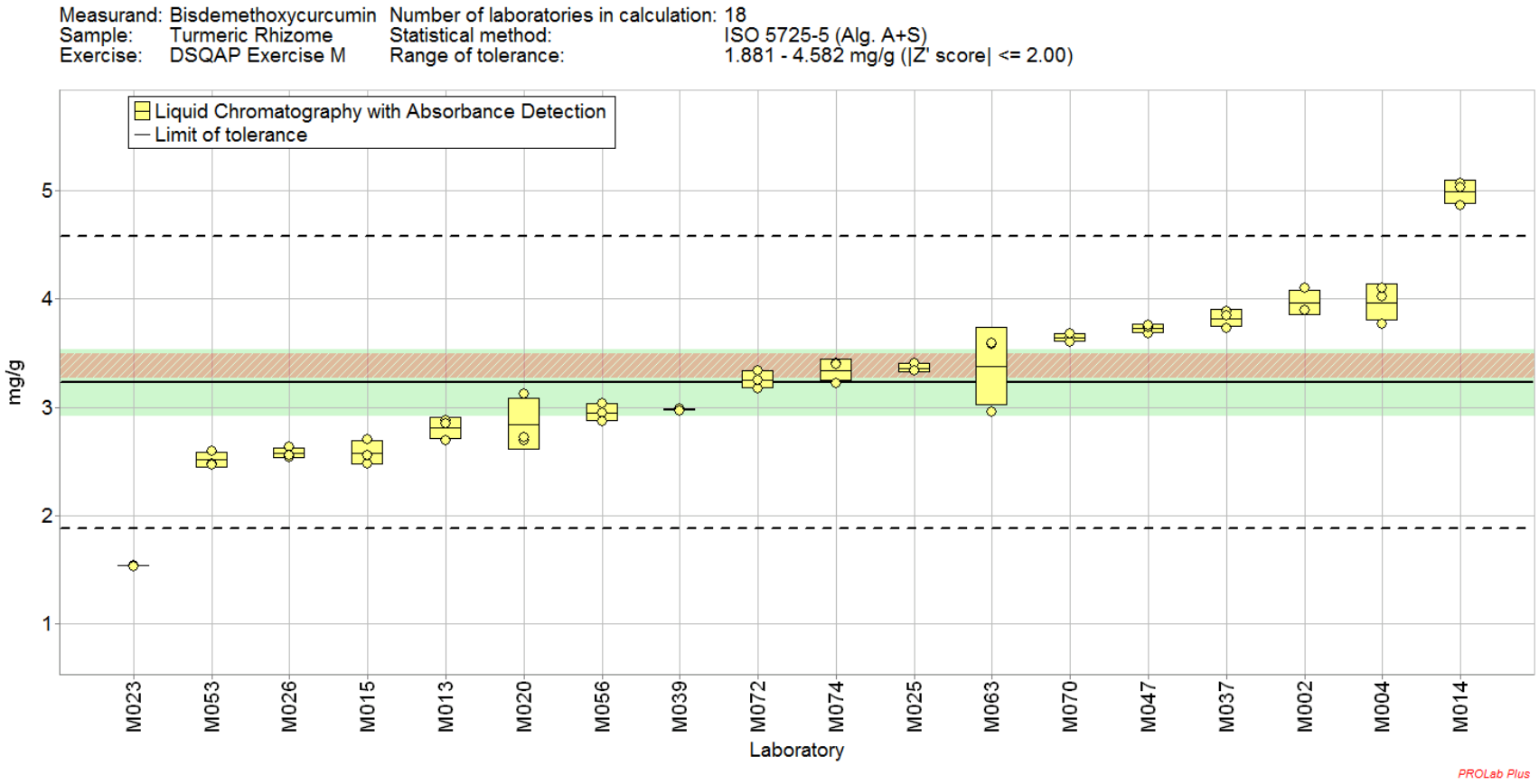

Figure 24. BDMC in turmeric rhizome (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents the consensus mean, and the green shaded region represents the consensus mean bounded by twice the consensus standard error. The black dashed lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{comm}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the NIST-determined value bounded by twice its standard deviation, and represents the range that results in an acceptable Z'_{NIST} score, $|Z'_{NIST}| \leq 2$.

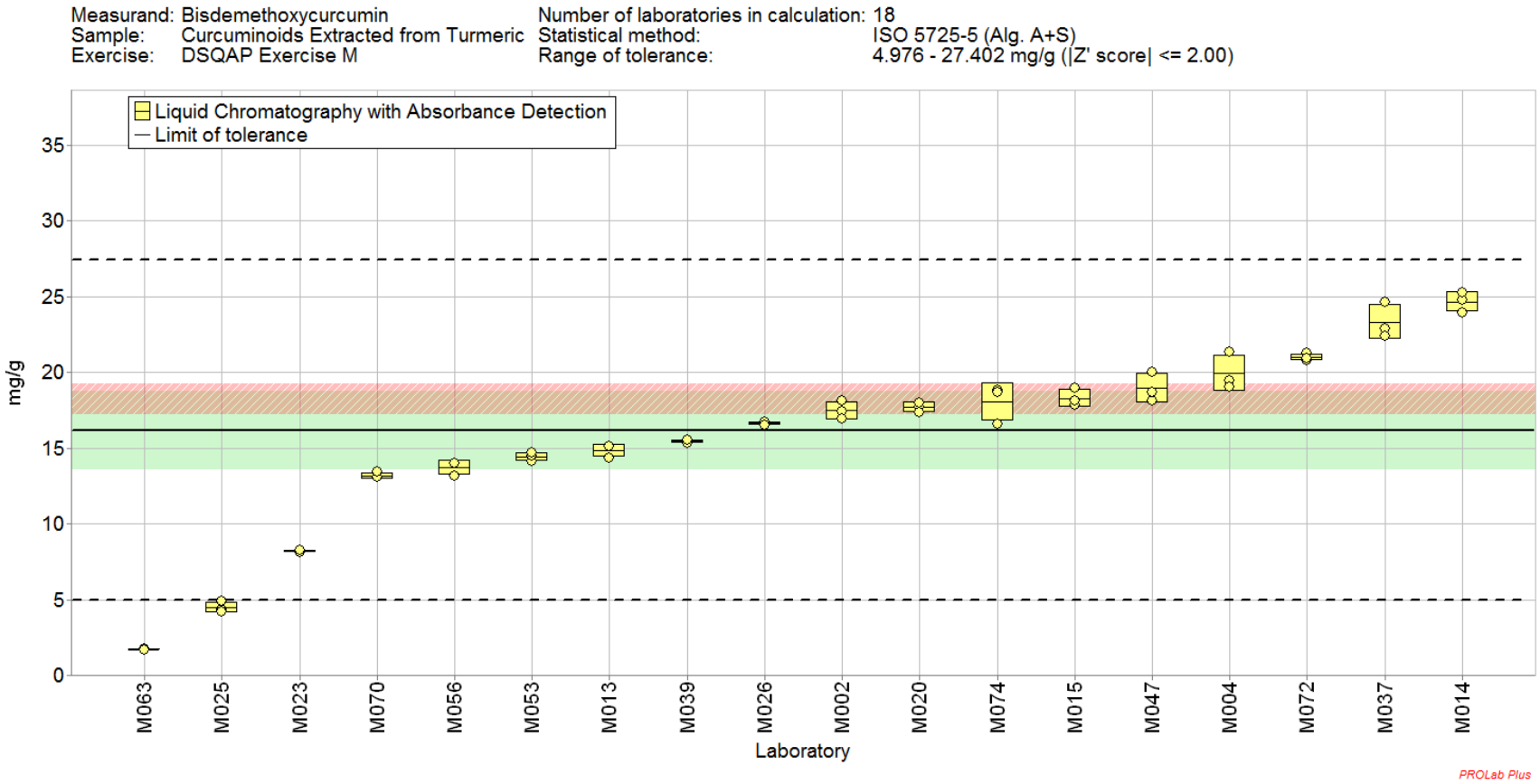

Figure 25. BDMC in curcuminoids extracted from turmeric (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents the consensus mean, and the green shaded region represents the consensus mean bounded by twice the consensus standard error. The black dashed lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{comm}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the NIST-determined value bounded by twice its standard deviation, and represents the range that results in an acceptable Z'_{NIST} score, $|Z'_{NIST}| \leq 2$.

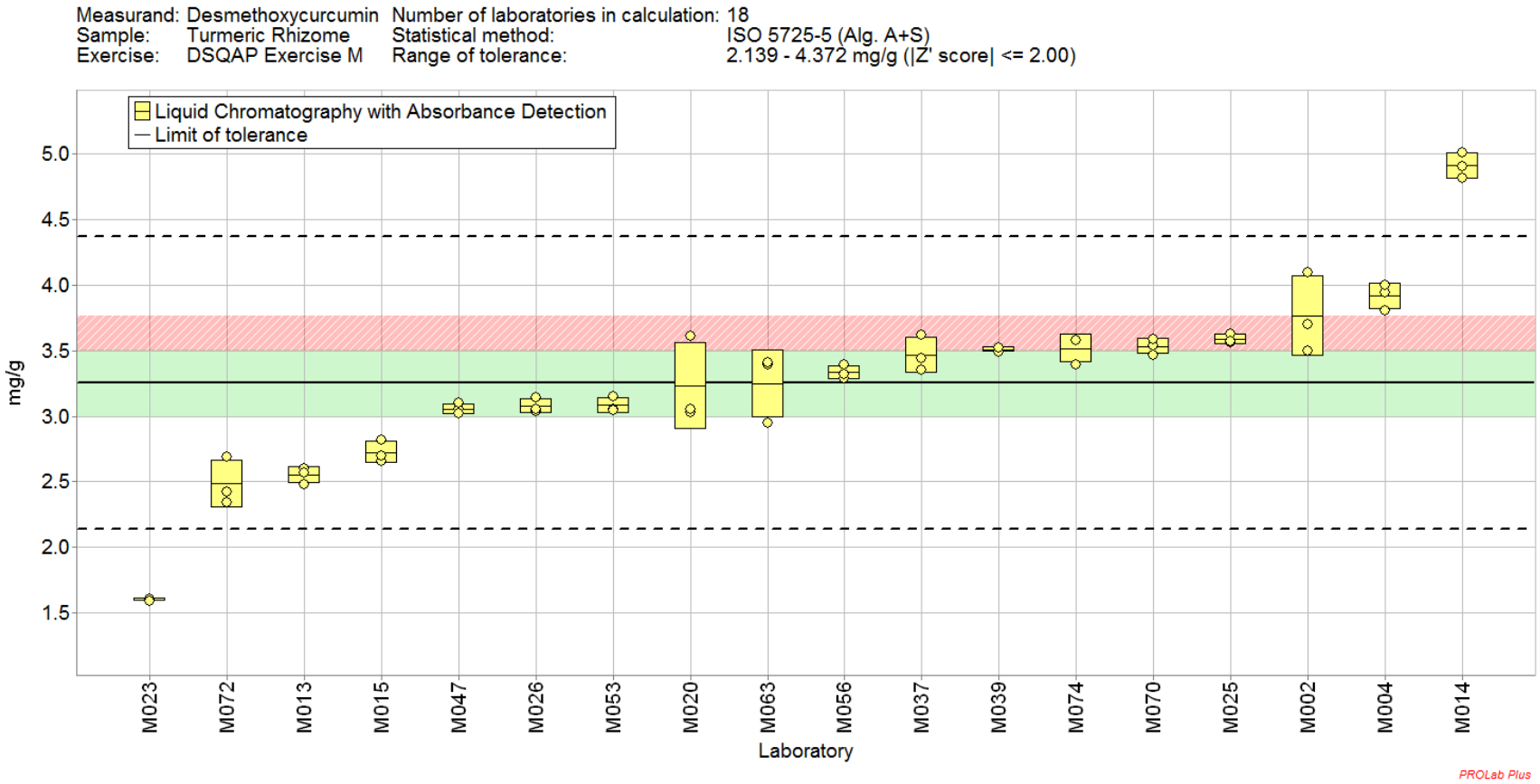

Figure 26. DMC in turmeric rhizome (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents the consensus mean, and the green shaded region represents the consensus mean bounded by twice the consensus standard error. The black dashed lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{comm}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the NIST-determined value bounded by twice its standard deviation, and represents the range that results in an acceptable Z'_{NIST} score, $|Z'_{NIST}| \leq 2$.

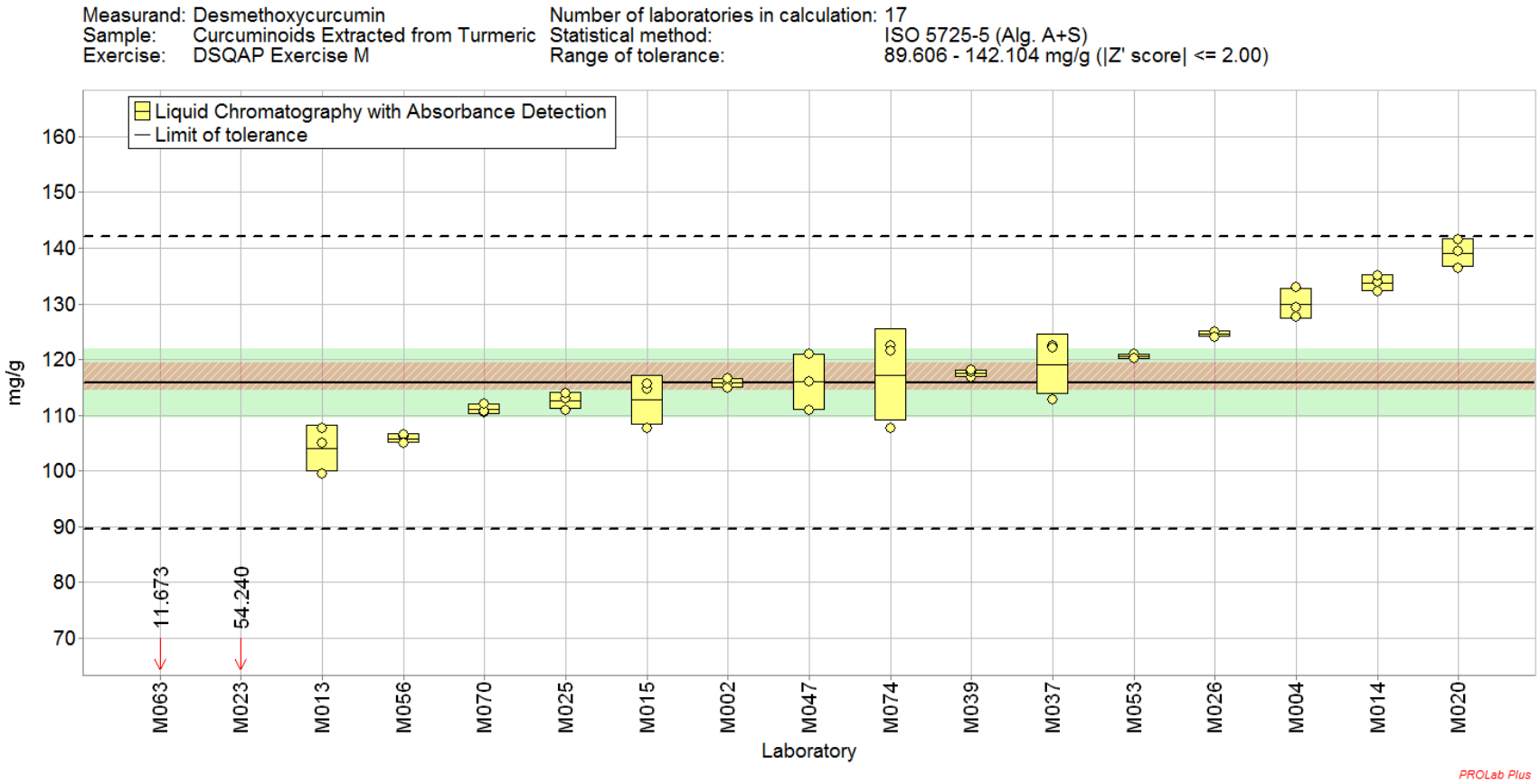

Figure 27. DMC in curcuminoids extracted from turmeric (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents the consensus mean, and the green shaded region represents the consensus mean bounded by twice the consensus standard error. The black dashed lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{comm}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the NIST-determined value bounded by twice its standard deviation, and represents the range that results in an acceptable Z'_{NIST} score, $|Z'_{NIST}| \leq 2$.

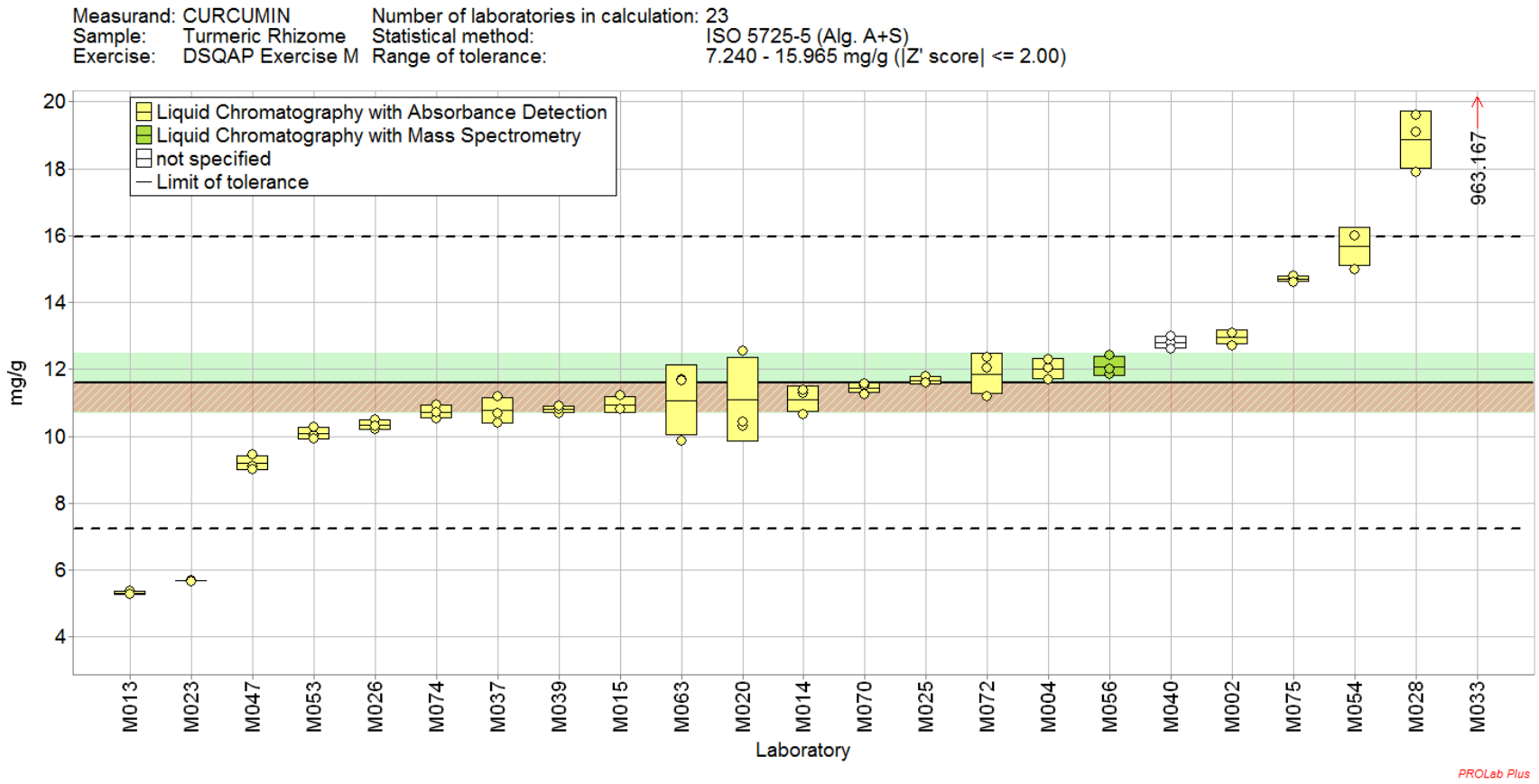

Figure 28. Curcumin in turmeric rhizome (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents the consensus mean, and the green shaded region represents the consensus mean bounded by twice the consensus standard error. The black dashed lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{comm}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the NIST-determined value bounded by twice its standard deviation, and represents the range that results in an acceptable Z'_{NIST} score, $|Z'_{NIST}| \leq 2$.

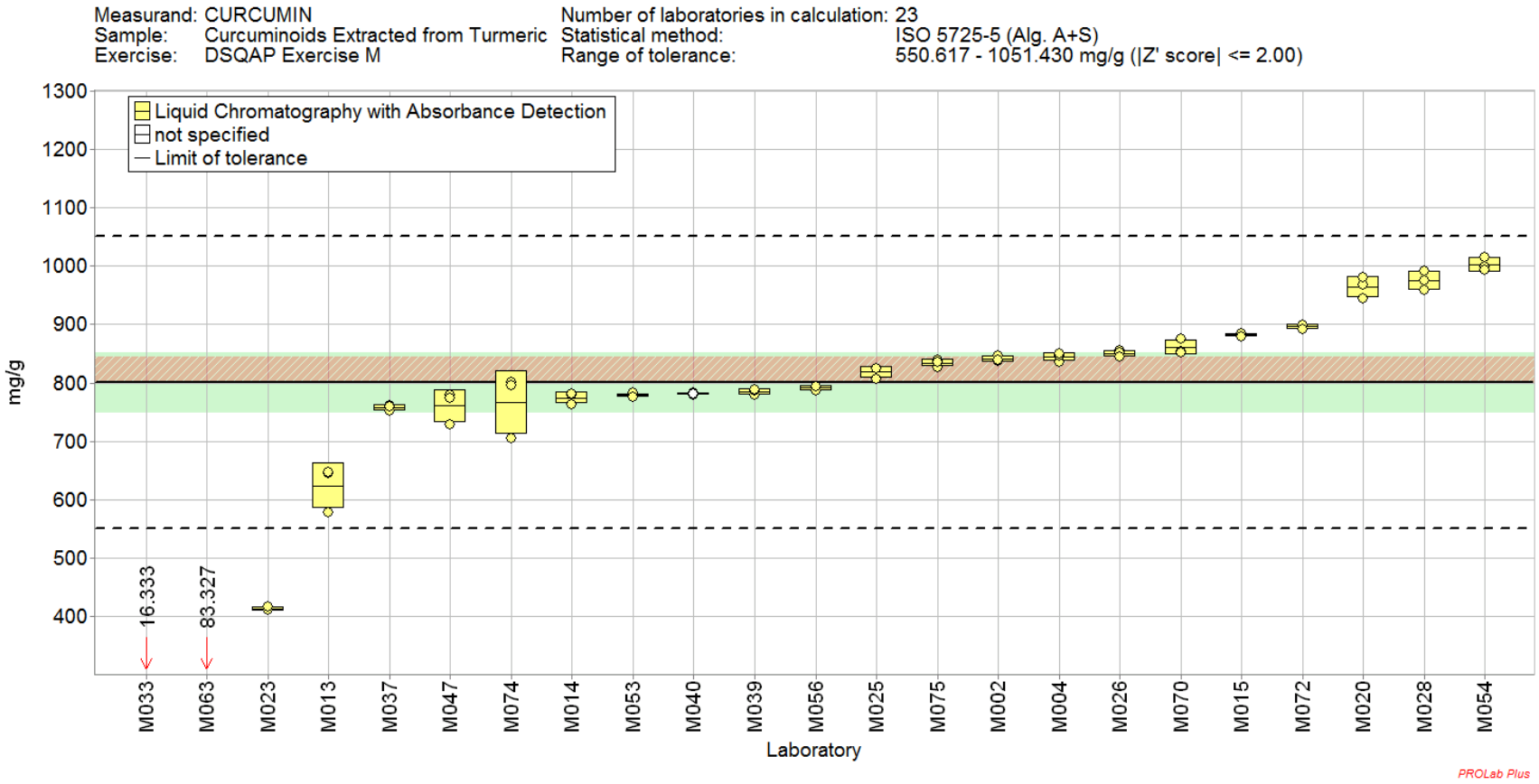

Figure 29. Curcumin in curcuminoids extracted from turmeric (data summary view – analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the analytical method employed. The solid black line represents the consensus mean, and the green shaded region represents the consensus mean bounded by twice the consensus standard error. The black dashed lines represent the consensus range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z'_{comm} score, $|Z'_{comm}| \leq 2$. The red shaded region represents the NIST range of tolerance, which encompasses the NIST-determined value bounded by twice its standard deviation, and represents the range that results in an acceptable Z'_{NIST} score, $|Z'_{NIST}| \leq 2$.

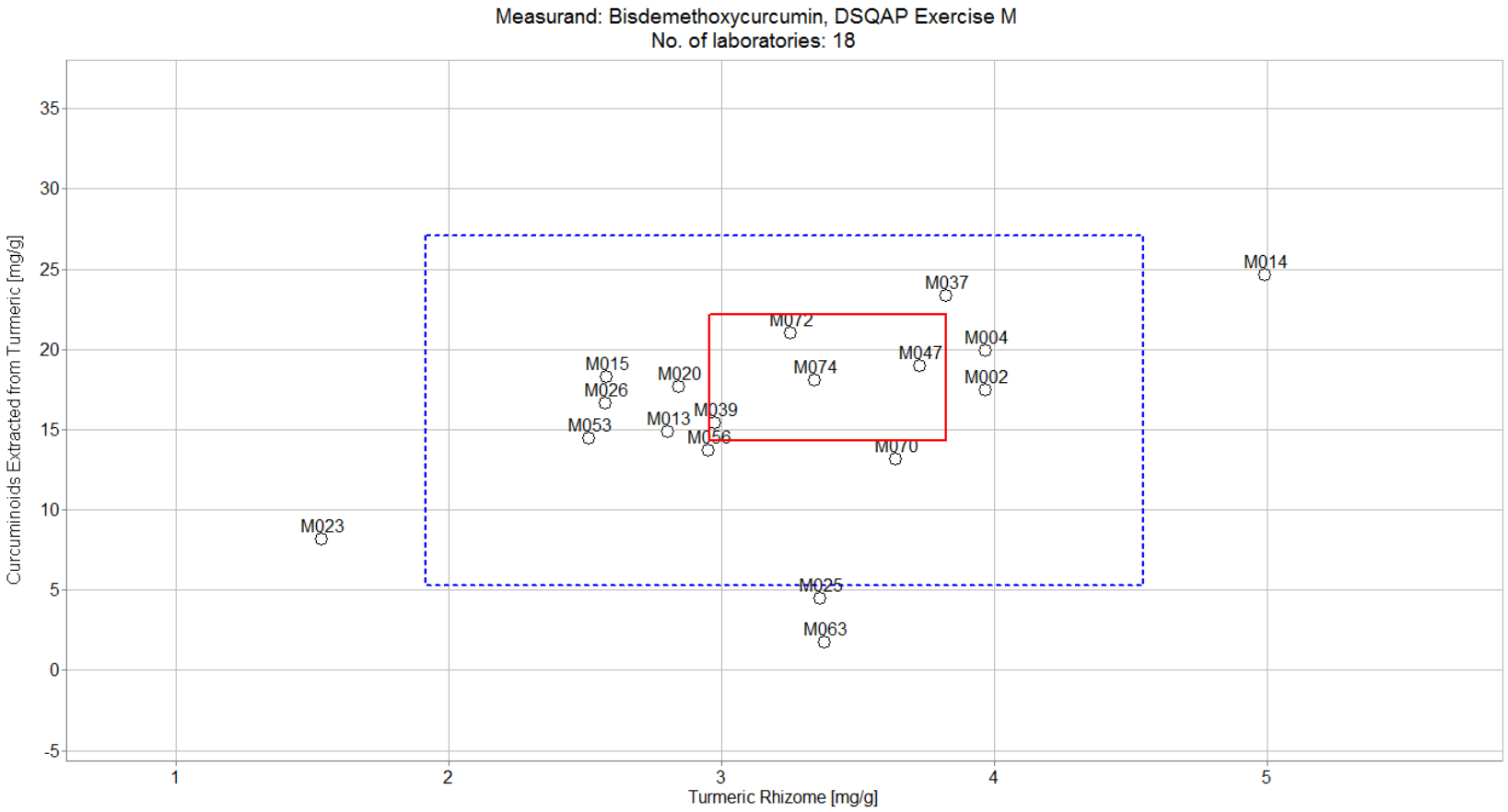

Figure 30. Laboratory means for BDMC in turmeric rhizome and curcuminoids extracted from turmeric (sample/sample comparison view). In this view, the individual laboratory mean for one sample (turmeric rhizome) is compared to the mean for a second sample (curcuminoids extracted from turmeric). The solid red box represents the NIST range of tolerance for the two samples, turmeric rhizome (x-axis) and curcuminoids extracted from turmeric (y-axis), which encompasses the NIST values bounded by twice their uncertainties (U_{95}), 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 turmeric rhizome (x-axis) and curcuminoids extracted from turmeric (y-axis), calculated as the values above and below the consensus means that result in an acceptable Z'_{comm} score, $|Z'_{comm}| \leq 2$.

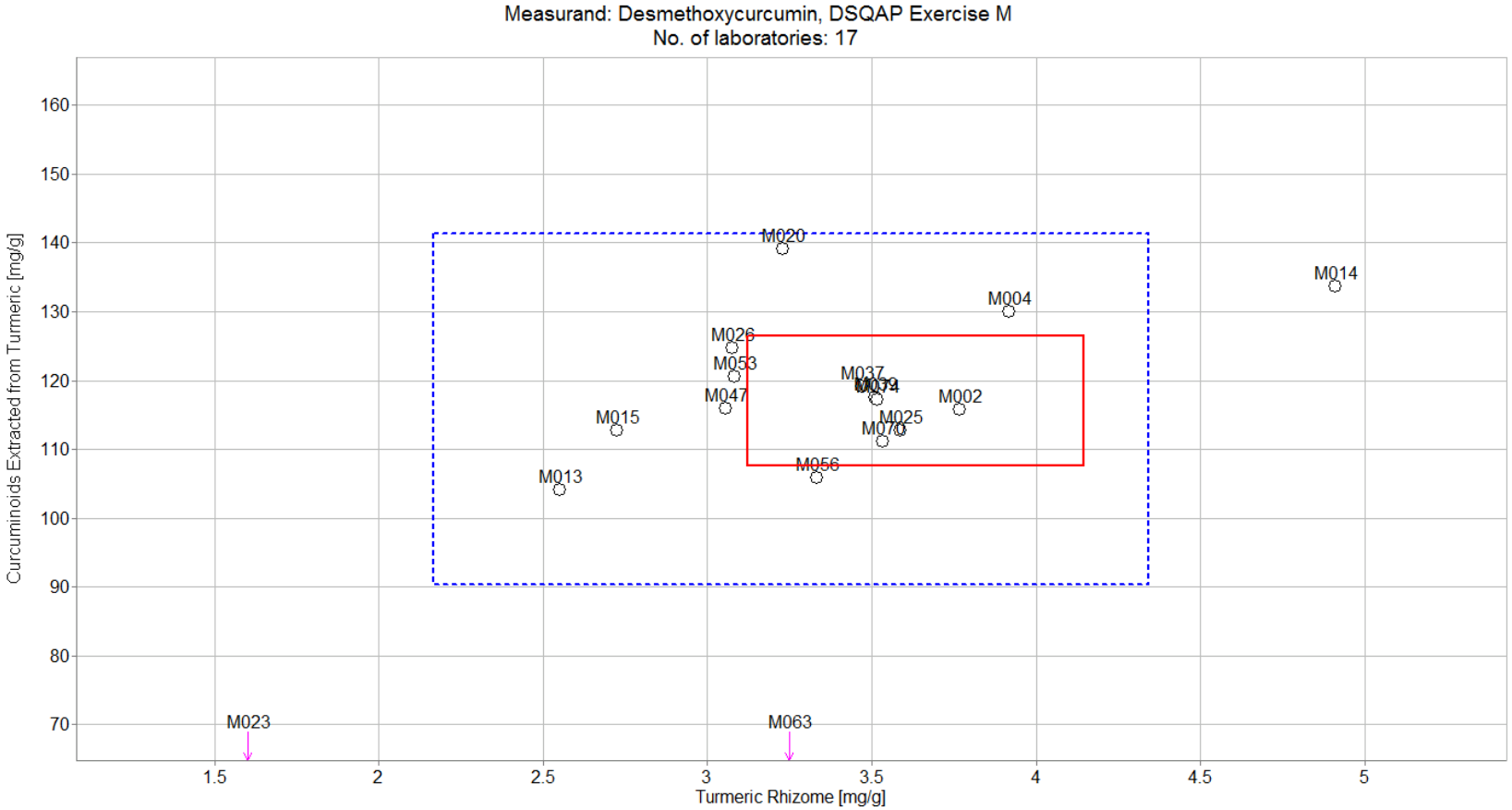

Figure 31. Laboratory means for DMC in turmeric rhizome and curcuminoids extracted from turmeric (sample/sample comparison view). In this view, the individual laboratory mean for one sample (turmeric rhizome) is compared to the mean for a second sample (curcuminoids extracted from turmeric). The solid red box represents the NIST range of tolerance for the two samples, turmeric rhizome (x-axis) and curcuminoids extracted from turmeric (y-axis), which encompasses the NIST values bounded by twice their uncertainties (U_{95}), 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 turmeric rhizome (x-axis) and curcuminoids extracted from turmeric (y-axis), calculated as the values above and below the consensus means that result in an acceptable Z'_{comm} score, $|Z'_{comm}| \leq 2$.

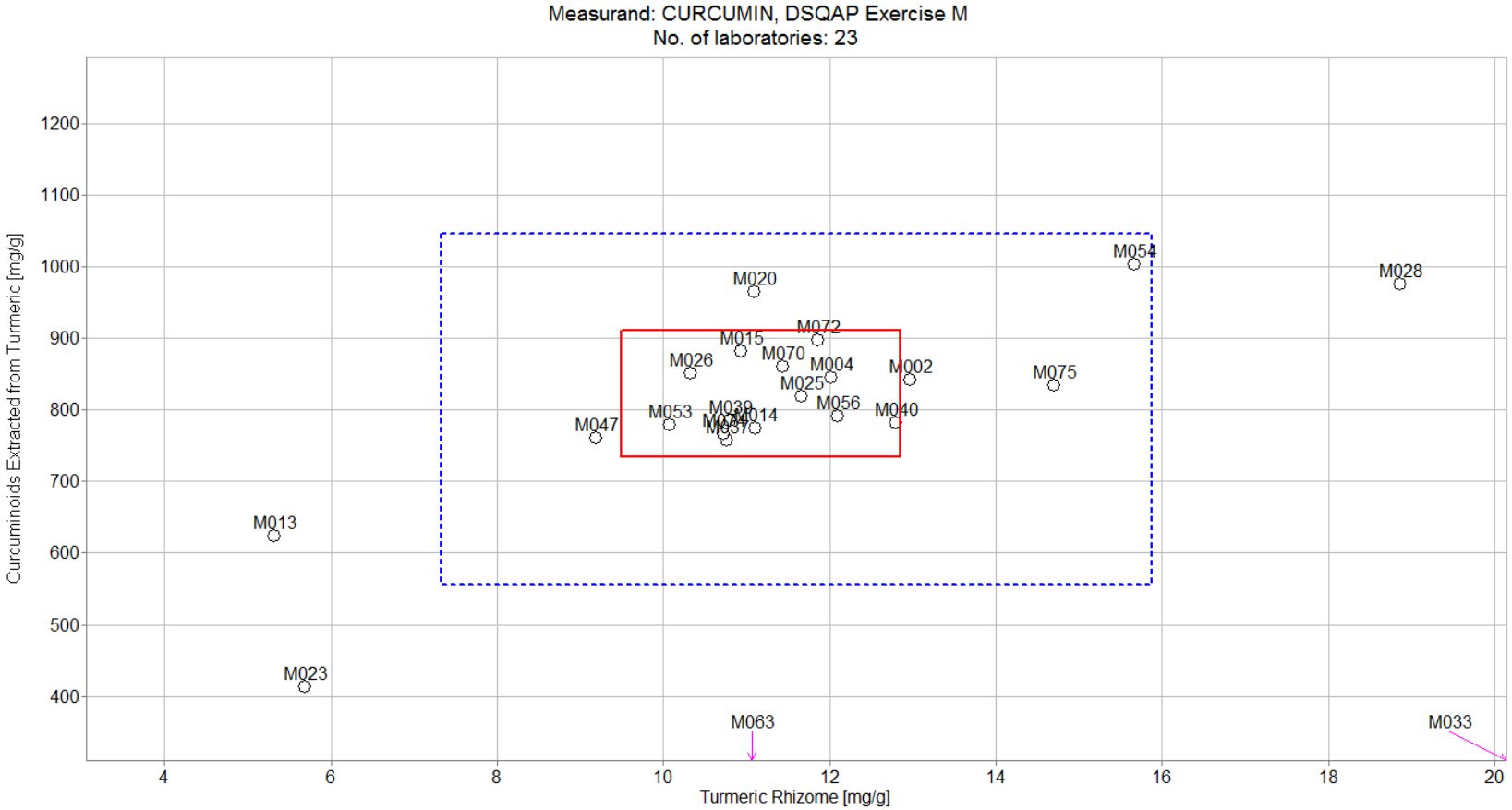

Figure 32. Laboratory means for curcumin in turmeric rhizome and curcuminoids extracted from turmeric (sample/sample comparison view). In this view, the individual laboratory mean for one sample (turmeric rhizome) is compared to the mean for a second sample (curcuminoids extracted from turmeric). The solid red box represents the NIST range of tolerance for the two samples, turmeric rhizome (x-axis) and curcuminoids extracted from turmeric (y-axis), which encompasses the NIST values bounded by twice their uncertainties (U_{95}), 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 turmeric rhizome (x-axis) and curcuminoids extracted from turmeric (y-axis), calculated as the values above and below the consensus means that result in an acceptable Z'_{comm} score, $|Z'_{comm}| \leq 2$.

CHONDROITIN SULFATE IN DIETARY SUPPLEMENT RAW MATERIALS

Study Overview

In this study, participants were provided with nine amber glass vials, each containing chondroitin sulfate from one of three sources (one bovine, two porcine). Participants were asked to use in-house analytical methods or AOAC First Action Official Method 2015.11 to determine the total mass fraction of chondroitin sulfate in each of the materials and report values on a dry-mass basis. Data from participants using AOAC 2015.11 will also be used as part of the collaborative study to evaluate method reproducibility. As part of this study, participants were asked to use their in-house analytical methods to determine the mass fractions of chondroitin sulfate A, chondroitin sulfate C, chondroitin sulfate D, and chondroitin sulfate E in each of the matrices to identify the source of chondroitin, and report values on a dry-mass basis.

Sample Information

Chondroitin Sodium Sulfate from Bovine Source. Participants were provided with three amber glass vials (labeled samples 3, 4, and 7), each containing approximately 4 g of chondroitin sulfate. Participants were asked to store the material at controlled room temperature, 20 °C to 25 °C, and to report a single value from each vial provided. Approximate analyte levels were not reported to participants prior to the study, and target values for these analytes have not been determined by NIST.

Chondroitin Sodium Sulfate from Porcine Source. Participants were provided with three amber glass vials (labeled samples 2, 5, and 6), each containing approximately 4 g of chondroitin sulfate. Participants were asked to store the material at controlled room temperature, 20 °C to 25 °C, and to report a single value from each vial provided. Approximate analyte levels were not reported to participants prior to the study, and target values for these analytes have not been determined by NIST.

Chondroitin Sulfate Calcium from Porcine Source. Participants were provided with three amber glass vials (labeled samples 1, 8, and 9), each containing approximately 4 g of chondroitin sulfate. Participants were asked to store the material at controlled room temperature, 20 °C to 25 °C, and to report a single value from each vial provided. Approximate analyte levels were not reported to participants prior to the study, and target values for these analytes have not been determined by NIST.

Study Results

- Fifteen laboratories enrolled in this exercise and received samples. Eight laboratories reported data for total chondroitin sulfate (53 % participation). Five laboratories reported data for chondroitin sulfate A and chondroitin sulfate C (33 % participation). No laboratories reported data for chondroitin sulfate D or chondroitin sulfate E.
- The results for total chondroitin were divided into two subsets. One group reported values in percent as requested by the shipping letter, while the other group reported values in µg/g as requested by the data entry page.
    - One subset of 3 laboratories reported results in µg/g as requested by the data entry page. The consensus results for subset 1 had excellent between-laboratory variability (4 % to 6 % RSD for the three samples).

- A second subset of 3 laboratories reported results in percent (%) as requested by the shipping letter. The consensus results for subset 2 had good between-laboratory variability (7 % to 11 % RSD for the three samples).
- When the values are adjusted to be on the same order of magnitude, assuming that the reporting units are the source of variability in the original data set, the results between the two subsets are consistent, with excellent between-laboratory variability (5 % to 7 % RSD for the three samples).

- Limited data was reported for chondroitin sulfate A and chondroitin sulfate C (five laboratories), and between-laboratory variability was very high (130 % to 150 % RSD).

Technical Recommendations

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

- No method information was collected as part of this study. In future studies, the ability to select the AOAC Official Method of Analysis will be clearly available on the reporting website.
- Inconsistent requests for reporting units between the shipping letter and data entry website led to results being divided into two subsets for chondroitin sulfate. Despite this issue, the results show promise for future studies with good between-laboratory variability after unit correction. In future studies, requests from NIST will be more consistent.
- Limited data gathered for chondroitin sulfate A and chondroitin sulfate C may indicate challenges with such speciation.

Table 25. Individualized data summary table (NIST) for chondroitin sulfate in dietary supplement raw materials.

National Institute of Standards & Technology

Exercise M - March 2016 - Chondroitin Sulfate

		Lab Code:	**NIST**	**1. Your Results**				**2. Community Results**			**3. Target**	
Analyte	Sample	Units	x_i	s_i	Z'_{comm}	Z_{NIST}	N	x*	s*	x_{NIST}	U_{95}
Total Chondroitin Sulfate	Bovine Sodium Sulfate	µg/g					8	392490	614314		
Total Chondroitin Sulfate	Porcine sodium sulfate	µg/g					8	379105	593879		
Total Chondroitin Sulfate	Porcine sulfate calcium	µg/g					8	356732	558577		
Total Chondroitin Sulfate (Subset 1)	Bovine Sodium Sulfate	µg/g					3	1046239	46235		
Total Chondroitin Sulfate (Subset 1)	Porcine sodium sulfate	µg/g					3	1010577	62101		
Total Chondroitin Sulfate (Subset 1)	Porcine sulfate calcium	µg/g					3	950933	50896		
Total Chondroitin Sulfate (Subset 2)	Bovine Sodium Sulfate	%					3	106	8		
Total Chondroitin Sulfate (Subset 2)	Porcine sodium sulfate	%					3	102	12		
Total Chondroitin Sulfate (Subset 2)	Porcine sulfate calcium	%					3	95	9		
Chondroitin Sulfate A	Bovine Sodium Sulfate	µg/g					5	381868	485968		
Chondroitin Sulfate A	Porcine sodium sulfate	µg/g					5	354194	462623		
Chondroitin Sulfate A	Porcine sulfate calcium	µg/g					5	337934	444910		
Chondroitin Sulfate C	Bovine Sodium Sulfate	µg/g					5	197883	303402		
Chondroitin Sulfate C	Porcine sodium sulfate	µg/g					5	144061	220869		
Chondroitin Sulfate C	Porcine sulfate calcium	µg/g					5	133615	204888		
Chondroitin Sulfate D	Bovine Sodium Sulfate	µg/g									
Chondroitin Sulfate D	Porcine sodium sulfate	µg/g									
Chondroitin Sulfate D	Porcine sulfate calcium	µg/g									
Chondroitin Sulfate E	Bovine Sodium Sulfate	µg/g									
Chondroitin Sulfate E	Porcine sodium sulfate	µg/g									
Chondroitin Sulfate E	Porcine sulfate calcium	µg/g									

x_i Mean of reported values
s_i Standard deviation of reported values
Z'_{comm} Z'-score with respect to community consensus
Z_{NIST} Z-score with respect to NIST value
N Number of quantitative values reported
x* Robust mean of reported values
s* Robust standard deviation
x_{NIST} NIST-assessed value
U_{95} ±95% confidence interval about the assessed value or standard deviation (s_{NIST})

Table 26. Data summary table for total chondroitin sulfate in dietary supplement raw materials.

		Total Chondroitin Sulfate														
		Bovine sodium sulfate (µg/g)					Porcine sodium sulfate (µg/g)					Porcine sulfate calcium (µg/g)				
	Lab	3	4	7	Avg	SD	5	2	6	Avg	SD	9	1	8	Avg	SD
Individual Results	NIST															
	M003															
	M006															
	M016	107	106	106	106	1	105	105	105	105	0	97	97	96	97	1
	M017	112	113	112	112	1	110	111	110	110	1	102	102	101	102	1
	M023															
	M025															
	M027	97	99	100	99	2	90	94	88	91	3	87	86	86	87	0
	M028															
	M033	1051600	1053900	1083900	1063133	18021	1084300	1062100	1066900	1071100	11681	990300	1019400	990500	1000067	16743
	M041															
	M042	1147754	1046531	1021969	1072085	66672	1022570	919861	960261	967564	51742	871114	947198	954487	924266	46175
	M054	0.08			0.08			0.09		0.09			0.08		0.08	
	M055															
	M056	861	865	923	883	35	788	815	807	803	14	791	748	763	767	22
	M065	993500	1013000	1004000	1003500	9760	993200	993900	992100	993067	907	924300	934300	926800	928467	5204
Community Results		Consensus Mean			392490		Consensus Mean			379105		Consensus Mean			356732	
		Consensus Standard Deviation			614314		Consensus Standard Deviation			593879		Consensus Standard Deviation			558577	
		Maximum			1072085		Maximum			1071100		Maximum			1000067	
		Minimum			0.08		Minimum			0.09		Minimum			0.08	
		N			8		N			8		N			8	

Table 27. Data summary table (subset 1) for total chondroitin sulfate in dietary supplement raw materials. Data in this group were reported on the order of magnitude consistent with the units requested by the data reporting site.

		Total Chondroitin Sulfate (Subset 1)														
		Bovine sodium sulfate (µg/g)					Porcine sodium sulfate (µg/g)					Porcine sulfate calcium (µg/g)				
	Lab	3	4	7	Avg	SD	5	2	6	Avg	SD	9	1	8	Avg	SD
Individual Results	NIST															
	M033	1051600	1053900	1083900	1063133	18021	1084300	1062100	1066900	1071100	11681	990300	1019400	990500	1000067	16743
	M042	1147754	1046531	1021969	1072085	66672	1022570	919861	960261	967564	51742	871114	947198	954487	924266	46175
	M065	993500	1013000	1004000	1003500	9760	993200	993900	992100	993067	907	924300	934300	926800	928467	5204
Community Results		Consensus Mean			1046239		Consensus Mean			1010577		Consensus Mean			950933	
		Consensus Standard Deviation			46235		Consensus Standard Deviation			62101		Consensus Standard Deviation			50896	
		Maximum			1072085		Maximum			1071100		Maximum			1000067	
		Minimum			1003500		Minimum			967564		Minimum			924266	
		N			3		N			3		N			3	

Table 28. Data summary table (subset 2) for total chondroitin sulfate in dietary supplement raw materials. Data in this group were reported roughly four orders of magnitude lower than the data in subset 1. An error in reporting units is suspected and based on conflicting information in the Exercise M Shipping Letter, which requested data reported in units of percent. This data has been modified based on this assumption in the set on the bottom for comparison of values.

		Total Chondroitin Sulfate (Subset 2)														
		Bovine sodium sulfate (µg/g)					Porcine sodium sulfate (µg/g)					Porcine sulfate calcium (µg/g)				
	Lab	3	4	7	Avg	SD	5	2	6	Avg	SD	9	1	8	Avg	SD
Individual Results	NIST															
	M016	107	106	106	106	1	105	105	105	105	0	97	97	96	97	1
	M017	112	113	112	112	1	110	111	110	110	1	102	102	101	102	1
	M027	97	99	100	99	2	90	94	88	91	3	87	86	86	87	0
Community Results		Consensus Mean			106		Consensus Mean			102		Consensus Mean			95	
		Consensus Standard Deviation			8		Consensus Standard Deviation			12		Consensus Standard Deviation			9	
		Maximum			112		Maximum			110		Maximum			102	
		Minimum			99		Minimum			91		Minimum			87	
		N			3		N			3		N			3	

		Total Chondroitin Sulfate (Subset 2) modified by 10^4														
		Bovine sodium sulfate (µg/g)					Porcine sodium sulfate (µg/g)					Porcine sulfate calcium (µg/g)				
	Lab	3	4	7	Avg	SD	5	2	6	Avg	SD	9	1	8	Avg	SD
Individual Results	NIST															
	M016	1068900	1062700	1057800	1063130	5563	1052000	1053700	1045500	1050400	4328	971600	973300	961900	968930	6150
	M017	1120000	1130000	1120000	1123330	5774	1100000	1110000	1100000	1103330	5774	1020000	1020000	1010000	1016670	5774
	M027	966000	992000	999000	985670	17388	896000	940000	883000	906330	29872	871000	863000	864000	866000	4359
Community Results		Consensus Mean			1057380		Consensus Mean			1020020		Consensus Mean			950530	
		Consensus Standard Deviation			78550		Consensus Standard Deviation			115800		Consensus Standard Deviation			87380	
		Maximum			1123330		Maximum			1103330		Maximum			1016670	
		Minimum			985670		Minimum			906330		Minimum			866000	
		N			3		N			3		N			3	

Table 29. Data summary table for chondroitin sulfate A in dietary supplement raw materials.

		Chondroitin A														
		Bovine sodium sulfate (µg/g)					Porcine sodium sulfate (µg/g)					Porcine sulfate calcium (µg/g)				
	Lab	3	4	7	Avg	SD	5	2	6	Avg	SD	9	1	8	Avg	SD
Individual Results	NIST															
	M003															
	M006															
	M016															
	M017	225832	230079	228784	228232	2177	183585	181364	182727	182559	1120	166716	165483	165345	165848	755
	M023															
	M025															
	M027	805000	827000	831000	821000	14000	778000	814000	764000	785333	25794	758000	749000	750000	752333	4933
	M028															
	M041															
	M042	919130	840597	816585	858771	53634	848148	762797	794256	801734	43164	724978	789227	796352	770186	39313
	M054	656			656			700		700			687		687	
	M055															
	M056	691	670	691	684	12	635	653	650	646	10	635	601	611	616	17
	M065															
Community Results		Consensus Mean			381868		Consensus Mean			354194		Consensus Mean			337934	
		Consensus Standard Deviation			485968	127.26%	Consensus Standard Deviation			462623	130.61%	Consensus Standard Deviation			444910	131.66%
		Maximum			858771		Maximum			801734		Maximum			770186	
		Minimum			656		Minimum			646		Minimum			616	
		N			5		N			5		N			5	

Table 30. Data summary table for chondroitin sulfate C in dietary supplement raw materials.

		Chondroitin C														
		Bovine sodium sulfate (µg/g)					Porcine sodium sulfate (µg/g)					Porcine sulfate calcium (µg/g)				
	Lab	3	4	7	Avg	SD	5	2	6	Avg	SD	9	1	8	Avg	SD
Individual Results	NIST															
	M003															
	M006															
	M016															
	M017	877191	881844	873319	877451	4268	899685	908480	899983	902716	4994	838860	838446	831469	836258	4153
	M023															
	M025															
	M027	161000	165000	168000	164667	3512	118000	126000	119000	121000	4359	113000	114000	114000	113667	577
	M028															
	M041															
	M042	185613	165720	165760	172364	11473	130438	118103	124427	124323	6168	107780	116433	117101	113771	5200
	M054	116			116			125		125			122		122	
	M055															
	M056	122	138	138	133	9	99	102	100	101	2	97	92	96	95	2
	M065															
Community Results		Consensus Mean			197883		Consensus Mean			144061		Consensus Mean			133615	
		Consensus Standard Deviation			303402	153.32%	Consensus Standard Deviation			220869	153.32%	Consensus Standard Deviation			204888	153.34%
		Maximum			877451		Maximum			902716		Maximum			836258	
		Minimum			116		Minimum			101		Minimum			95	
		N			5		N			5		N			5	

Table 31. Data summary table for chondroitin sulfate D in dietary supplement raw materials.

		Chondroitin D														
		Bovine sodium sulfate (µg/g)					Porcine sodium sulfate (µg/g)					Porcine sulfate calcium (µg/g)				
	Lab	3	4	7	Avg	SD	5	2	6	Avg	SD	9	1	8	Avg	SD
Individual Results	NIST															
	M003															
	M006															
	M016															
	M017															
	M023															
	M025															
	M027															
	M028															
	M041															
	M042															
	M054															
	M055															
	M056															
	M065															
Community Results		Consensus Mean					Consensus Mean					Consensus Mean				
		Consensus Standard Deviation					Consensus Standard Deviation					Consensus Standard Deviation				
		Maximum			0		Maximum			0		Maximum			0	
		Minimum			0		Minimum			0		Minimum			0	
		N			0		N			0		N			0	

Table 32. Data summary table for chondroitin sulfate E in dietary supplement raw materials.

		Chondroitin E														
		Bovine sodium sulfate (µg/g)					Porcine sodium sulfate (µg/g)					Porcine sulfate calcium (µg/g)				
	Lab	3	4	7	Avg	SD	5	2	6	Avg	SD	9	1	8	Avg	SD
Individual Results	NIST															
	M003															
	M006															
	M016															
	M017															
	M023															
	M025															
	M027															
	M028															
	M041															
	M042															
	M054															
	M055															
	M056															
	M065															
Community Results		Consensus Mean					Consensus Mean					Consensus Mean				
		Consensus Standard Deviation					Consensus Standard Deviation					Consensus Standard Deviation				
		Maximum			0		Maximum			0		Maximum			0	
		Minimum			0		Minimum			0		Minimum			0	
		N			0		N			0		N			0	

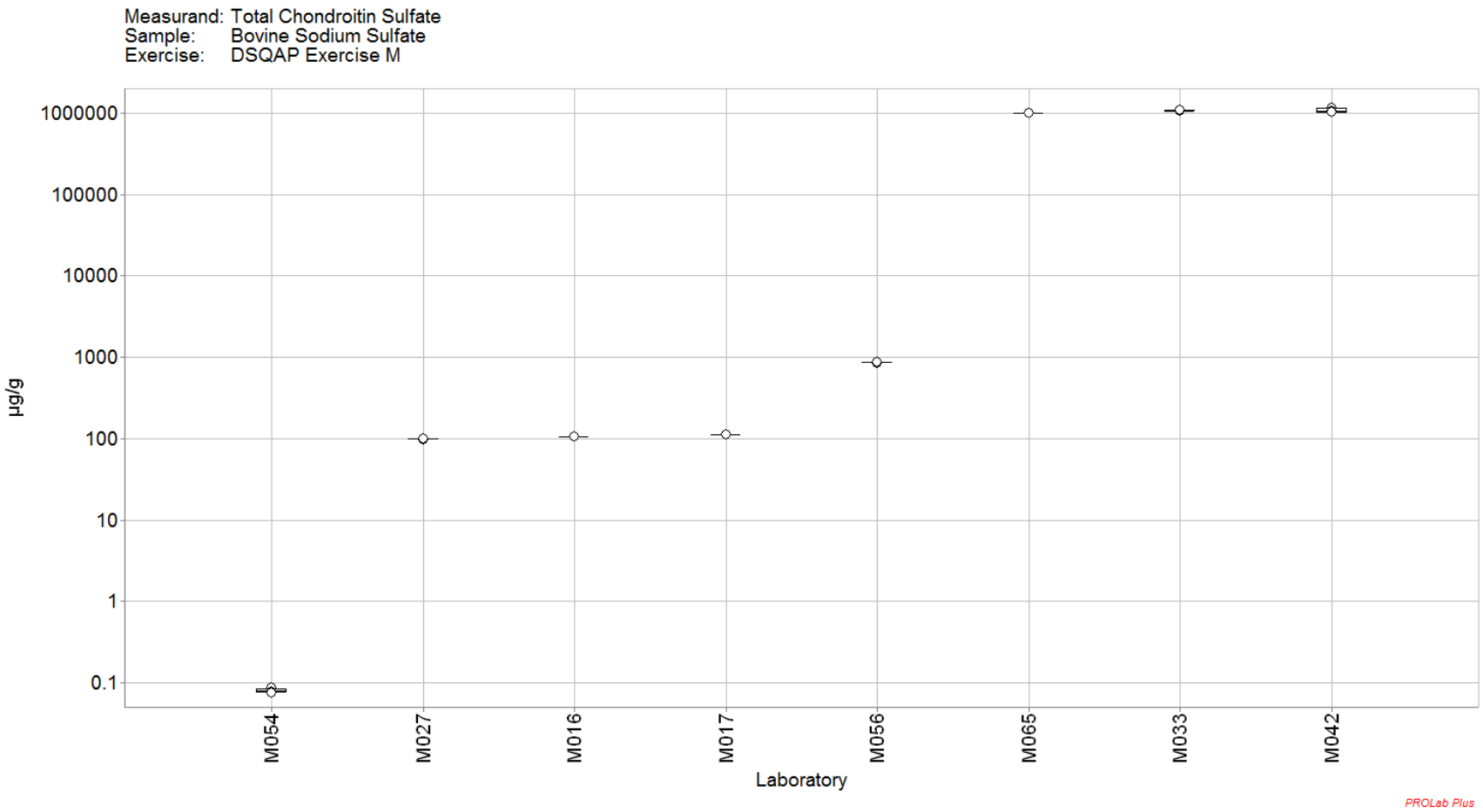

Figure 33. Total chondroitin sulfate in a bovine chondroitin sodium sulfate sample (data summary view). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). No consensus data is provided, as the data were dramatically different. No NIST value has been determined in this material.

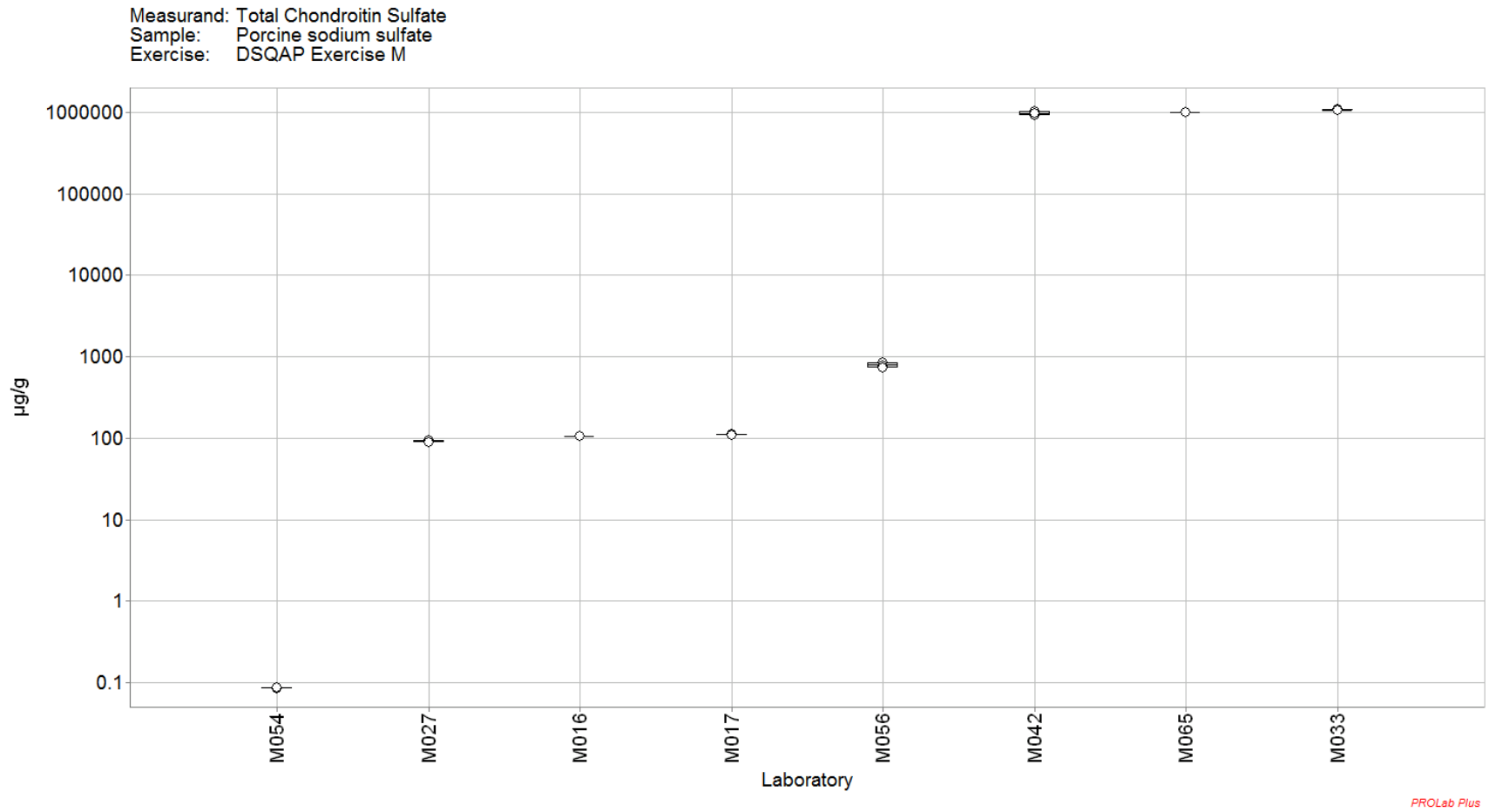

Figure 34. Total chondroitin sulfate in a porcine chondroitin sodium sulfate sample (data summary view). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). No consensus data is provided, as the data were dramatically different. No NIST value has been determined in this material.

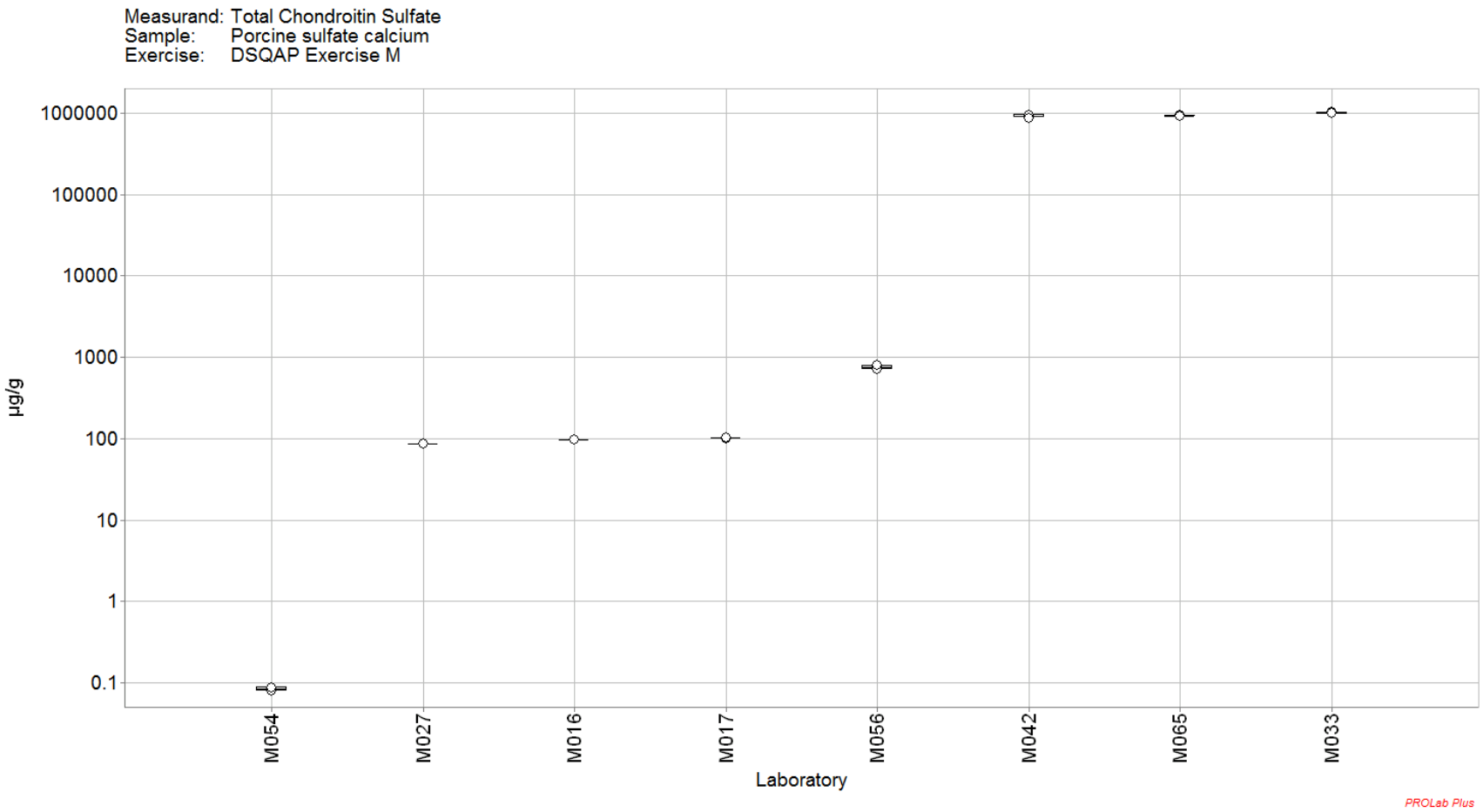

Figure 35. Total chondroitin sulfate in a porcine chondroitin sulfate calcium sample (data summary view). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). No consensus data is provided, as the data were dramatically different. No NIST value has been determined in this material.