NISTIR 8032

Dietary Supplement Laboratory Quality Assurance Program: Exercise K Final Report

Melissa M. Phillips
Catherine A. Rimmer
Laura J. Wood

This publication is available free of charge
from: http://dx.doi.org/10.6028/NIST.IR.8032

National Institute of
Standards and Technology
U.S. Department of Commerce

NISTIR 8032

Dietary Supplement Laboratory Quality Assurance Program: Exercise K Final Report

Melissa M. Phillips
Catherine A. Rimmer
Laura J. Wood

Chemical Sciences Division
Material Measurement Laboratory

This publication is available free of charge
from: http://dx.doi.org/10.6028/NIST.IR.8032

November 2014

U.S. Department of Commerce
Penny Pritzker, Secretary

National Institute of Standards and Technology
Willie May, Acting Under Secretary of Commerce for Standards and Technology and Acting Director

TABLE OF CONTENTS

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 K of this program offered the opportunity for laboratories to assess their in-house measurements of nutritional elements (P and Fe), contaminants (mercury and acrylamide), water-soluble vitamins (vitamins B_1, B_2, and B_3), fat-soluble vitamins (vitamin K_1), and phytosterols in foods and/or botanical dietary supplement ingredients and finished products.

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 $25 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

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

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 eleventh exercise of the DSQAP, Exercise K. Eighty-one laboratories responded to the call for participants distributed in December 2013. Samples were shipped to participants in February 2014, and results were returned to NIST by May 2014. This report contains the final data and information that was disseminated to the participants in November 2014.

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. The consensus mean and standard deviation are calculated according to the robust algorithm outlined in ISO 13528:2005(E), Annex C.[2] 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 $x_i^* = x^* - \delta$.

[2] ISO 13528:2005(E), *Statistical methods for use in proficiency testing by interlaboratory comparisons*, pp 14-15.

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^*:

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

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

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.[2]

Also in Section 1 are two Z-scores. The first Z-score, Z_{comm}, is calculated with respect to the community consensus value, using x* and s*:

$$Z_{comm} = \frac{x_i - x^*}{s^*}.$$

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 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.[3] Consensus means and 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, by collaborating laboratories, or in some combination. Reference values are assigned using NIST values obtained from the average and standard deviation of measurements made using a single analytical method 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 a single 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). Data points that are unfilled represent laboratories that reported a single value for that analyte and therefore were not included in the consensus mean. The black solid line represents the consensus mean, and the black dotted lines represent the consensus variability calculated as one standard deviation about 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 gray shaded region represents the target zone for "acceptable" performance, which encompasses the NIST certified, reference, or estimated value bounded by twice its uncertainty (U_{95}) or standard deviation. For the purpose of the DSQAP, a target range spanning twice the uncertainty in the NIST value is selected because participants are only asked to make a limited number of observations. The size of the y-axis on the data summary view graph represents the consensus

[3] ISO 13528:2005(E), *Statistical methods for use in proficiency testing by interlaboratory comparisons*, Annex C.

mean bounded by 2δ. 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 results for one sample (NIST SRM with a certified or reference value) are compared to the results for another sample (another NIST SRM with a more challenging matrix, a commercial sample, etc.). The error bars represent the individual laboratory standard deviation. 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 zero and twice the consensus mean. 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. This view emphasizes 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 (P, Fe) IN CRANBERRY AND BLUEBERRY

Study Overview

In this study, participants were provided with two NIST SRMs, SRM 3281 Cranberry (Fruit) and SRM 3287 Blueberry (Fruit). Participants were asked to use in-house analytical methods to determine the mass fractions of two nutritional elements (phosphorus and iron) in each of the matrices and report values on an as-received basis.

Sample Information

Cranberry. Participants were provided with one packet containing approximately 6 g of freeze-dried, powdered cranberries. The cranberry powder 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 use a sample size of at least 0.5 g. Participants were asked to store the material at controlled room temperature, 10 °C to 30 °C, and to prepare three samples and report three values from the single packet provided. Approximate analyte levels were not reported to participants prior to the study. The reference values in SRM 3281 Cranberry (Fruit) were determined at NIST using inductively coupled plasma optical emission spectroscopy (ICP-OES). The reference values and uncertainties for P and Fe are provided in the table below, both on a dry-mass basis and on an as-received basis accounting for moisture of the material (2.39 %).

	Reference Mass Fraction in SRM 3281 (mg/kg)	
Analyte	(dry-mass basis)	(as-received basis)
Phosphorus (P)	835 ± 17	815 ± 17
Iron (Fe)	27.7 ± 0.7	27.0 ± 0.7

Blueberry. Participants were provided with one packet containing approximately 5 g of freeze-dried, powdered blueberries. The blueberry powder 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 use a sample size of at least 0.5 g. Participants were informed that this material was packaged as a powder; however, over time the powder may have become a solid mass, and for hardened samples, an appropriate test portion should be removed and subdivided using a knife. Participants were asked to store the material at controlled room temperature, 10 °C to 30 °C, and to prepare three samples and report three values from the single packet provided. Approximate analyte levels were not reported to participants prior to the study. The certified values in SRM 3287 Blueberry (Fruit) were determined at NIST using ICP-OES in combination with data from numerous collaborating laboratories. The certified values and uncertainties for P and Fe are provided in the table below, both on a dry-mass basis and on an as-received basis accounting for moisture of the material (1.41 %).

Analyte	Certified Mass Fraction in SRM 3287 (mg/kg) (dry-mass basis)	(as-received basis)
Phosphorus (P)	671 ± 21	662 ± 21
Iron (Fe)	12.2 ± 0.7	12.0 ± 0.7

Study Results

- Fifty-one laboratories enrolled in this exercise and received samples. Thirty-eight laboratories reported results for phosphorus (75 % participation). Forty-two laboratories reported results for iron (82 % participation).
- The consensus means for phosphorus in both materials and for iron in the cranberries were below the target range.
    - The between-laboratory variability for phosphorus determination was acceptable in both materials (14 % to 16 % RSD).
    - The between-laboratory variability for iron determination was high in the cranberry material (22 % RSD).
- The consensus mean for iron in the blueberry material was at the upper limit of the target range with a high between-laboratory variability (32 % RSD).
- A majority of the laboratories reported using either microwave digestion (63 %) or open-beaker digestion (28 %) for sample preparation. The remaining laboratories reported using hot block digestion or dry ashing. Two laboratories did not report the type of sample preparation technique that was used.
- A majority of the laboratories reported using either ICP-MS (52 %) or ICP-OES (42 %) as their analytical method. Three laboratories reported using atomic absorption spectroscopy (AAS, 7 %), and one laboratory did not report the type of analytical technique that was used.
- A majority of the laboratories reported using an external standard approach to calibration (88 %). Four laboratories reported using a standard addition approach (10 %), and two laboratories reported using an internal standard approach (5 %). One laboratory did not report the type of calibration approach that was used.

Technical Recommendations

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

- No difference was apparent between results obtained using either open beaker or microwave digestion for phosphorus or iron. Too few results were reported by other methods to identify any additional trends.
- No difference was apparent in the iron results based on analytical method used (ICP-OES or ICP-MS).
    - As shown in **Figure 10**, some laboratories reported acceptable or high values for one sample but low values for the second sample.
        - This may indicate more difficulty in the digestion of one material over the other. Laboratories that reported using a more aggressive digestion, using high heat and concentrated acid, generally had values in the middle of the consensus range or target range.

    - This may also be an indicator of a larger matrix interference with one material versus the other during analysis. The use of an internal standard may alleviate this problem, especially when using ICP-OES.
- A slight difference in phosphorous results based on analytical method was identified.
    - In **Figure 9**, results that are low for phosphorous for both materials were analyzed using ICP-MS.
    - Phosphorous may be difficult to analyze by ICP-MS. Since there is only one mass available for phosphorous the use of a collision cell or reaction cell is needed to eliminate polyatomic interferences.
    - There may also be calibration issues for phosphorus that were not evident for iron. To avoid calibration problems, be sure to include the lowest and highest expected values in the calibration curve, plus one or two intermediate concentration points.
    - Ensure that the calibration curve is linear and surrounds expected sample concentrations following digestion and/or dilution. Samples should not go beyond the linear range of the calibration curve, as this results in extrapolation of calibration curves and the possibility of obtaining false values.
- Quality assurance samples should always be used. These can be commercially available reference materials (CRMs, SRMs, or RMs) or prepared in-house, but need to be of known concentration.
    - They are used to ensure that your method is performing as expected.
    - They are useful in finding where errors are occurring, including calculation errors.
    - After checking for calculation errors, make sure results are reported correctly.

Table 1. Individualized data summary table (NIST) for nutritional elements in cranberry and blueberry.

National Institute of Standards & Technology

Exercise K - February 2014 - Nutritional Elements

	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}
P	Cranberries	mg/kg	815	17	0.8	0.0	38	726	105	815	17
P	Blueberries	mg/kg	662	21	0.7	0.0	38	593	94	662	33
Fe	Cranberries	mg/kg	27.0	0.7	0.4	0.0	42	24.7	5.4	27.0	0.7
Fe	Blueberries	mg/kg	12.0	0.7	-0.3	0.0	42	13.5	4.3	12.0	0.7

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 phosphorus in cranberry and blueberry.

		Phosphorus									
		SRM 3281 Cranberries (mg/kg)					SRM 3287 Blueberries (mg/kg)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	NIST				815	17				662	21
	K002										
	K003	776	774	776	775	1	654	652	641	649	7
	K004	1035	1060	1043	1046	13	1028	1010	1003	1014	13
	K005	756	755	752	754	2	623	618	625	622	4
	K007	623	621	626	623	2	494	491	497	494	3
	K008										
	K010										
	K011	828	867	807	834	30	723	671	705	700	26
	K012	757	755	759	757	2	611	610	617	613	4
	K013	739	721	737	732	10	577	593	586	585	8
	K014										
	K015	647	694	654	665	25	556	658	642	618	55
	K016	698	686	694	693	6	546	533	529	536	9
	K018	913	918	928	920	8	755	749	753	752	3
	K024	630	642	606	626	18	468	482	496	482	14
	K025	786	789	743	773	26	628	639	600	622	20
	K027										
	K028	226	259	292	259	33	213	215	221	216	4
	K029	21	21	20	21	1	11	10	10	10	0
	K031	718	735	749	734	16	578	589	571	579	9
	K034	859	874	877	870	10	690	701	702	698	7
	K038	828	802	874	835	36	666	637	679	661	21
	K039	770	770	770	770	0	610	630	620	620	10
	K040										
	K042	737	710	702	717	18	578	550	562	563	14
	K045	796	764	809	790	23	628	653	622	634	16
	K046										
	K048	751	741	745	746	5	598	600	583	594	9
	K049	749	768	772	763	12	659	659	662	660	2
	K051	861	919	900	893	30	676	708	681	688	17
	K054										
	K056	791	786	709	762	46	622	646	601	623	23
	K057	775	779	776	777	2	608	638	622	623	15
	K058	610	630	620	620	10	620	660	690	657	35
	K060	617	604	626	616	11	768	754	762	761	7
	K061										
	K064	828	844	830	834	8	665	676	665	669	6
	K066	775	759	794	776	17	598	589	599	596	6
	K068										
	K069										
	K071	706	727	715	716	11	563	559	565	562	3
	K072	617	637	617	624	12	483	462	467	471	11
	K073	722	731	728	727	5	531	546	537	538	8
	K074	677	688	699	688	11	528	537	531	532	4
	K075	682	684	688	685	3	543	543	544	543	1
	K076										
	K077										
	K078	706	679	676	687	17	564	543	541	549	13
	K079	687	687	668	681	11	551	572	547	556	14
	K080	21	19	20	20	1	11	10	10	10	0
	K081	24	24	25	24	1	13	14		14	1
Community Results		Consensus Mean			726		Consensus Mean			593	
		Consensus Standard Deviation			105		Consensus Standard Deviation			94	
		Maximum			1046		Maximum			1014	
		Minimum			20		Minimum			10	
		N			38		N			38	

Table 3. Data summary table for iron in cranberry and blueberry.

		Iron									
		SRM 3281 Cranberries (mg/kg)					SRM 3287 Blueberries (mg/kg)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	NIST				27.0	0.7				12.0	0.7
	K002	14.0	13.0	22.0	16.3	4.9	38.0	45.0	32.0	38.3	6.5
	K003	24.9	24.5	24.3	24.6	0.3	12.3	11.1	11.6	11.7	0.6
	K004	12.9	12.5	12.9	12.8	0.3	13.9	14.7	14.0	14.2	0.4
	K005	33.5	35.0	33.7	34.1	0.8	28.6	31.0	27.2	28.9	1.9
	K007	23.3	22.6	23.5	23.1	0.5	11.7	10.6	11.5	11.3	0.6
	K008	23.4	22.8	21.5	22.6	1.0	11.7	12.1	11.0	11.6	0.6
	K010										
	K011	15.4	17.3	14.2	15.6	1.6	4.6	3.7	4.7	4.3	0.5
	K012	22.6	22.1	23.0	22.6	0.5	10.7	10.7	11.2	10.9	0.3
	K013	20.0	22.1	20.6	20.9	1.1	10.3	10.6	11.1	10.7	0.4
	K014	21.9	20.3	20.8	21.0	0.8	9.5	8.9	9.4	9.3	0.3
	K015	40.3	34.1	34.3	36.2	3.5	26.2	21.5	27.6	25.1	3.2
	K016	32.2	22.1	23.3	25.9	5.5	13.1	11.7	10.8	11.9	1.2
	K018	21.6	21.8	22.4	21.9	0.4	9.9	9.9	9.9	9.9	0.0
	K024	29.0	29.4	29.8	29.4	0.4	17.1	17.5	17.5	17.4	0.3
	K025	20.9	20.3	20.4	20.5	0.3	17.8	14.6	10.6	14.3	3.6
	K027	18.9	19.4	19.9	19.4	0.5	23.2	22.7	22.3	22.8	0.5
	K028	21.5	20.0	20.4	20.6	0.8	13.0	12.8	13.8	13.2	0.5
	K029	758.7	769.1	732.0	753.2	19.1	579.1	581.7	537.0	565.9	25.1
	K031	24.3	24.5	24.9	24.6	0.3	9.6	9.9	10.3	10.0	0.3
	K034	31.7	27.9	37.6	32.4	4.9	16.7	20.9	18.4	18.7	2.1
	K038	23.2	30.1	25.0	26.1	3.6	7.1	6.8	7.0	6.9	0.2
	K039	25.4	22.3	23.4	23.7	1.6	11.2	10.9	11.0	11.0	0.2
	K040										
	K042	31.7	27.0	26.7	28.5	2.8	10.0	10.4	10.6	10.3	0.3
	K045	29.5	28.7	28.4	28.9	0.6	11.2	12.2	10.9	11.4	0.7
	K046										
	K048	22.1	22.9	24.1	23.0	1.0	11.5	10.5	9.5	10.5	1.0
	K049	22.8	23.9	23.5	23.4	0.6	10.9	10.9	10.6	10.8	0.2
	K051	28.4	23.7	23.1	25.1	2.9	18.8	11.8	13.1	14.6	3.7
	K054	16.1	16.3	16.2	16.2	0.1	12.1	12.3	12.2	12.2	0.1
	K056	22.8	22.7	22.5	22.7	0.2	10.7	10.0	10.1	10.3	0.4
	K057	25.0	24.6	24.1	24.5	0.5	10.6	11.4	11.1	11.0	0.4
	K058	21.0	24.0	17.0	20.7	3.5	14.0	12.0	12.0	12.7	1.2
	K060	27.8	25.3	27.7	26.9	1.4	21.7	11.6	18.3	17.2	5.2
	K061										
	K064	24.4	24.8	24.3	24.5	0.3	11.2	11.0	10.9	11.0	0.2
	K066										
	K068										
	K069										
	K071	29.0	36.0	44.0	36.3	7.5	17.0	16.0	19.0	17.3	1.5
	K072	23.8	24.0	23.6	23.8	0.2	10.8	10.8	10.4	10.6	0.2
	K073	22.3	23.6	23.7	23.2	0.8	12.2	15.5	10.8	12.8	2.4
	K074	28.8	25.3	23.1	25.7	2.9	13.7	12.2	16.8	14.2	2.4
	K075	22.2	23.3	35.2	26.9	7.2	12.4	13.2	12.4	12.7	0.5
	K076										
	K077										
	K078	22.9	24.6	29.9	25.8	3.6	13.8	13.0	16.5	14.4	1.8
	K079	21.7	22.4	21.5	21.9	0.5	12.5	14.2	11.9	12.8	1.2
	K080	714.0	681.3	720.6	705.3	21.1	578.1	544.4	555.8	559.4	17.1
	K081	691.7	682.1	715.4	696.4	17.1	554.0	557.7	562.4	558.0	4.2
Community Results		Consensus Mean			24.7		Consensus Mean			13.5	
		Consensus Standard Deviation			5.4		Consensus Standard Deviation			4.3	
		Maximum			753.2		Maximum			565.9	
		Minimum			12.8		Minimum			4.3	
		N			42		N			42	

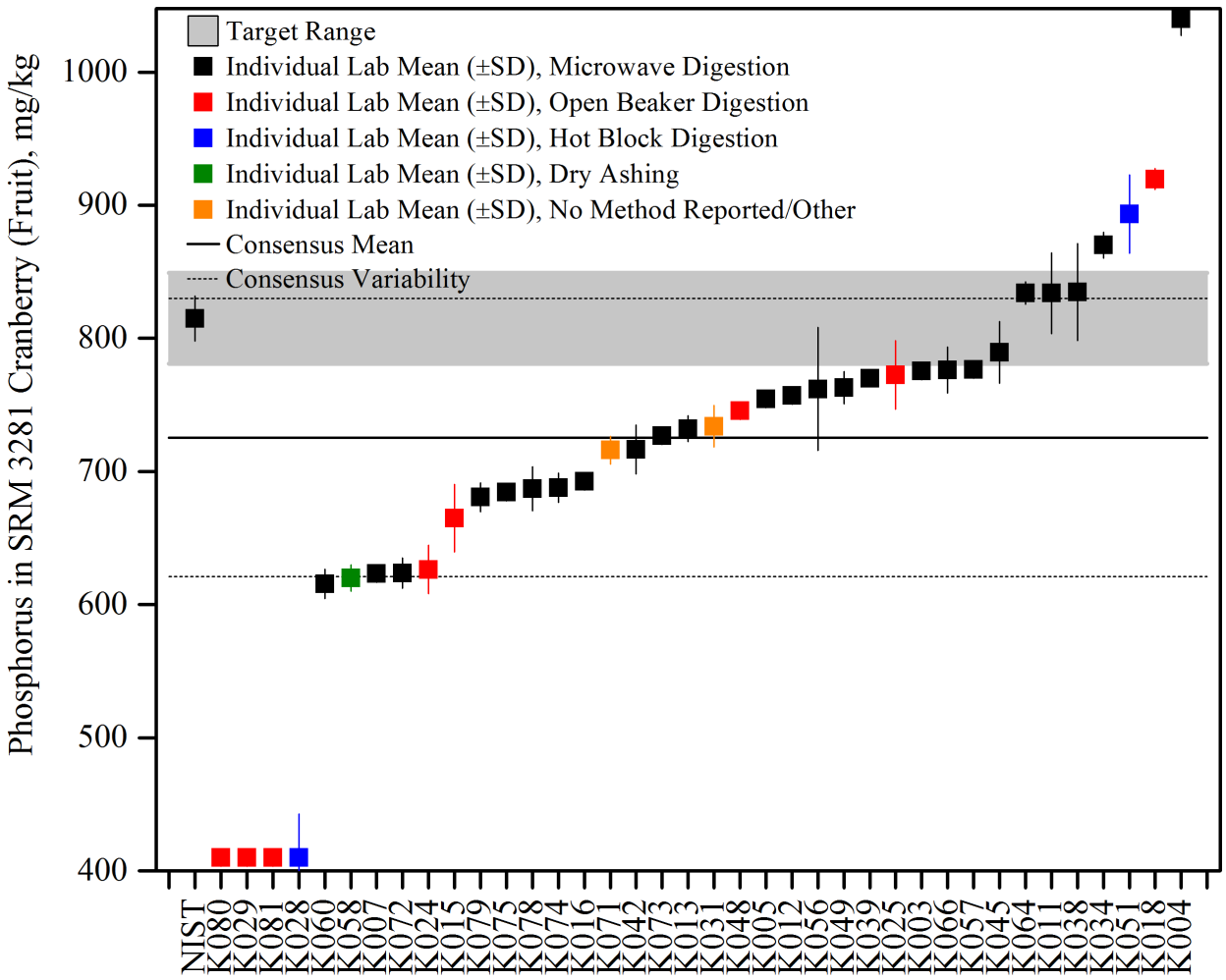

Figure 1. Phosphorus in SRM 3281 Cranberry (Fruit) (data summary view – digestion method). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The color of the data point represents the sample preparation (digestion) procedure employed. The black solid line represents the consensus mean, and the black dotted lines represent the consensus variability calculated as one standard deviation about the consensus mean. The gray shaded region represents the target zone for "acceptable" performance, which encompasses the NIST reference value bounded by twice its uncertainty (U_{95}).

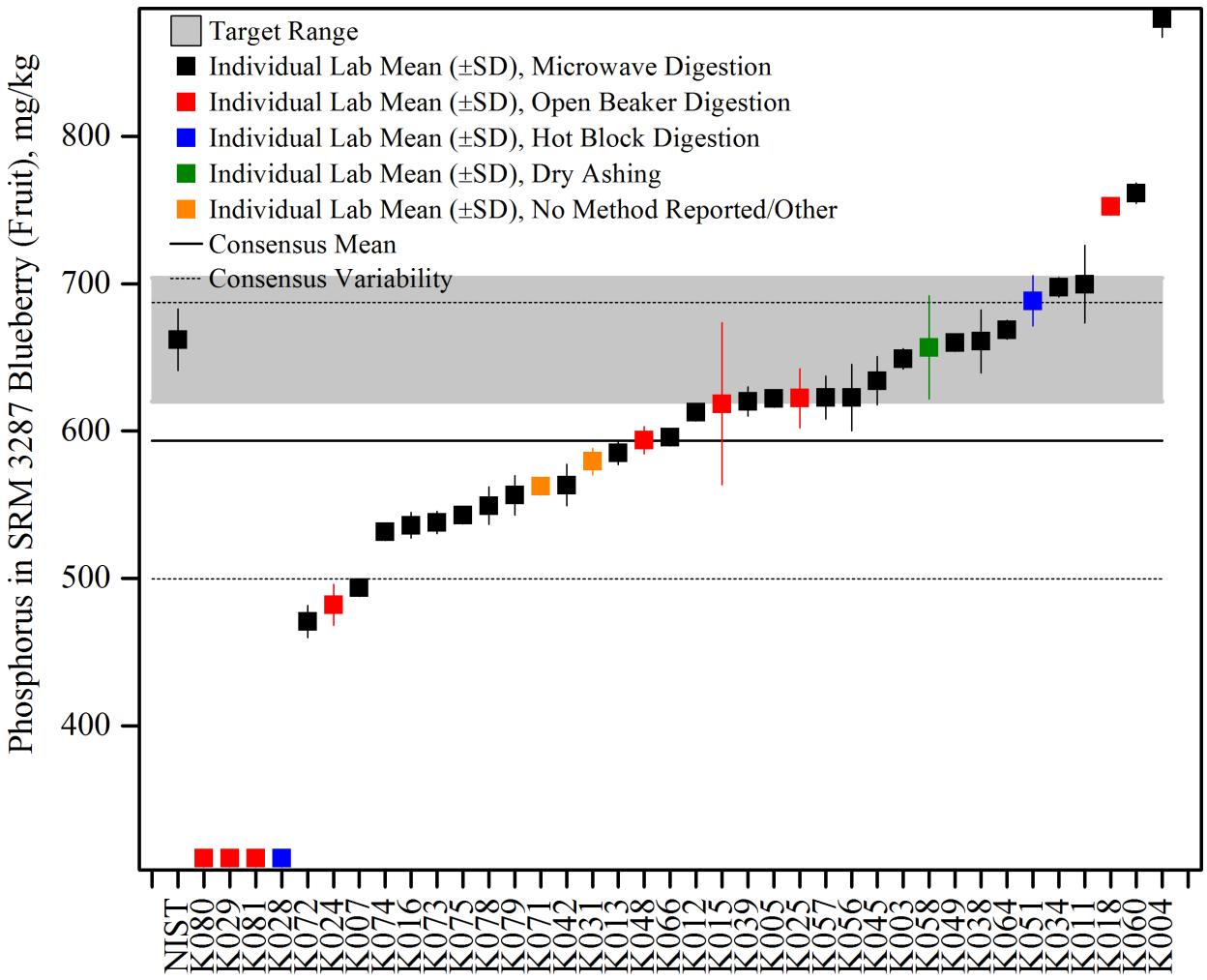

Figure 2. Phosphorus in SRM 3287 Blueberry (Fruit) (data summary view – digestion method). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The color of the data point represents the sample preparation (digestion) procedure employed. The black solid line represents the consensus mean, and the black dotted lines represent the consensus variability calculated as one standard deviation about the consensus mean. The gray shaded region represents the target zone for "acceptable" performance, which encompasses the NIST certified value bounded by twice its uncertainty (U_{95}).

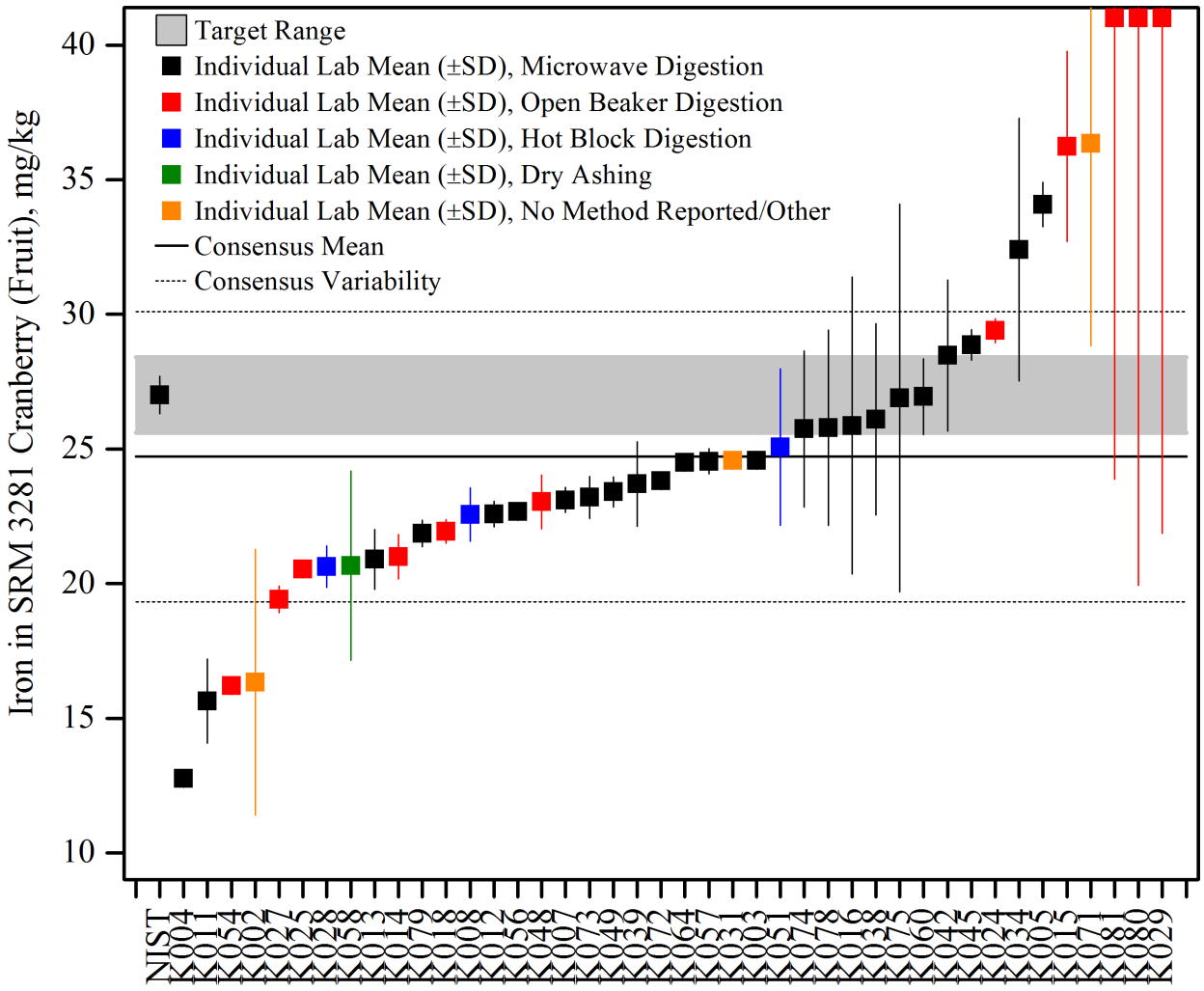

Figure 3. Iron in SRM 3281 Cranberry (Fruit) (data summary view – digestion method). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The color of the data point represents the sample preparation (digestion) procedure employed. The black solid line represents the consensus mean, and the black dotted lines represent the consensus variability calculated as one standard deviation about the consensus mean. The gray shaded region represents the target zone for "acceptable" performance, which encompasses the NIST reference value bounded by twice its uncertainty (U_{95}).

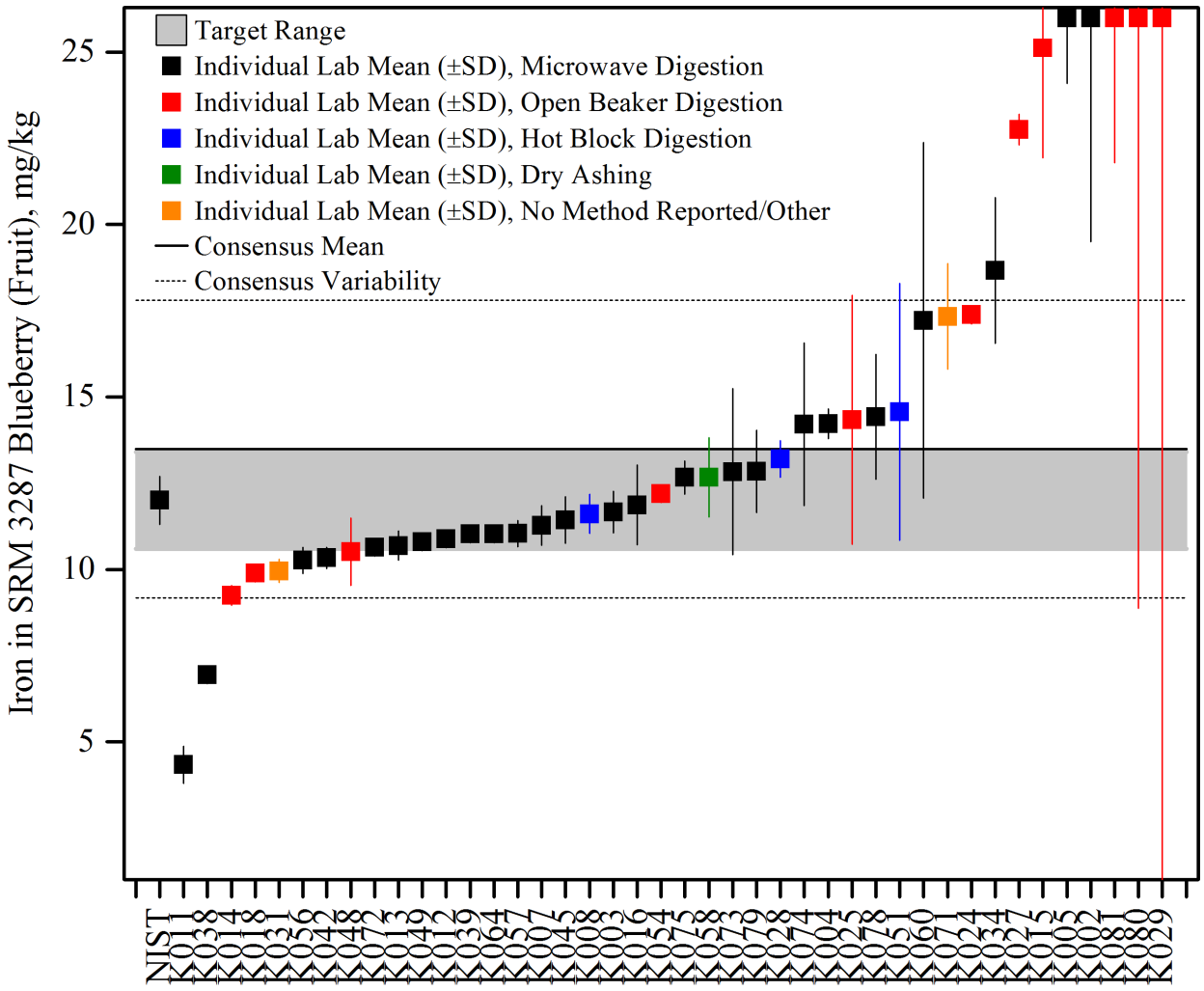

Figure 4. Iron in SRM 3287 Blueberry (Fruit) (data summary view – digestion method). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The color of the data point represents the sample preparation (digestion) procedure employed. The black solid line represents the consensus mean, and the black dotted lines represent the consensus variability calculated as one standard deviation about the consensus mean. The gray shaded region represents the target zone for "acceptable" performance, which encompasses the NIST certified value bounded by twice its uncertainty (U_{95}).

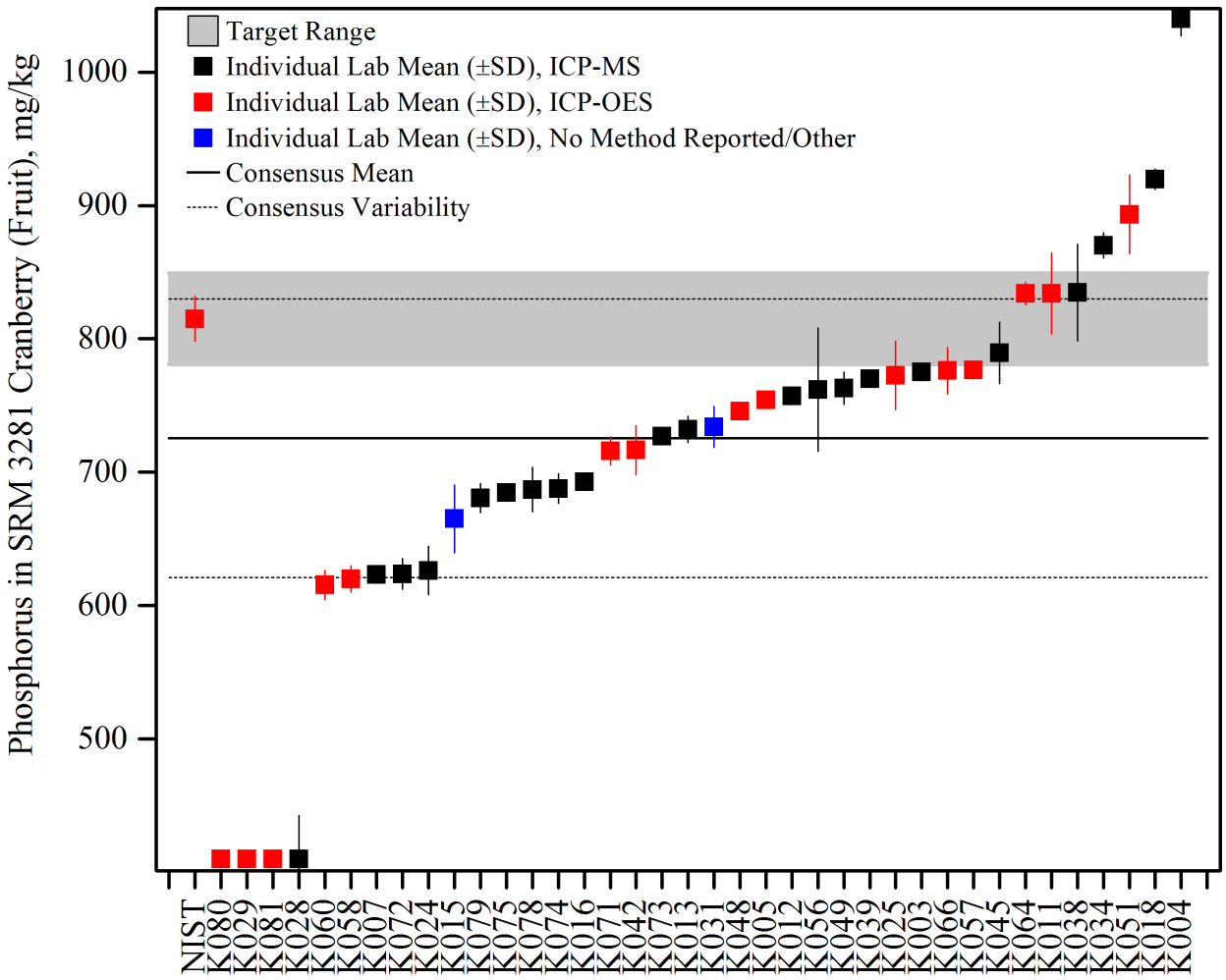

Figure 5. Phosphorus in SRM 3281 Cranberry (Fruit) (data summary view – instrumental method). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The color of the data point represents the instrumental method employed. The black solid line represents the consensus mean, and the black dotted lines represent the consensus variability calculated as one standard deviation about the consensus mean. The gray shaded region represents the target zone for "acceptable" performance, which encompasses the NIST reference value bounded by twice its uncertainty (U_{95}).

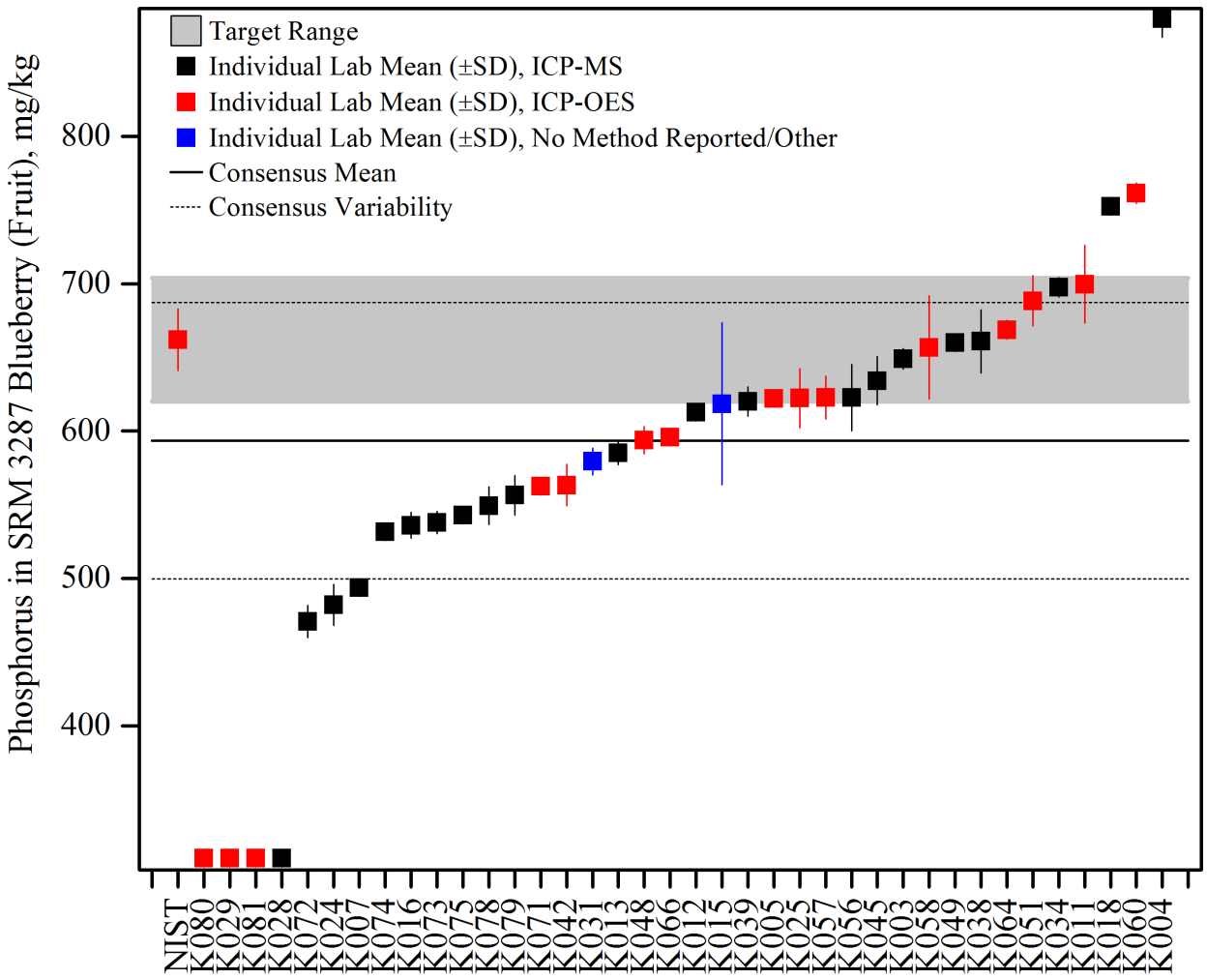

Figure 6. Phosphorus in SRM 3287 Blueberry (Fruit) (data summary view – instrumental method). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The color of the data point represents the instrumental method employed. The black solid line represents the consensus mean, and the black dotted lines represent the consensus variability calculated as one standard deviation about the consensus mean. The gray shaded region represents the target zone for "acceptable" performance, which encompasses the NIST certified value bounded by twice its uncertainty (U_{95}).

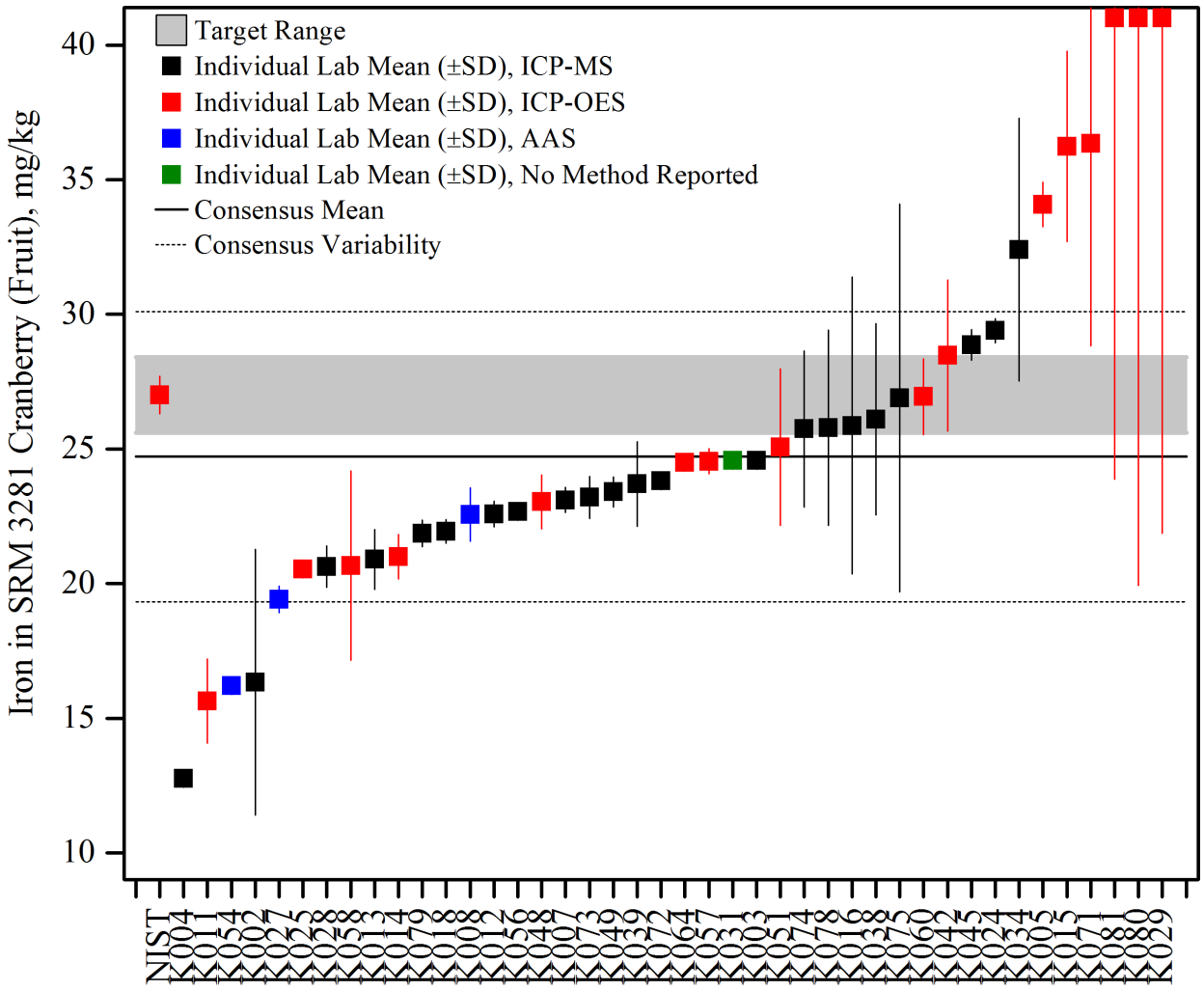

Figure 7. Iron in SRM 3281 Cranberry (Fruit) (data summary view – instrumental method). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The color of the data point represents the instrumental method employed. The black solid line represents the consensus mean, and the black dotted lines represent the consensus variability calculated as one standard deviation about the consensus mean. The gray shaded region represents the target zone for "acceptable" performance, which encompasses the NIST reference value bounded by twice its uncertainty (U_{95}).

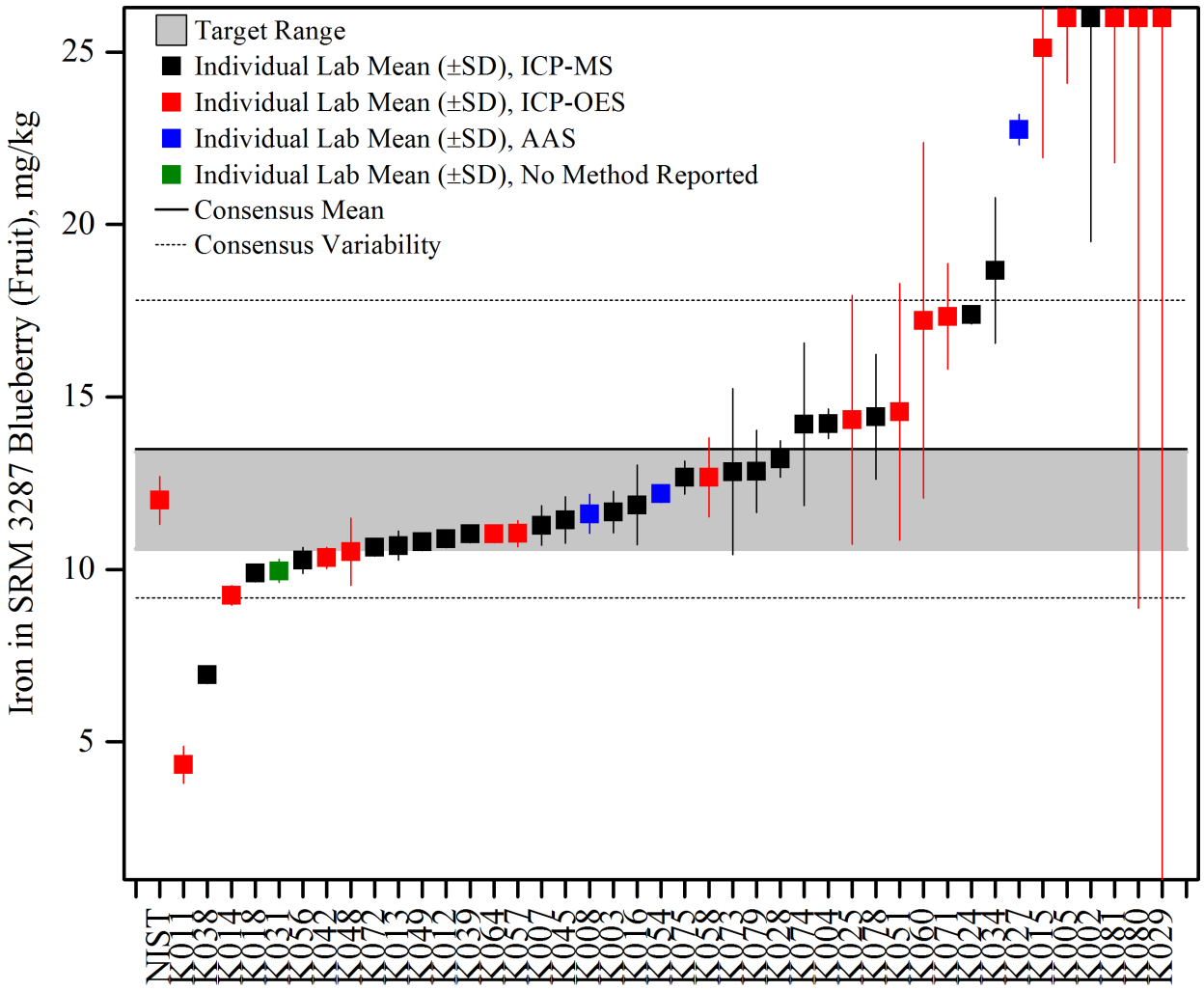

Figure 8. Iron in SRM 3287 Blueberry (Fruit) (data summary view – instrumental method). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The color of the data point represents the instrumental method employed. The black solid line represents the consensus mean, and the black dotted lines represent the consensus variability calculated as one standard deviation about the consensus mean. The gray shaded region represents the target zone for "acceptable" performance, which encompasses the NIST certified value bounded by twice its uncertainty (U_{95}).

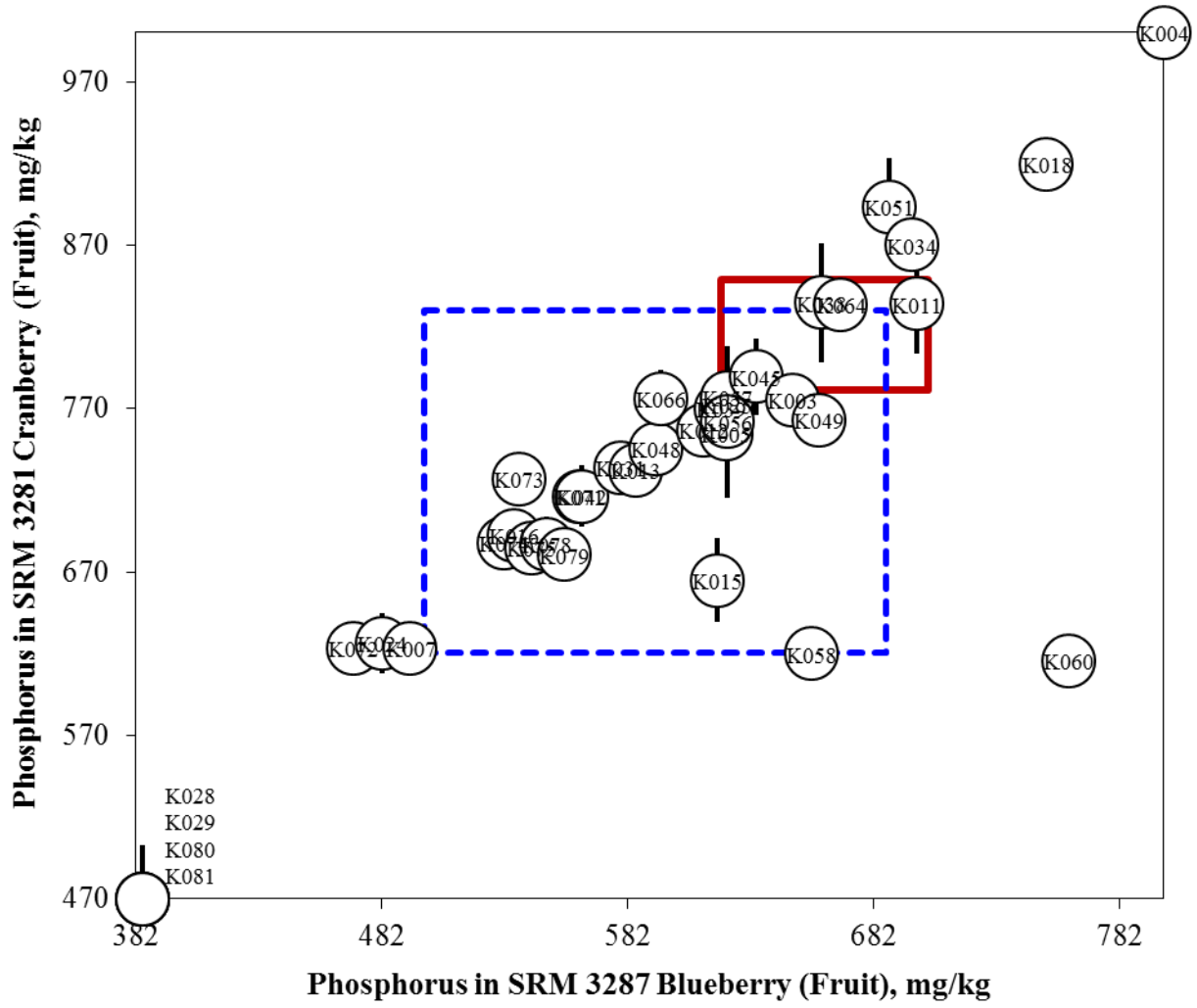

Figure 9. Phosphorus in SRM 3287 Blueberry (Fruit) and SRM 3281 Cranberry (Fruit) (sample/sample comparison view). In this view, the individual laboratory results for one sample (blueberry) are compared to the results for a second sample (cranberry). The solid red box represents the target zone for the two samples, blueberry (x-axis) and cranberry (y-axis). The dotted blue box represents the consensus zone for blueberry (x-axis) and cranberry (y-axis).

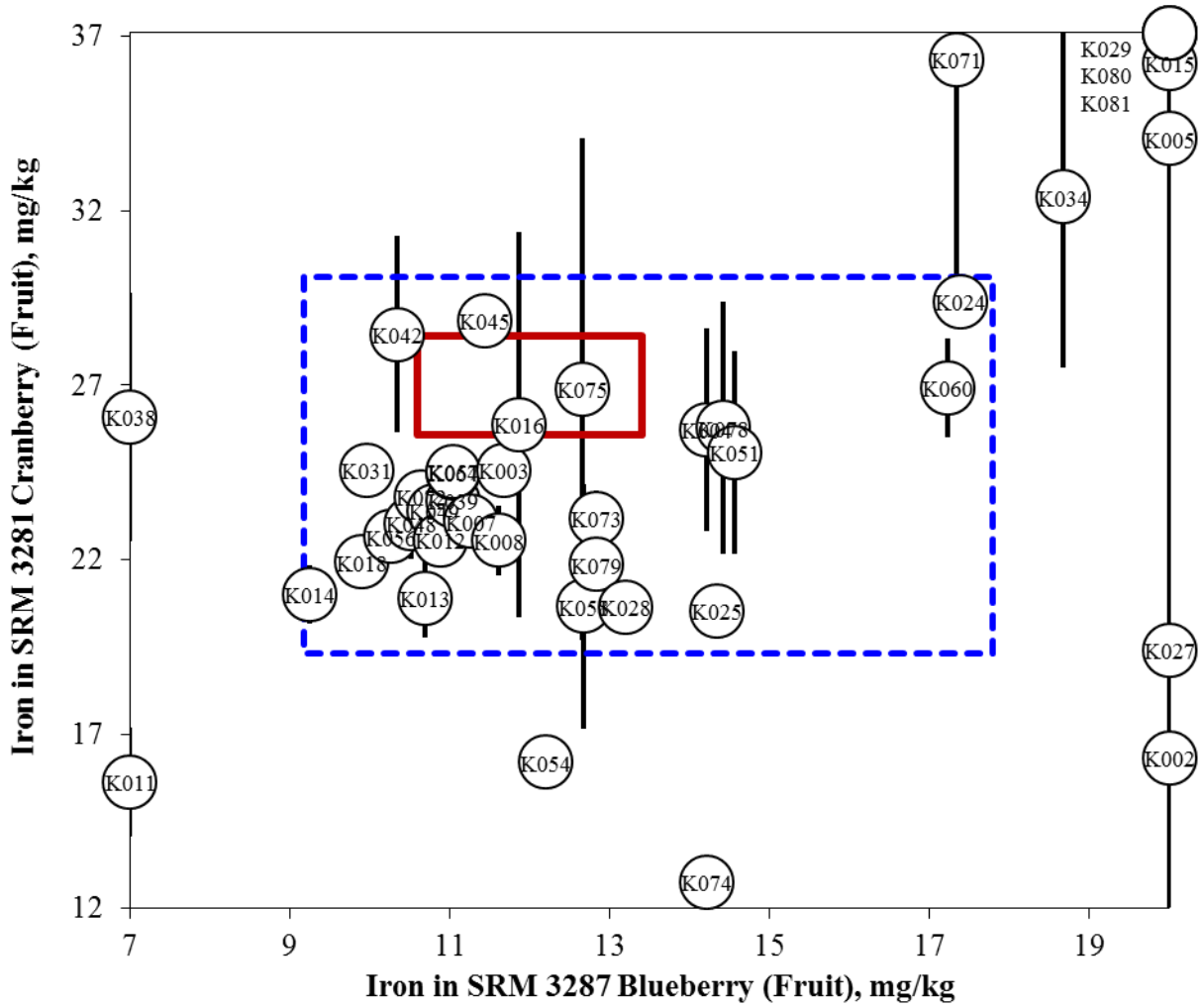

Figure 10. Iron in SRM 3287 Blueberry (Fruit) and SRM 3281 Cranberry (Fruit) (sample/sample comparison view). In this view, the individual laboratory results for one sample (blueberry) are compared to the results for a second sample (cranberry). The solid red box represents the target zone for the two samples, blueberry (x-axis) and cranberry (y-axis). The dotted blue box represents the consensus zone for blueberry (x-axis) and cranberry (y-axis).

TOXIC ELEMENTS (Hg) IN EPHEDRA AND GINKGO DIETARY SUPPLEMENTS

Study Overview

In this study, participants were provided with two NIST SRMs, SRM 3240 *Ephedra sinica* Stapf Aerial Parts and SRM 3246 *Ginkgo biloba* (Leaves). Participants were asked to use in-house analytical methods to determine the mass fractions of mercury (Hg) in each of the matrices and report values on an as-received basis.

Sample Information

Ephedra Aerial Parts. Participants were provided with one bottle containing approximately 5 g of dried *Ephedra sinica* Stapf aerial parts. The dried leaves were ground, homogenized, and packaged under nitrogen inside amber high-density polyethylene bottles with polypropylene screw caps. Before use, participants were instructed to thoroughly mix the contents of the bottle and use a sample size of at least 0.2 g. Participants were asked to store the material at controlled room temperature, 10 °C to 30 °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 value for mercury in SRM 3240 *Ephedra sinica* Stapf Aerial Parts was determined at NIST using cold-vapor generation isotope dilution inductively coupled plasma mass spectrometry (CV-ID-ICP-MS) and at the US Food and Drug Administration (FDA) using ICP-MS. The certified value and uncertainty for Hg are provided in the table below, both on a dry-mass basis and on an as-received basis accounting for moisture of the material (4.52 %).

	Certified Mass Fraction in SRM 3240 (ng/g)	
Analyte	(dry-mass basis)	(as-received basis)
Mercury (Hg)	16.7 ± 0.5	15.9 ± 0.5

Ginkgo Leaves. Participants were provided with one bottle containing approximately 3 g of dried *Ginkgo biloba* leaves. The dried leaves were ground, homogenized, and packaged under nitrogen inside amber high-density polyethylene bottles with polypropylene screw caps. Before use, participants were instructed to thoroughly mix the contents of the bottle and use a sample size of at least 0.25 g. Participants were asked to store the material at controlled room temperature, 10 °C to 30 °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 value for mercury in SRM 3246 *Ginkgo biloba* (Leaves) was determined at NIST using CV-ID-ICP-MS. The certified value and uncertainty for Hg are provided in the table below, both on a dry-mass basis and on an as-received basis accounting for moisture of the material (4.82 %).

	Certified Mass Fraction in SRM 3246 (ng/g)	
Analyte	(dry-mass basis)	(as-received basis)
Mercury (Hg)	23.08 ± 0.17	21.97 ± 0.16

Study Results

- Fifty-four laboratories enrolled in this exercise and received samples. Forty-three laboratories reported results for mercury in Ephedra aerial parts (80 % participation). Forty-four laboratories reported results for mercury in Ginkgo leaves (82 % participation).

- The consensus means for mercury in both matrices were within the target range but with high variability (20 % and 30 % RSD for the Ephedra and Ginkgo, respectively).
- A majority of the laboratories reported using microwave digestion (68 %) for sample preparation. Ten laboratories reported using open beaker digestion (25 %). Hot block digestion (3 %) and thermal decomposition (3 %) were also reported as methods of sample preparation. One laboratory reported doing no sample preparation, and one laboratory did not report the type of sample preparation used.
- Most laboratories reported using ICP-MS as their analytical method for analysis (85 %). Laboratories also reported using AAS (7 %), cold vapor AAS (2 %), ICP-OES (2 %), and a direct mercury analyzer (2 %). One laboratory did not report the analytical method used.
- A majority of the laboratories reported using an external standard approach to calibration (90 %). One laboratory reported using a standard addition approach (2 %), and three laboratories reported using an internal standard approach (7 %). Two laboratories did not report the type of calibration approach that was used.

Technical Recommendations

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

- Mercury is volatile, so care must be taken to not lose Hg during sample preparation. Microwave digestion is the best method for sample preparation.
- Plant materials can be difficult to digest without the use of HF.
- Low concentrations of Hg are not stable in solution over long periods of time. Samples are best prepared close to the time of analysis.
- Samples containing low concentrations of Hg may be more stable in dilute HCl than in dilute HNO_3.
- Mercury is at very low levels in both materials and may be close to method detection limits.
  - Use a good calibration curve with low concentrations to help with accuracy.
  - Mercury blanks and backgrounds may be large, making it difficult to determine low-level samples.
  - Use a sufficient number of blanks so an accurate method detection limit and limit of quantitation can be determined.
- Values reported at the higher end of the range had more within-laboratory variability. This was most likely due to contamination issues, or problems with sample analysis such as memory effects.
- Mercury has a poor washout (long memory effect) and can give erratic answers if an adequate washout time is not used after each measurement. Use of dilute HCl may decrease the length of necessary washout time.
- The sensitivity of Hg is low when using ICP-MS or ICP-OES. Using cold vapor mercury generation increases sensitivity allowing for lower levels of Hg to be measured.
- Run a quality assurance sample of known concentration to ensure your method is performing as expected. An appropriate control is one that will mirror both the sample matrix and the mass fraction levels expected to be found in the sample.
- Double-check all calculations for any errors.

Table 4. Individualized data summary table (NIST) for mercury in Ephedra and Ginkgo dietary supplements.

National Institute of Standards & Technology

Exercise K - February 2014 - Toxic Elements

	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}
Hg	Ephedra Aerial Parts	ng/g	15.9	0.5	-0.1	0.0	43	16.5	4.9	15.9	0.5
Hg	Ginkgo Leaves	ng/g	22.0	0.2	-0.1	0.0	44	22.3	4.6	22.0	0.2

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 mercury in Ephedra and Ginkgo dietary supplements.

		Mercury									
		SRM 3240 Ephedra Aerial Parts (ng/g)					SRM 3246 Ginkgo Leaves (ng/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	NIST				15.9	0.5				22.0	0.2
	K002	15.3	14.3	15.2	14.9	0.5	25.4	23.6	21.9	23.6	1.8
	K003	15.5	14.3	15.2	15.0	0.6	26.1	24.5	20.9	23.8	2.7
	K004										
	K005						16.2	14.1	14.7	15.0	1.1
	K007	19.1	22.5	19.4	20.3	1.9	25.5	37.7	29.5	30.9	6.2
	K010										
	K011	285.0	285.0	271.0	280.3	8.1	314.0	302.0	295.0	303.7	9.6
	K013	17.9	15.9	16.1	16.6	1.1	24.2	28.1	22.7	25.0	2.8
	K016	15.1	13.0	12.0	13.4	1.6	18.4	17.0	17.6	17.7	0.7
	K018	18.1	17.2	18.4	17.9	0.6	27.4	26.5	27.3	27.1	0.5
	K023	15.7	15.8	15.6	15.7	0.1	23.1	22.7	23.2	23.0	0.3
	K024	26.2	22.8	19.1	22.7	3.6	21.6	21.5	20.3	21.1	0.7
	K025	19.8	19.9	20.0	19.9	0.1	19.7	19.9	19.7	19.8	0.1
	K027	24.7	24.6	30.1	26.5	3.2	14.5	15.2	14.0	14.6	0.6
	K028	4.6	4.2	4.0	4.3	0.3	4.5	4.5	4.6	4.5	0.1
	K029	11.4	10.9	11.4	11.2	0.3	19.3	19.3	19.4	19.3	0.1
	K030	13.2	14.7	13.4	13.8	0.8	22.6	20.5	26.0	23.0	2.8
	K031	14.0	15.0	14.7	14.6	0.5	21.2	21.4	22.0	21.5	0.4
	K034	16.4	16.4	16.4	16.4	0.0	23.0	23.0	24.0	23.3	0.6
	K037	42.0	37.0	40.0	39.7	2.5	28.0	25.0	25.0	26.0	1.7
	K039	20.4	20.8	21.5	20.9	0.6	19.6	21.2	21.0	20.6	0.9
	K040										
	K042	21.5	20.3	20.7	20.8	0.6	24.8	28.4	27.3	26.8	1.8
	K045	16.8	17.9	19.3	18.0	1.3	24.4	27.5	25.8	25.9	1.6
	K046	16.1	15.7	16.4	16.1	0.4	22.2	22.0	21.1	21.8	0.6
	K047	17.6	17.9		17.7	0.2	24.9	23.8		24.3	0.8
	K048	12.9	14.0	12.4	13.1	0.8	17.7	17.9	16.7	17.4	0.6
	K049	32.4	18.6	16.7	22.6	8.6	44.2	29.8	26.0	33.3	9.6
	K050	18.0	18.9	18.4	18.4	0.5	23.3	22.1	22.5	22.6	0.6
	K051	5.4	7.0	7.5	6.6	1.1	17.8	18.7	18.1	18.2	0.4
	K052	14.5	13.8	14.0	14.1	0.3	20.5	21.0	20.6	20.7	0.3
	K056	12.0	11.0	12.0	11.7	0.6	22.0	22.0	22.0	22.0	0.0
	K057	16.4	16.6	16.3	16.4	0.2	23.0	24.2	24.3	23.8	0.7
	K058	14.0	15.0	17.0	15.3	1.5	23.0	21.0	22.0	22.0	1.0
	K059	14.4	13.7	11.9	13.3	1.3	34.9	21.0	24.6	26.8	7.2
	K060	18.0	13.0	24.0	18.3	5.5	22.0	19.0	51.0	30.7	17.7
	K061										
	K062	10.9	8.9	10.0	9.9	1.0	14.4	15.9	15.6	15.3	0.8
	K063	25.8	25.2	24.3	25.1	0.8	17.1	16.0	17.2	16.8	0.7
	K064										
	K065	18.1	21.3	24.3	21.2	3.1	25.3	25.6	29.1	26.6	2.1
	K066	12.0	13.7	14.0	13.2	1.1	22.9	23.2	20.9	22.3	1.2
	K067										
	K069										
	K070										
	K071	45.0	43.0	42.0	43.3	1.5	48.0	44.0	40.0	44.0	4.0
	K072	14.8	13.3	13.5	13.9	0.8	21.4	21.8	22.0	21.7	0.3
	K073	12.1	13.3	12.7	12.7	0.6	21.0	21.6	19.2	20.6	1.2
	K074	14.0	14.3	14.1	14.2	0.2	23.0	22.2	22.9	22.7	0.4
	K075	12.5	13.2	12.4	12.7	0.4	18.3	17.8	18.8	18.3	0.5
	K076										
	K077										
	K078	12.5	12.6	12.9	12.7	0.2	21.0	19.0	18.5	19.5	1.3
	K079	12.5	12.6	12.9	12.7	0.2	21.0	19.0	18.5	19.5	1.3
Community Results		Consensus Mean			16.5		Consensus Mean			22.3	
		Consensus Standard Deviation			4.9		Consensus Standard Deviation			4.6	
		Maximum			280.3		Maximum			303.7	
		Minimum			4.3		Minimum			4.5	
		N			43		N			44	

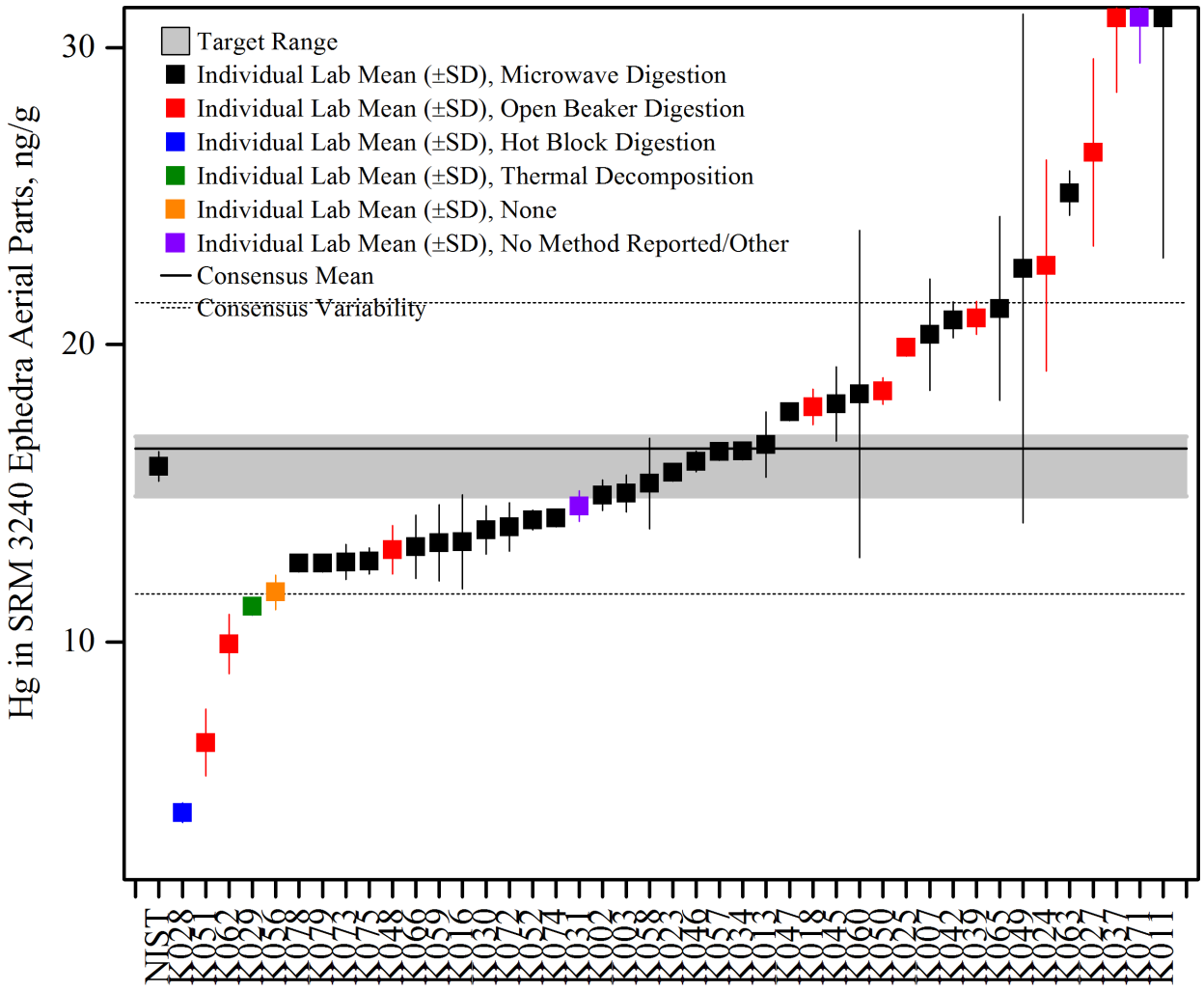

Figure 11. Mercury in SRM 3240 *Ephedra sinica* Stapf Aerial Parts (data summary view – digestion method). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The color of the data point represents the sample preparation (digestion) procedure employed. The black solid line represents the consensus mean, and the black dotted lines represent the consensus variability calculated as one standard deviation about the consensus mean. The gray shaded region represents the target zone for "acceptable" performance, which encompasses the NIST certified value bounded by twice its uncertainty (U_{95}).

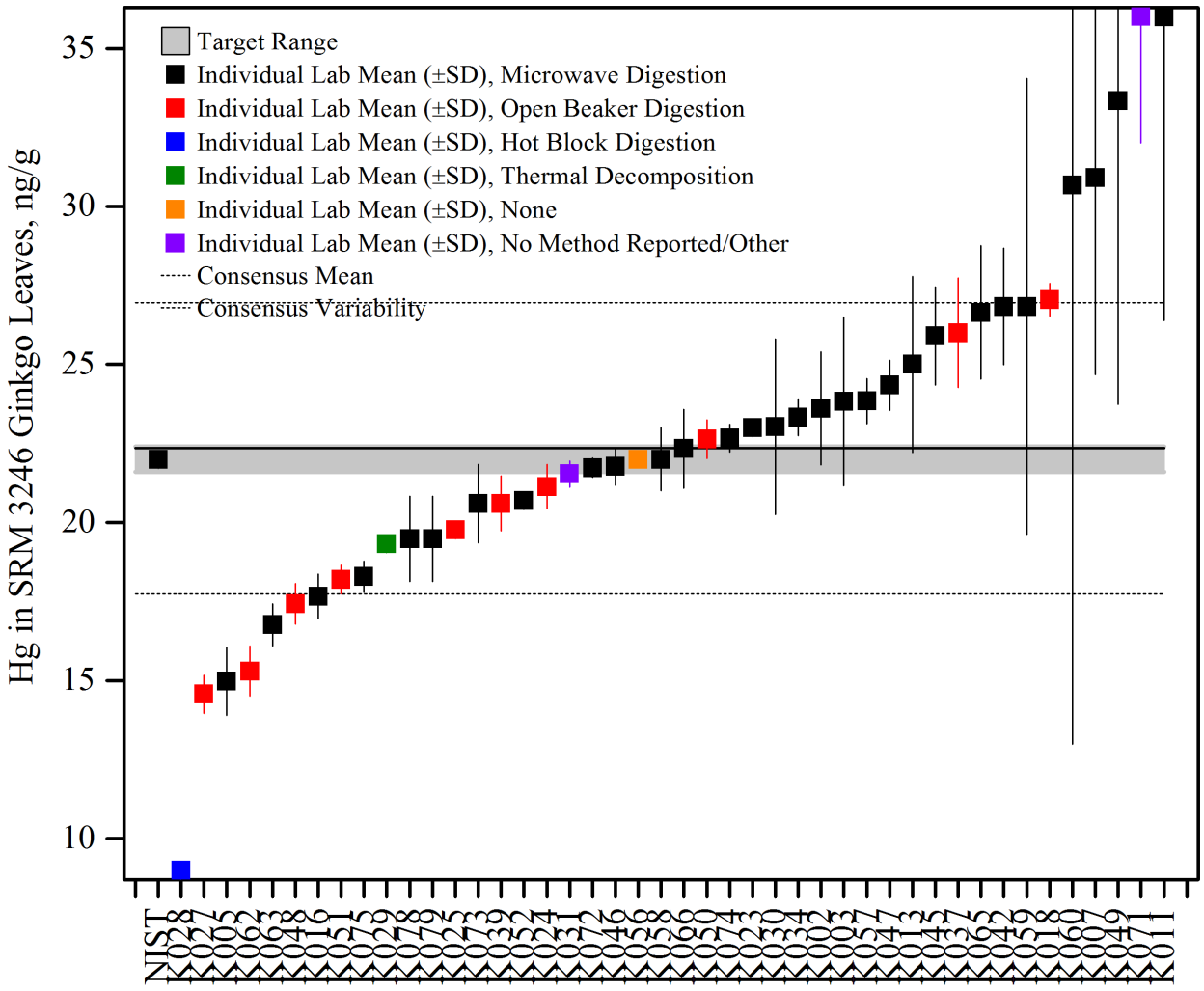

Figure 12. Mercury in SRM 3246 *Ginkgo biloba* (Leaves) (data summary view – digestion method). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The color of the data point represents the sample preparation (digestion) procedure employed. The black solid line represents the consensus mean, and the black dotted lines represent the consensus variability calculated as one standard deviation about the consensus mean. The gray shaded region represents the target zone for "acceptable" performance, which encompasses the NIST certified value bounded by twice its uncertainty (U_{95}).

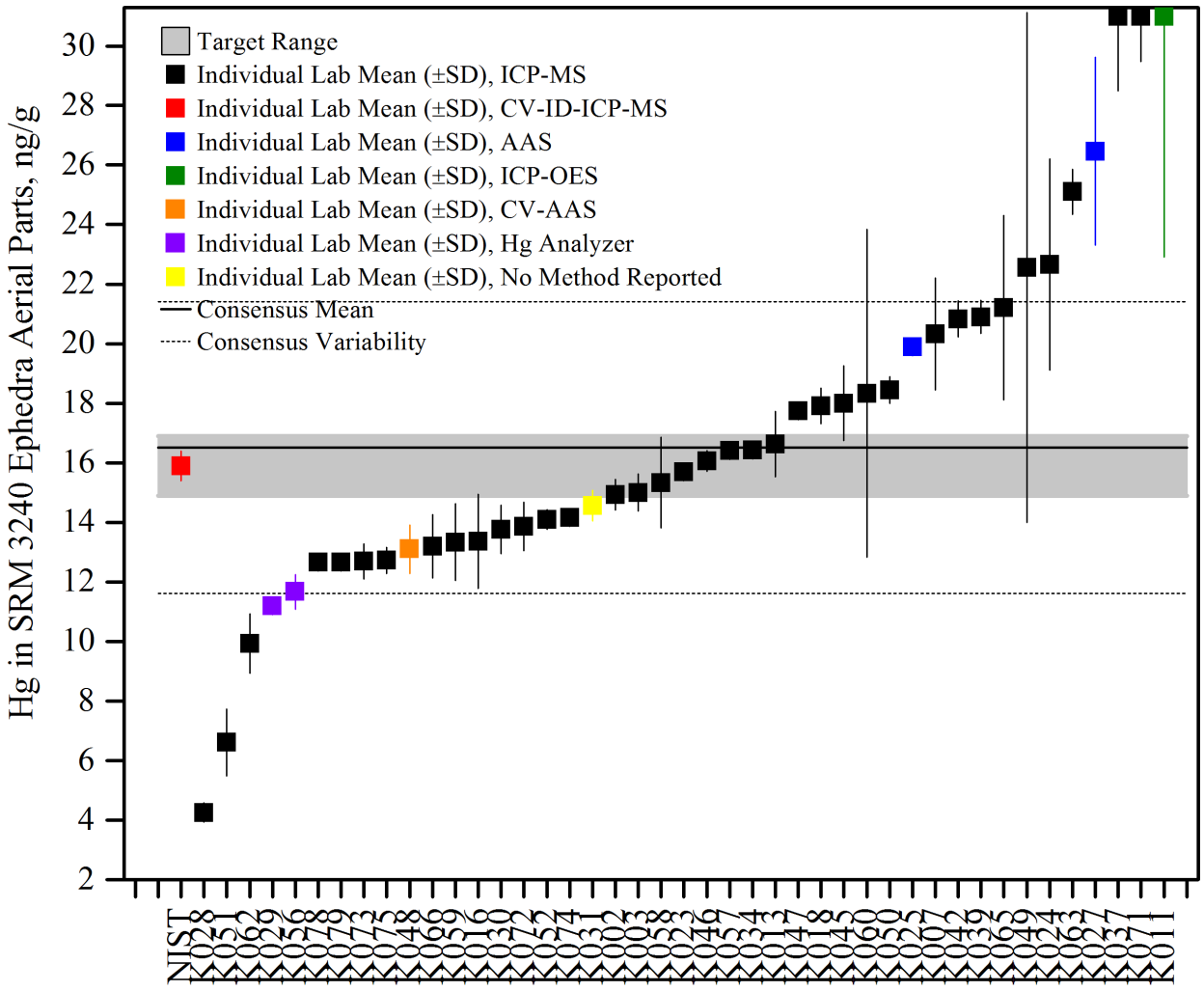

Figure 13. Mercury in SRM 3240 *Ephedra sinica* Stapf Aerial Parts (data summary view – instrumental method). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The color of the data point represents the instrumental method employed. The black solid line represents the consensus mean, and the black dotted lines represent the consensus variability calculated as one standard deviation about the consensus mean. The gray shaded region represents the target zone for "acceptable" performance, which encompasses the NIST certified value bounded by twice its uncertainty (U_{95}).

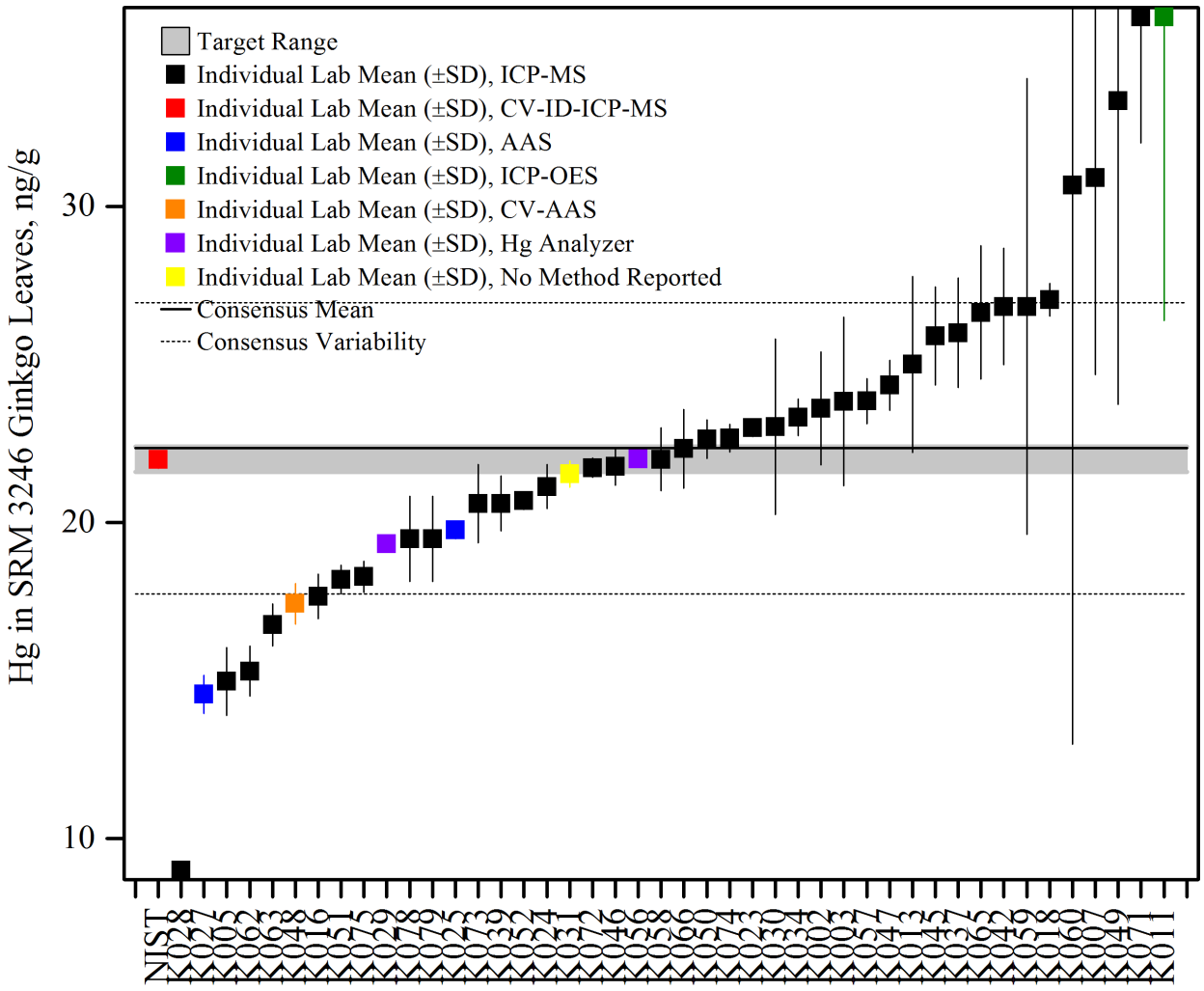

Figure 14. Mercury in SRM 3246 *Ginkgo biloba* (Leaves) (data summary view – instrumental method). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The color of the data point represents the instrumental method employed. The black solid line represents the consensus mean, and the black dotted lines represent the consensus variability calculated as one standard deviation about the consensus mean. The gray shaded region represents the target zone for "acceptable" performance, which encompasses the NIST certified value bounded by twice its uncertainty (U_{95}).

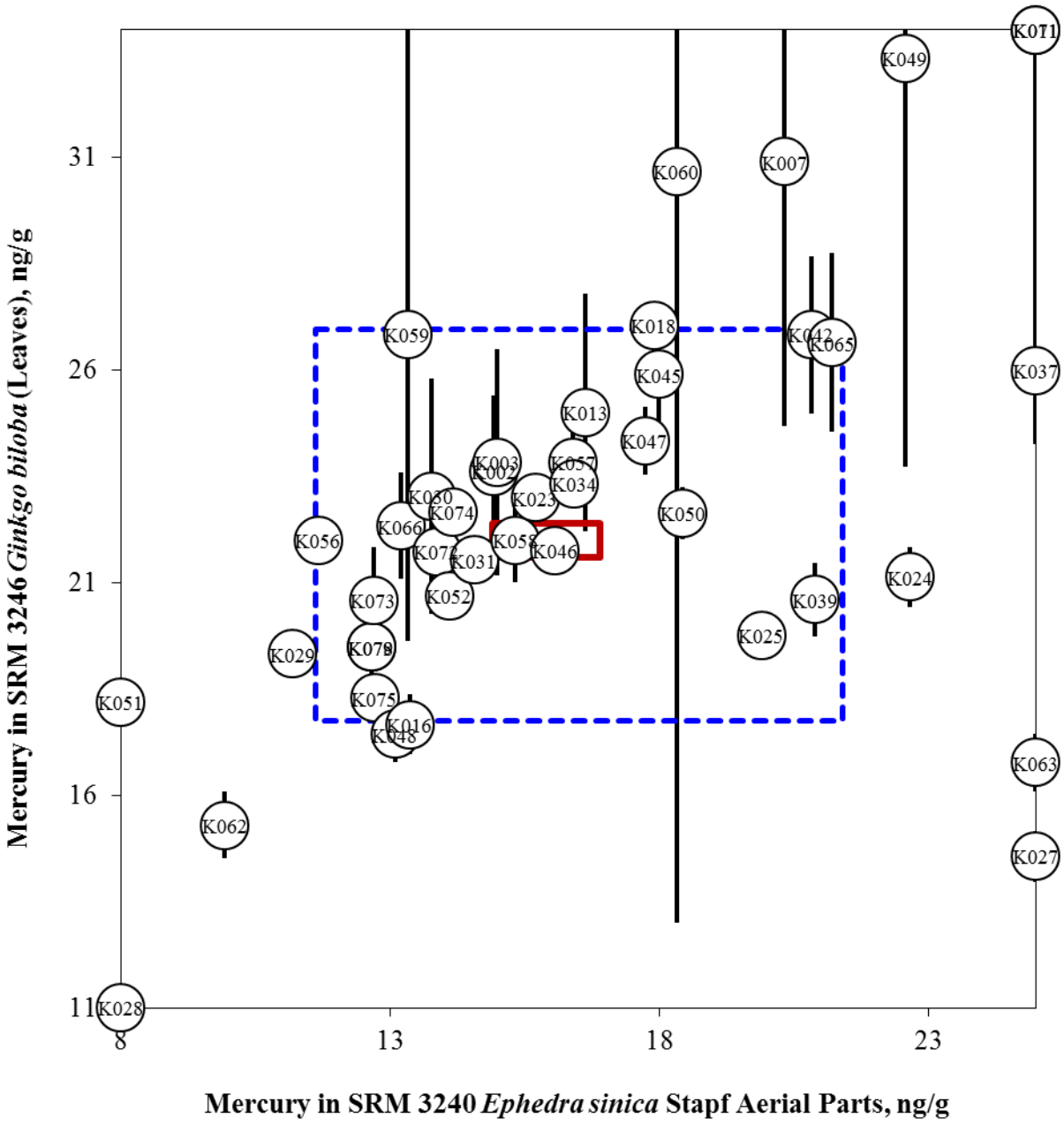

Figure 15. Mercury in SRM 3240 *Ephedra sinica* Stapf Aerial Parts and SRM 3246 *Ginkgo biloba* (Leaves) (sample/sample comparison view). In this view, the individual laboratory results for one sample (Ephedra leaves) are compared to the results for a second sample (Ginkgo leaves). The solid red box represents the target zone for the two samples, Ephedra leaves (x-axis) and Ginkgo leaves (y-axis). The dotted blue box represents the consensus zone for Ephedra leaves (x-axis) and Ginkgo leaves (y-axis).

WATER-SOLUBLE VITAMINS (B_1, B_2, B_3) IN DIETARY SUPPLEMENTS

Study Overview

In this study, participants were provided with one NIST SRM, SRM 3280 Multivitamin/ Multielement Tablets, and one NIST candidate SRM, SRM 3252 Protein Drink Mix. Participants were asked to use in-house analytical methods to determine the mass fractions of vitamins B_1, B_2, and B_3 in each of the matrices and report values on an as-received basis. Participants were asked to report the vitamin B_1, B_2, and B_3 content as thiamine hydrochloride, riboflavin, and niacinamide, respectively.

Sample Information

Multivitamin/Multielement Tablets. Participants were provided with one bottle containing 30 multivitamin/multielement tablets. Before use, participants were instructed to grind all 30 tablets, mix the resulting powder thoroughly, and use a sample size of at least 0.25 g. Participants were asked to store the material at controlled room temperature, 10 °C to 30 °C, 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 NIST certified values and uncertainties for vitamins B_1 and B_3 in SRM 3280 were determined at NIST by LC with absorbance detection (LC-Abs) and isotope dilution liquid chromatography with mass spectrometric detection (ID-LC-MS) following solvent extraction, in combination with data from numerous collaborating laboratories. The NIST certified value and uncertainty for vitamin B_2 in SRM 3280 were determined at NIST by LC-Abs and LC-MS following solvent extraction, in combination with data from numerous collaborating laboratories. The certified values and uncertainties are reported in the table below, both on a dry-mass basis and on an as-received basis accounting for moisture of the material (1.37 %).

Analyte	Certified Mass Fraction in SRM 3280 (mg/g) (dry-mass basis)	Certified Mass Fraction in SRM 3280 (mg/g) (as-received basis)
Thiamine Hydrochloride (B_1)	1.06 ± 0.12	1.05 ± 0.12
Riboflavin (B_2)	1.32 ± 0.17	1.30 ± 0.17
Niacinamide (B_3)	14.10 ± 0.23	13.91 ± 0.23

Protein Powder. Participants were provided with one packet containing approximately 10 g of protein powder. A mixture of commercially available chocolate protein drink mix powders was blended and heat-sealed inside nitrogen-flushed 4-mil plastic bags, which were heat-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 a sample size of at least 0.5 g was recommended. Participants were asked to store the material at controlled room temperature, 10 °C to 30 °C, prepare three samples, and report three values from the single packet provided. Approximate analyte levels were not reported to participants prior to the study. Certified values are not available for this material at the time of the report; NIST provided values for vitamins B_1, B_2, and B_3 based on duplicate analysis from 10 packets using ID-LC-MS/MS. The NIST values in SRM 3252 Protein Drink Mix are reported in the table below with an estimated uncertainty based on twice the method standard deviation.

Analyte	Estimated Mass Fraction in SRM 3252 Protein Drink Mix (mg/kg)
Thiamine Hydrochloride (B_1)	15.81 ± 0.66
Riboflavin (B_2)	26.9 ± 2.9
Niacinamide (B_3)	258.1 ± 8.7

Study Results

- Sixty laboratories enrolled in this exercise and received samples. Forty-one laboratories reported results for vitamins B_1 and B_3 in SRM 3280 (68 % participation) and 46 laboratories reported results for vitamin B_2 in SRM 3280 (77 % participation). For the protein powder, the results of 25 laboratories were used in consensus calculations for vitamin B_1 (42 % participation), 30 laboratories for vitamin B_2 (50 % participation), and 29 laboratories for vitamin B_3 (48 % participation).
- The consensus mean was within the target range for vitamins B_1 and B_2 in SRM 3280. The variability in these measurements was excellent, with approximately 12 % RSD for both vitamins in the multivitamin sample.
- The consensus mean for vitamin B_3 in the multivitamin was above the target range, but with excellent variability at 4 % RSD.
- A number of significantly outlying results were reported for the vitamins in the protein powder. These values varied from twice up to 100 times the expected value based on NIST data. As a result, target ranges were compared only to consensus means determined after these outlying data points had been excluded.
  - Consensus means for vitamins B_2 and B_3 in the protein powder were within the target ranges. The between-laboratory variability for vitamin B_3 was excellent (12 % RSD); the variability for vitamin B_2 was significantly higher (31 % RSD).
  - The consensus mean for vitamin B_1 in the protein powder was slightly higher than the target range, with high between-laboratory variability (48 % RSD).
  - The consensus means for the outlying data points were 18 times, 10 times, and 23 times greater than the target means for vitamins B_1, B_2, and B_3, respectively.
  - The consensus means for the outlying data points included 4 to 6 laboratory results, and had high to extremely high between-laboratory variability (23 % RSD, 57 % RSD, and more than 100 % RSD for vitamins B_1, B_2, and B_3, respectively).
- A majority of the laboratories reported using solvent extraction (71 %) as the sample preparation method. Laboratories also reported using acid hydrolysis (19 %), base hydrolysis (7 %), and enzymatic hydrolysis (2 %). Two laboratories did not report the type of sample preparation used.
- A majority of the laboratories reported using LC-Abs (87 %) as their instrumental method for analysis. Use of spectrophotometry (8 %), LC-FL (3%), and LC/MS (3 %) were also reported. Two laboratories did not report the type of instrumental method used.
- A majority of the laboratories reported using an external standard approach to calibration (91 %). Laboratories also reported using standard addition (7 %) and internal standard (2 %) approaches to quantitation. Four laboratories did not report the quantitation approach used.

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.
- For the laboratories included in the determination of consensus mean for the protein drink mix, no methods presented as significantly better or worse than any other. No systematic biases were noted.
- Extreme outliers in the measurement of vitamins B_1, B_2, and B_3 are likely a result of lack of specificity in the instrumental method.
  - All of the outlying laboratories used LC-Abs for all three analytes.
  - Some laboratories using LC-Abs may be experiencing a co-elution that would cause a high bias in the results.
  - No specific wavelengths were identified as being problematic; because this is likely a coelution, the problem can likely be corrected by alteration of the chromatographic conditions.
  - The following recommendations can help identify and avoid potential coelutions.
    - A chromatographic method with alternate selectivity (different retention order) can be used as a check for each new sample type that is run. Ideally, the retention of coeluting compounds would also be affected and the results from the two chromatographic systems would be different. Two different responses would indicate a possible bias in one approach.
    - A different detector can be used in series with an absorbance detector (as confirmation), such as a fluorescence detector or mass spectrometer. If a coeluting compound is present, the response from these detectors would be different than the response from the absorbance detector. Two different responses would indicate a possible bias in one approach.
    - Considerations of potential interferences can assist in troubleshooting. For example, on many C_{18} systems, caffeine and thiamine have similar retention characteristics. Understanding the matrix that is being tested and possible coeluting compounds can be evaluated before a sample is analyzed for additional confidence in the result.

Table 6. Individualized data summary table (NIST) for water-soluble vitamins in dietary supplements.

National Institute of Standards & Technology

Exercise K - February 2014 - Water-Soluble 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}
B1	Multivitamin	mg/kg	1050	118	-0.6	0.0	41	1140	145	1045	118
B1	Protein Drink	mg/kg	15.8	0.7	-0.6	0.0	25.0	37.2	36.3	15.8	0.7
B2	Multivitamin	mg/kg	1300	168	-0.4	0.0	46	1370	164	1302	168
B2	Protein Drink	mg/kg	26.9	2.9	-0.3	0.0	30	30.4	13.0	26.9	2.9
B3	Multivitamin	mg/kg	13900	227	-1.5	0.0	41	14800	615	13907	227
B3	Protein Drink	mg/kg	258	9	-0.6	0.0	29	291	55	258	9

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 7. Data summary table for vitamin B_1 (thiamine hydrochloride) in dietary supplements.

		Thiamine Cl HCl									
		SRM 3280 Multivitamin/Multielement Tablets (mg/kg)					SRM 3252 Protein Drink Mix (mg/kg)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	NIST				1045	118				15.8	0.7
	K001	1124	1119	1143	1129	13					
	K002										
	K003	1200	1200	1200	1200	0					
	K004	1136	1052	1116	1101	44					
	K006	994	975	981	983	10					
	K008	998	1009	1000	1002	6	285.0	250.0	255.0	263.3	18.9
	K009	939	945	937	940	4					
	K010										
	K011	1218	1203	1202	1208	9	327.5	303.5	326.3	319.1	13.5
	K012										
	K013										
	K014										
	K015	710	1159	946	938	224		19.1	39.1	29.1	14.2
	K016										
	K018	1240			1240		40.0			40.0	
	K019										
	K020										
	K022	1109	1090	1018	1072	48	13.8	13.8	12.8	13.5	0.6
	K024	2262	2132	2018	2137	122	118.1	143.0	125.2	128.8	12.8
	K025	1200	1220	1210	1210	10	28.4	27.4	29.9	28.6	1.3
	K026	1150	1140	1130	1140	10	16.6	15.8	15.0	15.8	0.8
	K028	1809	1800	1790	1800	10	355.0	350.0	350.0	351.7	2.9
	K029	1080	1090	1100	1090	10					
	K031	1110	1100	1110	1107	6	12.1	11.7	12.7	12.2	0.5
	K032	1152	1145	1139	1145	7	13.7	11.4	12.6	12.6	1.2
	K033	890	880	900	890	10	9.4	9.4	11.0	9.9	0.9
	K034										
	K035										
	K036	954	848	849	884	61	23.3	16.4	25.7	21.8	4.8
	K037	1150	1180	1200	1177	25					
	K040										
	K042	1060	1110	1040	1070	36					
	K043										
	K044	1100	1100	1100	1100	0	200.0	200.0	200.0	200.0	0.0
	K045	930	930	930	930	0					
	K046										
	K047	1162	1161	1158	1160	2					
	K048										
	K049	1690	1550	1280	1507	208	14.0	10.0	13.0	12.3	2.1
	K051	1230	1260	1250	1247	15	12.4	11.9	11.4	11.9	0.5
	K056	1163	1174	1177	1171	7	16.4	17.4	14.4	16.1	1.5
	K057	1319	1221	1277	1272	49					
	K058	1210	1250	1240	1233	21	14.5	13.1	13.6	13.7	0.7
	K059	1099	1089	1075	1088	12	265.0	303.0	313.0	293.7	25.3
	K062	1100	1020	1030	1050	44	19.0	19.0	15.0	17.7	2.3
	K063	1330	1310	1290	1310	20					
	K064	1250	1180	1200	1210	36					
	K065	1130	1133	1143	1135	7					
	K066	1101	1139	1103	1115	21	10.3	14.6	11.7	12.2	2.2
	K068										
	K069										
	K071	970	980	960	970	10					
	K073										
	K074	1109	1083	1118	1103	18	33.0	34.0	34.0	33.7	0.6
	K075	1223	1217	1242	1227	13	30.0	31.0	30.0	30.3	0.6
	K076	1391	1361	1391	1381	17	35.0	33.0	31.0	33.0	2.0
	K077	1098	1096	1116	1103	11	29.0	29.0	29.0	29.0	0.0
	K078	1394	1368	1354	1372	20	28.0	28.0	27.0	27.7	0.6
	K079										
	K080	1100	1120	1110	1110	10	13.3			13.3	

		Thiamine Cl HCl									
		SRM 3280 Multivitamin/Multielement Tablets (mg/kg)					SRM 3252 Protein Drink Mix (mg/kg)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Community Results		Consensus Mean			1138		Consensus Mean			37.2	
		Consensus Standard Deviation			145		Consensus Standard Deviation			36.3	
		Maximum			2137		Maximum			351.7	
		Minimum			884		Minimum			9.9	
		N			41		N			25	
						Consensus Group A	Consensus Mean			20.9	
							Consensus Standard Deviation			10.2	
							Maximum			40.0	
							Minimum			9.9	
							N			19	
						Consensus Group B	Consensus Mean			286	
							Consensus Standard Deviation			66	
							Maximum			352	
							Minimum			129	
							N			6	

Table 8. Data summary table for vitamin B_2 (riboflavin) in dietary supplements.

		Riboflavin									
		SRM 3280 Multivitamin/Multielement Tablets (mg/kg)					SRM 3252 Protein Drink Mix (mg/kg)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	NIST				1302	168				26.9	2.9
	K001	1254	1291	1279	1275	19					
	K002										
	K003	1330	1370	1380	1360	26					
	K004	1466	1421	1268	1385	104					
	K006	1190	1190	1150	1177	23	12.5	12.1	10.3	11.6	1.2
	K008	1117	1192	1156	1155	38	510.0	482.0	496.0	496.0	14.0
	K009	1255	1219	1270	1248	26	256.1	199.0	205.4	220.2	31.3
	K010										
	K011	1289	1310	1308	1302	12	31.3	31.6	31.1	31.3	0.3
	K012										
	K013										
	K014	1420	1490	1400	1437	47					
	K015	1242	1291	1288	1274	27	49.4	34.3	47.0	43.5	8.1
	K016										
	K018	1370			1370		50.0			50.0	
	K019										
	K020	1360	1360	1330	1350	17	227.0	233.0	232.0	230.7	3.2
	K022	1283	1305	1317	1302	17	19.7	23.2	19.7	20.8	2.0
	K024	4834	5313	5414	5187	310	224.0	64.6	87.4	125.3	86.2
	K025	1410	1480	1470	1453	38	24.7	28.8	31.4	28.3	3.4
	K026	1450	1520	1440	1470	44	24.0	23.9	24.6	24.2	0.4
	K028	1590	1630	1590	1603	23	320.0	310.0	310.0	313.3	5.8
	K029	1320	1290	1310	1307	15	15.2			15.2	
	K031	1350	1430	1350	1377	46	31.6	31.5	33.5	32.2	1.1
	K032	1201	1227	1184	1204	22	25.0	29.1	23.5	25.9	2.9
	K033	1100	1200	1100	1133	58	25.0	22.0	18.0	21.7	3.5
	K034										
	K035	1212	1184	1153	1183	29					
	K036	1356	1261	1313	1310	48					
	K037	1370	1360	1370	1367	6					
	K040										
	K042	1430	1520	1620	1523	95					
	K043	1248	1263	1277	1263	15	24.1	25.5	28.4	26.0	2.2
	K044	1300	1400	1400	1367	58	30.0	30.0	40.0	33.3	5.8
	K045	1300	1300	1300	1300	0					
	K046	1597	1480	1504	1527	62	21.9	7.2	13.7	14.2	7.4
	K047	1373	1429	1421	1408	30					
	K048										
	K049	1890	1790	1570	1750	164	27.0	21.0	27.0	25.0	3.5
	K051	1290	1270	1320	1293	25	19.0	17.6	16.3	17.6	1.4
	K056	1316	1342	1280	1313	31	27.3	22.4	20.4	23.4	3.6
	K057	1208	1285	1283	1258	44	15.1	15.9	19.2	16.7	2.2
	K058	1460	1500	1450	1470	26	24.6	23.7	25.2	24.5	0.8
	K059	1243	1243	1248	1245	3	26.6	26.2	25.9	26.2	0.4
	K062	1450	1440	1510	1467	38	24.0	25.0	28.0	25.7	2.1
	K063	2030	2020	2020	2023	6					
	K064	1320	1200	1230	1250	62					
	K065	1594	1581	1609	1595	14					
	K066	1211	1169	1138	1173	36	27.6	23.0	21.8	24.1	3.1
	K068										
	K069										
	K071	1250	1240	1190	1227	32					
	K073										
	K074	1447	1465	1483	1465	18	27.0	23.0	23.0	24.3	2.3
	K075	1609	1562	1592	1588	24	40.0	41.0	36.0	39.0	2.6
	K076	2007	1980	1984	1990	15	41.0	39.0	35.0	38.3	3.1
	K077	1302	1365	1303	1323	36	25.0	25.0	22.0	24.0	1.7
	K078	1796	1779	1744	1773	27	39.0	43.0	39.0	40.3	2.3
	K079										
	K080	1310	1320	1300	1310	10	22.0			22.0	

		Riboflavin									
		SRM 3280 Multivitamin/Multielement Tablets (mg/kg)					SRM 3252 Protein Drink Mix (mg/kg)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Community Results		Consensus Mean			1372		Consensus Mean			30.4	
		Consensus Standard Deviation			164		Consensus Standard Deviation			13.0	
		Maximum			5187		Maximum			496.0	
		Minimum			1133		Minimum			11.6	
		N			46		N			30	
						Consensus Group A	Consensus Mean			26.3	
							Consensus Standard Deviation			8.9	
							Maximum			50.0	
							Minimum			11.6	
							N			25	
						Consensus Group B	Consensus Mean			277	
							Consensus Standard Deviation			158	
							Maximum			496	
							Minimum			125	
							N			5	

Table 9. Data summary table for vitamin B_3 (niacinamide) in dietary supplements.

		Niacinamide									
		SRM 3280 Multivitamin/Multielement Tablets (mg/kg)					SRM 3252 Protein Drink Mix (mg/kg)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	NIST				13907	227				258	9
	K001										
	K002										
	K003	15500	15500	15500	15500	0					
	K004	14521	14298	14346	14388	118					
	K006	13400	13100	13300	13267	153	163	192	173	176	15
	K008	19000	19800	20000	19600	529	5394	5540	5500	5478	75
	K009	15149	15036	15151	15112	66	31930	32186	31872	31996	167
	K010										
	K011	14980	15301	15577	15286	299	298	294	296	296	2
	K012										
	K013										
	K014	15100	14700	15100	14967	231	262	245	264	257	10
	K015	13448	14121	13679	13749	342	752	376	256	461	259
	K016										
	K018	18870			18870		290			290	
	K019										
	K020	15500	15600	15300	15467	153					
	K022	14375	14316	14312	14334	35	245	251	247	248	3
	K024	14570	15059	14770	14800	246					
	K025	16600	16200	16200	16333	231	288	315	303	302	14
	K026	14290	15000	14750	14680	360	250	248	15	171	135
	K028	13808	13900	13850	13853	46	293	300	290	294	5
	K029	14520	14380	14580	14493	103	217			217	
	K031	15000	14700	14800	14833	153	279	273	274	275	3
	K032	15345	15149	15372	15289	121	275	276	297	283	12
	K033										
	K034										
	K035	14883	15352	15202	15146	239					
	K036	16916	14916	14144	15325	1431					
	K037	15120	14660	14760	14847	242	240	230	210	227	15
	K040										
	K042	13900	13900	14160	13987	150					
	K043										
	K044	14000	15000	15000	14667	577	1300	1200	1200	1233	58
	K045	14000	14000	14000	14000	0	260	260	270	263	6
	K046										
	K047	14211	14102	13956	14090	128					
	K048										
	K049	15000	15500	14900	15133	321	230	220	240	230	10
	K051	15100	15200	15200	15167	58	268	252	248	256	10
	K056	15035	14813	14720	14856	162	266	285	263	271	12
	K057	3669	3834	3913	3805	124	238	269	268	258	18
	K058	14500	14900	14600	14667	208	280	262	263	268	10
	K059	14851	14826	14899	14859	37	439	469	458	455	15
	K062	15100	15200	15400	15233	153	270	240	270	260	17
	K063	22100	22400	21500	22000	458					
	K064	14300	12300	14200	13600	1127	250	300	265	272	26
	K065	14333	14938	14432	14568	324					
	K066										
	K068										
	K069										
	K071	14710	14700	14640	14683	38	336	342	330	336	6
	K073										
	K074	14799	15068	14838	14902	145	314	316	316	315	1
	K075	15106	14734	14973	14938	189	287	277	283	282	5
	K076	14700	14767	14953	14807	131	355	342	336	344	10
	K077	14583	14564	14460	14536	66	259	271	260	263	7
	K078	14832	14801	14693	14775	73	308	302	310	307	4
	K079										
	K080	14950	14970	14860	14927	59	20			20	

		Niacinamide									
		SRM 3280 Multivitamin/Multielement Tablets (mg/kg)					SRM 3252 Protein Drink Mix (mg/kg)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Community Results		Consensus Mean			14770		Consensus Mean			290	
		Consensus Standard Deviation			615		Consensus Standard Deviation			61	
		Maximum			22000		Maximum			31996	
		Minimum			3805		Minimum			20	
		N			41		N			29	
						Consensus Group A	Consensus Mean			273	
							Consensus Standard Deviation			32	
							Maximum			455	
							Minimum			130	
							N			25	
						Consensus Group B	Consensus Mean			6051	
							Consensus Standard Deviation			8669	
							Maximum			31996	
							Minimum			461	
							N			4	

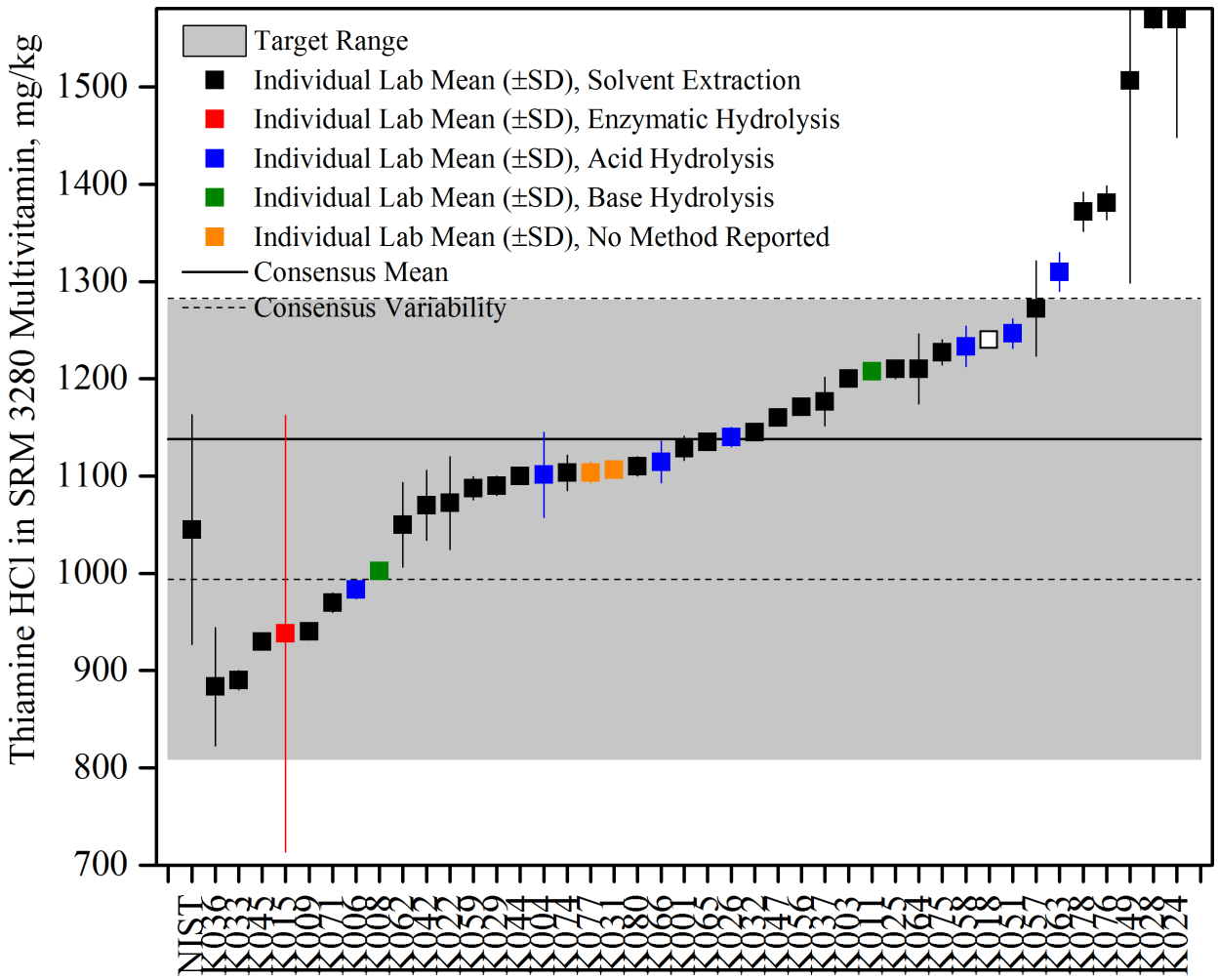

Figure 16. Thiamine hydrochloride in SRM 3280 Multivitamin/Multielement Tablets (data summary view – sample preparation method). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The color of the data point represents the sample preparation procedure employed. The black solid line represents the consensus mean, and the black dotted lines represent the consensus variability calculated as one standard deviation about the consensus mean. The gray shaded region represents the target zone for "acceptable" performance, which encompasses the NIST certified value bounded by twice its uncertainty (U_{95}).

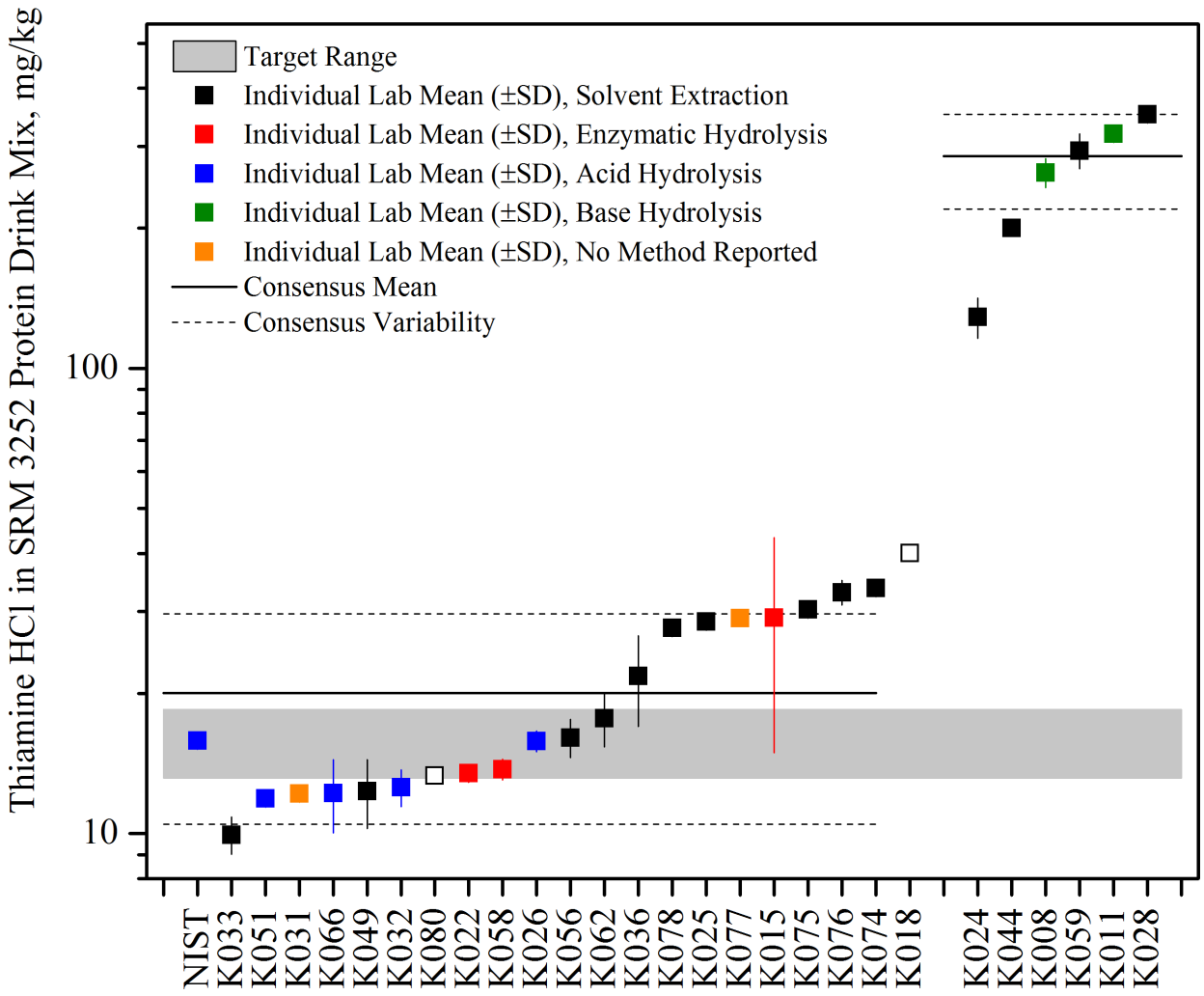

Figure 17. Thiamine hydrochloride in SRM 3252 Protein Drink Mix (data summary view – sample preparation method). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The color of the data point represents the sample preparation procedure employed. The black solid lines represents the consensus means, and the black dotted lines represent the consensus variability calculated as one standard deviation about each consensus mean. The gray shaded region represents the target zone for "acceptable" performance, which encompasses the NIST value determined by ID-LC-MS/MS from duplicate measurements of ten packets, bounded by an estimated uncertainty based on twice the method standard deviation.

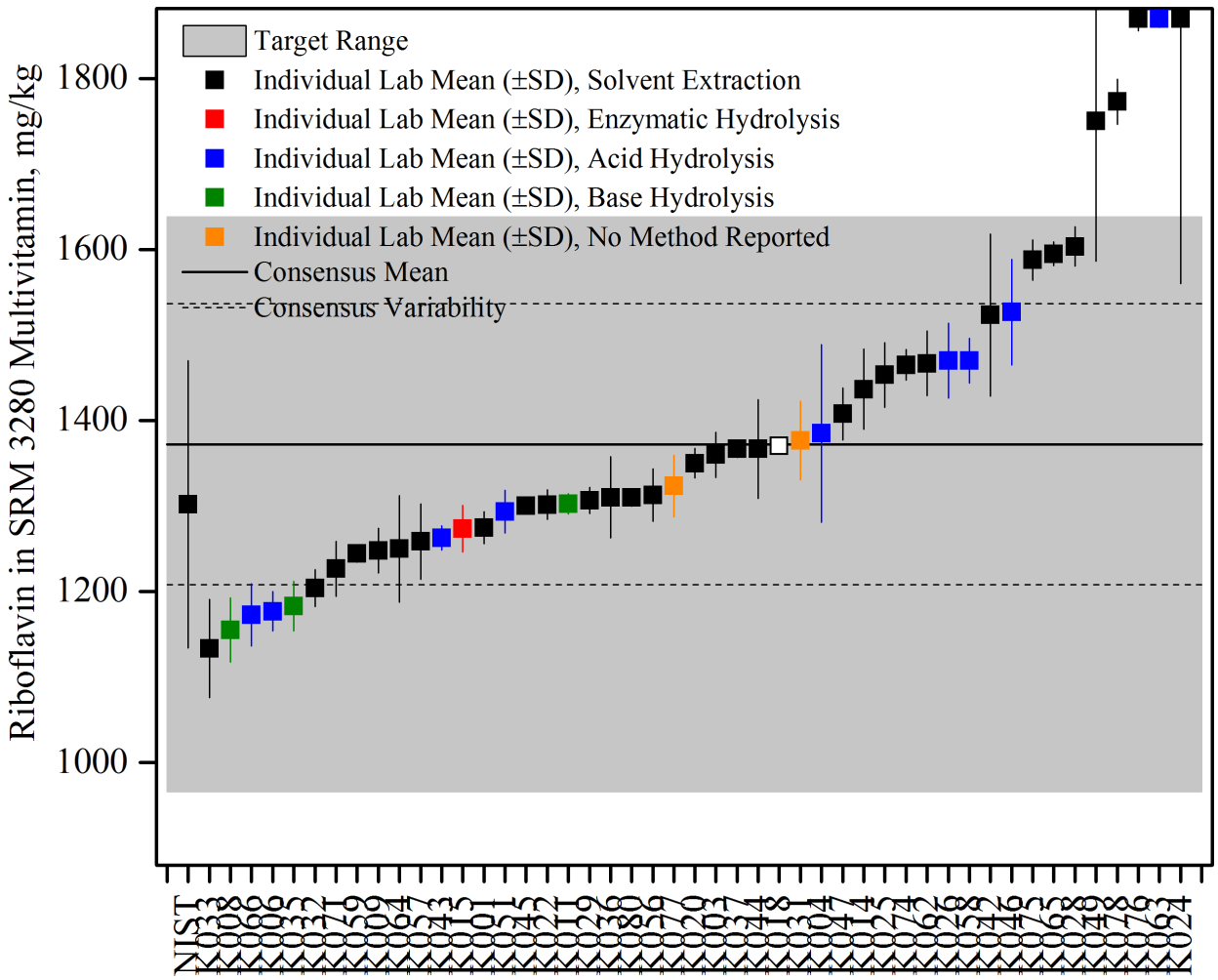

Figure 18. Riboflavin in SRM 3280 Multivitamin/Multielement Tablets (data summary view – sample preparation method). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The color of the data point represents the sample preparation procedure employed. The black solid line represents the consensus mean, and the black dotted lines represent the consensus variability calculated as one standard deviation about the consensus mean. The gray shaded region represents the target zone for "acceptable" performance, which encompasses the NIST certified value bounded by twice its uncertainty (U_{95}).

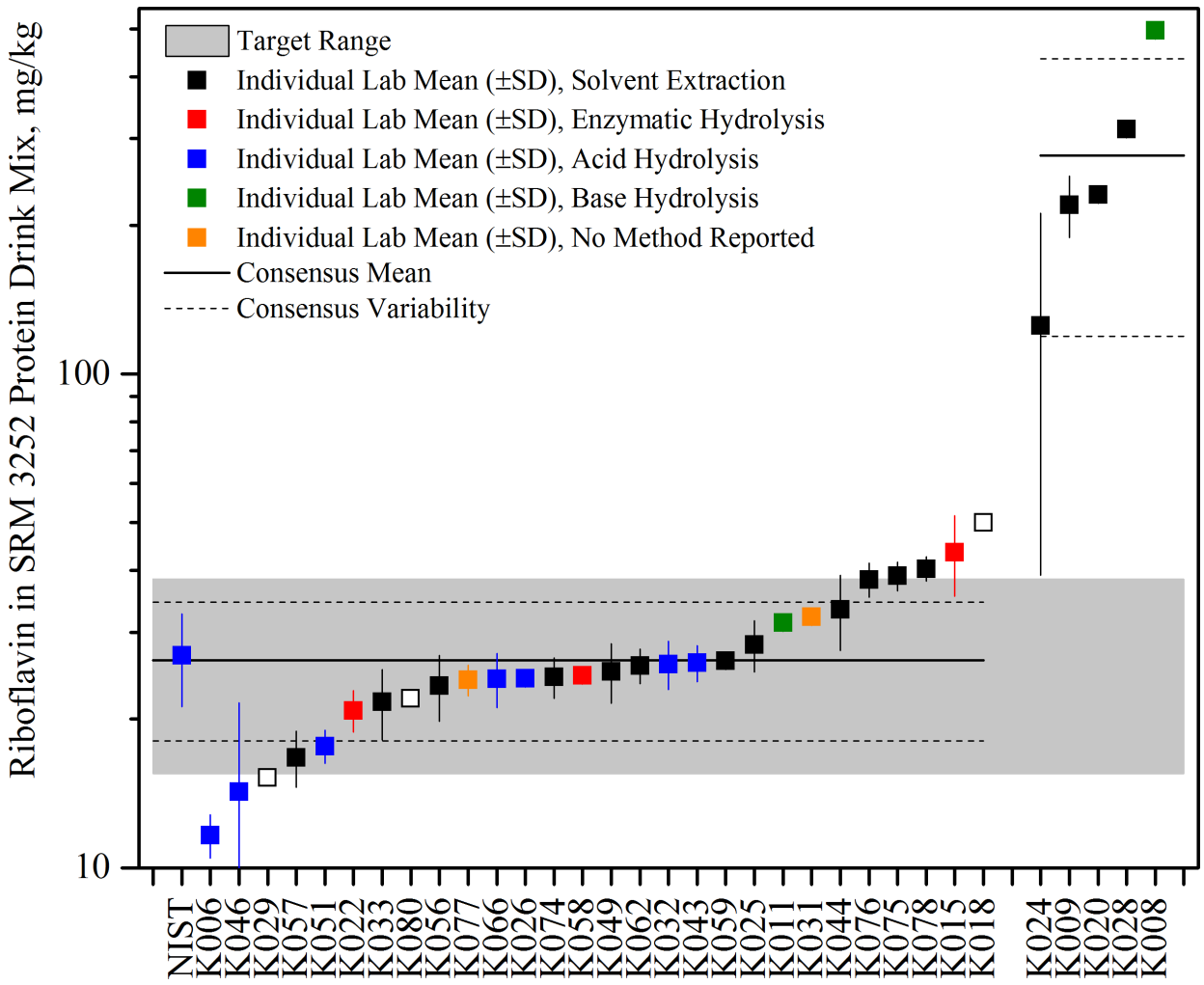

Figure 19. Riboflavin in SRM 3252 Protein Drink Mix (data summary view – sample preparation method). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The color of the data point represents the sample preparation procedure employed. The black solid lines represents the consensus means, and the black dotted lines represent the consensus variability calculated as one standard deviation about each consensus mean. The gray shaded region represents the target zone for "acceptable" performance, which encompasses the NIST value determined by ID-LC-MS/MS from duplicate measurements of ten packets, bounded by an estimated uncertainty based on twice the method standard deviation.

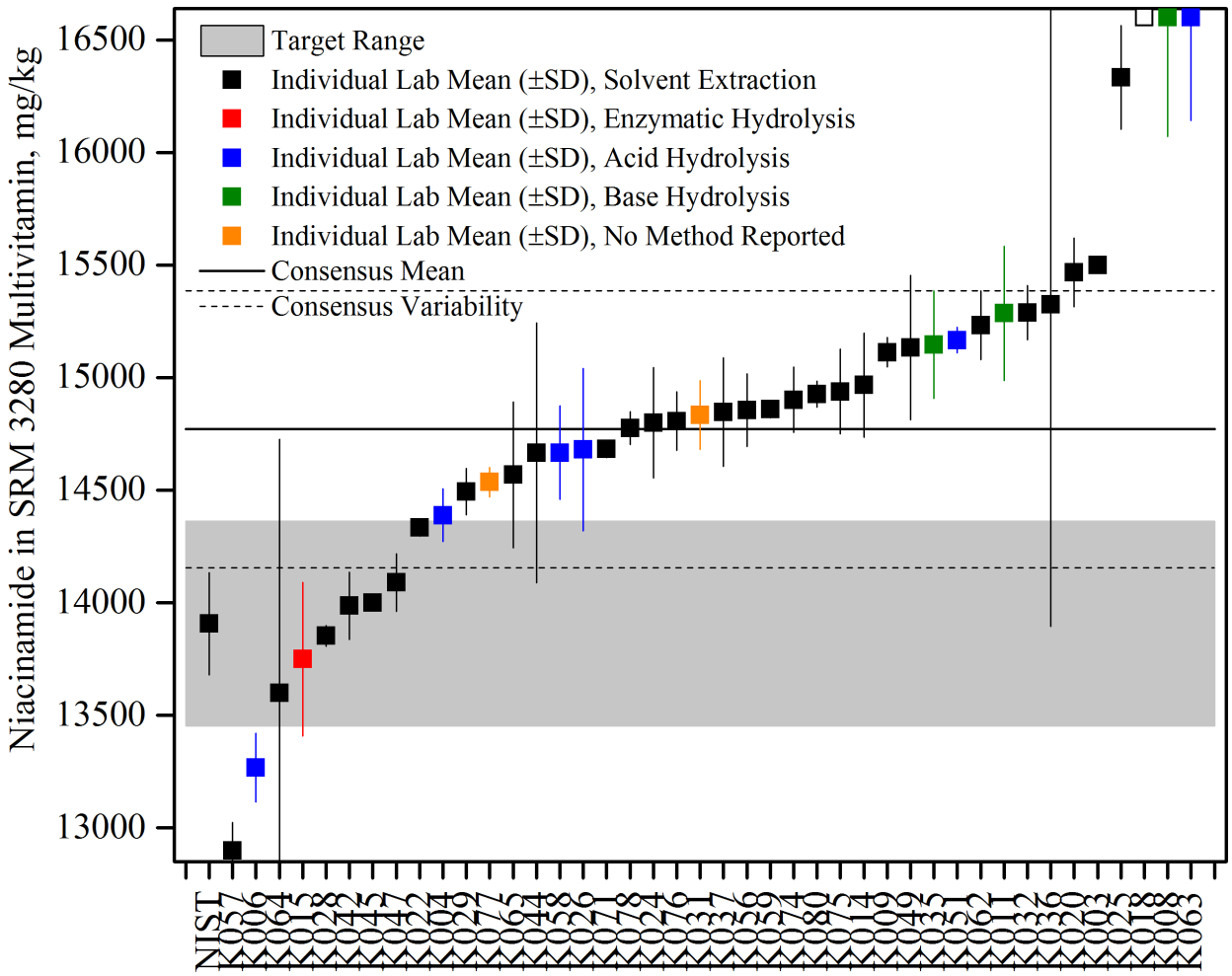

Figure 20. Niacinamide in SRM 3280 Multivitamin/Multielement Tablets (data summary view – sample preparation method). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The color of the data point represents the sample preparation procedure employed. The black solid line represents the consensus mean, and the black dotted lines represent the consensus variability calculated as one standard deviation about the consensus mean. The gray shaded region represents the target zone for "acceptable" performance, which encompasses the NIST certified value bounded by twice its uncertainty (U_{95}).

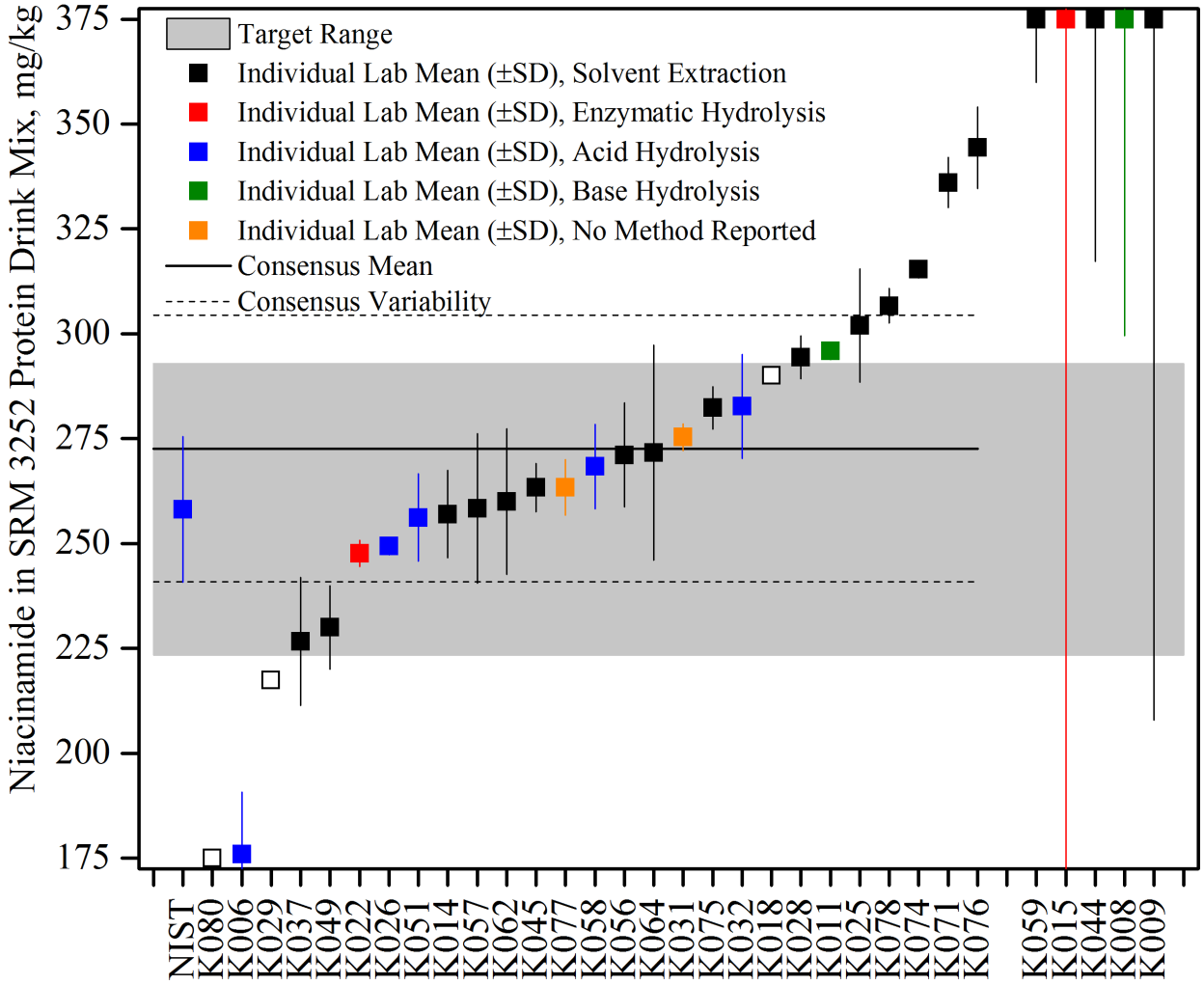

Figure 21. Niacinamide in SRM 3252 Protein Drink Mix (data summary view – sample preparation method). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The color of the data point represents the sample preparation procedure employed. The black solid lines represents the consensus means, and the black dotted lines represent the consensus variability calculated as one standard deviation about each consensus mean. The gray shaded region represents the target zone for "acceptable" performance, which encompasses the NIST value determined by ID-LC-MS/MS from duplicate measurements of ten packets, bounded by an estimated uncertainty based on twice the method standard deviation.

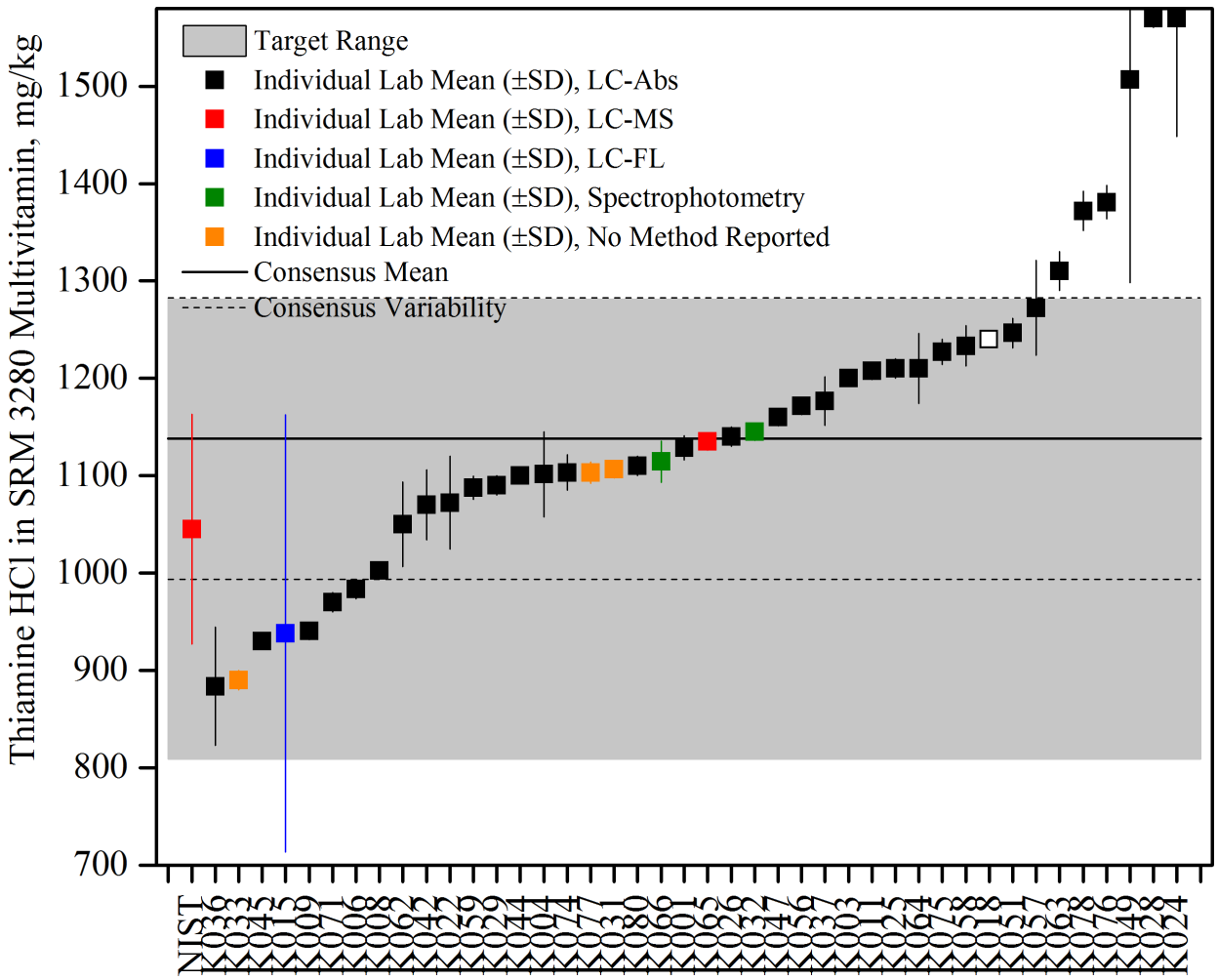

Figure 22. Thiamine hydrochloride in SRM 3280 Multivitamin/Multielement Tablets (data summary view – instrumental method). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The color of the data point represents the instrumental method employed. The black solid line represents the consensus mean, and the black dotted lines represent the consensus variability calculated as one standard deviation about the consensus mean. The gray shaded region represents the target zone for "acceptable" performance, which encompasses the NIST certified value bounded by twice its uncertainty (U_{95}).

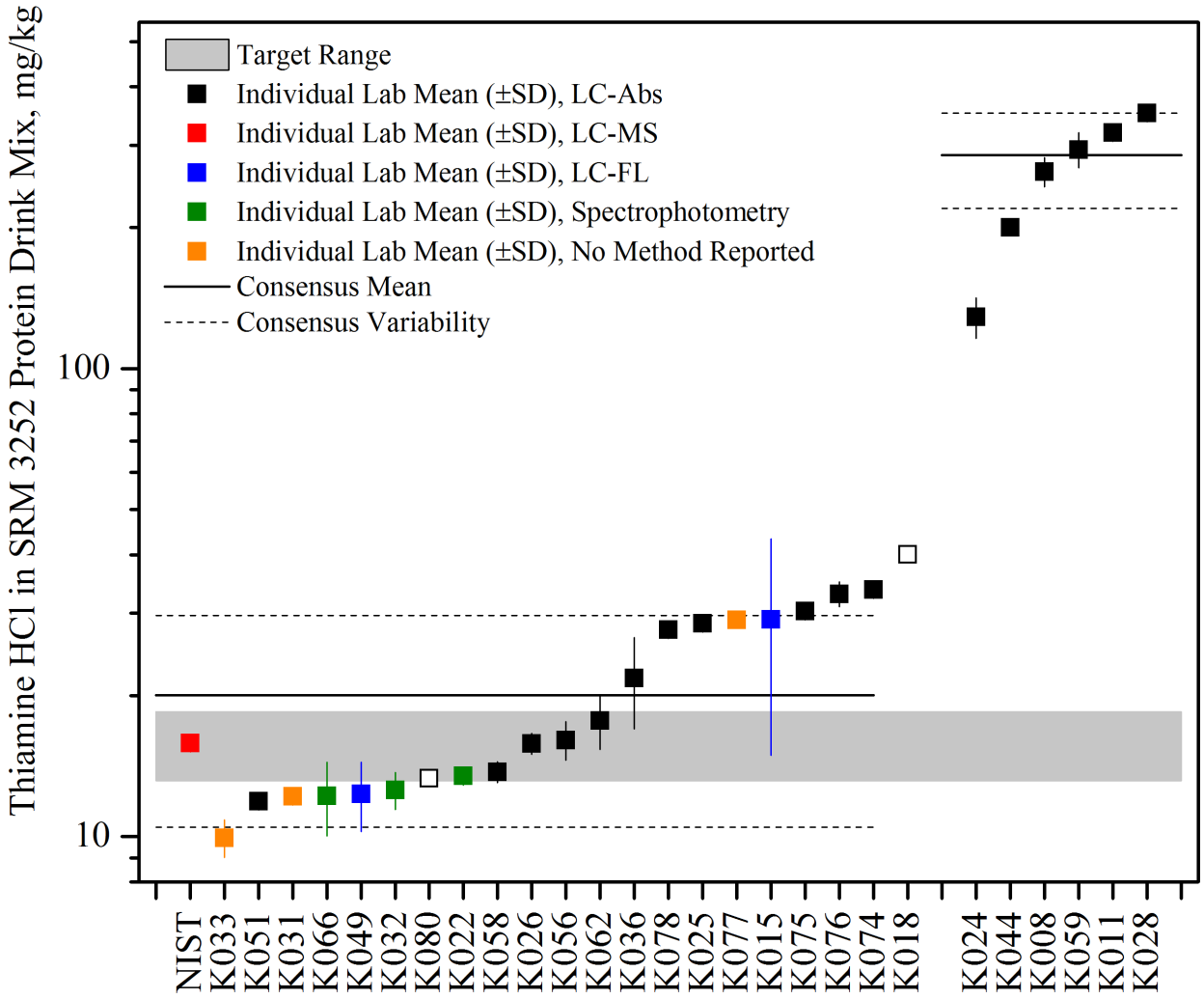

Figure 23. Thiamine hydrochloride in SRM 3252 Protein Drink Mix (data summary view – instrumental method). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The color of the data point represents the instrumental method employed. The black solid lines represents the consensus means, and the black dotted lines represent the consensus variability calculated as one standard deviation about each consensus mean. The gray shaded region represents the target zone for "acceptable" performance, which encompasses the NIST value determined by ID-LC-MS/MS from duplicate measurements of ten packets, bounded by an estimated uncertainty based on twice the method standard deviation.

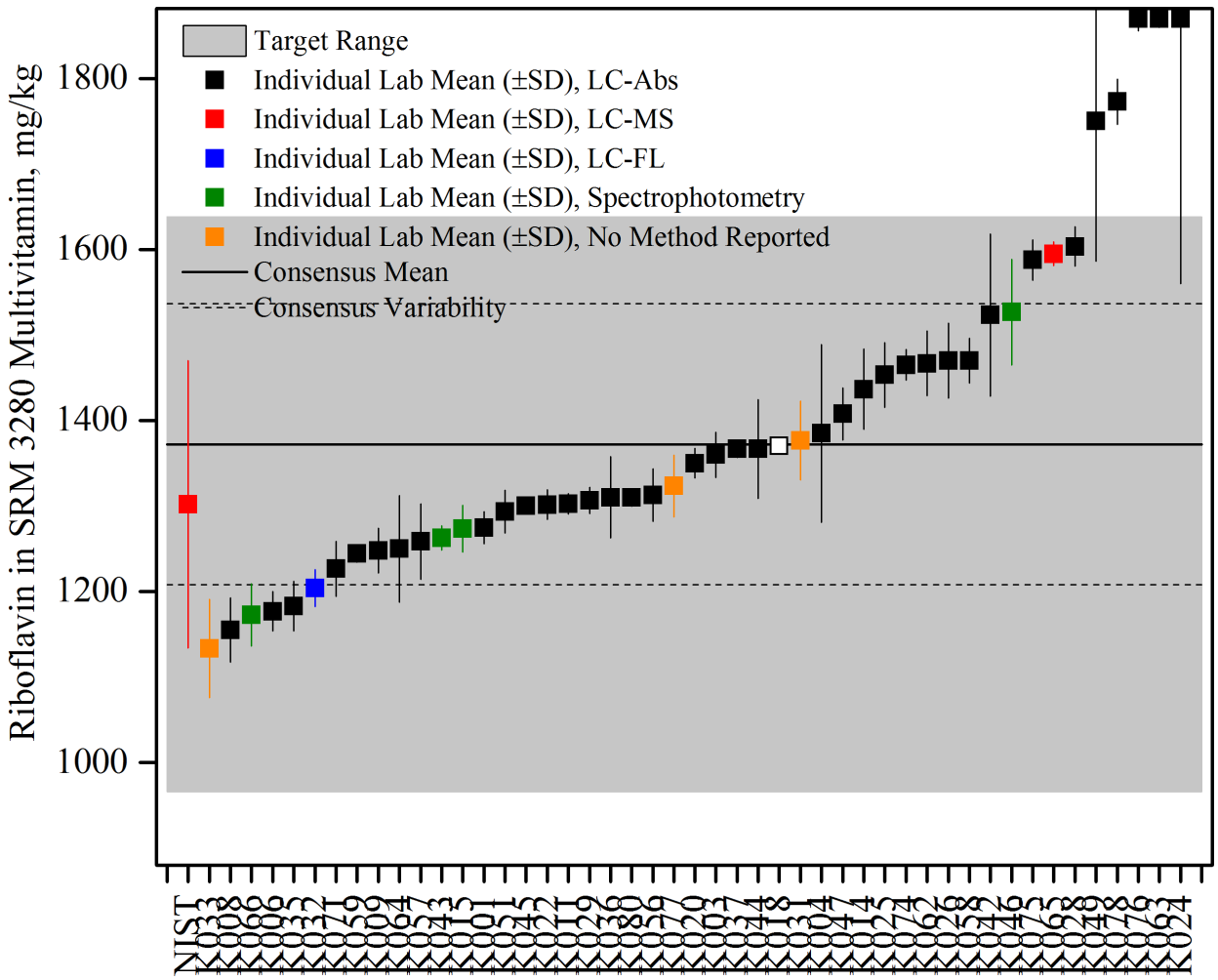

Figure 24. Riboflavin in SRM 3280 Multivitamin/Multielement Tablets (data summary view – instrumental method). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The color of the data point represents the instrumental method employed. The black solid line represents the consensus mean, and the black dotted lines represent the consensus variability calculated as one standard deviation about the consensus mean. The gray shaded region represents the target zone for "acceptable" performance, which encompasses the NIST certified value bounded by twice its uncertainty (U_{95}).

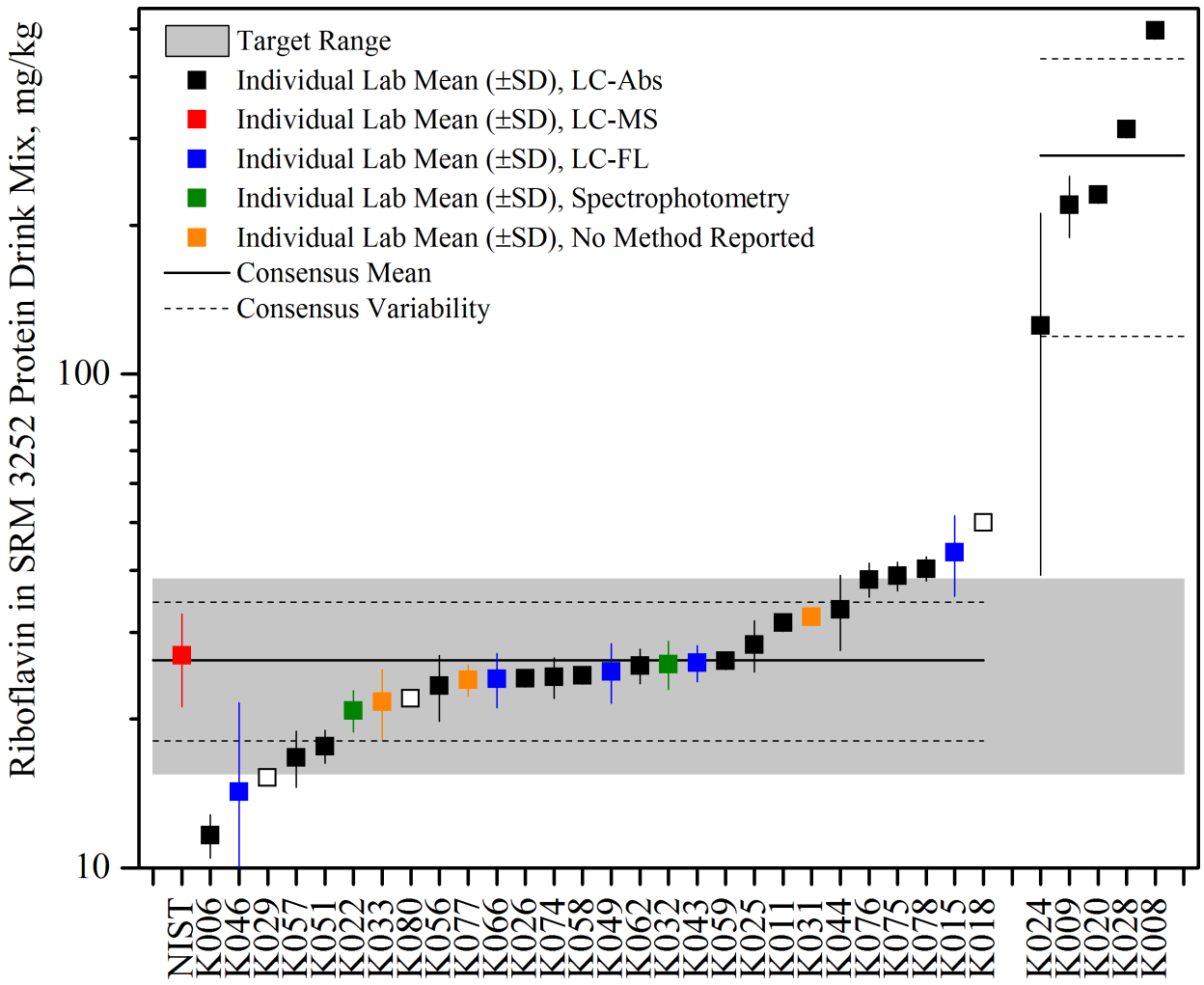

Figure 25. Riboflavin in SRM 3252 Protein Drink Mix (data summary view – instrumental method). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The color of the data point represents the instrumental method employed. The black solid lines represents the consensus means, and the black dotted lines represent the consensus variability calculated as one standard deviation about each consensus mean. The gray shaded region represents the target zone for "acceptable" performance, which encompasses the NIST value determined by ID-LC-MS/MS from duplicate measurements of ten packets, bounded by an estimated uncertainty based on twice the method standard deviation.

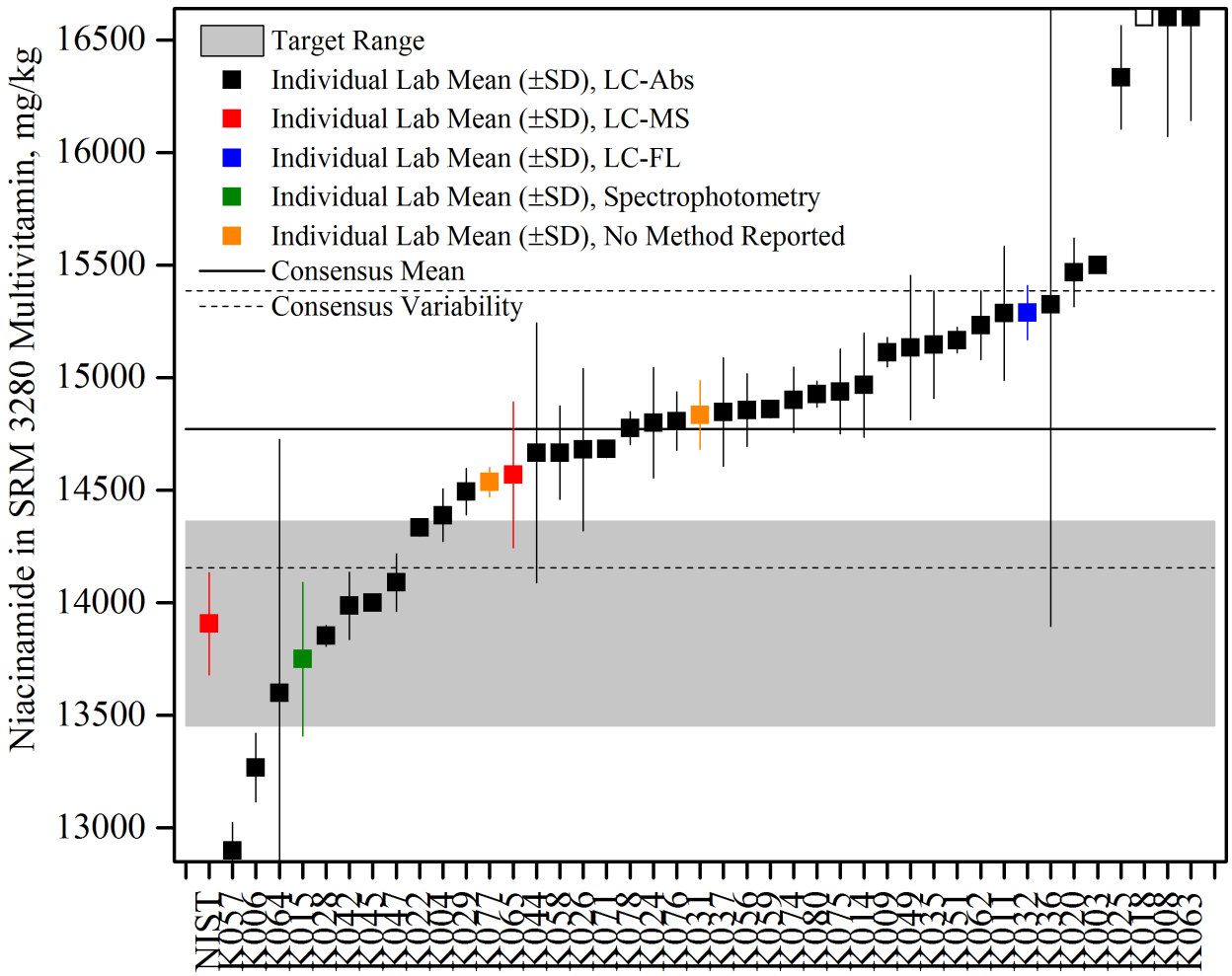

Figure 26. Niacinamide in SRM 3280 Multivitamin/Multielement Tablets (data summary view – instrumental method). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The color of the data point represents the instrumental method employed. The black solid line represents the consensus mean, and the black dotted lines represent the consensus variability calculated as one standard deviation about the consensus mean. The gray shaded region represents the target zone for "acceptable" performance, which encompasses the NIST certified value bounded by twice its uncertainty (U_{95}).

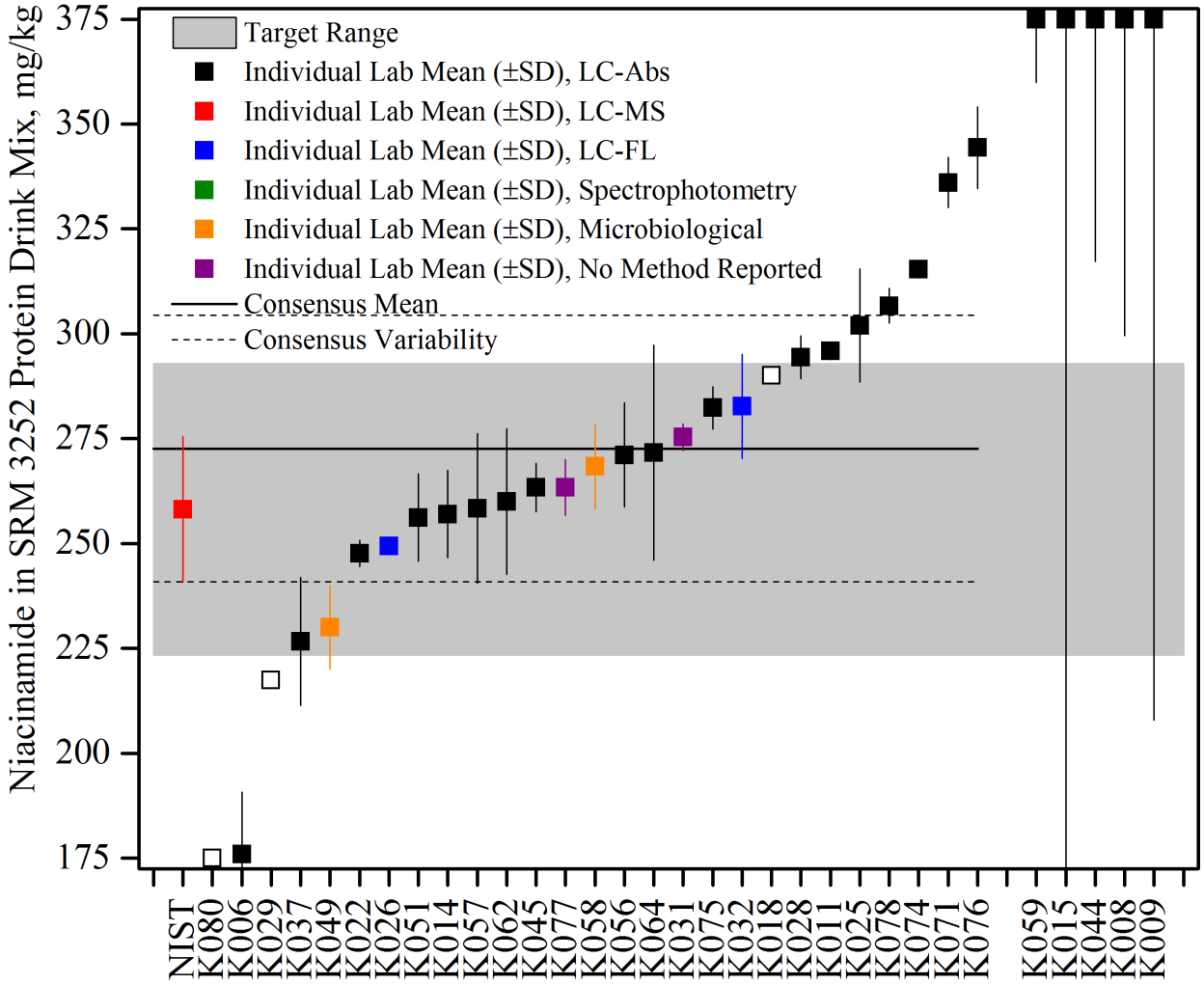

Figure 27. Niacinamide in SRM 3252 Protein Drink Mix (data summary view – instrumental method). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The color of the data point represents the instrumental method employed. The black solid lines represents the consensus means, and the black dotted lines represent the consensus variability calculated as one standard deviation about each consensus mean. The gray shaded region represents the target zone for "acceptable" performance, which encompasses the NIST value determined by ID-LC-MS/MS from duplicate measurements of ten packets, bounded by an estimated uncertainty based on twice the method standard deviation.

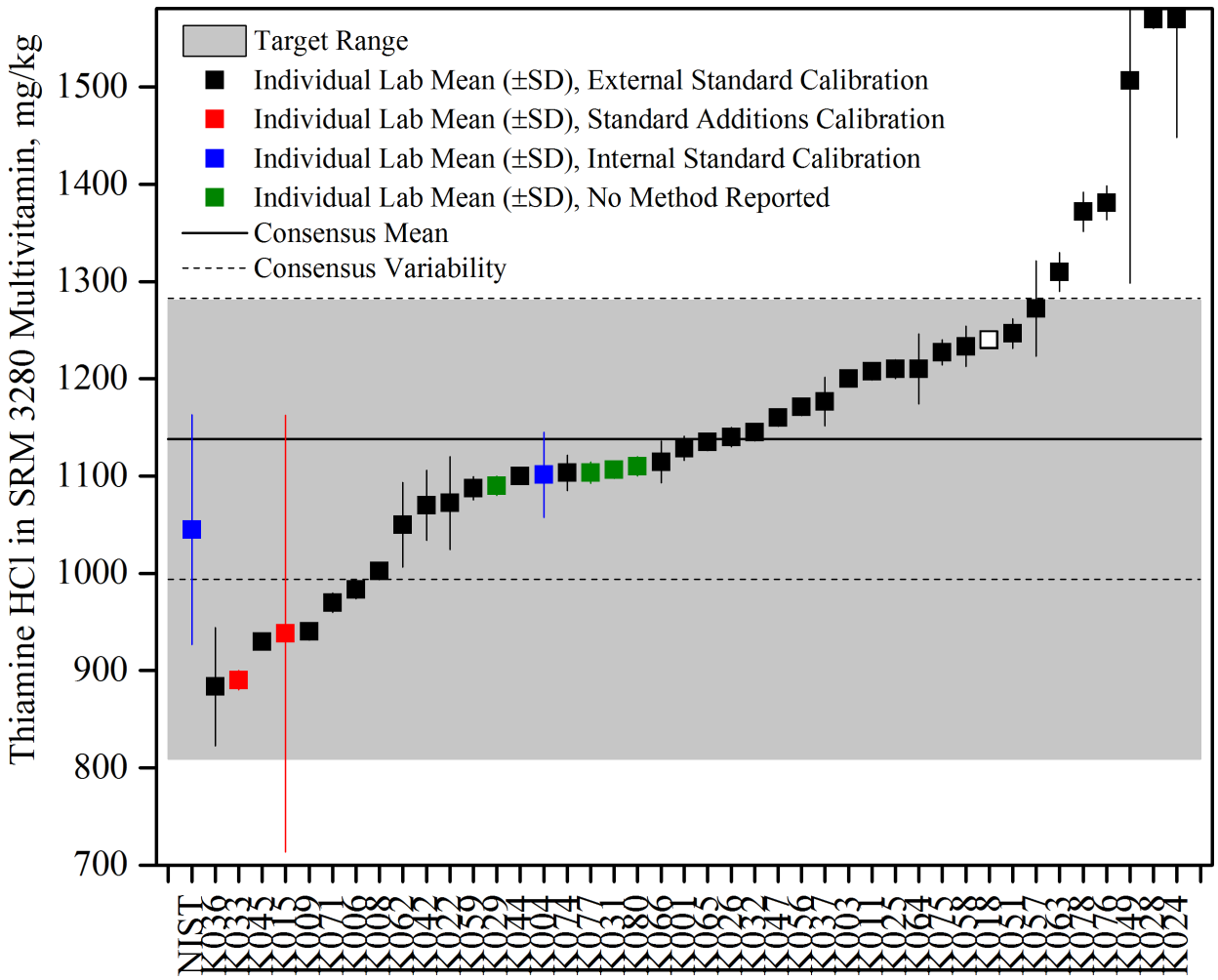

Figure 28. Thiamine hydrochloride in SRM 3280 Multivitamin/Multielement Tablets (data summary view – calibration method). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The color of the data point represents the calibration approach employed. The black solid line represents the consensus mean, and the black dotted lines represent the consensus variability calculated as one standard deviation about the consensus mean. The gray shaded region represents the target zone for "acceptable" performance, which encompasses the NIST certified value bounded by twice its uncertainty (U_{95}).

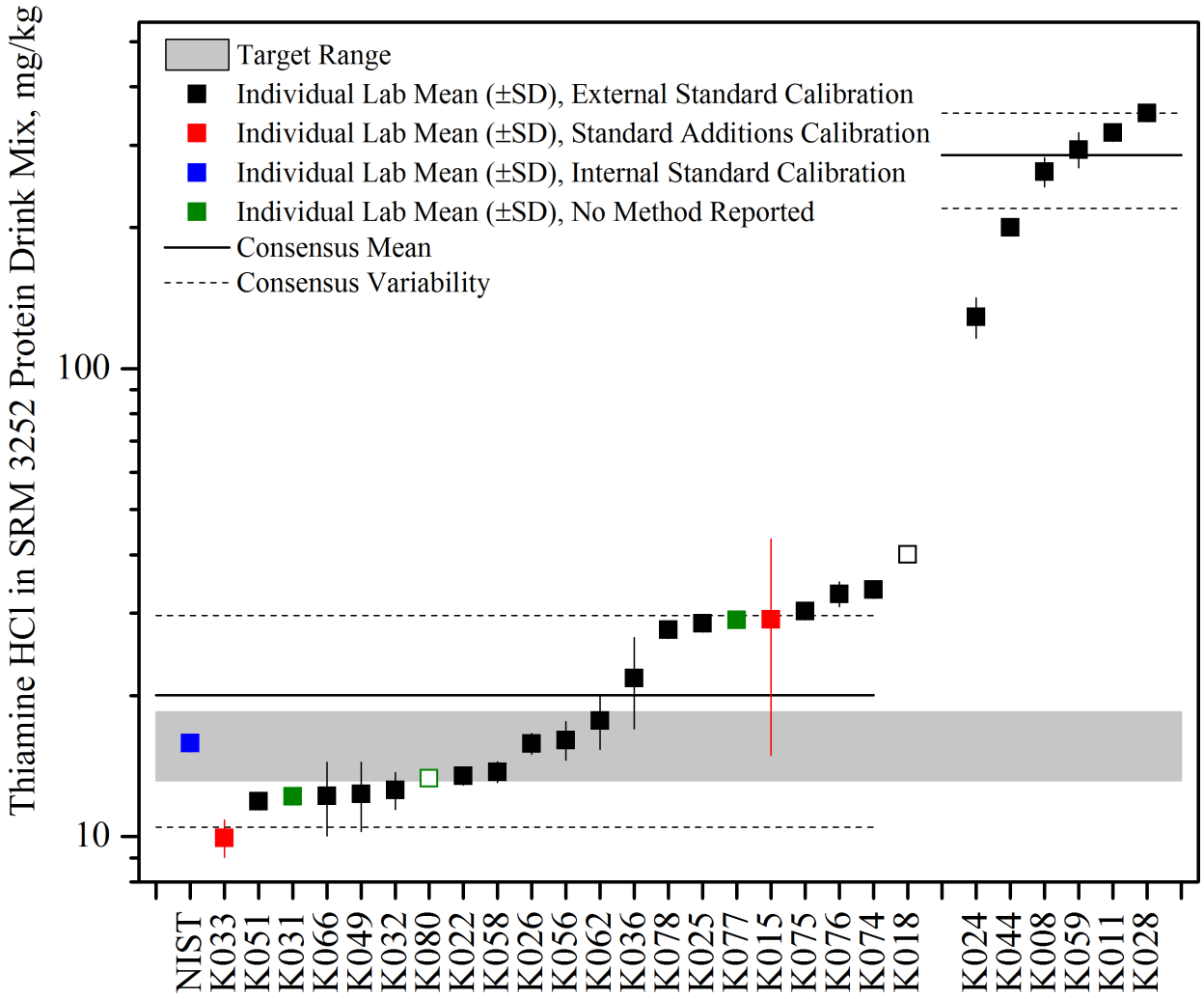

Figure 29. Thiamine hydrochloride in SRM 3252 Protein Drink Mix (data summary view – calibration method). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The color of the data point represents the calibration approach employed. The black solid lines represents the consensus means, and the black dotted lines represent the consensus variability calculated as one standard deviation about each consensus mean. The gray shaded region represents the target zone for "acceptable" performance, which encompasses the NIST value determined by ID-LC-MS/MS from duplicate measurements of ten packets, bounded by an estimated uncertainty based on twice the method standard deviation.

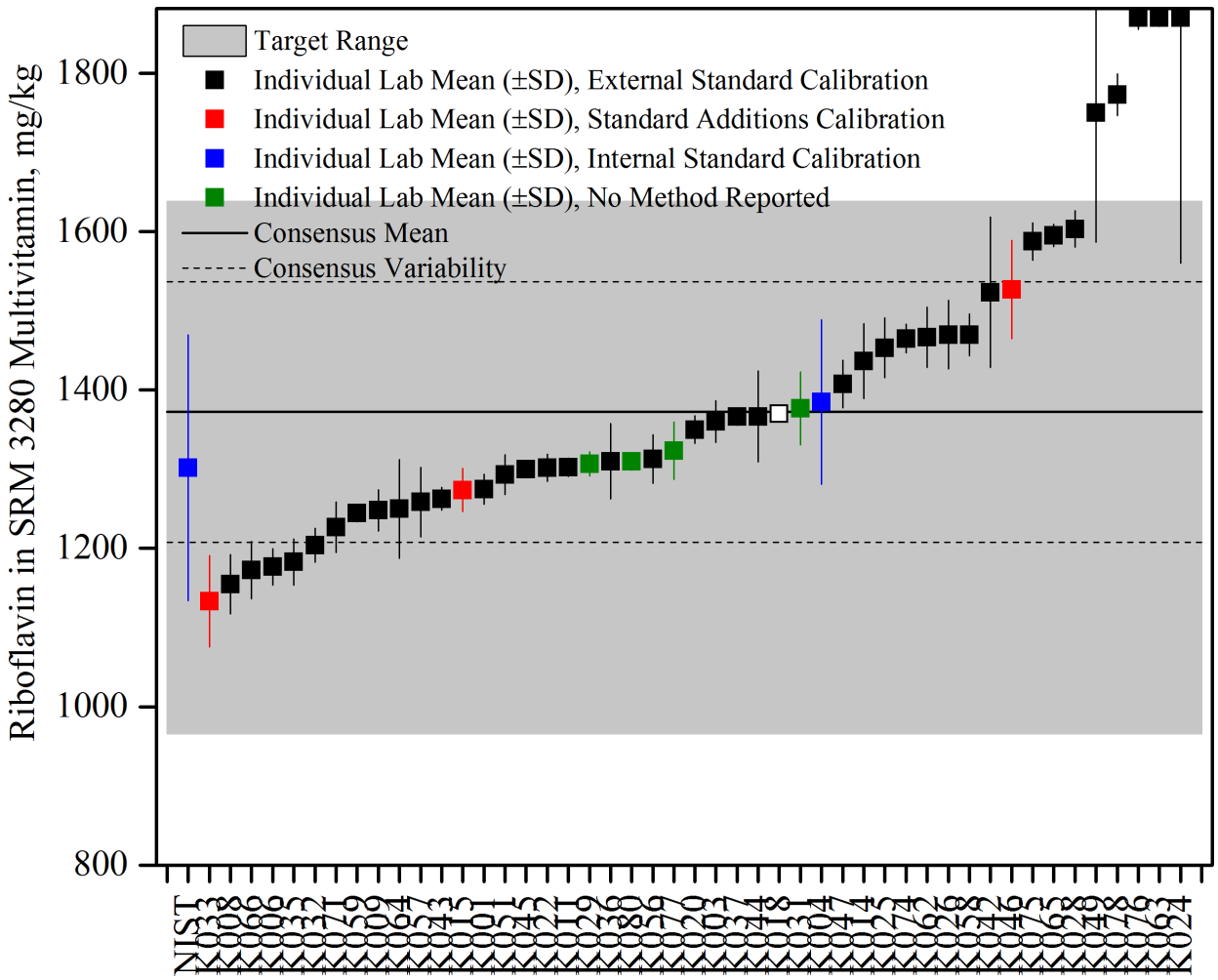

Figure 30. Riboflavin in SRM 3280 Multivitamin/Multielement Tablets (data summary view – calibration method). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The color of the data point represents the calibration approach employed. The black solid line represents the consensus mean, and the black dotted lines represent the consensus variability calculated as one standard deviation about the consensus mean. The gray shaded region represents the target zone for "acceptable" performance, which encompasses the NIST certified value bounded by twice its uncertainty (U_{95}).

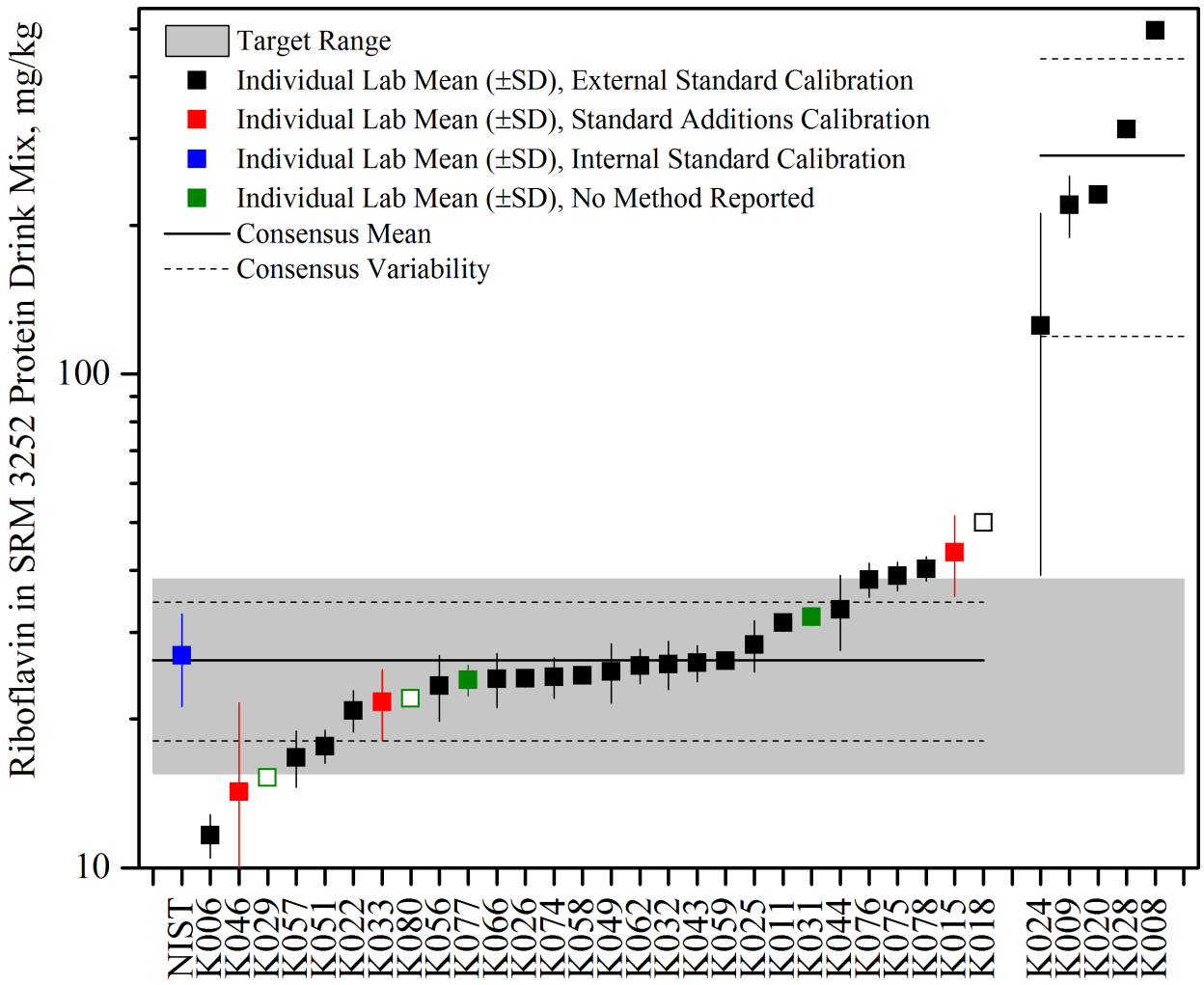

Figure 31. Riboflavin in SRM 3252 Protein Drink Mix (data summary view – calibration method). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The color of the data point represents the calibration approach employed. The black solid lines represents the consensus means, and the black dotted lines represent the consensus variability calculated as one standard deviation about each consensus mean. The gray shaded region represents the target zone for "acceptable" performance, which encompasses the NIST value determined by ID-LC-MS/MS from duplicate measurements of ten packets, bounded by an estimated uncertainty based on twice the method standard deviation.

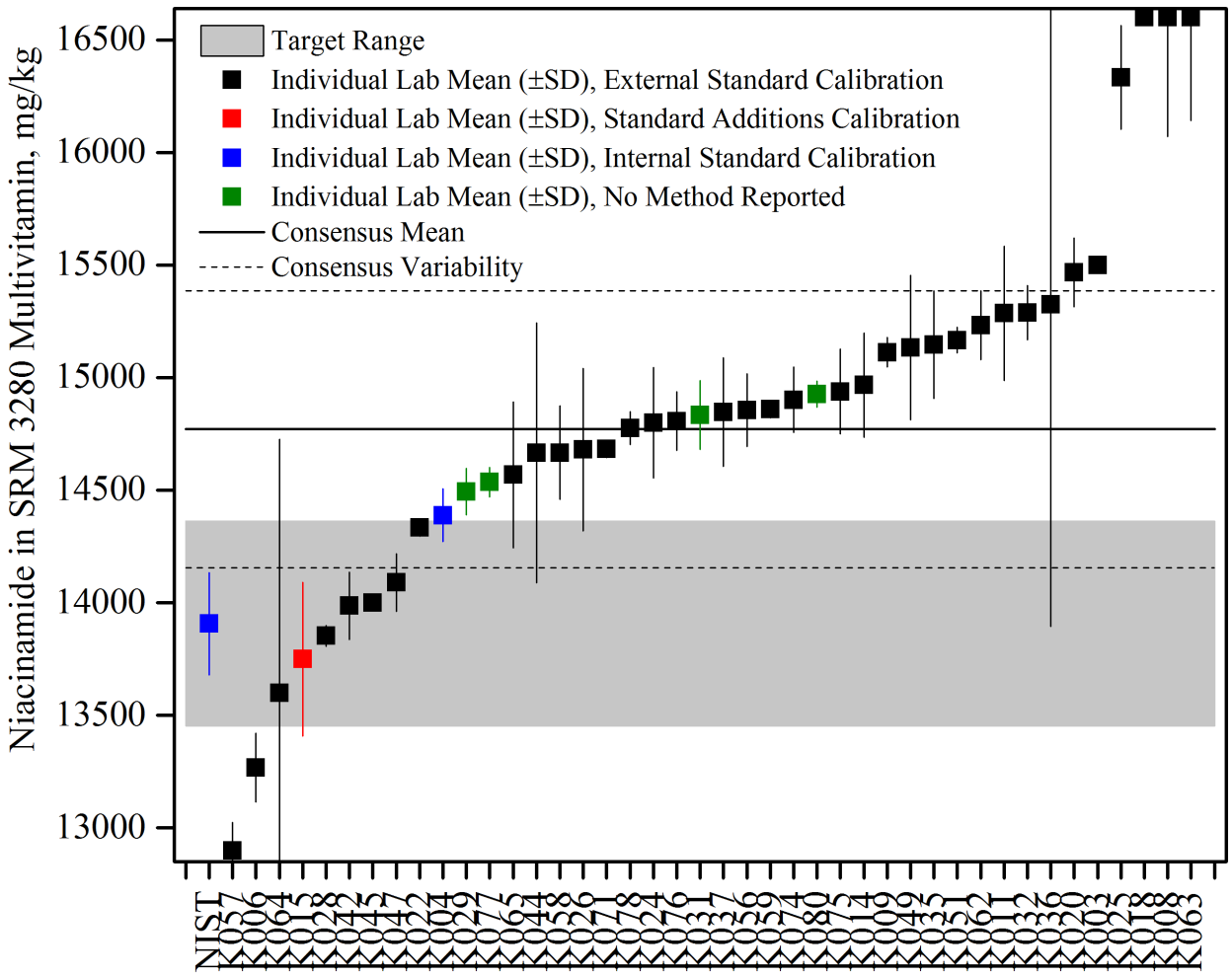

Figure 32. Niacinamide in SRM 3280 Multivitamin/Multielement Tablets (data summary view – calibration method). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The color of the data point represents the calibration approach employed. The black solid line represents the consensus mean, and the black dotted lines represent the consensus variability calculated as one standard deviation about the consensus mean. The gray shaded region represents the target zone for "acceptable" performance, which encompasses the NIST certified value bounded by twice its uncertainty (U_{95}).

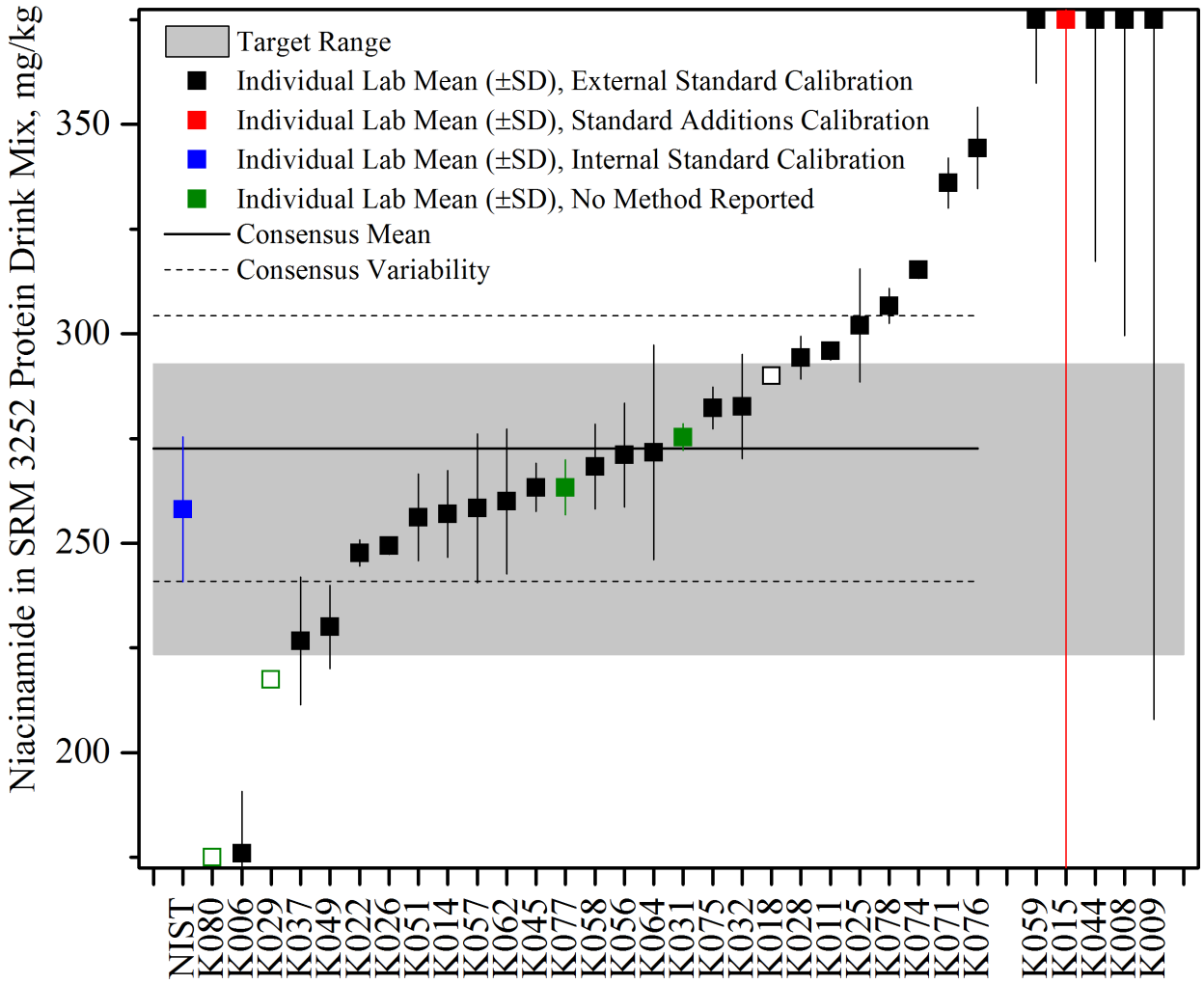

Figure 33. Niacinamide in SRM 3252 Protein Drink Mix (data summary view – calibration method). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The color of the data point represents the calibration approach employed. The black solid lines represents the consensus means, and the black dotted lines represent the consensus variability calculated as one standard deviation about each consensus mean. The gray shaded region represents the target zone for "acceptable" performance, which encompasses the NIST value determined by ID-LC-MS/MS from duplicate measurements of ten packets, bounded by an estimated uncertainty based on twice the method standard deviation.

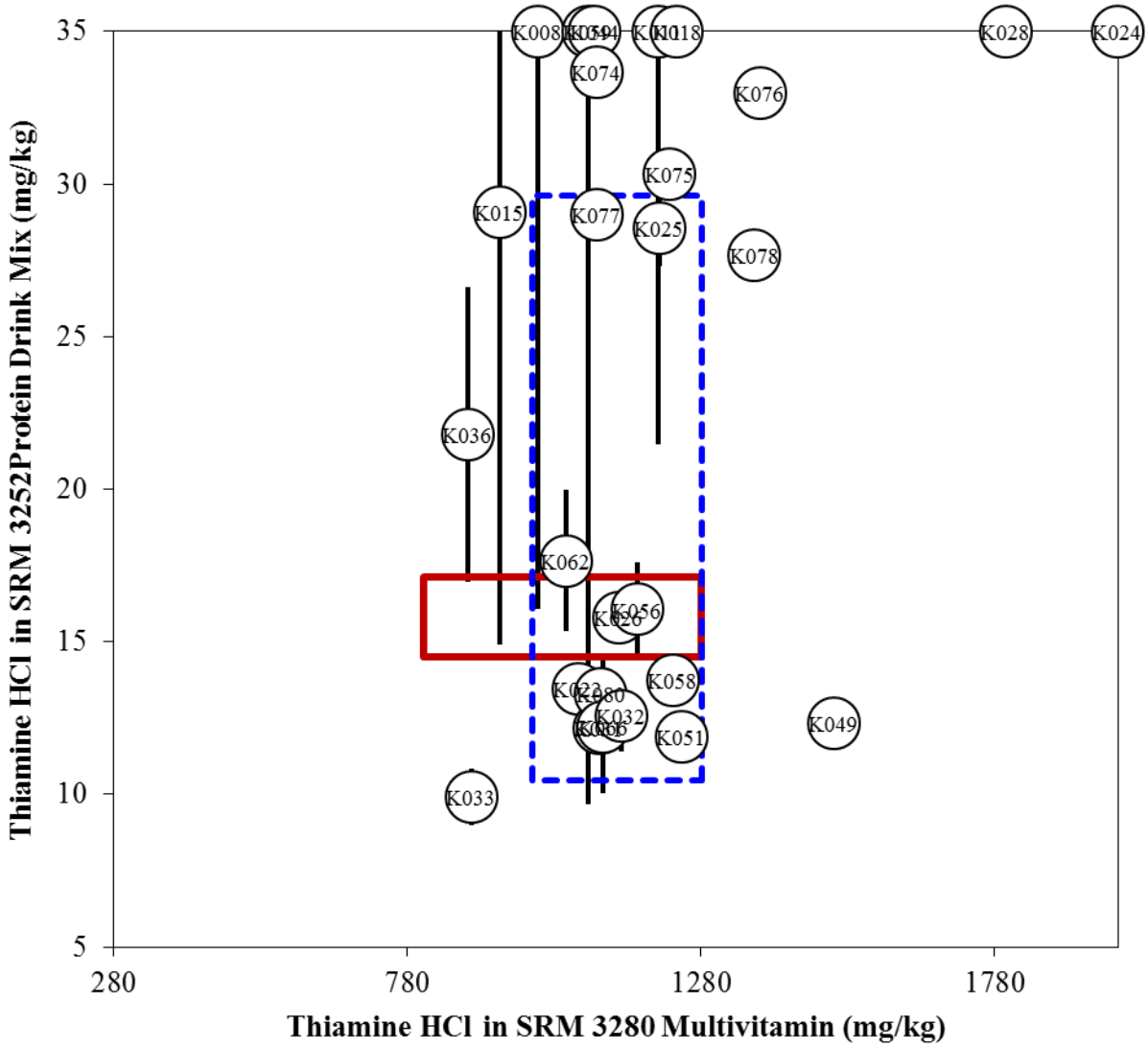

Figure 34. Thiamine HCl in SRM 3280 Multivitamin/Multielement Tablets and SRM 3252 Protein Drink Mix (sample/sample comparison view). In this view, the individual laboratory results for one sample (SRM 3280) are compared to the results for another sample (SRM 3252). The solid red box represents the target zone for the control (x-axis) and unknown sample (y-axis). The dotted blue box represents the consensus zone for the control (x-axis) and the unknown sample (y-axis).

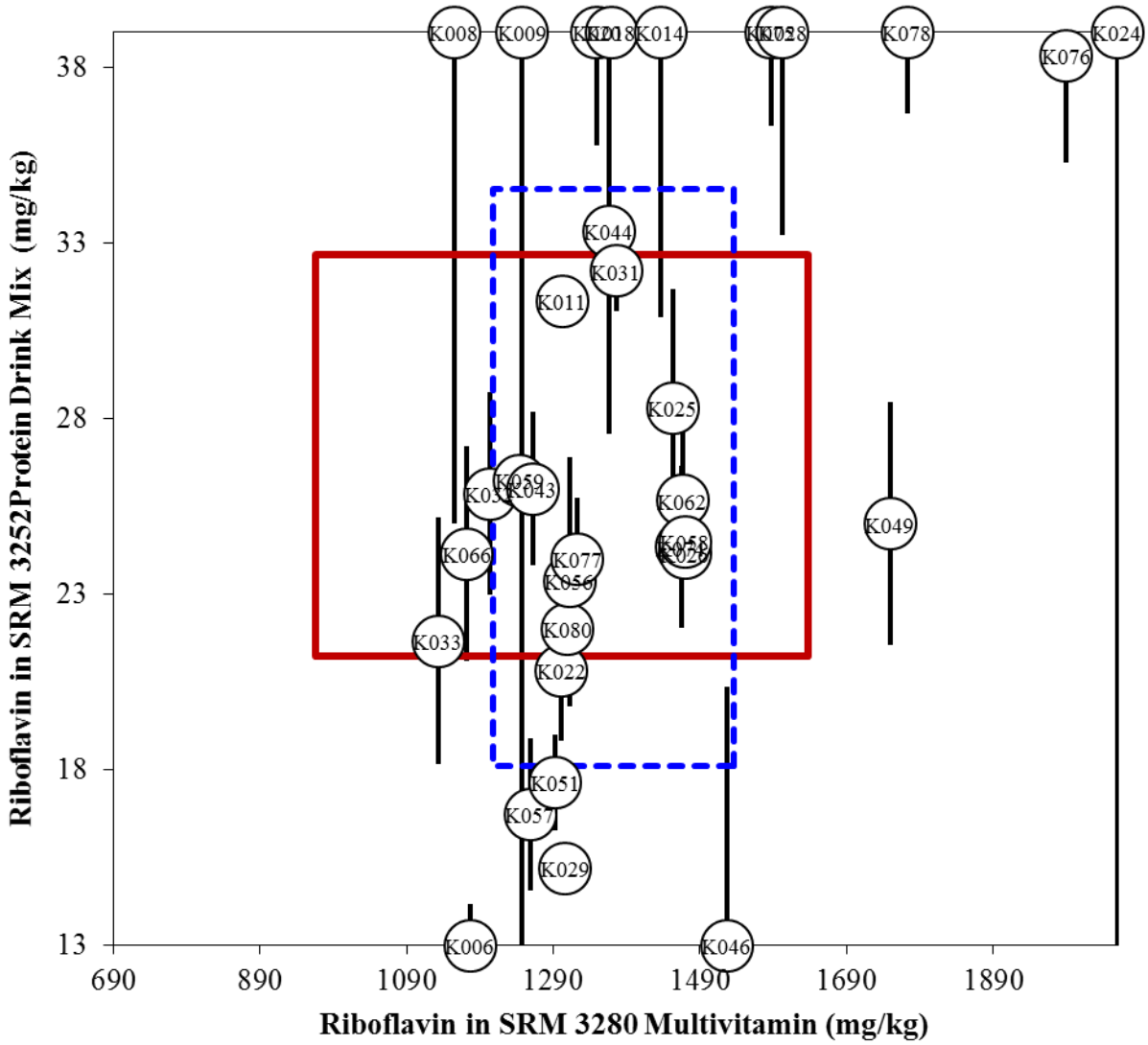

Figure 35. Riboflavin in SRM 3280 Multivitamin/Multielement Tablets and SRM 3252 Protein Drink Mix (sample/sample comparison view). In this view, the individual laboratory results for one sample (SRM 3280) are compared to the results for another sample (SRM 3252). The solid red box represents the target zone for the control (x-axis) and unknown sample (y-axis). The dotted blue box represents the consensus zone for the control (x-axis) and the unknown sample (y-axis).

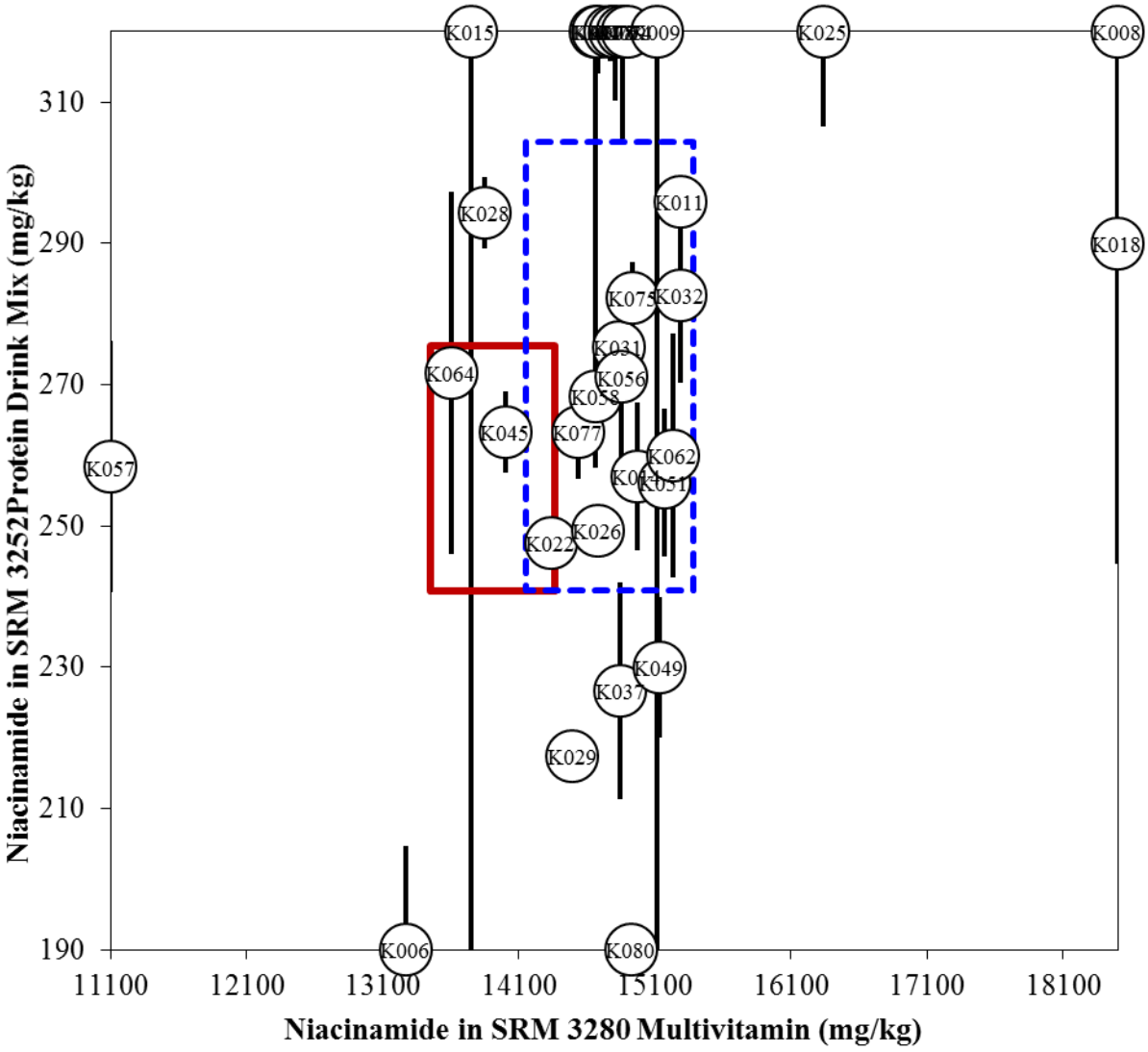

Figure 36. Niacinamide in SRM 3280 Multivitamin/Multielement Tablets and SRM 3252 Protein Drink Mix (sample/sample comparison view). In this view, the individual laboratory results for one sample (SRM 3280) are compared to the results for another sample (SRM 3252). The solid red box represents the target zone for the control (x-axis) and unknown sample (y-axis). The dotted blue box represents the consensus zone for the control (x-axis) and the unknown sample (y-axis).

VITAMIN K IN DIETARY SUPPLEMENTS

Study Overview

In this study, participants were provided with one NIST SRM, SRM 3280 Multivitamin/ Multielement Tablets, and one NIST candidate SRM, SRM 3252 Protein Drink Mix. Participants were asked to use in-house analytical methods to determine the mass fraction of vitamin K_1 in each of the matrices and report values on an as-received basis as phylloquinone.

Sample Information

Multivitamin/Multielement Tablets. Participants were provided with one bottle containing 30 multivitamin/multielement tablets. Before use, participants were instructed to grind all 30 tablets, mix the resulting powder thoroughly, and use a sample size of at least 0.6 g. Participants were asked to store the material at controlled room temperature, 10 °C to 30 °C, 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 NIST certified value and uncertainty for vitamin K_1 (phylloquinone) in SRM 3280 was determined by LC/MS following solvent extraction, in combination with data from numerous collaborating laboratories. The certified value and uncertainty are reported in the table below as phylloquinone on a dry-mass basis and after correction for moisture of the material (1.37 %).

Analyte	Certified Mass Fraction in SRM 3280 (mg/g) (dry-mass basis)	Certified Mass Fraction in SRM 3280 (mg/g) (as-received basis)
Phylloquinone (K_1)	22.8 ± 2.2	22.5 ± 2.2

Protein Powder. Participants were provided with one packet containing approximately 10 g of protein powder. A mixture of commercially available chocolate protein drink mix powders was blended and heat-sealed inside nitrogen-flushed 4-mil plastic bags, which were heat-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 a sample size of at least 0.5 g was recommended. Participants were asked to store the material at controlled room temperature, 10 °C to 30 °C, prepare three samples, and report three values from the single packet provided. Approximate analyte levels were not reported to participants prior to the study. Certified values are not available for this material at the time of the report.

Study Results

- Forty-eight laboratories enrolled in this exercise and received samples. Nineteen laboratories reported results for vitamin K in the multivitamin (40 % participation) and 6 laboratories reported results for vitamin K in the protein powder (15 % participation).
- The consensus mean was within the target range for vitamin K in the multivitamin, but with high variability (43 % RSD).
- The limited results reported for vitamin K in the protein powder were widely varied, with values ranging from 0.020 mg/kg to 1.555 mg/kg (128 % RSD).
- A majority of the laboratories reported using solvent extraction (79 %) as the sample preparation method. Some laboratories also reported using saponification (16 %). One laboratory did not report a sample preparation technique.

- A majority of the laboratories reported using LC-Abs (68 %) as their instrumental method for analysis. LC-Fluorescence (LC-FL) was also reported by some laboratories (21 %). Two laboratories did not report an analytical method.
- External standard was the most popular calibration approach (79 %), with some laboratories reporting using an internal standard calibration approach (11 %). Two laboratories did not report the type of calibration method used.

Technical Recommendations

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

- Care should be taken to minimize losses during the extraction process, during solvent evaporation, and by carefully washing down container walls with several rinses during each step to ensure complete dissolution of any residues.
- In general, laboratories reporting more vigorous extraction procedures, i.e. those using hexanes and longer extraction times, reported results closer to the target value.
- Since loss by photodecomposition is possible, care should be taken to prevent such losses (use of amber vials, aluminum foil, and/or reduced lighting).
- When using LC-Abs, chromatographic coelutions may cause results to be biased high. This is particularly important if monitoring the absorbance in the UV where many other compounds may also have chromophores. To avoid a high bias, more selective detectors (fluorescence, mass spectrometry) or chromatography with alternate selectivity may be used.
- When making calibration solutions make sure they are of known quality. These may need to be tested before running samples, which may include determination of purity by chromatographic and spectroscopic methods.
- If using an internal standard, the internal standard must behave similarly to the analyte of interest in extraction, chromatographic analysis, and detection.

Table 10. Individualized data summary table (NIST) for vitamin K_1 in dietary supplements.

National Institute of Standards & Technology

Exercise K - February 2014 - Vitamin K

	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}
Vitamin K1	Multivitamin	mg/kg	22.5	2.2	0.3	0.0	19	20.1	8.7	22.5	2.2
Vitamin K1	Protein Drink	mg/kg					6	0.6	0.7		

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 K_1 in dietary supplements.

		Phylloquinone									
		SRM 3280 Multivitamin/Multielement Tablets (mg/kg)					SRM 3252 Protein Drink Mix (mg/kg)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	NIST				22.5	2.2					
	K001										
	K002										
	K003	70.3	71.2	55.2	65.6	9.0					
	K006										
	K010										
	K014	21.3	21.4	21.2	21.3	0.1					
	K016										
	K017										
	K019										
	K020	27.5	23.0	24.0	24.8	2.4	0.049	0.088	0.042	0.060	0.025
	K024	17.2	16.3	14.0	15.8	1.6					
	K025	0.4	0.5	0.5	0.5	0.0	0.950	0.986	0.935	0.957	0.026
	K026	24.8	24.6	24.4	24.6	0.2	0.027	0.026	0.028	0.027	0.001
	K028										
	K029	21.6	20.5	19.5	20.5	1.1	0.022	0.023		0.023	0.001
	K031	22.0	21.7	22.3	22.0	0.3					
	K033	20.0	21.0	21.0	20.7	0.6	0.020			0.020	
	K034										
	K035	18.3	17.9	18.7	18.3	0.4					
	K037	8.0	7.0	6.0	7.0	1.0	0.800	0.800	0.600	0.733	0.115
	K040										
	K042										
	K043										
	K045										
	K046	19.6	22.4	20.1	20.7	1.5					
	K047										
	K048										
	K049	98.0	99.3	99.6	99.0	0.9					
	K051	21.0	18.6	20.9	20.2	1.4					
	K056										
	K057	13.3	12.3	9.2	11.6	2.2					
	K058	11.9	12.9	11.7	12.1	0.7					
	K059	31.4	30.9	34.8	32.4	2.1					
	K062	20.6	18.9	23.7	21.1	2.4					
	K063										
	K064										
	K065	15.5	16.1	15.7	15.8	0.3	1.661	1.563	1.441	1.555	0.110
	K066										
	K068										
	K069										
	K071										
	K073										
	K074										
	K075										
	K076										
	K077										
	K078										
	K079										
Community Results		Consensus Mean			20.1		Consensus Mean			0.559	
		Consensus Standard Deviation			8.7		Consensus Standard Deviation			0.717	
		Maximum			99.0		Maximum			1.555	
		Minimum			0.5		Minimum			0.020	
		N			19		N			6	

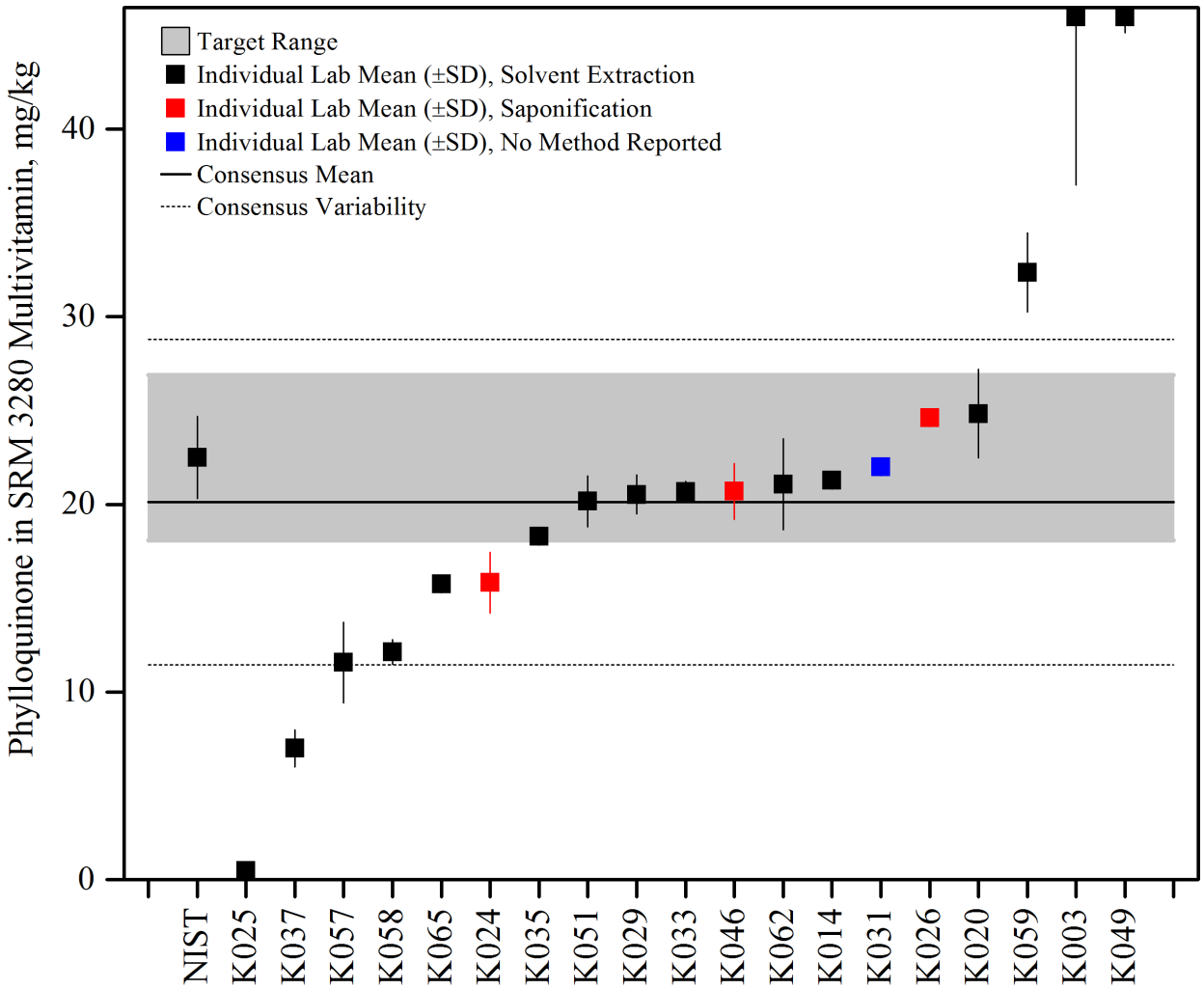

Figure 37. Phylloquinone in SRM 3280 Multivitamin/Multielement Tablets (data summary view – sample preparation method). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The color of the data point represents the sample preparation procedure employed. The black solid line represents the consensus mean, and the black dotted lines represent the consensus variability calculated as one standard deviation about the consensus mean. The gray shaded region represents the target zone for "acceptable" performance, which encompasses the NIST certified value bounded by twice its uncertainty (U_{95}).

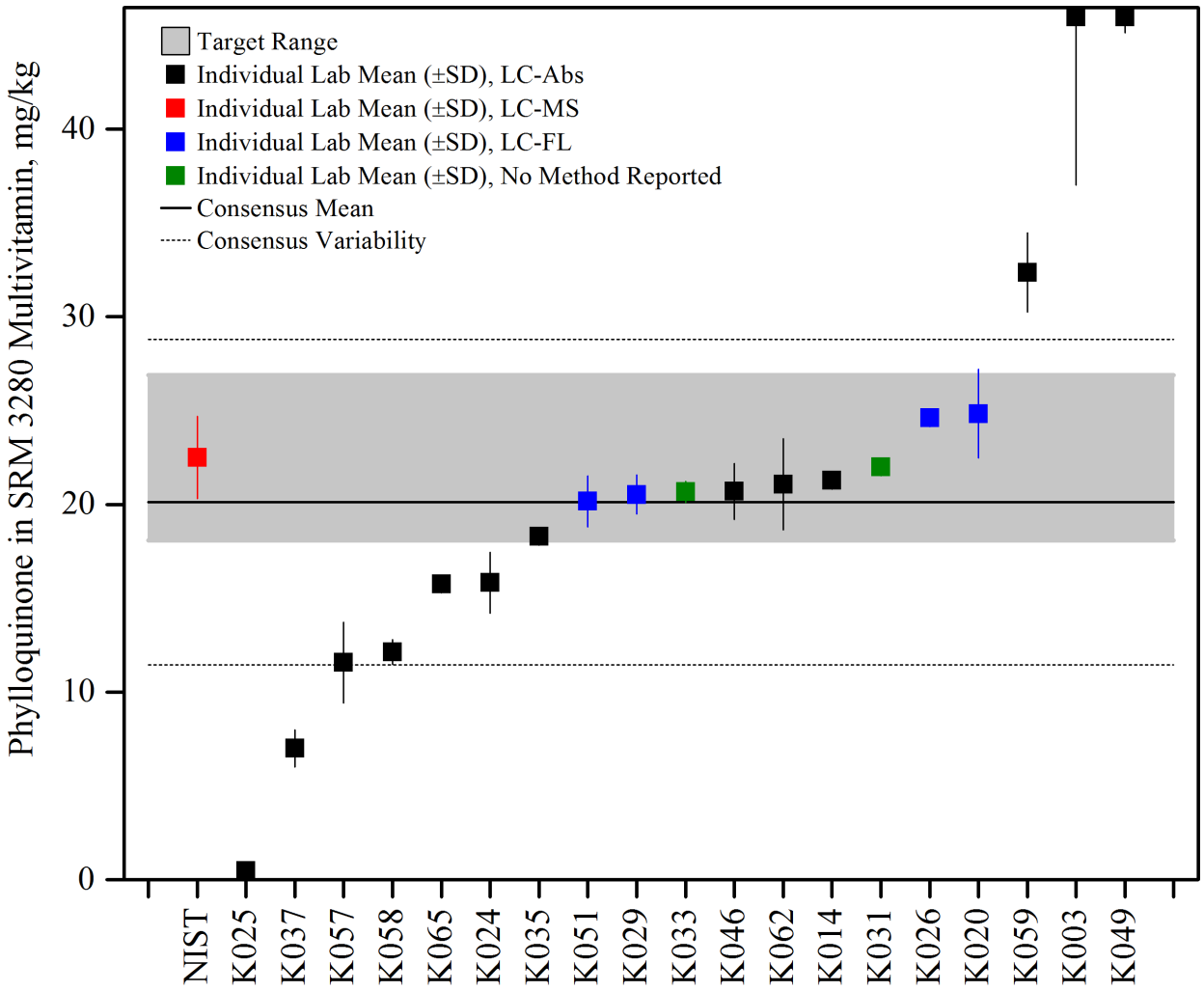

Figure 38. Phylloquinone in SRM 3280 Multivitamin/Multielement Tablets (data summary view – instrumental method). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The color of the data point represents the instrumental method employed. The black solid line represents the consensus mean, and the black dotted lines represent the consensus variability calculated as one standard deviation about the consensus mean. The gray shaded region represents the target zone for "acceptable" performance, which encompasses the NIST certified value bounded by twice its uncertainty (U_{95}).

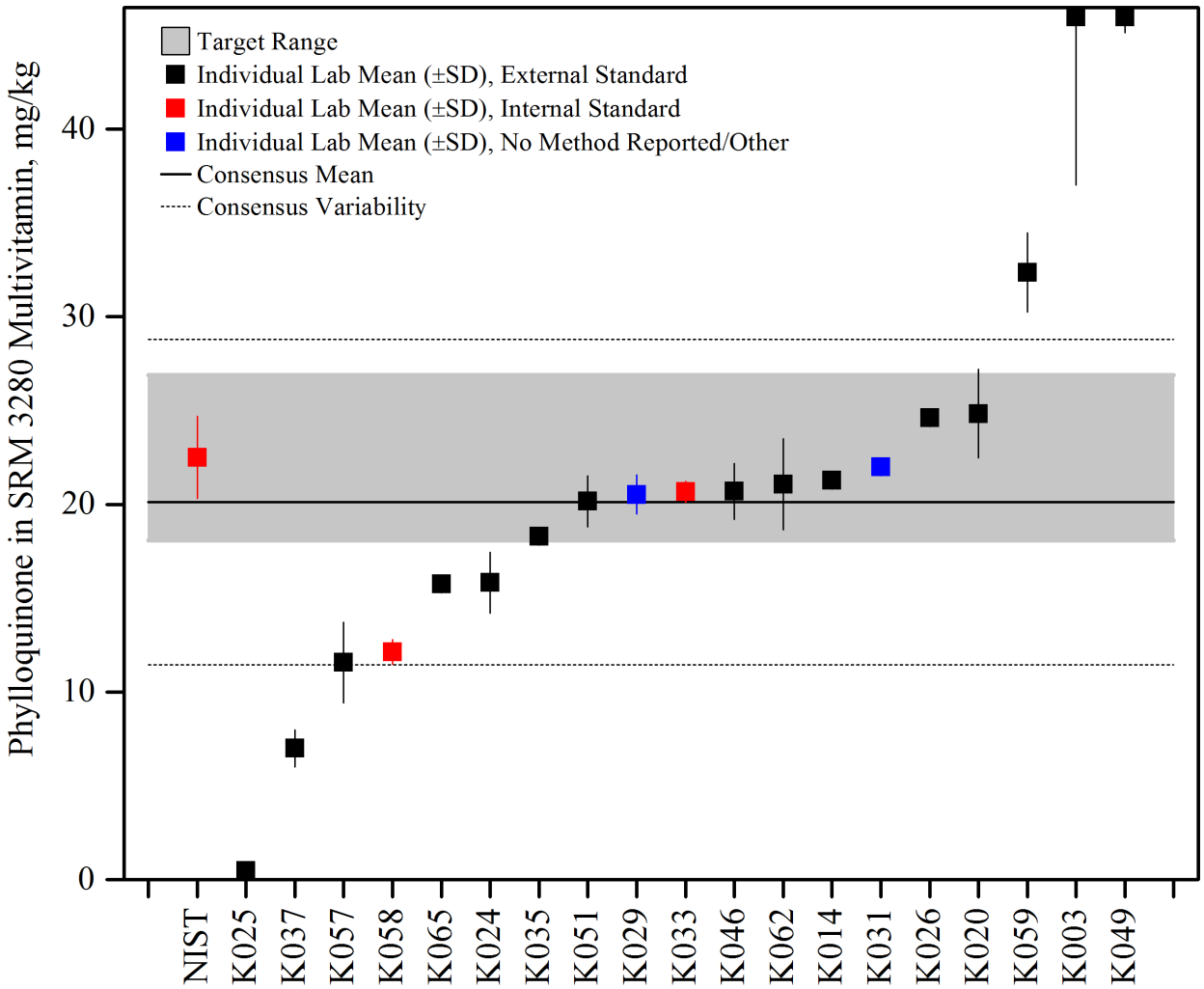

Figure 39. Phylloquinone in SRM 3280 Multivitamin/Multielement Tablets (data summary view – calibration method). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The color of the data point represents the calibration approach employed. The black solid line represents the consensus mean, and the black dotted lines represent the consensus variability calculated as one standard deviation about the consensus mean. The gray shaded region represents the target zone for "acceptable" performance, which encompasses the NIST certified value bounded by twice its uncertainty (U_{95}).

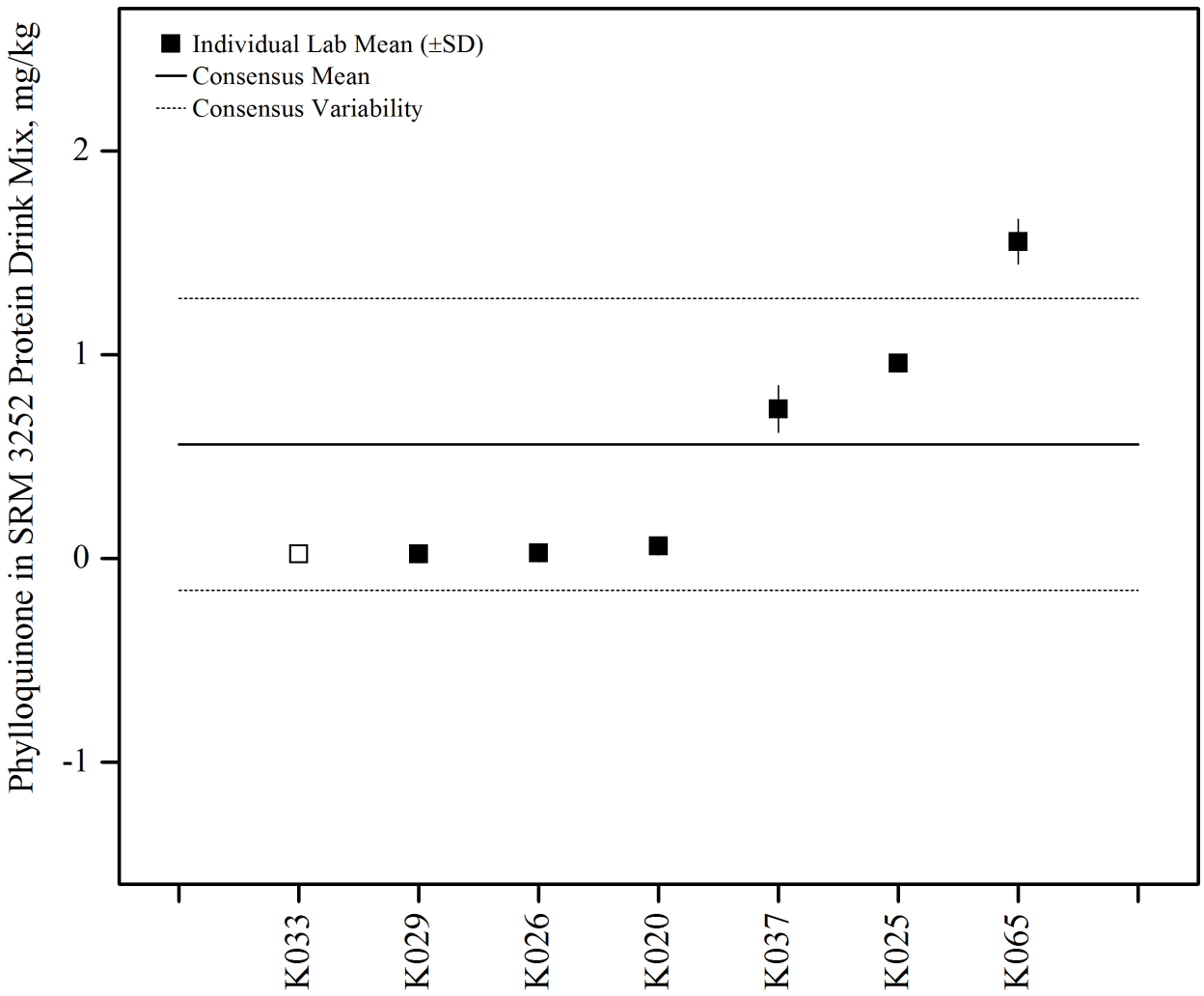

Figure 40. Phylloquinone in SRM 3252 Protein Drink Mix (data summary view). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The black solid line represents the consensus mean, and the black dotted lines represent the consensus variability calculated as one standard deviation about the consensus mean.

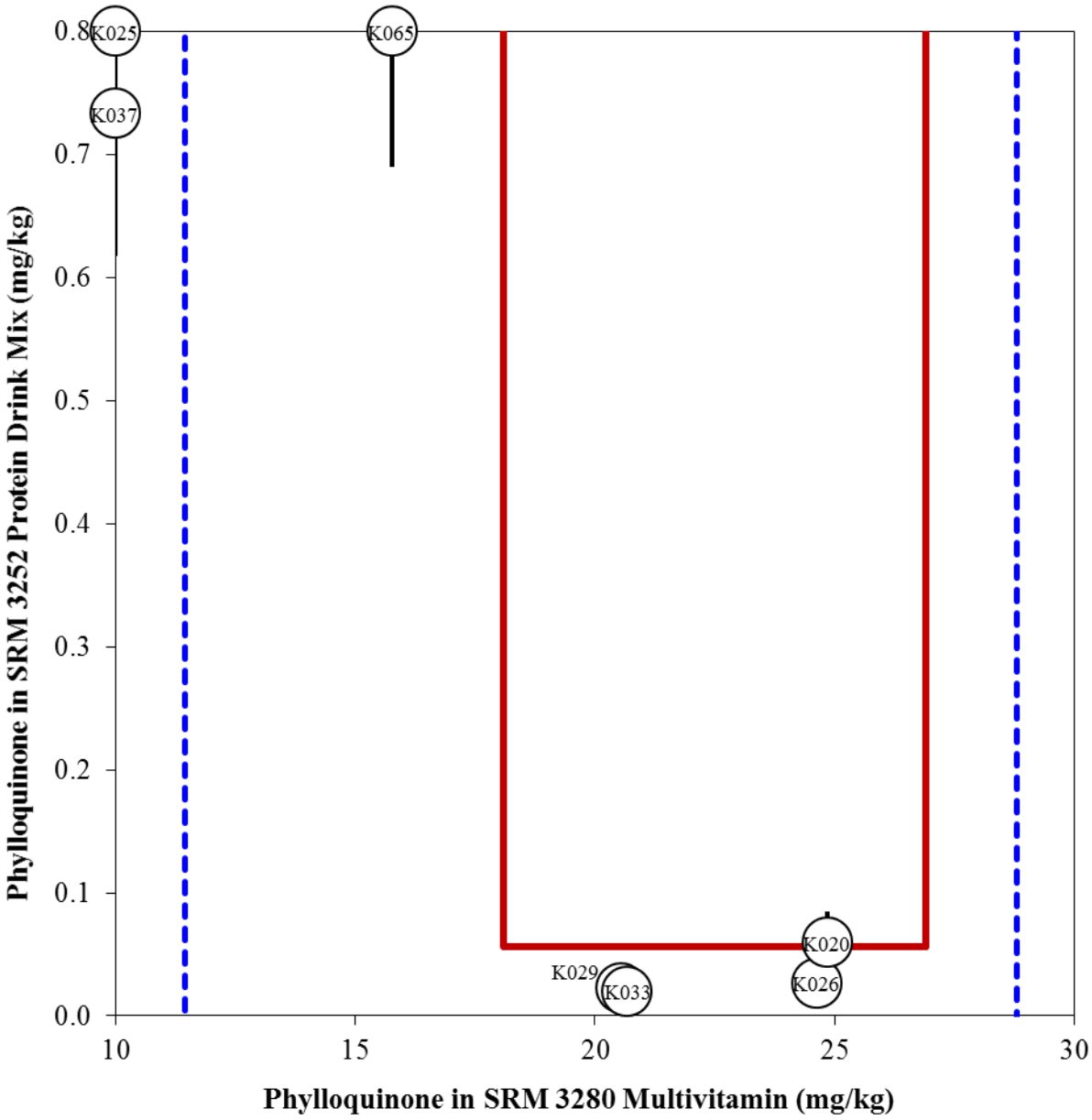

Figure 41. Phylloquinone in SRM 3280 Multivitamin/Multielement Tablets and SRM 3252 Protein Drink Mix (sample/sample comparison view). In this view, the individual laboratory results for one sample (SRM 3280) are compared to the results for a second sample (SRM 3252). The solid red box represents the target zone for the control (x-axis). The dotted blue lines represents the bounds of the consensus zone for the control (x-axis).

ACRYLAMIDE IN CHOCOLATE AND COFFEE

Study Overview

In this study, participants were provided with one NIST SRM, SRM 2384 Baking Chocolate, and a sample of whole roasted coffee beans. Participants were asked to use in-house analytical methods to determine the mass fraction of acrylamide in each of the matrices and report values on an as-received basis.

Sample Information

Baking Chocolate. Participants were provided with one bar containing 91 g of baking chocolate prepared from 100 % cocoa beans from a single production lot. Participants were instructed to use a sample size of at least 10 g. Participants were asked to store the material under controlled room temperature, 10 °C to 30 °C, and prepare three samples and report three values from the single bar provided. Approximate analyte levels were not reported to participants prior to the study. The NIST reference value and uncertainty for acrylamide, determined by collaborating laboratories, are provided in the table below.

Analyte	Reference Mass Fraction in SRM 2384 (ng/g)
Acrylamide	138 ± 17

Coffee. Participants were provided with one packet containing 100 g of roasted coffee beans. Before use, participants were instructed to grind and thoroughly mix the contents of the packet, and a sample size of at least 5 g was recommended. Participants were asked to store the material under controlled room temperature, 10 °C to 30 °C, and prepare three samples and report three values from the single packet provided. Approximate analyte levels were not reported to participants prior to the study. The NIST target value for acrylamide determined by collaborating laboratories, and uncertainty (estimated as twice the standard deviation from 5 laboratories), are provided in the table below.

Analyte	Estimated Mass Fraction in Roasted Coffee Beans (ng/g)
Acrylamide	141 ± 9

Study Results

- Nine laboratories enrolled in this exercise and received samples. Four laboratories reported results for acrylamide in the chocolate (44 % participation), and three laboratories reported results for acrylamide in the coffee (33 % participation).
- Limited conclusions can be drawn about the quality of the collaborative data from this study because very few laboratories returned results.
  - The consensus mean for acrylamide was within the target range for the chocolate sample, but with very high variability (111 % RSD).
  - The consensus mean for acrylamide was below the target range for the coffee sample, with high variability (46 % RSD).

- Two of the four laboratories reported using derivatization following sample extraction (50 %). One laboratory reported using only solvent extraction (25 %), and one laboratory reported using QuEChERS for sample cleanup (25 %).
- Two laboratories reported using gas chromatography with mass spectrometric detection (GC-MS) as their analytical method for analysis (50 %), and two laboratories reported using LC-MS or LC-MS/MS (50 %).
- Two laboratories reported using an external standard approach to quantitation (50 %), and two laboratories reported using an internal standard approach (50 %).

Technical Recommendations

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

- Because the data for this study was very limited (only 3 or 4 laboratories reporting data), drawing extensive technical conclusions is difficult. Similarly, the high level of between-laboratory variability may be exaggerated as a result of the low number of participants.
- No trends were identified indicating that any particular sample preparation method or instrumental technique provided more accurate results than another.

Table 12. Individualized data summary table (NIST) for acrylamide in chocolate and coffee.

National Institute of Standards & Technology

Exercise K - February 2014 - Acrylamide

	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}
Acrylamide	Chocolate	ng/g	138	17	0.0	0.0	4	142	157	138	17
Acrylamide	Coffee	ng/g	141	9	1.0	0.0	3	96	45	141	9

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 13. Data summary table for acrylamide in chocolate and coffee.

		Acrylamide									
		SRM 2384 Baking Chocolate (ng/g)					Coffee (ng/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	NIST				138	17				141	9
	K002										
	K003	34	38	39	37	3					
	K010										
	K024	140	121	123	128	10	54	50	56	53	3
	K040										
	K048	339	343	341	341	2	107	104	103	105	2
	K055										
	K061										
	K062	60	63	59	61	2	131	132	129	131	2
Community Results		Consensus Mean			142		Consensus Mean			96	
		Consensus Standard Deviation			157		Consensus Standard Deviation			45	
		Maximum			341		Maximum			131	
		Minimum			37		Minimum			53	
		N			4		N			3	

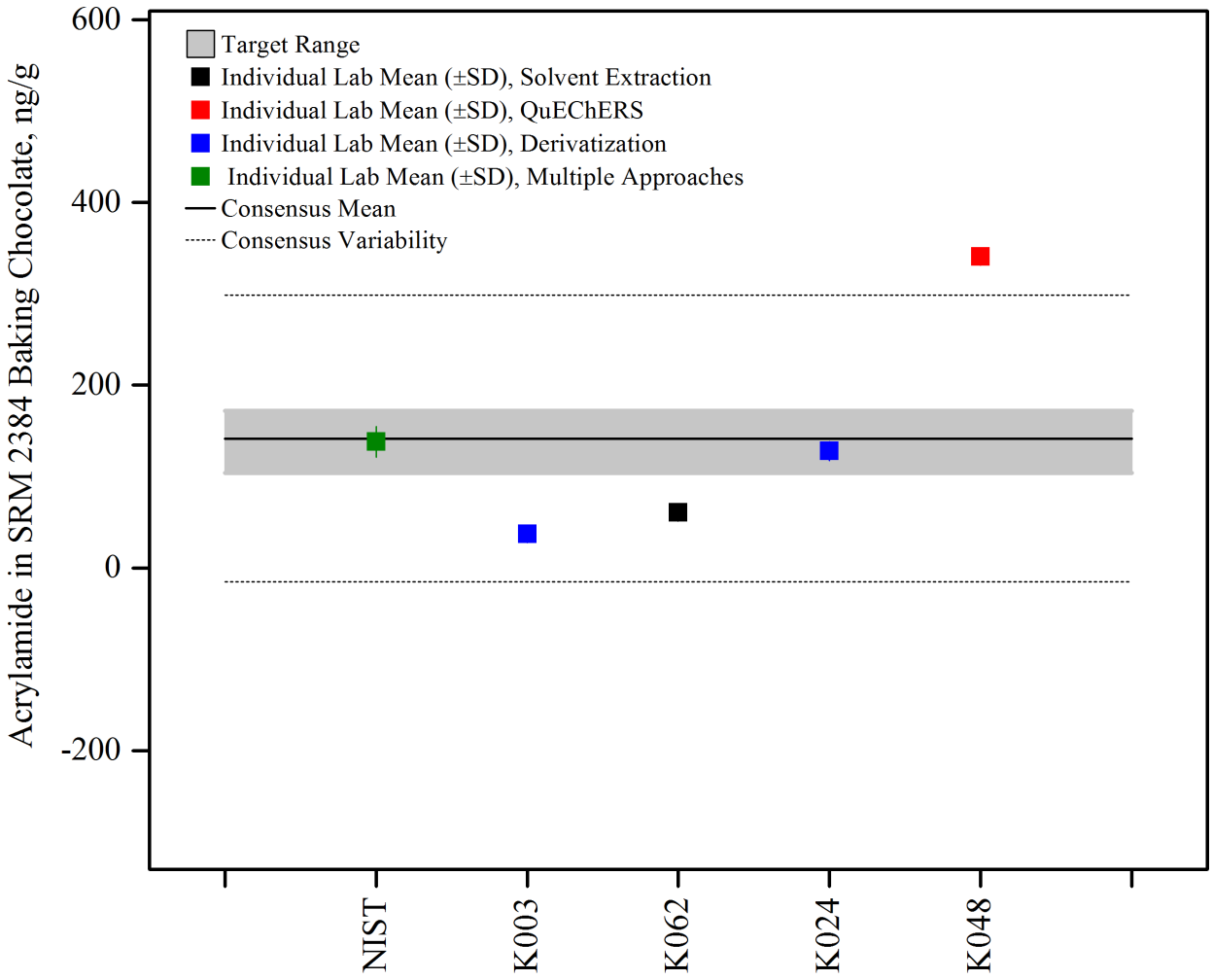

Figure 42. Acrylamide in SRM 2384 Baking Chocolate (data summary view – sample preparation method). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The color of the data point represents the sample preparation method employed. The black solid line represents the consensus mean, and the black dotted lines represent the consensus variability calculated as one standard deviation about the consensus mean. The gray shaded region represents the target zone for "acceptable" performance, which encompasses the NIST reference value bounded by twice its uncertainty (U_{95}).

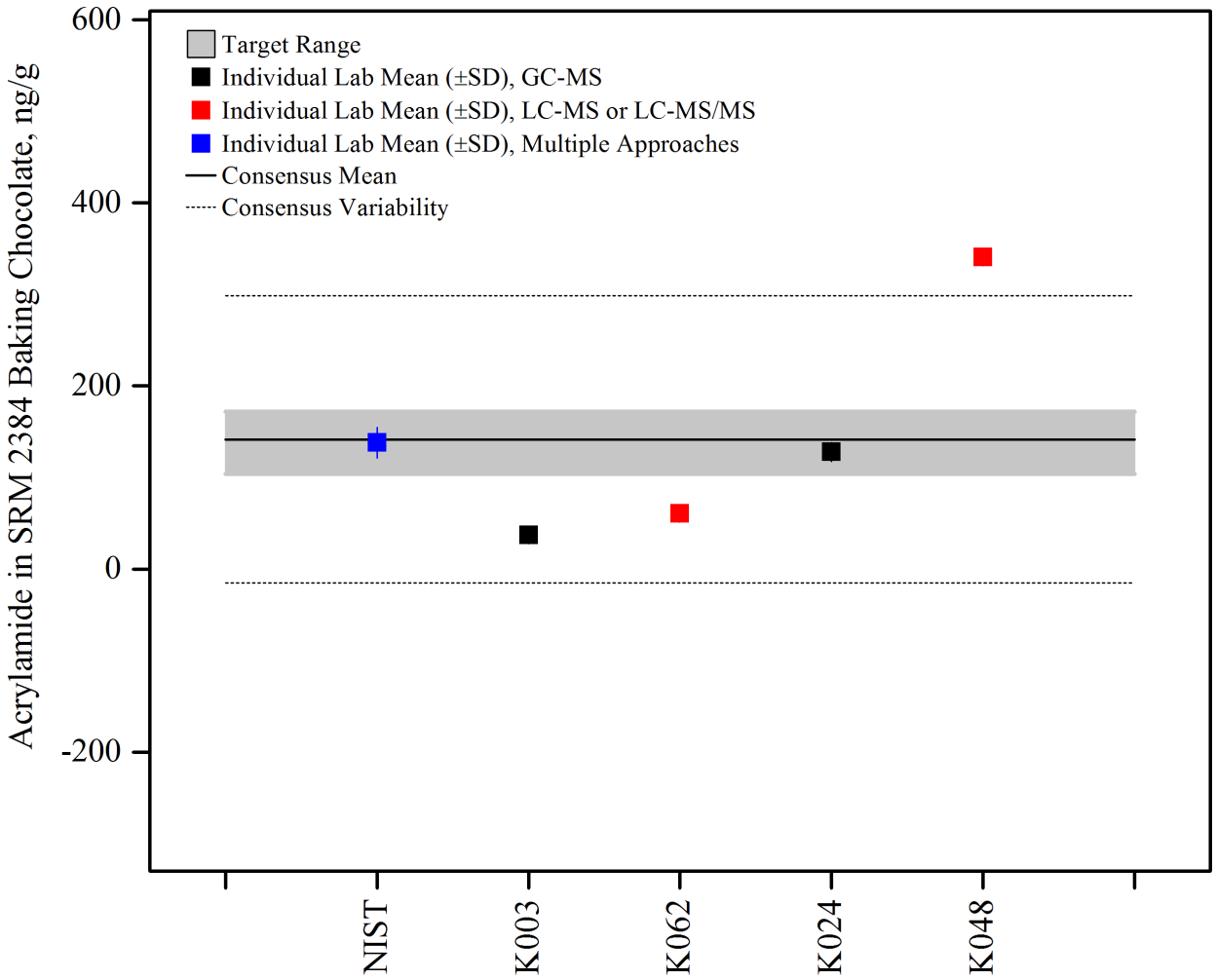

Figure 43. Acrylamide in SRM 2384 Baking Chocolate (data summary view – instrumental method). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The color of the data point represents the instrumental method employed. The black solid line represents the consensus mean, and the black dotted lines represent the consensus variability calculated as one standard deviation about the consensus mean. The gray shaded region represents the target zone for "acceptable" performance, which encompasses the NIST reference value bounded by twice its uncertainty (U_{95}).

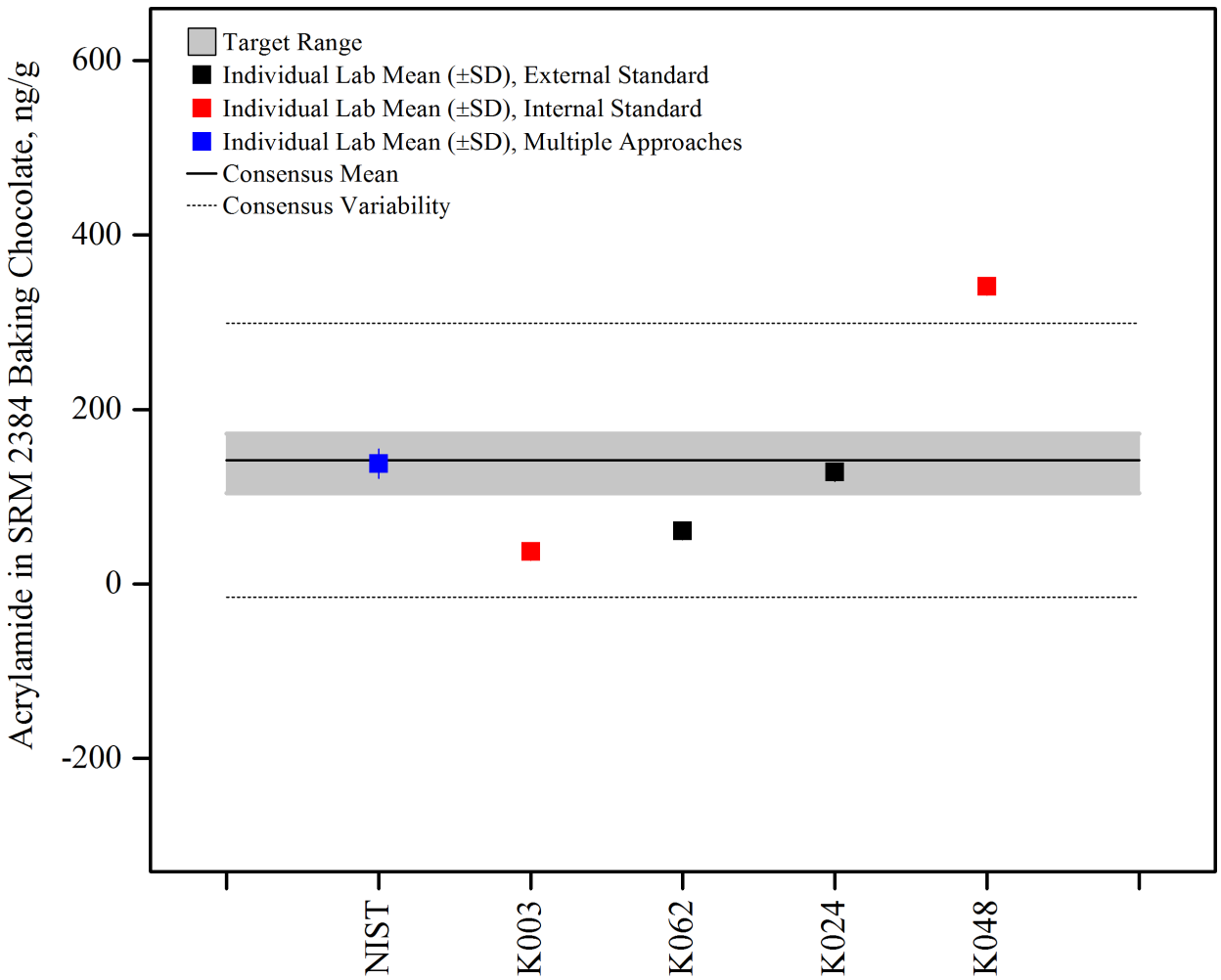

Figure 44. Acrylamide in SRM 2384 Baking Chocolate (data summary view – calibration method). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The color of the data point represents the calibration approach employed. The black solid line represents the consensus mean, and the black dotted lines represent the consensus variability calculated as one standard deviation about the consensus mean. The gray shaded region represents the target zone for "acceptable" performance, which encompasses the NIST reference value bounded by twice its uncertainty (U_{95}).

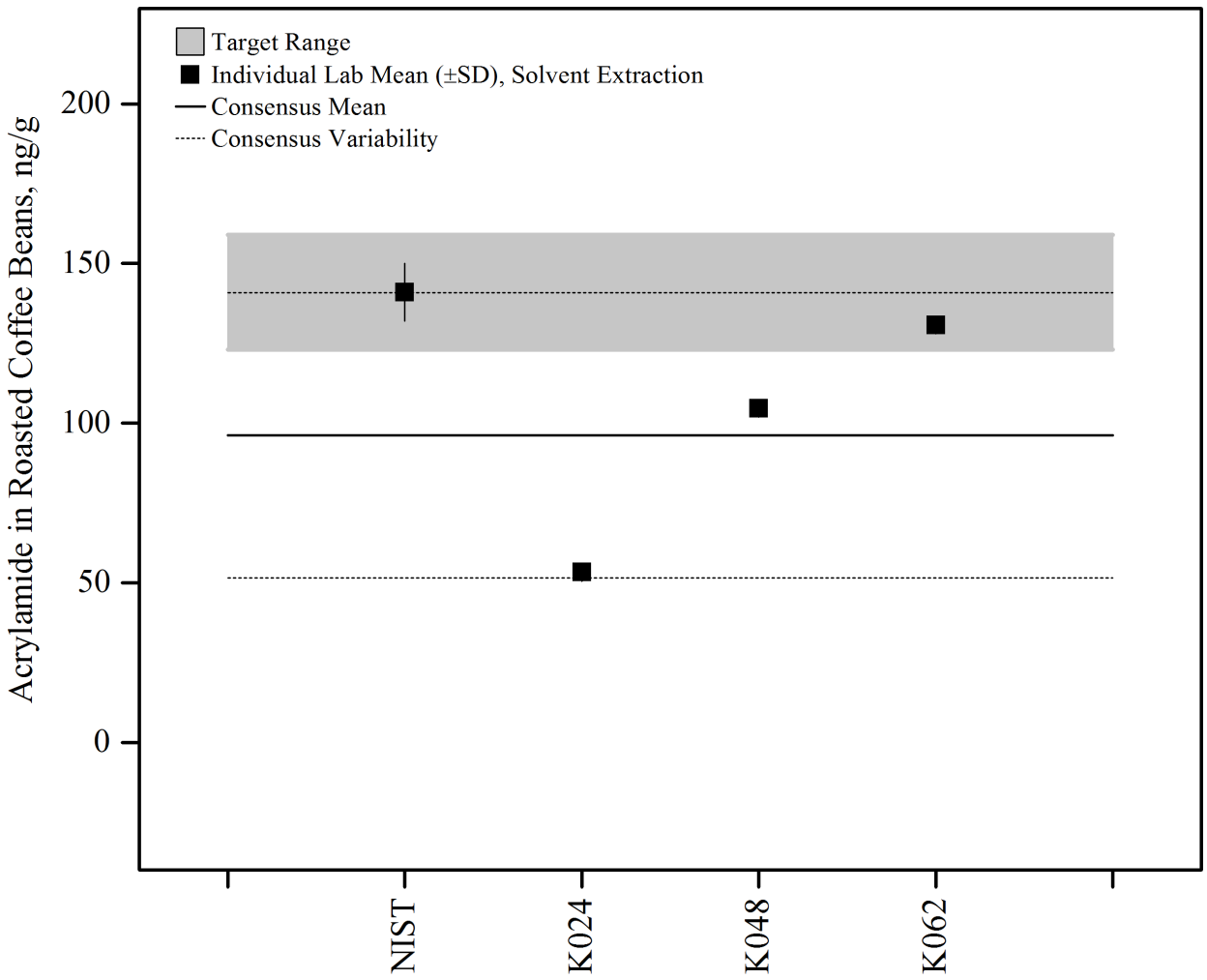

Figure 45. Acrylamide in roasted coffee beans (data summary view). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The black solid line represents the consensus mean, and the black dotted lines represent the consensus variability calculated as one standard deviation about the consensus mean. The gray shaded region represents the target zone for "acceptable" performance, which encompasses the NIST estimated value (based on data from 5 collaborating laboratories) bounded by twice its uncertainty (twice the standard deviation of the data from collaborating laboratories).

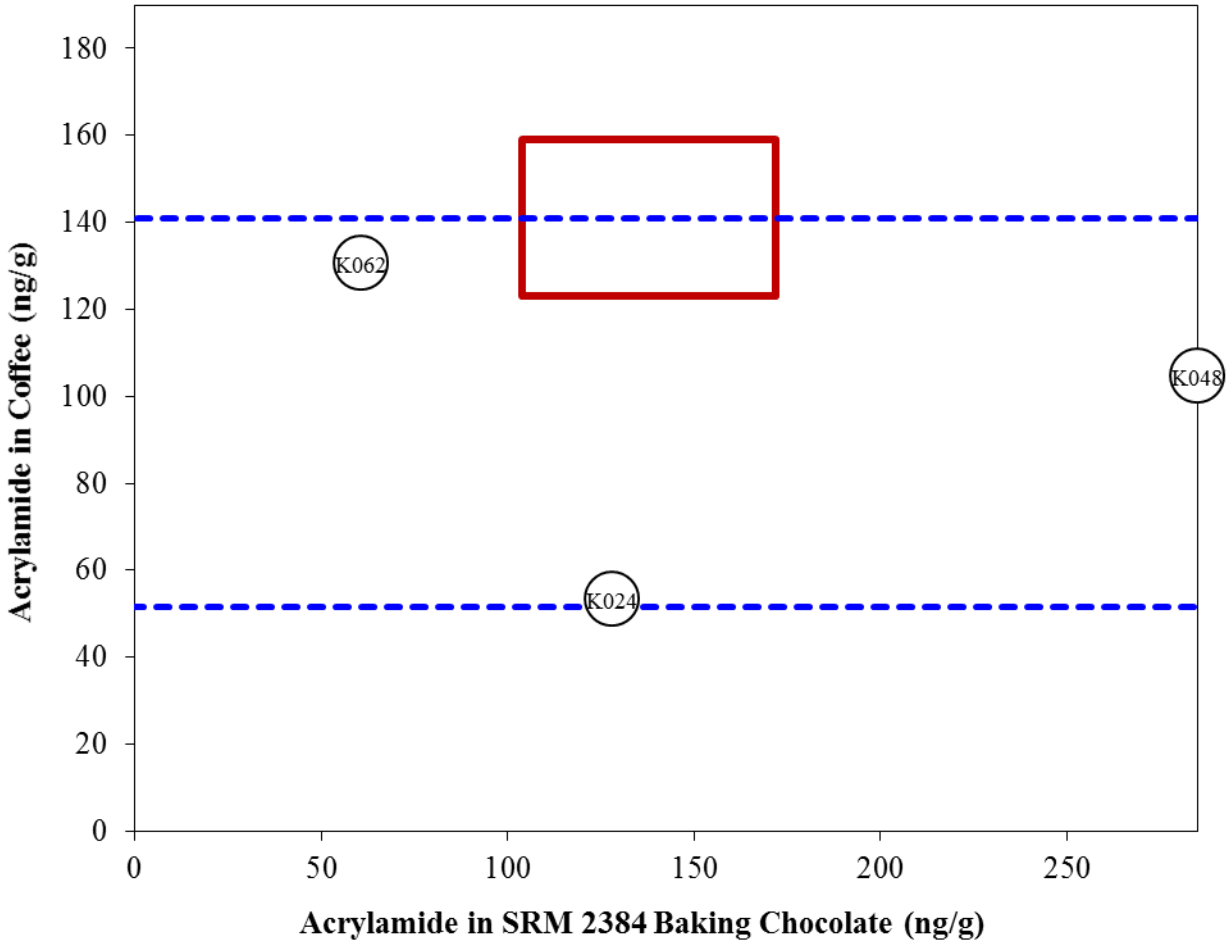

Figure 46. Acrylamide in SRM 2384 Baking Chocolate and roasted coffee beans (sample/sample comparison view). In this view, the individual laboratory results for one sample (SRM 2384) are compared to the results for a second sample (roasted coffee beans). The solid red box represents the target zone for the control (x-axis). The dotted blue box represents the consensus zone for the control (x-axis) and the unknown sample (y-axis).

PHYTOSTEROLS IN SAW PALMETTO

Study Overview

In this study, participants were provided with two NIST SRMs, SRM 3250 *Serenoa repens* Fruit and SRM 3251 *Serenoa repens* Extract. Participants were asked to use in-house analytical methods to determine the mass fractions of phytosterols (campesterol, β-sitosterol, and stigmasterol) in each of the matrices and report values on an as-received basis.

Sample Information

Saw Palmetto Fruit. Participants were provided with one packet containing 6 g of *Serenoa repens* (saw palmetto) berries that had been freeze-dried, ground, and heat-sealed inside nitrogen-flushed 4-mil polyethylene bags, which were then sealed inside aluminized plastic bags with 2 packets of silica gel. Before use, participants were instructed to mix each packet thoroughly and a sample size of at least 0.5 g was recommended. Participants were asked to store the material at room temperature, 10 °C to 30 °C, and to prepare three samples, and report three measurements of each analyte from the single packet provided. Approximate analyte levels were not reported to participants prior to the study. The NIST certified values and uncertainties for phytosterols in SRM 3250 were determined using LC-MS following solvent extraction and basic hydrolysis, and by gas chromatography with flame ionization detection (GC-FID) following solvent extraction, basic hydrolysis, and derivatization. The values and uncertainties are reported in the table below both on a dry-mass basis and after correction for moisture of the material (6.42 %).

Analyte	Certified Mass Fraction in SRM 3250 (mg/g) (dry-mass basis)	Certified Mass Fraction in SRM 3250 (mg/g) (as-received basis)
Campesterol	0.1175 ± 0.0025	0.1100 ± 0.0023
β-sitosterol	0.454 ± 0.018	0.425 ± 0.017
Stigmasterol	0.0477 ± 0.0020	0.0446 ± 0.0019

Saw Palmetto Extract. Participants were provided with three ampoules, each containing 1 mL of a carbon dioxide extract of *Serenoa repens* (saw palmetto) berries. Before use, participants were instructed to mix each ampoule thoroughly and a sample size of at least 125 mg was recommended. Participants were asked to store the material at room temperature, 10 °C to 30 °C, and to prepare one sample and report one measurement of each analyte from each ampoule provided. Approximate analyte levels were not reported to participants prior to the study. The NIST certified values and uncertainties for phytosterols in SRM 3251 were determined using LC-MS following solvent extraction and basic hydrolysis, and by GC-FID following solvent extraction, basic hydrolysis, and derivatization; the values and uncertainties are reported in the table below.

Analyte	Certified Mass Fraction in SRM 3251 (mg/g) (as-received basis)
Campesterol	0.533 ± 0.031
β-sitosterol	1.666 ± 0.064
Stigmasterol	0.247 ± 0.040

Study Results

- Twenty-six laboratories enrolled in this exercise and received samples. Ten laboratories reported data for phytosterols in the saw palmetto fruit (39 % participation), and eleven laboratories reported data for phytosterols in the saw palmetto extract (42 % participation).
- The consensus means were within the target ranges with acceptable between-laboratory variability for all three phytosterols in the saw palmetto extract (less than 15 % RSD for all analytes).
- The consensus mean for stigmasterol in the saw palmetto fruit was equivalent to the minimum of the target range, but the between-laboratory variability was high (30 % RSD).
- The consensus means were below the target ranges for campesterol and β–sitosterol in the saw palmetto fruit with acceptable between-laboratory variability (18 % and 12 % RSD, respectively).
- Laboratories reported using either solvent extraction with hydrolysis (37 %) or solvent extraction with hydrolysis and derivatization (55 %) as the sample preparation method. One laboratory did not report a sample preparation technique.
- A majority of the laboratories reported using GC-FID (82 %) for phytosterols determination. One laboratory reported using GC-MS (9 %) as their instrumental method, and one laboratory did not report the type of analytical method used.
- Laboratories reported using both external standard and internal standard approaches to quantitation of phytosterols (36 % and 55 %, respectively). One laboratory did not report the calibration approach used.

Technical Recommendations

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

- The low results for campesterol and β–sitosterol in the saw palmetto fruit are consistent with the results from Exercise H, conducted in 2012 (**Figure 68** and **Figure 69**). More details on Exercise H can be found in the final report, available at http://dx.doi.org/10.6028/NIST.IR.7903.
    - As shown in **Figure 68** and **Figure 69**, the consensus means from Exercise H and Exercise K are nearly identical. Between-laboratory precision has improved for both analytes. For both analytes in both exercises, the consensus means are below the target ranges.
    - The low results could be caused by incomplete extraction of these phytosterols from the saw palmetto berry matrix. All commercial saw palmetto products are sold as extracts, so laboratory protocols may not be developed to fully extract phytosterols from the plant material. In addition, many methods for phytosterols that involve the use of an internal standard suggest its addition after the extraction steps are complete. Addition of the internal standard this late will not compensate for extraction

inefficiencies. If an internal standard approach is used, it is best to add the internal standard at the earliest possible point (i.e., prior to extraction, saponification, and/or derivatization).

- The low results could also indicate instability of these analytes in this matrix. NIST will investigate this possible instability further.

- A slight calibration error is apparent in the sample/sample comparison graphs. Calibrant materials should be subjected to the same preparation procedure as the samples (derivatization, hydrolysis, etc.) to avoid calibration bias.

Table 14. Individualized data summary table (NIST) for phytosterols in saw palmetto dietary supplements.

National Institute of Standards & Technology

Exercise K - February 2014 - Phytosterols

	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}
Campesterol	SP Fruit	mg/g	0.110	0.002	1.8	0.0	10	0.083	0.015	0.110	0.002
Campesterol	SP Extract	mg/g	0.533	0.031	-0.4	0.0	11	0.568	0.078	0.533	0.031
β-sitosterol	SP Fruit	mg/g	0.425	0.017	4.0	0.0	10	0.285	0.035	0.425	0.017
β-sitosterol	SP Extract	mg/g	1.67	0.06	0.4	0.0	11	1.60	0.16	1.67	0.06
Stigmasterol	SP Fruit	mg/g	0.0446	0.0019	0.3	0.0	10	0.0410	0.0123	0.0446	0.0019
Stigmasterol	SP Extract	mg/g	0.247	0.040	-0.2	0.0	11	0.254	0.038	0.247	0.040

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 15. Data summary table for campesterol in saw palmetto dietary supplements.

		Campesterol									
		SRM 3250 Saw Palmetto Fruit (mg/g)					SRM 3251 Saw Palmetto Extract (mg/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	NIST				0.1100	0.0020				0.533	0.031
	K002										
	K006										
	K008										
	K009	0.0904	0.0841	0.0842	0.0862	0.0036	0.576	0.574	0.538	0.563	0.021
	K010										
	K012	0.0850	0.0822	0.0816	0.0829	0.0018	0.571	0.568	0.547	0.562	0.013
	K014										
	K015										
	K017										
	K019										
	K021										
	K024	0.0520	0.0634	0.0820	0.0658	0.0151	0.362	0.339	0.324	0.342	0.019
	K031	0.0850	0.0900	0.0900	0.0883	0.0029	0.612	0.610	0.600	0.607	0.006
	K040										
	K043	0.0810	0.0840	0.0800	0.0817	0.0021	0.650	0.645	0.639	0.645	0.006
	K044	0.1100	0.1100	0.1100	0.1100	0.0000	0.720	0.730	0.700	0.717	0.015
	K045										
	K053	0.0452	0.0316	0.0420	0.0396	0.0071	0.685	0.700	0.512	0.632	0.104
	K055										
	K057	0.0910	0.1087	0.0909	0.0969	0.0102	0.538	0.523	0.517	0.526	0.011
	K059						0.504	0.499	0.511	0.505	0.006
	K062										
	K064	0.0771	0.0777		0.0774	0.0004	0.525	0.524	0.522	0.524	0.001
	K066	0.0810	0.0813	0.0812	0.0812	0.0002	0.537	0.541	0.557	0.545	0.011
	K067										
	K070										
Community Results		Consensus Mean			0.0826		Consensus Mean			0.568	
		Consensus Standard Deviation			0.0145		Consensus Standard Deviation			0.078	
		Maximum			0.1100		Maximum			0.717	
		Minimum			0.0396		Minimum			0.342	
		N			10		N			11	

Table 16. Data summary table for β–sitosterol in saw palmetto dietary supplements.

		β-sitosterol									
		SRM 3250 Saw Palmetto Fruit (mg/g)					SRM 3251 Saw Palmetto Extract (mg/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	NIST				0.425	0.017				1.67	0.06
	K002										
	K006										
	K008										
	K009	0.334	0.320	0.310	0.321	0.012	1.74	1.72	1.61	1.69	0.07
	K010										
	K012	0.291	0.287	0.294	0.290	0.003	1.69	1.68	1.64	1.67	0.03
	K014										
	K015										
	K017										
	K019										
	K021										
	K024	0.272	0.323	0.302	0.299	0.026	1.53	1.42	1.38	1.44	0.08
	K031	0.296	0.318	0.315	0.310	0.012	1.84	1.81	1.73	1.79	0.06
	K040										
	K043	0.225	0.226	0.223	0.225	0.002	1.29	1.28	1.23	1.27	0.03
	K044	0.300	0.300	0.310	0.303	0.006	1.81	1.82	1.70	1.78	0.07
	K045										
	K053	0.203	0.147	0.188	0.179	0.029	1.64	1.66	1.19	1.50	0.26
	K055										
	K057	0.295	0.328	0.295	0.306	0.019	1.52	1.52	1.48	1.50	0.02
	K059						1.62	1.62	1.64	1.63	0.01
	K062										
	K064	0.277	0.277		0.277	0.000	1.70	1.71	1.71	1.71	0.01
	K066	0.271	0.282	0.275	0.276	0.006	1.55	1.55	1.57	1.56	0.01
	K067										
	K070										
Community Results		Consensus Mean			0.285		Consensus Mean			1.60	
		Consensus Standard Deviation			0.035		Consensus Standard Deviation			0.16	
		Maximum			0.321		Maximum			1.79	
		Minimum			0.179		Minimum			1.27	
		N			10		N			11	

Table 17. Data summary table for stigmasterol in saw palmetto dietary supplements.

		Stigmasterol									
		SRM 3250 Saw Palmetto Fruit (mg/g)					SRM 3251 Saw Palmetto Extract (mg/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	NIST				0.0446	0.0019				0.247	0.040
	K002										
	K006										
	K008										
	K009	0.0669	0.0591	0.0594	0.0618	0.0044	0.390	0.388	0.402	0.393	0.008
	K010										
	K012	0.0342	0.0339	0.0351	0.0344	0.0006	0.253	0.261	0.243	0.252	0.009
	K014										
	K015										
	K017										
	K019										
	K021										
	K024	0.0345	0.0426	0.0383	0.0385	0.0041	0.257	0.262	0.217	0.245	0.025
	K031	0.0470	0.0520	0.0510	0.0500	0.0026	0.298	0.296	0.288	0.294	0.005
	K040										
	K043	0.0291	0.0266	0.0268	0.0275	0.0014	0.168	0.160	0.153	0.160	0.008
	K044	0.0400	0.0400	0.0400	0.0400	0.0000	0.290	0.290	0.270	0.283	0.012
	K045										
	K053	0.0396	0.0244	0.0434	0.0358	0.0101	0.253	0.270	0.197	0.240	0.038
	K055										
	K057	0.0546	0.0669	0.0501	0.0572	0.0087	0.287	0.269	0.266	0.274	0.011
	K059						0.245	0.235	0.215	0.232	0.015
	K062										
	K064	0.0319	0.0320		0.0320	0.0001	0.234	0.229	0.229	0.231	0.003
	K066	0.0370	0.0358	0.0341	0.0356	0.0015	0.235	0.224	0.235	0.231	0.006
	K067										
	K070										
Community Results		Consensus Mean			0.0410		Consensus Mean			0.254	
		Consensus Standard Deviation			0.0123		Consensus Standard Deviation			0.037	
		Maximum			0.0618		Maximum			0.393	
		Minimum			0.0275		Minimum			0.160	
		N			10		N			11	

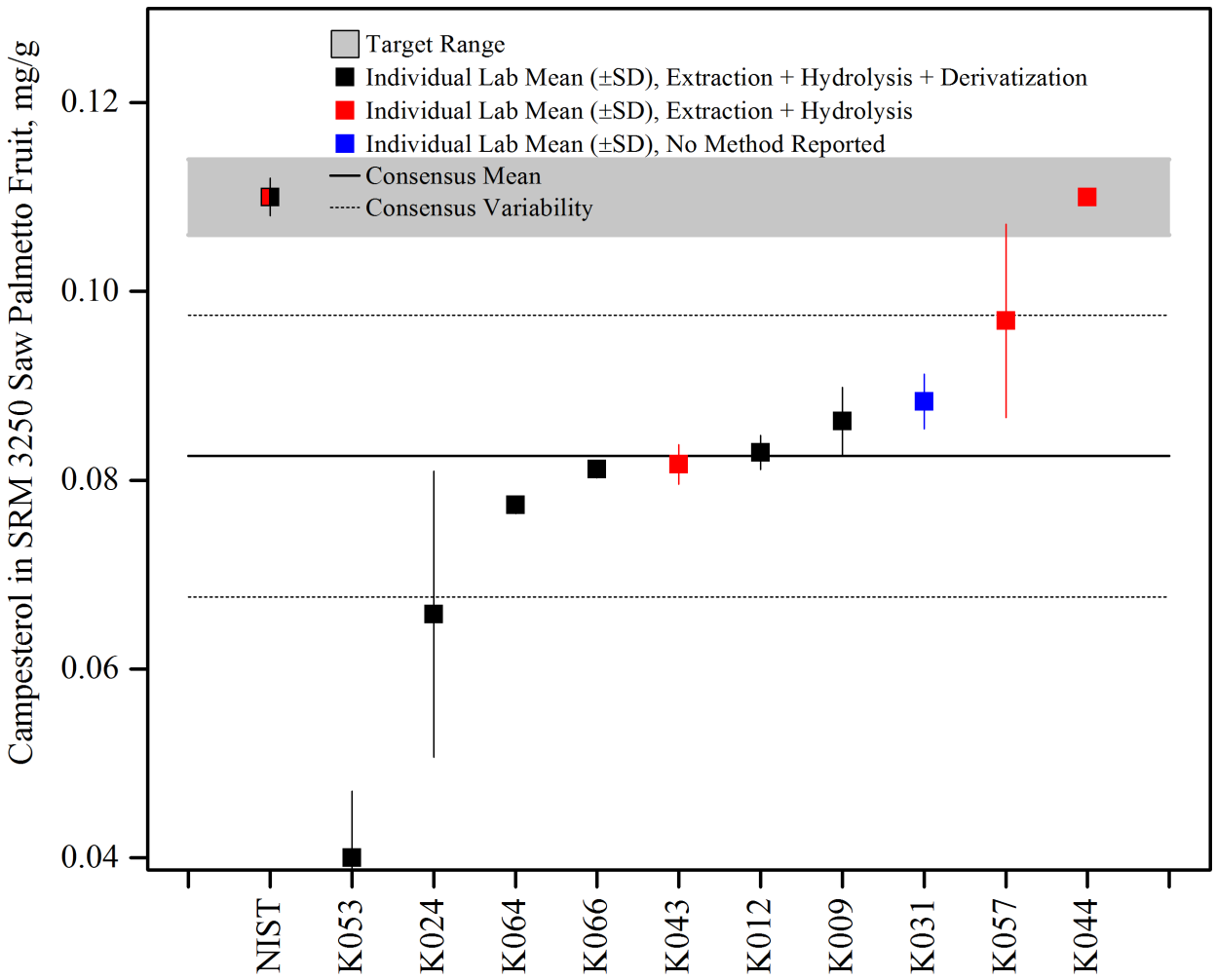

Figure 47. Campesterol in SRM 3250 *Serenoa repens* Fruit (data summary view – sample preparation method). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The color of the data point represents the sample preparation method employed. The black solid lines represent the consensus means for each sample preparation method, and the black dotted lines represent the consensus variability calculated as one standard deviation about the consensus mean for that sample preparation method. The gray shaded region represents the target zone for "acceptable" performance, which encompasses the NIST certified value bounded by twice its uncertainty (U_{95}).

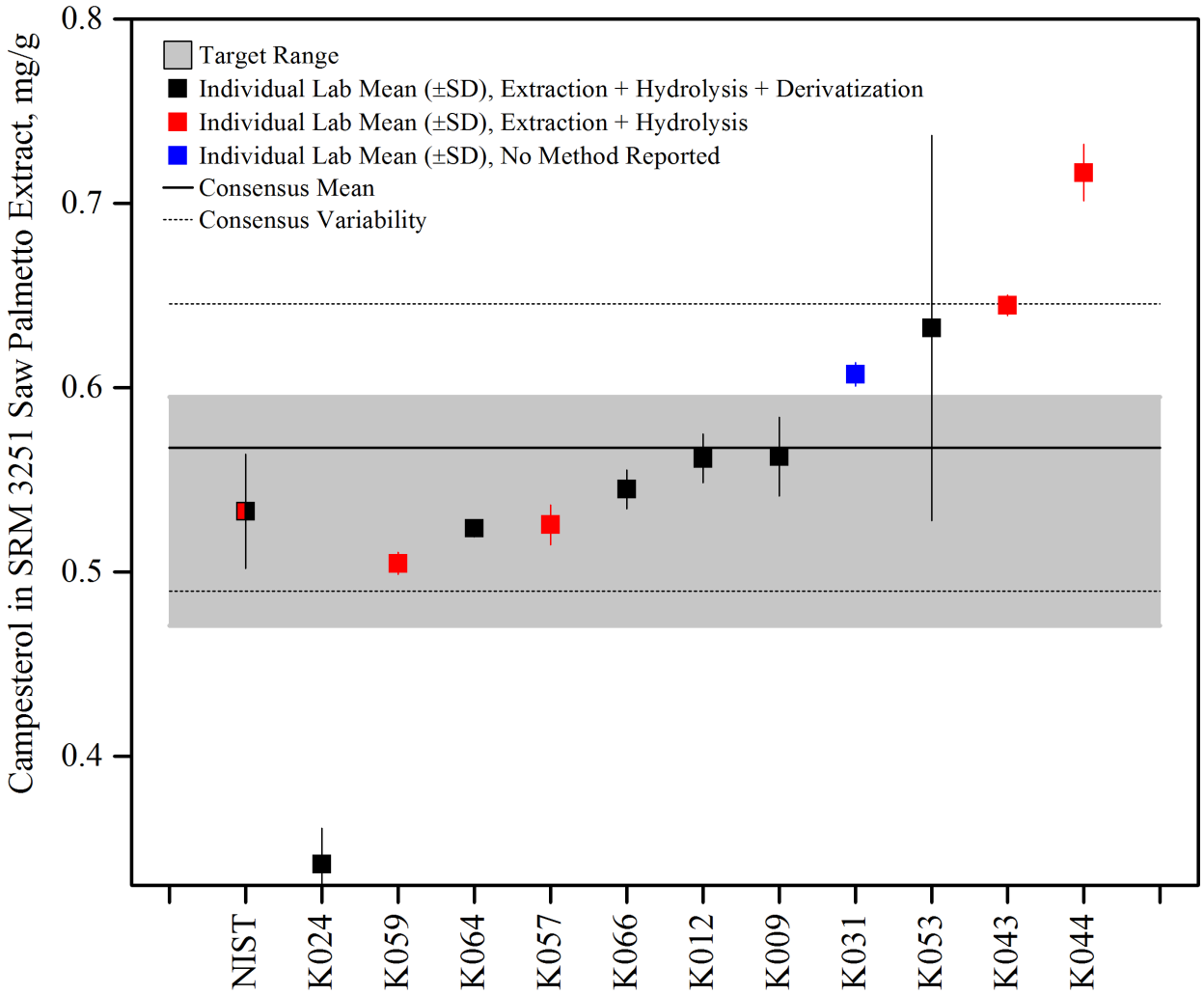

Figure 48. Campesterol in SRM 3251 *Serenoa repens* Extract (data summary view – sample preparation method). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The color of the data point represents the sample preparation method employed. The black solid lines represent the consensus means for each sample preparation method, and the black dotted lines represent the consensus variability calculated as one standard deviation about the consensus mean for that sample preparation method. The gray shaded region represents the target zone for "acceptable" performance, which encompasses the NIST certified value bounded by twice its uncertainty (U_{95}).

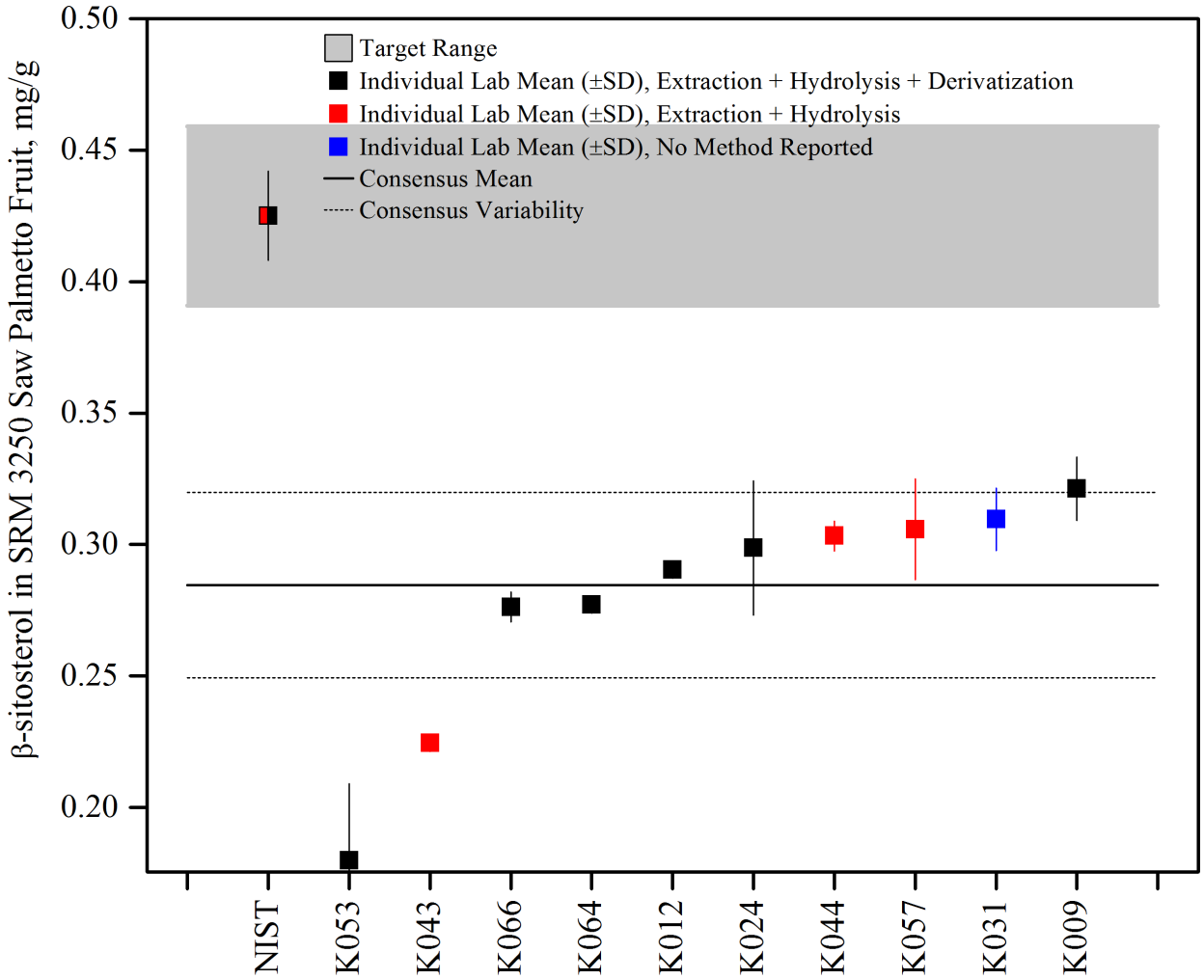

Figure 49. β-sitosterol in SRM 3250 *Serenoa repens* Fruit (data summary view – sample preparation method). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The color of the data point represents the sample preparation method employed. The black solid lines represent the consensus means for each sample preparation method, and the black dotted lines represent the consensus variability calculated as one standard deviation about the consensus mean for that sample preparation method. The gray shaded region represents the target zone for "acceptable" performance, which encompasses the NIST certified value bounded by twice its uncertainty (U_{95}).

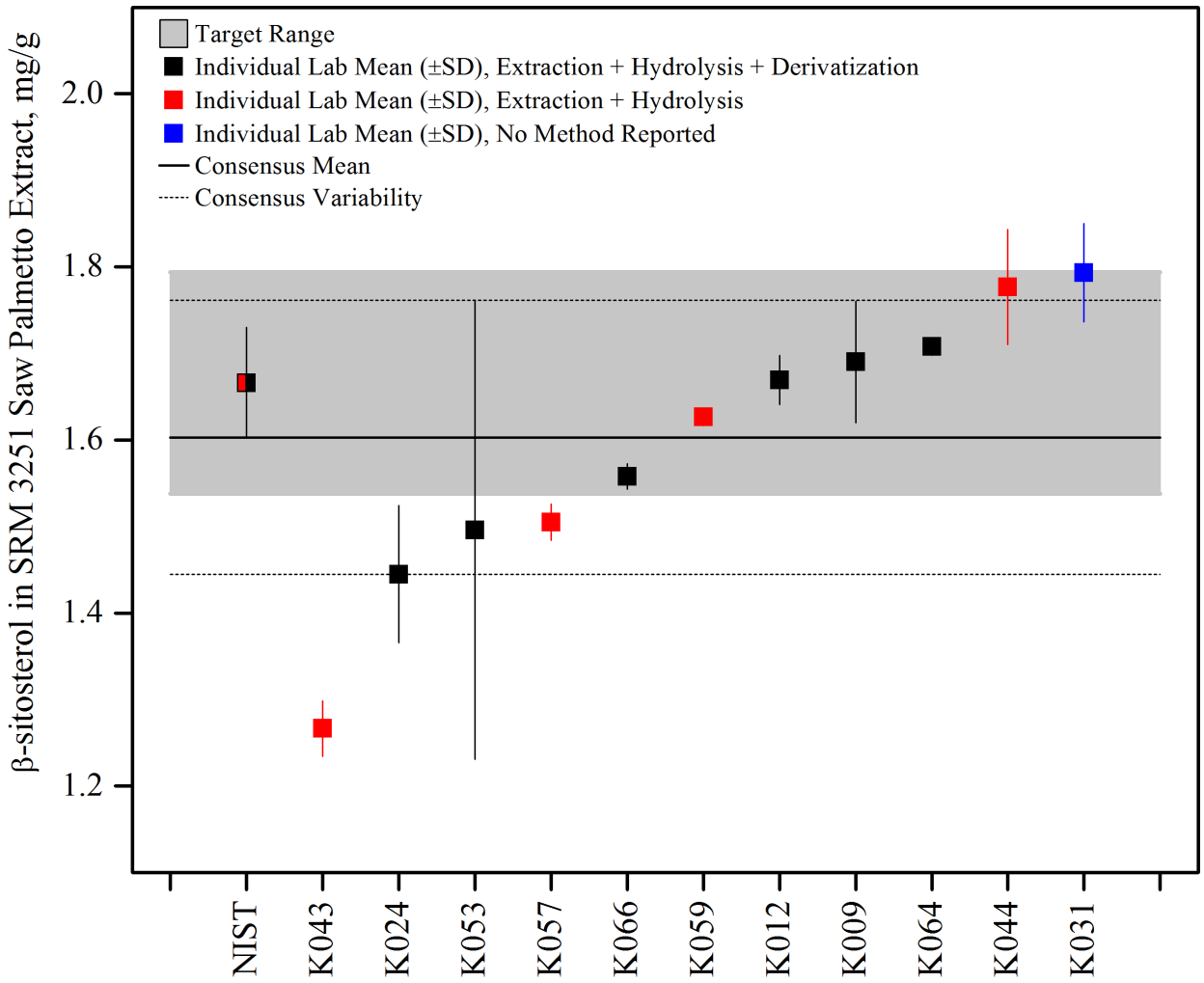

Figure 50. β-sitosterol in SRM 3251 *Serenoa repens* Extract (data summary view – sample preparation method). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The color of the data point represents the sample preparation method employed. The black solid lines represent the consensus means for each sample preparation method, and the black dotted lines represent the consensus variability calculated as one standard deviation about the consensus mean for that sample preparation method. The gray shaded region represents the target zone for "acceptable" performance, which encompasses the NIST certified value bounded by twice its uncertainty (U_{95}).

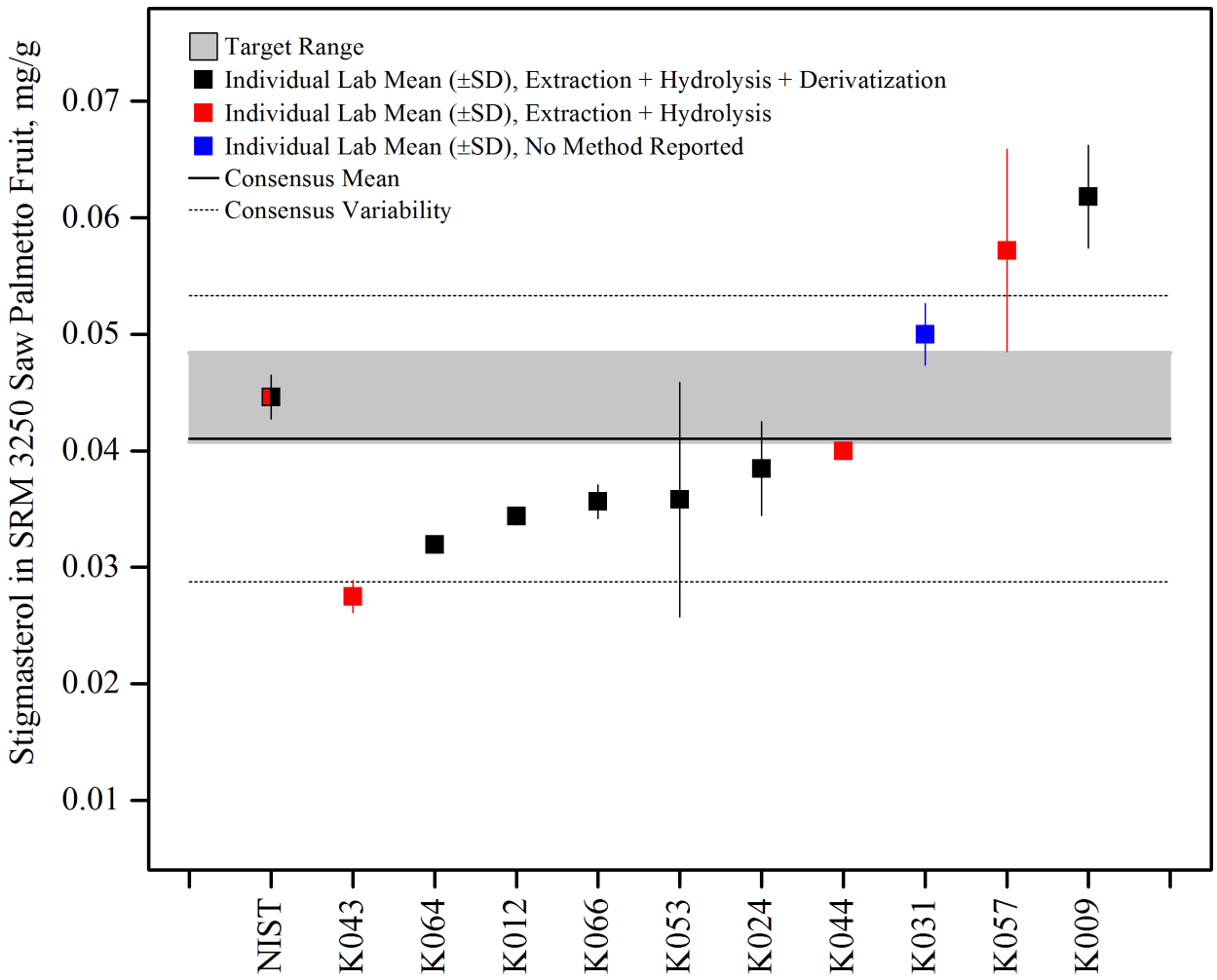

Figure 51. Stigmasterol in SRM 3250 *Serenoa repens* Fruit (data summary view – sample preparation method). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The color of the data point represents the sample preparation method employed. The black solid lines represent the consensus means for each sample preparation method, and the black dotted lines represent the consensus variability calculated as one standard deviation about the consensus mean for that sample preparation method. The gray shaded region represents the target zone for "acceptable" performance, which encompasses the NIST certified value bounded by twice its uncertainty (U_{95}).

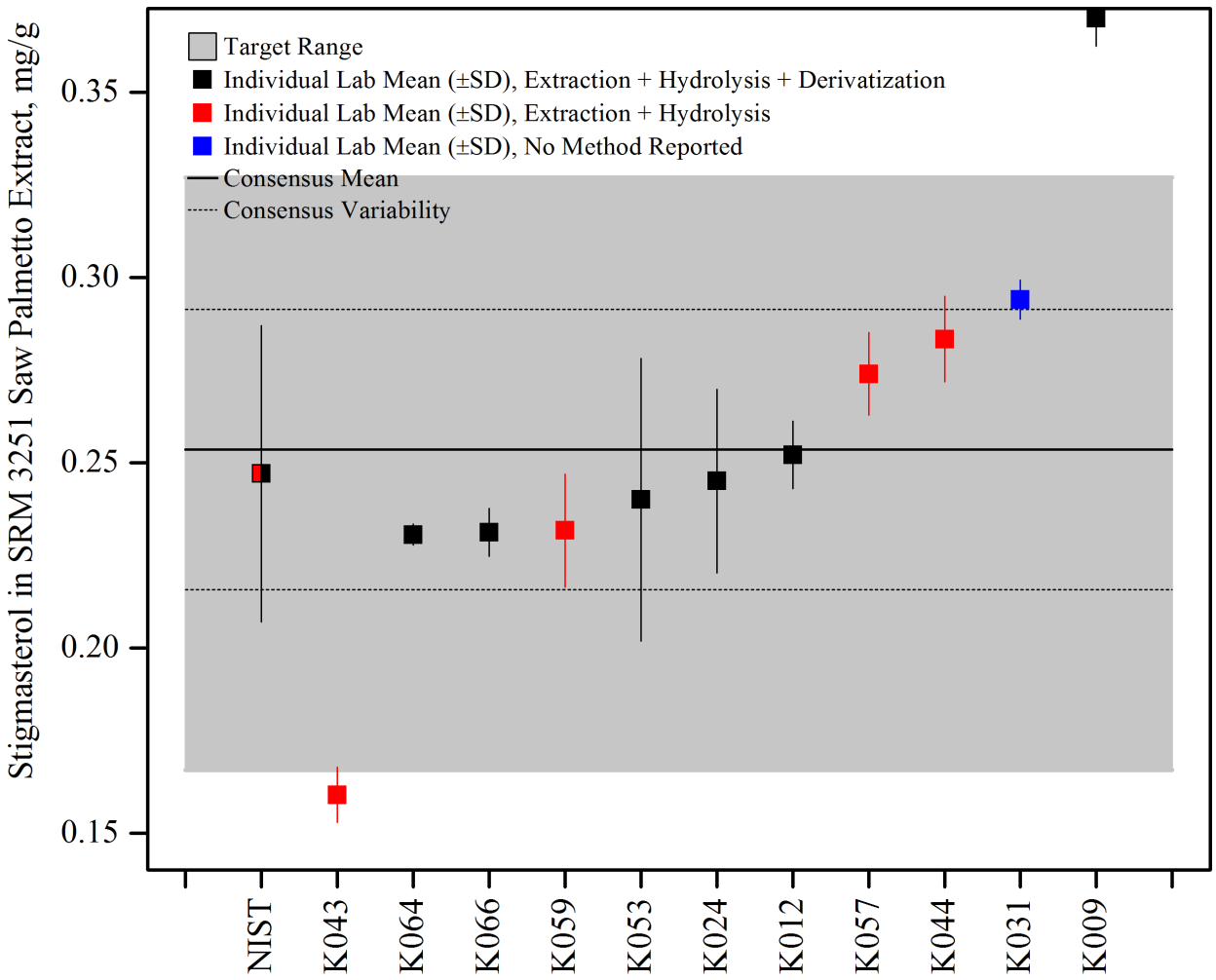

Figure 52. Stigmasterol in SRM 3251 *Serenoa repens* Extract (data summary view – sample preparation method). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The color of the data point represents the sample preparation method employed. The black solid lines represent the consensus means for each sample preparation method, and the black dotted lines represent the consensus variability calculated as one standard deviation about the consensus mean for that sample preparation method. The gray shaded region represents the target zone for "acceptable" performance, which encompasses the NIST certified value bounded by twice its uncertainty (U_{95}).

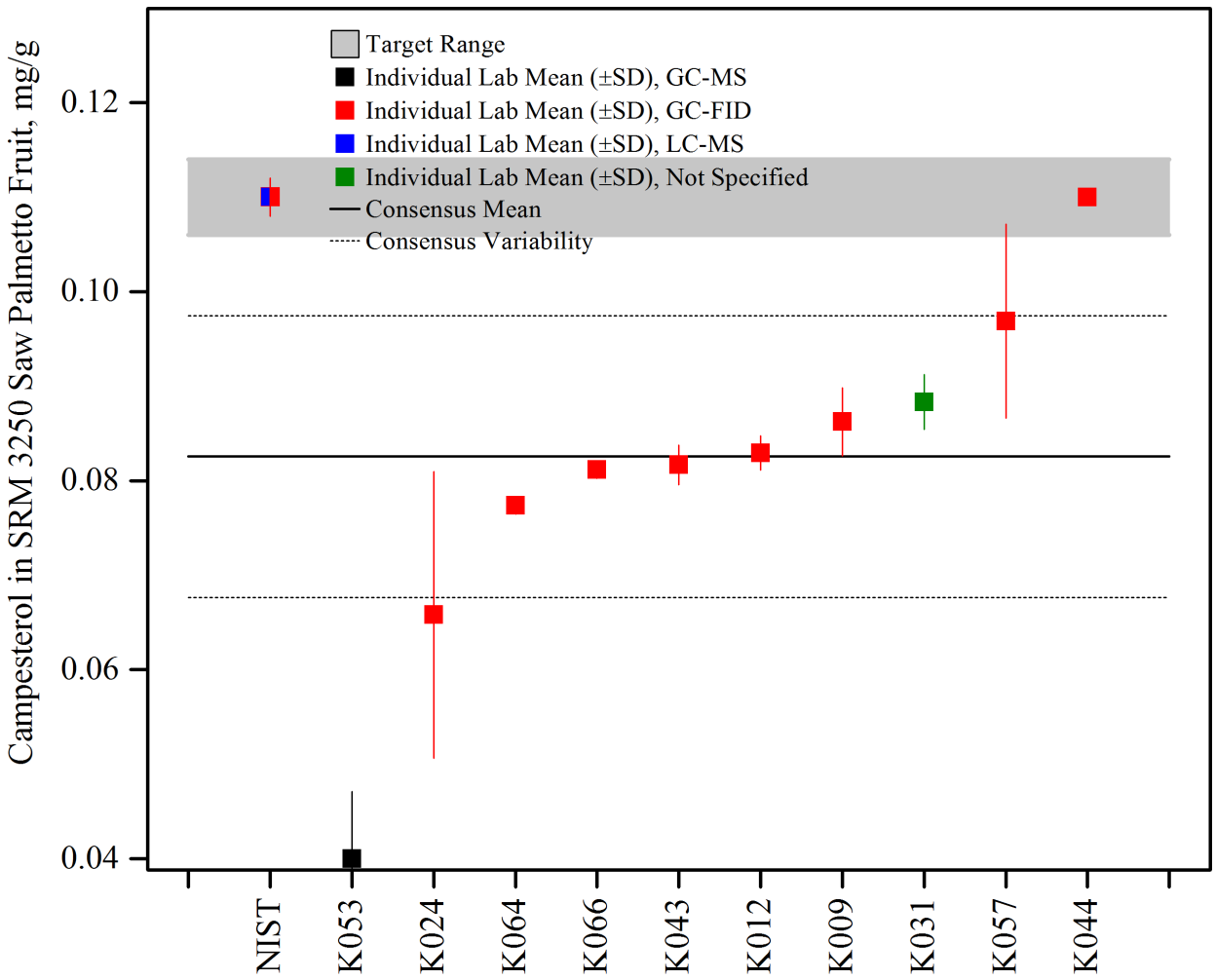

Figure 53. Campesterol in SRM 3250 *Serenoa repens* Fruit (data summary view –instrumental method). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The color of the data point represents the instrumental method employed. The black solid lines represent the consensus means for each sample preparation method, and the black dotted lines represent the consensus variability calculated as one standard deviation about the consensus mean for that sample preparation method. The gray shaded region represents the target zone for "acceptable" performance, which encompasses the NIST certified value bounded by twice its uncertainty (U_{95}).

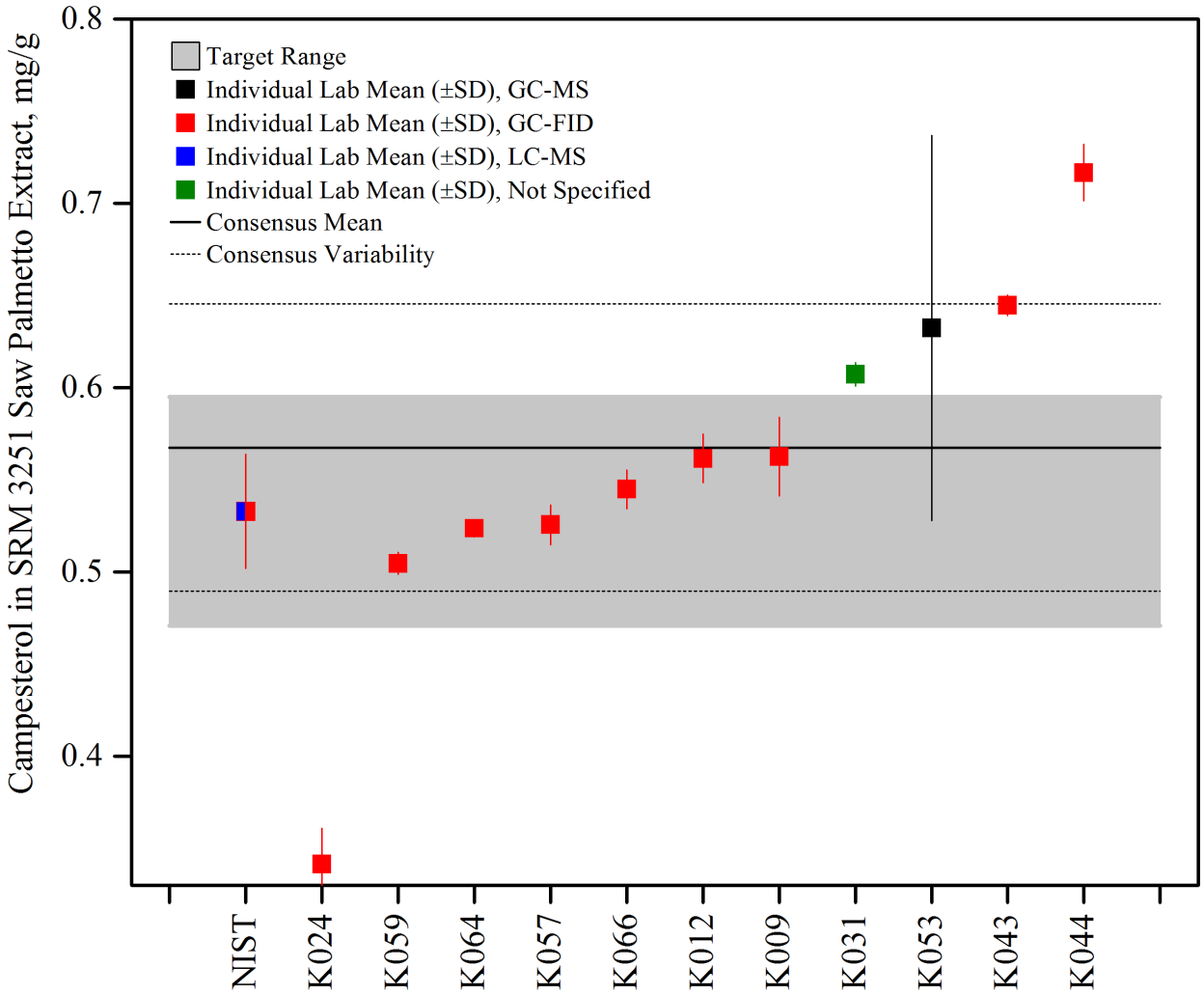

Figure 54. Campesterol in SRM 3251 *Serenoa repens* Extract (data summary view –instrumental method). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The color of the data point represents the instrumental method employed. The black solid lines represent the consensus means for each sample preparation method, and the black dotted lines represent the consensus variability calculated as one standard deviation about the consensus mean for that sample preparation method. The gray shaded region represents the target zone for "acceptable" performance, which encompasses the NIST certified value bounded by twice its uncertainty (U_{95}).

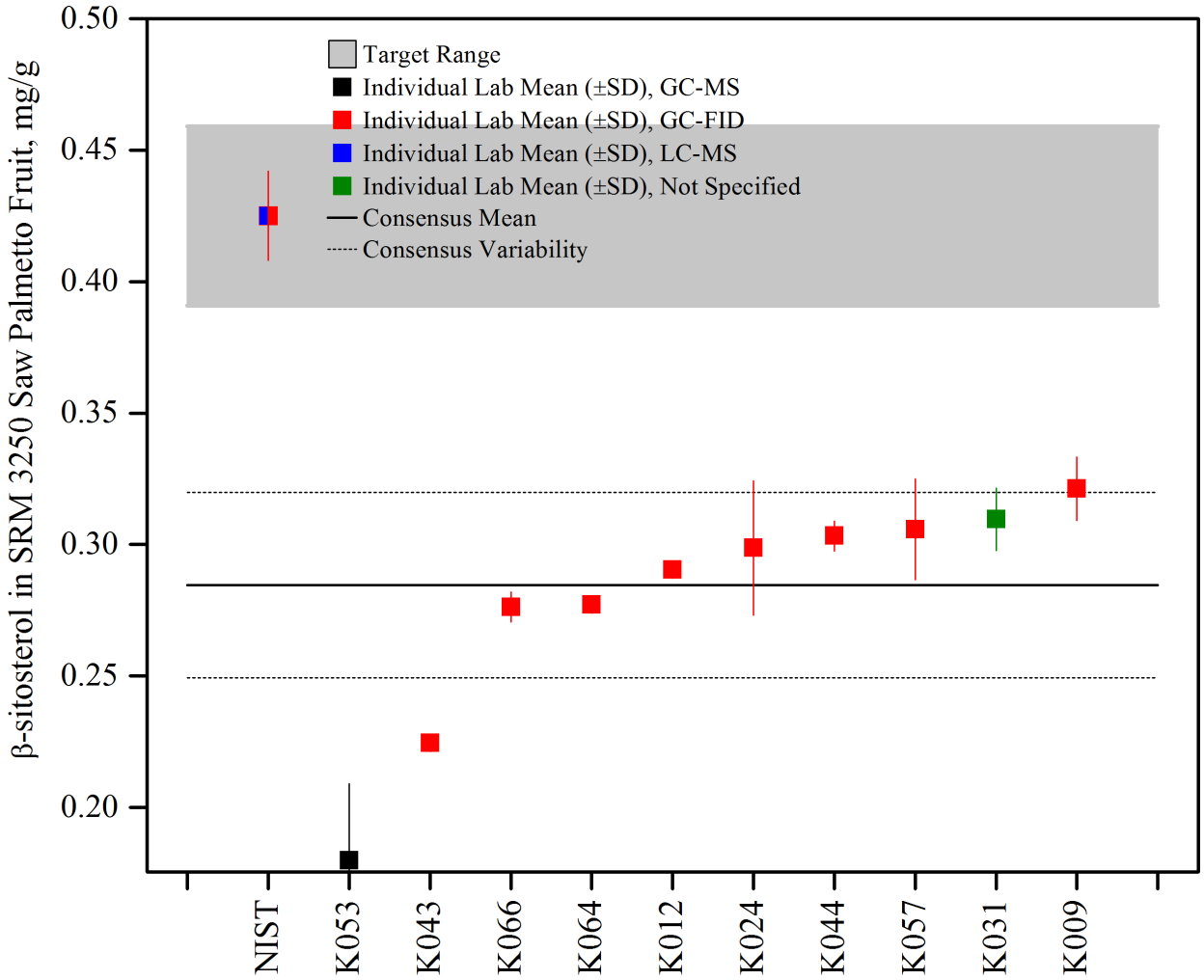

Figure 55. β-sitosterol in SRM 3250 *Serenoa repens* Fruit (data summary view –instrumental method). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The color of the data point represents the instrumental method employed. The black solid lines represent the consensus means for each sample preparation method, and the black dotted lines represent the consensus variability calculated as one standard deviation about the consensus mean for that sample preparation method. The gray shaded region represents the target zone for "acceptable" performance, which encompasses the NIST certified value bounded by twice its uncertainty (U_{95}).

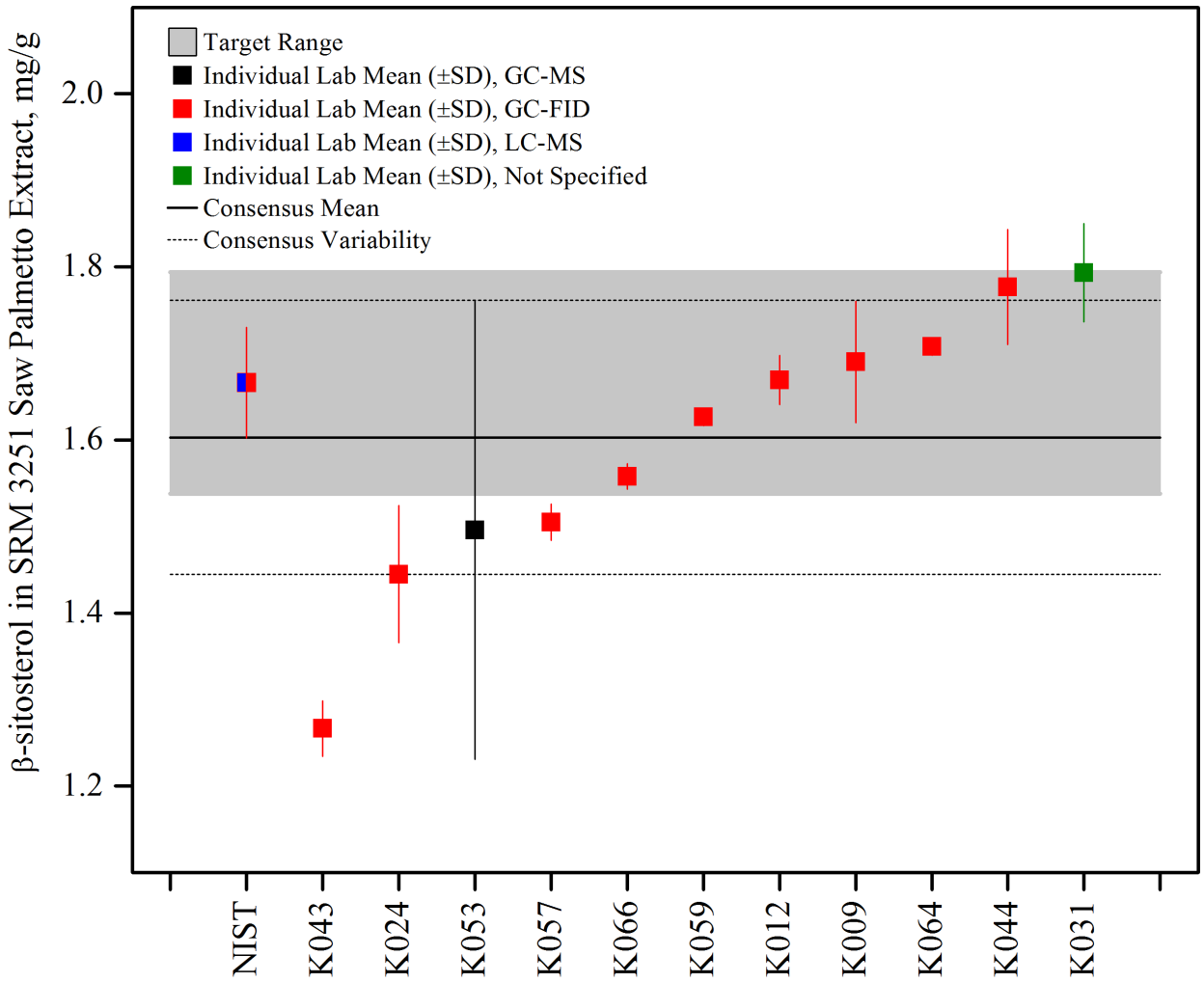

Figure 56. β-sitosterol in SRM 3251 *Serenoa repens* Extract (data summary view –instrumental method). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The color of the data point represents the instrumental method employed. The black solid lines represent the consensus means for each sample preparation method, and the black dotted lines represent the consensus variability calculated as one standard deviation about the consensus mean for that sample preparation method. The gray shaded region represents the target zone for "acceptable" performance, which encompasses the NIST certified value bounded by twice its uncertainty (U_{95}).

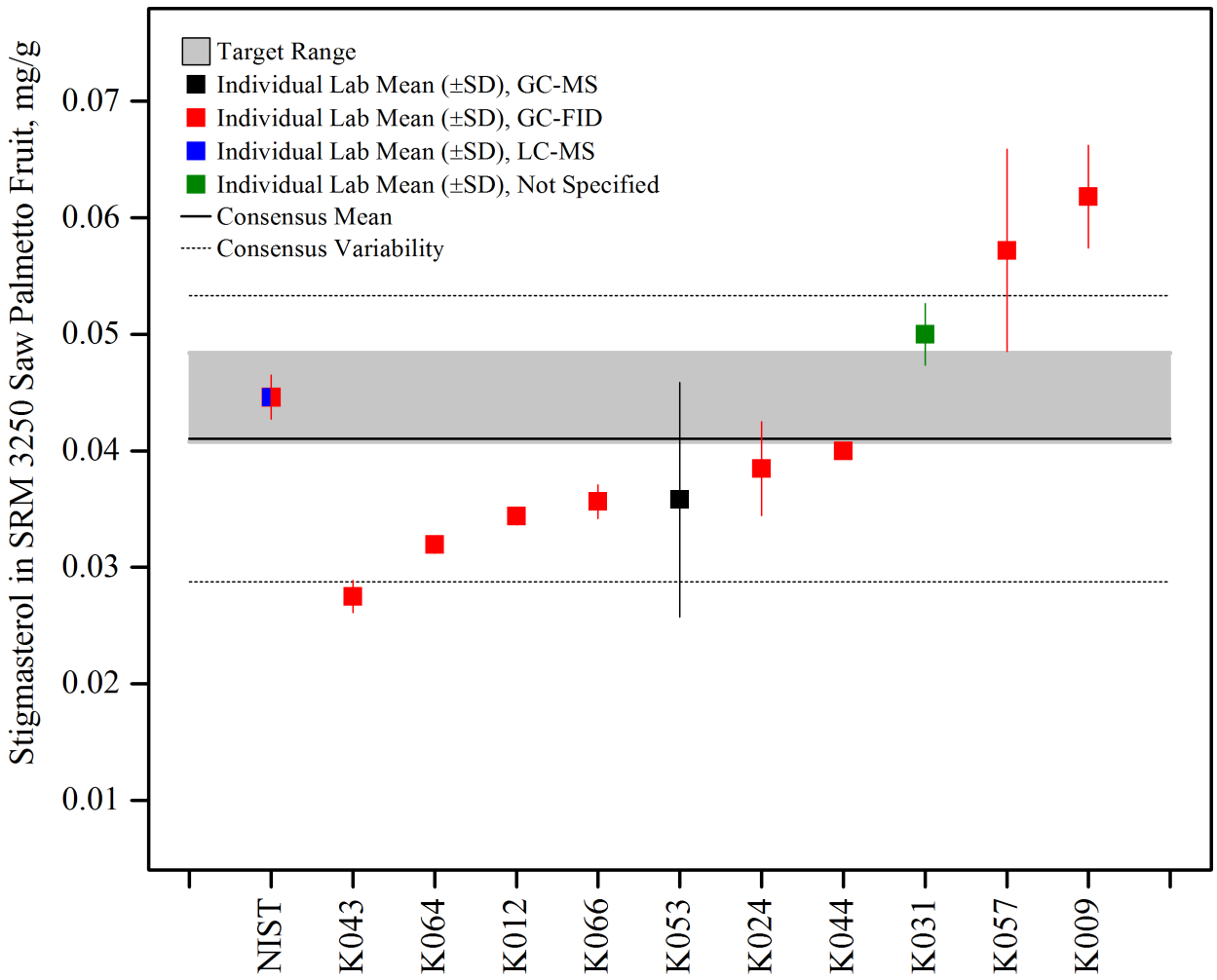

Figure 57. Stigmasterol in SRM 3250 *Serenoa repens* Fruit (data summary view –instrumental method). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The color of the data point represents the instrumental method employed. The black solid lines represent the consensus means for each sample preparation method, and the black dotted lines represent the consensus variability calculated as one standard deviation about the consensus mean for that sample preparation method. The gray shaded region represents the target zone for "acceptable" performance, which encompasses the NIST certified value bounded by twice its uncertainty (U_{95}).

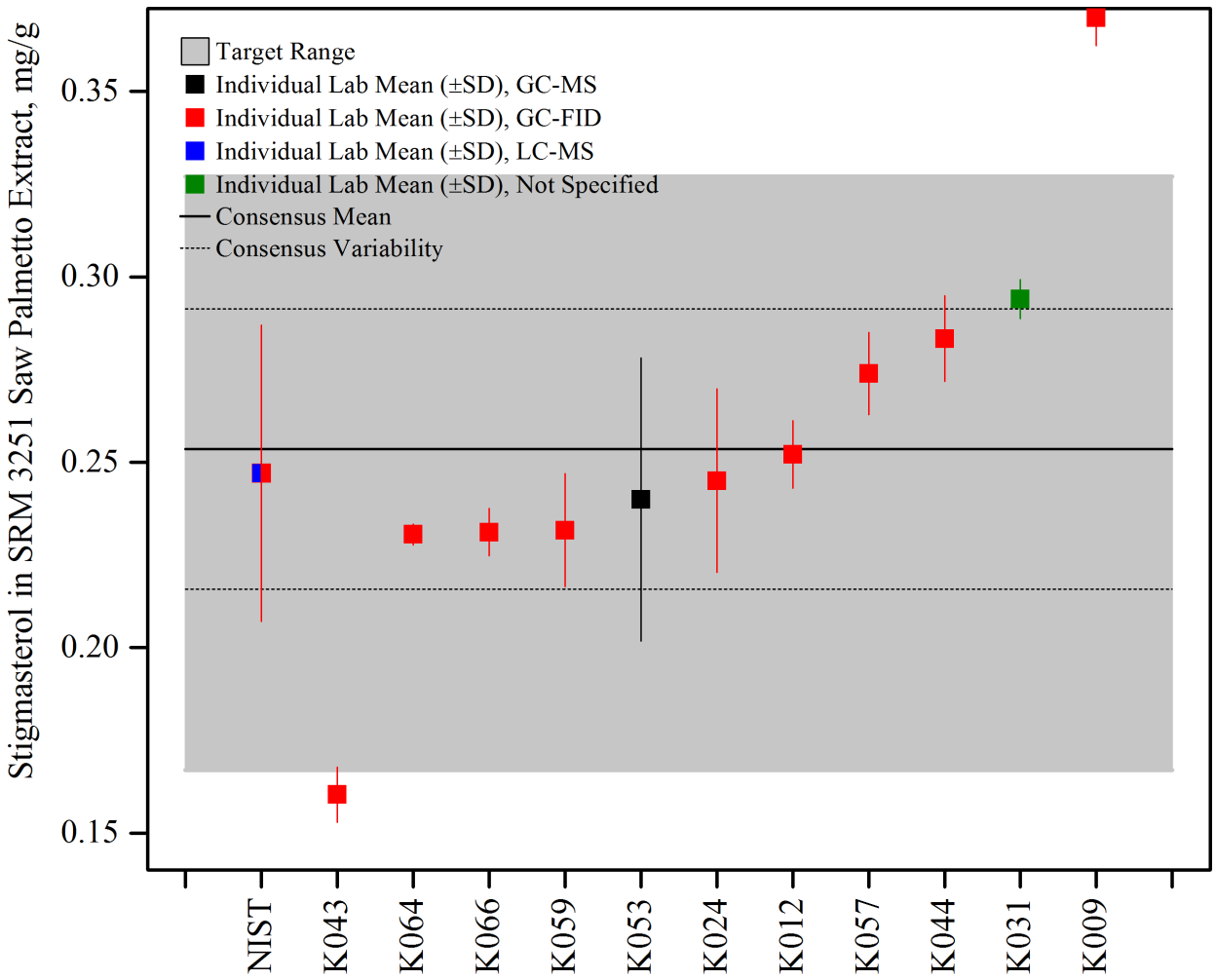

Figure 58. Stigmasterol in SRM 3251 *Serenoa repens* Extract (data summary view –instrumental method). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The color of the data point represents the instrumental method employed. The black solid lines represent the consensus means for each sample preparation method, and the black dotted lines represent the consensus variability calculated as one standard deviation about the consensus mean for that sample preparation method. The gray shaded region represents the target zone for "acceptable" performance, which encompasses the NIST certified value bounded by twice its uncertainty (U_{95}).

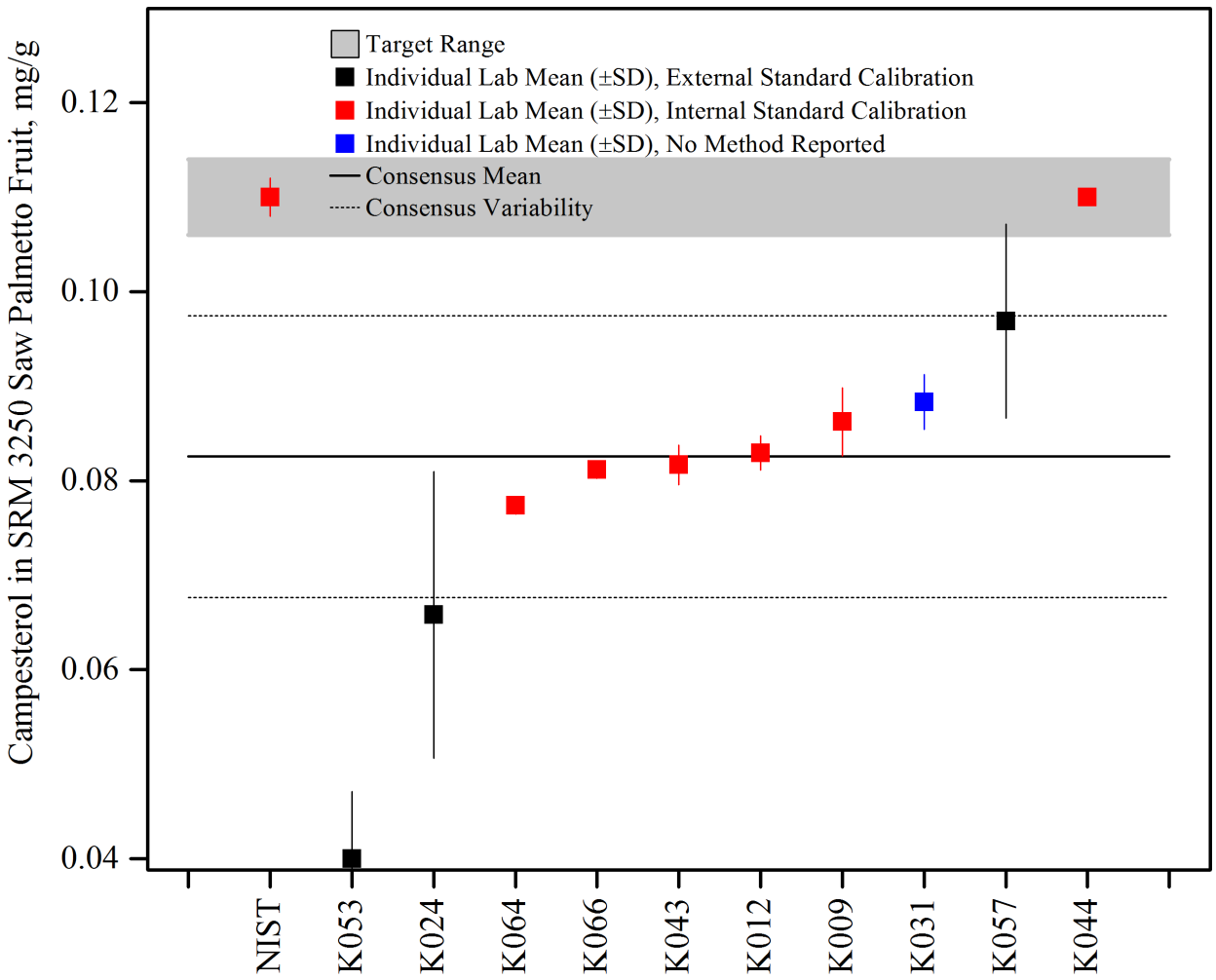

Figure 59. Campesterol in SRM 3250 *Serenoa repens* Fruit (data summary view – calibration approach). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The color of the data point represents the calibration approach employed. The black solid lines represent the consensus means for each sample preparation method, and the black dotted lines represent the consensus variability calculated as one standard deviation about the consensus mean for that sample preparation method. The gray shaded region represents the target zone for "acceptable" performance, which encompasses the NIST certified value bounded by twice its uncertainty (U_{95}).

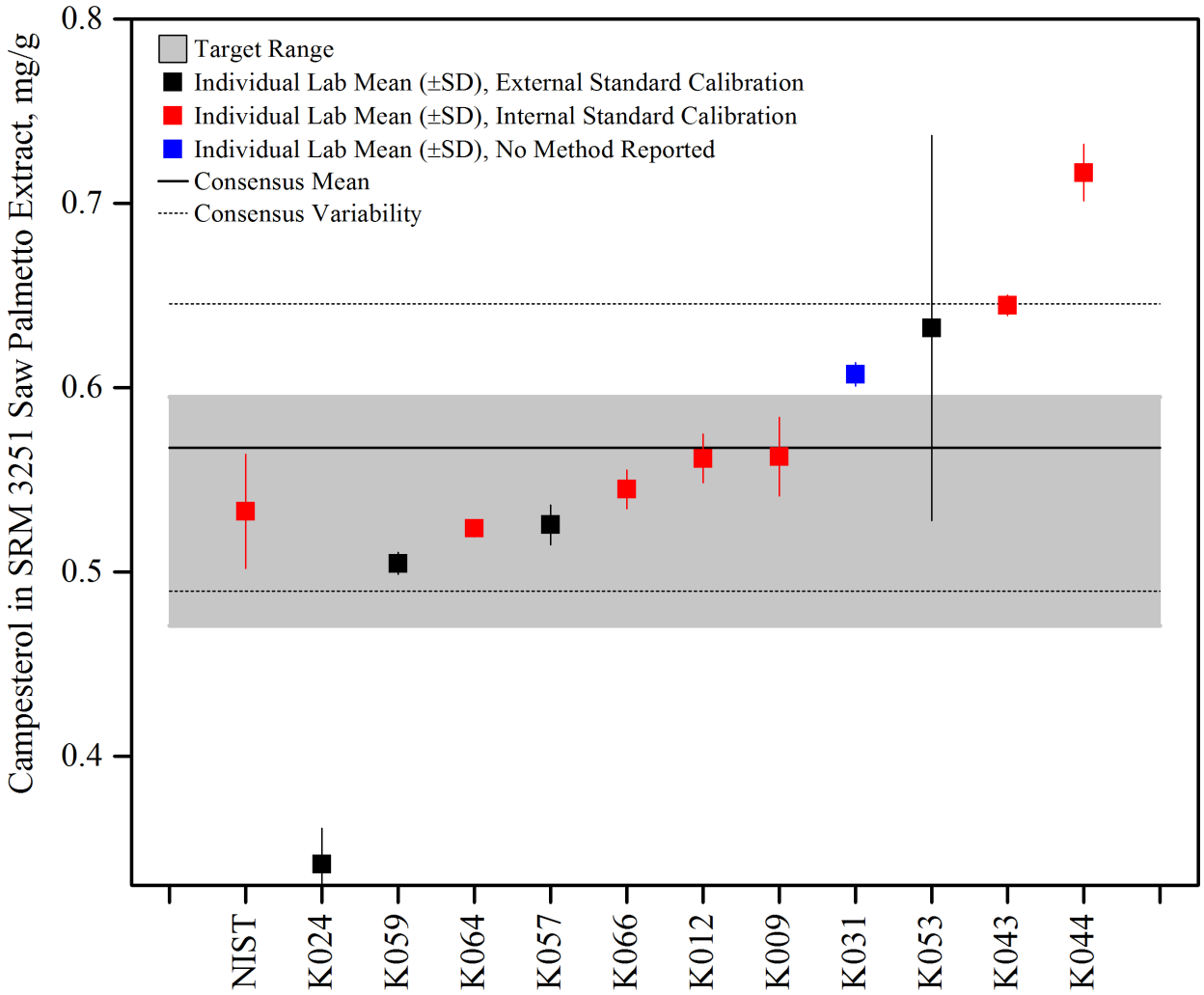

Figure 60. Campesterol in SRM 3251 *Serenoa repens* Extract (data summary view – calibration approach). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The color of the data point represents the calibration approach employed. The black solid lines represent the consensus means for each sample preparation method, and the black dotted lines represent the consensus variability calculated as one standard deviation about the consensus mean for that sample preparation method. The gray shaded region represents the target zone for "acceptable" performance, which encompasses the NIST certified value bounded by twice its uncertainty (U_{95}).

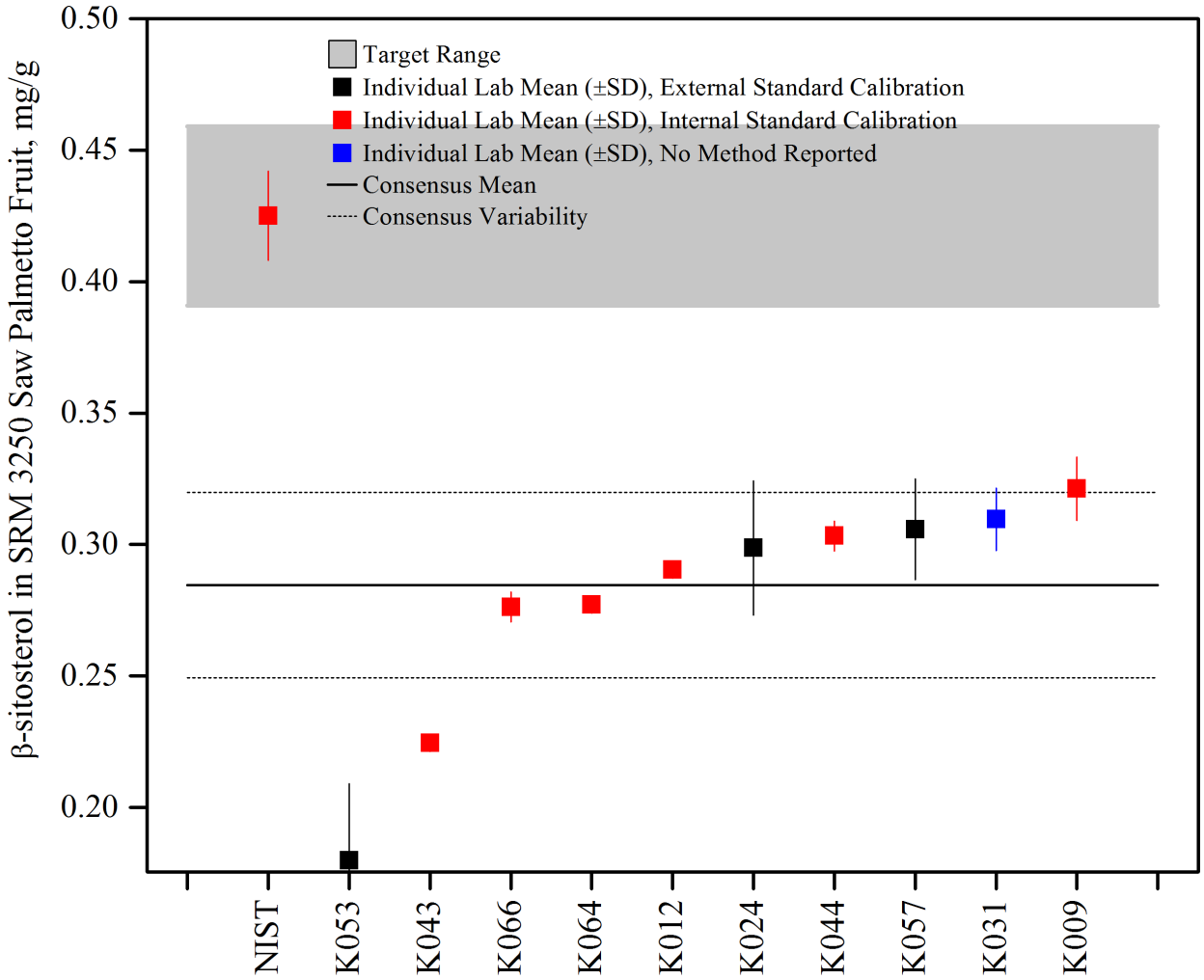

Figure 61. β-sitosterol in SRM 3250 *Serenoa repens* Fruit (data summary view – calibration approach). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The color of the data point represents the calibration approach employed. The black solid lines represent the consensus means for each sample preparation method, and the black dotted lines represent the consensus variability calculated as one standard deviation about the consensus mean for that sample preparation method. The gray shaded region represents the target zone for "acceptable" performance, which encompasses the NIST certified value bounded by twice its uncertainty (U_{95}).

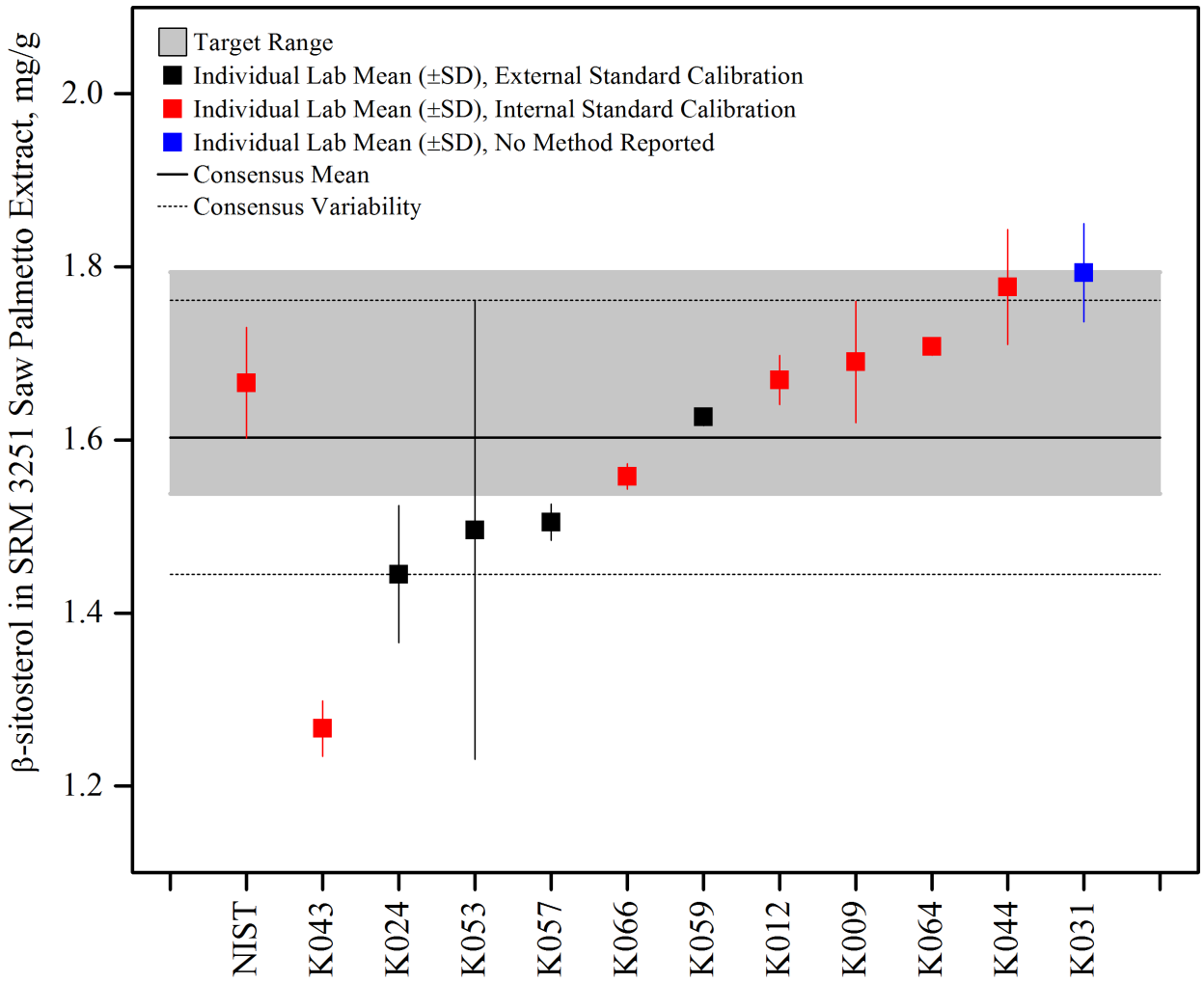

Figure 62. β-sitosterol in SRM 3251 *Serenoa repens* Extract (data summary view – calibration approach). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The color of the data point represents the calibration approach employed. The black solid lines represent the consensus means for each sample preparation method, and the black dotted lines represent the consensus variability calculated as one standard deviation about the consensus mean for that sample preparation method. The gray shaded region represents the target zone for "acceptable" performance, which encompasses the NIST certified value bounded by twice its uncertainty (U_{95}).

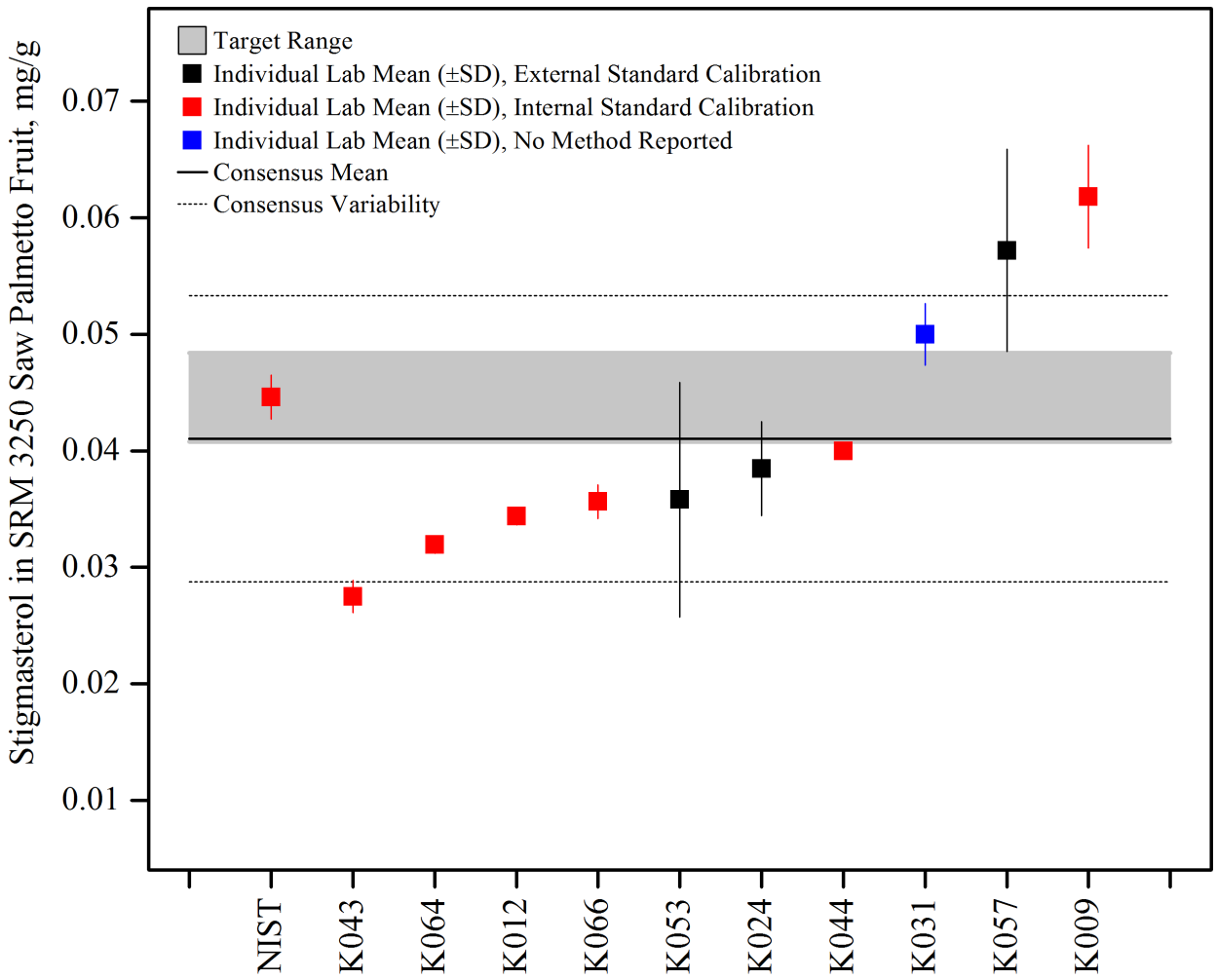

Figure 63. Stigmasterol in SRM 3250 *Serenoa repens* Fruit (data summary view – calibration approach). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The color of the data point represents the calibration approach employed. The black solid lines represent the consensus means for each sample preparation method, and the black dotted lines represent the consensus variability calculated as one standard deviation about the consensus mean for that sample preparation method. The gray shaded region represents the target zone for "acceptable" performance, which encompasses the NIST certified value bounded by twice its uncertainty (U_{95}).

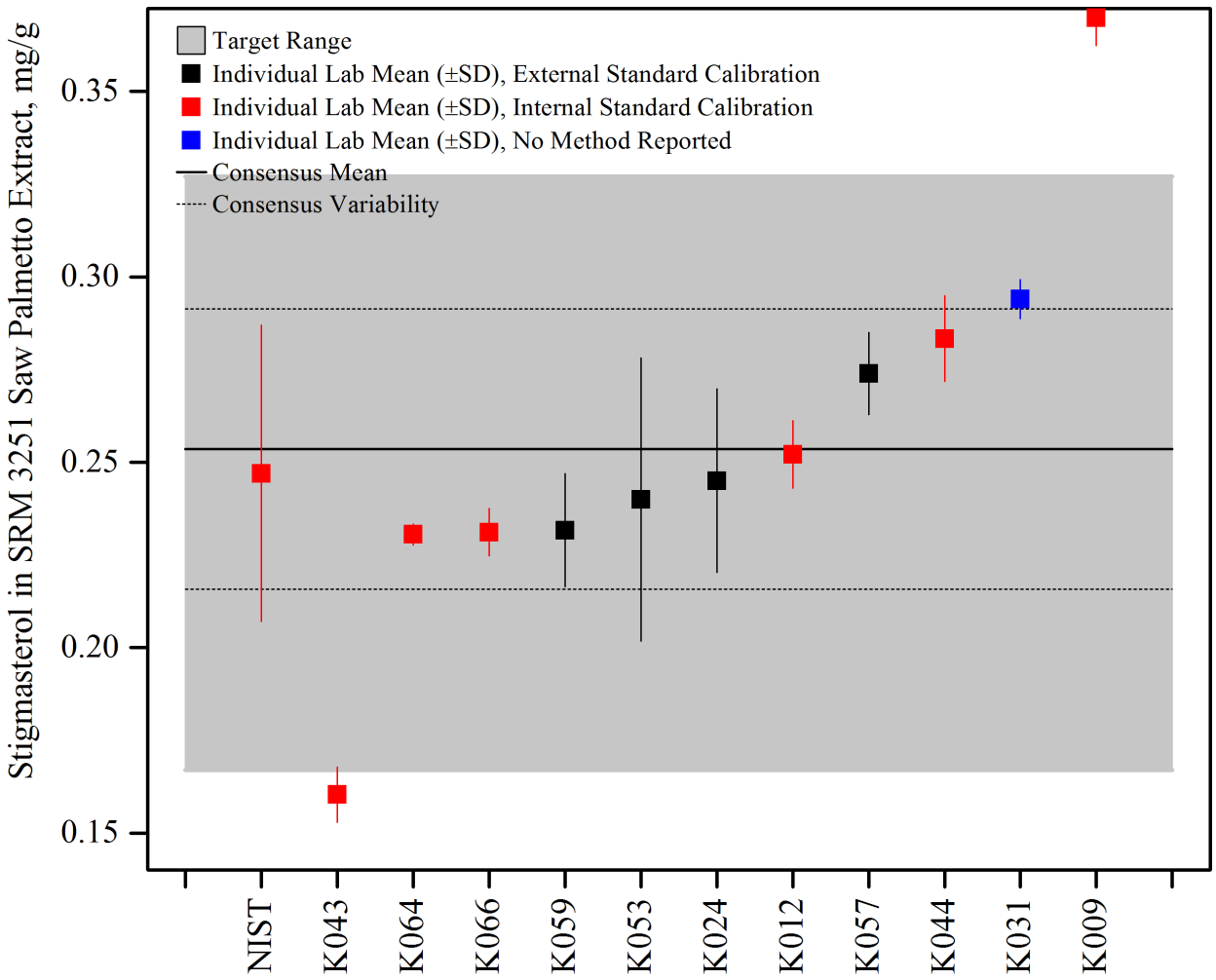

Figure 64. Stigmasterol in SRM 3251 *Serenoa repens* Extract (data summary view – calibration approach). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The color of the data point represents the calibration approach employed. The black solid lines represent the consensus means for each sample preparation method, and the black dotted lines represent the consensus variability calculated as one standard deviation about the consensus mean for that sample preparation method. The gray shaded region represents the target zone for "acceptable" performance, which encompasses the NIST certified value bounded by twice its uncertainty (U_{95}).

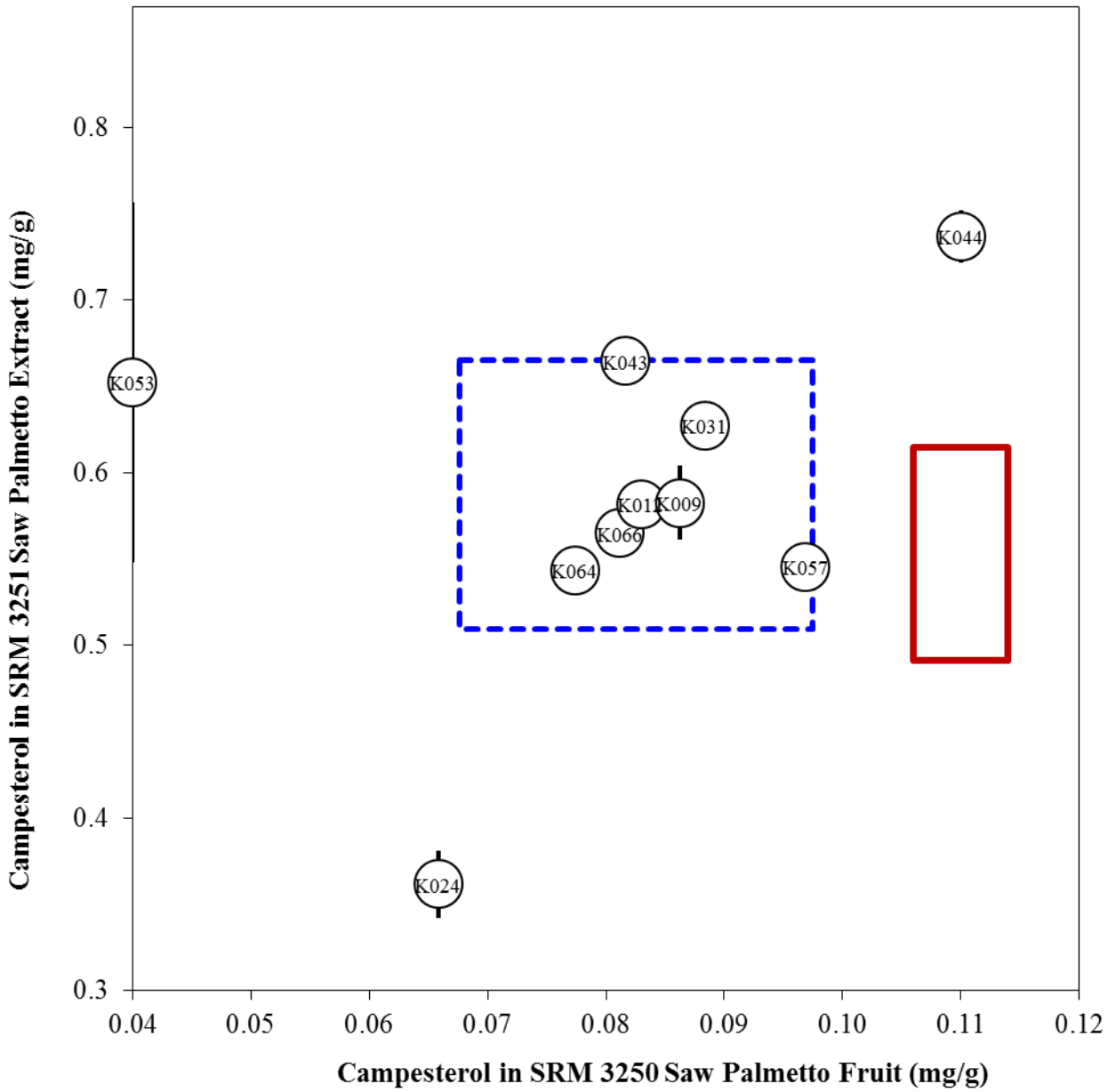

Figure 65. Campesterol in SRM 3250 *Serenoa repens* Fruit and SRM 3251 *Serenoa repens* Extract (sample/sample comparison view). In this view, the individual laboratory results for one sample (SRM 3250) are compared to the results for a second sample (SRM 3251). The solid red box represents the target zone for the control (x-axis) and unknown sample (y-axis). The dotted blue box represents the consensus zone for the control (x-axis) and the unknown sample (y-axis).

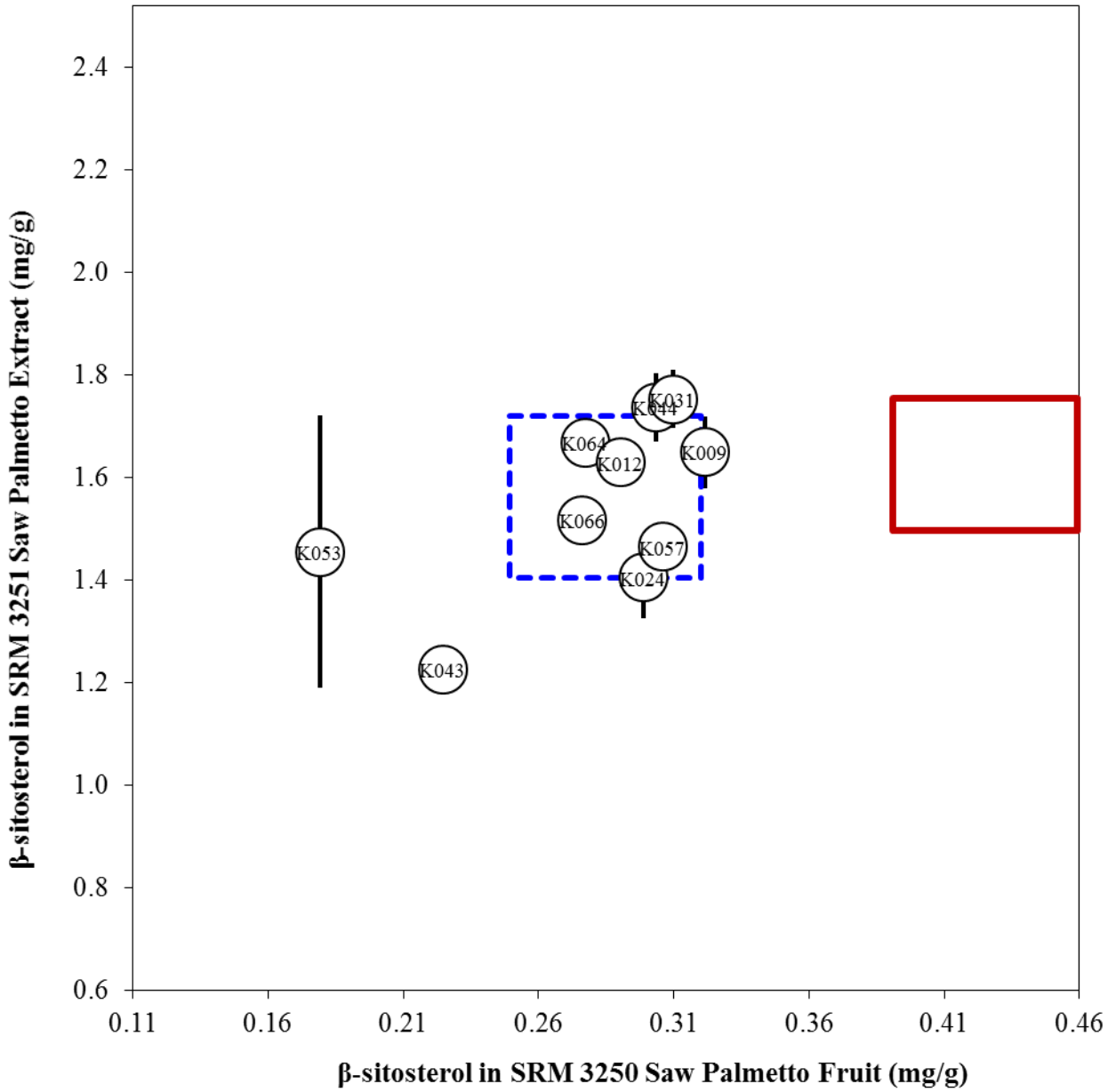

Figure 66. β-sitosterol in SRM 3250 *Serenoa repens* Fruit and SRM 3251 *Serenoa repens* Extract (sample/sample comparison view). In this view, the individual laboratory results for one sample (SRM 3250) are compared to the results for a second sample (SRM 3251). The solid red box represents the target zone for the control (x-axis) and unknown sample (y-axis). The dotted blue box represents the consensus zone for the control (x-axis) and the unknown sample (y-axis).

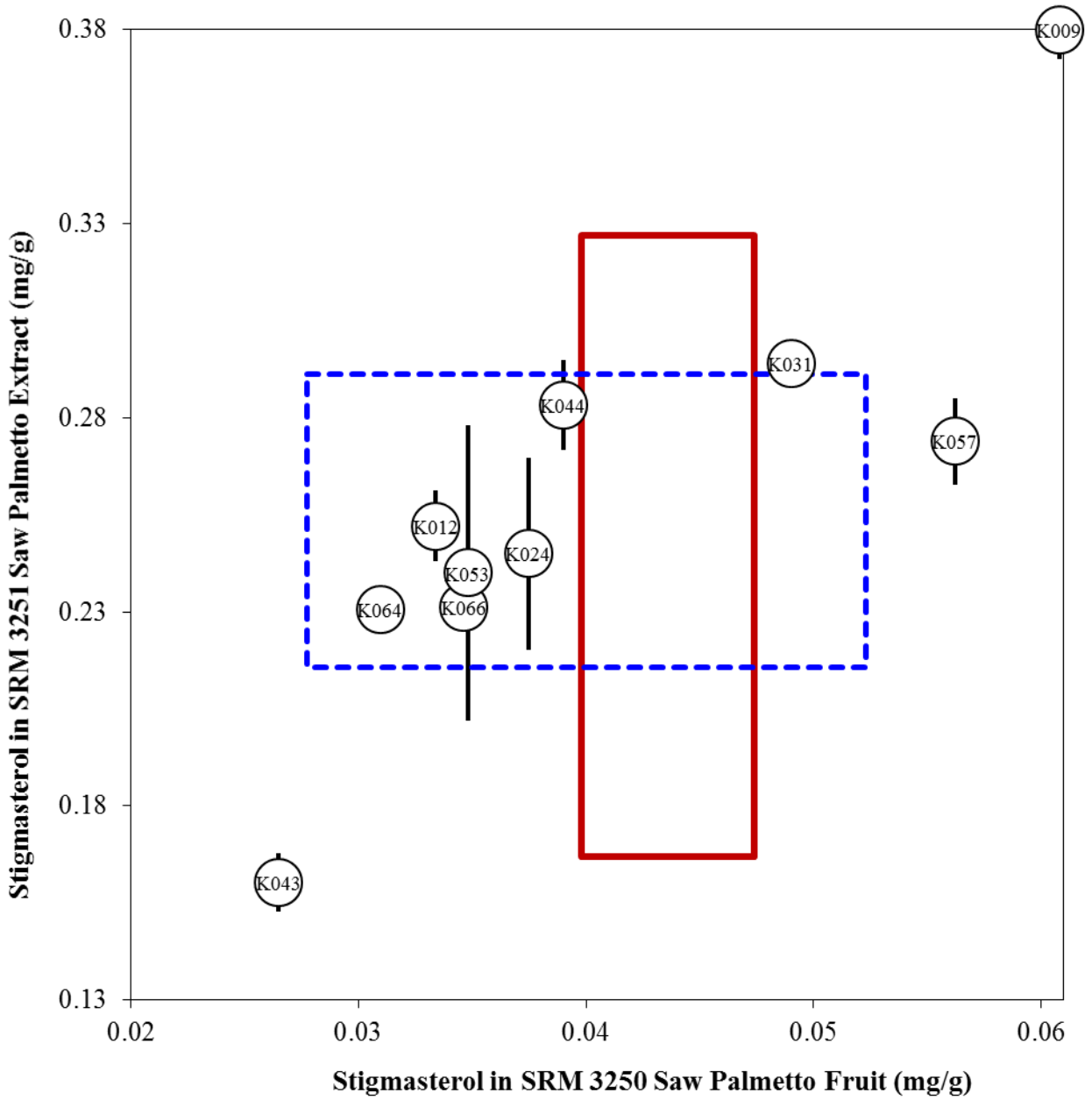

Figure 67. Stigmasterol in SRM 3250 *Serenoa repens* Fruit and SRM 3251 *Serenoa repens* Extract (sample/sample comparison view). In this view, the individual laboratory results for one sample (SRM 3250) are compared to the results for a second sample (SRM 3251). The solid red box represents the target zone for the control (x-axis) and unknown sample (y-axis). The dotted blue box represents the consensus zone for the control (x-axis) and the unknown sample (y-axis).

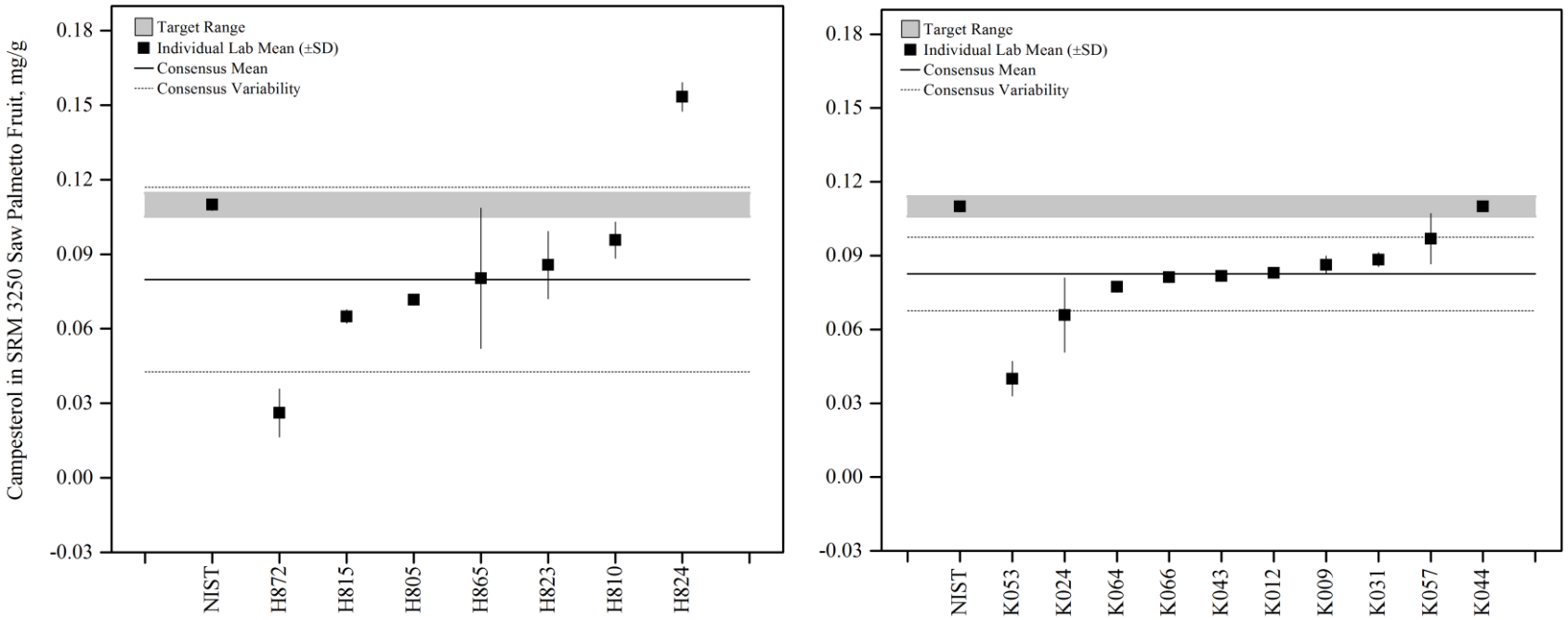

Figure 68. Comparison of results for campesterol in SRM 3250 Saw Palmetto Fruit from Exercise H (left) and Exercise K (right). In both graphs, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The black solid lines represent the consensus means for each sample preparation method, and the black dotted lines represent the consensus variability calculated as one standard deviation about the consensus mean for that sample preparation method. The gray shaded region represents the target zone for "acceptable" performance, which encompasses the NIST certified value bounded by twice its uncertainty (U_{95}). NOTE: The participant laboratory numbers are changed for each exercise, so no correlation can be made between laboratory numbers from Exercise H and Exercise K.

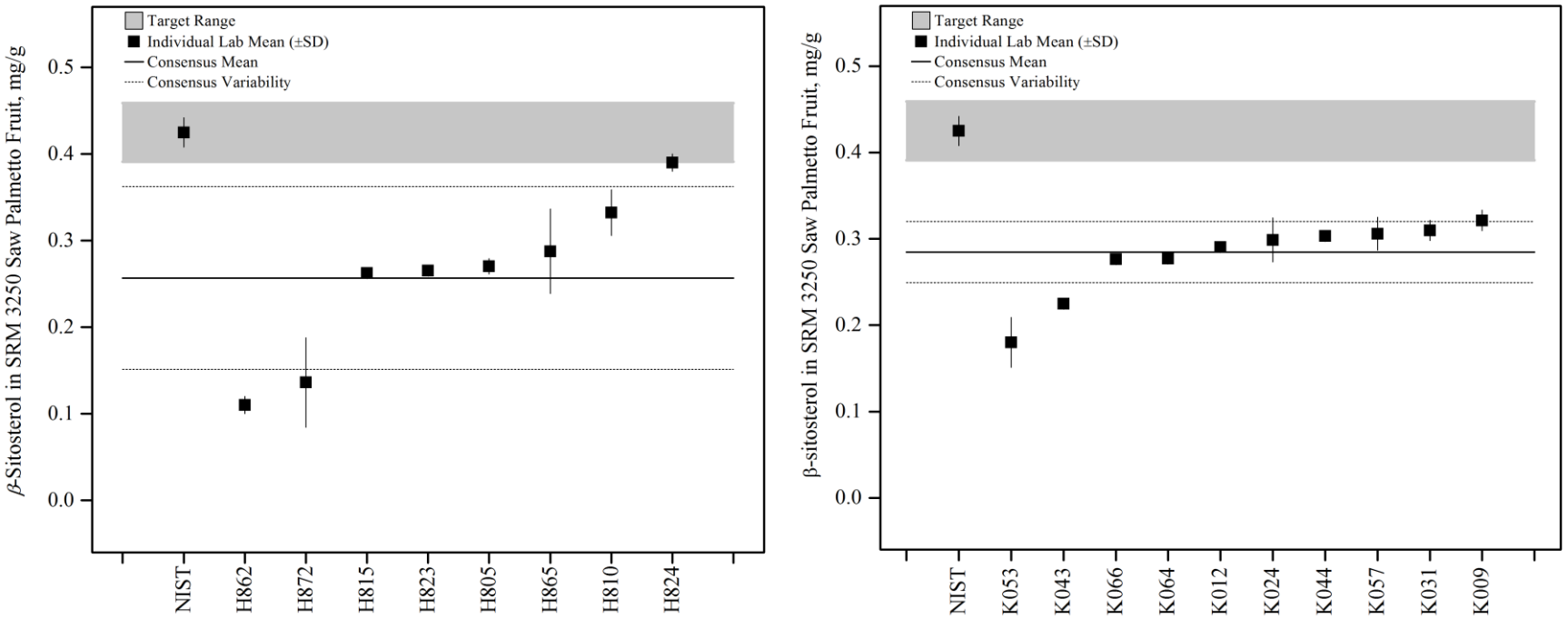

Figure 69. Comparison of results for β-sitosterol in SRM 3250 Saw Palmetto Fruit from Exercise H (left) and Exercise K (right). In both graphs, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The black solid lines represent the consensus means for each sample preparation method, and the black dotted lines represent the consensus variability calculated as one standard deviation about the consensus mean for that sample preparation method. The gray shaded region represents the target zone for "acceptable" performance, which encompasses the NIST certified value bounded by twice its uncertainty (U_{95}). NOTE: The participant laboratory numbers are changed for each exercise, so no correlation can be made between laboratory numbers from Exercise H and Exercise K.