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National Institute of Standards and Technology
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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. Exercise H of this program offered the opportunity for laboratories to assess their in-house measurements of nutritional elements (Ca, Cu, and Mn), contaminants (polycyclic aromatic hydrocarbons [PAHs]) water-soluble vitamins (choline), fat-soluble vitamins (tocopherols), fatty acids, 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 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 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. 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 Official Method of Analysis would benefit the dietary supplement community.

NIST has experience in the area 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 laboratory performance. In contrast, the

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

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 eighth exercise of the DSQAP, Exercise H. Seventy-five laboratories responded to the call for participants distributed in January 2011. Samples were shipped to participants in March 2012, and results were returned to NIST by June 2012. This report contains the final data and information to be disseminated to the participants in October 2012.

OVERVIEW OF DATA TREATMENT AND REPRESENTATION

Statistics

The individual data table and graphs 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 $x_i^* = x^* - \delta$.
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^*:

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

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

Individual 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}).

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

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.[1] Additional information on calculation of the consensus mean and standard deviation can be found in the previous section.

Section 3 of the data table contains the target values for each analyte. When possible, the target 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. 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 commercial products, the analytes are measured at NIST using an appropriate method. The NIST value represents the mean of at least three replicates.

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 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

In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). Data points that are unfilled represent laboratories that only 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. 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 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. One program goal is 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.

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

Sample/Control Comparison View (Sample/Sample Comparison View)
In this view, the individual laboratory results for a control (NIST SRM with a certified value) are compared to the results for an unknown (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 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). 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 IN FOODS AND SUPPLEMENTS

Study Overview

In this study, participants were provided with two NIST SRMs, SRM 1566b Oyster Tissue and candidate SRM 3532 Calcium Dietary Supplement. Participants were asked to use in-house analytical methods to determine the mass fractions of three nutritional elements (calcium, copper, and manganese) in each of the matrices and report values on an as-received basis.

Sample Information

Oyster tissue. Participants were provided with six vials, each containing approximately 1 g of freeze-dried, powdered oyster tissue. The material was prepared from oysters collected in the Gulf of Mexico that had been shucked, rinsed, and blended both before and after freeze drying. Before use, participants were instructed to thoroughly mix the contents of the vial and use a sample size of at least 0.25 g. Participants were asked to report a single value from each pair of vials and store the material at controlled room temperature, 10 °C to 30 °C. Approximate analyte levels were not reported prior to the study. NIST certified values in SRM 1566b were determined using inductively coupled plasma mass spectrometry (ICP-MS), isotope dilution ICP-MS, instrumental neutron activation analysis (INAA), and radiochemical neutron activation analysis (RNAA). The certified values and uncertainties for Ca, Cu, and Mn in SRM 1566b are outlined in the table below, both on a dry-mass basis and an as-received basis following adjustment for the moisture content of the material, 2.9 %.

Analyte	Certified Mass Fraction (mg/kg) (dry-mass basis)	Adjusted Mass Fraction (mg/g) (as-received basis)
Ca	838 ± 20	0.813 ± 0.019
Cu	71.6 ± 1.6	0.0695 ± 0.0016
Mn	18.5 ± 0.2	0.0180 ± 0.0002

Ca supplement. Participants were provided with one packet containing approximately 10 g of calcium dietary supplement powder. The calcium supplements were purchased commercially, then ground, sieved, and heat-sealed inside nitrogen-flushed 0.1 mm (4 mil) polyethylene bags, which were then sealed inside aluminized plastic bags with 2 packets of silica gel. Before use, participants were instructed to thoroughly mix the contents of the packet and use a sample size of at least 0.25 g. Participants were asked to report three values from the single packet provided and store the material at controlled room temperature, 10 °C to 30 °C. Approximate analyte levels were not reported prior to the study. NIST values in candidate SRM 3532 will be certified using X-ray fluorescence (XRF) and inductively coupled plasma optical emission spectrometry (ICP-OES) following microwave digestion using standard additions as the method of quantitation. The preliminary NIST values in candidate SRM 3532, estimated from the mean of these two methods of analysis, are reported in the table below with an estimated uncertainty of 5 %.

Analyte	Estimated Certified Mass Fraction (mg/g) (as-received basis)
Ca	170 ± 8.5
Cu	0.270 ± 0.014
Mn	0.530 ± 0.027

Study Results

- Forty-nine laboratories enrolled in this exercise and received samples. Thirty-seven laboratories reported results for calcium (76 % participation), 38 laboratories reported results for copper (78 % participation), and 34 laboratories reported results for manganese (71 % participation).
- The consensus means for calcium and copper in the dietary supplement were within the target range with acceptable variability (6 % and 14 % relative standard deviation (RSD), respectively). The consensus mean for manganese in the dietary supplement was within but near the low end of the target range, again with acceptable variability (11 % RSD).
- The consensus mean for calcium in the oyster tissue was within but at the high end of the target range, while the consensus mean for copper was within but near the low end of the target range. Again, both had acceptable variability (15 % and 9 % RSD, respectively). The consensus mean for manganese in the oyster tissue was within the target range with acceptable variability (9 % RSD).
- A majority of the laboratories reported using either open-beaker digestion (29 % to 41 %) or microwave digestion (32 % to 41 %) for sample preparation. Some laboratories used hot block digestion (15 % to 16 %). Other laboratories reported using dry ashing or partial digestion within plastic bottles.
- A majority of the laboratories reported using either ICP-OES (46 %) or ICP-MS (41 %) as their analytical method. Other laboratories reported using atomic absorption spectroscopy (AAS), titrimetry, or total reflection X-ray fluorescence (TXRF).

Technical Recommendations

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

- While there seemed to be only a slight difference in results between open-beaker digestions and microwave digestions, it did appear that the open-beaker digestions were slightly more effective, with more results in the consensus range. Open-beaker digestions work well for these three elements since they are neither easily volatilized nor found as contaminants in most laboratories. Participants would be able to digest materials until they could actually see that the material was fully dissolved.
- When using ICP-OES for value assignment, there are usually several wavelengths available for each analyte. Using several wavelengths for each analyte helps in the determination of interferences or background shifts due to matrix effects at any one wavelength.
- With both ICP-OES and ICP-MS, it is important to check the calibration curve for linearity.
    - With ICP-OES, some elements will only be linear within a specific range. Solution concentrations need to fall within that linear range.
    - With ICP-MS, many instruments run in pulse mode, which is more sensitive. If the calibration curve goes outside of the dynamic range for pulse mode then the instrument will use both the pulse and analog mode. The ICP-MS must be calibrated for both modes in this case. It is often easier and more accurate to have a narrower range of calibration points, making sure the calibration curve is linear in the pulse mode.

- More accurate measurements can be achieved by making sure the sample concentrations fall within the middle of the calibration curve.
- Double-check all calculations; this is a cause for many errors.

Table 1. Individual data table (NIST) for nutritional elements in foods and dietary supplements.

National Institute of Standards & Technology

Exercise H - March 2012 - 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}
Ca	Ca Supplement	mg/g	170	8.48	0.0	0.1	36	170	10.5	170	8.5
Ca	Oyster Tissue	mg/g	0.813	0.019	-0.3	0.0	37	0.847	0.126	0.813	0.019
Cu	Ca Supplement	mg/g	0.270	0.014	-0.1	0.0	37	0.273	0.040	0.270	0.014
Cu	Oyster Tissue	mg/g	0.0695	0.0016	0.4	0.0	38	0.0672	0.0063	0.0695	0.0016
Mn	Ca Supplement	mg/g	0.530	0.027	0.8	0.0	34	0.488	0.054	0.530	0.027
Mn	Oyster Tissue	mg/g	0.0180	0.0002	-0.1	0.2	34	0.0182	0.0017	0.0180	0.0002

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 calcium in foods and dietary supplements.

		Calcium									
		SRM 1566b Oyster Tissue (mg/g)					SRM 3532 Calcium Dietary Supplement (mg/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	NIST				0.813	0.019				170	8.5
	H802										
	H803										
	H804	0.760	0.716	0.748	0.741	0.023	165	168	170	168	2.8
	H805	0.816	0.782	0.775	0.791	0.022	178	180	179	179	1.0
	H806	0.928	0.893	0.877	0.899	0.026	182	180	179	180	1.7
	H807	0.887	0.846	0.885	0.873	0.023	181	188	186	185	4.0
	H808	0.778	0.753	0.754	0.762	0.014	172	156	158	162	8.7
	H809										
	H810	0.832	0.821	0.815	0.823	0.009	165	164	163	164	1.0
	H811	1.626	1.627	1.610	1.621	0.010	178	178	180	179	0.9
	H812	1.010	1.000	1.000	1.003	0.006	179	181	180	180	1.0
	H814	3.570	3.480	3.520	3.523	0.045	167	168	171	169	2.1
	H816	4.370	5.080	3.490	4.313	0.797	163	165	154	161	5.8
	H817	0.810	0.770	0.790	0.790	0.020	153	156	167	159	7.3
	H819	0.824	0.938	0.798	0.853	0.074	163	163	159	162	2.3
	H820	1.071	0.793	0.888	0.918	0.141	164	166	166	165	1.3
	H821	0.870	0.880	0.870	0.873	0.006	159	160	159	159	0.6
	H822	1.120	1.040	1.040	1.067	0.046	164	165	164	164	0.6
	H825	0.840	0.840	0.860	0.847	0.012	170	170	170	170	0.0
	H828										
	H829										
	H831	0.830	0.820	0.830	0.827	0.006	177	172	160	169	8.9
	H833	0.880	0.860	0.860	0.867	0.012	177	177	174	176	1.9
	H836	0.787	0.855	0.752	0.798	0.052	168	170	170	170	1.4
	H840	0.878	0.874	0.863	0.872	0.008	179	164	176	173	7.6
	H843										
	H844	0.780	0.773	0.775	0.776	0.003	169	171	170	170	1.0
	H847	0.727	0.678	0.724	0.710	0.027	169	169	169	169	0.2
	H848	0.751	0.764	0.757	0.757	0.007	168	169	170	169	0.8
	H849	0.855	0.867	0.873	0.865	0.009	169	174		172	3.6
	H850	0.833	0.846	0.841	0.840	0.007	173	172	171	172	0.8
	H852	0.857	0.852	0.860	0.857	0.004	184	189	183	185	3.4
	H853	0.813	0.822	0.786	0.807	0.018	214	216	212	214	2.3
	H854										
	H857	0.573	0.585	0.577	0.578	0.006	192	181	177	184	7.8
	H858	1.441	1.420	1.365	1.409	0.039	267	268	269	268	0.7
	H861										
	H862	1.001	0.917	1.026	0.981	0.057	162	167	159	163	3.9
	H863	1.800	1.760	1.600	1.720	0.106	173	174	174	173	0.7
	H864										
	H866										
	H867	0.680	0.620	0.667	0.656	0.031	71	70	88	76	10.0
	H868	0.666	0.646	0.690	0.667	0.022	119	117	119	118	1.2
	H869										
	H870	0.864	0.770	0.910	0.848	0.071	188	105	188	160	47.9
	H871	0.670	0.620	0.660	0.650	0.026	167	166	165	166	0.7
	H873										
	H874	0.800	0.800	0.800	0.800	0.000	186			186	
	H875	0.830	0.770	0.800	0.800	0.030	152	151	138	147	8.1
Community Results		Consensus Mean			0.852		Consensus Mean			170	
		Consensus Standard Deviation			0.137		Consensus Standard Deviation			10.6	
		Maximum			4.313		Maximum			268	
		Minimum			0.578		Minimum			76	
		N			37		N			36	

Table 3. Data summary table for copper in foods and dietary supplements.

		Copper									
		SRM 1566b Oyster Tissue (mg/g)					SRM 3532 Calcium Dietary Supplement (mg/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	NIST				0.0695	0.0016				0.270	0.014
	H802										
	H803										
	H804	0.0619	0.0595	0.0618	0.0611	0.0014	0.245	0.245	0.242	0.244	0.002
	H805	0.0676	0.0667	0.0674	0.0672	0.0005	0.274	0.286	0.278	0.279	0.006
	H806	0.0749	0.0743	0.0744	0.0745	0.0003	0.311	0.314	0.317	0.314	0.003
	H807	0.0657	0.0660	0.0676	0.0664	0.0010	0.256	0.255	0.258	0.256	0.002
	H808	0.0640	0.0654	0.0642	0.0645	0.0008	0.235	0.233	0.237	0.235	0.002
	H809										
	H810	0.0707	0.0704	0.0702	0.0704	0.0003	0.277	0.272	0.272	0.274	0.003
	H811	0.0600	0.0600	0.0596	0.0599	0.0002	0.211	0.212	0.215	0.213	0.002
	H812	0.0760	0.0770	0.0750	0.0760	0.0010	0.299	0.301	0.300	0.300	0.001
	H814	0.0800	0.0740	0.0780	0.0773	0.0031	0.331	0.326	0.322	0.326	0.005
	H816	0.0650	0.0650	0.0650	0.0650	0.0000	0.270	0.280	0.280	0.277	0.006
	H817	0.0700	0.0700	0.0700	0.0700	0.0000	0.270	0.260	0.270	0.267	0.006
	H819	0.0728	0.0827	0.0713	0.0756	0.0062	0.309	0.305	0.301	0.305	0.004
	H820	0.0647	0.0637	0.0655	0.0646	0.0009	0.314	0.320	0.315	0.316	0.003
	H821	0.0790	0.0770	0.0780	0.0780	0.0010	0.245	0.253	0.246	0.248	0.004
	H822	0.0714	0.0718	0.0683	0.0705	0.0019	0.245	0.266	0.259	0.257	0.011
	H825	0.0710	0.0720	0.0740	0.0723	0.0015	0.220	0.220	0.220	0.220	0.000
	H828										
	H829										
	H831	0.0700	0.0700	0.0700	0.0700	0.0000	0.280	0.280	0.280	0.280	0.000
	H833	0.0700	0.0700	0.0700	0.0700	0.0000	0.270	0.270	0.270	0.270	0.000
	H836	0.0674	0.0692	0.0663	0.0676	0.0015	0.276	0.273	0.277	0.275	0.002
	H840	0.0759	0.0759	0.0190	0.0569	0.0329	0.295	0.288	0.286	0.290	0.005
	H843										
	H844	0.0721	0.0719	0.0720	0.0720	0.0001	0.307	0.311	0.315	0.311	0.004
	H847	0.0610	0.0620	0.0600	0.0610	0.0010	0.359	0.375	0.370	0.368	0.008
	H848	0.0550	0.0550	0.0570	0.0557	0.0012	0.308	0.327	0.329	0.321	0.012
	H849	0.0729	0.0744	0.0749	0.0741	0.0010	0.273	0.266		0.270	0.005
	H850	0.0726	0.0731	0.0732	0.0730	0.0003	0.298	0.299	0.300	0.299	0.001
	H852	0.0701	0.0690	0.0700	0.0697	0.0006	0.267	0.270	0.267	0.268	0.002
	H853	0.0653	0.0642	0.0619	0.0638	0.0017	0.324	0.327	0.326	0.326	0.002
	H854										
	H857	0.0700	0.0710	0.0690	0.0700	0.0010	0.280	0.269	0.264	0.271	0.008
	H858	0.0664	0.0677	0.0665	0.0668	0.0007	0.193	0.192	0.193	0.193	0.001
	H861										
	H862	0.0700	0.0700	0.0696	0.0699	0.0002	0.302	0.304	0.314	0.307	0.006
	H863	0.0531	0.0531	0.0575	0.0546	0.0025	0.308	0.298	0.292	0.299	0.008
	H864										
	H866										
	H867	0.0679	0.0735	0.0648	0.0687	0.0044	0.237	0.171	0.323	0.244	0.076
	H868	0.0640	0.0641	0.0626	0.0636	0.0008	0.231	0.245	0.239	0.238	0.007
	H869										
	H870	0.0516	0.0551	0.0538	0.0535	0.0018	0.149	0.143	0.120	0.137	0.015
	H871	0.0590	0.0610	0.0620	0.0607	0.0015	0.260	0.260	0.230	0.250	0.017
	H873	0.0671	0.0579	0.0589	0.0613	0.0050	0.202	0.205	0.210	0.206	0.004
	H874	0.0672	0.0657	0.0669	0.0666	0.0008	0.242			0.242	
	H875	0.0640	0.0660	0.0640	0.0647	0.0012	0.290	0.280	0.290	0.287	0.006
Community Results		Consensus Mean			0.0673		Consensus Mean			0.273	
		Consensus Standard Deviation			0.0065		Consensus Standard Deviation			0.040	
		Maximum			0.0780		Maximum			0.368	
		Minimum			0.0535		Minimum			0.137	
		N			38		N			37	

Table 4. Data summary table for manganese in foods and dietary supplements.

		Manganese									
		SRM 1566b Oyster Tissue (mg/g)					SRM 3532 Calcium Dietary Supplement (mg/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	NIST				0.0180	0.0002				0.530	0.027
	H802										
	H803										
	H804	0.0178	0.0169	0.0173	0.0173	0.0004	0.470	0.506	0.465	0.480	0.022
	H805	0.0179	0.0178	0.0180	0.0179	0.0001	0.469	0.451	0.446	0.455	0.012
	H806	0.0190	0.0183	0.0183	0.0185	0.0004	0.463	0.455	0.455	0.458	0.005
	H807	0.0160	0.0163	0.0159	0.0161	0.0002	0.535	0.503	0.522	0.520	0.016
	H808	0.0158	0.0164	0.0159	0.0160	0.0003	0.462	0.473	0.461	0.465	0.007
	H809										
	H810	0.0181	0.0181	0.0179	0.0180	0.0001	0.533	0.500	0.498	0.510	0.020
	H811	0.0220	0.0209	0.0206	0.0212	0.0007	0.503	0.495	0.489	0.495	0.007
	H812										
	H814	0.0840	0.0820	0.0820	0.0827	0.0012	0.529	0.522	0.535	0.529	0.007
	H816	0.0450	0.0200	0.0200	0.0283	0.0144	0.520	0.500	0.610	0.543	0.059
	H817	0.0199	0.0195	0.0194	0.0196	0.0003	0.409	0.430	0.433	0.424	0.013
	H819	0.0187	0.0213	0.0184	0.0195	0.0016	0.482	0.513	0.453	0.483	0.030
	H820						0.470	0.460	0.478	0.470	0.009
	H821	0.0200	0.0200	0.0200	0.0200	0.0000	0.475	0.475	0.452	0.467	0.013
	H822										
	H825	0.0180	0.0180	0.0180	0.0180	0.0000	0.500	0.500	0.530	0.510	0.017
	H828										
	H829										
	H831	0.0200	0.0200	0.0200	0.0200	0.0000	0.440	0.430	0.430	0.433	0.006
	H833	0.0200	0.0200	0.0200	0.0200	0.0000	0.440	0.430	0.440	0.437	0.006
	H836	0.0169	0.0163	0.0160	0.0164	0.0005	0.434	0.446	0.439	0.440	0.006
	H840	0.0190	0.0190	0.0187	0.0189	0.0002	0.430	0.423	0.403	0.419	0.014
	H843										
	H844	0.0175	0.0175	0.0175	0.0175	0.0000	0.479	0.478	0.496	0.484	0.010
	H847	0.0160	0.0160	0.0160	0.0160	0.0000	0.573	0.521	0.558	0.551	0.027
	H848	0.0160	0.0170	0.0170	0.0167	0.0006	0.565	0.614	0.638	0.606	0.037
	H849	0.0187	0.0190	0.0189	0.0189	0.0002	0.475	0.486		0.481	0.008
	H850	0.0181	0.0183	0.0182	0.0182	0.0001	0.542	0.510	0.490	0.514	0.026
	H852	0.0183	0.0182	0.0183	0.0183	0.0001	0.525	0.540	0.548	0.538	0.012
	H853	0.0154	0.0151	0.0145	0.0150	0.0004	0.522	0.586	0.567	0.558	0.033
	H854										
	H857	0.0190	0.0190	0.0180	0.0187	0.0006	0.523	0.535	0.559	0.539	0.018
	H858	0.0189	0.0188	0.0183	0.0187	0.0003	0.524	0.527	0.584	0.545	0.034
	H861										
	H862	0.0190	0.0185	0.0188	0.0188	0.0003	0.452	0.518	0.488	0.486	0.033
	H863										
	H864										
	H866										
	H867	0.0132	0.0185	0.0198	0.0172	0.0035	0.391	0.312	0.234	0.312	0.079
	H868	0.0168	0.0170	0.0166	0.0168	0.0002	0.490	0.509	0.511	0.503	0.012
	H869										
	H870	0.0183	0.0178	0.0224	0.0195	0.0025	0.640	0.594	0.706	0.647	0.056
	H871	0.0160	0.0160	0.0160	0.0160	0.0000	0.440	0.480	0.450	0.457	0.021
	H873	0.0189	0.0169	0.0171	0.0176	0.0011	0.410	0.425	0.436	0.423	0.013
	H874	0.0181	0.0175	0.0178	0.0178	0.0003	0.415			0.415	
	H875	0.0160	0.0170	0.0170	0.0167	0.0006	0.430	0.420	0.420	0.423	0.006
Community Results		Consensus Mean			0.0182		Consensus Mean			0.488	
		Consensus Standard Deviation			0.0017		Consensus Standard Deviation			0.054	
		Maximum			0.0827		Maximum			0.647	
		Minimum			0.0150		Minimum			0.312	
		N			34		N			34	

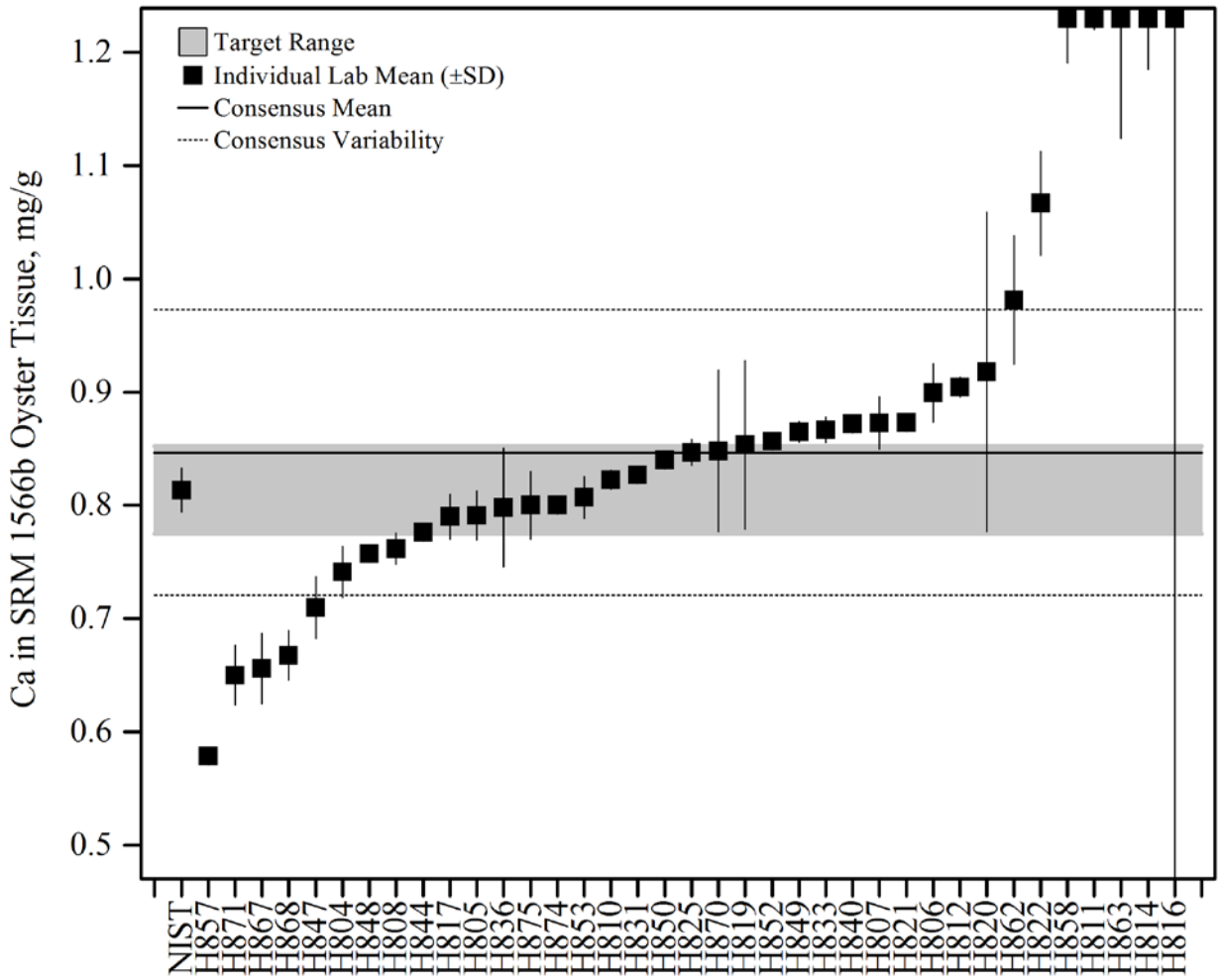

Figure 1. Calcium in SRM 1566b Oyster Tissue (data summary view). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The black solid line represents the consensus mean, and the black dotted lines represent the consensus variability calculated as one standard deviation about the consensus mean. The gray shaded region represents the target zone for "acceptable" performance, which encompasses the NIST certified value bounded by twice its uncertainty (U_{95}).

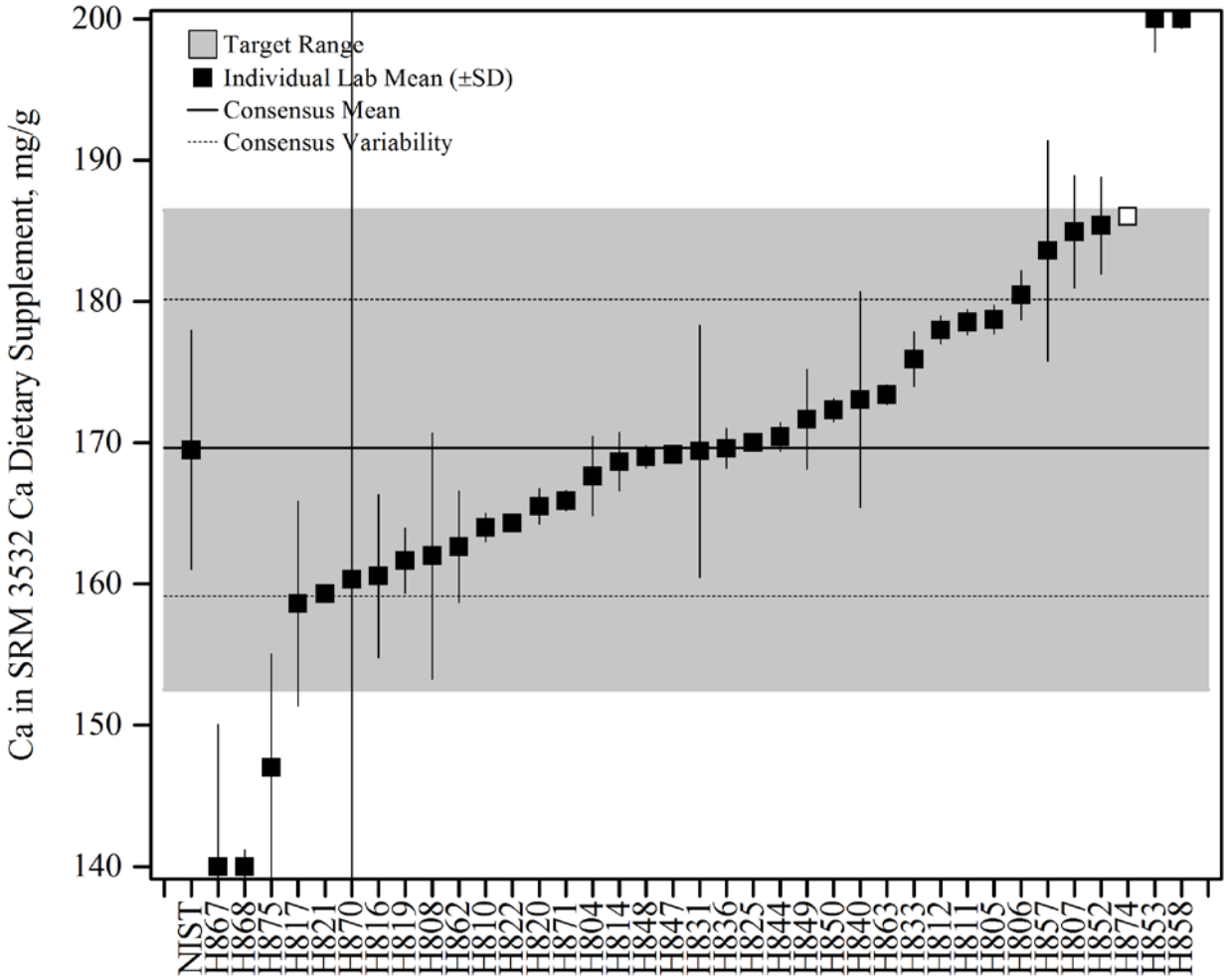

Figure 2. Calcium in candidate SRM 3532 Calcium Dietary Supplement (data summary view). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). Data points that are unfilled represent laboratories that only reported a single value for that 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. The gray shaded region represents the target zone for "acceptable" performance, which encompasses an approximation of the NIST certified value bounded by an approximated uncertainty of 5 %. The approximate certified value is the mean of results from ICP-OES and XRF.

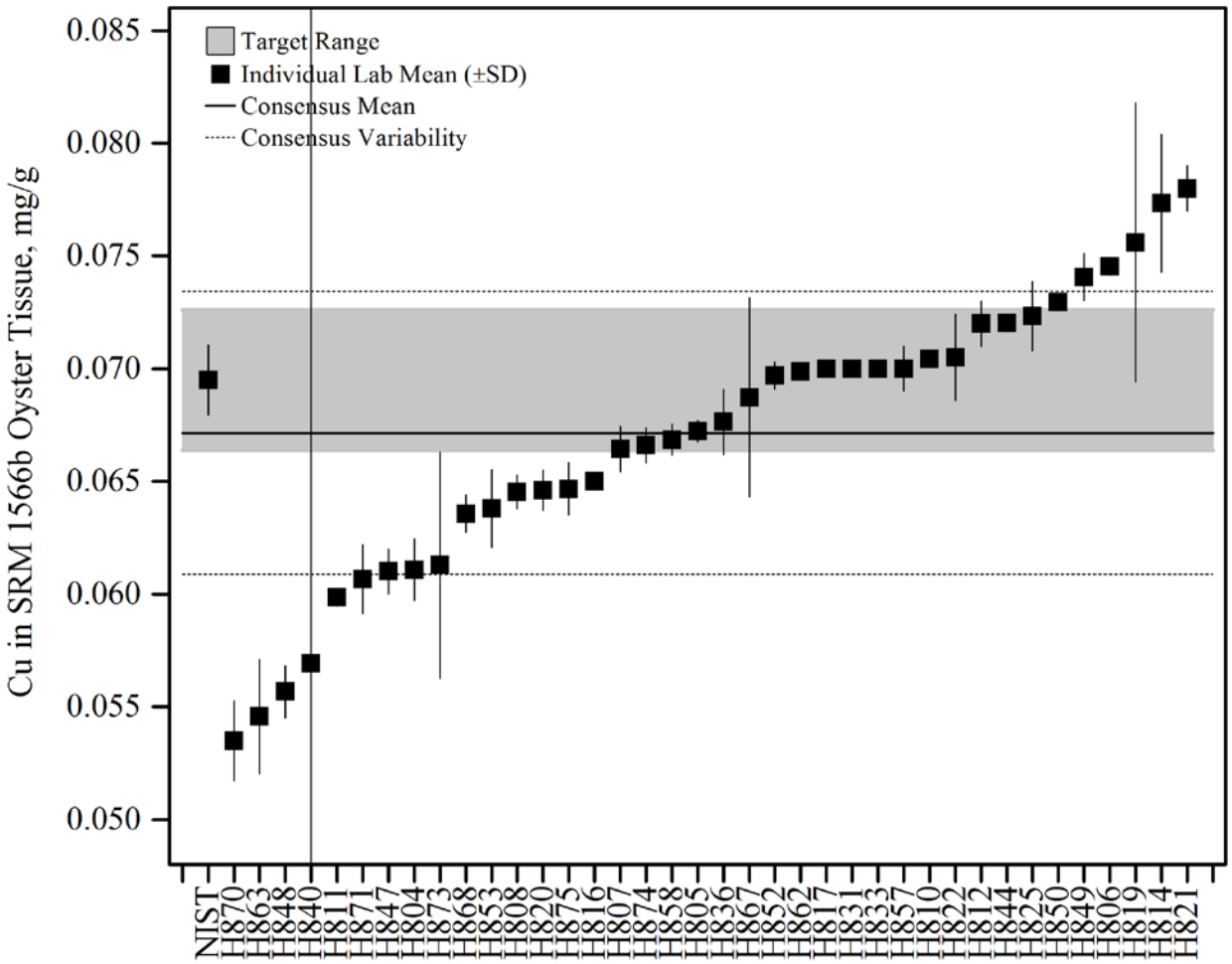

Figure 3. Copper in SRM 1566b Oyster Tissue (data summary view). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The black solid line represents the consensus mean, and the black dotted lines represent the consensus variability calculated as one standard deviation about the consensus mean. The gray shaded region represents the target zone for "acceptable" performance, which encompasses the NIST certified value bounded by twice its uncertainty (U_{95}).

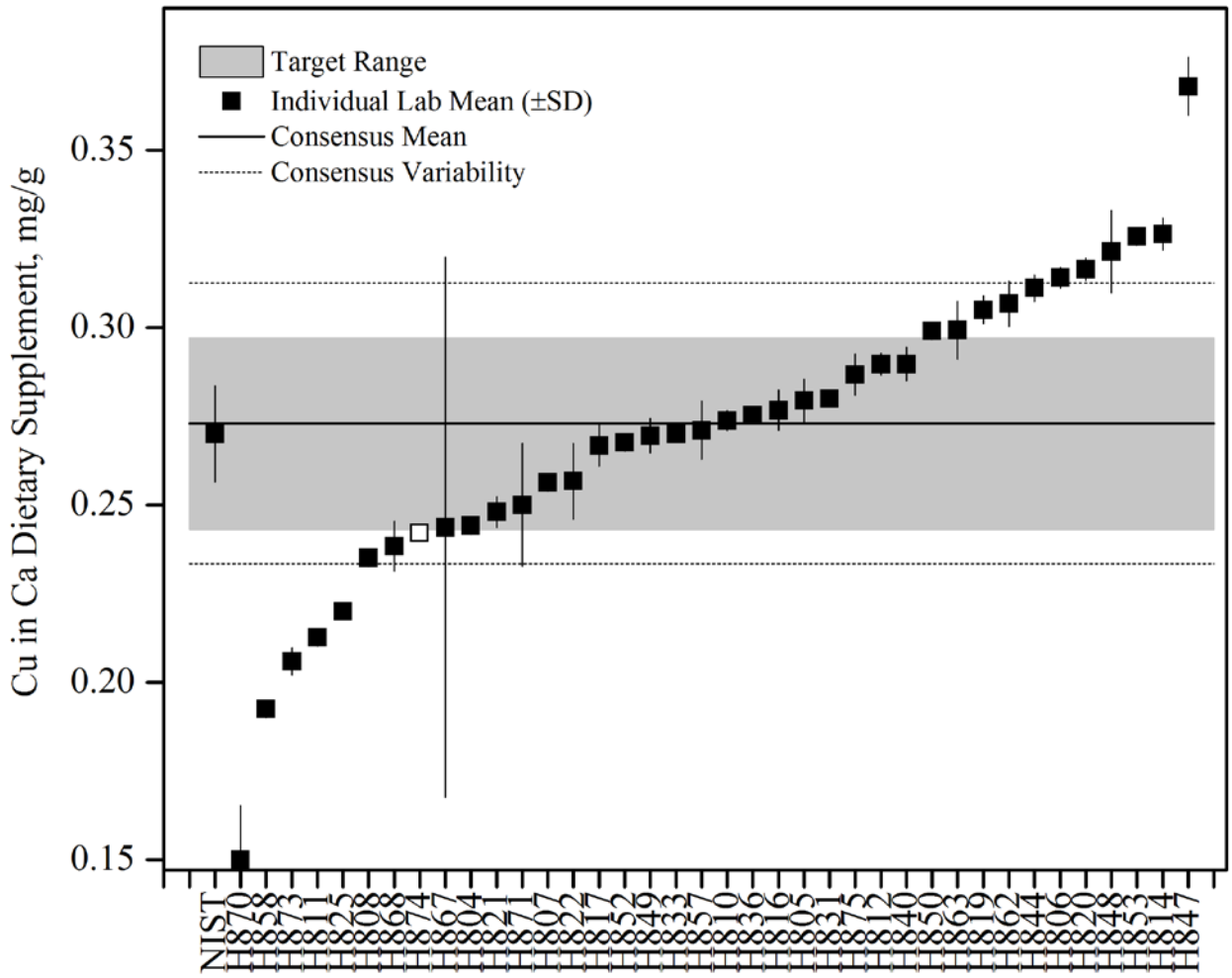

Figure 4. Copper in candidate SRM 3532 Calcium Dietary Supplement (data summary view). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). Data points that are unfilled represent laboratories that only reported a single value for that analyte 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. The gray shaded region represents the target zone for "acceptable" performance, which encompasses an approximation of the NIST certified value bounded by an approximated uncertainty of 5 %. The approximate certified value is the mean of results from ICP-OES and XRF.

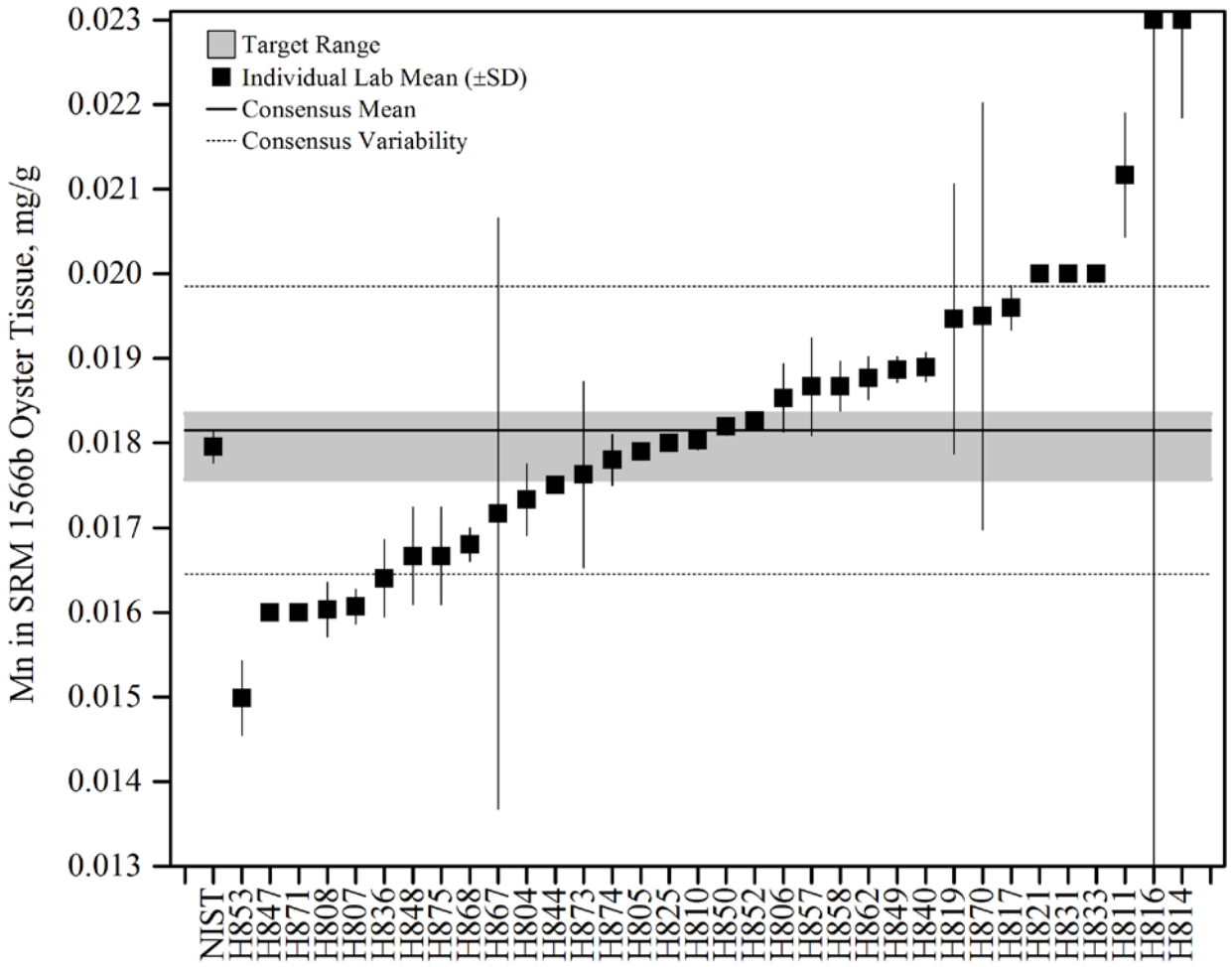

Figure 5. Manganese in SRM 1566b Oyster Tissue (data summary view). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The black solid line represents the consensus mean, and the black dotted lines represent the consensus variability calculated as one standard deviation about the consensus mean. The gray shaded region represents the target zone for "acceptable" performance, which encompasses the NIST certified value bounded by twice its uncertainty (U_{95}).

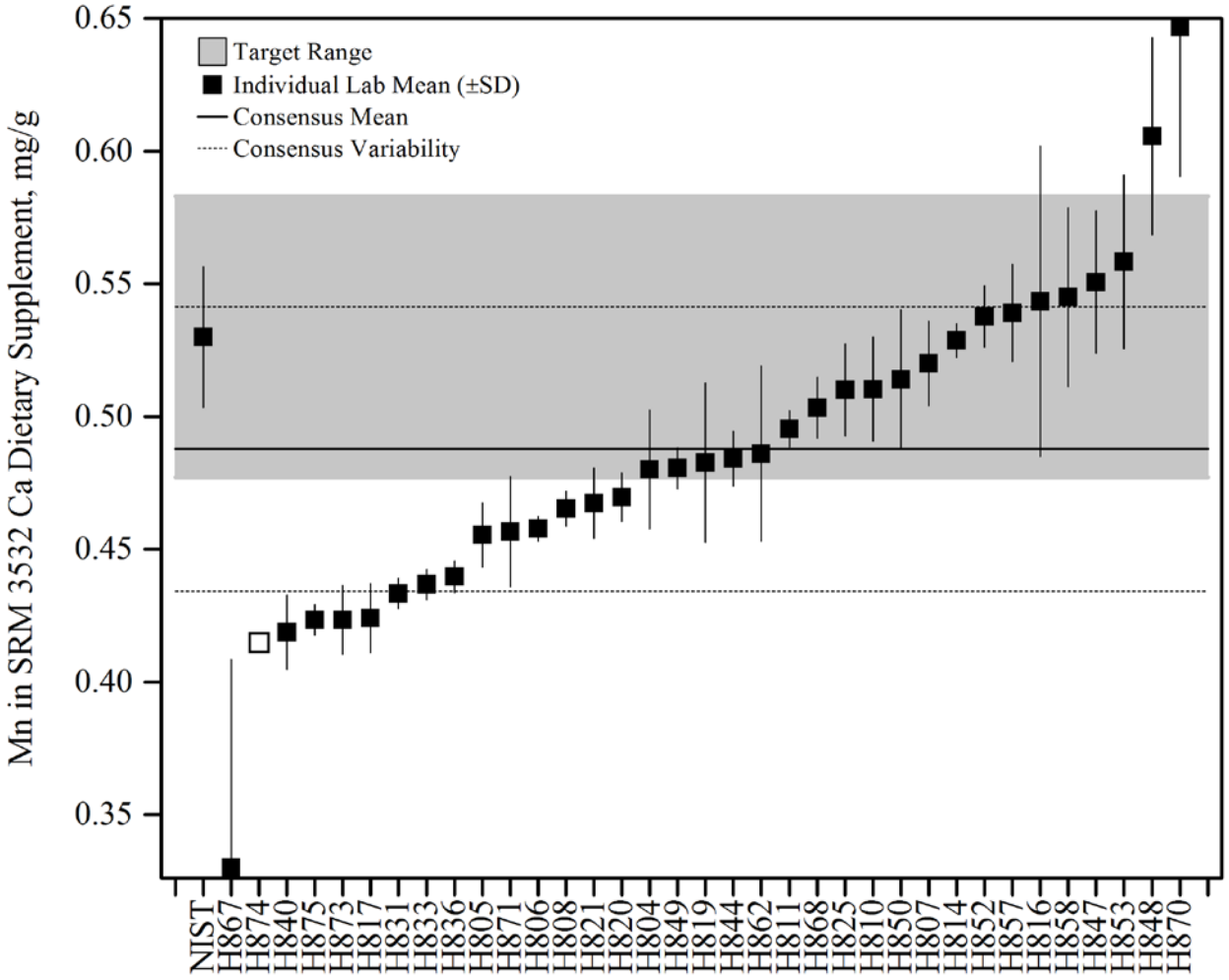

Figure 6. Manganese in candidate SRM 3532 Calcium Dietary Supplement (data summary view). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). Data points that are unfilled represent laboratories that only reported a single value for that 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. The gray shaded region represents the target zone for "acceptable" performance, which encompasses an approximation of the NIST certified value bounded by an approximated uncertainty of 5 %. The approximate certified value is the mean of results from ICP-OES and XRF.

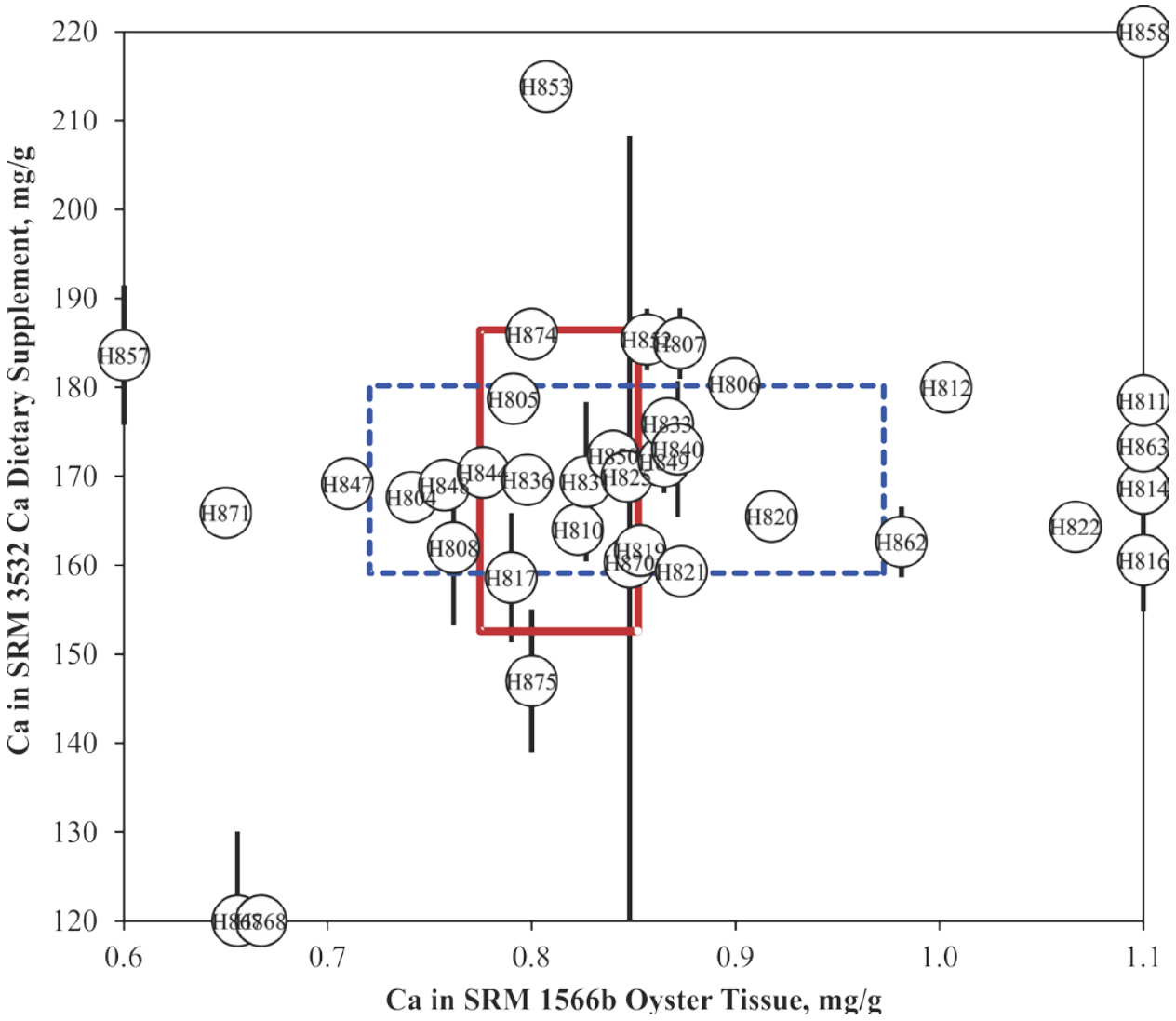

Figure 7. Calcium in SRM 1566b Oyster Tissue and candidate SRM 3532 Calcium Dietary Supplement (sample/sample comparison view). In this view, the individual laboratory results for one sample (SRM 1566b Oyster Tissue) with a certified value for the analyte are compared to the results for a second sample (candidate SRM 3532 Calcium Dietary Supplement). The error bars represent the individual laboratory standard deviation. The solid red lines represent the target zone for the control (x-axis) and unknown (y-axis). The dotted blue box represents the consensus zone for the control (x-axis) and the unknown sample (y-axis).

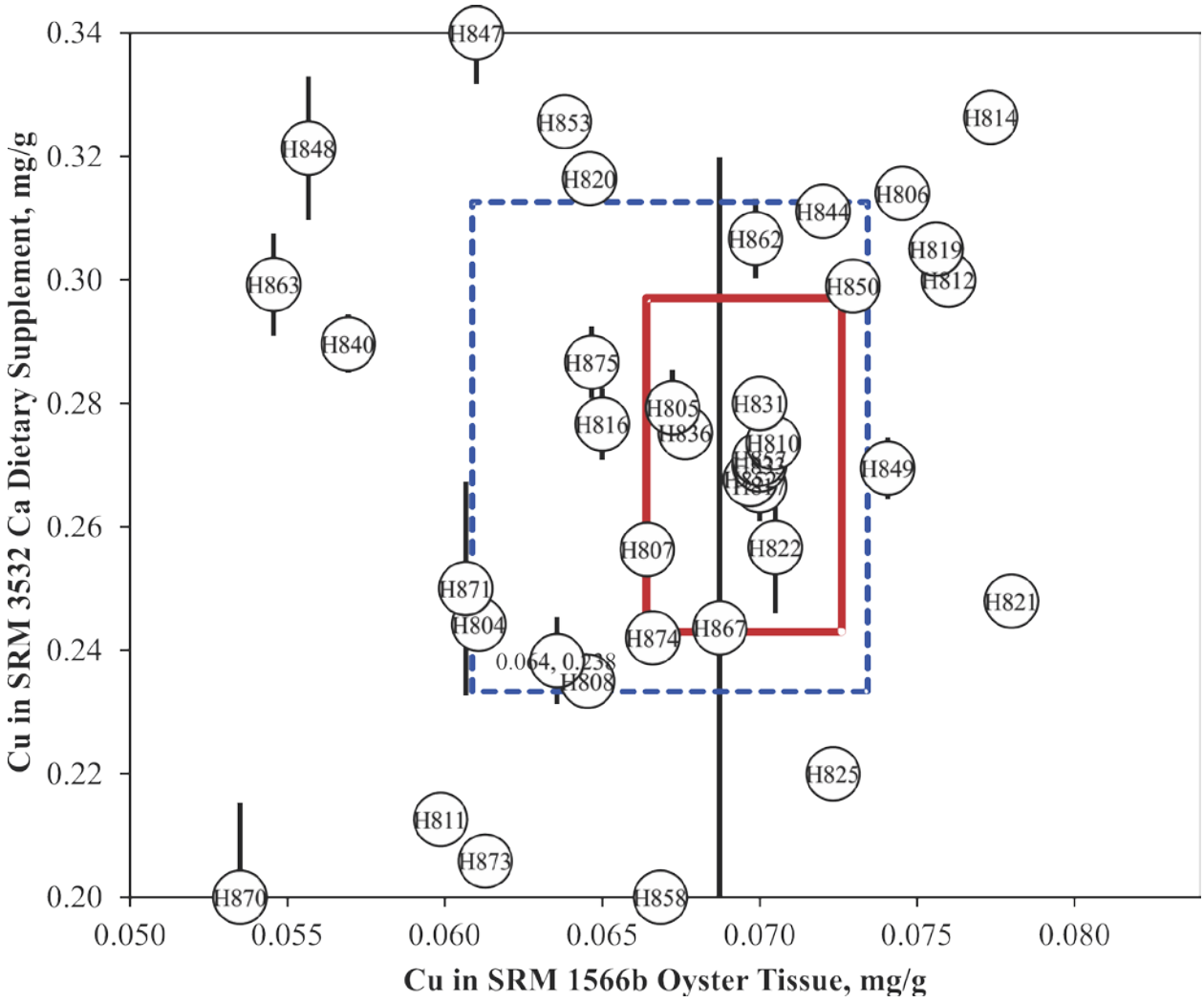

Figure 8. Copper in SRM 1566b Oyster Tissue and candidate SRM 3532 Calcium Dietary Supplement (sample/sample comparison view). In this view, the individual laboratory results for one sample (SRM 1566b Oyster Tissue) with a certified value for the analyte are compared to the results for a second sample (candidate SRM 3532 Calcium Dietary Supplement). The error bars represent the individual laboratory standard deviation. The solid red lines represent the target zone for the control (x-axis) and unknown (y-axis). The dotted blue box represents the consensus zone for the control (x-axis) and the unknown sample (y-axis).

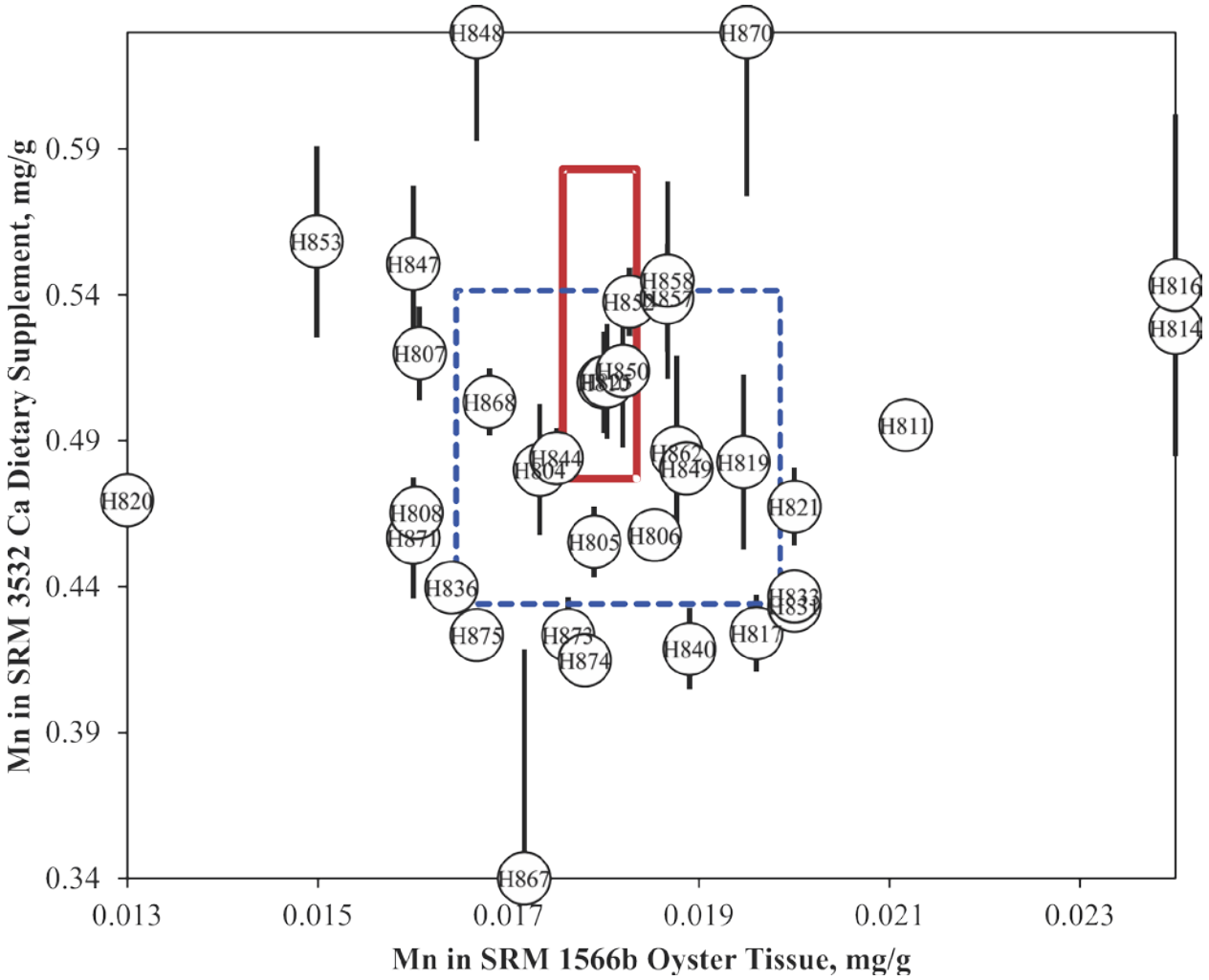

Figure 9. Manganese in SRM 1566b Oyster Tissue and candidate SRM 3532 Calcium Dietary Supplement (sample/sample comparison view). In this view, the individual laboratory results for one sample (SRM 1566b Oyster Tissue) with a certified value for the analyte are compared to the results for a second sample (candidate SRM 3532 Calcium Dietary Supplement). The error bars represent the individual laboratory standard deviation. The solid red lines represent the target zone for the control (x-axis) and unknown (y-axis). The dotted blue box represents the consensus zone for the control (x-axis) and the unknown sample (y-axis).

PAHs IN GREEN TEA

Study Overview

In this study, participants were provided with two NIST SRMs, SRM 1647e PAH Solution and SRM 3254 *Camellia sinensis* (Green Tea) Leaves. Participants were asked to use in-house analytical methods to determine the mass fractions of ten polycyclic aromatic hydrocarbons (PAHs) – naphthalene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benz[*a*]anthracene, chrysene, triphenylene, and benzo[*a*]pyrene) – in each of the matrices and report values on an as-received basis.

Sample Information

Neat solution. Participants were provided with three ampoules, each containing approximately 1.2 mL of an acetonitrile solution of 16 PAHs. The solution was prepared gravimetrically from individual compounds and aliquotted into 2 mL amber glass ampoules, which were purged with argon prior to adding the solution. Before use, participants were instructed to mix thoroughly the contents of the ampoule. Participants were asked to report a single value from each ampoule and store the material at controlled room temperature, 10 °C to 30 °C. Approximate analyte levels were not reported prior to the study. NIST certified values in SRM 1647e were based on the gravimetric preparation with purity assessment of the neat PAHs and analysis using liquid chromatography (LC) with absorbance detection. The certified values and uncertainties for each PAH in SRM 1647e are reported in the table below.

Green tea. Participants were provided with three packets, each containing approximately 3 g of green tea (*Camellia sinensis*) leaves. The green tea leaves were ground, sieved, and heat-sealed inside nitrogen-flushed 0.1 mm (4 mil) polyethylene bags, which were then sealed inside aluminized plastic bags with 2 packets of silica gel. Before use, participants were instructed to thoroughly mix the contents of the packet and use a sample size of at least 0.3 g. Participants were asked to report a single value from each packet and store the material at controlled room temperature, 10 °C to 30 °C. Approximate analyte levels were not reported prior to the study. Values in SRM 3254 were determined by gas chromatography (GC) with MS detection following pressurized-fluid extraction. The estimated values are based on an average and standard deviation of single measurements from three packets and are provided on an as-received basis in the table below.

Analyte	Certified Mass Fraction in SRM 1647e (mg/kg)	NIST-Determined Mass Fraction in SRM 3254 (ng/g)
Naphthalene	25.48 ± 0.58	48.1 ± 4.0
Fluorene	6.09 ± 0.14	12.7 ± 2.6
Phenanthrene	4.52 ± 0.11	102 ± 13
Anthracene	1.01 ± 0.02	4.22 ± 0.69
Fluoranthene	9.73 ± 0.21	47.4 ± 5.1
Pyrene	10.88 ± 0.22	27.7 ± 1.5
Benz[*a*]anthracene	5.25 ± 0.11	4.24 ± 0.25
Chrysene	4.62 ± 0.10	
Chrysene + Triphenylene	15.9 ± 0.4	
Benzo[*a*]pyrene	6.25 ± 0.15	

Study Results

- Twelve laboratories enrolled in this exercise and received samples, and six laboratories reported results for at least some of the PAHs (50 % participation).
- The consensus means for all PAHs in the neat solution were lower than the target range with high variability (35 % to over 100 % RSD).
- The consensus means for all PAHs in the green tea were higher than the target range with high variability (53 % to over 100 % RSD).
- Two laboratories (40 %) reported using pressurized-fluid extraction for sample preparation, two laboratories (40 %) reported using direct injection, and one laboratory (20 %) reported using Soxhlet extraction.
- Three laboratories (60 %) used GC-MS as their analytical method. Two laboratories (40 %) reported using GC with flame ionization detection (FID).
- Three laboratories (60 %) reported using an internal standard approach to calibration, and two laboratories (40 %) reported using an external standard approach to calibration.

Technical Recommendations

While more data is needed to draw strong conclusions about results of this study, the following recommendations are based on results that were obtained by the participants in this study.

- Low values obtained for the neat solution could be the result of improper calibration.
- Low values obtained for the neat solution could also be the result of excessive sample preparation. The neat solution only required dilution prior to injection.
- High results for the green tea sample could be a result of improper calibration. This is especially true if the differences between certified values and measured values for the control (SRM 1647e) were used to calculate a correction factor. Correction factors frequently lead to biased results due to differences in matrix effects.

Table 5. Individual data table (NIST) for PAHs in green tea.

National Institute of Standards & Technology

Exercise H - March 2012 - Contaminants (PAHs)

	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}
Naphthalene	Solution	ng/g	25500	580	1.0	0.0	4	15100	10300	25480	580
Naphthalene	Green Tea	ng/g	48.1	4.0	-1.1	0.0	2	464	362	48.1	4.0
Fluorene	Solution	ng/g	6090	140	1.2	0.0	5	2910	2730	6090	140
Fluorene	Green Tea	ng/g	12.7	2.6	-1.5	0.0	4	319	203	12.7	2.6
Phenanthrene	Solution	ng/g	4520	110	1.0	0.0	5	2330	2200	4520	110
Phenanthrene	Green Tea	ng/g	102.0	13.0	-0.5	0.0	4	188.0	178.0	102.0	13.0
Anthracene	Solution	ng/g	1010	20	0.9	0.0	5	550	522	1010	20
Anthracene	Green Tea	ng/g	4.22	0.69	-1.8	0.0	3	56.10	28.50	4.22	0.69
Fluoranthene	Solution	ng/g	9730	210	0.6	0.0	3	8090	2790	9730	210
Fluoranthene	Green Tea	ng/g	47.4	5.1	-0.6	0.0	2	146.0	167.0	47.4	5.1
Pyrene	Solution	ng/g	10900	220	0.9	0.1	5	5690	5720	10880	220
Pyrene	Green Tea	ng/g	27.7	1.5	-0.6	0.0	2	97.5	111.0	27.7	1.5
Benz(a)anthracene	Solution	ng/g	5250	110	0.9	0.0	6	2950	2610	5250	110
Benz(a)anthracene	Green Tea	ng/g	4.24	0.25	-1.0	0.0	5	49.50	44.50	4.24	0.25
Chrysene	Solution	ng/g	4620	100	0.7	0.0	6	2820	2460	4620	100
Chrysene	Green Tea	ng/g					5	70	87		
Triphenylene	Solution	ng/g					1				
Triphenylene	Green Tea	ng/g					1				
Chrysene+Triphenyl	Solution	ng/g	4620	100	0.5	0.0	6	3290	2800	4620	100
Chrysene+Triphenyl	Green Tea	ng/g	15.9	0.4	-0.7	0.0	5	72.4	84.5	15.9	0.4
Benzo(a)pyrene	Solution	ng/g	6250	150	0.7	0.0	5	4300	2730	6250	150
Benzo(a)pyrene	Green Tea	ng/g					2	6	6		

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 6. Data summary table for naphthalene in green tea.

		Naphthalene									
		SRM 1647e PAH Solution (ng/g)					SRM 3254 Green Tea (ng/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	NIST				25480	580				48.1	4.0
	H803										
	H810										
	H813										
	H818	12968	12199	12308	12492	416	115	330	270	238	111
	H819	21700	20400	20300	20800	781					
	H821										
	H843										
	H847	3288	3580	3759	3542	238					
	H848										
	H862										
	H865										
	H873	23310	23920	23930	23720	355	660	720	690	690	30
Community Results		Consensus Mean			15138		Consensus Mean			464	
		Consensus Standard Deviation			10293		Consensus Standard Deviation			362	
		Maximum			23720		Maximum			690	
		Minimum			3542		Minimum			238	
		N			4		N			2	

Table 7. Data summary table for fluorene in green tea.

		Fluorene									
		SRM 1647e PAH Solution (ng/g)					SRM 3254 Green Tea (ng/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	NIST				6090	140				12.7	2.6
	H803										
	H810										
	H813										
	H818	3636	3497	3442	3525	100	90	90	100	93.3	5.8
	H819	5080	5100	5260	5147	99					
	H821										
	H843										
	H847	328	357	363	349	19	534	446	499	493.1	44.4
	H848	464	339	379	394	64	445	424	408	425.7	18.6
	H862										
	H865										
	H873	4920	5570	4910	5133	378	240	280	270	263.3	20.8
Community Results		Consensus Mean			2910		Consensus Mean			319	
		Consensus Standard Deviation			2732		Consensus Standard Deviation			203	
		Maximum			5147		Maximum			493	
		Minimum			349		Minimum			93	
		N			5		N			4	

Table 8. Data summary table for phenanthrene in green tea.

		Phenanthrene									
		SRM 1647e PAH Solution (ng/g)					SRM 3254 Green Tea (ng/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	NIST				4520	110				102.0	13.0
	H803										
	H810										
	H813										
	H818	3054	2836	2821	2903	130	95	105	95	98.3	5.8
	H819	4130	4020	4200	4117	91					
	H821										
	H843										
	H847	261	258	259	259	2	76	120	143	113.0	34.0
	H848	331	255	268	285	40	122	109	119	116.7	6.8
	H862										
	H865										
	H873	4010	4060	4190	4087	93	390	440	440	423.3	28.9
Community Results		Consensus Mean			2330		Consensus Mean			188	
		Consensus Standard Deviation			2202		Consensus Standard Deviation			178	
		Maximum			4117		Maximum			423	
		Minimum			259		Minimum			98	
		N			5		N			4	

Table 9. Data summary table for anthracene in green tea.

		Anthracene									
		SRM 1647e PAH Solution (ng/g)					SRM 3254 Green Tea (ng/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	NIST				1010	20				4.2	0.7
	H803										
	H810										
	H813										
	H818	793	800	668	754	74	ND	ND	ND		
	H819	923	897	949	923	26					
	H821										
	H843										
	H847	53	52	50	52	2	124.4	23.0	28.0	58.5	57.2
	H848	65	52	50	56	8	30.8	29.4	29.7	30.0	0.7
	H862										
	H865										
	H873	890	990	1020	967	68	80.0	70.0	90.0	80.0	10.0
Community Results		Consensus Mean			550		Consensus Mean			56.1	
		Consensus Standard Deviation			522		Consensus Standard Deviation			28.5	
		Maximum			967		Maximum			80.0	
		Minimum			52		Minimum			30.0	
		N			5		N			3	

Table 10. Data summary table for fluoranthene in green tea.

		Fluoranthene									
		SRM 1647e PAH Solution (ng/g)					SRM 3254 Green Tea (ng/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	NIST				9730	210				47.4	5.1
	H803										
	H810										
	H813										
	H818	5742	4965	5035	5247	430	40.0	45.0	40.0	42	3
	H819	9200	9460	10100	9587	463					
	H821										
	H843										
	H847										
	H848										
	H862										
	H865										
	H873	9950	9300	9030	9427	473	260.0	250.0	240.0	250	10
Community Results		Consensus Mean			8087		Consensus Mean			146	
		Consensus Standard Deviation			2790		Consensus Standard Deviation			167	
		Maximum			9587		Maximum			250	
		Minimum			5247		Minimum			41.7	
		N			3		N			2	

Table 11. Data summary table for pyrene in green tea.

		Pyrene									
		SRM 1647e PAH Solution (ng/g)					SRM 3254 Green Tea (ng/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	NIST				10880	220				27.7	1.5
	H803										
	H810										
	H813										
	H818	6301	5478	5517	5765	465	25.0	30.0	30.0	28.3	2.9
	H819	10600	10800	11400	10933	416					
	H821										
	H843										
	H847	644	620	570	611	38					
	H848	753	583	609	648	92					
	H862										
	H865										
	H873	10370	10680	10380	10477	176	180.0	160.0	160.0	166.7	11.5
Community Results		Consensus Mean			5687		Consensus Mean			97.5	
		Consensus Standard Deviation			5716		Consensus Standard Deviation			111	
		Maximum			10933		Maximum			167	
		Minimum			611		Minimum			28.3	
		N			5		N			2	

Table 12. Data summary table for benz[*a*]anthracene in green tea.

		Benz(a)anthracene									
		SRM 1647e PAH Solution (ng/g)					SRM 3254 Green Tea (ng/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	NIST				5250	110				4.2	0.3
	H803										
	H810										
	H813										
	H818	2890	2580	2587	2686	177	15.0	ND	10.0	12.5	3.5
	H819	5330	5620	5900	5617	285					
	H821										
	H843										
	H847	334	270	228	277	53	66.4	79.0	99.0	81.5	16.4
	H848	352	272	271	298	46	63.7	72.5	56.2	64.1	8.2
	H862										
	H865	3731	3852	3855	3813	71	2.8	2.9	3.0	2.9	0.1
	H873	5320	4640	5110	5023	348	90.0	80.0	90.0	86.7	5.8
Community Results		Consensus Mean			2952		Consensus Mean			49.5	
		Consensus Standard Deviation			2606		Consensus Standard Deviation			44.5	
		Maximum			5617		Maximum			86.7	
		Minimum			277		Minimum			2.9	
		N			6		N			5	

Table 13. Data summary table for chrysene in green tea.

		Chrysene									
		SRM 1647e PAH Solution (ng/g)					SRM 3254 Green Tea (ng/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	NIST				4620	100					
	H803										
	H810										
	H813										
	H818	2929	2735	2852	2839	98	10.0	ND	10.0	10.0	0.0
	H819	4330	4540	4460	4443	106					
	H821										
	H843										
	H847	299	251	207	253	46	39.5	46.0	68.0	51.2	14.9
	H848	331	252	258	280	44	149.1	228.0	206.0	194.4	40.7
	H862										
	H865	3470	3551	3661	3561	96	9.4	9.4	10.0	9.6	0.4
	H873	6110	5570	4890	5523	611	90.0	80.0	90.0	86.7	5.8
Community Results		Consensus Mean			2816		Consensus Mean			70.4	
		Consensus Standard Deviation			2460		Consensus Standard Deviation			86.6	
		Maximum			5523		Maximum			194	
		Minimum			253		Minimum			9.6	
		N			6		N			5	

Table 14. Data summary table for triphenylene in green tea.

		Triphenylene									
		SRM 1647e PAH Solution (ng/g)					SRM 3254 Green Tea (ng/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	NIST										
	H803										
	H810										
	H813										
	H818	2929	2735	2852	2839	98	10.0	ND	10.0	10.00	0.00
	H819										
	H821										
	H843										
	H847										
	H848										
	H862										
	H865										
	H873										
Community Results		Consensus Mean					Consensus Mean				
		Consensus Standard Deviation					Consensus Standard Deviation				
		Maximum					Maximum				
		Minimum					Minimum				
		N			1		N			1	

Table 15. Data summary table for chrysene + triphenylene in green tea.

		Chrysene + Triphenylene									
		SRM 1647e PAH Solution (ng/g)					SRM 3254 Green Tea (ng/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	NIST				4620	100				15.9	0.4
	H803										
	H810										
	H813										
	H818	5859	5470	5703	5677	196	20.0		20.0	20.0	0.0
	H819	4330	4540	4460	4443	106					
	H821										
	H843										
	H847	299	251	207	253	46	39.5	46.0	68.0	51.2	14.9
	H848	331	252	258	280	44	149.1	228.0	206.0	194.4	40.7
	H862										
	H865	3470	3551	3661	3561	96	9.4	9.4	10.0	9.6	0.4
	H873	6110	5570	4890	5523	611	90.0	80.0	90.0	86.7	5.8
Community Results		Consensus Mean			3290		Consensus Mean			72.4	
		Consensus Standard Deviation			2795		Consensus Standard Deviation			84.5	
		Maximum			5677		Maximum			194	
		Minimum			253		Minimum			9.6	
		N			6		N			5	

Table 16. Data summary table for benzo[*a*]pyrene in green tea.

		Benzo(a)pyrene									
		SRM 1647e PAH Solution (ng/g)					SRM 3254 Green Tea (ng/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	NIST				6250	150					
	H803										
	H810										
	H813										
	H818	4033	3629	3800	3820	203	10.0	10.0	10.0	10.0	0.0
	H819	6020	6740	6980	6580	500					
	H821										
	H843										
	H847	497	378	330	402	86					
	H848										
	H862										
	H865	4722	5041	5057	4940	189	1.8	2.2	2.2	2.1	0.2
	H873	5910	5860	5480	5750	235					
Community Results		Consensus Mean			4298		Consensus Mean			6.0	
		Consensus Standard Deviation			2727		Consensus Standard Deviation			6.4	
		Maximum			6580		Maximum			10.0	
		Minimum			402		Minimum			2.1	
		N			5		N			2	

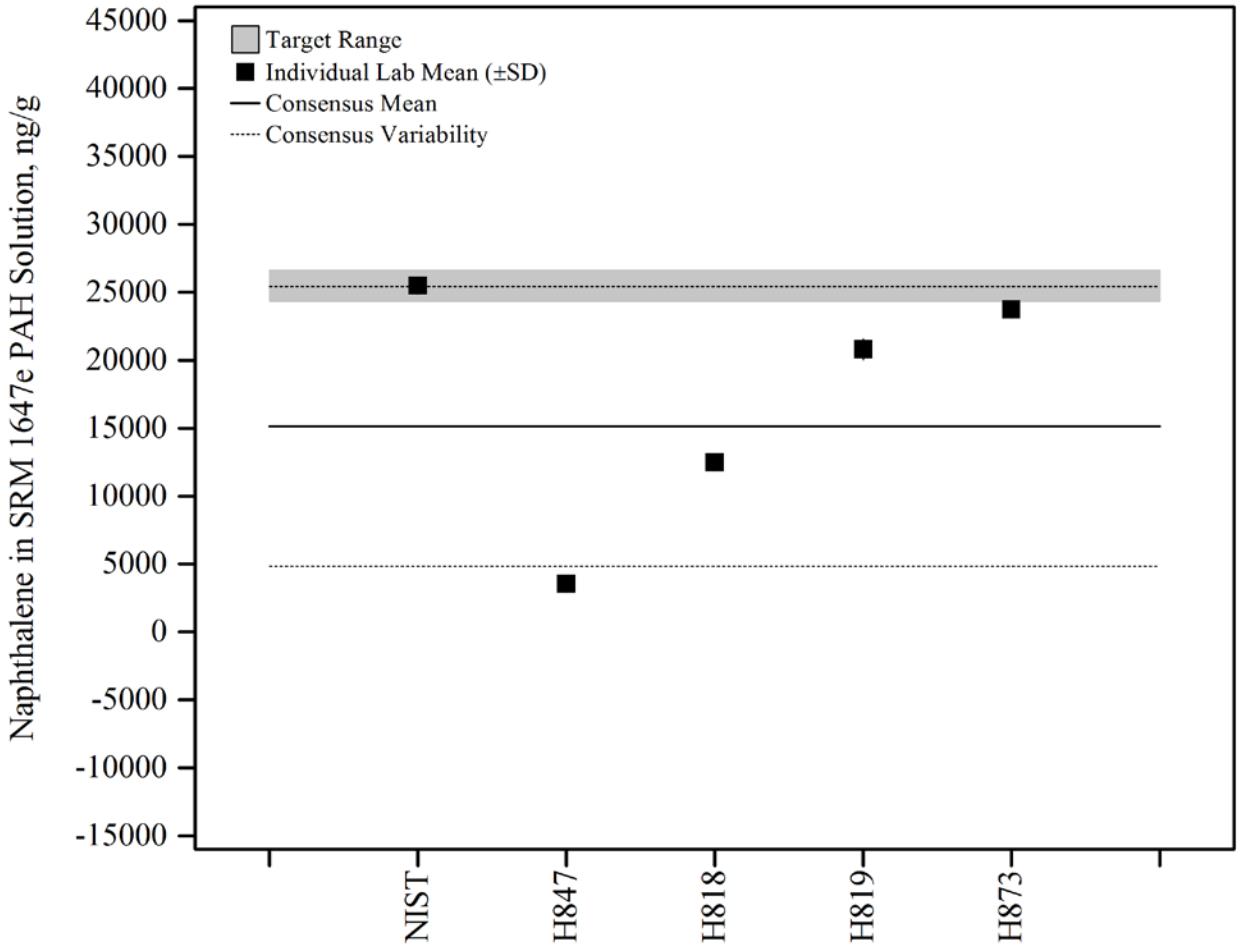

Figure 10. Naphthalene in SRM 1647e PAH Solution (data summary view). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The black solid line represents the consensus mean, and the black dotted lines represent the consensus variability calculated as one standard deviation about the consensus mean. The gray shaded region represents the target zone for "acceptable" performance, which encompasses the NIST certified value bounded by twice its uncertainty (U_{95}).

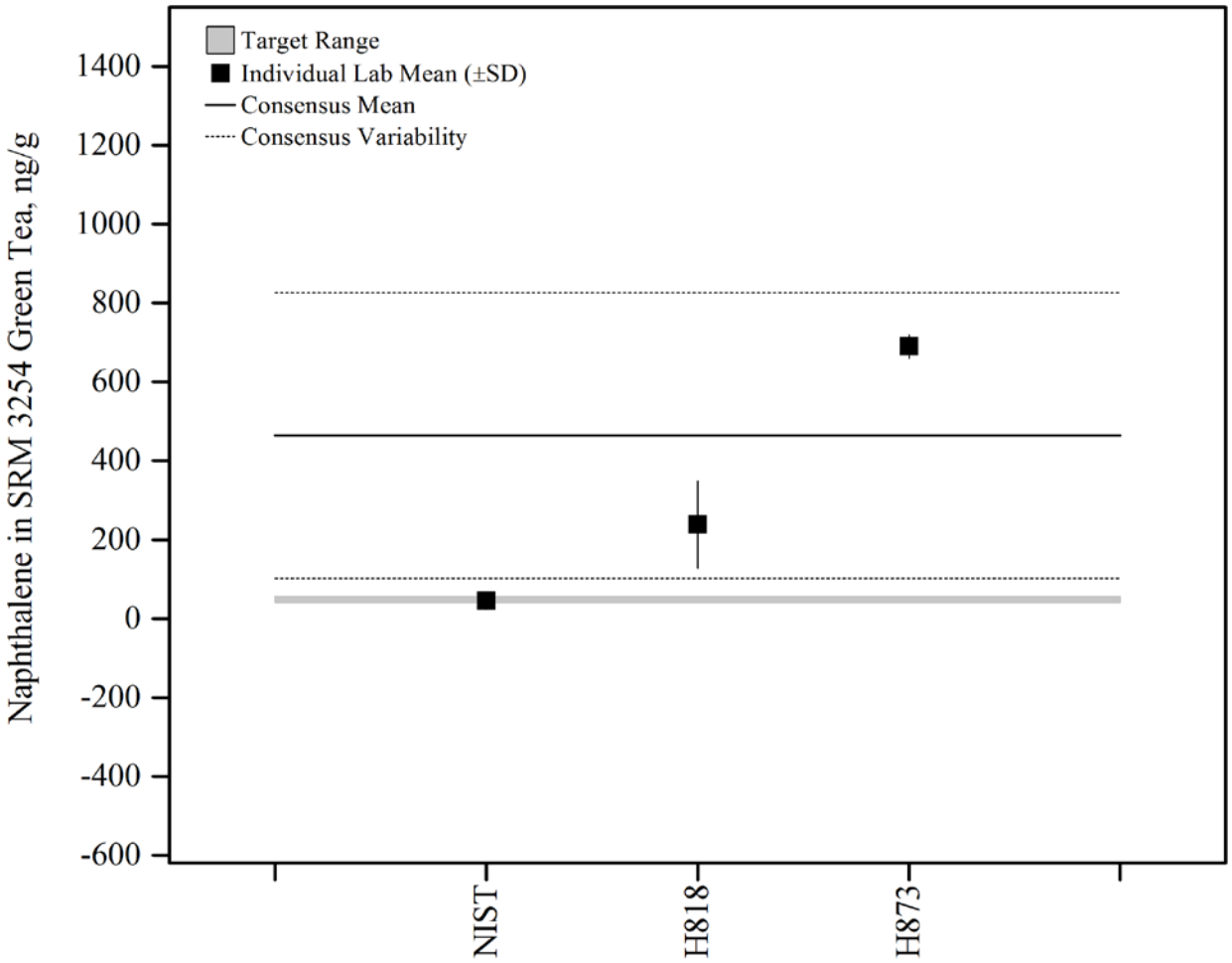

Figure 11. Naphthalene in SRM 3254 *Camellia sinensis* (Green Tea) Leaves (data summary view). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The black solid line represents the consensus mean, and the black dotted lines represent 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 value determined by GC-MS, bounded by twice the standard deviation observed for three measurements.

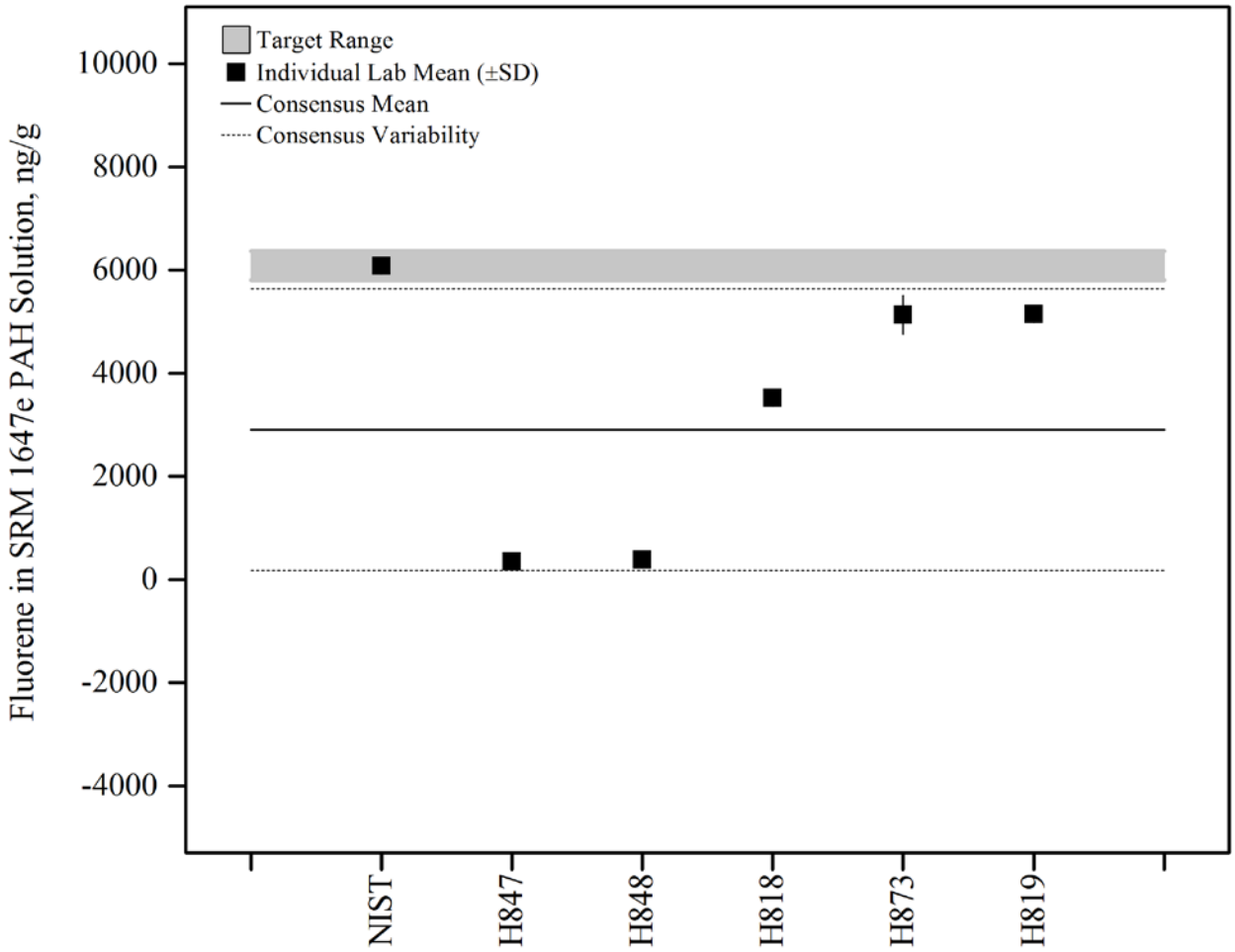

Figure 12. Fluorene in SRM 1647e PAH Solution (data summary view). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The black solid line represents the consensus mean, and the black dotted lines represent the consensus variability calculated as one standard deviation about the consensus mean. The gray shaded region represents the target zone for "acceptable" performance, which encompasses the NIST certified value bounded by twice its uncertainty (U_{95}).

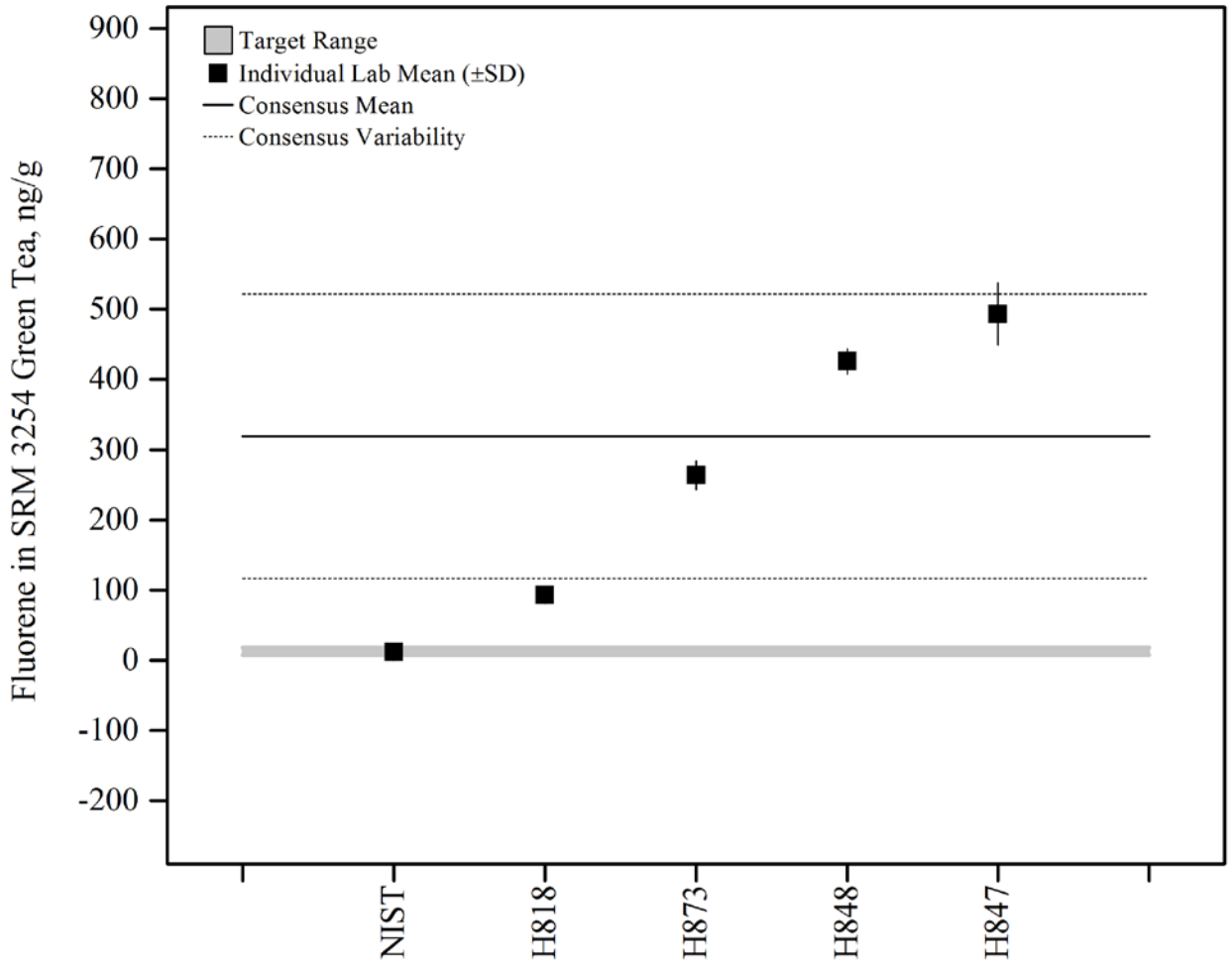

Figure 13. Fluorene in SRM 3254 *Camellia sinensis* (Green Tea) Leaves (data summary view). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The black solid line represents the consensus mean, and the black dotted lines represent the 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 value determined by GC-MS, bounded by twice the standard deviation observed for three measurements.

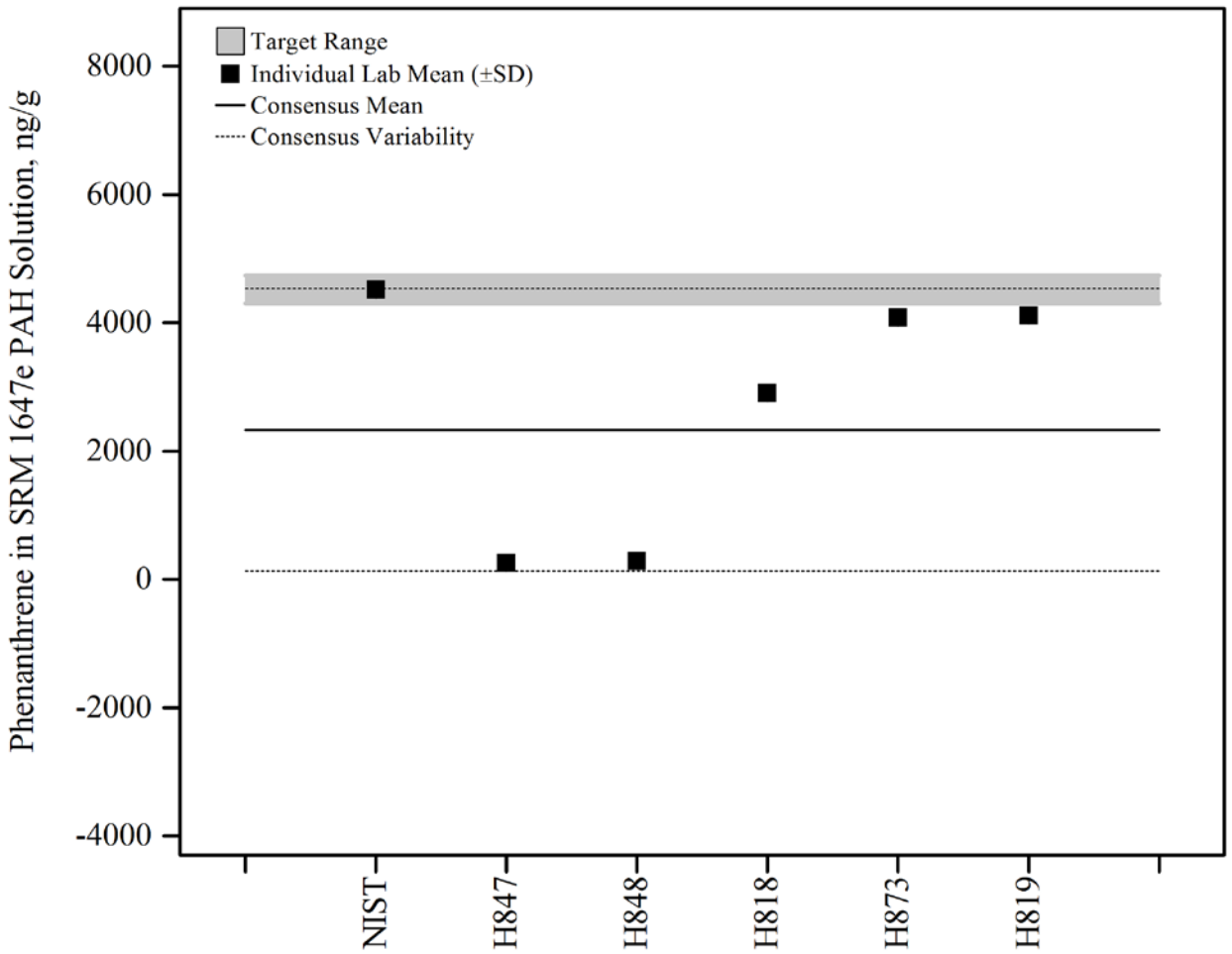

Figure 14. Phenanthrene in SRM 1647e PAH Solution (data summary view). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The black solid line represents the consensus mean, and the black dotted lines represent the consensus variability calculated as one standard deviation about the consensus mean. The gray shaded region represents the target zone for "acceptable" performance, which encompasses the NIST certified value bounded by twice its uncertainty (U_{95}).

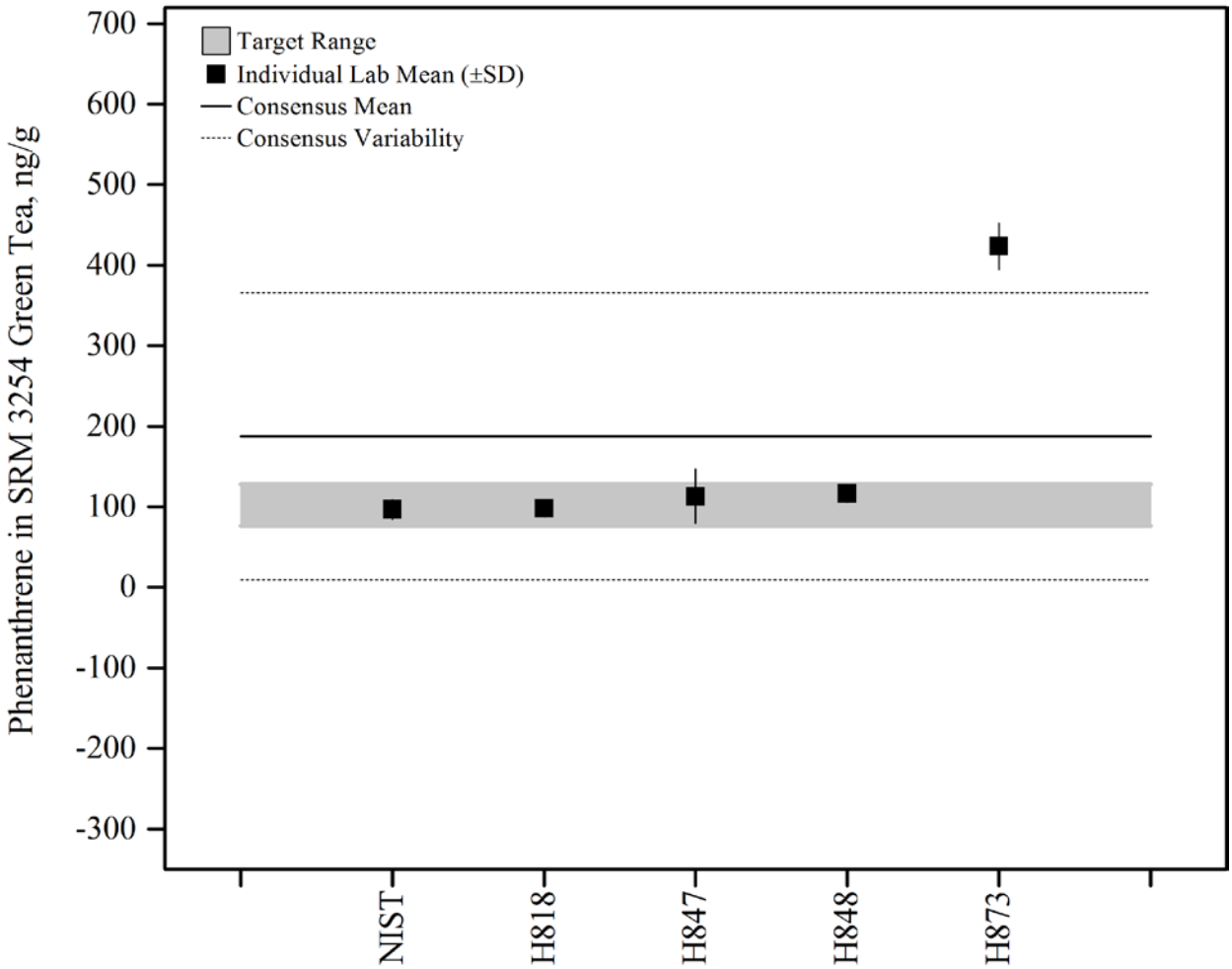

Figure 15. Phenanthrene in SRM 3254 *Camellia sinensis* (Green Tea) Leaves (data summary view). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The black solid line represents the consensus mean, and the black dotted lines represent 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 value determined by GC-MS, bounded by twice the standard deviation observed for three measurements.

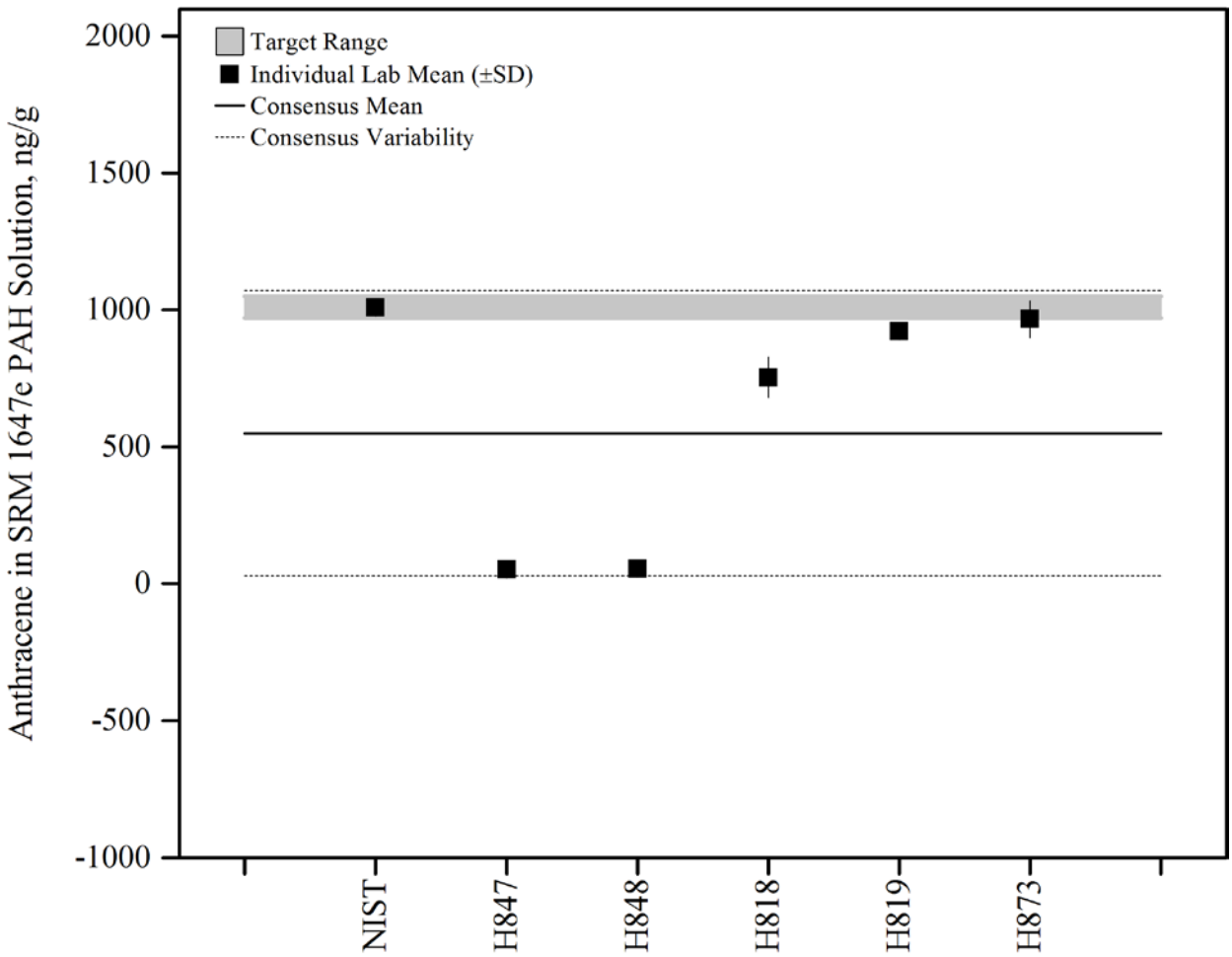

Figure 16. Anthracene in SRM 1647e PAH Solution (data summary view). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The black solid line represents the consensus mean, and the black dotted lines represent the consensus variability calculated as one standard deviation about the consensus mean. The gray shaded region represents the target zone for "acceptable" performance, which encompasses the NIST certified value bounded by twice its uncertainty (U_{95}).

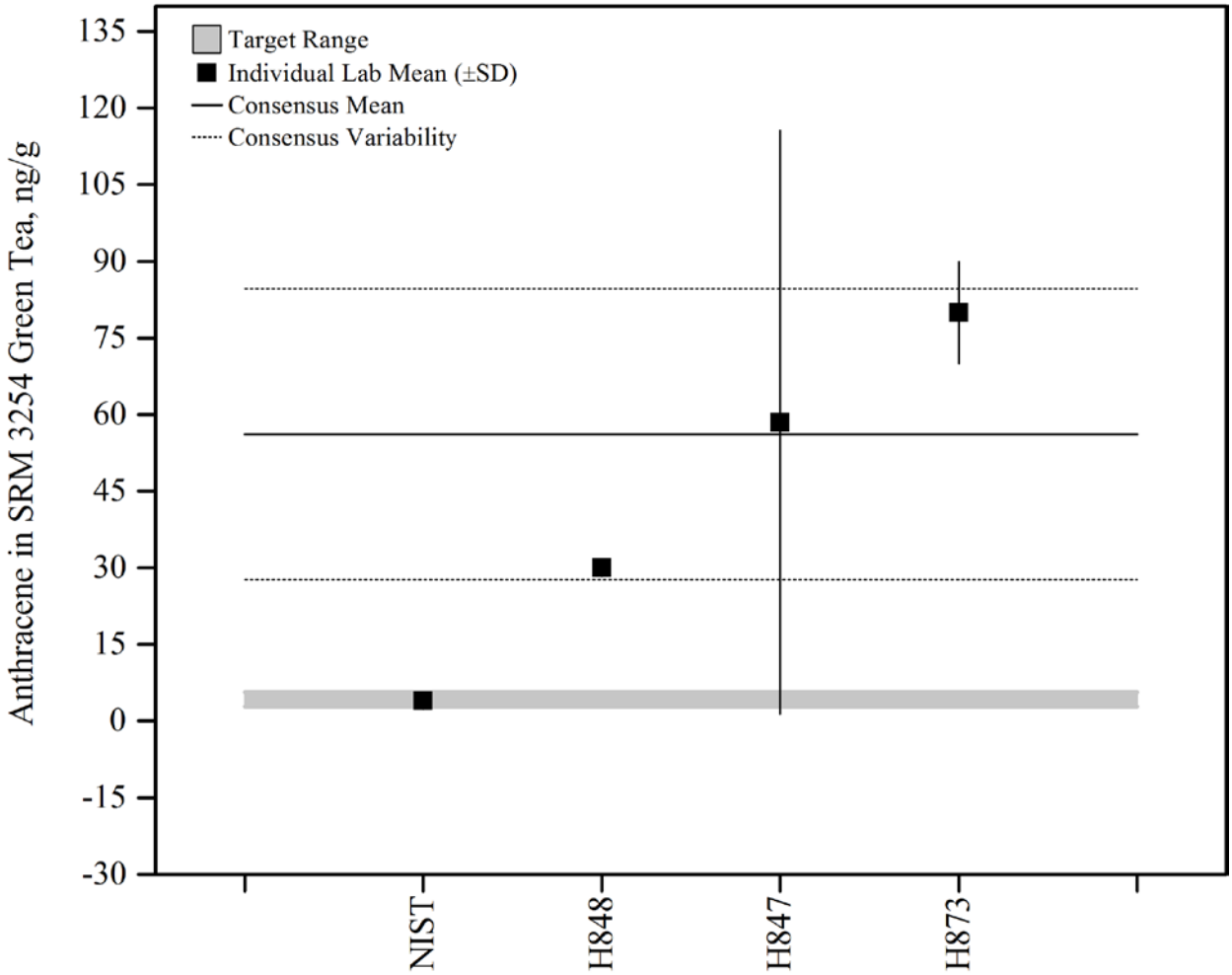

Figure 17. Anthracene in SRM 3254 *Camellia sinensis* (Green Tea) Leaves (data summary view). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The black solid line represents the consensus mean, and the black dotted lines represent the 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 value determined by GC-MS, bounded by twice the standard deviation observed for three measurements.

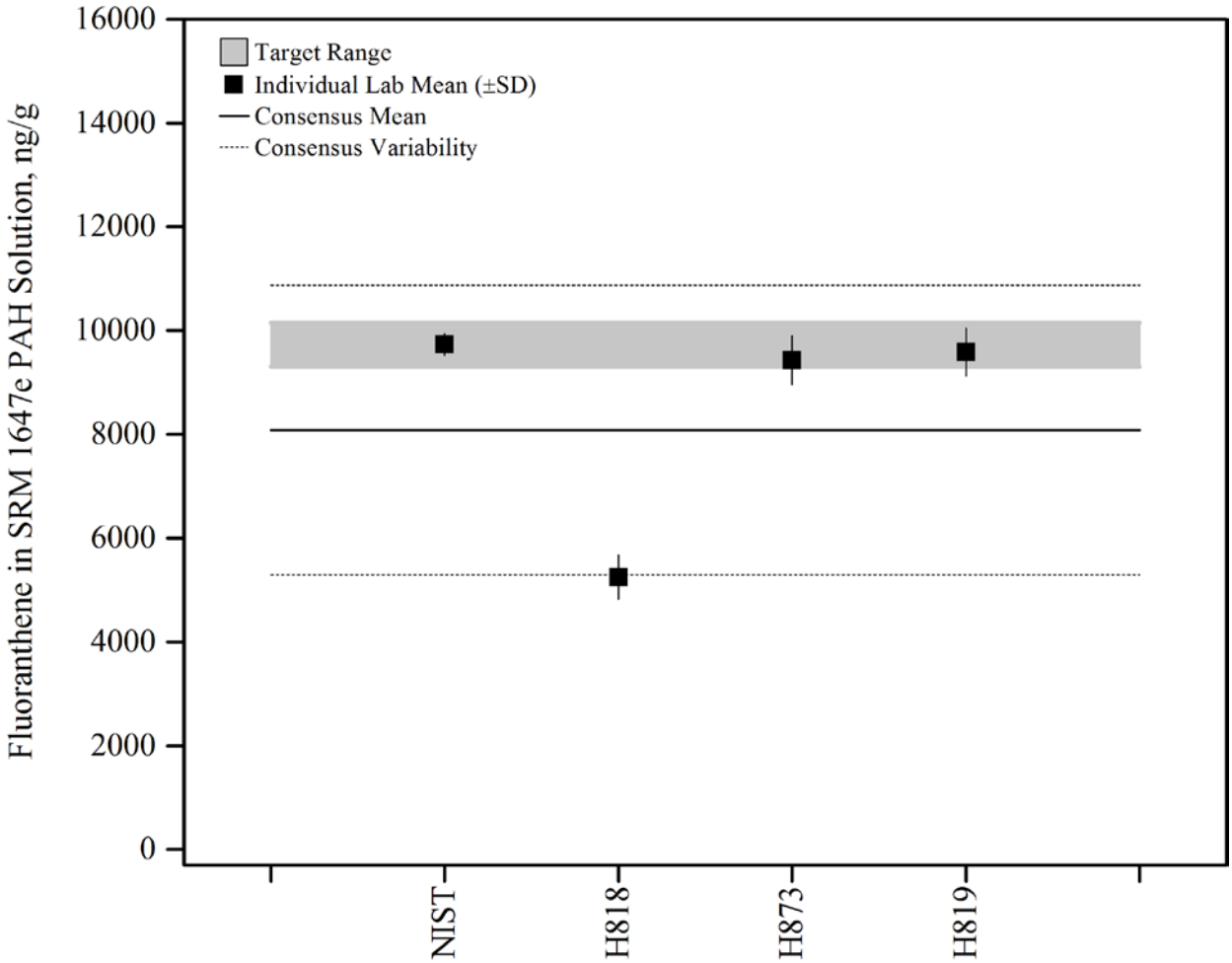

Figure 18. Fluoranthene in SRM 1647e PAH Solution (data summary view). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The black solid line represents the consensus mean, and the black dotted lines represent the consensus variability calculated as one standard deviation about the consensus mean. The gray shaded region represents the target zone for "acceptable" performance, which encompasses the NIST certified value bounded by twice its uncertainty (U_{95}).

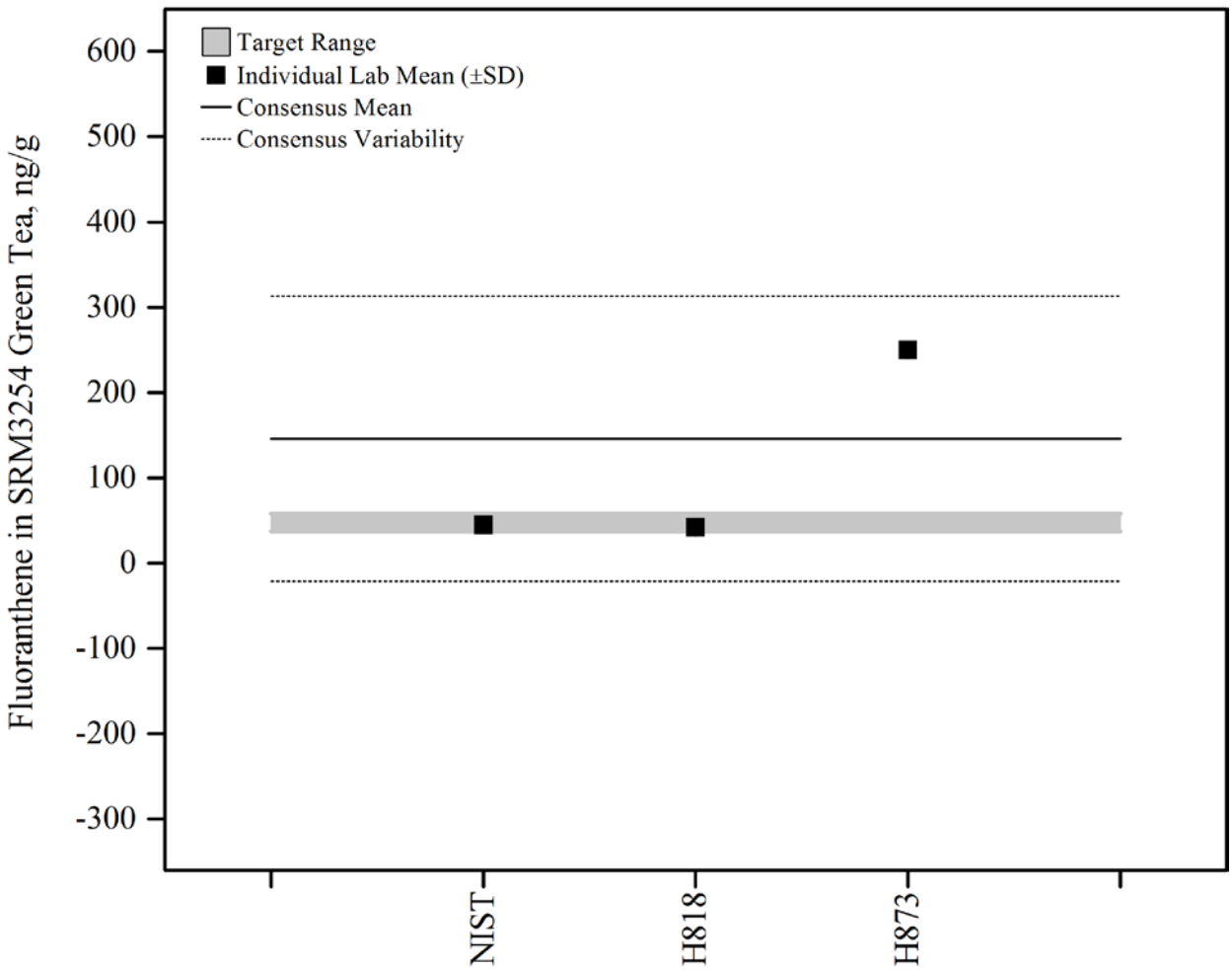

Figure 19. Fluoranthene in SRM 3254 *Camellia sinensis* (Green Tea) Leaves (data summary view). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The black solid line represents the consensus mean, and the black dotted lines represent 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 value determined by GC-MS, bounded by twice the standard deviation observed for three measurements.

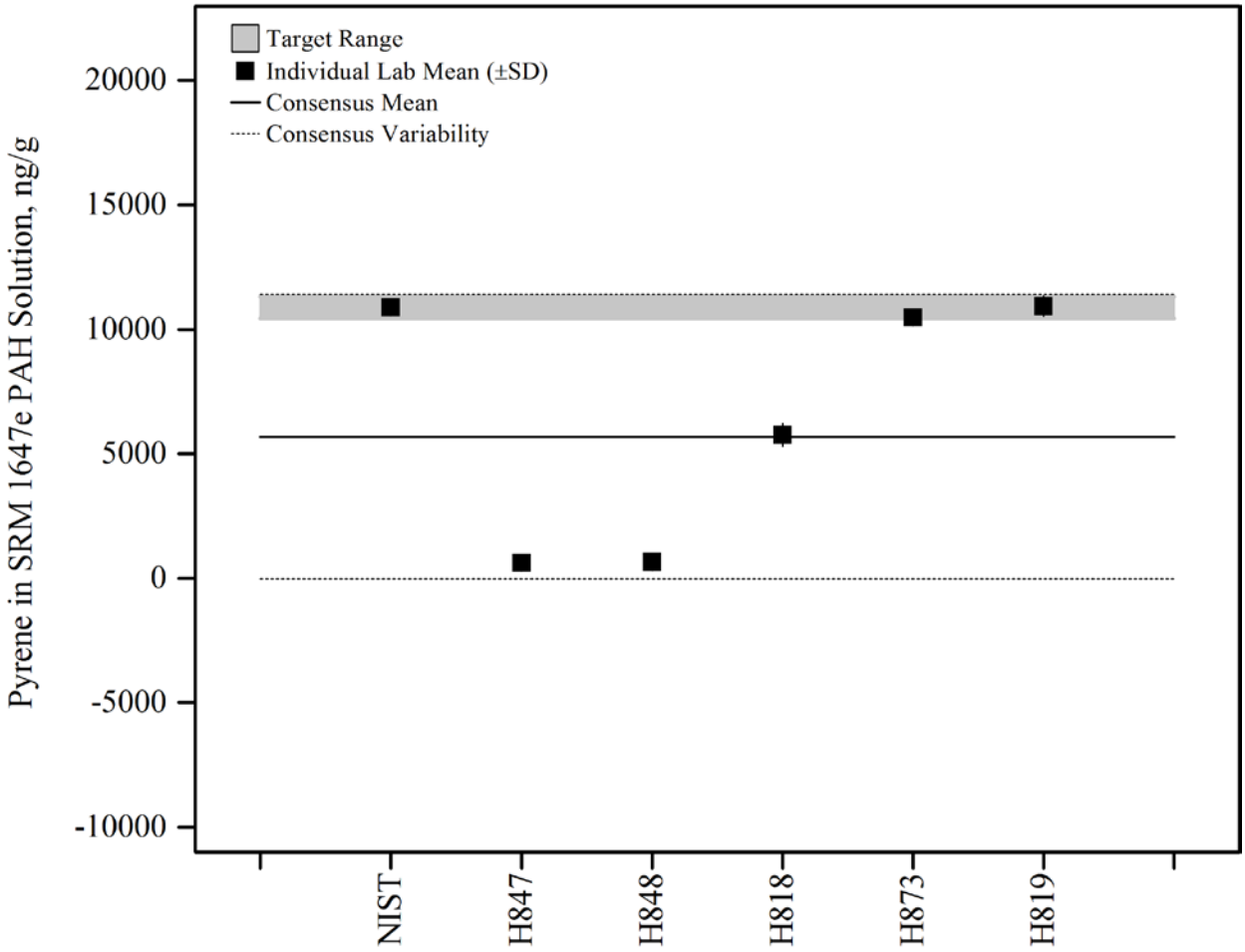

Figure 20. Pyrene in SRM 1647e PAH Solution (data summary view). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The black solid line represents the consensus mean, and the black dotted lines represent the consensus variability calculated as one standard deviation about the consensus mean. The gray shaded region represents the target zone for "acceptable" performance, which encompasses the NIST certified value bounded by twice its uncertainty (U_{95}).

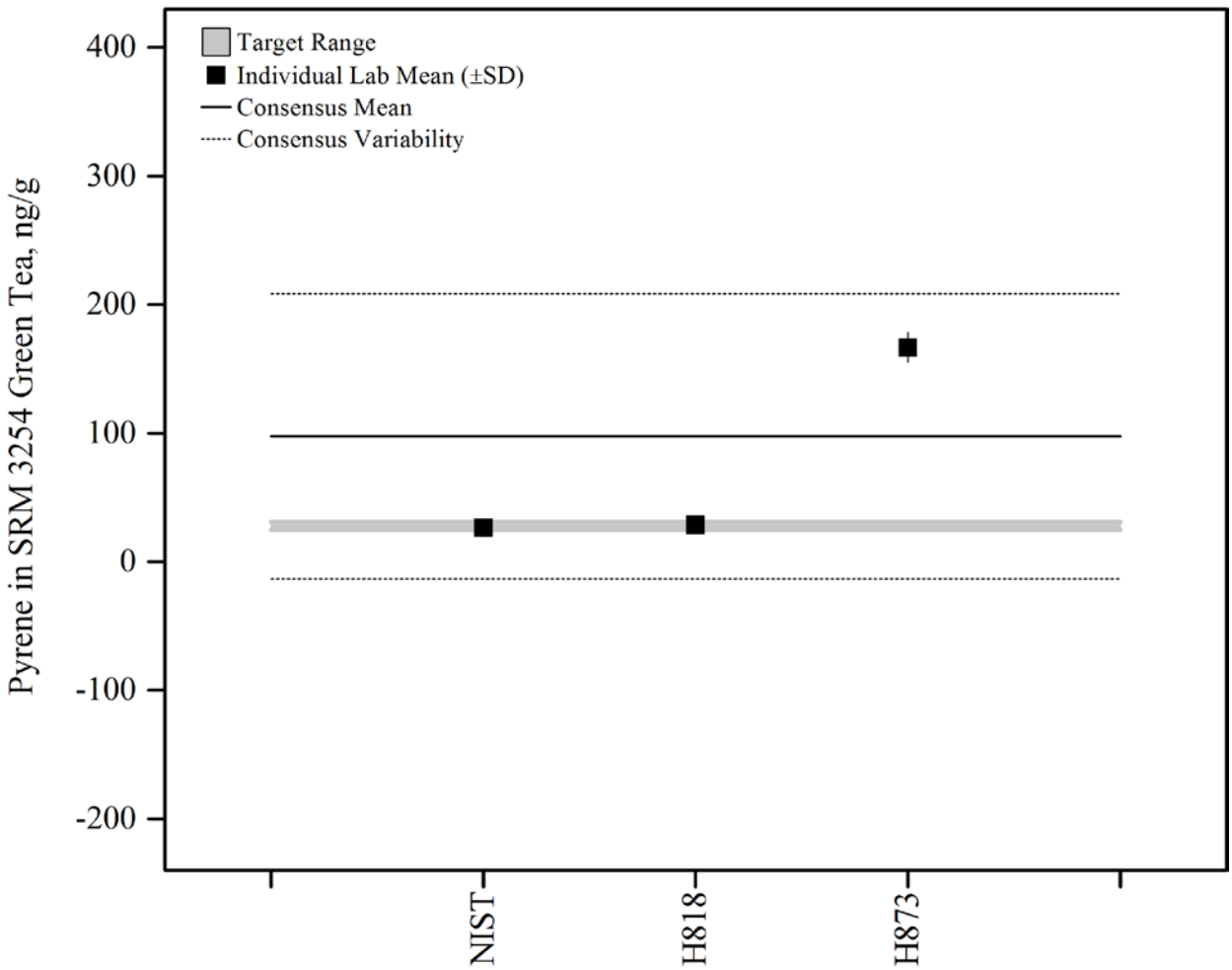

Figure 21. Pyrene in SRM 3254 *Camellia sinensis* (Green Tea) Leaves (data summary view). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The black solid line represents the consensus mean, and the black dotted lines represent the consensus 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 value determined by GC-MS, bounded by twice the standard deviation observed for three measurements.

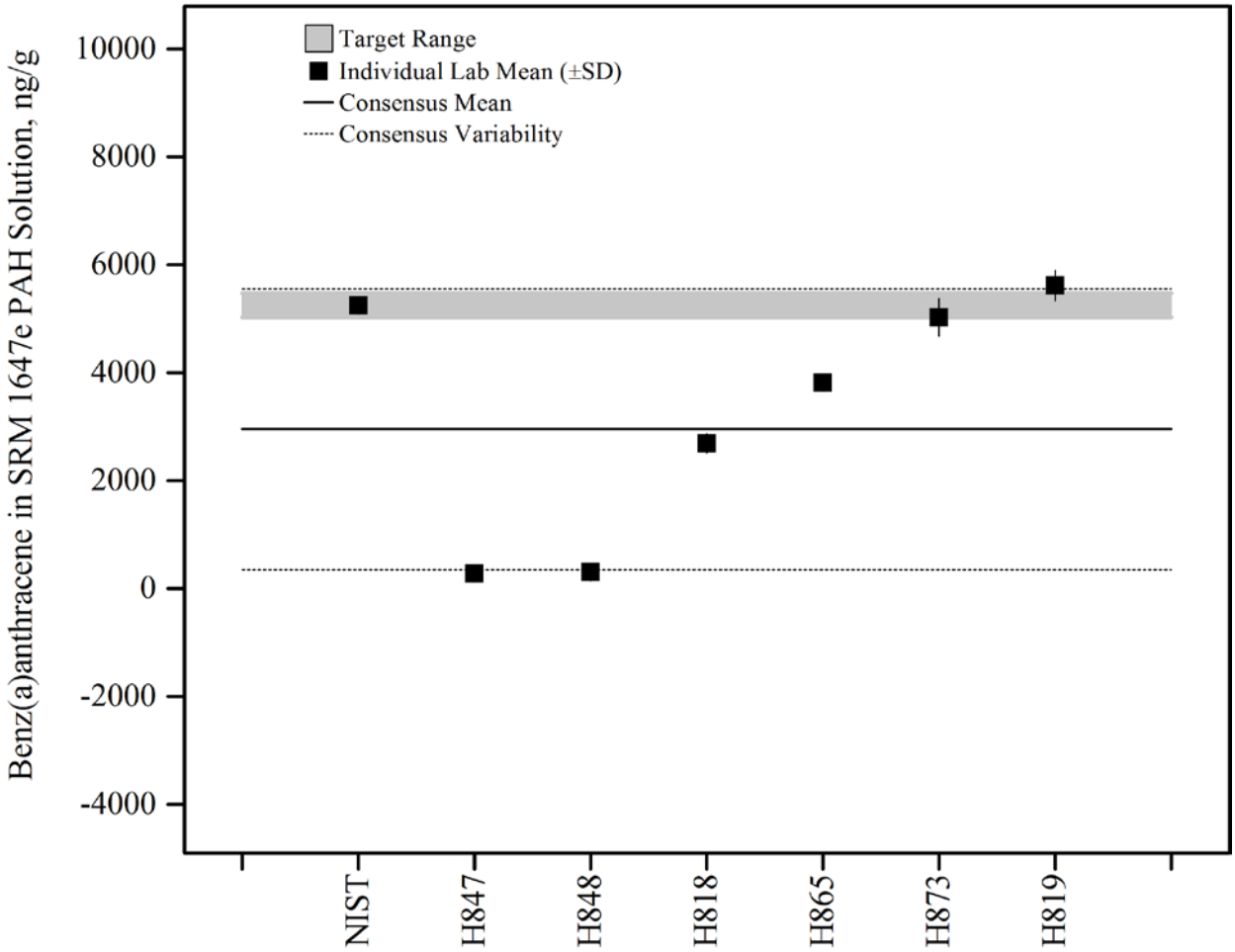

Figure 22. Benz[*a*]anthracene in SRM 1647e PAH Solution (data summary view). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The black solid line represents the consensus mean, and the black dotted lines represent the consensus variability calculated as one standard deviation about the consensus mean. The gray shaded region represents the target zone for "acceptable" performance, which encompasses the NIST certified value bounded by twice its uncertainty (U_{95}).

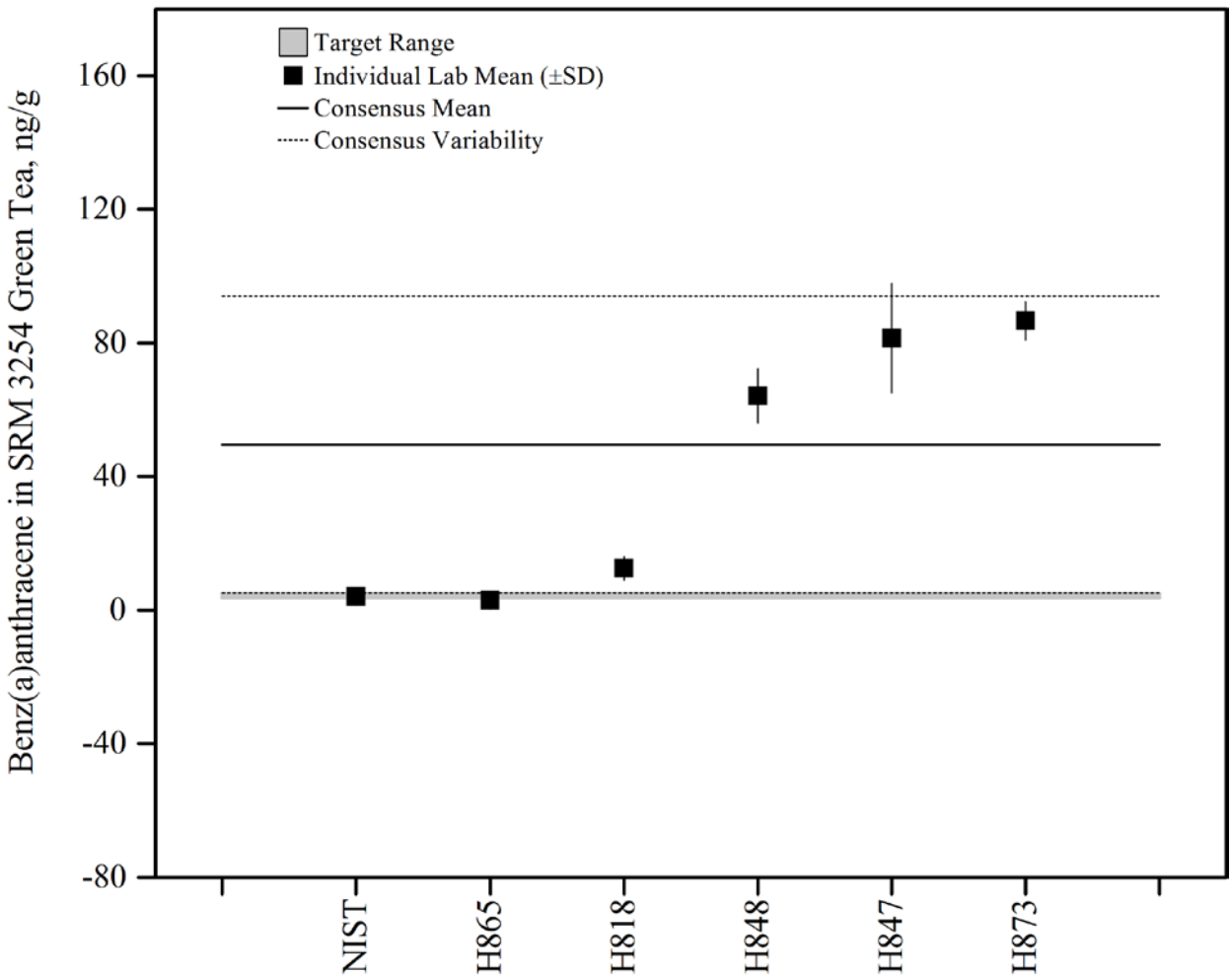

Figure 23. Benz[*a*]anthracene in SRM 3254 *Camellia sinensis* (Green Tea) Leaves (data summary view). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The black solid line represents the consensus mean, and the black dotted lines represent 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 value determined by GC-MS, bounded by twice the standard deviation observed for three measurements.

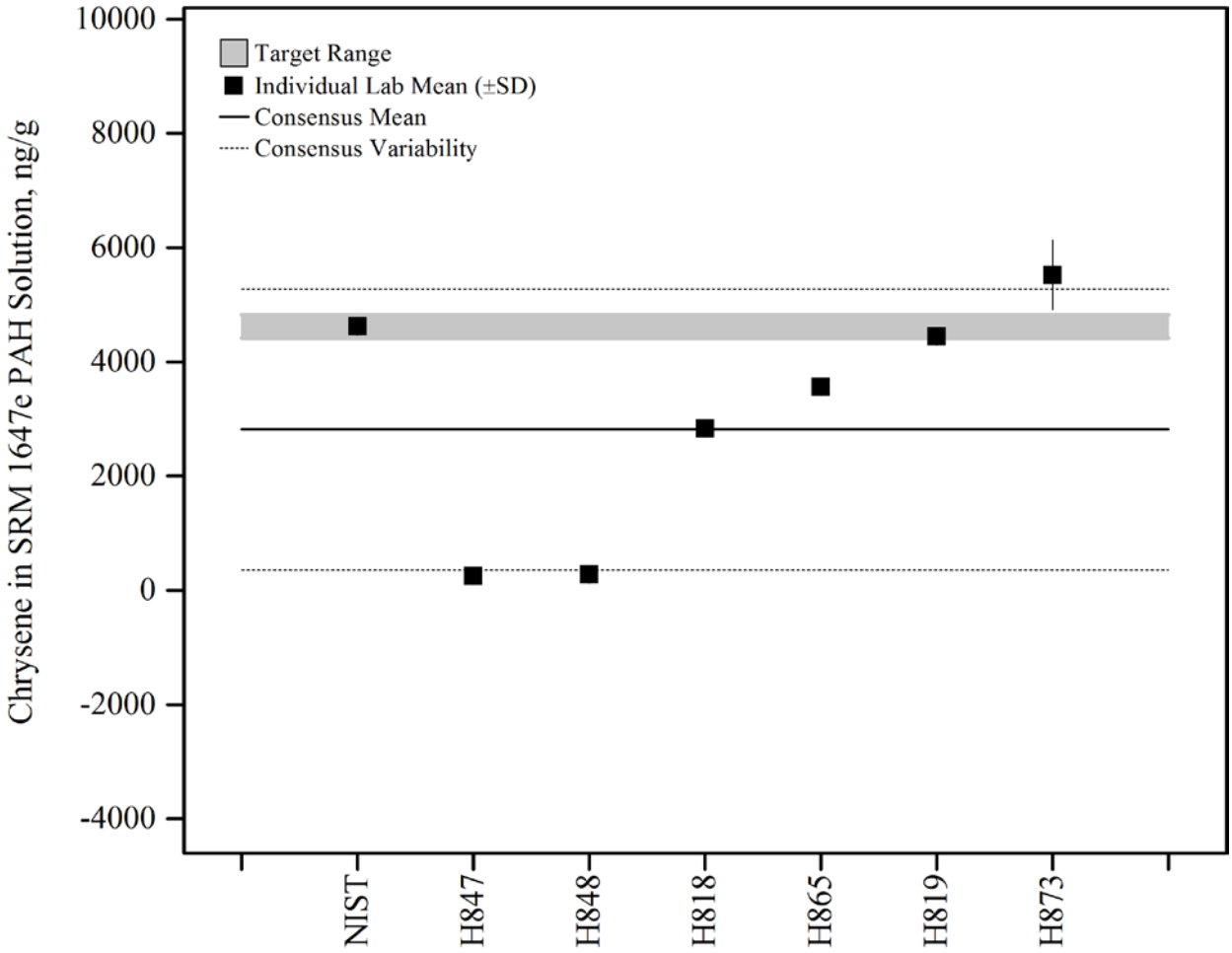

Figure 24. Chrysene in SRM 1647e PAH Solution (data summary view). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The black solid line represents the consensus mean, and the black dotted lines represent the consensus variability calculated as one standard deviation about the consensus mean. The gray shaded region represents the target zone for "acceptable" performance, which encompasses the NIST certified value bounded by twice its uncertainty (U_{95}).

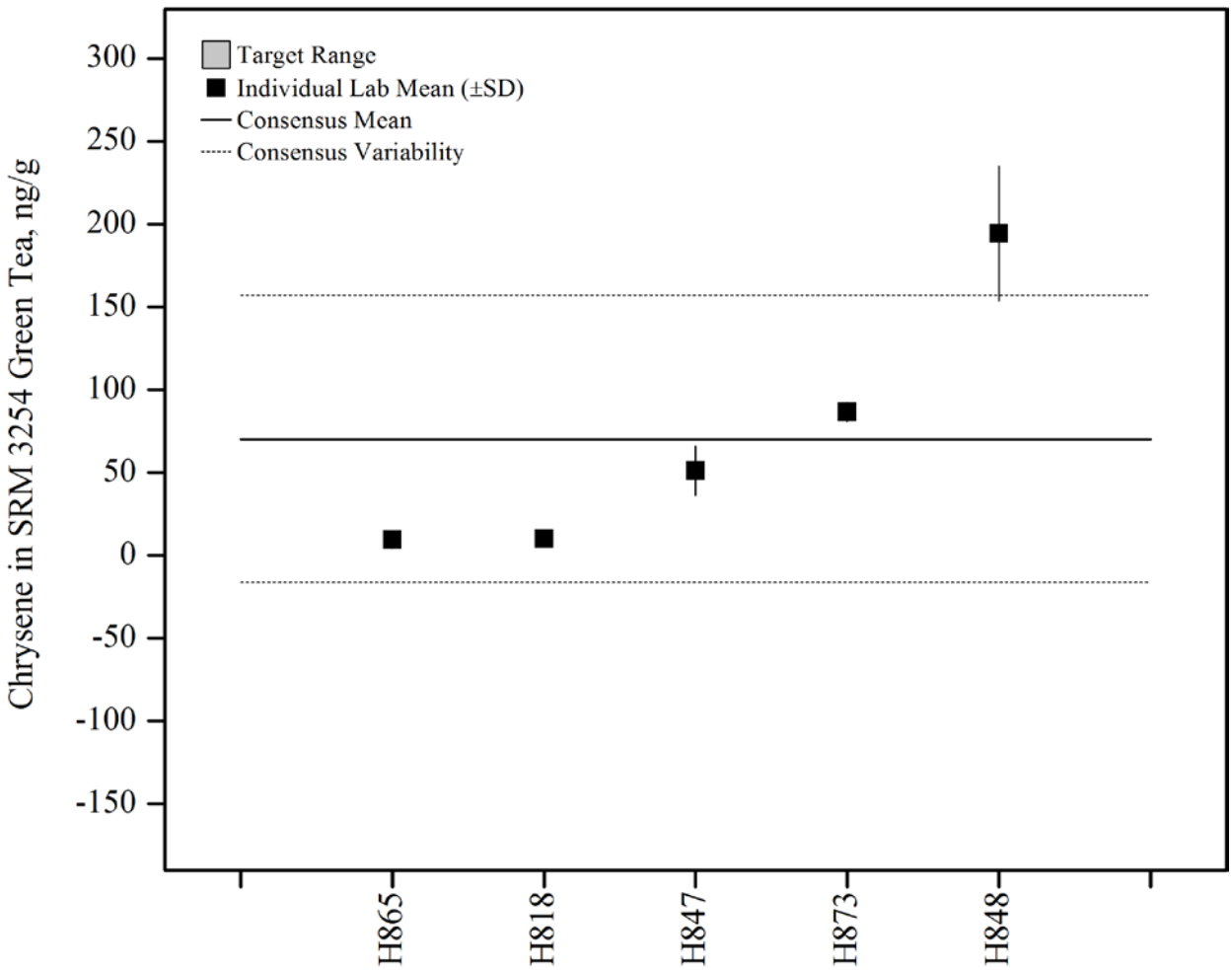

Figure 25. Chrysene in SRM 3254 *Camellia sinensis* (Green Tea) Leaves (data summary view). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The black solid line represents the consensus mean, and the black dotted lines represent the consensus variability calculated as one standard deviation about the consensus mean.

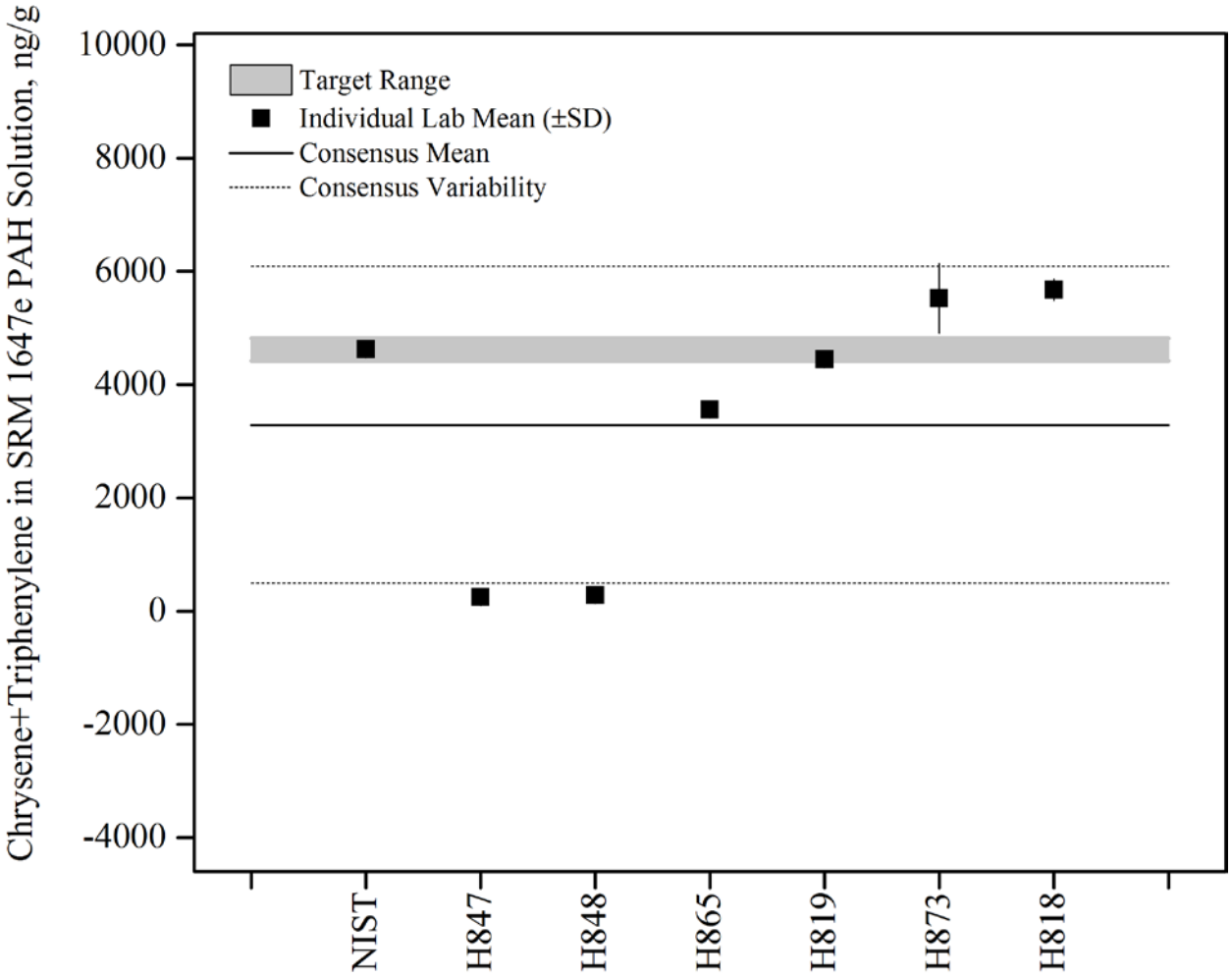

Figure 26. Sum of chrysene and triphenylene in SRM 1647e PAH Solution (data summary view). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The black solid line represents the consensus mean, and the black dotted lines represent the 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 certified value bounded by twice its uncertainty (U_{95}).

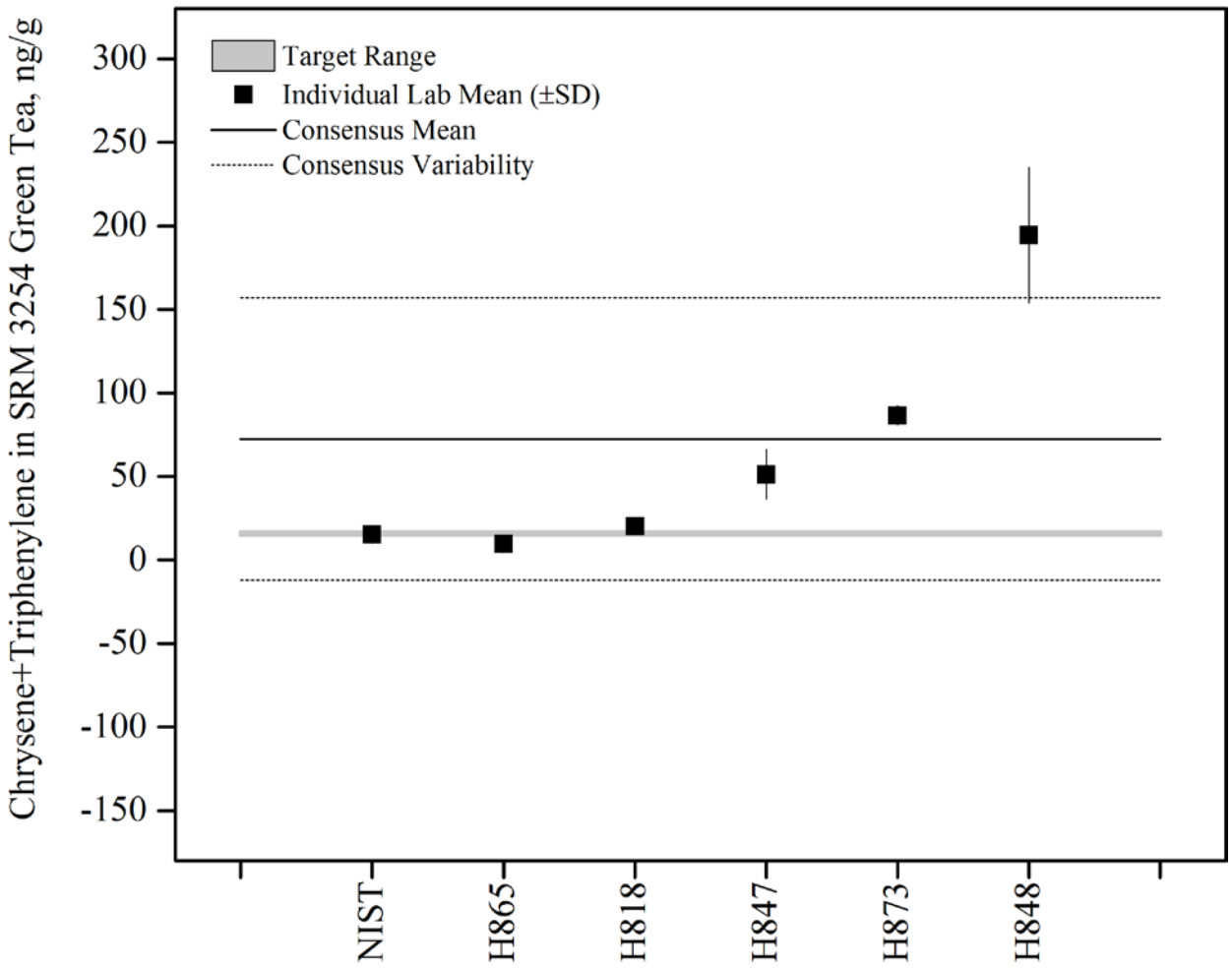

Figure 27. Sum of chrysene and triphenylene in SRM 3254 *Camellia sinensis* (Green Tea) Leaves (data summary view). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The black solid line represents the consensus mean, and the black dotted 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 value determined by GC-MS, bounded by twice the standard deviation observed for three measurements.

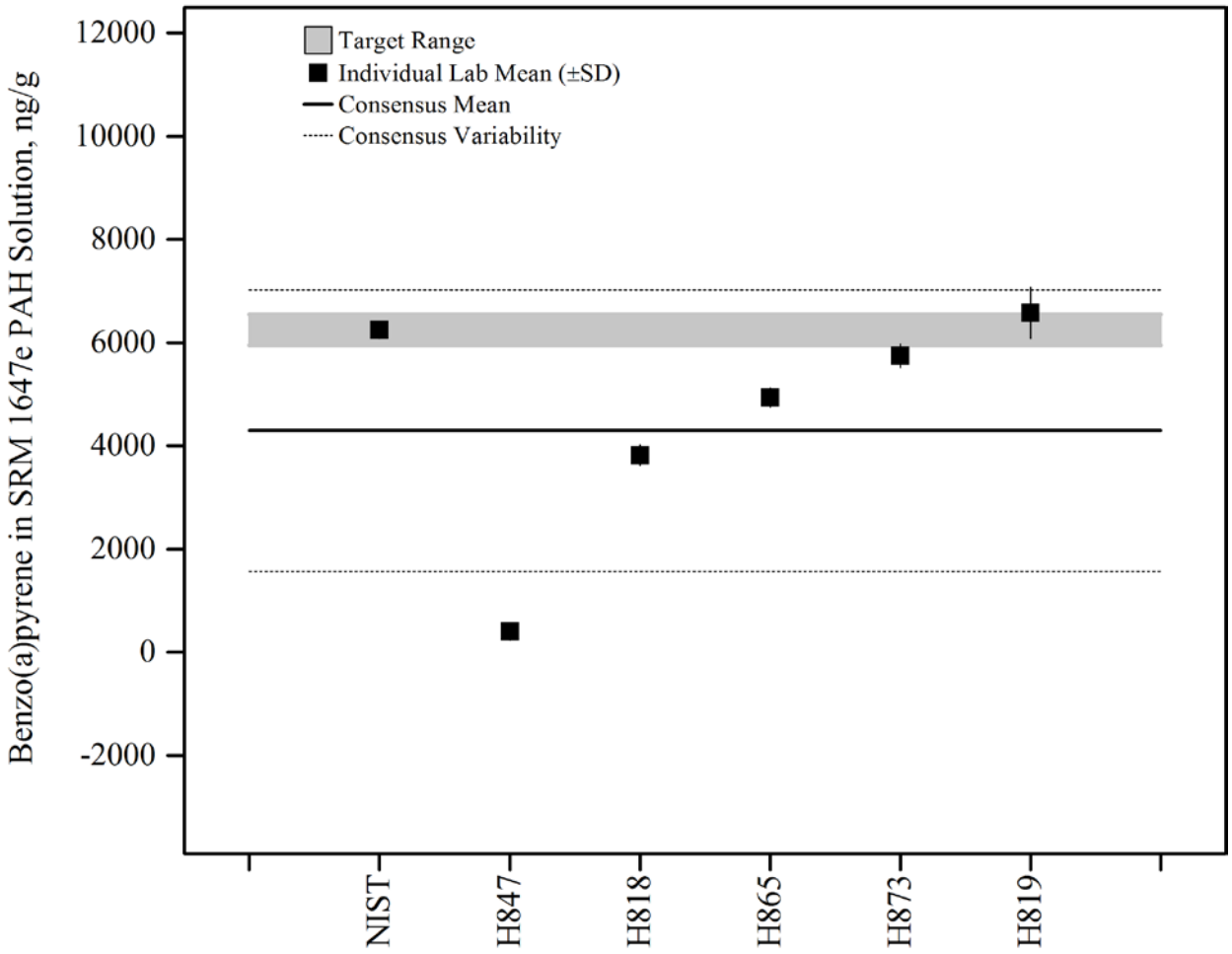

Figure 28. Benzo[*a*]pyrene in SRM 1647e PAH Solution (data summary view). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The black solid line represents the consensus mean, and the black dotted lines represent the consensus variability calculated as one standard deviation about the consensus mean. The gray shaded region represents the target zone for "acceptable" performance, which encompasses the NIST certified value bounded by twice its uncertainty (U_{95}).

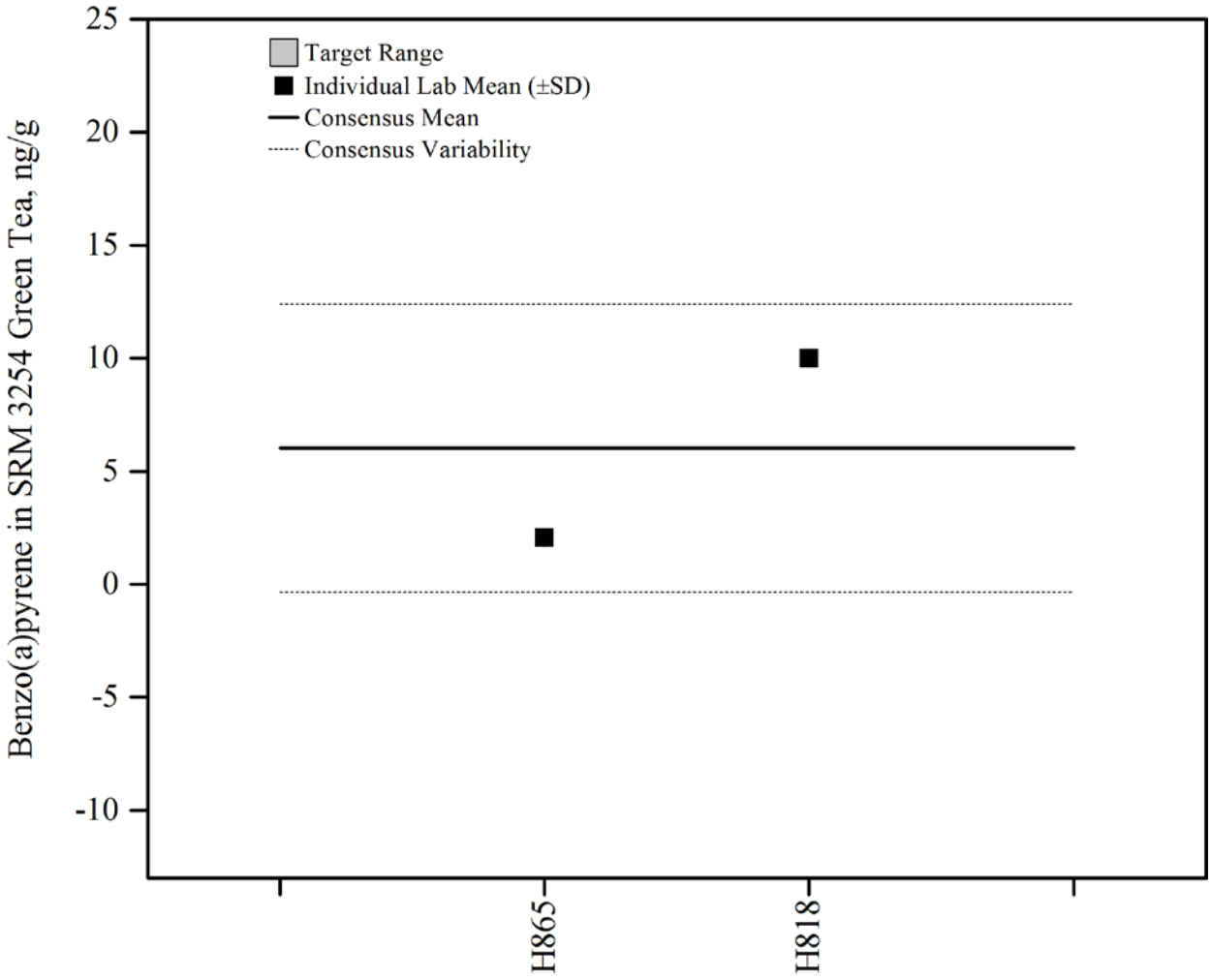

Figure 29. Benzo[*a*]pyrene in SRM 3254 *Camellia sinensis* (Green Tea) Leaves (data summary view). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The black solid line represents the consensus mean, and the black dotted lines represent the consensus variability calculated as one standard deviation about the consensus mean.

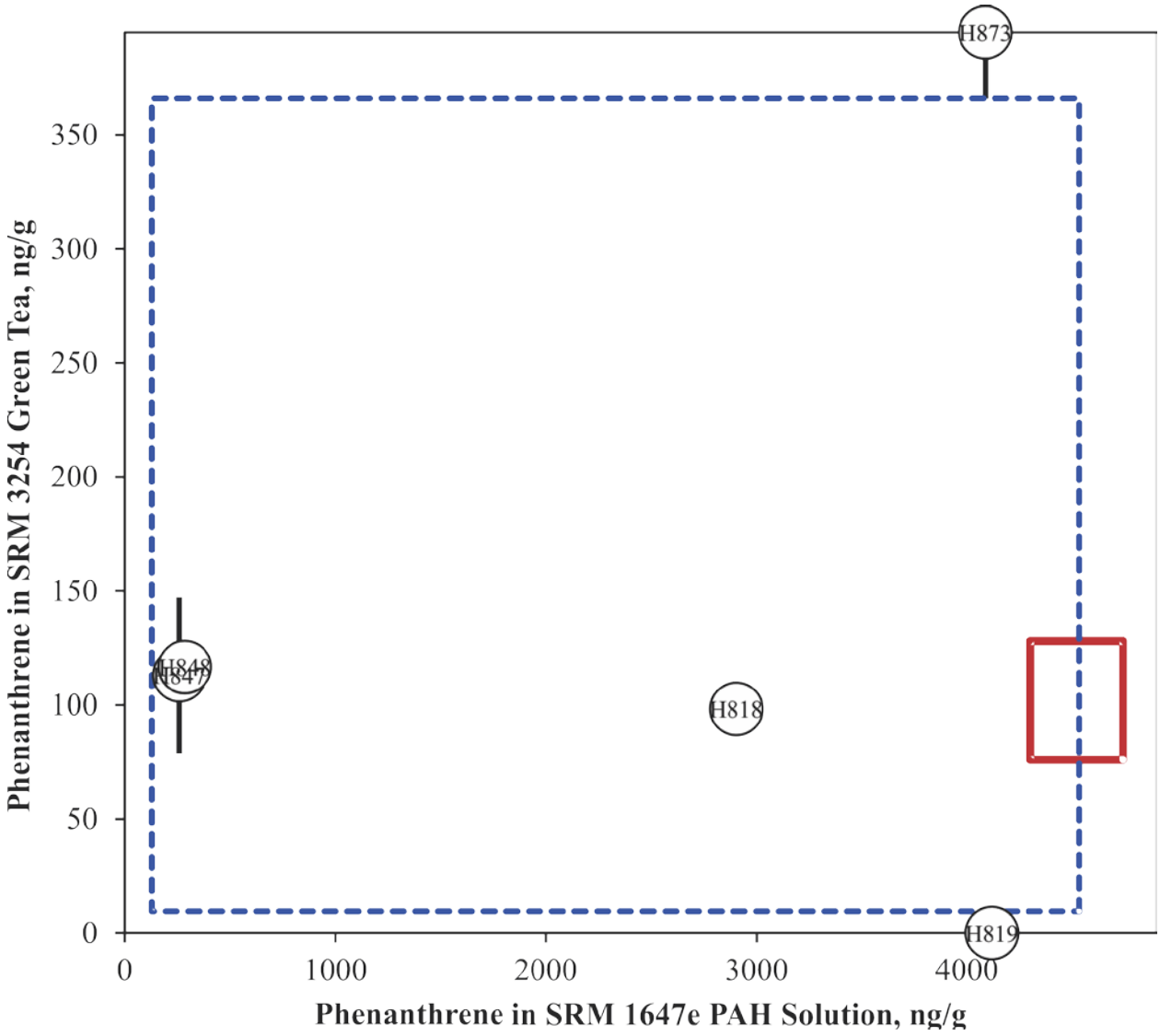

Figure 30. Phenanthrene in SRM 1647e PAH Solution and SRM 3254 *Camellia sinensis* (Green Tea) Leaves (sample/control comparison view). In this view, the individual laboratory results for the control (SRM 1647e PAH Solution) with a certified value for the analyte are compared to the results for an unknown (SRM 3254 *Camellia sinensis* (Green Tea) Leaves). The error bars represent the individual laboratory standard deviation. The solid red lines represent 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).

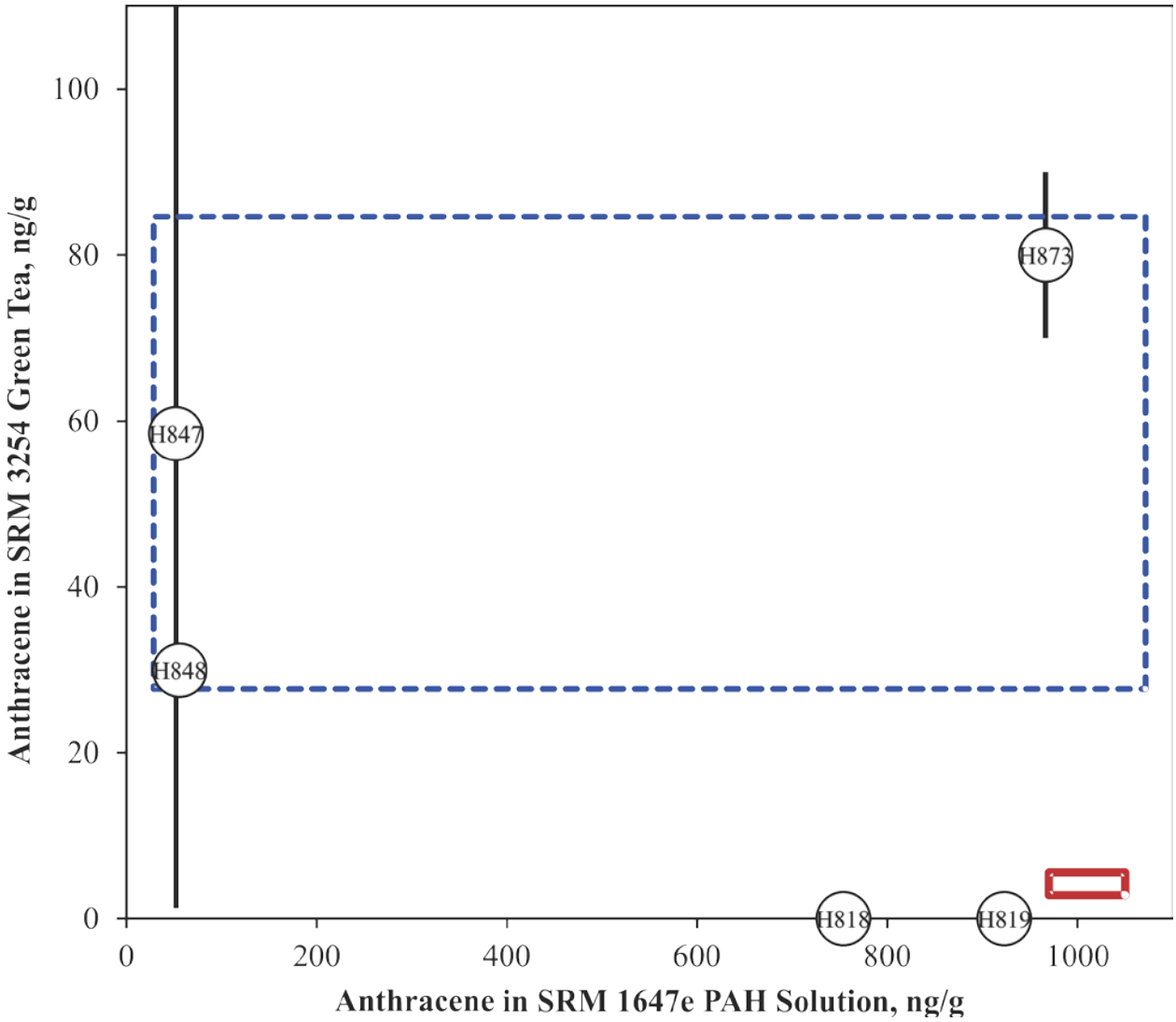

Figure 31. Anthracene in SRM 1647e PAH Solution and SRM 3254 *Camellia sinensis* (Green Tea) Leaves (sample/control comparison view). In this view, the individual laboratory results for the control (SRM 1647e PAH Solution) with a certified value for the analyte are compared to the results for an unknown (SRM 3254 *Camellia sinensis* (Green Tea) Leaves). The error bars represent the individual laboratory standard deviation. The solid red lines represent 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).

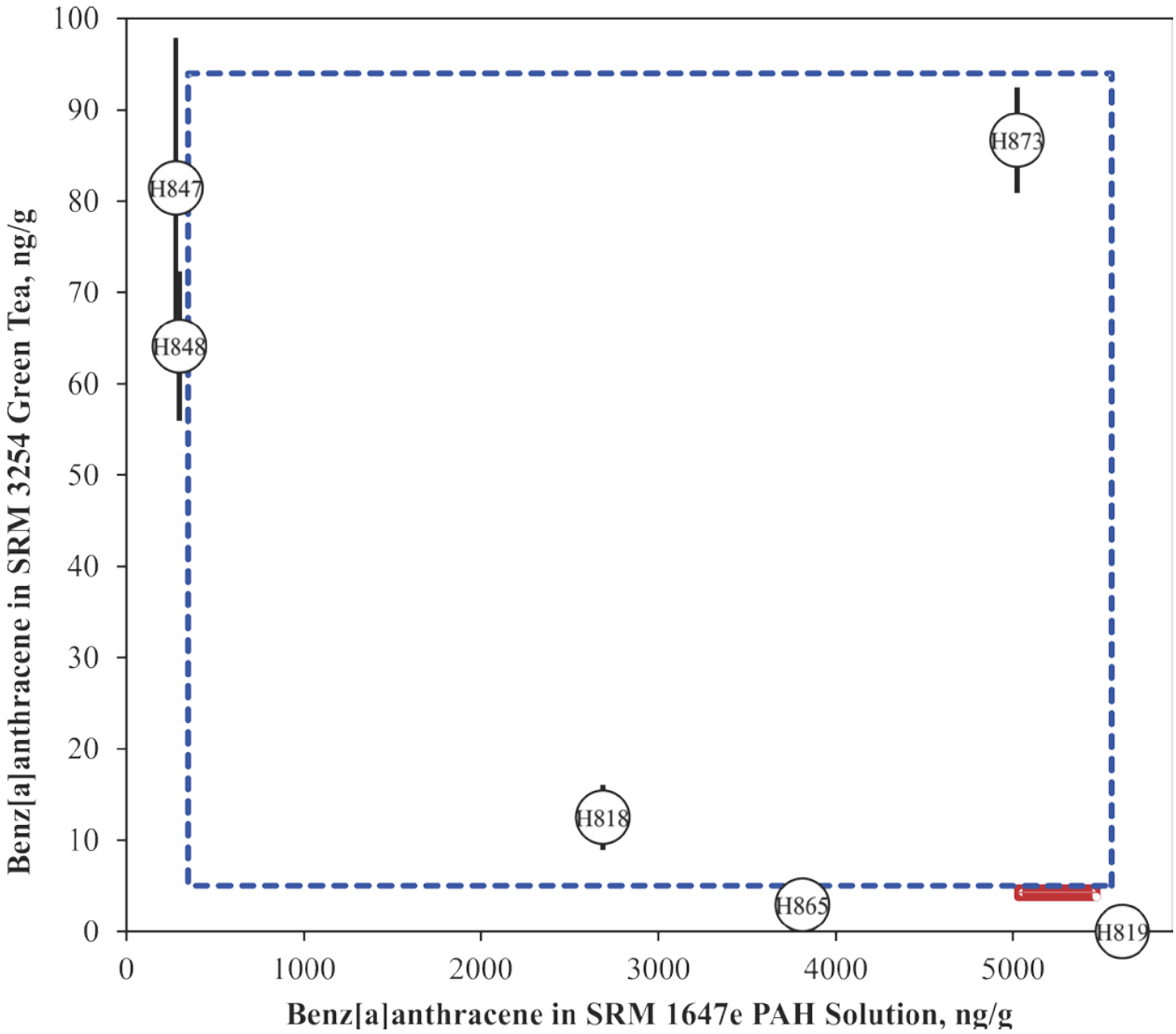

Figure 32. Benz[*a*]anthracene in SRM 1647e PAH Solution and SRM 3254 *Camellia sinensis* (Green Tea) Leaves (sample/control comparison view). In this view, the individual laboratory results for the control (SRM 1647e PAH Solution) with a certified value for the analyte are compared to the results for an unknown (SRM 3254 *Camellia sinensis* (Green Tea) Leaves). The error bars represent the individual laboratory standard deviation. The solid red lines represent 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).

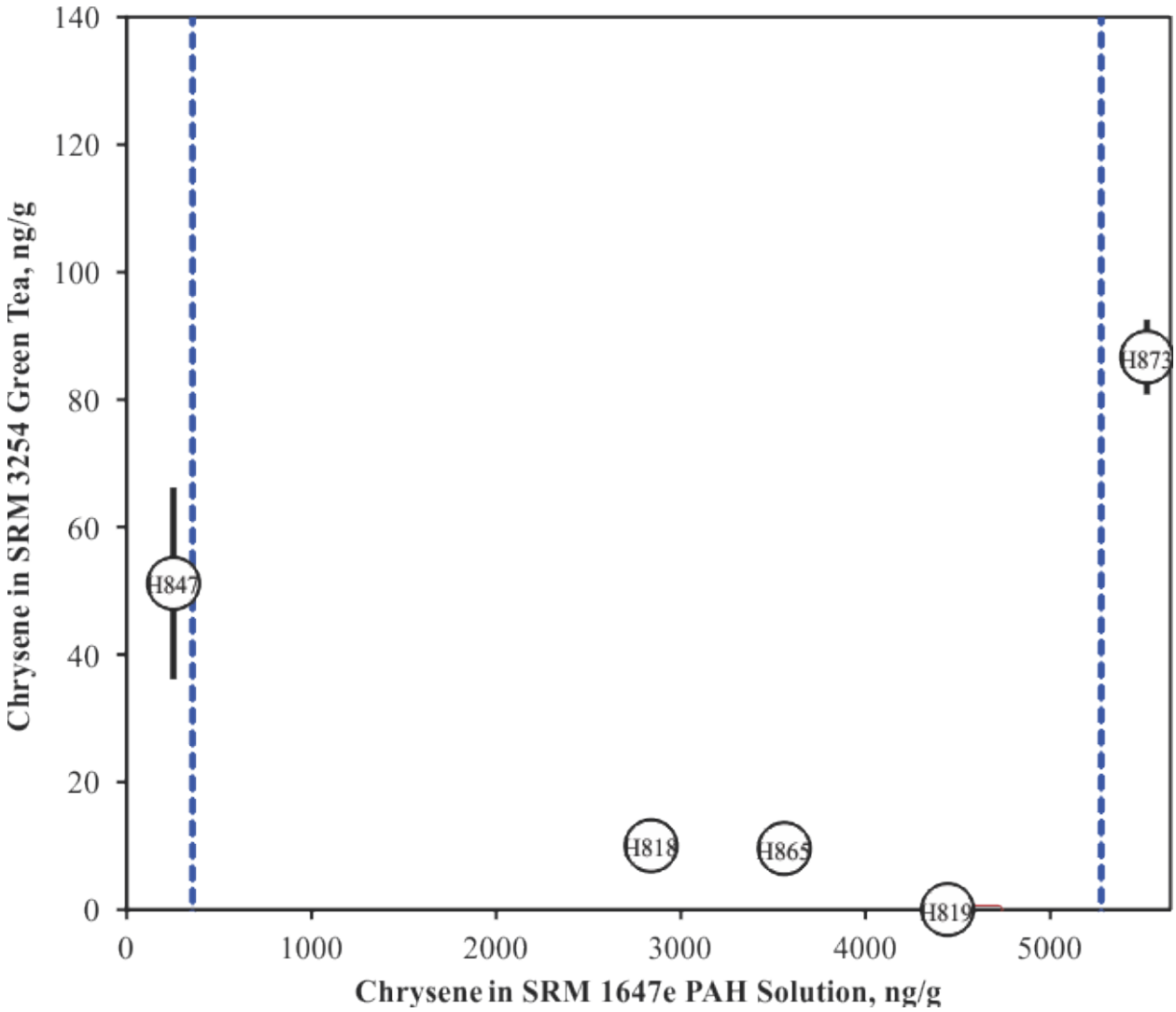

Figure 33. Chrysene in SRM 1647e PAH Solution and SRM 3254 *Camellia sinensis* (Green Tea) Leaves (sample/control comparison view). In this view, the individual laboratory results for the control (SRM 1647e PAH Solution) with a certified value for the analyte are compared to the results for an unknown (SRM 3254 *Camellia sinensis* (Green Tea) Leaves). The error bars represent the individual laboratory standard deviation. The dotted blue box represents the consensus zone for the control (x-axis) and the unknown sample (y-axis).

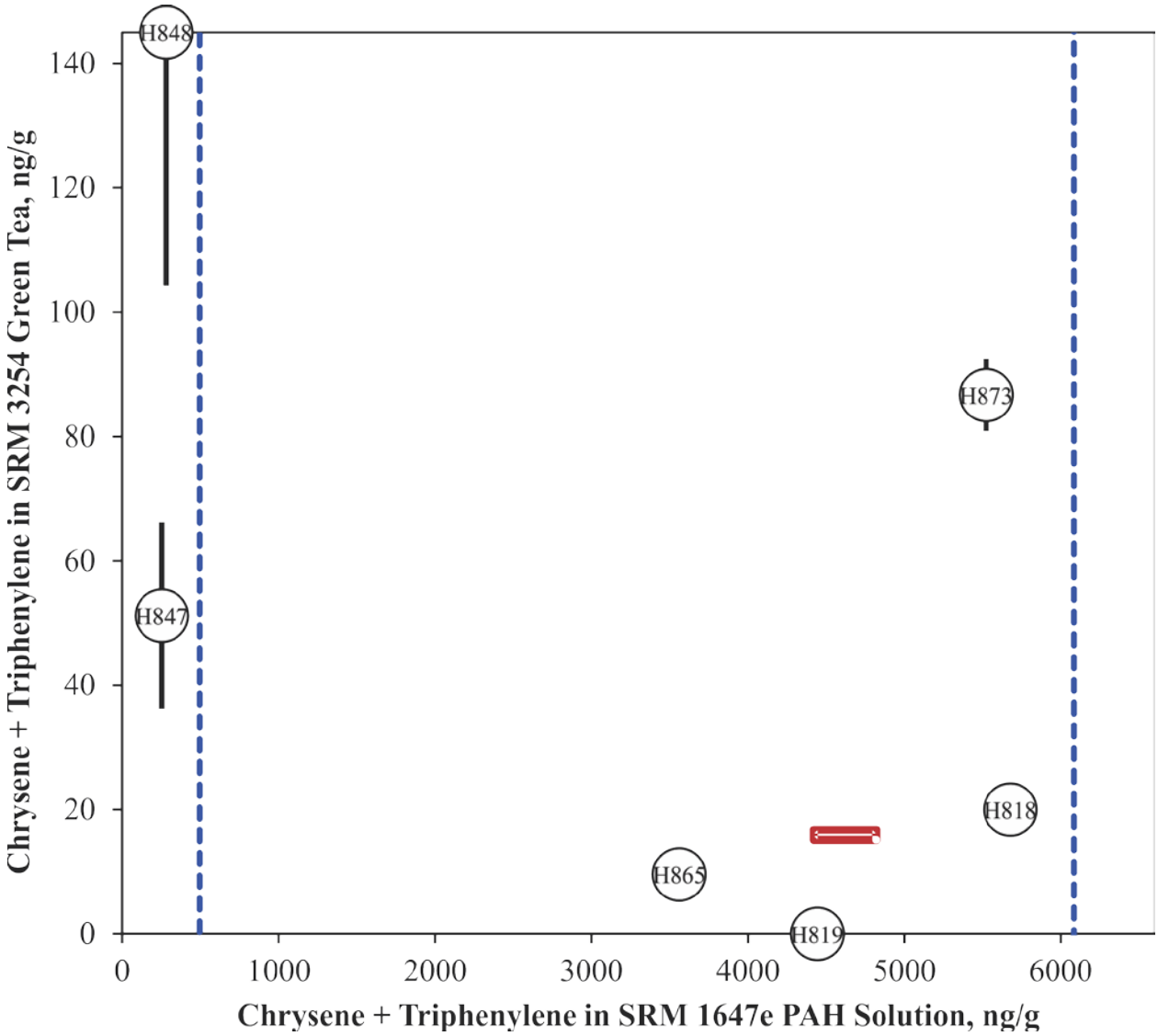

Figure 34. Sum of chrysene and triphenylene in SRM 1647e PAH Solution and SRM 3254 *Camellia sinensis* (Green Tea) Leaves (sample/control comparison view). In this view, the individual laboratory results for the control (SRM 1647e PAH Solution) with a certified value for the analyte are compared to the results for an unknown (SRM 3254 *Camellia sinensis* (Green Tea) Leaves). The error bars represent the individual laboratory standard deviation. The solid red lines represent 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).

CHOLINE IN FOODS

Study Overview

In this study, participants were provided with two NIST SRMs, candidate SRM 1845a Whole Egg Powder and candidate SRM 3234 Soy Flour, neither of which has been fortified with choline. Participants were asked to use in-house analytical methods to determine the mass fractions of total choline in each of the matrices and report values on an as-received basis. Participants were not asked to report the choline content in any particular form; NIST values are reported as the choline ion.

Sample Information

Whole egg powder. Participants were provided with three vials, each containing approximately 1.5 g of unfortified whole egg powder from a single production lot. The material is a free-flowing, fine powder prepared from USDA-inspected whole eggs. Before use, participants were instructed to thoroughly mix and homogenize the contents of the vial and use a sample size of at least 100 mg. Participants were asked to report a single value from each vial provided and to store the egg powder at controlled room temperature, 10 °C to 30 °C. Approximate analyte levels were not reported prior to the study. The NIST certified value for choline in candidate SRM 1845a will be determined using microwave acid digestion followed by ID-LC-MS in combination with data from external collaborating laboratories. An estimation of the certified value of the choline ion, (14. 71 ± 0.33) mg/g, is provided as the mean and standard deviation of duplicate ID-LC-MS measurements from 10 packets.

Soy Flour. Participants were provided with three vials, each containing approximately 1.5 g of defatted soy flour. Before use, participants were instructed to thoroughly mix and homogenize the contents of the vial and use a sample size of at least 400 mg. Participants were asked to report a single value from each vial provided and to store the egg powder at controlled room temperature, 10 °C to 30 °C. Approximate analyte levels were not reported prior to the study. The NIST certified value for choline in candidate SRM 3234 will be determined using microwave acid digestion followed by isotope dilution LC-MS in combination with data from external collaborating laboratories. An estimation of the certified value of the choline ion, (2.663 ± 0.023) mg/g, is provided as the mean and standard deviation of duplicate ID-LC-MS measurements from 12 packets.

Study Results

- Thirteen laboratories enrolled in this exercise and received samples, and seven laboratories reported results for the egg powder and soy flour (54 % participation).
- For both materials, the consensus ranges were very wide and were higher than the NIST target range (Figures 35 and 36).
    - The dispersion of the data could be a result of challenges in completely extracting and hydrolyzing the samples.
    - In the soy flour, five of the seven laboratories (71 %) reported values that were reasonably close to the target range. The remaining two laboratories reported values that were significantly higher than the target range (5 times higher and almost 100 times higher). This could indicate an interference in the analytical method (titration

and spectrophotometry) caused by matrix components. More information is needed about the analytical methods to draw more conclusive inferences.

- In the egg powder, four of the seven laboratories (57 %) reported values that were reasonably close to the target range. Two of the remaining laboratories reported values that were significantly lower than the target range (2 times lower and 500 times lower). This could indicate incomplete extraction (both laboratories reported using solvent extraction). Another laboratory reported a value that was 5 times higher than the target value. This laboratory also reported very high values for the soy flour, indicating a possible calibration error.
- Laboratories that reported low values for the egg powder did not report low values for the soy flour. This indicates that the egg powder may contain more choline esters that require hydrolysis prior to analytical determination.
- The NIST values were determined using microwave acid digestion. As a result, the NIST target ranges and the consensus means may not overlap when participating laboratories use less extensive extraction procedures. This may result in a discrepancy between laboratories (such as NIST) determining the "total" choline content and laboratories determining "free" choline content.
- In general, the instrumental method used did not correlate with any trend in the data. In this case, variability in the data is more likely related to sample preparation than to instrumental method. A larger data set and more information from participants is necessary to draw any strong correlations between method and result.

Technical Recommendations

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

- The literature indicates that some proportion of the total choline is present in these matrices as choline esters that require acidic or basic hydrolysis to release choline ion. Participants should be clear as to what form of choline is reported and whether a sample preparation procedure will yield total or free choline.
- No analytical method was identified as being exceptionally good or problematic. For these types of samples, the extraction method seems to be more critical than the instrumental methods used by participants.
- Participants were not asked to report choline results in any specific molecular form. The NIST estimation of the certified value is reported as the choline ion. Conversion to the choline hydroxide form would increase the values by 16 %. Two laboratories reported values 13.5 % and 17.7 % greater than the NIST value for the soy flour, which could be explained by a difference in the reported form. However, these same two laboratories reported values for the egg powder that were only 8.2 % and 4.1 % greater than the NIST value, respectively. While a small error due to inconsistent reporting of results is possible, it does not completely explain the outlying results.

Table 17. Individual data table (NIST) for choline in foods.

National Institute of Standards & Technology

Exercise H - March 2012 - Choline

	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}
Choline	Soy Flour	mg/g	2.66	0.023	-0.5	-0.1	7	5.59	6.1	2.66	0.023
Choline	Egg Powder	mg/g	14.7	0.33	0.2	0.0	7	13.200	9.150	14.7	0.33

x_i Mean of reported values

s_i Standard deviation of reported values

Z_{comm} Z-score with respect to community consensus

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

N Number of quantitative values reported

x* Robust mean of reported values

s* Robust standard deviation

x_{NIST} NIST-assessed value

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

Table 18. Data summary table for choline in foods.

		Choline									
		SRM 3234 Soy Flour (mg/g)					SRM 1845a Whole Egg Powder (mg/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	NIST				2.66	0.02				14.7	0.3
	H801										
	H803										
	H810	3.1	3.1	2.9	3.02	0.1	15.7	15.7	16.3	15.9	0.3
	H816	129	130	133	131	2.5	76.1	78.2	81.2	78.5	2.6
	H821										
	H824										
	H826	2.4	2.4	2.4	2.41	0.02	14.8	14.8	14.7	14.8	0.1
	H829										
	H845	2.0	2.0	2.2	2.07	0.08	13.4	13.2	13.6	13.4	0.2
	H846										
	H860	3.1	3.1	3.1	3.13	0.02	15.1	15.4	15.3	15.3	0.1
	H862	12.2	12.3	11.3	11.93	0.53	6.7	6.6	7.2	6.8	0.3
	H870	2.2	1.8	1.8	1.95	0.25	0.028	0.028	0.028	0.028	0.000
Community Results		Consensus Mean			5.59		Consensus Mean			13.2	
		Consensus Standard Deviation			6.05		Consensus Standard Deviation			9.1	
		Maximum			131		Maximum			78.5	
		Minimum			1.95		Minimum			0.028	
		N			7		N			7	

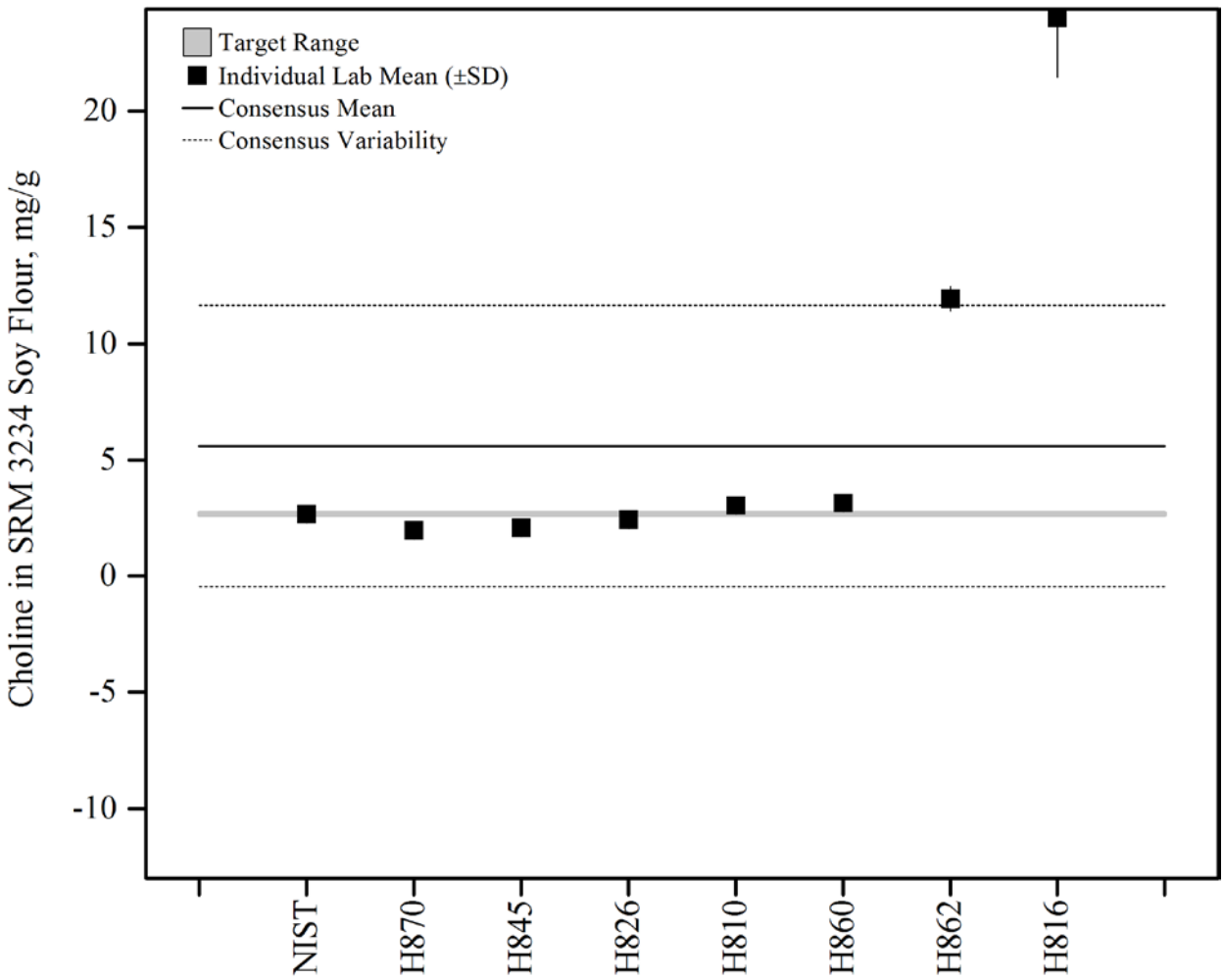

Figure 35. Choline in candidate SRM 3234 Soy Flour (data summary view). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The black solid line represents the consensus mean, and the black dotted lines represent the consensus variability calculated as one standard deviation about the consensus mean. The gray shaded region represents the target zone for "acceptable" performance, which encompasses the NIST value determined by ID-LC-MS bounded by twice the standard deviation observed for 24 measurements.

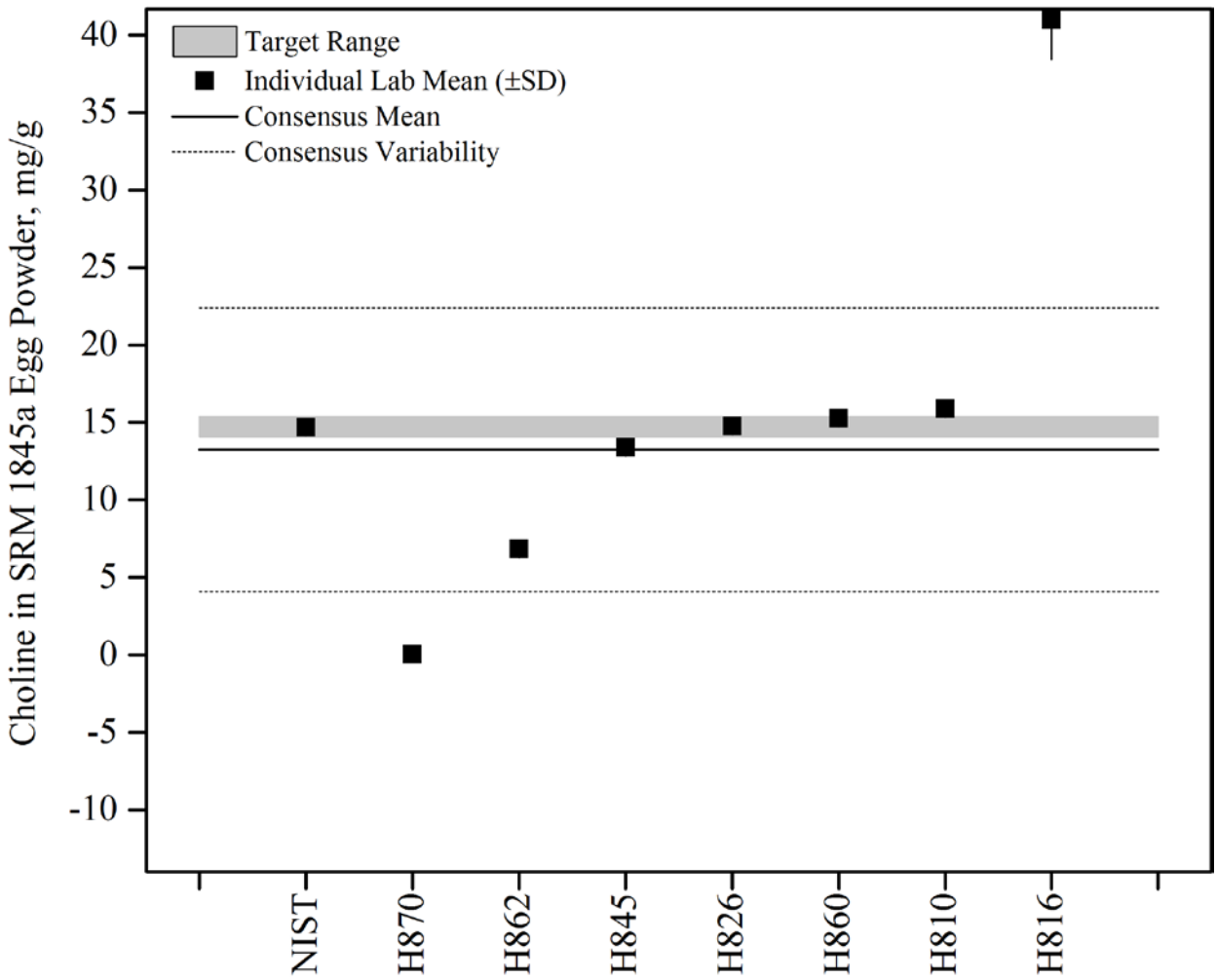

Figure 36. Choline in candidate SRM 1845a Whole Egg Powder (data summary view). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The black solid line represents the consensus mean, and the black dotted lines represent the consensus variability calculated as one standard deviation about the consensus mean. The gray shaded region represents the target zone for "acceptable" performance, which encompasses the NIST value determined by ID-LC-MS bounded by twice the standard deviation observed for 20 measurements.

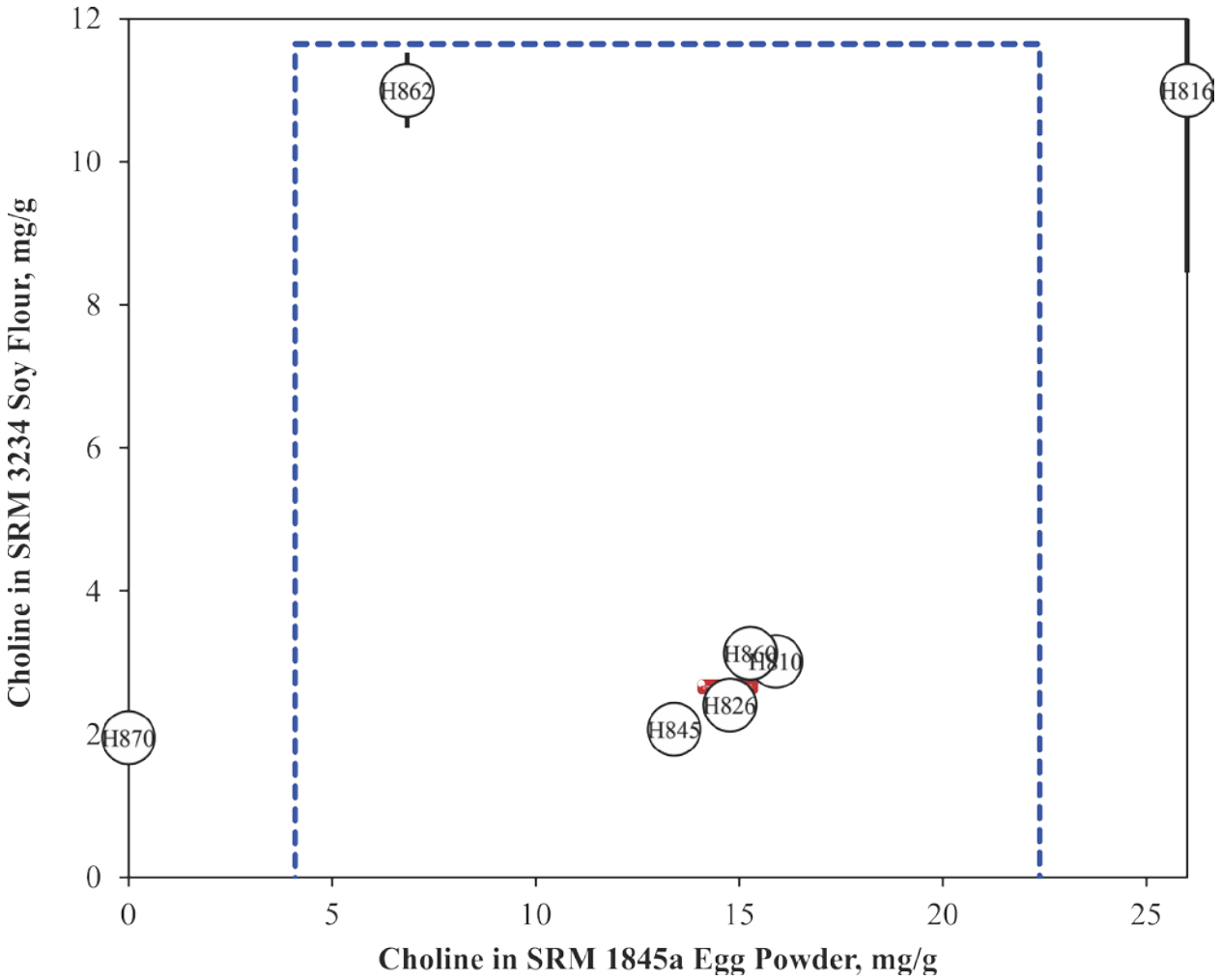

Figure 37. Choline in SRM 3234 Soy Flour and candidate SRM 1845a Whole Egg Powder (sample/sample comparison view). In this view, the individual laboratory results for one sample (candidate SRM 1845a Whole Egg Powder) with a certified value for the analyte are compared to the results for a second sample (candidate SRM 3234 Soy Flour). The error bars represent the individual laboratory standard deviation. The solid red lines represent the target zone for the control (x-axis) and the unknown sample (y-axis). The dotted blue box represents the consensus zone for the control (x-axis) and the unknown sample (y-axis).

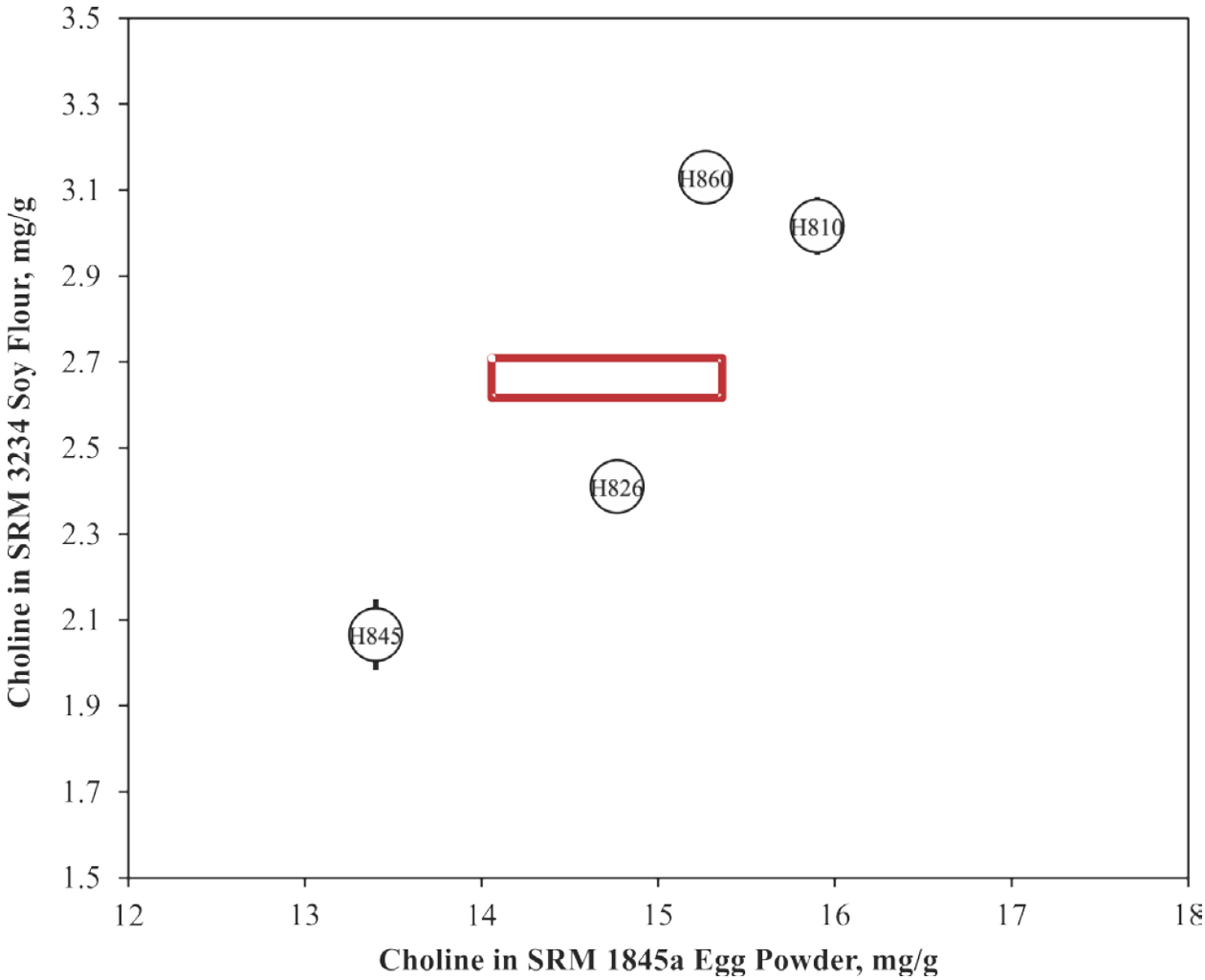

Figure 38. Expanded view of choline in SRM 3234 Soy Flour and candidate SRM 1845a Whole Egg Powder (sample/sample comparison view). In this view, the individual laboratory results for one sample (candidate SRM 1845a Whole Egg Powder) with a certified value for the analyte are compared to the results for a second sample (candidate SRM 3234 Soy Flour). The error bars represent the individual laboratory standard deviation. The solid red lines represent the target zone for the control (x-axis) and the unknown sample (y-axis).

TOCOPHEROLS IN FOODS

Study Overview

In this study, participants were provided with two NIST SRMs, SRM 3276 Carrot Extract in Oil and candidate SRM 1845a Whole Egg Powder. Participants were asked to use in-house analytical methods to determine the mass fractions of four tocopherols (α-tocopherol, β-tocopherol, γ-tocopherol, and δ-tocopherol) as well as the total amount of tocopherols in each of the matrices and report values on an as-received basis.

Sample Information

Carrot extract. Participants were provided with three ampoules, each containing approximately 1 mL of carrot extract in oil. The carrot extract in oil was mixed with butylated hydroxytoluene (BHT, approximately 670 µg/g) and ampouled under argon. Before use, participants were instructed to mix thoroughly the contents of the ampoule and use a sample size of at least 50 mg. Participants were asked to report a single value from each ampoule and store the extract at controlled room temperature, 10 °C to 30 °C. Approximate analyte levels were not reported prior to the study. The NIST certified values and uncertainties in SRM 3276 were determined by LC-fluorescence following solvent extraction and are reported in the table below.

Egg powder. Participants were provided with three vials, each containing approximately 1.5 g of whole egg powder. The material is a free-flowing, fine powder prepared from USDA-inspected whole eggs. Before use, participants were instructed to mix thoroughly the contents of the vial and use a sample size of at least 0.5 g. Participants were asked to report a single value from each vial and store the material at controlled room temperature, 10 °C to 30 °C. Approximate analyte levels were not reported prior to the study. The NIST certified values and uncertainties in candidate SRM 1845a Whole Egg Powder will be determined by a combination of LC-fluorescence data and data from external collaborating laboratories. An estimation of the values and uncertainties is provided in the table below as the mean and standard deviation of duplicate measurements performed by three (γ-tocopherol) or six (α-tocopherol) external collaborating laboratories. All laboratories used saponification in the sample preparation and liquid chromatography with either absorbance or fluorescence detection to measure the tocopherols in candidate SRM 1845a.

Analyte	Certified Mass Fraction in SRM 3276 (µg/g)	NIST-Determined Mass Fraction in Candidate SRM 1845a (µg/g)
α-tocopherol		36.0 ± 7.0
β-tocopherol		
γ-tocopherol	373 ± 34	12.0 ± 5.6
δ-tocopherol	443 ± 64	

Study Results

- Forty-one laboratories enrolled in this exercise and received samples, and 20 laboratories reported results for at least some of the tocopherols (49 % participation).
- NIST target values are available for γ-tocopherol and δ-tocopherol in the carrot oil sample.

    - The consensus mean for γ-tocopherol was within the target range, while the consensus mean for δ-tocopherol was slightly above the target range.
    - The consensus ranges were quite wide for both compounds in the carrot extract (33 % and 70 % RSD, respectively).
- NIST target values are available for α-tocopherol and γ-tocopherol in the egg powder sample. The consensus means for both compounds were within the target range, and the consensus ranges were both quite wide (48 % and 60 % RSD, respectively).
- Results for total tocopherols were calculated as the sum of all four tocopherol values reported for each sample.
    - In the carrot oil, the consensus mean was lower than the target range with a wide uncertainty (125 % RSD).
    - In the egg powder, the consensus mean was within the target range, but had a wide uncertainty (59 % RSD).
    - Many laboratories reported values for tocopherols not known to be present at quantifiable levels in the materials.
- Eleven laboratories (55 %) reported using saponification followed by extraction, while eight laboratories (40 %) reported using solvent extraction to prepare samples. One laboratory (5 %) reported using derivatization in the sample preparation.
- A majority of laboratories (75 %) used LC-absorbance for analysis. Three laboratories (15 %) reported using LC-fluorescence, one laboratory (5 %) reported using LC-MS, and one laboratory (5 %) reported using GC-MS.
- A majority of laboratories (88 %) reported using an internal standard approach to calibration. Two laboratories (12 %) reported using a standard addition approach to calibration.

<u>Technical Recommendations</u>

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

- A calibration error is possible, based on the sample/control comparison graphs, but more data for the entire sample set is needed to conclusively determine the source of error.
- Spiking studies or subjecting calibrant materials to the same preparation procedure as the samples (extraction, hydrolysis, etc.) can help to identify if tocopherols are being degraded during sample preparation.
- Tocopherol calibrant mass fraction should always be determined spectrophotometrically.
- If saponification is used for the sample preparation and an internal standard approach is taken to quantitation, it is imperative that laboratories check the stability of the internal standard.

Table 19. Individual data table (NIST) for tocopherols in foods.

National Institute of Standards & Technology

Exercise H - March 2012 - Tocopherols

	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}
α-tocopherol	Carrot Oil	µg/g					11	16.7	14.9		
α-tocopherol	Egg Powder	µg/g	36.0	7.0	-0.6	0.0	16	49.5	24.0	36.0	7.0
β-tocopherol	Carrot Oil	µg/g					3	6.02	1.63		
β-tocopherol	Egg Powder	µg/g					1				
γ-tocopherol	Carrot Oil	µg/g	443	64	0.7	0.0	10	365	117	443	64
γ-tocopherol	Egg Powder	µg/g	12	5.6	-0.5	0.0	8	17.8	10.7	12.0	5.6
δ-tocopherol	Carrot Oil	µg/g	373	34	-0.3	0.0	9	452	316	373	34
δ-tocopherol	Egg Powder	µg/g					2	14.4	21.4		
Total tocopherols	Carrot Oil	µg/g	816	73	0.7	0.0	20	446	561	816	72
Total tocopherols	Egg Powder	µg/g	48.0	9.0	-0.3	0.0	20	56.9	33.8	48.0	9.0

x_i Mean of reported values

s_i Standard deviation of reported values

Z_{comm} Z-score with respect to community consensus

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

N Number of quantitative values reported

x* Robust mean of reported values

s* Robust standard deviation

x_{NIST} NIST-assessed value

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

Table 20. Data summary table for α–tocopherol in foods.

		α-tocopherol									
		SRM 3276 Carrot Extract in Oil (µg/g)					SRM 1845a Whole Egg Powder (µg/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	NIST									36.0	7.0
	H801										
	H803										
	H805										
	H807										
	H809										
	H810						63.4	62.4	66.2	64.0	2.0
	H812	25.6	22.3	25.0	24.3	1.8	66.0	78.0	62.0	68.7	8.3
	H814						53.1	55.0	50.0	52.7	2.5
	H815	44.5	46.2	46.1	45.6	0.9	47.1	48.9	49.6	48.5	1.3
	H816		0.6	0.7	0.7	0.0	0.7	0.8	0.6	0.7	0.1
	H820										
	H821										
	H823	20.5	21.0	20.0	20.5	0.5	63.1	62.2	63.7	63.0	0.8
	H824	6560	6440	6470	6490	62	31.1	27.8	30.1	29.7	1.7
	H826	14.0	14.0	16.0	14.7	1.2	56.0	61.0	63.0	60.0	3.6
	H828										
	H829										
	H830										
	H832										
	H834										
	H835										
	H839										
	H842										
	H843										
	H846										
	H847	3.9	3.8	6.7	4.8	1.7	27.6	22.1	32.3	27.3	5.1
	H848	4.1	3.9	6.8	4.9	1.7	25.8	20.6	30.2	25.5	4.8
	H850										
	H852										
	H853						69.7	66.1	65.2	67.0	2.4
	H855			41.9	41.9		73.1	107.0	83.0	87.7	17.4
	H856										
	H857	8.1	7.3	25.4	13.6	10.2	18.5	22.5	17.2	19.4	2.8
	H858										
	H861										
	H862						69.4	72.2	68.4	70.0	2.0
	H870						40.5	40.1	38.5	39.7	1.1
	H871										
	H872										
	H873	15.9	16.7	16.0	16.2	0.4	56.3	59.2	55.7	57.1	1.9
	H874										
Community Results		Consensus Mean			19.1		Consensus Mean			49.5	
		Consensus Standard Deviation			17.9		Consensus Standard Deviation			24.0	
		Maximum			6490		Maximum			87.7	
		Minimum			0.7		Minimum			0.70	
		N			10		N			16	

Table 21. Data summary table for β–tocopherol in foods.

		β-tocopherol									
		SRM 3276 Carrot Extract in Oil (µg/g)					SRM 1845a Whole Egg Powder (µg/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	NIST										
	H801										
	H803										
	H805										
	H807										
	H809										
	H810										
	H812										
	H814										
	H815	3.81	4.90	4.44	4.38	0.55					
	H816										
	H820										
	H821										
	H823	7.44	6.91	6.73	7.03	0.37	0.54	0.62	0.62	0.59	0.04
	H824										
	H826	8.00	6.00	6.00	6.67	1.15					
	H828										
	H829										
	H830										
	H832										
	H834										
	H835										
	H839										
	H842										
	H843										
	H846										
	H847										
	H848										
	H850										
	H852										
	H853										
	H855										
	H856										
	H857										
	H858										
	H861										
	H862										
	H870										
	H871										
	H872										
	H873										
	H874										
Community Results		Consensus Mean			6.02		Consensus Mean				
		Consensus Standard Deviation			1.63		Consensus Standard Deviation				
		Maximum			7.03		Maximum				
		Minimum			4.38		Minimum				
		N			3		N			1	

Table 22. Data summary table for γ–tocopherol in foods.

		γ-tocopherol									
		SRM 3276 Carrot Extract in Oil (µg/g)					SRM 1845a Whole Egg Powder (µg/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	NIST				443	64				12.0	5.6
	H801										
	H803										
	H805										
	H807										
	H809										
	H810	342	334	336	337	4	19.8	20.7	24.4	21.6	2.4
	H812										
	H814										
	H815	201	216	198	205	10	4.7	6.0	6.4	5.7	0.9
	H816										
	H820										
	H821										
	H823	467	452	456	459	7	23.3	23.2	23.6	23.4	0.2
	H824										
	H826	380	396	382	386	9	20.0	22.0	22.0	21.3	1.2
	H828										
	H829										
	H830										
	H832										
	H834										
	H835										
	H839										
	H842										
	H843										
	H846										
	H847										
	H848										
	H850										
	H852										
	H853	477	468	494	479	13	29.5	29.8	32.1	30.5	1.4
	H855	391	396	399	395	4	19.0	18.9	18.8	18.9	0.1
	H856										
	H857	15	20	20	18	3	0.6	0.6	0.3	0.5	0.2
	H858										
	H861										
	H862	467	459	460	462	4					
	H870	420	450	400	423	25	18.5	19.6	19.1	19.1	0.6
	H871										
	H872										
	H873										
	H874										
Community Results		Consensus Mean			370		Consensus Mean			17.8	
		Consensus Standard Deviation			123		Consensus Standard Deviation			10.7	
		Maximum			479		Maximum			30.5	
		Minimum			18		Minimum			0.52	
		N			9		N			8	

Table 23. Data summary table for δ–tocopherol in foods.

		δ-tocopherol									
		SRM 3276 Carrot Extract in Oil (µg/g)					SRM 1845a Whole Egg Powder (µg/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	NIST				373	34					
	H801										
	H803										
	H805										
	H807										
	H809										
	H810	264	262	261	262	2					
	H812										
	H814										
	H815	102	93	99	98	4					
	H816										
	H820										
	H821										
	H823	390	386	388	388	2	1.11	1.06	1.02	1.06	0.04
	H824										
	H826	331	350	338	340	10					
	H828										
	H829										
	H830										
	H832										
	H834										
	H835										
	H839										
	H842										
	H843										
	H846										
	H847										
	H848										
	H850										
	H852										
	H853	900	892	918	903	14					
	H855	440	445	445	443	3					
	H856										
	H857										
	H858										
	H861										
	H862	331	327	326	328	3					
	H870	2010	2120	2020	2050	61	27.00	34.50	21.90	27.80	6.34
	H871										
	H872										
	H873										
	H874										
Community Results		Consensus Mean			472		Consensus Mean			14.43	
		Consensus Standard Deviation			361		Consensus Standard Deviation			21.44	
		Maximum			2050		Maximum			27.80	
		Minimum			98		Minimum			1.06	
		N			8		N			2	

Table 24. Data summary table for total tocopherol in foods.

		Total tocopherols									
		SRM 3276 Carrot Extract in Oil (µg/g)					SRM 1845a Whole Egg Powder (µg/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	NIST				816	72				48.0	9.0
	H801										
	H803										
	H805										
	H807										
	H809										
	H810	606	596	597	600	6	83.2	83.1	90.6	85.6	4.3
	H812	25.6	22.3	25.0	24.3	1.8	66.0	78.0	62.0	68.7	8.3
	H814	0.00	0.00	0.00	0.00	0.00	53.1	55.0	50.0	52.7	2.5
	H815	351	360	347	353	7	51.8	54.9	56.0	54.2	2.2
	H816	0.00	0.64	0.66	0.43	0.38	0.73	0.77	0.59	0.70	0.09
	H820										
	H821										
	H823	884	867	871	874	9	88.1	87.0	89.0	88.0	1.0
	H824	6560	6440	6470	6490	62	31.1	27.8	30.1	29.7	1.7
	H826	733	766	742	747	17	76.0	83.0	85.0	81.3	4.7
	H828										
	H829										
	H830										
	H832										
	H834	21.5	21.0	19.8	20.8	0.9	60.6	61.4	53.8	58.6	4.2
	H835	17.2	18.2	18.0	17.8	0.5	62.0	56.2	62.9	60.4	3.6
	H839	18.7	20.5	20.0	19.7	0.9	56.2	53.7	55.2	55.0	1.3
	H842										
	H843										
	H846										
	H847	3.92	3.80	6.74	4.82	1.66	27.6	22.1	32.3	27.3	5.1
	H848	4.09	3.89	6.84	4.94	1.65	25.8	20.6	30.2	25.5	4.8
	H850										
	H852										
	H853	1378	1359	1412	1383	27	99.2	95.9	97.3	97.5	1.7
	H855	831	841	886	853	29	92.1	126.0	102.0	106.7	17.4
	H856										
	H857	22.8	27.4	45.7	32.0	12.1	19.1	23.1	17.5	19.9	2.9
	H858										
	H861										
	H862	798	786	786	790	7	69.4	72.2	68.4	70.0	2.0
	H870	2430	2570	2420	2473	84	86.0	94.2	79.5	86.6	7.4
	H871										
	H872										
	H873	15.9	16.7	16.0	16.2	0.4	56.3	59.2	55.7	57.1	1.9
	H874										
Community Results		Consensus Mean			433		Consensus Mean			59.9	
		Consensus Standard Deviation			572		Consensus Standard Deviation			30.9	
		Maximum			6490		Maximum			106.7	
		Minimum			0.00		Minimum			0.70	
		N			19		N			19	

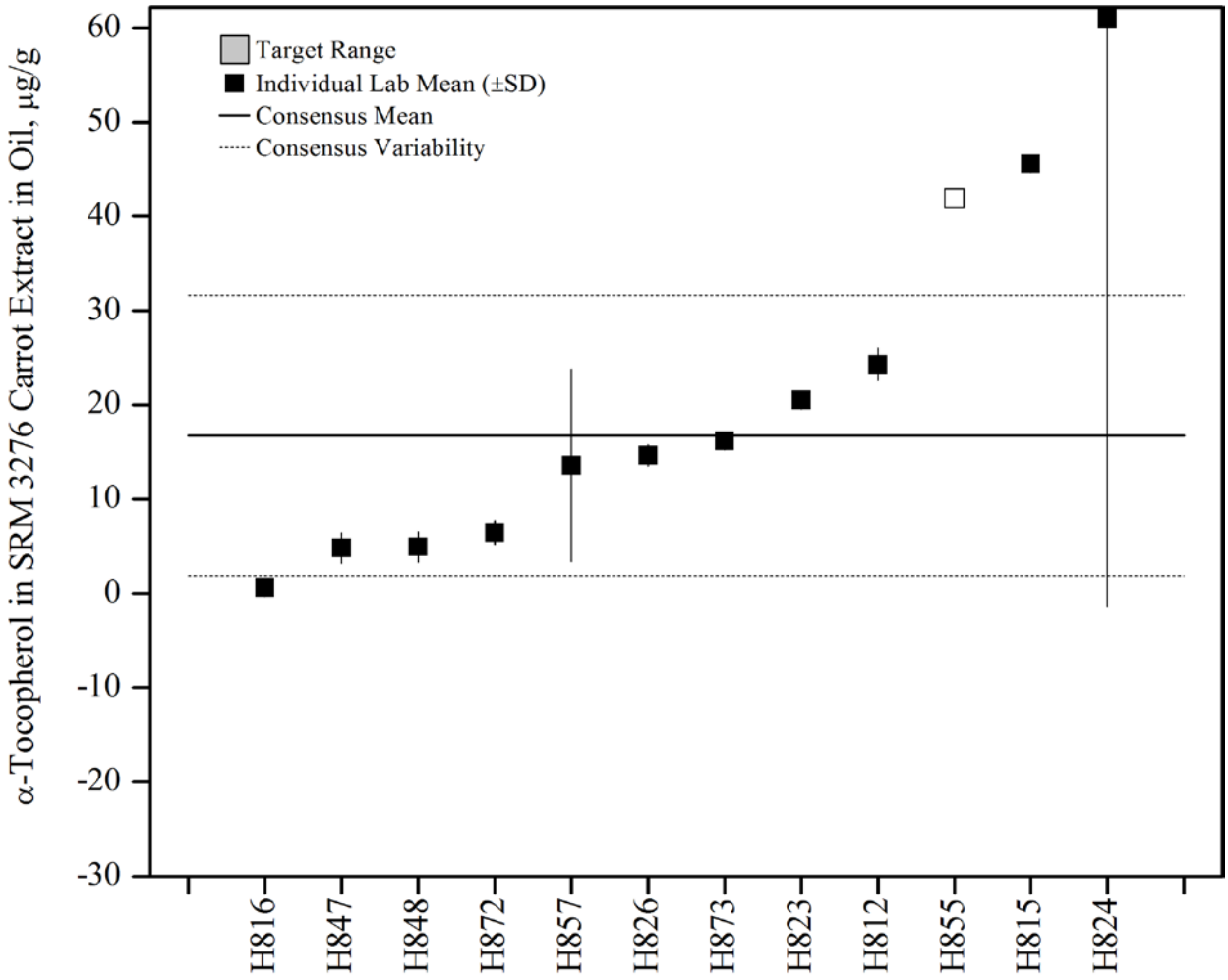

Figure 39. α-Tocopherol in SRM 3276 Carrot Extract in Oil (data summary view). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). Data points that are unfilled represent laboratories that only reported a single value for that analyte and 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.

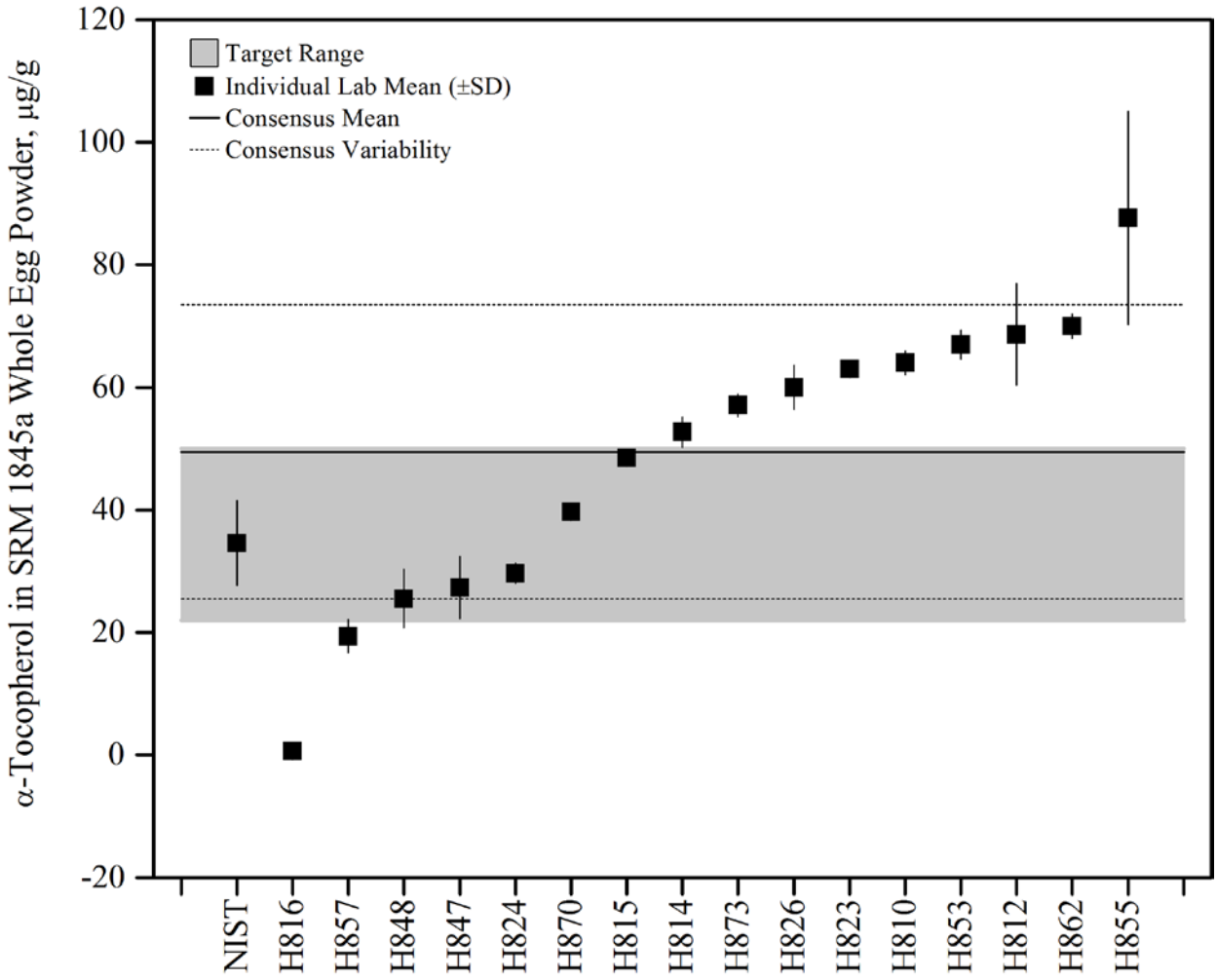

Figure 40. α-Tocopherol in candidate SRM 1845a Whole Egg Powder (data summary view). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The black solid line represents the consensus mean, and the black dotted lines represent the consensus variability calculated as one standard deviation about the consensus mean. The gray shaded region represents the target zone for "acceptable" performance, which encompasses an approximation of the NIST certified value based on LC-absorbance and LC-fluorescence data from six external collaborating laboratories, bounded by twice the standard deviation observed for 10 total measurements.

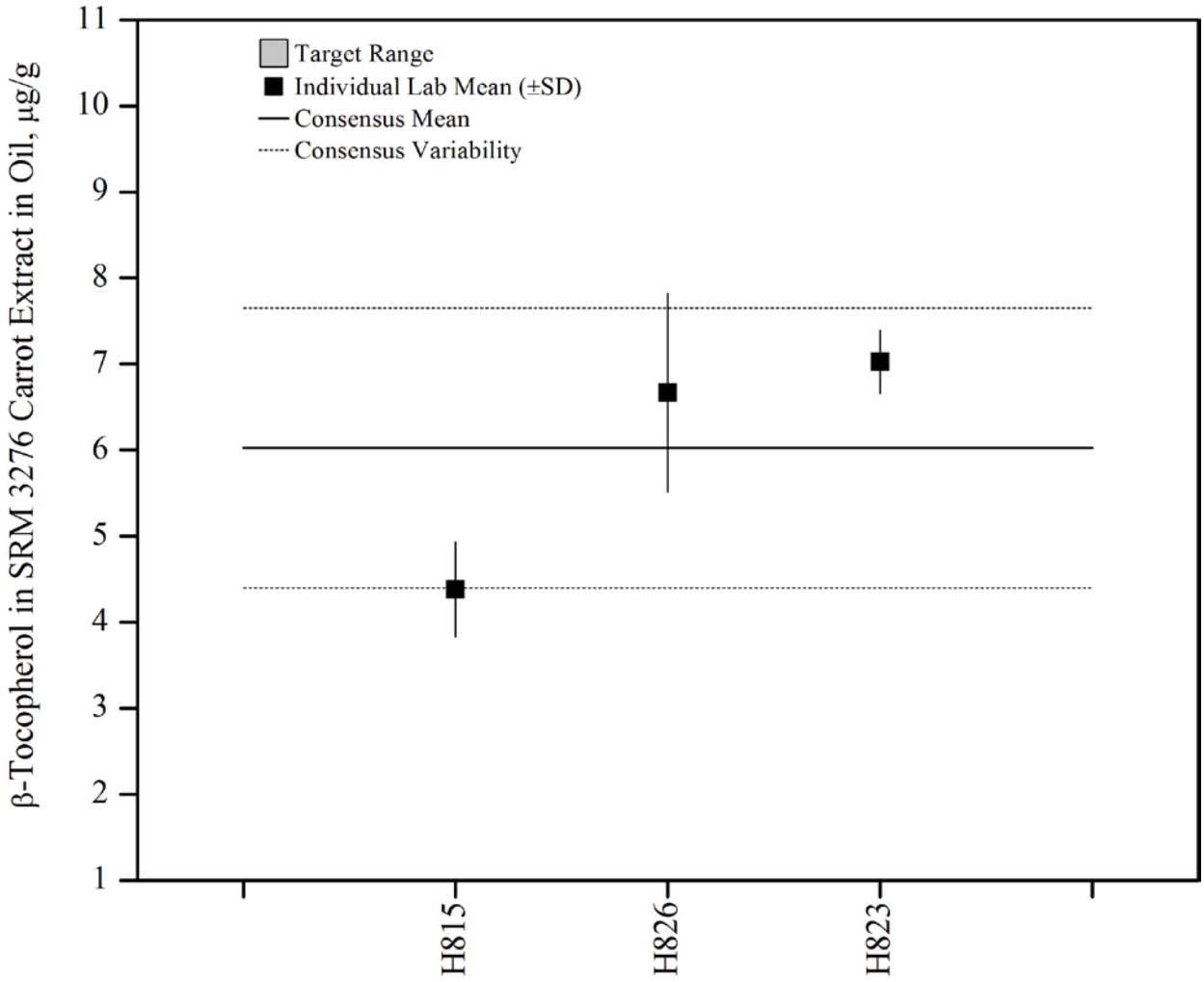

Figure 41. β-Tocopherol in SRM 3276 Carrot Extract in Oil (data summary view). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The black solid line represents the consensus mean, and the black dotted lines represent the consensus variability calculated as one standard deviation about the consensus mean.

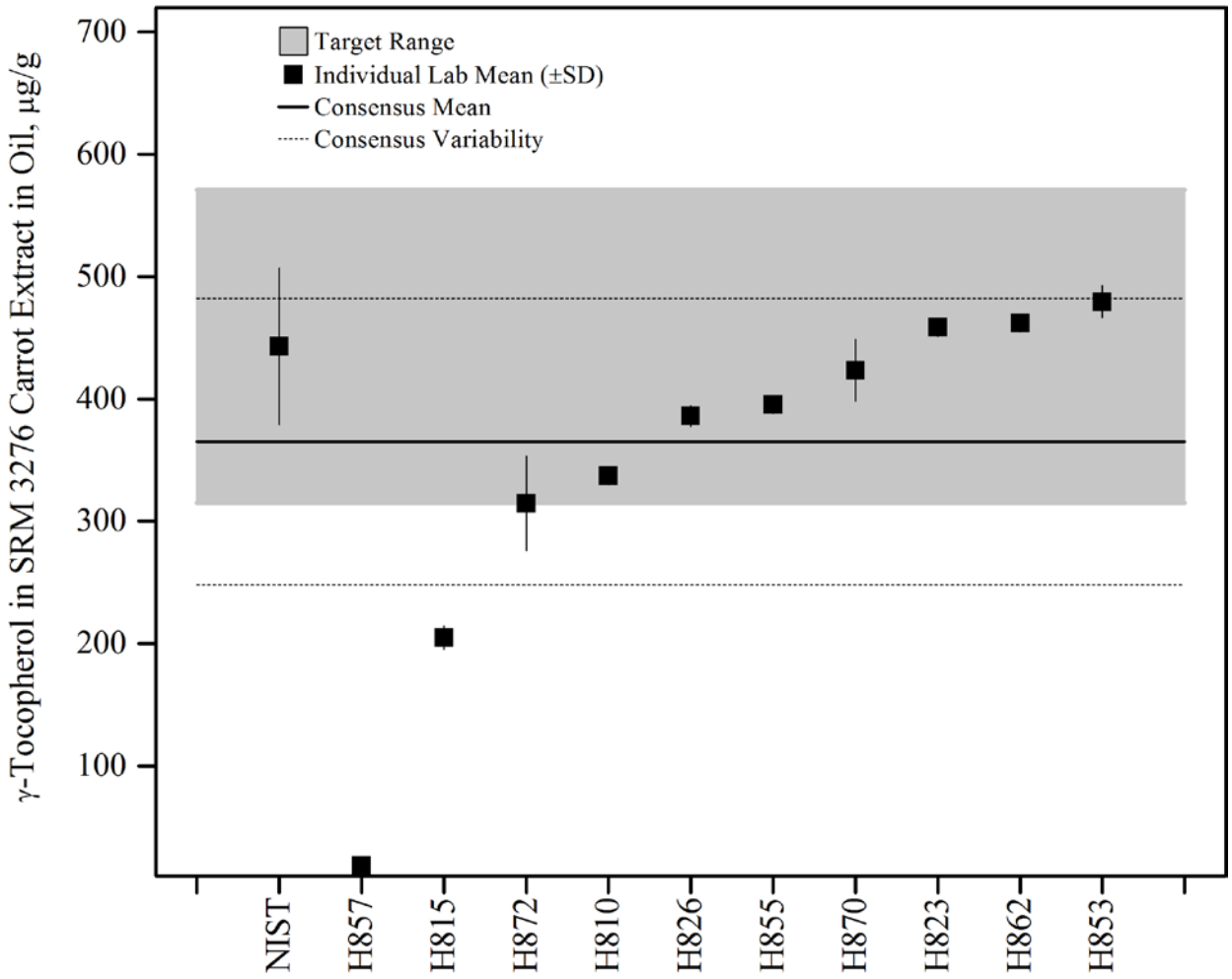

Figure 42. γ-Tocopherol in SRM 3276 Carrot Extract in Oil (data summary view). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The black solid line represents the consensus mean, and the black dotted lines represent the consensus variability calculated as one standard deviation about the consensus mean. The gray shaded region represents the target zone for "acceptable" performance, which encompasses the NIST certified value bounded by twice its uncertainty (U_{95}).

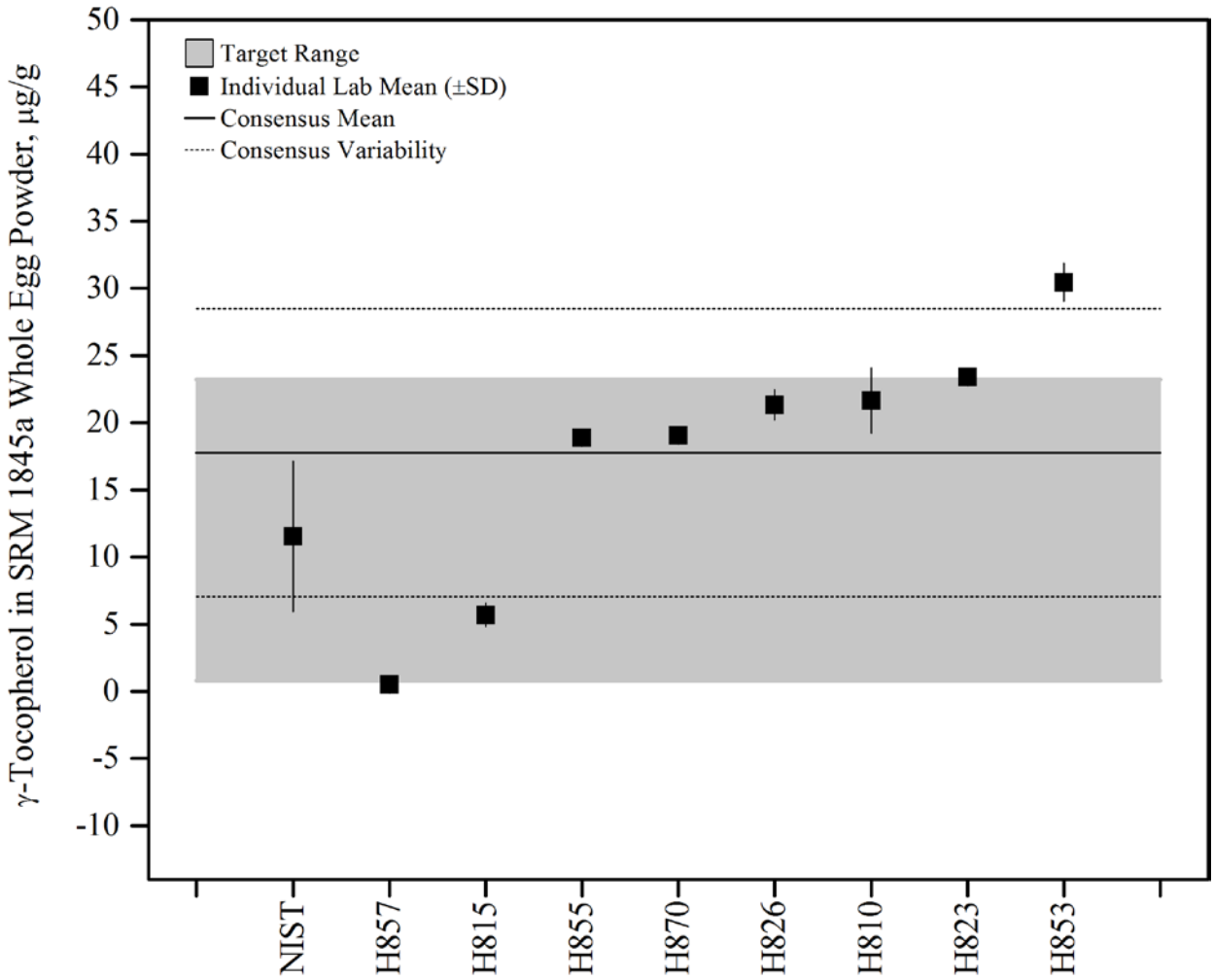

Figure 43. γ-Tocopherol in candidate SRM 1845a Whole Egg Powder (data summary view). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The black solid line represents the consensus mean, and the black dotted lines represent the consensus variability calculated as one standard deviation about the consensus mean. The gray shaded region represents the target zone for "acceptable" performance, which encompasses an approximation of the NIST certified value based on LC-absorbance and LC-fluorescence data from three external collaborating laboratories, bounded by twice the standard deviation observed for 5 total measurements.

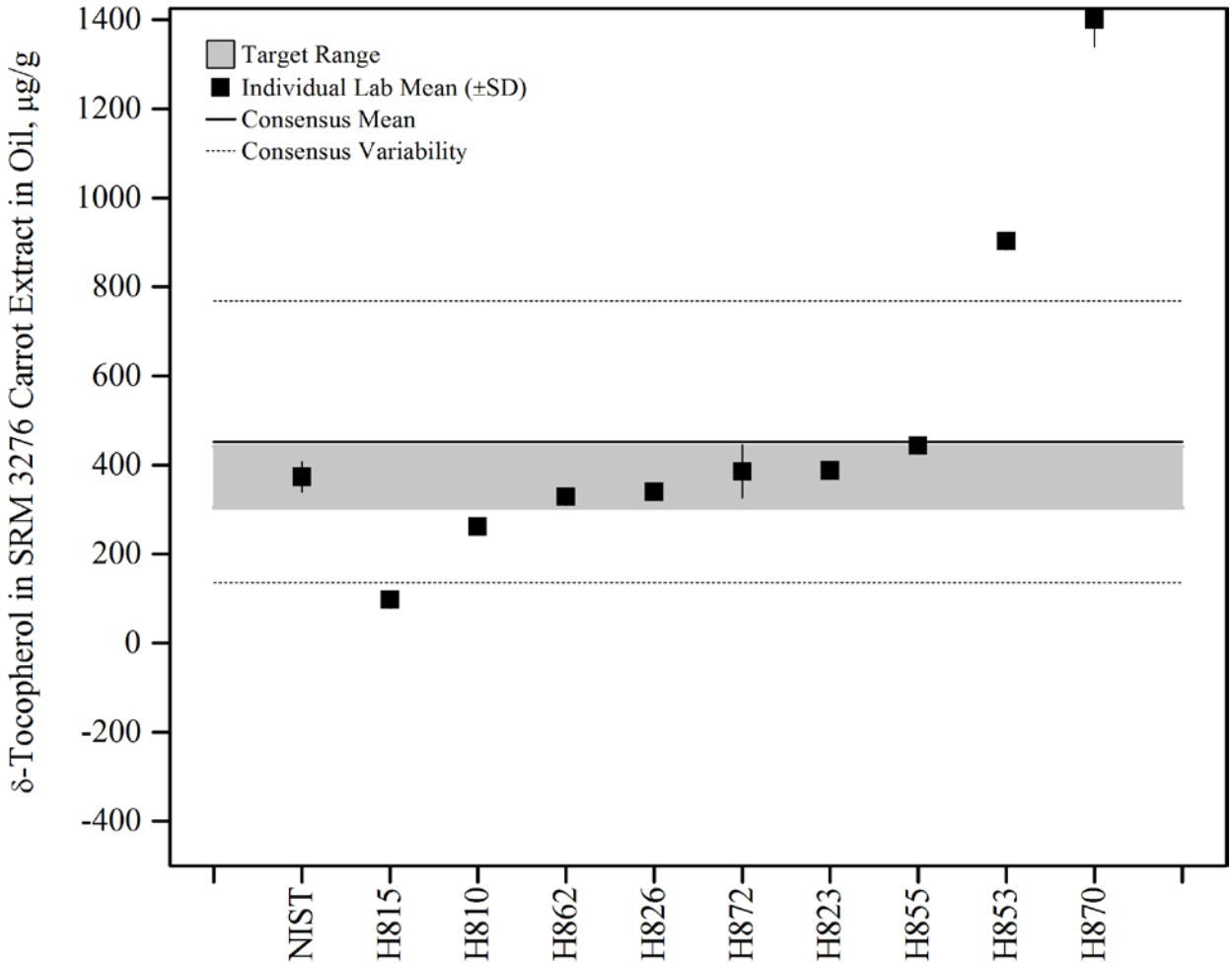

Figure 44. δ-Tocopherol in SRM 3276 Carrot Extract in Oil (data summary view). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The black solid line represents the consensus mean, and the black dotted lines represent the consensus variability calculated as one standard deviation about the consensus mean. The gray shaded region represents the target zone for "acceptable" performance, which encompasses the NIST certified value bounded by twice its uncertainty (U_{95}).

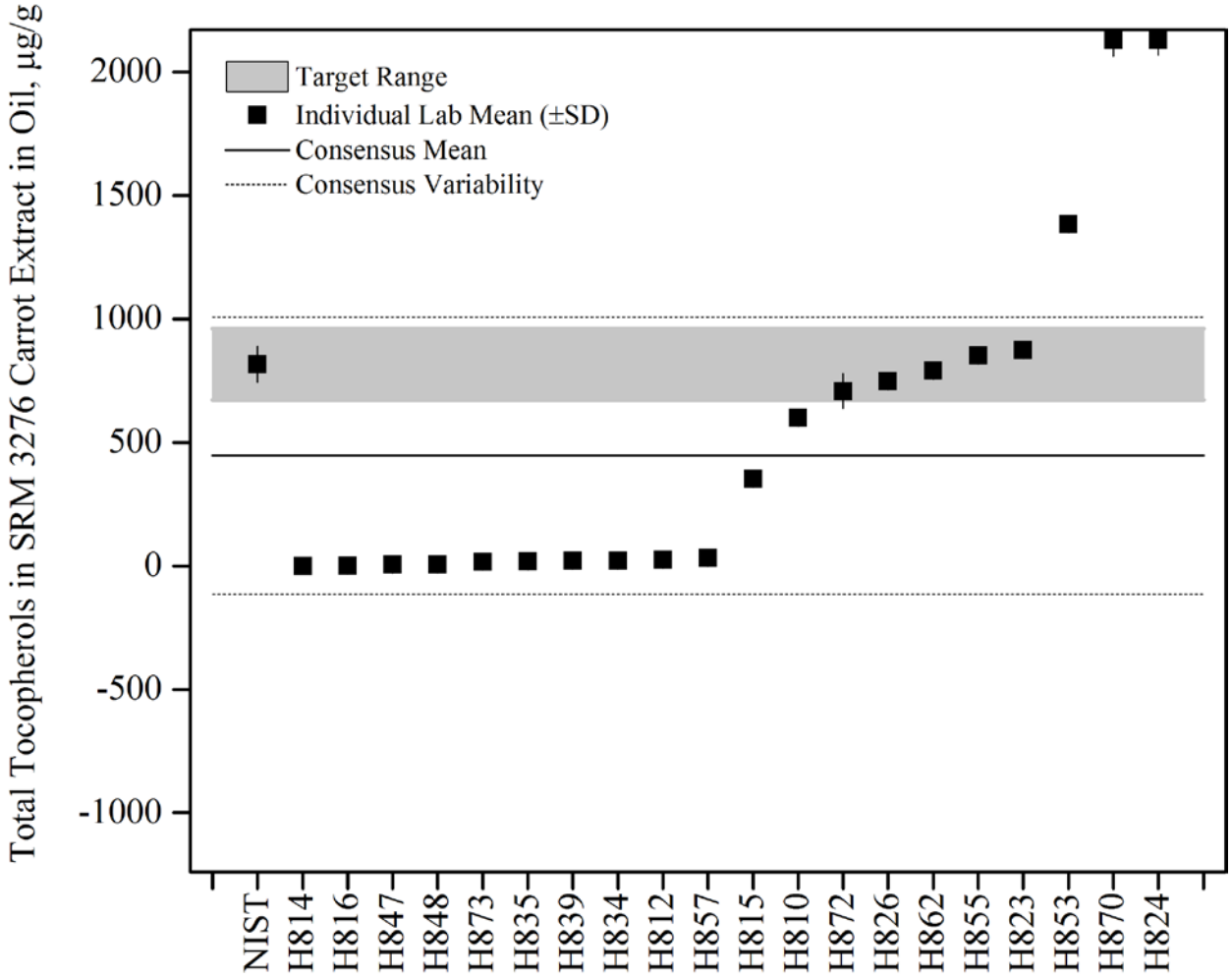

Figure 45. Total tocopherols in SRM 3276 Carrot Extract in Oil (data summary view). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The black solid line represents the consensus mean, and the black dotted lines represent the consensus 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 value bounded by twice its uncertainty (U_{95}), calculated as a combination of the certified values for γ-tocopherol and δ-tocopherol.

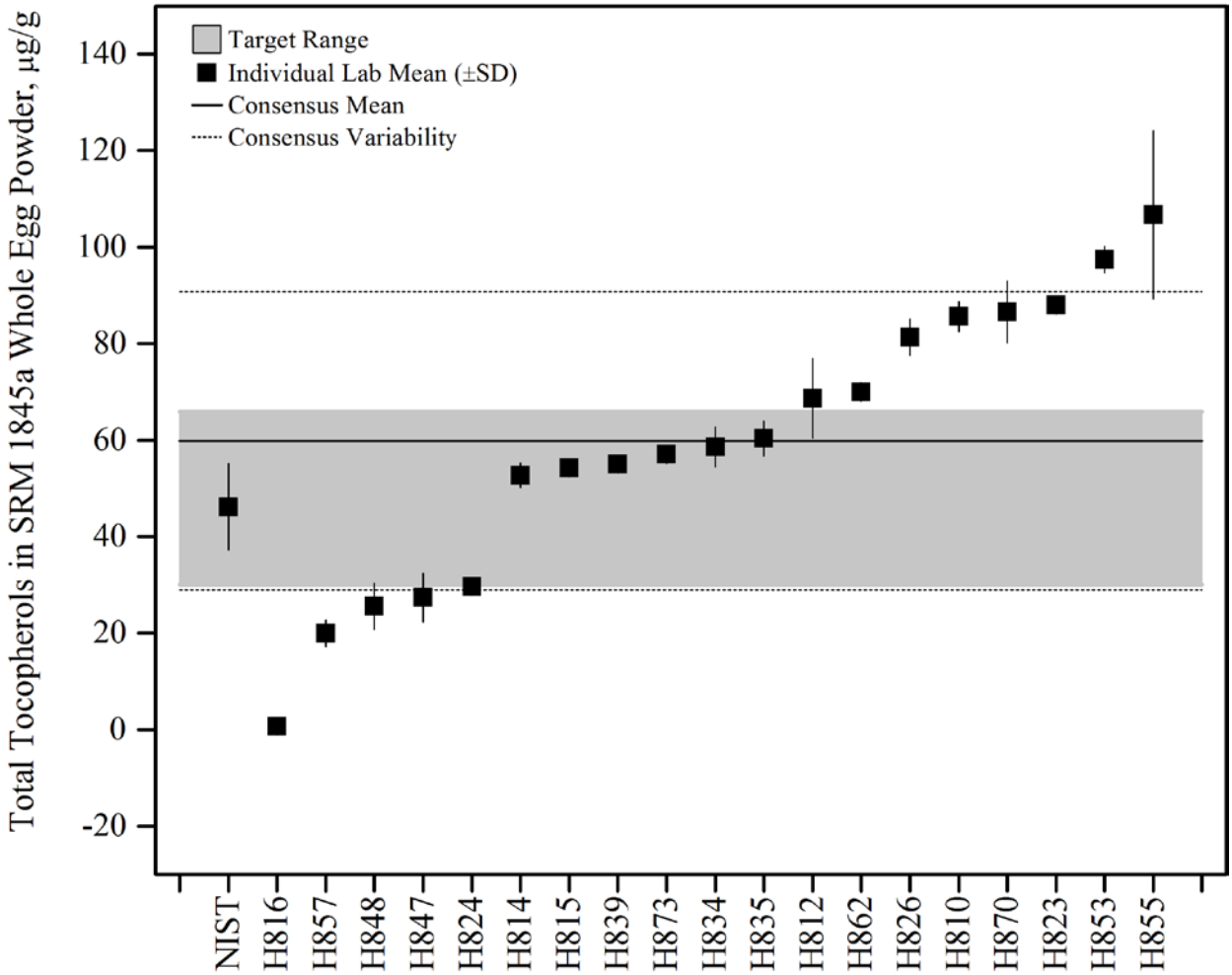

Figure 46. Total tocopherols in candidate SRM 1845a Whole Egg Powder (data summary view). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The black solid line represents the consensus mean, and the black dotted lines represent the consensus variability calculated as one standard deviation about the consensus mean. The gray shaded region represents the target zone for "acceptable" performance, which encompasses an approximation of the NIST certified value based on LC-absorbance and LC-fluorescence data from six external collaborating laboratories, bounded by twice the standard deviation observed for 10 total measurements, calculated as a combination of the values for α-tocopherol and γ-tocopherol.

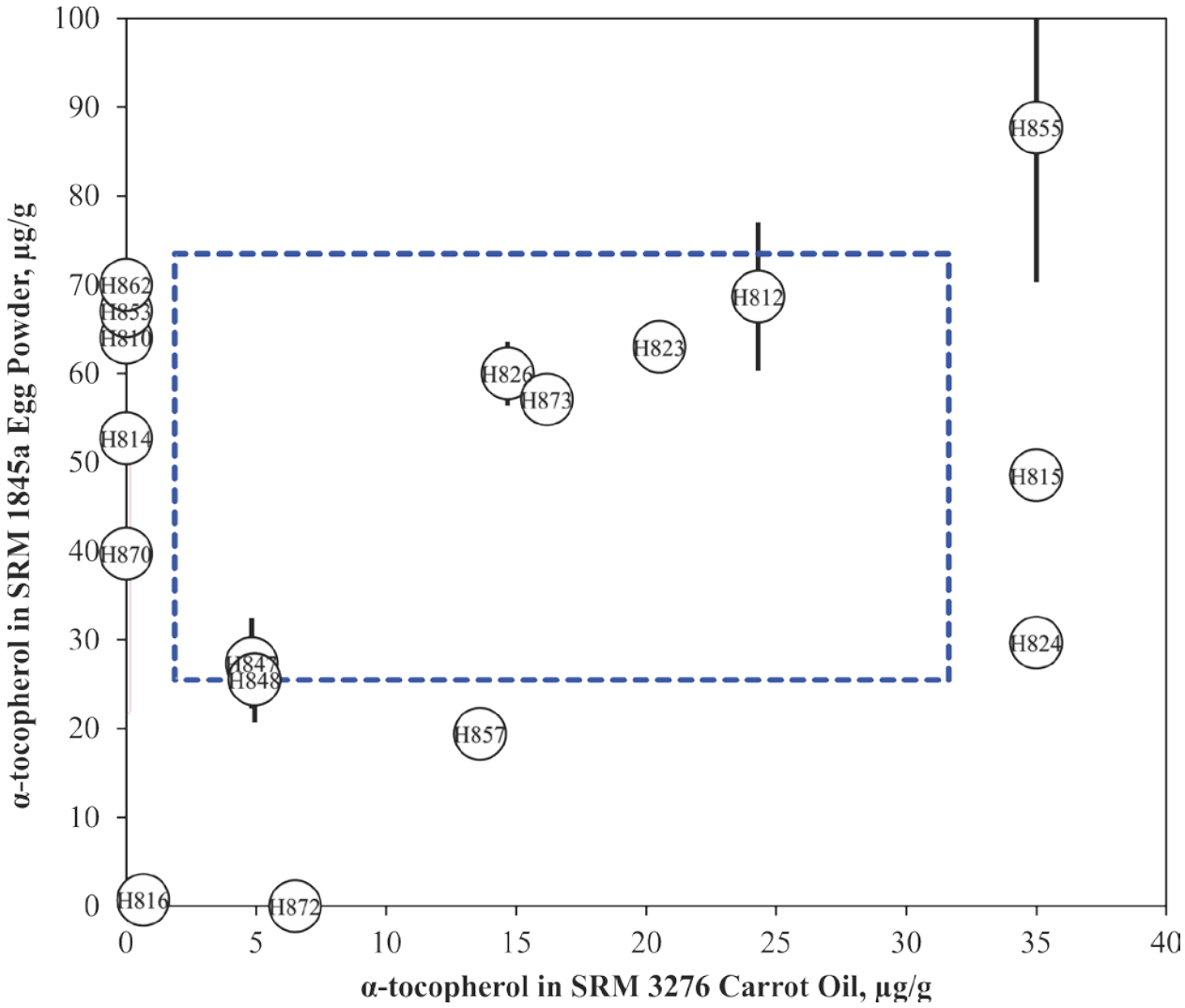

Figure 47. α-Tocopherol in candidate SRM 1845a Whole Egg Powder and SRM 3276 Carrot Extract in Oil (sample/control comparison view). In this view, the individual laboratory results for the control (SRM 3276 Carrot Extract in Oil) with a certified value for the analyte are compared to the results for an unknown (candidate SRM 1845a Whole Egg Powder). The error bars represent the individual laboratory standard deviation. The dotted blue box represents the consensus zone for the control (x-axis) and the unknown sample (y-axis).

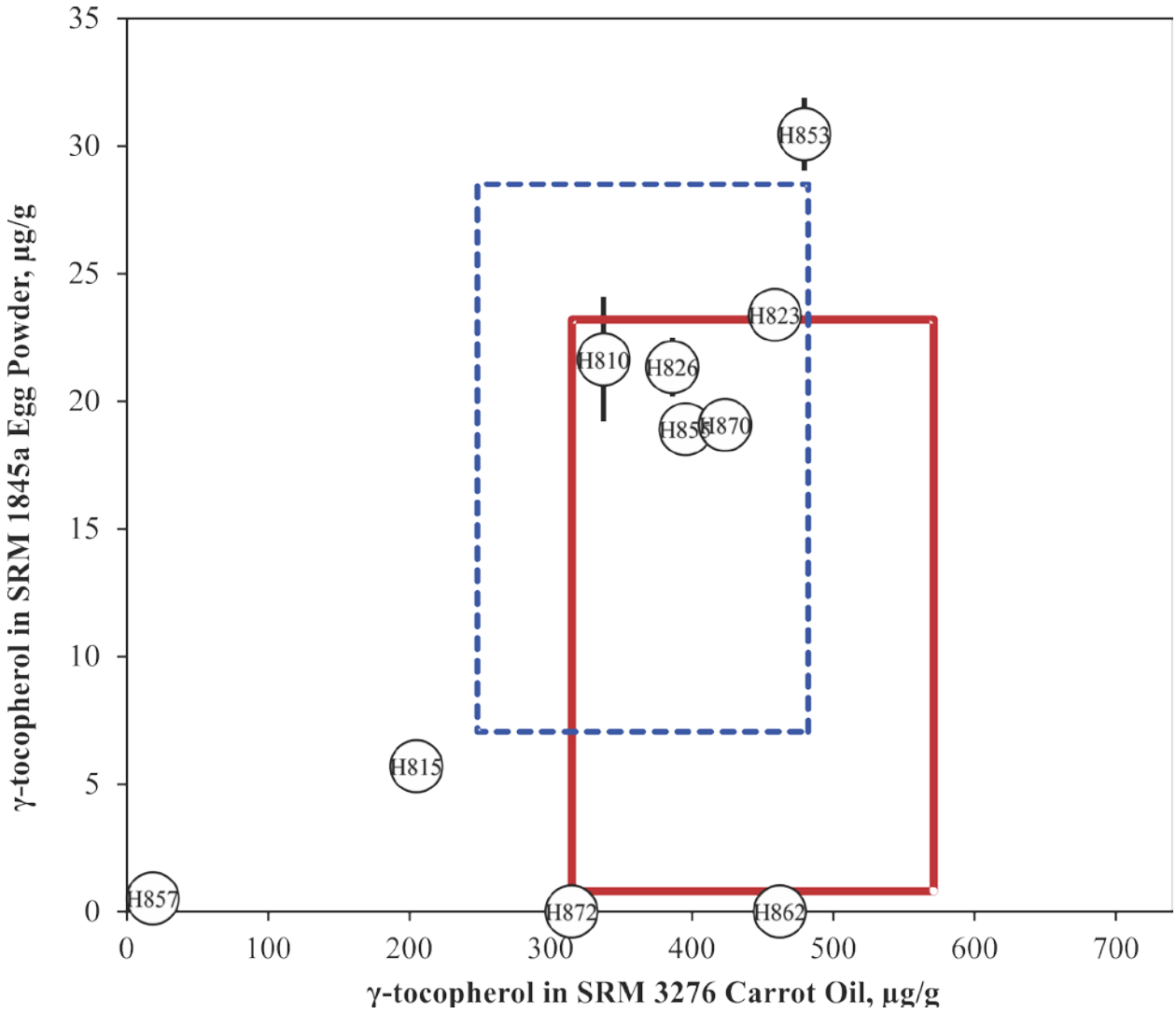

Figure 48. γ-Tocopherol in candidate SRM 1845a Whole Egg Powder and SRM 3276 Carrot Extract in Oil (sample/control comparison view). In this view, the individual laboratory results for the control (SRM 3276 Carrot Extract in Oil) with a certified value for the analyte are compared to the results for an unknown (candidate SRM 1845a Whole Egg Powder). The error bars represent the individual laboratory standard deviation. The solid red lines represent the target zone for the control (x-axis) and the unknown sample (y-axis). The dotted blue box represents the consensus zone for the control (x-axis) and the unknown sample (y-axis).

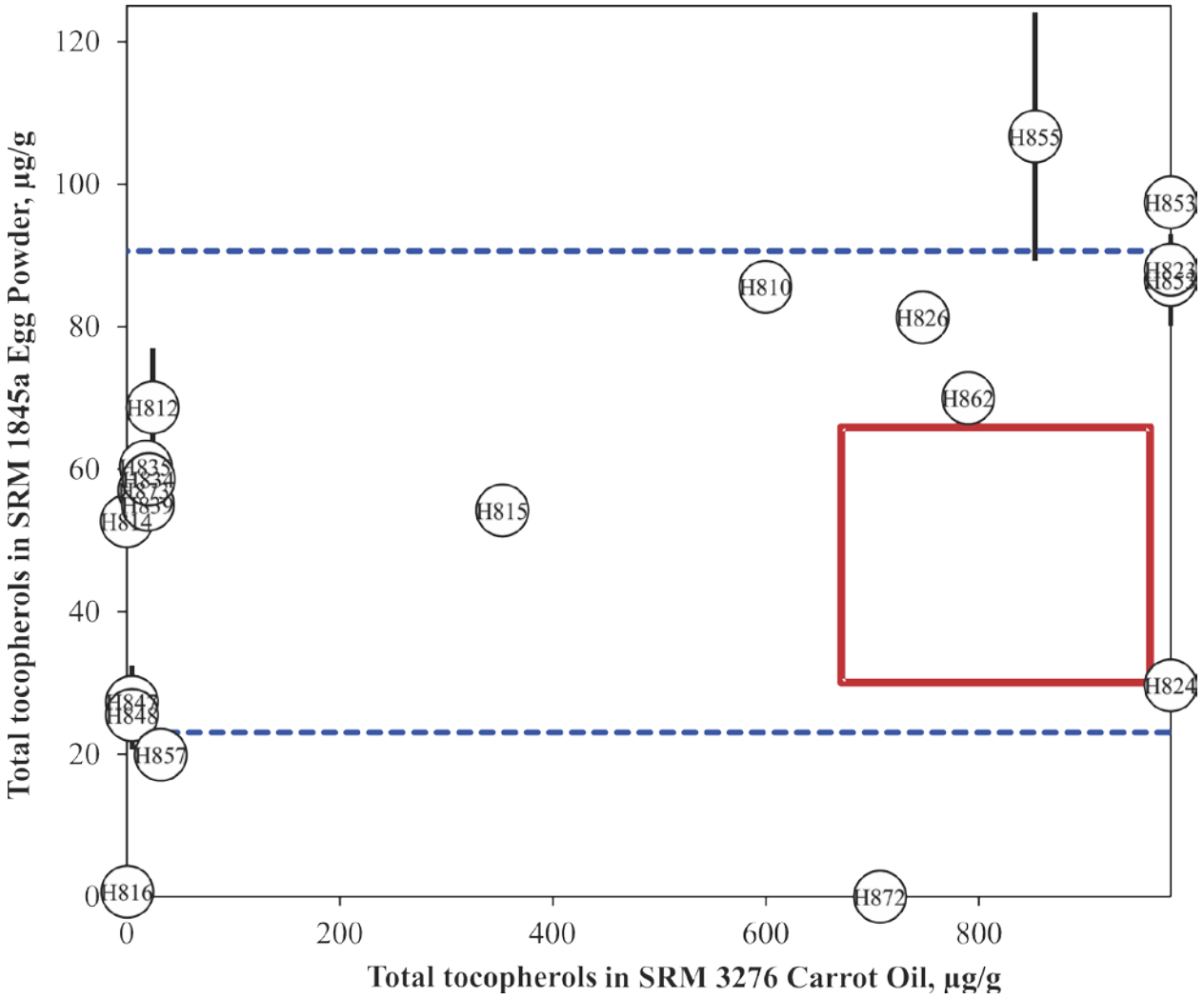

Figure 49. Total tocopherols in candidate SRM 1845a Whole Egg Powder and SRM 3276 Carrot Extract in Oil (sample/control comparison view). In this view, the individual laboratory results for the control (SRM 3276 Carrot Extract in Oil) with a certified value for the analyte are compared to the results for an unknown (candidate SRM 1845a Whole Egg Powder). The error bars represent the individual laboratory standard deviation. The solid red lines represent the target zone for the control (x-axis) and the unknown samples (y-axis). The dotted blue box represents the consensus zone for the control (x-axis) and the unknown sample (y-axis).

FATTY ACIDS IN BOTANICAL OILS

Study Overview

In this study, participants were provided with two NIST SRMs, SRM 3274-3 Flaxseed (*Linium usitatissimum*) Oil and SRM 3274-4 Perilla (*Perilla frutescens*) Oil. Participants were asked to use in-house analytical methods to determine the mass fractions of four fatty acids (linoleic acid, α-linolenic acid, γ-linolenic acid, and arachidonic acid) in each of the matrices and report values on an as-received basis. Participants were not instructed to report values for fatty acids in a certain form; NIST values are reported as triglycerides.

Sample Information

Flaxseed oil. Participants were provided with three ampoules, each containing approximately 1.2 mL of flaxseed oil from a single lot. The oil contained approximately 190 mg/L *tert*-butylhydroquinone (TBHQ) as an antioxidant and was packaged in amber glass ampoules under argon. Before use, participants were instructed to thoroughly mix the contents of the ampoule and use a sample size of at least 0.5 g. Participants were asked to report a single value from each ampoule and store the flaxseed oil in a refrigerator at 0 °C to 4 °C. Approximate analyte levels were not reported prior to the study. The NIST certified values and uncertainties in SRM 3274-3 were determined by GC-FID and GC/MS following multiple methods of hydrolysis and derivatization, and are summarized in the table below.

Perilla oil. Participants were provided with three ampoules, each containing approximately 1.2 mL of perilla oil from a single lot. The oil contained approximately 190 mg/L TBHQ as an antioxidant and was packaged in amber glass ampoules under argon. Before use, participants were instructed to mix thoroughly the contents of the ampoule and use a sample size of at least 0.5 g. Participants were asked to report a single value from each ampoule and store the perilla oil in a refrigerator at 0 °C to 4 °C. Approximate analyte levels were not reported prior to the study. The NIST certified values and uncertainties in SRM 3274-4 were determined by GC-FID and GC/MS following multiple methods of hydrolysis and derivatization, and are summarized in the table below.

Analyte	Certified Mass Fraction in SRM 3274-3 (mg/g)	Certified Mass Fraction in SRM 3274-4 (mg/g)
Linoleic acid	171 ± 11	160 ± 14
α-Linolenic acid	579 ± 30	629 ± 28
γ-Linolenic acid	1.55 ± 0.25*	2.08 ± 0.48*
Arachidonic acid	0.633 ± 0.029	

*reference value

Study Results

- Thirty-seven laboratories enrolled in this exercise and received samples, and 20 laboratories reported results for at least some of the fatty acids (54 % participation).
- The consensus mean for linoleic acid was lower than the target range in both study materials, and the consensus mean for α-linolenic acid was within the target range in both study materials. The consensus ranges were reasonable (less than the size of the NIST target range) for both compounds in both study materials.

    - Figures 58 and 59 indicate a possible calibration issue with these compounds. Laboratories that reported high values for flaxseed oil also reported high values for perilla oil. The same is true for laboratories reporting low values.
    - Five laboratories (25 %) reported using an external standard calibration approach, while 12 laboratories (60 %) reported using an internal standard calibration approach. When compared, the two approaches give similar results for these compounds.
- The consensus mean for γ-linolenic acid was well within the target range for both study materials. For perilla oil, the consensus range was also contained within the NIST target range.
- Not many laboratories were able to measure arachidonic acid (five laboratories for flaxseed oil and three laboratories for perilla oil). A certified value is only available in the flaxseed oil, and the consensus mean was higher and the consensus range significantly wider than the target range.
- Almost all laboratories (95 %) used a hydrolysis and derivatization procedure for sample preparation followed by GC-FID as their analytical method. One laboratory reported using GC-MS.

Technical Recommendations

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

- The trend observed in the sample/control comparison graph is indicative of a calibration error in the determination of linoleic acid and α–linolenic acid.
- Spiking studies or subjecting calibrant materials to the same preparation procedure as the samples (extraction, hydrolysis, derivatization, etc.) can help to identify if fatty acids are being degraded during sample preparation.
- Participants were not asked to report fatty acid results in any specific molecular form. The NIST certified values are reported as triglycerides. Conversion of fatty acid results between triglycerides and fatty acid methyl esters (FAMEs) free fatty acids would only result in a maximum of 5 % error. While a small error due to inconsistent reporting of results is possible, it does not completely explain the outlying results.

Table 25. Individual data table (NIST) for fatty acids in botanical oils.

National Institute of Standards & Technology

Exercise H - March 2012 - Fatty Acids

	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}
Linoleic Acid	Flax Oil	mg/g	171	11	0.8	0.0	20	159	16	171	11
Linoleic Acid	Perilla Oil	mg/g	160	14	1.8	0.0	20	137	13	160	14
α-Linolenic Acid	Flax Oil	mg/g	579	30	1.1	0.0	20	534	42	579	30
α-Linolenic Acid	Perilla Oil	mg/g	629	28	1.4	0.0	20	558	51	629	28
γ-Linolenic Acid	Flax Oil	mg/g	1.55	0.25	0.1	0.0	3	1.50	0.53	1.55	0.25
γ-Linolenic Acid	Perilla Oil	mg/g	2.08	0.48	0.4	0.0	14	2.00	0.20	2.08	0.48
Arachidonic Acid	Flax Oil	mg/g	0.633	0.029	-0.5	0.0	4	0.813	0.400	0.633	0.029
Arachidonic Acid	Perilla Oil	mg/g					3	0.732	0.569		

x_i Mean of reported values

s_i Standard deviation of reported values

Z_{comm} Z-score with respect to community consensus

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

N Number of quantitative values reported

x* Robust mean of reported values

s* Robust standard deviation

x_{NIST} NIST-assessed value

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

Table 26. Data summary table for linoleic acid in botanical oils.

		Linoleic Acid									
		SRM 3274-3 Flaxseed Oil (mg/g)					SRM 3274-4 Perilla Oil (mg/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	NIST				171	11.0				160	14.0
	H801										
	H803										
	H805	129	129	128	129	0.6	109	112	112	111	1.7
	H809										
	H810	162	164	162	163	1.2	140	140	139	140	0.6
	H815	181	183	182	182	1.2	156	157	157	157	0.4
	H817	168	168	168	168	0.1	144	144	144	144	0.1
	H818	180	143	167	163	18.8	142	138	140	140	2.4
	H819										
	H820	130	138	135	134	4.3	94	89	90	91	2.9
	H821										
	H823	145	144	144	144	0.4	126	126	126	126	0.4
	H824	158	158	159	158	0.6	137	136	136	136	0.6
	H825	160	160	159	160	0.6	136	136	136	136	0.0
	H827										
	H828										
	H829										
	H835	170	170	171	170	0.1	147	147	147	147	0.2
	H838	169	169	169	169	0.0	146	144	146	145	1.2
	H839	171	170	171	171	0.6	148	147	148	148	0.2
	H841	157	162	164	161	3.5	140	140	140	140	0.5
	H842										
	H843										
	H846										
	H850	78	77	77	77	0.4	68	68	66	67	1.0
	H852										
	H854										
	H855	157	155	155	156	1.0	133	132	135	133	1.5
	H857	169	175	179	174	5.0	149	155	146	150	4.6
	H858										
	H861										
	H862	163	163	163	163	0.3	140	138	139	139	1.1
	H864										
	H867	159	163	161	161	1.9	148	141	146	145	3.6
	H872	127	148	145	140	11.4	81	124	140	115	30.6
	H873	164	169	168	167	2.6	140	143	140	141	1.7
	H874										
Community Results		Consensus Mean			159		Consensus Mean			137	
		Consensus Standard Deviation			15		Consensus Standard Deviation			13	
		Maximum			182		Maximum			157	
		Minimum			77		Minimum			67	
		N			20		N			20	

Table 27. Data summary table for α-linolenic acid in botanical oils.

		α-Linolenic Acid									
		SRM 3274-3 Flaxseed Oil (mg/g)					SRM 3274-4 Perilla Oil (mg/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	NIST				579	30.0				629	28.0
	H801										
	H803										
	H805	394	400	391	395	4.6	400	415	403	406	7.9
	H809										
	H810	539	547	541	542	4.2	569	571	565	568	3.1
	H815	497	504	501	501	3.4	528	531	529	529	1.5
	H817	561	562	563	562	0.8	592	592	592	592	0.3
	H818	599	476	555	543	62.1	582	561	570	571	10.2
	H819										
	H820	502	531	519	517	14.7	461	439	442	447	11.8
	H821										
	H823	493	490	491	491	1.5	523	525	525	524	1.4
	H824	531	530	532	531	1.0	561	560	556	559	2.6
	H825	548	548	547	548	1	573	573	573	573	0.0
	H827										
	H828										
	H829										
	H835	575	574	575	575	0.3	603	604	604	604	0.5
	H838	571	574	577	574	3.0	609	608	601	606	4.4
	H839	578	575	578	577	1.6	607	609	609	608	1.1
	H841	523	537	548	536	12.4	567	571	574	571	3.3
	H842										
	H843										
	H846										
	H850	265	264	263	264	0.9	285	282	278	282	3.7
	H852										
	H854										
	H855	533	529	528	530	2.5	555	551	558	555	3.9
	H857	559	579	596	578	18.5	605	632	589	609	21.7
	H858										
	H861										
	H862	590	595	597	594	3.5	620	622	612	618	5.4
	H864										
	H867	492	506	495	497	7.3	578	542	553	558	18.5
	H872	461	513	510	495	29.2	355	532	599	495	126.1
	H873	554	561	555	557	3.8	566	585	572	574	9.7
	H874										
Community Results		Consensus Mean			535		Consensus Mean			558	
		Consensus Standard Deviation			42		Consensus Standard Deviation			51	
		Maximum			594		Maximum			618	
		Minimum			264		Minimum			282	
		N			20		N			20	

Table 28. Data summary table for γ-linolenic acid in botanical oils.

		γ-Linolenic Acid									
		SRM 3274-3 Flaxseed Oil (mg/g)					SRM 3274-4 Perilla Oil (mg/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	NIST				1.55	0.25				2.08	0.48
	H801										
	H803										
	H805										
	H809										
	H810						2.00	1.96	1.95	1.97	0.03
	H815	1.70	1.70	1.70	1.70	0.00	1.80	1.90	1.80	1.83	0.06
	H817	1.83	1.81	1.87	1.84	0.03	1.82	1.82	1.83	1.82	0.01
	H818	<0.01	<0.01	<0.01			<0.01	<0.01	<0.01		
	H819										
	H820										
	H821										
	H823						2.03	2.00	2.04	2.02	0.02
	H824	2.00	2.00	2.00	2.00	0.00	4.00	4.00	4.00	4.00	0.00
	H825	<1	<1	<1			2.20	2.20	2.20	2.20	0.00
	H827										
	H828										
	H829										
	H835						2.10	2.20	2.10	2.13	0.06
	H838						2.00	2.00	2.00	2.00	0.00
	H839						2.10	2.10	2.10	2.10	0.00
	H841						1.90	2.08	2.32	2.10	0.21
	H842										
	H843										
	H846										
	H850										
	H852										
	H854										
	H855						2.00	2.00	2.00	2.00	0.00
	H857										
	H858										
	H861										
	H862						1.70	1.70	1.80	1.73	0.06
	H864										
	H867	0.89	0.95	1.06	0.97	0.09	0.93	1.02	1.09	1.01	0.08
	H872										
	H873						2.29	2.11	2.02	2.14	0.14
	H874										
Community Results		Consensus Mean			1.63		Consensus Mean			2.00	
		Consensus Standard Deviation			0.52		Consensus Standard Deviation			0.20	
		Maximum			2.00		Maximum			4.00	
		Minimum			0.97		Minimum			1.01	
		N			4		N			14	

Table 29. Data summary table for arachidonic acid in botanical oils.

		Arachidonic Acid									
		SRM 3274-3 Flaxseed Oil (mg/g)					SRM 3274-4 Perilla Oil (mg/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	NIST				0.633	0.029					
	H801										
	H803										
	H805										
	H809										
	H810										
	H815										
	H817	1.120	1.150	1.110	1.127	0.021	0.230	0.220	0.210	0.220	0.010
	H818	1.000	<0.01	<0.01	1.000		<0.01	<0.01	<0.01		
	H819										
	H820										
	H821										
	H823	1.026	1.047	1.075	1.049	0.025	1.186	1.116	1.364	1.222	0.128
	H824										
	H825	<1	<1	<1			<1	<1	<1		
	H827										
	H828										
	H829										
	H835										
	H838										
	H839										
	H841										
	H842										
	H843										
	H846										
	H850										
	H852										
	H854										
	H855										
	H857										
	H858										
	H861										
	H862										
	H864										
	H867	0.319	0.344	0.400	0.354	0.041					
	H872										
	H873	0.730	0.710	0.720	0.720	0.010	0.790	0.720	0.750	0.753	0.035
	H874										
Community Results		Consensus Mean			0.850		Consensus Mean			0.732	
		Consensus Standard Deviation			0.359		Consensus Standard Deviation			0.569	
		Maximum			1.127		Maximum			1.222	
		Minimum			0.354		Minimum			0.220	
		N			4		N			3	

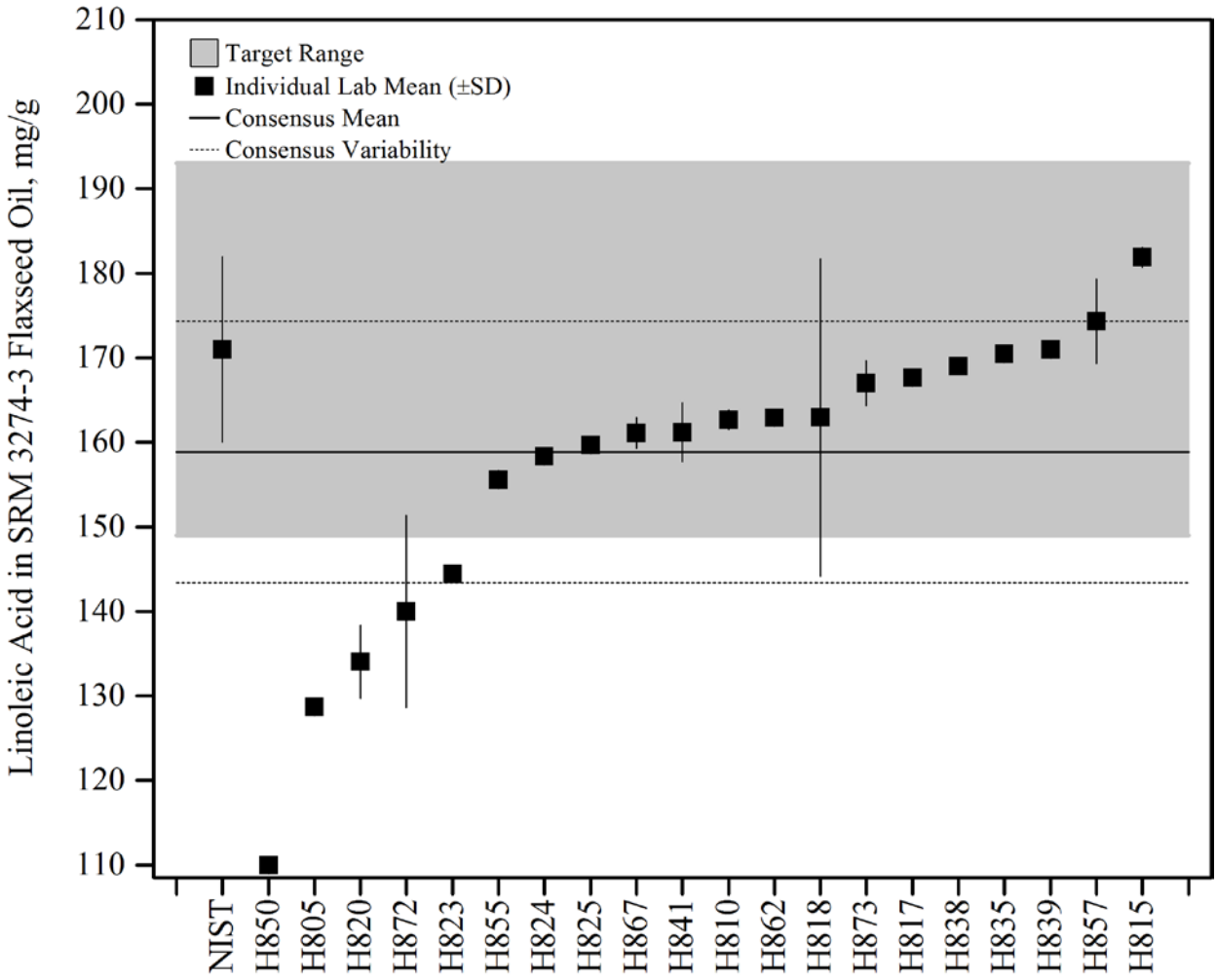

Figure 50. Linoleic Acid [C18:2, n-6] in SRM 3274-3 Flaxseed Oil (data summary view). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The black solid line represents the consensus mean, and the black dotted lines represent the consensus 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 certified value bounded by twice its uncertainty (U_{95}).

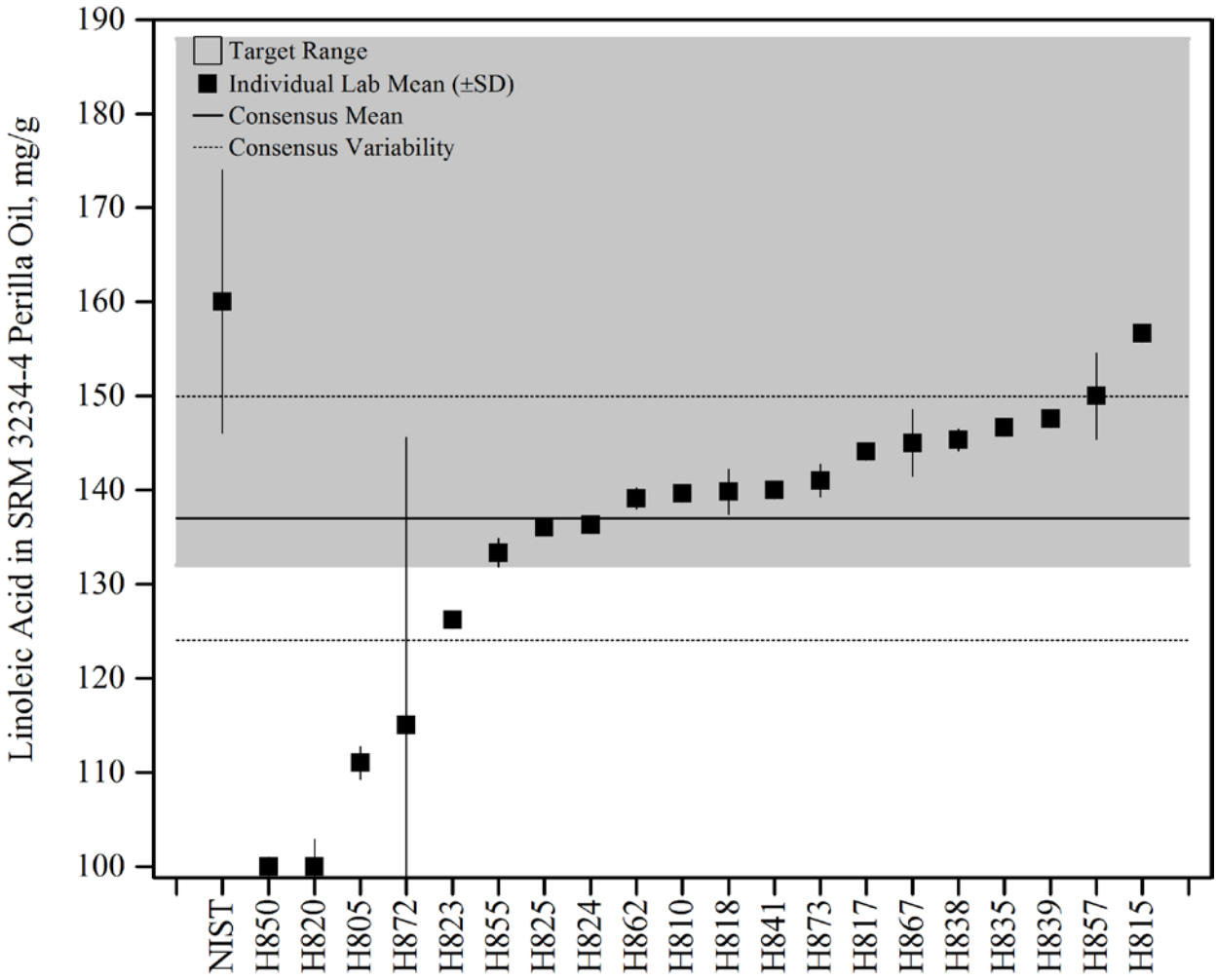

Figure 51. Linoleic Acid [C18:2, n-6] in SRM 3274-4 Perilla Oil (data summary view). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The black solid line represents the consensus mean, and the black dotted lines represent the consensus 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 certified value bounded by twice its uncertainty (U_{95}).

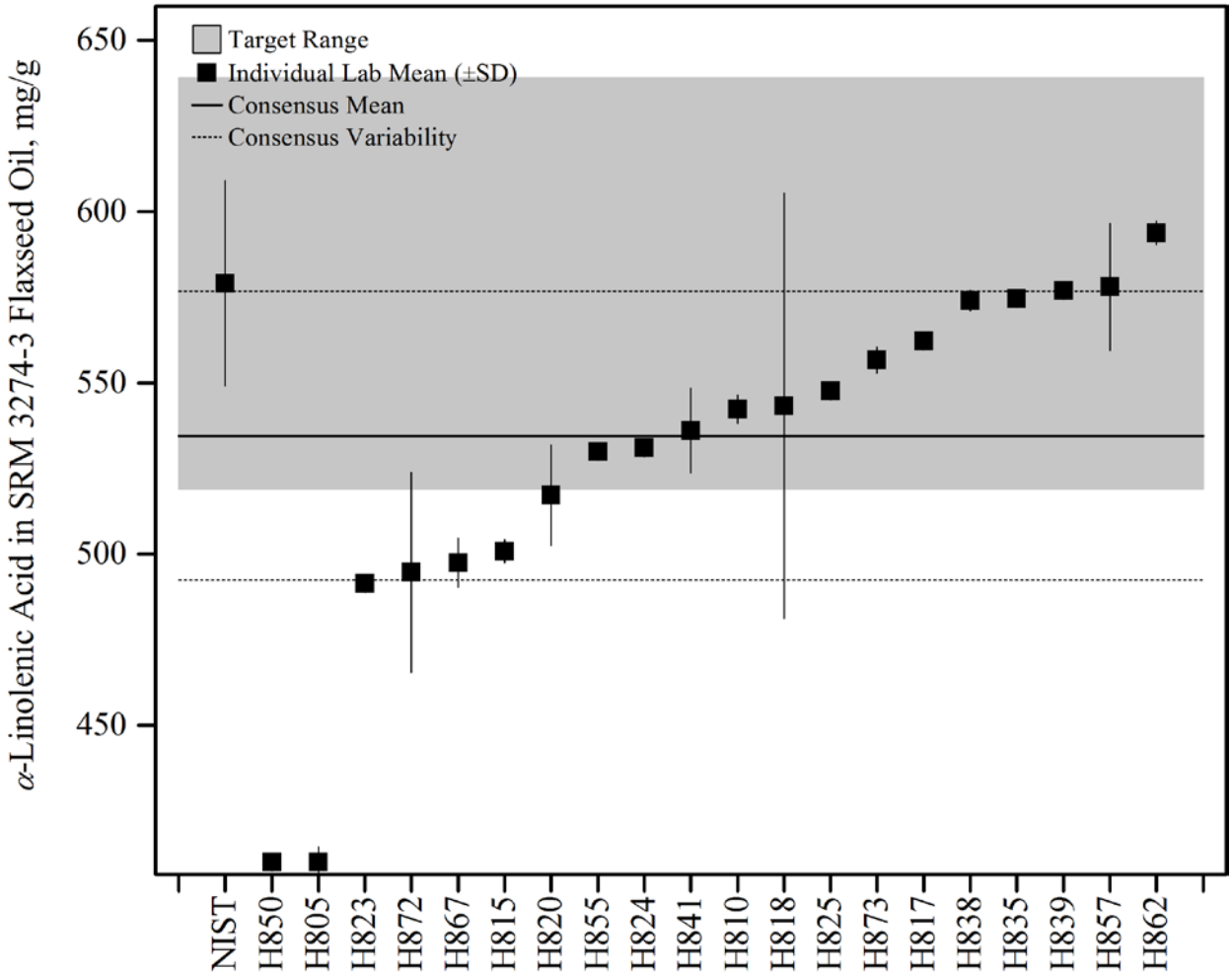

Figure 52. α-Linolenic Acid [C18:3, n-3] in SRM 3274-3 Flaxseed Oil (data summary view). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The black solid line represents the consensus mean, and the black dotted lines represent the consensus 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 certified value bounded by twice its uncertainty (U_{95}).

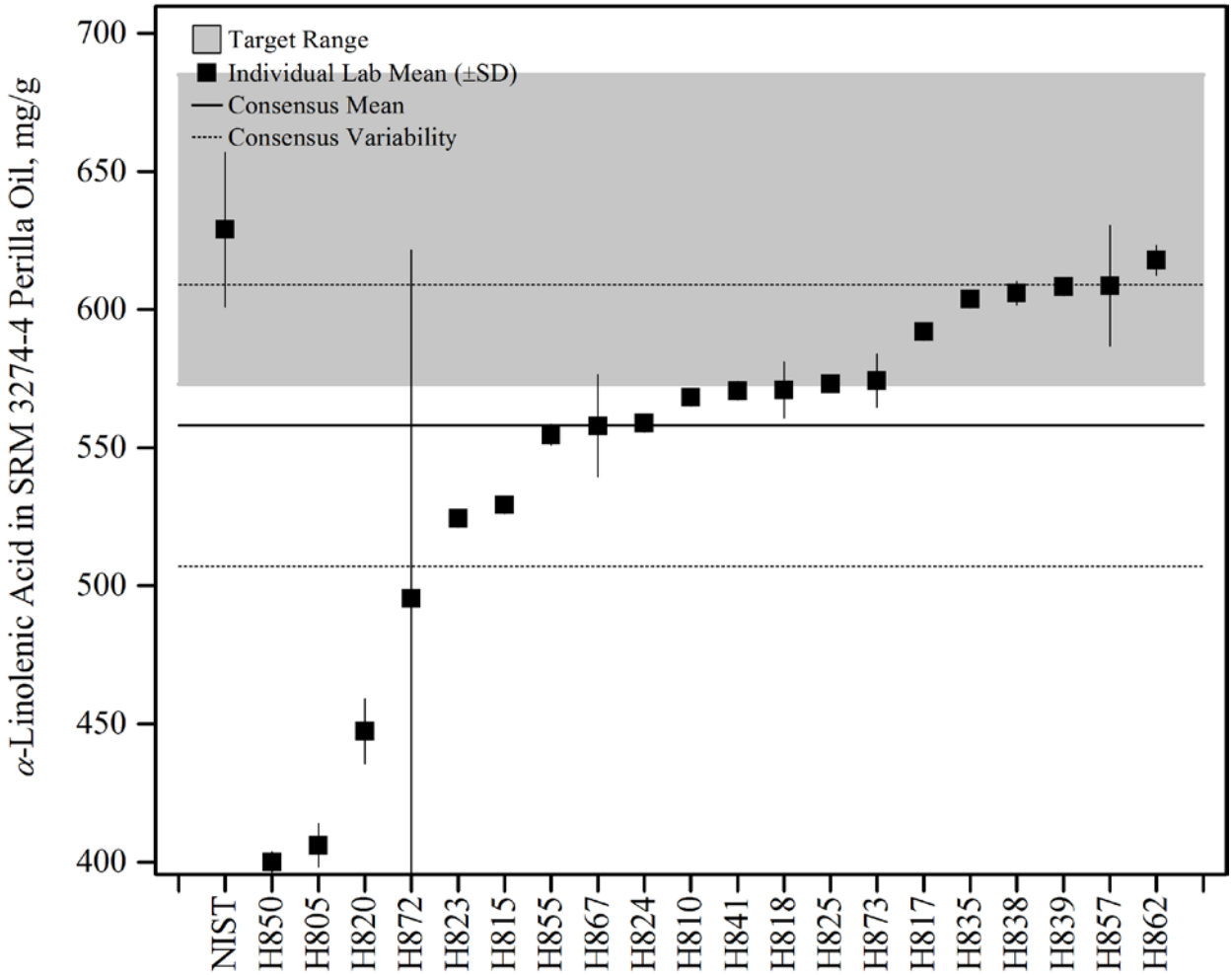

Figure 53. α-Linolenic Acid [C18:3, n-3] in SRM 3274-4 Perilla Oil (data summary view). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The black solid line represents the consensus mean, and the black dotted lines represent the consensus 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 certified value bounded by twice its uncertainty (U_{95}).

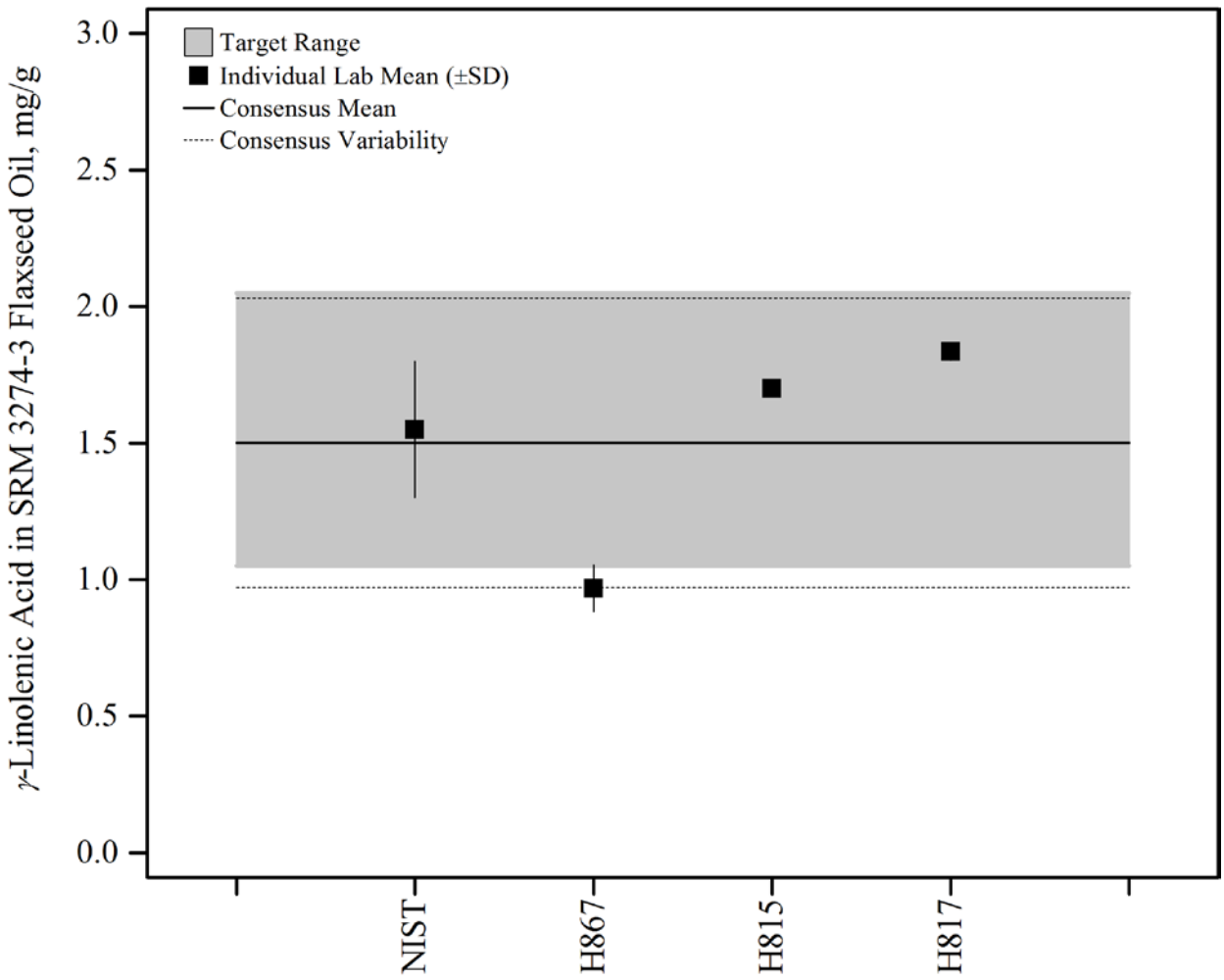

Figure 54. γ-Linolenic Acid [C18:3, n-6] in SRM 3274-3 Flaxseed Oil (data summary view). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The black solid line represents the consensus mean, and the black dotted lines represent the consensus 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 reference value bounded by twice its uncertainty (U_{95}).

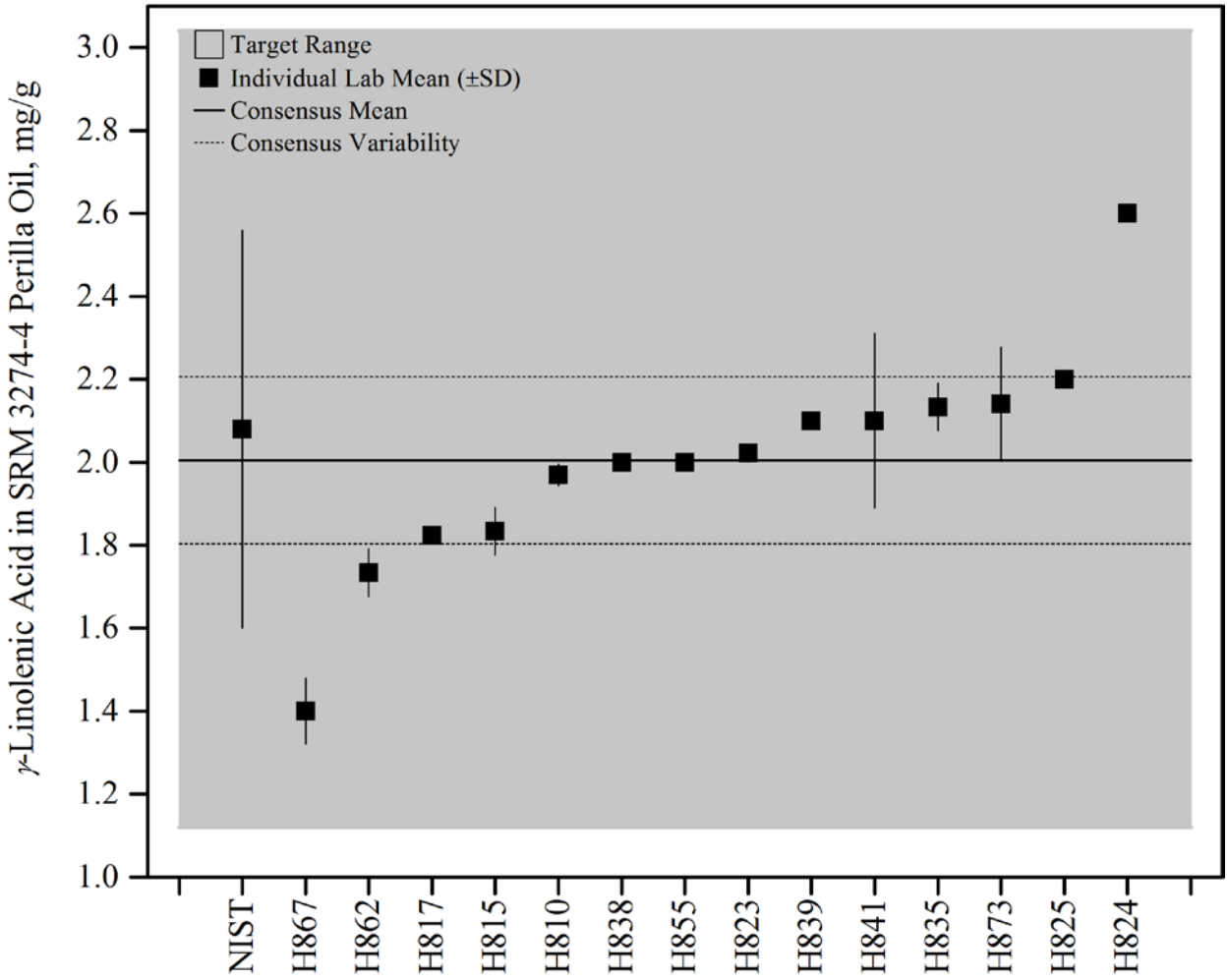

Figure 55. γ-Linolenic Acid [C18:3, n-6] in SRM 3274-4 Perilla Oil (data summary view). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The black solid line represents the consensus mean, and the black dotted lines represent the consensus 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 reference value bounded by twice its uncertainty (U_{95}).

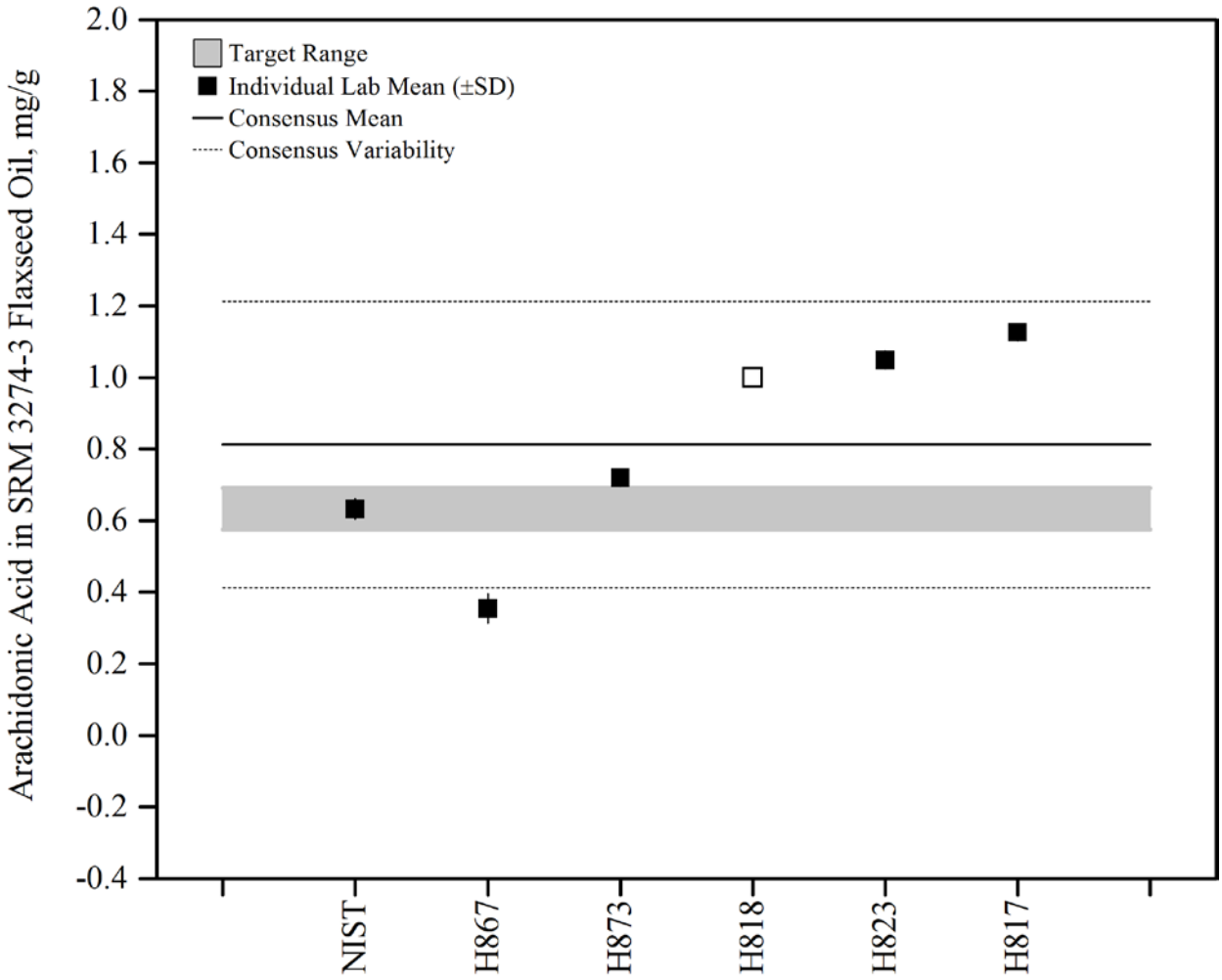

Figure 56. Arachidonic Acid [C20:4, n-6] in SRM 3274-3 Flaxseed Oil (data summary view). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). Data points that are unfilled represent laboratories that only reported a single value for that 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. The gray shaded region represents the target zone for "acceptable" performance, which encompasses the NIST certified value bounded by twice its uncertainty (U_{95}).

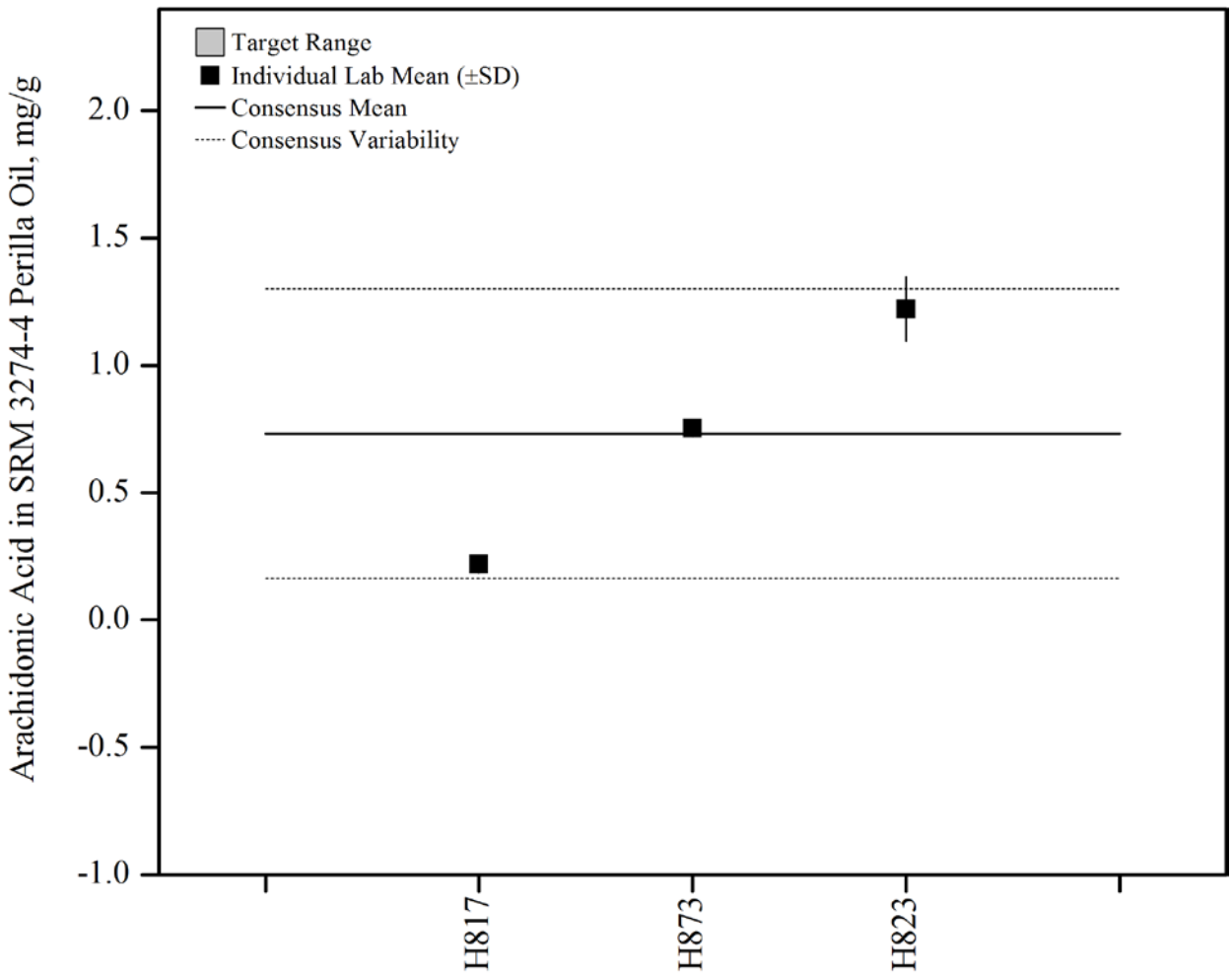

Figure 57. Arachidonic Acid [C20:4, n-6] in SRM 3274-4 Perilla Oil (data summary view). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The black solid line represents the consensus mean, and the black dotted lines represent the consensus variability calculated as one standard deviation about the consensus mean.

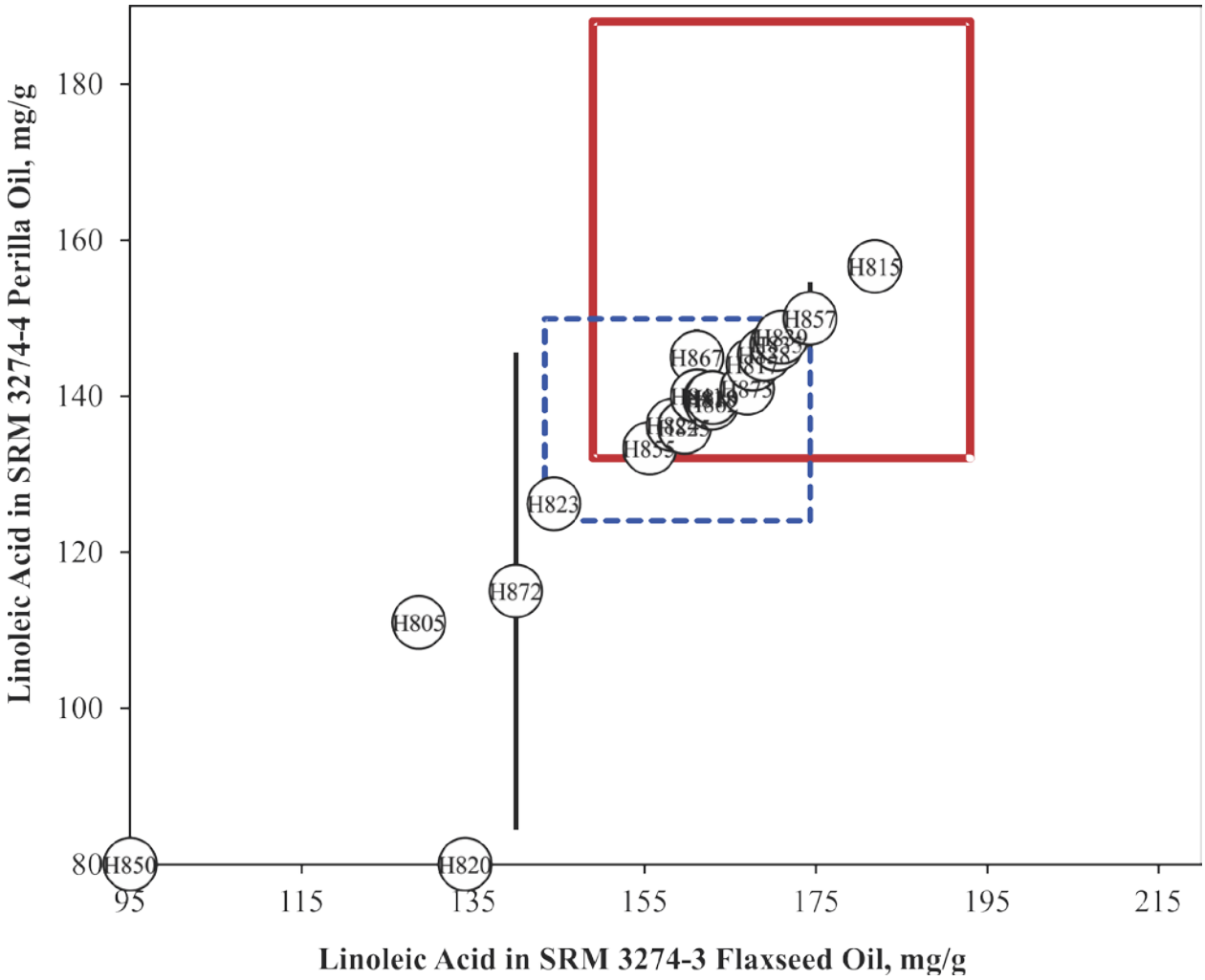

Figure 58. Linoleic acid in SRM 3274-3 Flaxseed Oil and SRM 3274-4 Perilla Oil (sample/control comparison view). In this view, the individual laboratory results for the control (SRM 3274-3 Flaxseed Oil) with a certified value for the analyte are compared to the results for an unknown (SRM 3274-4 Perilla Oil). The error bars represent the individual laboratory standard deviation. The solid red lines represent the target zone for the control (x-axis) and the unknown sample (y-axis). The dotted blue box represents the consensus zone for the control (x-axis) and the unknown sample (y-axis).

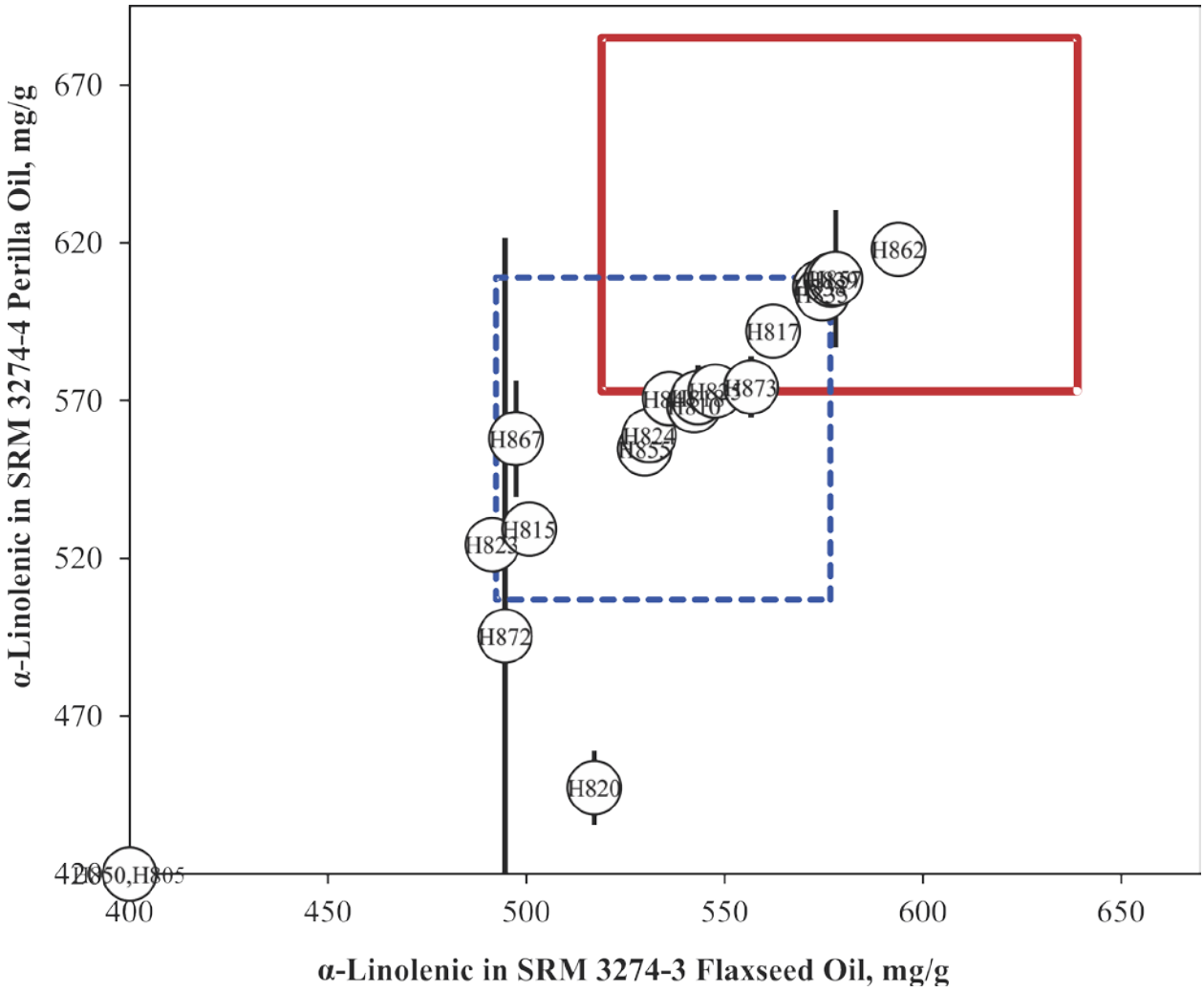

Figure 59. α-Linolenic acid in SRM 3274-3 Flaxseed Oil and SRM 3274-4 Perilla Oil (sample/control comparison view). In this view, the individual laboratory results for the control (SRM 3274-3 Flaxseed Oil) with a certified value for the analyte are compared to the results for an unknown (SRM 3274-4 Perilla Oil). The error bars represent the individual laboratory standard deviation. The solid red lines represent the target zone for the control (x-axis) and the unknown sample (y-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 3251 *Serenoa repens* Extract and SRM 3250 *Serenoa repens* (Fruit). Participants were asked to use in-house analytical methods to determine the mass fractions of three phytosterols (campesterol, β-sitosterol, and stigmasterol) in each of the matrices and report values on an as-received basis.

Sample Information

Saw palmetto extract. Participants were provided with three ampoules, each containing approximately 1 mL of a carbon dioxide extract of saw palmetto (*Serenoa repens*). The oil was packaged in amber glass ampoules under argon. Before use, participants were instructed to thoroughly mix the contents of the ampoule and use a sample size of at least 125 mg. Participants were asked to report a single value from each ampoule and store the extract at controlled room temperature, 10 °C to 30 °C. Approximate analyte levels were not reported prior to the study. The NIST certified values in SRM 3251 were determined by GC-FID (following hydrolysis and derivatization) and LC-MS (following hydrolysis). The certified values and their associated uncertainties are provided on an as-received basis in the table below.

Saw palmetto berries. Participants were provided with three packets, each containing approximately 6 g of saw palmetto (*Serenoa repens*) fruit. The ground saw palmetto berries were heat-sealed inside nitrogen-flushed 0.1 mm (4 mil) polyethylene bags, which were then sealed inside aluminized plastic bags with 2 packets of silica gel. Before use, participants were instructed to thoroughly mix the contents of the packet and use a sample size of at least 0.5 g. Participants were asked to report a single value from each packet and store the material at controlled room temperature, 10 °C to 30 °C. Approximate analyte levels were not reported prior to the study. The NIST certified values in SRM 3250 were determined by GC-FID (following extraction, hydrolysis and derivatization) and LC-MS (following extraction and hydrolysis). The certified values and their associated uncertainties, corrected for the moisture content of the material (6.42 %), are provided on an as-received basis in the table below.

Analyte	Certified Mass Fraction in SRM 3251 (mg/g)	Certified Mass Fraction in SRM 3250 (mg/g) (as-received basis)
Campesterol	0.533 ± 0.031	0.1100 ± 0.0023
β-Sitosterol	1.666 ± 0.064	0.425 ± 0.017
Stigmasterol	0.247 ± 0.040	0.0446 ± 0.0019

Study Results

- Twenty-four laboratories enrolled in this exercise and received samples, and eight laboratories reported results (33 % participation).
- The consensus means for campesterol, β-sitosterol, and stigmasterol in the extract were within the target range, but the consensus ranges were quite wide for all three (33 % to over 100 % RSD).

- The consensus means for campesterol and β-sitosterol in the ground berries were well below the target range, while the consensus mean for stigmasterol was within the target range. The consensus ranges were quite wide for all three (40 % to 50 % RSD).
- The sample/control comparison graphs (Figures 66-68) indicate a possible calibration error. Laboratories that reported high values for the extract also reported high values for the berries. The same is true for laboratories reporting low values.
- Half of the laboratories reported using a hydrolysis approach for sample preparation. Two laboratories (25 %) reported using solvent extraction, and one laboratory reported using a shaking/sonication extraction (13 %). Laboratories using solvent extraction reported values at or below the target value.
- Almost all laboratories (88 %) used GC-FID as their analytical method. One laboratory reported using GC-MS. The laboratory using GC-MS reported values that were below the target and consensus means.
- Half of the laboratories reported using an internal standard approach to calibration, and these laboratories consistently reported values at or above the target range in the extract material. Three laboratories (38 %) reported using an external standard approach to calibration, and these laboratories consistently reported values at or below the target range in the extract material. One laboratory (13 %) reported using a standard addition approach to calibration.

Technical Recommendations

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

- A calibration error is apparent in the sample/control comparison graphs. Calibrant materials should be subjected to the same preparation procedure as the samples (derivatization, hydrolysis, etc.).
- When sample preparation is extensive, an internal standard approach may be required to improve accuracy and precision.
- 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)

Table 30. Individual data table (NIST) for phytosterols in saw palmetto.

National Institute of Standards & Technology

Exercise H - March 2012 - 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	Extract	mg/g	0.533	0.031	0.0	0.0	8	0.529	0.320	0.533	0.031
Campesterol	Fruit	mg/g	0.110	0.002	0.8	0.0	7	0.080	0.037	0.110	0.002
β-sitosterol	Extract	mg/g	1.67	0.06	0.2	0.1	8	1.56	0.56	1.67	0.06
β-sitosterol	Fruit	mg/g	0.425	0.017	1.6	0.0	8	0.257	0.106	0.425	0.017
Stigmasterol	Extract	mg/g	0.247	0.0400	0.1	0.0	8	0.229	0.327	0.247	0.040
Stigmasterol	Fruit	mg/g	0.0446	0.0019	0.0	0.0	7	0.0453	0.0237	0.0446	0.0019

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 31. Data summary table for campesterol in saw palmetto.

		Campesterol									
		SRM 3251 Saw Palmetto Extract (mg/g)					SRM 3250 Saw Palmetto Fruit (mg/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	NIST				0.533	0.031				0.110	0.002
	H801										
	H803										
	H805	0.480	0.479	0.468	0.476	0.007	0.070	0.072	0.073	0.072	0.002
	H810	0.602	0.609	0.617	0.609	0.008	0.104	0.093	0.090	0.096	0.007
	H813										
	H814										
	H815	0.489	0.488	0.493	0.490	0.003	0.067	0.066	0.062	0.065	0.003
	H816										
	H821										
	H823	0.659	0.678	0.631	0.656	0.024	0.070	0.093	0.094	0.086	0.014
	H824	0.880	0.890	0.890	0.887	0.006	0.150	0.160	0.150	0.153	0.006
	H828										
	H835										
	H837										
	H845										
	H851										
	H852										
	H854										
	H858										
	H859										
	H862	0.070	0.060	0.070	0.067	0.006					
	H865	1.092	0.850	0.554	0.832	0.269	0.063	0.065	0.113	0.080	0.028
	H870										
	H872	0.225	0.213	0.216	0.218	0.006	0.015	0.031	0.032	0.026	0.010
Community Results		Consensus Mean			0.529		Consensus Mean			0.080	
		Consensus Standard Deviation			0.320		Consensus Standard Deviation			0.037	
		Maximum			0.887		Maximum			0.153	
		Minimum			0.067		Minimum			0.026	
		N			8		N			7	

Table 32. Data summary table for β-sitosterol in saw palmetto.

		β-Sitosterol									
		SRM 3251 Saw Palmetto Extract (mg/g)					SRM 3250 Saw Palmetto Fruit (mg/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	NIST				1.67	0.06				0.425	0.017
	H801										
	H803										
	H805	1.61	1.60	1.57	1.59	0.02	0.260	0.274	0.277	0.270	0.009
	H810	1.78	1.97	1.69	1.81	0.14	0.307	0.360	0.330	0.332	0.027
	H813										
	H814										
	H815	1.51	1.53	1.51	1.51	0.01	0.258	0.268	0.261	0.262	0.005
	H816										
	H821										
	H823	1.74	1.61	1.71	1.69	0.07	0.259	0.270	0.267	0.265	0.006
	H824	2.12	2.13	2.15	2.13	0.02	0.380	0.400	0.390	0.390	0.010
	H828										
	H835										
	H837										
	H845										
	H851										
	H852										
	H854										
	H858										
	H859										
	H862	0.26	0.25	0.26	0.26	0.01	0.100	0.110	0.120	0.110	0.010
	H865	1.79	1.83	2.54	2.06	0.42	0.323	0.308	0.232	0.288	0.049
	H870										
	H872	0.99	0.96	0.93	0.96	0.03	0.077	0.161	0.171	0.136	0.052
Community Results		Consensus Mean			1.56		Consensus Mean			0.257	
		Consensus Standard Deviation			0.56		Consensus Standard Deviation			0.106	
		Maximum			2.13		Maximum			0.390	
		Minimum			0.26		Minimum			0.110	
		N			8		N			8	

Table 33. Data summary table for stigmasterol in saw palmetto.

		Stigmasterol									
		SRM 3251 Saw Palmetto Extract (mg/g)					SRM 3250 Saw Palmetto Fruit (mg/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	NIST				0.247	0.040				0.0446	0.0019
	H801										
	H803										
	H805	0.259	0.259	0.244	0.254	0.009	0.0380	0.0380	0.0390	0.0383	0.0006
	H810	0.297	0.308	0.304	0.303	0.006	0.0600	0.0540	0.0530	0.0557	0.0038
	H813										
	H814										
	H815	0.174	0.176	0.176	0.175	0.001	0.0280	0.0290	0.0276	0.0282	0.0007
	H816										
	H821										
	H823	0.215	0.186	0.237	0.213	0.026	0.0440	0.0330	0.0680	0.0483	0.0179
	H824	0.290	0.290	0.290	0.290	0.000	0.0500	0.0500	0.0500	0.0500	0.0000
	H828										
	H835										
	H837										
	H845										
	H851										
	H852										
	H854										
	H858										
	H859										
	H862	0.030	0.030	0.030	0.030	0.000					
	H865	0.586	0.592	0.719	0.632	0.075	0.0920	0.0870	0.0750	0.0847	0.0087
	H870										
	H872	0.137	0.130	0.124	0.130	0.007	0.0079	0.0190	0.0198	0.0156	0.0067
Community Results		Consensus Mean			0.229		Consensus Mean			0.0453	
		Consensus Standard Deviation			0.327		Consensus Standard Deviation			0.0237	
		Maximum			0.632		Maximum			0.0847	
		Minimum			0.030		Minimum			0.0156	
		N			8		N			7	

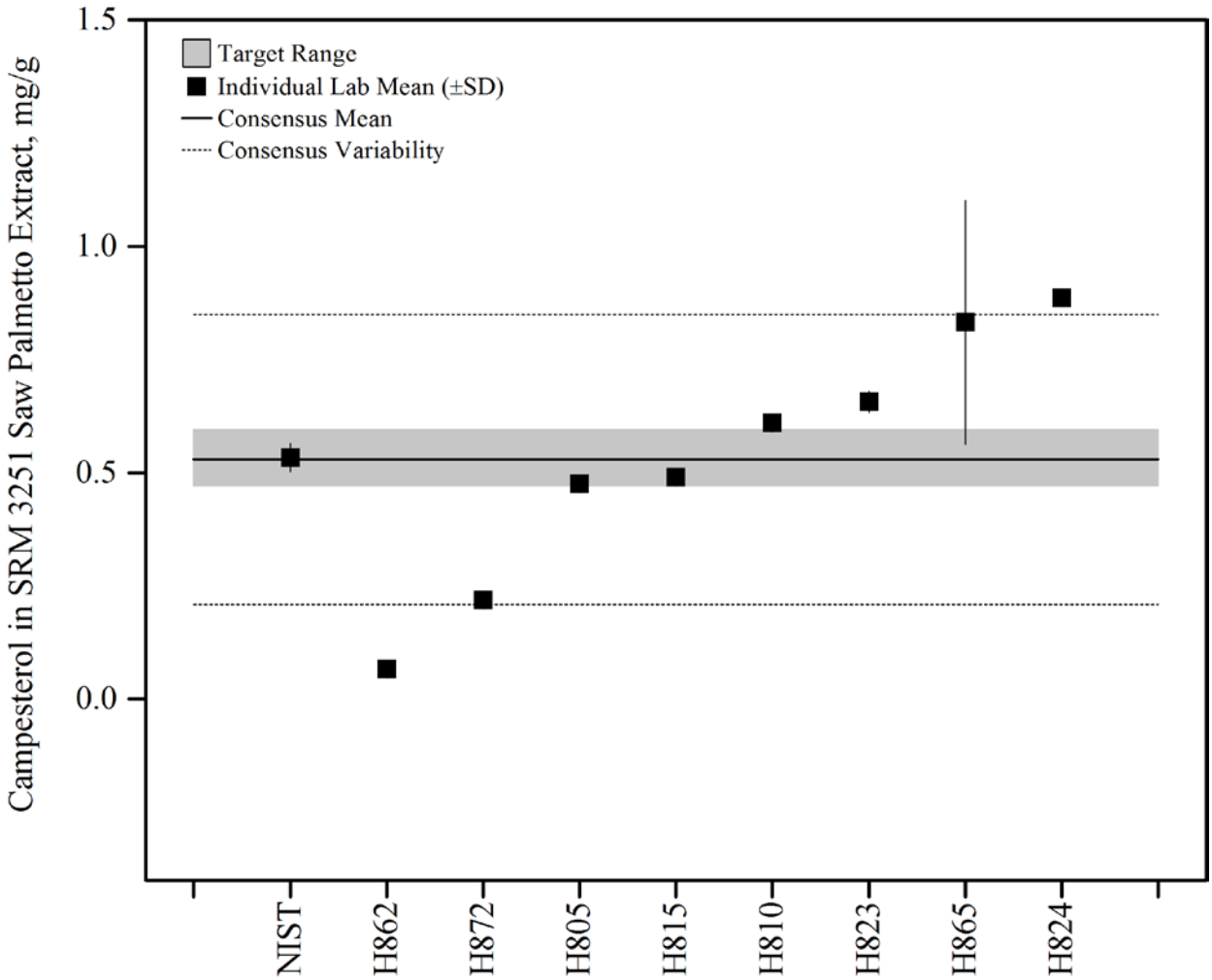

Figure 60. Campesterol in SRM 3251 *Serenoa repens* Extract (data summary view). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The black solid line represents the consensus mean, and the black dotted lines represent the consensus 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 certified value bounded by twice its uncertainty (U_{95}).

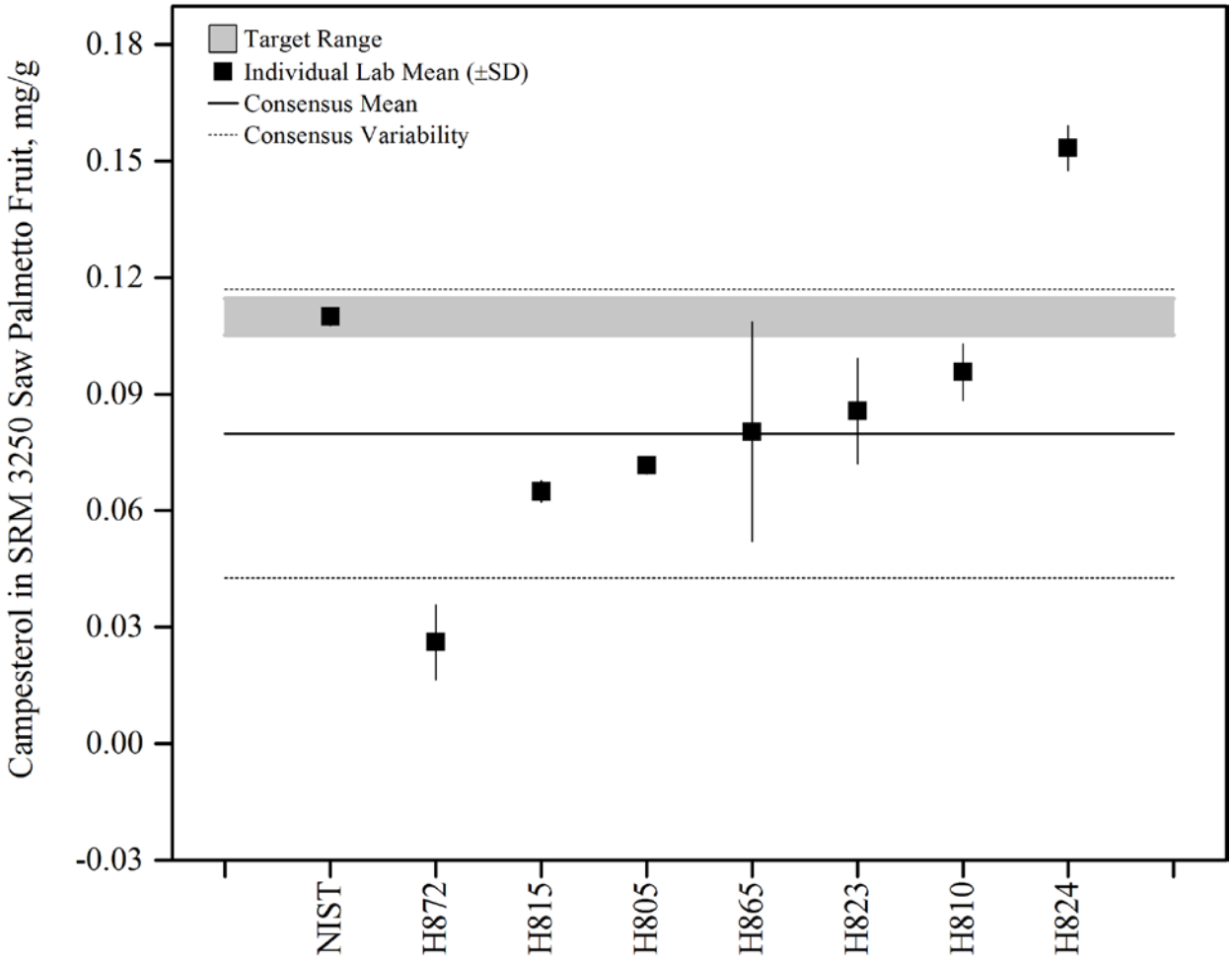

Figure 61. Campesterol in SRM 3250 *Serenoa repens* (Fruit) (data summary view). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The black solid line represents the consensus mean, and the black dotted lines represent the consensus 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 certified value bounded by twice its uncertainty (U_{95}).

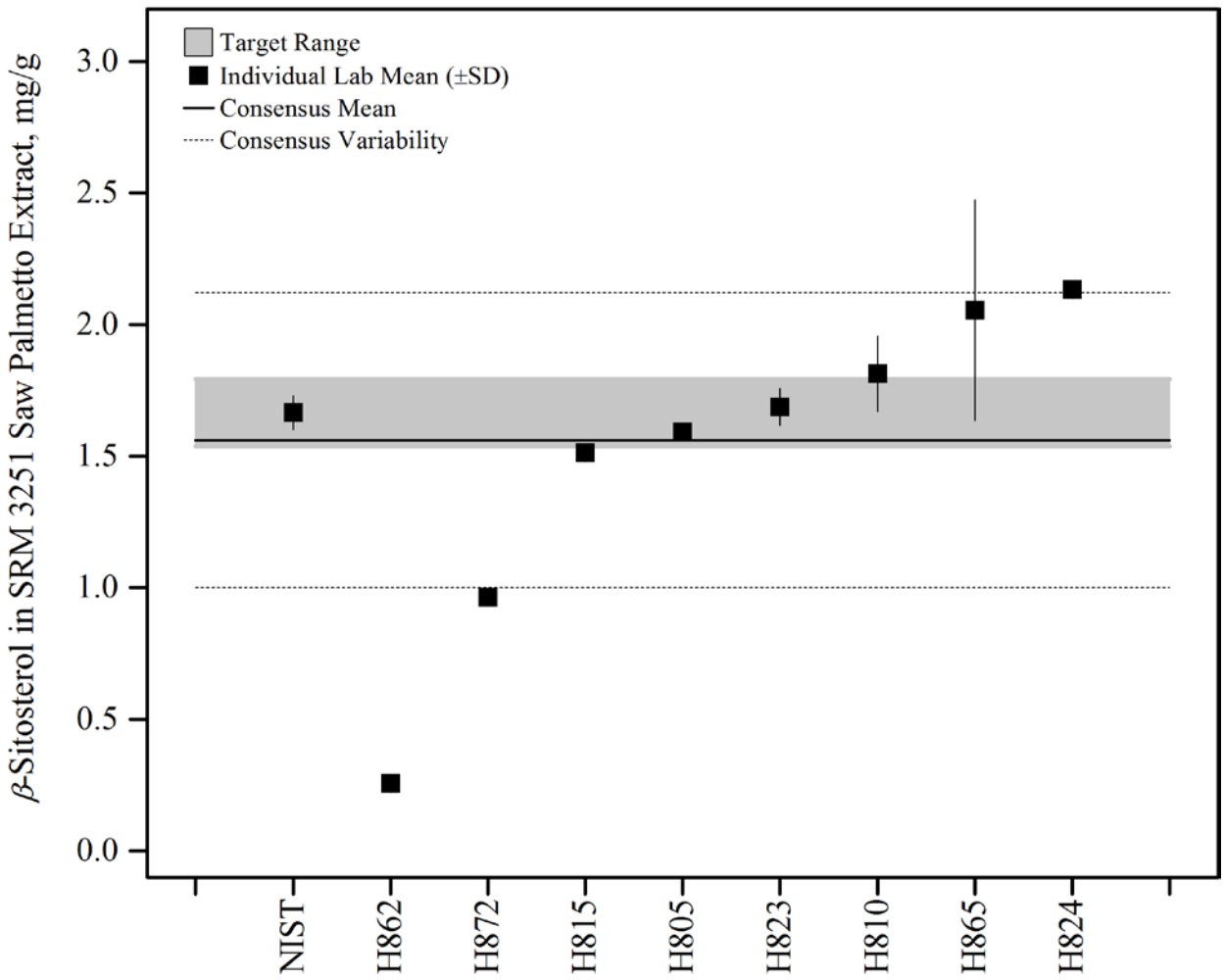

Figure 62. β-Sitosterol in SRM 3251 *Serenoa repens* Extract (data summary view). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The black solid line represents the consensus mean, and the black dotted lines represent the consensus 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 certified value bounded by twice its uncertainty (U_{95}).

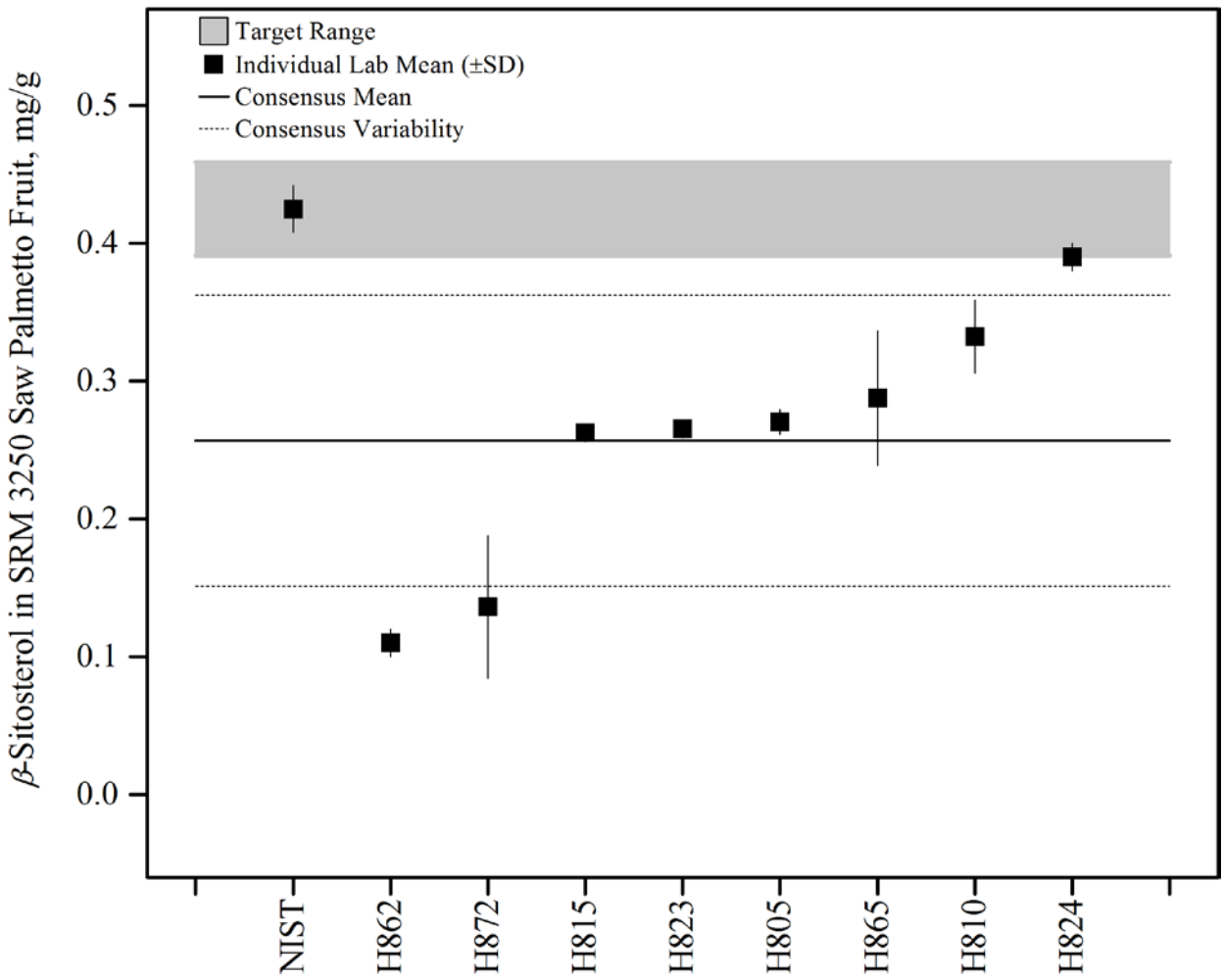

Figure 63. β-Sitosterol in SRM 3250 *Serenoa repens* (Fruit) (data summary view). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The black solid line represents the consensus mean, and the black dotted lines represent the consensus 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 certified value bounded by twice its uncertainty (U_{95}).

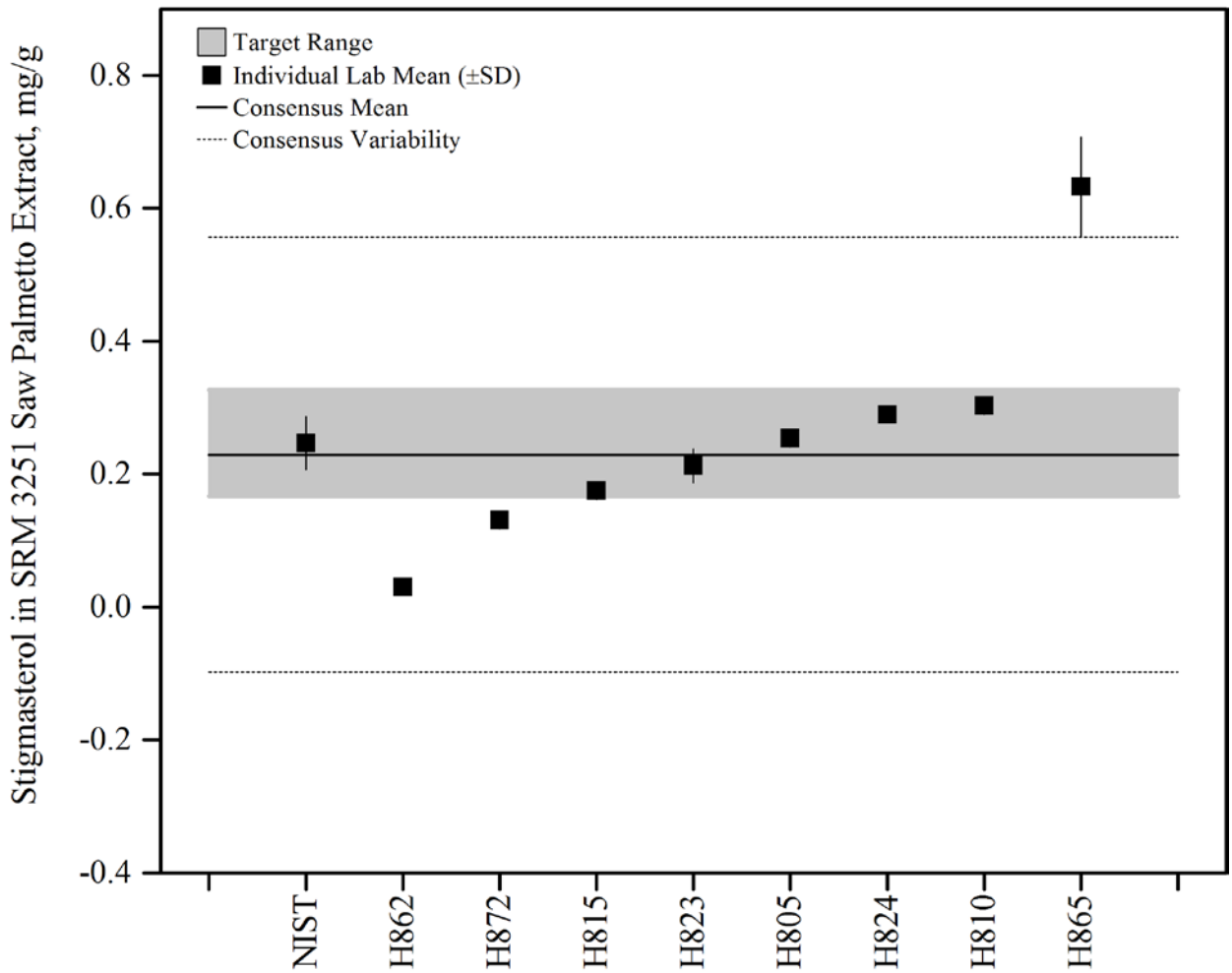

Figure 64. Stigmasterol in SRM 3251 *Serenoa repens* Extract (data summary view). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The black solid line represents the consensus mean, and the black dotted lines represent the consensus 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 certified value bounded by twice its uncertainty (U_{95}).

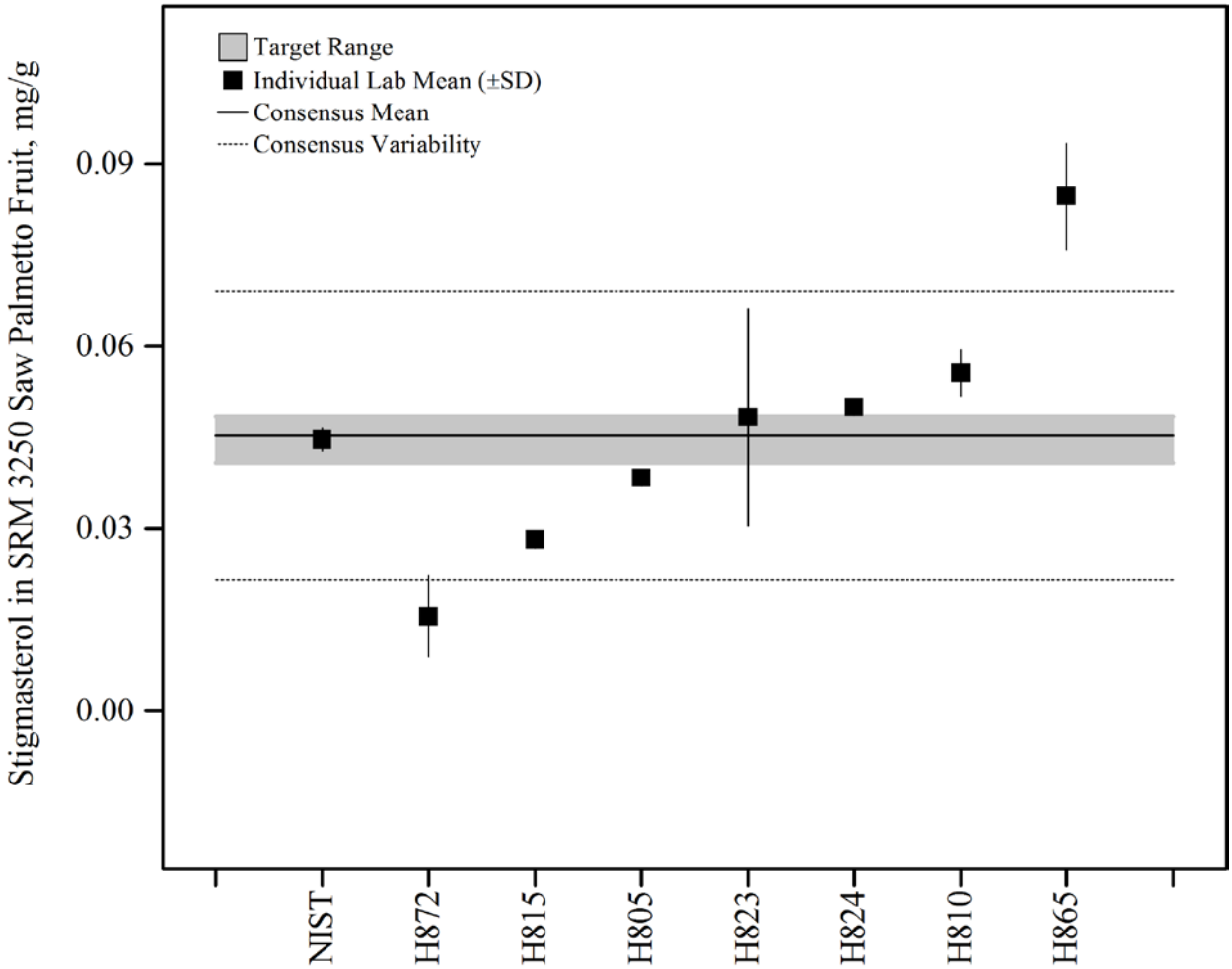

Figure 65. Stigmasterol in SRM 3250 *Serenoa repens* (Fruit) (data summary view). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The black solid line represents the consensus mean, and the black dotted lines represent the consensus 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 certified value bounded by twice its uncertainty (U_{95}).

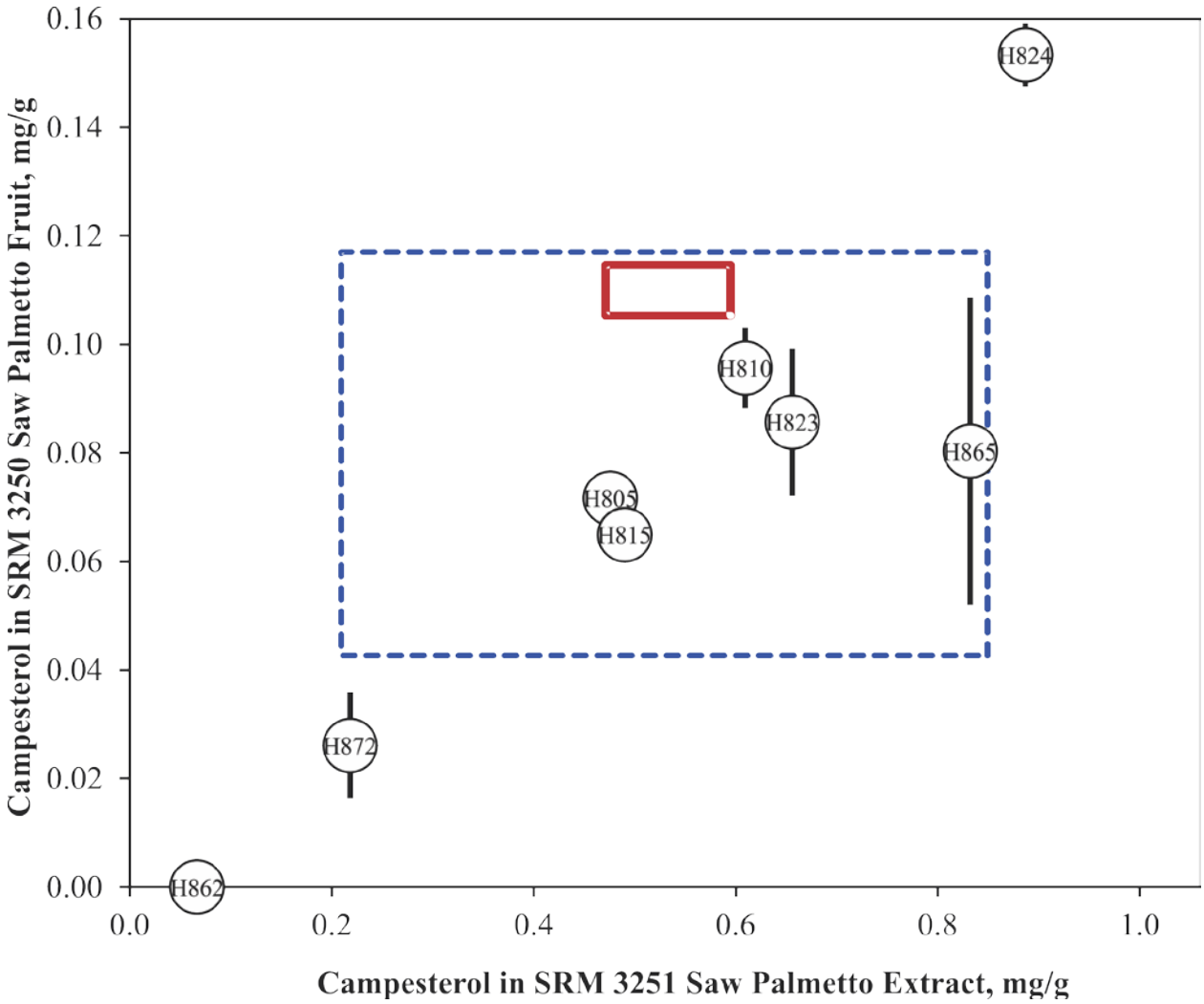

Figure 66. Campesterol in SRM 3250 *Serenoa repens* (Fruit) and SRM 3251 *Serenoa repens* Extract (sample/control comparison view). In this view, the individual laboratory results for the control (SRM 3251 *Serenoa repens* Extract) with a certified value for the analyte are compared to the results for an unknown (SRM 3250 *Serenoa repens* (Fruit)). The error bars represent the individual laboratory standard deviation. The solid red lines represent the target zone for the control (x-axis) and the unknown sample (y-axis). The dotted blue box represents the consensus zone for the control (x-axis) and the unknown sample (y-axis).

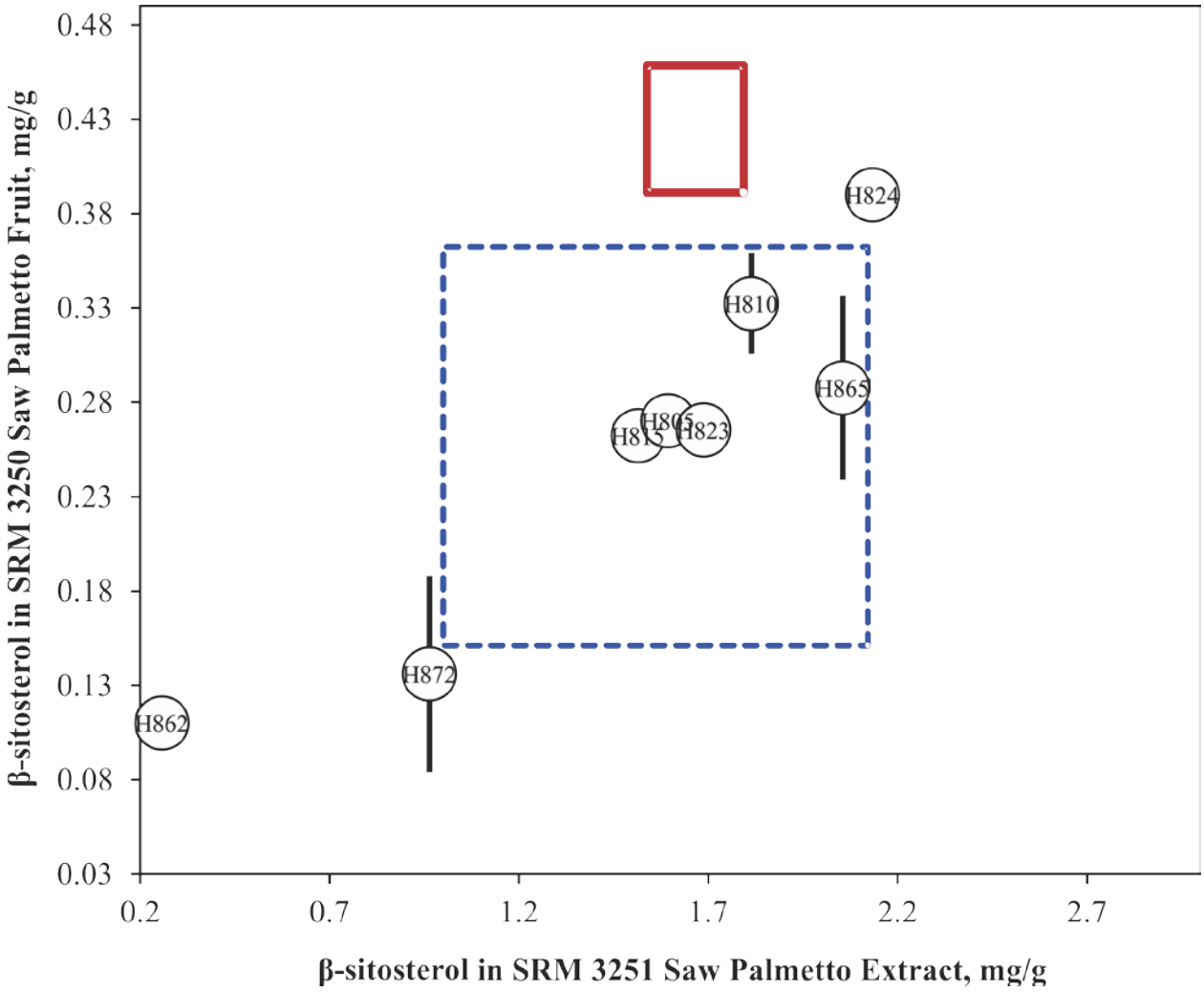

Figure 67. β-sitosterol in SRM 3250 *Serenoa repens* (Fruit) and SRM 3251 *Serenoa repens* Extract (sample/control comparison view). In this view, the individual laboratory results for the control (SRM 3251 *Serenoa repens* Extract) with a certified value for the analyte are compared to the results for an unknown (SRM 3250 *Serenoa repens* (Fruit)). The error bars represent the individual laboratory standard deviation. The solid red lines represent the target zone for the control (x-axis) and the unknown sample (y-axis). The dotted blue box represents the consensus zone for the control (x-axis) and the unknown sample (y-axis).

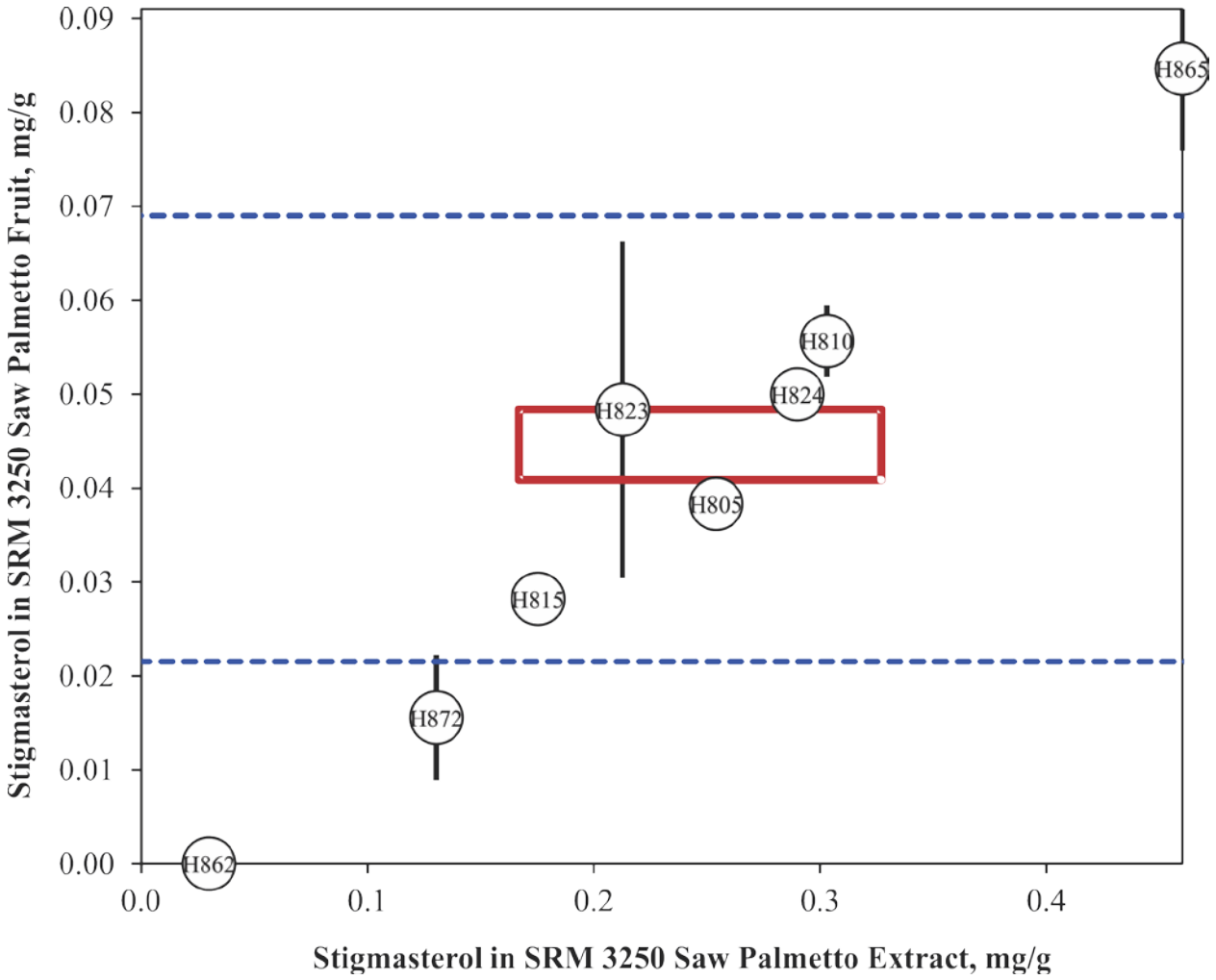

Figure 68. Stigmasterol in SRM 3250 *Serenoa repens* (Fruit) and SRM 3251 *Serenoa repens* Extract (sample/control comparison view). In this view, the individual laboratory results for the control (SRM 3251 *Serenoa repens* Extract) with a certified value for the analyte are compared to the results for an unknown (SRM 3250 *Serenoa repens* (Fruit)). The error bars represent the individual laboratory standard deviation. The solid red lines represent the target zone for the control (x-axis) and the unknown sample (y-axis). The dotted blue box represents the consensus zone for the control (x-axis) and the unknown sample (y-axis).