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Dietary Supplement Laboratory Quality Assurance Program: Exercise J Final Report

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National Institute of Standards and Technology
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Patrick D. Gallagher, Director

TABLE OF CONTENTS

ABSTRACT 1

INTRODUCTION.....1

OVERVIEW OF DATA TREATMENT AND REPRESENTATION
Statistics 2
Individual Data Table 3
Summary Data Table 4
Graphs 4
Data Summary View (Method Comparison Data Summary View) 4
Sample/Control Comparison View (Sample/Sample Comparison View) 5
Composition View 5
Bias View.....5

NUTRITIONAL ELEMENTS (Ca, Mg, Zn) IN NATURAL AND ENHANCED WATERS 6
Study Overview 6
Sample Information 6
Natural Water.....6
Enhanced Water 6
Study Results 6
Technical Recommendations 7
Table 1. Individual data summary table (NIST) for nutritional elements in water 8
Table 2. Data summary table for calcium in natural and enhanced waters.....9
Table 3. Data summary table for magnesium in natural and enhanced waters 10
Table 4. Data summary table for zinc in natural and enhanced waters 11
Figure 2. Calcium in enhanced water 13
Figure 3. Magnesium in SRM 1643e Trace Elements in Water 14
Figure 4. Magnesium in enhanced water 15
Figure 5. Zinc in SRM 1643e Trace Elements in Water 16
Figure 6. Zinc in enhanced water 17
Figure 7. Calcium in SRM 1643e Trace Elements in Water 18
Figure 8. Calcium in enhanced water 19
Figure 9. Magnesium in SRM 1643e Trace Elements in Water 20
Figure 10. Magnesium in enhanced water 21
Figure 11. Zinc in SRM 1643e Trace Elements in Water 22
Figure 12. Zinc in enhanced water 23
Figure 13. Calcium in SRM 1643e Trace Elements in Water and enhanced water 24
Figure 14. Magnesium in SRM 1643e Trace Elements in Water and enhanced water ...25
Figure 15. Zinc in SRM 1643e Trace Elements in Water and enhanced water 26

TOXIC ELEMENTS (As) IN ST. JOHN'S WORT DIETARY SUPPLEMENTS 27
Overview Sample.....27
St. John's Wort Aerial Parts.....*27*
St. John's Wort Methanol Extract.....27
Study Results 27
Technical Recommendations 28

Table 5. Individual data summary table (NIST) for arsenic in St. John's Wort dietary supplements....29
Table 6. Data summary table for arsenic in St. John's Wort dietary supplements....30
Figure 16. Arsenic in candidate SRM 3264 *Hypericum perforatum L.* (St. John's Wort) Methanol Extract (data summary view – digestion method)....31
Figure 17. Arsenic in candidate SRM 3262 *Hypericum perforatum L.* (St. John's Wort) Aerial Parts (data summary view – digestion method)....32
Figure 18. Arsenic in candidate SRM 3262 *Hypericum perforatum L.* (St. John's Wort) Aerial Parts and candidate SRM 3264 *Hypericum perforatum L.* (St. John's Wort) Methanol Extract (sample/sample comparison view)....33

WATER-SOLUBLE VITAMINS (B_5, B_6) IN DIETARY SUPPLEMENTS....34
Study Overview....34
Sample Information....34
Multivitamin/Multielement Tablets....*34*
Enhanced Water....34
Study Results....35
Technical Recommendations....35
Table 7. Individual data summary table (NIST) for water-soluble vitamins in dietary supplements....18
Table 8. Data summary table for vitamin B_5 (pantothenic acid) in dietary supplements 19
Table 9. Data summary table for vitamin B_6 (pyridoxine hydrochloride) in dietary supplements....20
Figure 19. Pantothenic acid in SRM 3280 Multivitamin/Multielement Tablets....39
Figure 20. Pantothenic acid in enhanced water....40
Figure 21. Pyridoxine hydrochloride in SRM 3280 Multivitamin/Multielement Tablets 41
Figure 22. Pyridoxine hydrochloride in enhanced water....42
Figure 23. Pantothenic acid in SRM 3280 Multivitamin/Multielement Tablets and enhanced water....43
Figure 24. Pyridoxine hydrochloride in SRM 3280 Multivitamin/Multielement Tablets and enhanced water....44

VITAMIN E IN DIETARY SUPPLEMENTS....45
Study Overview....45
Sample Information....45
Multivitamin/Multielement Tablets....*45*
Enhanced Water....45
Study Results....45
Technical Recommendations....46
Table 10. Individual data summary table (NIST) for α-tocopherol acetate in dietary supplements....47
Table 11. Data summary table for α-tocopherol acetate in dietary supplements....48
Figure 25. α-Tocopherol acetate in SRM 3280 Multivitamin/Multielement Tablets (data summary view)....49
Figure 26. α-Tocopherol acetate in enhanced water (data summary view)....50
Figure 27. α-Tocopherol acetate in SRM 3280 Multivitamin/Multielement Tablets and enhanced water (sample/control comparison view)....51

FATTY ACIDS IN BOTANICAL AND FISH OIL DIETARY SUPPLEMENTS.....52
Study Overview 52
Sample Information 52
Evening Primrose Oil*52*
Fish Oil.....52
Study Results 52
Technical Recommendations.....53
Table 12. Individual data summary table (NIST) for fatty acids in botanical and fish oil dietary supplements 54
Table 13. Data summary table for linoleic acid in botanical and fish oil dietary supplements.....55
Table 14. Data summary table for α-linolenic acid in botanical and fish oil dietary supplements.....56
Table 15. Data summary table for γ-linolenic acid in botanical and fish oil dietary supplements.....57
Table 16. Data summary table for arachidonic acid in botanical and fish oil dietary supplements.....58
Table 17. Data summary table for EPA in botanical and fish oil dietary supplements ...59
Table 18. Data summary table for DHA in botanical and fish oil dietary supplements ..60
Figure 28. Linoleic acid in SRM 3274-2 Evening Primrose Oil (data summary view) ..61
Figure 29. Linoleic acid in SRM 3275-2 Fish Oil (data summary view) 62
Figure 30. α-Linolenic acid in SRM 3274-2 Evening Primrose Oil (data summary view)63
Figure 31. α-Linolenic acid in SRM 3275-2 Fish Oil (data summary view).....64
Figure 32. γ-Linolenic acid in SRM 3274-2 Evening Primrose Oil (data summary view)65
Figure 33. γ-Linolenic acid in SRM 3275-2 Fish Oil (data summary view) 66
Figure 34. Arachidonic acid in SRM 3275-2 Fish Oil (data summary view).....67
Figure 35. EPA in SRM 3275-2 Fish Oil (data summary view).....68
Figure 36. DHA in SRM 3275-2 Fish Oil (data summary view).....69
Figure 37. Linoleic acid in SRM 3274-2 Evening Primrose Oil and SRM 3275-2 Fish Oil-2 (sample/control comparison view) 70
Figure 38. α-Linolenic acid in SRM 3274-2 Evening Primrose Oil and SRM 3275-2 Fish Oil-2 (sample/control comparison view) 71
Figure 39. γ-Linolenic acid in SRM 3274-2 Evening Primrose Oil and SRM 3275-2 Fish Oil-2 (sample/control comparison view) 72

AFLATOXINS IN PEANUT BUTTER.....73
Study Overview 73
Sample Information 73
Peanut Butter *73*
Peanut Butter Samples.....73
Study Results 73
Technical Recommendations.....74
Table 19. Individual data summary table (NIST) for aflatoxins in peanut butter.....75
Table 20. Data summary table for aflatoxin B1 in peanut butter.....76
Table 21. Data summary table for aflatoxin B2 in peanut butter.....76
Table 22. Data summary table for aflatoxin G1 in peanut butter.....77
Table 23. Data summary table for aflatoxin G2 in peanut butter.....77
Table 24. Data summary table for total aflatoxins in peanut butter.....78

Figure 40. Aflatoxin B1 in SRM 2387 Peanut Butter (data summary view).....79
Figure 41. Aflatoxin B2 in SRM 2387 Peanut Butter (data summary view).....80
Figure 42. Total aflatoxins in SRM 2387 Peanut Butter (data summary view).....81
Figure 43. Total aflatoxins in peanut butter sample 1 (low) (composition view).....82
Figure 44. Total aflatoxins in peanut butter sample 2 (medium) (composition view).....83
Figure 45. Total aflatoxins in peanut butter sample 3 (high) (composition view).....84
Figure 46. Aflatoxins in peanut butter sample 1 (low) (bias view) 85
Figure 47. Aflatoxins in peanut butter sample 3 (medium) (bias view) 86
Figure 48. Aflatoxins in peanut butter sample 3 (high) (bias view) 87

ISOFLAVONES IN SOY PRODUCTS 88
Study Overview 88
Sample Information 88
Soy Protein Isolate 88
Soy Flour.....88
Study Results 89
Technical Recommendations 90
Table 25. Individual data summary table (NIST) for isoflavones in soy dietary supplements.....92
Table 26. Data summary table for daidzin in soy dietary supplements 93
Table 27. Data summary table for glycitin in soy dietary supplements 94
Table 28. Data summary table for genistin in soy dietary supplements 95
Table 29. Data summary table for daidzein in soy dietary supplements.....96
Table 30. Data summary table for glycitein in soy dietary supplements 97
Table 31. Data summary table for genistein in soy dietary supplements.....98
Table 32. Data summary table for total isoflavones in soy dietary supplements.....99
Figure 49. Daidzin in SRM 3236 Soy Protein Isolate (data summary view, sample preparation comparison) 100
Figure 50. Daidzin in SRM 3234 Soy Flour (data summary view, sample preparation comparison).....101
Figure 51. Glycitin in SRM 3236 Soy Protein Isolate (data summary view, sample preparation comparison) 102
Figure 52. Glycitin in SRM 3234 Soy Flour (data summary view, sample preparation comparison).....103
Figure 53. Genistin in SRM 3236 Soy Protein Isolate (data summary view, sample preparation comparison) 104
Figure 54. Genistin in SRM 3234 Soy Flour (data summary view, sample preparation comparison).....105
Figure 55. Daidzein in SRM 3236 Soy Protein Isolate (data summary view, sample preparation comparison) 106
Figure 56. Daidzein in SRM 3234 Soy Flour (data summary view, sample preparation comparison).....107
Figure 57. Glycitein in SRM 3236 Soy Protein Isolate (data summary view, sample preparation comparison) 108
Figure 58. Glycitein in SRM 3234 Soy Flour (data summary view, sample preparation comparison).....109
Figure 59. Genistein in SRM 3236 Soy Protein Isolate (data summary view, sample preparation comparison) 110

Figure 60. Genistein in SRM 3234 Soy Flour (data summary view, sample preparation comparison).....111
Figure 61. Total isoflavones in SRM 3236 Soy Protein Isolate (data summary view, sample preparation comparison).....112
Figure 62. Total isoflavones in SRM 3234 Soy Flour (data summary view, sample preparation comparison).....113
Figure 63. Daidzin in SRM 3236 Soy Protein Isolate and SRM 3234 Soy Flour (sample/control comparison view).....114
Figure 64. Glycitin in SRM 3236 Soy Protein Isolate and SRM 3234 Soy Flour (sample/control comparison view).....115
Figure 65. Genistin in SRM 3236 Soy Protein Isolate and SRM 3234 Soy Flour (sample/control comparison view).....116
Figure 66. Daidzein in SRM 3236 Soy Protein Isolate and SRM 3234 Soy Flour (sample/control comparison view).....117
Figure 67. Glycitein in SRM 3236 Soy Protein Isolate and SRM 3234 Soy Flour (sample/control comparison view).....118
Figure 68. Genistein in SRM 3236 Soy Protein Isolate and SRM 3234 Soy Flour (sample/control comparison view).....119
Figure 69. Total isoflavones in SRM 3236 Soy Protein Isolate and SRM 3234 Soy Flour (sample/control comparison view).....120

BOTANICAL IDENTITY OF PURE AND ADULTERATED GINKGO BILOBA 121
Study Overview 121
Sample Information 121
Gingko biloba Extract.....121
Gingko biloba Leaf 121
Study Results 121
Technical Recommendations.....121
Table 33. Individual data summary table (NIST) for botanical identity of pure and adulterated *Ginkgo biloba*.....123
Table 34. Data summary table for *Ginkgo biloba* extracts (yes/no) 124
Table 35. Data summary table for *Ginkgo biloba* extracts (mass percentage). 125
Table 36. Data summary table for *Ginkgo biloba* leaves (yes/no).....126
Table 37. Data summary table for *Ginkgo biloba* leaves (mass percentage).....127
Figure 70. Adulterated *Ginkgo biloba* extract 128
Figure 71. Adulterated *Ginkgo biloba* leaves 129

ABSTRACT

The NIST Dietary Supplement Laboratory Quality Assurance Program (DSQAP) was established in collaboration with the National Institutes of Health (NIH) Office of Dietary Supplements (ODS) in 2007 to enable members of the dietary supplements community to improve the accuracy of measurements for demonstration of compliance with various regulations including the dietary supplement current good manufacturing practices (cGMPs). Exercise J of this program offered the opportunity for laboratories to assess their in-house measurements of nutritional elements (Ca, Mg, and Zn), contaminants (arsenic and aflatoxins), water-soluble vitamins (vitamins B_5 and B_6), fat-soluble vitamins (vitamin E), fatty acids, isoflavones, and botanical authenticity/identification in foods and/or botanical dietary supplement ingredients and finished products.

INTRODUCTION

The dietary supplement industry in the US is booming, with two-thirds of adults considering themselves to be supplement users.[1] Consumption of dietary supplements, which includes vitamin and mineral supplements, represents an annual US expenditure of more than $25 billion. These figures represent an increasing American and worldwide trend, and as a result, it is critically important that both the quality and safety of these products are verified and maintained.

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

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

NIST has experience in the administration of quality assurance programs, but the DSQAP takes a unique approach: In other NIST quality assurance programs, a set of analytes is measured repeatedly over time in the same or similar matrices to demonstrate laboratory performance. In contrast, the wide range of matrices and analytes under the "dietary supplement" umbrella means

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

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 tenth exercise of the DSQAP, Exercise J. Fifty-eight laboratories responded to the call for participants distributed in April 2013. Samples were shipped to participants in June 2013, and results were returned to NIST by September 2013. This report contains the final data and information to be disseminated to the participants in February 2014.

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^*:

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

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

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

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}).

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

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

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

Summary Data Table

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

Graphs

Data Summary View (Method Comparison Data Summary View)

In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). Data points that are unfilled represent laboratories that 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. Where appropriate, two consensus means may be calculated for the same sample if bimodality is identified in the data. In this case, two consensus means and ranges will be displayed in the data summary view. The gray shaded region represents the target zone for "acceptable" performance, which encompasses the NIST certified, reference, or estimated value bounded by twice its uncertainty (U_{95}) or standard deviation. For the purpose of the DSQAP, a target range spanning twice the uncertainty in the NIST value is selected because participants are only asked to make a limited number of observations. The size of the y-axis on the data summary view graph represents the consensus 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. In the case in which a method comparison is relevant, different colored data points may be used to indicate laboratories that used a specific approach to sample preparation, analytical method, or quantitation.

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). The axes of this graph are centered about the consensus mean values for each sample or control, to a limit of zero and twice the consensus mean. Depending on the variability in the data, the axes may be scaled proportionally to better display the individual data points for each laboratory. In some cases, when the consensus and target ranges have limited overlap, the solid red box may only appear partially on the graph. If the variability in the data is high (greater than 100 % RSD), the dotted blue box may also only appear partially on the graph. This view emphasizes trends in the data that may indicate potential calibration issues or method biases. One program goal is to identify such calibration or method biases and assist participants in improving analytical measurement capabilities. In some cases, when two equally challenging materials are provided, the same view (sample/sample comparison) can be helpful in identifying commonalities or differences in the analysis of the two materials.

Composition View
In this view, used for the aflatoxins in peanut butter study, total composition of the sample is plotted as a function of the measurement of individual components. This view allows comparison of data in which limited statistical information is available as a result of low participation and/or reporting of one data point per sample. This view is also useful in comparison of methods in which a total composition is reported by some laboratories, but individual components are reported by other laboratories. One program goal is to allow laboratories to demonstrate laboratory performance, regardless of the analytical approach.

Bias View
In this view, used for the aflatoxins in peanut butter study, the laboratory Z_{NIST}-score is overlaid for each component, and outlying Z_{NIST}-scores are highlighted in the marginally different range ($2 < |Z| < 3$) in orange, and in the significantly different range ($|Z| > 3$) in red. This view demonstrates visually which components result in poor Z_{NIST}-scores, and allows comparisons between problematic areas as a function of sample type. In an overall composition analysis, this view allows a laboratory to rapidly identify which analyte is resulting in erroneous results.

NUTRITIONAL ELEMENTS (Ca, Mg, Zn) IN NATURAL AND ENHANCED WATERS

Study Overview

In this study, participants were provided with SRM 1643e Trace Elements in Water and commercially available enhanced water. Participants were asked to use in-house analytical methods to determine the mass fractions of three nutritional elements (calcium, magnesium, and zinc) in each of the matrices and report values on an as-received basis.

Sample Information

Natural Water. Participants were provided with one polyethylene bottle containing 125 mL of acidified water. Nitric acid is present at a concentration of approximately 0.8 mol/L to stabilize the trace elements. Before use, participants were instructed to thoroughly mix the contents of the bottle, and a sample size of at least 1.0 mL was recommended. Participants were asked to store the material at controlled room temperature, 10 °C to 30 °C, to prepare three samples, and to report three values from the single bottle provided. Approximate analyte levels were not reported to participants prior to the study. The certified values in SRM 1643e Trace Elements in Water were determined at NIST using inductively coupled plasma mass spectrometry (ICP-MS) and inductively coupled plasma optical emission spectrometry (ICP-OES). The certified values and uncertainties for Ca, Mg, and Zn are outlined in the table below.

Enhanced Water. Participants were provided with one 600 mL bottle of commercially available enhanced water. Before use, participants were instructed to thoroughly mix the contents of the bottle, and a sample size of at least 1.0 mL was recommended. Participants were asked to store the material at controlled room temperature, 10 °C to 30 °C, to prepare three samples, and to report three values from the single bottle provided. Approximate analyte levels were not reported to participants prior to the study. Certified values are not available for this material; NIST provided values for Ca, Mg, and Zn based on triplicate analysis using ICP-OES using standard additions as the method of quantitation. The NIST values in enhanced water are reported in the table below with an estimated relative uncertainty of 5 %.

Analyte	Certified Mass Fraction in SRM 1643e (µg/g)	Estimated Mass Fraction in Commercial Enhanced Water (µg/g)
Ca	31.5 ± 1.1	201 ± 10
Mg	7.84 ± 0.096	86.4 ± 4.3
Zn	0.0765 ± 0.0021	6.79 ± 0.34

Study Results

- Thirty-five laboratories enrolled in this exercise and received samples. Twenty-nine laboratories reported results for calcium and magnesium (83 % participation). Twenty-six to 29 laboratories reported results for zinc (74 % to 83 % participation).
- The consensus means for calcium and magnesium in both materials and for zinc in the enhanced water were within the target range with an acceptable variability (5 % to 14 % relative standard deviation (RSD)).
- The consensus mean for zinc in SRM 1643e was above the target range with a high variability (59 % RSD).
- Zinc in the natural water is two orders of magnitude lower than that of the enhanced water, making this material near the method detection limit (MDL) or limit of

quantitation (LOQ) for some participants. Measuring near the MDL or LOQ can give erroneous values, either high or low. **Figure 15** demonstrates this by showing the consensus zone more tightly surrounding the target zone for enhanced water than for the natural water.

- A majority of the laboratories reported using either open-beaker digestion (34 %) or microwave digestion (41 %) for sample preparation. The remaining laboratories reported using hot block digestion, dilution, or other methods (14 %).
- A majority of the laboratories reported using either ICP-OES (55 %) or ICP-MS (41 %) as their analytical method. One laboratory reported using atomic absorption spectroscopy (AAS).

Technical Recommendations

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

- Both water samples should have been straightforward to digest; no trends were observed based on either the sample preparation method or analytical method used.
- The most likely source of error in this study is related to construction of the calibration curves. To avoid calibration problems, be sure to
  - Include the lowest and highest expected solution concentrations, plus one or two intermediate concentration points in the calibration curve.
  - Ensure that the calibration curve is linear and surrounds expected sample concentrations following digestion and/or dilution. Samples should not go beyond the linear range of the calibration curve resulting in extrapolation of calibration curves leading to false values.
  - Use a sufficient number of blanks to accurately determine MDL and LOQ.
- Run a quality control sample (either in-house or a commercially available reference material) of known concentration to ensure that your method is performing as expected.
- Double-check all calculations; miscalculations and reporting of wrong units were a cause for some errors.

Table 1. Individual data summary table (NIST) for nutritional elements in water.

National Institute of Standards & Technology

Exercise J - May 2013 - 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	Natural Water	µg/g	31.5	1.1	0.0	0.0	29	31.4	3.2	31.5	1.1
Ca	Enhanced Water	µg/g	201	3	-0.3	0.0	29	204	10	201	10
Mg	Natural Water	µg/g	7.84	0.10	-0.2	0.0	29	7.92	0.45	7.84	0.10
Mg	Enhanced Water	µg/g	86.4	0.1	0.0	0.0	29	86.4	5.1	86.4	4.3
Zn	Natural Water	µg/g	0.0765	0.0021	-0.6	0.0	26	0.1160	0.0679	0.0765	0.0021
Zn	Enhanced Water	µg/g	6.79	0.02	-0.3	0.0	29	7.09	0.99	6.79	0.34

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 natural and enhanced waters.

		Calcium									
		SRM 1643e Trace Elements in Water (µg/g)					Enhanced Water (µg/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	NIST				31.5	1.1	198	202	203	201	3
	J002	4.2	6.4	2.6	4.4	1.9	11	17	15	14	3
	J006	31.0	30.9	31.1	31.0	0.1	229	223	227	226	3
	J007	29.2	28.9	28.8	29.0	0.2	194	196	196	195	1
	J008	30.8	28.9	29.9	29.9	1.0	190	190	191	190	1
	J009										
	J014	34.4	34.0	33.2	33.9	0.6	211	205	210	209	3
	J016	30.9	30.7	32.0	31.2	0.7	203	203	197	201	3
	J017	28.7	28.2	28.3	28.4	0.3	210	208	210	209	1
	J018										
	J019	2679.3	2475.3	2497.2	2550.6	112.0	240	229	253	241	12
	J020	44.8	43.8	42.9	43.8	1.0	215	219	215	216	2
	J021										
	J022	32.8	30.4	31.2	31.5	1.2	195	190	201	195	5
	J024	34.8	35.0	34.8	34.9	0.1	219	230	216	222	7
	J025	55.4	55.2	54.4	55.0	0.5	199	200	204	201	3
	J026	29.1	28.5	31.1	29.6	1.3	187	202	201	197	8
	J029	31.4	31.4	30.5	31.1	0.5	204	205	209	206	3
	J031	30.0	30.0	30.0	30.0	0.0	204	204	202	203	1
	J033										
	J034	24.4	24.7	24.9	24.7	0.3	209	208	207	208	1
	J036	31.5	32.7	34.1	32.8	1.3	199	195	200	198	3
	J037	28.7	27.9	28.0	28.2	0.4	204	203	206	205	2
	J038	28.4	25.7	27.1	27.1	1.3	122	140	218	160	51
	J039										
	J041										
	J047	32.6	32.5	32.5	32.5	0.1	203	204	204	204	1
	J048	32.1	32.3	32.2	32.2	0.1	203	203	204	203	1
	J051	29.7	30.0	29.8	29.8	0.1	201	201	202	201	0
	J052	44.8	46.5	39.0	43.4	3.9	270	272	242	262	17
	J053	31.9	32.1	30.6	31.5	0.8	198	192	206	199	7
	J054	28.1	28.9	28.3	28.4	0.4	196	204	195	198	5
	J055	33.2	32.5	32.6	32.8	0.4	199	199	199	199	0
	J056	32.0	31.0	31.0	31.3	0.6	210	200	210	207	6
	J057	30.9	29.7	29.6	30.1	0.7	201	204	203	202	2
	J060	31.5	31.7	31.6	31.6	0.1	207	206	206	206	1
Community Results		Consensus Mean			31.4		Consensus Mean			204	
		Consensus Standard Deviation			3.2		Consensus Standard Deviation			10	
		Maximum			2550.6		Maximum			262	
		Minimum			4.4		Minimum			14	
		N			29		N			29	

Table 3. Data summary table for magnesium in natural and enhanced waters.

		Magnesium									
		SRM 1643e Trace Elements in Water (µg/g)					Enhanced Water (µg/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	NIST				7.84	0.10	86.4	86.6	86.4	86.4	0.1
	J002	6.61	6.35	6.80	6.59	0.22	72.9	71.8	72.7	72.5	0.6
	J006	7.82	7.84	7.80	7.82	0.02	84.6	87.3	85.7	85.9	1.4
	J007	7.64	7.52	7.60	7.59	0.06	86.3	86.8	88.7	87.3	1.3
	J008	7.93	7.67	7.82	7.81	0.13	81.3	82.1	82.0	81.8	0.4
	J009										
	J014	7.69	7.90	7.89	7.83	0.12	91.2	89.1	90.5	90.3	1.1
	J016	8.33	8.14	8.24	8.24	0.10	91.5	91.7	90.0	91.1	0.9
	J017	8.00	7.90	7.89	7.93	0.06	90.8	89.6	90.3	90.2	0.6
	J018										
	J019	835.20	812.00	819.10	822.10	11.89	107.4	94.0	90.7	97.4	8.8
	J020	12.60	12.60	12.20	12.47	0.23	91.5	82.6	92.8	89.0	5.6
	J021										
	J022	8.33	7.85	8.34	8.17	0.28	94.3	95.1	92.2	93.8	1.5
	J024	8.72	8.62	8.60	8.65	0.06	89.3	97.0	88.4	91.6	4.7
	J025	7.70	7.70	7.60	7.67	0.06	85.3	86.2	87.8	86.4	1.3
	J026	7.31	7.17	7.84	7.44	0.35	80.1	85.3	85.0	83.5	2.9
	J029	7.87	7.72	7.37	7.65	0.26	83.1	84.5	85.6	84.4	1.3
	J031	8.00	8.00	8.00	8.00	0.00	86.0	86.0	85.0	85.7	0.6
	J033										
	J034	7.72	7.80	7.83	7.78	0.06	82.5	81.8	82.1	82.1	0.4
	J036	8.16	8.58	10.80	9.18	1.42	89.1	87.9	88.6	88.5	0.6
	J037	7.70	7.48	7.51	7.56	0.12	89.5	88.4	89.5	89.1	0.7
	J038	7.06	6.41	6.80	6.76	0.33	44.6	55.2	90.9	63.6	24.2
	J039										
	J041										
	J047	8.39	8.49	8.39	8.42	0.06	88.8	89.0	89.6	89.1	0.4
	J048	8.00	8.10	8.05	8.05	0.05	82.5	82.3	82.1	82.3	0.2
	J051	7.73	7.82	7.76	7.77	0.04	87.5	87.1	87.8	87.5	0.4
	J052	8.06	8.07	8.11	8.08	0.02	75.7	75.7	75.5	75.6	0.1
	J053	7.94	8.20	7.68	7.94	0.26	85.7	82.0	80.8	82.8	2.6
	J054	7.54	7.52	7.61	7.56	0.05	86.3	88.2	89.1	87.9	1.4
	J055	7.51	7.30	7.29	7.37	0.12	82.3	82.2	82.1	82.2	0.1
	J056	8.20	7.80	8.20	8.07	0.23	95.0	92.0	94.0	93.7	1.5
	J057	8.04	7.88	7.97	7.96	0.08	86.1	85.6	85.6	85.8	0.3
	J060	7.30	8.10	8.07	7.82	0.45	86.6	86.6	86.9	86.7	0.2
Community Results		Consensus Mean			7.92		Consensus Mean			86.4	
		Consensus Standard Deviation			0.45		Consensus Standard Deviation			5.1	
		Maximum			822.10		Maximum			97.4	
		Minimum			6.59		Minimum			63.6	
		N			29		N			29	

Table 4. Data summary table for zinc in natural and enhanced waters.

		Zinc									
		SRM 1643e Trace Elements in Water (µg/g)					Enhanced Water (µg/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	NIST				0.0765	0.0021	6.77	6.82	6.79	6.79	0.02
	J002	842.5982	812.8184	1099.6997	918.3721	157.7387	824.04	842.77	862.72	843.18	19.34
	J006	0.0817	0.0797	0.0396	0.0670	0.0238	8.19	8.63	8.27	8.37	0.23
	J007	0.0710	0.0710	0.0700	0.0707	0.0006	6.40	6.43	6.55	6.46	0.08
	J008	0.1558	0.1578	0.1698	0.1611	0.0076	6.32	6.27	6.34	6.31	0.04
	J009										
	J014	0.0647	0.0660	0.0647	0.0651	0.0008	5.84	5.83	5.98	5.88	0.08
	J016	0.0893	0.0896	0.0848	0.0879	0.0027	6.71	6.71	6.70	6.71	0.01
	J017	0.0800	0.0755	0.0764	0.0773	0.0024	6.88	7.02	6.93	6.94	0.07
	J018										
	J019	13.0000	11.7000	11.8000	12.1667	0.7234		16.30	15.10	15.70	0.85
	J020	0.3400	0.2000	0.1300	0.2233	0.1069	6.80	6.60	6.50	6.63	0.15
	J021										
	J022	0.0770	0.0710	0.0750	0.0743	0.0031	6.07	6.24	6.21	6.17	0.09
	J024	0.0992	0.0948	0.1130	0.1023	0.0095	6.76	7.73	6.88	7.12	0.53
	J025	1.5691	1.5741	1.5496	1.5643	0.0129	6.52	6.90	6.54	6.65	0.21
	J026	0.1868	0.1776	0.1813	0.1819	0.0046	10.53	8.90	8.41	9.28	1.11
	J029	0.0850	0.0820	0.0900	0.0857	0.0040	6.93	7.07	6.67	6.89	0.20
	J031						7.00	7.00	7.00	7.00	0.00
	J033										
	J034						6.97	6.91	6.91	6.93	0.03
	J036						7.11	7.16	7.17	7.15	0.03
	J037	0.0711	0.0714	0.0710	0.0712	0.0002	6.53	6.42	6.50	6.48	0.06
	J038	0.3300	0.1800	0.1800	0.2300	0.0866	8.40	8.51	9.06	8.66	0.35
	J039										
	J041										
	J047	0.0770	0.0760	0.0780	0.0770	0.0010	6.79	6.82	6.86	6.82	0.04
	J048	0.0750	0.0760	0.0770	0.0760	0.0010	6.94	7.02	7.00	6.99	0.04
	J051	0.0776	0.0773	0.0778	0.0776	0.0002	6.70	6.69	6.69	6.69	0.01
	J052	0.0610	0.0610	0.0620	0.0613	0.0006	10.37	10.57	10.61	10.52	0.13
	J053	0.0920	0.0860	0.0970	0.0917	0.0055	5.91	6.06	6.46	6.14	0.28
	J054	0.1454	0.1453	0.1454	0.1454	0.0001	5.94	6.20	6.31	6.15	0.19
	J055	0.0998	0.0976	0.0977	0.0983	0.0012	7.83	7.97	7.90	7.90	0.07
	J056	0.0640	0.0640	0.0640	0.0640	0.0000	6.20	6.00	6.00	6.07	0.12
	J057	0.0835	0.0775	0.0795	0.0802	0.0031	7.03	7.07	7.01	7.03	0.03
	J060	0.0660	0.0660	0.0660	0.0660	0.0000	6.59	6.57	6.58	6.58	0.01
Community Results		Consensus Mean			0.1160		Consensus Mean			7.09	
		Consensus Standard Deviation			0.0679		Consensus Standard Deviation			0.99	
		Maximum			918.3721		Maximum			843.18	
		Minimum			0.0613		Minimum			5.88	
		N			26		N			29	

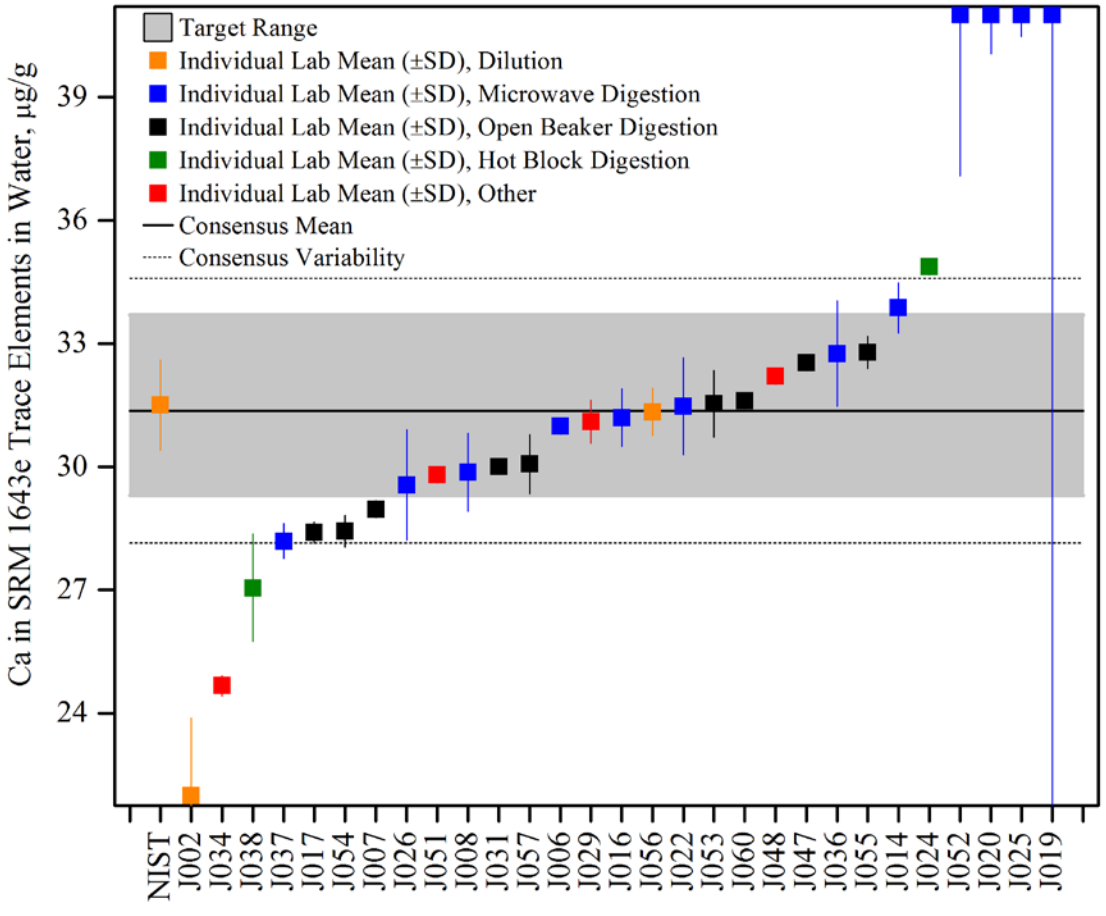

Figure 1. Calcium in SRM 1643e Trace Elements in Water (data summary view – digestion method). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The color of the data point represents the sample preparation (digestion) procedure employed. The black solid line represents the consensus mean, and the black dotted lines represent the consensus variability calculated as one standard deviation about the consensus mean. The gray shaded region represents the target zone for "acceptable" performance, which encompasses the NIST certified value bounded by twice its uncertainty (U_{95}).

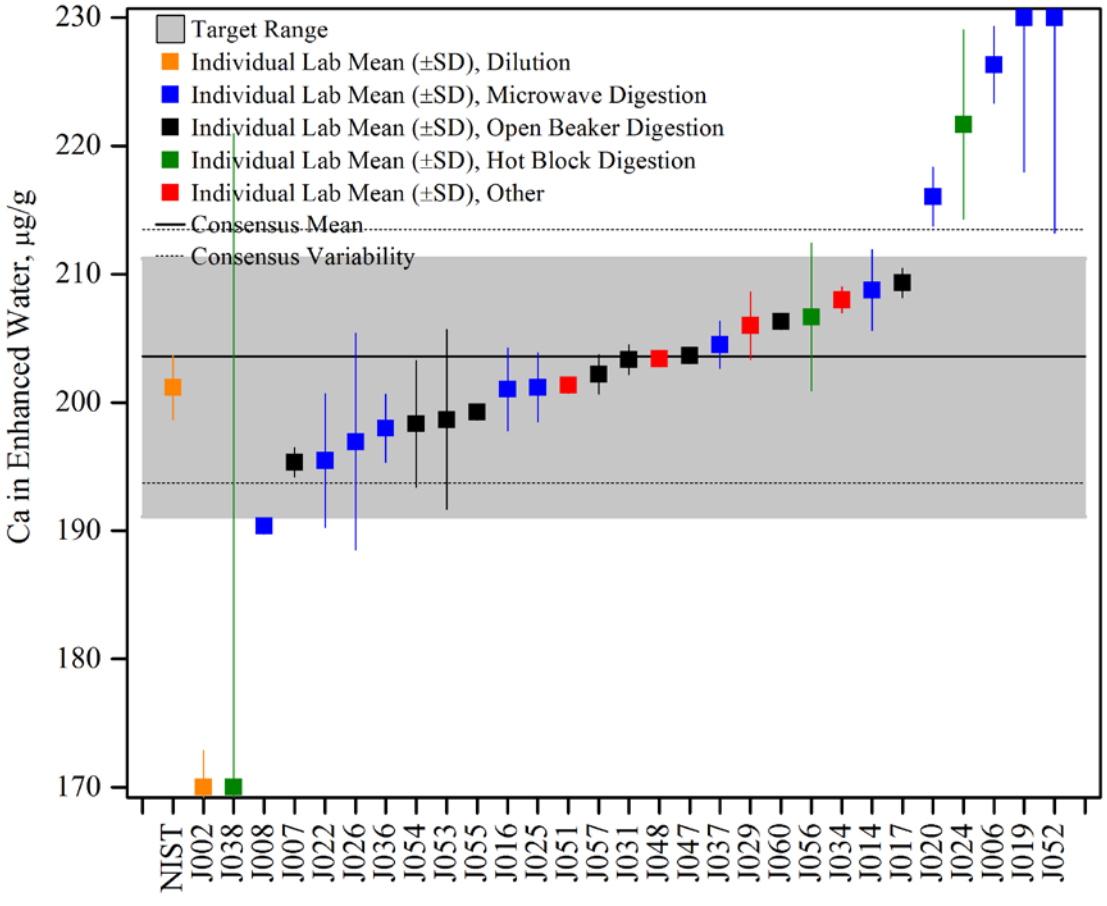

Figure 2. Calcium in enhanced water (data summary view – digestion method). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The color of the data point represents the sample preparation (digestion) procedure employed. The black solid line represents the consensus mean, and the black dotted lines represent the consensus variability calculated as one standard deviation about the consensus mean. The gray shaded region represents the target zone for "acceptable" performance, which encompasses the NIST value determined in triplicate by ICP-OES, bounded by an estimated relative uncertainty of 5 %.

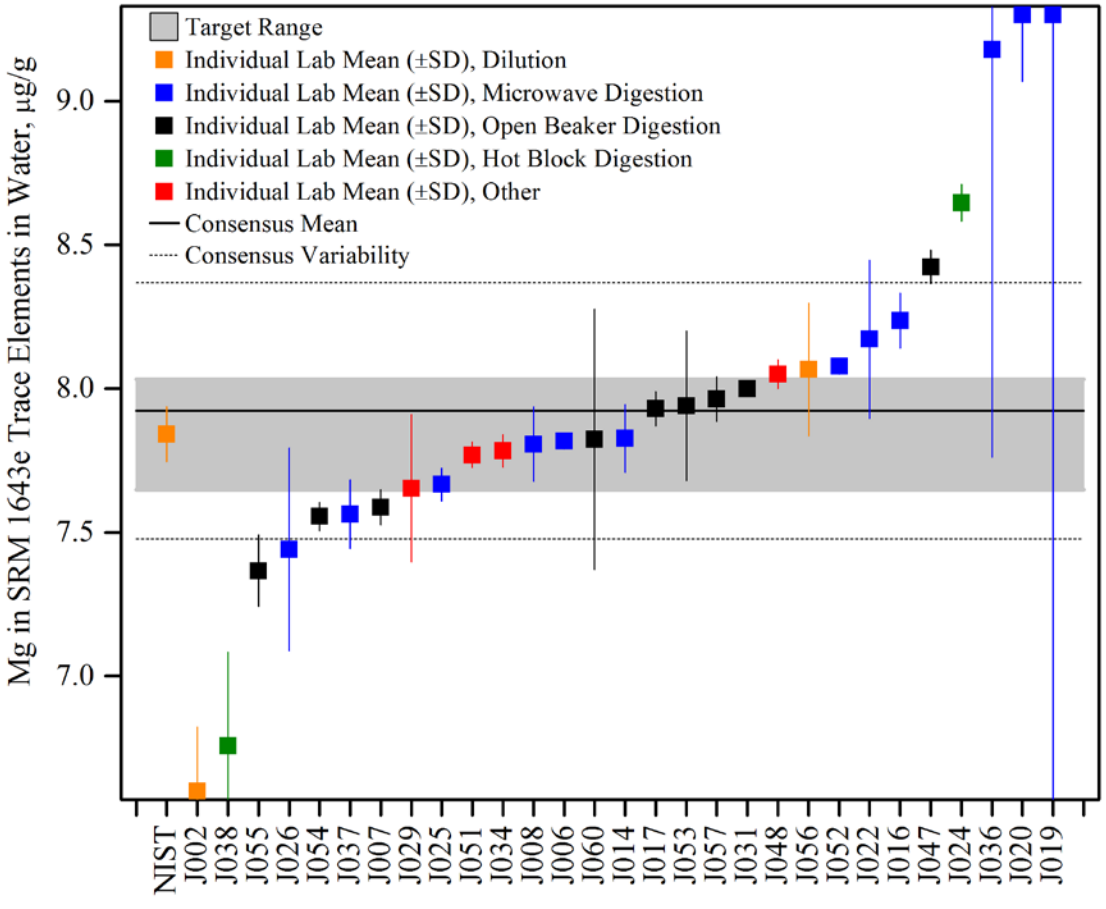

Figure 3. Magnesium in SRM 1643e Trace Elements in Water (data summary view – digestion method). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The color of the data point represents the sample preparation (digestion) procedure employed. The black solid line represents the consensus mean, and the black dotted lines represent the consensus variability calculated as one standard deviation about the consensus mean. The gray shaded region represents the target zone for "acceptable" performance, which encompasses the NIST certified value bounded by twice its uncertainty (U_{95}).

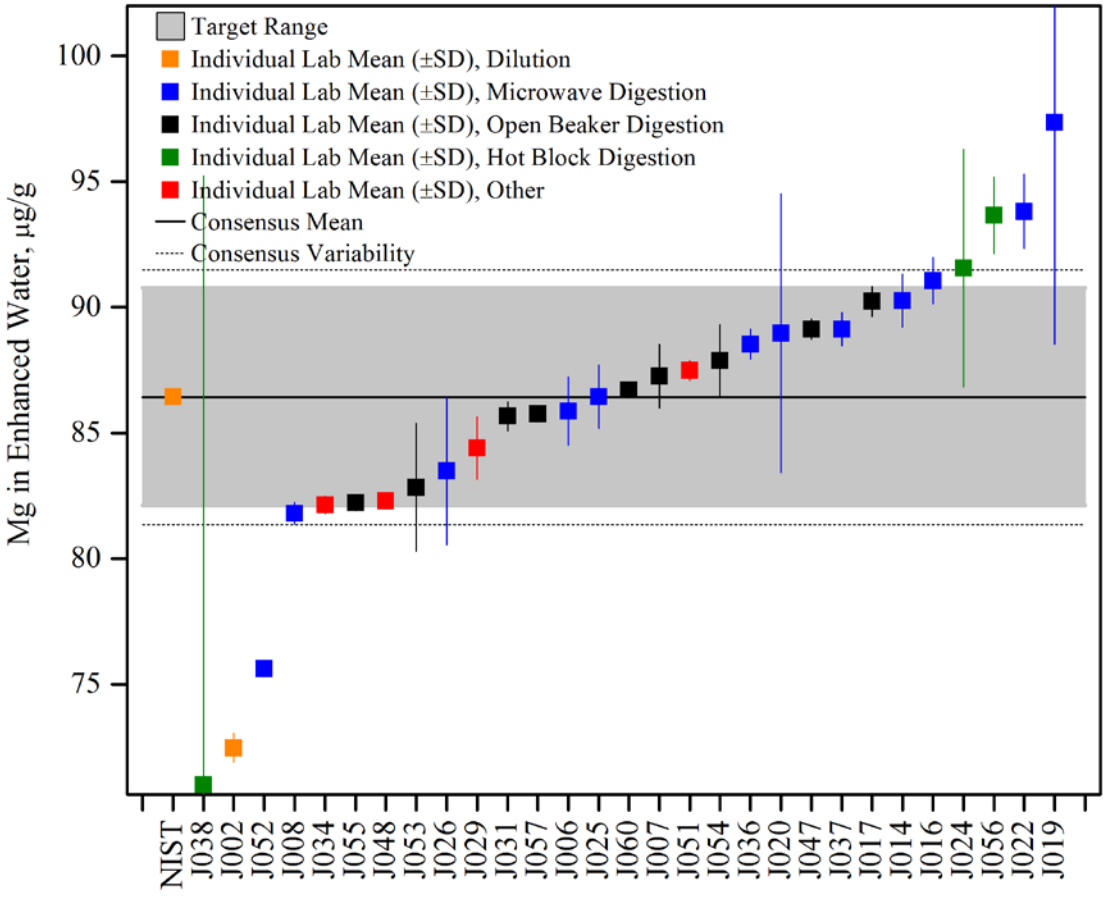

Figure 4. Magnesium in enhanced water (data summary view – digestion method). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The color of the data point represents the sample preparation (digestion) procedure employed. The black solid line represents the consensus mean, and the black dotted lines represent the consensus variability calculated as one standard deviation about the consensus mean. The gray shaded region represents the target zone for "acceptable" performance, which encompasses the NIST value determined in triplicate by ICP-OES, bounded by an estimated relative uncertainty of 5 %.

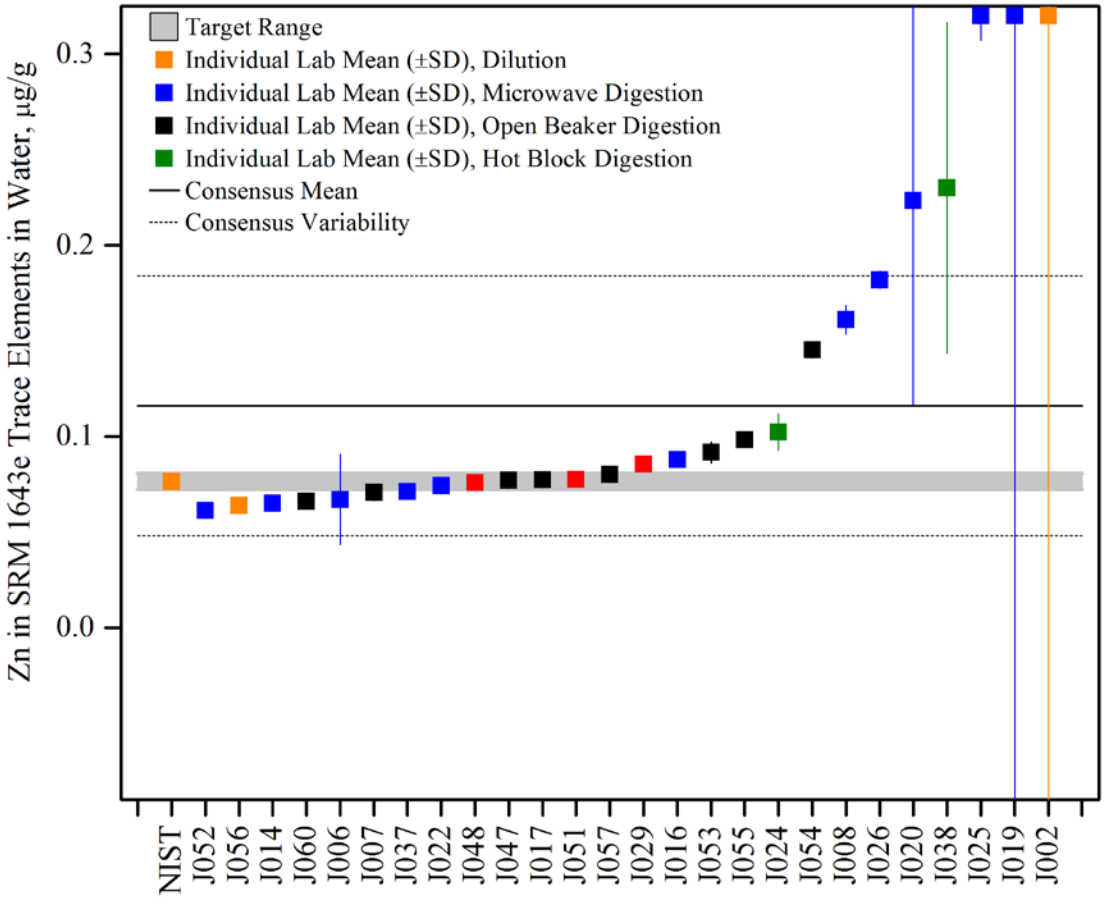

Figure 5. Zinc in SRM 1643e Trace Elements in Water (data summary view – digestion method). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The color of the data point represents the sample preparation (digestion) procedure employed. The black solid line represents the consensus mean, and the black dotted lines represent the consensus variability calculated as one standard deviation about the consensus mean. The gray shaded region represents the target zone for "acceptable" performance, which encompasses the NIST certified value bounded by twice its uncertainty (U_{95}).

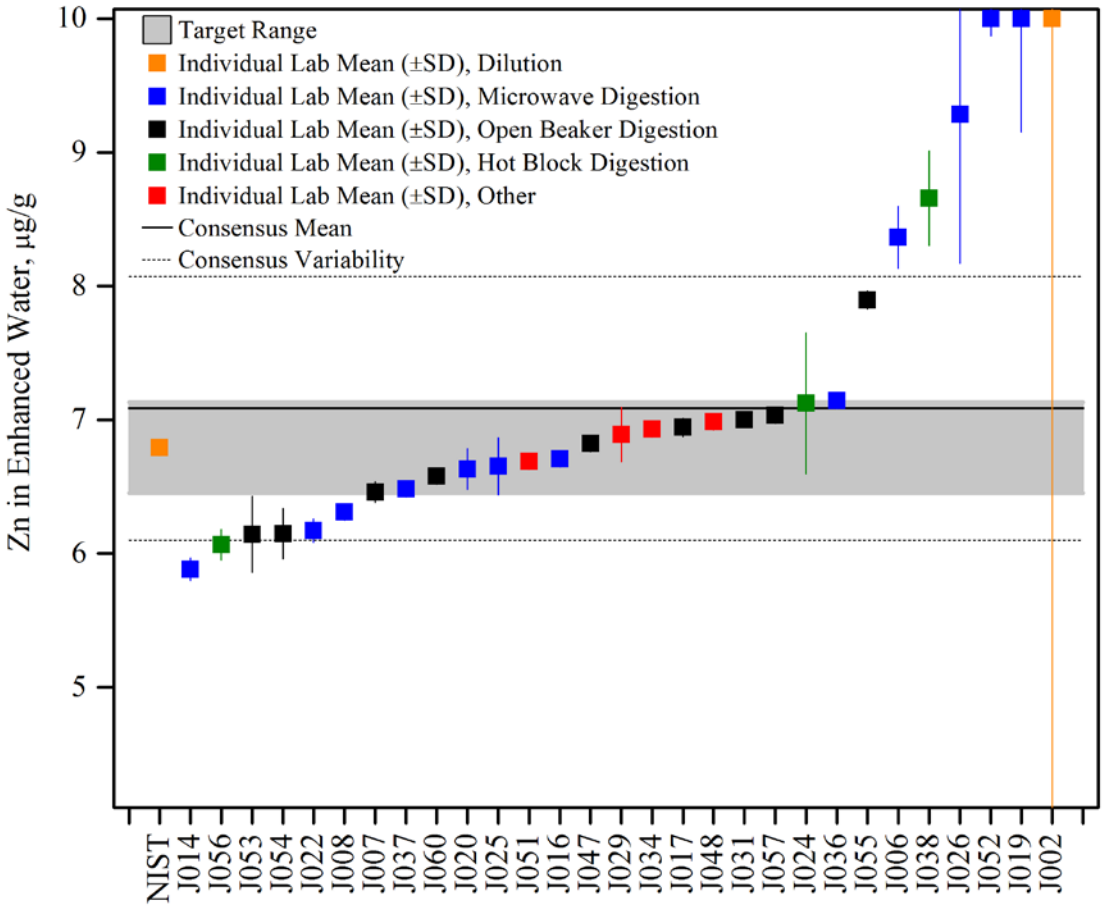

Figure 6. Zinc in enhanced water (data summary view – digestion method). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The color of the data point represents the sample preparation (digestion) procedure employed. The black solid line represents the consensus mean, and the black dotted lines represent the consensus variability calculated as one standard deviation about the consensus mean. The gray shaded region represents the target zone for "acceptable" performance, which encompasses the NIST value determined in triplicate by ICP-OES, bounded by an estimated relative uncertainty of 5 %.

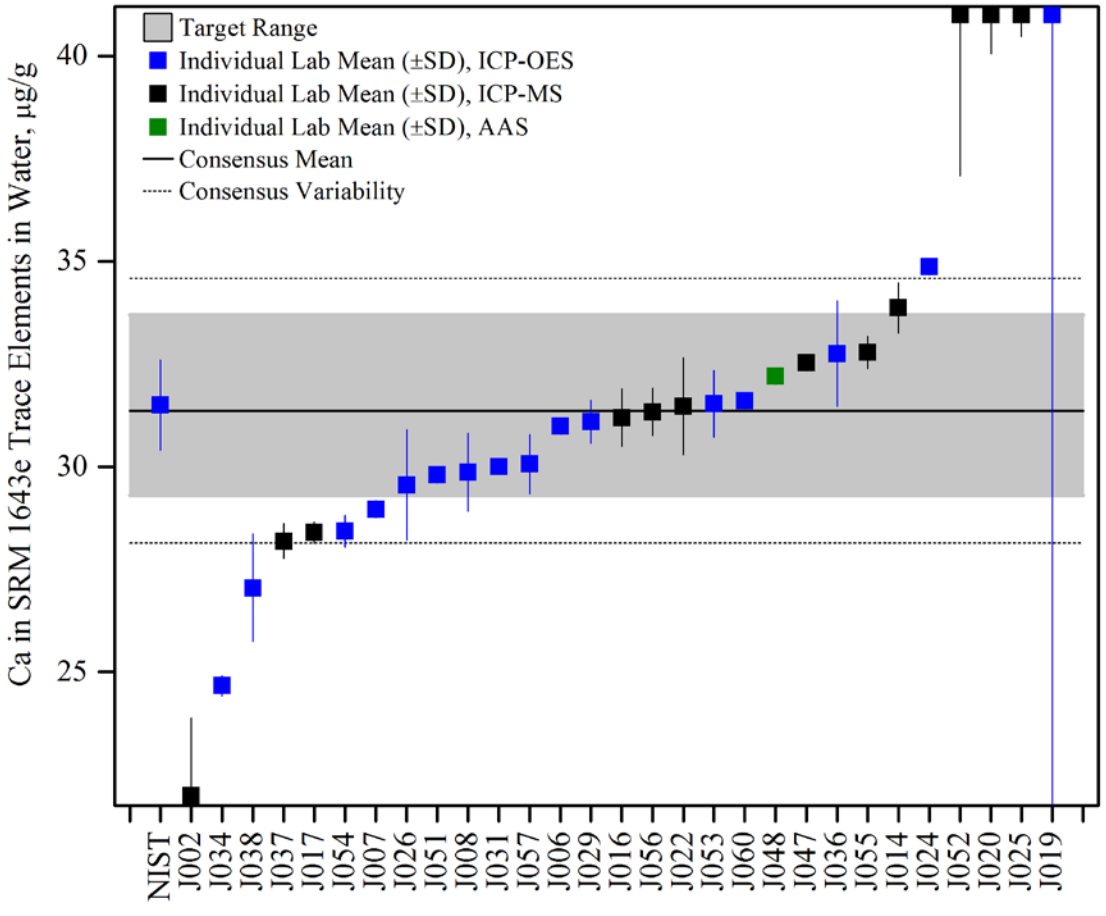

Figure 7. Calcium in SRM 1643e Trace Elements in Water (data summary view – instrumental method). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The color of the data point represents the instrumental method employed. The black solid line represents the consensus mean, and the black dotted lines represent the consensus variability calculated as one standard deviation about the consensus mean. The gray shaded region represents the target zone for "acceptable" performance, which encompasses the NIST certified value bounded by twice its uncertainty (U_{95}).

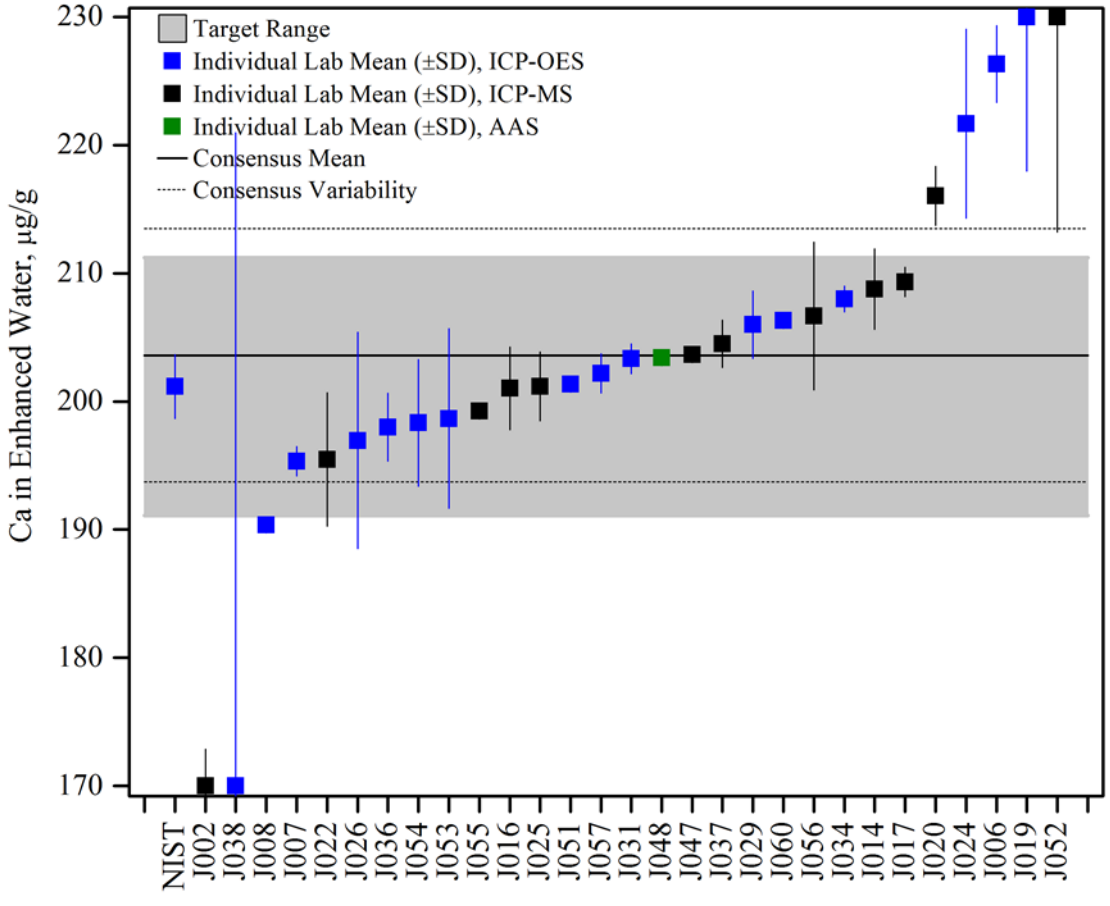

Figure 8. Calcium in enhanced water (data summary view – instrumental method). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The color of the data point represents the instrumental method employed. The black solid line represents the consensus mean, and the black dotted lines represent the consensus variability calculated as one standard deviation about the consensus mean. The gray shaded region represents the target zone for "acceptable" performance, which encompasses the NIST value determined in triplicate by ICP-OES, bounded by an estimated relative uncertainty of 5 %.

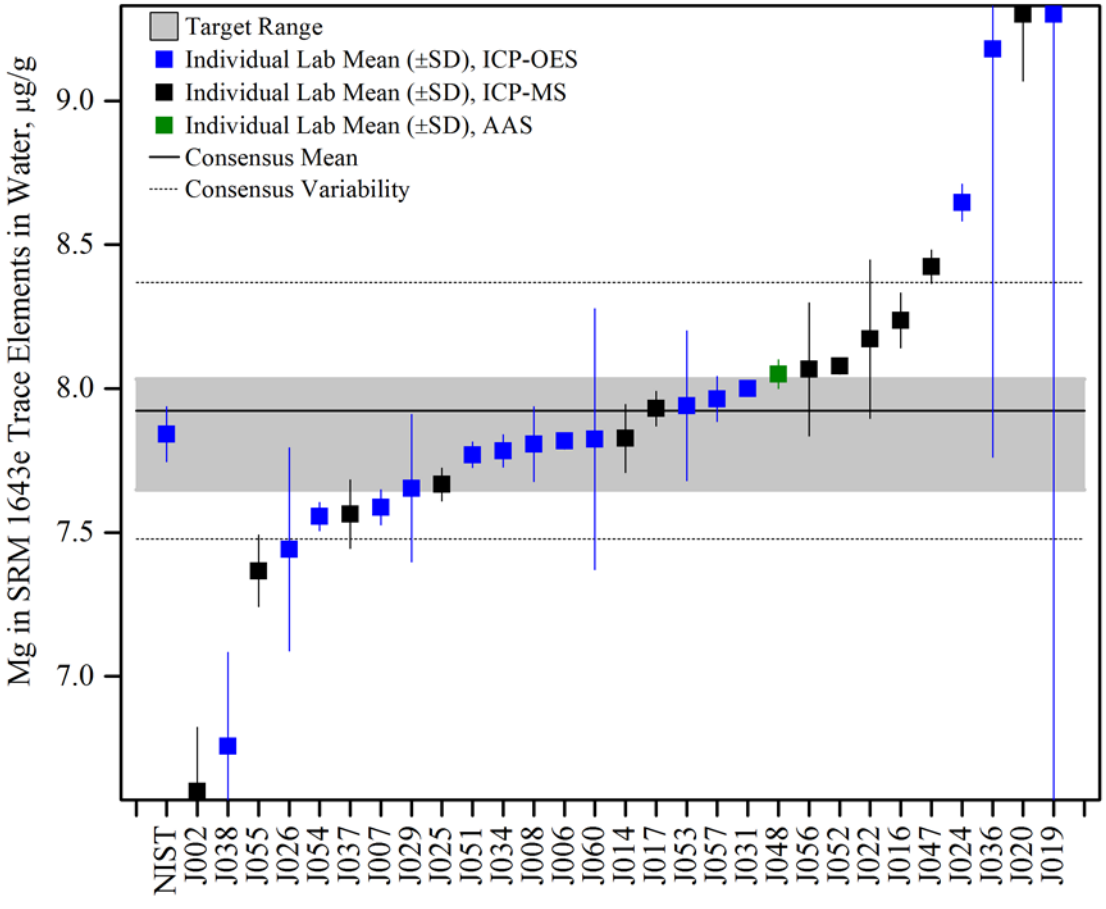

Figure 9. Magnesium in SRM 1643e Trace Elements in Water (data summary view – instrumental method). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The color of the data point represents the instrumental method employed. The black solid line represents the consensus mean, and the black dotted lines represent the consensus variability calculated as one standard deviation about the consensus mean. The gray shaded region represents the target zone for "acceptable" performance, which encompasses the NIST certified value bounded by twice its uncertainty (U_{95}).

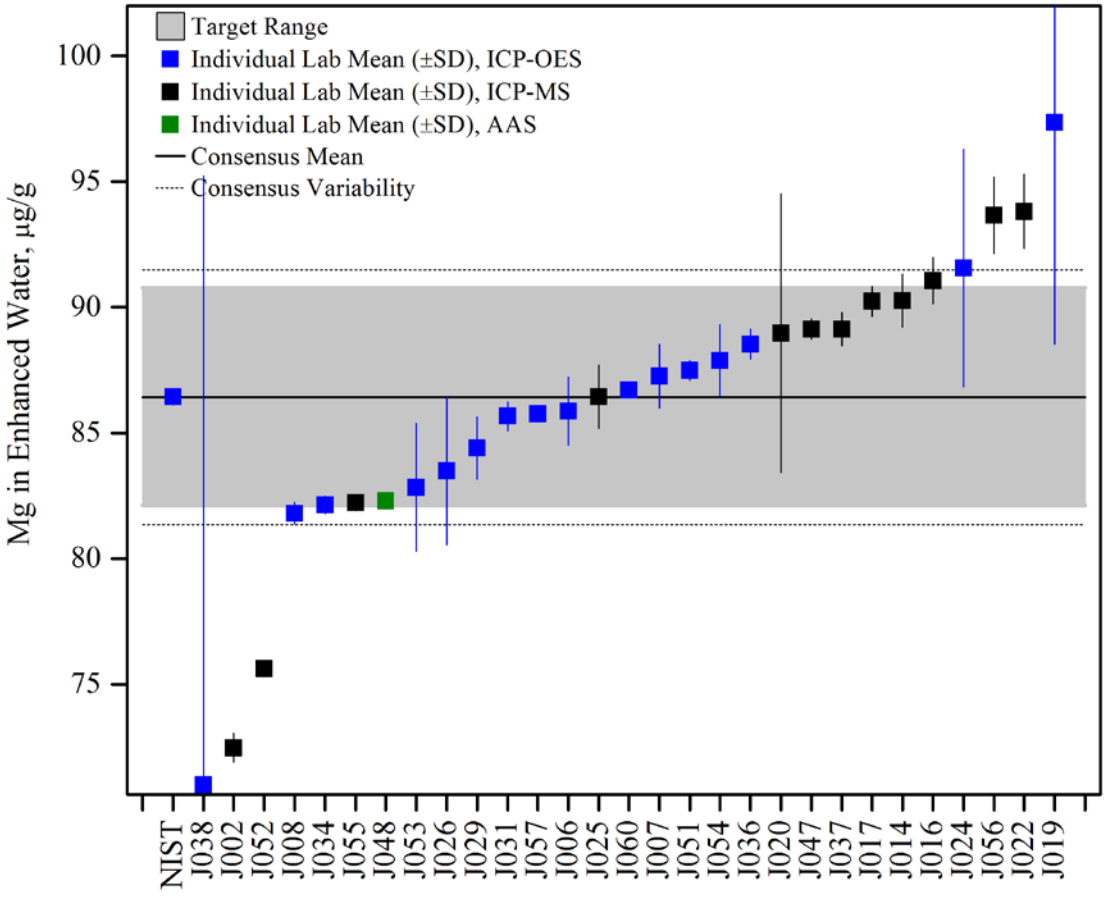

Figure 10. Magnesium in enhanced water (data summary view – instrumental method). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The color of the data point represents the instrumental method employed. The black solid line represents the consensus mean, and the black dotted lines represent the consensus variability calculated as one standard deviation about the consensus mean. The gray shaded region represents the target zone for "acceptable" performance, which encompasses the NIST value determined in triplicate by ICP-OES, bounded by an estimated relative uncertainty of 5 %.

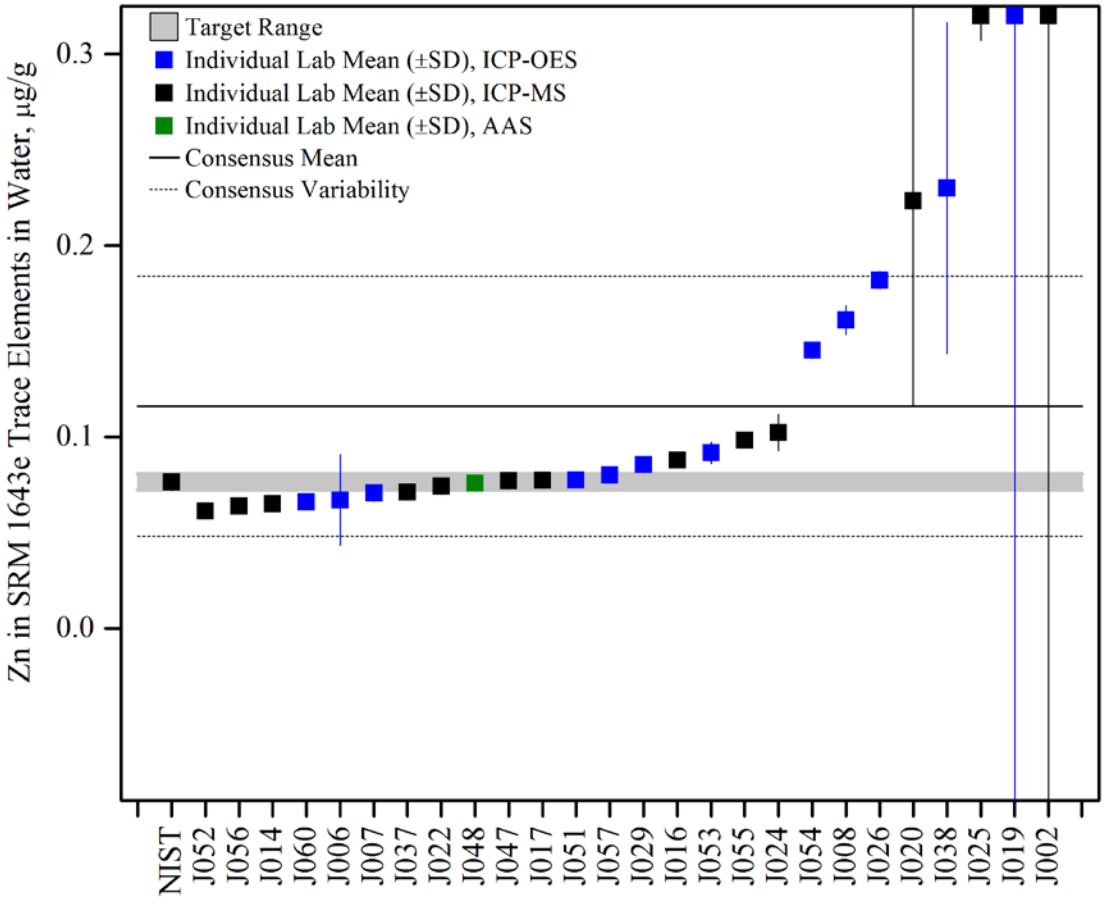

Figure 11. Zinc in SRM 1643e Trace Elements in Water (data summary view – instrumental method). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The color of the data point represents the instrumental method employed. The black solid line represents the consensus mean, and the black dotted lines represent the consensus variability calculated as one standard deviation about the consensus mean. The gray shaded region represents the target zone for "acceptable" performance, which encompasses the NIST certified value bounded by twice its uncertainty (U_{95}).

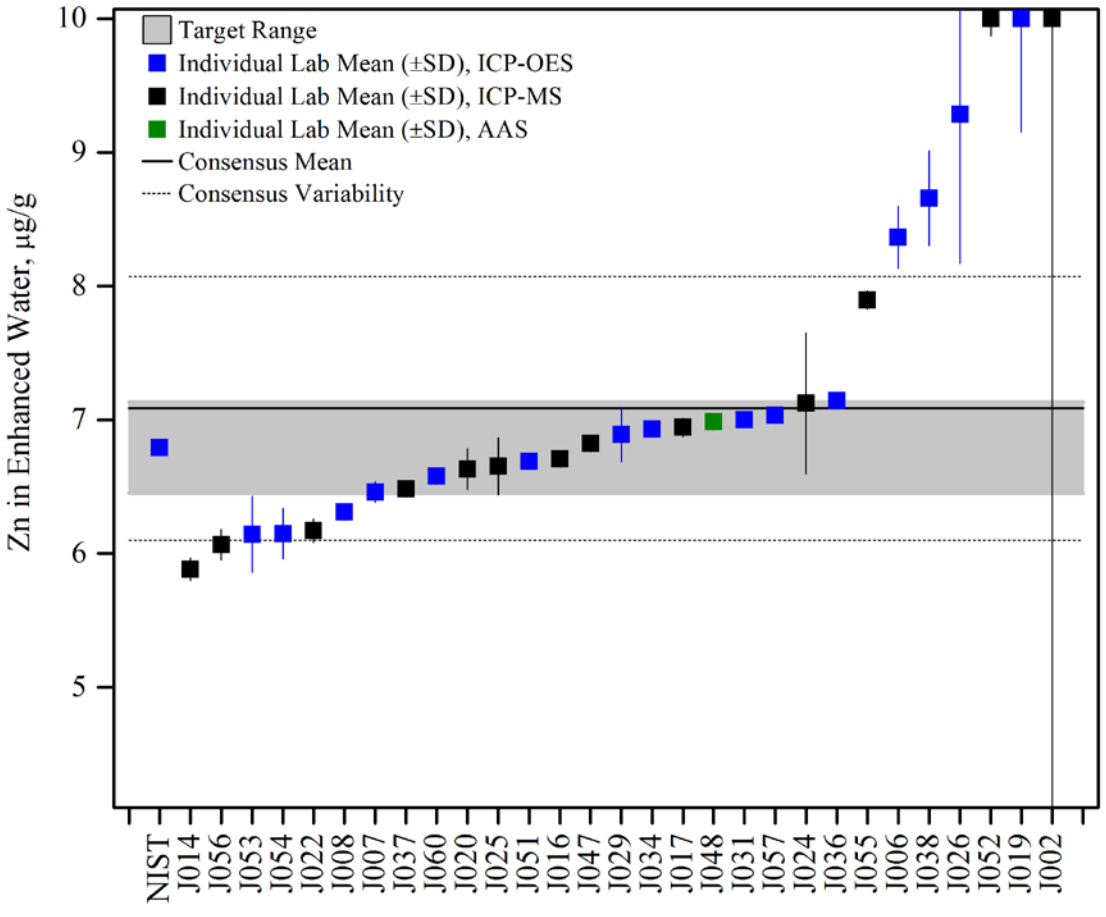

Figure 12. Zinc in enhanced water (data summary view – instrumental method). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The color of the data point represents the instrumental method employed. The black solid line represents the consensus mean, and the black dotted lines represent the consensus variability calculated as one standard deviation about the consensus mean. The gray shaded region represents the target zone for "acceptable" performance, which encompasses the NIST value determined in triplicate by ICP-OES, bounded by an estimated relative uncertainty of 5 %.

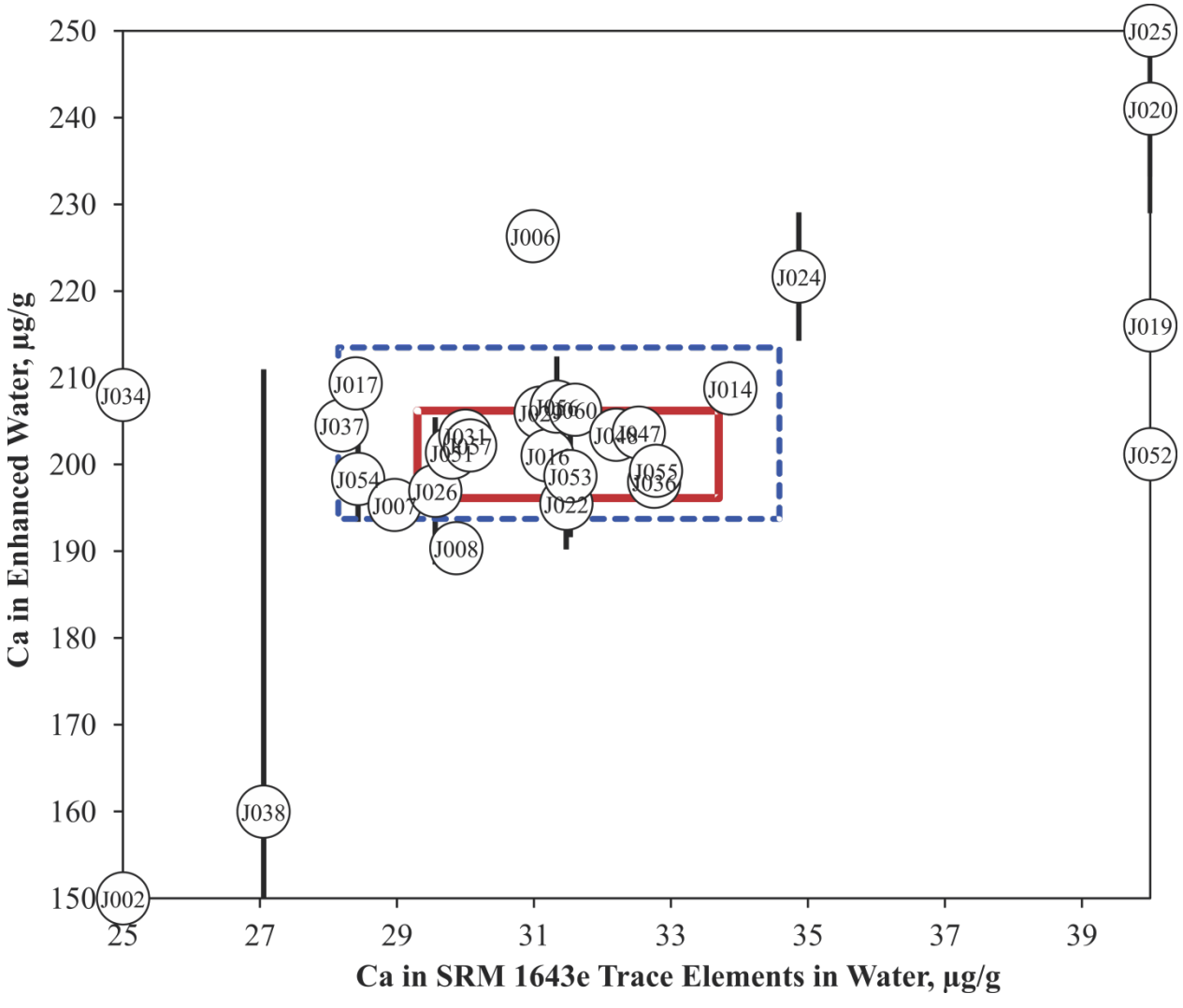

Figure 13. Calcium in SRM 1643e Trace Elements in Water and enhanced water (sample/control comparison view). In this view, the individual laboratory results for the control (SRM 1643e Trace Elements in Water) with a certified value for the analyte are compared to the results for an unknown (enhanced water). The solid red box represents 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).

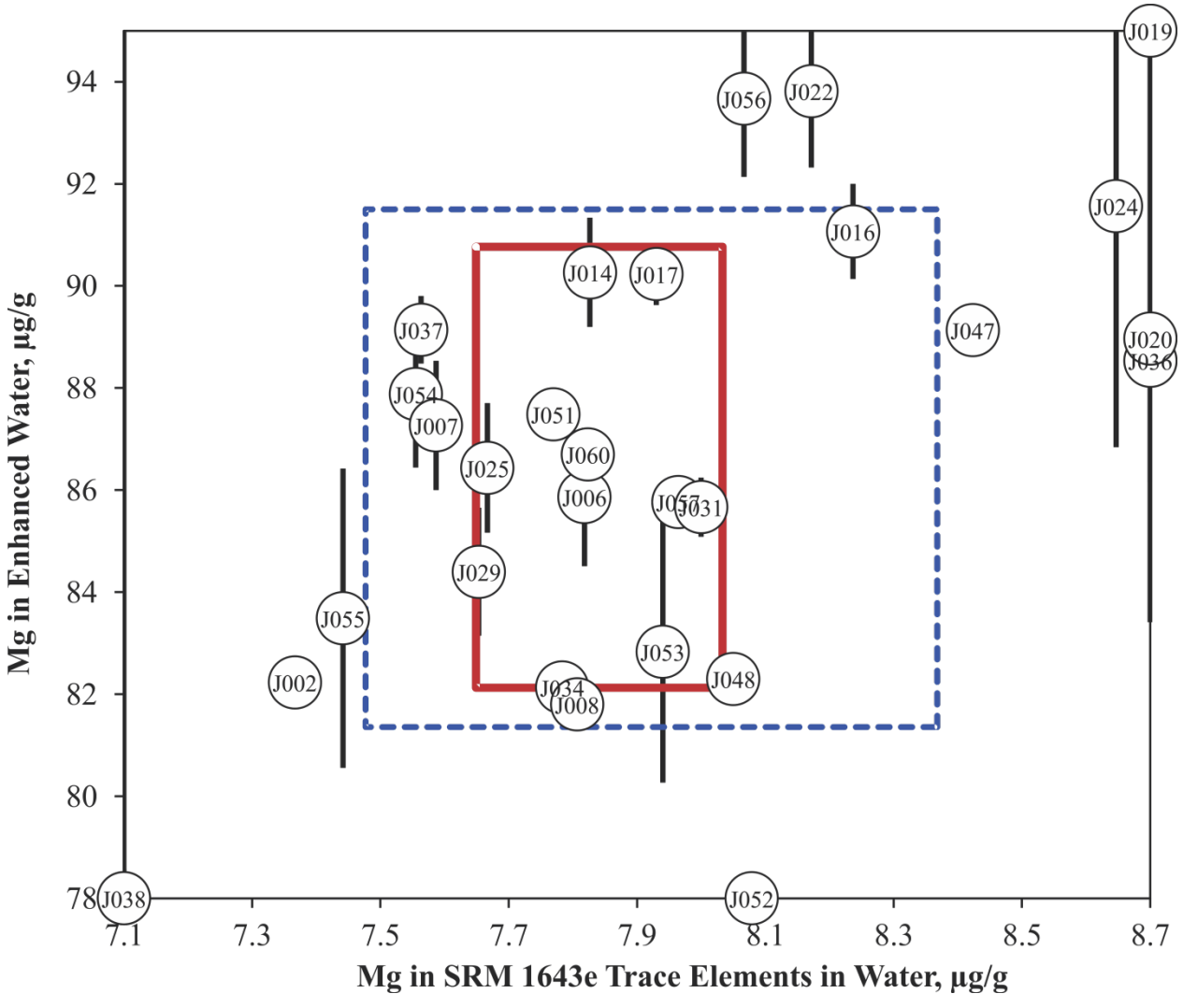

Figure 14. Magnesium in SRM 1643e Trace Elements in Water and enhanced water (sample/control comparison view). In this view, the individual laboratory results for the control (SRM 1643e Trace Elements in Water) with a certified value for the analyte are compared to the results for an unknown (enhanced water). The solid red box represents 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).

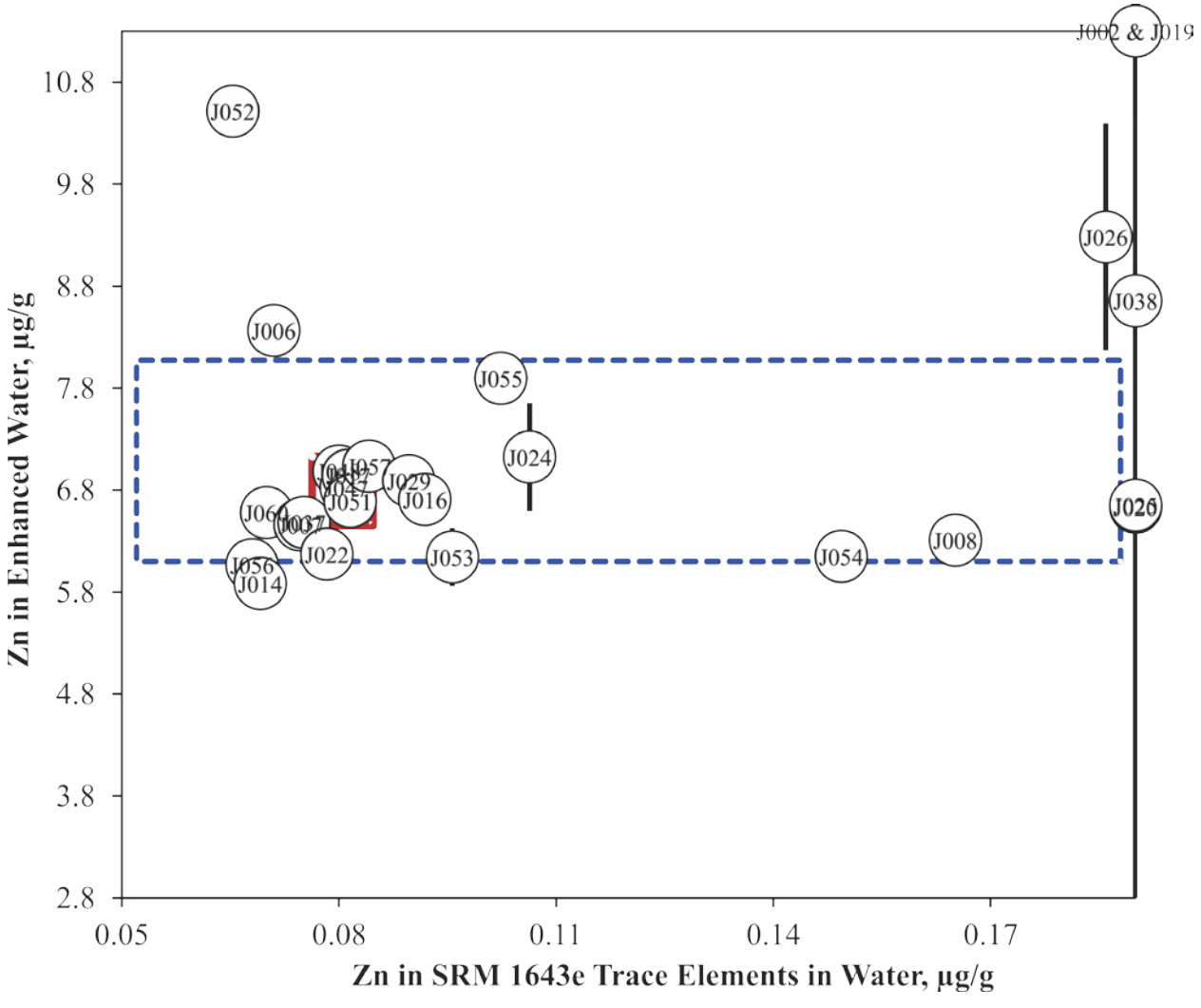

Figure 15. Zinc in SRM 1643e Trace Elements in Water and enhanced water (sample/control comparison view). In this view, the individual laboratory results for the control (SRM 1643e Trace Elements in Water) with a certified value for the analyte are compared to the results for an unknown (enhanced water). The solid red box represents 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).

TOXIC ELEMENTS (As) IN ST. JOHN'S WORT DIETARY SUPPLEMENTS

Study Overview

In this study, participants were provided with two NIST candidate SRMs, SRM 3262 *Hypericum perforatum L.* (St. John's Wort) Aerial Parts and SRM 3264 *Hypericum perforatum L.* (St. John's Wort) Methanol Extract. Participants were asked to use in-house analytical methods to determine the mass fractions of arsenic (As) in each of the matrices and report values on an as-received basis.

Sample Information

St. John's Wort Aerial Parts. Participants were provided with three packets, each containing approximately 3 g of St. John's Wort aerial parts. The plant was ground, homogenized, and heat-sealed inside 4 mil polyethylene bags, which were then sealed inside nitrogen-flushed aluminized plastic bags along with two packets of silica gel. Before use, participants were instructed to thoroughly mix the contents of each packet and use a sample size of at least 1.0 g. Participants were asked to store the material at controlled room temperature, 10 °C to 30 °C, and to prepare one sample and report one value from the each of the packets provided. Approximate analyte levels were not reported to participants prior to the study. The target value for arsenic in SRM 3262, (145 ± 8) ng/g, was determined at NIST using ICP-MS and instrumental neutron activation analysis (INAA).

St. John's Wort Methanol Extract. Participants were provided with three packets, each containing approximately 1.6 g of a methanol extract of St. John's Wort. The extract was ground, homogenized, and heat-sealed inside 4 mil polyethylene bags, which were then sealed inside nitrogen-flushed aluminized plastic bags along with two packets of silica gel. Before use, participants were instructed to thoroughly mix the contents of each packet and use a sample size of at least 1.0 g. Participants were asked to store the material at controlled room temperature, 10 °C to 30 °C, and to prepare one sample and report one value from the each of the packets provided. Approximate analyte levels were not reported to participants prior to the study. The target value for arsenic in SRM 3264, (34.8 ± 3.0) ng/g, was determined at NIST using ICP-MS.

Study Results

- Thirty-four laboratories enrolled in this exercise and received samples. Twenty-seven laboratories reported results for arsenic in St. John's Wort aerial parts (79 % participation). Twenty-six laboratories reported results for arsenic in St. John's Wort methanol extract (76 % participation).
- The consensus means for arsenic were within the target range with high variability (24 % and 34 % relative standard deviation (RSD) for the aerial parts and methanol extract, respectively).
- The greater number of low values reported for the aerial parts, compared to the extract, is most likely a result of incomplete digestions.
- The extremely high values reported for the extract material is most likely a result of incorrect calibration curves or dilutions of samples.
- A majority of the laboratories reported using microwave digestion (63 %) for sample preparation. Open beaker digestion (26 %) and hot block digestion (7 %) were also reported as methods of sample preparation.
- All of the laboratories reported using ICP-MS as their analytical method for analysis.

Technical Recommendations

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

- While twice as many laboratories reported using microwave digestion for sample preparation than other methods reported, results did not vary based on the sample preparation method used.
- Arsenic is volatile, so care must be taken to not lose arsenic during sample preparation.
- With a vigorous microwave digestion (high heat) organoarsenic should all be converted to As^{+5} and any subsequent heating will not result in loss of As.
- Plant materials can be difficult to digest without the use of HF.
- Arsenic in the methanol extract is at a very low level and may be close to detection limits.
    - Use a good calibration curve with low concentrations to help with accuracy.
    - Use a sufficient number of blanks so an accurate method detection limit and limit of quantitation can be determined.
- Run a quality control sample of known concentration to ensure your method is performing as expected.
- Double-check all calculations for any errors.

Table 5. Individual data summary table (NIST) for arsenic in St. John's Wort dietary supplements.

National Institute of Standards & Technology

Exercise J - May 2013 - Toxic Elements

	Lab Code:	**NIST**	**1. Your Results**				**2. Community Results**			**3. Target**	
Analyte	Sample	Units	x_i	s_i	Z_{comm}	Z_{NIST}	N	x*	s*	x_{NIST}	U_{95}
As	SJW Extract	ng/g	34.8	3.0	-0.2	0.0	26	38.0	13.0	34.8	3.0
As	SJW Aerial Parts	ng/g	145	8	0.0	0.0	27	146	35	145	8

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 arsenic in St. John's Wort dietary supplements.

		Arsenic									
		SRM 3264 St. John's Wort Extract (ng/g)					SRM 3262 St. John's Wort Aerial Parts (ng/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	NIST				34.8	3.0				145	8
	J002	29.9	23.5	30.3	27.9	3.8	123	128	118	123	5
	J006	27.4	43.5	26.4	32.4	9.6	140	153	154	149	8
	J007	42.0	38.8	46.8	42.5	4.0	136	122	116	125	10
	J008	76.5	73.0	66.8	72.1	4.9	197	216	214	209	10
	J009										
	J010	19.0	20.0	18.0	19.0	1.0	91	96	101	96	5
	J012	32.0	33.4	40.3	35.2	4.4	131	133	165	143	19
	J013	34.2	34.3	44.8	37.8	6.1	138	150	162	150	12
	J014	23.2	19.6	20.6	21.1	1.9	132	133	144	136	7
	J017						144	154	147	148	5
	J018										
	J019										
	J020	39.6	38.5	37.3	38.5	1.2	143	128	128	133	9
	J021										
	J022	54.4	59.8	53.7	56.0	3.3	209	192	213	205	11
	J024	46.4	30.3	48.5	41.7	10.0	130	130	143	134	8
	J025	19.6	22.4	14.6	18.9	4.0	95	91	114	100	12
	J026		35.5	32.6	34.1	2.0	176	190	183	183	7
	J029	46.2	50.8	51.6	49.5	2.9	168	135	134	146	19
	J030	40.0	40.0	40.0	40.0	0.0	170	190	170	177	12
	J031	114.0	117.0	121.0	117.3	3.5	124	116	113	118	6
	J033	37.3	38.8	38.8	38.3	0.9	165	169	174	169	5
	J035										
	J036	34.0	33.0	34.0	33.7	0.6	130	129	132	130	2
	J037	34.0	46.0	32.0	37.3	7.6	109	131	120	120	11
	J038										
	J041										
	J046	42.6	46.1	44.8	44.5	1.8	201	190	181	191	10
	J047	31.0	33.0	31.0	31.7	1.2	122	132	135	130	7
	J052	32.5	38.5	22.0	31.0	8.4	115	120	113	116	4
	J053	23.0	25.0	22.0	23.3	1.5	121	124	119	121	3
	J055	34.7	35.5	35.1	35.1	0.4	120	119	112	117	5
	J056	62.0	68.0	60.0	63.3	4.2	180	170	150	167	15
	J057	149.0	153.0	149.0	150.3	2.3	240	218	211	223	15
Community Results		Consensus Mean			38.0		Consensus Mean			146	
		Consensus Standard Deviation			13.0		Consensus Standard Deviation			35	
		Maximum			150.3		Maximum			223	
		Minimum			18.9		Minimum			96	
		N			26		N			27	

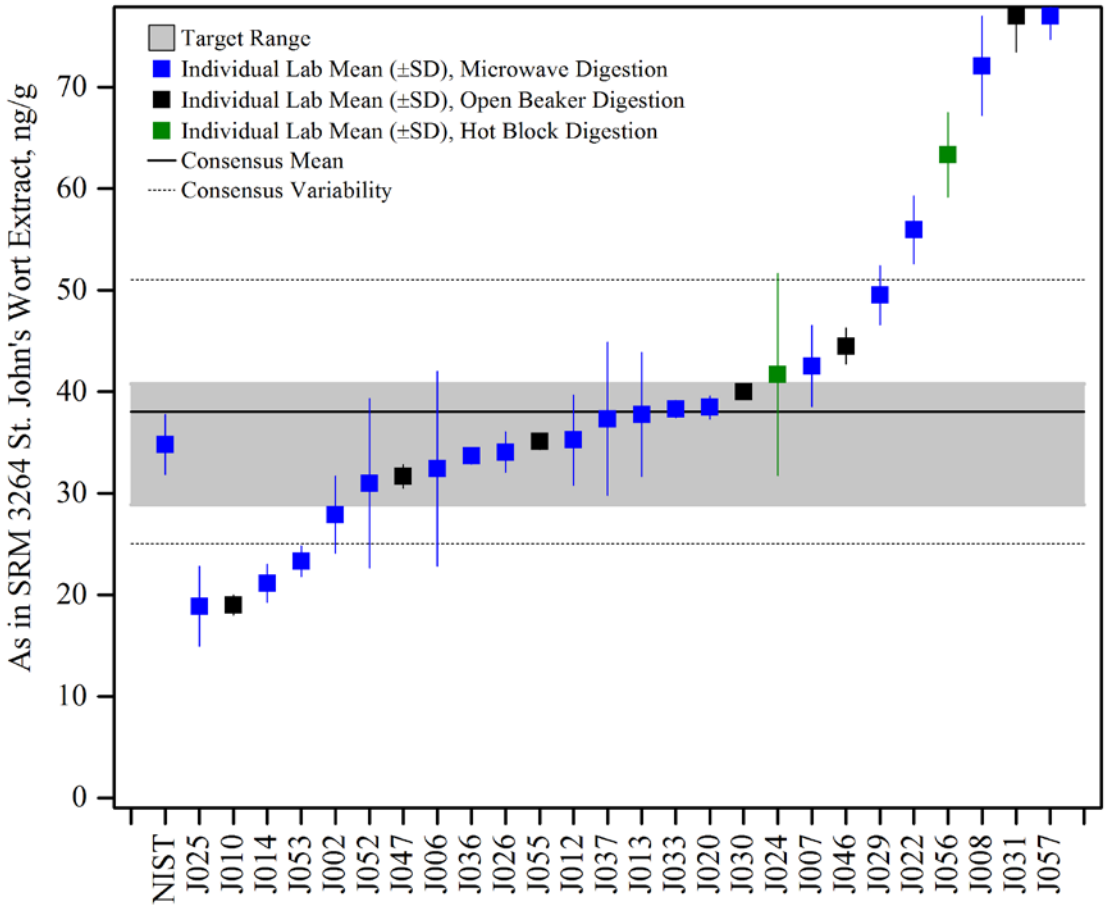

Figure 16. Arsenic in candidate SRM 3264 *Hypericum perforatum L.* (St. John's Wort) Methanol Extract (data summary view – digestion method). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The data are identified by digestion method in this graph. 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 ICP-MS, bounded by an uncertainty estimated as twice the standard deviation.

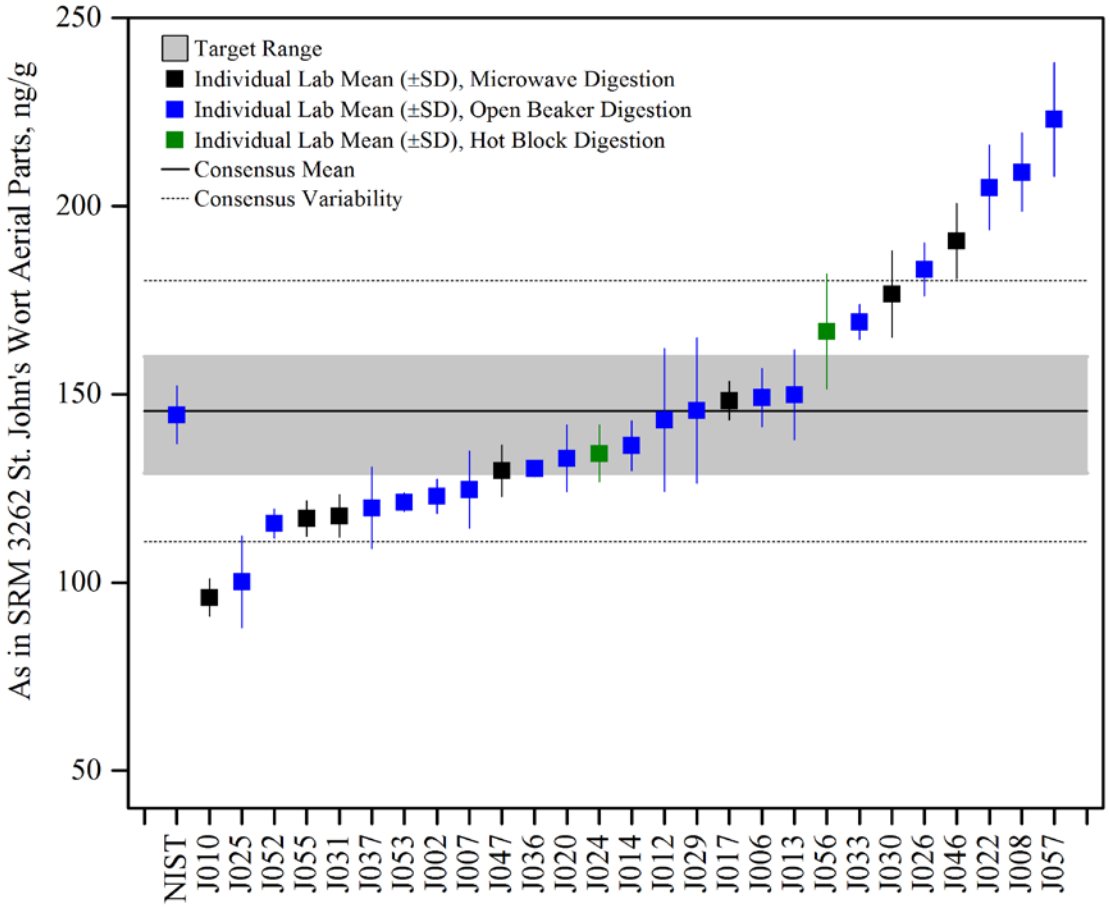

Figure 17. Arsenic in candidate SRM 3262 *Hypericum perforatum L.* (St. John's Wort) Aerial Parts (data summary view – digestion method). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The color of the data point represents the sample preparation (digestion) procedure employed. The black solid line represents the consensus mean, and the black dotted lines represent the consensus variability calculated as one standard deviation about the consensus mean. The gray shaded region represents the target zone for "acceptable" performance, which encompasses the mean of NIST value determined by ICP-MS and INAA, bounded by an uncertainty estimated as twice the combined standard deviation

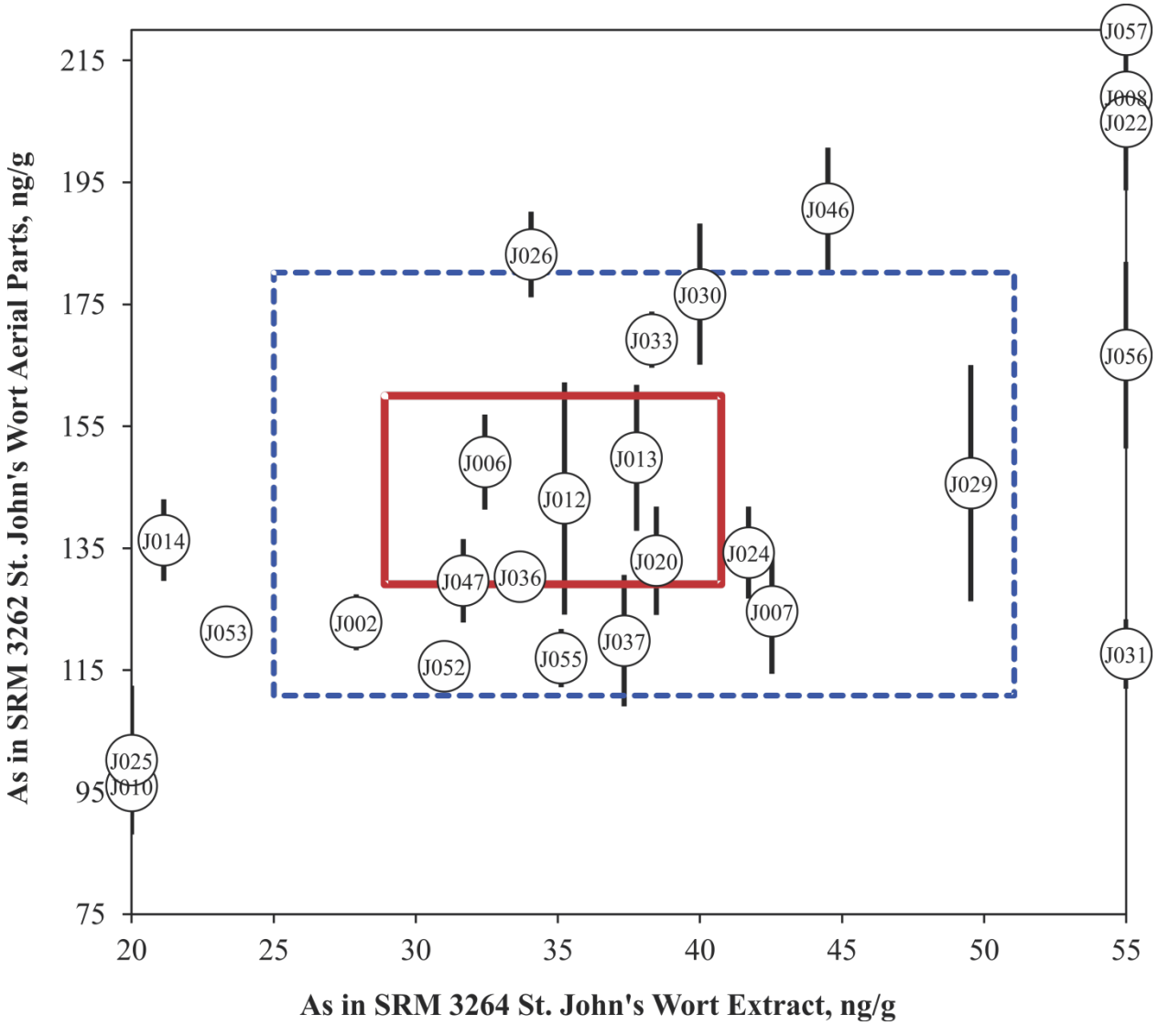

Figure 18. Arsenic in candidate SRM 3262 *Hypericum perforatum L.* (St. John's Wort) Aerial Parts and candidate SRM 3264 *Hypericum perforatum L.* (St. John's Wort) Methanol Extract (sample/sample comparison view). In this view, the individual laboratory results for one sample (St. John's Wort aerial parts) are compared to the results for a second sample (St. John's Wort extract). The solid red box represents the target zone for the two samples, St. John's Wort extract (x-axis) and St. John's Wort aerial parts (y-axis). The dotted blue box represents the consensus zone for St. John's Wort extract (x-axis) and St. John's Wort aerial parts (y-axis).

WATER-SOLUBLE VITAMINS (B_5, B_6) IN DIETARY SUPPLEMENTS

Study Overview

In this study, participants were provided with one NIST SRM, SRM 3280 Multivitamin/ Multielement Tablets, and one bottle of commercially available enhanced water. Participants were asked to use in-house analytical methods to determine the mass fractions of vitamins B_5 and B_6 in each of the matrices and report values on an as-received basis. Participants were asked to report the vitamin B_5 and B_6 content as pantothenic acid and pyridoxine hydrochloride, respectively.

Sample Information

Multivitamin/Multielement Tablets. Participants were provided with one bottle containing 30 multivitamin/multielement tablets. Before use, participants were instructed to grind all 30 tablets, mix the resulting powder thoroughly, and use a sample size of at least 0.25 g. Participants were asked to store the material at controlled room temperature, 10 °C to 30 °C, prepare three samples, and report three values from the single bottle provided. Approximate analyte levels were not reported to participants prior to the study. The NIST certified values and uncertainties for vitamin B_5 in SRM 3280 were determined by isotope dilution liquid chromatography with mass spectrometric detection (ID-LC/MS) following solvent extraction, in combination with data from numerous collaborating laboratories. The NIST certified values and uncertainties for vitamin B_6 in SRM 3280 were determined by LC with absorbance detection (LC/abs) and ID-LC/MS following solvent extraction, in combination with data from numerous collaborating laboratories. The certified values are reported in the table below, both on a dry-mass basis and after correction for moisture of the material (1.37 %).

Analyte	Certified Mass Fraction in SRM 3280 (mg/g) (dry-mass basis)	Certified Mass Fraction in SRM 3280 (mg/g) (as-received basis)
Pantothenic Acid (B_5)	7.30 ± 0.96	7.20 ± 0.95
Pyridoxine Hydrochloride (B_6)	1.81 ± 0.17	1.79 ± 0.17

Enhanced Water. Participants were provided with one 600 mL bottle of commercially available enhanced water. Before use, participants were instructed to thoroughly mix the contents of the bottle, and a sample size of at least 1.0 mL was recommended. Participants were asked to store the material at controlled room temperature, 10 °C to 30 °C, prepare three samples, and report three values from the single bottle provided. Approximate analyte levels were not reported to participants prior to the study. Certified values are not available for this material; NIST provided values for vitamins B_5 and B_6 based on triplicate analysis using ID-LC/MS. The NIST values in enhanced water are reported in the table below with an estimated uncertainty of 5 %.

Analyte	Estimated Mass Fraction in Commercial Enhanced Water (µg/g)
Pantothenic Acid	39.3 ± 0.1
Pyridoxine Hydrochloride	6.53 ± 0.02

Study Results

- Thirty-three laboratories enrolled in this exercise and received samples. Twenty-three laboratories reported results for vitamin B_5 in SRM 3280 (70% participation) and 25 laboratories reported results for vitamin B_6 in SRM 3280 (76% participation). Twenty-two laboratories reported results for both vitamins B_5 and B_6 in enhanced water (67 % participation).
- The consensus mean was within the target range for vitamins B_5 and B_6 in SRM 3280 and for vitamin B_6 in the enhanced water. The variability in these measurements was excellent, with 10 % RSD for both vitamins in the multivitamin sample and 17 % RSD for vitamin B_6 in the enhanced water.
- The consensus mean for vitamin B_5 in the enhanced water was slightly below the target range, and the variability was slightly higher at 23 % RSD.
- A majority of the laboratories reported using solvent extraction (88 %) as the sample preparation method. Laboratories also reported using acid hydrolysis (8 %).
- A majority of the laboratories reported using LC/Abs (88 %) as their instrumental method for analysis. Microbiological assay (8 %), LC/MS (4 %), and Direct Analysis in Real Time Mass Spectrometry (DART-MS) (4 %) were also reported.
- The level of vitamin B_5 in enhanced water sample was 200 times lower than that in the multivitamin tablet, which may have resulted in analytical challenges. Laboratories using LC/Abs may have had difficulty detecting pantothenic acid accurately.

Technical Recommendations

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

- In general, the results for this study were very good, and no analytical method was identified as being exceptionally good or problematic.
- When using LC-absorbance for a molecule like pantothenic acid without a chromophore, care must be taken to remove matrix interferences. Spiking of the sample used during method development may also be helpful to be sure that small chromatographic peaks are correctly identified.

Table 7. Individual data summary table (NIST) for water-soluble vitamins in dietary supplements.

National Institute of Standards & Technology

Exercise J - May 2013 - Water-Soluble Vitamins

	Lab Code:	**NIST**	**1. Your Results**				**2. Community Results**			**3. Target**	
Analyte	Sample	Units	x_i	s_i	Z_{comm}	Z_{NIST}	N	x*	s*	x_{NIST}	U_{95}
B5	Multivitamin	mg/g	7.20	0.95	-0.5	0.0	23	7.58	0.76	7.20	0.95
B5	Enhanced Water	µg/g	39.3	0.1	0.6	0.0	22	34.5	8.0	39.3	2.0
B6	Multivitamin	mg/g	1.79	0.17	0.3	0.0	25	1.73	0.18	1.79	0.17
B6	Enhanced Water	µg/g	6.53	0.02	-0.2	0.0	22	6.72	1.17	6.53	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 8. Data summary table for vitamin B_5 (pantothenic acid) in dietary supplements.

		Pantothenic Acid									
		SRM 3280 Multivitamin/Multielement Tablets (mg/g)					Enhanced Water (µg/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	NIST				7.20	0.95	39.1	39.4	39.3	39.3	0.1
	J002										
	J003										
	J004										
	J008	9.67	9.33	9.11	9.37	0.28	41.1	38.2	40.1	39.8	1.5
	J011	8.92	8.92	8.97	8.94	0.03	39.6	39.4	39.5	39.5	0.1
	J015	8.01	8.09	7.75	7.95	0.18	6.6	6.5	6.5	6.5	0.0
	J016										
	J017	7.49	7.48	7.63	7.53	0.08	35.5	42.1	35.0	37.5	4.0
	J018	8.00	8.00	8.00	8.00	0.00	49.0	50.0	50.0	49.7	0.6
	J020	6.79	6.51	6.65	6.65	0.14	27.1	27.0	26.9	27.0	0.1
	J021	3.84	4.05	3.79	3.89	0.14	14.1	14.0	14.1	14.0	0.1
	J022	7.50	7.40	7.40	7.43	0.06	29.3	29.2	29.3	29.3	0.1
	J025	7.40	7.50	7.40	7.43	0.06	35.0	33.0	35.0	34.3	1.2
	J028										
	J029	7.26	7.47	7.47	7.40	0.12	37.8	38.9	38.8	38.5	0.6
	J030	7.59	7.58	7.75	7.64	0.10	36.5	39.0	38.0	37.8	1.3
	J031	7.53	7.66	7.65	7.61	0.07	34.7	34.6	33.9	34.4	0.4
	J034	7.14	6.87	6.76	6.92	0.20	33.2	35.4	35.4	34.6	1.3
	J036	7.24	1.79	7.23	5.42	3.14	36.2	36.1	36.1	36.1	0.0
	J037										
	J038	8.26	7.93	7.89	8.03	0.20					
	J039										
	J042	7.95	7.90	7.34	7.73	0.34	30.9	35.6	36.9	34.5	3.2
	J043	8.23	7.99	8.12	8.12	0.12	43.0	42.4	46.6	44.0	2.3
	J044	7.30	7.20	7.19	7.23	0.06	32.9	31.1	36.2	33.4	2.6
	J045	6.80	6.80	6.80	6.80	0.00	34300.0	35200.0	34700.0	34733.3	450.9
	J047	7.13	7.13	7.30	7.19	0.10	34.9	36.0	35.8	35.6	0.6
	J052										
	J053										
	J054	11.40	11.20	11.70	11.43	0.25	20.1	20.3	20.1	20.2	0.1
	J057	8.45	7.99	7.95	8.13	0.28					
	J058	7.57	7.54	7.63	7.58	0.05	27.0	26.4	26.9	26.8	0.3
	J059						35.2	34.2	35.0	34.8	0.6
Community Results		Consensus Mean			7.58		Consensus Mean			34.5	
		Consensus Standard Deviation			0.76		Consensus Standard Deviation			8.0	
		Maximum			11.43		Maximum			34733.3	
		Minimum			3.89		Minimum			6.5	
		N			23		N			22	

Table 9. Data summary table for vitamin B_6 (pyridoxine hydrochloride) in dietary supplements.

		Pyridoxine Hydrochloride									
		SRM 3280 Multivitamin/Multielement Tablets (mg/g)					Enhanced Water (µg/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	NIST				1.79	0.17	6.51	6.52	6.55	6.53	0.02
	J002										
	J003										
	J004										
	J008	1.79	1.57	1.49	1.62	0.16	6.63	6.56	6.59	6.59	0.04
	J011	2.15	2.12	2.10	2.12	0.03	6.58	6.52	6.65	6.58	0.07
	J015	1.95	1.95	1.97	1.96	0.01	7.10	7.13	7.13	7.12	0.02
	J016										
	J017	1.65	1.62	1.67	1.65	0.03	7.61	7.43	7.60	7.55	0.10
	J018	1.54	1.57	1.51	1.54	0.03	7.00	7.00	7.00	7.00	0.00
	J020	1.80	1.79	1.77	1.79	0.02	6.28	6.33	6.28	6.30	0.03
	J021	1.74	1.75	1.82	1.77	0.04	8.24	8.20	8.13	8.19	0.06
	J022	1.84	1.82	1.81	1.82	0.02	7.00	7.00	7.00	7.00	0.00
	J025	1.96	1.92	1.94	1.94	0.02	6.70	6.80	6.70	6.73	0.06
	J028										
	J029	1.87	1.92	1.86	1.88	0.03	5.45	5.23	5.30	5.33	0.11
	J030	1.73	1.89	1.84	1.82	0.08	6.59	6.55	6.66	6.60	0.06
	J031	1.75	1.81	1.78	1.78	0.03	7.05	6.65	6.45	6.72	0.31
	J034	1.51	1.50	1.43	1.48	0.04	5.15	4.91	4.96	5.01	0.13
	J036	1.71	1.64	1.73	1.69	0.05	6.18	6.18	6.42	6.26	0.14
	J037										
	J038	1.87	1.84	1.81	1.84	0.03					
	J039										
	J042	1.83	1.81	1.81	1.82	0.01	5.57	5.55	5.55	5.56	0.01
	J042	1.70	1.69	1.72	1.70	0.02					
	J044	1.53	1.49	1.48	1.50	0.03	5.70	5.97	5.62	5.76	0.18
	J045	1.64	1.69	1.74	1.69	0.05	6800.80	6814.40	6814.80	6810.00	7.97
	J047	1.65	1.66	1.72	1.68	0.04	6.50	6.50	6.50	6.50	0.00
	J052	1.83	1.80		1.82	0.02					
	J053	1.35	1.37	1.30	1.34	0.03	5.10	5.31		5.21	0.15
	J054	1.27	1.25	1.29	1.27	0.02	31.24	32.12	30.13	31.16	1.00
	J057	1.71	1.67	1.67	1.68	0.02					
	J058	1.89	1.85	1.86	1.87	0.02	8.76	8.72	8.74	8.74	0.02
	J059						6.56	6.19	6.48	6.41	0.19
Community Results		Consensus Mean			1.73		Consensus Mean			6.72	
		Consensus Standard Deviation			0.18		Consensus Standard Deviation			1.17	
		Maximum			2.12		Maximum			6810.00	
		Minimum			1.27		Minimum			5.01	
		N			25		N			22	

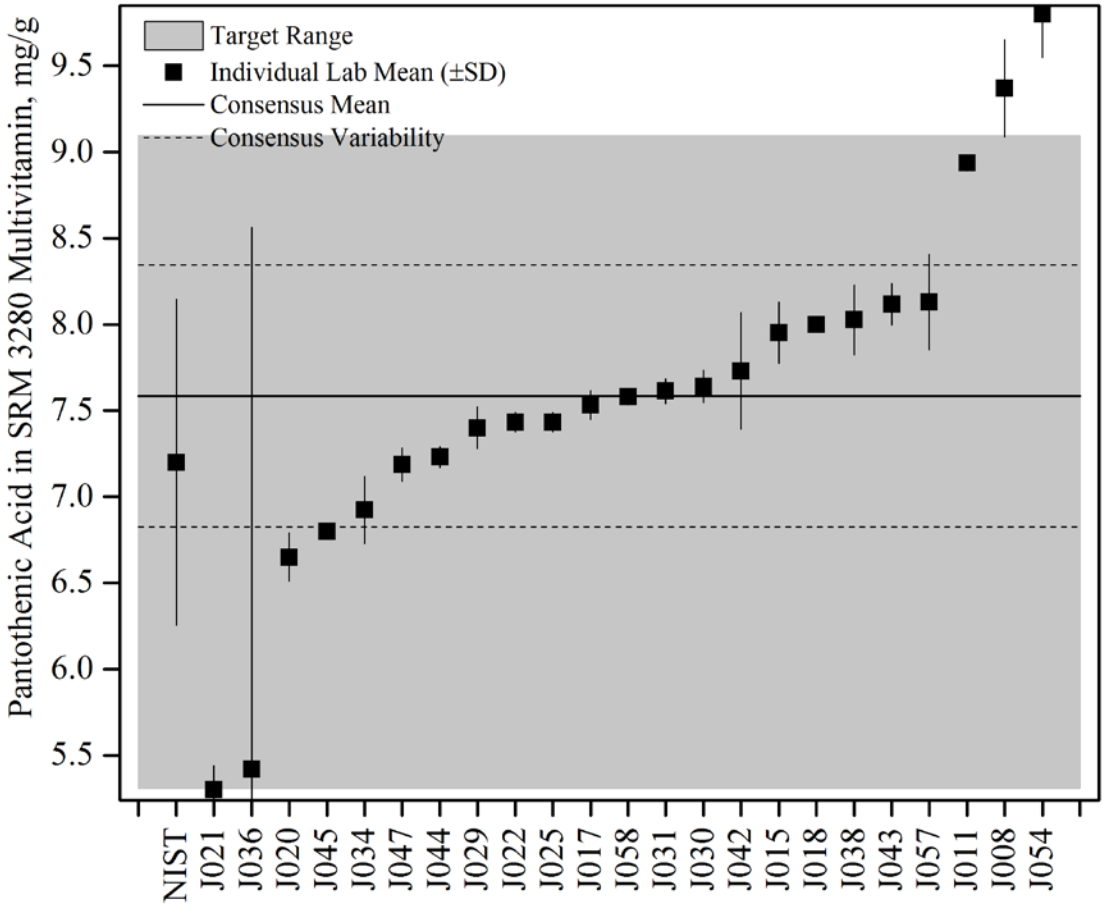

Figure 19. Pantothenic acid in SRM 3280 Multivitamin/Multielement Tablets (data summary view). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The black solid line represents the consensus mean, and the black dotted lines represent 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}).

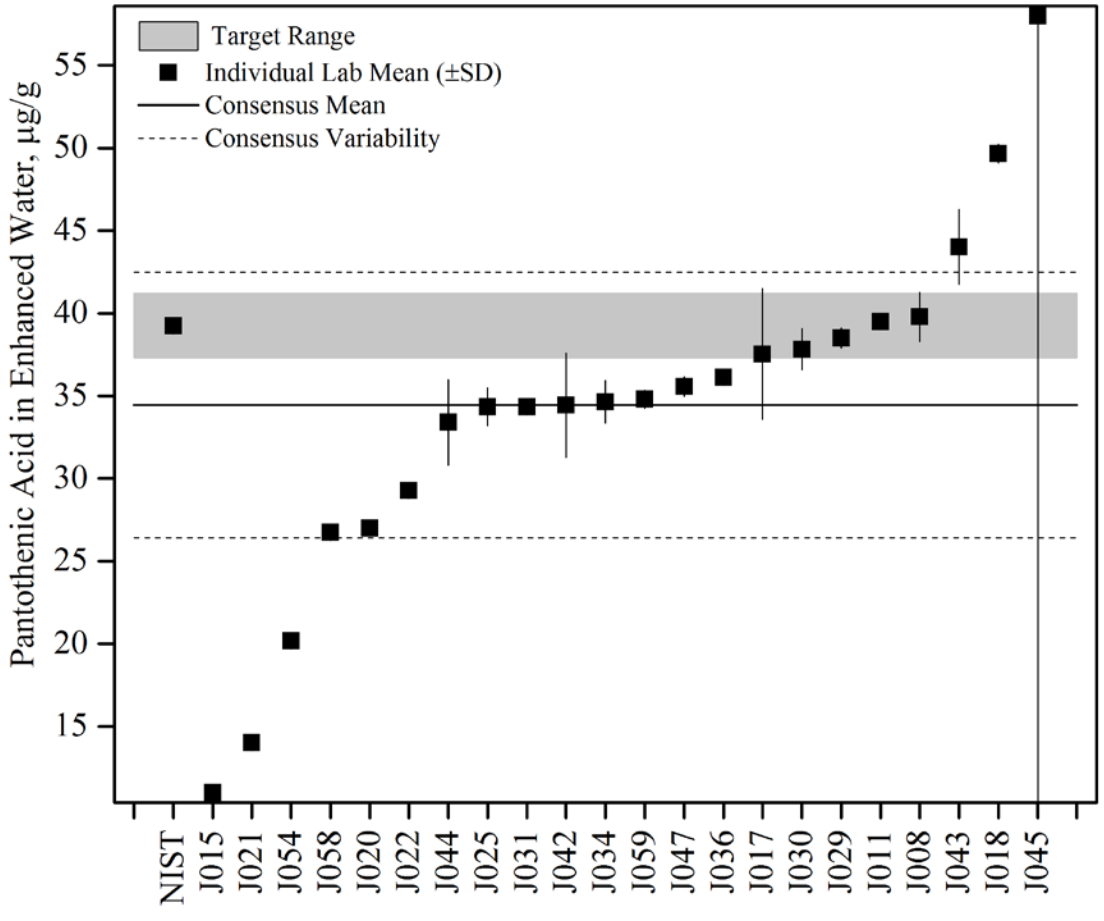

Figure 20. Pantothenic acid in enhanced water (data summary view). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The black solid line represents the consensus mean, and the black dotted lines represent the consensus variability calculated 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 in triplicate by ID-LC/MS, bounded by an estimated relative uncertainty of 5 %.

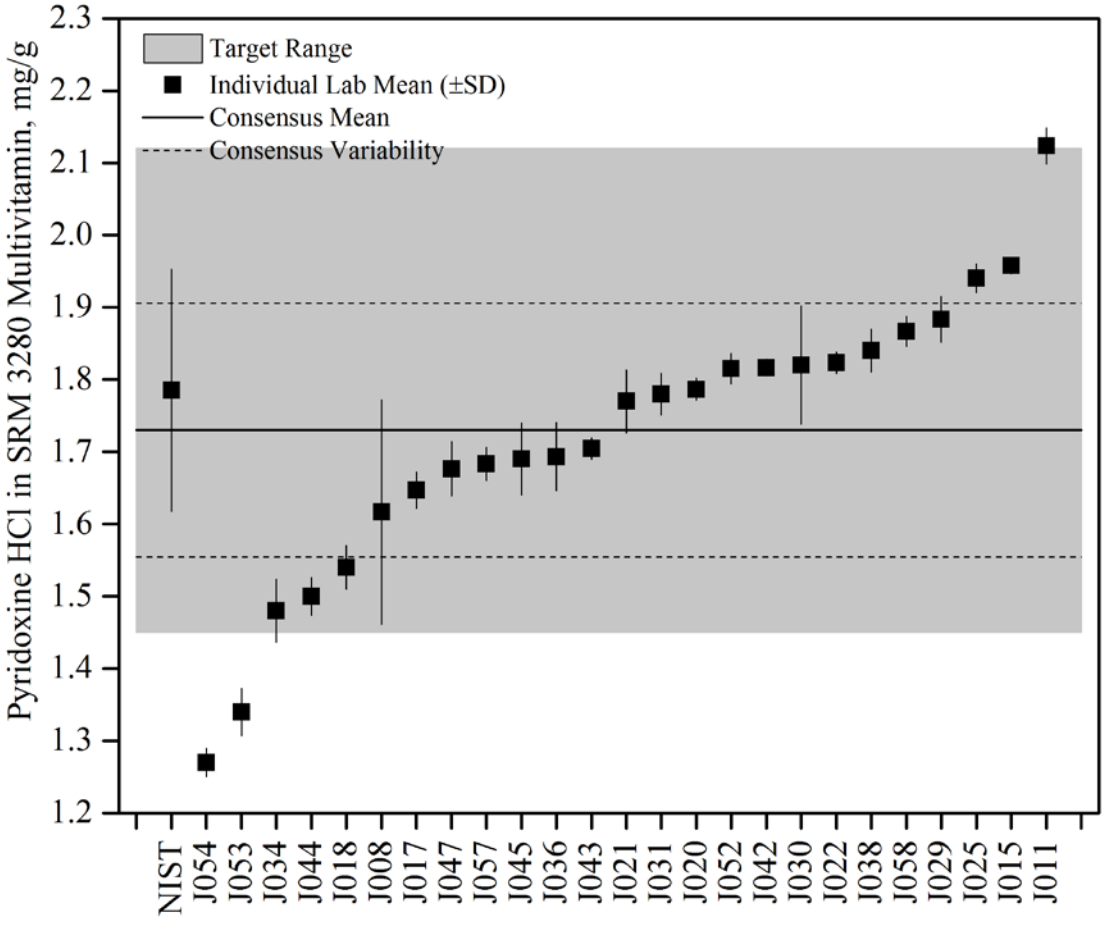

Figure 21. Pyridoxine hydrochloride in SRM 3280 Multivitamin/Multielement Tablets (data summary view). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The black solid line represents the consensus mean, and the black dotted lines represent 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}).

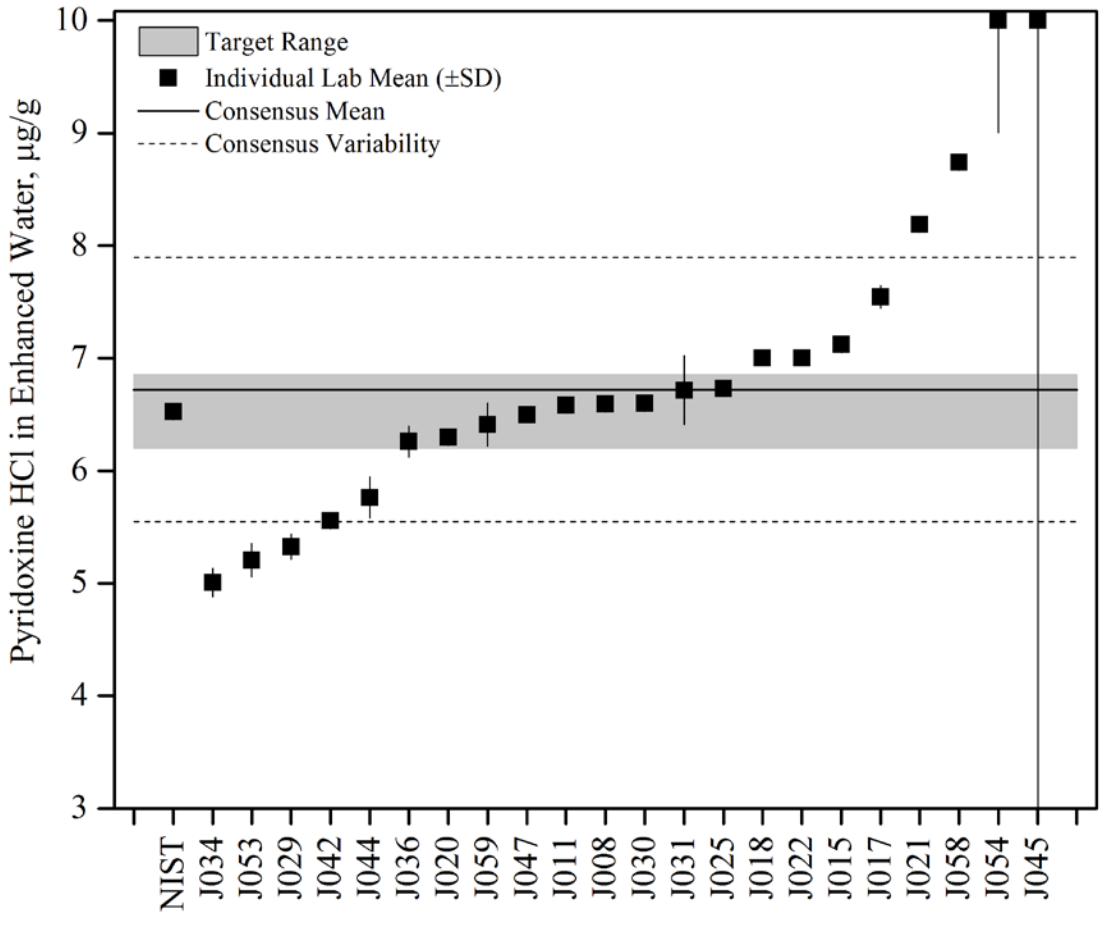

Figure 22. Pyridoxine hydrochloride in enhanced water (data summary view). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The black solid line represents the consensus mean, and the black dotted lines represent the consensus variability 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 in triplicate by ID-LC/MS, bounded by an estimated relative uncertainty of 5 %.

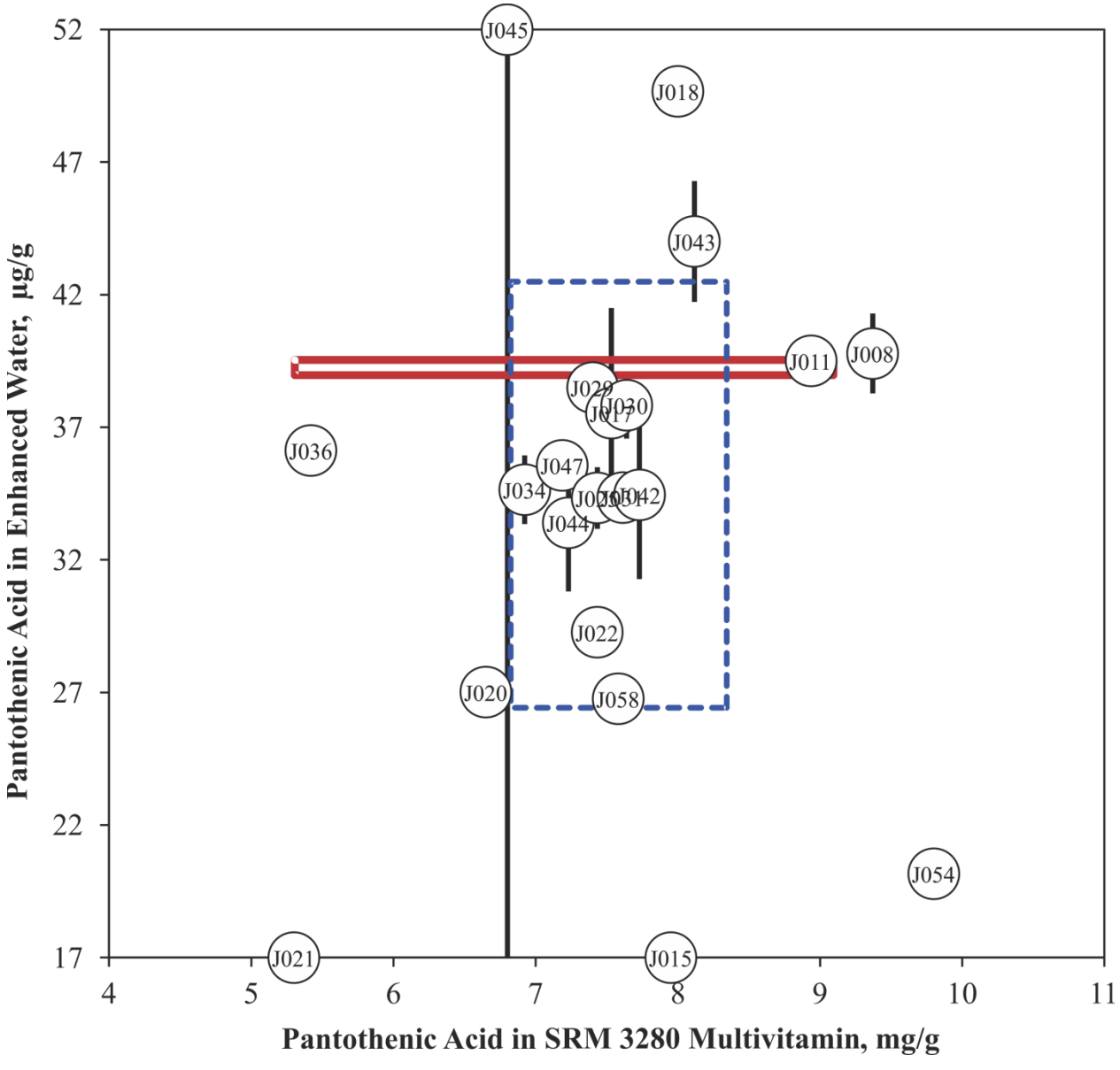

Figure 23. Pantothenic acid in SRM 3280 Multivitamin/Multielement Tablets and enhanced water (sample/control comparison view). In this view, the individual laboratory results for the control (SRM 3280) with a certified value for the analyte are compared to the results for an unknown (enhanced water). The solid red box represents the target zone for the control (x-axis) and unknown sample (y-axis). The dotted blue box represents the consensus zone for the control (x-axis) and the unknown sample (y-axis).

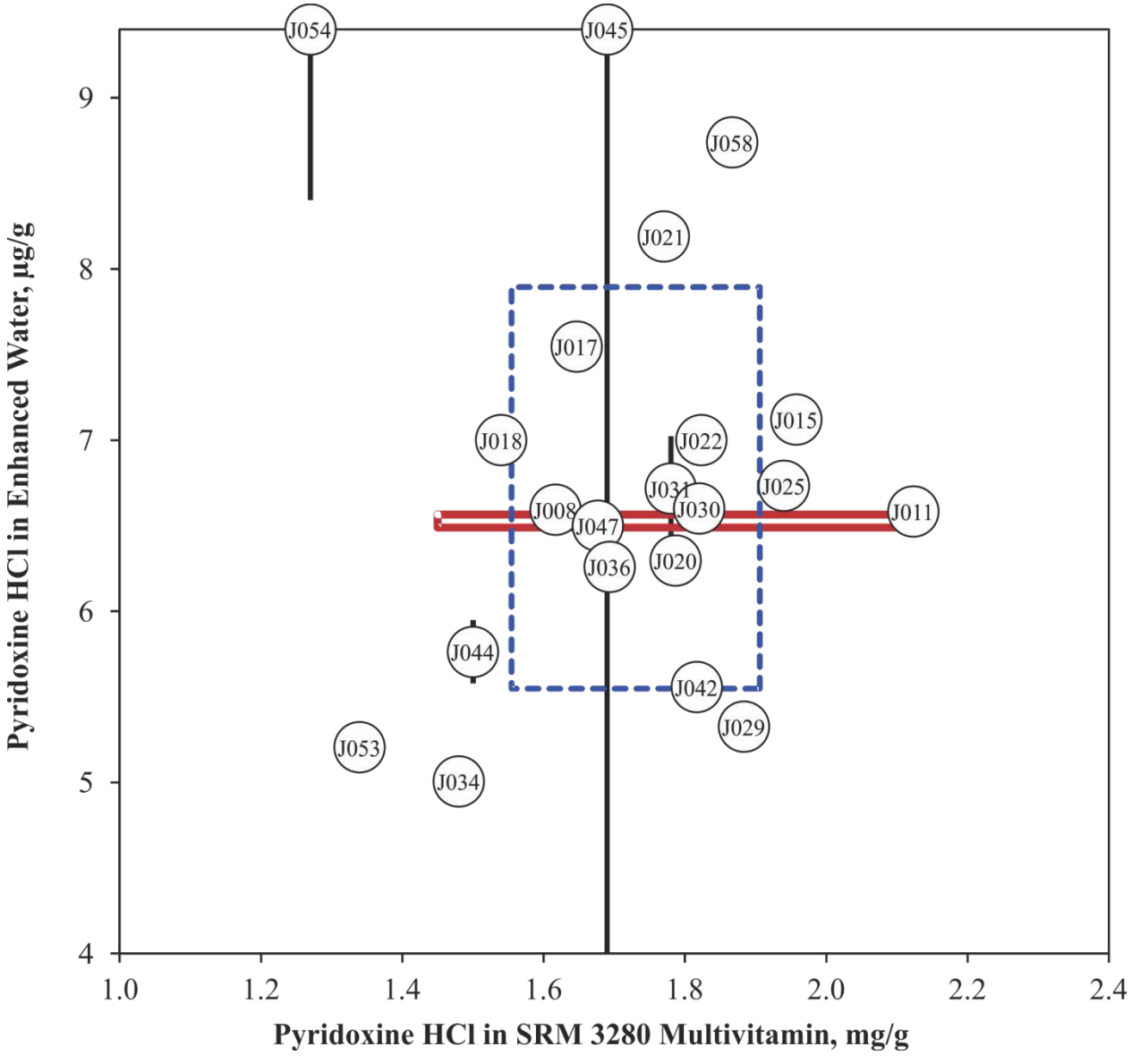

Figure 24. Pyridoxine hydrochloride in SRM 3280 Multivitamin/Multielement Tablets and enhanced water (sample/control comparison view). In this view, the individual laboratory results for the control (SRM 3280) with a certified value for the analyte are compared to the results for an unknown (enhanced water). The solid red box represents the target zone for the control (x-axis) and unknown sample (y-axis). The dotted blue box represents the consensus zone for the control (x-axis) and the unknown sample (y-axis).

VITAMIN E IN DIETARY SUPPLEMENTS

Study Overview

In this study, participants were provided with one NIST SRM, SRM 3280 Multivitamin/ Multielement Tablets, and one bottle of commercially available enhanced water. Participants were asked to use in-house analytical methods to determine the mass fraction of vitamin E (as α-tocopherol acetate) in each of the matrices and report values on an as-received basis.

Sample Information

Multivitamin/Multielement Tablets. Participants were provided with one bottle containing 30 multivitamin/multielement tablets. Before use, participants were instructed to grind all 30 tablets, mix the resulting powder thoroughly, and use a sample size of at least 0.6 g. Participants were asked to store the material at controlled room temperature, 10 °C to 30 °C, prepare three samples, and report three values from the single bottle provided. Approximate analyte levels were not reported to participants prior to the study. The NIST certified value and uncertainty for vitamin E (as α-tocopherol acetate) in SRM 3280 was determined by LC/abs and LC/MS following solvent extraction, in combination with data from numerous collaborating laboratories. The certified value is reported in the table below as α-tocopherol equivalents and as α-tocopherol acetate, both on a dry-mass basis and after correction for moisture of the material (1.37 %).

Analyte	Certified Mass Fraction in SRM 3280 (mg/g) (dry-mass basis)	Certified Mass Fraction in SRM 3280 (mg/g) (as-received basis)
α-tocopherol equivalents	21.4 ± 3.5	21.1 ± 3.5
α-tocopherol acetate	23.5 ± 3.8	23.2 ± 3.8

Enhanced Water. Participants were provided with one 600 mL bottle of commercially available enhanced water. Before use, participants were instructed to thoroughly mix the contents of the bottle, and a sample size of at least 1.0 mL was recommended. Participants were asked to store the material at controlled room temperature, 10 °C to 30 °C, prepare three samples, and report three values from the single bottle provided. Approximate analyte levels were not reported to participants prior to the study. Certified values are not available for this material.

Study Results

- Thirty-two laboratories enrolled in this exercise and received samples. Twenty laboratories reported results for vitamin E in SRM 3280 (62 % participation) and 18 laboratories reported results for vitamin E in enhanced water (56 % participation).
- The consensus mean was within the target range for vitamin E in SRM 3280 with acceptable variability (9 % RSD).
- The consensus variability was also acceptable for vitamin E in enhanced water (21 % RSD), a sample with a concentration 1000 times lower than that in the control material.
- A majority of the laboratories reported using solvent extraction (85 %) as the sample preparation method. Some laboratories also reported using saponification (15 %).
- A majority of the laboratories reported using LC/Abs (85 %) as their instrumental method for analysis. Liquid chromatography/fluorescence (LC/FL) was also reported by some laboratories (15 %).

Technical Recommendations

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

- When using saponification for sample preparation, it is important to determine that the saponification reaction is complete. Additionally, if an internal standard approach to calibration is used, the internal standard must be stable under the sample preparation conditions.
- Absorbance and fluorescence detection are both valuable tools for the determination of tocopherols. Fluorescence detection is especially sensitive and selective for α-, β-, γ-, and δ-tocopherol, however it is not very sensitive for the detection of α-tocopherol acetate. In this study, if the samples were saponified and the analyte was converted to α-tocopherol, fluorescence is an appropriate choice for detection.
- In general, the results reported for this study were very good, but there is a slight indication of calibration error in the sample/sample view. α-tocopherol acetate and α-tocopherol calibrant concentrations should be determined spectrophotometrically with a liquid chromatography purity correction. Also, be certain that the correct molar absorptivity is used.
- If laboratories would like further guidance on the use of calibrants traceable to molar absorptivity (rather than mass), please indicate the need. NIST staff will be happy to provide additional information and/or training as appropriate.

Table 10. Individual data summary table (NIST) for α-tocopherol acetate in dietary supplements.

National Institute of Standards & Technology

Exercise J - May 2013 - Vitamin E

	Lab Code:	**NIST**	**1. Your Results**				**2. Community Results**			**3. Target**	
Analyte	Sample	Units	x_i	s_i	Z_{comm}	Z_{NIST}	N	x*	s*	x_{NIST}	U_{95}
Vitamin E	Multivitamin	mg/g	23.2	3.8	0.1	0.0	20	23.0	2.2	23.2	3.8
Vitamin E	Enhanced Water	µg/g					18	23.8	4.9		

x_i Mean of reported values

s_i Standard deviation of reported values

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

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

N Number of quantitative values reported

x* Robust mean of reported values

s* Robust standard deviation

x_{NIST} NIST-assessed value

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

Table 11. Data summary table for α-tocopherol acetate in dietary supplements.

		Tocopherol Acetate									
		SRM 3280 Multivitamin/Multielement Tablets (mg/g)					Enhanced Water (µg/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	NIST				23.2	3.8					
	J002										
	J004										
	J008	22.4	22.2	23.3	22.6	0.6	17.6	18.6	18.6	18.3	0.6
	J011	18.9	18.1	18.6	18.5	0.4					
	J015	21.5	21.1	20.7	21.1	0.4	27.5	23.7	23.5	24.9	2.3
	J016	22.1	21.8	20.3	21.4	1.0	22.1	21.6	21.3	21.6	0.4
	J017	23.3	24.7	26.9	25.0	1.8	23.2	30.0	27.0	26.7	3.4
	J018										
	J020	36.0	34.0	35.0	35.0	1.0	19.6	21.6	21.3	20.8	1.1
	J021	22.5	22.4	22.6	22.5	0.1	13.1	13.4	13.7	13.4	0.3
	J022	23.2	23.8	24.0	23.7	0.4	25.1	26.2	25.7	25.7	0.6
	J023										
	J025	26.5	29.0	28.8	28.1	1.4	109.0	48.0	53.0	70.0	33.9
	J029	23.4	23.2	23.0	23.2	0.2	25.5	24.8	25.5	25.3	0.4
	J030										
	J031	24.6	24.3	24.4	24.4	0.2					
	J036	23.9	23.8	23.9	23.9	0.1	25.8	27.4	27.5	26.9	0.9
	J037										
	J038	26.1	26.0	25.1	25.7	0.5	28.6	30.6	27.4	28.9	1.6
	J039										
	J042	21.1	21.2	21.2	21.1	0.0	26.0	24.4	28.0	26.1	1.8
	J043	20.6	21.2	21.0	20.9	0.3	19.2	21.1	18.5	19.6	1.3
	J044	22.9	23.6	23.1	23.2	0.4	26.4	19.5	22.2	22.7	3.5
	J045										
	J047										
	J048	20.1	21.6		20.9	1.1	21.1	20.2		20.7	0.6
	J052										
	J053	20.3	23.1		21.7	2.0	25.0	28.5		26.7	2.5
	J054	24.2	24.1	24.6	24.3	0.3	30.0	27.1	29.3	28.8	1.5
	J057	20.7			20.7						
	J058	21.9	21.2	22.8	22.0	0.8	19.1	15.7	16.4	17.1	1.8
	J059										
Community Results		Consensus Mean			23.0		Consensus Mean			23.8	
		Consensus Standard Deviation			2.2		Consensus Standard Deviation			4.9	
		Maximum			35.0		Maximum			70.0	
		Minimum			18.5		Minimum			13.40	
		N			20		N			18	

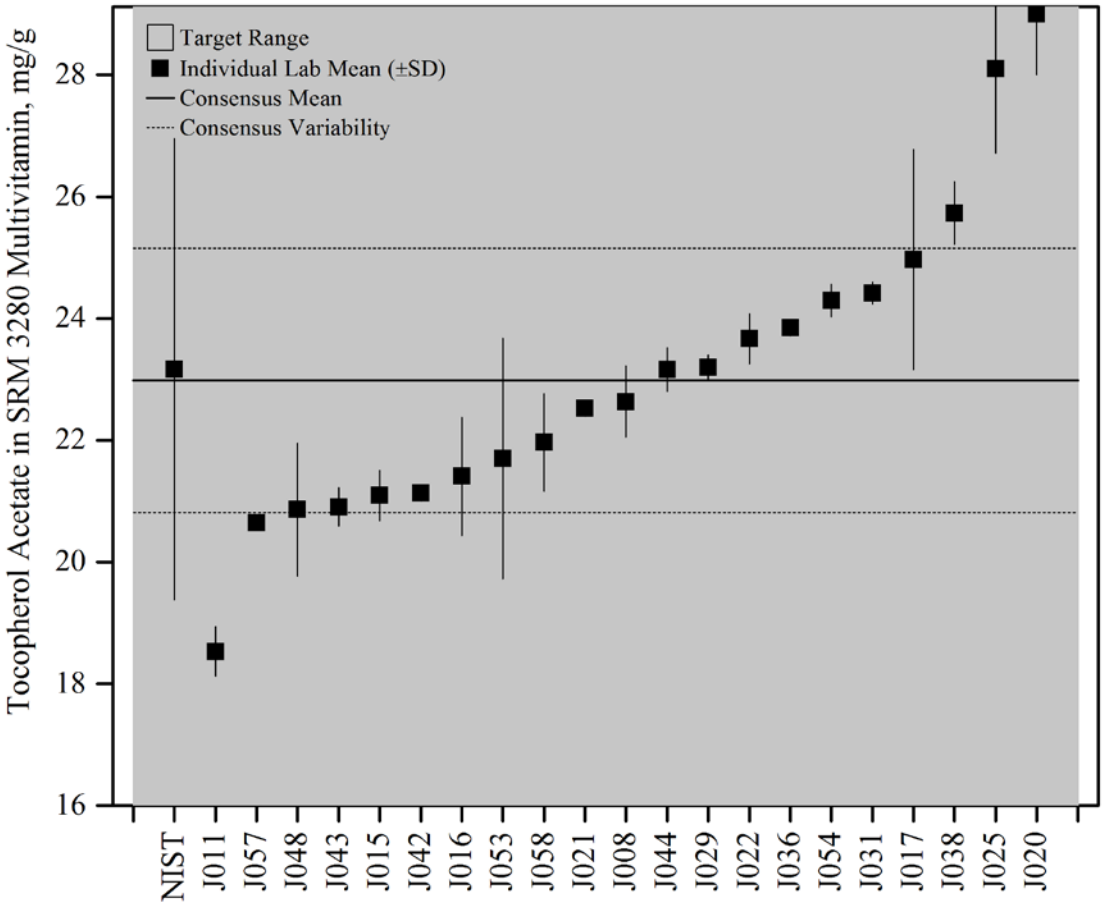

Figure 25. α-Tocopherol acetate in SRM 3280 Multivitamin/Multielement Tablets (data summary view). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The black solid line represents the consensus mean, and the black dotted lines represent 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}).

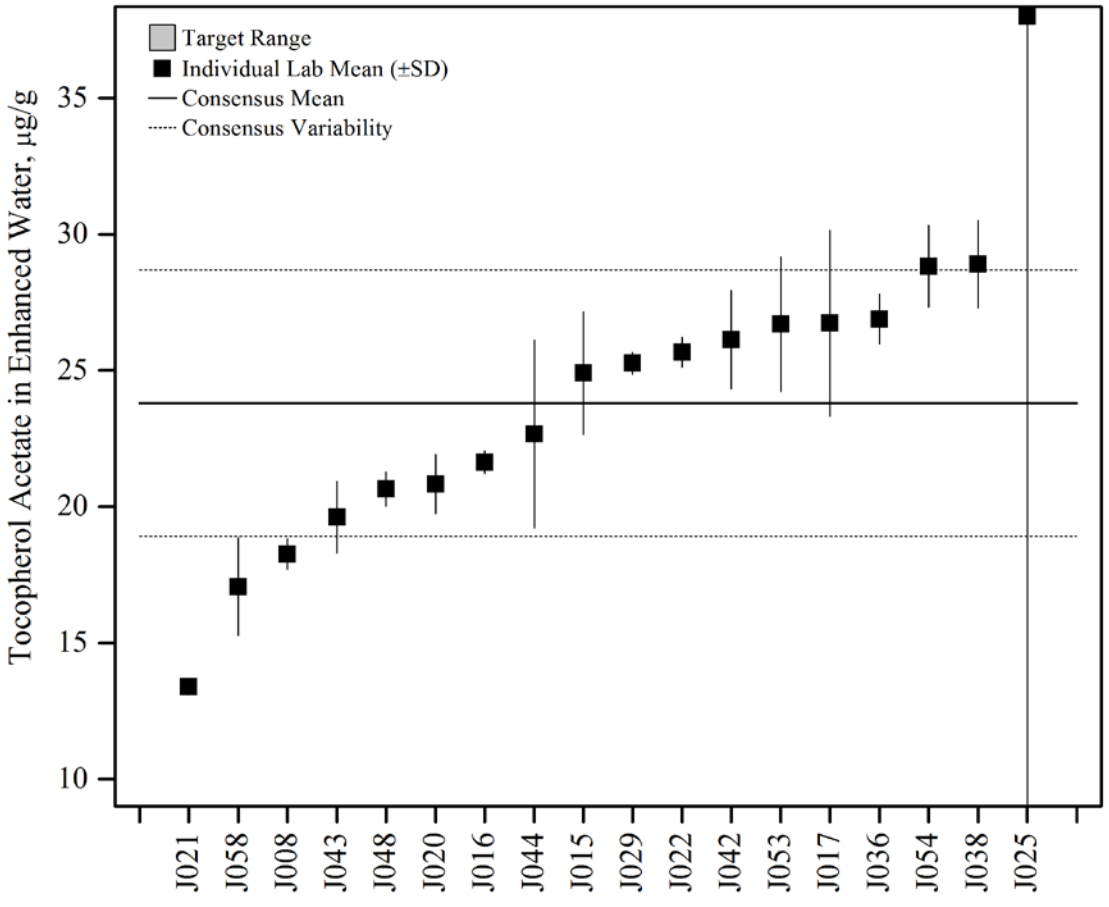

Figure 26. α-Tocopherol acetate in enhanced water (data summary view). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The black solid line represents the consensus mean, and the black dotted lines represent the consensus variability calculated as one standard deviation about the consensus mean.

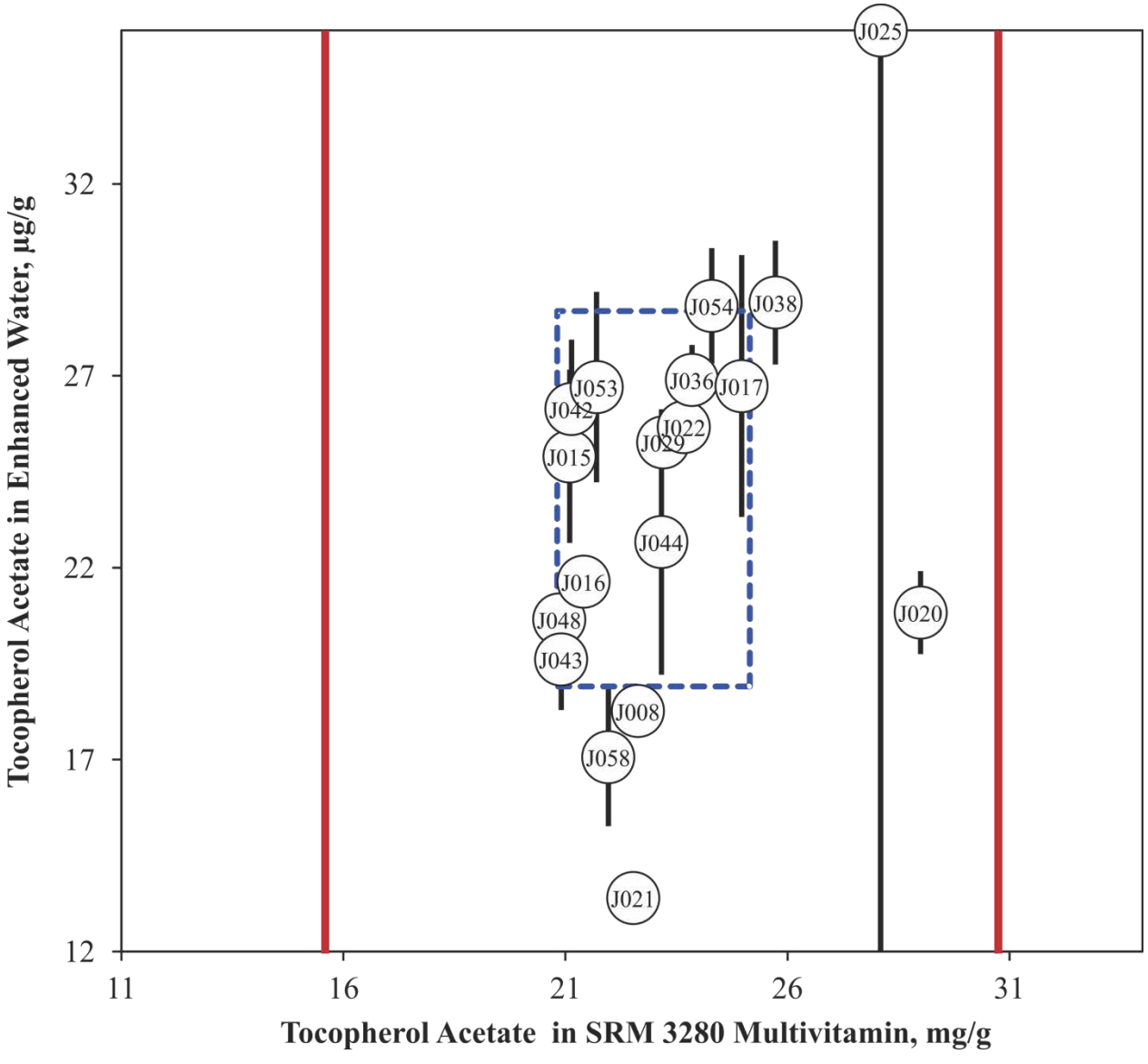

Figure 27. α-Tocopherol acetate in SRM 3280 Multivitamin/Multielement Tablets and enhanced water (sample/control comparison view). In this view, the individual laboratory results for the control (multivitamin/multielement tablets) with a certified value for the analyte are compared to the results for an unknown (enhanced water). The solid red box represents the target zone for the control (x-axis). The dotted blue box represents the consensus zone for the control (x-axis) and the unknown sample (y-axis).

FATTY ACIDS IN BOTANICAL AND FISH OIL DIETARY SUPPLEMENTS

Study Overview

In this study, participants were provided with two NIST SRMs, SRM 3274-2 Evening Primrose (*Oenothera biennis)* Oil and SRM 3275-2 Anchovy Oil. Participants were asked to use in-house analytical methods to determine the mass fractions of fatty acid methyl esters (FAMEs) in each of the matrices and report values on an as-received basis.

Sample Information

Evening Primrose Oil. Participants were provided with three ampoules, each containing 1.2 mL of evening primrose oil. 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 mix each ampoule thoroughly and a sample size of at least 0.5 g was recommended. Participants were asked to prepare one sample and report as many analytes as possible from each ampoule provided, and to store the oil under refrigeration at 0 °C to 4 °C. Approximate analyte levels were not reported to participants prior to the study. The NIST certified and reference values and uncertainties in SRM 3274-2 were determined by by gas chromatography-flame ionization detection (GC-FID) and gas chromatography/mass spectrometry (GC/MS) following multiple methods of hydrolysis and derivatization, and are summarized in the table below.

Fish Oil. Participants were provided with three ampoules, each containing 1.2 mL of anchovy oil. The oil contained mixed natural tocopherols at a minimum of 1 mg/g as an antioxidant and was packaged in amber glass ampoules under argon. Before use, participants were instructed to mix each ampoule thoroughly and a sample size of at least 0.5 g was recommended. Participants were asked to prepare one sample and report as many analytes as possible from each ampoule provided, and to store the oil under refrigeration at 0 °C to 4 °C. Approximate analyte levels were not reported to participants prior to the study. The NIST certified and reference values and uncertainties in SRM 3275-2 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 3274-2 (mg/g as FAME)	Certified Mass Fraction in SRM 3275-2 (mg/g as FAME)
Linoleic Acid	745 ± 24	3.00 ± 0.42
α- Linoleic Acid	1.61 ± 0.12	1.42 ± 0.12
γ- Linoleic Acid	100.4 ± 4.1	0.507 ± 0.043
Arachidonic Acid	0.0221 ± 0.0020	22.9 ± 1.0
EPA		394 ± 17
DHA		187 ± 8

Study Results

- Twenty-one laboratories enrolled in this exercise and received samples. Thirteen laboratories reported data for at least one FAME (62 % participation).
- A majority of the laboratories reported using derivatization/methylation as the sample preparation method (92 %). One laboratory also reported using hydrolysis as the sample preparation method.

- A majority of the laboratories reported using GC-FID (85 %) as their method for analysis. One laboratory reported using GC-MS and one laboratory reported using LC/absorbance.
- The consensus means for linoleic acid (LA) in both oils, α–linolenic acid (ALA) in the botanical oil, and EPA and DHA in the fish oil were within the target ranges with acceptable variabilities (5 % to 21 % RSD).
- The consensus mean for γ-linolenic acid (GLA) in the botanical oil was below the target range with low variability (5 % RSD).
- The consensus means for ALA, GLA, and AA in the fish oil were above the target range. The variabilities for ALA and GLA were high (31 % and 69 % RSD, respectively), while the variability for AA was acceptable (16 % RSD).

Technical Recommendations

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

- With a small number of laboratories reporting data for these fatty acids, and a majority reporting use of the same or very similar methods, drawing extensive technical conclusions is difficult.
- Participants were asked to report concentrations for fatty acids as fatty acid methyl esters (FAMEs). In this case, FAMEs should be used as calibrants or non-esterified fatty acids should be carried through the entire sample preparation procedure (hydrolysis and derivatization) to improve quantitation.
- Knowledge of calibrant response when carried through the derivatization procedure is necessary. For example, at NIST, calibrants for EPA and DPA give response factors of 1.3 and 1.6, respectively, corresponding to 30 % or 60 % low bias in the quantitation of these compounds if not considered.
- Similarly, for those laboratories using GC-MS, quantitation for some compounds may be inaccurate as a result of non-unity response factors from EI fragmentation.

Table 12. Individual data summary table (NIST) for fatty acids in botanical and fish oil dietary supplements.

National Institute of Standards & Technology

Exercise J - May 2013 - 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	Primrose	mg/g	745	24	1.0	0.0	10	710	34	745	24
Linoleic Acid	Fish	mg/g	3.00	0.42	-0.2	0.0	7	3.13	0.57	3.00	0.42
α-Linolenic Acid	Primrose	mg/g	1.61	0.12	0.2	0.0	8	1.54	0.33	1.61	0.12
α-Linolenic Acid	Fish	mg/g	1.42	0.12	-0.8	0.0	7	1.89	0.59	1.42	0.12
γ-Linolenic Acid	Primrose	mg/g	100	4	1.9	0.0	11	91	5	100	4
γ-Linolenic Acid	Fish	mg/g	0.507	0.043	-0.9	0.0	5	1.350	0.930	0.507	0.043
Arachidonic Acid	Primrose	mg/g	0.0221	0.0020		0.0	1			0.0221	0.0020
Arachidonic Acid	Fish	mg/g	22.9	1.0	-0.6	0.0	9	25.1	4.0	22.9	1.0
EPA	Primrose	mg/g					1				
EPA	Fish	mg/g	394	17	0.2	0.0	13	385	49	394	17
DHA	Primrose	mg/g					0				
DHA	Fish	mg/g	187	8	-0.1	0.0	13	188	16	187	8

x_i Mean of reported values

s_i Standard deviation of reported values

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

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

N Number of quantitative values reported

x* Robust mean of reported values

s* Robust standard deviation

x_{NIST} NIST-assessed value

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

Table 13. Data summary table for linoleic acid in botanical and fish oil dietary supplements.

		Linoleic Acid									
		SRM 3274-2 Evening Primrose Oil (mg/g)					SRM 3275-2 Fish Oil (mg/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	NIST				745	24				3.00	0.42
	J002										
	J004										
	J007										
	J009										
	J011	668	663	657	663	6					
	J016										
	J019	755	756	756	755	1	3.59	3.18	3.04	3.27	0.29
	J020										
	J023										
	J025										
	J027	739	738	739	739	1	3.28	3.30	3.28	3.29	0.01
	J029	717	718	718	718	1	3.05	3.03	3.07	3.05	0.02
	J031	714	714	719	716	3					
	J036	714	696	710	707	9	2.35	2.28	2.25	2.29	0.05
	J038										
	J040	706	706	706	706	0	5.00	5.00	5.00	5.00	0.00
	J043	726	709	714	716	9					
	J053	632	630	635	632	3	3.06	2.95	2.89	2.97	0.09
	J054										
	J057	719	720	719	719	0	3.06	3.07	3.08	3.07	0.01
	J059										
Community Results		Consensus Mean			710		Consensus Mean			3.13	
		Consensus Standard Deviation			34		Consensus Standard Deviation			0.57	
		Maximum			755		Maximum			5.00	
		Minimum			632		Minimum			2.29	
		N			10		N			7	

Table 14. Data summary table for α-linolenic acid in botanical and fish oil dietary supplements.

		α-Linolenic Acid									
		SRM 3274-2 Evening Primrose Oil (mg/g)					SRM 3275-2 Fish Oil (mg/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	NIST				1.61	0.12				1.42	0.12
	J002										
	J004										
	J007										
	J009										
	J011										
	J016										
	J019	1.25	1.23	1.30	1.26	0.04	1.69	1.38	1.46	1.51	0.16
	J020										
	J023										
	J025										
	J027	1.69	1.68	1.70	1.69	0.01	1.87	1.82	1.81	1.83	0.04
	J029	1.59	1.56	1.62	1.59	0.03	1.42	1.45	1.43	1.43	0.02
	J031										
	J036	1.60	1.54	1.59	1.58	0.03	2.57	2.53	2.50	2.53	0.04
	J038										
	J040	2.00	2.00	2.00	2.00	0.00	2.00	2.00	2.00	2.00	0.00
	J043	1.14	1.21	1.01	1.12	0.10					
	J053	1.36	1.32	1.39	1.36	0.04	1.30	1.33	1.30	1.31	0.02
	J054										
	J057	1.76	1.76	1.75	1.76	0.01	2.59	2.61	2.62	2.61	0.02
	J059										
Community Results		Consensus Mean			1.54		Consensus Mean			1.89	
		Consensus Standard Deviation			0.33		Consensus Standard Deviation			0.59	
		Maximum			2.00		Maximum			2.61	
		Minimum			1.12		Minimum			1.31	
		N			8		N			7	

Table 15. Data summary table for γ-linolenic acid in botanical and fish oil dietary supplements.

		γ-Linolenic Acid									
		SRM 3274-2 Evening Primrose Oil (mg/g)					SRM 3275-2 Fish Oil (mg/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	NIST				100.4	4.1				0.507	0.043
	J002										
	J004										
	J007										
	J009										
	J011	73.9	74.0	73.4	73.8	0.3					
	J016										
	J019	89.7	90.3	90.7	90.2	0.5					
	J020	89.0	89.0	89.0	89.0	0.0					
	J023										
	J025										
	J027	97.4	97.1	97.2	97.2	0.1	0.429	0.442	0.450	0.440	0.011
	J029	93.0	93.0	93.0	93.0	0.0					
	J031	92.5	92.6	93.2	92.8	0.4					
	J036	92.5	90.2	91.9	91.5	1.2	0.899	0.905	0.881	0.895	0.012
	J038										
	J040	91.0	91.0	91.0	91.0	0.0	1.000	1.000	1.000	1.000	0.000
	J043	102.5	90.8	93.2	95.5	6.2					
	J053	80.4	79.3	80.0	79.9	0.6	2.126	2.000	2.090	2.072	0.065
	J054										
	J057	94.4	94.4	94.4	94.4	0.0	2.350	2.360	2.360	2.357	0.006
	J059										
Community Results		Consensus Mean			91.1		Consensus Mean			1.353	
		Consensus Standard Deviation			4.9		Consensus Standard Deviation			0.930	
		Maximum			97.2		Maximum			2.357	
		Minimum			73.8		Minimum			0.440	
		N			11		N			5	

Table 16. Data summary table for arachidonic acid in botanical and fish oil dietary supplements.

		Arachidonic Acid									
		SRM 3274-2 Evening Primrose Oil (mg/g)					SRM 3275-2 Fish Oil (mg/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	NIST				0.0221	0.0020				22.9	1.0
	J002										
	J004										
	J007										
	J009										
	J011										
	J016										
	J019						24.8	24.4	23.3	24.1	0.8
	J020										
	J023										
	J025										
	J027						26.9	26.6	26.6	26.7	0.2
	J029						24.9	25.0	25.4	25.1	0.3
	J031						24.4	24.2	24.1	24.2	0.2
	J036										
	J038										
	J040						29.0	29.0	29.0	29.0	0.0
	J043						20.6	16.1	21.4	19.4	2.9
	J053						21.8	22.0	22.2	22.0	0.2
	J054	105.0000	98.0000	119.0000	107.3333	10.6927	38.0	44.0	31.0	37.7	6.5
	J057						24.1	24.1	24.1	24.1	0.0
	J059										
Community Results		Consensus Mean					Consensus Mean			25.1	
		Consensus Standard Deviation					Consensus Standard Deviation			4.0	
		Maximum					Maximum			37.7	
		Minimum					Minimum			19.4	
		N			1		N			9	

Table 17. Data summary table for EPA in botanical and fish oil dietary supplements.

		EPA									
		SRM 3274-2 Evening Primrose Oil (mg/g)					SRM 3275-2 Fish Oil (mg/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	NIST									394	17
	J002										
	J004										
	J007										
	J009										
	J011						354	359	362	358	4
	J016										
	J019	1.70	1.12	1.06	1.29	0.35	71	72	74	72	2
	J020						368	370	375	371	4
	J023						443	445	453	447	5
	J025										
	J027						412	408	408	409	3
	J029						384	386	385	385	1
	J031						386	385	384	385	1
	J036						387	385	380	384	4
	J038										
	J040						467	457	459	461	5
	J043						374	364	389	375	12
	J053						403	412	410	408	5
	J054						174	209	205	196	19
	J057						400	400	400	400	0
	J059										
Community Results		Consensus Mean					Consensus Mean			385	
		Consensus Standard Deviation					Consensus Standard Deviation			49	
		Maximum					Maximum			461	
		Minimum					Minimum			72	
		N			1		N			13	

Table 18. Data summary table for DHA in botanical and fish oil dietary supplements.

		DHA									
		SRM 3274-2 Evening Primrose Oil (mg/g)					SRM 3275-2 Fish Oil (mg/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	NIST									187	8
	J002										
	J004										
	J007										
	J009										
	J011						151	152	153	152	1
	J016										
	J019						189	204	208	200	10
	J020						175	176	178	176	2
	J023						208	209	213	210	3
	J025										
	J027						195	193	193	193	1
	J029						182	181	182	182	1
	J031						187	186	186	186	1
	J036						195	194	187	192	4
	J038										
	J040						212	208	209	210	2
	J043						199	181	187	189	9
	J053						164	165	165	165	1
	J054						175	206	202	194	17
	J057						185	186	185	185	0
	J059										
Community Results		Consensus Mean					Consensus Mean			188	
		Consensus Standard Deviation					Consensus Standard Deviation			16	
		Maximum					Maximum			210	
		Minimum					Minimum			152	
		N			0		N			13	

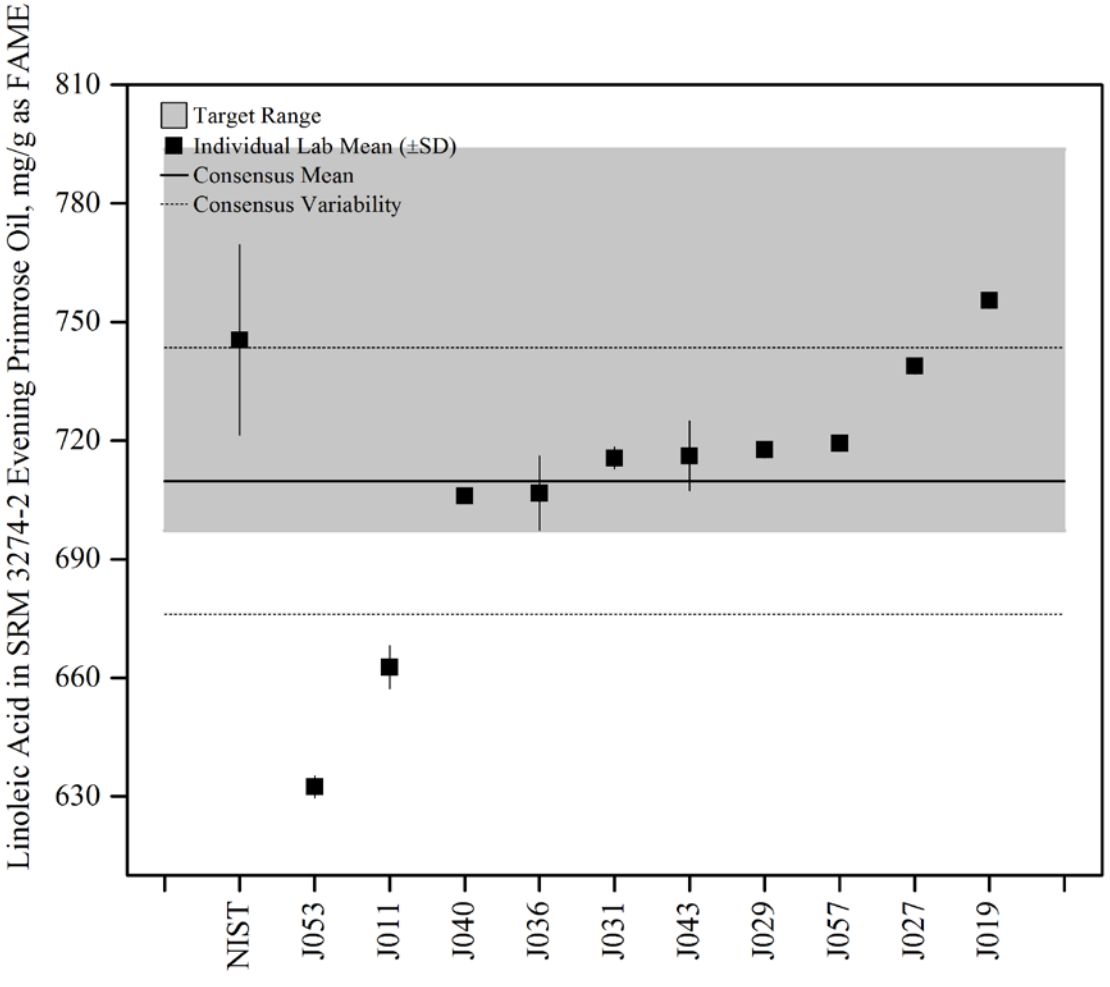

Figure 28. Linoleic acid in SRM 3274-2 Evening Primrose Oil (data summary view). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The black solid line represents the consensus mean, and the black dotted lines represent the consensus 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}).

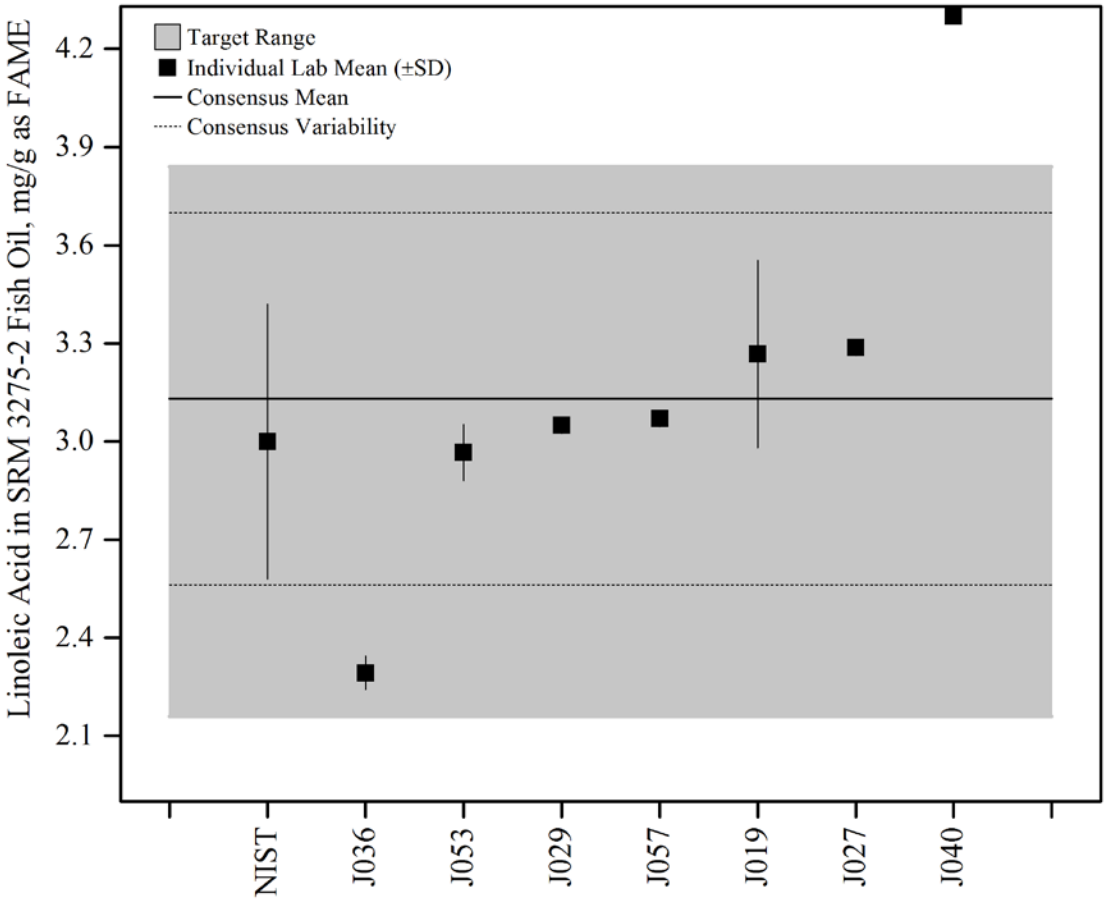

Figure 29. Linoleic acid in SRM 3275-2 Fish Oil (data summary view). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The black solid line represents the consensus mean, and the black dotted lines represent the consensus variability 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}).

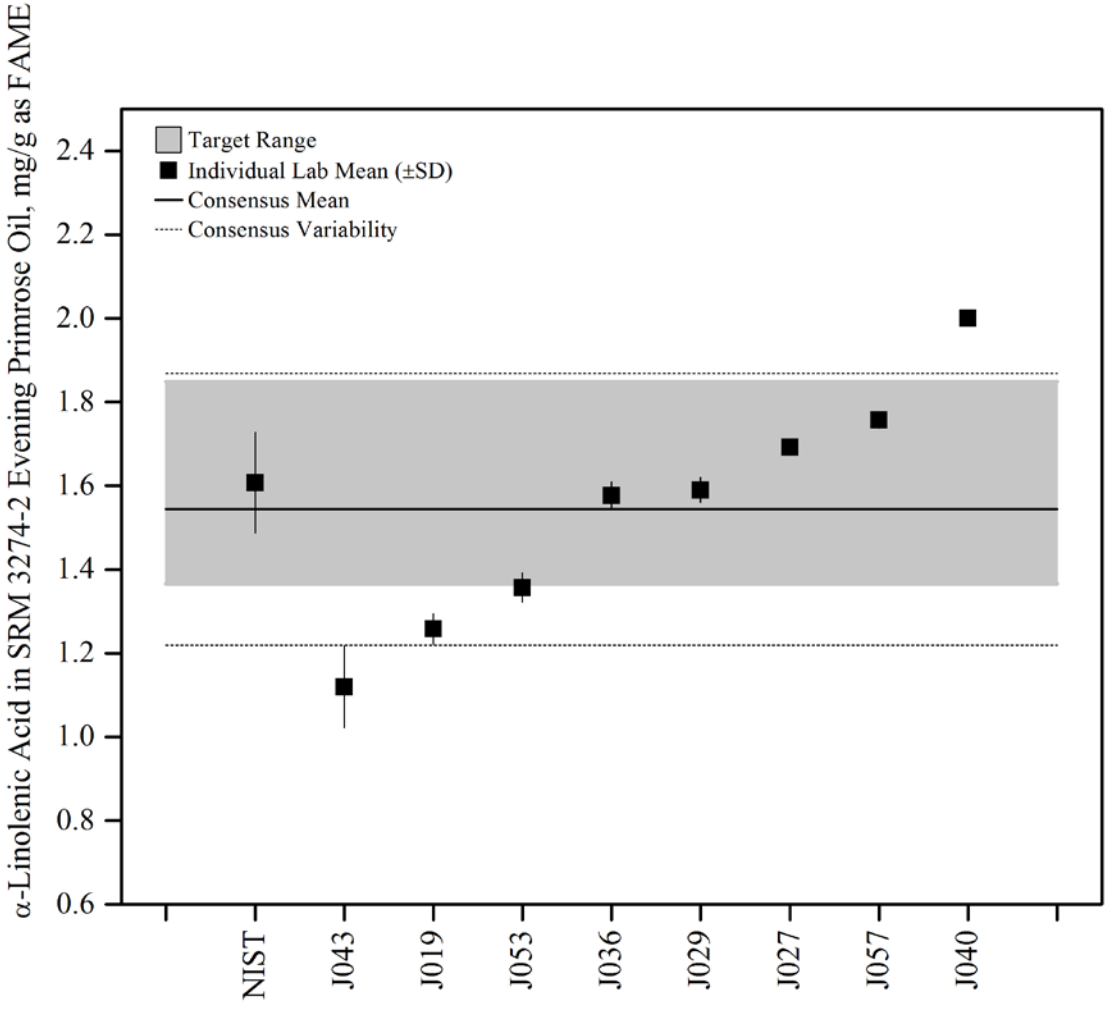

Figure 30. α-Linolenic acid in SRM 3274-2 Evening Primrose Oil (data summary view). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The black solid line represents the consensus mean, and the black dotted lines represent the consensus 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}).

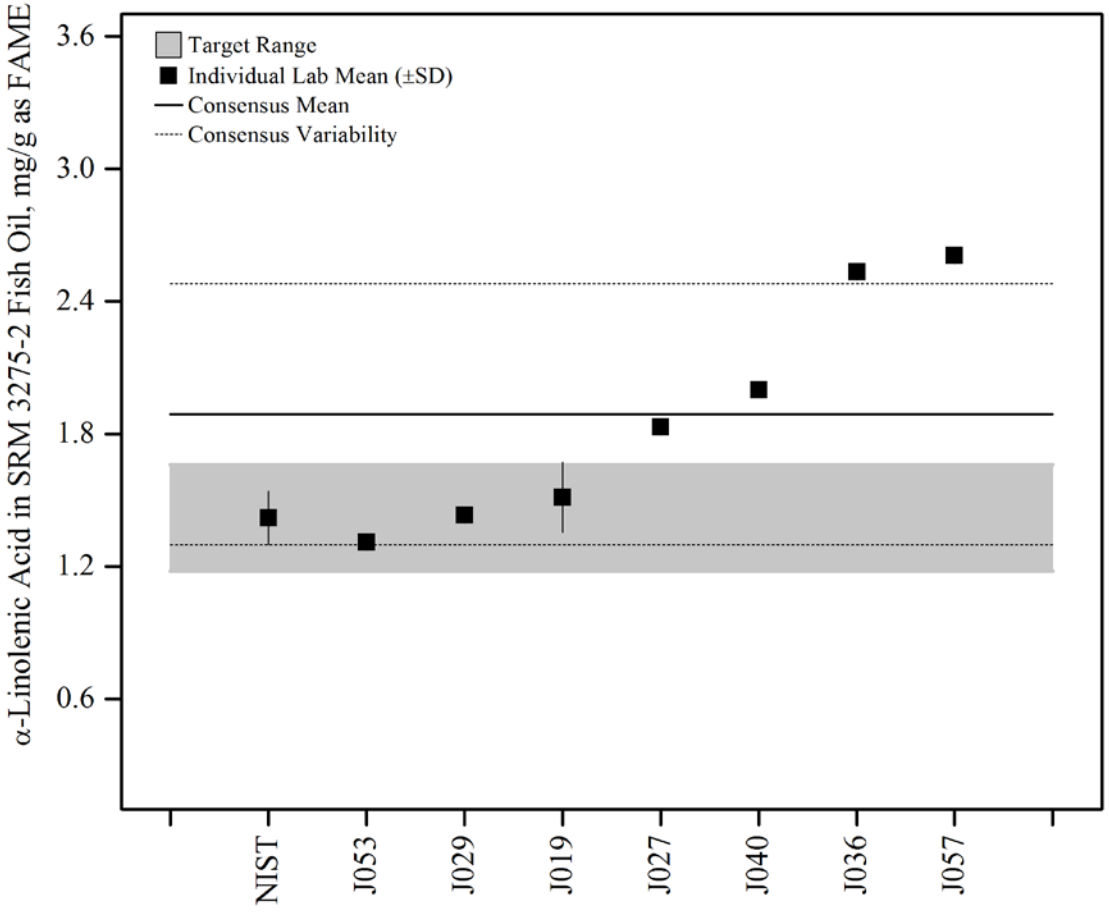

Figure 31. α-Linolenic acid in SRM 3275-2 Fish Oil (data summary view). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The black solid line represents the consensus mean, and the black dotted lines represent the consensus variability 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}).

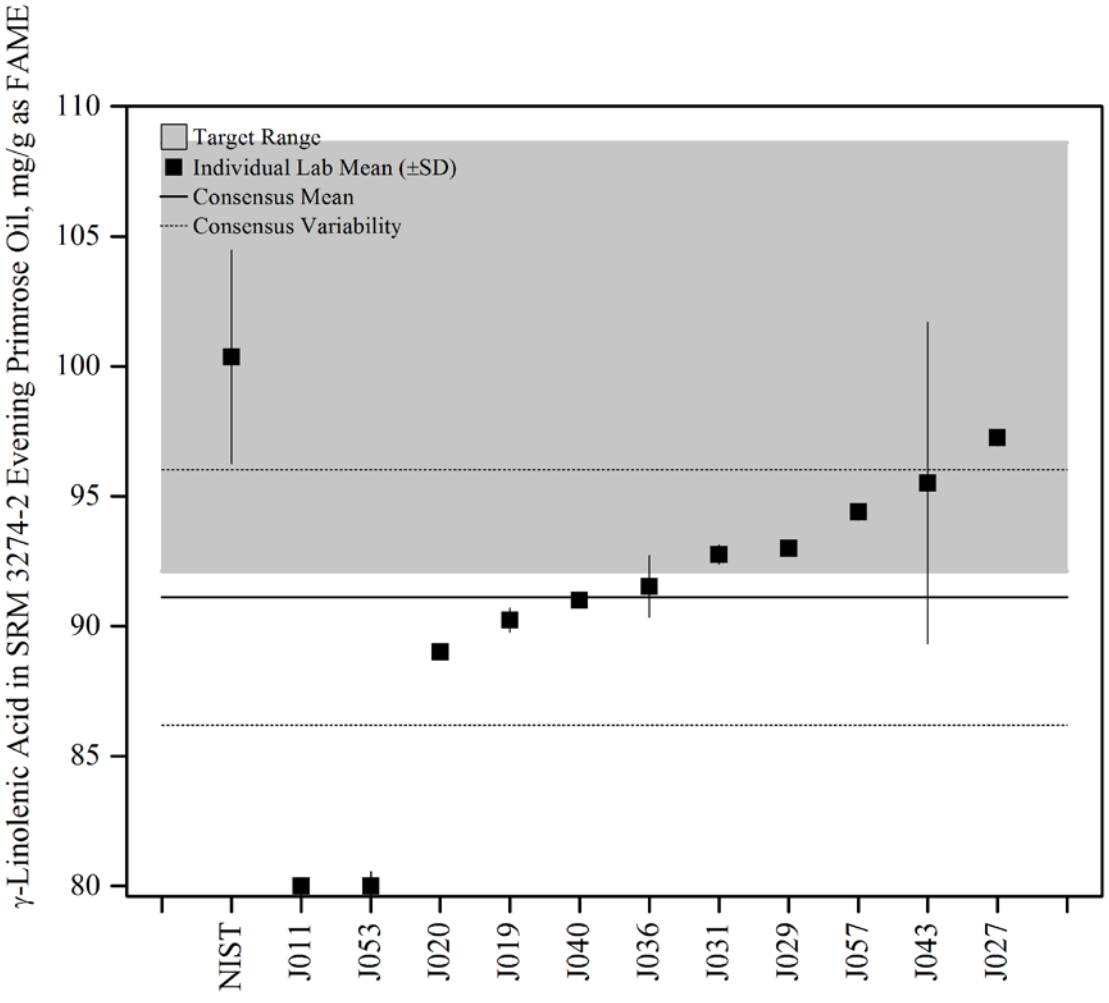

Figure 32. γ-Linolenic acid in SRM 3274-2 Evening Primrose Oil (data summary view). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The black solid line represents the consensus mean, and the black dotted lines represent the consensus 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}).

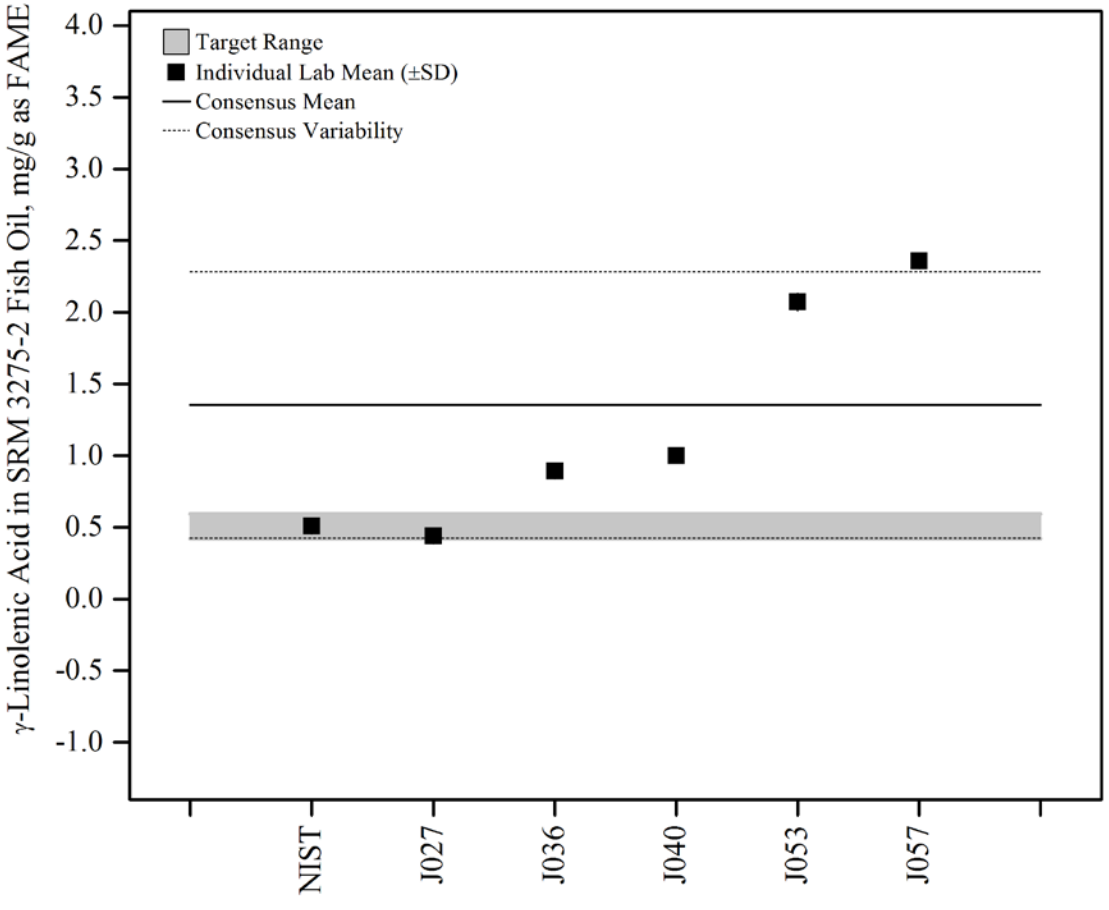

Figure 33. γ-Linolenic acid in SRM 3275-2 Fish Oil (data summary view). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The black solid line represents the consensus mean, and the black dotted lines represent the consensus variability 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}).

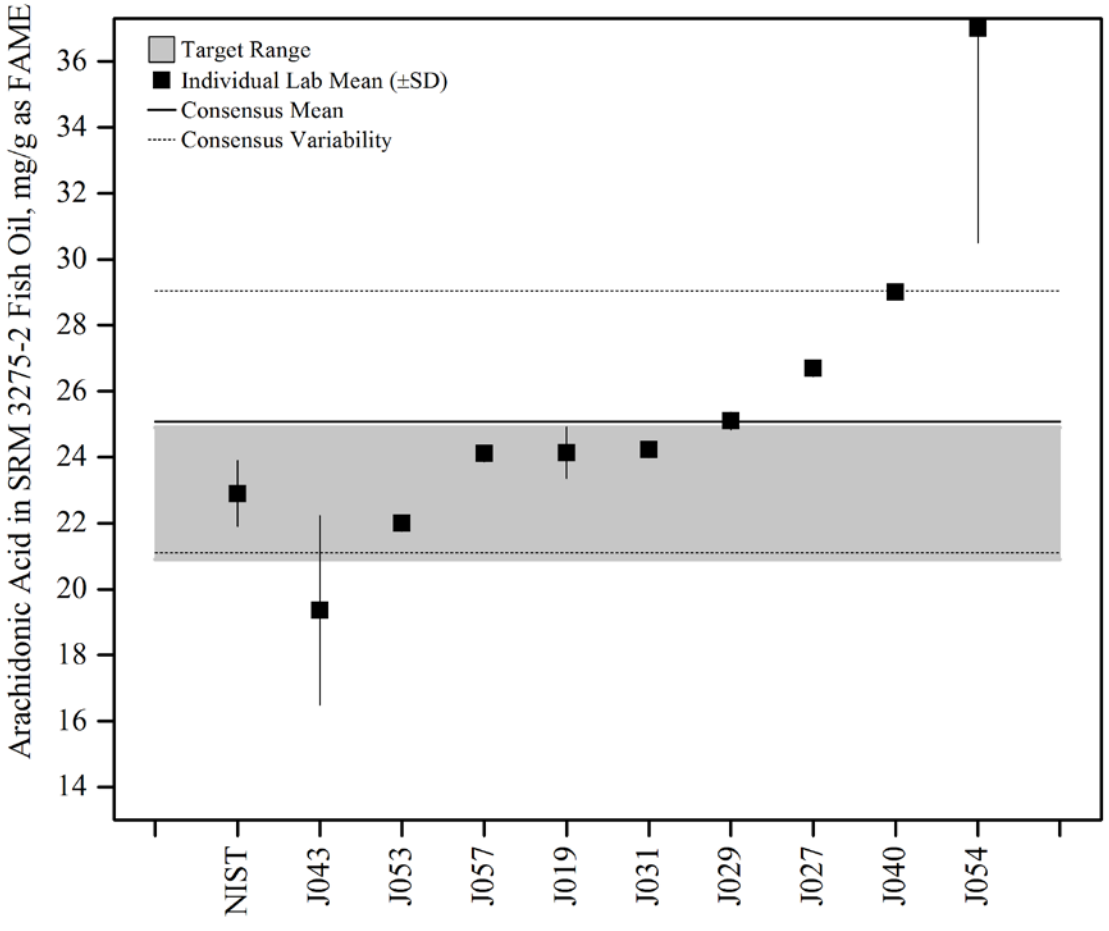

Figure 34. Arachidonic acid in SRM 3275-2 Fish Oil (data summary view). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The black solid line represents the consensus mean, and the black dotted lines represent the consensus variability 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}).

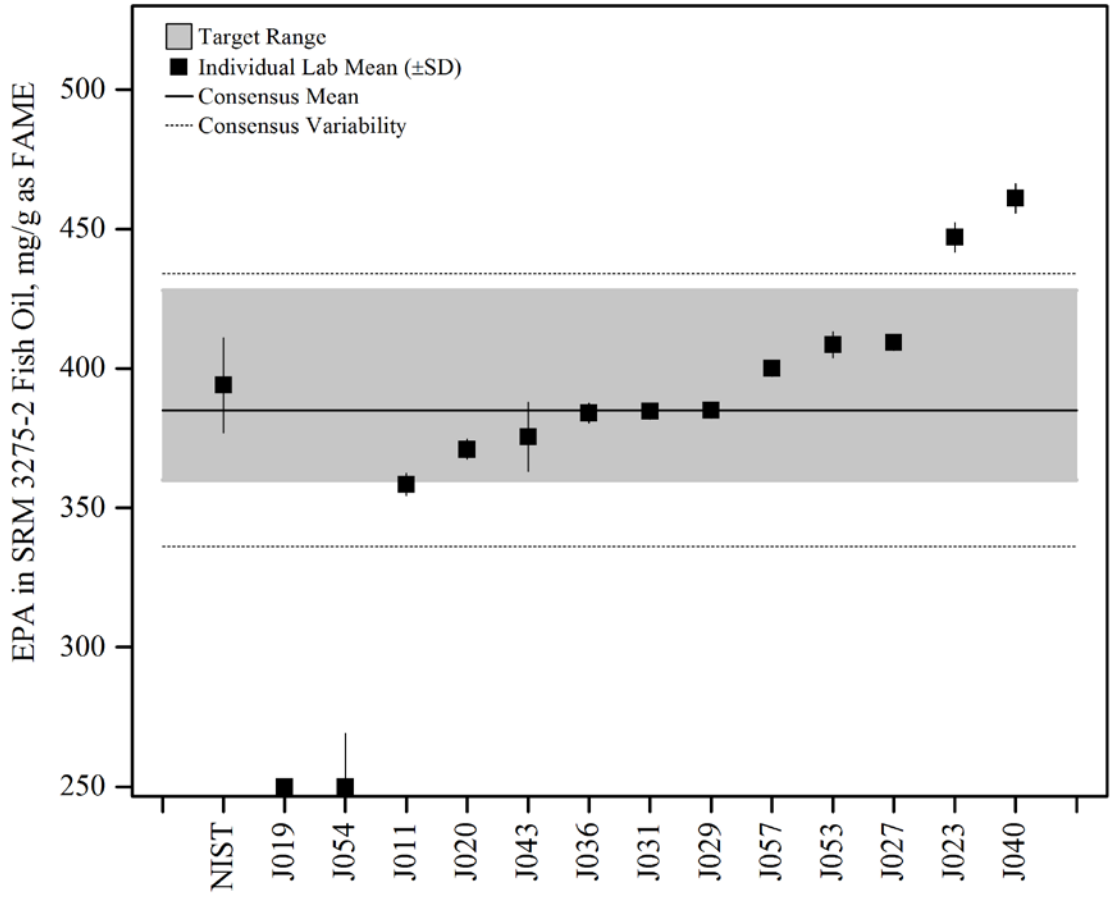

Figure 35. EPA in SRM 3275-2 Fish Oil (data summary view). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The black solid line represents the consensus mean, and the black dotted lines represent the consensus variability calculated 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}).

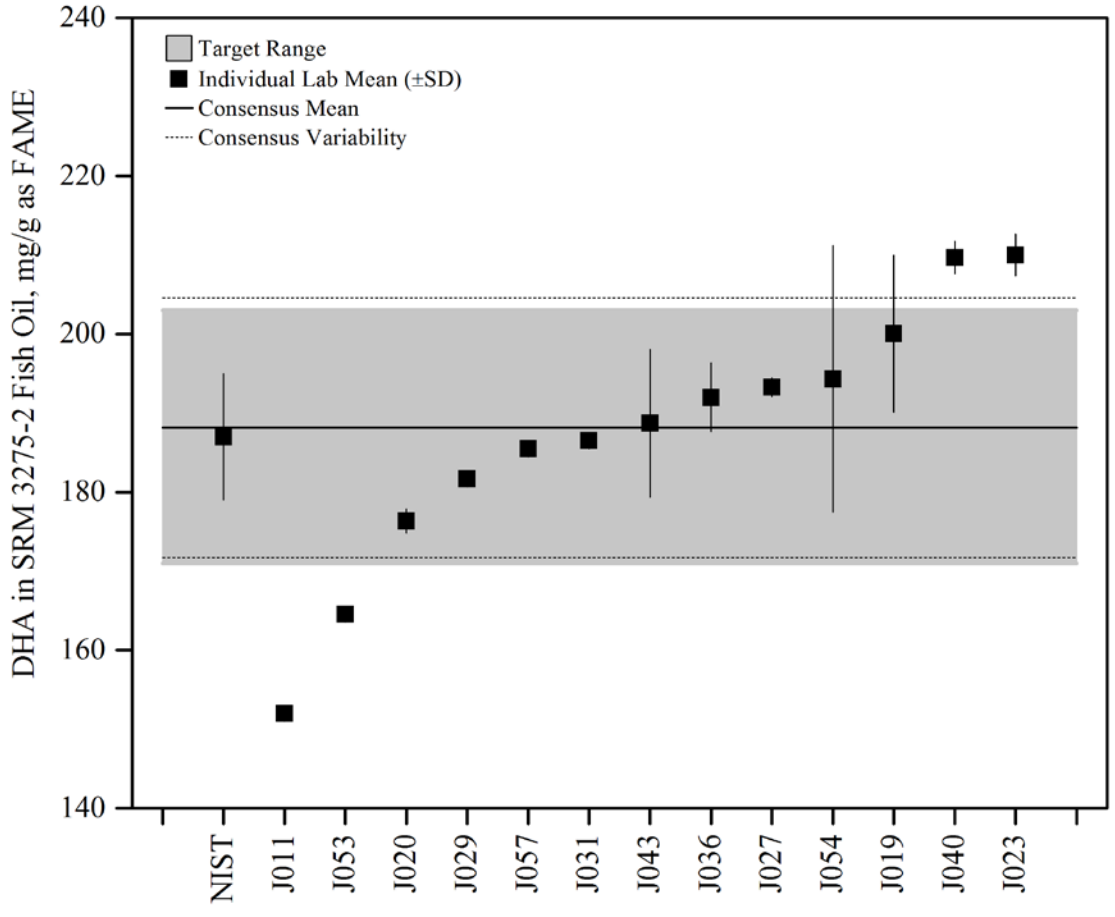

Figure 36. DHA in SRM 3275-2 Fish Oil (data summary view). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The black solid line represents the consensus mean, and the black dotted lines represent the consensus variability calculated 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}).

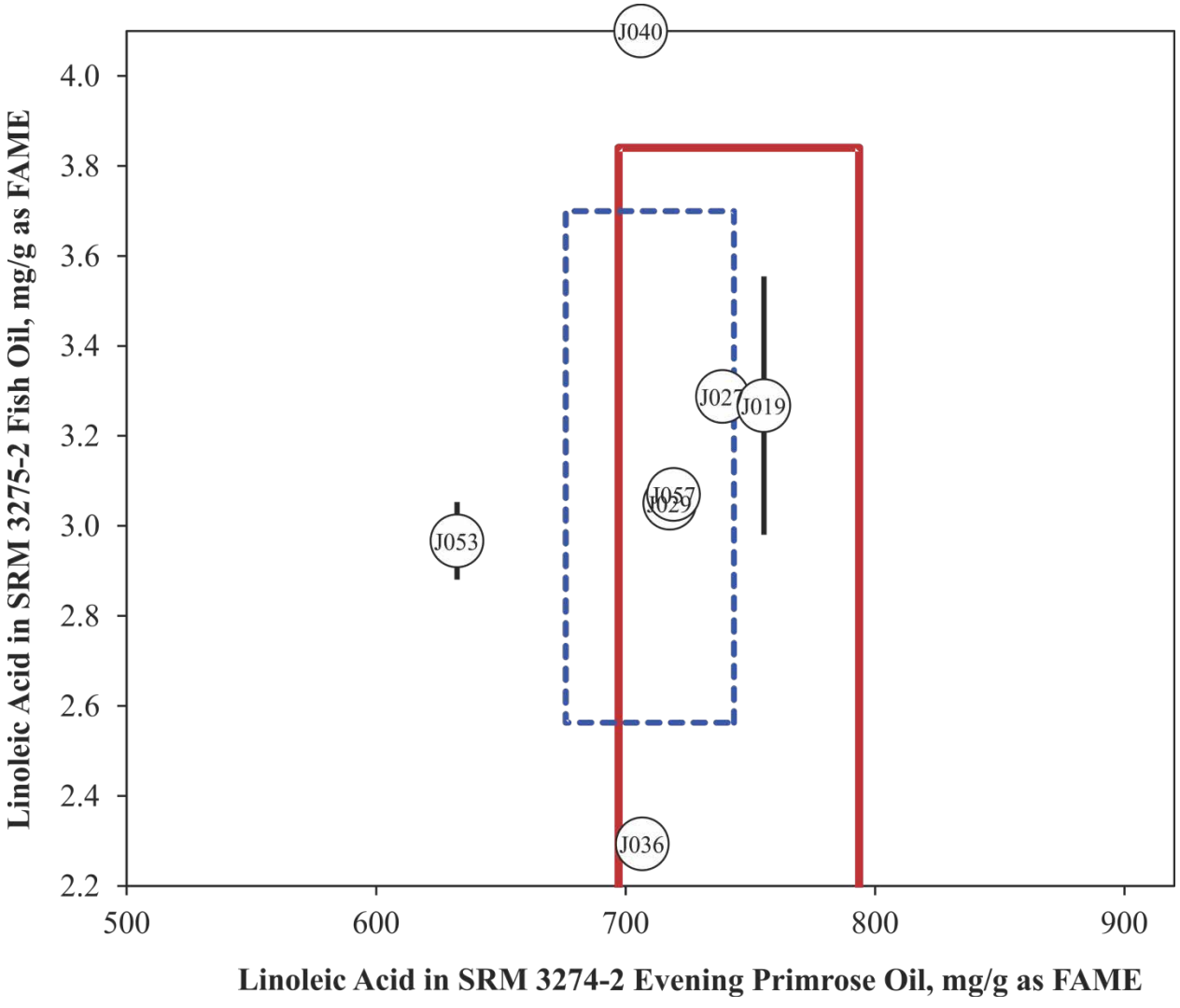

Figure 37. Linoleic acid in SRM 3274-2 Evening Primrose Oil and SRM 3275-2 Fish Oil-2 (sample/control comparison view). In this view, the individual laboratory results for the control (botanical oil) with a certified value for the analyte are compared to the results for an unknown (fish oil). The solid red box represents the target zone for the control (x-axis) and unknown sample (y-axis). The dotted blue box represents the consensus zone for the control (x-axis) and the unknown sample (y-axis).

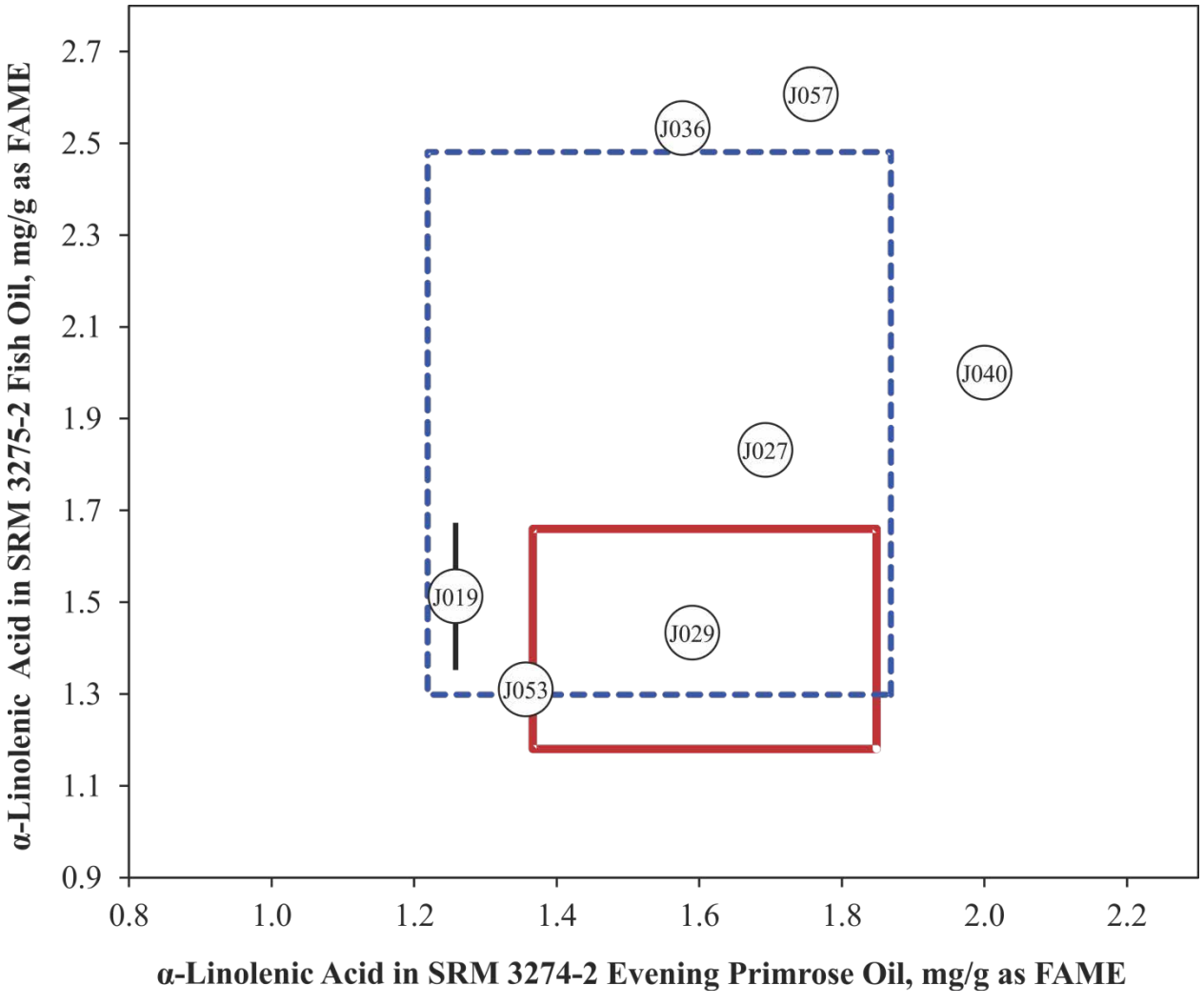

Figure 38. α-Linolenic acid in SRM 3274-2 Evening Primrose Oil and SRM 3275-2 Fish Oil-2 (sample/control comparison view). In this view, the individual laboratory results for the control (botanical oil) with a certified value for the analyte are compared to the results for an unknown (fish oil). The solid red box represents the target zone for the control (x-axis) and unknown sample (y-axis). The dotted blue box represents the consensus zone for the control (x-axis) and the unknown sample (y-axis).

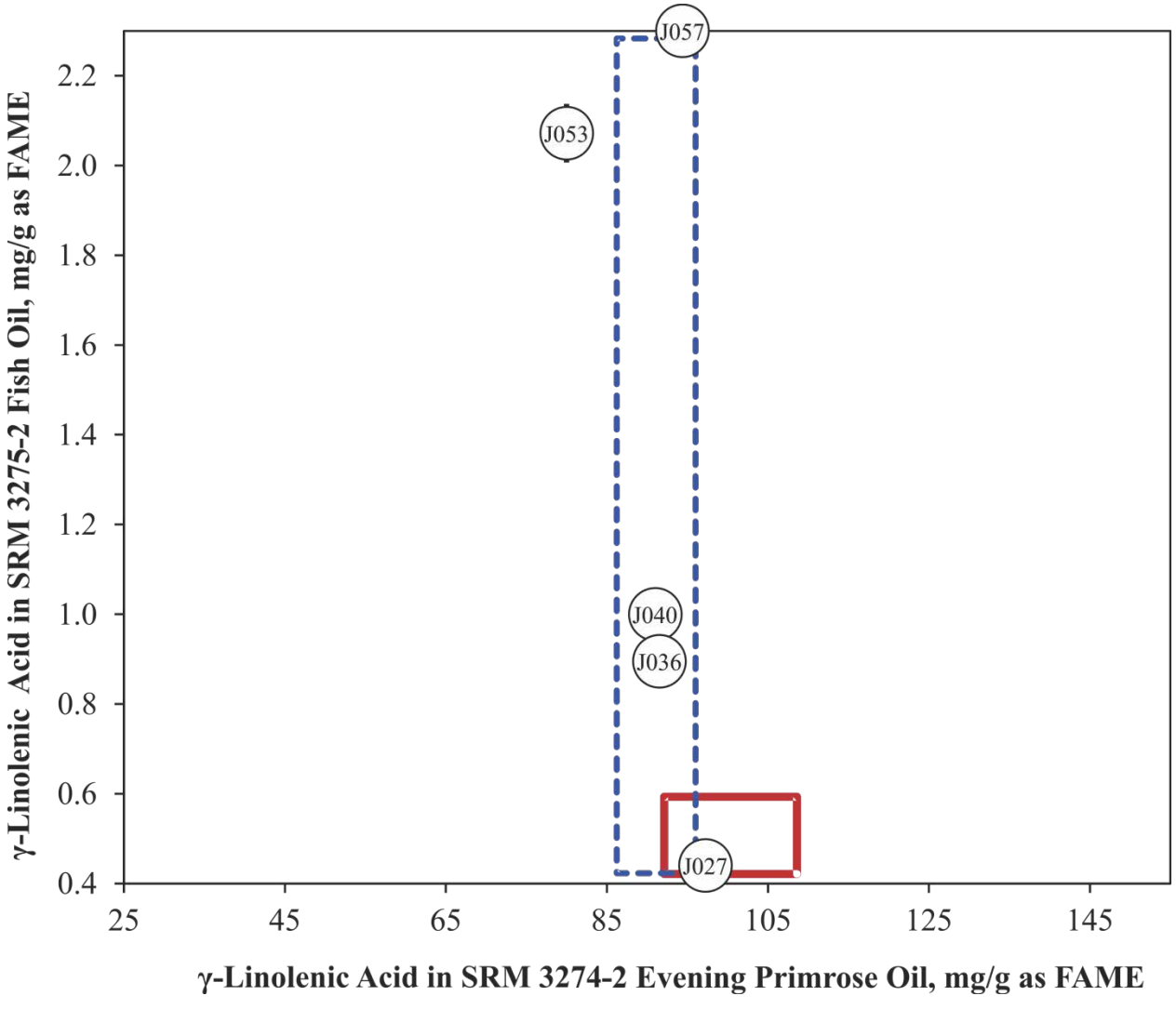

Figure 39. γ-Linolenic acid in SRM 3274-2 Evening Primrose Oil and SRM 3275-2 Fish Oil-2 (sample/control comparison view). In this view, the individual laboratory results for the control (botanical oil) with a certified value for the analyte are compared to the results for an unknown (fish oil). The 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).

AFLATOXINS IN PEANUT BUTTER

Study Overview

In this study, participants were provided with one NIST SRM, SRM 2387 Peanut Butter, and three peanut butter samples with low, medium, and high levels of aflatoxins. Participants were asked to use in-house analytical methods to determine the mass fractions of aflatoxins in each of the matrices and report values on an as-received basis. Participants were asked to report as many analytes as possible.

Sample Information

Peanut Butter. Participants were provided with one jar containing 170 g of peanut butter. Before use, participants were instructed to thoroughly mix the contents of the jar and a sample size of at least 50 g was recommended. Participants were asked to store the material under refrigeration, 0 °C to 4 °C, and prepare three samples and report three values for as many analytes as possible from the single jar provided. Approximate analyte levels were not reported to participants prior to the study. NIST reference values and uncertainties, determined by collaborating laboratories, are outlined in the table below.

Analyte	Reference Mass Fraction in SRM 2387 (ng/g)
Aflatoxin B1	4.2 ± 0.9
Aflatoxin B2	0.7 ± 0.3
Total Aflatoxins	5.0 ± 0.5

Peanut Butter Samples. Participants were provided with three 50 g jars of peanut butter labeled 1, 2, and 3. Before use, participants were instructed to thoroughly mix the contents of each jar, and a sample size of at least 50 g was recommended. Participants were asked to store the material under refrigeration, 0 °C to 4 °C, and prepare one sample and report one value for as many analytes as possible from each jar provided. Approximate analyte levels were not reported to participants prior to the study. NIST target values and uncertainties, determined by collaborating laboratories, are outlined in the table below.

Analyte	Estimated Mass Fraction in Peanut Butter 1 (Low) (ng/g)	Estimated Mass Fraction in Peanut Butter 2 (Med) (ng/g)	Estimated Mass Fraction in Peanut Butter 3 (High) (ng/g)
Aflatoxin B1	3.70 ± 0.63	7.17 ± 2.85	10.95 ± 2.22
Aflatoxin B2	1.01 ± 0.09	1.46 ± 1.08	2.16 ± 0.49
Aflatoxin G1		0.68 ± 0.29	1.81 ± 0.49
Aflatoxin G2		0.44 ± 0.62	0.43 ± 0.25
Total Aflatoxins	5.23 ± 2.22	9.95 ± 1.43	14.77 ± 2.85

Study Results

- Ten laboratories enrolled in this exercise and received samples. Five laboratories reported results for at least one aflatoxin per sample (50 % participation).
- The consensus means for aflatoxins B1 and B2 and total aflatoxins were within the target range with high variability (26 % to 36 % relative standard deviation (RSD)).

- In the low-level sample, consensus means for aflatoxin B1 and total aflatoxins were within the target range, while the consensus mean for aflatoxin B2 was slightly below the target range. All three analytes were measured with high variability (27 % to 38 % RSD).
- In the mid-level sample, consensus means for all analytes were within the target ranges, but with high variability (14 % to 31 % RSD).
- In the high-level sample, consensus means for all analytes were above the target ranges with high variability (33 % to 49 % RSD).
- Four of five laboratories reported using extraction for sample preparation (80 %). One laboratory reported using slurry blending sample preparation (20 %).
- Two laboratories reported using LC-fluorescence as their analytical method for analysis (40 %). Three laboratories reported using LC/MS/MS to measure aflatoxins (60 %).

Technical Recommendations

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

- Because the data for this study was very limited (only 4 or 5 laboratories reporting data), drawing extensive technical conclusions is difficult. Similarly, the high level of between-laboratory variability may be exaggerated as a result of the low number of participants.
- No trends were identified indicating that a particular sample preparation method or instrumental technique provided more accurate results than another.
- Always check that the levels of the analytes in the samples are within the calibration range. The high-biased results for the high-level sample may result from extrapolation of the calibration curves beyond their linear ranges.

Table 19. Individual data summary table (NIST) for aflatoxins in peanut butter.

National Institute of Standards & Technology

Exercise J - May 2013 - Aflatoxins

	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}
Aflatoxin B1	PB Control	ng/g	4.2	0.9	-0.1	0.0	4	4.4	1.6	4.2	0.9
Aflatoxin B1	PB Sample 1	ng/g	3.70	0.63	0.4	0.0	4	3.36	0.96	3.70	0.63
Aflatoxin B1	PB Sample 2	ng/g	7.17	3	-0.7	0.0	4	8.62	2.10	7.17	2.85
Aflatoxin B1	PB Sample 3	ng/g	11.00	2.22	-0.6	0.0	4	13.9	4.5	11.0	2.2
Aflatoxin B2	PB Control	ng/g	0.7	0.3	-0.1	0.0	4	0.7	0.2	0.7	0.3
Aflatoxin B2	PB Sample 1	ng/g	1.01	0.09	0.4	0.0	4	0.88	0.33	1.01	0.09
Aflatoxin B2	PB Sample 2	ng/g	1.46	1.08	-0.4	0.0	4	1.69	0.53	1.46	1.08
Aflatoxin B2	PB Sample 3	ng/g	2.16	0.49	-0.8	0.0	4	3.59	1.77	2.16	0.49
Aflatoxin G1	PB Control	ng/g					1				
Aflatoxin G1	PB Sample 1	ng/g					0				
Aflatoxin G1	PB Sample 2	ng/g	0.68	0.29	-0.8	0.0	3	0.77	0.11	0.68	0.29
Aflatoxin G1	PB Sample 3	ng/g	1.81	0.49	-0.7	0.0	3	2.29	0.66	1.81	0.49
Aflatoxin G2	PB Control	ng/g					0				
Aflatoxin G2	PB Sample 1	ng/g					0				
Aflatoxin G2	PB Sample 2	ng/g	0.44	0.62		0.0	0			0.44	0.62
Aflatoxin G2	PB Sample 3	ng/g	0.43	0.25	< -4	0.0	2	0.52	0.00	0.43	0.25
Total Aflatoxins	PB Control	ng/g	5.0	0.5	-0.1	0.0	5	5.2	1.4	5.0	0.5
Total Aflatoxins	PB Sample 1	ng/g	5.23	2.22	0.9	0.0	5	4.20	1.11	5.23	2.22
Total Aflatoxins	PB Sample 2	ng/g	9.95	1.43	-0.5	0.0	5	10.90	2.02	9.95	1.43
Total Aflatoxins	PB Sample 3	ng/g	14.80	2.85	-0.5	0.0	5	18.0	6.2	14.8	2.9

x_i Mean of reported values

s_i Standard deviation of reported values

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

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

N Number of quantitative values reported

x* Robust mean of reported values

s* Robust standard deviation

x_{NIST} NIST-assessed value

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

Table 20. Data summary table for aflatoxin B1 in peanut butter.

		Aflatoxin B1							
		SRM 2387 Peanut Butter (ng/g)					Peanut Butter Samples (ng/g)		
	Lab	A	B	C	Avg	SD	Lo	Med	Hi
Individual Results	NIST				4.2	0.9	3.70 ± 0.63	7.17 ± 2.85	11.0 ± 2.2
	J002								
	J007								
	J008								
	J029	5.4	5.4	5.8	5.5	0.2	2.60	7.85	13.2
	J030	2.2	2.0	3.6	2.6	0.9	2.67	7.04	9.6
	J035								
	J036								
	J052	3.9	4.0	3.8	3.9	0.1	3.90	8.30	13.4
	J053	5.4	5.6	5.3	5.4	0.2	4.25	11.29	19.2
	J059								
Community Results		Consensus Mean			4.4		3.36	8.62	13.9
		Consensus Standard Deviation			1.6		0.96	2.10	4.5
		Maximum			5.5		4.25	11.29	19.2
		Minimum			2.6		2.60	7.04	9.6
		N			4		4	4	4

Table 21. Data summary table for aflatoxin B2 in peanut butter.

		Aflatoxin B2							
		SRM 2387 Peanut Butter (ng/g)					Peanut Butter Samples (ng/g)		
	Lab	A	B	C	Avg	SD	Lo	Med	Hi
Individual Results	NIST				0.7	0.3	1.01 ± 0.09	1.46 ± 1.08	2.16 ± 0.49
	J002								
	J007								
	J008								
	J029	0.9	0.9	1.0	1.0	0.0	0.70	1.18	2.48
	J030	0.5	0.7	0.8	0.6	0.2	0.96	1.58	2.33
	J035								
	J036								
	J052	0.6	0.5	0.6	0.6	0.1	0.60	2.30	5.70
	J053	0.8	0.8	0.6	0.7	0.1	1.25	1.68	3.86
	J059								
Community Results		Consensus Mean			0.7		0.88	1.69	3.59
		Consensus Standard Deviation			0.2		0.33	0.53	1.77
		Maximum			1.0		1.25	2.30	5.70
		Minimum			0.6		0.60	1.18	2.33
		N			4		4	4	4

Table 22. Data summary table for aflatoxin G1 in peanut butter.

		Aflatoxin G1							
		SRM 2387 Peanut Butter (ng/g)					Peanut Butter Samples (ng/g)		
	Lab	A	B	C	Avg	SD	Lo	Med	Hi
Individual Results	NIST							0.68 ± 0.29	1.81 ± 0.49
	J002								
	J007								
	J008								
	J029							0.66	2.14
	J030	0.3	0.3	0.3	0.3	0.0		0.81	1.79
	J035								
	J036								
	J052								
	J053							0.84	2.93
	J059								
Community Results		Consensus Mean						0.77	2.29
		Consensus Standard Deviation						0.11	0.66
		Maximum						0.84	2.93
		Minimum						0.66	1.79
		N			1		0	3	3

Table 23. Data summary table for aflatoxin G2 in peanut butter.

		Aflatoxin G2							
		SRM 2387 Peanut Butter (ng/g)					Peanut Butter Samples (ng/g)		
	Lab	A	B	C	Avg	SD	Lo	Med	Hi
Individual Results	NIST							0.44 ± 0.62	0.43 ± 0.25
	J002								
	J007								
	J008								
	J029								0.52
	J030								0.52
	J035								
	J036								
	J052								
	J053								
	J059								
Community Results		Consensus Mean							0.52
		Consensus Standard Deviation							0.00
		Maximum							0.52
		Minimum							0.52
		N			0		0	0	2

Table 24. Data summary table for total aflatoxins in peanut butter.

		Total Aflatoxins							
		SRM 2387 Peanut Butter (ng/g)					Peanut Butter Samples (ng/g)		
	Lab	A	B	C	Avg	SD	Lo	Med	Hi
Individual Results	NIST				5.0	0.5	5.23 ± 2.22	9.95 ± 1.43	14.8 ± 2.9
	J002								
	J007								
	J008	5.5	6.0	4.3	5.3	0.9	3.80	11.00	12.0
	J029	6.4	6.3	6.8	6.5	0.3	3.30	9.69	18.3
	J030	2.9	3.0	4.7	3.5	1.0	3.63	9.43	14.3
	J035								
	J036								
	J052	4.5	4.5	4.4	4.5	0.1	4.50	10.60	19.1
	J053	6.2	6.4	5.9	6.2	0.3	5.77	13.89	26.3
	J059								
Community Results		Consensus Mean			5.2		4.20	10.92	18.0
		Consensus Standard Deviation			1.4		1.11	2.02	6.2
		Maximum			6.5		5.77	13.89	26.3
		Minimum			3.5		3.30	9.43	12.0
		N			5		5	5	5

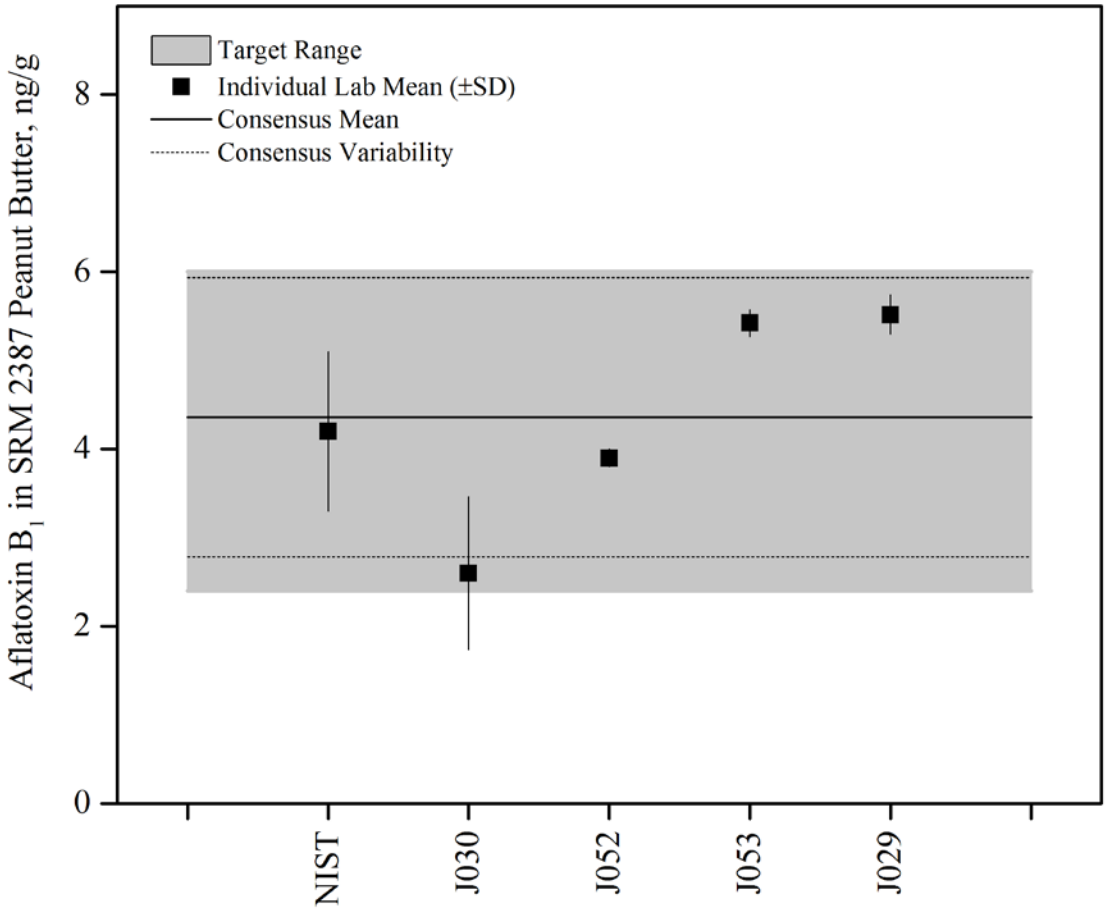

Figure 40. Aflatoxin B1 in SRM 2387 Peanut Butter (data summary view). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The black solid line represents the consensus mean, and the black dotted lines represent the consensus variability 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}).

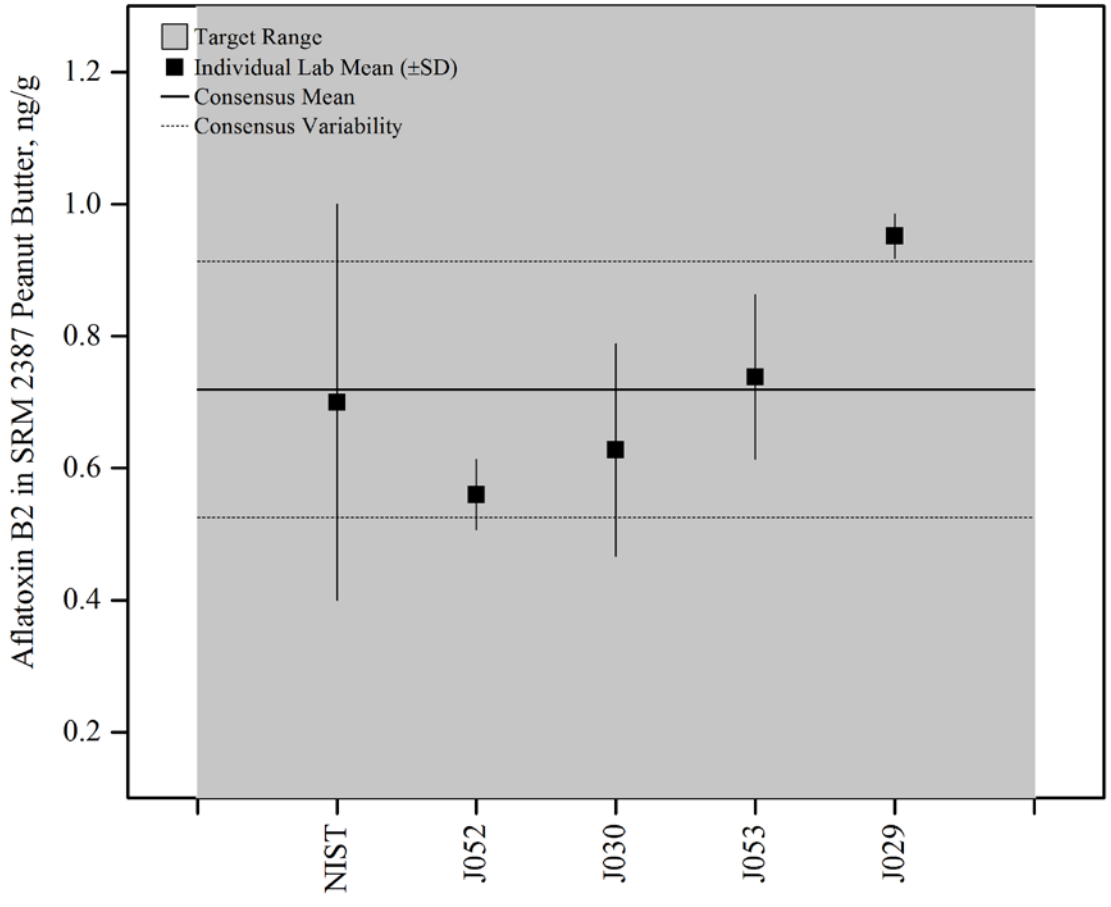

Figure 41. Aflatoxin B2 in SRM 2387 Peanut Butter (data summary view). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The black solid line represents the consensus mean, and the black dotted lines represent the consensus variability 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}).

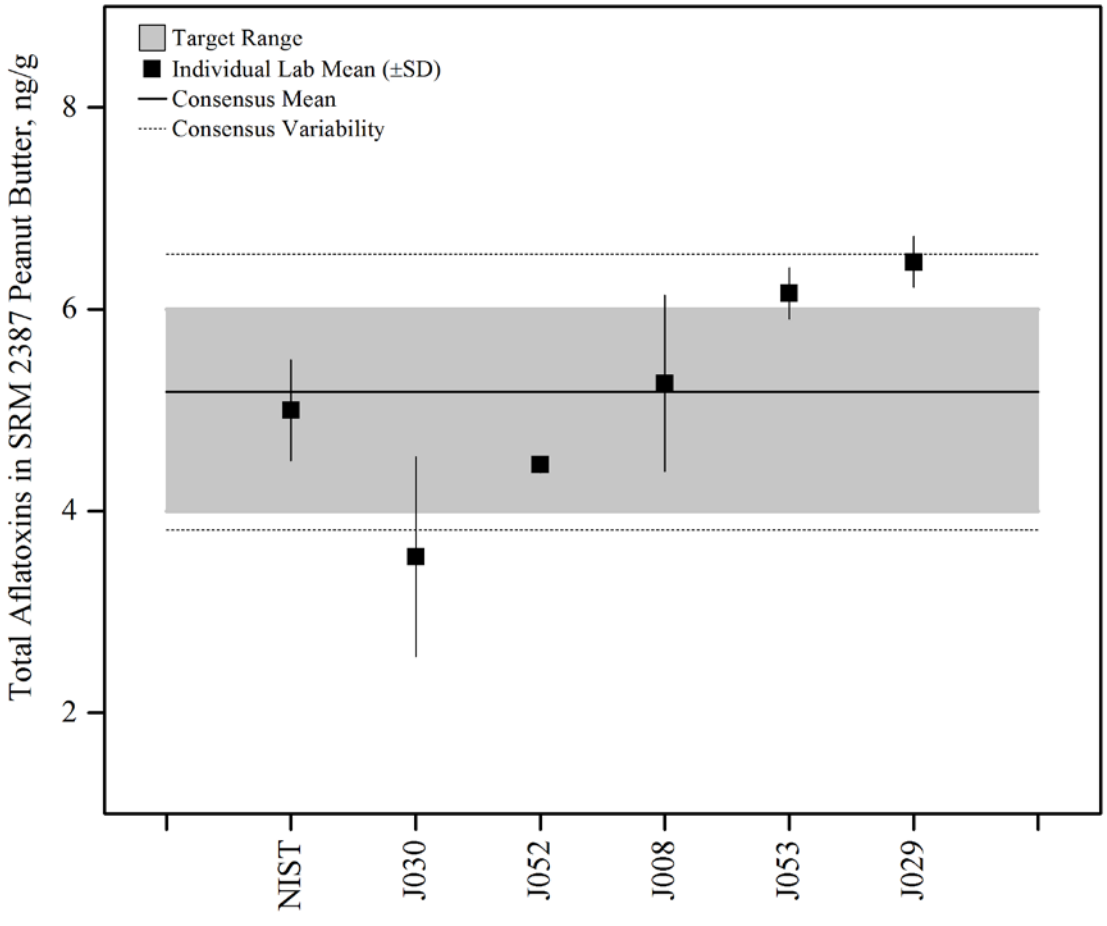

Figure 42. Total aflatoxins in SRM 2387 Peanut Butter (data summary view). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The black solid line represents the consensus mean, and the black dotted lines represent the consensus variability 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}).

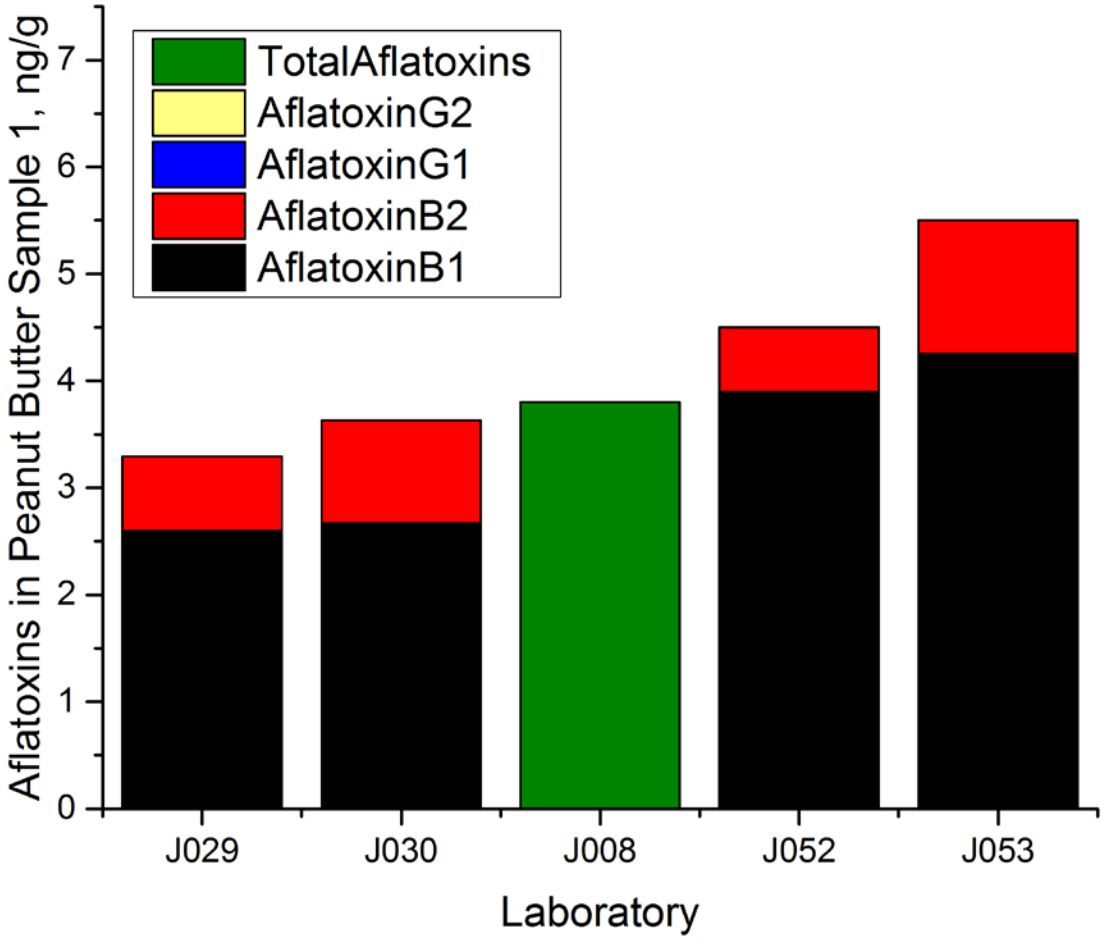

Figure 43. Total aflatoxins in peanut butter sample 1 (low) (composition view). In this view, total composition of the sample is plotted as a function of the measurement of individual components. The estimated value for total aflatoxins in this sample is (5.23 ± 2.22) ng/g.

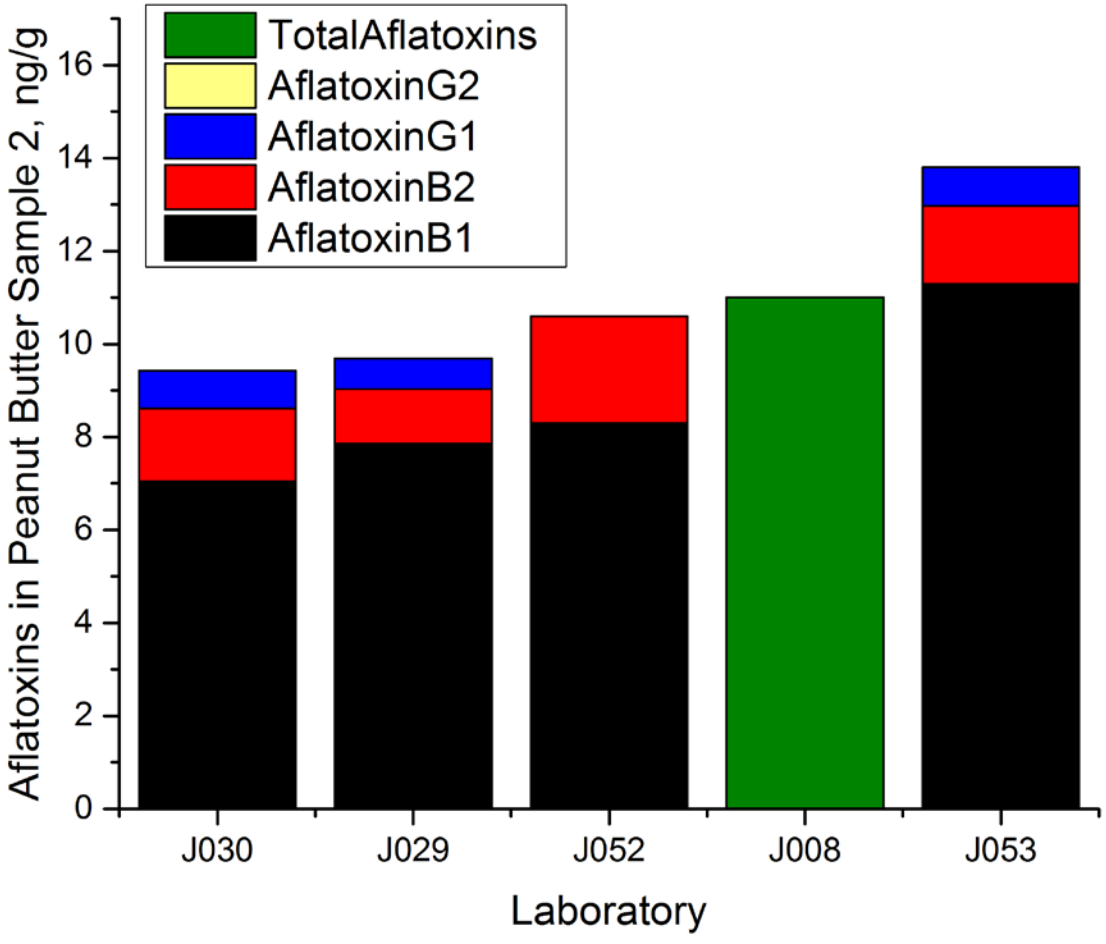

Figure 44. Total aflatoxins in peanut butter sample 2 (medium) (composition view). In this view, total composition of the sample is plotted as a function of the measurement of individual components. The estimated value for total aflatoxins in this sample is (9.95 ± 1.43) ng/g.

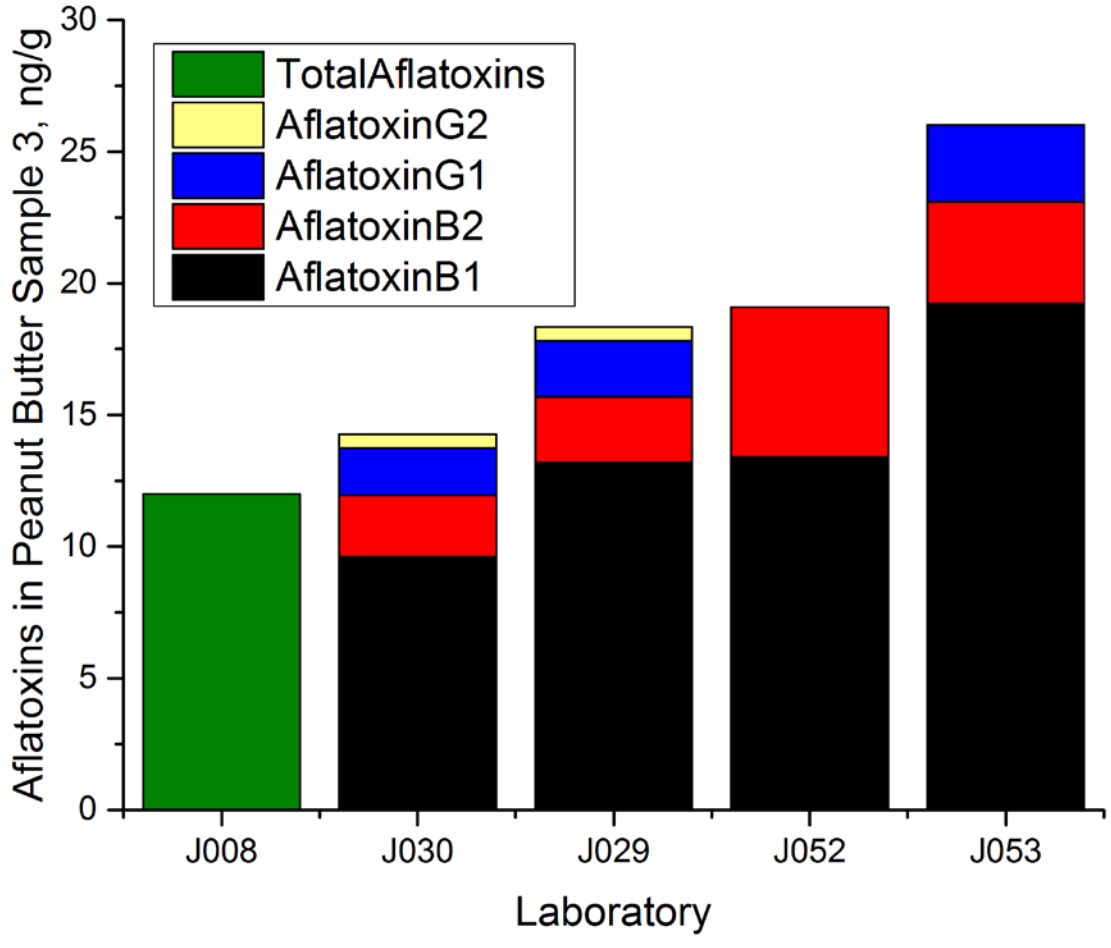

Figure 45. Total aflatoxins in peanut butter sample 3 (high) (composition view). In this view, total composition of the sample is plotted as a function of the measurement of individual components. The estimated value for total aflatoxins in this sample is (14.8 ± 2.9) ng/g.

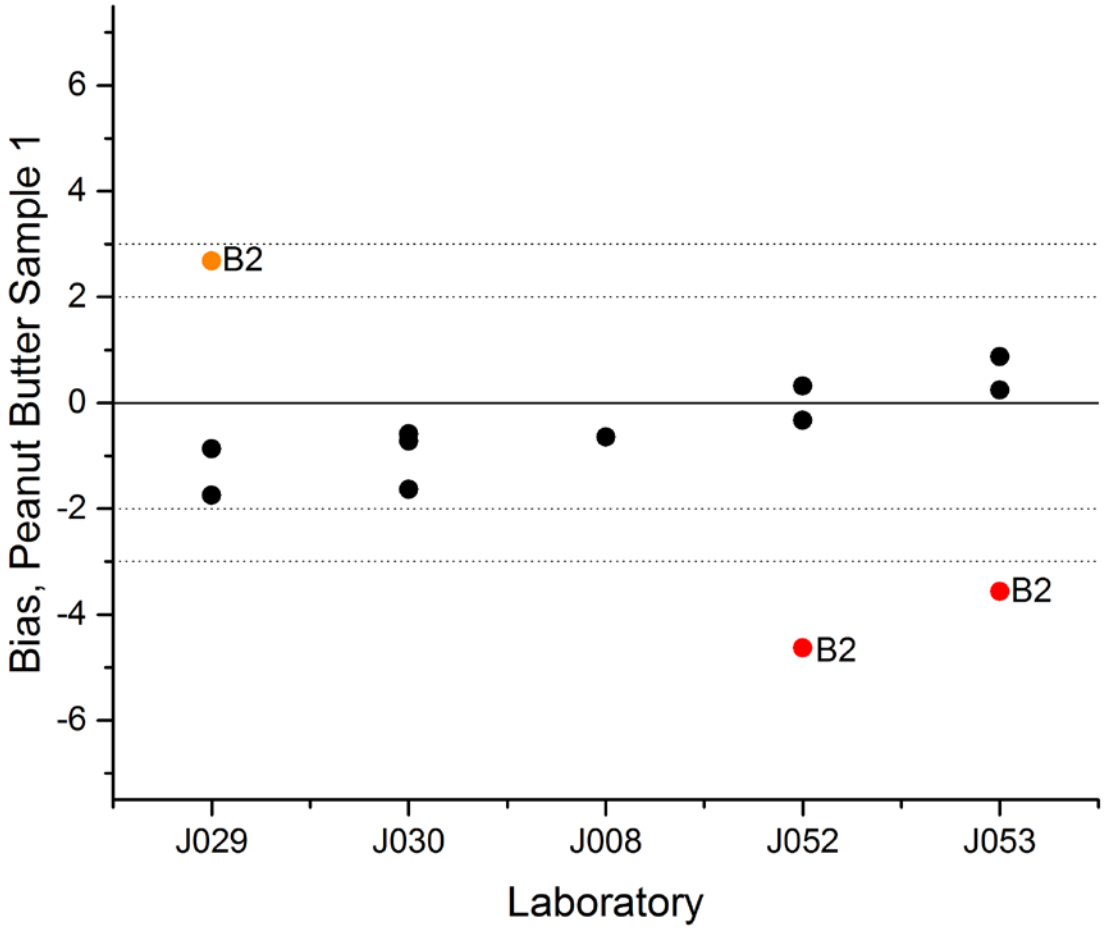

Figure 46. Aflatoxins in peanut butter sample 1 (low) (bias view). In this view, the Z_{NIST}-score for each individual component as well as total aflatoxins are plotted. Values determined to be marginally different than the NIST target value ($2 < |Z| < 3$) are colored in orange. Values determined to be significantly different than the NIST target value ($|Z| > 3$) are colored in red.

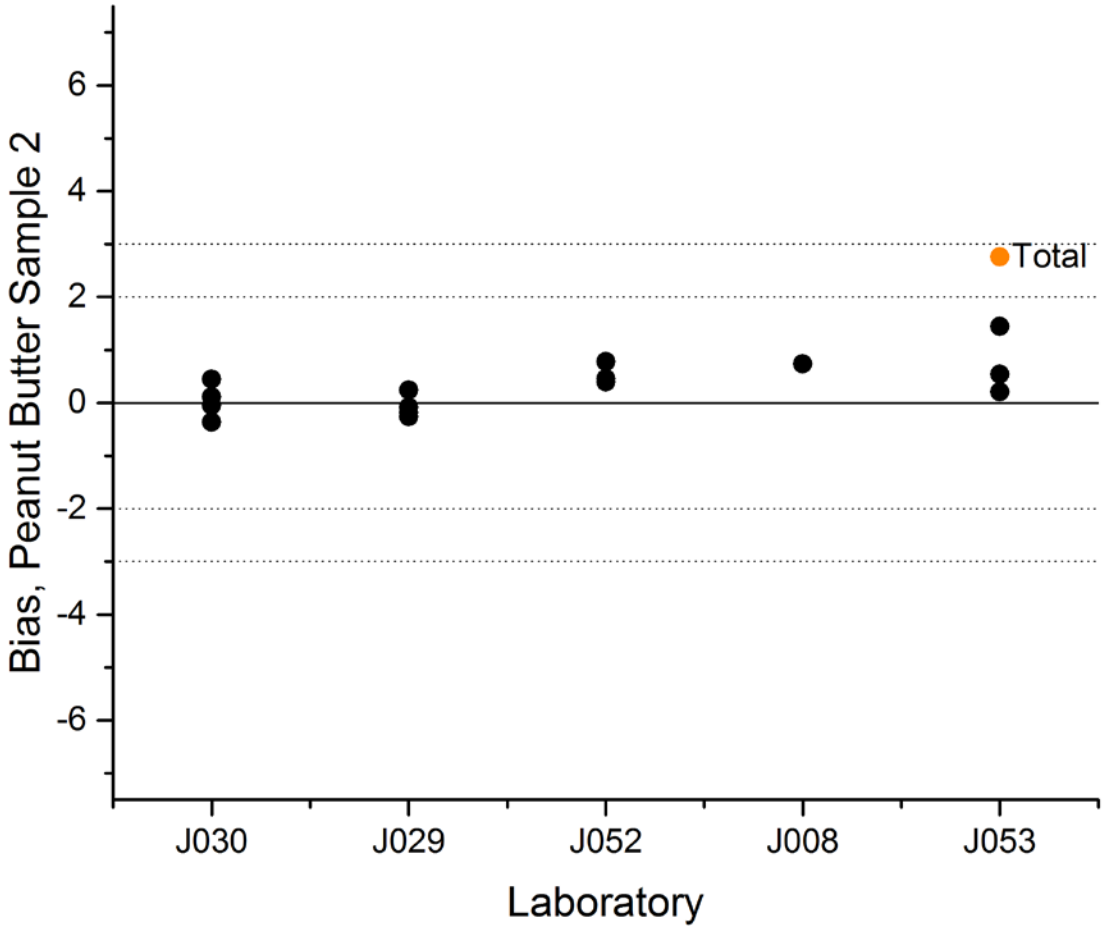

Figure 47. Aflatoxins in peanut butter sample 3 (medium) (bias view). In this view, the Z_{NIST}-score for each individual component as well as total aflatoxins are plotted. Values determined to be marginally different than the NIST target value ($2 < |Z| < 3$) are colored in orange. Values determined to be significantly different than the NIST target value ($|Z| > 3$) are colored in red.

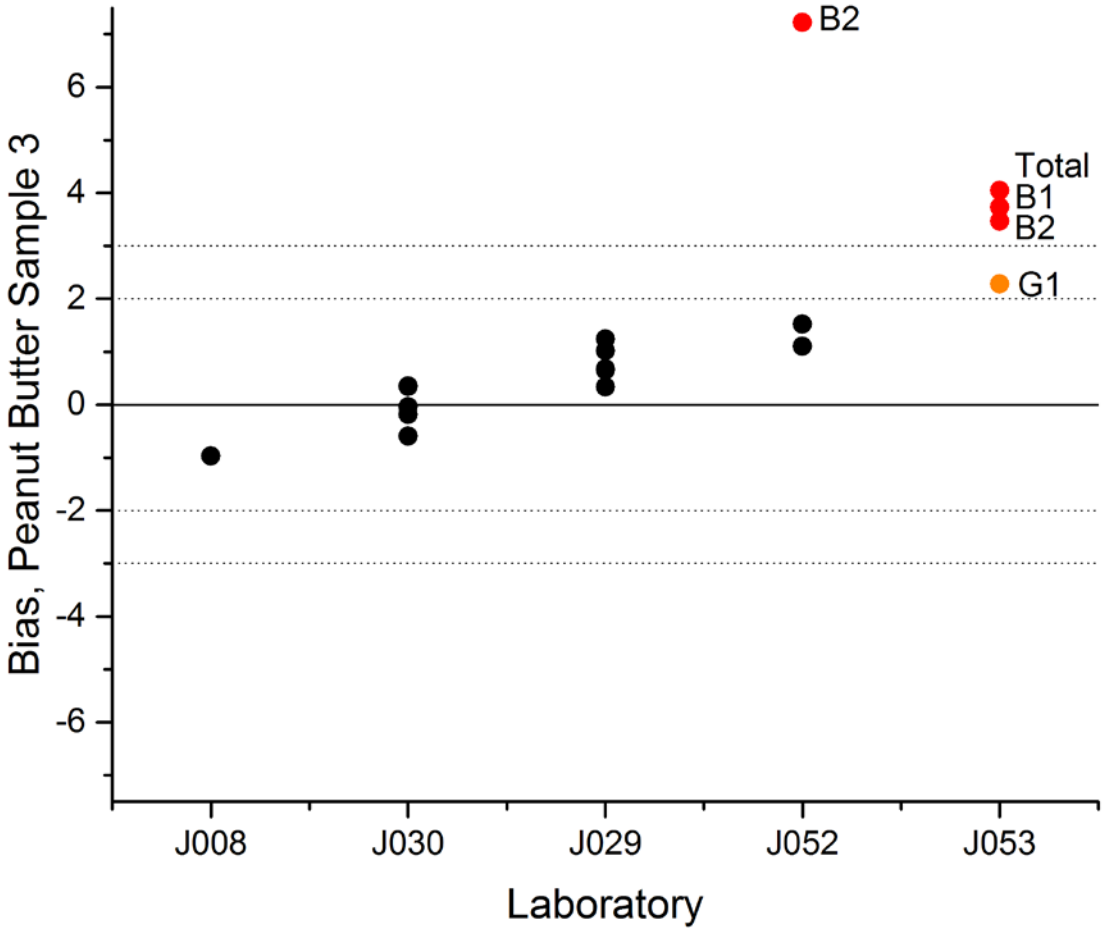

Figure 48. Aflatoxins in peanut butter sample 3 (high) (bias view). In this view, the Z_{NIST}-score for each individual component as well as total aflatoxins are plotted. Values determined to be marginally different than the NIST target value ($2 < |Z| < 3$) are colored in orange. Values determined to be significantly different than the NIST target value ($|Z| > 3$) are colored in red.

ISOFLAVONES IN SOY PRODUCTS

Study Overview

In this study, participants were provided with two NIST SRMs, SRM 3236 Soy Protein Isolate and SRM 3234 Soy Flour. Participants were asked to use in-house analytical methods to determine the mass fractions of isoflavones (daidzin, glycitin, genistin, daidzein, glycitein, and genistein) in each of the matrices and report values on an as-received basis.

Sample Information

Soy Protein Isolate. Participants were provided with three packets, each containing 10 g of soy protein isolate. Before use, participants were instructed to mix each packet thoroughly and a sample size of at least 100 mg was recommended. Participants were asked to prepare one sample and report as many analytes as possible from each packet provided, and to store the material at room temperature, 10 °C to 30 °C. Approximate analyte levels were not reported to participants prior to the study. The NIST certified values and uncertainties for isoflavones in SRM 3236 were determined using LC/abs and ID-LC/MS following solvent extraction and basic hydrolysis, and are reported in the table below both on a dry-mass basis and after correction for moisture of the material (4.83 %).

Analyte	Certified Mass Fraction in SRM 3236 (µg/g) (dry-mass basis)	Certified Mass Fraction in SRM 3236 (µg/g) (as-received basis)
Daidzin	174 ± 23	165 ± 22
Glycitin	31.37 ± 0.52	29.85 ± 0.49
Genistin	329 ± 10	313 ± 10
Daidzein	104.31 ± 0.48	99.27 ± 0.46
Glycitein	22.71 ± 0.19	21.61 ± 0.18
Genistein	183 ± 14	174 ± 13
Total Isoflavones	845 ± 29	804 ± 27

Soy Flour. Participants were provided one 50 g bottle of defatted soy flour. Before use, participants were instructed to mix the bottle thoroughly and a sample size of at least 100 mg was recommended. Participants were asked to prepare three samples and report as many analytes as possible from the bottle provided, and to store the material at room temperature, 10 °C to 30 °C. Approximate analyte levels were not reported to participants prior to the study. The NIST estimated values for isoflavones in SRM 3234 were determined using LC/abs and ID-LC/MS following solvent extraction and basic hydrolysis, and are reported in the table below on an as-received basis with an estimated uncertainty calculated as the standard deviation between the means of values determined by each method.

Analyte	NIST Estimated Mass Fraction in SRM 3234 (mg/g) (as-received basis)
Daidzin	1693 ± 427
Glycitin	241 ± 31
Genistin	2011 ± 387
Daidzein	13.5 ± 1.9
Glycitein	
Genistein	14.9 ± 0.1
Total Isoflavones	3973 ± 578

Study Results

- Sixteen laboratories enrolled in this exercise and received samples. Eleven laboratories reported data for at least some of the isoflavones in the study (69 % participation).
- Laboratories reported using either solvent extraction (64 %) or solvent extraction with hydrolysis (36 %) as the sample preparation method.
- A majority of the laboratories reported using LC/abs (91 %) for isoflavone determination. One laboratory reported using DART-MS as their instrumental method.
- The consensus means were within the target ranges with acceptable between-laboratory variability for daidzein and genistein in the soy protein isolate (4 % RSD for both analytes).
- The consensus means were above the target ranges for glycitein in the soy protein isolate and daidzein and genistein in the soy flour with high between-laboratory variability (29 % to 94 % RSD).
- A clear distinction was observed for the glycoside (daidzin, glycitin, and genistin) results in both materials between the laboratories using solvent extraction compared to those including a hydrolysis step in their sample preparation.
    - Separate consensus means and ranges were calculated for each sample preparation approach. The variances of each approach were found to be statistically equivalent, yet the mean values determined by each method for each analyte below were determined to be statistically different.
    - With the exception of glycitin in the soy protein isolate, the consensus means for all of the glycosides as well as total isoflavones determined using hydrolysis were within the target ranges in both materials with excellent between-laboratory variability (5 % to 14 % RSD).
    - The consensus mean for glycitin in the soy protein isolate for laboratories using hydrolysis was above the target range with slightly higher between-laboratory variability (24 % RSD).
    - For laboratories using an extraction approach (without hydrolysis), consensus means for all isoflavones were below the target ranges. Between-laboratory variability was higher than when hydrolysis was used (21 % to 58 % RSD), with the exception of total isoflavones in the soy protein isolate (7 % RSD).

Analyte	Consensus for Laboratories Using Extraction (µg/g)	Consensus for Laboratories Using Extraction with Hydrolysis (µg/g)
Soy Protein Isolate		
Daidzin	70 ± 18	172 ± 15
Glycitin	13.2 ± 5.6	32.8 ± 8.0
Genistin	105 ± 26	308 ± 14
Total Isoflavones	471 ± 31	750 ± 100
Soy Flour		
Daidzin	520 ± 100	1780 ± 170
Glycitin	91 ± 35	276 ± 25
Genistin	530 ± 200	2200 ± 130
Total Isoflavones	1040 ± 600	4220 ± 420

Technical Recommendations

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

- A clear distinction was observed for the glycoside (daidzin, glycitin, and genistin) results in both materials between the laboratories using solvent extraction compared to those including a hydrolysis step in their sample preparation.
  - The hydrolysis step is utilized to convert the acetyl- and malonyl-glycosides of daidzin, glycitin, and genistin to their glycoside forms.
  - If acetyl- and malonyl-glycosides are present, the use of hydrolysis in the sample preparation will result in determination of higher concentrations of the glycosides than if hydrolysis is not used.
  - Because the hydrolysis step does not cleave the glycosides from the isoflavone molecules, no increase in concentration is observed for the aglycones (daidzein, glycitein, and genistein) when hydrolysis is utilized.
  - Laboratories that do not use a hydrolysis step must take extra precautions to provide accurate results.
    - Ensure that acetyl- and malonyl-glycosides are not being cleaved during the extraction process, or that the extraction process being utilized is robust enough to provide consistent results with expected deviations in laboratory practice (extraction time, temperature, solvent concentration, etc.).
    - Ensure that the acetyl- and malonyl-glycosides are sufficiently stable so they do not degrade during the course of the sample preparation and chromatographic runs, leading to inconsistent results.
    - Ensure that the separation method is adequate to resolve malonyl- and acetyl-glycosides from aglycones and glycosides so that coelutions do not cause falsely high results.
    - The most accurate chromatographic peak identification and quantitation relies on pure reference standards. If standards are not available for every acetyl- and malonyl-glycoside, ensure that quantitation using response of another compound is appropriate.
  - Because the NIST target values were determined using a hydrolysis step, laboratories that also utilized the hydrolysis step were in better agreement. However, laboratories

using only extraction are not incorrect, and it is therefore important to specify method information when discussing and reporting isoflavone concentrations.

- A calibration issue may also be underlying the data for the glycosides (daidzin, glycitin, and genistin), as observed in the sample/control comparison views. The diagonal line observed is somewhat pronounced due to the effect of sample processing (described above), but for those laboratories using only solvent extraction, there may be some evidence of a calibration error.
  - Be sure to confirm the purity of all reference standards by measuring moisture as well as checking chromatographic purity. NMR can provide additional information.
  - The best approach is to use a reference standard for each isoflavone in the calibration.
  - If an internal standard approach is used, confirm that there are no chromatographic coelutions with the internal standard by running a blank.

Table 25. Individual data summary table (NIST) for isoflavones in soy dietary supplements.

National Institute of Standards & Technology

Exercise J - May 2013 - Isoflavones

	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}
Daidzin	Isolate	µg/g	165	22	0.9	0.0	10	111	62	165	22
Daidzin	Flour	µg/g	1693	427	0.9	0.0	10	1050	727	1693	427
Glycitin	Isolate	µg/g	29.9	0.5	0.9	0.0	9	19.2	11.4	29.9	0.5
Glycitin	Flour	µg/g	241	31	0.8	0.0	9	159	105	241	31
Genistin	Isolate	µg/g	313	10	1.1	0.0	10	189	118	313	10
Genistin	Flour	µg/g	2011	387	0.8	0.0	10	1220	968	2011	387
Daidzein	Isolate	µg/g	99.3	0.5	0.0	0.0	9	99.1	13.6	99.3	0.5
Daidzein	Flour	µg/g	13.5	1.9	-0.7	0.0	10	37.8	35.7	13.5	1.9
Glycitein	Isolate	µg/g	21.6	0.2	-0.4	0.0	9	24.9	8.1	21.6	0.2
Glycitein	Flour	µg/g					4	149.0	285.0		
Genistein	Isolate	µg/g	174	13	0.9	0.0	10	155	21	174	13
Genistein	Flour	µg/g	14.9	0.1	-0.4	0.0	10	16.6	4.8	14.9	0.1
Total Isoflavones	Isolate	µg/g	804	27	1.2	0.0	10	585	176	804	27
Total Isoflavones	Flour	µg/g	3973	578	0.9	0.0	11	2300	1800	3973	578

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 daidzin in soy dietary supplements.

		Daidzin									
		SRM 3236 Soy Protein Isolate (µg/g)					SRM 3234 Soy Flour (µg/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	NIST				165	22				1693	427
	J002										
	J004										
	J005										
	J011	162	163	155	160	4	1540	1590	1540	1557	29
	J019	50	52	50	51	1	384	410	532	442	79
	J029	204	174	178	185	16	1880	1870	1870	1873	6
	J030	189	175	181	181	7	1804	1771	1797	1791	17
	J031	76	78	77	77	1	606	606	610	607	2
	J035										
	J036	95	96	87	93	5	807	808	811	809	2
	J049	74	74	73	74	1	607	614		611	5
	J053	69	70	73	71	2	506	532		519	18
	J054	52	55	57	55	3	402	401	403	402	1
	J057										
	J058	164	155	162	160	5	1885	1863	1907	1885	22
	J059										
Community Results		Consensus Mean			111		Consensus Mean			1049	
		Consensus Standard Deviation			62		Consensus Standard Deviation			727	
		Maximum			185		Maximum			1885	
		Minimum			51		Minimum			402	
		N			10		N			10	

Table 27. Data summary table for glycitin in soy dietary supplements.

		Glycitin									
		SRM 3236 Soy Protein Isolate (µg/g)					SRM 3234 Soy Flour (µg/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	NIST				29.9	0.5				241	31
	J002										
	J004										
	J005										
	J011										
	J019	11.2	10.6	10.5	10.8	0.4	91	92	101	94	6
	J029	31.4	28.5	28.8	29.6	1.6	258	259	257	258	1
	J030	39.3	38.8	44.6	40.9	3.2	308	295	299	301	7
	J031	16.0	17.1	18.3	17.1	1.2	106	106	107	106	1
	J035										
	J036	17.1	17.5	15.1	16.6	1.3	153	154	150	152	2
	J049	13.0	12.1	11.1	12.1	1.0	81	85		83	3
	J053	17.0	16.5	20.2	17.9	2.0	128	125		126	3
	J054	5.0	5.0	5.0	5.0	0.0	44	43	45	44	1
	J057										
	J058	29.3	27.2	27.3	27.9	1.2	267	271	267	268	2
	J059										
Community Results		Consensus Mean			19.2		Consensus Mean			159	
		Consensus Standard Deviation			11.4		Consensus Standard Deviation			105	
		Maximum			40.9		Maximum			301	
		Minimum			5.0		Minimum			44	
		N			9		N			9	

Table 28. Data summary table for genistin in soy dietary supplements.

		Genisitn									
		SRM 3236 Soy Protein Isolate (µg/g)					SRM 3234 Soy Flour (µg/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	NIST				313	10				2011	387
	J002										
	J004										
	J005										
	J011	311	314	294	306	11	2110	2180	2100	2130	44
	J019	77	80	71	76	5	317	358	102	259	138
	J029	311	283	291	295	14	2080	2080	2080	2080	0
	J030	327	324	323	325	2	2260	2225	2258	2248	20
	J031	130	126	130	129	2	651	657	661	656	5
	J035										
	J036	126	127	116	123	6	677	681	675	678	3
	J049	134	136	136	135	1	750	766		758	11
	J053	114	111	111	112	1	592	620		606	20
	J054	84	88	91	88	4	464	463	465	464	1
	J057										
	J058	308	302	308	306	4	2344	2302	2352	2333	27
	J059										
Community Results		Consensus Mean			189		Consensus Mean			1221	
		Consensus Standard Deviation			118		Consensus Standard Deviation			968	
		Maximum			325		Maximum			2333	
		Minimum			76		Minimum			259	
		N			10		N			10	

Table 29. Data summary table for daidzein in soy dietary supplements.

		Daidzein									
		SRM 3236 Soy Protein Isolate (µg/g)					SRM 3234 Soy Flour (µg/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	NIST				99.3	0.5				13.5	1.9
	J002										
	J004										
	J005										
	J011										
	J019	119.6	120.6	117.6	119.2	1.6	63.4	66.7	81.9	70.7	9.9
	J029	79.7	83.8	87.3	83.6	3.8	15.6	15.0	15.4	15.3	0.3
	J030	116.0	117.1	120.3	117.8	2.2	35.2	29.1	28.9	31.1	3.6
	J031	91.9	92.0	92.1	92.0	0.1	16.5	13.2	13.9	14.5	1.7
	J035										
	J036	98.3	98.3	98.6	98.4	0.2	17.0	16.9	16.6	16.8	0.2
	J049	93.7	93.0	92.5	93.1	0.6	85.0	83.0		84.0	1.4
	J053	100.2	104.6	98.1	101.0	3.3	27.6	26.5		27.1	0.8
	J054	94.0	92.0	92.0	92.7	1.2	11.1	11.3	11.0	11.1	0.2
	J057										
	J058	94.9	91.9	95.0	93.9	1.8	16.8	15.9	15.7	16.1	0.6
	J059						103.0	109.0	102.0	104.7	3.8
Community Results		Consensus Mean			99.1		Consensus Mean			37.8	
		Consensus Standard Deviation			13.6		Consensus Standard Deviation			35.7	
		Maximum			119.2		Maximum			104.7	
		Minimum			83.6		Minimum			11.1	
		N			9		N			10	

Table 30. Data summary table for glycitein in soy dietary supplements.

		Glycitein									
		SRM 3236 Soy Protein Isolate (µg/g)					SRM 3234 Soy Flour (µg/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	NIST				21.6	0.2					
	J002										
	J004										
	J005										
	J011										
	J019	18.7	19.0	18.7	18.8	0.2	1.5	1.7	2.7	2.0	0.6
	J029	18.7	18.1	18.4	18.4	0.3					
	J030	28.4	20.4	25.8	24.9	4.1	10.8	10.2	10.6	10.5	0.3
	J031	24.9	23.1	22.1	23.4	1.4					
	J035										
	J036	47.2	46.6	46.3	46.7	0.5					
	J049	21.6	22.2	21.5	21.8	0.4					
	J053	23.9	25.7	25.8	25.1	1.1					
	J054	35.7	36.6	36.3	36.2	0.5	524.6	524.1	524.9	524.5	0.4
	J057										
	J058	17.8	19.3	19.0	18.7	0.8					
	J059						57.0	62.0	56.0	58.3	3.2
Community Results		Consensus Mean			24.9		Consensus Mean			148.8	
		Consensus Standard Deviation			8.1		Consensus Standard Deviation			285.4	
		Maximum			46.7		Maximum			524.5	
		Minimum			18.4		Minimum			2.0	
		N			9		N			4	

Table 31. Data summary table for genistein in soy dietary supplements.

		Genistein									
		SRM 3236 Soy Protein Isolate (µg/g)					SRM 3234 Soy Flour (µg/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	NIST				174	13				14.9	0.1
	J002										
	J004										
	J005										
	J011	169	170	159	166	6					
	J019	163	164	160	162	2	11.4	10.6	12.4	11.5	0.9
	J029	127	121	122	123	3	13.6	14.0	14.7	14.1	0.6
	J030	157	152	158	156	3	25.1	22.8	23.4	23.8	1.2
	J031	155	153	156	155	2	14.5	14.7	13.9	14.4	0.4
	J035										
	J036	164	164	167	165	2	14.1	14.7	15.1	14.6	0.5
	J049	162	162	162	162	0	16.0	15.8		15.9	0.1
	J053	121	120	123	121	1	17.9	18.2		18.1	0.2
	J054	150	153	157	153	3	12.7	12.5	12.9	12.7	0.2
	J057										
	J058	185	185	180	183	3	34.3	35.5	30.5	33.4	2.6
	J059						19.0	15.0	18.0	17.3	2.1
Community Results		Consensus Mean			155		Consensus Mean			16.6	
		Consensus Standard Deviation			21		Consensus Standard Deviation			4.8	
		Maximum			183		Maximum			33.4	
		Minimum			121		Minimum			11.5	
		N			10		N			10	

Table 32. Data summary table for total isoflavones in soy dietary supplements.

		Total Isoflavones									
		SRM 3236 Soy Protein Isolate (µg/g)					SRM 3234 Soy Flour (µg/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	NIST				804	27				3973	578
	J002										
	J004										
	J005										
	J011	642	647	608	632	21	3650	3770	3640	3687	72
	J019	440	446	427	438	10	869	939	832	880	54
	J029	772	708	726	735	33	4247	4238	4237	4241	6
	J030	857	828	852	845	15	4443	4353	4416	4404	46
	J031	494	489	495	493	3	1394	1397	1406	1399	6
	J035										
	J036	548	550	530	543	11	1668	1675	1668	1670	4
	J049	498	499	496	498	2	1539	1564		1551	18
	J053	445	448	451	448	3	1271	1321		1296	35
	J054	421	430	438	430	9	1459	1455	1462	1458	3
	J057										
	J058	798	780	792	790	9	4548	4487	4572	4535	43
	J059						179	186	176	180	5
Community Results		Consensus Mean			585		Consensus Mean			2300	
		Consensus Standard Deviation			176		Consensus Standard Deviation			1797	
		Maximum			845		Maximum			4535	
		Minimum			430		Minimum			180	
		N			10		N			11	

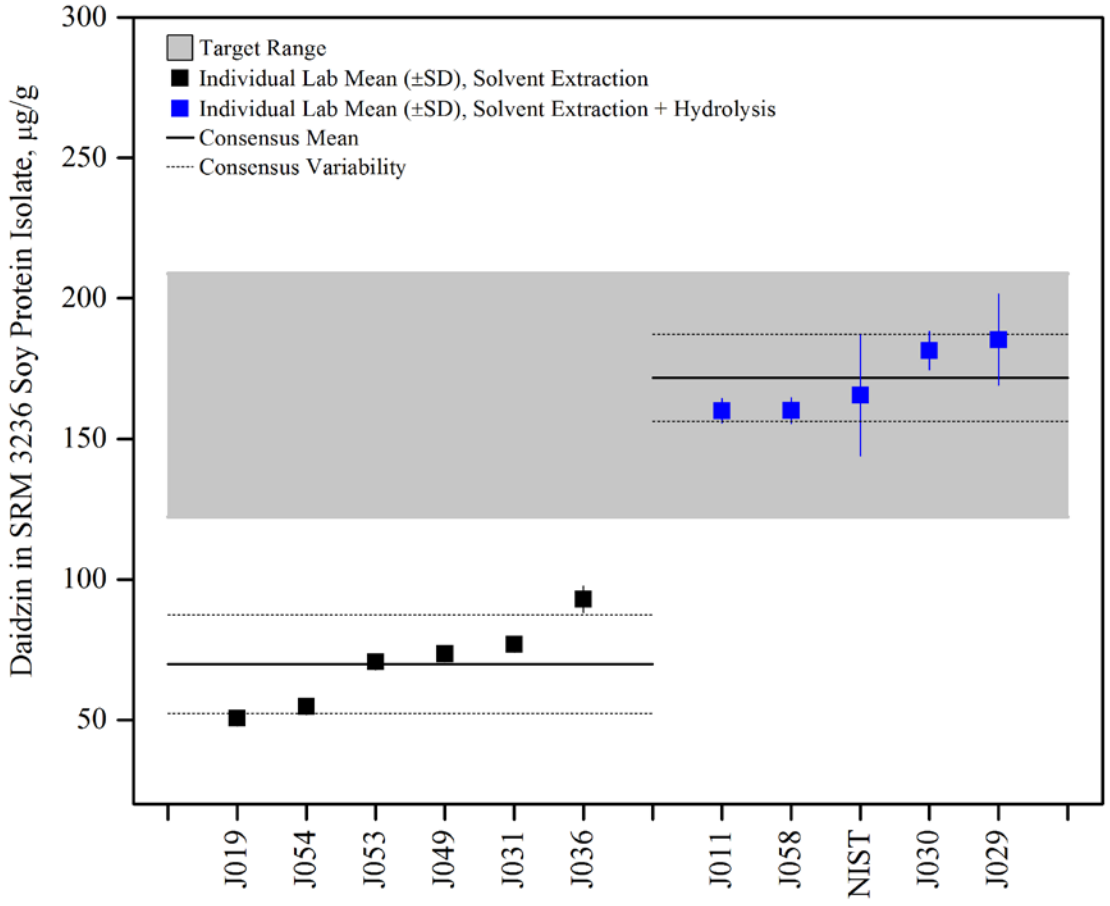

Figure 49. Daidzin in SRM 3236 Soy Protein Isolate (data summary view, sample preparation comparison). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The color of the data point represents the sample preparation method employed. The black solid lines represent the consensus means for each sample preparation method, and the black dotted lines represent the consensus variability calculated as one standard deviation about the consensus mean for that sample preparation method. The gray shaded region represents the target zone for "acceptable" performance, which encompasses the NIST certified value bounded by twice its uncertainty (U_{95}).

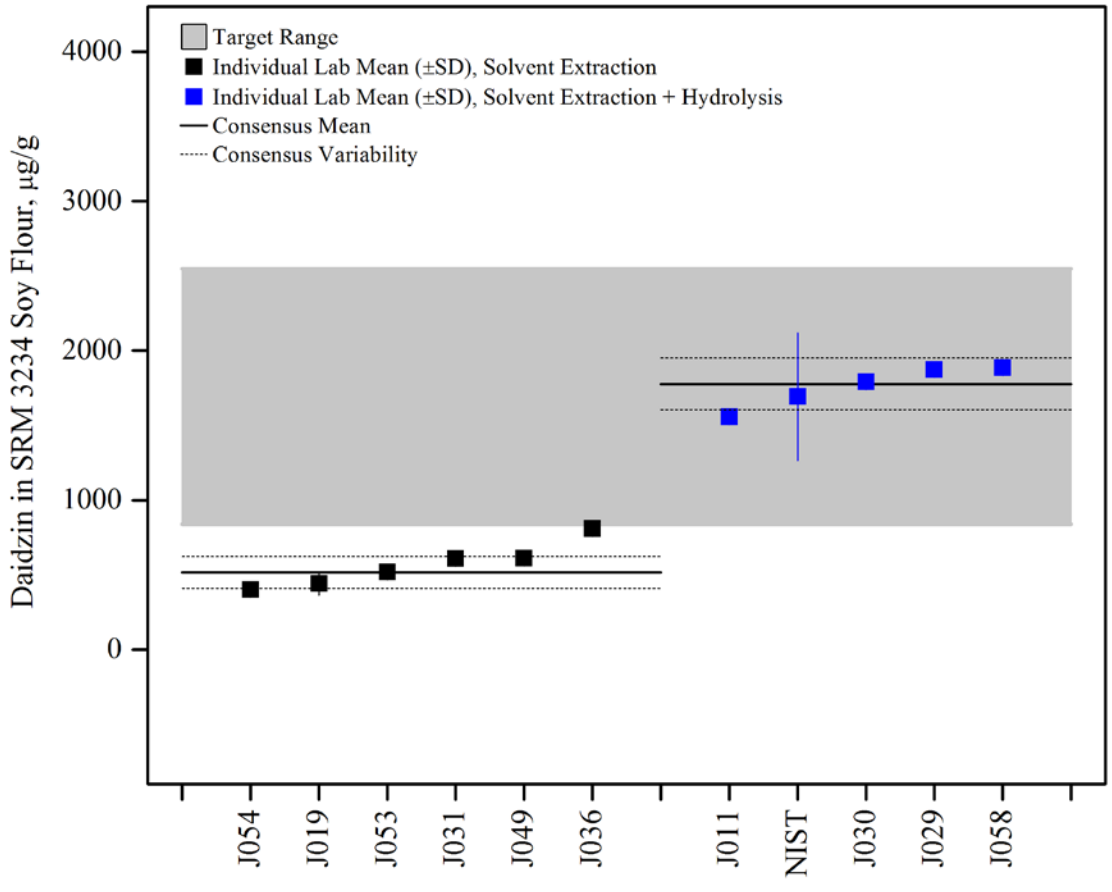

Figure 50. Daidzin in SRM 3234 Soy Flour (data summary view, sample preparation comparison). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The color of the data point represents the sample preparation method employed. The black solid lines represents the consensus mean for each sample preparation method, and the black dotted lines represent the consensus variability calculated as one standard deviation about the consensus mean for that sample preparation method. The gray shaded region represents the target zone for "acceptable" performance, which encompasses the NIST value determined by LC/abs and ID-LC/MS, bounded by an estimated uncertainty calculated as the standard deviation between the means of values determined by each method.

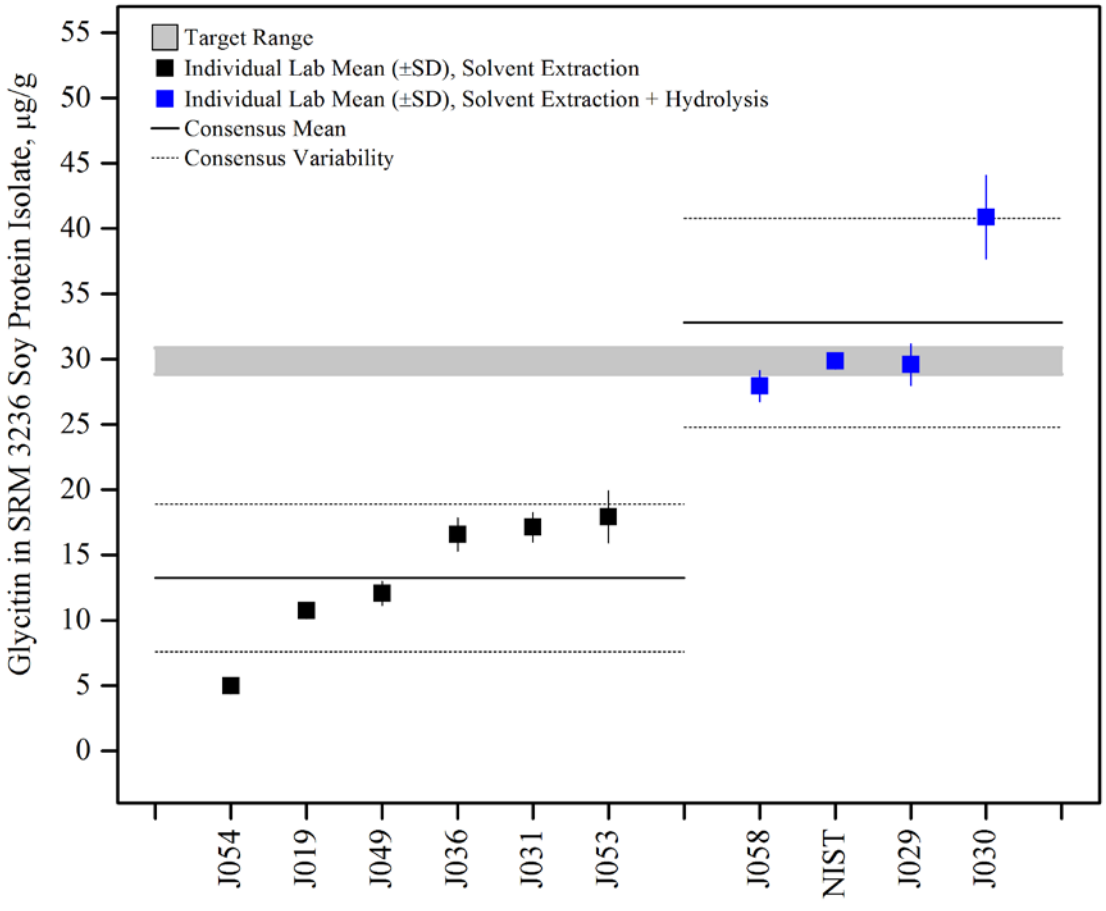

Figure 51. Glycitin in SRM 3236 Soy Protein Isolate (data summary view, sample preparation comparison). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The color of the data point represents the sample preparation method employed. The black solid lines represents the consensus mean for each sample preparation method, and the black dotted lines represent the consensus variability calculated as one standard deviation about the consensus mean for that sample preparation method. The gray shaded region represents the target zone for "acceptable" performance, which encompasses the NIST certified value bounded by twice its uncertainty (U_{95}).

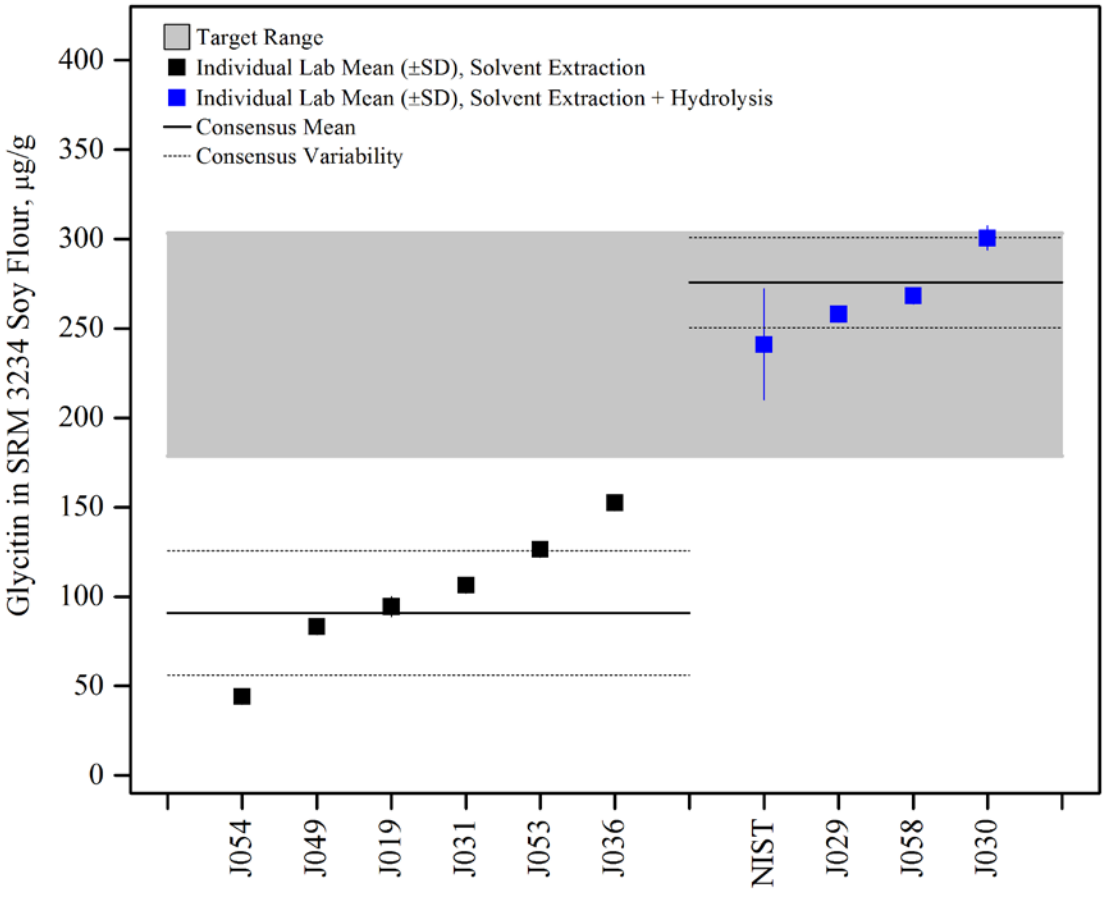

Figure 52. Glycitin in SRM 3234 Soy Flour (data summary view, sample preparation comparison). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The color of the data point represents the sample preparation method employed. The black solid lines represents the consensus mean for each sample preparation method, and the black dotted lines represent the consensus variability calculated as one standard deviation about the consensus mean for that sample preparation method. The gray shaded region represents the target zone for "acceptable" performance, which encompasses the NIST value determined by LC/abs and ID-LC/MS, bounded by an estimated uncertainty calculated as the standard deviation between the means of values determined by each method.

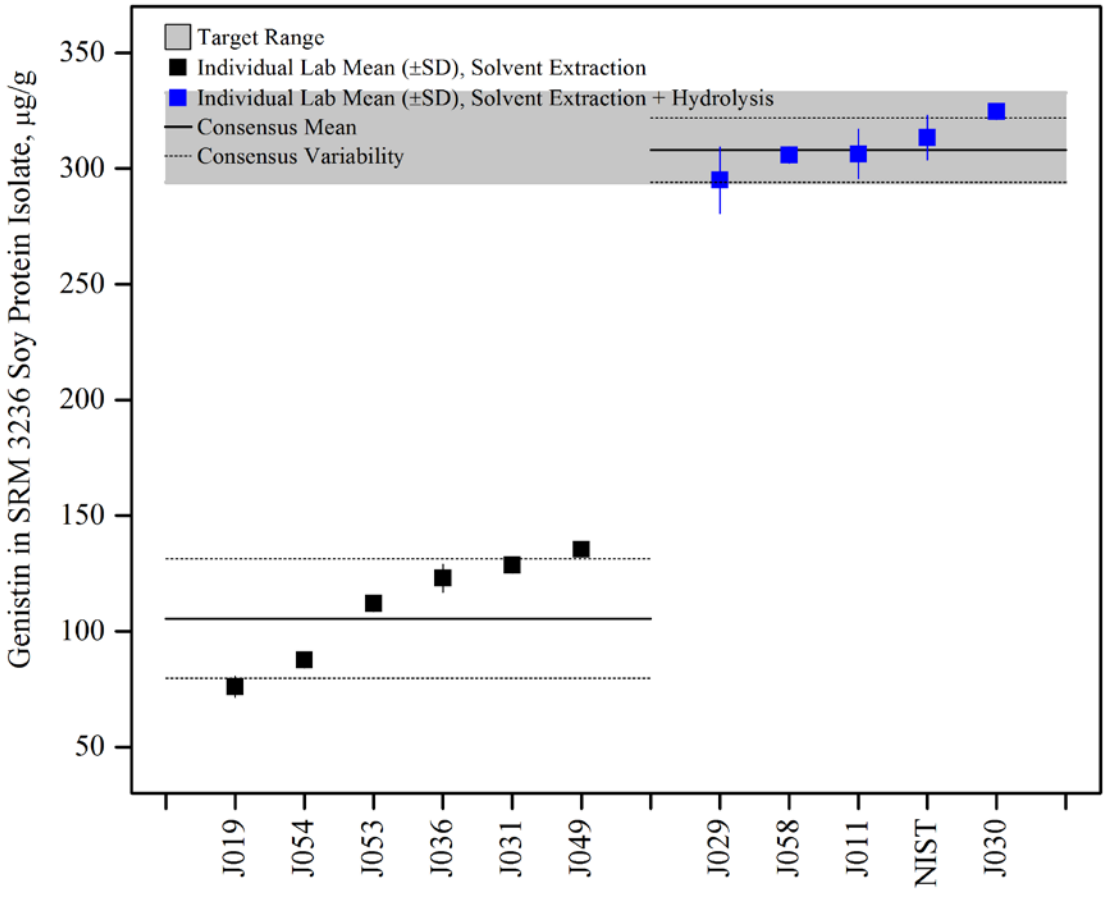

Figure 53. Genistin in SRM 3236 Soy Protein Isolate (data summary view, sample preparation comparison). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The color of the data point represents the sample preparation method employed. The black solid lines represents the consensus mean for each sample preparation method, and the black dotted lines represent the consensus variability calculated as one standard deviation about the consensus mean for that sample preparation method. The gray shaded region represents the target zone for "acceptable" performance, which encompasses the NIST certified value bounded by twice its uncertainty (U_{95}).

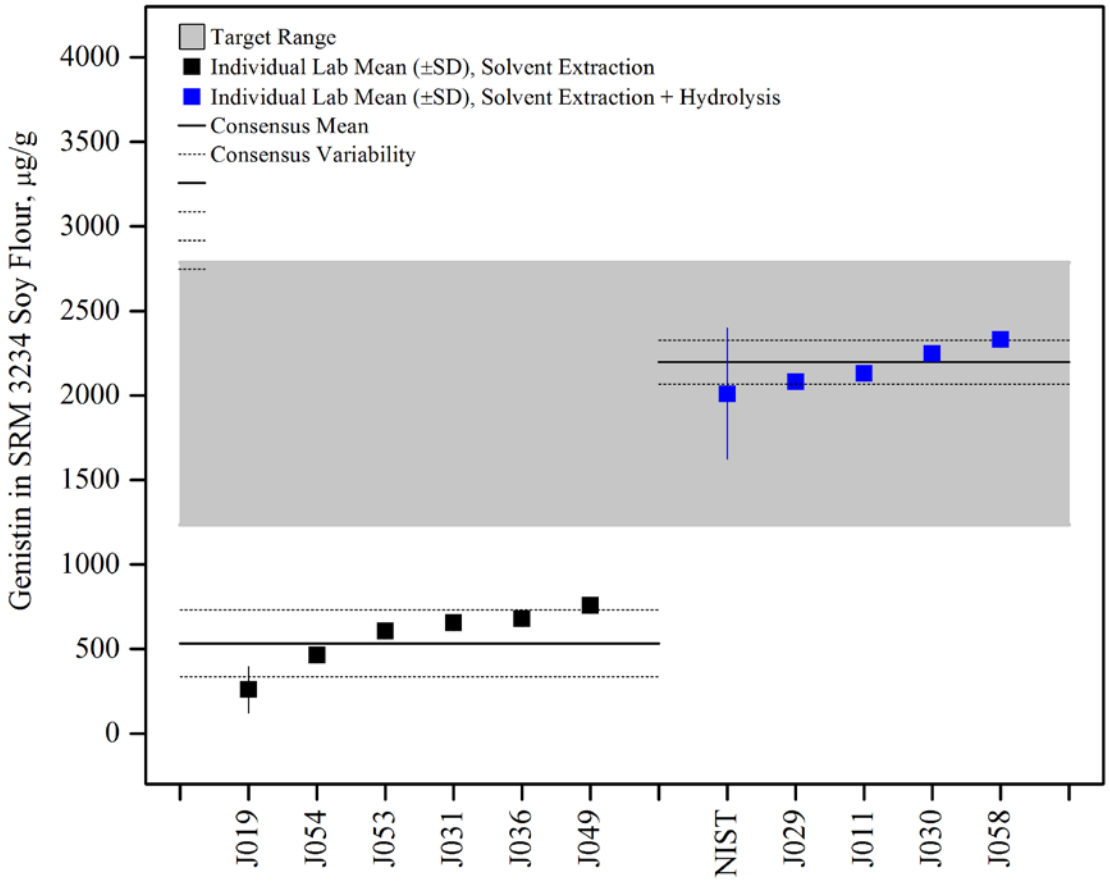

Figure 54. Genistin in SRM 3234 Soy Flour (data summary view, sample preparation comparison). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The color of the data point represents the sample preparation method employed. The black solid lines represents the consensus mean for each sample preparation method, and the black dotted lines represent the consensus variability calculated as one standard deviation about the consensus mean for that sample preparation method. The gray shaded region represents the target zone for "acceptable" performance, which encompasses the NIST value determined by LC/abs and ID-LC/MS, bounded by an estimated uncertainty calculated as the standard deviation between the means of values determined by each method.

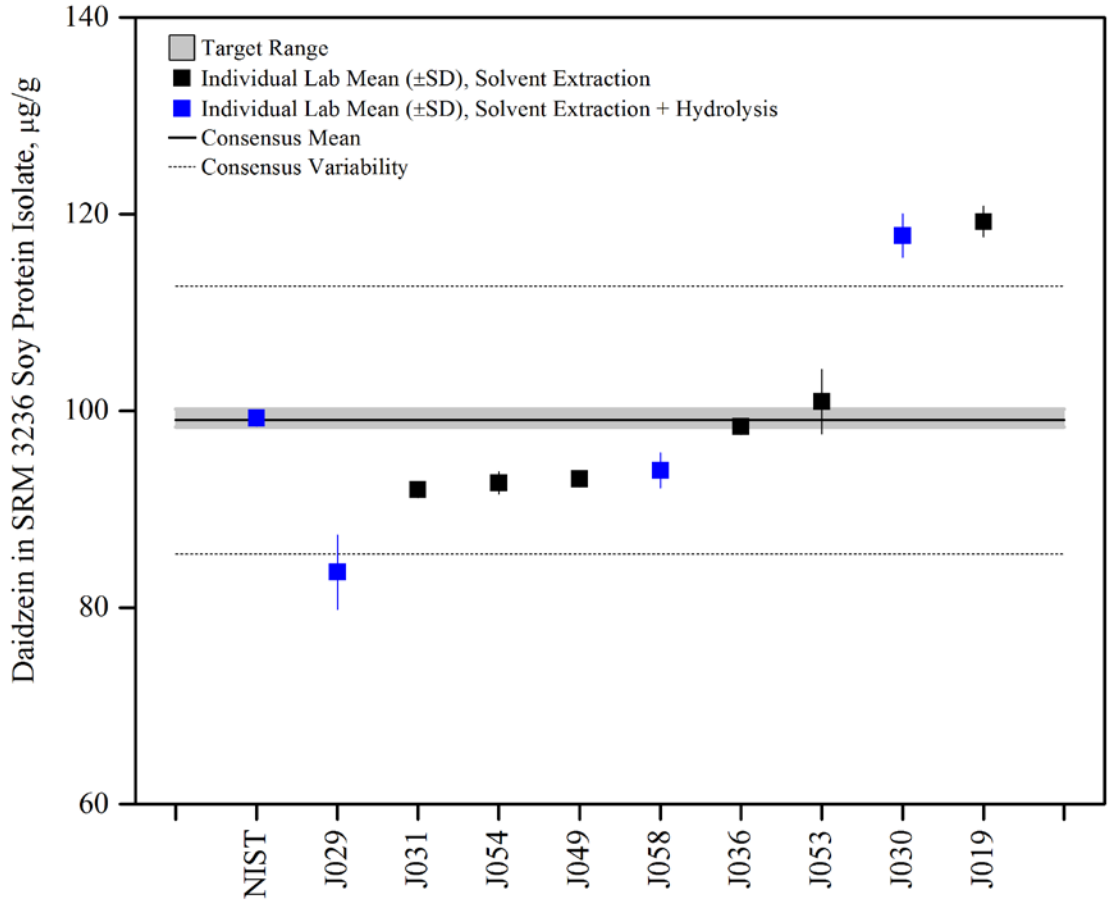

Figure 55. Daidzein in SRM 3236 Soy Protein Isolate (data summary view, sample preparation comparison). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The color of the data point represents the sample preparation method employed. The black solid line represents the consensus mean, and the black dotted lines represent the consensus variability calculated as one standard deviation about the consensus mean. The gray shaded region represents the target zone for "acceptable" performance, which encompasses the NIST certified value bounded by twice its uncertainty (U_{95}).

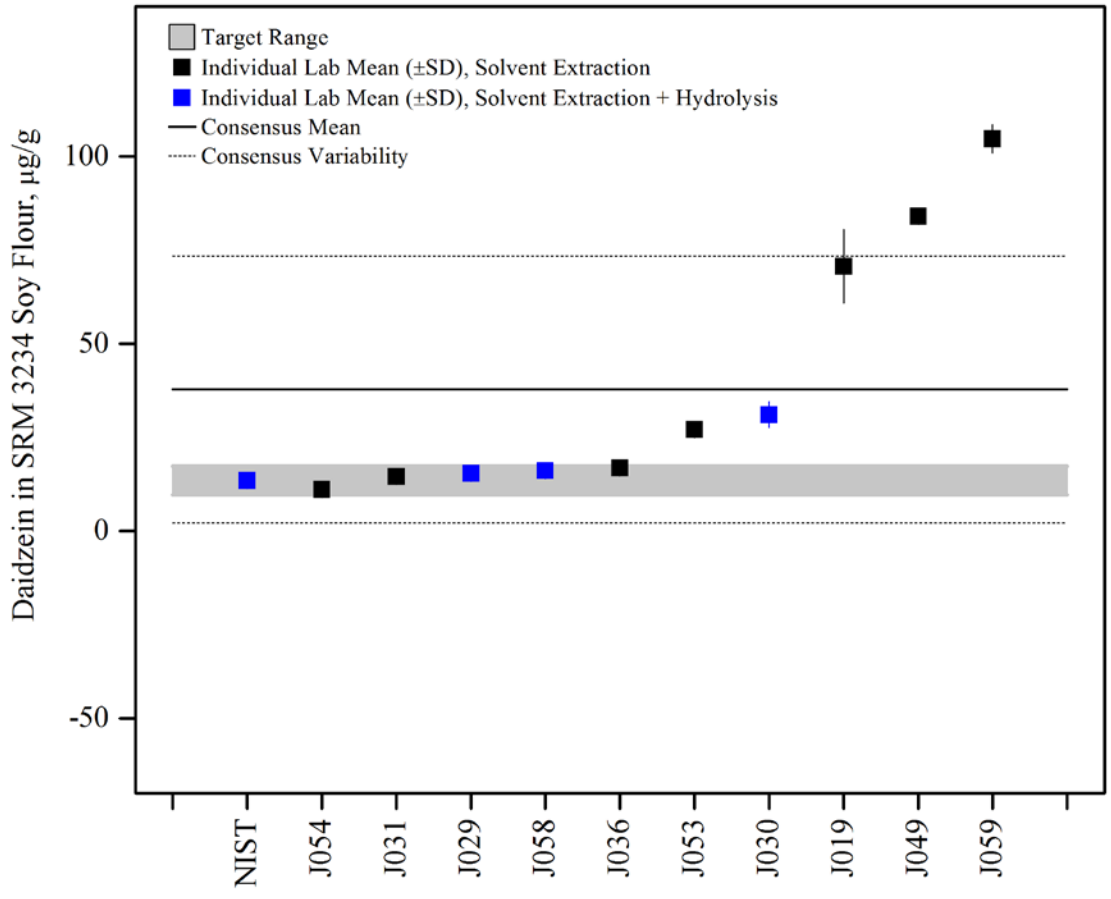

Figure 56. Daidzein in SRM 3234 Soy Flour (data summary view, sample preparation comparison). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The color of the data point represents the sample preparation method employed. The black solid line represents the consensus mean, and the black dotted lines represent the consensus variability calculated as one standard deviation about the consensus mean. The gray shaded region represents the target zone for "acceptable" performance, which encompasses the NIST value determined by LC/abs and ID-LC/MS, bounded by an estimated uncertainty calculated as the standard deviation between the means of values determined by each method.

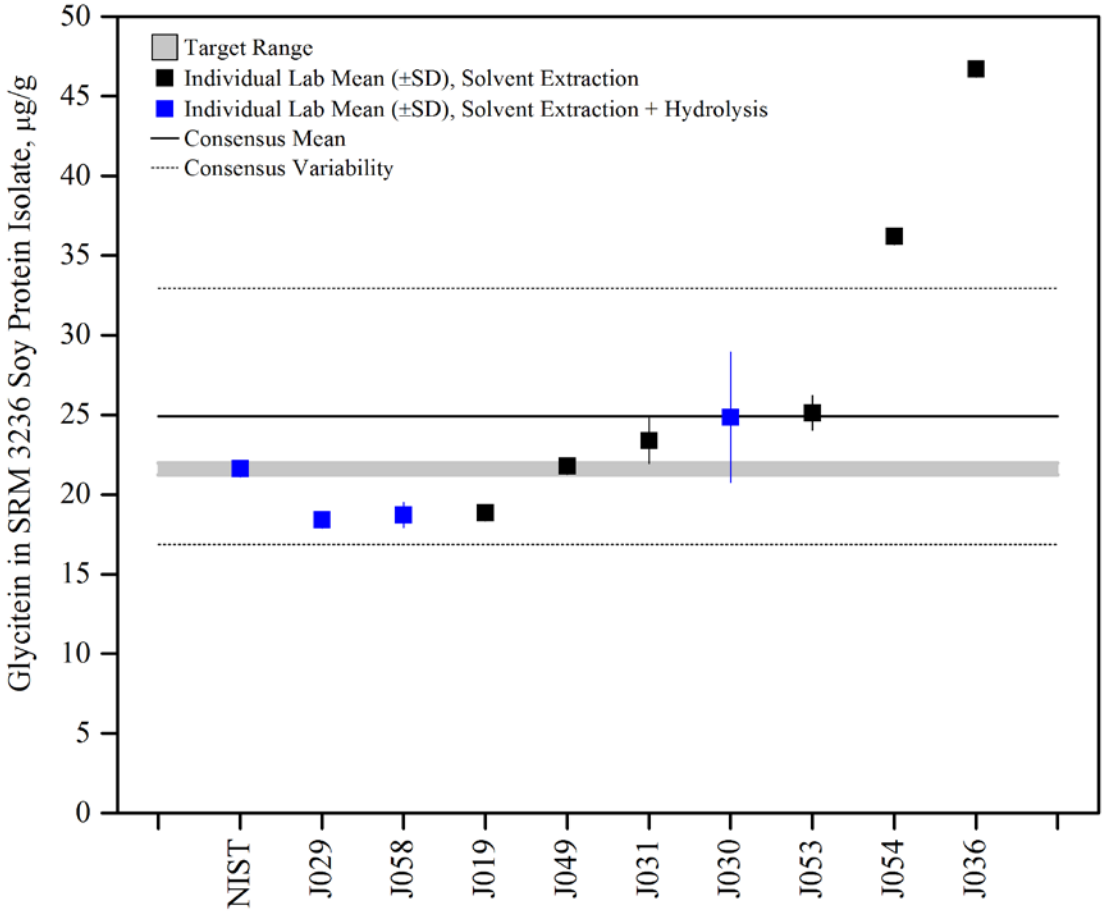

Figure 57. Glycitein in SRM 3236 Soy Protein Isolate (data summary view, sample preparation comparison). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The color of the data point represents the sample preparation method employed. The black solid line represents the consensus mean, and the black dotted lines represent the consensus variability calculated as one standard deviation about the consensus mean. The gray shaded region represents the target zone for "acceptable" performance, which encompasses the NIST certified value bounded by twice its uncertainty (U_{95}).

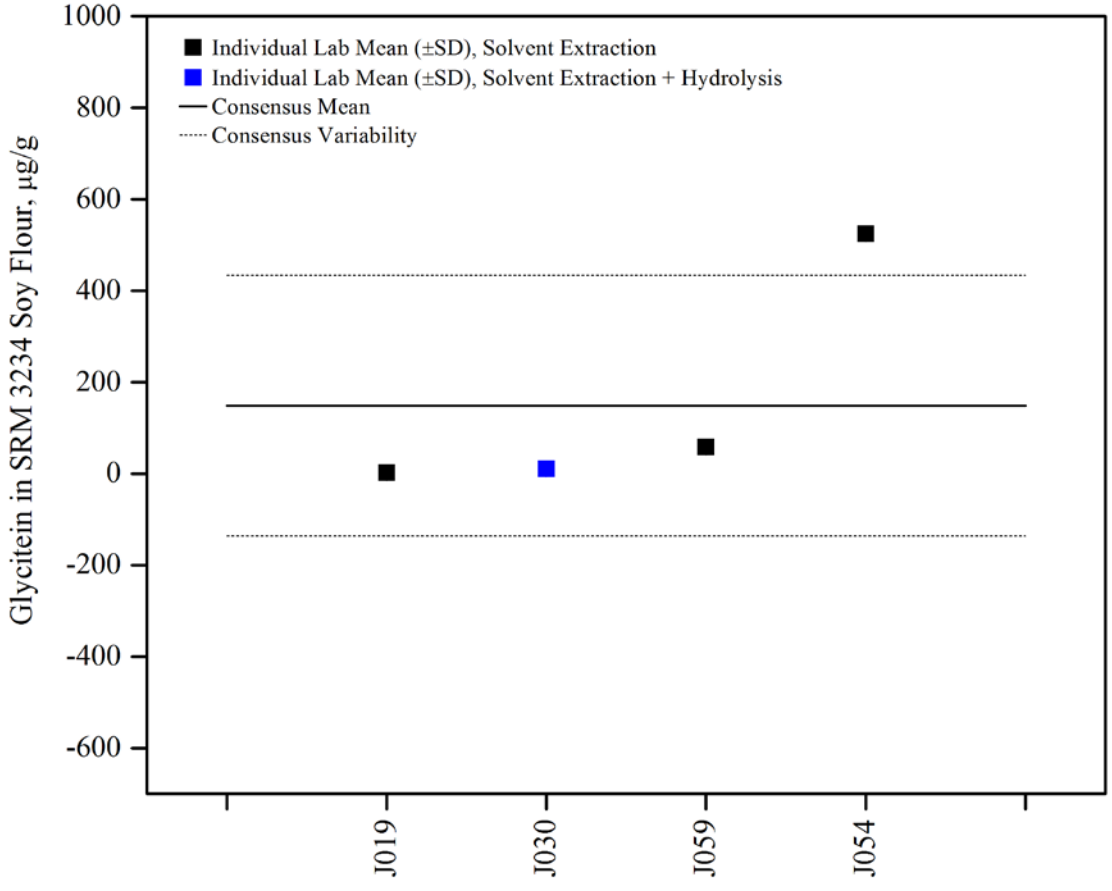

Figure 58. Glycitein in SRM 3234 Soy Flour (data summary view, sample preparation comparison). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The color of the data point represents the sample preparation method employed. The black solid line represents the consensus mean, and the black dotted lines represent the consensus variability calculated as one standard deviation about the consensus mean.

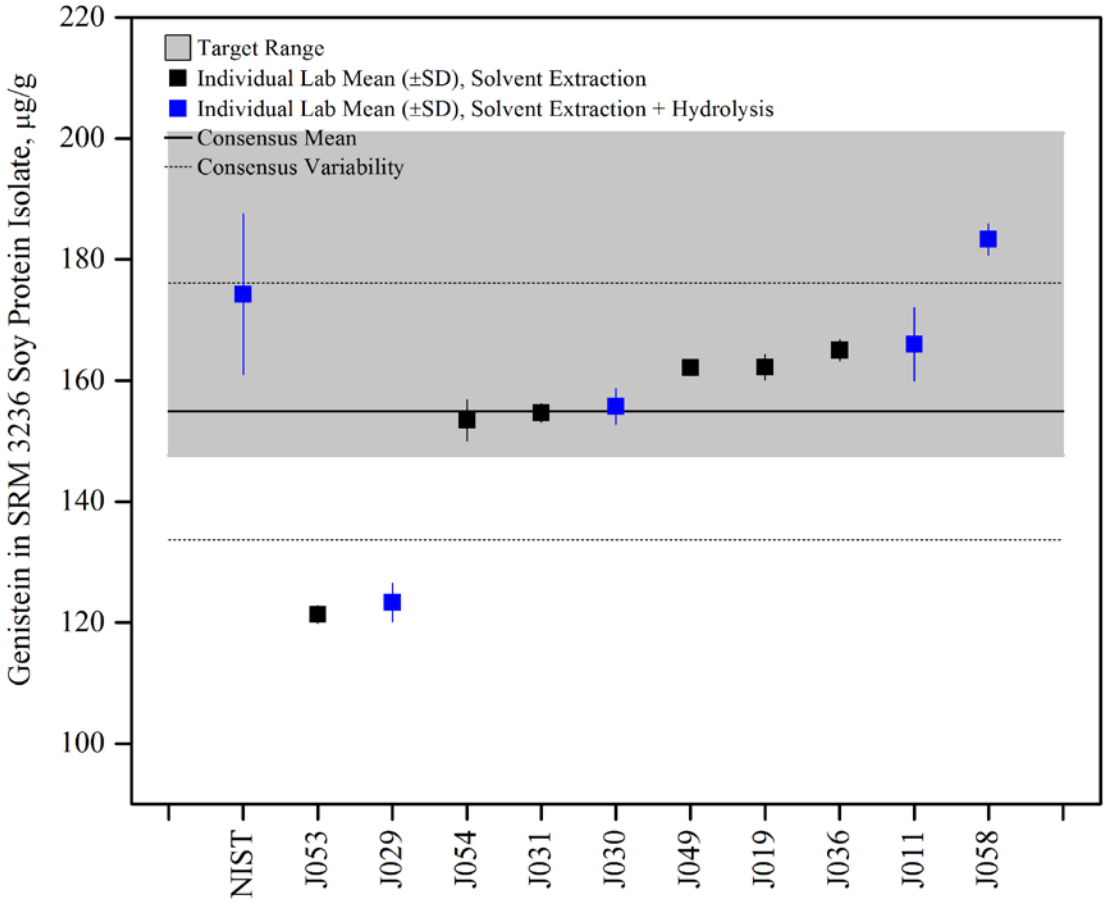

Figure 59. Genistein in SRM 3236 Soy Protein Isolate (data summary view, sample preparation comparison). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The color of the data point represents the sample preparation method employed. The black solid line represents the consensus mean, and the black dotted lines represent the consensus variability calculated as one standard deviation about the consensus mean. The gray shaded region represents the target zone for "acceptable" performance, which encompasses the NIST certified value bounded by twice its uncertainty (U_{95}).

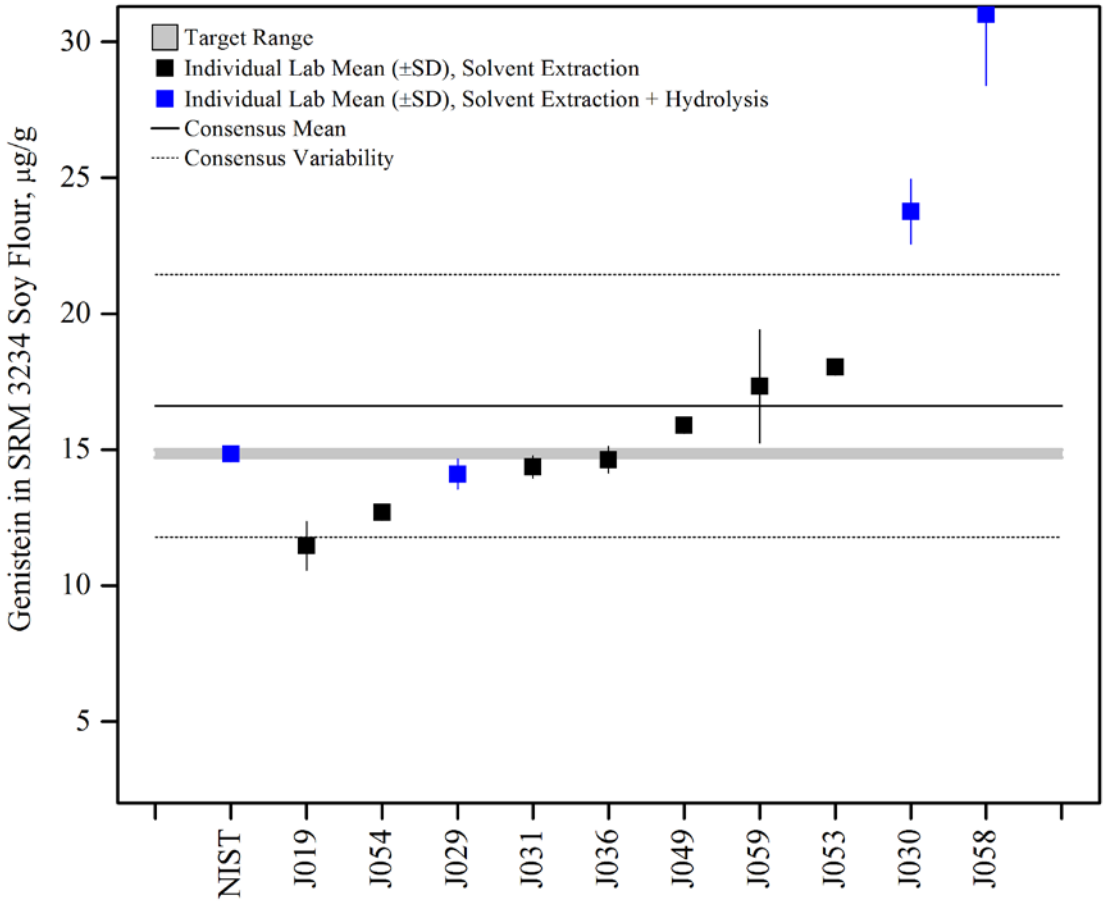

Figure 60. Genistein in SRM 3234 Soy Flour (data summary view, sample preparation comparison). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The color of the data point represents the sample preparation method employed. The black solid line represents the consensus mean, and the black dotted lines represent the consensus variability calculated as one standard deviation about the consensus mean. The gray shaded region represents the target zone for "acceptable" performance, which encompasses the NIST value determined by LC/abs and ID-LC/MS, bounded by an estimated uncertainty calculated as the standard deviation between the means of values determined by each method.

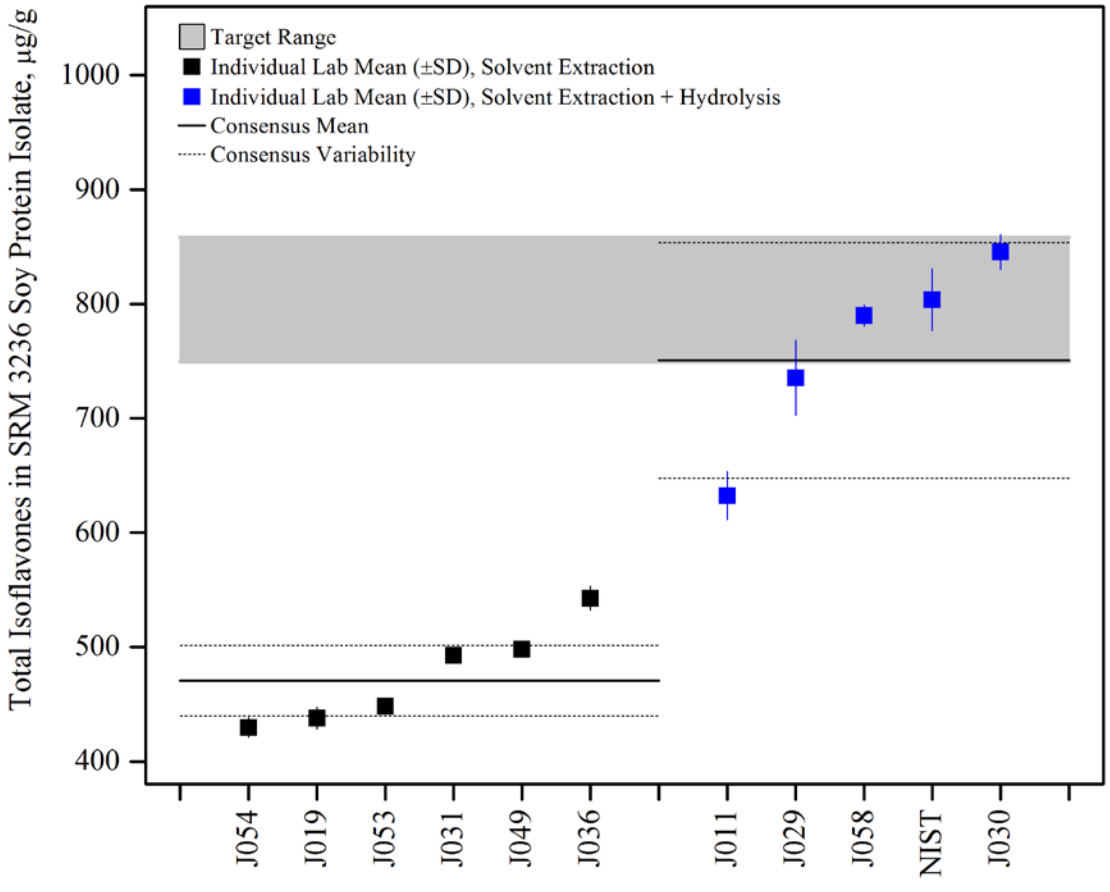

Figure 61. Total isoflavones in SRM 3236 Soy Protein Isolate (data summary view, sample preparation comparison). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The color of the data point represents the sample preparation method employed. The black solid lines represents the consensus mean for each sample preparation method, and the black dotted lines represent the consensus variability calculated as one standard deviation about the consensus mean for that sample preparation method. The gray shaded region represents the target zone for "acceptable" performance, which encompasses the NIST certified value bounded by twice its uncertainty (U_{95}).

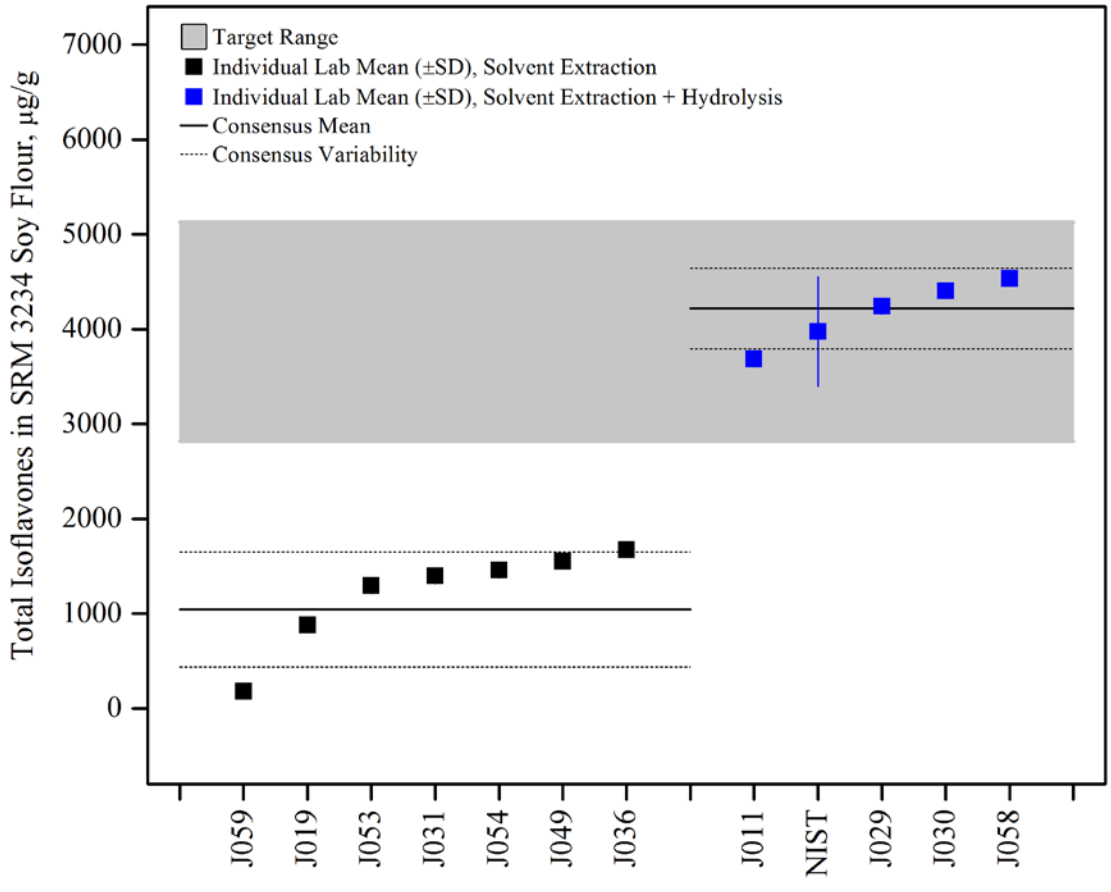

Figure 62. Total isoflavones in SRM 3234 Soy Flour (data summary view, sample preparation comparison). In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). The color of the data point represents the sample preparation method employed. The black solid lines represents the consensus mean for each sample preparation method, and the black dotted lines represent the consensus variability calculated as one standard deviation about the consensus mean for that sample preparation method. The gray shaded region represents the target zone for "acceptable" performance, which encompasses the NIST value determined by LC/abs and ID-LC/MS, bounded by an estimated uncertainty calculated as the standard deviation between the means of values determined by each method.

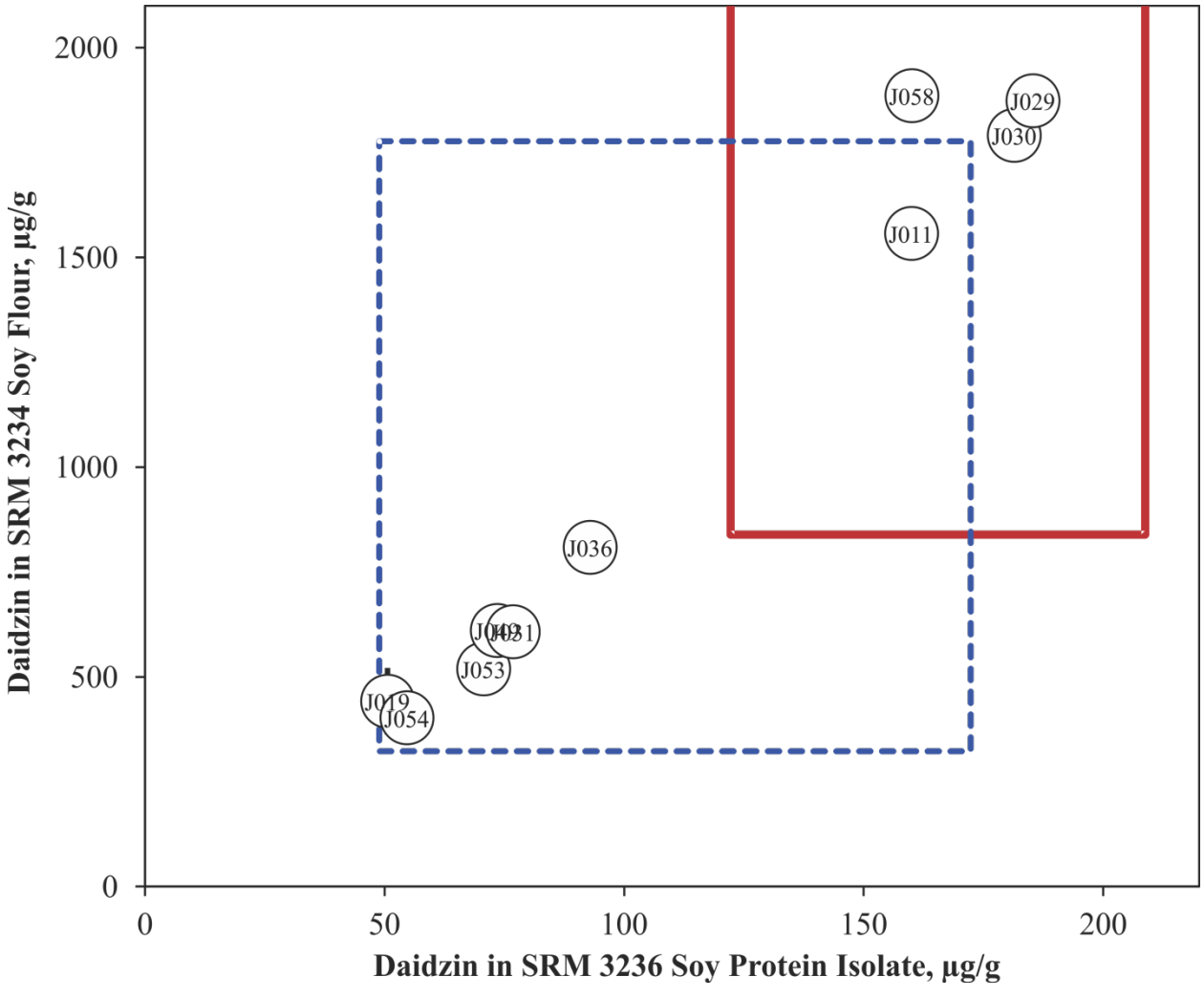

Figure 63. Daidzin in SRM 3236 Soy Protein Isolate and SRM 3234 Soy Flour (sample/control comparison view). In this view, the individual laboratory results for the control (soy protein isolate) with a certified value for the analyte are compared to the results for an unknown (soy flour). The solid red box represents the target zone for the control (x-axis) and unknown sample (y-axis). The dotted blue box represents the consensus zone for the control (x-axis) and the unknown sample (y-axis).

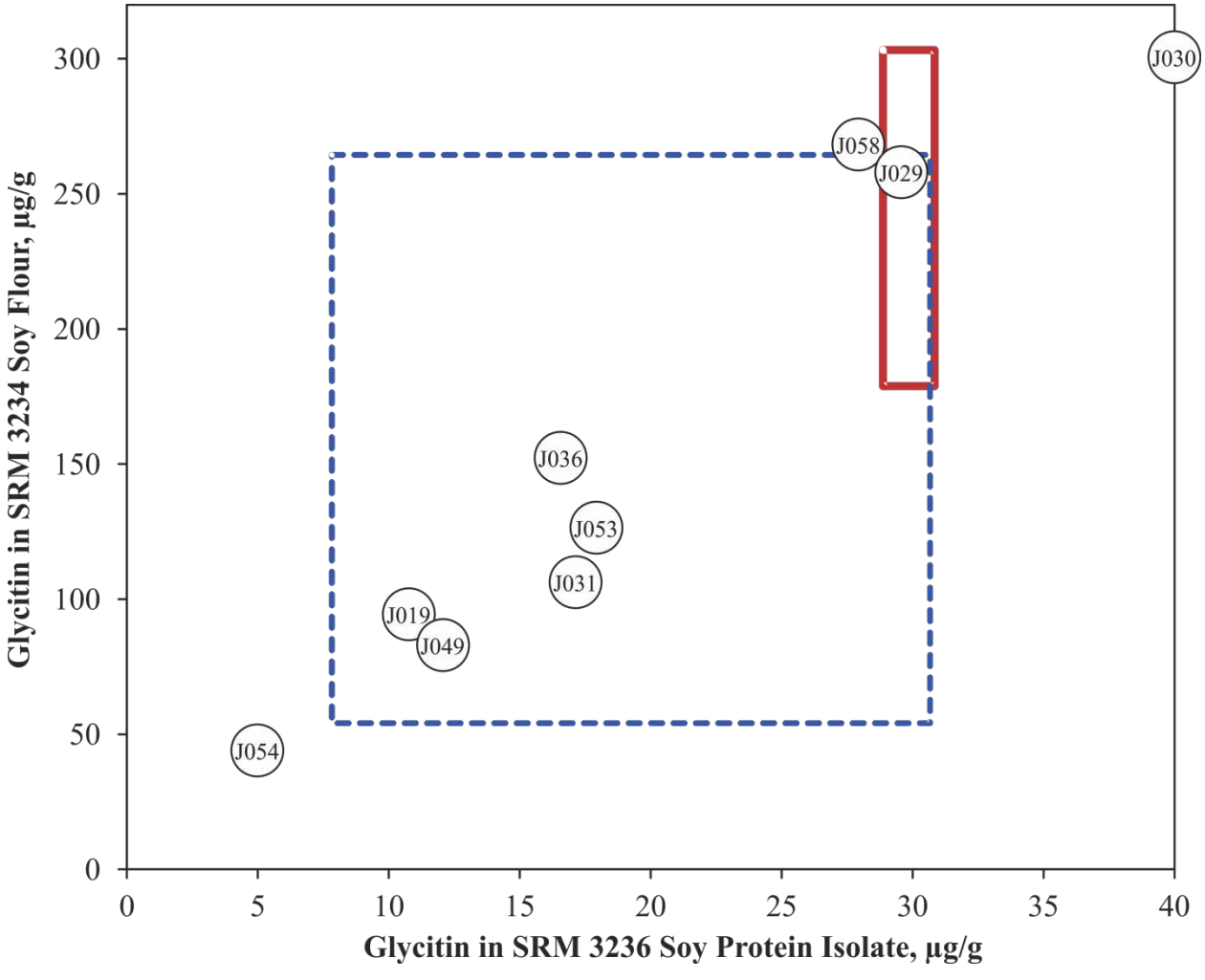

Figure 64. Glycitin in SRM 3236 Soy Protein Isolate and SRM 3234 Soy Flour (sample/control comparison view). In this view, the individual laboratory results for the control (soy protein isolate) with a certified value for the analyte are compared to the results for an unknown (soy flour). The solid red box represents the target zone for the control (x-axis) and unknown sample (y-axis). The dotted blue box represents the consensus zone for the control (x-axis) and the unknown sample (y-axis).

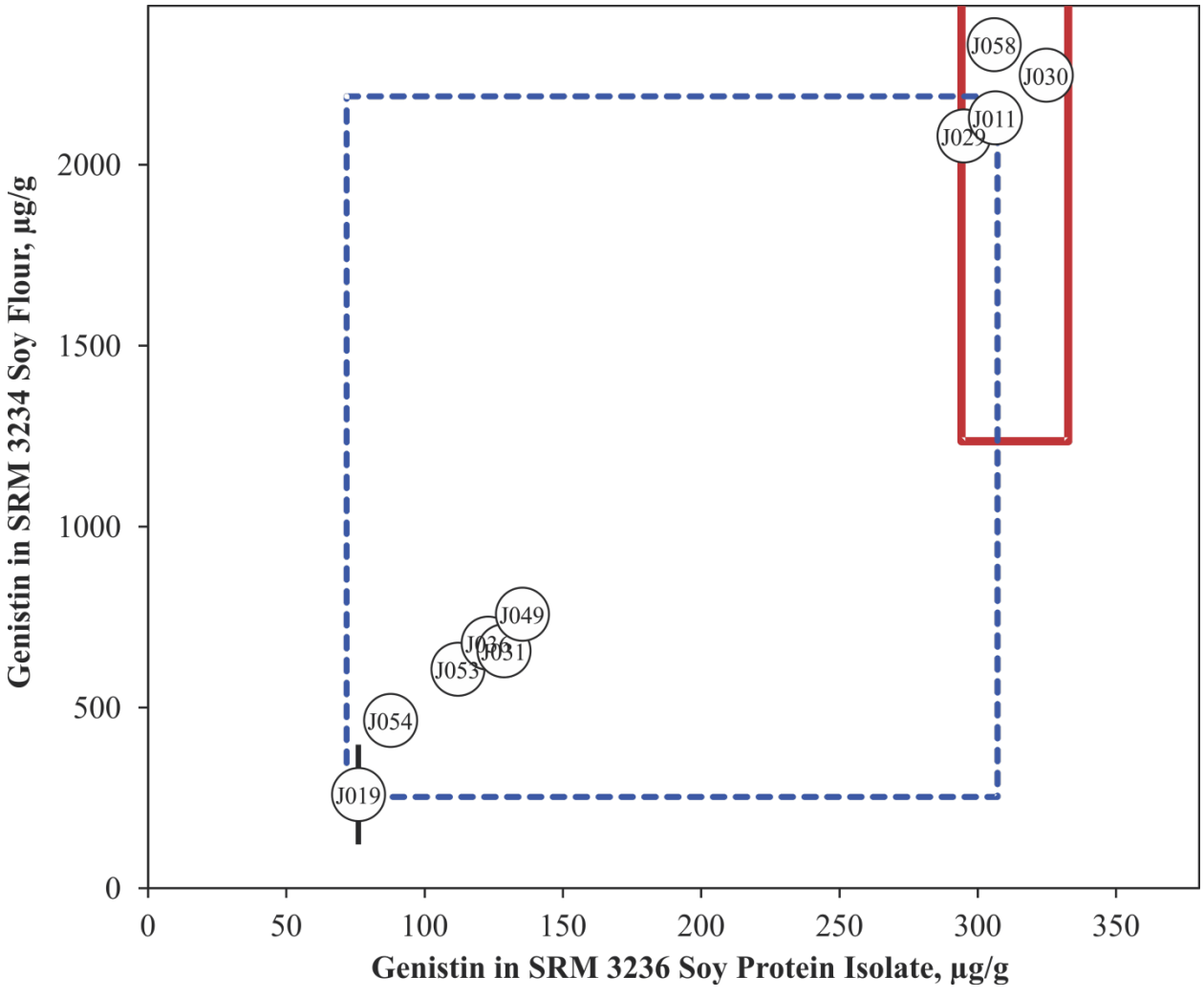

Figure 65. Genistin in SRM 3236 Soy Protein Isolate and SRM 3234 Soy Flour (sample/control comparison view). In this view, the individual laboratory results for the control (soy protein isolate) with a certified value for the analyte are compared to the results for an unknown (soy flour). The solid red box represents the target zone for the control (x-axis) and unknown sample (y-axis). The dotted blue box represents the consensus zone for the control (x-axis) and the unknown sample (y-axis).

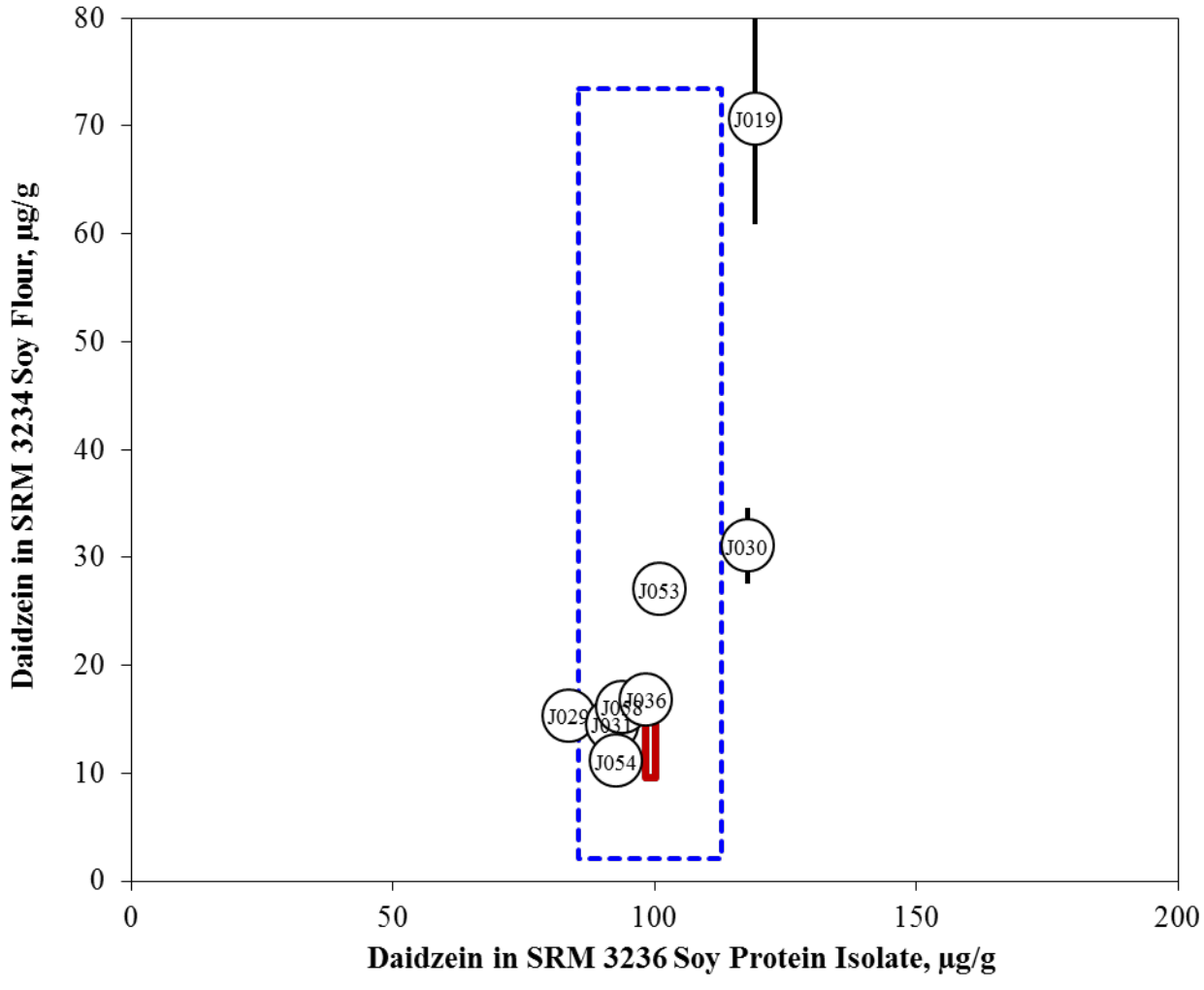

Figure 66. Daidzein in SRM 3236 Soy Protein Isolate and SRM 3234 Soy Flour (sample/control comparison view). In this view, the individual laboratory results for the control (soy protein isolate) with a certified value for the analyte are compared to the results for an unknown (soy flour). The solid red box represents the target zone for the control (x-axis) and unknown sample (y-axis). The dotted blue box represents the consensus zone for the control (x-axis) and the unknown sample (y-axis).

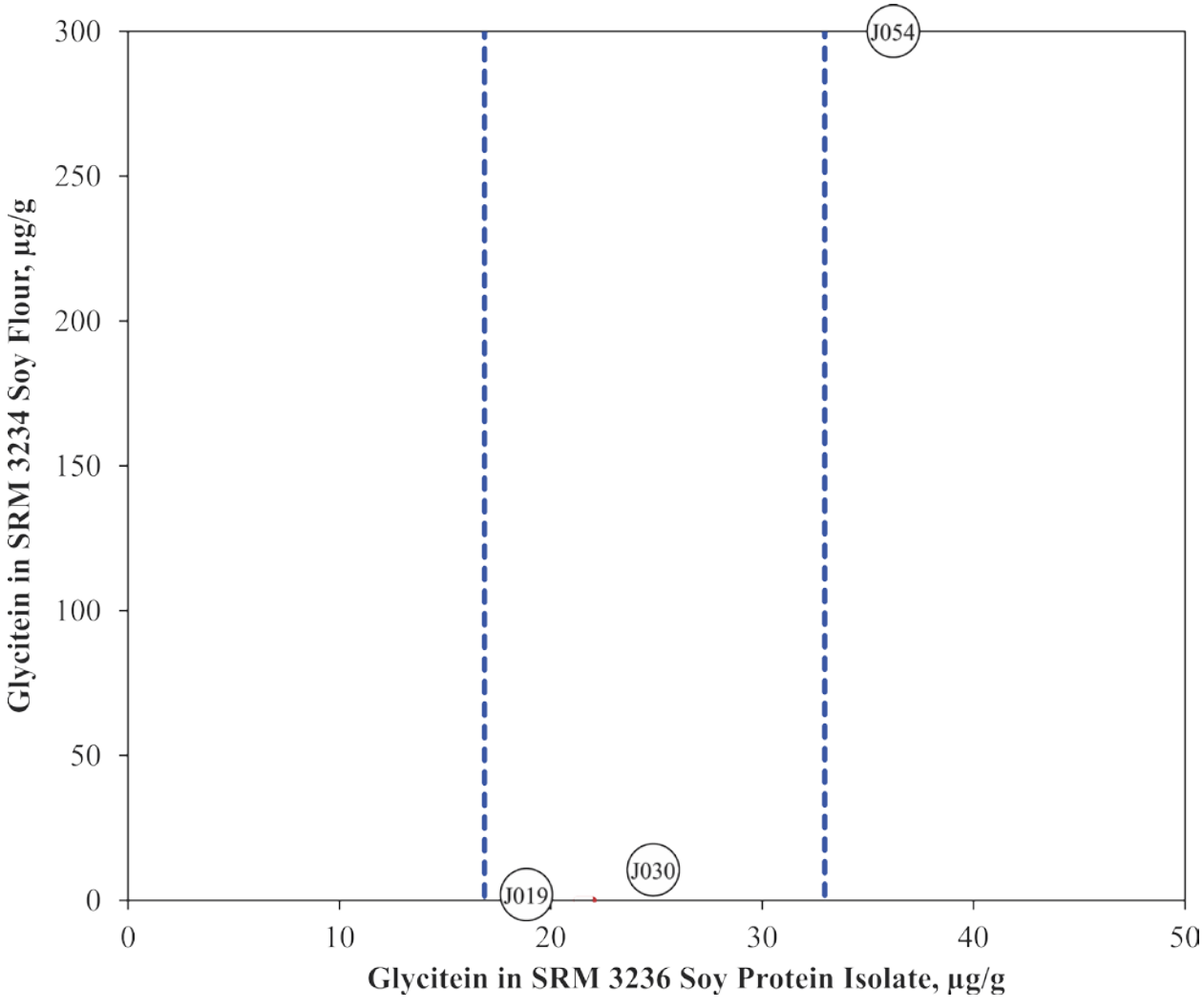

Figure 67. Glycitein in SRM 3236 Soy Protein Isolate and SRM 3234 Soy Flour (sample/control comparison view). In this view, the individual laboratory results for the control (soy protein isolate) with a certified value for the analyte are compared to the results for an unknown (soy flour). The dotted blue box represents the consensus zone for the control (x-axis) and the unknown sample (y-axis).

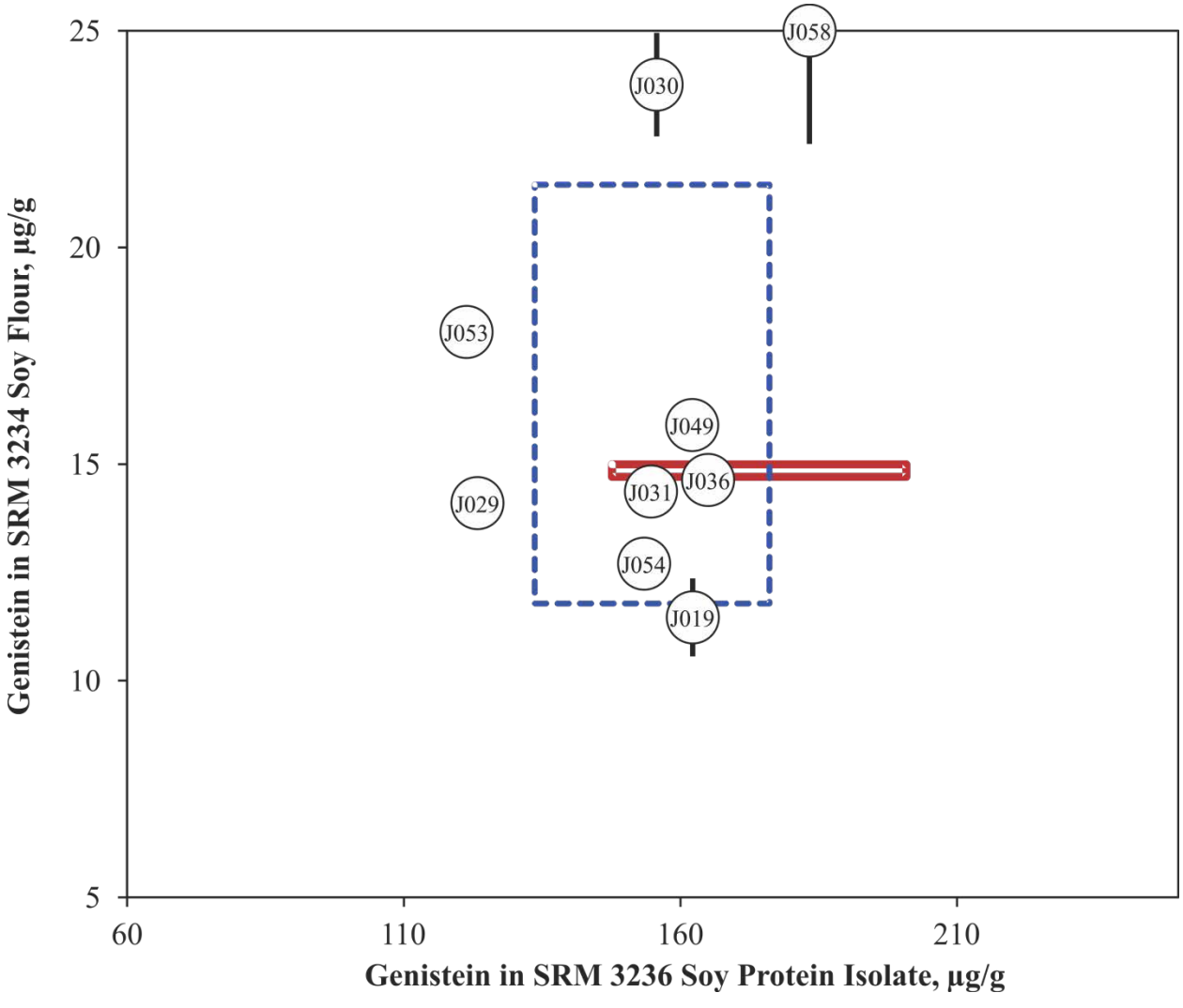

Figure 68. Genistein in SRM 3236 Soy Protein Isolate and SRM 3234 Soy Flour (sample/control comparison view). In this view, the individual laboratory results for the control (soy protein isolate) with a certified value for the analyte are compared to the results for an unknown (soy flour). The solid red box represents the target zone for the control (x-axis) and unknown sample (y-axis). The dotted blue box represents the consensus zone for the control (x-axis) and the unknown sample (y-axis).

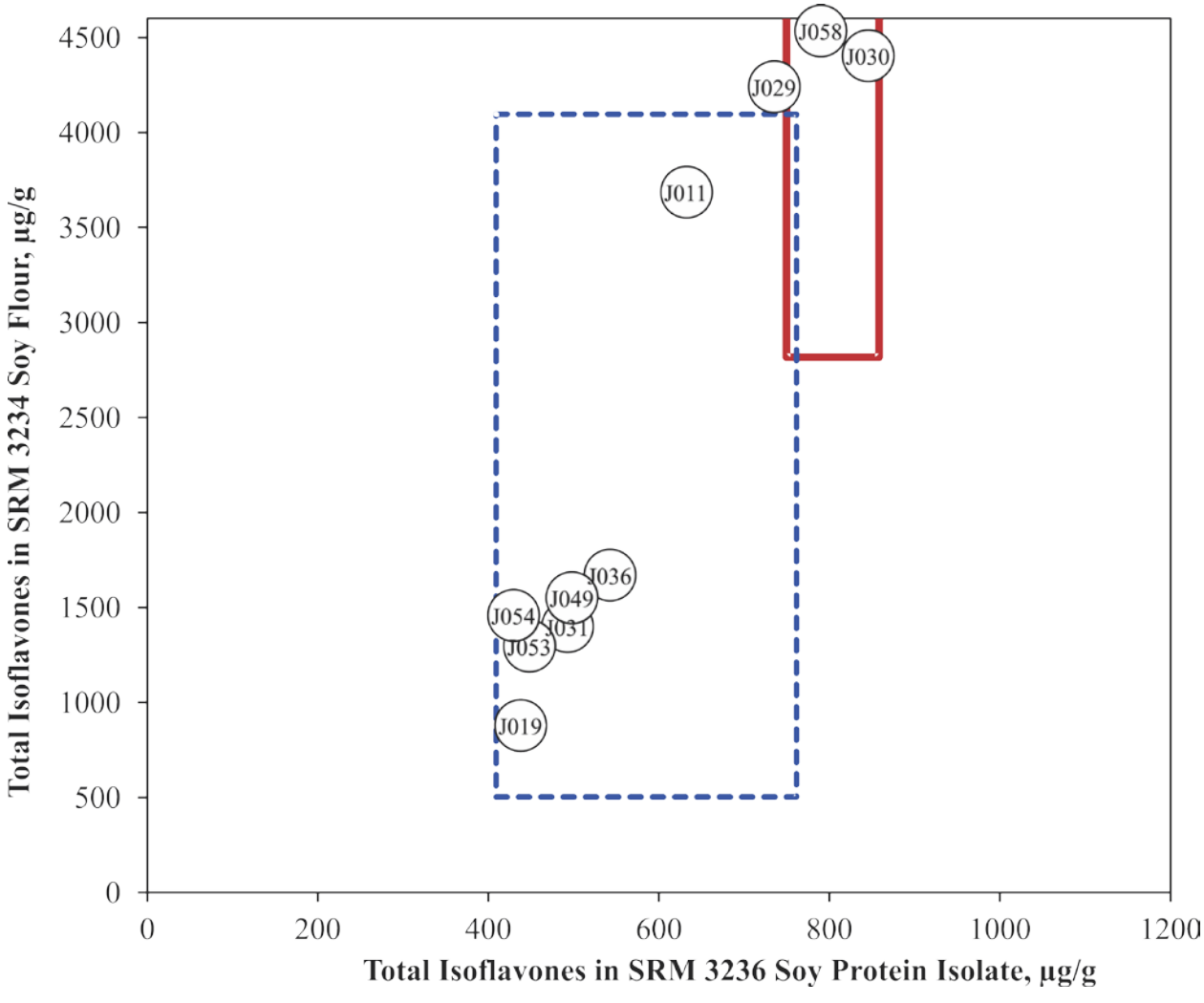

Figure 69. Total isoflavones in SRM 3236 Soy Protein Isolate and SRM 3234 Soy Flour (sample/control comparison view). In this view, the individual laboratory results for the control (soy protein isolate) with a certified value for the analyte are compared to the results for an unknown (soy flour). The 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).

BOTANICAL IDENTITY OF PURE AND ADULTERATED GINKGO BILOBA

Study Overview

In this study, ten vials labeled *Ginkgo biloba* extract and ten vials labeled *Ginkgo biloba* leaf were provided to the participants.

Sample Information

Gingko biloba Extract. Participants were provided with ten vials labeled *Ginkgo biloba* extract. Before use, participants were instructed to mix each vial thoroughly. Participants were asked to report "yes" for vials identified as *Ginkgo biloba*. If possible, reporting of the mass fraction (percentage) of *Ginkgo biloba* was requested. Participants were asked to report "no" for vials identified as not *Ginkgo biloba*. If possible, participants were asked to indicate the main constituent of the sample.

Gingko biloba Leaf. Participants were provided with ten vials labeled *Ginkgo biloba* leaf. Before use, participants were instructed to mix each vial thoroughly. Participants were asked to report "yes" for vials identified as *Ginkgo biloba*. If possible, reporting of the mass fraction (percentage) of *Ginkgo biloba* was requested. Participants were asked to report "no" for vials identified as not *Ginkgo biloba*. If possible, participants were asked to indicate the main constituent of the sample.

Study Results

- Twenty-five laboratories enrolled in this exercise and received samples. Nineteen laboratories reported results for some portion of the study (76 % participation).
- The *Gingko biloba* extract samples were adulterated with 0 %, 10 %, 25 %, 50 %, or 75 % green tea extract and 0 %, 10 %, 25 %, 50 %, or 75 % of microcellulose filler.
- Six laboratories attempted to identify the mass percentage of *Gingko biloba* extract in each sample.
- The *Gingko biloba* leaf samples were adulterated with 0 %, 10 %, 25 %, 50 %, or 75 % green tea leaves and 0 %, 10 %, 25 %, 50 %, or 75 % of microcellulose filler.
- Five laboratories attempted to identify the mass percentage of *Gingko biloba* leaves in each sample.
- Laboratories that used only thin-layer chromatography (TLC) for identity and detection of adulteration were better able to detect adulteration with other plants than with the microcellulose, which added no bands to the chromatogram.
- Laboratories were able to detect adulteration with green tea at lower levels in the plant material than in the extract material.
- Detection of adulteration with microcellulose was approximately the same in the plant material and the extract material.

Technical Recommendations

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

- TLC is a good screening method for identity and for finding adulteration, provided the adulterant has characteristic chromatographic bands that are different from those of the plant material under investigation.
- Laboratories that performed multiple methods (e.g., TLC and microscopy) were able to provide quantitative results for the percent adulteration as well as information as to whether or not the sample was an adulterated *Ginkgo* product.

- In future studies, more specific questions will be asked about testing methods.
- Laboratories will be given specific instructions on whether to test for authenticity/identity or adulteration.

Table 33. Individual data summary table (NIST) for botanical identity of pure and adulterated *Ginkgo biloba*.

National Institute of Standards & Technology

Exercise J - May 2013 - Botanical ID

Lab Code:	NIST		1. Your Results		2. Community Results						3. Target	
Sample		Adulterant	Y/N	%	$N_{Y/N}$	N_{Yes}	N_{No}	$N_{\%}$	Avg	SD	Y/N_{NIST}	$\%_{NIST}$
L8	*Ginkgo* Leaves	--	Yes	100%	19	17	2	3	98%	3%	Yes	100%
L5	*Ginkgo* Leaves	Green Tea Leaves	Yes	90%	19	9	10	5	82%	6%	Yes	90%
L9	*Ginkgo* Leaves	Green Tea Leaves	Yes	75%	19	7	12	4	69%	3%	Yes	75%
L2	*Ginkgo* Leaves	Green Tea Leaves	Yes	50%	19	7	12	5	58%	29%	Yes	50%
L6	*Ginkgo* Leaves	Green Tea Leaves	Yes	25%	19	8	11	5	28%	6%	Yes	25%
L4	*Ginkgo* Leaves	--	Yes	100%	19	17	2	4	84%	33%	Yes	100%
L1	*Ginkgo* Leaves	Microcellulose	Yes	90%	19	16	3	4	89%	5%	Yes	90%
L7	*Ginkgo* Leaves	Microcellulose	Yes	75%	19	16	3	3	77%	3%	Yes	75%
L3	*Ginkgo* Leaves	Microcellulose	Yes	50%	19	15	4	4	53%	5%	Yes	50%
L10	*Ginkgo* Leaves	Microcellulose	Yes	25%	19	15	4	3	18%	8%	Yes	25%
E5	*Ginkgo* Extract	--	Yes	100%	18	16	2	4	90%	20%	Yes	100%
E1	*Ginkgo* Extract	Green Tea Extract	Yes	90%	18	14	4	4	85%	7%	Yes	90%
E2	*Ginkgo* Extract	Green Tea Extract	Yes	75%	17	5	12	5	62%	15%	Yes	75%
E4	*Ginkgo* Extract	Green Tea Extract	Yes	50%	17	6	11	6	38%	26%	Yes	50%
E9	*Ginkgo* Extract	Green Tea Extract	Yes	25%	17	7	10	3	28%	41%	Yes	25%
E7	*Ginkgo* Extract	--	Yes	100%	18	17	1	4	98%	3%	Yes	100%
E8	*Ginkgo* Extract	Microcellulose	Yes	90%	18	7	11	4	68%	22%	Yes	90%
E3	*Ginkgo* Extract	Microcellulose	Yes	75%	17	14	3	4	73%	13%	Yes	75%
E6	*Ginkgo* Extract	Microcellulose	Yes	50%	17	12	5	4	43%	10%	Yes	50%
E10	*Ginkgo* Extract	Microcellulose	Yes	25%	17	12	5	2	10%	0%	Yes	25%

Table 34. Data summary table for *Ginkgo biloba* extracts (yes/no).

		***Ginkgo biloba* Extracts**									
	Adulterant	**Green Tea Extract**					**Inert Filler**				
	Mass % Ginkgo	**100%**	**90%**	**75%**	**50%**	**25%**	**100%**	**90%**	**75%**	**50%**	**25%**
	Lab/Sample	**E5**	**E1**	**E2**	**E4**	**E9**	**E7**	**E8**	**E3**	**E6**	**E10**
Individual Results	J001	Yes	Yes	No	No	No	Yes	No	Yes	Yes	Yes
	J002	Yes	Yes	No	No	No	Yes	No	Yes	Yes	Yes
	J003	Yes	No	No	No	No	Yes	No	No	No	No
	J005										
	J008	Yes	No	No	No	No	Yes	Yes	Yes	No	No
	J009										
	J010	Yes	Yes				Yes	Yes			
	J011										
	J015	Yes	Yes	No	No	No	Yes	No	Yes	Yes	Yes
	J018	no	no	no	yes	yes	no	no	no	no	no
	J020	Yes	Yes	No	No	No	Yes	No	Yes	Yes	Yes
	J027										
	J029	Yes	Yes	No	No	No	Yes	No	Yes	Yes	No
	J031	No	Yes	No	No	No	Yes	No	Yes	No	Yes
	J032	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
	J035										
	J036	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
	J038										
	J043	Yes	Yes	No	No	No	Yes	No	Yes	Yes	Yes
	J049	Yes	Yes	No	No	Yes	Yes	No	Yes	Yes	Yes
	J050										
	J052	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
	J053										
	J057	Yes	No	No	No	No	Yes	No	No	No	No
	J059										
Community Results	**N**	17	17	16	16	16	17	17	16	16	16
	Yes	15	13	4	5	6	16	6	13	11	11
	No	2	4	12	11	10	1	11	3	5	5
	% Yes	88%	76%	25%	31%	38%	94%	35%	81%	69%	69%
	% No	12%	24%	75%	69%	63%	6%	65%	19%	31%	31%

Table 35. Data summary table for *Ginkgo biloba* extracts (mass percentage).

		***Ginkgo biloba* Extracts**									
	Adulterant	**Green Tea Extract**					**Inert Filler**				
	Mass % Ginkgo	**100%**	**90%**	**75%**	**50%**	**25%**	**100%**	**90%**	**75%**	**50%**	**25%**
	Lab/Sample	**E5**	**E1**	**E2**	**E4**	**E9**	**E7**	**E8**	**E3**	**E6**	**E10**
Individual Results	J001										
	J002										
	J003	100%	80%	60%	30%	10%	100%	77%	60%	30%	10%
	J005										
	J008										
	J009										
	J010										
	J011										
	J015										
	J018				0.10%	0.08%					
	J020										
	J027										
	J029										
	J031	60%	80%	50%	70%				90%	50%	
	J032	100%	85%	65%	35%	10-15%	95%	80%	65%	40%	10-15%
	J035										
	J036										
	J038										
	J043										
	J049										
	J050										
	J052	12%	10%	8%	5%	2%	11%	10%	8%	5%	1%
	J053										
	J057	100%	95%	50%	25%	75%	100%	80%	75%	50%	10%
	J059										

Table 36. Data summary table for *Ginkgo biloba* leaves (yes/no).

		***Ginkgo biloba* Leaves**									
	Adulterant	**Green Tea Leaves**					**Inert Filler**				
	Mass % Ginkgo	**100%**	**90%**	**75%**	**50%**	**25%**	**100%**	**90%**	**75%**	**50%**	**25%**
	Lab/Sample	**L8**	**L5**	**L9**	**L2**	**L6**	**L4**	**L1**	**L7**	**L3**	**L10**
Individual Results	J001	Yes	No	No	No	No	Yes	Yes	Yes	Yes	Yes
	J002	Yes	No	No	No	No	Yes	Yes	Yes	Yes	Yes
	J003	Yes	No	No	No	No	Yes	No	No	No	No
	J005										
	J008	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	No
	J009										
	J010	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
	J011										
	J015	Yes	No	No	No	No	Yes	Yes	Yes	Yes	Yes
	J018	no	no	no	no	no	no	no	no	no	no
	J020	Yes	No	No	No	No	Yes	Yes	Yes	Yes	Yes
	J027										
	J029	Yes	No	No	No	No	Yes	Yes	Yes	Yes	Yes
	J031	No	Yes	No	No	Yes	No	Yes	Yes	Yes	Yes
	J032	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
	J035										
	J036	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
	J038										
	J043	Yes	No	No	No	No	Yes	Yes	Yes	Yes	Yes
	J049	Yes	Yes	No	No	No	Yes	Yes	Yes	Yes	Yes
	J050	Yes	No	No	No	No	Yes	Yes	Yes	Yes	Yes
	J052	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
	J053										
	J057	Yes	No	No	No	No	Yes	No	No	No	No
	J059	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Community Results	**N**	19	19	19	19	19	19	19	19	19	19
	Yes	17	9	7	7	8	17	16	16	15	15
	No	2	10	12	12	11	2	3	3	4	4
	% Yes	89%	47%	37%	37%	42%	89%	84%	84%	79%	79%
	% No	11%	53%	63%	63%	58%	11%	16%	16%	21%	21%

Table 37. Data summary table for *Ginkgo biloba* leaves (mass percentage).

		***Ginkgo biloba* Leaves**									
	Adulterant	**Green Tea Leaves**					**Inert Filler**				
	Mass % Ginkgo	**100%**	**90%**	**75%**	**50%**	**25%**	**100%**	**90%**	**75%**	**50%**	**25%**
	Lab/Sample	**L8**	**L5**	**L9**	**L2**	**L6**	**L4**	**L1**	**L7**	**L3**	**L10**
Individual Results	J001										
	J002										
	J003	100%	80%	70%	40%	30%	100%	85%	75%	50%	20%
	J005										
	J008										
	J009										
	J010										
	J011										
	J015										
	J018										
	J020										
	J027										
	J029										
	J031		75%		110%	35%	35%	85%		60%	
	J032	95%	85%	65%	45%	20%	100%	92%	75%	50%	25%
	J035										
	J036										
	J038										
	J043										
	J049										
	J050										
	J052	0.68%	0.68%	0.76%	0.59%	0.53%	0.69%	0.66%	0.50%	0.34%	0.28%
	J053										
	J057	100%	80%	70%	50%	25%	100%	95%	80%	50%	10%
	J059										

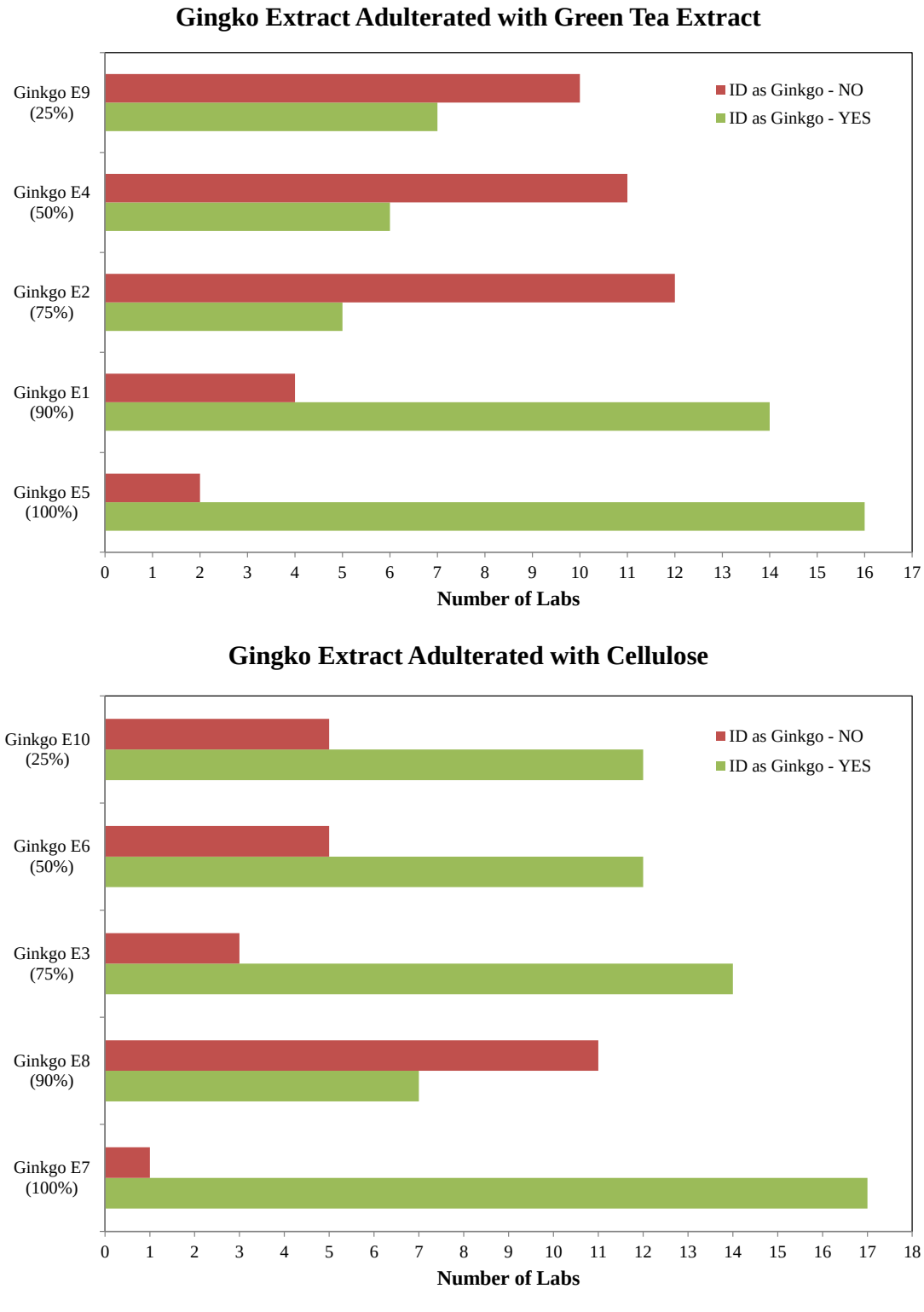

Figure 70. Adulterated *Ginkgo biloba* extract. These two charts show the number of laboratories reporting Ginkgo extract as being adulterated. The samples in the top chart were adulterated with varying amounts of green tea extract. The samples in the bottom chart were adulterated with varying amounts of cellulose.

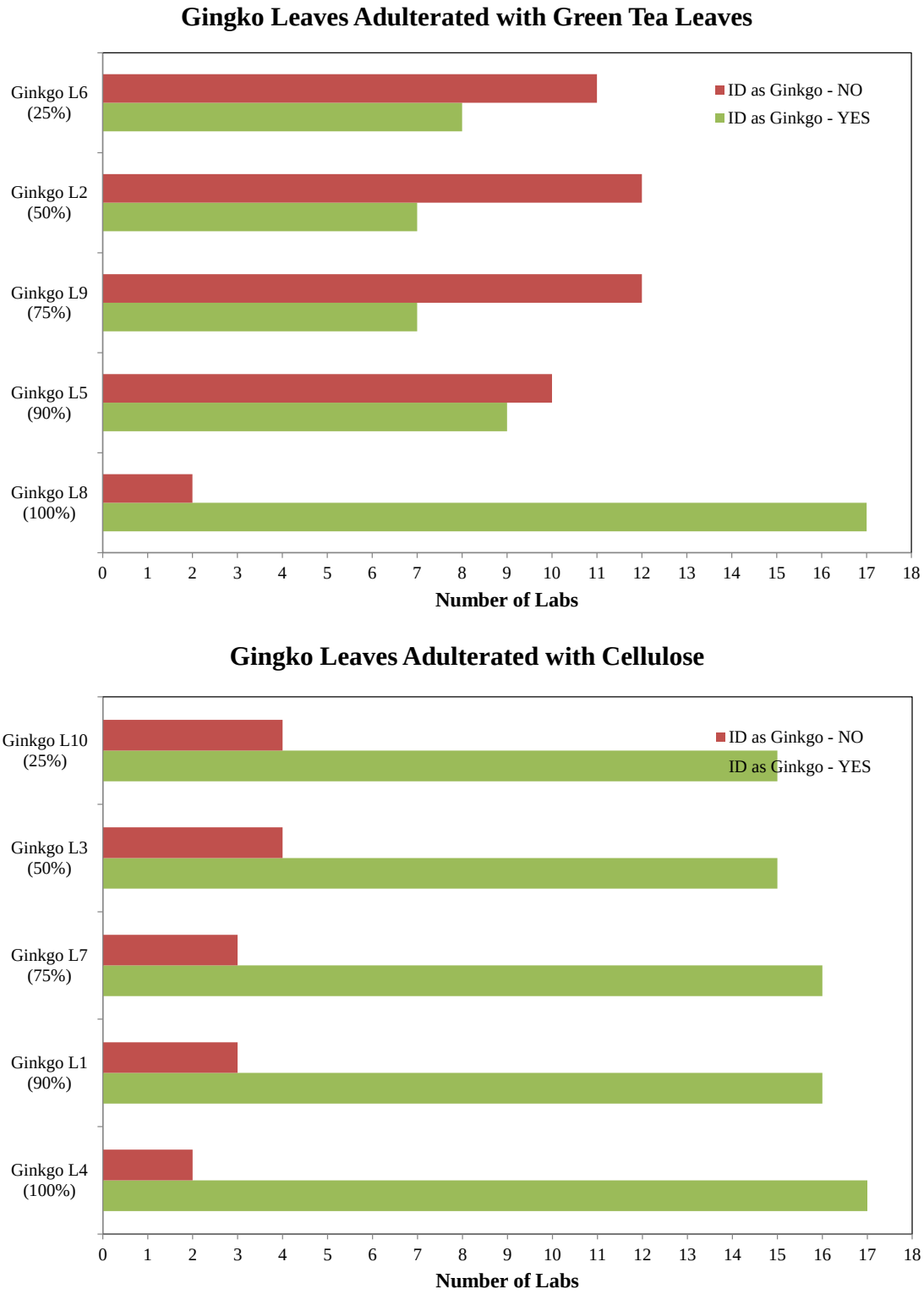

Figure 71. Adulterated *Ginkgo biloba* leaves. These two charts show the number of laboratories reporting Ginkgo leaves as being adulterated. The samples in the top chart were adulterated with varying amounts of green tea leaves. The samples in the bottom chart were adulterated with varying amounts of cellulose.