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**NIST Micronutrients Measurement
Quality Assurance Program
Winter 2005
Comparability Studies**

Results for Round Robin LVII
Fat-Soluble Vitamins and Carotenoids in Human Serum
and Round Robin 22 Ascorbic Acid in Human Serum

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U.S. Department of Commerce
Eco gtqp 'HOMgtt{, Acting Secretary

National Institute of Standards and Technology
Patrick D. Gallagher, Under Secretary of Commerce for Standards and Technology and Director

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Abstract

The National Institute of Standards and Technology coordinates the Micronutrients Measurement Quality Assurance Program (MMQAP) for laboratories that measure fat- and water-soluble vitamins and carotenoids in human serum and plasma. This report describes the design of and results for the Winter 2005 MMQAP measurement comparability improvement studies: 1) Round Robin LVII Fat-Soluble Vitamins and Carotenoids in Human Serum and 2) Round Robin 22 Total Ascorbic Acid in Human Serum. The materials for both studies were shipped to participants in November 2004; participants were requested to provide their measurement results by March 2, 2005.

Keywords

Human Serum
Retinol, α -Tocopherol, γ -Tocopherol, Total and *Trans*- β -Carotene
Total Ascorbic Acid

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Introduction

Beginning in 1988, the National Institute of Standards and Technology (NIST) has coordinated the Micronutrients Measurement Quality Assurance Program (MMQAP) for laboratories that measure fat- and water-soluble vitamins and carotenoids in human serum and plasma. The MMQAP provides participants with measurement comparability assessment through use of interlaboratory studies, Standard Reference Materials (SRMs) and control materials, and methods development and validation. Serum-based samples with assigned values for the target analytes (retinol, alpha-tocopherol, gamma/beta-tocopherol, *trans*- and total beta-carotene, and total ascorbic acid) and performance-evaluation standards are distributed by NIST to laboratories for analysis.

Participants use the methodology of their choice to determine analyte content in the control and study materials. Participants provide their data to NIST, where it is compiled and evaluated for trueness relative to the NIST value, within-laboratory precision, and concordance within the participant community. NIST provides the participants with a technical summary report concerning their performance for each exercise and suggestions for methods development and refinement. Participants who have concerns regarding their laboratory's performance are encouraged to consult with the MMQAP coordinators.

All MMQAP interlaboratory studies consist of individual units of batch-prepared samples that are distributed to each participant. For historical reasons these studies are referred to as "Round Robins". The MMQAP program and the nature of its studies are described elsewhere. [1,2]

Round Robin LVII: Fat-Soluble Vitamins and Carotenoids in Human Serum

Participants in the MMQAP Fat-Soluble Vitamins and Carotenoids in Human Serum Round Robin LVII comparability study (hereafter referred to as RR57) received four lyophilized and one liquid-frozen human serum test samples for analysis. Unless multiple vials were previously requested, participants received one vial of each serum. These sera were shipped on dry ice to participants in November 2004. The communication materials included in the sample shipment are provided in Appendix A.

Participants are requested to report values for all fat-soluble vitamin-related analytes that are of interest to their organizations. Not all participants report values for the target analytes, and many participants report values for non-target analytes.

The final report delivered to every participant in RR57 consists of three documents:

- A cover letter for the current study, a brief description of the other two documents, and a discussion of our analysis of the overall results that may be of broad interest. This cover letter is reproduced as Appendix B.
- The "All-Lab Report" that lists all of the reported measurement results, a number of consensus statistics for analytes reported by more than one participant, and the mean median and pooled SD from any prior distributions of the serum. This report also provides a numerical "score card" for each participant's measurement comparability for the more commonly reported analytes. This report is reproduced as Appendix C.

- An “Individualized Report” that graphically analyzes each participant’s results for all analytes reported by at least five participants. This report also provides a graphical summary of their measurement comparability. The graphical tools used in this report are described in detail elsewhere [3]. An example “Individualized Report” is reproduced as Appendix D.

Round Robin 22: Vitamin C in Human Serum

Participants in the MMQAP Vitamin C in Human Serum Round Robin 22 comparability study (hereafter referred to as RR22) received four frozen serum test samples, two frozen control sera, and a solid ascorbic acid control material for analysis. Unless multiple vials were previously requested, participants received one vial of each material. These sample materials were shipped on dry ice to participants in November 2004. The communication materials included in the sample shipment are provided in Appendix E.

The test and control serum materials were prepared by adding equal volumes of 10 % metaphosphoric acid (MPA) to human serum that had been spiked with ascorbic acid. While these samples contain some dehydroascorbic acid, its content is variable. Therefore, the participants report only total ascorbic acid (TAA, ascorbic acid plus dehydroascorbic acid). Participants are also encouraged to prepare calibration solutions from the supplied solid control to enable calibrating their serum measurements to the same reference standard.

The final report delivered to every participant in RR22 consists of three documents:

- A cover letter for the current study, a brief description of the other two documents, and a discussion of our analysis of overall results that may be of broad interest. This cover letter is reproduced as Appendix F.
- The “All-Lab Report” that summarizes all of the reported measurement results and provides several consensus statistics. This report is reproduced as Appendix G.
- An “Individualized Report” that graphically analyzes each participant’s results for TAA, including a graphical summary of their measurement comparability. The graphical tools used in this report are described in detail elsewhere [3]. An example “Individualized Report” is reproduced as Appendix H.

References

- 1 Duewer DL, Brown Thomas J, Kline MC, MacCrehan WA, Schaffer R, Sharpless KE, May WE, Crowell JA. NIST/NCI Micronutrients Measurement Quality Assurance Program: Measurement Repeatabilities and Reproducibilities for Fat-Soluble Vitamin-Related Compounds in Human Sera. *Anal Chem* 1997;69(7):1406-1413.
- 2 Margolis SA, Duewer DL. Measurement Of Ascorbic Acid in Human Plasma and Serum: Stability, Intralaboratory Repeatability, and Interlaboratory Reproducibility. *Clin Chem* 1996;42(8):1257-1262.
- 3 Duewer DL, Kline MC, Sharpless KE, Brown Thomas J, Gary KT, Sowell AL. Micronutrients Measurement Quality Assurance Program: Helping Participants Use Interlaboratory Comparison Exercise Results to Improve Their Long-Term Measurement Performance. *Anal Chem* 1999;71(9):1870-1878.

Appendix A. Shipping Package Inserts for RR57

The following three items were included in each package shipped to an RR57 participant:

- Cover letter
- Datasheet
- Packing List and Shipment Receipt Confirmation Form

The cover letter and datasheet were enclosed in a sealed waterproof bag along with the samples themselves. The packing list was placed at the top of the shipping box, between the cardboard covering and the foam insulation.



UNITED STATES DEPARTMENT OF COMMERCE
National Institute of Standards and Technology
Gaithersburg, Maryland 20899-0001

November 8, 2004

Dear Colleague:

Enclosed are the samples (Sera 309 – 313) for the first fat-soluble vitamins and carotenoids in serum round robin study (Round Robin LVII) for the fiscal year (FY) 05 NIST Micronutrients Measurement Quality Assurance Program. You will find one vial of each of one liquid-frozen and four lyophilized serum samples for analysis along with a form for reporting your results. When reporting your results, please submit one value for each analyte for a given serum sample. If a value is obtained below your limit of quantification, please indicate this result on the form by using NQ (*Not Quantified*). Results are due to NIST by **March 2, 2005**. Results received more than two weeks after the due date will not be included in the summary report for this round robin study. The feedback report concerning the study will be provided in April.

Lyophilized samples should be reconstituted with 1.0 mL of HPLC-grade water or equivalent. We recommend that dissolution be facilitated with 3 to 5 min agitation in an ultrasonic bath or at least 30 min at room temperature with intermittent swirling. (CAUTION: Vigorous shaking will cause foaming and possibly interfere with accurate measurement. The rubber stopper contains phthalate esters that may leach into the sample upon intermittent contact of the liquid sample with the stopper. These esters absorb strongly in the UV region and elute near retinol in most LC systems creating analytical problems.) Pipette a known volume of serum from the vial for analysis. The final volume of the reconstituted sample is greater than 1.0 mL. **Water should not be added to the liquid-frozen sample 313.**

For consistency, we request that laboratories use the following absorptivities (E 1% cm): retinol, 1843 at 325 nm (ethanol); retinyl palmitate, 975 at 325 nm (ethanol); α -tocopherol, 75.8 at 292 nm (ethanol); γ -tocopherol, 91.4 at 298 nm (ethanol); α -carotene, 2800 at 444 nm (hexane); β -carotene, 2560 at 450 nm (ethanol), 2592 at 452 nm (hexane); lycopene, 3450 at 472 nm (hexane).

Please mail or fax your results for Round Robin LVII to:

Micronutrients Measurement Quality Assurance Program
NIST
100 Bureau Drive Stop 8392
Gaithersburg, MD 20899-8392
Fax: (301) 977-0685

If you have questions or comments regarding this study, please call me at (301) 975-3120; e-mail me at jbthomas@nist.gov; or mail/fax queries to the above address.

Sincerely,

Jeanice Brown Thomas
Research Chemist
Analytical Chemistry Division
Chemical Science and Technology Laboratory

Enclosures

NIST

Participant #: _____

Date: _____

Round Robin LVII
NIST Micronutrients Measurement Quality Assurance Program

Analyte	309	310	311	312	313	Units*
total retinol						
trans-retinol						
didehydroretinol						
retinyl palmitate						
α -tocopherol						
γ/β -tocopherol						
δ -tocopherol						
total β -carotene						
trans- β -carotene						
total cis- β -carotene						
total α -carotene						
total lycopene						
trans-lycopene						
total β -cryptoxanthin						
total α -cryptoxanthin						
total lutein						
total zeaxanthin						
total lutein&zeaxanthin						
total Coenzyme Q10						
ubiquinol (QH ₂)						
ubiquinone (Qox)						
phylloquinone (K ₁)						
25-hydroxyvitamin D						
Other analytes?						

* we prefer $\mu\text{g/mL}$

Was serum 309 frozen when received? Yes | No

Comments:

Mail: M²QAP
 NIST, Stop 8392
 Gaithersburg, MD 20899-8392

Please return results **before**
 2-Mar-2005

A3

Fax: 301-977-0685
 Email: David.Duewer@NIST.gov

Participant #: _____

Date: _____

Fat-Soluble Vitamins Round Robin LVII
NIST Micronutrients Measurement Quality Assurance Program

Packing List and Shipment Receipt Confirmation Form

This box contains (we hope) one vial each of the following **five** FSV M²QAP sera:

Serum	Form	Reconstitute?
#309	Lyophilized	Yes (1 ml H ₂ O)
#310	Lyophilized	Yes (1 ml H ₂ O)
#311	Lyophilized	Yes (1 ml H ₂ O)
#312	Lyophilized	Yes (1 ml H ₂ O)
#313	Liquid frozen	No

- Please**
- 1) Open the pack immediately
 - 2) Check that it contains one vial each of the above samples
 - 3) Check if serum 313 arrived frozen
 - 4) Store the samples at -20 °C or below until analysis
 - 5) Complete the following information
 - 6) Fax the completed form to us at 301-977-0685
(or email requested information to david.duewer@nist.gov)

1) Date this shipment arrived: _____

2) Are all five vials intact? Yes | No
If "No", which one(s) were damaged?

3) Was there any dry-ice left in cooler? Yes | No

4) Did serum 313 arrive frozen? Yes | No

5) At what temperature are you storing the samples? _____ °C

6) When do you anticipate analyzing these samples? _____

Your prompt return of this information is appreciated.

The M²QAP Gang

Appendix B. Final Report for RR57

The following three pages are the final report as provided to all participants:

- Cover letter.
- An information sheet that:
 - describes the contents of the “All-Lab” report,
 - describes the content of the “Individualized” report,
 - describes the nature of the test samples and details their previous distributions, if any, and
 - summarizes aspects of the study that we believe may be of interest to the participants.



UNITED STATES DEPARTMENT OF COMMERCE
National Institute of Standards and Technology
Gaithersburg, Maryland 20899-0001

April 14, 2005

Dear Colleague:

Enclosed is the summary report of the results for round robin LVII (RR57) of the 2005 NIST Micronutrients Measurement Quality Assurance Program (M²QAP) for the fat-soluble vitamins and carotenoids in human serum. Included in this report are: (1) a summary of data and measurement comparability scores for all laboratories, (2) a detailed graphical analysis of your results; and (3) a graphical summary of your measurement comparabilities relative to the NIST assigned values. The NIST-assigned values are equally weighted means of the medians from this interlaboratory comparison exercise and the means from the analyses performed by NIST.

Data for evaluating laboratory performance in RR 57 are provided in the comparability summary (Score Card) on page 6 of the All Lab Report. Laboratory comparability is summarized as follows: results rated 1 to 3 are within 1 to 3 standard deviations of the assigned value, respectively; those rated 4 are >3 standard deviations from the assigned value.

If you have concerns regarding your laboratory's performance, we suggest that you obtain and analyze a unit of SRM 968c, Fat-Soluble Vitamins, Carotenoids, and Cholesterol in Human Serum. If your measured values do not agree with the certified values, we suggest that you contact us for consultation.

Samples for the second 2005 QA interlaboratory exercise will be shipped **starting the week of May 16**. We will send you a reminder via e-mail or fax a week prior to shipment. It is critical that you carefully inspect all samples upon arrival and that you promptly confirm to us that they have arrived. We will replace samples (lost or damaged in shipment or miss-packaged by us) only for participants who report the problem within one calendar week after the package arrives.

If you have any questions regarding this report, please contact Dave Duewer at david.duewer@nist.gov or me at jbthomas@nist.gov, tel: 301/975-3120, or fax: 301/977-0685.

Sincerely,

Jeanice Brown Thomas
Research Chemist
Analytical Chemistry Division
Chemical Science and Technology Laboratory

Enclosures

The NIST M²QAP Round Robin LVII (RR57) report consists of:

Page	“All Lab” Report
1-4	A listing of all results and statistics for analytes reported by at least two laboratories.
5a	A list of results for the analytes reported by only one laboratory.
5b	A legend for the above two lists.
6	The text version of the “Comparability Summary” (or “Score Card”).
Page	“Individualized” Report
1	Your values, the number of labs reporting values, and our assigned values.
2 to n	“Four Plot” summaries of your current and past measurement performance, one page for each analyte you report that is also reported by at least 8 other participants.
n+1	The “target” plot version of your “Comparability Summary” scores.

Samples. The five sera below were distributed in RR57.

Serum	Description	Prior Distributions
309	Lyophilized blended serum with native carotenoid levels, augmented with α - and δ -tocopherol; SRM 968c Level II.	#249:RR44-9/98, #256:RR46-6/99, #264:RR48-3/01, #284:RR52-9/02, #299:RR55-3/04
310	Lyophilized blend of the #311 augmented serum and stripped serum, in 1+3 ratio (<i>i.e.</i> , measurand levels should be $\frac{1}{4}$ those of #311).	#197:RR31-6/94, #211:RR35-9/95, #245:RR43-6/98
311	Lyophilized blended serum with native carotenoid levels, augmented with retinol, retinyl palmitate, and α - and γ -tocopherol.	#198:RR31-6/94, #212:RR35-9/95, #246:RR43-6/98
312	Lyophilized, native, single-donor, commercially obtained serum prepared in 2002. The same material was used to prepare #313.	#290:RR53-2/03, #300:RR55-3/04
313	Fresh-frozen, native, single-donor, commercially obtained serum prepared in 2002. The same material was used to prepare #312.	#292:RR53-2/03, #301:RR55-3/04

Results

- 1) Sera Stability. There was no significant change in the median level or increase in the variability of any measurand in any of the sera. Note that Sera 310 and 311 were prepared more than 10 years ago.
- 2) Relative Accuracy at Low Measurand Levels: Sera 310 and 311 were prepared to evaluate relative accuracy of measurements made at low measurand levels. The Serum 311 material was created by augmenting a blended serum pool having with fairly high native carotenoid levels with retinol, retinyl palmitate, α -tocopherol, and γ -tocopherol. The Serum #310 material was created by blending one part of the Serum 311 material with 3 parts stripped serum. The measurand levels in the

resulting Serum 310 pool should be $\approx 25\%$ of those in the Serum 311 pool. The Table below reports the observed ratios for the measurands reported by at least 8 participants. Only the ratio for retinyl palmitate is significantly different from the expected 0.25 value in Serum 310; this could result from systematic underestimation of low retinyl palmitate levels **or** about half of the retinyl palmitate in Serum 310 has been lost since the materials were prepared **or** about half of the retinyl palmitate that should be in Serum 310 was lost during preparation. The ratio data from earlier comparisons of these materials agree well with the current values, suggesting that retinyl palmitate has not degraded during storage. However, we cannot yet distinguish between the other two possibilities.

Measurand	Ratio, Sera 310/311		
	Mean	-SD	+SD
Retinyl Palmitate	0.15	0.13	0.17
<i>trans</i> - β -Carotene	0.21	0.19	0.24
<i>trans</i> -Lycopene	0.21	0.20	0.23
Total β -Carotene	0.22	0.18	0.27
Total Lycopene	0.23	0.19	0.29
Total α -Carotene	0.24	0.16	0.36
α -Tocopherol	0.24	0.23	0.26
Total Lutein&Zeaxanthin	0.25	0.21	0.29
Total Retinol	0.25	0.23	0.27
Total Lutein	0.26	0.21	0.32
γ/β -Tocopherol	0.26	0.24	0.28
Total β -Cryptoxanthin	0.28	0.22	0.38

If your serum 310/311 ratio for any measurand is much different from 0.25, you should review the manner by which you integrate low-level signals.

- 3) Matrix (Lyophilized vs. Fresh-Frozen) Differences. Sera 312 and 313 were prepared from the same serum pool. Since we suggest that you reconstitute our lyophilized samples with 1.0 mL water rather than to a total volume of 1.0 mL, the measurand levels in Serum 312 should be $\approx 95\%$ of those in Serum 313. The observed average ratio \pm SD over all measurands with 8 or more quantitative measurements is 0.952 ± 0.008 . If any of your Sera 312/313 ratios are much different than 0.95, you should look at your measurement system for those measurands. If your lyophilized/fresh frozen ratios are consistently much different from 0.95, you should review how you reconstitute lyophilized materials.

Appendix C. “All-Lab Report” for RR57

The following six pages are the “All-Lab Report” as provided to all participants, with two exceptions:

- the participant identifiers (Lab) have been altered.
- the order in which the participant results are listed has been altered.

The data summary in the “All-Lab Report” has been altered to ensure confidentiality of identification codes assigned to laboratories. The only attributed results are those reported by NIST. The NIST results are not used in the assessment of the consensus summary results of the study.

Round Robin LVII Laboratory Results
All Results in µg/mL

Lab	Total Retinol					trans-Retinol					Retinyl Palmitate					α-Tocopherol					γ/δ-Tocopherol					δ-Tocopherol									
	309	310	311	312	313	309	310	311	312	313	309	310	311	312	313	309	310	311	312	313	309	310	311	312	313	309	310	311	312	313	309	310	311	312	313
FSV-BA	0.489	0.204	0.787	0.649	0.670						0.088	0.031	0.219	0.110	0.115	16.63	3.46	14.75	10.05	10.40	1.589	0.706	2.693	1.843	1.915	0.581	0.046	0.154	0.055	0.057	0.581	0.046	0.154	0.055	0.057
FSV-BB	0.487	0.205	0.778	0.641	0.657						0.069	0.017	0.225	0.066	0.073	17.11	3.52	14.99	10.09	10.33	1.537	0.687	2.512	1.727	1.764	0.553	0.049	0.128	0.062	0.059	0.553	0.049	0.128	0.062	0.059
FSV-BC	0.477	0.111	0.730	0.613	0.647																														
FSV-BD	0.490	0.190	0.740	0.720	0.700																														
FSV-BE	0.520	0.250	0.790	0.600	0.690																														
FSV-BF	0.480	0.170	0.700	0.610	0.640																														
FSV-BG	0.477	0.191	0.760	0.595	0.627						0.081	0.036	0.250	0.123	0.123	16.41	3.42	14.28	9.64	10.09	1.561	0.678	2.575	1.754	1.833	1.449	0.639	2.461	1.694	1.678	1.449	0.639	2.461	1.694	1.678
FSV-BH	0.412	0.145	0.648	0.579	0.586						0.097	0.048	0.296	0.107	0.103	15.92	3.30	13.73	10.57	9.88	1.750	0.680	2.830	1.890	1.740	1.527	0.718	2.429	1.611	1.729	1.527	0.718	2.429	1.611	1.729
FSV-BI	0.459	0.179	0.707	0.567	0.542						0.086	ng	0.278	0.094	0.095	18.32	3.73	15.34	10.73	10.23	1.705	0.652	13.70	9.53	10.17	16.96	3.03	13.91	9.42	9.96	15.07	3.01	12.49	9.48	9.91
FSV-BJ	0.480	0.188	0.724	0.551	0.596											17.05	3.52	13.70	9.53	10.17	1.527	0.718	2.429	1.611	1.729	1.527	0.718	2.429	1.611	1.729	1.527	0.718	2.429	1.611	1.729
FSV-BK	0.509	0.190	0.784	0.643	0.679											16.40	3.60	15.20	10.30	10.90	1.289	0.587	2.013	1.299	1.489	0.466	ng	0.143	ng	0.466	ng	0.143	ng	0.048	
FSV-BL	0.490	0.110	0.460	0.690	0.720						0.073	0.048	0.272	0.086	0.092	14.69	3.02	12.15	8.37	9.25	1.289	0.587	2.013	1.299	1.489	0.466	ng	0.143	ng	0.466	ng	0.143	ng	0.048	
FSV-BM	0.486	0.180	0.752	0.623	0.646											15.69	4.05	13.70	9.48	9.86	1.289	0.587	2.013	1.299	1.489	0.466	ng	0.143	ng	0.466	ng	0.143	ng	0.048	
FSV-BN	0.456	0.190	0.693	0.545	0.601											16.65	-	-	10.70	9.57	1.289	0.587	2.013	1.299	1.489	0.466	ng	0.143	ng	0.466	ng	0.143	ng	0.048	
FSV-BO	0.470	-	0.705	0.690	0.667											16.60	3.50	14.30	10.10	10.70	1.289	0.587	2.013	1.299	1.489	0.466	ng	0.143	ng	0.466	ng	0.143	ng	0.048	
FSV-BP	0.482	-	-	0.596	0.632											14.84	3.10	14.35	9.92	10.93	1.289	0.587	2.013	1.299	1.489	0.466	ng	0.143	ng	0.466	ng	0.143	ng	0.048	
FSV-BQ	0.580	0.183	0.908	0.708	0.731											12.16	3.13	10.73	7.28	8.63	1.289	0.587	2.013	1.299	1.489	0.466	ng	0.143	ng	0.466	ng	0.143	ng	0.048	
FSV-BR	≥0.510	≥0.180	≥0.730	≥0.600	≥0.660											16.02	3.84	13.94	9.97	10.44	1.289	0.587	2.013	1.299	1.489	0.466	ng	0.143	ng	0.466	ng	0.143	ng	0.048	
FSV-BS	0.493	0.176	0.873	0.668	0.714											15.69	3.29	13.36	9.83	9.66	1.289	0.587	2.013	1.299	1.489	0.466	ng	0.143	ng	0.466	ng	0.143	ng	0.048	
FSV-BU	0.489	0.177	0.748	0.574	0.637											15.55	3.74	13.48	9.12	10.00	1.289	0.587	2.013	1.299	1.489	0.466	ng	0.143	ng	0.466	ng	0.143	ng	0.048	
FSV-BV	0.600	0.192	0.733	0.681	0.681											12.11	3.39	14.15	10.08	10.45	1.289	0.587	2.013	1.299	1.489	0.466	ng	0.143	ng	0.466	ng	0.143	ng	0.048	
FSV-BW	0.470	0.170	0.750	0.600	0.620						0.110	0.050	0.340	0.120	0.130	12.11	3.39	14.15	10.08	10.45	1.289	0.587	2.013	1.299	1.489	0.466	ng	0.143	ng	0.466	ng	0.143	ng	0.048	
FSV-BX	≥0.470	≥0.183	≥0.750	≥0.596	≥0.618											18.82	4.01	16.50	10.55	11.28	1.289	0.587	2.013	1.299	1.489	0.466	ng	0.143	ng	0.466	ng	0.143	ng	0.048	
FSV-CC	0.510	0.200	0.770	0.600	0.620						0.075	0.068	0.493	0.153	0.212	18.92	2.98	16.13	10.10	10.40	1.289	0.587	2.013	1.299	1.489	0.466	ng	0.143	ng	0.466	ng	0.143	ng	0.048	
FSV-CD	≥0.496	≥0.196	≥0.813	≥0.627	≥0.657											18.46	3.26	10.94	6.55	9.87	1.289	0.587	2.013	1.299	1.489	0.466	ng	0.143	ng	0.466	ng	0.143	ng	0.048	
FSV-CE	0.536	0.207	0.458	0.523	0.518											14.65	2.37	12.20	7.93	8.96	1.289	0.587	2.013	1.299	1.489	0.466	ng	0.143	ng	0.466	ng	0.143	ng	0.048	
FSV-CF	0.480	0.178	0.802	0.626	0.652						0.077	0.033	0.254	0.091	0.099	16.21	3.50	14.82	9.59	10.23	1.289	0.587	2.013	1.299	1.489	0.466	ng	0.143	ng	0.466	ng	0.143	ng	0.048	
FSV-CG	0.539	0.189	0.729	0.561	0.618											16.71	5.89	14.51	10.49	10.41	1.289	0.587	2.013	1.299	1.489	0.466	ng	0.143	ng	0.466	ng	0.143	ng	0.048	
FSV-CI	0.439	0.151	0.618	0.561	0.584											17.05	3.13	15.64	10.36	11.15	1.289	0.587	2.013	1.299	1.489	0.466	ng	0.143	ng	0.466	ng	0.143	ng	0.048	
FSV-CP																																			
FSV-CS	0.485	0.198	0.802	0.668	0.662						0.046	0.041	0.220	0.059	0.051	18.60	3.80	15.20	10.20	11.60	1.289	0.587	2.013	1.299	1.489	0.466	ng	0.143	ng	0.466	ng	0.143	ng	0.048	
FSV-CT																16.20	3.40	14.40	9.80	10.10	1.289	0.587	2.013	1.299	1.489	0.466	ng	0.143	ng	0.466	ng	0.143	ng	0.048	
FSV-CW	0.403	0.263	0.748	0.569	0.562						0.070	0.048	0.291	0.084	0.086	17.63	3.80	14.72	10.46	11.02	1.289	0.587	2.013	1.299	1.489	0.466	ng	0.143	ng	0.466	ng	0.143	ng	0.048	
FSV-CZ	≥0.471	≥0.213	≥0.758	≥0.601	≥0.633											16.12	3.89	13.57	9.44	9.99	1.289	0.587	2.013	1.299	1.489	0.466	ng	0.143	ng	0.466	ng	0.143	ng	0.048	
FSV-DA																																			
FSV-DB	0.481	0.171	0.712	0.582	0.617						0.076	0.048	0.263	0.093	0.097	16.6																			

Round Robin LVII Laboratory Results

All Results in µg/mL

Lab	Total β-Carotene					trans-β-Carotene					Total cis-β-Carotene					Total α-Carotene					Total Lycopene				
	309	310	311	312	313	309	310	311	312	313	309	310	311	312	313	309	310	311	312	313	309	310	311	312	313
FSV-BA	0.443	0.167	0.689	0.130	0.133	0.414	0.155	0.643	0.123	0.127	0.029	0.012	0.046	0.007	0.006	0.090	0.006	0.029	0.073	0.075	0.459	0.034	0.156	0.528	0.554
FSV-BB	0.424	0.148	0.692	0.111	0.117	0.394	0.139	0.643	0.106	0.111	0.030	0.009	0.048	0.005	0.006	0.090	0.008	0.035	0.064	0.068	0.418	0.037	0.156	0.468	0.483
FSV-BC																									
FSV-BD	0.531	0.124	0.800	0.136	0.142																				
FSV-BE	0.390	0.113	0.695	0.090	0.094																				
FSV-BF	0.461	0.137	0.729	0.144	0.157																				
FSV-BG	0.395	0.151	0.775	0.139	0.123																				
FSV-BH	0.395	0.151	0.775	0.139	0.123	0.369	0.138	0.718	0.139	0.123	0.026	0.013	0.057	nq	nq	0.076	nq	0.040	0.071	0.069	0.331	nq	0.141	0.434	0.463
FSV-BI	0.473	0.160	0.688	0.122	0.113																				
FSV-BJ	0.491	0.133	0.788	0.124	0.119																				
FSV-BK																									
FSV-BL																									
FSV-BM	0.401	0.139	0.606	0.103	0.114																				
FSV-BN	0.456	0.130	0.723	0.110	0.117																				
FSV-BO	0.444	-	-	0.109	0.122																				
FSV-BP																									
FSV-BQ																									
FSV-BR	≥0.414	≥0.150	≥0.633	≥0.130	≥0.144	0.414	0.150	0.633	0.130	0.144															
FSV-BS																									
FSV-BU	0.441	0.688	0.112	0.120																					
FSV-BV	0.403	0.160	0.637	0.106	0.113																				
FSV-BW	0.490	0.130	0.740	0.120	0.120																				
FSV-BX	≥0.380	≥0.134	≥0.628	≥0.115	≥0.122	0.380	0.134	0.628	0.115	0.122															
FSV-CC																									
FSV-CD	0.436	0.103	0.583	0.081	0.107																				
FSV-CE	0.496	0.132	0.547	0.049	0.123																				
FSV-CF																									
FSV-CG	0.369	0.111	0.569	0.097	0.111	0.339	0.099	0.519	0.091	0.104	0.030	0.012	0.050	0.006	0.008	0.106	0.008	0.040	0.075	0.086	0.398	0.028	0.143	0.408	0.469
FSV-CH	≥0.367	≥0.114	≥0.582	≥0.113	≥0.123	0.37	0.11	0.58	0.11	0.12						0.078	<0.016	0.031	0.061	0.067					
FSV-CI	0.417	0.165	0.665	0.122	0.121																				
FSV-CP	0.367	0.165	0.665	0.122	0.121																				
FSV-CS	0.449	0.180	0.684	0.117	0.124	0.449	0.180	0.684	0.117	0.124						0.105	0.011	0.043	0.082	0.081	0.403	0.042	0.143	0.466	0.459
FSV-CT	0.544	0.178	0.748	0.127	0.135											0.106	0.010	0.037	0.079	0.083	0.458	0.043	0.174	0.492	0.521
FSV-CW																									
FSV-CW																									
FSV-CW																									
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Round Robin LVII Laboratory Results

All Results in µg/mL

Lab	trans-Lycopene					Total β-Cryptoxanthin					Total α-Cryptoxanthin					Total Lutein					Total Zeaxanthin				
	309	310	311	312	313	309	310	311	312	313	309	310	311	312	313	309	310	311	312	313	309	310	311	312	313
FSV-BA	0.215	0.016	0.068	0.277	0.290	0.040	0.006	0.025	0.068	0.070	0.022	nq	0.013	0.033	0.033	0.107	0.013	0.057	0.078	0.072	0.023	0.007	0.027	0.026	0.031
FSV-BB	0.184	0.011	0.057	0.229	0.240	0.030	0.005	0.019	0.051	0.053	0.014	0.002	0.007	0.022	0.023	0.062	0.008	0.032	0.056	0.059	0.021	nq	0.012	0.027	0.030
FSV-BC						0.035	0.005	0.023	0.065	0.062						0.086	0.013	0.049	0.075	0.072	0.036	0.007	0.023	0.041	0.039
FSV-BD						0.032	nq	0.016	0.052	0.054						0.087	0.016	0.050	0.085	0.090					
FSV-BE						0.026	0.012	0.017	0.047	0.045						0.097	0.013	0.057	0.078	0.072	0.023	0.007	0.027	0.026	0.031
FSV-BF	0.229	0.017	0.078	0.300	0.307	0.030	0.006	0.020	0.054	0.057	0.030	nq	0.021	0.058	0.058	0.062	0.008	0.032	0.056	0.059	0.021	nq	0.012	0.027	0.030
FSV-BH						0.035	0.005	0.023	0.065	0.062						0.086	0.013	0.049	0.075	0.072	0.036	0.007	0.023	0.041	0.039
FSV-BI						0.035	0.005	0.023	0.065	0.062						0.086	0.013	0.049	0.075	0.072	0.036	0.007	0.023	0.041	0.039
FSV-BJ						0.032	nq	0.016	0.052	0.054						0.087	0.016	0.050	0.085	0.090					
FSV-BK						0.021	nq	0.010	0.043	0.048						0.062	0.015	0.039	0.055	0.062	0.021	nq	0.015	0.024	0.030
FSV-BL						0.023	nq	0.015	0.044	0.046	0.014	-	-	0.050	0.040	0.073	nq	0.050	0.071	0.073	0.011	nq	0.017	0.024	0.030
FSV-BM	0.187	0.011	0.052	0.224	0.245	0.048	0.008	0.019	0.073	0.060						0.086	0.012	0.053	0.082	0.085	0.018	0.003	0.015	0.028	0.028
FSV-BN						0.029	0.003	0.013	0.043	0.045						0.086	0.012	0.053	0.082	0.085	0.018	0.003	0.015	0.028	0.028
FSV-BO						0.027	0.007	0.012	0.049	0.055						0.086	0.012	0.053	0.082	0.085	0.018	0.003	0.015	0.028	0.028
FSV-BP						0.028	0.006	0.018	0.049	0.051						0.086	0.012	0.053	0.082	0.085	0.018	0.003	0.015	0.028	0.028
FSV-BQ						0.036	nq	0.014	0.056	0.062						0.086	0.012	0.053	0.082	0.085	0.018	0.003	0.015	0.028	0.028
FSV-BR						0.043	0.004	0.024	0.065	0.073						0.086	0.012	0.053	0.082	0.085	0.018	0.003	0.015	0.028	0.028
FSV-BS						0.039	0.007	0.023	0.065	0.066						0.086	0.012	0.053	0.082	0.085	0.018	0.003	0.015	0.028	0.028
FSV-BU						0.036	0.005	0.021	0.050	0.053						0.086	0.012	0.053	0.082	0.085	0.018	0.003	0.015	0.028	0.028
FSV-BV						0.039	0.007	0.018	0.050	0.056						0.086	0.012	0.053	0.082	0.085	0.018	0.003	0.015	0.028	0.028
FSV-BW						0.040	0.008	0.030	0.059	0.063						0.086	0.012	0.053	0.082	0.085	0.018	0.003	0.015	0.028	0.028
FSV-BX	0.179	0.014	0.065	0.244	0.253	0.044	0.007	0.025	0.064	0.067	0.019	0.002	0.008	0.029	0.030	0.086	0.012	0.053	0.082	0.085	0.018	0.003	0.015	0.028	0.028
FSV-CC						0.027	0.003	0.016	0.049	0.050						0.086	0.012	0.053	0.082	0.085	0.018	0.003	0.015	0.028	0.028
FSV-CD						0.043	0.004	0.024	0.065	0.073						0.086	0.012	0.053	0.082	0.085	0.018	0.003	0.015	0.028	0.028
FSV-CE						0.039	0.007	0.023	0.065	0.066						0.086	0.012	0.053	0.082	0.085	0.018	0.003	0.015	0.028	0.028
FSV-CF						0.036	0.005	0.021	0.050	0.053						0.086	0.012	0.053	0.082	0.085	0.018	0.003	0.015	0.028	0.028
FSV-CG	0.194	0.011	0.061	0.221	0.255	0.039	0.007	0.023	0.065	0.066						0.086	0.012	0.053	0.082	0.085	0.018	0.003	0.015	0.028	0.028
FSV-CI						0.036	0.005	0.021	0.050	0.053						0.086	0.012	0.053	0.082	0.085	0.018	0.003	0.015	0.028	0.028
FSV-CP						0.039	0.007	0.023	0.065	0.066						0.086	0.012	0.053	0.082	0.085	0.018	0.003	0.015	0.028	0.028
FSV-CS						0.036	0.005	0.021	0.050	0.053						0.086	0.012	0.053	0.082	0.085	0.018	0.003	0.015	0.028	0.028
FSV-CT						0.039	0.007	0.018	0.050	0.056						0.086	0.012	0.053	0.082	0.085	0.018	0.003	0.015	0.028	0.028
FSV-CW	0.211	0.022	0.080	0.289	0.306	0.040	0.008	0.030	0.059	0.063						0.086	0.012	0.053	0.082	0.085	0.018	0.003	0.015	0.028	0.028
FSV-CZ						0.044	0.007	0.025	0.064	0.067						0.086	0.012	0.053	0.082	0.085	0.018	0.003	0.015	0.028	0.028
FSV-DA	0.224	0.016	0.073	0.287	0.309	0.027	0.003	0.016	0.049	0.050						0.086	0.012	0.053	0.082	0.085	0.018	0.003	0.015	0.028	0.028
FSV-DB						0.044	0.007	0.025	0.064	0.067						0.086	0.012	0.053	0.082	0.085	0.018	0.003	0.015	0.028	0.028
FSV-DD						0.027	0.003	0.016	0.049	0.050						0.086	0.012	0.053	0.082	0.085	0.018	0.003	0.015	0.028	0.028
FSV-DE						0.044	0.007	0.025	0.064	0.067						0.086	0.012	0.053	0.082	0.085	0.018	0.003	0.015	0.028	0.028
FSV-DF						0.027	0.003	0.016	0.049	0.050						0.086	0.012	0.053	0.082	0.085	0.018	0.003	0.015	0.028	0.028
FSV-DI						0.044	0.007	0.025	0.064	0.067						0.086	0.012	0.053	0.082	0.085	0.018	0.003	0.015	0.028	0.028
FSV-DJ						0.027	0.003	0.016	0.049	0.050						0.086	0.012	0.053	0.082	0.085	0.018	0.003	0.015	0.028	0.028
FSV-DV						0.044	0.007	0.025	0.064	0.067						0.086	0.012	0.053	0.082	0.085	0.018	0.003	0.015	0.028	0.028
FSV-DW						0.027	0.003	0.016	0.049	0.050						0.086	0.012	0.053	0.082	0.085	0.018	0.003	0.015	0.028	0.028
FSV-DX						0.044	0.007	0.025	0.064	0.067						0.086	0.012	0.053	0.082	0.085	0.018	0.003	0.015	0.028	0.028
FSV-ET						0.026	0.006	0.016	0.044	0.046						0.086	0.012	0.053	0.082	0.085	0.018	0.003	0.015	0.028	0.028
N	8	8	8	8	8	24	17	23	24	24	4	2	3	4	4	15	13	15	15	15	13	8	13	13	13
Min	0.179	0.011	0.052	0.221	0.240	0.014	0.003	0.010	0.043	0.040	0.005	0.002	0.007	0.017	0.016	0.061	0.008	0.032	0.055	0.059	0.011	0.003	0.006	0.024	0.025
Median	0.203	0.015	0.067	0.260	0.273	0.030	0.006	0.018	0.052	0.055	0.016	0.002	0.008	0.026	0.027	0.086	0.015	0.050	0.078	0.073	0.021	0.006	0.017	0.027	0.030
Max	0.229	0.022	0.080	0.300	0.309	0.048	0.012	0.030	0.073	0.073	0.022	0.002	0.013	0.033	0.033	0.115	0.017	0.067	0.099	0.101	0.041	0.009	0.027	0.053	0.055
SD	0.023	0.004	0.010	0.044	0.041	0.009	0.001	0.004	0.008	0.009	0.006			0.007	0.007	0.018	0.002	0.011	0.016	0.015	0.003	0.002	0.005	0.005	0.004
CV	11	26	16	17	15	30	24	24	16	16	35			27</											

Round Robin LVII Laboratory Results

All Results in µg/mL

Lab	Total Lutein&Zeaxanthin						Coenzyme Q10						Ubiquinol						Ubiquinone						Phylloquinone (K1)					
	309	310	311	312	313		309	310	311	312	313		309	310	311	312	313		309	310	311	312	313		309	310	311	312	313	
FSV-BA	0.107	0.022	0.072	0.111	0.115																									
FSV-BB	0.148	0.021	0.092	0.152	0.156																									
FSV-BC																														
FSV-BD																														
FSV-BE	0.111	0.013	0.068	0.109	0.112																									
FSV-BF	0.109	0.016	0.069	0.091	0.086																									
FSV-BG	0.083	0.008	0.044	0.083	0.089																									
FSV-BH	0.122	0.020	0.072	0.116	0.111																									
FSV-BI																														
FSV-BJ							0.867	0.110	0.539	0.678	0.745																			
FSV-BK																														
FSV-BL																														
FSV-BM	0.083	0.015	0.055	0.079	0.092																									
FSV-BN	0.085	<i>nq</i>	0.0669	0.0940	0.0982																									
FSV-BO	0.116	-	-	0.099	0.115																									
FSV-BP																														
FSV-BQ																														
FSV-BR	0.105	0.029	0.071	0.116	0.107																									
FSV-BS	0.102		0.065	0.107	0.122																									
FSV-BU	0.096	0.014	0.060	0.106	0.111																									
FSV-BV	0.089	0.013	0.057	0.089	0.096																									
FSV-BW	0.105	0.015	0.068	0.110	0.114																									
FSV-BX	0.137	<i>nq</i>	0.074	0.118	0.110																									
FSV-CD																														
FSV-CE																														
FSV-CF	0.125	0.019	0.078	0.126	0.141																									
FSV-CG	0.079	<0.022	0.065	0.090	0.099																									
FSV-CI	0.131	0.023	0.082	0.136	0.137																									
FSV-CP	0.116	0.022	0.087	0.110	0.114																									
FSV-CS	0.139	<i>nq</i>	0.063	0.125	0.128																									
FSV-CT	0.106	0.024	0.080	0.114	0.111																									
FSV-CW							1.100	0.030	0.070	0.090	0.090																			
FSV-CZ							0.690	<0.2	0.360	0.390	0.430																			
FSV-DA	0.131	0.023	0.086	0.132	0.140																									
FSV-DB	0.093	0.013	0.058	0.103	0.115																									
FSV-DD																														
FSV-DF																														
FSV-DI																														
FSV-DV																														
FSV-DW	0.091	0.020	0.059	0.080	0.084																									
FSV-ET																														
N	24	18	23	24	24		6	5	6	6	6		1	1	2	2	2		2	2	2	2	2		3	3	3	3	3	
Min	0.079	0.008	0.044	0.079	0.084		0.690	0.030	0.070	0.090	0.090		0.780	0.053	0.208	0.138	0.234		0.150	0.045	0.120	0.170	0.340		0.71	0.19	0.65	0.18	0.16	
Median	0.107	0.020	0.068	0.109	0.112		0.948	0.090	0.530	0.645	0.690		0.780	0.053	0.304	0.319	0.292		0.533	0.046	0.286	0.420	0.471		0.83	0.21	0.86	0.33	0.31	
Max	0.148	0.029	0.092	0.152	0.156		1.100	0.110	0.660	0.808	0.836				0.400	0.500	0.350		0.916	0.047	0.452	0.670	0.602		1.32	0.24	1.20	0.36	0.41	
SD	0.022	0.006	0.012	0.017	0.013		0.081	0.041	0.115	0.170	0.175																			
CV	21	29	18	16	12		9	46	22	26	25																			
Npast	25	17	18	23	23		14	0	0	5	5		0	0	0	0	0		0	0	0	0	0		0	0	0	0	0	
Medianpast	0.109	0.021	0.074	0.107	0.112		0.950																							
SDpast	0.023	0.010	0.013	0.018	0.019		0.081			0.035	0.047																			
NIST	0.102	<i>nq</i>	±0.052	0.104	0.099																									
NNIST	3			3	3																									
Srep	0.003			0.005	0.002																									
Shet	0.002			0.005	0.002																									
SNIST	0.004			0.007	0.003																									
NAV	0.105	0.020	0.069	0.107	0.105		0.948	0.090	0.530	0.645	0.690																			
NAU	0.023	0.005	0.014	0.023	0.025		0.081	0.041	0.115	0.170	0.175																			

0.830	0.212	0.860	0.330	0.310
-------	-------	-------	-------	-------

Round Robin LVII Laboratory Results

All Results in µg/mL

Analytes Reported By One Laboratory

Analyte	Code	309	310	311	312	313
25-hydroxyvitamin D	FSV-BN	<i>nq</i>	0.0270	0.0170	0.0350	0.0230
trans-Lutein	FSV-DA	0.090	0.015	0.058	0.082	0.087
Phytofluene	FSV-DA	0.068	0.011	0.048	0.107	0.114
Phytoene	FSV-DA	0.051	0.009	0.052	0.125	0.132
Retinyl stearate	FSV-DA	0.030	0.005	0.037	0.033	0.034

Legend

Term	Definition
N	Number of (non-NIST) quantitative values reported for this analyte
Min	Minimum (non-NIST) quantitative value reported
Median	Median (non-NIST) quantitative value reported
Max	Maximum (non-NIST) quantitative value reported
SD	Standard deviation for (non-NIST) results: $0.741 \times (\text{3rd Quartile} - \text{1st Quartile})$
CV	Coefficient of Variation for (non-NIST) results: $100 \times \text{SD} / \text{Median}$
N_{past}	Mean of N(s) from past RR(s)
$\text{Median}_{\text{past}}$	Mean of Median(s) from past RR(s)
SD_{past}	Pooled SD from past RR(s)
NIST	Mean of all analyses (vials x duplicates) reported by a NIST analyst
N_{NIST}	Number of total vials analyzed in duplicate by NIST analysts
S_{rep}	Within-vial pooled standard deviation
S_{het}	Among-vial pooled standard deviation
S_{NIST}	Total standard deviation for NIST analyses: $(S_{\text{rep}}^2 + S_{\text{het}}^2)^{0.5}$
NAV	NIST Assigned Value = $(\text{Median} + \text{Mean}_{\text{NIST}}) / 2$ for analytes reported by NIST analyst(s) = Median for analytes reported by ≥ 10 labs but not NIST
NAU	NIST Assigned Uncertainty: $(S^2 + S_{\text{btw}}^2)^{0.5}$ S is the maximum of $(0.05 \times \text{NAV}, \text{SD}, S_{\text{NIST}}, \text{eSD})$ and S_{btw} is the standard deviation between $\text{Median}_{\text{part}}$ and $\text{Mean}_{\text{NIST}}$. The expected long-term SD, eSD, is defined in: Duewer, et al. Anal Chem 1997;69(7):1406-1413.
-	Not analyzed
<i>nd</i>	Not detected (i.e., no detectable peak for analyte)
<i>nq</i>	Detected but not quantitatively determined
<x	Concentration at or below the limit of quantification, x
$\geq x$	Concentration greater than or equal to x

Round Robin LVII Laboratory Results

Comparability Summary

Lab	TR	aT	g/bT	bC	tbC	aC	TLy	TbX	TLu	TZ	L&Z
FSV-BA	1	1	1	1	1	1	1	2	1	2	1
FSV-BB	1	1	1	1	1	1	1	1	1	1	1
FSV-BC	3										
FSV-BD	1	1									1
FSV-BE	2	1	2	2							
FSV-BF	1	3	3	2		2	1	2			1
FSV-BG	1	1	1	2		1	1	1	2	3	2
FSV-BH	2	1	1	1	1	1	1	1			
FSV-BI	2	1	1	1		1	1	1	2	1	2
FSV-BJ	1	1	1	1		1	1	1	1	1	1
FSV-BK	1	1									
FSV-BL	4	2							1		
FSV-BM	1	1									
FSV-BN	1	2	2	1	2	1	1	2			1
FSV-BO	1	1		1		1	1	1			1
FSV-BP	1	1		1		1	1	2	2	1	2
FSV-BQ	2	1									1
FSV-BR	1	1							2	2	1
FSV-BS	2	3	3	1	1	2	2	2	1	1	1
FSV-BU	1	1	1	1		1	1	1			
FSV-BV	2	1	1	1		2	1	1			
FSV-BW	1	1		1		2	1	1			1
FSV-BX	1	2	1	1	1	1		1			
FSV-CC	1	2									1
FSV-CD	1	2	3	2		1	2	1			1
FSV-CE	3	3		2							1
FSV-CF	1	2							1	2	2
FSV-CG	1	2	1	2	2	1	1	2			1
FSV-CI	2	1	1	1	1	2					
FSV-CP		3	4	1		1	1	1			1
FSV-CS	1	1	4	1		1	1	1			
FSV-CT				2			1	1	1	1	1
FSV-CW	3	2	1		2	2		2	1	1	1
FSV-CZ	1	1		2							
FSV-DA	1	1	1	1	1	1	1	2			
FSV-DB	1	1	1	1			1	1	1	1	1
FSV-DD	1										
FSV-DF	1								1		
FSV-DI	2	1	1	2			1		1	1	1
FSV-DV	4	2									
FSV-DW	2	1		3		2	2	1			
FSV-ET	1	1	2	1							
NISTa	1	1	1	1	1	1		1	1	1	1
n	41	39	24	30	11	24	23	25	16	14	25

	TR	aT	g/bT	bC	tbC	aC	TLy	TbX	TLu	TZ	L&Z
% 1	66	67	67	67	73	71	87	68	75	71	84
% 2	22	23	13	30	27	29	13	32	25	21	16
% 3	7	10	13	3	0	0	0	0	0	7	0
% 4	5	0	8	0	0	0	0	0	0	0	0

Label	Definition
Lab	Participant code
TR	Total Retinol
aT	α -Tocopherol
g/bT	γ/β -Tocopherol
bC	Total β -Carotene
tbC	trans- β -Carotene
aC	Total α -Carotene
TLy	Total Lycopene
TbX	Total β -Cryptoxanthin
TLu	Total Lutein
TZ	Total Zeaxanthin
L&Z	Total Lutein & Zeaxanthin

n	number of participants providing quantitative data
% 1	Percent of CS = 1 (within 1 SD of medians)
% 2	Percent of CS = 2 (within 2 SD of medians)
% 3	Percent of CS = 3 (within 3 SD of medians)
% 4	Percent of CS = 4 (3 or more SD from medians)

"Comparability Score"

The Comparability Score (CS) of summarizes your measurement performance for a given measurand, relative to the consensus medians. CS is the average distance, in standard deviation units, that your measurement performance characteristics are from the consensus performance. CS is calculated when the number of quantitative values you reported for a measurand, N_{you} , is at least two and the measurand has been reported by 10 or more participants.

$$CS = \text{MIN}(4, \text{INT}(1 + \sqrt{C^2 + AP^2}))$$

$$C = \text{Concordance} = \sum_i \frac{N_{you} \cdot \text{You}_i - \text{Median}_i}{NAU_i} / N_{you}$$

$$AP = \text{Apparent Precision} = \sqrt{\sum_i \left(\frac{\text{You}_i - \text{Median}_i}{NAU_i} \right)^2 / (N_{you} - 1)}$$

NAU = NIST Assigned Uncertainty, our estimate of the overall measurement standard deviation for each sample. The estimate includes serum heterogeneity, analytical repeatability, and among-participant reproducibility variance components.

For further details, please see: Duewer DL, Kline MC, Sharpless KE, Brown Thomas J, Gary KT. Micronutrients Measurement Quality Assurance Program: Helping participants use interlaboratory comparison exercise results to improve their long-term measurement performance. Anal Chem 1999;71(9):1870-8.

Appendix D. Representative “Individualized Report” for RR57

Each participant in RR57 received an “Individualized Report” reflecting their reported results. Each report included a detailed analysis for analytes that were assayed by at least five participants. The following analytes met this criterion in RR57:

- Total Retinol
- *trans*-Retinol
- Retinyl Palmitate
- α -Tocopherol
- γ/β -Tocopherol
- δ -Tocopherol
- Total β -Carotene
- *trans*- β -Carotene
- Total *cis*- β -Carotene
- Total α -Carotene
- Total Lycopene
- *trans*-Lycopene
- Total β -Cryptoxanthin
- Total Lutein
- Total Zeaxanthin
- Total Lutein & Zeaxanthin
- Coenzyme Q10

The following 15 pages are the “Individualized Report” for the analytes evaluated by participant FSV-BA.

Individualized Round Robin LVII Report: FSV-BA

Summary

Analyte	Serum 309			Serum 310			Serum 311			Serum 312			Serum 313		
	You	NAV	n	You	NAV	n	You	NAV	n	You	NAV	n	You	NAV	n
Total Retinol	0.489	0.491	36	0.204	0.188	34	0.787	0.769	35	0.649	0.650	36	0.670	0.658	36
Retinyl Palmitate	0.09	0.08	12	0.0	0.0	11	0.2	0.3	12	0.11	0.09	12	0.12	0.10	12
α-Tocopherol	16.63	16.69	38	3.46	3.49	37	14.75	14.42	37	10.05	10.51	38	10.40	10.37	38
γβ-Tocopherol	1.589	1.550	23	0.706	0.684	23	2.693	2.571	23	1.843	1.769	23	1.915	1.789	23
δ-Tocopherol	0.581	0.553	8	0.046	0.036	6	0.154	0.140	8	0.055	0.050	6	0.057	0.048	7
Total β-Carotene	0.443	0.436	26	0.167	0.140	24	0.689	0.679	25	0.130	0.118	26	0.133	0.123	26
trans-β-Carotene	0.414	0.386	10	0.155	0.136	10	0.643	0.638	10	0.123	0.116	10	0.127	0.123	10
Total cis-β-Carotene	0.029	0.034	6	0.012	0.012	6	0.046	0.052	6	0.007	0.006	5	0.006	0.006	5
Total α-Carotene	0.090	0.098	23	0.006	0.008	16	0.029	0.036	22	0.073	0.076	23	0.075	0.078	23
Total Lycopene	0.459	0.431	23	0.034	0.035	20	0.156	0.152	22	0.528	0.478	23	0.554	0.500	23
trans-Lycopene	0.215	0.203	8	0.016	0.015	8	0.068	0.067	8	0.277	0.260	8	0.290	0.273	8
Total β-Cryptoxanthin	0.040	0.030	24	0.006	0.006	17	0.025	0.019	23	0.068	0.051	24	0.070	0.053	24
Total α-Cryptoxanthin	0.022	0.016	4	nq		2	0.013	0.008	3	0.033	0.026	4	0.033	0.027	4
Total Lutein&Zeaxanthin	0.107	0.105	24	0.02	0.02	18	0.07	0.07	23	0.111	0.107	24	0.12	0.11	24

You : Your reported values for the listed analytes (micrograms/milliliter)

NAV : NIST Assigned Values, here equal to this RR's median

n : Number of non-NIST laboratories reporting quantitative values for this analyte in this serum

nq : Detected, but not quantitatively determined

Please check our records against your records. Send corrections and/or updates to...

Micronutrients Measurement Quality Assurance Program

National Institute of Standards and Technology

100 Bureau Drive Stop 8392

Gaithersburg, MD 20899-8392 USA

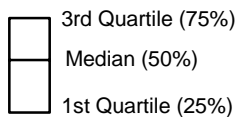
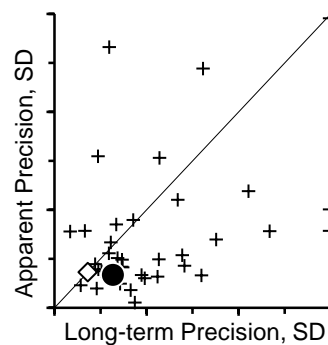
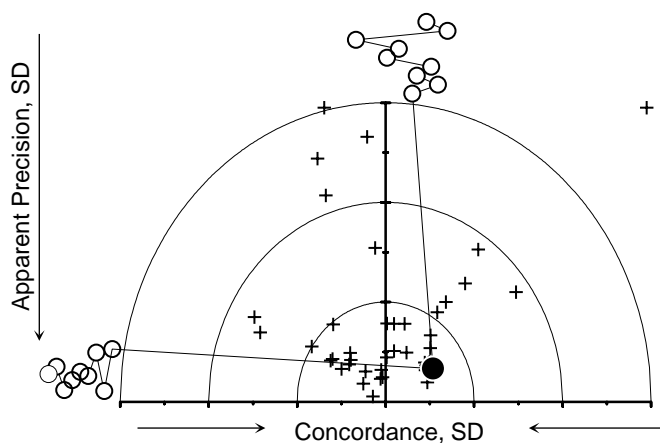
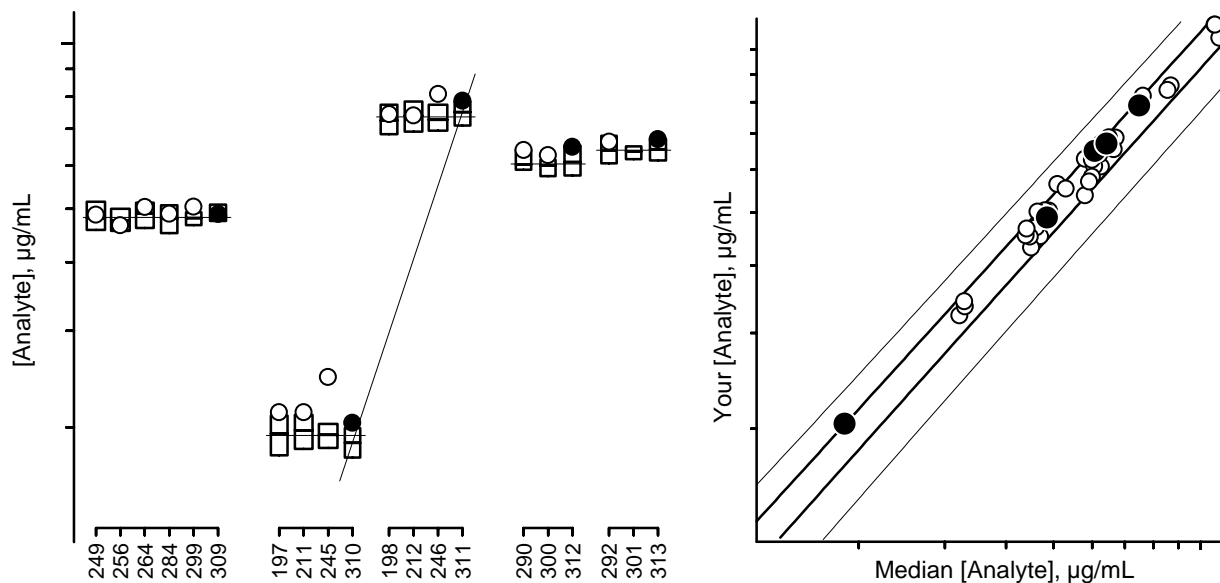
Tel: (301) 975-3935

Fax: (301) 977-0685

Email: david.duewer@nist.gov

Individualized RR LVII Report: FSV-BA

Total Retinol



● You, this RR
○ You, past RRs

▲ You, $\geq x$, this RR
△ You, $\geq x$, past RRs

◇ NIST, this RR
+ Others, this RR

Serum

#309 Lyophilized - 44:249, 46:256, 48:264, 52:284, 55:299
#310 Lyophilized - 31:197, 35:211, 43:245
#311 Lyophilized - 31:198, 35:212, 43:246
#312 Lyophilized - 53:290, 55:300
#313 Fresh-frozen: 53:292, 55:301

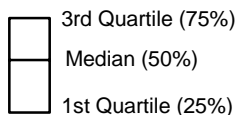
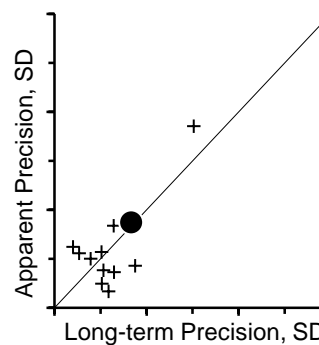
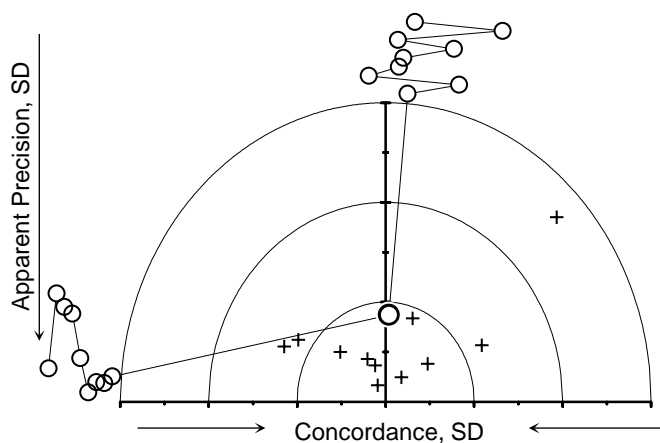
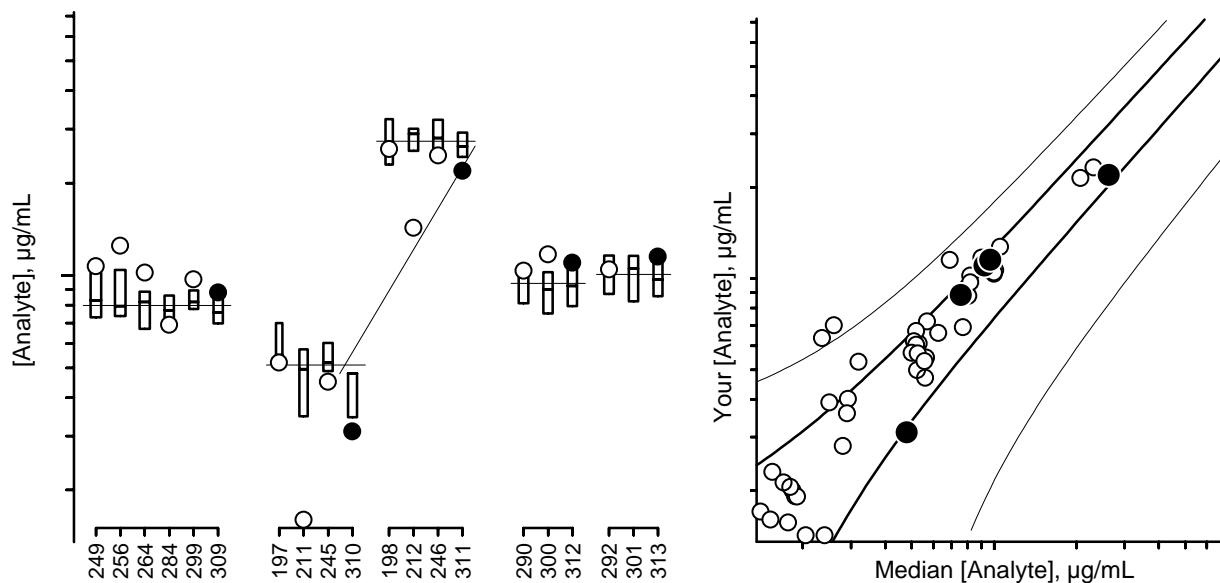
History

Comments

Augmented, multi-source (SRM 968c Level II)
Same as #311, 1:3 diluted with stripped serum
Augmented, multi-source
Native, single-source
Native, single-source, hemolyzed

Individualized RR LVII Report: FSV-BA

Retinyl Palmitate



● You, this RR
○ You, past RRs

▲ You, $\geq x$, this RR
△ You, $\geq x$, past RRs

◇ NIST, this RR
+ Others, this RR

Serum

#309 Lyophilized - 44:249, 46:256, 48:264, 52:284, 55:299
#310 Lyophilized - 31:197, 35:211, 43:245
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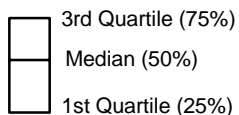
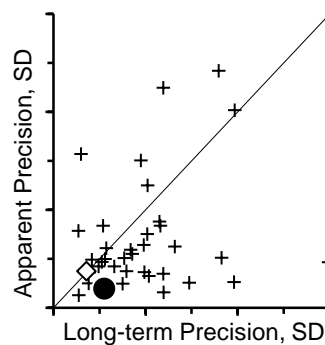
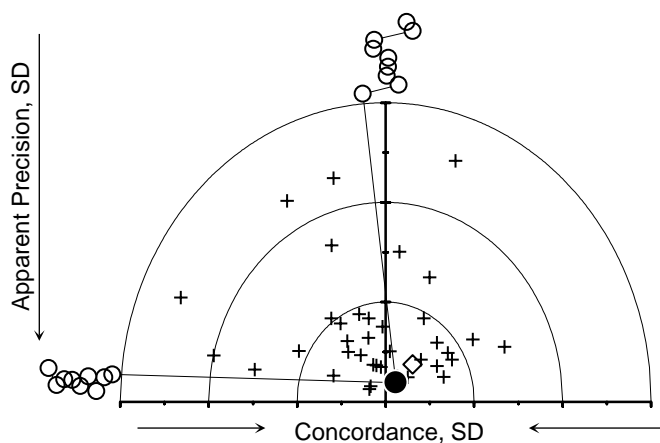
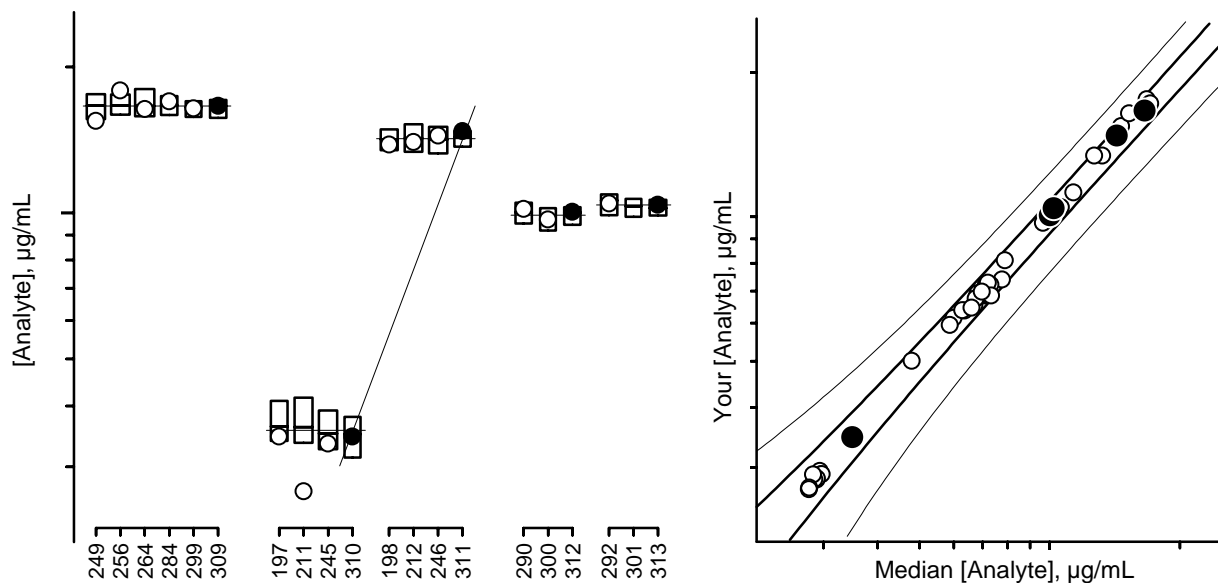
History

Comments

Augmented, multi-source (SRM 968c Level II)
Same as #311, 1:3 diluted with stripped serum
Augmented, multi-source
Native, single-source
Native, single-source, hemolyzed

Individualized RR LVII Report: FSV-BA

α -Tocopherol



● You, this RR
○ You, past RRs

▲ You, $\geq x$, this RR
△ You, $\geq x$, past RRs

◇ NIST, this RR
+ Others, this RR

Serum

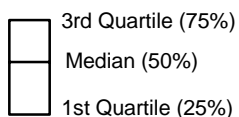
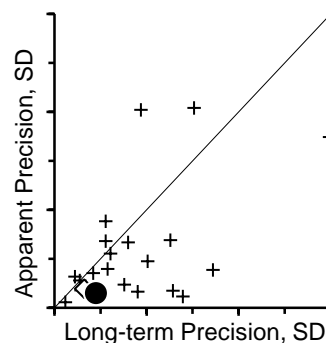
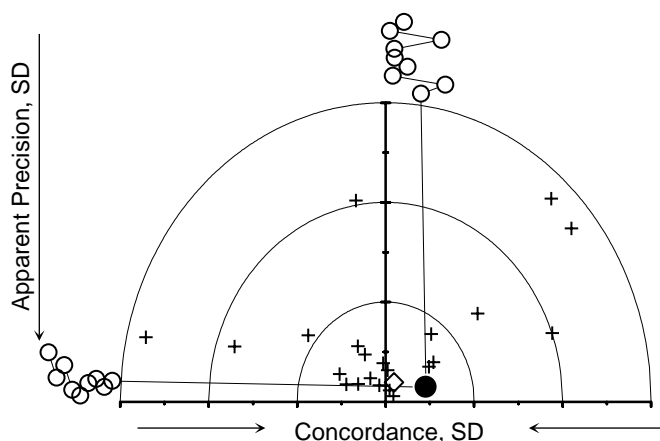
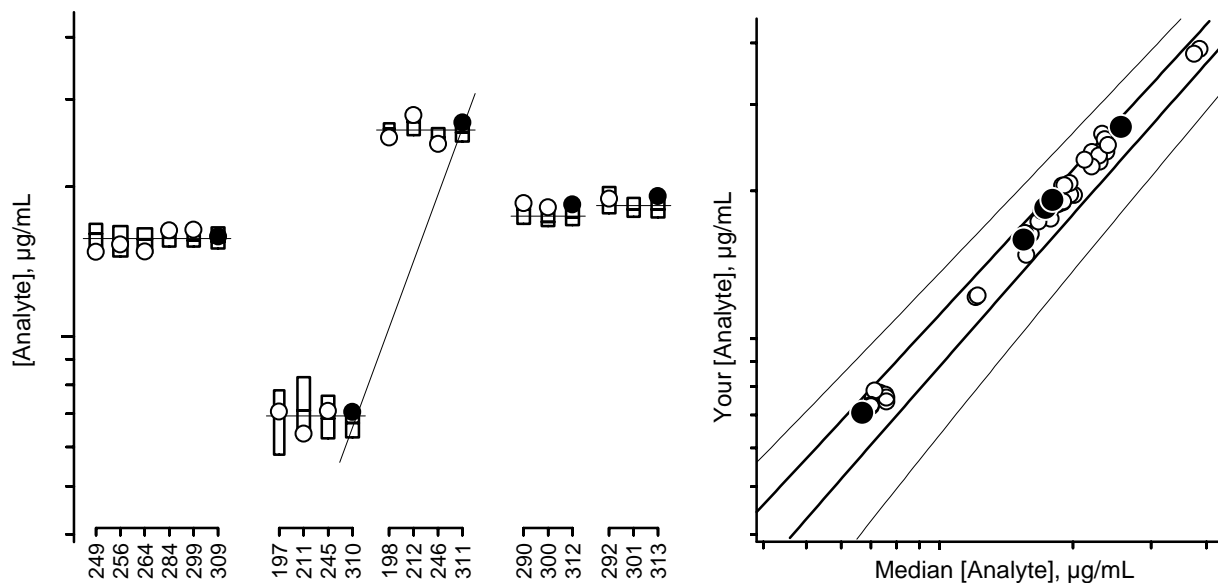
History

Comments

#309	Lyophilized - 44:249, 46:256, 48:264, 52:284, 55:299	Augmented, multi-source (SRM 968c Level II)
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#313	Fresh-frozen: 53:292, 55:301	Native, single-source, hemolyzed

Individualized RR LVII Report: FSV-BA

γ/β -Tocopherol



● You, this RR
○ You, past RRs

▲ You, $\geq x$, this RR
△ You, $\geq x$, past RRs

◇ NIST, this RR
+ Others, this RR

Serum

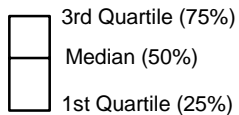
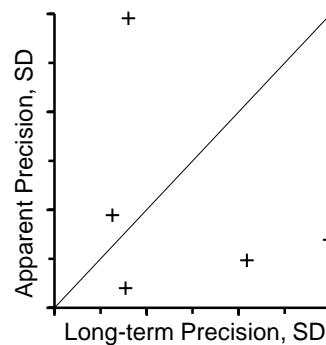
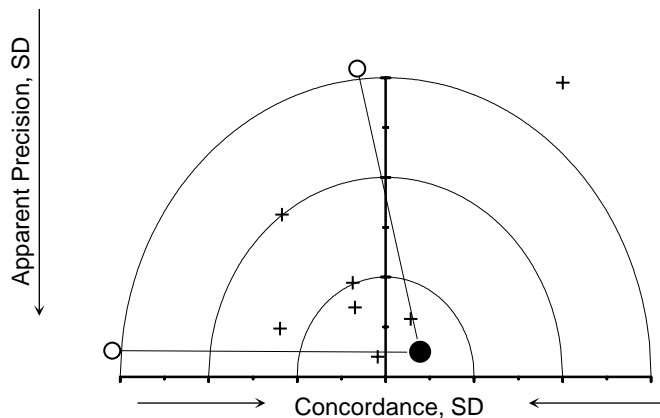
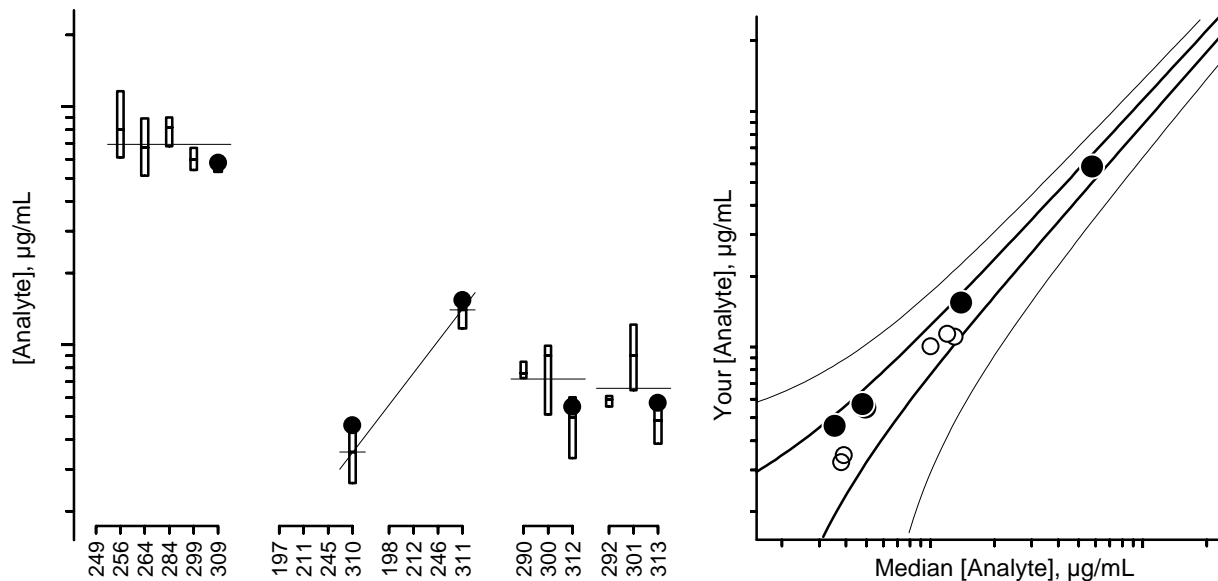
History

Comments

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#312	Lyophilized - 53:290, 55:300	Native, single-source
#313	Fresh-frozen: 53:292, 55:301	Native, single-source, hemolyzed

Individualized RR LVII Report: FSV-BA

δ-Tocopherol



● You, this RR
○ You, past RRs

▲ You, $\geq x$, this RR
△ You, $\geq x$, past RRs

◇ NIST, this RR
+ Others, this RR

Serum

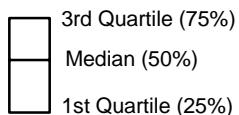
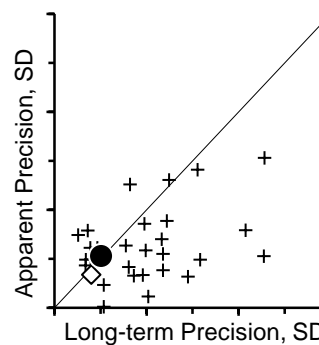
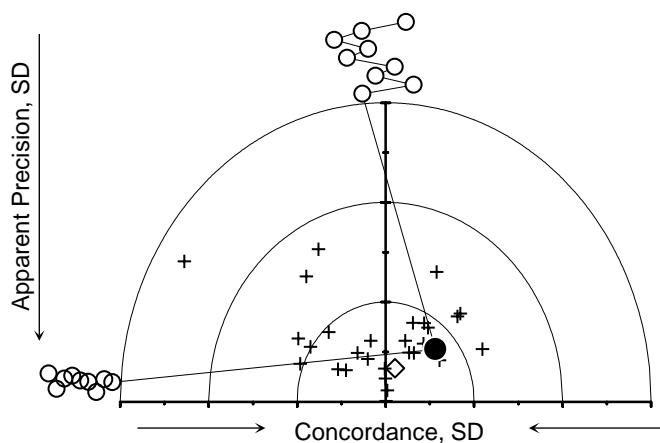
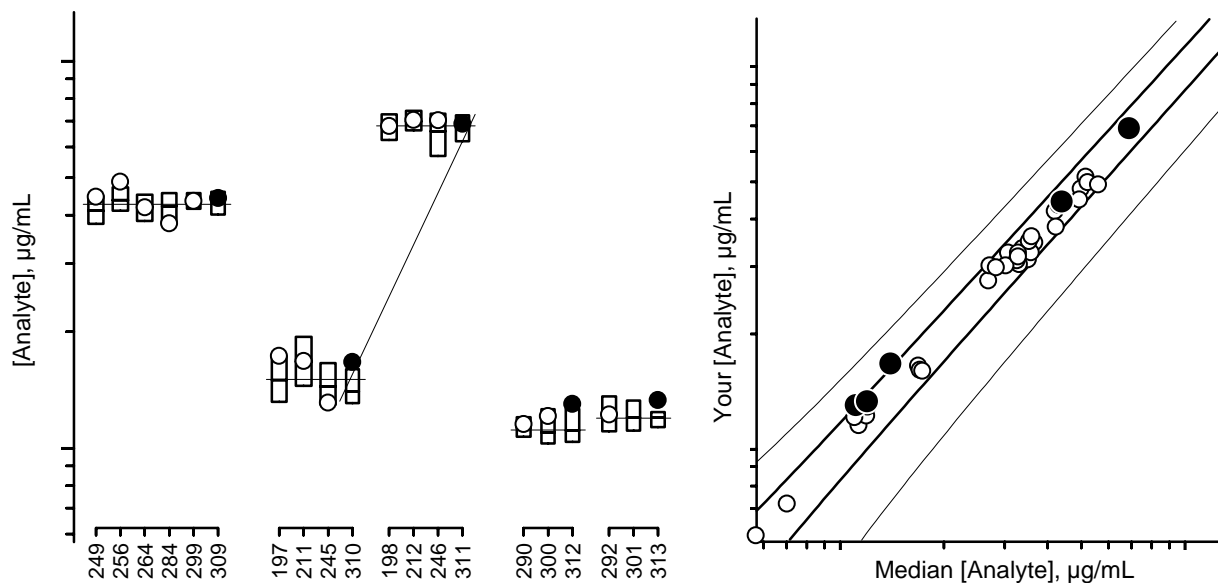
History

Comments

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#312	Lyophilized - 53:290, 55:300	Native, single-source
#313	Fresh-frozen: 53:292, 55:301	Native, single-source, hemolyzed

Individualized RR LVII Report: FSV-BA

Total β -Carotene



● You, this RR
○ You, past RRs

▲ You, $\geq x$, this RR
△ You, $\geq x$, past RRs

◇ NIST, this RR
+ Others, this RR

Serum

#309 Lyophilized - 44:249, 46:256, 48:264, 52:284, 55:299
#310 Lyophilized - 31:197, 35:211, 43:245
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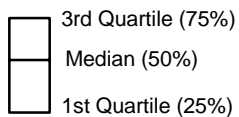
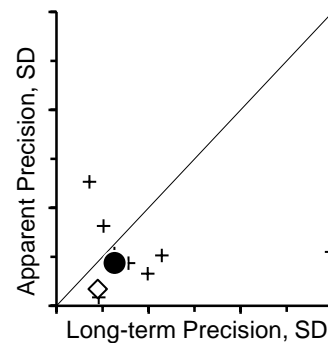
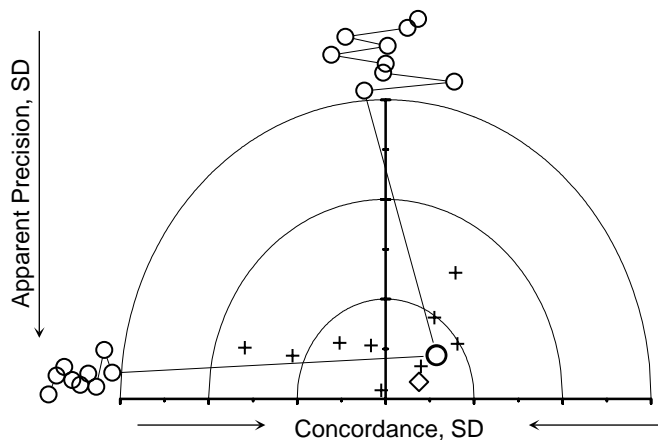
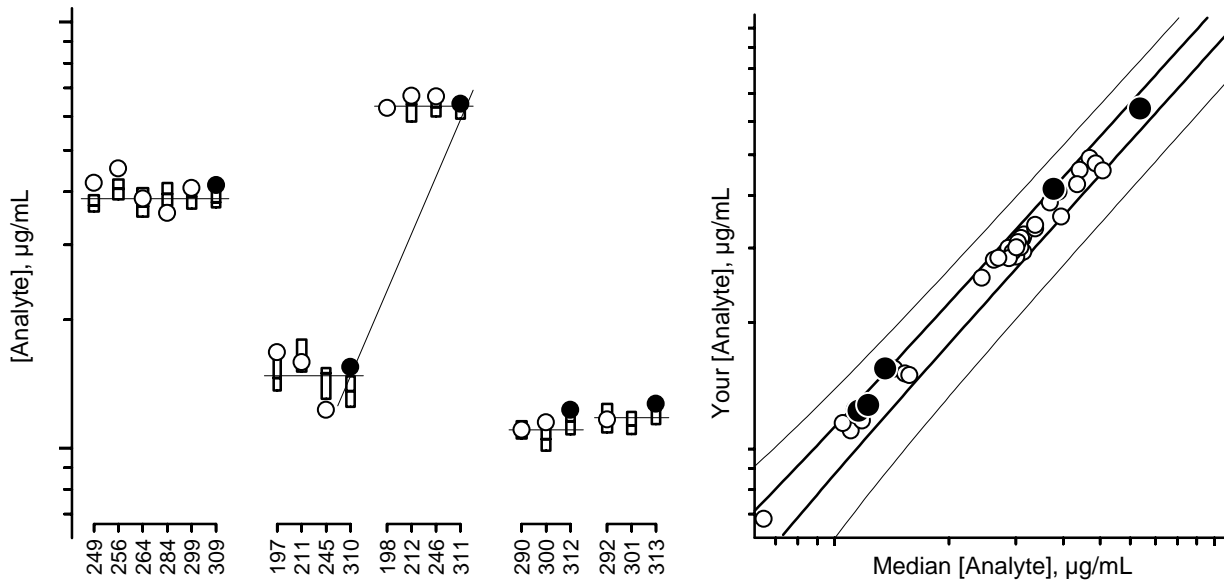
History

Comments

Augmented, multi-source (SRM 968c Level II)
Same as #311, 1:3 diluted with stripped serum
Augmented, multi-source
Native, single-source
Native, single-source, hemolyzed

Individualized RR LVII Report: FSV-BA

trans- β -Carotene



● You, this RR
○ You, past RRs

▲ You, $\geq x$, this RR
△ You, $\geq x$, past RRs

◇ NIST, this RR
+ Others, this RR

Serum

#309 Lyophilized - 44:249, 46:256, 48:264, 52:284, 55:299
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#313 Fresh-frozen: 53:292, 55:301

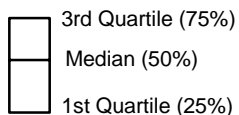
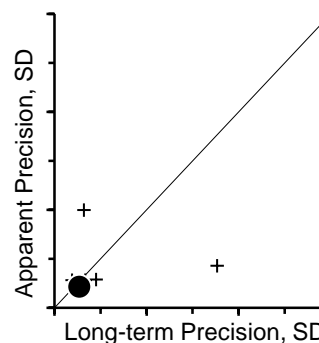
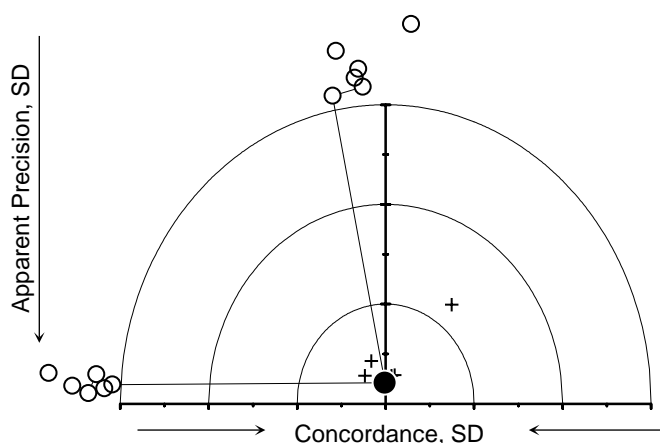
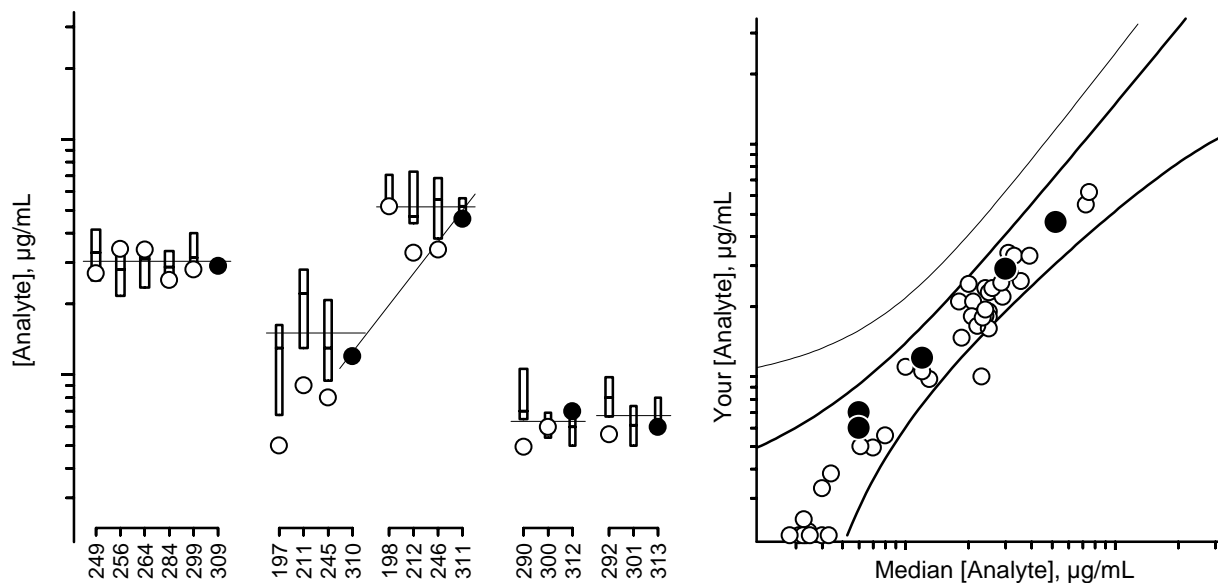
History

Comments

Augmented, multi-source (SRM 968c Level II)
Same as #311, 1:3 diluted with stripped serum
Augmented, multi-source
Native, single-source
Native, single-source, hemolyzed

Individualized RR LVII Report: FSV-BA

Total cis- β -Carotene



● You, this RR
○ You, past RRs

▲ You, $\geq x$, this RR
△ You, $\geq x$, past RRs

◇ NIST, this RR
+ Others, this RR

Serum

#309 Lyophilized - 44:249, 46:256, 48:264, 52:284, 55:299
#310 Lyophilized - 31:197, 35:211, 43:245
#311 Lyophilized - 31:198, 35:212, 43:246
#312 Lyophilized - 53:290, 55:300
#313 Fresh-frozen: 53:292, 55:301

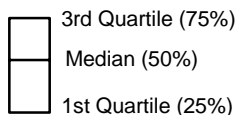
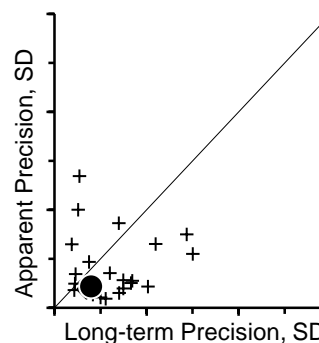
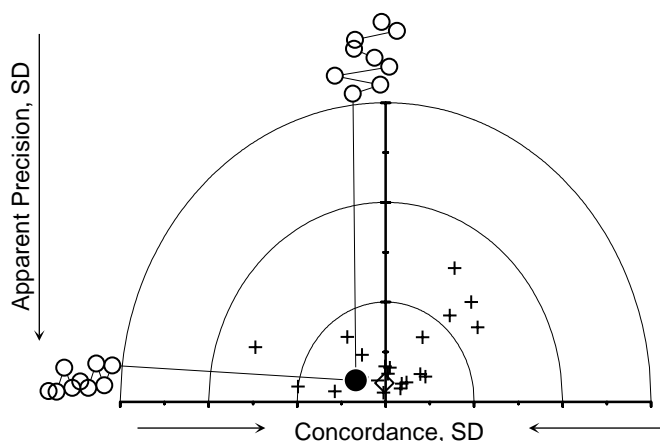
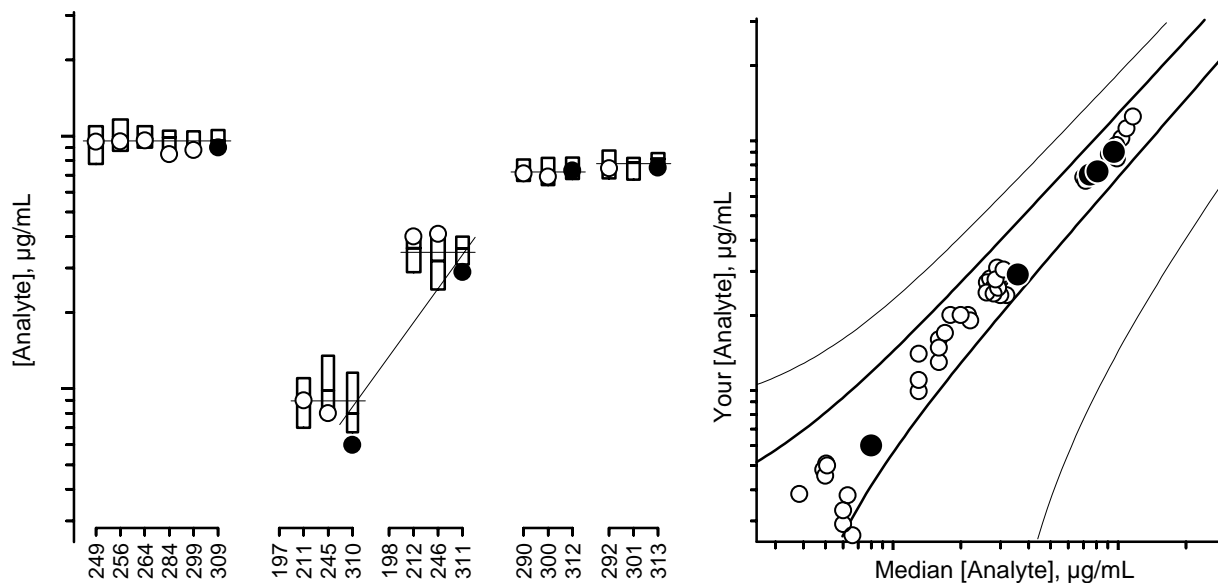
History

Comments

Augmented, multi-source (SRM 968c Level II)
Same as #311, 1:3 diluted with stripped serum
Augmented, multi-source
Native, single-source
Native, single-source, hemolyzed

Individualized RR LVII Report: FSV-BA

Total α -Carotene



● You, this RR
○ You, past RRs

▲ You, $\geq x$, this RR
△ You, $\geq x$, past RRs

◇ NIST, this RR
+ Others, this RR

Serum

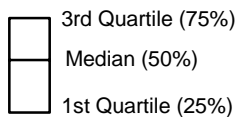
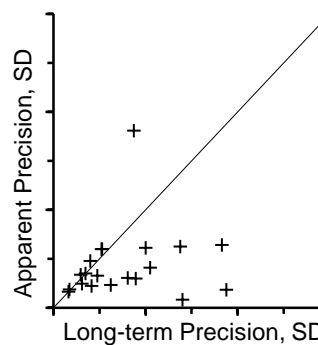
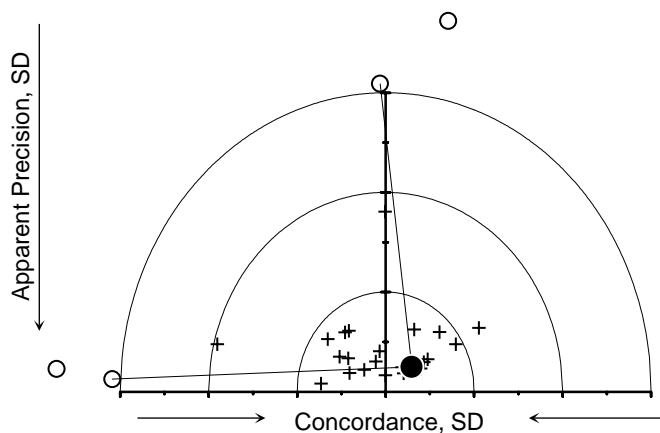
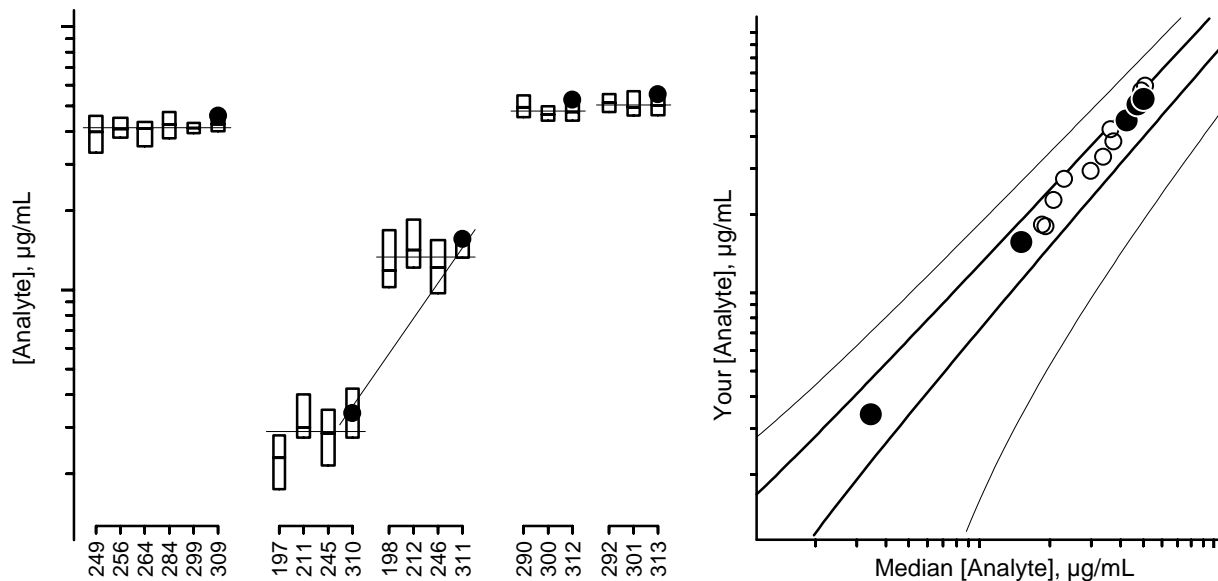
History

Comments

#309	Lyophilized - 44:249, 46:256, 48:264, 52:284, 55:299	Augmented, multi-source (SRM 968c Level II)
#310	Lyophilized - 31:197, 35:211, 43:245	Same as #311, 1:3 diluted with stripped serum
#311	Lyophilized - 31:198, 35:212, 43:246	Augmented, multi-source
#312	Lyophilized - 53:290, 55:300	Native, single-source
#313	Fresh-frozen: 53:292, 55:301	Native, single-source, hemolyzed

Individualized RR LVII Report: FSV-BA

Total Lycopene



● You, this RR
○ You, past RRs

▲ You, $\geq x$, this RR
△ You, $\geq x$, past RRs

◇ NIST, this RR
+ Others, this RR

Serum

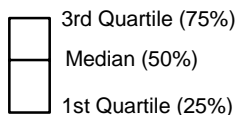
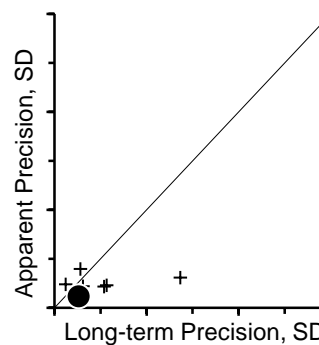
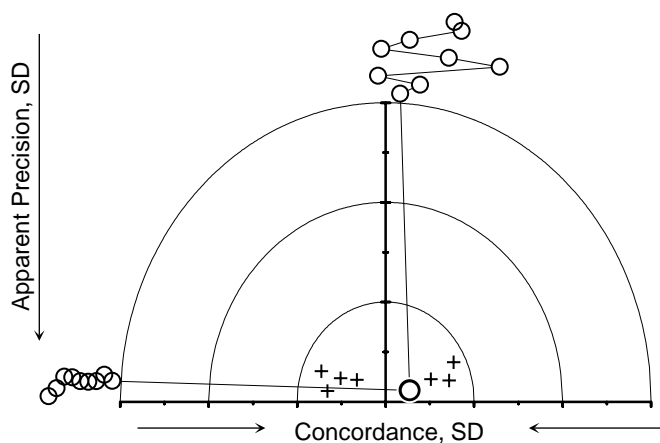
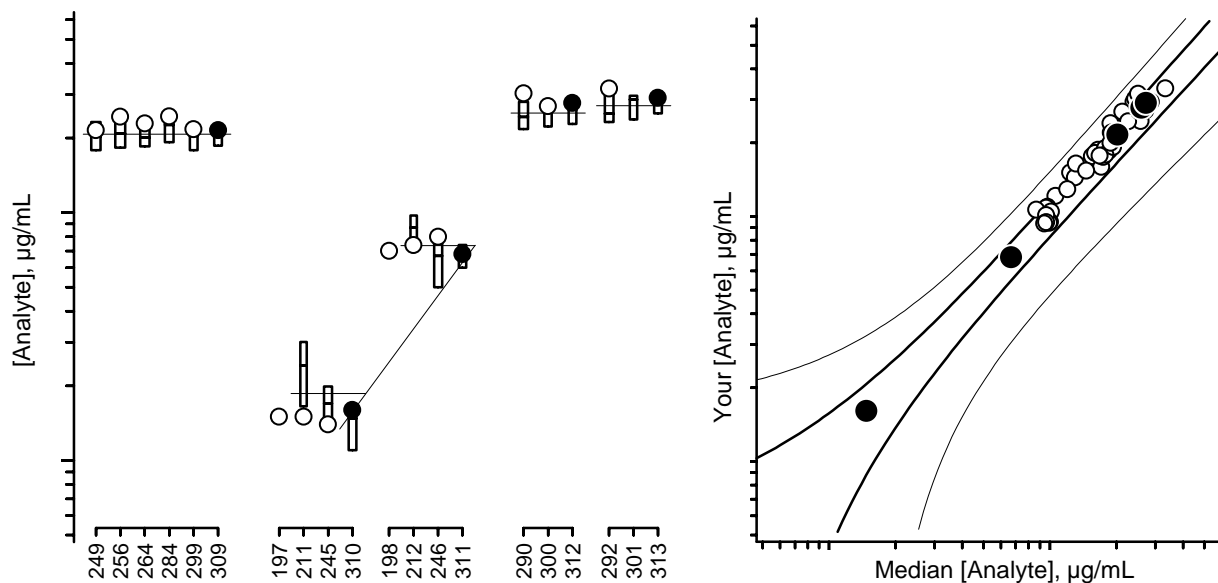
History

Comments

#309	Lyophilized - 44:249, 46:256, 48:264, 52:284, 55:299	Augmented, multi-source (SRM 968c Level II)
#310	Lyophilized - 31:197, 35:211, 43:245	Same as #311, 1:3 diluted with stripped serum
#311	Lyophilized - 31:198, 35:212, 43:246	Augmented, multi-source
#312	Lyophilized - 53:290, 55:300	Native, single-source
#313	Fresh-frozen: 53:292, 55:301	Native, single-source, hemolyzed

Individualized RR LVII Report: FSV-BA

trans-Lycopene



● You, this RR
○ You, past RRs

▲ You, $\geq x$, this RR
△ You, $\geq x$, past RRs

◇ NIST, this RR
+ Others, this RR

Serum

#309 Lyophilized - 44:249, 46:256, 48:264, 52:284, 55:299
#310 Lyophilized - 31:197, 35:211, 43:245
#311 Lyophilized - 31:198, 35:212, 43:246
#312 Lyophilized - 53:290, 55:300
#313 Fresh-frozen: 53:292, 55:301

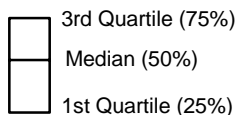
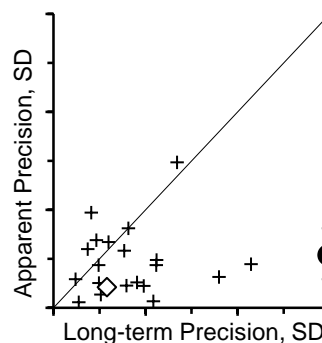
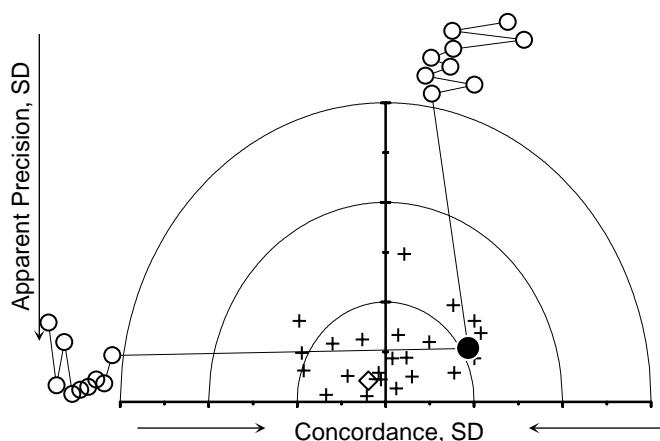
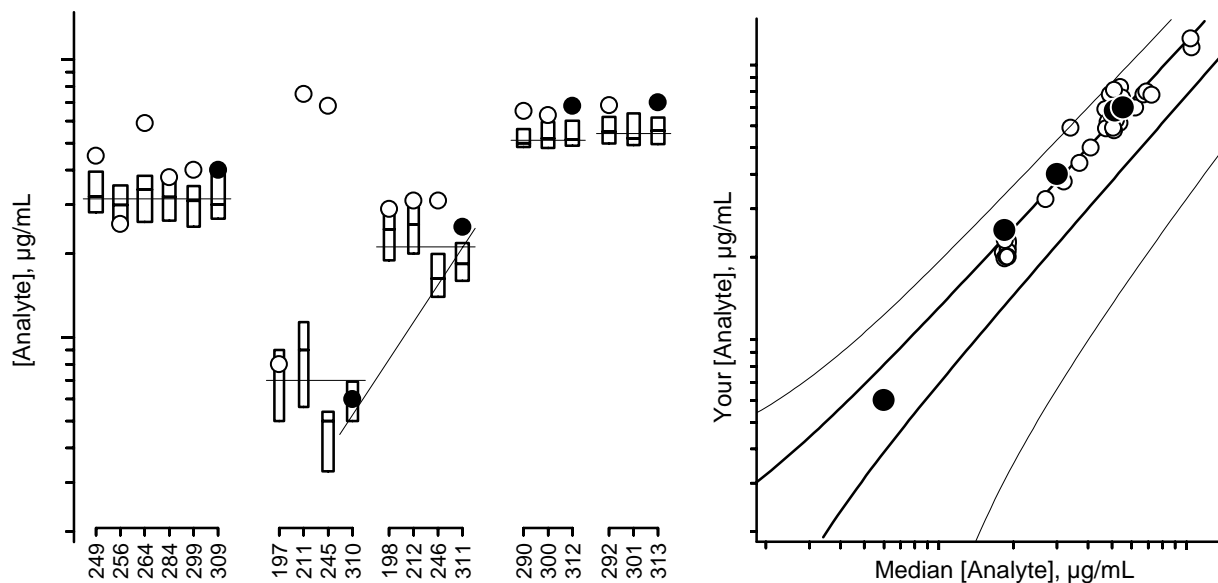
History

Comments

Augmented, multi-source (SRM 968c Level II)
Same as #311, 1:3 diluted with stripped serum
Augmented, multi-source
Native, single-source
Native, single-source, hemolyzed

Individualized RR LVII Report: FSV-BA

Total β -Cryptoxanthin



● You, this RR
○ You, past RRs

▲ You, $\geq x$, this RR
△ You, $\geq x$, past RRs

◇ NIST, this RR
+ Others, this RR

Serum

#309 Lyophilized - 44:249, 46:256, 48:264, 52:284, 55:299
#310 Lyophilized - 31:197, 35:211, 43:245
#311 Lyophilized - 31:198, 35:212, 43:246
#312 Lyophilized - 53:290, 55:300
#313 Fresh-frozen: 53:292, 55:301

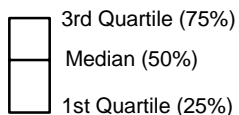
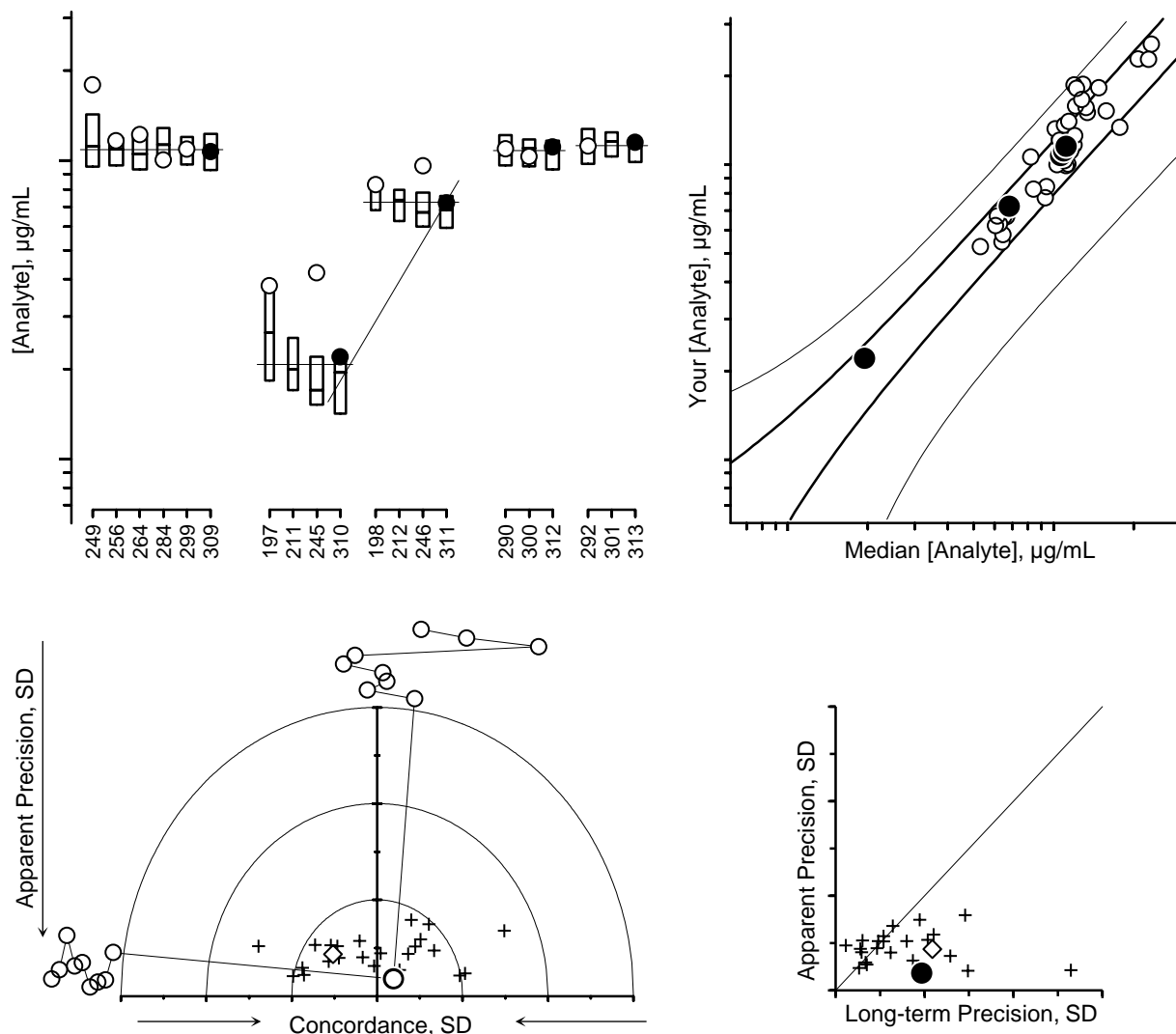
History

Comments

Augmented, multi-source (SRM 968c Level II)
Same as #311, 1:3 diluted with stripped serum
Augmented, multi-source
Native, single-source
Native, single-source, hemolyzed

Individualized RR LVII Report: FSV-BA

Total Lutein&Zeaxanthin



● You, this RR
○ You, past RRs

▲ You, ≥x, this RR
△ You, ≥x, past RRs

◇ NIST, this RR
+ Others, this RR

Serum

#309 Lyophilized - 44:249, 46:256, 48:264, 52:284, 55:299
#310 Lyophilized - 31:197, 35:211, 43:245
#311 Lyophilized - 31:198, 35:212, 43:246
#312 Lyophilized - 53:290, 55:300
#313 Fresh-frozen: 53:292, 55:301

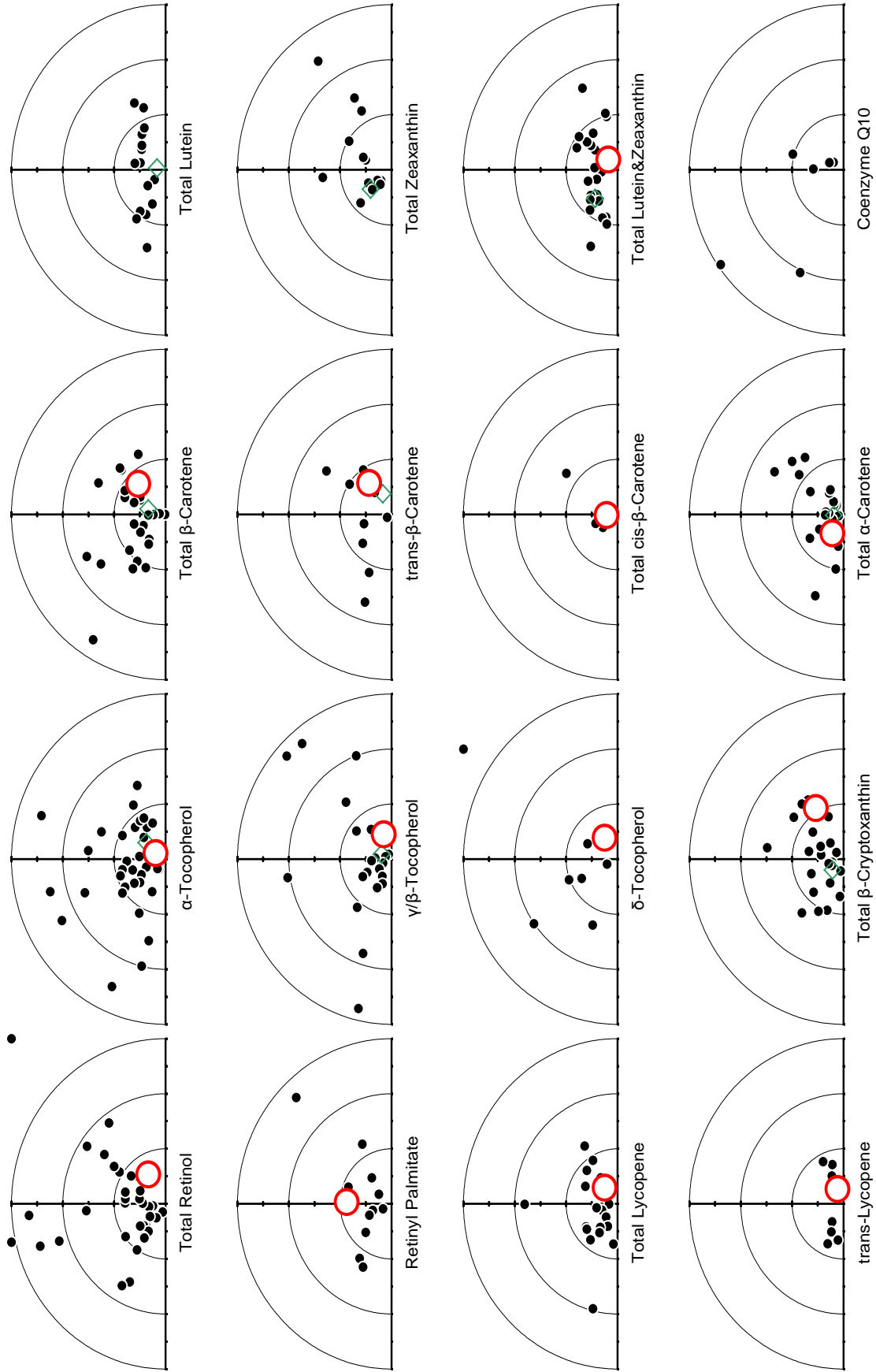
History

Comments

Augmented, multi-source (SRM 968c Level II)
Same as #311, 1:3 diluted with stripped serum
Augmented, multi-source
Native, single-source
Native, single-source, hemolyzed

Individualized Round Robin LVII Report: FSV-BA

Graphical Comparability Summary



Appendix E. Shipping Package Inserts for RR22

The following five items were included in each package shipped to an RR22 participant:

- Cover letter
- Protocol for Preparation and Analysis of the Ascorbic Acid Solid Control Material
- Preparation and Validation of Ascorbic Acid Solid Control Material Datasheet
- Analysis of Control Materials and Test Samples Datasheet
- Packing List and Shipment Receipt Confirmation Form

The cover letter, preparation protocol, and the two datasheets were enclosed in a sealed waterproof bag along with the samples themselves. The packing list was placed at the top of the shipping box, between the cardboard covering and the foam insulation.

November 8, 2004



UNITED STATES DEPARTMENT OF COMMERCE
National Institute of Standards and Technology
Gaithersburg, Maryland 20899-0001

Dear Colleague:

The samples within this package constitute Vitamin C Round Robin 22 (RR22) of the 2005 Micronutrients Measurement Quality Assurance Program.

RR22 consists of four vials of frozen serum *test samples* (#23, #33, #42, and #70), one vial of ascorbic acid *solid control material* (Control), and two vials of frozen *serum control materials* (Control #1 and Control #2). Please follow the attached protocols when you prepare and analyze these samples. If you cannot prepare the *solid control* solutions gravimetrically, please prepare equivalent solutions volumetrically and report the exact volumes used. (Routine 0.5 g gravimetric measurements are generally 10-fold more accurate than routine 0.5 mL volumetric measurements.)

The two *serum control materials* are a new component of the M²QAP for Vitamin C. Please use these materials to validate the performance of your measurement system before you analyze the *test samples*. The target value and $\approx 95\%$ confidence interval for *Control #1* is $8.41 \pm 0.61 \mu\text{mol/L}$ sample; the target value and $\approx 95\%$ confidence interval for *Control #2* is $28.05 \pm 0.49 \mu\text{mol/L}$ sample.

Please be aware that sample contact with any oxidant-contaminated surface (vials, glassware, etc.) may degrade your measurement system's performance (SA Margolis and E Park, "Stability of Ascorbic Acid in Solutions in Autosampler Vials", *Clinical Chemistry* 2001, 47(8), 1463-1464). You should suspect such degradation if you observe unusually large variation in replicate analyses.

The report for RR21 was mailed the week of Nov 1, 2004. If you find your results for RR21 unsatisfactory, we recommend that you obtain **Standard Reference Material (SRM) 970 Ascorbic Acid in Serum** to validate your methodology and value assign in-house control materials. This SRM may be purchased from the Standard Materials Reference Program at NIST (Tel: 301-975-6776, Fax: 301-948-3730, or e-mail: srminfo@nist.gov).

If you have any questions or concerns about the Vitamin C Micronutrients Measurement Quality Assurance Program please contact Jeanice Brown Thomas at tel: 301-975-3120, fax: 301-977-0685, or e-mail: jbthomas@nist.gov.

We ask that you return your results for these RR22 samples *before* **March 2, 2005**. We would appreciate receiving your results as soon as they become available. Please use the attached form. Your results will be kept confidential.

Sincerely,

Jeanice Brown Thomas
Research Chemist
Analytical Chemistry Division
Chemical Science and Technology Laboratory

Enclosures: Protocols, Preparation and Analysis of Control Materials and Analysis of Test Samples
RR22 Report Form for Ascorbic Acid Solid Control Material Preparation
RR22 Report Form for Control Material and Test Sample Analyses

NIST

Micronutrient Measurement Quality Assurance Program for Vitamin C

Please Read Through Completely BEFORE Analyzing Samples

Protocol for Preparation and Analysis of the Ascorbic Acid Solid Control Material

The *ascorbic acid solid control material* (in the amber vial) should be prepared and used in the following manner:

- 1) Prepare at least 500 mL of 5% mass fraction metaphosphoric acid (MPA) in distilled water. This solution will be referred to as the “Diluent” below.
- 2) Weigh 0.20 to 0.22 g of the ascorbic acid solid control material to 0.0001 g (if possible), dissolve it in the Diluent in a 100 mL volumetric flask, and dilute with the Diluent to the 100 mL mark. Weigh the amount of Diluent added to 0.1 g. Record the weights. The resulting material will be referred to as the “Stock Solution” below.
- 3) Prepare three dilute solutions of the Stock Solution as follows:

Dilute Solution 1: Weigh 0.500 mL of the Stock Solution to 0.0001 g into a 100 mL volumetric flask; dilute with Diluent to the 100 mL mark. Record the weight.

Dilute Solution 2: Weigh 0.250 mL of the Stock Solution to 0.0001 g into a 100 mL volumetric flask; dilute with Diluent to the 100 mL mark. Record the weight.

Dilute Solution 3: Weigh 0.125 mL of the Stock Solution to 0.0001 g into a 100 mL volumetric flask; dilute with Diluent to the 100 mL mark. Record the weight.

- 4) Calculate and record the total ascorbic acid concentrations, [TAA], in these Dilute Solutions. If you follow the above gravimetric preparation directions, the [TAA] in $\mu\text{mol/L}$ is calculated:

$$[\text{TAA}]_{\text{DS}} = \frac{(\text{g Stock Solution in Dilute Solution}) \cdot (\text{g AA in Stock Solution}) \cdot (56785 \mu\text{mol/g} \cdot \text{L})}{(\text{g AA in Stock Solution}) + (\text{g Diluent in Stock Solution})}$$

For example, if you prepared the Stock Solution with 0.2000 g of solid ascorbic acid and 103.0 g of Diluent, then 0.5 mL of the Stock Solution should weigh $(0.2+103)/200 = 0.52$ g and $[\text{TAA}]_{\text{DS1}} = (0.52 \text{ g})(0.2 \text{ g}) \cdot (56785 \mu\text{mol/g} \cdot \text{L}) / (0.2 + 103 \text{ g}) = 57.2 \mu\text{mol/L}$. Likewise, 0.25 mL of the Stock Solution should weigh 0.26 g and $[\text{TAA}]_{\text{DS2}} = 28.4 \mu\text{mol/L}$ and 0.125 mL should weigh 0.13 g and $[\text{TAA}]_{\text{DS3}} = 14.2 \mu\text{mol/L}$.

- 5) Measure the ultraviolet absorbance spectrum of Dilute Solution 1 against the Diluent as the blank using paired 1 cm path length cuvettes. Record the absorbance at 242, 243, 244, and 245 nm. Record the maximum absorbance (A_{max}) within this region. Record the wavelength (λ_{max}) at which this maximum occurs.

The extinction coefficient ($E^{1\%}$) of ascorbic acid at λ_{max} (using a cell with a 1 cm path length) of Dilute Solution #1 can be calculated:

$$E^{1\%} \left(\frac{\text{dL}}{\text{g} \cdot \text{cm}} \right) = \frac{(A_{\text{max}}) \cdot ((\text{g AA in Stock Solution}) + (\text{g Diluent in Stock Solution}))}{(\text{g Stock Solution in Dilute Solution 1}) \cdot (\text{g AA in Stock Solution})}$$

If your spectrophotometer is properly calibrated, λ_{max} should be between 243 and 244 nm and $E^{1\%}$ should be $550 \pm 30 \text{ dL/g} \cdot \text{cm}$. If they are not, you should calibrate the wavelength and/or absorbance axes of your spectrophotometer and repeat the measurements.

- 6) Measure and record the concentration of total ascorbic acid in all three dilute solutions and in the 5% MPA Diluent in duplicate using exactly the same method that you will use for the serum control materials and test samples, including any enzymatic treatment. We recommend that you analyze these solutions in the following order: Diluent, Dilute Solution 1, Dilute Solution 2, Dilute Solution 3, Dilute Solution 3, Dilute Solution 2, Dilute Solution 1, Diluent.
 - a) Compare the values of the duplicate measurements. *Are you satisfied that your measurement precision is adequate?*
 - b) Compare the measured with the calculated [TAA] values. This is most conveniently done by plotting the measured values on the y-axis of a scatterplot against the calculated values on the x-axis. The line through the four {calculated, measured} data pairs should go through the origin with a slope of 1.0. *Are you satisfied with the agreement between the measured and calculated values?*

Do **not** analyze the serum control materials or test samples until you are satisfied that your system is performing properly!

- 7) Once you have confirmed that your system is properly calibrated, analyze the serum control materials (see protocol below). The target values for these materials are:
 - Control #1: $8.5 \pm 0.5 \text{ } \mu\text{mol/L}$ of sample
 - Control #2: $28.1 \pm 1.0 \text{ } \mu\text{mol/L}$ of sample.

If your measured values are not close to these target values, please review your sample preparation procedure and whether you followed exactly the same measurement protocol the solutions prepared from the solid control material as you used for these serum controls. If the protocols differ, please repeat from Step 6 using the proper protocol. If the proper protocol was used, your measurement system may not be suitable for MPA-preserved samples. Please contact us: 301-975-3120 or Jeanice.BrownThomas@NIST.gov.

Do **not** analyze the test samples until you are satisfied that your system is performing properly and is suitable for the analysis of MPA-preserved serum!

Protocol for Analysis of the Serum Control Materials and Test Samples

The *serum control materials* and *test samples* are in sealed ampoules. They were prepared by adding equal volumes of 10% MPA to spiked human serum. We have checked the samples for stability and homogeneity. Only the total ascorbic acid is stable. While these samples contain some dehydroascorbic acid, its content is variable. Therefore, only total ascorbic acid should be reported. The *serum control materials* and *test samples* should be defrosted by warming at 20 °C for not more than 10 min otherwise some irreversible degradation may occur.

Each *serum test sample* contains between 0.0 and 80.0 μmol of total ascorbic acid/L of solution. The total ascorbic acid in each ampoule should be measured in duplicate. Please report your results in $\mu\text{mol}/(\text{L of the sample solution})$ rather than $\mu\text{mol}/(\text{L of serum NIST used to prepare the sample})$.

Participant #: _____

Date: _____

Vitamin C Round Robin 22
NIST Micronutrient Measurement Quality Assurance Program

Preparation and Validation of Ascorbic Acid Solid Control Material

STOCK SOLUTION

Mass of ascorbic acid in the Stock Solution g

Mass of 5% MPA Diluent added to the 100 mL volumetric flask..... g

DILUTE SOLUTION 1

Mass of added stock solution (0.5 mL)..... g

Mass of 5% MPA Diluent added to the 100 mL volumetric flask..... g

Absorbance of Dilute Solution 1 at 242 nm..... AU

Absorbance of Dilute Solution 1 at 243 nm..... AU

Absorbance of Dilute Solution 1 at 244 nm..... AU

Absorbance of Dilute Solution 1 at 245 nm..... AU

Absorbance of Dilute Solution absorbance maximum AU

Wavelength of maximum absorbance..... nm

Calculated $E^{1\%}$ dL/g·cm

Calculated [TAA]_{DS1} μmol/L

DILUTE SOLUTION 2

Mass of added stock solution (0.25 mL)..... g

Mass of 5% MPA Diluent added to the 100 mL volumetric flask..... g

Calculated [TAA]_{DS2} μmol/L

DILUTE SOLUTION 3

Mass of added stock solution (0.125 mL)..... g

Mass of 5% MPA Diluent added to the 100 mL volumetric flask..... g

Calculated [TAA]_{DS3} μmol/L

Please return *before* **March 2, 2005** to:

MMQAP
100 Bureau Drive, Stop 8392
Gaithersburg, MD 20899-8392

Fax: 301-977-0685
Email: david.duewer@nist.gov

Participant #: _____

Date: _____

Vitamin C Round Robin 22
NIST Micronutrient Measurement Quality Assurance Program

Analysis of Control Materials and Test Samples

Sample	Replicate 1	Replicate 2	Units
Dilute Solution 1			μmol/L of Dilute Solution
Dilute Solution 2			μmol/L of Dilute Solution
Dilute Solution 3			μmol/L of Dilute Solution
5% MPA Diluent			μmol/L of Diluent
Serum Control #1			μmol/L of Sample <i>Target: 8.5 ±0.5 μmol/L</i>
Serum Control #2			μmol/L of Sample <i>Target: 28.1 ±1.0 μmol/L</i>
Serum Test Sample #23			μmol/L of Sample
Serum Test Sample #33			μmol/L of Sample
Serum Test Sample #42			μmol/L of Sample
Serum Test Sample #70			μmol/L of Sample

Were samples frozen upon receipt? Yes | No

Analysis method: HPLC-EC | HPLC-Fluor DAB | HPLC-OPD | HPLC-UV | AO-OPD | Other
If "Other", please describe:

COMMENTS:

Please return *before* **March 2, 2005** to:

MMQAP
100 Bureau Drive, Stop 8392
Gaithersburg, MD 20899-8392

Fax: 301-977-0685
Email: david.duewer@nist.gov

Participant #: _____

Date: _____

Vitamin C Round Robin 22
NIST Micronutrients Measurement Quality Assurance Program
Packing List and Shipment Receipt Confirmation Form

This box contains one vial each of the following **seven** VitC M²QAP samples:

Sample	Form
VitC #23	Liquid frozen (1:1 serum:10% MPA)
VitC #33	Liquid frozen (1:1 serum:10% MPA)
VitC #42	Liquid frozen (1:1 serum:10% MPA)
VitC #70	Liquid frozen (1:1 serum:10% MPA)
Control #1	Liquid frozen (1:1 serum:10% MPA)
Control #2	Liquid frozen (1:1 serum:10% MPA)
Control	Solid AA

- Please**
- 1) Open the pack immediately
 - 2) Check that it contains one vial each of the above samples
 - 3) Check if the samples arrived frozen
 - 4) Store the samples at -20 °C or below until analysis
 - 5) Complete the following information
 - 6) Fax the completed form to us at 301-977-0685
(or email requested information to david.duewer@nist.gov)

1) Date this shipment arrived: _____

2) Are all of the vials intact? Yes | No
If "No", which one(s) were damaged?

3) Was there any dry-ice left in cooler? Yes | No

4) Did the samples arrive frozen? Yes | No

5) At what temperature are you storing the samples? _____ °C

6) When do you anticipate analyzing these samples? _____

Your prompt return of this information is appreciated.

The M²QAP Gang

Appendix F. Final Report for RR22

The following two pages are the final report as provided to all participants:

- Cover letter.
- An information sheet that:
 - describes the contents of the “All-Lab” report,
 - describes the content of the “Individualized” report,
 - describes the nature of the test samples and details their previous distributions, if any, and
 - summarizes aspects of the study that we believe may be of interest to the participants.



UNITED STATES DEPARTMENT OF COMMERCE
National Institute of Standards and Technology
Gaithersburg, Maryland 20899-0001

April 14, 2005

Dear Colleague:

Enclosed is the summary report of the results for Round Robin 22 (RR22) for the measurement of total ascorbic acid (TAA, ascorbic acid plus dehydroascorbic acid) in human serum. Included in this report are: a summary of data for all laboratories and a summary of individual laboratory performance and interlaboratory accuracy and repeatability. As in previous reports, the estimated standard deviations (eSD) for the measurements are defined as 0.74x interquartile range and the estimate coefficients of variation (eCV) are defined as 100x eSD/median.

RR 22 consists of four *test samples* (#23, #33, #42, and #70), two *serum control materials*, and one *solid control material* for preparation of TAA control solutions. Details regarding the samples can be found in the enclosed report.

If you have concerns regarding your laboratory's performance, we suggest that you obtain and analyze a unit of Standard Reference Material (SRM) 970, Vitamin C in Frozen Human Serum. SRM 970 can be purchased from the NIST SRM Program at phone: 301-975-6776; fax: 301-948-3730. If your measured values do not agree with the certified values, we suggest that you contact us for consultation.

Samples for the second vitamin C round robin study (RR 23) for the 2005 Vitamin C in Serum QA Program will be shipped (**during the week of May 16**). If you have questions or concerns regarding this report, please contact David Duewer at 301-975-3935; e-mail: david.duewer@nist.gov or me at 301-975-3120; e-mail: jbtthomas@nist.gov; or fax: 301-977-0685.

Sincerely,

Jeanice Brown Thomas
Research Chemist
Analytical Chemistry Division
Chemical Science and Technology Laboratory

Enclosures

The NIST M²QAP Vitamin C Round Robin 22 (RR22) report consists of

Page	“Individualized” Report
1	Summarizes your reported values for the nominal 55 mmol/L solution you prepared from the ascorbic acid solid control sample, the two serum control samples, and the four serum test samples.
2	Graphical summary of your RR 22 sample measurements.
Page	“All Lab” Report
1	A tabulation of results and summary statistics for Total Ascorbic Acid [TAA] in the RR22 samples and control/calibration solutions.

Serum-based Samples. Two serum controls and four unknowns were distributed in RR22.

- CS1 SRM 970 level 1, ampouled in mid-1998.
- CS2 SRM 970 level 2, ampouled in mid-1998.
- S22:1 Serum 23, ampouled in late 2001, previously distributed as sample S17:1 (RR17, Sep-02), S19:2 (RR19, Sep-03), and S21:2 (Sep-04). An augmented serum.
- S22:2 Serum 33, ampouled in late 2001, previously distributed as sample S17:2 (RR17, Sep-02), S18:1 (RR18, Mar-03) and S20:1 (RR20, Mar-04). An augmented serum.
- S22:3 Serum 42, ampouled in late 2001, previously distributed as sample S18:2 (RR18, Mar-03), S19:3 (RR19, Sep-03) and S21:3 (RR21, Sep-04). An augmented serum.
- S22:4 Serum 70, SRM 970 level 2, ampouled in mid-1998. This material was distributed with identification in RR11 (Oct-98) and RR12 (Mar-99) and as samples S13-2 (RR13, Mar-00), S14-4 (RR14, Mar-01), S15:2 (RR15, Sep-01), S18:3 (RR18, Mar-03), and S20:4 (RR20, Mar-04). An augmented serum.

Results.

- 1) All participants who prepared the four control/calibration solutions (the three “Dilute Solutions” and the 5% MPA “Diluent”) did so correctly. The criteria used to evaluate this success are: the density of the 5% MPA (≈ 1.03 g/mL), the observed wavelength maximum of “Dilute Solution 1” (≈ 244 nm), the observed absorbance at that maximum (≈ 0.55 OD), the calculated $E^{1\%}$ of Solution 1” (≈ 550 dL/g·cm).
- 2) Judging from the calibration parameters calculated for the control/calibration solutions (intercepts close to 0.0 and slopes close to 1.0), the measurement systems for most participants are well calibrated, although the slope for one participant was 11% lower than expected and the intercept for another participant was significantly different from zero.
- 3) Several participants reported values for high-level control that were well outside the target range of 26 $\mu\text{mol/L}$ to 30 $\mu\text{mol/L}$. If the measured values for the control samples are not close to the targets, even if your measured and calculated values for the calibration solutions agree, there is a problem with your measurement system.
- 4) There is no evidence of sample degradation with any of the samples distributed.

Appendix G. “All-Lab Report” for RR22

The following single page is the “All-Lab Report” as provided to all participants, with two exceptions:

- the participant identifiers (Lab) have been altered.
- the order in which the participant results are listed has been altered.

The data summary in the “All-Lab Report” has been altered to ensure confidentiality of identification codes assigned to laboratories.

Micronutrients Measurement Quality Assurance Program for Total Ascorbic Acid
"Round Robin" 22 - March 2005

Control / Calibration Samples													MPA			Dilute Solution 1 Spectrophotometry			Samples											
Lab	Date	Gravimetric, µmol/L			Measured, µmol/L					Calibration Parameters			Density g/mL	λ _{max}	A _{max}	E %	Measured, µmol/L				Corrected, µmol/L									
		Dil:1	Dil:2	Dil:3	Dil:1	Dil:2	Dil:3	MPA	Inter	Slope	R ²	SEE					CS#1	CS#2	S22:1	S22:2	S22:3	S22:4	CS#1	CS#2	S22:1	S22:2	S22:3	S22:4		
VC-MA	08/03/05	56.4	28.5	14.0	59.9	30.6	15.3	0.0	0.21	1.06	1.000	0.2	1.032	243.	0.5591	562.9	8.6	29.0	9.6	22.7	35.6	29.4	7.9	27.1	8.9	21.2	33.4	27.6		
VC-MB	02/03/05	56.6	28.0	14.3	53.7	26.1	13.7	0.0	-0.07	0.95	1.000	0.3	1.032	244.	0.5612	563.3	8.3	28.9	7.7	22.0	35.1	28.4	8.8	30.6	8.2	23.2	37.1	30.0		
VC-MC	29/11/04	57.2	27.5	13.5	50.8	25.5	12.5	0.0	0.40	0.89	0.999	0.6	1.030	243.	0.5553	551.1	8.3	27.0	10.3	23.9	37.6	27.0	8.9	30.0	11.1	26.4	42.0	30.0		
VC-ME	14/03/03	56.7	28.3	14.0	57.9	29.5	14.8	0.0	0.33	1.02	1.000	0.4	1.033	243.	0.5315	532.5	8.4	27.7	10.2	22.6	31.3	28.2	7.9	26.9	9.7	21.8	30.4	27.4		
VC-MG	08/02/05	57.6	29.1	14.5	58.4	28.1	13.3	0.0	-0.83	1.02	0.999	1.0	1.033	243.9	0.5700	561.6	8.7	30.4	11.4	24.7	40.6	34.1	9.3	30.6	12.0	25.1	40.7	34.3		
VC-MH	13/01/05	62.5	31.2	15.2	62.9	31.8	15.7	0.2	0.34	1.00	1.000	0.2	1.030	244.	0.6120	556.2	8.3	27.9	8.5	22.5	34.3	28.2	7.9	27.5	8.1	22.1	33.9	27.8		
VC-MI	12/01/05	56.0	28.0	14.3	55.2	26.8	13.4	1.0	0.15	0.97	0.999	1.0	1.033				7.1	26.5	4.1	16.1	26.4	26.0	7.1	27.0	4.0	16.4	26.9	26.5		
VC-MJ	15/12/04	58.7	28.8	15.0	57.4	30.4	15.0	0.4	0.80	0.97	0.998	1.3	1.023	244.	0.5854	564.0	10.3	27.2	11.6	22.6	31.1	26.5	9.7	27.0	11.1	22.3	31.1	26.4		
VC-MK	25/02/05	58.9	28.4	13.9	65.2	35.2	19.8	1.5	3.40	1.07	0.996	2.2	1.032	244.	0.5603	561.3	12.4	35.3	14.6	29.7	41.3	35.0	8.4	29.9	10.5	24.6	35.5	29.6		
VC-MT	01/03/05	56.7	27.8	14.4	56.9	28.4	13.9	0.0	-0.12	1.01	1.000	0.5	1.032				8.7	28.9	9.5	21.2	35.7	29.8	8.8	28.8	9.6	21.1	35.5	29.6		
VC-MY	22/02/05																11.5	49.8	11.1	34.5	54.9	46.0	11.5	49.8	11.1	34.5	54.9	46.0		
N	10	10	10	10	10	10	10	10	10				10	8	8	8	11	11	11	11	11	11	11	11	11	11	11	11		
Average	57.7	28.5	14.3	57.8	29.2	14.7	0.3						1.031	243.6	0.5669	556.6	9.1	30.8	9.9	23.8	36.7	30.8	8.8	30.5	9.5	23.5	36.5	30.5		
SD	1.9	1.0	0.5	4.2	2.9	2.0	0.5						0.003	0.5	0.0236	10.7	1.6	6.7	2.7	4.7	7.4	5.8	1.2	6.6	2.2	4.5	7.5	5.6		
Min	56.0	27.50	13.5	50.8	25.47	12.5	0.0						1.023	243.0	0.5315	532.5	7.1	26.5	4.1	16.1	26.4	26.0	7.1	26.9	4.0	16.4	26.9	26.4		
%25	56.6	27.98	14.0	55.6	27.09	13.4	0.0						1.031	243.0	0.5582	554.9	8.3	27.4	9.0	22.2	32.8	27.6	7.9	27.1	8.5	21.5	32.3	27.5		
Median	56.9	28.32	14.3	57.6	28.97	14.4	0.0						1.032	244.0	0.5608	561.5	8.6	28.9	10.2	22.6	35.6	28.4	8.8	28.8	9.7	22.3	35.5	29.6		
%75	58.5	28.73	14.5	59.5	30.53	15.2	0.3						1.033	244.0	0.5739	563.0	9.5	29.7	11.3	24.3	39.1	31.9	9.1	30.3	11.1	24.8	38.9	30.0		
Max	62.5	31.19	15.2	65.2	35.21	19.8	1.5						1.033	244.0	0.6120	564.0	12.4	49.8	14.6	34.5	54.9	46.0	11.5	49.8	12.0	34.5	54.9	46.0		
MADe	0.9	0.6	0.4	3.5	2.8	1.4	0.0						0.002	0.1	0.0109	3.3	0.4	2.1	1.8	1.9	6.4	2.1	1.3	2.6	2.1	1.8	6.5	3.0		
CV	2	2	3	6	10	10							0.19	0.03	1.9	0.6	5	7	18	8	18	7	14	9	22	8	18	10		

Appendix H. Representative “Individualized Report” for RR22

Each participant in RR22 received an “Individualized Report” reflecting their reported results. The following two pages are the “Individualized Report” for participant “VC-MA”.

Vitamin C "Round Robin" 22 Report: Participant VC-MA

Date	RR	Method	MPA	Dilute Solution 1			Control/Calibration Solutions			
			Density	Spectrophotometry			$Y_{\text{meas}} = \text{Inter} + \text{Slope} * X_{\text{grav}}$			
			g/mL	λ_{max}	A_{max}	$E^{1\%}$	Inter	Slope	R^2	SEE
12/12/02	17	HPLC-EC	1.026	242.0	0.552	551.0	-0.3	1.06	1.000	0.49
03/20/03	18	HPLC-EC	1.026	244.0	0.509	563.1	-0.1	1.02	1.000	0.18
11/13/03	19	HPLC-EC	1.026	243.0	0.584	561.9	1.1	1.03	0.998	1.24
02/23/04	20	HPLC-EC	1.031	243.0	0.552	560.7	-0.4	1.05	1.000	0.65
09/13/04	21	HPLC-EC	1.030	244.0	0.555	562.2	-0.1	0.99	1.000	0.10
03/08/05	22	HPLC-EC	1.034	243.0	0.559	562.9	0.2	1.06	1.000	0.24
		Mean	1.029	243.2	0.55	560.3				0.48
		SD	0.003	0.8	0.02	4.6				0.42
		CV	0.32	0.31	4.4	0.8				

Date	RR	Sample	[TAA] mmol/Lsample								
			Rep ₁	Rep ₂	F_{adj}	Mean	SD_{dup}	N	Mean	SD_{repeat}	SD_{reprod}
02/23/04	20	CS#1	7.8	8.0	1.0	7.9	0.1	3	8.2	0.1	0.4
09/13/04	21	CS#1	8.1	7.9	1.0	8.0	0.1				
03/08/05	22	CS#1	8.5	8.7	1.0	8.6	0.1				
02/23/04	20	CS#2	25.8	26.2	1.0	26.0	0.3	3	27.2	0.4	1.6
09/13/04	21	CS#2	26.2	27.2	1.0	26.7	0.7				
03/08/05	22	CS#2	29.0	29.0	1.0	29.0	0.0				
12/12/02	17	S17:1	9.9	9.1	1.0	9.5	0.6	4	9.3	0.3	0.4
11/13/03	19	S19:2	9.2	9.1	1.0	9.2	0.1				
09/13/04	21	S21:2	8.8	8.7	1.0	8.7	0.1				
03/08/05	22	S22:1	9.6	9.6	1.0	9.6	0.0				
12/12/02	17	S17:2	23.3	23.4	1.0	23.4	0.1	4	23.5	0.5	0.8
03/20/03	18	S18:1	22.7	23.7	1.0	23.2	0.7				
02/23/04	20	S20:1	25.1	24.1	1.0	24.6	0.7				
03/08/05	22	S22:2	22.7	22.7	1.0	22.7	0.0				
03/20/03	18	S18:2	35.1	36.0	1.0	35.6	0.6	4	35.0	0.3	1.3
11/13/03	19	S19:3	35.9	35.8	1.0	35.9	0.1				
09/13/04	21	S21:3	33.2	32.9	1.0	33.0	0.2				
03/08/05	22	S22:3	35.7	35.6	1.0	35.6	0.1				
09/23/98	11	S11:2	49.2	51.4	0.5	25.2	0.8	8	26.5	0.4	2.0
04/02/99	12	S12:2	47.7	47.0	0.5	23.7	0.2				
09/17/01	13	S13:2	27.6	27.7	1.0	27.7	0.1				
09/27/01	14	S14:4	25.7	26.4	1.0	26.0	0.5				
09/18/01	15	S15:2	25.4	25.6	1.0	25.5	0.2				
03/20/03	18	S18:3	28.8	29.2	1.0	29.0	0.3				
02/23/04	20	S20:4	25.9	25.2	1.0	25.5	0.5				
03/08/05	22	S22:4	29.4	29.4	1.0	29.4	0.0				

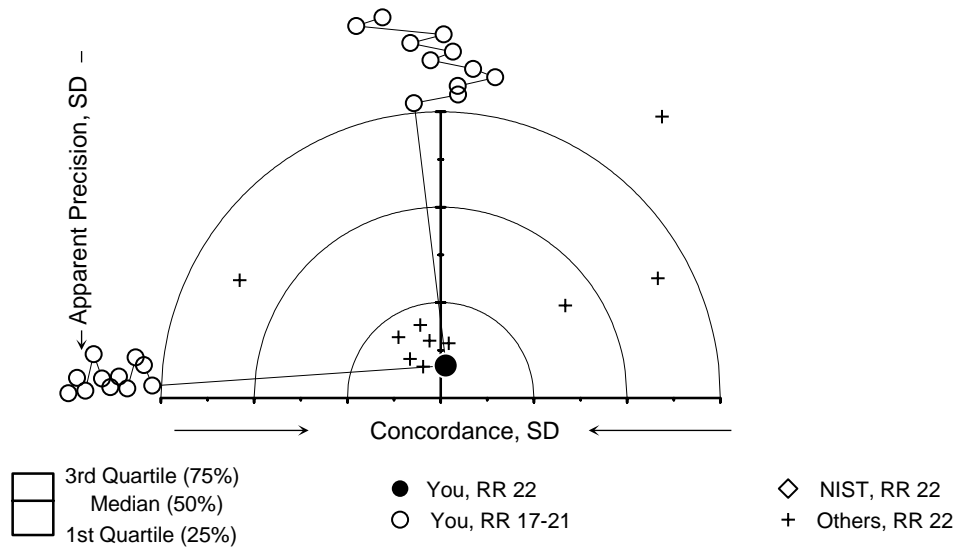
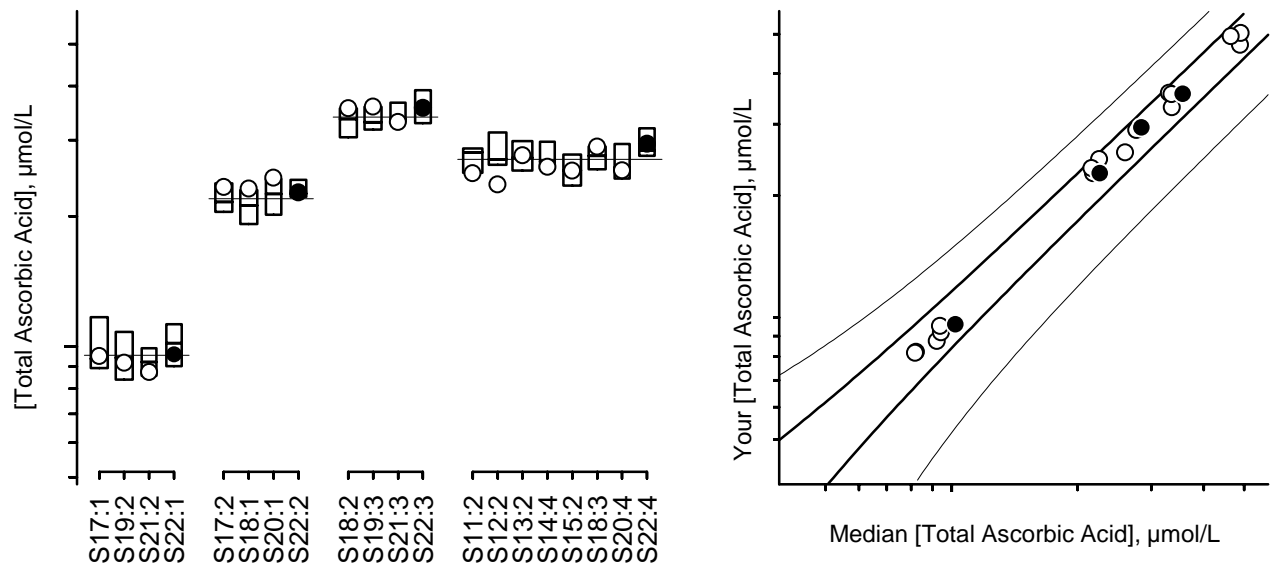
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Vitamin C "Round Robin" 22 Report: Participant VC-MA

Total Ascorbic Acid



For details of the construction and interpretation of these plots, see:
 Duewer, Kline, Sharpless, Brown Thomas, Gary, Sowell. Anal Chem 1999;71(9):1870-8.

Sample

Comments

- S21:1 Serum 23, previously distributed in RRs 17, 19, and 21
 S21:2 Serum 33, previously distributed in RRs 17, 18, and 20
 S21:3 Serum 42, previously distributed in RRs 18, 19, and 21
 S21:4 SRM 970 Level 2, previously distributed in RRs 11, 12, 13, 14, 15, 18, and 20