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NIST Micronutrients Measurement Quality Assurance Program Winter 2007 Comparability Studies

Results for Round Robin LXI Fat-Soluble Vitamins and Carotenoids in Human Serum and Round Robin 26 Ascorbic Acid in Human Serum

David L. Duewer
Jeanice B. Thomas

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National Institute of Standards and Technology
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April, 2013

U.S. Department of Commerce
Rebecca Blank, 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 2007 MMQAP measurement comparability improvement studies: 1) Round Robin LXI Fat-Soluble Vitamins and Carotenoids in Human Serum and 2) Round Robin 26 Total Ascorbic Acid in Human Serum. The materials for both studies were shipped to participants in November 2006; participants were requested to provide their measurement results by March 5, 2007.

Keywords

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

Table of Contents

Abstract iii
Keywords iii
Table of Contents iv
Introduction 1
Round Robin LXI: Fat-Soluble Vitamins and Carotenoids in Human Serum 1
Round Robin 26: Vitamin C in Human Serum 2
References 3

Appendix A. Shipping Package Inserts for RR61 ..... A1
Appendix B. Final Report for RR61 ..... B1
Appendix C. "All-Lab Report" for RR61 ..... C1
Appendix D. Representative "Individualized Report" for RR61 ..... D1
Appendix E. Shipping Package Inserts for RR26 ..... E1
Appendix F. Final Report for RR26 ..... F1
Appendix G. "All-Lab Report" for RR26 ..... G1
Appendix H. Representative "Individualized Report" for RR26 ..... H1

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 LXI: Fat-Soluble Vitamins and Carotenoids in Human Serum

Participants in the MMQAP Fat-Soluble Vitamins and Carotenoids in Human Serum Round Robin LXI comparability study (hereafter referred to as RR61) received one lyophilized and four 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 2006. 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 RR61 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 26: Vitamin C in Human Serum

Participants in the MMQAP Vitamin C in Human Serum Round Robin 26 comparability study (hereafter referred to as RR26) received four frozen serum test samples, one frozen control serum, 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 2006. 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 RR26 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 RR61

The following three items were included in each package shipped to an RR61 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-

November 8, 2006

Dear Colleague:

Enclosed are the samples (Sera 329-333) for the first fat-soluble vitamins and carotenoids in serum round robin study (Round Robin LXI) for the fiscal year (FY) 07 NIST Micronutrients Measurement Quality Assurance Program. You will find one vial of each of four liquid-frozen and one 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 5, 2007**. Results received more than two weeks after the due date will not be included in the summary report for this round robin study.

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 samples (Sera 329-332).**

For consistency, we request that laboratories use the following absorptivities (dL/g•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 LXI 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,

Jean B. Thomas

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

Enclosures

NIST

Participant #: _____ Date: _____

Round Robin LXI: Human Sera

NIST Micronutrients Measurement Quality Assurance Program

Analyte	329	330	331	332	333	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 measurands?						

* we prefer **µg/mL**

Were the liquid frozen samples #329, 330, 331, and 332 frozen when received? Yes | No

Comments:

Mail: M^2QAP
NIST, Stop 8392
Gaithersburg, MD 20899-8392

Please return results **before** 5-Mar-2007

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

Participant #: _____ Date: _____

Fat-Soluble Vitamins Round Robin LXI

NIST Micronutrients Measurement Quality Assurance Program

Packing List and Shipment Receipt Confirmation Form

This box contains: one vial each of the following five FSV M^2QAP sera

Serum	Form	Reconstitute?
#329	Liquid frozen	No
#330	Liquid frozen	No
#331	Liquid frozen	No
#332	Liquid frozen	No
#333	Lyophilized	Yes (1 ml H_2O)

Please
1) Open the pack immediately
2) Check that it contains all of the above samples
3) Check if the vials are intact
4) Store the sera 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 sera 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 liquid frozen samples arrive frozen? Yes | No

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

6) When do you anticipate analyzing these samples? _____

Your prompt return of this information is appreciated.

The M^2QAP Gang

Mail: M^2QAP
NIST, Stop 8392
Gaithersburg, MD 20899-8392

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

Appendix B. Final Report for RR61

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-

April 26, 2007

Dear Colleague:

Enclosed is the summary report of the results for round robin LXI (RR61) of the 2007 NIST Micronutrients Measurement Quality Assurance Program (M^2QAP) 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 comparability.

Data for evaluating laboratory performance in RR61 are provided in text "Score Card" summary, page 7 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. Similar information is presented in the graphical "target plot" summary, last page of your Individualized Report.

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 2007 QA interlaboratory exercise will be shipped **starting the week of June 4, 2007.** 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.

We look forward to meeting with you at the Micronutrients Measurement Quality Assurance Workshop that is being held in conjunction with the Experimental Biology meeting on **May 2, 2007** at the Convention Center in Washington, DC.

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 B Thomas

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

Cc: L.C. Sander
D.L. Duewer

NIST

The NIST M^2QAP Round Robin LXI (RR61) report consists of:

Page	"All Lab" Report
1-5	A listing of all results and statistics for all analytes.
6	A legend for the list of results and statistics.
7	The text Comparability Summary ("Score Card") of measurement performance.

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 graphical Comparability Summary (target plot) of measurement performance.

Samples. The five sera below were distributed in RR61.

Serum	Description	Prior Distributions
329	Fresh-frozen, native, single-donor serum prepared in Spring, 2006.	
330	Fresh-frozen, a 15:16 blend of the #329 and #331 sera (*i.e.*, #330 = (15×#324 + 16×#326)/31, prepared in Spring, 2006.	
331	Fresh-frozen, native, single-donor serum prepared in Spring, 2006.	#326:RR60-9/06
332	Fresh-frozen, native, single-donor, commercially obtained serum prepared in 2002. The same material was used to prepare #333.	#292:RR53-2/03, #301:RR55-3/04, #313:RR57-3/05, #323:RR59-3/06
333	Lyophilized, native, single-donor, commercially obtained serum prepared in 2002. The same material was used to prepare #332.	#290:RR53-2/03, #300:RR55-3/04, #312:RR57-3/05, #322:RR59-3/06

Results

1) Sera Stability. There was no significant change in the median level or variability of any measurand in either the fresh-frozen or lyophilized serum of the {332, 333} pair. These materials have been in --80 °C storage for more than four years. The stability of the fresh-frozen material in this and other sera pairs provides strong support for continued use of fresh-frozen rather than lyophilized materials in this program.

2) Measurand Additivity. Serum 330 was prepared as a mixture of two native, single-donor sera. Using the correct method of analysis, the median values in Serum 325 are as expected from the Sera 329 and 331 medians and their 15:16 blending ratio. (Previous additivity studies were incorrectly analyzed. The curved lines connecting the three sera in the current graphs are correct for reporting results on a logarithmic y-axis. This topic will be discussed in more detail at the Workshop.)

3) Report of Unusual Performance of High-purity Retinol Standards. Our colleague Fernando Granado-Lorencio, Hospital Universitario Puerta de Hierro (Madrid), reports unusually high chromatographic response per ng injected for recently purchased high-purity retinol standards from Sigma. The new material appears to be of about 20% higher purity than the older material. Similar behavior was noted by other members of the European Standardization Methods Committee during their revision of a candidate reference method for vitamin A in foods.

Appendix C. "All-Lab Report" for RR61

The following eight 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 LXI Laboratory Results

All Results in µg/mL

	Total Retinol					trans-Retinol					Retinyl Palmitate					α-Tocopherol				
Lab	329	330	331	332	333	329	330	331	332	333	329	330	331	332	333	329	330	331	332	333
FSV-BA	0.559	0.433	0.326	0.697	0.709						0.068	0.057	0.070	0.120	0.114	24.43	15.75	8.66	10.59	10.16
FSV-BB	0.485	0.388	0.284	0.628	0.617						0.055	0.033	0.011	0.077	0.073	25.17	16.33	9.00	10.42	10.07
FSV-BC	0.512	0.396	0.300	0.651	0.619															
FSV-BD	0.536	0.444	0.331	0.696	0.628											24.50	16.40	8.80	10.50	9.80
FSV-BE	0.553	0.390	0.314	0.635	0.607											28.58	17.63	10.12	12.16	11.47
FSV-BF	0.520	0.400	0.290	0.610	0.600											25.00	15.80	8.40	10.50	10.10
FSV-BG	0.563	0.431	0.316	0.702	0.653						0.106	0.084	0.066	0.172	0.152	26.48	17.00	9.00	11.50	10.58
FSV-BH	0.422	0.398	0.262	0.584	0.608											21.44	14.39	7.50	9.12	8.87
FSV-BI	0.472	0.364	0.276	0.577	0.561						0.098	*nd*	*nd*	0.110	0.105	23.41	14.99	8.18	10.15	9.54
FSV-BJ	0.496	0.379	0.280	0.593	0.581						0.050	0.026	0.012	0.096	0.097	27.06	16.60	8.26	10.39	10.07
FSV-BK	0.602	0.427	0.320	0.667	0.637											25.37	14.51	8.08	9.75	9.28
FSV-BL	0.520	0.370	0.290	0.630	0.600											21.50	14.20	8.20	9.50	9.50
FSV-BM	0.519	0.404	0.325	0.634	0.614											26.90	16.30	8.60	11.10	10.50
FSV-BN	0.421	0.352	0.251	0.553	0.536						0.050	0.032	0.013	0.100	0.098	22.46	15.93	8.23	10.79	10.44
FSV-BO	0.507	0.384	0.315	0.670	0.636											21.50	14.10	7.90	9.70	8.70
FSV-BQ	0.552	0.428	0.352	0.682	0.669											21.00	13.10	7.80	9.10	9.10
FSV-BR	0.527	0.402	0.305	0.663	0.661											23.06	16.09	9.19	11.51	11.49
FSV-BS	*≥0.469*	*≥0.366*	*≥0.274*	*≥0.588*	*≥0.557*	0.469	0.366	0.274	0.588	0.557						24.82	14.49	8.23	9.65	9.95
FSV-BT	0.599	0.471	0.342	0.666	0.604											23.26	15.22	8.22	10.79	10.49
FSV-BU	0.329	0.406	0.296	0.565	0.471											23.08	17.55	10.34	11.69	11.69
FSV-BV	0.424	0.325	0.262	0.645	0.605											22.95	14.72	9.25	11.26	10.70
FSV-BW	0.500	0.390	0.300	0.650	0.610						0.090	0.050	0.010	0.260	0.230	22.90	15.25	8.39	10.53	9.98
FSV-BX	*≥0.510*	*≥0.390*	*≥0.306*	*≥0.647*	*≥0.633*	0.510	0.390	0.306	0.647	0.633						23.69	15.39	8.90	10.79	10.39
FSV-CC	0.550	0.430	0.320	0.700	0.660	0.540	0.430	0.320	0.700	0.650						24.10	15.93	8.52	10.52	9.83
FSV-CE	0.480	0.360	0.300	0.610	0.710											25.00	15.30	8.70	10.50	5.80
FSV-CF	0.497	0.378	0.282	0.669	0.674											24.20	15.10	9.10	11.60	11.10
FSV-CG	0.530	0.413	0.322	0.647	0.638											23.43	15.04	8.28	10.01	9.66
FSV-CI	*≥0.462*	*≥0.362*	*≥0.289*	*≥0.596*	*≥0.581*	0.462	0.362	0.289	0.596	0.581	0.045	0.026	0.024	0.092	0.082	23.59	14.61	8.42	9.95	8.98
FSV-CS	0.585	0.437	0.329	0.789	0.650											22.54	15.88	9.38	11.40	9.68
FSV-CW	0.513	0.341	0.263	0.611	0.597						0.036	0.014	0.007	0.091	0.086	25.26	15.70	8.50	10.20	9.40
FSV-CZ	0.529	0.402	0.304	0.647	0.608											23.80	15.40	8.50	10.70	10.10
FSV-DD	≥0.600	≥0.340	≥0.380	≥0.730	≥0.680	0.600	0.340	0.380	0.730	0.680										
FSV-DF	0.500	0.376	0.303	0.637	0.765															
FSV-DI	0.523	0.418	0.324	0.642	0.624						0.059	0.033	0.010	0.094	0.091	24.20	15.90	8.67	10.50	10.20
FSV-DV	0.568	0.424	0.326	0.696	0.680											21.90	13.80	7.60	9.30	8.80
FSV-EE	0.454	0.309	0.266	0.550	0.508											19.90	13.60	7.10	9.20	8.50
N	32	32	32	32	32	5	5	5	5	5	10	9	9	10	10	33	33	33	33	33
Min	0.329	0.309	0.251	0.550	0.471	0.462	0.340	0.274	0.588	0.557	0.036	0.014	0.007	0.077	0.073	19.90	13.10	7.10	9.10	5.80
Median	0.520	0.399	0.304	0.646	0.618	0.510	0.366	0.306	0.647	0.633	0.057	0.033	0.012	0.098	0.098	23.69	15.39	8.50	10.50	9.98
Max	0.602	0.471	0.352	0.789	0.765	0.600	0.430	0.380	0.730	0.680	0.106	0.084	0.070	0.260	0.230	28.58	17.63	10.34	12.16	11.69
SD	0.042	0.035	0.029	0.043	0.038						0.026	0.018	0.010	0.019	0.018	1.56	0.98	0.51	0.62	0.77
CV	8	9	10	7	6						45	54	87	19	19	7	6	6	6	8
N_{past}	0	0	32	33	34	0	0	5	7	7	0	0	8	12	12	0	0	36	36	37
$Median_{past}$			0.309	0.641	0.605			0.310	0.643	0.603			0.037	0.099	0.093			8.32	10.39	9.87
SD_{past}			0.023	0.045	0.044			0.000	0.020	0.020			0.030	0.021	0.019			0.58	0.66	0.68
NAV	0.520	0.399	0.304	0.646	0.618	0.489	0.378	0.298	0.622	0.607	0.057	0.033	0.012	0.098	0.098	23.69	15.39	8.50	10.50	9.98
NAU	0.042	0.035	0.029	0.051	0.049	0.042	0.035	0.029	0.051	0.049	0.026	0.018	0.011	0.026	0.026	1.89	1.19	0.68	0.82	0.78

C2

Round Robin LXI Laboratory Results

All Results in µg/mL

	γ/β-Tocopherol					δ-Tocopherol					Total β-Carotene					trans-β-Carotene				
Lab	329	330	331	332	333	329	330	331	332	333	329	330	331	332	333	329	330	331	332	333
FSV-BA	1.06	2.569	3.83	1.94	1.87	0.065	0.065	0.079	0.064	0.052	0.580	0.351	0.158	0.132	0.127	0.548	0.330	0.148	0.126	0.121
FSV-BB	0.90	2.325	3.54	1.74	1.67	0.045	0.038	0.059	0.037	0.032	0.533	0.306	0.130	0.106	0.109	0.507	0.292	0.124	0.102	0.104
FSV-BC																				
FSV-BD																				
FSV-BE	1.10	2.743	4.33	2.06	1.95						0.528	0.356	0.169	0.134	0.106					
FSV-BF	0.81	2.340	3.54	1.79	1.77						0.436	0.272	0.121	0.102	0.093					
FSV-BG	0.86	2.630	4.07	2.14	1.98						0.674	0.410	0.179	0.172	0.177					
FSV-BH	0.67	2.181	3.16	1.57	1.53						0.580	0.362	0.145	0.119	0.116	0.548	0.344	0.145	0.119	0.116
FSV-BI	0.91	2.380	3.71	1.95	1.82						0.544	0.313	0.139	0.111	0.108					
FSV-BJ	0.94	2.573	3.84	1.76	1.72						0.617	0.359	0.128	0.130	0.126					
FSV-BK																				
FSV-BL																				
FSV-BM																				
FSV-BN	0.69	2.041	3.01	1.52	1.48						0.491	0.323	0.136	0.113	0.109	0.453	0.300	0.127	0.108	0.103
FSV-BO	0.90	2.400	3.60	1.70	1.60						0.573	0.358	0.177	0.165	0.114	0.500	0.309	0.150	0.139	0.114
FSV-BQ																				
FSV-BR																				
FSV-BS	1.24	2.387	3.45	1.80	1.84						*≥0.554*	*≥0.345*	*≥0.167*	*≥0.134*	*≥0.129*	0.554	0.345	0.167	0.134	0.129
FSV-BT	0.86	2.021	3.12	1.52	1.49	0.381	0.284	0.282	0.282	0.289	0.520	0.336	0.136	0.135	0.135	0.488	0.317	0.129	0.129	0.129
FSV-BU	1.05	2.690	4.26	2.04	2.25						0.466	0.278	0.097	0.126	0.110					
FSV-BV	0.83	2.181	3.46	1.71	1.60						0.584	0.337	0.160	0.121	0.114					
FSV-BW	1.24	3.470	5.41	2.50	2.39						0.520	0.340	0.160	0.140	0.130					
FSV-BX	1.01	2.475	3.56	1.77	1.72						*≥0.527*	*≥0.307*	*≥0.139*	*≥0.119*	*≥0.110*	0.527	0.307	0.139	0.119	0.110
FSV-CC																				
FSV-CE											0.780	0.440	0.150	0.220	0.040					
FSV-CF																				
FSV-CG	0.88	2.557	3.90	1.92	1.82	0.209	0.260	0.296	0.159	0.121	0.548	0.322	0.143	0.117	0.115	0.507	0.298	0.133	0.110	0.108
FSV-CI	0.80	2.349	4.12	1.82	1.69						*≥0.480*	*≥0.316*	*≥0.150*	*≥0.134*	*≥0.139*	0.480	0.316	0.150	0.134	0.139
FSV-CS	1.23	3.033	4.05	2.30	2.08						0.556	0.310	0.131	0.136	0.107					
FSV-CW	1.25	2.690	3.80	2.10	2.00	0.050	0.080	0.120	0.080	0.070	*≥0.531*	*≥0.284*	*≥0.141*	*≥0.138*	*≥0.134*	0.531	0.284	0.141	0.138	0.134
FSV-CZ											0.693	0.396	0.153	0.180	0.171					
FSV-DD																				
FSV-DF																				
FSV-DI	0.90	2.430	3.63	1.84	1.78	0.033	0.048	0.061	0.042	0.042	0.498	0.315	0.149	0.113	0.111					
FSV-DV																				
FSV-EE																				
N	21	21	21	21	21	6	6	6	6	6	19	19	19	19	19	11	11	11	11	11
Min	0.67	2.021	3.01	1.52	1.48	0.033	0.038	0.059	0.037	0.032	0.436	0.272	0.097	0.102	0.040	0.453	0.284	0.124	0.102	0.103
Median	0.90	2.430	3.71	1.82	1.78	0.058	0.073	0.100	0.072	0.061	0.548	0.337	0.145	0.130	0.114	0.507	0.309	0.141	0.126	0.116
Max	1.25	3.470	5.41	2.50	2.39	0.381	0.284	0.296	0.282	0.289	0.780	0.440	0.179	0.220	0.177	0.554	0.345	0.167	0.139	0.139
SD	0.15	0.215	0.38	0.22	0.21	0.094	0.121	0.131	0.068	0.047	0.046	0.033	0.019	0.017	0.013	0.034	0.018	0.013	0.015	0.015
CV	17	9	10	12	12	164	167	131	95	78	8	10	13	13	12	7	6	10	12	13
N_{past}	0	0	24	22	22	0	0	5	6	6	0	0	26	26	26	0	0	8	11	11
$Median_{past}$			3.62	1.83	1.74			0.076	0.063	0.067			0.147	0.120	0.114			0.126	0.116	0.109
SD_{past}			0.24	0.14	0.11			0.004	0.024	0.023			0.019	0.017	0.016			0.014	0.010	0.009
NAV	0.90	2.43	3.71	1.82	1.78	0.058	0.073	0.100	0.072	0.061	0.548	0.337	0.145	0.130	0.114	0.507	0.309	0.141	0.126	0.116
NAU	0.15	0.24	0.38	0.22	0.21	0.094	0.121	0.131	0.068	0.047	0.076	0.049	0.023	0.021	0.019	0.053	0.033	0.017	0.015	0.015

Round Robin LXI Laboratory Results

All Results in µg/mL

	Total cis-β-Carotene					Total α-Carotene					Total Lycopene					trans-Lycopene				
Lab	329	330	331	332	333	329	330	331	332	333	329	330	331	332	333	329	330	331	332	333
FSV-BA	0.033	0.021	0.010	0.006	0.006	0.316	0.154	0.016	0.084	0.082	0.262	0.427	0.577	0.507	0.486	0.121	0.231	0.327	0.300	0.286
FSV-BB	0.026	0.013	0.005	0.004	0.005	0.284	0.134	0.013	0.068	0.069	0.263	0.416	0.550	0.486	0.468	0.096	0.190	0.269	0.247	0.234
FSV-BC																				
FSV-BD																				
FSV-BE																				
FSV-BF						0.353	0.155	0.014	0.087	0.089	0.261	0.421	0.593	0.541	0.494					
FSV-BG						0.292	0.146	0.022	0.093	0.091	0.300	0.534	0.654	0.631	0.626	0.133	0.261	0.338	0.337	0.339
FSV-BH	0.032	0.018	*na*	*na*	*na*	0.319	0.160	0.010	0.078	0.077	0.280	0.499	0.636	0.566	0.563					
FSV-BI						0.304	0.145	0.016	0.075	0.070	0.228	0.374	0.487	0.438	0.389					
FSV-BJ						0.404	0.172	0.012	0.089	0.092	0.269	0.421	0.537	0.508	0.483					
FSV-BK																				
FSV-BL																				
FSV-BM																				
FSV-BN	0.040	0.026	0.012	0.009	0.009	0.290	0.151	0.014	0.080	0.078	0.236	0.445	0.542	0.494	0.489	0.100	0.225	0.290	0.274	0.264
FSV-BO	0.073	0.048	0.026	0.026	nd	0.325	0.162	0.017	0.105	0.089	0.307	0.490	0.737	0.710	0.525					
FSV-BQ																				
FSV-BR																				
FSV-BS						0.353	0.187	0.046	0.113	0.109	0.306	0.496	0.658	0.552	0.531					
FSV-BT	0.027	0.017	0.007	0.005	0.005	0.225	0.132	0.021	0.083	0.080	0.228	0.412	0.513	0.498	0.470					
FSV-BU						0.299	0.138	0.018	0.082	0.070	0.261	0.410	0.332	0.508	0.426					
FSV-BV						0.394	0.161	0.014	0.087	0.082	0.293	0.475	0.634	0.559	0.508					
FSV-BW						0.260	0.130	0.010	0.060	0.052	0.260	0.480	0.720	0.690	0.640					
FSV-BX						*≥0.285*	*≥0.140*	*≥0.021*	*≥0.081*	*≥0.075*						0.085	0.159	0.233	0.250	0.229
FSV-CC																				
FSV-CE																				
FSV-CF																				
FSV-CG	0.041	0.024	0.010	0.007	0.007	0.365	0.173	0.018	0.092	0.091	0.291	0.455	0.592	0.502	0.496	0.119	0.223	0.317	0.283	0.277
FSV-CI						0.302	0.180	0.055	0.120	0.113										
FSV-CS						0.327	0.148	0.012	0.094	0.077	0.301	0.433	0.569	0.601	0.522					
FSV-CW						0.348	0.143	0.007	0.083	0.082						0.100	0.148	0.195	0.205	0.199
FSV-CZ																				
FSV-DD																				
FSV-DF																				
FSV-DI						0.247	0.126	0.055	0.094	0.090	0.278	0.447	0.583	0.537	0.519					
FSV-DV																				
FSV-EE																				
N	7	7	6	6	5	19	19	19	19	19	17	17	17	17	17	7	7	7	7	7
Min	0.026	0.013	0.005	0.004	0.005	0.225	0.126	0.007	0.060	0.052	0.228	0.374	0.332	0.438	0.389	0.085	0.148	0.195	0.205	0.199
Median	0.033	0.021	0.010	0.007	0.006	0.316	0.151	0.016	0.087	0.082	0.269	0.445	0.583	0.537	0.496	0.100	0.223	0.290	0.274	0.264
Max	0.073	0.048	0.026	0.026	0.009	0.404	0.187	0.055	0.120	0.113	0.307	0.534	0.737	0.710	0.640	0.133	0.261	0.338	0.337	0.339
SD	0.008	0.006	0.003	0.002	0.001	0.044	0.015	0.005	0.009	0.010	0.024	0.044	0.070	0.047	0.031	0.016	0.040	0.053	0.032	0.037
CV	25	27	28	37	25	14	10	32	11	12	9	10	12	9	6	16	18	18	12	14
N_{past}	0	0	5	6	6	0	0	19	22	22	0	0	22	22	22	0	0	7	9	9
$Median_{past}$			0.009	0.007	0.007			0.017	0.079	0.074			0.587	0.506	0.479			0.306	0.262	0.247
SD_{past}			0.006	0.002	0.003			0.004	0.012	0.012			0.056	0.067	0.059			0.045	0.051	0.046
NAV	0.033	0.021	0.010	0.007	0.006	0.316	0.151	0.016	0.087	0.082	0.269	0.445	0.583	0.537	0.496	0.100	0.223	0.290	0.274	0.264
NAU	0.012	0.007	0.004	0.003	0.003	0.080	0.041	0.006	0.025	0.024	0.062	0.094	0.117	0.110	0.103	0.017	0.040	0.053	0.050	0.048

C4

Round Robin LXI Laboratory Results

All Results in µg/mL

	Total β-Cryptoxanthin					Total α-Cryptoxanthin					Total Lutein					Total Zeaxanthin				
Lab	329	330	331	332	333	329	330	331	332	333	329	330	331	332	333	329	330	331	332	333
FSV-BA	0.105	0.121	0.128	0.067	0.064	0.021	0.036	0.045	0.029	0.028										
FSV-BB	0.093	0.100	0.104	0.057	0.054	0.021	0.029	0.035	0.026	0.025	0.161	0.150	0.154	0.082	0.083	0.026	0.056	0.073	0.041	0.045
FSV-BC																				
FSV-BD																				
FSV-BE																				
FSV-BF	0.091	0.095	0.100	0.050	0.045															
FSV-BG	0.110	0.120	0.127	0.074	0.070						0.157	0.142	0.128	0.098	0.095	0.028	0.029	0.031	0.030	0.026
FSV-BH	0.119	0.135	0.130	0.065	0.061						0.113	0.109	0.100	0.057	0.057	0.023	0.054	0.070	0.030	0.028
FSV-BI	0.100	0.106	0.111	0.057	0.052						0.139	0.132	0.123	0.081	0.075	0.026	0.047	0.062	0.031	0.031
FSV-BJ	0.105	0.108	0.105	0.053	0.048						0.152	0.149	0.146	0.087	0.083					
FSV-BK																				
FSV-BL																				
FSV-BM																				
FSV-BN	0.079	0.097	0.099	0.051	0.049	0.013	0.026	0.033	0.022	0.021	0.157	0.174	0.167	0.100	0.094	0.032	0.107	0.151	0.041	0.040
FSV-BO	0.103	0.112	0.126	0.066	0.054						0.199	0.201	0.200	0.105	0.103	0.030	0.055	0.077	0.022	0.030
FSV-BQ																				
FSV-BR																				
FSV-BS	0.138	0.132	0.184	0.096	0.010															
FSV-BT	0.087	0.086	0.092	0.051	0.050	0.023	0.027	0.033	0.025	0.024	0.123	0.126	0.138	0.059	0.055	0.043	0.040	0.042	0.026	0.025
FSV-BU	0.076	0.079	0.062	0.057	0.055															
FSV-BV	0.072	0.074	0.078	0.037	0.035															
FSV-BW																				
FSV-BX	0.074	0.079	0.088	0.048	0.045						0.159	0.154	0.159	0.082	0.077	0.022	0.038	0.052	0.027	0.028
FSV-CC																				
FSV-CE																				
FSV-CF																				
FSV-CG	0.133	0.142	0.150	0.078	0.078															
FSV-CI											0.161	0.190	0.191	0.100	0.093	0.030	0.054	0.068	0.037	0.033
FSV-CS	0.082	0.081	0.086	0.054	0.047						0.122	0.115	0.116	0.072	0.061	0.026	0.043	0.066	0.042	0.039
FSV-CW	0.097	0.098	0.093	0.055	0.052						0.170	0.151	0.147	0.096	0.092	0.022	0.035	0.041	0.027	0.025
FSV-CZ																				
FSV-DD																				
FSV-DF																				
FSV-DI											0.149	0.166	0.179	0.090	0.085					
FSV-DV																				
FSV-EE																				
N	17	17	17	17	17	4	4	4	4	4	13	13	13	13	13	11	11	11	11	11
Min	0.072	0.074	0.062	0.037	0.010	0.013	0.026	0.033	0.022	0.021	0.113	0.109	0.100	0.057	0.055	0.022	0.029	0.031	0.022	0.025
Median	0.097	0.100	0.104	0.057	0.052	0.021	0.028	0.034	0.026	0.025	0.157	0.150	0.147	0.087	0.083	0.026	0.047	0.066	0.030	0.030
Max	0.138	0.142	0.184	0.096	0.078	0.023	0.036	0.045	0.029	0.028	0.199	0.201	0.200	0.105	0.103	0.043	0.107	0.151	0.042	0.045
SD	0.017	0.025	0.026	0.011	0.006	0.002	0.003	0.003	0.002	0.002	0.016	0.025	0.029	0.013	0.013	0.004	0.012	0.018	0.009	0.007
CV	18	25	25	19	11	9	11	10	7	8	10	17	20	15	16	16	25	27	29	23
N_{past}	0	0	23	22	22	0	0	4	5	7	0	0	14	15	15	0	0	11	13	13
$Median_{past}$			0.112	0.055	0.052			0.040	0.027	0.024			0.145	0.080	0.078			0.057	0.032	0.030
SD_{past}			0.027	0.009	0.008			0.004	0.007	0.007			0.042	0.015	0.015			0.018	0.007	0.007
NAV	0.097	0.100	0.104	0.057	0.052	0.021	0.028	0.034	0.026	0.025	0.157	0.150	0.147	0.087	0.083	0.026	0.047	0.066	0.030	0.030
NAU	0.022	0.025	0.026	0.014	0.013						0.029	0.027	0.029	0.017	0.016	0.007	0.013	0.018	0.009	0.008

C5

Round Robin LXI Laboratory Results

All Results in µg/mL

	Total Lutein&Zeaxanthin					Coenzyme Q10					Ubiquinol					Ubiquinone				
Lab	329	330	331	332	333	329	330	331	332	333	329	330	331	332	333	329	330	331	332	333
FSV-BA	0.192	0.220	0.252	0.126	0.119															
FSV-BB	0.187	0.206	0.228	0.123	0.128															
FSV-BC																				
FSV-BD																				
FSV-BE																				
FSV-BF	0.180	0.191	0.195	0.115	0.112															
FSV-BG	0.167	0.155	0.140	0.112	0.105															
FSV-BH	0.136	0.163	0.170	0.087	0.085															
FSV-BI	*0.165*	*0.179*	*0.185*	*0.112*	*0.106*															
FSV-BJ						0.781	1.119	1.194	0.804	0.740										
FSV-BK																				
FSV-BL																				
FSV-BM																				
FSV-BN	0.189	0.282	0.318	0.141	0.134															
FSV-BO	0.229	0.256	0.277	0.126	0.133															
FSV-BQ																				
FSV-BR																				
FSV-BS	0.191	0.229	0.256	0.119	0.102															
FSV-BT	0.164	0.190	0.205	0.102	0.096															
FSV-BU	0.152	0.166	0.116	0.101	0.093															
FSV-BV	0.201	0.219	0.236	0.127	0.118															
FSV-BW						0.436	0.595	1.032	0.715	0.709	0.179	0.211	0.814	0.528	0.619	0.257	0.384	0.218	0.187	0.090
FSV-BX	0.181	0.192	0.212	0.109	0.105															
FSV-CC																				
FSV-CE																				
FSV-CF																				
FSV-CG	0.238	0.265	0.293	0.152	0.149															
FSV-CI	*0.191*	*0.244*	*0.259*	*0.137*	*0.126*	0.700	0.880	1.100	0.650	0.590										
FSV-CS	*0.148*	*0.158*	*0.182*	*0.114*	*0.100*															
FSV-CW	*0.192*	*0.185*	*0.188*	*0.124*	*0.117*	0.630	0.730	0.820	0.660	0.500										
FSV-CZ						0.680	0.850	0.960	0.670	0.690										
FSV-DD																				
FSV-DF																				
FSV-DI						0.715	0.941	1.090	0.748	0.727	0.010	0.405	1.260	0.824	0.727	0.780	0.695	0.152	0.186	0.201
FSV-DV																				
FSV-EE						0.720	0.911	1.311	0.754	0.726										
N	17	17	17	17	17	7	7	7	7	7	2	2	2	2	2	2	2	2	2	2
Min	0.136	0.155	0.116	0.087	0.085	0.436	0.595	0.820	0.650	0.500	0.010	0.211	0.814	0.528	0.619	0.257	0.384	0.152	0.186	0.090
Median	0.187	0.192	0.212	0.119	0.112	0.700	0.880	1.090	0.715	0.709	0.095	0.308	1.037	0.676	0.673	0.519	0.540	0.185	0.187	0.146
Max	0.238	0.282	0.318	0.152	0.149	0.781	1.119	1.311	0.804	0.740	0.179	0.405	1.260	0.824	0.727	0.780	0.695	0.218	0.187	0.201
SD	0.020	0.037	0.053	0.011	0.018	0.046	0.101	0.112	0.064	0.064										
CV	11	19	25	9	16	7	11	10	9	9										
N_{past}	0	0	22	23	23	0	0	5	5	5	0	0	0	0	0	0	0	0	0	0
$Median_{past}$			0.211	0.113	0.108			1.190	0.760	0.699										
SD_{past}			0.056	0.017	0.018			0.060	0.169	0.162										
NAV	0.187	0.192	0.212	0.119	0.112	0.700	0.880	1.090	0.715	0.709										
NAU	0.039	0.040	0.053	0.025	0.023	0.070	0.101	0.112	0.072	0.071										

C6

Round Robin LXI Laboratory Results

All Results in µg/mL

	Phylloquinone (K1) x1000					25-hydroxyvitamin D				
Lab	329	330	331	332	333	329	330	331	332	333
FSV-BA						0.015	0.014	0.008	0.020	0.020
FSV-BB						0.016	0.012	0.010	0.020	0.025
FSV-BC										
FSV-BD										
FSV-BE	1.586	0.995	0.599	0.389	0.357					
FSV-BF										
FSV-BG										
FSV-BH						0.015	0.011	0.007	0.021	0.020
FSV-BI										
FSV-BJ										
FSV-BK										
FSV-BL										
FSV-BM										
FSV-BN						0.017	0.011	0.008	0.019	0.018
FSV-BO										
FSV-BQ										
FSV-BR										
FSV-BS										
FSV-BT										
FSV-BU										
FSV-BV										
FSV-BW										
FSV-BX										
FSV-CC										
FSV-CE										
FSV-CF										
FSV-CG										
FSV-CI	1.339	0.887	0.530	0.402	0.308					
FSV-CS										
FSV-CW										
FSV-CZ										
FSV-DD										
FSV-DF										
FSV-DI	0.880	0.700	0.500	0.370	0.330					
FSV-DV										
FSV-EE										
N	3	3	3	3	3	4	4	4	4	4
Min	0.880	0.700	0.500	0.370	0.308	0.015	0.011	0.007	0.019	0.018
Median	1.339	0.887	0.530	0.389	0.330	0.015	0.012	0.008	0.020	0.020
Max	1.586	0.995	0.599	0.402	0.357	0.017	0.014	0.010	0.021	0.025
SD						0.001	0.001	0.001	0.001	0.001
CV						5	10	7	5	7
N_{past}	0	0	0	0	0	0	0	0	5	4
$Median_{past}$										0.017
SD_{past}										0.004
NAV	1.339	0.887	0.530	0.389	0.330	0.015	0.012	0.008	0.020	0.020
NAU										

C7

Round Robin LXI Laboratory Results
All Results in µg/mL

Analytes Reported By One Laboratory

Analyte	Code	329	330	331	332	333
Total cis-β-Cryptoxanthin	FSV-BT	0.029	0.032	0.036	0.022	0.022
trans-α-Carotene	FSV-BX	0.285	0.140	0.021	0.081	0.075

Term	Legend
N	Number of (non-NIST) quantitative values reported for this analyte
Min	Minimum (non-NIST) quantitative value reported
$Median_{part}$	Median (non-NIST) quantitative value reported
Max	Maximum (non-NIST) quantitative value reported
SD	Standard deviation for (non-NIST) results: 0.741*(3rd Quartile - 1st Quartile)
CV	Coefficient of Variation for (non-NIST) results: 100*SD/Median
N_{past}	Mean of N(s) from past RR(s)
$Median_{past}$	Mean of Median(s) from past RR(s)
SD_{past}	Pooled SD from past RR(s)
NAV	NIST Assigned Value = $(Median_{part} + Mean_{NIST})/2$ for analytes reported by NIST analyst(s) = $Median_{part}$ for analytes reported by ≥ 10 labs but not NIST
NAU	NIST Assigned Uncertainty: $(S^2 + S_{btw}^2)^{0.5}$ S is the maximum of (0.05*NAV, SD, S_{NIST}, eSD) and S_{btw} is the standard deviation between $Median_{part}$ and $Mean_{NIST}$. The expected long-term SD, eS is defined in: Duewer, et al. Anal Chem 1997;69(7):1406-1413.
-	Not analyzed
nd	Not detected (i.e., no detectable peak for analyte)
nq	Detected but not quantitatively determined
≤x	Concentration at or below the limit of quantification, x
≥x	Concentration greater than or equal to x
?	Non-quantitative value: extrapolated beyond upper limit of calibration curve
*	Non-quantitative value: heterogeneous serum, damaged sample, malfunctio
italics	Not explictly reported but calculated by NIST from reported values

Comparability Summary

Lab	TR	aT	g/bT	bC	tbC	aC	TLy	TbX	TLu	TZ	L&Z
FSV-BA	2	1	1	1	1	1	1	1			1
FSV-BB	1	1	1	1	1	1	1	1	1	2	1
FSV-BC	1										
FSV-BD	1	1									
FSV-BE	1	3	2	1							
FSV-BF	1	1	1	2		1	1	1			1
FSV-BG	1	2	2	3		1	1	2	1	2	1
FSV-BH	2	2	2	1	1	1	1	1	2	1	2
FSV-BI	2	1	1	1		1	1	1	1	1	1
FSV-BJ	1	2	1	1		1	1	1	1		
FSV-BK	2	1									
FSV-BL	1	1									
FSV-BM	1	1									
FSV-BN	2	1	2	1	1	1	1	1	1	4	2
FSV-BO	1	2	1	2	1	1	2	1	2	1	2
FSV-BQ	2	2									
FSV-BR	1	2									
FSV-BS	2	1	2	1	2	3	1	3			1
FSV-BT	2	1	2	1	1	1	1	1	2	2	1
FSV-BU	3	2	2	2		1	2	1			2
FSV-BV	2	1	1	1		1	1	2			1
FSV-BW	1	1	4	1		1	2				
FSV-BX	1	1	1	1	1	1		1	1	1	1
FSV-CC	1	1									
FSV-CE	2	3		4							
FSV-CF	1	2									
FSV-CG	1	1	1	1	1	1	1	2			2
FSV-CI	1	1	1	1	1	4			2	1	1
FSV-CS	2	1	2	1		1	1	1	2	1	1
FSV-CW	2	1	2	1	1	1		1	1	1	1
FSV-CZ	1	1		3							
FSV-DD	3										
FSV-DF	2										
FSV-DI	1	1	1	1		4	1		1		
FSV-DV	2	2									
FSV-EE	2	2									
n	36	33	21	23	11	20	17	17	13	11	17

	TR	aT	g/bT	bC	tbC	aC	TLy	TbX	TLu	TZ	L&Z
% 1	53	64	52	74	91	85	82	76	62	64	71
% 2	42	30	43	13	9	0	18	18	38	27	29
% 3	6	6	0	9	0	5	0	6	0	0	0
% 4	0	0	5	4	0	10	0	0	0	9	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.

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

$$C = Concordance = \sum_{i}^{N_{you}} \frac{You_i - Median_i}{NAU_i} \Big/ N_{you}$$

$$AP = Apparent\ Precision = \sqrt{\sum_{i}^{N_{you}} \left(\frac{You_i - Median_i}{NAU_i} \right)^2 \Big/ (N_{you} - 1)}$$

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 RR61

Each participant in RR61 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 RR61:

- Total Retinol
- *trans*-Retinol
- Retinyl Palmitate
- α-Tocopherol
- γ/β-Tocopherol
- δ-Tocopherol
- *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 14 pages are the "Individualized Report" for the analytes evaluated by participant FSV-BA.

Individualized Round Robin LXI Report: FSV-BA

Summary

	Serum 329			Serum 330			Serum 331			Serum 332			Serum 333		
Analyte	You	NAV	n	You	NAV	n	You	NAV	n	You	NAV	n	You	NAV	n
Total Retinol	0.559	0.520	32	0.433	0.399	32	0.326	0.304	32	0.697	0.646	32	0.709	0.618	32
Retinyl Palmitate	0.07	0.06	10	0.1	0.0	9	0.1	0.0	9	0.12	0.10	10	0.11	0.10	10
α-Tocopherol	24.43	23.69	33	15.75	15.39	33	8.66	8.50	33	10.59	10.50	33	10.16	9.98	33
γ/β-Tocopherol	1.061	0.902	21	2.569	2.430	21	3.829	3.710	21	1.935	1.819	21	1.867	1.780	21
δ-Tocopherol	0.065	0.058	6	0.065	0.073	6	0.079	0.100	6	0.064	0.072	6	0.052	0.061	6
Total β-Carotene	0.580	0.548	19	0.351	0.337	19	0.158	0.145	19	0.132	0.130	19	0.127	0.114	19
trans-β-Carotene	0.548	0.507	11	0.330	0.309	11	0.148	0.141	11	0.126	0.126	11	0.121	0.116	11
Total cis-β-Carotene	0.033	0.033	7	0.021	0.021	7	0.010	0.010	6	0.006	0.007	6	0.006	0.006	5
Total α-Carotene	0.316	0.316	19	0.154	0.151	19	0.016	0.016	19	0.084	0.087	19	0.082	0.082	19
Total Lycopene	0.262	0.269	17	0.427	0.445	17	0.577	0.583	17	0.507	0.537	17	0.486	0.496	17
trans-Lycopene	0.121	0.100	7	0.231	0.223	7	0.327	0.290	7	0.300	0.274	7	0.286	0.264	7
Total β-Cryptoxanthin	0.105	0.097	17	0.121	0.100	17	0.128	0.104	17	0.067	0.057	17	0.064	0.052	17
Total α-Cryptoxanthin	0.021	0.021	4	0.036	0.028	4	0.045	0.034	4	0.029	0.026	4	0.028	0.025	4
Total Lutein&Zeaxanthin	0.192	0.187	17	0.220	0.192	17	0.252	0.212	17	0.126	0.119	17	0.119	0.112	17
25-hydroxyvitamin D	0.015	0.015	4	0.014	0.012	4	0.008	0.008	4	0.020	0.020	4	0.020	0.020	4

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

D2

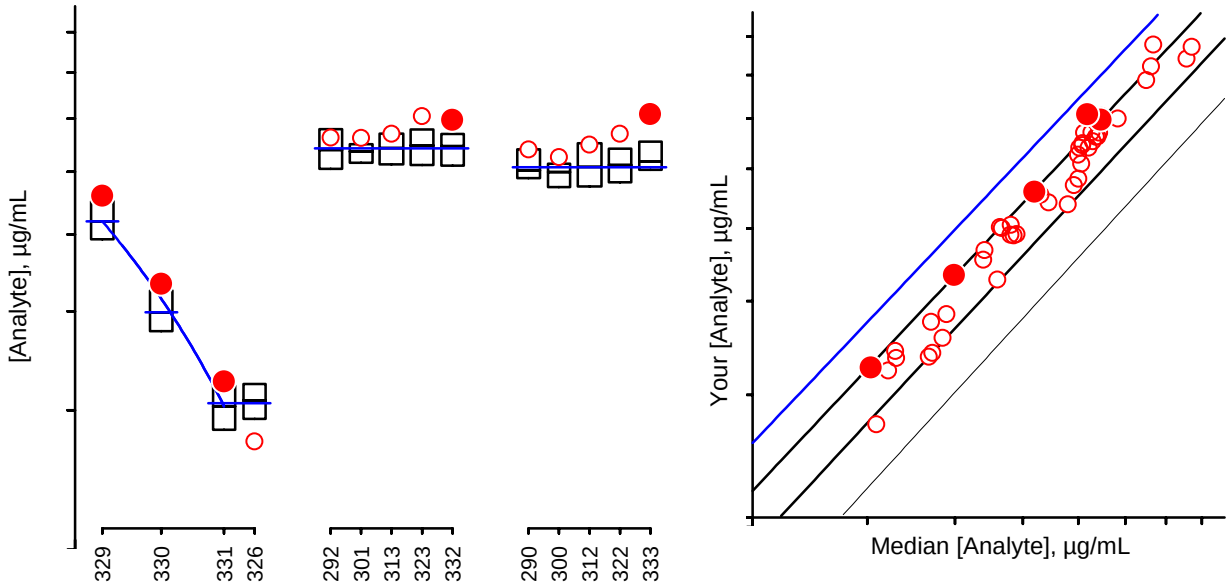
Please check our records against your records. Send corrections and/or updates to...

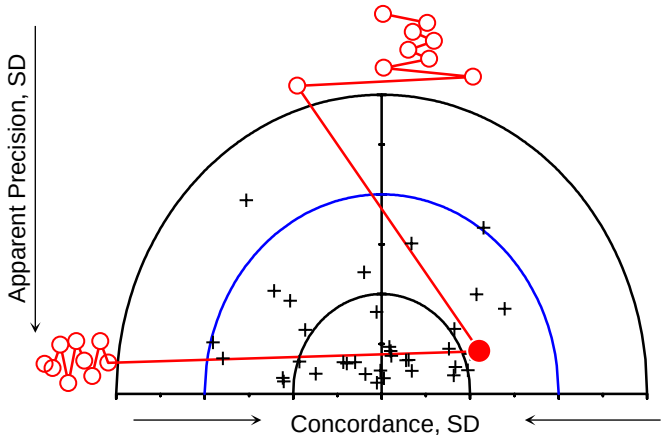
Micronutrients Measurement Quality Assurance Program
National Institute of Standards and Technology
100 Bureau Drive Stop 8392
Gaithersburg, MD 20899-8392 USA

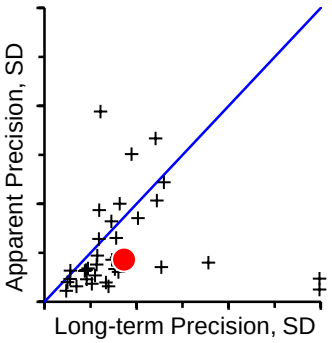
Tel: (301) 975-3935
Fax: (301) 977-0685
Email: david.duewer@nist.gov

Individualized RR LXI Report: FSV-BA

Total Retinol





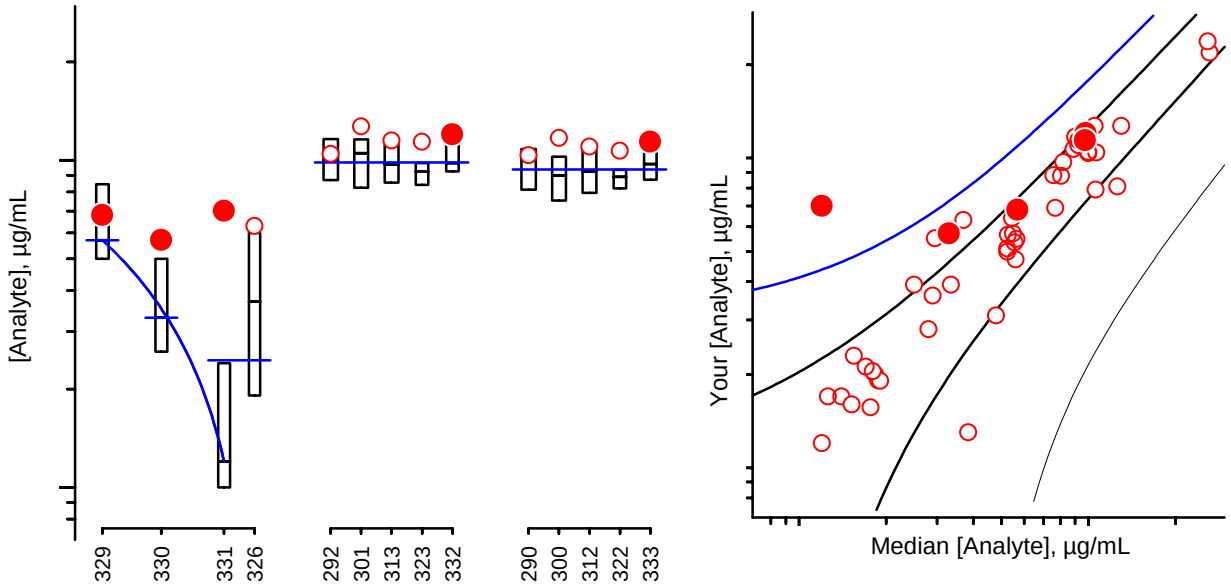


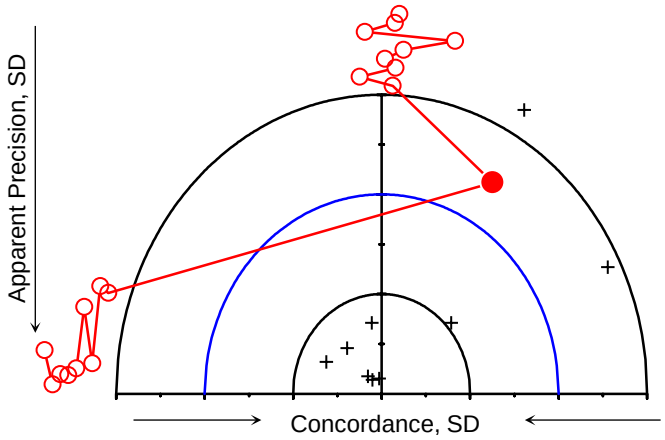
3rd Quartile (75%)
Median (50%)
1st Quartile (25%)
You, this RR
You, past RRs
Expectation
You, ≥x, this RR
You, ≥x, past RRs
Others, this RR

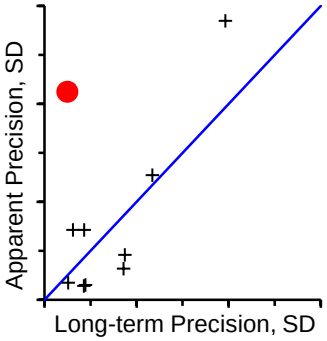
Serum	History	Comments
#329	Fresh frozen: New	Native, single-source
#330	Fresh frozen: New	15:16 blend of #329 and #331
#331	Fresh frozen: 60:#326	Native, single-source
#332	Fresh-frozen: 53:#292, 55:#301, 57:#313, 59:#323	Native, same single-source as #333
#333	Lyophilized: 53:#290, 55:#300, 57:#312, 59:#322	Native, same single-source as #332

Individualized RR LXI Report: FSV-BA

Retinyl Palmitate





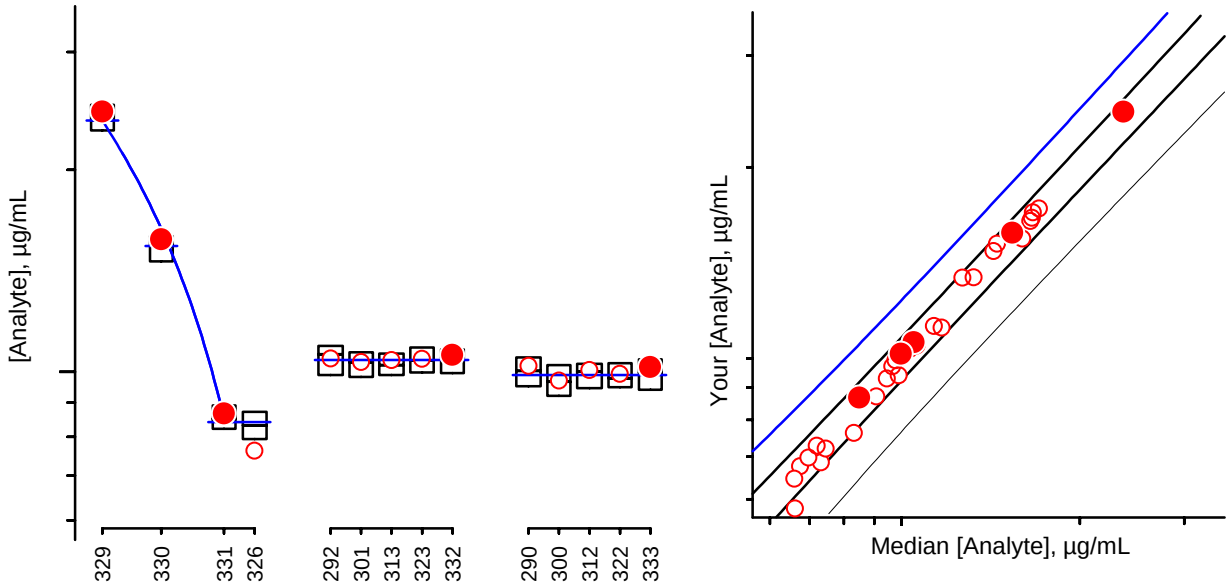


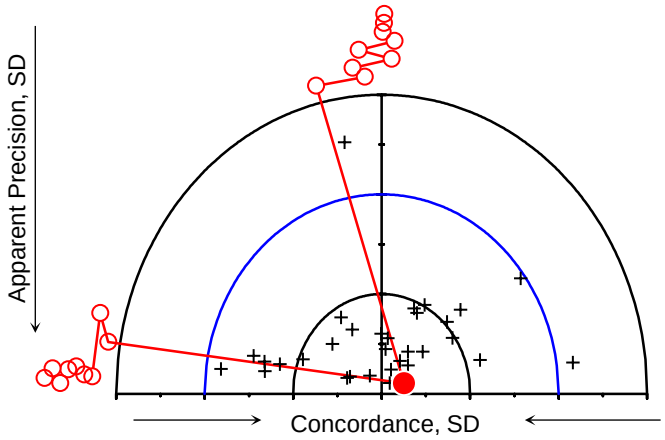
3rd Quartile (75%)
Median (50%)
1st Quartile (25%)
You, this RR
You, past RRs
Expectation
You, ≥x, this RR
You, ≥x, past RRs
Others, this RR

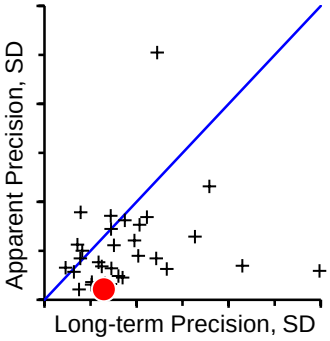
Serum	History	Comments
#329	Fresh frozen: New	Native, single-source
#330	Fresh frozen: New	15:16 blend of #329 and #331
#331	Fresh frozen: 60:#326	Native, single-source
#332	Fresh-frozen: 53:#292, 55:#301, 57:#313, 59:#323	Native, same single-source as #333
#333	Lyophilized: 53:#290, 55:#300, 57:#312, 59:#322	Native, same single-source as #332

Individualized RR LXI Report: FSV-BA

α-Tocopherol





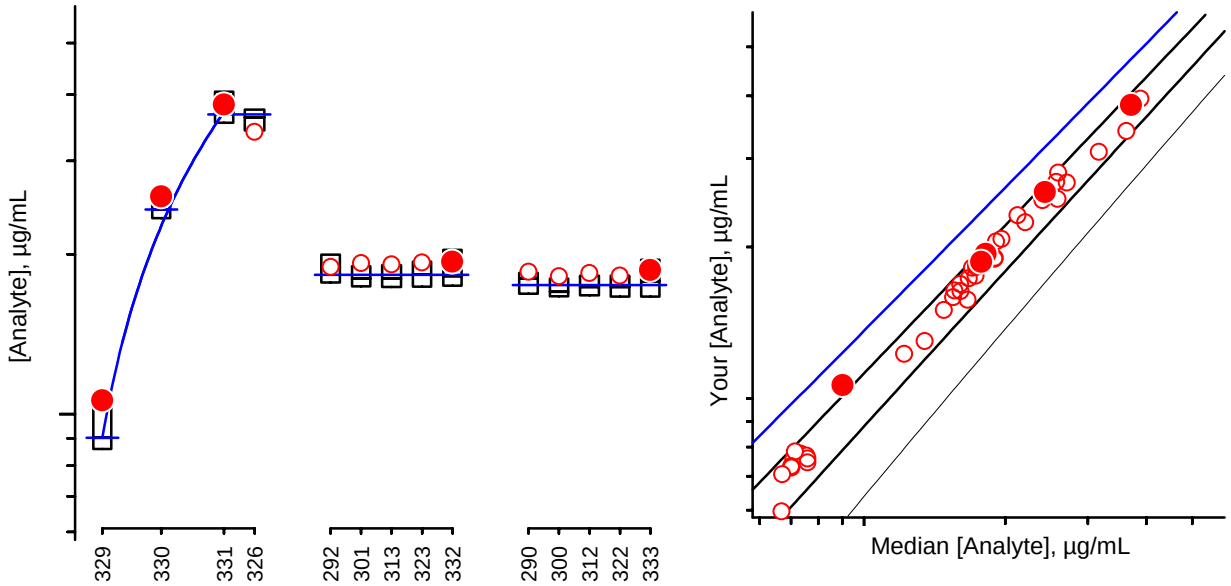


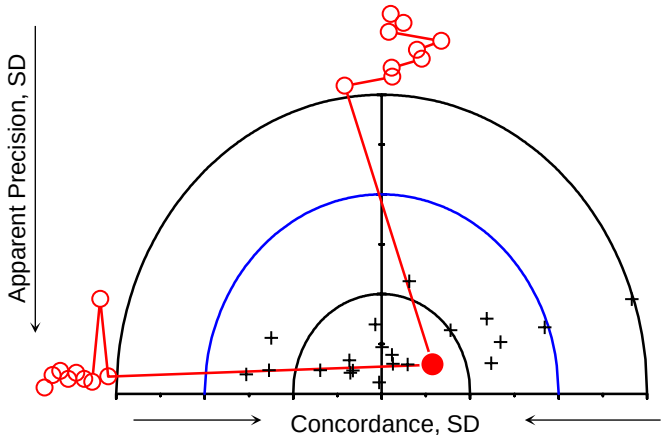
3rd Quartile (75%)
Median (50%)
1st Quartile (25%)
You, this RR
You, past RRs
Expectation
You, ≥x, this RR
You, ≥x, past RRs
Others, this RR

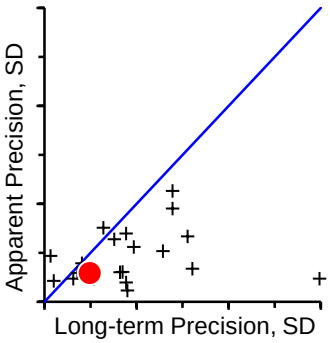
Serum	History	Comments
#329	Fresh frozen: New	Native, single-source
#330	Fresh frozen: New	15:16 blend of #329 and #331
#331	Fresh frozen: 60:#326	Native, single-source
#332	Fresh-frozen: 53:#292, 55:#301, 57:#313, 59:#323	Native, same single-source as #333
#333	Lyophilized: 53:#290, 55:#300, 57:#312, 59:#322	Native, same single-source as #332

Individualized RR LXI Report: FSV-BA

γ/β-Tocopherol







3rd Quartile (75%)
Median (50%)
1st Quartile (25%)
You, this RR
You, past RRs
Expectation
You, ≥x, this RR
You, ≥x, past RRs
Others, this RR

Serum	History	Comments
#329	Fresh frozen: New	Native, single-source
#330	Fresh frozen: New	15:16 blend of #329 and #331
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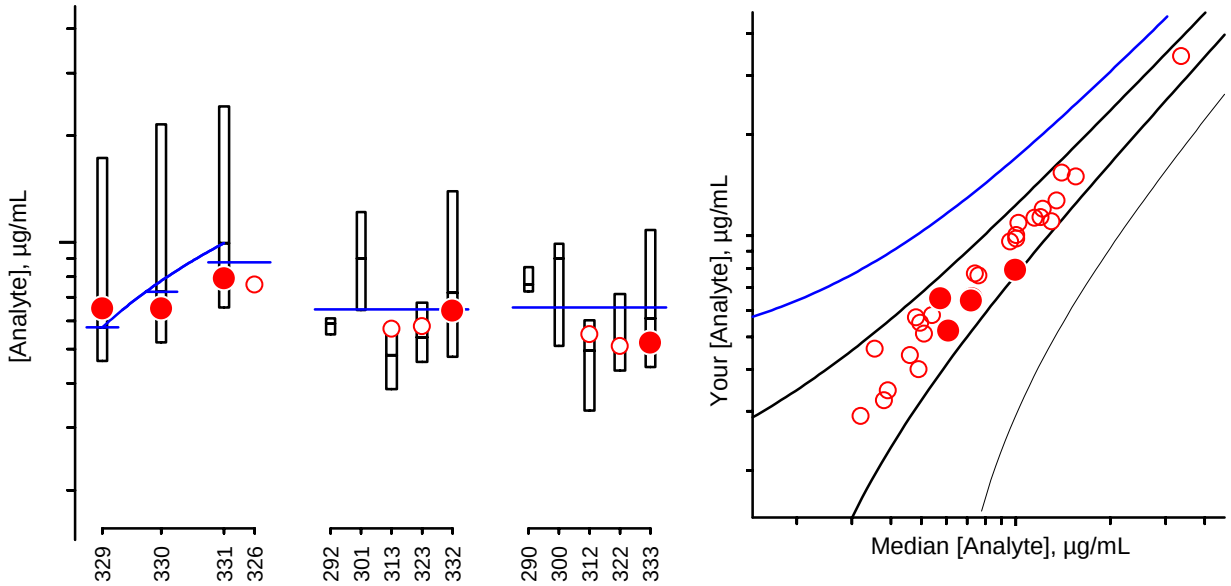
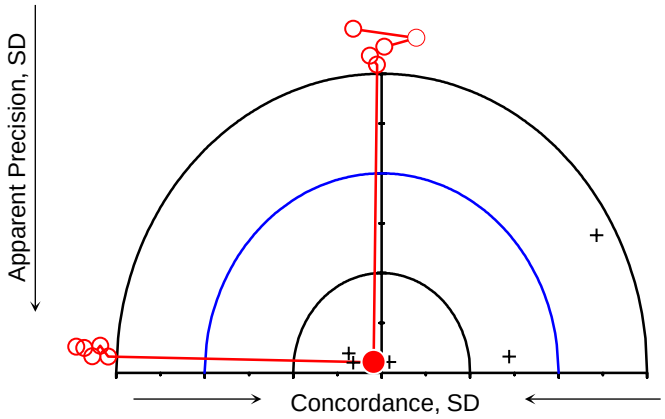
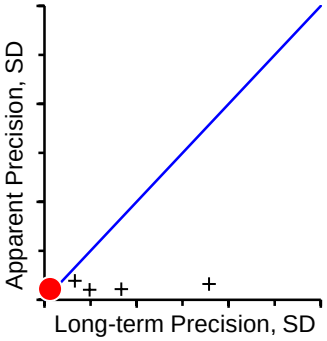
#333	Lyophilized: 53:#290, 55:#300, 57:#312, 59:#322	Native, same single-source as #332

Individualized RR LXI Report: FSV-BA

δ-Tocopherol







3rd Quartile (75%)
Median (50%)
1st Quartile (25%)
You, this RR
You, past RRs
Expectation
You, ≥x, this RR
You, ≥x, past RRs
Others, this RR

Serum	History	Comments
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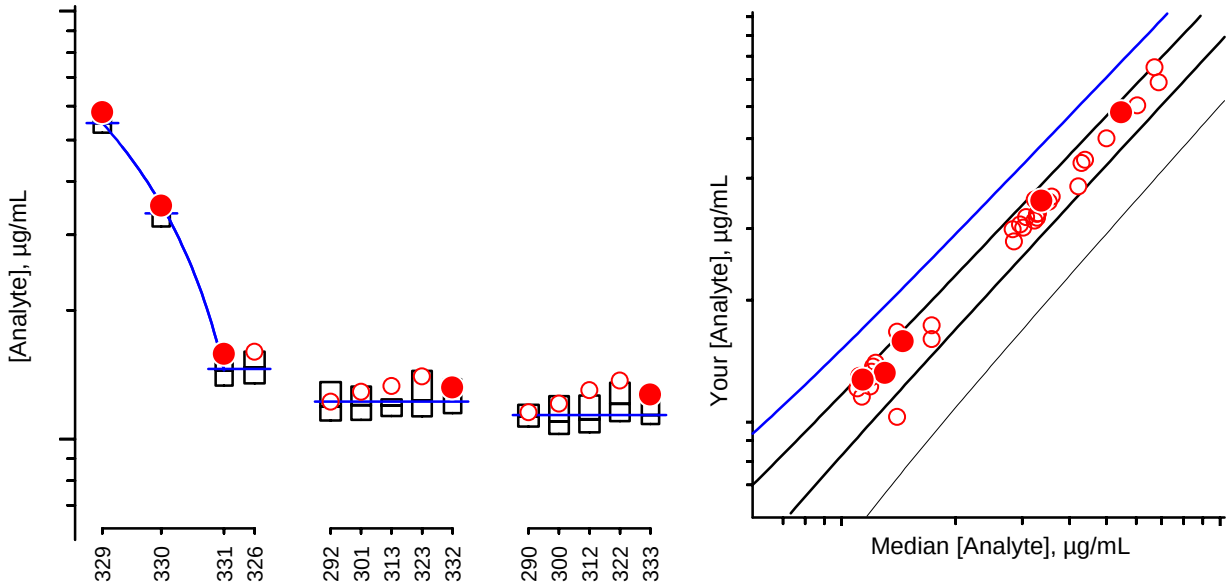
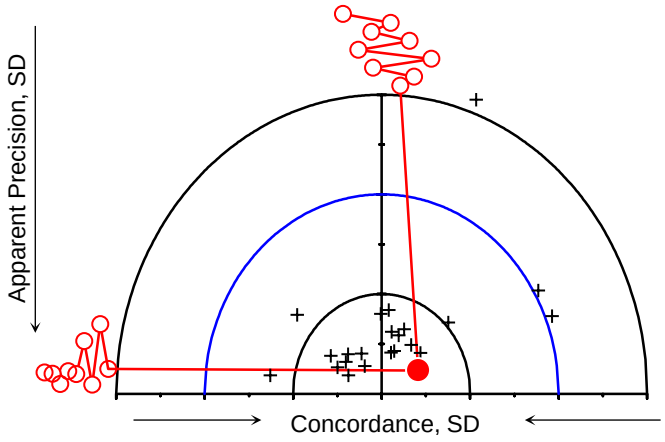
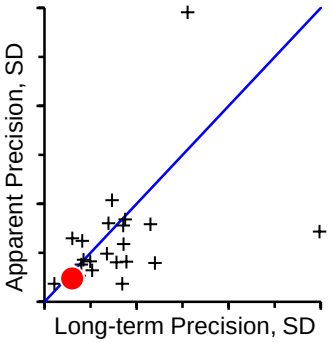
#333	Lyophilized: 53:#290, 55:#300, 57:#312, 59:#322	Native, same single-source as #332

Individualized RR LXI Report: FSV-BA

Total β-Carotene







3rd Quartile (75%)
Median (50%)
1st Quartile (25%)
You, this RR
You, past RRs
Expectation
You, ≥x, this RR
You, ≥x, past RRs
Others, this RR

Serum	History	Comments
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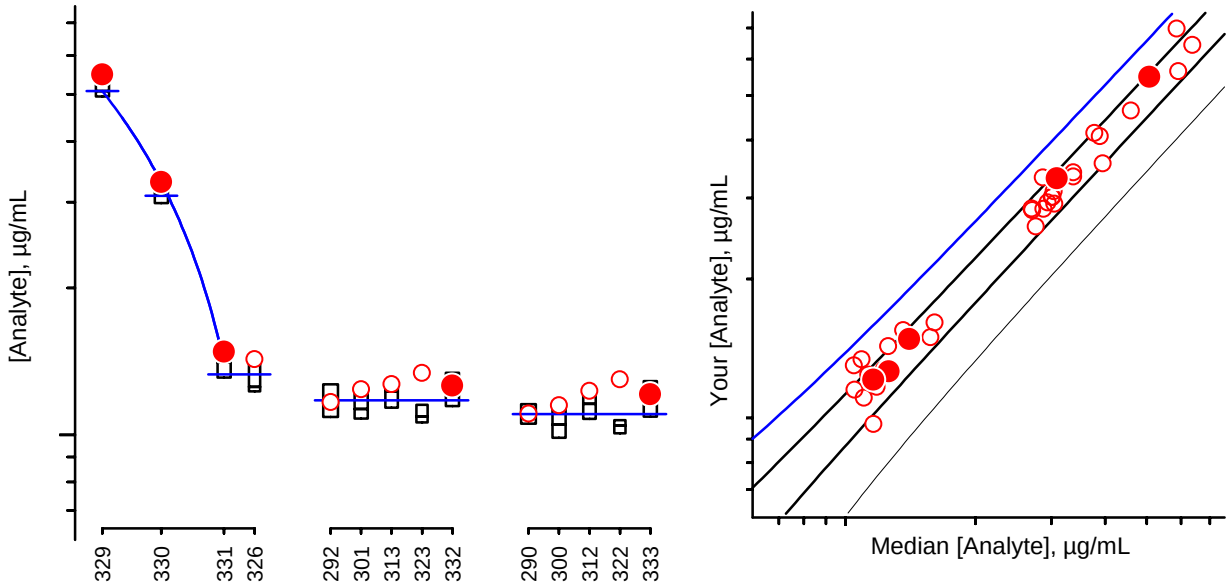
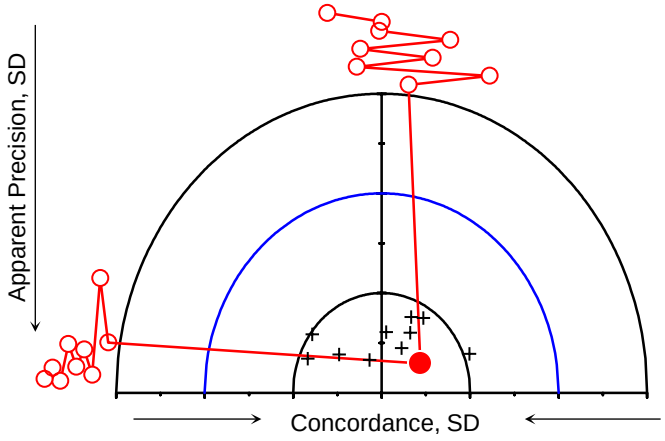
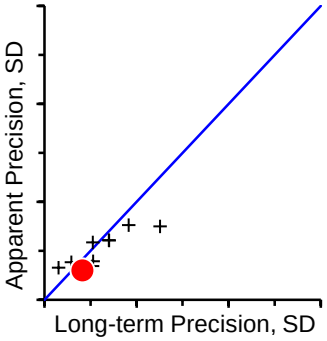
Individualized RR LXI Report: FSV-BA

trans-β-Carotene





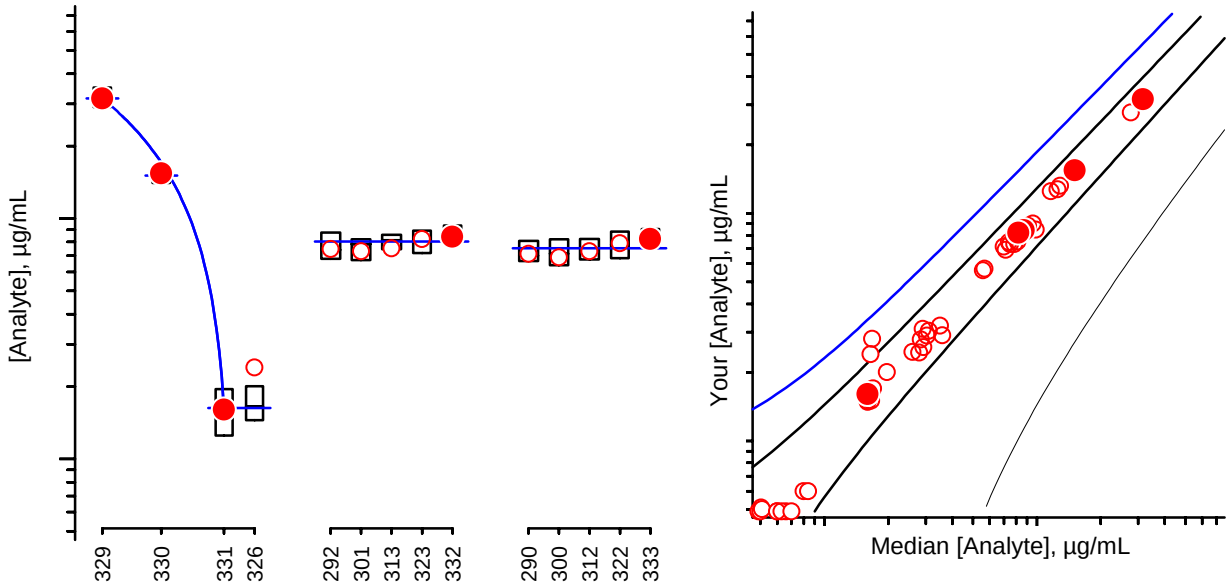


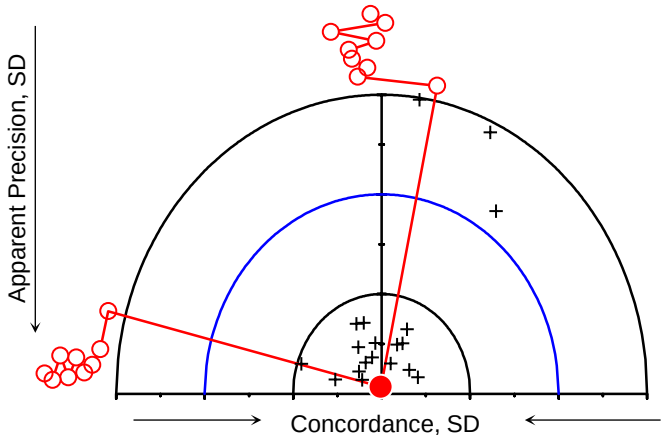
3rd Quartile (75%)
Median (50%)
1st Quartile (25%)
You, this RR
You, past RRs
Expectation
You, ≥x, this RR
You, ≥x, past RRs
Others, this RR

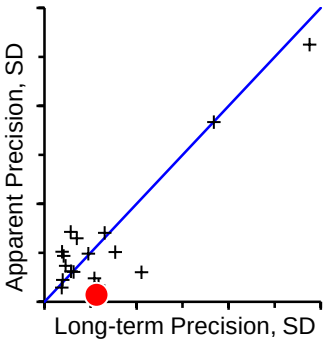
Serum	History	Comments
#329	Fresh frozen: New	Native, single-source
#330	Fresh frozen: New	15:16 blend of #329 and #331
#331	Fresh frozen: 60:#326	Native, single-source
#332	Fresh-frozen: 53:#292, 55:#301, 57:#313, 59:#323	Native, same single-source as #333
#333	Lyophilized: 53:#290, 55:#300, 57:#312, 59:#322	Native, same single-source as #332

Individualized RR LXI Report: FSV-BA

Total α-Carotene





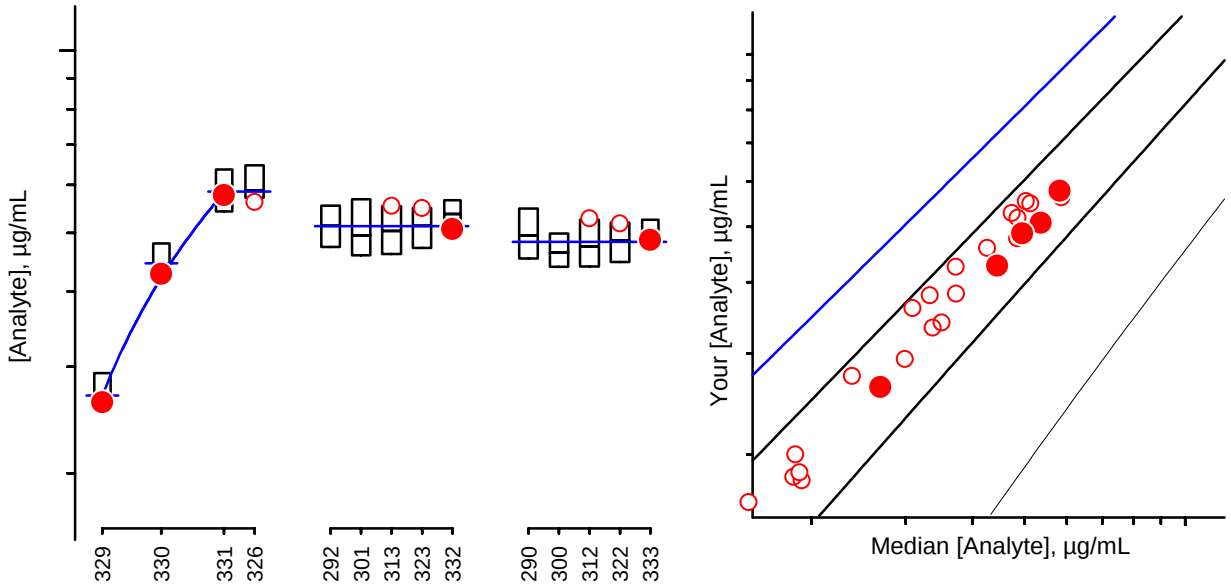


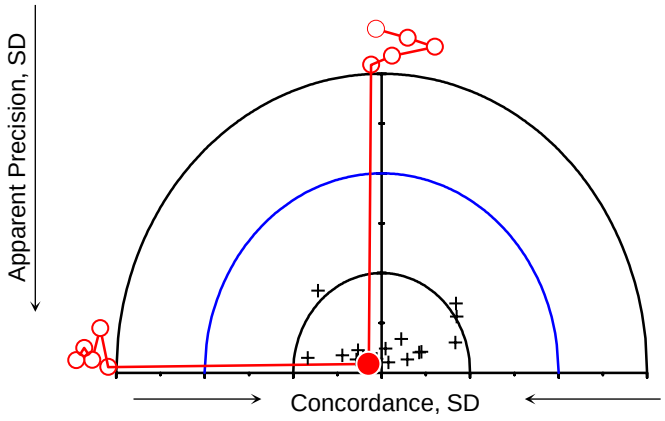
3rd Quartile (75%)
Median (50%)
1st Quartile (25%)
You, this RR
You, past RRs
Expectation
You, ≥x, this RR
You, ≥x, past RRs
Others, this RR

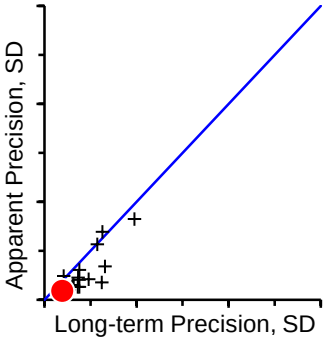
Serum	History	Comments
#329	Fresh frozen: New	Native, single-source
#330	Fresh frozen: New	15:16 blend of #329 and #331
#331	Fresh frozen: 60:#326	Native, single-source
#332	Fresh-frozen: 53:#292, 55:#301, 57:#313, 59:#323	Native, same single-source as #333
#333	Lyophilized: 53:#290, 55:#300, 57:#312, 59:#322	Native, same single-source as #332

Individualized RR LXI Report: FSV-BA

Total Lycopene





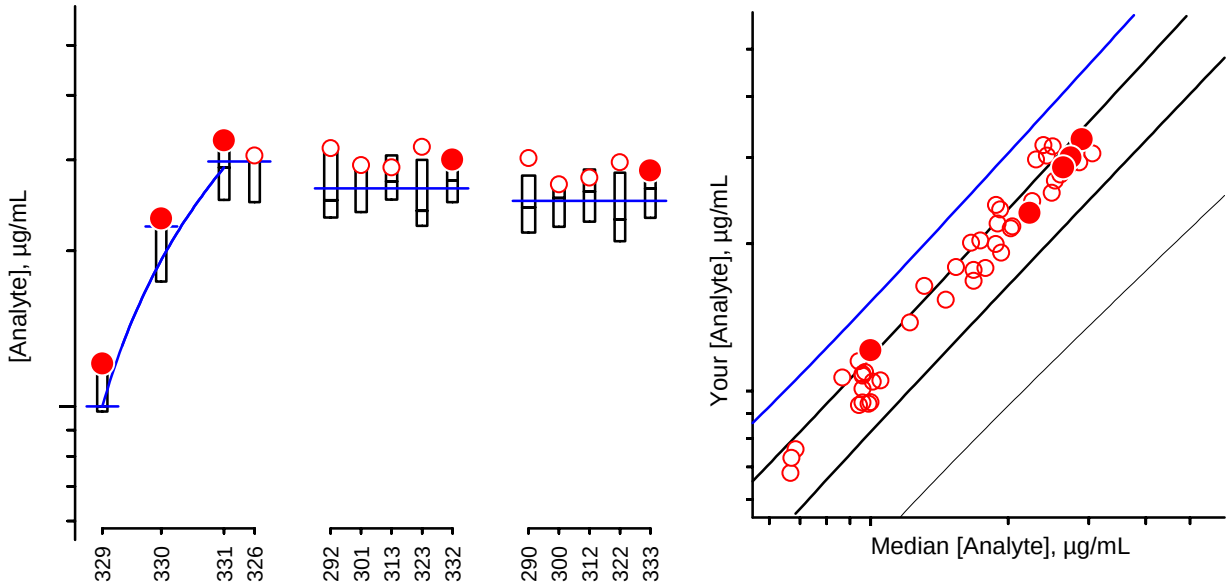


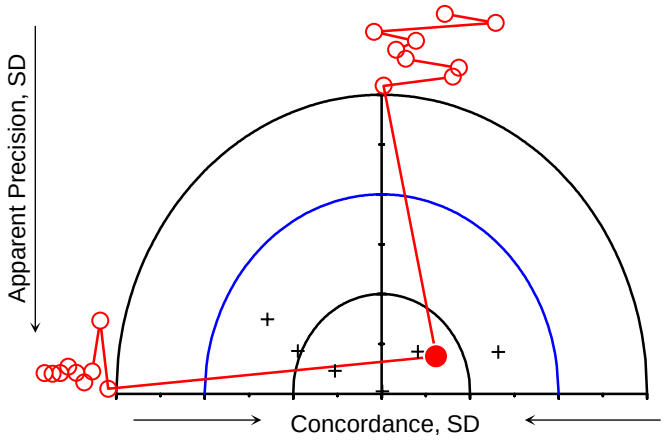
3rd Quartile (75%)
Median (50%)
1st Quartile (25%)
You, this RR
You, past RRs
Expectation
You, ≥x, this RR
You, ≥x, past RRs
Others, this RR

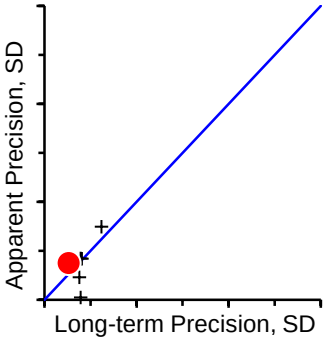
Serum	History	Comments
#329	Fresh frozen: New	Native, single-source
#330	Fresh frozen: New	15:16 blend of #329 and #331
#331	Fresh frozen: 60:#326	Native, single-source
#332	Fresh-frozen: 53:#292, 55:#301, 57:#313, 59:#323	Native, same single-source as #333
#333	Lyophilized: 53:#290, 55:#300, 57:#312, 59:#322	Native, same single-source as #332

Individualized RR LXI Report: FSV-BA

trans-Lycopene





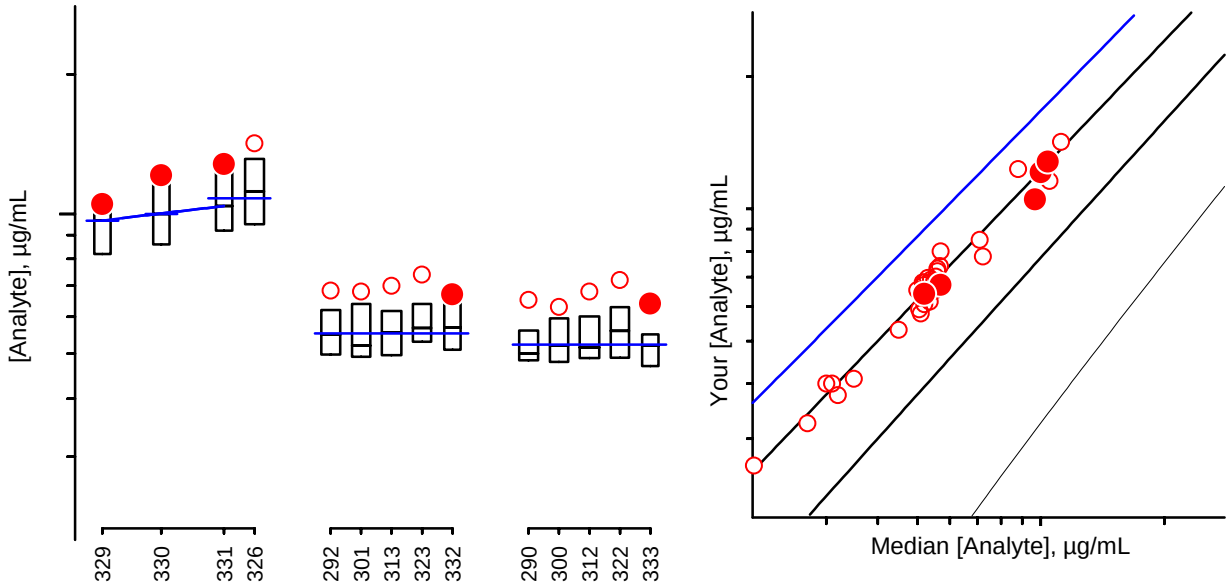


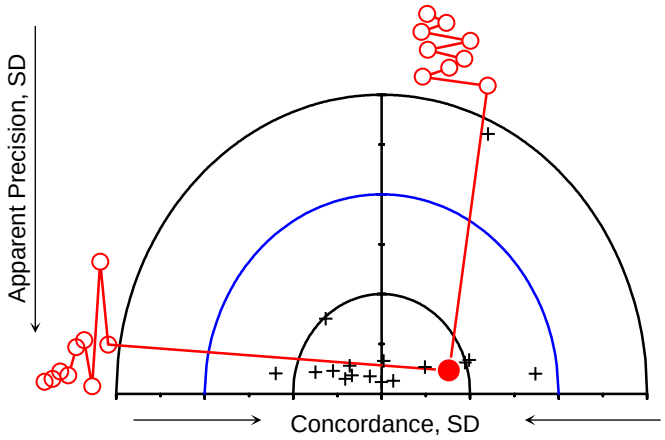
3rd Quartile (75%)
Median (50%)
1st Quartile (25%)
You, this RR
You, past RRs
Expectation
You, ≥x, this RR
You, ≥x, past RRs
Others, this RR

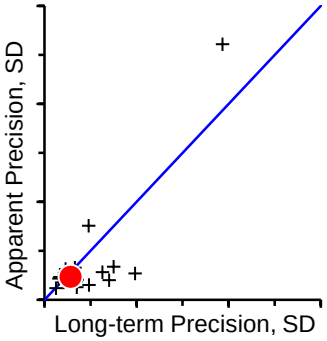
Serum	History	Comments
#329	Fresh frozen: New	Native, single-source
#330	Fresh frozen: New	15:16 blend of #329 and #331
#331	Fresh frozen: 60:#326	Native, single-source
#332	Fresh-frozen: 53:#292, 55:#301, 57:#313, 59:#323	Native, same single-source as #333
#333	Lyophilized: 53:#290, 55:#300, 57:#312, 59:#322	Native, same single-source as #332

Individualized RR LXI Report: FSV-BA

Total β-Cryptoxanthin





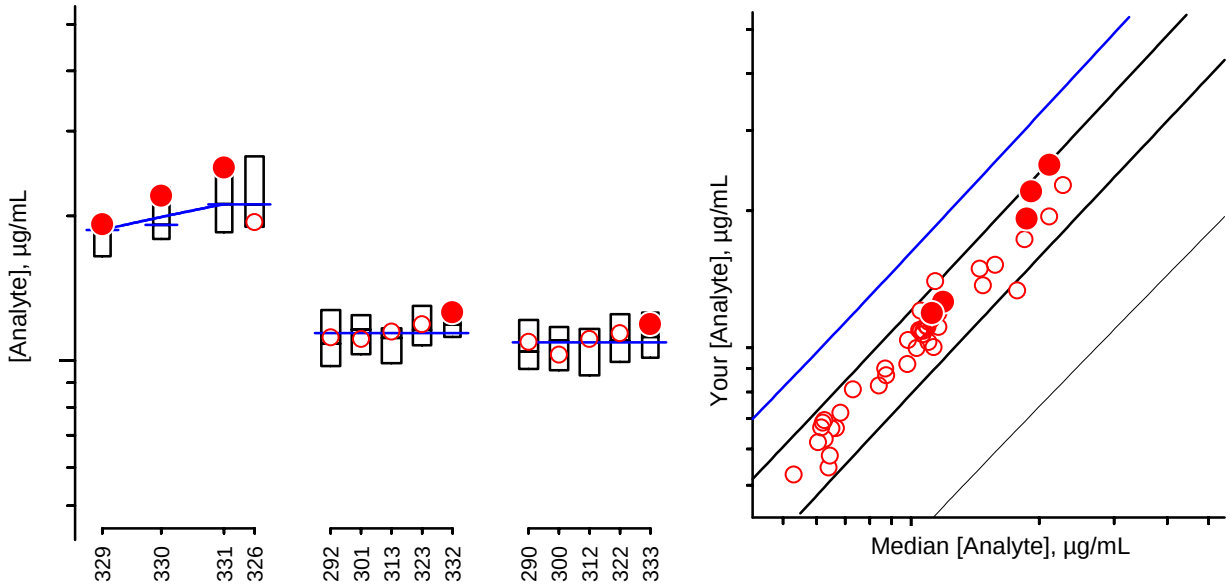


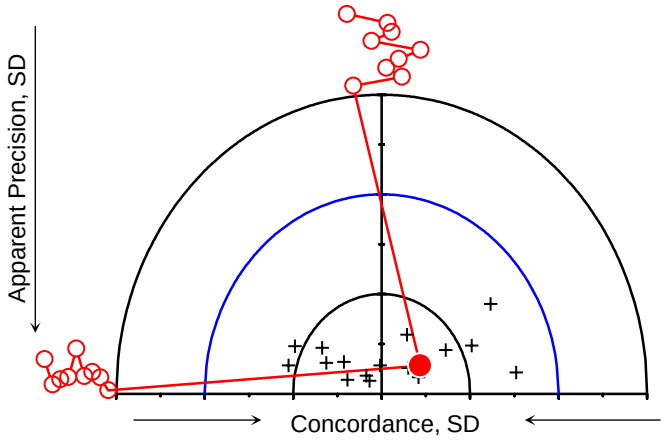
3rd Quartile (75%)
Median (50%)
1st Quartile (25%)
You, this RR
You, past RRs
Expectation
You, ≥x, this RR
You, ≥x, past RRs
Others, this RR

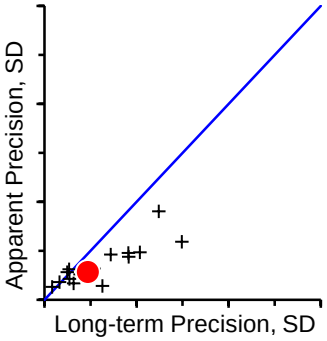
Serum	History	Comments
#329	Fresh frozen: New	Native, single-source
#330	Fresh frozen: New	15:16 blend of #329 and #331
#331	Fresh frozen: 60:#326	Native, single-source
#332	Fresh-frozen: 53:#292, 55:#301, 57:#313, 59:#323	Native, same single-source as #333
#333	Lyophilized: 53:#290, 55:#300, 57:#312, 59:#322	Native, same single-source as #332

Individualized RR LXI Report: FSV-BA

Total Lutein&Zeaxanthin







3rd Quartile (75%)
Median (50%)
1st Quartile (25%)
You, this RR
You, past RRs
Expectation
You, ≥x, this RR
You, ≥x, past RRs
Others, this RR

Serum	History	Comments
#329	Fresh frozen: New	Native, single-source
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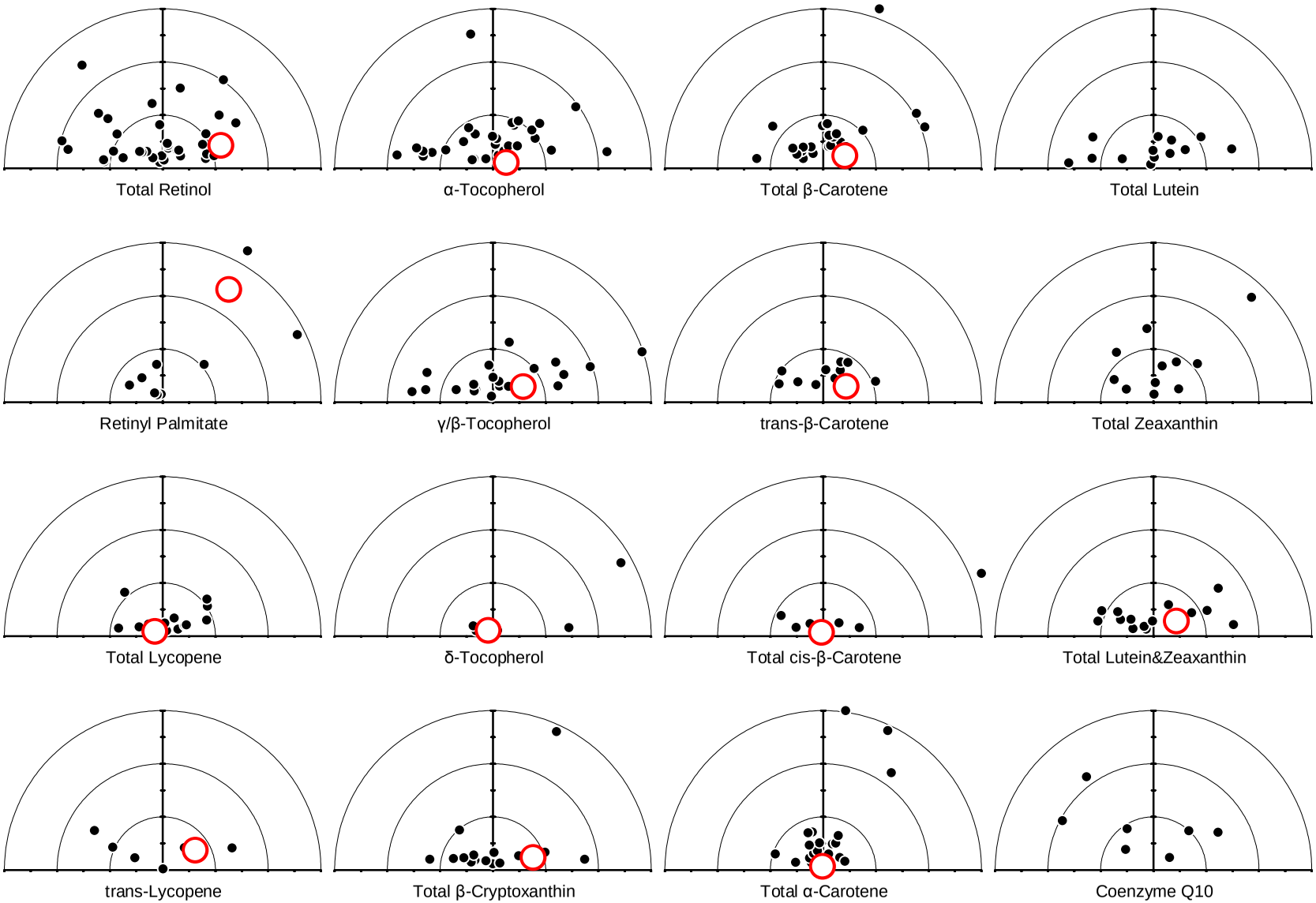
#333	Lyophilized: 53:#290, 55:#300, 57:#312, 59:#322	Native, same single-source as #332

Individualized Round Robin LXI Report: FSV-BA

Graphical Comparability Summary



D15

Appendix E. Shipping Package Inserts for RR26

The following five items were included in each package shipped to an RR26 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.

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

November 8, 2006

Dear Colleague:

The samples within this package constitute the first Vitamin C Round Robin (RR26) of the fiscal year (FY) 07 Micronutrients Measurement Quality Assurance Program. RR26 consists of four vials of frozen serum *test samples* (#13, #42, #112, and #113), one vial of ascorbic acid *solid control material*, and one vial of SRM 970 Ascorbic Acid in Serum-Level I to be used as the *serum control material*. 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.)

Please use SRM 970 to validate the performance of your measurement system before you analyze the *test samples*. The target value and ≈95% confidence interval for SRM 970-Level I is 8.41 ± 0.61 µmol/L of 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 RR25 was mailed mid-October. If you find your results for RR25 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 the RR26 samples by **March 5, 2007**. 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.
RR26 Report Form for Ascorbic Acid Solid Control Material Preparation
RR26 Report Form for Control Material and Test Sample Analyses

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 μmol/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 g)(0.2 g)·(56785 μmol/g·L)/(0.2 + 103 g) = 57.2 μmol/L. Likewise, 0.25 mL of the Stock Solution should weigh 0.26 g and $[\text{TAA}]_{\text{DS2}}$ = 28.4 μmol/L and 0.125 mL should weigh 0.13 g and $[\text{TAA}]_{\text{DS3}}$ = 14.2 μmol/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:

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

If your spectrophotometer is properly calibrated, λ_{max} should be between 243 and 244 nm and $E^{1\%}$ should be 550 ± 30 dL/g·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 CS #2 (see protocol below). The target values for this materials is 28.1 ±1.0 μmol/L of sample.

   If your measured values are not close to this value, 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 material* 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 material* 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 μmol/(L of the sample solution) rather than μmol/(L of serum NIST used to prepare the sample).

Participant #: _____ Date: _____

Vitamin C Round Robin 26

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 by **March 5, 2007**

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 26

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
SRM 970-Level I			µmol/L of Sample *Target:* 8.41 ±0.61 µmol/L
Serum Test Sample #13			µmol/L of Sample
Serum Test Sample #42			µmol/L of Sample
Serum Test Sample #112			µmol/L of Sample
Serum Test Sample #113			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 by **March 5, 2007**

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 26

NIST Micronutrients Measurement Quality Assurance Program

Packing List and Shipment Receipt Confirmation Form

This box contains one vial each of the following **six** VitC M^2QAP samples:

Label	Form
VitC #13	Liquid frozen (1:1 serum:10% MPA)
VitC #42	Liquid frozen (1:1 serum:10% MPA)
VitC #112	Liquid frozen (1:1 serum:10% MPA)
VitC #113	Liquid frozen (1:1 serum:10% MPA)
SRM 970 Lv1	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 M^2QAP Gang

Mail: M^2QAP
NIST, Stop 8392
Gaithersburg, MD 20899-8392

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

Appendix F. Final Report for RR26

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-

April 26, 2007

Dear Colleague:

Enclosed is the summary report of the results for Round Robin 26 (RR 26) 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 an individualized summary of your laboratory's measurement performance. The robust median is used to estimate the consensus value for all samples, the "median absolute deviation from the median" (MADe) is used to estimate the expected standard deviation, and the coefficient of variation (CV) is defined as 100×MADe/median.

RR 26 consisted of four test samples (13, 42, 112, and 113), one serum control material, 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 (RR 27) of the 2007 M^2QAP will be shipped **during the week of June 4, 2007.**

We look forward to meeting with you at the Micronutrients Measurement Quality Assurance Workshop that is being held in conjunction with the Experimental Biology meeting on **May 2, 2007** at the Convention Center in Washington, DC.

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: jbthomas@nist.gov; or fax: 301-977-0685.

Sincerely,

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

Enclosures

Cc: L. C. Sander
D.L. Duewer

The NIST M^2QAP Vitamin C Round Robin 26 (RR26) 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 serum control sample, and the four serum test samples.
2	Graphical summary of your RR26 sample measurements.

Page	"All Lab" Report
1	A tabulation of results and summary statistics for Total Ascorbic Acid [TAA] in the RR26 samples and control/calibration solutions.

Serum-based Samples. One serum control and four unknowns were distributed in RR26.

Control 1	SRM 970 level 1, ampouled in mid-1998.
Sample 1	Serum 13, ampouled in late 2001, previously distributed as sample S19:1 (RR19, Fall 03), S21:1 (RR21, Fall 04) and S23:1 (RR23, Fall 05). A "blank" stripped serum.
Sample 2	Serum 42, ampouled in late 2001, previously distributed as sample S18:2 (RR18, Spring 03), S19:3 (RR19, Fall 03), S21:3 (RR21, Fall 04), S22:3 (RR22, Spring 05) and S24:2 (RR24, Spring 06).
Sample 3	Serum 112, ampouled in 1993, previously distributed as sample 179B in RR4 (1993).
Sample 4	Serum 113, ampouled in 1993, previously distributed as sample 180 in RR4 (1993), RR6 (1994) and RR14 (2001).

Results.

1) All participants who prepared the four 5% MPA control/calibration solutions (the three "Dilute Solutions" and the "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 (≈0.58 OD), the calculated $E^{1\%}$ #1"(≈560 dL/g·cm).

2) Judging from the calibration parameters calculated for the control/calibration solutions (intercepts close to 0.0, slopes close to 1.0, R^2 close to 1, and RMS close to 0.0), the measurement systems for all participants are linear and reasonably well calibrated. However, several participants continue to have measurement systems that perform somewhat differently for the control solutions and the test samples.

3) Somewhat remarkably, the median and the MADe for sera 112 (S26:3) and 113 (S26:4) are effectively unchanged from the TAA results obtained for these materials in the 1993 RR4 study.

Appendix G. "All-Lab Report" for RR26

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" 26 - March 2007

		Control / Calibration Samples											MPA	Dilute Solution 1			Samples									
		Gravimetric, µmol/L			Measured, µmol/L				Calibration Parameters				Density	Spectrophotometry			Measured, µmol/L					Calibrated to Gravimetric, µmol/L				
Lab	Date	Dil:1	Dil:2	Dil:3	Dil:1	Dil:2	Dil:3	MPA	Inter	Slope	R^2	RMS	g/mL	λ_{max}	A_{max}	$E^{1\%}$	CS#1	S26:1	S26:2	S26:3	S26:4	CS#1	S26:1	S26:2	S26:3	S26:4
VC-MA	20/03/07	58.7	29.3	14.3	58.8	29.7	14.8	0.0	0.27	1.00	1.000	0.3	1.033	244.	0.5734	554.3	8.5	0.0	35.2	79.8	21.3	8.2	0.0	34.9	79.5	21.1
VC-MB	26/11/06	57.1	28.3	14.1	59.4	28.9	13.9	0.0	-0.39	1.04	1.000	0.4	1.027	244.	0.5630	560.2	8.3	0.0	33.0	71.0	19.4	8.3	0.4	32.0	68.4	18.9
VC-MC	23/01/07	55.6	28.2	14.2	60.7	27.0	14.0	0.0	-1.34	1.09	0.995	2.2	1.029	243.	0.5507	562.3	8.7	0.0	33.7	57.2	15.1	9.2	1.2	32.1	53.6	15.1
VC-ME	07/03/07	58.9	29.6	14.6	57.7	29.6	14.6	0.0	0.20	0.98	1.000	0.3	1.028	243.	0.5843	563.5	9.1	0.5	32.3	68.1	21.0	9.1	0.3	32.7	69.2	21.2
VC-MG	26/01/07	58.7	29.5	15.0	58.1	27.6	12.5	0.0	-1.24	1.00	0.998	1.4	1.029	243.6	0.5910	571.8	9.4	0.0	35.8	82.7	23.8	10.7	1.2	37.0	84.0	25.1
VC-MH	20/02/07	61.4	30.9	15.0	62.7	31.0	16.7	0.0	0.44	1.01	0.999	1.0	1.032	244.	0.6130	566.6	8.5	0.6	32.9	67.0	19.2	7.9	0.1	32.0	65.8	18.5
VC-MI	21/02/07	55.2	27.5	13.9	55.2	27.7	14.1	0.0	0.10	1.00	1.000	0.1	1.031				8.0	0.0	33.0	71.3	22.4	7.9	0.0	32.9	71.3	22.3
VC-MJ	05/02/07	59.7	29.9	15.5	62.1	32.4	16.1	0.0	0.24	1.04	0.999	0.8	1.025	*254* [a]	*0.369* [a]	*350.9* [a]	11.9	4.1	36.5	83.2	27.8	11.2	3.7	34.8	79.6	26.4
VC-MK	28/02/07	59.6	30.1	15.3	58.9	31.0	17.3	2.8	2.81	0.94	1.000	0.1	1.030	244.	0.5948	566.6	10.5	3.5	35.7	82.0	26.1	8.2	0.8	35.0	84.2	24.8
VC-MN	23/03/07	58.7	29.3	14.1	60.7	30.4	15.1	-1.1	-0.47	1.05	0.999	0.8	1.029	243.8	0.5616	543.3	8.9	0.0	34.1	75.9	23.0	9.0	0.4	33.0	73.0	22.5
VC-MU	02/03/07	61.6	31.0	17.6	59.1	30.1	17.0	0.0	0.13	0.96	1.000	0.2	1.034	244.	0.5881	542.0	7.7	0.3	29.0	68.7	20.2	7.9	0.2	30.1	71.5	20.9
	N	11	11	11	11	11	11	11					N	9	9	9	11	11	11	11	11	11	11	11	11	11
	Average	58.7	29.4	14.9	59.4	29.6	15.1	0.2					Average	243.7	0.5800	558.9	9.0	0.8	33.7	73.4	21.8	8.9	0.8	33.3	72.7	21.5
	SD	2.1	1.1	1.0	2.1	1.6	1.5	1.0					SD	0.4	0.0195	10.4	1.2	1.5	2.1	8.2	3.5	1.1	1.1	1.9	9.0	3.3
	Min	55.2	27.55	13.9	55.2	27.02	12.5	-1.1					Min	243.0	0.5507	542.0	7.7	0.0	29.0	57.2	15.1	7.9	0.0	30.1	53.6	15.1
	%25	57.9	28.76	14.2	58.5	28.28	14.0	0.0					%25	243.6	0.5630	554.3	8.4	0.0	32.9	68.4	19.8	8.0	0.1	32.1	68.8	19.9
	Median	58.7	29.51	14.6	59.1	29.72	14.8	0.0					Median	244.0	0.5843	562.3	8.7	0.0	33.7	71.3	21.3	8.3	0.4	32.9	71.5	21.2
	%75	59.7	29.95	15.2	60.7	30.68	16.4	0.0					%75	244.0	0.5910	566.6	9.3	0.5	35.4	80.9	23.4	9.2	1.0	34.8	79.5	23.6
	Max	61.6	31.02	17.6	62.7	32.37	17.3	2.8					Max	244.0	0.6130	571.8	11.9	4.1	36.5	83.2	27.8	11.2	3.7	37.0	84.2	26.4
	MADe	1.4	0.8	0.8	1.9	1.8	1.3	0.0					eSD	0	0.0162	6.4	0.7	0.0	2.1	6.8	2.9	0.6	0.6	1.4	8.6	3.4
	CV	2	3	5	3	6	9						CV	0.00	2.8	1.1	8		6	10	14	8		4	12	16

a) 5% Trichloroacetic acid solution

G2

Appendix H. Representative "Individualized Report" for RR26

Each participant in RR26 received an "Individualized Report" reflecting their reported results. The following two pages are the "Individualized Report" for participant "VC-MA".

Vitamin C "Round Robin" 26 Report: Participant VC-MA

Date	RR	Method	MPA Density g/mL	Dilute Solution 1 Spectrophotometry λ_{max}	A_{max}	$E^{1\%}$	Control/Calibration Solutions Y_{meas} = Inter + Slope* X_{grav} Inter	Slope	R^2	SEE
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
10/17/05	23	HPLC-EC	1.030	244.0	0.562	567.9	-0.6	1.09	0.998	1.47
03/09/06	24	HPLC-EC	1.031	244.0	0.568	586.7	0.2	1.13	1.000	0.41
08/28/06	25	HPLC-EC	1.039	242.0	0.555	557.4	0.8	0.95	0.999	0.92
03/20/07	26	HPLC-EC	1.033	244.0	0.573	554.3	0.3	1.00	1.000	0.31
		Mean	1.033	243.5	0.56	565.2				0.57
		SD	0.003	0.8	0.01	11.5				0.52
		CV	0.34	0.34	1.3	2.0				

[TAA] mmol/Lsample

Date	RR	Sample	Rep_1	Rep_2	F_{adj}	Mean	SD_{dup}	N	Mean	SD_{repeat}	SD_{reprod}
09/13/04	21	CS#1	8.1	7.9	1.0	8.0	0.1	7	8.8	0.1	0.6
03/08/05	22	CS#1	8.5	8.7	1.0	8.6	0.1				
10/17/05	23	CS#1	9.3	9.5	1.0	9.4	0.1				
10/17/05	23	CS#1	9.3	9.5	1.0	9.4	0.1				
03/09/06	24	CS#1	9.3	9.2	1.0	9.3	0.0				
08/28/06	25	CS#1	8.3	8.6	1.0	8.4	0.2				
03/20/07	26	CS#1	**8.6**	**8.3**	1.0	8.5	0.2				
11/13/03	19	S19:1	nd	nd	1.0			2	0.0	0.0	0.0
09/13/04	21	S21:1	nd	nd	1.0						
10/17/05	23	S23:1	0.0	0.0	1.0	0.0	0.0				
03/20/07	26	S26:1	**0.0**	**0.0**	1.0	0.0	0.0				
03/20/03	18	S18:2	35.1	36.0	1.0	35.6	0.6	6	35.1	0.3	1.1
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				
03/09/06	24	S24:2	35.8	35.5	1.0	35.6	0.2				
03/20/07	26	S26:2	**35.0**	**35.4**	1.0	35.2	0.3				
09/16/93	04	S04:2	2.27	2.27	28.4	64.4	0.0	2	72.1	0.1	10.8
03/20/07	26	S26:3	**79.7**	**79.8**	1.0	79.8	0.1				
09/16/93	04	S04:3	0.66	0.64	28.4	18.4	0.3	4	19.7	0.4	1.3
05/25/95	06	S06:2	0.7	0.7	28.4	19.2	0.2				
09/27/01	14	S14:1	20.4	19.6	1.0	20.0	0.6				
03/20/07	26	S26:4	**21.1**	**21.5**	1.0	21.3	0.3				

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

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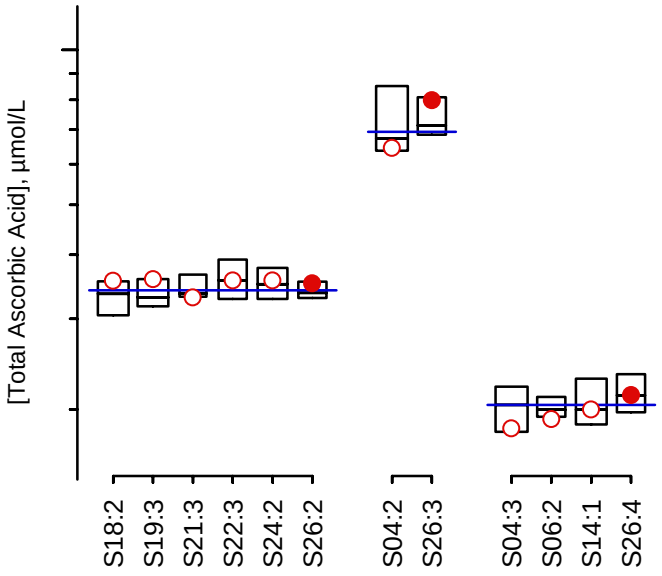
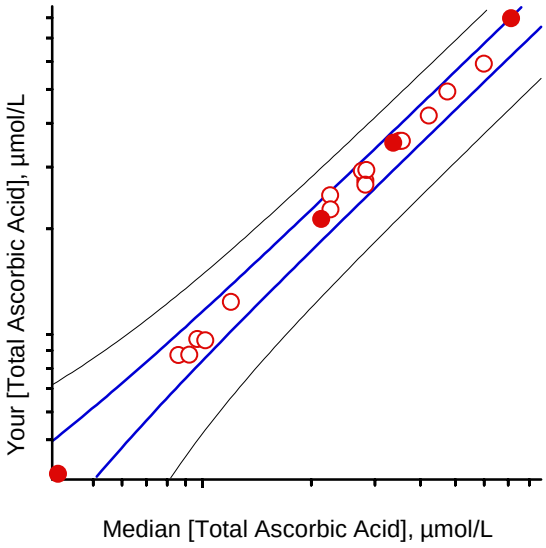
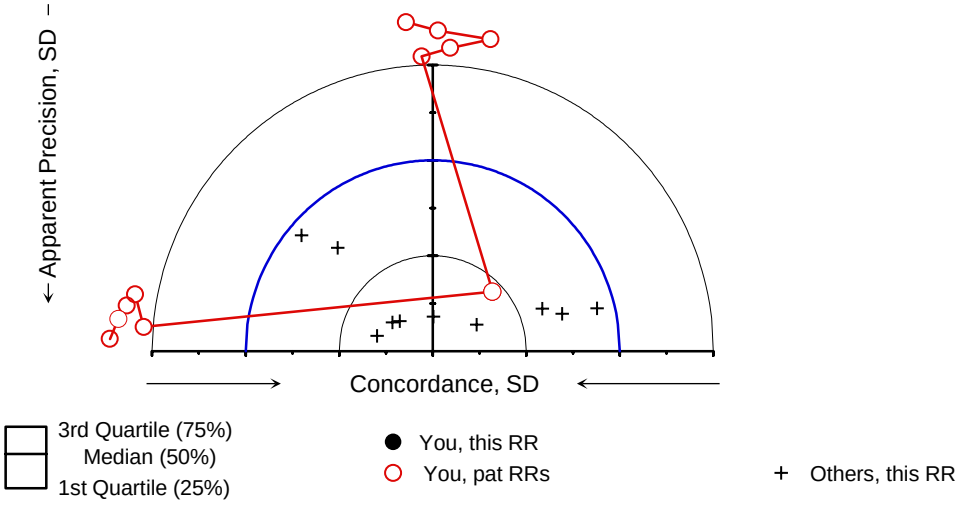
Vitamin C "Round Robin" 26 Report: Participant VC-MA

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
S26:1	VitC #13, a "blank" previously distributed in RRs 16, 19, 21, 22, and 24
S26:2	VitC #42, previously distributed in RRs 18, 19, 21, 22, and 24
S26:3	VitC #112, previous distributed in RR 4
S26:4	VitC #113, previous distributed in RR 4, 6 and 14