

**NISTIR 7880-11**

**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  
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David L. Duewer  
Jeanice B. Thomas  
*Chemical Sciences Division  
Materials Measurement Laboratory*

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,  $\alpha$ -Tocopherol,  $\gamma$ -Tocopherol, Total and *Trans*- $\beta$ -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 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.



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);  $\alpha$ -tocopherol, 75.8 at 292 nm (ethanol);  $\gamma$ -tocopherol, 91.4 at 298 nm (ethanol);  $\alpha$ -carotene, 2800 at 444 nm (hexane);  $\beta$ -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](mailto: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

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 <sub>2</sub> )						
ubiquinone (Qox)						
phylloquinone (K <sub>1</sub> )						
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:

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<sup>2</sup>QAP sera

<u>Serum</u>	<u>Form</u>	<u>Reconstitute?</u>
#329	Liquid frozen	No
#330	Liquid frozen	No
#331	Liquid frozen	No
#332	Liquid frozen	No
#333	Lyophilized	Yes (1 ml H <sub>2</sub> O)

- 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<sup>2</sup>QAP Gang

## **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.



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<sup>2</sup>QAP) 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](mailto:david.duewer@nist.gov) or me at [jbthomas@nist.gov](mailto: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

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

The NIST M<sup>2</sup>QAP 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>i.e.</i> , #330 = (15×#329 + 16×#331)/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

Lab	Total Retinol			trans-Retinol			Retinyl Palmitate			α-Tocopherol					
	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						24.43	15.75	8.66	10.59	10.16
FSV-BB	0.485	0.388	0.284	0.628	0.617						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						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						23.41	14.99	8.18	10.15	9.54
FSV-BJ	0.496	0.379	0.280	0.593	0.581						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						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						23.06	16.09	9.19	11.51	11.49
FSV-BR	0.527	0.402	0.305	0.663	0.661						24.82	14.49	8.23	9.65	9.95
FSV-BS	≥0.469	≥0.366	≥0.274	≥0.588	≥0.557	0.469	0.366	0.274	0.588	0.557	23.26	15.22	8.22	10.79	10.49
FSV-BT	0.599	0.471	0.342	0.666	0.604						22.95	14.72	9.25	11.26	10.70
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	0.507	0.384	0.315	0.670	0.636						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	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						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										
FSV-DV	0.568	0.424	0.326	0.696	0.680										
FSV-EE	0.454	0.309	0.266	0.550	0.508										
N	32	32	32	32	32	5	5	5	5	5	10	9	9	10	10
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
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
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
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
Npast	0	0	32	33	34	0	0	5	7	7	0	0	0	0	36
Medianpast			0.309	0.641	0.605			0.310	0.643	0.603					8.32
SDpast			0.023	0.045	0.044			0.000	0.020	0.020					0.58
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
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
											23.69	15.39	8.50	10.50	9.98
						1.89	1.19	0.68	0.82	0.78					

# Round Robin LXI Laboratory Results

All Results in µg/mL

Lab	γ/β-Tocopherol					δ-Tocopherol					Total β-Carotene					trans-β-Carotene				
	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											0.498	0.315	0.149	0.113	0.111					
FSV-DI																				
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 <sub>past</sub>	0	0	24	22	22	0	0	5	6	6	0	0	26	26	26	0	0	8	11	11
Median <sub>past</sub>			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 <sub>past</sub>			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

Lab	Total cis-β-Carotene					Total α-Carotene					Total Lycopene					trans-Lycopene				
	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						0.353	0.155	0.014	0.087	0.089	0.261	0.421	0.593	0.541	0.494					
FSV-BF						0.292	0.146	0.022	0.093	0.091	0.300	0.534	0.654	0.631	0.626					
FSV-BG						0.319	0.160	0.010	0.078	0.077	0.280	0.499	0.636	0.566	0.563					
FSV-BH	0.032	0.018	na	na	na	0.304	0.145	0.016	0.075	0.070	0.228	0.374	0.487	0.438	0.389					
FSV-BI						0.404	0.172	0.012	0.089	0.092	0.269	0.421	0.537	0.508	0.483					
FSV-BJ																				
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						0.225	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-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																				
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 <sub>past</sub>	0	0	5	6	6	0	0	19	22	22	0	0	22	22	22	0	0	0	7	9
Median <sub>past</sub>			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 <sub>past</sub>			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

# Round Robin LXI Laboratory Results

All Results in µg/mL

Lab	Total β-Cryptoxanthin					Total α-Cryptoxanthin					Total Lutein					Total Zeaxanthin				
	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	0.161	0.150	0.154	0.082	0.083	0.026	0.056	0.073	0.041	0.045
FSV-BB	0.093	0.100	0.104	0.057	0.054	0.021	0.029	0.035	0.026	0.025										
FSV-BC																				
FSV-BD																				
FSV-BE																				
FSV-BF	0.091	0.095	0.100	0.050	0.045						0.157	0.142	0.128	0.098	0.095	0.028	0.029	0.031	0.030	0.026
FSV-BG	0.110	0.120	0.127	0.074	0.070						0.113	0.109	0.100	0.057	0.057	0.023	0.054	0.070	0.030	0.028
FSV-BH	0.119	0.135	0.130	0.065	0.061						0.139	0.132	0.123	0.081	0.075	0.026	0.047	0.062	0.031	0.031
FSV-BI	0.100	0.106	0.111	0.057	0.052						0.152	0.149	0.146	0.087	0.083					
FSV-BJ	0.105	0.108	0.105	0.053	0.048															
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						0.123	0.126	0.138	0.059	0.055	0.043	0.040	0.042	0.026	0.025
FSV-BT	0.087	0.086	0.092	0.051	0.050	0.023	0.027	0.033	0.025	0.024										
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																				
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
Npast	0	0	23	22	22	0	0	4	5	7	0	0	14	15	15	0	0	11	13	13
Medianpast			0.112	0.055	0.052			0.040	0.027	0.024			0.145	0.080	0.078			0.057	0.032	0.030
SDpast			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	0.029	0.027	0.029	0.017	0.016	0.007	0.013	0.018	0.009	0.008

# Round Robin LXI Laboratory Results

## All Results in µg/mL

Lab	Total Lutein&Zeaxanthin				Coenzyme Q10				Ubiquinol				Ubiquinone								
	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	0.180	0.191	0.195	0.115	0.112																
FSV-BF	0.167	0.155	0.140	0.112	0.105																
FSV-BG	0.136	0.163	0.170	0.087	0.085																
FSV-BH	0.165	0.179	0.185	0.112	0.106																
FSV-BI																					
FSV-BJ																					
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	0.191	0.229	0.256	0.119	0.102																
FSV-BS	0.164	0.190	0.205	0.102	0.096																
FSV-BT	0.152	0.166	0.116	0.101	0.093																
FSV-BU	0.201	0.219	0.236	0.127	0.118																
FSV-BV																					
FSV-BW																					
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																
FSV-CS	0.148	0.158	0.182	0.114	0.100																
FSV-CW	0.192	0.185	0.188	0.124	0.117																
FSV-CZ																					
FSV-DD																					
FSV-DF																					
FSV-DI																					
FSV-DV																					
FSV-EE																					
N	17	17	17	17	17	7	7	7	7	7	2	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	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	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	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											
Npast	0	0	22	23	23	0	0	5	5	5	0	0	0	0	0	0	0	0	0	0	0
Medianpast			0.211	0.113	0.108			1.190	0.760	0.699											
SDpast			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	0.070	0.880	1.090	0.715	0.709	0.070	0.880	1.090	0.715	0.709	0.709
NAU	0.039	0.040	0.053	0.025	0.023	0.070	0.101	0.112	0.072	0.071	0.070	0.101	0.112	0.072	0.071	0.070	0.101	0.112	0.072	0.071	0.071

Round Robin LXI Laboratory Results  
All Results in µg/mL

Lab	Phylloquinone (K1) x1000				25-hydroxyvitamin D					
	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 <sub>past</sub>	0	0	0	0	0	0	0	0	5	4
Median <sub>past</sub>										0.017
SD <sub>past</sub>										0.004
NAV	1.339	0.887	0.530	0.389	0.330	0.015	0.012	0.008	0.020	0.020
NAU										

# 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 <sub>part</sub>	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 <sub>past</sub>	Mean of N(s) from past RR(s)
Median <sub>past</sub>	Mean of Median(s) from past RR(s)
SD <sub>past</sub>	Pooled SD from past RR(s)
NAV	NIST Assigned Value = (Median <sub>part</sub> + Mean <sub>NIST</sub> )/2 for analytes reported by NIST analyst(s) = Median <sub>part</sub> 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 <sub>NIST</sub> , eSD) and S <sub>btw</sub> is the standard deviation between Median <sub>part</sub> and Mean <sub>NIST</sub> . The expected long-term SD, eSD 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, malfunction
<i>italics</i>	Not explicitly reported but calculated by NIST from reported values

# Round Robin LXI Laboratory Results

## 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) 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.

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

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

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

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 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
- $\alpha$ -Tocopherol
- $\gamma/\beta$ -Tocopherol
- $\delta$ -Tocopherol
- *trans*- $\beta$ -Carotene
- Total *cis*- $\beta$ -Carotene
- Total  $\alpha$ -Carotene
- Total Lycopene
- *trans*-Lycopene
- Total  $\beta$ -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

Analyte	Serum 329			Serum 330			Serum 331			Serum 332			Serum 333		
	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

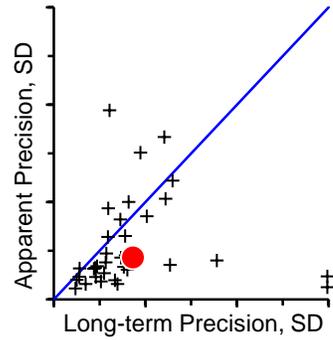
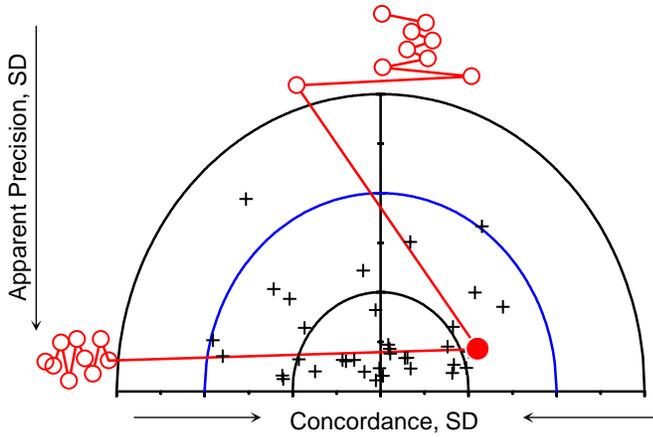
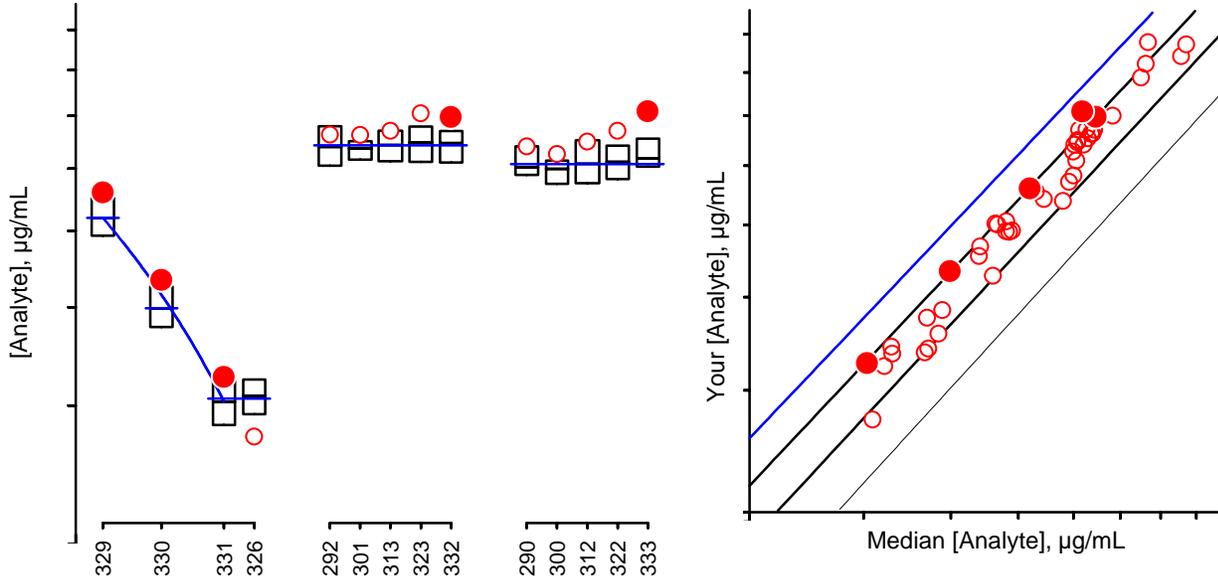
Please check our records against your records. Send corrections and/or updates to...

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



### Serum

#329	Fresh frozen: New
#330	Fresh frozen: New
#331	Fresh frozen: 60:#326
#332	Fresh-frozen: 53:#292, 55:#301, 57:#313, 59:#323
#333	Lyophilized: 53:#290, 55:#300, 57:#312, 59:#322

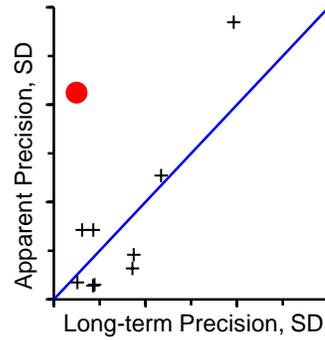
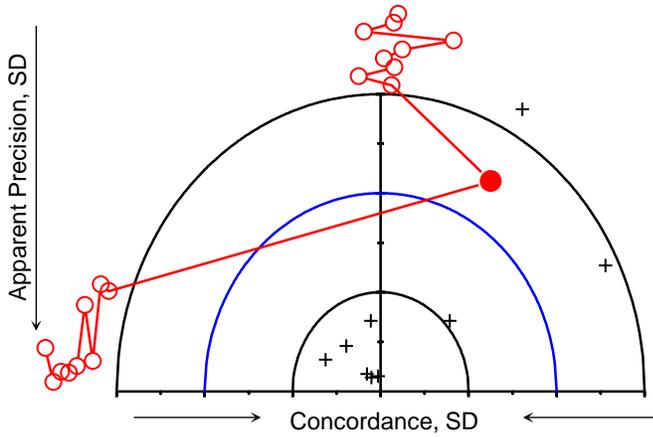
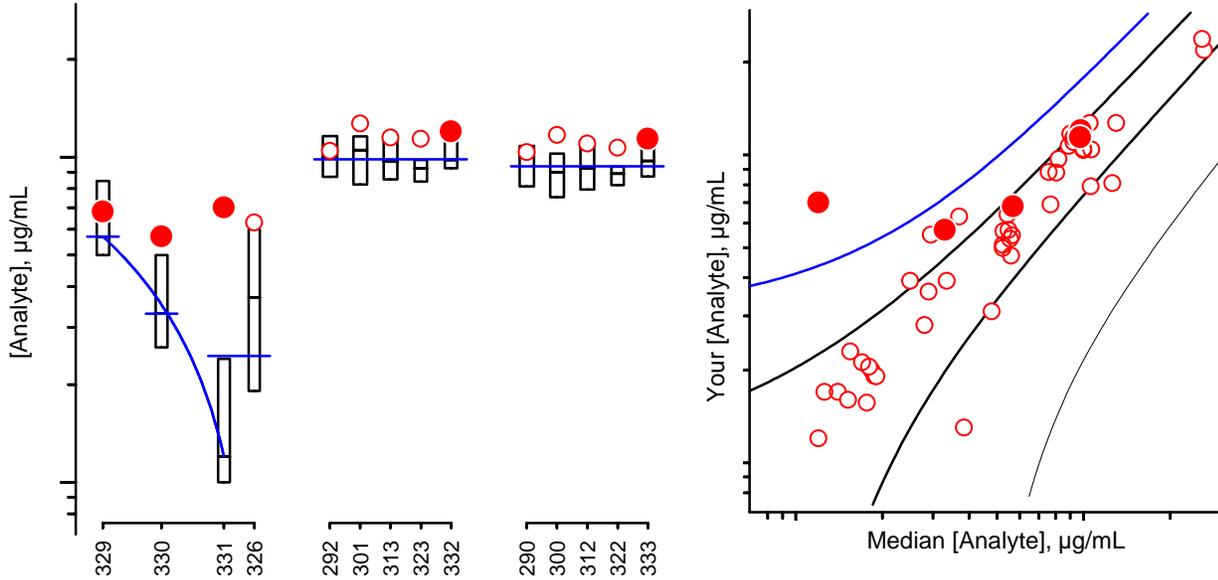
### History

### Comments

Native, single-source
15:16 blend of #329 and #331
Native, single-source
Native, same single-source as #333
Native, same single-source as #332

# Individualized RR LXI Report: FSV-BA

## Retinyl Palmitate



### Serum

#329	Fresh frozen: New
#330	Fresh frozen: New
#331	Fresh frozen: 60:#326
#332	Fresh-frozen: 53:#292, 55:#301, 57:#313, 59:#323
#333	Lyophilized: 53:#290, 55:#300, 57:#312, 59:#322

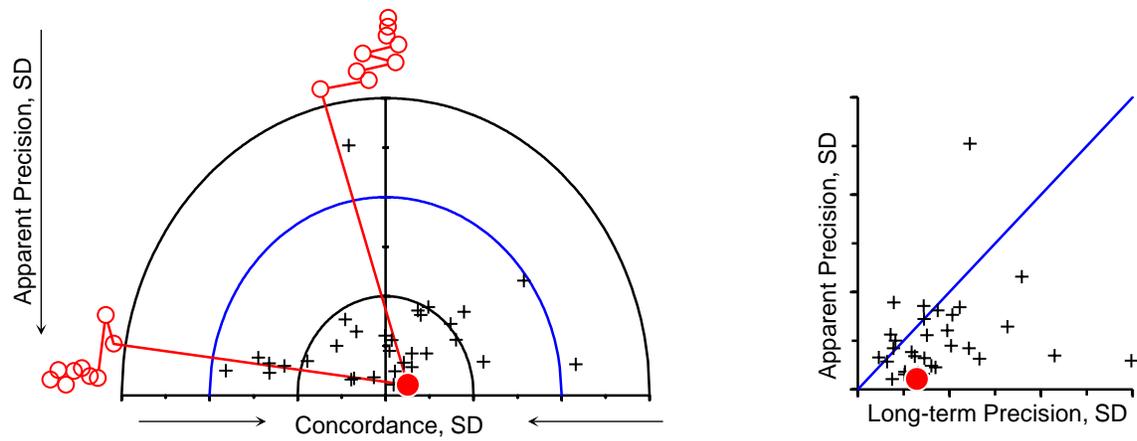
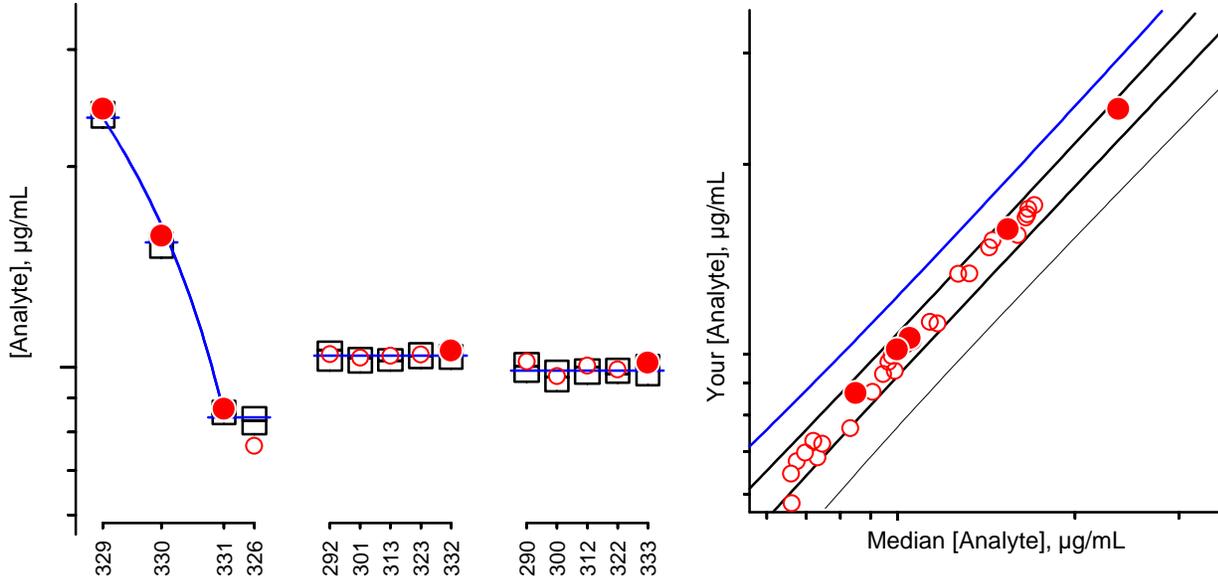
### History

### Comments

Native, single-source  
 15:16 blend of #329 and #331  
 Native, single-source  
 Native, same single-source as #333  
 Native, same single-source as #332

# Individualized RR LXI Report: FSV-BA

## α-Tocopherol



### Serum

#329	Fresh frozen: New
#330	Fresh frozen: New
#331	Fresh frozen: 60:#326
#332	Fresh-frozen: 53:#292, 55:#301, 57:#313, 59:#323
#333	Lyophilized: 53:#290, 55:#300, 57:#312, 59:#322

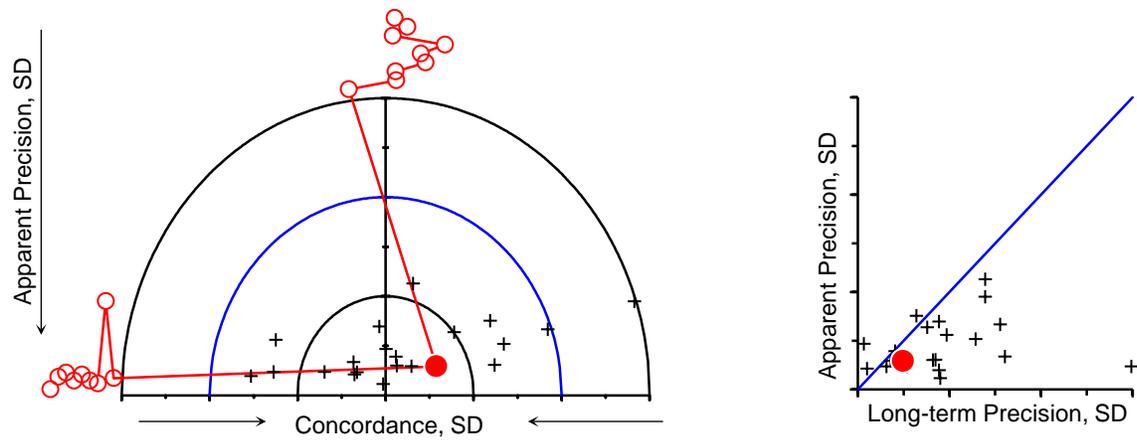
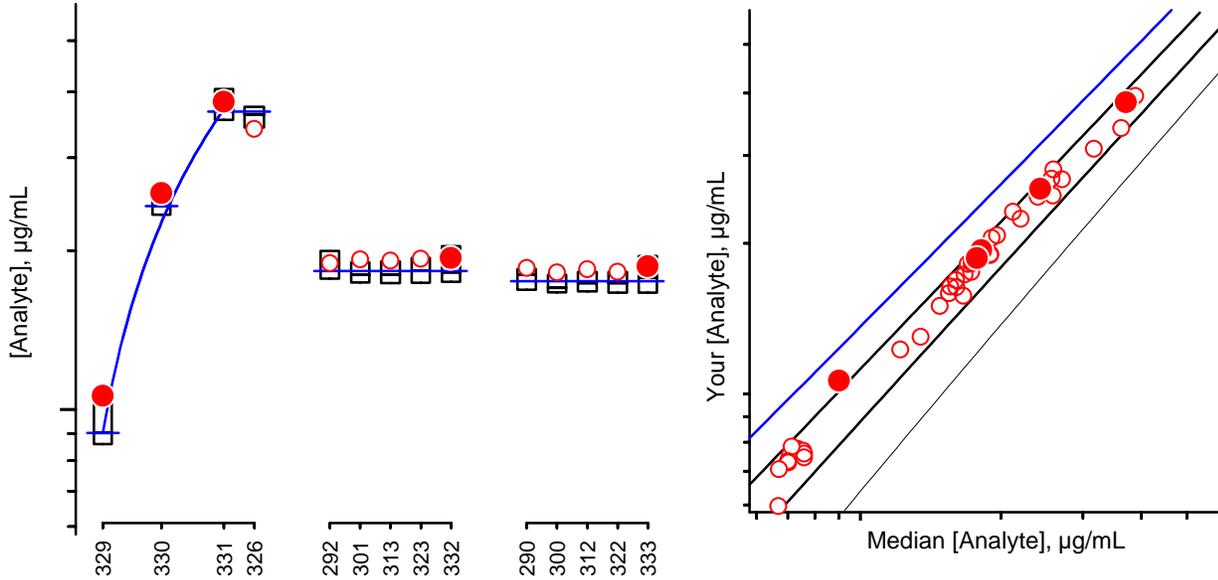
### History

### Comments

Native, single-source  
 15:16 blend of #329 and #331  
 Native, single-source  
 Native, same single-source as #333  
 Native, same single-source as #332

# Individualized RR LXI Report: FSV-BA

$\gamma/\beta$ -Tocopherol



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

Serum

#329  
#330  
#331  
#332  
#333

History

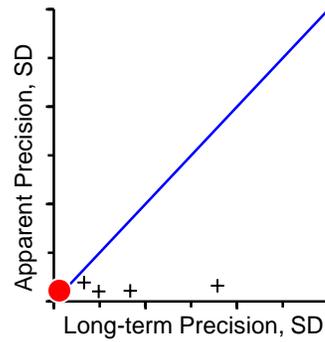
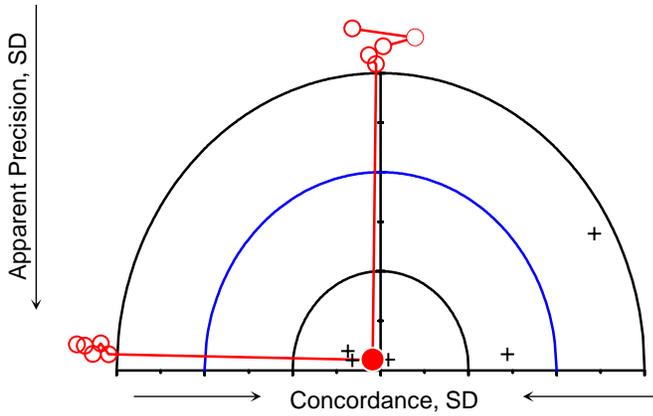
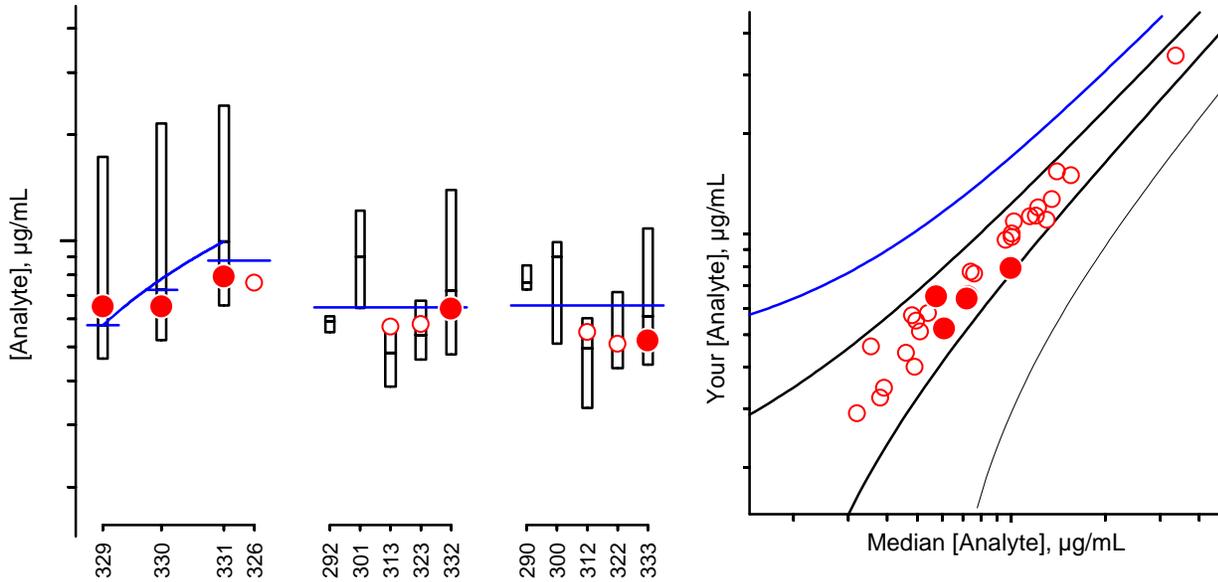
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Fresh frozen: New  
Fresh frozen: 60:#326  
Fresh-frozen: 53:#292, 55:#301, 57:#313, 59:#323  
Lyophilized: 53:#290, 55:#300, 57:#312, 59:#322

Comments

Native, single-source  
15:16 blend of #329 and #331  
Native, single-source  
Native, same single-source as #333  
Native, same single-source as #332

# Individualized RR LXI Report: FSV-BA

## δ-Tocopherol



### Serum

#329	Fresh frozen: New
#330	Fresh frozen: New
#331	Fresh frozen: 60:#326
#332	Fresh-frozen: 53:#292, 55:#301, 57:#313, 59:#323
#333	Lyophilized: 53:#290, 55:#300, 57:#312, 59:#322

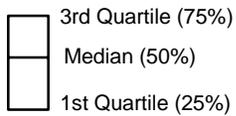
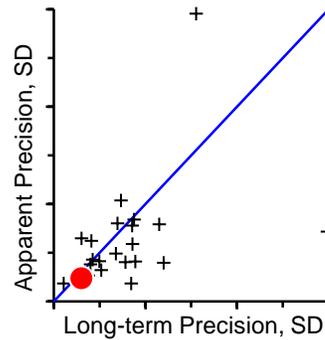
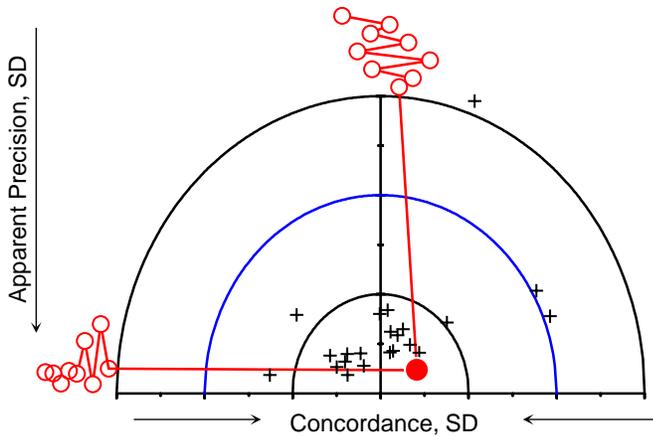
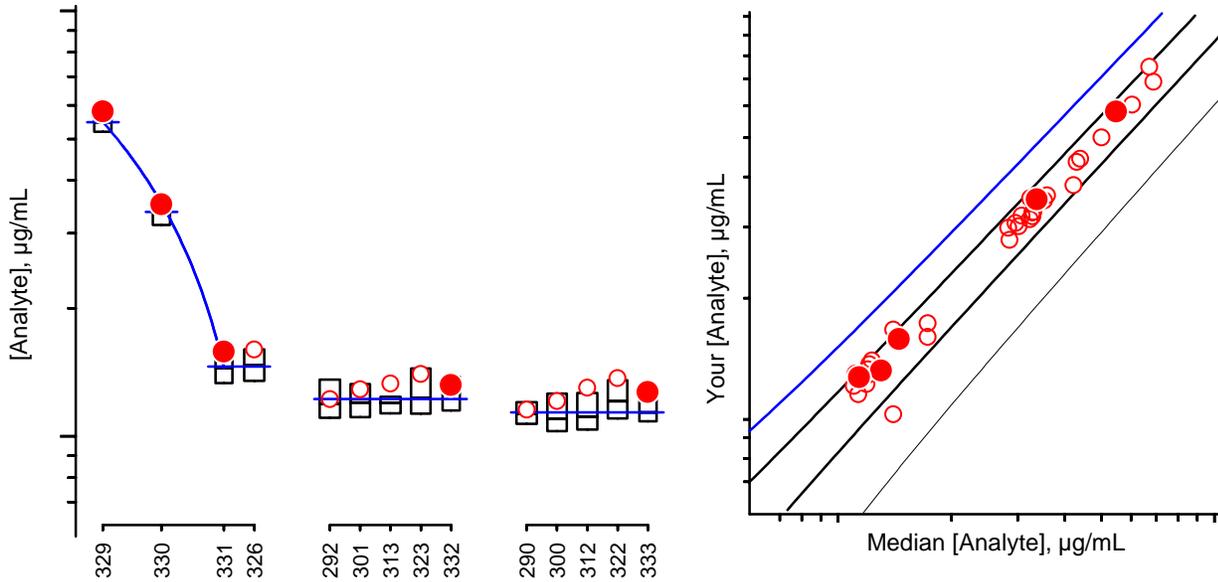
### History

### Comments

Native, single-source
15:16 blend of #329 and #331
Native, single-source
Native, same single-source as #333
Native, same single-source as #332

# Individualized RR LXI Report: FSV-BA

## Total β-Carotene



● You, this RR  
○ You, past RRs  
— Expectation

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

+ Others, this RR

### Serum

#329	Fresh frozen: New
#330	Fresh frozen: New
#331	Fresh frozen: 60:#326
#332	Fresh-frozen: 53:#292, 55:#301, 57:#313, 59:#323
#333	Lyophilized: 53:#290, 55:#300, 57:#312, 59:#322

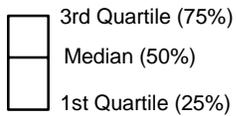
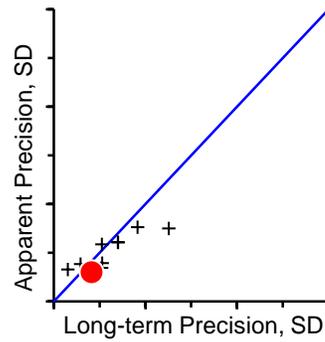
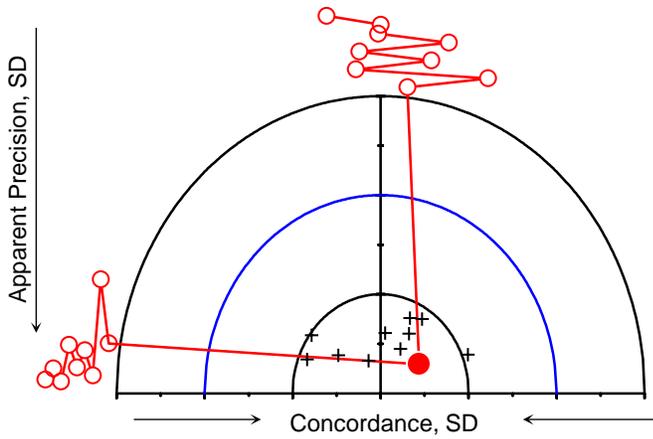
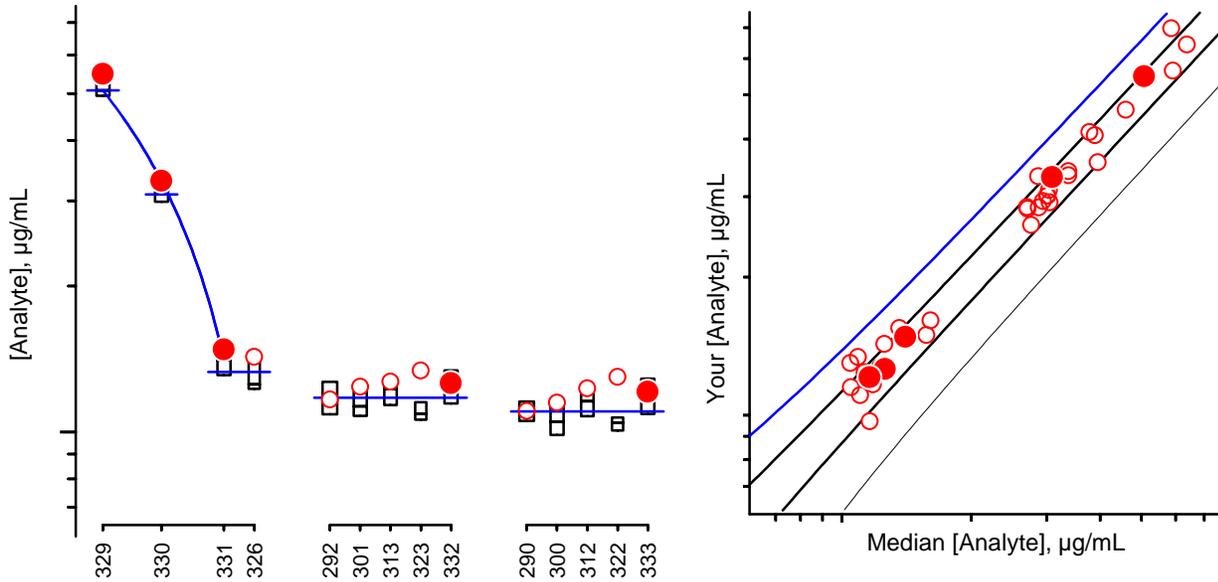
### History

### Comments

Native, single-source  
15:16 blend of #329 and #331  
Native, single-source  
Native, same single-source as #333  
Native, same single-source as #332

# Individualized RR LXI Report: FSV-BA

trans-β-Carotene



● You, this RR  
○ You, past RRs  
— Expectation

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

+ Others, this RR

Serum

Serum	History
#329	Fresh frozen: New
#330	Fresh frozen: New
#331	Fresh frozen: 60:#326
#332	Fresh-frozen: 53:#292, 55:#301, 57:#313, 59:#323
#333	Lyophilized: 53:#290, 55:#300, 57:#312, 59:#322

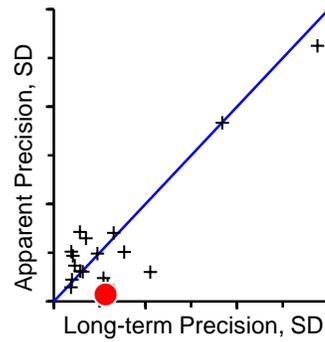
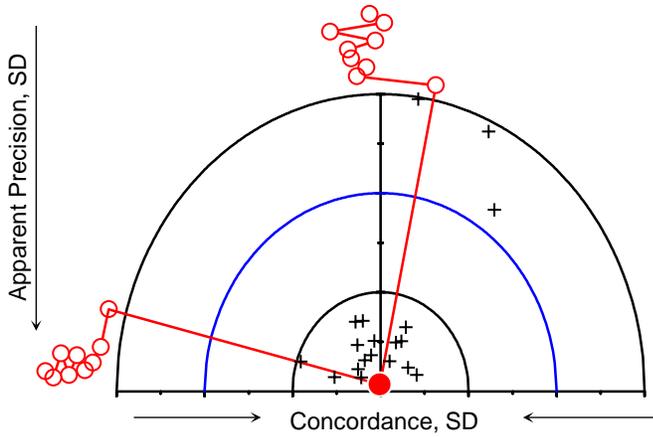
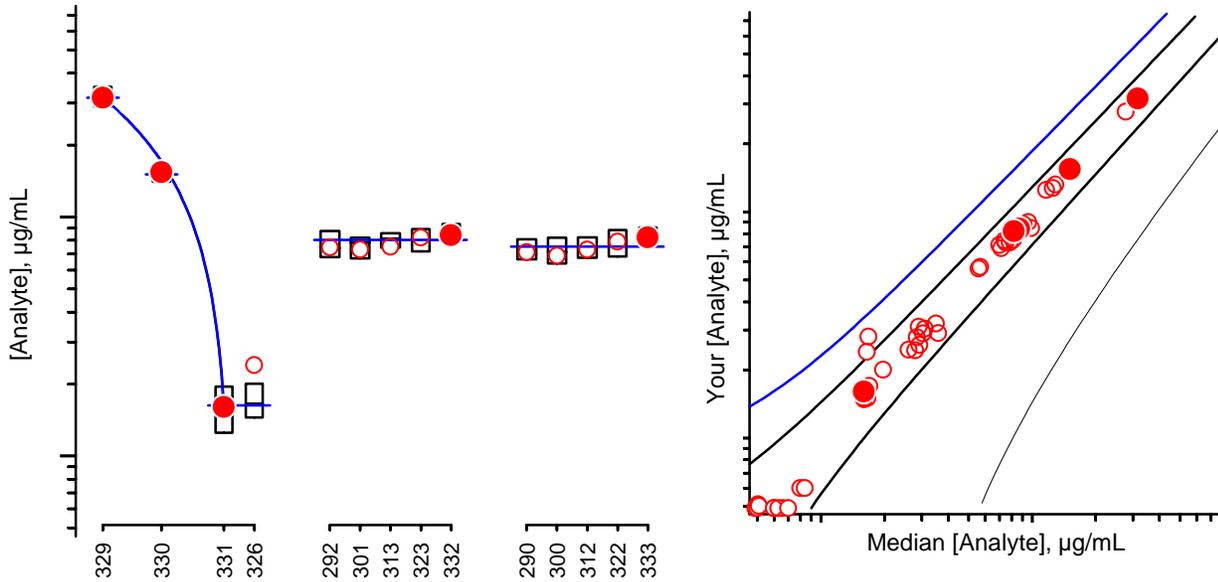
History

Comments

Native, single-source  
15:16 blend of #329 and #331  
Native, single-source  
Native, same single-source as #333  
Native, same single-source as #332

# Individualized RR LXI Report: FSV-BA

## Total $\alpha$ -Carotene



### Serum

#329	Fresh frozen: New
#330	Fresh frozen: New
#331	Fresh frozen: 60:#326
#332	Fresh-frozen: 53:#292, 55:#301, 57:#313, 59:#323
#333	Lyophilized: 53:#290, 55:#300, 57:#312, 59:#322

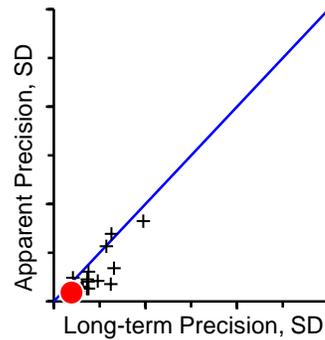
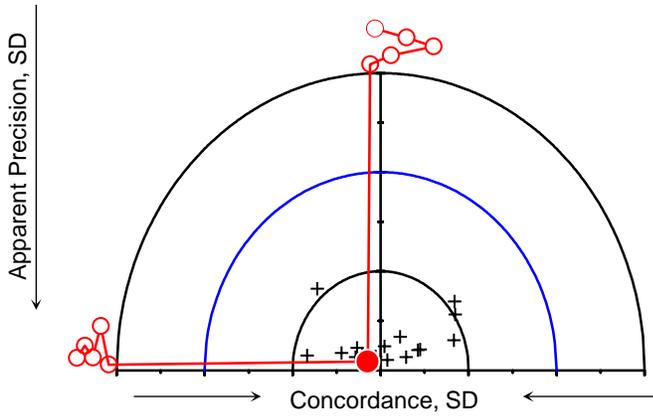
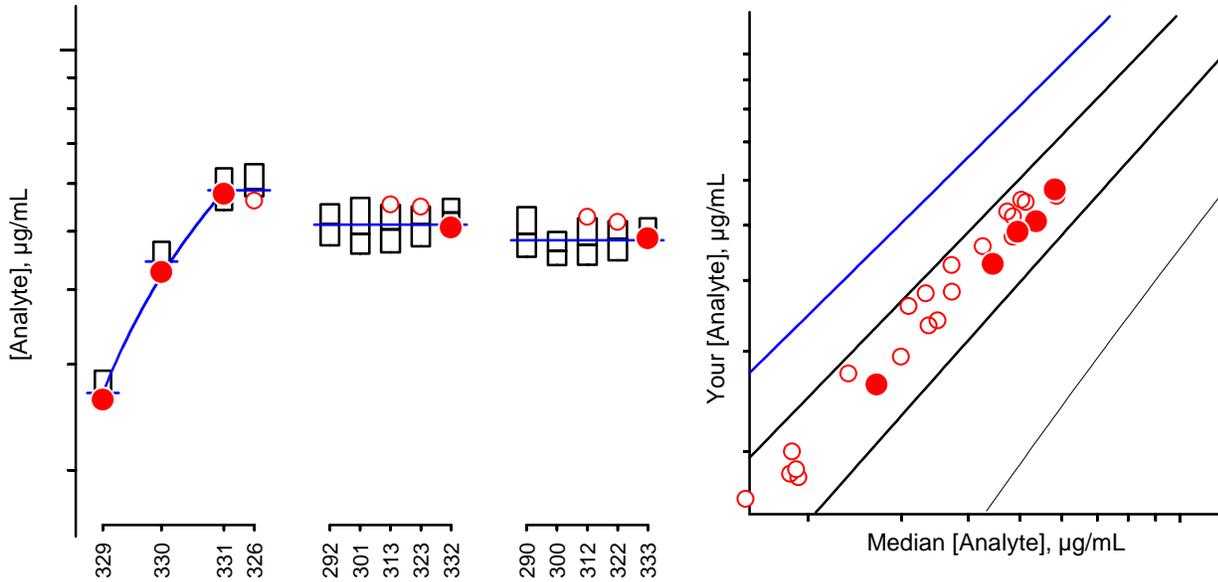
### History

### Comments

Native, single-source  
 15:16 blend of #329 and #331  
 Native, single-source  
 Native, same single-source as #333  
 Native, same single-source as #332

# Individualized RR LXI Report: FSV-BA

## Total Lycopene



### Serum

#329	Fresh frozen: New
#330	Fresh frozen: New
#331	Fresh frozen: 60:#326
#332	Fresh-frozen: 53:#292, 55:#301, 57:#313, 59:#323
#333	Lyophilized: 53:#290, 55:#300, 57:#312, 59:#322

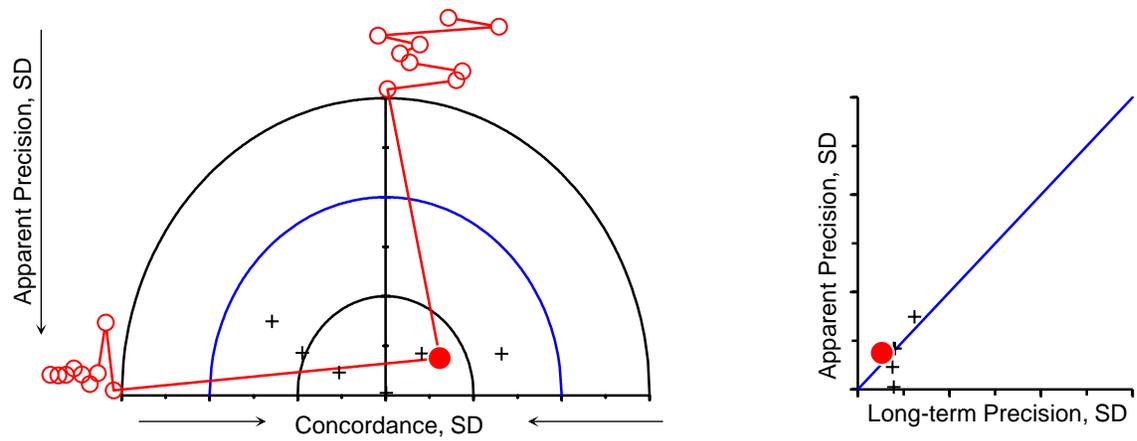
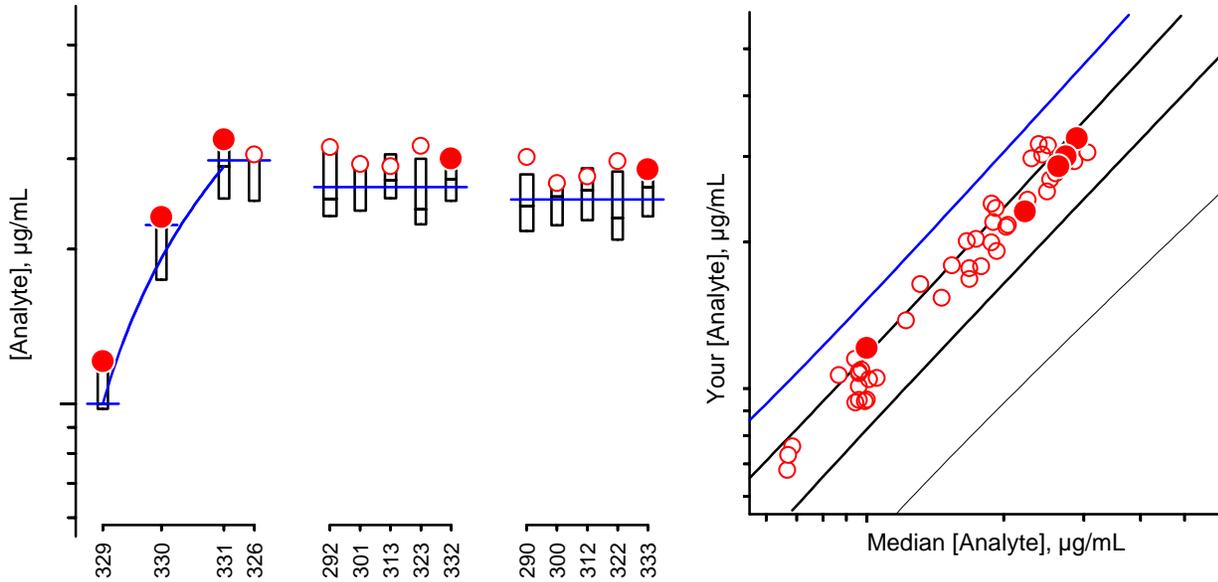
### History

### Comments

Native, single-source
15:16 blend of #329 and #331
Native, single-source
Native, same single-source as #333
Native, same single-source as #332

# Individualized RR LXI Report: FSV-BA

trans-Lycopene



Serum

#329  
#330  
#331  
#332  
#333

History

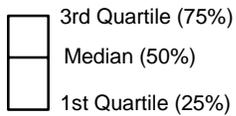
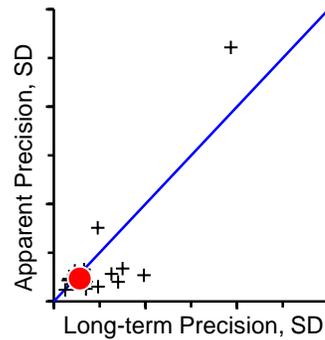
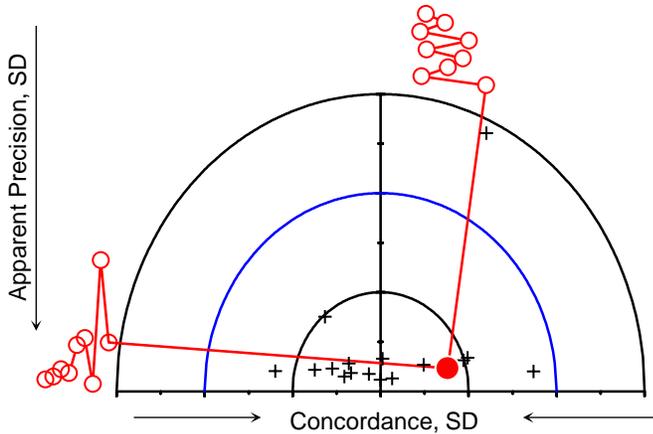
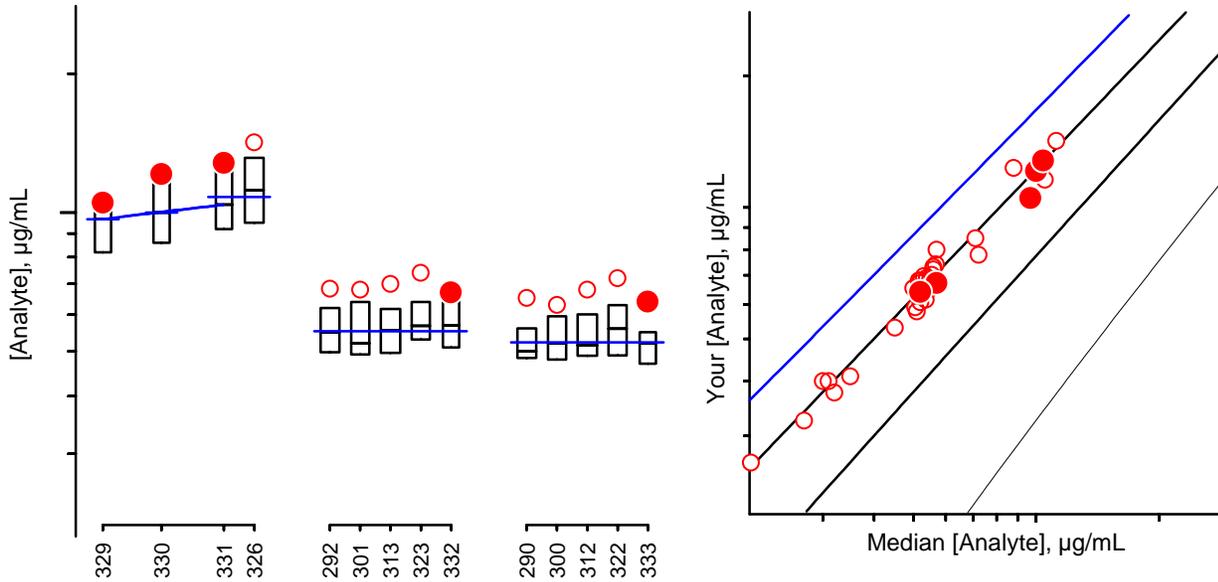
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Fresh frozen: New  
Fresh frozen: 60:#326  
Fresh-frozen: 53:#292, 55:#301, 57:#313, 59:#323  
Lyophilized: 53:#290, 55:#300, 57:#312, 59:#322

Comments

Native, single-source  
15:16 blend of #329 and #331  
Native, single-source  
Native, same single-source as #333  
Native, same single-source as #333

# Individualized RR LXI Report: FSV-BA

## Total $\beta$ -Cryptoxanthin



● You, this RR  
○ You, past RRs  
— Expectation

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

+ Others, this RR

### Serum

#329	Fresh frozen: New
#330	Fresh frozen: New
#331	Fresh frozen: 60:#326
#332	Fresh-frozen: 53:#292, 55:#301, 57:#313, 59:#323
#333	Lyophilized: 53:#290, 55:#300, 57:#312, 59:#322

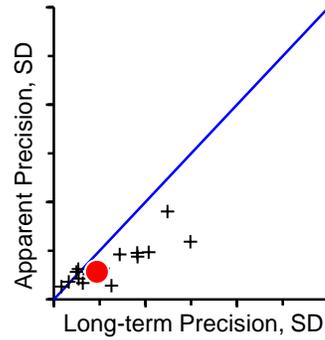
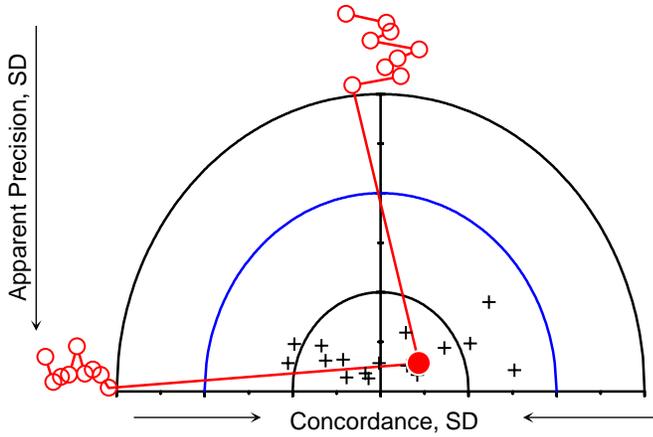
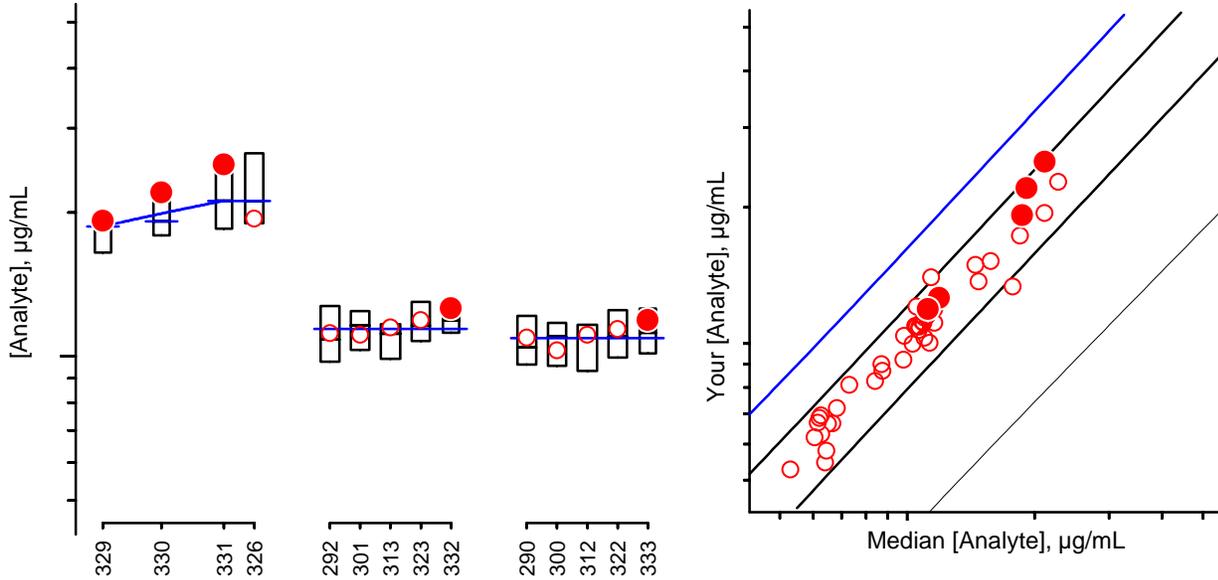
### History

### Comments

Native, single-source  
15:16 blend of #329 and #331  
Native, single-source  
Native, same single-source as #333  
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,  $\geq x$ , this RR
- You,  $\geq x$ , past RRs
- Others, this RR

Serum

#329	Fresh frozen: New
#330	Fresh frozen: New
#331	Fresh frozen: 60:#326
#332	Fresh-frozen: 53:#292, 55:#301, 57:#313, 59:#323
#333	Lyophilized: 53:#290, 55:#300, 57:#312, 59:#322

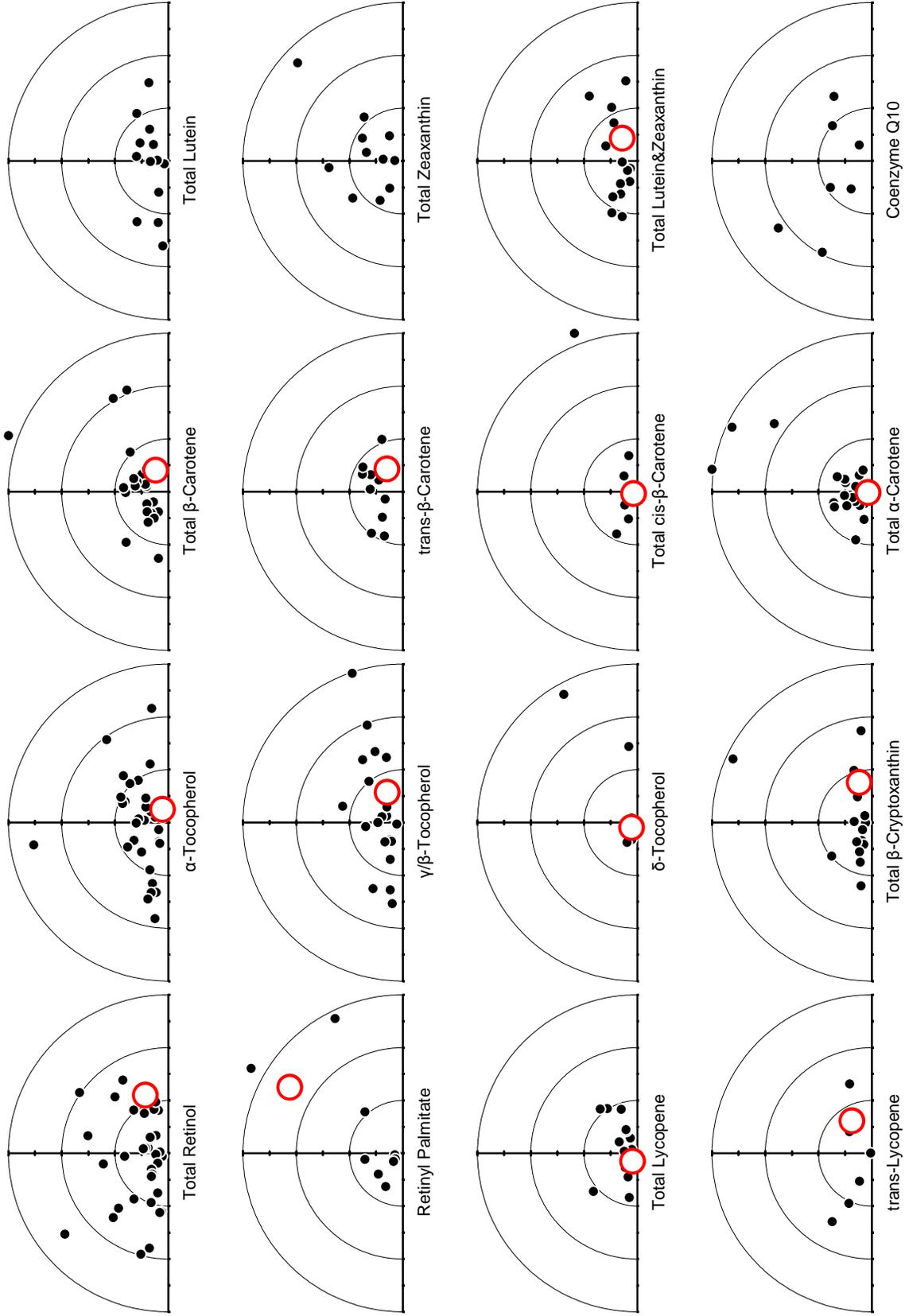
History

Comments

Native, single-source  
 15:16 blend of #329 and #331  
 Native, single-source  
 Native, same single-source as #333  
 Native, same single-source as #332

# Individualized Round Robin LXI Report: FSV-BA

## Graphical Comparability Summary



## **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.

November 8, 2006



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

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  $\approx 95\%$  confidence interval for SRM 970-Level I is  $8.41 \pm 0.61 \mu\text{mol/L}$  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](mailto: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](mailto: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,

A handwritten signature in black ink that reads "Jeanice Brown Thomas". The signature is written in a cursive style with a large, stylized initial "J".

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  $\mu\text{mol/L}$  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 ( $A_{\text{max}}$ ) within this region. Record the wavelength ( $\lambda_{\text{max}}$ ) at which this maximum occurs.

The extinction coefficient ( $E^{1\%}$ ) of ascorbic acid at  $\lambda_{\text{max}}$  (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,  $\lambda_{\text{max}}$  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 CS #2 (see protocol below). The target values for this materials is  $28.1 \pm 1.0 \mu\text{mol/L}$  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  $\mu\text{mol}$  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 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]<sub>DS1</sub> ..... \_\_\_\_\_ 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]<sub>DS2</sub> ..... \_\_\_\_\_ 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]<sub>DS3</sub> ..... \_\_\_\_\_ 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**

<b>Sample</b>	<b>Replicate 1</b>	<b>Replicate 2</b>	<b>Units</b>
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 <i>Target: 8.41 ±0.61 µmol/L</i>
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<sup>2</sup>QAP 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<sup>2</sup>QAP Gang

## **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.



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  $100 \times \text{MADe} / \text{median}$ .

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.

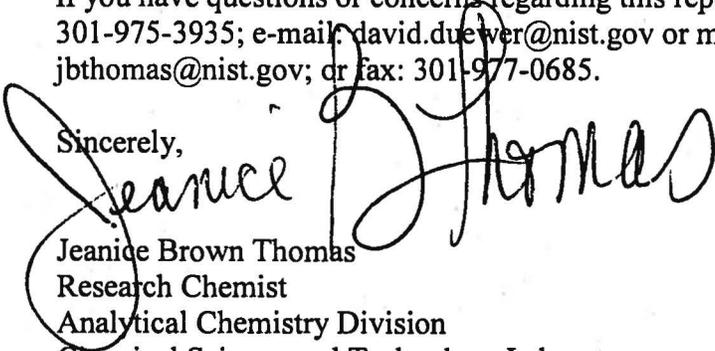
If you have concerns regarding your laboratory's performance, we suggest that you obtain and analyze a unit of Standard Reference Material (SRM) 970 Vitamin C in Frozen Human Serum. SRM 970 can be purchased from the NIST SRM Program at phone: 301-975-6776; fax: 301-948-3730. If your measured values do 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<sup>2</sup>QAP 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](mailto:david.duewer@nist.gov) or me at 301-975-3120; e-mail: [jbthomas@nist.gov](mailto: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<sup>2</sup>QAP 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 ( $\approx 1.03$  g/mL), the observed wavelength maximum of “Dilute Solution 1” ( $\approx 244$  nm), the observed absorbance at that maximum ( $\approx 0.58$  OD), the calculated  $E^{1\%}_{1\text{cm}}$  ( $\approx 560$  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**

Lab	Date	Control / Calibration Samples						MPA						Dilute Solution 1						Samples					
		Gravimetric, $\mu\text{mol/L}$		Measured, $\mu\text{mol/L}$		Calibration Parameters		Density		Spectrophotometry		Measured, $\mu\text{mol/L}$		Calibrated to Gravimetric, $\mu\text{mol/L}$		CS#1		CS#1		CS#1					
		Dil:1	Dil:2	Dil:3	Dil:1	Dil:2	Dil:3	MPA	Inter	Slope	R <sup>2</sup>	RMS	g/mL	$\lambda_{\text{max}}$	A <sub>max</sub>	E%	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	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	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	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	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	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	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				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 <sup>a</sup>	0.369 <sup>a</sup>	350.9 <sup>a</sup>	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	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	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	0.3	29.0	68.7	20.2	7.9	0.2	30.1	71.5	20.9

Lab	Date	Gravimetric, $\mu\text{mol/L}$		Measured, $\mu\text{mol/L}$		Calibration Parameters		Density		Spectrophotometry		Measured, $\mu\text{mol/L}$		Calibrated to Gravimetric, $\mu\text{mol/L}$			
		Dil:1	Dil:2	Dil:3	Dil:1	Dil:2	Dil:3	MPA	Inter	Slope	R <sup>2</sup>	RMS	g/mL	$\lambda_{\text{max}}$	A <sub>max</sub>	E%	
N	11	11	11	11	11	11	11	11	11	9	9	11	11	11	11		
Average	58.7	29.4	14.9	59.4	29.6	15.1	0.2		1.030	243.7	0.5800	558.9	0.8	33.7	73.4	21.8	8.9
SD	2.1	1.1	1.0	2.1	1.6	1.5	1.0	0.003	0.4	0.0195	10.4	1.2	1.5	2.1	8.2	3.5	1.1

Stat	Min		%25		Median		%75		Max		eSD		CV	
	N	Value	N	Value	N	Value	N	Value	N	Value	N	Value	N	Value
Min	11	55.2	11	27.02	11	12.5	11	-1.1	11	62.7	11	1.8	11	17.0
%25	11	57.9	11	28.28	11	14.0	11	0.0	11	31.0	11	1.6	11	15.1
Median	11	58.7	11	29.72	11	14.8	11	0.0	11	30.1	11	1.5	11	14.9
%75	11	59.7	11	30.68	11	16.4	11	0.0	11	31.0	11	1.8	11	15.1
Max	11	61.6	11	32.37	11	17.3	11	2.8	11	32.4	11	1.8	11	17.0
MADE	11	1.4	11	0.8	11	1.3	11	0.0	11	0.13	11	0.2	11	0.2
CV	2	3	5	3	6	9			0.003	0.000	2.8	0.003	0.4	0.0195

a) 5% Trichloroacetic acid solution

## **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	Dilute Solution 1			Control/Calibration Solutions			
			Density	Spectrophotometry			$Y_{\text{meas}} = \text{Inter} + \text{Slope} * X_{\text{grav}}$			
			g/mL	$\lambda_{\text{max}}$	$A_{\text{max}}$	$E^{1\%}$	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				

Date	RR	Sample	[TAA] mmol/Lsample					N	Mean	SD <sub>repeat</sub>	SD <sub>reprod</sub>				
			Rep <sub>1</sub>	Rep <sub>2</sub>	F <sub>adj</sub>	Mean	SD <sub>dup</sub>								
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	<b>8.6</b>	<b>8.3</b>	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	<b>0.0</b>	<b>0.0</b>	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	<b>35.0</b>	<b>35.4</b>	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	<b>79.7</b>	<b>79.8</b>	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	<b>21.1</b>	<b>21.5</b>	1.0	21.3	0.3								

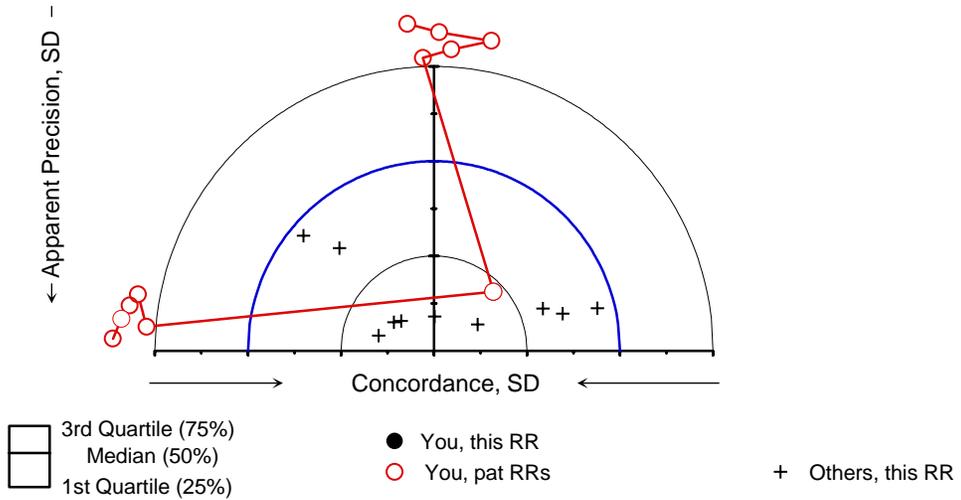
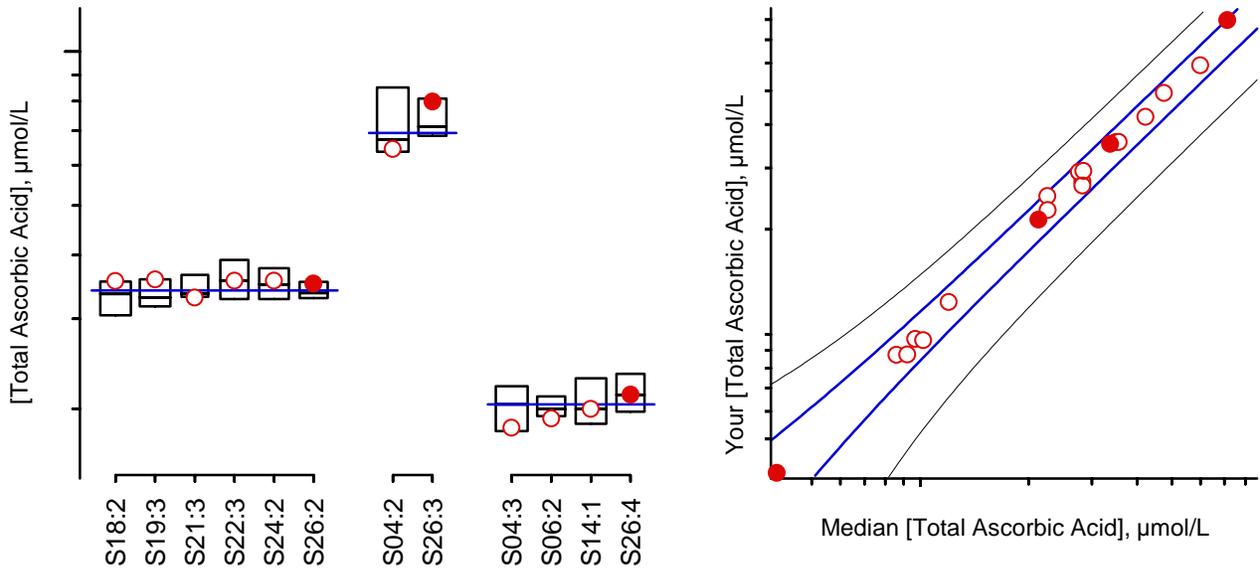
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Micronutrients Measurement Quality Assurance Program  
National Institute of Standards and Technology  
100 Bureau Drive Stop 8392  
Gaithersburg, MD 20899-8392 USA

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

# 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