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NIST Micronutrients Measurement Quality Assurance Program Summer 2006 Comparability Studies

Results for Round Robin LX
Fat-Soluble Vitamins and Carotenoids in Human Serum
and Round Robin 25 Ascorbic Acid in Human Serum

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National Institute of
Standards and Technology
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U.S. Department of Commerce
Cameron F. Kerry, 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 Summer 2006 MMQAP measurement comparability improvement studies: 1) Round Robin LX Fat-Soluble Vitamins and Carotenoids in Human Serum and 2) Round Robin 25 Total Ascorbic Acid in Human Serum. The materials for both studies were shipped to participants in May 2006; participants were requested to provide their measurement results by September 4, 2006.

Keywords

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

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Introduction

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

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

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

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

Participants in the MMQAP Fat-Soluble Vitamins and Carotenoids in Human Serum Round Robin LX comparability study (hereafter referred to as RR60) received two lyophilized and three 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 May 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 RR60 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 25: Vitamin C in Human Serum

Participants in the MMQAP Vitamin C in Human Serum Round Robin 25 comparability study (hereafter referred to as RR25) received four frozen serum test samples, two frozen control sera, and a solid ascorbic acid control material for analysis. Unless multiple vials were previously requested, participants received one vial of each material. These sample materials were shipped on dry ice to participants in May 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 RR25 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 RR60

The following three items were included in each package shipped to an RR60 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-

May 1, 2006

Dear Colleague:

Enclosed are the samples (Sera 324-328) for the second fat-soluble vitamins and carotenoids in serum round robin study (Round Robin LX) for the fiscal year (FY) 06 NIST Micronutrients Measurement Quality Assurance Program. You will find one vial of each of three liquid-frozen and two 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 **September 4, 2006**. Results received more than two weeks after the due date will not be included in the summary report for this round robin study. The feedback report concerning the study will be provided in April.

Lyophilized samples should be reconstituted with 1.0 mL of HPLC-grade water or equivalent. We recommend that dissolution be facilitated with 3 to 5 min agitation in an ultrasonic bath or at least 30 min at room temperature with intermittent swirling. (CAUTION: Vigorous shaking will cause foaming and possibly interfere with accurate measurement. The rubber stopper contains phthalate esters that may leach into the sample upon intermittent contact of the liquid sample with the stopper. These esters absorb strongly in the UV region and elute near retinol in most LC systems creating analytical problems.) Pipette a known volume of serum from the vial for analysis. The final volume of the reconstituted sample is greater than 1.0 mL. **Water should not be added to the liquid-frozen samples (Sera 324-326).**

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

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

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

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

Sincerely,

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

Enclosures

NIST

Participant #: _____ Date: _____

Round Robin LX: Human Sera

NIST Micronutrients Measurement Quality Assurance Program

Analyte	324	325	326	327	328	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 #324, 325, and 326 frozen when received? Yes | No

Comments:

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

Please return results **before** 4-Sep-2006

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

Participant #: _____ Date: _____

Fat-Soluble Vitamins Round Robin LX

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?
#324	Liquid frozen	No
#325	Liquid frozen	No
#326	Liquid frozen	No
#327	Lyophilized	Yes (1 ml H_2O)
#328	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 #324, 325, and 326 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 RR60

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-

October 12, 2006

Dear Colleague:

Enclosed is the summary report of the results for round robin LX (RR60) of the 2006 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 RR60 are provided in text "Score Card" summary, page 6 of the All Lab Report. Laboratory comparability is summarized as follows: results rated 1 to 3 are within 1 to 3 standard deviations of the assigned value, respectively; those rated 4 are >3 standard deviations from the assigned value. 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 first 2007 QA interlaboratory exercise will be shipped **during the week of November 13, 2006.** 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.

Please save the date! We will host the next Micronutrients Measurement Quality Assurance Workshop in conjunction with the Experimental Biology meeting on **May 2, 2007** at the Convention Center in Washington, DC. We will provide you with more details as our plans our finalized.

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

Sincerely,

Jeanice Brown Thomas

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 LX (RR60) report consists of:

Page	"All Lab" Report
1-4	A listing of all results and statistics for all analytes.
5	A legend for the list of results and statistics.
6	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 RR60.

Serum	Description	Prior Distributions
324	Fresh-frozen, native, single-donor serum prepared in Spring, 2006.	
325	Fresh-frozen, a 5:7 blend of the Sera 324 and 326 (*i.e.*, Serum 325 = (5 × Serum324 + 7 × Serum 326)/12, prepared in Spring, 2006.	
326	Fresh-frozen, native, single-donor serum prepared in Spring, 2006.	
327	Lyophilized, native, single-donor serum prepared in 1993.	Serum 184:RR28-6/93 Serum 319:RR59-3/06
328	Lyophilized, augmented, multi-donor serum prepared in 1994. This material is a 1:1 blend of stripped serum and a serum pool augmented with retinol, retinyl palmitate, and α-tocopherol.	Serum 195:RR31-6/94 Serum 214:RR35-9/95 Serum 244:RR43-6/98

Results

1) Sera Stability. There was no significant change in the median level or variability of any measurand in the two lyophilized sera. Both of these materials have been in storage for more than 12 years.

2) Measurand Additivity. Serum 325 was prepared as a mixture of two native, single-donor sera. With the exception of α- and β-carotene, the median values in Serum 325 are as expected from the Sera 324 and 326 medians and their 5:7 blending ratio. For these two measurands, the medians for Serum 325 are larger than expected. Serum 324 had natively high levels of these two measurands (particularly that of α-carotene) while the levels in Serum 326 were fairly low. We will continue to investigate whether these departures from expected additive behavior are related to measurand lipophilicity. Unfortunately, the retinyl palmitate levels in the Serum 326 material were below many participants' quantification limits; there are insufficient numbers of quantitative values for this measurand in both Sera 325 and326 for meaningful statistical assessment.

Appendix C. "All-Lab Report" for RR60

The following seven 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.

Round Robin LX Laboratory Results

All Results in µg/mL

	Total Retinol					trans-Retinol					Retinyl Palmitate					α-Tocopherol					γ/β-Tocopherol				
Lab	324	325	326	327	328	324	325	326	327	328	324	325	326	327	328	324	325	326	327	328	324	325	326	327	328
FSV-BA	0.427	0.341	0.274	0.337	0.357						0.057	0.055	0.063	0.127	0.081	9.40	8.70	7.62	4.27	5.80	2.49	3.093	3.40	1.57	1.30
FSV-BB	0.474	0.376	0.308	0.378	0.402						0.055	0.027	0.059	0.128	0.085	10.19	9.40	8.58	4.50	6.05	2.52	3.113	3.51	1.59	1.29
FSV-BC	0.458	0.373	0.296	0.358	0.384																				
FSV-BD	0.445	0.379	0.313	0.366	0.360											9.30	9.30	8.00	4.30	7.10					
FSV-BE	0.502	0.410	0.346	0.399	0.418											9.94	8.90	8.36	4.36	6.56	2.62	3.186	3.71	1.66	1.32
FSV-BF	0.460	0.360	0.280	0.360	0.290											10.50	9.40	8.70	4.70	6.10	2.79	3.340	3.68	1.75	1.22
FSV-BG	0.504	0.390	0.307	0.366	0.416						0.060	0.058	0.053	0.098	0.053	10.79	9.59	8.50	4.16	6.76	2.84	3.357	3.66	1.49	1.34
FSV-BH	0.378	0.314	0.233	0.289	0.346											9.31	8.20	7.97	4.21	6.63	2.55	3.141	3.73	1.64	1.37
FSV-BI	0.417	0.345	0.283	0.344	0.361						0.082	*nd*	*nd*	0.160	0.126	10.55	9.51	8.77	4.68	7.25	2.87	3.600	3.97	1.77	1.50
FSV-BJ	0.447	0.353	0.276	0.347	0.385						0.050	0.026	*nq*	0.139	0.129	10.03	9.13	7.96	4.19	7.41	2.45	3.212	3.56	1.53	1.32
FSV-BK	0.540	0.410	0.330	0.410	0.420											11.34	10.03	8.96	4.96	6.74					
FSV-BL	0.460	0.370	0.320	0.340	0.230											9.50	10.80	11.20	6.00	6.50					
FSV-BM	0.468	0.379	0.330	0.369	0.380											10.20	9.20	8.80	4.50	6.50					
FSV-BN	0.430	0.377	0.322	0.388	0.423						0.049	0.032	0.021	0.103	0.126	8.93	8.37	7.97	4.19	6.32	2.24	2.870	3.36	1.47	1.22
FSV-BO	0.400	0.350	0.300	0.320	0.290											7.43	4.04	1.08	3.68	5.54	1.27	1.710	2.21	0.85	0.70
FSV-BP	0.462	0.388	0.323	0.390	0.419											8.67	8.17	7.53	4.10	8.69					
FSV-BQ	0.601	0.496	0.446	0.457	0.475											8.61	7.82	7.74	3.91	6.50					
FSV-BR	*≥0.503*	*≥0.393*	*≥0.310*	*≥0.409*	*≥0.429*	0.503	0.393	0.310	0.409	0.429						9.67	8.81	8.30	4.03	6.53					
FSV-BS	*≥0.530*	*≥0.424*	*≥0.355*	*≥0.422*	*≥0.461*	0.530	0.424	0.355	0.422	0.461						11.03	9.04	8.06	4.43	6.79	2.77	3.089	3.71	1.67	1.41
FSV-BT	0.472	0.384	0.391	0.320	0.357											11.12	9.72	8.23	4.94	7.23	2.88	3.398	3.67	1.72	1.41
FSV-BU	0.468	0.368	0.303	0.367	0.315											9.86	8.59	7.78	4.30	6.73	2.51	2.921	3.41	1.56	1.55
FSV-BV	0.410	0.340	0.315	0.383	0.237											9.18	8.85	8.35	4.33	6.70	2.34	2.970	3.42	1.55	1.21
FSV-BW	0.410	0.310	0.260	0.310	0.330						0.080	0.050	0.020	0.130	0.170	9.59	8.36	7.84	4.01	6.11	4.19	4.930	5.44	2.40	1.93
FSV-CC	0.470	0.390	0.310	0.370	0.410	0.460	0.370	0.310	0.370	0.380						9.65	8.76	8.33	4.06	6.40					
FSV-CD	*≥0.400*	*≥0.350*	*≥0.260*	*≥0.320*	*≥0.300*	0.400	0.350	0.260	0.320	0.300	4.220	7.900	11.110	1.170	0.490	8.32	8.34	7.68	3.74	5.48	2.24	3.040	3.42	1.48	1.00
FSV-CE	0.431	0.371	0.310	0.357	0.386											11.13	9.44	8.47	4.54	6.63					
FSV-CF	0.494	0.388	0.314	0.396	0.453											11.80	10.60	9.60	5.20	8.60					
FSV-CG	0.463	0.363	0.304	0.368	0.400											9.39	8.51	7.65	3.99	6.21	2.75	3.323	3.79	1.73	1.36
FSV-CI	0.413	0.339	0.275	0.309	0.273						0.047	0.019	*nd*	0.148	0.103	9.59	8.51	8.18	4.42	7.02	2.53	3.000	3.43	1.68	1.42
FSV-CP																11.22	10.46	8.95	5.02	7.25	2.97	3.872	4.13	2.08	1.49
FSV-CS	0.460	0.370	0.382	0.396	0.285											10.24	11.52	8.79	7.89	5.85	3.62	3.289	3.59	1.67	2.19
FSV-CT																									
FSV-CW	0.353	0.331	0.273	0.381	0.453						0.033	0.023	0.016	0.092	0.109	10.50	9.51	9.31	4.27	7.01	2.92	3.499	4.10	1.96	1.65
FSV-CZ	0.478	0.380	0.307	0.374	0.395											10.10	9.10	8.40	4.50	7.50					
FSV-DB	0.498	0.372	0.335	0.375	0.412											11.35	9.51	8.93	4.74	7.26	2.81	3.305	4.00	1.83	1.39
FSV-DF	0.500	0.391	0.326	0.397	0.417																				
FSV-DI	0.429	0.360	0.296	0.357	0.380						0.048	0.024	0.010	0.194	0.129	9.48	8.68	8.24	4.12	6.38	2.51	3.110	3.58	1.57	1.29
FSV-DQ																9.85	8.50	5.29	4.24	6.13	2.52	2.920	3.12	1.68	1.30
FSV-DV	*≥0.544*	*≥0.422*	*≥0.359*	*≥0.429*	*≥0.466*	0.544	0.422	0.359	0.429	0.466						10.40	9.10	8.60	4.40	6.60					
N	32	32	32	32	32	5	5	5	5	5	11	10	8	11	11	36	36	36	36	36	24	24	24	24	24
Min	0.353	0.310	0.233	0.289	0.230	0.400	0.350	0.260	0.320	0.300	0.033	0.019	0.010	0.092	0.053	7.43	4.04	1.08	3.68	5.48	1.27	1.710	2.21	0.85	0.70
Median	0.460	0.372	0.309	0.367	0.385	0.503	0.393	0.310	0.409	0.429	0.055	0.030	0.037	0.130	0.126	9.90	9.07	8.32	4.32	6.61	2.59	3.164	3.62	1.66	1.35
Max	0.601	0.496	0.446	0.457	0.475	0.544	0.424	0.359	0.429	0.466	4.220	7.900	11.110	1.170	0.490	11.80	11.52	11.20	7.89	8.69	4.19	4.930	5.44	2.40	2.19
eSD	0.035	0.024	0.023	0.028	0.055						0.016	0.022	0.030	0.029	0.026	0.83	0.74	0.58	0.32	0.56	0.25	0.233	0.24	0.13	0.11
eCV	8	7	7	8	14	0	0	0	0	0	29	74	82	22	21	8	8	7	7	8	10	7	7	8	8
N_{past}	0	0	0	39	44	0	0	0	0	0	0	0	0	9	10	0	0	0	40	44	0	0	0	19	20
$Median_{past}$				0.365	0.380									0.124	0.111				4.333	7.182				1.630	1.320
SD_{past}				0.029	0.037									0.029	0.025				0.277	0.742				0.148	0.127
NAV	0.460	0.372	0.309	0.367	0.385	0.503	0.393	0.310	0.409	0.429	0.055	0.030	0.037	0.130	0.126	9.900	9.071	8.315	4.315	6.614	2.585	3.164	3.624	1.662	1.347
NAU	0.037	0.030	0.026	0.030	0.055	0.040	0.032	0.026	0.033	0.035	0.017	0.022	0.030	0.032	0.032	0.829	0.742	0.666	0.449	0.563	0.256	0.300	0.335	0.180	0.153

C2

Round Robin LX Laboratory Results

All Results in µg/mL

	δ-Tocopherol					Total β-Carotene					trans-β-Carotene					Total cis-β-Carotene					Total α-Carotene				
Lab	324	325	326	327	328	324	325	326	327	328	324	325	326	327	328	324	325	326	327	328	324	325	326	327	328
FSV-BA	0.127	0.096	0.076	0.044	0.077	0.501	0.307	0.160	0.320	0.327	0.463	0.282	0.143	0.293	0.301	0.038	0.026	0.017	0.027	0.026	0.277	0.132	0.024	0.028	0.020
FSV-BB	0.115	0.092	0.074	0.035	0.071	0.427	0.236	0.112	0.304	0.280	0.403	0.225	0.106	0.284	0.258	0.025	0.011	0.005	0.020	0.022	0.230	0.098	0.011	0.014	0.014
FSV-BC																									
FSV-BD																									
FSV-BE						0.494	0.228	0.131	0.284	0.319															
FSV-BF						0.483	0.288	0.136	0.334	0.469											0.340	0.147	0.017	0.014	0.021
FSV-BG						0.512	0.312	0.148	0.352	0.331											0.241	0.105	0.011	0.014	0.010
FSV-BH	0.141	0.096	0.076	0.081	0.070	0.533	0.296	0.144	0.328	0.336	0.506	0.282	0.144	0.308	0.312	0.027	0.014	*nq*	0.020	0.024	0.273	0.116	*nq*	*nq*	*nq*
FSV-BI						0.497	0.296	0.140	0.298	0.334											0.292	0.129	0.016	0.013	0.012
FSV-BJ						0.506	0.296	0.135	0.312	0.397											0.330	0.140	*nq*	*nq*	0.016
FSV-BK																									
FSV-BL																									
FSV-BM																									
FSV-BN	*nd*	*nd*	*nd*	*nd*	*nd*	0.402	0.249	0.131	0.270	0.294	0.370	0.231	0.123	0.248	0.261	0.038	0.025	0.015	0.028	0.039	0.246	0.119	0.021	0.018	0.025
FSV-BO						0.536	0.161	0.597	!0.115	0.121											0.263	0.124	0.017	!0.2736	0.023
FSV-BP						0.545	0.532	0.093	0.155	0.609											0.098	0.050	0.032	0.022	0.014
FSV-BQ																									
FSV-BR																									
FSV-BS						0.501	0.270	0.162	0.343	0.315											0.343	0.146	0.020	0.019	0.020
FSV-BT						0.506	0.296	0.149	0.337	0.388	0.478	0.281	0.141	0.316	0.360	0.031	0.017	0.009	0.021	0.029	0.273	0.128	0.025	0.019	0.019
FSV-BU						0.481	0.287	0.157	0.306	0.304											0.292	0.129	0.020	0.017	0.026
FSV-BV						0.477	0.290	0.148	0.321	0.355											0.302	0.135	0.017	0.014	0.018
FSV-BW						0.590	0.350	0.190	0.420	0.420											0.590	0.230	0.020	0.020	0.020
FSV-CC																									
FSV-CD						*nq*	0.610	0.340	0.690	0.430											*nq*	0.150	*nq*	*nq*	0.010
FSV-CE						0.548	0.307	0.160	0.307	0.289															
FSV-CF																									
FSV-CG	0.135	*nd*	*nd*	*nd*	*nd*	0.484	0.277	0.135	0.297	0.319	0.456	0.261	0.128	0.279	0.293	0.028	0.016	0.007	0.018	0.025	0.343	0.148	0.011	0.015	0.021
FSV-CI						*≥0.497*	*≥0.246*	*≥0.123*	*≥0.296*	*≥0.303*	0.497	0.246	0.123	0.296	0.303						0.234	0.102	0.014	0.012	0.014
FSV-CP						0.484	0.289	0.146	0.288	0.311											0.298	0.133	0.017	0.014	0.020
FSV-CS						0.524	0.279	0.131	0.347	0.374											0.309	0.122	0.011	0.016	0.020
FSV-CT						0.560	0.332	0.172	0.369	0.401															
FSV-CW	0.158	0.112	0.097	0.050	0.081	*≥0.424*	*≥0.282*	*≥0.122*	*≥0.361*	*≥0.411*	0.424	0.282	0.122	0.361	0.411						0.256	0.137	0.016	0.023	0.032
FSV-CZ						0.532	0.327	0.152	0.281	0.312															
FSV-DB	0.230	0.170	*nq*	*nq*	0.140	0.538	0.293	0.136	0.307	0.345															
FSV-DF																									
FSV-DI	0.110	0.090	0.076	0.046	0.064	0.488	0.307	0.189	0.306	0.329															
FSV-DQ						0.381	0.310	0.131	0.297	0.294											0.256	0.120	0.015	0.019	0.020
FSV-DV																									
N	7	6	5	5	6	25	26	26	25	26	8	8	8	8	8	6	6	5	6	6	21	22	19	18	21
Min	0.110	0.090	0.074	0.035	0.064	0.381	0.161	0.093	0.155	0.121	0.370	0.225	0.106	0.248	0.258	0.025	0.011	0.005	0.018	0.022	0.098	0.050	0.011	0.012	0.010
Median	0.135	0.096	0.076	0.046	0.074	0.501	0.296	0.147	0.307	0.330	0.460	0.271	0.126	0.295	0.302	0.030	0.017	0.009	0.021	0.026	0.277	0.129	0.017	0.017	0.020
Max	0.230	0.170	0.097	0.081	0.140	0.590	0.610	0.597	0.690	0.609	0.506	0.282	0.144	0.361	0.411	0.038	0.026	0.017	0.028	0.039	0.590	0.230	0.032	0.028	0.032
eSD	0.021	0.011	0.004	0.004	0.007	0.036	0.021	0.019	0.030	0.054	0.048	0.029	0.014	0.020	0.029	0.007	0.006	0.006	0.004	0.003	0.039	0.015	0.004	0.004	0.005
eCV	16	12	5	10	10	7	7	13	10	17	10	11	11	7	10	23	38	66	20	12	14	12	25	22	26
N_{past}	0	0	0	6	0	0	0	0	29	32	0	0	0	7	10	0	0	0	5	8	0	0	0	17	25
$Median_{past}$				0.049					0.338	0.345				0.306	0.326				0.021	0.027				0.016	0.018
SD_{past}				0.009					0.047	0.038				0.028	0.028				0.008	0.013				0.006	0.006
NAV	0.135	0.096	0.076	0.046	0.074	0.501	0.296	0.147	0.307	0.330	0.460	0.271	0.126	0.295	0.302	0.030	0.017	0.009	0.021	0.026	0.277	0.129	0.017	0.017	0.020
NAU	0.028	0.023	0.021	0.017	0.020	0.070	0.043	0.023	0.045	0.054	0.048	0.029	0.015	0.032	0.032	0.011	0.006	0.006	0.007	0.009	0.071	0.036	0.006	0.006	0.007

C3

Round Robin LX Laboratory Results

All Results in µg/mL

	Total Lycopene					trans-Lycopene					Total β-Cryptoxanthin					Total α-Cryptoxanthin					Total Lutein				
Lab	324	325	326	327	328	324	325	326	327	328	324	325	326	327	328	324	325	326	327	328	324	325	326	327	328
FSV-BA	0.340	0.477	0.562	0.186	0.077	0.168	0.254	0.306	0.105	0.034	0.080	0.123	0.142	0.073	0.012	0.033	0.050	0.058	0.038	0.006					
FSV-BB	0.308	0.405	0.456	0.191	0.091	0.139	0.191	0.226	0.089	0.024	0.059	0.085	0.103	0.056	0.010	0.023	0.030	0.036	0.028	0.003	0.128	0.137	0.154	0.096	0.037
FSV-BC																									
FSV-BD																									
FSV-BE																									
FSV-BF	0.366	0.544	0.659	0.185	0.086						0.057	0.095	0.120	0.055	0.008										
FSV-BG	0.362	0.519	0.602	0.206	0.081	0.185	0.282	0.335	0.116	0.034	0.050	0.074	0.092	0.051	0.009						0.180	0.137	0.096	0.078	0.032
FSV-BH	0.399	0.533	0.659	0.224	0.081						0.074	0.102	0.126	0.061	*nq*						0.105	0.108	0.111	0.068	0.018
FSV-BI	0.286	0.401	0.569	0.157	0.058						0.056	0.092	0.112	0.056	0.010										
FSV-BJ	0.400	0.544	0.589	0.210	0.106						0.048	0.075	0.087	0.045	*nq*						0.111	0.136	0.137	0.058	0.033
FSV-BK																									
FSV-BL																									
FSV-BM																									
FSV-BN	0.288	0.402	0.516	0.169	0.073	0.138	0.211	0.271	0.090	0.034	0.054	0.086	0.114	0.058	0.013	0.017	0.029	0.040	0.025	0.006	0.106	0.117	0.127	0.071	0.030
FSV-BO	0.346	0.529	0.693	!0.0123	0.102						0.047	0.074	0.103	!0.2165	0.010						0.127	0.134	0.153	!0.078	0.027
FSV-BP	0.215	0.408	0.585	0.154	0.137						0.057	0.088	0.106	0.054	0.030										
FSV-BQ																									
FSV-BR																									
FSV-BS	0.375	0.454	0.659	0.200	0.072						0.060	0.093	0.137	0.053	0.003										
FSV-BT	0.431	0.578	0.685	0.237	0.125	0.188	0.258	0.307	0.108	0.047	0.062	0.088	0.116	0.061	0.013	0.022	0.031	0.040	0.022	0.008	0.101	0.137	0.197	0.069	0.025
FSV-BU	0.351	0.480	0.585	0.187	0.083						0.053	0.074	0.088	0.051	0.028										
FSV-BV	0.358	0.500	0.580	0.190	0.075						0.040	0.063	0.080	0.038	0.005										
FSV-BW	0.340	0.490	0.610	0.190	0.070						0.080	0.125	0.177	0.077	0.006										
FSV-CC																									
FSV-CD	*nq*	0.080	*nq*	0.030	0.010						*nq*	0.210	0.340	0.120	*nq*						*nq*	1.100	1.620	0.480	0.120
FSV-CE																									
FSV-CF																									
FSV-CG	0.366	0.483	0.579	0.192	0.074	0.171	0.249	0.310	0.105	0.033	0.080	0.113	0.141	0.070	0.013										
FSV-CI																					0.115	0.123	0.132	0.077	0.023
FSV-CP	0.211	0.309	0.377	0.117	0.040						0.071	0.116	0.146	0.075	0.011										
FSV-CS	0.340	0.449	0.505	0.183	0.088						0.074	0.089	0.090	0.054	0.008						0.116	0.133	0.137	0.083	0.032
FSV-CT	0.324	0.485	0.602	0.213	0.085						0.051	0.079	0.098	0.050	0.011						0.167	0.208	0.224	0.166	0.038
FSV-CW						0.087	0.173	0.213	0.070	0.007	0.048	0.076	0.090	0.053	0.017						0.111	0.133	0.138	0.091	0.038
FSV-CZ																									
FSV-DB	0.361	0.494	0.585	0.199	0.079						0.072	0.093	0.114	0.063	*nq*										
FSV-DF																									
FSV-DI	0.377	0.504	0.590	0.202	0.061																0.132	0.153	0.170	0.085	0.024
FSV-DQ	0.349	0.628	0.697	0.136	0.002						0.045	0.079	0.101	0.056	*nd*						0.181	0.186	0.225	0.124	0.016
FSV-DV																									
N	22	23	22	22	23	7	7	7	7	7	22	23	23	22	18	4	4	4	4	4	13	14	14	13	14
Min	0.211	0.080	0.377	0.030	0.002	0.087	0.173	0.213	0.070	0.007	0.040	0.063	0.080	0.038	0.003	0.017	0.029	0.036	0.022	0.003	0.101	0.108	0.096	0.058	0.016
Median	0.350	0.485	0.587	0.190	0.079	0.168	0.249	0.306	0.105	0.034	0.057	0.088	0.112	0.056	0.011	0.023	0.031	0.040	0.027	0.006	0.116	0.137	0.145	0.083	0.031
Max	0.431	0.628	0.697	0.237	0.137	0.188	0.282	0.335	0.116	0.047	0.080	0.210	0.340	0.120	0.030	0.033	0.050	0.058	0.038	0.008	0.181	1.100	1.620	0.480	0.120
eSD	0.028	0.071	0.056	0.021	0.012	0.029	0.041	0.045	0.013	0.004	0.016	0.015	0.027	0.007	0.004	0.004	0.004	0.004	0.005	0.001	0.016	0.012	0.042	0.019	0.009
eCV	8	15	9	11	15	17	16	15	12	12	28	18	24	13	34	16	15	10	18	15	13	9	29	22	28
N_{past}	0	0	0	19	24	0	0	0	7	8	0	0	0	16	17	0	0	0	4	7	0	0	0	11	11
$Median_{past}$				0.185	0.068				0.094	0.041				0.060	0.012				0.025	0.007				0.076	0.026
SD_{past}				0.035	0.023				0.013	0.009				0.009	0.005				0.006	0.002				0.017	0.004
NAV	0.350	0.485	0.587	0.190	0.079	0.168	0.249	0.306	0.105	0.034	0.057	0.088	0.112	0.056	0.011	0.023	0.031	0.040	0.027	0.006	0.116	0.137	0.145	0.083	0.031
NAU	0.077	0.101	0.118	0.047	0.023	0.030	0.045	0.056	0.018	0.008	0.016	0.020	0.027	0.013	0.004						0.022	0.025	0.042	0.019	0.009

C4

Round Robin LX Laboratory Results

All Results in µg/mL

	Total Zeaxanthin					Total Lutein&Zeaxanthin					Coenzyme Q10					Ubiquinol					Ubiquinone				
Lab	324	325	326	327	328	324	325	326	327	328	324	325	326	327	328	324	325	326	327	328	324	325	326	327	328
FSV-BA						0.137	0.173	0.194	0.092	0.034															
FSV-BB	0.025	0.057	0.075	0.025	0.015	0.152	0.193	0.229	0.121	0.052															
FSV-BC																									
FSV-BD																									
FSV-BE																									
FSV-BF						0.153	0.182	0.197	0.098	0.034															
FSV-BG	0.044	0.033	0.027	0.024	0.009	0.198	0.152	0.106	0.087	0.034															
FSV-BH	0.012	0.050	0.077	0.024	0.007	0.117	0.158	0.188	0.092	0.025															
FSV-BI						0.180	0.221	0.249	0.112	0.037															
FSV-BJ											0.788	1.115	1.292	0.750	0.340										
FSV-BK																									
FSV-BL																									
FSV-BM																									
FSV-BN	0.007	0.054	0.085	0.019	0.004	*0.113*	*0.171*	*0.212*	*0.090*	*0.034*															
FSV-BO	0.011	0.034	0.054	!0.017	*nd*	0.138	0.169	0.206	!0.0951	0.027															
FSV-BP						0.061	0.093	0.107	0.032	0.040															
FSV-BQ																									
FSV-BR																									
FSV-BS						0.018	0.134	0.171	0.069	0.025															
FSV-BT	0.036	0.045	0.067	0.020	0.011	0.143	0.189	0.275	0.092	0.037															
FSV-BU						0.146	0.188	0.210	0.090	0.039															
FSV-BV						0.165	0.206	0.250	0.109	0.035															
FSV-BW						0.170	0.220	0.290	0.132	0.038	0.590	0.850	1.190	0.890	0.350	0.140	0.150	0.830	0.780	0.200	0.450	0.700	0.360	0.110	0.150
FSV-CC																									
FSV-CD																									
FSV-CE																									
FSV-CF																									
FSV-CG						0.192	0.242	0.283	0.120	0.043															
FSV-CI	0.018	0.041	0.057	0.019	<0.006	*0.133*	*0.164*	*0.189*	*0.096*	*>0.023*															
FSV-CP						0.237	0.298	0.333	0.149	0.050															
FSV-CS	0.014	0.042	0.055	0.027	0.006	*0.130*	*0.174*	*0.192*	*0.111*	*0.038*															
FSV-CT	0.009	0.029	0.047	0.016	0.007	0.176	0.237	0.271	0.182	0.045															
FSV-CW	0.022	0.037	0.049	0.021	0.016	*0.133*	*0.170*	*0.187*	*0.112*	*0.054*	0.779	0.913	1.007	0.653	0.403										
FSV-CZ											0.770	1.020	1.170	0.740	0.360										
FSV-DB						0.149	0.188	0.233	0.092	0.031															
FSV-DF																									
FSV-DI											0.684	0.986	1.210	0.702	0.299	0.051	0.522	1.042	0.658	0.192	0.561	0.381	0.115	0.058	0.069
FSV-DQ	0.029	0.058	0.080	0.022	0.009	*0.210*	*0.244*	*0.305*	*0.146*	*0.025*															
FSV-DV																									
N	11	11	11	10	9	22	22	22	21	21	5	5	5	5	5	2	2	2	2	2	2	2	2	2	2
Min	0.007	0.029	0.027	0.016	0.004	0.018	0.093	0.106	0.032	0.025	0.590	0.850	1.007	0.653	0.299	0.051	0.150	0.830	0.658	0.192	0.450	0.381	0.115	0.058	0.069
Median	0.018	0.042	0.057	0.022	0.009	0.148	0.185	0.211	0.098	0.037	0.770	0.986	1.190	0.740	0.350	0.096	0.336	0.936	0.719	0.196	0.506	0.541	0.238	0.084	0.110
Max	0.044	0.058	0.085	0.027	0.016	0.237	0.298	0.333	0.182	0.054	0.788	1.115	1.292	0.890	0.403	0.140	0.522	1.042	0.780	0.200	0.561	0.700	0.360	0.110	0.150
eSD	0.012	0.012	0.018	0.004	0.003	0.031	0.035	0.056	0.021	0.004	0.070	0.079	0.060	0.037	0.018										
eCV	65	29	32	16	33	21	19	27	22	12	9	8	5	5	5										
N_{past}	0	0	0	15	8	0	0	0	15	18	0	0	0	5	0	0	0	0	0	0	0	0	0	0	0
$Median_{past}$				0.023	0.011				0.110	0.040				0.694											
SD_{past}				0.005	0.006				0.016	0.009				0.084											
NAV	0.018	0.042	0.057	0.022	0.009	0.148	0.185	0.211	0.098	0.037	0.770	0.986	1.190	0.740	0.350										
NAU	0.012	0.012	0.018	0.006	0.004	0.031	0.039	0.056	0.021	0.008	0.077	0.099	0.119	0.074	0.035										

C5

Round Robin LX Laboratory Results

All Results in µg/mL

	Phylloquinone (K1) x1000					25-hydroxyvitamin D				
Lab	324	325	326	327	328	324	325	326	327	328
FSV-BA						0.0125	0.0093	0.0067	0.0048	0.0120
FSV-BB										
FSV-BC										
FSV-BD										
FSV-BE	2.608	1.466	0.593	0.212	0.656					
FSV-BF										
FSV-BG										
FSV-BH						0.0180	0.0120	0.0080	0.0050	0.0120
FSV-BI										
FSV-BJ										
FSV-BK										
FSV-BL										
FSV-BM										
FSV-BN						0.0154	0.0095	0.0077	0.0025	0.0221
FSV-BO										
FSV-BP										
FSV-BQ										
FSV-BR										
FSV-BS										
FSV-BT										
FSV-BU										
FSV-BV										
FSV-BW										
FSV-CC										
FSV-CD										
FSV-CE										
FSV-CF										
FSV-CG										
FSV-CI	1.890	1.110	0.520	0.230	0.510					
FSV-CP										
FSV-CS										
FSV-CT										
FSV-CW										
FSV-CZ										
FSV-DB										
FSV-DF										
FSV-DI	1.280	0.800	0.170	<0.05	0.410					
FSV-DQ										
FSV-DV										
N	3	3	3	2	3	3	3	3	3	3
Min	1.28	0.80	0.17	0.21	0.41	0.0125	0.0093	0.0067	0.0025	0.0120
Median	1.89	1.11	0.52	0.22	0.51	0.0154	0.0095	0.0077	0.0048	0.0120
Max	2.61	1.47	0.59	0.23	0.66	0.0180	0.0120	0.0080	0.0050	0.0221
eSD										
eCV										
N_{past}	0	0	0	0	0	0	0	0	4	0
$Median_{past}$									0.0042	
SD_{past}									0.0004	
NAV	1.890	1.110	0.520		0.510	0.0154	0.0095	0.0077	0.0048	0.0120
NAU										

C6

Round Robin LX Laboratory Results

Legend

Term	Definition
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
eSD	Standard deviation for (non-NIST) results: 0.741*(3rd Quartile - 1st Quartile)
eCV	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 NAV is the $Median_{part}$ for analytes reported by ≥ 5 labs.
NAU	NIST Assigned Uncertainty NAU is the maximum of (0.05*NAV, SD, eSD) where eSD is the expected long-term SD and is defined in: Duewer et al. Anal Chem 1997;69(7):1406-1413.
nd	Not detected (i.e., no detectable peak for analyte)
nq	Detected but not quantitatively determined
≥x	Concentration greater than or equal to x
!	Non-quantitative value: heterogeneous serum, damaged sample, malfunction, etc.
italics	Not explictly reported but calculated by NIST from reported values

Round Robin LX 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	2	1	2			1
FSV-BB	1	1	1	2	2	1	1	1	1	2	2
FSV-BC	1										
FSV-BD	1	1									
FSV-BE	2	1	1	1							
FSV-BF	1	1	1	2		1	1	1			1
FSV-BG	1	1	1	1		1	1	1	2	2	2
FSV-BH	3	1	1	1	1	1	1	1	2	1	1
FSV-BI	1	1	2	1		1	1	1			1
FSV-BJ	1	1	1	1		1	1	1	1		
FSV-BK	2	2									
FSV-BL	2	3									
FSV-BM	1	1									
FSV-BN	1	1	2	1	2	1	1	1	1	2	1
FSV-BO	2	4	4	4		1	1	1	1	1	1
FSV-BP	1	3		4		3	2	3			3
FSV-BQ	4	2									
FSV-BR	1	1									
FSV-BS	2	1	1	1		1	1	2			3
FSV-BT	2	2	1	1	2	1	2	1	1	1	1
FSV-BU	1	1	1	1		1	1	3			1
FSV-BV	2	1	1	1		1	1	2			1
FSV-BW	2	1	4	2		3	1	2			2
FSV-CC	1	1									
FSV-CD	2	2	2	4		2	4	4	4		
FSV-CE	1	1		1							
FSV-CF	1	3									
FSV-CG	1	1	1	1	1	1	1	2			2
FSV-CI	2	1	1	1	1	1			1	1	1
FSV-CP		2	2	1		1	2	2			3
FSV-CS	2	4	4	1		1	1	1	1	1	1
FSV-CT				2			1	1	3	1	3
FSV-CW	2	1	2	2	2	1		2	1	2	2
FSV-CZ	1	1		1							
FSV-DB	1	2	1	1			1	1			1
FSV-DF	1										
FSV-DI	1	1	1	1			1		1		
FSV-DQ		3	1	1		1	2	1	3	1	2
FSV-DV	2	1									
n	36	36	24	28	8	22	23	23	14	11	22

	TR	aT	g/bT	bC	tbC	aC	TLy	TbX	TLu	TZ	L&Z
% 1	56	67	67	71	50	82	78	57	64	64	55
% 2	39	17	21	18	50	9	17	30	14	36	27
% 3	3	11	0	0	0	9	0	9	14	0	18
% 4	3	6	13	11	0	0	4	4	7	0	0

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

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

"Comparability Score"

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

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

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

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

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 RR60

Each participant in RR60 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 RR60:

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

The following 14 pages are the "Individualized Report" for the analytes evaluated by participant FSV-BA.

Individualized Round Robin LX Report: FSV-BA

Summary

	Serum 324			Serum 325			Serum 326			Serum 327			Serum 328		
Analyte	You	NAV	n	You	NAV	n	You	NAV	n	You	NAV	n	You	NAV	n
Total Retinol	0.427	0.460	32	0.341	0.372	32	0.274	0.309	32	0.337	0.367	32	0.357	0.385	32
Retinyl Palmitate	0.06	0.06	11	0.1	0.0	10	0.1	0.0	8	0.13	0.13	11	0.08	0.13	11
α-Tocopherol	9.40	9.90	36	8.70	9.07	36	7.62	8.32	36	4.27	4.32	36	5.80	6.61	36
γ/β-Tocopherol	2.493	2.585	24	3.093	3.164	24	3.401	3.624	24	1.570	1.662	24	1.299	1.347	24
δ-Tocopherol	0.127	0.135	7	0.096	0.096	6	0.076	0.076	5	0.044	0.046	5	0.077	0.074	6
Total β-Carotene	0.501	0.501	25	0.307	0.296	26	0.160	0.147	26	0.320	0.307	25	0.327	0.330	26
trans-β-Carotene	0.463	0.460	8	0.282	0.271	8	0.143	0.126	8	0.293	0.295	8	0.301	0.302	8
Total cis-β-Carotene	0.038	0.030	6	0.026	0.017	6	0.017	0.009	5	0.027	0.021	6	0.026	0.026	6
Total α-Carotene	0.277	0.277	21	0.132	0.129	22	0.024	0.017	19	0.028	0.017	18	0.020	0.020	21
Total Lycopene	0.340	0.350	22	0.477	0.485	23	0.562	0.587	22	0.186	0.190	22	0.077	0.079	23
trans-Lycopene	0.168	0.168	7	0.254	0.249	7	0.306	0.306	7	0.105	0.105	7	0.034	0.034	7
Total β-Cryptoxanthin	0.080	0.057	22	0.123	0.088	23	0.142	0.112	23	0.073	0.056	22	0.012	0.011	18
Total α-Cryptoxanthin	0.033	0.023	4	0.050	0.031	4	0.058	0.040	4	0.038	0.027	4	0.006	0.006	4
Total Lutein&Zeaxanthin	0.137	0.148	22	0.173	0.185	22	0.194	0.211	22	0.092	0.098	21	0.034	0.037	21
25-hydroxyvitamin D	0.013	0.015	3	0.009	0.010	3	0.007	0.008	3	0.005	0.005	3	0.012	0.012	3

D2

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

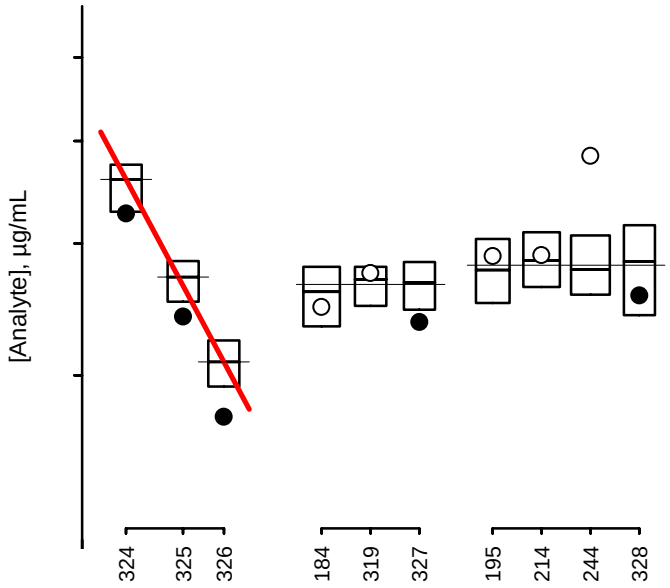
Please check our records against your records. Send corrections and/or updates to...

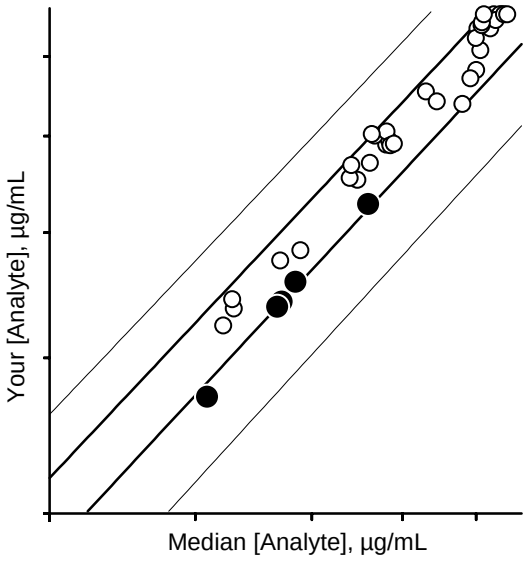
Micronutrients Measurement Quality Assurance Program
National Institute of Standards and Technology
100 Bureau Drive Stop 8392
Gaithersburg, MD 20899-8392 USA

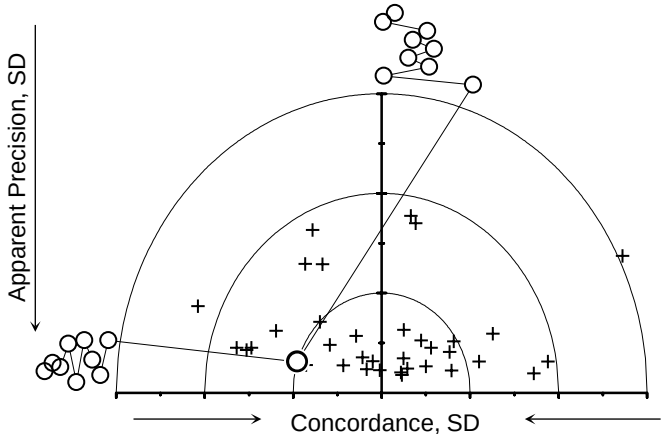
Tel: (301) 975-3935
Fax: (301) 977-0685
Email: david.duewer@nist.gov

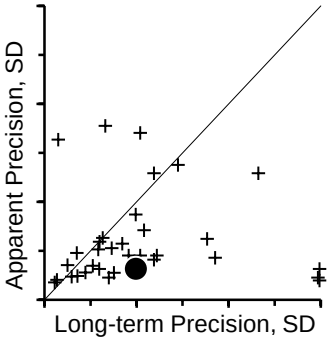
Individualized RR LX Report: FSV-BA

Total Retinol





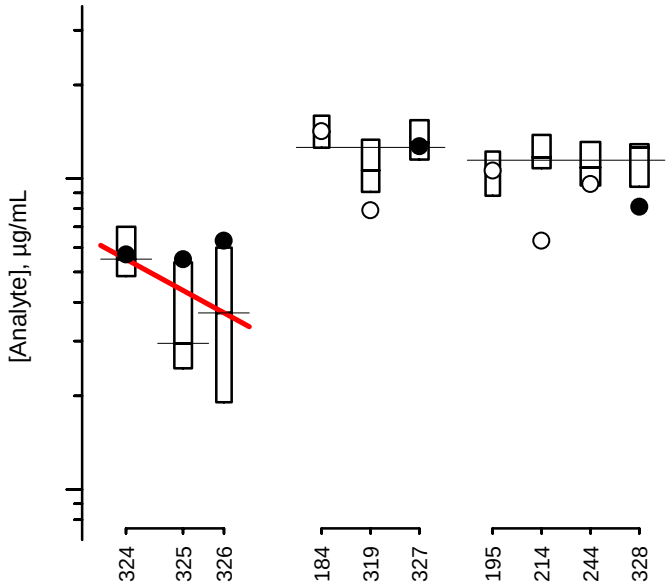


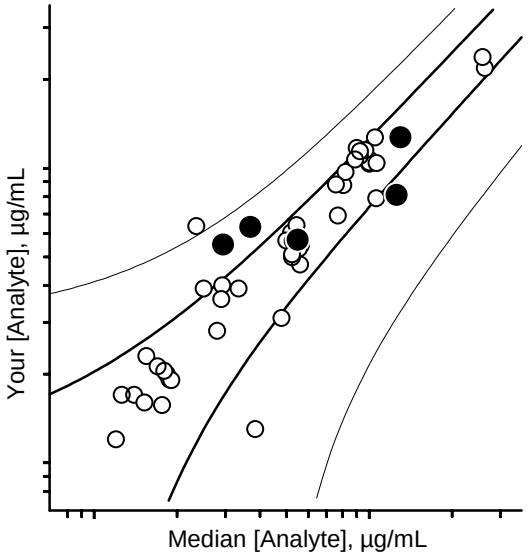


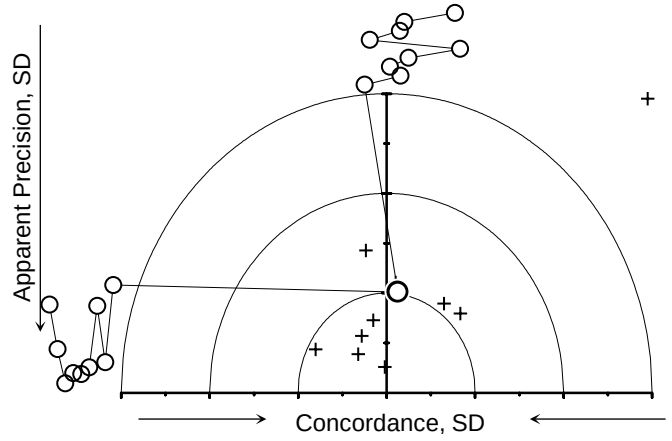
Serum	History	Comments
#324	Fresh frozen	Native, single-source
#325	Fresh frozen	25:35 blend of #324 and #326
#326	Fresh frozen	Native, single-source
#327	Lyophilized - 28:184, 59:319	Native, single-source
#328	Lyophilized - 31:195, 35:214, 43:244	Manipulated, multi-source

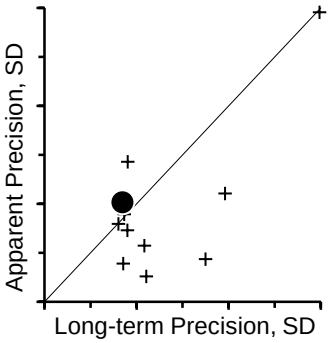
Individualized RR LX Report: FSV-BA

Retinyl Palmitate









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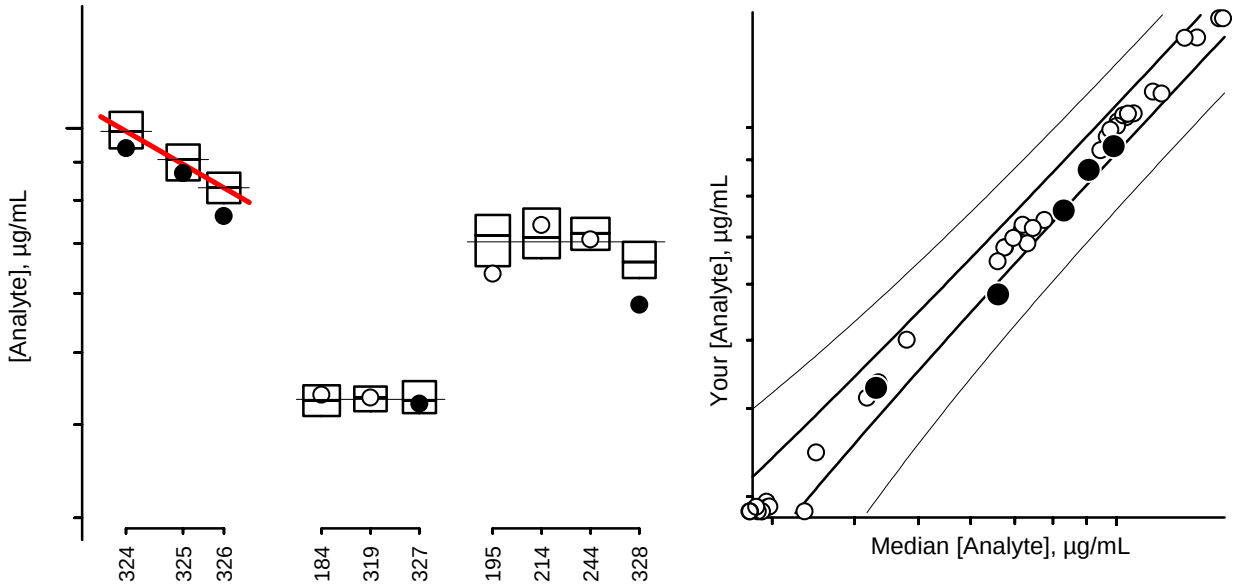
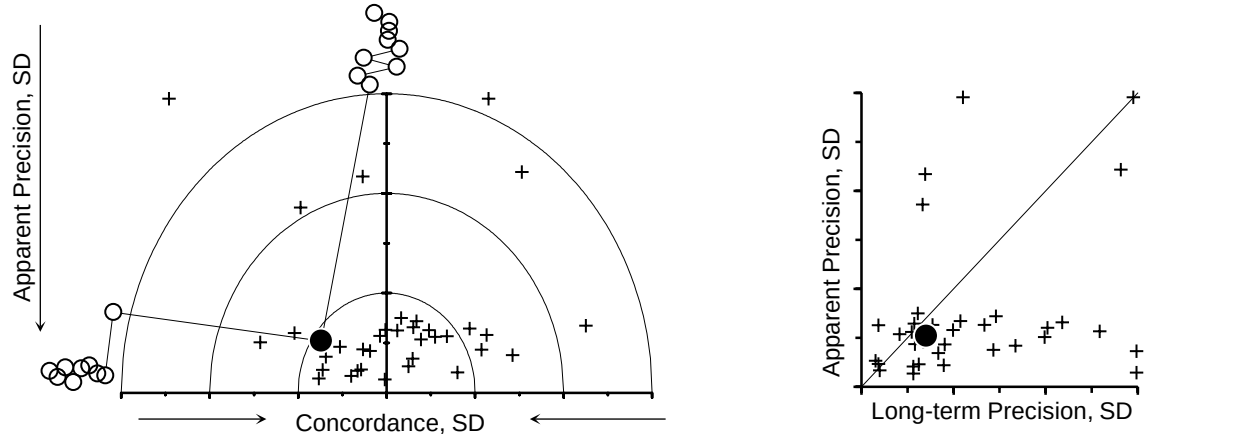
Individualized RR LX Report: FSV-BA

α-Tocopherol





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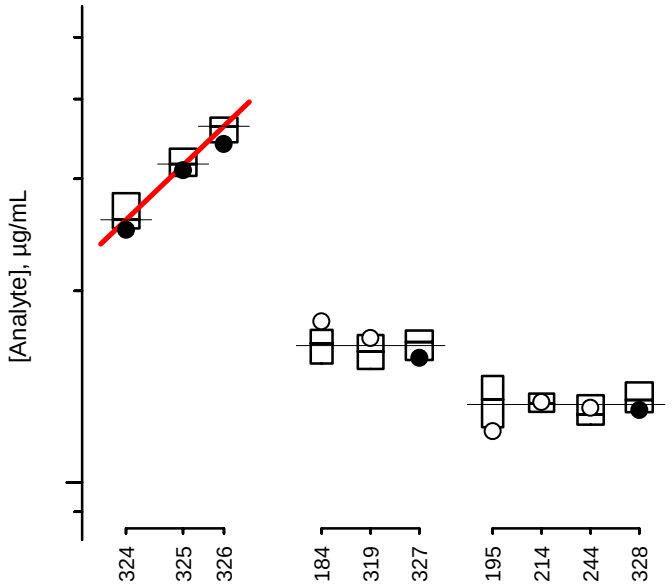
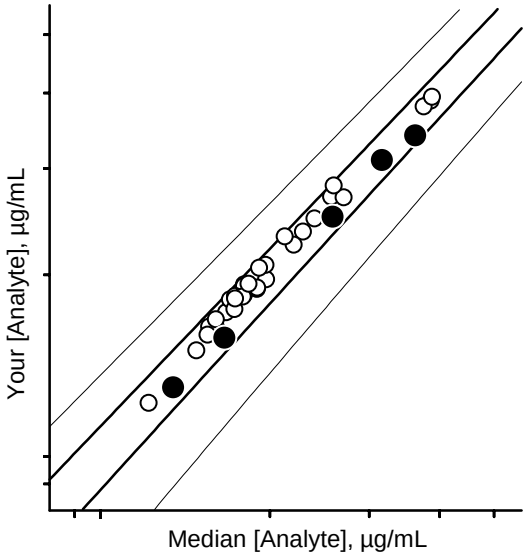
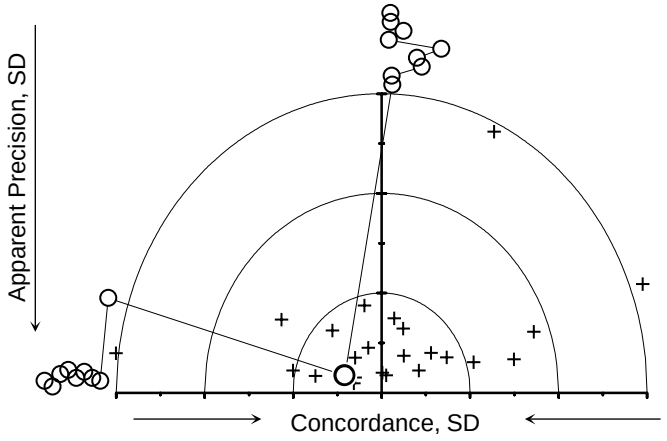
#327	Lyophilized - 28:184, 59:319	Native, single-source
#328	Lyophilized - 31:195, 35:214, 43:244	Manipulated, multi-source

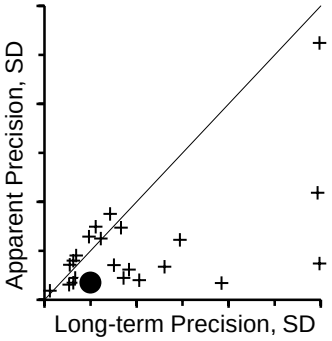
Individualized RR LX Report: FSV-BA

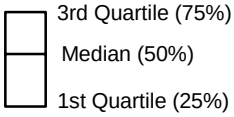
γ/β-Tocopherol











● You, this RR
▲ You, ≥x, this RR
○ You, past RRs
△ You, ≥x, past RRs
\+ Others, this RR

Serum	History	Comments
#324	Fresh frozen	Native, single-source
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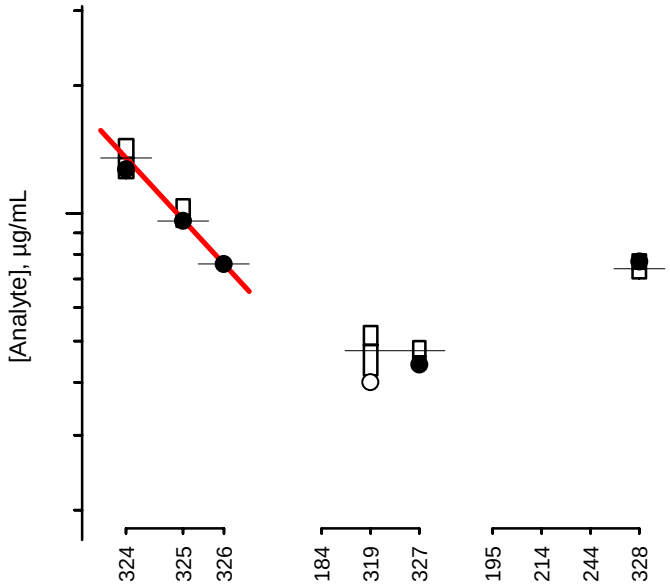
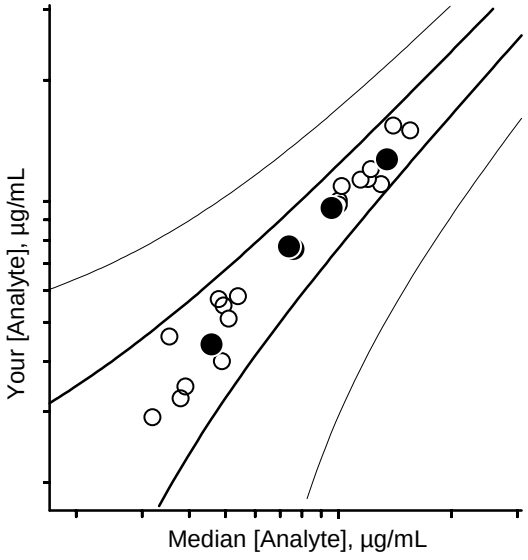
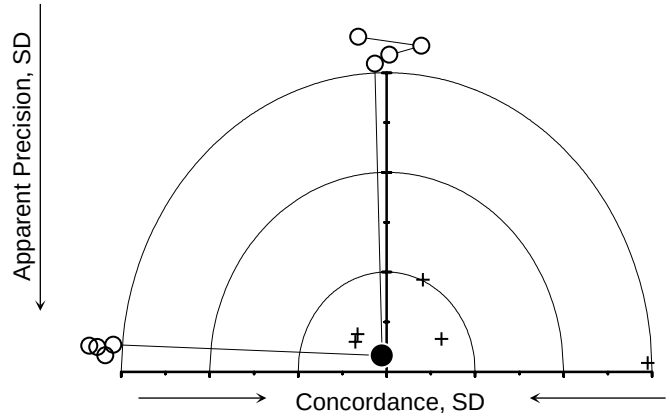
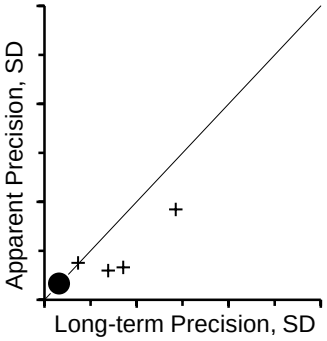
Individualized RR LX Report: FSV-BA

δ-Tocopherol









3rd Quartile (75%)
Median (50%)
1st Quartile (25%)

● You, this RR
○ You, past RRs
▲ You, ≥x, this RR
△ You, ≥x, past RRs
\+ Others, this RR

Serum	History	Comments
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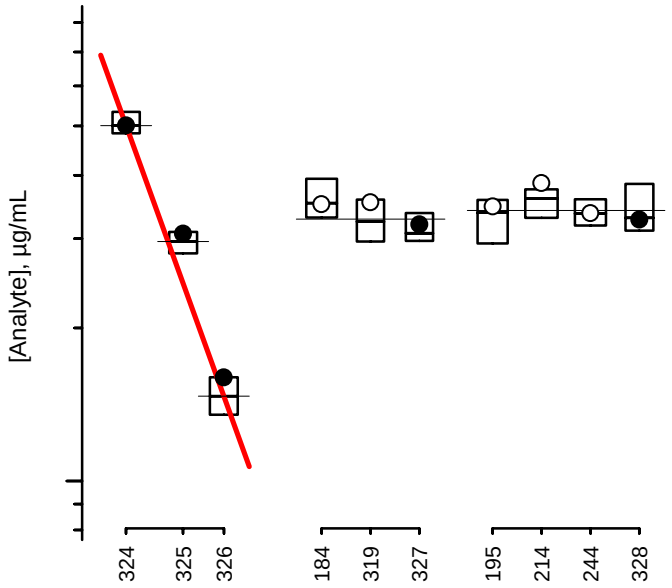
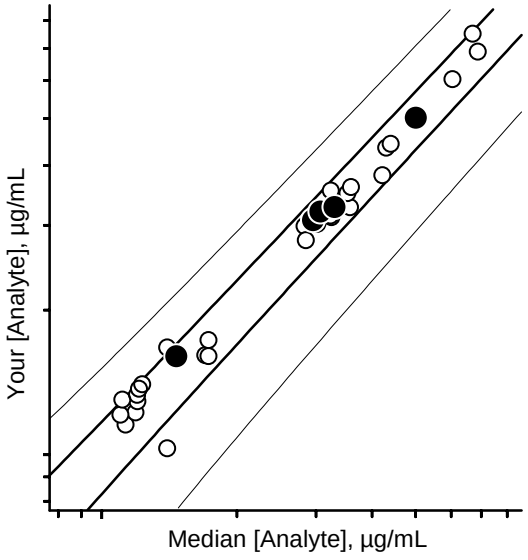
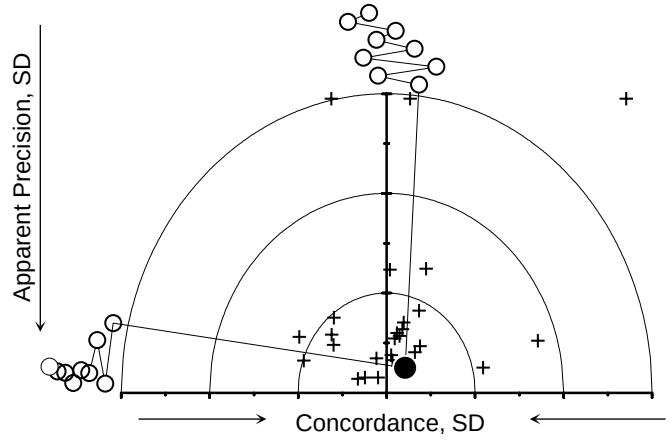
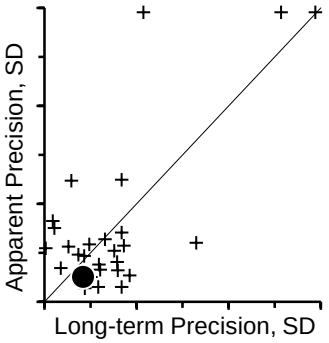
Individualized RR LX Report: FSV-BA

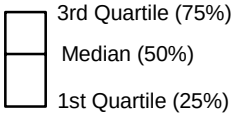
Total β-Carotene











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\+ Others, this RR

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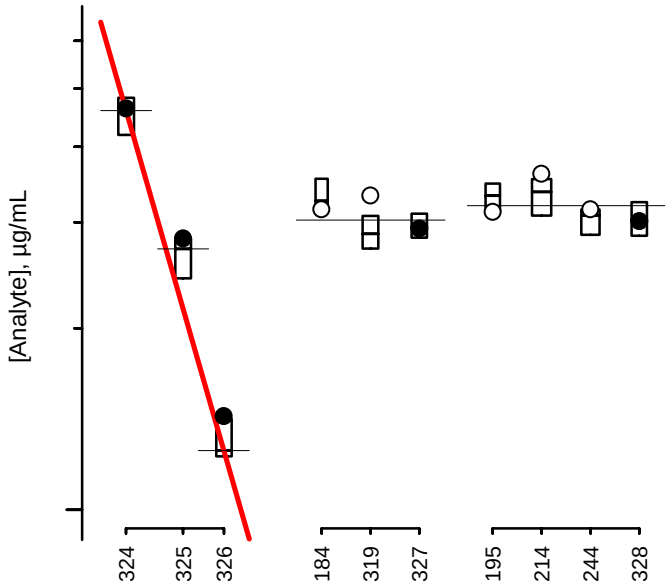
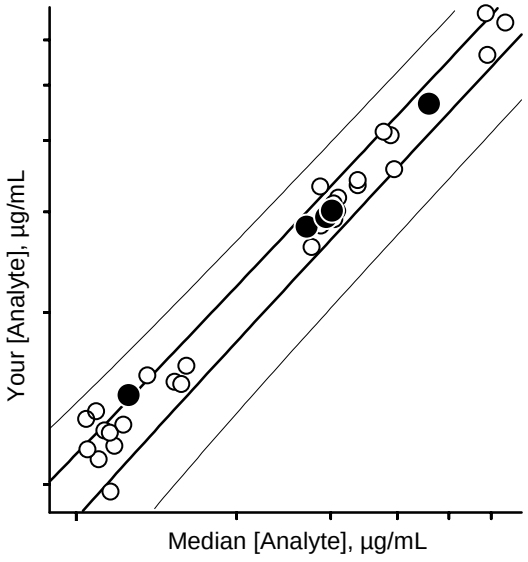
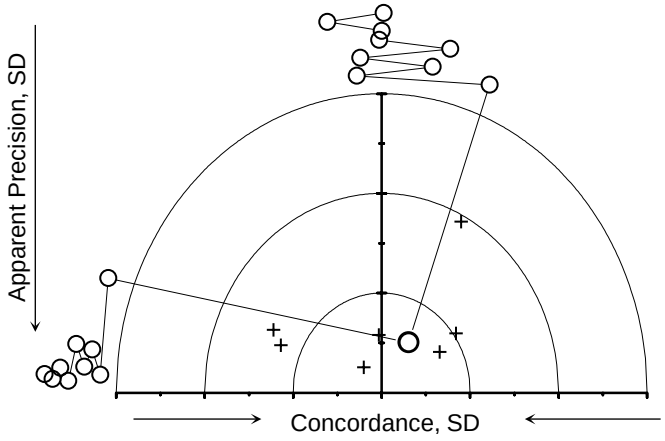
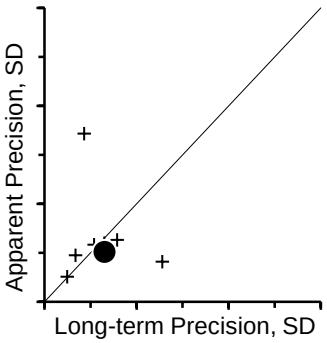
Individualized RR LX Report: FSV-BA

trans-β-Carotene









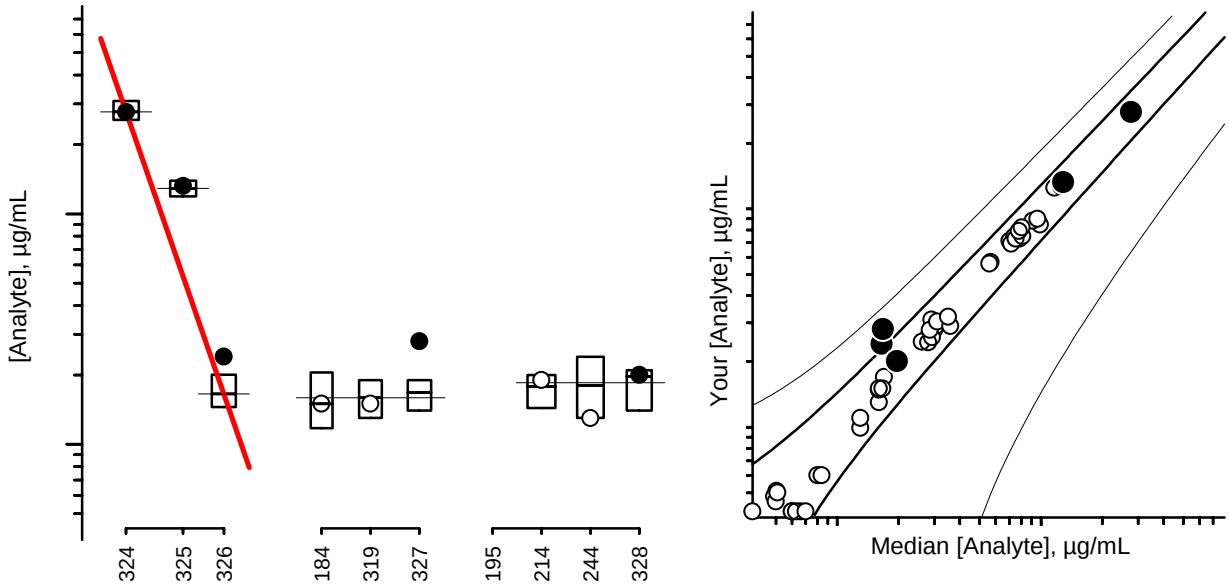
3rd Quartile (75%)
Median (50%)
1st Quartile (25%)

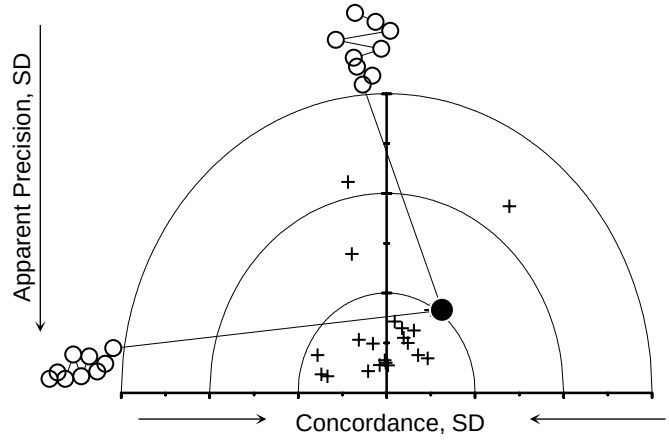
● You, this RR
○ You, past RRs
▲ You, ≥x, this RR
△ You, ≥x, past RRs
\+ Others, this RR

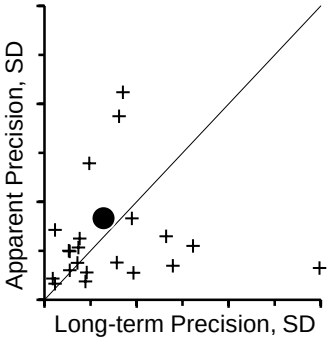
Serum	History	Comments
#324	Fresh frozen	Native, single-source
#325	Fresh frozen	25:35 blend of #324 and #326
#326	Fresh frozen	Native, single-source
#327	Lyophilized - 28:184, 59:319	Native, single-source
#328	Lyophilized - 31:195, 35:214, 43:244	Manipulated, multi-source

Individualized RR LX Report: FSV-BA

Total α-Carotene



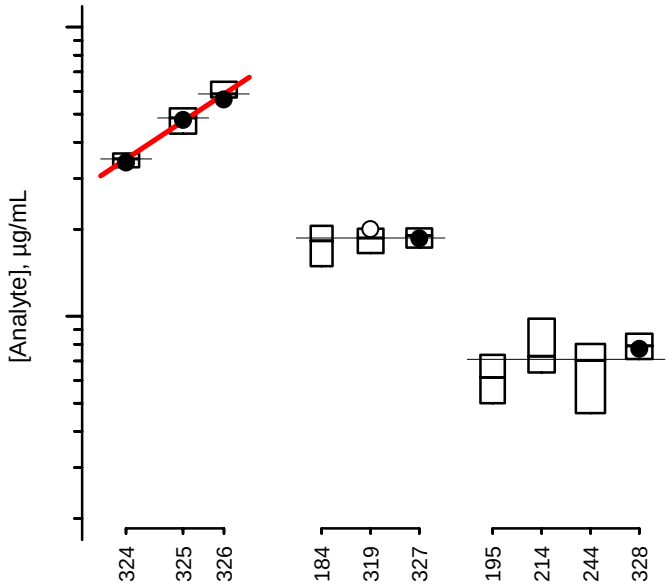


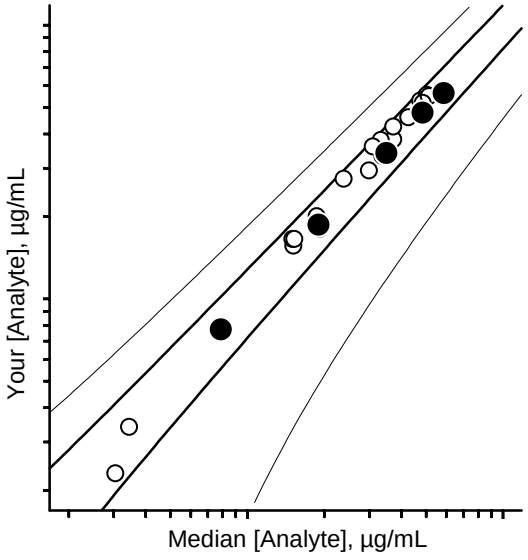


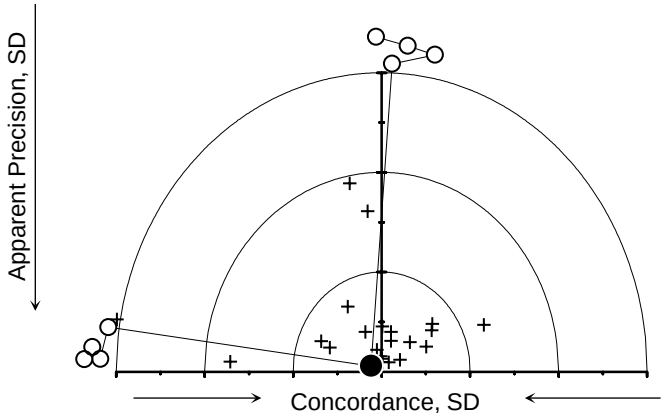
Serum	History	Comments
#324	Fresh frozen	Native, single-source
#325	Fresh frozen	25:35 blend of #324 and #326
#326	Fresh frozen	Native, single-source
#327	Lyophilized - 28:184, 59:319	Native, single-source
#328	Lyophilized - 31:195, 35:214, 43:244	Manipulated, multi-source

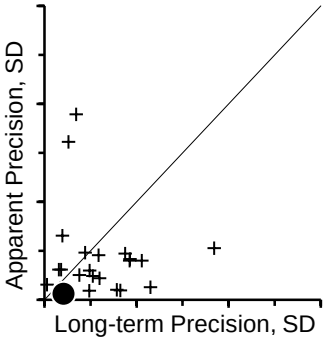
Individualized RR LX Report: FSV-BA

Total Lycopene





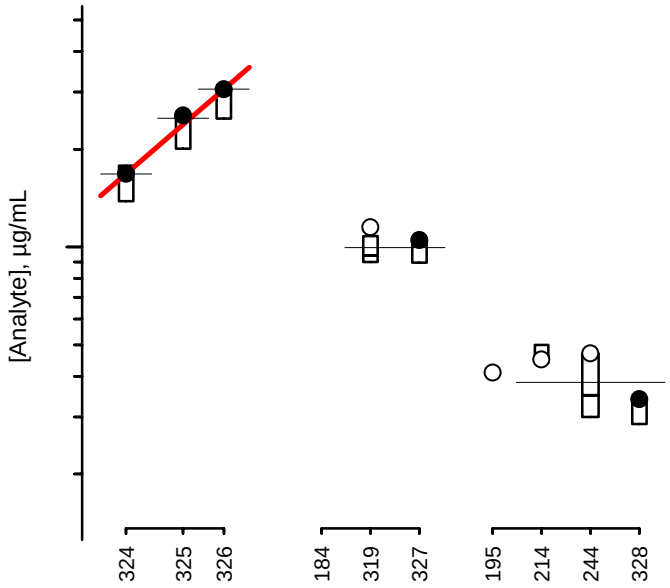


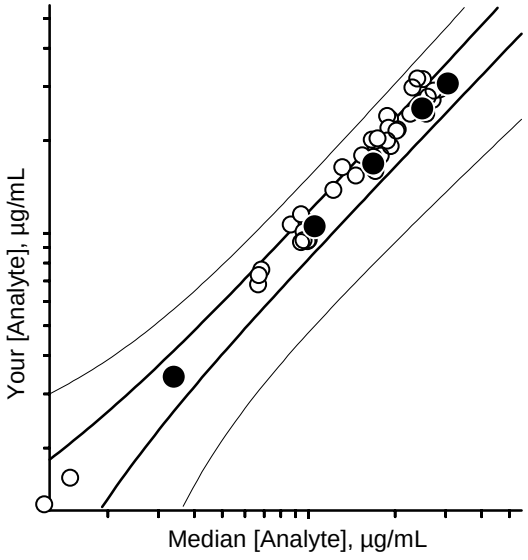


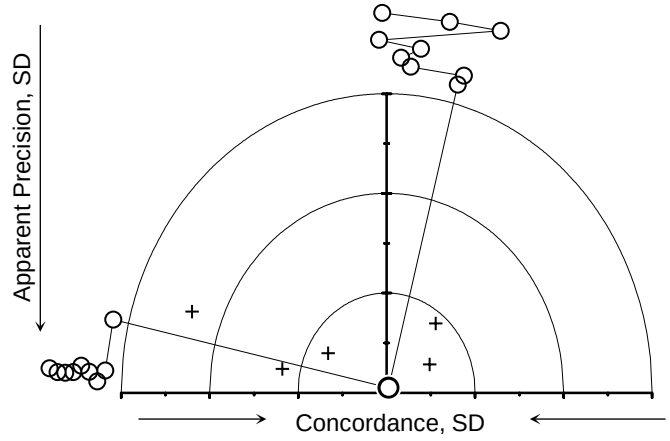
Serum	History	Comments
#324	Fresh frozen	Native, single-source
#325	Fresh frozen	25:35 blend of #324 and #326
#326	Fresh frozen	Native, single-source
#327	Lyophilized - 28:184, 59:319	Native, single-source
#328	Lyophilized - 31:195, 35:214, 43:244	Manipulated, multi-source

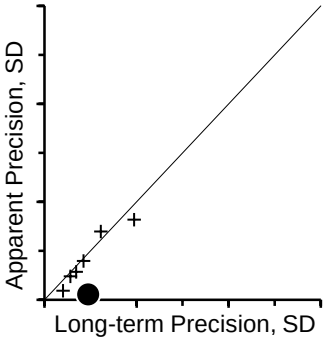
Individualized RR LX Report: FSV-BA

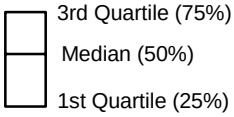
trans-Lycopene









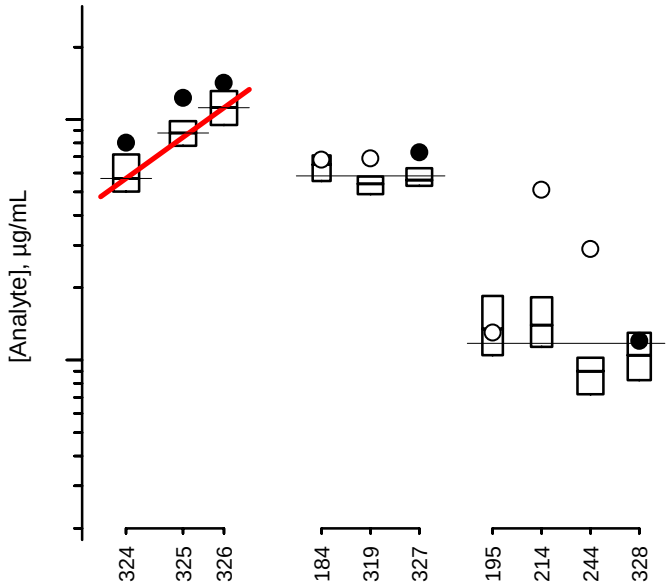


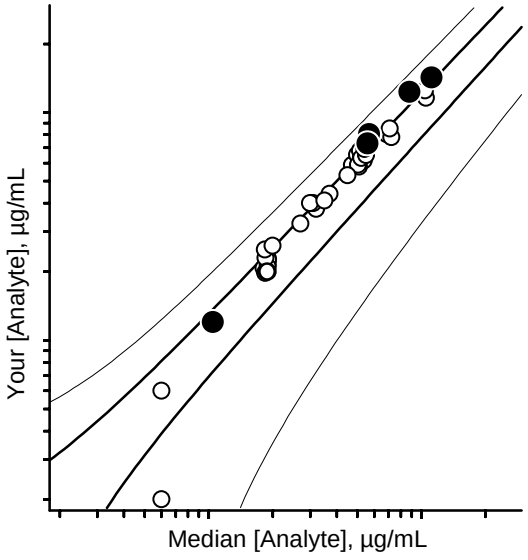
● You, this RR
▲ You, ≥x, this RR
○ You, past RRs
△ You, ≥x, past RRs
\+ Others, this RR

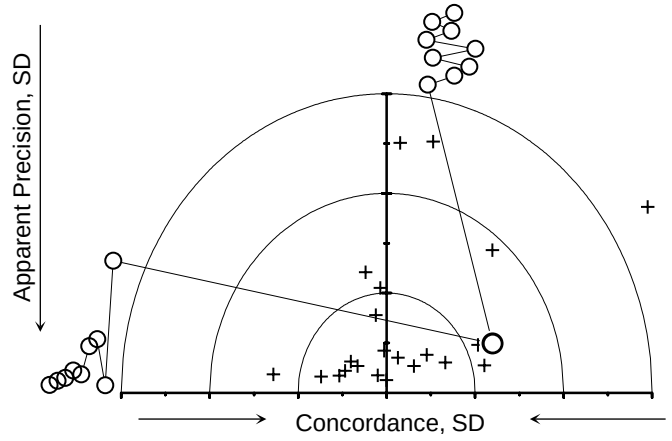
Serum	History	Comments
#324	Fresh frozen	Native, single-source
#325	Fresh frozen	25:35 blend of #324 and #326
#326	Fresh frozen	Native, single-source
#327	Lyophilized - 28:184, 59:319	Native, single-source
#328	Lyophilized - 31:195, 35:214, 43:244	Manipulated, multi-source

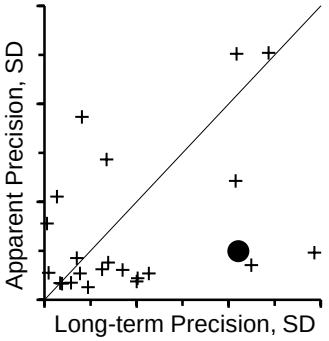
Individualized RR LX Report: FSV-BA

Total β-Cryptoxanthin





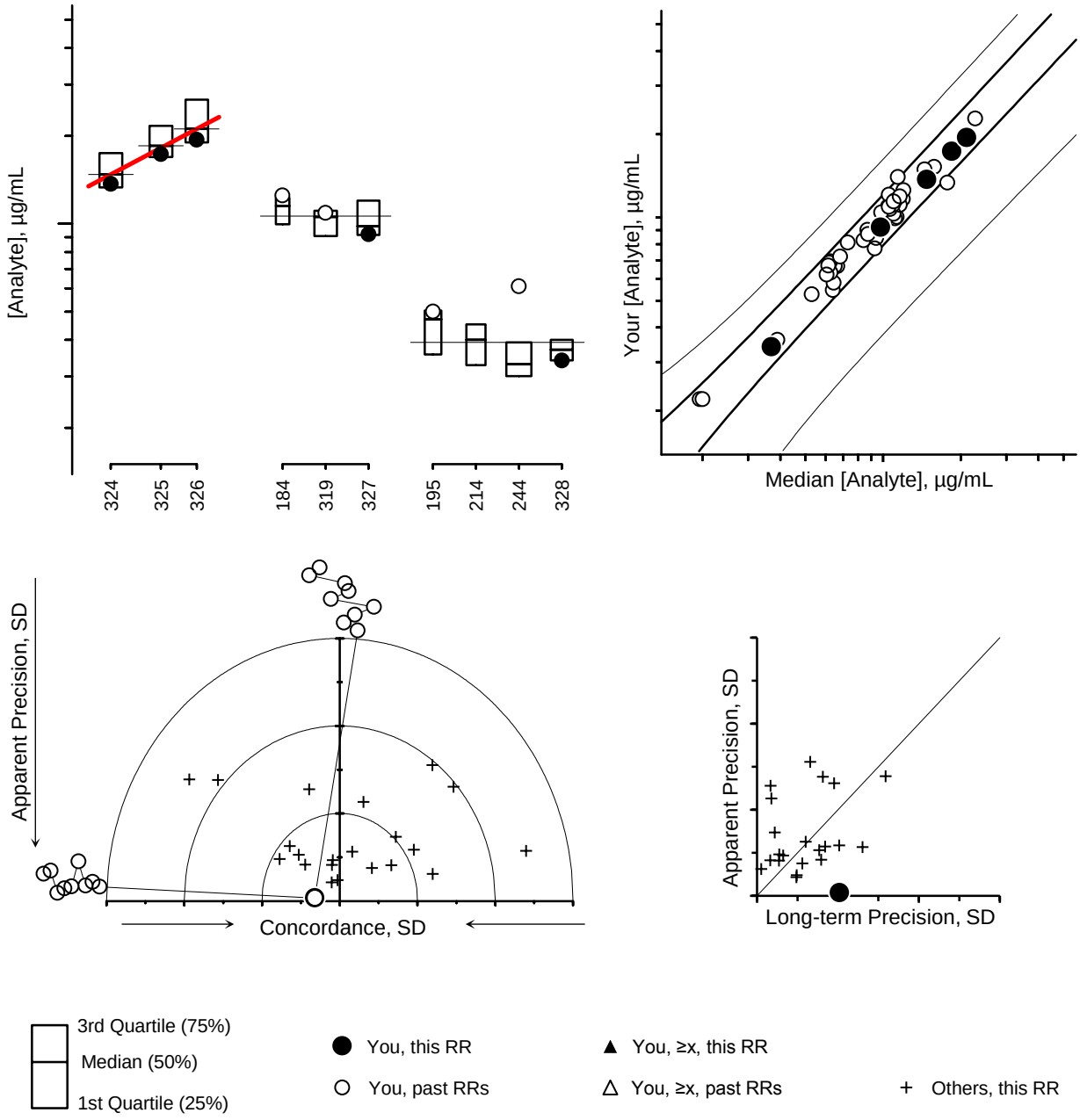




Serum	History	Comments
#324	Fresh frozen	Native, single-source
#325	Fresh frozen	25:35 blend of #324 and #326
#326	Fresh frozen	Native, single-source
#327	Lyophilized - 28:184, 59:319	Native, single-source
#328	Lyophilized - 31:195, 35:214, 43:244	Manipulated, multi-source

Individualized RR LX Report: FSV-BA

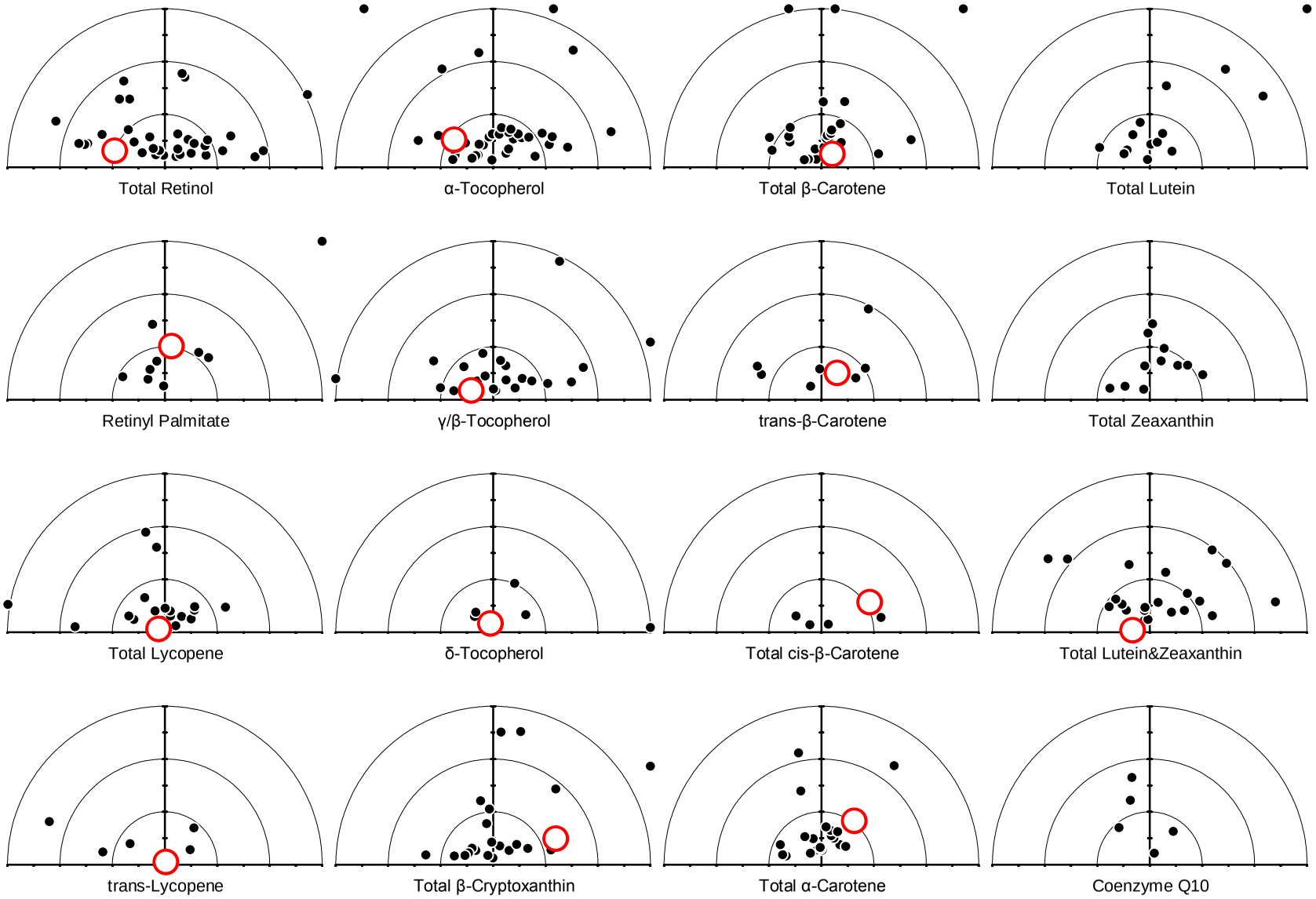
Total Lutein&Zeaxanthin



Serum	History	Comments
#324	Fresh frozen	Native, single-source
#325	Fresh frozen	25:35 blend of #324 and #326
#326	Fresh frozen	Native, single-source
#327	Lyophilized - 28:184, 59:319	Native, single-source
#328	Lyophilized - 31:195, 35:214, 43:244	Manipulated, multi-source

Individualized Round Robin LX Report: FSV-BA

Graphical Comparability Summary



D15

Appendix E. Shipping Package Inserts for RR25

The following five items were included in each package shipped to an RR25 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-

May 1, 2006

Dear Colleague:

The samples within this package constitute the second Vitamin C Round Robin (RR25) of the fiscal year (FY) 06 Micronutrients Measurement Quality Assurance Program.

RR25 consists of four vials of frozen serum *test samples* (#72, #89, #111, and #116), one vial of ascorbic acid *solid control material* (Control), and two vials of frozen *serum control materials* (Control #1 and Control #2). Please follow the attached protocols when you prepare and analyze these samples. If you cannot prepare the *solid control* solutions gravimetrically, please prepare equivalent solutions volumetrically and report the exact volumes used. (Routine 0.5 g gravimetric measurements are generally 10-fold more accurate than routine 0.5 mL volumetric measurements.)

The two *serum control materials* are a new component of the M^2QAP for Vitamin C. Please use these materials to validate the performance of your measurement system before you analyze the *test samples*. The target value and ≈95% confidence interval for *Control #1* is 8.41 ±0.61 μmol/L sample; the target value and ≈95% confidence interval for *Control #2* is 28.05 ±0.49 μmol/L sample.

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

The report for RR24 was mailed during the last week of April. If you find your results for RR24 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 RR25 samples by **September 4, 2006**. 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
RR25 Report Form for Ascorbic Acid Solid Control Material Preparation
RR25 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:

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

   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 $[TAA]_{DS1}$ = (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 $[TAA]_{DS2}$ = 28.4 μmol/L and 0.125 mL should weigh 0.13 g and $[TAA]_{DS3}$ = 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 25

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 **Sept 4, 2006**

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 25

NIST Micronutrient Measurement Quality Assurance Program

Analysis of Control Materials and Test Samples

Sample	Replicate 1	Replicate 2	Units
Dilute Solution 1			µmol/L of Dilute Solution
Dilute Solution 2			µmol/L of Dilute Solution
Dilute Solution 3			µmol/L of Dilute Solution
5% MPA Diluent			µmol/L of Diluent
Serum Control #1			µmol/L of Sample *Target:* 8.5 ±0.5 µmol/L
Serum Control #2			µmol/L of Sample *Target:* 28.1 ±1.0 µmol/L
Serum Test Sample #72			µmol/L of Sample
Serum Test Sample #89			µmol/L of Sample
Serum Test Sample #111			µmol/L of Sample
Serum Test Sample #116			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 **Sept 4, 2006**

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 25

NIST Micronutrients Measurement Quality Assurance Program

Packing List and Shipment Receipt Confirmation Form

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

Sample	Form
VitC #72	Liquid frozen (1:1 serum:10% MPA)
VitC #89	Liquid frozen (1:1 serum:10% MPA)
VitC #111	Liquid frozen (1:1 serum:10% MPA)
VitC #116	Liquid frozen (1:1 serum:10% MPA)
CS #1	Liquid frozen (1:1 serum:10% MPA)
CS #2	Liquid frozen (1:1 serum:10% MPA)
Control	Solid AA

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

1) Date this shipment arrived: _____

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

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

4) Did the samples arrive frozen? Yes | No

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

6) When do you anticipate analyzing these samples? _____

Your prompt return of this information is appreciated.

The 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 RR25

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-

October 12, 2006

Dear Colleague:

Enclosed is the summary report of the results for Round Robin 25 (RR 25) 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 25 consisted of four *test samples* (Sera 72, 89, 111, and 116), two *serum control materials*, and one *solid control material* for preparation of TAA control solutions. Details regarding the samples can be found in the enclosed report.

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

Samples for the first vitamin C round robin (RR 26) of the 2007 M^2QAP will be shipped **during the week of November 13, 2006.**

Please save the date! We will host the next Micronutrients Measurement Quality Assurance Workshop in conjunction with the Experimental Biology meeting on **May 2, 2007** at the Convention Center in Washington, DC. We will provide you with more details as our plans our finalized.

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

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

Enclosures

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

NIST

The NIST M^2QAP Vitamin C Round Robin 25 (RR25) report consists of

Page	"Individualized" Report
1	Summarizes your reported values for the nominal 55 mmol/L solution you prepared from the ascorbic acid solid control sample, the two serum control samples, and the four serum test samples.
2	Graphical summary of your RR25 sample measurements.

Page	"All Lab" Report
1	A tabulation of results and summary statistics for Total Ascorbic Acid [TAA] in the RR25 samples and control/calibration solutions.

Serum-based Samples. Two serum controls and four unknowns were distributed in RR25.

Control 1 (CS #1) SRM 970 level 1, ampouled in mid-1998.

Control 2 (CS #2) SRM 970 level 2, ampouled in mid-1998.

Serum 72, SRM 970 level 1, ampouled in mid-1998. An augmented serum.

Serum 89, SRM 970 level 2, ampouled in mid-1998. An augmented serum.

Serum 111, ampouled in 1989. It was used in some early experiments but has not previously been used in the M^2QAP program.

Serum 116, ampouled in 1995, previously distributed as sample 682a in RR8 (1996) and RR10 (1997).

Results.

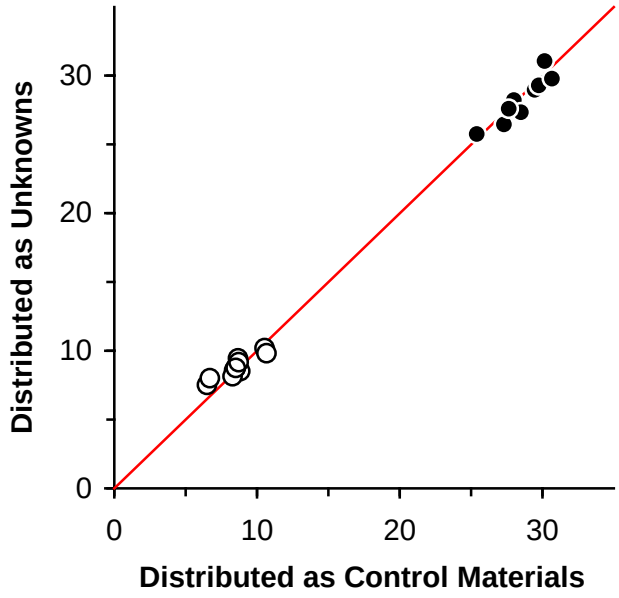
1) All participants who prepared the four 5% MPA control/calibration solutions (the three "Dilute Solutions" and the "Diluent") did so correctly. The criteria used to evaluate this success are: the density of the 5% MPA (≈1.03 gm/mL), the observed wavelength maximum of "Dilute Solution #1"(≈254 nm), the observed absorbance at that maximum (≈0.55 OD), the calculated $E^{1\%}$ #1"(≈550 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 root mean square error 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) While it appears that Serum 111 has not been previously distributed as a M^2QAP sample, the median value from RR25 agrees very well with the value assigned to the material by Dr. Margolis in 1989. There has been no significant change in the median value or the variability for Serum 116, ampouled in 1995.

4) Sera 72 and 89 are the same materials as CS #1 and CS #2, Levels 1 and 2 of SRM 970. The following Figure displays the reported {known Control Material, unknown Sample} pairs for the two levels. There is no significant difference in the results.

Reported Results for SRM 970 Levels 1 and 2
Distributed as Known Control Materials and as Unknown Samples.
All results are in mmol/L



Appendix G. "All-Lab Report" for RR25

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" 25 - September 2006

		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	CS#2	S25:1	S25:2	S25:3	S25:4	CS#1	CS#2	S25:1	S25:2	S25:3	S25:4
VC-MA	28/08/06	56.5	28.1	14.0	53.9	28.1	14.7	0.0	0.80	0.95	0.999	0.9	1.035	242.	0.5550	557.4	8.4	27.6	8.6	27.5	12.4	42.1	8.0	28.3	8.2	28.1	12.2	43.6
VC-MB	24/05/06	56.9	28.4	14.4	60.1	29.4	15.0	0.0	-0.21	1.06	1.000	0.3	1.031	243.	0.5580	556.5	8.5	29.9	8.8	29.4	10.8	45.7	8.3	28.5	8.5	28.0	10.5	43.4
VC-MC	12/05/06	56.6	28.4	14.0	55.4	28.2	14.3	0.0	0.36	0.98	1.000	0.4	1.031	243.	0.5677	569.2	8.8	28.0	8.5	28.2	11.0	38.7	8.7	28.3	8.4	28.6	10.9	39.3
VC-ME	05/09/06	57.6	29.3	14.2	55.8	31.1	14.3	0.0	0.77	0.97	0.997	1.6	1.028	244.	0.5251	517.6	6.5	27.3	7.5	26.5	11.6	42.0	5.9	27.4	7.0	26.5	11.1	42.5
VC-MG	25/08/06	59.0	29.6	15.2	60.1	28.4	13.6	0.0	-1.10	1.03	0.998	1.3	1.030	243.2	0.5990	576.1	8.7	30.1	9.4	31.1	12.9	49.9	9.5	30.5	10.3	31.4	13.6	49.7
VC-MH	01/09/06	62.3	31.1	15.3	62.2	30.9	15.2	0.0	-0.04	1.00	1.000	0.1	1.033	243.7	0.6210	565.6	8.3	25.4	8.2	25.8	12.0	40.7	8.4	25.5	8.2	25.8	12.1	40.8
VC-MI	05/09/06	56.3	27.8	13.6	57.8	26.0	12.6	0.0	-1.07	1.03	0.998	1.5	1.030				8.5	28.5	8.7	27.3	10.8	41.8	9.3	28.7	9.5	27.6	11.6	41.6
VC-MJ	10/08/06	60.0	31.1	13.8	56.4	29.7	14.5	0.4	0.96	0.93	1.000	0.6	1.022[a]	255[a]	0.355[a]	336.1[a]	10.5	29.5	10.2	29.0	14.6	*b*	10.3	30.8	10.0	30.2	14.8	*b*
VC-MK	19/07/06	57.4	28.4	14.1	56.7	28.1	14.3	0.9	0.72	0.97	1.000	0.2	1.031	244.	0.5690	563.2	10.7	30.7	9.8	29.8	18.4	44.5	10.2	30.8	9.4	29.9	18.1	45.0
VC-ML	02/08/06	56.3	28.4	14.2	55.7	25.6	12.6	0.0	-1.10	0.99	0.997	1.5	1.031	242.	0.5543	559.0	6.7	29.7	8.0	29.3	9.8	49.3	7.9	31.1	9.2	30.6	10.9	50.8
VC-MP	18/08/06																8.7	27.6	9.2	27.6	13.4	42.4						
N		10	10	10	10	10	10	10					N 9	8	8	8	11	11	11	11	11	10	10	10	10	10	10	9
Average		57.9	29.1	14.3	57.4	28.6	14.1	0.1					Average 1.031	243.1	0.5686	558.1	8.6	28.6	8.8	28.3	12.5	43.7	8.6	29.0	8.9	28.7	12.6	44.1
SD		2.0	1.2	0.5	2.6	1.8	0.9	0.3					SD 0.002	0.8	0.0294	17.6	1.3	1.6	0.8	1.6	2.4	3.6	1.3	1.8	1.0	1.8	2.4	3.9
Min		56.3	27.76	13.6	53.9	25.61	12.6	0.0					Min 1.028	242.0	0.5251	517.6	6.5	25.4	7.5	25.8	9.8	38.7	5.9	25.5	7.0	25.8	10.5	39.3
%25		56.6	28.37	14.0	55.7	28.12	13.8	0.0					%25 1.030	242.8	0.5548	557.2	8.4	27.6	8.3	27.4	10.9	41.8	8.1	28.3	8.3	27.7	11.0	41.6
Median		57.1	28.39	14.1	56.5	28.27	14.3	0.0					Median 1.031	243.1	0.5629	561.1	8.5	28.5	8.7	28.2	12.0	42.3	8.5	28.6	8.8	28.3	11.8	43.4
%75		58.7	29.51	14.4	59.5	29.66	14.6	0.0					%75 1.031	243.8	0.5765	566.5	8.8	29.8	9.3	29.4	13.2	45.4	9.5	30.7	9.5	30.1	13.3	45.0
Max		62.3	31.14	15.3	62.2	31.09	15.2	0.9					Max 1.035	244.0	0.6210	576.1	10.7	30.7	10.2	31.1	18.4	49.9	10.3	31.1	10.3	31.4	18.1	50.8
MADe		1.1	0.7	0.3	1.8	1.9	0.7	0.0					eSD 0.001	1.1	0.0122	6.7	0.3	1.7	0.9	1.6	1.7	2.8	1.1	2.3	0.9	2.5	1.3	2.7
CV		2	2	2	3	7	5						CV 0.12	0.46	2.2	1.2	4	6	10	6	14	7	13	8	11	9	11	6

a) 5% Trichloroacetic acid solution
b) Miss-labeled sample

G2

Appendix H. Representative "Individualized Report" for RR25

Each participant in RR25 received an "Individualized Report" reflecting their reported results. The following two pages are the "Individualized Report" for participant "VC-MA".

Vitamin C "Round Robin" 25 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
02/23/04	20	HPLC-EC	1.031	243.0	0.552	560.7	-0.4	1.05	1.000	0.65
09/13/04	21	HPLC-EC	1.030	244.0	0.555	562.2	-0.1	0.99	1.000	0.10
03/08/05	22	HPLC-EC	1.034	243.0	0.559	562.9	0.2	1.06	1.000	0.24
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
		Mean	1.032	243.3	0.56	566.3				0.63
		SD	0.004	0.8	0.01	10.6				0.50
		CV	0.35	0.34	1.0	1.9				

[TAA] mmol/Lsample

Date	RR	Sample	Rep_1	Rep_2	F_{adj}	Mean	SD_{dup}	N	Mean	SD_{repeat}	SD_{reprod}
02/23/04	20	CS#1	7.8	8.0	1.0	7.9	0.1	6	8.6	0.1	0.6
09/13/04	21	CS#1	8.1	7.9	1.0	8.0	0.1				
03/08/05	22	CS#1	8.5	8.7	1.0	8.6	0.1				
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				
02/23/04	20	CS#2	25.8	26.2	1.0	26.0	0.3	6	28.1	0.5	1.6
09/13/04	21	CS#2	26.2	27.2	1.0	26.7	0.7				
03/08/05	22	CS#2	29.0	29.0	1.0	29.0	0.0				
10/17/05	23	CS#2	29.4	30.5	1.0	30.0	0.8				
03/09/06	24	CS#2	29.2	29.1	1.0	29.2	0.1				
08/28/06	25	CS#2	**27.2**	**28.1**	1.0	27.6	0.6				
11/18/02	16	S16:1	8.8	8.8	1.0	8.8	0.0	5	8.5	0.3	0.3
11/13/03	19	S19:4	7.8	8.6	1.0	8.2	0.5				
02/23/04	20	S20:3	8.3	8.1	1.0	8.2	0.1				
10/17/05	23	S23:4	8.6	8.8	1.0	8.7	0.1				
08/28/06	25	S25:1	**8.7**	**8.5**	1.0	8.6	0.2				
03/20/03	18	S18:3	28.8	29.2	1.0	29.0	0.3	4	27.9	0.3	1.8
02/23/04	20	S20:4	25.9	25.2	1.0	25.5	0.5				
03/08/05	22	S22:4	29.4	29.4	1.0	29.4	0.0				
08/28/06	25	S25:2	**27.6**	**27.4**	1.0	27.5	0.1				
08/28/06	25	S25:3	**12.3**	**12.4**	1.0	12.4	0.1				
ND	08							2	61.6	0.4	27.6
08/20/97	10	S10:2	163.0	161.3	0.5	81.1	0.6				
08/28/06	25	S25:4	**42.0**	**42.2**	1.0	42.1	0.2				

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

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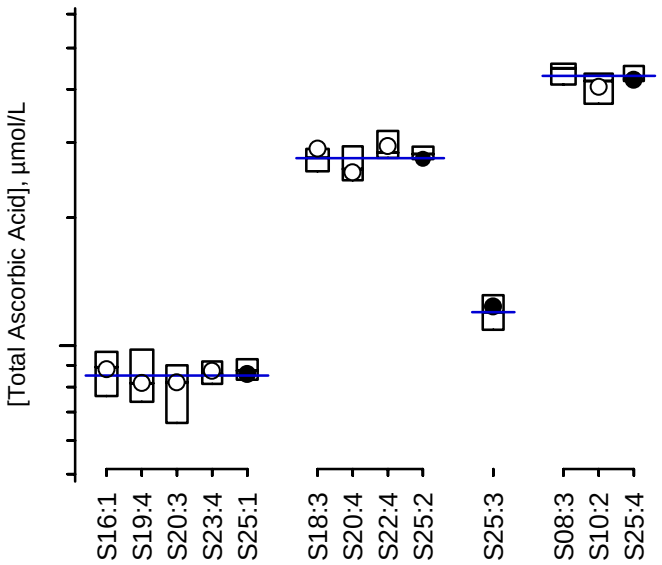
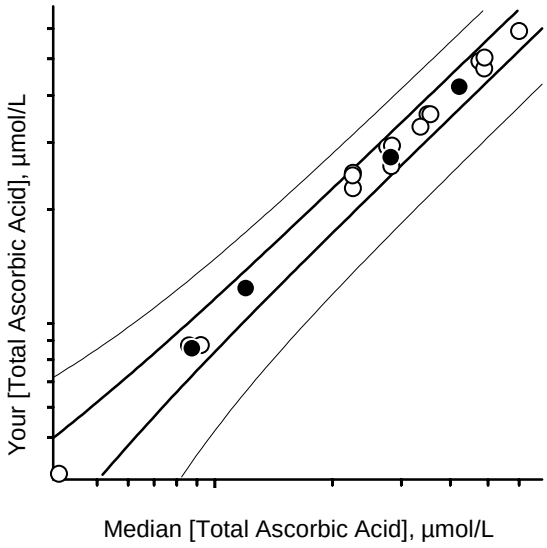
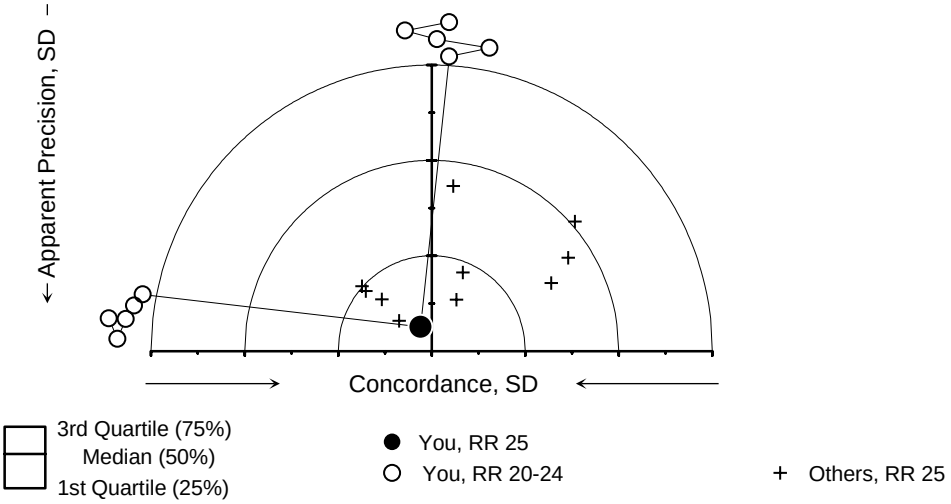
Vitamin C "Round Robin" 25 Report: Participant VC-MA

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
S25:1	VitC #72, previously distributed in RRs 16, 19, 20, and 23
S25:2	VitC #89, previously distributed in RRs 18, 20, and 22
S25:3	VitC #111
S25:4	VitC #116, previously distributed in RRs 8 and 10