NISTIR 7880-13

NIST Micronutrients Measurement Quality Assurance Program Winter 2006 Comparability Studies

Results for Round Robin LIX
Fat-Soluble Vitamins and Carotenoids in Human Serum
and Round Robin 24 Ascorbic Acid in Human Serum

David L. Duewer
Jeanice B. Thomas

http://dx.doi.org/10.6028/NIST.IR.7880-13

National Institute of Standards and Technology
U.S. Department of Commerce

NISTIR 7880-13

NIST Micronutrients Measurement Quality Assurance Program Winter 2006 Comparability Studies

Results for Round Robin LIX
Fat-Soluble Vitamins and Carotenoids in Human Serum
and Round Robin 24 Ascorbic Acid in Human Serum

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

http://dx.doi.org/10.6028/NIST.IR.7880-13

-XQH2013

U.S. Department of Commerce
&DPHURQ) . HUU, Acting Secretary

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

(This page intentionally blank)

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 2006 MMQAP measurement comparability improvement studies: 1) Round Robin LIX Fat-Soluble Vitamins and Carotenoids in Human Serum and 2) Round Robin 24 Total Ascorbic Acid in Human Serum. The materials for both studies were shipped to participants in November 2005; participants were requested to provide their measurement results by March 3, 2006.

Keywords

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

Table of Contents

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 LIX: Fat-Soluble Vitamins and Carotenoids in Human Serum

Participants in the MMQAP Fat-Soluble Vitamins and Carotenoids in Human Serum Round Robin LIX comparability study (hereafter referred to as RR59) received four lyophilized and one liquid-frozen human serum test samples for analysis. Unless multiple vials were previously requested, participants received one vial of each serum. These sera were shipped on dry ice to participants in November 2005. 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 RR59 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 24: Vitamin C in Human Serum

Participants in the MMQAP Vitamin C in Human Serum Round Robin 24 comparability study (hereafter referred to as RR24) received four frozen serum test samples, two frozen control sera, and a solid ascorbic acid control material for analysis. Unless multiple vials were previously requested, participants received one vial of each material. These sample materials were shipped on dry ice to participants in November 2005. 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 RR24 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 RR59

The following three items were included in each package shipped to an RR59 participant:

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

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

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

November 28, 2005

Dear Colleague:

Enclosed are the samples (Sera 319 – 323) for the first fat-soluble vitamins and carotenoids in serum round robin study (Round Robin LIX) for the fiscal year (FY) 06 NIST Micronutrients Measurement Quality Assurance Program. You will find one vial of each of one liquid-frozen and four lyophilized serum samples for analysis along with a form for reporting your results. When reporting your results, please submit one value for each analyte for a given serum sample. If a value is obtained below your limit of quantification, please indicate this result on the form by using NQ *(Not Quantified)*. Results are due to NIST by **March 3, 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 sample (Serum 323).**

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 LIX 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 B Thomas

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

Enclosures

NIST

Participant #: ________ Date: _____________

Round Robin LIX: Human Sera

NIST Micronutrients Measurement Quality Assurance Program

Analyte	319	320	321	322	323	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**

Was the liquid frozen sample #323 frozen when received? Yes | No

Comments:

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

Please return results **before**
3-Mar-2006

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

Participant #: ________ Date: ____________

Fat-Soluble Vitamins Round Robin LIX

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^{2}QAP sera

Serum	Form	Reconstitute?
#319	Lyophilized	Yes (1 ml H_2O)
#320	Lyophilized	Yes (1 ml H_2O)
#321	Lyophilized	Yes (1 ml H_2O)
#322	Lyophilized	Yes (1 ml H_2O)
#323	Liquid frozen	No

Please 1) Open the pack immediately
2) Check that it contains all of the above samples
3) Check if the ampoules and 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 sample #323 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^{2}QAP Gang

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

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

Appendix B. Final Report for RR59

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

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

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

April 10, 2006

Dear Colleague:

Enclosed is the summary report of the results for round robin LIX (RR59) 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 relative to the NIST assigned values. The NIST-assigned values are equally weighted means of the medians from this interlaboratory comparison exercise and the means from the analyses performed by NIST.

Data for evaluating laboratory performance in RR59 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 second 2006 QA interlaboratory exercise will be shipped **starting the week of May 8, 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 <u>one calendar week</u> after the package arrives.

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

Sincerely,

Jeanice B. Thomas

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

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

Enclosures

NIST

The NIST M^{2}QAP Round Robin LVII (RR59) report consists of:

Page	"All Lab" Report
1-4	A listing of all results and statistics for analytes reported by at least two laboratories.
5a	A list of results for the analytes reported by only one laboratory.
5b	A legend for the above two lists.
6	The text 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 RR59.

Serum	Description	Prior Distributions
319	Lyophilized, native, single-donor, commercially obtained serum.	#184:RR28-6/93
320	Lyophilized blend of the #311 augmented serum and stripped serum, in 1+3 ratio (*i.e.*, measurand levels should be ¼ those of #321).	#197:RR31-6/94, #211:RR35-9/95; #245:RR43-6/98; #310:RR57-3/05
321	Lyophilized blended serum with native carotenoid levels, augmented with retinol, retinyl palmitate, and α- and γ-tocopherol.	#198:RR31-6/94, #212:RR35-9/95; #246:RR43-6/98; #311:RR57-3/05
322	Lyophilized, native, single-donor, commercially obtained serum prepared in 2002. The same material was used to prepare #323.	#290:RR53-2/03, #300:RR55-3/04; #312:RR57-3/05
323	Fresh-frozen, native, single-donor, commercially obtained serum prepared in 2002. The same material was used to prepare #322.	#292:RR53-2/03, #301:RR55-3/04; #313:RR57-3/05

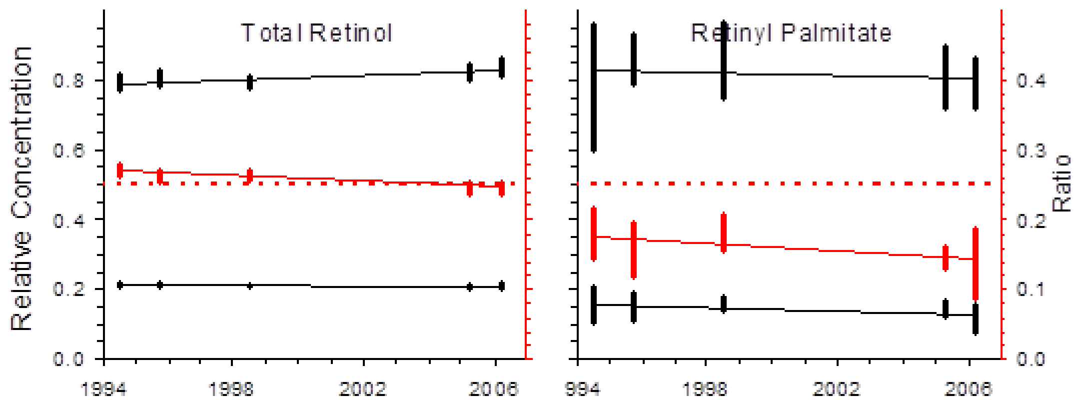
Results

1) Sera Stability. There was no significant change in the median level for any measurand in any of the sera. The measurement variability for some of the very low-level measurands in #320 was marginally higher than expected. Note that #319, 320, and 321 were prepared more than 10 years ago.

2) Follow-up on Retinyl Palmitate Issue from RR 57: Sera #320 and #321 provide the fifth set of paired data for an experiment begun in April, 1994. The material analyzed as serum #320 was prepared from a single-source pool spiked with retinol, α-tocopherol, and retinyl palmitate. The material analyzed as serum #321 was prepared as a 1:3 blend of the spiked pool and a stripped serum. These

materials were also analyzed in last year's RR57 as sera #310 and #311. At that time, we'd noted that the there was considerably less retinyl palmitate in the low-level material than expected and speculated on the cause. We are in the process of re-examining all of the available data for these materials, and have at least a tentative answer: retinyl palmitate was lost when the blended material was filtered.

The following Figure shows the trends with time for total retinol and retinyl palmitate. The trend for the spiked material is represented by the upper solid line), that for the "25%-of-spike" low level material is the lower solid line, and the trend of the ratio between the two is the middle solid line. The expected value for the ratio is 0.25 (dotted line). All of the bars represent an approximate 95% confidence interval on the estimated mean values. The ratios are estimated directly from the "raw" low-level / spiked pairs, not just from the summary statistics.



The pattern for total retinol is about as expected, although the small apparent increase in the values for the spiked material is curious. However, the retinyl palmitate ratio for the low-level/spike has been considerably less than the expected 0.25 from the beginning. Simplest explanation for the difference between the patterns is that some of the hydrophobic retinyl pal imitate spike was lost to filtration after blending the spiked-pool and the stripped serum. The variability in the estimates is too great to definitively judge whether the ratio actually changed over time.

We intend to provide a complete analysis of the results for other measurands in these materials within the next year. However, your values in serum #320 should be very close to 25% of those in #231 for total retinol, α and γ/β-tocopherol, and β-carotene; they should be at least "fairly" close for all of the other measurands except retinyl palmitate.

3) Matrix (Lyophilized vs. Fresh-Frozen) Differences. Sera 322 and 323 were prepared from the same serum pool. Since we suggest that you reconstitute our lyophilized samples with 1.0 mL water rather than to a total volume of 1.0 mL, the measurand levels in #322 should be $\approx$ 95% of those in #323. The observed average ratio $\pm$ 95% confidence interval over all measurands with 8 or more quantitative measurements is 0.953 ± 0.007. If any of your #322/#323 ratios are much different than 0.95, you should look at your measurement system for those measurands. If your lyophilized/fresh frozen ratios are consistently much different from 0.95, you should review how you reconstitute lyophilized materials.

Appendix C. “All-Lab Report” for RR59

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. 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 LIX Laboratory Results

All Results in µg/mL

	Total Retinol					trans-Retinol					Retinyl Palmitate					α-Tocopherol					γ/β-Tocopherol				
Lab	319	320	321	322	323	319	320	321	322	323	319	320	321	322	323	319	320	321	322	323	319	320	321	322	323
FSV-BA	0.375	0.230	0.878	0.670	0.705						0.079	0.013	0.238	0.107	0.114	4.35	1.80	15.14	9.92	10.46	1.69	0.486	2.81	1.82	1.93
FSV-BB	0.383	0.193	0.788	0.630	0.643						0.087	0.013	0.228	0.071	0.078	4.38	1.70	14.81	9.67	10.43	1.58	0.409	2.51	1.66	1.75
FSV-BC	0.371	0.211	0.834	0.637	0.680																				
FSV-BD	0.409	0.202	0.824	0.691	0.688											5.80	3.90	15.80	11.90	11.90					
FSV-BE	0.354	0.180	0.768	0.602	0.648											4.32	3.27	14.03	9.74	10.44	1.67	0.663	2.83	1.80	1.94
FSV-BF	0.340	0.170	0.760	0.590	0.630											3.20	3.00	15.60	9.90	10.50	1.59	0.620	2.58	1.70	1.80
FSV-BG	0.378	0.210	0.820	0.646	0.676						0.137	0.027	0.301	0.104	0.109	4.29	3.57	15.32	10.57	10.85	1.62	0.711	2.71	1.82	1.85
FSV-BH	0.354	0.124	0.728	0.622	0.638											4.50	3.41	13.04	9.99	10.69	1.88	0.740	2.70	1.88	1.99
FSV-BI	0.321	0.168	0.680	0.524	0.567						0.122	0.055	0.266	0.089	0.094	4.28	3.36	14.42	9.44	10.25	1.39	0.580	2.17	1.44	1.57
FSV-BJ	0.349	0.200	0.760	0.585	0.612						0.199	0.051	0.300	0.095	0.095	4.38	4.97	14.35	9.74	10.20	1.56	0.758	2.50	1.67	1.74
FSV-BK	0.400	0.140	0.843	0.725	0.764											3.79	2.52	13.45	12.04	9.98					
FSV-BL	0.370	0.170	0.740	0.600	0.630											4.30	3.40	13.40	9.90	9.90					
FSV-BM	0.327	0.169	0.715	0.542	0.574											4.30	3.30	14.50	9.60	11.10					
FSV-BN	0.381	0.207	0.814	0.652	0.678						0.101	0.044	0.236	0.089	0.091	4.17	3.33	12.81	9.38	9.35	1.50	0.732	2.39	1.73	1.80
FSV-BO	0.362	0.248	0.683	0.614	0.701											4.43	3.71	9.84	9.75	10.89					
FSV-BP	0.311	0.196	0.599	0.506	0.523											3.84	2.99	12.93	11.05	10.35					
FSV-BQ	0.372	0.195	0.797	0.635	0.670											4.17	4.70	14.50	9.67	9.70					
FSV-BR	≥0.395	≥0.194	≥0.813	≥0.697	≥0.689	0.395	0.194	0.813	0.697	0.689						3.91	3.64	12.60	8.90	9.02					
FSV-BS	≥0.533	≥0.226	≥1.272	≥0.951	≥1.001	0.533	0.226	1.272	0.951	1.001						2.83	2.82	1.55	6.98	6.24	1.17	0.513	0.33	1.40	1.25
FSV-BT	0.320	0.332	0.655	0.569	0.717											5.34	4.63	12.89	9.20	9.82	1.71	0.551	2.64	1.69	1.79
FSV-BU	0.353	0.145	0.733	0.570	0.611											4.41	2.77	15.21	9.59	11.01	1.44	0.415	2.42	1.55	1.92
FSV-BV	0.380	0.073	0.511	0.735	0.765											4.91	5.00	15.64	10.62	11.20	1.90	0.737	2.99	1.98	2.03
FSV-BW	0.380	0.190	0.810	0.640	0.670						0.077	0.034	0.260	0.084	0.089	4.23	3.10	13.30	9.40	9.97	1.66	0.673	2.57	1.76	1.82
FSV-CC	0.370	0.190	0.810	0.620	0.650	0.370	0.190	0.810	0.620	0.650						3.91	2.99	12.92	8.80	9.21					
FSV-CE	0.370	0.182	0.745	0.809	0.656											4.76	3.00	16.60	!6.66	10.20					
FSV-CF	0.340	0.211	0.775	0.586	0.579											4.50	3.90	15.10	9.80	10.10					
FSV-CG	0.364	0.185	0.753	0.605	0.631											3.87	2.12	13.06	8.92	9.38	1.50	0.602	2.54	1.70	1.73
FSV-CI	0.372	0.194	0.748	0.613	0.642						0.107	0.042	0.257	0.089	0.099	4.43	4.72	14.79	9.89	10.71	1.59	0.780	2.65	1.75	1.85
FSV-CP																5.02	5.16	16.24	11.21	11.67	2.32	0.820	3.18	2.34	2.47
FSV-CS	0.371	0.208	0.842	0.596	0.667											5.47	5.25	15.97	11.67	11.65	2.11	1.055	3.16	2.29	2.28
FSV-CT																									
FSV-CW	0.388	0.253	0.841	0.552	0.581						0.171	0.074	0.354	0.081	0.082	2.60	1.43	15.50	9.13	10.00	1.62	0.700	2.71	1.74	1.86
FSV-CZ	0.380	0.210	0.810	0.610	0.650											5.00	4.30	14.60	10.40	10.90					
FSV-DB	0.335	0.199	0.743	0.572	0.587											4.43	4.75	13.10	9.70	10.20	1.76	0.736	2.56	2.25	2.00
FSV-DF	0.354	0.158	0.797	!0.746	0.623																				
FSV-DI	0.390	0.205	0.870	0.672	0.686						0.105	0.035	0.254	0.073	0.075	4.25	4.30	14.65	10.01	10.40	1.50	0.590	2.61	1.64	1.69
FSV-DQ																4.67	3.14	14.66	10.42	11.04	1.52	0.658	2.39	1.55	1.62
FSV-DV	0.296	0.169	0.624	0.499	0.523											4.50	3.30	14.20	9.80	10.40					
N	33	33	33	32	33	3	3	3	3	3	10	10	10	10	10	35	35	35	34	35	22	22	22	22	22
Min	0.296	0.073	0.511	0.499	0.523	0.370	0.190	0.810	0.620	0.650	0.077	0.013	0.228	0.071	0.075	2.60	1.43	1.55	6.98	6.24	1.17	0.409	0.33	1.40	1.25
Median	0.370	0.194	0.768	0.612	0.648	0.395	0.194	0.813	0.697	0.689	0.106	0.039	0.259	0.089	0.093	4.35	3.36	14.50	9.78	10.40	1.61	0.668	2.60	1.74	1.83
Max	0.409	0.332	0.878	0.809	0.765	0.533	0.226	1.272	0.951	1.001	0.199	0.074	0.354	0.107	0.114	5.80	5.25	16.60	12.04	11.90	2.32	1.055	3.18	2.34	2.47
SD	0.023	0.029	0.060	0.044	0.049						0.032	0.015	0.037	0.008	0.010	0.25	0.97	1.55	0.61	0.66	0.15	0.114	0.15	0.12	0.15
CV	6	15	8	7	8						30	40	14	10	11	6	29	11	6	6	9	17	6	7	8
N_{past}	44	41	42	34	34	0	5	5	6	6	8	10	11	12	12	44	42	42	37	37	16	21	21	22	22
$Median_{past}$	0.360	0.193	0.735	0.603	0.639		0.190	0.750	0.603	0.643	0.142	0.051	0.274	0.094	0.101	4.32	3.57	14.22	9.90	10.39	1.66	0.693	2.60	1.75	1.83
SD_{past}	0.034	0.019	0.063	0.043	0.044		0.000	0.000	0.020	0.020	0.025	0.013	0.051	0.022	0.023	0.30	0.53	1.15	0.70	0.66	0.15	0.116	0.18	0.11	0.14
NIST	0.383	0.177	0.724	0.632	0.658											4.20	3.24	14.17	10.08	9.98	1.70	0.699	2.74	1.80	1.78
S_{rep}	0.002	0.006	0.016	0.014	0.005											0.07	0.14	0.50	0.33	0.17	0.018	0.017	0.056	0.046	0.004
S_{het}	0.003	0.001	0.015	0.004	0.009											0.07	0.13	0.06	0.21	0.11	0.004	0.008	0.030	0.015	0.000
S_{NIST}	0.004	0.006	0.022	0.014	0.010											0.10	0.19	0.50	0.39	0.20	0.018	0.019	0.064	0.048	0.004
NAV	0.376	0.185	0.744	0.622	0.653	0.395	0.194	0.813	0.697	0.689	0.106	0.039	0.259	0.089	0.093	4.28	3.30	14.34	9.93	10.19	1.65	0.68	2.66	1.77	1.80
NAU	0.032	0.031	0.069	0.051	0.052						0.032	0.015	0.059	0.024	0.025	0.46	0.97	1.57	0.80	0.86	0.19	0.12	0.27	0.19	0.20

Round Robin LIX Laboratory Results

All Results in µg/mL

	δ-Tocopherol					Total β-Carotene					trans-β-Carotene					Total cis-β-Carotene					Total α-Carotene				
Lab	319	320	321	322	323	319	320	321	322	323	319	320	321	322	323	319	320	321	322	323	319	320	321	322	323
FSV-BA	0.040	0.029	0.150	0.051	0.058	0.354	0.103	0.750	0.137	0.140	0.332	0.097	0.697	0.130	0.134	0.022	0.006	0.053	0.007	0.005	0.015	0.003	0.032	0.079	0.082
FSV-BB	0.052	0.020	0.123	0.039	0.042	0.321	0.075	0.669	0.113	0.122	0.299	0.069	0.617	0.108	0.116	0.022	0.006	0.051	0.005	0.006	0.010	0.004	0.035	0.067	0.072
FSV-BC																									
FSV-BD																									
FSV-BE						0.326	0.133	0.752	0.121	0.133															
FSV-BF						0.324	0.250	0.778	0.073	0.082											0.013	0.012	0.036	0.090	0.079
FSV-BG						0.369	0.135	0.803	0.154	0.156											0.022	0.007	0.044	0.076	0.075
FSV-BH	0.046	0.040	0.161	0.059	0.054	0.280	0.123	0.599	0.101	0.106	0.280	0.123	0.556	0.101	0.106	*nq*	*nq*	0.043	*nq*	*nq*	*nq*	*nq*	*nq*	0.069	0.070
FSV-BI						0.272	0.140	0.590	0.095	0.103											0.014	0.007	0.027	0.065	0.068
FSV-BJ						0.326	0.115	0.719	0.111	0.122											0.013	nq	0.035	0.079	0.083
FSV-BK																									
FSV-BL																									
FSV-BM																									
FSV-BN	0.072	0.048	0.198	0.084	0.077	0.296	0.147	0.611	0.108	0.113	0.269	0.127	0.553	0.102	0.106	0.032	0.025	0.063	0.011	0.013	0.019	0.012	0.031	0.076	0.081
FSV-BO						0.529	0.161	0.597	0.115	0.121											0.030	*nq*	0.045	0.085	0.090
FSV-BP						0.316	0.148	1.508	0.122	0.095											*nq*	*nq*	0.034	0.078	0.060
FSV-BQ																									
FSV-BR																									
FSV-BS						0.444	0.250	1.200	0.174	0.183															
FSV-BT						0.325	0.128	0.625	0.119	0.123	0.294	0.113	0.556	0.107	0.111	0.025	0.014	0.061	0.011	0.010	0.017	0.007	0.027	0.078	0.080
FSV-BU						0.299	0.171	0.582	0.121	0.145											0.013	nd	0.033	0.062	0.077
FSV-BV						0.367	0.175	0.775	0.129	0.130											0.011	0.006	0.027	0.061	0.062
FSV-BW						0.371	0.152	0.790	0.120	0.130											*nq*	*nq*	0.030	0.112	0.121
FSV-CC																									
FSV-CE						0.304	0.067	0.768	!0.057	0.112															
FSV-CF																									
FSV-CG	*nd*	*nd*	0.212	*nd*	*nd*	0.288	0.132	0.596	0.105	0.111	0.268	0.119	0.552	0.100	0.107	0.019	0.013	0.043	0.005	0.005	0.016	0.009	0.043	0.088	0.095
FSV-CI						≥0.272	≥0.079	≥0.624	≥0.093	≥0.102	0.272	0.079	0.624	0.093	0.102						0.013	0.003	0.026	0.058	0.063
FSV-CP						0.358	0.169	0.670	0.145	0.144											0.018	0.011	0.044	0.101	0.100
FSV-CS						0.424	0.234	0.844	0.148	0.149											0.017	0.011	0.041	0.091	0.094
FSV-CT						0.353	0.155	0.830	0.135	0.134															
FSV-CW	0.029	0.004	0.150	0.014	0.020	≥0.380	≥0.182	≥0.804	≥0.107	≥0.115	0.380	0.182	0.804	0.107	0.115						0.031	0.019	0.051	0.089	0.089
FSV-CZ						0.280	0.163	0.534	0.186	0.199															
FSV-DB	*nq*	*nq*	0.176	0.272	0.103	0.270	0.114	0.534	0.098	0.119															
FSV-DF																									
FSV-DI	0.055	0.035	0.144	0.048	0.050	0.300	0.091	0.663	0.125	0.122															
FSV-DQ						0.290	0.104	0.651	0.133	0.145											0.023	0.006	0.035	0.073	0.087
FSV-DV																									
N	6	6	8	7	7	25	25	25	24	25	8	8	8	8	8	5	5	6	5	5	17	14	19	20	20
Min	0.029	0.004	0.123	0.014	0.020	0.270	0.067	0.534	0.073	0.082	0.268	0.069	0.552	0.093	0.102	0.019	0.006	0.043	0.005	0.005	0.010	0.003	0.026	0.058	0.060
Median	0.049	0.032	0.156	0.051	0.054	0.324	0.140	0.670	0.121	0.123	0.287	0.116	0.587	0.105	0.109	0.022	0.013	0.052	0.007	0.006	0.016	0.007	0.035	0.078	0.081
Max	0.072	0.048	0.212	0.272	0.103	0.529	0.250	1.508	0.186	0.199	0.380	0.182	0.804	0.130	0.134	0.032	0.025	0.063	0.011	0.013	0.031	0.019	0.051	0.112	0.121
SD	0.009	0.012	0.025	0.021	0.016	0.046	0.036	0.133	0.019	0.023	0.027	0.023	0.065	0.005	0.007	0.002	0.006	0.010	0.004	0.004	0.004	0.004	0.008	0.015	0.013
CV	19	38	16	41	30	14	25	20	15	19	9	20	11	5	6	10	46	20	64	62	28	56	24	19	16
N_{past}	0	10	12	5	6	32	30	30	26	26	5	10	10	12	12	5	7	7	6	6	16	20	24	23	23
$Median_{past}$		0.036	0.140	0.072	0.066	0.353	0.150	0.681	0.111	0.120	0.326	0.148	0.635	0.110	0.118	0.019	0.015	0.052	0.006	0.007	0.015	0.009	0.035	0.072	0.078
SD_{past}		0.013	0.021	0.024	0.026	0.047	0.029	0.086	0.015	0.015	0.029	0.019	0.048	0.010	0.011	0.010	0.008	0.017	0.002	0.002	0.006	0.004	0.009	0.012	0.011
NIST						0.329	0.173	0.755	0.125	0.128															
S_{rep}						0.008	0.002	0.014	0.002	0.001															
S_{het}						0.002	0.000	0.012	0.001	0.000															
S_{NIST}						0.008	0.002	0.018	0.002	0.001															
NAV	0.049	0.032	0.156	0.051	0.054	0.326	0.156	0.713	0.123	0.125	0.287	0.116	0.587	0.105	0.109	0.022	0.013	0.052	0.007	0.006	0.016	0.007	0.035	0.078	0.081
NAU	0.017	0.016	0.031	0.021	0.018	0.047	0.042	0.146	0.020	0.024	0.031	0.023	0.065	0.013	0.014	0.008	0.006	0.021	0.004	0.004	0.006	0.004	0.011	0.023	0.024

Round Robin LIX Laboratory Results

All Results in µg/mL

Lab	Total Lycopene					trans-Lycopene					Total β-Cryptoxanthin					Total α-Cryptoxanthin				
	319	320	321	322	323	319	320	321	322	323	319	320	321	322	323	319	320	321	322	323
FSV-BA	0.200	0.023	0.165	0.518	0.549	0.115	0.010	0.073	0.297	0.318	0.069	0.002	0.026	0.072	0.074	0.032	*nd*	0.012	0.035	0.035
FSV-BB	0.178	0.027	0.137	0.429	0.453	0.082	0.010	0.051	0.210	0.226	0.050	*nd*	0.019	0.051	0.054	0.021	*nd*	0.006	0.022	0.023
FSV-BC																				
FSV-BD																				
FSV-BE																				
FSV-BF	0.139	0.035	0.144	0.499	0.526						0.045	0.009	0.015	0.046	0.047					
FSV-BG	0.214	0.043	0.202	0.542	0.550	0.106	0.014	0.077	0.281	0.287	0.056	0.006	0.024	0.064	0.075					
FSV-BH	0.202	0.035	0.151	0.532	0.555						0.058	*nq*	*nq*	0.061	0.064					
FSV-BI	0.157	0.027	0.125	0.375	0.397						0.054	0.004	0.018	0.048	0.051					
FSV-BJ	0.194	0.024	0.156	0.552	0.580						0.049	*nq*	0.015	0.049	0.053					
FSV-BK																				
FSV-BL																				
FSV-BM																				
FSV-BN	0.173	0.037	0.143	0.421	0.442	0.093	0.021	0.065	0.230	0.239	0.054	0.009	0.020	0.056	0.059	0.028	*nd*	0.012	0.030	0.031
FSV-BO	0.183	*nq*	0.163	0.467	0.509						0.021	*nq*	*nq*	0.018	0.018					
FSV-BP	0.161	*nq*	0.398	0.500	0.427						0.135	*nq*	0.098	0.102	0.092					
FSV-BQ																				
FSV-BR																				
FSV-BS	0.142	0.031	0.170	0.511	0.527															
FSV-BT	0.201	0.030	0.164	0.474	0.487	0.087	0.012	0.060	0.201	0.206	0.053	0.007	0.021	0.048	0.051	0.019	0.003	0.011	0.021	0.023
FSV-BU	0.198	0.067	0.169	0.434	0.541						0.058	*nd*	0.027	0.054	0.055					
FSV-BV	0.211	0.035	0.162	0.550	0.552						0.052	0.003	0.015	0.051	0.051					
FSV-BW	0.190	0.015	0.153	0.520	0.580						0.063	*nq*	0.015	0.060	0.063					
FSV-CC																				
FSV-CE																				
FSV-CF																				
FSV-CG	0.190	0.029	0.152	0.469	0.507	0.110	0.012	0.073	0.286	0.313	0.071	0.005	0.027	0.075	0.081					
FSV-CI																				
FSV-CP	0.175	0.033	0.128	0.477	0.483						0.077	0.005	0.025	0.079	0.082					
FSV-CS	0.282	0.060	0.249	0.705	0.718						0.054	0.007	0.023	0.055	0.057					
FSV-CT	0.201	*nq*	0.146	0.494	0.501						0.049	*nq*	0.020	0.057	0.057					
FSV-CW						0.094	0.022	0.067	0.208	0.221	0.056	<0.005	0.023	0.057	0.057					
FSV-CZ																				
FSV-DB	0.183	*nq*	0.134	0.441	0.517						0.049	*nq*	0.014	0.047	0.055					
FSV-DF																				
FSV-DI	0.163	0.030	0.120	0.466	0.468															
FSV-DQ	0.159	0.022	0.117	0.405	0.465						0.043	0.006	0.017	0.063	0.062					
FSV-DV																				
N	22	18	22	22	22	7	7	7	7	7	21	11	19	21	21	4	1	4	4	4
Min	0.139	0.015	0.117	0.375	0.397	0.082	0.010	0.051	0.201	0.206	0.021	0.002	0.014	0.018	0.018	0.019		0.006	0.021	0.023
Median	0.187	0.031	0.153	0.485	0.513	0.094	0.012	0.067	0.230	0.239	0.054	0.006	0.020	0.056	0.057	0.025	0.003	0.012	0.026	0.027
Max	0.282	0.067	0.398	0.705	0.718	0.115	0.022	0.077	0.297	0.318	0.135	0.009	0.098	0.102	0.092	0.032		0.012	0.035	0.035
SD	0.026	0.006	0.019	0.053	0.058	0.013	0.005	0.008	0.055	0.057	0.007	0.002	0.006	0.010	0.008	0.006		0.002	0.007	0.007
CV	14	19	13	11	11	14	40	12	24	24	12	30	32	19	14	26		15	27	25
N_{past}	16	21	24	22	22	0	8	8	9	9	11	15	20	23	23	0	7	10	6	10
$Median_{past}$	0.183	0.029	0.133	0.477	0.504		0.019	0.074	0.252	0.270	0.065	0.007	0.021	0.051	0.054		0.005	0.012	0.023	0.027
SD_{past}	0.042	0.010	0.041	0.060	0.070		0.007	0.017	0.043	0.049	0.012	0.003	0.006	0.008	0.010			0.002	0.007	0.007
NIST											0.042	*nq*	*nq*	0.049	0.051					
S_{rep}											0.003			0.002	0.004					
S_{het}											0.000			0.002	0.002					
S_{NIST}											0.003			0.003	0.005					
NAV	0.187	0.031	0.153	0.485	0.513	0.094	0.012	0.067	0.230	0.239	0.048	0.006	0.020	0.053	0.054	0.025		0.012	0.026	0.027
NAU	0.046	0.011	0.039	0.101	0.106	0.016	0.006	0.012	0.055	0.057	0.016	0.002	0.006	0.014	0.014					

Round Robin LIX Laboratory Results

All Results in µg/mL

	Total Lutein					Total Zeaxanthin					Total Lutein&Zeaxanthin				
Lab	319	320	321	322	323	319	320	321	322	323	319	320	321	322	323
FSV-BA											0.109	0.022	0.081	0.114	0.119
FSV-BB	0.089	0.012	0.065	0.085	0.089	0.024	0.004	0.022	0.041	0.043	0.113	0.016	0.087	0.126	0.132
FSV-BC															
FSV-BD															
FSV-BE															
FSV-BF											0.086	0.017	0.074	0.110	0.119
FSV-BG	0.086	0.015	0.060	0.114	0.134	0.018	0.005	0.020	0.029	0.032	0.093	0.016	0.066	0.126	0.147
FSV-BH	0.073	0.009	0.040	0.065	0.063	0.026	*nq*	0.017	0.034	0.033	0.099	0.009	0.054	0.099	0.096
FSV-BI	0.088	0.016	0.058	0.079	0.085	0.023	0.004	0.016	0.028	0.030	*0.111*	*0.020*	*0.074*	*0.107*	*0.115*
FSV-BJ	0.063	0.014	0.046	0.075	0.082										
FSV-BK															
FSV-BL															
FSV-BM															
FSV-BN	0.084	0.030	0.063	0.079	0.082	0.024	*nd*	*nd*	0.022	0.022	0.108	0.030	0.063	0.101	0.104
FSV-BO	0.096	*nq*	0.051	0.080	0.090	0.012	*nq*	0.019	0.054	0.055	0.109	*nq*	0.071	0.134	0.144
FSV-BP											0.090	*nq*	0.162	0.110	0.103
FSV-BQ															
FSV-BR															
FSV-BS											0.096	*nd*	0.089	0.094	0.117
FSV-BT	0.066	0.024	0.050	0.074	0.082	0.014	0.006	0.011	0.021	0.033	0.071	0.026	0.054	0.087	0.110
FSV-BU											0.089	0.017	0.068	0.106	0.113
FSV-BV											0.105	0.014	0.072	0.122	0.124
FSV-BW											0.095	*nq*	0.054	0.082	0.098
FSV-CC															
FSV-CE															
FSV-CF															
FSV-CG											0.118	0.021	0.083	0.136	0.147
FSV-CI	0.057	0.007	0.033	0.047	0.051	0.017	0.003	0.012	0.022	0.024	*0.074*	*0.010*	*0.045*	*0.069*	*0.075*
FSV-CP											0.144	0.025	0.099	0.171	0.173
FSV-CS	0.086	0.017	0.051	0.069	0.071	0.023	0.008	0.032	0.049	0.052	*0.110*	*0.025*	*0.083*	*0.119*	*0.123*
FSV-CT	0.116	*nq*	0.081	0.137	0.139	0.026	*nq*	*nq*	0.030	0.026	0.142	*nq*	0.081	0.167	0.165
FSV-CW	0.087	0.021	0.062	0.082	0.082	0.019	0.008	0.021	0.029	0.030	*0.106*	*0.029*	*0.083*	*0.112*	*0.111*
FSV-CZ															
FSV-DB											0.084	*nq*	0.060	0.093	0.112
FSV-DF															
FSV-DI	0.065	0.011	0.039	0.060	0.064										
FSV-DQ	0.070	*nd*	0.034	0.071	0.083	0.052	0.004	0.016	0.031	0.024	*0.122*	*≥0.004*	*0.050*	*0.102*	*0.107*
FSV-DV															
N	14	11	14	14	14	12	8	10	12	12	22	15	22	22	22
Min	0.057	0.007	0.033	0.047	0.051	0.012	0.003	0.011	0.021	0.022	0.071	0.009	0.045	0.069	0.075
Median	0.085	0.015	0.051	0.077	0.082	0.023	0.005	0.018	0.030	0.031	0.105	0.020	0.073	0.110	0.116
Max	0.116	0.030	0.081	0.137	0.139	0.052	0.008	0.032	0.054	0.055	0.144	0.030	0.162	0.171	0.173
SD	0.015	0.006	0.015	0.009	0.011	0.005	0.002	0.004	0.007	0.007	0.015	0.007	0.016	0.019	0.017
CV	18	38	29	12	13	22	40	21	23	24	14	34	23	17	14
N_{past}	7	11	13	15	15	0	8	10	13	13	8	18	20	23	23
$Median_{past}$	0.066	0.014	0.052	0.078	0.079		0.006	0.018	0.030	0.032	0.115	0.021	0.072	0.108	0.112
SD_{past}	0.019	0.004	0.011	0.017	0.016		0.003	0.007	0.007	0.007	0.017	0.009	0.013	0.017	0.017
NIST	0.058	*nq*	0.043	0.068	0.067	0.020	*nq*	*nq*	0.022	0.023	*0.077*	*nq*	*≥0.043*	*0.090*	*0.090*
S_{rep}	0.004		0.003	0.000	0.002	0.000			0.002	0.001	0.004			0.002	0.001
S_{het}	0.006		0.004	0.001	0.002	0.001			0.001	0.002	0.006			0.000	0.004
S_{NIST}	0.007		0.005	0.001	0.003	0.001			0.002	0.002	0.008			0.002	0.004
NAV	0.071	0.015	0.047	0.073	0.074	0.021	0.005	0.018	0.026	0.027	0.091	0.020	0.073	0.100	0.103
NAU	0.026	0.006	0.016	0.017	0.019	0.007	0.003	0.006	0.010	0.010	0.030	0.007	0.016	0.027	0.030

Round Robin LIX Laboratory Results

All Results in µg/mL

	Coenzyme Q10					Ubiquinol					Ubiquinone					25-hydroxyvitamin D				
Lab	319	320	321	322	323	319	320	321	322	323	319	320	321	322	323	319	320	321	322	323
FSV-BA																0.0048	0.0070	0.0166	0.0158	0.0154
FSV-BB																				
FSV-BC																				
FSV-BD																				
FSV-BE																				
FSV-BF																				
FSV-BG																				
FSV-BH																0.0040	0.0070	0.0200	0.0200	0.0210
FSV-BI																				
FSV-BJ	0.607	0.140	0.528	0.675	0.730															
FSV-BK																				
FSV-BL																				
FSV-BM																				
FSV-BN																0.0039	0.0274	0.0167	0.0176	*nd*
FSV-BO																				
FSV-BP																				
FSV-BQ																				
FSV-BR																				
FSV-BS																				
FSV-BT																				
FSV-BU																				
FSV-BV																				
FSV-BW	0.155	0.026	0.125	0.137	0.165	0.136	0.014	0.099	0.100	0.129	0.018	0.012	0.026	0.037	0.036					
FSV-CC																				
FSV-CE																				
FSV-CF																				
FSV-CG																				
FSV-CI																				
FSV-CP																				
FSV-CS																				
FSV-CT																				
FSV-CW	0.780	0.310	0.780	0.740	0.760															
FSV-CZ	0.720	0.230	0.620	0.730	0.770															
FSV-DB																				
FSV-DF																				
FSV-DI	0.694	0.110	0.595	0.660	0.655	0.700	0.076	0.533	0.622	0.749	0.140	0.050	0.188	0.263	0.262	0.0044	0.0033	0.0027	0.0017	0.0015
FSV-DQ																				
FSV-DV																				
N	5	5	5	5	5	2	2	2	2	2	2	2	2	2	2	4	4	4	4	3
Min	0.155	0.026	0.125	0.137	0.165	0.136	0.014	0.099	0.100	0.129	0.018	0.012	0.026	0.037	0.036	0.0039	0.0033	0.0027	0.0017	0.0015
Median	0.694	0.140	0.595	0.675	0.730	0.418	0.045	0.316	0.361	0.439	0.079	0.031	0.107	0.150	0.149	0.0042	0.0070	0.0167	0.0167	0.0154
Max	0.780	0.310	0.780	0.740	0.770	0.700	0.076	0.533	0.622	0.749	0.140	0.050	0.188	0.263	0.262	0.0048	0.0274	0.0200	0.0200	0.0210
SD	0.084	0.089	0.068	0.052	0.078											0.0004	0.0045	0.0033	0.0044	
CV	12	64	11	8	11											9	64	20	26	
N_{past}	0	6	7	5	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
$Median_{past}$		0.090	0.530	0.707	0.770															
SD_{past}		0.041	0.115	0.122	0.128															
NIST																				
S_{rep}																				
S_{het}																				
S_{NIST}																				
NAV	0.694	0.140	0.595	0.675	0.730											0.004	0.007	0.017	0.017	0.015
NAU	0.084	0.089	0.068	0.068	0.078															

Round Robin LIX Laboratory Results

All Results in µg/mL

Analytes Reported By One Laboratory

Analyte	Code	319	320	321	322	323
Phylloquinone (K1) x1000	FSV-CI	0.140	0.210	0.740	0.260	0.260
26-Cyclolycopene-15-diol	FSV-DQ	0.066	0.015	0.041	0.058	0.072

Legend

Term	Definition
N	Number of (non-NIST) quantitative values reported for this analyte
Min	Minimum (non-NIST) quantitative value reported
Median	Median (non-NIST) quantitative value reported
Max	Maximum (non-NIST) quantitative value reported
SD	Standard deviation for (non-NIST) results: 0.741*(3rd Quartile - 1st Quartile)
CV	Coefficient of Variation for (non-NIST) results: 100*SD/Median
N_{past}	Mean of N(s) from past RR(s)
$Median_{past}$	Mean of Median(s) from past RR(s)
SD_{past}	Pooled SD from past RR(s)
NIST	Mean of all analyses (vials x duplicates) reported by a NIST analyst
S_{rep}	Within-vial pooled standard deviation
S_{het}	Among-vial pooled standard deviation
S_{NIST}	Total standard deviation for NIST analyses: $(S_{rep}^2 + S_{het}^2)^{0.5}$
NAV	NIST Assigned Value = (Median + $Mean_{NIST}$)/2 for analytes reported by NIST analyst(s) = Median for analytes reported by ≥ 10 labs but not NIST
NAU	NIST Assigned Uncertainty: $(S^2 + S_{btw}^2)^{0.5}$ S is the maximum of (0.05*NAV, SD, S_{NIST}, eSD) and S_{btw} is the standard deviation between $Median_{part}$ and $Mean_{NIST}$. The expected long-term SD, eSD, 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 at or below the limit of quantification, x
≥x	Concentration greater than or equal to x
!	Non-quantitative value: heterogeneous serum, damaged sample, malfunction, etc.

Round Robin LIX Laboratory Results

Comparability Summary

Lab	TR	aT	g/bT	bC	tbC	aC	TLy	TbX	TLu	TZ	L&Z
FSV-BA	2	1	1	1	2	1	1	2	1	1	1
FSV-BB	1	1	2	1	2	1	1	1	2	1	1
FSV-BC	1										
FSV-BD	2	3									1
FSV-BE	1	1	1	1							
FSV-BF	1	2	1	2		1	1	1			1
FSV-BG	1	1	1	2		1	1	1	1	1	1
FSV-BH	2	1	1	1	1	1	1	1			
FSV-BI	2	1	2	1		1	1	1	1	1	1
FSV-BJ	1	1	1	1		1	1	1			
FSV-BK	2	2							1		
FSV-BL	1	1									
FSV-BM	2	1									2
FSV-BN	1	1	1	1	1	1	1	1			4
FSV-BO	2	2		3		2	1	3	2	1	1
FSV-BP	3	2		3		1	4	4			1
FSV-BQ	1	1							3	1	2
FSV-BR	1	2							1	3	1
FSV-BS	4	4	4	3			1		1	1	1
FSV-BT	3	2	1	1	1	1	1	1			
FSV-BU	1	1	2	1		1	2	1			
FSV-BV	4	2	2	1		1	1	1			1
FSV-BW	1	1	1	1		2	1	1			
FSV-CC	1	2									1
FSV-CE	2	1		2							1
FSV-CF	1	1									1
FSV-CG	1	2	1	1	1	1	1	2			
FSV-CI	1	1	1	2	1	1					1
FSV-CP		2	3	1		1	1	2			
FSV-CS	1	2	3	2		1	3	1	1	2	1
FSV-CT				1			1	1	1	3	2
FSV-CW	2	3	1	1	3	2		1	2	1	2
FSV-CZ	1	1		3							
FSV-DB	1	1	2	1			1	1			
FSV-DF	1								1		
FSV-DI	1	1	1	1			1		1	1	1
FSV-DQ		1	1	1		1	1	1			
FSV-DV	3	1									
NISTa	1	1	1	1				1	1	1	2
n	36	36	23	28	8	20	22	22	15	13	23

	TR	aT	g/bT	bC	tbC	aC	TLy	TbX	TLu	TZ	L&Z
% 1	61	61	65	68	63	85	86	77	73	77	74
% 2	25	31	22	18	25	15	5	14	20	8	22
% 3	8	6	9	14	13	0	5	5	7	15	0
% 4	6	3	4	0	0	0	5	5	0	0	4

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.

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

$$C = \mathrm{Concordance} = \sum_i^{N_{you}} \frac{\mathrm{You}_i - \mathrm{Median}_i}{\mathrm{NAU}_i} \Big/ N_{you}$$

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

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 RR59

Each participant in RR59 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 RR59:

- Total 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 LIX Report: FSV-BA

Summary

Analyte	Serum 319 You	Serum 319 NAV	Serum 319 n	Serum 320 You	Serum 320 NAV	Serum 320 n	Serum 321 You	Serum 321 NAV	Serum 321 n	Serum 322 You	Serum 322 NAV	Serum 322 n	Serum 323 You	Serum 323 NAV	Serum 323 n
Total Retinol	0.375	0.376	33	0.230	0.185	33	0.878	0.744	33	0.670	0.622	32	0.705	0.653	33
Retinyl Palmitate	0.08	0.11	10	0.0	0.0	10	0.2	0.3	10	0.11	0.09	10	0.11	0.09	10
α-Tocopherol	4.35	4.28	35	1.80	3.30	35	15.14	14.34	35	9.92	9.93	34	10.46	10.19	35
γ/β-Tocopherol	1.686	1.653	22	0.486	0.683	22	2.808	2.662	22	1.824	1.766	22	1.930	1.804	22
δ-Tocopherol	0.040	0.049	6	0.029	0.032	6	0.150	0.156	8	0.051	0.051	7	0.058	0.054	7
Total β-Carotene	0.354	0.326	25	0.103	0.156	25	0.750	0.713	25	0.137	0.123	24	0.140	0.125	25
trans-β-Carotene	0.332	0.287	8	0.097	0.116	8	0.697	0.587	8	0.130	0.105	8	0.134	0.109	8
Total cis-β-Carotene	0.022	0.022	5	0.006	0.013	5	0.053	0.052	6	0.007	0.007	5	0.005	0.006	5
Total α-Carotene	0.015	0.016	17	0.003	0.007	14	0.032	0.035	19	0.079	0.078	20	0.082	0.081	20
Total Lycopene	0.200	0.187	22	0.023	0.031	18	0.165	0.153	22	0.518	0.485	22	0.549	0.513	22
trans-Lycopene	0.115	0.094	7	0.010	0.012	7	0.073	0.067	7	0.297	0.230	7	0.318	0.239	7
Total β-Cryptoxanthin	0.069	0.048	21	0.002	0.006	11	0.026	0.020	19	0.072	0.053	21	0.074	0.054	21
Total α-Cryptoxanthin	0.032	0.025	4	nd		1	0.012	0.012	4	0.035	0.026	4	0.035	0.027	4
Total Lutein&Zeaxanthin	0.109	0.091	22	0.02	0.02	15	0.08	0.07	22	0.114	0.100	22	0.12	0.10	22
25-hydroxyvitamin D	0.005	0.004	4	0.007	0.007	4	0.017	0.017	4	0.016	0.017	4	0.015	0.015	3

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
nd : Not detected

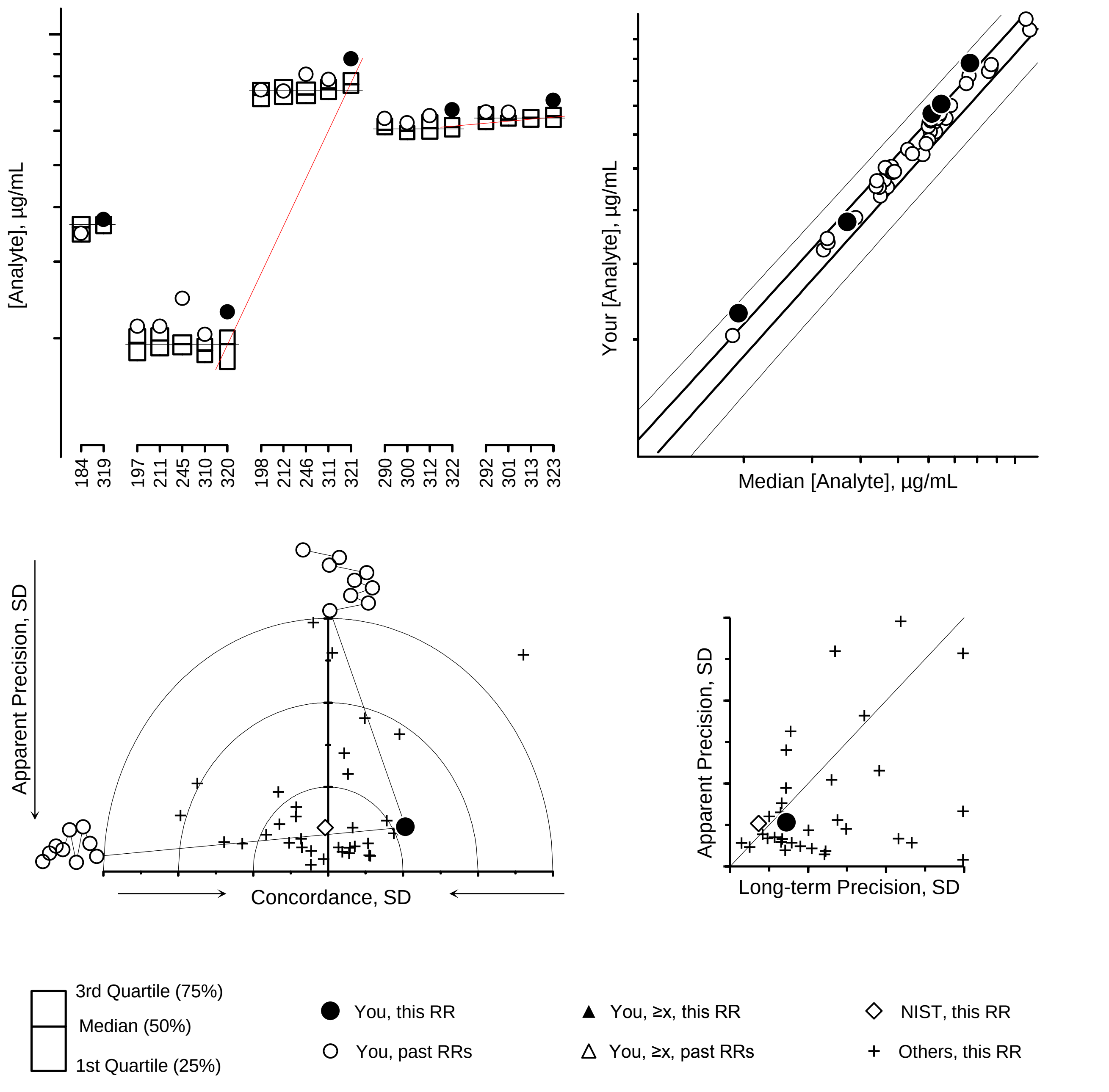
Please check our records against your records. Send corrections and/or updates to...

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 LIX Report: FSV-BA

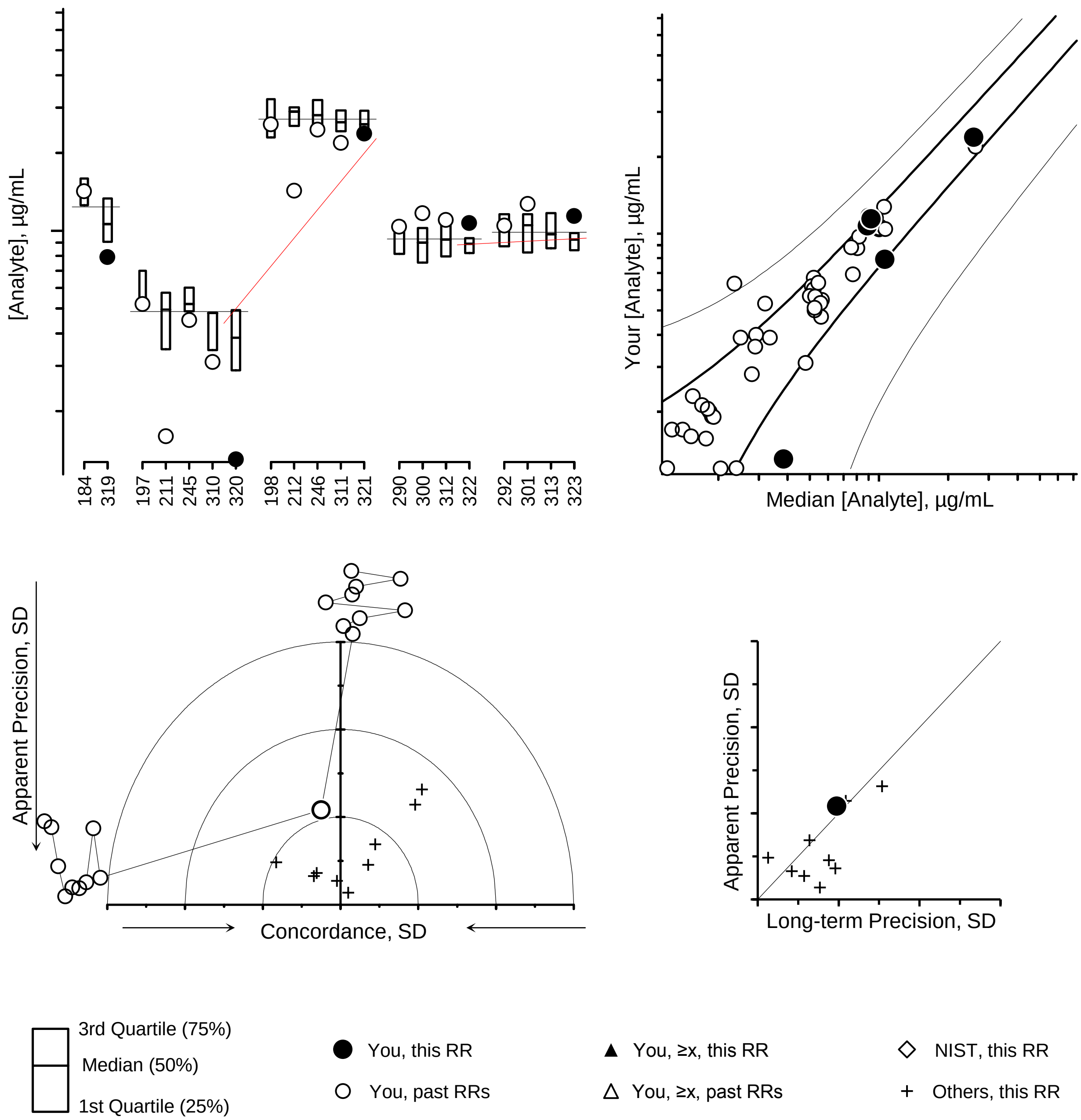
Total Retinol



Serum	History	Comments
#319	Lyophilized - 28:184	Native, single-source
#320	Lyophilized - 31:197, 35:211, 43:245, 57:310	Same as #321, 1:3 diluted with stripped serum
#321	Lyophilized - 31:198, 35:212, 43:246, 57:311	Augmented, multi-source
#322	Lyophilized - 53:290, 55:300, 57:312	Native, single-source
#323	Fresh-frozen: 53:292, 55:301, 57:313	Native, single-source, hemolyzed

Individualized RR LIX Report: FSV-BA

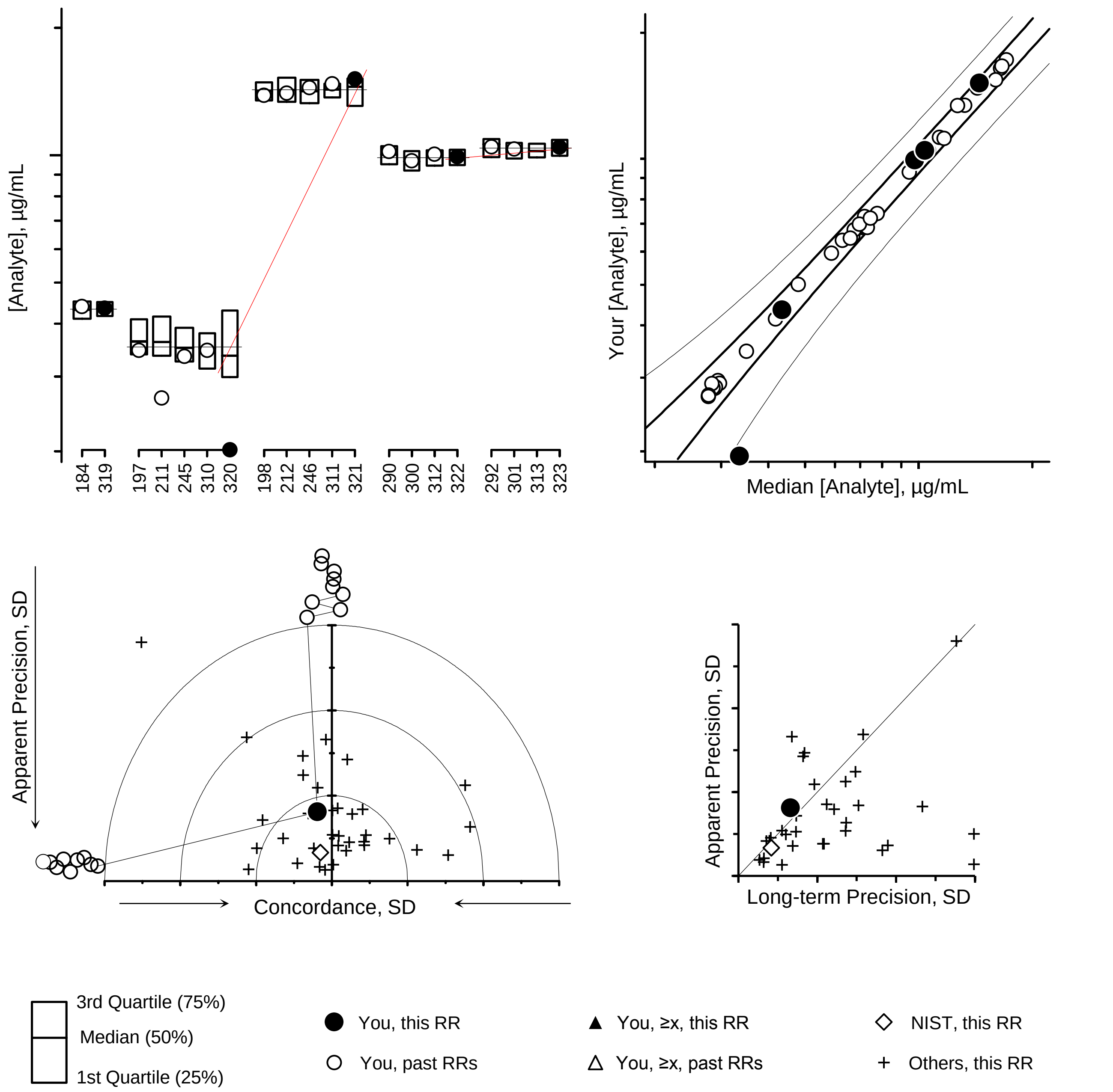
Retinyl Palmitate



Serum	History	Comments
#319	Lyophilized - 28:184	Native, single-source
#320	Lyophilized - 31:197, 35:211, 43:245, 57:310	Same as #321, 1:3 diluted with stripped serum
#321	Lyophilized - 31:198, 35:212, 43:246, 57:311	Augmented, multi-source
#322	Lyophilized - 53:290, 55:300, 57:312	Native, single-source
#323	Fresh-frozen: 53:292, 55:301, 57:313	Native, single-source, hemolyzed

Individualized RR LIX Report: FSV-BA

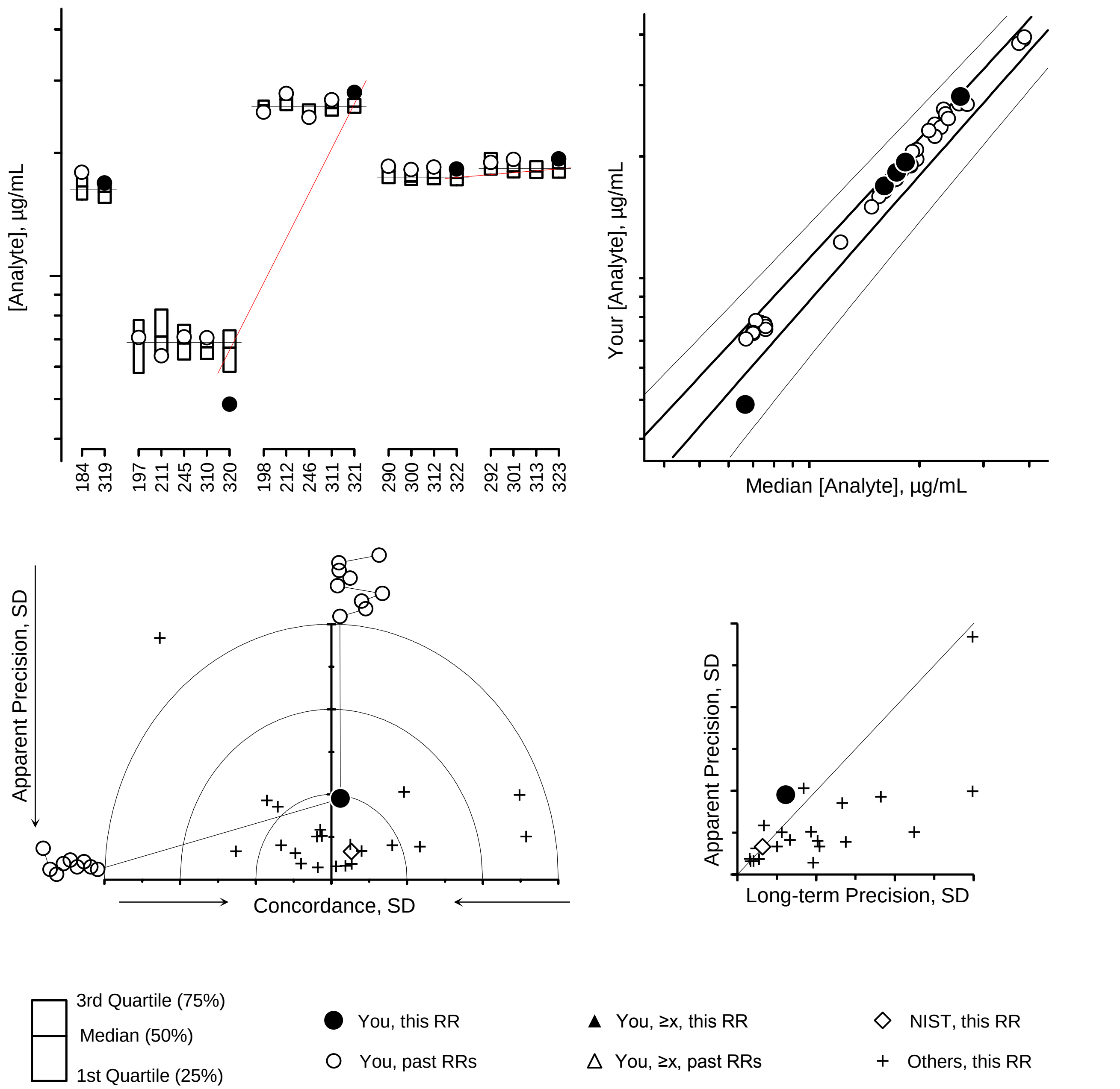
α-Tocopherol



Serum	History	Comments
#319	Lyophilized - 28:184	Native, single-source
#320	Lyophilized - 31:197, 35:211, 43:245, 57:310	Same as #321, 1:3 diluted with stripped serum
#321	Lyophilized - 31:198, 35:212, 43:246, 57:311	Augmented, multi-source
#322	Lyophilized - 53:290, 55:300, 57:312	Native, single-source
#323	Fresh-frozen: 53:292, 55:301, 57:313	Native, single-source, hemolyzed

Individualized RR LIX Report: FSV-BA

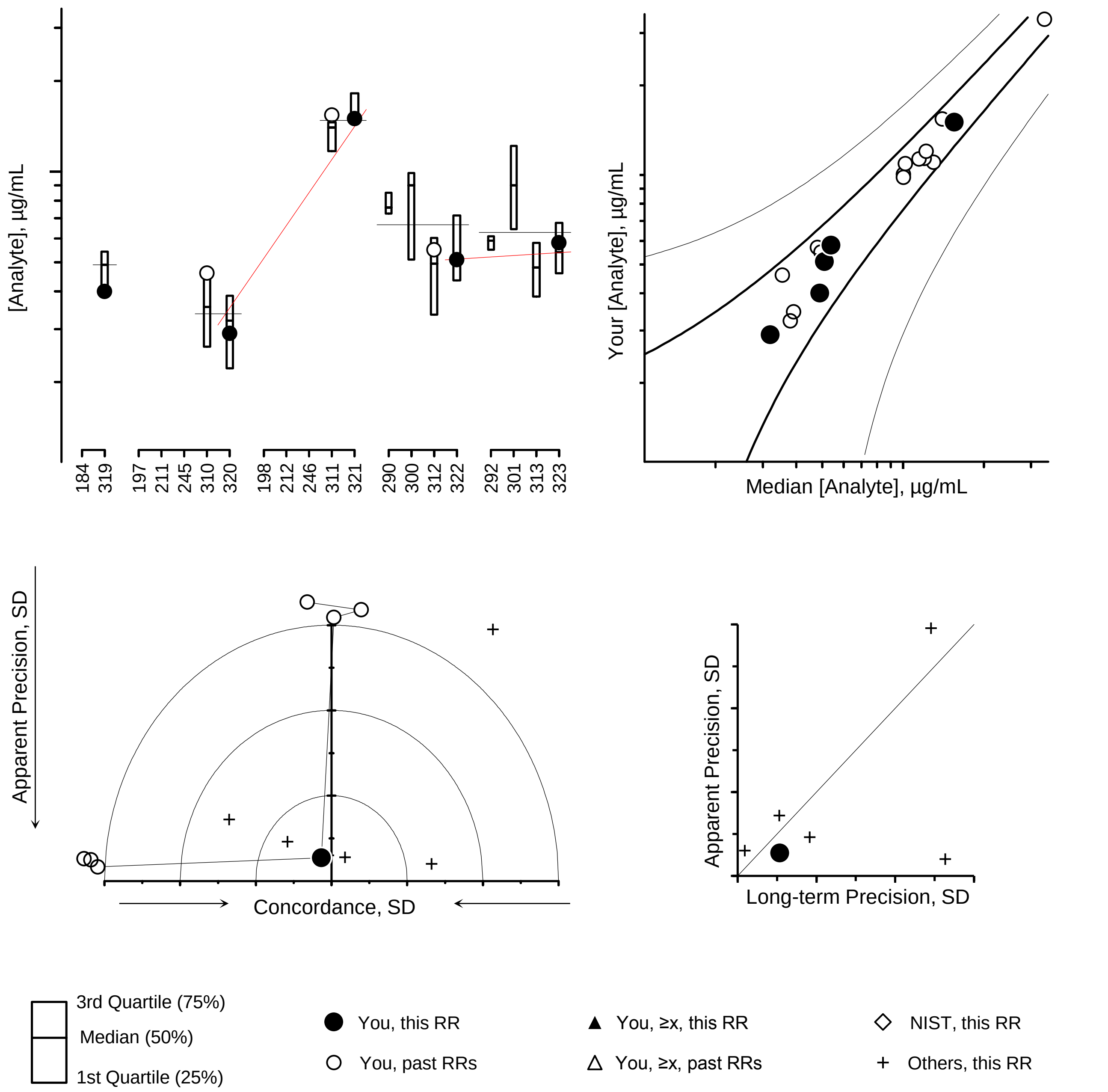
γ/β-Tocopherol



Serum	History	Comments
#319	Lyophilized - 28:184	Native, single-source
#320	Lyophilized - 31:197, 35:211, 43:245, 57:310	Same as #321, 1:3 diluted with stripped serum
#321	Lyophilized - 31:198, 35:212, 43:246, 57:311	Augmented, multi-source
#322	Lyophilized - 53:290, 55:300, 57:312	Native, single-source
#323	Fresh-frozen: 53:292, 55:301, 57:313	Native, single-source, hemolyzed

Individualized RR LIX Report: FSV-BA

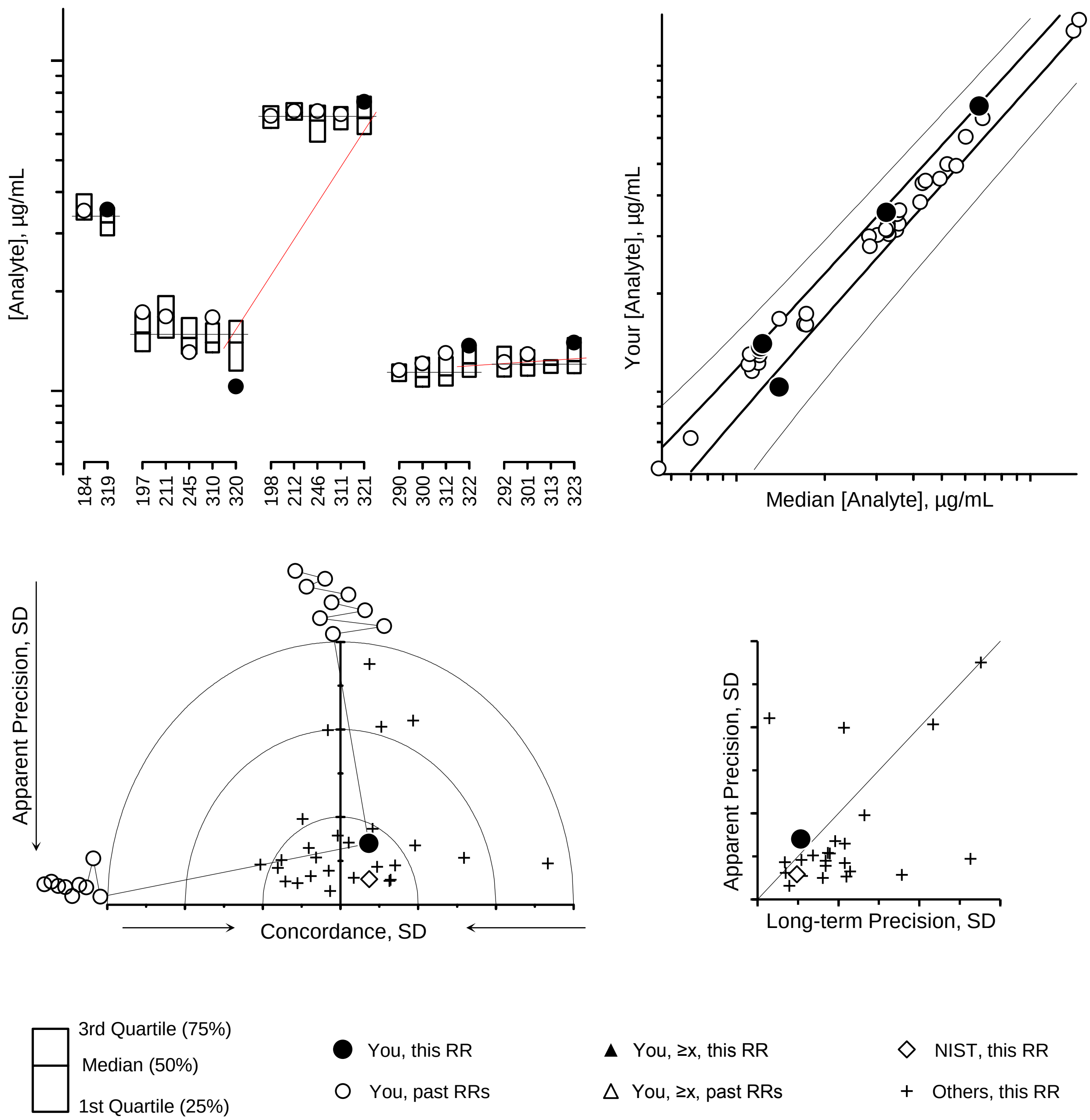
δ-Tocopherol



Serum	History	Comments
#319	Lyophilized - 28:184	Native, single-source
#320	Lyophilized - 31:197, 35:211, 43:245, 57:310	Same as #321, 1:3 diluted with stripped serum
#321	Lyophilized - 31:198, 35:212, 43:246, 57:311	Augmented, multi-source
#322	Lyophilized - 53:290, 55:300, 57:312	Native, single-source
#323	Fresh-frozen: 53:292, 55:301, 57:313	Native, single-source, hemolyzed

Individualized RR LIX Report: FSV-BA

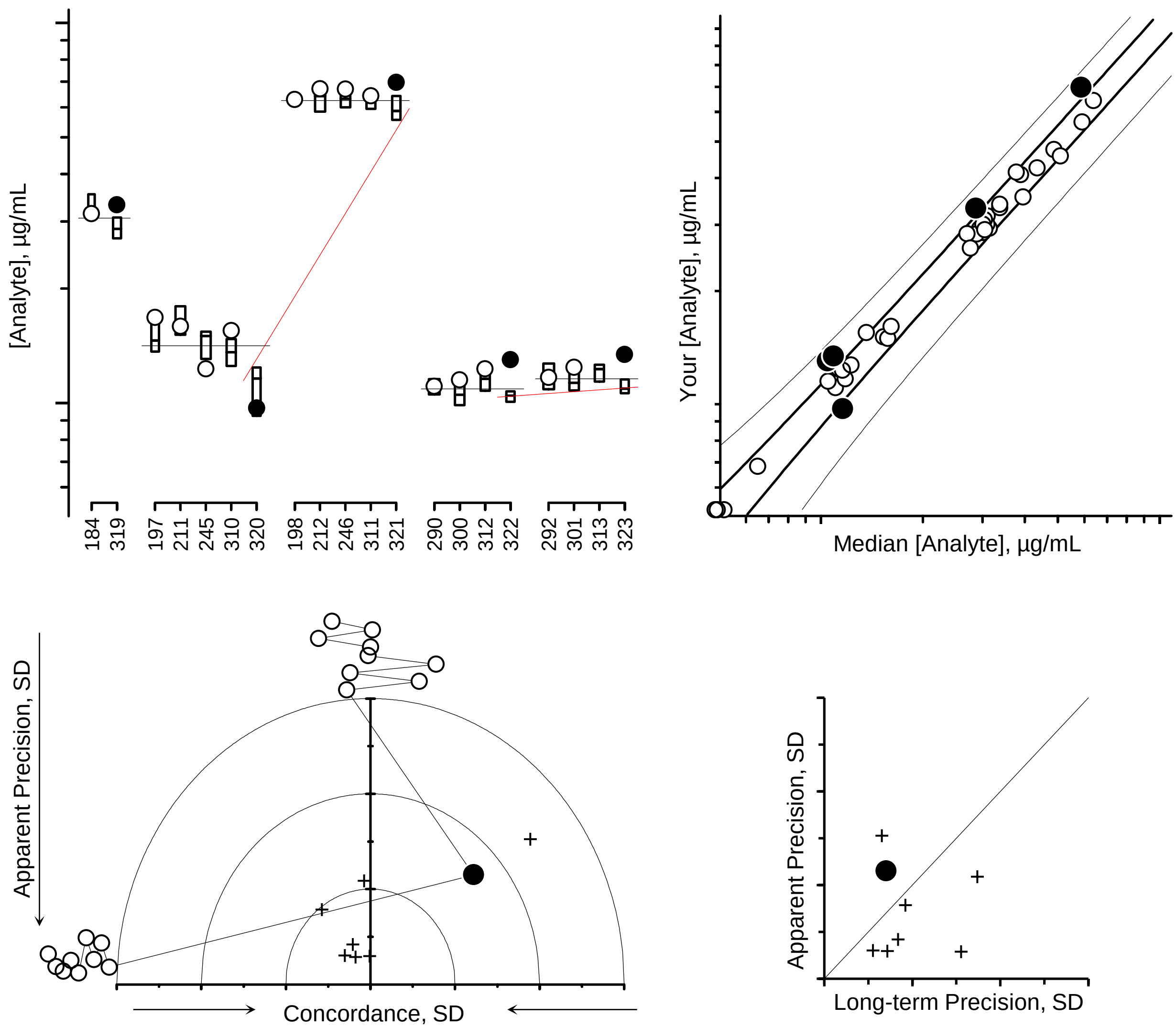
Total β-Carotene



Serum	History	Comments
#319	Lyophilized - 28:184	Native, single-source
#320	Lyophilized - 31:197, 35:211, 43:245, 57:310	Same as #321, 1:3 diluted with stripped serum
#321	Lyophilized - 31:198, 35:212, 43:246, 57:311	Augmented, multi-source
#322	Lyophilized - 53:290, 55:300, 57:312	Native, single-source
#323	Fresh-frozen: 53:292, 55:301, 57:313	Native, single-source, hemolyzed

Individualized RR LIX Report: FSV-BA

trans-β-Carotene



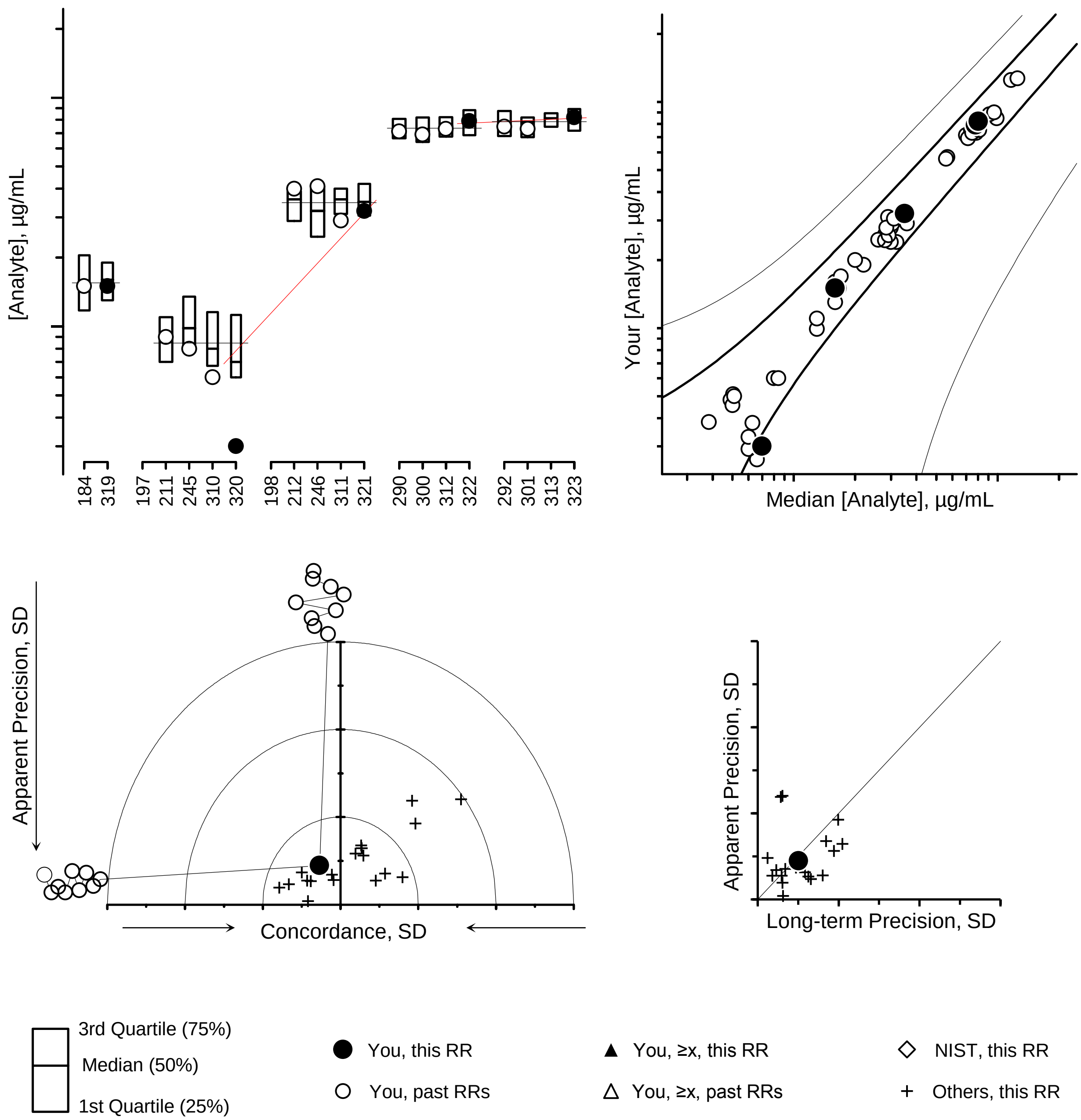
3rd Quartile (75%)
Median (50%)
1st Quartile (25%)

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

Serum	History	Comments
#319	Lyophilized - 28:184	Native, single-source
#320	Lyophilized - 31:197, 35:211, 43:245, 57:310	Same as #321, 1:3 diluted with stripped serum
#321	Lyophilized - 31:198, 35:212, 43:246, 57:311	Augmented, multi-source
#322	Lyophilized - 53:290, 55:300, 57:312	Native, single-source
#323	Fresh-frozen: 53:292, 55:301, 57:313	Native, single-source, hemolyzed

Individualized RR LIX Report: FSV-BA

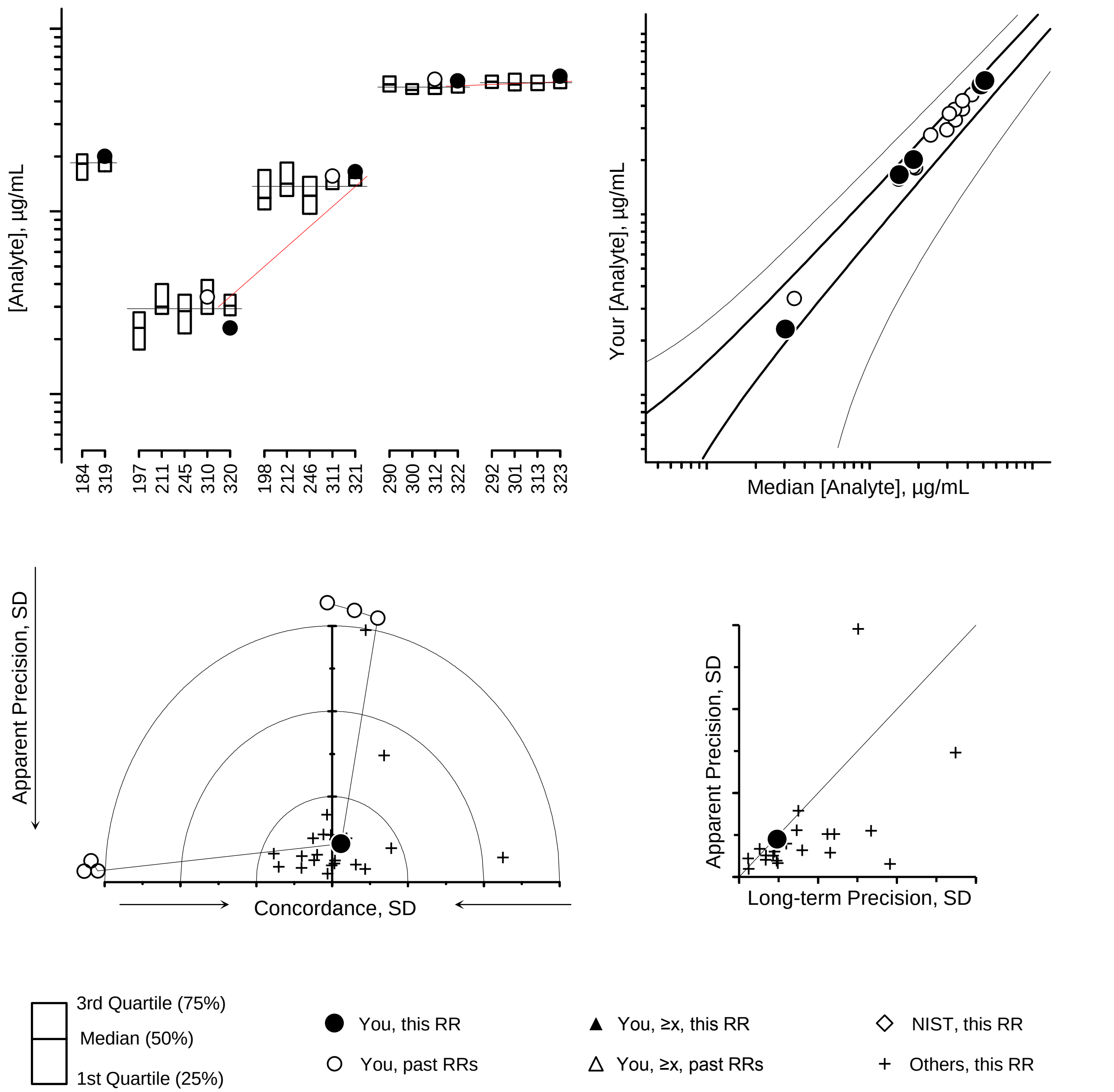
Total α-Carotene



Serum	History	Comments
#319	Lyophilized - 28:184	Native, single-source
#320	Lyophilized - 31:197, 35:211, 43:245, 57:310	Same as #321, 1:3 diluted with stripped serum
#321	Lyophilized - 31:198, 35:212, 43:246, 57:311	Augmented, multi-source
#322	Lyophilized - 53:290, 55:300, 57:312	Native, single-source
#323	Fresh-frozen: 53:292, 55:301, 57:313	Native, single-source, hemolyzed

Individualized RR LIX Report: FSV-BA

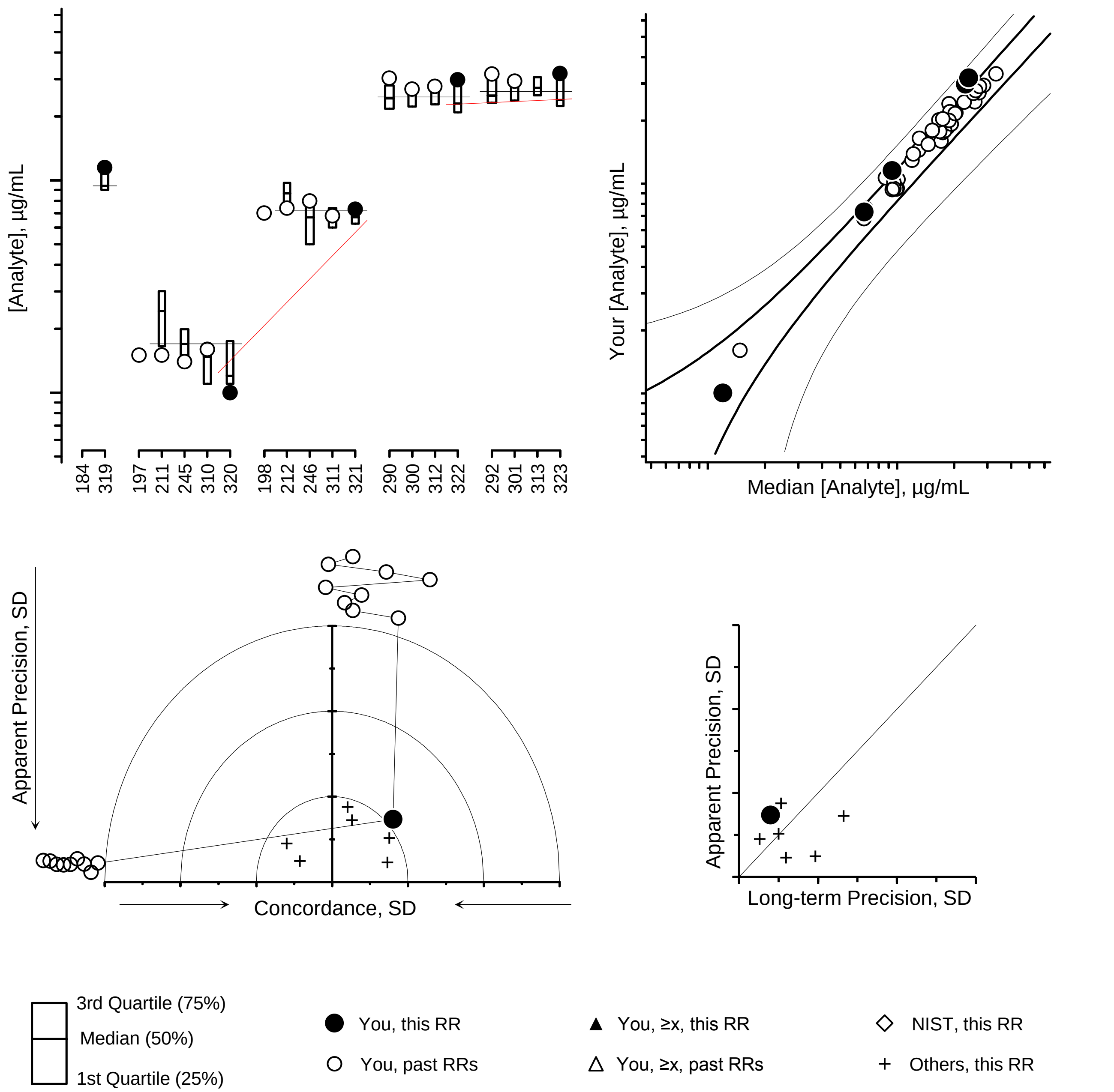
Total Lycopene



Serum	History	Comments
#319	Lyophilized - 28:184	Native, single-source
#320	Lyophilized - 31:197, 35:211, 43:245, 57:310	Same as #321, 1:3 diluted with stripped serum
#321	Lyophilized - 31:198, 35:212, 43:246, 57:311	Augmented, multi-source
#322	Lyophilized - 53:290, 55:300, 57:312	Native, single-source
#323	Fresh-frozen: 53:292, 55:301, 57:313	Native, single-source, hemolyzed

Individualized RR LIX Report: FSV-BA

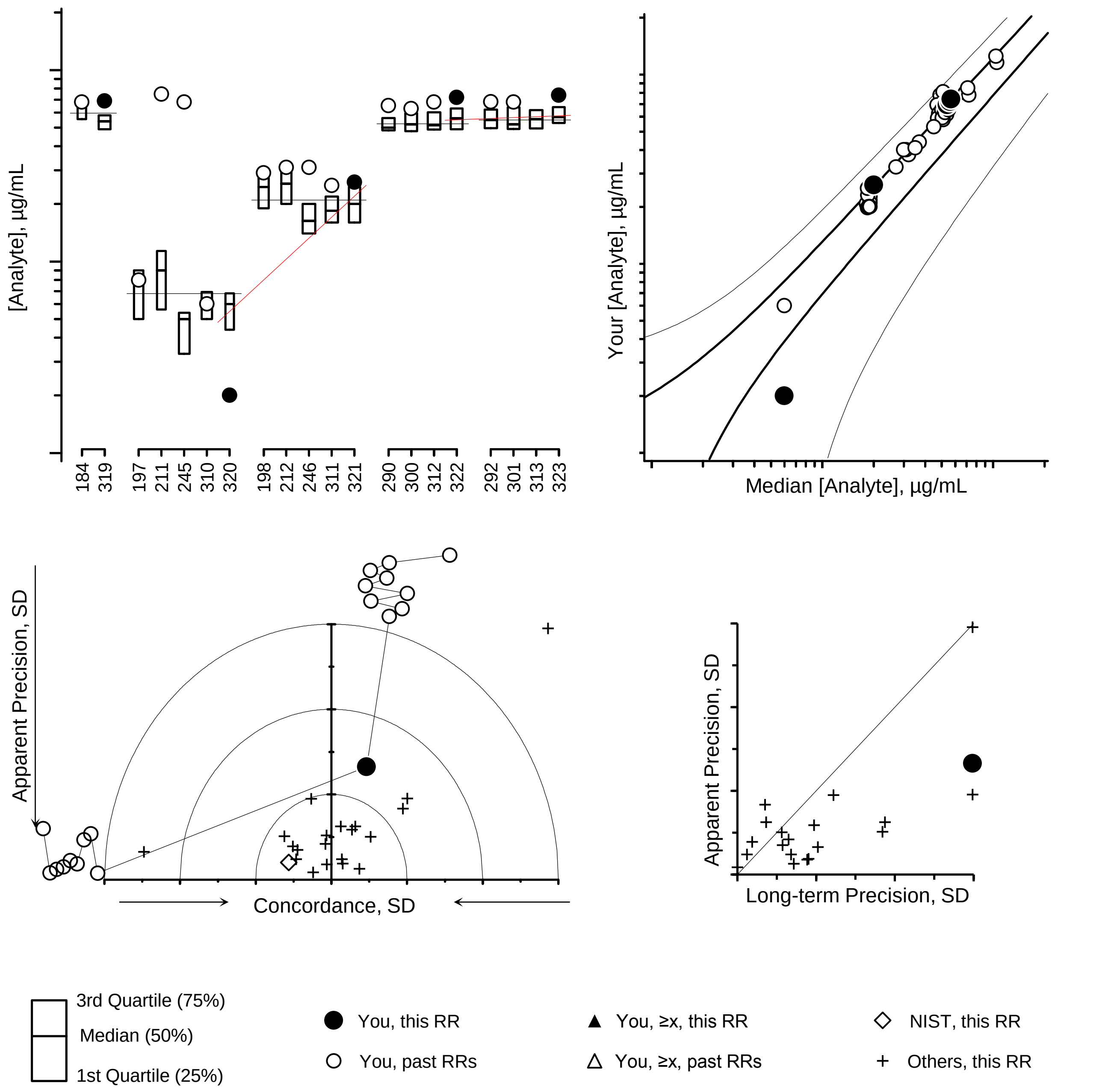
trans-Lycopene



Serum	History	Comments
#319	Lyophilized - 28:184	Native, single-source
#320	Lyophilized - 31:197, 35:211, 43:245, 57:310	Same as #321, 1:3 diluted with stripped serum
#321	Lyophilized - 31:198, 35:212, 43:246, 57:311	Augmented, multi-source
#322	Lyophilized - 53:290, 55:300, 57:312	Native, single-source
#323	Fresh-frozen: 53:292, 55:301, 57:313	Native, single-source, hemolyzed

Individualized RR LIX Report: FSV-BA

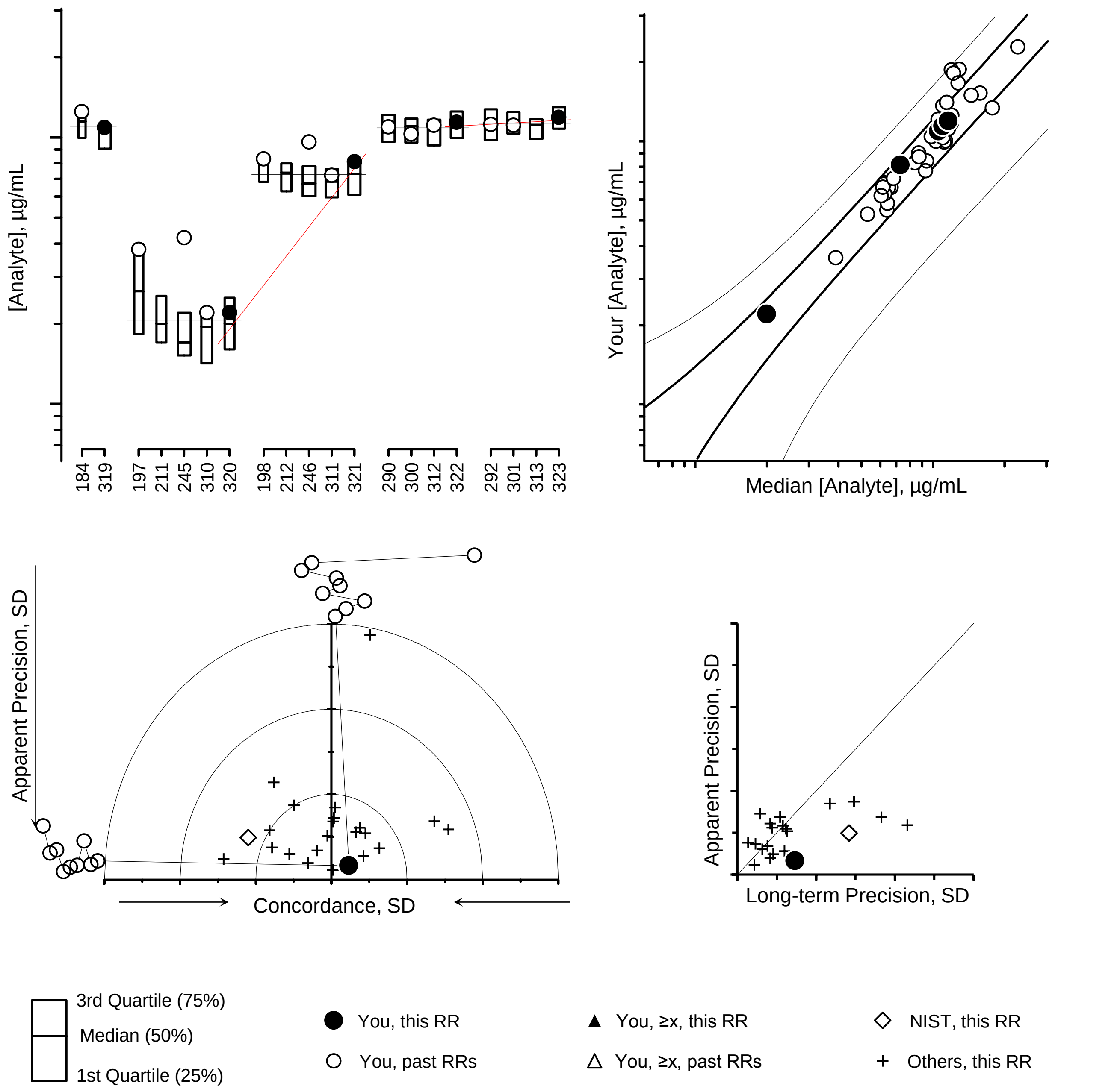
Total β-Cryptoxanthin



Serum	History	Comments
#319	Lyophilized - 28:184	Native, single-source
#320	Lyophilized - 31:197, 35:211, 43:245, 57:310	Same as #321, 1:3 diluted with stripped serum
#321	Lyophilized - 31:198, 35:212, 43:246, 57:311	Augmented, multi-source
#322	Lyophilized - 53:290, 55:300, 57:312	Native, single-source
#323	Fresh-frozen: 53:292, 55:301, 57:313	Native, single-source, hemolyzed

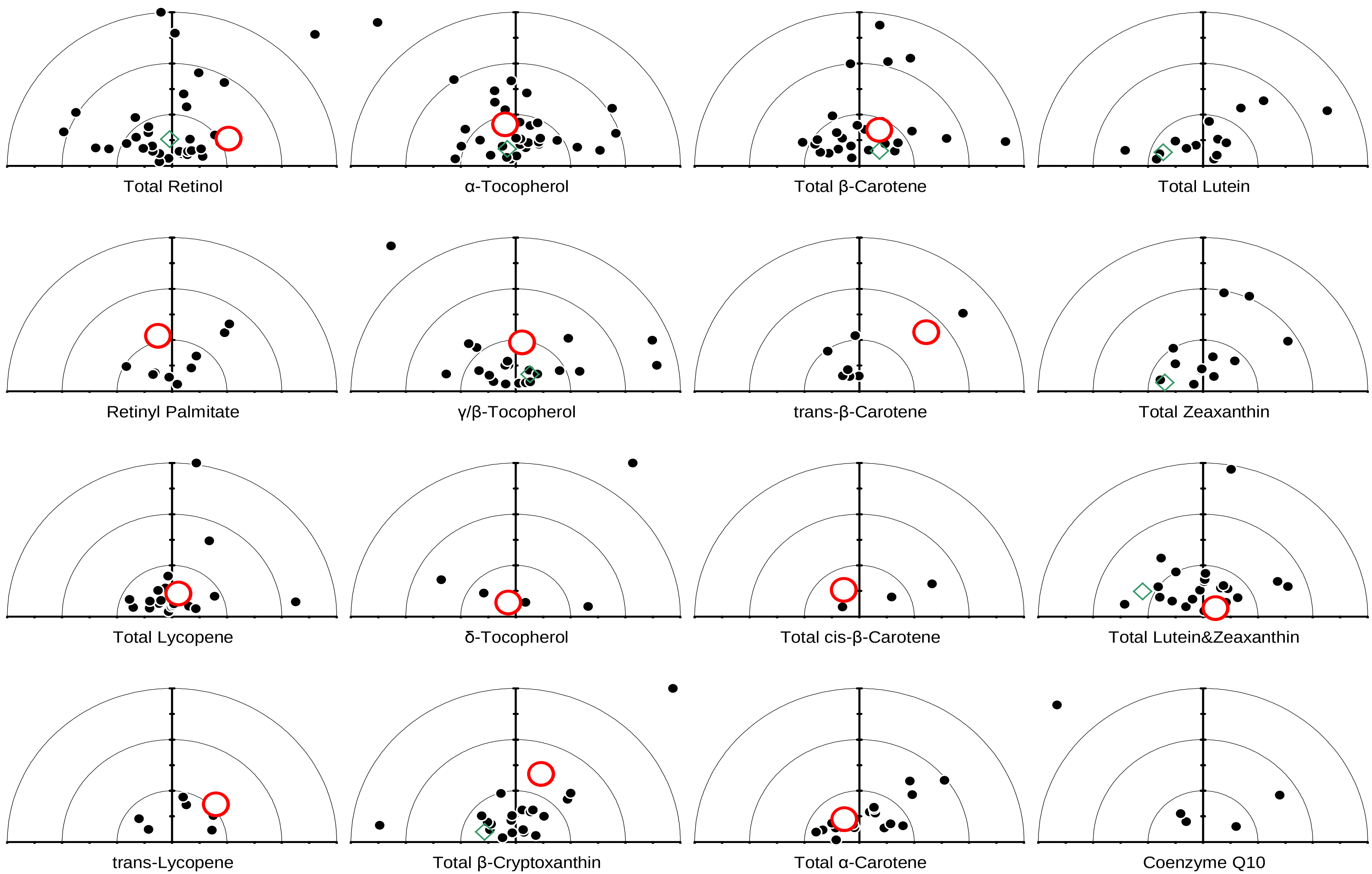
Individualized RR LIX Report: FSV-BA

Total Lutein&Zeaxanthin



Serum	History	Comments
#319	Lyophilized - 28:184	Native, single-source
#320	Lyophilized - 31:197, 35:211, 43:245, 57:310	Same as #321, 1:3 diluted with stripped serum
#321	Lyophilized - 31:198, 35:212, 43:246, 57:311	Augmented, multi-source
#322	Lyophilized - 53:290, 55:300, 57:312	Native, single-source
#323	Fresh-frozen: 53:292, 55:301, 57:313	Native, single-source, hemolyzed

Individualized Round Robin LIX Report: FSV-BA
Graphical Comparability Summary



D15

Appendix E. Shipping Package Inserts for RR24

The following five items were included in each package shipped to an RR24 participant:

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

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

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

November 28, 2005

Dear Colleague:

The samples within this package constitute Vitamin C Round Robin 24 (RR24) of the fiscal year (FY) 06 Micronutrients Measurement Quality Assurance Program.

RR24 consists of four vials of frozen serum *test samples* (#22, #43, #117, and #119), 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 RR23 was mailed during the first week of November. If you find your results for RR23 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 RR24 samples by **March 3, 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 B Thomas

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
RR24 Report Form for Ascorbic Acid Solid Control Material Preparation
RR24 Report Form for Control Material and Test Sample Analyses

NIST

Micronutrient Measurement Quality Assurance Program for
Vitamin C

Please Read Through Completely BEFORE Analyzing Samples

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

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

1) Prepare at least 500 mL of 5% mass fraction metaphosphoric acid (MPA) in distilled water. This solution will be referred to as the "Diluent" below.

2) Weigh 0.20 to 0.22 g of the ascorbic acid solid control material to 0.0001 g (if possible), dissolve it in the Diluent in a 100 mL volumetric flask, and dilute with the Diluent to the 100 mL mark. Weigh the amount of Diluent added to 0.1 g. Record the weights. The resulting material will be referred to as the "Stock Solution" below.

3) Prepare three dilute solutions of the Stock Solution as follows:

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

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

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

4) Calculate and record the total ascorbic acid concentrations, [TAA], in these Dilute Solutions. If you follow the above gravimetric preparation directions, the [TAA] in µmol/L is calculated:

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

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

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

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 24

NIST Micronutrient Measurement Quality Assurance Program

Preparation and Validation of Ascorbic Acid Solid Control Material

STOCK SOLUTION

Mass of ascorbic acid in the Stock Solution ....__________ g

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

DILUTE SOLUTION 1

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

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

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

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

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

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

Absorbance of Dilute Solution absorbance maximum ....__________ AU

Wavelength of maximum absorbance..__________ nm

Calculated $E^{1\%}$ ....__________ dL/g·cm

Calculated $[TAA]_{DS1}$ ....__________ µmol/L

DILUTE SOLUTION 2

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

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

Calculated $[TAA]_{DS2}$ ....__________ µmol/L

DILUTE SOLUTION 3

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

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

Calculated $[TAA]_{DS3}$ ....__________ µmol/L

Please return by **March 3, 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 24

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 #22			µmol/L of Sample
Serum Test Sample #43			µmol/L of Sample
Serum Test Sample #117			µmol/L of Sample
Serum Test Sample #119			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 3, 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 24

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^{2}QAP samples:

Sample	Form
VitC #22	Liquid frozen (1:1 serum:10% MPA)
VitC #43	Liquid frozen (1:1 serum:10% MPA)
VitC #117	Liquid frozen (1:1 serum:10% MPA)
VitC #119	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^{2}QAP Gang

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

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

Appendix F. Final Report for RR24

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

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

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

April 18, 2006

Dear Colleague:

Enclosed is the summary report of the results for Round Robin 24 (RR 24) 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 24 consisted of four *test samples* (#22, #43, #117, and #119), two *serum control materials*, and one *solid control material* for preparation of TAA control solutions. Details regarding the samples can be found in the enclosed report.

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

Samples for the second vitamin C round robin (RR 25) of the 2006 M^2QAP will be shipped **during the week of May 8, 2006.**

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 B Thomas

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

Enclosures

NIST

The NIST M^2QAP Vitamin C Round Robin 24 (RR24) 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 RR24 sample measurements.

Page	"All Lab" Report
1	A tabulation of results and summary statistics for Total Ascorbic Acid [TAA] in the RR24 samples and control/calibration solutions.

Serum-based Samples. Two serum controls and four unknowns were distributed in RR24.

- CS1 SRM 970 level 1, ampouled in mid-1998.
- CS2 SRM 970 level 2, ampouled in mid-1998.
- S24:1 Serum 22, ampouled in late 2001, previously distributed as sample S17:1 (RR17, Fall 02), S19:2 (RR19, Fall 03) and S21:2 (RR21, Fall 04).
- S24:2 Serum 43, ampouled in late 2001, previously distributed as sample S18:2 (RR18, Spring 03), S19:3 (RR19, Fall 03), S21:3 (RR21, Fall 04), and S22:3 (RR22, Spring 05).
- S24:3 Serum 117, ampouled in 1995, previously distributed sample 682b=S07:3 (RR7, Spring 95), 682b=S10:1 (RR10, 1997), and S13:4 (RR13, 2000).
- S24:4 Serum 119, ampouled in 1995, previously distributed sample 688b=S07:2 (RR7, Spring 1995), 688b=S08:2 (RR8, 1997), and S13:4 (RR13, 2000).

Results.

1) Nearly all participants who prepared the four 5% metaphosphoric acid (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"(≈244 nm), the observed absorbance at that maximum (≈0.55 OD), the calculated $E^{1\%}$ #1"(≈550 dL/g·cm).

   One participant chose to make the solutions in trichloroacetic acid (TCA) rather than MPA. One participant appears to have made simple aqueous solutions. Since the test samples are in 5% MPA (i.e., 1:1 dilutions of augmented serum in 10% MPA), their measurements of the test samples are not necessarily related to their control solution measurements.

2) Judging from the calibration parameters (intercepts close to 0.0, slopes close to 1.0, correlation (R^2) close to 1, and the root mean square error or "standard estimate of the error" (SEE) close to 0.0) calculated for the control/calibration solutions in the report summary, the measurement systems for all participants are linear and reasonably well calibrated. However, several participants have measurement systems that perform somewhat differently for the control solutions and the test samples. This suggests either that the serum matrix can induce a measurement bias and/or that the measurement system used for the control solutions isn't *exactly* the same as for the test materials. Figuring this out is one of our future projects.

3) Perhaps somewhat surprisingly, there is no evidence for sample degradation in any of the samples – including the two that were produced in 1995.

Appendix G. "All-Lab Report" for RR24

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" 24 - March 2006

		Control / Calibration Samples											MPA	Dilute Solution 1		
		Gravimetric, µmol/L			Measured, µmol/L				Calibration Parameters				Density	Spectrophotometry		
Lab	Date	Dil:1	Dil:2	Dil:3	Dil:1	Dil:2	Dil:3	MPA	Inter	Slope	R^2	RMSE	g/mL	λ_{max}	A_{max}	$E^{1\%}$
VC-MA	09/03/06	55.0	27.8	13.9	62.3	31.3	16.3	0.0	0.21	1.13	1.000	0.4	1.031	244.	0.5680	586.7
VC-MB	29/12/05	55.9	27.7	14.0	55.0	27.7	13.9	0.0	0.13	0.98	1.000	0.2	1.033	244.	0.5481	556.5
VC-MC	26/01/06	55.4	27.6	13.7	59.0	30.4	14.2	0.0	-0.01	1.07	0.999	0.7	1.031	243.	0.5757	589.7
VC-ME	03/03/06	56.8	29.2	14.1	58.0	28.9	14.3	0.9	0.33	1.01	0.999	0.8	1.029	243.	0.5712	571.2
VC-MG	09/02/06	58.8	30.4	16.0	59.8	27.7	13.7	0.0	-1.65	1.02	0.996	2.1	1.031	243.7	0.5730	553.1
VC-MH	09/03/06	62.2	31.1	15.4	63.9	32.2	15.9	0.1	0.15	1.02	1.000	0.1	1.031	244.1	0.6198	565.6
VC-MI	02/03/06	56.6	28.1	13.8	53.9	26.5	12.6	0.0	-0.31	0.95	1.000	0.3	1.031			
VC-MJ	31/01/06	59.4	29.1	15.1	58.3	30.5	15.3	0.7	0.98	0.97	0.999	1.0	1.022[a]	250[a]	0.3650[a]	349.1[a]
VC-MK	03/02/06	58.9	30.8	14.9	59.8	31.6	14.9	1.2	0.67	1.00	1.000	0.6	1.031	244.	0.5980	576.8
VC-ML	08/03/06	56.5	28.3	14.3	56.2	27.6	13.1	0.0	-0.56	1.00	1.000	0.6	1.026	242.	0.5580	560.9
VC-MP	03/03/06															
VC-MS	06/03/06	55.4	28.9	11.8	51.2	25.9	10.8	0.2	-0.10	0.92	1.000	0.5	0.992	243.	0.5135	526.6
	N	11	11	11	11	11	11	11				N	10	9	9	9
	Average	57.3	29.0	14.3	57.9	29.1	14.1	0.3				Average	1.026	243.4	0.5695	565.2
	SD	2.2	1.3	1.1	3.7	2.2	1.6	0.4				SD	0.012	0.7	0.0298	19.2
	Min	55.0	27.60	11.8	51.2	25.90	10.8	0.0				Min	0.992	242.0	0.5135	526.6
	%25	55.7	27.97	13.8	55.6	27.64	13.4	0.0				%25	1.029	243.0	0.5580	556.5
	Median	56.6	28.92	14.1	58.3	28.90	14.2	0.0				Median	1.031	243.7	0.5712	565.6
	%75	58.8	29.82	15.0	59.8	30.92	15.1	0.4				%75	1.031	244.0	0.5757	576.8
	Max	62.2	31.07	16.0	63.9	32.15	16.3	1.2				Max	1.033	244.1	0.6198	589.7
	MADe	1.9	1.6	0.6	3.1	2.4	1.6	0.0				eSD	0.000	0.6	0.0196	16.6
	CV	3	6	4	5	8	11					CV	0.04	0.24	3.4	2.9

	Samples											
	Measured, µmol/L						Calibrated to Gravimetric Solution, µmol/L					
Lab	CS#1	CS#2	S24:1	S24:2	S24:3	S24:4	CS#1	CS#2	S24:1	S24:2	S24:3	S24:4
VC-MA	9.3	29.2	9.7	35.6	59.1	29.2	8.0	25.7	8.4	31.4	52.2	25.7
VC-MB	7.5	26.6	8.5	33.3	60.9	27.6	7.5	26.9	8.6	33.7	61.9	28.0
VC-MC	7.5	30.0	11.1	38.3	60.4	27.1	7.0	28.0	10.4	35.8	56.4	25.3
VC-ME	8.7	27.5	9.6	32.7	59.7	27.5	8.3	27.0	9.2	32.1	58.9	27.0
VC-MG	8.1	30.2	10.7	38.2	69.7	31.3	9.5	31.1	12.1	38.9	69.6	32.2
VC-MH	8.9	29.0	9.8	34.6	*b*	27.6	8.5	28.1	9.4	33.6		26.8
VC-MI	8.5	28.0	8.3	29.4	62.5	27.4	9.2	29.6	9.0	31.1	65.8	29.0
VC-MJ	11.8	31.4	13.6	37.5	59.7	30.2	11.1	31.2	12.9	37.5	60.4	30.0
VC-MK	9.6	31.8	12.3	35.6	*c*	31.7	8.9	31.0	11.6	34.8		31.0
VC-ML	7.1	27.0	6.8	29.9	55.4	24.8	7.6	27.5	7.3	30.5	55.9	25.4
VC-MP	8.5	28.7		32.9	57.9	27.1						
VC-MS	6.7	32.2	7.9	40.8	78.8	32.8	7.4	35.1	8.6	44.4	85.7	35.8
N	12	12	11	12	10	12	11	11	11	11	9	11
Average	8.5	29.3	9.8	34.9	62.4	28.7	8.5	29.2	9.8	34.9	63.0	28.7
SD	1.4	1.9	2.0	3.5	6.8	2.4	1.2	2.7	1.7	4.2	10.0	3.3
Min	6.7	26.6	6.8	29.4	55.4	24.8	7.0	25.7	7.3	30.5	52.2	25.3
%25	7.5	27.9	8.4	32.8	59.3	27.3	7.5	27.2	8.6	31.7	56.4	26.2
Median	8.5	29.1	9.7	35.1	60.0	27.6	8.3	28.1	9.2	33.7	60.4	28.0
%75	9.0	30.5	10.9	37.7	62.1	30.5	9.1	31.0	11.0	36.7	65.8	30.5
Max	11.8	32.2	13.6	40.8	78.8	32.8	11.1	35.1	12.9	44.4	85.7	35.8
eSD	1.3	2.0	2.1	3.6	2.3	1.6	1.3	2.3	1.2	3.5	6.6	3.4
CV	16	7	21	10	4	6	15	8	13	10	11	12

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

Appendix H. Representative "Individualized Report" for RR24

Each participant in RR24 received an "Individualized Report" reflecting their reported results. The following two pages are the "Individualized Report" for participant "VC-MA".

Vitamin C "Round Robin" 24 Report: Participant VC-MA

			MPA Density	Dilute Solution 1 Spectrophotometry			Control/Calibration Solutions Y_{meas} = Inter + Slope* X_{grav}			
Date	RR	Method	g/mL	λ_{max}	A_{max}	$E^{1\%}$	Inter	Slope	R^2	SEE
11/13/03	19	HPLC-EC	1.026	243.0	0.584	561.9	1.1	1.03	0.998	1.24
02/23/04	20	HPLC-EC	1.031	243.0	0.552	560.7	-0.4	1.05	1.000	0.65
09/13/04	21	HPLC-EC	1.030	244.0	0.555	562.2	-0.1	0.99	1.000	0.10
03/08/05	22	HPLC-EC	1.034	243.0	0.559	562.9	0.2	1.06	1.000	0.24
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
		Mean	1.03	243.5	0.56	567.0				0.68
		SD	0.003	0.5	0.01	9.9				0.56
		CV	0.25	0.22	2.0	1.8				

			[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	5	8.6	0.1	0.7
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				
02/23/04	20	CS#2	25.8	26.2	1.0	26.0	0.3	5	28.2	0.5	1.7
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				
11/13/03	19	S19:2	9.2	9.1	1.0	9.2	0.1	4	9.3	0.1	0.4
09/13/04	21	S21:2	8.8	8.7	1.0	8.7	0.1				
03/08/05	22	S22:1	9.6	9.6	1.0	9.6	0.0				
03/09/06	24	S24:1	**9.8**	**9.6**	1.0	9.7	0.2				
11/13/03	19	S19:3	35.9	35.8	1.0	35.9	0.1	4	35.0	0.2	1.3
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				
03/09/06	24	S24:3	**59.1**	**59.1**	1.0	59.1	0.1				
03/09/06	24	S24:4	**29.0**	**29.5**	1.0	29.2	0.4				

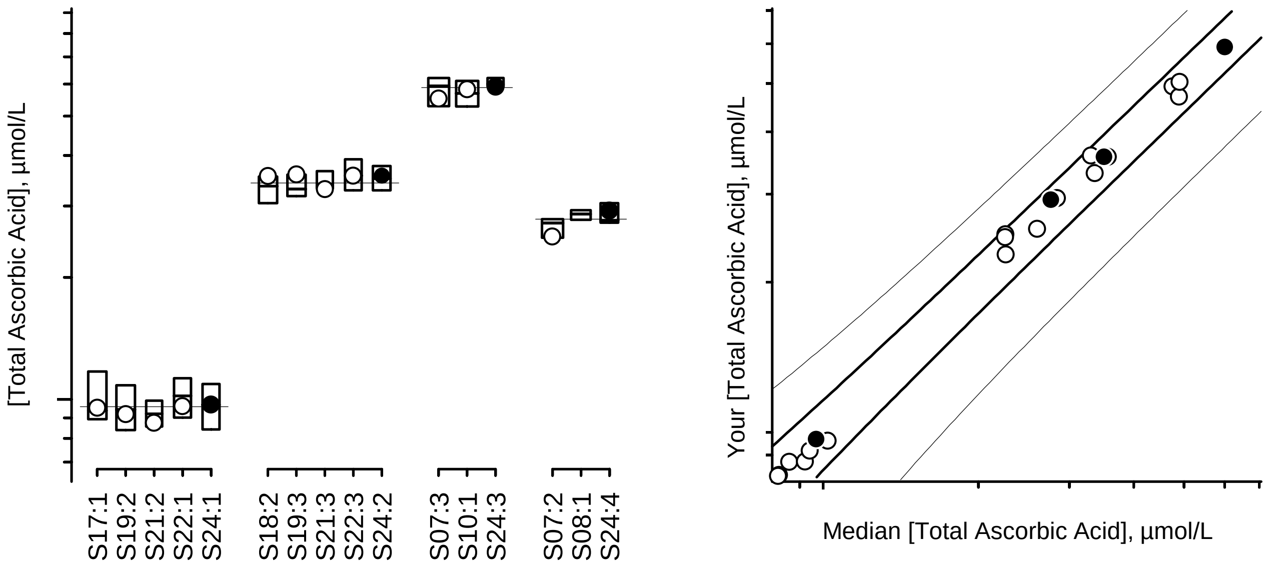
Please check our records against your records. Send corrections and/or updates to...

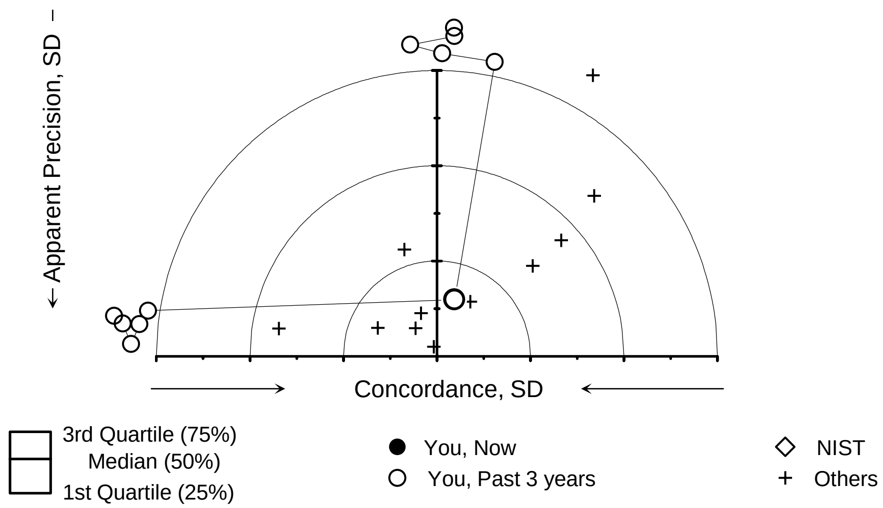
Micronutrients Measurement Quality Assurance Program
National Institute of Standards and Technology
100 Bureau Drive Stop 8392
Gaithersburg, MD 20899-8392 USA

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

Vitamin C "Round Robin" 24 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
S24:1	Serum 22, previously distributed in RRs 17, 19, 21, and 22
S24:2	Serum 43, previously distributed in RRs 18, 19, 21, and 22
S24:3	Serum 117, previously distributed in RRs 7 and 10
S24:4	Serum 119, previously distributed in RRs 7 and 8