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NIST Micronutrients Measurement Quality Assurance Program Summer 2001 Comparability Studies

Results for Round Robin L
Fat-Soluble Vitamins and Carotenoids in Human Serum
and Round Robin 15 Ascorbic Acid in Human Serum

David L. Duewer
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Katherine E. Sharpless
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NIST
National Institute of
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U.S. Department of Commerce

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June 2013

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 2001 MMQAP measurement comparability improvement studies: 1) Round Robin L Fat-Soluble Vitamins and Carotenoids in Human Serum and 2) Round Robin 15 Total Ascorbic Acid in Human Serum. The materials for both studies were shipped to participants in June 2001; participants were requested to provide their measurement results by mid-September, 2001.

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 L: Fat-Soluble Vitamins and Carotenoids in Human Serum

Participants in the MMQAP Fat-Soluble Vitamins and Carotenoids in Human Serum Round Robin L comparability study (hereafter referred to as RR15) received three lyophilized and two 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 June 2001. 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 RR15 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 15: Vitamin C in Human Serum

Participants in the MMQAP Vitamin C in Human Serum Round Robin 15 comparability study (hereafter referred to as RR15) received three frozen serum test samples 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 June 2001. The communication materials included in the sample shipment are provided in Appendix E.

The test 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 RR15 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 RR15

The following three items were included in each package shipped to an RR15 participant:

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

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

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

June 8, 2001

Dear Colleague:

Enclosed is the set of samples for the second quality assurance round robin exercise (Round Robin L) for 2001. You will find one vial of each of three lyophilized and two liquid-frozen 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 an obtained value is below your limit of quantitation, please indicate this result on the form by using NQ *(Not Quantitated)*. For analytes not measured, please leave a blank. Results are due to NIST by **September 14, 2001**. Results received 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 around mid-October.

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 will 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). Liquid-frozen samples 274 and 275 should **not be** reconstituted.

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

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

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

If you have questions regarding this round robin exercise, 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

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

Enclosures

NIST

Participant #: _____ Date: _____

Round Robin L

NIST Micronutrients Measurement Quality Assurance Program

Analyte	274	275	276	277	278	Units*
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						
trans-α-carotene						
total lycopene						
trans-lycopene						
total β-cryptoxanthin						
total α-cryptoxanthin						
total lutein						
total zeaxanthin						
total lutein&zeaxanthin						
ubiquinone-10 (Q_{10})						
phylloquinone (K_1)						
25-hydroxyvitamin D						

Other analytes?						

* we prefer μg/mL

Was sera 274 & 275 frozen when received? Yes | No

Comments:

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

Please return results on-or-before
14-Sep-2001

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

Participant #: _____ Date: _____

Round Robin L

NIST Micronutrients Measurement Quality Assurance Program

Packing List and Shipment Receipt Confirmation Form

The enclosed bubble-pack (should) contain one vial each of the following **five** sera:

Serum	Form	Reconstitute?
#274	Liquid frozen	No
#275	Liquid frozen	No
#276	Lyophilized	Yes (1 ml H_2O)
#277	Lyophilized	Yes (1 ml H_2O)
#278	Lyophilized	Yes (1 ml H_2O)

Please
1) Open the pack immediately
2) Check that it contains one vial each of the above samples
3) Check if sera #274 and #275 arrived frozen
4) Store the samples upright at -20 ˚C or below until analysis
5) Complete the following information
6) Fax the completed form to us at 301-977-0685
(or email requested information to david.duewer@nist.gov)

1) Date this shipment arrived: _____

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

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

4) Did sera #274 and #275 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 will help control M^2QAP expenses.

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 RR15

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.

NIST CENTENNIAL 1901-2001

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

October 31, 2001

Dear Colleague:

Enclosed is the summary report of the results for Round Robin L (RR 50) for fat-soluble vitamins and carotenoids. Included in this report are: a summary of data for all laboratories; the measurement comparability summary for evaluating laboratory performance; lyophilized vs. fresh-frozen commutability data, a summary of individual laboratory performance and interlaboratory accuracy and precision; and a summary of the NIST assigned value (NAV) vs. your laboratory value for the analytes that you measured. As in previous reports, the NIST-assigned values are equally weighted means of the medians from this interlaboratory comparison exercise and the means from the analyses performed by NIST.

Data for evaluating laboratory performance in RR 50 are provided in the comparability summary (Score Card) on page 6 of the "All Lab Report." Laboratory comparability is summarized as follows: results rated 1 to 3 are within 1 to 3 standard deviation(s) of the assigned value, respectively; those rated 4 are >3 standard deviations from the assigned value.

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

Intent-to-participate forms for the 2002 QA program were mailed about two weeks ago. This form will provide us with formal notification of your intent to participate in the program for the upcoming year. The program will consist of two round robin studies for the fat-soluble vitamins and carotenoids and one study for vitamin C in serum. To participate in the fat-soluble vitamins and carotenoids in serum studies, the participation fee is $1600 for U.S. laboratories and $2000 for non-U.S. laboratories. To participate in the vitamin C in serum study, the participation fee is $800 for U.S. laboratories and $1000 for non-U.S. laboratories. We ask that you return the form to us by **no later than November 30, 2001.**

NIST

The following publication has been released. Please contact the corresponding author for reprints.

"Stability of Ascorbic Acid in Solutions Stored in Autosampler Vials," Margolis, S. A. and Park, E., *Clin. Chem.*, 47, 1463-4, 2001.

If you have any questions 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; 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
S. A. Wise

Enclosures

The NIST M^2QAP Round Robin XLIX (RR50) report consists of:

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 10 other participants.
n+1	The "target" plot version of your "Comparability Summary" scores.

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 four analytes reported by only one laboratory.
5b	A legend for the above two lists.
6	The "Comparability Summary" (or "Score Card").

Samples. The five sera below were distributed in RR50.

Serum	Description	Prior Distribution
274	liquid-frozen material prepared from a native serum; partner to lyophilized serum 276	as serum 267 in RR48
275	liquid-frozen material prepared from a native serum; partner to lyophilized serum 277	as serum 271 in RR49
276	lyophilized material prepared from a native serum; partner to liquid-frozen serum 274	as serum 270 in RR49
277	lyophilized material prepared from a native serum; partner to liquid-frozen serum 275	as serum 266 in RR48
278	lyophilized material prepared from a native serum	as serum 192 in RR30 (3/94); serum 199 in RR32 (9/94); serum 218 in RR36 (3/96); and serum 250 in RR44 (9/98)

Qualitative Observations
Several participants noted the presence an "insoluble stringy clot" in Serum 275 and/or 277. We had similar reports from the earlier distributions of these sera. However, since the solids do **not** appear to affect measurements, we chose to redistribute this {liquid-frozen, lyophilized} pair to help complete our commutability study.

All participants received their samples still frozen. We thank all of you who promptly confirmed receipt of the samples. Such prompt confirmation greatly simplifies our package delivery tracking and thus helps contain M^2QAP costs. *We will be requesting similar confirmation in all future studies.*

Quantitative Results
There has been no significant change in the median level or in the variability of any measurand in serum 278 over eight years storage at –80 °C.

While the liquid frozen and lyophilized sera of the commutability pairs were prepared from the same serum pool, the measurand concentrations in the lyophilized sera are expected and generally observed to be about 0.96x that of their liquid-frozen analogues. This is a simple dilution effect, since the lyophilized sera are reconstituted with 1 mL of water rather than to their original 1 mL total volume.

Appendix C. "All-Lab Report" for RR15

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

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

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

Round Robin L Laboratory Results

All Results in µg/mL

Lab	Total Retinol					trans-Retinol					Retinyl Palmitate					α-Tocopherol					γ/β-Tocopherol					δ-Tocopherol				
	274	275	276	277	278	274	275	276	277	278	274	275	276	277	278	274	275	276	277	278	274	275	276	277	278	274	275	276	277	278
FSV-BA	0.654	0.452	0.608	0.431	1.050						0.012	0.067	0.003	0.062	0.053	6.38	6.84	5.94	6.53	6.89	2.61	2.01	2.40	1.91	2.55					
FSV-BB	0.657	0.455	0.602	0.432	1.049						0.024	0.049	0.024	0.045	0.034	6.41	7.08	5.98	6.78	7.12	2.34	1.91	2.20	1.80	2.28					
FSV-BD	0.624	0.435	0.607	0.429	1.043											6.00	5.50	5.40	6.20	6.50										
FSV-BE	0.670	0.470	0.670	0.420	1.120											6.50	7.30	6.50	6.90	7.50	2.40	2.00	2.40	1.80	2.40					
FSV-BF	0.671	0.464	0.629	0.460	1.121											5.97	7.38	6.16	7.37	8.04	2.19	1.90	2.20	1.90	2.46					
FSV-BG	0.634	0.485	0.622	0.443	1.145						0.043	0.083	0.049	0.054	0.047	5.56	7.03	5.63	6.56	6.86	2.04	1.89	2.00	1.67	2.27					
FSV-BH	0.745	0.524	0.720	0.522	1.200											6.59	7.45	6.27	7.04	7.69	2.30	1.92	2.17	1.83	2.31					
FSV-BI	0.664	0.460	0.618	0.440	1.119											6.06	7.00	5.61	6.70	7.13	2.33	2.04	2.16	1.93	2.38					
FSV-BJ	>0.638	>0.452	>0.632	>0.452	>1.077	0.638	0.452	0.632	0.452	1.077	*nq*	0.054	*nq*	0.055	*nq*	5.60	6.88	5.53	6.59	6.88	2.26	1.98	2.21	1.89	2.43					
FSV-BK	0.630	0.440	0.592	0.412	1.071											4.60	5.65	4.40	5.15	5.58										
FSV-BL	0.740	0.490	0.690	0.490	1.000											6.89	7.97	6.72	7.54	8.14										
FSV-BM	0.665	0.483	0.671	0.465	1.113											6.96	7.70	6.22	6.93	7.50										
FSV-BN	0.743	0.510	0.617	0.450	1.159						0.017	0.037	0.017	0.045	0.023	5.70	6.34	4.79	5.73	6.39	2.00	1.55	1.65	1.49	1.99	0.140	0.153	0.105	0.124	0.137
FSV-BO	0.713	0.484	0.581	0.444	1.054											6.00	6.60	5.80	6.90	6.80										
FSV-BP	0.675	0.464	0.636	0.451	1.033											7.61	6.90	5.69	6.22	6.70										
FSV-BQ	0.700	0.500	0.680	0.490	1.100											6.00	6.70	5.80	6.30	6.70										
FSV-BR	>0.62	>0.45	>0.61	>0.44	>1.06	0.62	0.45	0.61	0.44	1.06						5.77	6.71	5.61	6.32	7.27										
FSV-BU	0.585	0.384	0.540	0.351	1.022											4.88	5.78	4.41	5.47	5.69	2.36	2.04	2.09	1.81	2.20					
FSV-BV	0.684	0.470	0.567	0.472	0.958											6.64	7.15	6.20	6.41	6.76	2.40	1.93	2.30	1.82	2.26					
FSV-BW	0.710	0.500	0.680	0.480	1.190						0.023	0.066	0.021	0.055	0.030	6.45	7.54	6.10	7.07	7.50	2.26	1.90	2.12	1.79	2.29					
FSV-BX	>0.647	>0.463	>0.659	>0.436	>1.083	0.647	0.463	0.659	0.436	1.083						6.30	7.22	6.16	6.75	7.14	2.20	1.83	2.21	1.72	2.32					
FSV-CB	0.614	0.419	0.593	0.412	0.987											5.84	6.42	5.51	6.20	6.46										
FSV-CC	0.720	0.510	0.710	0.480	1.150	0.710	0.500	0.700	0.470	1.140						6.80	7.10	6.10	6.70	7.20										
FSV-CD	>0.661	>0.464	>0.633	>0.434	>1.116	0.661	0.464	0.633	0.434	1.116	0.035	0.098	0.028	0.091	0.037	6.09	6.75	5.66	6.36	7.03	2.39	1.95	2.21	1.88	2.31					
FSV-CE	0.568	0.394	0.570	0.384	0.986											5.58	6.27	5.32	5.95	6.42										
FSV-CF	0.665	0.465	0.642	0.432	1.050											7.00	7.60	6.60	7.00	7.20										
FSV-CG	0.705	0.495	0.682	0.497	1.099											5.57	6.26	5.11	5.72	5.33	2.54	2.09	2.32	1.94	2.45	0.283	0.340	0.248	0.260	0.364
FSV-CH	0.580	0.378	0.521	0.361	0.847											6.01	6.49	5.42	6.19	5.88	1.98	1.59	1.84	1.56	1.72					
FSV-CI	0.800	0.540	0.750	0.520	1.060						0.030	0.050	0.020	0.040	0.040	6.92	7.51	6.49	7.13	8.56	2.38	2.10	2.27	1.97	2.61					
FSV-CK	0.670	0.470	0.700	0.510	1.190											6.03	7.12	6.58	7.52	8.04	2.63	2.20	2.67	2.20	2.95					
FSV-CL	0.444	0.330	0.437	0.316	0.668											8.75	10.08	8.31	9.14	8.68	2.67	2.21	3.02	2.06	2.58					
FSV-CR	0.720	0.580	0.700	0.500	1.160											5.70	7.80	5.60	7.10	6.60										
FSV-CV	0.510	!0.88	0.467	0.217	1.463						0.050	0.050	*nd*	0.020	0.030	7.18	7.97	6.50	7.93	8.05	2.27	1.88	2.10	1.91	2.34	0.101	0.338	0.090	0.114	0.127
FSV-CW	>0.587	>0.397	>0.554	>0.387	>0.945	0.587	0.397	0.554	0.387	0.945	0.016	0.048	0.015	0.045	0.020	7.35	8.32	6.83	7.98	8.21	2.47	2.06	2.31	1.99	2.42	0.090	0.110	0.090	0.100	0.120
FSV-CX	>0.59	>0.40	>0.56	>0.38	>0.89	0.59	0.40	0.56	0.38	0.89						6.55	7.36	6.24	6.91	7.21										
FSV-CZ	0.700	0.470	0.650	0.460	1.210											2.40	2.70	2.20	2.60	2.80										
FSV-DB	0.640	0.470	0.620	0.460	1.110											6.74	7.98	6.25	7.71	7.15										
FSV-DD	>0.715	>0.486	>0.639	>0.322	>1.072	0.715	0.486	0.639	0.322	1.072																				
FSV-DF	0.660	0.470	0.620	0.440	1.096																									
FSV-DI	0.688	0.469	0.660	0.452	1.091						0.015	0.038	0.010	0.048	0.020	7.30	8.30	7.19	8.16	8.59	2.30	1.89	2.25	1.84	2.38	0.129	0.156	0.125	0.149	0.180
FSV-DQ																6.58	7.15	5.88	6.42	6.62	2.66	1.96	2.24	1.81	2.32					
FSV-DR	0.610	0.420	0.560	0.400	1.090											5.96	6.67	5.59	6.65	6.47										
FSV-DU	!0.93	0.510	0.690	0.470	1.120											!6.78	6.44	5.50	6.24	6.24										
FSV-EH	0.585	0.415	0.568	0.400	0.944						0.035	0.058	0.033	0.056	0.032	6.26	7.03	5.79	6.36	9.34	1.96	1.83	2.10	1.65	2.86	0.133	0.121	0.128	0.157	0.230
FSV-EQ	0.619	0.442	0.578	0.386	1.036											5.72	6.86	5.42	5.91	7.00										
FSV-FB	0.680	0.490	0.690	0.480	1.250											7.33	7.65	6.73	7.39	8.02										
FSV-FJ	0.700	0.500	0.670	0.480	1.190											7.33	8.38	6.92	8.51	8.55	2.31	2.00	2.22	1.92	2.36					
N	38	38	39	39	39	8	8	8	8	8	11	12	10	12	11	44	45	45	45	45	25	25	25	25	25	6	6	6	6	6
Min	0.444	0.330	0.437	0.217	0.668	0.587	0.397	0.554	0.322	0.890	0.012	0.037	0.003	0.020	0.020	2.40	2.70	2.20	2.60	2.80	1.96	1.55	1.65	1.49	1.72	0.090	0.110	0.090	0.100	0.120
Median	0.668	0.470	0.622	0.450	1.096	0.642	0.457	0.633	0.435	1.074	0.024	0.052	0.021	0.051	0.032	6.28	7.08	5.88	6.70	7.12	2.33	1.95	2.21	1.84	2.36	0.131	0.155	0.115	0.137	0.159
Max	0.800	0.580	0.750	0.522	1.463	0.715	0.500	0.700	0.470	1.140	0.050	0.098	0.049	0.091	0.053	8.75	10.08	8.31	9.14	9.34	2.67	2.21	3.02	2.20	2.95	0.283	0.340	0.248	0.260	0.364
SD	0.055	0.037	0.069	0.047	0.080	0.045	0.024	0.034	0.043	0.054	0.014	0.013	0.009	0.008	0.009	0.74	0.65	0.55	0.64	0.81	0.12	0.11	0.13	0.09	0.12	0.022	0.121	0.025	0.029	0.065
CV	8	8	11	11	7	7	5	5	10	5	57	25	42	15	28	12	9	9	10	11	5	6	6	5	5	17	79	22	21	41
N_{past}	44	35	35	44	45	11	12	12	11	0	11	14	12	15	8	52	44	44	52	45	29	25	25	29	21	6	7	7	6	9
$Median_{past}$	0.663	0.470	0.650	0.446	1.079	0.668	0.445	0.603	0.440		0.028	0.057	0.027	0.053	0.053	6.32	7.12	6.00	6.81	7.14	2.34	2.01	2.30	1.85	2.45	0.121	0.118	0.111	0.152	0.110
SD_{past}	0.081	0.051	0.050	0.045	0.106	0.033	0.023	0.031	0.030		0.014	0.014	0.013	0.014	0.035	0.64	0.58	0.54	0.68	0.55	0.27	0.16	0.19	0.15	0.27	0.201	0.065	0.066	0.180	0.020
NISTa	0.641	0.448	0.631	0.460	1.068	0.641	0.448	0.631	0.460	1.019						5.49	6.55	5.49	6.65	6.87	2.14	1.76	1.92	1.63	2.12					
NISTb	0.607	0.435	0.569	0.411	0.970	0.607	0.435	0.569	0.411	0.909						6.25	7.21	6.06	6.17	6.89	2.31	2.06	2.27	1.80	2.37	*nq*	0.214	0.109	0.139	0.136
N_{NIST}	4	4	4	4	4	4	4	4	4	4						4	4	4	4	4	4	4	4	4	4		1	1	1	1
Mean	0.624	0.441	0.599	0.435	1.016	0.624	0.441	0.599	0.435	0.959						5.83	6.88	5.75	6.41	6.88	2.22	1.91	2.10	1.71	2.25		0.214	0.109	0.139	0.136
S_{rep}	0.005	0.012	0.007	0.019	0.026	0.005	0.012	0.007	0.019	0.027						0.16	0.16	0.14	0.12	0.28	0.14	0.06	0.07	0.06	0.13		0.032	0.005	0.021	0.016
S_{het}	0.006	0.017	0.006	0.022	0.019	0.006	0.017	0.006	0.022	0.033						0.25	0.13	0.16	0.48	0.15	0.05	0.10	0.04	0.07	0.09					
S_{anl}	0.025	0.009	0.042	0.035	0.073	0.025	0.009	0.042	0.035	0.086						0.48	0.47	0.45	0.34	0.02	0.11	0.21	0.24	0.11	0.18					
S_{NIST}	0.026	0.023	0.043	0.046	0.080	0.026	0.023	0.043	0.046	0.096						0.56	0.51	0.50	0.60	0.32	0.18	0.24	0.25	0.15	0.24					
NAV	0.646	0.456	0.610	0.443	1.056	0.631	0.447	0.615	0.436	1.018	0.024	0.052	0.021	0.051	0.032	6.05	6.98	5.81	6.55	7.00	2.27	1.93	2.15	1.78	2.30	0.126	0.184	0.112	0.138	0.147
NAU	0.063	0.043	0.071	0.049	0.101	0.052	0.037	0.055	0.046	0.127	0.017	0.021	0.017	0.021	0.018	0.81	0.66	0.55	0.67	0.83	0.25	0.25	0.26	0.21	0.25	0.101	0.128	0.026	0.029	0.067

C2

Round Robin L Laboratory Results

All Results in µg/mL

	Total β-Carotene					trans-β-Carotene					Total cis-β-Carotene					Total α-Carotene					trans-α-Carotene				
Lab	274	275	276	277	278	274	275	276	277	278	274	275	276	277	278	274	275	276	277	278	274	275	276	277	278
FSV-BA	0.498	0.313	0.449	0.304	0.492	0.476	0.294	0.425	0.286	0.458	0.022	0.019	0.024	0.018	0.033	0.019	0.024	0.020	0.024	0.016					
FSV-BB	0.500	0.331	0.452	0.318	0.534	0.481	0.314	0.435	0.301	0.509	0.019	0.017	0.017	0.017	0.025	0.024	0.030	0.017	0.030	0.009					
FSV-BD																									
FSV-BE	0.478	0.349	0.471	0.327	0.530																				
FSV-BF	0.583	0.405	0.632	0.407	0.790											0.011	0.024	0.013	0.027	0.009					
FSV-BG	0.513	0.362	0.474	0.313	0.518											0.022	0.038	0.022	0.029	0.024					
FSV-BH	0.619	0.450	0.589	0.411	0.683	0.581	0.410	0.547	0.374	0.624	0.038	0.040	0.042	0.037	0.059	0.017	0.040	0.016	0.025	0.012					
FSV-BI	0.525	0.348	0.493	0.331	0.557											0.022	0.035	0.020	0.033	0.016					
FSV-BJ	0.573	0.357	0.546	0.323	0.578											0.025	0.038	0.025	0.038	0.016					
FSV-BK																									
FSV-BL																									
FSV-BM																									
FSV-BN	0.560	0.364	0.461	0.325	0.588	0.528	0.337	0.435	0.304	0.544	*0.032*	*0.027*	*0.026*	*0.021*	*0.044*	0.019	0.028	0.014	0.024	0.015					
FSV-BO	0.509	0.369	0.472	0.353	0.641											0.032	0.037	0.024	0.039	0.021					
FSV-BP	0.549	0.383	0.495	0.346	0.565											0.017	0.025	0.015	0.020	0.023					
FSV-BQ																									
FSV-BR																									
FSV-BU	0.576	0.359	0.502	0.400	0.596											0.026	0.032	0.025	0.033	0.022					
FSV-BV	0.542	0.349	0.498	0.340	0.552											0.017	0.024	0.015	0.023	0.012					
FSV-BW	0.540	0.360	0.510	0.330	0.560											0.024	0.041	0.023	0.038	0.030					
FSV-BX	>0.498	>0.336	>0.483	>0.313	>0.522	0.498	0.336	0.483	0.313	0.522						0.023	0.033	0.023	0.033	0.021					
FSV-CB	0.508	0.351	0.473	0.340	0.535											0.015	0.024	0.014	0.023	0.011					
FSV-CC																									
FSV-CD	0.522	0.331	0.509	0.310	0.566											0.018	0.031	0.019	0.027	0.013					
FSV-CE	0.487	0.311	0.438	0.308	0.491																				
FSV-CF																									
FSV-CG	0.580	0.393	0.529	0.360	0.618	0.548	0.365	0.498	0.335	0.571	0.031	0.028	0.030	0.026	0.041	0.024	0.035	0.021	0.032	0.021					
FSV-CH	0.466	0.283	0.425	0.292	0.427											0.014	0.024	0.016	0.023	0.012					
FSV-CI																									
FSV-CK	0.447	0.434	0.631	0.516	0.859											0.027	0.052	0.034	0.062	0.030					
FSV-CL	0.430	0.286	0.399	0.279	0.434											0.015	0.023	0.016	0.022	0.011					
FSV-CR																									
FSV-CV	0.568	0.386	0.593	0.388	0.741											0.032	0.063	0.024	0.051	0.169					
FSV-CW	0.512	0.346	0.485	0.338	0.526	0.488	0.324	0.460	0.313	0.492	*0.024*	*0.022*	*0.025*	*0.025*	*0.034*	0.023	0.031	0.022	0.031	0.017					
FSV-CX	>0.36	>0.24	>0.34	>0.21	>0.37	0.360	0.240	0.340	0.210	0.370						>0.02	>0.02	>0.02	>0.02	>0.02	0.020	0.020	0.020	0.020	0.020
FSV-CZ	0.600	0.440	0.560	0.430	0.680																				
FSV-DB	0.360	0.210	0.360	0.260	0.420																				
FSV-DD																									
FSV-DF																									
FSV-DI	>0.508	>0.319	>0.509	>0.342	>0.512	0.508	0.319	0.509	0.342	0.512															
FSV-DQ	0.550	0.347	0.508	0.307	0.547											0.025	0.036	0.025	0.036	0.019					
FSV-DR	0.410	0.300	0.400	0.280	0.590																				
FSV-DU		>0.25	>0.37	>0.24	>0.55	!0.40	0.25	0.37	0.24	0.55															
FSV-EH	0.424	0.299	0.406	0.297	0.447	0.395	0.274	0.377	0.265	0.408	*0.029*	*0.025*	*0.029*	*0.032*	*0.039*	0.021	0.036	0.015	0.036	0.014					
FSV-EQ	0.493	0.316	0.453	0.304	0.506	0.487	0.309	0.447	0.295	0.495	0.006	0.007	0.006	0.009	0.011	0.013	0.020	0.012	0.019	0.010					
FSV-FB	0.606	0.414	0.560	0.393	0.659	0.464	0.314	0.431	0.296	0.475	0.142	0.099	0.129	0.097	0.185	0.018	0.030	0.020	0.027	0.016					
FSV-FJ	1.270	0.910	1.310	0.780	1.380											0.030	0.060	0.030	0.050	0.010					
N	31	31	31	31	31	12	13	13	13	13	9	9	9	9	9	27	27	27	27	27	1	1	1	1	1
Min	0.360	0.210	0.360	0.260	0.420	0.360	0.240	0.340	0.210	0.370	0.006	0.007	0.006	0.009	0.011	0.011	0.020	0.012	0.019	0.009					
Median	0.522	0.351	0.493	0.330	0.560	0.488	0.314	0.435	0.301	0.509	0.029	0.025	0.026	0.025	0.039	0.022	0.032	0.020	0.030	0.016	0.020	0.020	0.020	0.020	0.020
Max	1.270	0.910	1.310	0.780	1.380	0.581	0.410	0.547	0.374	0.624	0.142	0.099	0.129	0.097	0.185	0.032	0.063	0.034	0.062	0.169					
SD	0.060	0.045	0.063	0.049	0.080	0.030	0.031	0.043	0.020	0.051	0.007	0.007	0.004	0.010	0.008	0.005	0.010	0.006	0.009	0.007					
CV	11	13	13	15	14	6	10	10	7	10	26	27	17	42	21	25	31	30	30	42					
N_{past}	33	32	32	33	33	17	14	14	17	11	11	10	10	11	8	28	24	23	29	22	0	0	0	0	0
$Median_{past}$	0.508	0.352	0.499	0.307	0.582	0.470	0.316	0.442	0.287	0.535	0.024	0.023	0.025	0.020	0.041	0.021	0.029	0.018	0.030	0.017					
SD_{past}	0.069	0.035	0.046	0.063	0.078	0.047	0.025	0.034	0.041	0.065	0.008	0.005	0.004	0.006	0.027	0.006	0.007	0.004	0.007	0.008					
NISTa	0.432	0.316	0.422	0.302	0.511											*nq*	0.036	nq	0.029	*nq*					
NISTb	0.574	0.375	0.523	0.348	0.562	0.531	0.345	0.494	0.292	0.518	*0.043*	*0.031*	*0.029*	*0.056*	*0.044*	>0.016	0.032	0.021	0.032	0.022	0.016	0.026	0.017	0.023	0.012
N_{NIST}	3	3	3	3	3	2	2	2	2	2							3	1	3	1	2	2	2	2	2
Mean	0.503	0.345	0.470	0.325	0.537	0.528	0.345	0.494	0.292	0.517							0.034	0.021	0.031	0.022	0.016	0.026	0.017	0.023	0.012
S_{rep}	0.009	0.007	0.011	0.007	0.010	0.008	0.015	0.020	0.016	0.006							0.003	0.002	0.001	0.003	0.003	0.000	0.001	0.005	0.000
S_{het}	0.019	0.001	0.011	0.005	0.008	0.013	0.004	0.003	0.022	0.003							0.001		0.001		0.001	0.001	0.001	0.002	0.001
S_{anl}	0.100	0.042	0.074	0.032	0.036												0.003		0.002						
S_{NIST}	0.102	0.043	0.076	0.033	0.038	0.015	0.015	0.020	0.027	0.007							0.004		0.003		0.003	0.001	0.002	0.005	0.001
NAV	0.513	0.348	0.482	0.327	0.548	0.508	0.329	0.464	0.296	0.513	0.027	0.024	0.026	0.023	0.040	0.022	0.033	0.021	0.030	0.019					
NAU	0.103	0.050	0.077	0.049	0.081	0.059	0.040	0.062	0.033	0.054	0.006	0.004	0.003	0.006	0.014	0.008	0.011	0.007	0.010	0.008					

C3

Round Robin L Laboratory Results

All Results in µg/mL

	Total Lycopene					trans-Lycopene					Total β-Cryptoxanthin					Total α-Cryptoxanthin					Total Lutein					trans-Lutein				
Lab	274	275	276	277	278	274	275	276	277	278	274	275	276	277	278	274	275	276	277	278	274	275	276	277	278	274	275	276	277	278
FSV-BA						0.144	0.185	0.129	0.181	0.333	0.078	0.076	0.069	0.081	0.062															
FSV-BB	0.222	0.330	0.207	0.311	0.572	0.117	0.152	0.107	0.141	0.273	0.052	0.055	0.049	0.052	0.049	0.024	0.025	0.023	0.023	0.019	0.102	0.092	0.092	0.088	0.077					
FSV-BD																														
FSV-BE																														
FSV-BF	0.199	0.374	0.208	0.386	0.768						0.035	0.042	0.036	0.043	0.040															
FSV-BG	0.227	0.362	0.192	0.252	0.549	0.130	0.208	0.126	0.143	0.335	0.046	0.045	0.042	0.043	0.048						0.081	0.089	0.083	0.086	0.089					
FSV-BH	0.223	0.337	0.207	0.307	0.542						0.078	0.090	0.073	0.086	0.090															
FSV-BI	0.183	0.281	0.172	0.256	0.509						0.062	0.065	0.056	0.062	0.048						0.092	0.104	0.085	0.097	0.081					
FSV-BJ	0.212	0.290	0.179	0.263	0.493						0.051	0.053	0.051	0.055	0.048						0.080	0.074	0.076	0.074	0.065					
FSV-BK																														
FSV-BL																														
FSV-BM																														
FSV-BN	0.313	0.456	0.267	0.425	0.823	0.175	0.239	0.146	0.226	0.470	0.061	0.060	0.049	0.057	0.052	0.027	0.024	0.021	0.023	0.018	0.112	0.104	0.092	0.098	0.087					
FSV-BO	0.270	0.368	0.237	0.227	0.722						0.044	0.045	0.042	0.045	0.047						0.147	0.144	0.132	0.144	0.139					
FSV-BP	0.297	0.407	0.276	0.363	0.632						0.057	0.055	0.049	0.050	0.047															
FSV-BQ																														
FSV-BR																														
FSV-BU	0.201	0.291	0.178	0.281	0.512						0.046	0.048	0.044	0.045	0.041															
FSV-BV	0.238	0.343	0.217	0.335	0.567						0.032	0.033	0.030	0.031	0.027															
FSV-BW	0.230	0.370	0.230	0.340	0.650																									
FSV-BX						0.120	0.155	0.113	0.151	0.290	0.045	0.045	0.044	0.042	0.041						0.095	0.084	0.097	0.081	0.069					
FSV-CB	0.198	0.308	0.184	0.302	0.524						0.047	0.049	0.044	0.048	0.045						0.094	0.079	0.072		0.072					
FSV-CC																														
FSV-CD	0.229	0.299	0.220	0.280	0.491						0.043	0.047	0.043	0.044	0.056															
FSV-CE																														
FSV-CF																														
FSV-CG	0.234	0.353	0.213	0.323	0.569	0.132	0.177	0.117	0.160	0.306	0.042	0.047	0.038	0.043	0.044						0.130	0.115	0.119	0.107	0.106					
FSV-CH	0.181	0.266	0.180	0.253	0.404																									
FSV-CI																														
FSV-CK	0.173	0.328	0.264	0.376	0.697						0.048	0.081	0.059	0.063	0.064	0.046	0.042	0.038	0.044	0.043	0.099	0.099	0.099	0.099	0.083					
FSV-CL	0.220	0.318	0.200	0.305	0.501						0.047	0.048	0.045	0.049	0.037	0.026	0.026	0.024	0.026	0.019										
FSV-CR																														
FSV-CV	0.304	0.473	0.312	0.490	0.927																									
FSV-CW	0.222	0.346	0.215	0.332	0.553	0.123	0.164	0.113	0.155	0.287	0.049	0.053	0.046	0.051	0.049	0.024	0.025	0.022	0.024	0.021	>0.084	>0.08	>0.079	>0.076	>0.061	0.084	0.080	0.079	0.076	0.061
FSV-CX	0.180	0.270	0.170	0.250	0.460	0.110	0.140	0.100	0.130	0.250	0.050	0.060	0.050	0.050	0.040						0.070	0.070	0.070	0.070	0.060					
FSV-CZ																														
FSV-DB	0.207	0.321	0.204	0.334	0.541						0.056	0.056	0.046	0.052	0.045															
FSV-DD																														
FSV-DF																														
FSV-DI						0.172	0.241	0.165	0.247	0.403											0.095	0.089	0.092	0.085	0.078					
FSV-DQ	0.280	0.402	0.236	0.366	0.599						0.065	0.063	0.061	0.059	0.054						0.095	0.108	0.102	0.102	0.095					
FSV-DR																														
FSV-DU																														
FSV-EH	0.230	0.359	0.226	0.349	0.622	0.124	0.193	0.120	0.206	0.373	0.048	0.059	0.051	0.054	0.052						0.088	0.081	0.084	0.079	0.071					
FSV-EQ	0.231	0.337	0.213	0.330	0.573						0.050	0.056	0.047	0.055	0.056															
FSV-FB	0.210	0.340	0.220	0.320	0.580	0.176	0.287	0.187	0.270	0.491	0.059	0.059	0.053	0.054	0.051						0.064	0.066	0.060	0.060	0.060					
FSV-FJ																														
N	26	26	26	26	26	11	11	11	11	11	25	25	25	25	25	5	5	5	5	5	15	15	15	14	15	1	1	1	1	1
Min	0.173	0.266	0.170	0.227	0.404	0.110	0.140	0.100	0.130	0.250	0.032	0.033	0.030	0.031	0.027	0.024	0.024	0.021	0.023	0.018	0.064	0.066	0.060	0.060	0.060					
Median	0.223	0.339	0.213	0.322	0.568	0.130	0.185	0.120	0.160	0.333	0.049	0.055	0.047	0.051	0.048	0.026	0.025	0.023	0.024	0.019	0.095	0.089	0.092	0.087	0.078	0.084	0.080	0.079	0.076	0.061
Max	0.313	0.473	0.312	0.490	0.927	0.176	0.287	0.187	0.270	0.491	0.078	0.090	0.073	0.086	0.090	0.046	0.042	0.038	0.044	0.043	0.147	0.144	0.132	0.144	0.139					
SD	0.023	0.042	0.026	0.049	0.085	0.027	0.048	0.018	0.051	0.074	0.008	0.010	0.005	0.008	0.006	0.002	0.001	0.001	0.002	0.001	0.012	0.018	0.014	0.014	0.013					
CV	10	12	12	15	15	21	26	15	32	22	17	18	12	15	12	9	5	6	9	8	13	20	15	16	17					
N_{past}	29	27	27	29	24	12	12	12	12	11	29	25	25	29	20	7	5	5	7	9	16	15	15	16	14	0	0	0	0	0
$Median_{past}$	0.228	0.363	0.230	0.330	0.575	0.135	0.189	0.124	0.165	0.380	0.050	0.057	0.051	0.054	0.061	0.029	0.027	0.024	0.026	0.027	0.092	0.089	0.088	0.083	0.083					
SD_{past}	0.042	0.046	0.037	0.057	0.135	0.018	0.042	0.032	0.015	0.083	0.010	0.007	0.013	0.014	0.016	0.006	0.007	0.009	0.007	0.009	0.017	0.025	0.029	0.012	0.024					
NISTa											0.048	0.046	0.048	0.042	0.044						0.108	0.094	0.087	0.107	0.086					
NISTb	0.288	0.370	0.227	0.334	0.621						0.065	0.058	0.049	0.052	0.047	0.032	0.030	0.030	0.029	0.024	*>0.076*	*>0.080*	*>0.073*	*>0.075*	*>0.069*	0.076	0.080	0.073	0.075	0.069
N_{NIST}	2	2	2	2	2						4	3	3	3	3	2	1	1	1	1	4	4	4	4	4	2	2	2	2	2
Mean	0.282	0.370	0.227	0.330	0.620						0.040	0.052	0.048	0.047	0.046	0.016	0.030	0.030	0.029	0.024	0.091	0.087	0.080	0.091	0.077	0.073	0.080	0.073	0.075	0.067
S_{rep}	0.010	0.006	0.031	0.001	0.015						0.004	0.003	0.004	0.004	0.005	0.003	0.002	0.004	0.000	0.007	0.004	0.004	0.004	0.003	0.006	0.000	0.006	0.003	0.004	0.003
S_{het}	0.024	0.004	0.029	0.015	0.001						0.032	0.001	0.002	0.000	0.003	0.023					0.007	0.001	0.003	0.004	0.009	0.010	0.000	0.004	0.004	0.006
S_{anl}											0.011	0.009	0.001	0.007	0.002						0.024	0.010	0.010	0.022	0.013					
S_{NIST}	0.026	0.007	0.043	0.015	0.015						0.034	0.009	0.005	0.008	0.006	0.023					0.026	0.011	0.011	0.022	0.017	0.010	0.006	0.005	0.006	0.007
NAV	0.252	0.354	0.220	0.326	0.594	0.130	0.185	0.120	0.160	0.333	0.045	0.053	0.048	0.049	0.047	0.024	0.027	0.026	0.026	0.023	0.093	0.088	0.086	0.089	0.077					
NAU	0.068	0.078	0.053	0.072	0.121	0.027	0.048	0.021	0.051	0.074	0.035	0.013	0.012	0.013	0.012	0.004	0.003	0.004	0.003	0.004	0.026	0.018	0.020	0.023	0.017					

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Round Robin L Laboratory Results

All Results in µg/mL

	Total Zeaxanthin					Total Lutein&Zeaxanthin					Coenzyme Q10					Phylloquinone (K1) x1000				
Lab	274	275	276	277	278	274	275	276	277	278	274	275	276	277	278	274	275	276	277	278
FSV-BA						0.187	0.186	0.166	0.181	0.136										
FSV-BB	0.046	0.037	0.040	0.037	0.048	*0.148*	*0.129*	*0.132*	*0.125*	*0.125*										
FSV-BD																				
FSV-BE																1.24	0.44	1.04	0.40	0.30
FSV-BF						0.138	0.131	0.128	0.122	0.119										
FSV-BG	0.030	0.024	0.026	0.021	0.024	0.101	0.102	0.098	0.098	0.104										
FSV-BH																				
FSV-BI	0.039	0.031	0.036	0.029	0.035	*0.131*	*0.135*	*0.121*	*0.126*	*0.116*										
FSV-BJ											0.546	0.545	0.551	0.480	0.733					
FSV-BK																				
FSV-BL																				
FSV-BM																				
FSV-BN	0.050	0.030	0.042	0.030	0.034	0.160	0.134	0.133	0.127	0.120										
FSV-BO	0.044	0.036	0.042	0.021	0.053	0.191	0.180	0.174	0.165	0.192										
FSV-BP						0.160	0.140	0.140	0.140	0.120										
FSV-BQ																				
FSV-BR																				
FSV-BU						0.082	0.120	0.132	0.117	0.110										
FSV-BV						0.120	0.100	0.112	0.095	0.089										
FSV-BW																				
FSV-BX	0.031	0.024	0.030	0.021	0.024	*0.127*	*0.108*	*0.128*	*0.102*	*0.093*										
FSV-CB	0.039	0.025	0.034		0.034	*0.134*	*0.104*	*0.106*	0.124	*0.106*										
FSV-CC																				
FSV-CD						0.117	0.110	0.117	0.105	0.098										
FSV-CE																				
FSV-CF																				
FSV-CG	0.020	0.020	0.018	0.017	0.018	0.150	0.135	0.137	0.124	0.124										
FSV-CH											0.545	0.562	0.415	0.572	0.449					
FSV-CI																0.59	0.19	0.59	0.20	0.10
FSV-CK	0.041	0.031	0.041	0.032	0.038	0.129	0.128	0.141	0.134	0.135										
FSV-CL						0.113	0.099	0.102	0.089	0.079										
FSV-CR																				
FSV-CV						0.116	0.109	0.107	0.108	0.096	0.683	0.873	0.830	0.947	0.935					
FSV-CW	0.039	0.028	0.037	0.027	0.031	>0.123	>0.108	>0.116	>0.103	>0.092	0.860	0.940	0.830	0.910	0.960					
FSV-CX	0.040	0.030	0.040	0.030	0.040	*0.110*	*0.100*	*0.110*	*0.100*	*0.100*										
FSV-CZ											1.090	0.990	0.930	0.920	1.030					
FSV-DB						0.128	0.117	0.123	0.108	0.100										
FSV-DD																				
FSV-DF																				
FSV-DI											0.420	0.480	0.517	0.609	0.549	1.07	0.28	1.10	0.32	0.11
FSV-DQ	0.036	0.023	0.029	0.020	0.023	*0.131*	*0.131*	*0.131*	*0.122*	*0.118*										
FSV-DR																				
FSV-DU																				
FSV-EH	0.026	0.022	0.026	0.020	0.021	*0.114*	*0.103*	*0.110*	*0.099*	*0.092*	0.882	0.960	0.845	0.858	1.043					
FSV-EQ						0.208	0.205	0.197	0.198	0.218										
FSV-FB	0.033	0.025	0.031	0.022	0.030	0.097	0.091	0.091	0.082	0.090										
FSV-FJ																				
N	14	14	14	13	14	23	23	23	23	23	7	7	7	7	7	3	3	3	3	3
Min	0.020	0.020	0.018	0.017	0.018	0.082	0.091	0.091	0.082	0.079	0.420	0.480	0.415	0.480	0.449	0.59	0.19	0.59	0.20	0.10
Median	0.039	0.027	0.035	0.022	0.032	0.129	0.120	0.128	0.122	0.110	0.683	0.873	0.830	0.858	0.935	1.07	0.28	1.04	0.32	0.11
Max	0.050	0.037	0.042	0.037	0.053	0.208	0.205	0.197	0.198	0.218	1.090	0.990	0.930	0.947	1.043	1.24	0.44	1.10	0.40	0.30
SD	0.007	0.005	0.008	0.007	0.010	0.025	0.023	0.019	0.019	0.019	0.242	0.294	0.225	0.241	0.263					
CV	17	20	23	31	30	20	19	15	15	17	35	34	27	28	28					
N_{past}	15	13	13	15	10	29	22	22	29	16	0	5	5	0	0	0	0	0	0	0
$Median_{past}$	0.035	0.024	0.034	0.027	0.034	0.133	0.121	0.132	0.114	0.117		0.950	0.830							
SD_{past}	0.009	0.006	0.007	0.005	0.011	0.025	0.027	0.029	0.017	0.030		0.151	0.162							
NISTa	0.031	0.015	0.026	*nq*	0.028	*0.139*	*0.109*	*0.113*	*>0.107*	*0.114*										
NISTb	*>0.043*	*>0.036*	*>0.037*	*>0.035*	*>0.044*	*>0.119*	*>0.115*	*>0.110*	*>0.110*	*>0.113*										
N_{NIST}	4	4	4	2	4	4	4	4	2	4										
Mean	0.036	0.025	0.031	0.035	0.037	0.127	0.112	0.111	0.110	0.113										
S_{rep}	0.003	0.003	0.003	0.007	0.002	0.006	0.003	0.005	0.010	0.009										
S_{het}	0.007	0.002	0.002	0.001	0.004	0.015	0.002	0.006	0.003	0.008										
S_{anl}	0.007	0.015	0.008		0.012	0.017	0.005	0.002		0.001										
S_{NIST}	0.011	0.015	0.009	0.007	0.013	0.024	0.006	0.008	0.010	0.012										
NAV	0.038	0.026	0.033	0.028	0.034	0.128	0.116	0.120	0.116	0.112	0.683	0.912	0.830	0.858	0.935					
NAU	0.011	0.015	0.010	0.011	0.014	0.027	0.025	0.029	0.027	0.023	0.242	0.166	0.139	0.241	0.263					

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Round Robin L Laboratory Results
All Results in µg/mL

Analytes Reported By One Laboratory

Analyte	Code	274	275	276	277	278
trans-β-Cryptoxanthin	NISTb	0.047	0.053	0.048	0.042	0.0160
trans-Zeaxanthin	NISTb	0.043	0.036	0.037	0.035	0.076
trans-Lutein&Zeaxanthin	NISTb	0.119	0.115	0.110	0.110	0.037
9-cis-β-Carotene	FSV-FB	0.02	0.02	0.02	0.02	0.14
13-cis-β-Carotene	FSV-FB	0.12	0.08	0.11	0.08	0.04
Phytofluene	FSV-CL	0.011	0.032	0.010	0.030	0.113
Phytoene	FSV-CL	0.059	0.079	0.058	0.077	0.044
25-hydroxyvitamin D	FSV-BN	0.0073	0.0079	0.0258	*nq*	0.041

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
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)
NISTa, NISTb	Mean of all analyses (vials x duplicates) reported by given NIST analyst
N_{NIST}	Number of total vials analyzed in duplicate by NIST analysts
$Mean_{NIST}$	Mean of the NIST-analyzed vial means
S_{rep}	Within-vial pooled standard deviation
S_{het}	Among-vial pooled standard deviation
S_{anl}	Between NIST analyst standard deviation
S_{NIST}	Total standard deviation for NIST analyses: $(S_{rep}^2 + S_{het}^2 + S_{anl}^2)^{0.5}$
NAV	NIST Assigned Value = $(Median_{part} + Mean_{NIST})/2$ for analytes reported by NIST analyst(s) = $Median_{part}$ for analytes reported by ≥ 10 labs but not NIST
NAU	NIST Assigned Uncertainty: $(S^2 + S_{btw}^2)^{0.5}$ S is the maximum of (0.05*NAV, SD, S_{NIST}, eSD) and S_{btw} is the standard deviation between $Median_{part}$ and $Mean_{NIST}$. The expected long-term SD, 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 greater than x
!	Non-quantitative value: heterogeneous serum, damaged sample, malfunction, etc.
italics	Not explicitly reported but calculated by NIST from reported values

Comparability Summary

Lab	TR	tR	RP	aT	g/bT	bC	tbC	aC	TLy	tLy	TbX	TLu	TZ	L&Z
FSV-BA	1		1	1	1	1	1	1		1	2	1	1	1
FSV-BB	1		1	1	1	1	1	1	1	1	1	1	1	1
FSV-BD	1			2										
FSV-BE	1			1	1	1								1
FSV-BF	1			1	1	2		1	1		1			
FSV-BG	1		2	1	1	1		1	1	1	1			2
FSV-BH	2			1	1	2	3	1	1		3	1	1	1
FSV-BI	1			1	1	1		1	1		1			
FSV-BJ		1	1	1	1	1		1	1		1	1	1	1
FSV-BK	1			3								1	1	1
FSV-BL	1			2										
FSV-BM	1			1								1		
FSV-BN	1		1	2	2	1	1	1	2	2	1			
FSV-BO	1			1		1		1	2		1			1
FSV-BP	1			1		1		1	1		1			
FSV-BQ	1			1										2
FSV-BR		1		1								1	1	1
FSV-BU	2			3	1	1		1	1		1	1	1	1
FSV-BV	1			1	1	1		1	1		2			1
FSV-BW	1		1	1	1	1		2	1			1	1	1
FSV-BX		1		1	1		1	1		1	1	3	1	3
FSV-CB	1			1		1		1	1		1			
FSV-CC	1	2		1										
FSV-CD		1	2	1	1	1		1	1		1	2	2	1
FSV-CE	2			2		1								
FSV-CF	1			1										
FSV-CG	1			2	1	1	2	1	1	1	1			1
FSV-CH	2			1	2	2		1	1					1
FSV-CI	2		1	2	1									1
FSV-CK	1			1	2	3		3	1		2			1
FSV-CL	4			4	2	2		1	1		1	1	1	1
FSV-CR	2			1										
FSV-CV	4		2	2	1	2		4	3					
FSV-CW		2	1	2	1	1	1	1	1	1	1			
FSV-CX		2		1			3		1	1	1			
FSV-CZ	1			4		2								
FSV-DB	1			2		2			1		1	1	1	1
FSV-DD		2												
FSV-DF	1													
FSV-DI	1		1	2	1		1			2				
FSV-DQ				1	1	1		1	1		1			4
FSV-DR	1			1		2								
FSV-DU	1			1			2					1		
FSV-EH	2		1	2	2	2	2	1	1	1	1		1	
FSV-EQ	1			1		1	1	2	1		1			
FSV-FB	1			2		2	1	1	1	3	1	2	1	2
FSV-FJ	1			2	1	4		2						
NISTa	1	1		1	1	1		1			1	1	1	1
NISTb	1	1		1	1	1	1	1	1		1			
n	41	10	12	47	27	33	14	29	27	11	27	16	15	24

	TR	tR	RP	aT	g/bT	bC	tbC	aC	TLy	tLy	TbX	TLu	TZ	L&Z
% 1	78	60	75	64	81	64	64	83	89	73	85	81	93	79
% 2	17	40	25	28	19	30	21	10	7	18	11	13	7	13
% 3	0	0	0	4	0	3	14	3	4	9	4	6	0	4
% 4	5	0	0	4	0	3	0	3	0	0	0	0	0	4

Label	Definition
Lab	Participant code
TR	Total Retinol
tR	trans-Retinol
RP	Retinyl palmitate
aT	α-Tocopherol
g/bT	γ/β-Tocopherol
bC	Total β-Carotene
tbC	trans-β-Carotene
aC	Total α-Carotene
TLy	Total Lycopene
tLy	trans-Lycopene
TbX	Total β-Cryptoxanthin
TLu	Total Lutein
TZ	Total Zeaxanthin
L&Z	Total Lutein&Zeaxanthin
n	number of participants providing quantitative data
% 1	Percent of CS = 1 (within 1 SD of medians)
% 2	Percent of CS = 2 (within 2 SD of medians)
% 3	Percent of CS = 3 (within 3 SD of medians)
% 4	Percent of CS = 4 (3 or more SD from medians)

"Comparability Score"

The Comparability Score (CS) summarizes your measurement performance for a given analyte relative to the consensus medians in this study. CS is the average distance (in units of standard deviation) of your measurement performance characteristics from the consensus performance. CS is calculated when the number of quantitative values you reported, N_{you}, is at least two and at least six participants reported quantitative values for the analyte.

We define CS as follows:

$$CS = \text{MINIMUM}\left(4, \text{INTEGER}\left(1+\sqrt{C^2+AP^2}\right)\right)$$

$$C = \text{Concordance} = \frac{\sum_{i=1}^{N_{you}} \frac{You_i - Median_i}{NAU_i}}{N_{you}}$$

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

NAU = NIST Assigned Uncertainty

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 RR15

Each participant in RR15 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 RR15:

- 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 α-Cryptoxanthin
- Total Lutein
- Total Zeaxanthin
- Total Lutein & Zeaxanthin
- Coenzyme Q10

The following 12 pages are the "Individualized Report" for the analytes evaluated by participant FSV-BA.

Individualized Round Robin L Report: FSV-BA

Summary

	Serum 274			Serum 275			Serum 276			Serum 277			Serum 278		
Analyte	You	NAV	n	You	NAV	n	You	NAV	n	You	NAV	n	You	NAV	n
Total Retinol	0.654	0.646	39	0.452	0.456	39	0.608	0.610	40	0.431	0.443	40	1.050	1.056	40
Retinyl Palmitate	0.01	0.02	11	0.1	0.1	12	0.0	0.0	10	0.06	0.05	12	0.05	0.03	11
α-Tocopherol	6.38	6.05	44	6.84	6.98	45	5.94	5.81	45	6.53	6.55	45	6.89	7.00	45
γ/β-Tocopherol	2.612	2.275	25	2.008	1.929	25	2.397	2.154	25	1.908	1.777	25	2.545	2.303	25
Total β-Carotene	0.498	0.513	31	0.313	0.348	31	0.449	0.482	31	0.304	0.327	31	0.492	0.548	31
trans-β-Carotene	0.476	0.508	12	0.294	0.329	13	0.425	0.464	13	0.286	0.296	13	0.458	0.513	13
Total cis-β-Carotene	0.022		9	0.019		9	0.024		9	0.018		9	0.033		9
Total α-Carotene	0.019	0.022	27	0.024	0.033	27	0.020	0.021	27	0.024	0.030	27	0.016	0.019	27
trans-Lycopene	0.144	0.130	11	0.185	0.185	11	0.129	0.120	11	0.181	0.160	11	0.333	0.333	11
Total β-Cryptoxanthin	0.078	0.045	25	0.076	0.053	25	0.069	0.048	25	0.081	0.049	25	0.062	0.047	25
Total Lutein&Zeaxanthin	0.187	0.128	23	0.186	0.116	23	0.166	0.120	23	0.181	0.116	23	0.136	0.112	23

You : Your reported values for the listed analytes (micrograms/milliliter)
NAV : NIST Assigned Values, equal to (NIST's average-of-averages + this RR's median) / 2
n : Number of non-NIST laboratories reporting quantitative values for this analyte in this serum

D2

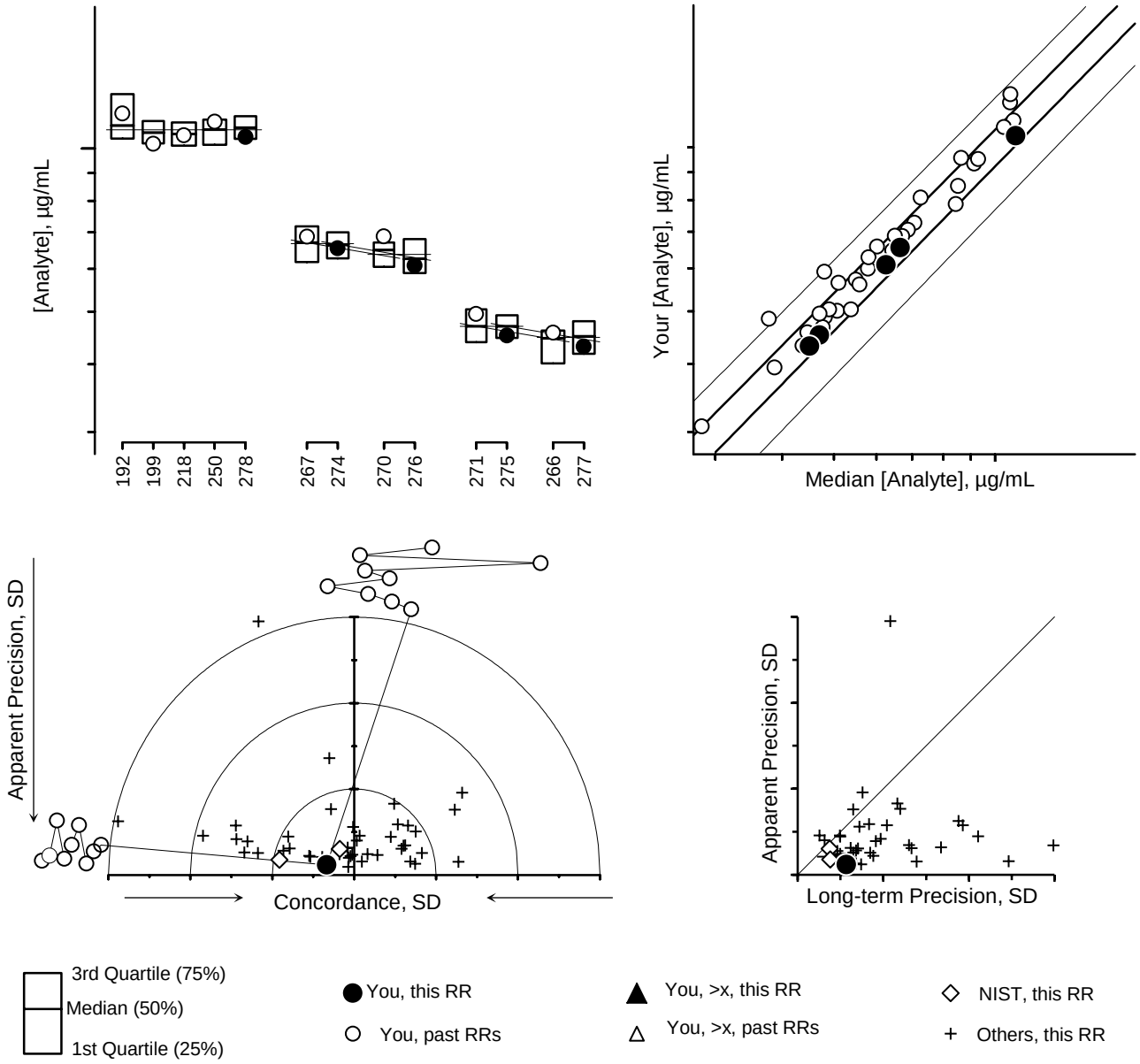
Please check our records against your records. Send corrections and/or updates to...

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

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

Individualized Round Robin L Report: FSV-BA

Total Retinol

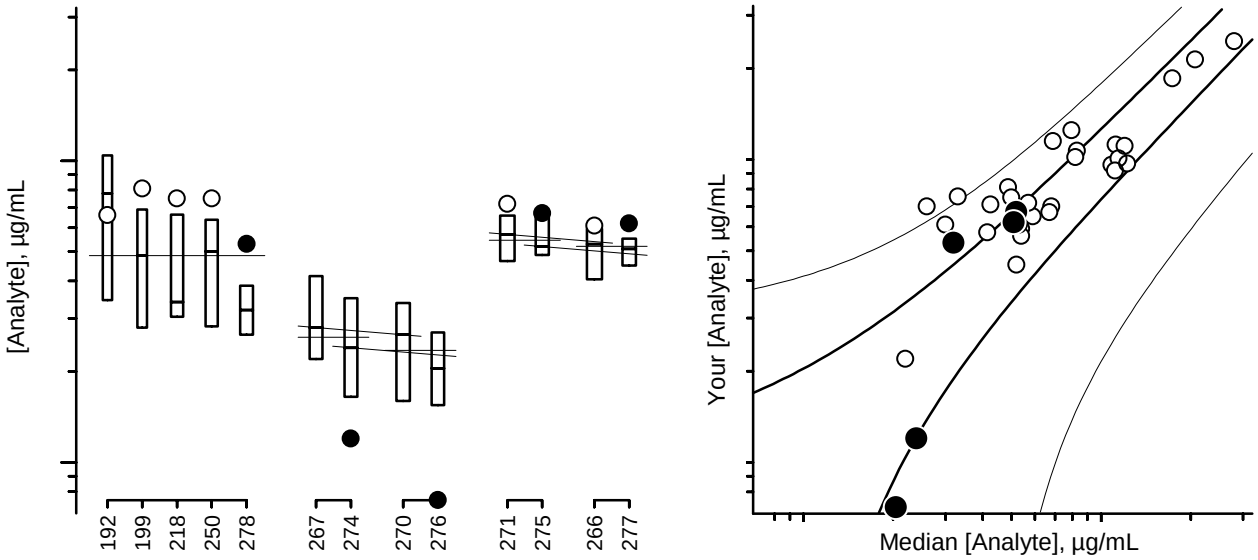


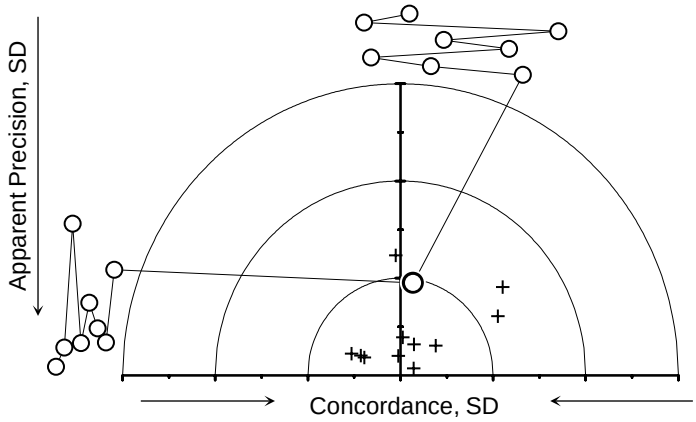
For details of the construction and interpretation of these plots, see:
Duewer, Kline, Sharpless, Brown Thomas, Gary, Sowell. Anal Chem 1999;71(9):1870-8.

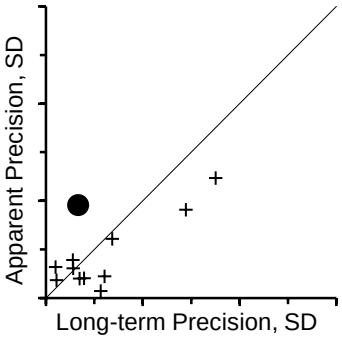
Serum	History	Comments
#274	#267 RR48, same pool as #276	Fresh frozen
#275	#271 RR49, same pool as #277	Fresh frozen
#276	#270 RR49, same pool as #274	Lyophilized
#277	#266 RR48, same pool as #275	Lyophilized
#278	#192 RR30, #199 RR32, #218 RR36, #250 RR44	Lyophilized

Individualized Round Robin L Report: FSV-BA

Retinyl Palmitate







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

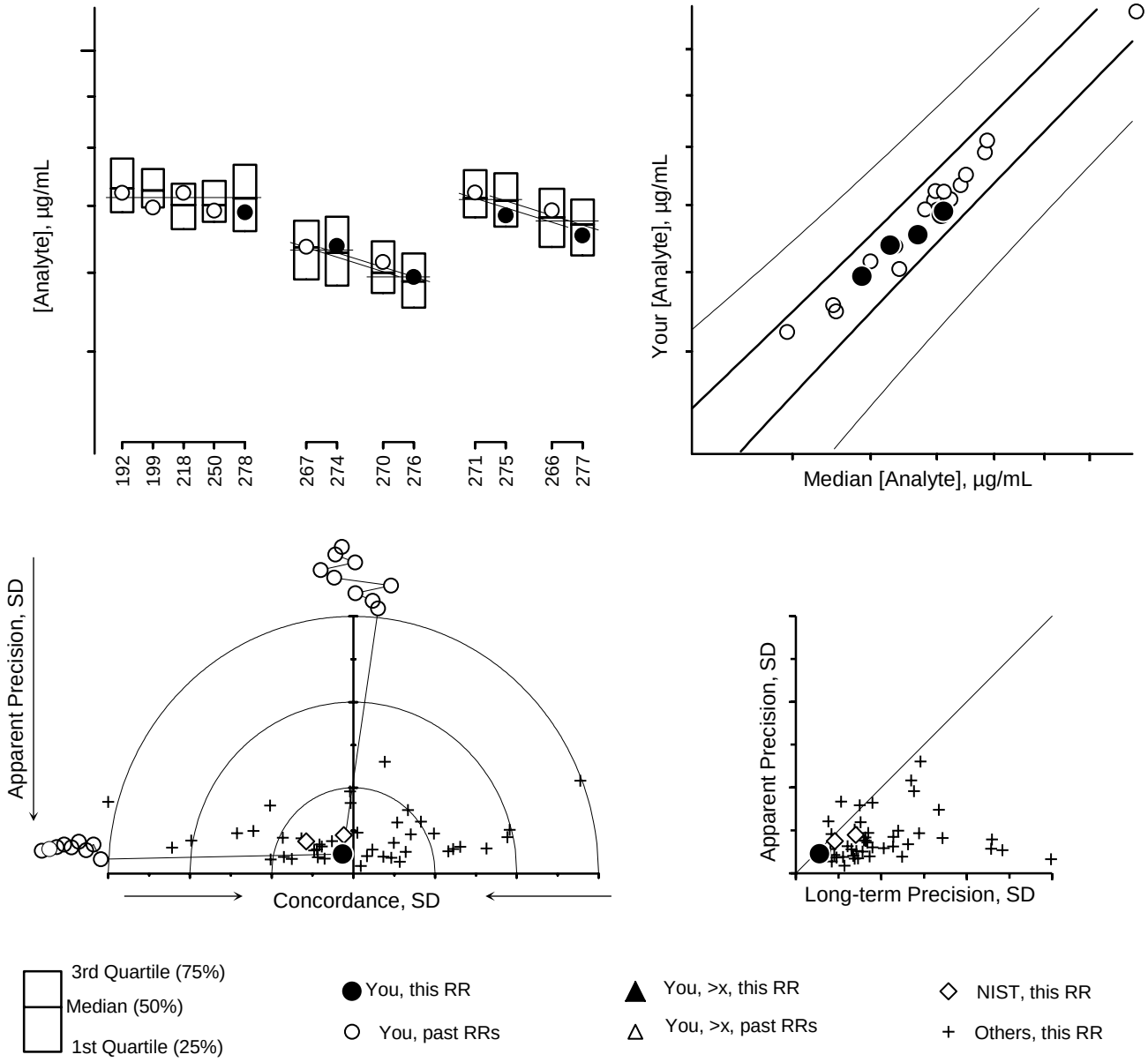
● You, this RR
○ You, past RRs
▲ You, >x, this RR
△ You, >x, past RRs
◇ NIST, this RR
+ Others, this RR

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.

Serum	History	Comments
#274	#267 RR48, same pool as #276	Fresh frozen
#275	#271 RR49, same pool as #277	Fresh frozen
#276	#270 RR49, same pool as #274	Lyophilized
#277	#266 RR48, same pool as #275	Lyophilized
#278	#192 RR30, #199 RR32, #218 RR36, #250 RR44	Lyophilized

Individualized Round Robin L Report: FSV-BA

α-Tocopherol

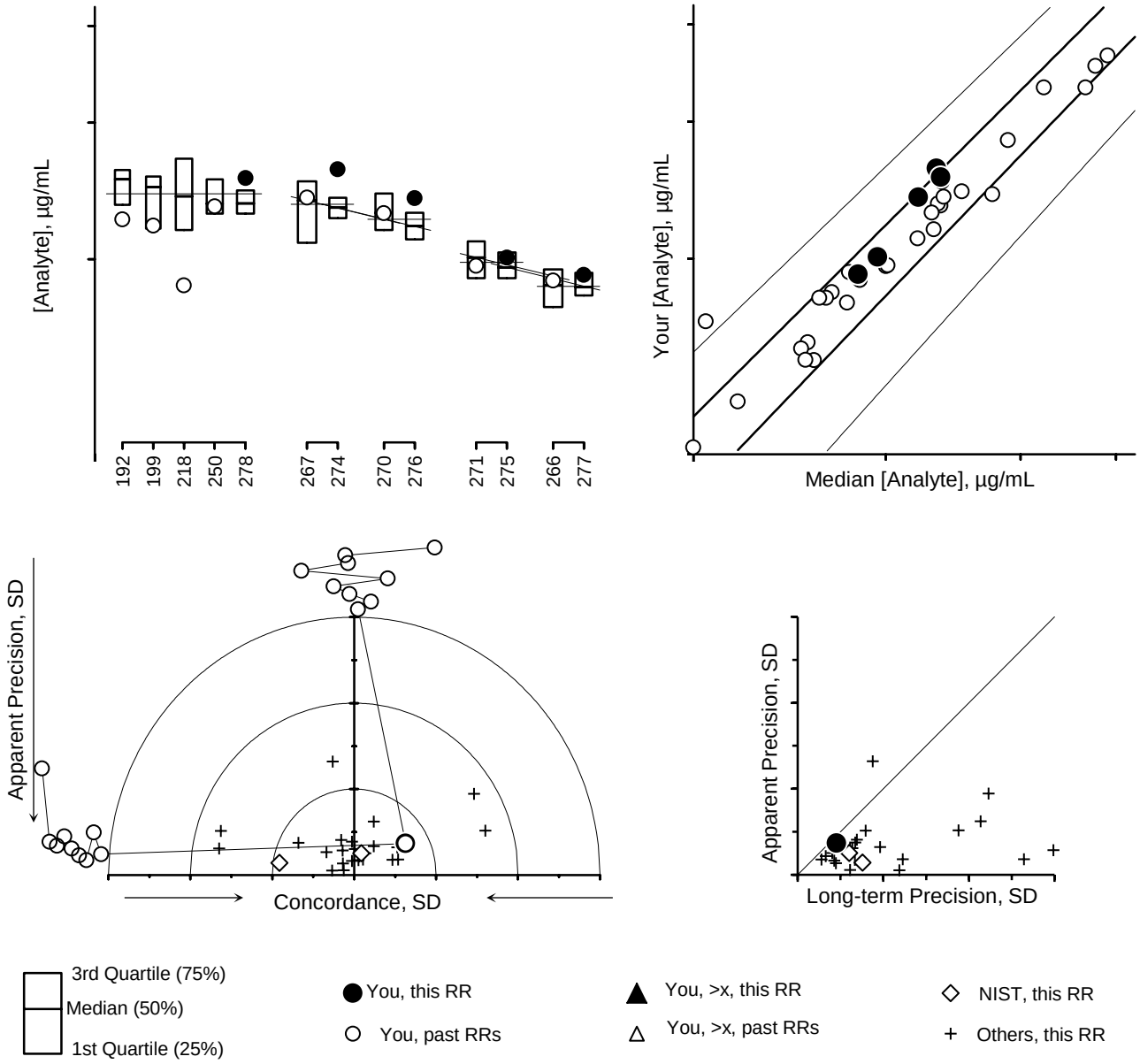


For details of the construction and interpretation of these plots, see:
Duewer, Kline, Sharpless, Brown Thomas, Gary, Sowell. Anal Chem 1999;71(9):1870-8.

Serum	History	Comments
#274	#267 RR48, same pool as #276	Fresh frozen
#275	#271 RR49, same pool as #277	Fresh frozen
#276	#270 RR49, same pool as #274	Lyophilized
#277	#266 RR48, same pool as #275	Lyophilized
#278	#192 RR30, #199 RR32, #218 RR36, #250 RR44	Lyophilized

Individualized Round Robin L Report: FSV-BA

γ/β-Tocopherol

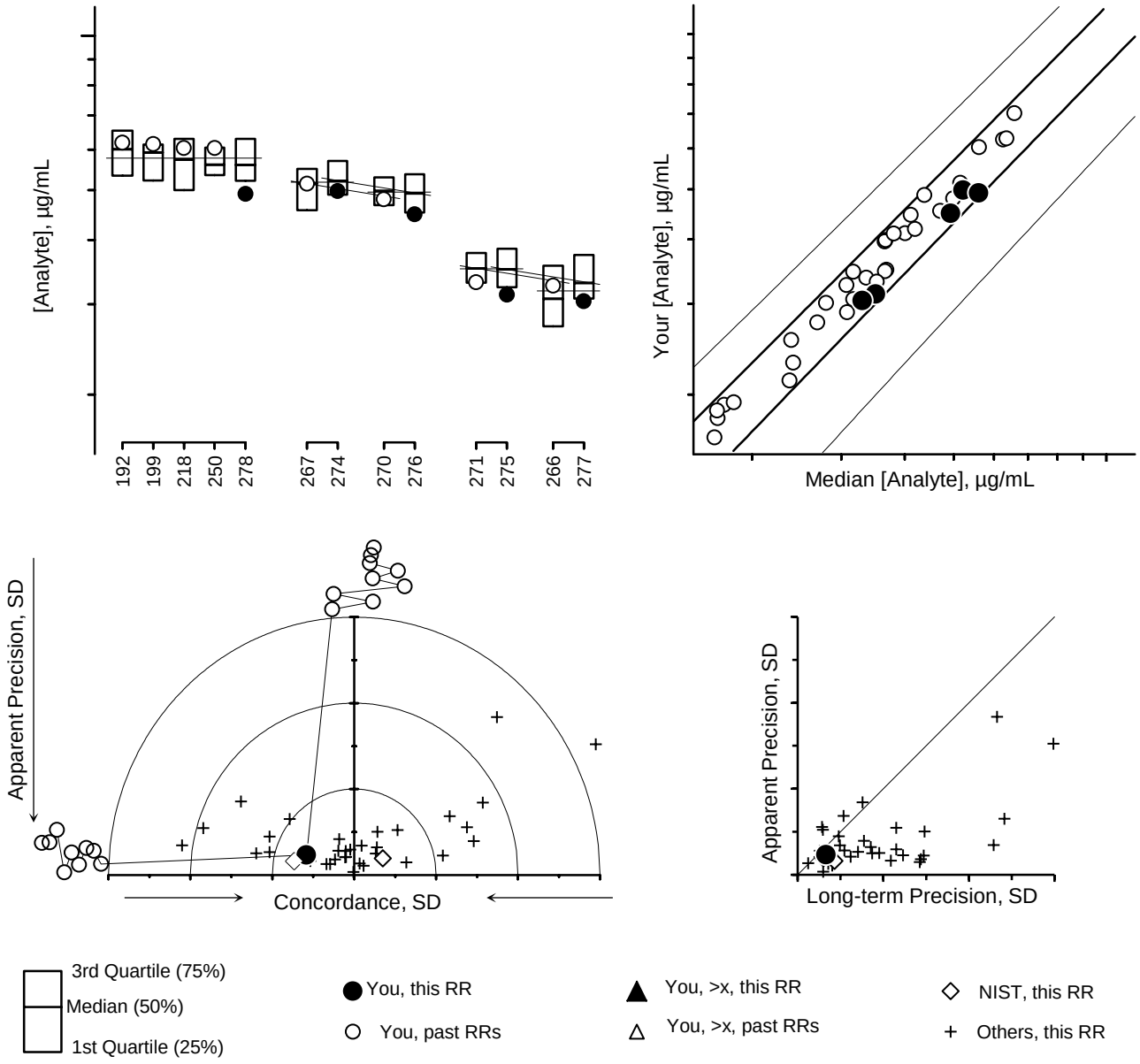


For details of the construction and interpretation of these plots, see:
Duewer, Kline, Sharpless, Brown Thomas, Gary, Sowell. Anal Chem 1999;71(9):1870-8.

Serum	History	Comments
#274	#267 RR48, same pool as #276	Fresh frozen
#275	#271 RR49, same pool as #277	Fresh frozen
#276	#270 RR49, same pool as #274	Lyophilized
#277	#266 RR48, same pool as #275	Lyophilized
#278	#192 RR30, #199 RR32, #218 RR36, #250 RR44	Lyophilized

Individualized Round Robin L Report: FSV-BA

Total β-Carotene

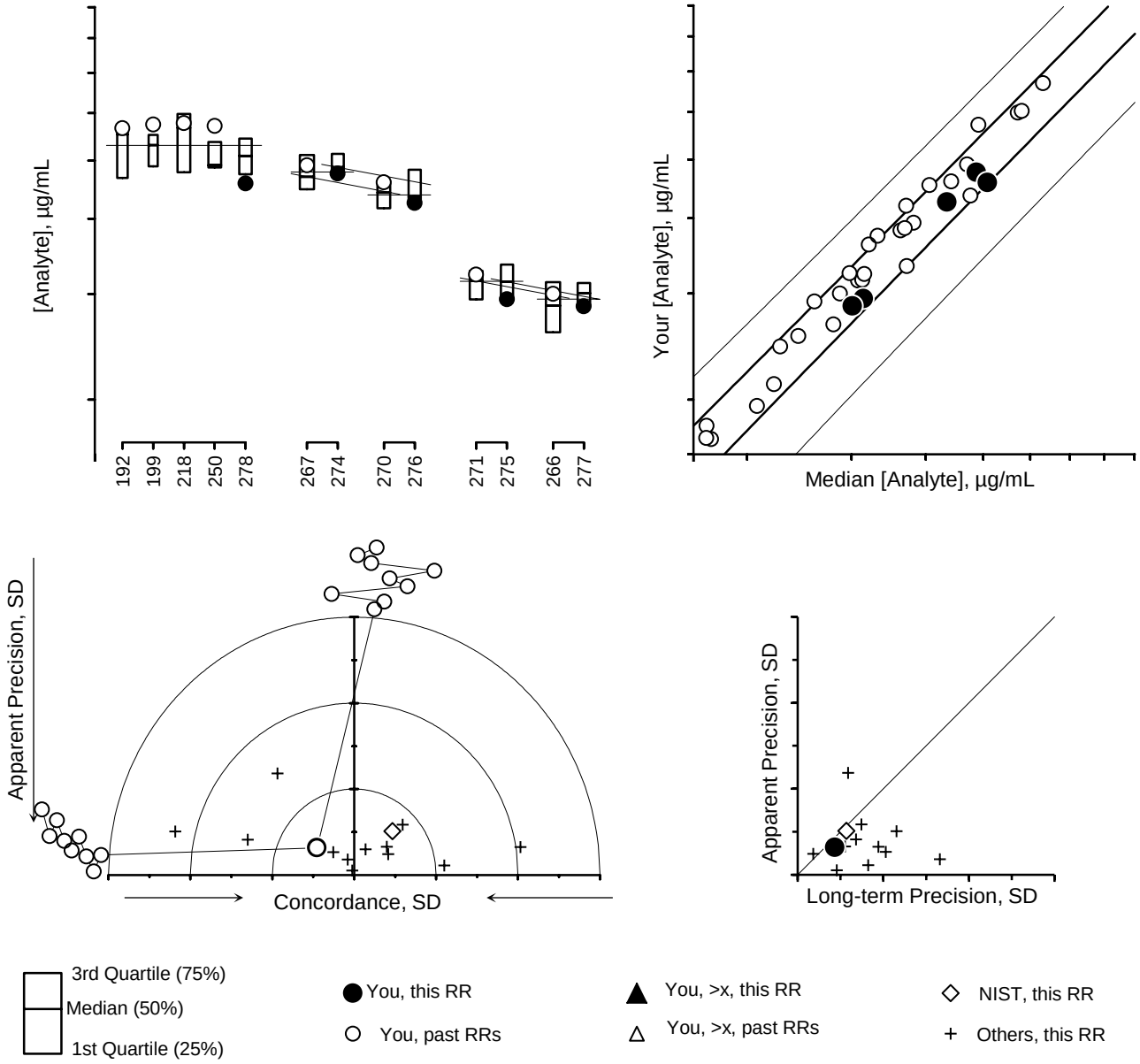


For details of the construction and interpretation of these plots, see:
Duewer, Kline, Sharpless, Brown Thomas, Gary, Sowell. Anal Chem 1999;71(9):1870-8.

Serum	History	Comments
#274	#267 RR48, same pool as #276	Fresh frozen
#275	#271 RR49, same pool as #277	Fresh frozen
#276	#270 RR49, same pool as #274	Lyophilized
#277	#266 RR48, same pool as #275	Lyophilized
#278	#192 RR30, #199 RR32, #218 RR36, #250 RR44	Lyophilized

Individualized Round Robin L Report: FSV-BA

trans-β-Carotene

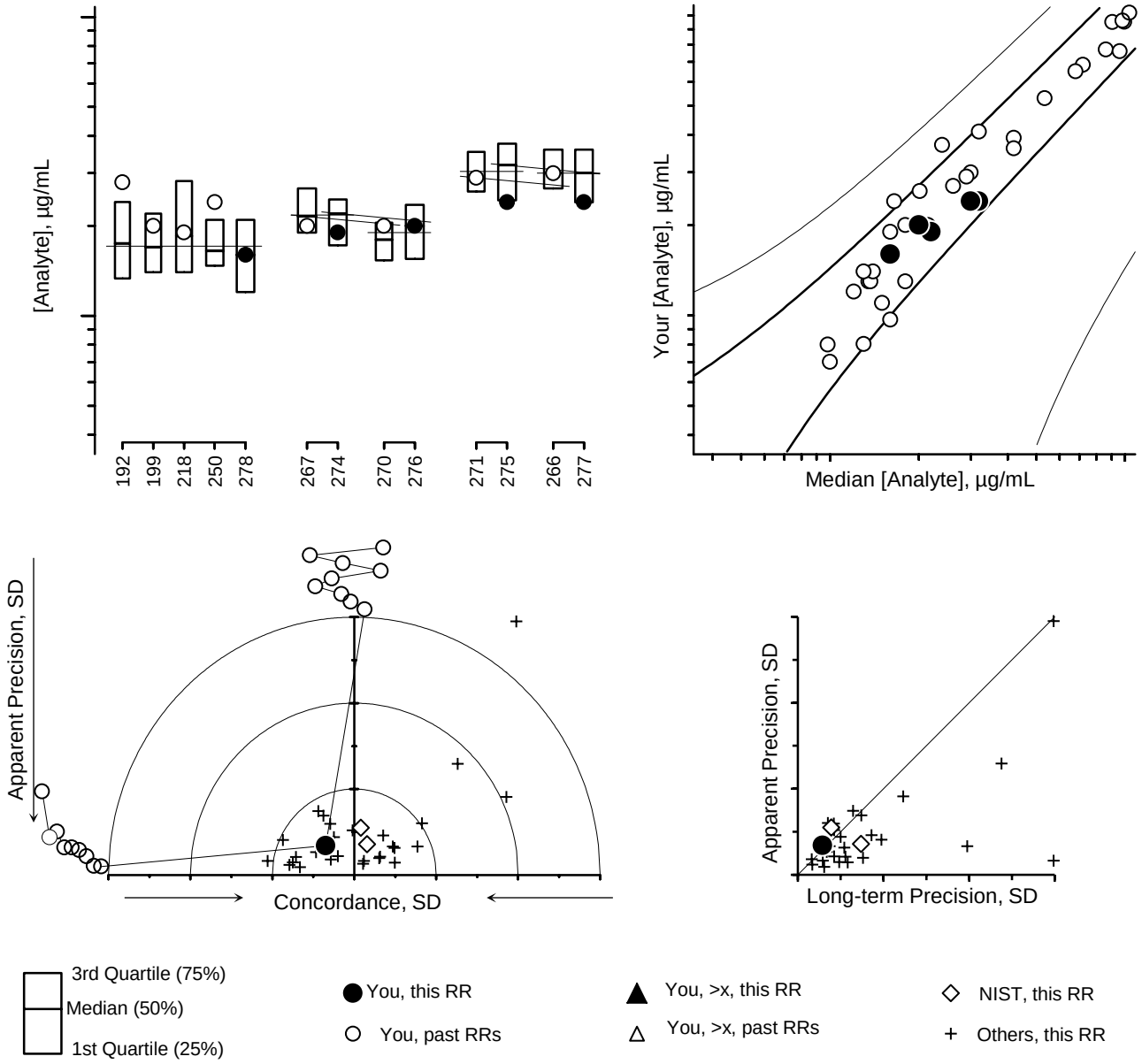


For details of the construction and interpretation of these plots, see:
Duewer, Kline, Sharpless, Brown Thomas, Gary, Sowell. Anal Chem 1999;71(9):1870-8.

Serum	History	Comments
#274	#267 RR48, same pool as #276	Fresh frozen
#275	#271 RR49, same pool as #277	Fresh frozen
#276	#270 RR49, same pool as #274	Lyophilized
#277	#266 RR48, same pool as #275	Lyophilized
#278	#192 RR30, #199 RR32, #218 RR36, #250 RR44	Lyophilized

Individualized Round Robin L Report: FSV-BA

Total α-Carotene

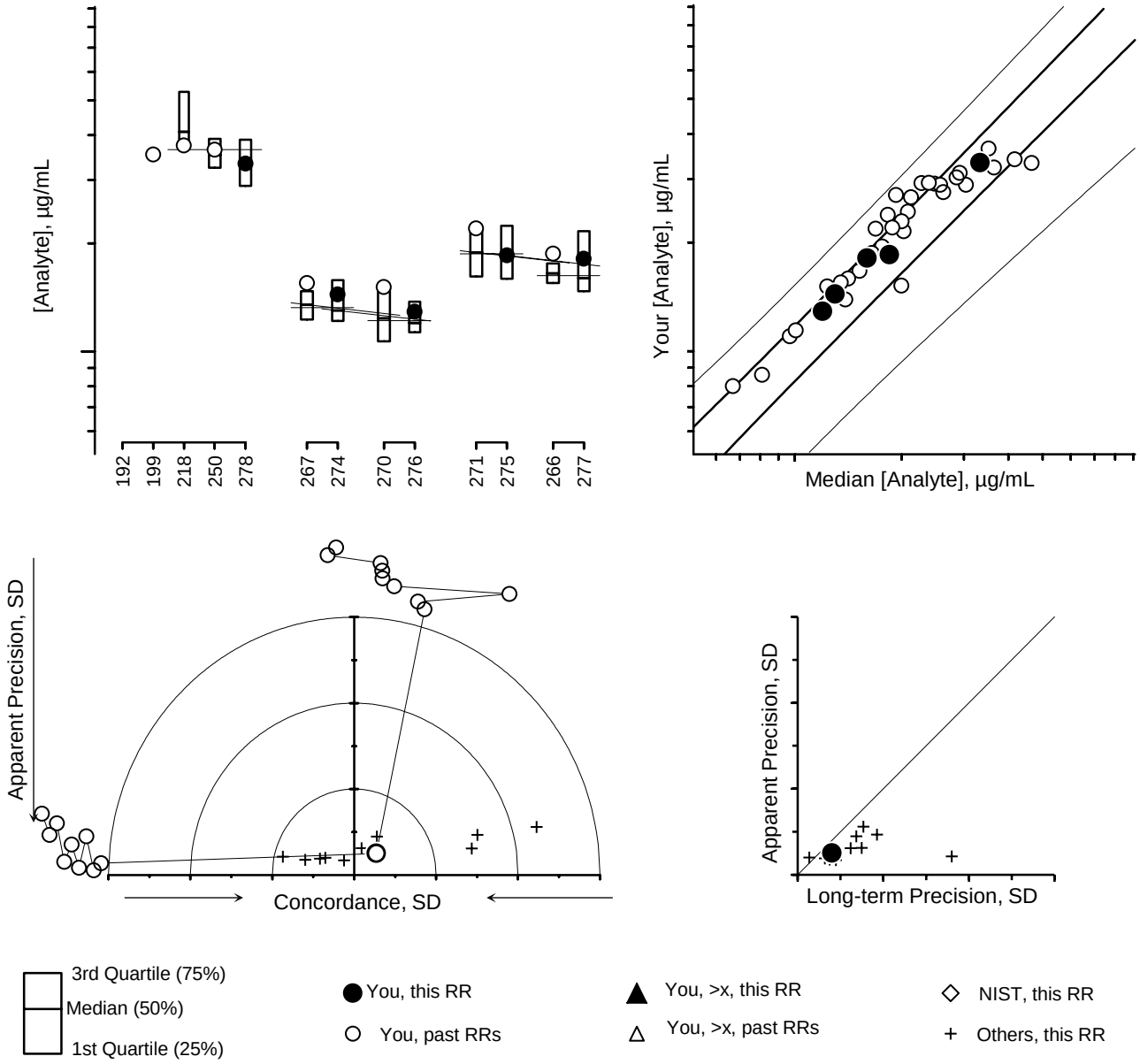


For details of the construction and interpretation of these plots, see:
Duewer, Kline, Sharpless, Brown Thomas, Gary, Sowell. Anal Chem 1999;71(9):1870-8.

Serum	History	Comments
#274	#267 RR48, same pool as #276	Fresh frozen
#275	#271 RR49, same pool as #277	Fresh frozen
#276	#270 RR49, same pool as #274	Lyophilized
#277	#266 RR48, same pool as #275	Lyophilized
#278	#192 RR30, #199 RR32, #218 RR36, #250 RR44	Lyophilized

Individualized Round Robin L Report: FSV-BA

trans-Lycopene

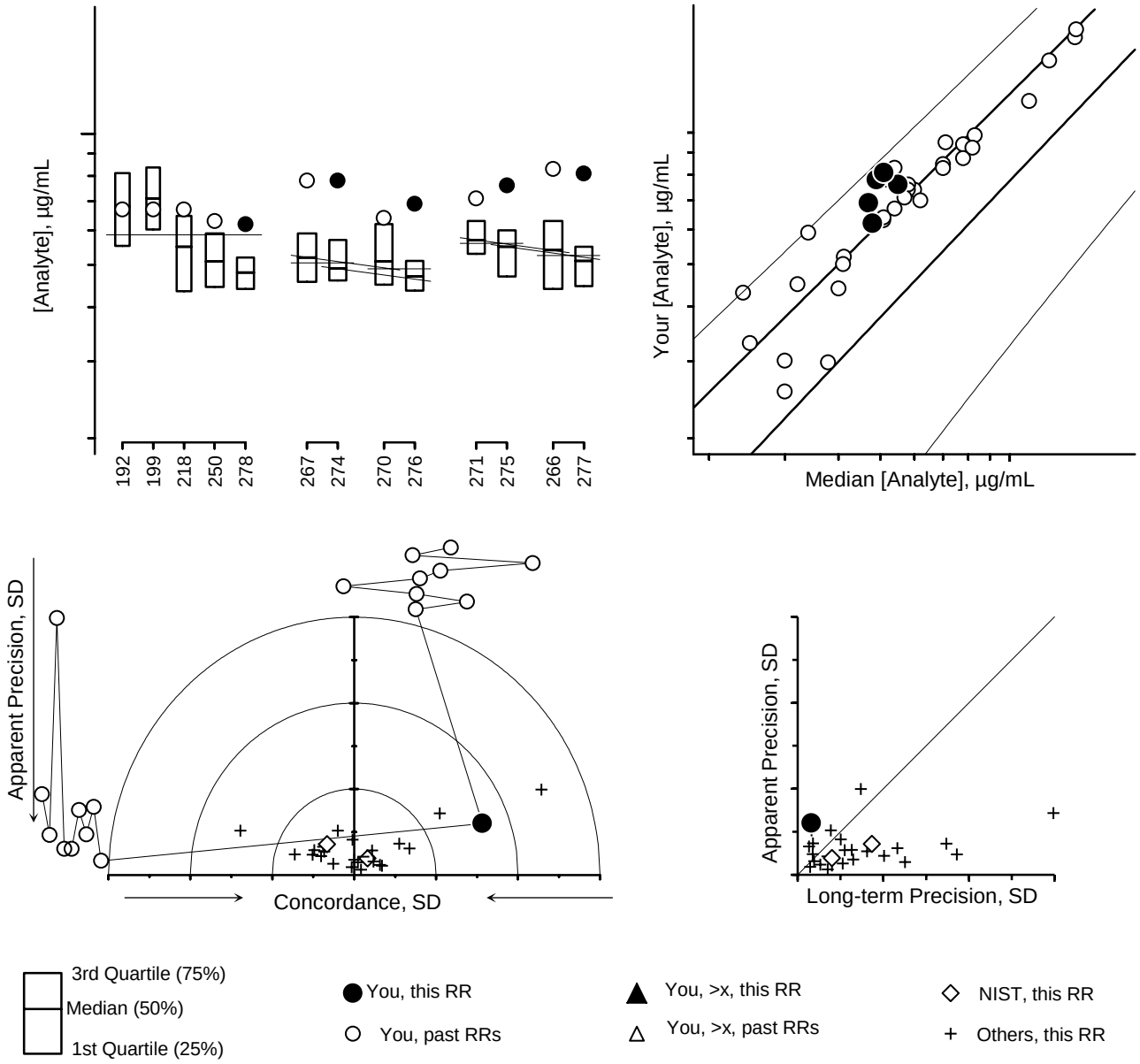


For details of the construction and interpretation of these plots, see:
Duewer, Kline, Sharpless, Brown Thomas, Gary, Sowell. Anal Chem 1999;71(9):1870-8.

Serum	History	Comments
#274	#267 RR48, same pool as #276	Fresh frozen
#275	#271 RR49, same pool as #277	Fresh frozen
#276	#270 RR49, same pool as #274	Lyophilized
#277	#266 RR48, same pool as #275	Lyophilized
#278	#192 RR30, #199 RR32, #218 RR36, #250 RR44	Lyophilized

Individualized Round Robin L Report: FSV-BA

Total β-Cryptoxanthin

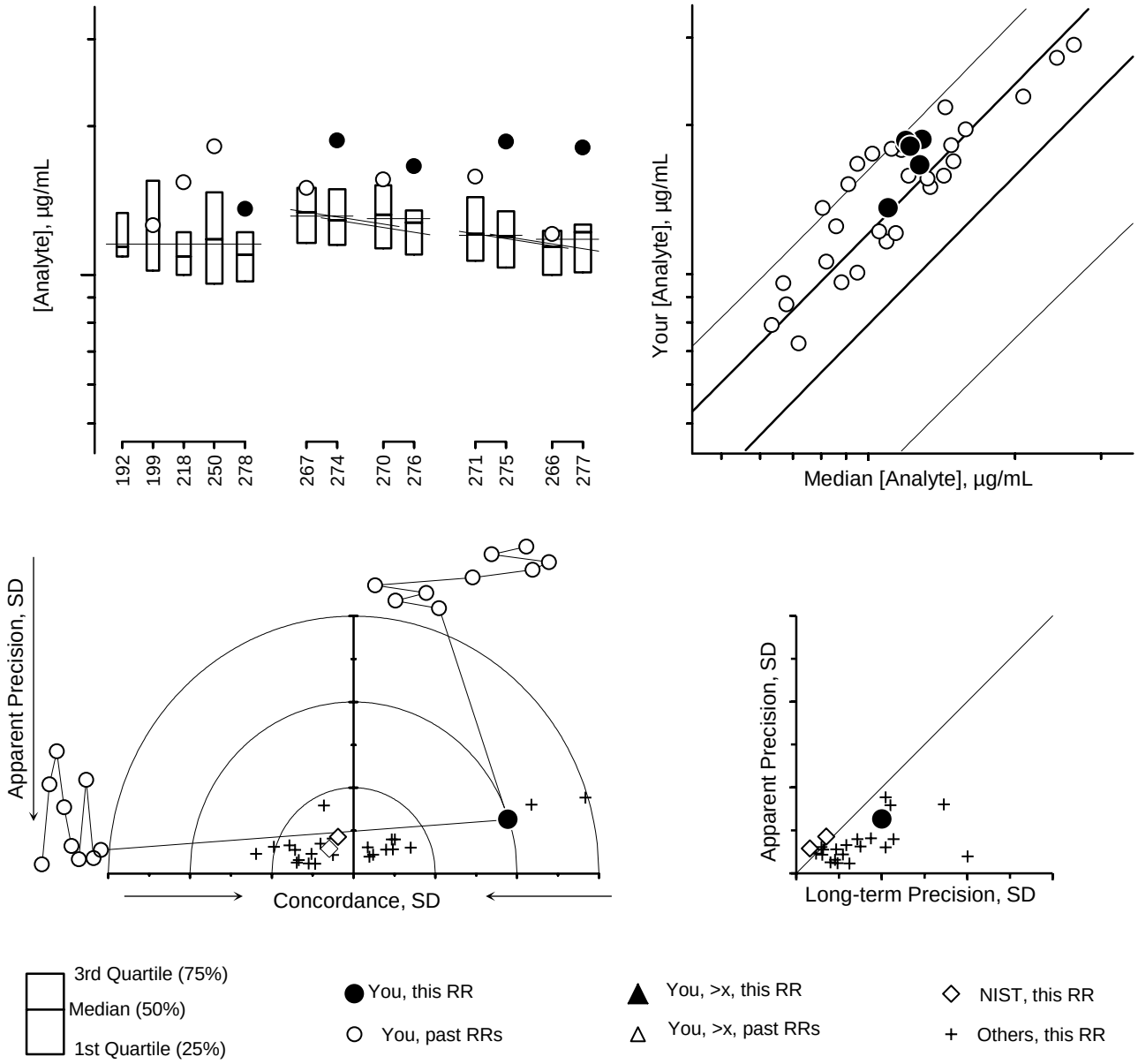


For details of the construction and interpretation of these plots, see:
Duewer, Kline, Sharpless, Brown Thomas, Gary, Sowell. Anal Chem 1999;71(9):1870-8.

Serum	History	Comments
#274	#267 RR48, same pool as #276	Fresh frozen
#275	#271 RR49, same pool as #277	Fresh frozen
#276	#270 RR49, same pool as #274	Lyophilized
#277	#266 RR48, same pool as #275	Lyophilized
#278	#192 RR30, #199 RR32, #218 RR36, #250 RR44	Lyophilized

Individualized Round Robin L Report: FSV-BA

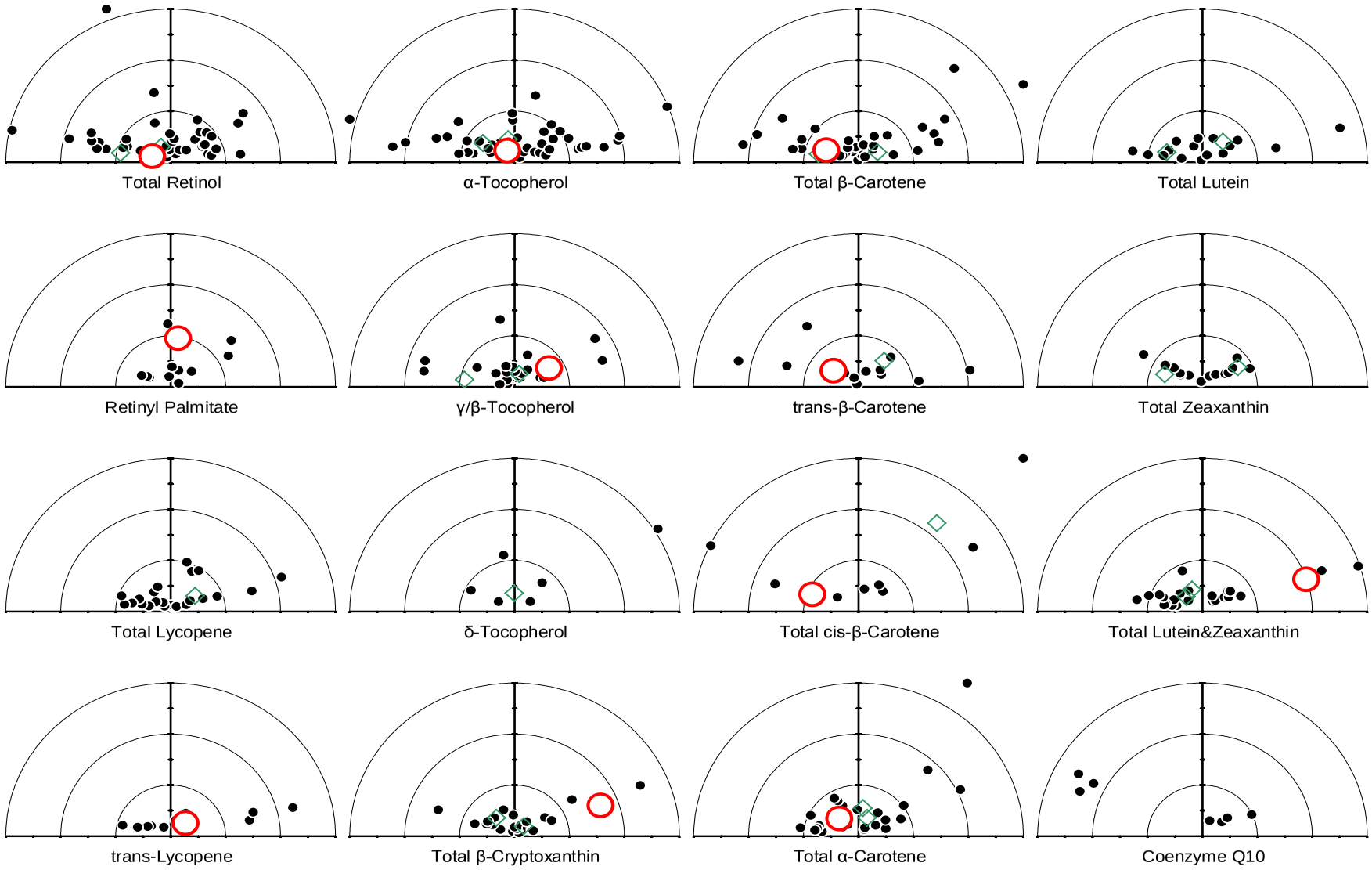
Total Lutein&Zeaxanthin



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.

Serum	History	Comments
#274	#267 RR48, same pool as #276	Fresh frozen
#275	#271 RR49, same pool as #277	Fresh frozen
#276	#270 RR49, same pool as #274	Lyophilized
#277	#266 RR48, same pool as #275	Lyophilized
#278	#192 RR30, #199 RR32, #218 RR36, #250 RR44	Lyophilized

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



D13

Appendix E. Shipping Package Inserts for RR15

The following five items were included in each package shipped to an RR15 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-

June 25, 2001

Dear Colleague:

The enclosed group of samples constitutes Vitamin C Round Robin XV of the 2001 Micronutrients Measurement Quality Assurance Program. Three vials of frozen serum (*test samples)* and a vial of solid ascorbic acid (*control sample)* are enclosed. Please follow the attached protocol when you analyze these samples.

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: srmminfo@nist.gov.

Return your results using the attached form by **September 17, 2001**. We also request that you send us a representative chromatogram from the analysis of each sample and indicate whether peak height or peak area was used in the calculation of the ascorbic acid concentration. Your results will be kept confidential.

Please send your results to:

Micronutrients Measurement Quality Assurance Program
NIST
100 Bureau Drive, Stop 8392
Gaithersburg, MD 20899-8392

If you have any questions or concerns please call me at (301) 975-3137, or contact me by Fax (301) 977-0685 or E-mail sam.margolis@nist.gov

Thank you for your participation. We look forward to receiving your results.

Sincerely,

Sam Margolis

Sam A. Margolis, Ph.D.
Research Chemist
Analytical Chemistry Division
Chemical Science and Technology Laboratory

Enclosures

NIST

Protocol for analyzing samples

The *control sample* consists of a sample of solid ascorbic acid in an amber vial and should be used in the following manner (please record your mass on the attached report form):

1. Prepare 250 mL of 5% metaphosphoric acid (MPA) in distilled water.

2. Weigh **180-220 mg** of the solid ascorbic acid sample to 0.1 mg (if possible), dissolve it in 5% MPA in a 100 mL volumetric flask, and dilute to the 100 mL mark. **Weigh the amount of MPA solution that was added.** This will be referred to as the Stock Solution.

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

   Dilute Solution 1: **Weigh** 0.500 mL of the stock solution into a 100 mL volumetric flask. Then dilute with 5% MPA solution to 100 mL mark and **weigh the amount of MPA solution that was added**.

   Dilute Solution 2: **Weigh** 0.250 mL of the stock solution into a 100 mL volumetric flask. Then dilute with 5% MPA solution to 100 mL mark and **weigh the amount of MPA solution that was added**.

   Dilute Solution 3: **Weigh** 0.125 mL of the stock solution into a 100 mL volumetric flask. Then dilute with 5% MPA solution to 100 mL mark and **weigh the amount ofMPA solution that was added**.

4. Record the ultraviolet absorbance spectrum of *Dilute Solution 1* against 5% MPA solution as the blank using paired cuvettes. Record the wavelength in the region of 240-245 nm at which you observe the maximum absorbance and record the absorbance at that wavelength.

5. Record the absorbance of the sample at 242, 243 and 244 nm.

6. Measure the concentration of the ascorbic acid in all three **dilute solutions** and the **5% MPA diluent** in duplicate along with the ampouled *test samples* using your usual methods.

The purpose of measuring the absorbance at the wavelength maximum is to check the concentration of your sample. If your spectrophotometer is properly calibrated, the maximum absorbance should be between 243 and 244 nm. If the concentration is correct, the molar extinction coefficient ($E_1^{\%}$) of ascorbic acid at this wavelength (using a cell with a 1 cm path length) should be close to 550 ± 30 nm. The extinction coefficient of your solution can be calculated using the following equation:

$$E1\%\ dl/g\cdot cm = \frac{\text{Observed Absorbance}_{\lambda max}}{\dfrac{(g\ AA/100\ mL\ stock)(g\ stock\ in\ 100\ mL\ dilute\ solution)}{(g\ AA\ stock\ solution) + (g\ MPA\ solution\ in\ 100\ mL\ dilute\ solution\ 1)}}$$

The *test samples* are in sealed ampoules and were prepared by adding equal volumes of 10% metaphosphoric acid 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 AA should be reported. The *test samples* sho_uld be defrosted by warming at 20 °C for not more than 10 min otherwise some irreversible degradation may occur.

Each *test sample* should contain between **0** and **100** µmol of ascorbic acid/L of solution. The total ascorbic acid in each ampoule should be measured in duplicate by the method(s) used in your laboratory. Please report your results in µg/L of sample.

REPORT OF ANALYSIS

NAME:
ADDRESS:

Method of Analysis:

Please note the type of method that you use. _____
Please attach representative chromatograms.
Method used for calculating ascorbic acid concentration.

Was SRM 970 used to validate your method or value-assign your in-house controls? ____
Peak height _____ Peak area _____
Manufacturer of ascorbic acid used to make in-house standards _____
Were samples frozen upon receipt? _____ Yes _____ No
Date of Analysis:_____

PREPARATION OF STOCK SOLUTION AND DILUTED SOLUTION

STOCK SOLUTION

Mass of ascorbic acid in the Stock Solution	_____	mg
Mass of 5% MPA added to the 100 mL volumetric flask	_____	g

DILUTE SOLUTION 1

Mass of added stock solution (0.5 mL)	_____	mg
Mass of 5% MPA 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
Wavelength of maximum absorbance	_____	nm
Calculated molar absorptivity	_____	dL/g·cm

DILUTE SOLUTION 2

Mass of added stock solution (0.250 mL)	_____	mg
Mass of 5% MPA added to the 100 mL volumetric flask	_____	g

DILUTE SOLUTION 3

Mass of added stock solution (0.125 mL)	_____	mg
Mass of 5% MPA added to the 100 mL volumetric flask	_____	g

COMMENTS: (use other side if necessary)

REPORT OF ANALYSIS

RESULTS (µmol/L of Sample)

DILUTE SOLUTION 1

REPLICATE 1 _____µmol/L of dilute solution 1
REPLICATE 2 _____ µmol/L of dilute solution 1

DILUTE SOLUTION 2

REPLICATE 1 _____µmol/L of dilute solution 2
REPLICATE 2 _____ µmol/L of dilute solution 2

DILUTE SOLUTION 3

REPLICATE 1 _____µmol/L of dilute solution 3
REPLICATE 2 _____ µmol/L of dilute solution 3

5% MPA SOLUTION (DILUENT)

REPLICATE 1 _____µmol/L of diluent
REPLICATE 2 _____ µmol/L of diluent

TEST SAMPLE #1

REPLICATE 1 _____µmol/L of Sample 1
REPLICATE 2 _____ µmol/L of Sample 1

TEST SAMPLE #2

REPLICATE 1 _____µmol/L of Sample 2
REPLICATE 2 _____ µmol/L of Sample 2

TEST SAMPLE #3

REPLICATE 1 _____µmol/L of Sample 3
REPLICATE 2 _____ µmol/L of Sample 3

Return by **September 17, 2001** to:
Micronutrients Measurement Quality Assurance Program
NIST, 100 Bureau Drive, Stop 8392
Gaithersburg, MD 20899-8392

Fax: 301-977-0685 Micronutrients
E-mail: sam.margolis@nist.gov

Participant #: _____ Date: _____

Vitamin C Round Robin 15

NIST Micronutrients Measurement Quality Assurance Program

Packing List and Shipment Receipt Confirmation Form

This box contains (we hope) one vial each of the following **four** VitC M^2QAP samples:

Sample	Form
#1	Liquid frozen (1:1 serum:10% MPA)
#2	Liquid frozen (1:1 serum:10% MPA)
#3	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 samples #1, #2, and #3 arrived frozen
4) Store the samples upright 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 four vials intact? Yes | No
If "No", which one(s) were damaged?

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

4) Did samples #1, #2, and #3 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 will help control M^2QAP expenses.

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 RR15

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-

January 10, 2002

Dear Colleague:

Enclosed is the summary report of the results for Round Robin 15 (RR15) for the measurement of total ascorbic acid (TAA, ascorbic acid plus dehydroascorbic acid) in human serum. Included in this report are: a summary of data for all laboratories and a summary of individual laboratory performance and interlaboratory accuracy and repeatability. As in previous reports, the estimated standard deviations (eSD) for the measurements are defined as 0.74 x interquartile range and the estimate coefficients of variation (eCV) are defined as 100 x eSD/median.

RR15 consists of three unknowns and one solid reference ascorbic acid for preparation of 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.

Also enclosed is a reprint of the newly released technical brief on the "Stability of Ascorbic Acid in Solutions Stored in Autosampler Vials" by S. A. Margolis and E. Park. If you have any questions regarding this report, please contact David Duewer at david.duewer@nist.gov, phone: 301-975-3935 or Sam Margolis at sam.margolis@nist.gov, phone: 301-975-3137, fax: 301-977-0685.

Sincerely,

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

Sam A. Margolis, Ph.D.
Research Chemist
Analytical Chemistry Division
Chemical Science and Technology Laboratory

Enclosures

Cc: S.A. Wise
L.C. Sander

The NIST M^2QAP Vitamin C Round Robin 15 (RR15) report consists of

Page	"Individualized" Report
1	Summarizes your reported values for the nominal 55 mmol/L control solution and the SRM 970 Level 1 and 2 samples distributed in RR11 through RR15.
2	Graphical summary of your RR15 sample measurements.
3	Graphical summary of your RR15 control solution measurements.

Page	"All Lab" Report
1	A listing of the • Total Ascorbic Acid concentration [TAA] for the four control/calibration solutions, calculated from your reported gravimetric measurements • Measured [TAA] values and summary statistics for the control/calibration solutions • Calibration parameters - intercept, slope, r2, and mean square error (MSE) of calibration, calculated by regressing the gravimetric [TAA] vs the measured [TAA] • Density of the 5% (nominal) metaphosphoric acid (MPA) used to prepare the control/calibration solutions, calculated from your reported gravimetric measurements, and summary statistics • Wavelength of maximum absorption, maximum absorbance, and molar extinction coefficient (El%), calculated from your gravimetric and UV/vis spectrophotometric measurements, and summary statistics • Measured [TAA] values and summary statistics for the three 1:1 serum:MPA samples • [TAA] values and summary statistics for the three samples after correction to the calibration curve defined by the control/calibration solutions

Samples. Three unknowns, one solid reference AA for control solutions. Sera samples 1 and 2 were prepared (05/98) as SRM 970, Level 1 and Level 2, respectively. Serum sample 3 was prepared (03/95) as sample 188b. Each serum sample was prepared by adding ascorbic acid to a serum pool that was depleted of ascorbic acid.

Observations.

1) Most of the molar extinction coefficients are within 2% of the literature value of 550 dL/gcm. (Most were also correctly reported, despite our factor-of-I0 error in the equation--our apologies.) However, there remain a few values outside this roughly limiting volumetric accuracy. Two of the relatively high values are attributable to known weighing difficulties. Some of the remaining errors may be attributable to the spectrophotometric measurements; given that there was some variability in the location of the absorbance maximum, instrument calibration (both wavelength and absorbance scales) and/or measurement practice (matching of sample and blank cuvettes?) could be an issue.

2) Almost everyone prepared their MPA within about 10% of the nominal 5% weight/weight target value. (One participant evidently prepared their control/calibration solutions in DI water.) We concur with the participant who suggested that our instructions should specify preparation of at least 500 mL of the 5% MPA.

3) We have finally demonstrated that some of the reported sample differences can be attributed to calibration inaccuracies. Regression of the calculated control/calibration solution [TAA] against the measured values enables "recalibration" of the reported sample [TAA] values against a common standard. Nearly all of these corrected values are "better" (closer to the consensus median) than the

original measurements. The improvement for the 27 µmol/L sample S15:2 is fairly dramatic, from a CV of 15% down to 5%.

4) There is no sign of degradation (change in median [TAA] or increase in estimated standard deviation) in either SRM 970 Level 1 or Level 2 since their certification in 1998. We will continue to periodically monitor these materials.

Appendix G. "All-Lab Report" for RR15

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.

NIST Micronutrients Measurement Quality Assurance Program for Total Ascorbic Acid
"Round Robin" 15 - Sep 2001

		Control / Calibration Samples												MPA	Dilute Solution 1			Samples					
		Gravimetric, μmol/L				Measured, μmol/L				Calibration Parameters				Density	Spectrophotometry			Measured, μmol/L			Corrected, μmol/L		
Lab	Date	[Dil1]	[Dil2]	[Dil3]	MPA	Ctrl:1	Ctrl:2	Ctrl:3	Ctrl:4	Inter	Slope	R^2	SEE	g/mL	λ_{max}	A_{max}	$E^{1\%}$	S15:1	S15:2	S15:3	S15:1	S15:2	S15:3
VC-MA	18/09/01	55.8	27.8	13.6	0	57.9	28.9	14.2	0.0	0.04	1.04	1.000	0.0	1.033	243.	0.5465	556.5	8.8	25.5	0.0	8.4	24.5	0.0
VC-MB	05/09/01	59.4	29.7	14.8	0	65.6	33.8	18.1	5.2	4.01	1.03	0.998	1.3	1.033	245.	0.5662	541.3	12.4	31.8	7.3	8.2	27.0	3.2
VC-MC	12/07/01	56.3	27.1	14.4	0	54.5	26.6	13.7	0.0	-0.04	0.97	1.000	0.3	1.030	243.	0.539	543.4	7.8	26.0	1.5	8.1	26.9	1.6
VC-ME	11/09/01	58.1	29.0	14.1	0	57.8	29.6	14.6	0.0	0.39	0.99	1.000	0.5	1.032	243.	0.5721	558.7	8.0		0.0	7.7		0.0
VC-MG	05/09/01	56.7	28.9	15.0	0	57.5	29.0	13.7	0.0	-0.67	1.02	0.999	0.9	1.033	243.6	0.544	544.4	9.4	28.2	0.0	9.8	28.3	0.7
VC-MH	12/07/01	55.2	27.5	13.5	0	53.3	26.9	13.0	0.0	0.01	0.97	1.000	0.2	1.032	244.	0.539	554.9	7.6	25.6	0.0	7.8	26.4	0.0
VC-MI	03/08/01	61.6	30.7	14.9	0	51.9	23.6	9.5	0.0	-1.66	0.86	0.996	1.9	1.028				5.1	23.1	0.0	7.9	28.9	1.9
VC-ML	06/09/01	55.9	27.9	14.0	0	46.4	23.3	12.6	0.0	0.41	0.82	0.999	0.6	1.034	243.	0.5905	599.6	8.2	22.8	4.0	9.5	27.1	4.3
VC-MO	17/09/01	57.9	28.4	14.2	0	56.6	28.2	14.4	0.0	0.30	0.98	1.000	0.3	1.031	243.	0.563	552.5	9.3	25.3	0.8	9.2	25.6	0.5
VC-MQ	12/07/01				0	55.5	28.3	14.4	0.0						243.6	0.592	572.0	6.4	20.5	0.0			
VC-MR	28/06/01	58.8	29.0	14.5	0	57.5	28.5	14.0	0.0	-0.06	0.98	1.000	0.2	1.031	244.	0.557	538.2	8.5	26.5	0.0	8.7	27.1	0.1
VC-MS	11/07/01	56.9	28.5	14.6	0	58.3	29.5	15.3	0.0	0.17	1.02	1.000	0.2	1.032	243.	0.5389	537.3	10.6	36.6	0.0	10.2	35.6	0.0
VC-NH	05/10/01	52.0	26.2	12.9	0	53.7	27.2	13.4	0.0	0.03	1.03	1.000	0.0	1.032	243.	0.5163	563.2	8.3	25.1	0.4	8.0	24.3	0.3
VC-NI	10/09/01	55.3	27.6	13.8	0	50.9	26.5	12.4	0.0	0.05	0.93	0.999	0.7	1.027	243.	0.559	574.0	6.3	22.5	0.0	6.7	24.2	0.0
VC-NK	14/09/01	57.8	28.7	14.4	0	67.6	41.4	32.4	15.3	16.94	0.88	0.992	2.4	1.000	243.	0.556	545.9	27.3	39.2	17.6	11.8	25.3	0.8
NIST	14/09/01	61.9	30.3	15.0	0	59.1	29.9	14.9	0.0	0.41	0.95	1.000	0.6	1.032	243.	0.6011	550.9	10.1	28.4	2.2	10.1	29.3	1.8
					N	15	15	15	15					N	14	14	14	14	15	14	15	14	13	14
					Average	56.3	28.7	15.0	1.4					Average	1.029	243.4	0.5557	555.9	9.6	27.0	2.1	8.7	27.0	1.0
					SD	5.3	4.3	5.1	4.1					SD	0.009	0.6	0.0207	17.2	5.2	5.4	4.7	1.3	3.0	1.3
					Min	46.4	23.28	9.5	0.0					Min	1.000	243.0	0.5163	537.3	5.1	20.5	0.0	6.7	24.2	0.0
					%25	53.5	26.75	13.2	0.0					%25	1.031	243.0	0.5403	543.7	7.7	23.6	0.0	7.9	25.3	0.0
					Median	56.6	28.30	14.0	0.0					Median	1.032	243.0	0.5565	553.7	8.3	25.6	0.0	8.3	26.9	0.4
					%75	57.9	29.24	14.5	0.0					%75	1.033	243.6	0.5654	562.1	9.3	27.8	1.2	9.4	27.1	1.4
					Max	67.6	41.40	32.4	15.3					Max	1.034	245.0	0.5920	599.6	27.3	39.2	17.6	11.8	35.6	4.3
					eSD	3.1	1.98	0.9	0.0					eSD	0.002		0.0208	14.7	1.5	3.8	0.0	0.8	2.0	0.6
					CV	6	7	7						CV	0		4	3	19	15		10	8	

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Appendix H. Representative "Individualized Report" for RR15

Each participant in RR15 received an "Individualized Report" reflecting their reported results. The following three pages are the "Individualized Report" for participant "VC-MA".

Vitamin C 'Round Robin' 15 Report: Participant VC-MA

Date	Method	RR	Control	AA mg	MPA g	Stock mg	MPA g	[AA] mmol/L Calc	[AA] mmol/L Obs	A242 OD	A243 OD	A244 OD	A245 OD	E1%max dL/gcm
09/23/98	HPLC-EC (Height)	11	55 mmol/L	200.0	103.09	526.0	102.45	57.8	6.1		0.0525	0.0527		
04/02/99	HPLC-EC (Height)	12	55 mmol/L	215.0	103.10	517.1	102.52	61.1	53.1		0.0721	0.0721		
09/17/01	HPLC-EC (Height)	13	55 mmol/L	200.8	103.16	508.0	102.58	0.0	55.9		0.5650	0.5232		
09/27/01	HPLC-EC (Height)	14	55 mmol/L, Crtl-1	200.3	103.23	510.0	102.31	56.1	57.1		0.5409	0.5193		548
09/18/01	HPLC-EC (Height)	15	55 mmol/L, Crtl:1	200.5	103.12	506.0	102.19	55.8	57.9	0.5461	0.5465	0.5461		557
														552 ±6

RR	Sample	[TAA] mmol/Lsample Rep1	Rep2	*Factor*	Mean	SDdup	Grand Average Mean	SDrepeat	SDreprod
11	SRM Lv 1, A	15.5	13.9	0.5	7.4	0.6	8.0	3.2	0.6
11	SRM Lv 1, B	14.0	14.5	0.5	7.1	0.2			
12	SRM Lv 1, A	14.5	15.8	0.5	7.6	0.5			
12	SRM Lv 1, B	16.1	15.1	0.5	7.8	0.3			
13	SRM Lv 1, S13:1			0.7	8.4	8.5			
14	SRM Lv 1, S14:3	8.0	7.7	1.0	7.8	0.2			
15	SRM Lv 1, S15:1	8.9	8.7	1.0	8.8	0.1			
11	SRM Lv 2, A	50.7	47.7	0.5	24.6	1.1	25.6	10.5	1.4
11	SRM Lv 2, B	48.8	54.0	0.5	25.7	1.8			
12	SRM Lv 2, A	49.5	45.9	0.5	23.9	1.3			
12	SRM Lv 2, B	46.2	47.9	0.5	23.5	0.6			
13	SRM Lv 2, S13:2	8.5	0.1	0.7	27.6	27.7			
14	SRM Lv 2, S14:4	25.7	26.4	1.0	26.0	0.5			
15	SRM Lv 2, S15:2	25.4	25.6	1.0	25.5	0.2			
14	S14:2 (188B)	0.0	0.0	1.0	0.0	0.0	0.0	0.0	0.0
15	S15:3 (188B)	0.0	0.0	1.0	0.0	0.0			

H2

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

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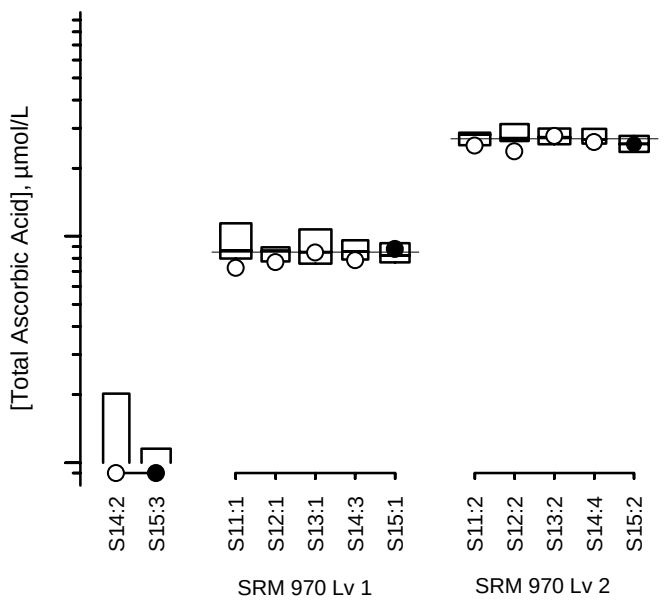
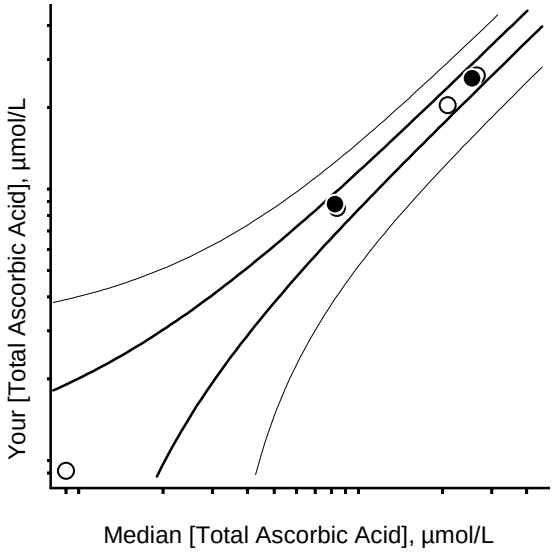
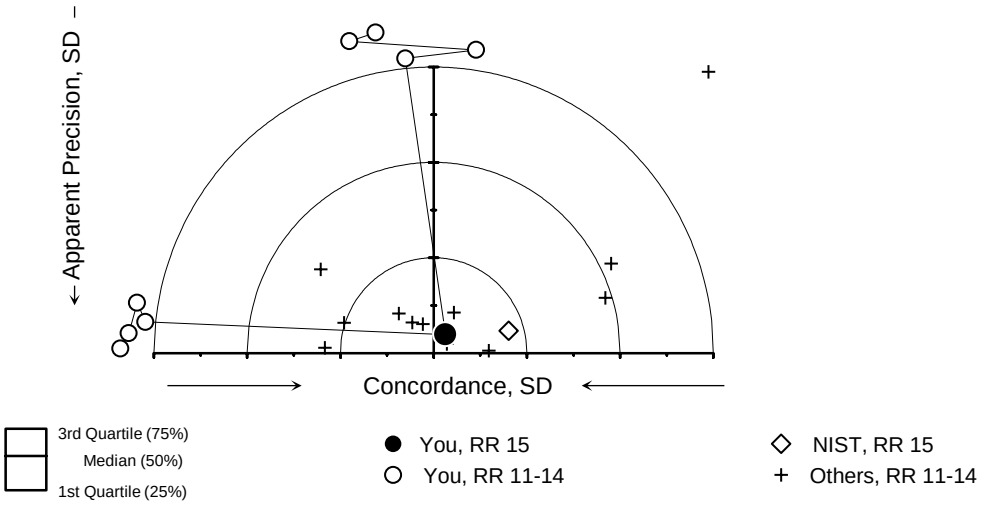
Vitamin C 'Round Robin' 15 Report: Participant VC-MA

Total Ascorbic Acid





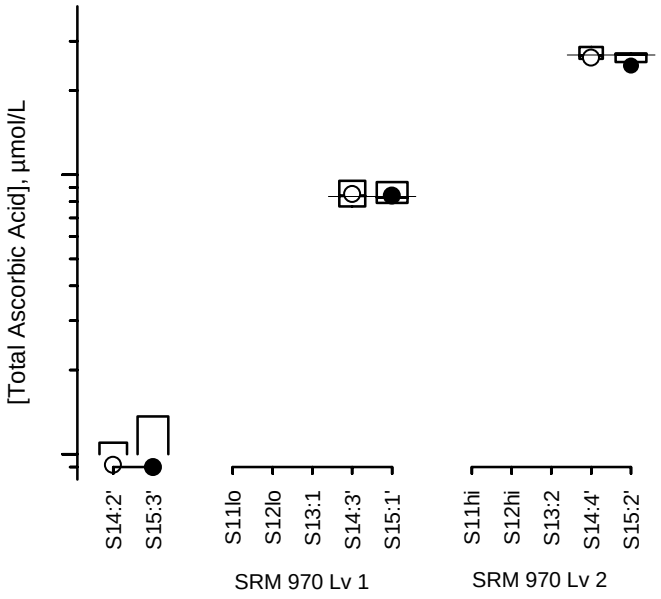


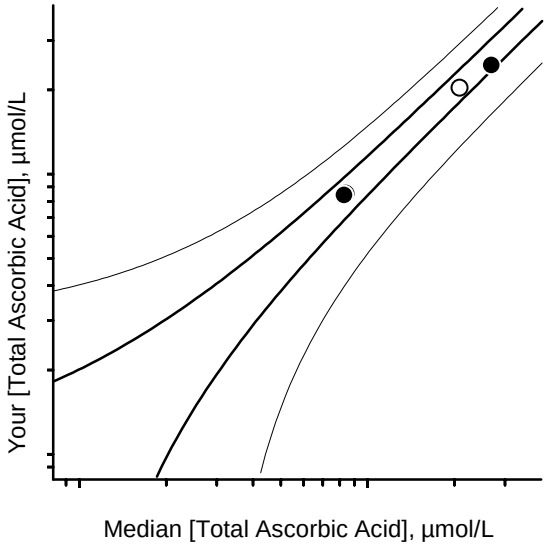
For details of the construction and interpretation of these plots, see:
Duewer, Kline, Sharpless, Brown Thomas, Gary, Sowell. Anal Chem 1999;71(9):1870-8.

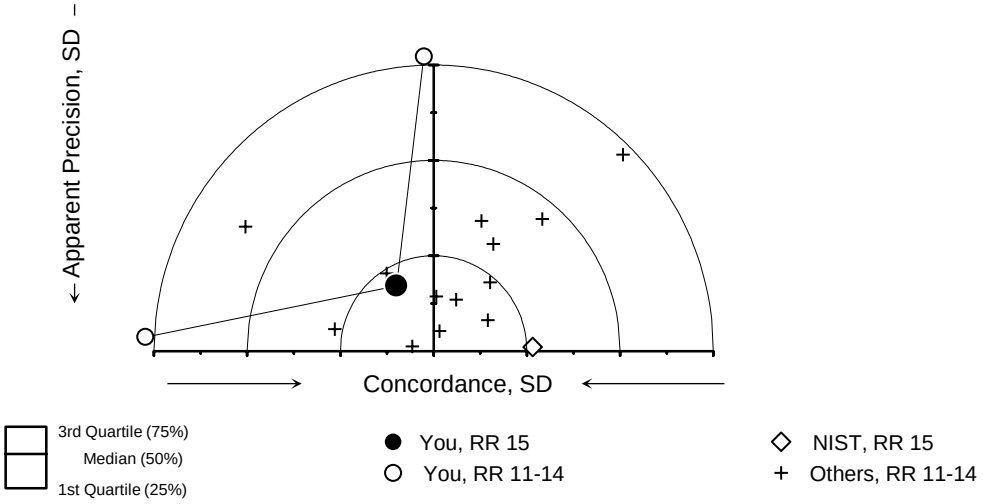
Sample		Comments
S15:1	SRM 970 Level 1	
S15:2	SRM 970 Level 2	
S15:3	Serum 188B, no augmentation	

Vitamin C 'Round Robin' 15 Report: Participant VC-MA

Total Ascorbic Acid Adjusted to Gravimetrically Prepared Calibrants







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
S15:1	SRM 970 Level 1	
S15:2	SRM 970 Level 2	
S15:3	Serum 188B, no augmentation	