

NISTIR 7880-22

**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
Sam A. Margolis (Retired)
Katherine E. Sharpless
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<http://dx.doi.org/10.6028/NIST.IR.7880-22>

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U.S. Department of Commerce
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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
Research Chemist
Analytical Chemistry Division
Chemical Science and Technology Laboratory

Enclosures

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: M²QAP
 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:

<u>Serum</u>	<u>Form</u>	<u>Reconstitute?</u>
#274	Liquid frozen	No
#275	Liquid frozen	No
#276	Lyophilized	Yes (1 ml H ₂ O)
#277	Lyophilized	Yes (1 ml H ₂ O)
#278	Lyophilized	Yes (1 ml H ₂ O)

- 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? _____

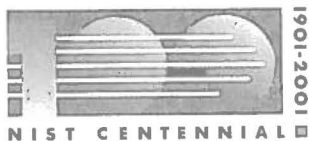
Your prompt return of this information will help control M²QAP expenses.

The M²QAP Gang

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.



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.**

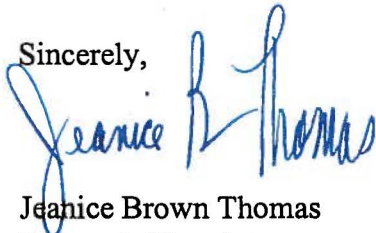
NIST

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

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

Enclosures

The NIST M²QAP 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 M²QAP 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/E-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	0.063		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								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								0.017	0.037	0.017	0.045	0.023		6.96	7.70	6.22	6.93	7.50		2.00	1.55	1.65	1.49	1.99		0.140	0.153	0.105	0.124	0.137	
FSV-BN	0.743	0.510	0.617	0.450	1.159														5.70	6.34	4.79	5.73	6.39													
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		0.62	0.45	0.61	0.44	1.06								6.00	6.70	5.80	6.30	6.70													
FSV-BR	>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								0.023	0.066	0.021	0.055	0.030		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		0.647	0.463	0.659	0.436	1.083								6.30	7.22	6.16	6.75	7.14		2.26	1.90	2.12	1.79	2.29							
FSV-BX	>0.647	>0.463	>0.659	>0.436	>1.083														6.84	7.54	6.10	7.07	7.50		2.20	1.83	2.21	1.72	2.32							
FSV-CB	0.614	0.419	0.593	0.412	0.987		0.710	0.500	0.700	0.470	1.140								6.30	7.22	6.16	6.75	7.14													
FSV-CC	0.720	0.510	0.710	0.480	1.150		0.661	0.464	0.633	0.434	1.116								5.84	6.42	5.51	6.20	6.46													
FSV-CD	>0.661	>0.464	>0.633	>0.434	>1.116														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								0.035	0.098	0.028	0.091	0.037		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	0.388	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	>0.597	>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	1.98	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		2.40	2.70	2.20	2.60	2.80							
FSV-CZ	0.700	0.470	0.620	0.460	1.110		0.715	0.486	0.639	0.322	1.072								6.74	7.98	6.25	7.71	7.15													
FSV-DD	>0.715	>0.486	>0.639	>0.322	>1.072																															
FSV-DE	0.660	0.470	0.620	0.440	1.096																															
FSV-DI	0.688	0.469	0.660	0.452	1.091																															
FSV-DQ	0.610	0.420	0.560	0.400	1.090																															
FSV-DR	0.93	0.510	0.690	0.470	1.120																															
FSV-DU	0.565	0.415	0.568	0.400	0.944																															
FSV-EH	0.619	0.442	0.578	0.386	1.036																															
FSV-EQ	0.680	0.490	0.690	0.480	1.250																															
FSV-FB	0.700	0.500	0.670	0.480	1.190																															
FSV-FV	0.38	38	39	39	39																															
N	38	38	39	39	39																															
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							

Round Robin L Laboratory Results
All Results in µg/mL

Lab	Total β-Carotene					trans-β-Carotene					Total cis-β-Carotene					Total α-Carotene					trans-α-Carotene				
	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	0.024	0.030	0.017	0.030	0.009
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										
FSV-BD																									
FSV-BE	0.478	0.349	0.471	0.327	0.530						0.038	0.040	0.042	0.037	0.059	0.011	0.024	0.013	0.027	0.009	0.011	0.024	0.013	0.027	0.009
FSV-BF	0.583	0.405	0.632	0.407	0.790											0.022	0.038	0.022	0.029	0.024	0.022	0.038	0.022	0.029	0.024
FSV-BG	0.513	0.362	0.474	0.313	0.518											0.019	0.040	0.016	0.025	0.012	0.019	0.040	0.016	0.025	0.012
FSV-BH	0.619	0.450	0.589	0.411	0.683											0.022	0.035	0.020	0.033	0.016	0.022	0.035	0.020	0.033	0.016
FSV-BI	0.525	0.348	0.493	0.331	0.557											0.025	0.038	0.025	0.038	0.016	0.025	0.038	0.025	0.038	0.016
FSV-BJ	0.573	0.357	0.546	0.323	0.578																				
FSV-BK																									
FSV-BL																									
FSV-BM	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	0.019	0.028	0.014	0.024	0.015
FSV-BN	0.509	0.369	0.472	0.353	0.641											0.032	0.037	0.024	0.039	0.021	0.032	0.037	0.024	0.039	0.021
FSV-BO	0.549	0.383	0.495	0.346	0.565											0.017	0.025	0.015	0.020	0.023	0.017	0.025	0.015	0.020	0.023
FSV-BP																									
FSV-BR																									
FSV-BR	0.576	0.359	0.502	0.400	0.596											0.026	0.032	0.025	0.033	0.022	0.026	0.032	0.025	0.033	0.022
FSV-BU	0.542	0.349	0.498	0.340	0.552											0.017	0.024	0.015	0.023	0.012	0.017	0.024	0.015	0.023	0.012
FSV-BV	0.540	0.360	0.510	0.330	0.560											0.024	0.041	0.023	0.038	0.030	0.024	0.041	0.023	0.038	0.030
FSV-BW	>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	0.023	0.033	0.023	0.033	0.021
FSV-BX	0.508	0.351	0.473	0.340	0.535											0.015	0.024	0.014	0.023	0.011	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	0.018	0.031	0.019	0.027	0.013
FSV-CE	0.487	0.311	0.438	0.308	0.491																				
FSV-CF	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	0.024	0.035	0.021	0.032	0.021
FSV-CG	0.466	0.283	0.425	0.292	0.427											0.014	0.024	0.016	0.023	0.012	0.014	0.024	0.016	0.023	0.012
FSV-CH																									
FSV-CI	0.447	0.434	0.631	0.516	0.859											0.027	0.052	0.034	0.062	0.030	0.027	0.052	0.034	0.062	0.030
FSV-CK	0.430	0.286	0.399	0.279	0.434											0.015	0.023	0.016	0.022	0.011	0.015	0.023	0.016	0.022	0.011
FSV-CL																									
FSV-CR	0.568	0.386	0.593	0.388	0.741											0.032	0.063	0.024	0.051	0.169	0.032	0.063	0.024	0.051	0.169
FSV-CV	0.512	0.346	0.485	0.338	0.526											0.023	0.031	0.022	0.031	0.017	0.023	0.031	0.022	0.031	0.017
FSV-CW	>0.36	>0.24	>0.34	>0.21	>0.37	0.468	0.324	0.460	0.313	0.492	0.024	0.022	0.025	0.025	0.034	>0.02	>0.02	>0.02	>0.02	>0.02	>0.02	>0.02	>0.02	>0.02	>0.02
FSV-CX	0.493	0.316	0.453	0.304	0.506	0.360	0.240	0.340	0.210	0.370															
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-DE																									
FSV-DF	>0.508	>0.319	>0.509	>0.342	>0.512	0.508	0.319	0.509	0.342	0.512						0.025	0.036	0.025	0.036	0.019	0.025	0.036	0.025	0.036	0.019
FSV-DI	0.550	0.347	0.508	0.307	0.547																				
FSV-DJ	0.410	0.300	0.400	0.280	0.590																				
FSV-DR	>0.25	>0.37	>0.24	>0.55	10.40	0.25	0.37	0.24	0.55																
FSV-DU	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	0.021	0.036	0.015	0.036	0.014
FSV-EH	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	0.013	0.020	0.012	0.019	0.010
FSV-EQ	0.606	0.414	0.560	0.383	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	0.018	0.030	0.020	0.027	0.016
FSV-FB	1.270	0.910	1.310	0.780	1.380											0.030	0.060	0.030	0.060	0.010	0.030	0.060	0.030	0.060	0.010
FSV-FJ	N	31	31	31	31	12	13	13	13	13	9	9	9	9	9	27	27	27	27	27	1	1	1	1	1
FSV-FK	N	31	31	31	31	12	13	13	13	13	9	9	9	9	9	27	27	27	27	27	1	1	1	1	1
FSV-FL	N	31	31	31	31	12	13	13	13	13	9	9	9	9	9	27	27	27	27	27	1	1	1	1	1
FSV-FM	N	31	31	31	31	12	13	13	13	13	9	9	9	9	9	27	27	27	27	27	1	1	1	1	1
FSV-FN	N	31	31	31	31	12	13	13	13	13	9	9	9	9	9	27	27	27	27	27	1	1	1	1	1
FSV-FO	N	31	31	31	31	12	13	13	13	13	9	9	9	9	9	27	27	27	27	27	1	1	1	1	1
FSV-FP	N	31	31	31	31	12	13	13	13	13	9	9	9	9	9	27	27	27	27	27	1	1	1	1	1
FSV-FQ	N	31	31	31	31	12	13	13	13	13	9	9	9	9	9	27	27	27	27	27	1	1	1	1	1
FSV-FR	N	31	31	31	31	12	13	13	13	13	9	9	9	9	9	27	27	27	27	27	1	1	1	1	1
FSV-FS	N	31	31	31	31	12	13	13	13	13	9	9	9	9	9	27	27	27	27	27	1	1	1	1	1
FSV-FT	N	31	31	31	31	12	13	13	13	13	9	9	9	9	9	27	27	27	27	27	1	1	1	1	1
FSV-FU	N	31	31	31	31	12	13	13	13	13	9	9	9	9	9	27	27	27	27	27	1	1	1	1	1
FSV-FV	N	31	31	31	31	12	13	13	13	13	9	9	9	9	9	27	27	27	27	27	1	1	1	1	1
FSV-FW	N	31	31	31	31	12	13	13	13	13	9	9	9	9	9	27	27	27	27	27	1	1	1	1	1
FSV-FX	N	31	31	31	31	12	13	13	13	13	9	9	9	9	9	27	27	27	27	27	1	1	1	1	1
FSV-FY	N	31	31	31	31	12	13	13	13	13	9	9	9	9	9	27	27	27	27	27	1	1	1	1	1
FSV-FZ	N	31	31	31	31	12																			

Round Robin L Laboratory Results
All Results in µg/mL

Lab	Total Lycopene					trans-Lycopene					Total β-Cryptoxanthin					Total α-Cryptoxanthin					Total Lutein					trans-Lutein				
	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	0.222	0.330	0.207	0.311	0.572	0.144	0.185	0.129	0.181	0.333	0.078	0.076	0.069	0.081	0.062	0.024	0.025	0.023	0.023	0.019	0.102	0.092	0.092	0.088	0.077					
FSV-BB	0.199	0.374	0.208	0.386	0.768	0.130	0.208	0.126	0.143	0.335	0.035	0.042	0.036	0.043	0.040						0.081	0.089	0.083	0.086	0.089					
FSV-BD	0.227	0.362	0.192	0.252	0.549	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-BE	0.223	0.337	0.207	0.307	0.542	0.297	0.407	0.276	0.363	0.632	0.044	0.045	0.042	0.045	0.047	0.057	0.055	0.049	0.050	0.047	0.147	0.144	0.132	0.144	0.139					
FSV-BG	0.183	0.281	0.172	0.256	0.509	0.120	0.155	0.113	0.151	0.290	0.046	0.048	0.044	0.045	0.041	0.045	0.045	0.044	0.042	0.041	0.095	0.084	0.097	0.081	0.069					
FSV-BH	0.212	0.290	0.179	0.263	0.493	0.176	0.287	0.187	0.270	0.491	0.047	0.049	0.044	0.048	0.045	0.026	0.026	0.024	0.026	0.019	0.080	0.074	0.076	0.074	0.065					
FSV-BI	0.201	0.291	0.178	0.281	0.512	0.132	0.177	0.117	0.160	0.306	0.043	0.047	0.043	0.044	0.056	0.024	0.025	0.022	0.024	0.021	0.099	0.099	0.099	0.099	0.083					
FSV-BJ	0.238	0.343	0.217	0.335	0.567	0.172	0.241	0.165	0.247	0.403	0.048	0.081	0.059	0.063	0.064	0.046	0.042	0.038	0.044	0.043	>0.084	>0.08	>0.079	>0.076	>0.061					
FSV-BK	0.220	0.340	0.210	0.330	0.560	0.110	0.140	0.100	0.130	0.250	0.047	0.048	0.045	0.049	0.037	0.026	0.026	0.024	0.026	0.019	0.070	0.070	0.070	0.070	0.060					
FSV-BL	0.230	0.370	0.230	0.340	0.650	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	0.088	0.081	0.084	0.079	0.071					
FSV-BM	0.210	0.340	0.220	0.320	0.580	0.110	0.140	0.100	0.130	0.250	0.050	0.060	0.050	0.050	0.040	0.026	0.026	0.024	0.026	0.019	0.064	0.066	0.060	0.060	0.060					
FSV-BN	0.207	0.337	0.207	0.307	0.542	0.176	0.287	0.187	0.270	0.491	0.056	0.056	0.046	0.052	0.045	0.046	0.042	0.038	0.044	0.043	0.095	0.089	0.092	0.085	0.078					
FSV-BO	0.207	0.337	0.207	0.307	0.542	0.176	0.287	0.187	0.270	0.491	0.065	0.063	0.061	0.059	0.054	0.024	0.025	0.022	0.024	0.021	0.095	0.108	0.102	0.102	0.095					
FSV-BP	0.207	0.337	0.207	0.307	0.542	0.176	0.287	0.187	0.270	0.491	0.048	0.059	0.051	0.054	0.052	0.006	0.007	0.008	0.007	0.009	0.088	0.081	0.084	0.079	0.071					
FSV-BQ	0.207	0.337	0.207	0.307	0.542	0.176	0.287	0.187	0.270	0.491	0.059	0.059	0.053	0.054	0.051	0.006	0.007	0.008	0.007	0.009	0.064	0.066	0.060	0.060	0.060					
FSV-BR	0.207	0.337	0.207	0.307	0.542	0.176	0.287	0.187	0.270	0.491	0.048	0.048	0.048	0.048	0.048	0.006	0.007	0.008	0.007	0.009	0.088	0.081	0.084	0.079	0.071					
FSV-BS	0.207	0.337	0.207	0.307	0.542	0.176	0.287	0.187	0.270	0.491	0.048	0.048	0.048	0.048	0.048	0.006	0.007	0.008	0.007	0.009	0.064	0.066	0.060	0.060	0.060					
FSV-BT	0.207	0.337	0.207	0.307	0.542	0.176	0.287	0.187	0.270	0.491	0.048	0.048	0.048	0.048	0.048	0.006	0.007	0.008	0.007	0.009	0.088	0.081	0.084	0.079	0.071					
FSV-BU	0.207	0.337	0.207	0.307	0.542	0.176	0.287	0.187	0.270	0.491	0.048	0.048	0.048	0.048	0.048	0.006	0.007	0.008	0.007	0.009	0.064	0.066	0.060	0.060	0.060					
FSV-BV	0.207	0.337	0.207	0.307	0.542	0.176	0.287	0.187	0.270	0.491	0.048	0.048	0.048	0.048	0.048	0.006	0.007	0.008	0.007	0.009	0.088	0.081	0.084	0.079	0.071					
FSV-BW	0.207	0.337	0.207	0.307	0.542	0.176	0.287	0.187	0.270	0.491	0.048	0.048	0.048	0.048	0.048	0.006	0.007	0.008	0.007	0.009	0.064	0.066	0.060	0.060	0.060					
FSV-BX	0.207	0.337	0.207	0.307	0.542	0.176	0.287	0.187	0.270	0.491	0.048	0.048	0.048	0.048	0.048	0.006	0.007	0.008	0.007	0.009	0.088	0.081	0.084	0.079	0.071					
FSV-BY	0.207	0.337	0.207	0.307	0.542	0.176	0.287	0.187	0.270	0.491	0.048	0.048	0.048	0.048	0.048	0.006	0.007	0.008	0.007	0.009	0.064	0.066	0.060	0.060	0.060					
FSV-CB	0.207	0.337	0.207	0.307	0.542	0.176	0.287	0.187	0.270	0.491	0.048	0.048	0.048	0.048	0.048	0.006	0.007	0.008	0.007	0.009	0.088	0.081	0.084	0.079	0.071					
FSV-CC	0.207	0.337	0.207	0.307	0.542	0.176	0.287	0.187	0.270	0.491	0.048	0.048	0.048	0.048	0.048	0.006	0.007	0.008	0.007	0.009	0.064	0.066	0.060	0.060	0.060					
FSV-CD	0.207	0.337	0.207	0.307	0.542	0.176	0.287	0.187	0.270	0.491	0.048	0.048	0.048	0.048	0.048	0.006	0.007	0.008	0.007	0.009	0.088	0.081	0.084	0.079	0.071					
FSV-CE	0.207	0.337	0.207	0.307	0.542	0.176	0.287	0.187	0.270	0.491	0.048	0.048	0.048	0.048	0.048	0.006	0.007	0.008	0.007	0.009	0.064	0.066	0.060	0.060	0.060					
FSV-CF	0.207	0.337	0.207	0.307	0.542	0.176	0.287	0.187	0.270	0.491	0.048	0.048	0.048	0.048	0.048	0.006	0.007	0.008	0.007	0.009	0.088	0.081	0.084	0.079	0.071					
FSV-CG	0.207	0.337	0.207	0.307	0.542	0.176	0.287	0.187	0.270	0.491	0.048	0.048	0.048	0.048	0.048	0.006	0.007	0.008	0.007	0.009	0.064	0.066	0.060	0.060	0.060					
FSV-CH	0.207	0.337	0.207	0.307	0.542	0.176	0.287	0.187	0.270	0.491	0.048	0.048	0.048	0.048	0.048	0.006	0.007	0.008	0.007	0.009	0.088	0.081	0.084	0.079	0.071					
FSV-CI	0.207	0.337	0.207	0.307	0.542	0.176	0.287	0.187	0.270	0.491	0.048	0.048	0.048	0.048	0.048	0.006	0.007	0.008	0.007	0.009	0.064	0.066	0.060	0.060	0.060					
FSV-CJ	0.207	0.337	0.207	0.307	0.542	0.176	0.287	0.187	0.270	0.491	0.048	0.048	0.048	0.048	0.048	0.006	0.007	0.008	0.007	0.009	0.088	0.081	0.084	0.079	0.071					
FSV-CK	0.207	0.337	0.207	0.307	0.542	0.176	0.287	0.187	0.270	0.491	0.048	0.048	0.048	0.048	0.048	0.006	0.007	0.008	0.007	0.009	0.064	0.066	0.060	0.060	0.060					
FSV-CL	0.207	0.337	0.207	0.307	0.542	0.176	0.287	0.187	0.270	0.491	0.048	0.048	0.048	0.048	0.048	0.006	0.007	0.008	0.007	0.009	0.088	0.081	0.084	0.079	0.071					
FSV-CM	0.207	0.337	0.207	0.307	0.542	0.176	0.287	0.187	0.270	0.491	0.048	0.048	0.048	0.048	0.048	0.006	0.007	0.008	0.007	0.009	0.064	0.066	0.060	0.060	0.060					
FSV-CN	0.207	0.337	0.207	0.307	0.542	0.176	0.287	0.187	0.270	0.491	0.048	0.048	0.048	0.048	0.048	0.006	0.007	0.008	0.007	0.009	0.088	0.081	0.084	0.079	0.071					
FSV-CO	0.207	0.337	0.207	0.307	0.542	0.176	0.287	0.187	0.270	0.491	0.048	0.048	0.048	0.048	0.048	0.006	0.007	0.008	0.007	0.009	0.064	0.066	0.060	0.060	0.060					
FSV-CP	0.207	0.337	0.207	0.307	0.542	0.176	0.287	0.187	0.270	0.491	0.048	0.048	0.048	0.048	0.048	0.006	0.007	0.008	0.007	0.009	0.088	0.081	0.084	0.079	0.071					
FSV-CQ	0.207	0.337	0.207	0.307	0.542	0.176	0.287	0.187	0.270	0.491	0.048	0.048	0.048	0.048	0.048	0.006	0.007	0.008	0.007	0.009	0.064	0.066	0.060	0.060	0.060					
FSV-CR	0.207	0.337	0.207	0.307	0.542	0.176	0.287	0.187	0.270	0.491	0.048	0.048	0.048	0.048	0.048	0.006	0.007	0.008	0.007	0.009	0.088	0.081	0.084	0.079	0.071					
FSV-CS	0.207	0.337	0.207	0.307	0.542	0.176	0.287	0.187	0.270	0.491	0.048	0.048	0.048	0.048	0.048	0.006	0.007	0.008	0.007	0.009	0.064	0.066								

Round Robin L Laboratory Results
All Results in µg/mL

Lab	Total Zeaxanthin				Total Lutein&Zeaxanthin				Coenzyme Q10				Phylloquinone (K1) x1000							
	274	275	276	277	278	274	275	276	277	278	274	275	276	277	278	274	275	276	277	278
FSV-BA	0.046	0.037	0.040	0.037	0.048	0.187	0.186	0.166	0.181	0.136										
FSV-BB						0.148	0.129	0.132	0.125	0.125										
FSV-BE																				
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						0.131	0.135	0.121	0.126	0.116										
FSV-BI	0.039	0.031	0.036	0.029	0.035															
FSV-BJ																				
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-BR						0.082	0.120	0.132	0.117	0.110										
FSV-BU						0.120	0.100	0.112	0.095	0.089										
FSV-BV																				
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	0.034	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																				
FSV-CI																				
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										
FSV-CW	0.039	0.028	0.037	0.027	0.031	>0.123	>0.108	>0.116	>0.103	>0.092										
FSV-CX	0.040	0.030	0.040	0.030	0.040	0.110	0.100	0.110	0.100	0.100										
FSV-CZ																				
FSV-DB						0.128	0.117	0.123	0.108	0.100										
FSV-DD																				
FSV-DF																				
FSV-DI																				
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										
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										
Min	0.020	0.020	0.018	0.017	0.018	0.082	0.091	0.091	0.082	0.079										
Median	0.039	0.027	0.035	0.022	0.032	0.129	0.120	0.128	0.122	0.110										
Max	0.050	0.037	0.042	0.037	0.053	0.208	0.205	0.197	0.198	0.218										
SD	0.007	0.005	0.008	0.007	0.010	0.025	0.023	0.019	0.019	0.019										
CV	17	20	23	31	30	20	19	15	15	17										
N _{pass}	15	13	13	15	10	29	22	22	29	16										
Median _{pass}	0.035	0.024	0.034	0.027	0.034	0.133	0.121	0.132	0.114	0.117										
SD _{pass}	0.009	0.006	0.007	0.005	0.011	0.025	0.027	0.029	0.017	0.030										
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										
NNIST	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										
Strep	0.003	0.003	0.003	0.007	0.002	0.006	0.003	0.005	0.010	0.009										
Sheet	0.007	0.002	0.002	0.001	0.004	0.015	0.002	0.006	0.003	0.008										
Slant	0.007	0.015	0.008	0.012	0.017	0.017	0.005	0.002	0.001	0.001										
SNIST	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										
NAU	0.011	0.015	0.010	0.011	0.014	0.027	0.025	0.029	0.027	0.023										

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	<i>nq</i>	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: $0.741 \times (\text{3rd Quartile} - \text{1st Quartile})$
CV	Coefficient of Variation for (non-NIST) results: $100 \times \text{SD} / \text{Median}$
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 = $(\text{Median}_{part} + \text{Mean}_{NIST}) / 2$ 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.
<i>nd</i>	Not detected (i.e., no detectable peak for analyte)
<i>nq</i>	Detected but not quantitatively determined
>x	Concentration greater than x
!	Non-quantitative value: heterogeneous serum, damaged sample, malfunction, etc.
<i>italics</i>	Not explicitly reported but calculated by NIST from reported values

Round Robin L Laboratory Results

Comparability Summary

Lab	TR	tR	RP	aT	g/bT	bC	tbC	aC	TLy	tLy	TbX	TLu	TZ	L&Z	Label	Definition
FSV-BA	1		1	1	1	1	1	1		1	2	1	1	1	Lab	Participant code
FSV-BB	1		1	1	1	1	1	1	1	1	1	1	1	1	TR	Total Retinol
FSV-BD	1			2											tR	trans-Retinol
FSV-BE	1			1	1	1								1	RP	Retinyl palmitate
FSV-BF	1			1	1	2		1	1		1				aT	α-Tocopherol
FSV-BG	1		2	1	1	1		1	1	1	1			2	g/bT	γ/β-Tocopherol
FSV-BH	2			1	1	2	3	1	1		3	1	1	1	bC	Total β-Carotene
FSV-BI	1			1	1	1		1	1		1				tbC	trans-β-Carotene
FSV-BJ		1	1	1	1	1		1	1		1	1	1	1	aC	Total α-Carotene
FSV-BK	1			3								1	1	1	TLy	Total Lycopene
FSV-BL	1			2											tLy	trans-Lycopene
FSV-BM	1			1								1			TbX	Total β-Cryptoxanthin
FSV-BN	1		1	2	2	1	1	1	2	2	1				TLu	Total Lutein
FSV-BO	1			1		1		1	2		1			1	TZ	Total Zeaxanthin
FSV-BP	1			1		1		1	1		1				L&Z	Total Lutein&Zeaxanthin
FSV-BQ	1			1										2		
FSV-BR		1		1								1	1	1	n	number of participants providing quantitative data
FSV-BU	2			3	1	1		1	1		1	1	1	1	% 1	Percent of CS = 1 (within 1 SD of medians)
FSV-BV	1			1	1	1		1	1		2			1	% 2	Percent of CS = 2 (within 2 SD of medians)
FSV-BW	1		1	1	1	1		2	1			1	1	1	% 3	Percent of CS = 3 (within 3 SD of medians)
FSV-BX		1		1	1		1	1		1	1	3	1	3	% 4	Percent of CS = 4 (3 or more SD from medians)
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										"Comparability Score"
FSV-CF	1			1												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.
FSV-CG	1			2	1	1	2	1	1	1	1			1		We define CS as follows:
FSV-CH	2			1	2	2		1	1					1		$CS = \text{MINIMUM} \left(4, \text{INTEGER} \left(1 + \sqrt{C^2 + AP^2} \right) \right)$
FSV-CI	2		1	2	1									1		$C = \text{Concordance} = \frac{\sum_{i=1}^{N_{you}} \frac{You_i - \text{Median}_i}{NAU_i}}{N_{you}}$
FSV-CK	1			1	2	3		3	1		2			1		$AP = \text{Apparent Precision} = \sqrt{\frac{\sum_{i=1}^{N_{you}} \left(\frac{You_i - \text{Median}_i}{NAU_i} \right)^2}{N_{you} - 1}}$
FSV-CL	4			4	2	2		1	1		1	1	1	1		NAU = NIST Assigned Uncertainty
FSV-CR	2			1												For further details, please see
FSV-CV	4		2	2	1	2		4	3							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.
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		

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

Analyte	Serum 274			Serum 275			Serum 276			Serum 277			Serum 278		
	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

D2

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

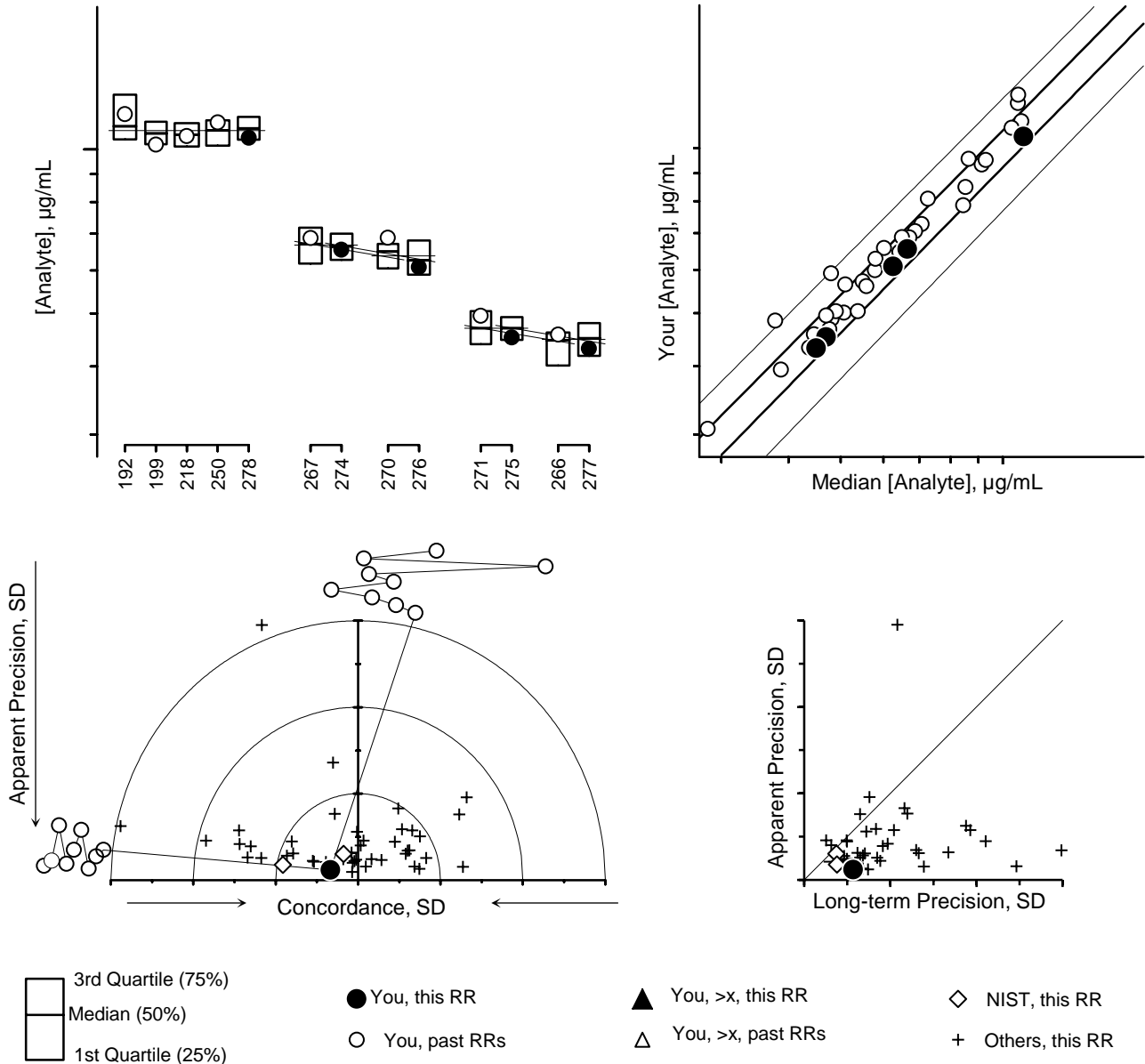
Please check our records against your records. Send corrections and/or updates to...

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

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

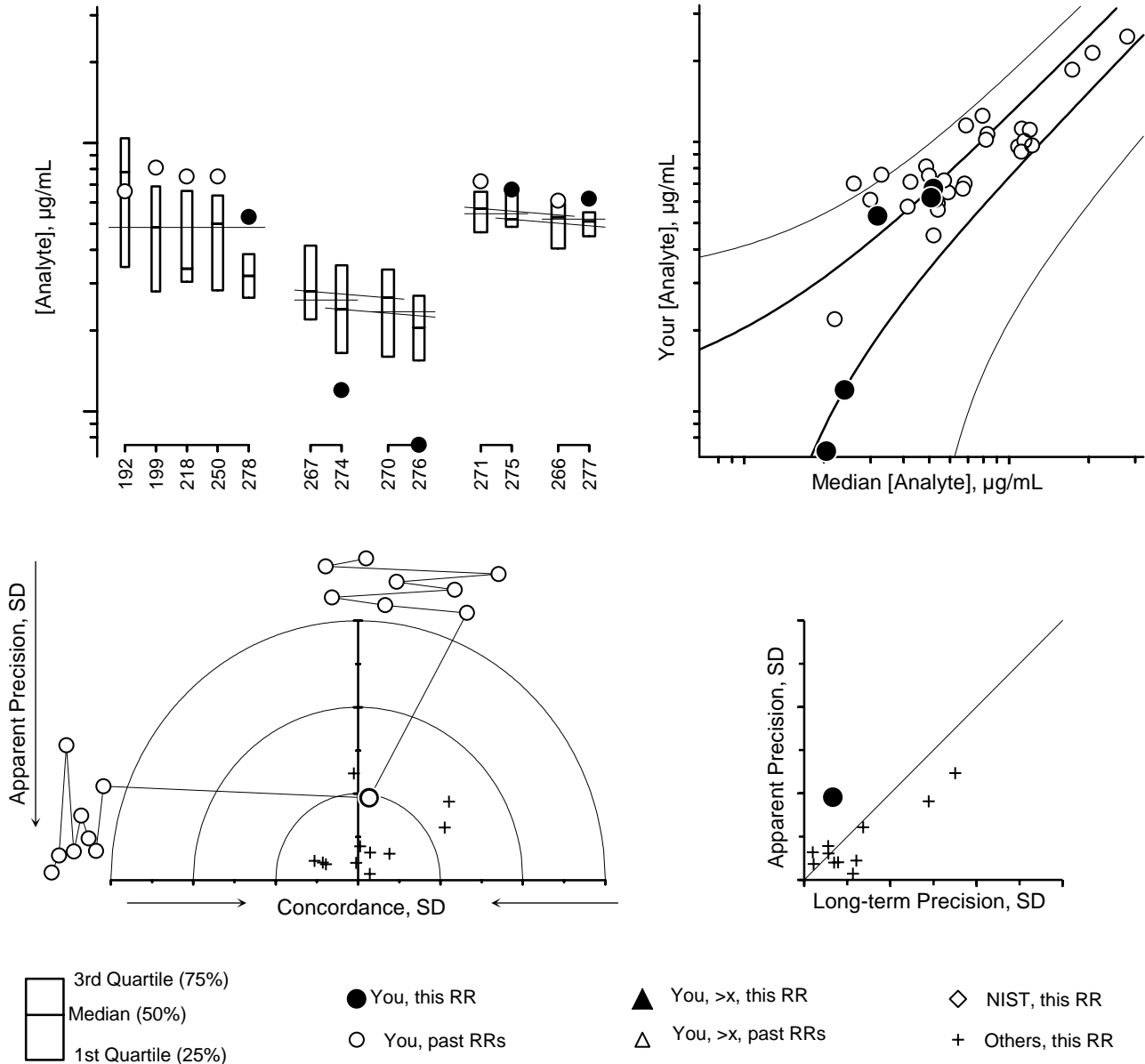
History

Comments

Fresh frozen
Fresh frozen
Lyophilized
Lyophilized
Lyophilized

Individualized Round Robin L Report: FSV-BA

Retinyl Palmitate



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

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

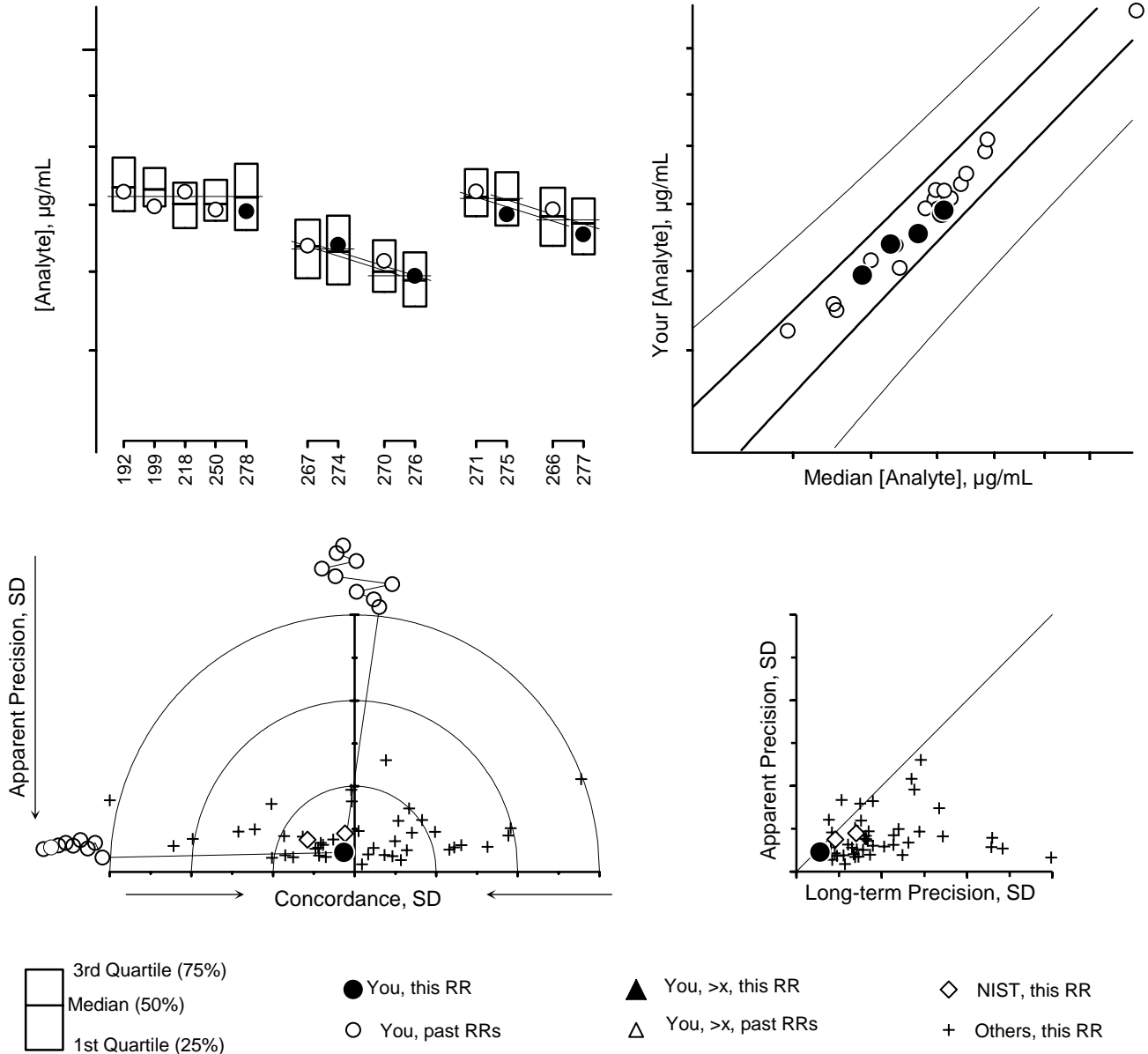
History

Comments

Fresh frozen
Fresh frozen
Lyophilized
Lyophilized
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

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

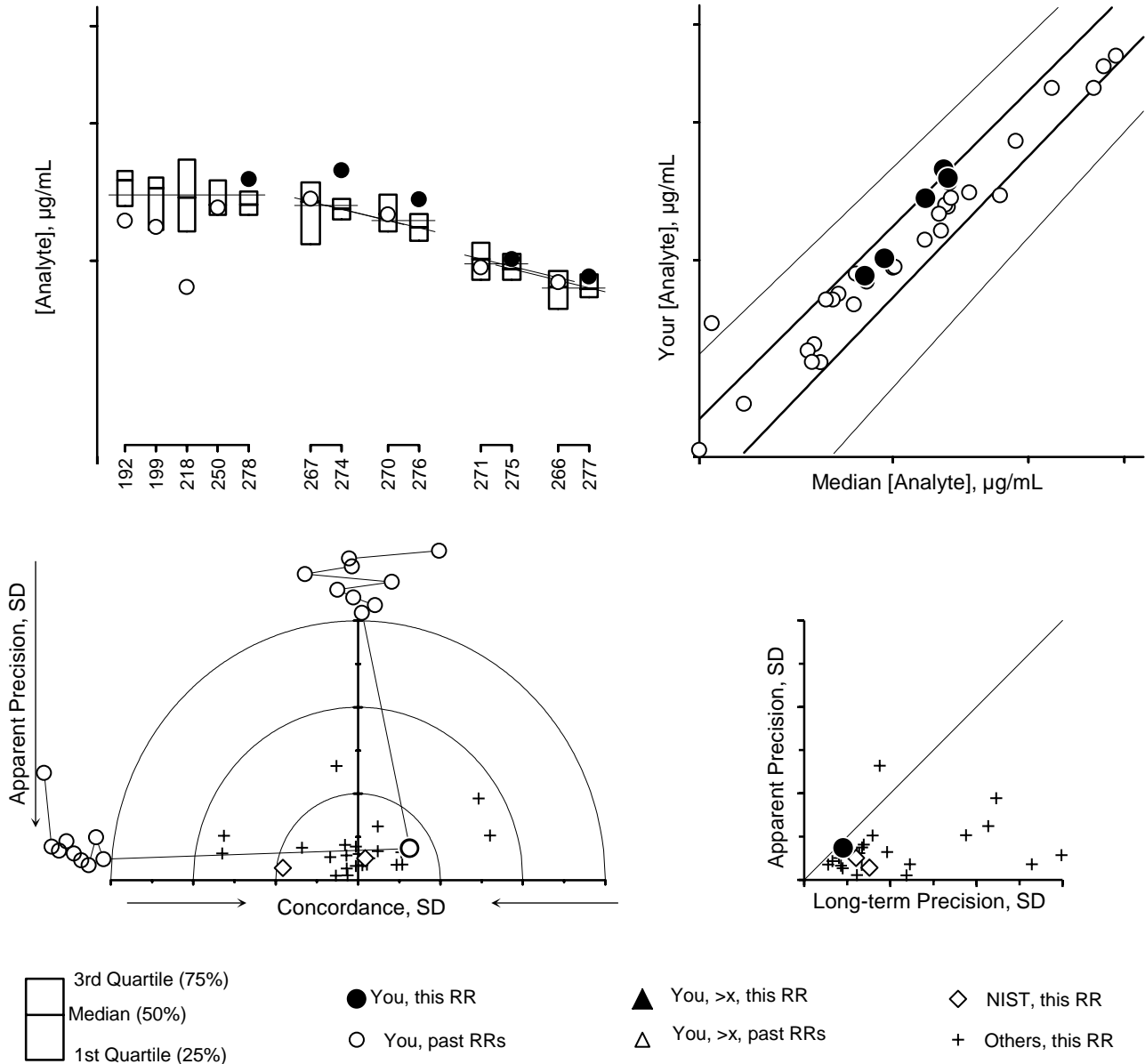
History

Comments

Fresh frozen
Fresh frozen
Lyophilized
Lyophilized
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

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

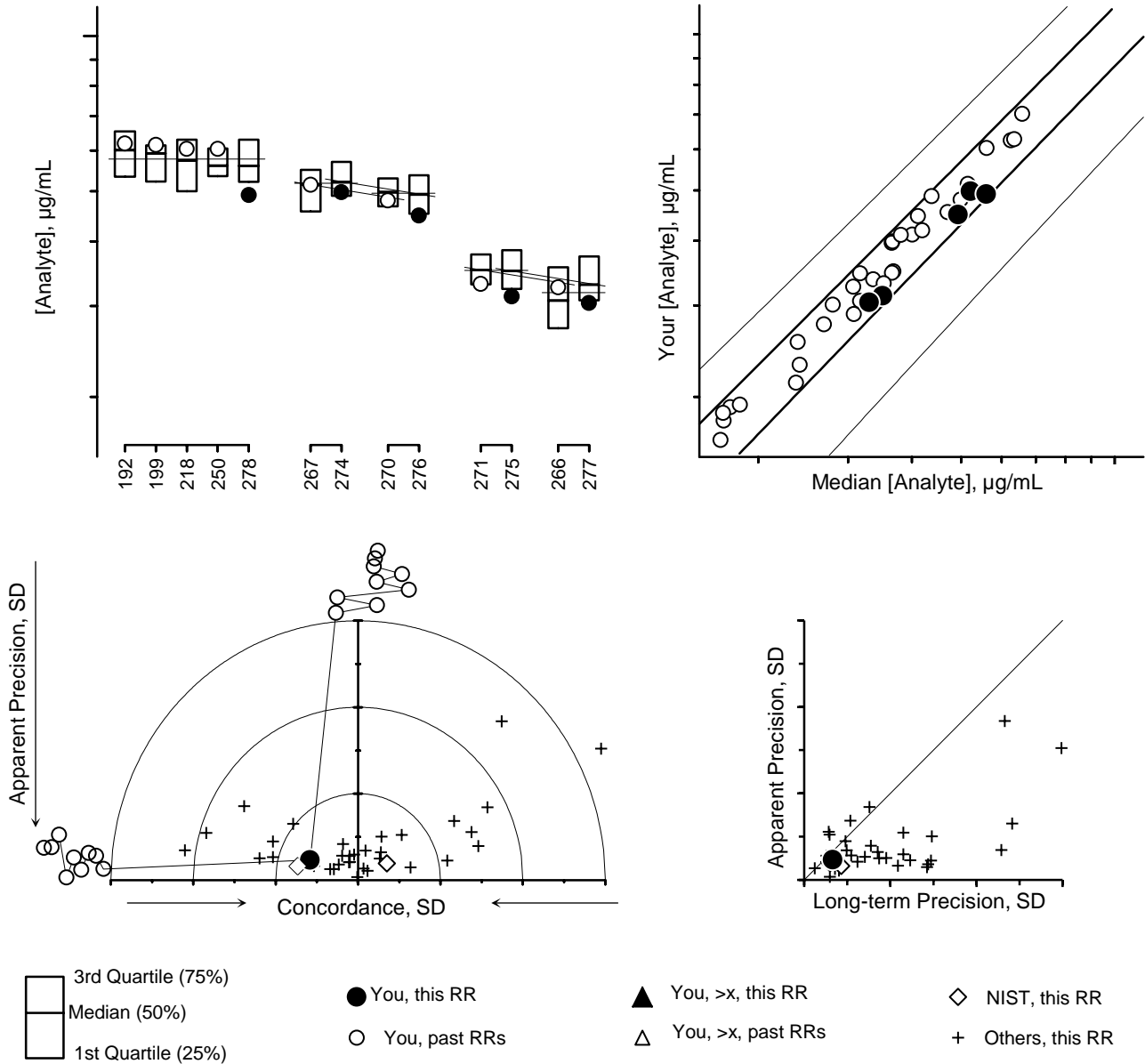
History

Comments

Fresh frozen
Fresh frozen
Lyophilized
Lyophilized
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

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

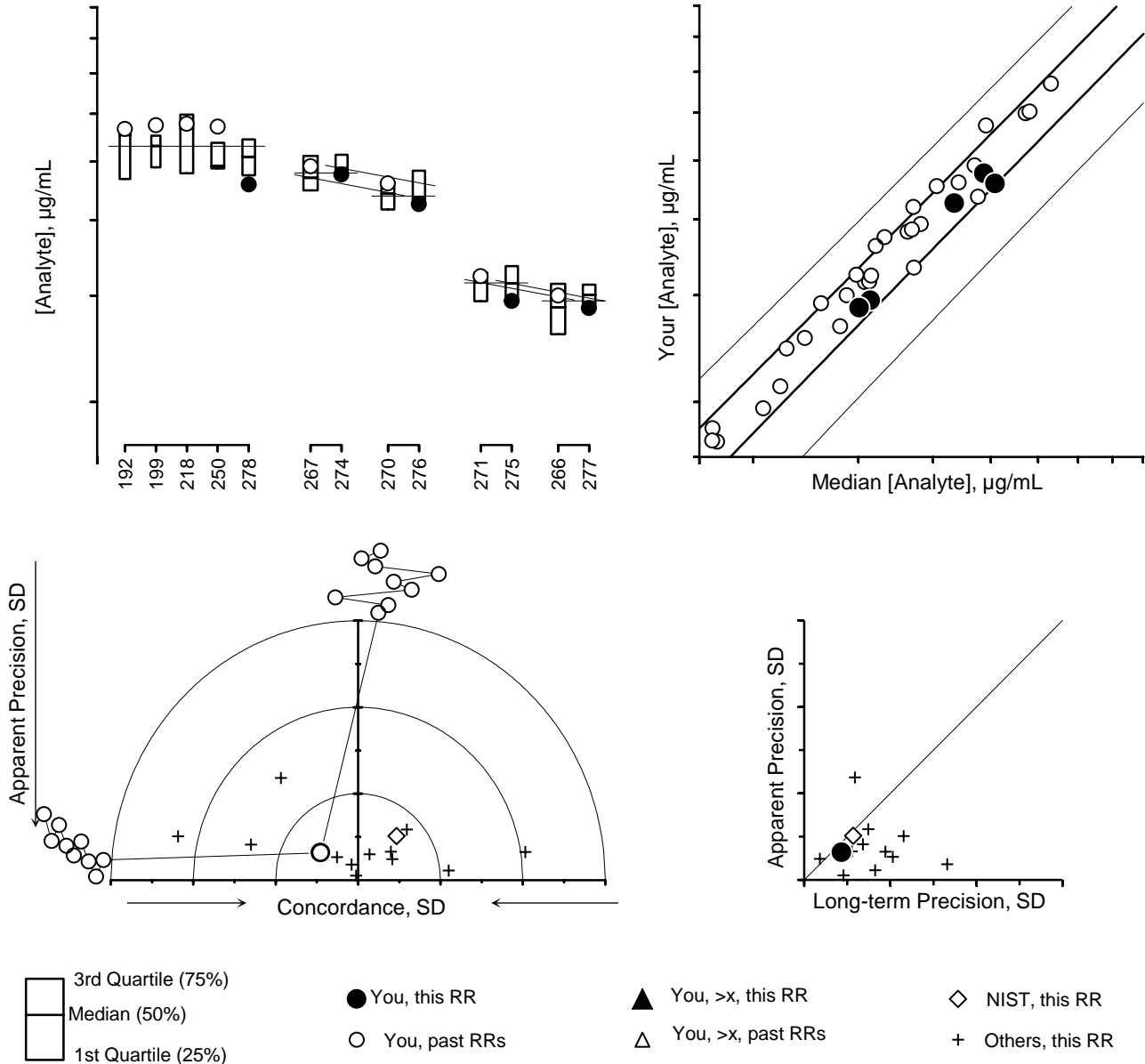
History

Comments

Fresh frozen
Fresh frozen
Lyophilized
Lyophilized
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

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

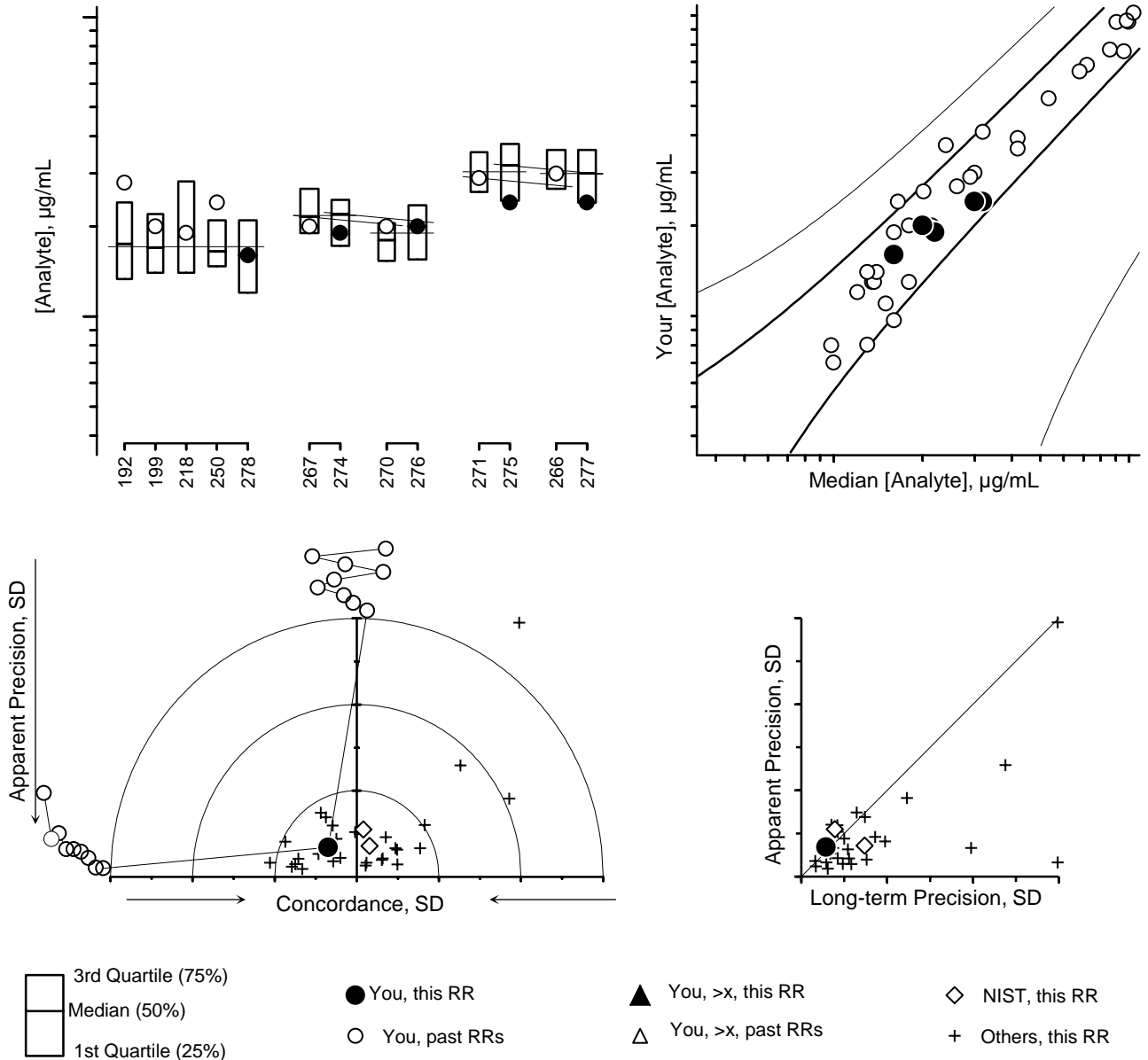
History

Comments

Fresh frozen
Fresh frozen
Lyophilized
Lyophilized
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

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

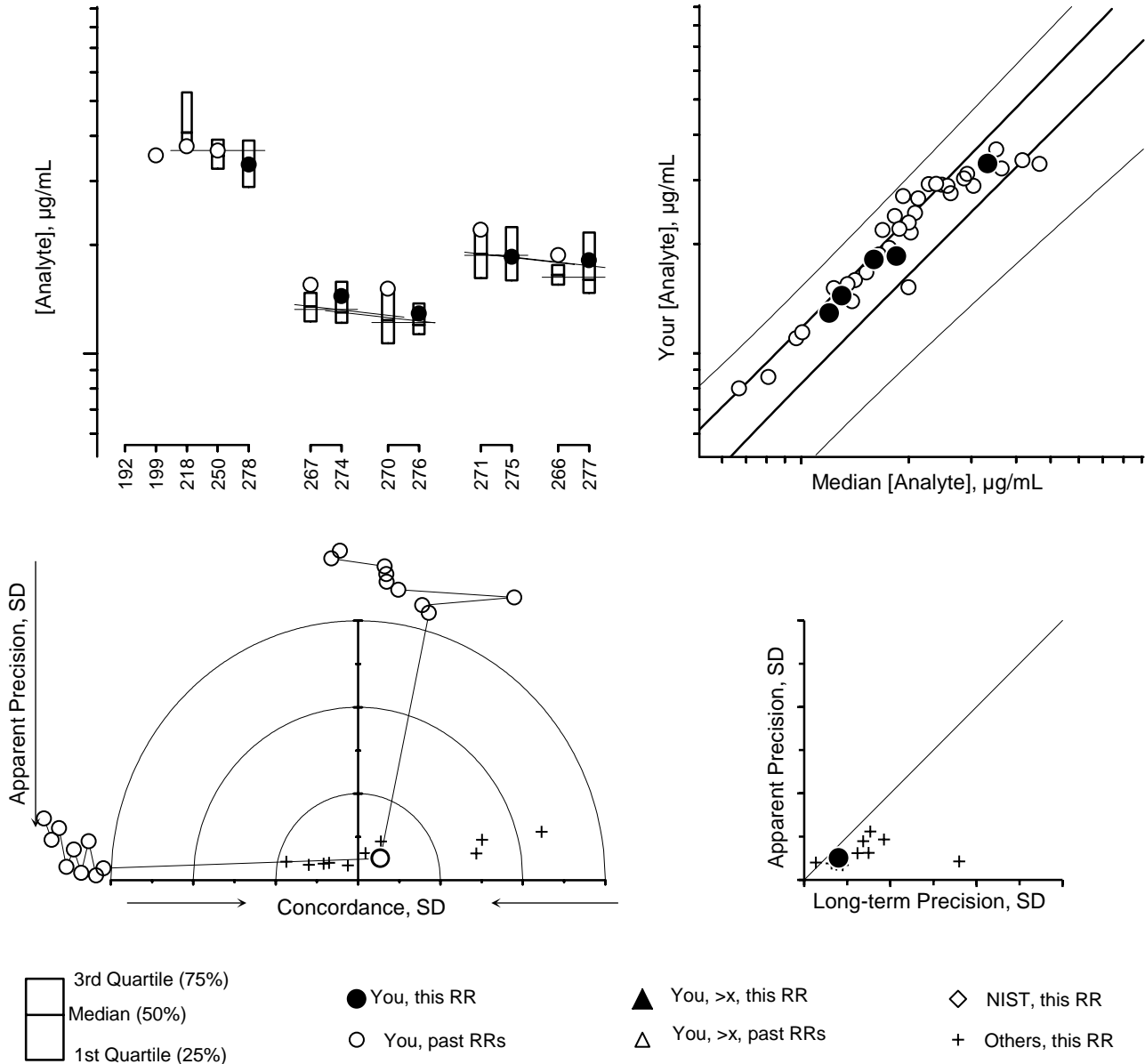
History

Comments

Fresh frozen
 Fresh frozen
 Lyophilized
 Lyophilized
 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

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

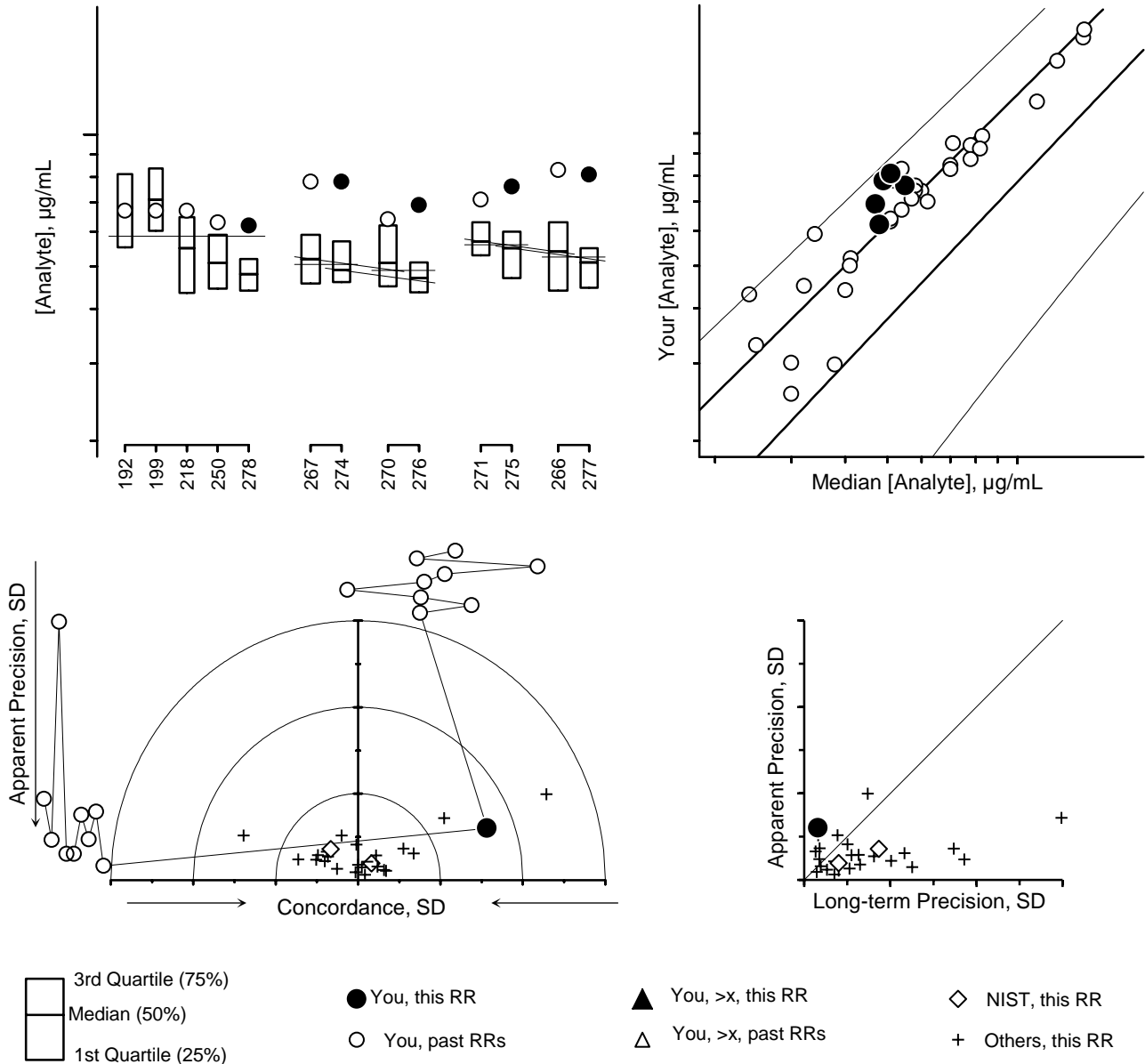
History

Comments

Fresh frozen
 Fresh frozen
 Lyophilized
 Lyophilized
 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

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

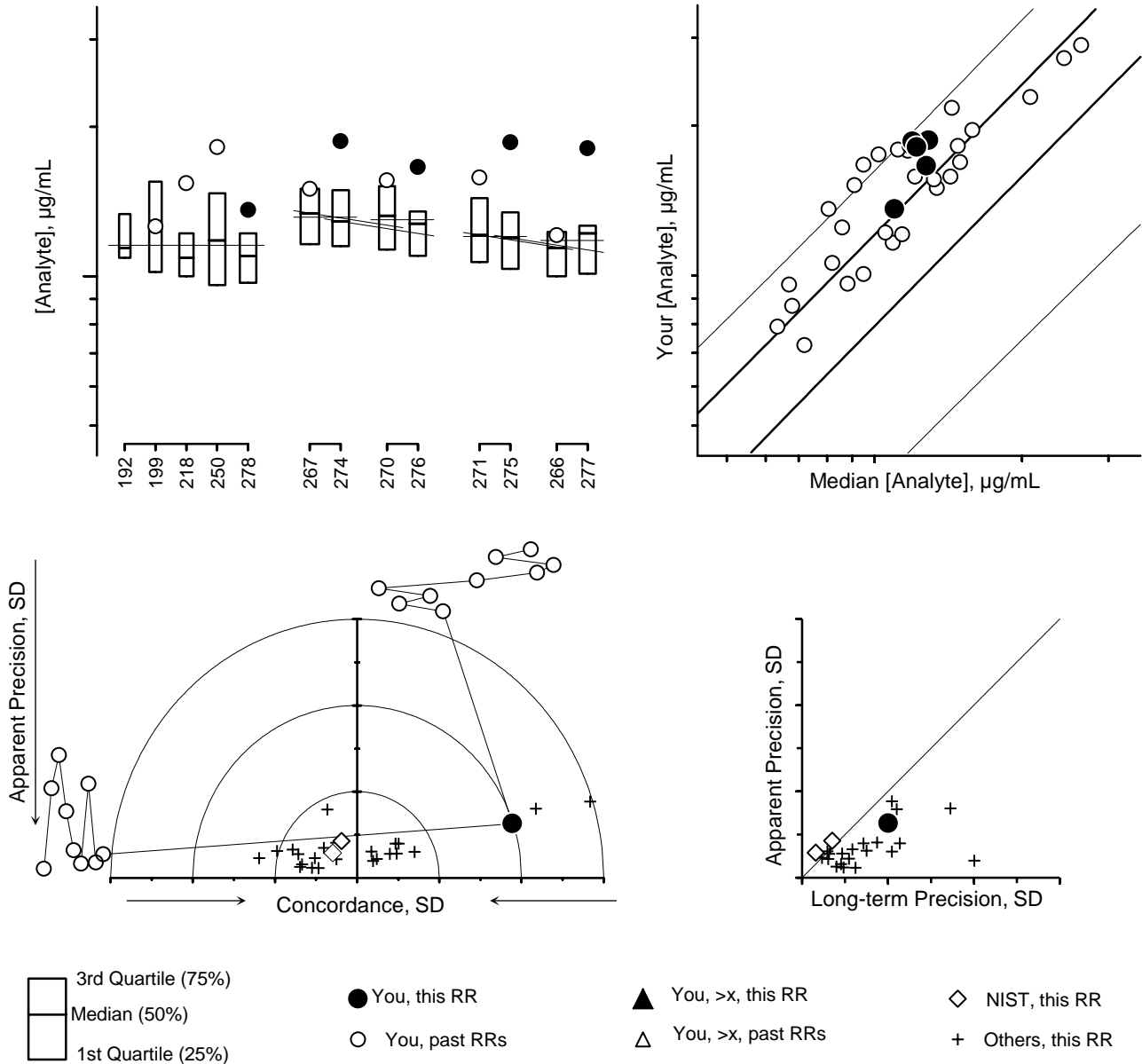
History

Comments

Fresh frozen
 Fresh frozen
 Lyophilized
 Lyophilized
 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

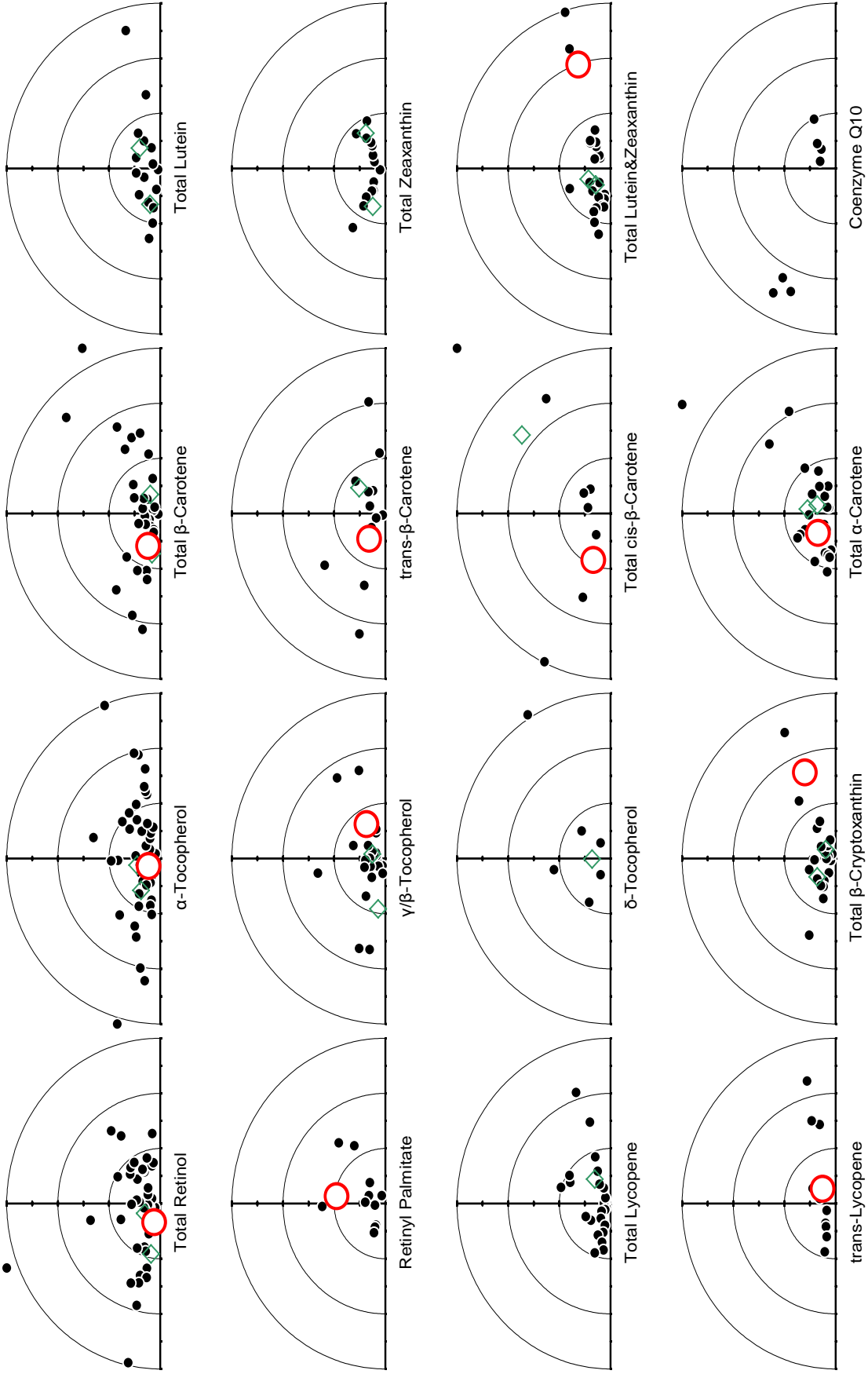
#274	#267 RR48, same pool as #276
#275	#271 RR49, same pool as #277
#276	#270 RR49, same pool as #274
#277	#266 RR48, same pool as #275
#278	#192 RR30, #199 RR32, #218 RR36, #250 RR44

History

Comments

Fresh frozen
 Fresh frozen
 Lyophilized
 Lyophilized
 Lyophilized

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



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,

A handwritten signature in black ink, appearing to read "Sam Margolis".

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

Enclosures

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 of MPA 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:

$$E_1\% \text{ dl/g}\cdot\text{cm} = \frac{\text{Observed Absorbance}_{\lambda_{\text{max}}}}{\frac{(\text{g AA}/100 \text{ mL stock})(\text{g stock in } 100 \text{ mL dilute solution})}{(\text{g AA stock solution}) + (\text{g MPA solution in } 100 \text{ mL dilute solution } 1)}}$$

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* should 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 $\mu\text{g/L}$ 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 ($\mu\text{mol/L}$ of Sample)

DILUTE SOLUTION 1

REPLICATE 1 _____ $\mu\text{mol/L}$ of dilute solution 1
REPLICATE 2 _____ $\mu\text{mol/L}$ of dilute solution 1

DILUTE SOLUTION 2

REPLICATE 1 _____ $\mu\text{mol/L}$ of dilute solution 2
REPLICATE 2 _____ $\mu\text{mol/L}$ of dilute solution 2

DILUTE SOLUTION 3

REPLICATE 1 _____ $\mu\text{mol/L}$ of dilute solution 3
REPLICATE 2 _____ $\mu\text{mol/L}$ of dilute solution 3

5% MPA SOLUTION (DILUENT)

REPLICATE 1 _____ $\mu\text{mol/L}$ of diluent
REPLICATE 2 _____ $\mu\text{mol/L}$ of diluent

TEST SAMPLE #1

REPLICATE 1 _____ $\mu\text{mol/L}$ of Sample 1
REPLICATE 2 _____ $\mu\text{mol/L}$ of Sample 1

TEST SAMPLE #2

REPLICATE 1 _____ $\mu\text{mol/L}$ of Sample 2
REPLICATE 2 _____ $\mu\text{mol/L}$ of Sample 2

TEST SAMPLE #3

REPLICATE 1 _____ $\mu\text{mol/L}$ of Sample 3
REPLICATE 2 _____ $\mu\text{mol/L}$ 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 M²QAP 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 M²QAP expenses.

The M²QAP Gang

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 M²QAP 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	<p>A listing of the</p> <ul style="list-style-type: none"> • 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 (E1%), 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-10 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 $\mu\text{mol/L}$ 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

Lab	Date	Gravimetric, $\mu\text{mol/L}$			Control / Calibration Samples						MPA			Dilute Solution 1 Spectrophotometry			Samples									
		[Dil1]	[Dil2]	[Dil3]	MPA	Ctri:1	Ctri:2	Ctri:3	Ctri:4	Inter	Slope	R ²	SEE	Density g/mL	λ_{max}	A_{max}	E %	S15:1	S15:2	S15:3	S15:1	S15:2	S15:3	Corrected, $\mu\text{mol/L}$		
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	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	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	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	26.0	0.0	7.7	26.9	0.0	7.7	26.9	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	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	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	243.	0.5905	599.6	5.1	23.1	0.0	7.9	28.9	1.9	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.	0.563	552.5	8.2	22.8	4.0	9.5	27.1	4.3	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	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	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	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	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	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	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	10.1	29.3	1.8

Control / Calibration Samples		MPA		Dilute Solution 1 Spectrophotometry		Samples	
Measured, $\mu\text{mol/L}$	MPA	λ_{max}	A_{max}	E %	S15:1	S15:2	S15:3
15	56.3	14	1.029	243.4	15	14	14
15	5.3	14	0.009	0.6	15	14	14
15	4.3	14	0.5557	243.0	15	14	14
15	4.3	14	0.0207	0.6	15	14	14
15	23.28	14	1.000	243.0	15	14	14
15	26.75	14	1.031	243.0	15	14	14
15	28.30	14	1.032	243.0	15	14	14
15	29.24	14	1.033	243.6	15	14	14
15	41.40	14	1.034	245.0	15	14	14
15	1.98	14	0.002	0.0208	15	14	14
15	7	14	0	4	15	14	14
15	7	14	0	3	15	14	14

Control / Calibration Samples		MPA		Dilute Solution 1 Spectrophotometry		Samples	
Average	SD	Average	SD	Min	Max	eSD	CV
56.3	5.3	1.029	0.009	243.0	245.0	0.0208	0
4.3	4.3	0.5557	0.0207	243.0	245.0	0.0208	0
23.28	46.4	1.000	1.000	243.0	245.0	0.0208	0
26.75	53.5	1.031	1.031	243.0	245.0	0.0208	0
28.30	56.6	1.032	1.032	243.0	245.0	0.0208	0
29.24	57.9	1.033	1.033	243.6	245.0	0.0208	0
41.40	67.6	1.034	1.034	245.0	245.0	0.0208	0
1.98	3.1	0.002	0.002	0.0208	0.0208	0.0208	0
7	6	0	0	4	7	0	0
7	7	0	0	3	7	0	0

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	E1%max dL/gcm
09/23/98	HPLC-EC (Height)	11	55 mmol/L	200.0	103.09	526.0	102.45	57.8	
04/02/99	HPLC-EC (Height)	12	55 mmol/L	215.0	103.10	517.1	102.52	61.1	
09/17/01	HPLC-EC (Height)	13	55 mmol/L	200.8	103.16	508.0	102.58	0.0	
09/27/01	HPLC-EC (Height)	14	55 mmol/L, Ctrl-1	200.3	103.23	510.0	102.31	56.1	548
09/18/01	HPLC-EC (Height)	15	55 mmol/L, Ctrl:1	200.5	103.12	506.0	102.19	55.8	557

552 ±6

Grand Average		
Mean	SDrepeat	SDreprod
8.0	3.2	0.6

RR	Sample	[TAA] mmol/L/sample				
		Rep1	Rep2	Factor	Mean	SDdup
11	SRM Lv 1, A	15.5	13.9	0.5	7.4	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	8.0	7.7	1.0	8.4	8.5
14	SRM Lv 1, S14:3	8.9	8.7	1.0	7.8	0.2
15	SRM Lv 1, S15:1			1.0	8.8	0.1

25.6	10.5	1.4
------	------	-----

0.0	0.0	0.0
-----	-----	-----

11	SRM Lv 2, A	50.7	47.7	0.5	24.6	1.1
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
15	S15:3 (188B)	0.0	0.0	1.0	0.0	0.0

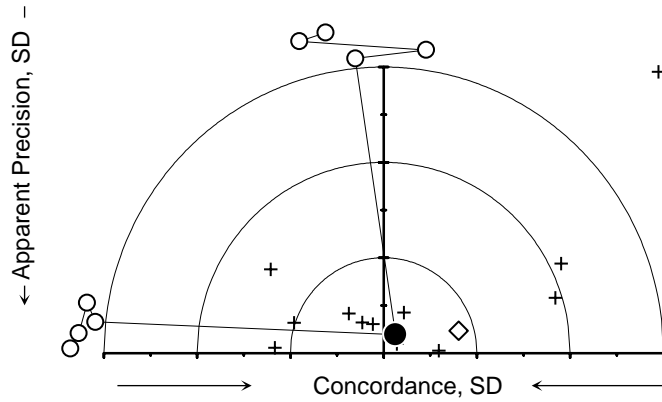
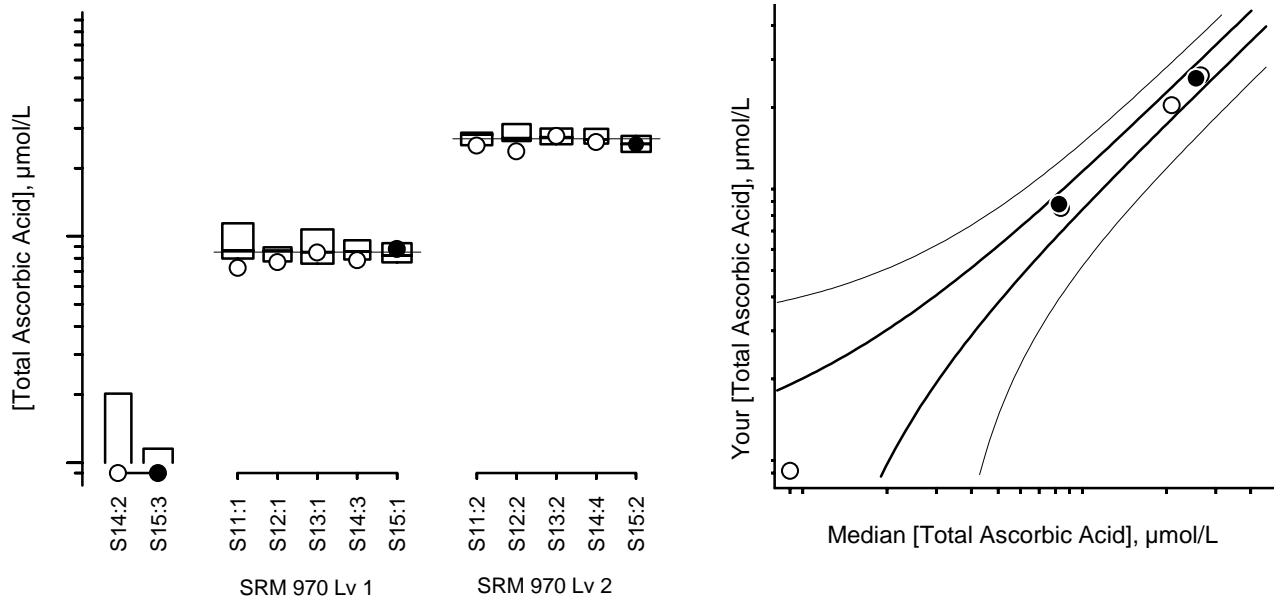
Please check our records against your records. Send corrections and/or updates to...


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

Total Ascorbic Acid



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

● You, RR 15
 ○ You, RR 11-14

◇ NIST, RR 15
 + Others, RR 11-14

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

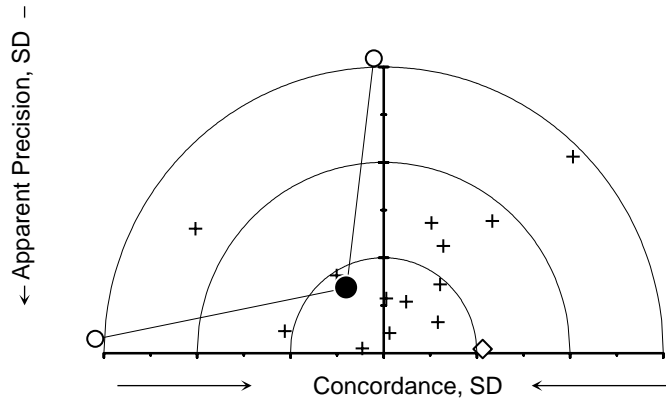
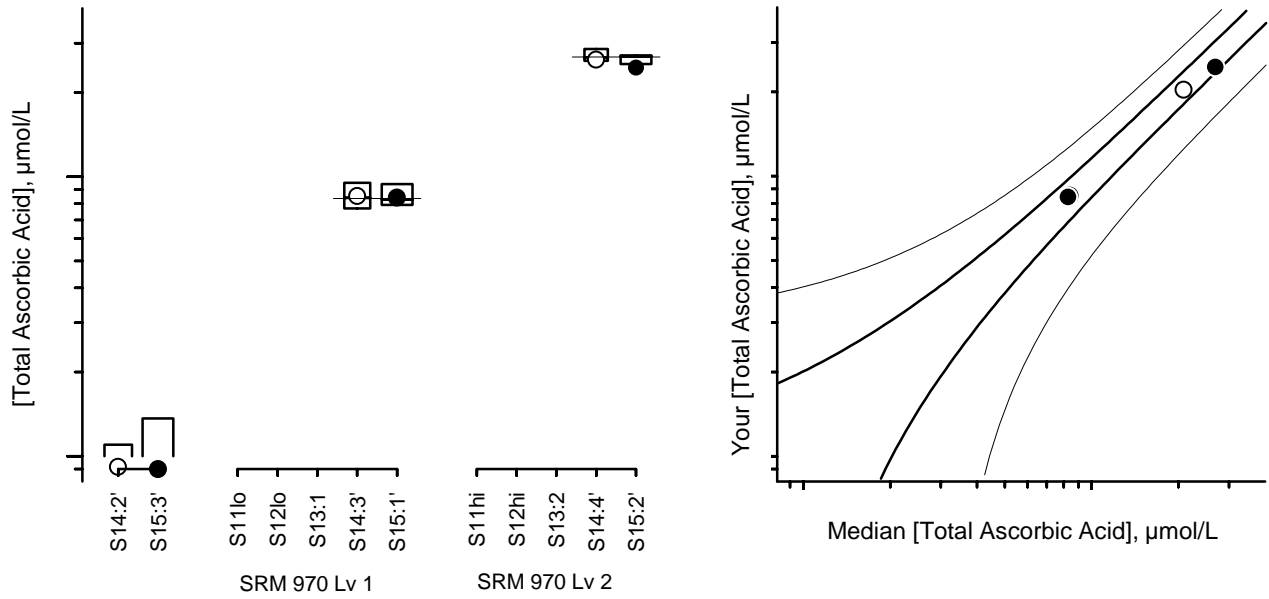
Sample






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

Comments

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

Total Ascorbic Acid Adjusted to Gravimetrically Prepared Calibrants



-  3rd Quartile (75%)
Median (50%)
1st Quartile (25%)
-  You, RR 15
-  You, RR 11-14
-  NIST, RR 15
-  Others, RR 11-14

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

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

Comments