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NIST Micronutrients Measurement Quality Assurance Program Winter 2010 Comparability Studies

Results for Round Robin LXVII
Fat-Soluble Vitamins and Carotenoids in Human Serum
and Round Robin 32 Ascorbic Acid in Human Serum

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

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April, 2013

U.S. Department of Commerce
Rebecca Blank, Acting Secretary

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

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Abstract

The National Institute of Standards and Technology coordinates the Micronutrients Measurement Quality Assurance Program (MMQAP) for laboratories that measure fat- and water-soluble vitamins and carotenoids in human serum and plasma. This report describes the design of and results for the Winter 2010 MMQAP measurement comparability improvement studies: 1) Round Robin LXVII Fat-Soluble Vitamins and Carotenoids in Human Serum and 2) Round Robin 32 Total Ascorbic Acid in Human Serum. The materials for both studies were shipped to participants in December 2009; participants were requested to provide their measurement results by March 8, 2010.

Keywords

Human Serum
Retinol, α-Tocopherol, γ-Tocopherol, Total and *Trans*-β-Carotene
SRM 968e
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 LXVII: Fat-Soluble Vitamins and Carotenoids in Human Serum

Participants in the MMQAP Fat-Soluble Vitamins and Carotenoids in Human Serum Round Robin LXVII comparability study (hereafter referred to as RR67) received one lyophilized and four liquid-frozen human serum test samples for analysis. Unless multiple vials were previously requested, participants received one vial of each serum. These sera were shipped on dry ice to participants in December 2009. 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 RR67 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 32: Vitamin C in Human Serum

Participants in the MMQAP Vitamin C in Human Serum Round Robin 32 comparability study (hereafter referred to as RR32) received four frozen serum test samples, one frozen control serum, and a solid ascorbic acid control material for analysis. Unless multiple vials were previously requested, participants received one vial of each material. These sample materials were shipped on dry ice to participants in December 2009. The communication materials included in the sample shipment are provided in Appendix E.

The test and control serum materials were prepared by adding equal volumes of 10 % metaphosphoric acid (MPA) to human serum that had been spiked with ascorbic acid. While these samples contain some dehydroascorbic acid, its content is variable. Therefore, the participants report only total ascorbic acid (TAA, ascorbic acid plus dehydroascorbic acid). Participants are also encouraged to prepare calibration solutions from the supplied solid control to enable calibrating their serum measurements to the same reference standard.

The final report delivered to every participant in RR32 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 RR67

The following three items were included in each package shipped to an RR67 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-

December 7, 2009

Dear Colleague:

Enclosed are samples for the first fat-soluble vitamins and carotenoids in serum study (Round Robin LXVII) for the 2010 NIST Micronutrients Measurement Quality Assurance Program. The set of samples (Sera 362 - 366) consists of one lyophilized sample and one vial each of four liquid-frozen serum samples for analysis along with a form for reporting your results. These samples should be stored in the dark at or below –20 °C upon receipt. When reporting your results, please submit one value for each analyte for a given serum sample. If a value obtained is below your limit of quantification, please indicate this result on the form by using NQ *(Not Quantified)*. Results are due to NIST by **March 8, 2010**. Results received more than <u>two weeks</u> after the due date may not be included in the summary report for this round robin study. The feedback report concerning the study will be distributed in April 2010.

Samples should be allowed to stand at room temperature under subdued light until thawed. We recommend that sample mixing be facilitated with 3 to 5 min agitation in an ultrasonic bath or at least 15 min at room temperature with intermittent swirling. (CAUTION: Vigorous shaking will cause foaming and possibly interfere with accurate measurement. The rubber stopper contains phthalate esters that may leach into the sample upon intermittent contact of the liquid sample with the stopper. These esters absorb strongly in the UV region and elute near retinol in most LC systems creating analytical problems.) **Water should not be added to the liquid-frozen samples.** Add water (1 mL) *only* to the lyophilized serum #362.

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

Please report your results for Round Robin LXVII by e-mail to david.duewer@nist.gov or fax to 301-977-0685. If you have questions or comments regarding this study, please call me at (301) 975-3120 or e-mail me at jbthomas@nist.gov.

Sincerely,

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

Enclosures

NIST

Participant #: ________ Date: ____________

Round Robin LXVII: Human Sera

NIST Micronutrients Measurement Quality Assurance Program

Analyte	362	363	364	365	366	Units*
total retinol						
trans-retinol						
didehydroretinol						
retinyl palmitate						
α-tocopherol						
γ/β-tocopherol						
δ-tocopherol						
total β-carotene						
trans-β-carotene						
total cis-β-carotene						
total α-carotene						
total lycopene						
trans-lycopene						
total β-cryptoxanthin						
total α-cryptoxanthin						
total lutein						
total zeaxanthin						
total lutein&zeaxanthin						
total coenzyme Q10						
ubiquinol (QH_2)						
ubiquinone (Qox)						
phylloquinone (K_1)						
25-hydroxyvitamin D						

Other measurands?						

* we prefer **µg/mL**

Were the liquid-frozen samples (363 to 366) frozen when received? Yes | No

Comments:

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

Please return results by
8-Mar-2010

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

Participant #: ________ Date: ____________

Fat-Soluble Vitamins Round Robin LXVII

NIST Micronutrients Measurement Quality Assurance Program

Packing List and Shipment Receipt Confirmation Form

This box contains: one vial each of the following five FSV M^{2}QAP sera

Serum	Form	Reconstitute?	Vial/Cap
#362	Lyophilized	Yes	2 mL clear or amber, blue cap
#363	Liquid frozen	No	2 mL amber, green cap
#364	Liquid frozen	No	2 mL amber, metallic-blue cap
#365	Liquid frozen	No	2 mL amber, red cap
#366	Liquid frozen	No	2 mL amber, silver cap

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

1) Date this shipment arrived: ___________________________

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

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

4) Did the liquid frozen samples (#363 to #366) arrive frozen? Yes | No

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

6) When do you anticipate analyzing these samples? ________________________

Your prompt return of this information is appreciated.

The M^{2}QAP Gang

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

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

Appendix B. Final Report for RR67

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

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

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

April 27, 2010

Dear Colleague:

Enclosed is the summary report of the results for round robin LXVII (RR67) of the 2010 NIST Micronutrients Measurement Quality Assurance Program (M^2QAP) for the fat-soluble vitamins and carotenoids in human serum. Included in this report are: 1) a summary of data and measurement comparability scores for all laboratories, 2) a detailed graphical analysis of your results; and 3) a graphical summary of your measurement comparability.

Your overall measurement comparability is summarized in the "Score Card" summary, page 6 of the All Lab Report. Combined results rated 1 to 3 are within 1 to 3 standard deviations of the assigned value, respectively; those rated 4 are >3 standard deviations from the assigned value. Similar information is presented graphically in the "target plots" that are the last page of your Individualized Report. If you have concerns regarding your laboratory's performance, please contact us for consultation.

Samples for the second 2010 QA interlaboratory exercise will be shipped **during the week of June 7, 2010.** If you have any questions regarding this report, please contact Dave Duewer at david.duewer@nist.gov or me at jbthomas@nist.gov, tel: 301/975-3120, or fax: 301/977-0685.

Sincerely,

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

David L. Duewer, Ph.D.
Research Chemometrician
Analytical Chemistry Division
Chemical Science and Technology Laboratory

Cc: L.C. Sander

The NIST M^{2}QAP Round Robin LXVII (RR67) report consists of:

Page	"All Lab" Report
1-4	A listing of all results and statistics for all analytes.
5	A legend for the list of results and statistics.
6	The text Comparability Summary ("Score Card") of measurement performance.

Page	"Individualized" Report
1	Your values, the number of labs reporting values, and our assigned values.
2 to n	"Four Plot" summaries of your current and past measurement performance, one page for each analyte you report that is also reported by at least 8 other participants.
n+1	The graphical Comparability Summary (target plot) of measurement performance.

Samples. Five samples were distributed in RR67.

Serum	Description	Prior Distributions
362	Lyophilized, native, single-donor, commercially obtained serum prepared in 2002. The same material was used to prepare #366.	#290:RR53-2/03, #300:RR55-3/04, #312:RR57-3/05, #322:RR59-3/06, #333:RR61-3/07, #348:RR64-9/08
363	Fresh-frozen, native, multi-donor, prepared in 2009. This is Level III of candidate SRM 968e.	#359:RR66 – 9/09
364	Fresh-frozen, native, multi-donor, prepared in 2009. This is Level II of candidate SRM 968e.	#358:RR66 – 9/09
365	Fresh-frozen, native, multi-donor, prepared in 2009. This is Level I of candidate SRM 968e.	#357:RR66 - 9/09
366	Fresh-frozen, native, single-donor, commercially obtained serum prepared in 2002. The same material was used to prepare #362.	#292:RR53-2/03, #301:RR55-3/04, #313:RR57-3/05, #323:RR59-3/06, #332:RR61-3/07, #349:RR64-9/08

Results

1) Candidate SRM 968e. Sera #363 to #365 are the components of candidate SRM 968e. All three materials were distributed last Fall in RR66; there have been no significant changes in the level nor variability of any of the reported analytes.

   These materials were prepared by blending commercially available materials without spiking. The materials were designed to represent relatively low, middle, and high levels of retinol, α-tocopherol and β-carotene. We will notify you when SRM 968e material becomes available for purchase.

2) Sera 362 and 366. These materials were prepared in 2003 from the same serum pool to help evaluate the commutability and relative stability of lyophilized and fresh-frozen sera. There have been no significant changes in the relative levels of any of the reported analytes. However, there is some evidence for increased variability of lutein and zeaxanthin in both materials.

Appendix C. "All-Lab Report" for RR67

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.

Sera 363, 364, and 365 are the components of the candidate NIST SRM 968e Fat-Soluable Vitamins, Carotenoids, and Cholesterol in Human Serum. Summary results for the NIST analysis of these materials are listed in the "All-Lab Report." These NIST results are not used in the assessment of the consensus results of the study.

Round Robin LXVII Laboratory Results

Lab	Total Retinol, µg/mL 362	363	364	365	366	trans-Retinol, µg/mL 362	363	364	365	366	Retinyl Palmitate, µg/mL 362	363	364	365	366
FSV-BA	0.616	0.665	0.517	0.366	0.645						0.107	0.163	0.071	0.022	0.115
FSV-BB	0.602	0.640	0.517	0.342	0.616						0.071	0.074	0.022	0.013	0.075
FSV-BC	0.579	0.627	0.495	0.349	0.639										
FSV-BD	0.613	0.653	0.530	0.359	0.640										
FSV-BE	0.661	0.723	0.560	0.400	0.725										
FSV-BF	0.650	0.680	0.530	0.360	0.650										
FSV-BG	0.610	0.659	0.521	0.376	0.649						0.101	0.179	0.054	0.017	0.103
FSV-BH	0.603	0.607	0.455	0.329	0.617										
FSV-BJ	0.571	0.611	0.480	0.352	0.615						0.076	0.074	*nq*	*nq*	0.075
FSV-BK	0.555	0.581	0.436	0.281	0.570										
FSV-BL	0.630	0.660	0.520	0.340	0.630										
FSV-BM	0.620	0.690	0.560	0.410	0.670										
FSV-BN	0.590	0.662	0.519	0.360	0.676										
FSV-BNa	0.582	0.643	0.497	0.354	0.638										
FSV-BO	0.547	0.571	0.451	0.300	0.575										
FSV-BP	0.630	0.650	0.520	0.370	0.660										
FSV-BQ	0.864	0.917	0.711	0.500	0.918										
FSV-BR	*≥0.590*	*≥0.750*	*≥0.490*	*≥0.390*	*≥0.590*	0.590	0.750	0.490	0.390	0.590					
FSV-BS	*≥0.616*	*≥0.687*	*≥0.566*	*≥0.416*	*≥0.710*	0.616	0.687	0.566	0.416	0.710					
FSV-BT	0.627	0.601	0.482	0.325	0.550										
FSV-BU	0.731	0.653	0.461	0.334	0.579										
FSV-BV	0.646	0.612	0.523	0.381	0.676										
FSV-BW	0.615	0.653	0.501	0.345	0.632						0.091	0.094	0.023	*nd*	0.100
FSV-CC	0.825	0.692	0.519	0.385	0.709	0.800	0.692	0.511	0.365	0.692					
FSV-CD	0.520	0.510	0.400	0.390	0.400						0.110	0.110	0.020	≤0.01	0.110
FSV-CE	0.668	0.654	0.517	0.368	0.707										
FSV-CF	0.591	0.722	0.496	0.364	0.616										
FSV-CG	0.534	0.612	0.512	0.356	0.584										
FSV-CI	0.645	0.712	0.547	0.391	0.677						0.094	0.093	0.035	0.013	0.097
FSV-CW	0.686	0.587	0.482	0.364	0.631						0.055	0.047	0.015	0.007	0.058
FSV-CZ	0.598	0.640	0.520	0.364	0.627										
FSV-DA	0.557	0.641	0.481	0.338	0.605	0.557	0.641	0.481	0.338	0.605	0.077	0.070	0.018	0.007	0.096
FSV-DD	0.650	0.570	0.430	0.300	0.550										
FSV-DI	0.690	0.720	0.580	0.400	0.700						0.080	0.070	0.015	0.010	0.085
FSV-DV	0.528	0.562	0.437	0.287	0.547										
FSV-EE	0.561	0.649	0.536	0.376	0.593										
FSV-EZ	*≥0.527*	*≥0.522*	*≥0.416*	*≥0.347*	*≥0.504*	0.527	0.522	0.416	0.347	0.504	0.073	0.070	*nq*	*nq*	0.070
N	34	34	34	34	34	5	5	5	5	5	11	11	9	7	11
Min	0.520	0.510	0.400	0.281	0.400	0.527	0.522	0.416	0.338	0.504	0.055	0.047	0.015	0.007	0.058
Median	0.614	0.650	0.517	0.360	0.632	0.590	0.687	0.490	0.365	0.605	0.080	0.074	0.022	0.013	0.096
Max	0.864	0.917	0.711	0.500	0.918	0.800	0.750	0.566	0.416	0.710	0.110	0.179	0.071	0.022	0.115
SD	0.053	0.056	0.032	0.028	0.057	0.049	0.069	0.031	0.038	0.129	0.016	0.029	0.010	0.006	0.021
CV	9	9	6	8	9	8	10	6	10	21	20	39	47	49	22
N_{past}	33	33	33	33	33	8	5	5	5	8	11	12	9	11	11
$Median_{past}$	0.610	0.654	0.497	0.351	0.642	0.611	0.549	0.471	0.330	0.644	0.092	0.090	0.021	0.008	0.097
SD_{past}	0.045	0.052	0.049	0.033	0.047	0.037	0.102	0.064	0.032	0.048	0.019	0.020	0.005	0.005	0.021
N_{NIST}		11	11	11											
$Mean_{NIST}$		0.657	0.490	0.346											
S_{rep}		0.017	0.011	0.013											
S_{het}		0.000	0.003	0.009											
S_{NIST}		0.017	0.011	0.016											
NAV	0.614	0.653	0.504	0.353	0.632	0.590	0.687	0.490	0.365	0.605	0.080	0.074	0.022	0.013	0.096
NAU	0.053	0.056	0.046	0.031	0.057	0.049	0.069	0.039	0.038	0.129	0.022	0.029	0.012	0.011	0.025

Lab	α-Tocopherol, µg/mL 362	363	364	365	366	γ/β-Tocopherol, µg/mL 362	363	364	365	366	δ-Tocopherol, µg/mL 362	363	364	365	366
FSV-BA	9.75	18.30	9.99	6.46	10.20	1.90	2.54	1.54	1.95	1.99	0.057	0.221	0.046	0.098	0.088
FSV-BB	10.30	18.40	10.40	7.13	10.90	1.78	2.34	1.48	1.86	1.87	0.056	0.201	0.120	0.120	0.083
FSV-BC															
FSV-BD	10.50	19.30	11.40	7.90	11.50										
FSV-BE	9.50	18.17	9.96	6.38	10.25	1.69	2.32	1.41	1.76	1.81					
FSV-BF	9.80	17.80	9.70	6.50	10.00										
FSV-BG	10.09	19.24	10.59	6.86	10.91	1.80	2.40	1.50	1.97	1.91					
FSV-BH	9.49	18.03	9.88	6.31	10.01	1.70	2.29	1.40	1.77	1.81					
FSV-BJ	9.35	18.06	10.48	6.43	9.86	1.57	2.12	1.38	1.78	1.69					
FSV-BK	9.37	18.51	9.50	5.86	9.62										
FSV-BL	10.40	18.60	10.80	7.00	10.90										
FSV-BM	9.70	18.80	10.70	6.90	10.20										
FSV-BN	10.90	21.57	11.66	7.44	12.08										
FSV-BNa	10.28	20.60	10.96	6.88	11.39										
FSV-BO	9.70	17.50	9.70	6.30	9.90	1.60	2.00	1.30	1.60	1.70					
FSV-BP	8.56	18.51	9.60	6.48	9.86										
FSV-BQ	13.20	24.30	13.00	8.90	13.60										
FSV-BR	11.54	22.80	9.64	6.83	11.33										
FSV-BS															
FSV-BT	10.81	17.88	9.76	6.43	10.28	1.93	2.31	1.38	1.77	1.74	0.132	0.162	0.108	0.120	0.087
FSV-BU	10.70	19.76	9.71	5.62	7.80	2.10	2.34	1.49	1.93	2.20					
FSV-BV	9.81	16.83	10.51	6.93	11.15	1.48	1.78	1.24	1.60	1.65					
FSV-BW	9.50	18.22	9.83	6.30	9.96	1.63	2.15	1.32	1.69	1.69					
FSV-CC	11.90	19.06	9.65	6.84	11.10										
FSV-CD	9.67	17.32	9.79	8.23	8.24	1.66	2.02	1.28	1.70	1.68					
FSV-CE	9.82	22.31	9.69	6.76	11.70										
FSV-CF	11.10	17.90	10.70	6.40	9.90										
FSV-CG	8.31	16.68	9.85	6.06	9.26	1.62	2.31	1.53	1.83	1.83	*nd*	0.216	0.125	0.124	*nd*
FSV-CI	9.94	20.03	11.18	7.46	11.26	1.66	2.26	1.44	1.96	1.83					
FSV-CW	10.52	20.87	11.79	7.84	12.03	2.21	3.01	1.88	2.40	2.41	0.090	0.258	0.106	0.114	0.066
FSV-CZ	9.13	15.47	6.86	4.42	7.20	1.70	2.22	1.40	1.69	1.78					
FSV-DA	9.77	19.31	10.00	6.63	10.66	1.73	2.50	1.41	1.90	1.92	0.119	0.381	0.106	0.146	*nd*
FSV-DD															
FSV-DI	10.80	19.60	11.20	7.10	10.90	1.91	2.48	1.58	2.01	1.90	0.065	0.250	0.080	0.105	0.065
FSV-DV	4.40	13.60	5.20	3.70	6.90										
FSV-EE	10.40	18.60	9.60	6.70	10.90										
FSV-EZ	9.59	16.92	9.53	7.48	9.47	1.68	2.26	1.41	2.12	1.71					
N	34	34	34	34	34	19	19	19	19	19	6	7	7	7	5
Min	4.40	13.60	5.20	3.70	6.90	1.48	1.78	1.24	1.60	1.65	0.056	0.162	0.046	0.098	0.065
Median	9.82	18.51	9.92	6.73	10.27	1.70	2.31	1.41	1.83	1.81	0.078	0.221	0.106	0.120	0.083
Max	13.20	24.30	13.00	8.90	13.60	2.21	3.01	1.88	2.40	2.41	0.132	0.381	0.125	0.146	0.088
SD	0.71	1.18	0.60	0.53	0.96	0.12	0.14	0.12	0.18	0.15	0.031	0.043	0.021	0.009	0.008
CV	7	6	6	8	9	7	6	9	10	8	40	19	20	7	10
N_{past}	35	34	34	34	35	21	21	21	21	21	6	5	4	4	7
$Median_{past}$	9.93	18.48	10.30	6.75	10.46	1.74	2.08	1.34	1.72	1.82	0.066	0.202	0.072	0.091	0.065
SD_{past}	0.71	1.39	0.86	0.52	0.65	0.14	0.38	0.16	0.18	0.15	0.030	0.034	0.022	0.023	0.037
N_{NIST}		11	11	11			11	11	11						
$Mean_{NIST}$		19.52	10.25	6.96			2.30	1.49	2.03						
S_{rep}		0.41	0.17	0.40			0.16	0.08	0.10						
S_{het}		0.13	0.11	0.00			0.00	0.00	0.00						
S_{NIST}		0.44	0.20	0.40			0.16	0.08	0.10						
NAV	9.82	19.01	10.09	6.84	10.27	1.70	2.30	1.45	1.93	1.81	0.078	0.221	0.106	0.120	0.083
NAU	0.77	1.61	0.81	0.59	0.96	0.18	0.23	0.17	0.24	0.19	0.031	0.043	0.024	0.026	0.021

Round Robin LXVII Laboratory Results

Lab	Total β-Carotene, µg/mL 362	363	364	365	366	trans-β-Carotene, µg/mL 362	363	364	365	366	Total cis-β-Carotene, µg/mL 362	363	364	365	366
FSV-BA	0.117	0.395	0.246	0.091	0.118	0.105	0.374	0.232	0.079	0.108	0.012	0.021	0.014	0.012	0.010
FSV-BB	0.090	0.285	0.159	0.065	0.100	0.086	0.276	0.151	0.063	0.092	0.005	0.009	0.008	0.002	0.008
FSV-BC															
FSV-BD															
FSV-BE	0.106	0.414	0.243	0.086	0.117										
FSV-BF	0.103	0.370	0.213	0.087	0.093										
FSV-BG	0.143	0.460	0.262	0.101	0.148										
FSV-BH	0.108	0.410	0.245	0.080	0.116	0.108	0.396	0.235	0.080	0.116	*nq*	0.014	0.010	*nq*	*nq*
FSV-BJ	0.127	0.416	0.218	0.097	0.113										
FSV-BK															
FSV-BL															
FSV-BM															
FSV-BN	0.128	0.449	0.269	0.110	0.139	0.122	0.428	0.253	0.101	0.130	*0.006*	*0.021*	*0.016*	*0.010*	*0.009*
FSV-BNa	0.137	0.470	0.272	0.114	0.150										
FSV-BO	0.113	0.331	0.219	0.091	0.114										
FSV-BP	0.129	0.347	0.230	0.096	0.128										
FSV-BQ															
FSV-BR															
FSV-BS	*≥0.135*	*≥0.439*	*≥0.311*	*≥0.081*	*≥0.155*	0.135	0.439	0.311	0.081	0.155					
FSV-BT	0.116	0.287	0.209	0.084	0.100	0.114	0.274	0.198	0.081	0.097	0.007	0.022	0.017	0.005	0.006
FSV-BU	0.118	0.417	0.263	0.108	0.120										
FSV-BV	0.135	0.444	0.291	0.112	0.149										
FSV-BW	0.109	0.466	0.260	0.096	0.123										
FSV-CC															
FSV-CD	0.120	0.240	0.170	0.110	0.110										
FSV-CE	0.080	0.422	0.161	0.073	0.100										
FSV-CF															
FSV-CG	0.095	0.358	0.232	0.081	0.108	0.090	0.341	0.218	0.076	0.103	0.005	0.017	0.014	0.005	0.005
FSV-CI	0.126	0.448	0.251	0.086	0.132										
FSV-CW	0.116	0.307	0.215	0.097	0.118										
FSV-CZ	0.119	0.281	0.191	0.077	0.104										
FSV-DA	0.108	0.336	0.215	0.086	0.122	0.101	0.311	0.195	0.074	0.115	0.007	0.025	0.019	0.013	0.007
FSV-DD															
FSV-DI	0.118	0.365	0.265	0.110	0.099										
FSV-DV															
FSV-EE															
FSV-EZ	*≥0.066*	*≥0.275*	*≥0.186*	*≥0.085*	*≥0.064*	0.066	0.275	0.186	0.085	0.064					
N	23	23	23	23	23	9	9	9	9	9	6	7	7	6	6
Min	0.080	0.240	0.159	0.065	0.093	0.066	0.274	0.151	0.063	0.064	0.005	0.009	0.008	0.002	0.005
Median	0.117	0.395	0.232	0.091	0.117	0.105	0.341	0.218	0.080	0.108	0.006	0.021	0.014	0.007	0.008
Max	0.143	0.470	0.291	0.114	0.150	0.135	0.439	0.311	0.101	0.155	0.012	0.025	0.019	0.013	0.010
SD	0.014	0.079	0.034	0.014	0.016	0.022	0.096	0.034	0.006	0.016	0.001	0.006	0.004	0.005	0.002
CV	12	20	15	15	14	21	28	16	7	15	23	28	32	73	31
N_{past}	24	24	24	24	24	10	9	9	9	10	6	8	8	5	6
$Median_{past}$	0.114	0.386	0.241	0.090	0.122	0.111	0.344	0.213	0.083	0.117	0.006	0.016	0.013	0.005	0.006
SD_{past}	0.017	0.070	0.036	0.010	0.018	0.011	0.039	0.016	0.006	0.011	0.003	0.002	0.001	0.001	0.002
N_{NIST}		11	11	11			11	11	11			11	11	11	
$Mean_{NIST}$		0.431	0.246	0.114			0.382	0.193	0.093			0.052	0.053	0.021	
S_{rep}		0.023	0.020	0.003			0.019	0.018	0.005			0.021	0.013	0.004	
S_{het}		0.000	0.000	0.002			0.000	0.000	0.000			0.000	0.000	0.001	
S_{NIST}		0.023	0.020	0.004			0.019	0.018	0.005			0.021	0.013	0.004	
NAV	0.117	0.413	0.239	0.102	0.117	0.105	0.361	0.206	0.086	0.108	0.006	0.036	0.034	0.014	0.008
NAU	0.019	0.083	0.036	0.022	0.019	0.022	0.101	0.038	0.014	0.016	0.003	0.030	0.030	0.011	0.004

Lab	Total α-Carotene, µg/mL 362	363	364	365	366	Total Lycopene, µg/mL 362	363	364	365	366	trans-Lycopene, µg/mL 362	363	364	365	366
FSV-BA	0.063	0.014	0.030	0.008	0.065	0.536	0.939	0.657	0.201	0.468	0.266	0.415	0.298	0.110	0.278
FSV-BB	0.057	0.005	0.017	0.002	0.062	0.394	0.733	0.433	0.187	0.411	0.209	0.320	0.228	0.097	0.224
FSV-BC															
FSV-BD															
FSV-BE															
FSV-BF															
FSV-BG	0.079	0.021	0.033	0.009	0.082	0.521	0.954	0.585	0.216	0.546	0.320	0.489	0.342	0.130	0.344
FSV-BH	0.072	*nq*	*nq*	*nq*	0.078	0.520	1.079	0.674	0.241	0.567					
FSV-BJ	0.090	0.032	0.052	*nq*	0.094	0.539	1.133	0.660	0.202	0.539					
FSV-BK															
FSV-BL															
FSV-BM															
FSV-BN	0.090	0.022	0.040	0.017	0.096	0.508	1.005	0.632	0.265	0.546	0.289	0.477	0.340	0.142	0.315
FSV-BNa	0.104	0.043	0.061	0.026	0.110	0.559	1.061	0.691	0.258	0.594					
FSV-BO	0.069	0.009	0.025	0.006	0.067	0.390	0.658	0.471	0.188	0.401					
FSV-BP	0.050	0.012	0.036	*nd*	0.053	0.448	0.830	0.565	0.254	0.414					
FSV-BQ															
FSV-BR															
FSV-BS	0.048	0.021	0.027	0.011	0.050	0.635	0.822	0.879	0.196	0.656	0.355	0.427	0.418	0.097	0.343
FSV-BT	0.055	0.016	0.025	0.008	0.048	0.444	0.667	0.508	0.205	0.372	0.244	0.268	0.257	0.112	0.213
FSV-BU	0.071	0.010	0.028	0.009	0.080	0.428	0.848	0.563	0.205	0.491					
FSV-BV	0.105	0.016	0.041	0.009	0.114	0.607	1.077	0.761	0.278	0.667					
FSV-BW	0.056	*nd*	0.009	0.003	0.062	0.520	1.080	0.680	0.240	0.590					
FSV-CC															
FSV-CD	0.060	0.010	0.020	0.030	0.030	0.180	0.310	0.210	0.160	0.160					
FSV-CE															
FSV-CF															
FSV-CG	0.078	0.021	0.041	0.010	0.088	0.446	0.914	0.630	0.220	0.503	0.255	0.444	0.355	0.125	0.294
FSV-CI	0.080	0.045	0.045	0.015	0.087										
FSV-CW	0.064	0.019	0.032	0.009	0.069						0.224	0.321	0.262	0.104	0.244
FSV-CZ															
FSV-DA	0.063	0.009	0.021	0.005	0.060	0.451	0.891	0.572	0.215	0.456	0.258	0.425	0.306	0.120	0.266
FSV-DD															
FSV-DI						0.580	0.950	0.689	0.275	0.603					
FSV-DV															
FSV-EE															
FSV-EZ															
N	19	17	18	16	19	18	18	18	18	18	9	9	9	9	9
Min	0.048	0.005	0.009	0.002	0.030	0.180	0.310	0.210	0.160	0.160	0.209	0.268	0.228	0.097	0.213
Median	0.069	0.016	0.031	0.009	0.069	0.514	0.927	0.631	0.216	0.521	0.258	0.425	0.306	0.112	0.278
Max	0.105	0.045	0.061	0.030	0.114	0.635	1.133	0.879	0.278	0.667	0.355	0.489	0.418	0.142	0.344
SD	0.017	0.008	0.014	0.004	0.023	0.098	0.177	0.088	0.037	0.105	0.046	0.077	0.065	0.019	0.055
CV	24	53	45	43	34	19	19	14	17	20	18	18	21	17	20
N_{past}	21	19	20	18	21	20	19	19	19	20	8	9	9	9	8
$Median_{past}$	0.076	0.016	0.030	0.008	0.081	0.480	0.965	0.594	0.236	0.511	0.253	0.374	0.287	0.115	0.268
SD_{past}	0.013	0.006	0.004	0.004	0.012	0.062	0.219	0.079	0.025	0.072	0.046	0.055	0.012	0.017	0.051
N_{NIST}			11				11	11	11			11	11	11	
$Mean_{NIST}$		*nq*	0.034	*nq*			0.717	0.394	0.173			0.602	0.326	0.156	
S_{rep}			0.003				0.032	0.015	0.004			0.022	0.023	0.004	
S_{het}			0.000				0.000	0.000	0.001			0.012	0.000	0.003	
S_{NIST}			0.003				0.032	0.015	0.004			0.025	0.023	0.005	
NAV	0.069	0.016	0.033	0.009	0.069	0.514	0.822	0.512	0.194	0.521	0.258	0.513	0.316	0.134	0.278
NAU	0.020	0.008	0.014	0.004	0.023	0.106	0.231	0.209	0.060	0.107	0.047	0.149	0.067	0.036	0.055

Round Robin LXVII Laboratory Results

	Total β-Cryptoxanthin, µg/mL					Total α-Cryptoxanthin, µg/mL					Total Lutein, µg/mL					Total Zeaxanthin, µg/mL					Total Lutein&Zeaxanthin, µg/mL					Coenzyme Q10, µg/mL				
Lab	362	363	364	365	366	362	363	364	365	366	362	363	364	365	366	362	363	364	365	366	362	363	364	365	366	362	363	364	365	366
FSV-BA	0.057	0.042	0.057	0.050	0.058	0.025	0.019	0.023	0.017	0.024											0.095	0.146	0.119	0.093	0.108					
FSV-BB	0.046	0.033	0.048	0.046	0.052	0.023	0.017	0.022	0.017	0.025	0.072	0.133	0.091	0.073	0.072	0.045	0.058	0.046	0.047	0.040	0.117	0.191	0.137	0.120	0.112					
FSV-BC																														
FSV-BD																														
FSV-BE																										0.507	1.190	0.800	0.710	0.644
FSV-BF																														
FSV-BG	0.059	0.033	0.057	0.053	0.063																0.112	0.188	0.139	0.104	0.119					
FSV-BH	0.064	0.053	0.065	0.057	0.068						0.054	0.093	0.072	0.059	0.060	0.028	0.027	0.035	0.035	0.029	0.082	0.120	0.107	0.094	0.089					
FSV-BJ	0.043	0.031	0.040	0.037	0.047						0.082	0.120	0.096	0.080	0.087											0.659	1.210	0.939	0.837	0.699
FSV-BK																														
FSV-BL																														
FSV-BM																														
FSV-BN	0.055	0.031	0.056	0.055	0.060	0.025	0.014	0.024	0.021	0.026	0.052	0.099	0.075	0.066	0.060	0.019	0.022	0.024	0.035	0.028	0.069	0.118	0.097	0.098	0.085					
FSV-BNa	0.051	0.019	0.046	0.041	0.052																0.064	0.121	0.103	0.105	0.096					
FSV-BO	0.053	0.029	0.053	0.051	0.055						0.143	0.154	0.141	0.152	0.121	0.013	0.013	0.011	0.007	0.010	0.156	0.167	0.151	0.159	0.131	0.469	0.976	0.466	0.467	0.380
FSV-BP	0.047	0.024	0.047	0.040	0.049																0.085	0.130	0.122	0.102	0.106					
FSV-BQ																														
FSV-BR																														
FSV-BS	0.041	0.014	0.071	0.048	0.069						0.061	0.126	0.101	0.082	0.077	0.027	0.032	0.029	0.037	0.036	0.088	0.158	0.130	0.119	0.113					
FSV-BT	0.045	0.029	0.040	0.038	0.041	0.029	0.031	0.028	0.020	0.027	0.044	0.053	0.049	0.047	0.039	0.030	0.031	0.029	0.029	0.027	0.089	0.100	0.093	0.090	0.079	0.715	1.145	0.837	0.760	0.735
FSV-BU	0.040	0.022	0.045	0.046	0.051																0.106	0.154	0.138	0.108	0.116					
FSV-BV	0.036	0.016	0.034	0.034	0.041																0.095	0.125	0.111	0.100	0.097					
FSV-BW	0.060	0.010	0.040	0.050	0.050																0.090	0.130	0.110	0.100	0.100	1.300	1.550	0.910	1.110	1.320
FSV-CC																														
FSV-CD	0.050	0.040	0.050	0.050	0.050	≤0.01	≤0.01	≤0.01	≤0.01	≤0.01											0.250	0.310	0.250	0.250	0.240					
FSV-CE																														
FSV-CF																														
FSV-CG	0.066	0.061	0.078	0.063	0.074																0.122	0.182	0.158	0.142	0.136					
FSV-CI											0.071	0.113	0.085	0.067	0.065	0.021	0.020	0.021	0.023	0.019	*0.092*	*0.133*	*0.107*	*0.090*	*0.084*	0.692	1.300	0.928	0.818	0.734
FSV-CW	0.036	0.022	0.035	0.037	0.039																0.117	0.139	0.117	0.114	0.108	0.407	1.102	0.799	0.649	0.625
FSV-CZ																										0.913	1.534	1.007	0.961	0.856
FSV-DA	0.060	0.051	0.067	0.054	0.063	0.026	0.018	0.027	0.018	0.026	0.070	0.129	0.090	0.058	0.077	0.030	0.039	0.037	0.034	0.036	0.100	0.168	0.127	0.092	0.113					
FSV-DD																														
FSV-DI											0.078	0.130	0.100	0.085	0.090											0.730	1.380	0.945	0.805	0.732
FSV-DV																														
FSV-EE																										0.715	1.352	0.936	0.817	0.745
FSV-EZ																														
N	18	18	18	18	18	5	5	5	5	5	10	10	10	10	10	8	8	8	8	8	18	18	18	18	18	10	10	10	10	10
Min	0.036	0.010	0.034	0.034	0.039	0.023	0.014	0.022	0.017	0.024	0.044	0.053	0.049	0.047	0.039	0.013	0.013	0.011	0.007	0.010	0.064	0.100	0.093	0.090	0.079	0.407	0.976	0.466	0.467	0.380
Median	0.051	0.030	0.049	0.049	0.052	0.025	0.018	0.024	0.018	0.026	0.070	0.123	0.091	0.070	0.075	0.028	0.029	0.029	0.035	0.028	0.095	0.143	0.121	0.103	0.108	0.703	1.255	0.919	0.811	0.733
Max	0.066	0.061	0.078	0.063	0.074	0.029	0.031	0.028	0.021	0.027	0.143	0.154	0.141	0.152	0.121	0.045	0.058	0.046	0.047	0.040	0.250	0.310	0.250	0.250	0.240	1.300	1.550	1.007	1.110	1.320
SD	0.012	0.013	0.013	0.008	0.010	0.001	0.002	0.003	0.001	0.001	0.016	0.015	0.015	0.017	0.020	0.007	0.012	0.010	0.006	0.011	0.018	0.032	0.022	0.016	0.016	0.178	0.175	0.080	0.112	0.091
CV	23	45	26	17	20	4	10	13	4	5	22	12	16	24	27	24	41	35	17	39	19	23	19	15	15	25	14	9	14	12
N_{past}	21	20	20	20	21	6	4	4	4	7	14	10	10	10	14	12	9	9	9	12	21	20	20	20	21	6	5	5	5	6
$Median_{past}$	0.052	0.029	0.049	0.047	0.055	0.024	0.015	0.021	0.016	0.026	0.079	0.115	0.088	0.072	0.081	0.030	0.034	0.032	0.037	0.031	0.109	0.142	0.130	0.110	0.114	0.706	1.440	0.990	0.887	0.747
SD_{past}	0.008	0.006	0.005	0.007	0.010	0.006	0.002	0.004	0.004	0.006	0.014	0.031	0.020	0.012	0.014	0.007	0.015	0.019	0.012	0.008	0.018	0.026	0.027	0.010	0.016	0.099	0.169	0.067	0.037	0.103
N_{NIST}		11	11	11								11	11	11			11	11	11			11	11	11						
$Mean_{NIST}$		0.017	0.036	0.041								0.131	0.100	0.069			0.027	0.029	0.029			0.157	0.129	0.098						
S_{rep}		0.001	0.002	0.002								0.007	0.006	0.005			0.004	0.002	0.003			0.010	0.007	0.006						
S_{het}		0.001	0.002	0.002								0.000	0.002	0.000			0.000	0.000	0.001			0.000	0.001	0.000						
S_{NIST}		0.001	0.003	0.003								0.007	0.006	0.005			0.004	0.002	0.003			0.010	0.007	0.006						
NAV	0.051	0.023	0.042	0.045	0.052	0.025	0.018	0.024	0.018	0.026	0.070	0.127	0.095	0.069	0.075	0.028	0.028	0.029	0.032	0.028	0.095	0.150	0.125	0.100	0.108	0.703	1.255	0.919	0.811	0.733
NAU	0.012	0.016	0.016	0.013	0.013	0.001	0.002	0.003	0.001	0.001	0.016	0.024	0.019	0.017	0.020	0.008	0.012	0.010	0.010	0.011	0.020	0.034	0.026	0.022	0.022	0.178	0.175	0.092	0.112	0.091

C4

Round Robin LXVII Laboratory Results

	25-hydroxyvitamin D, µg/mL					Phylloquinone (K1), ng/mL				
Lab	362	363	364	365	366	362	363	364	365	366
FSV-BA	0.021	0.021	0.014	0.007	0.022					
FSV-BB										
FSV-BC										
FSV-BD										
FSV-BE						0.455	3.050	0.572	0.464	0.519
FSV-BF										
FSV-BG										
FSV-BH	0.028	0.027	0.020	0.011	0.027					
FSV-BJ										
FSV-BK										
FSV-BL										
FSV-BM										
FSV-BN										
FSV-BNa	0.017	0.015	0.019	0.008	0.025					
FSV-BO	0.008	0.009	0.006	0.003	0.009					
FSV-BP										
FSV-BQ										
FSV-BR										
FSV-BS										
FSV-BT										
FSV-BU										
FSV-BV										
FSV-BW										
FSV-CC										
FSV-CD										
FSV-CE										
FSV-CF										
FSV-CG										
FSV-CI						0.453	2.562	0.564	0.396	0.480
FSV-CW										
FSV-CZ										
FSV-DA										
FSV-DD										
FSV-DI										
FSV-DV										
FSV-EE										
FSV-EZ										
N	4	4	4	4	4	2	2	2	2	2
Min	0.008	0.009	0.006	0.003	0.009	0.453	2.562	0.564	0.396	0.480
Median	0.019	0.018	0.016	0.008	0.024	0.454	2.806	0.568	0.430	0.500
Max	0.028	0.027	0.020	0.011	0.027	0.455	3.050	0.572	0.464	0.519
SD	0.009	0.009	0.004	0.003	0.004					
CV	45	49	25	34	16					
N_{past}	5	0	0	0	9	0	0	0	0	0
$Median_{past}$	0.018				0.020					
SD_{past}	0.003				0.001					
N_{NIST}										
$Mean_{NIST}$										
S_{rep}										
S_{het}										
S_{NIST}										
NAV	0.019	0.018	0.016	0.008	0.024					
NAU										

Round Robin LXVII Laboratory Results

Analytes Reported By One Laboratory

Analyte	Code	362	363	364	365	366
Phytofluene, µg/mL	FSV-DA	0.100	0.187	0.098	0.031	0.121
Phytoene, µg/mL	FSV-DA	0.159	0.143	0.109	0.017	0.197
Retinyl Stearate, µg/mL	FSV-DA	0.029	0.034	0.009	*nd*	0.033
Ubiquinol, µg/mL	FSV-BW	1.00	1.02	0.58	0.85	0.81
Ubiquinone, µg/mL	FSV-BW	0.30	0.53	0.33	0.26	0.51

Term	Legend
N	Number of (non-NIST) quantitative values reported for this analyte
Min	Minimum (non-NIST) quantitative value reported
Median	Median (non-NIST) quantitative value reported
Max	Maximum (non-NIST) quantitative value reported
SD	Standard deviation for (non-NIST) results: 0.741*(3rd Quartile - 1st Quartile)
CV	Coefficient of Variation for (non-NIST) results: 100*SD/Median
N_{past}	Mean of N(s) from past RR(s)
$Median_{past}$	Mean of Median(s) from past RR(s)
SD_{past}	Pooled SD from past RR(s)
N_{NIST}	Number of units evaluated at NIST
$Mean_{NIST}$	Mean of NIST results
S_{rep}	NIST's within-vial pooled standard deviation
S_{het}	NIST's among-vial pooled standard deviation
S_{NIST}	Combined standard deviation for NIST analyses: $\sqrt{(S_{rep}^2 + S_{het}^2)}$
NAV	NIST Assigned Value = (Median + $Mean_{NIST}$)/2 for analytes reported by NIST analyst(s) = Median for analytes reported by ≥ 5 labs but not NIST
NAU	NIST Assigned Uncertainty: $\sqrt{(S^2 + S_{btw}^2)}$ S is the maximum of (0.05*NAV, SD, S_{NIST}, eSD) and S_{btw} is the standard deviation between Median and $Mean_{NIST}$. The expected long-term SD, eSD, is defined in: Duewer et al., Anal Chem 1997;69(7):1406-1413.
nd	Not detected (i.e., no detectable peak for analyte)
nq	Detected but not quantitatively determined
≤x	Concentration at or below the limit of quantification, x
≥x	Concentration greater than or equal to x
italics	Not explicitly reported but calculated by NIST from reported values

Round Robin LXVII Laboratory Results

Comparability Summary

Lab	TR	aT	g/bT	bC	tbC	aC	TLy	TbX	TLu	TZ	L&Z
FSV-BA	1	1	1	1	1	1	1	1			1
FSV-BB	1	1	1	2	2	2	1	1	1	2	1
FSV-BC	1										
FSV-BD	1	2									
FSV-BE	2	1	1	1							
FSV-BF	1	1		1							
FSV-BG	1	1	1	2		1	1	1			1
FSV-BH	1	1	1	1	1	1	1	2	1	1	1
FSV-BJ	1	1	1	1		2	1	1	1		
FSV-BK	2	1									
FSV-BL	1	1									
FSV-BM	1	1									
FSV-BN	1	2		1	2	2	1	1	1	1	1
FSV-BNa	1	2		2		3	1	1			1
FSV-BO	2	1	1	1		1	1	1	4	2	3
FSV-BP	1	1		1		1	1	1			1
FSV-BQ	4	4									
FSV-BR	2	2									
FSV-BS	2			2	2	1	2	2	1	1	1
FSV-BT	2	1	1	1	1	1	1	1	3	1	2
FSV-BU	2	2	2	1		1	1	1			1
FSV-BV	1	1	2	2		2	1	2			1
FSV-BW	1	1	1	1		2	1	1			1
FSV-CC	3	2									
FSV-CD	3	2	1	2		3	3	1			4
FSV-CE	1	2		2							
FSV-CF	1	2									
FSV-CG	1	2	1	1	1	1	1	2			2
FSV-CI	1	2	1	1		2			1	1	1
FSV-CW	1	2	3	1		1		1			1
FSV-CZ	1	4	1	1							
FSV-DA	1	1	1	1	1	1	1	1	1	1	1
FSV-DD	2										
FSV-DI	2	2	1	1			1		1		
FSV-DV	2	4									
FSV-EE	1	1									
FSV-EZ	2	1	1	2	2						
NIST	1	1	1	1	1		1				
n	38	35	20	26	10	19	19	18	10	8	18

	TR	aT	g/bT	bC	tbC	aC	TLy	TbX	TLu	TZ	L&Z
% 1	63	54	85	69	60	58	89	78	80	75	78
% 2	29	37	10	31	40	32	5	22	0	25	11
% 3	5	0	5	0	0	11	5	0	10	0	6
% 4	3	9	0	0	0	0	0	0	10	0	6

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

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

"Comparability Score"

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

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

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

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

NAU = NIST Assigned Uncertainty, our estimate of the overall measurement standard deviation for each sample. The estimate includes serum heterogeneity, analytical repeatability, and among-participant reproducibility variance components.

For further details, please see: Duewer DL, Kline MC, Sharpless KE, Brown Thomas J, Gary KT. Micronutrients Measurement Quality Assurance Program: Helping participants use interlaboratory comparison exercise results to improve their long-term measurement performance. Anal Chem 1999;71(9):1870-8.

Appendix D. Representative "Individualized Report" for RR67

Each participant in RR67 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 RR67:

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

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

Individualized Round Robin LXVII Report: FSV-BA

Summary

	Serum 362			Serum 363			Serum 364			Serum 365			Serum 366		
Analyte	You	NAV	n	You	NAV	n	You	NAV	n	You	NAV	n	You	NAV	n
Total Retinol	0.616	0.614	34	0.665	0.653	34	0.517	0.504	34	0.366	0.353	34	0.645	0.632	34
Retinyl Palmitate	0.11	0.08	11	0.2	0.1	11	0.1	0.0	9	0.02	0.01	7	0.12	0.10	11
α-Tocopherol	9.750	9.815	34	18.300	19.014	34	9.990	10.087	34	6.460	6.843	34	10.200	10.266	34
γ/β-Tocopherol	1.900	1.696	19	2.540	2.302	19	1.540	1.448	19	1.950	1.931	19	1.990	1.814	19
δ-Tocopherol	0.057	0.078	6	0.221	0.221	7	0.046	0.106	7	0.098	0.120	7	0.088	0.083	5
Total β-Carotene	0.117	0.117	23	0.395	0.413	23	0.246	0.239	23	0.091	0.102	23	0.118	0.117	23
trans-β-Carotene	0.105	0.105	9	0.374	0.361	9	0.232	0.206	9	0.079	0.086	9	0.108	0.108	9
Total cis-β-Carotene	0.012	0.006	6	0.021	0.036	7	0.014	0.034	7	0.012	0.014	6	0.010	0.008	6
Total α-Carotene	0.063	0.069	19	0.014	0.016	17	0.030	0.033	18	0.008	0.009	16	0.065	0.069	19
Total Lycopene	0.536	0.514	18	0.939	0.822	18	0.657	0.512	18	0.201	0.194	18	0.468	0.521	18
trans-Lycopene	0.266	0.258	9	0.415	0.513	9	0.298	0.316	9	0.110	0.134	9	0.278	0.278	9
Total β-Cryptoxanthin	0.057	0.051	18	0.042	0.023	18	0.057	0.042	18	0.050	0.045	18	0.058	0.052	18
Total α-Cryptoxanthin	0.025	0.025	5	0.019	0.018	5	0.023	0.024	5	0.017	0.018	5	0.024	0.026	5
Total Lutein&Zeaxanthin	0.095	0.095	18	0.146	0.150	18	0.119	0.125	18	0.093	0.100	18	0.108	0.108	18
25-hydroxyvitamin D	0.021	0.019	4	0.021	0.018	4	0.014	0.016	4	0.007	0.008	4	0.022	0.024	4

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

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

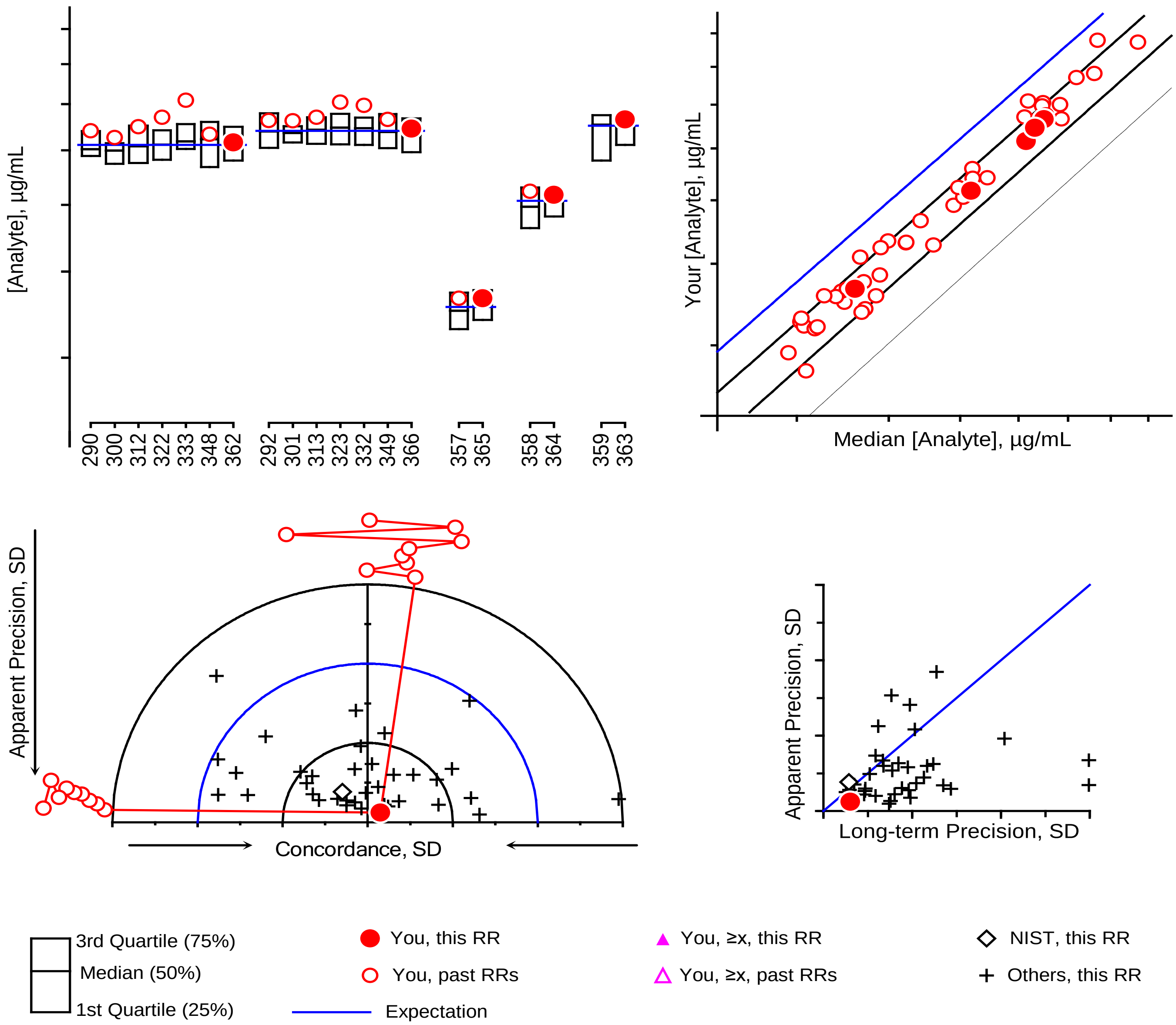
Micronutrients Measurement Quality Assurance Program
National Institute of Standards and Technology
100 Bureau Drive Stop 8392
Gaithersburg, MD 20899-8392 USA

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

D2

Individualized RR LXVII Report: FSV-BA

Total Retinol, µg/mL

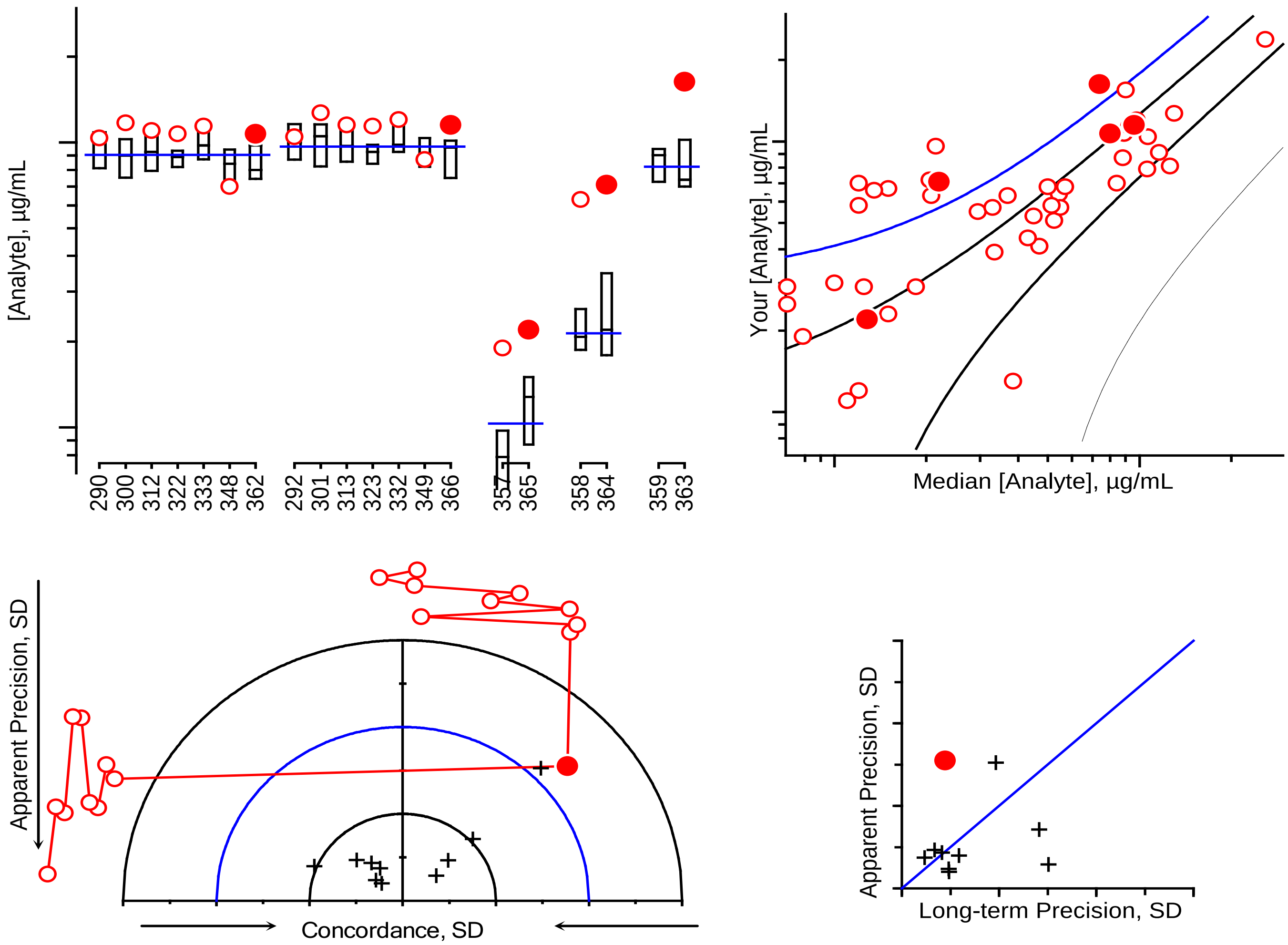


For details of the construction and interpretation of these plots, see:
Duewer, Kline, Sharpless, Brown Thomas, Gary, Sowell. Anal Chem 1999;71(9):1870-8.

Serum	History	Comments
#362	53#290, 55#300, 57#312, 59#322, 61#333, 64#348	Lyophilized, native, single-donor
#363	66#359	Fresh-frozen, native, single-donor
#364	66#358	Fresh-frozen, native, multi-donor
#365	66#357	Fresh-frozen, native, multi-donor
#366	53#292, 55#301, 57#313, 59#323, 61#332, 64#349	Fresh-frozen, native, single-donor

Individualized RR LXVII Report: FSV-BA

Retinyl Palmitate, µg/mL



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

You, this RR
You, past RRs
Expectation

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:
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Serum	History	Comments
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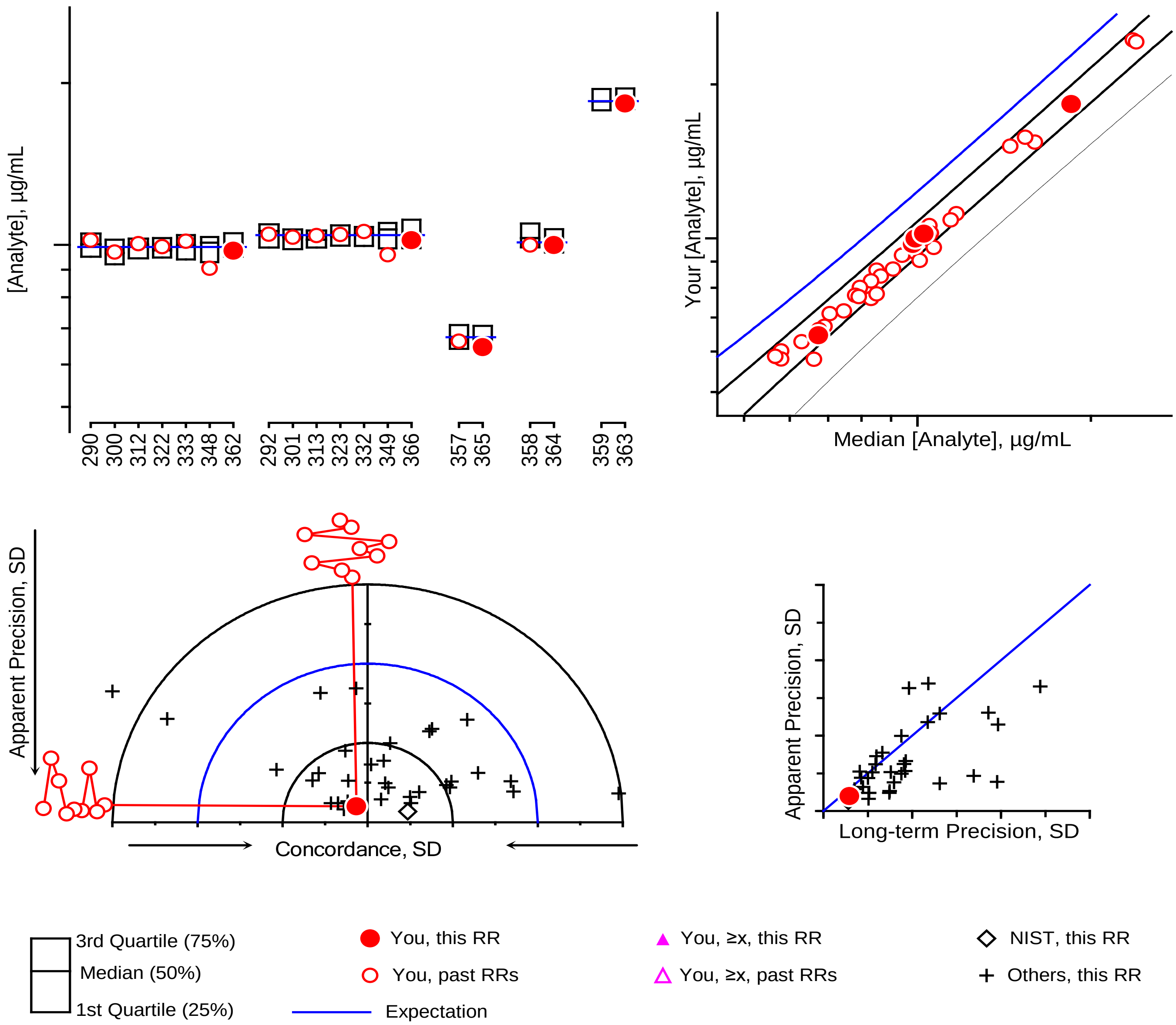
#364	66#358	Fresh-frozen, native, multi-donor
#365	66#357	Fresh-frozen, native, multi-donor
#366	53#292, 55#301, 57#313, 59#323, 61#332, 64#349	Fresh-frozen, native, single-donor

Individualized RR LXVII Report: FSV-BA

α-Tocopherol, µg/mL



For details of the construction and interpretation of these plots, see:
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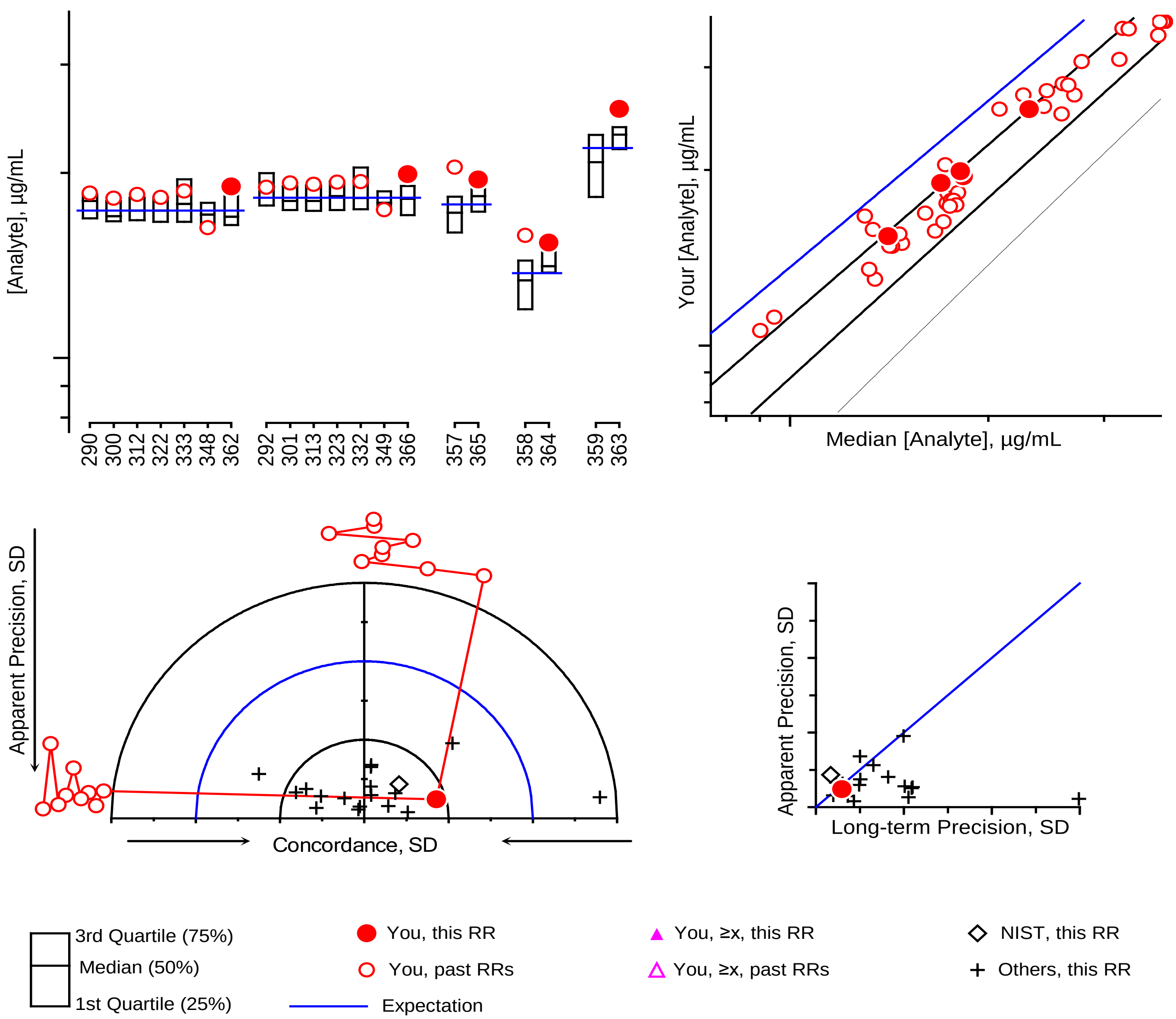
#365	66#357	Fresh-frozen, native, multi-donor
#366	53#292, 55#301, 57#313, 59#323, 61#332, 64#349	Fresh-frozen, native, single-donor

Individualized RR LXVII Report: FSV-BA

γ/β-Tocopherol, µg/mL



For details of the construction and interpretation of these plots, see:
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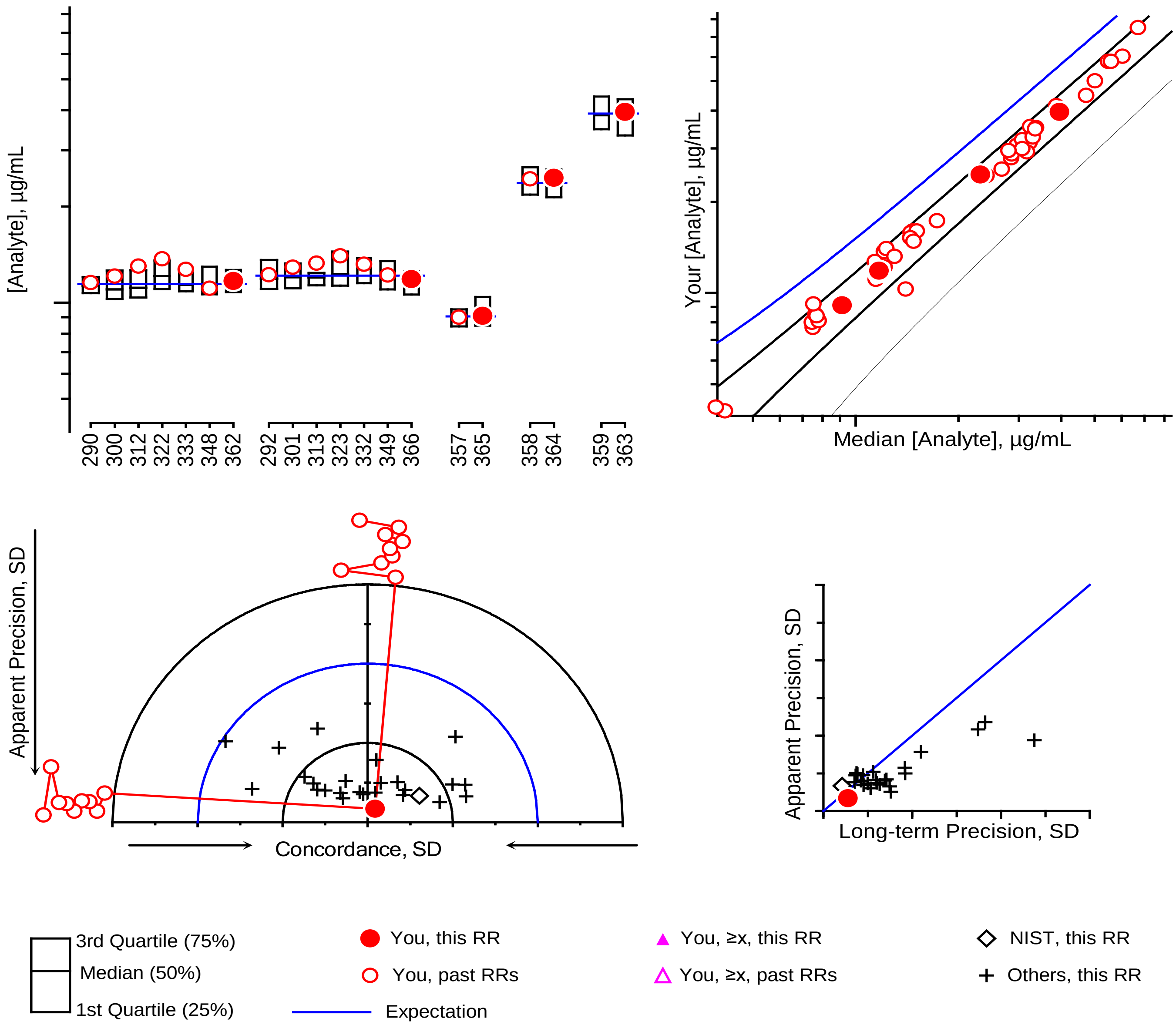
#365	66#357	Fresh-frozen, native, multi-donor
#366	53#292, 55#301, 57#313, 59#323, 61#332, 64#349	Fresh-frozen, native, single-donor

Individualized RR LXVII Report: FSV-BA

Total β-Carotene, µg/mL



For details of the construction and interpretation of these plots, see:
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Serum	History	Comments
#362	53#290, 55#300, 57#312, 59#322, 61#333, 64#348	Lyophilized, native, single-donor
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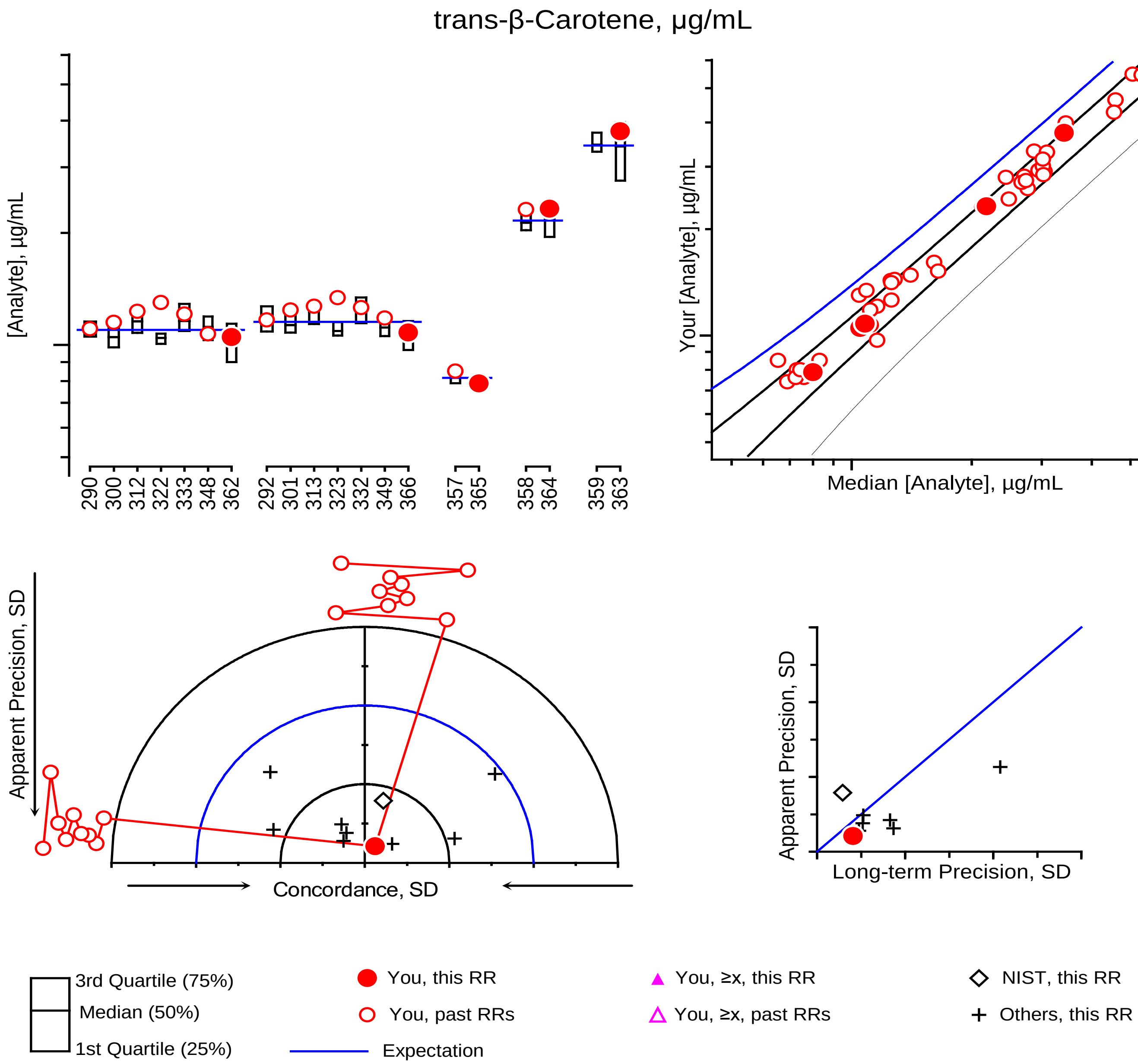
#364	66#358	Fresh-frozen, native, multi-donor
#365	66#357	Fresh-frozen, native, multi-donor
#366	53#292, 55#301, 57#313, 59#323, 61#332, 64#349	Fresh-frozen, native, single-donor

Individualized RR LXVII Report: FSV-BA



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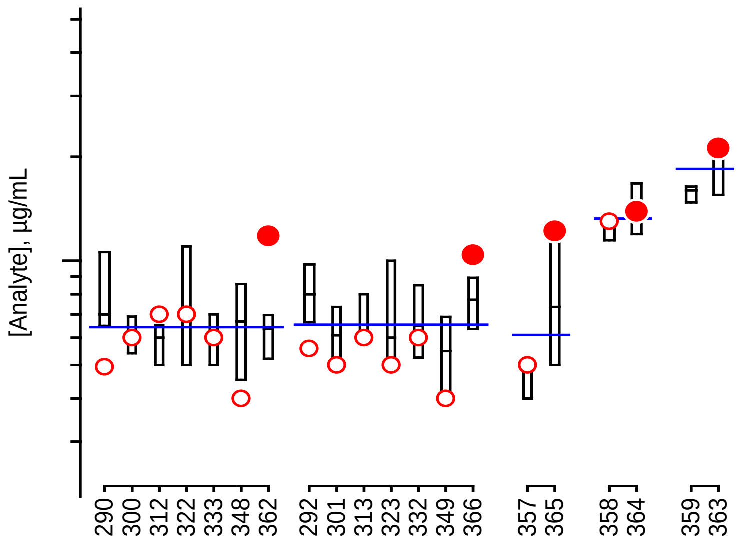
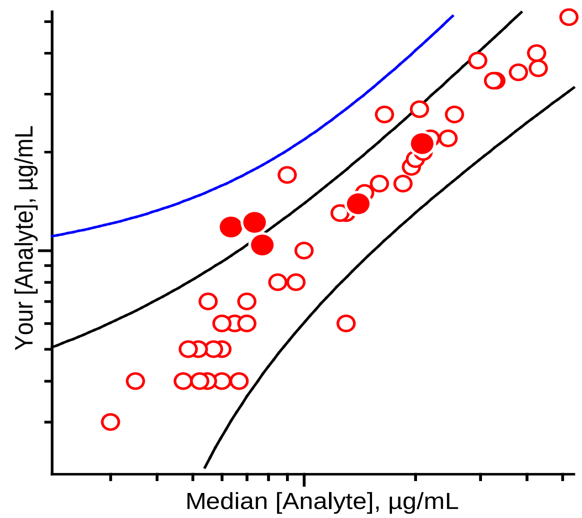
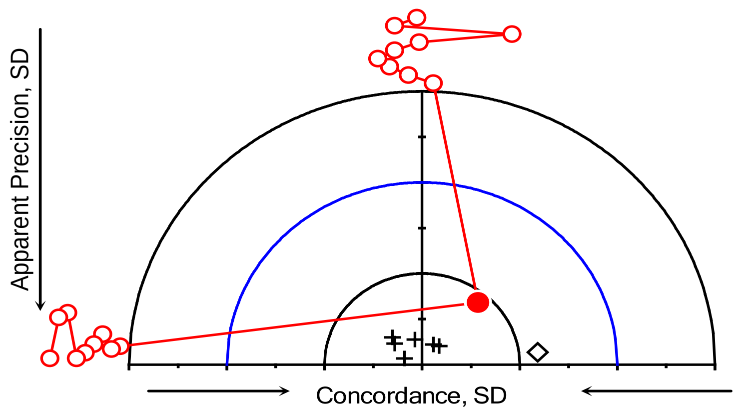
#366	53#292, 55#301, 57#313, 59#323, 61#332, 64#349	Fresh-frozen, native, single-donor

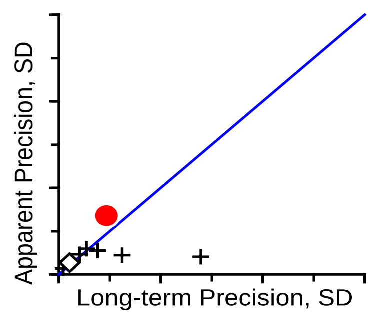
Individualized RR LXVII Report: FSV-BA

Total cis-β-Carotene, µg/mL









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

You, this RR
You, past RRs
Expectation

You, ≥x, this RR
You, ≥x, past RRs

NIST, this RR
Others, this RR

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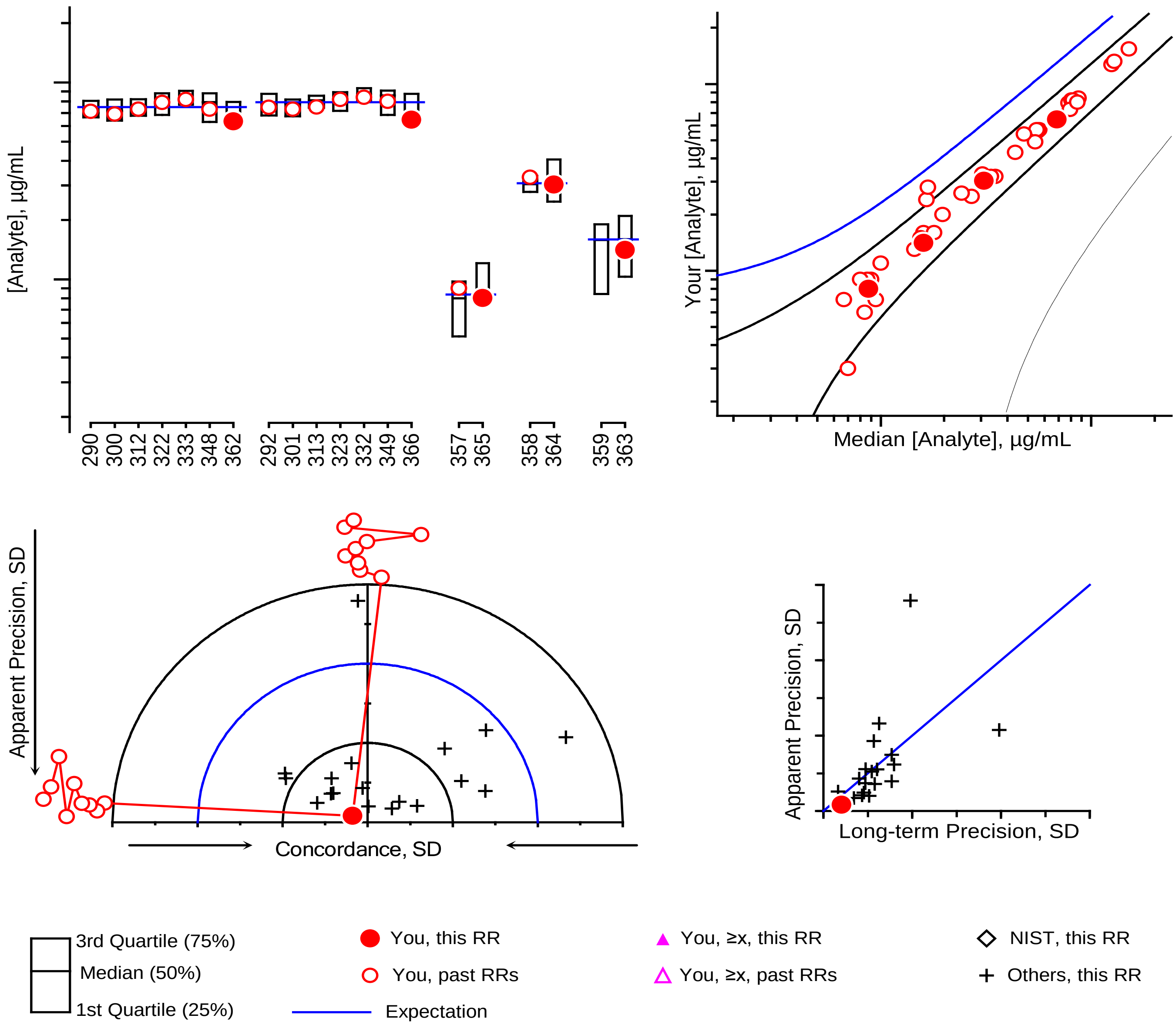
#366	53#292, 55#301, 57#313, 59#323, 61#332, 64#349	Fresh-frozen, native, single-donor

Individualized RR LXVII Report: FSV-BA

Total α-Carotene, µg/mL



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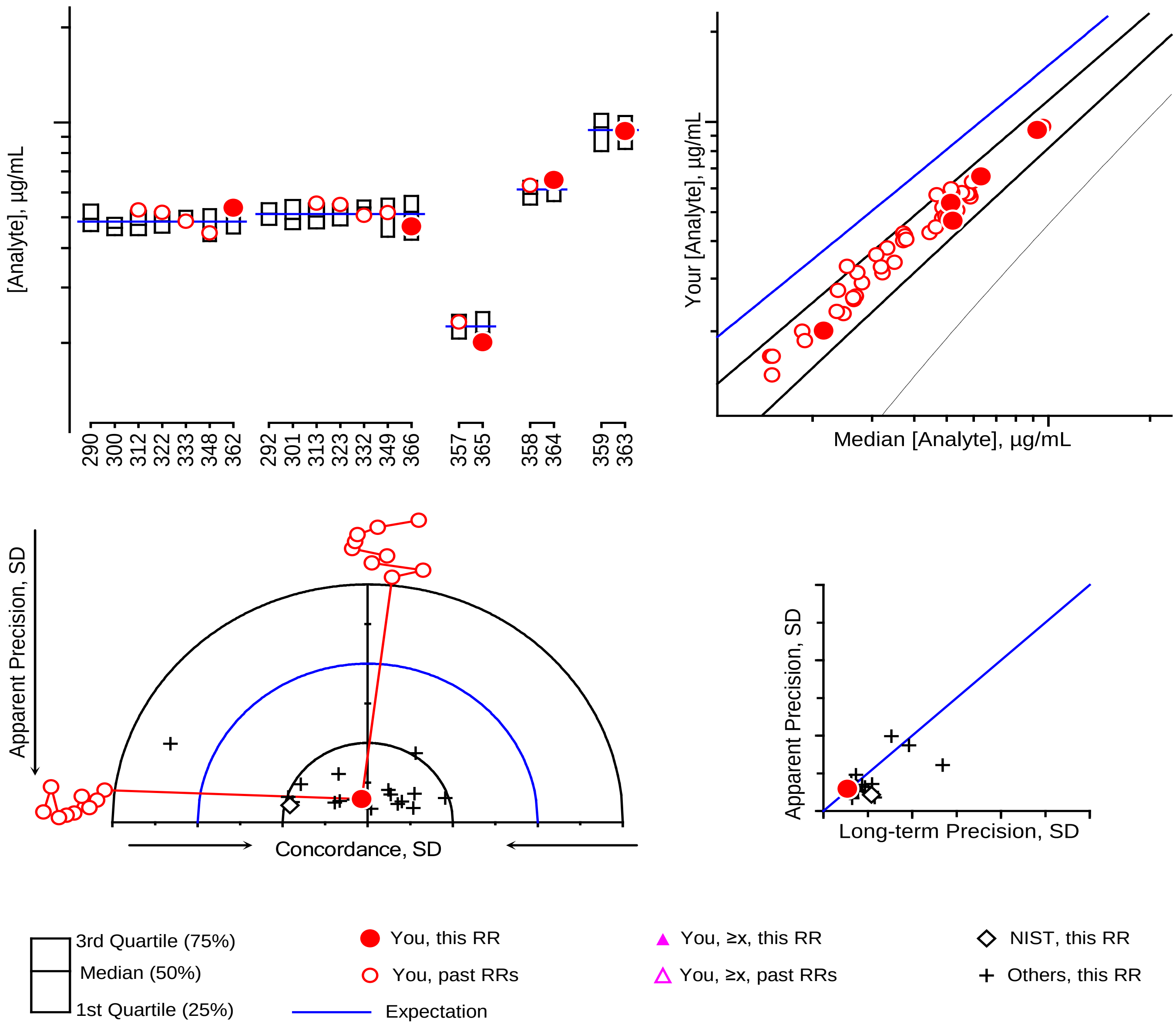
#362	53#290, 55#300, 57#312, 59#322, 61#333, 64#348	Lyophilized, native, single-donor
#363	66#359	Fresh-frozen, native, single-donor
#364	66#358	Fresh-frozen, native, multi-donor
#365	66#357	Fresh-frozen, native, multi-donor
#366	53#292, 55#301, 57#313, 59#323, 61#332, 64#349	Fresh-frozen, native, single-donor

Individualized RR LXVII Report: FSV-BA

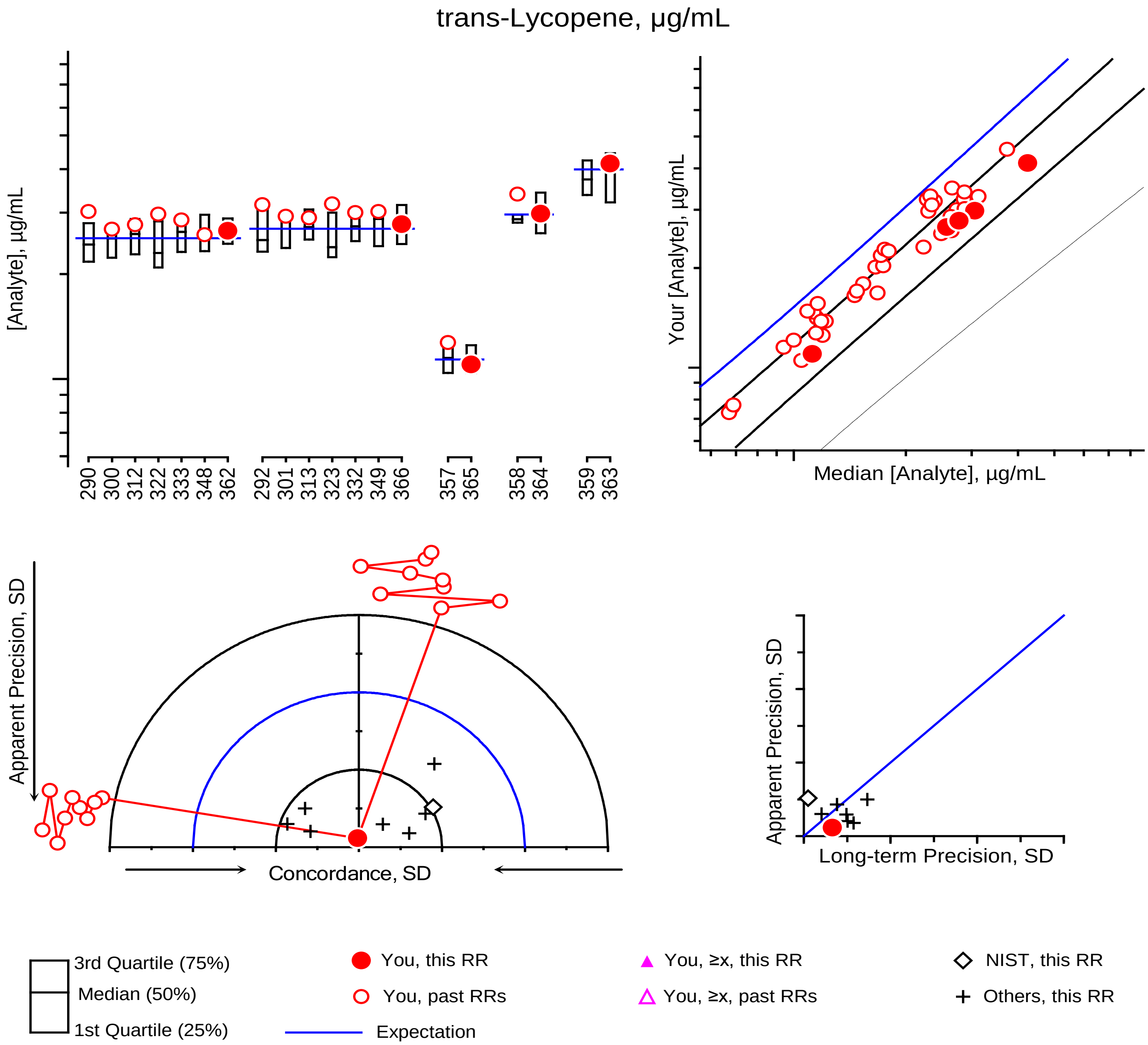
Total Lycopene, µg/mL



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
#362	53#290, 55#300, 57#312, 59#322, 61#333, 64#348	Lyophilized, native, single-donor
#363	66#359	Fresh-frozen, native, single-donor
#364	66#358	Fresh-frozen, native, multi-donor
#365	66#357	Fresh-frozen, native, multi-donor
#366	53#292, 55#301, 57#313, 59#323, 61#332, 64#349	Fresh-frozen, native, single-donor

Individualized RR LXVII Report: FSV-BA

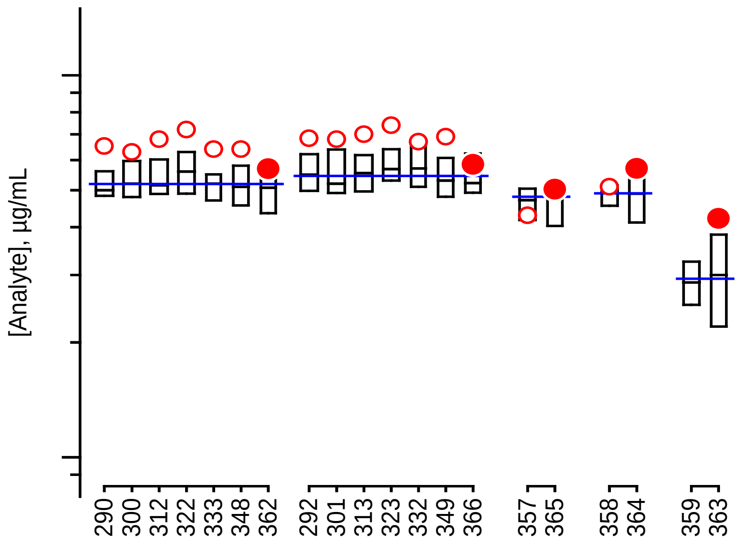


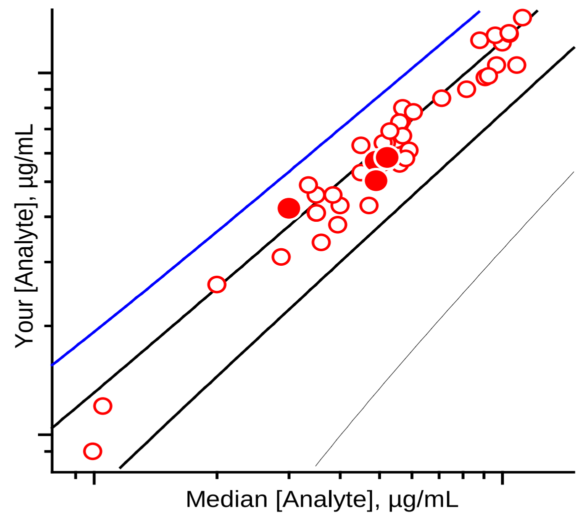
For details of the construction and interpretation of these plots, see:
Duewer, Kline, Sharpless, Brown Thomas, Gary, Sowell. Anal Chem 1999;71(9):1870-8.

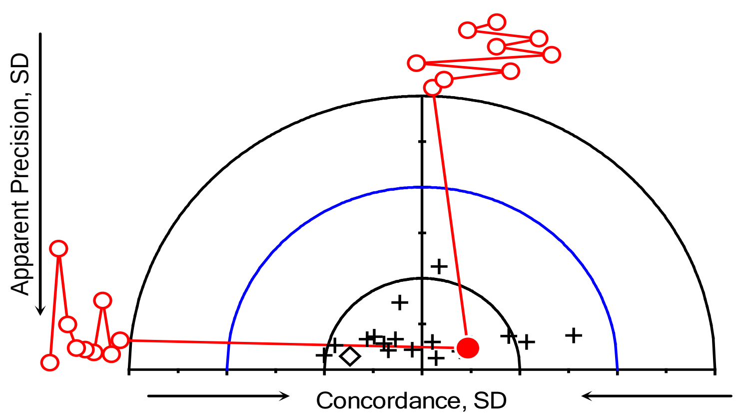
Serum	History	Comments
#362	53#290, 55#300, 57#312, 59#322, 61#333, 64#348	Lyophilized, native, single-donor
#363	66#359	Fresh-frozen, native, single-donor
#364	66#358	Fresh-frozen, native, multi-donor
#365	66#357	Fresh-frozen, native, multi-donor
#366	53#292, 55#301, 57#313, 59#323, 61#332, 64#349	Fresh-frozen, native, single-donor

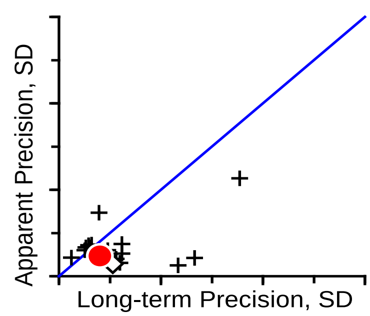
Individualized RR LXVII Report: FSV-BA

Total β-Cryptoxanthin, µg/mL









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

You, this RR
You, past RRs
Expectation

You, ≥x, this RR
You, ≥x, past RRs

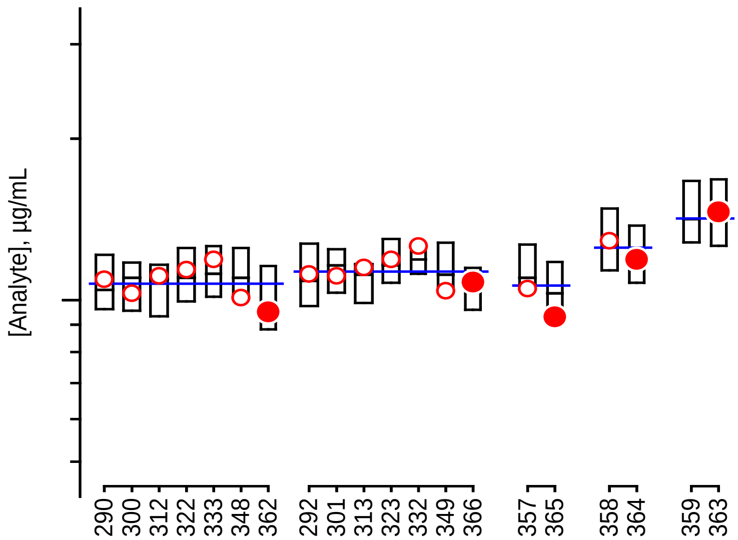
NIST, this RR
Others, this RR

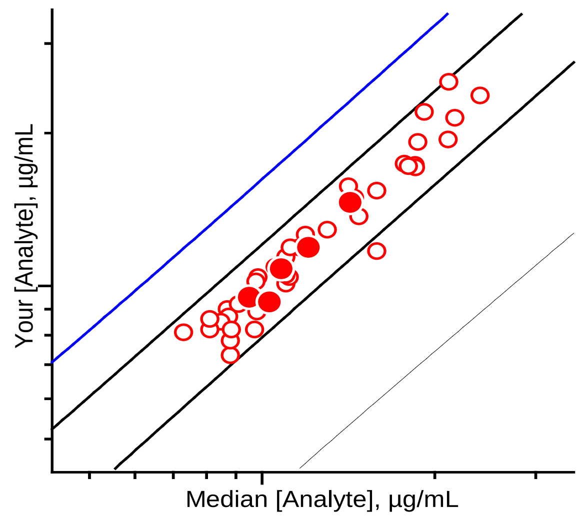
For details of the construction and interpretation of these plots, see:
Duewer, Kline, Sharpless, Brown Thomas, Gary, Sowell. Anal Chem 1999;71(9):1870-8.

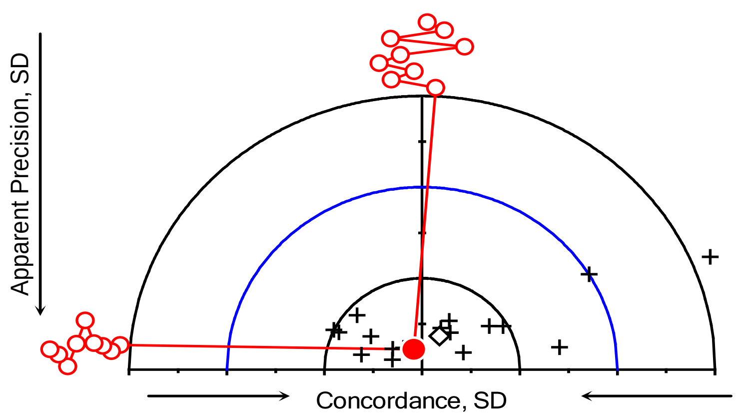
Serum	History	Comments
#362	53#290, 55#300, 57#312, 59#322, 61#333, 64#348	Lyophilized, native, single-donor
#363	66#359	Fresh-frozen, native, single-donor
#364	66#358	Fresh-frozen, native, multi-donor
#365	66#357	Fresh-frozen, native, multi-donor
#366	53#292, 55#301, 57#313, 59#323, 61#332, 64#349	Fresh-frozen, native, single-donor

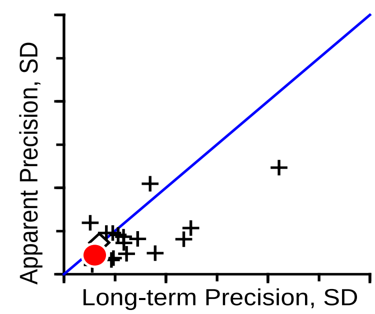
Individualized RR LXVII Report: FSV-BA

Total Lutein&Zeaxanthin, µg/mL









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

You, this RR
You, past RRs
Expectation

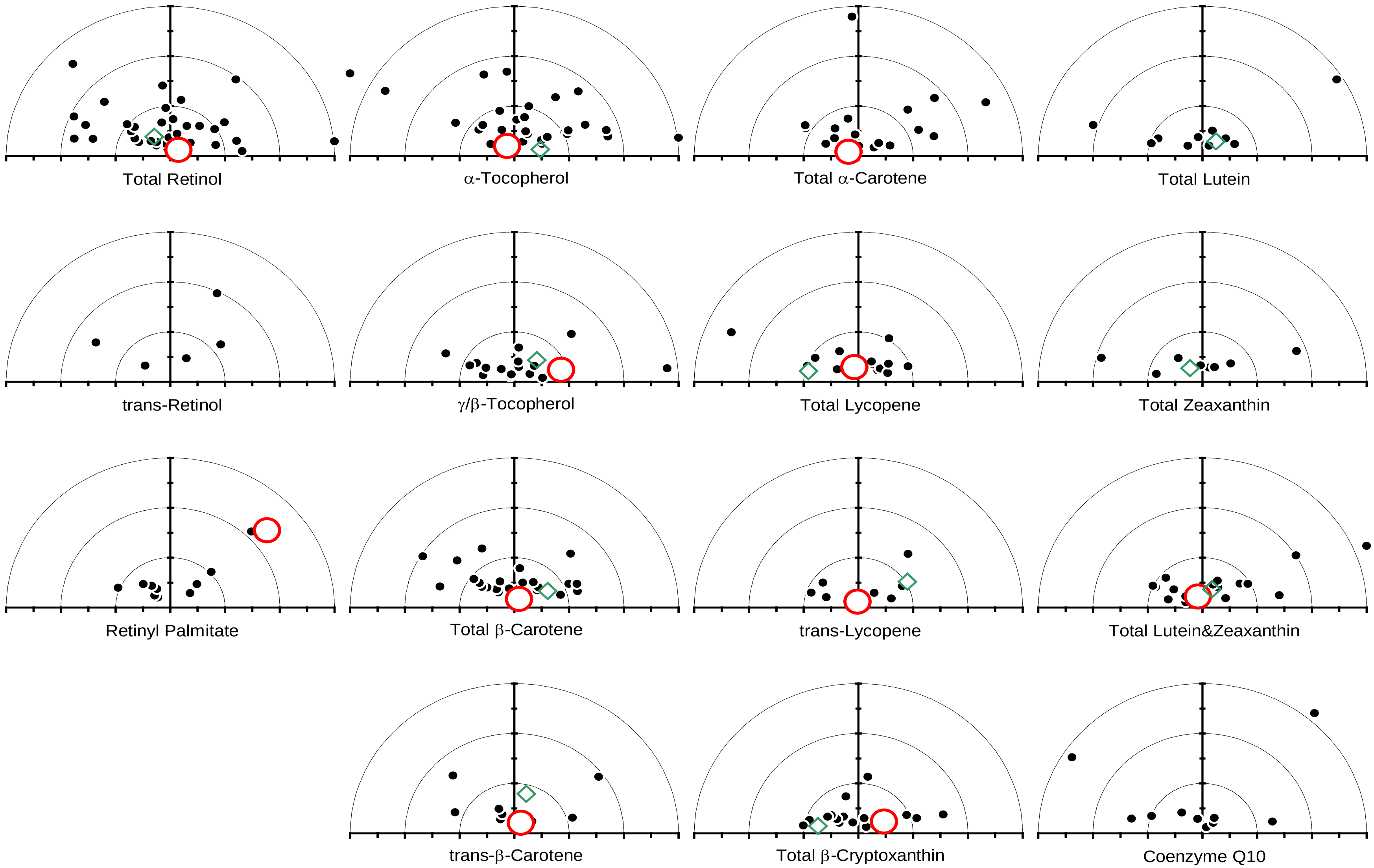
You, ≥x, this RR
You, ≥x, past RRs

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
#362	53#290, 55#300, 57#312, 59#322, 61#333, 64#348	Lyophilized, native, single-donor
#363	66#359	Fresh-frozen, native, single-donor
#364	66#358	Fresh-frozen, native, multi-donor
#365	66#357	Fresh-frozen, native, multi-donor
#366	53#292, 55#301, 57#313, 59#323, 61#332, 64#349	Fresh-frozen, native, single-donor

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



Appendix E. Shipping Package Inserts for RR32

The following five items were included in each package shipped to an RR32 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-

December 11, 2009

Dear Colleague:

The samples within this package constitute Vitamin C Round Robin 32 (RR32) of the 2010 Micronutrients Measurement Quality Assurance Program. RR32 consists of four vials of frozen serum *test samples* (#321, #322, #323, and #324), one vial of frozen *control serum* (CS #2), and one vial of ascorbic acid *solid control material* (Control). Please follow the attached protocols when you prepare and analyze these samples. If you cannot prepare the *solid control* solutions gravimetrically, please prepare equivalent solutions volumetrically and report the exact volumes used. (Routine 0.5 g gravimetric measurements are generally 10-fold more accurate than routine 0.5 mL volumetric measurements).

Please use the control serum to validate the performance of your measurement system before you analyze the *test samples*. The target value and ≈95% confidence interval for target value and ≈95% confidence interval for *CS #2* is 28.1 ±1.0 µmol/L of sample.

The report for RR31 was e-mailed in November. If you find your results for RR31 unsatisfactory, we recommend that you obtain Standard Reference Material (SRM) 970 Ascorbic Acid in Serum to validate your methodology and value assign in-house control materials. This SRM may be purchased from the Standard Materials Reference Program at NIST (Tel: 301-975-6776, Fax: 301-948-3730, or e-mail: srminfo@nist.gov).

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

If you have any questions or concerns about the Vitamin C Micronutrients Measurement Quality Assurance Program please contact Jeanice Brown Thomas at phone: 301-975-3120, fax: 301-977-0685, or e-mail: jbthomas@nist.gov.

We ask that you return your results for these RR32 samples by **March 15, 2010**. We would appreciate receiving your results as soon as they become available. Please use the attached form. Your results will be kept confidential.

Sincerely,

Jeanice Brown Thomas

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

Enclosures: Protocols, Preparation and Analysis of Control Materials and Analysis of Test Samples
RR32 Report Form for Ascorbic Acid Solid Control Material Preparation
RR32 Report Form for Control Material and Test Sample Analyses

Micronutrient Measurement Quality Assurance Program for
Vitamin C

Please Read Through Completely BEFORE Analyzing Samples

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

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

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

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

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

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

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

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

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

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

   For example, if you prepared the Stock Solution with 0.2000 g of solid ascorbic acid and 103.0 g of Diluent, then 0.5 mL of the Stock Solution should weigh (0.2+103)/200 = 0.52 g and $[\mathrm{TAA}]_{DS1}$ = (0.52 g)(0.2 g)·(56785 µmol/g·L)/(0.2 + 103 g) = 57.2 µmol/L. Likewise, 0.25 mL of the Stock Solution should weigh 0.26 g and $[\mathrm{TAA}]_{DS2}$ = 29.4 µmol/L and 0.125 mL should weigh 0.13 g and $[\mathrm{TAA}]_{DS3}$ = 14.2 µmol/L.

5) Measure the ultraviolet absorbance spectrum of Dilute Solution 1 against the Diluent as the blank using paired 1 cm path length cuvettes. Record the absorbance at 242, 243, 244, and 245 nm. Record the maximum absorbance (A_{max}) within this region. Record the wavelength (λ_{max}) at which this maximum occurs.

   The extinction coefficient ($E^{1\%}$) of ascorbic acid at λ_{max} (using a cell with a 1 cm path length) of Dilute Solution #1 can be calculated:

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

If your spectrophotometer is properly calibrated, λ_{max} should be between 243 and 244 nm and $E^{1\%}$ should be 550 ± 30 dL/g·cm. If they are not, you should recalibrate the wavelength and/or absorbance axes of your spectrophotometer and repeat the measurements.

6) Measure and record the concentration of total ascorbic acid in all three dilute solutions and in the 5% MPA Diluent in duplicate using <u>*exactly*</u> the same method that you will use for the serum control materials and test samples, including any enzymatic treatment. We recommend that you analyze these solutions in the following order: Diluent, Dilute Solution 1, Dilute Solution 2, Dilute Solution 3, Dilute Solution 3, Dilute Solution 2, Dilute Solution 1, Diluent.

   a) Compare the values of the duplicate measurements. *Are you satisfied that your measurement precision is adequate?*

   b) Compare the measured with the calculated [TAA] values. This is most conveniently done by plotting the measured values on the y-axis of a scatterplot against the calculated values on the x-axis. The line through the four {calculated, measured} data pairs should go through the origin with a slope of 1.0. *Are you satisfied with the agreement between the measured and calculated values?*

   Do <u>**not**</u> analyze the serum control materials or test samples until you are satisfied that your system is performing properly!

7) Once you have confirmed that your system is properly calibrated, analyze the serum control CS #2 (see protocol below). The target values for this materials is 28.1 ±1.0 µmol/L of sample.

   If your measured values are not close to this value, please review your sample preparation procedure and whether you followed *exactly* the same measurement protocol the solutions prepared from the solid control material as you used for these serum controls. If the protocols differ, please repeat from Step 6 using the proper protocol. If the proper protocol was used, your measurement system may not be suitable for MPA-preserved samples; please contact us at 301-975-3120 or jbthomas@NIST.gov.

   Do <u>**not**</u> analyze the test samples until you are satisfied that your system is performing properly and is suitable for the analysis of MPA-preserved serum!

Protocol for Analysis of the Serum Control Materials and Test Samples

The *serum control material* and *test samples* are in sealed ampoules. They were prepared by adding equal volumes of 10% MPA to spiked human serum. We have checked the samples for stability and homogeneity. Only the total ascorbic acid is stable. While these samples contain some dehydroascorbic acid, its content is variable. Therefore, only <u>total ascorbic acid</u> should be reported. The *serum control material* and *test samples* should be defrosted by warming at 20 °C for not more than 10 min otherwise some irreversible degradation may occur.

Each *serum test sample* contains between 0.0 and 80.0 µmol of total ascorbic acid/L of solution. The total ascorbic acid in each ampoule should be measured in duplicate. Please report your results in <u>µmol/(L of the sample solution)</u> rather than µmol/(L of serum NIST used to prepare the sample).

Participant #: _______ Date: __________

Vitamin C Round Robin 32

NIST Micronutrient Measurement Quality Assurance Program

Preparation and Validation of Ascorbic Acid Solid Control Material

STOCK SOLUTION

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

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

DILUTE SOLUTION 1

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

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

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

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

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

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

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

Wavelength of maximum absorbance... __________ nm

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

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

DILUTE SOLUTION 2

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

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

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

DILUTE SOLUTION 3

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

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

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

Please return by **March 15, 2010**

MMQAP
100 Bureau Drive, Stop 8392
Gaithersburg, MD 20899-8392

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

Participant #: _______ Date: __________

Vitamin C Round Robin 32

NIST Micronutrient Measurement Quality Assurance Program

Analysis of Control Materials and Test Samples

Sample	Replicate 1	Replicate 2	Units
Dilute Solution 1			μmol/L of Dilute Solution
Dilute Solution 2			μmol/L of Dilute Solution
Dilute Solution 3			μmol/L of Dilute Solution
5% MPA Diluent			μmol/L of Diluent
CS #1			μmol/L of Sample *Target:* 8.4 ±1.8 μmol/L
Serum Test Sample #321			μmol/L of Sample
Serum Test Sample #322			μmol/L of Sample
Serum Test Sample #323			μmol/L of Sample
Serum Test Sample #324			μmol/L of Sample

Were samples frozen upon receipt? Yes | No

Analysis method: HPLC-EC | HPLC-Fluor DAB | HPLC-OPD | HPLC-UV | AO-OPD | Other
If "Other", please describe:

COMMENTS:

Please return by **March 15, 2010**

MMQAP
100 Bureau Drive, Stop 8392
Gaithersburg, MD 20899-8392

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

Participant #: ________ Date: ____________

Vitamin C Round Robin 32

NIST Micronutrients Measurement Quality Assurance Program

Packing List and Shipment Receipt Confirmation Form

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

Label	Form
VitC #321	Liquid frozen (1:1 serum:10% MPA)
VitC #322	Liquid frozen (1:1 serum:10% MPA)
VitC #323	Liquid frozen (1:1 serum:10% MPA)
VitC #324	Liquid frozen (1:1 serum:10% MPA)
CS #2	Liquid frozen (1:1 serum:10% MPA)
Control	Solid AA

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

1) Date this shipment arrived: ___________________________

2) Are all of the vials intact? Yes | No

If "No", which one(s) were damaged?

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

4) Did the samples arrive frozen? Yes | No

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

6) When do you anticipate analyzing these samples? ________________________

Your prompt return of this information is appreciated.

The M^{2}QAP Gang

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

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

Appendix F. Final Report for RR32

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

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

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

April 27, 2010

Dear Colleague:

Enclosed is the summary report for Round Robin 32 (RR32) for the measurement of total ascorbic acid (TAA, ascorbic acid plus dehydroascorbic acid) in human serum. Included in this report are a summary of data for all laboratories and an individualized summary of your laboratory's measurement performance. The robust median is used to estimate the consensus value for all samples, the "median absolute deviation from the median" (MADe) is used to estimate the expected standard deviation, and the coefficient of variation (CV) is defined as 100×MADe/median.

RR32 consisted of four *test samples* (#321, #322, #323, and #324), one *serum control material* (CS#2), and one *solid control material* for preparation of TAA control solutions. Details regarding the samples can be found in the enclosed report.

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

Samples for the second vitamin C round robin (RR33) of the 2010 Micronutrients Measurement Quality Assurance Program (M^2QAP) will be shipped **during the week of June 7, 2010.**

If you have questions or concerns regarding this report, please contact David Duewer at 301-975-3935; e-mail: david.duewer@nist.gov or me at 301-975-3120; e-mail: jbthomas@nist.gov; or fax: 301-977-0685.

Sincerely,

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

David L. Duewer
Research Chemometrician
Analytical Chemistry Division
Chemical Science and Technology

Enclosures

Cc: L. C. Sander

NIST

The NIST M^2QAP Vitamin C Round Robin 32 (RR32) report consists of

Page	"Individualized" Report
1	Summarizes your reported values for the nominal 55 mmol/L solution you prepared from the ascorbic acid solid control sample, the serum control sample, and the four serum test samples.
2	Graphical summary of your RR32 sample measurements.

Page	"All Lab" Report
1	A tabulation of results and summary statistics for Total Ascorbic Acid [TAA] in the RR32 samples and control/calibration solutions.

Serum-based Samples. One serum control and four unknowns were distributed in RR32.

CS#2 SRM 970 level 2, ampuled in mid-1998.

S32:1 Serum 321, ampuled in late 2001, previously distributed as: S17:1 (RR17, Fall 02), S19:2 (RR19, Fall 03), S21:2 (RR21, Fall 04), S22:1 (RR22, Spring 05), S24:1 (RR24, Spring 06), S28:1 (RR28, Spring 07), S30:1 (RR30, Spring 08).

S32:2 Serum 322, ampuled in Fall 2009, initial distribution

S32:3 Serum 323, ampuled in Fall 2009, initial distribution

S32:4 Serum 324, ampuled in Fall 2009, initial distribution

Results.

1) All participants who prepared the four 5% MPA control/calibration solutions (the three "Dilute Solutions" and the "Diluent") did so correctly. The criteria used to evaluate this success are: the density of the 5% MPA (≈1.03 gm/mL), the observed wavelength maximum of "Dilute Solution #1"(≈244 nm), the observed absorbance at that maximum (≈0.58 OD), the calculated $E^{1\%}$ #1"(≈560 dL/g·cm).

2) The Measured = a+b*Gravimetric calibration parameters for the control/calibration solutions (columns 10 to 13 of the All Lab Report) indicate that the measurement systems for all participants are linear (R^2 close to 1 and RMS close to 0.0) and reasonably well calibrated (intercepts range from -0.5 to 1.7 and slopes range from 0.92 to 1.07).

3) The Measured = p+q*Median regression parameters for samples S32:1 to S32:4 confirm the linearity of most measurement systems (R^2 close to 1 and RMS close to 0.0).

4) There is no evidence of sample degradation in the CS#2 (ampuled in 1998) or S32:1 (ampuled in 2001) materials.

5) This was the first distribution of the S32:2, S32.3, and S32:4 materials. There were no reported issues in their analysis. The observed 8 % and 5 % relative standard deviations for the S32:2 and S32:3 are at or lower than the 8 % expected from past studies; the 11% is higher than expected but well "within the noise" given the number of participants.

Appendix G. "All-Lab Report" for RR32

The following single page is the "All-Lab Report" as provided to all participants, with two exceptions:

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

The data summary in the "All-Lab Report" has been altered to ensure confidentiality of identification codes assigned to laboratories.

Micronutrients Measurement Quality Assurance Program for Total Ascorbic Acid
"Round Robin" 32 - March 2010

		Control / Calibration Samples											MPA	Dilute Solution 1			Samples								
		Grav, µmol/L			Measured, µmol/L				Measured = a + b*Grav				Density	Spectrophotometry			Measured, µmol/L					Measured = p+q*Median			
Lab	Date	Dil:1	Dil:2	Dil:3	Dil:1	Dil:2	Dil:3	MPA	Inter	Slope	R^2	RMS	g/mL	λ_{max}	A_{max}	$E^{1\%}$	CS#2	S32:1	S32:2	S32:3	S32:4	Inter	Slope	R^2	RMS
VC-MA	24/02/10	59.0	29.8	14.8	60.9	31.5	15.8	0.0	0.34	1.03	1.000	0.5	1.034	242.	0.5660	545.1	28.8	9.2	26.1	33.1	48.8	-0.40	1.07	0.999	0.7
VC-MB	13/01/10	56.7	28.0	14.2	57.6	32.0	15.0	0.0	0.94	1.02	0.995	2.1	1.031	244.	0.5560	556.4	28.4	9.3	22.5	30.7	45.7	-0.51	0.99	0.998	0.8
VC-MC	25/03/10	58.3	29.3	14.5	57.5	28.0	14.2	0.0	-0.21	0.98	1.000	0.5	1.023	243.	0.5643	549.5	28.9	9.6	25.3	31.7	49.7	-0.93	1.08	0.998	0.8
VC-ME	02/03/10	57.2	28.5	14.5	57.4	28.8	14.4	0.0	-0.01	1.00	1.000	0.1	1.032	244.	0.5552	551.3	26.0	8.3	19.0	25.6	37.9	0.35	0.81	0.998	0.6
VC-MG	25/02/10	58.7	29.6	14.7	62.5	30.7	14.9	0.0	-0.45	1.07	1.000	0.5	1.028	243.7	0.5890	570.0	28.3	8.7	23.5	30.1	47.7	-1.69	1.05	0.998	0.9
VC-MH	07/02/10	62.1	31.6	14.8	61.8	31.0	14.8	0.0	-0.07	0.99	1.000	0.3	1.031	244.1	0.6121	559.9	28.1	9.7	24.0	31.5	46.0	0.44	0.99	1.000	0.3
VC-MI	25/02/10	55.3	27.7	13.9	56.6	27.5	14.5	0.0	-0.05	1.02	1.000	0.6	1.030				28.5	8.9	24.1	33.2	50.7	-2.37	1.14	0.999	0.8
VC-MJ	10/03/10	61.5	30.7	15.4	63.4	32.6	17.0	1.7	1.67	1.00	1.000	0.2	1.019	254a	0.373a	344.5a	31.3	14.4	29.9	37.0	51.8	5.18	1.01	0.999	0.5
VC-MN	05/03/10	58.5	28.4	14.0	54.2	27.0	12.8	0.0	0.06	0.93	1.000	0.5	1.029	243.6	0.6181	599.6	28.2	11.8	24.6	30.8	42.2	4.37	0.83	0.998	0.7
VC-MP	20/01/10																28.2	9.0	24.3	31.1	46.3	-0.32	1.01	1.000	0.3
VC-MU	18/02/10																25.6	*b*	19.0	25.3	40.0	-3.94	0.95	1.000	0.3
VC-NE	10/02/10	57.6	29.0	14.2	58.3	28.8	14.2	0.0	-0.16	1.01	1.000	0.3	1.034	244.	0.5840	575.2	28.8	9.6	25.4	33.6	50.1	-0.80	1.10	1.000	0.2
VC-NF	08/03/10	56.6	28.5	14.0	58.8	30.4	14.5	0.2	0.23	1.04	1.000	0.5	1.030	243.	0.5610	562.4	27.7	9.8	23.3	30.0	45.4	0.39	0.96	0.999	0.5
N		11	11	11	11	11	11	11					11	9	9	9	13	12	13	13	13				
Average		58.3	29.2	14.5	59.0	29.8	14.7	0.2					1.029	243.5	0.5784	563.3	28.2	9.9	23.9	31.0	46.3				
SD		2.0	1.2	0.5	2.8	1.9	1.0	0.5					0.004	0.7	0.0238	16.7	1.4	1.7	2.8	3.1	4.2				
Min		55.3	27.66	13.9	54.2	26.97	12.8	0.0					1.019	242.0	0.5552	545.1	25.6	8.3	19.0	25.3	37.9				
%25		57.0	28.46	14.1	57.4	28.38	14.3	0.0					1.028	243.0	0.5610	551.3	28.1	9.0	23.3	30.1	45.4				
Median		58.3	29.04	14.5	58.3	30.41	14.5	0.0					1.030	243.7	0.5660	559.9	28.3	9.4	24.1	31.1	46.3				
%75		58.8	29.68	14.8	61.3	31.26	14.9	0.0					1.032	244.0	0.5890	570.0	28.8	9.7	25.3	33.1	49.7				
Max		62.1	31.60	15.4	63.4	32.62	17.0	1.7					1.034	244.1	0.6181	599.6	31.3	14.4	29.9	37.0	51.8				
MADe / eSD		1.7	0.9	0.5	2.5	2.4	0.5	0.0					0.003	0.4	0.0160	15.0	0.8	0.6	1.8	1.5	5.1				
CV		3	3	3	4	8	4						0.29	0.18	2.8	2.7	3	6	8	5	11				

a) 5% Trichloroacetic acid solution
b) Mislabeled sample

Appendix H. Representative "Individualized Report" for RR32

Each participant in RR32 received an "Individualized Report" reflecting their reported results. The following two pages are the "Individualized Report" for participant "VC-MA".

Vitamin C "Round Robin" 32 Report: Participant VC-MA

Date	RR	Method	MPA Density g/mL	Dilute Solution 1 Spectrophotometry λ_{max}	A_{max}	$E^{1\%}$	Control/Calibration Solutions Y_{meas} = Inter + Slope* X_{grav} Inter	Slope	R^2	SEE
10/05/07	27	HPLC-EC	1.032	242.0	0.561	557.2	-0.1	0.99	1.000	0.14
03/04/08	28	HPLC-EC	1.035	243.0	0.572	562.2	0.7	1.03	0.999	0.99
08/11/08	29	HPLC-EC	1.037	243.0	0.567	553.2	0.3	1.03	1.000	0.64
03/03/09	30	HPLC-EC	1.037	242.0	0.569	555.6	0.2	1.03	1.000	0.40
09/10/09	31	HPLC-EC	1.036	244.0	0.566	546.1	-0.1	1.02	1.000	0.20
02/24/10	32	HPLC-EC	1.035	242.0	0.566	545.1	0.3	1.03	1.000	0.46
		Mean	1.035	242.7	0.57	553.2			Pooled SEE	0.55
		SD	0.002	0.8	0.00	6.6				
		CV	0.16	0.34	0.6	1.2				

[TAA] mmol/Lsample

Date	RR	Sample	Rep_1	Rep_2	F_{adj}	Mean	SD_{dup}	N	Mean	SD_{repeat}	SD_{reprod}
02/23/04	20	CS#2	25.8	26.2	1.0	26.0	0.3	10	28.4	0.5	1.7
09/13/04	21	CS#2	26.2	27.2	1.0	26.7	0.7				
03/08/05	22	CS#2	29.0	29.0	1.0	29.0	0.0				
10/17/05	23	CS#2	29.4	30.5	1.0	30.0	0.8				
03/09/06	24	CS#2	29.2	29.1	1.0	29.2	0.1				
08/28/06	25	CS#2	27.2	28.1	1.0	27.6	0.6				
10/05/07	27	CS#2	28.1	27.4	1.0	27.7	0.5				
08/11/08	29	CS#2	27.2	27.2	1.0	27.2	0.0				
09/10/09	31	CS#2	31.8	32.2	1.0	32.0	0.3				
02/24/10	32	CS#2	28.6	29.0	1.0	28.8	0.3				
12/12/02	17	S17:1	9.9	9.1	1.0	9.5	0.6	8	9.5	0.3	0.5
11/13/03	19	S19:2	9.2	9.1	1.0	9.2	0.1				
09/13/04	21	S21:2	8.8	8.7	1.0	8.7	0.1				
03/08/05	22	S22:1	9.6	9.6	1.0	9.6	0.0				
03/09/06	24	S24:1	9.8	9.6	1.0	9.7	0.2				
03/04/08	28	S28:1	10.4	10.3	1.0	10.4	0.1				
03/03/09	30	S30:1	9.5	9.4	1.0	9.4	0.1				
02/24/10	32	S32:1	9.5	8.9	1.0	9.2	0.5				
02/24/10	32	S32:2	26.2	26.0	1.0	26.1	0.1				
02/24/10	32	S32:3	33.4	32.8	1.0	33.1	0.5				
02/24/10	32	S32:4	49.0	48.6	1.0	48.8	0.3				

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

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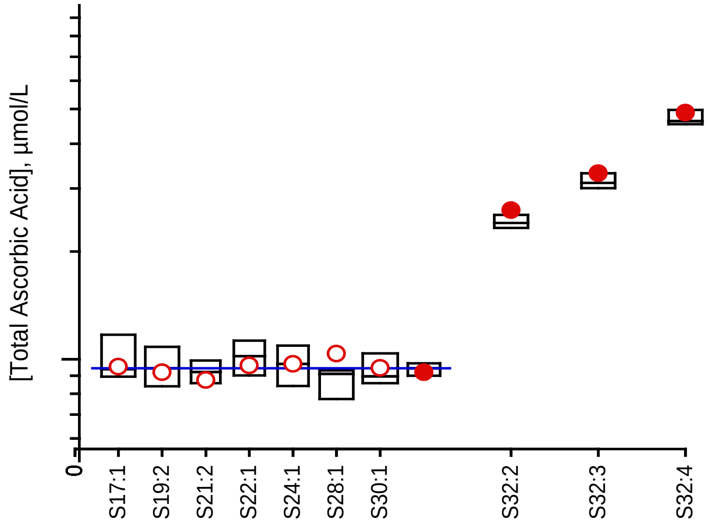
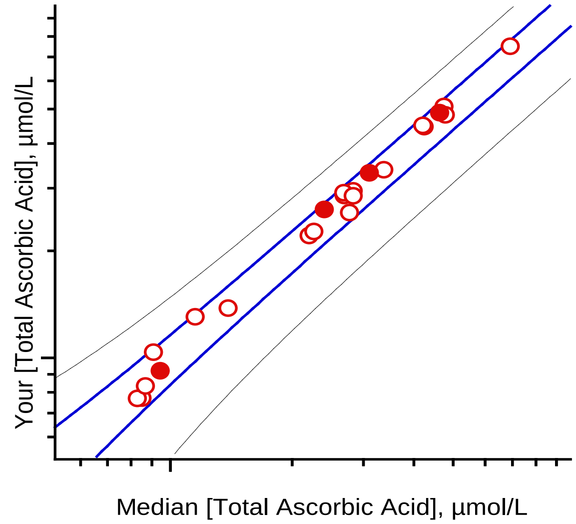
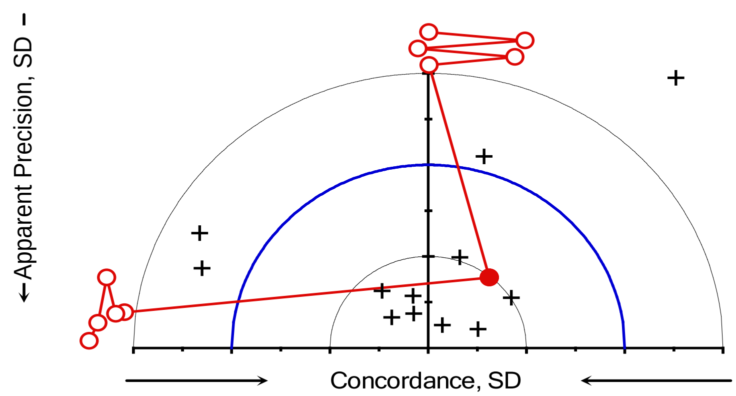
Vitamin C "Round Robin" 32 Report: Participant VC-MA

Total Ascorbic Acid, µmol/mL







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

You, this RR
You, past RRs

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.

Sample	Comments
S32:1	VitC #321 previously distributed in RRs 17, 19, 21, 22, 24, 28, 30
S32:2	VitC #322, first distribution
S32:3	VitC #323, first distribution
S32:4	VitC #324, first distribution