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NIST Micronutrients Measurement Quality Assurance Program Winter 2008 Comparability Studies

Results for Round Robin LXIII
Fat-Soluble Vitamins and Carotenoids in Human Serum
and Round Robin 28 Ascorbic Acid in Human Serum

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National Institute of Standards and Technology
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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 2008 MMQAP measurement comparability improvement studies: 1) Round Robin LXIII Fat-Soluble Vitamins and Carotenoids in Human Serum and 2) Round Robin 28 Total Ascorbic Acid in Human Serum. The materials for both studies were shipped to participants in November 2007; participants were requested to provide their measurement results by March 3, 2008.

Keywords

Human Serum
Retinol, α-Tocopherol, γ-Tocopherol, Total and *Trans*-β-Carotene
SRM 968d, SRM 1950
Total Ascorbic Acid

Table of Contents

Introduction

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

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

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

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

Participants in the MMQAP Fat-Soluble Vitamins and Carotenoids in Human Serum Round Robin LXIII comparability study (hereafter referred to as RR63) received eight liquid-frozen human serum test samples for analysis. Unless multiple vials were previously requested, participants received one vial of each serum. These sera were shipped on dry ice to participants in November 2007. 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 RR63 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 28: Vitamin C in Human Serum

Participants in the MMQAP Vitamin C in Human Serum Round Robin 28 comparability study (hereafter referred to as RR28) 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 November 2007. 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 RR28 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 RR63

The following three items were included in each package shipped to an RR63 participant:

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

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

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

November 5, 2007

Dear Colleague:

Enclosed are the samples (Sera 339-346) for the first fat-soluble vitamins and carotenoids in serum round robin study (Round Robin LXIII) for the fiscal year 2008 NIST Micronutrients Measurement Quality Assurance Program. You will find one vial of each of eight 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 a value is obtained below your limit of quantification, please indicate this result on the form by using NQ *(Not Quantified)*. Results are due to NIST by **March 3, 2008**. Results received more than two weeks after the due date will not be included in the summary report for this round robin study.

To ensure adequate mixing of the samples, we recommend 3 to 5 min agitation in an ultrasonic bath or about 10 min at room temperature with intermittent swirling. (CAUTION: Vigorous shaking will cause foaming and possibly interfere with accurate measurement. The rubber stopper contains phthalate esters that may leach into the sample upon intermittent contact of the liquid sample with the stopper. These esters absorb strongly in the UV region and elute near retinol in most LC systems creating analytical problems.) Pipette a known volume of serum from the vial for analysis. **Water should not be added to the liquid-frozen samples.**

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); lycopene, 3450 at 472 nm (hexane).

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

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

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

Sincerely,

Jeanice B Thomas

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

Enclosures

NIST

Participant #: ________ Date: ____________

Round Robin LXIII: Human Sera

NIST Micronutrients Measurement Quality Assurance Program

Analyte	339	340	341	342	343	344	345	346	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									
total lycopene									
trans-lycopene									
total β-cryptoxanthin									
total α-cryptoxanthin									
total lutein									
total zeaxanthin									
total lutein&zeaxanthin									
total coenzyme Q10									
phylloquinone (K_1)									
25-hydroxyvitamin D									

Other measurands?									

* we prefer **µg/mL**

Comments:

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

Please return results **before** March 3, 2008

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

Participant #: ________ Date: ____________

Fat-Soluble Vitamins Round Robin LXIII

NIST Micronutrients Measurement Quality Assurance Program

Packing List and Shipment Receipt Confirmation Form

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

Serum	Form	Reconstitute?	Vial & Cap
#339	Liquid frozen	No	2 mL amber, green
#340	Liquid frozen	No	2 mL amber, silver
#341	Liquid frozen	No	10 mL amber, silver
#342	Liquid frozen	No	10 mL amber, red
#343	Liquid frozen	No	10 mL amber, green
#344	Liquid frozen	No	10 mL amber, silver
#345	Liquid frozen	No	10 mL amber, red
#346	Liquid frozen	No	10 mL amber, green

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 eight 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 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 RR63

The following four 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-

May 2, 2008

Dear Colleague:

Enclosed is the summary report of the results for round robin LXIII (RR63) of the 2008 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.

We apologize for distributing four samples, sera #342, 343, 345, and 346 that proved to be quite heterogeneous in β-carotene. We have elected to exclude *all* results for these sera from further analysis, on the basis of possible heterogeneity in other analytes and that the observed low levels of most analytes in these materials are amply represented by the other RR63 samples. Sera {342, 345} and {343, 346} were each duplicates of two different serum pools, the first from the beginning of their bottling runs and the second from the end.

The "All Lab" and "Individualized" reports present results only for the other four RR63 samples, Sera 339, 340, 341 and 343. Your overall measurement comparability is summarized in the "Score Card" summary, page 5 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 2008 QA interlaboratory exercise will be shipped **starting the week of May 19, 2008.** We will send you a reminder via e-mail or fax a week prior to shipment. It is critical that you carefully inspect all samples upon arrival and that you promptly confirm to us that they have arrived. We will replace samples (lost or damaged in shipment or miss-packaged by us) only for participants who report the problem within one calendar week after the package arrives.

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

Sincerely,

Jeanice B Thomas

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

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

NIST

The NIST M^2QAP Round Robin LXI (RR63) report consists of:

Page	"All Lab" Report
1-3	A listing of all results and statistics for all analytes.
4	A legend for the list of results and statistics.
5	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. Eight samples were distributed in RR63.

Serum	Description	Prior Distributions
339	Fresh-frozen, native, single-donor serum prepared at NIST in Spring, 2006.	#326:RR60-9/06, #331:RR61-3/07, #338:RR62-9/07
340	Fresh-frozen, native, multi-donor plasma commercially prepared in Spring 2007.	
341	Fresh-frozen, native, multi-donor serum prepared in Fall, 2007. Vial from beginning of bottling run.	
342	Fresh-frozen, augmented in α-tocopherol and β-carotene, multi-donor serum prepared in Fall, 2007. Vial from beginning of bottling run.	Heterogeneous in (at least) β-carotene. Results not used.
343	Fresh-frozen; augmented in retinol, α-tocopherol, γ-tocopherol, and β-carotene; multi-donor serum prepared in Fall, 2007. Vial from beginning of bottling run.	Heterogeneous in (at least) β-carotene. Results not used.
344	Duplicate of #341; vial from end of bottling run.	
345	Duplicate of #342; vial from end of bottling run.	Results not used.
346	Duplicate of #343; vial from end of bottling run.	Results not used.

Results

1) Sample Heterogeneity: Figure 1 plots the ratio of the observed standard deviation (SD, estimated from the median absolute deviation from the median) to the SD expected from past results. The variability of β-carotene in sera {342,345} and {344,346} is many times that expected. While the variability of α-tocopherol is greater than expected for all of the RR63 samples, it is perhaps significantly higher for the augmented {342,345} and {344,346} than it is for the three native materials. There is no clear heterogeneity signal for the other analytes, but several (retinol,

γ/β-tocopherol, β-cryptoxanthin, and lutein&zeaxanthin) are equivocal. While the commercial preparer had achieved apparently homogenous β-carotene augmentation in several trial samples, it is clear that they were unable to scale up their process. The β-carotene in sera {342,345} and {344,346} appears to be largely in the form of globules of various size. These globules quite plausibly may have differentially absorbed some of other spiked compounds and/or the target analytes native to the materials. Given the generally low native levels of the target analytes in sera {342,345} and {344,346}, we chose to disregard *all* results for these samples.

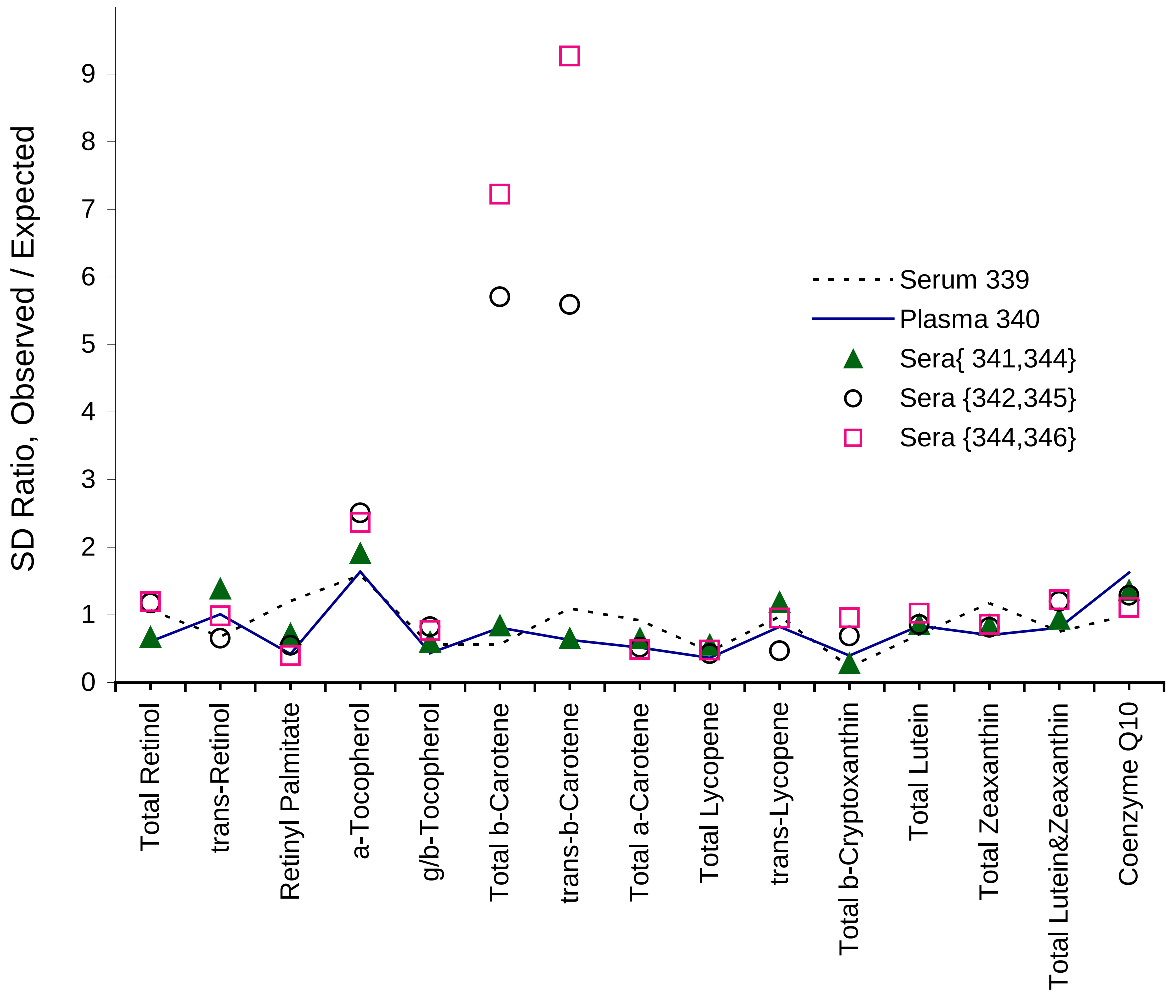


Figure 1: Heterogeneity Analysis, Observed SD / Expected SD,
for Analytes Having Seven or More Reported Results

2) Sera Stability. There was no significant change in the median level of any measurand in the fresh-frozen serum #339. There was no significant change in the measurement variability of any measurand in this material, with the possible exception of α-tocopherol. Since the α-tocopherol variability in plasma 340 and sera {341, 344} was also above historical expectations, this more likely reflects changes in the measurement processes used rather than the sample materials. We intend to explore in greater detail the results from the recent past to see if this increased variability is a "once off" or an evolving trend.

3) Plasma vs. serum. Plasma #340 is Candidate SRM 1950, Metabolites in Human Plasma. There were no analysis problems reported for this material. Your results will contribute to establishing reference and information values for the “Fat Soluble” measurands in this material. We hope to make this material available as an SRM soon.

4) Sera {341,344}: The sera {341, 344} material was intended to be the “Low Level” component of SRM 968d Fat-Soluble Vitamins and Carotenoids in Human Serum. Unfortunately, the intended “Middle” and “High” level components proved to be quite unacceptable. While resource constraints have delayed developing replacement materials for this quite high-priority SRM, we intend to make the {341,344} “Low” material available in some form. From our evaluation of the entire bottling run and from your evaluations of samples from the first and last of the run, the serum pool for Sera 341 and 344 appears to be adequately homogenous in all measurands. One laboratory, however, indicated that the level of α-tocopherol for these sera was near their limit of quantification. We will continue to look into this issue.

Appendix C. "All-Lab Report" for RR63

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 LXIII Laboratory Results

Lab	Total Retinol, µg/mL 339	340	341	344	trans-Retinol, µg/mL 339	340	341	344	Retinyl Palmitate, µg/mL 339	340	341	344
FSV-BA	0.330	0.430	0.349	0.363					0.058	0.022	0.030	0.029
FSV-BB	0.295	0.413	0.345	0.338					0.010	0.013	0.007	0.007
FSV-BC	0.331	0.459	0.361	0.361								
FSV-BD	0.300	0.405	0.339	0.308								
FSV-BE	0.305	0.423	0.339	0.336								
FSV-BF	0.280	0.430	0.350	0.330								
FSV-BG	0.298	0.409	0.348	0.348					0.009	0.012	0.009	0.008
FSV-BH	0.334	0.378	0.309	0.275								
FSV-BI	0.288	0.401	0.336	0.327								
FSV-BJ	0.280	0.402	0.326	0.336								
FSV-BK	0.372	0.509	0.408	0.412								
FSV-BL	0.230	0.320	0.260	0.260								
FSV-BM	0.326	0.392	0.331	0.359								
FSV-BN	0.289	0.388	0.308	0.313					0.019	0.026	0.014	0.016
FSV-BO	0.306	0.441	0.433	0.398								
FSV-BP	0.333	0.431	0.357	0.337								
FSV-BQ	0.344	0.465	0.379	0.394								
FSV-BR	*≥0.300*	*≥0.440*	*≥0.310*	*≥0.280*	0.300	0.440	0.310	0.280				
FSV-BS	*≥0.285*	*≥0.368*	*≥0.309*	*≥0.313*	0.285	0.368	0.309	0.313				
FSV-BT	*≥0.272*	*≥0.437*	*≥0.384*	*≥0.314*	0.272	0.437	0.384	0.314				
FSV-BU	0.301	0.428	0.317	0.354								
FSV-BV	0.291	0.422	0.339	0.345								
FSV-BW	0.310	0.430	0.350	0.360					0.009	0.013	0.006	0.004
FSV-CC	0.325	0.420	0.353	0.334	0.312	0.405	0.351	0.322				
FSV-CD	0.320	0.430	0.350	0.360								
FSV-CE	0.314	0.434	0.354	0.359								
FSV-CF	0.380	0.489	0.393	0.399								
FSV-CG	0.307	0.413	0.339	0.322								
FSV-CI	*≥0.303*	*≥0.455*	*≥0.363*	*≥0.381*	0.303	0.455	0.363	0.381	0.026	0.018	0.017	0.016
FSV-CW	0.258	0.485	0.353	0.385					0.010	0.016	0.007	*nq*
FSV-CZ	0.430	0.566	0.459	0.449								
FSV-DD	0.270	0.380	0.310	0.300								
FSV-DI	0.256	0.374	0.297	0.301					0.032	0.010	0.010	0.024
FSV-DQ												
FSV-DV	*≥0.282*	*≥0.371*	*≥0.288*	*≥0.287*	0.282	0.371	0.288	0.287				
FSV-EE	0.279	0.370	0.305	0.271								
FSV-EZ	*≥0.230*	*≥0.404*	*≥0.537*	*≥0.420*	0.230	0.404	0.537	0.420				
N	29	29	29	29	7	7	7	7	8	8	8	7
Min	0.230	0.320	0.260	0.260	0.230	0.368	0.288	0.280	0.009	0.010	0.006	0.004
Median	0.306	0.423	0.348	0.345	0.285	0.405	0.351	0.314	0.014	0.015	0.010	0.016
Max	0.430	0.566	0.459	0.449	0.312	0.455	0.537	0.420	0.058	0.026	0.030	0.029
SD	0.031	0.024	0.017	0.025	0.018	0.038	0.047	0.038	0.013	0.005	0.006	0.009
CV	10	6	5	7	6	9	14	12	92	32	60	58
N_{past}	32	0	0	0	5	0	0	0	8	0	0	0
$Median_{past}$	0.307				0.305				0.021			
SD_{past}	0.025				0.030				0.019			
NIST	0.295	0.407	0.331	0.331								
N_{NIST}	3	10	14	14								
S_{rep}	0.008	0.011	0.011	0.011								
S_{het}	0.011	0.005	0.007	0.007								
S_{NIST}	0.013	0.012	0.013	0.013								
NAV	0.301	0.415	0.342	0.342	0.285	0.405	0.351	0.351	0.014	0.015	0.010	0.010
NAU	0.032	0.036	0.030	0.030	0.024	0.038	0.047	0.047	0.013	0.011	0.011	0.011

Lab	α-Tocopherol, µg/mL 339	340	341	344	γ/β-Tocopherol, µg/mL 339	340	341	344	δ-Tocopherol, µg/mL 339	340	341	344
FSV-BA	8.25	8.01	5.87	6.02	3.65	1.77	1.48	1.55	0.089	0.085	0.081	0.086
FSV-BB	8.34	8.14	5.96	5.78	3.33	1.66	1.38	1.35	0.093	0.073	0.084	0.085
FSV-BC												
FSV-BD	8.90	8.80	7.40	6.10								
FSV-BE	7.68	7.61	5.08	5.01	3.83	1.88	1.54	1.49				
FSV-BF	8.80	9.20	4.80	2.80	3.70	2.00	1.50	1.30				
FSV-BG	8.34	7.89	5.96	5.95	3.76	1.73	1.54	1.48				
FSV-BH	7.87	7.65	5.53	5.25	3.29	1.58	1.31	1.26				
FSV-BI	8.13	8.00	5.86	5.91	3.66	1.83	1.48	1.58				
FSV-BJ	8.81	8.65	6.25	6.36	3.64	1.78	1.41	1.45				
FSV-BK	8.91	8.59	5.90	6.14								
FSV-BL	7.30	7.30	5.20	5.20								
FSV-BM	8.10	8.40	6.10	6.30								
FSV-BN	8.33	7.47	4.80	5.33	3.67	1.81	1.53	1.57				
FSV-BO	9.20	8.50	6.70	6.20	3.70	1.90	1.50	1.60				
FSV-BP	7.45	6.74	4.56	5.14								
FSV-BQ	7.74	7.14	5.65	5.24								
FSV-BR	8.30	7.40	*nq*	*nq*								
FSV-BS	7.73	7.60	5.32	12.17	2.29	1.41	1.15	1.96				
FSV-BT	7.30	6.54	4.75	4.95	3.02	1.31	1.10	1.13				
FSV-BU	8.50	8.82	5.30	5.51	3.58	1.67	1.30	1.29				
FSV-BV	8.80	8.55	6.15	6.44	3.61	1.79	1.46	1.50				
FSV-BW	8.46	8.29	5.75	5.90	3.59	1.67	1.55	1.52				
FSV-CC	10.05	9.47	6.62	6.16								
FSV-CD	9.45	9.13	6.27	6.37	3.71	1.77	1.43	1.44				
FSV-CE	7.57	7.51	4.83	5.05								
FSV-CF	9.10	9.00	6.60	6.40								
FSV-CG	7.70	7.65	1.13	*nd*	3.70	1.86	1.43	1.35	*nd*	*nd*	0.143	0.135
FSV-CI	7.56	7.60	5.09	5.76	3.20	1.89	1.25	1.38				
FSV-CW	10.75	10.48	6.35	5.82	5.14	2.46	1.96	1.89	0.130	0.150	0.130	0.120
FSV-CZ	5.28	6.21	2.73	2.93	2.58	1.00	0.77	0.81				
FSV-DD												
FSV-DI	8.85	8.94	6.55	6.48	3.25	1.70	1.40	1.36	0.068	0.075	0.077	0.075
FSV-DQ	7.63	7.15	4.35	4.99	3.98	1.83	1.32	1.49				
FSV-DV	8.60	7.80	4.70	4.80								
FSV-EE	8.50	8.50	5.70	5.70								
FSV-EZ	8.23	7.52	5.41	5.99	3.69	1.73	1.43	1.59				
N	35	35	34	33	23	23	23	23	4	4	5	5
Min	5.28	6.21	1.13	2.80	2.29	1.00	0.77	0.81	0.07	0.07	0.08	0.08
Median	8.33	8.00	5.68	5.82	3.65	1.77	1.43	1.48	0.09	0.08	0.08	0.09
Max	10.75	10.48	7.40	12.17	5.14	2.46	1.96	1.96	0.13	0.15	0.14	0.14
SD	0.81	0.82	0.92	0.71	0.29	0.13	0.14	0.16	0.01	0.02	0.04	0.03
CV	10	10	16	12	8	7	10	11	15	25	43	30
N_{past}	34	0	0	0	22	0	0	0	5	0	0	0
$Median_{past}$	8.486				3.673				0.086			
SD_{past}	0.514				0.322				0.076			
NIST	8.62	7.95	6.33	6.33	3.71	1.82	1.47	1.47				
N_{NIST}	3	10	14	14	3	10	14	14				
S_{rep}	0.105	0.208	0.245	0.245	0.052	0.045	0.044	0.044				
S_{het}	0.088	0.263	0.225	0.225	0.019	0.024	0.046	0.046				
S_{NIST}	0.136	0.335	0.332	0.332	0.055	0.051	0.064	0.064				
NAV	8.475	7.977	5.941	5.941	3.682	1.796	1.461	1.461	0.091	0.080	0.084	0.084
NAU	0.835	0.820	0.923	0.923	0.339	0.192	0.164	0.164			0.036	0.036

C2

Round Robin LXIII Laboratory Results

	Total β-Carotene, µg/mL				trans-β-Carotene, µg/mL				Total cis-β-Carotene, µg/mL			
Lab	339	340	341	344	339	340	341	344	339	340	341	344
FSV-BA	0.148	0.077	0.080	0.085	0.141	0.074	0.076	0.080	0.006	0.004	0.004	0.005
FSV-BB	0.133	0.080	0.083	0.081	0.126	0.074	0.077	0.075	0.007	0.006	0.005	0.006
FSV-BC												
FSV-BD												
FSV-BE	0.159	0.076	0.072	0.065								
FSV-BF												
FSV-BG	0.153	0.086	0.087	0.088								
FSV-BH	0.114	0.063	0.059	0.055	0.114	0.063	0.059	0.055	*nq*	*nq*	*nq*	*nq*
FSV-BI	0.123	0.068	0.075	0.075								
FSV-BJ	0.150	0.082	0.085	0.084								
FSV-BK												
FSV-BL												
FSV-BM												
FSV-BN	0.147	0.070	0.071	0.076	0.139	0.065	0.063	0.067	0.005	0.003	0.004	0.005
FSV-BO	*≥0.166*	*≥0.083*	*≥0.088*	*≥0.105*	0.166	0.083	0.088	0.105				
FSV-BP	0.150	0.060	0.060	0.070								
FSV-BQ												
FSV-BR												
FSV-BS	*≥0.117*	*≥0.069*	*≥0.077*	*≥0.073*	0.117	0.069	0.077	0.073				
FSV-BT	0.124	0.080	0.104	0.077	0.114	0.073	0.091	0.068	0.010	0.007	0.013	0.009
FSV-BU	0.141	0.068	0.079	0.079								
FSV-BV	0.148	0.075	0.078	0.078								
FSV-BW	0.167	0.077	0.064	0.064								
FSV-CC												
FSV-CD	0.220	0.190	0.190	0.170								
FSV-CE	0.116	0.056	0.064	0.059								
FSV-CF												
FSV-CG	0.135	0.075	0.074	0.071	0.126	0.068	0.074	0.070	0.009	0.007	*nd*	*nd*
FSV-CI	0.136	0.060	0.067	0.083								
FSV-CW	0.112	0.059	0.040	0.056								
FSV-CZ	0.157	0.116	0.104	0.108								
FSV-DD												
FSV-DI	0.133	0.050	0.067	0.067								
FSV-DQ	0.165	0.072	0.060	0.071								
FSV-DV												
FSV-EE												
FSV-EZ	*≥0.116*	*≥0.061*	*≥0.066*	*≥0.076*	0.116	0.061	0.066	0.076				
N	21	21	21	21	9	9	9	9	5	5	4	4
Min	0.112	0.050	0.040	0.055	0.114	0.061	0.059	0.055	0.005	0.003	0.004	0.005
Median	0.147	0.075	0.074	0.076	0.126	0.069	0.076	0.073	0.007	0.006	0.005	0.006
Max	0.220	0.190	0.190	0.170	0.166	0.083	0.091	0.105	0.010	0.007	0.013	0.009
SD	0.015	0.013	0.014	0.012	0.017	0.007	0.008	0.006	0.002	0.002	0.002	0.001
CV	10	17	19	16	14	10	11	8	32	37	42	19
N_{past}	21	0	0	0	10	0	0	0	6	0	0	0
$Median_{past}$	0.145				0.131				0.009			
SD_{past}	0.018				0.015				0.004			
NIST	0.147	0.078	0.077	0.077								
N_{NIST}	3	10	14	14								
S_{rep}	0.001	0.002	0.002	0.002								
S_{het}	0.001	0.002	0.003	0.003								
S_{NIST}	0.002	0.003	0.003	0.003								
NAV	0.147	0.077	0.075	0.075	0.126	0.069	0.076	0.076	0.007	0.006	0.005	0.005
NAU	0.023	0.014	0.014	0.014	0.017	0.010	0.011	0.011	0.003	0.003		

	Total α-Carotene, µg/mL				Total Lycopene, µg/mL				trans-Lycopene, µg/mL			
Lab	339	340	341	344	339	340	341	344	339	340	341	344
FSV-BA	0.013	0.025	0.009	0.007	0.581	0.313	0.290	0.314	0.323	0.165	0.138	0.148
FSV-BB	0.010	0.022	0.007	0.008	0.548	0.334	0.287	0.280	0.261	0.143	0.115	0.113
FSV-BC												
FSV-BD												
FSV-BE												
FSV-BF												
FSV-BG	0.015	0.027	0.012	0.013	0.578	0.344	0.293	0.289	0.315	0.169	0.137	0.138
FSV-BH					0.482	0.289	0.226	0.216				
FSV-BI	0.010	0.022	0.009	0.007	0.416	0.250	0.235	0.226				
FSV-BJ	0.014	0.030	*nq*	0.011	0.576	0.320	0.274	0.294				
FSV-BK												
FSV-BL												
FSV-BM												
FSV-BN	0.011	0.023	0.009	0.010	0.542	0.293	0.186	0.205	0.293	0.149	0.088	0.101
FSV-BO	0.022	0.027	*nd*	0.013	0.789	0.464	0.426	0.424				
FSV-BP	*nd*	0.024	*nd*	*nd*	0.264	0.336	0.306	0.238				
FSV-BQ												
FSV-BR												
FSV-BS					0.409	0.247	0.236	0.205				
FSV-BT	0.018	0.026	0.018	0.014	0.457	0.308	0.330	0.254	0.224	0.134	0.115	0.091
FSV-BU	0.012	0.022	0.009	0.006	0.552	0.278	0.271	0.269				
FSV-BV	0.016	0.027	0.009	0.010	0.611	0.332	0.288	0.279				
FSV-BW	*nq*	*nq*	*nq*	*nq*	0.730	0.370	0.200	0.240				
FSV-CC												
FSV-CD	0.030	0.070	0.030	0.030	0.580	0.440	0.430	0.390				
FSV-CE												
FSV-CF												
FSV-CG	0.017	0.031	0.009	0.009	0.556	0.325	0.263	0.198	0.309	0.163	0.122	0.057
FSV-CI	0.035	0.031	0.028	0.033								
FSV-CW	0.008	0.031	0.005	0.008					0.240	0.134	0.095	0.105
FSV-CZ												
FSV-DD												
FSV-DI					0.687	0.340	0.338	0.330				
FSV-DQ	0.038	0.034	0.010	0.008	0.497	0.285	0.229	0.274				
FSV-DV												
FSV-EE												
FSV-EZ												
N	15	16	13	15	18	18	18	18	7	7	7	7
Min	0.008	0.022	0.005	0.006	0.264	0.247	0.186	0.198	0.224	0.134	0.088	0.057
Median	0.015	0.027	0.009	0.010	0.554	0.323	0.281	0.272	0.293	0.149	0.115	0.105
Max	0.038	0.070	0.030	0.033	0.789	0.464	0.430	0.424	0.323	0.169	0.138	0.148
SD	0.006	0.005	0.002	0.004	0.070	0.036	0.050	0.047	0.046	0.019	0.018	0.022
CV	40	20	25	37	13	11	18	17	16	13	16	21
N_{past}	19	0	0	0	19	0	0	0	7	0	0	0
$Median_{past}$	0.016				0.582				0.288			
SD_{past}	0.006				0.068				0.044			
NIST		0.028										
N_{NIST}		10										
S_{rep}		0.002										
S_{het}		0.002										
S_{NIST}		0.003										
NAV	0.015	0.027	0.009	0.009	0.554	0.323	0.281	0.281	0.293	0.149	0.115	0.115
NAU	0.006	0.009	0.004	0.004	0.112	0.072	0.065	0.065	0.053	0.026	0.022	0.022

Round Robin LXIII Laboratory Results

Lab	Total β-Cryptoxanthin, µg/mL 339	340	341	344	Total α-Cryptoxanthin, µg/mL 339	340	341	344	Total Lutein, µg/mL 339	340	341	344
FSV-BA	0.105	0.034	0.038	0.043	0.036	0.014	0.015	0.018				
FSV-BB	0.098	0.036	0.040	0.039	0.031	0.014	0.015	0.015	0.162	0.072	0.063	0.061
FSV-BC												
FSV-BD												
FSV-BE												
FSV-BF												
FSV-BG	0.116	0.038	0.045	0.045					0.151	0.086	0.077	0.078
FSV-BH	0.110	0.036	0.039	0.038					0.118	0.056	0.043	0.045
FSV-BI	0.097	0.032	0.039	0.039					0.125	0.052	0.042	0.044
FSV-BJ	0.108	0.036	0.038	0.042					0.135	0.062	0.054	0.056
FSV-BK												
FSV-BL												
FSV-BM												
FSV-BN	0.102	0.027	0.025	0.026	0.028	0.007	0.005	0.006	0.185	0.079	0.062	0.063
FSV-BO	0.107	0.034	0.037	0.037					0.176	0.078	0.081	0.079
FSV-BP	0.109	0.069	0.037	0.040								
FSV-BQ												
FSV-BR												
FSV-BS												
FSV-BT	0.086	0.034	0.038	0.034	0.029	0.015	0.016	0.014				
FSV-BU	0.113	0.030	0.041	0.041								
FSV-BV	0.109	0.035	0.040	0.040								
FSV-BW	0.140	0.049	0.048	0.040								
FSV-CC												
FSV-CD	0.670	0.280	0.290	0.310	0.120	0.060	0.070	0.070				
FSV-CE												
FSV-CF												
FSV-CG	0.134	0.048	0.053	0.049								
FSV-CI									0.171	0.058	0.053	0.065
FSV-CW	0.108	0.040	0.042	0.045					0.174	0.092	0.086	0.094
FSV-CZ												
FSV-DD												
FSV-DI									0.144	0.059	0.052	0.054
FSV-DQ	0.104	0.039	0.031	0.037					0.183	0.068	0.069	0.059
FSV-DV												
FSV-EE												
FSV-EZ												
N	17	17	17	17	5	5	5	5	11	11	11	11
Min	0.086	0.027	0.025	0.026	0.028	0.007	0.005	0.006	0.118	0.052	0.042	0.044
Median	0.108	0.036	0.039	0.040	0.031	0.014	0.015	0.015	0.162	0.068	0.062	0.061
Max	0.670	0.280	0.290	0.310	0.120	0.060	0.070	0.070	0.185	0.092	0.086	0.094
SD	0.007	0.004	0.003	0.004	0.005	0.001	0.001	0.003	0.026	0.015	0.015	0.012
CV	6	12	8	9	17	5	5	20	16	22	25	20
N_{past}	19	0	0	0	4	0	0	0	13	0	0	0
$Median_{past}$	0.107				0.039				0.147			
SD_{past}	0.023				0.009				0.034			
NIST	0.083	0.039	0.025	0.025					0.165	0.083	0.054	0.054
N_{NIST}	3	10	14	14					3	10	14	14
S_{rep}	0.004	0.003	0.003	0.003					0.006	0.004	0.004	0.004
S_{het}	0.004	0.003	0.002	0.002					0.004	0.004	0.004	0.004
S_{NIST}	0.006	0.004	0.004	0.004					0.007	0.006	0.005	0.005
NAV	0.096	0.038	0.035	0.035	0.031	0.014	0.015	0.015	0.163	0.076	0.059	0.059
NAU	0.030	0.009	0.014	0.014	0.005	0.001	0.003	0.003	0.029	0.018	0.015	0.015

Lab	Total Zeaxanthin, µg/mL 339	340	341	344	Total Lutein&Zeaxanthin, µg/mL 339	340	341	344	Coenzyme Q10, µg/mL 339	340	341	344
FSV-BA					0.237	0.089	0.073	0.078				
FSV-BB	0.079	0.032	0.032	0.031	0.241	0.105	0.095	0.092				
FSV-BC												
FSV-BD												
FSV-BE												
FSV-BF												
FSV-BG	0.035	0.017	0.017	0.016	0.169	0.095	0.086	0.086				
FSV-BH	0.080	0.023	0.024	0.024	0.198	0.079	0.067	0.069				
FSV-BI	0.075	0.027	0.028	0.028	*0.200*	*0.079*	*0.070*	*0.072*				
FSV-BJ									1.351	0.781	0.657	0.630
FSV-BK												
FSV-BL												
FSV-BM												
FSV-BN	0.075	0.021	0.016	0.013	0.276	0.102	0.079	0.076				
FSV-BO	0.053	0.022	0.028	0.021	0.2292	0.0995	0.1092	0.0995				
FSV-BP					0.215	0.150	0.132	0.131				
FSV-BQ												
FSV-BR												
FSV-BS					0.270	0.069	0.040	0.046				
FSV-BT					0.295	0.156	0.152	0.134				
FSV-BU					0.245	0.084	0.089	0.088				
FSV-BV					0.240	0.098	0.088	0.088				
FSV-BW					0.250	0.107	0.088	0.090	1.190	0.660	0.390	0.330
FSV-CC												
FSV-CD					0.610	0.170	0.160	0.180				
FSV-CE												
FSV-CF												
FSV-CG					0.276	0.111	0.097	0.094				
FSV-CI	0.047	0.017	0.024	0.029	*0.218*	*0.075*	*0.077*	*0.094*	1.200	0.800	0.730	0.760
FSV-CW	0.037	0.014	0.019	0.022	0.210	0.106	0.105	0.116	0.880	0.518	0.537	0.535
FSV-CZ									1.449	1.096	0.853	0.907
FSV-DD												
FSV-DI									1.160	0.683	0.635	0.624
FSV-DQ	0.065	0.021	0.025	0.024	*0.248*	*0.089*	*0.094*	*0.083*				
FSV-DV												
FSV-EE									1.278	0.799	0.682	0.716
FSV-EZ									1.060	0.575	0.596	0.673
N	9	9	9	9	18	18	18	18	8	8	8	8
Min	0.035	0.014	0.016	0.013	0.169	0.069	0.040	0.046	0.880	0.518	0.390	0.330
Median	0.065	0.021	0.024	0.024	0.241	0.099	0.089	0.089	1.195	0.732	0.646	0.652
Max	0.080	0.032	0.032	0.031	0.610	0.170	0.160	0.180	1.449	1.096	0.853	0.907
SD	0.021	0.004	0.007	0.005	0.037	0.016	0.019	0.014	0.120	0.119	0.083	0.093
CV	32	21	28	22	15	16	21	16	10	16	13	14
N_{past}	11	0	0	0	19	0	0	0	6	0	0	0
$Median_{past}$	0.062				0.213				1.133			
SD_{past}	0.016				0.050				0.107			
NIST	0.067	0.019	0.025	0.025	*0.232*	*0.102*	*0.079*	*0.079*				
N_{NIST}	3	10	14	14	3	10	14	14				
S_{rep}	0.002	0.002	0.004	0.004	0.007	0.005	0.005	0.005				
S_{het}	0.004	0.001	0.002	0.002	0.008	0.005	0.004	0.004				
S_{NIST}	0.005	0.002	0.004	0.004	0.010	0.006	0.007	0.007				
NAV	0.066	0.020	0.024	0.024	0.236	0.101	0.086	0.086	1.195	0.732	0.646	0.646
NAU	0.021	0.006	0.007	0.007	0.051	0.021	0.020	0.020	0.120	0.119	0.093	0.093

Round Robin LXIII Laboratory Results

	Ubiquinol, µg/mL				Ubiquinone, µg/mL				Phylloquinone (K1), ng/mL				25-hydroxyvitamin D, µg/mL			
Lab	339	340	341	344	339	340	341	344	339	340	341	344	339	340	341	344
FSV-BA													0.011	0.024	0.014	0.013
FSV-BB																
FSV-BC																
FSV-BD																
FSV-BE									0.629	0.557	0.284	0.244				
FSV-BF																
FSV-BG																
FSV-BH													0.023	0.007	0.013	0.015
FSV-BI																
FSV-BJ																
FSV-BK																
FSV-BL																
FSV-BM																
FSV-BN																
FSV-BO																
FSV-BP																
FSV-BQ																
FSV-BR																
FSV-BS																
FSV-BT																
FSV-BU																
FSV-BV																
FSV-BW	0.890	0.270	*nd*	*nq*	0.310	0.390	0.390	0.330								
FSV-CC																
FSV-CD																
FSV-CE																
FSV-CF																
FSV-CG																
FSV-CI									0.620	0.520	0.220	0.200				
FSV-CW																
FSV-CZ																
FSV-DD																
FSV-DI	1.350	0.767	0.210	0.130	0.180	0.210	0.618	0.700	0.280	0.230	0.130	0.190				
FSV-DQ																
FSV-DV																
FSV-EE																
FSV-EZ																
N	2	2	1	1	2	2	2	2	3	3	3	3	2	2	2	2
Min	0.890	0.270			0.180	0.210	0.390	0.330	0.280	0.230	0.130	0.190	0.011	0.007	0.013	0.013
Median	1.120	0.519	0.210	0.130	0.245	0.300	0.504	0.515	0.620	0.520	0.220	0.200	0.017	0.015	0.013	0.014
Max	1.350	0.767			0.310	0.390	0.618	0.700	0.629	0.557	0.284	0.244	0.023	0.024	0.014	0.015
SD																
CV																
N_{past}	0	0	0	0	0	0	0	0	0	0	0	0	9	0	0	0
$Median_{past}$													0.008			
SD_{past}													0.001			
NIST																
N_{NIST}																
S_{rep}																
S_{het}																
S_{NIST}																
NAV									0.620	0.520	0.220	0.220				
NAU																

Round Robin LXIII Laboratory Results

Term	Legend
N	Number of quantitative values reported for this analyte
Min	Minimum quantitative value reported
Median	Median quantitative value reported
Max	Maximum quantitative value reported
SD	Standard deviation for results: (Median Absolute Difference from the median)/0.674
CV	Coefficient of Variation for results: 100*SD/Median
N_{past}	Mean of N(s) from past RR(s)
$Median_{past}$	Mean of Median(s) from past RR(s)
SD_{past}	Pooled SD from past RR(s)
NIST	Average of NIST measurements
N_{NIST}	Number of sample units analyzed by NIST
S_{rep}	NIST repeatability standard deviation
S_{het}	Material homogeniety
S_{NIST}	SQRT(SUMSQ(S_{rep},S_{het}))
NAV	NIST Assigned Value: Median
NAU	NIST Assigned Uncertainty: Max(0.05*NAV, SNIST, SD, eSD). The expected long-term SD, eSD, is defined in: Duewer, et al. Anal Chem 1997;69(7):1406-1413.
-	Not analyzed
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
italics	Not explictly reported but calculated by NIST from reported values

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	1	1	1	1	1	1	2	1
FSV-BC	1										
FSV-BD	1	2									
FSV-BE	1	1	1	1							
FSV-BF	1	3	1								
FSV-BG	1	1	1	1		1	1	1	1	2	1
FSV-BH	2	1	2	2	2		1	1	2	1	1
FSV-BI	1	1	1	1		1	1	1	2	1	1
FSV-BJ	1	1	1	1		1	1	1	1		
FSV-BK	3	1									
FSV-BL	3	1									
FSV-BM	1	1									
FSV-BN	2	1	1	1	1	1	1	1	1	2	1
FSV-BO	2	1	1	2	3	1	3	1	1	1	1
FSV-BP	1	2		1			2	2			3
FSV-BQ	2	1									
FSV-BR	2	1									
FSV-BS	2	4	4	1	1		2				2
FSV-BT	2	2	3	2	1	2	1	1			3
FSV-BU	1	1	1	1		1	1	1			1
FSV-BV	1	1	1	1		1	1	1			1
FSV-BW	1	1	1	1			2	1			1
FSV-CC	1	2									
FSV-CD	1	2	1	4		4	2	4			4
FSV-CE	1	1		2							
FSV-CF	2	1									
FSV-CG	1	1	1	1	1	1	1	1			1
FSV-CI	1	1	2	1		4			1	1	1
FSV-CW	2	3	4	2		1		1	2	1	1
FSV-CZ	4	4	4	3							
FSV-DD	2										
FSV-DI	2	1	1	2			1		1		
FSV-DQ		2	1	1		3	1	1	1	1	1
FSV-DV	2	1									
FSV-EE	2	1									
FSV-EZ	4	1	1		1						
NIST	1	1	1	1				1	1	1	1
n	36	36	24	24	9	15	18	18	12	10	19

	TR	aT	g/bT	bC	tbC	aC	TLy	TbX	TLu	TZ	L&Z
% 1	56	69	75	67	78	73	72	89	75	70	79
% 2	33	17	8	25	11	7	22	6	25	30	5
% 3	6	6	4	4	11	7	6	0	0	0	11
% 4	6	8	13	4	0	13	0	6	0	0	5

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 RR63

Each participant in RR63 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 RR63:

- Total Retinol
- *trans*-Retinol
- Retinyl Palmitate
- α-Tocopherol
- γ/β-Tocopherol
- Total β-Carotene
- *trans*-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 thirteen pages are the "Individualized Report" for the analytes evaluated by participant FSV-BA.

Individualized Round Robin LXIII Report: FSV-BA

Summary

	Serum 339			Serum 340			Serum 341			Serum 344		
Analyte	You	NAV	n	You	NAV	n	You	NAV	n	You	NAV	n
Total Retinol	0.330	0.301	31	0.430	0.415	31	0.349	0.342	31	0.363	0.342	31
Retinyl Palmitate	0.06	0.01	9	0.0	0.0	9	0.0	0.0	9	0.03	0.01	8
α-Tocopherol	8.25	8.47	36	8.01	7.98	36	5.87	5.94	34	6.02	5.94	34
γ/β-Tocopherol	3.651	3.682	24	1.774	1.796	24	1.479	1.461	24	1.552	1.461	24
δ-Tocopherol	0.089	0.091	5	0.085	0.080	5	0.081	0.084	6	0.086	0.084	6
Total β-Carotene	0.148	0.147	22	0.077	0.077	22	0.080	0.075	22	0.085	0.075	22
trans-β-Carotene	0.141	0.126	9	0.074	0.069	9	0.076	0.076	9	0.080	0.076	9
Total cis-β-Carotene	0.006	0.007	5	0.004	0.006	5	0.004	0.005	4	0.005	0.005	4
Total α-Carotene	0.013	0.015	16	0.025	0.027	17	0.009	0.009	14	0.007	0.009	16
Total Lycopene	0.581	0.554	18	0.313	0.323	18	0.290	0.281	18	0.314	0.281	18
trans-Lycopene	0.323	0.293	8	0.165	0.149	8	0.138	0.115	8	0.148	0.115	8
Total β-Cryptoxanthin	0.105	0.096	18	0.034	0.038	18	0.038	0.035	18	0.043	0.035	18
Total α-Cryptoxanthin	0.036	0.031	5	0.014	0.014	5	0.015	0.015	5	0.018	0.015	5
Total Lutein&Zeaxanthin	0.237	0.236	19	0.089	0.101	19	0.073	0.086	19	0.078	0.086	19
25-hydroxyvitamin D	0.011		2	0.024		2	0.014		2	0.013		2

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

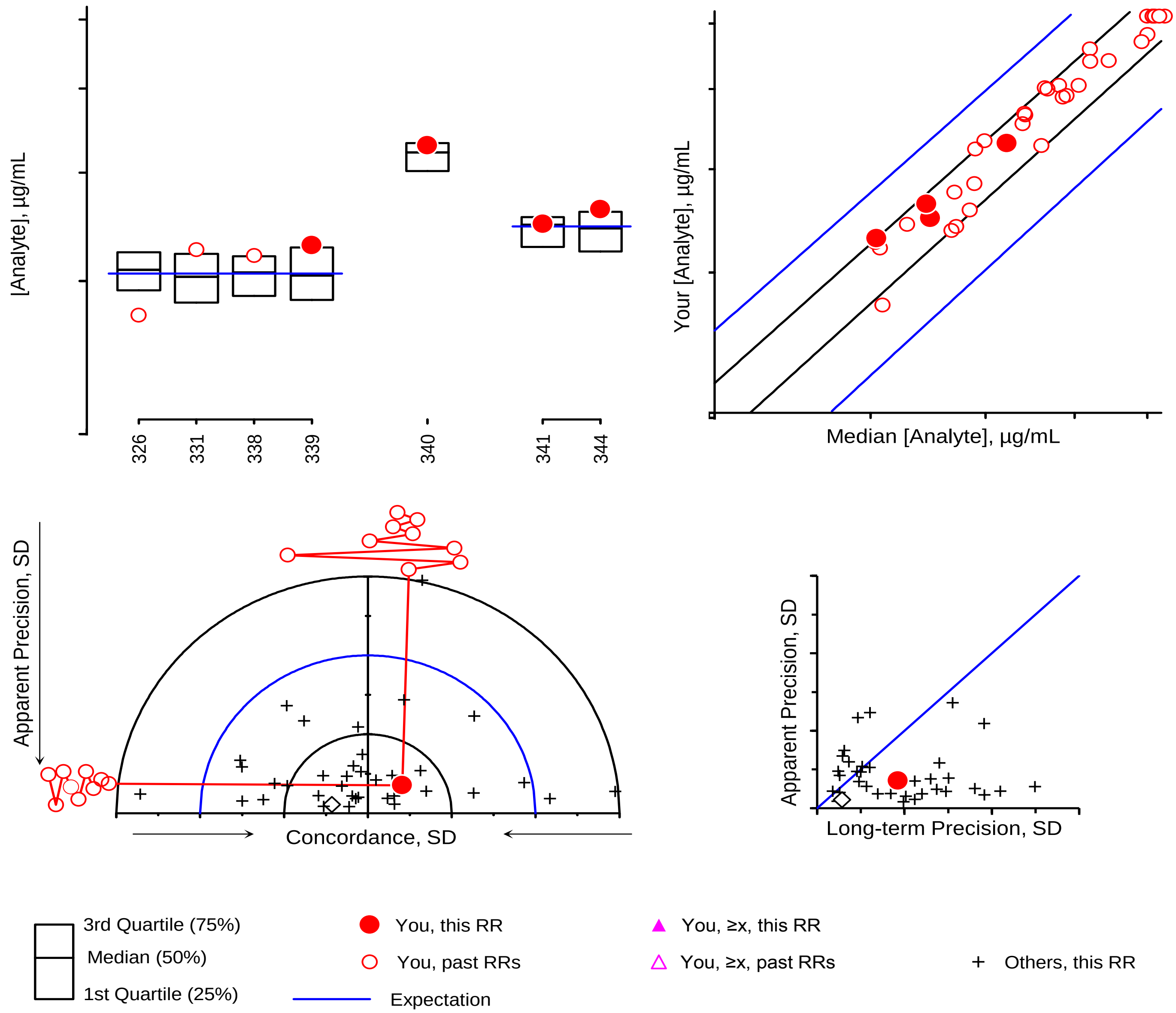
Please check our records against your records. Send corrections and/or updates to...

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

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

Individualized RR LXIII Report: FSV-BA

Total Retinol, µg/mL

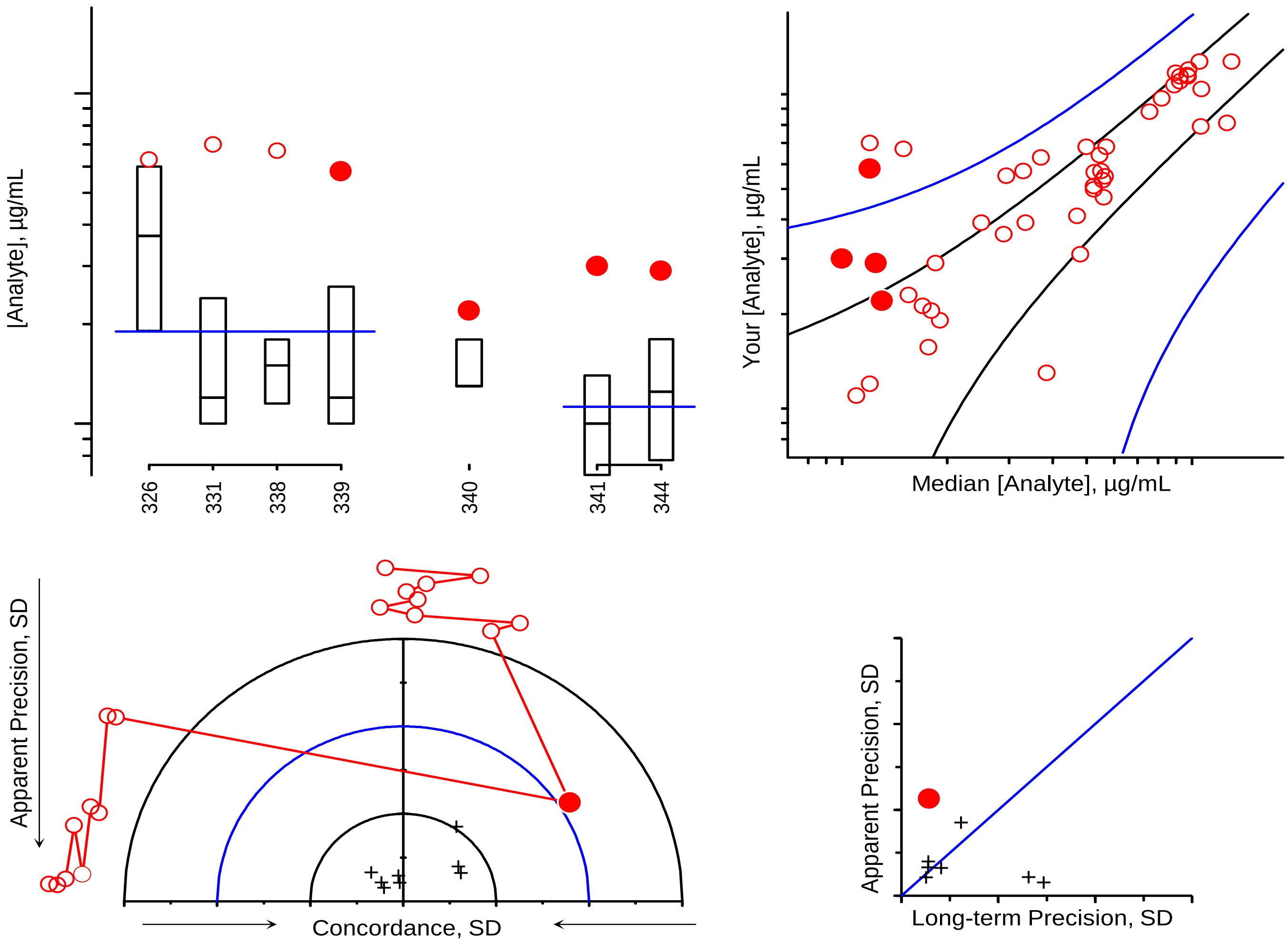


For details of the construction and interpretation of these plots, see:
Duewer, Kline, Sharpless, Brown Thomas, Gary, Sowell. Anal Chem 1999;71(9):1870-8.

Serum	History	Comments
#339	Fresh frozen: 60:#326, 61:#331, 62:#338	Native, single-source
#340	Fresh frozen: New	Native, multiple-source
#341	Fresh frozen: New, from beginning of production	Native, single-source
#344	Same as #341, but from end of production run	Native, single-source

Individualized RR LXIII 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

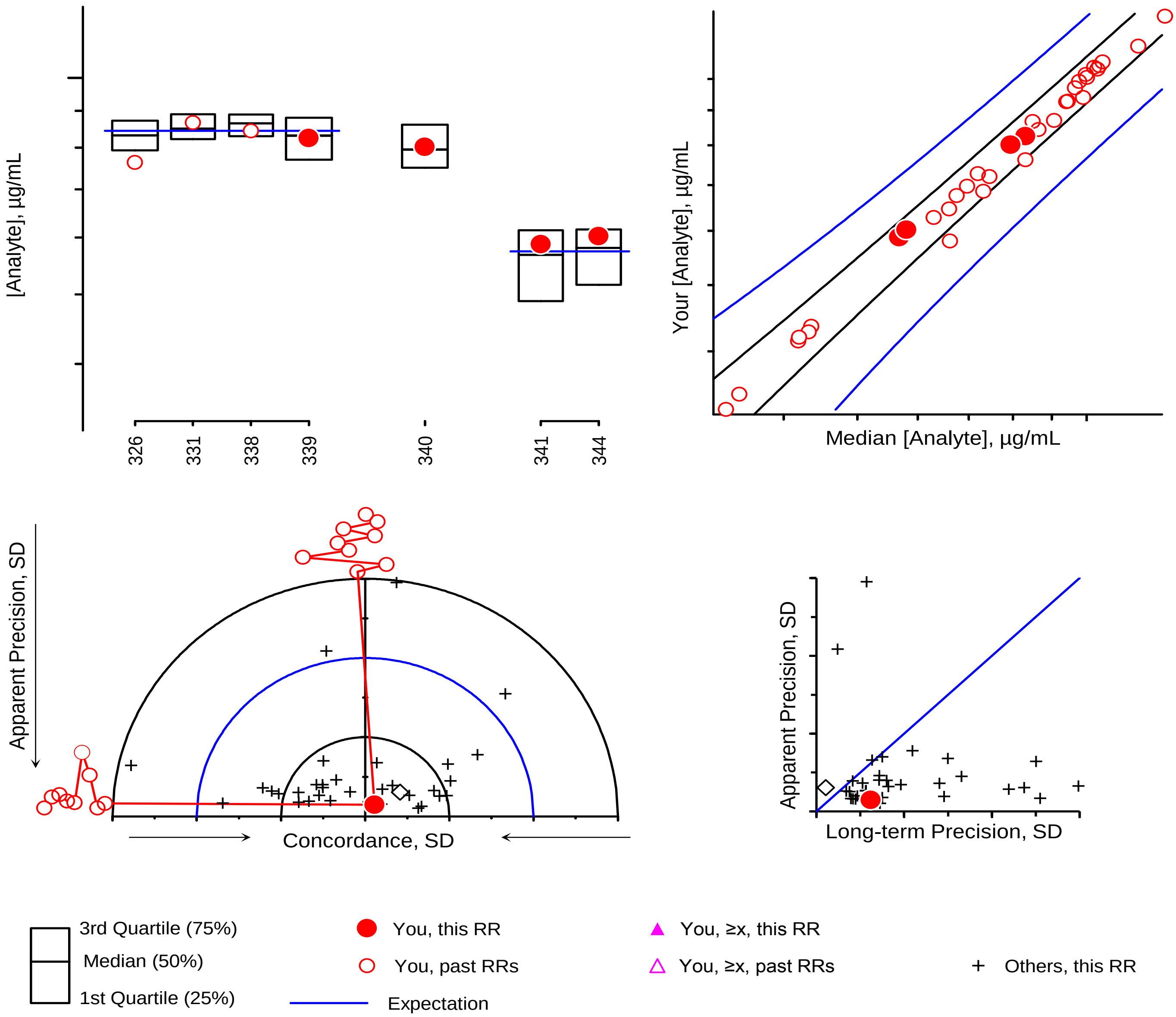
Others, this RR

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
#339	Fresh frozen: 60:#326, 61:#331, 62:#338	Native, single-source
#340	Fresh frozen: New	Native, multiple-source
#341	Fresh frozen: New, from beginning of production	Native, single-source
#344	Same as #341, but from end of production run	Native, single-source

Individualized RR LXIII Report: FSV-BA

α-Tocopherol, µg/mL

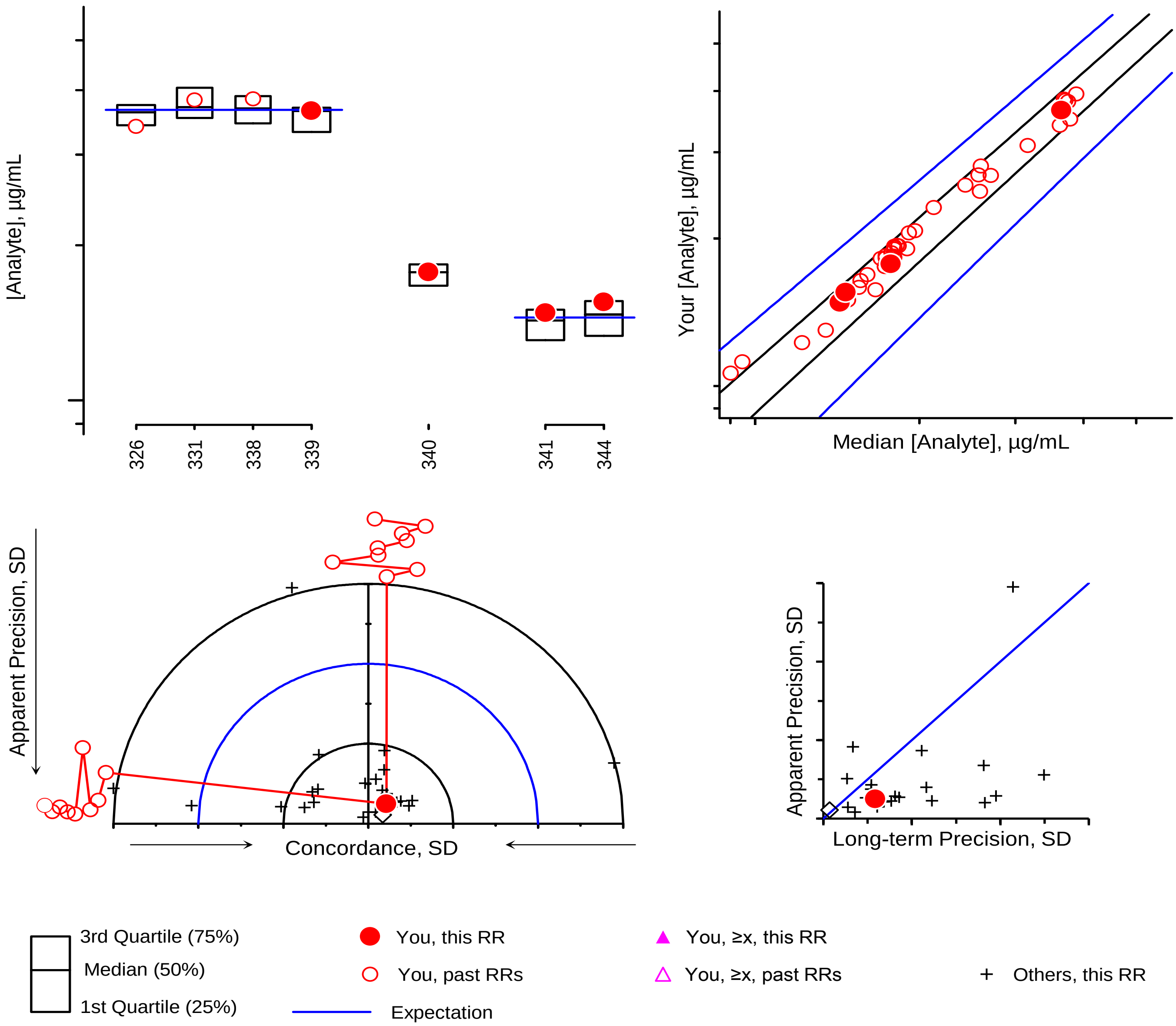


For details of the construction and interpretation of these plots, see:
Duewer, Kline, Sharpless, Brown Thomas, Gary, Sowell. Anal Chem 1999;71(9):1870-8.

Serum	History	Comments
#339	Fresh frozen: 60:#326, 61:#331, 62:#338	Native, single-source
#340	Fresh frozen: New	Native, multiple-source
#341	Fresh frozen: New, from beginning of production	Native, single-source
#344	Same as #341, but from end of production run	Native, single-source

Individualized RR LXIII Report: FSV-BA

γ/β-Tocopherol, µg/mL

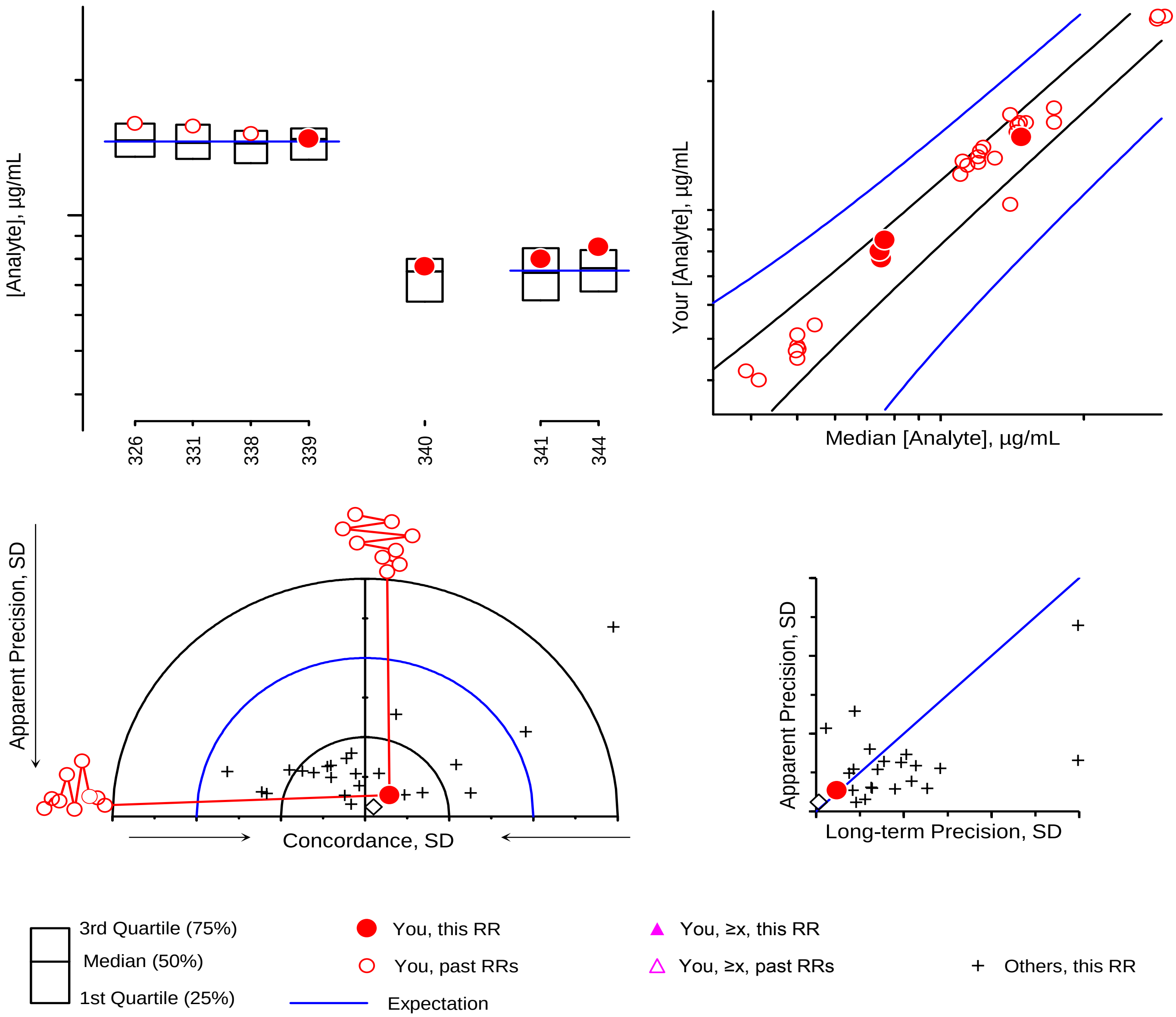


For details of the construction and interpretation of these plots, see:
Duewer, Kline, Sharpless, Brown Thomas, Gary, Sowell. Anal Chem 1999;71(9):1870-8.

Serum	History	Comments
#339	Fresh frozen: 60:#326, 61:#331, 62:#338	Native, single-source
#340	Fresh frozen: New	Native, multiple-source
#341	Fresh frozen: New, from beginning of production	Native, single-source
#344	Same as #341, but from end of production run	Native, single-source

Individualized RR LXIII Report: FSV-BA

Total β-Carotene, µg/mL

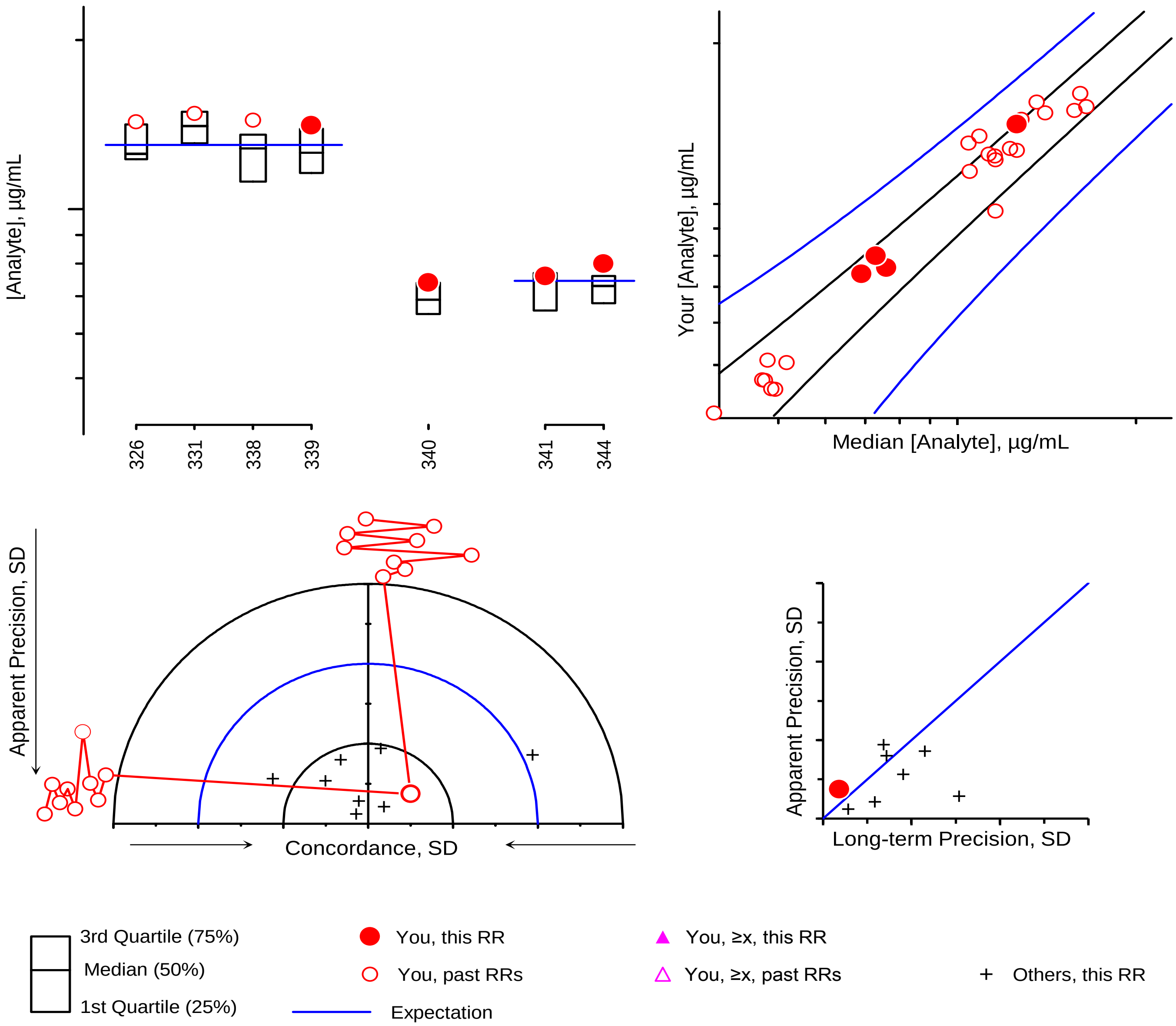


For details of the construction and interpretation of these plots, see:
Duewer, Kline, Sharpless, Brown Thomas, Gary, Sowell. Anal Chem 1999;71(9):1870-8.

Serum	History	Comments
#339	Fresh frozen: 60:#326, 61:#331, 62:#338	Native, single-source
#340	Fresh frozen: New	Native, multiple-source
#341	Fresh frozen: New, from beginning of production	Native, single-source
#344	Same as #341, but from end of production run	Native, single-source

Individualized RR LXIII Report: FSV-BA

trans-β-Carotene, µg/mL

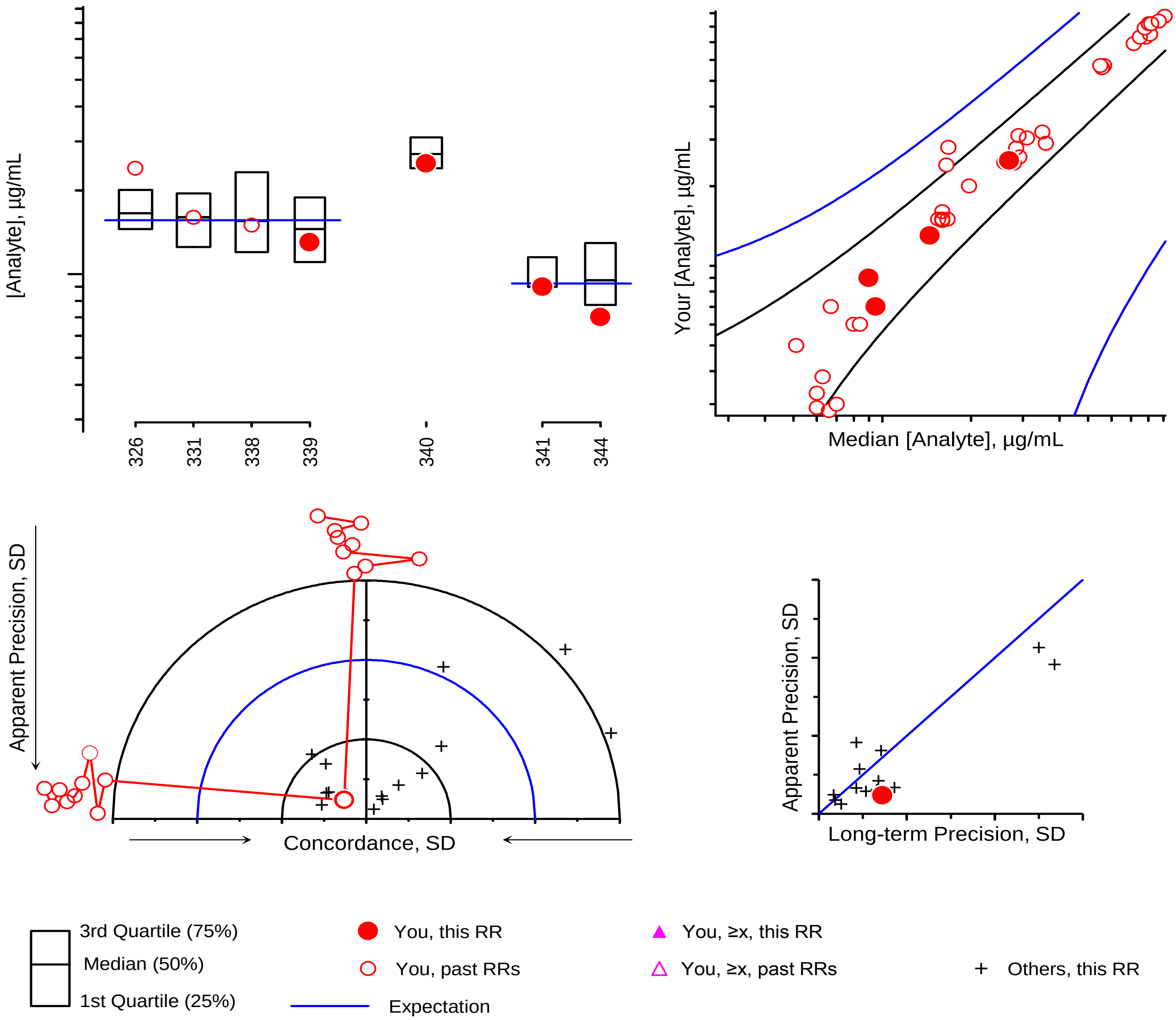


For details of the construction and interpretation of these plots, see:
Duewer, Kline, Sharpless, Brown Thomas, Gary, Sowell. Anal Chem 1999;71(9):1870-8.

Serum	History	Comments
#339	Fresh frozen: 60:#326, 61:#331, 62:#338	Native, single-source
#340	Fresh frozen: New	Native, multiple-source
#341	Fresh frozen: New, from beginning of production	Native, single-source
#344	Same as #341, but from end of production run	Native, single-source

Individualized RR LXIII Report: FSV-BA

Total α-Carotene, µg/mL

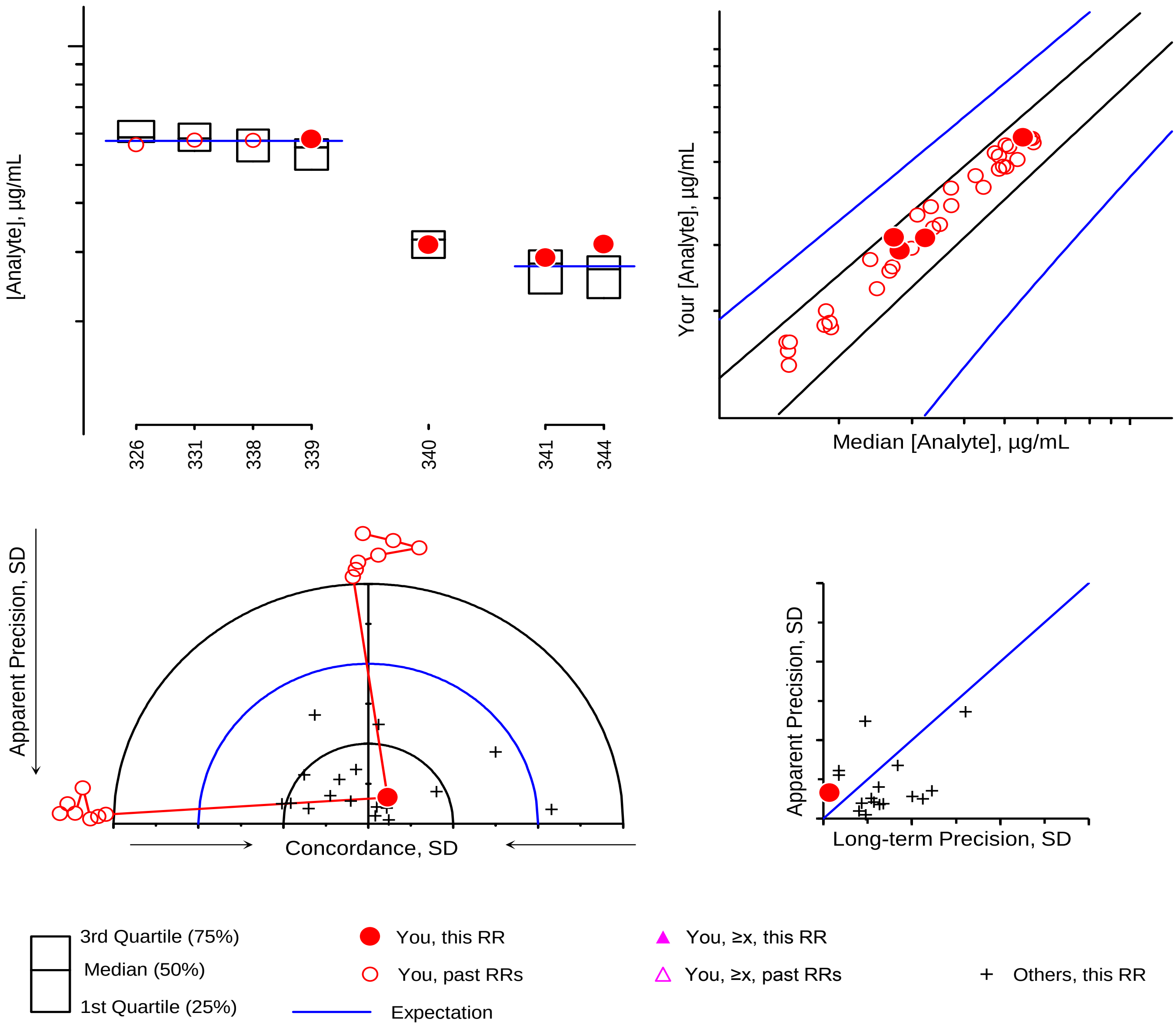


For details of the construction and interpretation of these plots, see:
Duewer, Kline, Sharpless, Brown Thomas, Gary, Sowell. Anal Chem 1999;71(9):1870-8.

Serum	History	Comments
#339	Fresh frozen: 60:#326, 61:#331, 62:#338	Native, single-source
#340	Fresh frozen: New	Native, multiple-source
#341	Fresh frozen: New, from beginning of production	Native, single-source
#344	Same as #341, but from end of production run	Native, single-source

Individualized RR LXIII Report: FSV-BA

Total Lycopene, µg/mL

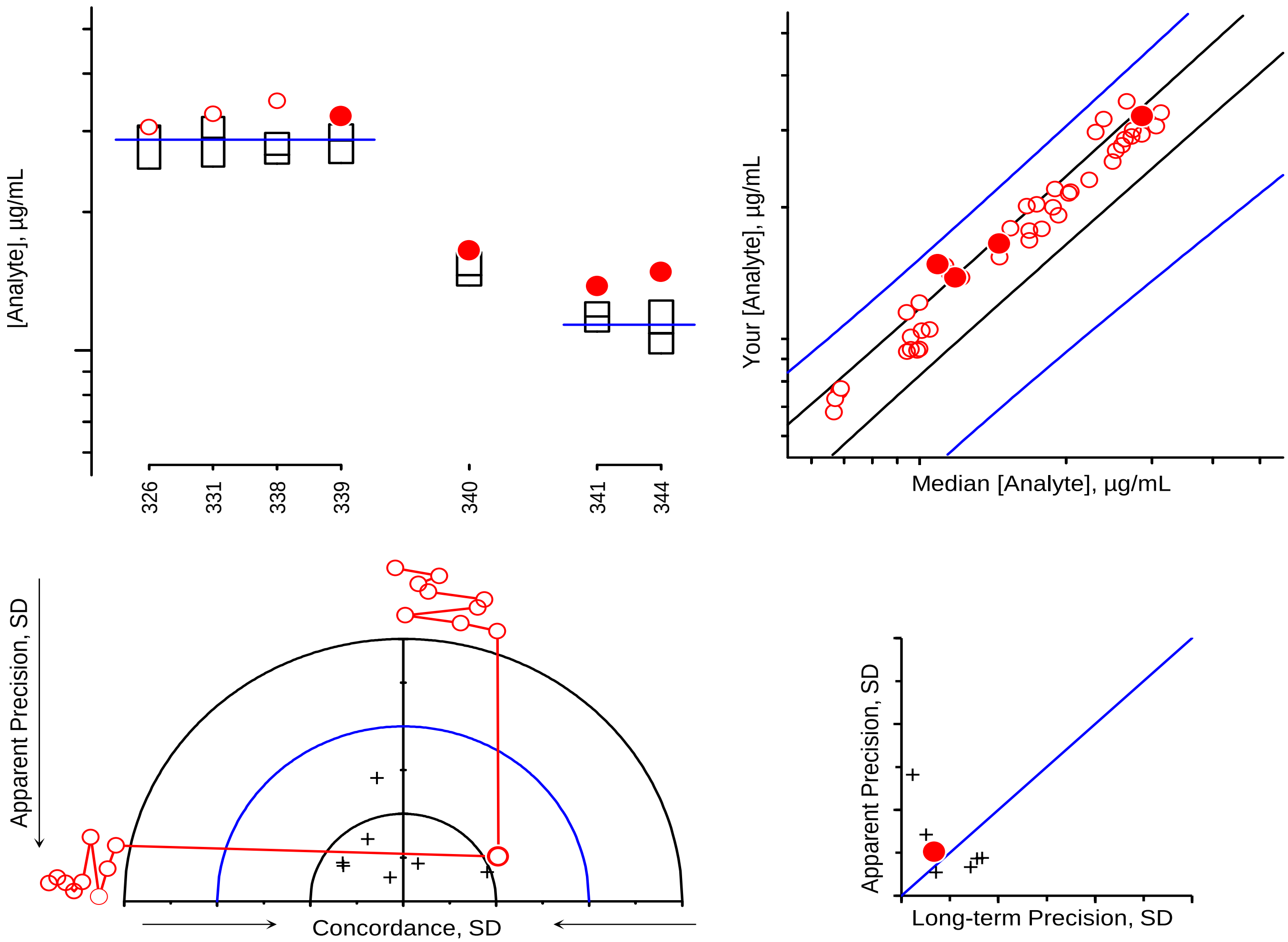


For details of the construction and interpretation of these plots, see:
Duewer, Kline, Sharpless, Brown Thomas, Gary, Sowell. Anal Chem 1999;71(9):1870-8.

Serum	History	Comments
#339	Fresh frozen: 60:#326, 61:#331, 62:#338	Native, single-source
#340	Fresh frozen: New	Native, multiple-source
#341	Fresh frozen: New, from beginning of production	Native, single-source
#344	Same as #341, but from end of production run	Native, single-source

Individualized RR LXIII Report: FSV-BA

trans-Lycopene, 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

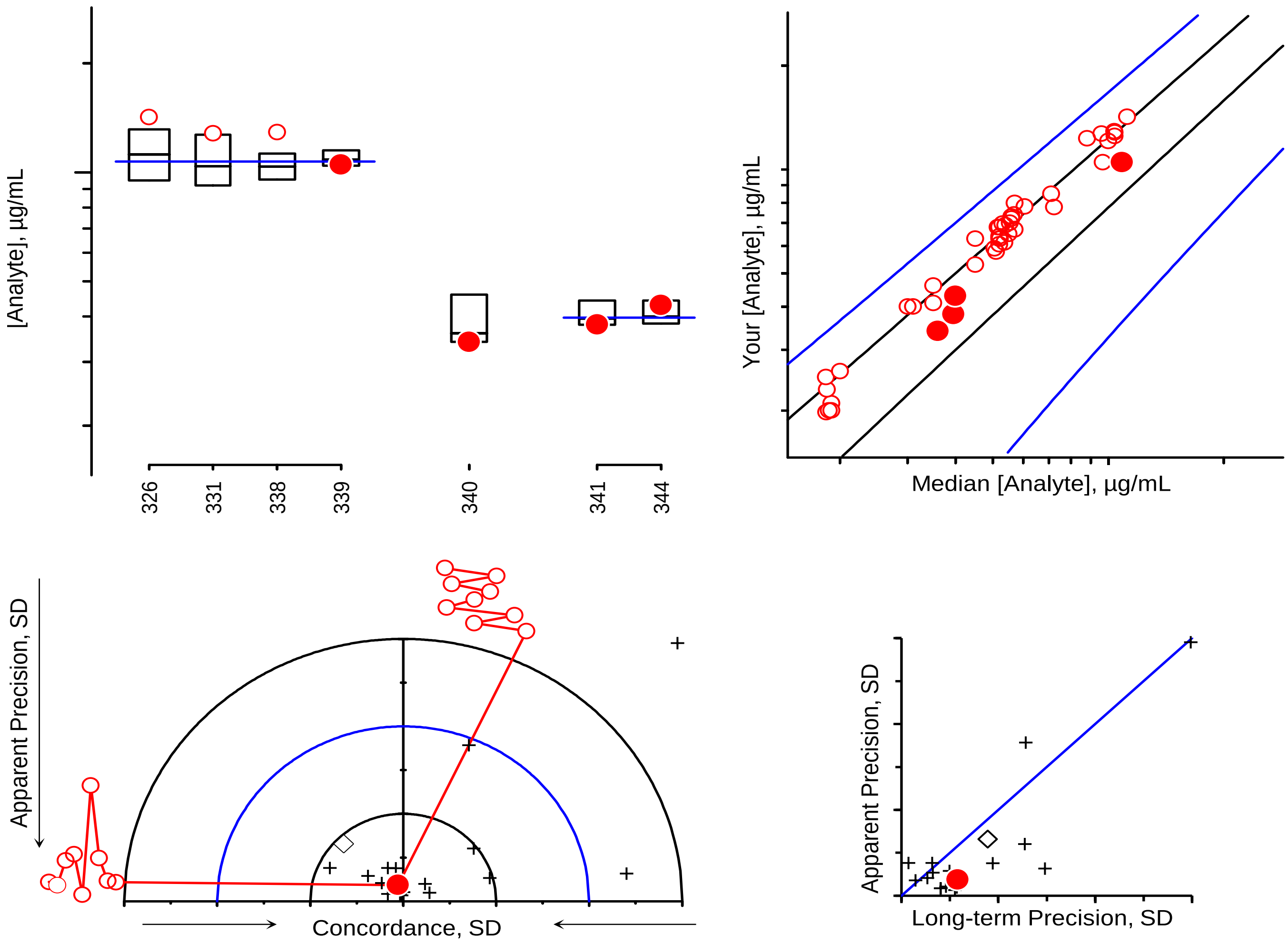
Others, this RR

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
#339	Fresh frozen: 60:#326, 61:#331, 62:#338	Native, single-source
#340	Fresh frozen: New	Native, multiple-source
#341	Fresh frozen: New, from beginning of production	Native, single-source
#344	Same as #341, but from end of production run	Native, single-source

Individualized RR LXIII 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

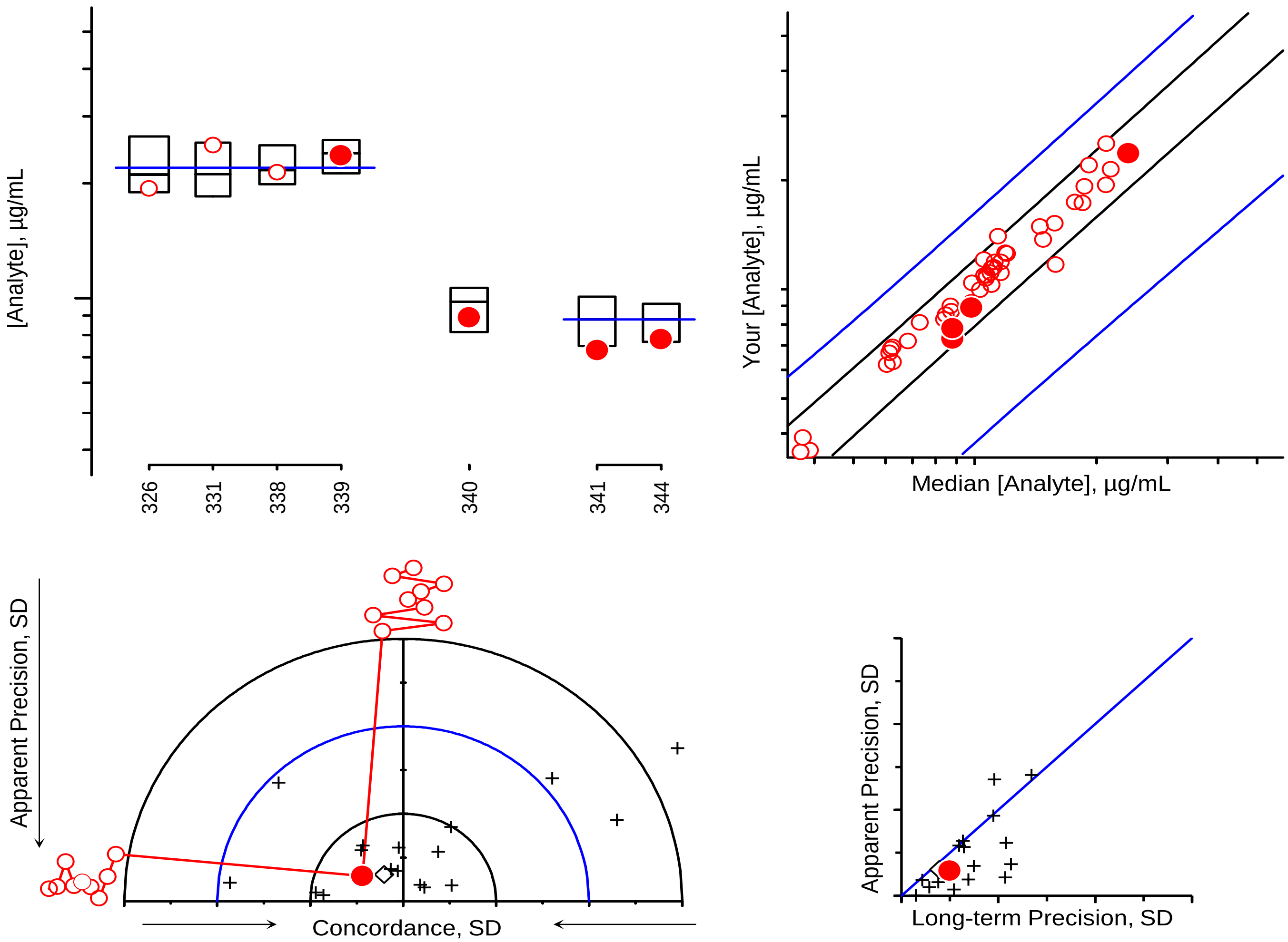
Others, this RR

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
#339	Fresh frozen: 60:#326, 61:#331, 62:#338	Native, single-source
#340	Fresh frozen: New	Native, multiple-source
#341	Fresh frozen: New, from beginning of production	Native, single-source
#344	Same as #341, but from end of production run	Native, single-source

Individualized RR LXIII Report: FSV-BA

Total Lutein&Zeaxanthin, µg/mL

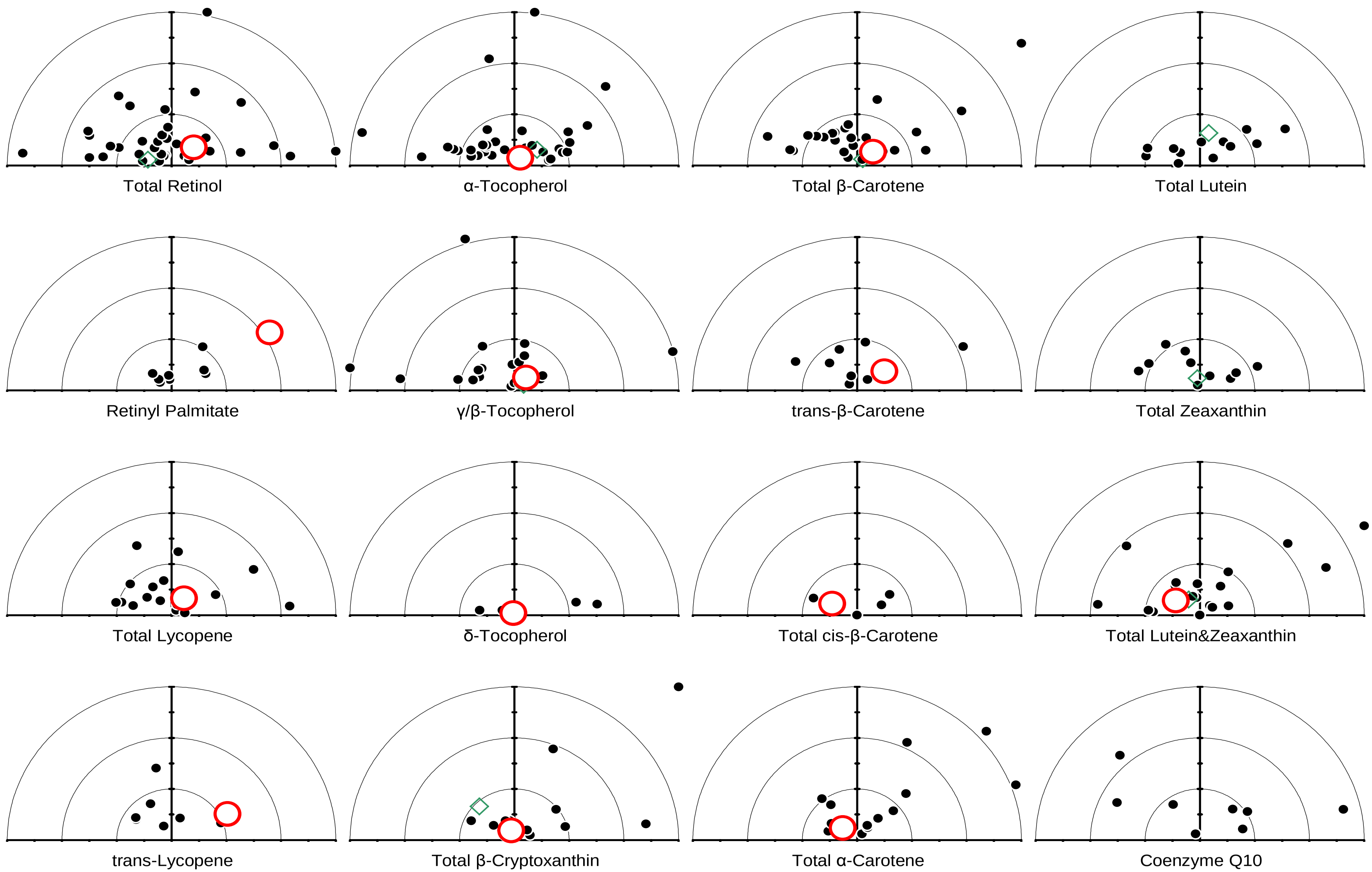


3rd Quartile (75%)
Median (50%)
1st Quartile (25%)
You, this RR
You, past RRs
Expectation
You, ≥x, this RR
You, ≥x, past RRs
Others, this RR

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
#339	Fresh frozen: 60:#326, 61:#331, 62:#338	Native, single-source
#340	Fresh frozen: New	Native, multiple-source
#341	Fresh frozen: New, from beginning of production	Native, single-source
#344	Same as #341, but from end of production run	Native, single-source

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



D14

Appendix E. Shipping Package Inserts for RR28

The following five items were included in each package shipped to an RR28 participant:

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

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

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

November 5, 2007

Dear Colleague:

The samples within this package constitute Vitamin C Round Robin 28 (RR28) of the 2008 Micronutrients Measurement Quality Assurance Program.

RR28 consists of four vials of frozen serum *test samples* (#26, #112, #116, and #119), one vial of frozen *control serum* (CS #1) 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 #1* is 8.4 ±0.7 µmol/L of sample.

The report for RR27 will be mailed shortly. If you find your results for RR27 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 tel: 301-975-3120, fax: 301-977-0685, or e-mail: jbthomas@nist.gov.

We ask that you return your results for these RR28 samples *before* **March 3, 2008**. We would appreciate receiving your results as soon as they become available. Please use the attached form. Your results will be kept confidential.

Sincerely,

Jeanice B Thomas

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

Enclosures: Protocols, Preparation and Analysis of Control Materials and Analysis of Test Samples
RR28 Report Form for Ascorbic Acid Solid Control Material Preparation
RR28 Report Form for Control Material and Test Sample Analyses

NIST

Micronutrient Measurement Quality Assurance Program for
Vitamin C

Please Read Through Completely BEFORE Analyzing Samples

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

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

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

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

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

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

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

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

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

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

   For example, if you prepared the Stock Solution with 0.2000 g of solid ascorbic acid and 103.0 g of Diluent, then 0.5 mL of the Stock Solution should weigh (0.2+103)/200 = 0.52 g and $[\mathrm{TAA}]_{DS1}$ = (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 $[\mathrm{TAA}]_{DS2}$ = 28.4 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:

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

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

6) Measure and record the concentration of total ascorbic acid in all three dilute solutions and in the 5% MPA Diluent in duplicate using <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 #1 (see protocol below). The target values for this materials is 8.4 ±0.7 µmol/L of sample.

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

   Do <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 28

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 *before* **March 3, 2008**

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 28

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 ±0.7 µmol/L
Serum Test Sample #26			µmol/L of Sample
Serum Test Sample #112			µmol/L of Sample
Serum Test Sample #116			µmol/L of Sample
Serum Test Sample #119			µmol/L of Sample

Were samples frozen upon receipt? Yes | No

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

COMMENTS:

Please return *before* **March 3, 2008**

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 28

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 #26	Liquid frozen (1:1 serum:10% MPA)
VitC #112	Liquid frozen (1:1 serum:10% MPA)
VitC #116	Liquid frozen (1:1 serum:10% MPA)
VitC #119	Liquid frozen (1:1 serum:10% MPA)
CS #1	Liquid frozen (1:1 serum:10% MPA)
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 RR28

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-

May 2, 2008

Dear Colleague:

Enclosed is the summary report of the results for Round Robin 28 (RR 28) for the measurement of total ascorbic acid (TAA, ascorbic acid plus dehydroascorbic acid) in human serum. Included in this report are a summary of data for all laboratories and an individualized summary of your laboratory's measurement performance. The robust median is used to estimate the consensus value for all samples, the "median absolute deviation from the median" (MADe) is used to estimate the expected standard deviation, and the coefficient of variation (CV) is defined as 100×MADe/median.

RR 28 consisted of four *test samples* (#26, #112, #116, and #119), one *serum control material* (CS #1), and one *solid control material* for preparation of TAA control solutions. Details regarding the samples can be found in the enclosed report.

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

Samples for the second vitamin C round robin (RR 29) of the 2007 M^2QAP will be shipped **during the week of May 19, 2008.**

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 Thomas

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

Enclosures

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

NIST

The NIST M^2QAP Vitamin C Round Robin 28 (RR28) 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 RR28 sample measurements.

Page	"All Lab" Report
1	A tabulation of results and summary statistics for Total Ascorbic Acid [TAA] in the RR28 samples and control/calibration solutions.

Serum-based Samples. One serum control and four unknowns were distributed in RR28.

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

S28:1 VitC #26, ampouled in late 2001, previously distributed as sample S17:1 (RR17, Fall 02), S19:2 (RR19, Fall 03), S21:2 (RR21, Fall 04), S22:1 (RR24, Spring 05), and S24:1 (RR24, Spring 06).

S28:2 VitC #112, ampouled in 1993, previously distributed as sample 179B=S04:2 (RR4, 1993) and S26:3 (RR26, Spring 07).

S28:3 VitC #116, ampouled in 1995, previously distributed sample 682a=S08:3 (RR8, 1996), 682a=S10:2 (RR10, 1997), and S25:4 (RR25, Fall 06).

S28:4 VitC #119, ampouled in 1995, previously distributed sample 688b=S07:2 (RR7, Spring 1995), 688b=S08:2 (RR8, 1997), and S24:4 (RR24, Spring 06).

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 -1.1 to 2.1 and slopes range from 0.90 to 1.09).

3) The Measured = p+q*Median regression parameters for samples S28:1 to S28:4 (columns 23 to 26 of the All Lab Report) mostly confirm the linearity of the measurement systems (R^2 close to 1 and RMS close to 0.0). There seems to be no strong relationship between the intercepts and slopes for the calibration solutions and for the regression-on-median for the unknown samples (see Figure 1). This implies that the performance of some measurement systems for [TAA] in 5% MPA:serum is different than it is in just 5% MPA – and that there may well be several causes for these differences.

4) The median and the MADe for Vit C #112 (S28:2), #116 (S28:3) and 119 (S28:4), all ampouled more than 10 years ago, are effectively unchanged from their initial values. The median [TAA] value for VitC #26 (S28:1) is somewhat lower than all previous estimates for this material; it is possible that the [TAA] level in the relatively new material has declined. Since sample #26 has a very low

[TAA] level, it is plausible that it could be less stable than materials with higher [TAA]. It is also plausible that measurements of low [TAA] levels are more susceptible to measurement system limitations (see above) than are the higher levels.

Comparison of the sample #26 measurements with those of the relatively low-[TAA] level control material CS#1 (see Figure 2) suggests measurement sensitivity is at least a contributing factor: participants with the lowest values for sample #26 also reported low values for the control. Since the measurement variability of sample #26 measurements does not appear to have increased with time, if the material has degraded then it degraded to about the same extent in all ampoules of the material. We will continue distribute this material in future studies.

Figure 1: Comparison of Intercepts and Slopes
Between Gravimetric Calibration (Measured = a+b*Participant Gravimetric)and
Regression-on-Median (Measured = p+q*Interlaboratory Median) Models

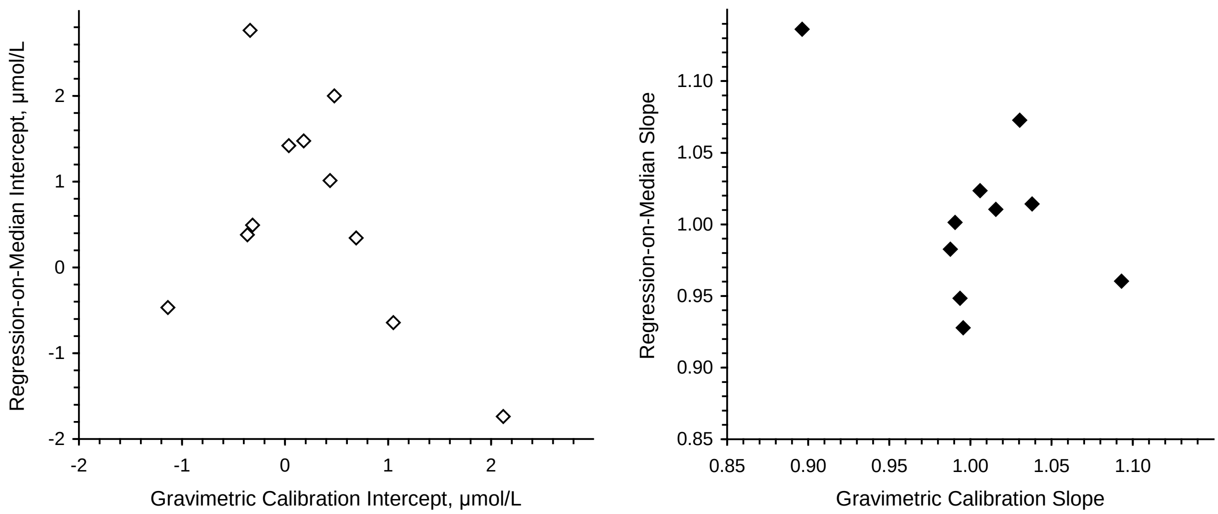
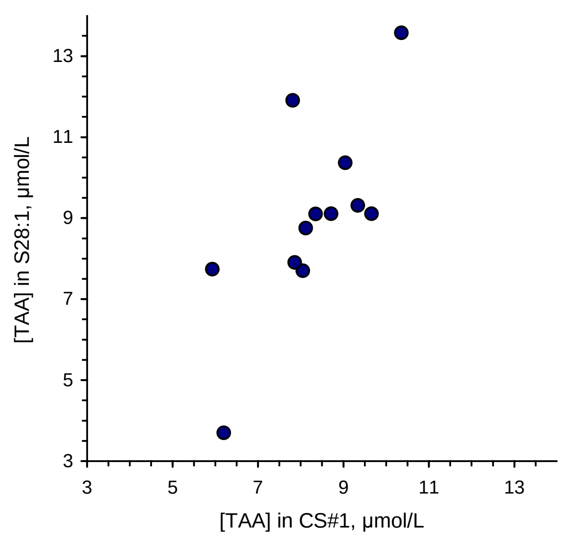


Figure 2: Comparison of Measured [TAA] in CS #1 and Vit C #26 = S28:1



Appendix G. "All-Lab Report" for RR28

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" 28 - March 2008

		Control / Calibration Samples												MPA
		Grav, µmol/L			Measured, µmol/L				Measured = a + b*Grav					Density
Lab	Date	Dil:1	Dil:2	Dil:3	Dil:1	Dil:2	Dil:3	MPA	Inter	Slope		R^2	RMS	g/mL
VC-MA	04/03/08	57.8	28.9	14.4	59.6	31.5	15.8	0.0	0.69	1.03		0.999	1.0	1.035
VC-MB	15/11/07	57.2	28.4	14.1	56.8	27.4	13.7	0.0	-0.31	0.99		1.000	0.5	1.030
VC-MC	05/12/07	54.9	27.5	14.3	55.3	26.3	14.2	0.0	-0.37	1.01		0.999	0.8	1.031
VC-ME	29/02/08	57.5	28.8	14.4	57.0	29.2	15.0	0.0	0.44	0.99		1.000	0.5	1.030
VC-MG	05/02/08	61.6	31.5	17.3	63.1	31.5	19.4	0.0	0.48	1.02		0.999	1.2	1.028
VC-MH	13/02/08	61.1	30.6	15.1	60.8	30.8	15.0	0.0	0.04	1.00		1.000	0.2	1.031
VC-MI	07/02/08	56.4	25.2	14.1	61.1	25.7	13.3	0.0	-1.14	1.09		0.999	1.2	1.029
VC-MJ	03/03/08	58.1	28.5	14.8	60.5	28.3	14.8	0.3	-0.34	1.04		0.999	0.9	1.027
VC-MK	03/01/08	57.6	29.0	14.2	53.5	27.9	16.2	1.2	2.12	0.90		0.998	1.1	1.033
VC-MN	04/03/08	60.8	31.0	15.7	61.2	30.6	18.7	0.0	1.05	0.99		0.997	1.8	1.026
VC-MP	06/03/08													
VC-MU	07/02/08													
VC-NF	27/02/08	59.6	29.4	15.1	59.3	30.0	15.1	0.0	0.18	1.00		1.000	0.5	1.034
N		11	11	11	11	11	11	11			N			11
Average		58.4	29.0	14.9	58.9	29.0	15.5	0.1			Average			1.030
SD		2.1	1.7	0.9	2.9	2.0	1.9	0.4			SD			0.003
Min		54.9	25.16	14.1	53.5	25.70	13.3	0.0			Min			1.026
%25		57.3	28.46	14.3	56.9	27.63	14.5	0.0			%25			1.029
Median		57.8	28.89	14.4	59.6	29.24	15.0	0.0			Median			1.030
%75		60.2	29.98	15.1	60.9	30.69	16.0	0.0			%75			1.032
Max		61.6	31.47	17.3	63.1	31.50	19.4	1.2			Max			1.035
MADe		2.1	0.7	0.5	2.4	2.2	1.2	0.0			eSD			0.0
CV		4	2	4	4	8	8				CV			0.27

	Dilute Solution 1			Samples								
	Spectrophotometry			Measured, µmol/L					Measured = p + q*Median			
Lab	λ_{max}	A_{max}	$E^{1\%}$	CS#1	S28:1	S28:2	S28:3	S28:4	Inter	Slope	R^2	RMS
VC-MA	243.	0.5720	562.2	9.0	10.4	75.0	45.0	29.1	0.34	1.07	1.000	0.5
VC-MB	243.	0.5710	567.1	5.9	7.7	65.0	42.1	26.8	0.49	0.95	0.996	1.9
VC-MC	243.	0.5220	539.4	9.3	9.3	70.9	44.1	28.0	0.38	1.02	1.000	0.6
VC-ME	244.	0.5234	516.7	8.7	9.1	68.6	42.8	28.3	1.01	0.98	0.999	1.0
VC-MG	243.4	0.6200	571.9	9.7	9.1	70.2	47.2	30.4	2.00	1.01	0.992	2.9
VC-MH	243.6	0.5985	555.9	8.4	9.1	65.0	41.6	26.7	1.42	0.93	0.999	1.1
VC-MI				8.1	7.7	64.6	43.6	23.8	-0.47	0.96	0.990	3.0
VC-MJ	*254* [a]	*0.367* [a]	*358.6* [a]	10.4	13.6	74.9	42.0	29.9	2.76	1.01	0.991	3.0
VC-MK	244.	0.5722	564.5	7.8	11.9	80.2	41.0	27.4	-1.74	1.14	0.981	5.0
VC-MN	243.4	0.6369	595.1	8.1	8.8	69.4	40.1	26.7	-0.64	1.00	0.999	1.1
VC-MP				*nd*	6.0	69.3	42.6	26.8	-2.34	1.05	0.998	1.4
VC-MU				6.2	3.7	62.2	37.8	21.9	-4.42	0.97	0.998	1.2
VC-NF	243.	0.5860	558.5	7.9	7.9	59.1	38.7	25.0	1.48	0.85	0.996	1.7
N	9	9	9	12	13	13	13	13				
Average	243.4	0.5780	559.0	8.3	8.8	68.8	42.2	27.0				
SD	0.4	0.0387	21.7	1.3	2.5	5.8	2.5	2.4				
Min	243.0	0.5220	516.7	5.9	3.7	59.1	37.8	21.9				
%25	243.0	0.5710	555.9	7.8	7.7	65.0	41.0	26.7				
Median	243.4	0.5722	562.2	8.2	9.1	69.3	42.1	26.8				
%75	243.6	0.5985	567.1	9.1	9.3	70.9	43.6	28.3				
Max	244.0	0.6369	595.1	10.4	13.6	80.2	47.2	30.4				
eSD	0.6	0.0390	9.3	1.0	1.9	6.4	2.2	2.1				
CV	0.24	6.8	1.7	12	21	9	5	8				

a) 5% Trichloroacetic acid solution

G2

Appendix H. Representative "Individualized Report" for RR28

Each participant in RR28 received an "Individualized Report" reflecting their reported results. The following two pages are the "Individualized Report" for participant "VC-MA".

Vitamin C "Round Robin" 28 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
10/17/05	23	HPLC-EC	1.030	244.0	0.562	567.9	-0.6	1.09	0.998	1.47
03/09/06	24	HPLC-EC	1.031	244.0	0.568	586.7	0.2	1.13	1.000	0.41
08/28/06	25	HPLC-EC	1.039	242.0	0.555	557.4	0.8	0.95	0.999	0.92
03/20/07	26	HPLC-EC	1.033	244.0	0.573	554.3	0.3	1.00	1.000	0.31
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
		Mean	1.034	243.2	0.57	564.3				0.71
		SD	0.003	1.0	0.01	12.0				0.50
		CV	0.32	0.40	1.3	2.1				

[TAA] mmol/Lsample

Date	RR	Sample	Rep_1	Rep_2	F_{adj}	Mean	SD_{dup}	N	Mean	SD_{repeat}	SD_{reprod}
10/17/05	23	CS#1	9.3	9.5	1.0	9.4	0.1	5	8.9	0.1	0.5
03/09/06	24	CS#1	9.3	9.2	1.0	9.3	0.0				
08/28/06	25	CS#1	8.3	8.6	1.0	8.4	0.2				
03/20/07	26	CS#1	8.6	8.3	1.0	8.5	0.2				
03/04/08	28	CS#1	**9.1**	**9.0**	1.0	9.0	0.1				
12/12/02	17	S17:1	9.9	9.1	1.0	9.5	0.6	6	9.5	0.2	0.6
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				
09/16/93	04	S04:2	2.3	2.3	28.4	64.4	0.0	3	73.1	0.5	7.8
03/20/07	26	S26:3	79.7	79.8	1.0	79.8	0.1				
03/04/08	28	S28:2	**75.6**	**74.3**	1.0	75.0	0.9				
ND	08							3	42.5	0.5	2.3
08/20/97	10	S10:2	81.5	80.6	0.5	40.5	0.3				
08/28/06	25	S25:4	42.0	42.2	1.0	42.1	0.2				
03/04/08	28	S28:3	**45.6**	**44.4**	1.0	45.0	0.9				
05/23/95	07	S07:2	51.05	49.75	0.5	25.2	0.5	3	27.8	0.5	2.3
ND	08										
03/09/06	24	S24:4	29.0	29.5	1.0	29.2	0.4				
03/04/08	28	S28:4	**29.5**	**28.7**	1.0	29.1	0.5				

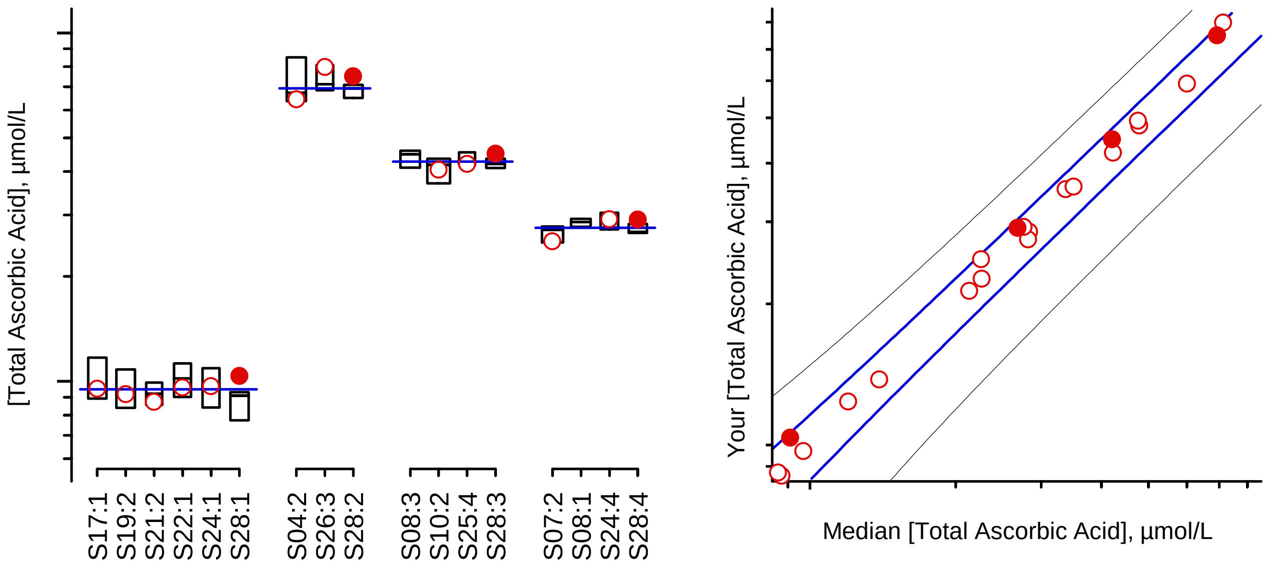
Please check our records against your records. Send corrections and/or updates to...

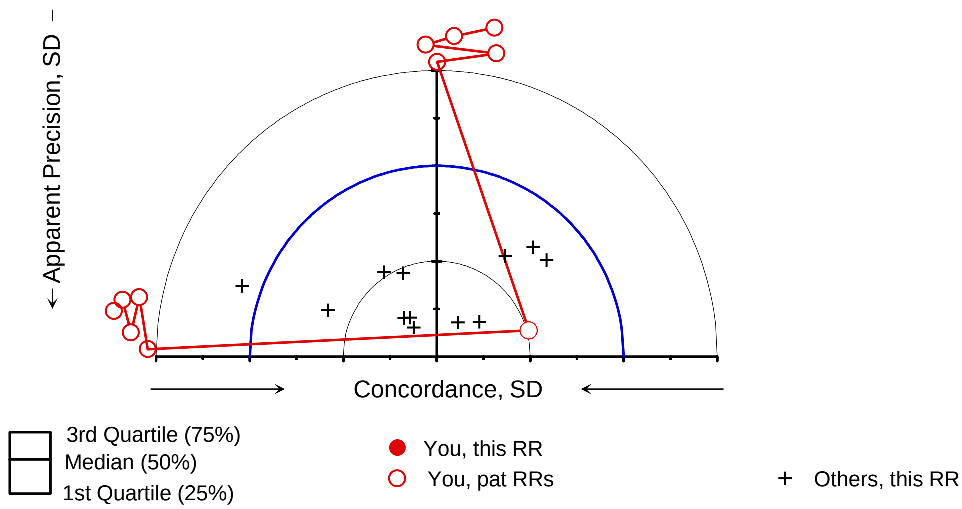
Micronutrients Measurement Quality Assurance Program
National Institute of Standards and Technology
100 Bureau Drive Stop 8392
Gaithersburg, MD 20899-8392 USA

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

Vitamin C "Round Robin" 28 Report: Participant VC-MA

Total Ascorbic Acid





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

Sample	Comments
S28:1	VitC #26, previously distributed in RRs 17 (Sep-02), 19 (Sep-03), 21 (Sep-04), 22 (Mar-05), 24 (Mar-06)
S28:2	VitC #112, previously distributed in RRs 4 (Sep-93), 26 (Mar-07)
S28:3	VitC #116, previously distributed in RRs 8 (Sep-95), 10 (Jan-97), 25 (Sep-06)
S28:4	VitC #119, previously distributed in RRs 7 (Jun-95), 8 (Sep-95), 24 (Mar-06)