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NIST Micronutrients Measurement Quality Assurance Program Winter 2004 Comparability Studies

Results for Round Robin LV Fat-Soluble Vitamins and Carotenoids in Human Serum and Round Robin 20 Ascorbic Acid in Human Serum

David L. Duewer
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National Institute of Standards and Technology
U.S. Department of Commerce

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U.S. Department of Commerce
Cameron F. Kerry, Acting Secretary

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

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Abstract

The National Institute of Standards and Technology coordinates the Micronutrients Measurement Quality Assurance Program (MMQAP) for laboratories that measure fat- and water-soluble vitamins and carotenoids in human serum and plasma. This report describes the design of and results for the Winter 2004 MMQAP measurement comparability improvement studies: 1) Round Robin LV Fat-Soluble Vitamins and Carotenoids in Human Serum and 2) Round Robin 20 Total Ascorbic Acid in Human Serum. The materials for both studies were shipped to participants in November 2003; participants were requested to provide their measurement results by March 5, 2004.

Keywords

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

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Introduction

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

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

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

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

Participants in the MMQAP Fat-Soluble Vitamins and Carotenoids in Human Serum Round Robin LV comparability study (hereafter referred to as RR55) received two lyophilized and three 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 2003. 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 RR55 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 20: Vitamin C in Human Serum

Participants in the MMQAP Vitamin C in Human Serum Round Robin 20 comparability study (hereafter referred to as RR20) received four frozen serum test samples, two frozen control sera, and a solid ascorbic acid control material for analysis. Unless multiple vials were previously requested, participants received one vial of each material. These sample materials were shipped on dry ice to participants in November 2003. 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 RR20 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 RR55

The following three items were included in each package shipped to an RR55 participant:

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

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

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

November 17, 2003

Dear Colleague:

Enclosed are the samples (Sera 299 – 303) for the first fat-soluble vitamins and carotenoids in serum round robin study (Round Robin LV) for the 2004 NIST Micronutrients Measurement Quality Assurance Program. You will find one vial of each of three liquid-frozen and two lyophilized serum samples for analysis along with a form for reporting your results. When reporting your results, please submit one value for each analyte for a given serum sample. If a value is obtained below your limit of quantification, please indicate this result on the form by using NQ *(Not Quantified)*. Results are due to NIST by **March 5, 2004**. Results received more than two weeks after the due date will not be included in the summary report for this round robin study. The feedback report concerning the study will be provided around mid-October.

Lyophilized samples should be reconstituted with 1.0 mL of HPLC-grade water or equivalent. We recommend that dissolution be facilitated with 3 to 5 min agitation in an ultrasonic bath or at least 30 min at room temperature with intermittent swirling. (CAUTION: Vigorous shaking will cause foaming and possibly interfere with accurate measurement. The rubber stopper contains phthalate esters that may leach into the sample upon intermittent contact of the liquid sample with the stopper. These esters absorb strongly in the UV region and elute near retinol in most LC systems creating analytical problems.) Pipette a known volume of serum from the vial for analysis. The final volume of the reconstituted sample is greater than 1.0 mL. **Water should not be added to the liquid-frozen samples 301, 302, and 303.**

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

Please mail or fax your results for Round Robin LV 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 Brown Thomas
Research Chemist
Analytical Chemistry Division
Chemical Science and Technology Laboratory

Enclosures

NIST

Participant #: _____ Date: _____

Round Robin LV

NIST Micronutrients Measurement Quality Assurance Program

Analyte	299	300	301	302	303	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 analytes?						

* we prefer μg/mL

Were sera {301,302,303} frozen when received? Yes | No

Comments:

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

Please return results **before**
5-Mar-2004

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

Participant #: _____ Date: _____

Fat-Soluble Vitamins Round Robin LV

NIST Micronutrients Measurement Quality Assurance Program

Packing List and Shipment Receipt Confirmation Form

This box contains (we hope) one vial each of the following **five** FSV M^2QAP sera:

Serum	Form	Reconstitute?
#299	Lyophilized	Yes (1 ml H_2O)
#300	Lyophilized	Yes (1 ml H_2O)
#301	Liquid frozen	No
#302	Liquid frozen	No
#303	Liquid frozen	No

Please 1) Open the pack immediately
2) Check that it contains one vial each of the above samples
3) Check if sera {301, 302, 303} 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 five vials intact? Yes | No
If "No", which one(s) were damaged?

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

4) Did sera {301, 302, 303} 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^2QAP Gang

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

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

Appendix B. Final Report for RR55

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 20899-0001

May 6, 2004

Dear Colleague:

Enclosed is the summary report of the results for the first round robin (RR 55) of the 2004 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 comparabilities relative to the NIST assigned values. For RR 55 only, the NIST-assigned values are the interlaboratory comparison exercise medians.

Data for evaluating laboratory performance in RR 55 are provided in the comparability summary (Score Card) on page 6 of the All Lab Report. Laboratory comparability is summarized as follows: results rated 1 to 3 are within 1 to 3 standard deviation(s) of the assigned value, respectively; those rated 4 are >3 standard deviations from the assigned value.

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

There were more than 40 attendees at the Micronutrients Measurement Quality Assurance Workshop, which was held at the Experimental Biology '04 meeting on April 21. A summary of the workshop will be sent in a future mailing.

Samples for the second 2004 QA interlaboratory exercises will be shipped **during the week of May 17**. 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

Enclosures

NIST

The NIST M^2QAP Round Robin LIV (RR55) report consists of:

Page	"All Lab" Report
1-4	A listing of all results and statistics for analytes reported by at least two laboratories.
5a	A list of results for the seven analytes reported by only one laboratory.
5b	A legend for the above two lists.
6	The text version of the "Comparability Summary" (or "Score Card").

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 "target" plot version of your "Comparability Summary" scores.

Samples. The five sera below were distributed in RR55.

Serum	Description	Prior Distributions
299	Lyophilized blended serum with native carotenoid levels, augmented with α- and δ-tocopherol; SRM 968c Level II.	#249 in RR44 (9/98), #256 in RR46 (6/99), #264 in RR48 (3/01), #284 in RR52 (9/02)
300	Lyophilized, native, single donor, commercially obtained serum prepared in 2002. The same material was used to prepare #301.	#290 in RR53 (2/03)
301	Fresh-frozen, native, single donor, commercially obtained serum prepared in 2002. The same material was used to prepare #300.	#292 in RR53 (2/03)
302	Fresh-frozen, native, single-donor, commercially obtained serum prepared in 2002. This material has rather low levels of most micronutrients.	#288 in RR52 (9/02), #293 in RR53 (2/03)
303	Fresh-frozen single-donor hemolyzed serum with endogenous augmented carotenoid levels. A gift to the M^2QAP from the CDC.	#287 in RR52 (9/02)

Results

1) Sera Stability. There was no significant change in the median level nor increase in the variability of any measurand in any of the sera. For most measurands in most of the sera, there may be a small decrease in the variability… Since the sera are unlikely to improve with age, we may hope that this indicates a general improvement in measurement concordance among the participants.

2) Matrix (Lyophilized Vs Fresh-Frozen) Differences. Sera 300 and 301 were prepared from the same serum pool. Since we suggest that you reconstitute our lyophilized samples with 1.0 mL water rather than to a total volume of 1.0 mL, the measurand levels in Serum 300 should be ≈ 95% of those in Serum 301. The observed average ratio ±SD over all measurands with 10 or more quantitative measurements is 0.952 ±0.008. If any of your Sera300/301 ratios are much different than 0.95, you may want to take a hard look at your measurement system for those measurands. If your ratios are consistently much different from 0.95, you should review how you reconstitute lyophilized materials.

3) Hemolysis. Serum 303 is somewhat hemolyzed. There was little or no increase in among-participant measurement variability for this serum; however, two participants reported chromatographic anomalies for α-tocopherol. If your results for this material are not consistent with those of the other four sera in RR55, you should evaluate the influence of sample hemolysis on your measurement system.

Appendix C. "All-Lab Report" for RR55

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 LV Laboratory Results

All values in µg/mL

	Total Retinol					trans-Retinol					Retinyl Palmitate					α-Tocopherol					γ/β-Tocopherol					δ-Tocopherol				
Lab	299	300	301	302	303	299	300	301	302	303	299	300	301	302	303	299	300	301	302	303	299	300	301	302	303	299	300	301	302	303
FSV-BA	0.505	0.626	0.662	0.343	0.645						0.097	0.117	0.127	0.023	0.039	16.44	9.70	10.34	2.90	13.4	1.64	1.82	1.93	0.784	2.31					
FSV-BB	0.474	0.607	0.610	0.323	0.617						0.081	0.073	0.080	0.012	0.063	18.44	10.47	10.84	2.87	14.8	1.56	1.67	1.72	0.722	2.10					
FSV-BD	0.473	0.567	0.601	0.288	0.588											16.50	8.60	9.30	2.40	12.6										
FSV-BE	≥0.48	≥0.59	≥0.63	≥0.34	≥0.62	0.480	0.590	0.630	0.340	0.620						16.30	9.60	10.20	3.00	13.0	1.50	1.70	1.80	0.700	2.10					
FSV-BF	0.425	0.575	0.610	0.292	0.591											15.99	9.99	10.84	2.97	14.3	1.64	1.80	1.96	0.680	2.27					
FSV-BG	0.493	0.610	0.643	0.326	0.626						0.085	0.092	0.202	0.034	0.049	16.98	9.90	10.53	2.92	13.4	1.59	1.70	1.79	0.720	2.08					
FSV-BH	0.464	0.548	0.569	0.287	0.513											16.93	10.08	10.30	2.75	11.9										
FSV-BI	0.491	0.618	0.670	0.349	0.645						0.082	0.099	0.113	*nd*	*nd*	18.46	10.78	11.30	3.08	14.2	1.76	1.87	1.98	0.790	2.28					
FSV-BJ	0.482	0.583	0.581	0.315	0.608						0.078	0.079	0.083	*nq*	*nq*	16.47	9.51	9.46	2.82	13.6	1.54	1.61	1.57	0.686	2.18					
FSV-BK	0.517	-	0.656	0.358	0.690											18.40	-	10.10	2.10	15.0										
FSV-BL	0.490	0.690	0.720	0.370	0.690											15.50	10.34	10.34	3.01	-										
FSV-BM	0.477	0.607	0.646	0.352	0.621											17.00	10.60	11.30	3.10	13.1										
FSV-BN	0.461	0.589	0.650	0.323	0.600						0.065	0.076	0.084	0.010	0.017	14.96	9.00	9.91	2.54	11.3	1.46	1.67	1.85	0.710	1.99	0.57	0.099	0.131	0.086	0.192
FSV-BO	≥0.453	≥0.573	≥0.647	≥0.315	≥0.590	0.453	0.573	0.647	0.315	0.590						15.68	8.96	9.56	2.64	11.9	1.38	1.47	1.70	0.654	1.83	0.50	*nq*	*nq*	*nq*	0.077
FSV-BP	0.481	0.595	0.642	0.349	0.629											15.77	10.46	10.19	2.84	12.6										
FSV-BQ	0.548	0.763	0.726	0.373	0.689											15.80	10.30	9.80	2.60	12.0										
FSV-BR	≥0.478	≥0.617	≥0.625	≥0.354	≥0.590	0.478	0.617	0.625	0.354	0.590						16.93	9.78	9.73	3.20	14.0										
FSV-BS	0.341	0.443	0.518	0.285	0.422																									
FSV-BU	0.446	0.599	0.629	!0.382	0.601											16.69	10.06	10.69	!3.058	12.6	1.57	1.80	1.97	!0.781	2.17					
FSV-BV	0.473	0.569	0.616	0.323	0.585											15.93	9.18	9.94	2.96	12.3	1.56	1.69	1.84	0.740	2.12					
FSV-BW	0.440	0.580	0.630	0.330	0.600						0.087	0.105	0.113	0.016	0.023	15.84	9.50	10.33	2.74	12.7	1.38	1.61	1.74	0.670	1.91					
FSV-BX	0.485	0.597	0.646	0.329	0.600											16.66	9.62	10.50	2.84	!6.19	1.52	1.70	1.77	0.716	!1.90					
FSV-CB	0.467	0.521	0.556	0.268	0.513											16.85	9.78	10.68	3.03	11.8										
FSV-CC	0.460	0.590	0.620	0.320	0.580	0.460	0.590	0.620	0.320	0.580						15.42	9.27	9.76	2.83	11.6										
FSV-CD	0.484	0.606	0.654	0.341	0.624						0.104	0.131	0.124	0.020	0.043	16.86	9.25	10.11	2.47	12.8	1.56	1.58	1.77	0.667	2.03					
FSV-CE	0.470	0.670	0.600	0.270	0.490											19.87	8.47	10.45	2.88	16.9										
FSV-CF	0.520	0.631	0.660	0.390	0.626											17.30	10.30	10.90	3.10	13.7										
FSV-CG	0.529	0.634	0.638	0.329	0.618											14.91	9.08	9.84	2.59	10.6	1.60	1.76	1.89	0.730	2.22	0.69	0.147	0.122	0.068	0.262
FSV-CI	0.467	0.613	0.632	0.365	0.625						0.066	0.069	0.075	0.013	0.024	15.73	9.19	9.55	2.55	11.5	1.45	1.60	1.66	0.620	2.05					
FSV-CS	0.487	0.656	0.665	0.353	0.675											16.92	10.24	10.48	2.74	13.6	1.55	1.70	1.76	0.740	2.13					
FSV-CT																														
FSV-CW	≥0.413	≥0.495	≥0.517	≥0.362	≥0.489	0.413	0.495	0.517	0.362	0.489	<0.010	0.020	0.027	0.034	0.026	15.30	8.60	8.70	2.60	10.8	1.60	1.70	1.70	0.800	2.00	0.75	0.090	0.090	0.160	0.160
FSV-CZ	0.530	0.560	0.630	0.310	0.560											18.40	9.50	10.50	2.80	10.8										
FSV-DA	0.473	0.608	0.643	0.333	0.590						0.092	0.102	0.112	0.015	0.022	17.12	10.28	10.82	2.93	11.5	1.66	1.89	1.97	0.733	2.14	0.53	0.051	0.064	0.037	0.097
FSV-DF	0.497	0.566	0.628	0.318	0.591																									
FSV-DI	0.481	0.606	0.636	0.329	0.628						0.078	0.088	0.098	0.015	0.022	17.40	10.20	10.80	2.90	13.6	1.57	1.73	1.82	0.710	2.13	0.63	0.051	0.054	0.040	0.126
FSV-DW	0.540	0.540	0.650	0.340	0.610											15.50	8.50	11.66	3.40	14.0										
FSV-ET	0.470	0.600	0.630	0.310	0.600											15.80	9.40	9.70	2.60	12.5	1.60	1.80	1.90	0.700	2.40					
N	33	32	33	32	33	5	5	5	5	5	11	12	12	10	10	35	34	35	34	33	21	21	21	20	20	6	5	5	5	6
Min	0.341	0.443	0.518	0.268	0.422	0.413	0.495	0.517	0.315	0.489	0.065	0.020	0.027	0.010	0.017	14.91	8.47	8.70	2.10	10.6	1.38	1.47	1.57	0.620	1.83	0.50	0.051	0.054	0.037	0.077
Median	0.481	0.600	0.636	0.329	0.608	0.460	0.590	0.625	0.340	0.590	0.082	0.090	0.105	0.016	0.025	16.50	9.66	10.33	2.84	12.7	1.56	1.70	1.80	0.713	2.13	0.60	0.090	0.090	0.068	0.143
Max	0.548	0.763	0.726	0.390	0.690	0.480	0.617	0.647	0.362	0.620	0.104	0.131	0.202	0.034	0.063	19.87	10.78	11.66	3.40	16.9	1.76	1.89	1.98	0.800	2.40	0.75	0.147	0.131	0.160	0.262
SD	0.024	0.030	0.032	0.026	0.030	0.023	0.030	0.031	0.025	0.030	0.009	0.020	0.025	0.007	0.015	0.89	0.76	0.64	0.27	1.3	0.08	0.10	0.12	0.037	0.11	0.10	0.036	0.043	0.034	0.059
CV	5	5	5	8	5	5	5	5	7	5	10	22	24	43	59	5	8	6	9	10	5	6	7	5	5	16	40	47	50	42
N_{past}	44	33	33	36	38	8	9	9	7	5	15	13	13	10	9	47	38	38	39	39	26	22	22	22	22	6	5	5	5	5
$Median_{past}$	0.482	0.602	0.639	0.326	0.606	0.486	0.620	0.670	0.332	0.616	0.080	0.100	0.100	0.017	0.028	16.64	10.03	10.62	2.93	13.2	1.58	1.80	1.90	0.731	2.21	0.74	0.076	0.059	0.045	0.165
SD_{past}	0.039	0.038	0.055	0.029	0.068	0.043	0.035	0.034	0.017	0.031	0.019	0.020	0.022	0.006	0.008	1.36	0.73	0.77	0.31	1.1	0.13	0.12	0.17	0.085	0.19	0.30	0.009	0.004	0.027	0.059
NIST	0.492	0.629	0.680	0.350	0.624	0.485	0.627	0.680	0.350	0.615						16.99	10.09	10.59	2.85	12.6	1.55	1.73	1.78	0.709	2.23	0.56	0.057	0.042	0.037	0.114
NAV	0.481	0.600	0.632	0.329	0.608	0.469	0.590	0.628	0.341	0.590	0.084	0.092	0.112	0.016	0.026	16.58	9.70	10.33	2.84	12.7	1.57	1.70	1.79	0.716	2.13	0.63	0.071	0.077	0.054	0.126
NAU	0.039	0.048	0.050	0.028	0.048	0.038	0.047	0.050	0.028	0.047	0.023	0.025	0.029	0.011	0.015	1.29	0.76	0.80	0.40	1.3	0.17	0.18	0.19	0.093	0.22	0.11				0.047

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Round Robin LV Laboratory Results

All values in µg/mL

	Total β-Carotene					trans-β-Carotene					Total cis-β-Carotene					Total α-Carotene					Total Lycopene				
Lab	299	300	301	302	303	299	300	301	302	303	299	300	301	302	303	299	300	301	302	303	299	300	301	302	303
FSV-BA	0.435	0.121	0.129	0.051	1.537	0.408	0.115	0.124	0.051	1.474	0.028	0.006	0.005	0.002	0.062	0.088	0.069	0.073	0.005	0.031					
FSV-BB	0.432	0.098	0.109	0.048	1.462	0.400	0.098	0.109	0.048	1.392	0.031	0.004	0.005	0.002	0.070	0.091	0.058	0.067	0.004	0.037	0.408	0.416	0.45	0.187	0.429
FSV-BD																									
FSV-BE	0.440	0.130	0.120	0.050	1.530																				
FSV-BF	0.421	0.122	0.140	0.062	1.811											0.111	0.093	0.105	0.020	0.033	0.403	0.506	0.57	0.203	0.502
FSV-BG	0.481	0.151	0.162	0.065	1.598											0.102	0.076	0.086	0.011	0.036	0.446	0.492	0.59	0.201	0.464
FSV-BH	0.413	0.105	0.110	0.044	1.223	0.389	0.105	0.110	0.044	1.167	0.024	*nq*	*nq*	*nq*	0.057	0.080	0.063	0.066	*nq*	*nq*	0.415	0.473	0.50	0.177	0.378
FSV-BI	0.460	0.127	0.135	0.056	1.490											0.092	0.071	0.078	*nd*	0.031	0.360	0.401	0.43	0.164	0.370
FSV-BJ	0.483	0.113	0.118	0.054	1.613											0.116	0.083	0.081	*nq*	0.028	0.504	0.564	0.57	0.213	0.492
FSV-BK																									
FSV-BL																									
FSV-BM																									
FSV-BN	0.415	0.104	0.113	0.042	1.397	0.377	0.104	0.113	0.042	1.316	0.038	*nq*	*nd*	*nd*	0.082	0.091	0.072	0.079	*nq*	0.023	0.369	0.419	0.48	0.159	0.360
FSV-BO	0.440	0.098	0.103	0.044	1.574											0.088	0.068	0.068	*nq*	0.027	0.394	0.462	0.50	0.188	0.413
FSV-BP	0.416	0.140	0.131	0.050	1.465											0.077	0.063	0.062	0.006	0.029	0.431	0.472	0.48	0.197	0.416
FSV-BQ																									
FSV-BR																									
FSV-BS	≥0.352	≥0.138	≥0.122	≥0.046	≥1.371	0.352	0.138	0.122	0.046	1.371						0.081	0.091	0.079	0.001	0.030	0.412	0.678	0.61	0.149	0.452
FSV-BU	0.416	0.135	0.134	!0.073	1.559											0.089	0.070	0.072	!0.0112	0.029	0.395	0.443	0.45	!0.176	0.401
FSV-BV	0.400	0.108	0.114	0.049	1.361											0.067	0.054	0.058	0.005	0.022	0.420	0.475	0.51	0.173	0.412
FSV-BW	0.430	0.102	0.111	0.040	1.580											0.105	0.094	0.103	*nq*	*nq*	0.440	0.510	0.58	0.180	0.224
FSV-BX	≥0.405	≥0.112	≥0.122	≥0.051	≥1.242	0.405	0.112	0.122	0.051	1.242						0.107	0.079	0.081	0.006	0.028					
FSV-CB	0.464	0.118	0.133	0.056	1.592											0.074	0.054	0.061	0.004	0.018	0.336	0.354	0.41	0.130	0.323
FSV-CC																									
FSV-CD	0.428	0.103	0.105	0.044	1.353											0.125	0.096	0.096	*nd*	0.037	0.454	0.463	0.46	0.168	0.485
FSV-CE	0.800	0.080	0.150	0.070	3.250																				
FSV-CF																									
FSV-CG	0.374	0.104	0.113	0.044	1.240	0.343	0.098	0.107	0.041	1.164	0.031	0.005	0.006	0.003	0.077	0.109	0.084	0.091	0.005	0.036	0.408	0.459	0.50	0.176	0.413
FSV-CI	≥0.440	≥0.097	≥0.095	≥0.036	≥1.240	0.440	0.097	0.095	0.036	1.240						0.089	0.061	0.059	<0.016	0.022					
FSV-CS	0.446	0.126	0.127	0.054	1.409	0.405	0.116	0.119	0.049	1.300	0.043	0.009	0.009	0.005	0.108	0.100	0.079	0.080	0.005	0.030	0.504	0.575	0.59	0.221	0.496
FSV-CT	0.331	0.079	0.089	0.035	1.425																0.371	0.445	0.49	0.152	0.406
FSV-CW	≥0.340	≥0.099	≥0.101	≥0.056	≥1.091	0.340	0.099	0.101	0.056	1.091						0.086	0.067	0.068	<0.007	0.029					
FSV-CZ	0.490	0.160	0.180	0.004	1.150																				
FSV-DA	0.425	0.121	0.129	0.057	1.343	0.383	0.115	0.121	0.052	1.270	0.042	0.007	0.007	0.005	0.075	0.097	0.077	0.082	0.006	0.028	0.425	0.498	0.52	0.199	0.397
FSV-DF																									
FSV-DI	0.456	0.108	0.120	0.046	1.489																0.413	0.439	0.49	0.180	0.386
FSV-DW	0.320	0.050	0.130	0.090	1.100																0.300	0.080	0.33	0.150	0.230
FSV-ET	0.410	0.110	0.110	0.050	1.440																				
N	25	25	25	24	25	11	11	11	11	11	7	5	5	5	7	22	22	22	12	20	21	21	21	20	21
Min	0.320	0.050	0.089	0.004	1.100	0.340	0.097	0.095	0.036	1.091	0.024	0.004	0.005	0.002	0.057	0.067	0.054	0.058	0.001	0.018	0.300	0.080	0.33	0.130	0.224
Median	0.430	0.110	0.120	0.050	1.465	0.389	0.105	0.113	0.048	1.270	0.031	0.006	0.006	0.003	0.075	0.091	0.072	0.078	0.005	0.029	0.412	0.463	0.50	0.179	0.412
Max	0.800	0.160	0.180	0.090	3.250	0.440	0.138	0.124	0.056	1.474	0.043	0.009	0.009	0.005	0.108	0.125	0.096	0.105	0.020	0.037	0.504	0.678	0.61	0.221	0.502
SD	0.030	0.017	0.016	0.009	0.158	0.030	0.012	0.010	0.006	0.104	0.008	0.001	0.002	0.002	0.010	0.013	0.013	0.011	0.001	0.003	0.027	0.044	0.08	0.026	0.055
CV	7	16	13	17	11	8	11	9	12	8	25	19	29	69	13	15	19	14	17	10	7	9	16	14	13
N_{past}	32	27	27	28	27	15	14	14	13	13	10	8	8	7	9	27	23	23	17	22	26	22	22	22	21
$Median_{past}$	0.422	0.113	0.119	0.050	1.405	0.385	0.110	0.118	0.048	1.330	0.030	0.007	0.008	0.003	0.073	0.097	0.070	0.074	0.005	0.030	0.410	0.495	0.51	0.191	0.422
SD_{past}	0.050	0.010	0.019	0.008	0.163	0.034	0.008	0.013	0.006	0.134	0.009	0.003	0.002	0.002	0.010	0.019	0.011	0.014	0.004	0.008	0.072	0.071	0.06	0.026	0.053
NIST	0.454	0.112	0.116	0.050	1.488	0.426	0.112	0.115	0.050	1.509	0.043	*nd*	0.002	*nd*	*0.083*	0.085	0.083	0.081	0.004	0.035	0.472	0.548	0.57	0.212	0.465
NAV	0.431	0.112	0.124	0.050	1.477	0.395	0.108	0.115	0.049	1.256	0.031	0.006	0.006	0.003	0.072	0.091	0.071	0.078	0.005	0.029	0.413	0.468	0.50	0.180	0.413
NAU	0.061	0.019	0.020	0.011	0.189	0.042	0.014	0.014	0.009	0.129	0.011	0.003	0.003	0.003	0.032	0.026	0.021	0.023	0.003	0.010	0.088	0.098	0.10	0.045	0.088

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Round Robin LV Laboratory Results

All values in µg/mL

	trans-Lycopene					Total β-Cryptoxanthin					Total α-Cryptoxanthin					Total Lutein					Total Zeaxanthin					Total Lutein&Zeaxanthin				
Lab	299	300	301	302	303	299	300	301	302	303	299	300	301	302	303	299	300	301	302	303	299	300	301	302	303	299	300	301	302	303
FSV-BA	0.217	0.269	0.293	0.101	0.220	0.040	0.063	0.068	0.023	0.125																0.109	0.103	0.111	0.063	0.152
FSV-BB	0.177	0.209	0.226	0.083	0.176	0.031	0.048	0.051	0.018	0.104	0.013	0.020	0.022	0.010	0.029	0.092	0.083	0.087	0.047	0.108	0.028	0.044	0.041	0.020	0.066	0.120	0.127	0.129	0.068	0.174
FSV-BD																														
FSV-BE																														
FSV-BF						0.022	0.041	0.049	0.015	0.106																0.099	0.111	0.121	0.058	0.164
FSV-BG	0.219	0.288	0.352	0.110	0.229	0.035	0.054	0.065	0.019	0.127						0.098	0.102	0.113	0.056	0.141	0.024	0.020	0.034	0.024	0.043	0.107	0.110	0.127	0.066	0.156
FSV-BH						0.043	0.068	0.069	0.020	0.131						0.070	0.058	0.065	0.039	0.066	0.024	0.029	0.032	0.019	0.057	0.094	0.087	0.097	0.058	0.123
FSV-BI						0.031	0.060	0.064	0.019	0.129						0.088	0.090	0.094	0.046	0.120	0.032	0.040	0.043	0.023	0.072	*0.120*	*0.130*	*0.137*	*0.069*	*0.192*
FSV-BJ						0.030	0.057	0.059	0.021	0.127						0.088	0.088	0.087	0.047	0.141										
FSV-BK																														
FSV-BL																														
FSV-BM																														
FSV-BN	0.192	0.240	0.279	0.090	0.182	0.024	0.050	0.056	0.012	0.112	0.008	0.019	0.021	0.004	0.027	0.065	0.059	0.065	0.034	0.080	*nd*	0.035	0.038	0.022	0.092	*≥0.065*	*0.094*	*0.103*	*0.056*	*0.172*
FSV-BO						0.028	0.051	0.049	0.015	0.097						0.089	0.080	0.076	0.046	0.108	0.031	0.036	0.037	0.023	0.069	0.120	0.116	0.113	0.069	0.176
FSV-BP						0.031	0.052	0.052	0.019	0.103																0.109	0.110	0.104	0.051	0.170
FSV-BQ																														
FSV-BR																														
FSV-BS						0.030	0.064	0.058	0.011	0.093																0.097	0.132	0.121	0.075	0.133
FSV-BU						0.040	0.060	0.063	!0.034	0.106																0.099	0.108	0.118	!0.059	0.193
FSV-BV						0.021	0.040	0.044	0.013	0.086																0.097	0.100	0.108	0.057	0.151
FSV-BW						0.025	0.052	0.048	0.050	0.100																0.083	0.085	0.090	0.053	0.150
FSV-BX	0.179	0.227	0.242	0.083	0.141	0.027	0.047	0.051	0.017	0.090						0.085	0.078	0.087	0.044	0.112	0.019	0.032	0.029	0.019	0.065	0.104	0.110	0.116	0.062	0.177
FSV-CB						0.022	0.042	0.046	0.015	0.098						-	-	-	-	0.067	-	-	-	-	0.044	0.080	0.084	0.089	0.053	0.111
FSV-CC																														
FSV-CD						0.032	0.058	0.065	0.021	0.100																0.107	0.111	0.125	0.061	0.153
FSV-CE																														
FSV-CF																														
FSV-CG	0.217	0.266	0.293	0.096	0.197																					0.130	0.140	0.151	0.076	0.210
FSV-CI																0.069	0.055	0.054	<0.033	0.055	0.021	0.024	0.024	0.013	0.053	0.090	0.079	0.078	<0.046	0.108
FSV-CS						0.034	0.051	0.051	0.017	0.093						0.090	0.084	0.086	0.047	0.086	0.019	0.030	0.030	0.017	0.048	0.109	0.114	0.116	0.064	0.134
FSV-CT						0.024	0.037	0.041	0.014	0.108						0.133	0.117	0.112	0.063	0.167	0.056	0.051	0.047	0.020	0.093	0.188	0.168	0.159	0.082	0.260
FSV-CW	0.167	0.208	0.218	0.096	0.151	0.036	0.052	0.052	0.026	0.091						0.064	0.062	0.063	0.032	0.076	0.024	0.024	0.024	0.014	0.054	*0.088*	*0.086*	*0.087*	*0.046*	*0.130*
FSV-CZ																														
FSV-DA	0.221	0.287	0.306	0.109	0.203	0.041	0.061	0.064	0.022	0.109	0.017	0.028	0.030	0.012	0.032	0.072	0.064	0.066	0.036	0.079	0.036	0.041	0.046	0.023	0.062	0.122	0.118	0.123	0.064	0.159
FSV-DF																														
FSV-DI																0.093	0.096	0.101	0.055	0.155										
FSV-DW																0.100	0.080	0.090	0.070	0.110										
FSV-ET																														
N	8	8	8	8	8	21	21	21	20	21	3	3	3	3	3	15	15	15	14	16	11	12	12	12	13	21	22	22	20	22
Min	0.167	0.208	0.218	0.083	0.141	0.021	0.037	0.041	0.011	0.086	0.008	0.019	0.021	0.004	0.027	0.064	0.055	0.054	0.032	0.055	0.019	0.020	0.024	0.013	0.043	0.080	0.079	0.078	0.046	0.108
Median	0.204	0.253	0.286	0.096	0.190	0.031	0.052	0.052	0.019	0.104	0.013	0.020	0.022	0.010	0.029	0.088	0.080	0.087	0.047	0.108	0.024	0.033	0.036	0.020	0.062	0.107	0.110	0.116	0.063	0.158
Max	0.221	0.288	0.352	0.110	0.229	0.043	0.068	0.069	0.050	0.131	0.017	0.028	0.030	0.012	0.032	0.133	0.117	0.113	0.070	0.167	0.056	0.051	0.047	0.024	0.093	0.188	0.168	0.159	0.082	0.260
SD	0.029	0.038	0.044	0.011	0.028	0.007	0.009	0.011	0.004	0.011						0.016	0.019	0.020	0.009	0.035	0.007	0.009	0.009	0.003	0.012	0.017	0.016	0.016	0.008	0.028
CV	14	15	15	11	15	24	17	21	24	10						18	24	23	20	32	28	28	25	17	19	16	15	14	14	18
N_{past}	12	11	11	11	11	27	23	23	23	23	6	4	0	7	5	16	15	15	17	17	14	13	13	14	14	26	23	23	22	20
$Median_{past}$	0.209	0.243	0.251	0.091	0.192	0.032	0.050	0.055	0.019	0.105	0.017	0.021		0.010	0.032	0.084	0.076	0.078	0.044	0.136	0.024	0.030	0.030	0.019	0.065	0.110	0.104	0.109	0.065	0.178
SD_{past}	0.038	0.047	0.061	0.015	0.042	0.008	0.006	0.009	0.003	0.022	0.006			0.002	0.017	0.014	0.015	0.012	0.014	0.053	0.007	0.006	0.008	0.003	0.014	0.024	0.019	0.022	0.014	0.051
NIST						0.035	0.066	0.069	0.020	0.116	0.024	0.032	0.034	0.015	0.056	0.083	0.091	0.096	0.048	0.120	0.037	0.044	0.041	0.023	0.059	0.102	0.124	0.120	0.064	0.179
NAV	0.217	0.266	0.293	0.096	0.197	0.031	0.052	0.052	0.019	0.104						0.088	0.082	0.087	0.047	0.108	0.024	0.032	0.034	0.020	0.060	0.107	0.110	0.116	0.063	0.156
NAU	0.039	0.048	0.053	0.017	0.036	0.008	0.013	0.013	0.005	0.023						0.017	0.016	0.018	0.010	0.039	0.007	0.011	0.010	0.006	0.016	0.022	0.023	0.024	0.013	0.033

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Round Robin LV Laboratory Results

All values in µg/mL

	Total Cryptoxanthin					Coenzyme Q10					Phylloquinone (K1) x1000				
Lab	299	300	301	302	303	299	300	301	302	303	299	300	301	302	303
FSV-BA															
FSV-BB	0.044	0.068	0.073	0.028	0.133										
FSV-BD															
FSV-BE															
FSV-BF															
FSV-BG															
FSV-BH															
FSV-BI															
FSV-BJ						0.911	0.697	0.727	0.426	1.076					
FSV-BK															
FSV-BL															
FSV-BM															
FSV-BN															
FSV-BO															
FSV-BP															
FSV-BQ															
FSV-BR															
FSV-BS															
FSV-BU															
FSV-BV															
FSV-BW						0.950	0.710	0.790	0.500	1.190					
FSV-BX															
FSV-CB															
FSV-CC															
FSV-CD															
FSV-CE															
FSV-CF															
FSV-CG	0.0509	0.0758	0.0818	0.0268	0.14										
FSV-CI											0.97	0.34	0.29	0.20	0.78
FSV-CS															
FSV-CT															
FSV-CW						0.900	0.600	0.600	0.500	1.100					
FSV-CZ						1.270	0.790	0.890	0.570	1.020					
FSV-DA	0.058	0.088	0.094	0.033	0.141										
FSV-DF															
FSV-DI						1.020	0.694	0.790	0.480	1.110	0.70	0.22	0.21	0.11	0.54
FSV-DW															
FSV-ET															
N	3	3	3	3	3	5	5	5	5	5	2	2	2	2	2
Min	0.04	0.07	0.07	0.03	0.13	0.900	0.600	0.600	0.426	1.020	0.70	0.22	0.21	0.11	0.54
Median	0.05	0.08	0.08	0.03	0.14	0.950	0.697	0.790	0.500	1.100	0.84	0.28	0.25	0.16	0.66
Max	0.06	0.09	0.09	0.03	0.14	1.270	0.790	0.890	0.570	1.190	0.97	0.34	0.29	0.20	0.78
SD						0.081	0.035	0.047	0.025	0.055					
CV						9	5	6	5	5					
N_{past}	0	0	0	0	0	0	4	4	7	0	0	0	0	0	0
$Median_{past}$							0.780	0.830	0.455						
SD_{past}															
NIST															
NAV	0.051	0.076	0.082	0.028	0.139	0.950	0.697	0.790	0.500	1.100					
NAU						0.081	0.035	0.047	0.025	0.055					

C5

Round Robin LV Laboratory Results

All values in µg/mL

Analytes Reported By One Laboratory

Analyte	Code	299	300	301	302	303
Retinyl stearate	FSV-DA	0.031	0.035	0.045	0.005	0.008
trans-Lutein	FSV-BB		0.068			
Ubiquinol	FSV-BW	0.720	0.490	0.580	0.420	0.860
Ubiquinone	FSV-BW	0.230	0.220	0.210	0.080	0.330
25-hydroxyvitamin D	FSV-CF	<0.007	<0.007	0.0070	0.0090	0.0210
Phytofluene	FSV-DA	0.070	0.107	0.120	0.035	0.029
Phytoene	FSV-DA	0.060	0.125	0.134	0.027	0.031

Legend

Term	Definition
N	Number of (non-NIST) quantitative values reported for this analyte
Min	Minimum (non-NIST) quantitative value reported
Median	Median (non-NIST) quantitative value reported
Max	Maximum (non-NIST) quantitative value reported
SD	Adjusted median absolute deviation from the median of the non-NIST results
CV	Coefficient of Variation for (non-NIST) results: 100*SD/Median
N_{past}	Mean of N(s) from past RR(s)
$Median_{past}$	Mean of Median(s) from past RR(s)
SD_{past}	Pooled SD from past RR(s)
NIST	Mean of NIST results
NAV	NIST Assigned Value = (Median + NIST)/2 for analytes reported by NIST = 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, SD_{past}, eSD) and S_{btw} is the standard deviation between Median and NIST. 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
≥x	Concentration greater than or equal to x
italics	Not explicitly reported but calculated by NIST from reported values

Round Robin LV 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		2	1	1	1
FSV-BB	1	2	1	1	1	1	1	1	2	1	1
FSV-BD	1	2									
FSV-BE	1	1	1	1							1
FSV-BF	1	1	1	2		3	1	1			
FSV-BG	1	1	1	2		1	1	1			1
FSV-BH	2	1		1	1	1	1	2	1	1	1
FSV-BI	1	2	1	1		1	1	1	2	1	1
FSV-BJ	1	1	1	1		1	1	1	1	1	1
FSV-BK	2	2									
FSV-BL	2	1							1		
FSV-BM	1	1									
FSV-BN	1	1	1	1	1	1	1	1			2
FSV-BO	1	1	2	1		1	1	1			1
FSV-BP	1	1		1		1	1	1	2	2	2
FSV-BQ	3	1									1
FSV-BR	1	1							2	3	3
FSV-BS	4			2	2	1	2	1	1	1	1
FSV-BU	1	1	1	1		1	1	1			
FSV-BV	1	1	1	1		1	1	1			
FSV-BW	1	1	1	1		1	2	3			2
FSV-BX	1	1	1	1	1	1		1			
FSV-CB	2	1		1		1	1	1			1
FSV-CC	1	1									1
FSV-CD	1	1	1	1		2	1	1	1	2	1
FSV-CE	2	3		4							1
FSV-CF	2	1									
FSV-CG	1	2	1	1	1	1	1				1
FSV-CI	1	1	1	2	2	2					
FSV-CS	1	1	1	1	1	1	1	1	1	1	1
FSV-CT				2			1	1	2	1	2
FSV-CW	3	2	1	2	2	1		1			
FSV-CZ	1	2		4							
FSV-DA	1	1	1	1	1	1	1	1	2		
FSV-DF	1								1		
FSV-DI	1	1	1	1			1		2	1	2
FSV-DW	2	2		3			3				
FSV-ET	1	1	1	1							
NISTa	1	1	1	1	2	1	1	1	1	2	1
n	38	36	22	30	12	23	22	22	16	13	23

	TR	aT	g/bT	bC	tbC	aC	TLy	TbX	TLu	TZ	L&Z
% 1	74	75	95	70	67	87	86	86	56	69	74
% 2	18	22	5	20	33	9	9	9	44	23	22
% 3	5	3	0	3	0	4	5	5	0	8	4
% 4	3	0	0	7	0	0	0	0	0	0	0

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

We define CS as follows:

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

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

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

NAU = NIST Assigned Uncertainty

For further details, please see

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

Appendix D. Representative "Individualized Report" for RR55

Each participant in RR55 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 RR55:

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

Individualized Round Robin LV Report: FSV-BA

Summary

	Serum 299			Serum 300			Serum 301			Serum 302			Serum 303		
Analyte	You	NAV	n	You	NAV	n	You	NAV	n	You	NAV	n	You	NAV	n
Total Retinol	0.505	0.481	33	0.626	0.600	32	0.662	0.632	33	0.343	0.329	32	0.645	0.608	33
Retinyl Palmitate	0.10	0.08	11	0.1	0.1	12	0.1	0.1	12	0.02	0.02	10	0.04	0.03	10
α-Tocopherol	16.44	16.58	35	9.70	9.70	34	10.34	10.33	35	2.90	2.84	34	13.40	12.74	33
γ/β-Tocopherol	1.639	1.565	21	1.818	1.700	21	1.925	1.794	21	0.784	0.716	20	2.312	2.130	20
Total β-Carotene	0.435	0.431	25	0.121	0.112	25	0.129	0.124	25	0.051	0.050	24	1.537	1.477	25
trans-β-Carotene	0.408	0.395	11	0.115	0.108	11	0.124	0.115	11	0.051	0.049	11	1.474	1.256	11
Total cis-β-Carotene	0.028	0.031	7	0.006	0.006	5	0.005	0.006	5	0.002	0.003	5	0.062	0.072	7
Total α-Carotene	0.088	0.091	22	0.069	0.071	22	0.073	0.078	22	0.005	0.005	12	0.031	0.029	20
trans-Lycopene	0.217	0.217	8	0.269	0.266	8	0.293	0.293	8	0.101	0.096	8	0.220	0.197	8
Total β-Cryptoxanthin	0.040	0.031	21	0.063	0.052	21	0.068	0.052	21	0.023	0.019	20	0.125	0.104	21
Total Lutein&Zeaxanthin	0.109	0.107	21	0.103	0.110	22	0.111	0.116	22	0.063	0.063	20	0.152	0.156	22

D2

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

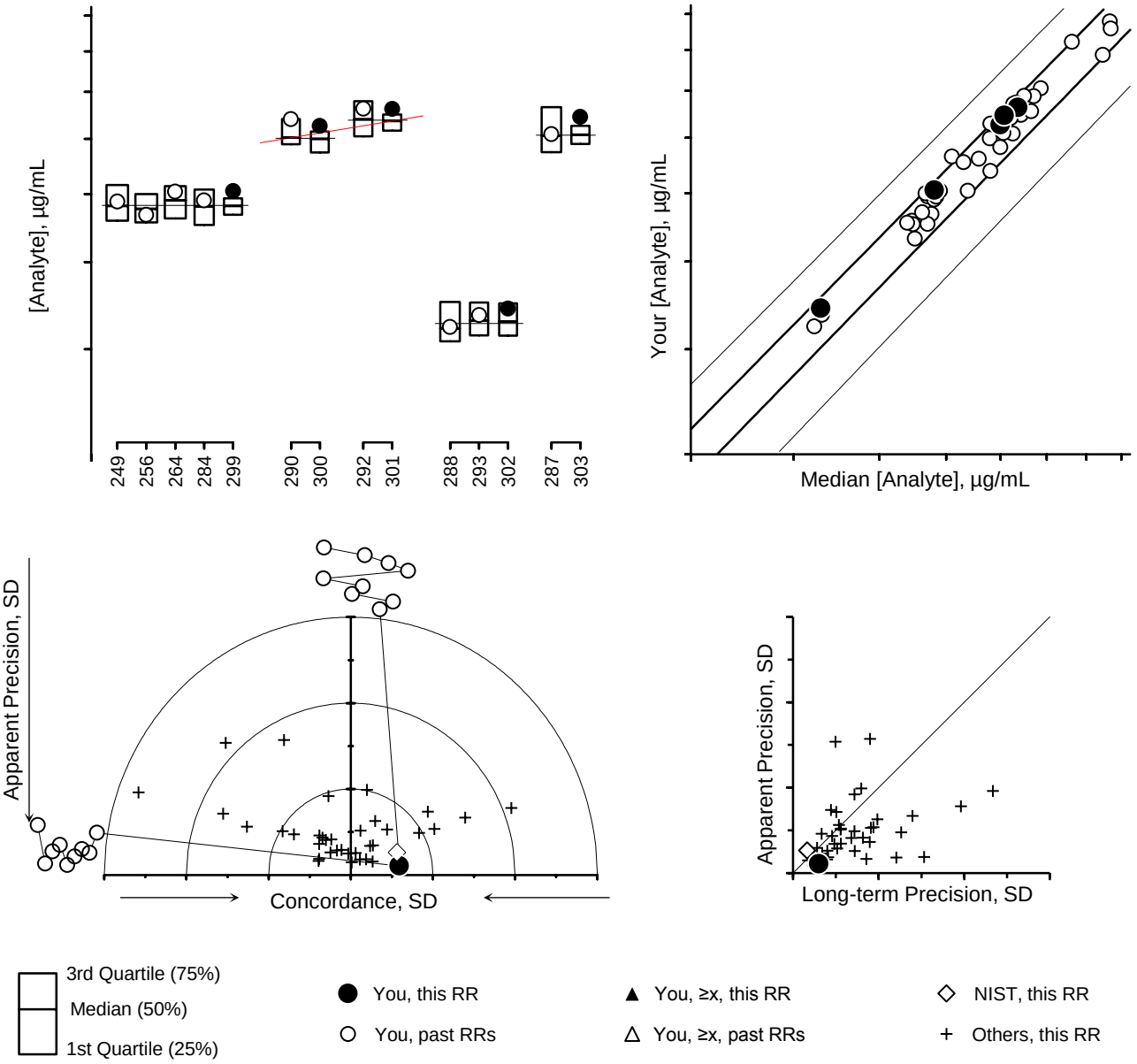
Please check our records against your records. Send corrections and/or updates to...

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 LV Report: FSV-BA

Total Retinol

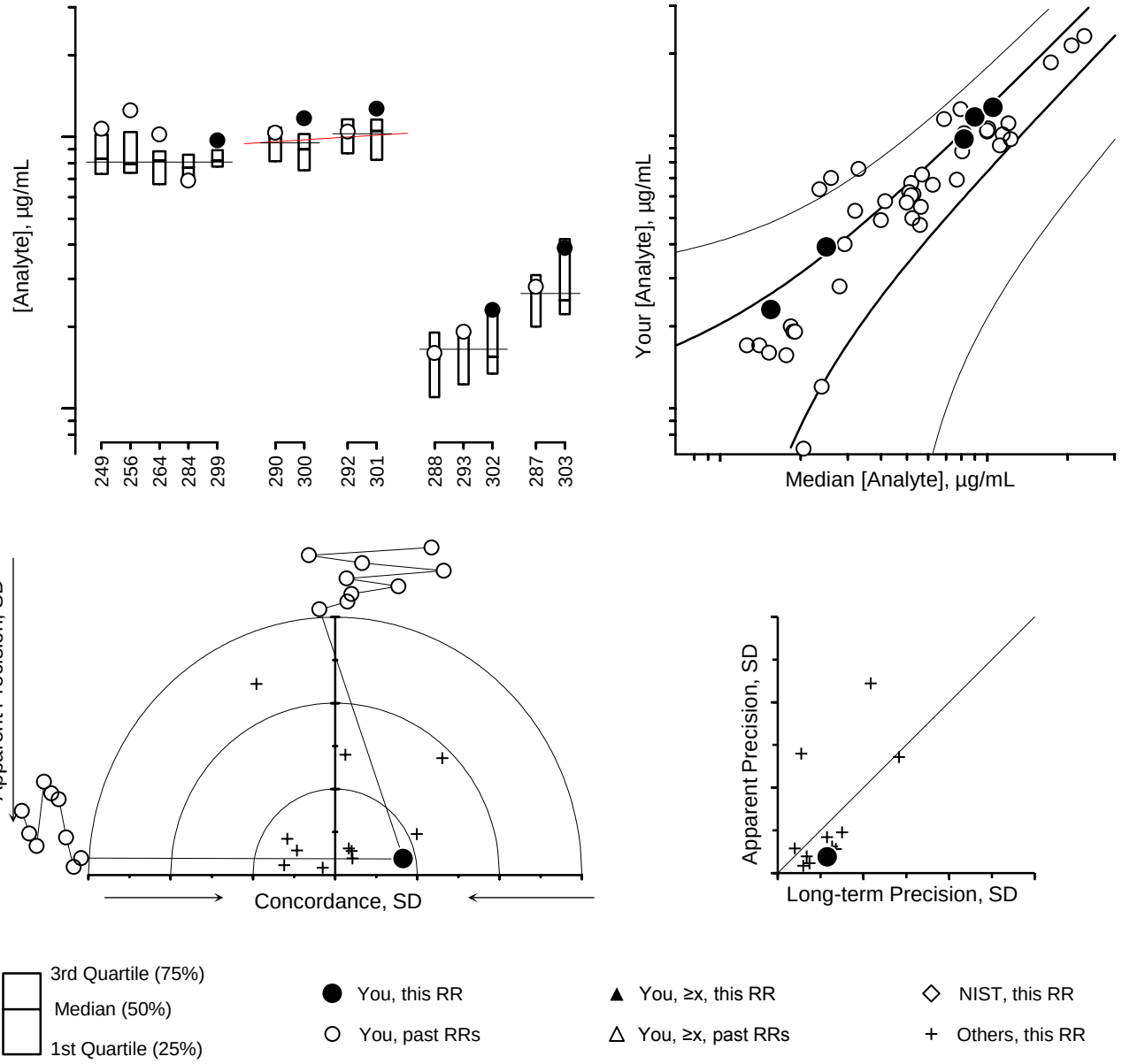


For details of the construction and interpretation of these plots, see:
Duewer, Kline, Sharpless, Brown Thomas, Gary, Sowell. Anal Chem 1999;71(9):1870-8.

Serum	History	Comments
#299	Lyophilized: #249(44), #256(46), #264(48), #284(52)	Augmented, multi-source (SRM 968c Level II)
#300	Lyophilized: #290(RR53)	Native, single-source
#301	Fresh-frozen: #292(RR53)	Same material as #300
#302	Fresh-frozen: #293(RR53)	Native, single-source
#303	Fresh-frozen: #287(RR52)	Native, single-source, hemolyzed

Individualized RR LV Report: FSV-BA

Retinyl Palmitate

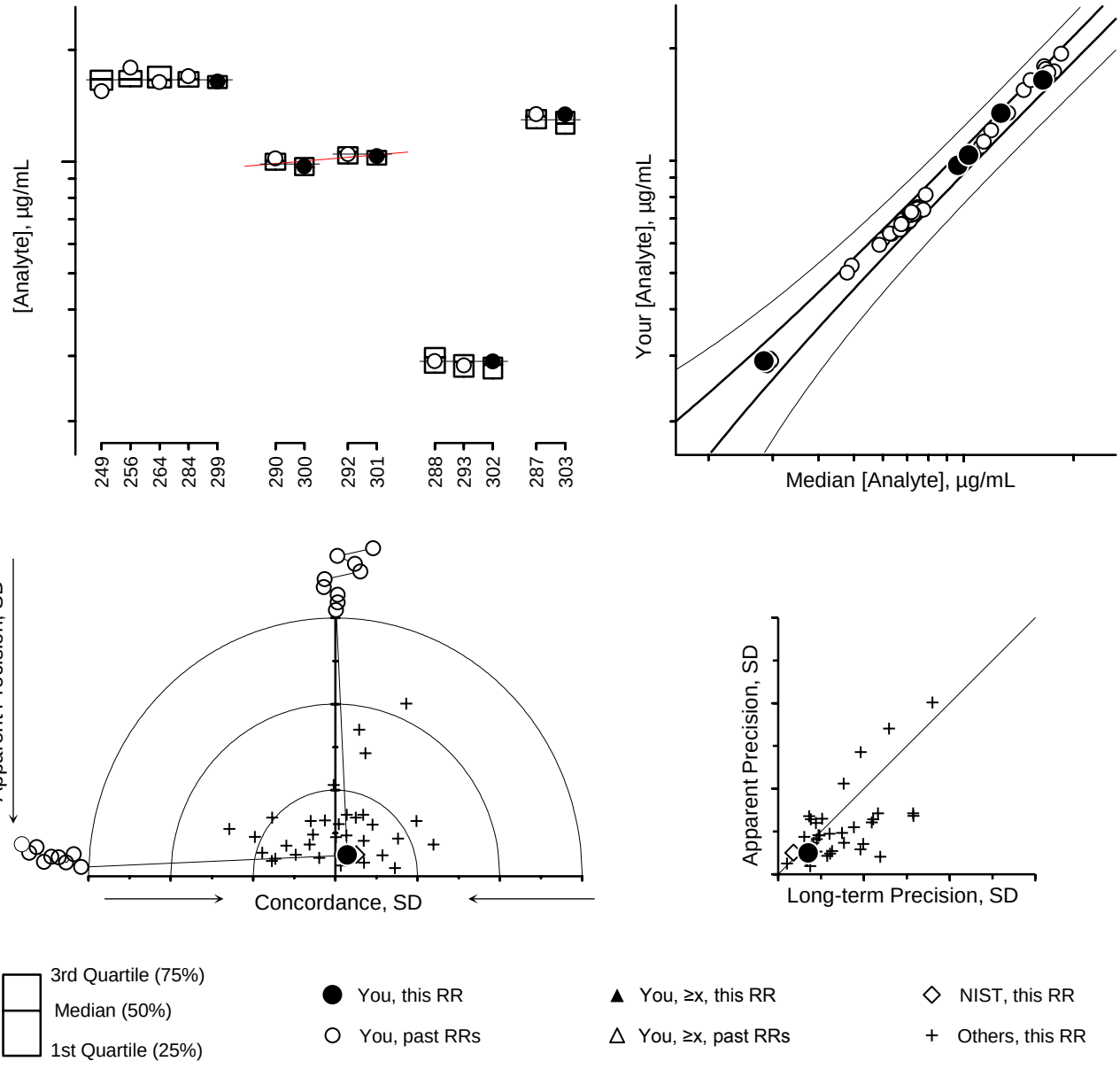


For details of the construction and interpretation of these plots, see:
Duewer, Kline, Sharpless, Brown Thomas, Gary, Sowell. Anal Chem 1999;71(9):1870-8.

Serum	History	Comments
#299	Lyophilized: #249(44), #256(46), #264(48), #284(52)	Augmented, multi-source (SRM 968c Level II)
#300	Lyophilized: #290(RR53)	Native, single-source
#301	Fresh-frozen: #292(RR53)	Same material as #300
#302	Fresh-frozen: #293(RR53)	Native, single-source
#303	Fresh-frozen: #287(RR52)	Native, single-source, hemolyzed

Individualized RR LV Report: FSV-BA

α-Tocopherol

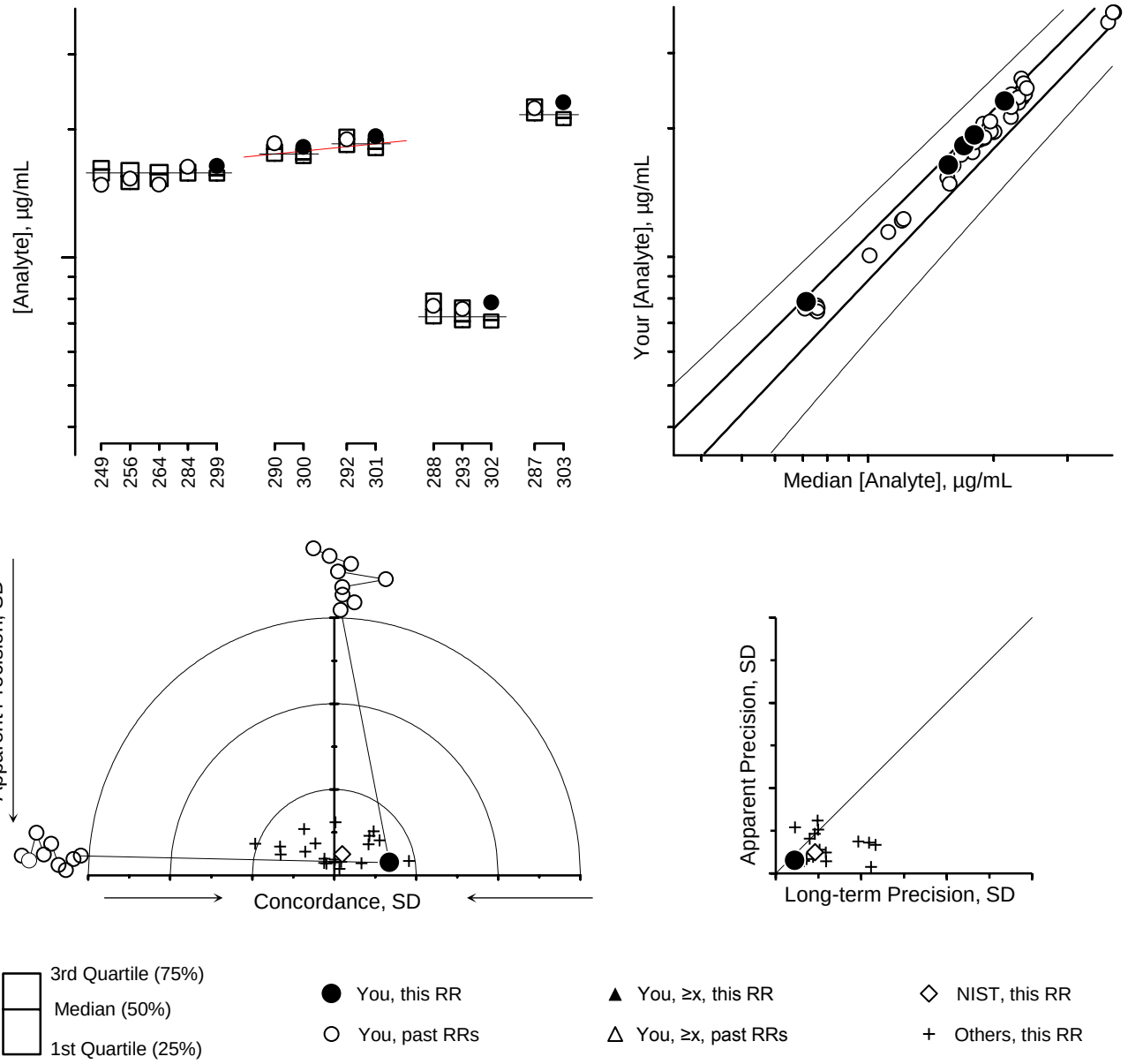


For details of the construction and interpretation of these plots, see:
Duewer, Kline, Sharpless, Brown Thomas, Gary, Sowell. Anal Chem 1999;71(9):1870-8.

Serum	History	Comments
#299	Lyophilized: #249(44), #256(46), #264(48), #284(52)	Augmented, multi-source (SRM 968c Level II)
#300	Lyophilized: #290(RR53)	Native, single-source
#301	Fresh-frozen: #292(RR53)	Same material as #300
#302	Fresh-frozen: #293(RR53)	Native, single-source
#303	Fresh-frozen: #287(RR52)	Native, single-source, hemolyzed

Individualized RR LV Report: FSV-BA

γ/β-Tocopherol



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

Serum	History	Comments
#299	Lyophilized: #249(44), #256(46), #264(48), #284(52)	Augmented, multi-source (SRM 968c Level II)
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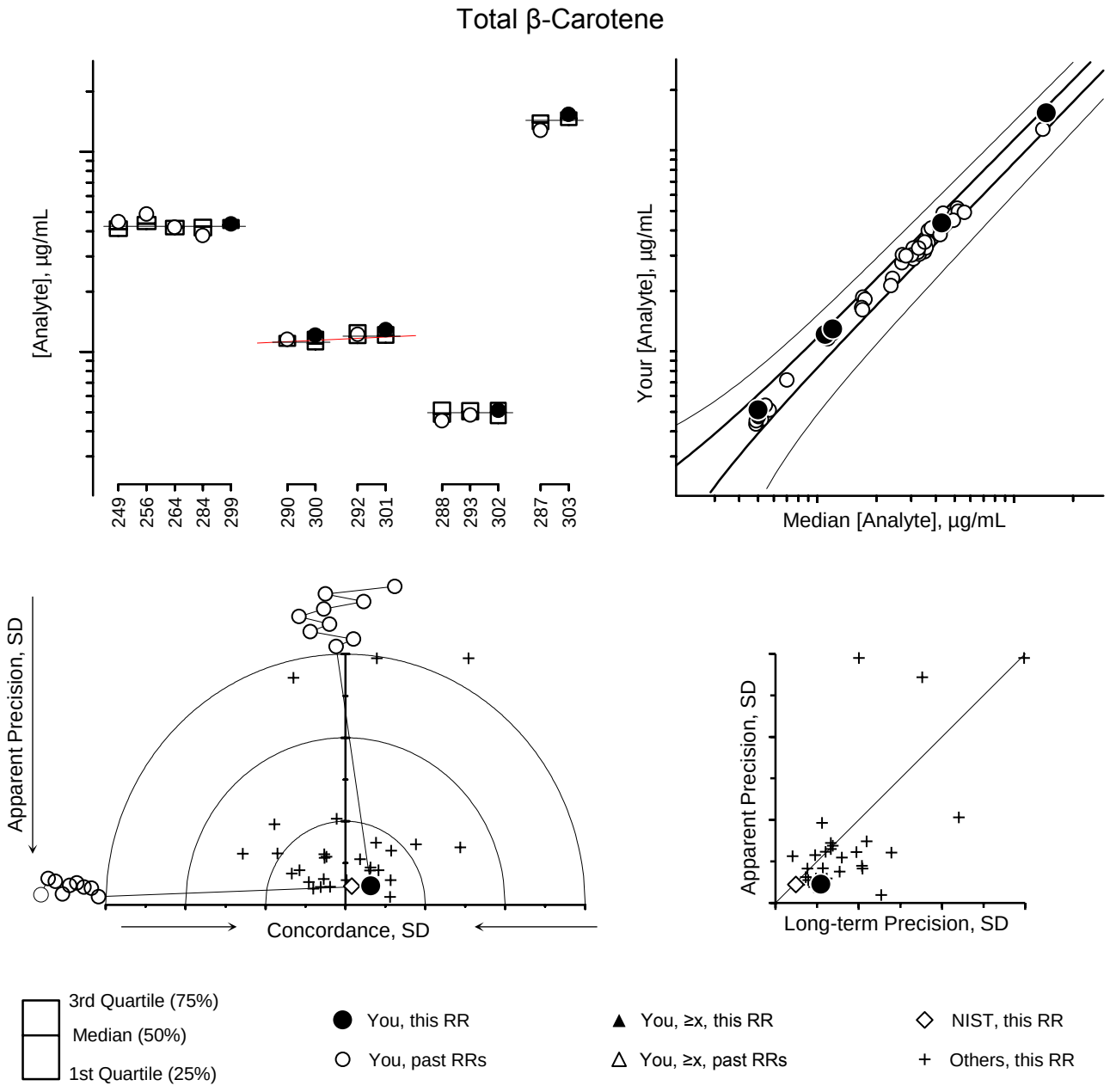
#301	Fresh-frozen: #292(RR53)	Same material as #300
#302	Fresh-frozen: #293(RR53)	Native, single-source
#303	Fresh-frozen: #287(RR52)	Native, single-source, hemolyzed

Individualized RR LV 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
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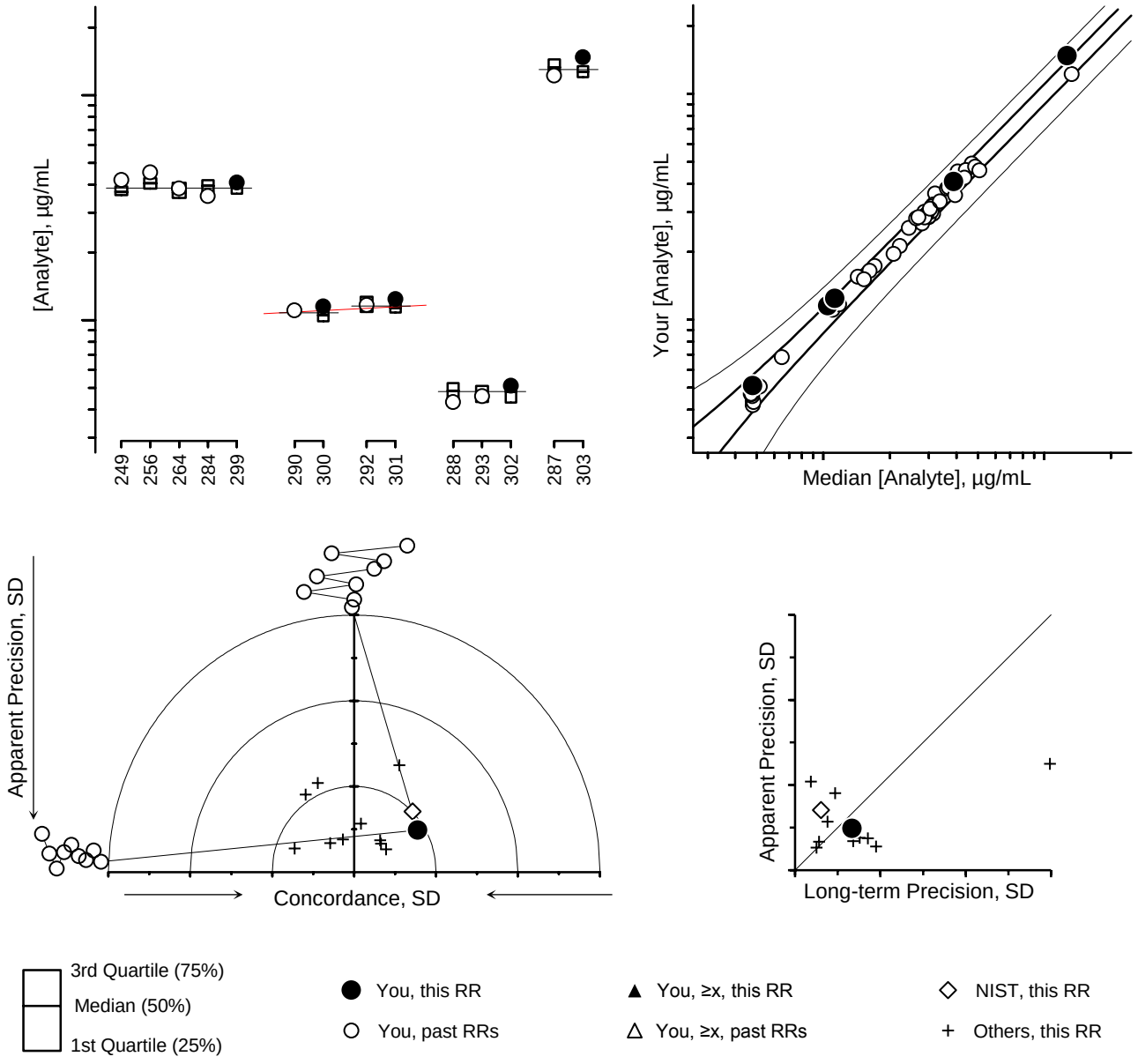
#301	Fresh-frozen: #292(RR53)	Same material as #300
#302	Fresh-frozen: #293(RR53)	Native, single-source
#303	Fresh-frozen: #287(RR52)	Native, single-source, hemolyzed

Individualized RR LV Report: FSV-BA

trans-β-Carotene



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

Serum	History	Comments
#299	Lyophilized: #249(44), #256(46), #264(48), #284(52)	Augmented, multi-source (SRM 968c Level II)
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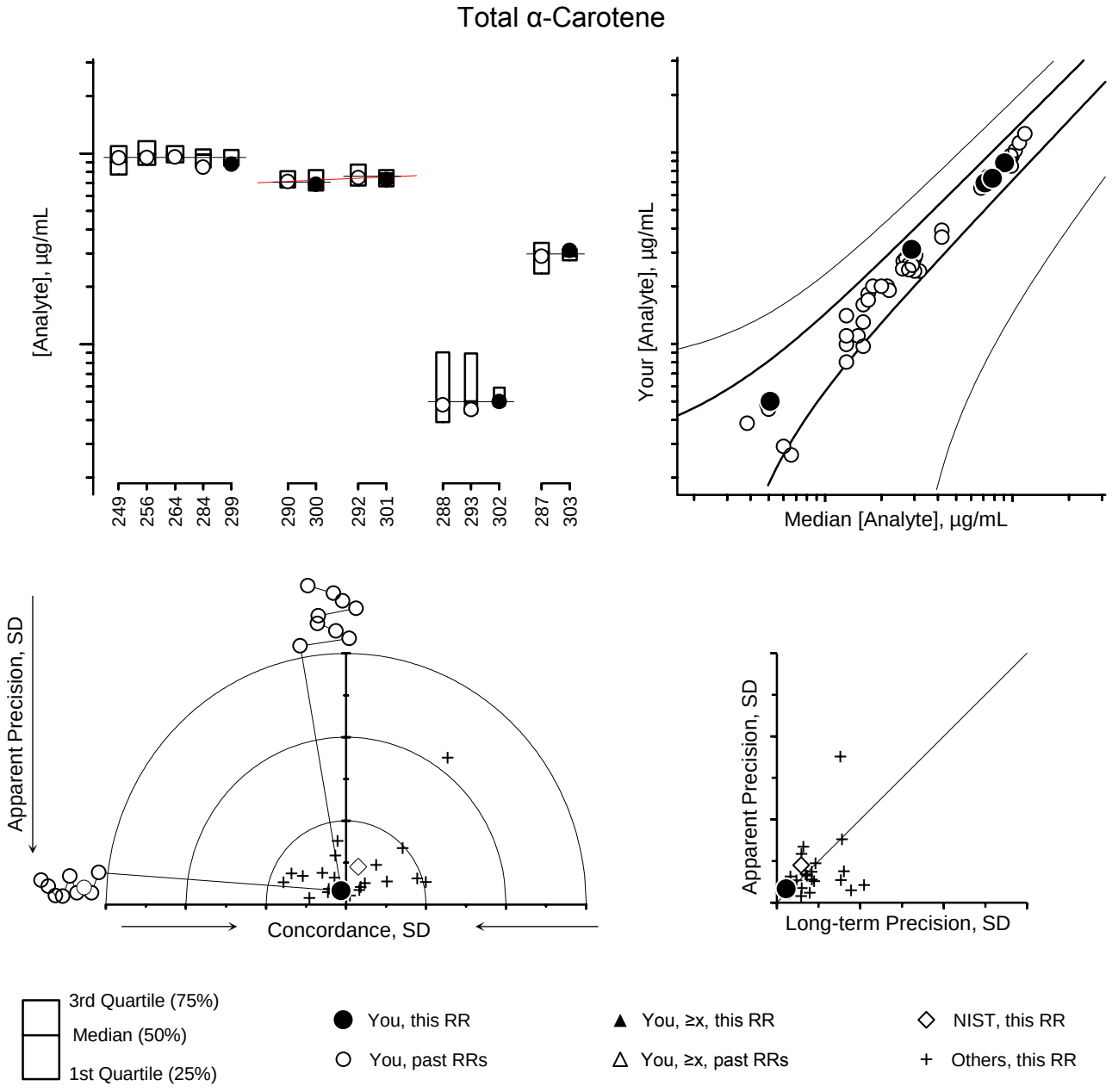
#303	Fresh-frozen: #287(RR52)	Native, single-source, hemolyzed

Individualized RR LV 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
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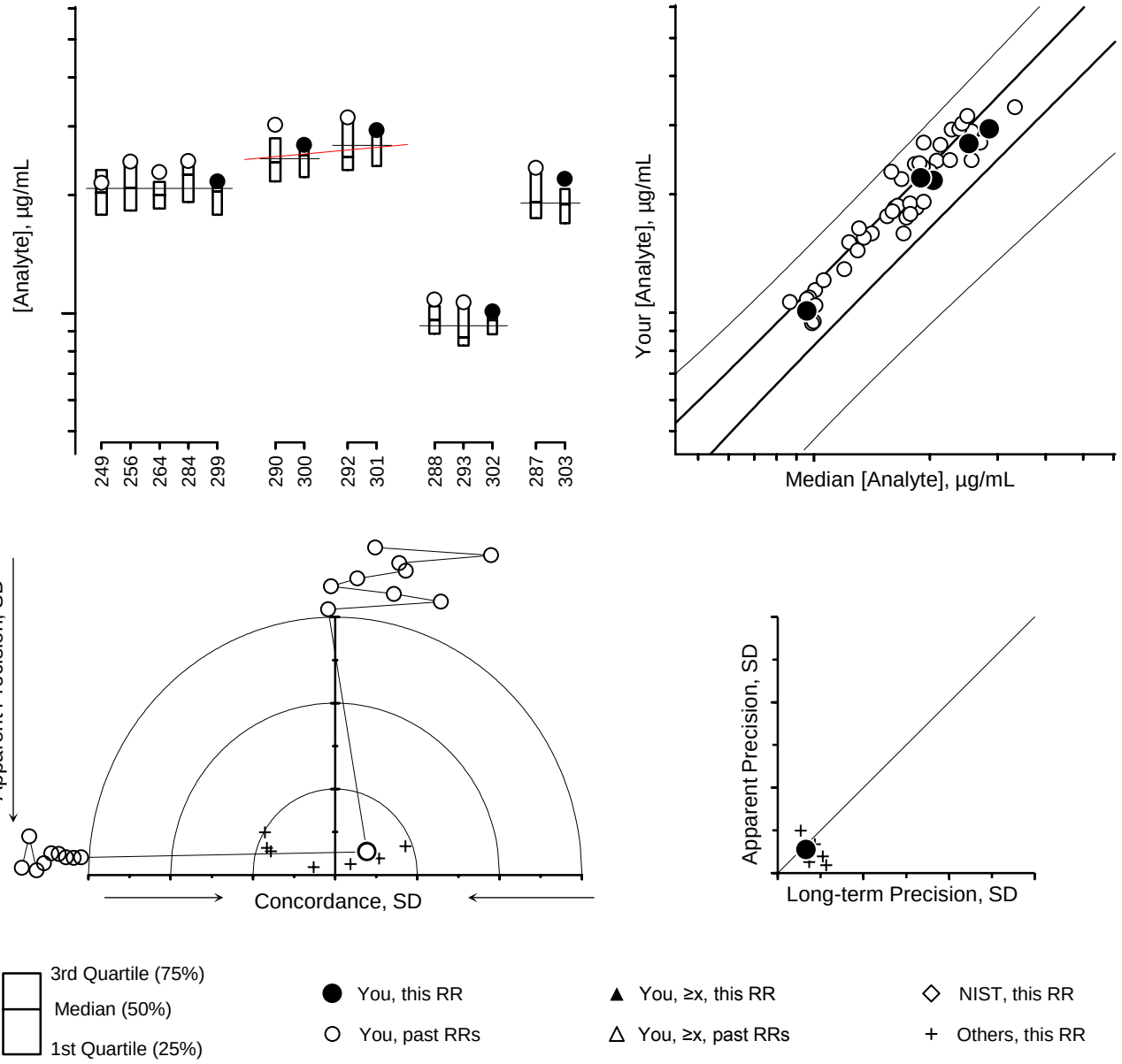
#301	Fresh-frozen: #292(RR53)	Same material as #300
#302	Fresh-frozen: #293(RR53)	Native, single-source
#303	Fresh-frozen: #287(RR52)	Native, single-source, hemolyzed

Individualized RR LV Report: FSV-BA

trans-Lycopene



For details of the construction and interpretation of these plots, see:
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Serum	History	Comments
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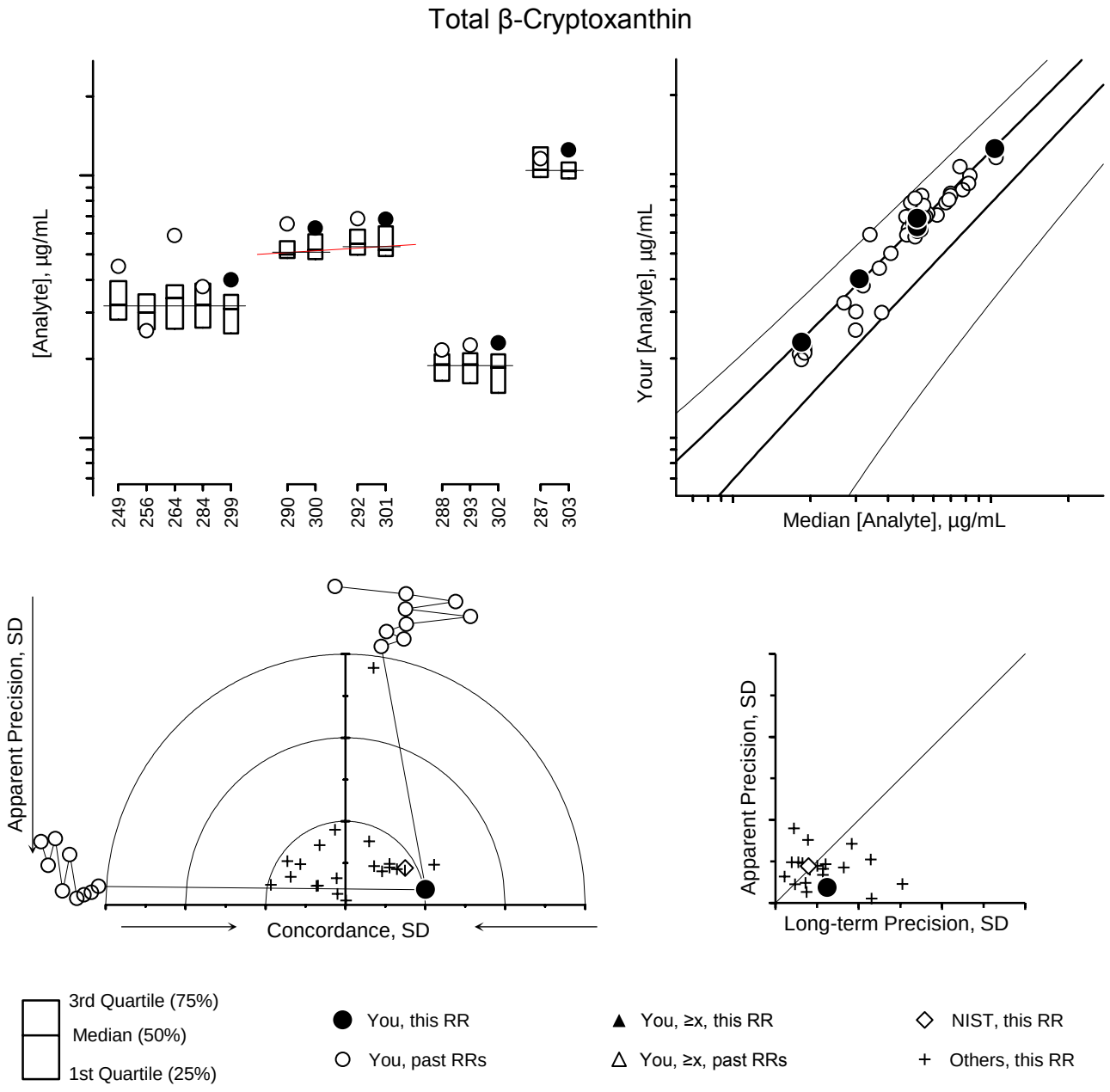
#301	Fresh-frozen: #292(RR53)	Same material as #300
#302	Fresh-frozen: #293(RR53)	Native, single-source
#303	Fresh-frozen: #287(RR52)	Native, single-source, hemolyzed

Individualized RR LV 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
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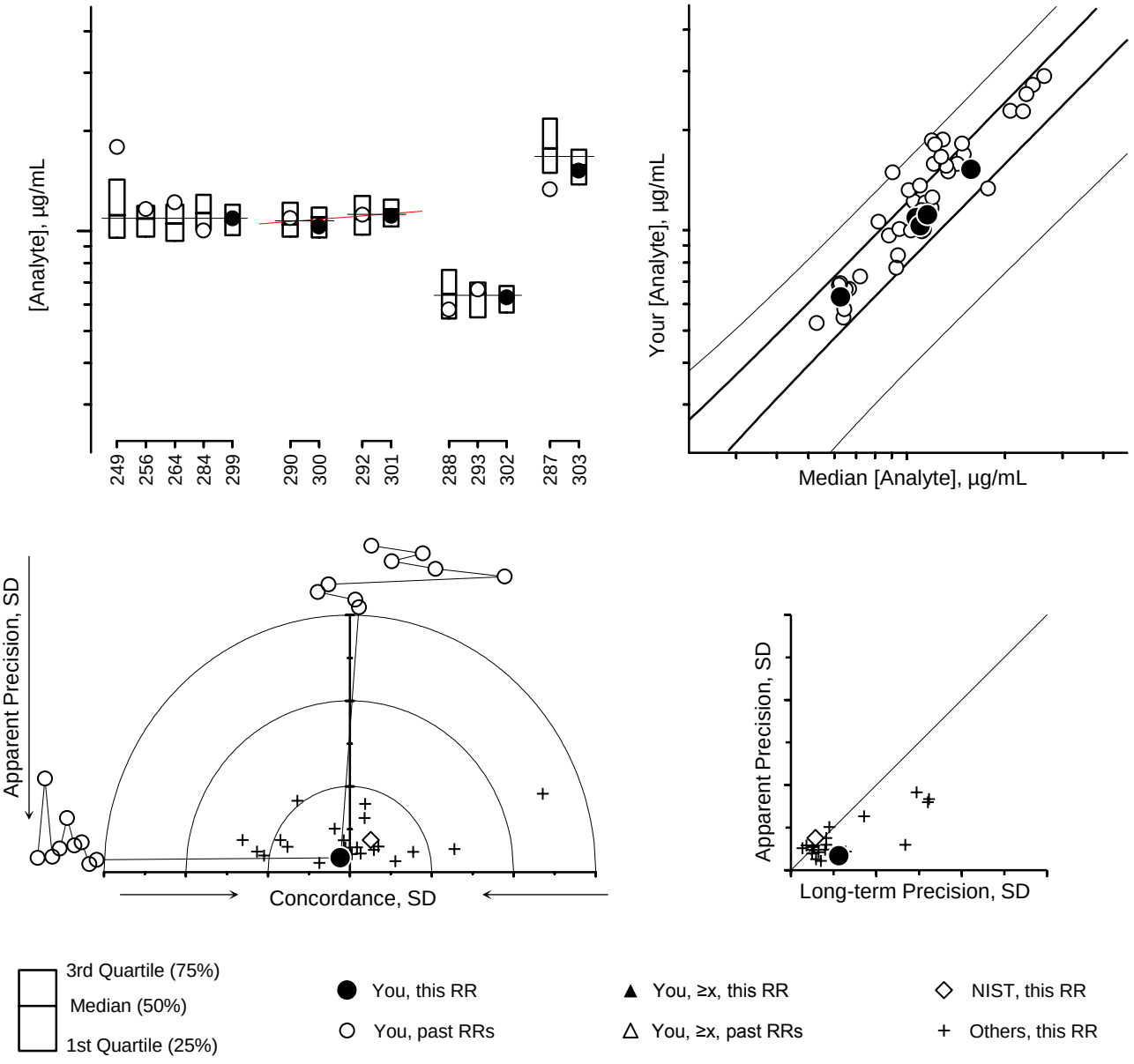
#301	Fresh-frozen: #292(RR53)	Same material as #300
#302	Fresh-frozen: #293(RR53)	Native, single-source
#303	Fresh-frozen: #287(RR52)	Native, single-source, hemolyzed

Individualized RR LV Report: FSV-BA

Total Lutein&Zeaxanthin



For details of the construction and interpretation of these plots, see:
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Serum	History	Comments
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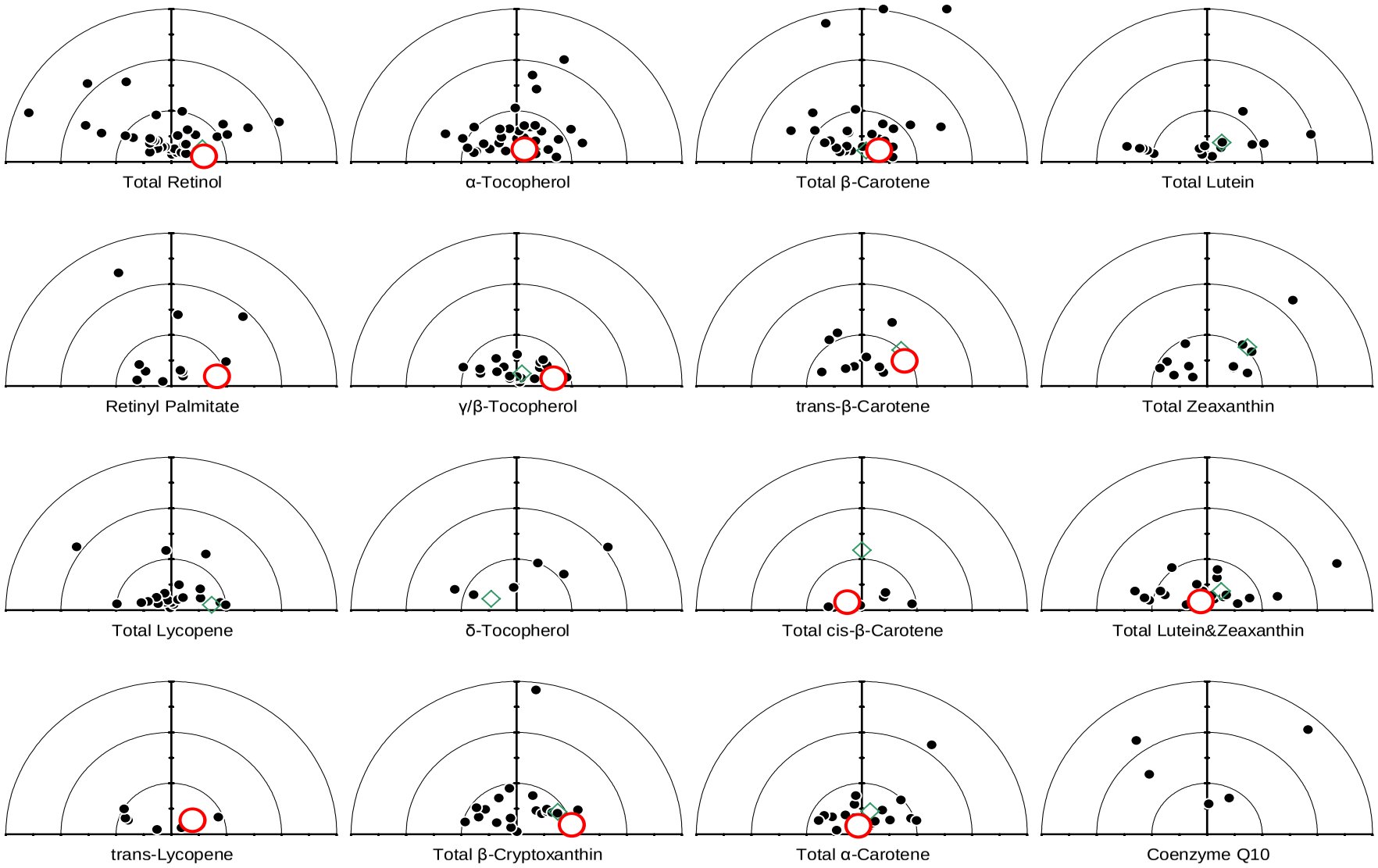
#301	Fresh-frozen: #292(RR53)	Same material as #300
#302	Fresh-frozen: #293(RR53)	Native, single-source
#303	Fresh-frozen: #287(RR52)	Native, single-source, hemolyzed

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



D13

Appendix E. Shipping Package Inserts for RR20

The following five items were included in each package shipped to an RR20 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 20899-0001

Nov 17, 2003

Dear Colleague:

The samples within this package constitute Vitamin C Round Robin 20 (RR20) of the 2004 Micronutrients Measurement Quality Assurance Program.

RR20 consists of four vials of frozen serum *test samples* (#35, #52, #68, and #69), one vial of ascorbic acid *solid control material* (Control), and two vials of frozen *serum control materials* (Control #1 and Control #2). Please follow the attached protocols when you prepare and analyze these samples. If you cannot prepare the *solid control* solutions gravimetrically, please prepare equivalent solutions volumetrically and report the exact volumes used. (Routine 0.5 g gravimetric measurements are generally 10-fold more accurate than routine 0.5 mL volumetric measurements.)

The two *serum control materials* are a new component of the M^2QAP for Vitamin C. Please use these materials to validate the performance of your measurement system before you analyze the *test samples*. The target value for *Control #1* is 8.5 ±0.5 µmol/L sample; the target value for *Control #2* is 28.1 ±1.0 µmol/L sample.

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

The report for RR19 will be mailed during the week of Nov 17, 2003. If you find your results for RR19 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).

We are pleased to announce that due to the generous funding support from the Centers for Disease Control in Atlanta, a second Vitamin C Round Robin (RR21) will be distributed, at no cost to you, during the week of **May 3, 2004**. As before, we will send you a reminder via e-mail or fax about a week prior to shipment.

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 RR20 samples *before* **March 5, 2004**. 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
Research Chemist
Analytical Chemistry Division
Chemical Science and Technology Laboratory

Enclosures: Protocols, Preparation and Analysis of Control Materials and Analysis of Test Samples
RR20 Report Form for Ascorbic Acid Solid Control Material Preparation
RR20 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:

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

   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 $[TAA]_{DS1}$ = (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 $[TAA]_{DS2}$ = 28.4 μmol/L and 0.125 mL should weigh 0.13 g and $[TAA]_{DS3}$ = 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 *exactly* the same method that you will use for the serum control materials and test samples, including any enzymatic treatment. We recommend that you analyze these solutions in the following order: Diluent, Dilute Solution 1, Dilute Solution 2, Dilute Solution 3, Dilute Solution 3, Dilute Solution 2, Dilute Solution 1, Diluent.
   a) Compare the values of the duplicate measurements. *Are you satisfied that your measurement precision is adequate?*
   b) Compare the measured with the calculated [TAA] values. This is most conveniently done by plotting the measured values on the y-axis of a scatterplot against the calculated values on the x-axis. The line through the four {calculated, measured} data pairs should go through the origin with a slope of 1.0. *Are you satisfied with the agreement between the measured and calculated values?*

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

7) Once you have confirmed that your system is properly calibrated, analyze the serum control materials (see protocol below). The target values for these materials are:
   Control #1: 8.5 ±0.5 μmol/L of sample
   Control #2: 28.1 ±1.0 μmol/L of sample.

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

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

Protocol for Analysis of the Serum Control Materials and Test Samples

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

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

Participant #: _____ Date: _____

Vitamin C Round Robin 20

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 5, 2004** to:

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 20

NIST Micronutrient Measurement Quality Assurance Program

Analysis of Control Materials and Test Samples

Sample	Replicate 1	Replicate 2	Units
Dilute Solution 1			µmol/L of Dilute Solution
Dilute Solution 2			µmol/L of Dilute Solution
Dilute Solution 3			µmol/L of Dilute Solution
5% MPA Diluent			µmol/L of Diluent
Serum Control #1			µmol/L of Sample *Target:* 8.5 ±0.5 µmol/L
Serum Control #2			µmol/L of Sample *Target:* 28.1 ±1.0 µmol/L
Serum Test Sample #35			µmol/L of Sample
Serum Test Sample #52			µmol/L of Sample
Serum Test Sample #68			µmol/L of Sample
Serum Test Sample #69			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 5, 2004** to:

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 20

NIST Micronutrients Measurement Quality Assurance Program

Packing List and Shipment Receipt Confirmation Form

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

Sample	Form
VitC #35	Liquid frozen (1:1 serum:10% MPA)
VitC #52	Liquid frozen (1:1 serum:10% MPA)
VitC #68	Liquid frozen (1:1 serum:10% MPA)
VitC #69	Liquid frozen (1:1 serum:10% MPA)
Control #1	Liquid frozen (1:1 serum:10% MPA)
Control #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^2QAP Gang

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

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

Appendix F. Final Report for RR20

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 20899-0001

May 5, 2004

Dear Colleague:

Enclosed is the summary report of the results for Round Robin 20 (RR20) for the measurement of total ascorbic acid (TAA, ascorbic acid plus dehydroascorbic acid) in human serum. Included in this report are: a summary of data for all laboratories and a summary of individual laboratory performance and interlaboratory accuracy and repeatability. As in previous reports, the estimated standard deviations (eSD) for the measurements are defined as 0.74x interquartile range and the estimate coefficients of variation (eCV) are defined as 100x eSD/median.

RR 20 consisted of four *test samples* (#35, #52, #68, and #69), two *serum control materials*, and one *solid control material* for preparation of TAA control solutions. Details regarding the samples can be found in the enclosed report.

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

Samples for the second vitamin C round robin study (RR 21) for the 2004 Vitamin C in Serum QA Program will be shipped **(during the week of May 17)**. If you have questions or concerns regarding this report, please contact 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

Enclosures

NIST

The NIST M^2QAP Vitamin C Round Robin 20 (RR20) report consists of

Page	"Individualized" Report
1	Summarizes your reported values for the nominal 55 mmol/L solution you prepared from the ascorbic acid solid control sample, the two serum control samples, and the four serum test samples.
2	Graphical summary of your RR 20 sample measurements.

Page	"All Lab" Report
1	A tabulation of results and summary statistics for Total Ascorbic Acid [TAA] in the RR20 samples and control/calibration solutions.

Serum-based Samples. Two serum controls and four unknowns were distributed in RR20.

CS1 SRM 970 level 1, ampouled in mid-1998.

CS2 SRM 970 level 2, ampouled in mid-1998.

S20:1 Serum 35, ampouled in late 2001, previously distributed as sample S12:2 (RR17, Sep-02) and S18:1 (RR18, Mar-03). An augmented serum.

S20:2 Serum 52, ampouled in late 2001, previously distributed as sample 16:3 (RR13, Mar-02) and S17:3 (RR17, Sep-02). An augmented serum.

S20:3 Serum 69, SRM 970 level 1, ampouled in mid-1998. This material was distributed with identification in RR11 (Oct-98) and RR12 (Mar-99) and as samples S13-1 (RR13, Mar-00), S14-3 (RR14, Mar-01), S15:1 (RR15, Sep-01), and 16:1 (RR16, Mar-02). An augmented serum.

S20:4 Serum 69, SRM 970 level 2, ampouled in mid-1998. This material was distributed with identification in RR11 (Oct-98) and RR12 (Mar-99) and as samples S13-2 (RR13, Mar-00), S14-4 (RR14, Mar-01), S15:2 (RR15, Sep-01), and 18:3 (RR18, Mar-03). An augmented serum.

Results.

1) All participants who prepared the four control/calibration solutions (the three "Dilute Solutions" and the 5% MPA "Diluent") did so correctly. The criteria used to evaluate this success are: the density of the 5% MPA (≈1.03 gm/mL), the observed wavelength maximum of "Dilute Solution #1"(≈244 nm), the observed absorbance at that maximum (≈0.55 OD), the calculated $E^{1\%}$ #1"(≈550 dL/g·cm).

2) Judging from the calibration parameters calculated for the control/calibration solutions (intercepts close to 0.0 and slopes close to 1.0), the measurement systems for most participants are well calibrated.

3) Everyone reported consistent results for the SRM 970 levels used as controls (CS1 and CS2) and as test samples (S20:3 and S20:4). This implies that all measurements systems are in statistical control. However, not all participants appear to have believed the results for the controls. Several participants reported values for the controls (and consequently for the corresponding test samples) that were well outside the target range. If the measured values for the control samples are not close to the targets, even if your measured and calculated values for the calibration solutions agree, there is problem with your measurement system.

4) Over the past five years, we have been monitoring the stability of [TAA] in both levels of SRM 970 through interlaboratory studies and NIST analyses. Based on the interlaboratory data and data from NIST, the assigned concentration levels of [TAA] have decreased (by about 10 – 20 %) in both levels of the SRM since certification in 1998, but have stabilized over time. We will be recertifying the material in the next few months and will issue a revised Certificate of Analysis for the SRM.

Appendix G. "All-Lab Report" for RR20

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. 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.

Micronutrients Measurement Quality Assurance Program for Total Ascorbic Acid
"Round Robin" 20 - March 2004

		Control / Calibration Samples											MPA	Dilute Solution 1			Samples								
		Gravimetric, µmol/L			Measured, µmol/L				Calibration Parameters				Density	Spectrophotometry			Measured, µmol/L						Correcte		
Lab	Date	Dil:1	Dil:2	Dil:3	Dil:1	Dil:2	Dil:3	MPA	Inter	Slope	R^2	SEE	g/mL	λ_{max}	A_{max}	$E^{1\%}$	CS#1	CS#2	S20:1	S20:2	S20:3	S20:4	CS#1	CS#2	S20:1
VC-MA	23/02/04	55.9	28.3	14.0	59.0	28.7	14.3	0.0	-0.39	1.05	1.000	0.6	1.030	243.	0.5524	560.7	7.9	26.0	24.6	50.3	8.2	25.5	7.9	25.0	23.7
VC-MB	01/03/04	51.6	28.6	14.3	46.2	24.0	13.7	3.6	2.39	0.82	0.993	1.9	1.033	244.	0.4769	524.4	10.3	29.7	23.8	49.1	9.3	24.8	9.6	33.0	25.9
VC-MC	19/02/04	57.2	28.6	14.3	59.1	30.1	16.9	0.0	0.96	1.02	0.998	1.2	1.031	243.	0.5829	578.8	6.9	28.3	20.7	43.2	7.5	29.9	5.8	26.7	19.3
VC-ME	04/03/04	58.4	28.8	14.2	52.9	26.0	12.5	0.4	0.12	0.90	1.000	0.4	1.027	244.	0.5906	574.0	5.5	20.7	16.9	34.9	5.7	21.5	6.0	22.8	18.6
VC-MG	02/03/04	59.7	30.6	16.2	61.0	30.4	14.5	0.0	-0.96	1.03	0.999	1.1	1.029	243.7	0.5640	536.1	20.7	44.2	34.6	65.4	21.1	44.8	21.0	43.8	34.5
VC-MH	12/12/03	59.7	29.8	14.5	59.9	29.9	14.6	0.0	0.02	1.00	1.000	0.0	1.031	243.7	0.5931	564.0	8.2	28.6	22.5	54.3	8.3	27.7	8.1	28.5	22.4
VC-MI	01/03/04	58.6	29.5	13.8	59.5	29.3	13.3	0.0	-0.43	1.02	1.000	0.5	1.032				7.9	31.1	24.3	52.4	8.7	-	8.1	31.0	24.3
VC-MK	03/03/04	61.8	30.3	15.3	60.5	29.6	15.7	0.3	0.44	0.97	1.000	0.4	1.033	244.	0.6023	553.1	17.8	36.7	26.0	53.1	13.0	33.2	17.9	37.3	26.3
VC-MR	25/11/03	58.4	29.2	14.4	57.0	28.8	14.1	0.0	0.09	0.98	1.000	0.2	1.029	244.	0.5860	569.3	7.9	27.5	22.3	47.4	7.7	26.4	8.0	28.0	22.7
VC-MT	08/03/04	24.1	12.3	5.7	25.3	12.6	5.2	0.0	-0.38	1.06	0.999	0.4	1.033	243.	0.2330	548.0	6.4	25.1	19.7	44.4	3.2	24.4	6.4	24.1	19.0
VC-MY	15/03/04																5.1	19.8	13.1	27.4	5.2	21.0	5.1	19.8	13.1
NIST	24/10/03	59.1	29.2	14.0	58.7	32.1	13.6	0.0	0.47	1.00	0.996	2.0	1.024	243.	0.5723	549.7	8.9	28.8	22.5	44.6	8.9	28.8	8.4	28.3	22.0
	N	10	10	10	10	10	10	10					N	10	9	9	9	11	11	11	11	11	10	11	11	11
	Average	54.6	27.6	13.7	54.0	26.9	13.5	0.4					Average	1.031	243.6	0.5312	556.5	9.5	28.9	22.6	47.4	8.9	27.9	9.4	29.1	22.7
	SD	11.0	5.4	2.9	11.1	5.4	3.2	1.1					SD	0.002	0.5	0.1181	17.9	5.1	6.9	5.5	10.1	4.8	7.0	5.2	6.9	5.5
	Min	24.1	12.31	5.7	25.3	12.60	5.2	0.0					Min	1.027	243.0	0.2330	524.4	5.1	19.8	13.1	27.4	3.2	21.0	5.1	19.8	13.1
	%25	56.2	28.57	14.0	53.9	26.71	13.4	0.0					%25	1.029	243.0	0.5524	548.0	6.6	25.6	20.2	43.8	6.6	24.5	6.2	24.6	19.1
	Median	58.4	28.98	14.3	59.1	29.04	14.2	0.0					Median	1.031	243.7	0.5829	560.7	7.9	28.3	22.5	49.1	8.2	26.0	8.0	28.0	22.7
	%75	59.4	29.73	14.5	59.8	29.84	14.6	0.2					%75	1.032	244.0	0.5906	569.3	9.2	30.4	24.4	52.8	9.0	29.4	8.9	32.0	25.1
	Max	61.8	30.61	16.2	61.0	30.41	16.9	3.6					Max	1.033	244.0	0.6023	578.8	20.7	44.2	34.6	65.4	21.1	44.8	21.0	43.8	34.5
	MADe	1.9	0.9	0.4	2.5	1.5	1.1	0.0					eSD	0.003	0.4	0.0280	18.8	2.3	4.1	3.0	6.9	1.6	4.2	2.4	5.8	5.0
	CV	3	3	3	4	5	7						CV	0.26	0.18	5	3	29	15	14	14	20	16	30	21	22

G2

Appendix H. Representative "Individualized Report" for RR20

Each participant in RR20 received an "Individualized Report" reflecting their reported results. The following two pages are the "Individualized Report" for participant "VC-MA".

Vitamin C "Round Robin" 20 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
09/18/01	15	HPLC-EC	1.027	243.0	0.547	556.5	0.0	1.04	1.000	0.05
11/18/02	16	HPLC-EC	1.032	242.0	0.575	576.5	-0.4	1.07	0.999	0.90
12/12/02	17	HPLC-EC	1.026	242.0	0.552	551.0	-0.3	1.06	1.000	0.49
03/20/03	18	HPLC-EC	1.026	244.0	0.509	563.1	-0.1	1.02	1.000	0.18
11/13/03	19	HPLC-EC	1.026	243.0	0.584	561.9	1.1	1.03	0.998	1.24
02/23/04	20	HPLC-EC	1.031	243.0	0.552	560.7	-0.4	1.05	1.000	0.65
		Mean	1.028	242.8	0.55	561.6				
		SD	0.003	0.8	0.03	8.5				
		CV	0.25	0.31	4.7	1.5				

[TAA] mmol/Lsample

Date	RR	Sample	Rep_1	Rep_2	F_{adj}	Mean	SD_{dup}	N	Mean	SD_{repeat}	SD_{reprod}
12/12/02	17	S17:2	23.3	23.4	1.0	23.4	0.1	3	23.7	0.6	0.8
03/20/03	18	S18:1	22.7	23.7	1.0	23.2	0.7				
02/23/04	20	S20:1	25.1	24.1	1.0	24.6	0.7				
11/18/02	16	S16:3	49.9	44.9	1.0	47.4	3.5	3	49.0	2.1	1.9
12/12/02	17	S17:3	49.7	49.1	1.0	49.4	0.4				
02/23/04	20	S20:2	50.6	50.0	1.0	50.3	0.4				
09/23/98	11	S11:1	14.7	14.3	0.5	7.2	0.2	8	8.2	0.2	0.6
04/02/99	12	S12:1	15.2	15.6	0.5	7.7	0.1				
09/17/01	13	S13:1	8.4	8.5	1.0	8.5	0.1				
09/27/01	14	S14:3	8.0	7.7	1.0	7.8	0.2				
09/18/01	15	S15:1	8.9	8.7	1.0	8.8	0.1				
11/18/02	16	S16:1	8.8	8.8	1.0	8.8	0.0				
11/13/03	19	S19:4	7.8	8.6	1.0	8.2	0.5				
02/23/04	20	S20:3	8.3	8.1	1.0	8.2	0.1				
09/23/98	11	S11:2	49.2	51.4	0.5	25.2	0.8	7	26.1	0.4	1.7
04/02/99	12	S12:2	47.7	47.0	0.5	23.7	0.2				
09/17/01	13	S13:2	27.6	27.7	1.0	27.7	0.1				
09/27/01	14	S14:4	25.7	26.4	1.0	26.0	0.5				
09/18/01	15	S15:2	25.4	25.6	1.0	25.5	0.2				
03/20/03	18	S18:3	28.8	29.2	1.0	29.0	0.3				
02/23/04	20	S20:4	25.9	25.2	1.0	25.5	0.5				

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

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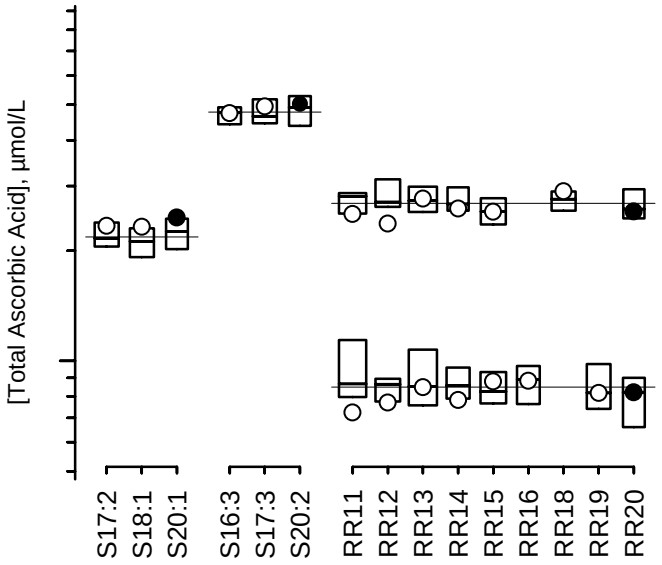
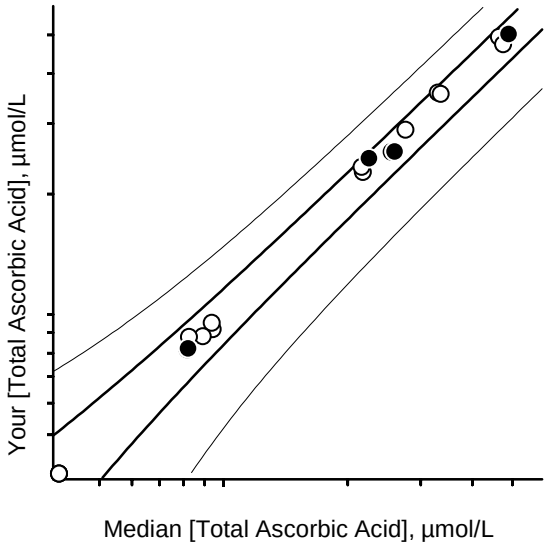
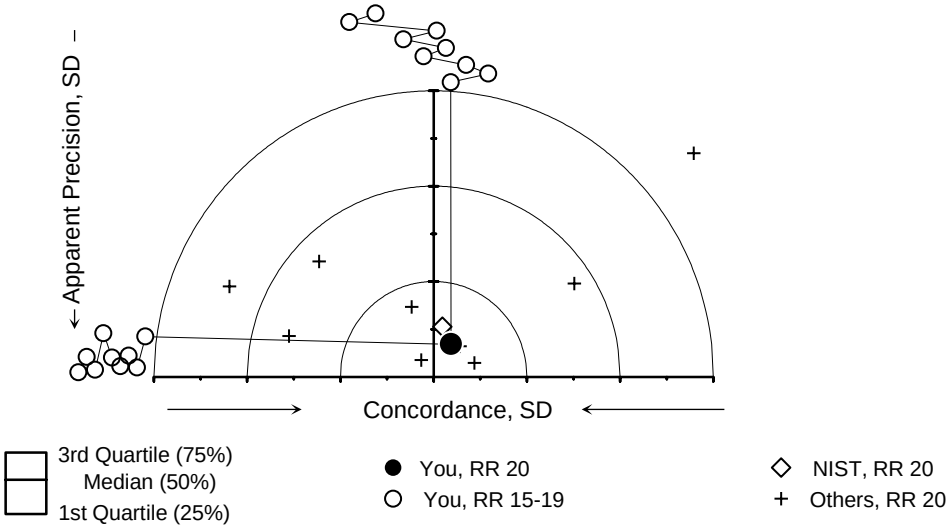
Vitamin C "Round Robin" 20 Report: Participant VC-MA

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
S20:1	Serum 35, previously distributed in RRs 17 and 18
S20:2	Serum 52, previously distributed in RRs 16 and 17
S20:3	SRM 970 Level 1, previously distributed in RRs 11, 12, 13, 14, 15, 16, and 19
S20:4	SRM 970 Level 2, previously distributed in RRs 11, 12, 13, 14, 15, and 18