NISTIR 7880-16

NIST Micronutrients Measurement Quality Assurance Program Summer 2004 Comparability Studies

Results for Round Robin LVI Fat-Soluble Vitamins and Carotenoids in Human Serum and Round Robin 21 Ascorbic Acid in Human Serum

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June 2013

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

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

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Abstract

The National Institute of Standards and Technology coordinates the Micronutrients Measurement Quality Assurance Program (MMQAP) for laboratories that measure fat- and water-soluble vitamins and carotenoids in human serum and plasma. This report describes the design of and results for the Summer 2004 MMQAP measurement comparability improvement studies: 1) Round Robin LVI Fat-Soluble Vitamins and Carotenoids in Human Serum and 2) Round Robin 21 Total Ascorbic Acid in Human Serum. The materials for both studies were shipped to participants in May 2004; participants were requested to provide their measurement results by September 13, 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 LVI: Fat-Soluble Vitamins and Carotenoids in Human Serum

Participants in the MMQAP Fat-Soluble Vitamins and Carotenoids in Human Serum Round Robin LVI comparability study (hereafter referred to as RR56) 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 May 2004. 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 RR56 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 21: Vitamin C in Human Serum

Participants in the MMQAP Vitamin C in Human Serum Round Robin 21 comparability study (hereafter referred to as RR21) 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 May 2004. 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 RR21 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 RR56

The following three items were included in each package shipped to an RR56 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

May 12, 2004

Dear Colleague:

Enclosed are the samples (Sera 304 – 308) for the second fat-soluble vitamins and carotenoids in serum round robin study (Round Robin LVI) 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 **September 13, 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 306, 307, and 308.**

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 LVI 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

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Participant #: _____ Date: _____

Round Robin LVI

NIST Micronutrients Measurement Quality Assurance Program

Analyte	304	305	306	307	308	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 {306,307,308} frozen when received? Yes | No

Comments:

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

Please return results **before**
13-Sep-2004

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

Participant #: _____ Date: _____

Fat-Soluble Vitamins Round Robin LVI

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?
#304	Lyophilized	Yes (1 ml H_2O)
#305	Lyophilized	Yes (1 ml H_2O)
#306	Liquid frozen	No
#307	Liquid frozen	No
#308	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 {306, 307, 308} 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 {306, 307, 308} 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 RR56

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

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

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

November 3, 2004

Dear Colleague:

Enclosed is the summary report of the results for round robin LVI (RR56) 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. The NIST-assigned values are equally weighted means of the medians from this interlaboratory comparison exercise and the means from the analyses performed by NIST.

Data for evaluating laboratory performance in RR 56 are provided in the comparability summary (Score Card) on page 5 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.

Samples for the first 2005 QA interlaboratory exercises will be shipped **starting the week of November 8**. 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 mis-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 Brown Thomas
Research Chemist
Analytical Chemistry Division
Chemical Science and Technology Laboratory

Enclosures

NIST

The NIST M^2QAP Round Robin LIV (RR56) report consists of:

Page	"All Lab" Report
1-3	A listing of all results and statistics for analytes reported by at least two laboratories.
4a	A list of results for the analytes reported by only one laboratory.
4b	A legend for the above two lists.
5	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 RR56.

Serum	Description	Prior Distributions
304	Lyophilized blended serum with native carotenoid levels, augmented with *trans*-retinol and γ/β-tocopherol; SRM 968c Level I.	#248 RR44 (9/98), #258 RR46 (6/99), #263 RR47 (5/00), #280 RR51 (3/02)
305	Lyophilized, native, single donor serum prepared in 1999. The same material was used for #308.	#266 RR48 (9/00), #277 RR50 (0/01) #282 RR51 (3/02), #295 RR54 (9/03)
306	The same fresh-frozen blended serum as #307, augmented with ≈0.3 µg/mL *trans*-retinol. It was prepared in 2002.	#285 RR52 (9/02), #297 RR54 (9/03)
307	The same fresh-frozen blended serum as #306, augmented with ≈0.3 µg/mL 13-*cis*-retinol. It was prepared in 2002.	#286 RR52 (9/02), #298 RR54 (9/03)
308	Fresh-frozen, native, single donor serum prepared in 1999. The same material was used for #305.	#271 RR49 (3/01), #275 RR50 (9/01) #279 RR51 (3/02), #296 RR54 (9/03)

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.

2) Matrix (Lyophilized vs. Fresh-Frozen) Differences. Sera 305 and 306 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 305 should be ≈ 95% of those in Serum 306. The observed average ratio ± SD over all measurands with 10 or more quantitative measurements is 0.937 ± 0.018. If any of your Sera 305/308 ratios are much different than 0.95, you should 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.

Appendix C. "All-Lab Report" for RR56

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 LVI Laboratory Results

All Values in µg/mL

	Total Retinol					trans-Retinol					Retinyl Palmitate					α-Tocopherol					γ/β-Tocopherol					δ-Tocopherol				
Lab	304	305	306	307	308	304	305	306	307	308	304	305	306	307	308	304	305	306	307	308	304	305	306	307	308	304	305	306	307	308
FSV-BA	0.841	0.467	0.653	0.571	0.502						0.036	0.057	0.021	0.021	0.053	6.85	6.46	2.70	2.72	6.97	3.80	1.90	0.73	0.73	2.05	0.110	0.100	0.032	0.035	0.113
FSV-BB	0.851	0.438	0.625	0.607	0.458						0.029	0.053	0.014	0.011	0.059	7.65	7.03	3.08	2.99	7.45	3.42	1.71	0.68	0.63	1.75	0.115	0.098	0.035	0.041	0.115
FSV-BC	0.812	0.433	0.619	0.609	0.467																									
FSV-BD	0.869	0.428	0.605	0.590	0.453											7.80	7.20	2.70	2.60	7.80										
FSV-BE	0.930	0.450	0.630	0.640	0.490											7.80	6.60	2.80	2.90	7.10	4.00	1.80	0.70	0.70	1.90					
FSV-BF	0.870	0.440	0.610	0.490	0.460											7.00	6.30	2.50	2.60	6.30	3.70	1.80	0.70	0.70	1.80					
FSV-BG	0.825	0.420	0.603	0.600	0.453						0.039	0.051	0.025	0.023	0.056	7.47	6.62	2.89	2.93	7.26	3.65	1.77	0.76	0.75	1.94					
FSV-BH	0.810	0.436	0.646	0.423	0.441											7.53	7.02	2.85	2.78	7.26										
FSV-BI	0.914	0.496	0.672	0.642	0.507						0.030	0.057	0.020	0.018	0.057	6.44	6.21	2.51	2.53	6.43	3.55	1.84	0.72	0.73	1.93					
FSV-BJ	0.847	0.435	0.613	0.602	0.454						*nq*	*nq*	*nq*	*nq*	*nq*	7.28	6.80	2.68	2.67	7.02	4.05	1.96	0.73	0.70	2.00					
FSV-BK	0.845	0.442	0.585	0.579	0.489											6.82	5.42	2.64	2.48	5.65										
FSV-BL	1.000	0.600	0.720	0.660	0.540											6.03	5.60	3.01	3.01	6.89										
FSV-BM	0.852	0.511	0.657	0.643	0.528											6.90	6.30	2.80	3.00	6.70										
FSV-BN	0.806	0.405	0.613	0.589	0.423						0.026	0.039	0.011	0.010	0.041	7.00	5.33	2.28	2.53	6.23	3.63	1.49	0.52	0.68	1.75	0.164	0.165	0.067	0.068	0.158
FSV-BO	0.934	0.398	0.622	0.593	0.438											6.63	5.69	2.58	2.54	6.16										
FSV-BP	0.917	0.448	0.723	0.687	0.555											7.35	6.58	2.88	2.80	6.90										
FSV-BQ	0.895	0.519	0.662	0.596	0.525											7.60	6.20	3.00	3.00	7.60										
FSV-BR	≥0.830	≥0.450	≥0.630	-	≥0.470	0.830	0.450	0.630	0.410	0.470						7.77	6.76	2.77	2.60	6.97										
FSV-BS	0.783	0.329	0.446	0.410	0.384											4.87	4.67	1.49	2.45	3.76	2.57	0.88	0.09	0.31	0.68					
FSV-BT	0.887	0.468	0.606	0.560	0.458											7.02	5.93	3.20	3.24	6.90	3.27	1.59	0.69	0.72	1.69					
FSV-BU	0.850	0.433	0.619	0.590	0.461											7.84	6.92	3.24	3.30	7.17	3.96	2.01	0.67	0.71	1.95					
FSV-BV	1.010	0.497	0.634	0.439	0.490											8.90	7.29	2.77	2.78	7.44	4.81	2.10	0.74	0.76	2.18					
FSV-BW	0.950	0.480	0.660	0.660	0.490						0.054	0.080	0.024	0.019	0.082	8.00	7.24	3.02	3.03	7.41	4.70	2.22	0.86	0.86	2.23					
FSV-BX	0.864	0.444	0.639	0.629	0.471											7.71	6.82	2.99	2.92	7.18	3.83	1.81	0.73	0.72	1.84					
FSV-CB	0.710	0.384	0.507	0.480	0.369											7.28	6.46	2.96	2.83	7.09										
FSV-CC	0.810	0.410	0.570	0.540	0.450	0.790	0.400	0.570	0.380	0.450						7.04	6.31	2.70	2.71	7.03										
FSV-CD	0.877	0.439	0.644	0.627	0.454						0.026	0.065	0.022	0.020	0.080	6.96	6.25	2.48	2.38	6.42	3.54	1.72	0.65	0.60	1.82					
FSV-CE	0.738	0.392	0.480	0.450	0.390											7.41	6.84	3.06	2.64	7.46										
FSV-CF	0.875	0.453	0.629	0.620	0.469											7.20	6.90	3.20	3.10	6.90										
FSV-CG	0.922	0.442	0.609	0.584	0.462											6.39	5.93	2.43	2.49	6.56	3.56	1.74	0.69	0.70	1.93	0.146	0.145	0.038	0.038	0.154
FSV-CI	0.844	0.453	0.669	0.487	0.476						0.036	0.048	0.017	0.021	0.047	5.86	5.37	2.05	2.10	5.43	3.09	1.50	0.53	0.55	1.54					
FSV-CS	0.777	0.397	0.569	0.540	0.411											9.58	8.53	2.59	2.48	6.89	4.11	1.92	0.69	0.63	1.94					
FSV-CT																														
FSV-CW	≥1.088	≥0.552	≥0.835	-	≥0.643	1.088	0.552	0.835	0.825	0.643	0.024	0.039	0.013	0.011	0.044	8.90	7.60	2.60	2.80	8.10	4.70	2.10	0.80	0.80	2.30	0.130	0.100	0.040	0.050	0.120
FSV-CZ	0.860	0.450	0.680	0.580	0.480											7.60	6.90	3.20	2.90	7.20										
FSV-DA	0.850	0.452	0.725	0.636	0.479	0.850	0.452	0.725	0.372	0.479	0.024	0.053	0.012	0.015	0.056	7.51	6.81	2.82	3.31	7.28	3.94	1.87	0.70	0.82	1.99	0.112	0.083	0.031	0.039	0.083
FSV-DD	1.497	0.780	1.081	1.031	0.765																									
FSV-DF	0.853	0.425	0.659	0.616	0.457																									
FSV-DI	0.950	0.432	0.680	0.590	0.466						0.022	0.039	0.011	0.008	0.040	7.88	7.01	2.96	2.91	7.28	3.84	1.77	0.68	0.67	1.83	0.138	0.116	0.039	0.038	0.120
FSV-DW	0.880	0.490	0.670	0.650	0.480											7.32	6.64	2.73	2.68	6.33										
FSV-ET	0.850	0.440	0.620	0.590	0.380											7.00	6.40	2.60	2.50	6.90	3.60	1.70	0.70	0.60	1.90					
N	38	38	38	38	38	4	4	4	4	4	11	11	11	11	11	37	37	37	37	37	22	22	22	22	22	7	7	7	7	7
Min	0.710	0.329	0.446	0.410	0.369	0.790	0.400	0.570	0.372	0.450	0.022	0.039	0.011	0.008	0.040	4.87	4.67	1.49	2.10	3.76	2.57	0.88	0.09	0.31	0.68	0.110	0.083	0.031	0.035	0.083
Median	0.857	0.441	0.630	0.592	0.464	0.840	0.451	0.677	0.395	0.474	0.029	0.053	0.017	0.018	0.056	7.32	6.60	2.77	2.78	6.97	3.75	1.80	0.70	0.70	1.91	0.130	0.100	0.038	0.039	0.120
Max	1.497	0.780	1.081	1.031	0.765	1.088	0.552	0.835	0.825	0.643	0.054	0.080	0.025	0.023	0.082	9.58	8.53	3.24	3.31	8.10	4.81	2.22	0.86	0.86	2.30	0.164	0.165	0.067	0.068	0.158
SD	0.050	0.026	0.039	0.049	0.027						0.008	0.010	0.007	0.007	0.009	0.56	0.51	0.29	0.29	0.52	0.32	0.15	0.04	0.07	0.13	0.021	0.023	0.004	0.006	0.017
CV	6	6	6	8	6	0	0	0	0	0	29	19	40	39	17	8	8	10	10	7	9	8	5	9	7	16	23	12	15	14
N_{past}	43	39	36	37	37	9	9	8	7	9	14	13	9	9	12	47	44	37	37	42	26	25	22	22	24	6	6	5	5	6
$Median_{past}$	0.858	0.446	0.624	0.590	0.467	0.839	0.437	0.608	0.389	0.451	0.035	0.052	0.016	0.016	0.054	7.53	6.76	2.92	2.90	7.14	3.850	1.847	0.746	0.752	1.974	0.150	0.144	0.065	0.065	0.135
SD_{past}	0.057	0.041	0.052	0.049	0.043	0.047	0.037	0.030	0.044	0.033	0.012	0.011	0.006	0.004	0.013	0.68	0.58	0.24	0.27	0.57	0.342	0.126	0.090	0.073	0.161	0.053	0.100	0.039	0.017	0.080
NIST	0.848	0.451	0.619	0.601	0.447											7.66	6.86	2.64	2.78	7.13	3.91	1.85	0.71	0.72	1.90					
N_{NIST}	3	3	3	3	3											3	3	3	3	3	3	3	3	3	3					
S_{rep}	0.031	0.016	0.042	0.066	0.041											0.18	0.23	0.13	0.13	0.61	0.05	0.04	0.05	0.02	0.04					
S_{het}	0.021	0.016	0.010	0.037	0.013											0.01	0.13	0.06	0.10	0.10	0.07	0.01	0.01	0.02	0.02					
S_{NIST}	0.037	0.022	0.043	0.076	0.043											0.18	0.26	0.15	0.16	0.62	0.09	0.04	0.05	0.03	0.05					
NAV	0.852	0.446	0.624	0.592	0.456	0.840	0.451	0.677	0.395	0.474	0.029	0.053	0.017	0.018	0.056	7.49	6.73	2.71	2.78	7.05	3.83	1.82	0.70	0.71	1.91	0.130	0.100	0.038	0.039	0.120
NAU	0.067	0.036	0.051	0.076	0.045						0.013	0.017	0.011	0.011	0.017	0.65	0.59	0.41	0.40	0.63	0.36	0.19	0.09	0.09	0.20	0.027	0.024	0.016	0.016	0.026

C2

Round Robin LVI Laboratory Results

All Values in µg/mL

	Total β-Carotene					trans-β-Carotene					Total cis-β-Carotene					Total α-Carotene					Total Lycopene				
Lab	304	305	306	307	308	304	305	306	307	308	304	305	306	307	308	304	305	306	307	308	304	305	306	307	308
FSV-BA	0.160	0.319	0.045	0.047	0.360	0.150	0.301	0.045	0.045	0.340	0.011	0.018	0.002	0.002	0.019	0.015	0.028	0.003	0.004	0.030	0.294	0.333	0.183	0.180	0.382
FSV-BB	0.179	0.347	0.044	0.051	0.390	0.169	0.323	0.044	0.051	0.366	0.010	0.024	*nd*	0.000	0.025	0.012	0.030	0.003	0.004	0.033	0.335	0.357	0.189	0.190	0.393
FSV-BC																									
FSV-BD																									
FSV-BE	0.172	0.321	0.042	0.045	0.340																				
FSV-BF	0.130	0.290	0.039	0.050	0.295											0.016	0.029	0.008	0.011	0.026	0.284	0.311	0.168	0.179	0.308
FSV-BG	0.160	0.366	0.055	0.055	0.379											0.019	0.026	0.008	0.009	0.031	0.267	0.363	0.204	0.210	0.370
FSV-BH	0.155	0.319	0.040	0.040	0.315	0.143	0.292	0.040	0.040	0.291	0.012	*0.027*	*nq*	*nq*	0.024	0.009	0.020	*nq*	*nq*	0.020	0.297	0.337	0.182	0.181	0.336
FSV-BI	0.197	0.409	0.057	0.056	0.429											0.016	0.033	0.004	0.004	0.036	0.263	0.311	0.155	0.158	0.319
FSV-BJ	0.191	0.381	0.033	0.032	0.389											0.016	0.030	*nq*	*nq*	0.035	0.360	0.378	0.185	0.198	0.410
FSV-BK																									
FSV-BL																									
FSV-BM																									
FSV-BN		0.325	0.047				0.299	0.043				0.021					0.023					0.317	0.179		
FSV-BO	0.183	0.359	0.052	0.041	0.422											0.010	0.019	*nq*	*nq*	0.031	0.359	0.379	0.220	0.195	0.419
FSV-BP	0.198	0.354	0.078	0.061	0.388											0.027	0.024	0.027	0.017	0.025	0.393	0.359	0.303	0.205	0.375
FSV-BQ																									
FSV-BR																									
FSV-BS	≥0.179	≥0.307	≥0.061	≥0.056	≥0.342	0.179	0.307	0.061	0.056	0.342						0.031	0.047	0.025	0.024	0.042	0.375	0.359	0.224	0.203	0.388
FSV-BT	0.168	0.277	0.057	0.053	0.323	0.149	0.252	0.052	0.048	0.293	0.014	0.019	0.004	0.004	0.022	0.017	0.024	0.008	0.007	0.029	0.300	0.278	0.197	0.193	0.330
FSV-BU	0.154	0.305	0.038	0.041	0.323											0.015	0.027	0.007	0.007	0.028	0.296	0.310	0.172	0.172	0.331
FSV-BV	0.212	0.352	0.050	0.051	0.364											0.013	0.019	0.003	0.003	0.021	0.418	0.402	0.196	0.204	0.417
FSV-BW	0.188	0.349	0.052	0.049	0.364											0.012	0.028	*nq*	*nq*	0.024	0.357	0.371	0.204	0.202	0.389
FSV-BX	≥0.154	≥0.292	≥0.049	≥0.049	≥0.324	0.154	0.292	0.049	0.049	0.324						0.020	0.030	0.008	0.008	0.032					
FSV-CB	0.115	0.322	0.047	0.049	0.344											0.006	0.015	0.002	0.003	0.022	0.175	0.309	0.141	0.169	0.315
FSV-CC																									
FSV-CD	0.162	0.273	0.044	0.041	0.312											0.021	0.036	*nd*	*nd*	0.045	0.298	0.291	0.147	0.148	0.333
FSV-CE	0.163	0.326	0.055	0.040	0.358																				
FSV-CF																									
FSV-CG	0.150	0.289	0.044	0.045	0.319	0.138	0.266	0.042	0.043	0.296	0.012	0.023	0.005	0.005	0.023	0.017	0.033	0.004	0.004	0.037	0.295	0.318	0.177	0.179	0.351
FSV-CI	0.172	0.305	0.066	0.065	0.318											0.034	0.041	<0.016	<0.016	0.046					
FSV-CS	0.187	0.355	0.053	0.052	0.372	0.168	0.319	0.050	0.046	0.334	0.020	0.036	0.003	0.006	0.038	0.014	0.030	0.005	0.005	0.031	0.325	0.371	0.188	0.182	0.387
FSV-CT	0.192	0.358	0.037	0.047	0.412																0.364	0.371	0.134	0.212	0.411
FSV-CW	≥0.226	≥0.409	≥0.057	≥0.058	≥0.490	0.226	0.409	0.057	0.058	0.490						0.021	0.035	0.009	0.006	0.043					
FSV-CZ	0.176	0.328	0.070	0.072	0.352																				
FSV-DA	0.180	0.353	0.056	0.067	0.383	0.161	0.327	0.051	0.061	0.357	0.019	0.026	0.004	0.007	0.026	0.014	0.030	0.006	0.006	0.032	0.334	0.367	0.201	0.240	0.397
FSV-DD																									
FSV-DF																									
FSV-DI	0.174	0.343	0.045	0.044	0.346																0.296	0.310	0.220	0.215	0.305
FSV-DW	0.110	0.220	0.050	0.050	0.220											0.013	0.024	0.006	0.007	0.024	0.190	0.210	0.140	0.150	0.210
FSV-ET	0.180	0.360	0.050	0.050	0.380																				
N	26	27	27	26	26	10	11	11	10	10	7	8	5	6	7	23	24	17	17	23	22	23	23	22	22
Min	0.110	0.220	0.033	0.032	0.220	0.138	0.252	0.040	0.040	0.291	0.010	0.018	0.002	0.000	0.019	0.006	0.015	0.002	0.003	0.020	0.175	0.210	0.134	0.148	0.210
Median	0.173	0.328	0.050	0.050	0.359	0.157	0.301	0.049	0.049	0.337	0.012	0.023	0.004	0.004	0.024	0.016	0.029	0.006	0.006	0.031	0.299	0.337	0.185	0.192	0.373
Max	0.212	0.409	0.078	0.072	0.429	0.226	0.409	0.061	0.061	0.490	0.020	0.036	0.005	0.007	0.038	0.034	0.047	0.027	0.024	0.046	0.418	0.402	0.303	0.240	0.419
SD	0.020	0.031	0.008	0.006	0.044	0.015	0.021	0.006	0.007	0.037	0.004	0.004	0.001	0.002	0.002	0.005	0.005	0.003	0.003	0.007	0.048	0.043	0.024	0.018	0.046
CV	11	10	16	13	12	9	7	12	15	11	31	18	26	58	9	31	18	49	42	24	16	13	13	10	12
N_{past}	31	30	27	27	30	15	14	12	12	14	9	9	6	6	9	27	26	16	15	25	26	25	21	21	24
$Median_{past}$	0.173	0.323	0.051	0.050	0.348	0.161	0.297	0.048	0.048	0.319	0.014	0.023	0.003	0.004	0.023	0.016	0.029	0.006	0.005	0.030	0.309	0.326	0.192	0.190	0.348
SD_{past}	0.022	0.047	0.007	0.007	0.038	0.018	0.028	0.006	0.004	0.023	0.007	0.008	0.002	0.002	0.006	0.004	0.007	0.003	0.002	0.007	0.052	0.053	0.031	0.029	0.053
NIST	≥0.155	≥0.308	≥0.050	≥0.054	≥0.306	0.155	0.308	0.050	0.054	0.306						*nq*	0.034	*nq*	*nq*	0.025					
N_{NIST}	3	3	3	3	3	3	3	3	3	3							3			3					
S_{rep}	0.008	0.017	0.003	0.002	0.016	0.008	0.017	0.003	0.002	0.016							0.003			0.003					
S_{het}	0.002	0.012	0.005	0.001	0.011	0.002	0.012	0.005	0.001	0.011							0.001			0.002					
S_{NIST}	0.008	0.021	0.006	0.002	0.020	0.008	0.021	0.006	0.002	0.020							0.003			0.003					
NAV	0.164	0.319	0.050	0.052	0.333	0.156	0.306	0.049	0.051	0.322	0.012	0.023	0.004	0.004	0.024	0.016	0.031	0.006	0.006	0.028	0.299	0.337	0.185	0.192	0.373
NAU	0.030	0.049	0.011	0.011	0.063	0.018	0.033	0.009	0.010	0.043	0.004	0.008	0.003	0.003	0.008	0.006	0.010	0.003	0.003	0.011	0.068	0.075	0.046	0.047	0.081

C3

Round Robin LVI Laboratory Results

All Values in µg/mL

	trans-Lycopene					Total β-Cryptoxanthin					Total α-Cryptoxanthin					Total Lutein					Total Zeaxanthin				
Lab	304	305	306	307	308	304	305	306	307	308	304	305	306	307	308	304	305	306	307	308	304	305	306	307	308
FSV-BA	0.154	0.177	0.095	0.094	0.200	0.078	0.059	0.020	0.020	0.070	0.023	0.029	0.012	0.012	0.035										
FSV-BB	0.144	0.160	0.087	0.084	0.172	0.069	0.049	0.019	0.018	0.053	0.018	0.022	0.010	0.010	0.024	0.057	0.097	0.053	0.049	0.099	0.034	0.040	0.019	0.018	0.039
FSV-BC																									
FSV-BD																									
FSV-BE																									
FSV-BF						0.063	0.043	0.014	0.015	0.044															
FSV-BG	0.144	0.194	0.111	0.108	0.209	0.080	0.050	0.018	0.019	0.047						0.058	0.091	0.045	0.051	0.097	0.020	0.024	0.016	0.016	0.022
FSV-BH						0.074	0.051	0.020	0.018	0.053						0.043	0.073	0.038	0.038	0.079	0.034	0.031	0.017	0.017	0.034
FSV-BI						0.087	0.066	0.020	0.020	0.066						0.048	0.077	0.041	0.040	0.081	0.030	0.029	0.022	0.018	0.030
FSV-BJ						0.083	0.055	0.017	0.018	0.059						0.063	0.087	0.043	0.044	0.089					
FSV-BK																									
FSV-BL																									
FSV-BM																									
FSV-BN		0.168	0.096				0.049	0.013				0.018	0.005				0.061	0.031	0.070	0.105		0.031	0.021	0.033	0.043
FSV-BO						0.059	0.037	0.012	0.009	0.047						0.050	0.075	0.044	0.036	0.086	0.026	0.021	0.014	0.013	0.017
FSV-BP						0.068	0.048	0.018	0.026	0.044															
FSV-BQ																									
FSV-BR																									
FSV-BS						0.088	0.066	0.033	0.031	0.065															
FSV-BT	0.148	0.138	0.096	0.095	0.162	0.064	0.047	0.018	0.018	0.047	0.026	0.029	0.014	0.014	0.032	0.068	0.083	0.041	0.040	0.078	0.031	0.023	0.015	0.015	0.025
FSV-BU						0.072	0.047	0.019	0.019	0.053															
FSV-BV						0.056	0.030	0.008	0.008	0.032															
FSV-BW						0.066	0.039	0.016	0.020	0.046															
FSV-BX	0.122	0.157	0.088	0.087	0.176	0.069	0.046	0.017	0.018	0.052						0.053	0.084	0.043	0.046	0.092	0.031	0.028	0.019	0.019	0.030
FSV-CB						0.084	0.056	0.025	0.024	0.060						0.051	0.057	0.033	0.048	0.075	0.038	0.019	0.014	0.022	0.022
FSV-CC																									
FSV-CD						0.072	0.051	0.023	0.022	0.058															
FSV-CE																									
FSV-CF																									
FSV-CG	0.166	0.182	0.099	0.100	0.202	0.088	0.067	0.026	0.026	0.074															
FSV-CI																0.055	0.074	0.042	0.036	0.078	0.041	0.030	0.021	0.018	0.032
FSV-CS						0.070	0.051	0.019	0.019	0.053	0.021	0.024	0.011	0.011	0.025	0.054	0.084	0.047	0.045	0.091	0.024	0.026	0.015	0.013	0.027
FSV-CT						0.077	0.049	0.013	0.016	0.059						0.052	0.078	0.047	0.048	0.086	0.030	0.022	0.011	0.021	0.029
FSV-CW	0.100	0.097	0.060	0.064	0.114	0.097	0.070	0.030	0.031	0.077						0.068	0.100	0.059	0.060	0.116	0.046	0.043	0.030	0.031	0.048
FSV-CZ																									
FSV-DA	0.174	0.197	0.108	0.130	0.217	0.077	0.058	0.020	0.025	0.062	0.020	0.025	0.011	0.014	0.026	0.055	0.088	0.046	0.055	0.094	0.039	0.034	0.022	0.025	0.035
FSV-DD																									
FSV-DF																									
FSV-DI																0.067	0.089	0.048	0.048	0.093					
FSV-DW						0.069	0.052	0.021	0.022	0.050						0.043	0.070	0.044	0.044	0.067	0.041	0.025	0.024	0.025	0.031
FSV-ET																									
N	8	9	9	8	8	23	24	24	23	23	5	6	6	5	5	16	17	17	17	17	14	15	15	15	15
Min	0.100	0.097	0.060	0.064	0.114	0.056	0.030	0.008	0.008	0.032	0.018	0.018	0.005	0.010	0.024	0.043	0.057	0.031	0.036	0.067	0.020	0.019	0.011	0.013	0.017
Median	0.146	0.168	0.096	0.094	0.188	0.072	0.051	0.019	0.019	0.053	0.021	0.024	0.011	0.012	0.026	0.054	0.083	0.044	0.046	0.089	0.032	0.028	0.019	0.018	0.030
Max	0.174	0.197	0.111	0.130	0.217	0.097	0.070	0.033	0.031	0.077	0.026	0.029	0.014	0.014	0.035	0.068	0.100	0.059	0.070	0.116	0.046	0.043	0.030	0.033	0.048
SD	0.014	0.019	0.008	0.012	0.025	0.010	0.007	0.003	0.004	0.011	0.003	0.004	0.001	0.002	0.005	0.006	0.010	0.004	0.006	0.011	0.006	0.006	0.005	0.005	0.007
CV	9	11	8	12	14	14	14	14	21	20	13	16	11	16	19	11	12	9	14	13	19	20	25	28	22
N_{past}	13	11	10	10	11	27	26	23	23	25	5	5	5	4	6	17	16	16	16	16	15	15	14	13	14
$Median_{past}$	0.161	0.169	0.098	0.098	0.186	0.073	0.051	0.019	0.018	0.054	0.021	0.024	0.011	0.010	0.025	0.055	0.084	0.045	0.044	0.089	0.032	0.025	0.018	0.019	0.027
SD_{past}	0.029	0.033	0.011	0.009	0.040	0.013	0.009	0.004	0.004	0.009	0.005	0.004	0.003	0.003	0.005	0.014	0.013	0.012	0.013	0.018	0.010	0.007	0.003	0.004	0.006
NIST						0.073	0.054	0.026	0.024	0.049						0.056	0.091	0.046	0.049	0.088	0.019	0.021	*nq*	0.019	0.016
N_{NIST}						3	3	2	3	3						3	3	3	3	3	3	3		3	3
S_{rep}						0.003	0.005	0.002	0.000	0.005						0.003	0.008	0.003	0.002	0.007	0.001	0.003		0.001	0.003
S_{het}						0.002	0.002	0.002	0.001	0.006						0.003	0.003	0.005	0.003	0.001	0.000	0.002		0.001	0.001
S_{NIST}						0.004	0.005	0.003	0.001	0.008						0.004	0.008	0.006	0.004	0.007	0.001	0.004		0.001	0.003
NAV	0.146	0.168	0.096	0.094	0.188	0.073	0.052	0.023	0.022	0.051	0.021	0.024	0.011	0.012	0.026	0.055	0.087	0.045	0.047	0.089	0.026	0.025	0.019	0.018	0.023
NAU	0.026	0.030	0.017	0.016	0.033	0.017	0.013	0.007	0.006	0.013	0.003	0.004	0.001	0.002	0.005	0.011	0.017	0.010	0.010	0.017	0.013	0.009	0.006	0.006	0.013

C4

Round Robin LVI Laboratory Results

All Values in µg/mL

	Total Lutein&Zeaxanthin					Coenzyme Q10					Phylloquinone (K1) x1000				
Lab	304	305	306	307	616	304	305	306	307	308	304	305	306	307	308
FSV-BA	0.083	0.121	0.067	0.062	0.140										
FSV-BB	0.099	0.142	0.077	0.073	0.147										
FSV-BC															
FSV-BD															
FSV-BE											0.84	0.51	0.19	0.18	0.49
FSV-BF	0.079	0.102	0.058	0.057	0.112	0.501	0.651	0.320	0.355	0.746					
FSV-BG	0.065	0.101	0.054	0.057	0.103										
FSV-BH	0.077	0.104	0.055	0.055	0.113										
FSV-BI	*0.078*	*0.106*	*0.063*	*0.058*	*0.111*										
FSV-BJ						0.634	0.769	0.459	0.395	0.726					
FSV-BK															
FSV-BL															
FSV-BM															
FSV-BN		0.076	0.042	0.082	0.122										
FSV-BO	0.0757	0.0950	0.0580	0.0489	0.1030										
FSV-BP	0.115	0.134	0.082	0.075	0.123										
FSV-BQ															
FSV-BR															
FSV-BS	0.107	0.162	0.089	0.091	0.198										
FSV-BT	0.102	0.109	0.058	0.056	0.106										
FSV-BU	0.082	0.098	0.054	0.055	0.104										
FSV-BV	0.116	0.109	0.058	0.059	0.116										
FSV-BW	0.074	0.092	0.049	0.051	0.108	0.970	1.110	0.660	0.660	1.230					
FSV-BX	0.084	0.113	0.061	0.064	0.122										
FSV-CB	*0.089*	*0.076*	*0.046*	*0.069*	*0.096*										
FSV-CC															
FSV-CD	0.096	0.104	0.062	0.058	0.105										
FSV-CE															
FSV-CF															
FSV-CG	0.110	0.128	0.075	0.076	0.142										
FSV-CI	*0.096*	*0.104*	*0.063*	*0.054*	*0.110*						0.82	0.39	0.15	0.17	0.45
FSV-CS	*0.077*	*0.110*	*0.062*	*0.058*	*0.118*										
FSV-CT	0.082	0.100	0.058	0.069	0.115										
FSV-CW	*0.114*	*0.143*	*0.089*	*0.091*	*0.164*	0.500	0.800	0.300	0.400	0.900					
FSV-CZ						0.680	0.700	0.590	0.530	0.930					
FSV-DA	0.094	0.122	0.068	0.080	0.130										
FSV-DD															
FSV-DF															
FSV-DI						0.688	0.806	0.476	0.458	0.810	0.72	0.45	0.16	0.18	0.37
FSV-DW	*0.084*	*0.095*	*0.068*	*0.069*	*0.098*										
FSV-ET															
N	23	24	24	24	24	6	6	6	6	6	3	3	3	3	3
Min	0.065	0.076	0.042	0.049	0.096	0.500	0.651	0.300	0.355	0.726	0.72	0.39	0.15	0.17	0.37
Median	0.084	0.105	0.061	0.061	0.114	0.657	0.785	0.468	0.429	0.855	0.82	0.45	0.16	0.18	0.45
Max	0.116	0.162	0.089	0.091	0.198	0.970	1.110	0.660	0.660	1.230	0.84	0.51	0.19	0.18	0.49
SD	0.016	0.016	0.008	0.012	0.014	0.113	0.065	0.153	0.086	0.119					
CV	19	15	13	20	12	17	8	33	20	14					
N_{past}	26	25	22	22	23	6	6	7	7	5	0	0	0	0	0
$Median_{past}$	0.094	0.114	0.065	0.063	0.120	0.617	0.831	0.470	0.475	0.895					
SD_{past}	0.018	0.015	0.015	0.013	0.020	0.329	0.219	0.062	0.072	0.195					
NIST	*0.075*	*0.112*	*≥0.046*	*0.068*	*0.105*										
N_{NIST}	3	3		3	3										
S_{rep}	0.002	0.006		0.002	0.009										
S_{het}	0.002	0.002		0.003	0.002										
S_{NIST}	0.003	0.006		0.004	0.009										
NAV	0.080	0.109	0.061	0.064	0.109	0.657	0.785	0.468	0.429	0.855	0.820	0.450	0.160	0.180	0.450
NAU	0.018	0.022	0.013	0.014	0.025	0.113	0.065	0.153	0.086	0.119					

C5

Round Robin LVI Laboratory Results
All Results in µg/mL

Analytes Reported By One Laboratory

Analyte	Code	304	305	306	307	308
25-hydroxyvitamin D	FSV-BN	0.0830	0.0590	0.0700	0.0730	0.0630
Phytoene	FSV-DA	0.017	0.026	0.010	0.010	0.027
Phytofluene	FSV-DA	0.042	0.048	0.027	0.032	0.053
Retinyl stearate	FSV-DA	0.007	0.012	0.004	0.004	0.014
trans-Lutein	FSV-DA	0.048	0.077	0.041	0.049	0.083
Ubiquinol	FSV-BW	0.800	1.000	0.500	0.500	0.940
Ubiquinone	FSV-BW	0.170	0.110	0.160	0.160	0.290

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	Standard deviation for (non-NIST) results: 0.741*(3rd Quartile - 1st Quartile)
CV	Coefficient of Variation for (non-NIST) results: 100*SD/Median
N_{past}	Mean of N(s) from past RR(s)
$Median_{past}$	Mean of Median(s) from past RR(s)
SD_{past}	Pooled SD from past RR(s)
NIST	Mean of all analyses (vials x duplicates) reported by a NIST analyst
N_{NIST}	Number of total vials analyzed in duplicate by NIST analysts
S_{rep}	Within-vial pooled standard deviation
S_{het}	Among-vial pooled standard deviation
S_{NIST}	Total standard deviation for NIST analyses: $(S_{rep}^2 + S_{het}^2)^{0.5}$
NAV	NIST Assigned Value = (Median + $Mean_{NIST}$)/2 for analytes reported by NIST analyst(s) = Median for analytes reported by ≥ 10 labs but not NIST
NAU	NIST Assigned Uncertainty: $(S^2 + S_{btw}^2)^{0.5}$ S is the maximum of (0.05*NAV, SD, S_{NIST}, eSD) and S_{btw} is the standard deviation between $Median_{part}$ and $Mean_{NIST}$. The expected long-term SD, eSD, is defined in: Duewer, et al. Anal Chem 1997;69(7):1406-1413.
-	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

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	1	1
FSV-BB	1	1	1	1	1	1	1	1	1	1	1
FSV-BC	1										
FSV-BD	1	1									1
FSV-BE	1	1	1	1							
FSV-BF	1	1	1	2		1	1	1			1
FSV-BG	1	1	1	1		1	1	1	1	1	2
FSV-BH	2	1		1	1	2	1	1			
FSV-BI	2	1	1	2		1	1	1	1	1	1
FSV-BJ	1	1	1	2		1	1	1	1	1	1
FSV-BK	1	2									
FSV-BL	3	2							1		
FSV-BM	2	1									
FSV-BN	1	2	2	1	1		1	1	1	1	1
FSV-BO	1	2		1		1	1	2			2
FSV-BP	2	1		2		4	2	1	2	2	2
FSV-BQ	2	1									3
FSV-BR		1							1	1	1
FSV-BS	3	4	4		1	4	1	2	1	1	1
FSV-BT	1	1	1	1	1	1	1	1	1	1	1
FSV-BU	1	1	1	1		1	1	1			
FSV-BV	2	2	2	1		1	1	2			
FSV-BW	1	1	3	1		1	1	1			2
FSV-BX	1	1	1		1	1		1			
FSV-CB	2	1		1		2	2	1			1
FSV-CC	1	1									1
FSV-CD	1	1	1	1		1	1	1	1	1	1
FSV-CE	3	1		1							1
FSV-CF	1	1									
FSV-CG	1	2	1	1	1	1	1	2			1
FSV-CI	1	3	2	2		3					
FSV-CS	2	3	1	1	1	1	1	1	1	1	1
FSV-CT				1			1	1	1	1	1
FSV-CW		2	2		3	1		2			
FSV-CZ	1	1		2							
FSV-DA	1	1	1	1	1	1	1	1	1	1	1
FSV-DD	4										
FSV-DF	1								1		
FSV-DI	1	1	1	1			1		2	2	2
FSV-DW	1	1		2		1	2	1			
FSV-ET	1	1	1	1							
NISTa	1	1	1		1	1		1	1	1	1
n	39	38	23	27	12	24	23	25	18	16	25

	TR	aT	g/bT	bC	tbC	aC	TLy	TbX	TLu	TZ	L&Z
% 1	69	74	74	74	92	79	87	80	89	88	76
% 2	21	18	17	26	0	8	13	20	11	13	20
% 3	8	5	4	0	8	4	0	0	0	0	4
% 4	3	3	4	0	0	8	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) 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 RR56

Each participant in RR56 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 RR56:

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

The following 15 pages are the "Individualized Report" for the analytes evaluated by participant FSV-BA.

Individualized Round Robin LVI Report: FSV-BA

Summary

	Serum 304			Serum 305			Serum 306			Serum 307			Serum 308		
Analyte	You	NAV	n	You	NAV	n	You	NAV	n	You	NAV	n	You	NAV	n
Total Retinol	0.841	0.852	38	0.467	0.446	38	0.653	0.624	38	0.571	0.592	38	0.502	0.456	38
Retinyl Palmitate	0.04	0.03	11	0.1	0.1	11	0.0	0.0	11	0.02	0.02	11	0.05	0.06	11
α-Tocopherol	6.85	7.49	37	6.46	6.73	37	2.70	2.71	37	2.72	2.78	37	6.97	7.05	37
γ/β-Tocopherol	3.801	3.831	22	1.900	1.823	22	0.733	0.703	22	0.728	0.709	22	2.051	1.906	22
δ-Tocopherol	0.110	0.130	7	0.100	0.100	7	0.032	0.038	7	0.035	0.039	7	0.113	0.120	7
Total β-Carotene	0.160	0.164	26	0.319	0.319	27	0.045	0.050	27	0.047	0.052	26	0.360	0.333	26
trans-β-Carotene	0.150	0.156	10	0.301	0.306	11	0.045	0.049	11	0.045	0.051	10	0.340	0.322	10
Total cis-β-Carotene	0.011	0.012	7	0.018	0.023	8	0.002	0.004	5	0.002	0.004	6	0.019	0.024	7
Total α-Carotene	0.015	0.016	23	0.028	0.031	24	0.003	0.006	17	0.004	0.006	17	0.030	0.028	23
Total Lycopene	0.294	0.299	22	0.333	0.337	23	0.183	0.185	23	0.180	0.192	22	0.382	0.373	22
trans-Lycopene	0.154	0.146	8	0.177	0.168	9	0.095	0.096	9	0.094	0.094	8	0.200	0.188	8
Total β-Cryptoxanthin	0.078	0.073	23	0.059	0.052	24	0.020	0.023	24	0.020	0.022	23	0.070	0.051	23
Total α-Cryptoxanthin	0.023	0.021	5	0.029	0.024	6	0.012	0.011	6	0.012	0.012	5	0.035	0.026	5
Total Lutein&Zeaxanthin	0.083	0.080	23	0.121	0.109	24	0.067	0.061	24	0.062	0.064	24	0.140	0.109	24

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

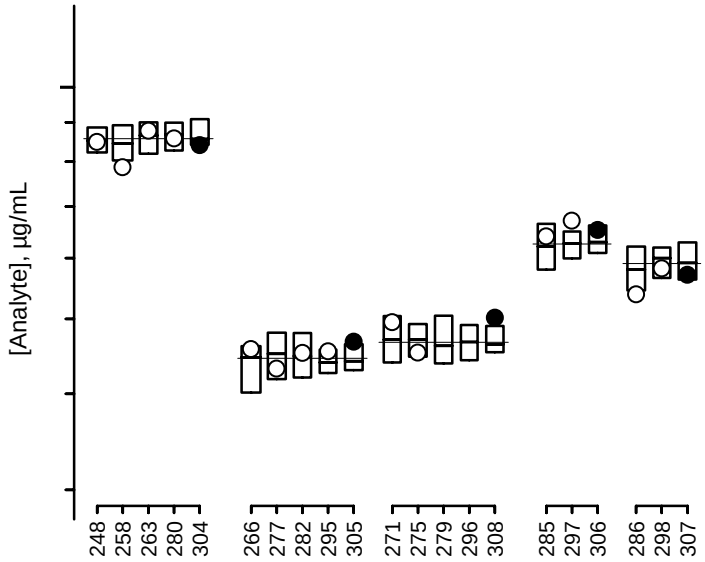
Please check our records against your records. Send corrections and/or updates to...

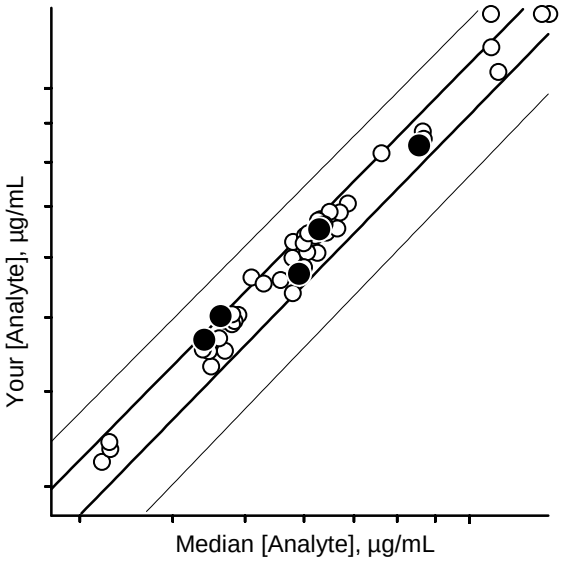
Micronutrients Measurement Quality Assurance Program
National Institute of Standards and Technology
100 Bureau Drive Stop 8392
Gaithersburg, MD 20899-8392 USA

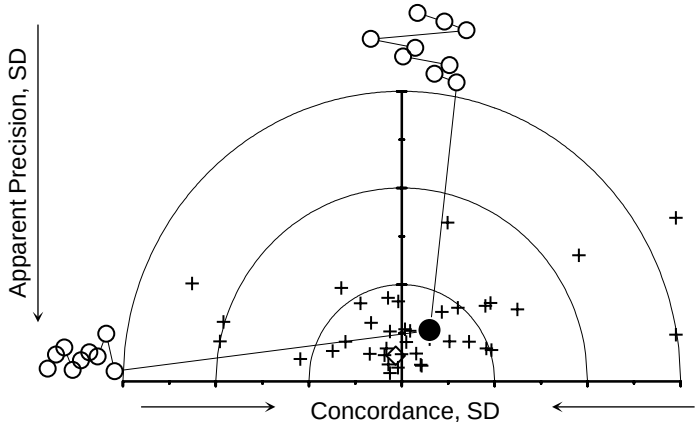
Tel: (301) 975-3935
Fax: (301) 977-0685
Email: david.duewer@nist.gov

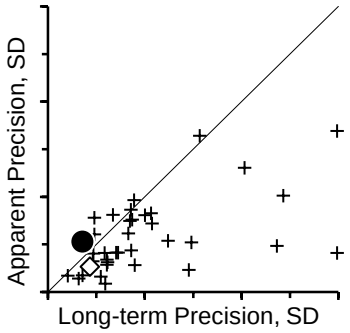
Individualized RR LVI Report: FSV-BA

Total Retinol







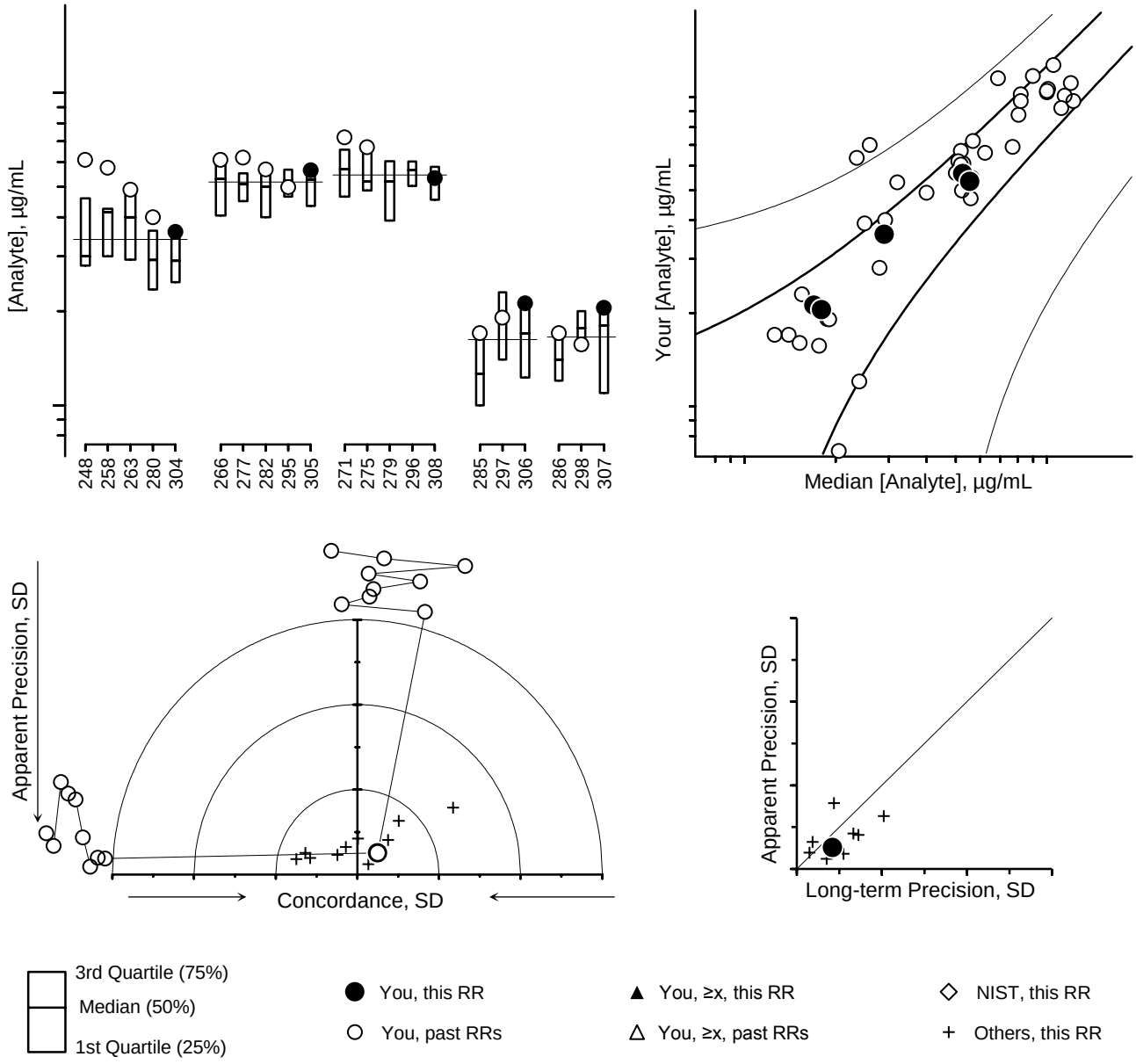


For details of the construction and interpretation of these plots, see:
Duewer, Kline, Sharpless, Brown Thomas, Gary, Sowell. Anal Chem 1999;71(9):1870-8.

Serum	History	Comments
#304	Lyophilized: #248(44), #258(46), #263(47), #280(51)	Augmented, multi-source (SRM 968c Level I)
#305	Lyophilized: #266(48), #277(50), #282(51), #295(54)	Same pool as #308, native
#306	Fresh-frozen: #285(52), #297(54)	Same pool as #307, trans-Retinol augmented
#307	Fresh-frozen: #286(52), #298(54)	Same pool as #306, cis-Retinol augmented
#308	Fresh-frozen: #271(49), #275(50), #279(51), #296(54)	Same pool as #305, native

Individualized RR LVI Report: FSV-BA

Retinyl Palmitate



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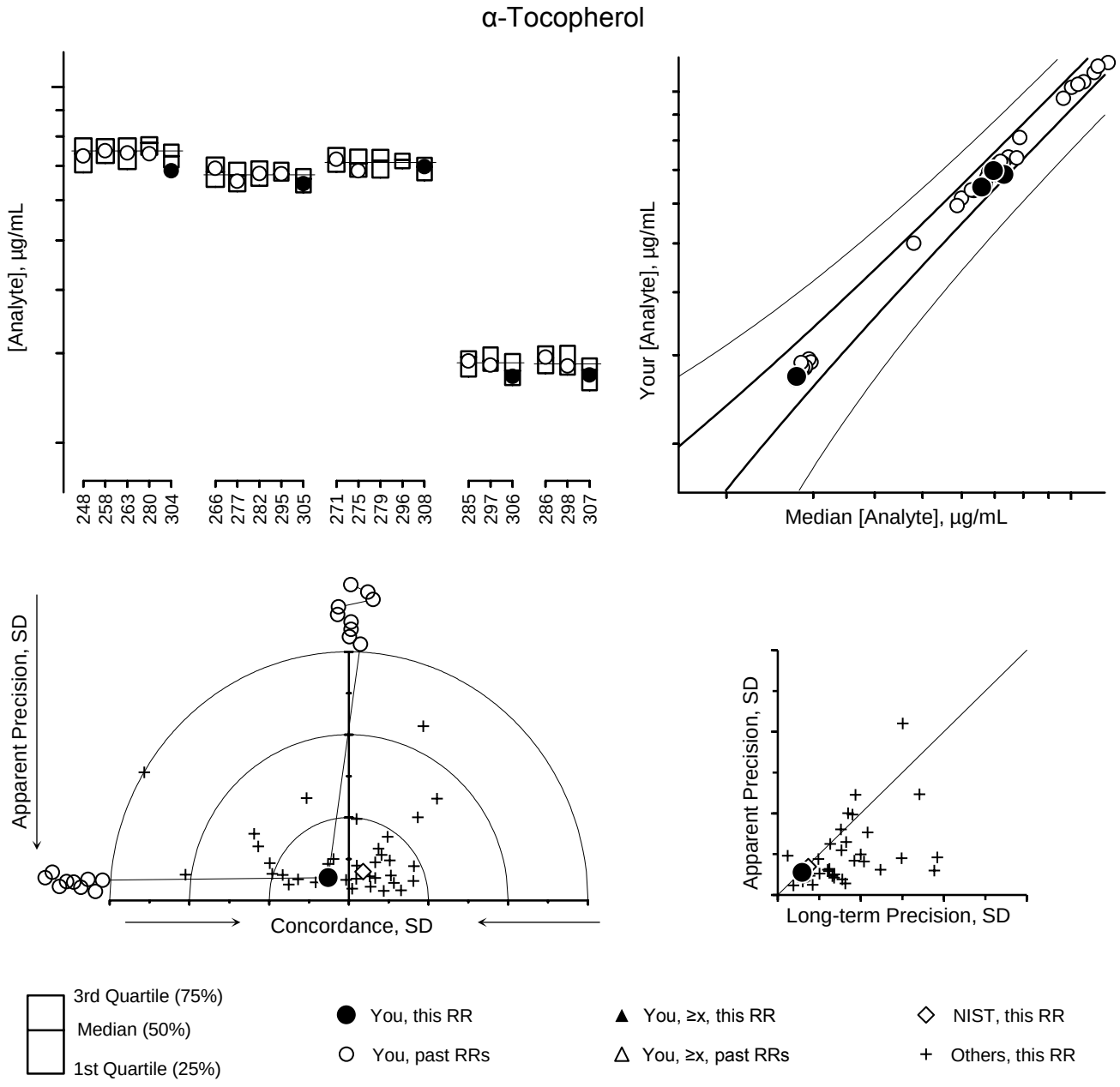
Individualized RR LVI Report: FSV-BA



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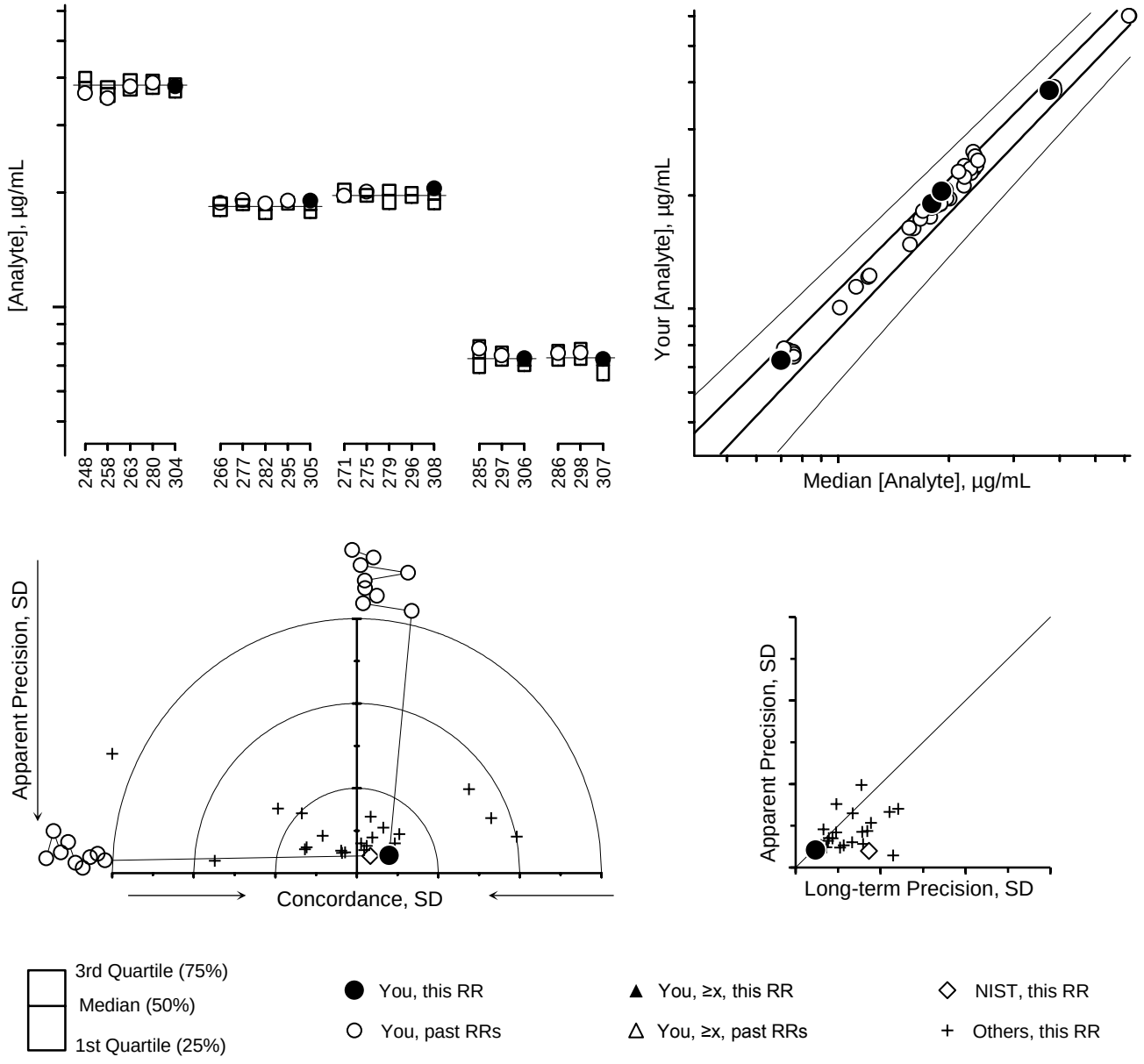
#308	Fresh-frozen: #271(49), #275(50), #279(51), #296(54)	Same pool as #305, native

Individualized RR LVI Report: FSV-BA

γ/β-Tocopherol



For details of the construction and interpretation of these plots, see:
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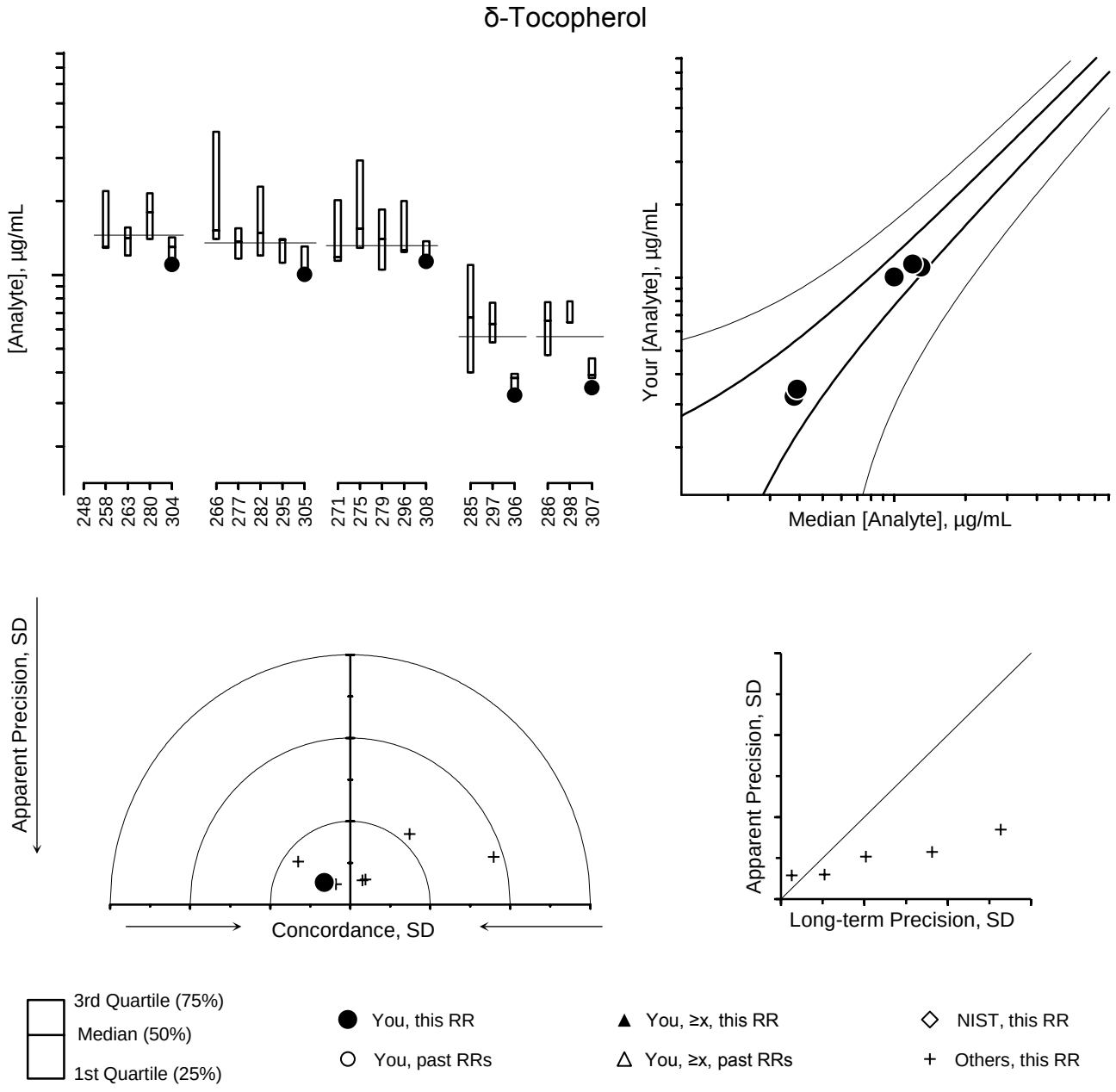
Individualized RR LVI Report: FSV-BA



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.

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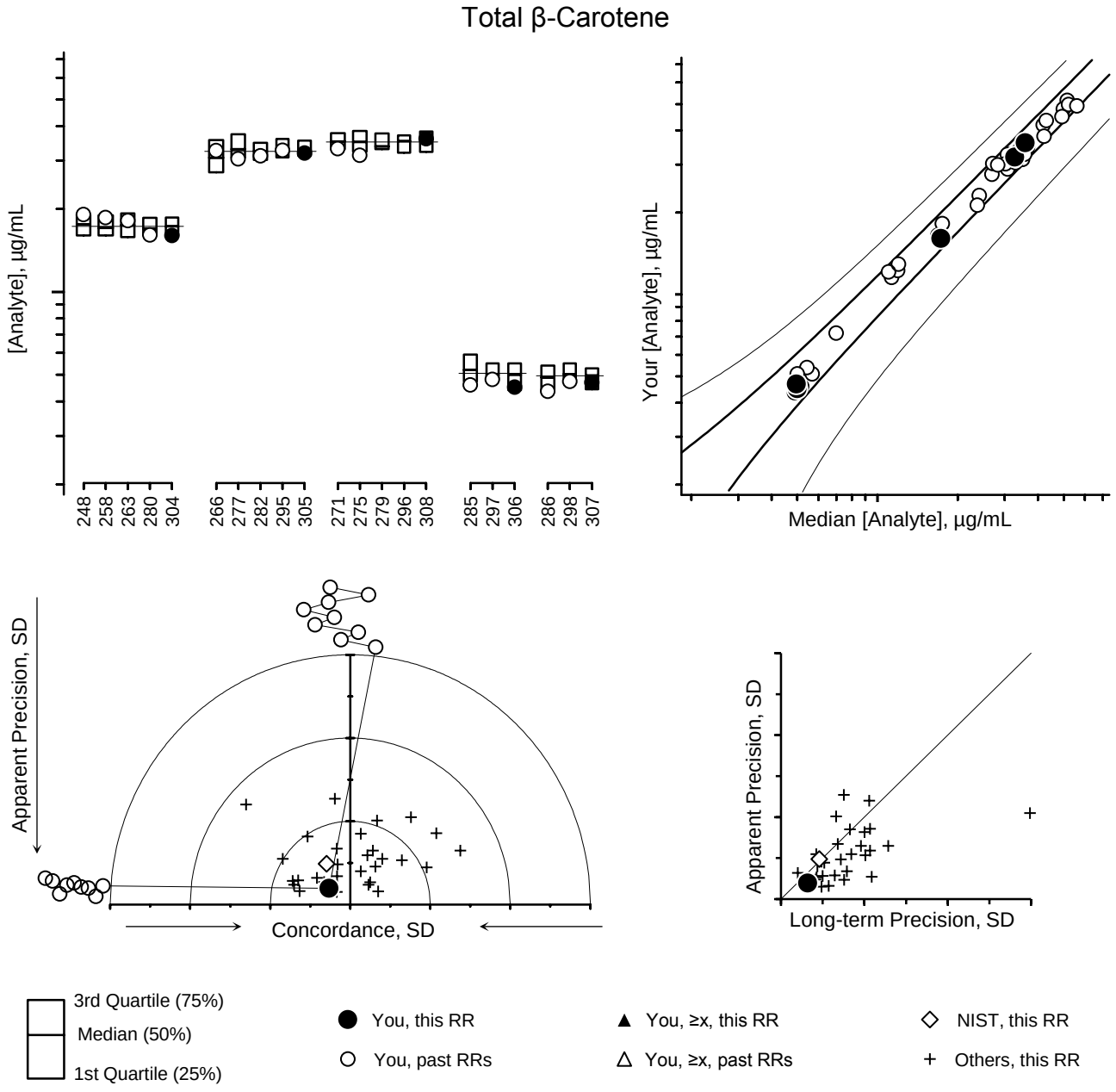
#308	Fresh-frozen: #271(49), #275(50), #279(51), #296(54)	Same pool as #305, native

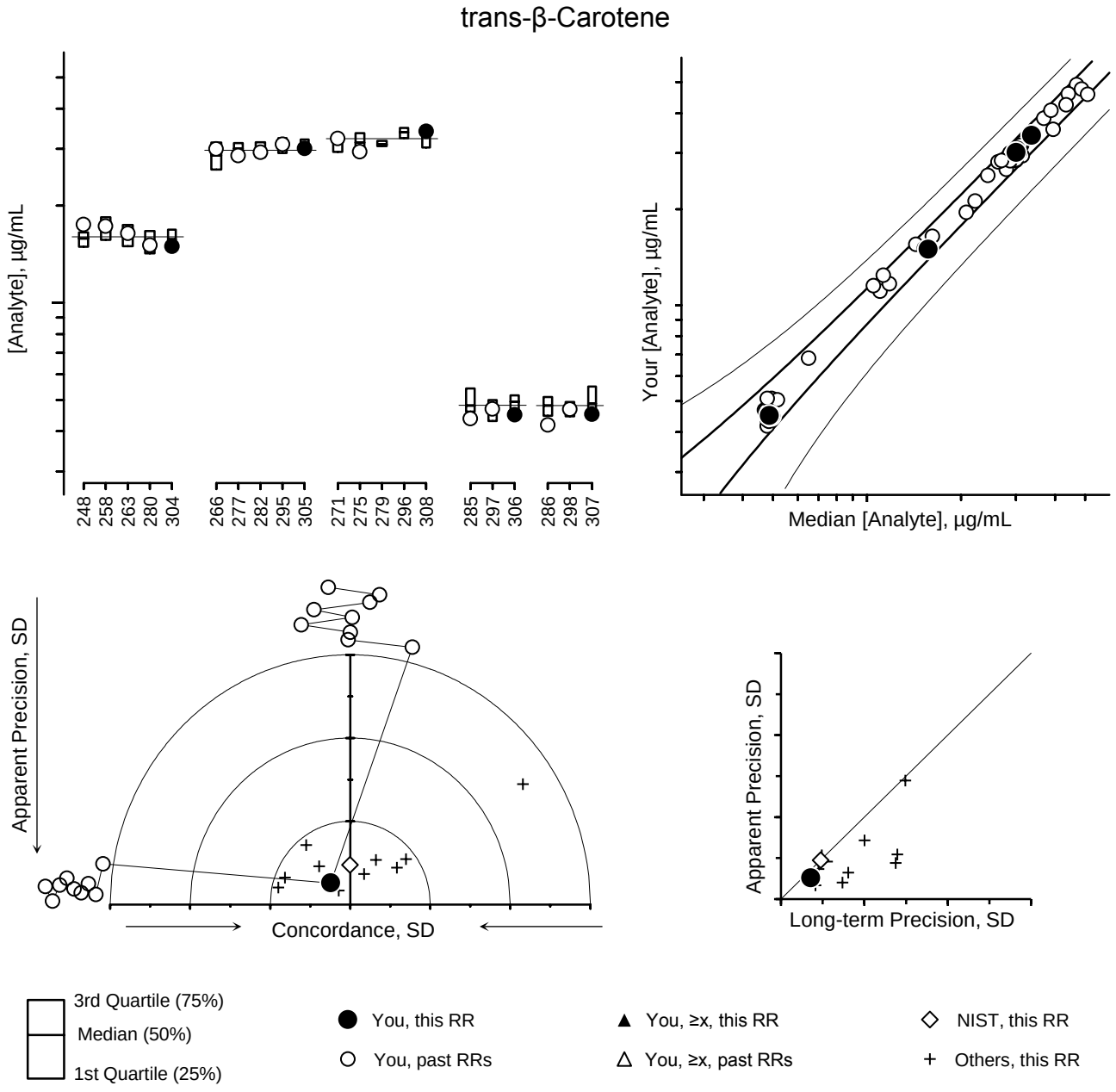
Individualized RR LVI Report: FSV-BA



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
#304	Lyophilized: #248(44), #258(46), #263(47), #280(51)	Augmented, multi-source (SRM 968c Level I)
#305	Lyophilized: #266(48), #277(50), #282(51), #295(54)	Same pool as #308, native
#306	Fresh-frozen: #285(52), #297(54)	Same pool as #307, trans-Retinol augmented
#307	Fresh-frozen: #286(52), #298(54)	Same pool as #306, cis-Retinol augmented
#308	Fresh-frozen: #271(49), #275(50), #279(51), #296(54)	Same pool as #305, native

Individualized RR LVI Report: FSV-BA



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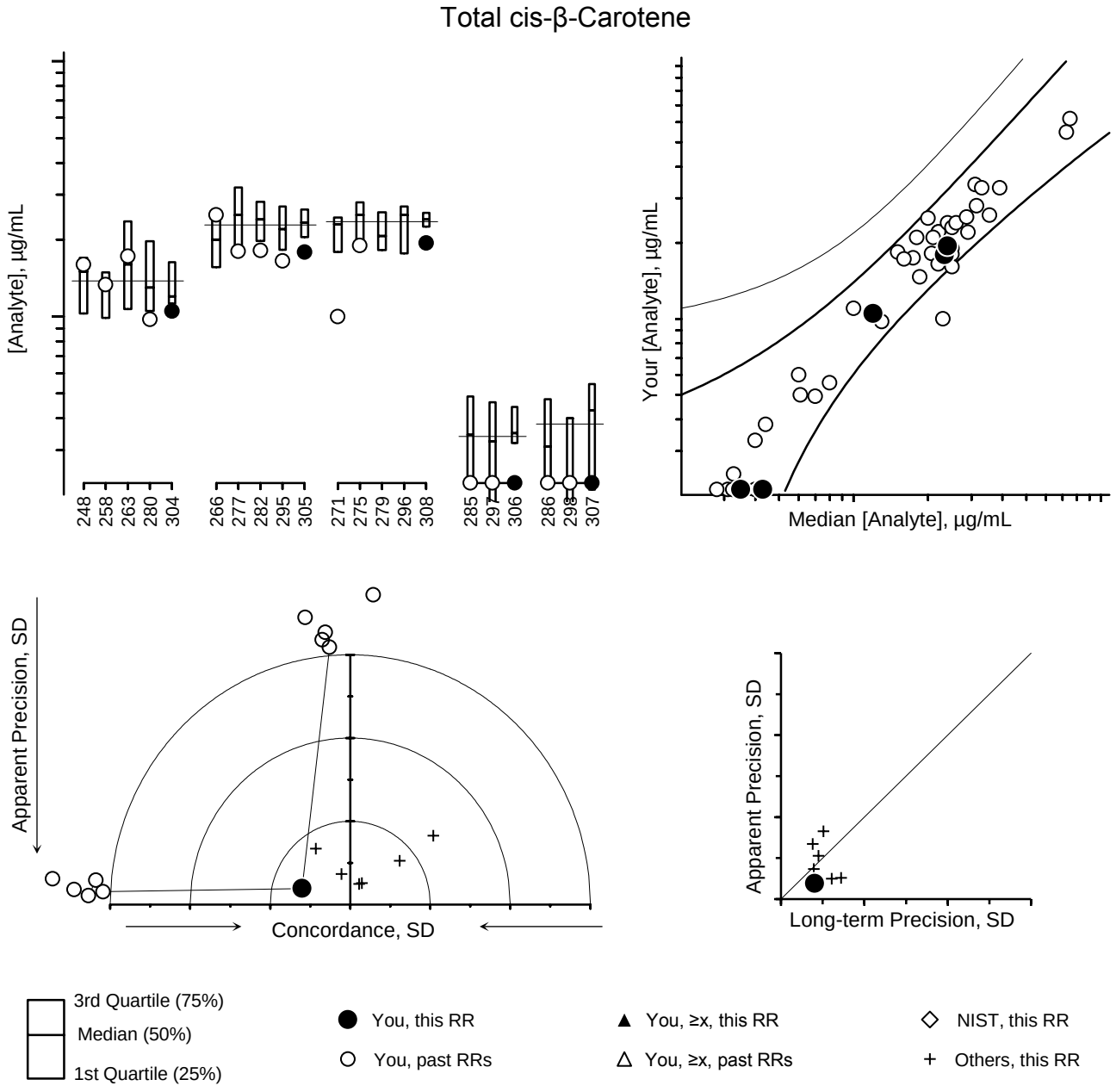
Individualized RR LVI Report: FSV-BA



For details of the construction and interpretation of these plots, see:
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Serum	History	Comments
#304	Lyophilized: #248(44), #258(46), #263(47), #280(51)	Augmented, multi-source (SRM 968c Level I)
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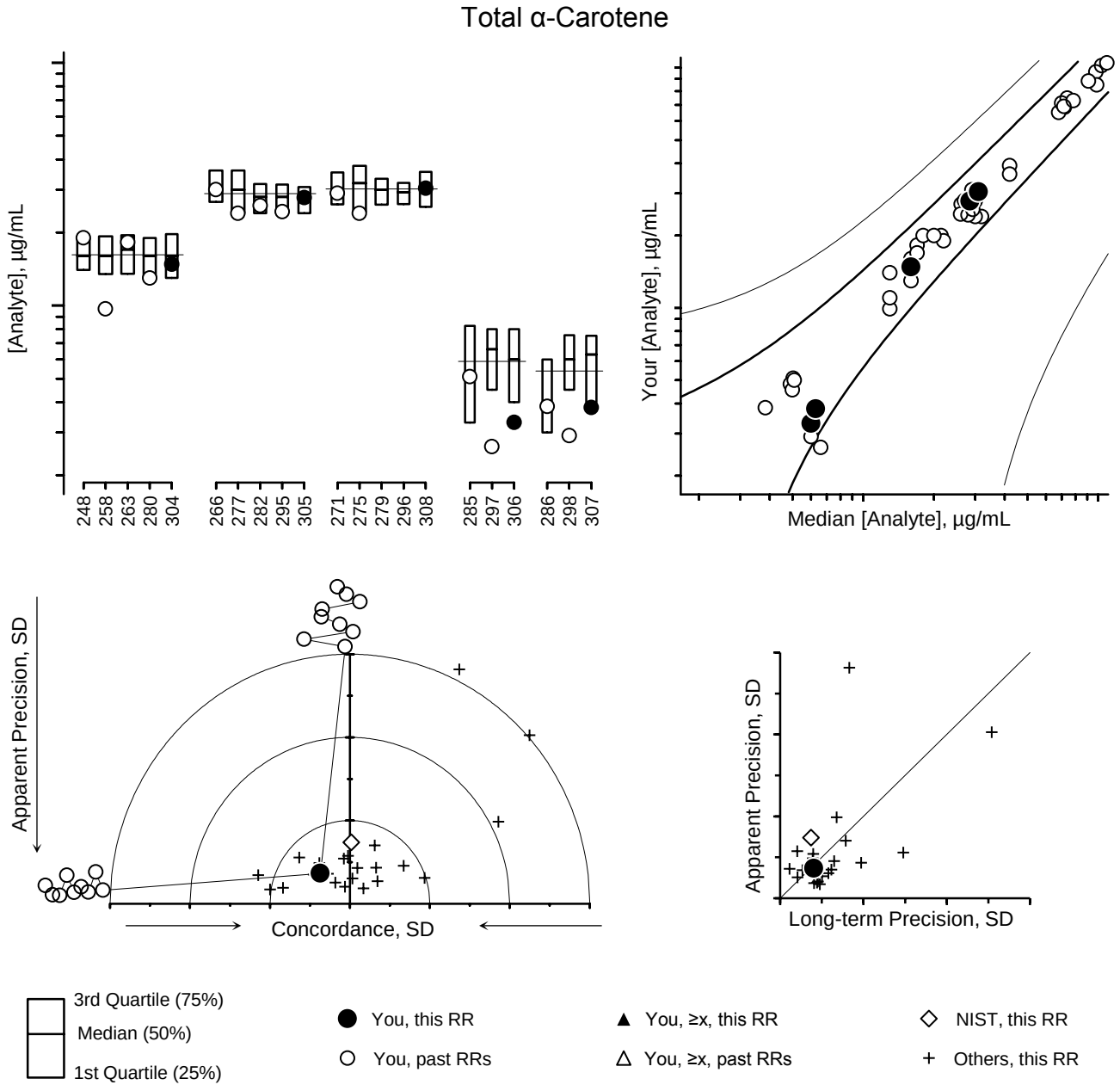
#308	Fresh-frozen: #271(49), #275(50), #279(51), #296(54)	Same pool as #305, native

Individualized RR LVI 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.

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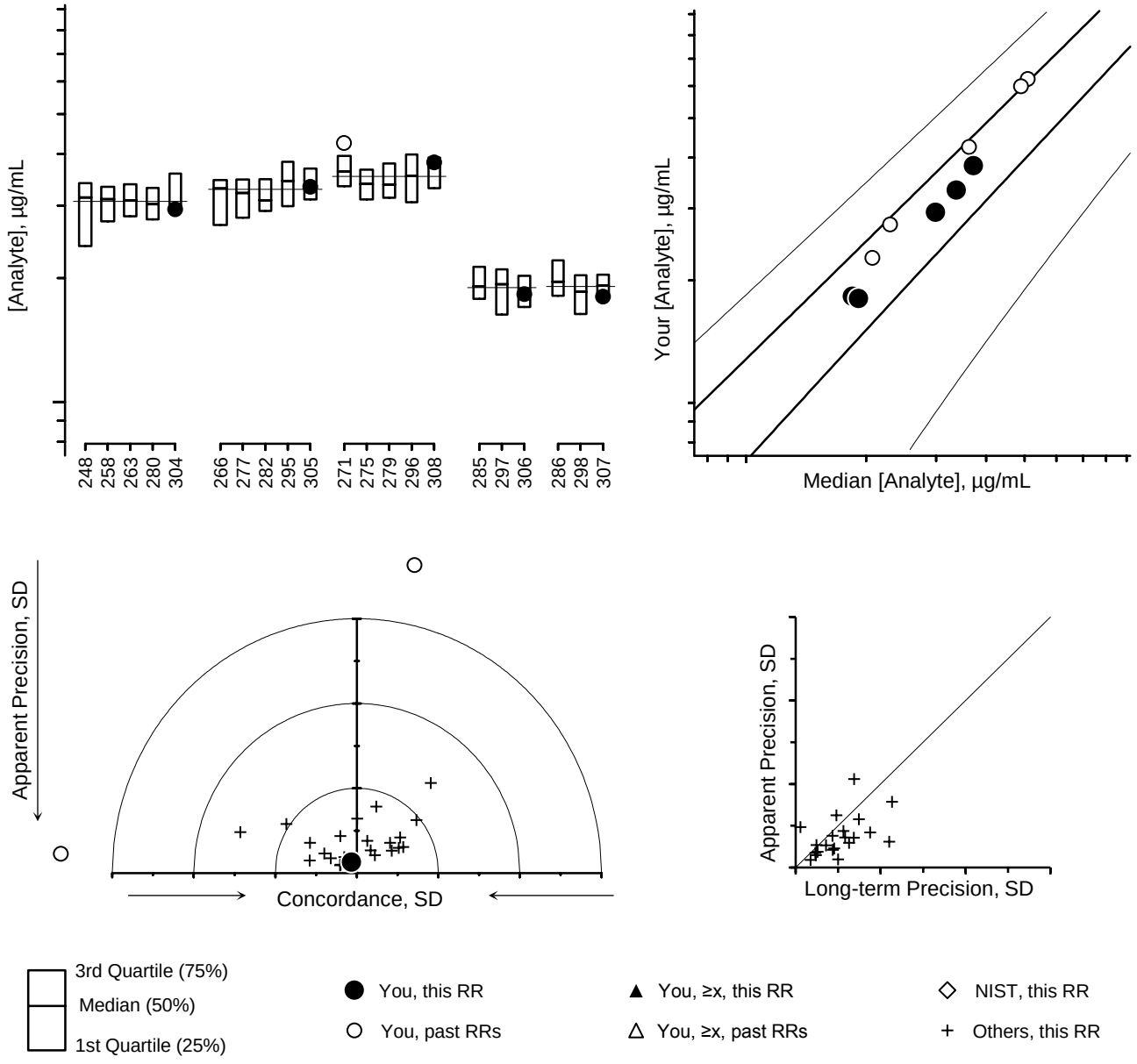
#308	Fresh-frozen: #271(49), #275(50), #279(51), #296(54)	Same pool as #305, native

Individualized RR LVI Report: FSV-BA

Total Lycopene



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

Serum	History	Comments
#304	Lyophilized: #248(44), #258(46), #263(47), #280(51)	Augmented, multi-source (SRM 968c Level I)
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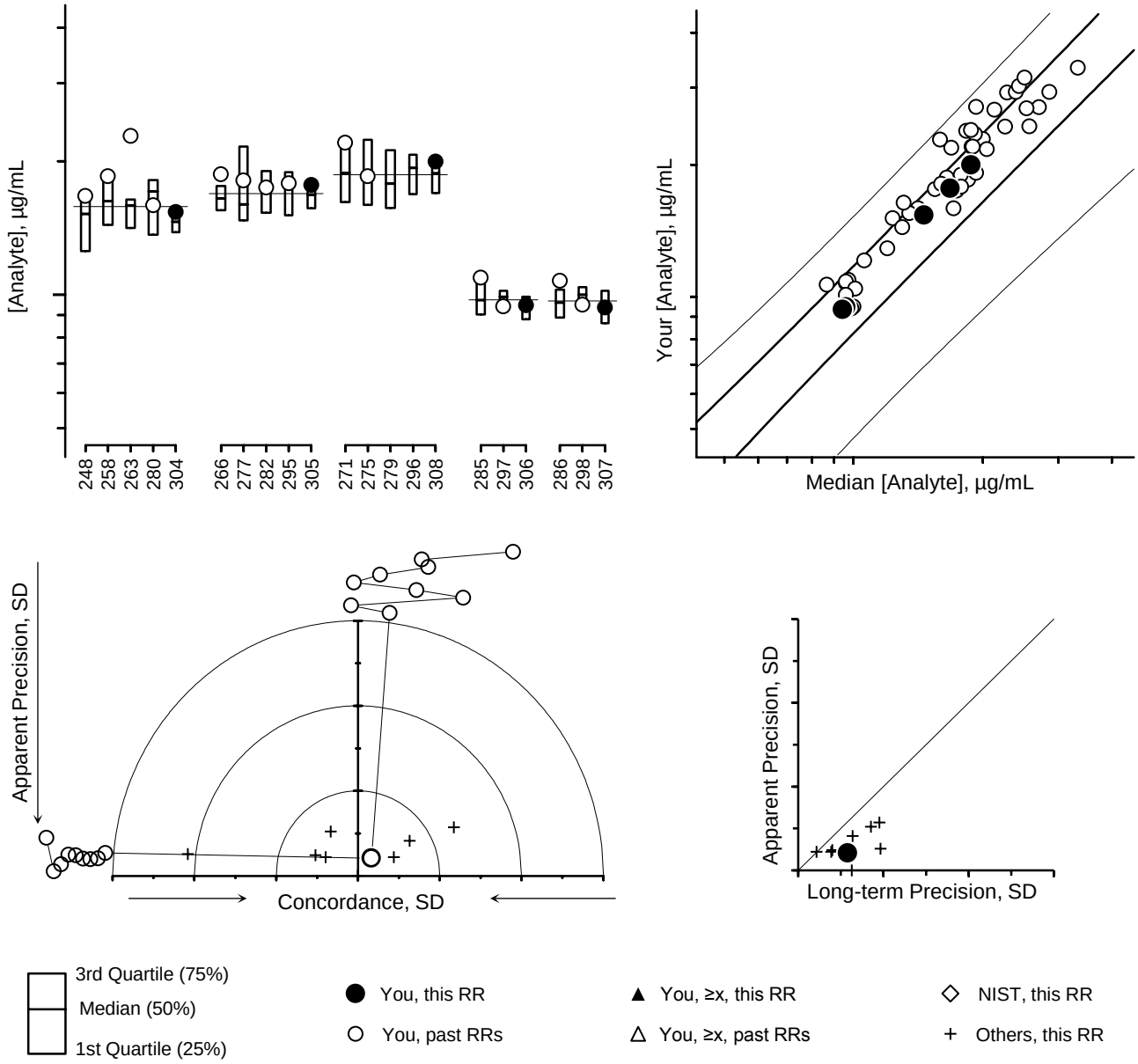
Individualized RR LVI Report: FSV-BA

trans-Lycopene



For details of the construction and interpretation of these plots, see:
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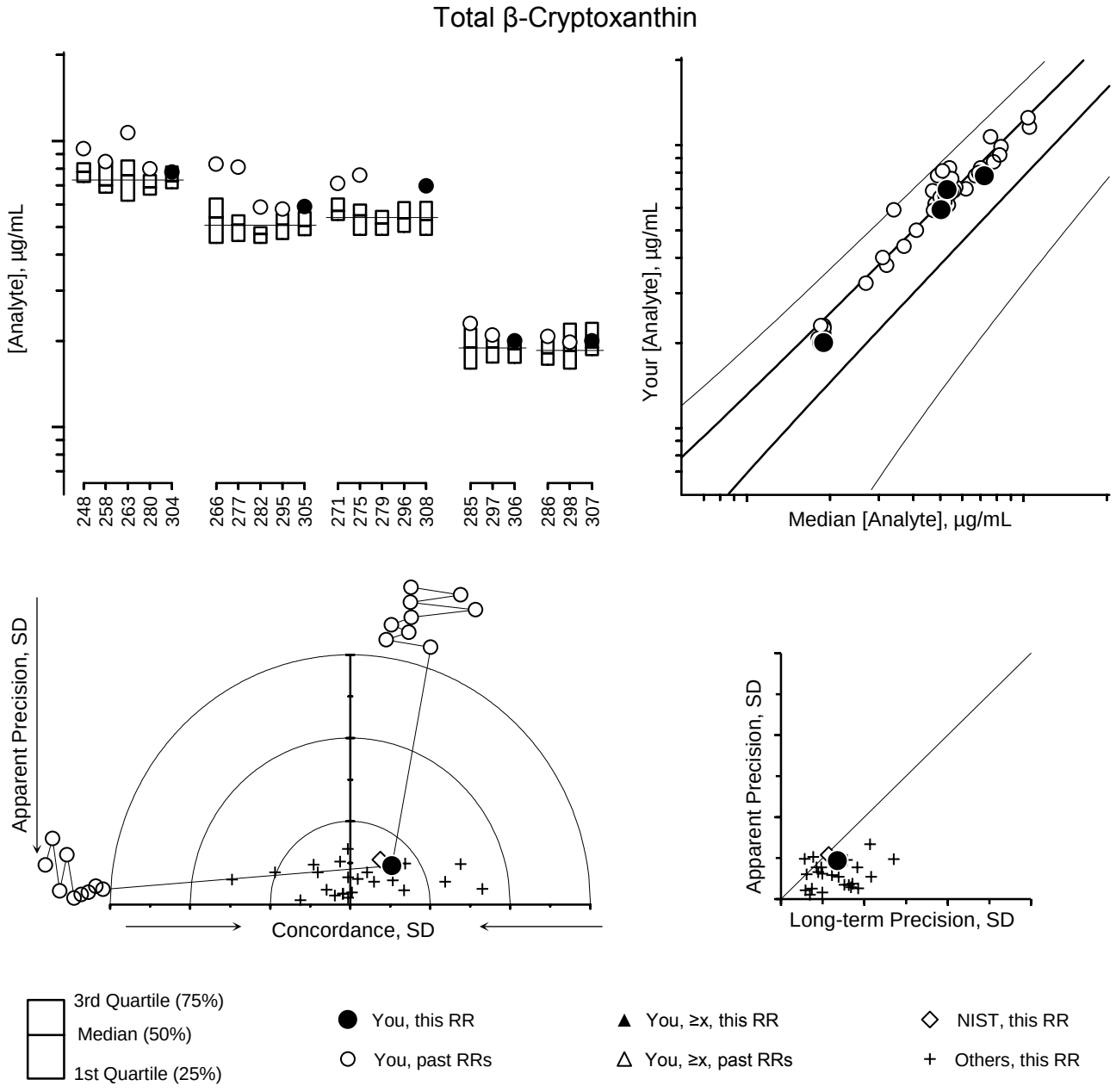
#308	Fresh-frozen: #271(49), #275(50), #279(51), #296(54)	Same pool as #305, native

Individualized RR LVI 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.

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#304	Lyophilized: #248(44), #258(46), #263(47), #280(51)	Augmented, multi-source (SRM 968c Level I)
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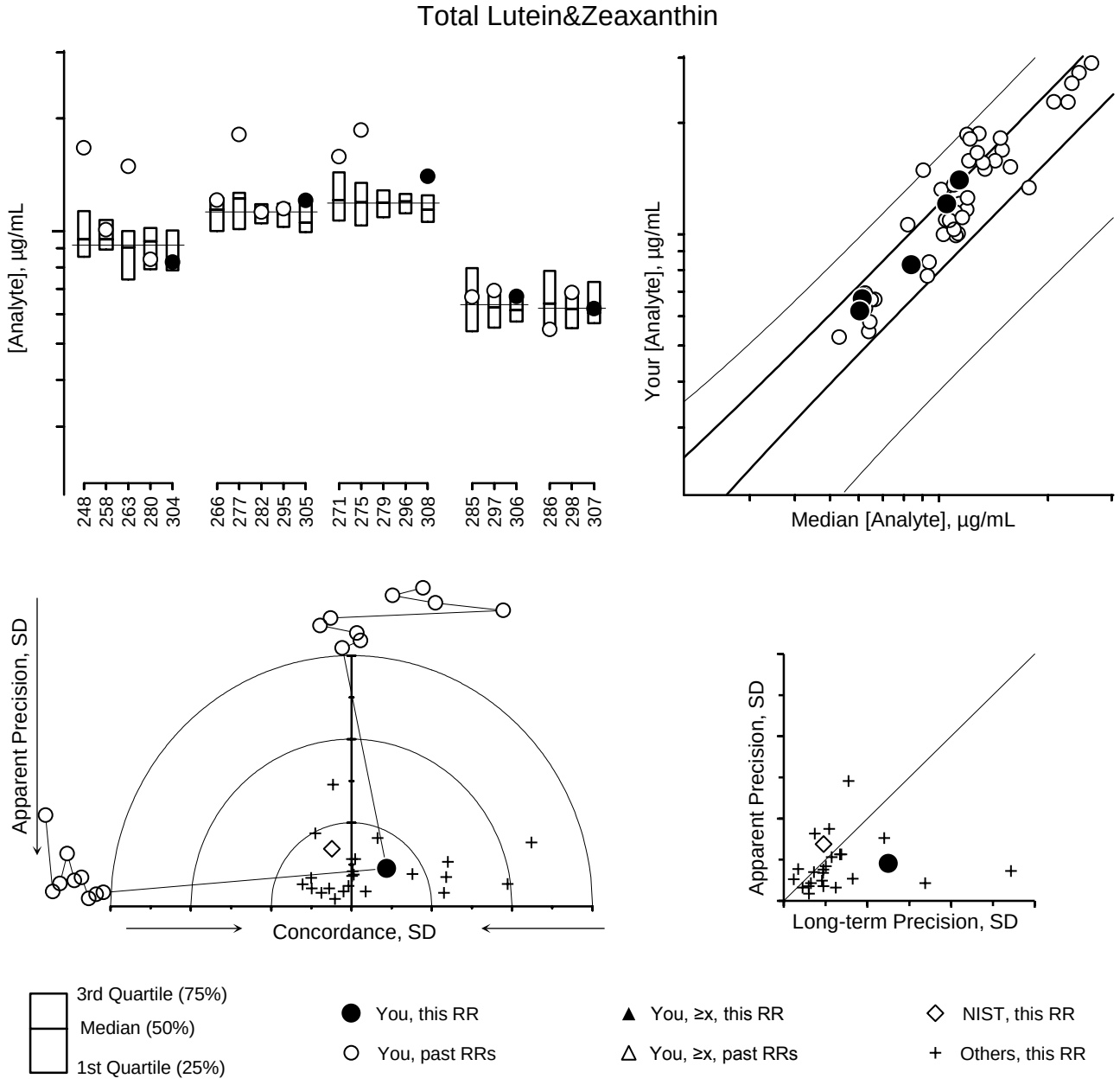
#308	Fresh-frozen: #271(49), #275(50), #279(51), #296(54)	Same pool as #305, native

Individualized RR LVI 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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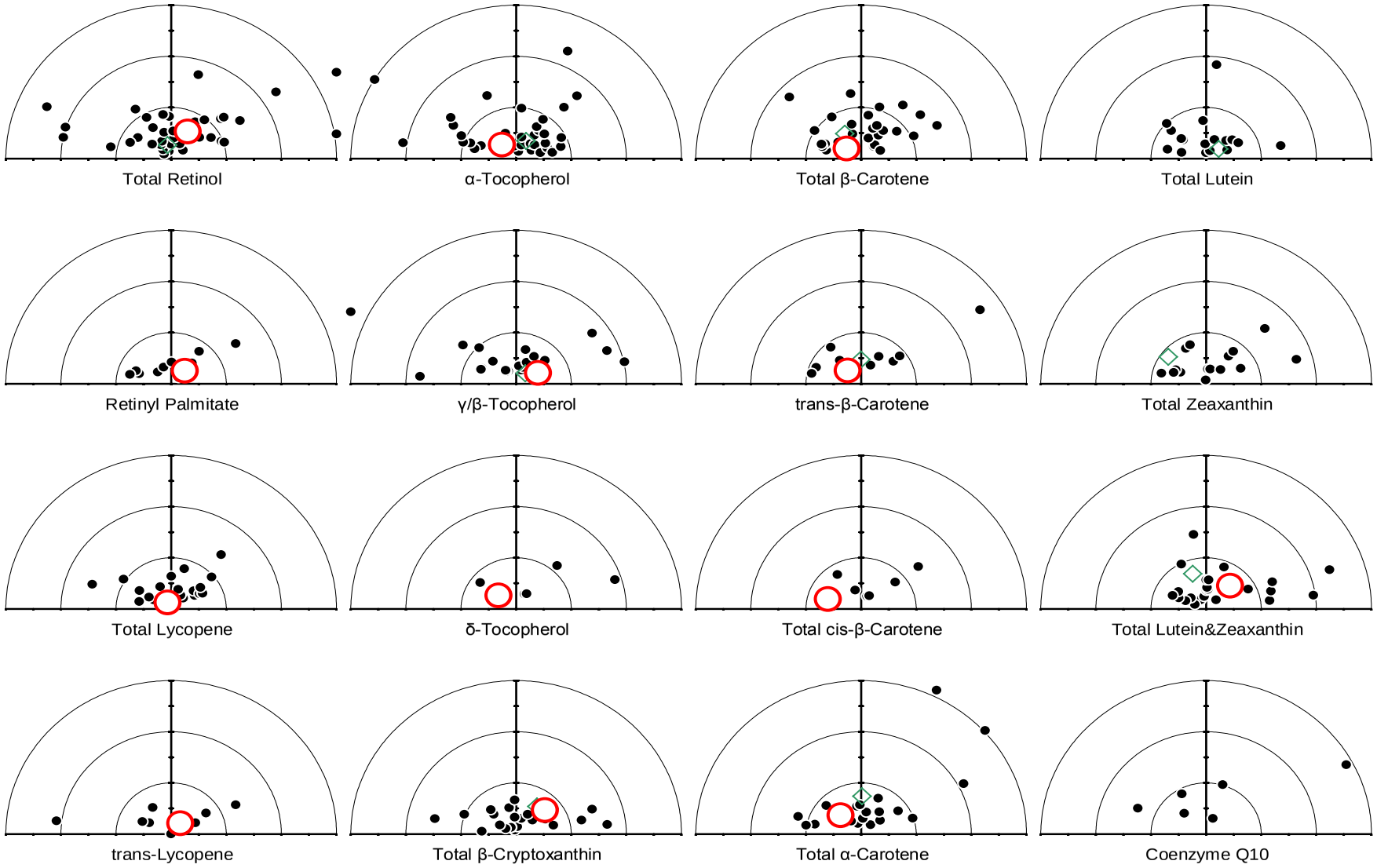
#308	Fresh-frozen: #271(49), #275(50), #279(51), #296(54)	Same pool as #305, native

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



D16

Appendix E. Shipping Package Inserts for RR21

The following five items were included in each package shipped to an RR21 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

May 12, 2004

Dear Colleague:

The samples within this package constitute Vitamin C Round Robin 21 (RR21) of the 2004 Micronutrients Measurement Quality Assurance Program.

RR21 consists of four vials of frozen serum *test samples* (#11, #24, #41, and #55), 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 RR20 was mailed during the week of May 3. If you find your results for RR20 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).

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

We ask that you return your results for the RR 21 samples *before* **September 13, 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,

Janice B Thomas

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

Enclosures

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 21

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* **September 13, 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 21

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 #11			µmol/L of Sample
Serum Test Sample #24			µmol/L of Sample
Serum Test Sample #41			µmol/L of Sample
Serum Test Sample #55			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* **September 13, 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 21

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 #11	Liquid frozen (1:1 serum:10% MPA)
VitC #24	Liquid frozen (1:1 serum:10% MPA)
VitC #41	Liquid frozen (1:1 serum:10% MPA)
VitC #55	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 RR21

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

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

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

November 2, 2004

Dear Colleague:

Enclosed is the summary report of the results for Round Robin 21 (RR21) 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 21 consisted of four *test samples* (#11, #24, #41, and #55), 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 first vitamin C round robin study (RR 22) for the 2005 Vitamin C in Serum QA Program will be shipped **starting the week of November 8**. 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 B. Thomas

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

Enclosures

The NIST M^2QAP Vitamin C Round Robin 21 (RR21) 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 21 sample measurements.

Page	"All Lab" Report
1	A tabulation of results and summary statistics for Total Ascorbic Acid [TAA] in the RR21 samples and control/calibration solutions.

Serum-based Samples. Two serum controls and four unknowns were distributed in RR21.

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

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

S21:1 Serum 11, stripped serum used in the preparation of the augmented sera for this study; ampouled in late 2001, previously distributed in RR19 (Sep-03).

S21:2 Serum 24, augmented serum ampouled in late 2001, previously distributed in RR17 (Sep-02) and RR19 (Sep-03).

S21:3 Serum 41, augmented serum ampouled in late 2001, previously distributed in RR18 (Mar-03) and RR19 (Sep-03).

S21:4 Serum 55, augmented serum ampouled in late 2001, previously distributed in RR 16 (Mar-02), RR17 (Sep-02) and RR20 (Mar-04).

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 g/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, although the slopes for two participants are 15% to 20% lower than expected.

3) One participant reported values for the controls that were well outside the target range and one participant did not report values for the controls. 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 a problem with your measurement system.

4) Please note that we have recertified the SRM 970 materials. The recertified values for total ascorbic acid are as follows:
Level I – 8.41 µmol/L, ≈95% confidence range of 7.75 µmol/L to 9.07 µmol/L.
Level II – 28.05 µmol/L, ≈95% confidence range of 27.56 µmol/L to 28.54 µmol/L.

Appendix G. "All-Lab Report" for RR21

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" 21 - September 2004

		Control / Calibration Samples											MPA	Dilute Solution 1			Samples											
		Gravimetric, µmol/L			Measured, µmol/L				Calibration Parameters				Density	Spectrophotometry			Measured, µmol/L						Corrected, µmol/L					
Lab	Date	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	S21:1	S21:2	S21:3	S21:4	CS#1	CS#2	S21:1	S21:2	S21:3	S21:4
VC-MA	13/09/04	56.1	28.3	13.9	55.5	27.9	13.6	0.0	-0.09	0.99	1.000	0.1	1.031	244.	0.5551	562.2	8.0	26.7	<0.68	8.7	33.0	47.0	8.2	27.1		8.9	33.4	47.5
VC-MB	14/07/04	57.5	28.6	8.9	55.5	25.8	8.0	0.0	-0.60	0.96	0.999	1.0	1.032	245.	0.5534	546.5	7.5	27.7	<5.2	9.6	35.9	50.1	8.3	29.3		10.5	37.8	52.6
VC-MC	07/09/04	57.2	28.6	13.7	57.9	30.0	15.0	0.0	0.64	1.01	0.999	0.8	1.031	243.	0.5386	534.8	8.3	34.7	*nq*	9.1	33.7	53.3	7.6	33.8		8.4	32.8	52.3
VC-MG	13/09/04	57.6	28.9	14.6	56.5	27.3	13.2	0.0	-0.62	0.99	0.999	0.7	1.030	243.	0.5550	547.2	7.8	28.5	*nq*	9.3	36.8	52.0	8.5	29.5		10.1	38.0	53.4
VC-MH	09/06/04	61.6	30.8	14.9	61.9	30.6	14.5	0.1	-0.21	1.00	1.000	0.3	1.030	243.8	0.6125	564.2	8.4	28.3	*0.3*	8.5	33.5	46.1	8.5	28.4	0.5	8.7	33.5	46.1
VC-MI	10/09/04	57.5	28.9	14.3	48.7	22.4	14.6		1.47	0.81	0.986	3.0	1.028				8.7	25.7	3.7	10.0	26.9	36.3	8.9	30.0	2.7	10.6	31.5	43.1
VC-MK	09/09/04	61.7	30.7	15.2	53.1	24.5	14.0	0.5	0.27	0.85	0.997	1.4	1.033	244.	0.6089	560.7	12.6	34.4	4.3	13.6	38.5	51.6	14.6	40.4	4.8	15.8	45.2	60.7
VC-MR	13/09/04	57.5	28.6	14.2	58.1	29.8	14.7	0.0	0.28	1.01	1.000	0.5	1.031	245.	0.5650	557.7	8.7	27.4	0.4	10.2	33.5	47.7	8.3	26.8	0.1	9.8	32.9	47.0
VC-MT	08/09/04	56.9	27.9	13.4	57.3	27.5	13.4	0.0	-0.15	1.01	1.000	0.4	1.031	243.	0.5580	557.3	8.7	28.3	0.3	8.3	27.4	42.6	8.7	28.2	0.4	8.4	27.4	42.5
VC-MY	13/09/04																		0.1	6.8	74.5	123.8			0.1	6.8	74.5	123.8
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	1.2	10.1	34.0	44.4	8.4	28.3	0.7	9.6	33.5	43.9
	N	9	9	9	9	9	9	8					N	9	8	8	8	9	6	10	10	10	9	9	6	10	10	10
	Average	58.2	29.0	13.7	56.0	27.3	13.4	0.1					Average	1.031	243.9	0.5683	553.8	8.7	29.1	1.5	9.4	37.4	55.0	9.1	30.4	1.4	9.8	38.7	56.9
	SD	2.0	1.0	1.9	3.6	2.7	2.1	0.2					SD	0.001	0.8	0.0272	10.1	1.5	3.2	1.9	1.8	13.5	24.7	2.1	4.3	1.9	2.4	13.4	24.1
	Min	56.1	27.87	8.9	48.7	22.41	8.0	0.0					Min	1.028	243.0	0.5386	534.8	7.5	25.7	0.1	6.8	26.9	36.3	7.6	26.8	0.1	6.8	27.4	42.5
	%25	57.2	28.58	13.7	55.5	25.80	13.4	0.0					%25	1.030	243.0	0.5546	547.0	8.0	27.4	0.3	8.6	33.1	46.3	8.3	28.2	0.2	8.5	32.8	46.3
	Median	57.5	28.64	14.2	56.5	27.45	14.0	0.0					Median	1.031	243.9	0.5566	557.5	8.4	28.3	0.4	9.2	33.6	48.9	8.5	29.3	0.5	9.3	33.5	49.9
	%75	57.6	28.91	14.6	57.9	29.75	14.6	0.0					%75	1.031	244.3	0.5760	561.1	8.7	28.5	2.8	9.9	36.6	51.9	8.7	30.0	2.2	10.4	38.0	53.2
	Max	61.7	30.80	15.2	61.9	30.62	15.0	0.5					Max	1.033	245.0	0.6125	564.2	12.6	34.7	4.3	13.6	74.5	123.8	14.6	40.4	4.8	15.8	74.5	123.8
	MADe	0.5	0.4	0.7	2.0	3.4	1.0	0.0					eSD	0.001	1.3	0.0086	8.5	0.5	1.4	0.2	1.1	4.1	4.3	0.3	1.5	0.5	1.4	4.7	5.4
	CV	1	1	5	4	12	7						CV	0.12	0.55	2	2	6	5		12	12	9	4	5		15	14	11

G2

Appendix H. Representative "Individualized Report" for RR21

Each participant in RR21 received an "Individualized Report" reflecting their reported results. The following two pages are the "Individualized Report" for participant "VC-MA".

Vitamin C "Round Robin" 21 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
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
09/13/04	21	HPLC-EC	1.030	244.0	0.555	562.2	-0.1	0.99	1.000	0.10
		Mean	1.028	243.0	0.55	562.6				
		SD	0.003	0.9	0.03	8.2				
		CV	0.26	0.37	4.7	1.4				

[TAA] mmol/Lsample

Date	RR	Sample	Rep_1	Rep_2	F_{adj}	Mean	SD_{dup}	N	Mean	SD_{repeat}	SD_{reprod}
11/13/03	19	S19:1	na	na	1.0			0			
09/13/04	21	S21:1	na	na	1.0						
12/12/02	17	S17:1	9.9	9.1	1.0	9.5	0.6	3	9.1	0.3	0.4
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				
03/20/03	18	S18:2	35.1	36.0	1.0	35.6	0.6	3	34.8	0.4	1.6
11/13/03	19	S19:3	35.9	35.8	1.0	35.9	0.1				
09/13/04	21	S21:3	33.2	32.9	1.0	33.0	0.2				
11/18/02	16	S16:3	49.9	44.9	1.0	47.4	3.5	4	48.5	1.8	1.8
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				
09/13/04	21	S21:4	47.1	47.0	1.0	47.0	0.0				

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

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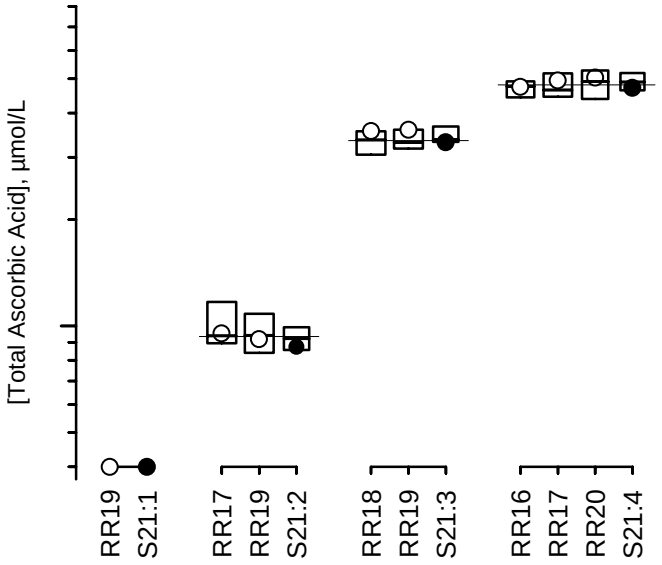
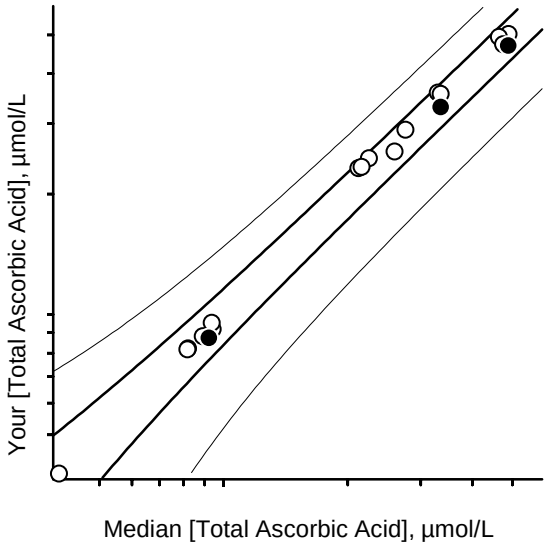
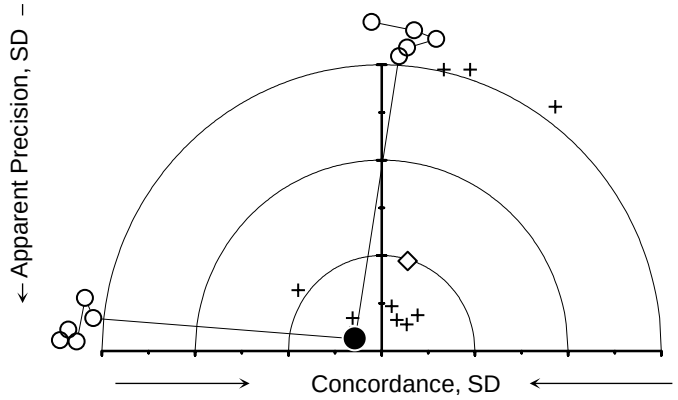
Vitamin C "Round Robin" 21 Report: Participant VC-MA

Total Ascorbic Acid







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

You, RR 21
You, RR 16-20

NIST, RR 21
Others, RR 21

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
S21:1	Serum 11, previously distributed in RR 19
S21:2	Serum 24, previously distributed in RRs 17 and 19
S21:3	Serum 41, previously distributed in RRs 18 and 19
S21:4	Serum 55, previously distributed in RRs 16, 17, and 20