NISTIR 7880-19

NIST Micronutrients Measurement Quality Assurance Program Winter 2003 Comparability Studies

Results for Round Robin LIII
Fat-Soluble Vitamins and Carotenoids in Human Serum
and Round Robin 18 Ascorbic Acid in Human Serum

David L. Duewer
Katherine E. Sharpless
Jeanice B. Thomas

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

NISTIR 7880-19

NIST Micronutrients Measurement Quality Assurance Program Winter 2003 Comparability Studies

Results for Round Robin LIII
Fat-Soluble Vitamins and Carotenoids in Human Serum
and Round Robin 18 Ascorbic Acid in Human Serum

David L. Duewer
Katherine E. Sharpless
Jeanice B. Thomas
Chemical Sciences Division
Materials Measurement Laboratory

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

(This page intentionally blank)

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 2003 MMQAP measurement comparability improvement studies: 1) Round Robin LIII Fat-Soluble Vitamins and Carotenoids in Human Serum and 2) Round Robin 18 Total Ascorbic Acid in Human Serum. The materials for both studies were shipped to participants in November 2002; participants were requested to provide their measurement results by March 3, 2003.

Keywords

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

Table of Contents

Introduction

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

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

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

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

Participants in the MMQAP Fat-Soluble Vitamins and Carotenoids in Human Serum Round Robin LIII comparability study (hereafter referred to as RR53) 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 2002. 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 RR53 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 18: Vitamin C in Human Serum

Participants in the MMQAP Vitamin C in Human Serum Round Robin 18 comparability study (hereafter referred to as RR18) received three frozen serum test samples and a solid ascorbic acid control material for analysis. Unless multiple vials were previously requested, participants received one vial of each material. These sample materials were shipped on dry ice to participants in November 2002. The communication materials included in the sample shipment are provided in Appendix E.

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

The final report delivered to every participant in RR18 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 RR53

The following three items were included in each package shipped to an RR53 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 13, 2002

Dear Colleague:

Enclosed are the samples for the first fat-soluble vitamins and carotenoids in serum round robin study (Round Robin LIII) for the 2003 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 an obtained value is below your limit of quantification, please indicate this result on the form by using NQ *(Not Quantified)*. Results are due to NIST by **March 3, 2003**. 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-April.

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 291-293.**

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 LIII to:

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

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

Sincerely,

Jeanice B Thomas

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

Enclosures

NIST

Participant #: ________ Date: ____________

Round Robin LIII

NIST Micronutrients Measurement Quality Assurance Program

Analyte	289	290	291	292	293	Units*
total retinol						
trans-retinol						
retinyl palmitate						
α-tocopherol						
γ/β-tocopherol						
δ-tocopherol						
total β-carotene						
trans-β-carotene						
total cis-β-carotene						
total α-carotene						
trans-α-carotene						
total lycopene						
trans-lycopene						
total β-cryptoxanthin						
total α-cryptoxanthin						
total lutein						
total zeaxanthin						
total lutein&zeaxanthin						
total Coenzyme Q10						
ubiquinol (QH_2)						
ubiquinone (Qox)						
phylloquinone (K_1)						
25-hydroxyvitamin D						

Other analytes?						

* we prefer μg/mL

Were sera {291,292,293} frozen when received? Yes | No

Comments:

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

Please return results **before** 3-Mar-2003

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

Participant #: ________ Date: ____________

Fat-Soluble Vitamins Round Robin LIII

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^{2}QAP sera:

Serum	Form	Reconstitute?
#289	Lyophilized	Yes (1 ml H_2O)
#290	Lyophilized	Yes (1 ml H_2O)
#291	Liquid frozen	No
#292	Liquid frozen	No
#293	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 {291,292,293} 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 {291,292,293} arrive frozen? Yes | No

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

6) When do you anticipate analyzing these samples? ________________________

Your prompt return of this information is appreciated.

The M^{2}QAP Gang

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

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

Appendix B. Final Report for RR53

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

March 26, 2003

Dear Colleague:

Enclosed is the summary report of the results for Round Robin LIII (RR 53) for fat-soluble vitamins and carotenoids. Included in this report are: a summary of data for all laboratories; the measurement comparability summary for evaluating laboratory performance; a summary of individual laboratory performance and interlaboratory accuracy and precision; and a summary of the NIST assigned value (NAV) vs. your laboratory value for the analytes that you measured. As in previous reports, the NIST-assigned values are equally weighted means of the medians from this interlaboratory comparison exercise and the means from the analyses performed by NIST.

Data for evaluating laboratory performance in RR 53 are provided in the comparability summary (Score Card) on page 6 of the "All Lab Report." Laboratory comparability is summarized as follows: results rated 1 to 3 are within 1 to 3 standard deviation(s) of the assigned value, respectively; those rated 4 are >3 standard deviations from the assigned value.

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

Intent-to-participate forms for the 2004 QA program will be mailed in May. This form will provide us with formal notification of your intent to participate in the program for the upcoming year. The program will consist of two round robin studies for the fat-soluble vitamins and carotenoids and one study for vitamin C in serum. To participate in the fat-soluble vitamins and carotenoids in serum studies, the participation fee is $1600 for U.S. laboratories and $2000 for non-U.S. laboratories. To participate in the vitamin C in serum study, the participation fee is $800 for U.S. laboratories and $1000 for non-U.S. laboratories. We ask that you return the form to us by **no later than September 1, 2003.**

Samples for the second round robin exercise (RR54) will be distributed during **the week of April 7, 2003.** We will send you a reminder via e-mail or fax a week prior to shipment. It is critical that you carefully inspect all samples upon arrival and that you promptly confirm to us that they have arrived. We will replace samples (lost or damaged in shipment or miss-packaged by us) **only** for participants who report the problem within <u>one calendar week</u> after the package arrives.

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

Sincerely,

Jeanice B Thomas

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

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

Enclosures

NIST

The NIST M^2QAP Round Robin LIII (RR53) report consists of:

Page	"Individualized" Report
1	Your values, the number of labs reporting values, and our assigned values.
2 to n	"Four Plot" summaries of your current and past measurement performance, one page for each analyte you report that is also reported by at least 10 other participants.
n+1	The "target" plot version of your "Comparability Summary" scores.

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

Samples. The five sera below were distributed in RR53.

Serum	Description	Prior Distributions
289	Lyophilized, native, single-donor serum prepared in 1987.	Serum 93 in RR13 (Jun-88) Serum 111 RR16 (Jun-89)
290	Lyophilized, native, single donor, commercially obtained serum prepared in 2002. The same material was used to prepare Serum 292.	Initial distribution.
291	Fresh-frozen, native, single-donor serum prepared in 2002.	Initial distribution.
292	Fresh-frozen, native, single donor, commercially obtained serum prepared in 2002. The same material was used to prepare Serum 290.	Initial distribution.
293	Fresh-frozen, native, single-donor, commercially obtained serum prepared in 2002. This material has rather low levels of most micronutrients.	Serum 288 RR52 (Nov-02)

Results

1) Sera Stability. There was no significant change in the median level or in the variability of retinol or α-tocopherol in the lyophilized Serum 289 after 15 years; however, the total β-carotene apparently has declined by $\approx$ 0.8% per year but with no significant change in variability. There has been no significant change in the median level or variability for any measurand in the fresh-frozen Serum 293 after six months.

2) Matrix (Lyophilized Vs Fresh-Frozen) Differences. Sera 290 and 292 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 290 should be ≈ 96% of those in Serum 292. The observed average ±SD over all measurands with 10 or more quantitative measurements is 95.1 ±0.8%. If any of your Sera 290/292 ratios are much different than 0.95, you may want to take a hard look at your measurement system for those measurands.

   For those of you who remember that we've been studying the potential interactions between measurement systems and the sample matrix – we're still working on it! We hope to finish the report within the year.

3) Didehydroretinol. A recent letter (Erhardt JC, Biesalski HK, Malaba LC, Craft NE. Improved Method for Measurement of Retinol and Didehydroretinol in the Modified Relative Dose Response Test to Detect Vitamin A Deficiency. Clin Chem 2003 49:338-339) suggested that didehydroretinol be used as a marker for Vitamin A deficiency. Participant 164 found measurable levels of this measurand in the (obtained more than five years ago) Serum 293. Sad to say, it's quite plausible that the individual who was paid by the commercial vendor for this material may well have been nutritionally deficient.

4) Total Retinol and trans-retinol: An Answer to the "Halfway Between" Values for Serum 286 in RR52. Thanks to one of our participants who supplied chromatograms and re-evaluated their numerical results, we have identified at least one cause for the anomalous "total retinol" values for the 13-*cis*-retinol spiked Serum 286 in the previous Round Robin: quantitation by *height* in combination with modest *chromatographic separation* between the 13-*cis*- and *trans*-retinol isomers.

Warnings!

1) A few samples in upcoming Round Robins should have measurable didehydroretinol levels.

2) We will soon distribute another pair of sera that have been spiked with *trans*- and 13-*cis*-retinol.

3) Whenever you quantitate by peak height, you should be very sure that all isomers you wish to group together as the same measurand (i.e, grouping all retinol isomers as "total retinol" and β- and γ-tocopherol as "γ/β-tocopherol) elute at *exactly* the same time. If there is any appreciable peak broadening for such a measurand, peak *area* will be a more appropriate metric than peak *height*.

4) It is critical that you ***carefully*** inspect all samples on arrival and that you ***promptly*** confirm to us that they have arrived. We will now replace samples (whether lost or damaged in shipment or miss-packed by us!) ***only*** for participants who report the problem within *one calendar week* after the package's arrival.

Appendix C. "All-Lab Report" for RR53

The following seven 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 analysts. The NIST results are not used in the assessment of the consensus summary results of the study.

Round Robin LIII Laboratory Results

All Results in µg/mL

Lab	Total Retinol 289	290	291	292	293	trans-Retinol 289	290	291	292	293	Retinyl Palmitate 289	290	291	292	293
FSV-BA	0.821	0.640	0.554	0.663	0.336						0.020	0.104	0.088	0.105	0.019
FSV-BB	0.774	0.599	0.497	0.634	0.325						0.017	0.081	0.081	0.084	0.010
FSV-BD	0.715	0.660	0.556	0.674	0.368										
FSV-BE	≥0.690	≥0.570	≥0.490	≥0.670	≥0.330	0.690	0.570	0.490	0.670	0.330					
FSV-BF	0.750	0.620	0.530	0.640	0.310										
FSV-BG	0.791	0.601	0.521	0.590	0.300						0.034	0.123	0.100	0.090	0.019
FSV-BH	0.715	0.604	0.509	0.605	0.317										
FSV-BI	0.761	0.593	0.537	0.649	0.328						*nd*	0.103	0.107	0.116	*nd*
FSV-BJ	0.771	0.599	0.506	0.621	0.324						*nq*	0.087	0.076	0.087	*nq*
FSV-BK	0.794	0.600	0.535	0.619	0.325										
FSV-BL	0.690	0.630	0.520	0.690	0.320										
FSV-BM	0.670	0.644	0.571	0.679	0.385										
FSV-BN	0.915	0.602	0.551	0.639	0.332						*nd*	0.068	0.060	0.062	*nq*
FSV-BO	≥0.624	≥0.647	≥0.481	≥0.564	≥0.281	0.624	0.647	0.481	0.564	0.281					
FSV-BP	0.703	0.574	0.494	0.613	0.343										
FSV-BQ	0.680	0.520	0.470	0.590	0.270										
FSV-BR	≥0.780	≥0.665	≥0.597	≥0.676	≥0.340	0.780	0.665	0.597	0.676	0.340					
FSV-BS	0.684	0.513	0.397	0.551	0.270										
FSV-BT	0.913	0.563	0.978	0.695	0.445						0.102	0.139	0.145	0.149	0.030
FSV-BU	-	0.589	0.519	0.620	0.403										
FSV-BV	0.859	0.700	0.591	0.714	0.370										
FSV-BW	0.779	0.609	0.532	0.643	0.335						0.013	0.137	0.128	0.152	0.021
FSV-BX	≥0.781	≥0.597	≥0.565	≥0.639	≥0.331	0.781	0.597	0.565	0.639	0.331					
FSV-CB	0.528	0.522	0.458	0.547	0.281										
FSV-CC	0.820	0.640	0.540	0.690	0.350	0.790	0.640	0.540	0.690	0.350					
FSV-CD	0.827	0.619	0.536	0.643	0.335						*nd*	0.108	0.110	0.120	0.019
FSV-CE	0.708	0.557	0.567	0.612	0.297										
FSV-CF	0.840	0.663	0.576	0.680	0.350										
FSV-CG	0.702	0.586	0.517	0.604	0.314										
FSV-CI	0.660	0.620	0.530	0.650	0.340						0.030	0.100	0.070	0.100	0.020
FSV-CL	0.707	0.516	0.407	0.548	0.300										
FSV-CR	0.730	0.660	0.600	0.700	0.340										
FSV-CS	≥0.864	≥0.644	≥0.573	≥0.685	≥0.359	0.864	0.644	0.573	0.685	0.359					
FSV-CT															
FSV-CW	≥0.696	≥0.513	≥0.450	≥0.536	≥0.316	0.696	0.513	0.450	0.536	0.316	<0.010	0.073	0.069	0.077	0.011
FSV-CZ	0.780	0.600	0.490	0.600	0.330										
FSV-DA	≥0.789	≥0.598	≥0.529	≥0.639	≥0.322	0.789	0.598	0.529	0.639	0.322	0.011	0.071	0.065	0.104	0.011
FSV-DD	≥0.802	≥0.620	≥0.559	≥0.719	≥0.315	0.802	0.620	0.559	0.719	0.315					
FSV-DF	0.769	0.654	0.588	0.685	0.366										
FSV-DI	0.885	0.734	0.616	0.730	0.404						0.012	0.100	0.071	0.099	0.016
FSV-DW	0.764	0.609	0.502	0.612	0.319										
FSV-ET	0.680	0.590	0.530	0.600	0.300										
N	32	33	33	33	33	9	9	9	9	9	8	13	13	13	10
Min	0.528	0.513	0.397	0.547	0.270	0.624	0.513	0.450	0.536	0.281	0.011	0.068	0.060	0.062	0.010
Median	0.763	0.602	0.530	0.639	0.330	0.781	0.620	0.540	0.670	0.330	0.018	0.100	0.081	0.100	0.019
Max	0.915	0.734	0.978	0.730	0.445	0.864	0.665	0.597	0.719	0.359	0.102	0.139	0.145	0.152	0.030
SD	0.073	0.038	0.037	0.055	0.027	0.070	0.035	0.056	0.034	0.018	0.013	0.020	0.027	0.022	0.006
CV	10	6	7	9	8	9	6	10	5	5	73	20	34	22	30
Npast	28	0	0	0	38	0	0	0	0	5	0	0	0	0	9
Medianpast	0.752				0.322					0.333					0.015
SDpast	0.041				0.032					0.017					0.006
NISTa	0.766	≥0.621	≥0.579	≥0.689	≥0.351	0.717	0.621	0.579	0.689	0.351					
NISTb	0.764	0.636	0.557	0.671	0.348	0.698	0.634	0.557	0.671	0.348					
NNIST	4	4	4	4	4	4	4	4	4	4					
Mean	0.765	0.629	0.568	0.680	0.350	0.707	0.627	0.568	0.680	0.350					
Srep	0.014	0.031	0.018	0.015	0.006	0.012	0.031	0.018	0.015	0.006					
Shet	0.038	0.014	0.004	0.004	0.002	0.050	0.013	0.004	0.004	0.002					
Sanl	0.001	0.011	0.016	0.012	0.002	0.013	0.009	0.016	0.012	0.002					
SNIST	0.041	0.036	0.024	0.020	0.006	0.053	0.035	0.024	0.020	0.006					
NAV	0.764	0.615	0.549	0.660	0.340	0.744	0.624	0.554	0.675	0.340		0.100	0.081	0.100	0.019
NAU	0.073	0.051	0.050	0.062	0.031	0.087	0.050	0.059	0.053	0.031		0.026	0.027	0.026	0.011

Lab	α-Tocopherol 289	290	291	292	293	γ/β-Tocopherol 289	290	291	292	293	δ-Tocopherol 289	290	291	292	293
FSV-BA	5.00	10.20	11.23	10.47	2.83	2.48	1.85	1.73	1.89	0.76					
FSV-BB	5.06	10.45	11.67	10.90	2.91	2.24	1.68	1.52	1.77	0.69					
FSV-BD	5.50	11.00	12.20	11.10	3.30										
FSV-BE	4.90	9.40	10.70	10.70	2.90	2.40	1.60	1.60	1.90	0.70					
FSV-BF	4.80	10.00	11.30	10.40	2.80	2.22	1.69	1.58	1.77	0.58					
FSV-BG	4.99	9.99	11.71	9.84	2.60	2.35	1.73	1.60	1.66	0.68					
FSV-BH	5.00	10.62	11.48	10.69	2.91	5.33	4.07	3.65	4.15	1.61					
FSV-BI	5.09	10.18	11.55	11.40	3.01	2.45	1.80	1.69	2.02	0.86					
FSV-BJ	4.61	10.07	12.52	10.57	2.76	2.37	1.62	1.65	1.67	0.66					
FSV-BK	4.70	9.90	11.30	10.30	2.50										
FSV-BL	4.31	11.63	12.92	12.92	3.45										
FSV-BM	5.30	10.80	12.00	11.00	3.40										
FSV-BN	4.43	8.64	10.44	9.13	2.30	2.06	1.32	1.37	1.43	0.56	0.131	0.073	0.124	0.055	0.023
FSV-BO	4.35	9.76	9.56	9.48	2.58										
FSV-BP	5.13	10.48	11.09	9.89	2.76										
FSV-BQ	4.60	9.00	9.90	10.30	2.60										
FSV-BR	4.12	10.52	11.40	10.88	2.88										
FSV-BS															
FSV-BT	5.15	11.13	10.70	11.91	3.24	2.40	1.81	1.51	1.93	0.77					
FSV-BU	-	9.04	10.11	9.45	2.55	-	1.98	1.86	2.07	0.74					
FSV-BV	4.44	9.60	9.66	10.45	3.58	2.34	1.68	1.46	1.82	0.72					
FSV-BW	5.00	10.20	11.54	10.81	3.00	2.66	2.05	1.85	2.11	0.83					
FSV-BX	4.65	9.75	12.20	10.51	2.92	2.26	1.70	1.70	1.77	0.69					
FSV-CB	4.48	9.05	10.32	9.58	2.67										
FSV-CC	5.02	10.50	11.52	10.93	3.15										
FSV-CD	4.64	10.52	11.73	11.02	2.62	2.40	1.82	1.69	1.84	0.67					
FSV-CE	4.66	9.56	12.75	10.87	2.73										
FSV-CF	5.50	10.50	12.30	11.10	3.40										
FSV-CG	4.25	9.18	10.58	9.18	2.37	2.06	1.66	1.53	1.70	0.64	0.083	0.076	0.111	0.059	0.011
FSV-CI	5.40	9.30	9.50	10.10	2.90	2.80	1.80	1.70	1.90	0.80					
FSV-CL	4.01	8.59	9.28	8.64	2.70	2.83	2.57	2.09	2.56	1.05					
FSV-CR	4.97	10.91	12.07	11.67	2.91										
FSV-CS	5.23	10.07	11.80	10.67	3.02										
FSV-CT															
FSV-CW	4.40	9.00	9.90	9.60	2.50	2.40	1.80	1.60	1.90	0.70	0.200	0.050	0.090	0.050	0.040
FSV-CZ	4.50	10.00	10.80	9.90	2.90										
FSV-DA	4.79	9.67	10.98	10.69	2.84	2.40	1.76	1.66	1.92	0.75	0.174	0.085	0.149	0.061	0.068
FSV-DD															
FSV-DF															
FSV-DI	5.90	11.30	12.10	11.20	3.46	3.26	2.23	1.97	2.21	0.97	0.315	0.085	0.134	0.087	0.073
FSV-DW	4.86	10.34	12.10	10.78	3.27										
FSV-ET	4.60	9.50	11.20	9.80	2.50	2.40	1.80	1.70	1.80	0.70					
N	37	38	38	38	38	21	22	22	22	22	5	5	5	5	5
Min	4.01	8.59	9.28	8.64	2.30	2.06	1.32	1.37	1.43	0.56	0.083	0.050	0.090	0.050	0.011
Median	4.80	10.03	11.35	10.62	2.89	2.40	1.80	1.67	1.90	0.71	0.174	0.076	0.124	0.059	0.040
Max	5.90	11.63	12.92	12.92	3.58	5.33	4.07	3.65	4.15	1.61	0.315	0.085	0.149	0.087	0.073
SD	0.42	0.73	0.99	0.77	0.28	0.12	0.12	0.10	0.17	0.08	0.051	0.009	0.017	0.004	0.033
CV	9	7	9	7	10	5	7	6	9	11	29	12	14	8	84
Npast	26	0	0	0	39	0	0	0	0	22	0	0	0	0	5
Medianpast	4.92				2.97					0.76					0.050
SDpast	0.38				0.33					0.09					0.019
NISTa	4.42	10.01	10.90	10.52	2.85	2.17	1.86	1.85	1.90	0.77					
NISTb	4.99	10.17	10.89	10.67	2.85	2.17	1.60	1.45	1.67	0.65	0.157	0.057	0.098	0.042	0.037
NNIST	4	4	4	4	4	4	4	4	4	4	2	2	2	2	2
Mean	4.70	10.09	10.89	10.59	2.86	2.17	1.73	1.65	1.78	0.71	0.157	0.057	0.098	0.042	0.033
Srep	0.30	0.31	0.22	0.12	0.07	0.13	0.04	0.06	0.03	0.05	0.006	0.004	0.004	0.007	0.002
Shet	0.14	0.42	0.25	0.25	0.05	0.06	0.02	0.05	0.03	0.00	0.005	0.003	0.002	0.001	0.016
Sanl	0.40	0.11	0.01	0.11	0.02	0.00	0.18	0.29	0.16	0.08					
SNIST	0.52	0.54	0.34	0.30	0.09	0.14	0.19	0.30	0.17	0.10	0.008	0.005	0.005	0.007	0.016
NAV	4.75	10.06	11.12	10.61	2.87	2.28	1.77	1.66	1.84	0.71	0.166	0.067	0.111	0.051	0.036
NAU	0.53	0.78	1.05	0.82	0.40	0.29	0.20	0.30	0.22	0.10	0.053	0.024	0.032	0.022	0.034

Round Robin LIII Laboratory Results

All Results in µg/mL

Lab	Total β-Carotene 289	290	291	292	293	trans-β-Carotene 289	290	291	292	293	Total cis-β-Carotene 289	290	291	292	293	Total α-Carotene 289	290	291	292	293	trans-α-Carotene 289	290	291	292	293
FSV-BA	0.302	0.115	0.299	0.122	0.048	0.284	0.111	0.284	0.117	0.046	0.018	0.005	0.015	0.006	0.002	0.017	0.071	0.125	0.075	0.005					
FSV-BB	0.299	0.110	0.266	0.116	0.049	0.280	0.105	0.254	0.111	0.046	0.020	0.005	0.012	0.005	0.003	0.015	0.069	0.114	0.071	0.004					
FSV-BD																									
FSV-BE	0.280	0.100	0.260	0.120	0.040																				
FSV-BF	0.410	0.098	0.294	0.115	0.049											0.021	0.089	0.150	0.091	0.013					
FSV-BG	0.327	0.123	0.318	0.139	0.054											0.025	0.081	0.122	0.088	0.011					
FSV-BH	0.291	0.106	0.283	0.107	0.043	0.271	0.106	0.270	0.107	0.043	0.020	*nq*	0.013	*nq*	*nq*	*nq*	0.068	0.116	0.068	*nq*					
FSV-BI	0.326	0.118	0.301	0.128	0.053											0.019	0.068	0.118	0.072	0.004					
FSV-BJ	0.256	0.109	0.303	0.116	0.044											*nq*	0.099	0.171	0.102	*nq*					
FSV-BK																									
FSV-BL																									
FSV-BM																									
FSV-BN	0.308	0.091	0.258	0.089	0.043	0.277	0.086	0.243	0.086	0.034	0.023	*nd*	0.005	*nq*	*nq*	0.012	0.060	0.116	0.060	*nq*					
FSV-BO	0.295	0.110	0.260	0.105	0.048											0.015	0.062	0.100	0.058	*nq*					
FSV-BP	0.286	0.114	0.219	0.135	0.073											0.007	0.042	0.075	0.047	0.005					
FSV-BQ																									
FSV-BR																									
FSV-BS	≥0.415	≥0.126	≥0.317	≥0.128	≥0.034	0.415	0.126	0.317	0.128	0.034						*nq*	≥0.071	≥0.112	≥0.062	*nq*	*nq*	0.071	0.112	0.062	*nq*
FSV-BT	0.321	0.119	0.294	0.137	0.061	0.297	0.112	0.275	0.129	0.058	0.024	0.007	0.018	0.008	0.003	0.018	0.073	0.113	0.084	0.010					
FSV-BU	0.351	0.133	0.340	0.147	0.064											0.019	0.083	0.150	0.089	0.006					
FSV-BV	0.310	0.121	0.256	0.132	0.057											0.012	0.068	0.093	0.073	0.004					
FSV-BW	0.302	0.114	0.285	0.121	0.050											0.012	0.091	0.159	0.095	*nq*					
FSV-BX	≥0.288	≥0.115	≥0.305	≥0.123	≥0.050	0.288	0.115	0.305	0.123	0.050						0.017	0.083	0.143	0.089	0.009					
FSV-CB	0.287	0.092	0.268	0.098	0.051											0.013	0.053	0.099	0.057	0.004					
FSV-CC																									
FSV-CD	0.300	0.117	0.271	0.117	0.041											0.017	0.071	0.112	0.079	*nd*					
FSV-CE	0.301	0.106	0.345	0.111	0.044																				
FSV-CF																									
FSV-CG	0.279	0.109	0.273	0.116	0.048	0.253	0.101	0.254	0.108	0.044	0.026	0.007	0.019	0.007	0.004	0.015	0.066	0.112	0.070	0.004					
FSV-CI	≥0.286	≥0.095	≥0.223	≥0.099	≥0.039	0.29	0.10	0.22	0.10	0.04						0.017	0.066	0.081	0.068	<0.009					
FSV-CL	0.261	0.108	0.208	0.097	0.048											0.015	0.067	0.091	0.062	0.005					
FSV-CR																									
FSV-CS	0.357	0.154	0.394	0.170	0.068	0.302	0.116	0.248	0.119	0.051	0.03	0.01	0.02	0.01	0.00	0.019	0.080	0.121	0.084	0.005					
FSV-CT	0.330	0.100	0.260	0.090	0.053																				
FSV-CW	0.312	0.113	0.282	0.119	0.052	0.288	0.106	0.263	0.111	0.049	0.024	0.007	0.019	0.008	0.003	0.021	0.070	0.120	0.074	0.007	0.021	0.070	0.120	0.074	0.007
FSV-CZ	0.260	0.170	0.340	0.170	0.050																				
FSV-DA	0.323	0.127	0.294	0.143	0.068	0.295	0.110	0.270	0.129	0.057	0.028	0.017	0.024	0.014	0.012	0.020	0.076	0.123	0.087	0.016					
FSV-DD																									
FSV-DF																									
FSV-DI	≥0.403	≥0.132	≥0.293	≥0.130	≥0.059	0.403	0.132	0.293	0.130	0.059															
FSV-DW	0.386	0.140	0.327	0.140	0.060	0.332	0.121	0.288	0.122	0.051	0.054	0.019	0.039	0.018	0.010										
FSV-ET	0.310	0.110	0.260	0.110	0.040																				
N	27	27	27	27	27	14	14	14	14	14	10	8	10	8	8	21	23	23	23	16	1	2	2	2	1
Min	0.256	0.091	0.208	0.089	0.040	0.253	0.086	0.223	0.086	0.034	0.018	0.005	0.005	0.005	0.002	0.007	0.042	0.075	0.047	0.004		0.070	0.112	0.062	
Median	0.302	0.113	0.283	0.119	0.050	0.288	0.110	0.270	0.118	0.048	0.024	0.007	0.019	0.008	0.003	0.017	0.070	0.116	0.074	0.005	0.021	0.071	0.116	0.068	0.007
Max	0.410	0.170	0.394	0.170	0.073	0.415	0.132	0.317	0.130	0.059	0.054	0.019	0.039	0.018	0.012	0.025	0.099	0.171	0.102	0.016		0.071	0.120	0.074	
SD	0.026	0.010	0.031	0.019	0.007	0.015	0.008	0.025	0.013	0.006	0.005	0.003	0.005	0.002	0.002	0.003	0.011	0.013	0.014	0.003					
CV	9	9	11	16	14	5	7	9	11	12	21	44	26	29	58	19	15	11	20	68					
Npast	18	0	0	0	28	7	0	0	0	12	0	0	0	0	6	0	0	0	0	18	0	0	0	0	0
Medianpast	0.343				0.049	0.330				0.049					0.003					0.005					
SDpast	0.035				0.008	0.024				0.006					0.001					0.004					
NISTa	0.337	≥0.115	0.272	≥0.113	≥0.053	0.288	0.115	0.238	0.113	0.053	0.049	*nd*	0.034	*nd*	*nd*	*nd*	0.083	0.099	0.077	*nd*					
NISTb	0.326	≥0.108	0.276	0.118	≥0.047	0.296	0.108	0.259	0.116	0.047	*0.030*	*nd*	*0.017*	*0.002*	*nd*	0.018	0.083	≥0.114	0.086	≥0.004	0.016	0.076	0.114	0.080	0.004
NNIST	4	4	4	4	4	4	4	4	4	4	4		4	1	1	2	4	4	4	2	2	2	2	2	2
Mean	0.332	0.112	0.274	0.115	0.050	0.292	0.112	0.248	0.115	0.050	0.040		0.025	0.002		0.016	0.080	0.106	0.079	0.004	0.016	0.076	0.114	0.080	0.004
Srep	0.011	0.003	0.007	0.003	0.001	0.020	0.003	0.005	0.003	0.001	0.013		0.005			0.002	0.003	0.002	0.003	0.001	0.001	0.001	0.003	0.001	0.001
Shet	0.009	0.002	0.006	0.001	0.000	0.005	0.002	0.004	0.001	0.000	0.011		0.003			0.002	0.006	0.004	0.004	0.001	0.001	0.006	0.006	0.004	0.001
Sanl	0.008	0.005	0.003	0.002	0.004	0.006	0.005	0.015	0.001	0.004	0.013		0.013				0.004	0.011	0.002						
SNIST	0.016	0.006	0.010	0.004	0.005	0.021	0.006	0.017	0.003	0.005	0.022		0.014			0.003	0.008	0.012	0.006	0.001	0.002	0.006	0.006	0.004	0.001
NAV	0.317	0.112	0.278	0.117	0.050	0.290	0.111	0.259	0.116	0.049	0.032		0.022	0.005		0.017	0.075	0.111	0.076	0.004					
NAU	0.049	0.019	0.042	0.020	0.011	0.031	0.014	0.033	0.015	0.009	0.025		0.015	0.006		0.006	0.022	0.033	0.022	0.004					

Round Robin LIII Laboratory Results

All Results in µg/mL

Lab	Total Lycopene 289	Total Lycopene 290	Total Lycopene 291	Total Lycopene 292	Total Lycopene 293	trans-Lycopene 289	trans-Lycopene 290	trans-Lycopene 291	trans-Lycopene 292	trans-Lycopene 293
FSV-BA						0.164	0.302	0.240	0.316	0.107
FSV-BB	0.268	0.449	0.368	0.473	0.189	0.127	0.237	0.180	0.251	0.087
FSV-BD										
FSV-BE										
FSV-BF	0.339	0.506	0.437	0.532	0.209					
FSV-BG	0.256	0.549	0.438	0.601	0.200	0.154	0.321	0.240	0.345	0.107
FSV-BH	0.266	0.489	0.406	0.495	0.179					
FSV-BI	0.218	0.388	0.315	0.418	0.156					
FSV-BJ	0.266	0.564	0.461	0.586	0.202					
FSV-BK										
FSV-BL										
FSV-BM										
FSV-BN	0.246	0.373	0.343	0.374	0.148	0.128	0.212	0.170	0.213	0.076
FSV-BO	0.281	0.506	0.392	0.472	0.193					
FSV-BP	0.244	0.508	0.324	0.518	0.203					
FSV-BQ										
FSV-BR										
FSV-BS	0.393	0.621	0.487	0.659	0.234					
FSV-BT	0.299	0.477	0.421	0.550	0.221	0.114	0.185	0.159	0.212	0.085
FSV-BU	0.314	0.561	0.456	0.606	0.227					
FSV-BV	0.302	0.581	0.399	0.628	0.214					
FSV-BW	0.255	0.474	0.367	0.509	0.179					
FSV-BX						0.122	0.206	0.182	0.228	0.080
FSV-CB	0.226	0.338	0.349	0.345	0.165					
FSV-CC										
FSV-CD	0.291	0.379	0.347	0.409	0.135					
FSV-CE										
FSV-CF										
FSV-CG	0.250	0.441	0.375	0.475	0.167	0.129	0.245	0.190	0.266	0.086
FSV-CI										
FSV-CL	0.268	0.501	0.348	0.477	0.195					
FSV-CR										
FSV-CS	0.293	0.547	0.394	0.546	0.219					
FSV-CT	0.330	0.580	0.440	0.540	0.230					
FSV-CW	0.289	0.468	0.408	0.489	0.203	0.131	0.222	0.172	0.236	0.088
FSV-CZ										
FSV-DA	0.288	0.464	0.387	0.556	0.204	0.153	0.257	0.203	0.311	0.107
FSV-DD										
FSV-DF										
FSV-DI						0.253	0.429	0.267	0.421	0.158
FSV-DW						0.136	0.243	0.188	0.248	0.032
FSV-ET										
N	22	22	22	22	22	11	11	11	11	11
Min	0.218	0.338	0.315	0.345	0.135	0.114	0.185	0.159	0.212	0.032
Median	0.275	0.495	0.393	0.514	0.201	0.131	0.243	0.188	0.251	0.087
Max	0.393	0.621	0.487	0.659	0.234	0.253	0.429	0.267	0.421	0.158
SD	0.031	0.071	0.059	0.060	0.025	0.019	0.047	0.034	0.061	0.018
CV	11	14	15	12	12	15	19	18	24	21
Npast	0	0	0	0	21	0	0	0	0	11
Medianpast					0.181					0.096
SDpast					0.027					0.012
NISTa										
NISTb	0.314	0.548	0.414	0.574	0.212					
NNIST	2	2	2	2	2					
Mean	0.314	0.548	0.414	0.574	0.213					
Srep	0.015	0.003	0.012	0.009	0.010					
Shet	0.012	0.001	0.010	0.011	0.002					
Sanl										
SNIST	0.019	0.003	0.016	0.014	0.010					
NAV	0.295	0.521	0.403	0.544	0.207	0.131	0.243	0.188	0.251	0.087
NAU	0.069	0.109	0.086	0.114	0.050	0.023	0.047	0.034	0.061	0.018

Lab	Total β-Cryptoxanthin 289	Total β-Cryptoxanthin 290	Total β-Cryptoxanthin 291	Total β-Cryptoxanthin 292	Total β-Cryptoxanthin 293	Total α-Cryptoxanthin 289	Total α-Cryptoxanthin 290	Total α-Cryptoxanthin 291	Total α-Cryptoxanthin 292	Total α-Cryptoxanthin 293
FSV-BA	0.032	0.065	0.067	0.068	0.023					
FSV-BB	0.026	0.052	0.052	0.055	0.018	0.010	0.022	0.042	0.024	0.010
FSV-BD										
FSV-BE										
FSV-BF	0.027	0.049	0.047	0.051	0.017					
FSV-BG	0.019	0.047	0.049	0.056	0.014					
FSV-BH	0.028	0.064	0.060	0.065	0.020					
FSV-BI	0.028	0.058	0.060	0.063	0.020					
FSV-BJ	0.024	0.049	0.045	0.053	0.014					
FSV-BK										
FSV-BL										
FSV-BM										
FSV-BN	0.012	0.033	0.029	0.030	*nq*	*nd*	0.005	0.024	*nq*	*nq*
FSV-BO	0.023	0.050	0.048	0.049	0.016					
FSV-BP	0.028	0.051	0.093	0.042	0.042					
FSV-BQ										
FSV-BR										
FSV-BS										
FSV-BT	0.029	0.056	0.062	0.063	0.023					
FSV-BU	0.026	0.047	0.041	0.050	0.015					
FSV-BV	0.021	0.048	0.036	0.051	0.015					
FSV-BW										
FSV-BX	0.024	0.043	0.045	0.048	0.018					
FSV-CB	0.025	0.056	0.050	0.061	0.021					
FSV-CC										
FSV-CD	0.024	0.044	0.036	0.047	0.017					
FSV-CE										
FSV-CF										
FSV-CG	0.028	0.049	0.055	0.056	0.020					
FSV-CI										
FSV-CL	0.027	0.062	0.058	0.061	0.020	0.008	0.033	0.058	0.033	0.010
FSV-CR										
FSV-CS	0.028	0.050	0.048	0.051	0.018					
FSV-CT	0.027	0.052	0.055	0.046	0.016					
FSV-CW	0.033	0.055	0.058	0.057	0.022					
FSV-CZ										
FSV-DA	0.036	0.050	0.056	0.066	0.021	0.013	0.021	0.041	0.027	0.011
FSV-DD										
FSV-DF										
FSV-DI										
FSV-DW	0.040	0.077	0.078	0.080	0.024					
FSV-ET										
N	23	23	23	23	22	3	4	4	3	3
Min	0.012	0.033	0.029	0.030	0.014	0.008	0.005	0.024	0.024	0.010
Median	0.027	0.050	0.052	0.055	0.019	0.010	0.021	0.041	0.027	0.010
Max	0.040	0.077	0.093	0.080	0.042	0.013	0.033	0.058	0.033	0.011
SD	0.003	0.006	0.010	0.009	0.004					
CV	12	11	19	17	19					
Npast	0	0	0	0	23	0	0	0	0	0
Medianpast					0.019					
SDpast					0.003					
NISTa	0.038	0.071	0.076	0.076	*nd*					
NISTb	0.028	0.060	0.049	0.062	0.020	0.016	0.032	0.053	0.034	0.015
NNIST	4	4	4	4	2	2	2	2	2	2
Mean	0.033	0.066	0.062	0.069	0.021	0.016	0.032	0.053	0.034	0.015
Srep	0.003	0.004	0.002	0.005	0.001	0.000	0.001	0.004	0.002	0.002
Shet	0.005	0.001	0.002	0.002	0.002	0.000	0.001	0.001	0.001	0.000
Sanl	0.007	0.008	0.019	0.010						
SNIST	0.009	0.009	0.019	0.011	0.002	0.000	0.001	0.004	0.002	0.002
NAV	0.030	0.058	0.057	0.062	0.020					
NAU	0.010	0.016	0.020	0.017	0.005					

Lab	Total Lutein 289	Total Lutein 290	Total Lutein 291	Total Lutein 292	Total Lutein 293	trans-Lutein 289	trans-Lutein 290	trans-Lutein 291	trans-Lutein 292	trans-Lutein 293
FSV-BA										
FSV-BB	0.041	0.078	0.188	0.080	0.046					
FSV-BD										
FSV-BE										
FSV-BF										
FSV-BG	0.052	0.073	0.172	0.078	0.037					
FSV-BH	0.031	0.052	0.138	0.055	0.034					
FSV-BI	0.041	0.090	0.211	0.105	0.054					
FSV-BJ	0.034	0.081	0.183	0.083	0.041					
FSV-BK										
FSV-BL										
FSV-BM										
FSV-BN	0.029	0.046	0.123	0.048	0.030					
FSV-BO	0.023	0.043	0.113	0.043	0.025					
FSV-BP										
FSV-BQ										
FSV-BR										
FSV-BS										
FSV-BT	0.035	0.055	0.214	0.068	0.049					
FSV-BU										
FSV-BV										
FSV-BW										
FSV-BX	0.039	0.077	0.206	0.082	0.045					
FSV-CB		0.065		0.064	0.031					
FSV-CC										
FSV-CD										
FSV-CE										
FSV-CF										
FSV-CG										
FSV-CI	0.039	0.065	0.137	0.068	0.041					
FSV-CL										
FSV-CR										
FSV-CS	0.046	0.078	0.139	0.078	0.042					
FSV-CT	0.047	0.076	0.206	0.081	0.051					
FSV-CW	*≥0.031*	*≥0.058*	*≥0.141*	*≥0.062*	*≥0.037*	0.031	0.058	0.141	0.062	0.037
FSV-CZ										
FSV-DA	*≥0.032*	*≥0.055*	*≥0.147*	*≥0.060*	*≥0.034*	0.032	0.055	0.147	0.060	0.034
FSV-DD										
FSV-DF										
FSV-DI	0.045	0.118	0.240	0.118	0.054					
FSV-DW	0.044	0.083	0.185	0.093	0.050					
FSV-ET										
N	14	15	14	15	15	2	2	2	2	2
Min	0.023	0.043	0.113	0.043	0.025	0.031	0.055	0.141	0.060	0.034
Median	0.040	0.076	0.184	0.078	0.042	0.032	0.057	0.144	0.061	0.036
Max	0.052	0.118	0.240	0.118	0.054	0.032	0.058	0.147	0.062	0.037
SD	0.008	0.015	0.050	0.012	0.010					
CV	19	19	27	16	25					
Npast	0	0	0	0	18	0	0	0	0	0
Medianpast					0.047					
SDpast					0.017					
NISTa	0.045	0.102	0.231	0.114	0.053					
NISTb	*≥0.038*	*≥0.080*	*≥0.174*	*≥0.079*	*≥0.042*	0.038	0.080	0.174	0.079	0.042
NNIST	2	2	2	2	2	2	2	2	2	2
Mean	0.045	0.102	0.231	0.114	0.053	0.038	0.080	0.174	0.079	0.041
Srep	0.009	0.017	0.009	0.005	0.007	0.001	0.004	0.002	0.004	0.001
Shet	0.002	0.009	0.001	0.002	0.005	0.003	0.001	0.002	0.003	0.001
Sanl										
SNIST	0.010	0.019	0.009	0.005	0.009	0.004	0.004	0.003	0.005	0.001
NAV	0.042	0.089	0.208	0.096	0.048					
NAU	0.010	0.027	0.060	0.029	0.013					

Round Robin LIII Laboratory Results

All Results in µg/mL

	Total Zeaxanthin					Total Lutein&Zeaxanthin				
Lab	289	290	291	292	293	289	290	291	292	586
FSV-BA						0.053	0.109	0.227	0.112	0.067
FSV-BB	0.023	0.050	0.046	0.050	0.027	*0.064*	*0.128*	*0.303*	*0.130*	*0.073*
FSV-BD										
FSV-BE										
FSV-BF						0.051	0.110	0.202	0.115	0.055
FSV-BG	0.012	0.019	0.047	0.028	0.015	0.059	0.083	0.203	0.096	0.045
FSV-BH	0.008	0.028	0.037	0.030	0.018	0.039	0.080	0.175	0.085	0.052
FSV-BI	0.013	0.033	0.038	0.037	0.019	*0.054*	*0.123*	*0.303*	*0.142*	*0.073*
FSV-BJ										
FSV-BK										
FSV-BL										
FSV-BM										
FSV-BN						0.033	0.055	0.135	0.058	0.036
FSV-BO	0.009	0.030	0.039	0.026	0.014	0.0316	0.0722	0.1519	0.0688	0.0387
FSV-BP						0.095	0.108	0.210	0.119	0.089
FSV-BQ										
FSV-BR										
FSV-BS						0.060	0.200	0.401	0.206	0.086
FSV-BT	0.013	0.034	0.070	0.037	0.018	0.048	0.088	0.285	0.105	0.068
FSV-BU						0.065	0.141	0.340	0.150	0.071
FSV-BV						0.050	0.120	0.231	0.125	0.065
FSV-BW										
FSV-BX	0.008	0.024	0.030	0.026	0.016	0.048	0.101	0.236	0.109	0.061
FSV-CB		0.033		0.030	0.029	0.057	*0.098*	0.260	*0.094*	*0.060*
FSV-CC										
FSV-CD						0.047	0.104	0.200	0.106	0.051
FSV-CE										
FSV-CF										
FSV-CG						0.053	0.128	0.249	0.137	0.069
FSV-CI	0.015	0.029	0.031	0.031	0.016	*0.054*	*0.094*	*0.168*	*0.099*	*0.057*
FSV-CL						0.045	0.101	0.189	0.012	0.055
FSV-CR										
FSV-CS	0.012	0.024	0.021	0.023	0.013	*0.058*	*0.101*	*0.161*	*0.101*	*0.055*
FSV-CT	*nq*	0.025	0.038	0.020	0.014	0.047	0.100	0.244	0.101	0.065
FSV-CW	0.015	0.033	0.056	0.035	0.019	*≥0.046*	*≥0.091*	*≥0.197*	*≥0.097*	*≥0.056*
FSV-CZ										
FSV-DA	*≥0.014*	*≥0.030*	*≥0.036*	*≥0.030*	*≥0.018*	0.065	0.111	0.214	0.122	0.068
FSV-DD										
FSV-DF										
FSV-DI										
FSV-DW	0.038	0.082	0.097	0.088	0.043	0.082	0.165	0.282	0.180	0.093
FSV-ET										
N	11	13	12	13	13	23	23	23	23	23
Min	0.008	0.019	0.021	0.020	0.013	0.032	0.055	0.135	0.012	0.036
Median	0.013	0.030	0.038	0.030	0.018	0.053	0.104	0.227	0.109	0.065
Max	0.038	0.082	0.097	0.088	0.043	0.095	0.200	0.401	0.206	0.093
SD	0.003	0.006	0.010	0.008	0.003	0.009	0.019	0.057	0.022	0.011
CV	26	20	27	27	16	17	18	25	20	17
Npast	0	0	0	0	14	0	0	0	0	20
Medianpast					0.020					0.064
SDpast					0.003					0.016
NISTa										
NISTb	0.019	0.044	0.051	0.041	0.023	*≥0.057*	*≥0.124*	*≥0.224*	*≥0.120*	*≥0.064*
NNIST	2	2	2	2	2	2	2	2	2	2
Mean	0.019	0.044	0.051	0.041	0.023	0.057	0.124	0.224	0.120	0.048
Srep	0.001	0.001	0.003	0.002	0.004	0.002	0.004	0.005	0.006	0.033
Shet	0.000	0.004	0.003	0.001	0.002	0.003	0.005	0.001	0.002	0.022
Sanl										
SNIST	0.001	0.004	0.004	0.003	0.004	0.004	0.007	0.005	0.006	0.039
NAV	0.016	0.037	0.044	0.036	0.020	0.055	0.114	0.226	0.114	0.057
NAU	0.006	0.013	0.014	0.012	0.007	0.012	0.026	0.057	0.024	0.041

	Coenzyme Q10					Phylloquinone (K1)					Phytofluene				
Lab	289	290	291	292	293	289	290	291	292	293	289	290	291	292	293
FSV-BA															
FSV-BB															
FSV-BD															
FSV-BE						0.23	0.29	0.49	0.17	0.21					
FSV-BF															
FSV-BG															
FSV-BH															
FSV-BI															
FSV-BJ															
FSV-BK															
FSV-BL															
FSV-BM															
FSV-BN															
FSV-BO															
FSV-BP															
FSV-BQ															
FSV-BR															
FSV-BS															
FSV-BT															
FSV-BU															
FSV-BV															
FSV-BW	0.470	0.640	0.560	0.700	0.410										
FSV-BX															
FSV-CB															
FSV-CC															
FSV-CD															
FSV-CE															
FSV-CF															
FSV-CG															
FSV-CI						0.36	0.33	0.72	0.19	0.16					
FSV-CL											0.027	0.097	0.042	0.090	0.027
FSV-CR															
FSV-CS															
FSV-CT															
FSV-CW	0.700	1.000	0.900	1.100	0.500										
FSV-CZ	0.570	0.920	0.580	0.960	0.660										
FSV-DA											0.067	0.154	0.088	0.189	0.055
FSV-DD															
FSV-DF															
FSV-DI	0.293	0.397	0.262	0.401	0.254	0.33	0.29	0.67	0.31	0.18					
FSV-DW															
FSV-ET															
N	4	4	4	4	4	3	3	3	3	3	2	2	2	2	2
Min	0.293	0.397	0.262	0.401	0.254	0.23	0.29	0.49	0.17	0.16	0.027	0.097	0.042	0.090	0.027
Median	0.520	0.780	0.570	0.830	0.455	0.33	0.29	0.67	0.19	0.18	0.047	0.126	0.065	0.140	0.041
Max	0.700	1.000	0.900	1.100	0.660	0.36	0.33	0.72	0.31	0.21	0.067	0.154	0.088	0.189	0.055
Npast	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Round Robin LIII Laboratory Results
All Results in µg/mL

Analytes Reported By One Laboratory

Analyte	Code	289	290	291	292	293
trans-Zeaxanthin	FSV-DA	0.014	0.030	0.036	0.030	0.054
Didehydroretinol	FSV-DF	*nd*	*nd*	*nd*	*nd*	0.090
Ubiquinol	FSV-BW	0.310	0.440	0.350	0.420	0.320
Ubiquinone	FSV-BW	0.160	0.200	0.210	0.280	0.105
Phytoene	FSV-CL	*nd*	0.120	0.070	0.103	0.018
Retinyl stearate	FSV-DA	0.010	0.030	0.050	0.030	0.018

Legend

Term	Definition
N	Number of (non-NIST) quantitative values reported for this analyte
Min	Minimum (non-NIST) quantitative value reported
$Median_{part}$	Median (non-NIST) quantitative value reported
Max	Maximum (non-NIST) quantitative value reported
SD	Standard deviation for (non-NIST) results: 0.741*(3rd Quartile - 1st Quartile)
CV	Coefficient of Variation for (non-NIST) results: 100*SD/Median
N_{past}	Mean of N(s) from past RR(s)
$Median_{past}$	Mean of Median(s) from past RR(s)
SD_{past}	Pooled SD from past RR(s)
N_{NIST}	Number of vials analyzed in duplicate by NIST analyst(s)
$Mean_{NIST}$	Mean of the NIST-analyzed vial means
S_{rep}	Within-vial pooled standard deviation
S_{het}	Among-vial pooled standard deviation
S_{anl}	Between NIST analyst standard deviation
S_{NIST}	Total standard deviation for NIST analyses: $(S_{rep}^2 + S_{het}^2 + S_{anl}^2)^{0.5}$
NAV	NIST Assigned Value = $(Median_{part} + Mean_{NIST})/2$ for analytes reported by NIST analyst(s) = $Median_{part}$ for analytes reported by ≥ 10 labs but not NIST
NAU	NIST Assigned Uncertainty: $(S^2 + S_{btw}^2)^{0.5}$ S is the maximum of (0.05*NAV, SD, S_{NIST}, eSD) and S_{btw} is the standard deviation between $Median_{part}$ and $Mean_{NIST}$. The expected long-term SD, eSD, is defined in: Duewer, et al. Anal Chem 1997;69(7):1406-1413.
-	Not analyzed
nd	Not detected (i.e., no detectable peak for analyte)
nq	Detected but not quantitatively determined
≥x	Concentration greater than or equal to x
italics	Not explictly reported but calculated by NIST from reported values

Comparability Summary

Lab	TR	aT	g/bT	bC	tbC	aC	TLy	tLy	TbX	TLu	TZ
FSV-BA	1	1	1	1	1	1		2	1	1	1
FSV-BB	1	1	1	1	1	1	1	1	1	1	1
FSV-BD	1	2									
FSV-BE		1	1	1					1		
FSV-BF	1	1	1	2		2	1				
FSV-BG	1	1	1	1		2	1	2	1		
FSV-BH	1	1	4	1	1	1	1		1	1	2
FSV-BI	1	1	1	1		1	1		1	1	1
FSV-BJ	1	1	1	1		2	1		1	1	1
FSV-BK	1	1									
FSV-BL	1	2							1	1	
FSV-BM	2	1									
FSV-BN	2	2	2	1	2	1	1	1	1		
FSV-BO		2		1		1	1		1	1	2
FSV-BP	1	1		2		2	1		3		
FSV-BQ	2	1							2	2	
FSV-BR		1									
FSV-BS	2				3		2		1	1	1
FSV-BT	4	2	1	1	1	1	1	1	1	2	1
FSV-BU	2	2	1	2		1	1		1	1	2
FSV-BV	2	2	1	1		1	1				
FSV-BW	1	1	2	1		2	1				
FSV-BX		1	1		1	1		1	1		
FSV-CB	3	1		1		1	2				
FSV-CC	1	1									
FSV-CD	1	1	1	1		1	1		1		
FSV-CE	1	1		1					1		
FSV-CF	1	2							1		
FSV-CG	1	2	1	1	1	1	1	1	1		
FSV-CI	1	2	1		2	1					
FSV-CL	2	2	3	2		1	1				
FSV-CR	2	1									
FSV-CS		1		3	1	1	1		1	1	1
FSV-CT				1			1			1	1
FSV-CW		2	1	1	1	1	1	1			
FSV-CZ	1	1		3							
FSV-DA		1	1	2	1	2	1	1	2	1	4
FSV-DF	1									2	
FSV-DI	3	2	3		3			4	1		1
FSV-DW	1	1		2	1			2			
FSV-ET	1	1	1	1							
NISTa		1	1	1	1	1			2	1	
NISTb	1	1	1	1	1	1	1		1		1
n	34	40	24	29	16	25	23	11	25	16	14

	TR	aT	g/bT	bC	tbC	aC	TLy	tLy	TbX	TLu	TZ
% 1	68	68	79	72	75	76	91	64	84	81	71
% 2	24	33	8	21	13	24	9	27	12	19	21
% 3	6	0	8	7	13	0	0	0	4	0	0
% 4	3	0	4	0	0	0	0	9	0	0	7

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
tLy	trans-Lycopene
TbX	Total β-Cryptoxanthin
TLu	Total Lutein
TZ	Total Zeaxanthin
TL&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 RR53

Each participant in RR53 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 RR53:

- 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

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

Individualized Round Robin LIII Report: FSV-BA

Summary

	Serum 289			Serum 290			Serum 291			Serum 292			Serum 293		
Analyte	You	NAV	n	You	NAV	n	You	NAV	n	You	NAV	n	You	NAV	n
Total Retinol	0.821	0.764	32	0.640	0.615	33	0.554	0.549	33	0.663	0.660	33	0.336	0.340	33
Retinyl Palmitate	0.02		8	0.1	0.1	13	0.1	0.1	13	0.10	0.10	13	0.02	0.02	10
α-Tocopherol	5.00	4.75	37	10.20	10.06	38	11.23	11.12	38	10.47	10.61	38	2.83	2.87	38
γ/β-Tocopherol	2.478	2.284	21	1.853	1.767	22	1.732	1.662	22	1.894	1.839	22	0.756	0.708	22
Total β-Carotene	0.302	0.317	27	0.115	0.112	27	0.299	0.278	27	0.122	0.117	27	0.048	0.050	27
trans-β-Carotene	0.284	0.290	14	0.111	0.111	14	0.284	0.259	14	0.117	0.116	14	0.046	0.049	14
Total cis-β-Carotene	0.018	0.032	10	0.005		8	0.015	0.022	10	0.006	0.005	8	0.002		8
Total α-Carotene	0.017	0.017	21	0.071	0.075	23	0.125	0.111	23	0.075	0.076	23	0.005	0.004	16
trans-Lycopene	0.164	0.131	11	0.302	0.243	11	0.240	0.188	11	0.316	0.251	11	0.107	0.087	11
Total β-Cryptoxanthin	0.032	0.030	23	0.065	0.058	23	0.067	0.057	23	0.068	0.062	23	0.023	0.020	22
Total Lutein&Zeaxanthin	0.053	0.055	23	0.109	0.114	23	0.227	0.226	23	0.112	0.114	23	0.067	0.057	23

You : Your reported values for the listed analytes (micrograms/milliliter)
NAV : NIST Assigned Values, equal to (NIST's average-of-averages + this RR's median) / 2
n : Number of non-NIST laboratories reporting quantitative values for this analyte in this serum

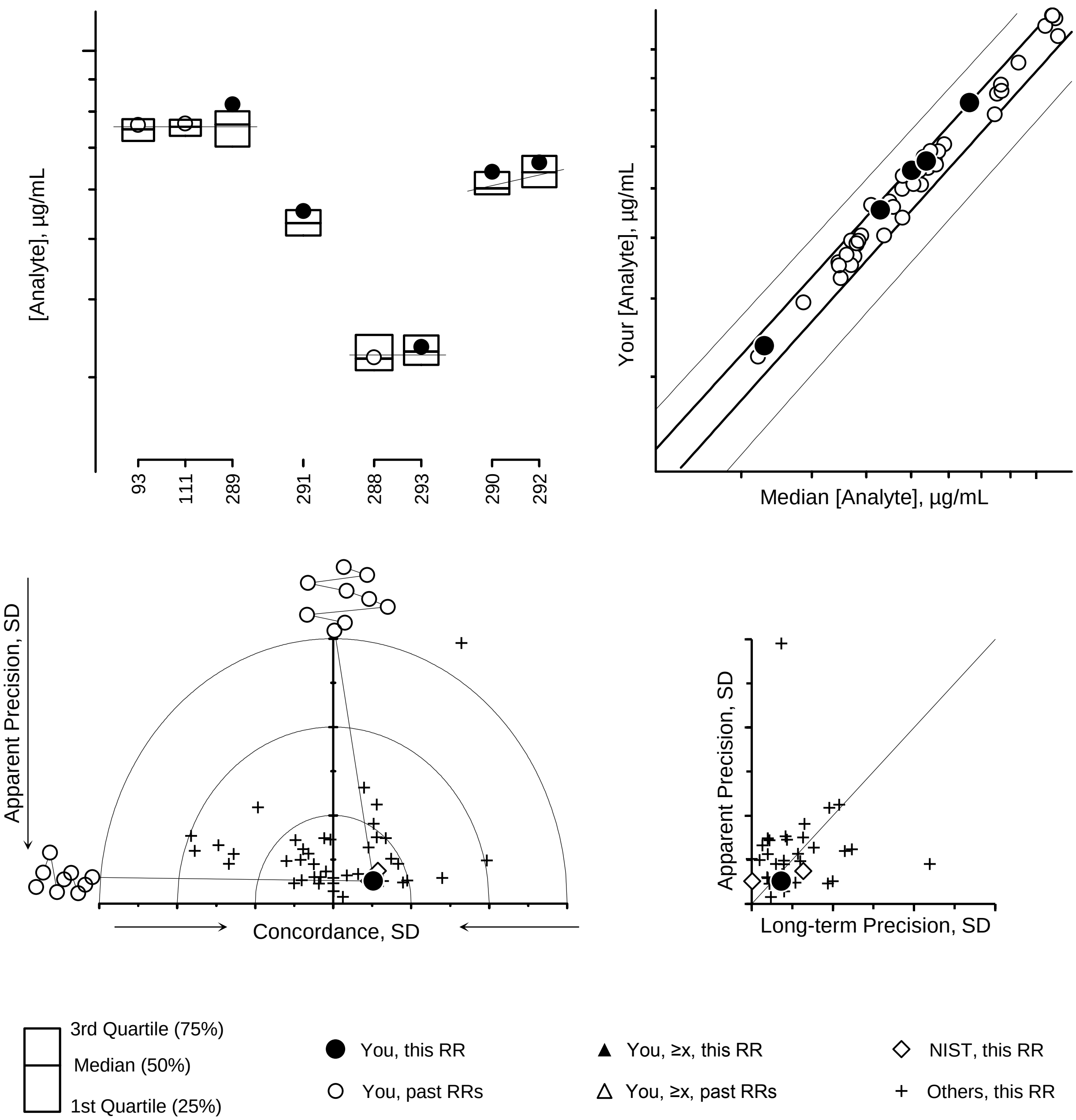
Please check our records against your records. Send corrections and/or updates to...

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

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

Individualized RR LIII Report: FSV-BA

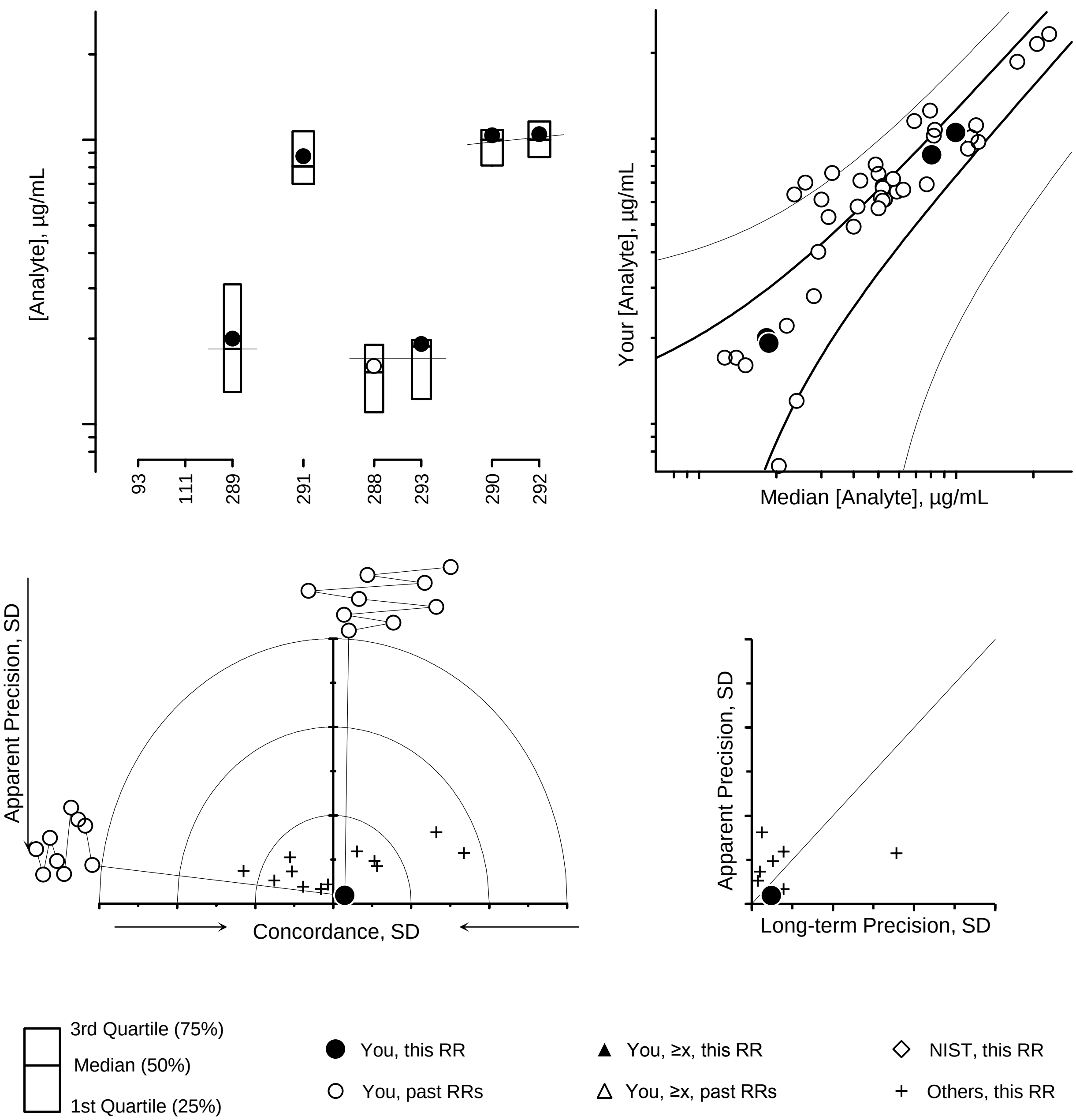
Total Retinol



Serum	History	Comments
#289	Lyophilized: #93 RR13 (Jun-88), #111 RR16 (Jun-89)	Native, single-source
#290	Lyophilized: new	Native, single-source
#291	Fresh-frozen: new	Native, single-source
#292	Fresh-frozen: new	Same pool as #290
#293	Fresh-frozen: #288 RR52	Native, single-source

Individualized RR LIII Report: FSV-BA

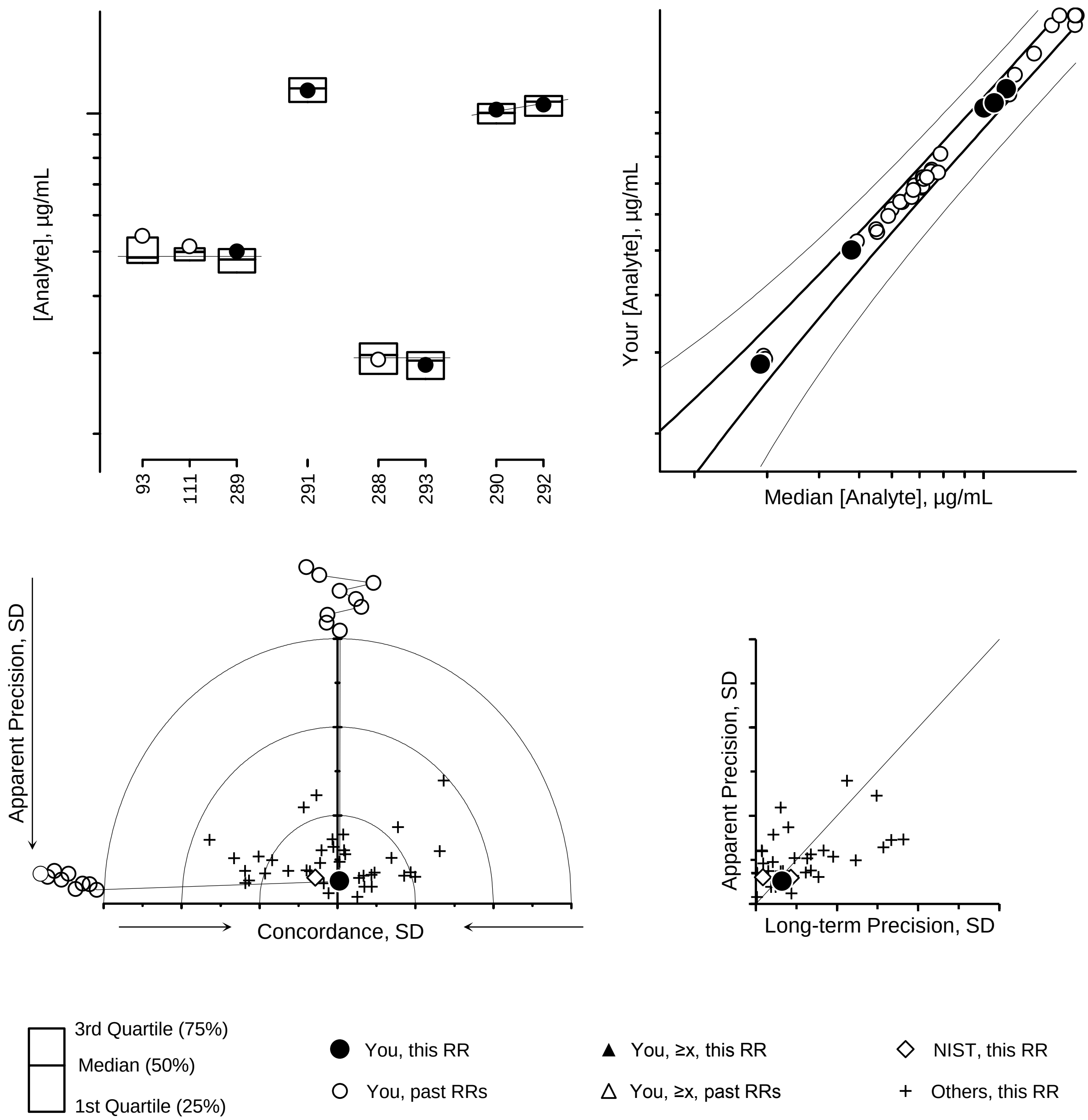
Retinyl Palmitate



Serum	History	Comments
#289	Lyophilized: #93 RR13 (Jun-88), #111 RR16 (Jun-89)	Native, single-source
#290	Lyophilized: new	Native, single-source
#291	Fresh-frozen: new	Native, single-source
#292	Fresh-frozen: new	Same pool as #290
#293	Fresh-frozen: #288 RR52	Native, single-source

Individualized RR LIII Report: FSV-BA

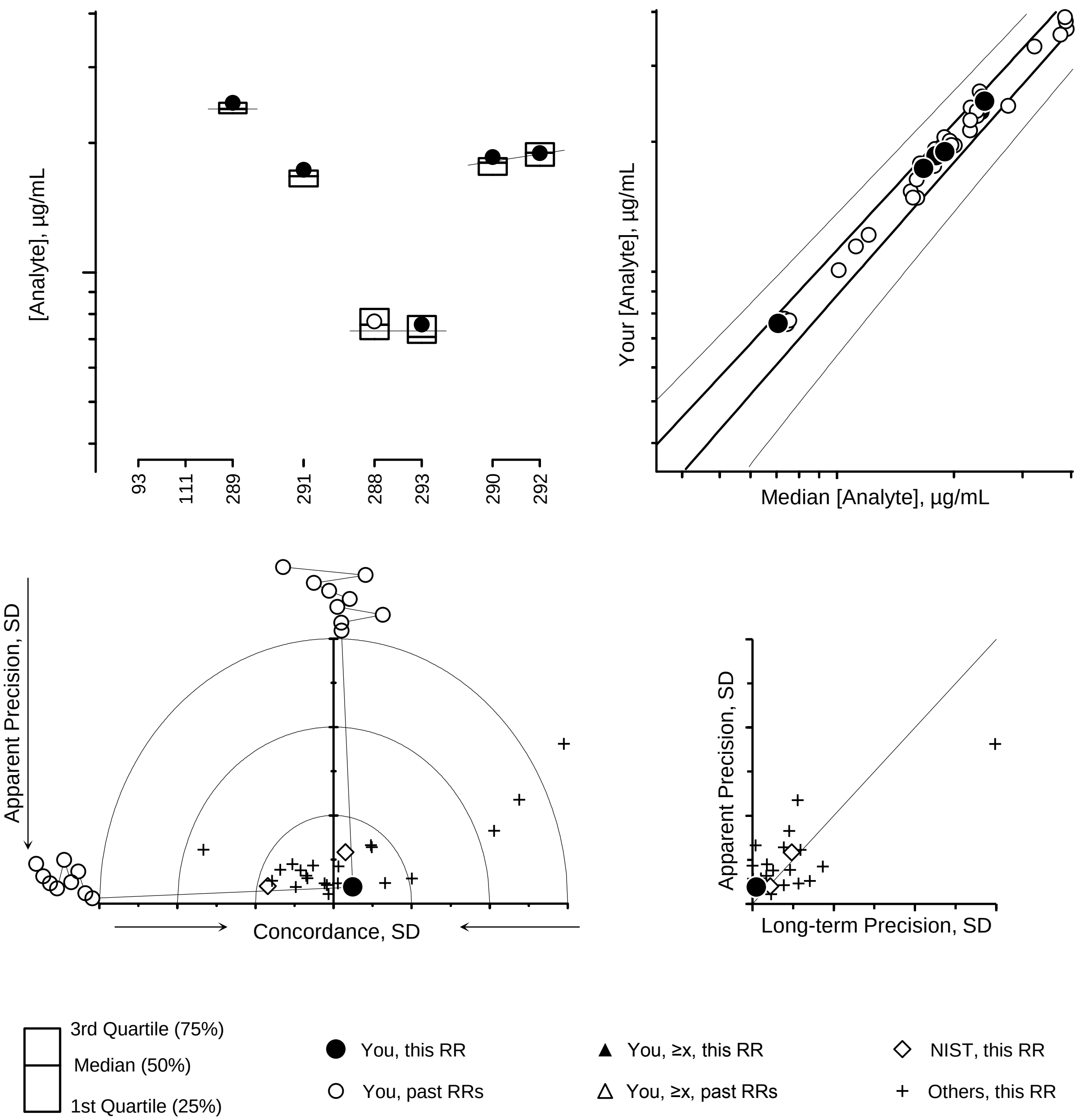
α-Tocopherol



Serum	History	Comments
#289	Lyophilized: #93 RR13 (Jun-88), #111 RR16 (Jun-89)	Native, single-source
#290	Lyophilized: new	Native, single-source
#291	Fresh-frozen: new	Native, single-source
#292	Fresh-frozen: new	Same pool as #290
#293	Fresh-frozen: #288 RR52	Native, single-source

Individualized RR LIII Report: FSV-BA

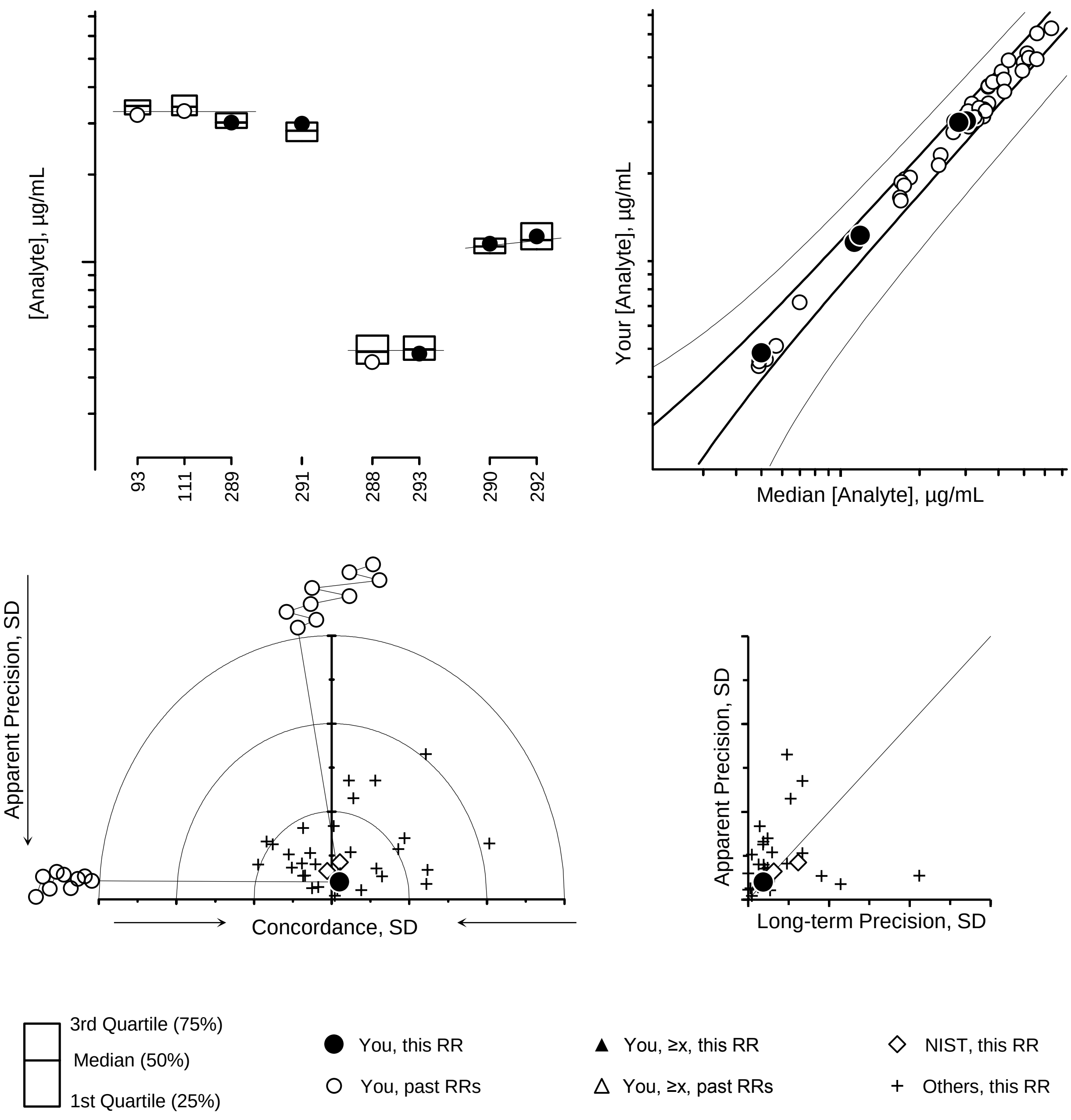
γ/β-Tocopherol



Serum	History	Comments
#289	Lyophilized: #93 RR13 (Jun-88), #111 RR16 (Jun-89)	Native, single-source
#290	Lyophilized: new	Native, single-source
#291	Fresh-frozen: new	Native, single-source
#292	Fresh-frozen: new	Same pool as #290
#293	Fresh-frozen: #288 RR52	Native, single-source

Individualized RR LIII Report: FSV-BA

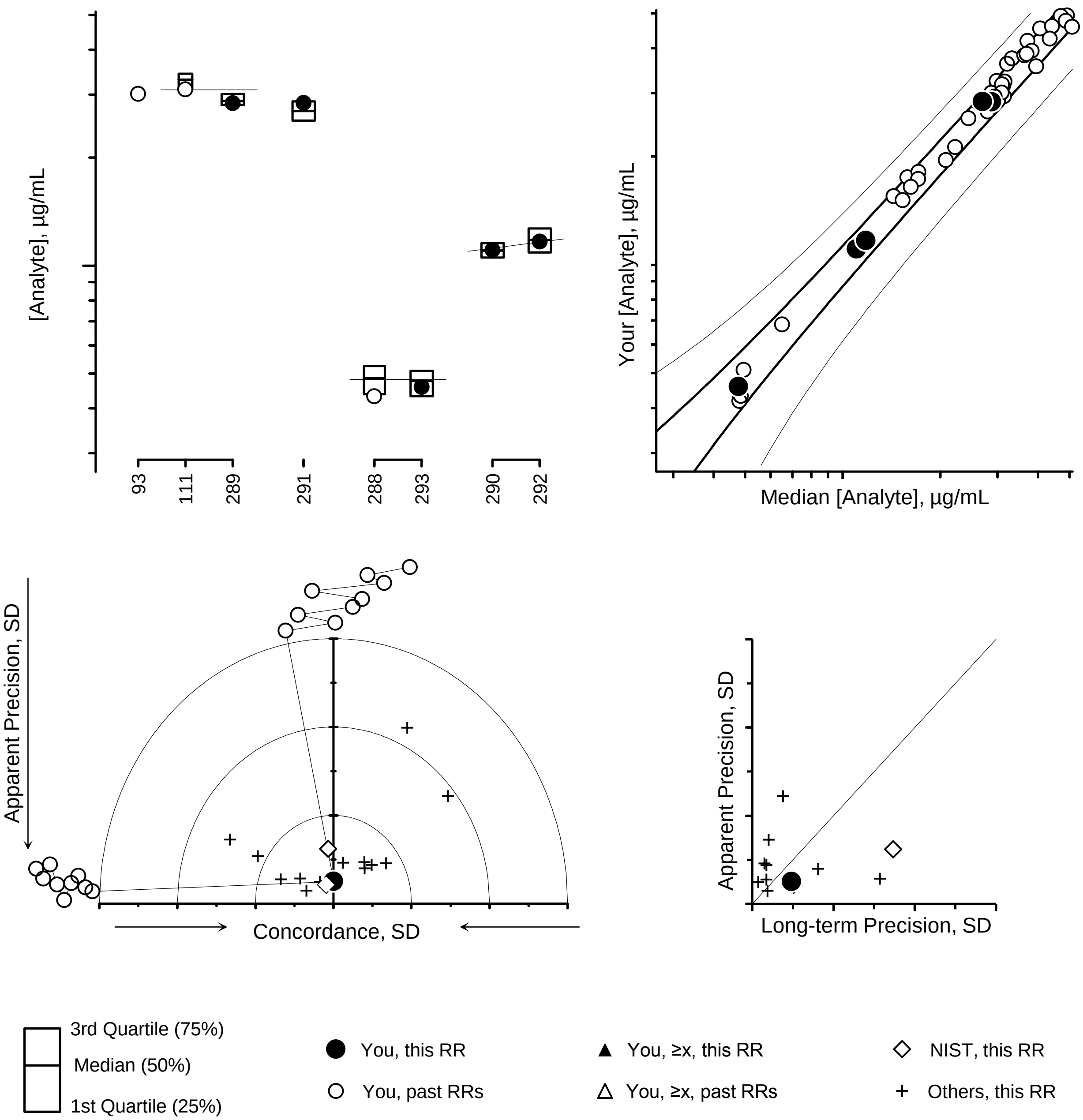
Total β-Carotene



Serum	History	Comments
#289	Lyophilized: #93 RR13 (Jun-88), #111 RR16 (Jun-89)	Native, single-source
#290	Lyophilized: new	Native, single-source
#291	Fresh-frozen: new	Native, single-source
#292	Fresh-frozen: new	Same pool as #290
#293	Fresh-frozen: #288 RR52	Native, single-source

Individualized RR LIII Report: FSV-BA

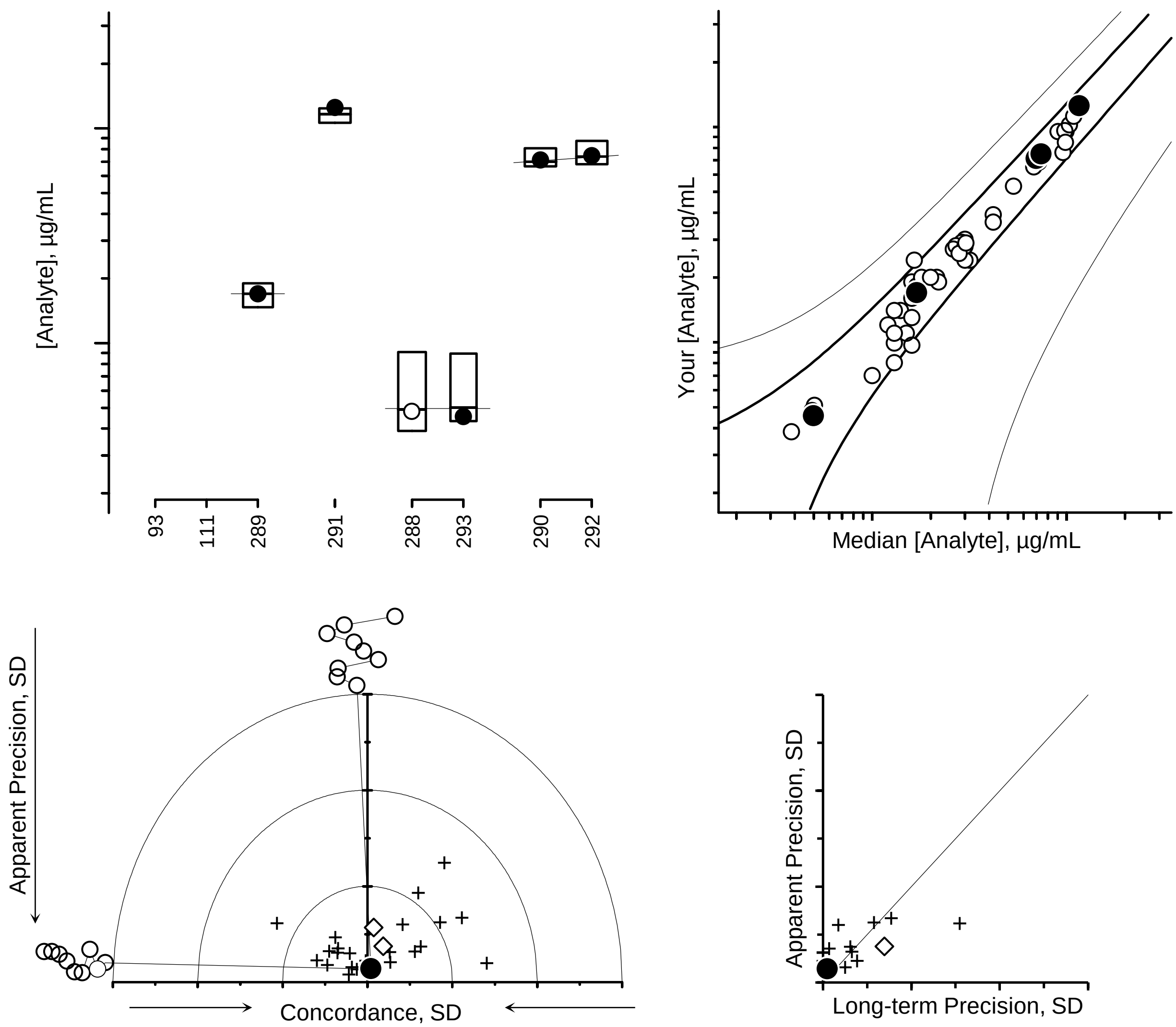
trans-β-Carotene



Serum	History	Comments
#289	Lyophilized: #93 RR13 (Jun-88), #111 RR16 (Jun-89)	Native, single-source
#290	Lyophilized: new	Native, single-source
#291	Fresh-frozen: new	Native, single-source
#292	Fresh-frozen: new	Same pool as #290
#293	Fresh-frozen: #288 RR52	Native, single-source

Individualized RR LIII Report: FSV-BA

Total α-Carotene



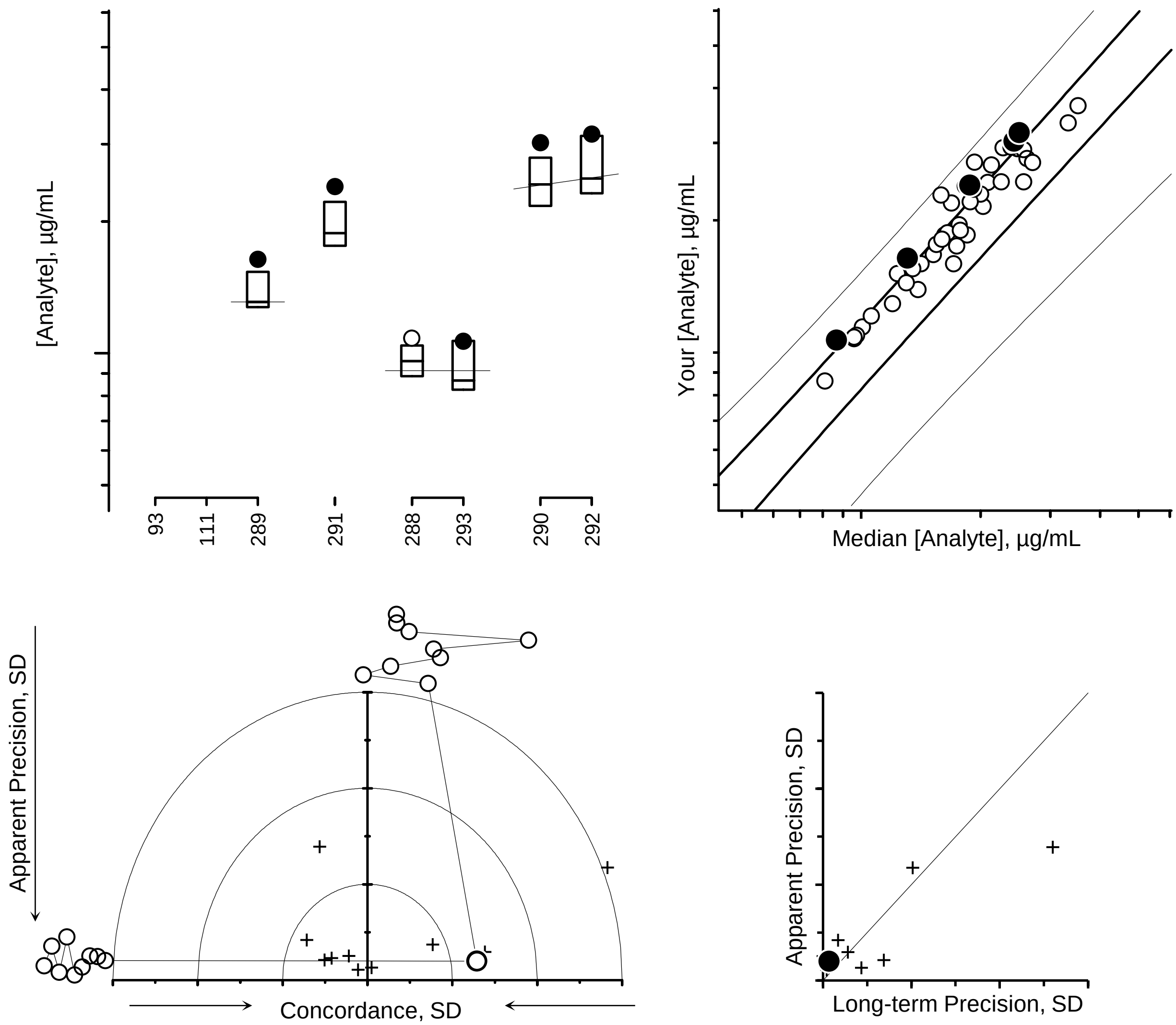
3rd Quartile (75%)
Median (50%)
1st Quartile (25%)

● You, this RR
○ You, past RRs
▲ You, ≥x, this RR
△ You, ≥x, past RRs
◇ NIST, this RR
+ Others, this RR

Serum	History	Comments
#289	Lyophilized: #93 RR13 (Jun-88), #111 RR16 (Jun-89)	Native, single-source
#290	Lyophilized: new	Native, single-source
#291	Fresh-frozen: new	Native, single-source
#292	Fresh-frozen: new	Same pool as #290
#293	Fresh-frozen: #288 RR52	Native, single-source

Individualized RR LIII Report: FSV-BA

trans-Lycopene



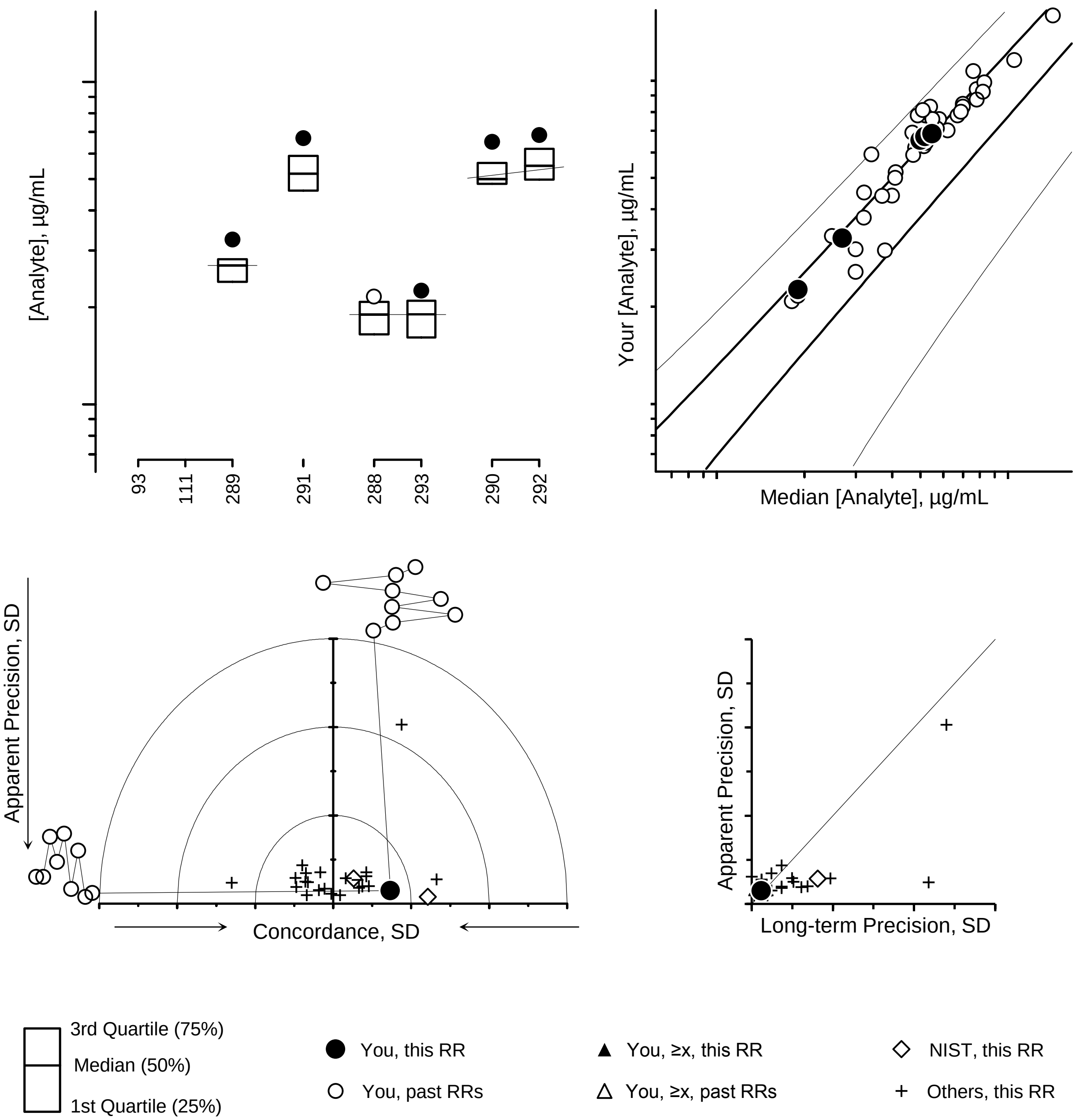
3rd Quartile (75%)
Median (50%)
1st Quartile (25%)

● You, this RR
○ You, past RRs
▲ You, ≥x, this RR
△ You, ≥x, past RRs
◇ NIST, this RR
+ Others, this RR

Serum	History	Comments
#289	Lyophilized: #93 RR13 (Jun-88), #111 RR16 (Jun-89)	Native, single-source
#290	Lyophilized: new	Native, single-source
#291	Fresh-frozen: new	Native, single-source
#292	Fresh-frozen: new	Same pool as #290
#293	Fresh-frozen: #288 RR52	Native, single-source

Individualized RR LIII Report: FSV-BA

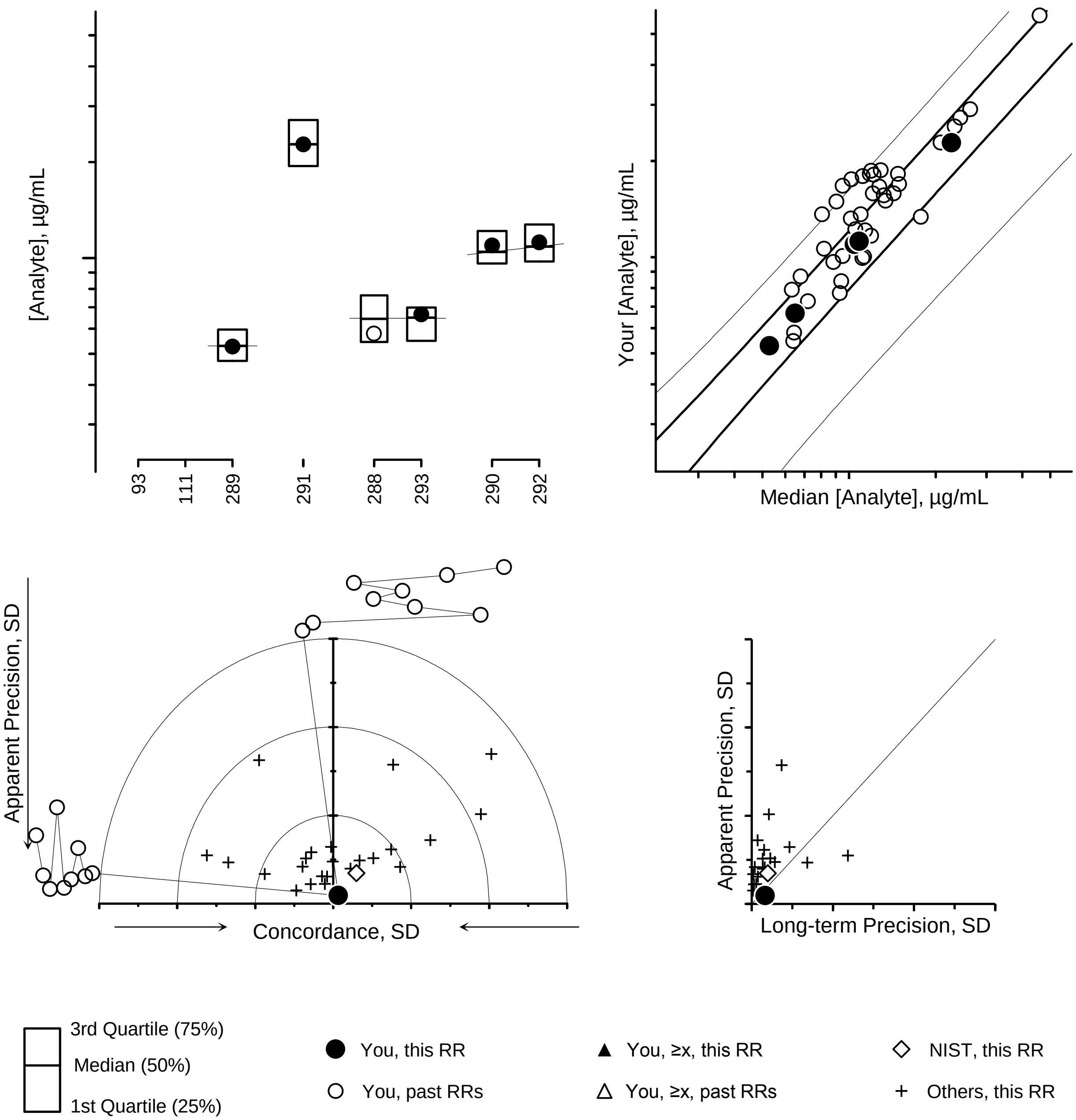
Total β-Cryptoxanthin



Serum	History	Comments
#289	Lyophilized: #93 RR13 (Jun-88), #111 RR16 (Jun-89)	Native, single-source
#290	Lyophilized: new	Native, single-source
#291	Fresh-frozen: new	Native, single-source
#292	Fresh-frozen: new	Same pool as #290
#293	Fresh-frozen: #288 RR52	Native, single-source

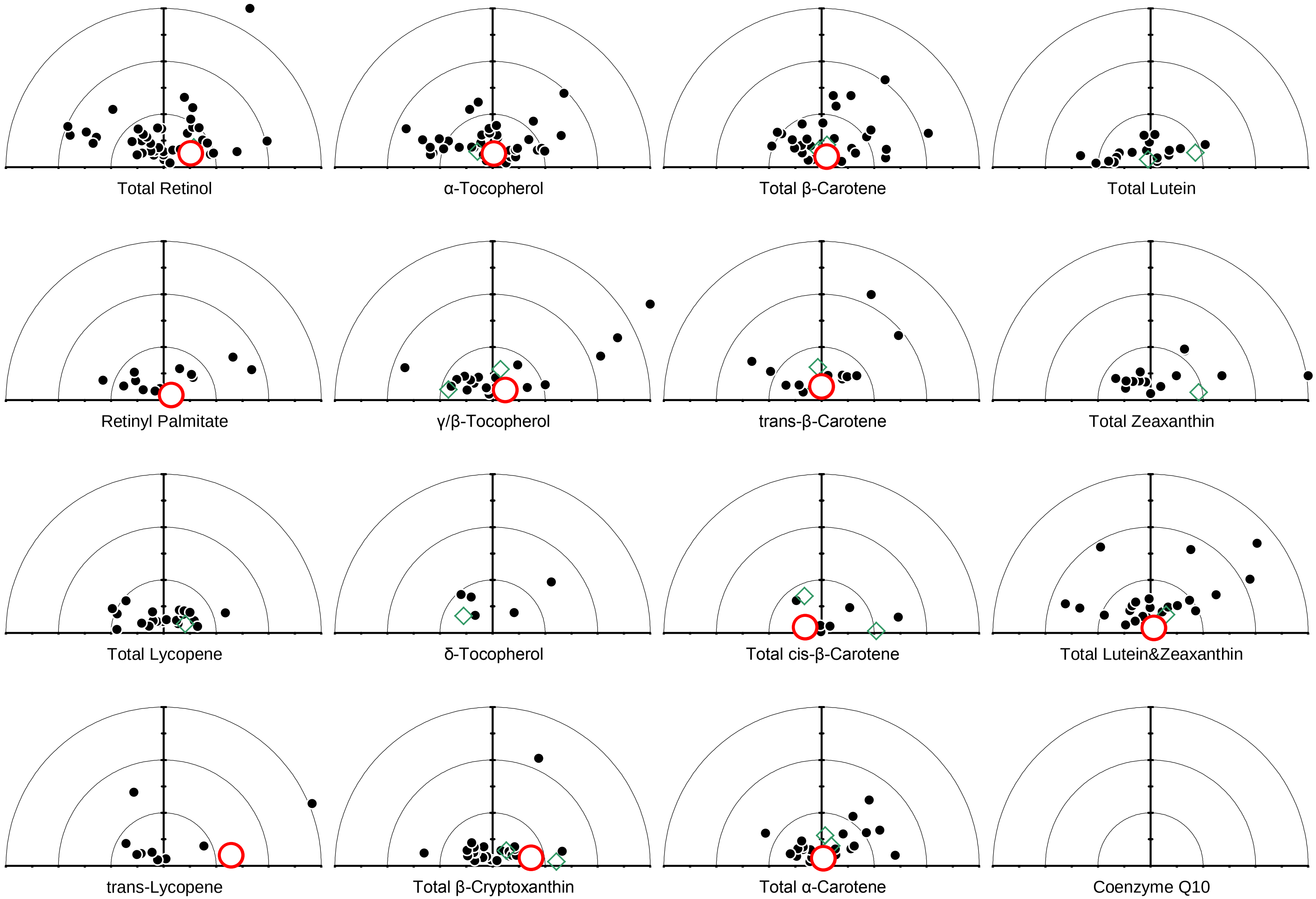
Individualized RR LIII Report: FSV-BA

Total Lutein&Zeaxanthin



Serum	History	Comments
#289	Lyophilized: #93 RR13 (Jun-88), #111 RR16 (Jun-89)	Native, single-source
#290	Lyophilized: new	Native, single-source
#291	Fresh-frozen: new	Native, single-source
#292	Fresh-frozen: new	Same pool as #290
#293	Fresh-frozen: #288 RR52	Native, single-source

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



D13

Appendix E. Shipping Package Inserts for RR18

The following five items were included in each package shipped to an RR18 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

November 13, 2002

Dear Colleague:

Enclosed are samples for the vitamin C round robin study (RR 18) for the 2003 NIST Micronutrients Measurement Quality Assurance Program. RR 18 consists of three vials of frozen serum (*test samples)* and one vial of solid ascorbic acid (*control sample*). Please follow the attached protocol when you prepare and analyze these samples. If you cannot prepare the control sample solutions gravimetrically, please prepare equivalent solutions volumetrically and report the exact volumes used.

Please be reminded that sample contact with any oxidant-contaminated surface (sample 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, particularly of your calibration solutions and/or control samples.

We recommend that you obtain **Standard Reference Material (SRM) 970 Ascorbic Acid in Serum** to validate your methodology and value assign in-house control materials. This SRM may be purchased from the Standard Reference Materials Program at NIST. (Tel: 301-975-6776, Fax: 301-948-3730, or e-mail: srminfo@nist.gov)

Please return your results for RR 18 using the attached form by **March 3, 2003** 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 concerns about this study, please contact me at 301-975-3120; e-mail: jbthomas@nist.gov; or mail/fax queries to the above address.

Sincerely,

Jeanice B Thomas

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

Enclosures

NIST

Micronutrient Measurement Quality Assurance Program for
Vitamin C

Please Read Through Completely BEFORE Analyzing Samples

Protocol for Preparation and Analysis of the Control Sample

The *control sample* consists of a sample of solid ascorbic acid in an amber vial. It 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 0.18 to 0.22 g of the solid ascorbic acid sample to 0.0001 g (if possible), dissolve it in the Diluent in a 100 mL volumetric flask, and dilute with the Diluent to the 100 mL mark. Weigh the amount of Diluent added to 0.1 g. Record the weights. The resulting material will be referred to as the "Stock Solution" below.

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

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

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

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

4. Calculate and record the total ascorbic acid concentrations, [TAA], in these Dilute Solutions.

   If you follow the above gravimetric preparation directions, the [TAA] in µmol/L is calculated:

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

   For example, if you prepared the Stock Solution with 0.2000 g of solid ascorbic acid and 103.0 g of Diluent, then 0.5 mL of the Stock Solution should weigh (0.2+103)/200 = 0.52 g and $[\mathrm{TAA}]_{\mathrm{DS1}}$ = (0.52 g)(0.2 g)·(56785 µmol/g·L)/(0.2 + 103 g) = 57.2 µmol/L. Likewise, 0.25 mL of the Stock Solution should weigh 0.26 g and $[\mathrm{TAA}]_{\mathrm{DS2}}$ = 28.4 µmol/L and 0.125 mL should weigh 0.13 g and $[\mathrm{TAA}]_{\mathrm{DS3}}$ = 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 of the sample 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:

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

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 used for the test samples, including any enzymatic treatment. Compare the replicate values. *Are you satisfied that your measurement precision is adequate?* Do **not** evaluate the test samples until you are satisfied that your system is performing properly!

7. Compare the measured with the calculated $[TAA]_{DS}$ 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** evaluate the test samples until you are satisfied that your system is performing properly!

Protocol for Analysis of the Test Samples

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

Each *test sample* 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).

Vitamin C Round Robin 18

NIST Micronutrient Measurement Quality Assurance Program

Preparation and Validation of Control Samples

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

Vitamin C Round Robin 18

NIST Micronutrient Measurement Quality Assurance Program

Analysis of Control 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
Test Sample #32			µmol/L of Sample
Test Sample #45			µmol/L of Sample
Test Sample #67			µmol/L of Sample

Were samples frozen upon receipt? Yes | No

Was SRM 970 used to validate your method or value-assign your in-house controls? Yes | No

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

COMMENTS:

Vitamin C Round Robin 18

NIST Micronutrients Measurement Quality Assurance Program

Packing List and Shipment Receipt Confirmation Form

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

Sample	Form
VitC #32	Liquid frozen (1:1 serum:10% MPA)
VitC #45	Liquid frozen (1:1 serum:10% MPA)
VitC #67	Liquid frozen (1:1 serum:10% MPA)
Control	Solid AA

Please 1) Open the pack immediately
2) Check that it contains one vial each of the above samples
3) Check if samples VitC #32, #45, and #67 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 four vials intact? Yes | No

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

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

4) Did samples VitC #32, #45, and #67 arrive frozen? Yes | No

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

6) When do you anticipate analyzing these samples? _________________________

Your prompt return of this information is appreciated.

The M^{2}QAP Gang

Appendix F. Final Report for RR18

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

March 28, 2003

Dear Colleague:

Enclosed is the summary report of the results for Round Robin 18 (RR18) 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.

RR18 consists of three unknowns (*test samples*) and one solid reference ascorbic acid for preparation of control solutions. Details regarding the samples can be found in the enclosed report.

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

Samples for a second vitamin C round robin study (RR 19) will be shipped, at no additional cost to you, during the **week of April 7.** We will send you a reminder via e-mail or fax a week prior to shipment. It is critical that you carefully inspect all samples upon arrival and that you promptly confirm to us that they have arrived. We will replace samples (lost or damaged in shipment or miss-packaged by us) **only** for participants who report the problem within one calendar week after the package arrives.

If you have 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 B Thomas

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

Enclosures

NIST

The NIST M^2QAP Vitamin C Round Robin 18 (RR18) report consists of

Page	"Individualized" Report
1	Summarizes your reported values for the nominal 55 mmol/L solution you prepared from the solid ascorbic acid control sample and for three recently prepared test samples.
2	Graphical summary of your RR 18 sample measurements.

Page	"All Lab" Report
1	A tabulation of results and summary statistics for Total Ascorbic Acid [TAA] in the RR18 samples and control/calibration solutions.

Test Samples. Three unknowns were distributed in RR18.

S18:1 Serum 32, previously distributed as sample S17:2 (RR17, Sep-02).

S18:2 Serum 45, initial distribution.

S18:3 Serum 67, SRM 970 level 2. 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), and S15:2 (RR15, Sep-01)

Results.

1) All participants successfully prepared the four control/calibration solutions (the three dilute solutions and the 5% MPA diluent). 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. The overall among-participant agreement (concordance) was pretty much unchanged by "correcting" the reported test sample results with the observed calibration parameters.

3) There has been little if any change in the TAA level in Serum 32 over the past six months. There has been little if any change in the TAA level in SRM 970 level 2 over the past five years.

4) The among-participant 10% to 14% relative standard deviations for these materials are as expected from past experience (see the RR17 report).

Appendix G. "All-Lab Report" for RR18

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

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

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

Micronutrients Measurement Quality Assurance Program for Total Ascorbic Acid
"Round Robin" 18 - March 2003

		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}	$E^{1\%}$	S18:1	S18:2	S18:3	S18:1	S18:2	S18:3
VC-MA	20/03/03	51.3	25.4	12.2	52.6	25.7	12.5	0.0	-0.054	1.02	1.000	0.2	1.030	244.	0.5093	563.1	23.2	35.6	29.0	22.7	34.8	28.4
VC-MB	19/12/02	59.5	30.0	14.9	50.1	23.5	13.9	0.3	0.363	0.83	0.997	1.5	1.032	245.	0.5922	564.6	21.4	33.6	27.6	25.5	40.2	33.0
VC-MC	02/12/02	55.6	25.8	11.9	53.2	26.9	13.2	0.0	1.196	0.95	0.997	1.4	1.027	243.	0.5178	528.5	23.3	34.3	28.5	23.3	34.9	28.8
VC-ME	14/03/03	56.7	28.0	13.8	55.8	26.9	13.6	1.4	0.734	0.96	0.999	0.9	1.028	243.	0.5709	572.0	18.7	30.5	25.1	18.7	30.9	25.3
VC-MG	26/02/03	57.0	29.4	14.6	55.0	27.8	14.1	0.0	-0.101	0.96	1.000	0.3	1.031	243.7	0.5410	539.3	23.0	35.5	30.4	24.0	36.9	31.7
VC-MH	14/02/03	61.4	31.0	14.9	59.7	32.1	14.4	0.0	0.334	0.98	0.999	1.2	1.031	243.4	0.6151	568.8	21.2	31.3	25.7	21.4	31.7	26.0
VC-MI	20/02/03	55.2	28.0	13.7	55.8	26.8	14.2	0.0	-0.160	1.01	0.999	1.1	1.030				19.6	28.1	27.6	19.7	28.1	27.6
VC-MO	28/02/03	58.6	29.0	14.4			17.3			1.20			1.032	243.	0.5720	554.4	26.2	36.0	34.5	21.8	29.9	28.7
VC-MQ	03/04/03	58.5	29.3	14.9	55.4	28.1	14.0	0.0	0.005	0.95	1.000	0.3	1.028	244.	0.5680	551.6	20.4	33.5	27.5	21.4	35.3	28.9
VC-MR	26/11/02	59.4	29.3	14.7	59.1	29.3	14.7	0.0	0.003	1.00	1.000	0.1	1.029	244.	0.5800	554.6	21.8	33.6	26.9	21.9	33.7	27.0
VC-MS	14/01/03	56.5	27.5	14.0		21.5	11.8	0.0	0.276	0.78	0.998	0.7	1.029	243.	0.5479	550.6	19.2	25.4	25.6	24.2	32.0	32.4
VC-MT	10/12/02	57.0	28.9	14.4	59.0	30.9	14.3	0.0	-0.032	1.04	0.999	0.8	1.031	244.	0.5677	565.0	16.3	30.3	22.8	15.7	29.2	21.9
VC-MY	20/11/02	56.9	28.1	14.1	62.5	24.4	12.5	0.0	-2.493	1.10	0.987	3.8	1.031				14.2	55.1	120.4	15.1	52.2	111.4
NIST	17/12/02	60.0	26.6	14.9	57.1	25.6	14.9	0.0	0.346	0.95	1.000	0.4	1.032	244.	0.5614	531.7	21.4	33.1	26.8	22.2	34.6	27.8
	N	13	13	13	11	12	13	12				N	13	11	11	11	13	13	13	13	13	13
	Average	57.2	28.4	14.0	56.2	27.0	13.9	0.1				Average	1.030	243.6	0.5620	555.7	20.7	34.0	34.7	21.2	34.6	34.7
	SD	2.5	1.6	1.0	3.6	3.0	1.3	0.4				SD	0.002	0.6	0.0311	13.1	3.2	7.1	25.9	3.1	6.3	23.2
	Min	51.3	25.38	11.9	50.1	21.55	11.8	0.0				Min	1.027	243.0	0.5093	528.5	14.2	25.4	22.8	15.1	28.1	21.9
	%25	56.5	28.03	13.8	54.1	25.39	13.2	0.0				%25	1.029	243.0	0.5445	551.1	19.2	30.5	25.7	19.7	30.9	27.0
	Median	57.0	28.93	14.4	55.8	26.95	14.0	0.0				Median	1.030	243.7	0.5680	554.6	21.2	33.6	27.6	21.8	33.7	28.7
	%75	58.6	29.32	14.7	59.0	28.39	14.3	0.0				%75	1.031	244.0	0.5760	564.8	23.0	35.5	29.0	23.3	35.3	31.7
	Max	61.4	31.01	14.9	62.5	32.05	17.3	1.4				Max	1.032	245.0	0.6151	572.0	26.2	55.1	120.4	25.5	52.2	111.4
	eSD	2.2	1.33	0.7	4.7	2.62	0.6	0.0				eSD	0.001	0.4	0.0298	14.8	3.0	3.3	2.8	3.1	4.2	4.1
	eCV	4	5	5	8	10	4					CV	0.12	0.18	5	3	14	10	10	14	12	14

Appendix H. Representative "Individualized Report" for RR18

Each participant in RR18 received an "Individualized Report" reflecting their reported results. The following two pages are the "Individualized Report" for participant "VC-MA".

Vitamin C "Round Robin" 18 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
09/17/01	13	HPLC-EC	1.031	244.0	0.523	572.5				
09/27/01	14	HPLC-EC	1.028	243.0	0.541	547.7	-0.9	1.03	0.999	1.09
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
		Mean	1.028	242.8	0.54	559.0				
		SD	0.002	0.8	0.02	11.4				
		CV	0.23	0.34	4.4	2.0				

[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	2	23.3	0.5	0.4
03/20/03	18	S18:1	22.7	23.7	1.0	23.2	0.7				
03/20/03	18	S18:2	35.1	36.0	1.0	35.6	0.6				
09/23/98	11	S11:2:A	50.7	47.7	0.5	24.6	1.1	8	25.7	0.9	1.9
09/23/98	11	S11:2:B	48.8	54.0	0.5	25.7	1.8				
04/02/99	12	S12:2:A	49.5	45.9	0.5	23.9	1.3				
04/02/99	12	S12:2:B	46.2	47.9	0.5	23.5	0.6				
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				

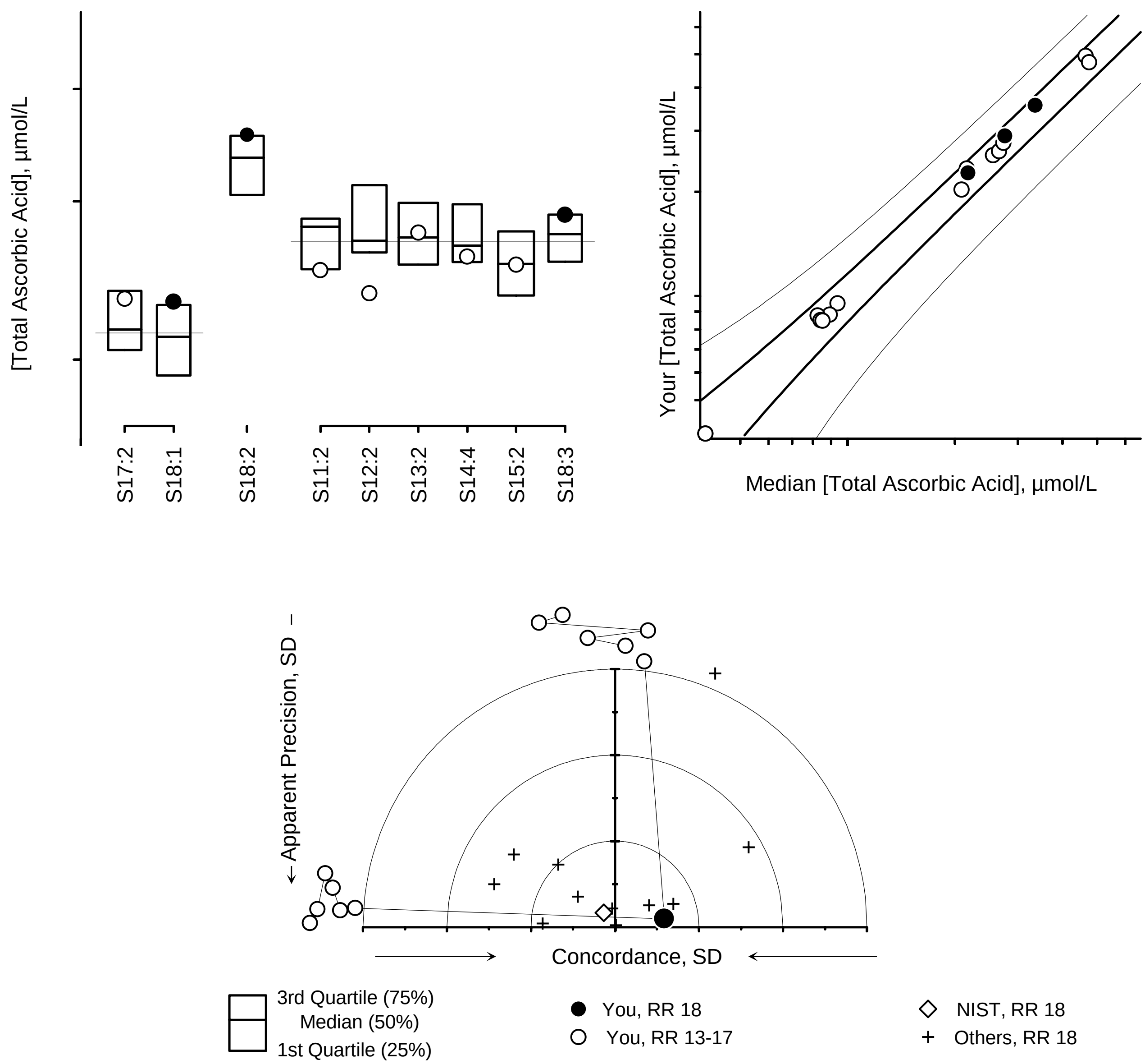
Please check our records against your records. Send corrections and/or updates to...

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

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

Vitamin C "Round Robin" 18 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
S18:1	Serum 32, previously distributed in RR 17
S18:2	Serum 45, initial distribution
S18:3	SRM 970 Level 2, previously distributed as in RRs 11, 12, 13, 14, and 15