NISTIR 7880-21

NIST Micronutrients Measurement Quality Assurance Program Winter 2002 Comparability Studies

Results for Round Robin LI Fat-Soluble Vitamins and Carotenoids in Human Serum and Round Robin 16 Ascorbic Acid in Human Serum

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

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Materials Measurement Laboratory

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

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

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Abstract

The National Institute of Standards and Technology coordinates the Micronutrients Measurement Quality Assurance Program (MMQAP) for laboratories that measure fat- and water-soluble vitamins and carotenoids in human serum and plasma. This report describes the design of and results for the Winter 2002 MMQAP measurement comparability improvement studies: 1) Round Robin LI Fat-Soluble Vitamins and Carotenoids in Human Serum and 2) Round Robin 16 Total Ascorbic Acid in Human Serum. The materials for both studies were shipped to participants in January 2002; participants were requested to provide their measurement results by April 8, 2002.

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 LI: Fat-Soluble Vitamins and Carotenoids in Human Serum

Participants in the MMQAP Fat-Soluble Vitamins and Carotenoids in Human Serum Round Robin LI comparability study (hereafter referred to as RR51) received four lyophilized and one 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 January 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 RR51 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 16: Vitamin C in Human Serum

Participants in the MMQAP Vitamin C in Human Serum Round Robin 16 comparability study (hereafter referred to as RR16) 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 January 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 RR16 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 RR51

The following three items were included in each package shipped to an RR51 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.

NIST CENTENNIAL 1901-2001

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

January 24, 2002

Dear Colleague:

Happy New Year! Enclosed is the set of samples for the first quality assurance round robin exercise (Round Robin LI) for 2002. You will find one vial of each of four lyophilized and one liquid-frozen serum samples for analysis along with a form for reporting your results. When reporting your results, please submit one value for each analyte for a given serum sample. If an obtained value is below your limit of quantitation, please indicate this result on the form by using NQ *(Not Quantified)*. Results are due to NIST by **April 8, 2002**. 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-May.

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 sample 279**.

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 LI to:

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

To improve the efficiency of our program for next year, the **intent-to-participate forms for the 2003 QA Program will be mailed in May 2002.** Please return all forms by September 1, 2002. Laboratories will be invoiced for the 2003 program at the end of September 2002. Samples for the first fat-soluble vitamins/carotenoids and vitamin C in serum round robins will be shipped during the first week of November 2002. Please call me at (301) 975-3120; e-mail me at jbthomas@nist.gov; or mail/fax queries to the above address if you have any questions or comments.

Sincerely,

Jeanice Brown Thomas

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

Enclosures

NIST

Participant #: _____ Date: _____

Round Robin LI

NIST Micronutrients Measurement Quality Assurance Program

Analyte	279	280	281	282	283	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						
ubiquinone-10 (Q_{10})						
phylloquinone (K_1)						
25-hydroxyvitamin D						
Other analytes?						

* we prefer μg/mL

Was serum 279 frozen when received? Yes | No

Comments:

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

Please return results **before** 8-Apr-2002

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

Participant #: _____ Date: _____

Fat-Soluble Vitamins Round Robin LI

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?
#279	Liquid frozen	No
#280	Lyophilized	Yes (1 ml H_2O)
#281	Lyophilized	Yes (1 ml H_2O)
#282	Lyophilized	Yes (1 ml H_2O)
#283	Lyophilized	Yes (1 ml H_2O)

Please 1) Open the pack immediately
2) Check that it contains one vial each of the above samples
3) Check if serum #279 arrived frozen
4) Store the samples upright 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 serum #279 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 will help control M^2QAP expenses.

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 RR51

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

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

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

May 21, 2002

Dear Colleague:

Enclosed is the summary report of the results for Round Robin LI (RR 51) 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; lyophilized vs. fresh-frozen commutability data, 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 51 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.

Intent-to-participate forms for the 2003 QA program will be mailed in about two weeks. 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, 2002.**

Shipping has become more difficult post September 11, 2001. It is important that you carefully inspect all samples on arrival and that you **promptly** confirm that they have arrived and that they have remained frozen. While we can (and do) determine the date of receipt (and the signature of the signee) of shipments when we don't receive direct confirmation, this costs us significant time and effort. We will now replace lost or damaged samples *only* for participants who have **promptly** reported the difficulty.

Samples for the next round robin (RR52) will be distributed during **the week of June 10, 2002.** We will send you a reminder via e-mail or fax a week prior to shipment. Please notify us, **preferably before the week of June 10**, if you have special shipping instructions.

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

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

NIST

The NIST M^2QAP Round Robin LI (RR51) 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
1-3	A listing of all results and statistics for analytes reported by at least two laboratories.
4a	A list of results for the four 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").

Samples. The five sera below were distributed in RR51.

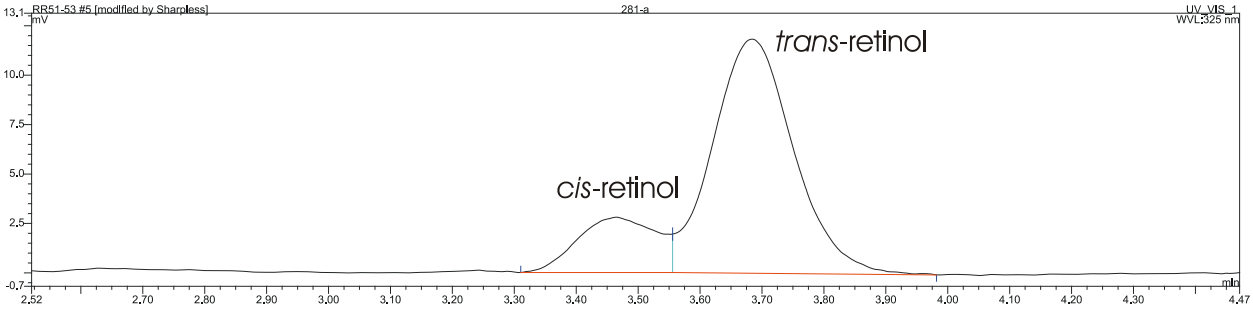
Serum	Description	Prior Distributions
279	Liquid-frozen native serum; partner to lyophilized serum 282.	#271 in RR49 (3/01), #275 in RR50 (9/01)
280	Lyophilized blended serum with native carotenoid levels, augmented with retinol and γ-tocopherol; SRM 968c level-I.	#248 in RR44 (9/98), #258 in RR46 (6/99), #263 in RR47 (5/00)
281	Lyophilized native serum apparently augmented with 13-*cis*- enriched "retinol".	#293 in RR30 (3/94), #254 in RR45 (3/99), #255 in RR46 (6/99), #269 in RR49 (3/01)
282	Lyophilized native serum; partner to liquid-frozen serum 279.	#266 in RR48 (9/00), #277 in RR50 (9/01)
283	Lyophilized native serum augmented with retinyl palmitate.	#181 in RR28 (6/93), #222 in RR37 (6/96), #243 in RR43 (6/98)

Observations

1) As in earlier distributions of these materials, several participants noted the presence an "insoluble stringy clot" in Serum 279 and/or 282. These solids do not appear to influence measurement performance.

2) Sera Stability. There has been no significant change in the median level or in the variability of any measurand in any of the five sera. The oldest serum distributed in RR 51 was produced in early 1993. All of these materials have been continuously stored –80 °C.

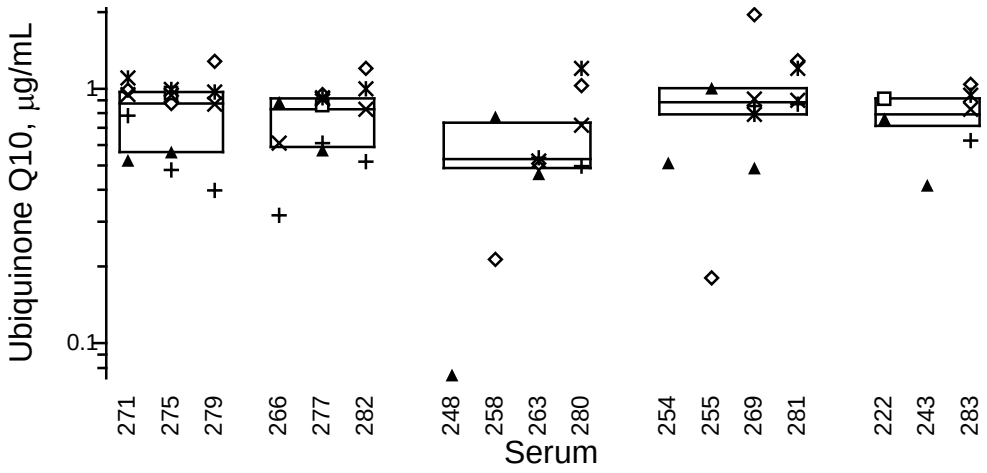
3) Total versus *trans*-retinol. Serum 281 has an unusually high level of *cis*-retinol, roughly 20% of the total retinol. The following is the relevant section of one of Kathy Sharpless's recent chromatograms, using a Bakerbond™ C_{18} column and method "B" described in the SRM 968c Certificate of Analysis (http://srmcatalog.nist.gov/srmcatalog/certificates/968c.pdf). Based upon the retention time of exemplar compounds, the leading peak appears to be entirely 13-*cis*-retinol.



We strongly urge all of you who report "retinol" to carefully evaluate the relevant sections of your chromatograms for serum #281. If you report *trans*-retinol and did not resolve the *cis*-retinol peak reasonably well, you should either confirm that your system is capable of identifying the *trans*-isomer in the presence of known 13-*cis* or you should consider reporting "total retinol." If you report "total retinol" and at least partially resolve the 13-*cis* peak but did not include its area, you should modify your integration parameters or consider reporting "*trans*-retinol."

We have prepared a series of sera with known high levels of 13-*cis*-retinol for future distribution. At least one of them will be distributed in the next round robin (RR 52).

4) Ubiquinone (Coenzyme Q10). While too few participants routinely report "ubiquinone" to enable regular feedback on measurement performance, there are enough historical data for the sera distributed in RR 51 to address several issues. The following Figure presents all available participant data for all of the distributions of the sera and summarizes the expected distributions as box-plots. Each symbol type represents values reported by a particular participant.



Are participants reporting the same measurand? Probably not. Coenzyme Q10 (CoQ10) is the sum of reduced (ubiquinol, QH2) and oxidized forms (ubiquinone, Qox). There appear to be fairly consistent among-participants biases which may in part arise from differing definitions of what's being measured. Starting with RR 52, the Report form will list all three forms.

Are the "Ubiquinone" measurands stable in the M^2QAP sera? Probably. The best evidence is provided by the {liquid frozen (271, 275, 279), lyophilized (266, 277, 282)} pairs: for most participants, the levels in these two materials have been nearly equal. Additionally, the values reported by about half of the participants reporting this measurand appear pretty consistent over time.

Appendix C. "All-Lab Report" for RR51

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 LI Laboratory Results

All Results in µg/mL

	Total Retinol					trans-Retinol					Retinyl Palmitate					α-Tocopherol					γ/β-Tocopherol					δ-Tocopherol				
Lab	279	280	281	282	283	279	280	281	282	283	279	280	281	282	283	279	280	281	282	283	279	280	281	282	283	279	280	281	282	283
FSV-BA	0.470	0.858	1.132	0.452	0.494						0.060	0.040	0.063	0.057	0.106	7.15	7.39	27.36	6.77	7.21	1.96	3.89	6.16	1.87	2.36					
FSV-BB	0.434	0.831	1.070	0.419	0.447						0.056	0.029	0.030	0.052	0.085	7.20	7.87	27.11	7.03	7.44	1.81	3.64	5.64	1.73	2.16					
FSV-BD	0.511	0.871	0.983	0.469	0.506											6.50	6.40	23.60	5.50	6.20										
FSV-BE	0.510	0.980	1.220	0.410	0.530											7.40	8.00	27.40	6.30	7.40	2.10	4.30	6.70	1.60	2.50					
FSV-BF	0.430	0.810	1.000	0.440	0.450											7.00	7.40	26.80	6.70	6.90	1.80	3.70	5.60	1.80	2.20					
FSV-BG	0.458	0.897	1.153	0.443	0.558						0.063	0.029	0.032	0.058	0.087	6.95	7.82	28.51	6.77	7.46	1.90	4.24	6.59	1.93	2.67					
FSV-BH	0.450	0.807	0.923	0.480	0.434											7.25	7.79	27.53	6.86	7.25	2.09	4.15	6.58	1.99	2.51	*nd*	*nd*	*nd*	*nd*	*nd*
FSV-BI	0.496	0.894	1.140	0.460	0.480						*nd*	*nd*	*nd*	*nd*	0.122	7.34	7.58	28.04	6.77	6.63	2.11	4.12	6.43	1.87	2.17					
FSV-BJ	0.432	0.827	1.046	0.414	0.437						0.035	*nq*	*nq*	0.049	0.147	7.52	7.91	29.16	6.85	7.29	2.04	4.41	6.96	1.88	2.51					
FSV-BK	0.444	0.893	1.144	0.423	0.474											6.80	7.46	27.26	6.53	6.84										
FSV-BL	0.520	0.860	0.860	0.490	0.460											8.01	9.04	27.61	7.28	7.54										
FSV-BM	0.469	0.848	1.089	0.475	0.488											7.20	7.99	29.20	7.20	8.50										
FSV-BN	0.462	0.866	1.028	0.416	0.479						0.038	0.024	0.008	0.039	0.059	6.05	6.74	23.32	5.47	6.14	1.50	3.08	4.45	1.30	1.75	0.049	*nd*	0.233	*nd*	*nd*
FSV-BNa	0.433	0.840	1.019	0.428	0.477						0.034	0.026	0.014	0.040	0.080	5.70	6.78	24.00	5.96	6.18	1.47	3.11	4.75	1.42	1.74	nd	*nd*	0.237	*nd*	*nd*
FSV-BO	0.415	0.773	0.776	0.478	0.390											6.40	7.70	28.60	6.40	7.60										
FSV-BP	0.460	0.838	1.114	0.464	0.506											6.56	7.56	28.10	7.61	7.12										
FSV-BQ	0.509	0.929	1.235	0.486	0.506											7.30	7.90	28.50	7.10	7.40										
FSV-BR	>0.466	>0.878	>1.020	>0.449	>0.493	0.466	0.878	1.020	0.449	0.493						6.80	7.55	27.83	6.73	7.47										
FSV-BS																														
FSV-BT	0.457	0.894	1.068	0.455	0.500						0.044	0.018	0.017	0.050	0.102	5.61	6.22	22.31	5.54	6.07	1.28	2.77	4.73	1.43	1.61	0.739	0.790	0.993	0.966	0.548
FSV-BU	0.436	0.774	0.968	0.426	0.502											6.48	7.46	24.53	7.04	7.98	1.79	3.43	5.57	1.64	2.31					
FSV-BV	0.487	0.880	1.078	0.465	0.532											7.28	7.71	26.93	7.16	7.64	1.85	3.78	6.05	1.77	2.20					
FSV-BW	0.507	0.905	0.853	0.443	0.475						0.066	0.035	0.030	0.059	0.115	7.71	7.80	30.64	6.87	7.48	2.11	4.00	6.94	1.88	2.29					
FSV-BX	>0.450	>0.835	>0.942	>0.438	>0.490	0.450	0.835	0.942	0.438	0.490						6.78	7.38	24.13	6.68	7.21	1.85	3.86	5.76	1.82	2.36					
FSV-CB	0.417	0.770	0.916	0.387	0.431											7.03	7.11	24.36	6.35	6.47										
FSV-CC	>0.450	>0.840	>0.950	>0.440	>0.480	0.450	0.840	0.950	0.440	0.480						7.32	7.78	26.95	6.55	7.24										
FSV-CD	0.496	0.920	1.218	0.484	0.516						0.052	0.031	0.010	0.038	0.100	7.62	7.84	30.27	7.43	7.57	2.08	4.10	6.38	2.14	2.68					
FSV-CE	0.461	0.856	1.003	0.429	0.491											6.61	7.31	25.84	6.39	6.77										
FSV-CF	0.547	0.911	1.148	0.506	0.527											7.90	8.10	29.00	7.30	7.80										
FSV-CG	0.498	0.879	1.113	0.506	0.516											6.66	6.43	24.50	6.09	6.42	2.15	3.81	6.02	1.91	2.33	0.193	0.215	0.586	0.229	0.213
FSV-CI	0.460	0.790	0.740	0.420	0.440						0.060	0.040	0.030	0.050	0.110	7.20	7.10	32.40	6.40	7.20	2.00	3.80	7.00	1.70	2.30					
FSV-CL	0.354	0.618	0.808	0.328	0.364											8.71	8.37	29.53	7.53	8.17	2.10	3.63	6.37	1.74	2.33					
FSV-CP																7.59	8.15	27.38	6.73	6.63	2.16	4.08	5.63	2.17	2.23					
FSV-CR	0.490	0.890	1.030	0.440	0.520											8.30	8.50	30.10	6.70	7.90										
FSV-CV	0.530	0.910	0.920	0.460	0.430						0.068	0.082	0.050	0.110	0.140	7.99	8.22	23.14	7.46	7.37	2.07	3.99	5.97	1.76	2.08	0.120	0.140	0.350	0.120	0.310
FSV-CW	>0.390	>0.779	>0.940	>0.382	>0.388	0.390	0.779	0.940	0.382	0.388	0.046	0.022	0.011	0.045	0.098	7.38	7.93	28.73	7.14	7.39	1.97	4.00	6.23	1.89	2.36	0.100	0.110	0.380	0.100	0.120
FSV-CZ	0.450	0.940	1.180	0.498	0.510											5.80	7.30	18.20	6.30	6.60										
FSV-DD	>0.477	>0.700	>0.782	>0.401	>0.447	0.477	0.700	0.782	0.401	0.447																				
FSV-DF	0.427	0.819	1.113	0.414	0.446																									
FSV-DI	>0.394	>0.808	>0.838	>0.343	>0.369	0.394	0.808	0.838	0.343	0.369	0.039	0.019	0.007	0.031	0.062	7.55	8.20	27.00	7.35	7.51	1.85	3.87	6.21	1.78	2.17	0.160	0.180	0.565	0.148	0.179
FSV-DQ																8.29	8.68	25.85	7.88	8.18	2.21	3.14	7.06	2.08	1.93					
FSV-DR	0.470	0.840	1.070	0.420	0.470											7.15	7.09	25.26	6.47	6.88										
FSV-DU	0.460	0.900	1.150	0.410	0.480											6.52	8.10	25.38	6.60	6.97										
FSV-DW	0.726	1.408	1.847	0.748	0.816											6.96	7.75	27.65	7.04	7.29										
FSV-EQ	0.553	0.956	1.254	0.516	0.557											8.40	7.80	30.10	7.10	7.90										
FSV-ET	0.380	0.720	0.850	0.370	0.370											6.30	6.80	22.50	6.10	6.50	1.60	3.10	4.70	1.40	1.80					
FSV-FB	0.510	0.890	1.300	0.510	0.580	0.510	0.890	1.300	0.510	0.580						7.09	7.97	30.29	7.53	7.68										
N	38	38	38	38	38	7	7	7	7	7	13	12	12	13	14	44	44	44	44	44	25	25	25	25	25	6	5	7	5	5
Min	0.354	0.618	0.740	0.328	0.364	0.390	0.700	0.782	0.343	0.369	0.034	0.018	0.007	0.031	0.059	5.61	6.22	18.20	5.47	6.07	1.28	2.77	4.45	1.30	1.61	0.049	0.110	0.233	0.100	0.120
Median	0.462	0.869	1.070	0.447	0.484	0.450	0.835	0.942	0.438	0.480	0.052	0.029	0.024	0.050	0.101	7.18	7.77	27.37	6.77	7.29	1.97	3.86	6.16	1.80	2.29	0.140	0.180	0.380	0.148	0.213
Max	0.726	1.408	1.847	0.748	0.816	0.510	0.890	1.300	0.510	0.580	0.068	0.082	0.063	0.110	0.147	8.71	9.04	32.40	7.88	8.50	2.21	4.41	7.06	2.17	2.68	0.739	0.790	0.993	0.966	0.548
SD	0.050	0.053	0.130	0.044	0.050	0.037	0.049	0.071	0.040	0.055	0.016	0.009	0.015	0.012	0.021	0.65	0.45	2.64	0.55	0.54	0.21	0.33	0.70	0.14	0.15	0.059	0.056	0.209	0.081	0.097
CV	11	6	12	10	10	8	6	8	9	11	31	32	63	25	21	9	6	10	8	7	11	9	11	8	6	42	31	55	55	46
N_{past}	38	44	45	42	46	9	10	9	9	0	13	14	11	14	12	45	48	46	49	44	25	26	26	27	23	7	7	9	6	8
$Median_{past}$	0.470	0.855	1.050	0.448	0.477	0.451	0.842	1.022	0.437		0.055	0.037	0.037	0.052	0.114	7.10	7.46	27.05	6.76	7.05	1.98	3.85	6.07	1.85	2.31	0.136	0.136	0.491	0.144	0.170
SD_{past}	0.042	0.058	0.104	0.045	0.051	0.031	0.046	0.098	0.035		0.014	0.012	0.035	0.011	0.028	0.61	0.74	2.26	0.66	0.57	0.14	0.35	0.53	0.13	0.28	0.097	0.051	0.205	0.129	0.045
NISTa	0.481	0.858	1.065	0.499	0.474	0.481	0.858	0.838	0.499	0.474						7.20	7.25	26.64	7.29	7.19	2.13	3.86	6.40	2.13	2.62					
NISTb	0.477	0.878	0.919	0.458	0.490	0.477	0.847	0.752	0.453	0.464						7.26	7.30	27.24	6.82	6.80	1.86	3.58	5.66	1.80	2.23	0.125	0.127	0.395	0.129	0.167
N_{NIST}	4	4	3	4	2	4	4	3	4	2						4	4	4	4	2	4	4	4	4	2	2	2	2	2	1
Mean	0.479	0.868	0.992	0.478	0.482	0.479	0.852	0.795	0.476	0.469						7.23	7.27	26.94	7.06	6.99	2.00	3.72	6.03	1.96	2.43	0.125	0.127	0.395	0.129	0.167
S_{rep}	0.006	0.020	0.038	0.010	0.013	0.006	0.019	0.028	0.008	0.013						0.26	0.12	0.81	0.24	0.30	0.10	0.24	0.11	0.10	0.08	0.009	0.021	0.022	0.019	0.010
S_{het}	0.008	0.009	0.008	0.007		0.008	0.010	0.009	0.007							0.23	0.08	1.26	0.17		0.13	0.08	0.10	0.13		0.030	0.023	0.012	0.032	
S_{anl}	0.003	0.014	0.103	0.029	0.011	0.003	0.008	0.061	0.032	0.007						0.04	0.03	0.43	0.33	0.28	0.19	0.20	0.52	0.23	0.28					
S_{NIST}	0.010	0.026	0.110	0.031	0.017	0.010	0.022	0.068	0.034	0.015						0.35	0.15	1.56	0.45	0.41	0.25	0.32	0.54	0.28	0.29	0.031	0.031	0.026	0.037	
NAV	0.470	0.868	1.031	0.463	0.483	0.465	0.843	0.869	0.457	0.475	0.052	0.029	0.024	0.050	0.101	7.20	7.52	27.16	6.91	7.14	1.98	3.79	6.10	1.88	2.36	0.133	0.154	0.387	0.139	0.190
NAU	0.051	0.068	0.141	0.049	0.050	0.042	0.066	0.127	0.048	0.056	0.021	0.018	0.017	0.020	0.029	0.66	0.72	2.66	0.61	0.64	0.25	0.37	0.71	0.31	0.30	0.060	0.067	0.209	0.082	0.103

C2

Round Robin LI Laboratory Results

All Results in µg/mL

	Total β-Carotene					trans-β-Carotene					Total cis-β-Carotene					Total α-Carotene					trans-α-Carotene				
Lab	279	280	281	282	283	279	280	281	282	283	279	280	281	282	283	279	280	281	282	283	279	280	281	282	283
FSV-BA	0.335	0.161	0.327	0.312	0.072	0.317	0.151	0.301	0.293	0.068	0.018	0.010	0.026	0.018	0.004	0.028	0.013	0.010	0.026	0.011					
FSV-BB	0.329	0.163	0.335	0.314	0.066	0.310	0.153	0.306	0.295	0.062	0.018	0.011	0.029	0.020	0.004	0.033	0.017	0.015	0.031	0.011					
FSV-BD																									
FSV-BE	0.410	0.210	0.420	0.380	0.080																				
FSV-BF	0.402	0.171	0.395	0.367	0.066											0.035	0.019	0.015	0.031	0.010					
FSV-BG	0.335	0.186	0.359	0.349	0.066											0.030	0.021	0.021	0.032	0.016					
FSV-BH	0.380	0.204	0.400	0.361	0.075	0.353	0.184	0.358	0.332	0.075	0.027	0.020	0.042	0.029	*nd*	0.038	0.018	0.014	0.033	0.016					
FSV-BI	0.401	0.201	0.400	0.364	0.075											0.034	0.018	0.011	0.032	0.012					
FSV-BJ	0.383	0.192	0.410	0.343	0.065											0.039	0.020	0.018	0.035	0.013					
FSV-BK																									
FSV-BL																									
FSV-BM																									
FSV-BN	0.354	0.174	0.365	0.327	0.069	0.329	0.169	0.327	0.303	0.068	*0.025*	*0.005*	*0.038*	*0.024*	*0.001*	0.030	0.013	0.008	0.028	0.010					
FSV-BNa	0.333	0.186	0.383	0.352	0.069	0.318	0.165	0.328	0.320	0.066	*0.015*	*0.021*	*0.055*	*0.032*	*0.003*	0.028	0.012	0.007	0.028	0.009					
FSV-BO	0.347	0.167	0.366	0.329	0.075											0.032	0.026	0.022	0.031	0.013					
FSV-BP	0.338	0.166	0.370	0.352	0.156											0.033	0.017	*nd*	0.032	*nd*					
FSV-BQ																									
FSV-BR																									
FSV-BS	>0.305	>0.143	>0.303	>0.293	>0.050	0.305	0.143	0.303	0.293	0.050						>0.018	>0.002	*nq*	>0.016	>0.006	0.018	0.002	*nd*	0.016	0.006
FSV-BT	0.260	0.117	0.253	0.246	0.061	0.239	0.105	0.225	0.225	0.054	0.021	0.012	0.028	0.021	0.008	0.023	0.012	0.011	0.020	0.014					
FSV-BU	0.364	0.173	0.357	0.336	0.078											0.027	0.015	0.012	0.027	0.014					
FSV-BV	0.351	0.189	0.373	0.323	0.075											0.026	0.014	0.010	0.024	0.011					
FSV-BW	0.354	0.168	0.371	0.305	0.072											0.023	0.009	*nd*	0.025	*nd*					
FSV-BX	>0.315	>0.162	>0.326	>0.316	>0.075	0.315	0.162	0.326	0.316	0.075						0.033	0.019	0.018	0.032	0.022					
FSV-CB	0.337	0.153	0.324	0.303	0.070											0.024	0.010	0.008	0.022	0.010					
FSV-CC																									
FSV-CD	0.266	0.153	0.354	0.268	0.056											0.032	0.016	0.013	0.035	0.013					
FSV-CE	0.331	0.167	0.327	0.303	0.067																				
FSV-CF																									
FSV-CG	0.333	0.161	0.349	0.301	0.074	0.307	0.142	0.303	0.275	0.065	0.026	0.019	0.046	0.026	0.008	0.030	0.015	0.014	0.028	0.015					
FSV-CI	>0.309	>0.182	>0.503	>0.327	>0.078	0.309	0.182	0.503	0.327	0.078						0.041	0.036	0.071	0.050	0.028					
FSV-CL	0.263	0.149	0.311	0.252	0.060											0.024	0.013	0.010	0.019	0.009					
FSV-CP																									
FSV-CR																									
FSV-CV	0.390	0.170	0.410	0.300	0.070											0.010	0.019	0.037	0.007	0.031					
FSV-CW	0.332	0.177	0.349	0.337	0.070	0.314	0.163	0.316	0.313	0.065	*0.018*	*0.014*	*0.033*	*0.024*	*0.005*	>0.028	>0.016	>0.015	>0.032	>0.013	0.028	0.016	0.015	0.032	0.013
FSV-CZ	0.290	0.190	0.300	0.300	0.120																				
FSV-DD																									
FSV-DF																									
FSV-DI	>0.266	>0.139	>0.284	>0.254	>0.025	0.266	0.139	0.284	0.254	0.025															
FSV-DQ	0.462	0.197	0.413	0.382	0.101											0.083	0.031	0.054	0.028	0.018					
FSV-DR	0.380	0.170	0.330	0.330	0.130																				
FSV-DU	>0.320	>0.170	>0.340	>0.290	>0.054	0.320	0.170	0.340	0.290	0.054															
FSV-DW	0.251	0.100	0.209	0.164	0.043	0.231	0.089	0.178	0.154	0.037	0.020	0.011	0.031	0.010	0.005										
FSV-EQ	0.309	0.147	0.290	0.272	0.059											0.023	0.009	0.008	0.020	0.007					
FSV-ET	0.330	0.160	0.330	0.310	0.070																				
FSV-FB	0.344	0.181	0.357	0.334	0.060	0.307	0.153	0.309	0.294	0.060	0.037	0.028	0.048	0.040	*nd*	0.026	0.013	0.006	0.021	0.008					
N	30	30	30	30	30	15	15	15	15	15	10	10	10	10	8	25	25	23	25	23	2	2	1	2	2
Min	0.251	0.100	0.209	0.164	0.043	0.231	0.089	0.178	0.154	0.025	0.015	0.005	0.026	0.010	0.001	0.010	0.009	0.006	0.007	0.007	0.018	0.002		0.016	0.006
Median	0.338	0.170	0.357	0.325	0.070	0.310	0.153	0.309	0.294	0.065	0.021	0.013	0.036	0.024	0.004	0.030	0.016	0.013	0.028	0.013	0.023	0.009	0.015	0.024	0.010
Max	0.462	0.210	0.420	0.382	0.156	0.353	0.184	0.503	0.332	0.078	0.037	0.028	0.055	0.040	0.008	0.083	0.036	0.071	0.050	0.031	0.028	0.016		0.032	0.013
SD	0.034	0.019	0.039	0.034	0.007	0.016	0.018	0.019	0.024	0.011	0.006	0.007	0.011	0.006	0.002	0.005	0.004	0.006	0.006	0.004					
CV	10	11	11	11	10	5	12	6	8	16	27	53	32	26	39	18	28	45	20	30					
N_{past}	32	31	34	32	31	14	15	14	15	10	10	9	10	10	7	26	27	25	28	20	0	0	0	0	0
$Median_{past}$	0.351	0.174	0.368	0.319	0.079	0.315	0.164	0.322	0.294	0.076	0.024	0.015	0.036	0.023	0.006	0.030	0.016	0.013	0.030	0.016					
SD_{past}	0.040	0.023	0.043	0.056	0.016	0.028	0.017	0.040	0.032	0.009	0.006	0.007	0.022	0.009	0.004	0.009	0.004	0.005	0.008	0.005					
NISTa	0.376	0.172	0.389	0.388	0.078																				
NISTb	0.393	0.181	0.397	0.373	0.070	0.365	0.171	0.348	0.344	0.070	*0.028*	*0.010*	*0.049*	*0.029*	*nd*	0.029	0.013	0.012	0.030	0.009	0.027	0.013	0.008	0.026	0.009
N_{NIST}	4	4	4	4	2	2	2	2	2	1						2	2	2	2	1	2	2	2	2	1
Mean	0.384	0.177	0.393	0.380	0.074	0.365	0.171	0.348	0.344	0.070						0.029	0.013	0.012	0.030	0.009	0.027	0.013	0.008	0.026	0.009
S_{rep}	0.014	0.016	0.016	0.014	0.005	0.005	0.008	0.003	0.008	0.001						0.001	0.003	0.004	0.001	0.000	0.001	0.003	0.002	0.001	0.000
S_{het}	0.013	0.004	0.008	0.015		0.006	0.003	0.000	0.009							0.004	0.002	0.000	0.002		0.001	0.001	0.000	0.002	
S_{anl}	0.011	0.006	0.006	0.011	0.005																				
S_{NIST}	0.022	0.017	0.019	0.023	0.007	0.008	0.009	0.003	0.012							0.004	0.003	0.004	0.002		0.001	0.003	0.002	0.002	
NAV	0.361	0.173	0.375	0.353	0.072	0.338	0.164	0.330	0.319	0.068	0.021	0.013	0.036	0.024		0.030	0.015	0.013	0.029	0.011					
NAU	0.059	0.027	0.057	0.061	0.014	0.050	0.020	0.042	0.047	0.011	0.007	0.007	0.013	0.008		0.010	0.006	0.006	0.009	0.006					

C3

Round Robin LI Laboratory Results

All Results in µg/mL

Lab	Total Lycopene					trans-Lycopene					Total β-Cryptoxanthin					Total α-Cryptoxanthin					Total Lutein					trans-Lutein				
	279	280	281	282	283	279	280	281	282	283	279	280	281	282	283	279	280	281	282	283	279	280	281	282	283	279	280	281	282	283
FSV-BA						0.190	0.159	0.244	0.175	0.271	0.063	0.080	0.044	0.059	0.064															
FSV-BB	0.323	0.295	0.437	0.305	0.429	0.164	0.137	0.207	0.152	0.223	0.051	0.068	0.037	0.049	0.052	0.023	0.018	0.015	0.022	0.024	0.094	0.054	0.063	0.090	0.079					
FSV-BD																														
FSV-BE																														
FSV-BF	0.397	0.344	0.562	0.370	0.559						0.051	0.068	0.037	0.044	0.040															
FSV-BG	0.350	0.334	0.557	0.309	0.447	0.178	0.181	0.293	0.174	0.254	0.066	0.095	0.049	0.063	0.078						0.098	0.066	0.086	0.101	0.099					
FSV-BH	0.377	0.341	0.526	0.347	0.477						0.074	0.104	0.060	0.070	0.088						0.083	0.045	0.051	0.078	0.065					
FSV-BI	0.319	0.278	0.447	0.290	0.400						0.059	0.080	0.037	0.053	0.051						0.110	0.069	0.071	0.103	0.091					
FSV-BJ	0.399	0.392	0.638	0.373	0.539						0.046	0.071	0.037	0.044	0.045						0.101	0.076	0.072	0.088	0.080					
FSV-BK																														
FSV-BL																														
FSV-BM																														
FSV-BN	0.345	0.308	0.481	0.304	0.514	0.197	0.178	0.257	0.168	0.290	0.042	0.062	0.014	0.030	0.032	0.016	*nd*	*nd*	*nd*	*nd*	0.083	0.056	0.054	0.078	0.062					
FSV-BNa	0.304	0.302	0.485	0.318	0.512	0.171	0.171	0.260	0.186	0.285	0.035	0.063	0.015	0.035	0.037	*nd*	*nd*	*nd*	*nd*	*nd*	0.091	0.057	0.049	0.078	0.072					
FSV-BO	0.336	0.251	0.463	0.266	0.482						0.053	0.065	0.037	0.048	0.050						0.076	0.037	0.046	0.074	0.058					
FSV-BP	0.386	0.322	0.438	0.476	0.514						0.052	0.073	0.046	0.059	0.063															
FSV-BQ																														
FSV-BR																														
FSV-BS						0.266	0.240	0.304	0.253	0.295	0.039	0.047	0.035	0.035	0.040						0.048	0.036	0.052	0.063	0.062					
FSV-BT	0.310	0.245	0.418	0.292	0.425	0.146	0.114	0.196	0.134	0.195	0.064	0.092	0.056	0.067	0.080	0.034	0.029	0.024	0.028	0.037	0.098	0.087	0.069	0.095	0.094					
FSV-BU	0.317	0.264	0.427	0.288	0.457						0.047	0.066	0.032	0.042	0.051															
FSV-BV	0.390	0.373	0.555	0.358	0.542						0.043	0.065	0.030	0.039	0.043															
FSV-BW	0.341	0.310	0.531	0.317	0.508																									
FSV-BX						0.142	0.129	0.219	0.157	0.251	0.053	0.068	0.036	0.048	0.054						0.088	0.054	0.058	0.089	0.083					
FSV-CB	0.301	0.239	0.392	0.271	0.401						0.051	0.069	0.036	0.047	0.053						0.092	0.052	0.066	0.084	0.089					
FSV-CC																														
FSV-CD	0.287	0.277	0.510	0.287	0.336						0.050	0.058	0.039	0.047	0.055															
FSV-CE																														
FSV-CF																														
FSV-CG	0.382	0.327	0.537	0.347	0.522	0.212	0.174	0.284	0.190	0.291	0.050	0.059	0.037	0.045	0.052						0.112	0.082	0.079	0.099	0.099					
FSV-CI																					0.075	0.046	0.062	0.062	0.063					
FSV-CL	0.343	0.297	0.508	0.303	0.465						0.057	0.071	0.034	0.047	0.051	0.028	0.017	0.013	0.024	0.026										
FSV-CP																														
FSV-CR																														
FSV-CV	0.310	0.290	0.520	0.310	0.460																									
FSV-CW	0.337	0.314	0.464	0.340	0.468	0.157	0.141	0.206	0.153	0.217	0.053	0.070	0.042	0.049	0.054	0.022	0.017	0.015	0.020	0.020	>0.074	>0.048	>0.048	>0.071	>0.058	0.074	0.048	0.048	0.071	0.058
FSV-CZ																														
FSV-DD																														
FSV-DF																														
FSV-DI						0.240	0.221	0.358	0.232	0.304											0.137	0.110	0.117	0.139	0.120					
FSV-DQ	0.387	0.454	0.288	0.570	0.786						0.051	0.076	0.040	0.048	0.053						0.094	0.047	0.064	0.085	0.089					
FSV-DR																														
FSV-DU																														
FSV-DW						0.136	0.101	0.161	0.104	0.151	0.065	0.087	0.061	0.046	0.078						0.079	0.058	0.074	0.074	0.075					
FSV-EQ	0.324	0.291	0.449	0.294	0.442						0.054	0.074	0.048	0.050	0.059															
FSV-ET																														
FSV-FB	0.289	0.259	0.422	0.281	0.407	0.251	0.226	0.367	0.244	0.362	0.047	0.065	0.031	0.045	0.047						0.086	0.060	0.060	0.082	0.071					
N	23	23	23	23	23	13	13	13	13	13	25	25	25	25	25	5	4	4	4	4	18	18	18	18	18	1	1	1	1	1
Min	0.287	0.239	0.288	0.266	0.336	0.136	0.101	0.161	0.104	0.151	0.035	0.047	0.014	0.030	0.032	0.016	0.017	0.013	0.020	0.020	0.048	0.036	0.046	0.062	0.058					
Median	0.337	0.302	0.481	0.309	0.468	0.178	0.171	0.257	0.174	0.271	0.051	0.069	0.037	0.047	0.052	0.023	0.017	0.015	0.023	0.025	0.092	0.057	0.063	0.085	0.079	0.074	0.048	0.048	0.071	0.058
Max	0.399	0.454	0.638	0.570	0.786	0.266	0.240	0.367	0.253	0.362	0.074	0.104	0.061	0.070	0.088	0.034	0.029	0.024	0.028	0.037	0.137	0.110	0.117	0.139	0.120					
SD	0.049	0.040	0.067	0.042	0.058	0.041	0.033	0.064	0.027	0.051	0.007	0.008	0.007	0.004	0.009	0.004					0.011	0.015	0.012	0.012	0.018					
CV	15	13	14	14	12	23	19	25	16	19	14	12	18	9	17	19					12	26	20	14	22					
N_{past}	27	26	28	28	23	12	12	13	12	8	25	27	25	27	19	5	5	5	6	7	15	16	16	15	12	0	0	0	0	0
$Median_{past}$	0.351	0.311	0.504	0.326	0.447	0.187	0.158	0.253	0.163	0.302	0.056	0.075	0.043	0.053	0.066	0.026	0.022	0.019	0.025	0.035	0.089	0.055	0.063	0.085	0.077					
SD_{past}	0.044	0.055	0.102	0.053	0.100	0.045	0.027	0.058	0.038	0.071	0.009	0.014	0.009	0.011	0.016	0.005	0.006	0.010	0.005	0.007	0.021	0.014	0.025	0.013	0.016					
NISTa											0.062	0.077	*nq*	0.068	0.061						0.105	0.064	0.075	0.107	0.080					
NISTb	0.403	0.347	0.569	0.379	0.532						0.060	0.064	0.039	0.056	0.056	0.029	0.028	0.020	0.035	*nd*	>0.085	>0.042	>0.067	>0.084	>0.070	0.085	0.042	0.067	0.084	0.070
N_{NIST}	2	2	2	2	1						4	4	2	4	2	1	1	1	1		4	4	4	4	3	2	2	2	2	1
Mean	0.403	0.347	0.569	0.379	0.532						0.061	0.071	0.039	0.062	0.058	0.029	0.028	0.020	0.035		0.095	0.054	0.071	0.096	0.058	0.085	0.042	0.067	0.084	0.070
S_{rep}	0.012	0.024	0.015	0.004	0.031						0.005	0.007	0.003	0.003	0.003	0.000		0.003	0.002		0.005	0.008	0.005	0.004	0.004	0.007	0.011	0.006	0.002	0.009
S_{het}	0.000	0.019	0.009	0.024							0.009	0.003	0.001	0.001							0.007	0.006	0.003	0.005	0.035	0.007	0.008	0.004	0.001	
S_{anl}											0.001	0.010		0.009	0.003						0.014	0.017	0.006	0.016	0.032					
S_{NIST}	0.012	0.031	0.017	0.024							0.011	0.012	0.003	0.009	0.005						0.016	0.019	0.008	0.017	0.048	0.010	0.013	0.007	0.002	
NAV	0.370	0.325	0.525	0.344	0.500	0.174	0.165	0.251	0.171	0.262	0.056	0.070	0.038	0.055	0.056	0.026					0.094	0.055	0.067	0.090	0.069					
NAU	0.088	0.076	0.118	0.086	0.108	0.035	0.033	0.059	0.030	0.051	0.014	0.016	0.009	0.016	0.013						0.018	0.019	0.014	0.019	0.050					

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Round Robin LI Laboratory Results

All Results in µg/mL

	Total Zeaxanthin					Total Lutein&Zeaxanthin					Coenzyme Q10					Phylloquinone (K1)				
Lab	279	280	281	282	283	279	280	281	282	283	279	280	281	282	283	279	280	281	282	283
FSV-BA						0.117	0.084	0.077	0.112	0.099										
FSV-BB	0.037	0.040	0.045	0.036	0.048	*0.131*	*0.094*	*0.108*	*0.126*	*0.127*										
FSV-BD																				
FSV-BE																0.530	0.800	0.410	0.520	0.560
FSV-BF						0.123	0.086	0.093	0.118	0.115										
FSV-BG	0.020	0.017	0.013	0.018	0.018	0.110	0.075	0.094	0.110	0.111										
FSV-BH	0.034	0.038	0.029	0.031	0.039	0.117	0.083	0.080	0.109	0.104										
FSV-BI	0.036	0.036	0.032	0.034	0.035	*0.146*	*0.105*	*0.103*	*0.137*	*0.126*										
FSV-BJ											0.748	0.587	0.883	0.635	0.743					
FSV-BK																				
FSV-BL																				
FSV-BM																				
FSV-BN	0.017	0.016	0.013	0.011	0.015	0.100	0.072	0.067	0.090	0.077										
FSV-BNa	0.017	0.022	0.017	0.018	0.016	0.108	0.079	0.065	0.097	0.088										
FSV-BO	0.027	0.060	0.055	0.027	0.039	0.103	0.097	0.101	0.101	0.097										
FSV-BP						0.109	0.098	0.089	0.112	0.108										
FSV-BQ																				
FSV-BR																				
FSV-BS	0.020	0.020	0.022	0.020	0.024	*0.068*	*0.056*	*0.074*	*0.083*	*0.086*										
FSV-BT	0.030	0.040	0.026	0.030	0.033	0.129	0.127	0.095	0.125	0.127										
FSV-BU						0.138	0.113	0.099	0.133	0.142										
FSV-BV						0.122	0.098	0.085	0.115	0.108										
FSV-BW																				
FSV-BX	0.028	0.032	0.023	0.025	0.030	*0.115*	*0.086*	*0.081*	*0.114*	*0.113*										
FSV-CB	0.027	0.034	0.026	0.025	0.032	*0.120*	*0.086*	*0.092*	*0.109*	*0.121*										
FSV-CC																				
FSV-CD						0.122	0.094	0.088	0.108	0.100										
FSV-CE																				
FSV-CF																				
FSV-CG	0.021	0.022	0.015	0.018	0.020	0.133	0.104	0.094	0.117	0.119										
FSV-CI	0.028	0.030	0.031	0.018	0.027	*0.103*	*0.076*	*0.093*	*0.080*	*0.090*						0.180	0.320	0.170	0.180	0.220
FSV-CL						0.098	0.079	0.074	0.090	0.091										
FSV-CP																				
FSV-CR																				
FSV-CV						0.160	0.102	0.101	0.150	0.120	1.280	1.030	1.290	1.200	1.040					
FSV-CW	0.031	0.036	0.028	0.030	0.034	>0.105	>0.084	>0.076	>0.101	>0.092	0.870	0.720	0.900	0.830	0.830					
FSV-CZ											0.970	1.200	1.200	1.000	0.940					
FSV-DD																				
FSV-DF																				
FSV-DI											0.398	0.495	0.868	0.515	0.626	0.590	0.670	0.480	0.410	0.520
FSV-DQ	0.027	0.029	0.030	0.024	0.028	*0.121*	*0.076*	*0.094*	*0.109*	*0.117*										
FSV-DR																				
FSV-DU																				
FSV-DW	0.041	0.048	0.055	0.036	0.059	*0.119*	*0.105*	*0.128*	*0.110*	*0.134*										
FSV-EQ						0.198	0.200	0.120	0.177	0.190										
FSV-ET																				
FSV-FB	0.023	0.028	0.022	0.023	0.029	0.109	0.098	0.082	0.105	0.100										
N	17	17	17	17	17	25	25	25	25	25	5	5	5	5	5	3	3	3	3	3
Min	0.017	0.016	0.013	0.011	0.015	0.068	0.056	0.065	0.080	0.077	0.398	0.495	0.868	0.515	0.626	0.180	0.320	0.170	0.180	0.220
Median	0.027	0.032	0.026	0.025	0.030	0.119	0.094	0.093	0.110	0.111	0.870	0.720	0.900	0.830	0.830	0.530	0.670	0.410	0.410	0.520
Max	0.041	0.060	0.055	0.036	0.059	0.198	0.200	0.128	0.177	0.190	1.280	1.200	1.290	1.200	1.040	0.590	0.800	0.480	0.520	0.560
SD	0.008	0.012	0.007	0.009	0.008	0.015	0.017	0.013	0.010	0.016	0.165	0.329	0.235	0.271	0.146					
CV	28	38	25	35	27	12	18	14	9	14	19	46	26	33	18					
N_{past}	14	15	12	14	14	23	26	22	26	17	6	0	8	10	0	0	0	0	0	0
$Median_{past}$	0.025	0.032	0.025	0.025	0.035	0.120	0.094	0.087	0.118	0.112	0.912		0.855	0.858						
SD_{past}	0.006	0.009	0.007	0.006	0.013	0.025	0.018	0.019	0.018	0.034	0.234		0.089	0.241						
NISTa																				
NISTb	>0.032	>0.034	>0.042	>0.036	>0.041	*>0.117*	*>0.075*	*>0.108*	*>0.121*	*>0.111*										
N_{NIST}	2	2	2	2	2	2	2	2	2	2										
Mean	0.032	0.034	0.042	0.036	0.020	0.117	0.075	0.108	0.121	0.055										
S_{rep}	0.002	0.008	0.002	0.001	0.001	0.009	0.017	0.004	0.003	0.007										
S_{het}	0.007	0.003	0.000	0.002	0.029	0.014	0.011	0.004	0.002	0.078										
S_{anl}																				
S_{NIST}	0.007	0.009	0.002	0.002	0.029	0.017	0.020	0.006	0.004	0.079										
NAV	0.030	0.033	0.035	0.031	0.026	0.118	0.085	0.101	0.116	0.084										
NAU	0.008	0.009	0.013	0.012	0.030	0.025	0.024	0.022	0.024	0.088										

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Round Robin LI Laboratory Results

All Results in µg/mL

Analytes Reported By One Laboratory

Analyte	Code	279	280	281	282	283
trans-β-Cryptoxanthin	NISTb	0.051	0.061	0.033	0.050	0.073
trans-Zeaxanthin	NISTb	0.032	0.034	0.042	0.036	0.043
Phytofluene	FSV-CL	0.027	0.028	0.042	0.029	0.041
Phytoene	FSV-CL	0.051	0.052	0.060	0.051	0.048

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.
nd	Not detected (i.e., no detectable peak for analyte)
nq	Detected but not quantitatively determined
<x	Concentration at or below the limit of quantification, x
italics	Not explictly reported but calculated by NIST from reported values

Round Robin LI Laboratory Results

Comparability Summary

Lab	TR	tR	RP	aT	g/bT	bC	tbC	aC	TLy	tLy	TbX	TLu	TZ	L&Z
FSV-BA	1		2	1	1	1	1	1		1	1	1	1	1
FSV-BB	1		1	1	1	1	1	1	1	1	1	1	2	1
FSV-BD	1			2										
FSV-BE	2			1	1	2								1
FSV-BF	1			1	1	1		1	1		1			
FSV-BG	1		1	1	1	1		1	1	1	2			1
FSV-BH	1			1	1	1	2	1	1		3	1	2	1
FSV-BI	1			1	1	1		1	1		1	1	1	1
FSV-BJ	1		2	1	1	1		1	1		1	1	1	1
FSV-BK	1			1										
FSV-BL	2			2								1		
FSV-BM	1			2										
FSV-BN	1		1	2	2	1	1	1	1	1	2			2
FSV-BNa	1		1	2	2	1	1	1	1	1	2			
FSV-BO	2			1		1		2	1		1			1
FSV-BP	1			1		4		1	2		1	1	1	1
FSV-BQ	1			1										1
FSV-BR		1		1								1	1	1
FSV-BT	1		1	3	3	2	2	1	1	2	2	1	2	1
FSV-BU	1			1	1	1		1	1		1	1	2	1
FSV-BV	1			1	1	1		1	1		1	1	1	1
FSV-BW	1		1	1	1	1		1	1					
FSV-BX		1		1	1		1	1		1	1			
FSV-CB	2			1		1		1	1		1	2	1	1
FSV-CC		1		1										
FSV-CD	1		1	1	1	1		1	1		1			
FSV-CE	1			1		1								1
FSV-CF	2			1										1
FSV-CG	1			2	1	1	1	1	1	1	1			1
FSV-CI	2		1	2	1		3	4						
FSV-CL	3			2	1	1		1	1		1			
FSV-CP				1	1									
FSV-CR	1			2										
FSV-CV	2		3	2	1	1		3	1					
FSV-CW		2	1	1	1	1	1		1	1	1	1	1	1
FSV-CZ	1			2		2						1	1	1
FSV-DD		2												
FSV-DF	1													
FSV-DI		2	1	1	1		2			2		1	2	1
FSV-DQ				2	2	2		4	3		1			3
FSV-DR	1			1		3								
FSV-DU	1			1			1					3		
FSV-DW	4			1		3	3			2	2		1	
FSV-EQ	2			2		1		1	1		1	1	1	1
FSV-ET	2			2	2	1								
FSV-FB	2	2		1		1	1	1	1	2	1			
NISTa	1	1		1	1	1					1	1		
NISTb	1	1		1	1	1	1	1	1		1			
n	40	9	13	46	27	32	15	26	24	12	26	18	16	24

	TR	tR	RP	aT	g/bT	bC	tbC	aC	TLy	tLy	TbX	TLu	TZ	L&Z
% 1	70	56	77	67	81	78	67	85	92	67	77	89	69	92
% 2	25	44	15	30	15	13	20	4	4	33	19	6	31	4
% 3	3	0	8	2	4	6	13	4	4	0	4	6	0	4
% 4	3	0	0	0	0	3	0	8	0	0	0	0	0	0

Label	Definition
Lab	Participant code
TR	Total Retinol
tR	trans-Retinol
RP	Retinyl palmitate
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
L&Z	Total Lutein&Zeaxanthin
n	number of participants providing quantitative data
% 1	Percent of CS = 1 (within 1 SD of medians)
% 2	Percent of CS = 2 (within 2 SD of medians)
% 3	Percent of CS = 3 (within 3 SD of medians)
% 4	Percent of CS = 4 (3 or more SD from medians)

"Comparability Score"

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

We define CS as follows:

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

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

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

NAU = NIST Assigned Uncertainty

For further details, please see

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

Appendix D. Representative "Individualized Report" for RR51

Each participant in RR51 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 RR51:

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

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

Individualized Round Robin LI Report: FSV-BA

Summary

	Serum 279			Serum 280			Serum 281			Serum 282			Serum 283		
Analyte	You	NAV	n	You	NAV	n	You	NAV	n	You	NAV	n	You	NAV	n
Total Retinol	0.470	0.470	38	0.858	0.868	38	1.132	1.031	38	0.452	0.463	38	0.494	0.483	38
Retinyl Palmitate	0.06	0.05	13	0.0	0.0	12	0.1	0.0	12	0.06	0.05	13	0.11	0.10	14
α-Tocopherol	7.15	7.20	44	7.39	7.52	44	27.36	27.16	44	6.77	6.91	44	7.21	7.14	44
γ/β-Tocopherol	1.964	1.983	25	3.885	3.789	25	6.161	6.097	25	1.869	1.882	25	2.357	2.358	25
Total β-Carotene	0.335	0.361	30	0.161	0.173	30	0.327	0.375	30	0.312	0.353	30	0.072	0.072	30
trans-β-Carotene	0.317	0.337	15	0.151	0.162	15	0.301	0.328	15	0.293	0.319	15	0.068	0.068	15
Total cis-β-Carotene	0.018	0.021	10	0.010	0.013	10	0.026	0.036	10	0.018	0.024	10	0.004		8
Total α-Carotene	0.028	0.030	25	0.013	0.015	25	0.010	0.013	23	0.026	0.029	25	0.011	0.011	23
trans-Lycopene	0.190	0.178	13	0.159	0.171	13	0.244	0.257	13	0.175	0.174	13	0.271	0.271	13
Total β-Cryptoxanthin	0.063	0.056	25	0.080	0.070	25	0.044	0.038	25	0.059	0.055	25	0.064	0.055	25
Total Lutein&Zeaxanthin	0.117	0.118	25	0.084	0.085	25	0.077	0.101	25	0.112	0.116	25	0.099	0.083	25

D2

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

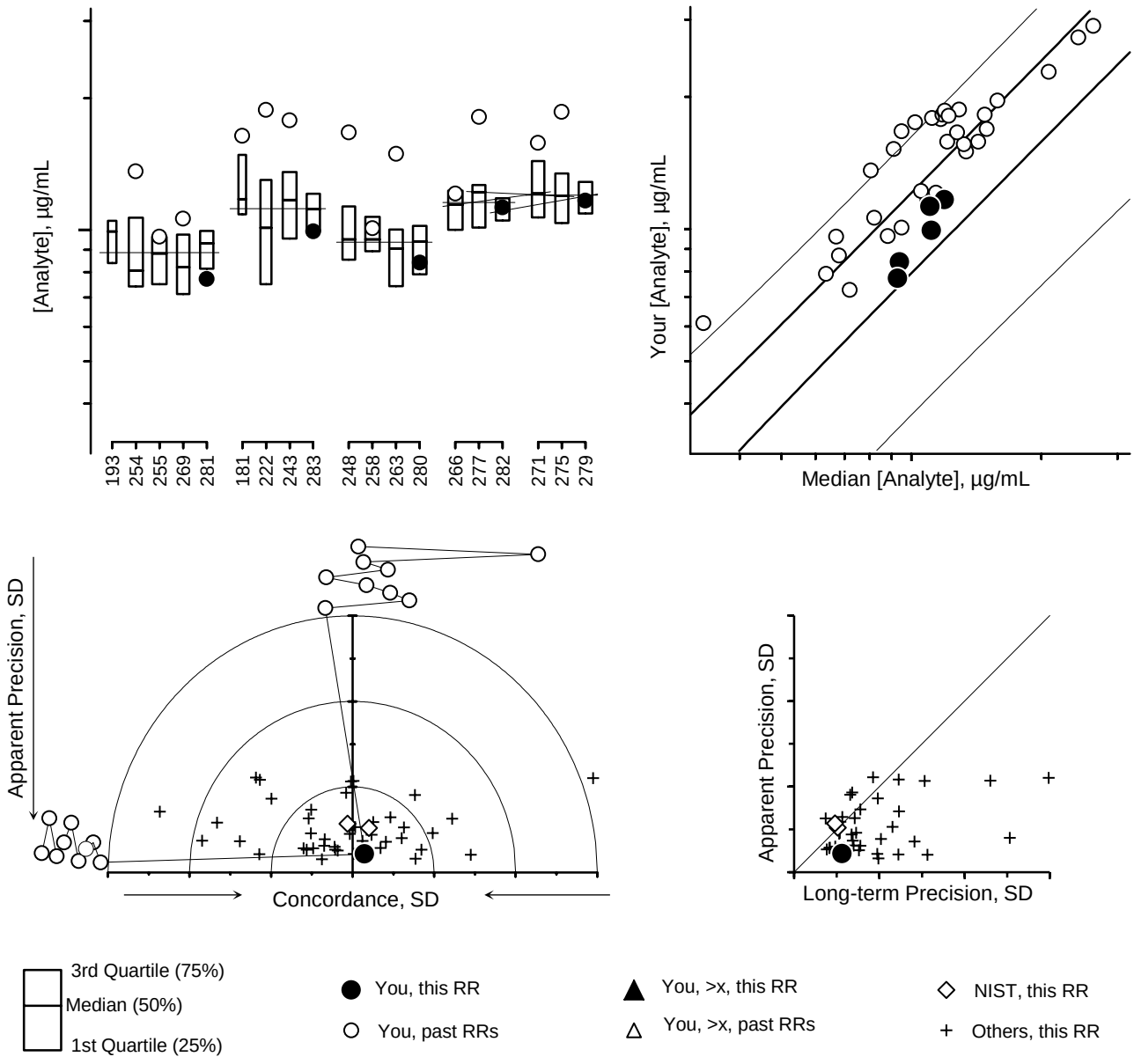
Please check our records against your records. Send corrections and/or updates to...

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 LI 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
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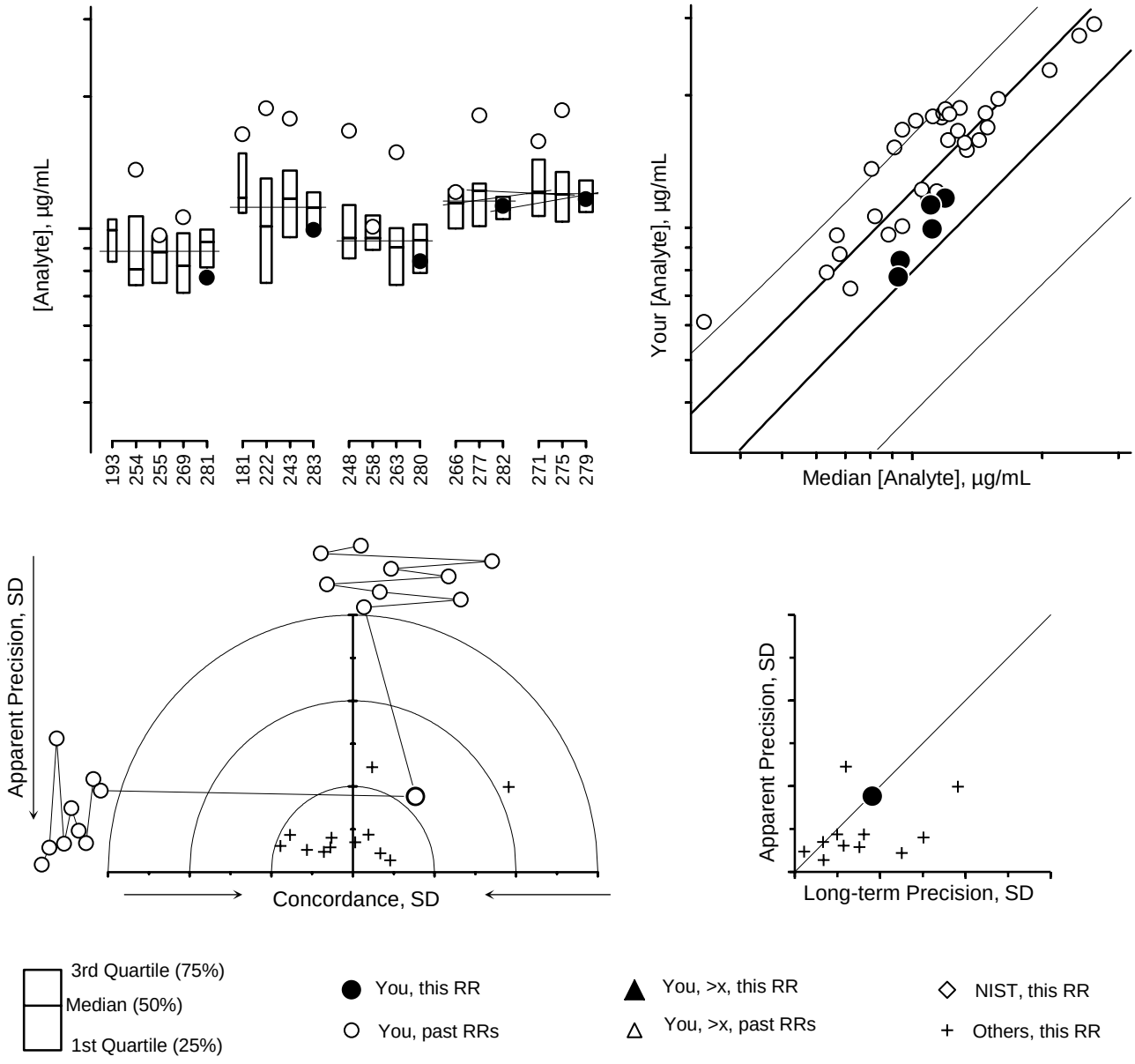
#280	#248 RR44, #258 RR46, #263 RR47	SRM 968c Lv I
#281	#193 RR30, #254 RR45, #255 RR46, #269 RR49	~30% of retinol as 13-cis isomer
#282	#266 RR48, #277 RR50	Lyophilized pair of #279
#283	#181 RR28, #222 RR37, #243 RR43	

Individualized RR LI Report: FSV-BA

Retinyl Palmitate



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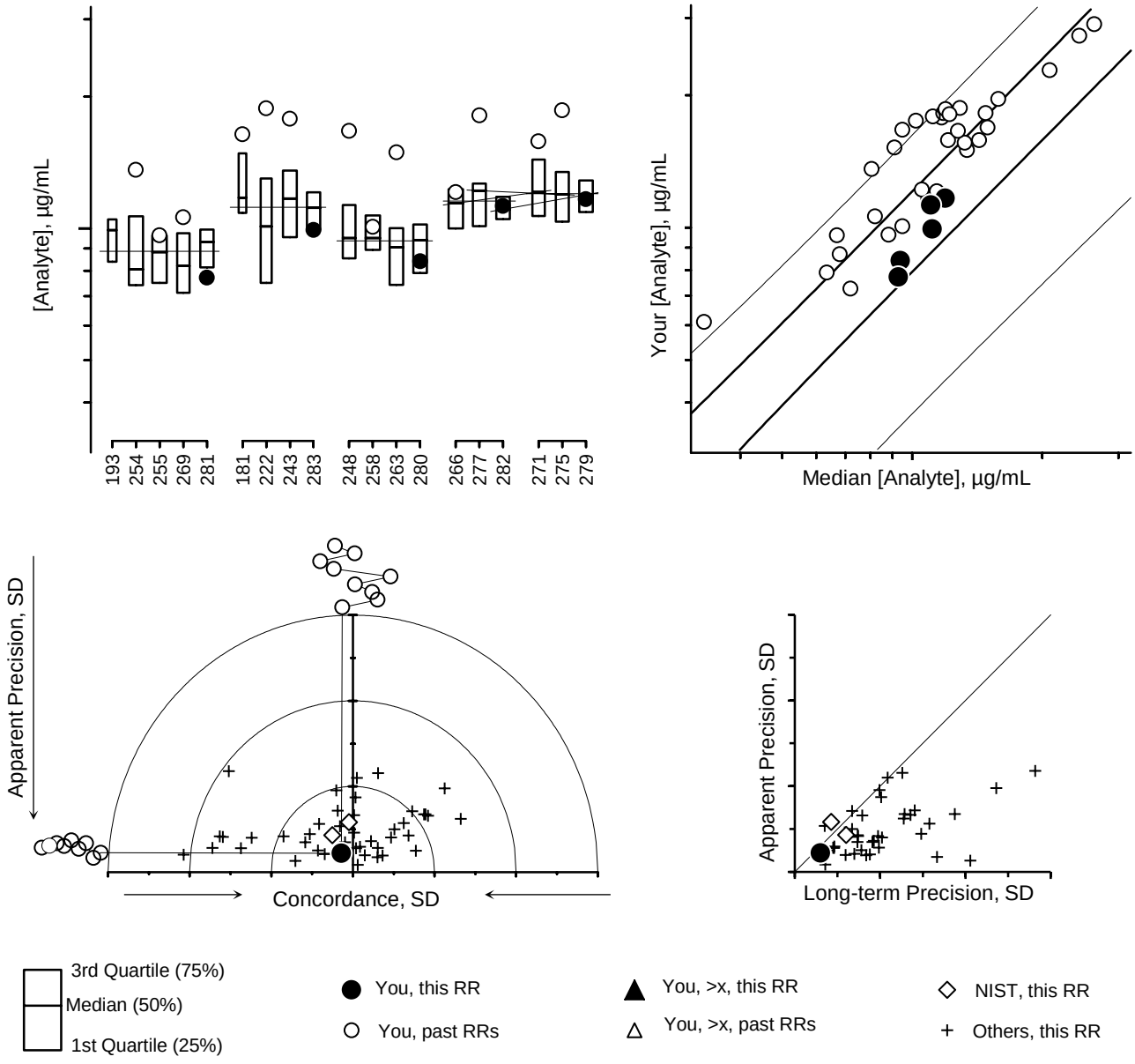
Individualized RR LI Report: FSV-BA

α-Tocopherol



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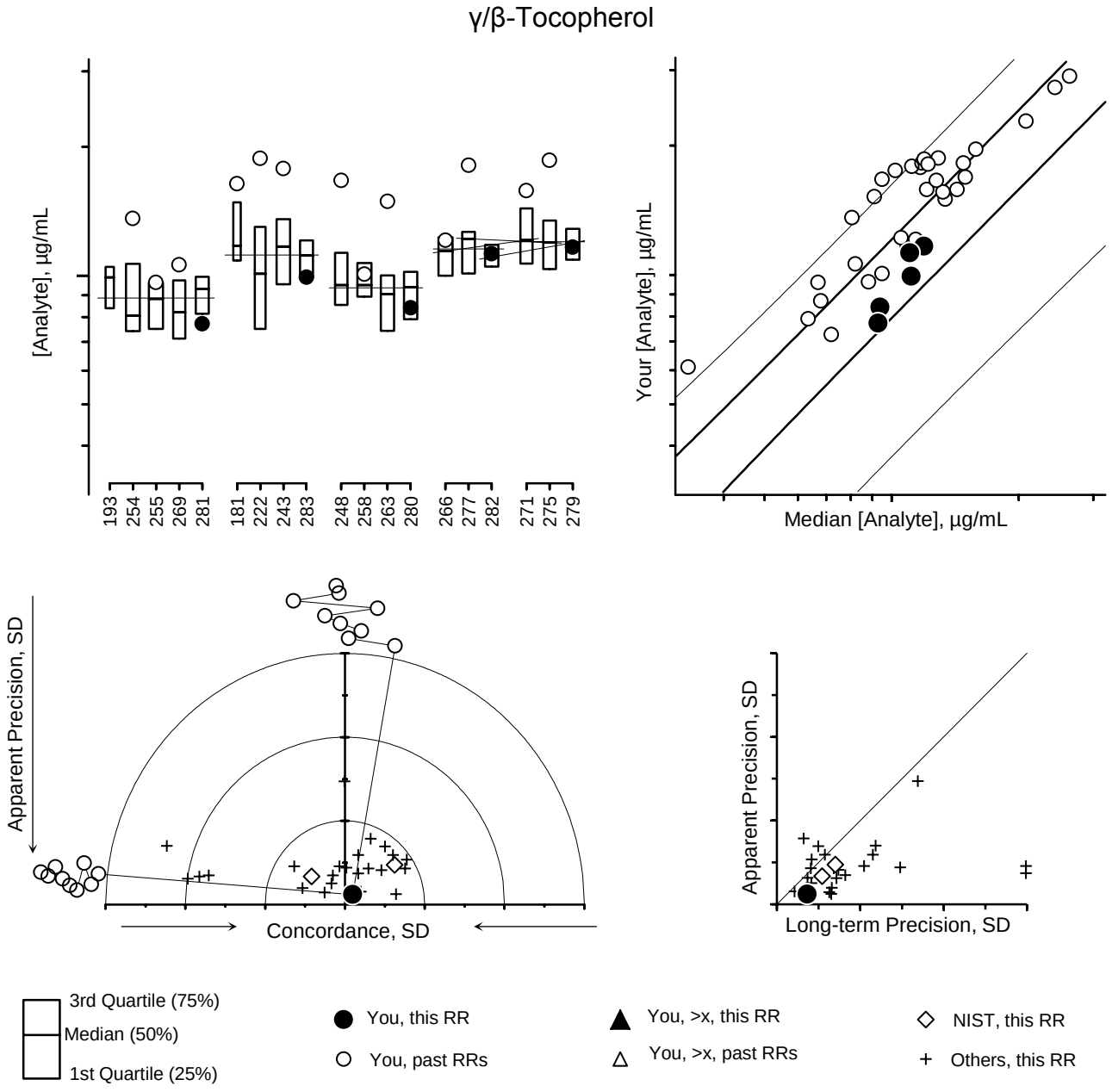
Individualized RR LI Report: FSV-BA



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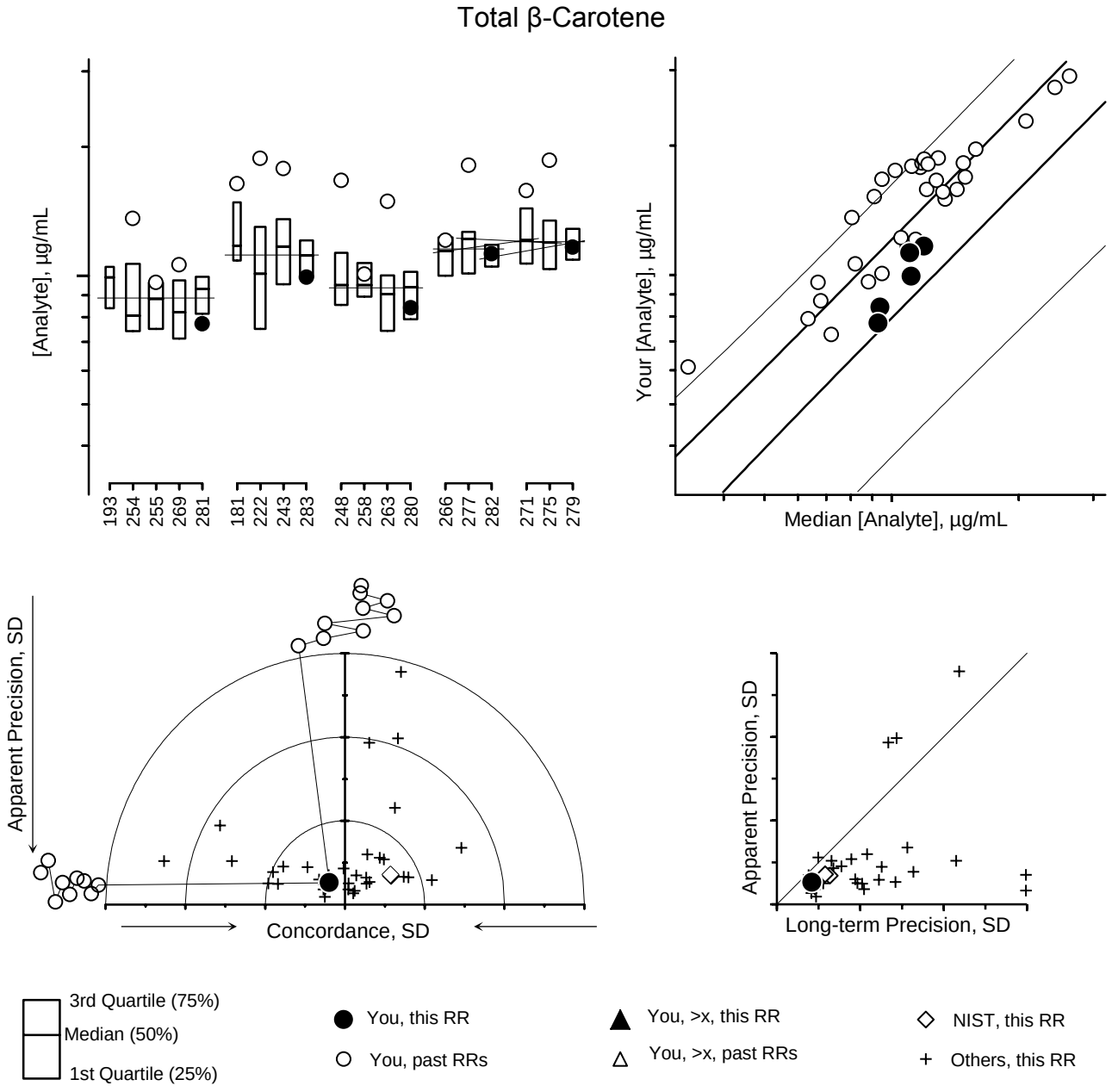
Individualized RR LI Report: FSV-BA



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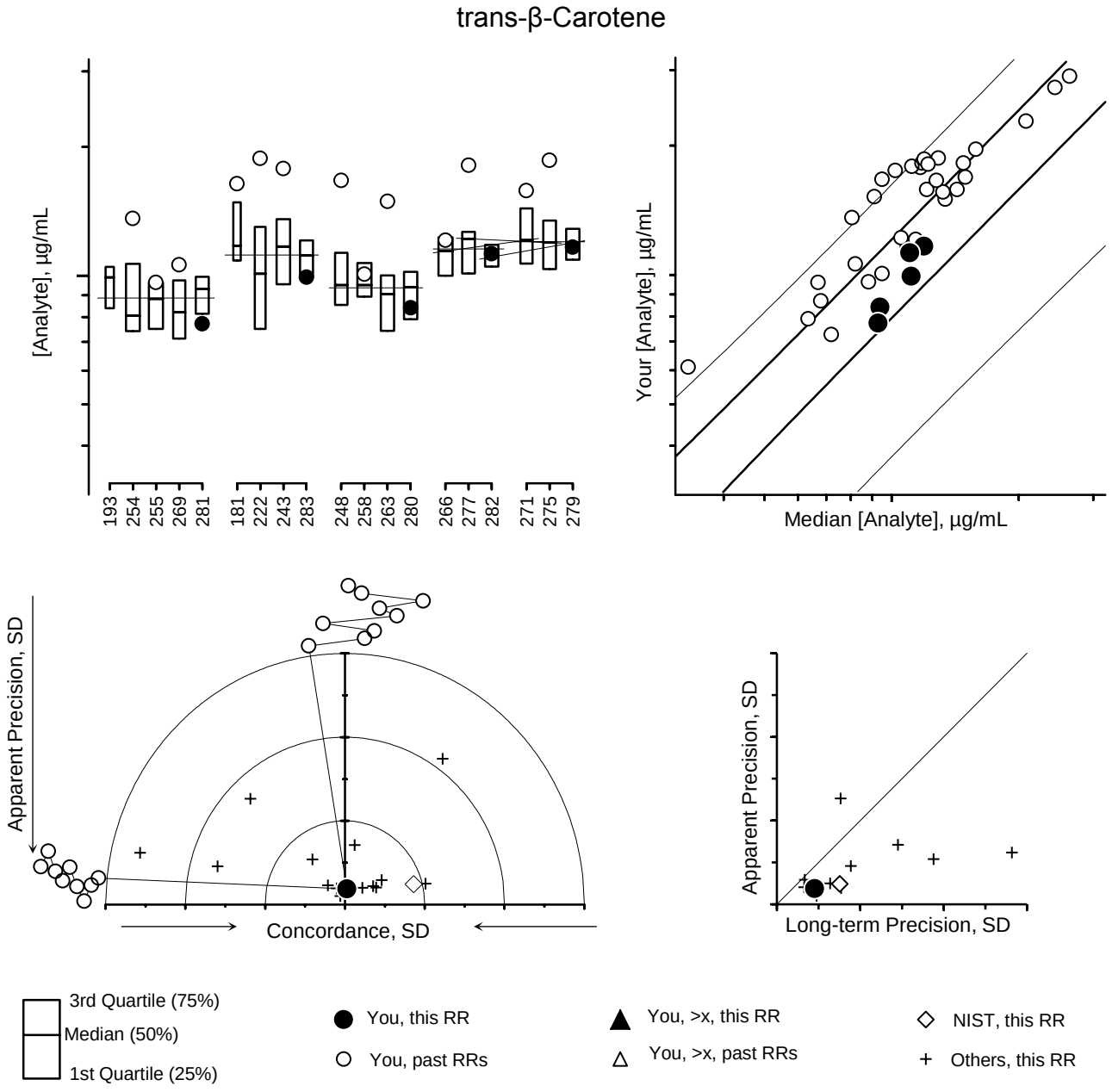
Individualized RR LI Report: FSV-BA



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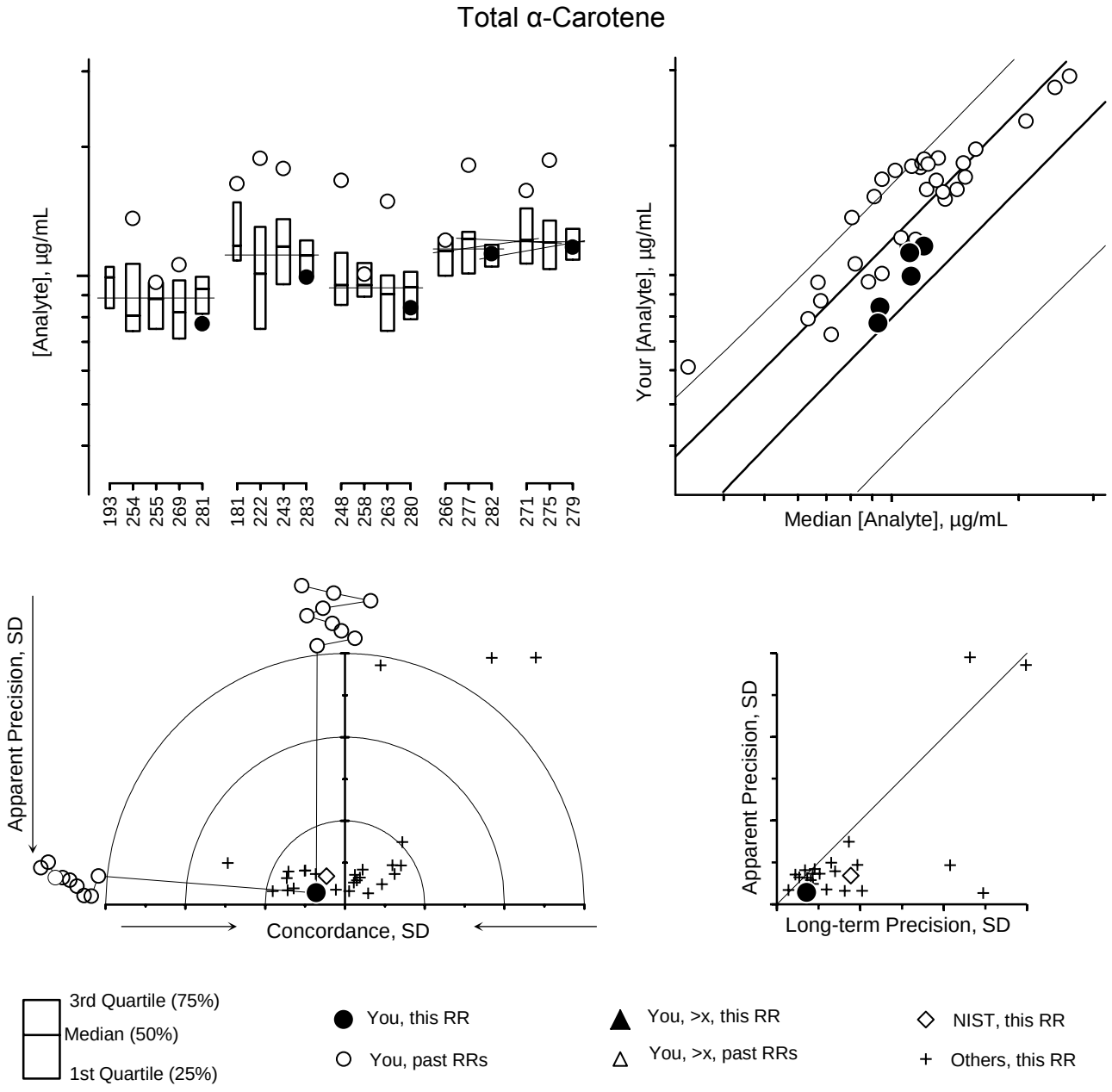
Individualized RR LI Report: FSV-BA



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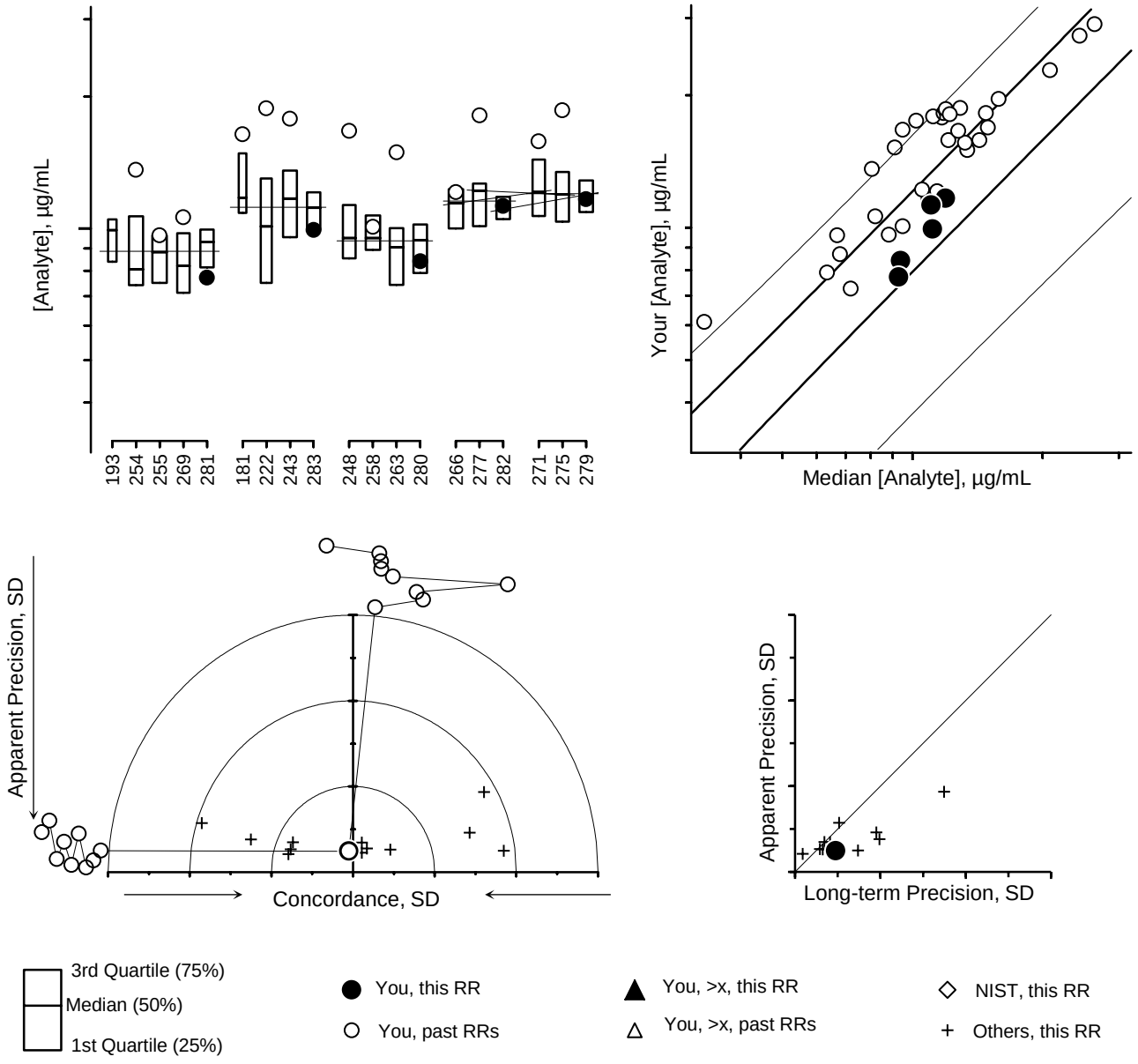
Individualized RR LI Report: FSV-BA

trans-Lycopene



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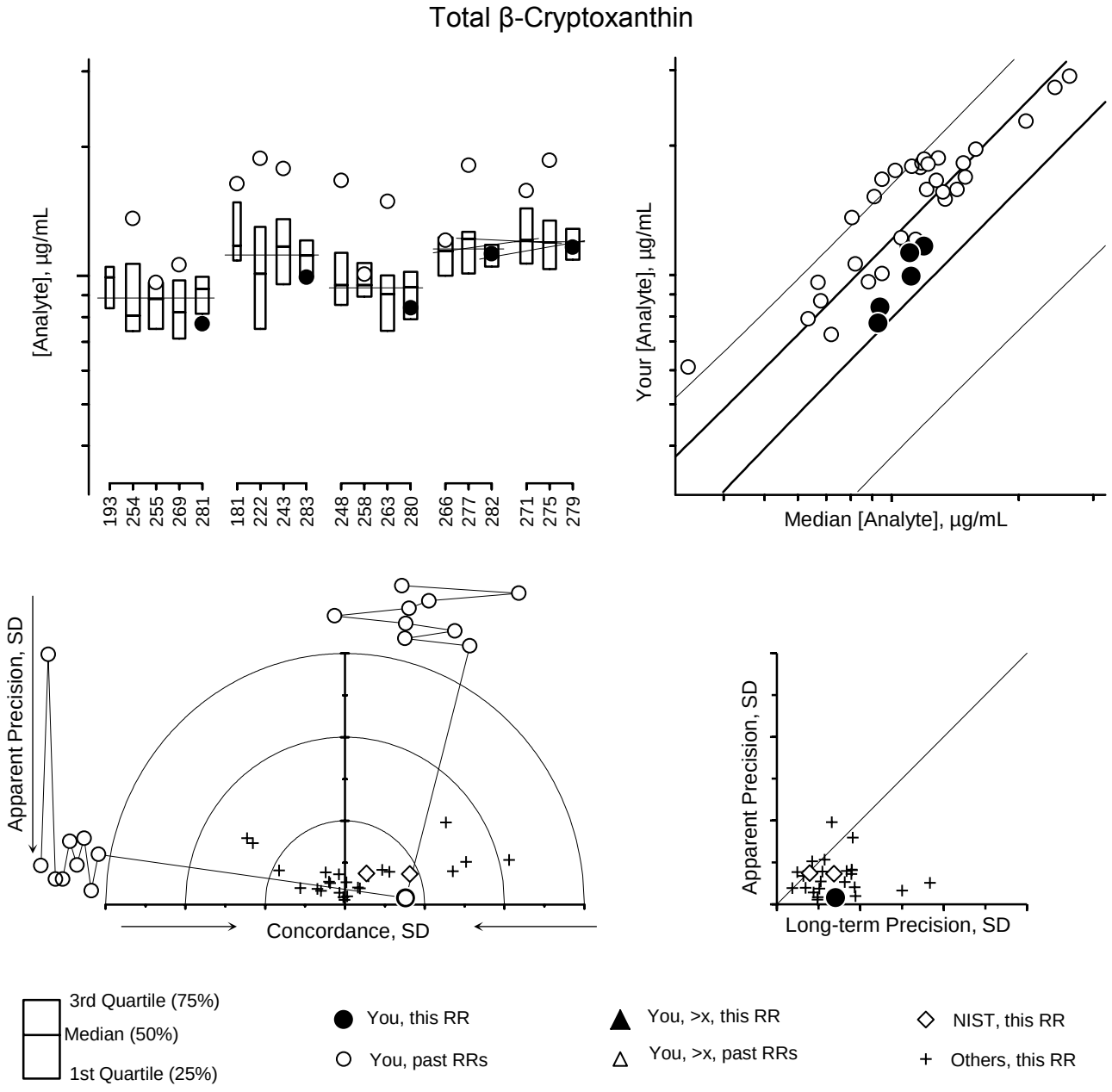
Individualized RR LI Report: FSV-BA



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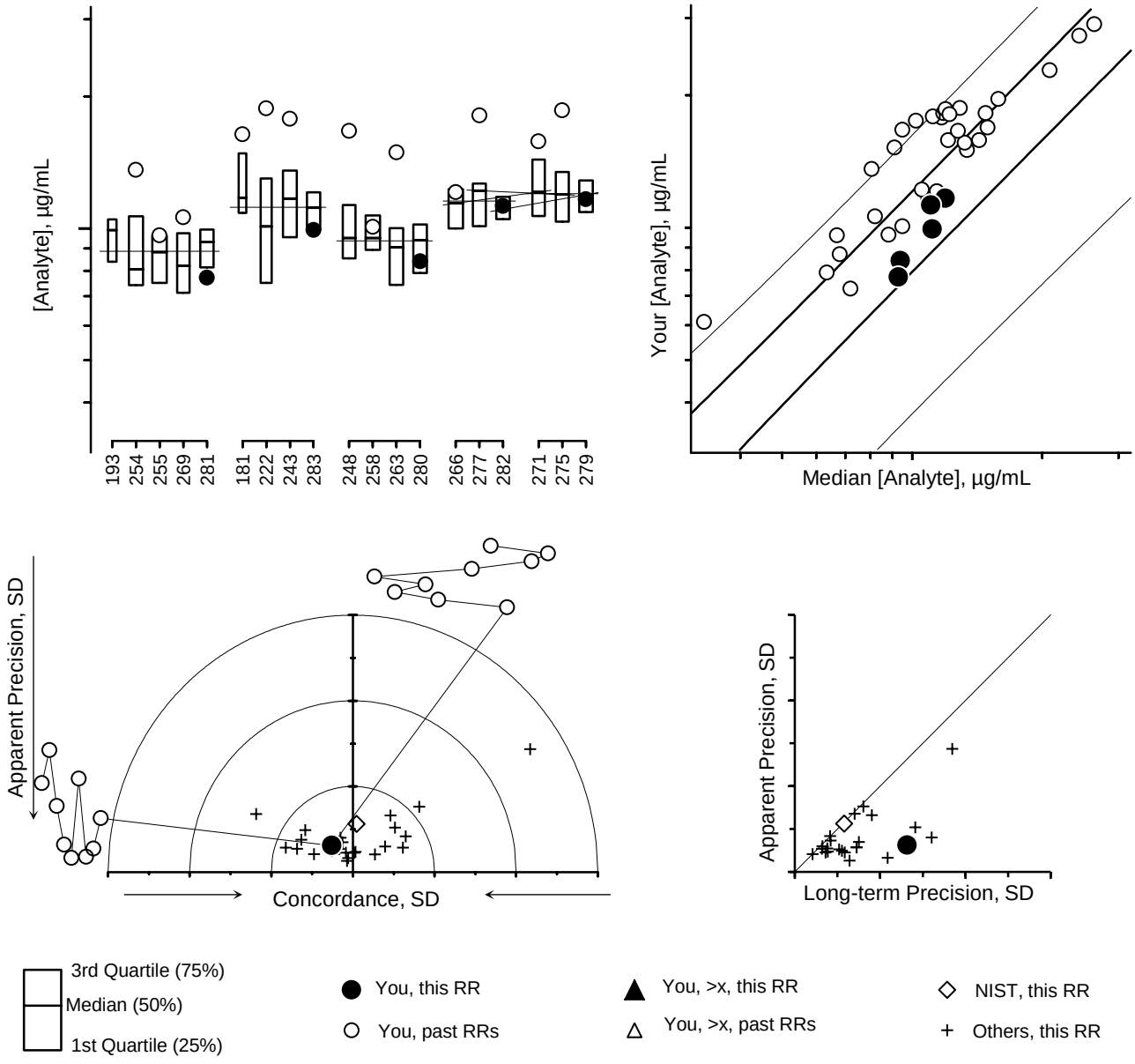
Individualized RR LI Report: FSV-BA

Total Lutein&Zeaxanthin



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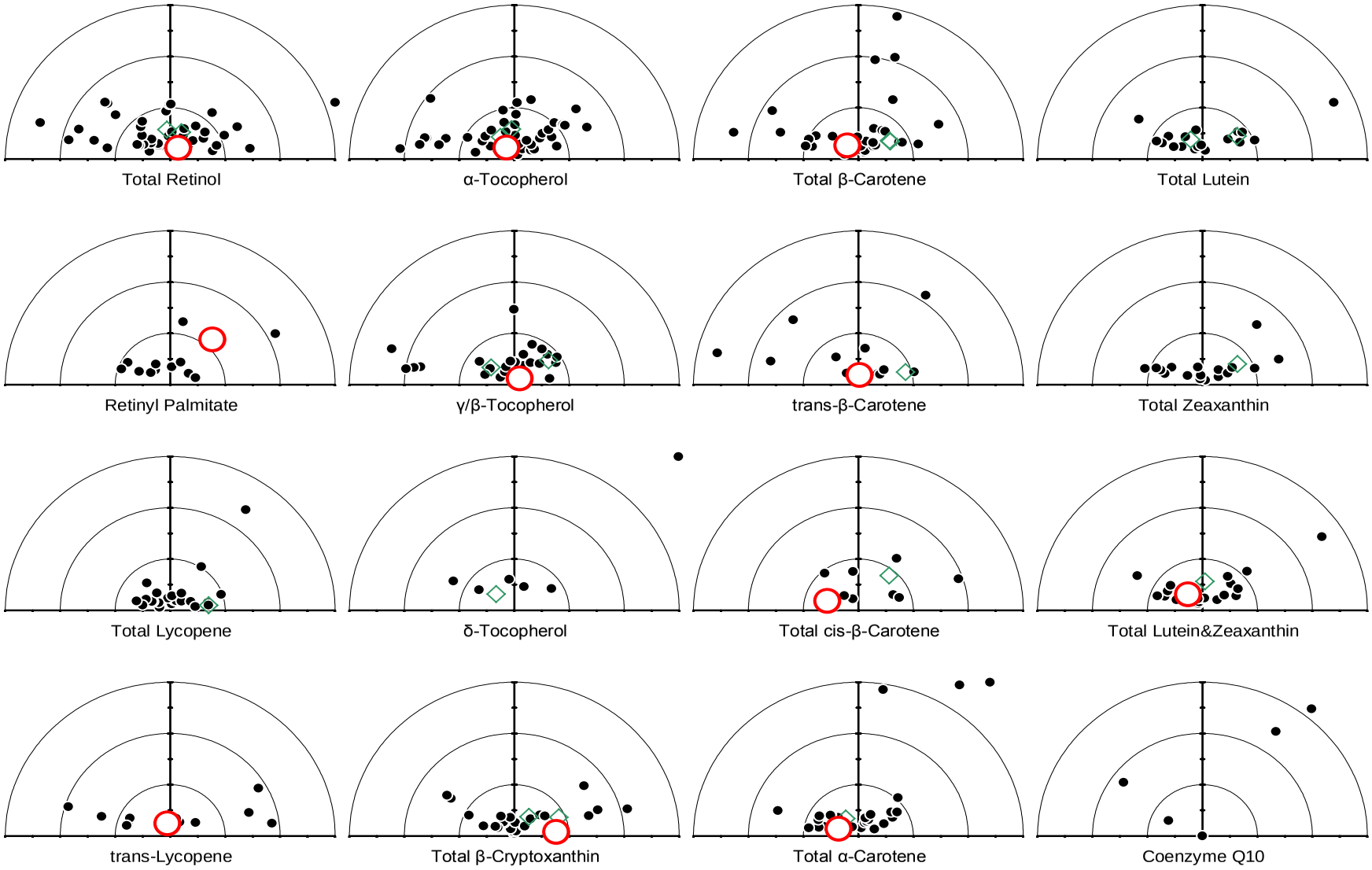
Individualized Round Robin LI Report: FSV-BA
Graphical Comparability Summary



D13

Appendix E. Shipping Package Inserts for RR16

The following five items were included in each package shipped to an RR16 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.

NIST CENTENNIAL 1901-2001

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

January 25, 2002

Dear Colleague:

The enclosed group of samples constitutes Vitamin C Round Robin 16 of the 2002 Micronutrients Measurement Quality Assurance Program. Three vials of frozen serum (*test samples)* and a vial of solid ascorbic acid (*control sample)* are enclosed. Please follow the attached protocol when you analyze these samples.

In RR15 we noted that several of the laboratories reported high blank values. We also noted that by correcting for the discrepancy between the assay values and the calculated values for the calibration solutions we were able to reduce the CV for the entire group by a factor of three. This would suggest that some laboratories need to examine their assay methods for errors due to high solvent blanks and that the standards should be prepared by weight as the calibration solutions are prepared. We hope that by drawing attention to these techniques we can improve the overall laboratory performance.

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)

Return your results using the attached form by **April 8, 2002**. We also request that you send us a representative chromatogram from the analysis of each sample and indicate whether peak height or peak area was used in the calculation of the ascorbic acid concentration. Your results will be kept confidential.

Please send your results to:

Micronutrients Measurement Quality Assurance Program
NIST
100 Bureau Drive, Stop 8392
Gaithersburg, MD 20899-8392

If you have any questions or concerns please call me at 301-975-3137, or contact me by Fax: 301- 977-0685 or e-mail: sam.margolis@nist.gov.

Thank you for your participation. We look forward to receiving your results.

Sincerely,

Sam Margolis, Ph.D.
Research Chemist
Analytical Chemistry Division
Chemical Science and Technology Laboratory

Enclosure

NIST

Protocol for analyzing samples

The *control sample* consists of a sample of solid ascorbic acid in an amber vial and should be used in the following manner (please record your mass on the attached report form):

1. Prepare 250 mL of 5% metaphosphoric acid (MPA) in distilled water.

2. Weigh **180-220 mg** of the solid ascorbic acid sample to 0.1 mg (if possible), dissolve it in 5% MPA in a 100 mL volumetric flask, and dilute to the 100 mL mark. **Weigh the amount of MPA solution that was added.** This will be referred to as the Stock Solution.

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

   Dilute Solution 1: **Weigh** 0.500 mL of the stock solution into a 100 mL volumetric flask. Then dilute with 5% MPA solution to 100 mL mark and **weigh the amount of MPA solution that was added**.

   Dilute Solution 2: **Weigh** 0.250 mL of the stock solution into a 100 mL volumetric flask. Then dilute with 5% MPA solution to 100 mL mark and **weigh the amount of MPA solution that was added**.

   Dilute Solution 3: **Weigh** 0.125 mL of the stock solution into a 100 mL volumetric flask. Then dilute with 5% MPA solution to 100 mL mark and **weigh the amount ofMPA solution that was added**.

4. Record the ultraviolet absorbance spectrum of *Dilute Solution 1* against 5% MPA solution as the blank using paired cuvettes. Record the wavelength in the region of 240-245 nm at which you observe the maximum absorbance and record the absorbance at that wavelength.

5. Record the absorbance of the sample at 242, 243 and 244 nm.

6. Measure the concentration of the ascorbic acid in all three **dilute solutions** and the **5% MPA diluent** in duplicate along with the ampouled *test samples* using your usual methods.

The purpose of measuring the absorbance at the wavelength maximum is to check the concentration of your sample. If your spectrophotometer is properly calibrated, the maximum absorbance should be between 243 and 244 nm. If the concentration is correct, the molar extinction coefficient ($E_1^{\%}$) of ascorbic acid at this wavelength (using a cell with a 1 cm path length) should be close to 550 ± 30 nm. The extinction coefficient of your solution can be calculated using the following equation:

$$E1\%\ dl/g\cdot cm = \frac{\text{Observed Absorbance}_{\lambda max}}{\frac{(\text{g AA}/100\text{ mL stock})(\text{g stock in 100 mL dilute solution})}{(\text{g AA stock solution}) + (\text{g MPA solution in 100 mL dilute solution 1})}}$$

The *test samples* are in sealed ampoules and 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 AA should be reported. The *test samples* sho_uld be defrosted by warming at 20 °C for not more than 10 min otherwise some irreversible degradation may occur.

Each *test sample* should contain between **0** and **100** µmol of ascorbic acid/L of solution. The total ascorbic acid in each ampoule should be measured in duplicate by the method(s) used in your laboratory. Please report your results in µg/L of sample.

REPORT OF ANALYSIS

NAME:
ADDRESS:

Method of Analysis:

Please note the type of method that you use. _____
Please attach representative chromatograms.
Method used for calculating ascorbic acid concentration.

Was SRM 970 used to validate your method or value-assign your in-house controls? ___
Peak height _____ Peak area _____
Manufacturer of ascorbic acid used to make in-house standards _____
Were samples frozen upon receipt? _____ Yes _____ No
Date of Analysis:_____

PREPARATION OF STOCK SOLUTION AND DILUTED SOLUTION

STOCK SOLUTION

Mass of ascorbic acid in the Stock Solution	_____ mg
Mass of 5% MPA added to the 100 mL volumetric flask	_____ g

DILUTE SOLUTION 1

Mass of added stock solution (0.5 mL)	_____ mg
Mass of 5% MPA 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
Wavelength of maximum absorbance	_____ nm
Calculated molar absorptivity	_____ dL/g·cm

DILUTE SOLUTION 2

Mass of added stock solution (0.250 mL)	_____ mg
Mass of 5% MPA added to the 100 mL volumetric flask	_____ g

DILUTE SOLUTION 3

Mass of added stock solution (0.125 mL)	_____ mg
Mass of 5% MPA added to the 100 mL volumetric flask	_____ g

COMMENTS: (use other side if necessary)

REPORT OF ANALYSIS

RESULTS (µmol/L of Sample)

DILUTE SOLUTION 1

REPLICATE 1 _____µmol/L of dilute solution 1
REPLICATE 2 _____ µmol/L of dilute solution 1

DILUTE SOLUTION 2

REPLICATE 1 _____µmol/L of dilute solution 2
REPLICATE 2 _____ µmol/L of dilute solution 2

DILUTE SOLUTION 3

REPLICATE 1 _____µmol/L of dilute solution 3
REPLICATE 2 _____ µmol/L of dilute solution 3

5% MPA SOLUTION (DILUENT)

REPLICATE 1 _____µmol/L of diluent
REPLICATE 2 _____ µmol/L of diluent

TEST SAMPLE #1

REPLICATE 1 _____µmol/L of Sample 1
REPLICATE 2 _____ µmol/L of Sample 1

TEST SAMPLE #14

REPLICATE 1 _____µmol/L of Sample 14
REPLICATE 2 _____ µmol/L of Sample 14

TEST SAMPLE #53

REPLICATE 1 _____µmol/L of Sample 53
REPLICATE 2 _____ µmol/L of Sample 53

Return by **April 8, 2002** to:
Micronutrients Measurement Quality Assurance Program
NIST, 100 Bureau Drive, Stop 8392
Gaithersburg, MD 20899-8392

Fax: 301-977-0685 Micronutrients
E-mail: sam.margolis@nist.gov

Participant #: _____ Date: _____

Vitamin C Round Robin 16

NIST Micronutrients Measurement Quality Assurance Program

Packing List and Shipment Receipt Confirmation Form

This box contains (we hope) one vial each of the following **four** VitC M^2QAP samples:

Sample	Form
VitC:1	Liquid frozen (1:1 serum:10% MPA)
VitC:11	Liquid frozen (1:1 serum:10% MPA)
VitC:51	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:1, VitC:11, and VitC:51 arrived frozen
4) Store the samples upright 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:1, VitC:11, and VitC:51 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 will help control M^2QAP expenses.

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 RR16

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

June 19, 2002

Dear Colleague:

Enclosed is the summary report of the results for Round Robin 16 (RR16) 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.

RR16 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.

As a reminder, please return the intent-to-participate forms for the 2003 QA program to us by **no later than September 1, 2002.** 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.

Please contact us if you have questions.

Sincerely,

Jeanice Brown Thomas
Research Chemist
Analytical Chemistry Division
Chemical Science and Technology Laboratory
Phone: 301-975-3120
E-mail: jbthomas@nist.gov
Fax: 301-977-0685

Sam A. Margolis, Ph.D.
Research Chemist
Analytical Chemistry Division
Chemical Science and Technology Laboratory
Phone: 301-975-3137
E-mail: sam.margolis@nist.gov
Fax: 301-977-0685

Enclosures

NIST

The NIST M^2QAP Vitamin C Round Robin 16 (RR16) 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, the SRM 970 Level 1 test samples distributed in RR11 through RR16, and for two recently prepared test samples.
2	Graphical summary of your RR 16 sample measurements.
3	Graphical summary of your RR 16 control solution measurements.

Page	"All Lab" Report
1	A listing of all results and statistics for Total Ascorbic Acid [TAA] in the RR16 samples and control solutions, the density of the 5% metaphosphoric acid (MPA) used to prepare the control solutions, the maximum absorbance reported between 243 nm and 245 nm for control solution #1, and the molar extinction coefficient.

Test Samples. Three unknowns were distributed in RR16.

S16:1 SRM 970 Level 1

S16:2 Serum 11, a "blank" prepared from an unaugmented serum pool.

S16:3 Serum 51, prepared by augmentation with solid, high-purity ascorbic acid from a serum pool.

Qualitative Observations.

1) Nearly all participants successfully prepared the four (three "Dilute Solutions" and the 5% MPA "Diluent") control solutions. The criteria used to evaluate this success are: the weight of 100 mL of 5% MPA (it should be about 103 gm), the observed wavelength maximum, the observed absorbance at that maximum, and the calculated $E^{1\%}$ of "Dilute Solution #1" (they should be: 243 to 244 nm, 0.5 to 0.6 OD, and 530 to 590 dL/g·cm), and the extent of correlation between the expected and observed [TAA] for the four materials (R^2 as close to 1.0 as possible).

2) Two participants initially reported results for the three Dilute Solutions that differed significantly from those expected from the material weights. Starting with RR17, we are asking that you calculate the expected [TAA] for all control solutions and compare them to your measured results. We ask that you analyze the test samples only *after* you are satisfied that your measurement system is performing properly.

3) There has been a general improvement in measurement system calibration, as judged by the Calibration Parameters (intercepts close to 0.0 and slopes close to 1.0) calculated from the control samples. For the first time, the overall among-participant agreement (concordance) was *not* improved by "correcting" the reported test sample results with the observed Calibration Parameters.

Quantitative Results

1) There is no sign of degradation (change in median [TAA] or increase in estimated standard deviation) in SRM 970 Level 1 since their Certification in 1998. We will continue to periodically monitor these materials.

Appendix G. "All-Lab Report" for RR16

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.

NIST Micronutrients Measurement Quality Assurance Program for Total Ascorbic Acid
"Round Robin" 16 - March 2002

		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	[Dil1]	[Dil2]	[Dil3]	MPA	Ctrl:1	Ctrl:2	Ctrl:3	Ctrl:4	Inter	Slope	R^2	SEE	g/mL	λ_{max}	A_{max}	$E^{1\%}$	S16:1	S16:2	S16:3	S16:1	S16:2	S16:3
VC-MA	18/11/02	56.7	28.3	13.8	0	60.9	28.9	14.6	0.0	-0.35	1.07	0.999	0.9	1.032	242.	0.5754	576.5	8.8	0.0	47.4	8.6	0.4	44.5
VC-MB	08/02/02	58.1	29.1	14.7	0	55.5	25.1	13.2	1.4	-0.03	0.94	0.995	2.0	1.033	245.	0.6023	589.0	9.4	1.3	48.3	10.0	1.4	51.6
VC-MC	04/02/02	56.4	28.1	14.1	0	57.7	27.7	14.3		-0.56	1.03	0.999	0.8	1.032	243.	0.5715	575.0	6.7	1.4	44.3	7.1	1.9	43.6
VC-ME	05/04/02	55.4	28.4	14.4	0	55.4	28.2	13.8	0.0	-0.28	1.00	1.000	0.3	1.032	243.6	0.5648	578.6	-	*nq*	12.4[a]			12.6[a]
VC-MG	26/03/02	55.7	28.5	14.3	0	50.7	25.5	12.7	0.0	-0.24	0.91	1.000	0.3	1.033	243.7	0.533	543.3	12.4	*nq*	48.2	13.8		53.1
VC-MH	11/03/02	56.0	27.8	13.8	0	55.4	27.5	13.8	0.0	0.05	0.99	1.000	0.0	1.033	243.	0.553	560.7	7.6	0.1	47.0	7.6	0.1	47.5
VC-MI	26/03/02	56.7	28.6	13.9	0	61.5	29.5	13.9	0.0	-0.77	1.09	0.999	1.0	1.031				7.5	0.8	47.8	7.6	1.4	44.6
VC-ML	10/06/02	59.1	29.5	14.8	0	57.7	35.9	15.9	0.0	2.00	0.98	0.982	4.1	1.034	242.	0.5907	567.6	7.1[b]	*nq*	33.2[b]	5.2[b]		31.8[b]
VC-MM	10/06/02																	6.4[b]	*nq*	38.0[b]	4.4[b]		36.6[b]
VC-MO	04/04/02	56.3	28.4	14.0	0	67.1	26.0	15.3	0.0	-2.07	1.18	0.983	4.6	1.038	242.	0.5269	531.0	8.0	0.4	42.1	8.5	2.1	37.4
VC-MQ	23/04/02	2.8	5.6	11.2		2.7	5.6	12.2	0.0	-0.21	1.09	0.998	0.3		243.4	0.520	518.7	9.4	1.7	44.2	8.8	1.8	40.7
VC-MR	26/02/02	56.6	28.9	14.0	0	59.0	35.0	17.0	0.0	1.93	1.04	0.991	2.9	1.032	244.	0.557	558.6	9.0	*nq*	55.0	6.8		51.1
VC-MT	05/04/02	55.2	27.7	14.2	0	44.0	23.7	12.9	2.1	2.23	0.76	1.000	0.3	1.031	244.	0.578	594.8	nq	*nq*	*nq*			
VC-MY	08/04/02	56.5	27.1	14.0	0	83.9[c]	74.1[c]	75.0[c]	83.5[c]					1.107[c]	244.	1.220	1226	11.6	*nq*	185.7			
VC-NH	19/02/02	52.6	26.2	13.1	0	51.2	25.7	12.9		0.08	0.97	1.000	0.1	1.032	243.	0.5231	564.4	7.7	<0.4	43.6	7.8		44.7
VC-NI	08/04/02	55.6	27.8	13.9	0	47.9	25.0	12.6	0.0	0.45	0.86	0.999	0.6	1.023	243.	0.531	542.7	10.6	*nq*	52.0	11.7		59.9
NIST	27/02/02	54.4	27.3	13.5	0	54.6	26.0	14.7	0.0	0.20	0.99	0.998	1.2	1.031	243.	0.5381	561.3	8.6	0.3	47.4	8.4	0.1	47.6
	N					15	15	15	13					14	15	15	15	13	8	13	12	8	12
	Average					52.5	26.5	14.0	0.3					1.032	243.2	0.6015	606.9	9.0	0.7	57.9	8.9	1.2	46.9
	SD					14.9	6.7	1.4	0.7					0.003	0.9	0.1730	172.6	1.7	0.7	38.5	2.0	0.8	6.1
	Min					2.7	5.62	12.2	0.0					1.023	242.0	0.5200	518.7	6.7	0.0	42.1	6.8	0.1	37.4
	%25					51.0	25.27	12.9	0.0					1.032	242.5	0.5320	551.0	7.7	0.1	44.3	7.6	0.4	44.3
	Median					55.5	27.50	13.8	0.0					1.032	243.0	0.5648	567.6	8.8	0.6	47.4	8.5	1.4	44.7
	%75					59.9	28.86	14.6	0.0					1.033	243.9	0.5767	577.6	9.4	1.3	48.3	9.1	1.8	51.2
	Max					67.1	35.90	17.0	2.1					1.038	245.0	1.2200	1226	12.4	1.7	185.7	13.8	2.1	59.9
	eSD					7.0	2.89	1.3	0.0					0.001	1.5	0.0384	16.3	1.7	0.8	4.6	1.4	0.9	5.0
	CV					13	11	10						0		7	3	20		10	17		11

a Suspect sample; data not included in statistical summaries.
b Samples thawed in shipment; data not included in statistical summaries.
c Contol sample preparation error; data not included in statistical summaries.

Appendix H. Representative "Individualized Report" for RR16

Each participant in RR16 received an "Individualized Report" reflecting their reported results. The following two pages are the "Individualized Report" for participant "VC-MA".

Vitamin C 'Round Robin' 16 Report: Participant VC-MA

Date	Method	RR	Control	AA mg	MPA g	Stock mg	MPA g	[AA] mmol/L Calc	[AA] mmol/L Obs	A242 OD	A243 OD	A244 OD	A245 OD	E1%max dL/gcm
09/23/98	HPLC-EC (Height)	11	55 mmol/L	200.0	103.09	526.0	102.45	57.8	6.1		0.0525	0.0527		
04/02/99	HPLC-EC (Height)	12	55 mmol/L	215.0	103.10	517.1	102.52	61.1	53.1		0.0721	0.0721		
09/17/01	HPLC-EC (Height)	13	55 mmol/L	200.8	103.16	508.0	102.58	56.0	55.4		0.5650	0.5232		572
09/27/01	HPLC-EC (Height)	14	55 mmol/L, Crtl:1	200.3	103.23	510.0	102.31	56.1	57.1		0.5409	0.5193		548
09/18/01	HPLC-EC (Height)	15	55 mmol/L, Crtl:1	200.5	103.12	506.0	102.19	55.8	57.9	0.5461	0.5465	0.5461		557
11/18/02	HPLC-EC (Height)	16	55 mmol/L, Crtl:1	200.0	103.00	515.0	102.65	56.7	60.9	0.5754	0.5449	0.5465		577
														563 ±14

[TAA] mmol/Lsample

RR	Sample	Rep1	Rep2	*Factor*	Mean	SDdup
11	SRM Lv 1, A	15.5	13.9	0.5	7.4	0.6
11	SRM Lv 1, B	14.0	14.5	0.5	7.1	0.2
12	SRM Lv 1, A	14.5	15.8	0.5	7.6	0.5
12	SRM Lv 1, B	16.1	15.1	0.5	7.8	0.3
13	SRM Lv 1, S13-1	8.4	8.5	1.0	8.5	0.1
14	SRM Lv 1, S14-3	8.0	7.7	1.0	7.8	0.2
15	SRM Lv 1, S15:1	8.9	8.7	1.0	8.8	0.1
16	SRM Lv 1, S16:1	8.8	8.8	1.0	8.8	0.0
16	S16:2, Serum 11	0.0	0.1	1.0	0.0	0.0
16	S16:3, Serum 51	49.9	44.9	1.0	47.4	3.5

Grand Average

Mean	SDrepeat	SDreprod
8.1	0.3	0.6

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

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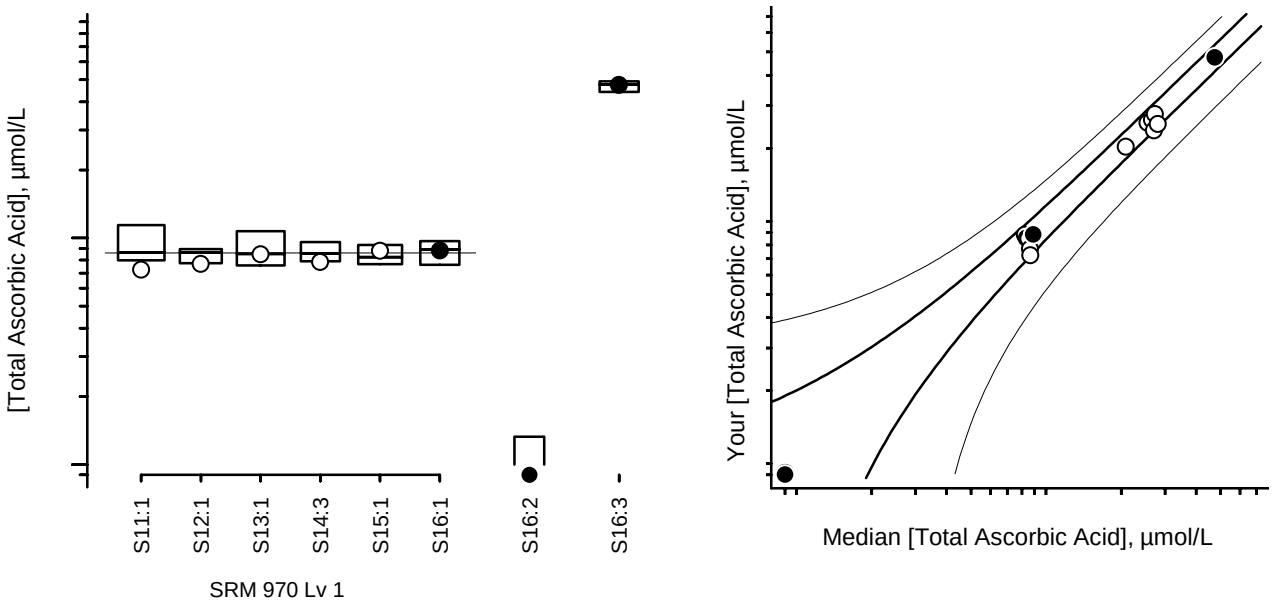
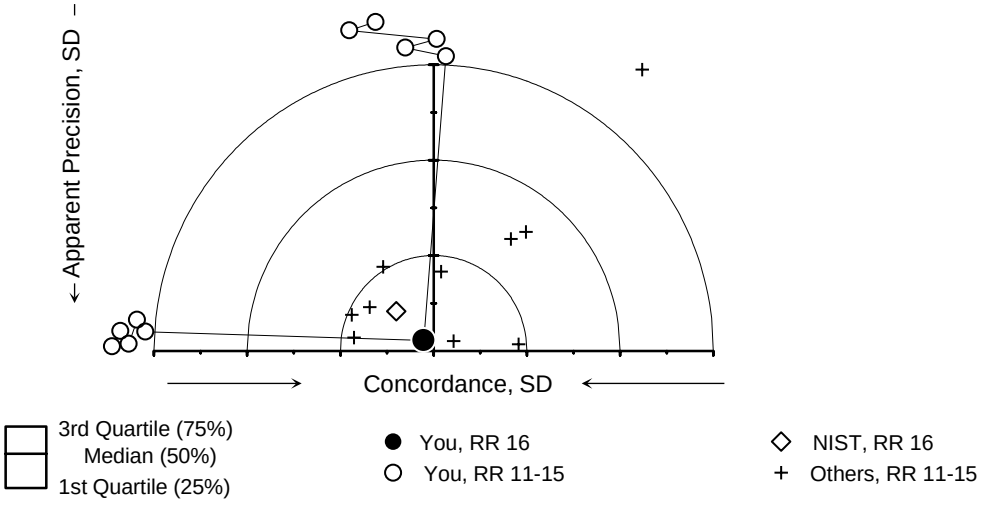
Vitamin C 'Round Robin' 16 Report: Participant VC-MA

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
S16:1	SRM 970 Level 1
S16:2	Serum 11, no augmentation
S16:3	Serum 51, augmented to 47.92 µmol/L sample