# **NISTIR 7880-23**

# NIST Micronutrients Measurement Quality Assurance Program Winter 2001 Comparability Studies

Results for Round Robin XLIX Fat-Soluble Vitamins and Carotenoids in Human Serum and Round Robin 14 Ascorbic Acid in Human Serum

David L. Duewer Sam A. Margolis (Retired) Katherine E. Sharpless Jeanice B. Thomas



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July, 2013



U.S. Department of Commerce *Penny Pritzker, Secretary* 

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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 2001 MMQAP measurement comparability improvement studies: 1) Round Robin XLIX Fat-Soluble Vitamins and Carotenoids in Human Serum and 2) Round Robin 14 Total Ascorbic Acid in Human Serum. The materials for both studies were shipped to participants in January 2001. Participants in Round Robin XLIX were requested to provide their measurement results by March 16, 2001. Participants in Round Robin 14 were requested to provide their measurement results by May 12, 2001.

### **Keywords**

Human Serum Retinol,  $\alpha$ -Tocopherol,  $\gamma$ -Tocopherol, Total and  $\mathit{Trans}$ - $\beta$ -Carotene Total Ascorbic Acid

# **Table of Contents**

Abstract	ii
Keywords	ii
Table of Contents	iv
Introduction	1
Round Robin XLIX: Fat-Soluble Vitamins and Carotenoids in Human Serum	1
Round Robin 14: Vitamin C in Human Serum	2
References	3
Appendix A. Shipping Package Inserts for RR49	A1
Appendix B. Final Report for RR49	B1
Appendix C. "All-Lab Report" for RR49	C1
Appendix D. Representative "Individualized Report" for RR49	D1
Appendix E. Shipping Package Inserts for RR14	E1
Appendix F. Final Report for RR14	
Appendix G. "All-Lab Report" for RR14	G1
Appendix H. Representative "Individualized Report" for RR14	

### 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, alphatocopherol, 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 XLIX: Fat-Soluble Vitamins and Carotenoids in Human Serum

Participants in the MMQAP Fat-Soluble Vitamins and Carotenoids in Human Serum Round Robin XLIX comparability study (hereafter referred to as RR49) 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 2001. 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 RR49 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 selected analytes. 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 14: Vitamin C in Human Serum

Participants in the MMQAP Vitamin C in Human Serum Round Robin 14 comparability study (hereafter referred to as RR14) received four 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 2001. 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 RR14 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 RR49

The following three items were included in each package shipped to an RR49 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-

January 16, 2001

Dear Colleague:

Happy New Year! Enclosed is the first set of samples for the quality assurance round robin exercise (Round Robin XLIX) for 2001. You will find one vial of each of one liquid-frozen and four 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 quantitation, please indicate this result on the form by using NQ (Not Quantitated). For analytes not measured, please leave a blank. Results are due to NIST by March 16, 2001. Results received 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 during the week of April 9, 2001.

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 will 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). Liquid-frozen sample 271 should **not be** reconstituted.

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

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

Micronutrients Measurement Quality Assurance Program

NIST

100 Bureau Drive Stop 8392

Gaithersburg, MD 20899-8392

Fax: (301) 977-0685

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

Sincerely.

Jegfice Brown Thomas

Research Chemist

Analytical Chemistry Division

Chemical Science and Technology Laboratory

Enclosures



Participant #:	Date:
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# Round Robin XLIX NIST Micronutrients Measurement Quality Assurance Program

Analyte	269	270	271	272	273	Units*
total retinol						
trans-retinol						
retinyl palmitate						
lpha-tocopherol						
γ-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 <sub>10</sub> )						
phylloquinone (K <sub>1</sub> )						
25-hydroxyvitamin D						
cholesterol						
Other analytes?						•

\* we prefer μg/mL

Fax: 301-977-0685

Email: David.Duewer@NIST.gov

Was serum 271 frozen when received? Yes | No

# Round Robin XLIX

# NIST Micronutrients Measurement Quality Assurance Program

# Packing List and Shipment Receipt Confirmation Form

The enclosed bubble-pack (should) contain one vial each of the following five sera:

Serum	Form	Reconstitute?
#269	Lyophilized	Yes (1 ml H <sub>2</sub> O)
#270	Lyophilized	Yes (1 ml H <sub>2</sub> O)
#271	Liquid frozen	No
#272	Lyophilized	Yes (1 ml H <sub>2</sub> O)
#273	Lyophilized	Yes (1 ml H <sub>2</sub> O)

- Please 1) Open the pack immediately
  - 2) Check that it contains one vial each of the above samples
  - 3) Check if serum #271 arrived frozen
  - 4) Store the RR XLIX 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 #271 arrive frozen? Yes | No
- 5) At what temperature are you storing the samples? \_\_\_\_\_ °C
- 6) When do you anticipate analyzing these samples? \_\_\_\_\_

Thank you in advance for your prompt response.

The M<sup>2</sup>QAP Gang

### Appendix B. Final Report for RR49 and Folate Questionnaire

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

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

The fifth page of this section is a questionnaire that was distributed in the same mailing as the final report. This questionnaire was intended to assess the level of interest in the measurement of folates in serum and plasma.





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

April 25, 2001

### Dear Colleague:

Enclosed is the summary report of the results for Round Robin XLIX (RR 49) 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 49 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.

The following are newly released or forthcoming publications. Reprints will be provided upon request.

"Preparation and Value Assignment of Standard Reference Material 968c, Fat-Soluble Vitamins, Carotenoids, and Cholesterol in Human Serum," Brown Thomas et al., Clinica Chimica Acta 305 (2001) 141-155.

"The Stability of Ascorbic Acid in Autosampler Vials," Margolis, S.A. and Park, E., accepted by Clinical Chemistry, March 2001.

The NIST Micronutrients Measurement Quality Assurance Workshop was held on April 4, 2001 as a symposium at the Experimental Biology (EB) meeting in Orlando, Florida. At the workshop, results from the past three interlaboratory comparison exercises (Round Robins 47-49), methodology updates, and program needs were discussed. There were 41 attendees at the workshop. As discussed at the session, the "better-than-expected" attendance was thought to be due to the workshop being held at the end of the conference instead of at the beginning. Since the EB meetings now begin a day earlier (on Saturday) than in the past, having the QA workshop at the end of the conference allows conferees to attend the EB meetings first and focus on the QA workshop afterwards. It was agreed that the next workshop will be held in conjunction with EB, which provides the appropriate audience, and will be held at the end of the conference. It was also suggested that our next QA workshop be held at EB 2003 in San Diego, CA. You will be notified about the next QA workshop as plans are made.

Most of the discussion for the afternoon session at the workshop concerned the measurement of folates in serum. Attendees expressed that quality assurance of folate measurements in serum and a folate-in-serum/plasma reference material are needed, and that they would like to start participating in round robin studies for folates in serum/plasma. To help us assess the level of interest in the measurement of folates in serum/plasma among the current QA program participants, please return the enclosed questionnaire by May 31.

Another topic discussed at the workshop was the need to have more than one vitamin C round robin study so that laboratories can meet the requirements of the Clinical Laboratory Improvement Amendments (CLIA). Since there were not enough representatives from laboratories that routinely measure vitamin C present at the workshop, a decision about increasing the number of studies could not be made at that time. Again, we would appreciate your feedback regarding this issue by completing the enclosed questionnaire. Additionally, please be reminded that SRM 970, Vitamin C in Frozen Human Serum, is now available. This material can be purchased through the Standard Reference Materials Program at 301-975-6776; fax: 301-948-3730; or at srminfo@nist.gov.

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

**Enclosures** 

The NIST M<sup>2</sup>QAP Round Robin XLIX (RR49) 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.
n+2	An experimental "Commutability" plot summarizing your measurements in the five liquid-frozen / lyophilized sample pairs distributed in RR47, RR48, and RR49.
Page	"All Lab" Report
1-4	A listing of all results and statistics for analytes reported by at least two laboratories
5	A list of results for the four analytes reported by only one laboratory.  A legend for the above two lists
6	The "Comparability Summary" (or "Score Card")

### **Samples**. Five sera were distributed in RR49.

Serum #269 is a lyophilized material that has been distributed in three previous studies, as #193 in RR30 (3/94), #254 in RR45 (3/99), and #255 in RR46 (6/99). It was prepared from a native serum pool augmented with "retinol" that we now believe was highly enriched in *cis*-isomers. Up to 30% of the total retinol in this sample may be in the form of *cis*-retinols.

Serum #270 is a lyophilized material prepared from a native serum pool. The liquid-frozen partner of this sample was distributed as #267 in RR48. Sera #267 and #270 were prepared and aliquoted into vials as a single batch.

Serum #271 is a liquid-frozen material prepared from a native serum pool. The lyophilized partner of this sample was distributed as #266 in RR48. Sera #266 and #271 were prepared and aliquoted into vials as a single batch.

Serum #272 is a lyophilized material that has been distributed in two previous studies, as #223 and #225 in RR38 (9/96) and #228 in RR39 (3/97). It was prepared from a native serum pool augmented with retinyl palmitate.

Serum #273 is a lyophilized material that was distributed as #203 in RR33 (3/95). It was prepared from a native carotenoid-deficient serum augmented with retinol, retinyl palmitate,  $(\alpha, \gamma, \delta)$ -tocopherols,  $(\alpha, \beta)$ -carotenes, and lycopene.

### **Qualitative Observations.**

- 1) Several participants noted the presence an "insoluble stringy clot" in Serum #271. As noted in the RR48 report for #266, we failed to re-filter the final blend of the serum pool used for this sample. As with its lyophilized partner, the resulting solids do **not** appear to have affected measurements.
- 2) One participant noted that what we've been reporting as  $\gamma$ -tocopherol is "really"  $\gamma$  and/or  $\beta$ -tocopherol. We now denote this measurand as " $\gamma/\beta$ -tocopherol.
- 3) All participants received their samples still frozen. We thank all of you who promptly confirmed receipt of the samples. Such prompt confirmation greatly simplifies our package delivery tracking and thus helps contain M<sup>2</sup>QAP costs. We will be requesting similar confirmation in all future studies.

### **Quantitative Results**

- 1) With the possible exception of zeaxanthin, there have been no changes in the median level of any measurand in sera #269 (7 years storage), #272 (5 years), or #273 (6 years). There is, however, evidence of increased variability in retinol and α-tocopherol measurements, particularly in sera #272 and #273. We believe that this increased variability may be related to contamination of many of the lyophilized sera prepared in the past 6 years. We will discuss this more fully in the RR50 report.
  - As discussed in the RR45 report for serum #254, perhaps 30% of the retinol in Serum #269 is in the form of *cis*-isomers. While we did not intentionally spike this serum with *cis*-retinol, our methods indicate an unusually large retinol shoulder peak that is compatible with the presence of one or more of the *cis*-forms.
- 2) The distribution of reported "retinol-related measurand" interlaboratory values is bimodal, with approximately equal numbers centered at about 0.9 μg/mL and 1.1 μg/mL. Somewhat distressingly, many of your are **not** correctly specifying the form you are actually measuring. Many of the reported "Total Retinol" values are more compatible with being "trans-Retinol"; many of reported "trans-Retinol" values are more compatible with being "Total".
  - If you report "Total Retinol" and your #269 value isn't closer to  $0.9 \,\mu\text{g/mL}$  than to  $1.1 \,\mu\text{g/mL}$ , we suggest that you check your integration protocol to ensure that you are including *cis*-retinol isomers. If you report "*trans*-Retinol" and your #269 value isn't closer to  $1.1 \,\mu\text{g/mL}$  than to  $0.9 \,\mu\text{g/mL}$ , we suggest that you confirm that your separation system does adequately resolve *cis* and *trans*-retinol isomers. We intend to address this issue in the near future with explicitly *cis*-augmented samples.
- 3) A total of five matched {liquid-frozen, lyophilized} matrix pairs have now been distributed: {261,259} and {262,260} in RR47, {265,268} in RR48, and {266,271} and {270,267} in RR48 and RR49. Since these pairs were prepared at the same time from the same serum pools, the ratio of the values in the two matrices (liquid-frozen / lyophilized) is a direct assessment of measurement commutability between the two matrices. The last page of your "Individualized Report" summarizes the community's and your individual commutability ratios.

We have not yet completed the detailed analysis of these data; however, a number of interesting results are already apparent:

The average (liquid-frozen / lyophilized) ratio is 0.94, essentially identical to the 0.95 expected from serum density consideration. There is no systematic variation in this ratio across measurands. While there is considerable variability about this average with most of the "minor" carotenoids, much of this variability in the ratios is a consequence of limited reporting precision at low measurand levels (e.g., 0.0034/0.0036 = 0.94 but 0.003/0.004 = 0.75).

There are several participants with retinol and/or tocopherol commutability ratios that are consistently above or below 0.94, indicating a systematic difference between the matrices in their measurement systems. We believe that this arises from contamination of the lyophilized samples used rather than a lyophilization-specific matrix effect.

Note: since the {266,271} and {270,267} ratios involve measurements made half a year apart, the average of these two ratios (in logarithmic form) estimates commutability while the difference between the ratios estimates within-laboratory calibration differences between RR48 and RR49.

Lab number:	
Phone:	
Fax:	
E-mail:	

# Questionnaire

### **NIST Micronutrients Measurement Quality Assurance Program**

Please provide us with your input by completing this form and returning it by May 31, 2001 to:

NIST Micronutrients Measurement Quality Assurance Program **NIST** 100 Bureau Drive, Stop 8392 Gaithersburg, MD 20899-8392 Fax: 301-977-0685 Folates in serum/plasma are currently measured in my organization by: Name of the contact Phone Fax E-mail address I would participate and be willing to pay for a second annual QA interlaboratory comparison study for vitamin C if it were available. I am required to comply with the Clinical Laboratory Improvement Amendments and/or another laboratory standards program for the measurement of: □ vitamin A □ vitamin D □ carotenes  $\square$  vitamin  $K_1$ □ vitamin C  $\square$  coenzyme  $Q_{10}$ □ vitamin E  $\square$  other (please specify)

Comments:

# Appendix C. "All-Lab Report" for RR49

The following eight 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.

		To	otal Retin	nol		trans-Retinol					Retinyl Palmitate						α-Tocopherol					
Lab	269	270	271	272	273	269	270	271	272	273	269	270	271	272	273	269	270	271	272	273		
FSV-BA	1.25	0.688	0.495	0.564	1.35						0.070	nd	0.072	0.115	0.066	28.9		7.21	8.11	17.6		
FSV-BB	1.27	0.714	0.519	0.542	1.32						0.017	0.008	0.054	0.068	0.050	29.8	6.52	8.01	8.82	18.6		
FSV-BD	1.02	0.619	0.451	0.487	1.15											26.6	6.50	6.50	7.40			
FSV-BE	1.04	0.660	0.450	0.570	1.37											25.1	5.50	6.40	7.40			
FSV-BF	1.01	0.640	0.430	0.500	1.26											29.1	6.20	7.10	7.90			
FSV-BG	1.24	0.653	0.483	0.533	1.35						0.046	0.047	0.065	0.049	0.055	30.1		7.67	8.28			
FSV-BH		0.612		0.425												27.2	6.00	6.85	7.50			
FSV-BI	1.22	0.680	0.503	0.582									0.066			27.7			8.56			
FSV-BJ	1.11	0.634	0.460	0.509							nq	nq	0.049	0.091	0.074		5.94	7.14	7.85			
FSV-BK	1.03	0.649	0.421	0.516													5.25	5.70	6.87			
FSV-BL	0.40	0.600	0.460	0.400													10.34	9.04	8.61			
FSV-BM	0.98	0.668	0.511	0.516							0.040	0.040	0.040	0.050	0.040	32.8		7.40	8.40			
FSV-BN FSV-BO	1.08	0.630	0.470	0.494							0.018	0.016	0.048	0.050	0.043		5.89	7.30	7.68			
FSV-BO FSV-BP	1.01	0.610 0.590	0.513 0.436	0.504 0.460												24.9 27.2	5.30	7.30	7.20			
FSV-BP				0.460												24.4	5.07	7.07	7.28 7.44			
FSV-BQ FSV-BR						1 04	0.600	0.440	0.500	1 15						24.4	4.90	6.22 5.86	6.96			
FSV-BS																24.5	4.30	3.00	0.90	14.5		
FSV-BT		0.599	0.643	0.506		1.03	0.530	0.440	0.300	1.10	0.026	0.014	0.046	0.081	0.070	26.8	5.83	5.56	7.59	16.6		
FSV-BU		0.603		0.413							0.020	0.014	0.040	0.001	0.070	27.6	5.43	6.83	7.35			
FSV-BV	0.94	0.570	0.440	0.450												26.7	5.94	7.71	7.71			
FSV-BW		0.660		0.540	-						0.022	0.018	0.062	0.071	0.076		6.04	7.32	8.10			
FSV-BX	-	>0.62		>0.50		1.02	0.620	0.455	0.501	1.14	0.022	0.010	0.002	0.07	0.070	25.0	5.65	6.90	7.54			
FSV-CB		0.591	0.419	0.473			0.020	000	0.00.							25.7	5.96	7.03	7.67			
FSV-CC		0.670		0.510		1.03	0.630	0.450	0.490	1.21						27.9		7.00	7.50			
FSV-CD						1					0.013	0.033	0.022	0.179	0.041		5.94	6.99	8.36			
FSV-CE	1.16	0.670	0.510	0.570	1.30											29.5	6.70	8.07	9.08			
FSV-CF	1.08	0.663	0.474	0.512												25.5	6.50	7.90	8.20	16.5		
FSV-CG	1.13	0.715	0.510	0.560	1.28											24.7	5.59	6.75	7.19	13.8		
FSV-CH	0.94	0.542	0.376	0.431	1.01											26.3	5.92	7.01	7.92	15.3		
FSV-CI	0.88	0.710	0.520	0.550	1.42						0.030	0.030	0.060	0.060	0.050	31.2	5.60	6.70	7.60	16.9		
FSV-CL	0.99	0.534	0.389	0.396	1.08											31.6	7.54	9.01	9.89	19.3		
FSV-CR	1.14	0.720	0.490	0.550	1.35											28.3	6.00	6.90	7.90	18.2		
FSV-CV	0.49	0.488	0.283	0.205									0.103			30.3	7.69	7.82	9.04	16.9		
FSV-CW	>0.99	>0.575	>0.401	>0.441	>1.15	0.99	0.575	0.401	0.441	1.15	0.012	0.016	0.046	0.065	0.065	27.4	5.82	6.86	7.60	16.7		
FSV-CZ	1.12	0.690	0.510	0.550	1.47											15.9	1.60	2.38	2.67	10.7		
FSV-DB			0.470													28.1	6.43	7.57	8.60	18.0		
FSV-DD						1.04	0.600	0.417	0.525	1.09												
FSV-DF			0.433																			
	>0.98	>0.606	>0.428	>0.466	>1.14	0.98	0.606	0.428	0.466	1.14	0.031	0.036	0.028	0.046	0.040				8.11			
FSV-DQ																36.8		10.24				
FSV-DR			0.490													31.9	6.58	7.99	8.23			
FSV-DU																24.4		5.51	6.19			
FSV-EH						1.07	0.549	0.468	0.508	1.26	0.031	0.032	0.070	0.070	0.062		5.75	6.59	7.00			
FSV-EQ			0.523			4 00	0.700	0.000	0.770	4 70						31.3	6.90		8.80			
FSV-FB						1.33	0.760	0.660	0.773	1./6								7.15				
FSV-FJ		0.640	0.470	0.470		11	11	11	11	11	12	12	11	11	1.1	27.7	6.08	7.43	8.06			
N Min	36 0.40	36 0.488	36 0.283	36 0.205		11 0 98	11 0 549		11 0 441		13 0.012			14	14		44 1.60	44 2.38	2.67	44 10.7		
Median	1.07	0.466	0.263	0.205							0.012							7.12				
Max		0.030	0.643	0.510							0.020											
eSD	0.13	0.050	0.050	0.059							0.012					2.8	0.56	0.62	0.70	1.2		
eCV	12	8	11	12		4	6	5	3		46	56	26	34	28	10	9	9	9	7		
Npast	47	44	44	49	49	7		11	0	0	11	11	15	15	9	47	52	52	47	47		
Medianpast		0.671	0.446	0.498				0.440					0.053			26.9	6.36	6.81	7.72			
SDpast	0.10	0.073	0.044	0.039	0.11	0.15	0.028	0.010			0.040	0.010	0.016	0.044	0.061	2.2	0.70	0.67	0.59	1.5		
NISTa	0.91	0.599	0.454	0.478	1.13	0.71	0.599	0.454	0.478	1.13						23.9	5.28	6.76	7.14	16.3		
NISTb	0.92	0.621	0.403	0.468	1.11											28.5	5.55	5.94	7.21	15.5		
NNIST	3	4	4	4	4	2	2	2	2	2						3	4	4	4	4		
Mean	0.92	0.610	0.428	0.473	1.13	0.71	0.599	0.454	0.478	1.13						26.2	5.41	6.35	7.18	15.9		
Srep	0.02	0.017	0.016	0.008					0.010							0.3	0.14	0.29	0.12			
Shet		0.038	0.004	0.037		0.02	0.009	0.003	0.009	0.01						0.4	0.32	0.10	0.40			
Sanl	0.01	0.016	0.036	0.007												3.3	0.19	0.58	0.05			
Snist	0.02	0.045	0.040	0.038	0.06	0.03	0.025	0.019	0.014	0.01						3.3	0.39	0.65	0.42	0.6		
NAV	0.99	0.630	0.449	0.491	1.20	0.88	0.599	0.447	0.489	1.16	0.026	0.027	0.057	0.069	0.063	26.9	5.71	6.74	7.52	16.3		
NAU		0.059	0.058	0.065							0.012						0.69		0.86			
						•					•					•						

		γ/β-Τ	ocoph	nerol			δ-Τ	ocophe	erol				trans-β-Carotene							
Lab	269				273	269	270	271	272	273	269	270	271	272	273	269	270	271	272	273
FSV-BA	6.42			_							0.347			0.165			0.460		0.155	0.051
FSV-BB	6.18	2.30	2.03	1.78	1.21						0.366			0.158	0.063	0.338	0.470	0.338	0.155	0.045
FSV-BD											0.376									
FSV-BE				1.70							0.357		0.295	0.156						
FSV-BF FSV-BG	6.72				1.10						0.440	0.586 0.543		0.169 0.174	0.031 0.058					
FSV-BH	6.56										0.366 0.375					0 342	O 481	0 336	0.159	0.048
FSV-BI	6.47										0.360			0.172		0.542	0.401	0.550	0.155	0.040
FSV-BJ	6.23											0.565			0.060					
FSV-BK																				
FSV-BL																				
FSV-BM																				
FSV-BN	4.72	1.79	1.62	1.37	0.96	0.700	0.250	0.310	0.340	3.39				0.148		0.300	0.442	0.324	0.140	0.052
FSV-BO											0.367									
FSV-BP											0.380	0.453	0.310	0.183	nd					
FSV-BQ																				
FSV-BR FSV-BS											. 0 222	- 0 202	- 0 100	- 0 002	- 0 024	0 222	0.202	0.100	0.000	0.034
FSV-BS	5.86	2 22	1 56	1 69	1 20	0.757	0.748	0 333	0 336	3 38					>0.034			0.160	0.082 0.135	
FSV-BU	6.16					0.737	0.740	0.233	0.550	3.30			0.362			0.200	0.410	0.230	0.155	0.000
FSV-BV	6.44											0.506			0.057					
FSV-BW	6.75											0.500		0.160						
FSV-BX	6.02	2.22	1.96	1.75	1.13						>0.317	>0.467			>0.076	0.317	0.467	0.336	0.165	0.076
FSV-CB											0.367	0.497	0.345	0.170	0.056					
FSV-CC																				
FSV-CD	5.77	2.24	1.94	1.90	1.24						0.390		0.332							
FSV-CE											0.390	0.570	0.430	0.200	0.050					
FSV-CF	0.00	0.00	0.44	4 00	4 04	0.400	0.400	0.474	0.000	0.40	0.000	0.470	0.050	0.404	0.050	0.004	0.440	0.000	0.454	0.050
FSV-CG						0.492	0.123	0.171	0.228	2.48		0.472		0.164		0.334	0.446	0.328	0.154	0.052
FSV-CH FSV-CI	5.65 6.00										0.233	0.440	0.322	0.153	0.051					
FSV-CL	6.84										0.321	0 432	0.292	0 153	0 049					
FSV-CR	0.04	2.75	2.00	1.55	0.30						0.521	0.432	0.232	0.100	0.043					
FSV-CV	16.17	5.81	4.36	4.15	2.53	0.432	0.111	0.118	0.166	2.64	0.467	0.691	0.394	0.173	0.038					
FSV-CW						0.390					0.344				0.055	0.310	0.442	0.308	0.147	0.051
FSV-CZ											0.380	0.530								
FSV-DB											0.400	0.530	0.360	0.170	0.070					
FSV-DD																				
FSV-DF																				
FSV-DI						0.378	0.084	0.106	0.153	3.22	>0.320					0.320	0.439	0.293	0.135	0.039
FSV-DQ	9.39	2.76	2.73	2.83	1.92								0.483							
FSV-DR FSV-DU													0.390		>0.070	0.274	0 276	0.252	0.118	0.021
FSV-EH	6.06	2 01	1 02	1 65	1 2/	0.313	0.106	0 118	0 196	2 15		0.463			0.070			0.232	0.116	0.021 0.048
FSV-EQ	0.00	2.01	1.52	1.00	1.27	0.515	0.100	0.110	0.130	2.10			0.328	0.165			0.463		0.162	0.052
FSV-FB												0.390			0.073				0.134	0.042
FSV-FJ	5.53	2.18	1.89	1.63	0.95						0.310	0.320	0.150	0.160						
N	25	25	25	25	25	7					32	32	32	32	31	14	14		14	14
						0.313					0.233	0.320	0.150	0.119		0.215			0.082	0.021
Median											0.366	0.499	0.352	0.169		0.313			0.144	0.050
						0.757					0.485			0.337		0.342			0.165	0.076
eSD						0.089					0.030	0.047				0.034			0.016	0.005
eCV	8	9	9	9	9	21	28	15	27		8	9	10	9	16	11	8	9	11	10
Npast	26	29	29	25	22	10	6	6			35	33	33	34	35	13	17	17	12	11
Medianpast											0.369	0.515	0.307	0.171		0.325			0.158	0.056
SDpast	0.65	0.25	0.17	0.15	0.17	0.249	U.U48	0.062	0.038		0.051			0.022					0.020	0.012
NISTa						0.268	•	nq	•	2.06	0.327				0.046					
NISTb						0.403									>0.048				0.153	0.048
NNIST	3	4	-	-	-	3				-	3	2	2	2	2	3	4	4	2	1
Mean						0.337					0.349	0.402		0.139					0.153	0.048
Srep Shot						0.023					0.005	0.015		0.005		0.005			0.003	0.003
Shet Sanl						0.010 0.093	0.001	0.011	0.011	0.10	0.001 0.031	0.008	0.006	0.007	0.001	0.008	0.026		0.009	0.000
SNIST						0.093	0.004	0.014	0.014		0.031	0.017	0.011	0.008	0.007	0.069			0.010	0.003
г															1					
NAV						0.385					0.358	0.450		0.154		0.304			0.148	0.049
NAU	0.52	0.29	0.35	0.22	U. IÖ	0.117	0.042	0.027	0.008	0.70	0.054	0.097	0.075	0.034	0.014	0.070	U.U8/	0.048	0.018	0.009

		Total c	is-β-Ca	arotene	<b>:</b>		Tota	l α-Caro	tene			tra	ns-α-Car	otene			Tota	ıl Lycor	ene	
Lab	269	270	271	272	273	269	270	271	272	273	269	270	271	272	273	269	270	271	272	273
FSV-BA	0.033	0.023	0.010	0.011	nd	0.014	0.020	0.029	0.027	0.028						0.62	0.274	0.425	0.60	0.227
FSV-BB	0.028	0.020	0.018	0.003	0.019	0.009	0.018	0.029	0.020	0.017						0.59	0.260	0.421	0.57	0.237
FSV-BD																				
FSV-BE																				
FSV-BF						0.015	0.020	0.031	0.030	0.035								0.377		
FSV-BG	0.000	0.004	0.000	0.040	n al	0.023	0.034	0.043		0.030								0.415		
FSV-BH	0.033	0.024	0.022	0.010	na		0.015	0.027		0.024								0.376		
FSV-BI FSV-BJ							0.022	0.028		0.031								0.338		
FSV-BJ						0.015	0.026	0.042	0.034	0.028						0.49	0.100	0.330	0.45	0.169
FSV-BL																				
FSV-BM																				
FSV-BN	0.032	0.026	0 024	0 004	nd	0.001	0.011	0.023	0.018	0.025						0.48	0 217	0.363	0 47	0.218
FSV-BO	0.002	0.020	0.024	0.004	770	0.014	0.018	0.023		0.029								0.395		-
FSV-BP						0.009	0.015		0.022	nd								0.362		
FSV-BQ						0.000	0.0.0	0.020	0.022							0.0.	00	0.002	0	
FSV-BR																				
FSV-BS						nd	>0.009	>0.015	>0.017	>0.012	nd	0.009	0.015	0.017	0.012	0.48	0.278	0.330	0.42	0.276
FSV-BT	0.024	0.019	0.018	0.008	0.003				0.026	0.026								0.311		
FSV-BU						0.020	0.021	0.035	0.032	0.034						0.57	0.134	0.346	0.57	0.142
FSV-BV						0.006	0.012	0.021	0.017	0.019						0.40	0.181	0.299	0.40	0.173
FSV-BW						>0.020	>0.016				0.020	0.016	< 0.015	< 0.015	< 0.015	0.53	0.230	0.350	0.50	0.240
FSV-BX						0.019	0.023	0.038	0.037	0.035										
FSV-CB						0.010	0.016	0.026	0.022	0.025						0.45	0.200	0.305	0.44	0.177
FSV-CC																				
FSV-CD						0.012	0.018	0.032	0.023	0.027						0.60	0.231	0.338	0.41	0.158
FSV-CE																				
FSV-CF																				
FSV-CG	0.034	0.026	0.025	0.010	0.007	0.016	0.021	0.036	0.030	0.029						0.54	0.233	0.381	0.50	0.207
FSV-CH						0.013	0.018	0.038	0.023	0.029						0.40	0.196	0.297	0.40	0.179
FSV-CI																				
FSV-CL						0.013	0.020	0.027	0.026	0.022						0.49	0.230	0.324	0.46	0.180
FSV-CR																				
FSV-CV									0.203									0.632		
FSV-CW	0.034	0.027	0.024	0.010	0.004	>0.013	>0.021	>0.031	>0.025	>0.028	0.013	0.021	0.031	0.025	0.028	0.46	0.203	0.339	0.45	0.200
FSV-CZ																				
FSV-DB																0.65	0.280	0.440	0.61	0.270
FSV-DD																				
FSV-DF																				
FSV-DI						0.005	0.047	0.045	0.040	0.040						0.00	0.440	0.504	4.07	0.004
FSV-DQ FSV-DR						0.025	0.017	0.045	0.042	0.049						0.89	0.149	0.594	1.07	0.361
FSV-DR FSV-DU																				
FSV-EH	0.050	0.044	0.041	0 022	0.022	0.000	0.013	0.026	0.010	0.020						0.51	0 224	0.262	0.40	0.208
FSV-EQ									0.018									0.364		
FSV-FB											0.003	0.012	0.020	0.014	0.018					
FSV-FJ	0.111	0.073	0.007	0.044	0.001	0.010	nq		0.020		0.000	0.012	0.020	0.014	0.010	0.40	0.272	0.550	0.50	0.223
N	10	10	10	10	6	24	23	24	24	23	3	3 4	3	3	3	27	27	27	27	26
		0.008				0.001	0.011	0.020	0.017			0.009	0.015	0.014	0.012					
Median						0.013	0.018	0.029	0.026			0.014		0.017	0.012					
		0.073				0.115	0.034	0.045		0.049		0.021	0.031	0.025	0.028					
		0.005				0.005	0.004	0.005	0.008	0.006		0.005						0.049		
eCV		21	33	63		38	25	17	29	22		35				18	19	13	21	20
		11	11					29	27	28	0		0	0	0		29	29		
Npast Medianpast	10			7		25	28		0.027		U	0	0	0	0	28		0.330	29	29
Niedianpast SDpast						0.013 0.005	0.022 0.007	0.030		0.028 0.006								0.330		
					0.004											V. 1 I	0.049	0.000	0.10	0.000
NISTa				٠.	nq	nq	nq	nq	>0.034		nq	nq	nq	0.034						
NISTb	0.028	nd	nd	nd	nd	>0.017		>0.021	>0.018					0.018						0.184
NNIST							1		2	2	1	_	2	2	1	1	2	2	2	1
Mean							0.012		0.034			0.014	0.021	0.018				0.296		
Srep							0.000		0.001			0.004	0.003	0.001				0.019		
Shet							0.000		0.001	0.001	0.000	0.001	0.001	0.002	0.000	0.00	0.013	0.003	0.04	0.000
Sanl							0.000		0.001	0.000	0.00-		0.00:	0.005	0.005		0.615	0.645	0.05	0.000
SNIST							0.000		0.001	0.001	0.000	0.004	0.004	0.002	0.000	0.05	0.016	0.019	0.05	0.038
NAV	0.033	0.025	0.023	0.010		0.013	0.015	0.029	0.030	0.028						0.45	0.208	0.329	0.48	0.196
NAU	0.012	0.009	0.008	0.006		0.005	0.008	0.010	0.011	0.009						0.13	0.063	0.093	0.10	0.053

	trans-Lycopene						Total β-Cryptoxanthin					Total α-Cryptoxanthin					Total Lutein				
Lab	269		271	272	273	269	270	271	272	273	269	270	271	272		269	270	271	272	273	
FSV-BA				0.267		0.050				0.069	0.040	0.004	0.005	0.040	0.004	0.000	0.404	0.007	0.440	0.004	
FSV-BB FSV-BD	0.235	0.128	0.186	0.219	0.101	0.039	0.049	0.056	0.052	0.055	0.016	0.024	0.025	0.019	0.031	0.062	0.101	0.097	0.118	0.064	
FSV-BE																					
FSV-BF						0.036	0.037	0.043	0.043	0.041											
FSV-BG	0.322	0.172	0.221	0.256	0.126	0.032	0.042	0.042	0.044	0.033						0.065	0.058	0.074	0.077	0.060	
FSV-BH									0.083	0.080						0.040				0.054	
FSV-BI FSV-BJ							0.061		0.059	0.066 0.055						0.070 0.062		0.113	0.143	0.082	
FSV-BJ						0.034	0.043	0.054	0.044	0.055						0.062	0.062	0.090	0.119	0.000	
FSV-BL																					
FSV-BM																					
FSV-BN	0.217	0.119	0.191	0.207	0.117						0.009	0.023	0.024	0.014	0.033		0.088	0.084	0.104	0.068	
FSV-BO FSV-BP							0.049			0.059						0.059	0.111	0.108	0.205	0.080	
FSV-BQ						0.035	0.064	0.059	0.064	0.032											
FSV-BR																					
FSV-BS	0.243	0.103	0.122	0.156	0.103	0.042	0.051	0.052	0.064	0.058						nd	0.033	0.033	0.052	0.023	
FSV-BT	0.188	0.088	0.138	0.178	0.084						0.033	0.035	0.035	0.039	0.043	0.072	0.105	0.115	0.119	0.095	
FSV-BU							0.056			0.062											
FSV-BV FSV-BW						0.021	0.029	0.033	0.030	0.032											
FSV-BX	0.220	0.111	0.170	0.199	0.100	0.039	0.051	0.054	0.051	0.051						0.057	0.097	0.092	0.118	0.069	
FSV-CB						0.041	0.046	0.054	0.049	0.053						0.048	0.074	0.084	0.133	0.063	
FSV-CC																					
FSV-CD						0.039	0.045	0.054	0.054	0.048											
FSV-CE FSV-CF																					
FSV-CG	0.283	0.139	0.208	0.245	0.116	0.047	0.048	0.058	0.058	0.051						0.082	0.119	0.115	0.138	0.099	
FSV-CH																0.00=					
FSV-CI																					
FSV-CL						0.047	0.059	0.050	0.043	0.056	0.037	0.041	0.034	0.029	0.042						
FSV-CR FSV-CV																					
FSV-CW	0.204	0.106	0.155	0.185	0.101	0.042	0.045	0.053	0.051	0.052	0.020	0.023	0.027	0.022	0.029	>0.048	>0.073	>0.073	>0.093	>0.056	
FSV-CZ																					
FSV-DB						0.045	0.063	0.060	0.056	0.072											
FSV-DD																					
FSV-DF FSV-DI	0 242	0.150	0.217	0 204	0.100											0.038	0.064	0.063	0.086	0.052	
FSV-DQ	0.542	0.130	0.217	0.304	0.103	0.049	0.067	0.087	0.093	0.107						0.038			0.000		
FSV-DR						0.010	0.001	0.007	0.000	0.107						0.017	0.207	0.000	0.012	0.000	
FSV-DU																					
FSV-EH	0.215	0.107	0.164	0.197	0.089					0.062						>0.051	>0.080	>0.077	>0.092	>0.061	
FSV-EQ	0 442	0.200	0 245	0 502	0.406		0.045			0.052						0.052	0.063	0.065	0.100	0.044	
FSV-FB FSV-FJ	0.413	0.206	0.343	0.503	0.190	0.036	0.062	0.063	0.057	0.067						0.052	0.063	0.065	0.100	0.044	
N	12	12	12	12	12	25	25	25	25	25	5	5	5	5	5	14	15	15	15	15	
Min	0.188	0.088	0.122	0.156	0.084	0.021	0.029	0.033	0.030	0.032	0.009	0.023			0.029	0.038	0.033	0.033	0.052	0.023	
Median											0.020					0.058	0.088	0.089	0.118	0.064	
						0.061					0.037					0.082	0.207	0.115	0.205	0.099	
eSD eCV	24	23	24	23	14	0.006 14	23	14	13	21	0.016 82	0.001	16	47	18	0.014 25	0.030	0.029	0.031 26	0.024 37	
Npast	14	12	12	8	5	24	29	29	22	22	6	7	7	0	0	16	16	16	14	8	
Medianpast											0.019			U	U	0.065	0.092	0.083	0.125	0.079	
						0.010				0.016						0.026	0.017			0.014	
NISTa							0.029			0.036						0.055	0.101	0.110	0.137	0.075	
NISTb						,			,	>0.030	0.030	0.047	0.047	0.038	0.036				>0.137		
NNIST						1	4	4	2	2	1	2	2	2	1	2	2	2	2	2	
Mean									0.065	0.036						0.055	0.101	0.110	0.137	0.075	
Srep Shet							0.001				0.000					0.001	0.010	0.005	0.004	0.004 0.000	
Snet Sant						0.000	0.007	0.004	0.003	0.002	0.000	0.002	0.002	0.001	0.000	0.006	0.002	0.002	0.003	0.000	
SNIST						0.000	0.026		0.015	0.003	0.000	0.002	0.009	0.007	0.007	0.006	0.010	0.006	0.005	0.004	
	0.239	0.124	0.189	0.213	0.106	0.041					0.025					0.057	0.094	0.099	0.127	0.069	
						0.010					0.018					0.015	0.031			0.025	
											_						•	•			

		tra	ıns-Lute	ein			Tota	ıl Zeaxar	nthin			trans	-Zeaxa	nthin	
Lab	269	270	271	272	273	269	270	271	272	273	269	270	271	272	273
FSV-BA FSV-BB FSV-BD FSV-BE						0.051	0.048	0.041	0.054	0.035					
FSV-BF FSV-BH FSV-BI FSV-BJ FSV-BK						0.019 0.020 0.026	0.033	0.020 0.023 0.033	0.025	0.020 0.035 0.039					
FSV-BL FSV-BM FSV-BN FSV-BO FSV-BP FSV-BQ FSV-BR						0.017 0.020		0.022 0.018	0.023 0.019	0.032 0.027					
FSV-BS FSV-BT FSV-BU FSV-BV						>0.028	>0.033	>0.038	>0.044	>0.041	0.028	0.033	0.038	0.044	0.041
FSV-BW FSV-BX FSV-CB FSV-CC FSV-CD						0.022 0.020	0.034 0.035	0.024 0.033		0.034 0.036					
FSV-CE FSV-CF FSV-CG FSV-CH FSV-CI FSV-CL						0.018	0.025	0.024	0.034	0.022					
FSV-CV FSV-CW FSV-CZ FSV-DB FSV-DD FSV-DF	0.048	0.073	0.073	0.093	0.056	0.023	0.034	0.025	0.026	0.033					
FSV-DI FSV-DQ FSV-DR FSV-DU						0.019	0.064	0.017	0.008	0.025					
FSV-EH FSV-EQ FSV-FB	0.051	0.080	0.077	0.092	0.061	0.023	0.036	0.028	0.029 0.012	0.033					
FSV-FJ						0.020	0.007	0.010	0.012	0.000					
N Min	2 0.048	2	0.072	2	0.056	13	13	13	13	13 0.020	1	1	1	1	1
Median		0.077	0.075	0.093	0.059	0.017 0.020 0.051 0.002 12	0.012 0.034 0.064 0.010 31	0.016 0.024 0.041 0.007 28	0.007 0.025 0.054 0.010 42		0.028	0.033	0.038	0.044	0.041
Npast Medianpast SDpast	0	0	0	0	0	12 0.027 0.010	15 0.035 0.009	15 0.027 0.005	11 0.032 0.012	8 0.035 0.008	0	0	0	0	0
NISTa NISTb Nnist	0.054	0.067	0.068	0.091	0.050	nq >0.029	<i>nq</i> >0.036	0.020 >0.026 2	0.015 >0.034 2	0.024 >0.033 2	0.029	0.036	0.026	0.034	0.033
Srep	0.054 0.009 0.000	0.004	0.005	0.004	0.001			0.020 0.001 0.000	0.015 0.001 0.003	0.001	0.029 0.000 0.000	0.002	0.002	0.004	0.003
Sanl SNIST NAV NAU	0.009	0.006	0.006	0.007	0.001	0.020 0.006	0.034 0.010	0.001 0.022 0.007	0.003 0.020 0.012	0.001 0.028 0.011	0.000	0.004	0.004	0.007	0.003

		Total Lu	tein&Zea	axanthin			Coer	nzyme	Q10		Phyl	loquir	none (	K1) x	1000
Lab	269	270	271	272	273	269	270	271	272	273	269	270	271	272	273
FSV-BA	0.106	0.156	0.158	0.182	0.132										
FSV-BB	0.113	0.149	0.138	0.172	0.099										
FSV-BD											0.00	0.04	0.00	0.40	0.40
FSV-BE FSV-BF	0.107	0.157	0.153	0.203	0.138						0.38	0.91	0.38	0.42	0.16
FSV-BG	0.107	0.137	0.102	0.203	0.136										
FSV-BH	0.060	0.101	0.086	0.110	0.089										
FSV-BI	0.096	0.141	0.145	0.187	0.130										
FSV-BJ	-	• • • • • • • • • • • • • • • • • • • •	• • • • • • • • • • • • • • • • • • • •												
FSV-BK															
FSV-BL															
FSV-BM															
FSV-BN	0.060	0.104	0.098	0.118	0.087										
FSV-BO	0.079	0.134	0.126	0.224	0.107										
FSV-BP	0.080	0.120	0.110	0.130	0.006										
FSV-BQ															
FSV-BR															
FSV-BS FSV-BT	0.192	0.191	0.217	0.263	0.192										
FSV-BU	0.192	0.153	0.217	0.203	0.132										
FSV-BV	0.071	0.113	0.109	0.136	0.099										
FSV-BW	0.07	0.110	0.100	0.100	0.000										
FSV-BX	0.079	0.130	0.115	0.144	0.103										
FSV-CB	0.069	0.109	0.117	0.140	0.099										
FSV-CC															
FSV-CD	0.080	0.116	0.143	0.130	0.089										
FSV-CE															
FSV-CF															
FSV-CG	0.100	0.143	0.139	0.171	0.120										
FSV-CH						0.487	0.45	0.52	0.335	0.48					
FSV-CI	0.004	0.444	0.400	0.405	0.400										
FSV-CL FSV-CR	0.084	0.114	0.100	0.125	0.100										
FSV-CK FSV-CV	0.092	0.146	0.125	0.172	0.110	1.948	0.06	0 00	0 042	1 70					
FSV-CW		>0.140				0.910									
FSV-CZ	20.071	<b>-0.107</b>	<b>&gt;</b> 0.030	<b>-0.113</b>	<b>~0.003</b>				0.540						
FSV-DB	0.098	0.129	0.115	0.152	0.120	0.700	0.0 .	0	0.010	1.00					
FSV-DD					***										
FSV-DF															
FSV-DI						0.855	0.72	0.79	0.493	0.85	0.68	1.37	0.53	0.47	nd
FSV-DQ	0.066	0.271	0.106	0.080	0.058										
FSV-DR															
FSV-DU															
FSV-EH															
FSV-EQ	0.017		0.183	0.245	0.178										
FSV-FB FSV-FJ		0.100	0.081	0.112	0.074										
F3V-F3		22	22	22	22	5	5	5	5	5	2	2	2	2	1
Min	0.017	0.073	0.081	0.080		0.487									
Median	0.082	0.132	0.121	0.148		0.855									0.16
Max		0.271	0.217	0.263	0.192				0.942						-
eSD	0.020	0.030	0.030	0.047	0.024	0.096	0.16	0.22	0.070	0.22					
eCV	25	22	25	32	24	11	20	23	13	22					
Npast	22	29	29	22	21	0	0	0	0	0	0	0	0	0	0
Medianpast		0.134	0.114	0.154	0.117		,	,	,	-	,	,	,	,	-
SDpast		0.027	0.017	0.034	0.018										
NISTa	>0.055		0.130	0.151	0.098										
		>0.101													
NNIST	1	2	4	4	3										
Mean		0.103	0.112	0.138	0.090										
Srep	0.000	0.005	0.007	0.008	0.004										
Shet		0.009	0.005	0.000	0.000										
Sanl			0.026	0.019	0.011										
Snist	0.000	0.010	0.028	0.020	0.012										
NAV	0.080	0.118	0.117	0.143	0.096										
NAU	0.021	0.036	0.030	0.048	0.026										

# Round Robin XLIX Laboratory Results

# Analytes Reported By One Laboratory

Values in µg/mL

Analyte	Code	269	270	271	272	273
25-hydroxyvitamin D	FSV-BN	0.01	0.06	0.02	0.02	0.01
3'-dehydro-Lutein	FSV-BH	0.04	0.03	0.04	0.06	0.03
Phytofluene	FSV-CL	0.059	0.014	0.042	0.078	0.024
Total cis-Lutein&Zeaxanthin	FSV-BT	0.092	0.052	0.064	0.100	0.056
trans-Lutein&Zeaxanthin	FSV-BT	0.100	0.138	0.153	0.163	0.136
trans-β-Cryptoxanthin	NISTb	0.040	0.056	0.059	0.053	0.045

# Legend

Term	Definition
N	Number of (non-NIST) quantitative values reported for this analyte
Min	Minimum (non-NIST) quantitative value reported
Median	, , , ,
Max	Maximum (non-NIST) quantitative value reported
SD CV	Adjusted median absolute deviation from the median of the non-NIST results Coefficient of Variation for (non-NIST) results: 100*SD/Median
CV	Coefficient of Variation for (non-initial) results. Too 3D/Median
N <sub>past</sub>	Mean of N(s) from past RR(s)
Medianpast	Mean of Median(s) from past RR(s)
$SD_past$	Pooled SD from past RR(s)
\o=	March (NIOT and Its
NIST	Mean of NIST results
NAV	NIST Assigned Value
	= (Median + NIST)/2 for analytes reported by NIST
	= Median for analytes reported by ≥ 5 labs but not NIST
NAU	NIST Assigned Uncertainty: √(S <sup>2</sup> + S <sub>btw</sub> <sup>2</sup> )
	S is the maximum of (0.05*NAV, SD, SD <sub>past</sub> , eSD) and S <sub>btw</sub> is the standard
	deviation between Median and NIST. The expected long-term SD, eSD,
	is defined in: Duewer et al., Anal Chem 1997;69(7):1406-1413.
N <sub>NIST</sub>	Number of total vials analyzed in duplicate by NIST analysts
Mean	
	Within-vial pooled standard deviation
	Among-vial pooled standard deviation
Sanl	Between NIST analyst standard deviation
Snist	Total standard deviation for NIST analyses: $(S_{rep}^2 + S_{het}^2 + S_{anl}^2)^{0.5}$
1	Net detected (i.e., we detected a real-few cook to)
nd	Not detected (i.e., no detectable peak for analyte)  Detected but not quantitatively determined
nq <x< td=""><td>Concentration less than the limit of quantification, x</td></x<>	Concentration less than the limit of quantification, x
<x &gt;X</x 	Concentration greater than x
<b>/</b>	Concentration greater than x
italics	Not explicitly reported but calculated by NIST from reported values

### Round Robin XLIX Laboratory Results

### Comparability Summary

										•	
Lab	TR	аΤ	g/bT	bC	tbC	аC	TLy	TbX	TLu	ΤZ	L&Z
FSV-BA	1	1	1	1	1	1	1	1		1	1
FSV-BB	1	1	1	1	1	1	1	1	1	2	2
FSV-BD	1	1		1							
FSV-BE	1	1	1	1							2
FSV-BF	1	1	1	2		1	1	1			
FSV-BG	1	1	1	1		2	1	1	1		2
FSV-BH	1	1	1	1	1	1	1	2	1	3	1
FSV-BI	1	1	1	1		1	1	1	1	1	1
FSV-BJ	1	1	1	1		1	1	1	1	1	1
FSV-BK	1	2									
FSV-BL	3	4									
FSV-BM	1	2									
FSV-BN	1	1	2	1	1	2	1	1	1		1
FSV-BO	1	1		1		1	2	1	2		
FSV-BP	1	1		1		1	3	1			1
FSV-BQ	1	1								2	1
FSV-BR	1	2									2
FSV-BS	1			3	3	2	1	1	2	1	1
FSV-BT	2	1	1	1	1	1	1	1	1		
FSV-BU	1	1	1	1		1	2	1		1	1
FSV-BV	2	1	1	1		1	1	2		2	4
FSV-BW	1	1	1	1		2	1				
FSV-BX	1	1	1	1	2	1		1	1		
FSV-CB	1	1		1		1	1	1	1	1	1
FSV-CC	1	1									
FSV-CD	1	1	1	1		1	1	1			
FSV-CE	1	1		1							1
FSV-CF	1	1									1
FSV-CG	1	2	1	1	1	1	1	1	2		1
FSV-CH	2	1	1	2		1	1				1
FSV-CI	2	1	2							1	1
FSV-CL	2	2	2	1		1	1	1			
FSV-CR	1	1									
FSV-CV	4	2	4	2		4	4				
FSV-CW	2	1	1	1	1	1	1	1	1		
FSV-CZ	1	4		2							
FSV-DB	1	1		1			2	1		2	3
FSV-DD	1										
FSV-DF	1										
FSV-DI	1	1	2	1	1				1		
FSV-DQ		4	4	4		2	4	2	3		3
FSV-DR	1	2		1							
FSV-DU	1	2		2	2						
FSV-EH	1	1	1	1	1	1	1	1	1	1	1
FSV-EQ	1	3		1	1	1	1	1			
FSV-FB	3	1		1	2	2	1	1	1	1	1
FSV-FJ	1	1	1	2		1					
NISTa	1	1	1	1	1	1		1	1	1	1
NISTb	1	1	1	1	1	1	1	1	1	1	1
n	48	46	27	38	16	30	28	27	19	16	26
		_	/ı <del>-</del>	L C		_	<b>-</b> .	<b>T</b> I V			

Label	Definition
Lab	Participant code
TR	Total Retinol
аТ	$\alpha$ -Tocopherol
g/bT	γ/β-Tocopherol
bC	Total β-Carotene
tbC	trans-β-Carotene
aC	Total α-Carotene
TLy	Total Lycopene
TbX	Total β-Cryptoxanthin
TLu	Total Lutein
TZ	Total Zeaxanthin
L&Z	Total Lutein & Zeaxanthin
n	number of participants providing quantitative data
% 1	Percent of CS = 1 (within 1 SD of medians)
% 2	Percent of CS = 2 (within 2 SD of medians)
% 3	Percent of CS = 3 (within 3 SD of medians)
% 4	Percent of CS = 4 (3 or more SD from medians)

### "Comparability Score"

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

We define CS as follows:

$$\begin{split} & \text{CS = MINIMUM} \bigg( 4, \text{INTEGER} \bigg( 1 + \sqrt{C^2 + AP^2} \bigg) \bigg) \\ & \text{C = Concordance} = \frac{\displaystyle \sum_{i=1}^{N_{you}} \frac{You_i - Median_i}{NAU_i}}{N_{you}} \\ & \text{AP = Apparent Precision} = \sqrt{\frac{\displaystyle \sum_{i=1}^{N_{you}} \bigg( \frac{You_i - Median_i}{NAU_i} \bigg)^2}{N_{you} - 1}} \end{split}$$

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.

74

All Lab Report

TR aT g/bT bC tbC aC TLy TbX TLu TZ L&Z

C9

### Appendix D. Representative "Individualized Report" for RR49

Each participant in RR49 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 RR49:

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

The following 13 pages are the "Individualized Report" for the analytes evaluated by participant FSV-BA.

# Individualized Round Robin XLIX Report: FSV-BA

# Summary

Serum 269 Serum 270				Ser	um 270	<b>,</b>	Seri	Jm 271		Seri	um 272		Seri	um 273	
(# '1 ( ) Y			9		7 / / / /				9		1 / 1 / 1	9		7 / 1 / 1	9
Analyte	You	NAV	u	rou	NAV		You	NAV	u	rou	NAV	_	You	NAV	L
Total Retinol	1.252	0.979	36	0.688	0.630	36	0.495	0.449	36	0.564	0.491	36	1.350	1.204	36
Retinyl Palmitate	0.07	0.03	13	pu	0.0	12	0.1	0.1	4	0.12	0.07	4	0.07	90.0	4
a-Tocopherol	28.85	26.88	44	6.15	5.71		7.21	7.21 6.72	44	8.11	8.11 7.52	44	$\overline{}$	7.58 16.33	44
y/β-Tocopherol	6.423	6.124	25	2.292	2.179	22	1.961	1.890	25	1.755	1.705	25	1.215	1.165	25
Total 8-Carotene	0.347	0.358	32	0.480	0.452			0.314	32		0.154	32	_	0.052	31
trans-β-Carotene	0.316	0.305	14	0.460	0.420	4		0.288	4		0.150	4	_	0.048	4
Total cis-β-Carotene	0.033	0.035	10	0.023	0.025	10	0.010	0.022	10	0.011	0.011	10		0.010	9
Total α-Carotene	0.014	0.013	24	0.020	0.015	23	0.029	0.030	24	0.027	0.030		_	0.028	23
Total Lycopene	0.624	0.465	27	0.274	0.206	27		0.329	27		0.484	27	_	0.194	56
trans-Lycopene	0.293	0.259	12	0.151	0.134	12	0.220	0.197	12		0.232		_	0.105	12
Total 8-Cryptoxanthin	0.050	0.041	25	0.064	0.049			0.062	25		0.060		_	0.046	22
Total Lutein&Zeaxanthin	0.106	0.082	22	0.156	0.118	22		0.117	22	0.182	0.143	22	0.132	960.0	22

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

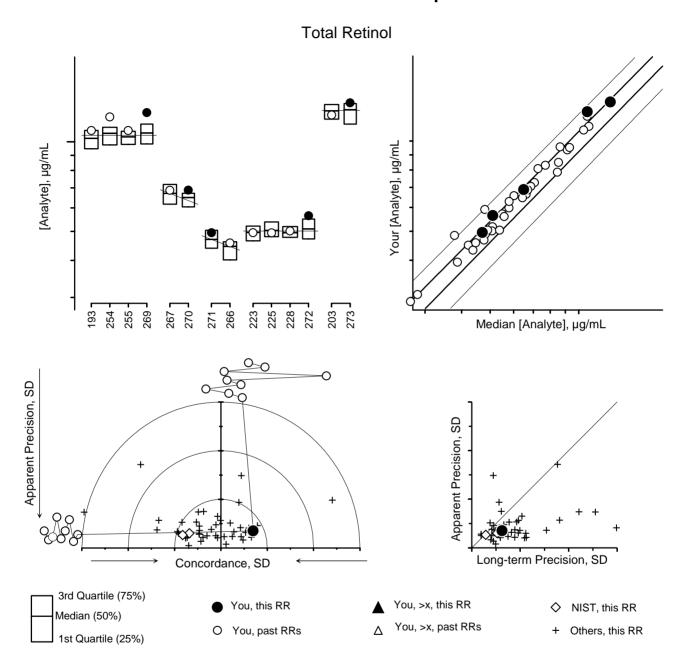
n: Number of non-NIST laboratories reporting quantitative values for this analyte in this serum

nd: Not detected

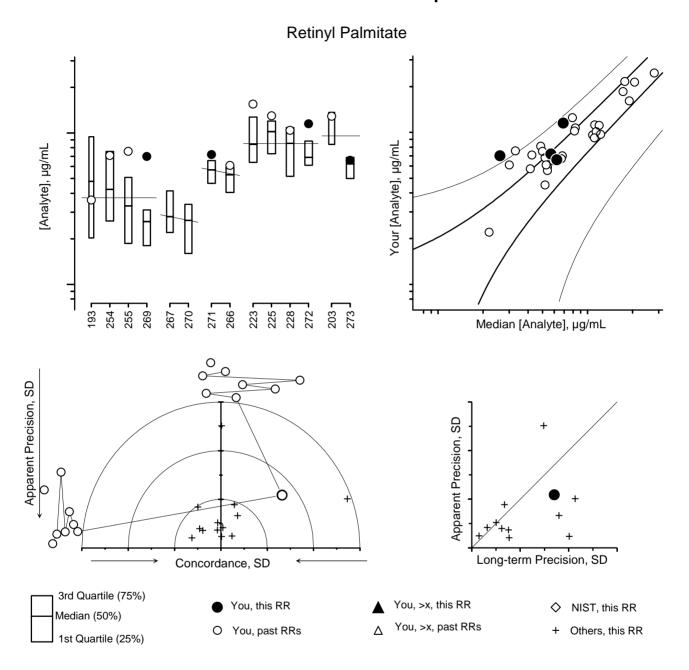
Please check our records against your records. Send corrections and/or updates to...

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

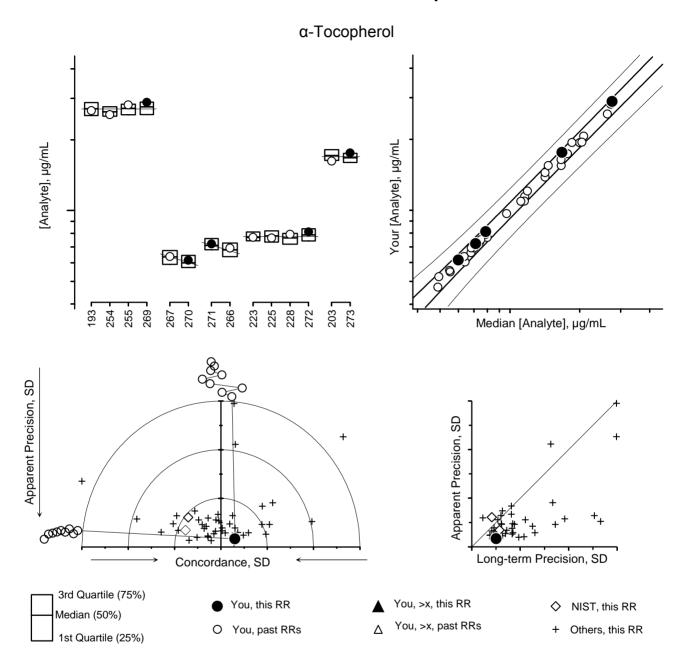
Tel: (301) 975-3935 Fax: (301) 977-0685 Email: david.duewer@nist.gov Page 1 / 13



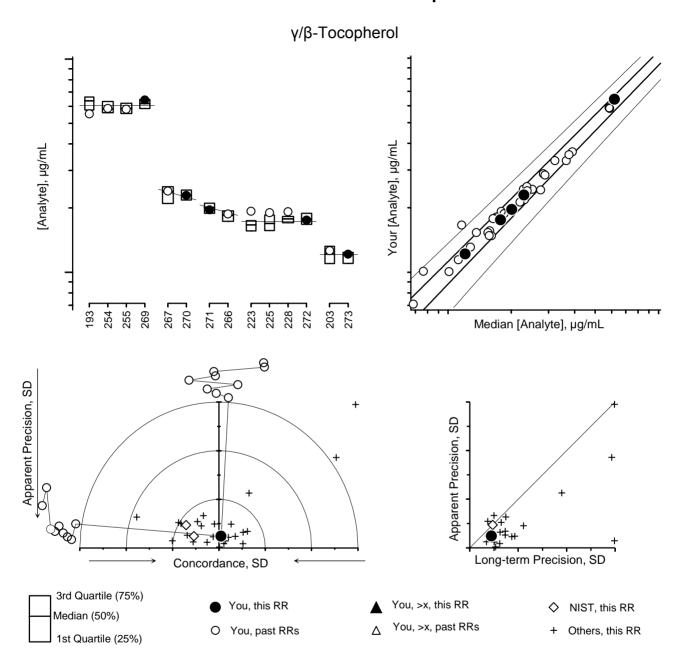
<u>Serum</u>	<u>History</u>	<u>Comments</u>
#269	#193 RR30 3/94, #254 RR45 3/99, #255 RR46 6/99	Lyophilized
#270	Same pool as #267 (lq fz) RR48 9/00	Lyophilized
#271	Same pool as #266 (ly) RR48 9/00	Fresh frozen
#272	#223 & #225 RR38 9/96, #228 RR39 3/97	Lyophilized
#273	#203 RR33 3/95	Lyophilized



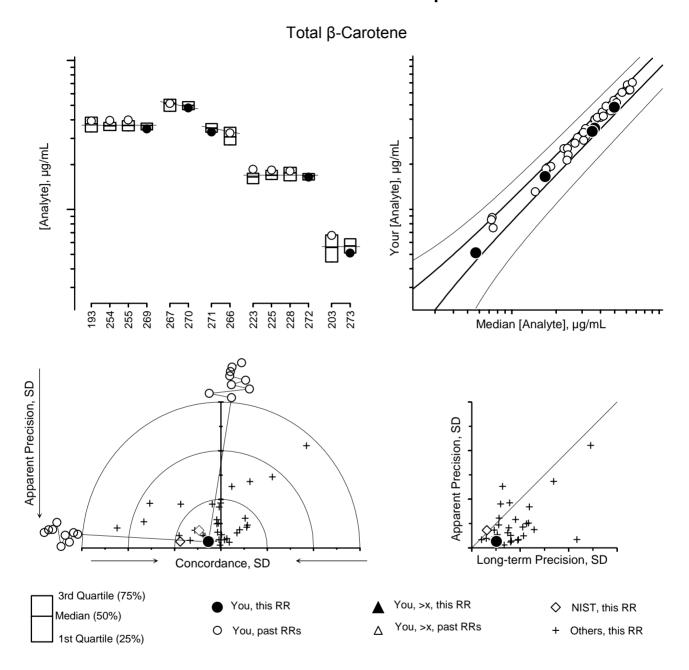
<u>Serum</u>	<u>History</u>	<u>Comments</u>
#269	#193 RR30 3/94, #254 RR45 3/99, #255 RR46 6/99	Lyophilized
#270	Same pool as #267 (lq fz) RR48 9/00	Lyophilized
#271	Same pool as #266 (ly) RR48 9/00	Fresh frozen
#272	#223 & #225 RR38 9/96, #228 RR39 3/97	Lyophilized
#273	#203 RR33 3/95	Lyophilized



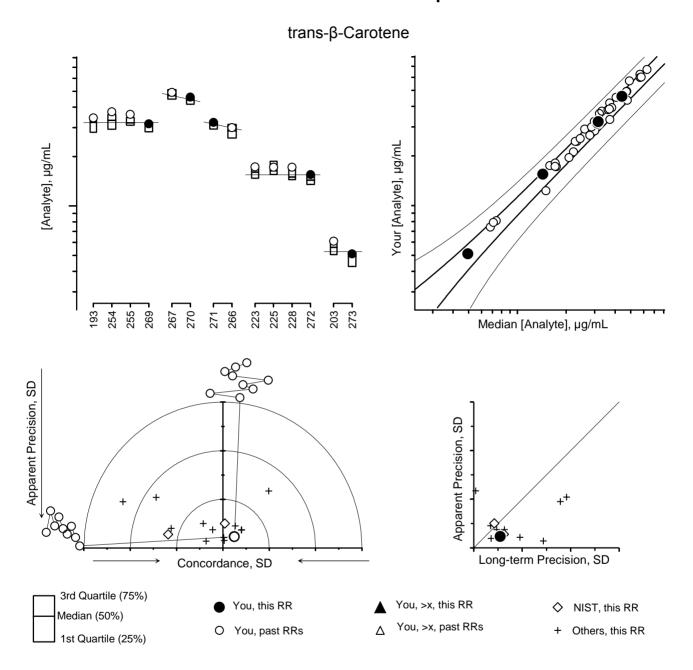
<u>Serum</u>	<u>History</u>	<u>Comments</u>
#269	#193 RR30 3/94, #254 RR45 3/99, #255 RR46 6/99	Lyophilized
#270	Same pool as #267 (lq fz) RR48 9/00	Lyophilized
#271	Same pool as #266 (ly) RR48 9/00	Fresh frozen
#272	#223 & #225 RR38 9/96, #228 RR39 3/97	Lyophilized
#273	#203 RR33 3/95	Lyophilized



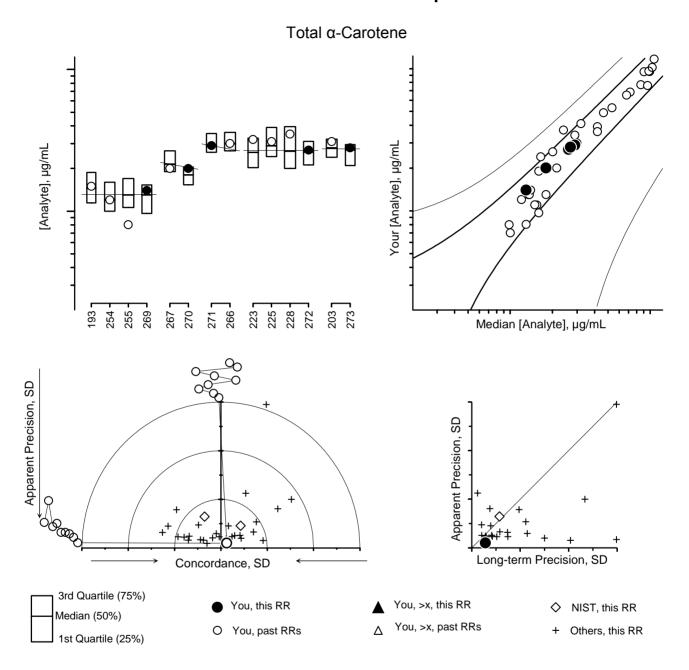
<u>Serum</u>	<u>History</u>	<u>Comments</u>
#269	#193 RR30 3/94, #254 RR45 3/99, #255 RR46 6/99	Lyophilized
#270	Same pool as #267 (lq fz) RR48 9/00	Lyophilized
#271	Same pool as #266 (ly) RR48 9/00	Fresh frozen
#272	#223 & #225 RR38 9/96, #228 RR39 3/97	Lyophilized
#273	#203 RR33 3/95	Lyophilized



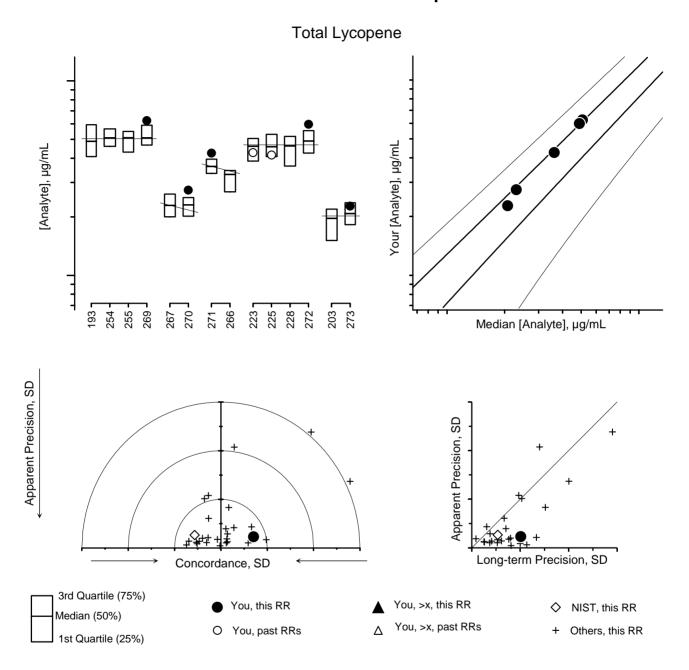
<u>Serum</u>	<u>History</u>	<u>Comments</u>
#269	#193 RR30 3/94, #254 RR45 3/99, #255 RR46 6/99	Lyophilized
#270	Same pool as #267 (lq fz) RR48 9/00	Lyophilized
#271	Same pool as #266 (ly) RR48 9/00	Fresh frozen
#272	#223 & #225 RR38 9/96, #228 RR39 3/97	Lyophilized
#273	#203 RR33 3/95	Lyophilized



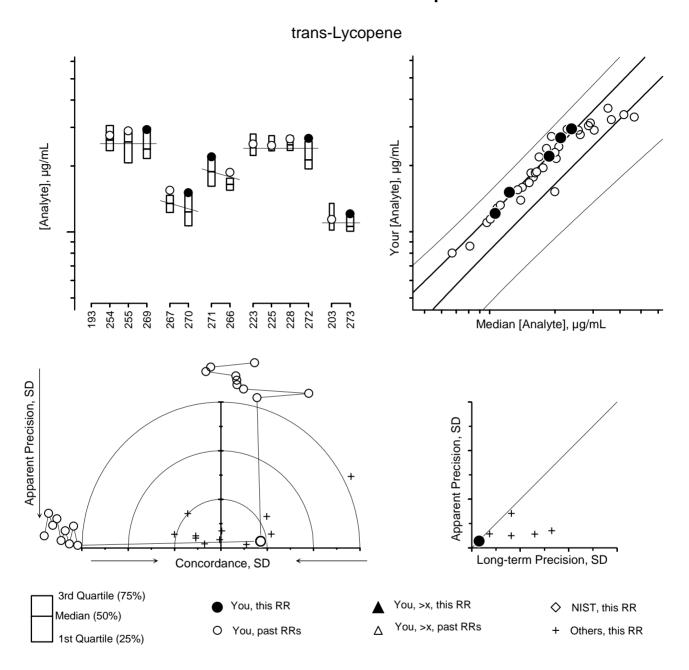
<u>Serum</u>	<u>History</u>	<u>Comments</u>
#269	#193 RR30 3/94, #254 RR45 3/99, #255 RR46 6/99	Lyophilized
#270	Same pool as #267 (lq fz) RR48 9/00	Lyophilized
#271	Same pool as #266 (ly) RR48 9/00	Fresh frozen
#272	#223 & #225 RR38 9/96, #228 RR39 3/97	Lyophilized
#273	#203 RR33 3/95	Lyophilized



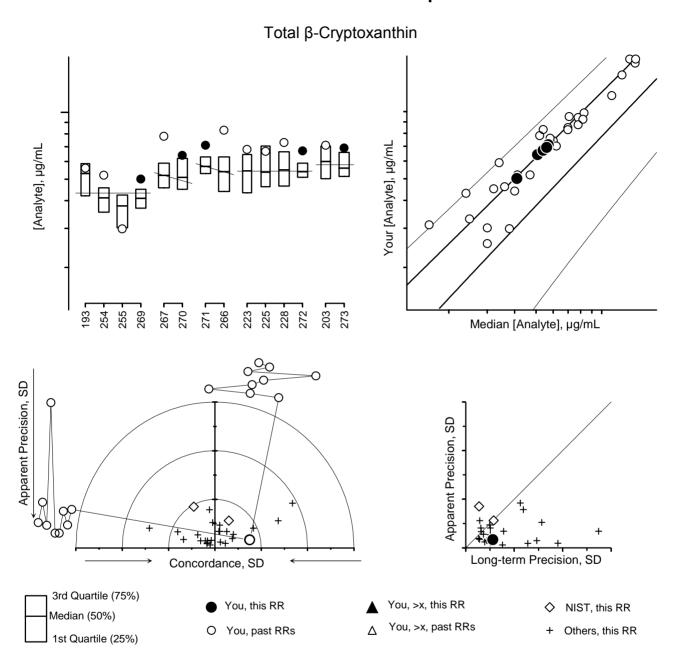
<u>Serum</u>	<u>History</u>	<u>Comments</u>
#269	#193 RR30 3/94, #254 RR45 3/99, #255 RR46 6/99	Lyophilized
#270	Same pool as #267 (lq fz) RR48 9/00	Lyophilized
#271	Same pool as #266 (ly) RR48 9/00	Fresh frozen
#272	#223 & #225 RR38 9/96, #228 RR39 3/97	Lyophilized
#273	#203 RR33 3/95	Lyophilized



<u>Serum</u>	<u>History</u>	<u>Comments</u>
#269	#193 RR30 3/94, #254 RR45 3/99, #255 RR46 6/99	Lyophilized
#270	Same pool as #267 (lq fz) RR48 9/00	Lyophilized
#271	Same pool as #266 (ly) RR48 9/00	Fresh frozen
#272	#223 & #225 RR38 9/96, #228 RR39 3/97	Lyophilized
#273	#203 RR33 3/95	Lyophilized



<u>Serum</u>	<u>History</u>	<u>Comments</u>
#269	#193 RR30 3/94, #254 RR45 3/99, #255 RR46 6/99	Lyophilized
#270	Same pool as #267 (lq fz) RR48 9/00	Lyophilized
#271	Same pool as #266 (ly) RR48 9/00	Fresh frozen
#272	#223 & #225 RR38 9/96, #228 RR39 3/97	Lyophilized
#273	#203 RR33 3/95	Lyophilized

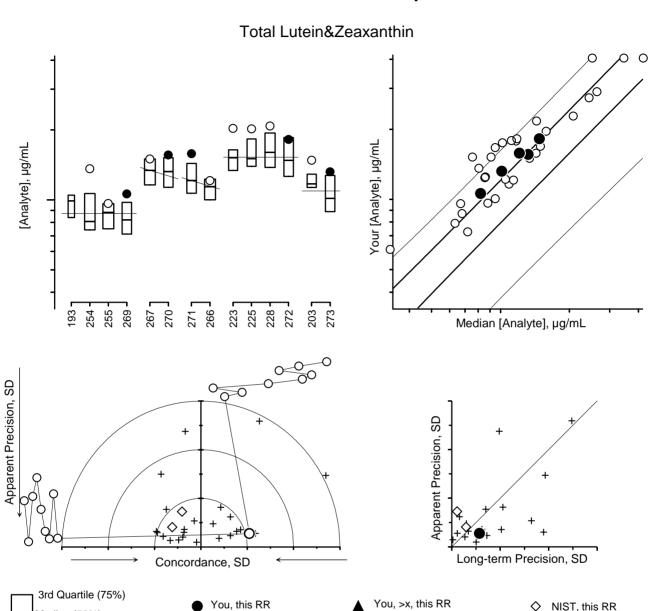


<u>Serum</u>	<u>History</u>	<u>Comments</u>
#269	#193 RR30 3/94, #254 RR45 3/99, #255 RR46 6/99	Lyophilized
#270	Same pool as #267 (lq fz) RR48 9/00	Lyophilized
#271	Same pool as #266 (ly) RR48 9/00	Fresh frozen
#272	#223 & #225 RR38 9/96, #228 RR39 3/97	Lyophilized
#273	#203 RR33 3/95	Lyophilized

Median (50%)

1st Quartile (25%)

# Individualized RR XLIX Report: FSV-BA



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

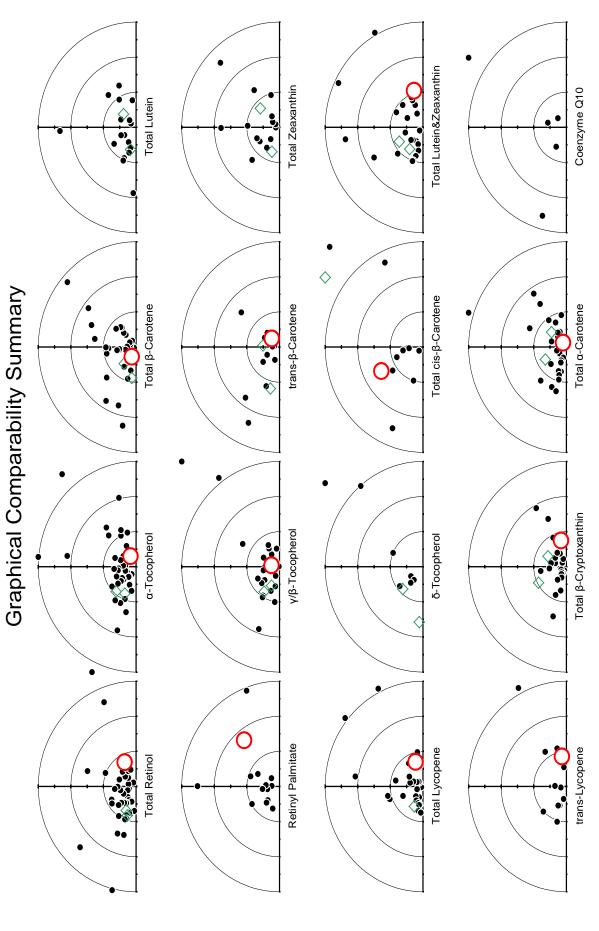
You, >x, past RRs

Others, this RR

<u>Serum</u>	<u>History</u>	<u>Comments</u>
#269	#193 RR30 3/94, #254 RR45 3/99, #255 RR46 6/99	Lyophilized
#270	Same pool as #267 (lq fz) RR48 9/00	Lyophilized
#271	Same pool as #266 (ly) RR48 9/00	Fresh frozen
#272	#223 & #225 RR38 9/96, #228 RR39 3/97	Lyophilized
#273	#203 RR33 3/95	Lyophilized

You, past RRs

Set 1 of 49



### Appendix E. Shipping Package Inserts for RR14

The following three items were included in each package shipped to an RR14 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

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.

We have no record, either electronic or hardcopy, of a packing list for the RR14 study.

January, 2001

### Dear Colleague:

For the past 16 years the National Institute of Standards and Technology (NIST) has coordinated a Micronutrients Measurement Quality Assurance (QA) Program for laboratories making vitamin measurements in human serum. Frozen and/or freeze dried sera are sent to laboratories for analysis as an interlaboratory comparison exercise. Results are returned to NIST for data tabulation and evaluation. Value-assignment of the sample pools is based on the median of all the laboratory results, with confirmation based on measurements at NIST. We provide consultation and trouble-shooting regarding methods of analysis, and a certificate of participation in the QA program is issued at the end of each calendar year. We also host a micronutrient analysis QA workshop for fat-soluble vitamin, carotenoid, and ascorbic acid measurements in serum.

The enclosed set of samples constitute the round robin exercise for vitamin C (Round Robin XIV) for 2001. Four vials of frozen serum (*test samples*), and a vial of solid ascorbic acid (*a control sample*), are enclosed. Please follow the attached protocol when you analyze these samples.

Report your results using the attached form by May 12, 2001. 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. Results received two weeks after the due date will not be included in the summary report of this round robin study. The summary report concerning this study will be provided near the end of June.

Please mail your results to:

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

or Fax: 301.977.0685 E-mail: sam.margolis@nist.gov

If you have any questions or concerns please call me at 301.975.3137 or contact me by Fax or E-mail.

Thank you for your participation and we look forward to receiving your results.

Sincerely,

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

**Enclosures** 

### **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 weights 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:

<u>Dilute Solution 1:</u> **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.** 

<u>Dilute Solution 2:</u> **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**.

<u>Dilute Solution 3:</u> **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 of MPA solution that was added**.

- 4. Record the ultraviolet absorbance spectrum of <u>Dilute Solution 1</u> 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 <u>all three **dilute solutions**</u> 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  $550 \pm 30$  nm. The extinction coefficient of your solution can be calculated using the following equation:

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

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* should 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  $100 \, \mu \text{mol}$  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  $\mu g/L$  of sample.

### REPORT OF ANALYSIS

NAME:	
ADDRESS:	
Telephone no.: Fax no.:	
Method of Analysis:	
Please note the type of method that you use.	
Please attach representative chromatograms.	
Method used for calculating ascorbic acid concentration.	
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 SO	LUTIONS
STOCK SOLUTION	
	mg g
DILUTE SOLUTION 1	
W. 1. 650 N.D. 11.1. 1.100 Y. 1	mg
Absorbance of Dilute Solution 1 at <b>243 nm</b> Absorbance of Dilute Solution 1 at <b>244 nm</b>	AU AU
Wavelength of maximum absorbance  Calculated molar absorptivity	nm dL/g·c

# **DILUTE SOLUTION 2**

Weight of added stock solution (0.25 mL)	mg
Weight of 5% MPA added to the 100 mL volumetric flask	g
DILUTE SOLUTION 3	
Weight of added stock solution (0.125 mL)	mg
Weight of 5% MPA added to the 100 mL volumetric flask	g
<u>COMMENTS</u> : (use other side if necessary)	

Mail by May 12, 2001 to: Micronutrients Measurement Quality Program NIST, 100 Bureau Drive, Stop 8392 Gaithersburg, MD 20899-8392

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

# REPORT OF ANALYSIS

# RESULTS (µmol/L of Sample)

DILUTE SOLUTION 1	
REPLICATE 1	umol/L of dilute solution 1
REPLICATE 2	μmol/L of dilute solution 1
DILUTE SOLUTION 2	
REPLICATE 1	umol/L of dilute solution 2
REPLICATE 2	μmol/L of dilute solution 2
DILUTE SOLUTION 3	
REPLICATE 1	umol/L of dilute solution 3
REPLICATE 2	μmol/L of dilute solution 3
TEST SAMPLE #1	
REPLICATE 1	umol/L of Sample 1
REPLICATE 2	μmol/L of Sample 1
TEST SAMPLE #2	
REPLICATE 1	umol/L of Sample 2
REPLICATE 2	μmol/L of Sample 2
TEST SAMPLE #3	
REPLICATE 1	umol/L of Sample 3
REPLICATE 2	μmol/L of Sample 3
TEST SAMPLE #4	
REPLICATE 1	umol/L of Sample 4
REPLICATE 2	μmol/L of Sample 4

Mail by May 12, 2001 to: Micronutrients Measurement Quality Program NIST, 100 Bureau Drive, Stop 8392 Gaithersburg, MD 20899-8392

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

# **Appendix F. Final Report for RR14**

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

- Cover letter.
- An information sheet that:
  - o describes the contents of the "All-Lab" report,
  - o describes the content of the "Individualized" report,
  - o describes the nature of the test samples and details their previous distributions, if any, and
  - o 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-

August 8, 2001

### Dear Colleague:

Enclosed is the summary report of the results for Round Robin 14 (RR14) 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; the measurement comparability summary for evaluating laboratory performance; and a summary of individual laboratory performance and interlaboratory accuracy and precision. As in previous reports, the estimated standard deviations (eSD) for the measurements are defined as 0.74 x interquartile range and the estimate coefficients of variation (eCV) are defined as 100 x eSD/median. NIST data are not included in the statistical analysis.

RR14 consists of four unknowns and one solid reference ascorbic acid for control solutions. Details regarding the samples can be found in the enclosed report.

Data for evaluating laboratory performance in RR14 are provided in the comparability summary (Score Card) on page 1 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 values; 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 970, Vitamin C in Frozen Human Serum. SRM 970 can be purchased from the NIST SRM Office 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.

If you have any questions regarding this report, please contact David Duewer at <u>david.duewer@nist.gov</u>, phone: 301-975-3935 or Sam Margolis at <u>sam.margolis@nist.gov</u>; phone: 301-975-3137; fax: 301-977-0685.

Sincerely,

Jeanice Brown Thomas

Research Chemist

Analytical Chemistry Division

Chemical Science and Technology Laboratory

Sam A. Margolis, Ph.D.

Research Chemist

Analytical Chemistry Division

Chemical Science and Technology Laboratory

enclosure



The NIST M<sup>2</sup>QAP Vitamin C Round Robin 14 (RR14) report consists of

Page	"Individualized" Report												
1	Summarizes your reported values for the nominal 55 mmol/L control solution and the SRM 970 Level 1 and 2 samples distributed in RR11 through RR14.												
2	Graphical summary of your RR 14 sample measurements.												
3	Graphical summary of your RR 14 control solution measurements.												
Page	"All Lab" Report												
1	A listing of all results and statistics for Total Ascorbic Acid [TAA] in the RR14 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.												

**Samples.** Four unknowns and a control material (ascorbic acid) were distributed in RR14. . Serum sample 1 was prepared (04/93) as sample 180a. Serum sample 2 was prepared (03/95) as sample 188b. Sera samples 3 and 4 were prepared (05/98) as SRM 970, Level 1 and Level 2, respectively. Each serum sample was prepared by adding ascorbic acid to a serum pool that was depleted of ascorbic acid.

### **Qualitative Observations.**

- 1) Too few sets of data have been returned to place a great deal of faith in the statistical summaries. If the program is to continue, the number of active participants must be increased. Suggestions are more than welcome!
- 2) Many participants that reported "Not detected" for the "blank" (S14-2) sample had significantly non-zero intercepts for their external calibration curve. While these intercepts averaged about 1 μmol/L (the expected value for [TAA] in S14-2), they ranged as high as 13 μmol/L. Future exercises will request a "true" 5% MPA blank to help sort things out.
- 3) Almost all participants correctly prepared the control solutions, although one participant appears to be using about 3% MPA and one about 10%. While the actual concentration of MPA in the nominal 5% is not believed to be a critical parameter, these two participants reported the most extreme (low and high) molar extinction coefficients (E<sup>1%</sup>). These differences may influence calibrant purity corrections.
- 4) Based upon 1% to 10% differences between reported and calculated E1% values, several participants based their calculations on volume rather than weight of stock solution delivered to control solution #1. In general, 0.500 g gravimetric measurements are more accurate than are 0.500 mL volumetric measurements. These differences may influence calibrant purity corrections.
- 5) All participants are now reporting "reasonable" maximum absorbance values ( $OD_{max}$ ) for the nominal 55 µmol/L control solution #1. However, several participants report the location of maximal absorbance 1 nm to 2 nm offset from its nominal location of 244 nm. For most accurate results, spectrophotometers require periodic calibration of both their absorbance and wavelength axes.

### Quantitative Results.

1) There is no sign of degradation (change in median [TAA] or increase in estimated standard deviation) in either SRM 970 Level 1 or Level 2 since their Certification in 1998. We will continue to periodically monitor these materials.

### Appendix G. "All-Lab Report" for RR14

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.

Vitamin C 'Round Robin' 14 Laboratory Results

	E <sub>1%</sub>	dL/g·cm	548	266	572	268	584	269		256	527	538	542	292	543	572	12	222	527	543	561	268	584	18	3.2
Ctrl1 Properties	ODmax	AU	0.5409	0.5908	0.5773	0.6280	0.5870	0.5461		0.5617	0.5090	0.5520	0.5369	0.5215	0.5470	0.5643	12	0.558	0.509	0.540	0.550	0.580	0.628	0.030	5.4
Ctrl1 P	$A_{max}$	nm	243.0	245.0	243.0	243.6	243.6	243.0		243.0	242.0	244.0	244.0	243.0	243.0	243.6	12	243.4	242.0	243.0	243.0	243.7	245.0	0.44	0
	$D_{5\%MPA}$	g/mL	1.034	1.033	1.032	1.032	1.032	1.033	1.035	1.032	1.043	1.032	1.031	1.034	1.029	1.031	13	1.033	1.029	1.032	1.032	1.034	1.043	0.001	0.1
A] <sub>calc</sub> )	14-Ctrl3	lution	13.83	15.38	14.60	13.42	10.91	13.97	15.30	15.45		15.25		13.73	23.63	13.31	11	15.0	10.91	13.78	14.60	15.34	23.63	1.15	∞
[AA] <sub>nom</sub> ([AA] <sub>obs</sub> / [AA] <sub>calc</sub> )	14-Ctrl2	Nominal µmol/L solution	26.54	29.32	30.72	26.83	24.49	27.59	33.67	29.50		28.67		27.11	36.12	25.79	11	29.1	24.49	26.97	28.67	30.11	36.12	2.71	6
[AA] <sub>nom</sub> (	14-Ctrl1	Nomina	56.03	57.54	80.09	54.03	55.35	54.95	78.76	54.47	53.78	57.17		53.49	58.25	52.58	12	57.8	53.49	54.36	55.69	57.72	78.76	2.60	2
	S14:4		26.0	30.2	33.0	25.7	27.5		31.4	23.7	24.8	28.5	25.7		21.0	27.1	11	27.0	21.0	25.2	26.0	29.4	33.0	3.4	13
rbic Acid	S14:3	olution	7.8	9.8	11.4	6.5	9.1	7.9		8.1	7.9	9.0	7.9	9.2	12.2	9.1	12	8.9	6.5	7.9	8.6	9.6	12.2	1.1	13
Total Ascorbic Acid	S14:2	µmol/L solution	0.00	3.60	6.32	0.04	0.00	0.00	0.00	0.26	1.49	0.00	0.00		0.00	1.7	12	1.0	0.00	0.00	0.00	0.57	6.32	0.00	
Tc	S14:1		20.0	23.0	26.0	18.7	18.0	19.8	29.3	16.7	18.7	20.5	19.0	22.8	36.7	20.6	13	22.2	16.7	18.7	20.0	23.0	36.7	3.0	15
		Method		obas Fara		luoro	onpair	OPD				4		DAB			z	Average	Min	%52	Median	%75	Max	eSD	eCV
		Me	HPLC-EC	AO-OPD, Cobas Fara	HPLC-EC	Cobas Bio Fluoro	HPLC-UV-ionpair	HPLC-Fluor OPD	HPLC-UV	HPLC-OPD	HPLC-EC	HPLC-OPD/	HPLC-EC	HPLC-Fluor DAB	24DNPH	HPLC-EC									
		Date	27/09/01	26/03/01	02/05/01	17/05/01	02/03/01	11/04/01	11/05/01	15/02/01	05/06/01	13/03/01	13/04/01	23/04/01	27/04/01	18/04/01									
		Lab	VC-MA	VC-MB	VC-MC	VC-ME	VC-MG	VC-MH	VC-MI	VC-MO	VC-MQ	VC-MR	VC-MS	VC-NH	VC-NK	NIST									

# Appendix H. Representative "Individualized Report" for RR14

Each participant in RR14 received an "Individualized Report" reflecting their reported results. The following three pages are the "Individualized Report" for participant "VC-MA".

# Vitamin C 'Round Robin' 14 Report: Participant VC-MA

Set 1 of 13

				₹	MPA	Stock	AA MPA Stock MPA [AA] µmol/L	[AA] µm	J/Joi	A <sub>243</sub>	$A_{244}$	$A_{245}$	E <sup>1%</sup> max
Ф	Method	RR	Control	mg	ರಾ	mg	D	Calc	sqC	ОО	ОО	ОО	dL/gcm
86/8	HPLC-EC (Height)	1	55 mmol/L	200.0	200.0 103.09	526.0	526.0 102.45 57.8 6.1	57.8	6.1	0.0525	0.0525 0.0527		0
04/02/99 F	HPLC-EC (Height)	12	55 µmol/L	215.0	215.0 103.10 517.1	517.1	102.5	61.1	53.1	52 61.1 53.1 0.0721 0.0721	0.0721		29
7/01	HPLC-EC	13	55 µmol/L	200.8	200.8 103.16 508.0	508.0	102.5	26.0	55.4	0.5650	0.5232		572
701	HPLC-EC (Height)	4	55 µmol/L, Crtl-1	200.3	200.3 103.23	510.0	102.3	56.1	57.1	57.1 0.5409 (	0.5193		548
													297 ±305

Grand Average	Mean SD <sub>repeat</sub> SD <sub>reprod</sub>	7.8 0.3 0.5						25.6 1.1 1.7							
	$SD_{dup}$	9.0	0.2	0.5	0.3	0.1	0.2	1.1	1.8	1.3	9.0	0.1	0.5	9.0	0.0
[TAA] µmol/L <sub>sample</sub>	Mean 3	7.4	7.1	9.7	7.8	8.5	7.9	24.6	25.7	23.9	23.5	27.7	26.0	20.0	0.0
	Factor	0.5	0.5	0.5	0.5	1.0	1.0	0.5	0.5	0.5	0.5	1.0	1.0	1.0	1.0
	$Rep_2$	13.9	14.5	15.8	15.1	8.5	7.8	47.7	54.0	45.9	47.9	27.7	26.4	19.6	
	Rep <sub>1</sub>	15.5	14.0	14.5	16.1	8.4	8.0	20.7	48.8	49.5	46.2	27.6	25.7	20.4	
	Sample	SRM Lv 1, A	SRM Lv 1, B	SRM Lv 1, A	SRM Lv 1, B	SRM Lv 1, S13-1	SRM Lv 1, S14-3	SRM Lv 2, A	SRM Lv 2, B	SRM Lv 2, A	SRM Lv 2, B	SRM Lv 2, S13-2	SRM Lv 2, S14-4	S14-1 (180B)	S14-2 (188B)
	RR	7	7	12	12	13	4	7	7	12	12	13	4	4	14

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

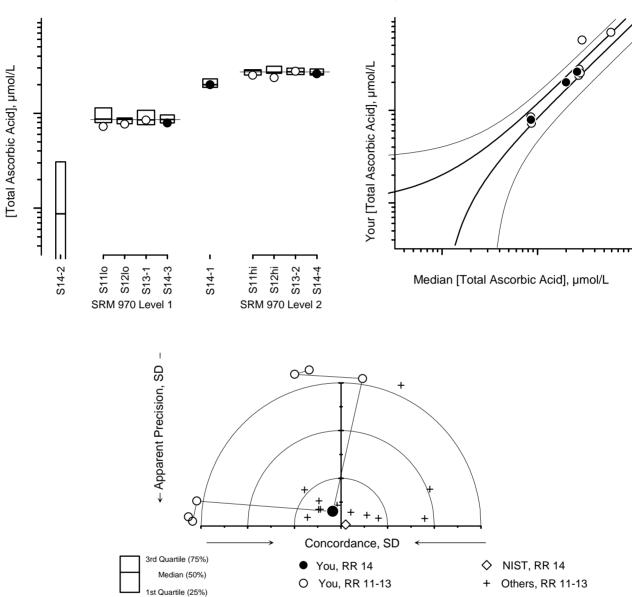
Micronutrients Measurement Quality Assurance Program National Institute of Standards and Technology

100 Bureau Drive Stop 8392 Gaithersburg, MD 20899-8392 USA

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# Vitamin C 'Round Robin' 14 Report: Participant VC-MA

Total Ascorbic Acid: Samples



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.

Comments

### Sample

S14-1 Serum 180B, augmented

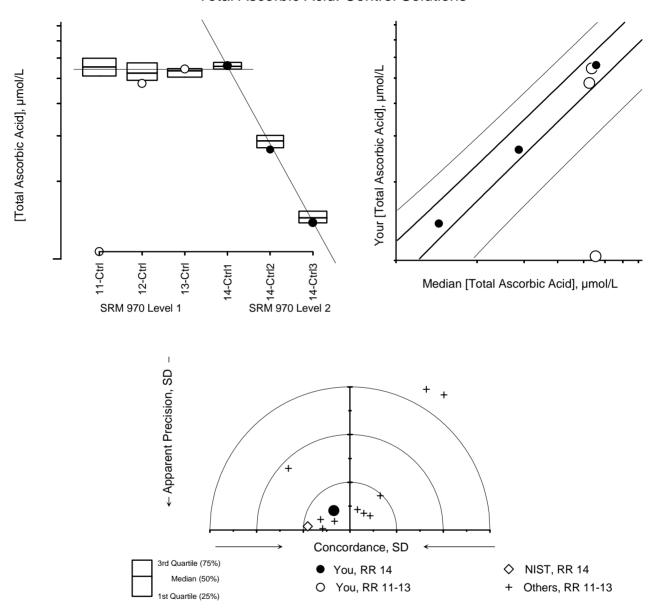
S14-2 Serum 188B, no augmentation

S14-3 SRM 970 Level 1

S14-4 SRM 970 Level 2

# Vitamin C 'Round Robin' 14 Report: Participant VC-MA

### Total Ascorbic Acid: Control Solutions



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

14Ctrl-1 Control Solution 1, nominally 55.0 μm/mL. Displayed Value = (Nominal)(Measured)/(Calculated)

14Ctrl-2 Control Solution 2, nominally 27.5 µm/mL. Displayed Value = (Nominal)(Measured)/(Calculated) 14Ctrl-3 Control Solution 3, nominally 13.75 μm/mL. Displayed Value = (Nominal)(Measured)/(Calculated)