PKV'O ket qpwt kgpwi'O gcuwt go gpv'' S wcrkx{ 'Cunwt cpeg'Rt qi t co '' Y kpvgt '4235'' E qo r ct cdktkx{ 'Uvwf kgu''

Results for Round Robin LXXIII Fat-Soluble Vitamins and Carotenoids in Human Serum and Round Robin 38 Ascorbic Acid in Human Serum



Р **К**/**УКГ** '9:: 2/63

NIST Micronutrients Measurement Quality Assurance Program Winter 2013 Comparability Studies

Results for Round Robin LXXIII Fat-Soluble Vitamins and Carotenoids in Human Serum and Round Robin 38 Ascorbic Acid in Human Serum

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November 2014



U.S. Department of Commerce Penny Pritzker, Secretary

National Institute of Standards and Technology Willie E. May, Acting Under Secretary of Commerce for Standards and Technology and Acting Director (This page intentionally blank)

Abstract

The National Institute of Standards and Technology coordinates the Micronutrients Measurement Quality Assurance Program (MMQAP) for laboratories that measure fat- and water-soluble vitamins and carotenoids in human serum and plasma. This report describes the design of and results for the Summer 2013 MMQAP measurement comparability improvement studies: 1) Round Robin LXXIII Fat-Soluble Vitamins and Carotenoids in Human Serum and 2) Round Robin 38 Total Ascorbic Acid in Human Serum. The materials for both studies were shipped to participants in January 2013; participants were requested to provide their measurement results by April 1, 2013.

Keywords

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

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Introduction

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

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

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

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

Participants in the MMQAP Fat-Soluble Vitamins and Carotenoids in Human Serum Round Robin LXXIII comparability study (hereafter referred to as RR73) received one lyophilized and four 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 2013. 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 RR73 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 All-Lab 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 the Individualized Report are described in detail elsewhere [3]. An example Individualized Report is reproduced as Appendix D.

Round Robin 38: Vitamin C in Human Serum

Participants in the MMQAP Vitamin C in Human Serum Round Robin 38 comparability study (hereafter referred to as RR38) received four frozen serum test samples and two frozen control sera. 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 2013. The communication materials included in the sample shipment are provided in Appendix E.

The test and control serum materials were prepared by adding equal volumes of 10 % metaphosphoric acid (MPA) to human serum that had been spiked with ascorbic acid. While these samples contain some dehydroascorbic acid, its content is variable. Therefore, the participants report only total ascorbic acid (TAA, ascorbic acid plus dehydroascorbic acid).

The final report delivered to every participant in RR38 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 All-Lab 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 the Individualized Report are described in detail elsewhere [3]. An example Individualized Report is reproduced as Appendix H.

References

- 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 RR73

The following three items were included in each package shipped to an RR73 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.



January 21, 2013

Dear Colleague:

Enclosed are samples for the first fat-soluble vitamins and carotenoids in serum study (Round Robin LXXIII) for the 2013 NIST Micronutrients Measurement Quality Assurance Program. The set of samples (Sera 392- 396) consists of one vial of lyophilized serum and one vial each of four liquid-frozen serum samples for analysis along with a form for reporting your results. These samples should be stored in the dark at or below -20 °C upon receipt. When reporting your results, please submit one value for each analyte for a given serum sample. If a value obtained is below your limit of quantification, please indicate this result on the form by using NQ (*Not Quantified*). Results are due to NIST by April 1, 2013. Results received more than two weeks after the due date may not be included in the summary report for this round robin study. The feedback report concerning the study will be distributed in May 2013.

Samples should be allowed to stand at room temperature under subdued light until thawed. We recommend that sample mixing be facilitated with 3 to 5 min agitation in an ultrasonic bath or at least 15 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.) Water should not be added to the liquid-frozen samples.

For consistency, we request that laboratories use the following absorptivities (dL/g · 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); and lycopene, 3450 at 472 nm (hexane).

Please report your results for Round Robin LXXIII by e-mail to david.duewer@nist.gov or fax to 301-977-0685. If you have questions or comments regarding this study, please call me at (301) 975-3120 or email me at jbthomas@nist.gov

Sincerely.

Jeanice Brown Thomas Program Coordinator/Research Chemist: Analytical Chemistry Division Material Measurement Laboratory



Date: _____

Round Robin LXXIII: Human Sera

NIST Micronutrients Measurement Quality Assurance Program

Analyte	392	393	394	395	396	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						
total lycopene						
trans-lycopene						
total β-cryptoxanthin						
total α -cryptoxanthin						
total lutein						
total zeaxanthin						
total lutein&zeaxanthin						
total coenzyme Q10						
ubiquinol (QH ₂)						
ubiquinone (Qox)						
phylloquinone (K ₁)						
25-hydroxyvitamin D						
Phytoene						
Phytofluene						

* we prefer μg/mL

Were the samples frozen when received? Yes | No

Comments:

Fat-Soluble Vitamins Round Robin LXXIII NIST Micronutrients Measurement Quality Assurance Program

Packing List and Shipment Receipt Confirmation Form

This box contains: one vial each of the following five FSV M²QAP sera

Serum	Form	Reconstitute?	Vial/Cap
#392	Lyophilized	Yes	5 mL clear / silver
#393	Liquid frozen	No	5 mL clear / silver
#394	Liquid frozen	No	2 mL amber / blue
#395	Liquid frozen	No	2 mL clear / green
#396	Liquid frozen	No	2 mL clear / black

Please 1) Open the pack immediately

- 2) Check that it contains all of the above samples
- 3) Check if the vials are intact
- 4) Store the sera at -20 °C or below until analysis
- 5) Email (david.duewer@nist.gov) or fax (301-977-0685) us the following information:

1) Date this shipment arrived: _____

- 2) Are all five sera vials intact? Yes | No If "No", which one(s) were damaged?
- 3) Was there any dry-ice left in cooler? Yes | No
- 4) Did the samples arrive frozen? Yes | No
- 5) At what temperature are you storing the serum samples? _____ °C
- 6) When do you anticipate analyzing these samples? _____

Your prompt return of this information is appreciated.

The M²QAP Gang

Appendix B. Final Report for RR73

The following three pages are the final report for RR73 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,
 - 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.



May 16, 2013

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

Dear Colleague:

Enclosed is the summary report of the results for "Round Robin" LXXIII (RR73) of the 2013 NIST Micronutrients Measurement Quality Assurance Program (M²QAP) for the fat-soluble vitamins and carotenoids in human serum. Included in this report are: 1) a summary of data and measurement comparability scores for all laboratories, 2) a detailed graphical analysis of your results; and 3) a graphical summary of your measurement comparability. RR73 (Sera 392 - 396) consisted of one vial of lyophilized serum and one vial each of four liquid-frozen serum samples. Details regarding the samples can be found in the enclosed report.

Your overall measurement comparability is summarized in the "Score Card" summary, page 7 of the All Lab Report. Combined results rated 1 to 3 are within 1 to 3 standard deviations of the assigned value, respectively; those rated 4 are >3 standard deviations from the assigned value. Similar information is presented graphically in the "target plots" that are the last page of your Individualized Report. If you have concerns regarding your laboratory's performance, please contact us for consultation.

Samples for the second set of fat-soluble vitamins and carotenoids in serum interlaboratory exercise (RR74) will be shipped starting June 10, 2013. Please contact us immediately if this schedule is problematic for your laboratory.

We are currently in the process of providing on-line documentation of data and reports from the MMQAP studies (past and present). The results for Round Robin 71 Fat-Soluble Vitamins and Carotenoids in Human Serum and and Round Robin 36 Ascorbic Acid in Human Serum are available at: http://www.nist.gov/manuscript-publication-search.cfm?pub_id=911458. Click on the DOI link to access the manuscript. Data summaries have been altered to ensure confidentiality of identification codes assigned to laboratories. We will update you as additional on-line documents become available.

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

Sincerely,

Jeanice Brown Thomas, M.B.A. Research Chemist Analytical Chemistry Division Material Measurement Laboratory

Enclosures

Cc: L.C. Sander

David L. Duewer, Ph.D. Research Chemometrician Analytical Chemistry Division Material Measurement Laboratory



The NIST MMQAP Round Robin LXXIII	(RR73) report consists of:
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Page	All-Lab Report
1-5	A listing of all results and statistics for analytes reported by more than one participant.
6	A listing of the analytes reported by only one participant and a legend for the list of results and statistics.
7	The text Comparability Summary ("Score Card") of measurement performance.
Page	Individualized Report
Page 1	Individualized Report Your values, the number of labs reporting values, and our assigned values.
Page 1 2 to n	Individualized Report Your values, the number of labs reporting values, and our assigned values. "Four Plot" summaries of your current and past measurement performance, one page for each analyte you report that is also reported by at least eight other participants.

Samples. Five samples were distributed to each participant in RR73. Unfortunately, the sample distributed as Serum 394 was a mixture of two materials. Fortunately, the two materials have distinctive levels for several analytes and we have been able to unambiguously identify who got which material. We have *post-facto* designated these two materials Serum 394a and Serum 394b. The All Lab Report therefore lists values for six samples. The Individualized Reports for every participant reflect the results just for the five materials that they evaluated.

Serum	Description	Prior Distributions
392	Lyophilized, multi-donor stripped serum augmented with retinol, retinyl palmitate, α - and γ -tocopherol, α - and β -carotene, lycopene, β -cryptoxanthin, lutein, and zeaxanthin prepared in 1995.	#224:RR38-9/96
393	Liquid-frozen, multi-donor heparin-treated plasma material augmented with glycyrrhetinic acid prepared in 1989.	First MMQAP distribution
394a	Fresh-frozen, native, single donor serum prepared in 1999.	#271:RR49-3/01, #275:RR50-9/01, #279:RR51-3/02, #296:RR54-9/03, #308:RR56-9/04
394b	Liquid-frozen, multi-donor serum augmented with <i>trans</i> - β -carotene in a lipoprotein carrier prepared in 2008.	#353:RR65-3/09, #371:RR68-9/10
395	Fresh-frozen, native, multi-donor serum prepared in 2011. This material has relatively high contents of α - and β -carotene.	#382:RR71-3/12
396	Fresh-frozen, native, multi-donor serum prepared in 2011.	#385:RR71-3/12

Results

- Serum 392. This serum was constructed to test whether it was practical to spike sera with very lipophilic analytes. While we did not fully achieve the targeted levels for the carotenoids, the augmentation method used appeared to be relatively effective. This distribution of the material was intended to access the material's stability over time and storage. The median value for many, but not all, of the analytes is lower than that of 18 years ago. We speculate that due to the aging of the material, the augmented analytes in this lyophilized material have become more difficult to reconstitute and/or extract.
- 2) Serum 393. This material was prepared as part of an investigation into the chemopreventative potential of glycyrrhetinic acid (the main metabolite of the glucoside glycyrrhizin obtained from licorice root, *Glycyrriza glabra*). This relatively polar compound's absorbance maxima is at 248 nm and should not interfere with the analysis of the usual MMQAP analytes. However, if any of your results are uniquely high in Serum 393, please check the chromatogram for evidence of interference. If you find such evidence, please contact JBThomas@nist.gov.
- 3) <u>Sera 394a and 394b</u>. We apologize for the confusion. We unintentionally combined amber vials with blue caps that were labeled "271" and "371". The median levels and variability of the results for both "Serum 394" materials are essentially unchanged from their prior levels.
- 4) <u>Sera 395 and 396</u>. The median levels and variability of the results for both of these relatively new materials are essentially unchanged from their prior levels.

Appendix C. "All-Lab Report" for RR73

The following seven pages are the "All-Lab Report" for RR73 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.

/mL	395 396	1.42 1.90	1.41 1.75		1.30 1.60		1.41 1.84	1.37 1.70	1.36 1.81						1.50 1.80					1.42 1.80	1.43 1.85	1.21 1.71	1.35 1.85	1.46 1.98		1.39 1.86	0.92 1.23	1.51 1.89	1.12 1.70				1.40 1.75		1.35 1.76	18 18	0.92 1.23	1.39 1.80	1.51 1.98	D.06 0.08	4 5	16 16	1.36 1.77	
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	396	7.11 4	7.14 3	6 10	7.80 3	6.40	7.53 4	7.15 4	7.87 4	6.80	7.80	8.70	6.46	7.13	7.70 3	7.72	7.00	7.40		7.21 3	7.63 4	7.04 3	7.15 4	9.49 4	3.96	7.04 3	6.57 2	7.40 4	8.50 3		8.00		7.73 3	7.30	7.53 4	31	3.96 2	7.30 3	9.49 4	0.59 0	8	29	7.51 3	
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	Lab	FSV-B	FSV-B	FSV-B	FSV-B	FSV-B	FSV-B	FSV-B	FSV-E	FSV-B	FSV-B	FSV-BI	FSV-B	FSV-BN	FSV-B	FSV-B	FSV-B	FSV-B	FSV-B	FSV-B	FSV-B	FSV-B	FSV-B	FSV-C	FSV-C	FSV-C	FSV-(ESV-C	FSV-C	FSV-D	FSV-D	FSV-E	FSV-E	FSV-F	FSV-F		Z	Media	Ma	eS	eC	Npat	Median _{pa}	0

All Lab Report

Page 1 / 7

		ð-Toc	opherol,	, µg/mL			Total (3-Caroter	ne, µg/m	_		trai	ıs-β-Caro	tene, µg	/mL		Total c	cis-β-Carot	tene, µg	/mL	
Lab	392	393 3	94a 39	14b 395	396	392	393	394a 3	94b 3	95 3.	96 3	92 393	3 394a	394b	395 396	392	393	394a 3	94b 3	95 3	96
FSV-BA	0.749	0.040 0	.117	0.08	3 0.126	0.299	0.019	0.332	Ö	.636 0.	.052 0.	286 0.01	4 0.309	0	.597 0.04	0.013	0.005	0.023	0	039 0.	200
FSV-BB	0.686	0.046 0	.129	0.09	3 0.093	0.323	0.014	0.319	Ö	.705 0.	.048 0.	311 0.01	1 0.301	0	.668 0.04	0.011	0.003	0.018	Ö	037 0.	001
FSV-BC																					
								c		0 023	050										
FSV-BE						0.108	20.02		255 0.	39.3 0.	050										
FSV-BG						0.350	0.016	0.372	0	0 969	057										
FSV-BH						0.381	bu	0.338	o o	739 0.	.046										
FSV-BJ						0.192	bu	0.280	0	.555 0.	.034										
FSV-BK																					
FSV-BL																					
FSV-BM																					
FSV-BN						0.293	0.012	0.318	Ó	.0 609	.045										
FSV-BNa						0.282	0.031	0.307	Ö	564 0.	.047 0.	230 0.01	7 0.250	0	.468 0.03	3 0.052	0.014	0.057	0	006 0.	014
FSV-BO						0.363	0.013	Ö	311 0.	616 0.	.053										
FSV-BP						0.222	0.053	0.203	Ö	651 0.	.329										
FSV-BQ																					
FSV-BR																					
FSV-BS						≥0.443	₹ bu	÷0.448	02	.762 ≥	0.13 0.	443 ng	0.448	0	.762 0.130						
FSV-BT	0.485	0.079 0	.111	0.09	0 0.070	0.360	0.015	0.328	Ö	.683 0.	.053 0.	317 0.01	3 0.281	0	.582 0.048	3 0.012	bu	0.017	Ö	037 0.	001
FSV-BU						0.335	0.018	0.316	Ó	664 0.	.065										
FSV-BV						0.240	0.019	Ö	.249 0.	545 0.	.048										
FSV-BW						0.221	0.006	0.388	Ö	.643 0.	.065										
FSV-CD						0.427	0.066	Ö	.387 0.	.801 0.	.114										
FSV-CE	bu	bu	0.1	167 0.65	bu 6																
FSV-CG	0.629	0.128	0.1	167 0.13	9 0.141	0.328	0.015	Ö	.294 0.	.667 0.	.054 0.	317 0.01	3	0.278 0	.626 0.050	0.011	0.005	Ö	.016 0.	040 0.	005
FSV-CI						0.259	0.009	0.343	Ö	.720 0.	.041										
FSV-CO						0.347	0.021	0.354	Ö	.705 0.	.061										
FSV-CZ						0.632	0.088	Ö	.279 0.	.674 0.	.120										
FSV-DD																					
FSV-DV																					
FSV-EE																					
FSV-EZ						0.473 <	:0.059	0.321	Ő	.593 <0.	059										
FSV-FK									c												
77-72						180.0	0.020	0.3/ 0	D	0 021	0/0	,			,					1	
z	4	4	က	0	5	22	17	15	2	22	21	9	5	~	9	2	4	4	-	2	5
MIN	0.485	0.040 0	.111 0.	167 0.08	3 0.070	0.108	0.006	0.203 0	.249 0	.393 0	.034 0.	230 0.01	1 0.250	0	.468 0.03	3 0.011	0.003	0.017	Ö	037 0	.001
Median	0.658	0.062 0	.117 0.1	167 0.09	3 0.109	0.325	0.018	0.328 0	.294 0	.666 0.	.053 0.	314 0.01	3 0.301	0.278 0	.612 0.04	0.012	0.005	0.020 0.	.016 0.	039 0	.005
Max	0.749	0.128 0	.129 0.1	167 0.65	9 0.141	0.632	0.088	0.388 0.	.387 0	.801 0.	.329 0.	443 0.01	7 0.448	0	.762 0.130	0.052	0.014	0.057	Ö	0 960	.014
eSD	0.089	0.029		0.01	5 0.035	0.073	0.006	0.022 0.	.025 0	.066 0.	.011 0.	023 0.00	2 0.030	0	.064 0.00	t 0.001	0.002	0.004	Ö	002 0	.005
eCV	14	46		~	6 32	23	35	7	6	10	20	7	3 10		10	3 10	37	21		5	107
Npast	5	0	9	4	4 4	32	0	29	21	19	18	12	0 13	7	2	8	0	6	9	4	0
Medianpast	0.750	Ó	.132 0.0	387 0.08	1 0.067	0.624		0.350 0.	.305 0.	.653 0.	.055 0.	587	0.323	0.288 C	.634 0.05	t 0.027		0.024 0.	.016 0.	038	
SDpast	0.119	0	.053 0.0	0.06	5 0.048	0.103		0.040 0.	.053 0.	.122 0.	.007 0.	103	0.027	0.027 0	.126 0.00	t 0.019		0.007 0.	.003 0.	005	
NAV	0.658	0.062 0	.117	0.09	3 0.109	0.325	0.018	0.328 0.	.294 0.	.666 0.	.053 0.	314 0.01	3 0.301	0	612 0.04	0.012	0.005	0.020	0	039 0	005
NAU				0.02	3	0.073	0.008	0.047 0	.043 0	.0 060.	.011 0.	034 0.00	7 0.032	0	.064 0.00	0.004			Ö	015 0.	.005

		Total (α-Carot	tene, µ	g/mL			Tota	Lycope	ne, µg/i	шĻ		tra	ans-Lyc	opene, µ	g/mL		Total	3-Crypto	oxanthin,	hg/mL	
Lab	392	393	394a	394b	395	396	392	393	394a 3	394b	395 3	96	392 39	3 394	a 394b	395	396	392 393	394a	394b	395 3	396
FSV-BA	0.372	0.004	0.030		0.340	0.020	0.299	0.135	0.307	0	0.260 0.	214 0	204 0.07	71 0.17	۲.	0.130	0.104	0.059 0.01	1 0.061	-	0.071 0	.101
FSV-BB	0.347	0.003	0.025		0.343	0.018	0.296	0.125	0.289	0	.284 0.	208 0	173 0.05	59 0.13	12	0.110	0.085	0.051 0.01	0 0.050	-	0.066 0	.091
FSV-BC FSV-BD																						
FSV-BE																						
FSV-BF																						
FSV-BG	0.344	0.008	0.033		0.326	0.024	0.338	0.147	0.340	0	.280 0.	248 0	230 0.07	78 0.15	5	0.138	0.123	0.055 0.01	1 0.054		0.067 0	.103
FSV-BH	0.466	bu	0.022		0.367	0.018	0.469	0.165	0.387	0	.352 0.	272						0.082 nq	0.067		0.088 0	.126
FSV-BJ FSV-RK	0.413	bu	bu		0.383	bu	0.305	0.201	0.328	0	.276 0.	258						bu bu	bu	•	0.066 0	.080
					0.055			77	220.0	C	0 200	764									0 130 0	100
FSV-BNa	0.374	con.o	0.041		0.333	0.023	0.327	0.164	0.369	00		284 0	153 0.06	38 0.18	0	0.127	0.120	0.044 0.01	7 0.048		0.057 0	.082
FSV-BO																						
FSV-BP	0.051	pu	0.010		0.350	0.024	0.742	0.220	0.143	0	0.271 0.	232						0.094 0.09	0 0.046		0.062 0	.143
FSV-BQ																						
FSV-BR																						
FSV-BS	0.620	0.073	0.068		0.432	0.041	0.565	0.267	0.815	0 (0.399 0.	452 0	315 0.13	36 0.34	φ	0.159	0.188	0.123 0.06	9 0.185		0.124 0	.190
FSV-BI	0.417	0.007	0.031		0.347	0.026	0.315	0.123	0.297		.292 0.	219 0	168 0.05	0.13	5	0.121	0.099	0.057 0.01	2 0.053		0.058 0	G 60.
FSV-BU	0.434	0.005	0.026		0.376	0.029	0.335	0.133	0.305	0	.274 0.	231						0.052 0.01	2 0.056	-	0.071 0	.092
FSV-BV	0.355	0.006		0.026	0.340	0.023	0.242	0.099	0	0.362 0	.224 0.	206						0.029 0.00	ő	0.058	0.042 0	.071
FSV-BW	0.684	0.015	0.017		0.540	0.015	0.275	0.136	0.332	0	.226 0.	234										
FSV-CD	0.597	bu		0.050	0.525	0.033	0.385	0.152	0	0.543 0	.309 0.	280						0.081 0.01	o	0.128	0.105 0	.124
FSV-CE																						
FSV-CG	0.471	0.005		0.042	0.459	0.030	0.367	0.154	0	0.513 0	.340 0.	273 0	264 0.08	33	0.274	0.157	0.131	0.069 0.02	0	0.129	0.104 0	.129
FSV-CI	0.333	0.008	0.028		0.338	0.020																
FSV-CO							0.376	0.140	0.336	0	0.276 0.	239										
FSV-CZ																						
FSV-DD																						
FSV-DV																						
FSV-EE																						
FSV-EZ																						
FSV-FK FSV-FZ																						
z	16	11	12	e	16	15	16	16	13	e	16	16	7	7	6 1	7	2	13 1	2 10	e	14	14
Min	0.051	0.003	0.010	0.026	0.326	0.015	0.242	0.099	0.143 0	0.362 0	0.224 0.	206 0	153 0.05	56 0.13	12	0.110	0.085	0.029 0.00	6 0.046	0.058	0.042 0	.071
Median	0.403	0.006	0.029	0.042	0.353	0.023	0.331	0.144	0.332 0	0.513 0	0.282 0.	243 0	204 0.07	71 0.17	6 0.274	0.130	0.120	0.057 0.01	2 0.054	0.128	0.066 0	.098
Max	0.684	0.073	0.068	0.050	0.540	0.041	0.742	0.267	0.815 0	0.543 0	.399 0.	452 0	315 0.13	36 0.34	œ	0.159	0.188	0.123 0.09	0 0.185	0.129	0.124 0	.190
eSD	0.086	0.002	0.008		0.025	0.005	0.052	0.022	0.052	0	0.035 0.	039 0	053 0.0'	17 0.03	80	0.014	0.024	0.018 0.00	5 0.008		0.013 0	.025
eCV	21	41	29		7	21	16	15	16		12	16	26	24	2	10	20	32 4	3 14		20	25
Npast	28	0	24	17	15	15	28	0	24	16	13	13	8	0	1 8	9	9	20	0 24	17	14	14
Medianpast	0.428		0.030	0.033	0.384	0.025	0.377		0.353 0	0.506 0	0.278 0.	237 0	288	0.18	6 0.234	0.132	0.119	0.060	0.054	0.089	0.065 0	.106
SD _{past}	0.082		0.007	0.007	0.117	0.004	0.086		0.054 0	0.078 0	0.053 0.	056 0	.063	0.04	1 0.028	0.021	0.022	0.015	0.009	0.013	0.005 0	.034
NAV	0.403	0.006	0.029	0.042	0.353	0.023	0.331	0.144	0.332 0	0.513 0	.282 0.	243 0	204 0.07	71 0.17	6	0.130	0.120	0.057 0.01	2 0.054	0.128	0.066 0	.098
NAU	0.100	0.003	0.010		0.088	0.008	0.074	0.038	0.074	0	0.065 0.	058 0	.053 0.0	17 0.03	88	0.023	0.024	0.018 0.00	5 0.013		0.016 0	.025

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	μ	otal α-(Cryptox	anthin,	ng/mL			Tota	al Lutein, _I	Jm/pr			Total Zea	axanthin,	ng/mL		Tot	al Lutei	n&Zeaxantl	hin, µg/n	Ļ
Lab	392	393	394a	394b	395	396	392	393	394a 394	tb 395	396	392	393 39	4a 394b	395	396	392	393 3	394a 394t	395	396
FSV-BA FSV-BB	0.046	0.006 0.007	0.031 0.023		0.026 0.020	0.014 0.015	0.128	0.031	0.097	0.15	7 0.097	0.083 (0.014 0.0	043	0.043	0.042	0.179 (0.211 (0.033 (0.045 (0.0	0.103 0.140	0.151 0.199	0.105
FSV-BC FSV-BD																					
FSV-BE FSV-BF																					
FSV-BG FSV-BH							0.116	0.015	0.077	0.12	3 0.069	0.093 (0.0 0.0	33	0.027	0.035	0.193 (0.209 (0.040 (0.025 (0.0	0.118 0.110	0.158 0.015	0.116
FSV-BJ							0.144	bu	0.081	0.13	5 0.085										
FSV-BK																					
FSV-BM																					
FSV-BN																	0.196 (0.032		0.11	0.105
FSV-BNa							0.143	0.040		0.13	3 0.087	0.064 (0.020		0.028	0.032	0.208 (0.061		0.161	0.119
FSV-BP																	0.160 (0.152 (0.096	0.150	0.113
FSV-BQ																					
FSV-BS							0.212	0.054	0.258	0.25	2 0.170	0.126 (0.095 0.1	121	0.094	0.102	0.338 (.149 (.379	0.346	0.272
FSV-BT	0.040	0.006	0.024		0.022	0.019								·			0.201	0.042 (0.110	0.165	0.119
FSV-BU																	0.216 (0.197 (0.040 (0.118 0.21	3 0.178 3 0.181	0.124
FSV-BW																	20.0	040.	1.0	5	5
FSV-CD	0.064	0.011	-	0.058	0.039	0.027											0.284 (0.086	0.28	8 0.238	0.201
FSV-CG																	0.237 (0.051	0.25	1 0.229	0.158
FSV-CI							0.135	0.019	0.070	0.12	9 0.068	0.077 (0.011 0.0	121	0.015	0.024	0.212 (030 0	.091	0.144	0.092
רט-יעכד FSV-CZ																					
FSV-DD																					
FSV-DV																					
FSV-EZ FSV-EZ																					
FSV-FK FSV-FZ																					
z	4	4	с	-	4	4	9	5	5	0	6 6	5	5	4) 5	5	14	14	о	3 14	14
Min	0.040	0.006	0.023		0.020	0.014	0.116	0.015	0.070	0.12	3 0.068	0.064 (0.009 0.0	121	0.015	0.024	0.160 (0.025 (0.091 0.21	3 0.015	0.092
Median	0.043	0.007	0.024	0.058	0.024	0.017	0.139	0.031	0.081	0.13	4 0.086	0.083 (0.014 0.0	338	0.028	0.035	0.209 (0.041 (0.110 0.25	1 0.163	0.119
Max	0.064	0.011	0.031		0.039	0.027	0.212	0.054	0.258	0.25	2 0.170	0.126 (0.095 0.1	121	0.094	0.102	0.338 (0.152 (0.379 0.28	8 0.346	0.272
eSD	0.005	00000			0.004 16	0.003	0.012 8	0.017 56	0.016 20	0.01	2 0.021 9 24	0.015 (18	0.008 0.0 56)16 43	0.019 69	0.011 31	0.018 o	0.016 ().012 11	0.027	0.021
Nnast	4		9	ŝ	5 4	4	0 T	0	16	œ	• 4 • 4	; ;	0	14	0		20	0	23 1.	7	4
Medianpast	0.042		0.025 (0.031	0.023	0.021	0.148		0.089 0.1	24 0.13	7 0.086	0.082	0.0	0.061	_		0.222	U	0.119 0.18	2 0.160	0.120
SDpast	0.006		0.005	0.008	0.008	0.010	0.030		0.019 0.0	22 0.02	2 0.024	0.012	0.0	07 0.021	_		0.044	0	0.019 0.02	9 0.062	0.044
NAV NAU	0.043	0.007	0.024		0.024	0.017	0.139 0.026	0.031	0.081 0.016	0.13 0.02	4 0.086 5 0.021	0.083 0.022 0).014 0.0).008	338	0.028 0.019	0.035 0.011	0.209 (0.044 ().041 ().016 (0.110 0.25 0.023	1 0.163 0.034	0.119
						1															

		Coer) zyme	Q10, µ	g/mL			Phyllo	quinone	e (K1),	Jm/pr	
Lab	392	393	394a	394b	395	396	392	393	394a	394b	395	396
FSV-BA												
	, ,	500			040	5				0300	1 665	
FSV-BF	1.12	0.00		0.880	0.70	1.02	1.203	0.333		0.009	4.000	0.2/4
ESV-RG												
FSV-BH												
	1 03	02.0	0 850		0 71	1 15						
ESV-RK	c0.1	00	000.0		0	<u>.</u>						
FSV-BN												
FSV-BNa												
FSV-BO	0.80	0.49		0.890	0.62	0.79						
FSV-BP												
FSV-BQ												
FSV-BR												
FSV-BS												
FSV-RT												
PSV-BV					i							
FSV-BW	1.33	0.65	0.840		0.71	1.08						
FSV-CD												
FOV-CE												
FSV-CG												
FSV-CI	0.73	0.48	0.670		0.53	0.77	0.613	0.161	0.504		1.727	0.125
FSV-CO												
FSV-CZ	1.08	0.79		1.227	0.94	1.09						
FSV-DD												
FSV-DV												
FSV-EE	0.88	0.56	0.860		0.66	1.02						
FSV-EZ												
FSV-FK												
FSV-FZ												
z	7	7	4	e	7	7	2	2	-	-	2	2
Min	0.73	0.48	0.670	0.890	0.53	0.77	0.613	0.161			1.727	0.125
Median	1.03	0.65	0.845	066.0	0.71	1.02	0.911	0.280	0.504	0.869	3.196	0.200
Max	1.33	0.79	0.860	1.227	0.94	1.15	1.209	0.399			4.665	0.274
eSD	0.23	0.13	0.015		0.10	0.10						
eCV	22	20	2		15	10						
Npast	0	0	2	8	о	о	0	0	0	0	0	0
Medianeset			0 887	1 044	0.73	0 00						
SD _{past}			0.171	0.136	0.07	0.03						
NAV	1.03	0.65	0.845	066.0	0.71	1.02						
NAU	0.23	0.13	0.0	0000	0.10	0.10						

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Analytes Reported By One Laboratory Values in µg/mL

 Analyte
 Code
 392
 393
 394a
 394b
 395
 396

 25-hydroxyvitamin D
 FSV-BH
 0.021
 0.023
 0.008
 0.021
 0.026

 Phytoene
 FSV-BS
 0.113
 nq
 0.140
 nq
 0.124

 Phytofluene
 FSV-BS
 0.171
 0.068
 0.296
 0.203
 0.196

 trans-Retinol
 FSV-BR
 0.650
 0.225
 0.460
 0.440
 0.450

Legend

Term	Definition
N	Number of (non-NIST) quantitative values reported for this analyte
Min	Minimum (non-NIST) quantitative value reported
Median	Median (non-NIST) quantitative value reported
Max	Maximum (non-NIST) quantitative value reported
eSD	Adjusted median absolute deviation from the median of the non-NIST results
eCV	Coefficient of Variation for (non-NIST) results: 100*SD/Median
Npast	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)
ΝΙΔΥ	
NAV	Modion (NIST)/2 for analyton reported by NIST
	= (Median + MST)/2 for analytes reported by NIST
NIALL	= Median for analytes reported by ≥ 5 rabs but not initial
NAU	NIST Assigned Uncertainty: $\sqrt{S^2 + S_{btw}^2}$
	S is the maximum of (0.05°NAV, SD, SD _{past} , eSD) and Sbtw 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.
nd	Not detected (i.e., no detectable neak for analyte)
na	Detected but not quantitatively determined
11q ~Y	Concentration at or below the limit of quantification x
~~	Concentration at or below the limit of quantification, x
∠×	Discrepant value: damaged sample, interferent, instrument malfunction, etc.
:	Discrepant value. damaged sample, interferent, instrument manufiction, etc.
italics	Not explicitly reported but calculated by NIST from reported values

Comparability Summary

Lab	TR	аT	g/bT	bC	tbC	aC	TLy	TbX	TLu	ΤZ	L&Z	Label	Definition
FSV-BA	1	1	1	1	1	1	1	1			1	Lab	Participant code
FSV-BB	1	1	1	1	1	1	1	1	1	1	1	TR	Total Retinol
FSV-BC	2											aT	α-Tocopherol
FSV-BD	2	2										g/bT	γ/β-Tocopherol
FSV-BE	1	1	1	1								bC	Total β-Carotene
FSV-BF	4	2		3								tbC	trans-β-Carotene
FSV-BG	1	1	1	1		1	1	1			1	aC	Total α-Carotene
FSV-BH	1	1	1	1		1	2	2	1	1	3	TLy	Total Lycopene
FSV-BJ	1	1	1	2		1	1	1	1			TbX	Total β-Cryptoxanthin
FSV-BK	1	1										TLu	Total Lutein
FSV-BL	1	1										TZ	Total Zeaxanthin
FSV-BM	2	2										L&Z	Total Lutein & Zeaxanthin
FSV-BN	1	2		1		1	1	1			1		
FSV-BNa	1	1		2	2	1	1	1	1	1	1	n	number of participants providing quantitative data
FSV-BO	1	1	1	1								% 1	Percent of CS = 1 (within 1 SD of medians)
FSV-BP	1	2		4		3	4	4			4	% 2	Percent of CS = 2 (within 2 SD of medians)
FSV-BQ	1	2										% 3	Percent of CS = 3 (within 3 SD of medians)
FSV-BR	1	1										% 4	Percent of $CS = 4$ (3 or more SD from medians)
FSV-BS				4	4	4	4	4	4	4	4		
FSV-BT	2	1	1	1	1	1	1	1			1		
FSV-BU	3	2	2	1		1	1	1			1		"Comparability Score"
FSV-BV	4	2	1	1		1	2	2			1	The Compa	arability Score (CS) summarizes your measurement
FSV-BW	1	1	1	2		3	1					performanc	the for a given analyte relative to the consensus
FSV-CD	1	3	2	4		2	1	2			3	standard de	eviation) of your measurement performance
FSV-CE	4	4										characteris	tics from the consensus performance. CS is
FSV-CG	1	2	1	1	1	1	1	2			2	calculated v	when the number of quantitative values you reported,
FSV-CI	1	1	3	1		1			1	1	1	N _{you} , is at le	east two and at least six participants reported
FSV-CO		1	1	1			1					quantitative	values for the analyte.
FSV-CZ	3	2	2	4								We define (CS as follows:
FSV-DD	2											CS = M	$IINIMUM \left(4.INTEGER \left(1 + \sqrt{C^2 + AP^2} \right) \right)$
FSV-DV	2	1											······································
FSV-EZ	4	1	1	3									$\sum_{i=1}^{N_{you}} You_i - Median_i$
FSV-FK	1	1										C = Cor	$ncordance = \frac{\frac{1}{i=1}}{ncordance} NAU_i$
FSV-FZ	3	1	1	2									N _{you}
n	32	31	18	23	6	16	16	14	6	5	14		$\sqrt{\frac{N_{you}}{You}}$ (You, -Median,) ²
		_				-							$\sum_{i=1}^{n} \left(\frac{\sum_{i=1}^{n} \left(\frac{1}{NAU_i} \right) \right)$
.	TR	aT	g/bT	bC	tbC	aC	TLy	TbX	TLu	TZ	L&Z	AP = Ap	$Precision = \sqrt{\frac{N_{vol}}{N_{vol}} - 1}$
% 1	59	61	/8	57	67	/5	/5	57	83	80	64 -	NAU =	NIST Assigned Uncertainty
% 2	19	32	1/	1/	1/	6	13	29	0	0	(<u> </u>
% 3	9	3	6	9	0	13	0	0	0	0	14	For further	details, please see
% 4	13	3	0	17	17	6	13	14	17	20	14	Duewer	DL, MILLE MU, STATPLESS KE, BROWN TROMAS J, GAR

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 RR73

Each participant in RR73 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:

- Total Retinol
- Retinyl Palmitate
- α-Tocopherol
- γ/β -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 fourteen pages are the "Individualized Report" for the analytes evaluated by participant FSV-BA.

Set 6 of 35

Individualized Round Robin LXXIII Report: FSV-BA

		c	31	9	31	18	4	20	9	S	44	15	~	13	4	13
	im 396	NAV	0.475	0.02	7.21	1.800	0.109	0.053	0.047	0.005	0.023	0.249	0.120	0.095	0.017	0.119
	Seru	You	0.484	0.03	7.11	1.897	0.126	0.052	0.045	0.007	0.020	0.214	0.104	0.101	0.014	0.105
		c	31	∞	31	18	S	21	9	S	15	15	2	13	4	13
	m 395	NAV	0.463	0.05	11.21	1.387	0.093	0.666	0.612	0.039	0.355	0.288	0.130	0.067	0.024	0.165
	Seru	You	0.452	0.06	10.78	1.416	0.083	0.636	0.597	0.039	0.340	0.260	0.130	0.071	0.026	0.151
	-	c	10		ი	9	2	~	~	~	ო	ო	~	ო	~	ო
	um 394b	NAV	0.317		8.40	2.950		0.294			0.042	0.513		0.128		0.251
	Seru	You														
		c	21	~	22	12	ო	1 4	2	4	5	12	9	ი	ო	ω
	Serum 394a	NAV	0.470	0.0	6.88	1.890	0.117	0.328	0.301	0.020	0.030	0.332	0.176	0.054	0.024	0.114
ımary		You	0.470	0.0	6.95	2.002	0.117	0.332	0.309	0.023	0.030	0.307	0.171	0.061	0.031	0.103
Sun	Serum 393	L	31	5	31	18	4	16	5	4	1	15	7	1	4	13
		NAV	0.243	0.0	4.15	0.800	0.062	0.016	0.013	0.005	0.006	0.144	0.071	0.012	0.007	0.040
		You	0.262	0.0	4.09	0.839	0.040	0.019	0.014	0.005	0.004	0.135	0.071	0.011	0.006	0.033
		L	31	ω	31	18	4	21	9	2	15	15	2	12	4	13
	m 392	NAV	0.675	0.16	8.37	3.913	0.704	0.476	0.450	0.019	0.421	0.352	0.246	0.058	0.042	0.216
	Seru	You	0.724	0.08	8.53	4.331	0.749	0.299	0.286	0.013	0.372	0.299	0.204	0.059	0.046	0.179
		Analyte	Total Retinol	Retinyl Palmitate	α-Tocopherol	γ/β-Tocopherol	ō-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&Zeaxanthin

You : Your reported values for the listed analytes (micrograms/milliliter)

NAV : NIST Assigned Values, here equal to this RR's median

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

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

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 Page 1 / 14

Individualized Report



<u>Serum</u>	<u>Comments</u>	<u>History</u>
#392	Lyophilized, augmented, multi-donor	38#224
#393	Fresh-frozen, native, multi-donor	New
#394a	Fresh-frozen, augmented, single-donor	49#271, 50#275, 51#279, 54#296, 56#308
#394b	Fresh-frozen, augmented, multi-donor	65#353, 68#371
#395	Fresh-frozen, native, multi-donor	71#382
#396	Fresh-frozen, native, multi-donor	71#385



<u>Serum</u>	<u>Comments</u>	<u>History</u>
#392	Lyophilized, augmented, multi-donor	38#224
#393	Fresh-frozen, native, multi-donor	New
#394a	Fresh-frozen, augmented, single-donor	49#271, 50#275, 51#279, 54#296, 56#308
#394b	Fresh-frozen, augmented, multi-donor	65#353, 68#371
#395	Fresh-frozen, native, multi-donor	71#382
#396	Fresh-frozen, native, multi-donor	71#385



<u>Serum</u>	<u>Comments</u>	<u>History</u>
#392	Lyophilized, augmented, multi-donor	38#224
#393	Fresh-frozen, native, multi-donor	New
#394a	Fresh-frozen, augmented, single-donor	49#271, 50#275, 51#279, 54#296, 56#308
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#395	Fresh-frozen, native, multi-donor	71#382
#396	Fresh-frozen, native, multi-donor	71#385



<u>Serum</u>	<u>Comments</u>	<u>History</u>
#392	Lyophilized, augmented, multi-donor	38#224
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<u>Serum</u>	<u>Comments</u>	<u>History</u>
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<u>Serum</u>	<u>Comments</u>	<u>History</u>
#392	Lyophilized, augmented, multi-donor	38#224
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#394a	Fresh-frozen, augmented, single-donor	49#271, 50#275, 51#279, 54#296, 56#308
#394b	Fresh-frozen, augmented, multi-donor	65#353, 68#371
#395	Fresh-frozen, native, multi-donor	71#382
#396	Fresh-frozen, native, multi-donor	71#385





<u>Serum</u>	<u>Comments</u>	<u>History</u>
#392	Lyophilized, augmented, multi-donor	38#224
#393	Fresh-frozen, native, multi-donor	New
#394a	Fresh-frozen, augmented, single-donor	49#271, 50#275, 51#279, 54#296, 56#308
#394b	Fresh-frozen, augmented, multi-donor	65#353, 68#371
#395	Fresh-frozen, native, multi-donor	71#382
#396	Fresh-frozen, native, multi-donor	71#385



<u>Serum</u>	<u>Comments</u>	<u>History</u>
#392	Lyophilized, augmented, multi-donor	38#224
#393	Fresh-frozen, native, multi-donor	New
#394a	Fresh-frozen, augmented, single-donor	49#271, 50#275, 51#279, 54#296, 56#308
#394b	Fresh-frozen, augmented, multi-donor	65#353, 68#371
#395	Fresh-frozen, native, multi-donor	71#382
#396	Fresh-frozen, native, multi-donor	71#385



<u>Serum</u>	<u>Comments</u>	<u>History</u>
#392	Lyophilized, augmented, multi-donor	38#224
#393	Fresh-frozen, native, multi-donor	New
#394a	Fresh-frozen, augmented, single-donor	49#271, 50#275, 51#279, 54#296, 56#308
#394b	Fresh-frozen, augmented, multi-donor	65#353, 68#371
#395	Fresh-frozen, native, multi-donor	71#382
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<u>Serum</u>	<u>Comments</u>	<u>History</u>
#392	Lyophilized, augmented, multi-donor	38#224
#393	Fresh-frozen, native, multi-donor	New
#394a	Fresh-frozen, augmented, single-donor	49#271, 50#275, 51#279, 54#296, 56#308
#394b	Fresh-frozen, augmented, multi-donor	65#353, 68#371
#395	Fresh-frozen, native, multi-donor	71#382
#396	Fresh-frozen, native, multi-donor	71#385

Total β-Cryptoxanthin, µg/mL



<u>Serum</u>	<u>Comments</u>	<u>History</u>
#392	Lyophilized, augmented, multi-donor	38#224
#393	Fresh-frozen, native, multi-donor	New
#394a	Fresh-frozen, augmented, single-donor	49#271, 50#275, 51#279, 54#296, 56#308
#394b	Fresh-frozen, augmented, multi-donor	65#353, 68#371
#395	Fresh-frozen, native, multi-donor	71#382
#396	Fresh-frozen, native, multi-donor	71#385

Total Lutein&Zeaxanthin, µg/mL 0 Your [Analyte], µg/mL [Analyte], µg/mL 0 0 353 -371 394b 382 . 395 . Median [Analyte], µg/mL 224 392 271 275 279 296 308 394a 385 396 393 Apparent Precision, SD Apparent Precision, SD + Long-term Precision, SD Concordance, SD 3rd Quartile (75%) You, this RR You, ≥x, this RR Median (50%) You, past RRs △ You, ≥x, past RRs Others, this RR O 1st Quartile (25%) Expectation

<u>Serum</u>	<u>Comments</u>	<u>History</u>
#392	Lyophilized, augmented, multi-donor	38#224
#393	Fresh-frozen, native, multi-donor	New
#394a	Fresh-frozen, augmented, single-donor	49#271, 50#275, 51#279, 54#296, 56#308
#394b	Fresh-frozen, augmented, multi-donor	65#353, 68#371
#395	Fresh-frozen, native, multi-donor	71#382
#396	Fresh-frozen, native, multi-donor	71#385

[ILR_RR73_FSV-BA.XLSx]Target , Page 14 / 14



D15

5/17/2013

Appendix E. Shipping Package Inserts for RR38

The following three items were included in each package shipped to an RR38 participant:

- Cover letter
- Analysis of Control Materials and Test Samples 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.



January 21, 2013

Dear Colleague:

The samples within this package constitute Vitamin C Round Robin 38 (RR38) of the 2013 Micronutrients Measurement Quality Assurance Program. RR38 consists of one vial each of four frozen serum *test samples* (#381, #382 #383, and #384) and one vial each of two frozen *control sera* (CS#3 and CS#4). These materials are in sealed ampoules. They were prepared by adding equal volumes of 10% MPA to spiked human serum. We have checked the samples for stability and homogeneity. Only total ascorbic acid is stable. While these samples contain some dehydroascorbic acid, its content is variable. Therefore, only <u>total ascorbic acid</u> should be analyzed and reported.

Please use the *control sera* to validate the performance of your measurement system <u>before</u> you analyze the *test samples*. The target value for CS#3 is $(15.5 \pm 1.6; 13.9 \text{ to } 17.1) \mu \text{mol/L}$ and the target for CS#4 is $(46.1 \pm 4.6; 41.5 \text{ to } 50.7) \mu \text{mol/L}$. We expect your results for both of these controls to be within this $\pm 10\%$ target range. If your results are significantly outside this range, your analysis system may not be suited to the analysis of MPA-preserved samples. In this case, please do **not** proceed to the analysis of the *test samples* but contact us at 301-975-3120 or jbthomas@nist.gov.

The test samples and control sera should be defrosted by warming at 20 °C for not more than 10 min otherwise some irreversible degradation may occur. Please be aware that sample contact with any oxidant-contaminated surface (vials, glassware, etc.) may degrade your measurement system's performance (SA Margolis and E Park, "Stability of Ascorbic Acid in Solutions in Autosampler Vials", *Clinical Chemistry* 2001, 47(8), 1463-1464). You should suspect such degradation if you observe unusually large variation in replicate analyses.

Please measure the total ascorbic acid in each ampoule in duplicate, reporting in units of $\mu mol/(L sample solution)$ rather than $\mu mol/(L serum used to prepare the sample)$. Please email (david.duewer@nist.gov) or fax (301-977-0685) your results to us as soon as possible but no later than April 1, 2013.

If you have questions or comments regarding this study, please e-mail me at jbthomas@nist.gov or call me at (301) 975₁₃3120.

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

Sincerely.

Enclosure: RR38 Report Form for Control Material and Test Sample Analyses



Vitamin C Round Robin 38 NIST Micronutrients Measurement Quality Assurance Program

Packing List and Shipment Receipt Confirmation Form

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

Label	Form							
VitC #381	Liquid frozen (1:1 serum:10% MPA)							
VitC #382	Liquid frozen (1:1 serum:10% MPA)							
VitC #383	Liquid frozen (1:1 serum:10% MPA)							
VitC #384	Liquid frozen (1:1 serum:10% MPA)							
CS #3	Liquid frozen (1:1 serum:10% MPA)							
CS #4	Liquid frozen (1:1 serum:10% MPA)							

Please 1) Open the pack immediately

- 2) Check that it contains one vial each of the above samples
- 3) Check if the samples arrived frozen
- 4) Store the samples at -20 °C or below until analysis
- 5) Email (david.duewer@nist.gov) or fax (301-977-0685) us the following information:

1) Date this shipment arrived: _____

2) Are all of the vials intact? Yes | No If "No", which one(s) were damaged?

- 3) Was there any dry-ice left in cooler? Yes | No
- 4) Did the samples arrive frozen? Yes | No
- 5) At what temperature are you storing the samples? _____ °C
- 6) When do you anticipate analyzing these samples? _____

Your prompt return of this information is appreciated.

The M²QAP Gang

Appendix F. Final Report for RR38

The following three pages are the final report for RR38 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,
 - 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.



May 16, 2013

Dear Colleague:

Enclosed is the summary report of the results for Round Robin 38 (RR38) 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 an individualized summary of your laboratory's measurement performance. The robust median is used to estimate the consensus value for all samples, the "adjusted median absolute deviation from the median" (MADe) is used to estimate the expected standard deviation, and we estimate the coefficient of variation (CV) as 100×MADe/median.

RR38 consisted of four test samples (#381, #382,, #383, and #384), one vial each of two frozen control serum control samples (CS #3 and CS #4), and one vial of solid control material (Control) for preparation of TAA control solutions. Details regarding the samples can be found in the enclosed report.

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

Samples for the second vitamin C round robin (RR39) of the 2013 MMQAP will be shipped starting June 10, 2013. Please contact us immediately if this schedule is problematic for your laboratory.

We are currently in the process of providing on-line documentation of data and reports from the MMQAP studies (past and present). The results for Round Robin 71 Fat-Soluble Vitamins and Carotenoids in Human Serum and and Round Robin 36 Ascorbic Acid in Human Serum are available at: http://www.nist.gov/manuscript-publication-search.cfm?pub_id=911458. Click on the DOI link to access the manuscript. Data summaries have been altered to ensure confidentiality of identification codes assigned to laboratories. We will update you as additional documents become available on-line.

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

Sincerely,

Jeanice Brown Thomas, M.B.A . Research Chemist Analytical Chemistry Division Material Measurement Laboratory

Enclosures

cc: L. C. Sander

UNITED STATES DEPARTMENT OF COMMERCE National Institute of Standards and Technology

Gaithersburg, Maryland 20899-

David L. Duewer, Ph.D. Research Chemometrician Analytical Chemistry Division Material Measurement Laboratory



The NIST MMQAP Vitamin C Round Robin 38 (RR38) report consists of:

	Page	"Individualized" Report										
	1	Summary of your reported values for the two serum control and four serum test samples.										
	2	Graphical summary of your RR38 measurements.										
	Page	"All-Lab" Report										
	1 A tabulation of results and summary statistics for total ascorbic acid [TAA] in the RR38 control and test samples. Results and summary statistics are also presented for the test samples calibrated to the results for the control samples											
Sei	rum-Ba	ased Samples. Two serum controls and four test samples were distributed in RR38.										
	CS#3	a (15.4 \pm 0.4) µmol/L material ampouled in 2009										
	CS#4	a (46.2 \pm 1.2) µmol/L material ampouled in 2009										
	S38:1	an unaugmented material (i.e., the [TAA] content is zero or nearly so), ampouled in 2001, previously distributed in RRs 16, 19, 21, 23, 26, and 29										
	S38:2	Ampouled in late 2009, previously distributed in RRs 32, 33, and 35										
	S38:3	Ampouled in late 2009, previously distributed in RRs 32, 35, and 36										
	S38:4	Ampouled in late 2009, previously distributed in RRs 34 and 36										

Results.

- 1) The reported [TAA] contents of the two control sera, CS#3 and CS#4, are unchanged from the values estimated for these materials when they were distributed as unknowns. The expected [TAA] content and inter-participant standard deviation are estimated with the robust median and adjusted median absolute deviation (eSD) statistics.
- 2) All participants reported either "zero" or credible limit-of-quantification (LoQ) values for the unaugmented (nominal zero-level) material, S38:1. We regard providing LoQ (i.e., "<*x*") values to be the more informative reporting practice.
- 3) The reported [TAA] contents of the three non-blank test samples, S38:2 to S38:4, are the same as estimated in previous studies. The robust inter-participant estimated coefficient of variation

$$eCV = 100 eSD/Median$$

pooled over these three materials is 8%.

4) The results for two control sera with well-separated [TAA] levels enable calibration of the reported results for the unknowns to the function:

$$[TAA]_{reportedCS} = a + b*[TAA]_{referenceCS}$$

where [TAA]_{reportedCS} are the reported values for the two control sera and [TAA]_{referenceCS} are the established reference values for these materials. The calibrated values for unknown samples are then given by:

$$[TAA]_{calibrated} = ([TAA]_{reported} - a) / b$$
.

Figure 1 displays the eSD as a function of the Median values for the test samples distributed in RR37 and RR38 for both the "as reported" and "after calibration" results. While there is as yet too little data to draw strong conclusions, calibration to the control materials appears to improve the pooled eCV of the seven test samples from about 7% to 5%.



Figure 1: Estimated Standard Deviation as a Function of Median Value Each symbol represents the summary statistics for one of the four test samples distributed in RR37 or one of the three non-blank test samples distributed in RR38.

Appendix G. "All-Lab Report" for RR38

The following two pages are the "All-Lab Report" for RR38 as provided to all participants, with the following exceptions:

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

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

Micronutrients Measurement Quality Assurance Program for Total Ascorbic Acid "Round Robin" 38 - Winter 2013

<i>q/</i> (:	S38:4'	57.4	54.6	57.0	53.1	56.2	55.2	61.6	56.7	56.7	6	56.5	2.4		53.1	55.2	56.7	57.0	61.6	1.0	2
[TAA]-a	mol/L	S38:3'	31.3	33.9	31.1	29.0	31.9	29.5	32.6	31.4	31.6	6	31.4	1.5		29.0	31.1	31.4	31.9	33.9	0.8	7
TAA]' = (<u>I AAJ', µ</u>	S38:2' ;	23.0	22.9	23.7	22.0	23.6	21.6	24.1	24.8	24.3	6	23.3	1.0		21.6	22.9	23.6	24.1	24.8	0.9	4
kesults: [:	S38:1'	<7.0	pu	0.8	<0.9	<6.4	<1.3	-1.4	<0.9	<8.4	2	-0.3	1.6		-1.4		-0.3		0.8		
	ers	٩	2.0	0.1	0.8	0.1	7.8	8.7	1.3	0.9	2.4	<u>. </u>				<u>. </u>						
alibra	amete	•••		2 2	' ო	' 7	4	~	2	ო												
C)	rara.	q	1.0	1.0	1.0	1.0	4. 4	0.9	0.9	0.0	0.9											
		S38:4	57.0	57.2	57.8	53.9	73.3	62.2	57.7	53.6	52.8	6	58.4	6.3		52.8	53.9	57.2	57.8	73.3	4.8	ø
), µmol/	S38:3	30.2	35.5	31.1	29.4	38.3	37.3	31.2	30.1	28.3	6	32.4	3.7		28.3	30.1	31.1	35.5	38.3	2.6	ω
amples	<u>a ([I AA]</u>	S38:2	21.7	24.0	23.5	22.4	26.2	29.7	23.3	24.0	21.2	6	24.0	2.6		21.2	22.4	23.5	24.0	29.7	1.7	7
Ŭ,	rbic Acid	S38:1	<5.2	pu	0.0	<0.9	<1. 4	<10	0.0	<1.7	<5.7	2	0.0	0.0		0.0		0.0		0.0		
	al Asco	CS#4	45.4	48.3	46.6	46.8	58.8	53.4	43.5	43.7	42.5	6	47.7	5.3		42.5	43.7	46.6	48.3	58.8	4.3	0
F	lot	CS#3 (13.9	16.2	15.1	15.7	14.6	23.8	15.5	15.3	12.7	6	15.9	3.1		12.7	14.6	15.3	15.7	23.8	1.1	7
L		Date	27/02/13	15/05/13	03/04/13	18/03/13	29/03/13	05/03/13	05/04/13	28/03/13	11/03/13	Z	Average	SD	L	Min	%25	Median	%75	Max	eSD	eCV
	-	Lab	VC-MB	VC-MC	VC-MG	VC-MH	VC-MI	VC-MJ	VC-MN	VC-NF	VC-NM											

All Lab Report

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Legend

Definition	Participant code Date results received at NIST Slope and Intercept of the line connecting the expected values for control samples CS#3 and CS#4 with the results reported by each participant	Number of quantitative values reported for this analyte Mean of the reported quantitative values Standard deviation of the reported quantitative values	Minimum quantitative value reported First quartile of the reported quantitative values Median (second quartile) of the reported quantitative values Third quartile of the reported quantitative values Maximum quantitative value reported Robust standard deviation, estimated using the adjusted median absolute deviation, estimated as 100*eSD/Median Robust Coefficient of Variation, estimated as 100*eSD/Median Not determined
Term	Lab Date <i>b</i> a	N Average SD	Min %25 Median %75 Max eSD eSD eCV ad

Appendix H. Representative "Individualized Report" for RR38

Each participant in RR38 received an "Individualized Report" reflecting their reported results. The following two pages are the "Individualized Report" for participant "VC-MB".

				[TAA] m	imol/Ls	ample						
Date	RR	Sample	Rep₁	Rep ₂	F_{adj}	Mean	SD_{dup}	N		Mean	SD _{repeat}	SD _{reprod}
07/23/10	33	S33:3	13.4	13.4	1.0	13.4	0.0		5	13.8	1.5	1.2
01/14/11	34	S34:2	13.9	13.9	1.0	13.9	0.0					
06/13/11	35	S35:1	15.0	15.5	1.0	15.2	0.4					
08/06/12	37	CS#3	10.3	15.0	1.0	12.6	3.3					
02/27/13	38	CS#3	13.9	13.9	1.0	13.9	0.0					
01/13/10	32	S32·4	15 A	15.0	1.0	15.7	0.4		5	/6.1	11	1.0
01/13/10	3/	S34·3	45.4	45.5 45.0	1.0	45.7	0.4		5	40.1	1.1	1.0
06/13/11	35	\$35·4	43.4	43.3	1.0	47.5	0.4					
08/06/12	37	CS#4	47.5	48.0	1.0	47.5	2.0					
02/27/13	38	CS#4	44.5	40.0 11 Q	1.0	40.5	0.7					
02/21/15	50	00#4	40.0	44.3	1.0	40.4	0.7					
02/08/02	16	S16:2	3.1	2.0	0.5	1.3	0.4		5	0.9	0.4	1.4
05/01/03	19	S19:1	5.2	7.2	0.5	3.1	0.7					
07/14/04	21	S21:1	<5.2	<5.2	1.0							
05/25/05	23	S23:1	0.0	0.0	1.0	0.0	0.0					
11/26/06	26	S26:1	0.0	0.0	1.0	0.0	0.0					
06/20/08	29	S29:1	0.0	0.0	1.0	0.0	0.0					
01/02/00	38	S38:1	<5.16	<5.16	1.0							
												1
01/13/10	32	S32:2	22.2	22.7	1.0	22.5	0.4		4	22.7	0.4	0.9
07/23/10	33	S33:4	23.2	22.7	1.0	23.0	0.4					
06/13/11	35	S35:2	23.7	23.7	1.0	23.7	0.0					
02/27/13	38	S38:2	21.2	22.2	1.0	21.7	0.7					
01/13/10	32	S32:3	30.5	31.0	1.0	30.7	0.4		4	30.9	0.5	0.7
06/13/11	35	S35:3	32.0	31.5	1.0	31.7	0.4	L				-
03/07/12	36	S36:2	31.5	30.5	1.0	31.0	0.7					
02/27/13	38	S38:3	29.9	30.5	1.0	30.2	0.4					
		0 0 / /										
01/14/11	34	S34:4	56.8	57.3	1.0	57.0	0.4		4	56.8	0.7	0.7
03/07/12	36	S36:1	57.8	56.8	1.0	57.3	0.7					
03/07/12	36	S36:4	56.8	55.2	1.0	56.0	1.1					
02/27/13	38	S38:4	57.3	56.8	1.0	57.0	0.4					

Vitamin C "Round Robin" 38 Report: Participant VC-MB

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

Micronutrients Measurement Quality Assurance Program National Institute of Standards and Technology

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Vitamin C "Round Robin" 38 Report: Participant VC-MB

Total Ascorbic Acid, µmol/mL



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

CS#3 Distributed as an unknown in RRs 33, 34, and 35. Distributed as a control in RR37.
CS#4 Distributed as an unknown in RRs 32, 34, and 35. Distributed as a control in RR37.
S38:1 Zero-level control serum, distributed in RRs 16, 19, 21, 23, 26, and 29
S38:2 Distributed in RRs 32, 33, and 35

- S38:3 Distributed in RRs 32, 35, and 36 $\,$
- S38:4 Distributed in RRs 34 and 36