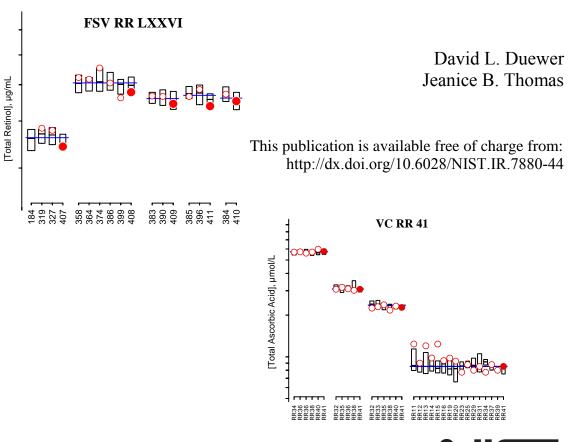
NIST Micronutrients Measurement Quality Assurance Program Summer 2014 Comparability Studies

Results for Round Robin LXXVI Fat-Soluble Vitamins and Carotenoids in Human Serum and Round Robin 41 Ascorbic Acid in Human Serum



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

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U.S. Department of Commerce Penny Pritzker, Secretary (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 2014 MMQAP measurement comparability improvement studies: 1) Round Robin LXXVI Fat-Soluble Vitamins and Carotenoids in Human Serum and 2) Round Robin 41 Total Ascorbic Acid in Human Serum. To avoid increasing participation fees, the overhead costs for these programs were minimized by shipping the materials in January 2014 together with the samples for FSV Round Robin LXXV and VC Round Robin 40. Participants were requested not to analyze any of the Summer samples before June 1, 2014 but to provide their measurement results by September 15, 2014. Participants were reminded of the due-date by e-mail on August 4, 2014.

Keywords

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

Table of Contents

Abstract	ii
Keywords	ii
Table of Contents	iv
Introduction	1
Round Robin LXXVI: Fat-Soluble Vitamins and Carotenoids in Human Serum	1
Round Robin 41: Vitamin C in Human Serum	2
References	3
Appendix A. Shipping Package Inserts for RR76	A1
Appendix B. Final Report for RR76	B1
Appendix C. All-Lab Report for RR76	C1
Appendix D. Representative Individualized Report for RR76	D1
Appendix E. Shipping Package Inserts for RR41	E1
Appendix F. Final Report for RR41	
Appendix G. "All-Lab Report" for RR41	G1
Appendix H. Representative "Individualized Report" for RR41	H1

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 LXXVI: Fat-Soluble Vitamins and Carotenoids in Human Serum

Participants in the MMQAP Fat-Soluble Vitamins and Carotenoids in Human Serum Round Robin LXXVI comparability study (hereafter referred to as RR76) 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 2014 in the same shipping package as the RR75 materials but in separate clearly labeled plastic bags. Participants were requested not to analyze any of the RR76 samples before June 1, 2014 but to provide their measurement results by September 15, 2014. 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 RR76 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 41: Vitamin C in Human Serum

Participants in the MMQAP Vitamin C in Human Serum Round Robin 40 comparability study (hereafter referred to as RR41) 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 materials were shipped on dry ice to participants in January 2014 in the same shipping package as the RR40 materials but in separate clearly labeled plastic bags. Participants were requested not to analyze any of the RR41 samples before June 1, 2014 but to provide their measurement results by September 15, 2014. 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 RR41 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

- 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 RR76

The following items were included in each package shipped to an RR76 participant:

- Combined cover letter for Round Robin LXXV (RR75) and RR76. See Appendix A in NISTIR-43.
- Datasheet for RR76. This was enclosed in the same sealed waterproof bag that contained the cover letter and the data sheet for RR75.
- Packing List and Shipment Receipt Confirmation Form for RR76.

This RR76 samples were enclosed in a bubble-wrapped sealed plastic bag that was labeled:

NIST MMQAP-FSV: RR LXXVI

Micronutrients Measurement Fat-Soluble
Vitamin
Quality Assurance Program

Summer 2014 Samples

Analyze after: **June 1, 2014**Results due on or before: **September 15, 2014**

The packing list was placed at the top of the shipping box, between the cardboard covering and the foam insulation.

Participant #:	Date:
----------------	-------

Round Robin LXXVI: Human Sera NIST Micronutrients Measurement Quality Assurance Program

Analyte	407	408	409	410	411	Units*
total retinol						
trans-retinol						
retinyl palmitate						
lpha-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 (K₁)						
25-hydroxyvitamin D						
Phytoene						
Phytofluene						
-						

we prefer μg/ml	we	prefer	μg/ml
-----------------	----	--------	-------

Were the samples frozen when received? Yes | No

Comments:

Date:		
Date		

Fat-Soluble Vitamins Round Robin LXXVI 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
#407	Lyophilized	Yes	5 mL clear / silver
#408	Liquid frozen	No	3 mL amber / blue
#409	Liquid frozen	No	2 mL amber / gold
#410	Liquid frozen	No	2 mL amber / purple
#411	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 RR76

The following three pages are the final report for RR76 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.

Dear Colleague:

Enclosed is the summary report of the results for Round Robin LXXVI (RR76) of the 2014 NIST Micronutrients Measurement Quality Assurance Program (MMQAP) 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. RR76 (Sera 407 - 411) 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 6 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.

If you returned your intent-to-participate form for the 2015 MMQAP, you should have received a confirmation receipt. Please notify us if you have not received your confirmation receipt.

Laboratories participating in the upcoming program will receive samples for the two fat-soluble vitamins and carotenoids in serum studies (RR LXXVII and LXXVIII) in February 2015. Please note that we will ship the samples for both studies at the same time in February. Results will be due in May 2015 for the first study and in September 2015 for the second study. We will send you a reminder in late July 2015 about the reporting deadline for the second study. Please contact us immediately if this schedule is problematic for your laboratory.

If you have questions or concerns regarding this report, please contact David Duewer at 301-976-3935; e-mail: david.duewer@nist.gov or me at 301-976-3120; e-mail: jbthomas@nist.gov; or fax: 301-977-0685.

Sincerely,

Jeanice Brown Thomas, M.B.A.

Research Chemist

Chemical Sciences Division

Material Measurement Laboratory

David L. Duewer, Ph.D. Research Chemometrician Chemical Sciences Division Material Measurement Laboratory

Enclosures



The NIST MMQAP Round Robin LXXVI (RR76) report consists of:

Page	All-Lab Report
1-4	A listing of all results and statistics for analytes reported by more than one participant.
5	The legend for the list of results and statistics.
6	The text Comparability Summary ("Score Card") of measurement performance.
Page	Individualized Report
1	Your values, the number of labs reporting values, and our assigned values.
2 to	"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.
n+1	The graphical Comparability Summary (target plot) of measurement performance.

Samples. Five samples were distributed to each participant in RR76.

Serum	Description	Prior Distributions
407	Lyophilized, native, single-source, prepared in 1992.	#184:RR28-6/93, #319:RR59-3/96; #327:RR60-9/96
408	Fresh-frozen, native, multi-donor, prepared in 2009. This is Level II of SRM 968e.	#358:RR66-9/09, #364:RR67-3/10, #374:RR69-3/11, #386:RR71-3/12; #399:RR74-9/13
409	Fresh-frozen, native, single donor, prepared in 2011	#383:RR71-3/12;#390:RR72-9/12
410	Fresh-frozen, native, single donor, prepared in 2011	#384:RR71-3/12
411	Fresh-frozen, two donor, prepared in 2011. All analytes in this material are expected to be about half-way between those for sera 409 and 410.	#385:RR71-3/12;#396:RR73-3/13

Results

- 1) <u>Stability</u>: There have been no appreciable changes in either the level nor the variability in any analytes in the 22-year old lyophilized serum 407, the five-year old fresh-frozen SRM 968e serum 408, or the three-year old fresh-frozen sera 409, 410, and 411.
- 2) <u>Linear additivity</u>: Serum 411 was prepared as a 31:26 blend of the serum pools used to prepare sera 409 and 410. Assuming that the concentration of an analyte in the blend is the simple sum of the concentrations in the individual pools, the result for any analyte in the blended material is expected to be the weighted average of the results in the parent materials: Serum 411 = ((31 × #409) + (26 × Serum 410))/57.

Figure 1 plots values calculated from the sera 409 and 410 medians as a function of the medians of the serum 411 measurements. The measured and calculated values agree very well.

Therefore, if the result for any analyte in serum 411 is much different from the average of sera 409 and 410 results, you may want to carefully re-evaluate your measurement procedure for that analyte.

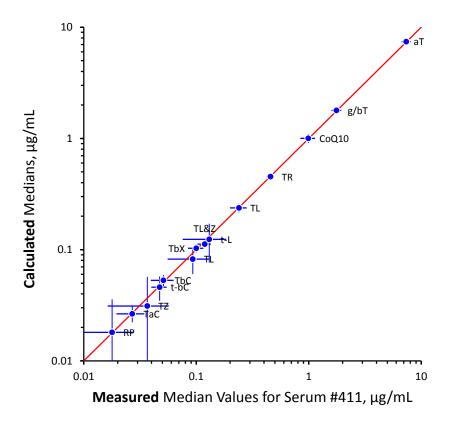


Figure 1: Measured vs Calculated Medians for the Blended Serum 411

The solid blue circles represent the {Measured, Calculated} median values for all RR76 analytes reported by at least five participants. The red line represents the ideal relationship: Estimated = Measured. The blue error-bars represent approximate 95 % confidence intervals on the medians, Median $\pm t_{95\%,n-1} \times \text{eSD}/\sqrt{n}$, where n is the number of participants reporting quantitative values for the analyte, eSD is estimated from the median absolute deviation from the median, and $t_{95\%,n-1}$ is the Student t 95 % confidence expansion factor.

Appendix C. "All-Lab Report" for RR76

The following six pages are the "All-Lab Report" for RR76 as provided to all participants, with two exceptions:

- the participant identifiers (Lab) have been altered and
- 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.

	411	1.79			1.70		1.81	1.50	1.93								1.56	1.71	2.21	1.75	1.88		1.51	1.78	1.83	1.65				0	67.7	<u>;</u> t	16	1.50	1.77	2.21	0.10	2	17	1.78	60.0	1.77
µg/mL	410 4	1.83 1			1.70				2.04								na 1	2.09 1		2.06 1			1.52 1			1.73 1							15		1.87			10		1.81		1.85 1
γ/β-Tocopherol, μg/ml	409 4	1.73 1			1.60				1.77 2								1.71		2.33 2	1.86 2			1.51		1.83	1.58 1					00.7		16		1.72 1	2.33 2	0.14 0	∞		1.75 1		1.72 1
Tocop	408 4	1.41			1.10				1.44								1.53 1		1.67	1.49	1.41		1.19 1		1.47 1	1.19					77.		16		1.41			6				1.41
-β/λ	407	1.63			1.60		_		1.65								1.49	1.68	2.11	1.73			1.31	_		1.52					5 n		16			2.11		7		1.65		1.64
	411	7.84		7.70	8.30	2.90	7.46	7.28	7.76	7.21	6.50	6.50	7.29	7.36	8.99		6.98	7.50	8.77	7.43	7.99	7.41	2.67	6.13	7.40	92.9		6.10	7	0.10	7.0.7	17.76	28	2.67	7.38	17.76	0.64	6	30	7.41	0.68	7.36
g/mL	410	8.20		8.40	9.00	7.20	8.00	8.58	8.65	7.80	7.30	7.40	7.86	7.72	9.52		na	9.31	8.23	8.50	76.7	8.39	6.13	7.02	8.20	7.27		7.40	7	00.7	0.43					21.62	0.85	7	59	8.00	99.0	8.00
α-Tocopherol, µg/ml	409 ,	7.26		7.50	7.40	5.80	6.94	6.93	6.82		6.50		7.05	7.27	7.13		7.62	8.28	8.64	6.94	7.42		5.38			5.88		5.80		5.30		(6.94	23.61 2	0.67	10		7.02		6.93
Focoph	408 4	10.63								10.11	9.50	9.00	10.43		. 11.58		10.26	10.48	11.63		10.35		8.16	9.25				8.80		0.70		C				33.53 23	0.89	6			0.77	10.35
α-J	407 4	4.62 10										3.90			53		4.55 10		5.72 11			3.58 13			4.31 10	20		40		3.30	7			3.35			0.34	œ		4.33 10		4.31 10
				4	4			4	4	4	<u>ო</u>	<u>е</u>	4	4	4		4	4	2		4	<u>ო</u>	<u>ო</u>		4	4		<u>හ</u>				12	2			_		82				
/mL	411	3 0.030					2 0.018													4 0.006				7 0.008									5		2 0.018	3 0.030	0.0	32 8			2 0.011	2 0.018
Retinyl Palmitate, µg/ml	410	0.033					0.022													0.004				0.017						000							0.0				0.012	0.022
almita	409	0.030					0.015													0.00				0.014						5	0.0		2				0.003	20	2	0.016	0.009	0.015
tinyl P	408	0.076					0.030													0.016				0.019						46.0	5.0.0		2	0.016	0.030	0.076	0.021	70	7	0.027	0.009	0.030
Re	407	960.0					0.122													0.072				0.087						0	9.0.0		2			0.122		26			0.034	0.087
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	407															0.34																			0.344	0.345				0.409		0.348
	411	0.438	0.441	0.505	0.530	0.420	0.462	0.365	0.468	0.454	0.430	0.450	0.495	0.460	0.473	0.467	0.595	0.472	0.471	0.430	0.480	0.457	0.393	0.410	_	0.553	0.430	0.438	7	0.410	20.40	0.687	29	0.365	0.457	0.687	0.034	7	31	0.474	0.035	0.456
_1	410	0.452	0.432	0.491	0.550	0.450	0.465	0.356	0.464	0.451	0.400	0.440	0.489	0.460	0.475	0.483	na	0.363	0.380	0.520	0.460	0.467	0.387	0.377		0.511	0.420	0.440		0.445		0.564	28	0.356	0.451	0.564	0.041	6	31	0.470	0.034	0.451
, µg/ml	4																													/	NI.							œ				
Total Retinol, µg/ml	409	0.444	0.428	0.486	0.530	0.450	0.459	0.346	0.468	0.444	0.430	0.480	0.481	0.460	0.390	0.471	0.450	0.379	0.519	0.500	0.460	0.457	0.397	0.342		0.568	0.400	0.431	0	0.439	10.44	0.670	2	0.342	0.454	0.670	0.039		7	0.462	0.029	0.452
Total F	408	0.477	0.491	0.539	0.580	0.460	0.496	0.442	0.498	0.489	0.490	0.480	0.533	0.500	0.525	0.499	0.524	0.511	0.491	0.500	0.510	0.559	0.425	0.458		909.0	0.500	0.468		0.450		0.685	29	0.425	0.498	0.685	0.031	9	31	0.507	0.041	0.497
	407	0.342	0.339	0.369	0.390	0.350	0.362	0.272	0.351	0.348	0.340	0.340	0.377	0.330	0.300	0.345	0.389	0.275	0.405	0.570	0.360	0.351	0.313	0.312		0.430	0.340	0.347		0.308	VI.				0.348		0.027	∞		998.0		0.348
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Saroter	409	0.014		0.015	<i>nq</i> 0.017			0.013	0.010	0.074	0.014	0.01	0.0	5.5	5	0.010		0.009					5		14	0.009	0.014	0.074	30	13	0.013		0.015
Total α-Carotene, μg/mL	408	0.032		0.031	0.025			0.052	0.021	0.101	0.034	0.026	0.033	0.030	5	0.030	0.045	0.024						0.032	15	0.021	0.032	0.101	0.008	16	0.032	- 6	0.032
		0.021		7	<i>nq</i> 0.006				0.012				0.013	0.020	<i>b</i> :	0.013		0.012						0.020	14				33	17	0.016	0000	0.016
٦Ļ	411	0.003								pu	0.003					0.005							900		4	0.003			0.002 45	∞	0.005		0.005
ie, µg/i	410	0.001								pu	па					0.005									3	0.001	0.005	0.008		0			0.005
Caroter		0.002								pu	0.003					0.005							900		4				0.002	0			0.004
Total cis-β-Carotene, µg/mL		0.011									0.015					0.021 0.011 0.005 0.005 0.005									4				0.003	2	0.013		0.013
Tota		0.017									0.022					0.021								0.030	4	0.017			0.004 18	2	0.021		0.022
	411	0.047								0.118	0.055					0.041							770	0.044	2	0.041	0.047	0.118	0.009	9	0.051	1,00	0.047
µg/mL		0.054								0.126	na					.049							0.00		4				0.003	2	0.056		0.054
trans-β-Carotene, μg/mL		0.040 0									0.046					0.037 0.049 0.041								0000	2				0.004 11	2	0.042		0.040 0
s-β-ca		0.231 0									0.236 0					0.208 0							2,00		2				0.027 u 13	7	0.218 0		0.213 0
		0.304 0.									0.295 0.					0.258 0.							0000		2				0.043 U. 15	7	0.302 0.		0.295 0.
		0.050	0.050	0.062	0.036			0.049	0.041			0.052	0.004	5 5	0 03		0.037	0.056	690.0			0.034	0.055		20				0.010	70	0.054		0.050
µg/mL		0.055 0	0.060	9	0.045 C 0.063 C			0.062		0.126			100.0						0.080				0.062		70			∞ α	0.010 17	48	0.063		0.061 0.012 0
Total β-Carotene, μg/mL		0.042 0	0.050.0		0.034 0 0.039 0			0.043 0	0.038 0				0.000		ď				0.650.0				0.046 0		20				0.009 20	17	0.049 0		0.046 0
al β-Ca		0.242 0	0.250 0		0.234 0 0.233 0			0.248 0	0.203 0				0.280 0						0.230 0				0.234 0		22				0.019 8	7	0.241 0		0.234 0
		0.320 (0.300	0.346					0.269				0.410						0.269 (0.304		22				0.032 0	27	0.328		0.303 0.044 0
		0.049																							2			0.200		4	0.088		
		0.083 0														.193												0.193 C		4	0.069 0		
erol, μ		0.065 0														264 0									2			0.264 0		7	0.063 0		
ð-Tocopherol, µg/mL																.0 88									2			0.088 0.0		9	0.073 0.0		
	407 40	0.044 0.083														0.038 0.088 0.264 0.193 0.200									2			0.044 0.0		9	0.048 0.0		
			BE Fa	ဗ္ဘ	표 교	ᇫ	ĭ≅	3N	<u>ک</u> ۳	38	BT:	<u>ر</u>		<u> </u>	<u>ئ</u> ب		<u></u> ب	<u>,</u>	7 5	3 2	> Ш	片片	7 5	2 H	z	Min 0.0			esn ecv	ast			NAV NAU
	Lab	FSV-BB FSV-BC FSV-BD	FSV-BE FSV-BFa	FSV-BG	FSV-BH FSV-BJ	FSV-BK	FSV-BM	FSV-BN	FSV-BO FSV-BR	FSV-BS	FSV-B1	FSV-BU	73-751 707 BW	70.70	FSV-CE	FSV-CG	FSV-CI	FSV-CO	FSV-CZ	70-70-7	FSV-EE	FSV-FK	74-754	FSV-GE		2	Median	≥ `	ĕ ĕ	Npast	Medianpast	1	żŻ
	ļ													(C 3																_		

	411	7 0.080	8 0.069	9 0.093	7 0.134 0.095		7 0.072			6 7 7 0.069 8 0.093 7 0.134 0 0.024 3 26	4 6 6 0.086 6 0.023	9 0.093 1 0.024
Total Lutein, µg/mL	410	'3 0.087	8 0.068 8 0.095	0.089	10 0.137 16 na		7 0.067			0.06 0.08 0.13 0.02	0.07 0.01	7 0.089
Luteir	409	3 0.073	0.058	0.104	5 0.130		7200			0.0 0.0 0.1 0.0	0.09	0.028
Total	408	0.083	0.080	0.106	0.136		0.081			7 0.080 0.086 0.136 0.009	7 0.092 0.020	0.086
	407	0.082	0.066	0.080	0.132		0.068			7 0.066 0.076 0.132 0.009	12 0.080 0.017	0.076
/mL	411	0.016			0.011	bu				2 0.011 0.013 0.016	4 0.019 0.007	
Total α-Cryptoxanthin, μg/mL	410	0.012			na	bu				0.012	4 0.013 0.004	
ptoxant	409	0.020 0.020			0.010	bu				2 0.010 0.015 0.020	6 0.025 0.013	
α-Cry	408	0.020			0.018 0.010	bu				2 0.018 0.019 0.020	5 0.026 0.011	
Total	407	0.022			0.012	bu				2 0.012 0.017 0.022	4 0.026 0.006	
/mL	411	0.098	0.106 0.104 0.120	0.107	0.143 0.064 0.109 0.080	0.100	0.097	0.088		13 0.064 0.100 0.143 0.010	15 0.100 0.027	0.100
Total β-Cryptoxanthin, μg/mL	410	0.022	0.026 0.023 0.023	0.017	0.076 <i>na</i> 0.019 0.009	bu	0.034	0.021		11 0.009 0.022 0.076 0.006	13 0.027 0.014	0.023
otoxant	409	0.164	0.179 0.182 0.194	0.183	0.186 0.096 0.179 0.167	0.160	0.155	0.144		13 0.096 0.170 0.194 0.018	12 0.168 0.037	0.170
β-Cryp	408	0.051	0.054 0.060 0.055	0.053	0.108 0.046 0.053 0.033	bu	0.063	0.049		12 0.033 0.053 0.108 0.007	15 0.053 0.016	0.053
Total	407	0.053 (0.057 0.060 0.049	0.054 (0.059 (0.095 (0.039 (0.056 (0.040 (0.050	0.058	0.050		13 0.039 (0.054 (0.095 (0.006 (18 0.058 0.010	0.056 (
	411	0.105	0.130		0.209		0.102			5 0.102 0.130 0.209 0.038	7 0.120 0.023	0.125
µg/mL	410	0.133	0.174		0.251 na					4 0.133 0.157 0.251 0.031 20	6 0.148 0.033	0.174
opene,	409	0.073	0.097		0.165		0.077			5 0.073 0.096 0.165 0.028	6 0.081 0.015	0.096
trans-Lycopene, µg/	408	0.263	0.337		0.408		0.279			5 0.263 0.312 0.408 0.049	7 0.292 0.048	0.312
	407	0.088	0.117		0.168		0.091 0.279 0.077 0.139			5 0.088 0.092 0.168 0.006	8 0.100 0.017	0.092
	411	0.214	0.248 0.216 0.227	0.292	0.280 0.152 0.271 0.297	0.330	0.218	0.240	0.227	15 0.152 0.240 0.330 0.038	15 0.238 0.048	0.240
Total Lycopene, µg/mL	410	0.292	0.333 0.290 0.342	0.431	0.342 na 0.368 0.345		0.294	0.312	0.291	14 0.245 0.323 0.431 0.046	13 0.327 0.060	0.312
copene	409	0.152	0.185 0.164 0.148	0.202	0.212 0.111 0.189 0.235		0.166	0.182	0.156	15 0.111 0.166 0.235 0.028	12 0.174 0.030	0.166
otal Lyc	408	0.54 (0.58 0.71 0.59	0.81	0.57 0.35 0.58 0.68		0.55 (0.59	0.56	15 0.35 0.58 0.81 0.06	15 0.60 0.09	0.58
	407	0.156	0.196 0.198 0.170	0.206 0.136	0.210 0.102 0.184 0.232 0.168	0.210	0.171	0.184	0.158	15 0.102 0.184 0.232 0.033	20 0.186 0.033	0.184
L	Lab	FSV-BB FSV-BC FSV-BD FSV-BE FSV-BFa					FSV-CG FSV-CI			Min Median Max eSD eCV	Npast Medianpast SDpast	NAV
						C4					2	

All Lab Report

	411		902	2003							0.003 0.004 0.005	0	
25-hydroxyvitamin D, µg/mL			0.006 0.012 0.006 0.004 0.005	0.005 0.003							2 0.004 0.0 0.004 0.0 0.005 0.0	0	
min D,	9 410		0.0 90									0	
xyvita	409		2 0.00	0.006 0.015 0.005							2 2 2 0.005 3 0.006 5 0.006	o o 4	
-hydro	408		0.01	0.01							0.01	0.07	
25	407		0.006	0.006							2 0.006 0.006 0.006	7 0.004 0.000	
٦L	411	0.135					0.095		0.120		3 0.095 0.120 0.135	0	0.120
), ng/n	410	0.189					0.129		0.130		3 0.129 0.130 0.189	0	0.130
Phylloquinone (K1), ng/mL	409	0.110					0.066		0.100		3 0.066 0.100 0.110	0	0.100
oduino	408	0.312					0.221		0.290		3 0.221 0.290 0.312	0	0.290
Phyll	407 4	0.140 0					0.109 0		0.110 0		3 0.109 0.110 0.140	0	0.110 0
		0.99	0.88	0.91	1.12	1.08	1.21	1.01	0.97	0.98	9 0.88 0. 0.99 0. 1.21 0. 12	8 1.01 0.07	0.98 0.
g/mL	410 411	0.910 0.9	0.919 0.8	0.874 0.3		1.090 1.	1.130 1.	0.927 1.	0.931 0.8	0.847 0.9	8 0.847 0.0 0.923 0.1 1.130 1.1 0.046 0.	9 0.955 1.0 0.052 0.0	0.919 0.
Coenzyme Q10, µg/mL	409 4	1.07 0.9	0.69 0.8	0.92 0.8	0.97 na	1.47 1.0	1.21 1.1	0.98 0.9	1.08 0.9	1.10 0.8	9 0.69 0.8 1.07 0.9 1.47 1.1 0.15 0.0	9 1.07 0.9 0.12 0.0	1.07 0.9 0.15 0.0
ızyme	408 40	0.910 1.	0.863 0.	0.908 0.	0.778 0.	1.020 1.	1.060 1.	0.999 0.	0.921	0.952 1.	9 0.778 0. 0.921 1. 1.060 1. 0.086 0.	8 0.966 1. 0.122 0.	0.921 1.0.092 0.
Coer	407 40	0.76 0.9	0.47 0.8	0.63 0.9	0.57 0.7	0.96 1.0	0.82 1.0	0.79 0.9	0.74 0.9	0.68 0.9	9 0.47 0.74 0.74 0.96 0.11 0.11	5 0.72 0.9 0.10 0.	0.74 0.9
		0.119	0.153	0.111 0.		0.130			O .	o o	0.094 0.119 0.213 0.012 0.012	15 0.119 0. 0.035 0.	0.119 0.025 0.
µg/mL	411												
ınthin,	410	0.119	0.088	0.103	0	0.090	0.094				0.0	0.0	0.101
,Zeaxa	409	0.122	0.118	0.140	0.218 0.114 0.122	0.126	0.113				13 0.101 0.122 0.218 0.012	12 0.124 0.031	0.122
Total Lutein&Zeaxanthin, µg/mL	408	0.128	0.137	0.133	0.211 0.114 0.119	0.111	0.114				13 0.101 0.120 0.211 0.012	16 0.127 0.032	0.120
Total	407	0.108	0.100	0.104		0.115	0.083				13 0.083 0.097 0.204 0.017	17 0.106 0.018	0.097
	411	0.039	0.084	0.018	0.079		0.022				6 0.018 0.037 0.084 0.025	9 0.034 0.014	0.038
µg/mL	410	0.031	0.028 0.035 0.043 0.020 0.084	0.020	0.067 C		0.011				5 0.011 0 0.020 0 0.067 0 0.013 0	0	0.025 0.008
anthin,	409	0.049 0	043 0	0.012 0	0.088 0		033 0				6 0.012 0.041 0.088 0.012 28	7 0.044 0.026	0.041 0
Total Zeaxanthin, µg/mL	408 4	0.046 0.	035 0.	0.015 0.	0.075 0. 0.028 0.		0.020 0.033				6 0.015 0. 0.032 0. 0.075 0. 0.019 0.	8 0.028 0. 0.012 0.	0.032 0.0.019 0.0
Total		0.026 0.0	28 0.0										
	407			∑ Z O 0	35 0.072 3T 0.021	<u>></u> ≥ □ L	CI 0.015	ΝΩ	 	ΝÖШ	0.0	0.0	V 0.024 U 0.010
	Lab	FSV-BB FSV-BC FSV-BD FSV-BE	FSV-BFa FSV-BG FSV-BH FSV-BJ FSV-BK FSV-BK	FSV-BM FSV-BN FSV-BO FSV-BD	FSV-BS FSV-BT FSV-BU	FSV-BV FSV-BW FSV-CD	FSV-CG FSV-CG FSV-CI	FSV-CZ FSV-DD	FSV-DV FSV-EE FSV-FK	FSV-FZ FSV-GD FSV-GE	Min Median Max eSD eCV	Npast Medianpast SDpast	N N N N N N N N N N N N N N N N N N N

Analytes Reported By One Laboratory

Values in µg/mL

Analyte	Code	407	408	409	410	411
Phytofluene	FSV-BS	0.091	0.327	0.113	0.197	0.162
Total ζ-Carotene	FSV-BS	0.082	0.157	0.095	0.120	0.110

Table Legend

Term	Definition
Ν	Number of (non-NIST) quantitative values reported for this analyte
Min	Minimum (non-NIST) quantitative value reported
Median	Median (non-NIST) quantitative value reported
Max	Maximum (non-NIST) quantitative value reported
SD	Adjusted median absolute deviation from the median of the non-NIST results
CV	Coefficient of Variation for (non-NIST) results: 100*SD/Median
N_{past}	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)
NAV	NIST Assigned Value
	= Median for analytes reported by ≥ 5 labs
NAU	NIST Assigned Uncertainty
	= the maximum of (0.05*NAV, SD, SD _{past} , eSD). The expected long-term SD,
	eSD, is defined in: Duewer et al., Anal Chem 1997;69(7):1406-1413.
na	Not analyzed
nd	Not detected (i.e., no detectable peak for analyte)
nq	Detected but not quantitatively determined
≥x	Concentration greater than or equal to x
italics	Not explicitly reported but calculated by NIST from reported values

Comparability Summary

Lab	TR	аТ	g/bT	bC	tbC	аC	TLy	TbX	TLu	ΤZ	L&Z
FSV-BB	1	1	1	1	1	1	1	1	1	1	1
FSV-BC	1										
FSV-BD	2	1									
FSV-BE	3	2	2	1							
FSV-BFa	1	2									
FSV-BG	1	1	1	1		1	1	1			1
FSV-BH	3	1	2	2		1	1	1	1	1	1
FSV-BJ	1	1	1	1		1	1	1	1		
FSV-BK	1	1									
FSV-BL	1	1									
FSV-BM	1	2									
FSV-BN	1	1		1		3	2	1			1
FSV-BO	1	1		1		1	2	1	1	2	1
FSV-BR	2	2									
FSV-BS	1			4	4	4	1	4	3	4	4
FSV-BT	3	1	1	1	1	1	2	2	1	1	1
FSV-BU	2	2	1	1		1	1	1			1
FSV-BV	2	3	3	2		1	2	2			1
FSV-BW	4	1	1	1		2	1				
FSV-CD	1	1	1	1			2	1			1
FSV-CE	1	2		2							
FSV-CG	2	3	2	1	1	1	1	1			1
FSV-CI	2	2	1	2		1			1	1	1
FSV-CO		1	1	1		1	1	1			1
FSV-CZ	3	2	1	2							
FSV-DD	1										
FSV-DV	1	2									
FSV-FK	1	2		4							
FSV-FZ	1	1	1	1							
FSV-GD	1	1	1	1	1	1	1				
FSV-GE	4	4		4							
n	30	28	16	22	5	15	15	13	7	6	13
	TR	аТ	g/bT	bC	tbC	аC	TLy	TbX	TLu	ΤZ	L&Z
% 1	60	54	75	68	80	80	67	69	86	67	92
% 2	20	36	19	18	0	7	33	23	0	17	0

0

14 20

6

7

7

0

0

0

0

8

14 0 0

0 17 8

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

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

We define CS as follows:

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

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.

% 3 13

Appendix D. Representative Individualized Report for RR76

Each participant in RR76 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
- 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-BB.

Individualized Round Robin LXXVI Report: FSV-BB

Summary

						,									
	Seri	um 407		Seru	Serum 408		Ser	Serum 409		Ser	Serum 410		Ser	Serum 411	
Analyte You	You	NAV	_	You	NAV	_	You	NAV	_	You	NAV	_	You	NAV	_
Total Retinol	0.342	0.348	59	0.477	0.497	29	0.444	0.452	29	0.452	0.451	28	0.438	0.456	29
Retinyl Palmitate	0.10	0.09	2	0.1	0.0		0.0	0.0	2	0.03	0.02	2	0.03	0.02	2
α-Tocopherol	4.62	4.31	28	10.63	10.35		7.26	6.93	28	8.20	8.00	27	7.84	7.36	28
γ/β-Tocopherol	1.629		16	1.408	1.409	16	1.726	1.721	16	1.835	1.854	15	1.793	1.767	16
ō-Tocopherol	0.044		7	0.083			0.065		7	0.083		7	0.049		7
Total β-Carotene	0.320		22	0.242	0.234	22	0.042	0.046	20	0.055	0.061	20	0.050	0.050	20
trans-β-Carotene	0.304		2	0.231	0.213		0.040	0.040	2	0.054	0.054	4	0.047	0.047	2
Total cis-β-Carotene	0.017		4	0.011	0.013		0.002	0.004	4	0.001	0.005	က	0.003	0.005	4
Total α-Carotene	0.021		14	0.032	0.032		0.014	0.015	4	0.042	0.041	4	0.029	0.027	14
Total Lycopene	0.156		15	0.543	0.585		0.152	0.166	15	0.292	0.312	4	0.214	0.240	15
trans-Lycopene	0.088		2	0.263	0.312		0.073	0.096	2	0.133	0.174	4	0.105	0.125	2
Total β-Cryptoxanthin	0.053		13	0.051	0.053	12	0.164	0.170	13	0.022	0.023	7	0.098	0.100	13
Total α-Cryptoxanthin	0.022		7	0.020			0.020		7	0.012		_	0.016		7
Total Lutein	0.082		7	0.083	0.086		0.073	0.077	7	0.087	0.089	9	0.080	0.093	7
Total Zeaxanthin	0.026		9	0.046	0.032	9	0.049	0.041	9	0.031	0.025	2	0.039	0.038	9
Total Lutein&Zeaxanthin	0.108		13	0.128	0.120	13	0.122	0.122	13	0.119	0.101	12	0.119	0.119	13

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

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

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

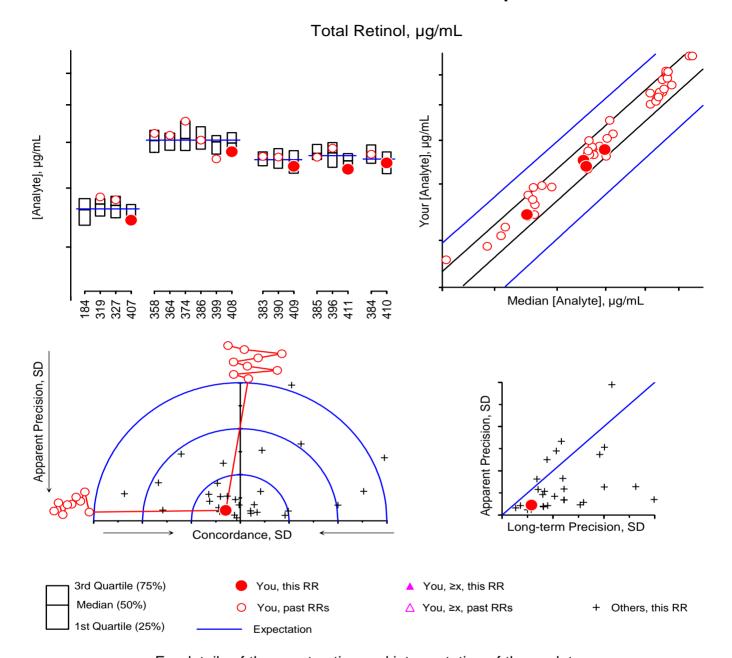
Please check our records against your records. Send corrections and/or updates to...

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

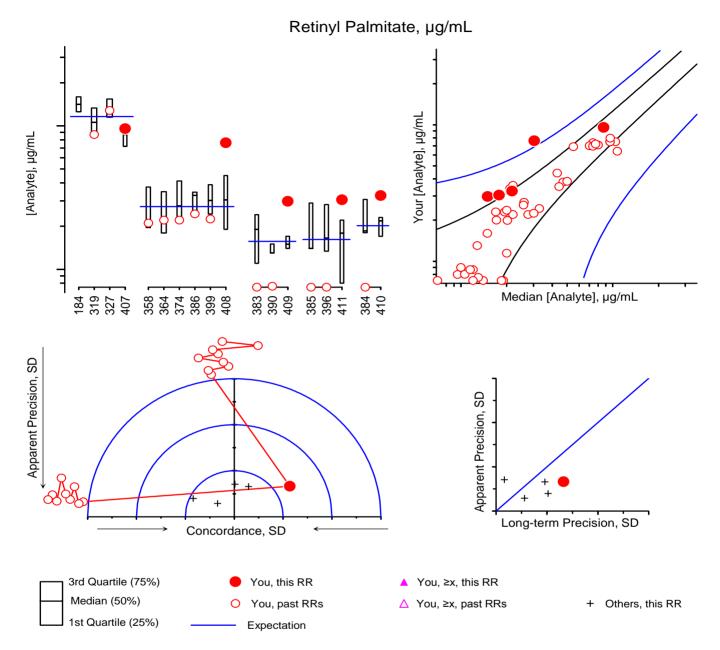
Tel: (301) 975-3935 Fax: (301) 977-0685

Email: david.duewer@nist.gov

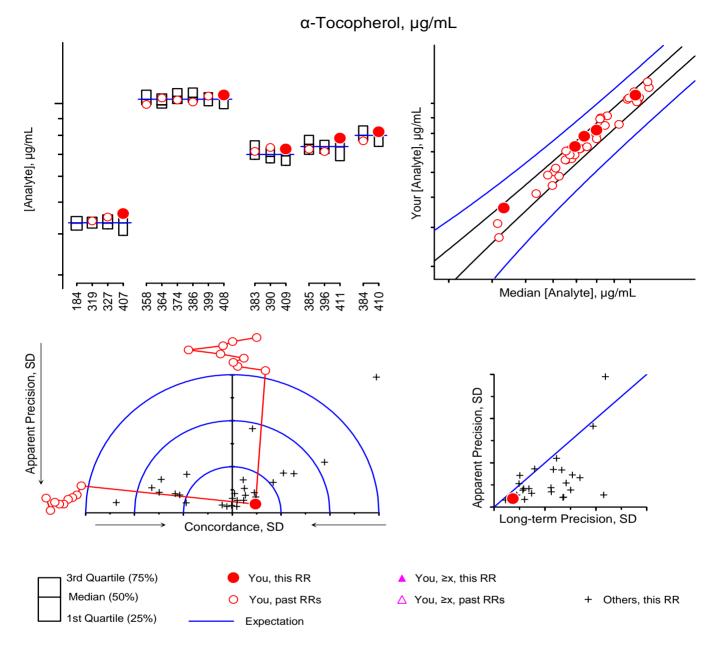
Page 1 / 14



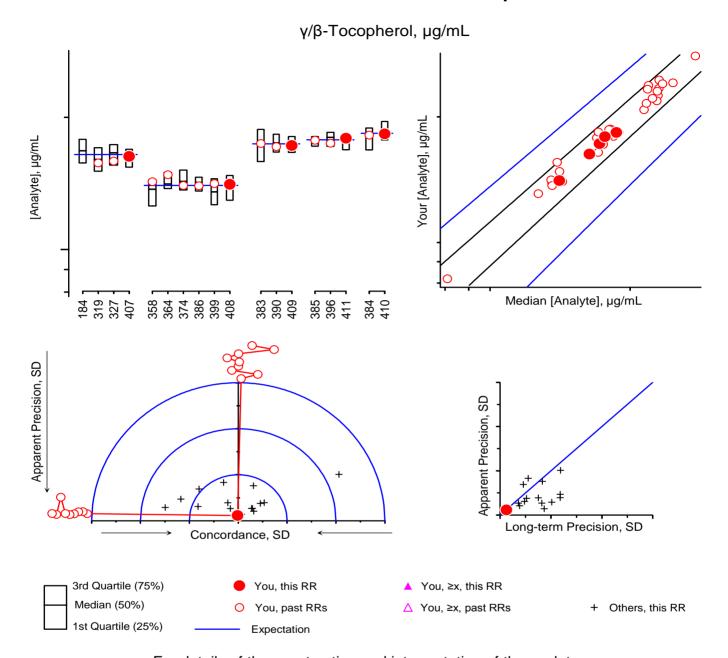
<u>Serum</u>	<u>Comments</u>	<u>History</u>
#407	Lyophilized, native, single-source	28#184, 59#319, 60#327
#408	Fresh-frozen, native, multi-donor: SRM 968e II	66#358, 67#364, 69#374, 71#386, 74#399
#409	Fresh-frozen, native, single-donor	71#386, 72#390
#410	Fresh-frozen, native, single-donor	71#384
#411	Mixture of (310 mL #409) and (260 mL #410)	71#385, 73#396



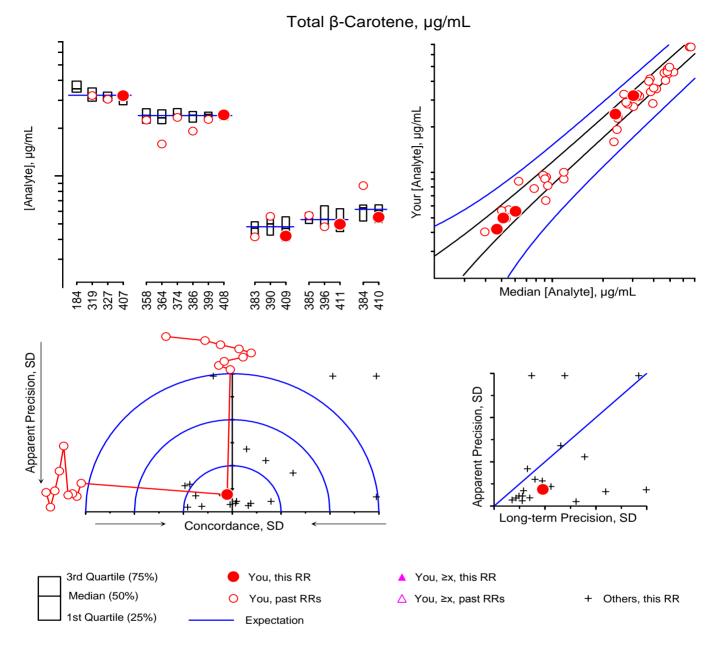
<u>Serum</u>	<u>Comments</u>	<u>History</u>
#407	Lyophilized, native, single-source	28#184, 59#319, 60#327
#408	Fresh-frozen, native, multi-donor: SRM 968e II	66#358, 67#364, 69#374, 71#386, 74#399
#409	Fresh-frozen, native, single-donor	71#386, 72#390
#410	Fresh-frozen, native, single-donor	71#384
#411	Mixture of (310 mL #409) and (260 mL #410)	71#385, 73#396



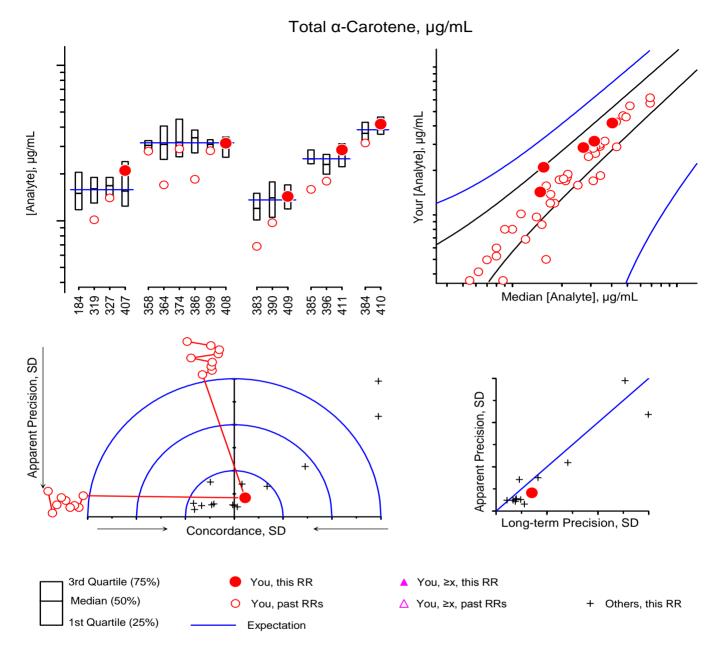
<u>Serum</u>	<u>Comments</u>	<u>History</u>
#407	Lyophilized, native, single-source	28#184, 59#319, 60#327
#408	Fresh-frozen, native, multi-donor: SRM 968e II	66#358, 67#364, 69#374, 71#386, 74#399
#409	Fresh-frozen, native, single-donor	71#386, 72#390
#410	Fresh-frozen, native, single-donor	71#384
#411	Mixture of (310 mL #409) and (260 mL #410)	71#385, 73#396



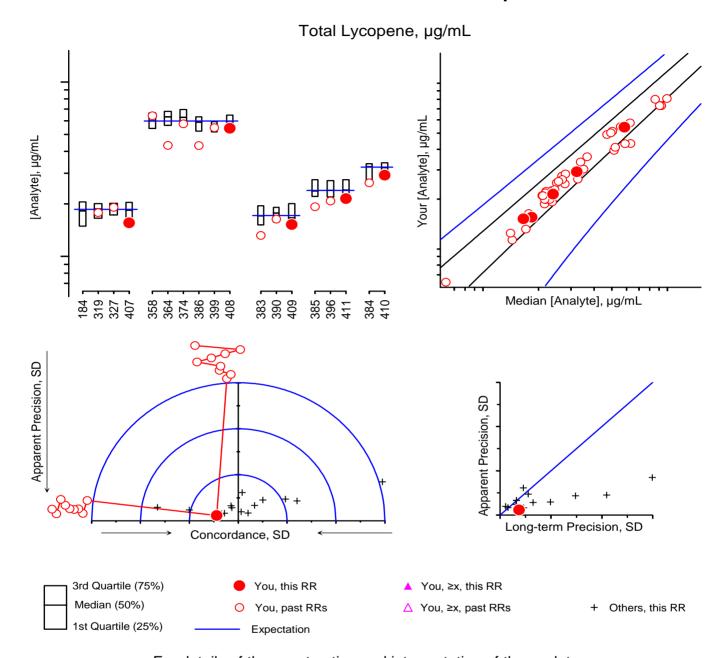
<u>Serum</u>	<u>Comments</u>	<u>History</u>
#407	Lyophilized, native, single-source	28#184, 59#319, 60#327
#408	Fresh-frozen, native, multi-donor: SRM 968e II	66#358, 67#364, 69#374, 71#386, 74#399
#409	Fresh-frozen, native, single-donor	71#386, 72#390
#410	Fresh-frozen, native, single-donor	71#384
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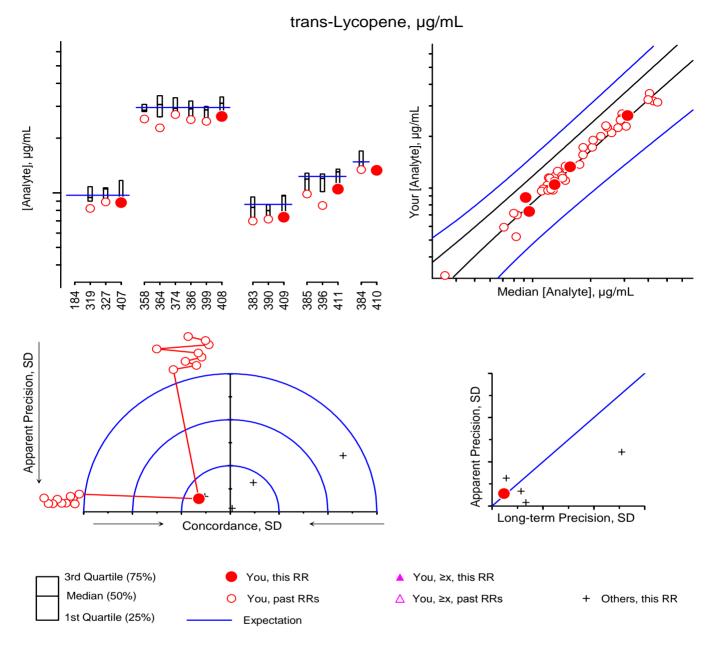
<u>Serum</u>	<u>Comments</u>	<u>History</u>
#407	Lyophilized, native, single-source	28#184, 59#319, 60#327
#408	Fresh-frozen, native, multi-donor: SRM 968e II	66#358, 67#364, 69#374, 71#386, 74#399
#409	Fresh-frozen, native, single-donor	71#386, 72#390
#410	Fresh-frozen, native, single-donor	71#384
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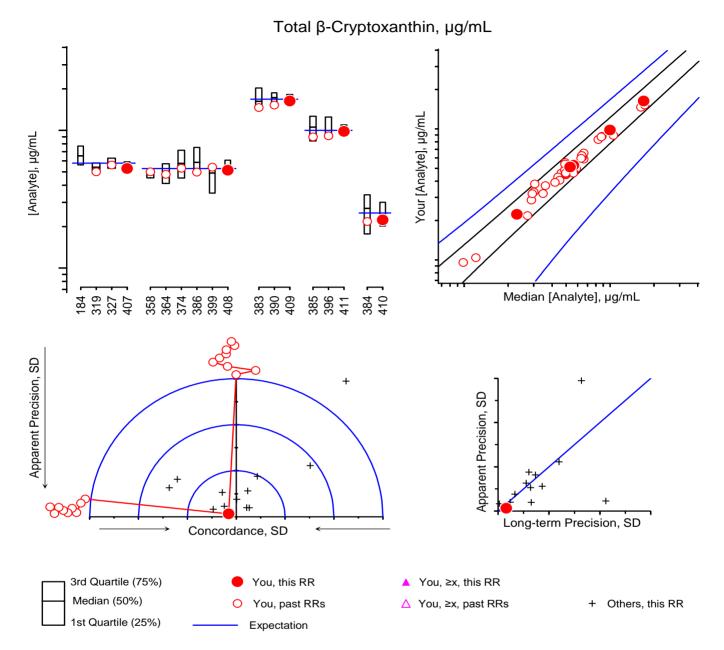
<u>Serum</u>	<u>Comments</u>	<u>History</u>
#407	Lyophilized, native, single-source	28#184, 59#319, 60#327
#408	Fresh-frozen, native, multi-donor: SRM 968e II	66#358, 67#364, 69#374, 71#386, 74#399
#409	Fresh-frozen, native, single-donor	71#386, 72#390
#410	Fresh-frozen, native, single-donor	71#384
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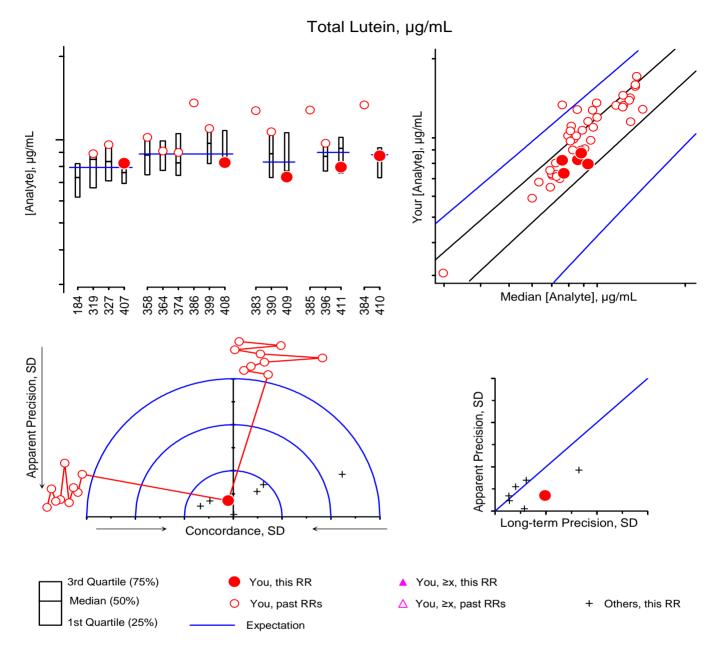
<u>Serum</u>	<u>Comments</u>	<u>History</u>
#407	Lyophilized, native, single-source	28#184, 59#319, 60#327
#408	Fresh-frozen, native, multi-donor: SRM 968e II	66#358, 67#364, 69#374, 71#386, 74#399
#409	Fresh-frozen, native, single-donor	71#386, 72#390
#410	Fresh-frozen, native, single-donor	71#384
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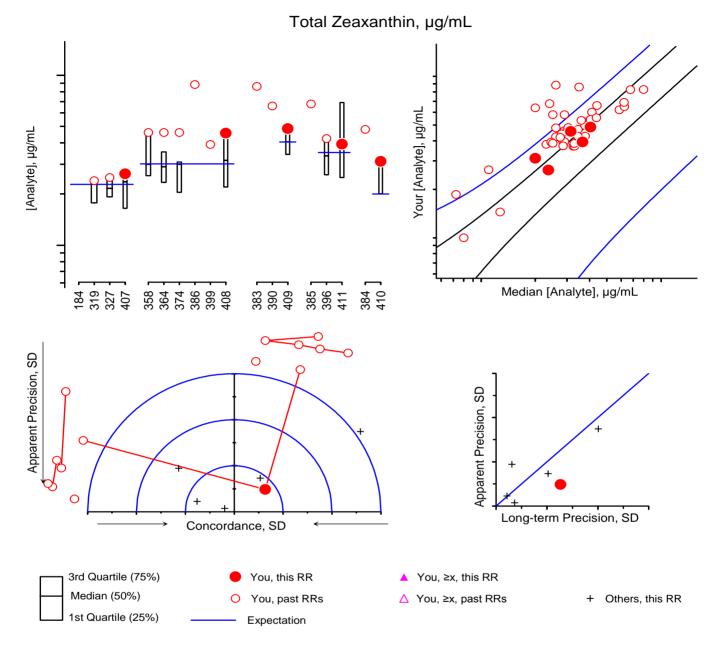
<u>Serum</u>	<u>Comments</u>	<u>History</u>
#407	Lyophilized, native, single-source	28#184, 59#319, 60#327
#408	Fresh-frozen, native, multi-donor: SRM 968e II	66#358, 67#364, 69#374, 71#386, 74#399
#409	Fresh-frozen, native, single-donor	71#386, 72#390
#410	Fresh-frozen, native, single-donor	71#384
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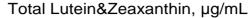
<u>Serum</u>	<u>Comments</u>	<u>History</u>
#407	Lyophilized, native, single-source	28#184, 59#319, 60#327
#408	Fresh-frozen, native, multi-donor: SRM 968e II	66#358, 67#364, 69#374, 71#386, 74#399
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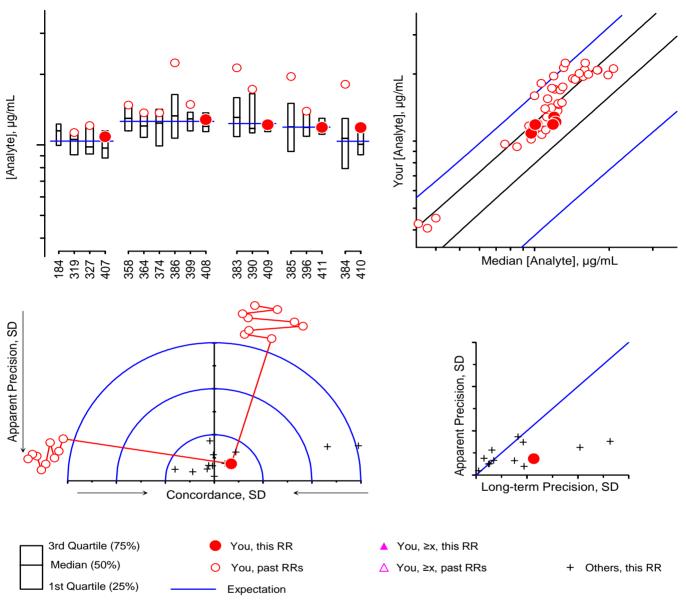


<u>Serum</u>	<u>Comments</u>	<u>History</u>
#407	Lyophilized, native, single-source	28#184, 59#319, 60#327
#408	Fresh-frozen, native, multi-donor: SRM 968e II	66#358, 67#364, 69#374, 71#386, 74#399
#409	Fresh-frozen, native, single-donor	71#386, 72#390
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<u>Serum</u>	<u>Comments</u>	<u>History</u>
#407	Lyophilized, native, single-source	28#184, 59#319, 60#327
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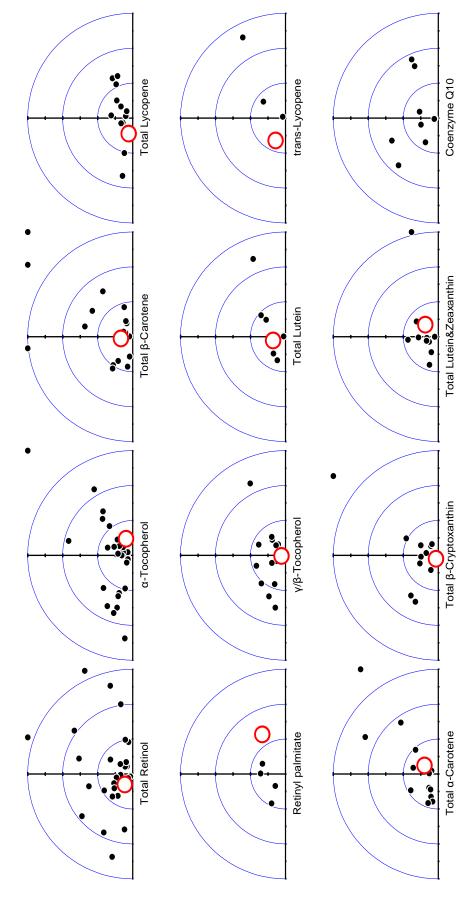




<u>Serum</u>	<u>Comments</u>	<u>History</u>
#407	Lyophilized, native, single-source	28#184, 59#319, 60#327
#408	Fresh-frozen, native, multi-donor: SRM 968e II	66#358, 67#364, 69#374, 71#386, 74#399
#409	Fresh-frozen, native, single-donor	71#386, 72#390
#410	Fresh-frozen, native, single-donor	71#384
#411	Mixture of (310 mL #409) and (260 mL #410)	71#385, 73#396

Individualized Round Robin LXXVI Report: FSV-BB Graphical Comparability Summary

Set 6 of 33



Appendix E. Shipping Package Inserts for RR41

The following three items were included in each package shipped to an RR41 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. This bag was labeled:

NIST MMQAP-VC: RR 41

Micronutrients Measurement Vitamin C Quality Assurance Program

Summer 2014 Controls & Samples

Analyze after: **June 1, 2014**Results due on or before: **September 15, 2014**

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-

February 10, 2014

Dear Colleague:

The samples within this package constitute Vitamin C Round Robin 41 (RR41) of the 2014 Micronutrients Measurement Quality Assurance Program. RR41 consists of one vial each of four frozen serum test samples (#411, #412 #413, and #414) 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 total ascorbic acid 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 <u>jbthomas@nist.gov</u> or 301-975-3120.

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 μ mol/(L sample solution) rather than μ 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 **September 15, 2014**.

Please report your results by e-mail to david.duewer@nist.gov or fax to 301-977-0685. If you have questions or comments regarding the studies, please contact us at 301-975-3120 (Jeanice); jbthomas@nist.gov or 301-975-3935 (Dave); david.duewer@nist.gov.

Sincerely,

Jeanice B. Thomas, M.B.A.

Research Chemist

Chemical Sciences Division

Material Measurement Laboratory

David L. Duewer, Ph.D.

Research Chemometrician

Chemical Sciences Division

Material Measurement Laboratory

Enclosure: RR41 Report Form for Control and Test Sample Analyses



Participant #:	Date:
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Vitamin C Round Robin 41 NIST Micronutrient Measurement Quality Assurance Program

Analysis of Control Materials and Test Samples

Sample	Replicate 1	Replicate 2	Units
Control serum CS#3			μmol/L of Sample T arget: (15.5 \pm 1.6) $μ$ mol/L
Control serum CS#4			μ mol/L of Sample Target: (46.1 ±4.6) μ mol/L
Test sample #411			μmol/L of Sample
Test sample #412			μmol/L of Sample
Test sample #413			μmol/L of Sample
Test sample #414			μmol/L of Sample

Were samples frozen upon receipt? Yes | No

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

Nature of samples you typically analyze: native | MPA-preserved | DTT-preserved | Other If "Other", please describe:

COMMENTS:

Please return by **September 15, 2014**

Fax: 301-977-0685

Email: david.duewer@nist.gov

Vitamin C Round Robin 41 NIST Micronutrients Measurement Quality Assurance Program

Packing List and Shipment Receipt Confirmation Form

This box contains one vial each of the following **six** VitC M²QAP samples:

Label	Form
VitC #411	Liquid frozen (1:1 serum:10% MPA)
VitC #412	Liquid frozen (1:1 serum:10% MPA)
VitC #413	Liquid frozen (1:1 serum:10% MPA)
VitC #414	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

Your prompt return of this information is appreciated.

The M²QAP Gang

Appendix F. Final Report for RR410

The following three pages are the final report for RR41 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.

Dear Colleague:

Enclosed is the summary report of the results for Round Robin 41 (RR41) 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 $100 \times MADe/median$.

RR41 consisted of one vial each of four test samples (#411, #412 #413, and #414) and one vial each of two control samples (CS#3 and CS#4). Both the test and control samples are 1:1 mixtures of serum and 10 % metaphosphoric acid augmented with high-purity ascorbic acid. 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 www.nist.gov/srm; 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 returned your intent-to-participate form for the 2015 NIST Micronutrients Measurement Quality Assurance Program (MMQAP), you should have received a confirmation receipt. Please notify us if you have not received your confirmation receipt.

Laboratories participating in the upcoming program will receive samples for the two vitamin C in serum studies (RR42 and RR43) in February 2015. Please note that we will ship the samples for both studies at the same time in February. Results will be due in May 2015 for the first study and in September 2015 for the second study. We will send you a reminder in late July 2015 about the reporting deadline for the second study. Please contact us immediately if this schedule is problematic for your laboratory.

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

Chemical Sciences Division

Material Measurement Laboratory

Enclosures

David L. Duewer, Ph.D. Research Chemometrician Chemical Sciences Division Material Measurement Laboratory



The NIST MMQAP Vitamin C Round Robin 41 (RR41) 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 RR41 measurements.
Page	"All-Lab" Report
1	A tabulation of results and summary statistics for total ascorbic acid [TAA] in the RR41 control and test samples. Results and summary statistics are also presented for the test samples calibrated to the results for the control samples using both 2-point proportional and linear models.

Serum-Based Samples. Two serum controls and four test samples were distributed in RR41.

- CS#3 Material ampouled in 2009 containing 15.4 ±0.4 μmol/L [TAA]
- CS#4 Material ampouled in 2009 containing $46.2 \pm 1.2 \mu mol/L$ [TAA]
- S41:1 Ampouled in late 2009, previously distributed in RRs 34, 36 (as two samples), 38 and 40
- S41:2 Ampouled in late 2009, previously distributed in RRs 32, 35, 36 and 38
- S41:3 Ampouled in late 2009, previously distributed in RRs 32, 33, 35, 38 and 40
- S41:4 SRM 970 level 1, ampouled in mid-1998, previously distributed as a test sample in RRs 11 to 16, 19, 20 23, 25, 29, 31, 34, 37 and 39

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 deviations are estimated with the robust median and adjusted median absolute deviation (eSD) statistics.
- 2) There is no evidence for any significant change in the [TAA] level for any of the four test materials.
- 3) The results for two control sera with well separated [TAA] levels enable calibration of the reported results for the unknowns to the both proportional and linear models. The proportional model is:

$$[TAA]_{reportedCS} = b \times [TAA]_{referenceCS}$$

where $[TAA]_{reportedCS}$ are the reported values for the two control sera, $[TAA]_{referenceCS}$ are the established reference values for these materials, and b is estimated using least-squares regression. The inverse model for estimating calibrated values for unknown samples is then:

$$[TAA]_{calibrated} = [TAA]_{reported} / b.$$

The linear model and its inverse are:

$$[TAA]_{reportedCS} = a + b \times [TAA]_{referenceCS}; [TAA]_{calibrated} = ([TAA]_{reported} - a) / b$$
.

where the *a* and *b* parameters are defined by the line between the {reference, reported} values for the two control sera.

Figure 1 displays eSD as a function of the Median values for the test samples distributed in RR34 through RR41 where the CS#3 and CS#4 were distributed as either test or control samples.

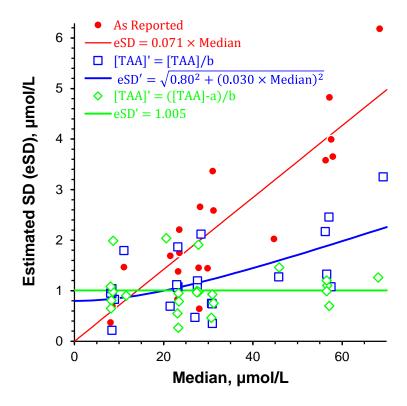


Figure 1: Estimated Standard Deviation as a Function of Median Value

Each symbol represents the summary statistics for one of the test samples distributed in RR34 through RR41. The solid red circles represent the results as they were reported (i.e., uncalibrated); the red line represents a "best fit" functional relationship between the eSD and the median. The open blue squares and blue line represent the reported test-sample results after calibration to the control samples using the proportional model. The open green diamonds and green line represent the results after calibration to the linear model.

The relationship between the eSD and the median values for the "As Reported" results is adequately described as a constant coefficient of variation, eCV = 100*eSD/Median, of 7.1 %. The best-fit relationship for the results calibrated to the proportional model is more complex, with a fairly constant component of 0.80 μ mol/L for [TAA] levels of 20 μ mol/L or less and a constant eCV of 3.0 % above 20 μ mol/L. The best-fit relationship with the linear model calibration is a constant eSD of 1.0 μ mol/L regardless of [TAA].

Since calibration using the proportional model tends to increase the variability for the lower [TAA] samples but does not completely stabilize it at higher levels, the proportional calibration model appears to be inferior to the linear model. While calibration to the two control samples dramatically reduces interlaboratory variability for the higher [TAA] materials, it increases variability at the lower [TAA] levels.

The addition of the RR41 results to those from the previous studies do not significantly impact the above analysis. Consequently, we will not distribute control materials in the 2015 vitamin C studies.

Appendix G. "All-Lab Report" for RR41

The following two pages are the "All-Lab Report" for RR41 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" 41 - Fall 2014

51.9 30.9 22.8 8.0 1.03 -1.5 56.0 30.4 23.6 7.7 0.6	57.9 30.9 22.8 8.6 1.03 -1.5 56.9 30.4 23.6 7.7 0.97 0.5 57.5 28.2 22.2 7.8 1.09 -1.2	57.9 30.9 22.8 8.6 1.03 -1.5 56.9 30.4 23.6 7.7 0.97 0.5 57.5 28.2 22.2 7.8 1.09 -1.2 55.1 30.4 23.1 8.4 0.93 1.5	56.9 30.4 23.6 7.7 0.97 0.5 57.5 28.2 22.2 7.8 1.09 -1.2 55.1 30.4 23.1 8.4 0.93 1.5 57.7 31.5 23.7 9.3 1.01 -0.6	56.9 30.4 22.8 8.6 1.03 -1.5 56.9 30.4 23.6 7.7 0.97 0.5 57.5 28.2 22.2 7.8 1.09 -1.2 55.1 30.4 23.1 8.4 0.93 1.5 57.7 31.5 23.7 9.3 1.01 -0.6 61.1 35.0 28.0 8.4 0.98 3.9	56.9 30.9 22.8 8.6 56.9 30.4 23.6 7.7 0.97 0.5 57.5 28.2 22.2 7.8 1.09 -1.2 55.1 30.4 23.1 8.4 0.93 1.5 67.7 31.5 23.7 9.3 1.01 -0.6 61.1 35.0 28.0 8.4 0.98 3.9 56.8 30.9 20.8 7.6 0.88 1.6	57.9 30.9 22.8 8.6 1.03 -1.5 56.9 30.4 23.6 7.7 0.97 0.5 57.5 28.2 22.2 7.8 1.09 -1.2 55.1 30.4 23.1 8.4 0.93 1.5 57.7 31.5 23.7 9.3 1.01 -0.6 61.1 35.0 28.0 8.4 0.98 3.9 56.8 30.9 20.8 7.6 0.88 1.6 58.3 30.7 22.9 9.2 0.97 -1.3
57.9 30.9 22.8 8.6 1.03 56.0 20.4 23.6 7.7 0.07	57.9 30.9 22.8 8.6 1.03 56.9 30.4 23.6 7.7 0.97 57.5 28.2 22.2 7.8 1.09	57.9 30.9 22.8 8.6 1.03 56.9 30.4 23.6 7.7 0.97 57.5 28.2 22.2 7.8 1.09 55.1 30.4 23.1 8.4 0.93	57.9 30.9 22.8 8.6 56.9 30.4 23.6 7.7 0.97 57.5 28.2 22.2 7.8 1.09 55.1 30.4 23.1 8.4 0.93 57.7 31.5 23.7 9.3 1.01	57.9 30.9 22.8 8.6 1.03 56.9 30.4 23.6 7.7 0.97 57.5 28.2 22.2 7.8 1.09 55.1 30.4 23.1 8.4 0.93 57.7 31.5 23.7 9.3 1.01 61.1 35.0 28.0 8.4 0.98	57.9 30.9 22.8 8.6 1.03 56.9 30.4 23.6 7.7 0.97 57.5 28.2 22.2 7.8 1.09 55.1 30.4 23.1 8.4 0.93 57.7 31.5 23.7 9.3 1.01 61.1 35.0 28.0 8.4 0.98 56.8 30.9 20.8 7.6 0.88	57.9 30.9 22.8 8.6 56.9 30.4 23.6 7.7 0.97 57.5 28.2 22.2 7.8 1.09 55.1 30.4 23.1 8.4 0.93 57.7 31.5 23.7 9.3 1.01 61.1 35.0 28.0 8.4 0.98 56.8 30.9 20.8 7.6 0.88 58.3 30.7 22.9 9.2 0.97
57.9 30.9 22.8	57.9 30.9 22.8 56.9 30.4 23.6 57.5 28.2 22.2	57.9 30.9 22.8 56.9 30.4 23.6 57.5 28.2 22.2 55.1 30.4 23.1	57.9 30.9 22.8 56.9 30.4 23.6 57.5 28.2 22.2 55.1 30.4 23.1 57.7 31.5 23.7	57.9 30.9 22.8 56.9 30.4 23.6 57.5 28.2 22.2 55.1 30.4 23.1 57.7 31.5 23.7 61.1 35.0 28.0	57.9 30.9 22.8 56.9 30.4 23.6 57.5 28.2 22.2 55.1 30.4 23.1 57.7 31.5 23.7 61.1 35.0 28.0 56.8 30.9 20.8	57.9 30.9 22.8 56.9 30.4 23.6 57.5 28.2 22.2 55.1 30.4 23.1 57.7 31.5 23.7 61.1 35.0 28.0 56.8 30.9 20.8 58.3 30.7 22.9
66.0	0.99	0.99 0.98 1.06 0.97	96.0 90.0 76.0	99 90 90 90 80		
			00404	0 0 + 0 + +	0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	0.98 0.98 0.97 0.97 0.093 0.93
14.2 45.9 57.6 30.7 22.7 8.	14.2 45.9 57.6 30.7 22.7 15.1 44.8 55.6 29.7 23.1 15.4 48.8 60.8 29.8 23.5	14.2 45.9 57.6 30.7 22.7 15.1 44.8 55.6 29.7 23.1 15.4 48.8 60.8 29.8 23.5 15.7 44.1 53.3 29.4 22.4	14.2 45.9 57.6 30.7 22.7 15.1 44.8 55.6 29.7 23.1 15.4 48.8 60.8 29.8 23.5 15.7 44.1 53.3 29.4 22.4 14.8 45.9 57.5 31.4 23.7	14.2 45.9 57.6 30.7 22.7 15.1 44.8 55.6 29.7 23.1 15.4 48.8 60.8 29.8 23.5 15.7 44.1 53.3 29.4 22.4 14.8 45.9 57.5 31.4 23.7 18.8 49.0 66.3 38.0 30.4	14.2 45.9 57.6 30.7 22.7 15.1 44.8 55.6 29.7 23.1 15.4 48.8 60.8 29.8 23.5 15.7 44.1 53.3 29.4 22.4 14.8 45.9 57.5 31.4 23.7 18.8 49.0 66.3 38.0 30.4 15.1 42.2 52.5 28.6 19.3	14.2 45.9 57.6 30.7 22.7 15.1 44.8 55.6 29.7 23.1 15.4 48.8 60.8 29.8 23.5 15.7 44.1 53.3 29.4 22.4 14.8 45.9 57.5 31.4 23.7 18.8 49.0 66.3 38.0 30.4 15.1 42.2 52.5 28.6 19.3 13.6 43.5 54.9 28.9 21.6
14.7 43.9	4 4 .8 8 .8 .8	12/09/14 15.1 44.8 22/08/14 15.4 48.8 14/08/14 15.7 44.1	12/09/14 15.1 44.8 22/08/14 15.4 48.8 14/08/14 15.7 44.1 15/09/14 14.8 45.9	15.1 44.8 15.4 48.8 15.7 44.1 14.8 45.9 18.8 49.0	12/09/14 15.1 44.8 22/08/14 15.1 44.8 14/08/14 15.7 44.1 15/09/14 14.8 45.9 09/09/14 15.1 42.2	12/09/14 15.1 44.8 22/08/14 15.1 44.8 14/08/14 15.7 44.1 15/09/14 14.8 45.9 05/08/14 15.1 42.2 05/08/14 13.6 43.5
	15.4 48.8 60.8 29.8 23.5	15.4 48.8 60.8 29.8 23.5 15.7 44.1 53.3 29.4 22.4	15.4 48.8 60.8 29.8 23.5 15.7 44.1 53.3 29.4 22.4 14.8 45.9 57.5 31.4 23.7	15.4 48.8 60.8 29.8 23.5 15.7 44.1 53.3 29.4 22.4 14.8 45.9 57.5 31.4 23.7 18.8 49.0 66.3 38.0 30.4	15.4 48.8 60.8 29.8 23.5 15.7 44.1 53.3 29.4 22.4 14.8 45.9 57.5 31.4 23.7 18.8 49.0 66.3 38.0 30.4 15.1 42.2 52.5 28.6 19.3	15.4 48.8 60.8 29.8 23.5 15.7 44.1 53.3 29.4 22.4 14.8 45.9 57.5 31.4 23.7 18.8 49.0 66.3 38.0 30.4 15.1 42.2 52.5 28.6 19.3 13.6 43.5 54.9 28.9 21.6

G2

Micronutrients Measurement Quality Assurance Program for Total Ascorbic Acid "Round Robin" 41 - Summer 2014

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		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 from the median (MADe)	Robust Coefficient of Variation, estimated as 100*eSD/Median
Term	Lab Date b	3	N Average	SD	Min	%52	Median	%75	Max	eSD		eCV

Appendix H. Representative "Individualized Report" for RR41

Each participant in RR41 received an "Individualized Report" reflecting their reported results. The following three pages are the "Individualized Report" for participant "VC-MB".

Vitamin C "Round Robin" 41 Report: Participant VC-MB

[TAA] mmol/Lsample											
Date	RR	Sample	Rep₁	Rep ₂	F_{adj}	Mean	SD_dup	N	Mean	SD _{repeat}	SD_{reprod}
01/14/11	34	S34:4	56.8	57.3	1.0	57.0	0.4	6	57.5	0.6	1.3
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				
02/24/14	40	S40:4	59.9	59.9	1.0	59.9	0.0				
08/14/14	41	S41:1	57.3	57.8	1.0	57.6	0.4				
0.1.1.0.1.0											
01/13/10	32	S32:3	30.5	31.0		30.7	0.4	5	30.9	0.5	0.6
06/13/11	35	S35:3	32.0	31.5		31.7	0.4				
03/07/12	36	S36:2	31.5	30.5		31.0	0.7				
02/27/13	38	S38:3	29.9	30.5		30.2	0.4				
08/14/14	41	S41:2	30.5	31.0	1.0	30.7	0.4				
01/13/10	32	S32:2	22.2	22.7	1.0	22.5	0.4	6	22.8	0.6	0.7
07/23/10	33	S33:4	23.2	22.7	1.0	23.0	0.4		22.0	0.0	0.7
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				
02/24/14	40	S40:3	23.7	22.7	1.0	23.2	0.7				
08/14/14	41	S41:3	22.2	23.2	1.0	22.7	0.7				
00, 1 1, 1 1	• •	011.0					0				
09/25/98	11	S11:1	25.0	25.0	0.5	12.5	0.0	16	9.4	0.6	1.6
02/26/99	12	S12:1	18.0	18.0	0.5	9.0	0.0				
03/03/00	13	S13:1	24.0	24.0	0.5	12.0	0.0				
03/26/01	14	S14:3	19.6	19.6	0.5	9.8	0.0				
09/05/01	15	S15:1	25.8	23.7	0.5	12.4	0.7				
02/08/02	16	S16:1	18.7	18.7	0.5	9.4	0.0				
05/01/03	19	S19:4	19.6	19.6	0.5	9.8	0.0				
03/01/04	20	S20:3	7.8	10.9		9.3	2.2				
05/25/05	23	S23:4	7.7	7.7	1.0	7.7	0.0				
05/24/06	25	S25:1	8.8	8.8	1.0	8.8	0.0				
06/20/08	29	S29:2	8.3	7.7	1.0	8.0	0.4				
08/05/09	31	S31:3	8.8	8.3	1.0	8.5	0.4				
01/14/11	34	S34:1	7.7	7.7	1.0	7.7	0.0				
08/06/12	37	S37:1	8.8	na	1.0	8.8					
08/01/13	39	S39:1	8.3	7.7	1.0	8.0	0.4				
08/14/14	41	S41:4	8.8	8.3	1.0	8.5	0.4				

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

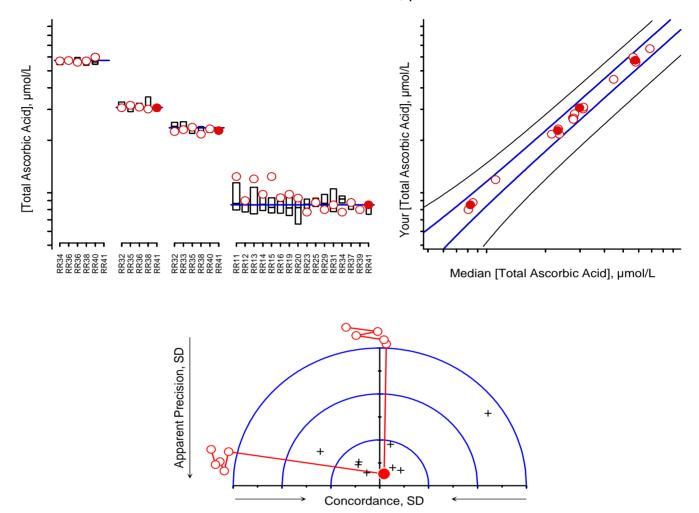
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Individualized Report Page 1/2

Vitamin C "Round Robin" 41 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.

You, this RR

You, past RRs

Others, this RR

<u>Sample</u> <u>Comments</u>

3rd Quartile (75%)

1st Quartile (25%)

Median (50%)

S41:1 Prepared 2009; distributed RRs 34,36(dups),38,40

S41:2 Prepared 2009; distributed RRs 32,33,35,38

S41:3 Prepared 2009; distributed RRs 32,33,35,38,40

S41:4 SRM970 Lv I; prepared 1998; distributed as unknowns RRs 11 - 16,19,20,23,25,29,31,34,37,39