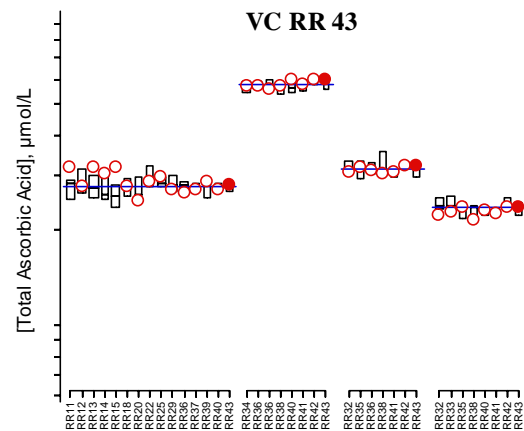
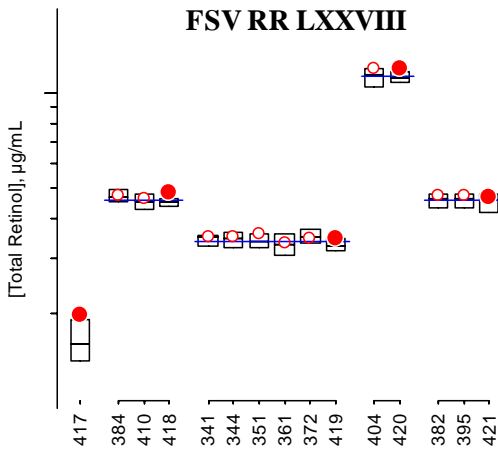


NIST Micronutrients Measurement Quality Assurance Program Summer 2015 Comparability Studies

Results for Round Robin LXXVIII
Fat-Soluble Vitamins and Carotenoids in Human Serum
and Round Robin 43 Ascorbic Acid in Human Serum

David L. Duewer
Jeanice B. Thomas

This publication is available free of charge from:
<http://dx.doi.org/10.6028/NIST.IR.7880-46>



NISTIR 7880-46

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and Round Robin 43 Ascorbic Acid in Human Serum

David L. Duewer
Jeanice B. Thomas
*Chemical Sciences Division
Materials Measurement Laboratory*

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July 2016



U.S. Department of Commerce
Penny Pritzker, Secretary

National Institute of Standards and Technology
Willie E. May, Under Secretary of Commerce for Standards and Technology and Director

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Abstract

The National Institute of Standards and Technology coordinates the Micronutrients Measurement Quality Assurance Program (MMQAP) for laboratories that measure fat- and water-soluble vitamins and carotenoids in human serum and plasma. This report describes the design of and results for the Summer 2015 MMQAP measurement comparability improvement studies: 1) Round Robin LXXVIII Fat-Soluble Vitamins and Carotenoids in Human Serum and 2) Round Robin 43 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 2015 together with the samples for FSV Round Robin LXXVII and VC Round Robin 42. Participants were requested not to analyze any of the Summer samples before June 22, 2015 but to provide their measurement results by September 1, 2015. Participants were reminded of the due-date by e-mail in early August, 2015.

Keywords

Human Serum

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

Table of Contents

Abstract	iii
Keywords	iii
Table of Contents	iv
Introduction	1
Round Robin LXXVIII: Fat-Soluble Vitamins and Carotenoids in Human Serum	1
Round Robin 43: Vitamin C in Human Serum	2
References	3
Appendix A. Shipping Package Inserts for RR78	A1
Appendix B. Final Report for RR78	B1
Appendix C. All-Lab Report for RR78	C1
Appendix D. Representative Individualized Report for RR78	D1
Appendix E. Shipping Package Inserts for RR43	E1
Appendix F. Final Report for RR43	F1
Appendix G. “All-Lab Report” for RR43	G1
Appendix H. Representative “Individualized Report” for RR43	H1

Introduction

Beginning in 1984, 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 LXXVIII: Fat-Soluble Vitamins and Carotenoids in Human Serum

Participants in the MMQAP Fat-Soluble Vitamins and Carotenoids in Human Serum Round Robin LXXVIII comparability study (hereafter referred to as RR78) received five 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 2015 in the same shipping package as the RR77 materials but in separate clearly labeled plastic bags. Participants were requested not to analyze any of the RR78 samples before June 22, 2015 but to provide their measurement results by September 1, 2015. 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 RR78 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 43: Vitamin C in Human Serum

Participants in the MMQAP Vitamin C in Human Serum Round Robin 43 comparability study (hereafter referred to as RR43) 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 2015 in the same shipping package as the RR42 materials but in separate clearly labeled plastic bags. Participants were requested not to analyze any of the RR43 samples before June 22, 2015 but to provide their measurement results by September 1, 2015. 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 RR43 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.

Note: RR43 was the last of the MMQAP Vitamin C comparability studies.

References

- 1 Diewer 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, Diewer DL. Measurement Of Ascorbic Acid in Human Plasma and Serum: Stability, Intralaboratory Repeatability, and Interlaboratory Reproducibility. *Clin Chem* 1996;42(8):1257-1262.
- 3 Diewer 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 RR78

The following items were included in each package shipped to an RR78 participant:

- Combined cover letter for Round Robin LXXV (RR77) and RR78.
- Datasheet for RR78. This was enclosed in the same sealed waterproof bag that contained the cover letter and the data sheet for RR77.
- Packing List and Shipment Receipt Confirmation Form for RR78.

This RR78 samples were enclosed in a bubble-wrapped sealed plastic bag that was labeled:

NIST MMQAP-FSV: RR LXXVIII
Micronutrients Measurement Fat-Soluble Vitamins
Quality Assurance Program
Summer 2015 Samples
Analyze after: **June 22, 2015**
Results due on or before:
September 1, 2015

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



February 13, 2015

Dear Colleague:

Enclosed are samples for the fat-soluble vitamins and carotenoids in serum studies for the 2015 NIST Micronutrients Measurement Quality Assurance Program. Sample details are provided below.

<u>Comparability study/Round Robin (RR)</u>	<u>Sample description</u>	<u>Results due</u>
RR77	Sera 412 - 416	May 18, 2015
RR78	Sera 417 - 421	September 1, 2015

RR77 consists of one vial of lyophilized serum and one vial each of four liquid-frozen serum samples for analysis; RR78 consists of four liquid-frozen serum samples. Samples should be stored in the dark at or below -20°C upon receipt. A form for each study is also included for reporting your results. When reporting your results, please submit one value for each analyte for each serum sample. If a value obtained is below your limit of quantification, please indicate this result on the form as "nq" (*Not Quantified*) or " $<x$ " where x is your established limit of quantification. Results are due to NIST for each study as indicated above. 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 each study will be distributed in June and October 2015, respectively. Please contact us immediately if this schedule is problematic for your laboratory.

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 ($\text{dL/g} \cdot \text{cm}$): 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 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

Participant #: _____

Date: _____

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

Analyte	417	418	419	420	421	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:

Please return results by
1-Sep-2015

Mail: M²QAP
NIST, Stop 8392
Gaithersburg, MD 20899-8392

A3

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

This publication is available free of charge from: <http://dx.doi.org/10.6028/NIST.IR.7880-46>

Participant #: _____

Date: _____

Fat-Soluble Vitamins Round Robin LXXVIII
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
#417	Liquid frozen	No	5 mL clear / silver
#418	Liquid frozen	No	2 mL amber / purple
#419	Liquid frozen	No	10 mL amber, silver
#420	Liquid frozen	No	2 mL clear / green
#421	Liquid frozen	No	2 mL clear / forest green

- 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 RR78

The following three pages are the final report for RR78 as provided to all participants:

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



UNITED STATES DEPARTMENT OF COMMERCE
National Institute of Standards and Technology
Gaithersburg, Maryland 20899-8390

September 25, 2015

Dear Colleague:

Enclosed is the summary report of the results for “Round Robin” LXXVIII (RR78) of the 2015 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. RR78 (Sera 417 to 421) consisted of one vial each of five 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 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: 301-978-0685.

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 LXXVIII (RR78) 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 n	“Four Plot” summaries of your current and past measurement performance, one page for each analyte you report that is also reported by at least eight other participants.
n+1	The graphical Comparability Summary (target plot) of measurement performance.

Samples. Five samples were distributed to each participant in RR78.

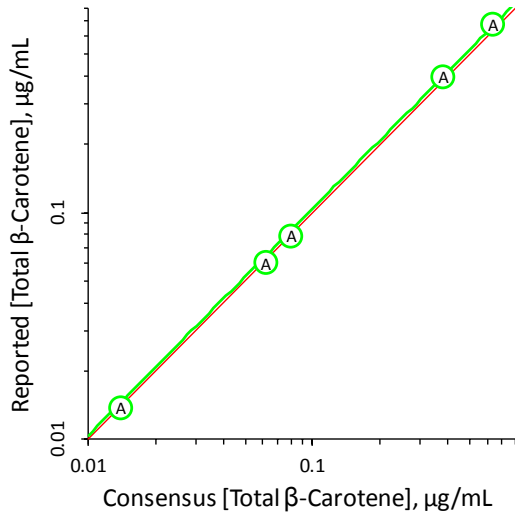
Serum	Description	Prior Distributions
417	Liquid-frozen, multi-donor heparin-treated plasma augmented with glycyrrhetic acid prepared in 1989.	First MMQAP FSV distribution.
418	Fresh-frozen, native, single donor, prepared in 2011	#384:RR71-3/12, #410:RR76 9/14
419	Fresh-frozen, native, multi-donor serum prepared in Fall, 2007. This was SRM 968d.	#341 & #344:RR63-3/08, #351:RR64-9/08, #361:RR66-9/09 #372:RR69-3/11
420	Fresh-frozen, augmented, single donor, prepared in 2013. This material was augmented with <i>trans</i> -retinol, retinyl palmitate, and β -tocopherol.	#404:RR75-3/14
421	Fresh-frozen, native, multi-donor serum prepared in 2011. This material has relatively high content of α - and β -carotene.	#382:RR71-3/12, #395:RR73-3-13

Results

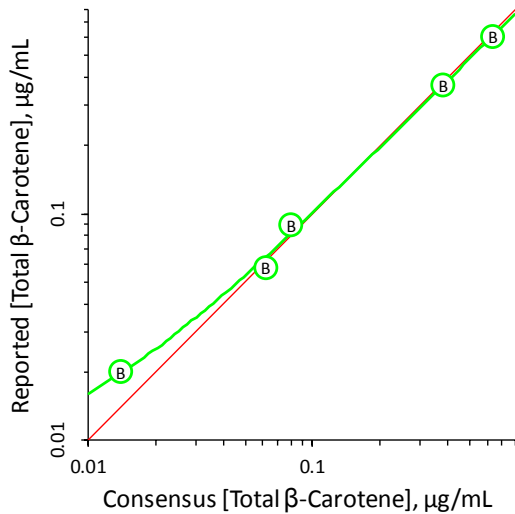
- 1) Stability: There has been no significant change in the concentration nor variability of any of the sera.
- 2) Serum 417: This material was prepared in 1989 as part of an investigation into the chemopreventative potential of glycyrrhetic acid (GRA). We distributed the material to further explore whether GRA interferes with the analysis of FSV analytes. However, the confounding of very low analyte levels, the presence of fibrin clots in some vials, and our mislabeling the vial as needing to be reconstituted precludes meaningful interpretation. While the results reported for this material have relatively large estimated relative variability (eCV) because of the very low analyte levels, the absolute variability (eSD) agrees well with our expectations. This material will not be distributed in future studies.
- 3) Serum 420: This material was spiked with β -tocopherol: this doesn't appear to have affected any of the reported “ γ/β -tocopherol” estimates.

- 4) α - and β -carotene: The levels of these analytes in the RR78 sera span unusually large ranges. For those who reported quantitative values for either or both of these analytes, this provides you with an opportunity to check your assays by plotting your reported values against the consensus medians on a log-log plot.

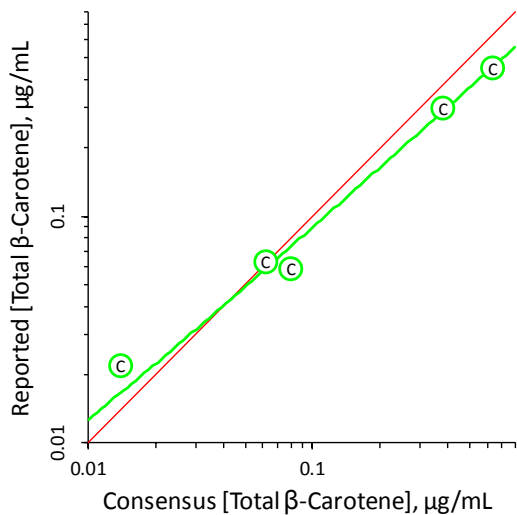
The interpretation of the such diagnostic plots depends on whether the consensus medians accurately reflect the true analyte levels. Assuming that they do, here's how we interpret a few examples. In all of the following, each symbol represents the {median, reported value} for one serum, the red line marks ideal agreement, and the thicker line is an approximate "best fit" sigmoidal curve.



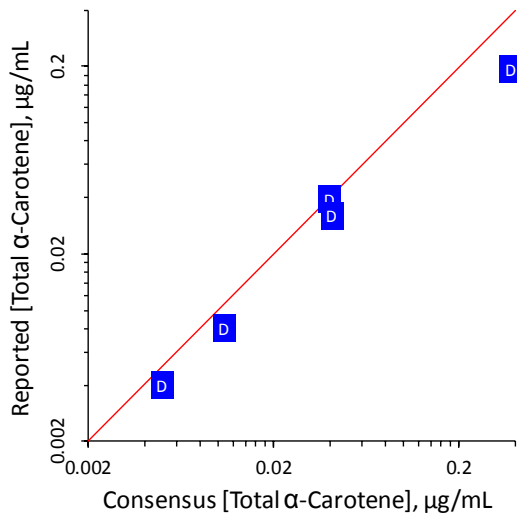
Excellent agreement from bottom to top!



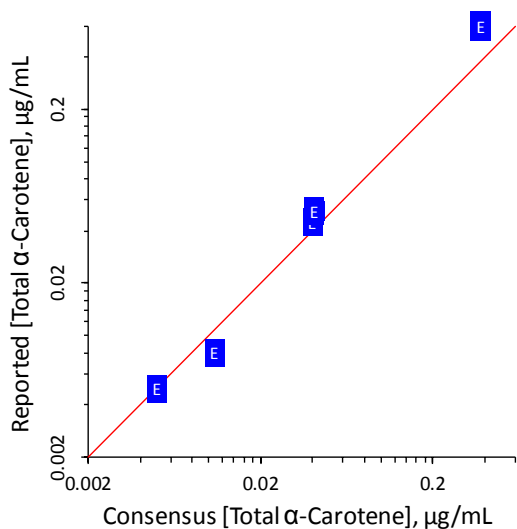
Good agreement above about 0.07 $\mu\text{g/mL}$. However, it's possible that the lab's limit of quantification for this analyte should be re-evaluated.



There's a strong linear relationship between the reported values and the medians. However, the lab's calibration function for this analyte is suspect.



Good agreement below about 0.04 $\mu\text{g/mL}$. However, it's possible that the unusually high α -carotene of serum 421 may have exceeded the assay's upper limit.



Well, it's a nice sigmoidal curve. It is, however, unlikely to represent the lab's real calibration function. A head scratcher, but also an invitation to look closely at the assay.

Appendix C. “All-Lab Report” for RR78

The following six pages are the “All-Lab Report” for RR78 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.

Round Robin LXXVIII Laboratory Results

Lab	Total β-Carotene, µg/mL				trans-β-Carotene, µg/mL				Total cis-β-Carotene, µg/mL				Total α-Carotene, µg/mL				Total Lycopene, µg/mL				
	417	418	419	420	417	418	419	420	417	418	419	420	417	418	419	420	417	418	419	420	
FSV-BC																					
FSV-BD																					
FSV-BE	<0.02	0.090	0.080	0.520	0.660																
FSV-BFa																					
FSV-BG	0.014	0.060	0.079	0.399	0.674												0.127	0.344	0.287	0.268	
FSV-BH																					
FSV-BJ	0.062	0.047	0.106	0.378	0.570												0.104	0.307	0.245	0.274	
FSV-BK																					
FSV-BL																					
FSV-BM																					
FSV-BN	na	0.064	na	0.450	0.688												na	0.289	na	0.214	
FSV-BR																					
FSV-BS	≤0.004	≤0.053	≤0.044	≤0.333	≤0.404	0.004	0.053	0.044	0.333	0.404								0.099	0.340	0.192	0.256
FSV-BT	0.017	0.072	0.092	0.365	0.607	0.011	0.060	0.068	0.334	0.546	0.006	0.012	0.024	0.031	0.061						
FSV-BU	0.010	0.053	0.039	0.386	0.757													0.003	0.043	0.013	0.326
FSV-BV	0.013	0.074	0.084	0.448	0.736													0.003	0.037	0.005	0.034
FSV-BW	0.004	0.050	0.055	0.472	0.595													0.005	0.045	0.008	0.052
FSV-CD	ng	0.070	0.102	0.429	0.795													0.005	0.047	0.022	0.036
FSV-CE	0.010	0.040	0.080	0.370	0.660													ng	ng	ng	ng
FSV-CF																					
FSV-CG	0.020	0.058	0.089	0.369	0.609	0.016	0.053	0.082	0.345	0.571	0.004	0.004	0.004	0.023	0.038						
FSV-CI	<0.02	0.051	0.056	0.352	0.593													0.011	0.040	0.014	0.039
FSV-CO	0.015	0.067	0.088	0.404	0.720													0.005	0.038	0.008	0.039
FSV-CZ	0.022	0.063	0.059	0.299	0.450																
FSV-DD																					
FSV-DV																					
FSV-EE																					
FSV-FK	ng	≤0.031	≤0.073	≤0.331	≤0.607	ng	0.031	0.073	0.331	0.607											
FSV-FZ																					
FSV-GD	na	0.063	0.069	0.349	0.582	na	0.055	0.060	0.308	0.512	na	0.008	0.009	0.041	0.070						
FSV-GF																					
FSV-GG																					
n	10	15	14	15	15	3	5	5	5	5	2	3	3	3	3	10	12	10	12		
Min	0.004	0.040	0.039	0.299	0.450	0.004	0.031	0.044	0.308	0.404	0.004	0.004	0.004	0.023	0.038	0.003	0.027	0.005	0.032		
Median	0.014	0.063	0.080	0.386	0.660	0.011	0.053	0.068	0.333	0.546	0.005	0.008	0.009	0.031	0.061	0.005	0.040	0.009	0.041		
Max	0.062	0.090	0.106	0.520	0.795	0.016	0.060	0.082	0.345	0.607	0.006	0.012	0.024	0.041	0.070	0.025	0.047	0.022	0.071		
eSD	0.007	0.013	0.017	0.050	0.096	0.003	0.012	0.003	0.050	0.050	0.002	0.004	0.005	0.003	0.065	0.002	0.004	0.005	0.003		
eCV	45	21	21	13	15	6	17	1	9	9	44	9	49	7	17	44	9	49	7		
Npast	0	19	22	21	21	0	5	8	6	6	0	0	6	4	5	0	15	16	14		
Medianpast	0.062	0.077	0.380	0.659	0.623	0.055	0.074	0.367	0.623	0.623	0.005	0.031	0.038	0.038	0.367	0.038	0.009	0.043	0.367		
SDpast	0.012	0.016	0.047	0.098	0.100	0.005	0.011	0.042	0.100	0.100	0.002	0.011	0.004	0.004	0.085	0.007	0.003	0.005	0.085		
NAV	0.014	0.063	0.080	0.386	0.660	0.011	0.053	0.068	0.333	0.546	0.008	0.009	0.031	0.061	0.061	0.005	0.040	0.009	0.041		
NAU	0.008	0.013	0.017	0.055	0.096	0.009	0.012	0.036	0.057	0.057	0.003	0.013	0.005	0.013	0.097	0.003	0.013	0.005	0.013		

Round Robin LXXVIII Laboratory Results

Lab	trans-Lycopene, µg/mL			Total β-Cryptoxanthin, µg/mL			Total α-Cryptoxanthin, µg/mL			Total Lutein, µg/mL			Total Zeaxanthin, µg/mL		
	417	418	419	420	421	417	418	419	420	421	417	418	419	420	421
FSV-BC															
FSV-BD															
FSV-BE															
FSV-BFa															
FSV-BG	0.070	0.168	0.134	0.146	0.137	0.009	0.027	0.046	0.071	0.072	0.015	0.079	0.049	0.061	0.128
FSV-BH															
FSV-BJ						<i>nq</i>	<i>nq</i>	0.048	0.071	0.083	<i>nq</i>	0.083	0.061	0.090	0.150
FSV-BK															
FSV-BL															
FSV-BM															
FSV-BN						<i>na</i>	0.026	<i>na</i>	0.074	0.059					
FSV-BR															
FSV-BS	0.048	0.129	0.070	0.104	0.073	0.001	0.025	0.028	0.058	0.069	0.009	0.068	0.039	0.055	0.108
FSV-BT	0.026	0.133	0.086	0.111	0.142	0.007	0.024	0.030	0.041	0.043	0.021	0.090	0.078	0.065	0.118
FSV-BU						0.007	0.013	0.030	0.062	0.062					
FSV-BV						0.005	0.013	0.044	0.088	0.086					
FSV-BW						<i>nq</i>	<i>nq</i>	<i>nq</i>	0.070	0.070	<i>nq</i>	<i>nq</i>	<i>nq</i>	<i>nq</i>	<i>nq</i>
FSV-CD															
FSV-CE															
FSV-CF															
FSV-CG	0.095	0.159	0.149	0.135	0.141	0.028	0.032	0.060	0.074	0.077	0.022	0.067	0.050	0.063	0.105
FSV-CI															
FSV-CO						0.016	0.025	0.045	0.060	0.060					
FSV-CZ															
FSV-DD															
FSV-DV															
FSV-EE															
FSV-FK															
FSV-FZ															
FSV-GD															
FSV-GF															
FSV-GG															
n	4	4	4	4	4	7	8	8	10	10	1	1	1	2	1
Min	0.026	0.129	0.070	0.104	0.073	0.001	0.013	0.028	0.041	0.043	0.004	0.011	0.013	0.025	0.015
Median	0.059	0.146	0.110	0.123	0.139	0.007	0.025	0.045	0.071	0.070	0.020	0.079	0.050	0.063	0.118
Max	0.095	0.168	0.149	0.146	0.142	0.028	0.032	0.060	0.088	0.086	0.030	0.090	0.078	0.090	0.150
eSD	0.032	0.022	0.047	0.023	0.003	0.003	0.002	0.013	0.009	0.013					
eCV	55	15	42	19	2	46	10	30	13	18	29	21	33	5	13
Npast	0	5	8	6	7	0	12	18	13	15	0	5	10	8	6
Medianpast	0.152	0.117	0.142	0.131		0.025	0.040	0.064	0.065		0.013	0.017	0.072	0.136	
SDpast	0.032	0.019	0.019	0.018		0.011	0.008	0.007	0.010		0.004	0.004	0.014	0.017	0.018
NAV	0.059	0.146	0.110	0.123	0.139	0.007	0.025	0.045	0.071	0.070	0.018	0.079	0.050	0.063	0.118
NAU						0.003	0.007	0.013	0.017	0.016	0.016	0.016	0.016	0.013	0.022

Round Robin LXXVIII Laboratory Results

Lab	Total Lutein&Zeaxanthin, µg/mL				Coenzyme Q10, µg/mL				Phylloquinone (K1), ng/mL				25-hydroxyvitamin D, µg/mL			
	417	418	419	420	421	417	418	419	420	421	417	418	419	420	421	
FSV-BC																
FSV-BD																
FSV-BE																
FSV-BFa																
FSV-BG	0.030	0.117	0.078	0.106	0.169	0.37	0.70	0.57	0.610	0.39	0.164	0.135	0.151	0.405	1.447	
FSV-BH	0.023	0.101	0.076	0.105	0.153											
FSV-BJ						0.69	0.97	0.77	0.873	0.68						
FSV-BK																
FSV-BL																
FSV-BM																
FSV-BN	na	0.097	na	0.099	0.122											
FSV-BR																
FSV-BS	0.011	0.078	0.054	0.078	0.124											
FSV-BT	0.031	0.110	0.111	0.095	0.140	0.41	0.87	0.46	0.760	0.68						
FSV-BU	0.025	0.096	0.078	0.100	0.158											
FSV-BV	0.030	0.094	0.078	0.123	0.205	0.26	0.61	0.41	0.620	0.33						
FSV-BW																
FSV-CD	0.050	0.130	0.090	0.140	0.190											
FSV-CE																
FSV-CF																
FSV-CG	0.050	0.107	0.087	0.114	0.164											
FSV-CI	0.030	0.077	0.065	0.086	0.116	0.55	0.86	0.66	0.770	0.64	0.295	0.225	0.204	0.568	1.936	
FSV-CO	0.015	0.062	0.051	0.070	0.105	0.51	0.87	0.54	0.811	0.50						
FSV-CZ																
FSV-DD																
FSV-DV																
FSV-EE						0.56	0.96	0.70	0.838	0.65	0.190	0.180	0.190	0.470	2.280	
FSV-FK																
FSV-FZ						na	0.80	0.69	0.711	0.66						
FSV-GD						0.49	0.89	0.70	0.740	0.57						
FSV-GF						0.60	1.00	0.90	0.900	0.50						
FSV-GG																
n	10	11	10	11	11	9	10	10	10	10	3	3	3	3	3	
Min	0.011	0.062	0.051	0.070	0.105	0.26	0.61	0.41	0.610	0.33	0.164	0.135	0.151	0.405	1.447	
Median	0.030	0.097	0.078	0.100	0.153	0.51	0.87	0.68	0.765	0.61	0.190	0.180	0.190	0.470	1.936	
Max	0.050	0.130	0.111	0.140	0.205	0.69	1.00	0.90	0.900	0.68	0.295	0.225	0.204	0.568	2.280	
eSD	0.009	0.019	0.016	0.021	0.043	0.13	0.12	0.15	0.094	0.11	0.020	0.004	0.014	0.019	0.019	
eCV	30	20	20	21	28	25	13	22	12	17	0	0	0	0	0	
Npast	0	13	18	13	15	0	9	8	10	8	0	0	0	0	0	
Medianpast		0.104	0.083	0.102	0.162		0.94	0.65	0.830	0.72						
SDpast		0.033	0.020	0.012	0.049		0.05	0.12	0.142	0.09						
NAV	0.030	0.097	0.078	0.100	0.153	0.51	0.87	0.68	0.765	0.61	0.190	0.180	0.190	0.470	1.936	
NAU	0.009	0.020	0.016	0.021	0.043	0.13	0.12	0.15	0.094	0.11						

Round Robin LXXVIII Laboratory Results

Analytes Reported By One Laboratory

Values in µg/mL

Analyte	Code	417	418	419	420	421
Phytofluene	FSV-BS	0.032	0.099	0.061	0.060	0.073
Vitamin D3	FSV-BH	≤0.0005	≤0.0005	0.002	≤0.0005	≤0.0005
β-Tocopherol	FSV-BU	0.058	0.095	0.103	2.219	0.103

Table 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
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)
Median _{past}	Mean of Median(s) from past RR(s)
SD _{past}	Pooled SD from past RR(s)
NAV	NIST Assigned Value, the 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: Duewer et al., Anal Chem 1997;69(7):1406-1413.
na	Discrepant value: heterogeneous serum, damaged sample, malfunction, etc.
nd	Not detected (i.e., no detectable peak for analyte)
nq	Detected but not quantitatively determined
≤x	Concentration at or below the limit of quantification, x
<i>italics</i>	Not explicitly reported but calculated by NIST from reported values

Round Robin LXXVIII Laboratory Results

Comparability Summary

Lab	TR	aT	g/bT	bC	aC	TLy	TbX	TLu	L&Z
FSV-BC	1								
FSV-BD	2	1							
FSV-BE	2	2	1	2					
FSV-BFa	1	1							
FSV-BG	1	1	1	1	1	1			1
FSV-BH	2	1						1	1
FSV-BJ	1	1	1	4	4	1	1	2	
FSV-BK	2	1							
FSV-BL	1	1							
FSV-BM	2	1							
FSV-BN	1	1		1	2	1	1		1
FSV-BR	1	1							
FSV-BS	1	4		2	2	1	2	1	2
FSV-BT	3	1	2	1	1	2	2	2	2
FSV-BU	2	1	1	2	1	2	2		1
FSV-BV	3	2	2	1	2	1	2		1
FSV-BW	2	4	3	2	2	2			
FSV-CD	2	1	1	2		2	1		2
FSV-CE	1	1		1					
FSV-CF	2	2							
FSV-CG	2	2	2	1	1	1	4		2
FSV-CI	2	2	1	1	2			1	1
FSV-CO	1	1	1	1	1	1	2		2
FSV-CZ	1	2	3	2					
FSV-DD	2								
FSV-DV	1	3							
FSV-FK	1	2		2					
FSV-FZ	1	1	1						
FSV-GD	2	1	1	1	1	1			
n	29	27	14	17	12	12	10	5	11

	TR	aT	g/bT	bC	aC	TLy	TbX	TLu	L&Z
% 1	48	63	64	53	50	67	40	60	55
% 2	45	26	21	41	42	33	50	40	45
% 3	7	4	14	0	0	0	0	0	0
% 4	0	7	0	6	8	0	10	0	0

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

"Comparability Score"

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

We define CS as follows:

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

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

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

NAU = NIST Assigned Uncertainty

For further details, please see

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

Appendix D. Representative Individualized Report for RR78

Each participant in RR78 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
- Total β -Cryptoxanthin
- Total Lutein
- Total Lutein & Zeaxanthin
- Coenzyme Q10

The following eleven pages are the “Individualized Report” for the analytes evaluated by participant FSV-BG.

Individualized Round Robin LXXVIII Report: FSV-BG

Summary

Analyte	Serum 417			Serum 418			Serum 419			Serum 420			Serum 421		
	You	NAV	n	You	NAV	n	You	NAV	n	You	NAV	n	You	NAV	n
Total Retinol	0.198	0.160	26	0.483	0.450	29	0.347	0.329	28	1.192	1.123	29	0.470	0.456	29
Retinyl Palmitate	nd	0.01	3	0.0	0.0	4	0.0	0.0	4	0.55	0.55	5	0.06	0.06	5
α -Tocopherol	3.50	3.63	24	7.27	7.80	27	5.50	5.53	26	8.23	8.07	27	11.40	10.82	27
γ/β -Tocopherol	0.757	0.730	13	1.821	1.934	14	1.443	1.482	14	3.943	3.840	14	1.401	1.480	14
Total β -Carotene	0.014	0.014	10	0.060	0.063	15	0.079	0.080	14	0.399	0.386	15	0.674	0.660	15
Total α -Carotene	0.005	0.005	10	0.036	0.040	12	0.011	0.009	10	0.042	0.041	11	0.357	0.393	13
Total Lycopene	0.127	0.102	10	0.344	0.304	12	0.287	0.255	11	0.268	0.262	12	0.281	0.278	12
trans-Lycopene	0.070	0.059	4	0.168	0.146	4	0.134	0.110	4	0.146	0.123	4	0.137	0.139	4
Total β -Cryptoxanthin	0.009	0.007	7	0.027	0.025	8	0.046	0.045	8	0.071	0.071	10	0.072	0.070	10
Total Lutein&Zeaxanthin	0.030	0.030	10	0.117	0.097	11	0.078	0.078	10	0.106	0.100	11	0.169	0.153	11

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

nd : Not detected

D2

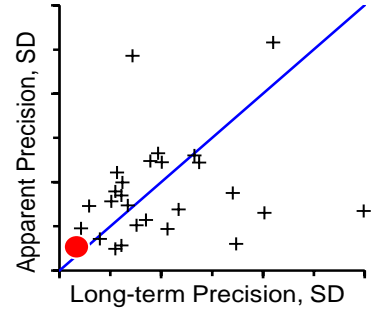
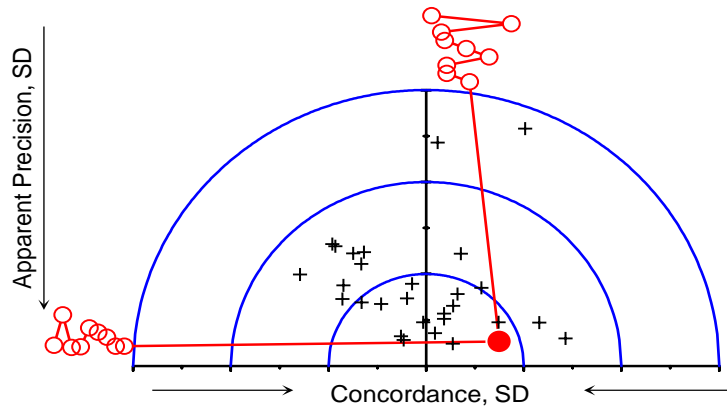
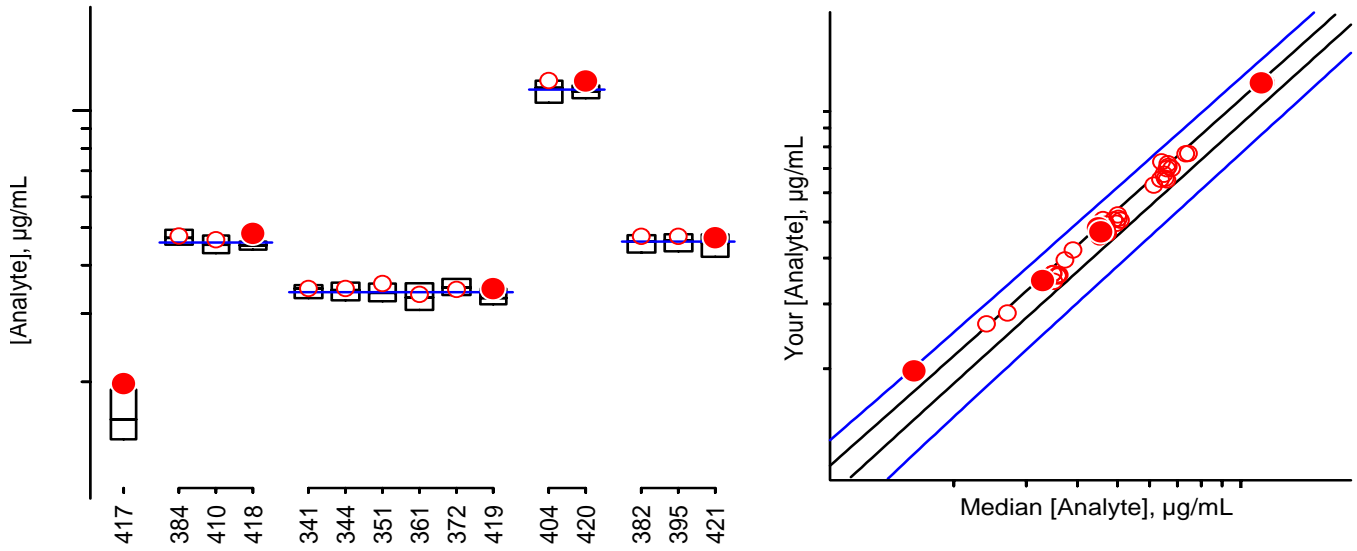
Please check our records against your records. Send corrections and/or updates to...

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

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

Individualized RR LXXVIII Report: FSV-BG

Total Retinol, $\mu\text{g/mL}$



- 3rd Quartile (75%)
- Median (50%)
- 1st Quartile (25%)
- You, this RR
- You, past RRs
- Expectation
- You, $\geq x$, this RR
- You, $\geq x$, past RRs
- Others, this RR

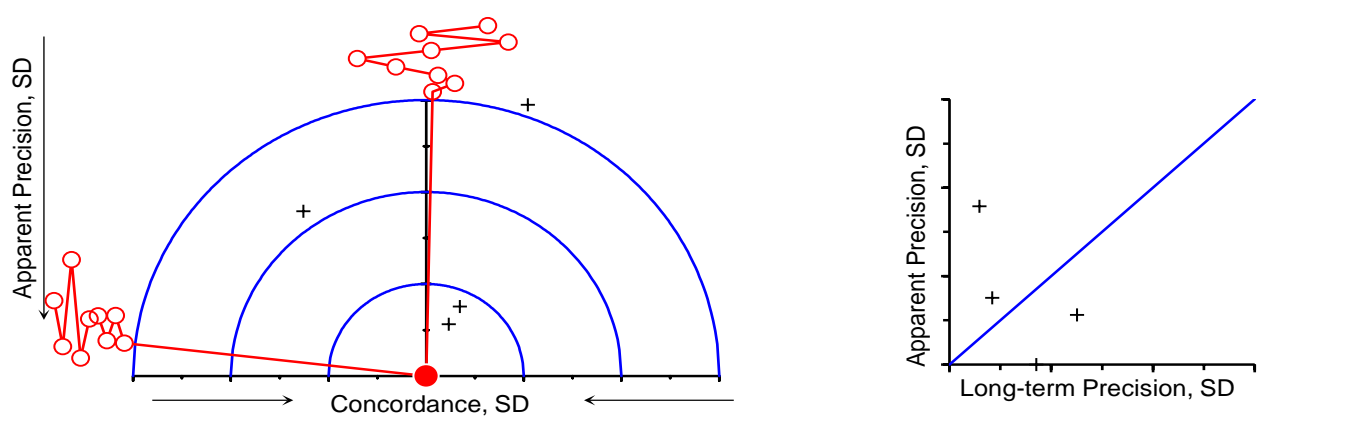
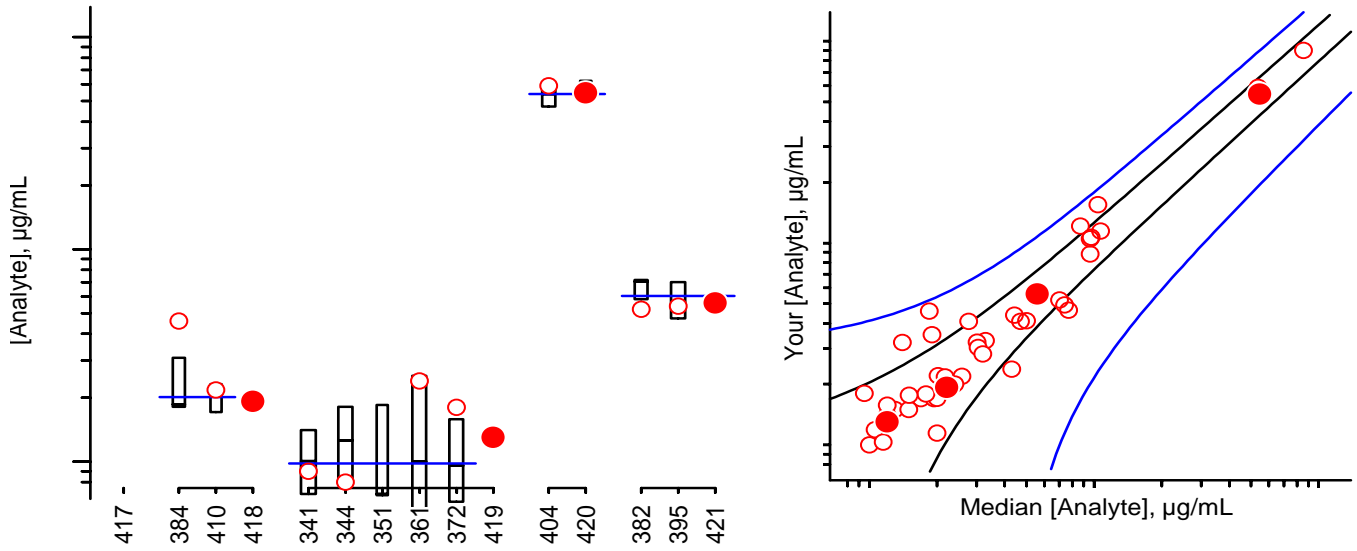
For details of the construction and interpretation of these plots, see:
 Duewer, Kline, Sharpless, Brown Thomas, Gary, Sowell. Anal Chem 1999;71(9):1870-8.

<u>Serum</u>	<u>Comments</u>	<u>History</u>
#417	Fresh-frozen, native, multi-donor	First (and last) distribution
#418	Fresh-frozen, native, multi-donor	RR71#384, RR76#410
#419	Fresh-frozen, native, multi-donor: SRM968d	RR63#341 & #344, RR64#351, RR66#361, RR69#372
#420	Fresh-frozen, augmented {tR,RP, β T}, single-donor	RR75#404
#421	Fresh-frozen, native, multi-donor	RR71#382, RR73#395

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Individualized RR LXXVIII Report: FSV-BG

Retinyl Palmitate, µg/mL



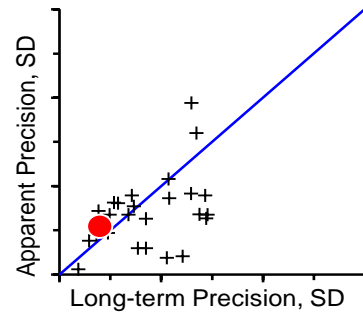
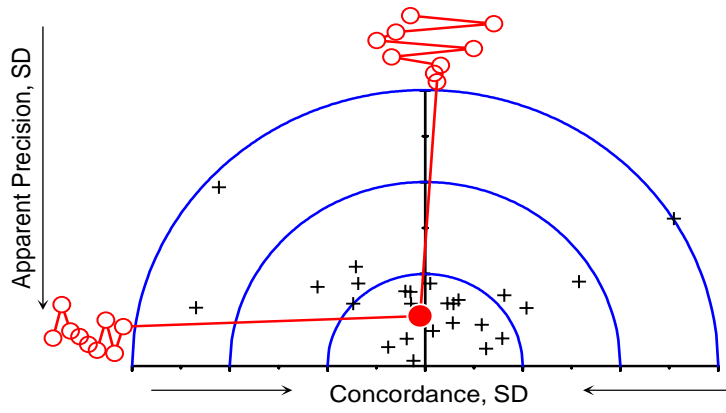
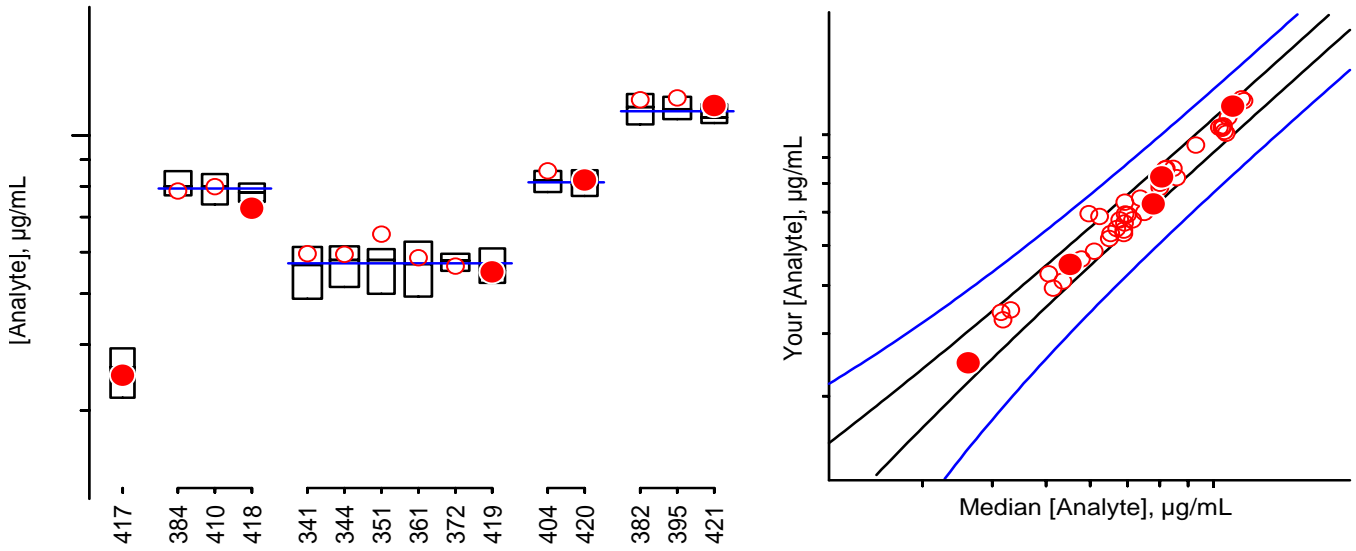
For details of the construction and interpretation of these plots, see:
 Duewer, Kline, Sharpless, Brown Thomas, Gary, Sowell. Anal Chem 1999;71(9):1870-8.

Serum	Comments	History
#417	Fresh-frozen, native, multi-donor	First (and last) distribution
#418	Fresh-frozen, native, multi-donor	RR71#384, RR76#410
#419	Fresh-frozen, native, multi-donor: SRM968d	RR63#341 & #344, RR64#351, RR66#361, RR69#372
#420	Fresh-frozen, augmented {tR,RP,βT}, single-donor	RR75#404
#421	Fresh-frozen, native, multi-donor	RR71#382, RR73#395

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Individualized RR LXXVIII Report: FSV-BG

α-Tocopherol, µg/mL



- 3rd Quartile (75%)
 ● You, this RR
▲ You, ≥x, this RR
- Median (50%)
 ○ You, past RRs
△ You, ≥x, past RRs
+ Others, this RR
- 1st Quartile (25%)
 — Expectation

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

Serum

Comments

History

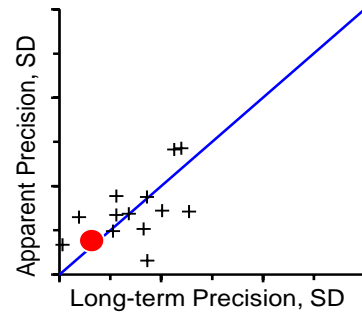
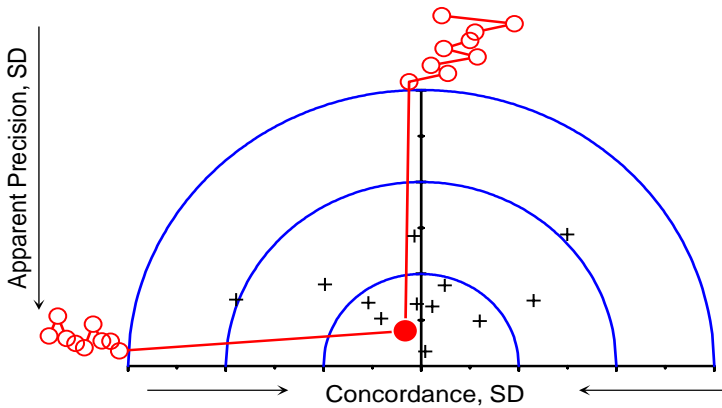
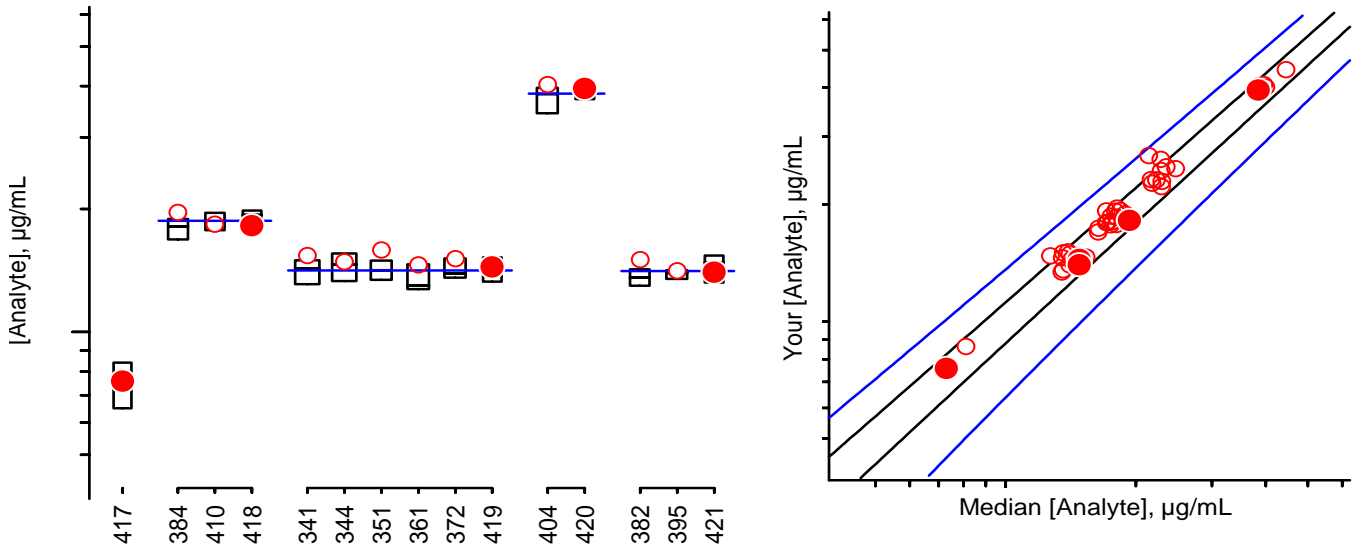
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- #418 Fresh-frozen, native, multi-donor
- #419 Fresh-frozen, native, multi-donor: SRM968d
- #420 Fresh-frozen, augmented {tR,RP,βT}, single-donor
- #421 Fresh-frozen, native, multi-donor

- First (and last) distribution
- RR71#384, RR76#410
- RR63#341 & #344, RR64#351, RR66#361, RR69#372
- RR75#404
- RR71#382, RR73#395

This publication is available free of charge from: <http://dx.doi.org/10.6028/NIST.JR.7880-46>

Individualized RR LXXVIII Report: FSV-BG

γ/β -Tocopherol, $\mu\text{g/mL}$



- 3rd Quartile (75%)
- Median (50%)
- 1st Quartile (25%)
- You, this RR
- You, past RRs
- Expectation
- You, $\geq x$, this RR
- You, $\geq x$, past RRs
- Others, this RR

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

Serum

Comments

History

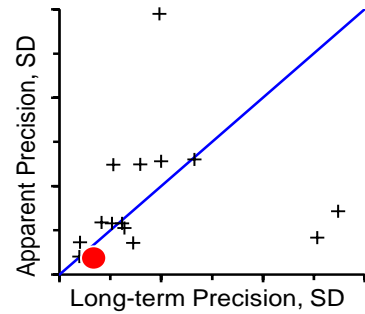
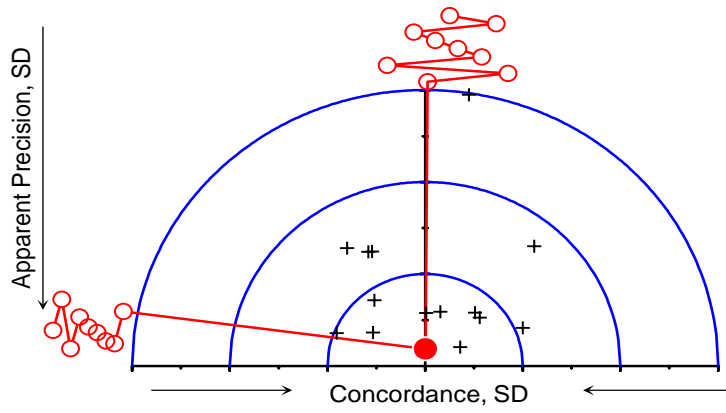
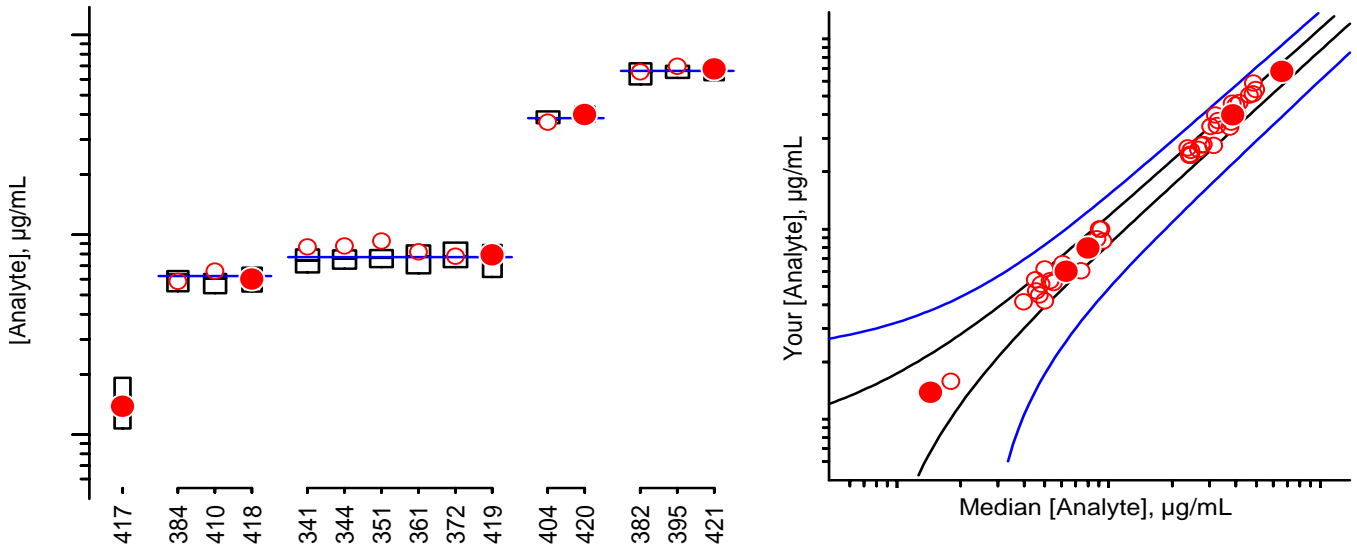
- #417 Fresh-frozen, native, multi-donor
- #418 Fresh-frozen, native, multi-donor
- #419 Fresh-frozen, native, multi-donor: SRM968d
- #420 Fresh-frozen, augmented {tR,RP, β T}, single-donor
- #421 Fresh-frozen, native, multi-donor

- First (and last) distribution
- RR71#384, RR76#410
- RR63#341 & #344, RR64#351, RR66#361, RR69#372
- RR75#404
- RR71#382, RR73#395

This publication is available free of charge from: <http://dx.doi.org/10.6028/NIST.JR.7880-46>

Individualized RR LXXVIII Report: FSV-BG

Total β -Carotene, $\mu\text{g/mL}$



- 3rd Quartile (75%)
 ● You, this RR
▲ You, $\geq x$, this RR
- Median (50%)
 ○ You, past RRs
○ You, $\geq x$, past RRs
+ Others, this RR
- 1st Quartile (25%)
 — Expectation

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

Serum

Comments

History

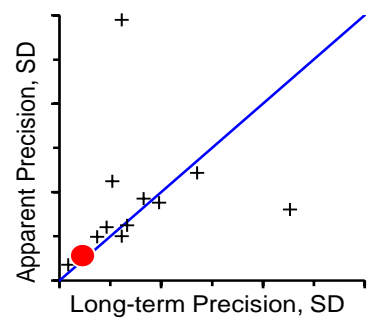
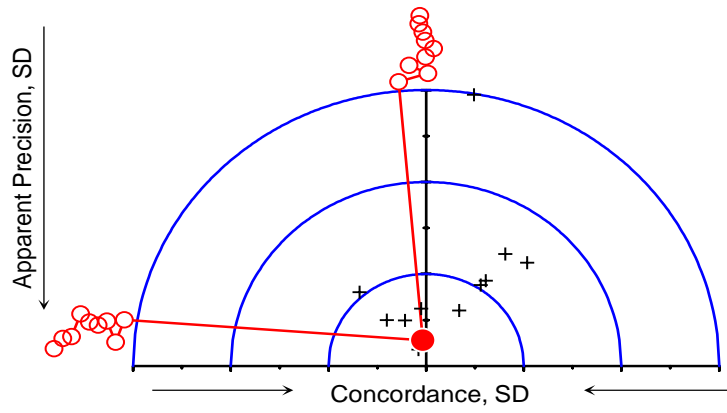
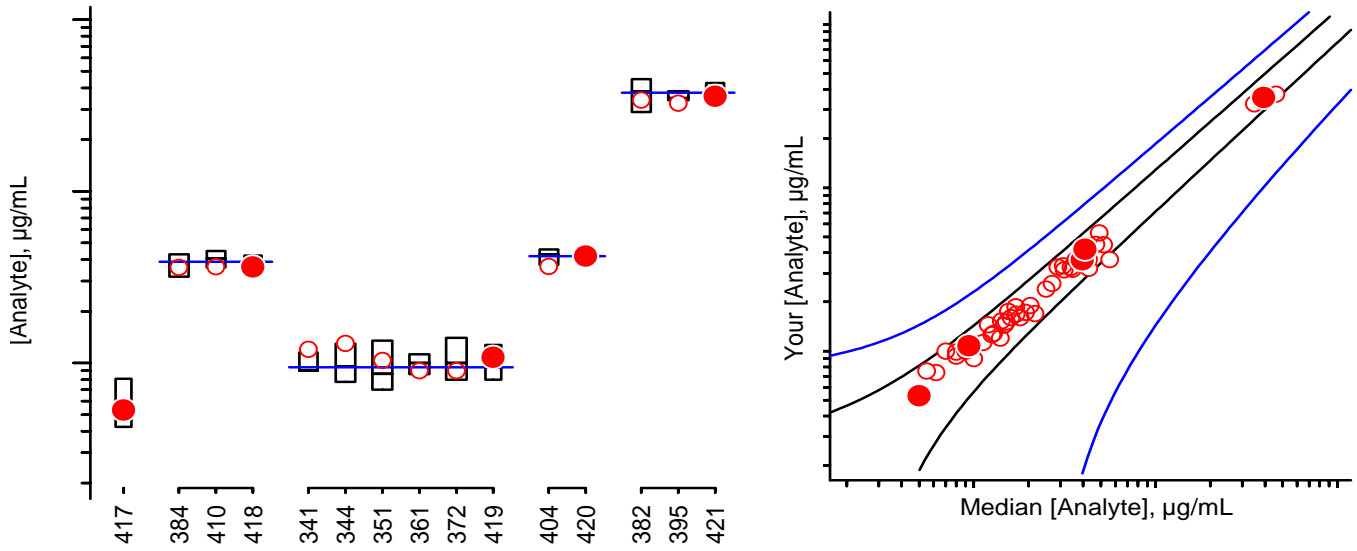
- #417 Fresh-frozen, native, multi-donor
- #418 Fresh-frozen, native, multi-donor
- #419 Fresh-frozen, native, multi-donor: SRM968d
- #420 Fresh-frozen, augmented {tR,RP, β T}, single-donor
- #421 Fresh-frozen, native, multi-donor

- First (and last) distribution
- RR71#384, RR76#410
- RR63#341 & #344, RR64#351, RR66#361, RR69#372
- RR75#404
- RR71#382, RR73#395

This publication is available free of charge from: <http://dx.doi.org/10.6028/NIST.IR.7880-46>

Individualized RR LXXVIII Report: FSV-BG

Total α -Carotene, $\mu\text{g/mL}$



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

● You, this RR
○ You, past RRs
— Expectation

▲ You, $\geq x$, this RR
△ You, $\geq x$, past RRs
+ Others, this RR

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

Serum

Comments

History

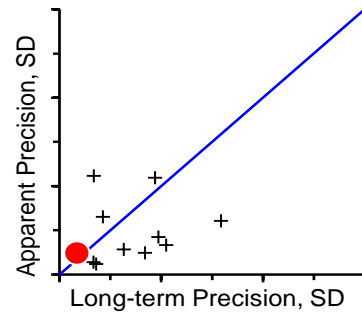
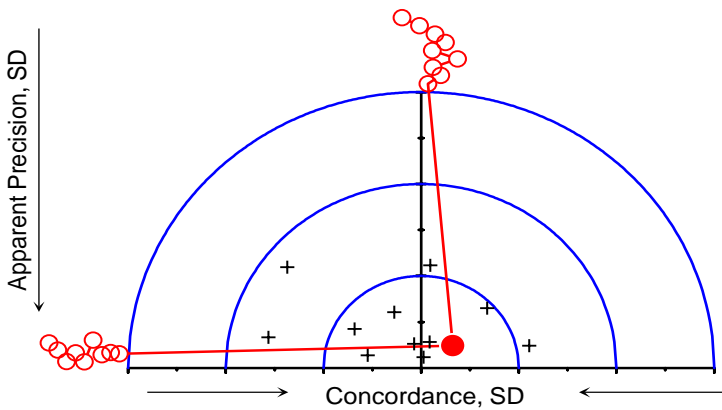
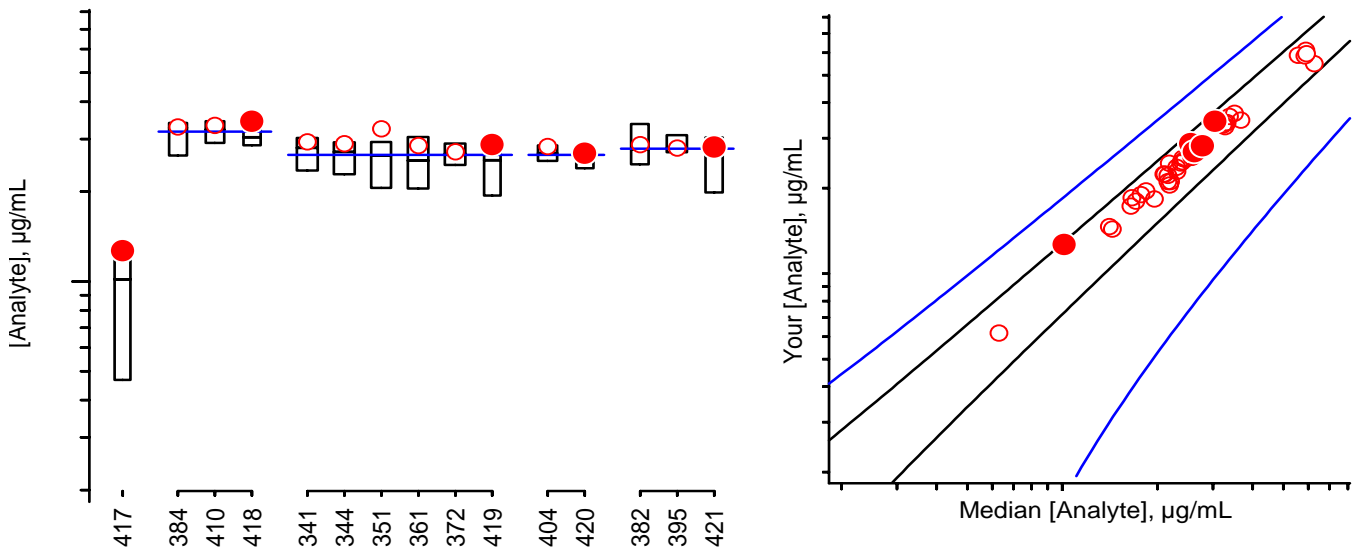
#417 Fresh-frozen, native, multi-donor
 #418 Fresh-frozen, native, multi-donor
 #419 Fresh-frozen, native, multi-donor: SRM968d
 #420 Fresh-frozen, augmented {tR,RP, β T}, single-donor
 #421 Fresh-frozen, native, multi-donor

First (and last) distribution
 RR71#384, RR76#410
 RR63#341 & #344, RR64#351, RR66#361, RR69#372
 RR75#404
 RR71#382, RR73#395

This publication is available free of charge from: <http://dx.doi.org/10.6028/NIST.IR.7880-46>

Individualized RR LXXVIII Report: FSV-BG

Total Lycopene, µg/mL



- 3rd Quartile (75%)
 ● You, this RR
▲ You, ≥x, this RR
- Median (50%)
 You, past RRs
 You, ≥x, past RRs
+ Others, this RR
- 1st Quartile (25%)
 — Expectation

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

Serum

Comments

History

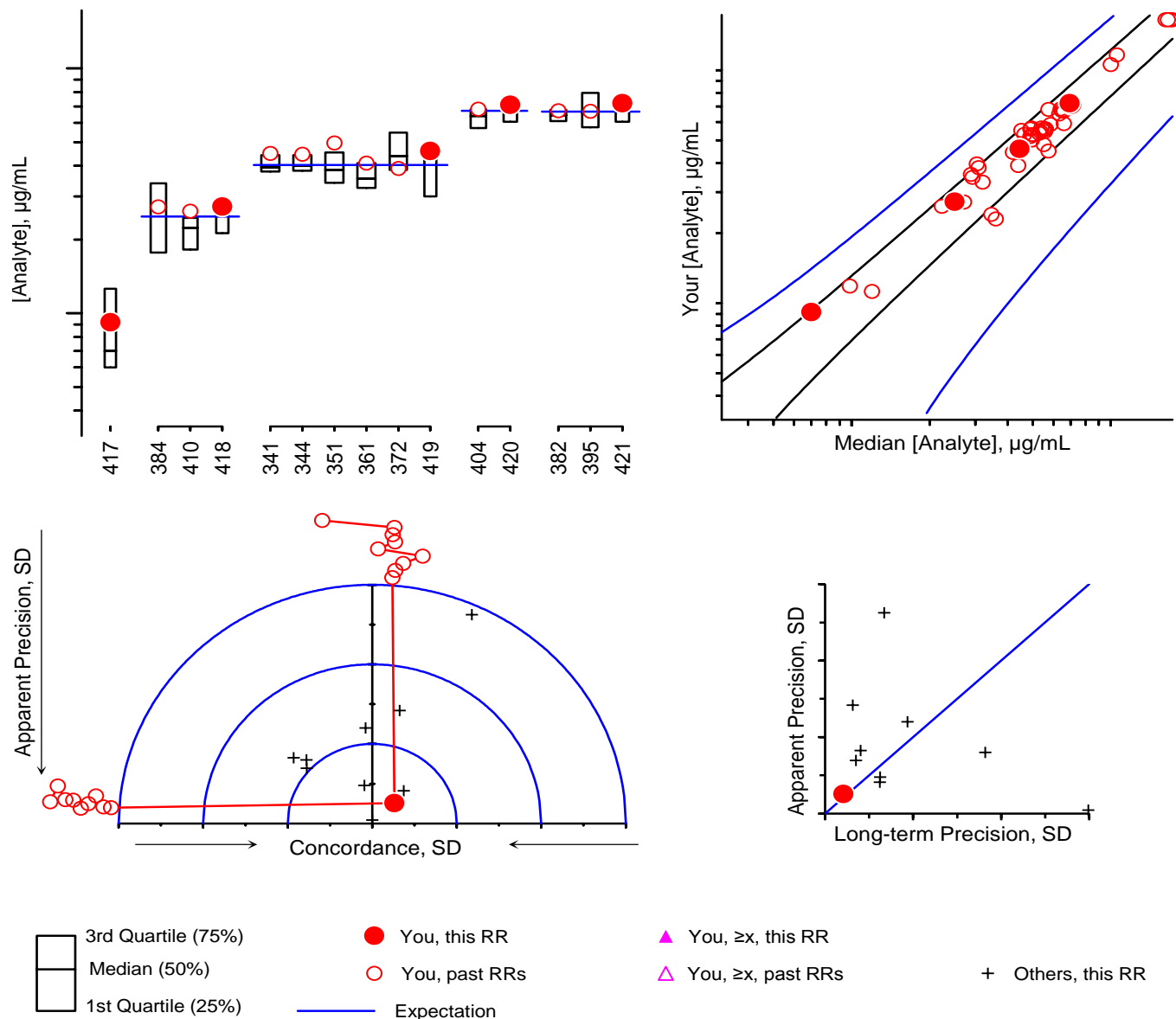
- #417 Fresh-frozen, native, multi-donor
- #418 Fresh-frozen, native, multi-donor
- #419 Fresh-frozen, native, multi-donor: SRM968d
- #420 Fresh-frozen, augmented {tR,RP,βT}, single-donor
- #421 Fresh-frozen, native, multi-donor

- First (and last) distribution
- RR71#384, RR76#410
- RR63#341 & #344, RR64#351, RR66#361, RR69#372
- RR75#404
- RR71#382, RR73#395

This publication is available free of charge from: <http://dx.doi.org/10.6028/NIST.IR.7880-46>

Individualized RR LXXVIII Report: FSV-BG

Total β -Cryptoxanthin, $\mu\text{g/mL}$



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.

Serum

Comments

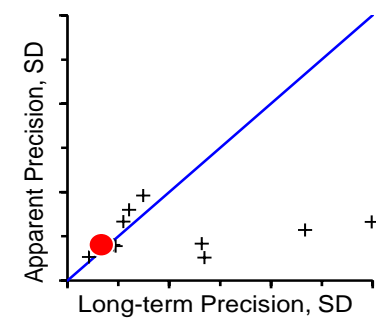
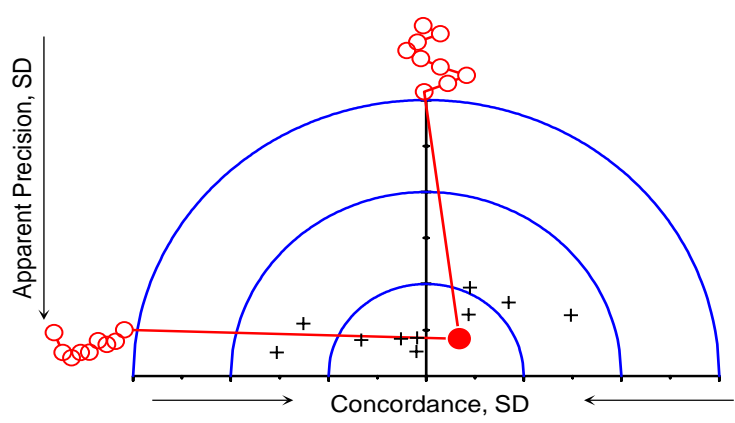
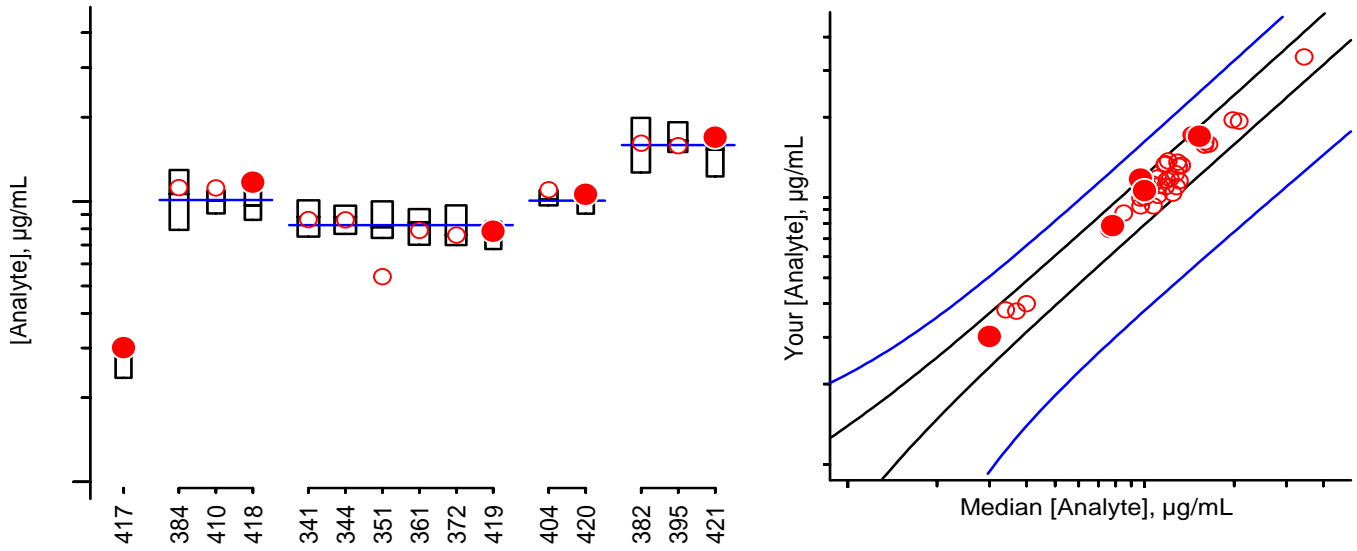
History

- #417 Fresh-frozen, native, multi-donor
- #418 Fresh-frozen, native, multi-donor
- #419 Fresh-frozen, native, multi-donor: SRM968d
- #420 Fresh-frozen, augmented {tR,RP, β T}, single-donor
- #421 Fresh-frozen, native, multi-donor

- First (and last) distribution
- RR71#384, RR76#410
- RR63#341 & #344, RR64#351, RR66#361, RR69#372
- RR75#404
- RR71#382, RR73#395

Individualized RR LXXVIII Report: FSV-BG

Total Lutein&Zeaxanthin, µg/mL



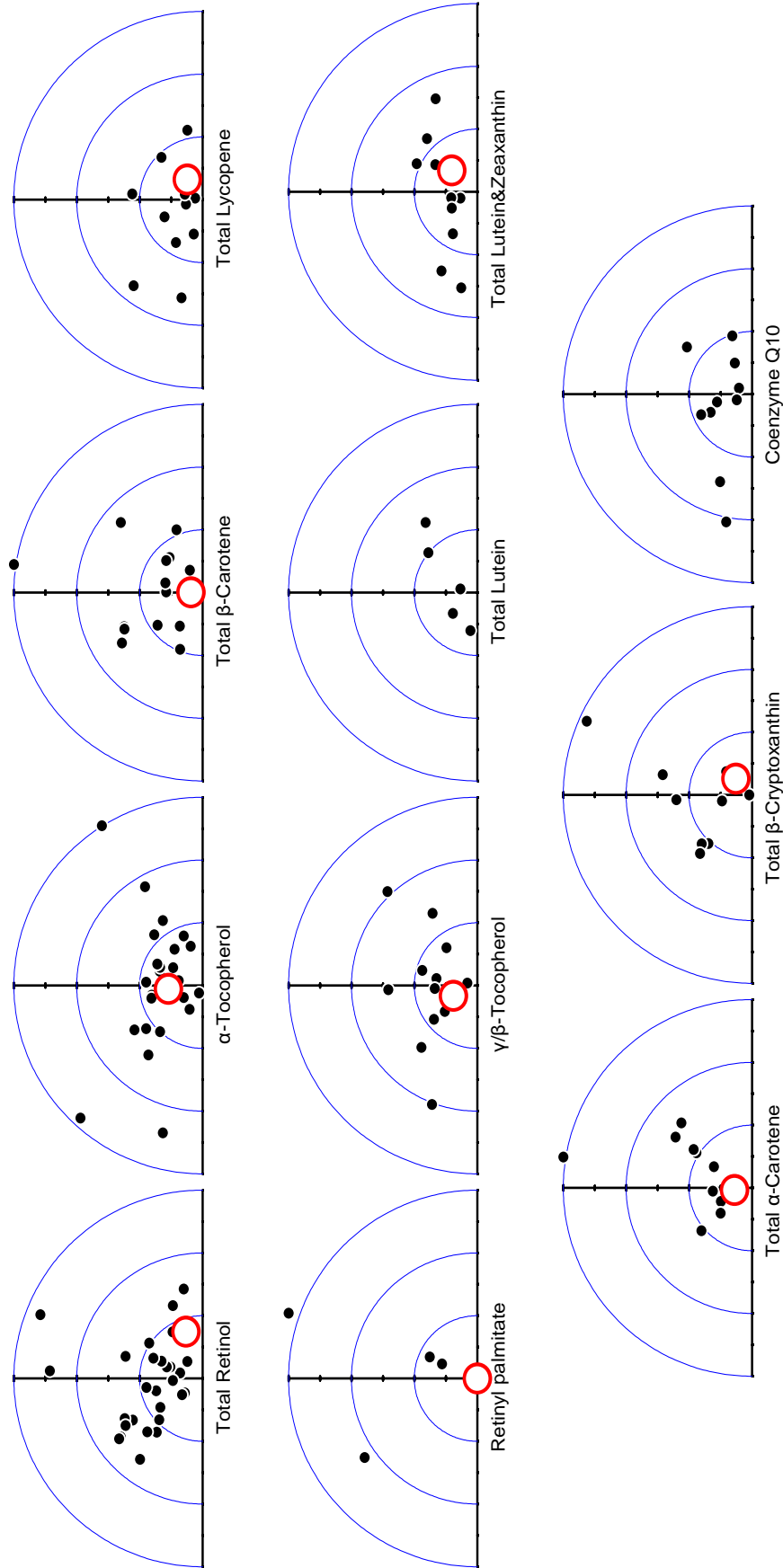
3rd Quartile (75%)
 Median (50%)
 1st Quartile (25%)
 ● You, this RR
 ○ You, past RRs
 — Expectation
 ▲ You, ≥x, this RR
 △ You, ≥x, past RRs
 + Others, this RR

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

Serum	Comments	History
#417	Fresh-frozen, native, multi-donor	First (and last) distribution
#418	Fresh-frozen, native, multi-donor	RR71#384, RR76#410
#419	Fresh-frozen, native, multi-donor: SRM968d	RR63#341 & #344, RR64#351, RR66#361, RR69#372
#420	Fresh-frozen, augmented {tR,RP,βT}, single-donor	RR75#404
#421	Fresh-frozen, native, multi-donor	RR71#382, RR73#395

This publication is available free of charge from: <http://dx.doi.org/10.6028/NIST.JR.7880-46>

Individualized Round Robin LXXVIII Report: FSV-BG Graphical Comparability Summary



Appendix E. Shipping Package Inserts for RR43

The following three items were included in each package shipped to an RR43 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 43
Micronutrients Measurement Vitamin C
Quality Assurance Program
Summer 2015 Controls & Samples
Analyze after: **June 22, 2015**
Results due on or before:
September 1, 2015

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



February 10, 2015

Dear Colleague:

The samples within this package constitute Vitamin C Round Robin 43 (RR43) of the 2015 Micronutrients Measurement Quality Assurance Program. RR43 consists of one vial each of four frozen serum *test samples* (#431, #432 #433, and #434) 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.

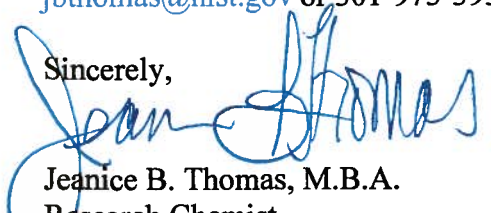
Please use the *control sera* to validate the performance of your measurement system before you analyze the *test samples*. The target value for CS#3 is $(15.5 \pm 1.6; 13.9 \text{ to } 17.1) \mu\text{mol/L}$ and the target for CS#4 is $(46.1 \pm 4.6; 41.5 \text{ to } 50.7) \mu\text{mol/L}$. We expect 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 jbthomas@nist.gov 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 $\mu\text{mol}/(\text{L sample solution})$ rather than $\mu\text{mol}/(\text{L serum used to prepare the sample})$. Please email (david.duewer@nist.gov) or fax (301-977-0685) your results to us as soon as possible but no later than **September 1, 2015**.

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: RR43 Report Form for Control and Test Sample Analyses

Participant #: _____

Date: _____

Vitamin C Round Robin 43
NIST Micronutrient Measurement Quality Assurance Program

Analysis of Control Materials and Test Samples

Sample	Replicate 1	Replicate 2	Units
Control serum CS#3	$\mu\text{mol/L}$ of Sample <i>Target: (15.5 \pm 1.6) $\mu\text{mol/L}$</i>
Control serum CS#4	$\mu\text{mol/L}$ of Sample <i>Target: (46.1 \pm 4.6) $\mu\text{mol/L}$</i>
Test sample #431	$\mu\text{mol/L}$ of Sample
Test sample #432	$\mu\text{mol/L}$ of Sample
Test sample #433	$\mu\text{mol/L}$ of Sample
Test sample #434	$\mu\text{mol/L}$ 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 1, 2015**

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

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

Participant #: _____

Date: _____

Vitamin C Round Robin 43
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 #431	Liquid frozen (1:1 serum:10% MPA)
VitC #432	Liquid frozen (1:1 serum:10% MPA)
VitC #433	Liquid frozen (1:1 serum:10% MPA)
VitC #434	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 RR43

The following three pages are the final report for RR43 as provided to all participants:

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



UNITED STATES DEPARTMENT OF COMMERCE
National Institute of Standards and Technology
Gaithersburg, Maryland 20899-8390

September 24, 2015

Dear Colleague:

Enclosed is the summary report of the results for Round Robin 43 (RR43) 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 $100 \times \text{MADe} / \text{median}$.

RR43 consisted of four test samples (#431, #432, #433, and #434) and one vial each of two frozen control serum control samples (CS #3 and CS #4). Details regarding the samples can be found in the enclosed report.

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.

Due to steadily declining enrollment, RR43 was the Vitamin C Quality Assurance Program's last study. One of our vitamin C participants has identified a similar program (INSTAND e. V.) that addresses ascorbic acid in serum. You can obtain more information about this program at: <http://www.instandev.de/en/eqas/program.html>.

If you should know of any other QA or proficiency testing programs for vitamin C in serum or plasma, please let us know and we will share that information with all recent participants.

We sincerely thank you for your participation over these past 25 years.

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 Vitamin C Round Robin 43 (RR43) 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 measurements made on the RR43 test samples.
3	Your RR43 measurements as a function of their expected values.
Page	“All-Lab” Report
1	A tabulation of results and summary statistics for total ascorbic acid [TAA] in the RR43 control and test samples. Results and summary statistics are also presented for the test samples calibrated to the results for the control samples. The consensus [TAA] content and inter-participant standard deviations are estimated using robust estimators.

Serum-Based Samples. Two serum controls and four test samples were distributed in RR43.

- CS#3 Ampouled in late 2009, a (15.4 ±0.4) µmol/L control material
- CS#4 Ampouled in late 2009, a (46.2 ±1.2) µmol/L control material
- S43:1 SRM 970 level II, ampouled in mid-1998, previously distributed as a test sample in RRs 11 to 15, 18, 20, 22, 25, 29, 36, 37, 39, 40
- S43:2 Ampouled in late 2009, previously distributed in RRs 34, 36 (two samples), 38, 40, 41, 42
- S43:3 Ampouled in late 2009, previously distributed in RRs 32, 33, 35, 40, 41, 42
- S43:4 Ampouled in late 2009, previously distributed in RRs 32, 33, 35, 38, 40, 41, 42

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 test samples.
- 2) There is no evidence for any significant change in the [TAA] level or interlaboratory variability for any of the samples.
- 3) The primary focus of these VitC QAP studies has been improving the interlaboratory comparability of [TAA] measurements. In addition to providing you with objective assessment of your results against the community’s consensus, we used these studies to explore other ways to further improve comparability. Chief among these was the use of various control materials to linearly recalibrate your measurement systems. We explored three different systems: ascorbic acid in 5 % MPA solutions that you prepared, SRM 970 Level I (8.5 µmol/L) and II (27 µmol/L), and CS#3 (15 µmol/L) and CS#3 (46 µmol/L). Comparability was significantly improved only with this final system, using the transform:

$$[\text{TAA}]_{\text{TestCalibrated}} = [\text{TAA}]_{\text{TestReported}} / b$$

where $b = \text{Slope of the regression } \{ \text{CS\#3, CS\#4} \}_{\text{Reported}} = b \times \{ \text{CS\#3, CS\#4} \}_{\text{Reference}}$.

Figure 1 displays the summary statistics for the 32 test samples that can be recalibrated with these materials.

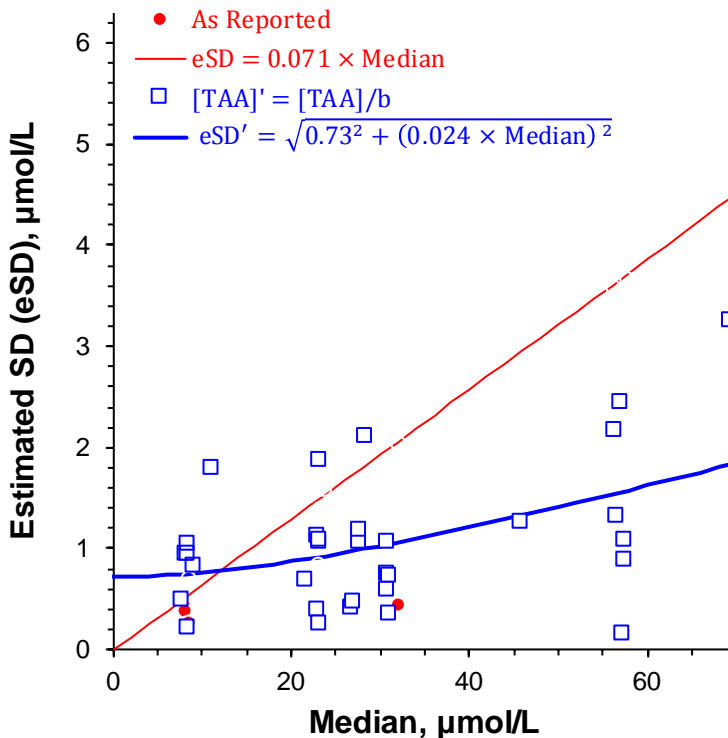


Figure 1: Estimated Standard Deviation as a Function of Median Value before and after Recalibration with CS#3 and CS#4

The interlaboratory variability of the “As Reported” results are well described as a constant percent relative standard deviation (coefficient of variation or CV) of 7.1 %. The variability of the recalibrated results are well described by the non-linear function:

$$eSD = \sqrt{\alpha^2 + (\beta \times \text{Median})^2}$$

where $\alpha = 0.73 \mu\text{mol/L}$ is the expected constant variability and $\beta = 2.4 \%$ is the expected proportional variability. This or similar functional relationships are routinely observed in interlaboratory studies whenever the analyte content in some samples approaches the quantification limits of a sizable minority of the measurement systems.

We hypothesize that interlaboratory comparability is limited by small differences in how your measurement processes respond to components of the sample matrix other than TAA itself. At least for the samples distributed in the VitC QAP, these biases were significantly reduced using matrix-matched calibrants having appropriately designed [TAA] content.

Appendix G. “All-Lab Report” for RR43

The following two pages are the “All-Lab Report” for RR43 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" 43 - Summer 2015

Lab	Date	Samples						Calibrated Results: [TAA] = TAA/b						Calibrated Results: [TAA]' = (TAA-a)/b					
		Total Ascorbic Acid ([TAA]), μmol/L						[TAA]', μmol/L						Parameters					
		CS#3	CS#4	S43:1	S43:2	S43:3	S43:4	S43:1'	S43:2'	S43:3'	S43:4'	b	a	S43:1'	S43:2'	S43:3'	S43:4'		
VC-MB	26/08/15	15.0	48.3	27.9	59.9	32.0	23.7	1.03	26.9	57.9	30.9	22.9	1.07	-1.4	27.3	57.2	31.2	23.5	
VC-MD	12/05/15	15.9	48.0	28.2	59.3	32.4	24.3	1.03	27.3	57.3	31.3	23.4	1.03	0.0	27.3	57.3	31.3	23.4	
VC-MG	13/08/15	14.5	47.5	28.2	59.4	30.6	23.6	1.02	27.7	58.4	30.1	23.2	1.06	-1.8	28.2	57.5	30.5	23.9	
VC-MI	25/08/15	16.2	49.5	28.7	62.1	32.2	24.8	1.07	26.9	58.3	30.2	23.3	1.07	-0.2	26.9	58.2	30.2	23.3	
VC-MJ	09/07/15	21.9	53.9	31.2	64.5	39.0	30.7	1.19	26.3	54.3	32.8	25.8	1.03	6.1	24.4	56.7	31.9	23.9	
VC-MN	21/08/15	15.3	43.3	25.4	50.7	27.5	20.2	0.94	27.0	53.9	29.2	21.5	0.90	1.6	26.5	54.6	28.8	20.7	
VC-NM	01/09/15	12.7	42.0	24.9	52.8	28.0	21.0	0.90	27.7	58.8	31.2	23.4	0.94	-1.8	28.2	57.9	31.6	24.1	

N	7	7	7	7	7	7
Average	15.9	47.5	27.8	58.4	31.7	24.0
SD	2.9	3.9	2.1	4.9	3.8	3.4

Min	12.7	42.0	24.9	50.7	27.5	20.2
%25	14.7	45.4	26.6	56.1	29.3	22.3
Median	15.3	48.0	28.2	59.4	32.0	23.7
%75	16.0	48.9	28.5	61.0	32.3	24.5
Max	21.9	53.9	31.2	64.5	39.0	30.7
eSD	1.3	2.3	0.7	4.1	2.0	1.6
eCV	8	5	3	7	6	7

7	7	7	7	7
27.1	57.0	30.8	23.4	
0.5	2.0	1.1	1.3	

26.3	53.9	29.2	21.5
26.9	55.8	30.2	23.1
27.0	57.9	30.9	23.3
27.5	58.3	31.2	23.4
27.7	58.8	32.8	25.8
0.4	0.9	1.1	0.3
2	2	3	1

7	7	7	7
27.0	57.0	30.8	23.3
1.3	1.2	1.1	1.1

24.4	54.6	28.8	20.7
26.7	56.9	30.4	23.4
27.3	57.3	31.2	23.5
27.8	57.7	31.4	23.9
28.2	58.2	31.9	24.1
1.2	0.9	1.1	0.5
4	1	3	2

Appendix H. Representative “Individualized Report” for RR43

Each participant in RR43 received an “Individualized Report” reflecting their reported results. The following three pages are the “Individualized Report” for participant “VC-MB”.

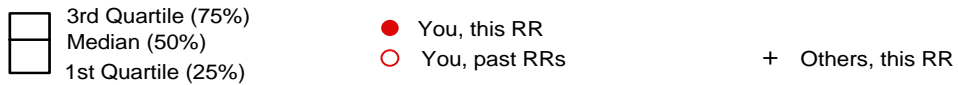
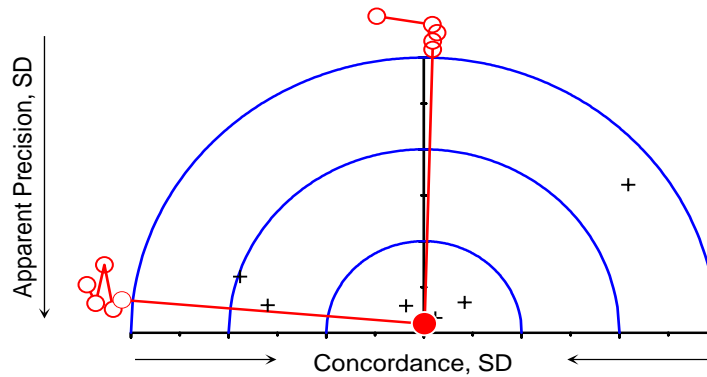
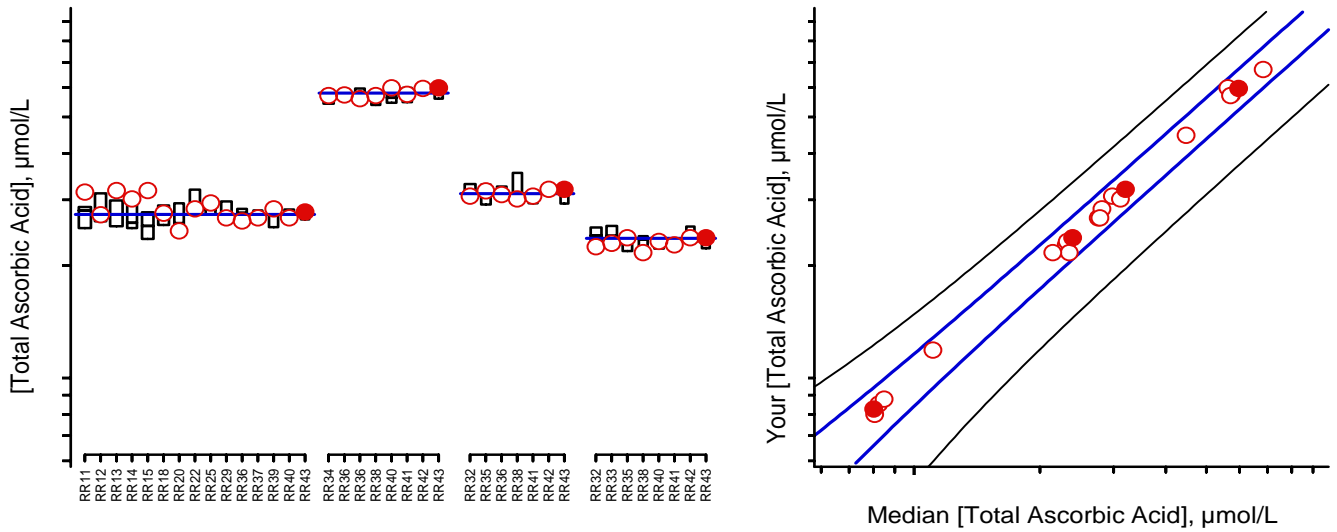
Vitamin C "Round Robin" 43 Report: Participant VC-MB

Date	RR	Sample	[TAA] mmol/Lsample					N	Mean	SD _{repeat}	SD _{reprod}				
			Rep ₁	Rep ₂	F _{adj}	Mean	SD _{dup}								
07/23/10	33	S33:3	13.4	13.4	1.0	13.4	0.0	10	14.3	1.1	1.0				
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								
08/01/13	39	CS#3	15.5	15.5	1.0	15.5	0.0								
02/24/14	40	CS#3	13.9	13.9	1.0	13.9	0.0								
08/14/14	41	CS#3	13.9	14.5	1.0	14.2	0.4								
03/11/15	42	CS#3	15.5	15.5	1.0	15.5	0.0								
08/26/15	43	CS#3	15.0	15.0	1.0	15.0	0.0								
01/13/10	32	S32:4	45.4	45.9	1.0	45.7	0.4	10	46.8	0.8	1.1				
01/14/11	34	S34:3	45.4	45.9	1.0	45.7	0.4								
06/13/11	35	S35:4	47.5	47.5	1.0	47.5	0.0								
08/06/12	37	CS#4	44.9	48.0	1.0	46.5	2.2								
02/27/13	38	CS#4	45.9	44.9	1.0	45.4	0.7								
08/01/13	39	CS#4	48.0	47.5	1.0	47.7	0.4								
02/24/14	40	CS#4	47.5	48.0	1.0	47.7	0.4								
08/14/14	41	CS#4	45.9	45.9	1.0	45.9	0.0								
03/11/15	42	CS#4	48.0	48.0	1.0	48.0	0.0								
08/26/15	43	CS#4	48.0	48.5	1.0	48.3	0.4								
09/25/98	11	S11:2	63.0	62.0	0.5	31.3	0.4	15	28.4	1.2	2.1				
02/26/99	12	S12:2	55.0	55.0	0.5	27.5	0.0								
03/03/00	13	S13:2	63.0	64.0	0.5	31.8	0.4								
03/26/01	14	S14:4	60.9	59.9	0.5	30.2	0.4								
09/05/01	15	S15:2	63.0	64.0	0.5	31.8	0.4								
12/19/02	18	S18:3	55.8	54.7	0.5	27.6	0.4								
03/01/04	20	S20:4	21.7	27.9	1.0	24.8	4.4								
03/02/05	22	S22:4	28.4	28.4	1.0	28.4	0.0								
05/24/06	25	S25:2	29.4	29.4	1.0	29.4	0.0								
06/20/08	29	S29:3	26.8	26.8	1.0	26.8	0.0								
03/07/12	36	S36:3	25.8	26.8	1.0	26.3	0.7								
08/06/12	37	S37:3	25.8	27.9	1.0	26.8	1.5								
08/01/13	39	S39:2	28.4	28.4	1.0	28.4	0.0								
02/24/14	40	S40:1	26.8	26.8	1.0	26.8	0.0								
08/26/15	43	S43:1	27.9	27.9	1.0	27.9	0.0								
01/14/11	34	S34:4	56.8	57.3	1.0	57.0	0.4					8	58.0	0.5	1.5
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								
03/11/15	42	S42:3	59.4	59.9	1.0	59.6	0.4								
08/26/15	43	S43:2	59.9	59.9	1.0	59.9	0.0								
01/13/10	32	S32:3	30.5	31.0	1.0	30.7	0.4	7	31.2	0.4	0.7				
06/13/11	35	S35:3	32.0	31.5	1.0	31.7	0.4								
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								
08/14/14	41	S41:2	30.5	31.0	1.0	30.7	0.4								
03/11/15	42	S42:2	32.0	32.0	1.0	32.0	0.0								
08/26/15	43	S43:3	32.0	32.0	1.0	32.0	0.0								
01/13/10	32	S32:2	22.2	22.7	1.0	22.5	0.4					8	23.0	0.5	0.8
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								
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								
03/11/15	42	S42:1	23.7	23.7	1.0	23.7	0.0								
08/26/15	43	S43:4	23.2	24.3	1.0	23.7	0.7								

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

Vitamin C "Round Robin" 43 Report: Participant VC-MB

Total Ascorbic Acid, $\mu\text{mol/mL}$



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.

Sample

Comments

- S43:1 SRM970 Lv II; prepared 1998; distributed as unknowns RRs 11 - 15,18,20,22,25,29,36,37,39,40
- S43:2 Prepared 2009; distributed RRs 34,36(dups),38,40,41,42
- S43:3 Prepared 2009; distributed RRs 32,33,35,38,41,42
- S43:4 Prepared 2009; distributed RRs 32,33,35,38,40,41,42

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