NISTIR 7880-29

NIST Micronutrients Measurement Quality Assurance Program Winter, Spring, and Fall 1995 Comparability Studies

Results for Round Robins XXXIII, XXXIV, and XXXV Fat-Soluble Vitamins and Carotenoids in Human Serum and Round Robins 7 and 8 Ascorbic Acid in Human Serum

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April 2014

U.S. Department of Commerce
Penny Pritzker, Secretary

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

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Abstract

The National Institute of Standards and Technology coordinates the Micronutrients Measurement Quality Assurance Program (MMQAP) for laboratories that measure fat- and water-soluble vitamins and carotenoids in human serum and plasma. This report describes the design of and results for the Winter, Spring and Fall 1995 MMQAP measurement comparability improvement studies: 1) Round Robin XXXIII Fat-Soluble Vitamins and Carotenoids in Human Serum, 2) Round Robin XXXIV Fat-Soluble Vitamins and Carotenoids in Human Serum, 3) Round Robin XXXV Fat-Soluble Vitamins and Carotenoids in Human Serum, 4) Round Robin 7 Ascorbic Acid in Human Serum, and 5) Round Robin 8 Ascorbic Acid in Human Serum. The materials for Round Robin XXXIII were shipped to participants in January 1995; participants were requested to provide their measurement results by March 20, 1995. The materials for Round Robin XXXIV were shipped to participants in April 1995; participants were requested to provide their measurement results by July 24, 1995. The materials for Round Robin XXXV were shipped to participants in July 1995; participants were requested to provide their measurement results by September 15, 1995. The sample materials for Round Robin 7 were distributed in January 1995 with results due by late May 1995. The sample materials for Round Robin 8 were distributed in October 1995 with results due by December 18, 1995.

Keywords

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

Table of Contents

Abstract iii
Keywords iii
Table of Contents iv
Introduction 1
Round Robin XXXIII: Fat-Soluble Vitamins and Carotenoids in Human Serum 1
Round Robin XXXIV: Fat-Soluble Vitamins and Carotenoids in Human Serum 2
Round Robin XXXV: Fat-Soluble Vitamins and Carotenoids in Human Serum 2
Round Robin 7: Vitamin C in Human Serum 3
Round Robin 8: Vitamin C in Human Serum 3
References 4

Appendix A. Shipping Package Inserts for RR33 ..... A1
Appendix B. Final Report for RR33 ..... B1
Appendix C. "All-Lab Report" for RR33 ..... C1
Appendix D. "Individualized Report" for RR33 ..... D1
Appendix E. Shipping Package Inserts for RR34 ..... E1
Appendix F. Final Report for RR34 ..... F1
Appendix G. "All-Lab Report" for RR34 ..... G1
Appendix H. "Individualized Report" for RR34 ..... H1
Appendix I. Shipping Package Inserts for RR35 ..... I1
Appendix J. Final Report for RR35 ..... J1
Appendix K. "All-Lab Report" for RR35 ..... K1
Appendix L. "Individualized Report" for RR35 ..... L1
Appendix M. Shipping Package Inserts for RR07 ..... M1
Appendix N. Final Report for RR06 and RR07 ..... N1
Appendix O. "All Lab Report" for RR07 ..... O1
Appendix P. Shipping Package Inserts for RR08 ..... P1
Appendix Q. "All Lab Report" for RR08 ..... Q1

Introduction

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

Participants in the MMQAP Fat-Soluble Vitamins and Carotenoids in Human Serum Round Robin XXXIII comparability study (hereafter referred to as RR33) received four lyophilized 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 1995. 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 RR33 has three sections:

- A cover letter, a "Lies, Damned Lies, and Statistics" summary report that describes the samples and our analysis of the participants' results, and a "Report of (Meta)Analysis" that presents a detailed analysis of measurements made by NIST analysts. This cover letter and associated reports are reproduced as Appendix B.

- The "All-Lab Report" that lists all of the reported measurement results and a number of consensus statistics for analytes reported by more than one participant. This report also provides a numerical "score card" for each participant's measurement comparability for the more commonly reported analytes. This report is reproduced as Appendix C.

- An "Individualized Report" that graphically analyzes each participant's results for selected analytes. This report also provides a graphical summary of their measurement comparability. The graphical tools used in this report are described in detail elsewhere [3]. An example "Individualized Report" is reproduced as Appendix D.

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

Participants in the MMQAP Fat-Soluble Vitamins and Carotenoids in Human Serum Round Robin XXXIV comparability study (hereafter referred to as RR34) received four lyophilized human serum test samples for analysis. Unless multiple vials were previously requested, participants received one vial of each material. These sample materials were shipped on dry ice to participants in April 1995. The communication materials included in the sample shipment are provided in Appendix E.

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 RR34 has three sections:

- A cover letter, a "Lies, Damned Lies, and Statistics" summary report that describes the samples and our analysis of the participants' results, and a "Report of (Meta)Analysis" that presents a detailed analysis of measurements made by NIST analysts. This cover letter and associated reports are reproduced as Appendix F.

- The "All-Lab Report" that lists all of the reported measurement results and a number of consensus statistics for analytes reported by more than one participant. This report also provides a numerical "score card" for each participant's measurement comparability for the more commonly reported analytes. This report is reproduced as Appendix G.

- An "Individualized Report" that graphically analyzes each participant's results for selected analytes. This report also provides a graphical summary of their measurement comparability. The graphical tools used in this report are described in detail elsewhere [3]. An example "Individualized Report" is reproduced as Appendix H.

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

Participants in the MMQAP Fat-Soluble Vitamins and Carotenoids in Human Serum Round Robin XXXV comparability study (hereafter referred to as RR35) received four lyophilized human serum test samples for analysis. Unless multiple vials were previously requested, participants received one vial of each material. These sample materials were shipped on dry ice to participants in July 1995. The communication materials included in the sample shipment are provided in Appendix I.

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 RR35 has three sections:

- A cover letter, a "Lies, Damned Lies, and Statistics" summary report that describes the samples and our analysis of the participants' results, and a "Report of (Meta)Analysis" that presents a detailed analysis of measurements made by NIST analysts. This cover letter and associated reports are reproduced as Appendix J.

- The "All-Lab Report" that lists all of the reported measurement results and a number of consensus statistics for analytes reported by more than one participant. This report also provides a numerical "score card" for each participant's measurement comparability for the more commonly reported analytes. This report is reproduced as Appendix K.

- An "Individualized Report" that graphically analyzes each participant's results for selected analytes. This report also provides a graphical summary of their measurement comparability. The graphical tools used in this report are described in detail elsewhere [3]. An example "Individualized Report" is reproduced as Appendix L.

Round Robin 7: Vitamin C in Human Serum

Participants in the MMQAP Vitamin C in Human Serum Round Robin 7 comparability study (hereafter referred to as RR07) received four frozen serum test samples and a frozen serum control material for analysis. These materials were shipped on dry ice to participants in January 1995. The available communication materials included in the sample shipment are provided in Appendix M.

The test and control materials were prepared by adding equal volumes of 10 % metaphosphoric acid (MPA) to human serum that had been spiked with ascorbic acid. Participants were asked to provide two results for each vial.

The final report delivered to all participants in RR06 and RR07 consists of a cover letter and a series of Tables and Figures that summarize the results of the study. This report is reproduced as Appendix N.

While not distributed to the participants in RR07, Appendix O is a modified "All Lab Report" that lists the results for the test materials transformed into units of µmol/mL sample.

No "Individualized Report" was provided to the participants in RR07.

Round Robin 8: Vitamin C in Human Serum

Participants in the MMQAP Vitamin C in Human Serum Round Robin 8 comparability study (hereafter referred to as RR08) received four frozen serum test samples and a frozen serum control material for analysis. These sample materials were shipped on dry ice to participants in October 1995. The communication materials included in the sample shipment are provided in Appendix P.

The test and control materials were prepared by adding equal volumes of 10 % metaphosphoric acid (MPA) to human serum that had been spiked with ascorbic acid. Participants were asked to provide two results for each vial.

As described in NISTIR 7880-28, the data and results for RR08 were reported November 1996 in combination with the data and results for RR09.

While not distributed to the participants in RR08, Appendix Q is a modified "All Lab Report" that lists the results for the test materials transformed into units of µmol/mL sample.

No "Individualized Report" was provided to the participants in RR08.

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 1995;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 1995;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 RR33

The following two items were included in each package shipped to RR33 participants:

- Cover letter
- Datasheet

The cover letter and datasheet were enclosed in a sealed waterproof bag along with the samples themselves.

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

January 17, 1995

Dear Colleague:

The 1995 Micronutrients Measurement Quality Assurance Program will include three studies of fat-soluble vitamins and carotenoids in serum. The core analytes in the program will be: retinol, retinyl palmitate, α-tocopherol, γ-tocopherol, and total β-carotene. We will continue to encourage you to provide results for other fat-soluble analytes and carotenoids that you routinely analyze.

Enclosed is the set of samples for the first 1995 Round Robin exercise (Round Robin XXXIII). You will find duplicate vials of three lyophilized serum samples and a single vial of one sample for analysis, along with a form for reporting your results. When reporting your results, please submit one value for each analyte for a given serum sample. If an obtained value is below your detection limit, please indicate this on the form by using NQ *(Not Quantified)*. Results are due to NIST by March 20, 1995. Results received after April 12 will not be included in the summary report for this round robin study nor in the permanent database. Written feedback concerning the study will be mailed to you by April 24.

Samples should be reconstituted with 1.0 mL of HPLC-grade water or equivalent. We recommend that dissolution be facilitated with 3 to 5 minutes agitation in an ultrasonic bath or at least 30 minutes at room temperature with intermittent swirling. (CAUTION: Vigorous shaking will cause foaming and possibly interfere with accurate measurement. The rubber stopper contains phthalate esters which will leach into the sample upon intermittent contact of the liquid sample with the stopper. These esters absorb strongly in the UV region and elute very near retinol in most HPLC systems, creating analytical problems.) Pipette a known volume of serum from the vial for analysis since the final volume of the reconstituted sample is greater than 1.0 mL. For consistency, we request that laboratories use the following absorptivities (E 1% cm) in ethanol: retinol, 1850 at 325 nm; retinyl palmitate, 97.5 at 325 nm; α-tocopherol, 75.8 at 292 nm; γ-tocopherol, 91.4 at 298 nm; α-carotene, 2800 at 444 nm; β-carotene, 2560 at 450 nm; lycopene, 3450 at 472 nm.

Please mail or FAX your results for Round Robin XXXIII to the address below or e-mail your results to DLDuewer@enh.nist.gov. A receipt of results will be mailed to you shortly thereafter.

Ms. Donna Sirk
NIST
Bldg. 222, Rm. B208
Gaithersburg, MD 20899
FAX: (301) 977-0685

If you have questions regarding this round robin exercise, please call me at (301) 975-3120 or mail/FAX queries to the above address.

Sincerely,

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

Attachment

cc: W. May
S. Wise

NIST/NCI

Micronutrients Measurement Quality Assurance Program

Round Robin **XXXIII** Results from Laboratory #_____

Analyte	Serum 203	Serum 204	Serum 205	Serum 206	Units*
retinol					
retinyl palmitate					
a-tocopherol					
g-tocopherol					
total b-carotene					
trans-b-carotene					
cis-b-carotene					
total a-carotene					
total lycopene					
trans-lycopene					
b-cryptoxanthin					
lutein					
zeaxanthin					
lutein&zeaxanthin					

* We prefer results as microgram/milliliter.

Comments?

Mail: Donna Sirk, 222/B208
NIST
Gaithersburg, MD 20899

Please return results on-or-before
March 20, 1995

Fax: 301-977-0685
Email: DLDuewer@enh.NIST.gov

Appendix B. Final Report for RR33

The following 11 pages are the final report for RR33 as provided to all participants:

- Cover letter
- A discussion entitled "Lies, Damn Lies, and Statistics" that:
  - describes the nature of the test samples and details any previous distributions
  - summarizes aspects of the study that we believe may be of interest to the participants
- A "Report of (Meta)Analysis" that details the analysis of NIST measurements

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

April 24, 1995

Dear Colleague:

Enclosed is the summary report of the results for Round Robin XXXIII (Sera 203-206). Included in this report are: a summary of data for all laboratories; the measurement comparability summary for evaluating your laboratory's performance relative to the other participants'; a summary of your individual laboratory performance for the past three years; a summary of the interlaboratory accuracy and precision over the same period of time for the measurement of retinol, α- and γ-tocopherol, and β-carotene; and a graphical summary of the NIST assigned value vs. your laboratory value for these analytes. The NIST assigned values are derived from the equally weighted values for the combined results from the analyses performed by NIST and the laboratories that participated in this round robin exercise (more details are provided in the attached Appendix).

In this round robin exercise, Serum 205 is a 1:1 volumetric mix of Serum 203 and stripped serum; Serum 204 is a 1:2 volumetric mix of Serum 203 and the stripped serum. Serum 206 was previously distributed in Round Robin XVIII as Serum 120 and in Round Robin XXI as Serum 147.

The overall laboratory performance for retinol, γ- and α-tocopherol, and β-carotene for this round robin exercise is good and is comparable to that of the last round robin exercise (Round Robin XXXII). The average estimated coefficient of variation (eCV) is about 9% for retinol, 8% for α-tocopherol, and 14% for γ-tocopherol. The levels of β-carotene in this round robin exercise were significantly lower (≤100 ng/mL in Sera 203-205) than those in the previous exercise. The interlaboratory variation for total β-carotene is about 32% for this round robin, as compared to 14% in Round Robin XXXII. The higher variation is due to the difficulty of making measurements at levels (≤100 ng/mL vs ≥300 ng/mL) which appear to be at or below the limit of quantification for most laboratories. Similar findings are reported for retinyl palmitate, which were also at low concentrations in this exercise.

The overall interlaboratory precision for retinol and γ- and α-tocopherol measurements has remained at an average eCV of approximately 10% over the past three years. The eCV for β-carotene has averaged around 20% during the same period provided that the levels are above the limit of quantification. Interlaboratory precision for β-cryptoxanthin, total lycopene, lutein, and zeaxanthin is continuing to improve. δ-Tocopherol was enriched at measurable levels in the Round Robin XXXIII samples. However, only one laboratory reported results for this analyte. We encourage you to report values for as many quantifiable analytes as possible.

Data for evaluating your laboratory's performance in Round Robin XXXIII are provided in the comparability summary on page 6 of the report. The criteria used to summarize laboratory performance is as follows: results rated **1** (within ± 1 SD of the assigned value) indicate **EXCEPTIONAL** performance, those rated **2** (within ± 2 SD) indicate **ACCEPTABLE** performance, a rating of **3** (within ± 3 SD of the assigned value) is **MARGINAL** performance, and **4** (>3 SD of the assigned value) indicates **POOR** performance relative to the current state-of-the-practice for these measurements.

NIST

As you are probably aware, Standard Reference Material (SRM) 968a (Fat-Soluble Vitamins in Human Serum), which we recommend that you use to validate your methods, is sold out. The renewal material, SRM 968b, is not yet available for purchase. If your laboratory is out of SRM 968a, we suggest that you use for method validation control materials that are available in your laboratory or previously distributed (≤1 year ago) round robin samples. If you do not have access to either of these options, please give me a call.

If you still have some SRM 968a and have concerns regarding your performance, or are a lab whose performance would be rated "**POOR**" based on the convention stated above, we suggest that you analyze all three levels of the SRM. If with minor method modifications, your measured values do not agree with the certified values, please contact us for consultation.

Samples for the food round robin (shipped only to those labs that requested these samples) and for Round Robin XXXIV were shipped during the last week of April. Results are due June 16 and feedback to labs is expected by July 24. We will attempt to follow this schedule as closely as possible.

The methods manual, Methods for Analysis of Cancer Chemopreventive Agents in Human Serum, is now available and has been distributed to all QA participants. If you have not received a copy, please notify Ms. Donna Sirk at 301/975-3174 or FAX: 301/977-0685. If you have further questions or concerns, please contact me at 301/975-3120.

Sincerely,

Jeanice B Thomas

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

"Lies, Damned Lies, and Statistics"
Mark Twain

Well, we've done it again... the attached Round Robin XXXIII Report incorporates a major change in the NIST/NCI Micronutrients Measurement Quality Assurance Program (NNMMQAP – spreadsheets just don't do N^2M^2QAP). Most of the Tables look much the same, but we now value- and uncertainty-assign many serum analytes. We'll explain how this is done in the Appendix, but the bottom line is that we provide you with something approaching "true" concentration values – and we make more emphatic statements about your measurement performance.

As before, the Report includes a fairly exhaustive analysis of your results (the "Individualized" report) and a complete listing of everyone's results (the "All Lab" report). Your "Individualized" has the following elements:

Page	Contents
1	Your values, our assigned values, and the %bias between us.
2	%Bias barchart for retinol for your last 3 years' results
3	%Bias barchart for α- and γ-tocopherol for your last 3 years' results
4	%Bias barchart for total and *trans*-β-carotene for your last 3 years' results
5	Our assigned value vs. your value scatterplots for retinol, α- and γ-tocopherol, total and *trans*-β-carotene, again for your last 3 years' results
6	Accuracy/Precision Summary, yet again for your last 3 years' results. Note: this replaces the "(Provisional) Performance Summary" page!
7	Comparison-to-Known-Relationship plots for retinol, total α-carotene, total and *trans*-β-carotene, and α- and γ- tocopherol
8	Comparison-to-Known-Relationship plots for total lycopene, β-cryptoxanthin, lutein, zeaxanthin, and lutein & zeaxanthin

The Comparison-to-Known-Relationship plots on pages 7 and 8 show your individual results and a box-plot summary of the group's results, plotted against either historical results or known dilution ratios. In detail:

Serum #206 was #120 in Round Robin XVIII and #147 in Round Robin XXI. The "true value" line is the average of the three Round Robin results.

Sera #203–205 are a dilution series. Serum #205 is a 1:1 mixture of serum #203 and a delipidized serum diluent. Serum #204 is a 1:2 mixture of serum #203 and the diluent. The "true value" has the slope of the designed-in ratios, and is pegged at the average (dilution corrected) assigned value over the three sera.

The "All Lab" report has the following elements:

Page	Contents
1-4	A complete listing of all results provided by every laboratory and both NIST analysts, plus essential summary statistics.
5	A Legend for the above.
6	The "Measurement Comparability Summary"

The "Measurement Comparability Summary" (¿popularly? known as the "Score Card") provides a four-level score for each laboratory for retinol, α- and γ-tocopherol, and total and *trans*-β-carotene. The score is based upon the worst-case differences between your reported values and our assigned values, after scaling the values by the total measurement uncertainty for each serum. Details of the calculations are provided along side the scores; for those of you who don't want to dig through the details, note the following two points:

1) The NIST Assigned Value (NAV) is calculated as:

$$\frac{\text{(the average of all NIST analyses)} + \text{(the median from this Round Robin)}}{2}$$

As the two summary values are equally weighted, <u>our</u> biases (who, us?) don't dominate the evaluation.

2) The NIST Assigned Uncertainty (NAU) is calculated as the maximum of:
   a) 5% of NAV (this experience-based lower bound prevents too-low estimates)
   b) the combined repeatability and material heterogeneity standard deviation estimated from NIST replicate analysis of multiple samples
   c) the total estimated standard deviation from this Round Robin, estimated as before: eSD = 0.74(IQR), where IQR is the "InterQuartile Range" or central 50% span of all non-NIST values

   This insures that NAU is at least as large as the observed interlaboratory estimate used last year, and (since we give ourselves worst-case samples) that any sample heterogeneity is fully accounted for.

The attached Appendix details the value and uncertainty assignments for all analytes determined by either of the NIST analysts. As patience and time are limited, our promised approach to documenting measurement consistency over time will (hopefully!) be part of Round Robin XXXIV's Report.

We do wish to briefly address a data-reporting issue*: how many significant digits are appropriate for reporting analyte values?* There are two simple rules-of-thumb ("R-o-T") and a slightly complex but pretty good answer.

R-o-T #1: As many as fit **readably** in the little box on the report form. As far as the *NNMMQAP Reports* are concerned, any excess digits are just invisible. Too few digits, and you may introduce an unnecessary bias. Too many digits are data-entry pains, but they cause no harm to the analysis. This rule doesn't apply to providing results to your *customers*, though... too many digits and people get cynical.

R-o-T #2: Three significant figures, or four if you're compulsive. Experience suggests that none of the fat-soluble vitamin-related analytes can reproducibly be measured to better than 1-part-in-1000, so three-to-four significant figures will suffice.

Good Answer: Depends on your measurement repeatability for the particular sample. If you could make the measurement many times over the course of a week, what would the standard deviation of the repeated measurements be? We would like to see the standard deviation stated so that it can read it to about 10% of its calculated value, thus two (one is generally too few, three is always too many) significant digits. Report the mean to the same number of decimal places.

What, you don't always know what that standard deviation is? So estimate it from previous experience (say, 5% of the measured level?) Using 5%: "9.8765..." is "9.88 ±0.49", "0.54321..." is "0.543 ±0.027", and"0.012345..." is "0.01234 ±0.00062".

Two notes on the analytical results:

We intentionally spiked δ-tocopherol into sera #203 — albeit, maybe we spiked a tad too much... While the resulting high levels of this analyte in sera #203–205 caused many of you to scratch your heads and mutter, there is no evidence that it interfered with the quantitative determination of any reported analyte in any laboratory.

The carotenoid levels in serum #203 were lower than designed. We'd intended to challenge your carotenoid detection limits, but not with all three sera of the dilution series! However, the multiple challenges do provide enough information to define your true analytical capabilities near your detection limits: if your values for a given analyte in the series do not decline monotonically, your real detection limit for this analyte is not what you think it is. If they decline but the trend is not parallel to the expected line, you probably need to re-examine how you are defining baseline, peak start, and/or peak end for both samples and calibrants.

Once again, your comments and suggestions are welcome. If you discover any errors in our recording or interpretation of your data, please let us know!

Dave Duewer
Research Chemometrician
DLDuewer@enh.NIST.gov, 301-975-3935

Margaret Kline
Research Biologist

Appendix

Value- and Uncertainty-Assignments for Round Robin XXXIII Sera #203–206

Four sera were distributed in Round Robin XXXIII (RR33). Serum #203 was prepared from stock pools, with effective augmentation of: retinol and α-, γ-, and δ-tocopherol. Serum 204 and 205 were prepared as 1:2 and 1:1 mixtures of serum 203 and delipidized serum. Serum 206 was from the archives, having been previously distributed as sera 120 in Round Robin XVIII (RR18) and 147 in Round Robin XXI (RR21).

NIST analysts NIST1 and NIST3 extracted and analyzed two aliquots of three vials of each of the four sera, providing duplicate results for a total of six samples of each serum. Both researchers provided quantitative results for: retinol; α-, γ-, and δ-tocopherol; total and *trans*-β-carotene; and total α-carotene. NIST3 also provided quantitative results for: *trans*-α-carotene; total and *trans*-lycopene; β-cryptoxanthin; lutein; and zeaxanthin. Retinyl palmitate levels in all four sera were too low for either researcher to quantify.

Table 1 presents the values and measurement uncertainties assigned to sera #203–206 for all analytes characterized by either NIST analyst.

Calculations: The following assumptions and procedures have been used to arrive at the various values presented in Table1:

1) NIST Assigned Values (NAV)

Analytes	Formula
trans-α-carotene	$NAV = NIST3_{AV}$
δ-tocopherol	$NAV = (NIST1_{AV} + NIST3_{AV}) / 2$
total and *trans*-lycopene; β-cryptoxanthin; lutein, zeaxanthin, lutein & zeaxanthin	$NAV = (RR_{MED} + NIST3_{AV}) / 2$
retinol; α- and γ-tocopherol; total and *trans*-β-carotene; total α-carotene	$NAV = \frac{RRmed + (NIST1_{AV} + NIST3_{AV}) / 2}{2}$

where: $NIST1_{AV}$ = mean of 6 analyses by NIST1
$NIST3_{AV}$ = mean of 6 analyses by NIST3
RR_{MED} = median of all RR33 participants' data.

2) NIST Repeatability Standard Deviation (SDrep)
This "within samples" or "repeatability" pooled variance is closely related to the standard deviation of the paired-differences between the replicate extractions / measurements of the samples analyzed at NIST. It arises in within-vial differences associated with extraction, chromatography, peak area determination, etc. Standard analysis-of-variance (anova) calculations were performed as in Eqs. 19.6-4, 19.6-5, and 19.6-6, Mathematical Handbook for Scientists and Engineers, G. and T. Korn (1968). Any proportional systematic bias between results for the two NIST analysts was removed prior to anova analysis.

3) NIST Heterogeneity Standard Deviation (SDhet)
This "between samples" or "heterogeneity" pooled variance is closely related to the standard deviation of the mean values of the unique vials analyzed at NIST. It arises in vial-to-vial differences associated with preparing and reconstituting the serum samples. Standard anova calculations were performed as in Eqs. 19.6-4, 19.6-5, and 19.6-6, Mathematical Handbook for Scientists and Engineers, G. and T. Korn (1968). Any proportional systematic bias between results for the two NIST analysts was removed prior to anova analysis.

4) NIST Material Standard Deviation (SDmat)
This is the combination of SDrep and SDhet as an estimate of the total variation observed in the NIST analyses:

$$SDmat = \sqrt{SDrep^2 + SDhet^2}$$

This value is reported explicitly only for δ-tocopherol and trans-α-carotene, as these analytes were not well characterized by RR33 participants. This value can, however, be directly calculated for all analytes from the provided information.

5) Interlaboratory Bias Standard Deviation (SDlab)
This estimates the residual variance from (non-NIST) laboratory-to-laboratory biases after correction for SDmat, assuming that the population of these systematic biases can be approximated as a normal distribution centered at the NIST assigned value.

The observed interlaboratory standard deviation for the results of RR33 is estimated by eSD = 0.7421(InterQuartile Range), as previously described ("Lies, Damn Lies, and Statistics" report for RR31). This estimate assumes a) that at least 50% of the laboratories that report values for a given analyte are "performing" (they basically know what they're doing), and b) that "non-performing" labs are about equally likely to report low values as high values.

The variance among single-sample results reported by different laboratories is a composite of at least three variation sources: the repeatability of any given method, the heterogeneity among supplied samples, and systematic biases among analysts and/or methods & materials. As we have NIST-based estimates for the first two sources (SDrep and SDhet), we can model laboratory differences as the residual of the observed interlaboratory variance (eSD) after subtraction of the measurement and heterogeneity components:

$$SDlab = \sqrt{MAX[0, eSD^2 - SDrep^2 - SDhet^2]} = \sqrt{MAX[0, eSD^2 - SDmat^2]}$$

The "MAX" function is required if the observed eSD is smaller than the calculated SDmat.

6) NIST Assigned Uncertainty (NAU)
Using the same assumptions as described above for SDlab, the "total" expected interlaboratory variance can be described as:

$$NAU = \sqrt{SDrep^2 + SDhet^2 + SDlab^2} = MAX[eSD, SDmat]$$

Note that this is constrained to be at least as large as the total observed interlaboratory variance, eSD. On the basis of our experience with these analytes in human serum, we also force NAU to be at least 5% of NAV.

7) <u>95% Material</u>
This is the 95% confidence range on the "true" analyte concentration:

NAV ±2 SDmat

The value "nq" (for "not quantitated") is used for the lower 95% bound when 2 SDmat is greater than or equal to NAV.

8) <u>95% Laboratory</u>
This is the 95% confidence range on the RR33 reports of analyte concentration:

NAU ±2 NAU

The value "nq" (for "not quantitated") is used for the lower 95% bound when 2 NAU is greater than or equal to NAV.

Table 1

Value- and Uncertainty Assignments for Round Robin XXXIII

	Sera			
Retinol	203	204	205	206
NAV	1.26	0.43	0.65	0.26
SDrep	0.05	0.01	0.02	0.01
SDhet	0.05	0.02	0.03	0.02
SDlab	0.09	0.05	0.03	0.02
NAU	0.12 (9%)	0.06 (12%)	0.05 (8%)	0.03 (10%)
95% Material	1.11 — 1.41	0.38 — 0.48	0.56 — 0.73	0.22 — 0.30
95% Laboratory	1.03 — 1.50	0.33 — 0.54	0.54 — 0.75	0.21 — 0.32
a-Tocopherol				
NAV	16.5	5.2	8.2	4.7
SDrep	0.3	0.1	0.2	0.1
SDhet	1.2	0.7	0.9	0.3
SDlab	0.9	0.0	0.0	0.3
NAU	1.6 (9%)	0.8 (14%)	0.9 (11%)	0.5 (9%)
95% Material	14.0 — 18.9	3.7 — 6.7	6.4 — 10.0	4.1 — 5.4
95% Laboratory	13.4 — 19.5	3.7 — 6.7	6.4 — 10.0	3.9 — 5.6
g-Tocopherol				
NAV	1.18	0.43	0.65	2.26
SDrep	0.03	0.02	0.04	0.03
SDhet	0.12	0.04	0.09	0.11
SDlab	0.08	0.07	0.03	0.26
NAU	0.15 (13%)	0.09 (19%)	0.11 (16%)	0.29 (13%)
95% Material	0.92 — 1.44	0.34 — 0.53	0.45 — 0.85	2.02 — 2.50
95% Laboratory	0.88 — 1.48	0.26 — 0.61	0.44 — 0.86	1.69 — 2.83
Total b-Carotene				
NAV	0.052	0.020	0.031	0.402
SDrep	0.004	0.003	0.002	0.007
SDhet	0.010	0.007	0.012	0.040
SDlab	0.014	0.010	0.000	0.032
NAU	0.018 (34%)	0.013 (63%)	0.013 (41%)	0.052 (13%)
95% Material	0.030 — 0.074	0.004 — 0.035	0.005 — 0.056	0.320 — 0.483
95% Laboratory	0.016 — 0.088	nq — 0.045	0.005 — 0.056	0.298 — 0.505
trans-b-Carotene				
NAV	0.052	0.018	0.030	0.369
SDrep	0.003	0.003	0.002	0.013
SDhet	0.011	0.004	0.011	0.035
SDlab	0.000	0.008	0.000	0.000
NAU	0.012 (22%)	0.010 (52%)	0.012 (38%)	0.038 (10%)
95% Material	0.028 — 0.076	0.007 — 0.028	0.006 — 0.053	0.294 — 0.444
95% Laboratory	0.028 — 0.076	nq — 0.037	0.006 — 0.053	0.294 — 0.444

Table 1

Value- and Uncertainty Assignments for Round Robin XXXIII

	Sera			
Total a-Carotene	203	204	205	206
NAV	0.026	0.010	0.017	0.023
SDrep	0.002	0.001	0.003	0.003
SDhet	0.007	0.001	0.004	0.006
SDlab	0.000	0.005	0.009	0.000
NAU	0.008 (29%)	0.006 (49%)	0.011 (65%)	0.007 (28%)
95% Material	0.011 — 0.042	0.006 — 0.015	0.006 — 0.027	0.010 — 0.037
95% Laboratory	0.011 — 0.042	0.000 — 0.021	nq — 0.038	0.010 — 0.037
Total Lycopene				
NAV	0.194	0.047	0.096	0.271
SDrep	0.008	0.008	0.010	0.011
SDhet	0.044	0.025	0.038	0.051
SDlab	0.035	0.000	0.000	0.052
NAU	0.057 (29%)	0.027 (56%)	0.040 (41%)	0.075 (27%)
95% Material	0.105 — 0.283	nq — 0.101	0.016 — 0.175	0.166 — 0.377
95% Laboratory	0.081 — 0.307	nq — 0.101	0.016 — 0.175	0.123 — 0.420
trans-Lycopene				
NAV	0.113	0.027	0.053	0.149
SDrep	0.006	0.002	0.000	0.002
SDhet	0.025	0.008	0.021	0.018
SDlab	0.039	0.000	0.000	0.022
NAU	0.047 (41%)	0.009 (30%)	0.021 (39%)	0.029 (19%)
95% Material	0.062 — 0.165	0.010 — 0.044	0.011 — 0.096	0.112 — 0.187
95% Laboratory	0.019 — 0.208	0.010 — 0.044	0.011 — 0.096	0.092 — 0.207
b-Cryptoxanthin				
NAV	0.052	0.017	0.027	0.040
SDrep	0.002	0.001	0.001	0.001
SDhet	0.008	0.004	0.008	0.005
SDlab	0.010	0.004	0.005	0.013
NAU	0.013 (25%)	0.006 (36%)	0.010 (36%)	0.014 (35%)
95% Material	0.035 — 0.069	0.007 — 0.026	0.011 — 0.044	0.030 — 0.050
95% Laboratory	0.026 — 0.078	0.004 — 0.029	0.007 — 0.047	0.012 — 0.068
Lutein				
NAV	0.078	0.026	0.042	0.051
SDrep	0.001	0.001	0.002	0.002
SDhet	0.003	0.001	0.001	0.004
SDlab	0.009	0.004	0.005	0.006
NAU	0.010 (13%)	0.005 (16%)	0.006 (13%)	0.008 (15%)
95% Material	0.071 — 0.084	0.022 — 0.030	0.036 — 0.047	0.041 — 0.060
95% Laboratory	0.058 — 0.097	0.018 — 0.035	0.031 — 0.053	0.035 — 0.066

Table 1

Value- and Uncertainty Assignments for Round Robin XXXIII

	Sera			
Zeaxanthin	203	204	205	206
NAV	0.039	0.013	0.022	0.015
SDrep	0.001	0.001	0.002	0.002
SDhet	0.002	0.002	0.003	0.003
SDlab	0.007	0.006	0.002	0.004
NAU	0.007 (18%)	0.007 (48%)	0.004 (17%)	0.006 (38%)
95% Material	0.034 — 0.043	0.009 — 0.017	0.016 — 0.028	0.006 — 0.023
95% Laboratory	0.025 — 0.053	0.000 — 0.026	0.014 — 0.030	0.003 — 0.027
ıtein&Zeaxanthin				
NAV	0.120	0.040	0.063	0.065
SDrep	0.002	0.001	0.004	0.004
SDhet	0.001	0.004	0.004	0.009
SDlab	0.011	0.009	0.007	0.009
NAU	0.012 (9%)	0.010 (24%)	0.010 (14%)	0.014 (21%)
95% Material	0.115 — 0.125	0.032 — 0.047	0.052 — 0.074	0.044 — 0.086
95% Laboratory	0.097 — 0.143	0.020 — 0.059	0.044 — 0.082	0.038 — 0.093
d-Tocopherol				
NAV	3.25	1.08	1.66	0.18
SDrep	0.04	0.06	0.03	0.03
SDhet	0.03	0.02	0.09	0.04
NAU = SDmat	0.06 (2%)	0.07 (6%)	0.10 (6%)	0.05 (28%)
95% Material	3.13 — 3.36	0.94 — 1.22	1.46 — 1.85	0.07 — 0.28
trans-a-Carotene				
NAV	0.022	0.007	0.012	0.014
SDrep	0.003	0.002	0.004	0.001
SDhet	0.008	0.001	0.005	0.004
NAU = SDmat	0.009 (37%)	0.003 (35%)	0.007 (53%)	0.004 (27%)
95% Material	0.006 — 0.039	0.002 — 0.012	nq — 0.024	0.006 — 0.022

Appendix C. "All-Lab Report" for RR33

The following 6 pages are the "All-Lab Report" as provided to all participants, with two exceptions:

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

The data summary in the "All-Lab Report" has been altered to ensure confidentiality of identification codes assigned to laboratories. The only attributed results are those reported by NIST. The NIST results are not used in the assessment of the consensus summary results of the study.

Round Robin XXXIII Laboratory Results

Values in µg/mL

	Retinol				Retinyl Palmitate				α-Tocopherol				γ-Tocopherol			
Lab	203	204	205	206	203	204	205	206	203	204	205	206	203	204	205	206
FSV-BA	1.23	0.414	0.632	0.267	0.129	0.073	0.118	0.059	16.2	5.37	8.16	4.76	1.26	0.54	0.73	2.23
FSV-BD	1.31	0.426	0.636	0.270					16.0	5.43	8.27	4.84				
FSV-BE	1.29	0.512	0.632	0.275					17.6	6.36	8.56	5.03	1.22	0.49	0.63	2.48
FSV-BG	1.20	0.400	0.590	0.250	0.066	0.045	0.080	*nq*	16.7	5.48	8.25	4.74				
FSV-BH	1.19	0.415	0.617	0.261	*nq*	*nq*	*nq*	*nq*	18.1	6.22	9.18	5.28	1.04	0.37	0.55	1.95
FSV-BI	1.05	0.352	0.492	0.186	0.114	0.042	0.123	*nd*	18.3	6.02	9.25	4.88	1.34	0.52	0.69	2.47
FSV-BJ	1.27	0.418	0.610	0.269	0.131	0.086	0.117	*nq*	17.5	6.98	9.70	5.16	1.45	0.62	0.80	2.61
FSV-BK	1.34	0.423	0.636	0.263					16.7	5.47	8.04	4.31				
FSV-BM	1.25	0.425	0.645	0.266					18.2	5.80	8.80	5.30				
FSV-BN	1.34	0.455	0.654	0.329	0.068		0.072	0.066	18.0	5.16	8.95	4.57	0.96	0.18	0.52	2.13
FSV-BO	1.18	0.352	0.550	0.227					17.7	5.87	8.00	4.92				
FSV-BP	1.35	0.478	0.716	0.308					19.4	7.14	10.67	6.08				
FSV-BQ	1.29	0.450	0.650	0.270					18.1	4.10	9.30	5.40				
FSV-BR	1.29	0.445	0.674	0.283												
FSV-BS																
FSV-BT	1.19	0.725	0.653	0.271	0.084	0.079	0.091	*nd*	15.5	5.35	8.02	4.43	1.17	0.45	0.59	2.06
FSV-BU	1.36	0.434	0.652	0.286	*nq*	*nq*	*nq*	*nq*	16.1	4.73	8.05	4.15	1.09	0.32	0.50	2.06
FSV-BV	1.25	0.405	0.706	0.228					17.5	6.07	10.14	5.25	1.31	0.50	0.77	2.53
FSV-BX	1.36	0.456	0.654	0.283					17.3	5.55	8.47	4.91	1.21	0.48	0.62	2.27
FSV-BY	1.20	0.404	0.623	0.269	0.172	0.049	0.085	*nd*	16.8	4.83	8.38	4.84	1.54	0.45	0.74	2.30
FSV-CA	1.20	0.380	0.620	0.260					17.1	5.74	8.75	4.64				
FSV-CB	1.57	0.518	0.770	0.327					20.0	7.28	10.19	6.38				
FSV-CD	1.37	0.461	0.684	0.259	*nq*	0.027	0.022	0.035	21.7	7.02	10.35	5.58	1.07	0.37	0.54	2.03
FSV-CH	1.33	0.428	0.608	0.249					19.4	5.90	8.54	4.81	1.23	0.41	0.55	2.29
FSV-CJ	1.40	0.435	0.611	0.254					16.8	5.76	7.80	5.66				
FSV-CK	1.15	0.353	0.586	0.244					14.7	5.25	7.79	4.60	1.11	0.48	0.66	2.49
FSV-CL	1.14	0.431	0.586	0.310					15.3	6.27	8.36	5.89				
FSV-CM									17.1	5.60	8.90	5.10				
FSV-CN	1.19	0.388	0.613	0.276					17.4	5.48	9.50	4.78	1.09	0.22	0.43	2.02
FSV-CP	0.99	0.321	0.519	0.209					11.7	3.81	5.98	3.28	1.19		0.26	1.26
FSV-CR	1.48	0.480	0.730	0.300					16.8	5.90	8.80	5.10				
FSV-CT	1.28	0.426	0.642	0.246					17.0	5.69	8.32	5.32				
FSV-CU	1.18	0.416	0.611	0.259	0.137	0.018	0.028	0.080	15.5	4.87	7.51	4.72				
FSV-CV	1.24	0.443	0.661	0.263					17.2	5.32	8.80	4.75	1.05	0.62	0.57	2.83
FSV-CX	1.22	0.470	0.660	0.270	0.170	0.040	0.030	0.030	15.5	5.96	8.49	4.81	1.13	0.39	0.61	2.39
FSV-CY	1.27	0.375	0.649	0.270					22.1	5.30	8.84	4.82				
FSV-DA	1.21	0.470	0.680	0.280					16.6	5.71	8.87	4.75	1.23	0.45	0.67	2.22
FSV-DB	1.29	0.511	0.667	0.648					15.6	5.59	8.42	4.99				
FSV-DJ	1.24	0.440	0.730	0.290					17.0	6.60	8.80	5.20				
FSV-DK	1.17	0.300	0.440	0.196					18.3	6.72	9.20	5.30				
FSV-DL	1.29	0.318	0.695	0.299					17.1	5.10	8.66	5.16				
FSV-DM	1.13	0.367	0.493	0.248					18.7	5.56	8.30	5.20				
FSV-DP	1.19	0.404	0.586	0.242												
FSV-DX	1.03	0.398	0.563	0.258					15.2	5.17	8.10	4.60				
FSV-EC	1.32	0.491	0.701	0.299					13.5	5.04	7.20	4.12				
FSV-EH	1.44	0.558	0.756	0.303	*nd*	*nd*	*nd*	*nd*	19.6	6.63	10.02	5.49	1.52	0.45	0.66	2.45
FSV-EJ	1.31	0.553	0.718	0.253					18.8	5.80	9.18	5.17				
FSV-EK	1.50	0.653	0.909	0.454					19.0	5.34	9.14	4.40	1.45	0.65	0.93	2.68
FSV-EL	1.32	0.410	0.640	0.290												
FSV-FD	1.71	0.507	0.713	0.291					16.4	5.95	9.22	4.49				
FSV-FP	0.81	0.446	0.829	0.272					17.3	6.77	8.61	5.26	1.33	0.46	0.67	2.33
n	49	49	49	49	9	9	10	5	47	47	47	47	22	21	22	22
Min	0.81	0.300	0.440	0.186	0.066	0.018	0.022	0.030	11.7	3.81	5.98	3.28	0.96	0.18	0.26	1.26
Median	1.27	0.428	0.645	0.270	0.129	0.045	0.083	0.059	17.1	5.69	8.66	4.91	1.22	0.45	0.63	2.30
Max	1.71	0.725	0.909	0.648	0.172	0.086	0.123	0.080	22.1	7.28	10.67	6.38	1.54	0.65	0.93	2.83
eSD	0.11	0.044	0.052	0.025	0.061	0.027	0.052	0.031	1.5	0.55	0.74	0.43	0.17	0.09	0.11	0.28
eCV	8	10	8	9	47	59	63	53	9	10	9	9	14	20	18	12
NISTa	1.22	0.415	0.611	0.254	*nq*	*nq*	*nq*	*nq*	16.4	4.94	8.08	4.77	1.08	0.42	0.70	2.21
NISTb	1.29	0.460	0.688	0.265	*nq*	*nd*	*nd*	*nd*	15.2	4.63	7.62	4.50	1.23	0.40	0.63	2.25
NAV	1.26	0.433	0.647	0.265					16.5	5.24	8.26	4.77	1.19	0.43	0.65	2.26
NAU	0.12	0.049	0.059	0.029					1.6	0.87	0.92	0.51	0.17	0.11	0.11	0.30

C2

Round Robin XXXIII Laboratory Results

Values in µg/mL

	δ-Tocopherol				Total β-Carotene				trans-β-Carotene				Total cis-β-Carotene			
Lab	203	204	205	206	203	204	205	206	203	204	205	206	203	204	205	206
FSV-BA					0.067	0.019	0.035	0.425	0.061	0.019	0.035	0.392	0.006	*nd*	*nd*	0.033
FSV-BD																
FSV-BE					0.040	0.013	0.027	0.383								
FSV-BG					0.055	0.024	0.030	0.398								
FSV-BH					0.060	0.021	0.033	0.405	0.060	0.021	0.033	0.379	*nq*	*nq*	*nq*	0.026
FSV-BI					0.062	0.022	0.033	0.408								
FSV-BJ					0.075	0.031	0.037	0.432								
FSV-BK																
FSV-BM																
FSV-BN					0.095	0.043	0.042	0.455	0.077	0.026	0.042	0.410	0.037	*0.017*	0.020	0.081
FSV-BO					0.050	0.017	0.026	0.352								
FSV-BP					0.044	0.025	0.037	0.325								
FSV-BQ					0.140	0.070	0.070	0.450								
FSV-BR																
FSV-BS					0.068	0.027	0.037	0.420								
FSV-BT					0.059	0.019	0.031	0.362	0.056	0.018	0.029	0.338	0.003	0.001	0.001	0.025
FSV-BU					0.069	0.099	0.037	0.400	*nq*	0.083	*nq*	0.384	*nq*	0.018	*nq*	0.016
FSV-BV					*>0.044*	*>0.018*	*>0.031*	*>0.377*	0.044	0.018	0.031	0.377				
FSV-BX					0.037	0.018	0.029	0.358								
FSV-BY					0.058	*nd*	0.028	0.411								
FSV-CA																
FSV-CB					0.040	0.010	0.018									
FSV-CD					0.045	0.016	0.027	0.336								
FSV-CH					0.055	0.016	0.024	0.345								
FSV-CJ																
FSV-CK					0.049	0.022	0.031	0.427								
FSV-CL					0.069	0.035	0.042	0.360								
FSV-CM																
FSV-CN					*>0.030*	*nd*	*nd*	*>0.321*	0.030	*nd*	*nd*	0.321				
FSV-CP					0.043	0.013	0.019	0.268								
FSV-CR																
FSV-CT					0.063	0.019	0.027	0.341								
FSV-CU					0.110	0.037	0.048	0.497	0.098	0.037	0.038	0.394	*0.012*	*0.000*	*0.010*	*0.103*
FSV-CV					0.066	0.010	0.020	0.380								
FSV-CX					0.050	0.020	0.030	0.410								
FSV-CY					0.042	0.117	0.138	0.339								
FSV-DA	2.70	0.910	1.400	0.130	0.068	0.032	0.038	0.441	0.064	0.030	0.034	0.396	0.004	0.002	0.004	0.045
FSV-DB					0.021	*nq*	*nq*	0.426								
FSV-DJ																
FSV-DK																
FSV-DL					0.061	0.013	0.030	0.412	0.055	0.011	0.026	0.390	*0.006*	*0.002*	*0.004*	*0.022*
FSV-DM					0.042	0.009	0.024	0.314								
FSV-DP																
FSV-DX					*>0.046*	*>0.008*	*>0.025*	*>0.309*	0.046	0.008	0.025	0.309	*nq*	*nq*	*nq*	0.014
FSV-EC					0.045	0.022	0.033	0.413								
FSV-EH					0.056	0.025	0.031	0.403	0.056	0.025	0.031	0.366	*nd*	*nd*	*nd*	*nd*
FSV-EJ					0.162	0.121	0.126	3.016								
FSV-EK						*nd*	0.120	0.398								
FSV-EL																
FSV-FD					0.047	0.018	0.029	0.440								
FSV-FP					0.042	0.031	0.057	0.415								
n	1	1	1	1	35	33	35	35	11	11	10	12	6	6	5	9
Min					0.021	0.009	0.018	0.268	0.030	0.008	0.025	0.309	0.003	0.000	0.001	0.014
Median					0.056	0.022	0.031	0.405	0.056	0.021	0.032	0.382	0.006	0.002	0.004	0.026
Max					0.162	0.121	0.138	3.016	0.098	0.083	0.042	0.410	0.037	0.018	0.020	0.103
eSD					0.017	0.009	0.009	0.040	0.012	0.007	0.004	0.020	0.004	0.002	0.004	0.015
eCV					31	40	29	10	21	35	14	5	59	100	96	59
NISTa	3.24	1.07	1.63	0.18	0.047	0.023	0.038	0.450	0.043	0.017	0.035	0.384	*0.004*	*0.006*	*0.004*	*0.066*
NISTb	3.26	1.09	1.69	0.17	0.044	0.012	0.018	0.351	0.044	0.012	0.018	0.313	*0.000*	*0.000*	*0.000*	*0.039*
NAV					0.051	0.020	0.030	0.403	0.050	0.018	0.029	0.365	0.004	0.002	0.003	0.039
NAU					0.018	0.011	0.016	0.076	0.013	0.009	0.014	0.063	0.005	0.009	0.005	0.029

C3

Round Robin XXXIII Laboratory Results

Values in µg/mL

	Total α-Carotene				Total Lycopene				trans-Lycopene				β-Cryptoxanthin			
Lab	203	204	205	206	203	204	205	206	203	204	205	206	203	204	205	206
FSV-BA	0.031	0.010	0.014	0.023					0.114	0.042	0.057	0.159	0.071	0.033	0.040	0.050
FSV-BD																
FSV-BE																
FSV-BG	0.028	0.013	0.014	0.023	0.234	0.082	0.106	0.326								
FSV-BH	0.029	0.010	0.015	0.020	0.203	0.064	0.102	0.293					0.083	0.027	0.041	0.052
FSV-BI	0.028	0.009	0.018	0.019	0.144	0.037	0.074	0.190					0.077	0.026	0.040	0.045
FSV-BJ	0.052	0.025	0.033	0.034	0.132	0.038	0.065	0.184								
FSV-BK																
FSV-BM																
FSV-BN	0.046	0.017	0.029	0.024	0.262	0.062	0.151	0.415	0.232	0.030	0.103	0.227	0.050	0.023	0.046	0.055
FSV-BO	0.026	0.009	0.013	0.015	0.197	0.060	0.088	0.279					0.059	0.021	0.030	0.036
FSV-BP	0.024	0.011	0.017	0.020	0.086	0.038	0.063	0.150					0.051	0.022	0.031	0.035
FSV-BQ																
FSV-BR																
FSV-BS	0.024	0.012	0.017	0.016	0.120	0.040	0.010	0.140					0.042	0.020	0.051	0.074
FSV-BT	0.030	0.014	0.019	0.021	0.167	0.051	0.094	0.232	0.135	0.044	0.077	0.187	0.069	0.027	0.034	0.040
FSV-BU	0.025	0.016	0.016	0.016	0.219	0.055	0.092	0.263	*nq*	*nq*	*nq*	*nq*	0.055	0.012	0.029	0.038
FSV-BV	0.036	0.014	0.024	0.028	0.314	0.102	0.184	0.450					0.038	0.016	0.025	0.029
FSV-BX	0.082	0.019	0.031	0.033	0.172	0.052	0.079	0.207					0.110	0.043	0.059	0.058
FSV-BY	0.024	*nd*	0.013	0.018	0.139	0.043	0.071	0.310					0.060	0.018	0.031	0.043
FSV-CA																
FSV-CB	0.019	0.005	0.008		0.095	0.013	0.033						0.041	0.013	0.021	
FSV-CD	0.020	0.008	0.012	0.014	0.161	0.053	0.078	0.223					0.043	0.015	0.020	0.025
FSV-CH	0.028	0.010	0.012	0.013	0.199	0.037	0.076	0.234								
FSV-CJ	0.030	0.014	0.018	0.017	0.209	0.032	0.105	0.304					0.064	0.016	0.033	0.045
FSV-CK	0.033	0.012	0.019	0.026	0.225	0.063	0.106	0.368					0.067	0.022	0.032	0.067
FSV-CL	0.047	0.033	0.036	0.038	0.330	0.178	0.209	0.392					0.119	0.069	0.082	0.088
FSV-CM																
FSV-CN	*nd*	*nd*	*nd*	*nd*	0.205	*nd*	*nd*	0.182								
FSV-CP	0.022	0.009	0.014	0.015	0.172	0.052	0.077	0.214					0.063	0.022	0.034	0.036
FSV-CR																
FSV-CT																
FSV-CU																
FSV-CV					0.215	0.046	0.095	0.271								
FSV-CX	0.030	0.010	0.020	0.020	0.230	0.070	0.120	0.350					0.060	0.020	0.030	0.040
FSV-CY																
FSV-DA	0.027	0.013	0.018	0.024	0.168	0.042	0.070	0.255	0.088	0.023	0.040	0.139	0.039	0.014	0.022	0.032
FSV-DB																
FSV-DJ																
FSV-DK																
FSV-DL	0.032	0.007	0.015	0.021	0.208	0.040	0.090	0.304					0.104	0.027	0.046	0.062
FSV-DM																
FSV-DP																
FSV-DX	0.014	0.003	0.009	0.013	0.151	0.043	0.075	0.246								
FSV-EC																
FSV-EH	0.020	0.012	0.012	0.015	0.191	0.063	0.100	0.267	0.102	0.037	0.055	0.145	0.054	0.015	0.023	0.033
FSV-EJ	0.105	0.054	0.064	0.091	0.262	0.163	0.175	0.394								
FSV-EK								0.504								
FSV-EL																
FSV-FD																
FSV-FP	0.020	0.015	0.028	0.014	0.093	0.061	0.087	0.193								
n	28	27	28	27	29	28	28	29	5	5	5	5	22	22	22	21
Min	0.014	0.003	0.008	0.013	0.086	0.013	0.010	0.140	0.088	0.023	0.040	0.139	0.038	0.012	0.020	0.025
Median	0.028	0.012	0.017	0.020	0.197	0.052	0.089	0.267	0.114	0.037	0.057	0.159	0.060	0.021	0.033	0.043
Max	0.105	0.054	0.064	0.091	0.330	0.178	0.209	0.504	0.232	0.044	0.103	0.227	0.119	0.069	0.082	0.088
eSD	0.006	0.004	0.005	0.006	0.050	0.017	0.023	0.087	0.031	0.010	0.025	0.030	0.016	0.008	0.011	0.013
eCV	21	37	31	30	25	32	26	33	27	27	44	19	26	38	34	31
NISTa	0.025	0.010	0.019	0.030												
NISTb	0.022	0.007	0.012	0.022	0.192	0.043	0.104	0.272	0.084	0.020	0.037	0.121	0.041	0.011	0.021	0.032
NAV	0.026	0.010	0.016	0.023	0.194	0.048	0.096	0.269	0.099	0.028	0.047	0.140	0.050	0.016	0.027	0.038
NAU	0.009	0.005	0.006	0.009	0.052	0.021	0.031	0.083	0.039	0.017	0.023	0.051	0.021	0.011	0.013	0.016

C4

Round Robin XXXIII Laboratory Results

Values in µg/mL

	Lutein				Zeaxanthin				Lutein&Zeaxanthin			
Lab	203	204	205	206	203	204	205	206	203	204	205	206
FSV-BA									0.148	0.074	0.092	0.088
FSV-BD												
FSV-BE												
FSV-BG												
FSV-BH	0.079	0.031	0.042	0.052	0.038	*nq*	0.028	0.011	*0.117*	*0.031*	*0.070*	*0.063*
FSV-BI	0.078	0.027	0.038	0.045	0.042	0.012	0.020	0.015	0.122	0.039	0.056	0.057
FSV-BJ												
FSV-BK												
FSV-BM												
FSV-BN	0.054	0.014	0.022	0.038	0.036	0.007	0.016	0.008	*0.090*	*0.021*	*0.038*	*0.046*
FSV-BO									0.090	0.032	0.047	0.056
FSV-BP												
FSV-BQ												
FSV-BR												
FSV-BS												
FSV-BT	0.089	0.056	0.050	0.054	0.023	0.019	0.020	0.010	0.113	0.075	0.070	0.064
FSV-BU	*nq*	*nq*	*nq*	*nq*	*nq*	*nq*	*nq*	*nq*	0.126	0.032	0.055	0.064
FSV-BV									0.096	0.035	0.061	0.061
FSV-BX	0.084	0.033	0.044	0.045	0.032	0.013	0.017	0.012	*0.116*	*0.033*	*0.061*	*0.057*
FSV-BY	0.090	0.027	0.045	0.057	0.024	0.008	0.012	0.020	*0.114*	*0.035*	*0.057*	*0.077*
FSV-CA												
FSV-CB									0.120	0.044	0.061	
FSV-CD									0.167	0.057	0.080	0.089
FSV-CH												
FSV-CJ									0.148	0.049	0.064	0.073
FSV-CK									0.133	0.042	0.066	0.081
FSV-CL									0.129	0.047	0.064	0.088
FSV-CM												
FSV-CN												
FSV-CP									0.113	0.047	0.068	0.060
FSV-CR												
FSV-CT												
FSV-CU												
FSV-CV												
FSV-CX									0.120	0.040	0.060	0.070
FSV-CY												
FSV-DA	0.069	0.026	0.038	0.053	0.033	0.019	0.022	0.023	*0.102*	*0.045*	*0.060*	*0.076*
FSV-DB												
FSV-DJ												
FSV-DK												
FSV-DL									0.139	0.034	0.071	0.080
FSV-DM												
FSV-DP												
FSV-DX									0.099	0.035	0.052	0.061
FSV-EC												
FSV-EH	0.069	0.026	0.037	0.047	0.046	0.017	0.023	0.021	*0.115*	*0.042*	*0.060*	*0.068*
FSV-EJ												
FSV-EK				0.092								
FSV-EL												
FSV-FD												
FSV-FP												
n	8	8	8	9	8	7	8	8	21	21	21	20
Min	0.054	0.014	0.022	0.038	0.023	0.007	0.012	0.008	0.090	0.021	0.038	0.046
Median	0.079	0.027	0.040	0.052	0.035	0.013	0.020	0.014	0.117	0.040	0.061	0.066
Max	0.090	0.056	0.050	0.092	0.046	0.019	0.028	0.023	0.167	0.075	0.092	0.089
eSD	0.014	0.004	0.006	0.007	0.008	0.007	0.004	0.007	0.018	0.010	0.007	0.013
eCV	18	15	14	14	24	57	22	49	15	25	12	20
NISTa												
NISTb	0.076	0.026	0.042	0.049	0.044	0.014	0.024	0.018	*0.120*	*0.040*	*0.065*	*0.067*
NAV	0.077	0.026	0.041	0.051	0.039	0.013	0.022	0.016	0.119	0.040	0.063	0.066
NAU	0.020	0.009	0.012	0.014	0.012	0.006	0.007	0.008	0.027	0.012	0.016	0.017

C5

Round Robin XXXIII Laboratory Results

Analytes Reported By One Laboratory

Values in µg/mL

Analyte	Code	203	204	205	206
trans-α-Carotene	NISTb	0.022	0.007	0.012	0.014

Legend

Term	Definition
n	Number of (non-NIST) quantitative values reported for this analyte
Min	Minimum (non-NIST) quantitative value reported
Median	Median (non-NIST) quantitative value reported
Max	Maximum (non-NIST) quantitative value reported
eSD	Estimated standard deviation, calculated from the median absolute deviation from the median of the non-NIST results
eCV	Coefficient of Variation for (non-NIST) results: 100*eSD/Median
NAV	NIST Assigned Value, our estimate of the "true" analyte concentration
NAU	NIST Assigned Uncertainty, our estimate of the total (serum hetergeniety and inter- and intra-laboratory) standard deviation For details on how we assign these quantities, see the "Analysis of Results."
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

C6

Round Robin XXXIII Laboratory Results

Comparability Summary

Lab	R	aT	gT	bC	tbC
FSV-BA	1	1	2	1	
FSV-BD	1	1	1	1	
FSV-BE	2	2		1	
FSV-BG	1	1			
FSV-BH	1	2			
FSV-BI	3	2			
FSV-BJ	1	2	1	1	
FSV-BK	1	1	1	1	1
FSV-BM	1	2	2	1	1
FSV-BN	3	1	1	1	
FSV-BO	2	1	1	1	
FSV-BP	2	3		4	
FSV-BQ	1	2			
FSV-BR	1		2	2	
FSV-BS					
FSV-BT	4	1	2	1	
FSV-BU	1	2		3	3
FSV-BV	2	3	3	2	
FSV-BX	1	1		1	1
FSV-BY	1	1	1	1	
FSV-CA	2	1		4	
FSV-CB	3	3		2	
FSV-CD	1	4		2	
FSV-CH	1	2	1	1	
FSV-CJ	2	2		1	
FSV-CK	2	2		2	
FSV-CL	2	3	3	3	2
FSV-CM		1		1	
FSV-CN	1	2		1	
FSV-CP	3	3		1	
FSV-CR	2	1	1	1	1
FSV-CT	1	2		1	
FSV-CU	1	1			
FSV-CV	1	1		4	
FSV-CX	1	1			
FSV-CY	2	4			
FSV-DA	1	1			
FSV-DB	4	1			2
FSV-DJ	2	2	3	4	
FSV-DK	4	2	1	1	
FSV-DL	3	1			
FSV-DM	3	2		1	
FSV-DP	2			2	
FSV-DX	2	1	2	4	4
FSV-EC	2	2	1	2	
FSV-EH	3	2	1	2	2
FSV-EJ	3	2			
FSV-EK	4	2			
FSV-EL	1		2		1
FSV-FD	4	2	2	1	1
FSV-FP	4	2	2		2
NISTa	1	1	1	1	1
NISTb	1	1	1	1	1
n	49	47	22	36	12

Label	Definition
Lab	laboratory number
R	"Standard Score" for Retinol
aT	"Standard Score" for α-Tocopherol
gT	"Standard Score" for γ-Tocopherol
bC	"Standard Score" for Total β-Carotene
tbC	"Standard Score" for trans-β-Carotene
n	number of (non-NIST) laboratories providing data for this analyte

"Standard Score"

Given that our knowledge of the shape, location, and width of the measurement distributions is approximate and that a limited number of labs are involved, we summarize comparability with the following four-level "Standard Score" (StS)…

StS	Definition	
1	All StV within $\pm t$ (1-0.683,n-1)	{i.e., ±1 SD}
2	All StV within $\pm t$ (1-0.954,n-1)	{i.e., ±2 SD}
3	All StV within $\pm t$ (1-0.997,n-1)	{i.e., ±3 SD}
4	At least one StV > $\pm t$ (1-0.997,n-1)	{i.e., >3 SD}

where:	
StV	Standardized Value, the distance in standard deviation units your value is from the "true" concentration: StV = (your value - NAV) / NAU
NAV	NIST Assigned Value, our estimate of the "true" analyte concentration
NAU	NIST Assigned Uncertainty, our estimate of the total (serum heterogeniety and iter- and intra-laboratory) measurement standard deviation.
t (1-α,n-1)	Two-tailed Student's t for coverage of ±1, ±2, and ±3 NAU about NAV, assuming a normal population of size n

For details on the NIST Assigned quantities, see this Round Robin's "Report of (Meta)Analysis."

StS	% Observed					Expected
1	43	40	50	58	50	68.2 %
2	29	45	36	22	33	27.3 %
3	16	11	14	6	8	4.3 %
4	12	4	0	14	8	0.3 %

These are the observed and normal-population-expected proportions of each Standard Score (StS), based upon each laboratory's largest StV for the four sera.

C7

Appendix D. Representative "Individualized Report" for RR33

Each participant in RR33 received an "Individualized Report" reflecting their reported results. Each report included a detailed analysis of the results they reported for some or all of the following analytes:

- Retinol
- Retinol palmitate
- α-Tocopherol
- γ-Tocopherol
- Total β-Carotene
- *trans*-β-Carotene
- Total α-Carotene
- Total Lycopene
- β-Cryptoxanthin
- Lutein
- Zeaxanthin
- Lutein & Zeaxanthin

The following 8 pages are the "Individualized Report" for the analytes evaluated by participant FSV-BA.

Individualized Round Robin XXXIII Report to: FSV-BA

Your Data, NIST Assigned Values, and %Differences

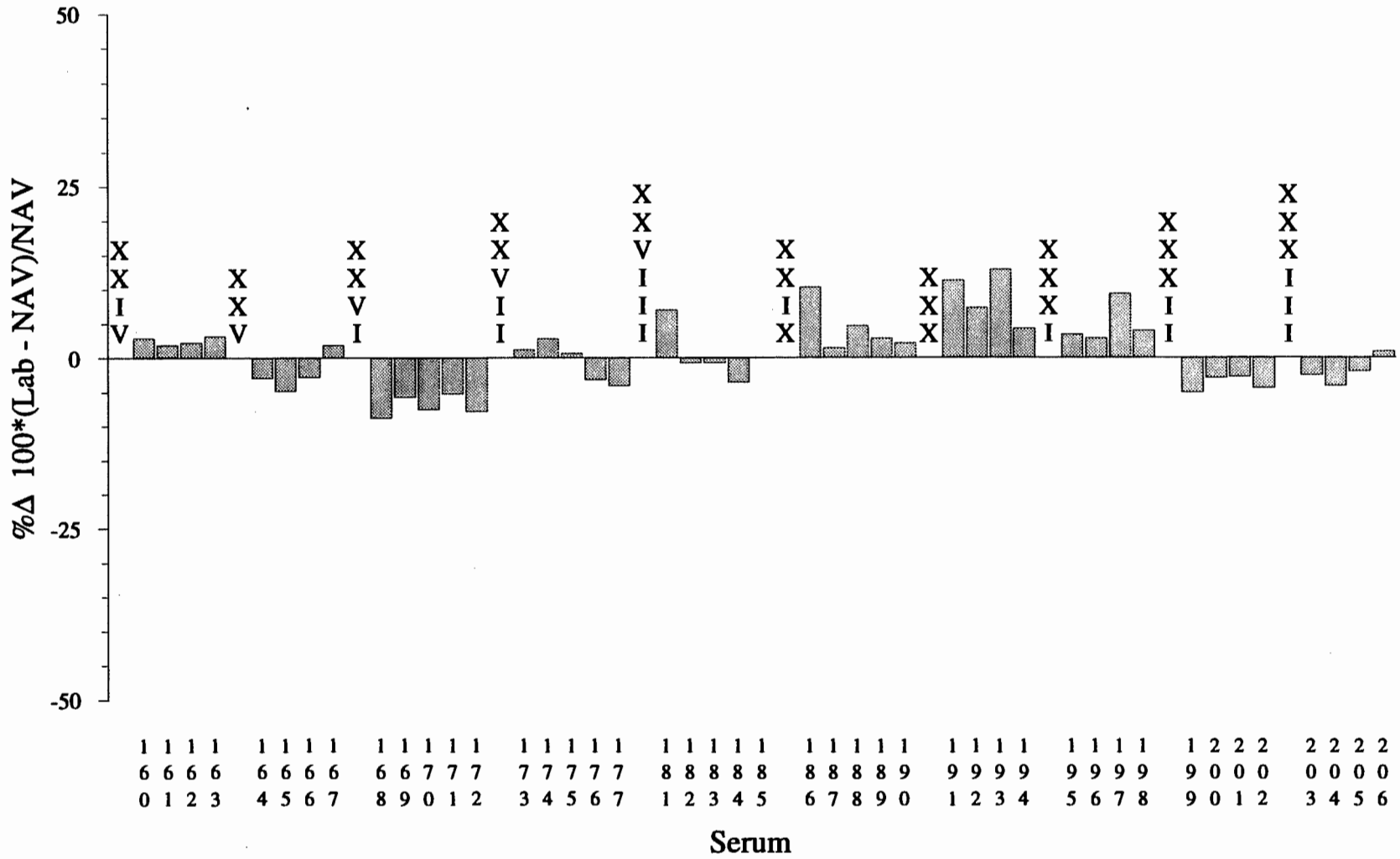
	Serum 203				Serum 204				Serum 205				Serum 206			
Analyte	You	NAV	%Δ	n	You	NAV	%Δ	n	You	NAV	%Δ	n	You	NAV	%Δ	n
Retinol	1.23	1.26	-3	44	.41	.43	-4	44	.63	.65	-2	44	.27	.26	1	44
Retinyl Palmitate	.129			9	.073			8	.118			9	.059			4
a-Tocopherol	16.23	16.47	-1	42	5.37	5.19	3	42	8.16	8.20	0	42	4.76	4.75	0	42
g-Tocopherol	1.26	1.18	7	19	.54	.43	24	17	.73	.65	13	18	2.23	2.26	-1	19
Total b-Carotene	.067	.052	29	32	.019	.020	-3	31	.035	.031	14	33	.425	.402	6	32
trans-b-Carotene	.061	.052	17	8	.019	.018	8	9	.035	.030	17	8	.392	.369	6	9
Total cis-b-Carotene	.006			4	*nd*			3	*nd*			3	.033			7
Total a-Carotene	.031	.026	18	25	.010	.010	-4	24	.014	.017	-15	25	.023	.023	-1	24
trans-Lycopene	.114	.113	1	4	.042	.027	55	4	.057	.053	7	4	.159	.149	6	4
b-Cryptoxanthin	.071	.052	37	19	.033	.017	99	19	.040	.027	47	19	.050	.040	26	18
Lutein & Zeaxanthin	.148	.120	23	18	.074	.040	87	18	.092	.063	46	18	.088	.065	35	17

You : Your reported values for the listed analytes (micrograms/milliliter)
NAV : NIST Assigned Values, equal to (NIST's average-of-averages + this Round Robin's median) / 2
%Δ : Percent difference between your value and the NAV
n : Number of non-NIST laboratories reporting quantitative values for this analyte in this serum
nd : Not detected

Please check our recorded values against your records.
Send corrections to: NNMMQAP, 222/B208, NIST, Gaithersburg, MD 20899 fax: 301-977-0685

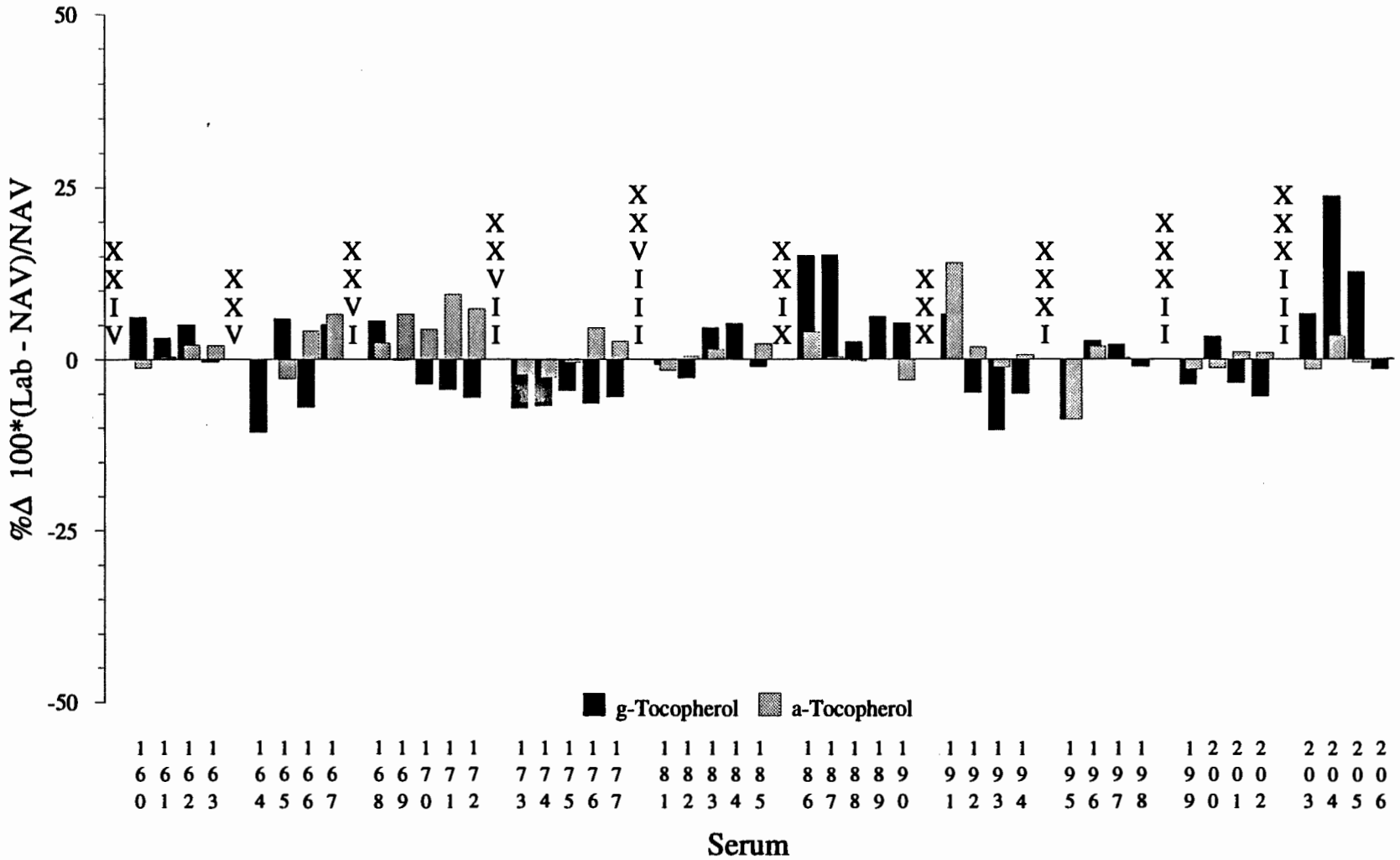
Individualized Round Robin XXXIII Report to: FSV-BA

Retinol, %Δ in Round Robin XXIV - XXXIII



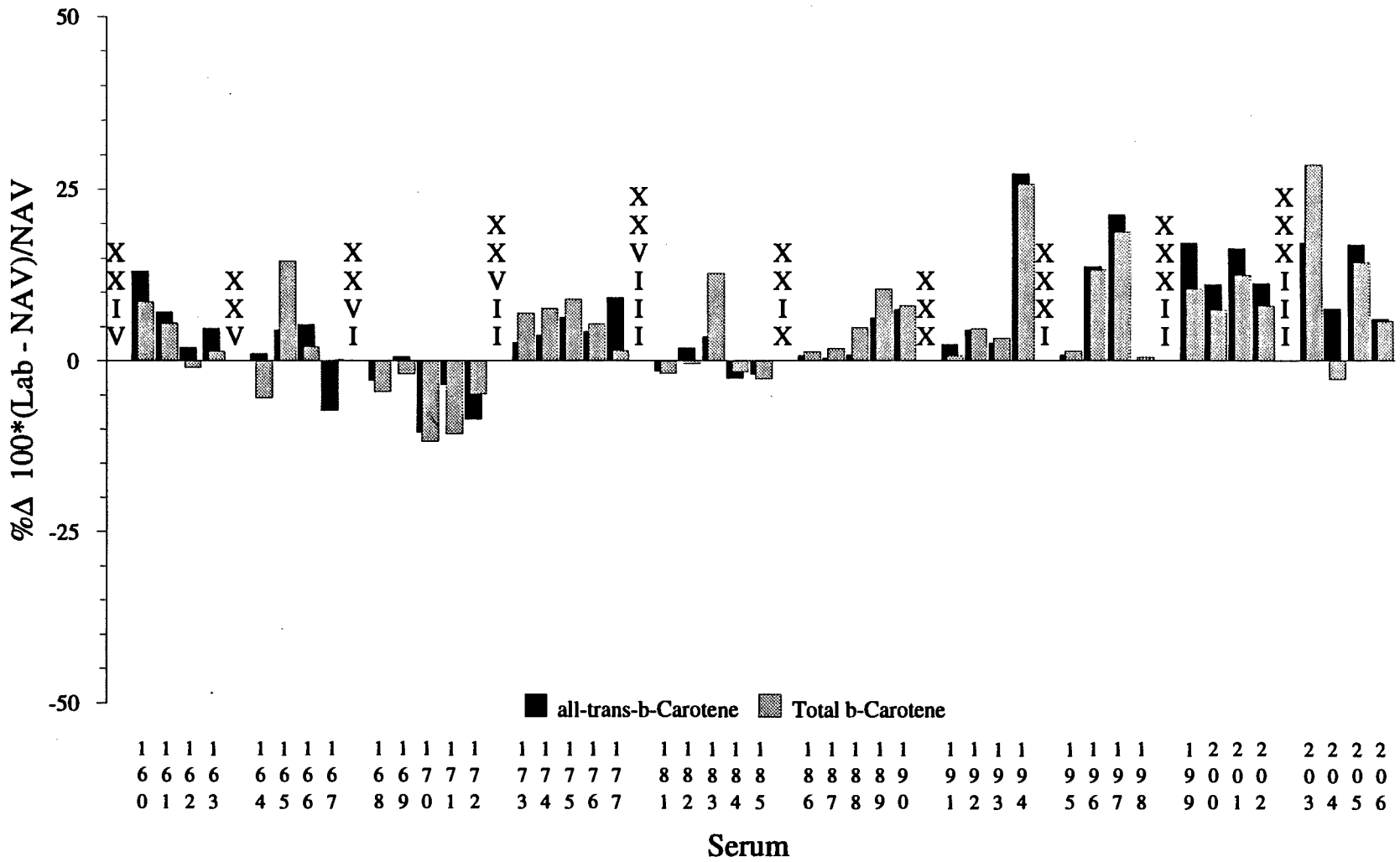
Individualized Round Robin XXXIII Report to: FSV-BA

Tocopherols, %Δ in Round Robin XXIV - XXXIII



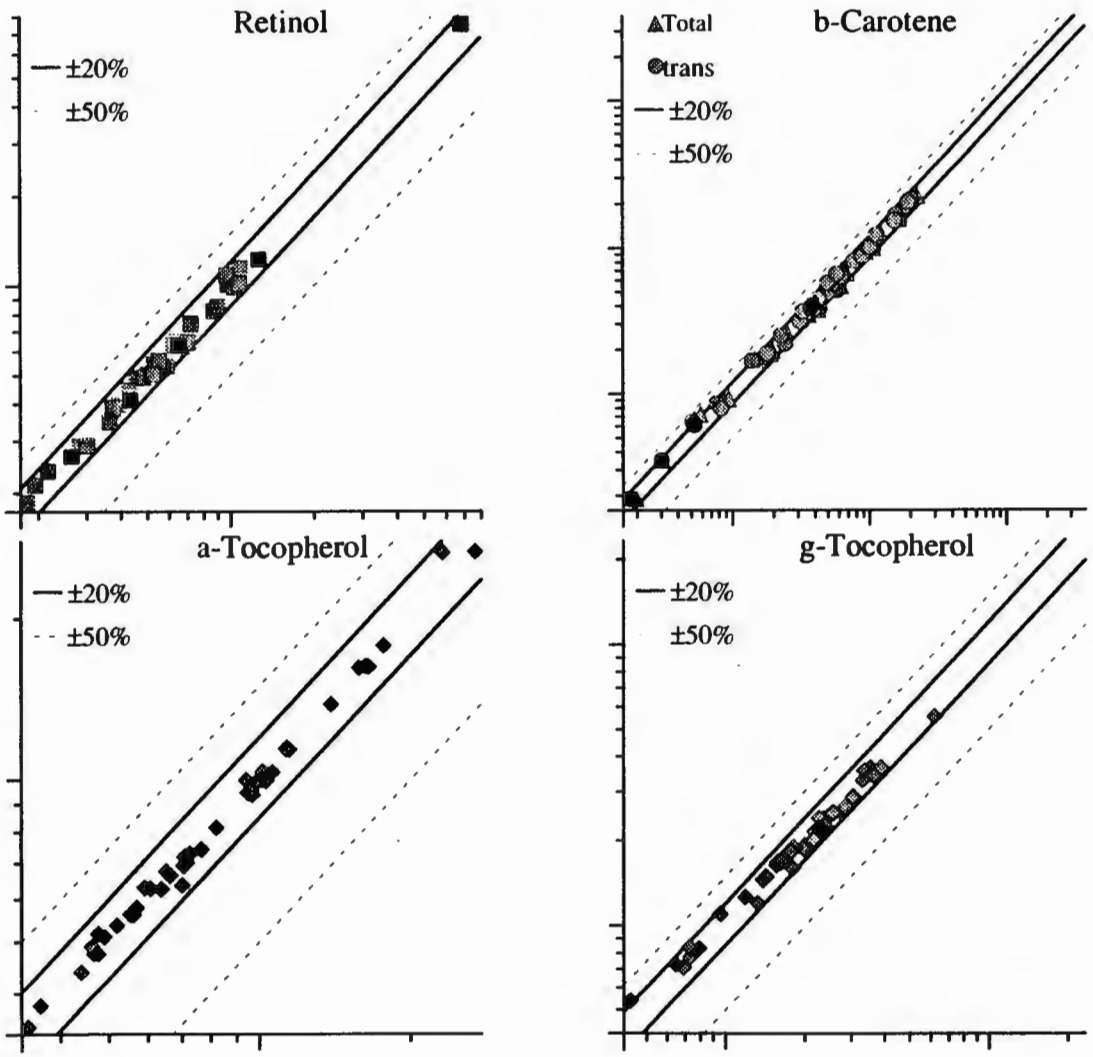
Individualized Round Robin XXXIII Report to: FSV-BA

b-Carotene, %Δ in Round Robin XXIV - XXXIII



Individualized Round Robin XXXIII Report to: FSV-BA

NIST Assigned Values Vs Laboratory Values



Legend

Shaded Symbols: Round Robin XXIV-XXXII
Black Symbols: Round Robin XXXIII

Ordinate: NIST Assigned Value
Abscissa: Your reported concentration

Interpretation

Adequately intercomparable data are within ±20% lines. If you have data scattered outside ±50% lines, your measurement system is not consistent with those of most participating laboratories. If your data are systematically higher or lower than the NAV, your system may be consistent but your results are biased.

If your data show increased scatter at low concentrations, your "limit of quantification" may not be what you think it is.

If there are one or two "wild" outliers, they might be calculation or transcription errors. We would appreciate hearing from you about any such problems.

Individualized Round Robin XXXIII Report to: FSV-BA

Accuracy/Precision Summary

	Ret		aToc		gToc		Total		trans	
	mΔ	vΔ	mΔ	vΔ	mΔ	vΔ	mΔ	vΔ	mΔ	vΔ
XXIV	3	1	1	2	4	3	4	4	7	5
XXV	-2	3	2	4	-2	8	3	8	1	6
XXVI	-7	2	6	3	-2	5	-7	4	-5	5
XXVII	-1	3	0	3	-6	1	6	3	5	3
XXVIII	0	4	1	2	1	4	1	7	0	3
XXIX	4	4	0	3	9	6	5	4	3	3
XXX	9	4	4	7	-3	7	9	12	9	12
XXXI	5	3	-2	5	-1	5	9	9	9	10
XXXII	-4	1	0	1	-2	4	10	2	14	3
XXXIII	-2	2	0	2	11	11	12	13	12	6

Legend

Ret	Retinol
aToc	a-Tocopherol
gToc	g-Tocopherol
Total	Total b-Carotene
trans	all-trans-b-Carotene
mΔ	Mean difference, the average %Δ for all sera of a given RR, where %Δ = 100(Your value - NAV) / NAV
vΔ	Difference variability, one standard deviation of %Δ for all sera of a RR
NAV	NIST Assigned Value, our best estimate of analyte concentration... NAV = (NIST's average-of-averages + Round Robin median) / 2

(Traditional) Performance Criteria

The absolute value of %Δ of every measurement has traditionally been evaluated as follows...

%Δ	Evaluation
0-5%	Exceptional
6-10%	Acceptable
11-20%	Marginal
> 20%	Poor

More representative criteria need to be established, factoring in each serum's analyte level and the analyte distribution in adult human populations. Stay tuned, we're working on it...

Interpretation

Accuracy and precision are separate but kindred aspects of measurement comparison. We estimate accuracy as mΔ, the average %Δ, and precision as vΔ, the standard deviation of %Δ, for all sera of a Round Robin.

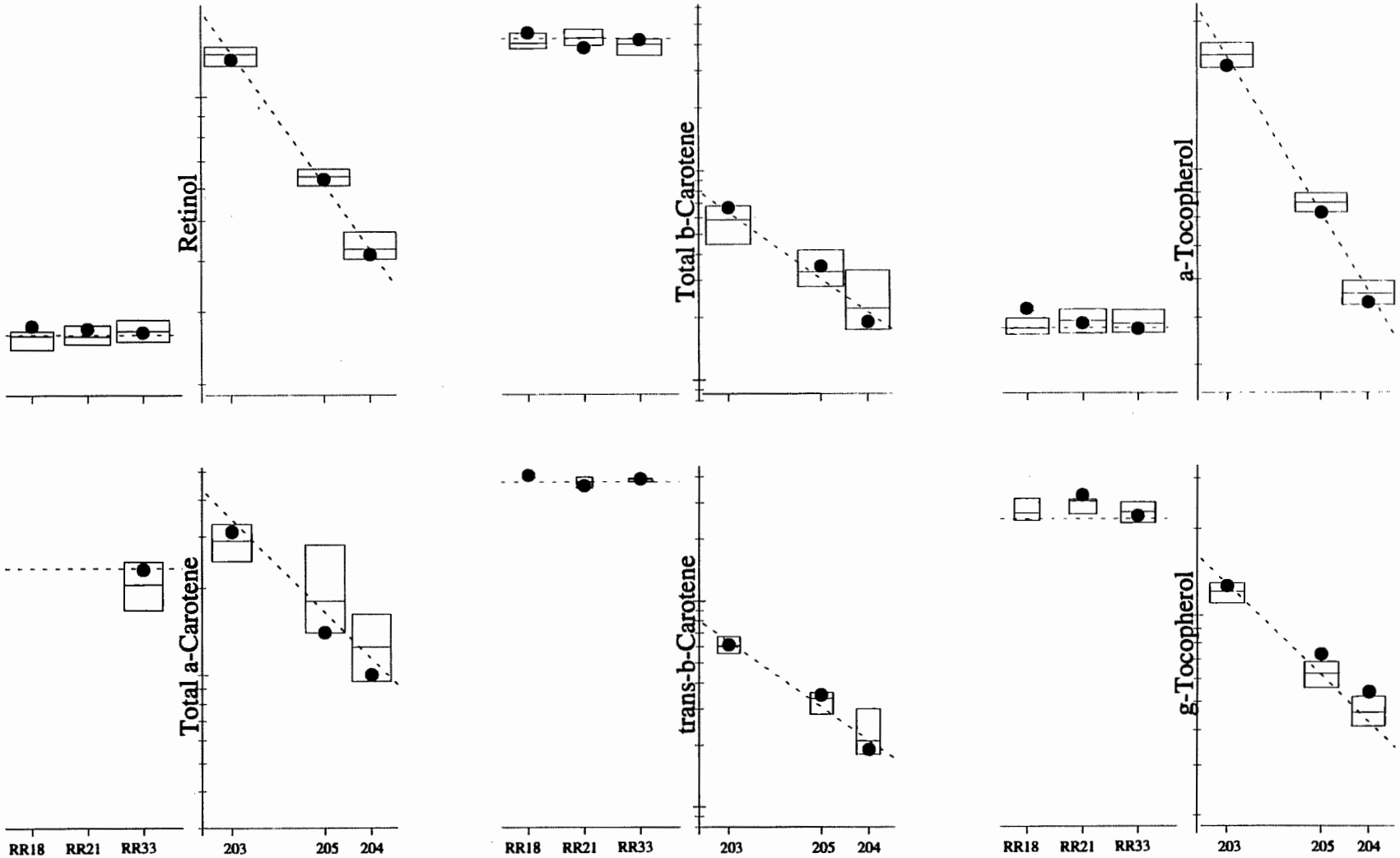
It's best to be accurate and precise (small mΔ, small vΔ)!

Good precision (small vΔ) with poor accuracy (large mΔ) is better than the converse: at least such values are internally consistent and may be relatable to others' values once the relative biases have been determined.

Poor precision (large vΔ) suggests that your measurement system is not in adequate control for the analyte levels examined.

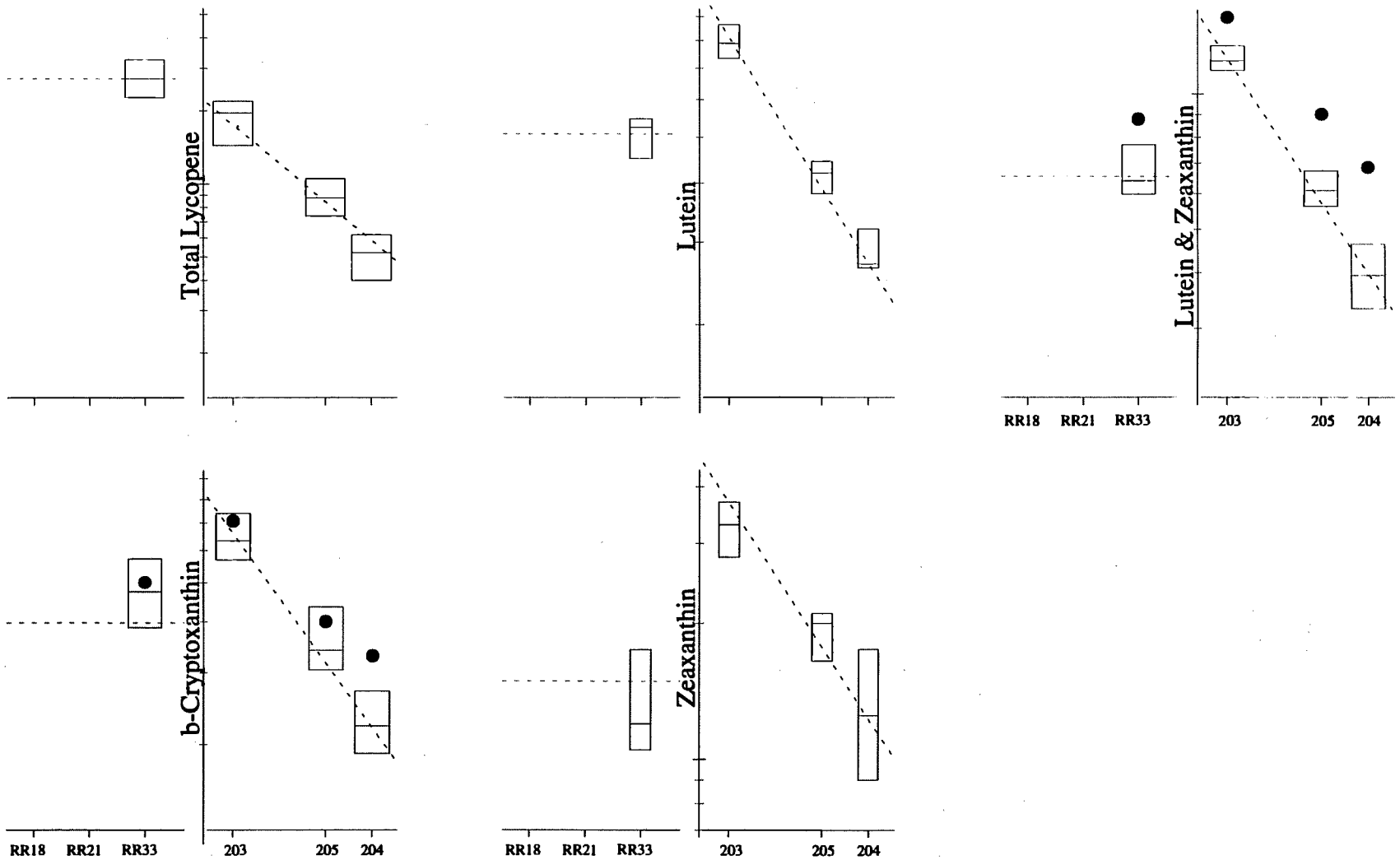
Individualized Round Robin XXXIII Report to: FSV-BA

Comparisons to Known Relationships



Individualized Round Robin XXXIII Report to: FSV-BA

Comparisons to Known Relationships (Continued)



Appendix E. Shipping Package Inserts for RR34

The following two items were included in each package shipped to RR34 participants:

- Cover letter
- Datasheet

The cover letter and datasheet were enclosed in a sealed waterproof bag along with the samples themselves.

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

April 13, 1995

Dear Colleague:

Enclosed is the set of samples for the second round robin exercise (Round Robin XXXIV). You will find one vial of four lyophilized sera samples for analysis along with a form for reporting your results. When reporting your results, please submit one value for each analyte for a given serum sample. If an obtained value is below the detection limit, please indicate this result on the form by using ND *(Not Detected)*. For values not obtained, please leave a blank for the given analyte. Results will be due to NIST by June 16, 1995. Results received two weeks after the due date will not be included in the summary report for this round robin study. Written feedback concerning the study will be provided to you by July 24, 1994.

Samples should be reconstituted with 1.0 mL of HPLC-Grade water or equivalent. We recommend that dissolution be facilitated with 3 to 5 minutes agitation in an ultrasonic bath or at least 30 min at room temperature with intermittent swirling. (CAUTION: Vigorous shaking will cause foaming and possibly interfere with accurate measurement. The rubber stopper contains phthalate esters which will leach into the sample upon intermittent contact of the liquid sample with the stopper. These esters absorb strongly in the UV region and elute very near retinol in most HPLC systems creating analytical problems.) Pipette a known volume of serum from the vial for analysis since the final volume of the reconstituted sample is greater than 1.0 mL. For consistency, we request that laboratories use the following absorptivities (E 1% cm) in ethanol: retinol, 1850 at 325 nm; retinyl palmitate, 975 at 325 nm; α-tocopherol, 75.8 at 292 nm; γ-tocopherol, 91.4 at 298 nm; α-carotene, 2800 at 444 nm; β-carotene, 2560 at 450 nm; lycopene, 3450 at 472 nm.

Please mail or FAX your results for Round Robin XXXIV to:

Micronutrients Measurement Quality Assurance Program
NIST
Bldg. 222, Rm. B208
Gaithersburg, MD 20899
FAX: (301) 977-0685

If you have questions regarding this round robin exercise, please call me at (301) 975-3120 or mail/FAX queries to the above address.

Sincerely,

Jeanice B Thomas

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

Attachment

cc: W. May
S. Wise

NIST

NIST/NCI

Micronutrients Measurement Quality Assurance Program

Round Robin **XXXIV** Results from Laboratory #_____

Analyte	Serum 207	208	209	210	Units*
retinol					
retinyl palmitate					
a-tocopherol					
g-tocopherol					
total b-carotene					
trans-b-carotene					
total cis-b-carotene					
total a-carotene					
total lycopene					
trans-lycopene					
b-cryptoxanthin					
lutein					
zeaxanthin					
lutein&zeaxanthin					

Other Analytes?					

* We prefer results as microgram/milliliter.

Comments?

Mail: NNMMQAP, 222/B208
NIST
Gaithersburg, MD 20899

Please return results on-or-before
June 16, 1995

Fax: 301-977-0685
Email: DLDuewer@enh.NIST.gov

Appendix F. Final Report for RR34

The following 13 pages are the final report for RR34 as provided to all participants:

- Cover letter
- A discussion entitled "Lies, Damn Lies, and Statistics" that:
  - describes the nature of the test samples and details any previous distributions
  - summarizes aspects of the study that we believe may be of interest to the participants
- A "Report of (Meta)Analysis" that details the analysis of NIST measurements

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

July 28, 1995

Dear Colleague:

Enclosed is the summary report of the results for Round Robin XXXIV (Sera 207-210). Included in this report are: a summary of data for all laboratories; a summary of your individual laboratory performance for the past three years; a summary of the interlaboratory accuracy and precision over the same period of time for the measurement of retinol, α- and γ-tocopherol, and β-carotene; a graphical summary of the NIST assigned value vs. your laboratory value for these analytes; and the measurement comparability summary for evaluating your laboratory's performance relative to the other participants'. The NIST assigned values (described in the attached Appendix) are derived from the equally weighted values for the combined results from the analyses performed by NIST and the laboratories that participated in this round robin exercise.

In this round robin exercise, Serum 207, 209, and 210 were previously distributed in Round Robin XXXII as sera 200, 201, and 202, respectively. These serum samples are also the low, medium, and high levels of SRM 968b. Serum 208 (identified as "Control B" during the initial stages of the QA program), was previously distributed as serum 81 in Round Robin XII, serum 94 in Round Robin XIII, and serum 112 in Round Robin XVI.

The overall interlaboratory performance for retinol, γ- and α-tocopherol, and β-carotene for Round Robin XXXIV is comparable to that of Round Robin XXXII. The average estimated coefficient of variation (eCV) is about 8.5% for retinol, 10% for α-tocopherol, 9% for γ-tocopherol, and about 17% for β-carotene. The eCV for β-carotene was about 14% in round robin XXXII. The median values obtained in this round robin exercise are also in agreement with those from the previous round robin exercises and the NIST-assigned values. The results for retinol, retinyl palmitate, γ- and α-tocopherol, total and *trans*-β-carotene, total α-carotene, and lutein in this round robin exercise are comparable to the proposed certified values in SRM 968b (See Figure 1 in Appendix).

The overall interlaboratory measurement comparability for β-cryptoxanthin, total lycopene, and zeaxanthin continues to improve. Laboratories are beginning to report additional values for new analyte(s) of interest (i.e., one laboratory has reported values for co-enzyme Q10). We encourage you to continue to report values for as many quantifiable analytes as possible.

Data for evaluating your laboratory's performance in Round Robin XXXIV are provided in the comparability summary on page 6 of the report. The criteria used to summarize laboratory performance are as follows: results rated **1** (within ± 1 SD of the assigned value) indicate **EXCEPTIONAL** performance, those rated **2** (within ± 2 SD) indicate **ACCEPTABLE** performance, a rating of **3** (within ± 3 SD of the assigned value) is **MARGINAL** performance, and **4** (>3 SD of the assigned value) indicates **POOR** performance relative to the current state-of-the-practice for these measurements.

NIST

SRM 968b: Fat-Soluble Vitamins and Cholesterol in Human Serum, the renewal material for SRM 968a, which we recommend that you use to validate your methods, will be available for purchase in August. If your laboratory is out of SRM 968a, we suggest that you use for method validation control materials that are available in your laboratory or previously distributed (≤1 year ago) round robin samples. If neither of these options is available, please give me a call.

If you have concerns regarding your performance, or are a lab whose performance would be rated **"POOR"** based on the convention stated above, we suggest that you validate your method using SRM 968a or available control materials. If with minor method modifications your measured values do not agree with the assigned values, please contact us for consultation.

Samples for Round Robin XXXV were shipped during the last week of July. Results are due September 15 and feedback to labs is expected by October 20. Feedback for the food round robin (shipped only to those labs that requested these samples) will be mailed within two weeks.

As was discussed at the QA workshop last fall, future workshops will be held on a bi-annual basis. The next workshop will be held in April as a pre-meeting in conjunction with Experimental Biology '96 in Washington, DC. You will be provided with updated information regarding the QA workshop as plans for this meeting are finalized.

We will hold our annual tutorial session at NIST this fall, provided there is sufficient interest. As usual, this session will include a discussion of calibration, sample preparation, and chromatographic techniques for measuring fat-soluble vitamins and carotenoids in serum. We will ask you to indicate your plans for attending this tutorial in a future mailing. If you have further questions or concerns, please contact me at 301/975-3120; fax: 301/977-0685; or e-mail: jbthomas@enh.nist.gov.

Sincerely,

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

cc: W. May

"Lies, Damned Lies, and Statistics"

Mark Twain

The attached NNMMQAP Round Robin XXXIV Report is (ta da!) essentially unchanged from RR XXXIII's. It includes a fairly exhaustive analysis of your results (the "Individualized" report) and a complete listing of everyone's results (the "All Lab" report). Your "Individualized" has the following elements:

Page	Contents
1	Your values, our assigned values, and the %bias between us
2	%Bias barchart for retinol for your last 3 years' results
3	%Bias barchart for α- and γ-tocopherol for your last 3 years' results
4	%Bias barchart for total and *trans*-β-carotene for your last 3 years' results
5	Our assigned value vs. your value scatterplots for retinol, α- and γ-tocopherol, total and *trans*-β-carotene, again for your last 3 years' results
6	Accuracy/Precision Summary, yet again for your last 3 years' results
7	Comparison-to-Prior-Analyses plots for retinol, retinyl palmitate, α- and γ-tocopherol, and total and *trans*-β-carotene
8	Comparison-to-Prior-Analyses plots for total α-carotene, total lycopene, β-cryptoxanthin, lutein, zeaxanthin, and lutein & zeaxanthin

The Comparison-to- Prior-Analyses plots on pages 7 and 8 show your individual results and a box-plot summary of the group's results, plotted against results from prior Round Robins. In detail:

Serum #208 is "Control B." It was distributed as #81 in Round Robin XII, #94 in Round Robin XIII, and #112 in Round Robin XVI. The "true value" lines are averages of the four Round Robin results.

Serum #207, 209, and 210 were distributed as #200, 201, and 202 in Round Robin XXXII. These sera are the "low", "medium", and "high" components of the candidate SRM 968b! The "true value" lines are the proposed certified or informational values for the SRM.

The "All Lab" report has the following elements:

Page	Contents
1-4	A listing of all results for analytes reported by at least two laboratories, plus essential summary statistics.
5a	A list of results for the four analytes reported by only one laboratory.
5b	A Legend for the above two lists.
6	The "Measurement Comparability Summary"

The "Measurement Comparability Summary" (the "score card") provides a four-level score for each laboratory for: retinol, α- and γ-tocopherol, and total and *trans*-β-carotene. The score is based upon the worst-case differences between your reported values and our assigned values, after scaling the values by the total measurement uncertainty. The attached Appendix details our value and uncertainty assignment methods.

If you dig through the Appendix, you may note that we intend to *certify* values for retinol, retinyl palmitate, α- and γ-tocopherol, total and *trans*-β-carotene, total α-carotene, and lutein (also cholesterol, 9-*cis*-, 15-*cis*-, and 13- & 15-*cis*-β-carotene) in SRM 968b. We will also provide *non-certified* values for: δ-tocopherol, *trans*-α-carotene, total and *trans*-lycopene, β-cryptoxanthin, and zeaxanthin. What's the difference between "certified" and "non-certified" analytes? Well...

In addition to your Round Robin results, we analyze SRM-to-be samples using three separate and distinct methodologies. We believe that our "normal method" results for total and *trans*-lycopene, zeaxanthin, and probably β-cryptoxanthin are confounded with one-or-more co-eluting compounds. (Results for δ-tocopherol and *trans*-α-carotene are mostly detector-sensitivity limited.) Judging from the high-bias discord between the Round Robin and our best-guess non-certified values, many of you who report these analytes also are plagued with co-eluting compounds...

We recognize that the levels of these and other analytes in the lyophilized sera are too low to be reliably measured. Resources permitting (it's not so much the $ cost, but the ~0.3L plasma/sample set that's the hassle), we still hope to eventually obtain more interesting "natural" sera. But to really nail down what's going on, we need methods for augmenting plasma with known analytes and their potential co-eluters. Ideas?

Your comments and suggestions are welcome. If you discover any errors in our recording or interpretation of your data, please let us know!

Dave Duewer
Research Chemometrician
DLDuewer@enh.NIST.gov

Margaret Kline
Research Biologist
Kline@enh.NIST.gov

From: David Duewer Date: July 22, 1995

To: Jeanice Brown Thomas, Margaret Kline, Willie E. May, Katherine E. Sharpless
cc: Laurie Locascio, Dennis J. Reeder, Stephen A. Wise

Re: Value Assignment and Analysis of Variance for RR XXXIV Sera: 207 – 210

Purpose: We now compare individual laboratory values to a NIST value-assigned analyte concentration rather than to the interlaboratory median. Analysts NIST1 and NIST3 provide replicate measurements on sufficient samples to permit confident value-assignment. This reports the statistical / chemometrical analysis of their analytical results for the sera distributed in Round Robin XXXIV (RR34).

Background: Four sera were distributed in RR34. Sera 207, 209, and 210 were distributed as sera 200, 201, and 202, respectively, in RR32 (i.e., the low, medium, and high components of SRM 968b). Serum 208 is "Control B" from the archives, having been previously distributed as sera 81 (RR12), 94 (RR13), and 112 (RR16).

NIST1 and NIST3 analyzed all sera using their normal procedures. Both researchers extracted and analyzed two aliquots of three vials, providing duplicate results for a total of six samples of each serum. NIST1 provided quantitative results for: retinol, α-, and γ-tocopherol, total and *trans*-β-carotene, and total α-carotene. NIST3 provided quantitative results for these analytes, plus: retinyl palmitate, δ-tocopherol, *trans*-α-carotene, total and *trans*-lycopene, β-cryptoxanthin, lutein, and zeaxanthin.

Results: Table 1 presents the values and uncertainties assigned to sera 207 – 210. Table 2 compares average results for the two NIST analysts in RR34, the median results from RR34, and the median results from previous RRs. Table 3 lists the linear bias models between the NIST1 and NIST3 data.

The following assumptions and definitions have been used in calculating the various statistics:

1) NIST Assigned Value (NAV): (RR + (N1 + N3) / 2) / 2 (Both NIST analysts, ≥2 RR labs)
(RR + N3) / 2 (One NIST analyst, ≥2 RR labs)
(N3) (One NIST analyst)

where: N1 = mean of NIST1's data,
N3 = mean of NIST3's data, and
RR = median of all RR34 participants' data.

2) NIST Repeatability Standard Deviation (SDrep): "measurement" standard deviation (sd), within-vial differences associated with extraction, chromatography, peak area determination, etc. This "within samples" pooled sd is closely related to the sd of the paired-differences between the replicate extractions / measurements of the samples analyzed at NIST. For the analytes reported by both NIST analytes, any proportional systematic bias between results for the two analysts was removed as part of the combined-data ANOVA. These bias models are listed in Table 3.

3) NIST Heterogeneity Standard Deviation (SDhet): "heterogeneity" sd, vial-to-vial differences associated with preparing and reconstituting the serum samples. This "between samples" pooled sd is closely related to the sd of the mean values of the unique vials analyzed at NIST.

For the analytes reported by both NIST analysts, any systematic proportional bias between results for the two analysts was removed as part of the combined-data ANOVA. These bias models are listed in Table 3. See Eqs. 19.6-4, 19.6-5, and 19.6-6, Mathematical Handbook for Scientists and Engineers, G. and T. Korn (1968), for details of the ANOVA calculations.

4) Interlaboratory Bias Standard Deviation (SDlab): "Laboratory bias" sd, the residual sd from (non-NIST) laboratory-to-laboratory biases after correction for SDrep and SDhet. This assumes that the population of these systematic biases can be approximated as a normal distribution centered at the NAV.

   The observed interlaboratory sd for the results of RR34 is estimated as

   eSD = MAX(0.7421*InterQuartile Range, 0.05*NAV),

   as previously described ("Lies, Damn Lies, and Statistics" report for RR31). This estimate assumes a) that at least 50% of the laboratories that report values for a given analyte are "performing" (they basically know what they're doing), b) that "non-performing" labs are about equally likely to report low values as high values, and c) that standard deviations less than 5% of NAV are unrealistically low.

   The sd among single-sample results reported by different laboratories is a composite of at least three variation sources: the repeatability of any given method, the heterogeneity among supplied samples, and systematic biases among analysts and/or methods and materials. As we have NIST-based estimates for the first two sources (SDrep and SDhet), we can model laboratory differences as the residual of the observed interlaboratory sd (eSD) after subtraction of the measurement and heterogeneity components:

   $$SDlab = MAX(\sqrt{eSD^2 - SDrep^2 - SDhet^2}, 0).$$

   The "MAX" function is required if eSD is smaller than $\sqrt{SDrep^2 + SDhet^2}$.

5) NIST Assigned Uncertainty (NAU)
   Using the same assumptions as described above for SDlab, the "total" expected interlaboratory sd can be described as:

   $$NAU = \sqrt{SDrep^2 + SDhet^2 + SDlab^2}.$$

   Note that this is constrained to be at least as large as the observed interlaboratory sd, eSD.

6) 95% Material, the 95% confidence range on the "true" analyte concentration:

   $$NAV \pm 2\sqrt{SDrep^2 + SDhet^2}.$$

   The value "nq" (for "not quantitative") is used if the uncertainty is large relative to the value.

7) 95% Laboratory, the 95% confidence range on the RR34 reports of analyte concentration:

   NAV ±2 NAU.

   The value "nq" (for "not quantitative") is used if the uncertainty is large relative to the value.

Conclusions: The RR34 median values agree well with the NIST-analyst values and with the median values from recent Round Robin exercises (Table 2). The retinol, α-tocopherol, total and *trans*-β-carotene RR 34 values for serum 208 ("Control B") are remarkably unchanged from the RR12, RR13, and RR16 (1988 – 1989) results.

Figure 1 compares the NAVs and NAUs for sera 207, 209, and 210 to the proposed values for SRM 968b. RR34 results for analytes with *certified* values in SRM 968b agree very well with the proposed certified values and material uncertainty regions (top segment of Figure 1). For all levels of all these analytes: 1) the RR34 NAVs are within the certified 95% confidence region and 2) the RR34 95% interlaboratory uncertainty region is slightly larger than the (NIST-analysis-based) certified 95% confidence region.

Note: The two 95% confidence region definitions reflect philosophical differences between the SRM and QA programs. SRM value/uncertainty statements reflect NIST's state-of-knowledge of the *composition* of the "stuff in the vials". The NAV/NAU in this report reflect the QA community's *ability to measure* the "stuff in the vials".

RR34 results for analytes with *non-certified* values in SRM 968b are biased and generally have large NAUs (bottom segment of Figure 1). While there is qualitative agreement between the "informational" SRM and RR-assigned values, the putative 95% confidence ranges for δ-tocopherol, *trans*-α-carotene, and zeaxanthin do not overlap for some/all levels. The current routine methods for these analytes do not yet provide adequately quantitative information.

The systematic biases between the N1 and N3 data (Table 3) continue to be intriguing. As we now have at least three data sets to intercompare, it may be possible to separate intrinsic differences between the two methods from "accidental" differences between the analyses. Should we invest the resources?

This memo will be attached as the Appendix to RR34's "Lies, Damn Lies, and Statistics" report.

David Lee Duewer
Research Chemist

Table 1
RR XXXIV Analyte Value and Uncertainty Assignments

Sera

	207	208	209	210
Retinol				
NAV	0.28	0.43	0.52	0.88
SDrep	0.01	0.01	0.01	0.02
SDhet	0.02	0.02	0.02	0.01
SDlab	0.02	0.04	0.04	0.07
NAU	0.03 (10%)	0.05 (10%)	0.05 (8%)	0.08 (8%)
95% Material	0.24 — 0.32	0.38 — 0.47	0.47 — 0.56	0.83 — 0.94
95% Laboratory	0.22 — 0.34	0.34 — 0.52	0.43 — 0.61	0.74 — 1.03
Retinyl palmitate				
NAV	0.088	- 0.030	0.177	0.263
SDrep	0.016		0.003	0.001
SDhet	0.021	0.018	0.014	0.024
NAU	0.027 (30%)	0.018 (59%)	0.015 (8%)	0.024 (9%)
95% Material	0.050 — 0.120		0.160 — 0.190	0.260 — 0.270
95% Laboratory	0.030 — 0.150	nq — 0.070	0.140 — 0.210	0.210 — 0.320
a-Tocopherol				
NAV	7.0	6.0	10.1	17.7
SDrep	0.2	0.2	0.2	0.3
SDhet	0.2	0.1	0.3	0.2
SDlab	0.9	0.9	0.7	0.9
NAU	1.0 (13%)	0.9 (15%)	0.8 (8%)	1.0 (6%)
95% Material	6.5 — 7.6	5.6 — 6.4	9.3 — 10.8	17.0 — 18.5
95% Laboratory	5.1 — 9.0	4.2 — 7.8	8.5 — 11.7	15.7 — 19.7
g-Tocopherol				
NAV	1.67	2.93	2.38	3.76
SDrep	0.08	0.12	0.11	0.15
SDhet	0.07	0.21	0.14	0.15
SDlab	0.21	0.22	0.17	0.15
NAU	0.24 (14%)	0.33 (11%)	0.25 (10%)	0.26 (7%)
95% Material	1.45 — 1.89	2.44 — 3.42	2.02 — 2.74	3.33 — 4.19
95% Laboratory	1.18 — 2.15	2.27 — 3.59	1.88 — 2.87	3.24 — 4.28
d-Tocopherol				
NAV	0.130	0.181	0.181	0.186
SDrep	0.009	0.011	0.018	0.008
SDhet	0.004	0.018	0.014	0.013
NAU	0.011 (8%)	0.022 (12%)	0.023 (13%)	0.016 (8%)
95% Material	0.100 — 0.150	0.130 — 0.230	0.130 — 0.230	0.150 — 0.220

Table 1
RR XXXIV Analyte Value and Uncertainty Assignments

	Sera			
	207	208	209	210
Total b-Carotene				
NAV	0.252	0.194	0.635	1.239
SDrep	0.013	0.017	0.024	0.058
SDhet	0.015	0.018	0.011	0.038
SDlab	0.050	0.033	0.099	0.147
NAU	0.054 (21%)	0.042 (21%)	0.103 (16%)	0.163 (13%)
95% Material	0.213 — 0.292	0.144 — 0.244	0.582 — 0.687	1.099 — 1.378
95% Laboratory	0.145 — 0.360	0.111 — 0.277	0.430 — 0.840	0.913 — 1.564
trans-b-Carotene				
NAV	0.241	0.175	0.584	1.165
SDrep	0.012	0.018	0.023	0.039
SDhet	0.015	0.015	0.012	0.034
SDlab	0.009	0.014	0.045	0.048
NAU	0.022 (9%)	0.028 (16%)	0.052 (9%)	0.071 (6%)
95% Material	0.202 — 0.279	0.127 — 0.222	0.532 — 0.636	1.061 — 1.268
95% Laboratory	0.197 — 0.284	0.120 — 0.230	0.479 — 0.688	1.023 — 1.306
Total a-Carotene				
NAV	0.022	0.017	0.034	0.047
SDrep	0.003	0.005	0.003	0.006
SDhet	0.005	0.006	0.006	0.002
SDlab	0.000	0.000	0.009	0.012
NAU	0.006 (26%)	0.008 (43%)	0.012 (34%)	0.014 (29%)
95% Material	0.010 — 0.034	0.002 — 0.033	0.020 — 0.049	0.034 — 0.059
95% Laboratory	0.010 — 0.034	0.002 — 0.033	0.011 — 0.058	0.019 — 0.075
trans-a-Carotene				
NAV	0.022	0.017	0.040	0.039
SDrep	0.003	0.002	0.003	0.003
SDhet	0.002	0.002	0.002	0.002
NAU	0.004 (15%)	0.003 (17%)	0.004 (8%)	0.004 (8%)
95% Material	0.015 — 0.030	0.010 — 0.023	0.033 — 0.048	0.032 — 0.046
Total Lycopene				
NAV	0.201	0.229	0.301	0.345
SDrep	0.034	0.017	0.012	0.027
SDhet	0.018	0.017	0.026	0.008
SDlab	0.033	0.042	0.052	0.078
NAU	0.051 (25%)	0.049 (21%)	0.060 (20%)	0.084 (24%)
95% Material	0.124 — 0.278	0.181 — 0.278	0.243 — 0.359	0.287 — 0.402
95% Laboratory	0.100 — 0.302	0.131 — 0.327	0.182 — 0.420	0.178 — 0.511

Table 1
RR XXXIV Analyte Value and Uncertainty Assignments

	Sera			
	207	208	209	210
trans-Lycopene				
NAV	0.138	0.178	0.196	0.215
SDrep	0.006	0.004	0.002	0.006
SDhet	0.005	0.003	0.003	0.002
SDlab	0.129	0.156	0.151	0.152
NAU	0.130 (94%)	0.157 (88%)	0.152 (77%)	0.152 (71%)
95% Material	0.123 — 0.153	0.167 — 0.189	0.189 — 0.204	0.203 — 0.227
95% Laboratory	nq — 0.398	nq — 0.492	nq — 0.500	nq — 0.519
b-Cryptoxanthin				
NAV	0.029	0.040	0.040	0.039
SDrep	0.002	0.003	0.002	0.002
SDhet	0.002	0.003	0.001	0.001
SDlab	0.012	0.014	0.009	0.010
NAU	0.012 (41%)	0.015 (36%)	0.009 (23%)	0.010 (25%)
95% Material	0.023 — 0.035	0.031 — 0.048	0.036 — 0.044	0.034 — 0.044
95% Laboratory	0.005 — 0.054	0.011 — 0.069	0.021 — 0.058	0.019 — 0.059
Lutein				
NAV	0.061	0.074	0.064	0.037
SDrep	0.002	0.004	0.000	0.001
SDhet	0.002	0.002	0.001	0.002
SDlab	0.011	0.011	0.011	0.008
NAU	0.011 (18%)	0.012 (15%)	0.011 (17%)	0.009 (22%)
95% Material	0.054 — 0.067	0.066 — 0.082	0.061 — 0.067	0.032 — 0.042
95% Laboratory	0.038 — 0.083	0.051 — 0.097	0.042 — 0.086	0.020 — 0.054
Zeaxanthin				
NAV	0.023	0.041	0.030	0.019
SDrep	0.002	0.001	0.001	0.002
SDhet	0.001	0.002	0.001	0.004
SDlab	0.002	0.008	0.003	0.000
NAU	0.004 (14%)	0.009 (22%)	0.003 (10%)	0.005 (24%)
95% Material	0.018 — 0.028	0.035 — 0.046	0.026 — 0.033	0.009 — 0.029
95% Laboratory	0.016 — 0.030	0.023 — 0.059	0.023 — 0.036	0.009 — 0.029
Lutein&Zeaxanthin				
NAV	0.083	0.124	0.092	0.056
SDrep	0.004	0.002	0.001	0.003
SDhet	0.002	0.004	0.001	0.005
SDlab	0.015	0.023	0.021	0.010
NAU	0.016 (19%)	0.024 (19%)	0.022 (23%)	0.012 (20%)
95% Material	0.074 — 0.092	0.114 — 0.133	0.089 — 0.095	0.044 — 0.068
95% Laboratory	0.050 — 0.115	0.077 — 0.171	0.049 — 0.135	0.033 — 0.079

Table 2
Summary of Data Used in Assigning Values

	207				209				210			
	N1	N3	RR34	RR32	N1	N3	RR34	RR32	N1	N3	RR34	RR32
Retinol	0.22	0.30	0.30	0.30	0.51	0.51	0.52	0.51	0.88	0.88	0.89	0.88
Retinyl palmitate		0.08	0.09	0.10		0.16	0.20	0.18		0.24	0.28	0.26
a-Tocopherol	7.27	6.92	7.00	7.15	10.60	9.97	9.83	10.00	18.07	17.56	17.67	17.80
g-Tocopherol	1.65	1.68	1.67	1.69	2.34	2.39	2.39	2.41	3.60	3.80	3.83	3.76
d-Tocopherol		0.11	0.15			0.15	0.21			0.23	0.14	
Total b-Carotene	0.264	0.263	0.241	0.248	0.631	0.658	0.625	0.619	1.204	1.270	1.240	1.232
trans-b-Carotene	0.222	0.230	0.255	0.231	0.535	0.594	0.603	0.565	1.066	1.150	1.221	1.117
Total a-Carotene	0.023	0.027	0.019	0.020	0.035	0.044	0.029	0.031	0.059	0.056	0.036	0.039
trans-a-Carotene		0.018	0.026			0.030	0.051			0.033	0.045	
Total Lycopene		0.221	0.181	0.179	- -	0.325	0.277	0.268		0.358	0.331	0.314
trans-Lycopene		0.084	0.193			0.139	0.254			0.148	0.282	
b-Cryptoxanthin		0.026	0.033	0.041		0.033	0.047	0.053		0.033	0.045	0.049
Lutein		0.055	0.066	0.064		0.060	0.068	0.064		0.037	0.037	0.036
Zeaxanthin		0.024	0.023	0.020		0.032	0.027	0.028		0.019	0.020	0.014
Lutein&Zeaxanthin		0.079	0.087	0.094		0.092	0.093	0.099		0.056	0.056	0.061

	208					
	N1	N3	RR34	RR16	RR13	RR12
Retinol	0.41	0.42	0.44	0.43	0.43	0.44
Retinyl palmitate		*nq*	0.03			
a-Tocopherol	6.28	5.91	5.87	6.02	5.90	5.98
g-Tocopherol	2.53	3.06	3.06			
d-Tocopherol		0.17	0.20			
Total b-Carotene	0.208	0.198	0.185	0.205	0.20	0.207
trans-b-Carotene	0.155	0.173	0.185	0.196		0.203
Total a-Carotene	*nd*	0.022	0.013			
trans-a-Carotene		0.014	0.019			
Total Lycopene		0.242	0.216			
trans-Lycopene		0.112	0.245			
b-Cryptoxanthin		0.032	0.047			
Lutein		0.075	0.073			
Zeaxanthin		0.048	0.034			
Lutein&Zeaxanthin		0.123	0.125			

Table 3
Summary of Linear Calibration of N1 to N3 Average Values

Bias Models

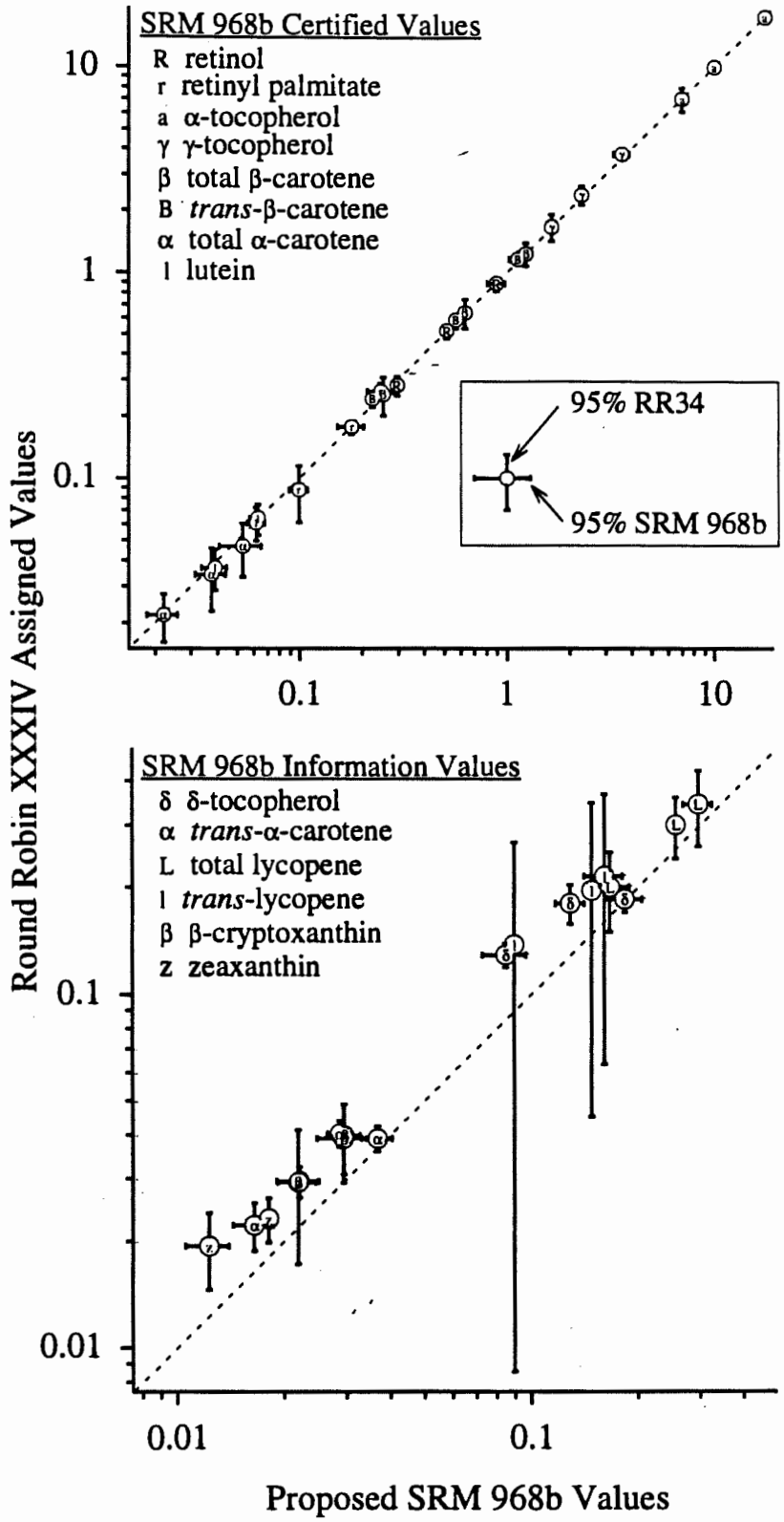
N1 = Intercept + Slope*N3 ± SDreg

	Slope	Inter	SDreg	CVreg	R2	F	df
Retinol	0.92 (0.06)	0.06 (0.04)	0.03	3 – 10%	0.990	207	2
a-Tocopherol	0.99 (0.01)	-0.3 (0.2)	0.1	1 – 2%	1.000	4604	2
g-Tocopherol	1.08 (0.04)	0	0.2	6 – 13%	0.942	49	3
Total b-Carotene	1.07 (0.00)	-0.023 (0.002)	0.003	0 – 1%	1.000	104034	2
trans-b-Carotene	1.08 (0.01)	0	0.011	1 – 7%	0.999	4636	3
Total a-Carotene	0.76 (0.24)	0.012 (0.010)	0.006	11 – 29%	0.912	10	1

Legend

Slope: Estimate of slope ("standard error" of estimate)
Inter: Estimate of intercept ("standard error" of estimate)
SDreg: "Standard error" (sd of residuals) of N1 regression onto N3 values
CVreg: 100*SDreg/(maximum N3 value) – 100*SDreg/(minimum N3 value)
R2: Square of the correlation between N1 and N3
F: F statistic (= variance accounted for by the model / variance not accounted for)
df: Degrees of freedom (= number of data - number of parameters in model)

Figure 1
Comparison of SRM 968b to RR34 Results



Appendix G. "All-Lab Report" for RR34

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

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

The data summary in the "All-Lab Report" has been altered to ensure confidentiality of identification codes assigned to laboratories. The only attributed results are those reported by NIST. The NIST results are not used in the assessment of the consensus summary results of the study.

Round Robin XXXIV Laboratory Results

Values in µg/mL

	Retinol				Retinyl Palmitate				α-Tocopherol				γ-Tocopherol				δ-Tocopherol			
Lab	207	208	209	210	207	208	209	210	207	208	209	210	207	208	209	210	207	208	209	210
FSV-BA	0.299	0.459	0.517	0.891	0.110	0.032	0.199	0.275	6.80	5.75	9.53	17.3	1.75	3.09	2.39	3.87				
FSV-BD	0.264	0.416	0.467	0.832					5.60	4.97	8.68	16.5								
FSV-BE	0.266	0.417	0.489	0.838					6.81	5.72	9.83	17.5	1.74	3.09	2.45	3.97				
FSV-BG	0.290	0.420	0.540	0.920	0.074	0.021	0.181	0.276	6.23	5.29	9.24	16.3								
FSV-BH	0.299	0.385	0.499	0.847	0.041	*nd*	0.196	0.287	7.18	6.21	10.30	18.1	1.77	3.21	2.46	3.95				
FSV-BI	0.283	0.432	0.481	0.839	0.112	*nd*	0.207	0.304	7.00	6.12	10.07	17.0	1.65	3.04	2.35	3.74				
FSV-BJ	0.296	0.451	0.547	0.936	0.091	0.056	0.191	0.253	7.68	6.49	10.22	17.8	1.96	3.37	2.56	4.02				
FSV-BK	0.318	0.489	0.567	0.900					7.41	6.44	9.88	17.9								
FSV-BL	0.401	0.573	0.659	1.003					8.18	6.85	11.41	20.7								
FSV-BM	0.306	0.395	0.532	0.895					7.45	6.53	10.16	18.1								
FSV-BN	0.358	0.454	0.617	1.103	0.085	*nd*	0.224	0.377	7.57	6.81	10.79	20.7	1.41	2.68	2.03	3.60				
FSV-BO	0.273	0.367	0.493	0.854					6.55	5.94	9.48	17.5								
FSV-BP	0.322	0.484	0.565	0.939					8.09	6.34	10.18	17.6								
FSV-BQ	0.302	0.429	0.540	0.822					5.80	5.70	9.30	14.9								
FSV-BR	0.294	0.458	0.503	0.812																
FSV-BS																				
FSV-BT	0.378	0.501	0.496	0.856	0.108	0.019	0.186	0.269	7.52	7.42	10.66	17.7	1.66	3.08	2.44	3.83	0.203	0.271	0.297	0.178
FSV-BU	0.277	0.408	0.478	0.844					6.28	5.56	9.40	16.2	1.45	3.03	2.26	3.66				
FSV-BV	0.283		0.453	0.802					6.81		9.86	16.9	1.44		2.26	3.77				
FSV-BY	0.302	0.420	0.515	0.872	0.093	*nd*	0.175	0.279	7.04	5.53	9.87	17.8	1.69	2.66	2.34	3.83				
FSV-BZ									5.61	4.32	8.98	19.7	2.21	3.43	2.92	4.45				
FSV-CB	0.461	0.620	0.657	1.191					7.72	5.74	8.96	17.1								
FSV-CD	0.299	0.445	0.511	0.929	0.086	0.040	0.168	0.275	8.50	6.93	11.18	21.0	1.57	2.74	2.09	3.38				
FSV-CH	0.284	0.433	0.504	0.851					5.84	4.84	8.04	14.4	1.45	2.80	2.18	3.32				
FSV-CJ	0.357	0.465	0.531	0.910					7.77	6.45	10.00	17.5								
FSV-CK	0.358	0.498	0.566	1.024					8.52	6.62	10.91	19.6	2.13	3.56	2.74	4.23				
FSV-CM									6.86	5.87	9.99	18.6								
FSV-CN	0.299		0.540	0.799					6.85		10.62	17.5	1.46		2.25	3.89				
FSV-CQ	0.278	0.464	0.492	0.886					6.08	5.21	9.39	18.2								
FSV-CR	0.330	0.560	0.630	0.980					7.50	6.30	9.70	15.0								
FSV-CT	0.272	0.407	0.480	0.889					6.96	5.93	9.35	16.9								
FSV-CU	0.280	0.439	0.496	0.822	0.143	0.056	0.194	0.318	6.97	6.16	10.48	18.1								
FSV-CV	0.289	0.439	0.551	0.903					7.04	5.20	10.50	17.9								
FSV-CX	0.280	0.410	0.490	0.850	0.120	0.030	0.210	0.310	6.67	5.77	9.69	17.3	1.58	2.90	2.39	3.83				
FSV-CY	0.280	0.450	0.530	0.900					7.23	5.98	9.31	19.2								
FSV-DA	0.309	0.451	0.513	0.934	0.092	0.018	0.203	0.288	7.22	6.52	10.10	17.7	1.73	3.31	2.50	3.71	0.100	0.120	0.120	0.110
FSV-DB	0.320	0.500	0.560	0.930					6.18	4.97	9.11	15.8								
FSV-DJ	0.320	0.460	0.550	0.930					7.16	5.68	11.39	20.2								
FSV-DK	0.315	0.475	0.575	1.030	0.026	*nd*	0.072	0.089	7.90	7.50	10.10	17.0								
FSV-DL	0.312	0.365	0.396	0.832					6.85	5.31	8.39	18.0	1.34	2.03	1.70	2.91				
FSV-DM	0.309	0.485	0.548	0.934					6.83	5.76	9.56	16.9								
FSV-DP	0.300	0.441	0.535	0.900																
FSV-DS	0.250	0.360	0.420	0.700					6.01	4.76	7.47	13.4								
FSV-DX	0.383	0.541	0.642	1.109					9.62	7.89	12.97	23.1								
FSV-EA	0.283	0.374	0.455	0.789					8.10	6.80	11.30	19.2	1.50	2.80	2.10	3.70				
FSV-EH	0.310		0.550	0.940					7.70		10.90	18.7	1.77		2.58	3.92	*nd*		0.150	0.182
FSV-EK	0.417	0.578	0.730	1.060					9.29	7.98	11.70	23.3	2.65	2.02	2.48	4.50				
FSV-EL	0.280	0.430	0.540	0.840																
FSV-FC	0.304	0.438	0.519	0.863					7.33	6.31	10.64	18.1	1.78	3.21	2.54	3.94				
FSV-FD	0.270	0.500	0.480	1.030					6.43	5.26	9.81	17.4								
FSV-FP	0.420	0.652	0.864	1.652					5.13	4.02	6.85	13.2	1.12	2.02	1.55	2.68				
n	48	45	48	48	13	8	13	13	47	44	47	47	23	20	23	23	2	2	3	3
Min	0.250	0.360	0.396	0.700	0.026	0.018	0.072	0.089	5.13	4.02	6.85	13.2	1.12	2.02	1.55	2.68	0.10	0.12	0.12	0.11
Median	0.300	0.450	0.531	0.898	0.092	0.031	0.194	0.279	7.04	5.94	9.88	17.7	1.66	3.03	2.39	3.83	0.15	0.20	0.15	0.18
Max	0.461	0.652	0.864	1.652	0.143	0.056	0.224	0.377	9.62	7.98	12.97	23.3	2.65	3.56	2.92	4.50	0.20	0.27	0.30	0.18
eSD	0.029	0.050	0.052	0.073	0.027	0.016	0.019	0.014	0.79	0.84	0.86	1.1	0.24	0.38	0.20	0.19				
eCV	10	11	10	8	29	52	10	5	11	14	9	6	14	13	8	5				
NISTa	0.224	0.407	0.511	0.876					7.27	6.28	10.60	18.1	1.65	2.53	2.34	3.60				
NISTb	0.295	0.423	0.506	0.885	0.083	*nd*	0.158	0.242	6.92	5.91	9.97	17.6	1.68	3.06	2.39	3.80	0.108	0.167	0.154	0.228
NAV	0.280	0.432	0.519	0.890	0.092	0.031	0.194	0.279	7.07	6.02	10.08	17.7	1.67	2.92	2.38	3.76				
NAU	0.059	0.054	0.052	0.077	0.027	0.016	0.019	0.014	0.80	0.77	0.89	1.4	0.21	0.43	0.25	0.38				

G2

Round Robin XXXIV Laboratory Results

Values in µg/mL

Lab	Total β-Carotene				trans-β-Carotene				Total cis-β-Carotene			
	207	208	209	210	207	208	209	210	207	208	209	210
FSV-BA	0.267	0.213	0.66	1.29	0.252	0.200	0.634	1.24	0.015	0.013	0.025	0.050
FSV-BD												
FSV-BE	0.243	0.194	0.70	1.42								
FSV-BG	0.226	0.164	0.57	1.17								
FSV-BH	0.234	0.194	0.65	1.31	0.221	0.183	0.606	1.22	0.013	0.011	0.041	0.082
FSV-BI	0.232	0.182	0.61	1.15								
FSV-BJ	0.291	0.206	0.72	1.31								
FSV-BK												
FSV-BL												
FSV-BM												
FSV-BN	0.279	0.190	0.63	1.16	0.239	0.175	0.540	1.03	0.101	0.077	0.142	0.186
FSV-BO	0.222	0.154	0.50	0.97								
FSV-BP	0.168	0.115	0.46	0.90								
FSV-BQ	0.316	0.254	0.77	1.51								
FSV-BR												
FSV-BS	0.291	0.187	0.64	1.29	0.258	0.169	0.582	1.22	0.032	0.018	0.057	0.069
FSV-BT	0.259	0.196	0.64	1.25	0.242	0.185	0.599	1.16	0.017	0.011	0.041	0.090
FSV-BU	0.212	0.155	0.70	1.06								
FSV-BV	>0.22		>0.66	>1.077	0.220		0.660	1.08				
FSV-BY	0.264	0.172	0.62	1.21								
FSV-BZ	0.241	0.146	0.24	0.42								
FSV-CB												
FSV-CD	0.234	0.170	0.55	1.17								
FSV-CH	0.193	0.161	0.54	1.03								
FSV-CJ	0.189	0.161	0.56	1.12								
FSV-CK	0.203	0.233	0.74	1.49								
FSV-CM												
FSV-CN	>0.238		>0.636	>1.164	0.238		0.636	1.16				
FSV-CQ	0.338	0.262	0.85	1.48								
FSV-CR												
FSV-CT	0.232	0.165	0.61	1.22								
FSV-CU	0.314	0.222	0.65	1.29	0.263	0.185	0.599	1.22	*0.051*	*0.037*	*0.046*	*0.069*
FSV-CV	0.226	0.131	0.57	1.05								
FSV-CX	0.230	0.160	0.52	1.04								
FSV-CY	0.220	0.180	0.61	1.24								
FSV-DA	0.292	0.251	0.79	1.54	0.272	0.233	0.727	1.41	0.020	0.018	0.058	0.130
FSV-DB	0.140	0.130	0.55	1.24								
FSV-DJ												
FSV-DK	0.326	0.144	0.44									
FSV-DL	0.193	0.125	0.44	1.04								
FSV-DM	0.320	0.232	0.85	1.60								
FSV-DP												
FSV-DS	0.216	0.209	0.57	1.14								
FSV-DX	>0.337	>0.24	>0.869	>1.714	0.337	0.240	0.869	1.71				
FSV-EA	0.262	0.178	0.61	1.20	*0.237*	*0.166*	*0.581*	*1.16*	0.025	0.012	0.029	0.041
FSV-EH	0.262		0.64	1.21	0.236		0.573	1.09				
FSV-EK	0.248	0.179	0.68	1.43								
FSV-EL												
FSV-FC	0.236	0.185	0.70	1.34								
FSV-FD	0.210	0.140	0.59	1.06								
FSV-FP	0.342	0.234	0.82	1.80								
n	37	36	37	36	12	9	12	12	8	8	8	8
Min	0.140	0.115	0.24	0.42	0.220	0.166	0.540	1.03	0.013	0.011	0.025	0.041
Median	0.236	0.180	0.62	1.22	0.241	0.185	0.603	1.19	0.023	0.016	0.044	0.076
Max	0.342	0.262	0.85	1.80	0.337	0.240	0.869	1.71	0.101	0.077	0.142	0.186
eSD	0.039	0.037	0.10	0.16	0.022	0.022	0.045	0.06	0.013	0.006	0.021	0.030
eCV	16	20	16	13	9	12	8	5	56	38	48	39
NISTa	0.264	0.208	0.63	1.20	0.222	0.155	0.535	1.07	*0.042*	*0.053*	*0.097*	*0.138*
NISTb	0.263	0.198	0.66	1.27	0.230	0.173	0.594	1.15	*0.033*	*0.025*	*0.064*	*0.120*
NAV	0.249	0.191	0.63	1.23	0.239	0.175	0.582	1.16	0.030	0.027	0.062	0.103
NAU	0.051	0.039	0.11	0.17	0.034	0.029	0.067	0.14	0.019	0.026	0.039	0.046

G3

Round Robin XXXIV Laboratory Results

Values in µg/mL

Lab	Total α-Carotene				trans-α-Carotene				Total Lycopene				trans-Lycopene				β-Cryptoxanthin			
	207	208	209	210	207	208	209	210	207	208	209	210	207	208	209	210	207	208	209	210
FSV-BA	0.025	0.013	0.036	0.047									0.113	0.159	0.177	0.188	0.040	0.054	0.049	0.048
FSV-BD																				
FSV-BE																				
FSV-BG	0.015	0.010	0.025	0.034					0.200	0.213	0.294	0.357								
FSV-BH	0.018	0.011	0.032	0.048					0.181	0.252	0.304	0.344					0.038	0.052	0.052	0.050
FSV-BI	0.022	0.013	0.030	0.035					0.117	0.163	0.191	0.208					0.032	0.047	0.040	0.038
FSV-BJ	0.034	0.026	0.057	0.079					0.141	0.190	0.232	0.240								
FSV-BK																				
FSV-BL																				
FSV-BM																				
FSV-BN	0.047	0.019	0.050	0.059					0.217	0.246	0.314	0.348	0.108	0.139	0.152	0.171	0.047	0.060	0.050	0.056
FSV-BO	0.017	0.009	0.022	0.032					0.190	0.252	0.336	0.432					0.024	0.033	0.034	0.031
FSV-BP									0.143	0.088	0.149	0.153					0.010	0.023	0.019	0.016
FSV-BQ																				
FSV-BR																				
FSV-BS	0.155	0.157	0.160	0.130					0.623	0.788	0.906	1.355	0.504	0.620	0.613	0.644	*nd*	*nd*	*nd*	*nd*
FSV-BT	0.020	0.015	0.030	0.037					0.186	0.216	0.277	0.308	0.144	0.178	0.216	0.241	0.034	0.044	0.043	0.043
FSV-BU	0.013	0.009	0.026	0.035					0.166	0.214	0.265	0.280					0.021	0.028	0.038	0.022
FSV-BV	0.029		0.046	0.051					0.280		*nd*	0.347					0.018		0.038	0.021
FSV-BY	0.019	0.010	0.043	0.044					0.180	0.185	0.276	0.311					0.041	0.050	0.050	0.051
FSV-BZ	0.020	0.030	0.020	0.017																
FSV-CB																				
FSV-CD	0.020	0.009	0.026	0.039					0.160	0.200	0.239	0.286					0.017	0.022	0.022	0.022
FSV-CH	0.011	0.011	0.021	0.032					0.118	0.183	0.215	0.231								
FSV-CJ	0.016	0.012	0.029	0.035					0.168	0.231	0.299	0.345					0.033	0.047	0.051	0.057
FSV-CK	0.021	0.023	0.054	0.073					0.191	0.349	0.460	0.587					0.043	0.065	0.065	0.078
FSV-CM																				
FSV-CN	*nd*		*nd*	*nd*					0.160		*nd*	0.337								
FSV-CQ																				
FSV-CR																				
FSV-CT																				
FSV-CU																				
FSV-CV																				
FSV-CX	0.020	0.010	0.010	0.020					0.150	0.190	0.240	0.260					0.020	0.030	0.030	0.040
FSV-CY																				
FSV-DA	0.018	0.006	0.018	0.042					0.183	0.224	0.215	0.331	0.096	0.127	0.113	0.168	0.030	0.036	0.047	0.045
FSV-DB																				
FSV-DJ																				
FSV-DK	0.010	0.003	0.020																	
FSV-DL	0.027	0.013	0.035	0.057					0.166	0.156	0.238	0.319					0.024	0.026	0.028	0.032
FSV-DM	0.015	0.016	0.027	0.035					0.145	0.199	0.252	0.267								
FSV-DP																				
FSV-DS																				
FSV-DX					0.026	0.019	0.051	0.045	0.214	0.246	0.344	0.378								
FSV-EA									0.219	0.255	0.316	0.373								
FSV-EH					0.018		0.029	0.038	0.195		0.282	0.303	0.099		0.148	0.160	0.026		0.033	0.028
FSV-EK																				
FSV-EL																				
FSV-FC									0.240	0.330	0.430	0.470								
FSV-FD																				
FSV-FP	0.017	0.015	0.021	0.026					0.111	0.115	0.159	0.188								
n	23	22	23	22	2	1	2	2	26	23	24	26	6	5	6	6	17	15	17	17
Min	0.010	0.003	0.010	0.017	0.02		0.03	0.04	0.111	0.088	0.149	0.153	0.096	0.127	0.113	0.160	0.010	0.022	0.019	0.016
Median	0.020	0.013	0.029	0.038	0.02	0.02	0.04	0.04	0.181	0.214	0.276	0.325	0.111	0.159	0.165	0.180	0.030	0.044	0.040	0.040
Max	0.155	0.157	0.160	0.130	0.03		0.05	0.05	0.623	0.788	0.906	1.355	0.504	0.620	0.613	0.644	0.047	0.065	0.065	0.078
eSD	0.006	0.004	0.012	0.011					0.038	0.047	0.058	0.069	0.019	0.030	0.050	0.023	0.013	0.015	0.015	0.016
eCV	30	35	41	30					21	22	21	21	17	19	30	13	43	35	37	41
NISTa	0.023	*nd*	0.035	0.059																
NISTb	0.027	0.022	0.044	0.056	0.018	0.014	0.030	0.033	0.221	0.242	0.325	0.358	0.084	0.112	0.139	0.148	0.026	0.032	0.033	0.033
NAV	0.022	0.017	0.034	0.047					0.201	0.228	0.301	0.339	0.098	0.136	0.158	0.168	0.029	0.038	0.038	0.038
NAU	0.008	0.010	0.014	0.018					0.055	0.058	0.075	0.080	0.034	0.046	0.054	0.059	0.013	0.018	0.015	0.016

Round Robin XXXIV Laboratory Results

Values in µg/mL

	Lutein				Zeaxanthin				Lutein&Zeaxanthin			
Lab	207	208	209	210	207	208	209	210	207	208	209	210
FSV-BA									0.112	0.162	0.111	0.064
FSV-BD												
FSV-BE												
FSV-BG												
FSV-BH	0.057	0.058	0.054	0.030	*nd*	0.029	*nd*	*nd*	*0.057*	*0.087*	*0.054*	*0.030*
FSV-BI	0.053	0.070	0.056	0.031	0.020	0.042	0.029	0.020	0.083	0.126	0.086	0.052
FSV-BJ												
FSV-BK												
FSV-BL												
FSV-BM												
FSV-BN	0.048	0.065	0.056	0.034	0.029	0.035	0.027	0.015	0.080	0.103	0.089	0.051
FSV-BO												
FSV-BP												
FSV-BQ												
FSV-BR												
FSV-BS	0.068	0.061	0.095	0.171								
FSV-BT	0.075	0.103	0.069	0.037	0.023	0.034	0.022	0.020	0.098	0.137	0.092	0.057
FSV-BU									0.080	0.110	0.046	0.026
FSV-BV									0.060		0.083	0.042
FSV-BY	0.065	0.083	0.067	0.037	0.022	0.019	0.027	0.022	*0.087*	*0.102*	*0.094*	*0.059*
FSV-BZ	0.090	0.075	0.070	0.040								
FSV-CB												
FSV-CD									0.120	0.158	0.135	0.071
FSV-CH												
FSV-CJ									0.105	0.124	0.096	0.056
FSV-CK									0.114	0.146	0.112	0.071
FSV-CM												
FSV-CN												
FSV-CQ												
FSV-CR												
FSV-CT	0.058	0.075	0.061	0.036								
FSV-CU												
FSV-CV												
FSV-CX									0.060	0.090	0.070	0.040
FSV-CY												
FSV-DA	0.069	0.077	0.072	0.057	0.025	0.054	0.033	0.020	0.094	0.131	0.105	0.077
FSV-DB												
FSV-DJ												
FSV-DK												
FSV-DL									0.080	0.089	0.065	0.067
FSV-DM									0.087	0.130	0.107	0.055
FSV-DP												
FSV-DS												
FSV-DX									0.102	0.134	0.096	0.070
FSV-EA												
FSV-EH	0.053		0.055	0.029	0.027		0.025	0.010	0.084		0.081	0.042
FSV-EK												
FSV-EL												
FSV-FC												
FSV-FD												
FSV-FP												
n	10	9	10	10	6	6	6	6	17	15	17	17
Min	0.048	0.058	0.054	0.029	0.020	0.019	0.022	0.010	0.057	0.087	0.046	0.026
Median	0.062	0.075	0.064	0.037	0.024	0.034	0.027	0.020	0.087	0.126	0.092	0.056
Max	0.090	0.103	0.095	0.171	0.029	0.054	0.033	0.022	0.120	0.162	0.135	0.077
eSD	0.012	0.012	0.012	0.007	0.004	0.010	0.003	0.002	0.016	0.030	0.020	0.021
eCV	19	16	19	18	16	28	11	9	18	24	22	37
NISTa												
NISTb	0.055	0.075	0.060	0.037	0.024	0.048	0.032	0.019	*0.079*	*0.123*	*0.092*	*0.056*
NAV	0.060	0.075	0.063	0.037	0.023	0.041	0.030	0.019	0.083	0.124	0.093	0.056
NAU	0.016	0.017	0.016	0.008	0.007	0.014	0.009	0.006	0.020	0.027	0.021	0.013

G5

Round Robin XXXIV Laboratory Results

Analytes Reported By One Laboratory

Values in µg/mL

Analyte	Code	207	208	209	210
cis-Lutein&Zeaxanthin	FSV-BT	0.053	0.058	0.072	0.066
Coenzyme Q10	FSV-CH	0.751	1.718	1.243	0.682
Total Carotenoids	FSV-BT	0.748	0.767	1.252	1.852
α-Cryptoxanthin	FSV-BT	0.020	0.032	0.023	0.016

Legend

Term	Definition
n	Number of (non-NIST) quantitative values reported for this analyte
Min	Minimum (non-NIST) quantitative value reported
Median	Median (non-NIST) quantitative value reported
Max	Maximum (non-NIST) quantitative value reported
eSD	Estimated standard deviation, calculated from the median absolute deviation from the median of the non-NIST results
eCV	Coefficient of Variation for (non-NIST) results: 100*eSD/Median
NAV	NIST Assigned Value, our estimate of the "true" analyte concentration
NAU	NIST Assigned Uncertainty, our estimate of the total (serum hetergeniety and inter- and intra-laboratory) standard deviation For details on how we assign these quantities, see the "Analysis of Results."
nd	Not detected (i.e., no detectable peak for analyte)
>x	Concentration greater than or equal to x
italics	Not explicitly reported but calculated by NIST from reported values

G6

Round Robin XXXIV Laboratory Results

Comparability Summary

Lab	R	aT	gT	bC	tbC
FSV-BA	1	1	1	1	
FSV-BD	1	2	1	1	
FSV-BE	1	1		1	
FSV-BG	1	2		2	
FSV-BH	1	1			
FSV-BI	1	1		3	
FSV-BJ	1	1	1	2	
FSV-BK	2	1	1	1	1
FSV-BL	3	3	1	1	1
FSV-BM	1	1	3	2	
FSV-BN	3	3	3	4	
FSV-BO	2	1		1	
FSV-BP	1	2			
FSV-BQ	1	2			
FSV-BR	1		2	1	
FSV-BS					
FSV-BT	2	2		2	
FSV-BU	1	2		2	1
FSV-BV	2	1	3	2	
FSV-BY	1	1	2	2	
FSV-BZ		3		3	
FSV-CB	4	2	2	1	1
FSV-CD	1	3	1	2	
FSV-CH	1	3			
FSV-CJ	2	1		2	
FSV-CK	2	2	2	1	1
FSV-CM		1		1	1
FSV-CN	2	1		1	
FSV-CQ	1	2		2	
FSV-CR	3	2		1	
FSV-CT	1	1	1	1	1
FSV-CU	1	1	1	1	
FSV-CV	1	2			
FSV-CX	1	1		2	
FSV-CY	1	2			
FSV-DA	1	1			
FSV-DB	2	2			
FSV-DJ	1	2			4
FSV-DK	2	2	4	2	
FSV-DL	3	2	2	1	
FSV-DM	1	1		2	
FSV-DP	1			2	
FSV-DS	3	3		3	
FSV-DX	3	4	2	1	
FSV-EA	2	2	3	4	
FSV-EH	1	1	1	2	2
FSV-EK	4	4			
FSV-EL	1				
FSV-FC	1	1	2		2
FSV-FD	2	1	1	1	1
FSV-FP	4	4	1		1
NISTa	1	1	1	1	1
NISTb	1	1	1	1	1
n	48	47	23	37	12

Label	Definition
Lab	laboratory number
R	"Standard Score" for Retinol
aT	"Standard Score" for α-Tocopherol
gT	"Standard Score" for γ-Tocopherol
bC	"Standard Score" for Total β-Carotene
tbC	"Standard Score" for trans-β-Carotene
n	number of (non-NIST) laboratories providing data for this analyte

"Standard Score"

Given that our knowledge of the shape, location, and width of the measurement distributions is approximate and that a limited number of labs are involved, we summarize comparability with the following four-level "Standard Score" (StS)…

StS	Definition	
1	All StV within $\pm t$ (1-0.683,n-1)	{i.e., ±1 SD}
2	All StV within $\pm t$ (1-0.954,n-1)	{i.e., ±2 SD}
3	All StV within $\pm t$ (1-0.997,n-1)	{i.e., ±3 SD}
4	At least one StV > $\pm t$ (1-0.997,n-1)	{i.e., >3 SD}

where:	
StV	Standardized Value, the distance in standard deviation units your value is from the "true" concentration: StV = (your value - NAV) / NAU
NAV	NIST Assigned Value, our estimate of the "true" analyte concentration
NAU	NIST Assigned Uncertainty, our estimate of the total (serum heterogeniety and iter- and intra-laboratory) measurement standard deviation.
t (1-α,n-1)	Two-tailed Student's t for coverage of ±1, ±2, and ±3 NAU about NAV, assuming a normal population of size n

For details on the NIST Assigned quantities, see this Round Robin's "Report of (Meta)Analysis."

StS	% Observed					Expected
1	58	45	48	46	75	68.2 %
2	23	36	30	41	17	27.3 %
3	13	13	17	8	0	4.3 %
4	6	6	4	5	8	0.3 %

These are the observed and normal-population-expected proportions of each Standard Score (StS), based upon each laboratory's largest StV for the four sera.

G7

Appendix H. Representative "Individualized Report" for RR34

Each participant in RR34 received an "Individualized Report" reflecting their reported results. Each report included a detailed analysis of the results they reported for some or all of the following analytes:

- Retinol
- Retinol palmitate
- α-Tocopherol
- γ-Tocopherol
- Total β-Carotene
- *trans*-β-Carotene
- Total α-Carotene
- Total Lycopene
- β-Cryptoxanthin
- Lutein
- Zeaxanthin
- Lutein & Zeaxanthin

The following 8 pages are the "Individualized Report" for the analytes evaluated by participant FSV-BA.

Individualized Round Robin XXXIV Report to: FSV-BA

Your Data, NIST Assigned Values, and %Differences

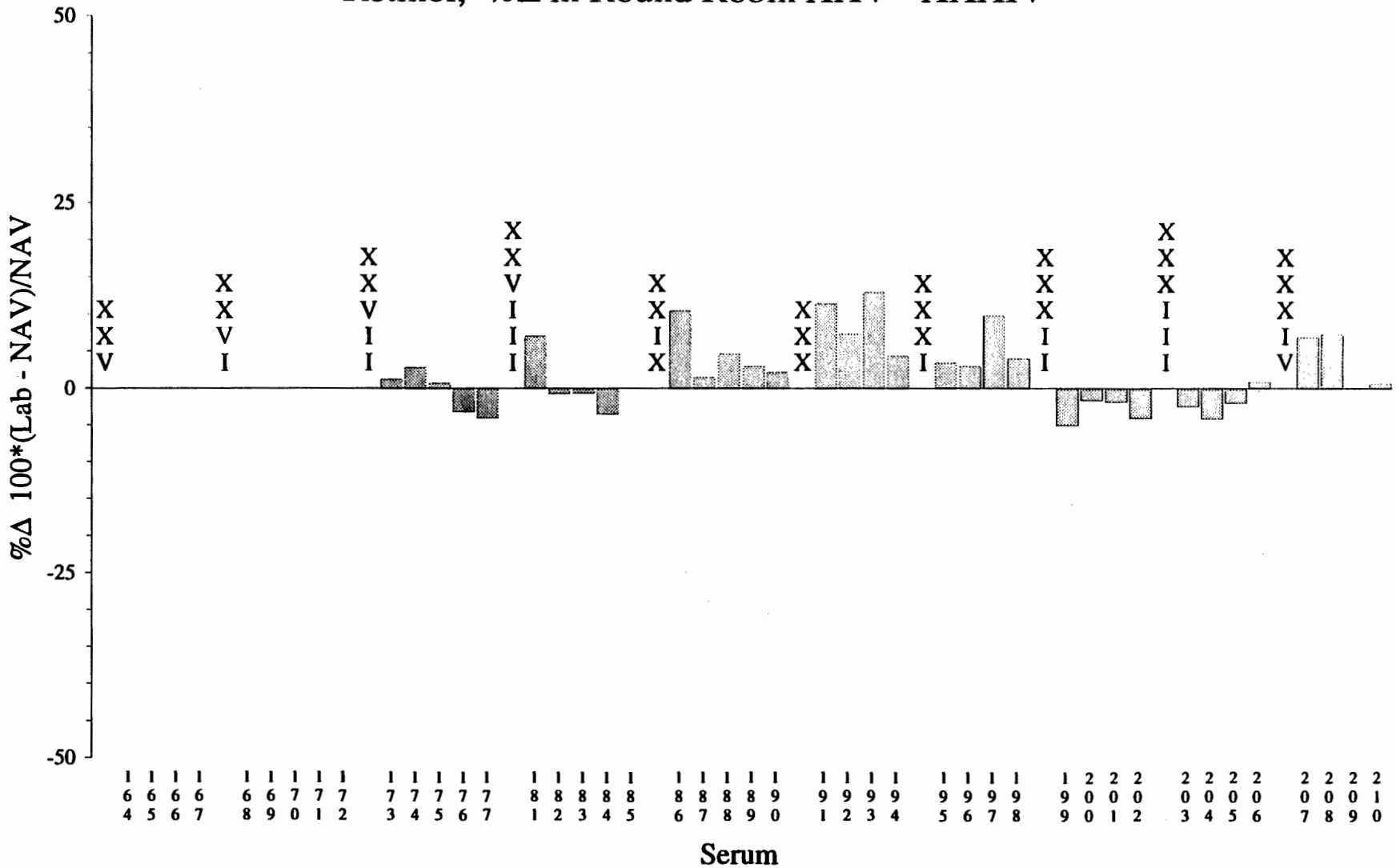
	Serum 207				Serum 208				Serum 209				Serum 210			
Analyte	You	NAV	%Δ	n	You	NAV	%Δ	n	You	NAV	%Δ	n	You	NAV	%Δ	n
Retinol	.30	.28	7	38	.46	.43	7	38	.52	.52	0	38	.89	.88	1	38
Retinyl Palmitate	.110	.088	26	12	.032	.030	7	7	.199	.177	13	12	.275	.263	5	12
a-Tocopherol	6.80	7.05	-4	37	5.75	5.98	-4	37	9.53	10.06	-5	37	17.28	17.74	-3	37
g-Tocopherol	1.75	1.67	5	18	3.09	2.93	5	18	2.39	2.38	0	18	3.87	3.76	3	18
Total b-Carotene	.267	.252	6	31	.213	.194	10	31	.659	.635	4	31	1.290	1.239	4	30
trans-b-Carotene	.252	.241	5	8	.200	.175	15	8	.634	.584	9	8	1.240	1.165	6	8
Total cis-b-Carotene	.015			7	.013			7	.025			7	.050			7
Total a-Carotene	.025	.022	14	21	.013	.017	-25	21	.036	.034	5	21	.047	.047	0	20
trans-Lycopene	.113	.138	-18	5	.159	.178	-11	5	.177	.196	-10	5	.188	.215	-13	5
b-Cryptoxanthin	.040	.029	37	13	.054	.040	36	13	.049	.040	23	13	.048	.039	23	13
Lutein&Zeaxanthin	.112	.083	35	14	.162	.124	31	14	.111	.092	20	14	.064	.056	14	14

You : Your reported values for the listed analytes (micrograms/milliliter)
NAV : NIST Assigned Values, equal to (NIST's average-of-averages + this Round Robin's median) / 2
%Δ : Percent difference between your value and the NAV
n : Number of non-NIST laboratories reporting quantitative values for this analyte in this serum

Please check our recorded values against your records.
Send corrections to: NNMMQAP 222/B208, NIST, Gaithersburg, MD 20899; fax 301-977-0685; email DLDuewer@enh.NIST.gov

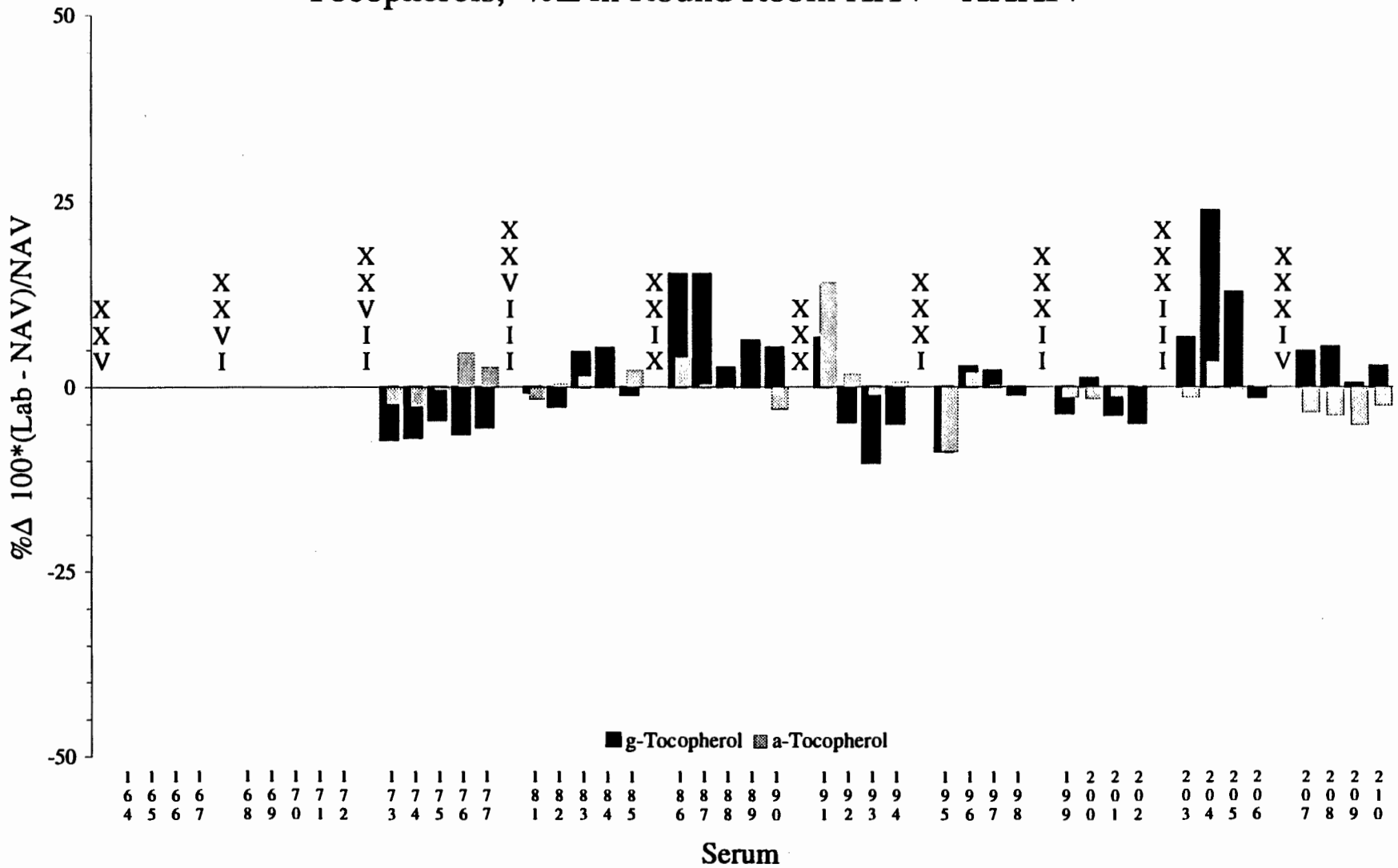
Individualized Round Robin XXXIV Report to: FSV-BA

Retinol, %Δ in Round Robin XXV - XXXIV



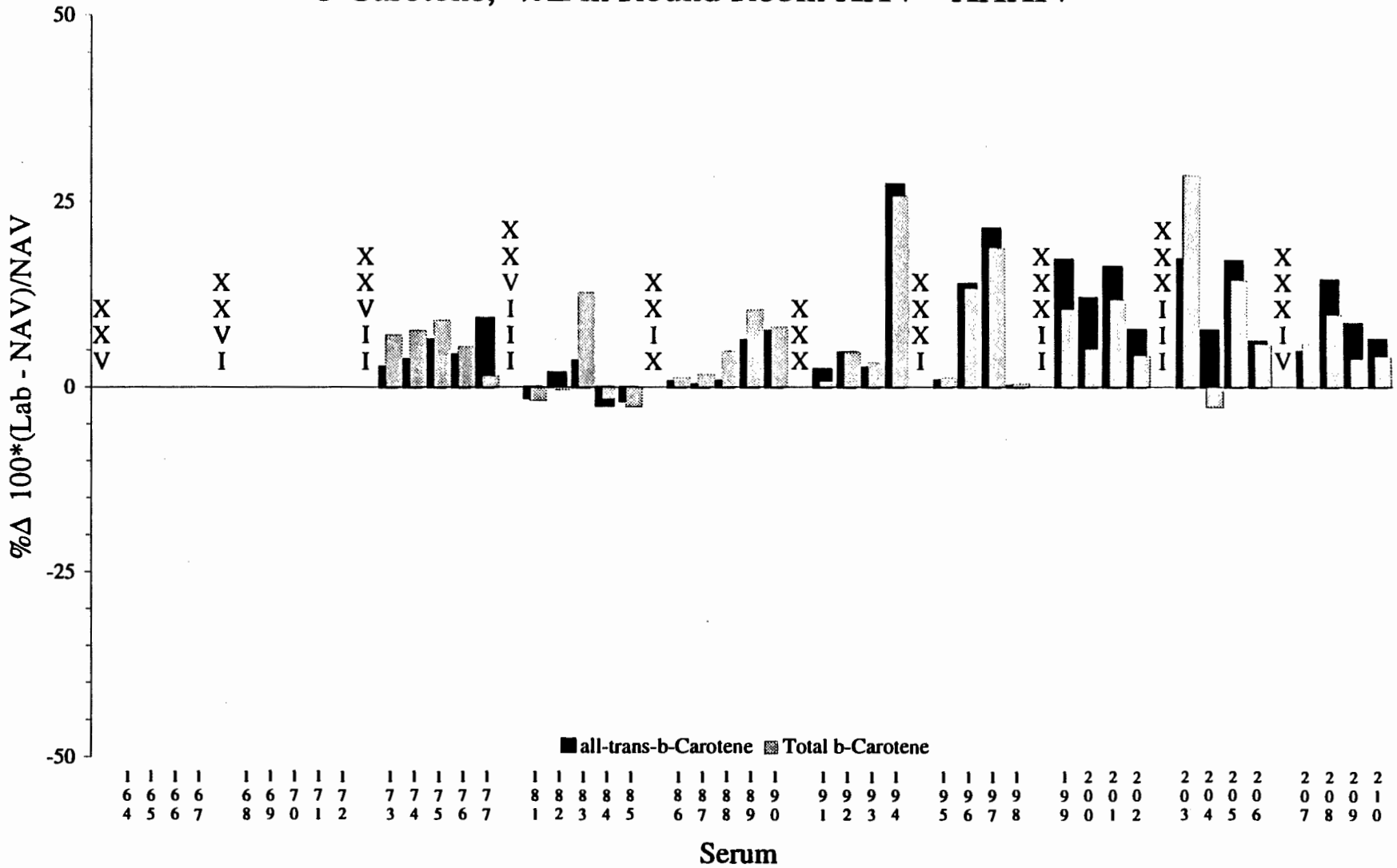
Individualized Round Robin XXXIV Report to: FSV-BA

Tocopherols, %Δ in Round Robin XXV - XXXIV



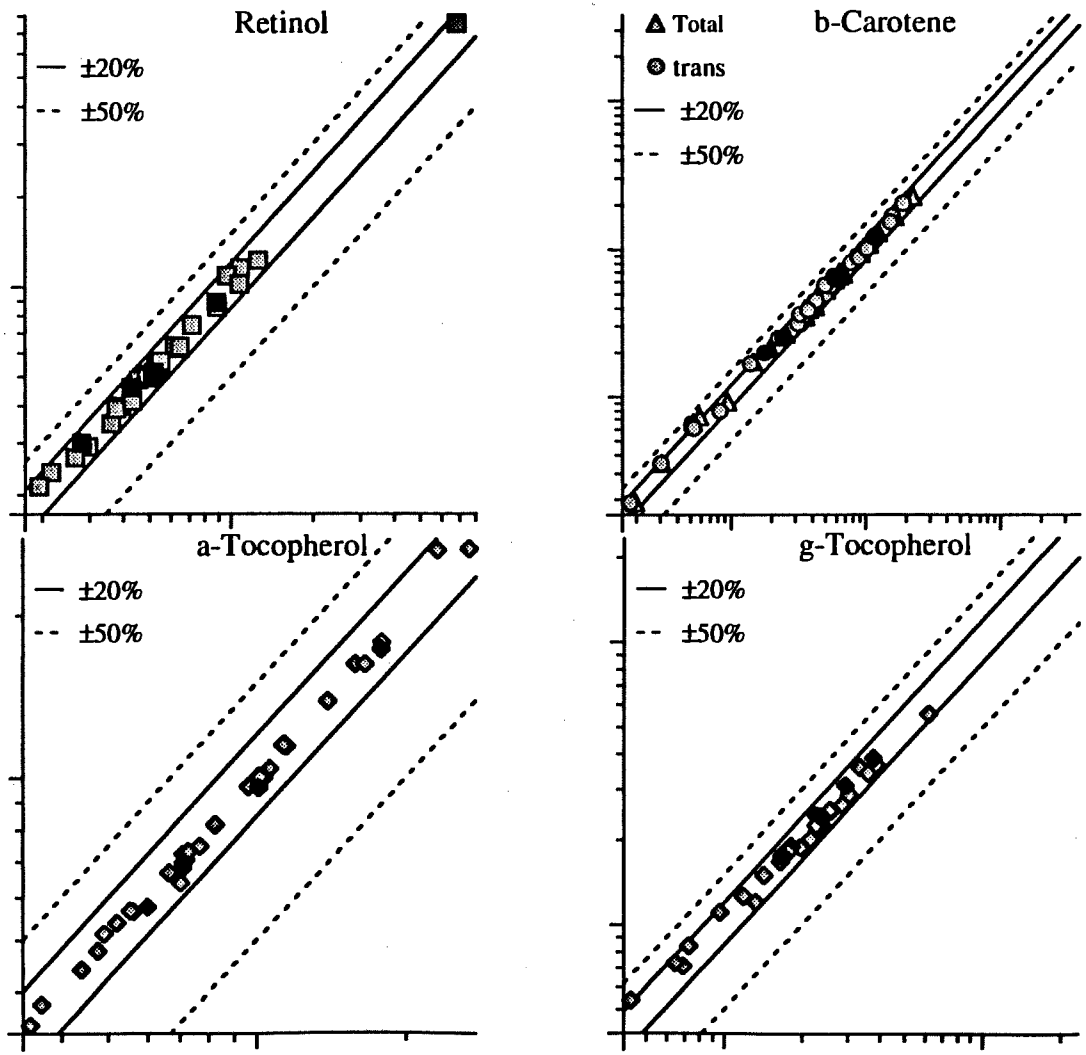
Individualized Round Robin XXXIV Report to: FSV-BA

b-Carotene, %Δ in Round Robin XXV - XXXIV



Individualized Round Robin XXXIV Report to: FSV-BA

NIST Assigned Values Vs Laboratory Values



Legend

Shaded Symbols: Round Robin XXV-XXXIII
Black Symbols: Round Robin XXXIV

Ordinate: NIST Assigned Value
Abscissa: Your reported concentration

Interpretation

Adequately intercomparable data are within ±20% lines. If you have data scattered outside ±50% lines, your measurement system is not consistent with those of most participating laboratories. If your data are systematically higher or lower than the NAV, your system may be consistent but your results are biased.

If your data show increased scatter at low concentrations, your "limit of quantification" may not be what you think it is.

If there are one or two "wild" outliers, they might be calculation or transcription errors. We would appreciate hearing from you about any such problems.

H6

Individualized Round Robin XXXIV Report to: FSV-BA

Accuracy/Precision Summary

	Ret		aToc		gToc		Total		trans	
	mΔ	vΔ	mΔ	vΔ	mΔ	vΔ	mΔ	vΔ	mΔ	vΔ
XXV										
XXVI										
XXVII	-1	3	0	3	-6	1	6	3	5	3
XXVIII	0	4	1	2	1	4	1	7	0	3
XXIX	4	4	0	3	9	6	5	4	3	3
XXX	9	4	4	7	-3	7	9	12	9	12
XXXI	5	3	-2	5	-1	5	9	9	9	10
XXXII	-3	2	-1	1	-3	3	8	4	13	4
XXXIII	-2	2	0	2	11	11	12	13	12	6
XXXIV	4	4	-4	1	3	2	6	3	9	4

Legend

Ret	Retinol
aToc	a-Tocopherol
gToc	g-Tocopherol
Total	Total b-Carotene
trans	all-trans-b-Carotene
mΔ	Mean difference, the average %Δ for all sera of a given RR, where
	%Δ = 100(Your value - NAV) / NAV
vΔ	Difference variability, one standard deviation of %Δ for all sera of a RR
NAV	NIST Assigned Value, our best estimate of analyte concentration...
	NAV = (NIST's average-of-averages + Round Robin median) / 2

(Traditional) Performance Criteria

The absolute value of %Δ of every measurement has traditionally been evaluated as follows...

%Δ	Evaluation
0-5%	Exceptional
6-10%	Acceptable
11-20%	Marginal
> 20%	Poor

More representative criteria need to be established, factoring in each serum's analyte level and the analyte distribution in adult human populations. Stay tuned, we're working on it...

Interpretation

Accuracy and precision are separate but kindred aspects of measurement comparison. We estimate accuracy as mΔ, the average %Δ, and precision as vΔ, the standard deviation of %Δ, for all sera of a Round Robin.

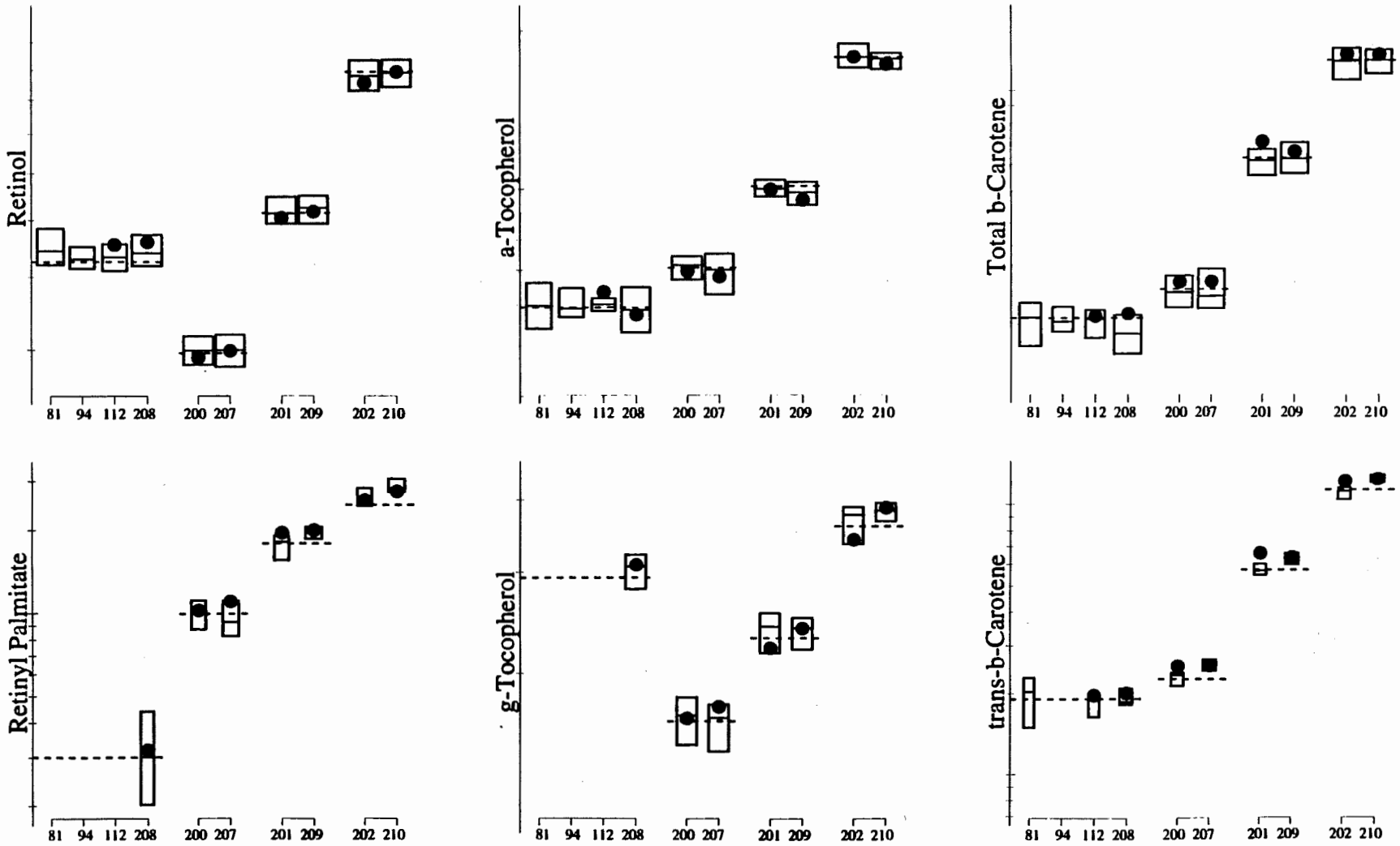
It's best to be accurate and precise (small mΔ, small vΔ)!

Good precision (small vΔ) with poor accuracy (large mΔ) is better than the converse: at least such values are internally consistent and may be relatable to others' values once the relative biases have been determined.

Poor precision (large vΔ) suggests that your measurement system is not in adequate control for the analyte levels examined.

Individualized Round Robin XXXIV Report to: FSV-BA

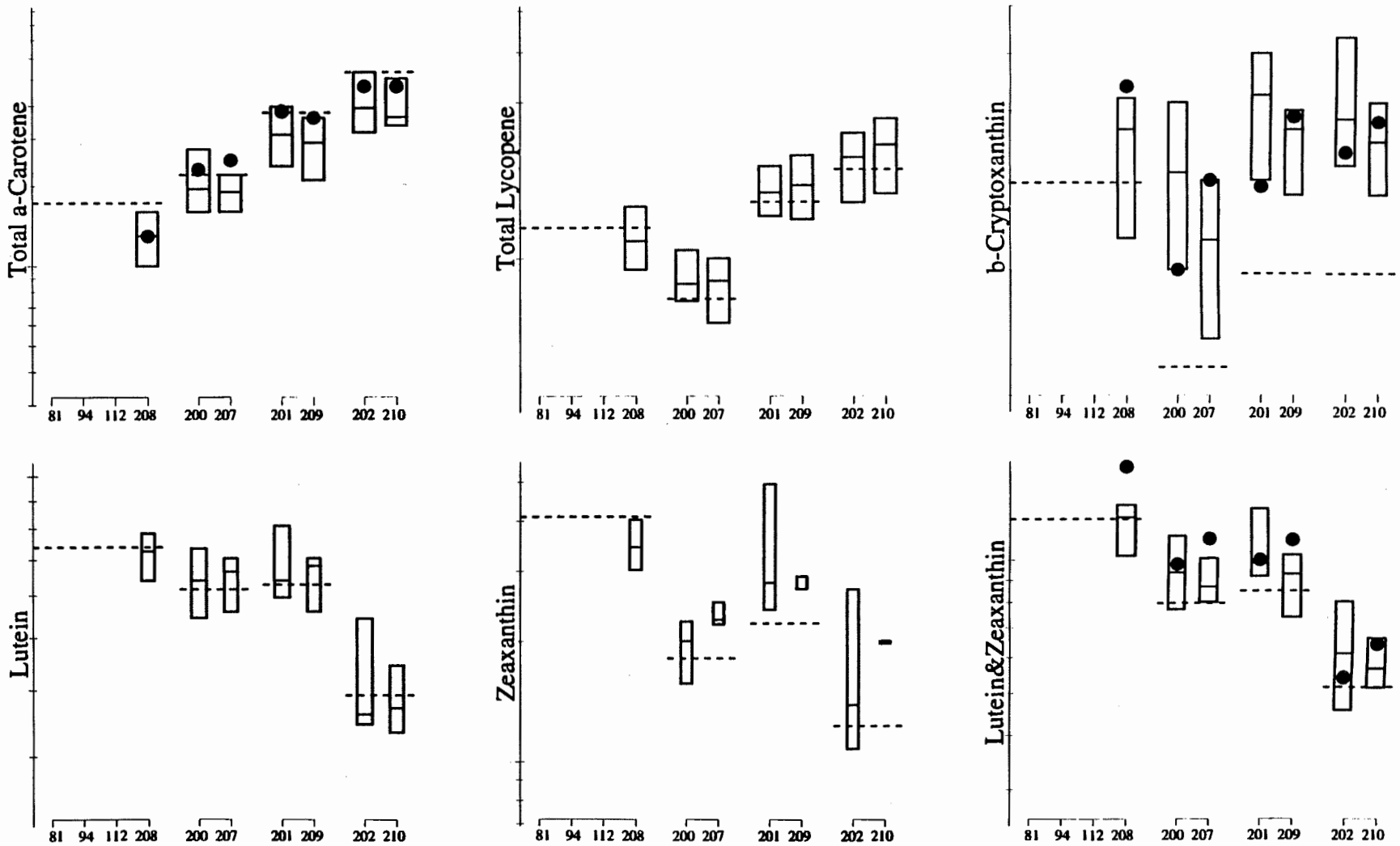
Comparisons to Prior Analyses



H8

Individualized Round Robin XXXIV Report to: FSV-BA

Comparisons to Prior Analyses (Continued)



H9

Appendix I. Shipping Package Inserts for RR35

The following two items were included in each package shipped to RR35 participants:

- Cover letter
- Datasheet

The cover letter and datasheet were enclosed in a sealed waterproof bag along with the samples themselves.

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

July 14, 1995

Dear Colleague:

Enclosed is the set of samples for the third round robin exercise (Round Robin XXXV). You will find one vial each of four lyophilized sera samples for analysis along with a form for reporting your results. When reporting your results, please submit one value for each analyte for a given serum sample. If an obtained value is below the detection limit, please indicate this result on the form by using ND *(Not Detected)*. For values not obtained, please leave a blank for the given analyte. Results will be due to NIST by September 15. Results received two weeks after the due date will not be included in the summary report for this round robin study. Written feedback concerning the study will be provided to you by October 20.

Samples should be reconstituted with 1.0 mL of HPLC-Grade water or equivalent. We recommend that dissolution be facilitated with 3 to 5 minutes agitation in an ultrasonic bath or at least 30 minutes at room temperature with intermittent swirling. (CAUTION: Vigorous shaking will cause foaming and possibly interfere with accurate measurement. The rubber stopper contains phthalate esters which will leach into the sample upon intermittent contact of the liquid sample with the stopper. These esters absorb strongly in the UV region and elute very near retinol in most HPLC systems creating analytical problems.) Pipette a known volume of serum from the vial for analysis since the final volume of the reconstituted sample is greater than 1.0 mL. For consistency, we request that laboratories use the following absorptivities (E 1 % cm) in ethanol: retinol, 1850 at 325 nm; retinyl palmitate, 97 5 at 325 nm; a-tocopherol, 75.8 at 292 nm; y-tocopherol, 91.4 at 298 nm; a-carotene, 2800 at 444 nm; f3-carotene, 2560 at 450 nm; lycopene, 3450 at 472 nm.

Please mail or FAX your results for Round Robin XXXV to:

Micronutrients Measurement Quality Assurance Program
NIST
Bldg. 222, Rm. B208
Gaithersburg, MD 20899
FAX: (301) 977-0685

If you have questions regarding this round robin exercise, please call me at (301) 975-3120 or mail/FAX queries to the above address.

Sincerely,

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

Enclosures

cc: W. May
S. Wise

NIST

NIST/NCI

Micronutrients Measurement Quality Assurance Program

Round Robin **XXXV** Results from Laboratory #_____

Analyte	Serum 211	212	213	214	Units*
retinol					
retinyl palmitate					
a-tocopherol					
g-tocopherol					
total b-carotene					
trans-b-carotene					
total cis-b-carotene					
total a-carotene					
total lycopene					
trans-lycopene					
b-cryptoxanthin					
lutein					
zeaxanthin					
lutein&zeaxanthin					

Other Analytes?					

* We prefer results as microgram/milliliter.

Comments?

Mail: NNMMQAP, 222/B208
NIST
Gaithersburg, MD 20899

Please return results on-or-before
September 15, 1995

Fax: 301-977-0685
Email: DLDuewer@enh.NIST.gov

Appendix J. Final Report for RR35

The following 19 pages are the final report for RR35 as provided to all participants:

- Cover letter
- A discussion entitled "Lies, Damn Lies, and Statistics" that:
  - describes the nature of the test samples and details any previous distributions
  - summarizes aspects of the study that we believe may be of interest to the participants
- A "Report of (Meta)Analysis" that details the analysis of NIST measurements

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

November 3, 1995

Dear Colleague:

The tabular summaries for Round Robins XXXIII and XXXIV mentioned below are provided in previous Appendices of this document and are not repeated here.

Enclosed is the summary report of the results for Round Robin XXXV. This report summarizes both overall and individual laboratory performance for the three round robin exercises conducted during 1995. Included in this report are: tabular summaries of data for Round Robins XXXIII, XXXIV, and XXXV; a graphical summary of the interlaboratory median vs. individualized laboratory data for retinol, α- and γ-tocopherol, and total and *trans*-β-carotene; percent bias charts for retinol, α- and γ-tocopherol, and *trans*- and total β-carotene; and a summary of individual laboratory performance for the past three years. Tabular data only are provided for α-carotene, β-cryptoxanthin, lutein, lycopene, retinyl palmitate, and zeaxanthin.

The overall interlaboratory precision of retinol and α-tocopherol measurements has remained at an average estimated coefficient of variation (eCV) of approximately 10% over the past three years, and around 21% for β-carotene during the same period of time. The eCV for γ-tocopherol has remained at about 10% over the past 18 months.

Round Robin XXXIII (Sera 203-206) consisted of a dilution series where Serum 205 was a 1:1 volumetric mix of Serum 203 and stripped serum; Serum 204 was a 1:2 volumetric mix of Serum 203 and the stripped serum. Serum 206 was previously distributed in Round Robin XVIII as Serum 120 and in Round Robin XXI as Serum 147. The results from this exercise indicate that the measurement capability for retinol, α- and γ-tocopherol, and β-carotene is adequate.

Round Robin XXXIV consisted of Sera 207-210 where Sera 207, 209, and 210 were previously distributed as Sera 200, 201, and 202 , respectively in Round Robin XXXII and are the three concentration levels of SRM 968b. Serum 208 (also identified as Control B in earlier studies) was previously distributed as Serum 81 in Round Robin XII, Serum 94 in Round Robin XIII, and Serum 112 in Round Robin XVI. In this study, the average eCV for retinol and α- and γ-tocopherol was 9-10% and 17% for β-carotene. Results from this study demonstrated that the interlaboratory measurement reproducibility over the past 18 months has improved.

Round Robin XXXV (Sera 211-214) consisted of a replicate dilution series. Sera 211, 212, and 214 were previously distributed in Round Robin XXXI as Sera 197, 198, and 195, respectively. Serum 213 was distributed in Round Robin XXX as Serum 191. Sera 195, 197, and 198 are a dilution series. Serum 198 was blended from native sera that was high in β-carotene. Serum 195 is a 1:1 blend of Serum 198 and stripped serum. Serum 197 is a 1:3 blend of Serum 198 and stripped serum. Serum 191 is a blend of native serum that was supplemented with a high level of retinol.

NIST

EXCEPTIONAL performance, those rated **2** (within ± 2 SD) indicate **ACCEPTABLE** performance, a rating of **3** (within ± 3 SD of the assigned value) is **MARGINAL** performance, and **4** (>3 SD of the assigned value) indicates **POOR** performance relative to the current state-of-the-practice for these measurements.

If you have concerns regarding your laboratory performance or were rated "**POOR**" based on the criteria stated above, we suggest that you obtain a unit of SRM 968b, Fat-Soluble Vitamins and Cholesterol in Human Serum, and analyze all three levels. If with minor method modifications, your measured values do not agree with the certified values, we suggest that you contact us for consultation.

As last year, the fees for non-NCI funded laboratories will be $300 for US labs and $600 for non-US labs. An invoice to that effect will be mailed in January.

The 1996 QA Program will consist of three round robin exercises for the analysis of fat-soluble vitamins and carotenoids in serum, two studies for the analysis of ascorbic acid in serum, and two exercises for the analysis of fat-soluble vitamins and carotenoids in food. The FY96 program thrusts will continue to include methods development for the measurement of difluoromethylornithine, 4-hydroxyphenylretinamide, and selenomethionine in serum. Attention will also be focused on agents that are currently in preclinical efficacy and toxicity trials (e.g.,epigallocatechin gallate, phenethyl isothiocyanate, indole-3-carbinol, dimethyl fumarate, and curcumin).

The first set of samples for the fat-soluble vitamins in serum analysis will be distributed the week of January 22. Results are due by March 15; written feedback will be provided to labs by April 12. The second set of samples will be shipped the week of April 29 with results due by June 14 and feedback to labs by July 26. The third set of samples will be shipped the week of July 29. Results will be due by September 20. Feedback will be provided to the laboratories by October 25.

The round robin studies for the analysis of fat-soluble vitamins and carotenoids in food will also be scheduled in April and July to coincide with the second and third serum round robin studies. The coordinator of these exercises is Dr. Katherine Sharpless (301/975-3121).

The first set of samples for the analysis of ascorbic acid in serum will be distributed in February. The second set will be distributed in May. These round robin studies are being coordinated by Dr. Sam Margolis (301/975-3137).

The next QA workshop will be held Saturday, April 13 as a pre-meeting in conjunction with Experimental Biology '96 in Washington, DC. Details of the workshop will be provided as plans are finalized.

Certificates of participation in the FY95 QA Program will be distributed again in January. If you have suggestions regarding improvements to the certificates, please contact us. We are also in the process of developing a home page through the Internet for the QA program. You will be updated as to when it becomes available.

Sincerely,

Jeanice Brown Thomas

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

cc: W. May

"Lies, Damned Lies, and Statistics"

Mark Twain

The attached N^2M^2QAP Round Robin XXXV Report is identical (!) in form to RR XXXIV's. It includes an exhaustive analysis of your results (the "Individualized" report), a complete listing of everyone's results (the "All Lab" report), and the details of the NIST value and uncertainty assignments (the "Report of (Meta)Analysis for RR XXXV Sera: 211-214").

Your "Individualized" report has the following elements:

Page	Contents
1	Your values, our assigned values, and the %bias between you and us
2	%Bias barchart for retinol for your last 3 years' results
3	%Bias barchart for α- and γ-tocopherol for your last 3 years' results
4	%Bias barchart for total and *trans*-β-carotene for your last 3 years' results
5	Our assigned value vs. your value scatterplots for retinol, α- and γ-tocopherol, and total and *trans*-β-carotene, again for your last 3 years' results
6	Accuracy/Precision Summary, yet again for your last 3 years' results
7	Comparison-to-Prior-Analyses plots for retinol, retinyl palmitate, α- and γ-tocopherol, and total and *trans*-β-carotene
8	Comparison-to-Prior-Analyses plots for *cis*-β-carotene, total α-carotene, total lycopene, β-cryptoxanthin, lutein, and lutein & zeaxanthin

The Comparison-to-Prior-Analyses plots on pages 7 and 8 show your individual results and a box-plot summary of the group's results, plotted against results from prior Round Robins:

> Serum #213 was distributed as #191 in Round Robin XXX. This serum was prepared to have high levels of the retinoids, tocopherols, and *trans*-β-carotene. (It has a *very* high level of retinol, one representative of extreme retinol poisoning.) The "true value" line is the average of the assigned values for the two Round Robins.
>
> Serum #211, 212, and 214 were distributed as #197, 198, and 195, respectively, in Round Robin XXXI. These sera were prepared as a concentration series by dilution of a blended native serum with delipidized serum. The level of analytes in serum 214 is 50% of that in serum 212 and the level in serum 211 is 25% of that in 212. There are thus two sets of "true value" lines: the horizontal lines are the average of the assigned values for the two Round Robins. The sloping lines represent the expected dilution relationship.

The "All Lab" report has the following elements:

Page	Contents
1-4	A listing of all results for analytes reported by at least two laboratories, plus essential summary statistics.
5a	A list of results for the four analytes reported by only one laboratory.
5b	A Legend for the above two lists.
6	The "Measurement Comparability Summary" (or "Score card")

The Legend for the data summary lists all the "flag codes" we currently use. Two new codes have been added: "?" and "*". The "?" indicates non-quantitative values resulting from the analyte level being above the limit of quantification of the routine calibration curve. The "*" indicates non-quantitative values resulting from a miscellany of causes: broken vial, instrument malfunction, etc. These codes have been introduced to insure that values you report as somehow suspect are not included in the statistical and "Score card" calculations. We want to keep as accurate a record of measurement results as possible, but do not wish to in any way "punish" anyone for honest reporting of results recognized as non-quantitative.

The "?" code for non-quantitative high values was suggested by Laboratory 70. They recognized that the value from the routine analysis was beyond the range of their calibration, but were unable to rerun a properly diluted sample. We believe that "?" is a better code for such extrapolated values than is the upper-limit of quantification code ">x". We believe that differentiating extrapolation-related from other sources of non-quantitative values is important, as the recognition of "toxic high level!" is the critical aspect of the retinol in serum 213.

Note that the dilution series ([212], [214] = [212]/2, [211] = [212]/4) enables you to estimate your effective limits of quantification ("LOQ"s) for some of the analytes in these lyophilized sera. For example, as described in the "Report of (Meta)Analysis", the NIST LOQs are below the serum 211 levels for retinol, α- and γ-tocopherol, total and *trans*-β-carotene, and lutein. The LOQs are between the serum 212 and 211 levels for retinyl palmitate, total and *trans*-α-carotene, total and *trans*-lycopene, β-cryptoxanthin, and zeaxanthin. The LOQ for δ-tocopherol is at least as high as the serum 214 level. The Comparison-to-Prior-Analyses plots present all the values required for you to make your own LOQ determinations. We encourage you to report "<x" (where x is your LOQ) for values below your LOQ; "*nq*" is acceptable, but is less informative. Please reserve "*nd*" (or "0") for situations where you find no evidence for the analyte's presence in the sample.

The interlaboratory medians for nearly all analytes reported in this Round Robin are very similar to those reported previously, and the observed distribution of values among reporting laboratories is generally tighter. Thus, we believe that interlaboratory measurement performance for most analytes is again improving. (We're *still* working on methods to more quantitatively document such trends...) However, the lutein and especially the zeaxanthin values are suspect.

The poorer performance for lutein and zeaxanthin is not surprising, as some of you measure a combined "lutein and zeaxanthin" peak, others measure lutein and zeaxanthin independently, some just estimate lutein, and a few laboratories differentiate all-*trans*- and *cis*-isomers. Our separation system may provide resolved peaks for *trans*-lutein and *trans*-zeaxanthin, but we have

thus far not determined whether any of the *cis*-isomers are included under these dominant peaks. There are many peaks eluting in this region; most of them remain to be identified.

The retinol level in serum 213, while about that of an actual polar-bear-liver poisoning case, represents an extreme "Challenge Sample." We promise not to distribute it again for a few years... As we should have freeze-dryer capability again soon, we anticipate introducing a number of new and "more interesting" samples in '96. We intend to explore the expected high levels for many of the carotenoids. The attached Table summarizes the analyte distributions we have gleaned from recent literature.

Your comments and suggestions are welcome. If you discover any errors in our recording or interpretation of your data, please let us know!

Dave Duewer
Research Chemometrician
DLDuewer@enh.NIST.gov

Margaret Kline
Research Biologist
Kline@enh.NIST.gov

Recent Literature Statistics[a] for Selected Analytes

Study	n	β-Carotene				α-Carotene				β-Cryptoxanthin			
Bieri {1}	5	0.28±.13	[0.13	0.33	0.46]	0.071±.032	[0.029	0.082	0.11]	0.15±.09	[0.07	0.13	0.30]
Stacewicz {2}	110	0.20±.19	[0.02		0.69]	0.034±.023	[0.0		0.12]	0.09±.06	[0.020		0.27]
Cavina {3}	14	0.31±.17	[0.08	0.26	0.58]	0.022±.017	[0.005	0.023	0.06]	0.20±.17	[0.051	0.15	0.70]
Cantilena {4}	33	0.14±.08				0.045±.034				0.10±.04			
Olmedilla {5}	307	0.29±.18				0.062±.054							
Ascherio {6}	43	0.18±.08	[0.05	0.17	0.44]	0.043±.023	[0.007	0.039	0.12]	0.28±.23	[0.052	0.21	1.1]
Stacewicz {7}	160	0.19±.14	[0.02		0.80]	0.048±.037	[0.003		0.24]	0.10±.07	[0.0		0.47]
Sowell {8}	3480	0.18	[0.04		0.37]*c*	0.043	[0.011		0.12]*c*	0.12	[0.03		0.29]*c*
Sharpless {9}	1402	0.17±.19	[0.02	0.13	0.43]*c*	0.036±.036	[0.006	0.026	0.09]*c*	0.09±.11	[0.001	0.053	0.29]*c*

	Lycopene				Lutein				Zeaxanthin			
Bieri	0.32±.17	[0.20	0.29	0.62]	0.28±.10	[0.17	0.32	0.41]	*b*			
Stacewicz	0.20±.12	[0.015		0.71]	0.18±.09	[0.044		0.45]	*b*			
Cavina	0.40±.18	[0.010	0.37	0.81]	0.28±.09	[0.13	0.27	0.46]	*b*			
Cantilena	0.14±.05				0.20±.08				*b*			
Olmedilla	0.42±.19				0.16±.06				0.036±.017			
Ascherio	0.31±.15	[0.068	0.28	0.65]	0.15±.07	[0.063	0.14	0.43]	0.045±.022	[0.015	0.041	0.10]
Stacewicz	0.29±.16	[0.009		0.80]	0.12±.06	[0.020		0.38]	0.030±.017	[0.0		0.11]
Sowell	0.21	[0.06		0.43]*c*	0.20	[0.08		0.42]*c*	*b*			
Sharpless	0.13±.08	[0.025	0.11	0.49]*c*	0.13±.06	[0.053	0.12	0.25]*c*	0.037±.018	[0.014	0.035	0.07]*c*

	α-Tocopherol				γ-Tocopherol				Retinol			
Bieri												
Stacewicz									0.70±.19	[0.38		1.3]
Cavina	13±3	[7.0	13	19]					0.65±.22	[0.35	0.58	1.2]
Cantilena												
Olmedilla	11±4				0.6±0.3							
Ascherio	15±3	[9.0	15	25]					0.57±.14	[0.31	0.54	0.86]
Stacewicz	14±5	[6.3		34]	3.1±1.6	[0.3		10]	0.64±.18	[0.30		1.2]
Sowell	11	[6.0		21]*c*					0.55	[0.30		0.85]*c*
Sharpless	11±6	[5.8	9.8	110]*c*	1.8±1.1	[0.47	1.6	3.9]*c*	0.56±.17	[0.33	0.54	0.87]*c*

a Mean±SD and [minimum median maximum], expressed in μg/mL. Values recalculated from literature data as necessary.

b The corresponding lutein values report lutein + zeaxanthin.

c [5% median 95%] range reported rather than [minimum median maximum].

References

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3 Cavina G, Gallinella B, Porrá R, Pecora P, Suraci C. **1988**. Carotenoids, Retinoids, and alpha-Tocopherol in Human Serum: Identification and Determination by Reversed-phase HPLC. *Journal of Pharmaceutical and Biomedical Analysis* **6** 259-269.

4 Cantilena LR, Nierenberg DW. **1989**. Simultaneous Analysis of Five Carotenoids in Human Plasma by Isocratic High Performance Liquid Chromatography. *Journal of Micronutrient Analysis* **6** 127-145.

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6 Ascherio A, Stampfer MJ, Colditz GA, Rimm EB, Litin L, Willett WC. **1992**. Correlations of Vitamin A and E Intakes with the Plasma Concentrations of Carotenoids and Tocopherols among American Men and Women. *Journal of Nutrition* **122** 1792-1801.

7 Stacewicz-Sapuntzakis M, Bowen PE, Mares-Perlman, JA. **1993**. Serum Reference Values for Lutein and Zeaxanthin Using a Rapid Separation Technique. Carotenoids in Human Health. Eds: Canfield LM, Krinsky NI, Olson JA. *Annals of the New York Academy of Sciences*, Vol. 691: 207-209.

8 Sowell AL, Huff DL, Yeager PR, Caudill SP, Gunter EW. **1994**. Retinol, α-Tocopherol, Lutein/Zeaxanthin, β-Cryptoxanthin, Lycopene, α-Carotene, *trans*-β-Carotene, and Four Retinyl Esters in Serum Determined Simultaneously by Reversed-Phase HPLC with Multiwavelength Detection. *Clinical Chemistry* **40** 411-416.

9 Sharpless KS, Duewer DL. **1995**. Population Distributions and Intralaboratory Reproducibility for Fat-Soluble Vitamin-related Compounds in Human Serum. *Analytical Chemistry*, scheduled for publication Dec 1, 1995.

From: David L. Duewer Date: October 31, 1995

To: Jeanice Brown Thomas, Margaret Kline, Willie E. May, Katherine E. Sharpless
cc: Gary W. Kramer, Dennis J. Reeder, Stephen A. Wise

Re: Value and Uncertainty Assignment for the NIST/NCI Micronutrient Measurement Quality Assurance Program's Round Robin XXXV (RR35) Sera: #211 to 214.

Background: Four sera were distributed in RR35. Sera 211, 212, and 214 had been distributed as sera 197, 198, and 195, respectively, in Round Robin XXXI (RR31); serum 213 had been distributed as serum 191 in Round Robin XXX (RR30). Sera 212, 214, and 211 are a dilution series: serum 212-stock was blended from native sera high in β-carotene, serum 214 is a 50:50 blend of 212-stock and delipidized serum, and serum 211 is a 25:75 blend of 212-stock and delipidized serum. Serum 213 is a blend of native sera augmented to a very high retinol level.

Analysts NIST1 and NIST3 independently extracted and analyzed two aliquots of three vials of each serum using normal procedures. Both analysts reported quantitative results for retinol, α- and γ-tocopherol, total and *trans*-β-carotene, and total α-carotene. In addition, analyst NIST3 reported results for retinyl palmitate, δ-tocopherol, *trans*-α-carotene, total and *trans*-lycopene, β-cryptoxanthin, "lutein", and "zeaxanthin". (NIST3 is uncertain about the isomeric composition of the single peaks observed for lutein and zeaxanthin: while mostly the respective all-*trans* isomers, they may include some as yet unknown proportion of the *cis* isomers.)

Results: Table 1 presents the NIST data, summary statistics for the NIST data, summary interlaboratory results for serum 191 in RR30 and sera 195, 197, and 198 in RR31, summary interlaboratory results for sera 211 – 214 in RR35, and the NIST assigned values and uncertainties.

Table 2 presents a compact summary of the assigned values and uncertainties for each analyte in each serum. The sera are listed in order of decreasing retinol concentration, facilitating interpretation of the dilution series: [212], [214] = [212]/2, [211] = [212]/4.

The entries in Table 1 are as follows:

- Individual NIST Analyst Data and Summary Statistics

A:1 to C:2	two aliquots ("1" and "2") of three vials (A, B, and C) of each serum were extracted and analyzed. Each analyst analyzed a separate set of three vials.
n_x	number of quantitative values for this analyte for this serum for this analyst
$Mean_x$	arithmetic average
SD_x	simple standard deviation
SD_{repx}	within-vial pooled standard deviation, reflecting variation in extraction, chromatography, peak integration, etc.
SD_{hetx}	among-sample standard deviation, reflecting heterogeneity in preparing and reconstituting the serum samples
SD_{NISTx}	$\sqrt{SD_{repx}^2 + SD_{hetx}^2}$, total standard deviation. This value is $\geq SD_x$, as sample replicates reduce the true degrees of freedom.
CV_{NISTx}	$100*SD_{NISTx}/Mean_x$

- NIST Summary Statistics

n	number of quantitative values for this analyte for this serum
Mean	$(Mean_{NIST1} + Mean_{NIST3})/2$ or $Mean_{NIST3}$ for analytes that NIST1 did not report
SD_{rep}	within-vial pooled standard deviation
SD_{het}	among-sample standard deviation
SD_{anl}	between-analyst standard deviation. This is estimated as the residual standard deviation for the linear regression of NIST1's $Mean_x$ values onto NIST3's. For analytes that NIST1 did not report, SD_{anl} is estimated from the regression of the RR35 interlaboratory Median (see below) onto NIST3's $Mean_x$ values. The regression model used to determine SD_{anl} is defined to the right of this block. Details include: the model used, the parameters and standard errors on the parameters, and R^2 for the regression.
SD_{NIST}	$\sqrt{SD_{rep}^2 + SD_{het}^2 + SD_{anl}^2}$, total standard deviation for NIST analyses.
CV_{NIST}	$100*SD_{NIST}/Mean$

- Previous Round Robin Summary Statistics

RR	identifier of the Round Robin in which this serum was distributed
Serum	identifier of the serum
n_p	number of non-NIST laboratories reporting quantitative values for this analyte for this serum in this Round Robin
$Median_p$	median of the reported values. The median is a robust location estimate
eSD_p	0.7421*InterQuartile Range (IQR). The IQR is a robust dispersion estimate. The scale factor 0.7421 is the expected ratio between SD and IQR for normal distributions.

- Round Robin XXXV Summary Statistics

n_n	number of non-NIST laboratories reporting quantitative values for this analyte for this serum in this Round Robin
$Median_n$	median of the reported values
eSD_n	0.7421*InterQuartile Range (IQR)

P(n=p) $$\mathrm{TDIST}\left(\frac{\left|Median_n - Median_p\right|\sqrt{n_n + n_p - 2}}{\sqrt{\left((n_n - 1)eSD_n^2 + (n_p - 1)eSD_p^2\right)\left(\frac{1}{n_n} + \frac{1}{n_p}\right)}},\ n_p+n_n-2,\ 2\ \text{tail}\right)$$

This is the approximate probability that the median in RR35 is the same as it was in the previous RR. Where the hypothesis that $Median_n = Median_p$ could be rejected with 95% confidence, the P(n=p) value would be flagged with an "*". TDIST is Excel®'s student's t function.

P(n<p) $$\mathrm{FDIST}\left(\frac{eSD_n^2}{eSD_o^2},\ n_n-1,\ n_p-1\right)$$

This represents the approximate probability that the interlaboratory variance in RR35 is smaller than it was in the previous RR. Where the hypothesis that $eSD_n < eSD_p$ can be rejected with 95% confidence, the P(n<p) value is flagged with an "*". FDIST is Excel®'s F-distribution function.

SD_{labs} $\sqrt{eSD_n^2 - SD_{NIST}^2}$, the residual non-NIST interlaboratory biases after correction for measurement-, sample-, and NIST-analyst-related sources of variance. When SD_{NIST} is greater than eSD_n, $SD_{labs} = 0$.

CV_{labs} $100*SD_{labs}/Median_n$

- NIST Assigned Values and Uncertainties

  NAV (Mean + Median_n) / 2, our best guess of the "true" analyte level

  NAU $\text{Maximum}\left(0.05*\text{NAV}, \sqrt{\text{SD}^2_{\text{NIST}} + \text{SD}^2_{\text{labs}}}\right)$, our best guess for the "true" interlaboratory standard deviation characterizing measurement, sample heterogeneity, inter-analyst, and interlaboratory sources of variation. When SD_{labs} could not be determined, NAU is estimated as $\text{Maximum}\left(0.10*\text{NAV}, \sqrt{2\ \text{SD}^2_{\text{NIST}}}\right)$.

  CV 100*NAU/NAV

Conclusions: The retinol, α- and γ-tocopherol, total and *trans*-β-carotene, and "lutein" values are fully compatible with the known dilution series ([214] = [212]/2, [211] = [212]/4); NIST's limits of quantification (LOQ) for these analytes are below the levels of serum 211. The retinyl palmitate, total and *trans*-α-carotene, total and *trans*-lycopene, β-cryptoxanthin, and "zeaxanthin" values are compatible with the dilution series for sera 214 and 212, but the serum 211 values are higher than expected; NIST's LOQ for these analytes are therefore somewhere between the levels of sera 214 and 211. The δ-tocopherol values are not compatible with the dilution series; NIST's LOQ for this analyte is at least as high as the level in serum 212.

All median values from RR35 (Median_n) are statistically indistinguishable at the 95% confidence level from the corresponding values (Median_p) determined in RR30 and RR31. Nearly all of the interlaboratory standard deviations from RR35 (eSD_n) are smaller than the corresponding previous values (eSD_p). Most of the few increases can be accounted for by insufficient or contradictory data in the earlier RR: only the α- and γ-tocopherol eSD_n values for serum 212 may have actually increased (9.9 vs. 7.0% and 8.5 vs. 5.3%, respectively). Therefore: 1) none of the sera degraded during storage and 2) the interlaboratory reproducibility has improved somewhat over the past 12 to 18 months.

Systematic constant and/or proportional biases are again apparent between the NIST1 and NIST3 analyses for many analytes. These biases are statistically significant when the standard errors for the intercept "a" and/or slope "b" regression parameters are small relative to the differences between the observed and expected 0.0 (intercept) and 1.0 (slope) values.

To better account for the NIST versus non-NIST sources of variation, the standard estimate of error for the regression has been included into the total NIST standard deviation. In general, this "analyst" or "method" component is greater than or equal to the analytical repeatability and sample heterogeneity components. For low levels of all analytes, it is the dominant component. For total α-carotene and γ-tocopherol in these sera, the NIST analyses are now revealed as being at least as variable as the interlaboratory values. For α-carotene, this probably just reflects the quantification limit: we need sera with higher native "minor" carotenoid levels and/or methods for selectively augmenting their levels. For γ-tocopherol, it is another indication that we need to re-evaluate our methodologies. This modification of the analysis of variance does NOT affect either the NIST assigned value (NAV) or the NIST assigned uncertainty (NAU); it only redistributes part of what was previously attributed to interlaboratory biases onto NIST. The SD_{NIST} can be interpreted as defining NIST's "true" measurement uncertainty.

This memo will be attached as the Appendix to RR35's "Lies, Damn Lies, and Statistics" report.

Table 1
NIST Data and Calculations

Retinol

	NIST1				NIST3			
	211	212	213	214	211	212	213	214
A:1	0.210	0.734	6.63	0.353	0.195	0.736	7.71	0.363
A:2	0.191	0.712	6.86	0.369	0.180	0.725	6.98	0.373
B:1	0.192	0.687	6.63	0.369	0.172	0.684	6.71	0.339
B:2	0.179	0.733	6.42	0.353	0.176	0.683	6.79	0.346
C:1	0.198	0.723	6.57	0.380	0.169	0.702	6.96	0.365
C:2	0.190	0.684	6.45	0.381	0.174	0.711	6.96	0.376
n_x	6	6	6	6	6	6	6	6
$Mean_x$	0.193	0.712	6.59	0.368	0.178	0.707	7.02	0.360
SD_x	0.010	0.022	0.16	0.012	0.009	0.022	0.36	0.015
SD_{repx}	0.010	0.026	0.14	0.010	0.006	0.006	0.30	0.007
SD_{hetx}	0.008	0.010	0.13	0.011	0.009	0.024	0.30	0.016
SD_{NISTx}	0.013	0.028	0.19	0.015	0.011	0.024	0.42	0.017
CV_{NISTx}	6.5	3.9	2.9	4.0	6.1	3.4	6.0	4.7

Retinyl Palmitate

	NIST1				NIST3			
	211	212	213	214	211	212	213	214
A:1					0.01	0.213	0.139	0.074
A:2						0.237	0.113	0.091
B:1						0.203	0.114	0.088
B:2						0.172	0.099	0.097
C:1						0.194	0.106	0.079
C:2						0.209	0.109	0.061
n_x	0	0	0	0	1	6	6	6
$Mean_x$					0.01	0.205	0.113	0.082
SD_x						0.022	0.014	0.013
SD_{repx}						0.017	0.012	0.011
SD_{hetx}						0.019	0.011	0.011
SD_{NISTx}						0.026	0.016	0.016
CV_{NISTx}						13	14	19

	NIST			
n	12	12	12	12
Mean	0.185	0.709	6.81	0.364
SD_{rep}	0.008	0.019	0.23	0.008
SD_{het}	0.009	0.019	0.21	0.013
SD_{anl}	0.014	0.014	0.01	0.014
SD_{NIST}	0.019	0.030	0.32	0.021
CV_{NIST}	10	4.2	4.6	5.7

NIST3=a+b*NIST1

a: -0.039 ±0.009
b: 1.070 ±0.003
R^2: 1.000

	NIST			
n	1	6	6	6
Mean	0.01	0.205	0.113	0.082
SD_{rep}		0.021	0.014	0.009
SD_{het}		0.019	0.011	0.011
SD_{anl}	0.02	0.019	0.019	0.019
SD_{NIST}		0.034	0.026	0.024
CV_{NIST}		17	23	29

NIST3=a+b*Median

a: 0
b: 0.696 ±0.054
R^2: 0.892

RR	XXXI	XXXI	XXX	XXXI
Serum	197	198	191	195
n_p	38	39	41	38
$Median_p$	0.195	0.725	6.84	0.378
eSD_p	0.024	0.067	0.87	0.039

<— Previous Results —>

	XXXI	XXXI	XXX	XXXI
Serum	197	198	191	195
n_p	6	8	10	7
$Median_p$	0.06	0.262	0.156	0.106
eSD_p	0.01	0.069	0.031	0.025

	RRXXXV			
	211	212	213	214
n_n	42	42	40	42
$Median_n$	0.196	0.737	6.93	0.383
eSD_n	0.021	0.068	0.77	0.035
P(n=p)	1.00	0.97	0.98	0.97
P(n<p)	0.81	0.46	0.79	0.77
SD_{labs}	0.008	0.061	0.70	0.028
CV_{labs}	4.2	8.3	10	7.3

<— Current Results —>

	RRXXXV			
	211	212	213	214
n_n	10	11	11	11
$Median_n$	0.05	0.297	0.135	0.126
eSD_n	0.02	0.036	0.022	0.022
P(n=p)	0.92	0.76	0.73	0.68
P(n<p)	0.26	0.97	0.87	0.66
SD_{labs}		0.012	0	0
CV_{labs}		4.1	0	0

NAV	0.190	0.723	6.87	0.374
NAU	0.021	0.068	0.77	0.035
CV	11	9.4	11	9.3

<—— Assignments ——>

	0.251	0.124	0.104
	0.036	0.026	0.024
	14	21	23

Table 1
NIST Data and Calculations

	α-Tocopherol								γ-Tocopherol							
	NIST1				NIST3				NIST1				NIST3			
	211	212	213	214	211	212	213	214	211	212	213	214	211	212	213	214
A:1	3.83	14.8	23.8	7.35	3.27	13.5	22.6	6.63	0.67	2.70	2.97	1.25	0.61	2.38	3.23	1.24
A:2	3.67	14.8	23.8	7.25	2.86	13.7	22.1	6.64	0.70	2.78	3.68	1.51	0.60	2.37	3.19	1.21
B:1	3.61	14.5	23.5	7.68	2.71	12.8	21.6	6.41	0.69	2.75	3.36	1.38	0.55	2.30	3.05	1.18
B:2	3.52	14.1	24.2	7.28	2.88	12.7	21.9	6.49	0.63	2.50	3.21	1.41	0.56	2.30	3.11	1.20
C:1	3.58	14.5	24.7	7.18	3.05	13.1	25.3	7.20	0.66	2.64	3.53	1.36	0.57	2.33	3.33	1.20
C:2	4.09	13.7	22.7	7.03	2.77	13.1	24.4	7.07	0.79	2.87	3.21	1.41	0.56	2.32	3.23	1.18
n_x	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6
$Mean_x$	3.72	14.4	23.8	7.29	2.92	13.2	23.0	6.74	0.69	2.71	3.33	1.39	0.58	2.33	3.19	1.20
SD_x	0.21	0.5	0.7	0.22	0.21	0.4	1.5	0.32	0.06	0.13	0.25	0.08	0.02	0.04	0.10	0.02
SD_{repx}	0.22	0.4	0.9	0.18	0.21	0.1	0.4	0.06	0.06	0.15	0.32	0.11	0.01	0.01	0.05	0.02
SD_{hetx}	0.14	0.4	0.1	0.19	0.14	0.4	1.6	0.35	0.03	0.07	0.04	0.01	0.03	0.04	0.10	0.02
SD_{NISTx}	0.26	0.5	0.9	0.26	0.25	0.4	1.7	0.36	0.07	0.16	0.33	0.11	0.03	0.04	0.11	0.02
CV_{NISTx}	7.0	3.8	3.7	3.6	8.7	3.2	7.3	5.3	10	6.0	9.8	7.8	4.8	1.8	3.5	2.0

	NIST				NIST3=a+b*NIST1	NIST				NIST3=a+b*NIST1
n	12	12	12	12		12	12	12	12	
Mean	3.32	13.8	23.4	7.02	a: -0.73 ±0.32	0.63	2.52	3.26	1.29	a: 0
SD_{rep}	0.21	0.3	0.6	0.13	b: 0.99 ±0.02	0.04	0.10	0.23	0.08	b: 0.91 ±0.03
SD_{het}	0.12	0.4	1.0	0.29	R^2: 0.998	0.04	0.09	0.11	0.04	R^2: 0.976
SD_{anl}	0.34	0.3	0.3	0.34		0.13	0.13	0.13	0.13	
SD_{NIST}	0.42	0.6	1.3	0.47		0.14	0.19	0.29	0.16	
CV_{NIST}	13	4.4	5.4	6.6		22	7.5	8.8	12	

RR	XXXI	XXXI	XXX	XXXI		XXXI	XXXI	XXX	XXXI
Serum	197	198	191	195	<— Previous Results —>	197	198	191	195
n_p	39	40	43	39		15	16	20	15
$Median_p$	3.63	14.2	24.0	7.18		0.71	2.62	3.58	1.35
eSD_p	0.53	1.0	2.9	0.83		0.15	0.14	0.27	0.18

	RRXXXV					RRXXXV			
	211	212	213	214	<— Current Results —>	211	212	213	214
n_n	43	42	43	43		20	19	19	19
$Median_n$	3.62	14.1	24.4	7.10		0.74	2.72	3.62	1.34
eSD_n	0.58	1.4	2.4	0.83		0.14	0.23	0.27	0.10
P(n=p)	1.00	0.99	0.97	0.98		0.95	0.85	0.96	0.98
P(n<p)	0.29	0.03*	0.89	0.51		0.62	0.03*	0.50	0.99
SD_{labs}	0.40	1.3	2.0	0.69		0	0.13	0	0
CV_{labs}	11	9.0	8.2	9.7		0	4.6	0	0
NAV	3.47	13.9	23.9	7.06	<—— Assignments ——>	0.68	2.62	3.44	1.32
NAU	0.58	1.4	2.4	0.83		0.14	0.23	0.29	0.16
CV	17	10	9.9	12		21	8.6	8.3	12

Table 1
NIST Data and Calculations

	δ-Tocopherol								Total β-Carotene							
	NIST1				NIST3				NIST1				NIST3			
	211	212	213	214	211	212	213	214	211	212	213	214	211	212	213	214
A:1					0.04	0.162	0.218	0.07	0.177	0.717	1.65	0.355		0.705	1.97	0.374
A:2						0.175	0.230	0.09	0.175	0.710	1.64	0.384		0.713		
B:1					0.06	0.161	0.213	0.09	0.175	0.729	1.64	0.416		0.681	1.57	0.337
B:2					0.04	0.156	0.216	0.08	0.177	0.749	1.77	0.383		0.693	1.63	0.357
C:1					0.06	0.153	0.212	0.08	0.175	0.785	1.74	0.392		0.671		0.308
C:2					0.04	0.146	0.223	0.09	0.148	0.753	1.64	0.404		0.676	1.61	0.307
n_x	0	0	0	0	5	6	6	6	6	6	6	6	0	6	4	5
$Mean_x$					0.05	0.159	0.219	0.08	0.171	0.741	1.68	0.389		0.690	1.69	0.337
SD_x					0.01	0.010	0.007	0.01	0.011	0.028	0.06	0.021		0.017	0.18	0.029
SD_{repx}					0.01	0.006	0.007	0.01	0.011	0.016	0.07	0.019		0.006	0.03	0.008
SD_{hetx}					0.00	0.010	0.005	0.00	0.008	0.028	0.03	0.017		0.018	0.21	0.033
SD_{NISTx}					0.01	0.011	0.008	0.01	0.014	0.032	0.08	0.025		0.019	0.21	0.034
CV_{NISTx}					21	7.2	3.8	16	8.1	4.3	4.5	6.5		2.7	12	10

	NIST				NIST3=a+b*Median	NIST				NIST3=a+b*NIST1
n	5	6	6	6		6	12	10	11	
Mean	0.05	0.159	0.219	0.08	a: 0.078 ±0.053	0.171	0.715	1.69	0.363	a: -0.082 ±0.015
SD_{rep}	0.01	0.006	0.005	0.01	b: 0.351 ±0.285	0.011	0.012	0.06	0.015	b: 1.056 ±0.014
SD_{het}	0.00	0.010	0.005	0.00	R^2: 0.187	0.009	0.022	0.14	0.025	R^2: 1.000
SD_{anl}	0.07	0.071	0.071	0.07		0.013	0.013	0.01	0.013	
SD_{NIST}	0.07	0.072	0.071	0.07		0.019	0.029	0.15	0.032	
CV_{NIST}	150	45	32	86		11	4.0	8.7	8.8	

RR	XXXI	XXXI	XXX	XXXI		XXXI	XXXI	XXX	XXXI
Serum	197	198	191	195	<— Previous Results —>	197	198	191	195
n_p	0	0	0	0		30	31	35	30
$Median_p$						0.150	0.678	1.65	0.338
eSD_p						0.029	0.076	0.21	0.047

	RRXXXV					RRXXXV			
	211	212	213	214	<— Current Results —>	211	212	213	214
n_n	1	1	1	1		32	32	33	33
$Median_n$	0.05	0.330	0.170	0.01		0.166	0.704	1.69	0.360
eSD_n						0.037	0.057	0.08	0.035
P(n=p)						0.90	0.92	0.96	0.89
P(n<p)						0.09	0.95	1.00	0.95
SD_{labs}						0.032	0.049	0	0.014
CV_{labs}						19	6.9	0	3.8
NAV					<—— Assignments ——>	0.169	0.710	1.69	0.361
NAU						0.037	0.057	0.15	0.035
CV						22	8.0	8.7	9.7

Table 1
NIST Data and Calculations

trans-β-Carotene

	NIST1				NIST3			
	211	212	213	214	211	212	213	214
A:1	0.154	0.65	1.57	0.302		0.64	1.82	0.325
A:2	0.152	0.65	1.47	0.335		0.64	1.57	0.308
B:1	0.152	0.65	1.44	0.343		0.61	1.47	0.295
B:2	0.154	0.65	1.59	0.364		0.62	1.51	0.309
C:1	0.149	0.65	1.42	0.328		0.60	1.47	0.275
C:2	0.134	0.63	1.47	0.356		0.61	1.48	0.276
n_x	6	6	6	6	0	6	6	6
$Mean_x$	0.149	0.65	1.49	0.338		0.62	1.55	0.298
SD_x	0.008	0.01	0.07	0.022		0.02	0.14	0.020
SD_{repx}	0.006	0.01	0.07	0.020		0.00	0.11	0.009
SD_{hetx}	0.007	0.00	0.04	0.018		0.02	0.12	0.021
SD_{NISTx}	0.009	0.01	0.08	0.027		0.02	0.16	0.022
CV_{NISTx}	6.1	1.3	5.5	7.9		3.3	10	7.5

	NIST			
n	6	12	12	12
Mean	0.149	0.63	1.52	0.318
SD_{rep}	0.006	0.01	0.09	0.016
SD_{het}	0.007	0.01	0.08	0.018
SD_{anl}	0.010	0.01	0.01	0.010
SD_{NIST}	0.014	0.02	0.12	0.026
CV_{NIST}	9.4	2.9	8.0	8.3

NIST3=a+b*NIST1

a: -0.077 ±0.012
b: 1.091 ±0.012
R^2: 1.000

RR	XXXI	XXXI	XXX	XXXI
Serum	197	198	191	195
n_p	7	7	10	7
$Median_p$	0.160	0.63	1.44	0.332
eSD_p	0.021	0.01	0.17	0.026

<— Previous Results —>

	RRXXXV			
	211	212	213	214
n_n	11	11	11	11
$Median_n$	0.159	0.64	1.59	0.335
eSD_n	0.022	0.12	0.06	0.041
P(n=p)	0.99	0.95	0.59	0.97
P(n<p)	0.47	0.00*	1.00	0.14
SD_{labs}	0.017	0.12	0	0.032
CV_{labs}	11	19	0	9.5

<— Current Results —>

NAV	0.154	0.64	1.56	0.326
NAU	0.022	0.12	0.12	0.041
CV	14	19	7.9	13

<—— Assignments ——>

Total α-Carotene

	NIST1				NIST3			
	211	212	213	214	211	212	213	214
A:1		0.043	0.046	0.022			0.065	0.020
A:2		0.042	0.046	0.018		0.038	0.064	0.018
B:1		0.046	0.048	0.016		0.042	0.041	0.022
B:2		0.042	0.048	0.016		0.043	0.058	0.023
C:1		0.045	0.050	0.019		0.041	0.049	0.017
C:2		0.042	0.040	0.023		0.042	0.049	0.020
n_x	0	6	6	6	0	5	6	6
$Mean_x$		0.043	0.046	0.019		0.041	0.054	0.020
SD_x		0.002	0.004	0.003		0.002	0.010	0.002
SD_{repx}		0.002	0.004	0.002		0.000	0.007	0.002
SD_{hetx}		0.001	0.001	0.003		0.002	0.009	0.002
SD_{NISTx}		0.002	0.005	0.003		0.002	0.011	0.003
CV_{NISTx}		4.6	9.7	18		5.6	21	14

	NIST			
n	0	11	12	12
Mean		0.042	0.050	0.020
SD_{rep}		0.001	0.007	0.002
SD_{het}		0.003	0.006	0.002
SD_{anl}		0.005	0.005	0.005
SD_{NIST}		0.006	0.011	0.006
CV_{NIST}		15	22	29

NIST3=a+b*NIST1

a: 0
b: 1.064 ±0.074
R^2: 0.843

	XXXI	XXXI	XXX	XXXI
	197	198	191	195
	18	22	21	21
	0.010	0.037	0.026	0.017
	0.006	0.011	0.015	0.004

	211	212	213	214
	22	25	25	25
	0.010	0.036	0.033	0.018
	0.003	0.008	0.010	0.004
	0.97	0.98	0.87	0.95
	1.00	0.90	0.98	0.53
		0.005	0	0
		14	0	0

		0.039	0.042	0.019
		0.008	0.011	0.006
		21	26	30

Table 1
NIST Data and Calculations

trans-α-Carotene

	NIST1				NIST3			
	211	212	213	214	211	212	213	214
A:1							0.03	0.011
A:2						0.02	0.02	0.013
B:1						0.02	0.02	0.012
B:2						0.02	0.02	0.014
C:1						0.03	0.02	0.011
C:2						0.03	0.02	0.011
n_x	0	0	0	0	0	5	6	6
$Mean_x$						0.03	0.02	0.012
SD_x						0.00	0.00	0.001
SD_{repx}						0.00	0.00	0.001
SD_{betx}						0.00	0.00	0.001
SD_{NISTx}						0.00	0.00	0.001
CV_{NISTx}						4.8	14	12

Total Lycopene

	NIST1				NIST3			
	211	212	213	214	211	212	213	214
A:1						0.155	0.206	
A:2						0.159	0.206	0.087
B:1						0.154	0.163	0.088
B:2						0.169	0.194	0.092
C:1						0.158	0.171	0.071
C:2						0.149	0.191	0.089
n_x	0	0	0	0	0	6	6	5
$Mean_x$						0.157	0.189	0.085
SD_x						0.007	0.018	0.008
SD_{repx}						0.007	0.015	0.007
SD_{betx}						0.004	0.015	0.005
SD_{NISTx}						0.008	0.021	0.009
CV_{NISTx}						5.3	11	11

	NIST			
n	0	5	6	6
Mean		0.03	0.02	0.012
SD_{rep}		0.00	0.00	0.001
SD_{bet}		0.00	0.00	0.001
SD_{anl}				
SD_{NIST}				
CV_{NIST}				

	NIST			
n	0	6	6	5
Mean		0.157	0.189	0.085
SD_{rep}		0.008	0.018	0.003
SD_{bet}		0.004	0.015	0.005
SD_{anl}		0.002	0.002	0.002
SD_{NIST}		0.010	0.024	0.006
CV_{NIST}		6.1	13	7.4

NIST3=a+b*Median

a: 0.017 ±0.004
b: 0.948 ±0.031
R^2: 0.998

RR	XXXI	XXXI	XXX	XXXI
Serum	197	198	191	195
n_p	0	0	0	0
$Median_p$				
eSD_p				

<— Previous Results —>

	XXXI	XXXI	XXX	XXXI
Serum	197	198	191	195
n_p	19	22	23	20
$Median_p$	0.02	0.118	0.167	0.062
eSD_p	0.01	0.049	0.045	0.018

	RRXXXV			
	211	212	213	214
n_n	0	0	0	0
$Median_n$				
eSD_n				
P(n=p)				
P(n<p)				
SD_{labs}				
CV_{labs}				

<— Current Results —>

	RRXXXV			
	211	212	213	214
n_n	22	22	22	22
$Median_n$	0.03	0.146	0.183	0.073
eSD_n	0.01	0.054	0.062	0.027
P(n=p)	0.82	0.87	0.93	0.88
P(n<p)	0.16	0.33	0.07	0.03*
SD_{labs}		0.053	0.058	0.027
CV_{labs}		36	32	37

NAV				
NAU				
CV				

<—— Assignments ——>

	0.152	0.186	0.079
	0.054	0.062	0.027
	35	34	35

Table 1
NIST Data and Calculations

trans-Lycopene

	NIST1				NIST3			
	211	212	213	214	211	212	213	214
A:1						0.060	0.091	
A:2						0.064	0.081	0.031
B:1						0.061	0.077	0.031
B:2						0.062	0.080	0.031
C:1						0.058	0.082	0.027
C:2						0.060	0.081	0.031
n_x	0	0	0	0	0	6	6	5
$Mean_x$						0.061	0.082	0.030
SD_x						0.002	0.005	0.002
SD_{repx}						0.002	0.004	0.002
SD_{hetx}						0.002	0.004	0.001
SD_{NISTx}						0.002	0.006	0.002
CV_{NISTx}						4.0	6.9	7.5

β-Cryptoxanthin

	NIST1				NIST3			
	211	212	213	214	211	212	213	214
A:1						0.019	0.047	
A:2						0.018	0.041	0.010
B:1						0.017	0.038	0.006
B:2						0.017	0.039	0.007
C:1						0.014	0.033	0.007
C:2						0.013	0.034	0.006
n_x	0	0	0	0	0	6	6	5
$Mean_x$						0.016	0.039	0.007
SD_x						0.002	0.005	0.002
SD_{repx}						0.001	0.002	0.000
SD_{hetx}						0.002	0.005	0.002
SD_{NISTx}						0.002	0.006	0.002
CV_{NISTx}						15	15	30

	NIST			
n	0	6	6	5
Mean		0.061	0.082	0.030
SD_{rep}		0.002	0.004	0.000
SD_{het}		0.002	0.004	0.001
SD_{anl}		0.004	0.004	0.004
SD_{NIST}		0.004	0.007	0.004
CV_{NIST}		7.2	8.3	13

NIST3=a+b*Median

a: -0.009 ±0.007
b: 0.838 ±0.083
R^2: 0.981

	NIST			
n	0	6	6	5
Mean		0.016	0.039	0.007
SD_{rep}		0.001	0.002	0.000
SD_{het}		0.002	0.005	0.002
SD_{anl}		0.001	0.001	0.001
SD_{NIST}		0.002	0.006	0.002
CV_{NIST}		15	15	31

NIST3=a+b*Median

a: -0.005 ±0.001
b: 0.817 ±0.024
R^2: 0.998

RR	XXXI	XXXI	XXX	XXXI	<— Previous Results —>	XXXI	XXXI	XXX	XXXI
Serum	197	198	191	195		197	198	191	195
n_p	1	1		1		12	15	18	13
$Median_p$	0.02	0.070		0.041		0.008	0.024	0.050	0.013
eSD_p						0.003	0.008	0.008	0.007

	RRXXXV				<— Current Results —>	RRXXXV			
	211	212	213	214		211	212	213	214
n_n	6	6	6	6		11	15	15	14
$Median_n$	0.02	0.087	0.107	0.046		0.010	0.026	0.053	0.014
eSD_n	0.01	0.017	0.013	0.004		0.004	0.007	0.008	0.005
P(n=p)						0.77	0.92	0.91	0.95
P(n<p)						0.32	0.65	0.42	0.94
SD_{labs}		0.016	0.011	0.002			0.007	0.005	0.004
CV_{labs}		19	11	4.6			26	10	30
NAV		0.074	0.094	0.038	<—— Assignments ——>		0.021	0.046	0.011
NAU		0.017	0.013	0.004			0.007	0.008	0.005
CV		23	14	12			34	18	44

Table 1
NIST Data and Calculations

"Lutein"

	NIST1				NIST3			
	211	212	213	214	211	212	213	214
A:1					0.010	0.048	0.020	
A:2					0.009	0.051	0.024	0.020
B:1					0.013	0.050	0.024	0.025
B:2					0.010	0.050	0.020	0.023
C:1					0.011	0.051	0.023	0.027
C:2					0.011	0.049	0.021	0.026
n_x	0	0	0	0	6	6	6	5
$Mean_x$					0.011	0.050	0.022	0.024
SD_x					0.001	0.001	0.002	0.003
SD_{repx}					0.001	0.001	0.002	0.001
SD_{hetx}					0.001	0.001	0.000	0.004
SD_{NISTx}					0.001	0.001	0.002	0.004
CV_{NISTx}					13	3.0	11	15

	NIST			
n	6	6	6	5
Mean	0.011	0.050	0.022	0.024
SD_{rep}	0.001	0.001	0.003	0.001
SD_{het}	0.001	0.001	0.000	0.004
SD_{anl}	0.001	0.001	0.001	0.001
SD_{NIST}	0.002	0.002	0.003	0.004
CV_{NIST}	18	3.2	12	16

NIST3=a+b*Median

a: -0.006 ±0.001
b: 1.034 ±0.029
R^2: 0.997

RR	XXXI	XXXI	XXX	XXXI
Serum	197	198	191	195
n_p	6	8	13	7
$Median_p$	0.011	0.057	0.035	0.020
eSD_p	0.002	0.019	0.018	0.003

<— Previous Results —>

	RRXXXV			
	211	212	213	214
n_n	9	10	10	10
$Median_n$	0.016	0.054	0.026	0.030
eSD_n	0.005	0.007	0.007	0.004
P(n=p)	0.53	0.92	0.79	0.21
P(n<p)	0.03*	1.00	1.00	0.24
SD_{labs}	0.005	0.007	0.006	0.001
CV_{labs}	29	12	24	3.1

<— Current Results —>

NAV	0.013	0.052	0.024	0.027
NAU	0.005	0.007	0.007	0.004
CV	37	13	28	15

<—— Assignments ——>

"Zeaxanthin"

	NIST1				NIST3			
	211	212	213	214	211	212	213	214
A:1						0.017	0.013	
A:2						0.021	0.012	0.006
B:1						0.016	0.008	0.010
B:2						0.018	0.007	0.010
C:1						0.022	0.009	0.012
C:2						0.018	0.006	0.011
n_x	0	0	0	0	0	6	6	5
$Mean_x$						0.019	0.009	0.010
SD_x						0.002	0.003	0.002
SD_{repx}						0.002	0.001	0.001
SD_{hetx}						0.001	0.003	0.003
SD_{NISTx}						0.003	0.003	0.003
CV_{NISTx}						14	35	27

	NIST			
n	0	6	6	5
Mean		0.019	0.009	0.010
SD_{rep}		0.002	0.001	0.001
SD_{het}		0.001	0.003	0.003
SD_{anl}		0.000	0.000	0.000
SD_{NIST}		0.002	0.003	0.003
CV_{NIST}		12	33	27

NIST3=a+b*Median

a: -0.007 ±0.001
b: 1.324 ±0.082
R^2: 0.992

	XXXI	XXXI	XXX	XXXI
	197	198	191	195
n_p	4	4	6	4
$Median_p$	0.009	0.025	0.011	0.017
eSD_p			0.006	

211	212	213	214
6	6	6	6
0.006	0.019	0.012	0.012
0.003	0.004	0.004	0.002
		0.85	
		0.88	
	0.003	0.002	0
	17	16	0

0.019	0.011	0.011
0.004	0.004	0.003
21	34	24

Table 2
Summary of NIST Assigned Values and Uncertainties

	213			212			214			211		
Analyte	NAV	NAU	CV	NAV	NAU	CV	NAV	NAU	CV	NAV	NAU	CV
Retinol	6.87	0.77	11	0.723	0.068	9.4	0.374	0.035	9.3	0.190	0.021	11
Retinyl Palmitate	0.124	0.025	20	0.251	0.036	14	0.102	0.027	26	0.03		
α-Tocopherol	23.9	2.4	9.9	13.9	1.4	10	7.06	0.83	12	3.47	0.58	17
γ-Tocopherol	3.44	0.29	8.3	2.62	0.23	8.6	1.32	0.16	12	0.68	0.14	21
δ-Tocopherol	0.194	0.100	52	0.245	0.101	41	0.05	0.10	210	0.05	0.10	210
Total β-Carotene	1.69	0.15	8.7	0.710	0.057	8.0	0.361	0.035	9.7	0.169	0.037	22
trans-β-Carotene	1.56	0.12	7.9	0.64	0.12	19	0.326	0.041	13	0.154	0.022	14
Total α-Carotene	0.042	0.011	26	0.039	0.008	21	0.019	0.006	30			
trans-α-Carotene	0.02			0.03			0.012					
Total Lycopene	0.186	0.062	34	0.152	0.054	35	0.079	0.027	35			
trans-Lycopene	0.094	0.013	14	0.074	0.017	23	0.038	0.004	12			
β-Cryptoxanthin	0.046	0.008	18	0.021	0.007	34	0.011	0.005	44			
"Lutein"	0.024	0.007	28	0.052	0.007	13	0.027	0.004	15	0.013	0.005	37
"Zeaxanthin"	0.011	0.004	34	0.019	0.004	21	0.011	0.003	24			

Appendix K. "All-Lab Report" for RR35

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

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

The data summary in the "All-Lab Report" has been altered to ensure confidentiality of identification codes assigned to laboratories. The only attributed results are those reported by NIST. The NIST results are not used in the assessment of the consensus summary results of the study.

Round Robin XXXV Laboratory Results

Values in µg/mL

	Retinol				Retinyl Palmitate				α-Tocopherol				γ-Tocopherol				δ-Tocopherol			
Lab	211	212	213	214	211	212	213	214	211	212	213	214	211	212	213	214	211	212	213	214
FSV-BA	0.213	0.740	7.21	0.390	0.016	0.143	0.079	0.063	2.67	14.0	25.8	7.41	0.64	2.79	3.85	1.34				
FSV-BD	0.182	0.733	5.74	0.375					3.70	12.8	22.5	6.60								
FSV-BE	0.183	0.718	2.49	0.354					3.24	13.3	23.6	6.61	0.69	2.54	3.51	1.29				
FSV-BF	0.200	0.780	6.87	0.430					3.50	15.3	28.7	7.30	0.60	2.70	3.80	1.30				
FSV-BG	0.200	0.780	7.80	0.370	0.043	0.282	0.146	0.113	3.36	13.3	21.6	6.01								
FSV-BH	0.156	0.680	6.96	0.364	0.053	0.303	0.133	0.117	4.05	14.6	24.9	7.80	0.82	2.90	3.84	1.48				
FSV-BI	0.183	0.796	8.14	0.395	0.050	0.337	0.199	0.148	3.81	15.7	27.3	6.59	0.73	2.85	3.85	1.38				
FSV-BJ	0.197	0.739	5.92	0.419	*nd*	0.241	0.133	0.126	4.81	15.0	22.5	8.19	1.16	2.93	3.62	1.75				
FSV-BK	0.180	0.750	7.30	0.388					2.66	14.3	25.4	7.50								
FSV-BM	0.184	0.689	7.61	0.363					3.40	14.7	25.1	6.70								
FSV-BN	0.200	0.938	9.31	0.459	0.059	0.372	0.188	0.187	4.18	15.3	27.1	7.58	0.55	2.21	3.29	1.08				
FSV-BO	0.094	0.622	5.29	0.316					3.06	14.0	25.2	7.55								
FSV-BP	0.248	0.811		0.455					4.40	15.8	27.9	8.54								
FSV-BQ	0.180	0.760	6.54	0.400					2.80	10.1	21.5	6.00								
FSV-BR	0.194	0.761	6.99	0.388																
FSV-BS																				
FSV-BT	!0.34	0.718	7.06	!0.41	!0.07	0.297	0.135	!0.13	3.57	12.9	23.0	6.85	0.63	2.72	3.54	1.29	0.047	0.330	0.170	0.015
FSV-BU	0.223	0.783	7.78	0.385					2.88	13.8	24.4	6.07	0.57	2.43	3.67	1.30				
FSV-BV									4.16	19.5	17.1	9.24	0.86	3.43	4.21	1.87				
FSV-BY	0.187	0.734	6.94	0.369	0.064	0.256	0.126	0.128	3.49	14.2	24.1	7.14	0.68	2.68	3.55	1.34				
FSV-BZ									5.30	16.1	26.0	9.80	4.00	3.00	3.50	3.00				
FSV-CA	0.149	0.580	5.96	0.309					3.76	15.2	27.2	8.00								
FSV-CB	0.279	0.844	8.01	0.468					6.69	15.2	22.9	9.05								
FSV-CD	0.201	0.832	8.09	0.391	0.049	0.260	0.114	0.111	4.38	17.1	28.2	8.06	0.65	2.52	3.17	1.22				
FSV-CH	0.172	0.662	6.55	0.340					3.15	12.2	24.5	7.04	0.65	2.62	3.85	1.28				
FSV-CK	0.238	0.717	6.94	0.356					4.40	13.4	19.6	6.44	0.82	2.76	3.38	1.31				
FSV-CM									<4.0	14.6	26.7	7.50								
FSV-CN	0.223	0.754	6.65	0.403					3.48	15.0	22.8	7.27	0.65	2.60	3.16	1.19				
FSV-CP									3.29	10.3	17.1	5.36	1.79	1.79	2.78	0.93				
FSV-CQ	0.169		5.95	0.328					3.65		26.4	6.93								
FSV-CR	0.220	0.820	7.88	0.420					3.70	13.4	25.8	7.30								
FSV-CT	0.170	0.735	8.28	0.336					3.45	13.5	27.1	6.91								
FSV-CU	0.193	0.679	6.21	0.366	0.032	0.299	0.164	0.164	3.40	14.9	25.0	7.08								
FSV-CV	0.191	0.690	7.23	0.375					3.38	15.1	24.3	7.10								
FSV-CX	0.210	0.690	6.47	0.460	0.030	0.300	0.080	0.100	3.89	14.0	25.3	7.90	0.83	2.74	3.71	1.64				
FSV-CY	0.170	0.770	5.2?	0.380					3.89	15.0	25.0	7.59								
FSV-DA	0.198	0.730	6.89	0.396	0.062	0.250	0.153	0.105	3.73	13.5	24.2	7.05	0.74	2.60	3.43	1.33				
FSV-DB	0.240	0.930	7.09	0.330					3.54	16.9	22.9	5.73								
FSV-DJ	0.200	0.840	Hi?	0.420					3.80	15.3	22.9	7.80								
FSV-DK	0.190	0.800	6.92	0.410					3.62	13.9	24.3	6.89								
FSV-DM	0.125	0.477	4.77	0.263					2.86	13.1	22.5	6.69								
FSV-DP	0.188	0.729	7.29	0.389																
FSV-DS	0.340	0.510	4.72	0.410					5.07	12.7	21.4	8.27								
FSV-DX	0.207	0.692	6.54	0.382					3.19	13.5	21.4	6.66								
FSV-EC	0.193	0.757	6.37	0.377					3.44	12.7	21.4	6.73								
FSV-EH	0.210	0.788	7.75	0.386					4.38	17.1	28.8	8.39	0.66	2.91	3.83	1.36				
FSV-EK	0.228	0.845	7.77	0.430					4.30	15.2	24.0	8.32	0.83	2.33	3.04	1.37				
FSV-EL	0.210	0.680	6.26	0.350																
FSV-FC	0.218	0.707	6.60	0.382					4.58				0.74							
FSV-FD	0.210	0.771	6.85	0.420					3.24	13.0	18.1	6.61								
n	44	44	42	44	10	12	12	11	45	44	45	45	22	21	21	21	1	1	1	1
Min	0.094	0.477	2.49	0.263	0.016	0.143	0.079	0.063	2.66	10.1	17.1	5.36	0.55	1.79	2.78	0.93				
Median	0.198	0.740	6.93	0.385	0.050	0.289	0.134	0.117	3.62	14.2	24.3	7.14	0.71	2.70	3.55	1.33	0.05	0.33	0.17	0.01
Max	0.340	0.938	9.31	0.468	0.064	0.372	0.199	0.187	6.69	19.5	28.8	9.80	4.00	3.43	4.21	3.00				
eSD	0.022	0.071	0.91	0.035	0.016	0.047	0.029	0.018	0.56	1.4	2.5	0.78	0.14	0.24	0.38	0.07				
eCV	11	10	13	9	33	16	22	15	15	10	10	11	20	9	11	6				
NISTa	0.193	0.712	6.59	0.368					3.72	14.4	23.8	7.30	0.69	2.71	3.33	1.39				
NISTb	0.178	0.707	7.02	0.360	0.010	0.205	0.113	0.082	2.92	13.2	23.0	6.74	0.58	2.33	3.19	1.20	0.05	0.16	0.22	0.08
NAV	0.192	0.725	6.87	0.375	0.050	0.289	0.134	0.117	3.47	14.0	23.8	7.08	0.67	2.61	3.40	1.31				
NAU	0.022	0.072	0.85	0.038	0.016	0.047	0.029	0.018	0.65	1.4	2.5	0.83	0.15	0.32	0.40	0.16				

K2

Round Robin XXXV Laboratory Results

Values in µg/mL

	Total β-Carotene				trans-β-Carotene				Total cis-β-Carotene			
Lab	211	212	213	214	211	212	213	214	211	212	213	214
FSV-BA	0.168	0.704	1.69	0.386	0.159	0.671	1.62	0.361	0.009	0.033	0.068	0.025
FSV-BD												
FSV-BE	0.129	0.759	1.88	0.361								
FSV-BF	0.237	0.672	1.70	0.399								
FSV-BG	0.212	0.712	1.67	0.374								
FSV-BH	0.150	0.689	1.60	0.341	0.140	0.642	1.50	0.315	0.010	0.047	0.097	0.026
FSV-BI	0.147	0.740	1.75	0.360								
FSV-BJ	0.176	0.746	1.72	0.385								
FSV-BK												
FSV-BM												
FSV-BN	0.213	0.818	1.68	0.411	0.159	0.690	1.53	0.335	0.054	0.124	0.150	0.076
FSV-BO	0.131	0.656	1.69	0.358								
FSV-BP	0.120	0.518	1.29	0.377								
FSV-BQ	0.190	1.120	2.63	0.500								
FSV-BR												
FSV-BS	*0.225*	*0.477*	*2.04*	*0.284*	0.203	0.451	2.02	0.264	0.022	0.026	0.022	0.020
FSV-BT	0.167	0.686	1.70	0.356	0.153	0.641	1.59	0.333	0.013	0.045	0.108	0.024
FSV-BU	0.132	0.681	1.41	0.298								
FSV-BV	*>0.179*	*>0.779*	*>1.639*	*>0.409*	0.179	0.779	1.64	0.409				
FSV-BY	0.159	0.655	1.63	0.330								
FSV-BZ	*>0.225*	*>0.423*	*>1.78*	*>0.466*	0.225	0.423	1.78	0.466	0.034	0.073	0.140	0.060
FSV-CA												
FSV-CB												
FSV-CD	0.177	0.634	1.36	0.360								
FSV-CH	0.118	0.606	1.41	0.270								
FSV-CK	0.070	0.663	1.53	0.334								
FSV-CM												
FSV-CN	*>0.152*	*>0.603*	*>1.698*	*>0.342*	0.152	0.603	1.70	0.342				
FSV-CP	0.155	0.572	1.67	0.373								
FSV-CQ	0.060		3.11	0.294								
FSV-CR												
FSV-CT	0.155	0.692	1.72	0.334								
FSV-CU	0.195	0.704	1.62	0.365	0.182	0.660	1.57	0.339	*0.013*	*0.044*	*0.045*	*0.026*
FSV-CV	0.181	0.730	1.63	0.369								
FSV-CX	0.180	0.920	2.15	0.440								
FSV-CY		0.790	1.80	0.250								
FSV-DA	0.176	0.662	1.74	0.288	0.148	0.597	1.61	0.249	0.028	0.065	0.130	0.039
FSV-DB	0.150	0.850	1.86	0.340								
FSV-DJ												
FSV-DK	0.198	0.722	1.73	0.381								
FSV-DM	0.099	0.647	1.52	0.317								
FSV-DP												
FSV-DS	0.206	0.671	0.15	0.360								
FSV-DX	*>0.126*	*>0.546*	*>1.046*	*>0.286*	0.126	0.546	1.05	0.286				
FSV-EC	0.166	0.745	1.63	0.328								
FSV-EH	0.202	0.756	1.72	0.395	0.177	0.680	1.58	0.351	*0.025*	*0.076*	*0.143*	*0.044*
FSV-EK	0.196	0.682	1.67	0.369								
FSV-EL												
FSV-FC	0.144	0.724	1.73	0.369								
FSV-FD	0.170	0.720	1.68	0.330								
n	34	34	35	35	12	12	12	12	9	9	9	9
Min	0.060	0.477	0.15	0.250	0.126	0.423	1.05	0.249	0.009	0.026	0.022	0.020
Median	0.167	0.698	1.69	0.360	0.159	0.642	1.60	0.337	0.022	0.047	0.108	0.026
Max	0.237	1.120	3.11	0.500	0.225	0.779	2.02	0.466	0.054	0.124	0.150	0.076
eSD	0.038	0.062	0.09	0.039	0.027	0.062	0.08	0.034	0.013	0.027	0.052	0.009
eCV	23	9	5	11	17	10	5	10	61	57	48	33
NISTa	0.171	0.741	1.68	0.389	0.149	0.646	1.49	0.338	*0.022*	*0.095*	*0.186*	*0.051*
NISTb	*nq*	0.690	1.70	0.337	*nq*	0.620	1.56	0.298	*nq*	*0.070*	*0.140*	*0.039*
NAV	0.169	0.707	1.69	0.363	0.154	0.637	1.56	0.328	0.022	0.064	0.133	0.036
NAU	0.036	0.095	0.21	0.053	0.022	0.066	0.16	0.040	0.011	0.039	0.087	0.024

K3

Round Robin XXXV Laboratory Results

Values in µg/mL

	Total α-Carotene				Total Lycopene				trans-Lycopene				β-Cryptoxanthin			
Lab	211	212	213	214	211	212	213	214	211	212	213	214	211	212	213	214
FSV-BA	0.009	0.040	0.036	0.019					0.015	0.074	0.100	0.045	0.075	0.031	0.073	0.051
FSV-BD																
FSV-BE																
FSV-BF	0.015	0.049	0.040	0.020	0.029	0.203	0.253	0.092								
FSV-BG	0.014	0.036	0.038	0.018	0.033	0.164	0.174	0.072								
FSV-BH	0.007	0.038	0.029	0.018	0.029	0.144	0.172	0.070					*nd*	0.024	0.052	*nd*
FSV-BI	0.007	0.039	0.035	0.018	0.021	0.096	0.120	0.048					0.008	0.025	0.052	0.011
FSV-BJ	0.032	0.059	0.048	0.038	0.068	0.228	0.264	0.124								
FSV-BK																
FSV-BM																
FSV-BN	0.011	0.060	0.048	0.047	0.066	0.229	0.218	0.104	0.031	0.097	0.096	0.047		0.032	0.055	0.032
FSV-BO	0.006	0.031	0.033	0.014	0.029	0.148	0.214	0.074					*nd*	0.015	0.033	0.009
FSV-BP	0.010	0.036	0.034	0.025	0.041	0.200	0.228	0.116					0.003	0.029	0.061	0.014
FSV-BQ																
FSV-BR																
FSV-BS	0.026	0.045	0.088	0.029	0.073	0.051	0.087	0.042					0.057	0.027	0.063	0.027
FSV-BT	0.007	0.027	0.030	0.013	0.028	0.140	0.154	0.073	0.021	0.098	0.123	0.051	0.011	0.033	0.057	0.019
FSV-BU					0.016	0.120	0.150	0.050								
FSV-BV	0.009	0.039	0.026	0.020	0.079	0.292	0.303	0.142					0.013	0.020	0.018	0.018
FSV-BY	0.010	0.039	0.040	0.019	0.028	0.124	0.168	0.062					0.007	0.026	0.038	0.013
FSV-BZ	0.022	0.013	0.033	0.034	0.026	0.014	0.323	0.201								
FSV-CA																
FSV-CB																
FSV-CD	0.007	0.033	0.020	0.017	0.032	0.122	0.134	0.066					0.003	0.012	0.030	0.006
FSV-CH	*nd*	0.026	0.024	0.012	0.019	0.099	0.120	0.053								
FSV-CK	0.005	0.049	0.050	0.023	0.022	0.250	0.378	0.131					0.003	0.039	0.053	0.016
FSV-CM																
FSV-CN	*nd*	*nd*	*nd*	*nd*	*nd*	0.106	0.177	*nd*								
FSV-CP	0.008	0.028	0.026	0.018	0.039	0.122	0.173	0.073					0.006	0.021	0.046	0.013
FSV-CQ																
FSV-CR																
FSV-CT																
FSV-CU																
FSV-CV																
FSV-CX	<0.01	0.020	0.020	0.010	0.030	0.150	0.170	0.070					0.010	0.020	0.050	0.010
FSV-CY																
FSV-DA	0.011	0.037	0.037	0.017	0.031	0.137	0.356	0.051	0.015	0.066	0.107	0.025	0.010	0.017	0.617	0.014
FSV-DB																
FSV-DJ																
FSV-DK	*nd*	0.032	0.020	0.018												
FSV-DM	0.004	0.020	0.020	0.011	0.027	0.138	0.161	0.070								
FSV-DP																
FSV-DS																
FSV-DX	0.010	0.025	0.016	0.012					0.044	0.138	0.125	0.083				
FSV-EC																
FSV-EH	0.007	0.034	0.027	0.016	0.049	0.153	0.191	0.083	0.027	0.077	0.106	0.044	*nd*	0.028	0.054	0.012
FSV-EK																
FSV-EL																
FSV-FC	0.005	0.032	0.025	0.015	0.036	0.180	0.223	0.091								
FSV-FD	0.010	0.038	0.016	0.014												
n	23	26	26	26	23	24	24	23	6	6	6	6	12	16	16	15
Min	0.004	0.013	0.016	0.010	0.016	0.014	0.087	0.042	0.015	0.066	0.096	0.025	0.003	0.012	0.018	0.006
Median	0.009	0.036	0.032	0.018	0.030	0.142	0.176	0.073	0.024	0.087	0.107	0.046	0.009	0.026	0.052	0.014
Max	0.032	0.060	0.088	0.047	0.079	0.292	0.378	0.201	0.044	0.138	0.125	0.083	0.075	0.039	0.617	0.051
eSD	0.003	0.007	0.010	0.005	0.009	0.043	0.062	0.028	0.012	0.017	0.013	0.005	0.005	0.008	0.011	0.006
eCV	33	19	33	27	30	30	35	39	49	20	12	12	54	32	21	42
NISTa	*nd*	0.043	0.046	0.019												
NISTb	*nq*	0.041	0.054	0.020	*nq*	0.157	0.189	0.085	*nq*	0.061	0.082	0.030	*nq*	0.016	0.039	0.007
NAV		0.039	0.041	0.019		0.150	0.182	0.079		0.074	0.094	0.038		0.021	0.046	0.011
NAU		0.012	0.017	0.006		0.048	0.056	0.027		0.026	0.028	0.015		0.010	0.017	0.007

K4

Round Robin XXXV Laboratory Results

Values in µg/mL

Lab	Lutein 211	Lutein 212	Lutein 213	Lutein 214	Zeaxanthin 211	Zeaxanthin 212	Zeaxanthin 213	Zeaxanthin 214	Lutein&Zeaxanthin 211	Lutein&Zeaxanthin 212	Lutein&Zeaxanthin 213	Lutein&Zeaxanthin 214
FSV-BA												
FSV-BD												
FSV-BE												
FSV-BF												
FSV-BG												
FSV-BH	*nd*	0.035	0.025	0.031	*nd*	*nd*	*nd*	*nd*	*nd*	*0.035*	*0.025*	*0.031*
FSV-BI	0.014	0.054	0.026	0.026	0.004	0.023	0.017	0.014	0.021	0.077	0.046	0.040
FSV-BJ												
FSV-BK												
FSV-BM												
FSV-BN	0.017	0.048	0.026	0.028	0.013	0.031	0.013	0.010	*0.030*	*0.078*	*0.039*	*0.038*
FSV-BO									0.014	0.048	0.025	0.027
FSV-BP												
FSV-BQ												
FSV-BR												
FSV-BS	0.078	0.053	0.186	0.222								
FSV-BT	0.022	0.059	0.029	0.031	0.009	0.017	0.030	0.012	0.030	0.076	0.059	0.043
FSV-BU												
FSV-BV									0.020	0.086	0.016	0.044
FSV-BY	0.011	0.045	0.026	0.023	0.005	0.017	0.011	0.009	*0.016*	*0.062*	*0.037*	*0.032*
FSV-BZ	0.032	0.055	0.040	0.040								
FSV-CA												
FSV-CB												
FSV-CD									0.028	0.101	0.056	0.056
FSV-CH												
FSV-CK									0.028	0.087	0.045	0.047
FSV-CM												
FSV-CN												
FSV-CP									0.021	0.072	0.046	0.046
FSV-CQ												
FSV-CR												
FSV-CT	0.015	0.048	0.037	0.025								
FSV-CU												
FSV-CV												
FSV-CX									0.020	0.070	0.040	0.040
FSV-CY												
FSV-DA	0.016	0.064	0.024	0.032	0.007	0.021	0.011	0.013	*0.023*	*0.085*	*0.035*	*0.045*
FSV-DB												
FSV-DJ												
FSV-DK												
FSV-DM									0.018	0.063	0.034	0.037
FSV-DP												
FSV-DS												
FSV-DX									0.014	0.054	0.015	0.026
FSV-EC												
FSV-EH	0.015	0.058	0.026	0.028	0.004	0.018	0.012	0.039	*0.019*	*0.076*	*0.037*	*0.067*
FSV-EK												
FSV-EL												
FSV-FC												
FSV-FD									0.016	0.064	0.034	0.033
n	9	10	10	10	6	6	6	6	15	16	16	16
Min	0.011	0.035	0.024	0.023	0.004	0.017	0.011	0.009	0.014	0.035	0.015	0.026
Median	0.016	0.054	0.026	0.030	0.006	0.019	0.012	0.012	0.020	0.074	0.037	0.040
Max	0.078	0.064	0.186	0.222	0.013	0.031	0.030	0.039	0.030	0.101	0.059	0.067
eSD	0.003	0.008	0.002	0.004	0.003	0.004	0.002	0.003	0.006	0.016	0.012	0.009
eCV	19	15	9	15	52	18	15	24	30	22	33	23
NISTa												
NISTb	0.011	0.050	0.022	0.024	*nq*	0.019	0.009	0.010	*>0.011*	*0.069*	*0.031*	*0.034*
NAV	0.013	0.052	0.024	0.027		0.019	0.011	0.011	0.016	0.071	0.034	0.036
NAU	0.006	0.012	0.007	0.008		0.006	0.005	0.005	0.009	0.016	0.011	0.011

K5

Round Robin XXXV Laboratory Results

Analytes Reported By One Laboratory

Values in µg/mL

Analyte	Code	211	212	213	214
cis-Lutein&Zeaxanthin	FSV-BT	0.018	0.052	0.021	0.029
Total Carotenoids	FSV-BT	0.284	1.084	2.080	0.571
α-Cryptoxanthin	FSV-BT	0.006	0.013	0.012	0.007
trans-α-Carotene	NISTb	*nq*	0.02	0.02	0.01

Legend

Term	Definition
n	Number of (non-NIST) quantitative values reported for this analyte
Min	Minimum (non-NIST) quantitative value reported
Median	Median (non-NIST) quantitative value reported
Max	Maximum (non-NIST) quantitative value reported
eSD	Estimated standard deviation, calculated from the median absolute deviation from the median of the non-NIST results
eCV	Coefficient of Variation for (non-NIST) results: 100*eSD/Median
NAV	NIST Assigned Value, our estimate of the "true" analyte concentration
NAU	NIST Assigned Uncertainty, our estimate of the total (serum hetergeniety and inter- and intra-laboratory) standard deviation For details on how we assign these quantities, see the "Analysis of Results."
nd	Not detected (i.e., no detectable peak for analyte)
nq	Detected but not quantitatively determined
>x	Concentration greater than or equal to x
!	Discrepant value: heterogeneous serum, damaged sample, malfunction, etc.
italics	Not explicitly reported but calculated by NIST from reported values

K6

Round Robin XXXV Laboratory Results
Comparability Summary

Lab	R	aT	gT	bC	tbC
FSV-BA	1	2	1	1	
FSV-BD	2	1	2	1	
FSV-BE	4	1		2	
FSV-BF	2	2			
FSV-BG	2	2			
FSV-BH	2	1	1	2	
FSV-BI	2	2		4	
FSV-BJ	2	2	1	2	
FSV-BK	1	2	2	1	1
FSV-BM	1	1	2	1	1
FSV-BN	3	2	2	3	
FSV-BO	4	1	4		3
FSV-BP	3	2		3	
FSV-BQ	1	3			
FSV-BR	1		3	1	
FSV-BS					
FSV-BT	1	1		1	
FSV-BU	2	2		1	2
FSV-BV		4	4	2	
FSV-BY	1	1	2	2	
FSV-BZ		4		2	
FSV-CA	2	2	2	3	
FSV-CB	4	4			
FSV-CD	2	3		2	
FSV-CH	1	2	2	2	1
FSV-CK	3	2		3	3
FSV-CM		2		1	
FSV-CN	2	1		2	
FSV-CP		3		4	
FSV-CQ	2	2	1	1	1
FSV-CR	2	1		1	
FSV-CT	2	2	1	1	
FSV-CU	1	1			
FSV-CV	1	1		4	
FSV-CX	3	1			
FSV-CY	1	1			
FSV-DA	1	1			
FSV-DB	3	2			3
FSV-DJ	2	1	2	1	
FSV-DK	2	1	1	2	
FSV-DM	4	1		1	
FSV-DP	1			1	
FSV-DS	4	3		2	
FSV-DX	1	1	1	2	
FSV-EC	1	1	1	2	2
FSV-EH	2	3			
FSV-EK	2	2			
FSV-EL	1		3		2
FSV-FC	2	2	2	1	1
FSV-FD	2	3	1		1
NISTa	1	1	1	1	1
NISTb	1	1	1	1	1
n	45	46	22	35	12

Label	Definition
Lab	laboratory number
R	"Standard Score" for Retinol
aT	"Standard Score" for α-Tocopherol
gT	"Standard Score" for γ-Tocopherol
bC	"Standard Score" for Total β-Carotene
tbC	"Standard Score" for trans-β-Carotene
n	number of (non-NIST) laboratories providing data for this analyte

"Standard Score"

Given that our knowledge of the shape, location, and width of the measurement distributions is approximate and that a limited number of labs are involved, we summarize comparability with the following four-level "Standard Score" (StS)…

StS	Definition	
1	All StV within $\pm t\,(1\text{-}0.683,n\text{-}1)$	{i.e., ±1 SD}
2	All StV within $\pm t\,(1\text{-}0.954,n\text{-}1)$	{i.e., ±2 SD}
3	All StV within $\pm t\,(1\text{-}0.997,n\text{-}1)$	{i.e., ±3 SD}
4	At least one StV > $\pm t\,(1\text{-}0.997,n\text{-}1)$	{i.e., >3 SD}

where:	
StV	Standardized Value, the distance in standard deviation units your value is from the "true" concentration: StV = (your value - NAV) / NAU
NAV	NIST Assigned Value, our estimate of the "true" analyte concentration
NAU	NIST Assigned Uncertainty, our estimate of the total (serum heterogeniety and iter- and intra-laboratory) measurement standard deviation.
$t\,(1\text{-}\alpha,n\text{-}1)$	Two-tailed Student's t for coverage of ±1, ±2, and ±3 NAU about NAV, assuming a normal population of size n

For details on the NIST Assigned quantities, see this Round Robin's "Report of (Meta)Analysis."

StS	% Observed					Expected
1	36	41	41	43	50	68.2 %
2	42	39	41	37	25	27.3 %
3	11	13	9	11	25	4.3 %
4	11	7	9	9	0	0.3 %

These are the observed and normal-population-expected proportions of each Standard Score (StS), based upon each laboratory's largest StV for the four sera.

K7

Appendix L. Representative "Individualized Report" for RR35

Each participant in RR35 received an "Individualized Report" reflecting their reported results. Each report included a detailed analysis of the results they reported for some or all of the following analytes:

- Retinol
- Retinol palmitate
- α-Tocopherol
- γ-Tocopherol
- Total β-Carotene
- *trans*-β-Carotene
- Total α-Carotene
- Total Lycopene
- β-Cryptoxanthin
- Lutein
- Zeaxanthin
- Lutein & Zeaxanthin

The following 8 pages are the "Individualized Report" for the analytes evaluated by participant FSV-BA.

Individualized Round Robin XXXV Report to: FSV-BA

Your Data, NIST Assigned Values, and %Differences

	Serum 211				Serum 212				Serum 213				Serum 214			
Analyte	You	NAV	%Δ	n	You	NAV	%Δ	n	You	NAV	%Δ	n	You	NAV	%Δ	n
Retinol	.21	.19	12	42	.74	.72	2	42	7.21	6.87	5	40	.39	.37	4	42
Retinyl Palmitate	.016			9	.143	.251	-43	11	.079	.124	-36	11	.063	.104	-39	10
a-Tocopherol	2.67	3.47	-23	43	14.01	13.90	1	42	25.76	23.90	8	43	7.41	7.05	5	43
g-Tocopherol	.64	.68	-6	20	2.79	2.62	6	19	3.85	3.44	12	19	1.34	1.32	1	19
Total b-Carotene	.168	.169	-1	32	.704	.710	-1	32	1.686	1.690	0	33	.386	.361	7	33
trans-b-Carotene	.159	.154	3	11	.671	.640	5	11	1.618	1.560	4	11	.361	.326	11	11
Total cis-b-Carotene	.009			7	.033			7	.068			7	.025			7
Total a-Carotene	.009			22	.040	.039	3	25	.036	.042	-14	25	.019	.019	0	25
trans-Lycopene	.015			6	.074	.074	0	6	.100	.094	6	6	.045	.038	18	6
b-Cryptoxanthin	.075			11	.031	.021	48	15	.073	.046	59	15	.051	.011	364	14

L2

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

NAV : NIST Assigned Values, equal to (NIST's average-of-averages + this Round Robin's median) / 2

%Δ : Percent difference between your value and the NAV

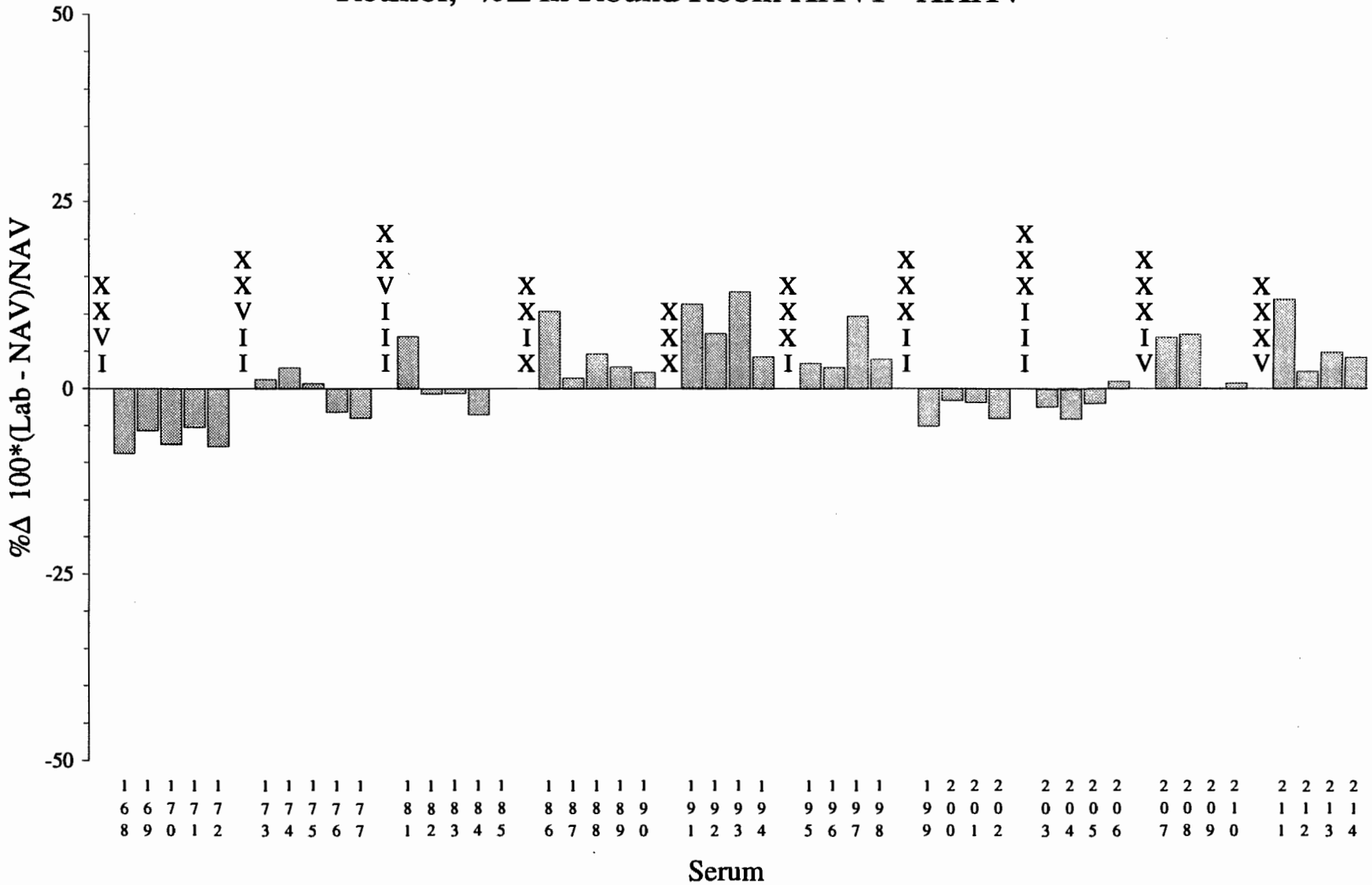
n : Number of non-NIST laboratories reporting quantitative values for this analyte in this serum

Please check our recorded values against your records.

Send corrections to: NNMMQAP 222/B208, NIST, Gaithersburg, MD 20899; fax 301-977-0685; email DLDuewer@enh.NIST.gov

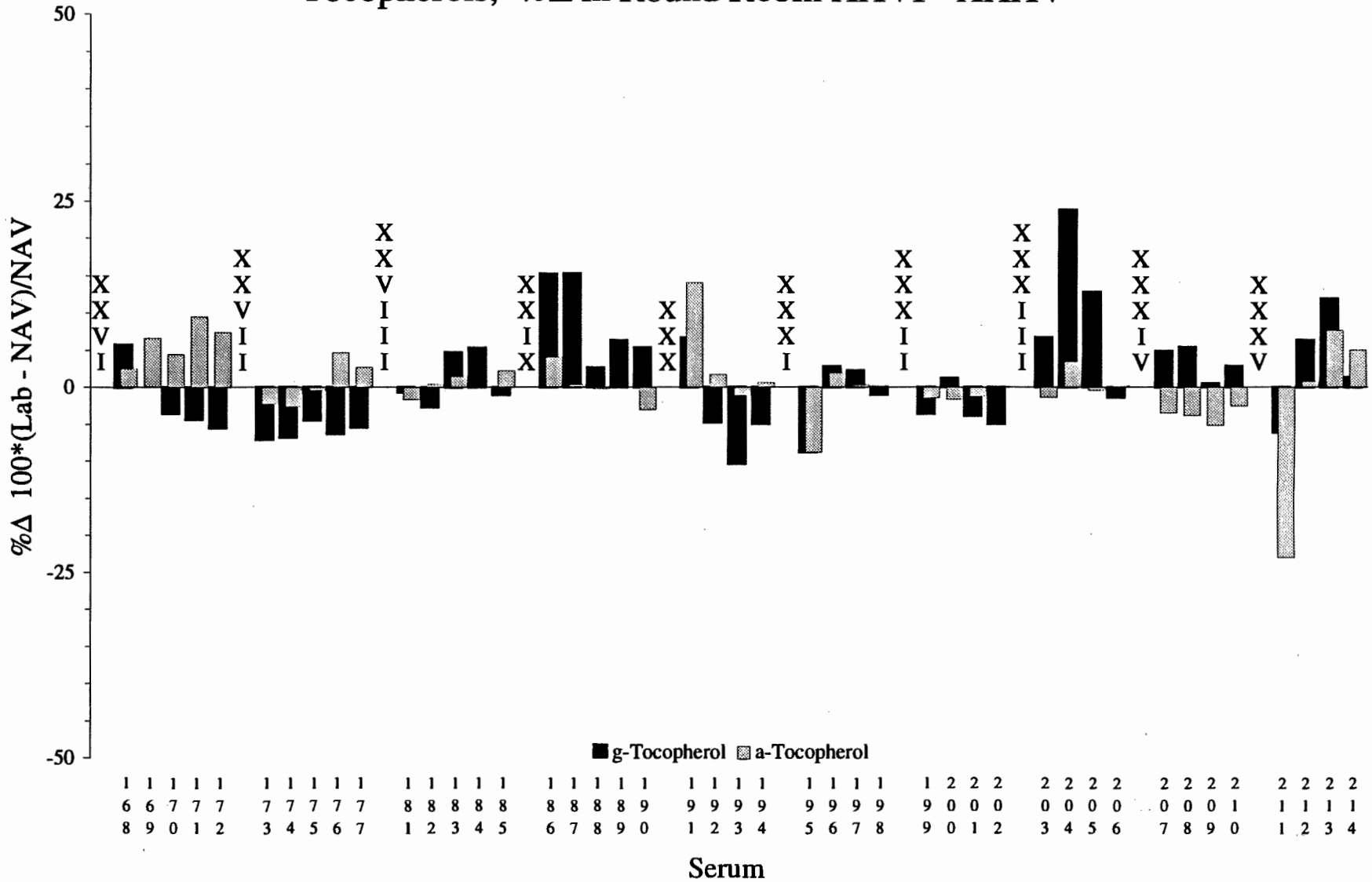
Individualized Round Robin XXXV Report to: FSV-BA

Retinol, %Δ in Round Robin XXVI - XXXV



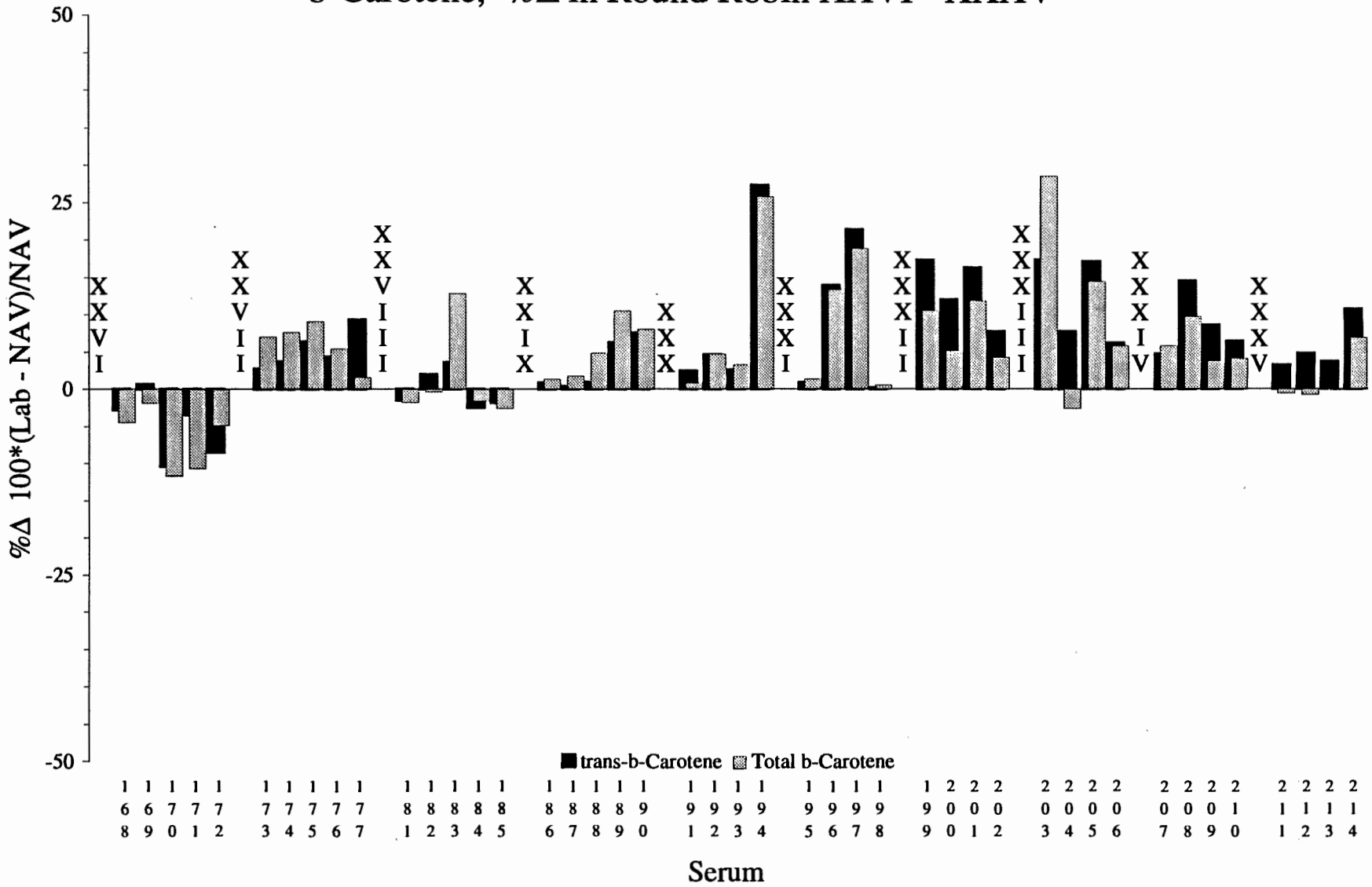
Individualized Round Robin XXXV Report to: FSV-BA

Tocopherols, %Δ in Round Robin XXVI - XXXV



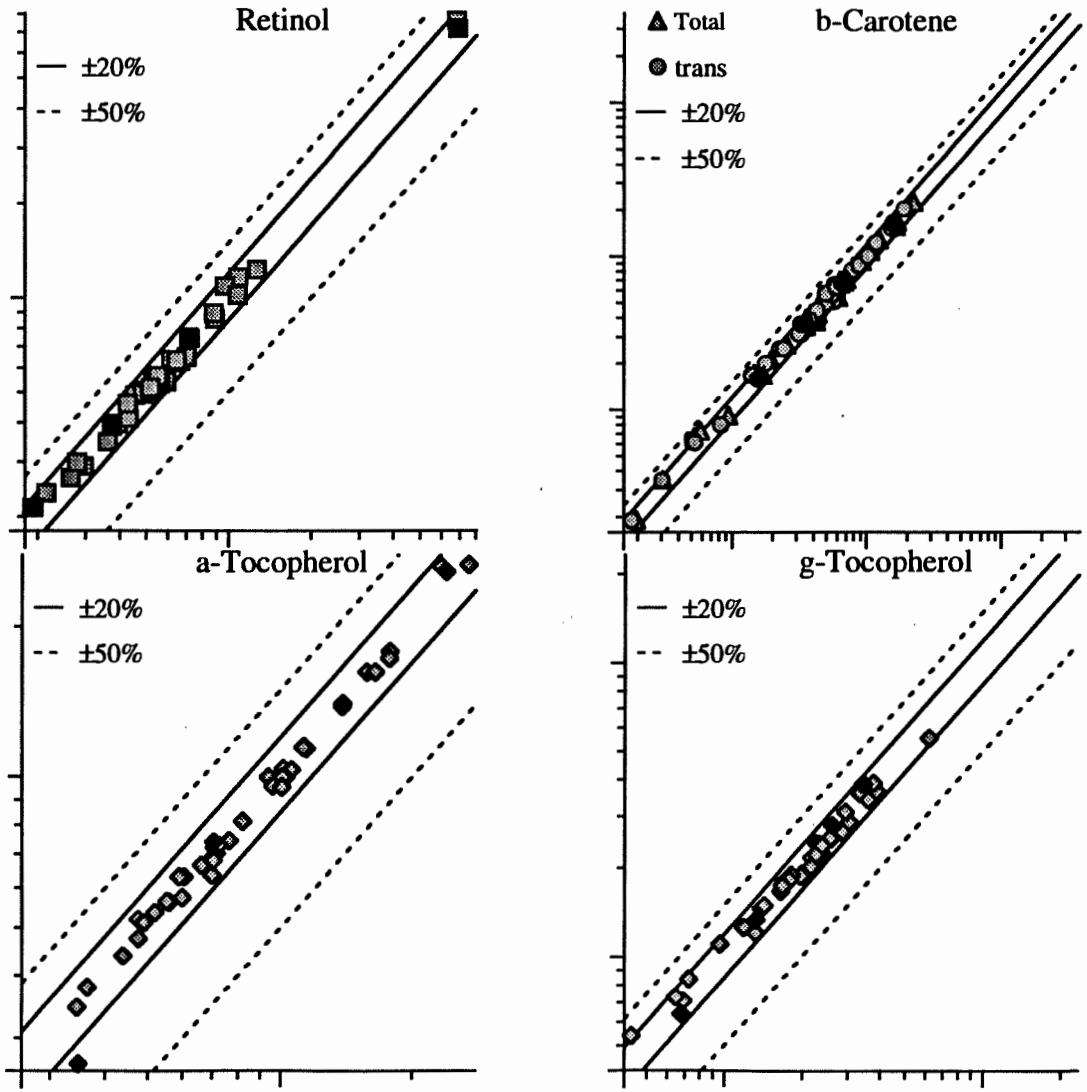
Individualized Round Robin XXXV Report to: FSV-BA

b-Carotene, %Δ in Round Robin XXVI - XXXV



Individualized Round Robin XXXV Report to: FSV-BA

NIST Assigned Values Vs Laboratory Values



Legend

Shaded Symbols: Round Robin XXVI-XXXIV
Black Symbols: Round Robin XXXV

Ordinate: NIST Assigned Value
Abscissa: Your reported concentration

Interpretation

Adequately intercomparable data are within ±20% lines. If you have data scattered outside ±50% lines, your measurement system is not consistent with those of most participating laboratories. If your data are systematically higher or lower than the NAV, your system may be consistent but your results are biased.

If your data show increased scatter at low concentrations, your "limit of quantification" may not be what you think it is.

If there are one or two "wild" outliers, they might be calculation or transcription errors. We would appreciate hearing from you about any such problems.

Individualized Round Robin XXXV Report to: FSV-BA

Accuracy/Precision Summary

	Ret		aToc		gToc		Total		trans	
	mΔ	vΔ	mΔ	vΔ	mΔ	vΔ	mΔ	vΔ	mΔ	vΔ
XXVI	-7	2	6	3	-2	5	-7	4	-5	5
XXVII	-1	3	0	3	-6	1	6	3	5	3
XXVIII	0	4	1	2	1	4	1	7	0	3
XXIX	4	4	0	3	9	6	5	4	3	3
XXX	9	4	4	7	-3	7	9	12	9	12
XXXI	5	3	-2	5	-1	5	9	9	9	10
XXXII	-3	2	-1	1	-3	3	8	4	13	4
XXXIII	-2	2	0	2	11	11	12	13	12	6
XXXIV	4	4	-4	1	3	2	6	3	9	4
XXXV	6	4	-2	14	3	8	1	4	6	3

Legend

Ret	Retinol
aToc	a-Tocopherol
gToc	g-Tocopherol
Total	Total b-Carotene
trans	all-trans-b-Carotene
mΔ	Mean difference, the average %Δ for all sera of a given RR, where %Δ = 100(Your value - NAV) / NAV
vΔ	Difference variability, one standard deviation of %Δ for all sera of a RR
NAV	NIST Assigned Value, our best estimate of analyte concentration... NAV = (NIST's average-of-averages + Round Robin median) / 2

(Traditional) Performance Criteria

The absolute value of %Δ of every measurement has traditionally been evaluated as follows...

%Δ	Evaluation
0-5%	Exceptional
6-10%	Acceptable
11-20%	Marginal
> 20%	Poor

More representative criteria need to be established, factoring in each serum's analyte level and the analyte distribution in adult human populations. Stay tuned, we're working on it...

Interpretation

Accuracy and precision are separate but kindred aspects of measurement comparison. We estimate accuracy as mΔ, the average %Δ, and precision as vΔ, the standard deviation of %Δ, for all sera of a Round Robin.

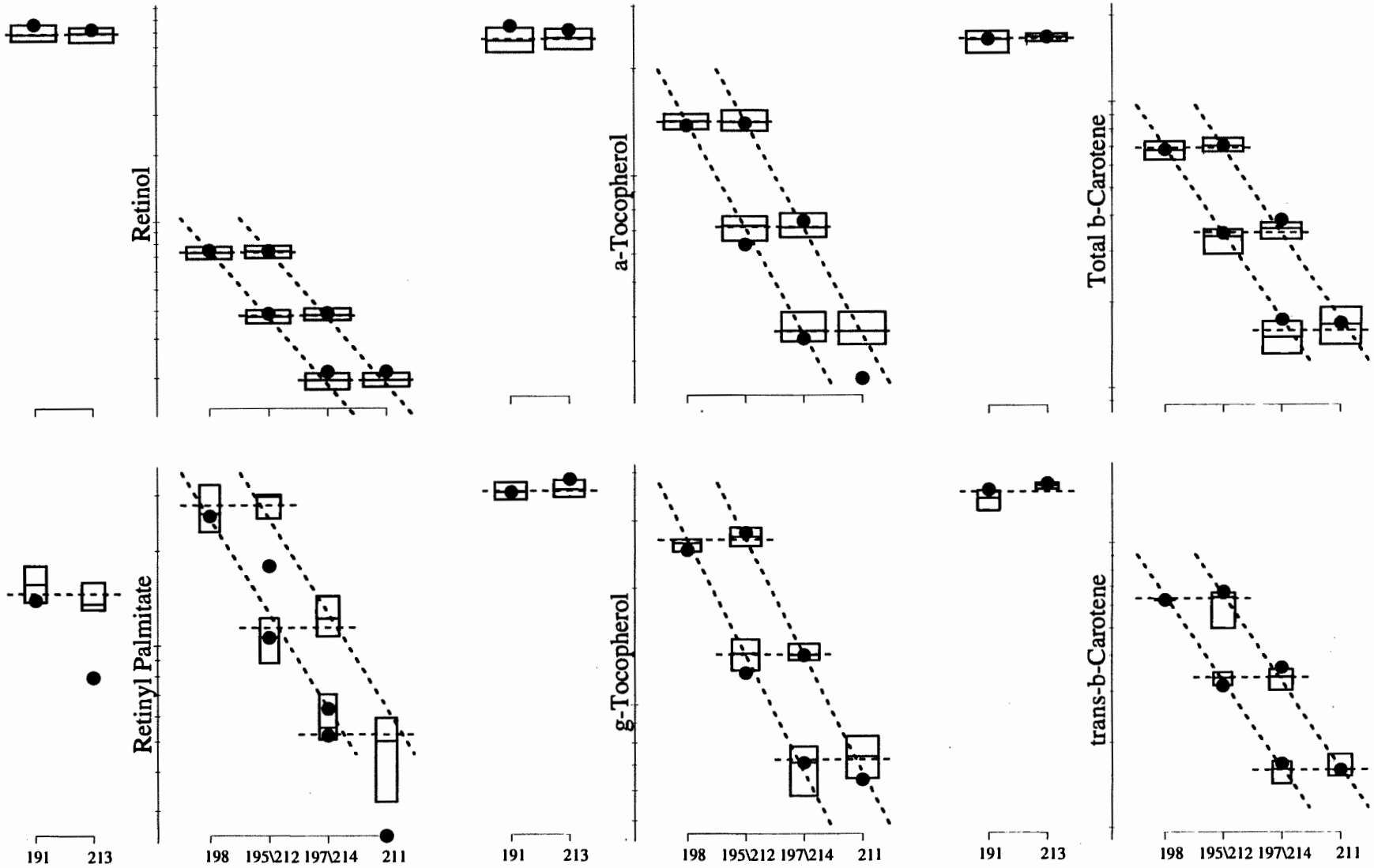
It's best to be accurate and precise (small mΔ, small vΔ)!

Good precision (small vΔ) with poor accuracy (large mΔ) is better than the converse: at least such values are internally consistent and may be relatable to others' values once the relative biases have been determined.

Poor precision (large vΔ) suggests that your measurement system is not in adequate control for the analyte levels examined.

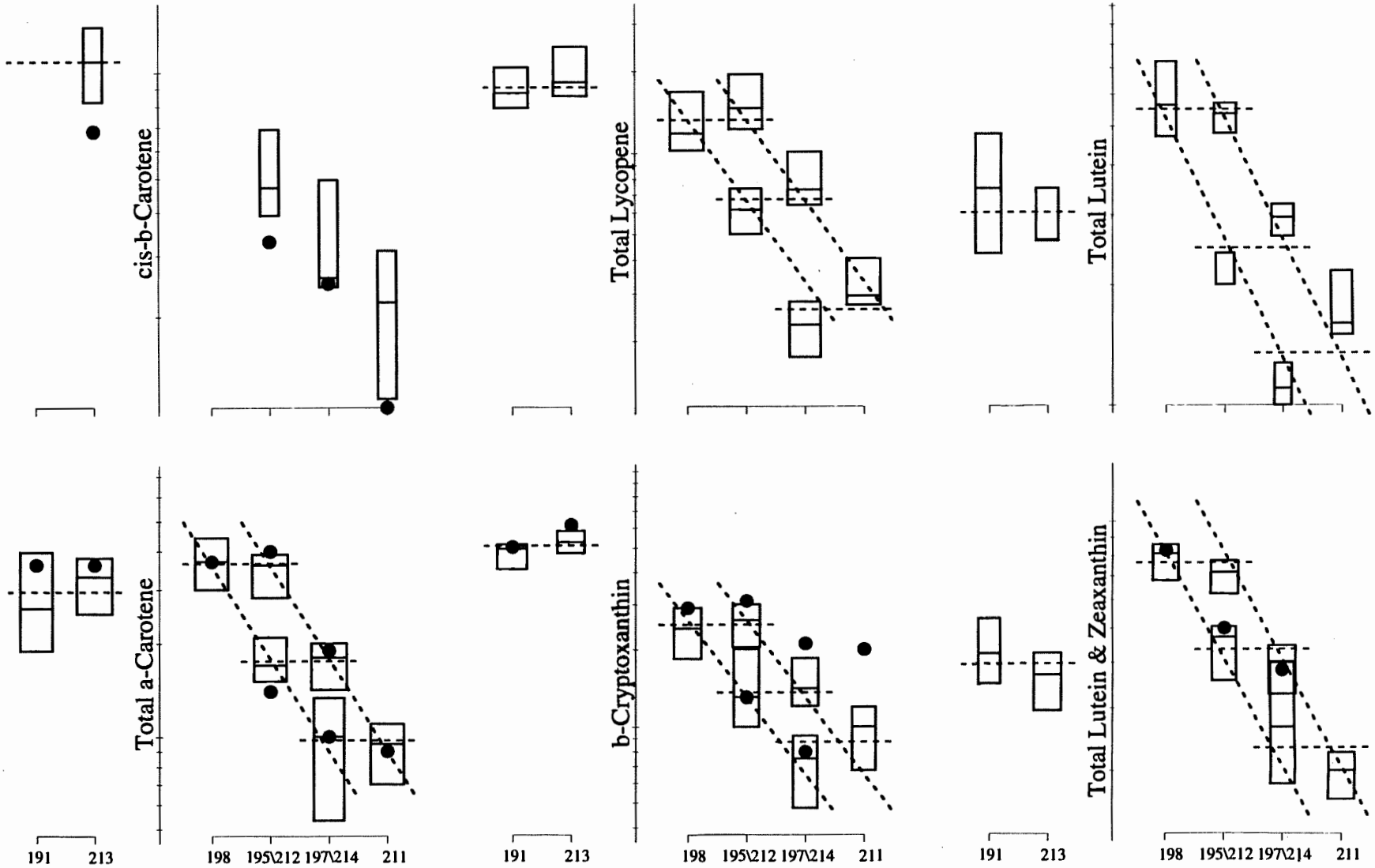
Individualized Round Robin XXXV Report to: FSV-BA

Comparisons to Prior Analyses



Individualized Round Robin XXXV Report to: FSV-BA

Comparisons to Prior Analyses (Continued)



Appendix M. Shipping Package Inserts for RR07

Three items were included in each package shipped to a RR07 participant:

- Cover letter
- Methodology questionnaire
- Report of Analysis datasheet for the analysis of the Control and Serum Samples

The cover letter, questionnaire, and datasheet were enclosed in a sealed waterproof bag along with the samples themselves.

Dr. Margolis's records did not preserve these materials. By inference, the cover letter was similar to that for RR08 (see Appendix Q) with the addition of information and instructions relating to the questionnaire. The questionnaire and Report of Analysis have been recreated from the preserved participant responses.

Survey of Methods used in the Round Robin Studies
on Ascorbic Acid in Plasma

Please complete the sections in the following list which apply to the method(s) you are using in the Round robin study and return it to with the attached report of analysis form.

I. Method of preparing calibration standards: _____

_____

_____

A. Solvent: _____

B. Source of standard: _____

C. Ascorbic acid concentration in stock solution; in working solution:

_____

II. Method for preparing quality conrol standards: _____

_____

_____

III. Method for preparing plasma sample for analysis: _____

_____

_____

IV. Method of analysis: _____

_____

A. Calorimetric: _____

1. Dinitrophenylhydrazine
   Reference for method: _____

2. Dichlorophenol
   Reference for method: _____

B. Enzymatic: _____

C. HPLC: _____

1. Column brand size: _____
2. Solvent(s) composition: _____
3. Method of detection: _____
   a. UV-wavelength: _____
   b. Electrochemical-voltage: _____
4. Method of sample introduction: _____
5. Literature reference for method (if published): _____
   _____

D. Other: _____
_____
_____
_____
_____

REPORT OF ANALYSIS

Name and mailing address specified on sheet set to each participant

LAB NO.: *Assigned code number*

PHONE NO.: *specified*

FAX NO.: *specified*

Method of Analysis: _____

Date of Analysis: _____

RESULTS (µmol/L)

SERUM 179, VIAL# _____

REPLICATE 1 _____ µmol/L

REPLICATE 2 _____ µmol/L

SERUM 688b, VIAL# _____

REPLICATE 1 _____ µmol/L

REPLICATE 2 _____ µmol/L

SERUM 688b, VIAL# _____

REPLICATE 1 _____ µmol/L

REPLICATE 2 _____ µmol/L

SERUM 682b, VIAL# _____

REPLICATE 1 _____ µmol/L

REPLICATE 2 _____ µmol/L

SERUM 682b, VIAL# _____

REPLICATE 1 _____ µmol/L

REPLICATE 2 _____ µmol/L

Appendix N. Final Report for RR07

The following 13 pages are the final report for RR07 and for Round Robin 6 (RR06); the samples for RR06 were distributed in August 1994 and are described in NISTIR 7880-30. This report was provided to all participants in either study.

This report contains:

- Cover letter and analysis of results.
- Table 1 "Results of Round Robin RR06 Measurement of Ascorbic Acid in Human Plasma".
- Table 2 "Results of Round Robin RR07 Measurement of Ascorbic Acid in Human Plasma".
- Table 3 "NIST Results of Measurement AA in Human Plasma".
- Figure 1 "Box Plot of the Round Robin RR06 Results"
- Figure 2 "Box Plot of the Round Robin RR07 Results"
- Figure 3. "Box Plot Comparing the Results of Round Robins RR04, RR05 and RR06".
- Figure 4. "Distribution of Round Robin RR06 Results for Lot 179".
- Figure 5. "Distribution of Round Robin RR06 Results for Lot 180".
- Figure 6. "Distribution of Round Robin RR07 Results for Lots 179 and 688b".
- Figure 7. "Distribution of Round Robin RR07 Results for Lot 682b".

A number of the results reported in the Tables were later revised to correct for miscommunication of the reporting units. Since the listed results do not necessarily represent measurement performance, the Lab identifiers used by Dr. Margolis have been redacted from these Tables rather than re-coded. The reporting unit confusion impacts some of the conclusions discussed in the cover letter. However, the results discussed in the Dr. Margolis's text have **not** been updated or corrected.

The "All Lab Report" in Appendix O lists the corrected results and provides more extensive statistical summaries.

August 22, 1995

Title~ First~ Last~
Company~
Address~

Dear Title~ Last~:

This report describes both the overall-group and your laboratory's performance in Round Robin VI and Round Robin VII for the measurement of ascorbic acid in human plasma. The studies involve the duplicate analysis of four unknown samples (two each from lots 179 and 180 and two each from lots 688b and 682b). Round Robin VII also contained two vials of lot 179 which served as a control material. Specifically, your package contains tabular presentations of all data submitted for ascorbic acid for Round Robins VI and VII. Your results are designated Lab No.~ in the tables and figures.

Tables 1 and 2 provide a summary of the data submitted by the participating laboratories (the NIST data were not included in the statistical analysis). Two laboratories submitted two sets of measurements, each done by a different method. As shown in Tables 1 and 2, the percent Relative Standard Deviation (RSD) for both lots ranged from 18.0 - 24.8. The intra-laboratory %RSD ranged from 0.3 - 4.0 with three exception in Round robin VII indicating that the major source of variation was the interlaboratory variation. The box plots in Figures 1 and 2 graphically summarize the results. The highest and lowest 10% of the measurements for each lot are plotted as small open circles, the two simple lines each span the next 15% intervals and the center box contains the values from the remaining data sets. The NIST mean value for ascorbic acid (AA) + dehydro-AA is represented by a solid circle and the NIST mean for reduced AA is represented by a solid square. The horizontal line in the 50% boxes represents the median interlaboratory values which are slightly different from the NIST values. Finally, the results of the previous round robins are compared to the results of this round robin and show a similar distribution of results (Figure 3).

The basis of the distribution of the majority of the LC measurements below the NIST mean requires further evaluation along with the large variation in the results (%RSD = 18-24.8%). In Round Robin VII we added in a control material to help you evaluate the capability of your method to measure the AA in a sample of known concentration. Three laboratories were between 12.5 and 14% lower and one 11% higher than the stated value of the control sample. The results of remaining laboratories were in agreement with the stated value. The assay done by the DNPH method was also high as would be expected from the previous round robin studies. Further examination of the data indicates that three laboratories, which were

able to measure AA in the control material, varied quite widely from the values established for the unknown samples. The fact that all of the gravimetrically added AA was accounted for (Table 3) also supports the need to continue these round robins in order to reduce the measurement variation. We would appreciate it if each of you, whose results deviated widely from the assigned values, would reexamine your methods for possible systematic errors. The distribution of the results of each laboratory are graphically illustrated in figures 4-7.

The next set of samples, Round Robin VIII, will be shipped around September 25, 1995. If you have any questions concerning Round Robin VI and VII please indicate it on the enclose sheet and return it to us via mail (Donna Sirk, NIST, Chemistry B208, Gaithersburg, MD 20899) or FAX (301/977-0685), or contact me at 301/975-3137.

Sincerely,

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

Enclosures

Table 1. Results of the Round Robin VI Measurement of Ascorbic Acid in Human Plasma.

Lab	Data Set[b]	Method	Ascorbic Acid (µmol/L Plasma)[a] Lot 179	Lot 180
	2	DNPH	54.8 ± 1.1	52.8 ± 1.5
	6	LC	48.6 ± 1.9	47.4 ± 1.9
	12	LC-EC	45.4 ± 2.9	44.3 ± 2.8
	3	DNPH		
	5	ENZ	40.9 ± 4.0	40.6 ± 1.4
	4	DNPH		
	1	DCIP	47.0 ± 1.3	47.3 ± 1.2
	13	LC-EC	71.3 ± 1.0	79.2 ± 3.0
	14	LC-EC	39.6 ± 1.2	39.9 ± 1.7
	15	LC-EC	45.6 ± 0.5	44.0 ± 0.3
	11	LC	39.3 ± 0.3	38.3 ± 0.7
	8	LC-EC	39.2 ± 0.5	38.1 ± 0.5
	18	LC	39.2 ± 1.3	37.6 ± 0.9
	19	LC	28.2 ± 0.9	38.0 ± 1.3
	20	LC-UV	58.1 ± 4.7	40.7 ± 2.7
	21	AUTOAN	41.5 ± 0.8	39.2 ± 1.8
	17	LC-EC	42.2 ± 0.3	40.3 ± 1.2
MEAN			45.4	43.8
SD			10.1	11.3
%RSD			22.2	24.8
NIST AA + DHAA		LC-EC	43.5 ± 1.5[c]	42.7 ± 1.5[a]
NIST AA		LC-EC	12.1 ± 0.3[c]	15.1 ± 0.4[a]

[a] Values represent the mean and SD of replicate measurements on two samples (total of 4 measurements).
[b] The Data Set numbers correspond to those in the figures 2-4.
[c] Values represent the mean and SD of replicate measurements on five samples (total of 10 measurements).

Table 2. Results of the Round Robin VII Measurement of Ascorbic Acid in Human Plasma.

Lab	Data Set[b]	Method	Ascorbic Acid (µmol/L Plasma)[a] Lot 179	Lot 688b	Lot 682b
	2	DNPH	50.3 ± 0.1	65.5 ± 0.9	122.2 ± 4.0
	6	LC	48.6 ± 0.7	56.0 ± 3.7	122.5 ± 5.2
	12	LC-EC	41.7 ± 2.1	63.1 ± 19.8	111.8 ± 6.2
	3	LC-UV	37.7 ± 0.9	31.3 ± 3.5	105.9 ± 3.3
	5	ENZ	41.0 ± 1.4	55.5 ± 6.2	127.3 ± 12.0
	4	DNPH	54.0	60.5 ± 0.6	124.3 ± 0.5
	13		46.7 ± 5.2	72.2 ± 0.6	141.2 ± 1.5
	14	LC-EC	42.5 ± 0.7	55.0 ± 2.0	122.8 ± 3.1
	11	LC	40.1 ± 0.4	50.4 ± 1.0	110.5 ± 1.8
	8	LC-EC	41.8 ± 1.7	56.5 ± 3.1	122.4 ± 2.4
	18	LC	37.9 ± 0.1	52.9 ± 0.3	128.9 ± 16.1
	19	LC	46.0 ± 2.3	52.2 ± 1.8	119.3 ± 5.0
	20	LC-UV	41.4 ± 7.5	33.1 ± 2.0	55.7 ± 29.1
	21	AUTOAN	42.0 ± 0.2	54.7 ± 0.3	118.1 ± 0.9
	22	LC-EC	40.9 ± 0.2	55.7 ± 0.5	118.7 ± 1.3
	23	LC-EC	42.7 ± 1.1	50.1 ± 0.5	105.2 ± 0.2
	24	LC-OPD	38.5 ± 0.7	39.3 ± 1.2	74.7 ± 2.1
MEAN			43.2	53.2	113.6
SD			4.5	10.6	20.5
%RSD			10.4	19.9	18.0
NIST AA + DHAA		LC-EC	43.5 ± 1.5[c]	56.4 ± 1.6[a]	115.4 ± 2.7
NIST AA		LC-EC	12.1 ± 0.3[c]	43.2 ± 3.3[a]	100.1 ± 1.4

[a] Values represent the mean and SD of replicate measurements on two samples (total of 4 measurements).

[b] The Data Set numbers correspond to those in the figures 2-4.

[c] Values represent the mean and SD of replicate measurements on five samples (total of 10 measurements).

Table 3. NIST Results of Measurement of AA in Human Plasma.

Lot No.	Ascorbic Acid (μmol/L) without DTT (AA)	with DTT (AA+DHAA)	Supplemented Amount
Plasma 1	5.1 ± 1.1 (4)[a]	11.5 ± 0.2 (4)	
178	10.2 ± 0.4 (4)	27.0 ± 0.5 (4)	15.8
179	12.1 ± 3.9 (10)	43.5 ± 1.5 (10)	32.1
180	15.1 ± 0.4 (4)	42.7 ± 0.3 (4)	31.6
Plasma 2	18.9 ± 6.5 (4)	52.2 ± 1.6 (4)	
179B	67.2 ± 26.4 (5)	154.4 ± 1.1 (10)	101.3

[a] The value in parentheses is the number of measurements made.

Figure 1. Box Plot of the Round Robin VI Results.
Figure 2. Box Plot of the Round Robin VII Results.
Figure 3. Box Plot Comparing the Results of Round Robins IV, V and VI.
Figure 4. Distribution of Round Robin VI Results for Lot 179.
Figure 5. Distribution of Round Robin VI Results for Lot 180.
Figure 6. Distribution of Round Robin VII Results for Lots 179 and 688b.
Figure 7. Distribution of Round Robin VII Results for Lot 682b.

Key for Figures 1 and 2:

The small open circles represent the highest and lowest 10% of the measurements, the two simple lines each span the next 15% intervals and the center box contains the values from the remaining data sets. The NIST mean value for ascorbic acid (AA) + dehydro-AA is represented by a solid circle and the NIST mean for reduced AA is represented by a solid square. The horizontal line in the 50% boxes represents the median interlaboratory values which are slightly different from the NIST values.

Key for Figure 3:

The highest and lowest 10% of the measurements are not represented in this figure, the two simple lines each span the next 15% intervals and the center box contains the values from the remaining data sets. The horizontal line in the 50% boxes represents the median interlaboratory values. The dashed lines represent the 95% confidence range of the NIST values for total AA.

Key for Figures 4, 5 and 7:

■ First Vial; First Measurement
♦ Second Vial; First Measurement
□ First Vial; Second Measurement
◊ Second Vial; Second Measurement

Key for Figure 6:

■ First Vial; First Measurement Lot 179
□ First Vial; Second Measurement Lot 179
♦ First Vial; First Measurement Lot 688b
◊ First Vial; Second Measurement Lot 688b
● Second Vial; First Measurement Lot 688b
○ Second Vial; Second Measurement Lot 688b

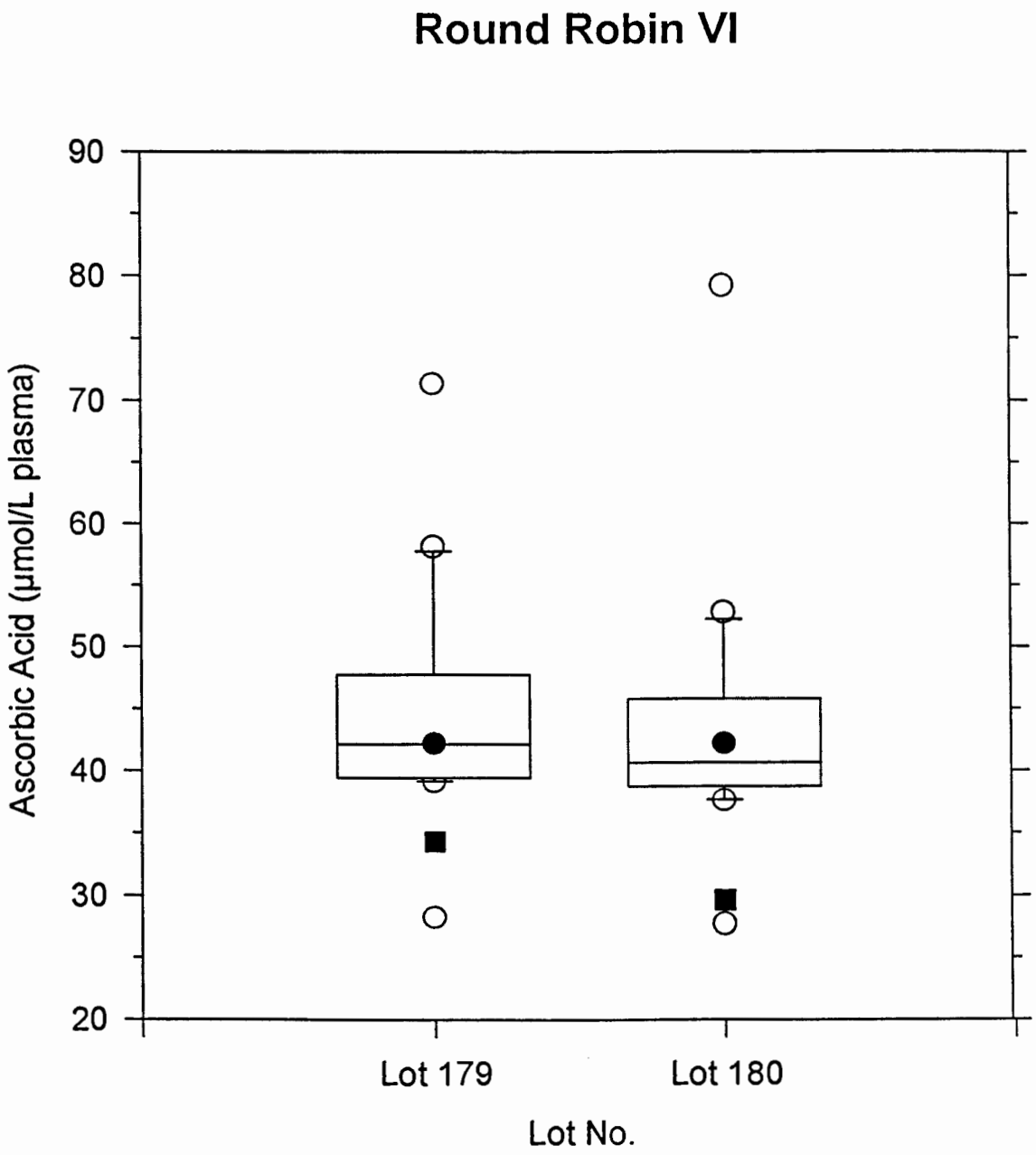

Round Robin VI
90
80
70
60
50
40
30
20
Ascorbic Acid (µmol/L plasma)
Lot 179
Lot 180
Lot No.

Fig. 1

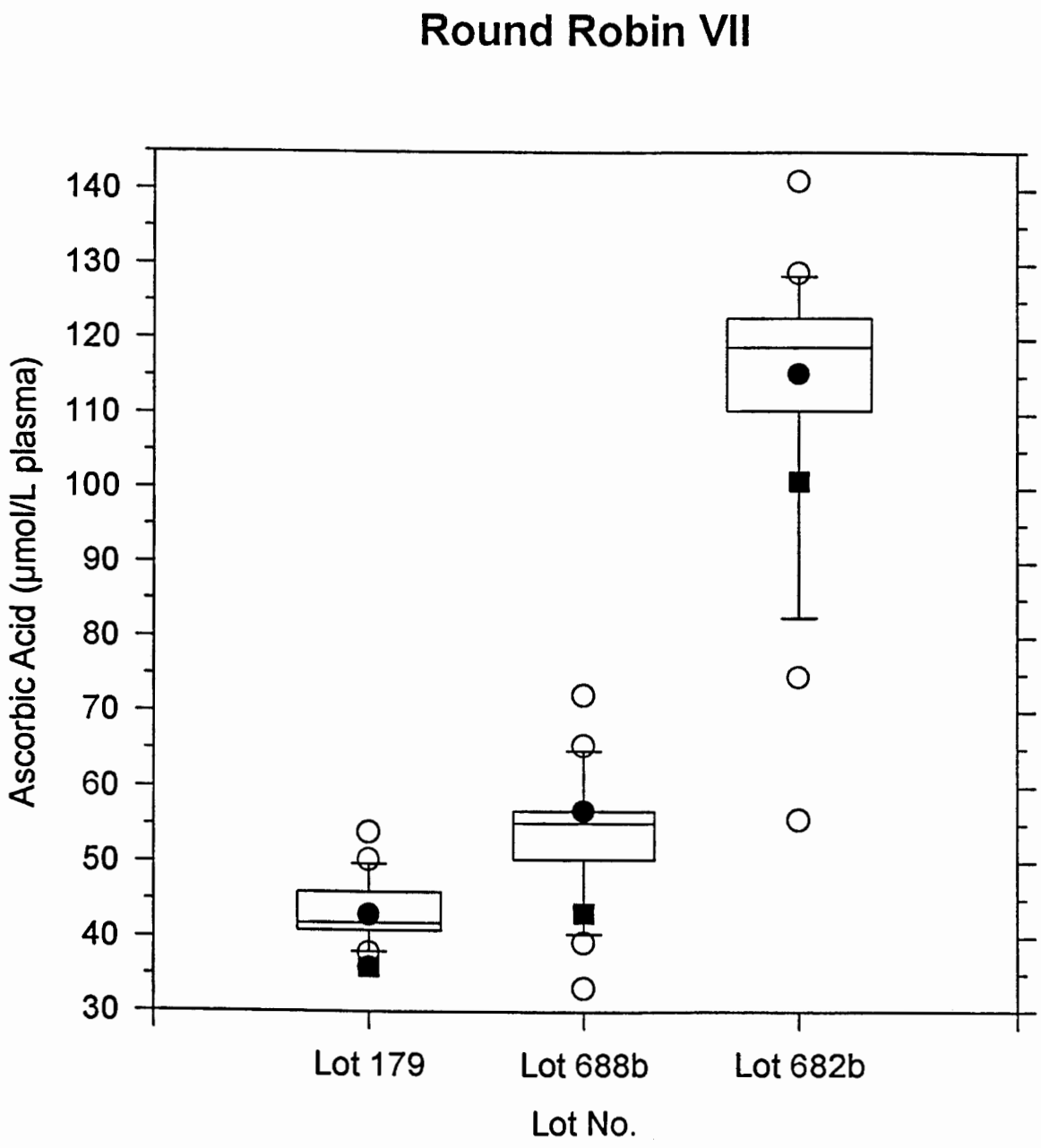

Round Robin VII
Ascorbic Acid (µmol/L plasma)
140
130
120
110
100
90
80
70
60
50
40
30
Lot 179
Lot 688b
Lot 682b
Lot No.

Fig. 2

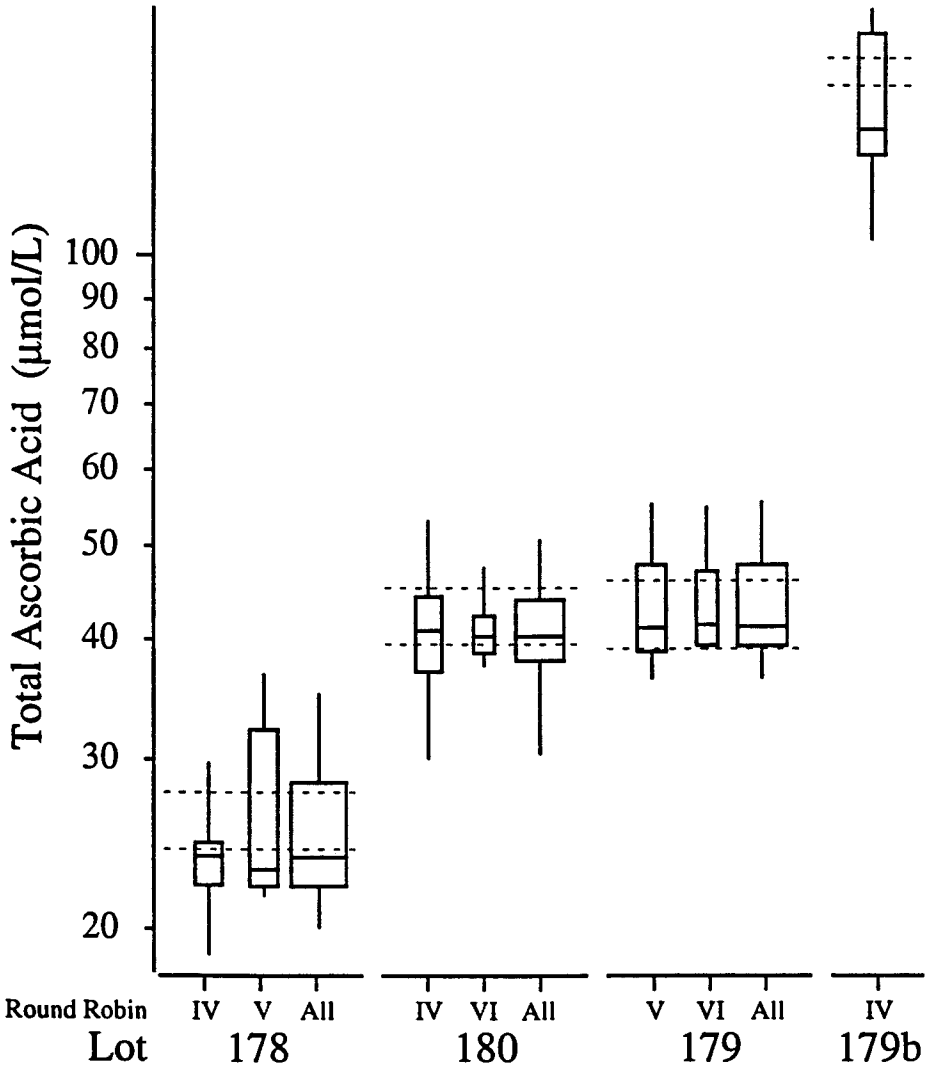

Total Ascorbic Acid (µmol/L)
100
90
80
70
60
50
40
30
20
Round Robin
IV
V
All
IV
VI
All
V
VI
All
IV
Lot
178
180
179
179b

Fig. 3

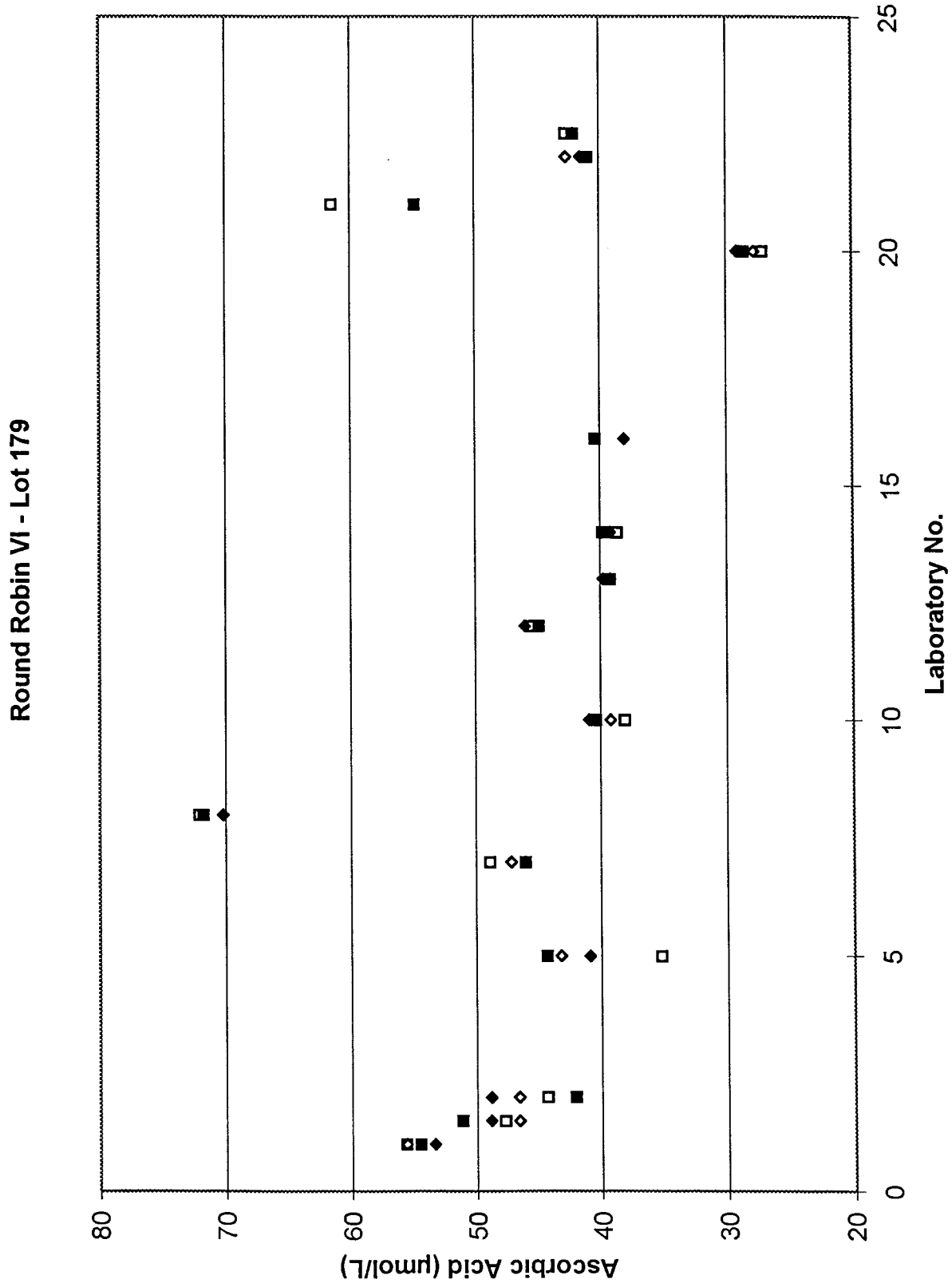

Round Robin VI - Lot 179
Ascorbic Acid (µmol/L)
80
70
60
50
40
30
20
0
5
10
15
20
25
Laboratory No.

Fig. 4

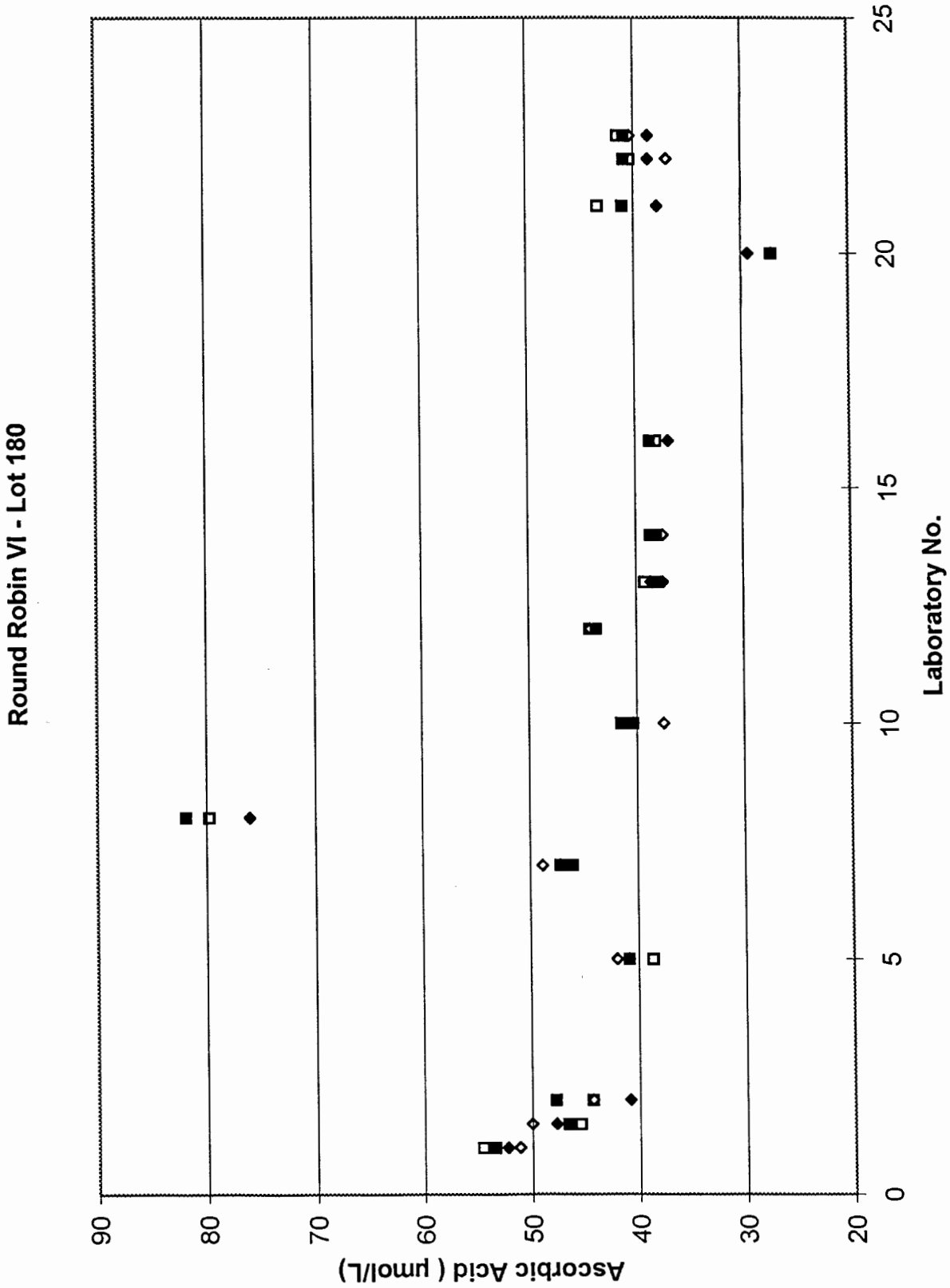

Round Robin VI - Lot 180
Ascorbic Acid (µmol/L)
90
80
70
60
50
40
30
20
0
5
10
15
20
25
Laboratory No.

Fig. 5

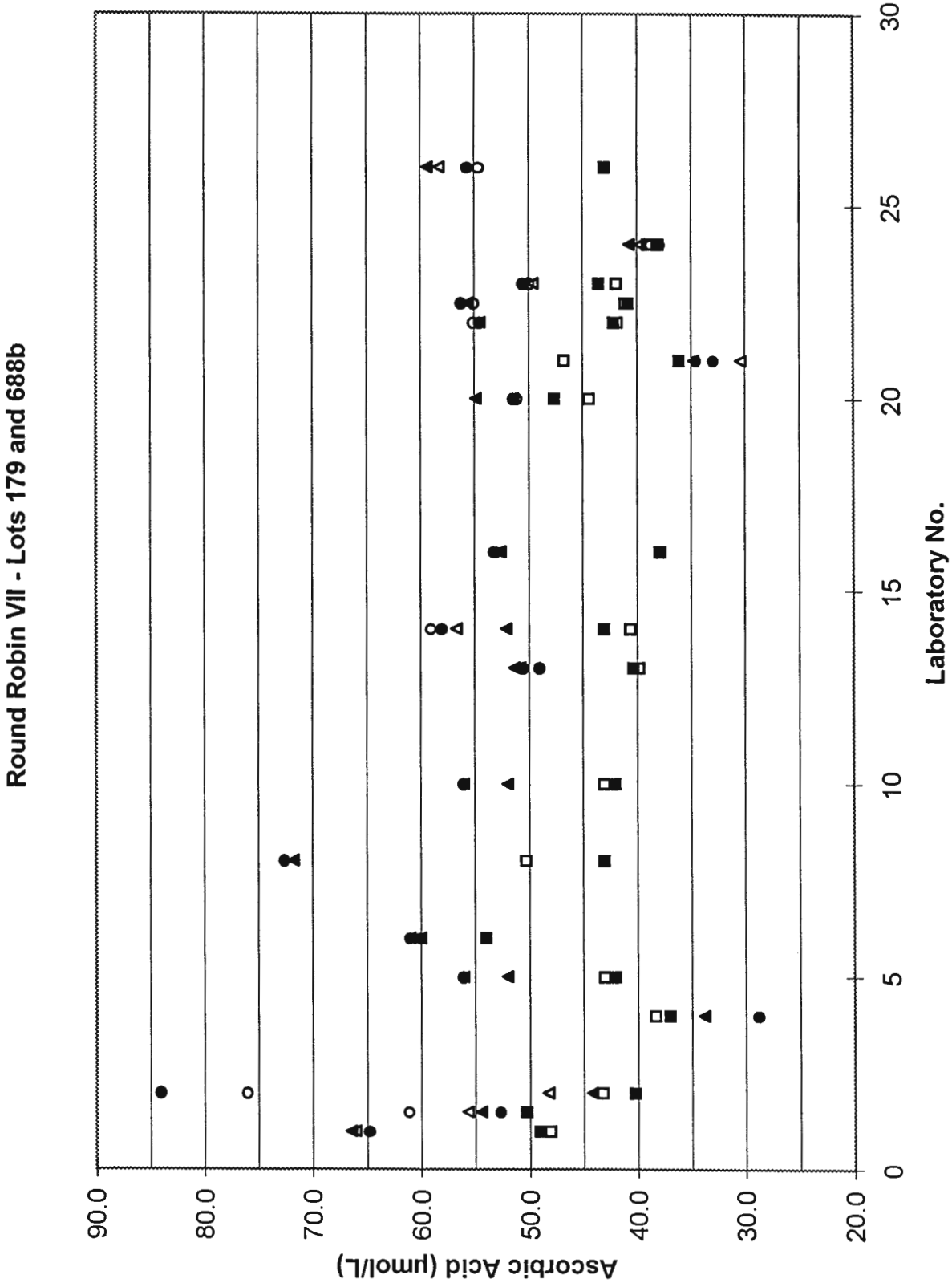

Round Robin VII - Lots 179 and 688b
Ascorbic Acid (µmol/L)
90.0
80.0
70.0
60.0
50.0
40.0
30.0
20.0
0
5
10
15
20
25
30
Laboratory No.

Fig. 6

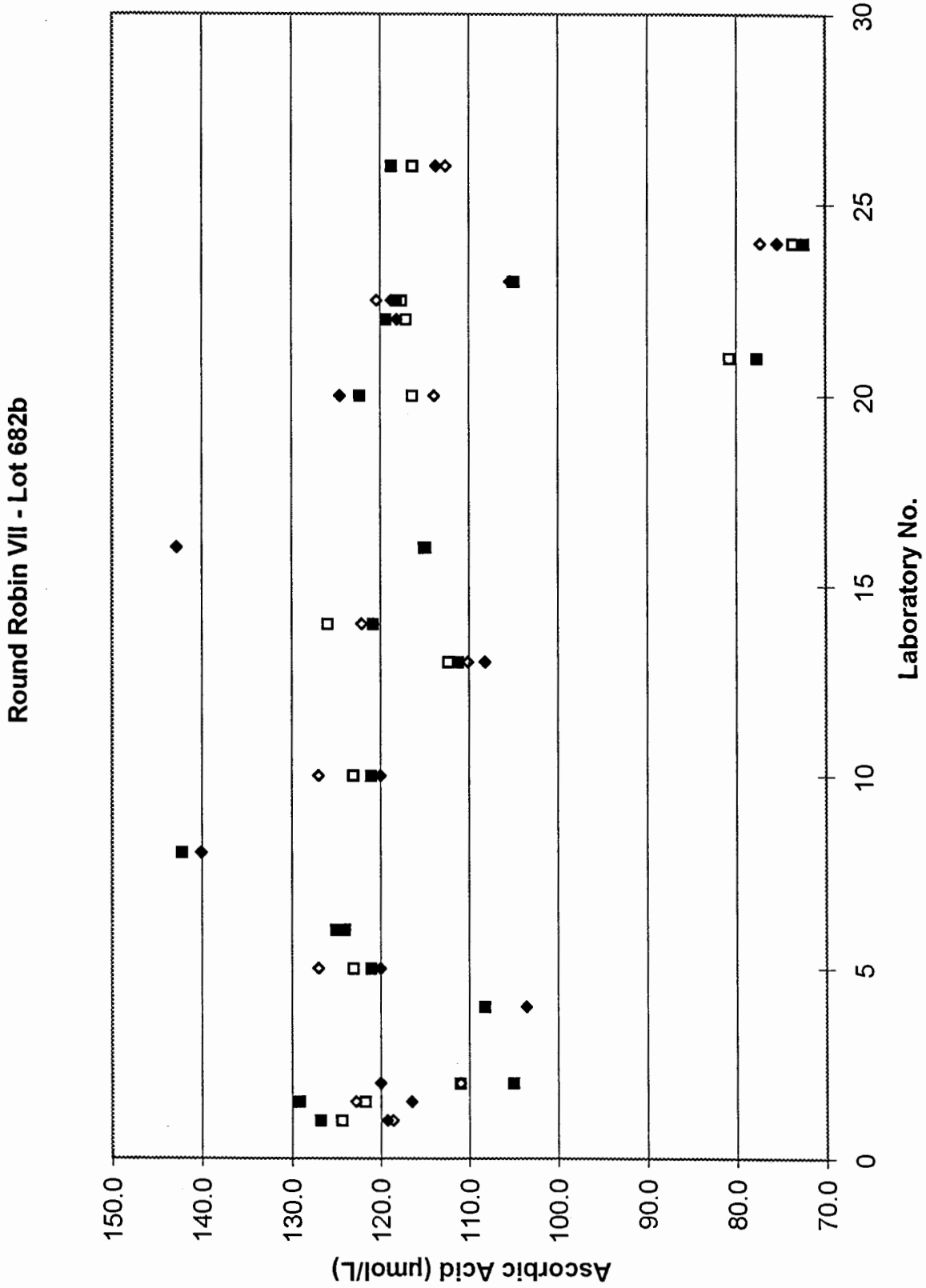

Round Robin VII - Lot 682b
Ascorbic Acid (µmol/L)
150.0
140.0
130.0
120.0
110.0
100.0
90.0
80.0
70.0
0
5
10
15
20
25
30
Laboratory No.

Fig. 7

Appendix O. "All-Lab Report" for VC-RR07

The following is a single page "All-Lab Report" that contains the same information as originally provided to all participants, with the following exceptions:

- the participant identifiers (Lab) have been altered to ensure confidentiality of identification codes assigned to laboratories..
- the order in which the participant results are listed has been altered.
- results for the Serum Samples have been corrected and transformed to have units of µmol/mL sample.
- results for the Control and Serum Samples have been consolidated.
- additional summary statistics have been included.

Vitamin C Round Robin 7

		Control														
		179, [AA] mmol/mL					688B, [AA] mmol/mL					682B, [AA] mmol/mL				
Lab	Method	Av	S_{dup}	S_{rep}	S_{het}	S_{tot}	Av	S_{dup}	S_{rep}	S_{het}	S_{tot}	Av	S_{dup}	S_{rep}	S_{het}	S_{tot}
VC-MA	24DNPH	20.0		0.2		0.2	25.2	0.5	0.4	0.4	0.5	55.2	0.9	0.5	0.8	1.0
VC-MB	AO	20.5		0.7		0.7	27.8	2.8	2.6	2.2	3.4	63.6	5.1	5.3	3.5	6.3
VC-MC	AO-OPD	22.3	2.8	0.4	2.8	2.8	31.4	2.0	0.6	2.0	2.0	62.2	2.2	0.8	2.2	2.3
VC-MD	HPLC-EC	24.3	1.1	0.1	1.1	1.1	27.2	1.0	1.7	0.5	1.8	63.6	4.4	4.7	3.7	5.9
VC-ME	HPLC-EC	21.1	0.2	0.1	0.2	0.2	27.3	0.1	0.1	0.0	0.1	59.1	0.0	0.6	0.0	0.6
VC-MF	HPLC-EC	20.6	0.1	0.4	0.0	0.4	27.8	0.0	0.3	0.0	0.3	59.3	0.6	0.5	0.5	0.7
VC-ML	HPLC-EC	20.7		3.8		3.8	16.6	0.4	1.2	0.0	1.2	27.8	16.6	6.4	16.0	17.2
VC-MQ	HPLC-UV	23.3		2.6		2.6	18.1	0.1	0.0		0.0	35.3	0.4	0.0		0.0
VC-MV	24DNPH	21.3		0.4		0.4	27.5	0.7	1.0	0.0	1.0	61.4	0.5	1.8	0.0	1.8
VC-MX	24DNPH	18.9		0.0		0.0	26.4	0.2	0.1	0.2	0.2	64.4	9.9	0.1	9.9	9.9
VC-MZ	HPLC-UV	20.9		0.8		0.8	28.2	1.5	1.1	1.3	1.7	61.2	0.7	1.3	0.0	1.3
VC-NA	HPLC-EC	23.0		1.1		1.1	26.1	0.7	0.9	0.3	0.9	59.6	0.0	3.0	0.0	3.0
VC-NB	HPLC-EC	21.4		0.6		0.6	25.1	0.1	0.3		0.3	52.6	0.1	0.0		0.0
VC-NC	HPLC-EC	27.0		0.0		0.0	30.3	0.0	0.4	0.0	0.4	62.1	0.2	0.3	0.0	0.3
VC-ND	HPLC-UV	19.3		0.4		0.4	19.6	0.7	0.3	0.6	0.7	37.3	1.2	0.6	1.1	1.2
VC-NR	HPLC-UV	20.9		1.1		1.1	31.6	11.9	2.2	11.8	12.0	55.9	2.7	2.7	1.8	3.3
VC-NU	AutoAnal	18.8		0.5		0.5	15.7	1.8	0.0	1.8	1.8	53.0	1.6	0.0	1.6	1.6
NIST	HPLC-EC	21.8				0.1	28.2				0.1	57.7				1.4
	N	17					17					17				
	Min	18.8	0.1	0.0	0.0	0.0	15.7	0.0	0.0	0.0	0.0	27.8	0.0	0.0	0.0	0.0
	Median	20.9	0.7	0.4	0.7	0.6	27.2	0.7	0.4	0.4	0.9	59.3	0.9	0.6	1.1	1.6
	Max	27.0	2.8	3.8	2.8	3.8	31.6	11.9	2.6	11.8	12.0	64.4	16.6	6.4	16.0	17.2
	eSD	1.3					3.0					6.1				
	eCV	6					11					10				

AO	Ascorbate oxidase
AutoAnal	Auto Analyzer
24DNPH	2,4-Dinitrophenylhydrazine
EC	Electrochemical detector
HPLC	Liquid chromatography
OPD	Orthophenlyenediamine
UV	Ultraviolet absorbance

Appendix M. Shipping Package Inserts for RR08

The following two items were included in each package shipped to a RR08 participant:

- Cover letter and instructions for preparing the Control Sample
- Report of Analysis datasheets for the preparation of the Control Sample and for the analysis of the Control and Serum Samples

The cover letter and datasheets were enclosed in a sealed waterproof bag along with the samples themselves.

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

Dr. Margolis sent individual letters to invited study participants. His original form letter is no longer available. This has been redacted from a letter that was prepared but never sent.

Dear

Thank you for agreeing to measure the ascorbic acid in the accompanying samples. Enclosed are two sets of samples, one set consisting of two ampules is a Control Sample, and the second set consisting of four ampules, is the Test Samples.

The control materials consist of two ampules (57 µmol ascorbic acid/L).

We ask that you use this control material to test your method before you analyze the Round Robin (RR) samples. We request that the following testing pattern be used.

- Analyze a single vial of the Control Sample. If these results are within 10% of the assigned values, proceed with the analysis of the unknown samples.
- If the results of your Control Sample is biased by 10-20%, we suggest that you review your method, make adjustments as necessary, then analyze the second Control Sample.
- If your results for the Control Sample exceeds 20%, we ask that you thoroughly examine your procedures and make necessary adjustments, and then analyze the second Control. If your data remains significantly biased, feel free to contact us to discuss possible solutions to your measurement problems.

The samples are in sealed ampules and were prepared by adding equal volumes of spiked human serum to 10% metaphosphoric acid (MPA). All samples have been stored at -70 °C and should be kept at this temperature. We have checked them for stability and the ascorbic acid appears sufficiently stable.

Each ampul contains between **20 and 120 µmol of ascorbic acid/L** of diluted serum and each ampul should be analyzed in duplicate by the method(s) used in your laboratory (preferably one measuring total ascorbic acid).

The Samples should be defrosted by warming at 20 °C for not more than 10 min, otherwise some oxidation of ascorbic acid may occur.

A report form is attached and we would appreciate it if you would make your measurements and return your report to me by **December 18, 1995.** We also request that you send us a representative chromatogram for each lot and indicate whether you used the peak area or the peak height for calculating the concentration of ascorbic acid in your samples. Your results will be kept confidential. We will use these results in a study to demonstrate the comparative accuracy and precision of the laboratories currently measuring ascorbic acid. However, values will not be assigned to individual labs. If you wish to fax your results to me. The fax number is: (301) 977-0685. If you have any questions I can be reached at (301) 975-3137.

Thank you for your participation is this exercise.

Sincerely,

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

Enclosures

REPORT OF ANALYSIS

Name: *specified on sheet set to each participant*
Address: *specified on sheet set to each participant*

Telephone no: _____
Fax no: _____

Method of Analysis: _____

- Please attach representative chromatograms.
- Method used for calculating ascorbic acid concentration
  Peak Height _____ Peak Area _____

Date of Analysis: _____

RESULTS (mg/dL)

TEST SAMPLE, VIAL# ____

REPLICATE 1 _____ µmol/L
REPLICATE 2 _____ µmol/L

TEST SAMPLE, VIAL# ____

REPLICATE 1 _____ µmol/L
REPLICATE 2 _____ µmol/L

SERUM 688A, VIAL# _____

REPLICATE 1 _____ µmol/L
REPLICATE 2 _____ µmol/L

SERUM 688A, VIAL# _____

REPLICATE 1 _____ µmol/L
REPLICATE 2 _____ µmol/L

SERUM 688A, VIAL# _____

REPLICATE 1 _____ µmol/L
REPLICATE 2 _____ µmol/L

SERUM 682A, VIAL# _____

REPLICATE 1 _____ µmol/L
REPLICATE 2 _____ µmol/L

Appendix Q. "All-Lab Report" for VC-RR08

The following is a single page "All-Lab Report" that contains the same information as originally provided to all participants, with the following exceptions:

- the participant identifiers (Lab) have been altered to ensure confidentiality of identification codes assigned to laboratories..
- the order in which the participant results are listed has been altered.
- results for the Serum Samples have been corrected and transformed to have units of µmol/mL sample.
- results for the Control and Serum Samples have been consolidated.
- additional summary statistics have been included.

Vitamin C Round Robin 8

		Control														
		688B, [AA] mmol/mL					688A, [AA] mmol/mL					682A, [AA] mmol/mL				
Lab	Method	Av	S_{dup}	S_{rep}	S_{het}	S_{tot}	Av	S_{dup}	S_{rep}	S_{het}	S_{tot}	Av	S_{dup}	S_{rep}	S_{het}	S_{tot}
VC-MB	AO	30.4	0.4	0.4	0.3	0.5	15.8	0.2	0.0	0.2	0.2	45.9	0.2	0.3	0.0	0.3
VC-MC	AO-OPD	28.6	1.4	0.3	1.4	1.4	13.4	0.4	0.4	0.3	0.5	45.9	0.8	0.8	0.7	1.0
VC-MD	HPLC-EC	28.9	0.8	1.3	0.0	1.3	14.0	0.6	1.2	0.1	1.2	46.4	0.8	0.8	0.6	1.0
VC-ME	24DNPH	29.5	0.1	1.0	0.0	1.0	14.4	0.7	0.3	0.7	0.7	43.7	1.5	1.0	1.3	1.6
VC-MF	HPLC-EC	27.1	0.2	0.0	0.2	0.2	13.6	0.0	0.0	0.0	0.0	42.1	0.1	0.4	0.0	0.4
VC-MQ	HPLC-EC	28.7	1.1		1.1	1.1	17.1	1.0	0.8	0.8	1.1	47.3	2.3	4.1	0.0	4.1
VC-MV	24DNPH	30.8	0.2	1.2	0.0	1.2	17.1	0.7	0.2	0.7	0.7	49.3	0.3	0.8	0.0	0.8
VC-MX	24DNPH	26.6	0.1	0.0	0.1	0.1	13.3	0.0	0.0	0.0	0.0	41.2	0.5	0.1	0.5	0.5
VC-MZ	HPLC-UV	28.7	0.3	0.2	0.3	0.3	14.7	0.1	0.3	0.0	0.3	44.8	0.4	0.4	0.2	0.5
VC-NA	HPLC-UV	29.1	0.9	0.4	0.8	0.9	12.9	0.6	0.4	0.5	0.7	45.0	0.8	0.6	0.7	1.0
VC-NB	HPLC-EC	16.9	0.2		0.2	0.2	10.9					34.1				
VC-NC	HPLC-UV	31.1	0.8	0.0	0.8	0.8	16.0	0.1	0.1	0.1	0.1	46.0	0.1	0.1	0.1	0.2
VC-ND	AutoAnal	28.5		0.4		0.5	13.5	1.4	1.4	1.0	1.8	39.0	0.2	0.8	0.0	0.8
VC-NG	HPLC-UV	28.4	0.9	0.7	0.7	1.0	6.7	2.3	0.4	2.3	2.4	24.8	3.2	0.5	3.2	3.3
VC-NL	HPLC-EC	25.8	0.6		0.6	0.6	13.1	0.6		0.6	0.6	40.9	0.3		0.3	0.3
NIST	HPLC-EC	27.7				0.8	14.0				1.6	41.6				2.8
	N	15					15					15				
	Min	16.9	0.1	0.0	0.0	0.1	6.7	0.0	0.0	0.0	0.0	24.8	0.1	0.1	0.0	0.2
	Median	28.7	0.5	0.4	0.3	0.8	13.6	0.6	0.3	0.4	0.6	44.8	0.4	0.6	0.3	0.8
	Max	31.1	1.4	1.3	1.4	1.4	17.1	2.3	1.4	2.3	2.4	49.3	3.2	4.1	3.2	4.1
	eSD	1.2					1.2					3.7				
	eCV	4					8					8				

AO	Ascorbate oxidase
AutoAnal	Auto Analyzer
24DNPH	2,4-Dinitrophenylhydrazine
EC	Electrochemical detector
HPLC	Liquid chromatography
OPD	Orthophenlyenediamine
UV	Ultraviolet absorbance

Q2