Newborn Screening Quality Assurance Program

2003 TANDEM MASS SPECTROMETRY ANNUAL SUMMARY REPORT

Volume 3 February 2004

INTRODUCTION

The Centers for Disease Control and Prevention (CDC), in partnership with the Association of Public Health Laboratories (APHL), operates the Newborn Screening Quality Assurance Program (NSQAP) to help screening laboratories achieve excellence in technical proficiency and maintain confidence in their performance while processing large volumes of specimens daily. Our program continually strives to produce certified dried-blood-spot (DBS) materials for reference and quality control (QC) analysis, to improve the quality and scope of our services, and to provide immediate consultative assistance. Through interactive efforts with the program's participants, we aspire to meet their growing and changing needs. Tandem mass spectrometry is a multiplexing platform for detecting more than 30 disorders. This report is an overview of the specimen preparation and reported results for the 2003 Tandem Mass Spectrometry Proficiency Testing (PT) Program. Comments and suggestions on how we may better serve the newborn screening laboratories are always welcomed.

Contents

Newborn screening for detection of treatable, inherited metabolic diseases is a major public health responsibility consisting of six parts: education, screening, follow-up, diagnosis, management, and evaluation. Effective screening of newborns using DBS specimens collected at birth, combined with follow-up diagnostic studies and treatment, helps prevent mental retardation and premature death. These blood specimens are routinely collected from more than 95% of all newborns in the United States. State public health laboratories and their associated laboratories screen DBS specimens for inborn errors of metabolism and other disorders that require intervention.

For more than 25 years, CDC has conducted research on materials development and assisted laboratories with both QC and PT issues. The quality assurance (QA) services primarily support state laboratories performing newborn screening; however, privately owned and foreign laboratories can also be accepted into the voluntary program. Currently, the program provides QA services in the form of quarterly PT panels that include amino acids and acylcarnitines enrichments. In July of this year, DBS QC materials for both amino acids and acylcarnitines were sent to participants. A summary of the QC data can be found in the NSQAP 2003 Annual Summary Report. All DBS materials for QC and PT are certified for homogeneity, accuracy, stability, and performance by most methods.

Along with the quarterly PT panels, which use blind-coded DBS specimens, the PT program provides to each laboratory an independent external assessment report of its performance. PT specimen panels are shipped to the laboratories in January, April, July, and

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Association of Public Health Laboratories

October of each year. The laboratories have a three-week deadline for submitting the results. A quarterly summary report contains the certified enrichment values and participant statistics showing the mean, minimum, and maximum cutoff value for each specimen. At the end of every year, the program publishes an annual report to summarize the assessment outcomes over the year and serve as a resource of accumulated information that could benefit all laboratories involved in newborn screening efforts.

TANDEM MASS SPECTROMETRY PROFICIENCY TESTING

In 2003, NSQAP operated a pilot PT program for laboratories performing newborn screening tests using DBS specimens by tandem mass spectrometry (MS/MS). MS/MS is being used to detect amino acid metabolic disorders, urea cycle disorders, fatty acid oxidation disorders, and organic acid metabolic disorders. Over this year, the program distributed four five-specimen panels to 85 active participants in the MS/MS PT program. Of these 85 participants, 31 were domestic laboratories (Figure 1) and 54 were foreign laboratories from 24 countries around the world (Figure 2). Quarterly reports were prepared using results received by the deadlines and then distributed to all participant laboratories. Late-results data were not used in the quarterly reports; however, late data are included in the statistics of this annual MS/MS report. This report summarizes the mean and median cutoff values for amino acids and acylcarnitines from data received for the Quarter 3, 2003 event. Individual participant results were plotted against the calculated mean and median cutoff value for each analyte and specimen. A table summary showing the percentages of false-negative and false-positive errors represents the overall performance of each quarter's participant results for amino acids and acylcarnitines.

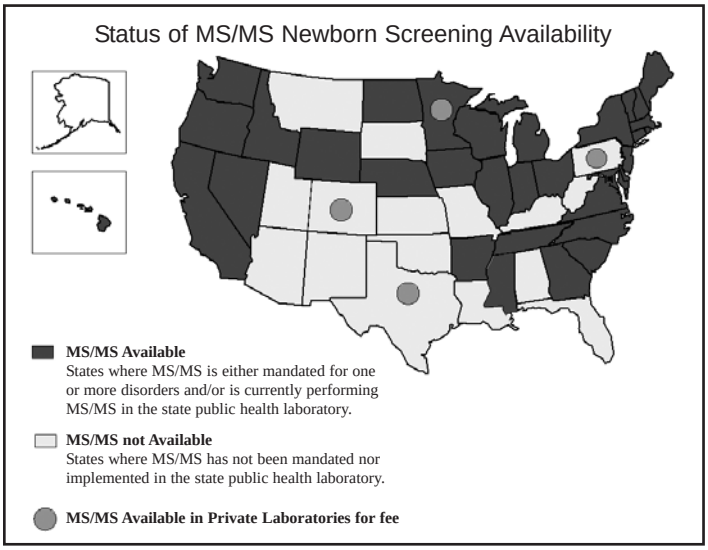


FIGURE 1. States that participated in the NSQAP tandem mass spectrometry pilot PT in 2003.

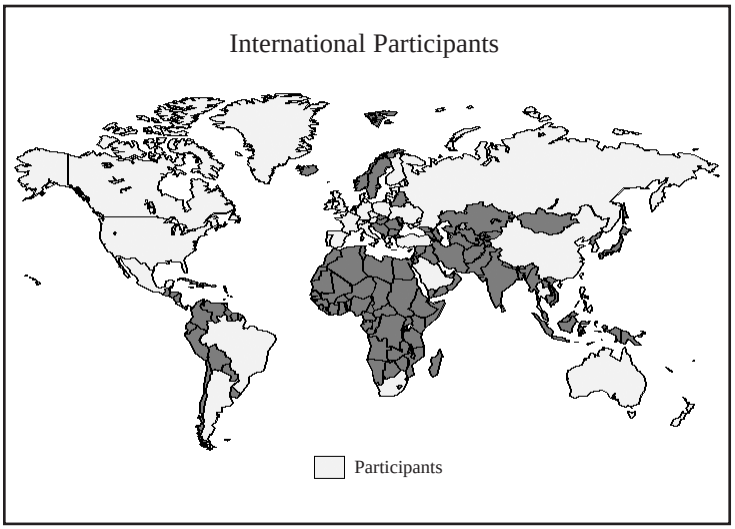


FIGURE 2. Worldwide participants that participated in the NSQAP tandem mass spectrometry pilot PT program in 2003.

SPECIMEN PREPARATION

The amino acid and acylcarnitine PT panels distributed to participants in the 2003 PT program contain five blind-coded DBS specimens containing either 75 uL or

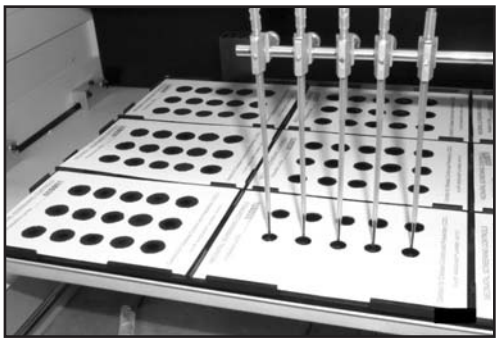


FIGURE 3. Automated blood spotting.

Program Information Web site:
http://www.cdc.gov/nceh/dls/newborn_screening.htm

100 uL whole blood. The whole blood specimens were derived from two sources: blood with a 55 ± 1 % hematocrit of lysed red cells, or blood with a 55 ± 1 % hematocrit of intact red cells. The PT specimens were made using blood from single donors with natural endogenous levels or by enriching single-donor whole blood specimens with one or more purified analytes at predetermined levels. The amino acid PT specimens were dispensed on S&S Grade 903 Lots W941, W961, W001, and W011 filter papers (Figure 3). The acylcarnitine PT specimens were dispensed on S&S Grade 903 Lots W961, W001, and W011 filter papers. The DBS specimens were wrapped in weighing paper and packaged in zip-closed metallized plastic bags along with desiccant. The specimen bags along with instructions for analysis, and data-report forms were all enclosed with the shipment (Figure 4).

FIGURE 4. Packing cards.

CUTOFF VALUES

Participants were asked to provide their cutoff value for each analyte tested. The cutoff value is defined as the decision level for sorting test results that are reported as presumptive positive (outside limits) from results reported as negative (within limits).

The distributions of reported cutoff values from participating laboratories are illustrated. Figures 5a-5f show the participant cutoff values for amino acids and Figures 6a-6i show the cutoff values for acylcarnitines. Mean and median cutoff values were calculated from the cutoff data submitted by laboratories for Quarter 3, 2003. Each analyte graphic shows the overall combined mean cutoff value represented by a solid line and the overall median cutoff value represented by a dotted line. The decision to reference the "median" cutoff value along with the "mean" cutoff value was to show possible skewing of the mean due to one very high or very low cutoff value. Some of the cutoff values appear to be outliers, and those individual laboratories should evaluate and justify the wide variations.

It is observed that most of the mean cutoff values are close between domestic and foreign laboratories with the exception of C3 and C16.

Following the cutoff graphics are Tables 1 and 2 showing the domestic and foreign amino acid cutoff value statistics and Tables 3 and 4 showing the domestic and foreign acylcarnitine cutoff value statistics. The tables also show the minimum and maximum cutoff values. The minimum-maximum data indicate the lowest and highest cutoff value submitted by the participants. This range points out the spread of established cutoff values for reference. All laboratories must establish and refine their own cutoff values.

The statistical tables on page 8 were created so that comparisons could be observed between the domestic and the foreign laboratories. Table 5 shows side-by-side comparisons of the mean and median cutoffs for the amino acids in mg/dL. Table 6 shows the same comparisons for mean and median cutoff values for the amino acids in µmol/L. Table 7 shows the mean and median cutoff values for acylcarnitines in µmol/L as comparisons between domestic, foreign, and combined laboratories.

It is observed that most of the mean cutoff values are fairly close between domestic and foreign laboratories with the exception of C3 and C16. The cutoff means and medians for the domestic laboratories appear to be higher than the cutoff means for the foreign laboratories. Even though some outliers are still evident, the distributions of cutoff values for amino acids and acylcarnitines are moving closer in agreement among participants and among countries in the world. The cutoff values are expected to vary somewhat due to differences in derivatization methodologies, instrumentation, and population ethnicity; however, this cutoff data can be used as a reference guide while laboratories are establishing and refining their own cutoff values.

Figures 5a-5f. Reported Cutoff (Domestic and Foreign) vs. Calculated Mean Cutoff Value for Amino Acids (mg/dL whole blood)

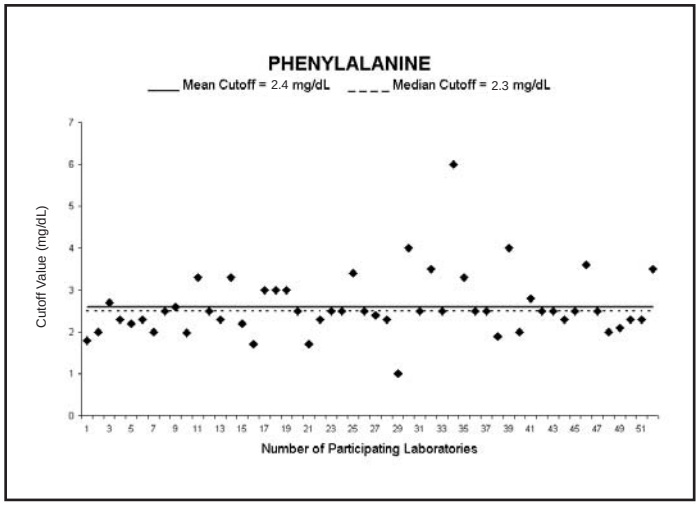


Figure 5a.

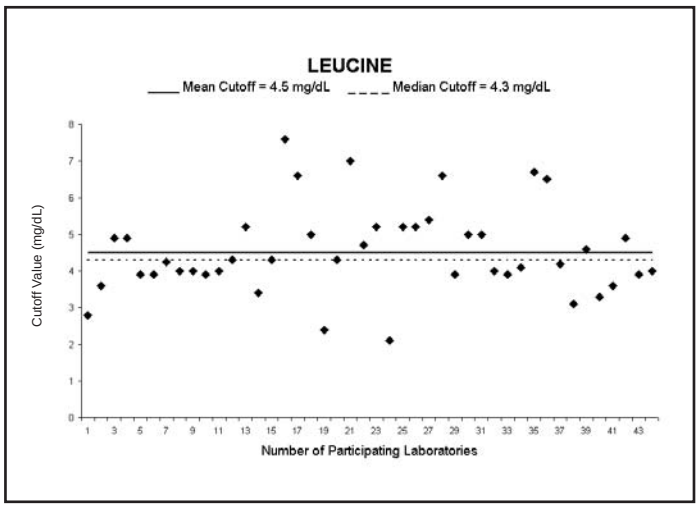


Figure 5b.

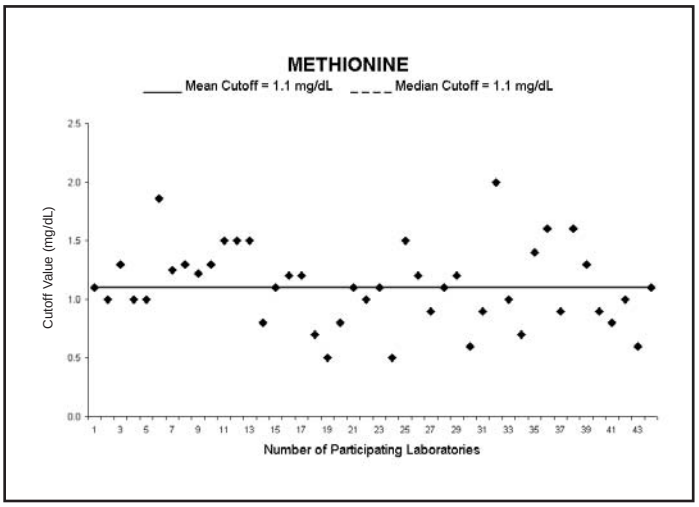


Figure 5c.

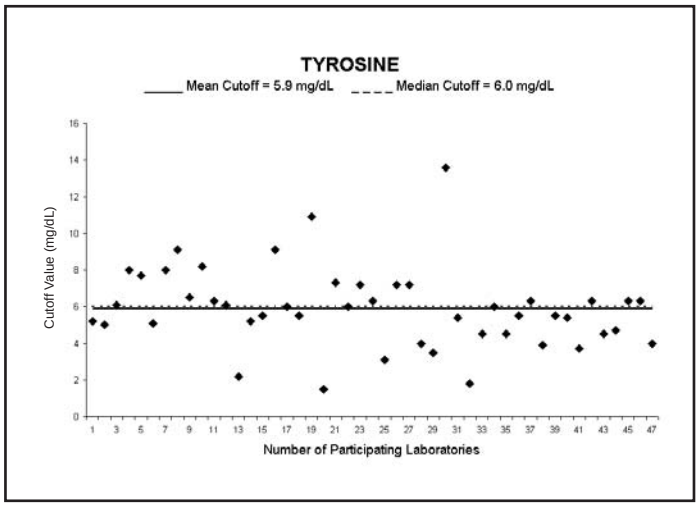


Figure 5d.

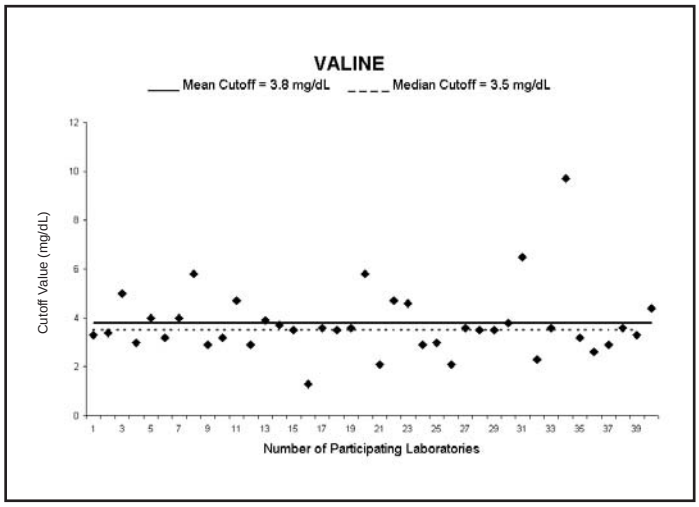


Figure 5e.

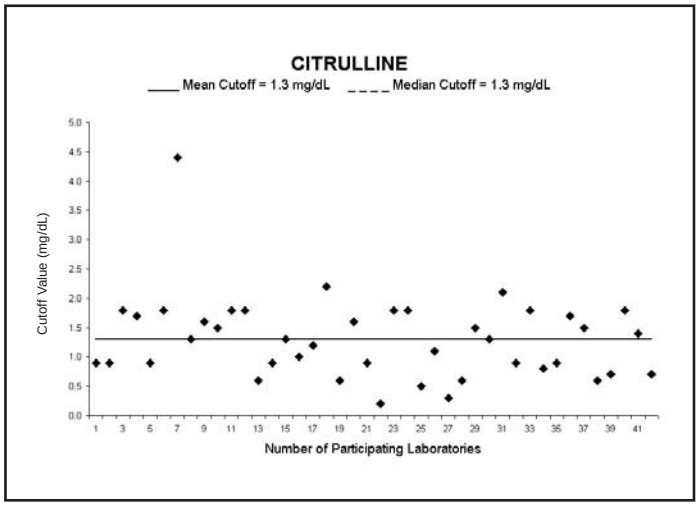


Figure 5f.

Figures 6a-6f. Reported Cutoff (Domestic and Foreign) vs. Calculated Mean Cutoff Value for Acylcarnitines (µmol/L whole blood)

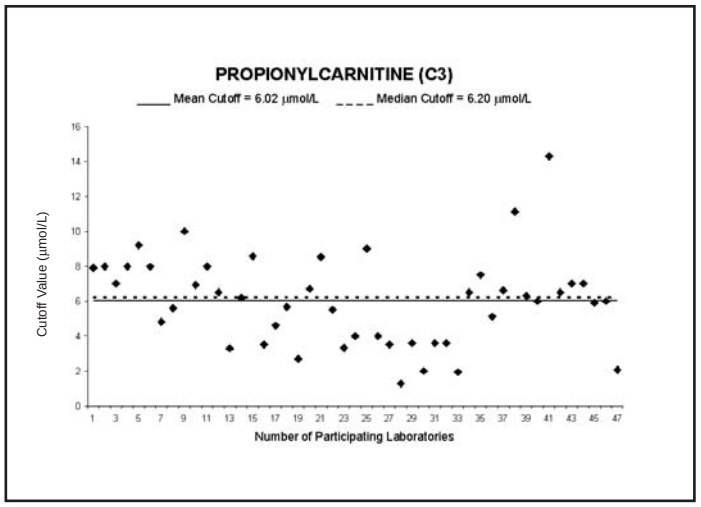


Figure 6a.

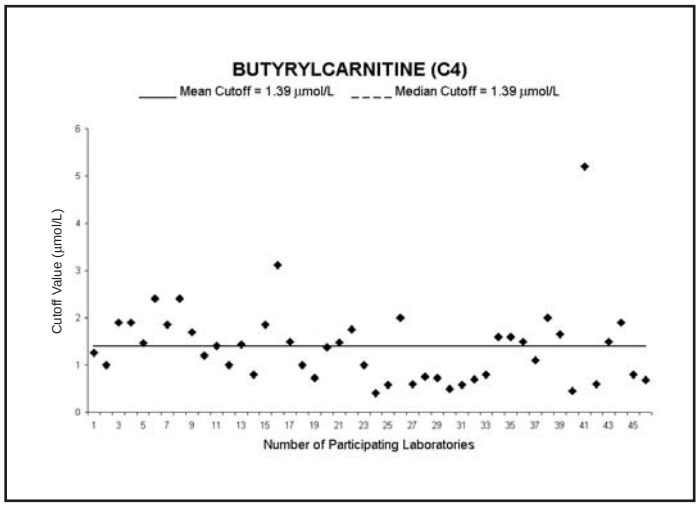


Figure 6b.

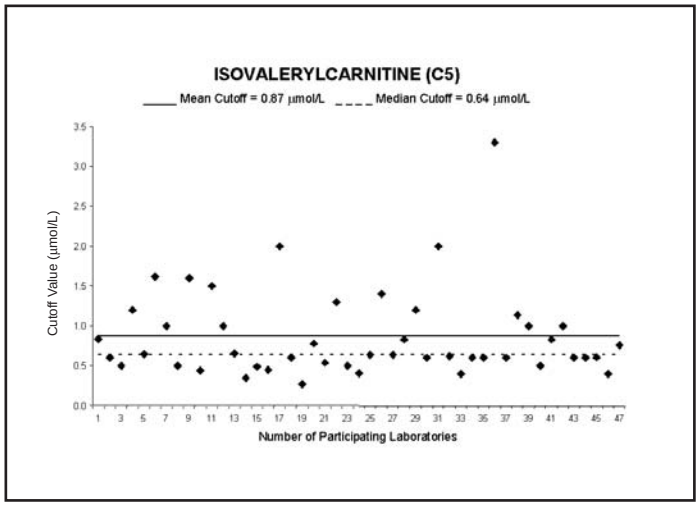


Figure 6c.

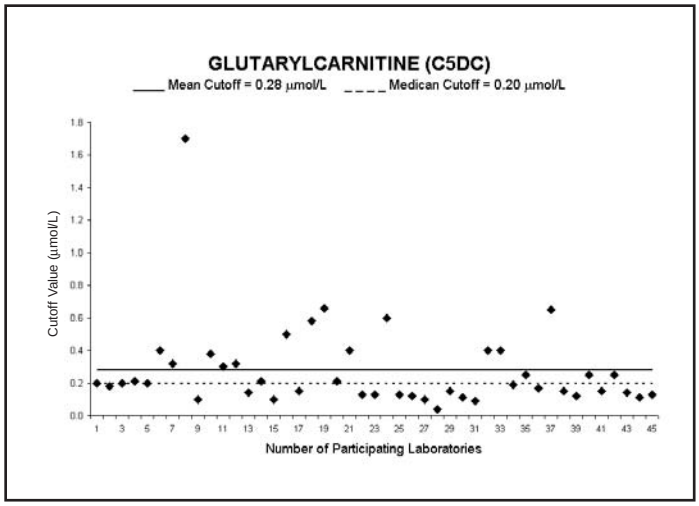


Figure 6d.

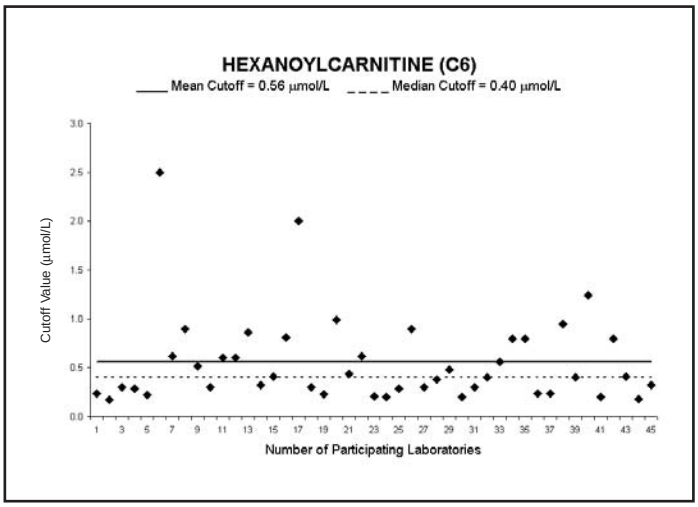


Figure 6e.

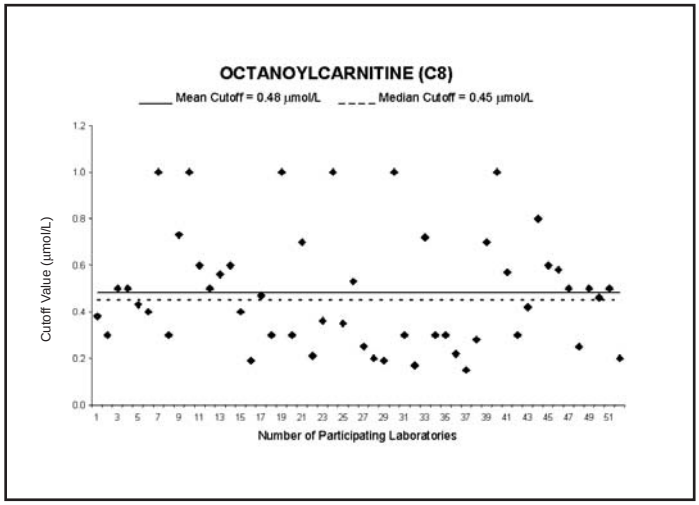


Figure 6f.

Figures 6g-6i. Reported Cutoff (Domestic and Foreign) vs. Calculated Mean Cutoff Value for Acylcarnitines (µmol/L whole blood)

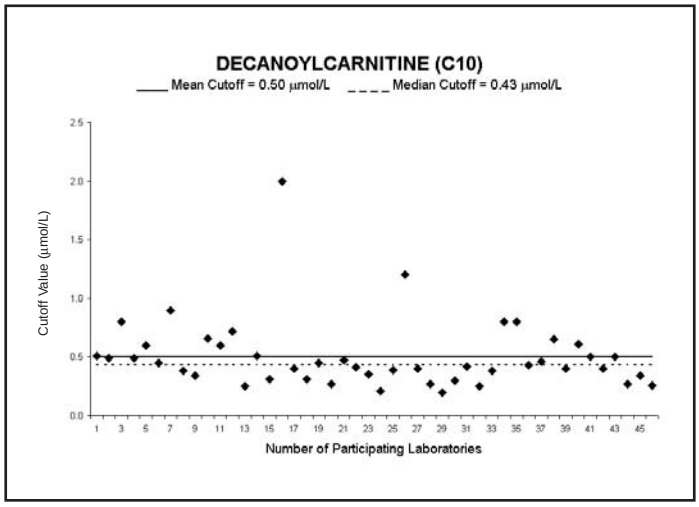


Figure 6g.

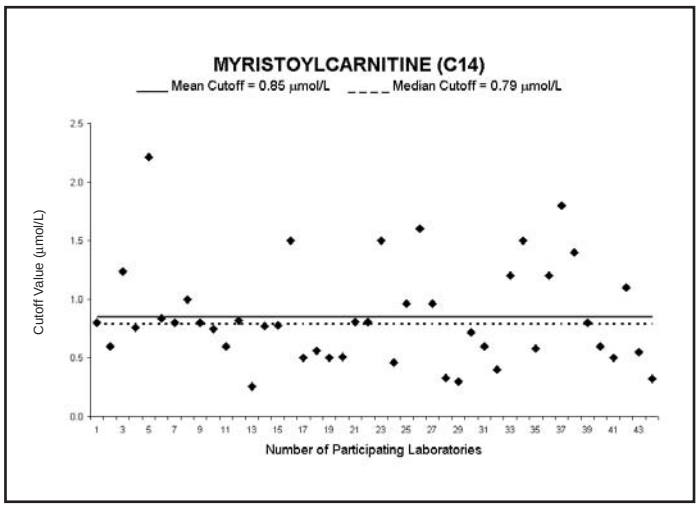


Figure 6h.

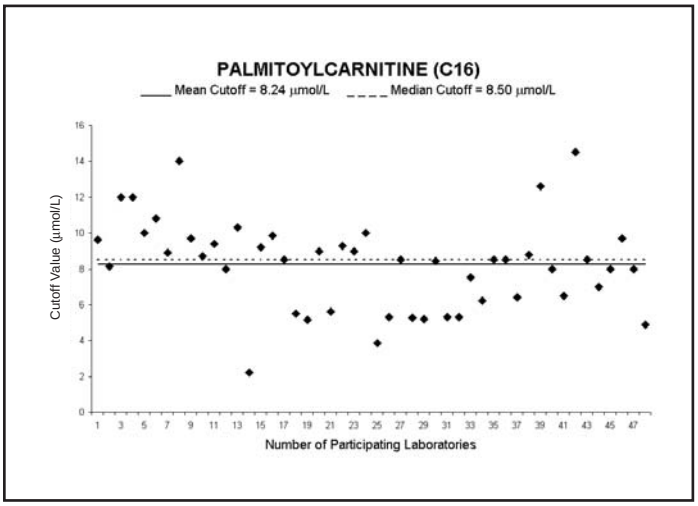


Figure 6i.

TABLE 1. Domestic Amino Acid Cutoff Value Statistics.

	N	MEAN µmol/L	MEDIAN µmol/L	MIN/MAX µmol/L		MEAN mg/dL	MEDIAN mg/dL	MIN/MAX mg/dL
Phenylalanine	17	145	139	103-200		2.4	2.3	1.7-3.3
Leucine	15	313	305	214-397		4.1	4.0	2.8-5.2
Methionine	15	81	113	54-127		1.2	1.3	0.8-1.9
Tyrosine	14	348	342	121-502		6.3	6.2	2.2-9.1
Valine	14	325	308	248-496		3.8	3.6	2.9-5.8
Citrulline	14	91	91	34-251		1.6	1.6	0.6-4.4

TABLE 2. Foreign Amino Acid Cutoff Value Statistics.

	N	MEAN µmol/L	MEDIAN µmol/L	MIN/MAX µmol/L		MEAN mg/dL	MEDIAN mg/dL	MIN/MAX mg/dL
Phenylalanine	35	164	152	61-364		2.7	2.5	1.0-6.0
Leucine	29	366	359	160-580		4.8	4.7	2.1-7.6
Methionine	29	74	67	34-134		1.1	1.0	0.5-2.0
Tyrosine	33	315	304	83-751		5.7	5.5	1.5-13.6
Valine	26	316	299	111-829		3.7	3.5	1.3-9.7
Citrulline	28	69	69	11-126		1.2	1.2	0.2-2.2

TABLE 3. Domestic Acylcarnitine Cutoff Value Statistics.

	N	MEAN µmol/L	MEDIAN µmol/L	MIN/MAX µmol/L
C3	14	7.1	7.45	3.3-10.00
C4	15	1.57	1.46	0.80-2.40
C5	15	0.86	0.66	0.35-1.62
C5DC	15	0.35	0.21	0.10-1.70
C6	17	0.59	0.41	0.17-2.50
C8	14	0.52	0.50	0.19-1.00
C10	14	0.55	0.51	0.25-0.90
C14	15	0.88	0.80	0.26-2.21
C16	14	9.53	9.63	2.20-14.00

TABLE 4. Foreign Acylcarnitine Cutoff Value Statistics.

	N	MEAN µmol/L	MEDIAN µmol/L	MIN/MAX µmol/L
C3	33	5.56	5.67	1.27-14.30
C4	31	1.30	1.00	0.41-5.21
C5	32	0.87	0.62	0.27-3.30
C5DC	31	0.24	0.15	0.04-0.66
C6	30	0.54	0.40	0.18-2.00
C8	35	0.46	0.36	0.15-1.00
C10	32	0.48	0.40	0.20-2.00
C14	30	0.85	0.75	0.30-1.80
C16	33	7.65	8.00	3.84-14.50

TABLE 5. Comparison of Amino Acid Cutoff Means and Medians in mg/dL between Domestic and Foreign Laboratories including the Combined Laboratory Means and Medians

	Domestic Laboratory Cutoffs			Foreign Laboratory Cutoffs			Domestic and Foreign (Combined) Cutoffs		
	N	Means	Medians	N	Means	Medians	N	Means	Medians
Phenylalanine	17	2.4	2.3	35	2.7	2.5	52	2.6	2.5
Leucine	15	4.1	4.0	29	4.8	4.7	44	4.5	4.3
Methionine	15	1.3	1.3	29	1.1	1.0	44	1.1	1.1
Tyrosine	14	6.3	6.2	33	5.7	5.5	47	5.9	6.0
Valine	14	3.8	3.6	26	3.7	3.5	40	3.8	3.5
Citrulline	14	1.6	1.6	28	1.2	1.2	42	1.3	1.3

Source: Quarter 3, 2003 NSQAP Data

TABLE 6. Comparison of Amino Acid Cutoff Means and Medians in µmol/L between Domestic and Foreign Laboratories including the Combined Laboratory Means and Medians

	Domestic Laboratory Cutoffs			Foreign Laboratory Cutoffs			Domestic and Foreign (Combined) Cutoffs		
	N	Means	Medians	N	Means	Medians	N	Means	Medians
Phenylalanine	17	145	139	35	164	152	52	158	152
Leucine	15	313	305	29	476	359	44	343	328
Methionine	15	87	87	29	74	67	44	75	74
Tyrosine	14	348	342	33	315	304	47	326	331
Valine	14	325	308	26	316	299	40	325	299
Citrulline	14	91	91	28	69	69	42	74	74

Source: Quarter 3, 2003 NSQAP Data

TABLE 7. Comparison of Acylcarnitine Cutoff Means and Medians in µmol/L between Domestic and Foreign Laboratories including the Combined Cutoff Means and Medians

	Domestic Laboratory Cutoffs µmol/L blood			Foreign Laboratory Cutoffs µmol/L blood			Domestic and Foreign (Combined) Cutoffs µmol/L blood		
	N	Means	Medians	N	Means	Medians	N	Means	Medians
C3	48	7.10	7.45	33	5.56	5.67	81	6.02	6.20
C4	46	1.57	1.46	31	1.30	1.00	77	1.39	1.39
C5	47	0.86	0.66	32	0.87	0.62	79	0.87	0.64
C6	45	0.59	0.41	30	0.54	0.40	75	0.56	0.40
C8	51	0.52	0.50	35	0.46	0.36	86	0.48	0.45
C10	46	0.55	0.51	32	0.48	0.48	78	0.50	0.43
C14	44	0.88	0.80	30	0.85	0.85	74	0.85	0.79
C16	48	9.53	9.63	33	7.65	7.65	81	8.24	8.50
C5DC	45	0.35	0.21	31	0.24	0.24	76	0.28	0.20

The circles indicate cutoff value differences >1.0 µmol/L whole blood.

Source: Quarter 3, 2003 NSQAP Data

This reference can also be used to provide general information about presumptive classification decisions used by newborn screening laboratories. The amino acids pilot PT program expanded to include the qualitative presumptive clinical assessment data in 2002. The acylcarnitine assessment component was added as a requirement for third quarter of 2003. PT specimen pools for acylcarnitines presently contain enrichments of one or more acylcarnitine levels. Calculated ratios are not consistent with acylcarnitine disorders and the use of ratios for detection is not standardized among laboratories. The grading component of our PT program is based on cutoff values for individual analytes. The NSQAP will apply laboratory-reported specific cutoff values, when available, to our grading algorithm for evaluating the clinical assessments. Clinical assessments are used to evaluate proficiency. If none are reported, grading cannot be done. The quantitative data are not used in the evaluation process; their statistics are provided for information only. Our grading algorithm is utilized only when a reported clinical assessment differs from the expected clinical assessment. When that occurs, the CDC certified reference value is measured against the CDC cutoff value and the participant laboratory's reported cutoff value. If the two clinical assessments found as a result of this process are the same, the laboratory's reported assessment is false-positive or false-negative. If the two resulting assessments are not the same, it is because of the large difference in the two cutoff values. A clinical assessment cannot be graded as incorrect when the discrepancy is due to the reported cutoff value. If a laboratory does not report cutoff values, the CDC cutoff is used for both steps of the algorithm.

The reporting of cutoff values is highly encouraged since the cutoff plays an important role in the evaluation process. The cutoff value is also used by NSQAP to guide the analytical enrichment levels for production of the PT specimens for future use.

PARTICIPANT RESULTS

Amino Acids

The following graphics (Figures 7-12) illustrate the assayed values submitted for each amino acid analyte by participant laboratories, domestic and foreign combined.

Figures 7a-7e. Participants' Results vs. Calculated Cutoff Mean and Median Values (Domestic and Foreign)
Phenylalanine

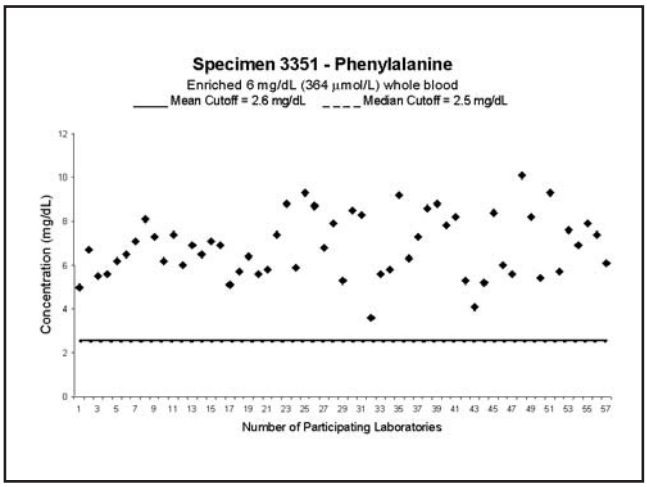


Figure 7a.

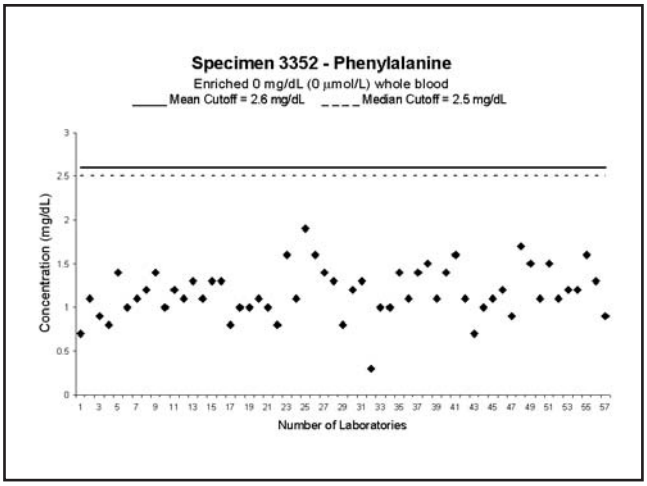


Figure 7b.

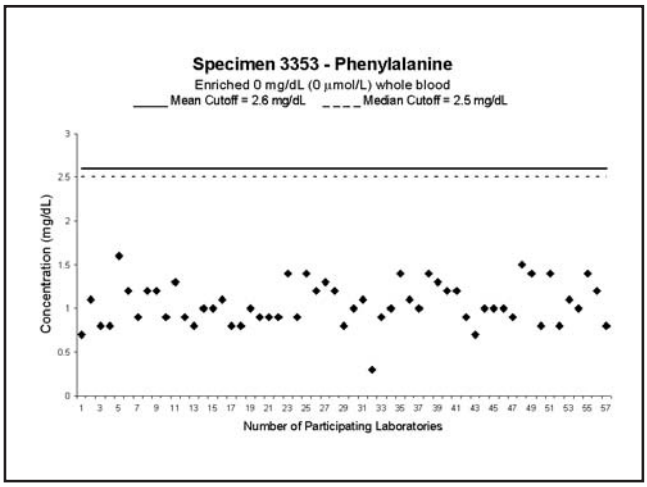


Figure 7c.

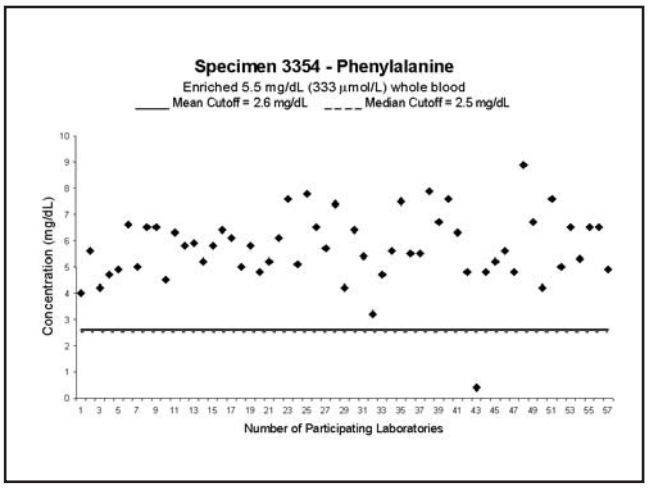


Figure 7d.

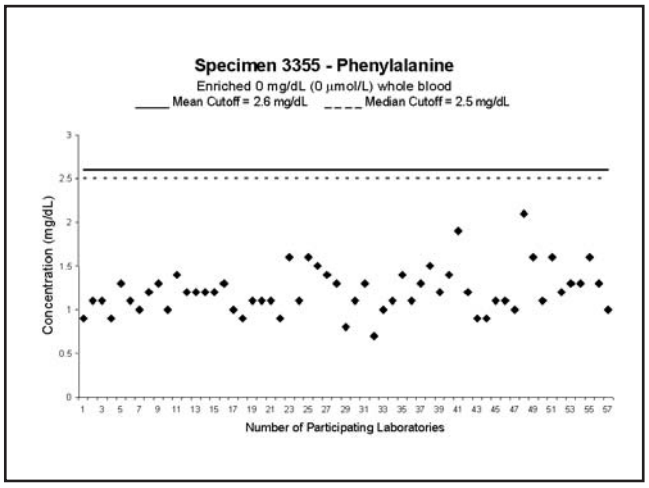


Figure 7e.

Figures 8a-8e. Participants' Results vs. Calculated Cutoff Mean and Median Values (Domestic and Foreign)
Leucine

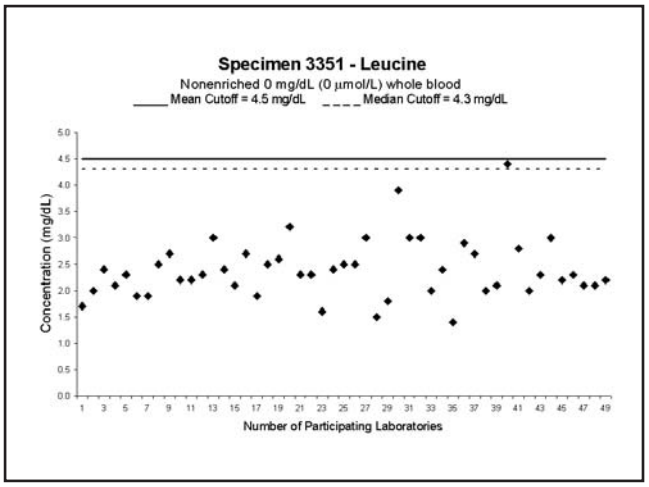


Figure 8a.

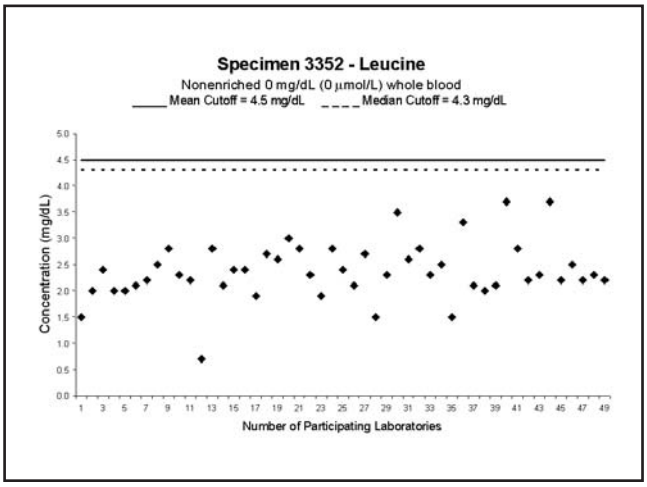


Figure 8b.

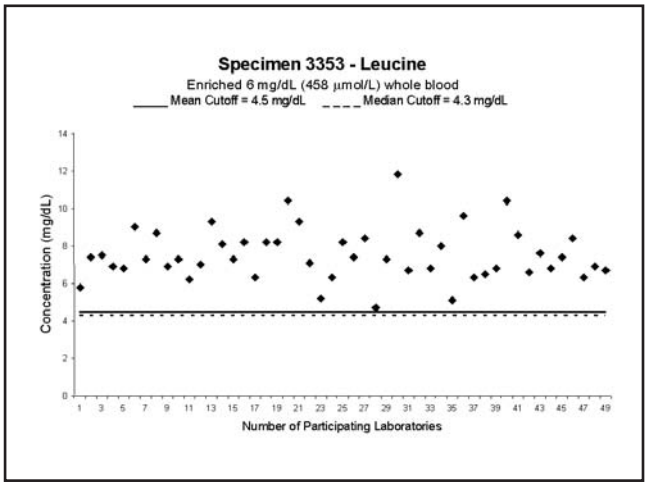


Figure 8c.

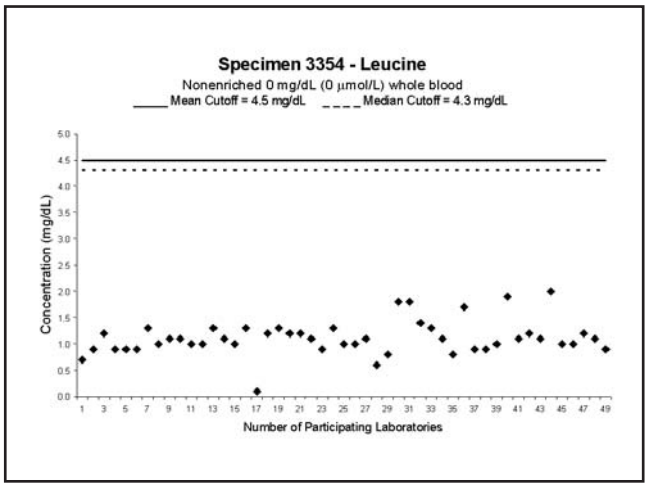


Figure 8d.

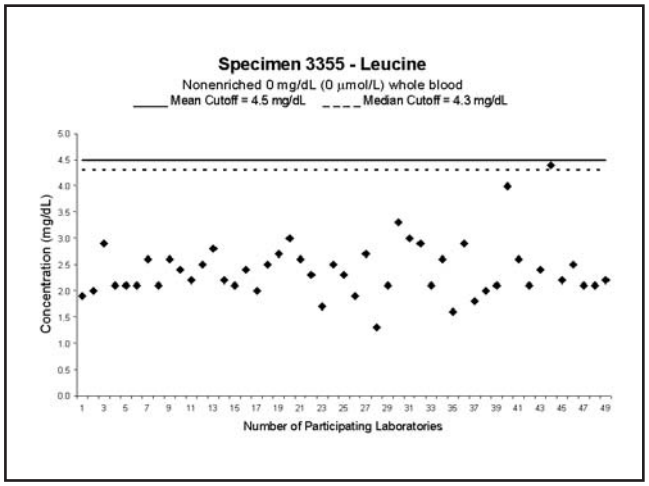


Figure 8e.

Figures 9a-9e. Participants' Results vs. Calculated Cutoff Mean and Median Values (Domestic and Foreign)
Methionine

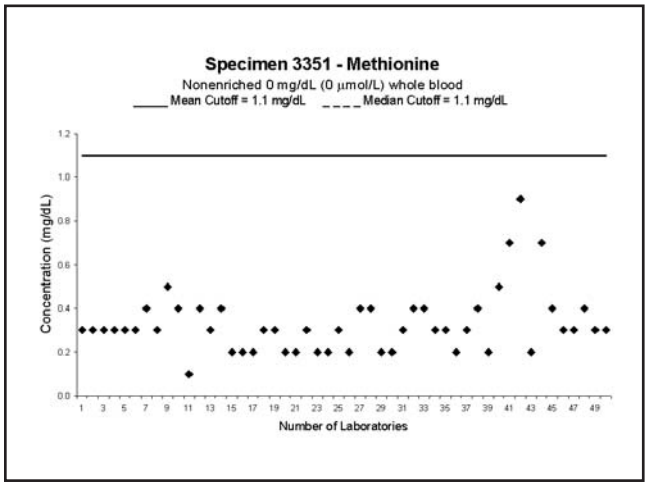


Figure 9a.

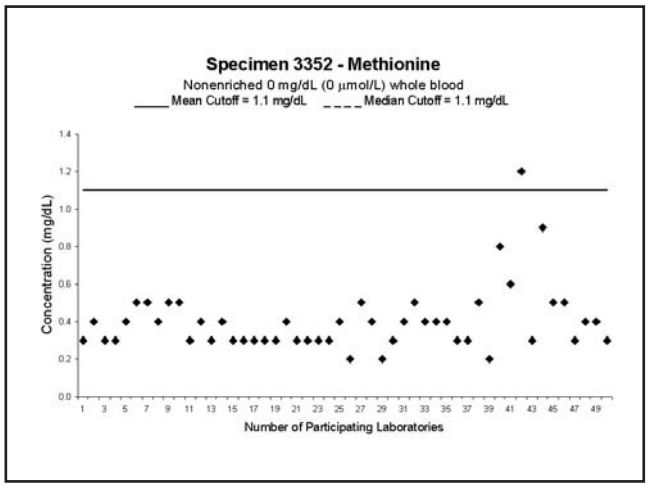


Figure 9b.

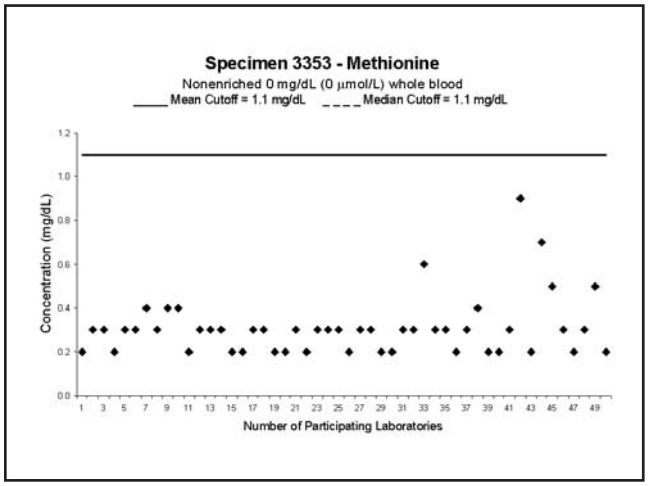


Figure 9c.

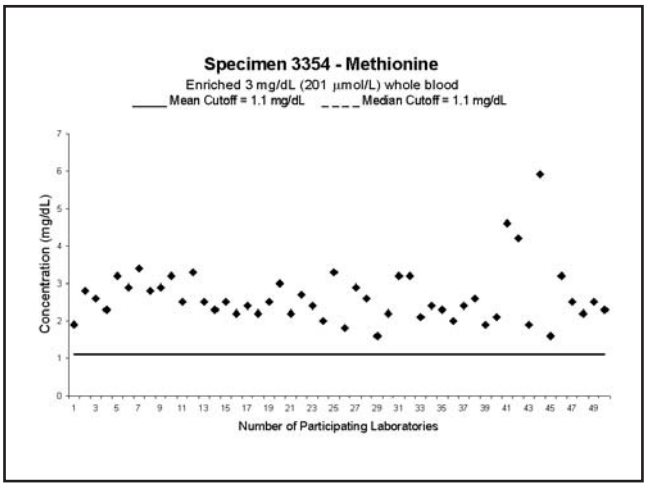


Figure 9d.

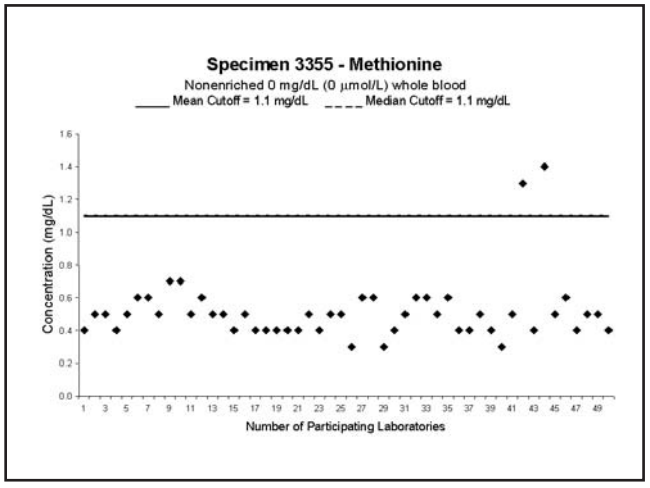


Figure 9e.

Figures 10a-10e. Participants' Results vs. Calculated Cutoff Mean and Median Values (Domestic and Foreign)
Tyrosine

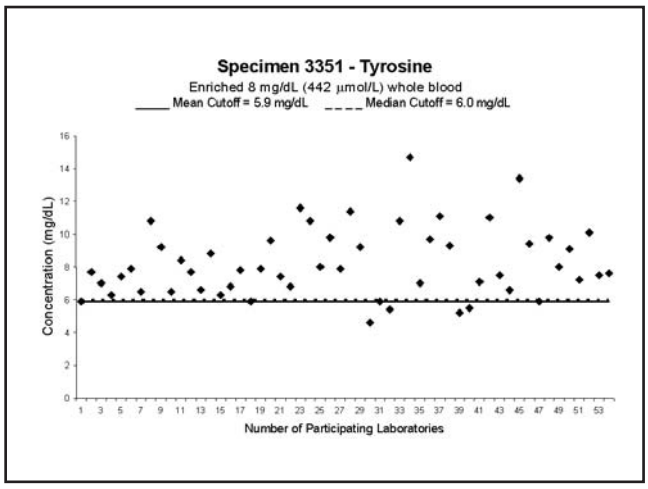


Figure 10a.

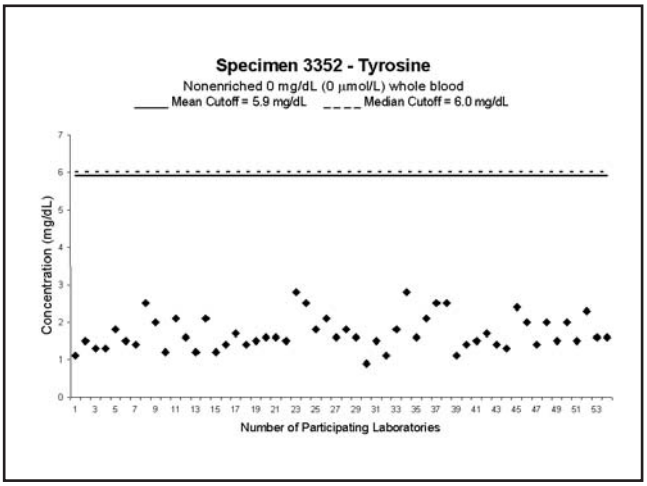


Figure 10b.

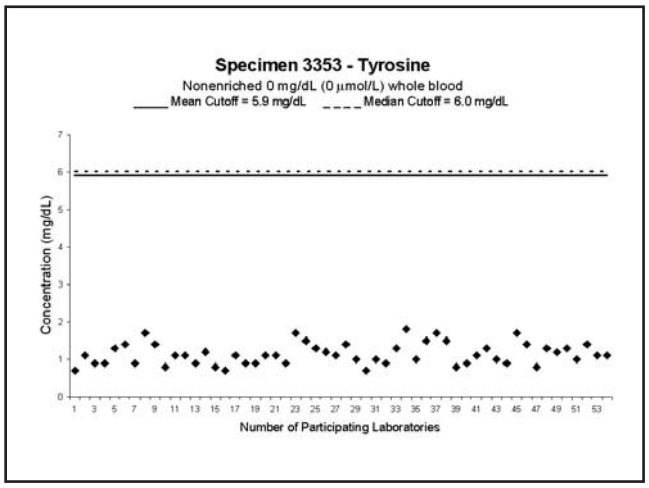


Figure 10c.

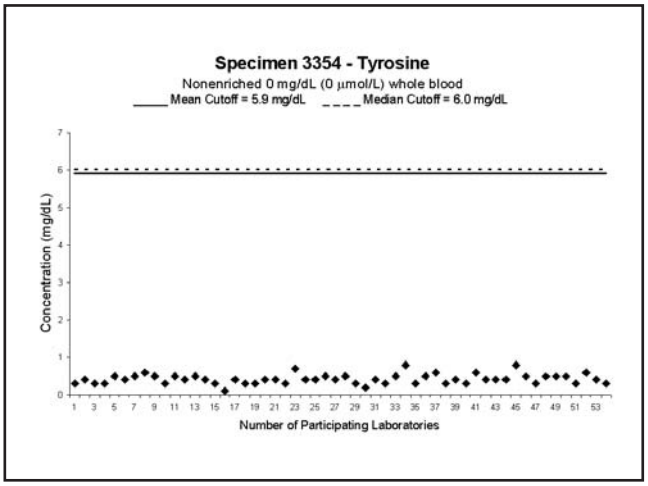


Figure 10d.

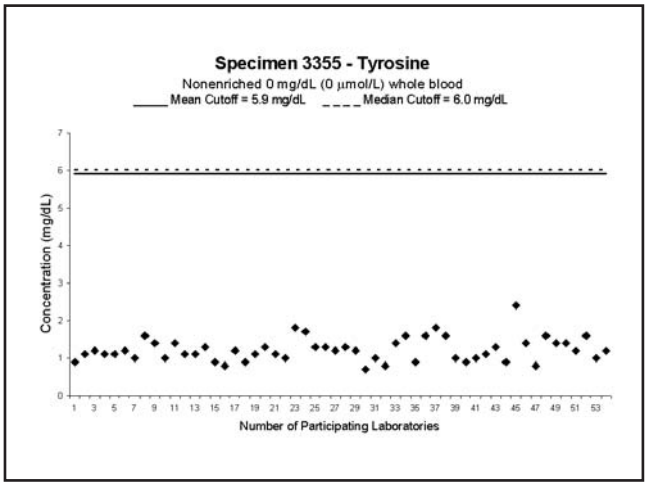


Figure 10e.

Figures 11a-11e. Participants' Results vs. Calculated Cutoff Mean and Median Values (Domestic and Foreign)
Valine

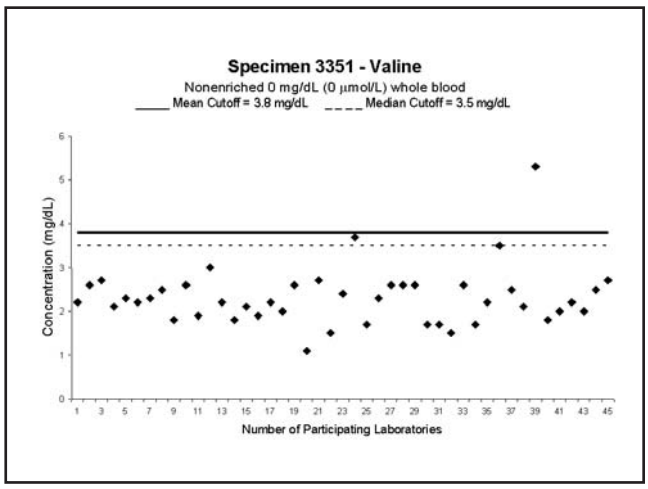


Figure 11a.

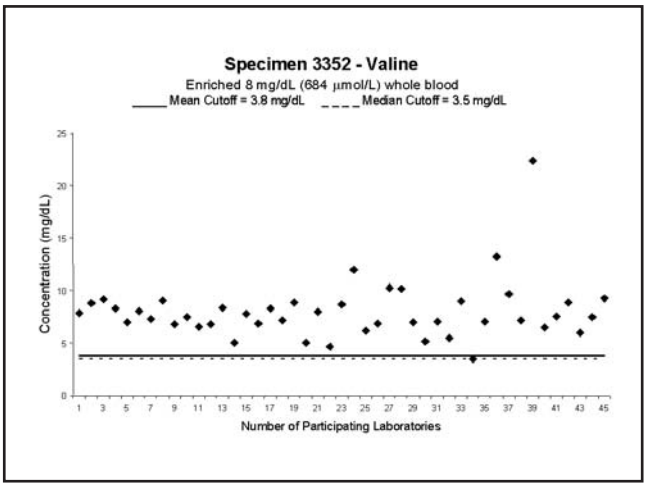


Figure 11b.

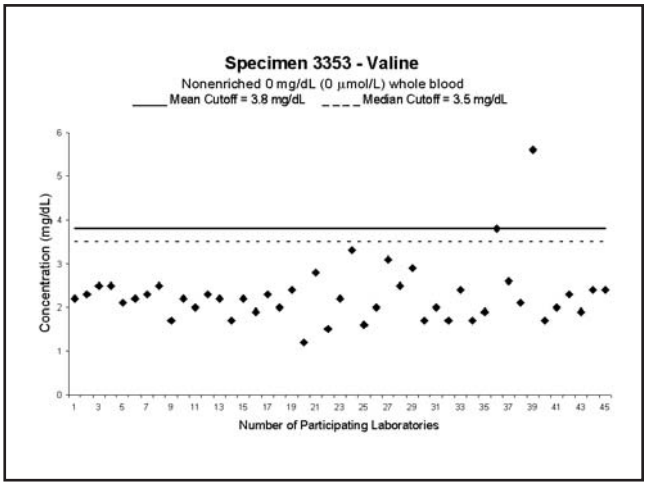


Figure 11c.

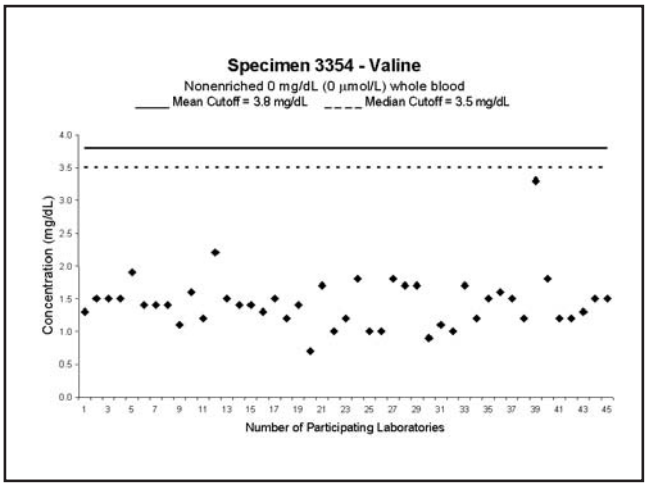


Figure 11d.

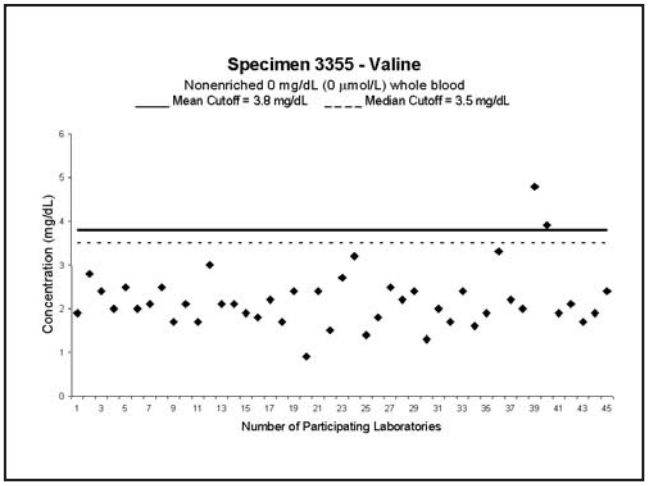


Figure 11e.

Figures 12a-12e. Participants' Results vs. Calculated Cutoff Mean and Median Values (Domestic and Foreign)
Citrulline

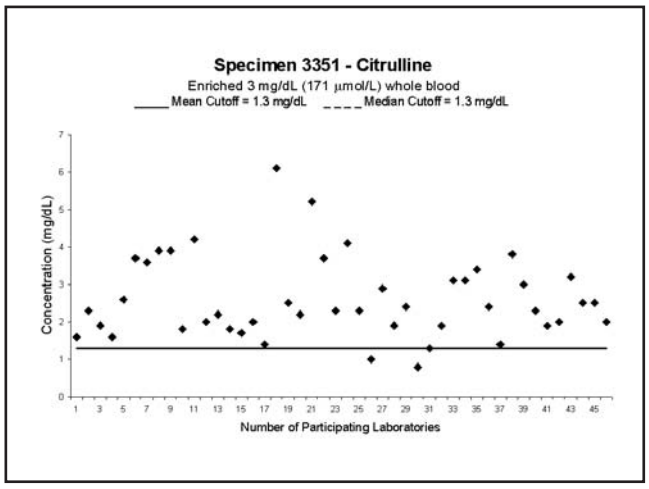


Figure 12a.

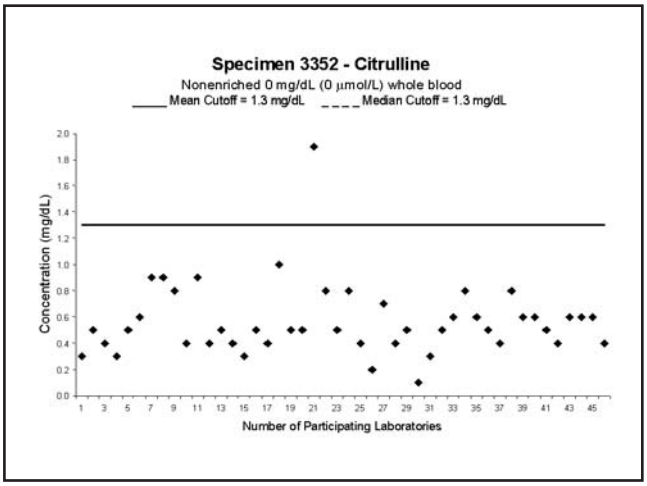


Figure 12b.

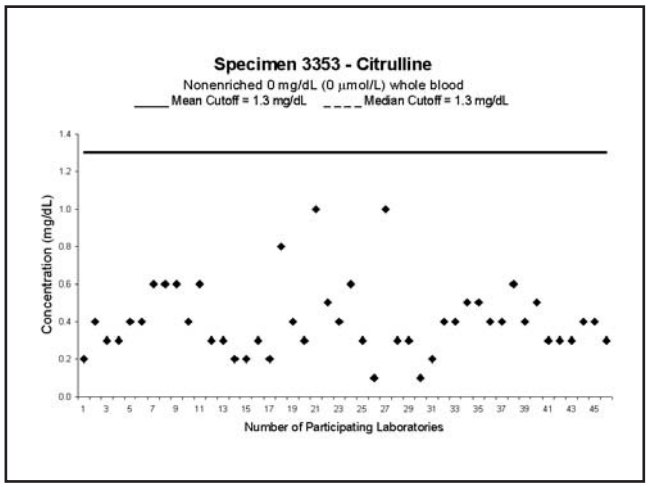


Figure 12c.

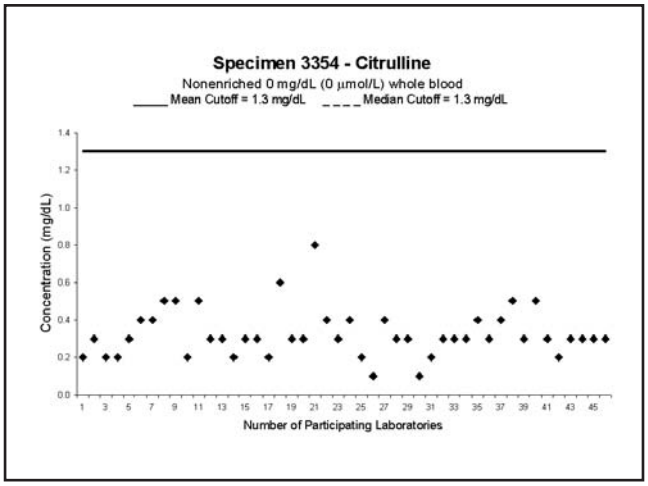


Figure 12d.

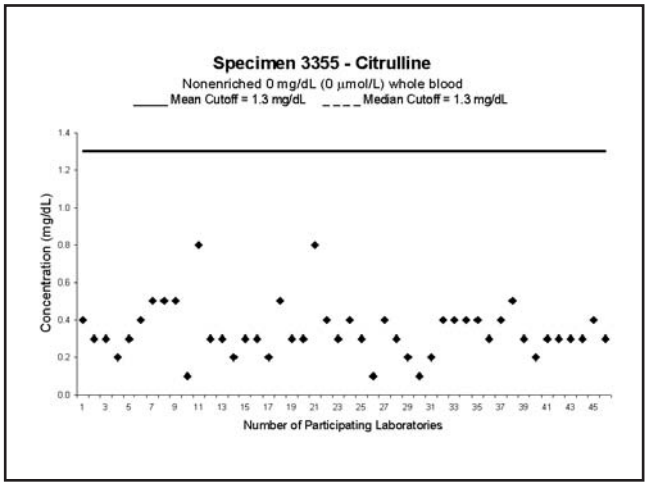


Figure 12e.

The solid line represents the mean cutoff and the dotted line represents the median cutoff for each analyte. (See section on determining appropriate cutoffs). Assayed values from the PT event for Quarter 3, 2003, were plotted against the overall mean and median cutoff values. The values for the nonenriched specimens "0 mg/dL" show the measured endogenous concentrations for the analyte. When specimens are enriched with predetermined levels of pure analyte, the overall concentration can be higher due to the contribution of endogenous levels. Even though the inherent characteristics of DBS cause some variation among data values, differences in pre-analytic derivatization methods and internal standard materials also influence the measured concentrations. Inquiries in the form of questionnaires are periodically added to the NSQAP data-report forms as a means of collecting procedural information that will enable the sorting of data by these differences.
Quarter 3, 2003, participant results for amino acids show that reported Phenylalanine (Phe) values for all specimens, in reference to the mean cutoff value for Phe, are in good agreement with regard to classifications (Figures 7a-7e). The nonenriched specimens 3352, 3353, and 3355 reported Phe values well below the mean cutoff values. The enriched specimen 3351 containing 6 mg Phe/dL shows all results above the mean cutoff value while specimen 3354 enriched at 5.5 mg Phe/dL shows all values above the cutoff except for one value that fell below.

The nonenriched Leucine (Leu) specimens (Figures 8a-8e) show results that are below the cutoff value and the one enriched specimen 3353 of 6 mg Leu/dL showed all participant results falling above the cutoff as expected.

The pattern of Methionine (Met) results (Figures 9a-9e) was similar to that of Leucine. The majority of values for the nonenriched specimens fall below the mean cutoff value with a few outliers seen in specimens 3352 and 3355. The reported values for specimen 3354 enriched with 3 mg Met/dL are clearly above the mean cutoff value. The nonenriched specimens for tyrosine (Tyr), 3352, 3353, 3354, and 3355 all show results well below the cutoff value. Specimen 3351 enriched with 8 mg Tyr/dL shows the majority of participant results fell above the mean cutoff; however, results from five foreign laboratories fell below the mean cutoff value.
Valine (Val) results (Figures 11a-11e) show at least one laboratory above the mean cutoff for all the nonenriched Val specimens. The specimen enriched with 8 mg Val/dL of blood shows all but one laboratory above the mean cutoff value.

Citrulline (Cit) results for all nonenriched specimens are below the mean cutoff with the exception of one result for specimen 3352. Specimen 3351 enriched with 3.0 mg Cit/dL of blood showed most values above the cutoff mean. There were four laboratories that gave false-negative assessments which were considered misses. In cases where the distribution of participant values is evenly distributed above and below the mean cutoff value, and if the consensus of values is not greater than 80% either way, the specimen would be classified as a not evaluated specimen.

Acylcarnitines

The acylcarnitine participant results are shown in reference to the calculated cutoff means and medians (Figures 14-20). The graphs were produced using the Quarter 3, 2003 results.

The C3 (Propionylcarnitine) results show that quantitative values reported for the nonenriched C3 specimens (3361, 3362, 3363, 3364) were all well below the cutoff mean of 6.02 µmol C3/L blood. Specimen 3365, which was enriched with 7.5 µmol C3/L blood, showed most values above the cutoff mean. There were a few reported values that fell below the cutoff range; however, the assessments were outside normal limits based on their laboratory-specific cutoff values.

The nonenriched C4 (Butyrylcarnitine) results for specimens 3361, 3362, 3363, and 3364 show all laboratories falling below the mean cutoff of 1.39 µmol C4/L blood. Results for specimen 3365 enriched with 2.5 µmol C4/L blood show all but one laboratory reporting above the cutoff mean of 1.39 µmol C4/L blood.

The C5 (Isovalerylcarnitine) results for the nonenriched specimens 3361 and 3363 show all but two laboratories reporting well below the cutoff value of 0.87 µmol C5/L blood. Specimen 3364 shows all results well below the cutoff. The two enriched specimens 3362 with 2.0 µmol C5/L blood and 3365 with 1.5 µmol C5/L blood, show all results above the mean cutoff value.

Specimen results for C5DC (Glutarylcarnitine) show most laboratories falling below the cutoff value for the nonenriched specimens.

Figures 13a-13e. Participants' Results vs. Calculated Cutoff Mean and Median Values (Domestic and Foreign)
Propionylcarnitine (C3)

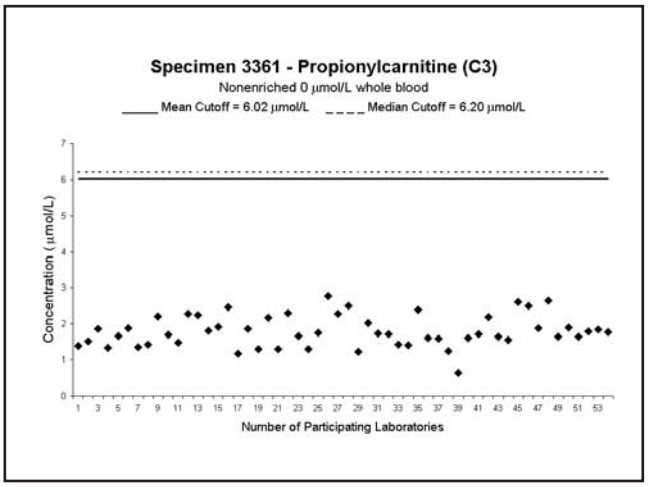


Figure 13a.

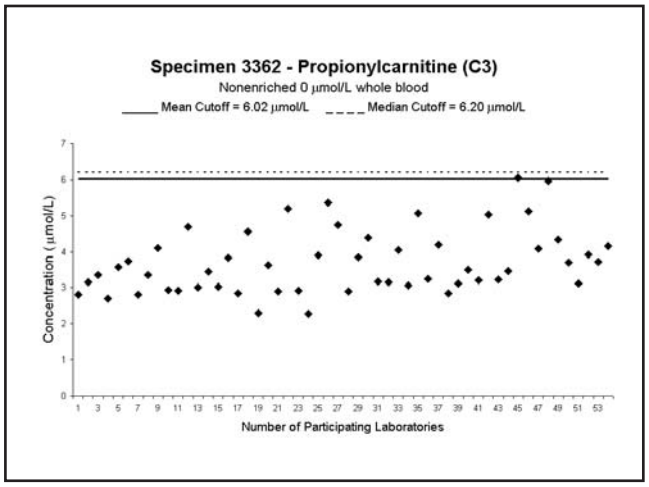


Figure 13b.

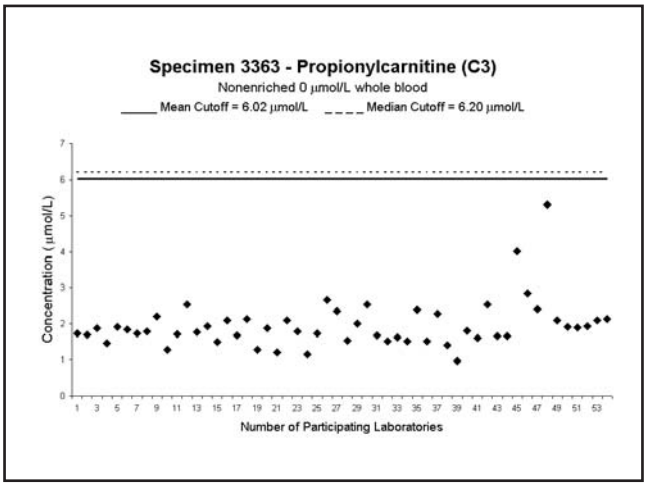


Figure 13c.

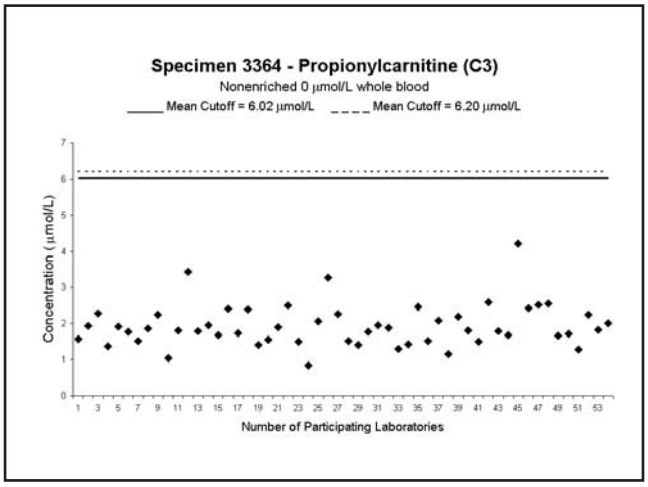


Figure 13d.

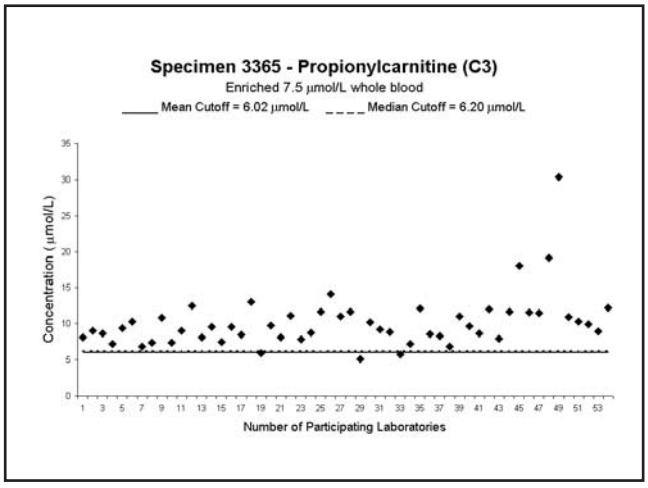


Figure 13e.

Figures 14a-14e. Participants' Results vs. Calculated Cutoff Mean and Median Values (Domestic and Foreign)
Butyrylcarnitine (C4)

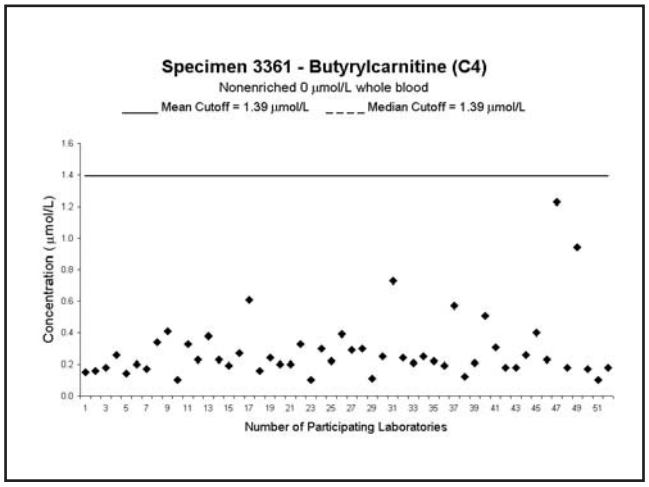


Figure 14a.

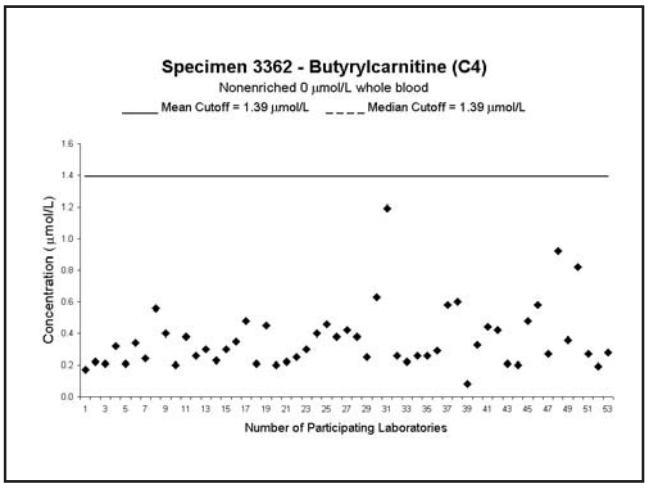


Figure 14b.

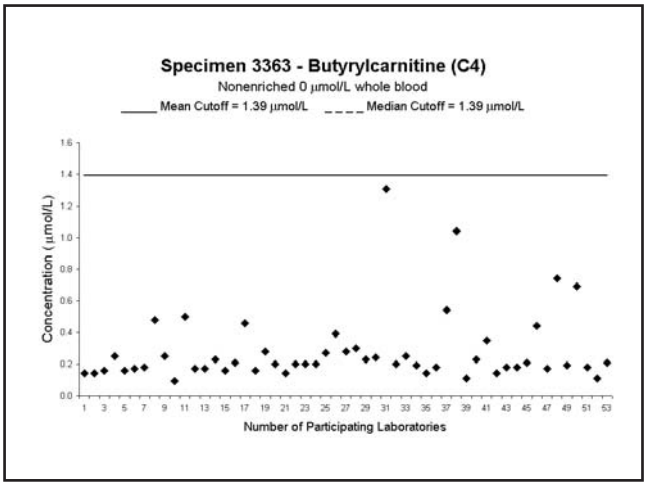


Figure 14c.

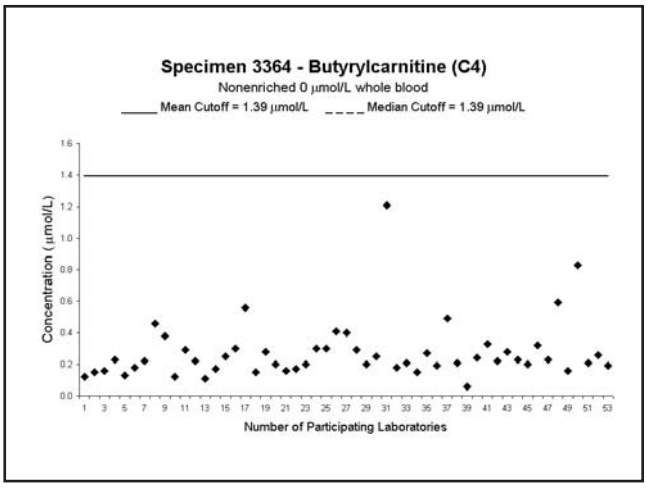


Figure 14d.

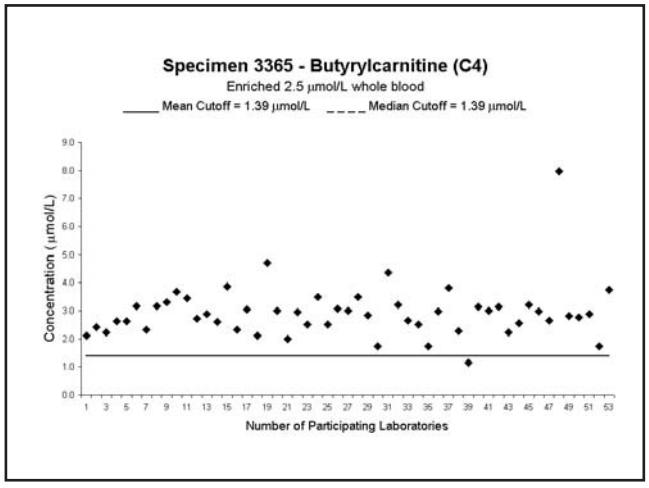


Figure 14e.

Figures 15a-15e. Participants' Results vs. Calculated Cutoff Mean and Median Values (Domestic and Foreign)
Isovalerylcarnitine (C5)

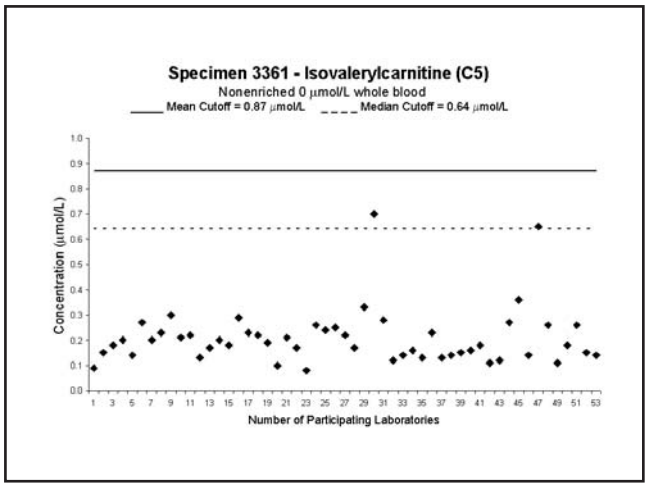


Figure 15a.

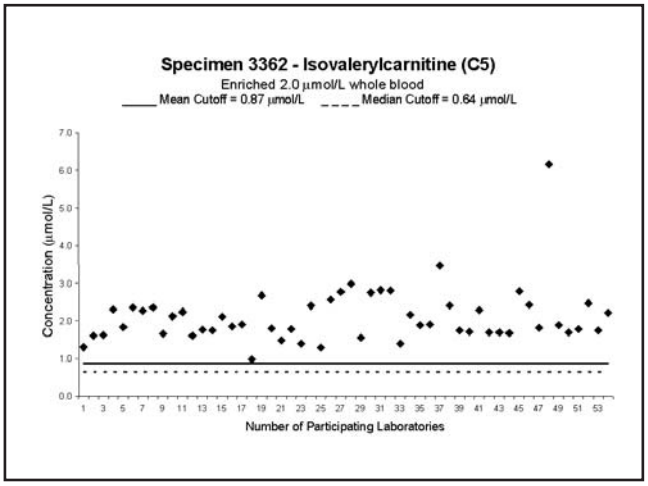


Figure 15b.

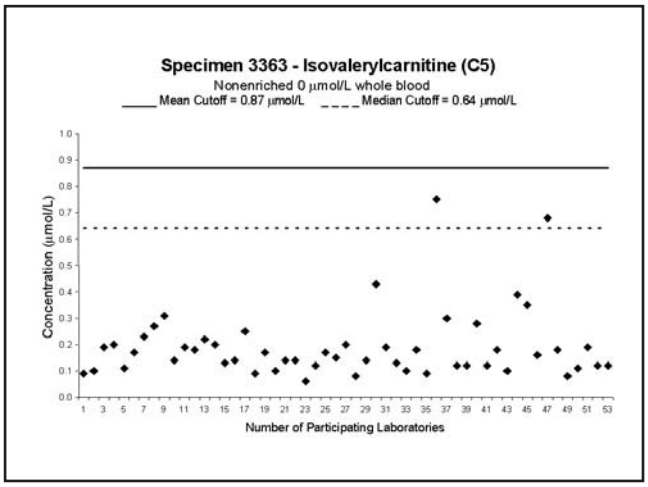


Figure 15c.

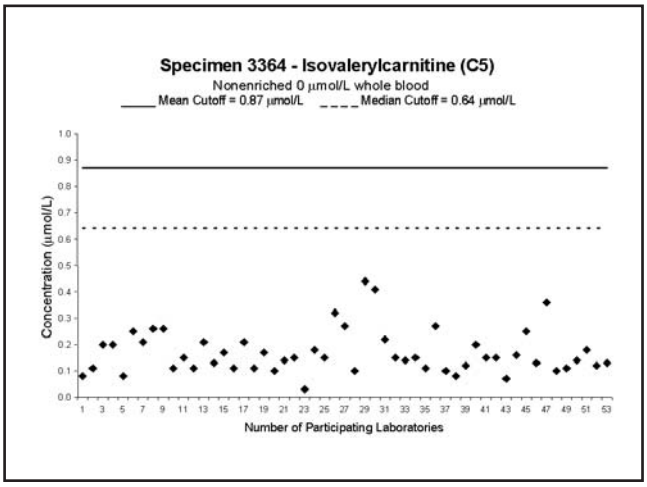


Figure 15d.

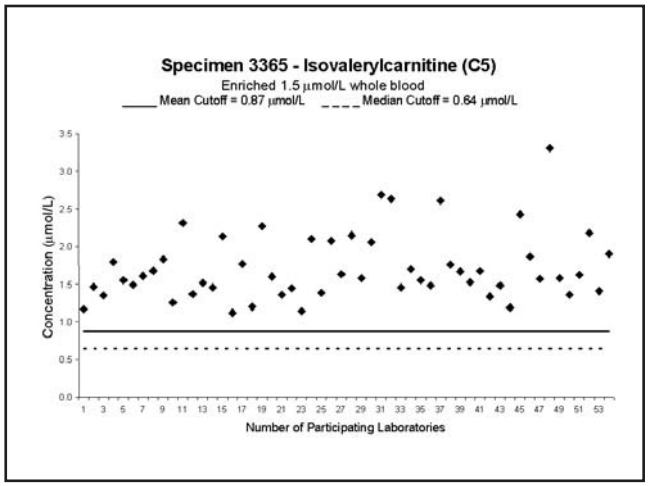


Figure 15e.

Figures 16a-16e. Participants' Results vs. Calculated Cutoff Mean and Median Values (Domestic and Foreign)
Glutarylcarnitine (C5DC)

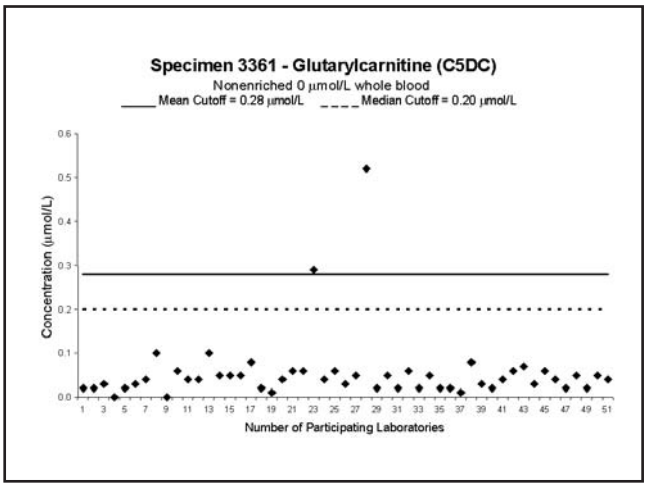


Figure 16a.

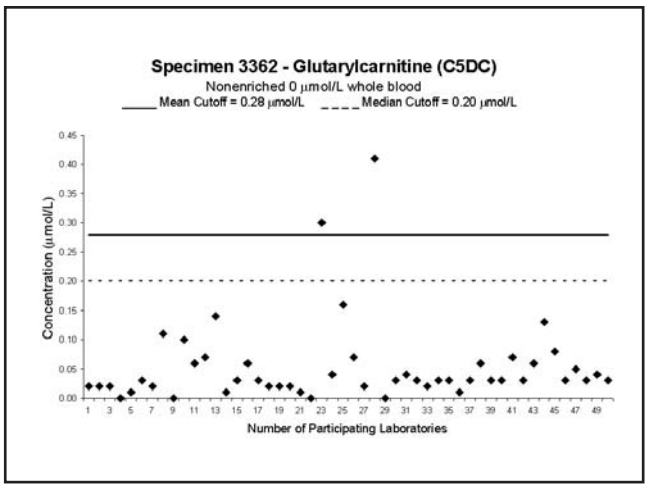


Figure 16b.

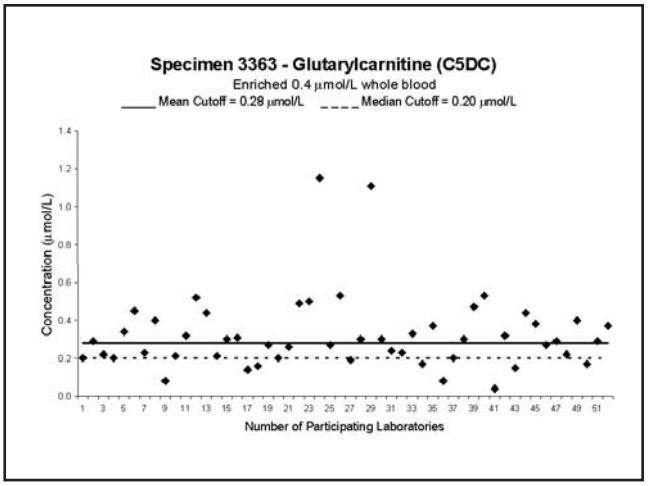


Figure 16c.

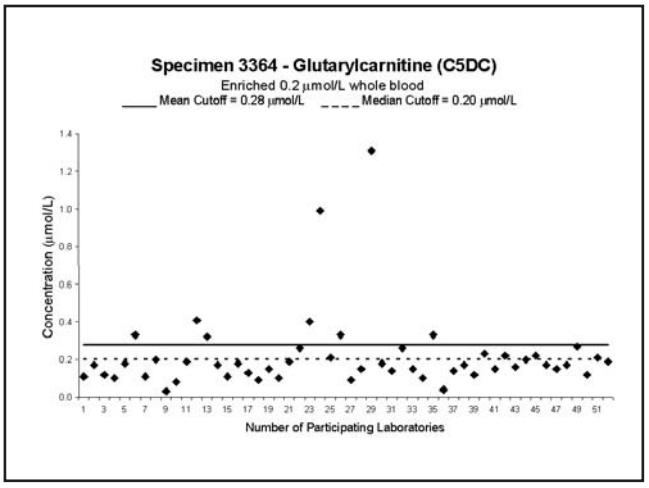


Figure 16d.

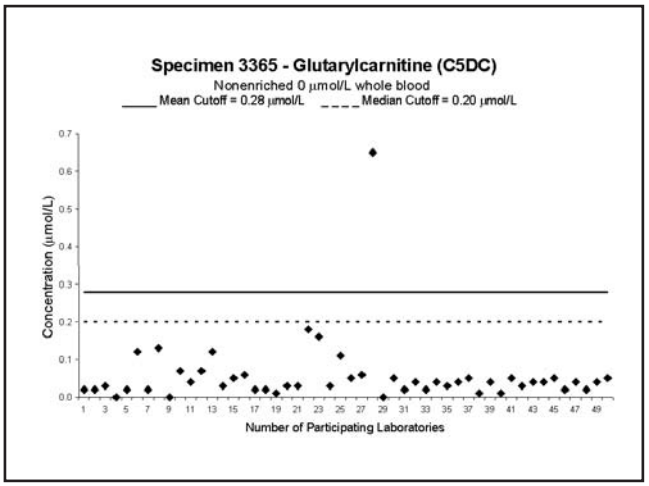


Figure 16e.

Figures 17a-17e. Participants' Results vs. Calculated Cutoff Mean and Median Values (Domestic and Foreign)
Hexanoylcarnitine (C6)

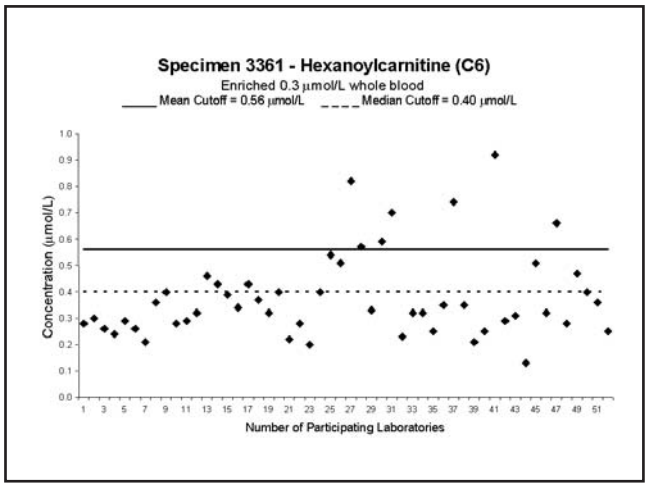


Figure 17a.

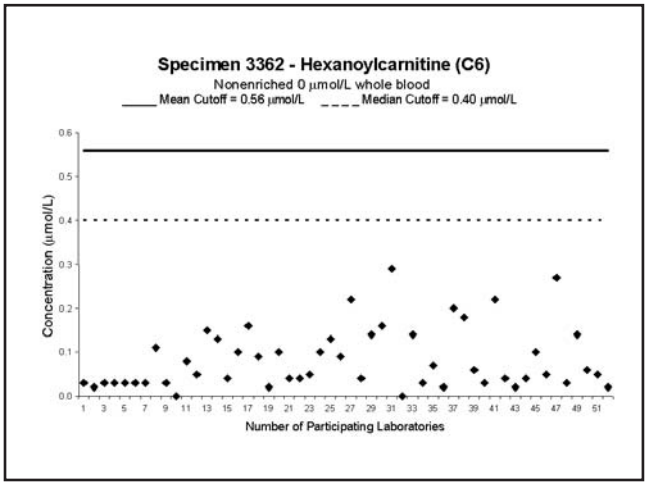


Figure 17b.

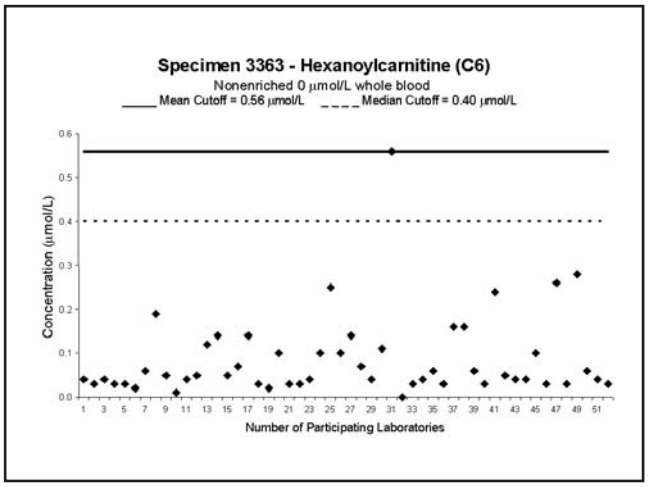


Figure 17c.

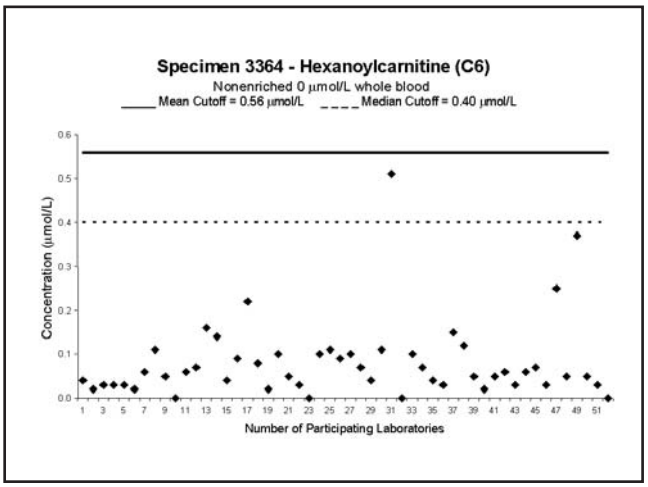


Figure 17d.

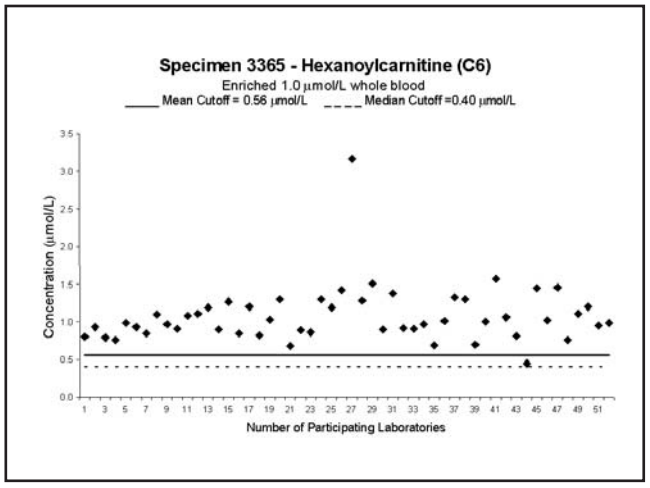


Figure 17e.

Figures 18a-18e. Participants' Results vs. Calculated Cutoff Mean and Median Values (Domestic and Foreign)
Octanoylcarnitine (C8)

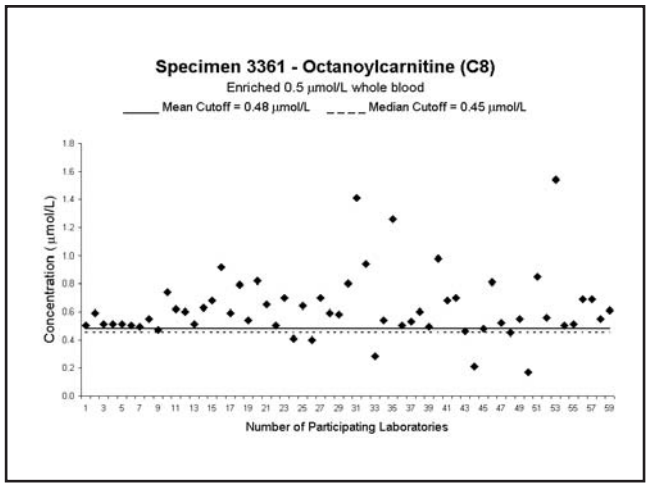


Figure 18a.

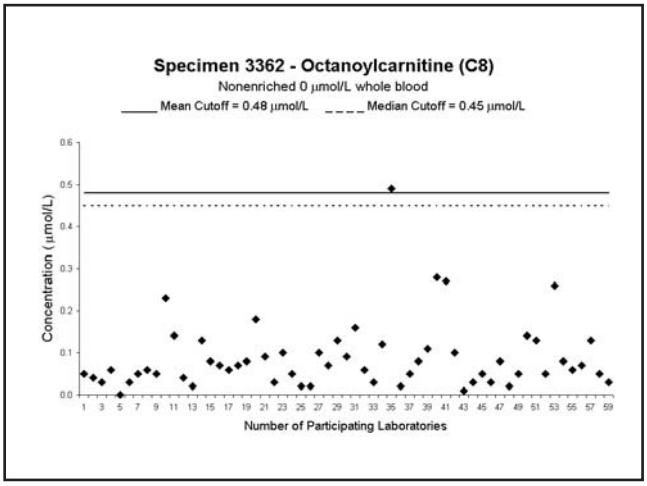


Figure 18b.

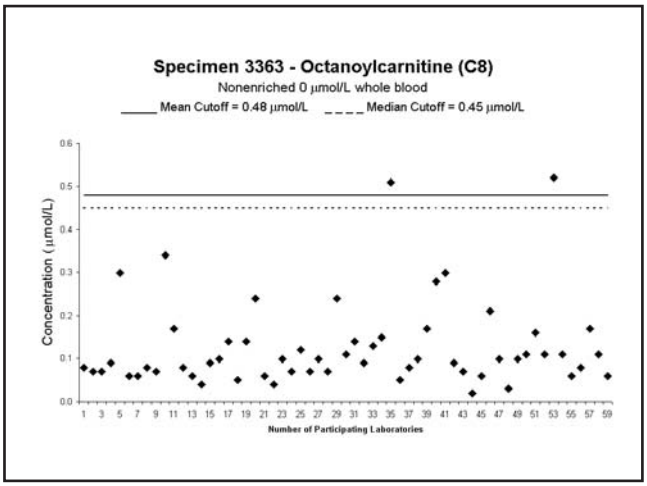


Figure 18c.

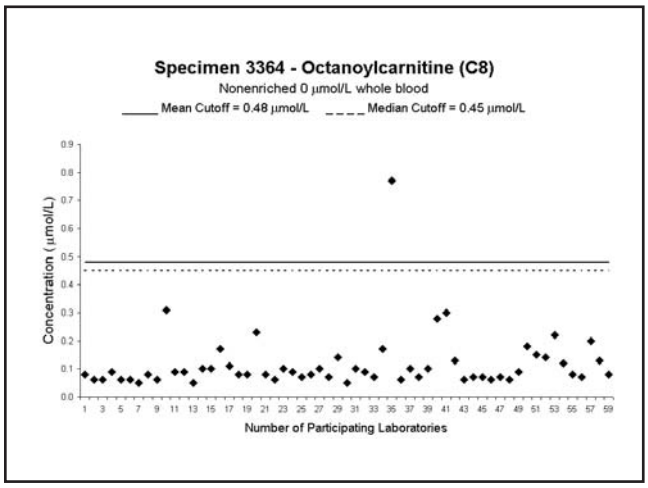


Figure 18d.

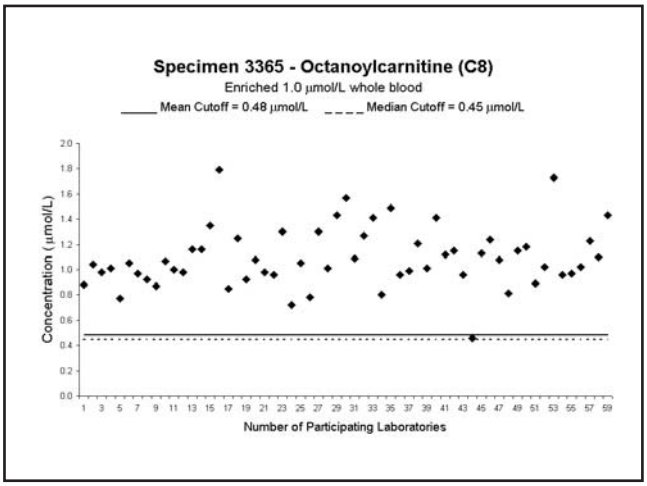


Figure 18e.

Figures 19a-19e. Participants' Results vs. Calculated Cutoff Mean and Median Values (Domestic and Foreign)
Decanoylcarnitine (C10)

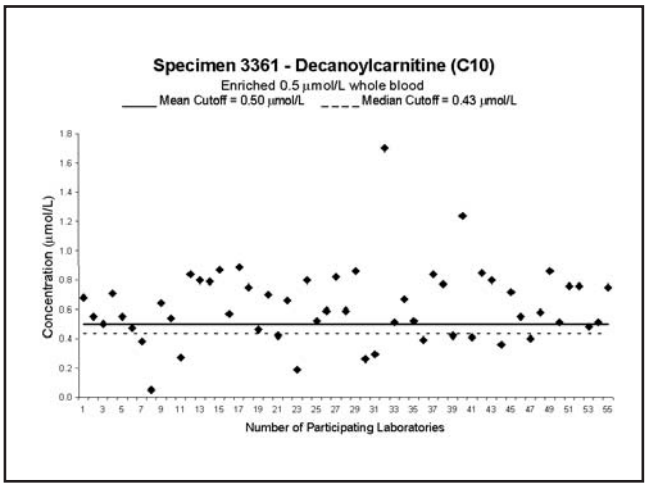


Figure 19a.

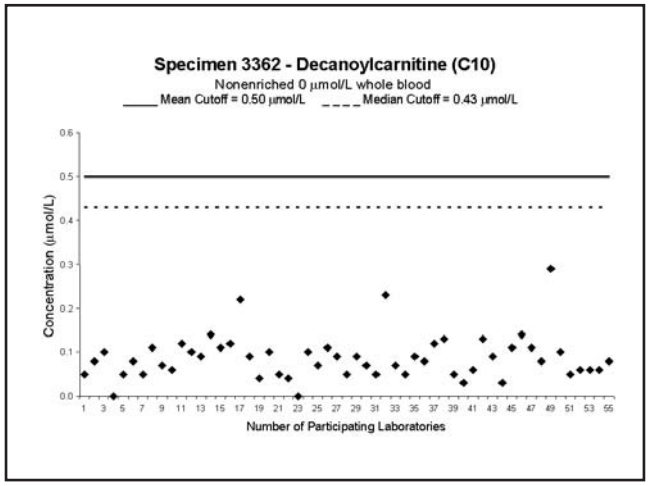


Figure 19b.

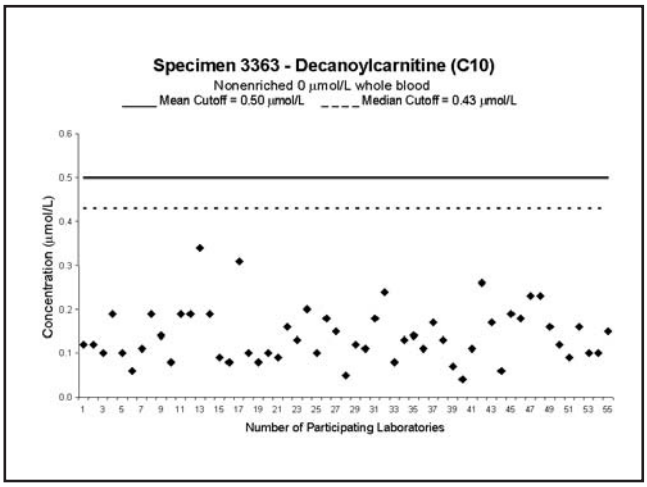


Figure 19c.

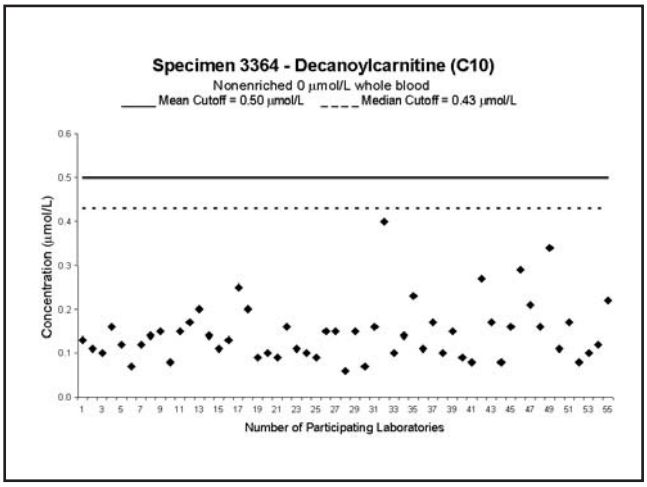


Figure 19d.

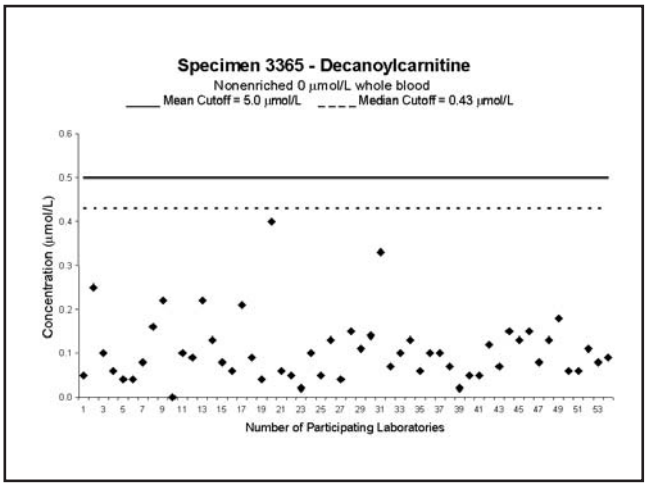


Figure 19e.

Figures 20a-20e. Participants' Results vs. Calculated Cutoff Mean and Median Values (Domestic and Foreign)
Myristoylcarnitine (C14)

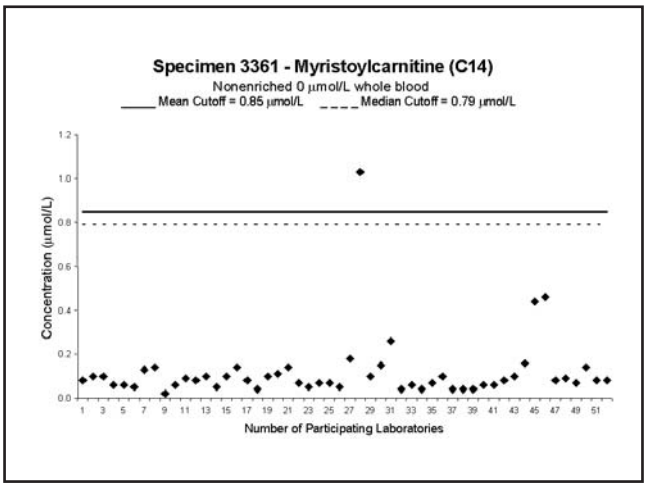
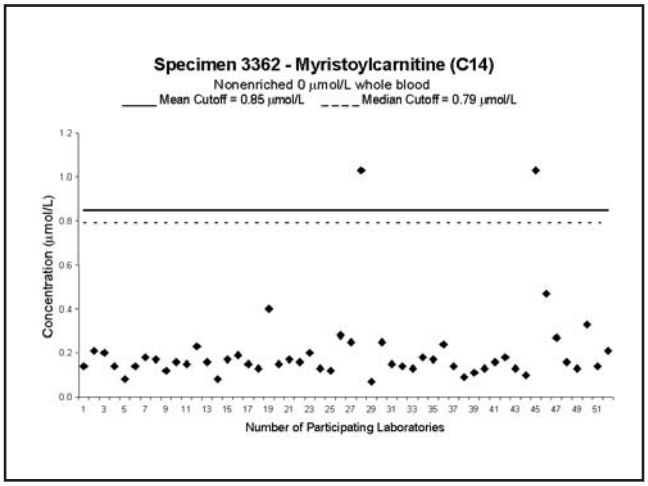


Figure 20a.



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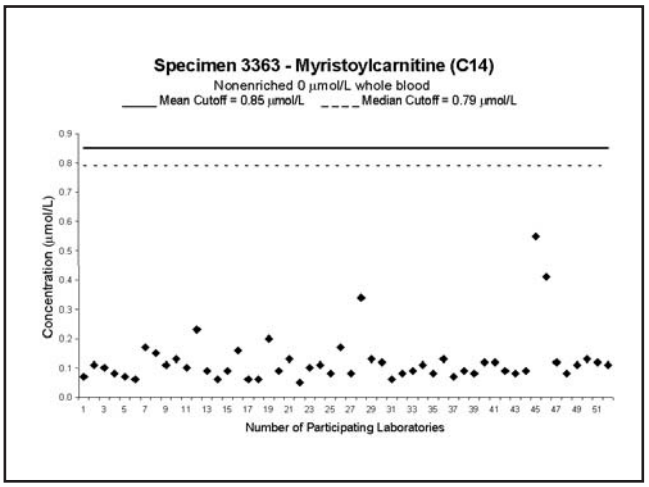

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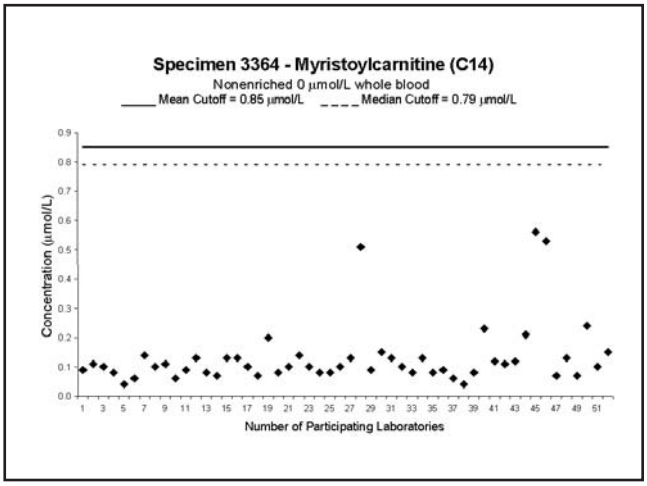

Figure 20d.

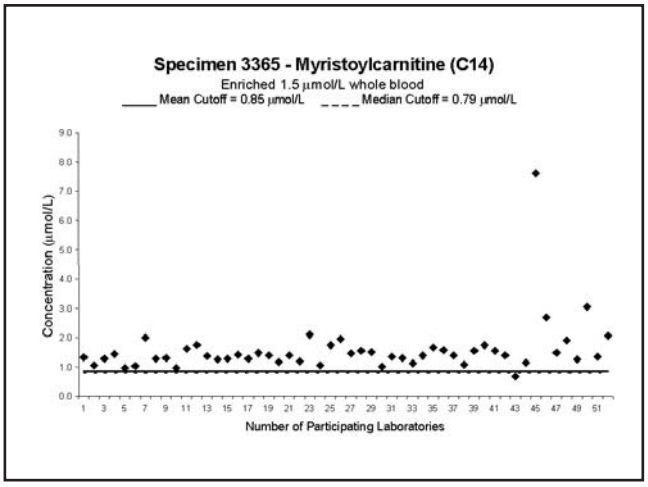

Figure 20e.

Figures 21a-21e. Participants' Results vs. Calculated Cutoff Mean and Median Values (Domestic and Foreign)
Palmitoylcarnitine (C16)

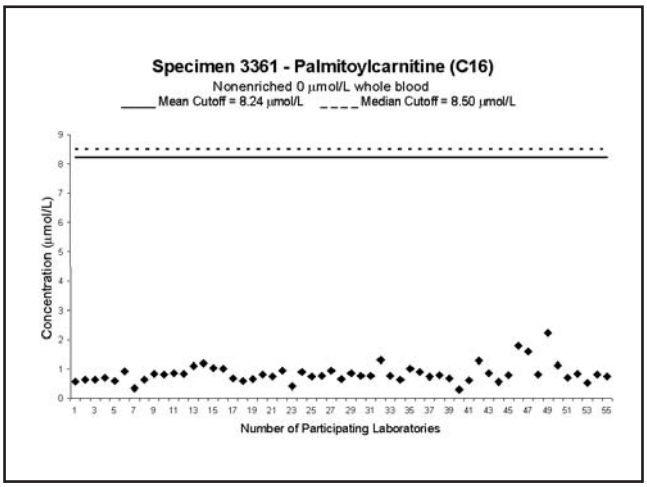

Figure 21a.

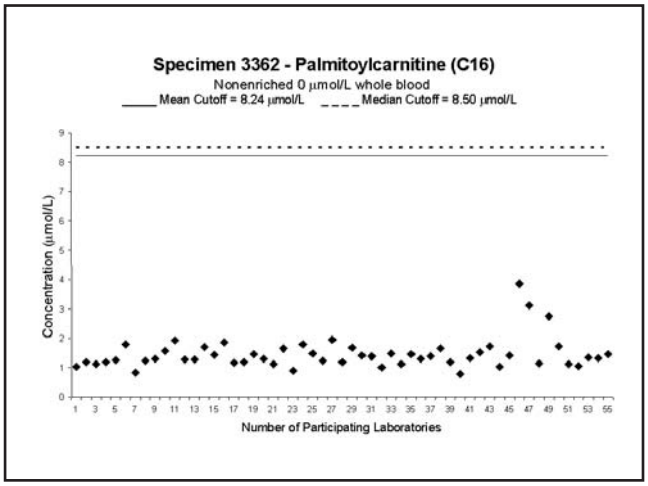

Figure 21b.

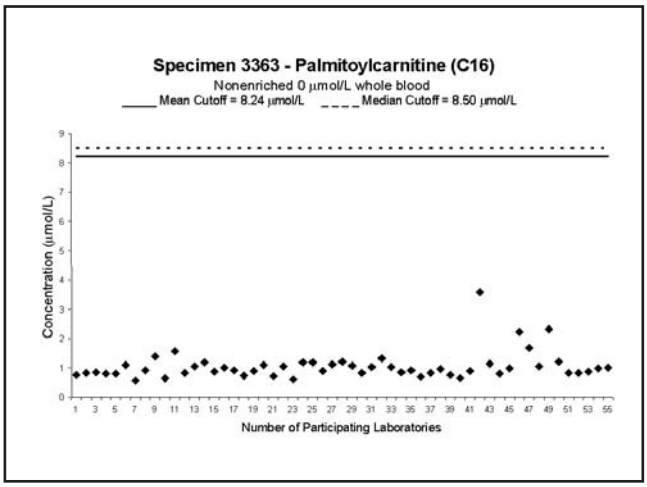

Figure 21c.

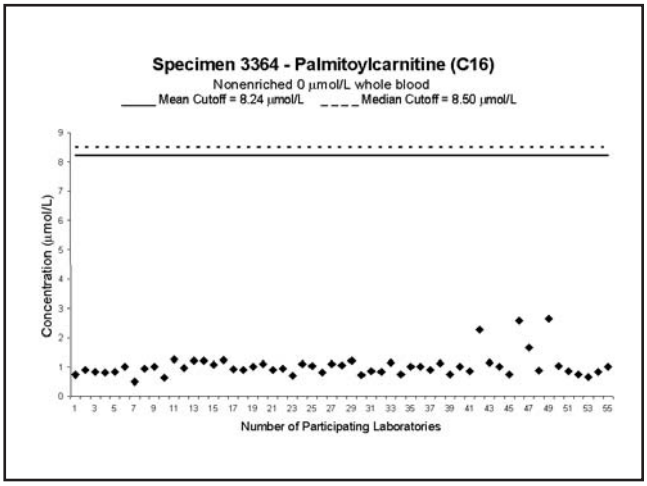

Figure 21d.

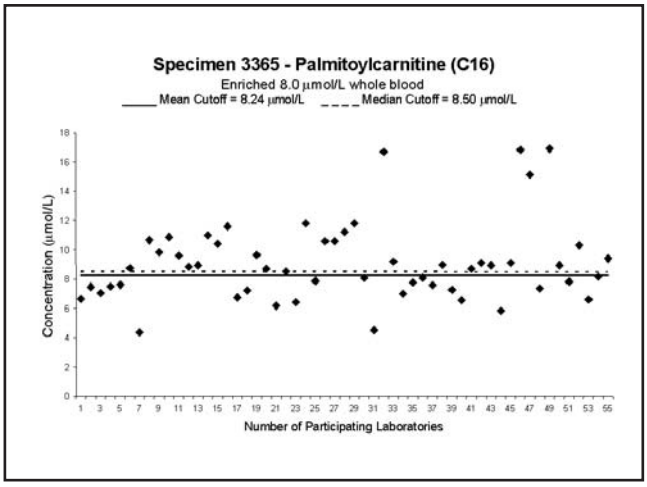

Figure 21e.

TABLE 8. 2003 Summary of Performance Evaluation Errors by Domestic and Foreign Laboratories

Domestic	Positive Specimens Assayed (N)	False-Negative Errors (%)	Negative Specimens Assayed (N)	False-Positive Errors (%)
Phenylalanine Screen	218	0.0	174	0.0
Leucine Screen	126	0.8	203	1.3
Methionine Screen	132	0.0	220	1.4
Tyrosine Screen	87	5.7	240	1.5
Valine Screen	109	1.8	228	1.6
Citrulline Screen	87	2.3	200	0.0
C3 Screen	37	0.0	148	1.3
C4 Screen	37	0.0	148	0.0
C5 Screen	74	0.0	92	0.0
C5DC Screen	18	0.0	123	0.0
C6 Screen	56	0.0	111	0.0
C8 Screen	65	0.0	129	0.0
C10 Screen	20	0.0	148	0.0
C14 Screen	35	0.0	140	0.0
C16 Screen	19	10.5	148	0.0
Foreign	Positive Specimens Assayed (N)	False-Negative Errors (%)	Negative Specimens Assayed (N)	False-Positive Errors (%)
Phenylalanine Screen	379	0.5	316	1.3
Leucine Screen	200	3.5	341	0.9
Methionine Screen	200	0.5	358	0.6
Tyrosine Screen	163	3.1	472	1.3
Valine Screen	161	1.2	386	1.3
Citrulline Screen	132	5.3	403	1.0
C3 Screen	69	0.0	276	3.6
C4 Screen	64	0.0	256	2.3
C5 Screen	136	0.7	170	0.0
C5DC Screen	33	3.0	225	1.8
C6 Screen	96	1.0	189	0.5
C8 Screen	107	0.9	213	0.9
C10 Screen	34	0.0	260	0.8
C14 Screen	64	1.6	256	0.0
C16 Screen	34	2.9	268	0.0

When reporting cutoff values, we requested the decision level for sorting test results that are reported as presumptive positive (outside limits) from results reported as negative (within limits).

There appears to be a few outliers among the nonenriched samples. Specimens 3363 and 3364 are enriched with 0.4 µmol/L blood and 0.2 µmol/L blood respectively. The results are scattered above and below the mean cutoff value of 0.28 µmol/L blood.

All results for the nonenriched C6 (Hexanoylcarnitine) specimens 3362, 3363, and 3364 are well below the cutoff mean value of 0.56 µmol C6/L blood. Specimen 3361, with an enrichment of 0.3 µmol/L blood, shows the majority of laboratories falling below the mean cutoff value of 0.48 µmol/L blood; however, there are at least seven laboratories reporting values greater than the mean cutoff. Specimen 3361 was classified as a not evaluated specimen. Specimen 3365 was enriched with 1.0 C6 µmol/L blood and shows all laboratories reporting in the expected range.

Each of the nonenriched C8 (Octanoylcarnitine) specimens 3362, 3363, and 3364 show results well below the mean cutoff with the exception of two laboratories. The results for specimen 3361 enriched with 0.5 µmol C8/L blood shows a tight scatter above and below the mean cutoff value of 4.8 µmol C8/L blood.

All of the nonenriched C10 (Decanoylcarnitine) specimens, 3362, 3363, 3364, and 3365 show that all laboratories reported results well below the cutoff values as expected. Results for the enriched specimen 3361 of 0.5 µmol C10/L blood show both domestic and foreign laboratories reporting values above and below the mean cutoff of 0.50 µmol C10/L blood. Consensus of at least 80% was not met; therefore, specimen 3361 was classified as a not evaluated specimen.

There are four nonenriched specimens for C14 (Myristoylcarnitine), 3361, 3362, 3363, and 3364. Most of the participant results for these specimens were below the mean cutoff value of 0.85 µmol C14/L blood. There were three laboratories, however, that got results above the cutoff value. Specimen 3365 was enriched with 1.5 µmol C14/L blood, and all but one result was above the mean cutoff value.

All reported values for the nonenriched C16 (Palmitoylcarnitine) specimens fell below the mean cutoff value of 8.24 µmol C16/L blood. Results for the specimen 3365, enriched with 8 µmol C16/L blood, show an even distribution below and above the mean cutoff value. Consensus for specimen 3365 was not met and was classified as a not evaluated specimen.

A summary of the performance evaluation assessment errors is shown in Table 8. The percentage of error for each amino acid screen is shown separately for domestic and foreign laboratory participants. The rates for false-positive misclassifications are based on the number of distributed negative specimens, and the rates for false-negative misclassifications are based on the number of positive specimens. False positive rates of error ranged from 0 % - 1.6 % for domestic laboratories and 0 % - 3.6 % for foreign laboratories. Screening programs are designed to set cutoff values cautiously to avoid false-negative reports, and this design may contribute to more false-positive misclassifications. Even though false-negative rates are expected to be zero, the range of errors went from 0 % to 10.5 % among domestic laboratories, and 0 % to 5.3 % among the foreign laboratories.

A presumptive-classification grading component was added to the MS/MS PT program for acylcarnitines for Quarter 3, 2003.

The highest false-negative rate of 10.5% occurred in the C16 results among the domestic laboratories. This high percentage of error is partially due to the low number of C16-enriched challenges over only two quarters of grading for acylcarnitines.

PARTICIPANT SURVEY RESULTS

NSQAP sent a survey with the Quarter 4, 2003, PT shipment to all MS/MS PT participants. This survey was used to gather information about instrument, internal standards, and methods used to analyze the amino acids and acylcarnitines. Sixty-three of 85 participants responded to the survey. Tables 9 and 10 show the percentages of common parameters reported by participants on the survey. NSQAP does not endorse any manufacturer or method, but the information is provided to help laboratories that are starting up mass spectrometry programs to be better informed about what has been successful for the other MS/MS laboratories.

TABLE 9. Perkin Elmer Sciex Tandem Mass Spectrometer
Users = 32 laboratories

Software	Analyst	34%
	Neogram	31%
	Generations	6%
	Chemo View	6%
	Neonatal Script	6%
	Multiview	3%
	Other	13%
Auto Sampler	PerkinElmer	56%
	Gilson	25%
	Agilent	13%
	Other	6%
Sample Prep	Derivitization	78%
	Non-derivitized	22%
Solvent	Methanol	52%
	MEOH/H2O	48%
Punch Size	3mm	77%
	5mm	23%
	6mm	0%
Plate Material	Polypropylene	59%
	Polystyrene	41%
Plate Type	Round Bottom	20%
	Flat Bottom	37%
	Conical Bottom	13%
	Both	30%
Method	Non Kit	68%
	PE Neogram Kit	32%
Calibrator	Cambridge	70%
	PerkinElmer	17%
	Adelaide	10%
	ten Brink	3%
Calibrator Mix	Inhouse Mix	41%
	PreMixed	59%
Mobile Phase	80% ACN	47%
	50% ACN/H2O	53%
Sample Drying	Nitrogen	50%
	Heat/Air	28%
	None	22%
Scan	Parent	75%
	Multiple Reaction	91%
	Neutral	69%
	Daughter	6%
	Full	22%

*Results reported by participants in response to a survey. Accuracy of results cannot be validated.

TABLE 10. Waters Micromass Tandem Mass Spectrometer
Users = 29 laboratories

Software	Masslynx	45%	**Plate Type**	Round Bottom	73%
	Neolynx	31%		Flat Bottom	23%
	Masslynx/Neolynx	21%		Conical Bottom	5%
			Method	Non Kit	100%
				PE Neogram Kit	0%
Auto Sampler	Waters	36%	**Calibrator**	Cambridge	79%
	Gilson	36%		ten Brink	38%
	Jasco	7%		In-house	17%
	CTC-Pal	14%		Adelaide	3%
	Agilent	7%			
Sample Prep	Derivitization	97%	**Calibrator Mix**	Inhouse Mix	72%
	Non-derivitized	3%		PreMixed	28%
Solvent	Methanol	93%	**Mobile Phase**	80% ACN	74%
	MEOH/H2O	7%		50% ACN/H2O	26%
Punch Size	3mm	81%	**Sample Drying**	Nitrogen	46%
	5mm	4%		Heat/Air	50%
	6mm	15%		None	4%
Plate Material	Polypropylene	83%	**Scan**	Parent	69%
	Polystyrene	17%		Multiple Reaction	62%
				Neutral	59%
				Daughter	21%
				Full	10%

*Results reported by participants in response to a survey. Accuracy of results cannot be validated.

ACTIVITIES: PAST, PRESENT, AND FUTURE

- A two-session Tandem Mass Spectrometry QA/QC Web Net Conference was held January 21, 2004, and February 4, 2004. The audio portion, as well as the PowerPoint slides will be available soon at: http://www.cdc.gov/nceh/dls/newborn_screening.htm Contact Nancy Meredith at 770-488-7897 or email nmeredith@cdc.gov for information.

- *Newborn Screening by Tandem Mass Spectrometry: A Course in Understanding Laboratory Issues and Interpreting Test Results.* This five-day course is co-sponsored by APHL, NNSGRC, and CDC. The last course was held January 26-30, 2004, at Duke University Medical Center, Durham, North Carolina. The next course will be held March 1-5, 2004, at Baylor University Medical Center, Dallas, Texas. Contact Jelili A. Ojodu at APHL at 202-822-5227 ext. 235 or email at jojodu@aphl.org for information.

- *Newborn Screening by Tandem Mass Spectrometry: A course in Translating Tandem Mass Spectrometry Results from Laboratory to Follow-up.* This course was held February 23-27, 2004, at Biochemical Laboratory of Duke University Medical Center in Durham, North Carolina. Contact Jelili A. Ojodu at APHL at 202-822-5227 ext. 235 or email at jojodu@aphl.org for information.

- The 2004 Newborn Screening and Genetic Testing Symposium will be held at the Crowne Plaza Ravinia Hotel in Atlanta, Georgia from May 3-6, 2004. It will consist of a keynote session, general and breakout sessions, a poster and exhibit hall over the course of 2.5 days. See the APHL Web site for registration information: http://www.aphl.org/National_Conferences/2004_Newborn_Symposium

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2. Chace DH, Adam BW, Smith SJ, Alexander JR, Hillman SL, Hannon WH. Validation of accuracy-based amino acid reference materials in dried-blood spots by tandem mass spectrometry for newborn screening assays. Clinical Chemistry 1999; 45:1269-77.

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This ***NEWBORN SCREENING QUALITY ASSURANCE PROGRAM*** report is an internal publication distributed to program participants and selected program colleagues. The laboratory quality assurance program is a project cosponsored by the **Centers for Disease Control and Prevention (CDC) and the Association of Public Health Laboratories.**

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