Newborn Screening Quality Assurance Program

Filter Paper Comparison Study

May 2009

The U.S. Food and Drug Administration (FDA) has registered two sources of filter paper for blood collection as Class II Medical Devices (21 CFR §862.1675) based on sustained compliance with the performance parameters specified in the Clinical and Laboratory Standards Institute (CLSI) LA4-A5 Approved Standard.[1] Newborn screening programs requested a comparative assessment of the filter papers by analyte testing. This study was designed to examine the comparative properties of the two FDA-cleared/approved filter paper sources and grades (Whatman 903 [Lot No. W071] and Ahlstrom 226 [Lot Nos. 8040201 and 6460701]) by analyzing a large array of newborn screening analytes.

Analytes were spiked into adult whole blood (type O) with the hematocrit adjusted to 50%. Aliquots of 75 µL of analyte-enriched blood were applied in tandem to blind-coded strips of each filter paper grade (903 and 226) with preprinted broken-line circles of 12 mm. The blood spots were dried at ambient temperature overnight and then placed into zip-close, low gas-permeable plastic bags with desiccant packs to maintain humidity below 30%. The dried-blood spots (DBS) were stored at -20°C until pulled for distribution.

DBS were prepared with multiple-analyte mixtures at a single level within a spot (Table 1a); for some analytes, a dose-response (dilution) series was prepared to contain multiple levels of analytes within a spot (Table 1b). When the dose-response series (Table 1b) was prepared for assessment by immunoassay, octanoylcarnitine (C8) was added to the stock analyte mixture before dilution as an internal standard to monitor linearity and accuracy of the dilution series by highly specific, nonimmunomethodology tandem mass spectrometry (MS/MS). The dilutions were made with a split aliquot of the original nonenriched blood.

All sets of blind-coded DBS were sent by next-day-delivery express mail to the testing laboratories along with assay and data reporting instructions. Each participating laboratory assayed specimens in duplicate for two analytic runs by using routine testing methods. Each participant entered results on a data report form and faxed it to the Centers for Disease Control and Prevention (CDC).

Study participants included laboratories in the United States and Europe. The study comprised two separate specimen distributions: one in October 2008 and the other in December 2008. The first shipment included analytes measured by MS/MS, and the second one covered analytes measured by immunoassays. Table 2 identifies by analyte the variety of methods used by study participants. For the comparative studies, galactose, galactose-1-phosphate uridylyltransferase, biotinidase, and hemoglobins were not examined. Biotinidase and hemoglobins data are routinely reported qualitatively.

The compiled results of this study are based on analysis of all reported data and are shown in Figures 1–18. All data show a strong overlap at one standard deviation for each analyte. Table 3a shows an estimate of the lot-to-lot variance for the production of eight different lots of Whatman filter paper over approximately10 years. Data for the Ahlstrom paper are presented in Table 3b and show lot-to-lot variance and serum volumes similar to the Whatman paper; however, the Ahlstrom data encompass fewer lot numbers and a shorter time span. Tables 3a and 3b include data for serum volume of each lot of filter paper. Lots used in this study are indicated by an asterisk in Tables 3a and 3b and are also identified in Figures 1–18. The lot-to-lot data in Tables 3a and 3b were the same data used to generate the charts in Figures 19 and 20 that were replicated from the Newborn Screening Quality Assurance Program Annual Report (January 2009).

The study data indicate that the difference between manufacturers could be at least 4–5% for comparability or, at a minimum, equal to the lot-to-lot variance of a single manufacturer's filter paper products (Tables 3a-b). Data support the conclusion that the performance of filter paper grades (903 and 226) from two FDA-cleared/approved sources is essentially equivalent. The conclusion is based on the analysis by multiple laboratories of an array of analytes in DBS prepared as enriched-blood pools and identically spotted and dried on the two grades of papers.

1. Clinical and Laboratory Standards Institute (CLSI). Blood collection on filter paper for newborn screening programs; approved standard—fifth edition. CLSI document LA4-A5. Wayne, PA: Clinical and Laboratory Standards Institute; 2007.

CDC/APHL

This program is cosponsored by the Centers for Disease Control and Prevention (CDC) and the Association of Public Health Laboratories (APHL).

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Table 1a. Enrichment Values of Single Analyte Specimens Amino Acids and Acylcarnitines (October 2008)

Specimen Number	Analyte	Enrichment µmol/L
E&F 2	Tyr	828
E&F 5	Cit	200
E&F 6	Phe	424
E&F 8	Leu	496
E&F 8	Val	513
E&F 10	C0	75
E&F 10	C2	30
E&F 10	C3	12
E&F 10	C4	5
E&F 10	C5	3
E&F 10	C6	2.5
E&F 10	C8	**2.5***
E&F 10	C10	1.5
E&F 10	C14	3
E&F 10	C16	12
E&F 10	C18	5
E&F 10	C3DC	3
E&F 10	C5DC	2
E&F 10	C5OH	3

Table 1b. Enrichment Values of Dose-Response Series (December 2008)

Specimen Number	T4 (µg/dL)	TSH (µIU/mL)	17-OHP (ng/mL)	IRT (ng/mL)	C8 (µmol/L)
E&F101	0.00	0.00	0.00	0.00	0.00
E&F102	0.00	1.56	3.13	7.80	0.15
E&F103	0.00	3.13	6.25	15.60	0.30
E&F104	2.50	6.25	12.50	31.25	0.60
E&F105	5.00	12.50	25.00	62.50	1.25
E&F106	10.00	25.00	50.00	125.00	**2.5***
E&F107	15.00	50.00	100.00	250.00	5.00
E&F108	30.00	100.00	200.00	500.00	10.00

E = Whatman Paper
F = Ahlstrom Paper

* See C8 comparisons in Figure 13.

Table 2. Methods Used by Participants

Analyte	Method
T4	AutoDelfia Delfia
TSH	AutoDelfia Delfia
17-OHP	AutoDelfia
IRT	AutoDelfia Delfia MP Biomedicals Elisa
Amino Acids and Acylcarnitines	Derivatized-MS/MS Non-kit Non-derivatized-MS/MS Non-kit Derivatized-MS/MS PerkinElmer NeoGram MS2 Kit Derivatized-MS/MS Chromsystems Kit

Table 3a. Whatman Filter Paper Lot-to-Lot Variance

Intact Red Blood Cells (RBC)

Year of Manufacture	Lots	Serum Volume Intact Cell	Mean Serum Volume	SD
1998	W981	1.460	1.474	0.061
2000	W001	1.400		
2001	W011	1.571	n	8
2003	W031	1.510	CV	**4.13%**
2004	W041	1.440		
2005	W051	1.489		
2007	**W071***	**1.397**		
2008	W081	1.521		

Lysed RBC

Year of Manufacture	Lots	Serum Volume Lysed Cell	Mean Serum Volume	SD
1998	W981	1.381	1.362	0.051
2000	W001	1.300		
2001	W011	1.450	n	8
2003	W031	1.400	CV	**3.78%**
2004	W041	1.350		
2005	W051	1.309		
2007	W071	1.320		
2008	W081	1.383		

Among-paper lot variance is 4% by examining the variances using two different and independent tests [lysed and intact cells] for filter paper lots produced over approximately 10 years.

Table 3b. Ahlstrom Filter Paper Lot-to-Lot Variance

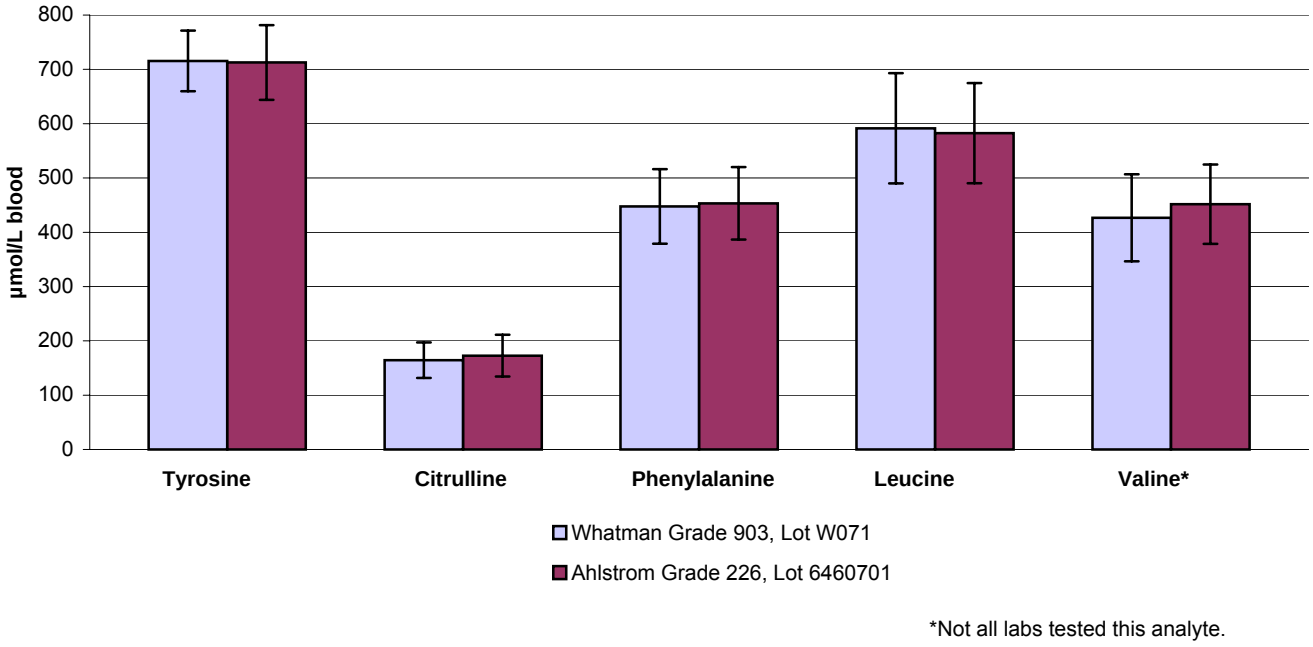
Intact RBC

Year of Manufacture	Lots	Serum Volume Intact Cell	Mean Serum Volume	SD
2005	5431001	1.416	1.472	0.069
2006	6050501	1.465		
2007	**6460701***	**1.488**	n	6
2007	7181001	1.440	CV	**4.66%**
2007	7231001	1.423		
2008	**8040201***	**1.601**		

*Filter paper lot used in study.

Note: Serum volumes were measured by CDC according to test description in CLSI LA4-A5, Appendix C.

Figure 1. Whatman vs. Ahlstrom
Amino Acids - Single Level Multiple-Analyte Specimens
All Laboratories Combined (5 labs, n=20 results for all analytes except valine)
(valine 4 labs, n=16 results)
error bar = one standard deviation



*Not all labs tested this analyte.

Figure 2. Whatman vs. Ahlstrom
Amino Acid - Single Level Multiple-Analyte Specimens
Results per Laboratory (n=4 results per analyte per lab)

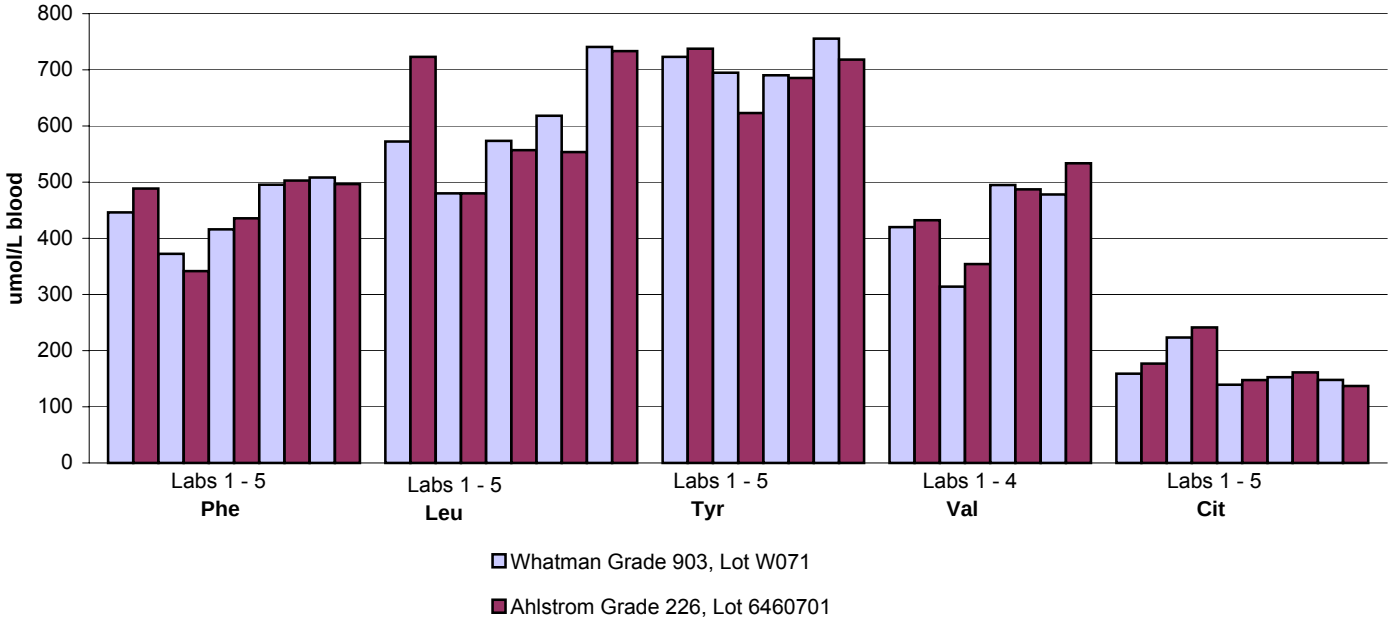


Figure 3. Whatman vs. Ahlstrom
Acylcarnitines C0 and C2 - Single Level Multiple-Analyte Specimens
All Laboratories Combined (5 labs, n=20 results)
error bar = one standard deviation

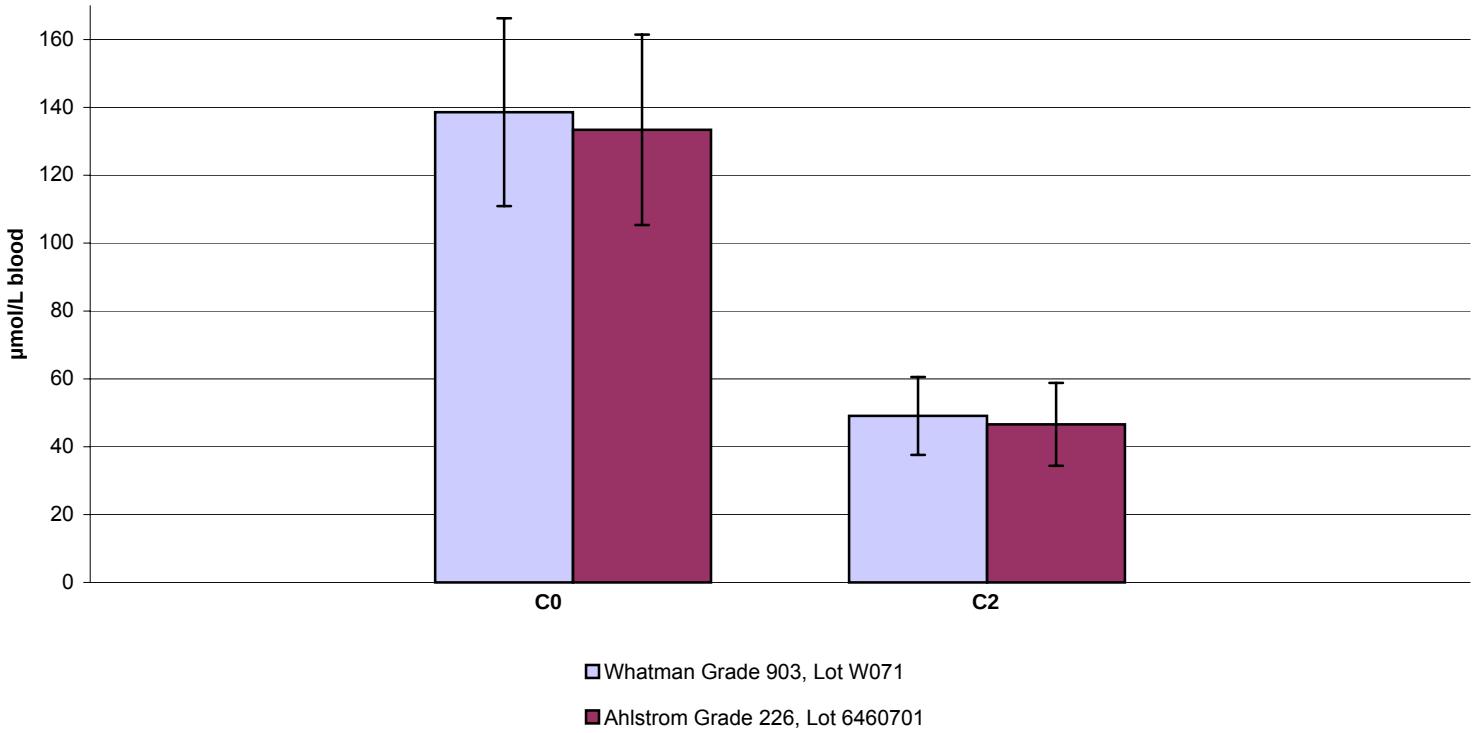
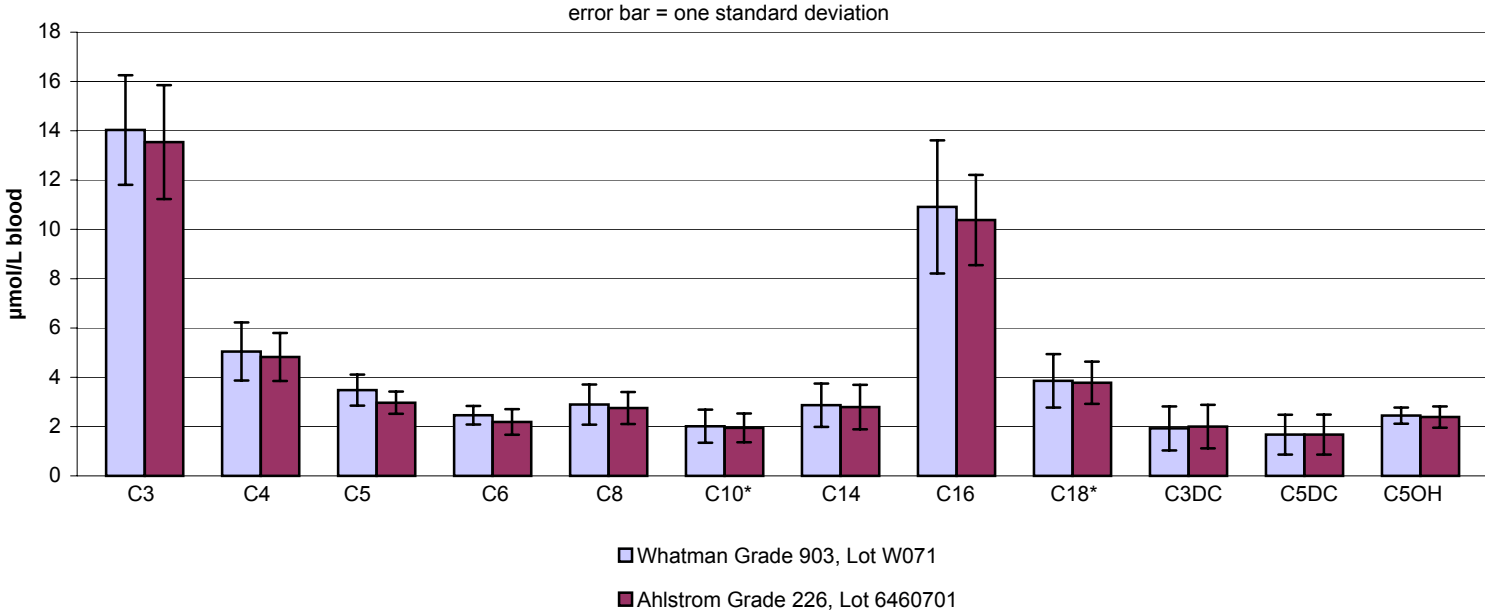


Figure 4. Whatman vs. Ahlstrom
Acylcarnitines - Single Level Multiple-Analyte Specimens
All Laboratories Combined (5 labs, n=20 results for all analytes except C10 and C18)
(C10 and C18 4 labs, n=16 results)
error bar = one standard deviation



*Not all labs tested this analyte.

Figure 5. Whatman vs. Ahlstrom
C0 & C2 - Single Level Multiple-Analyte Specimens
Results per Laboratory (n=4 results per analyte per lab)

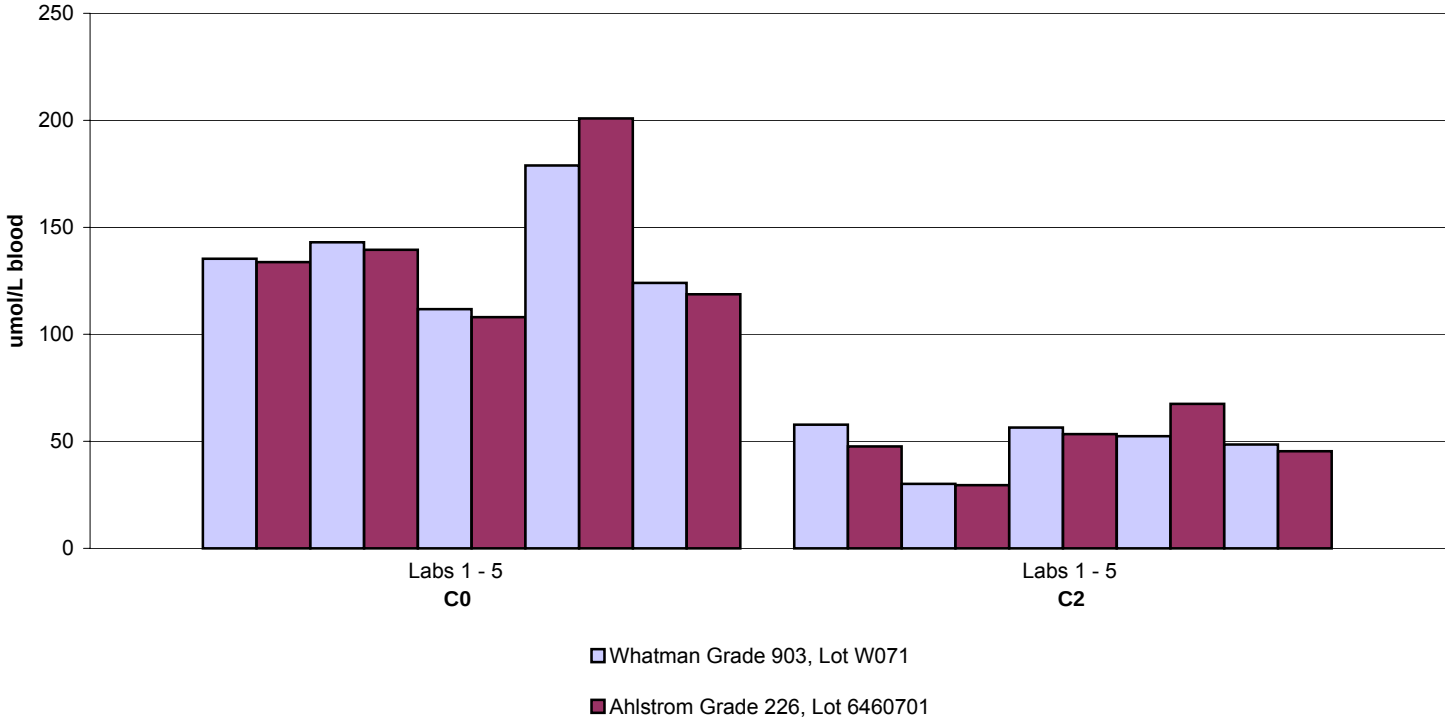


Figure 6. Whatman vs. Ahlstrom
Acylcarnitines - Single Level Multiple-Analyte Specimens
Results per Laboratory (n=4 results per analyte per lab)

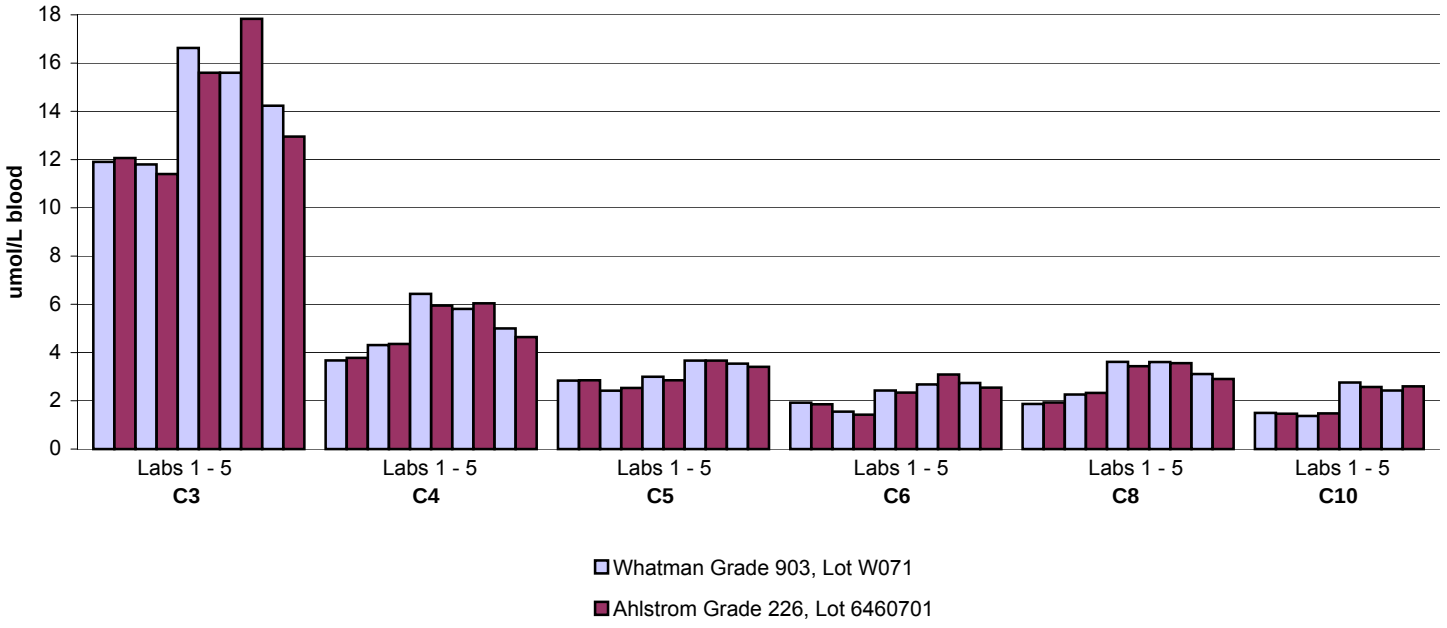


Figure 7. Whatman vs. Ahlstrom
Acylcarnitines - Single Level Multiple-Analyte Specimens
Results per Laboratory (n=4 results per analyte per lab)

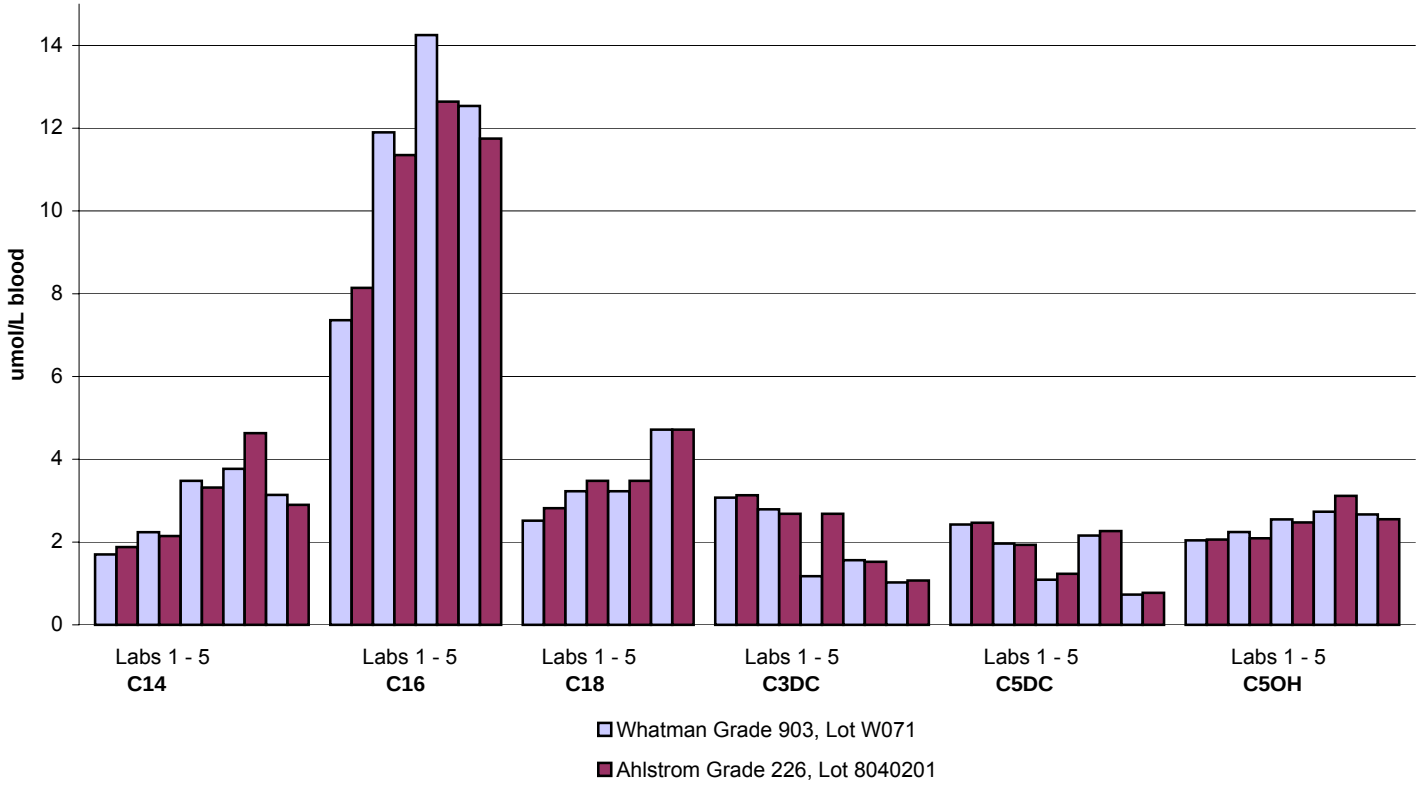
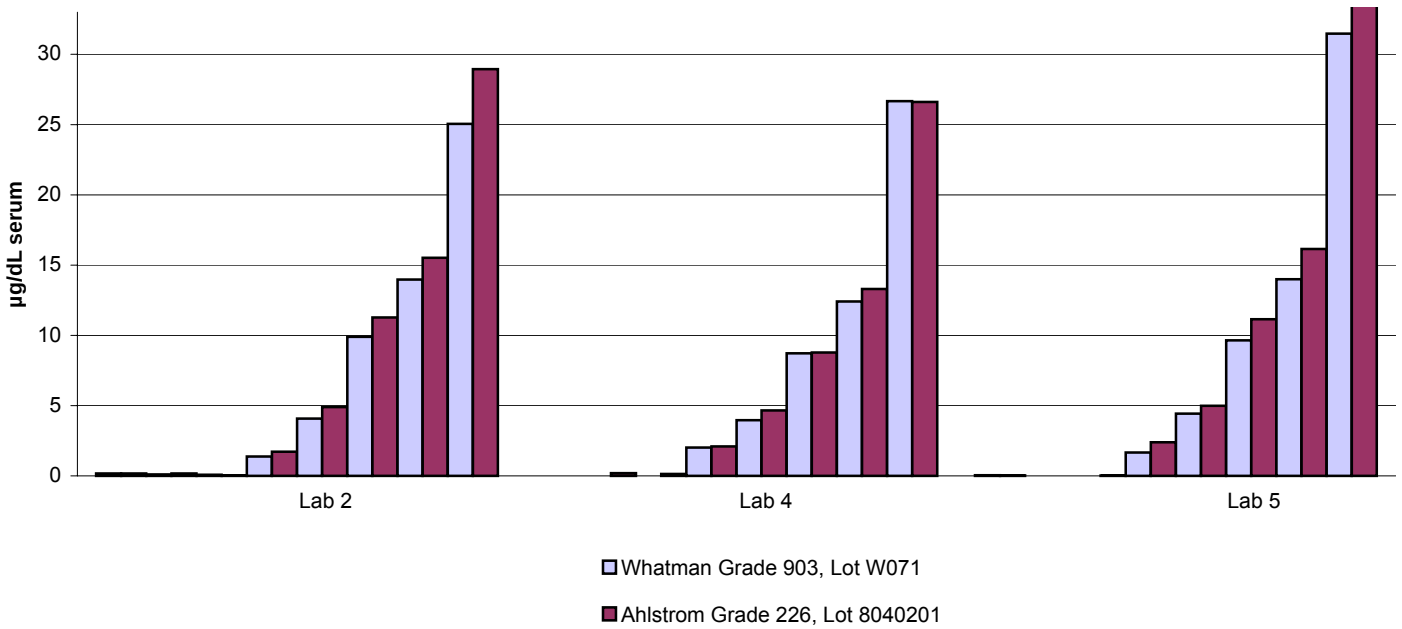


Figure 8. Whatman vs. Ahlstrom
T4 - Dose-Response Series
Results per Laboratory (n=4 results per specimen per lab)*



*Not all labs tested this analyte.

Figure 9. Whatman vs. Ahlstrom
TSH - Dose-Response Series
Results per Laboratory (n=4 results per specimen per lab)

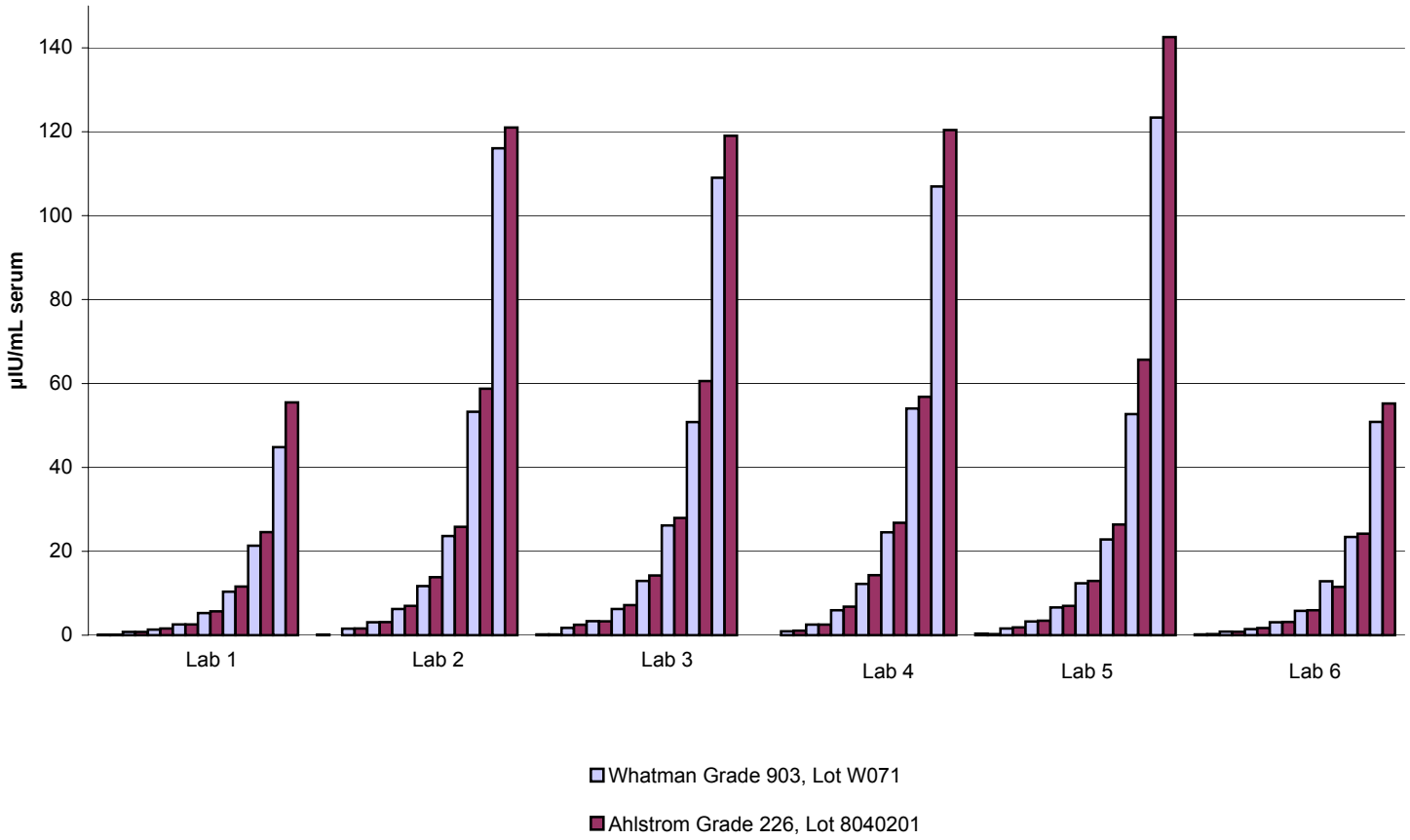
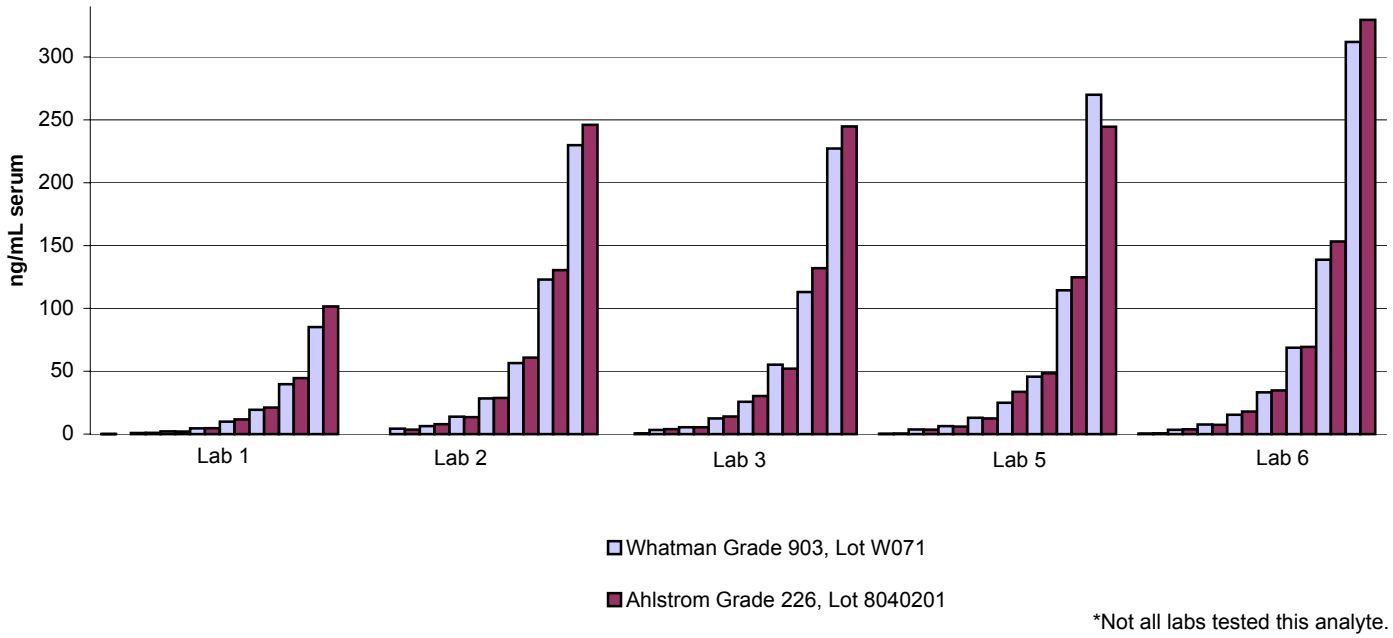
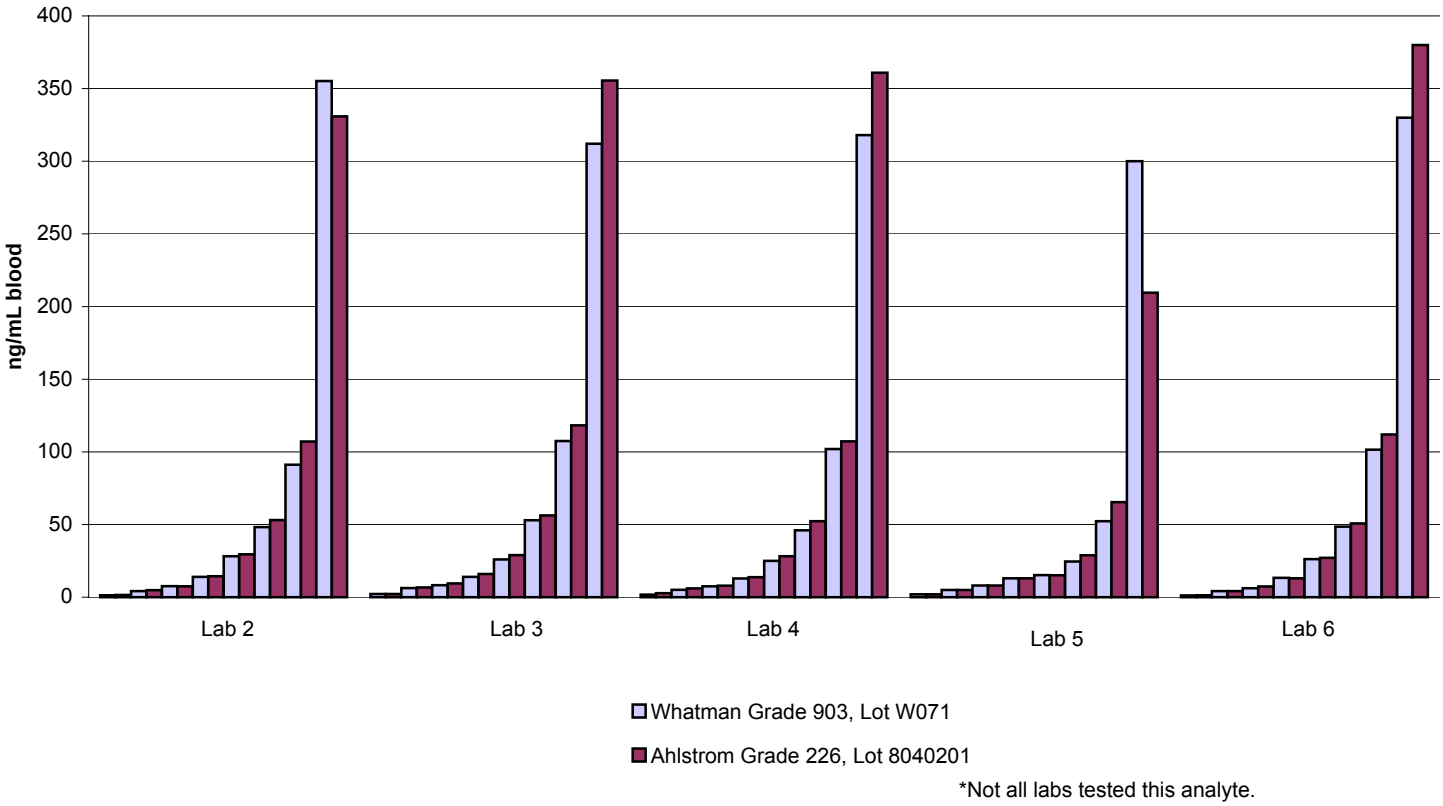


Figure 10. Whatman vs. Ahlstrom
17-OHP - Dose-Response Series
Results per Laboratory (n=4 results per specimen per lab)*



*Not all labs tested this analyte.

Figure 11. Whatman vs. Ahlstrom
IRT - Dose-Response Series
Results per Laboratory (n=4 results per specimen per lab)*



*Not all labs tested this analyte.

Figure 12. Whatman vs. Ahlstrom
C8 - Dose-Response Series
Results per Laboratory (n=4 results per specimen per lab)

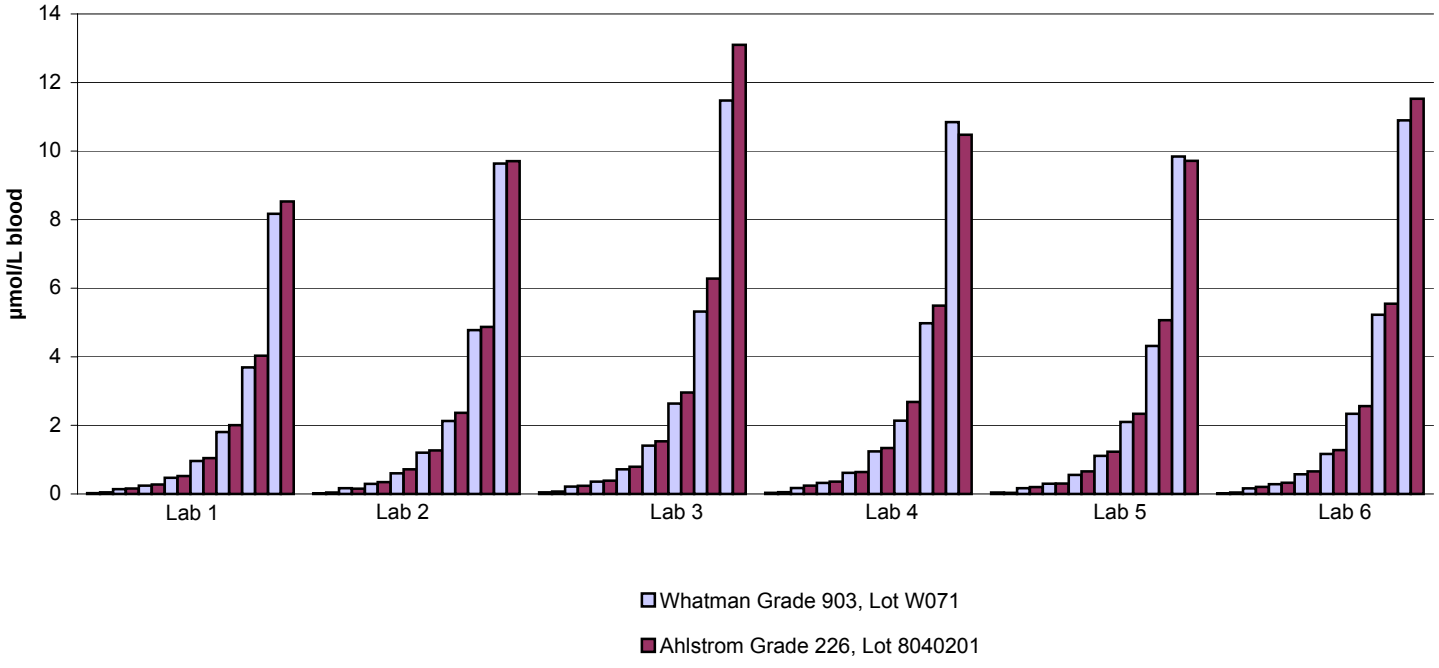
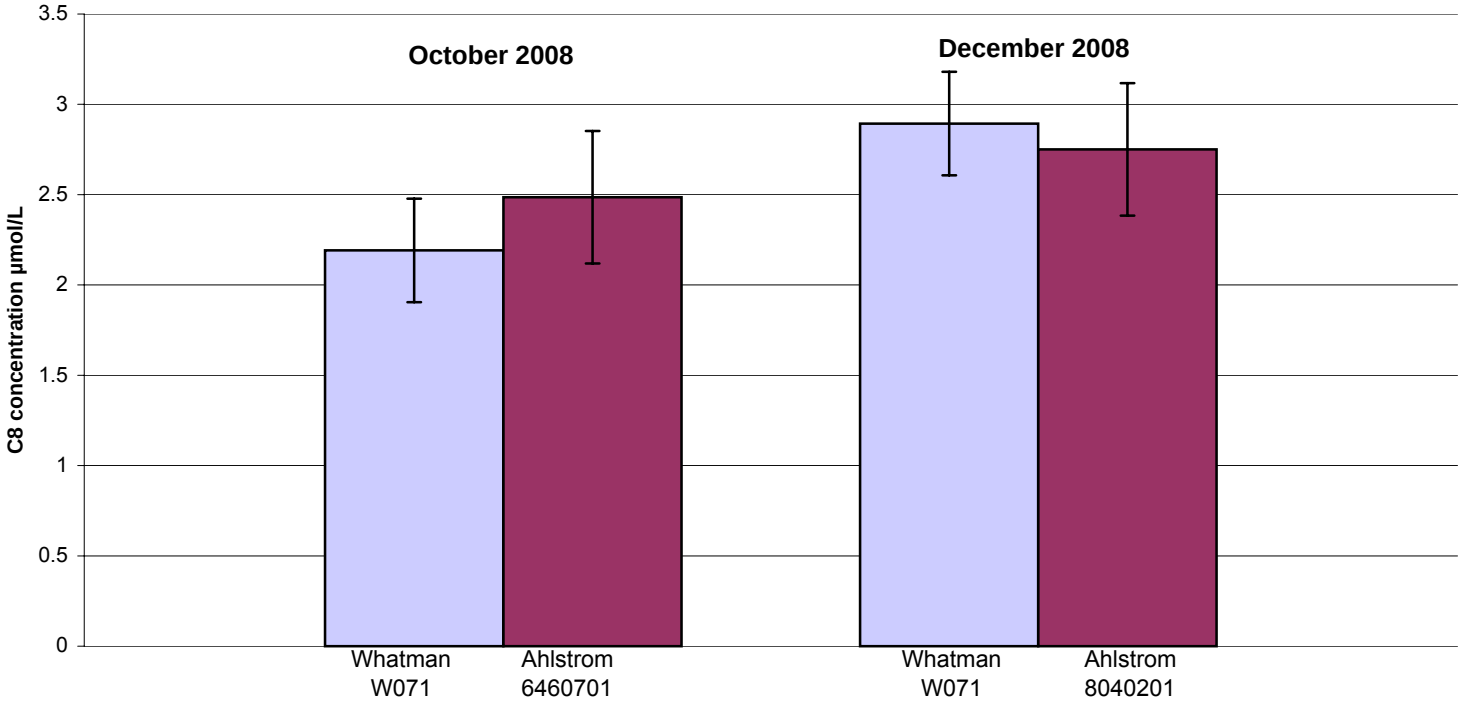


Figure 13. Comparison of Octanoylcarnitine (C8) Replicate Measurements

All laboratories combined (5 labs, n=20 results)

error bar = one standard deviation

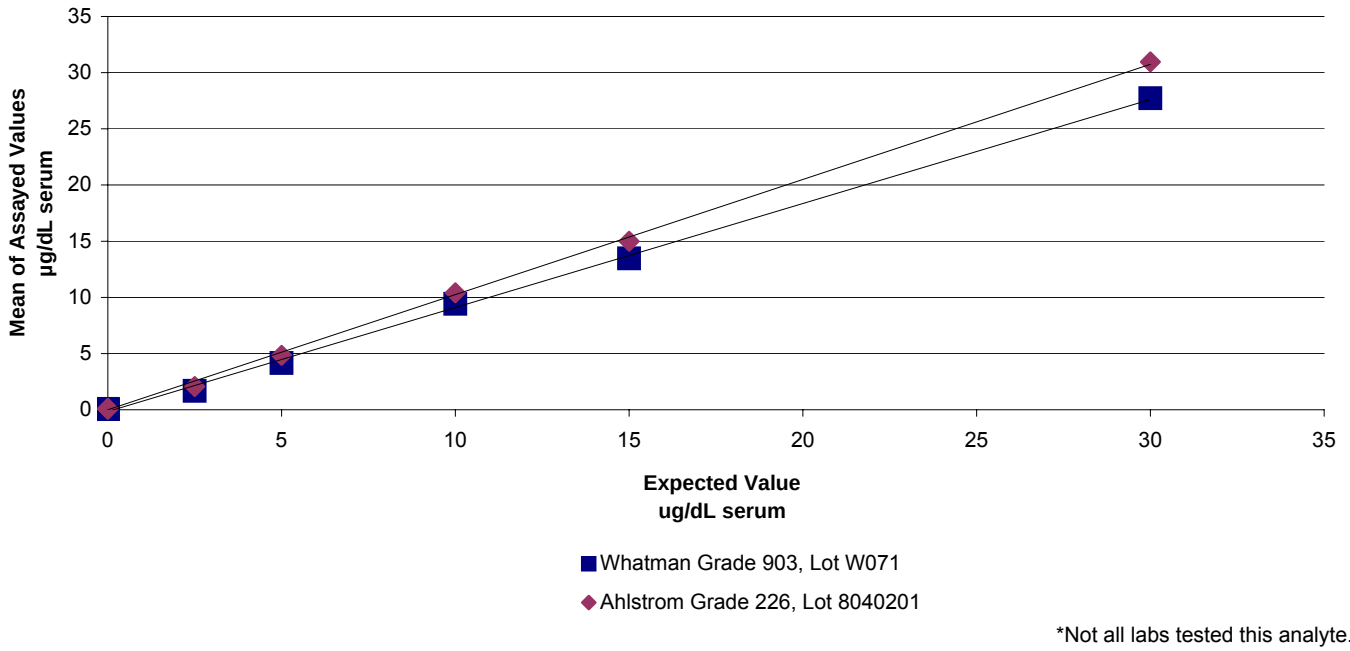


Note: The blood pools for October and December 2008 distributions were prepared on two separate occasions using different base pools enriched at the same concentration (C8 = 2.5 µmol/L) and spotted and dried on filter paper. For Ahlstrom Grade 226 filter paper, two different lot numbers were used. Target value is analyte-enriched quantity plus endogenous-analyte quantity.

Figure 14. Whatman vs. Ahlstrom
T4 - Dose-Response Series

All Laboratories Combined (3 Labs, n=12)*

(See Figure 8 for individual lab results)



*Not all labs tested this analyte.

Figure 15. Whatman vs. Ahlstrom
TSH - Dose-Response Series
All Laboratories Combined (6 Labs, n=24)
(See Figure 9 for individual lab results)

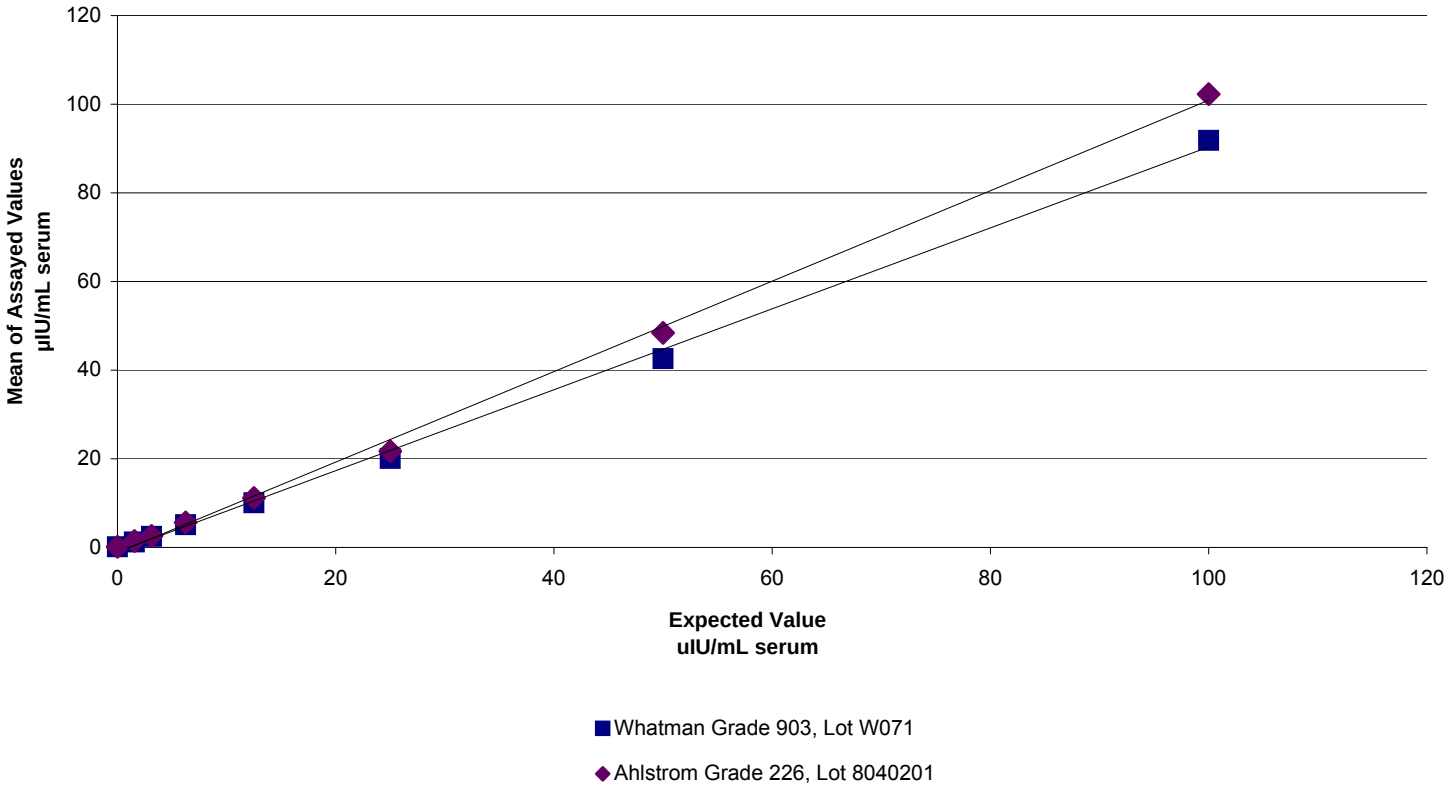
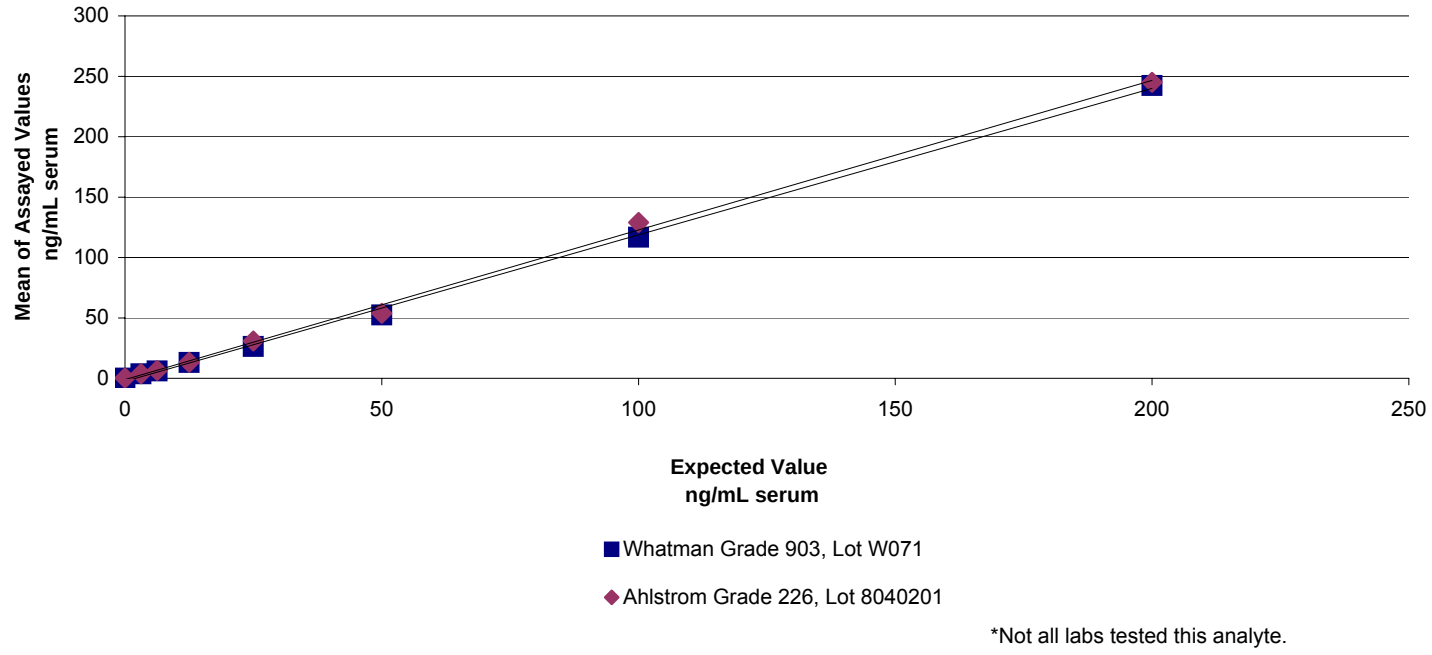


Figure 16. Whatman vs. Ahlstrom
17-OHP - Dose-Response Series
All Laboratories Combined (5 Labs, n=20)*
(See Figure 10 for individual lab results)



*Not all labs tested this analyte.

Figure 17. Whatman vs. Ahlstrom
C8 - Dose-Response Series
All Laboratories Combined (6 Labs, n=24)
(See Figure 12 for individual lab results)

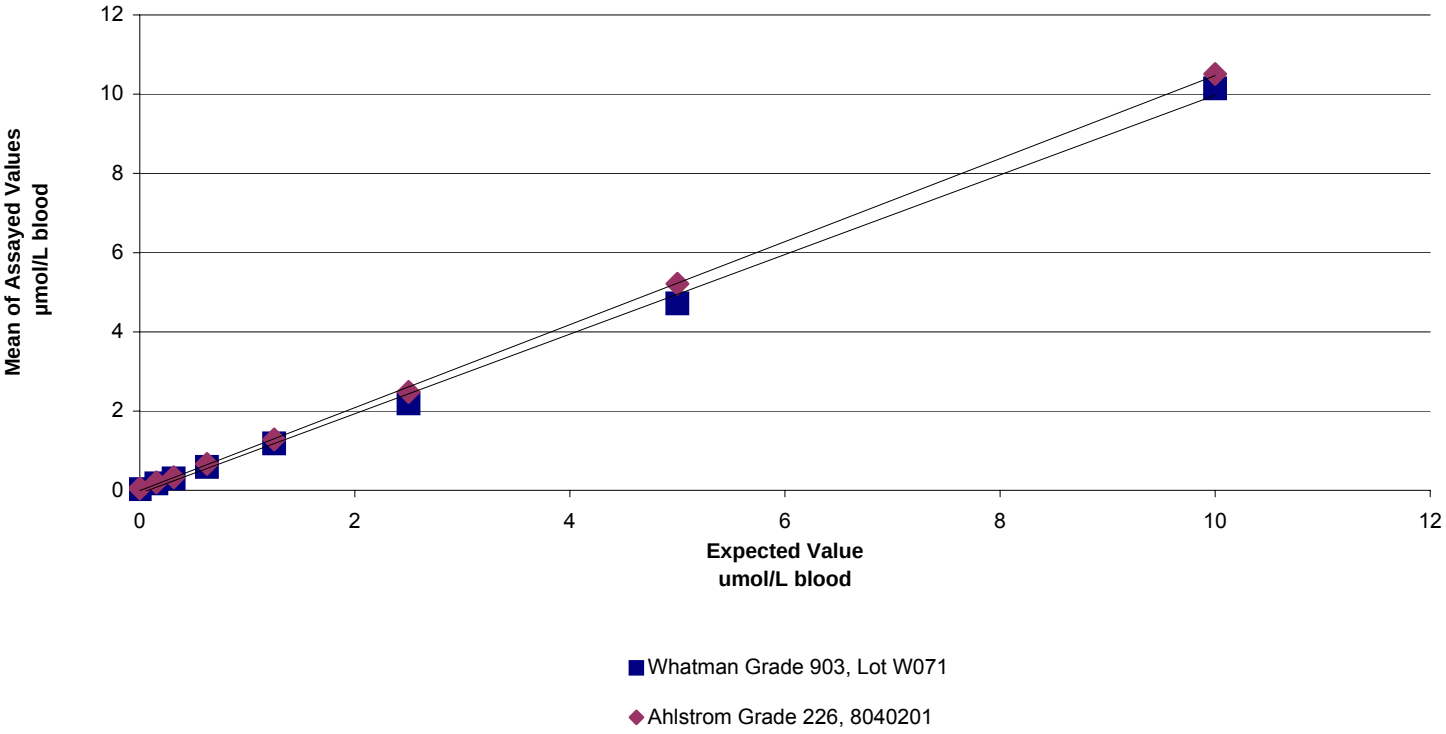
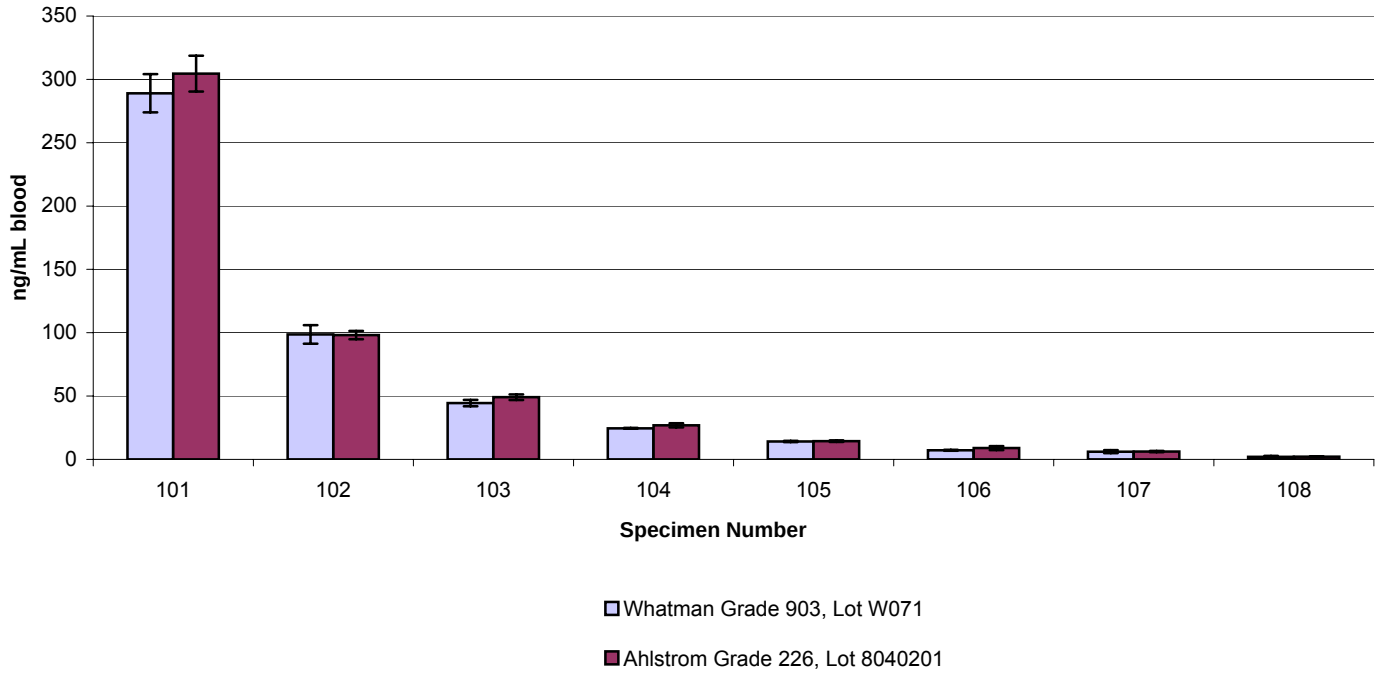


Figure 18. Whatman vs. Ahlstrom
IRT Summary - Dose-Response Series - Combined Data
All Laboratories Combined (5 Labs, n=20)*
error bar = one standard deviation



*Not all labs tested this analyte.

Figure 19. Whatman Grade 903® Specimen Collection Paper
Serum Volume by Lot Number - Intact Red Blood Cells

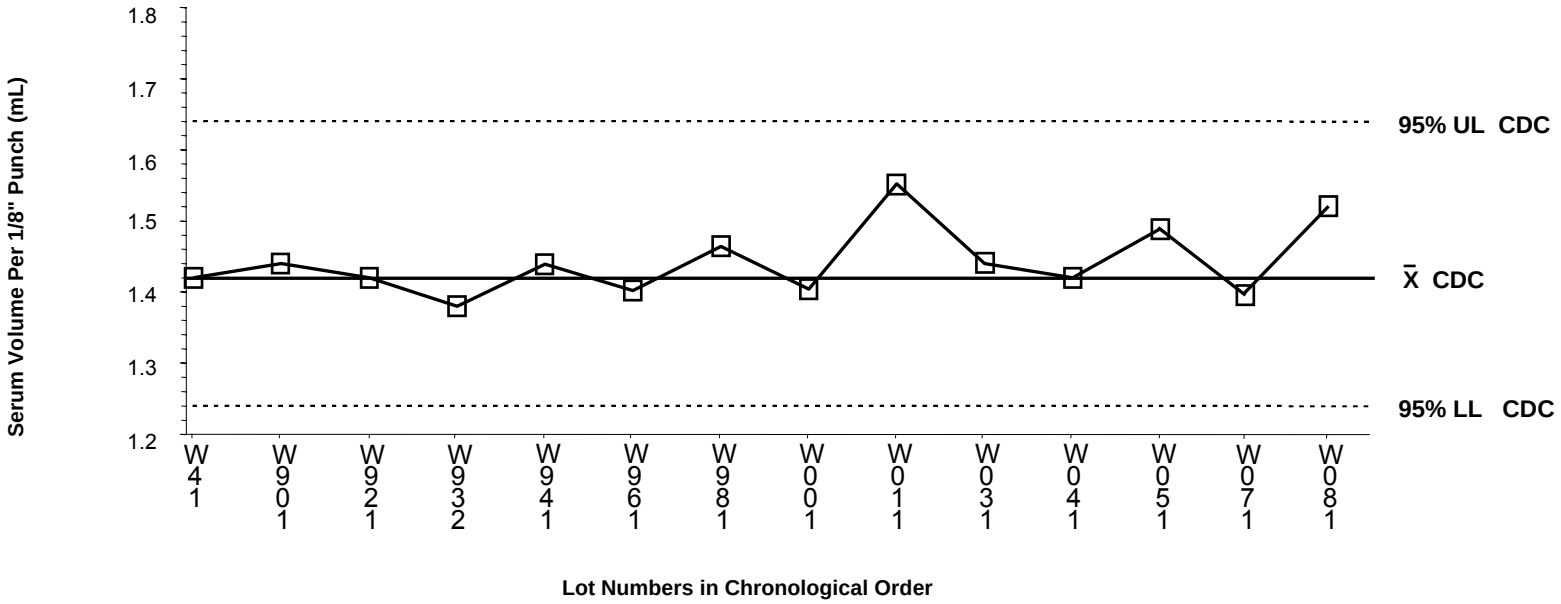
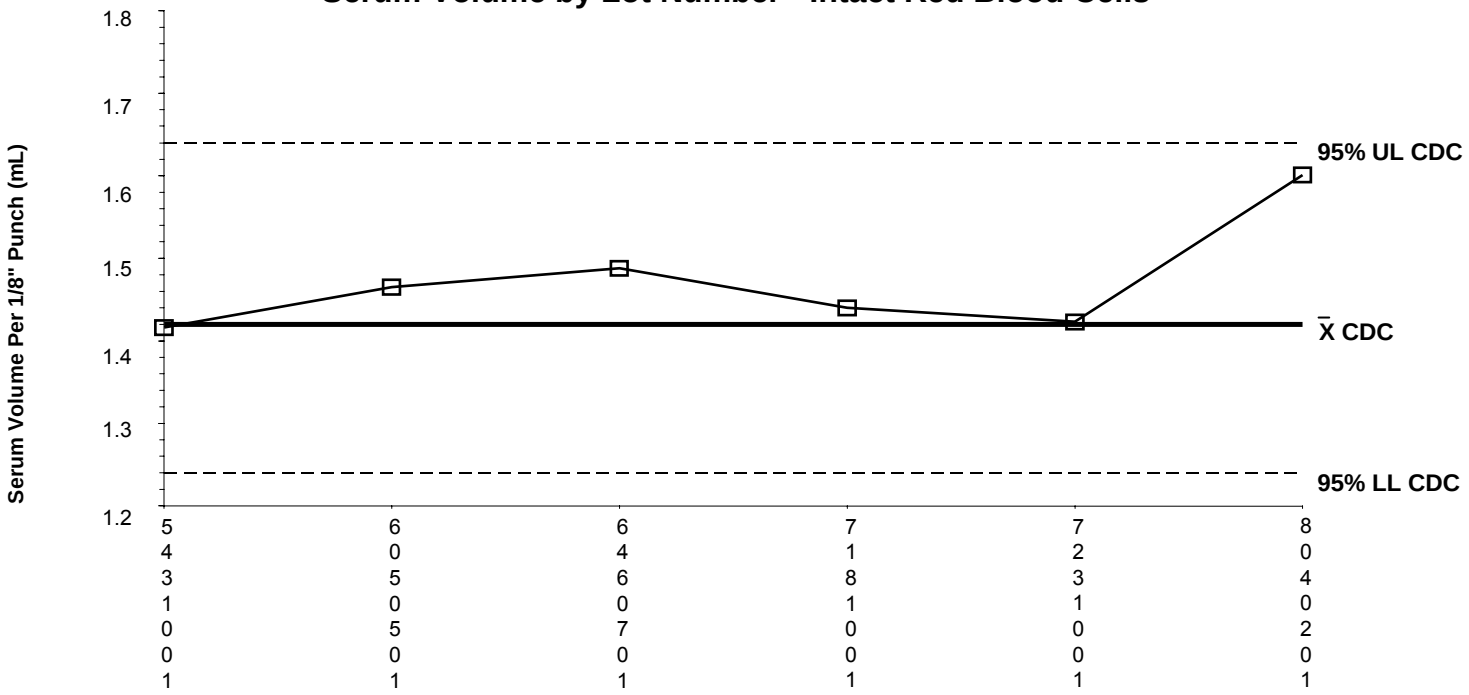


Figure 20. Ahlstrom Grade 226 Specimen Collection Paper
Serum Volume by Lot Number - Intact Red Blood Cells



This *Filter Paper Comparison Study Report* is a special internal report of the **Newborn Screening Quality Assurance Program** and is made available 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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