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. 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 approximately 10 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.

### Table 1a. Enrichment Values of Single Analyte Specimens

**Amino Acids and Acylcarnitines (October 2008)**

<table>
<thead>
<tr>
<th>Specimen Number</th>
<th>Analyte</th>
<th>Enrichment µmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>E&amp;F 2</td>
<td>Tyr</td>
<td>828</td>
</tr>
<tr>
<td>E&amp;F 5</td>
<td>Cit</td>
<td>200</td>
</tr>
<tr>
<td>E&amp;F 6</td>
<td>Phe</td>
<td>424</td>
</tr>
<tr>
<td>E&amp;F 8</td>
<td>Leu</td>
<td>496</td>
</tr>
<tr>
<td>E&amp;F 8</td>
<td>Val</td>
<td>513</td>
</tr>
<tr>
<td>E&amp;F 10</td>
<td>C0</td>
<td>75</td>
</tr>
<tr>
<td>E&amp;F 10</td>
<td>C2</td>
<td>30</td>
</tr>
<tr>
<td>E&amp;F 10</td>
<td>C3</td>
<td>12</td>
</tr>
<tr>
<td>E&amp;F 10</td>
<td>C4</td>
<td>5</td>
</tr>
<tr>
<td>E&amp;F 10</td>
<td>C5</td>
<td>3</td>
</tr>
<tr>
<td>E&amp;F 10</td>
<td>C6</td>
<td>2.5</td>
</tr>
<tr>
<td>E&amp;F 10</td>
<td>C8</td>
<td>2.5*</td>
</tr>
<tr>
<td>E&amp;F 10</td>
<td>C10</td>
<td>1.5</td>
</tr>
<tr>
<td>E&amp;F 10</td>
<td>C14</td>
<td>3</td>
</tr>
<tr>
<td>E&amp;F 10</td>
<td>C16</td>
<td>12</td>
</tr>
<tr>
<td>E&amp;F 10</td>
<td>C18</td>
<td>5</td>
</tr>
<tr>
<td>E&amp;F 10</td>
<td>C3DC</td>
<td>3</td>
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<tr>
<td>E&amp;F 10</td>
<td>C5DC</td>
<td>2</td>
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<tr>
<td>E&amp;F 10</td>
<td>C5OH</td>
<td>3</td>
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### Table 1b. Enrichment Values of Dose-Response Series (December 2008)

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

E = Whatman Paper
F = Ahlstrom Paper

* See C8 comparisons in Figure 13.
## Table 2. Methods Used by Participants

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>T4</td>
<td>AutoDelfia</td>
</tr>
<tr>
<td></td>
<td>Delfia</td>
</tr>
<tr>
<td>TSH</td>
<td>AutoDelfia</td>
</tr>
<tr>
<td></td>
<td>Delfia</td>
</tr>
<tr>
<td>17-OHP</td>
<td>AutoDelfia</td>
</tr>
<tr>
<td>IRT</td>
<td>AutoDelfia</td>
</tr>
<tr>
<td></td>
<td>Delfia</td>
</tr>
<tr>
<td></td>
<td>MP Biomedicals Elisa</td>
</tr>
<tr>
<td>Amino Acids and Acylcarnitines</td>
<td>Derivatized-MS/MS Non-kit</td>
</tr>
<tr>
<td></td>
<td>Non-derivatized-MS/MS Non-kit</td>
</tr>
<tr>
<td></td>
<td>Derivatized-MS/MS PerkinElmer NeoGram MS2 Kit</td>
</tr>
<tr>
<td></td>
<td>Derivatized-MS/MS Chromsystems Kit</td>
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</table>
Table 3a. Whatman Filter Paper Lot-to-Lot Variance

Intact Red Blood Cells (RBC)

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

Lysed RBC

<table>
<thead>
<tr>
<th>Year of Manufacture</th>
<th>Lots</th>
<th>Serum Volume Lysed Cell</th>
<th>Mean Serum Volume</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1998</td>
<td>W981</td>
<td>1.381</td>
<td>1.362</td>
<td>0.051</td>
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<tr>
<td>2000</td>
<td>W001</td>
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<tr>
<td>2004</td>
<td>W041</td>
<td>1.350</td>
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<tr>
<td>2005</td>
<td>W051</td>
<td>1.309</td>
<td></td>
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<td>2007</td>
<td>W071</td>
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<td>2008</td>
<td>W081</td>
<td>1.383</td>
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</table>

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

<table>
<thead>
<tr>
<th>Year of Manufacture</th>
<th>Lots</th>
<th>Serum Volume Intact Cell</th>
<th>Mean Serum Volume</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>2005</td>
<td>5431001</td>
<td>1.416</td>
<td>1.472</td>
<td>0.069</td>
</tr>
<tr>
<td>2006</td>
<td>6050501</td>
<td>1.465</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2007</td>
<td>6460701*</td>
<td>1.488</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2007</td>
<td>7181001</td>
<td>1.440</td>
<td>CV</td>
<td>4.66%</td>
</tr>
<tr>
<td>2007</td>
<td>7231001</td>
<td>1.423</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2008</td>
<td>8040201*</td>
<td>1.601</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*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

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

*Not all labs tested this analyte.
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

0 20 40 60 80 100 120 140 160
µmol/L blood

C0  C2

Whatman Grade 903, Lot W071
Ahlstrom Grade 226, Lot 6460701

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

0 2 4 6 8 10 12 14 16 18
µmol/L blood

C3  C4  C5  C6  C8  C10*  C14  C16  C18*  C3DC  C5DC  C5OH

Whatman Grade 903, Lot W071
Ahlstrom Grade 226, Lot 6460701

*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)

0 20 40 60 80 100 120 140 
µIU/mL serum

Whatman Grade 903, Lot W071
Ahlstrom Grade 226, Lot 8040201

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

0 50 100 150 200 250 300 
ng/mL serum

Whatman Grade 903, Lot W071
Ahlstrom Grade 226, Lot 8040201

*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)*

Lab 2 Lab 3 Lab 4 Lab 5 Lab 6

*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)

Lab 1 Lab 2 Lab 3 Lab 4 Lab 5 Lab 6
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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