The Adequacy of Capillary Specimens for Determining Whole Blood Lead*†

NATHAN H. JOHNSON,‡ K. OVEN ASH, Ph.D.,§ KERN L. NUTTALL, M.D., Ph.D.,§ and EDWARD R. ASHWOOD, M.D.§

‡81 MIDOS/SHOKI, Clinical Research Laboratory, Keesler AFB, MS 39534
§ARUP Laboratories, Salt Lake City, UT 84108

ABSTRACT

In response to demands for reliable alternatives to collection of venous specimens for determination of whole blood lead levels in children, the Centers for Disease Control has called for increased research into capillary methodologies. In this study, a three tiered approach was developed to assess the adequacy of capillary specimens for determining whole blood lead. Patient blood lead results from capillary and venous specimens were compared for obvious differences. Next, follow-up specimens for patients with elevated lead levels were compared with the initial results. In addition, experiments were conducted to determine whether or not handwashing eliminates gross contamination. Although the differences are not clinically important, the mean, 3.83 µg/dL for 5,100 venous specimens, was significantly lower (p < 0.005) then the mean of 4.6 µg/dL for 1,100 capillary specimens. Gross contamination was rare. Lead levels in follow-up specimens on patients whose initial screens were elevated were generally low. Handwashing greatly reduced the amount of external lead contamination. It is concluded that capillary specimens are an acceptable alternative to venous specimens for whole blood screening programs provided the patient and collector meticulously follow the prescribed collection protocol. Nevertheless, all elevated whole blood lead screening results, venous or capillary, should be confirmed with a venous collection before follow-up action is taken.

Introduction

In recent years, the detection and subsequent prevention of lead poisoning in children has become a major public issue. Until 1991, when the danger level for blood lead was considered to be 25 µg/dL, measurement of free erythrocyte protoporphyrin (FEP) or zinc protoporphyrin (ZPP) was common, but...
the results correlated only with blood lead levels greater than about 25 μg/dL. Both venous and capillary collections are acceptable for determining blood lead levels, but venous specimens are less prone to exogenous lead contamination. Venipuncture has also been demonstrated to be less expensive owing to the limited number of repeat analyses required. However, venipuncture is often difficult and time consuming, especially on younger children. Fingerstick collections are often much easier to obtain than venous specimens. For parents, a fingerstick is often viewed as less invasive.

The majority of all microspecimens for lead are collected into 500 μL ethylenediamine tetraacetic acid (EDTA) capillary collection tubes. Filter paper analysis is also available requiring only a few drops of blood and the filter paper is stable for six months. A capillary sampling protocol was issued by the CDC but not endorsed as a definitive, as contamination during the collection of capillary samples has been shown to be a definite problem. With this in mind, several rules must be applied to capillary collections.

First, and most importantly, is strict adherence to the blood collection technique by the collector. Second, no capillary sample should be considered definitive because there will always be a chance of contamination. Most current studies indicate a positive bias for capillary samples when compared with venous lead results, although falsely lowered capillary values have also been noted. Gross contamination, although not prominent, is still found in most studies and in the field. Several reports conclude that handwashing techniques have been found to be effective in preventing most external contamination.

Studies have also found that when children who have elevated capillary results return for venous confirmation, the lead values are lower, suggesting less contamination with venous collections. However, not all studies find this to be the case. One study found that repeat capillary analysis, after initial capillary analysis, showed almost the same decrease as a venous repeat. This indicates that other factors may contribute to the decrease. This study examines the adequacy of capillary specimens for whole blood lead determinations.

Material and Methods

Distribution of Capillary and Venous Lead Values

Lead result logs were reviewed from October, 1993, to June, 1994. Subjects six months to six years old were classified as either having a venous sample or a capillary sample. Other important demographic data, such as race, duration, and continuity of lead exposure, m3 health, and socioeconomic status were not available. Descriptive statistics were performed for both venous results and capillary results. Selected children with elevated venous or capillary blood lead results were followed for four months. This was done to compare values of repeat to initial analysis.

Soil-Lead Mixture Preparation

Lead, 200 mesh and finer was obtained from a commercial vendor. Soil was obtained from a freshly plowed suburban garden. The lead was mixed with soil at approximately a 1:12 ratio. The resulting mixture was analyzed for lead content at a certified environmental testing laboratory. The lead-soil mixture was measured at 7.90% lead.

Collection of Specimens

Volunteers were solicited at the 649th Medical Group Hospital, Hill Air Force Base, Utah. Thirty volunteers were solicited. Each signed a consent form approved by the University of Utah Medical School Institutional

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* Associated Regional University Pathologists, Salt Lake City, UT 84108.
† Manufactured by Spectrum Chemical Corporation, 14422 South San Pedro St, Gardena, CA 90248.
‡ Rocky Mountain Geochemical Corporation, 1323 W 7900 S, West Jordan, UT 84097.
Review Board. Volunteers received no compensation, other than knowledge of their lead values.

Venous specimens were collected in the normal manner. Capillary specimens were collected using a modified Centers for Disease Control (CDC) method (with and without handwashing). Collection of all specimens was performed by an accomplished phlebotomist, Senior Airman Craig Woodall, for the investigation of handwashing. Venous samples were collected first after both the subject and collector washed their hands. The subject then “covered” his/her finger over a test tube filled with the soil/lead mixture, inverted the tube, and deposited small amount of soil/lead on his/her finger. The collector then cleansed the finger with two alcohol pads and collected the sample (wiping off the first drop). Another sample was taken from the opposite hand in the same manner (with the exception that the subjects washed their hands after the soil/lead mixture was deposited on the finger).

Preparation and Analysis of Samples

Samples were prepared in the ARUP metals laboratory using established operating instructions. All samples were quantitated on an ICP-MS.* The testing was performed using a standardized procedure.19 Testing was performed on the same day to eliminate day to day analytical variability.

Statistical Evaluation

Statistical evaluation was accomplished by the use of a computer program entitled NCSS.† This computer program uses standard computations.20 Differences between the large capillary and venous databases were determined by use of pooled variance (two sample) t-tests.21 Differences between follow up tests and handwashing experiments were determined by use of paired t-tests.21

Results

Over 5,100 venous and 1,100 capillary samples were reviewed. The mean (3.83 µg/dL) of the venous samples was statistically less (p < 0.0005) than the mean (4.61 µg/dL) of the capillary samples (table I). Although a larger percentage of venous specimens were in CDC group I (0 to 10 µg/dL), the percentage of values less than 15 µg/dL was the same in both groups, 98.5%.

Sixteen children who had elevated initial capillary results underwent repeated tests within the four month follow up period. Repeat results were lower than the initial results (mean difference 11.81 µg/dL, p < 0.0001). Thirty six children whose initial venous results were elevated were followed for four months. This group was divided into two groups according to the initial lead value. In the 10 to 15 µg/dL group, 15 were less on repeat (mean difference 2.74 µg/dL, p = 0.0004). In the >15 µg/dL group, 17 were less on repeat (mean difference 5.82 µg/dL, p = 0.0003). These results are summarized in table II.

In the handwashing experiment, both venous and two capillary specimens were collected on the 30 subjects. Figures 1 and 2 display the relationship between the three groups. Summary statistics for each group are listed in table III. Statistical analysis revealed

<table>
<thead>
<tr>
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<th>Venous</th>
<th>Capillary</th>
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<tbody>
<tr>
<td>n</td>
<td>5129</td>
<td>1164</td>
</tr>
<tr>
<td>Mean, µg/dL</td>
<td>3.83</td>
<td>4.61</td>
</tr>
<tr>
<td>% &lt; 5 µg/dL</td>
<td>81.6</td>
<td>70.3</td>
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<tr>
<td>% &lt; 10 µg/dL</td>
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<td>94.1</td>
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<tr>
<td>% &lt; 15 µg/dL</td>
<td>98.5</td>
<td>98.5</td>
</tr>
</tbody>
</table>

* Elan 5000, 761 Main Ave Perkin-Elmer, Norwalk, CT 06859.
† Written by Dr. Jerry Hientze (326 N 1000 E, Kaysville UT, 84037).
TABLE II
Analysis of Elevated Lead Followups

<table>
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<tr>
<th></th>
<th>n</th>
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<th>10-15</th>
<th>15.1-20</th>
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<td>Cappillary initial</td>
<td>16</td>
<td>0</td>
<td>5</td>
<td>2</td>
<td>9</td>
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<tr>
<td>Cappillary repeat</td>
<td>16</td>
<td>9</td>
<td>6</td>
<td>0</td>
<td>1</td>
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<tr>
<td>Venous (10-15) initial</td>
<td>18</td>
<td>0</td>
<td>18</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Venous (10-15) repeat</td>
<td>18</td>
<td>7</td>
<td>10</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Venous (&gt; 15) initial</td>
<td>18</td>
<td>–</td>
<td>–</td>
<td>7</td>
<td>11</td>
</tr>
<tr>
<td>Venous (&gt; 15) repeat</td>
<td>18</td>
<td>3</td>
<td>6</td>
<td>3</td>
<td>6</td>
</tr>
</tbody>
</table>

All results in μg/dL.

significant differences between venous and capillary handwashing (p = 0.045), venous and capillary non-handwashing (p = 0.005), and capillary handwashing and nonhandwashing (p = 0.006)

Discussion

There is continuing debate over capillary sampling methodologies. Since contamination can never be completely eliminated, and some believe that any lead is potentially harmful to children, any contamination will exacerbate the results. The CDC has taken a different approach wherein a cutoff level of greater than 10 μg/dL is set to establish lead poisoning; this is different from the poison at all levels concept. Using the CDC guidelines, any lead result under 10 μg/dL is considered acceptable, and contamination that does not increase the blood lead levels above 10 μg/dL is not an issue.

Thirty years ago, it was common to have a considerable percentage of children with lead levels above 25 μg/dL. Twenty years ago, the average adult lead level, which historically has been less than child levels, was 16 μg/dL. Today, most studies find the average child in the U.S. to have levels less than 4 μg/dL. This dramatic decrease in blood lead levels has two important impacts on the credibility of capillary samples.

Figure 1. Distribution of venous and capillary lead values obtained from volunteers (the volunteer washed his/her hands before collection of the capillary sample). All values listed are μg/dL.

Figure 2. Distribution of venous and capillary lead values obtained from volunteers (the volunteer did not wash his/her hands before collection of the capillary sample). All values listed are μg/dL.
TABLE III
Effect of Handwashing in Eliminating Gross Contamination

<table>
<thead>
<tr>
<th></th>
<th>V</th>
<th>CHW</th>
<th>CNHW</th>
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</thead>
<tbody>
<tr>
<td>n</td>
<td>30</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>Mean, μg/dl</td>
<td>1.30</td>
<td>4.27</td>
<td>168.6</td>
</tr>
<tr>
<td>SD, μg/dl</td>
<td>0.56</td>
<td>8.00</td>
<td>303.7</td>
</tr>
<tr>
<td>Range, μg/dl</td>
<td>1.88</td>
<td>41.77</td>
<td>1181</td>
</tr>
<tr>
<td>100% tile</td>
<td>2.63</td>
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<td>1.86</td>
<td>3.28</td>
<td>112.0</td>
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<tr>
<td>50% tile</td>
<td>1.09</td>
<td>1.92</td>
<td>43.68</td>
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<tr>
<td>25% tile</td>
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<td>1.40</td>
<td>22.37</td>
</tr>
<tr>
<td>0% tile</td>
<td>0.75</td>
<td>1.03</td>
<td>1.83</td>
</tr>
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</table>

V = venous.
CHW = capillary + handwashing.
CNHW = capillary – no handwashing.

First, because lead levels dropped, so too has the importance of contamination. For example, if the average lead value was 9.5 μg/dL and the average contamination was 2.0 μg/dL, it is easy to see why many samples would be above the cutoff level of 10 μg/dL. However, the current averages around 4.0 μg/dL, contamination at 2.0 μg/dL, does not alter the action to be taken. Second, with decreasing levels of lead in the environment, a corresponding drop in the amount of contamination is inevitable.

Gross contamination is mentioned in the literature as being a major deterrent to capillary methodologies. In the past, there is no doubt that contamination of capillary specimens was present and affected many samples. When proper collection techniques are followed, gross contamination is not a major problem. A major portion of the decrease seen at retest is likely due to the increased awareness of the potential for contamination and, hence, closer compliance with the collection instructions. Follow up lead testing on those having elevated results shows a statistically significant difference between the means of repeat capillary and venous and their initial results. All capillary repeats and 89% of venous repeats were lower than the initial results with the difference in means greater in capillary than venous samples. Some examples of gross contamination are obvious, such as one seen that had an initial result of 35 μg/dL and a repeat within 7 days of 3 μg/dL.

The analysis of a large database of venous and capillary results was performed to evaluate contamination. Comparing the percentages greater than 5 and 10 μg/dL (table I), it appears some contamination occurred resulting in a higher percentage of capillary and venous specimens in the CDC group II. The percentage greater than 15 μg/dL was the same for both capillary and venous specimens. Gross contamination is not a major problem.

Minor contamination may be either uniform for all samples, suggesting a process bias such as lead contamination in the collection media, reagents, tubes, pipet tips, etc., or variable which would be more likely if contamination took place during blood collection. Adjustments can be made to correct a bias. In this case, the difference between the means of the capillary and venous collected specimens, is 0.78 μg/dL, a relatively small difference.

After adjusting for this mean difference, there are still significant differences between the percentages in each group (table I). If uniform contamination occurred, the percentages would be the same. However, the percentages are different, although closer together, suggesting that this low level contamination is variable.

The major advantage to capillary testing is the ease of collection. Even the most skilled pediatric phlebotomist can have difficulty obtaining a venous specimen from a small child. Capillary specimens, as a general rule, are much easier to collect and less traumatic to parent and child. However, capillary samples are harder to work with in the laboratory and do not offer the volume of blood often necessary for repeat analysis.

The main issue is, “Will the blood lead levels determined on capillary specimens falsely categorize subjects?”

It is concluded that with proper handwashing most lead contamination in the field can be avoided. Some studies find that use of an
alcohol wipe alone does this. Of the studies examined, only one introduced external contamination (1% lead soil). However, no handwashing was performed in this study. The mean of capillary handwashing specimens was 4.61 µg/dL, compared to over 168 µg/dL for nonhandwashing specimens. This difference was statistically significant (p = 0.045). The venous baseline was 1.30 µg/dL. Of the 30 volunteers, 28 were white collar hospital employees and two were machinist shop workers. The phlebotomist noted that the hands of the machinists looked dirty, even after proper handwashing. The two machinists had capillary handwashing results of 42.8 and 17.85 µg/dL and nonhandwashing results of 492.5 and 1,172.0 µg/dL. This problem should not occur in children.

This study clearly indicates that proper handwashing and meticulous attention to detail by the phlebotomist can reduce contamination to manageable levels in the collection of capillary specimens for determination of whole blood lead.

Acknowledgments

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References