Some Sources of Error in Thyroxine Screening in Neonates by Radioimmunoassay of Whole Blood Spotted on Blotter Paper

To the Editor:

The “GammaCoat T4 Kit” (Clinical Assays, Inc.) is a valuable blood thyroxine assay for early detection of thyroid insufficiency in neonates (1–6). Essentially, it involves the following steps:

- absorption of a few drops of the neonate’s capillary blood onto a piece of blotter paper
- extraction of thyroxine from the dried blood spot into an aqueous buffer
- assay of the thyroxine concentration by conventional radioimmunoassay methods.

Generally, the analysis is done on a small paper disk cut from the blood spot with an ordinary hole puncher. During our developmental work, we looked into sources of error that may interfere with the paper disk method. Somewhat unexpectedly, we found that the size of the blood spot on the paper and the region of the spot from which the disk is punched affect the apparent concentration of thyroxine. When these factors are ignored, the resulting thyroxine concentration determination can be as much as 50% in error.

We undertook two independent types of experiments, to establish the precision and accuracy problems caused by “paper effects” in this assay:

(a) 125I-labeled thyroxine was added to heparinized venous blood and various volumes of blood were spotted onto blotter paper to produce different spot sizes. After the blood had dried, 3.5-mm disks were punched from the central and peripheral regions of these spots. Blood volume per disk was determined by counting the radioactivity in the disk and comparing the count per minute (cpm) per disk with the cpm of an aliquot of known volume. Table 1 gives representative results.

(b) Capillary or venous blood was spotted on blotter paper and dried. Disks of 3.5- or 5-mm diameter were punched, extracted, and assayed by a modification of the Clinical Assay’s GammaCoat T4 Kit. These disks were punched from spots of various sizes and from various regions (inner or edge) of a given blood spot. Typical data are given in Table 2.

These data show that the quantity of thyroxine per disk is not constant but appears to be influenced by two factors: a chromatographic effect, which causes a buildup of T4 on the periphery of the spot; and an apparent “buildup” of blood, which increases with the total volume of blood added to the same spot on the paper and which is probably related to over-saturation of the absorbent capacity of the paper.

For a given blood specimen, the most severe discrepancies in assay value were observed in replicate determinations of thyroxine from the inner regions of a small spot and the edge region of a large spot. Widely differing spot sizes have been reported (3, 7) by investigators analyzing batches of samples for routine screening, and the precision and accuracy of the assay as it is now done is put into question. Collection and sampling of the disk from the spots needs to be standardized.

Other results not shown here demonstrate that comparable effects occur for several different lots of Schleicher & Schuell blotter paper and for other manufacturers’ papers as well—for synthetic whole bloods of various hematocrits used as assay standards and for both heparinized blood and blood containing no anticoagulant (capillary or venous). The technique used in applying the blood to the paper (dripping, squirting, touching, etc.) has not been shown to result in any systematic variation in assay value or in the spread of thyroxine in the blood spot. Very typically, greater coefficients of variations were found for disks taken from close to the edge of the spot as indicat-

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**Table 1. Apparent Volume of Blood per 3.5-mm Disk**

<table>
<thead>
<tr>
<th>Region of spot taken</th>
<th>35 (10.5)</th>
<th>50 (12)</th>
<th>100 (20)</th>
<th>200 (25.4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Center</td>
<td>2.61 μl (CV 5.79%)</td>
<td>2.82 μl (CV 2.96%)</td>
<td>3.03 μl (CV 1.39%)</td>
<td>3.18 μl (CV 2.41%)</td>
</tr>
<tr>
<td>Intermediate</td>
<td>—</td>
<td>—</td>
<td>3.11 μl (CV 4.86%)</td>
<td>2.14 μl</td>
</tr>
<tr>
<td>Outer edge</td>
<td>3.21 μl (CV 7.49%)</td>
<td>3.15 μl (CV 9.17%)</td>
<td>3.38 μl (CV 6.51%)</td>
<td>3.33 μl (CV 4.82%)</td>
</tr>
</tbody>
</table>

* Blood spotted on Schleicher & Schuell no. 903 paper.

**Table 2. Assay of Thyroxine in Fingertip Capillary Blood Dried on S&S Paper**

<table>
<thead>
<tr>
<th>Spot diam.</th>
<th>Region</th>
<th>Subjects</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Thyroxine, ng/two 3.5-mm disks</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Small (10.5 mm)</td>
<td>Inner</td>
<td>0.12</td>
<td>0.11</td>
<td>0.11</td>
<td>0.11</td>
<td>0.12</td>
<td>0.07</td>
<td></td>
</tr>
<tr>
<td>Medium (12 mm)</td>
<td>Inner</td>
<td>0.13</td>
<td>0.12</td>
<td>0.21</td>
<td>0.11</td>
<td>0.14</td>
<td>0.09</td>
<td></td>
</tr>
<tr>
<td>Large (25.4 mm)</td>
<td>Inner</td>
<td>0.15</td>
<td>0.15</td>
<td>0.12</td>
<td>0.13</td>
<td>0.15</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Outer</td>
<td>0.17</td>
<td>0.15</td>
<td>0.17</td>
<td>0.16</td>
<td>0.20</td>
<td>0.12</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Outer</td>
<td>0.19</td>
<td>0.18</td>
<td>0.18</td>
<td>0.18</td>
<td>0.19</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Values given are generally averages of two or three replicate assays.
* Inner: Disk punched from middle or center region of the blood spot. Outer: Disk punched from within 2 mm of the edge of the blood spot.
* Subjects A–F are normal adult men and women laboratory personnel.
* We question this result but have no basis for excluding it.

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ed in Table 1. (In all cases, of course, the total area of the punched disks appeared to be completely blood-soaked.)

For a quantitative test of this sort, variations as great as those given in the Tables can introduce a serious degree of uncertainty in the estimated thyroxine concentration. Fortunately, the error can be decreased (but not eliminated) by following a few simple rules of technique in the nursery and in the laboratory:

1. Blood should be applied to the blotter paper so that spot size is fairly constant at a diameter about 13 to 20 mm in all cases. (The blood spot standards used for standard curve preparation should be of the same diameter). Larger and smaller spots should be avoided; very small spots of 10-mm diameter or less should not be offered for assay.

2. The assayer should punch disks from the central region of the blood spot, not from the edge.

By strict adherence to these simple rules, the error-range implied by our tabulated data can be decreased markedly and confidence in the assay results correspondingly increased.

References


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