Improved Micro-Radioimmunoassay of Digoxin in Serum, with Use of $^{125}$I-Labeled Digoxin

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A micro-radioimmunoassay of $^{125}$I-labeled digoxin is described in which 10-μl rather than 50-μl aliquots of sera are required. The method is a modification of a simplified, rapid, and accurate procedure, which is commercially available as a kit. There is excellent correlation between results by these two methods, in which lyophilized digoxin standards and insoluble antibody polymer are used. The digoxin concentration in capillary sera was not significantly different from that in the corresponding venous blood. It is clinically useful to monitor digoxin therapy in capillary sera, particularly in patients in whom blood sampling by venipuncture is inconvenient or difficult.

Additional Keyphrases: "kit" methods • pediatric chemistry

Knowledge of concentrations of drugs in the blood is important to forensic scientists, toxicologists, medical investigators, and clinicians. However, the significance of these data depends on the accuracy of the methods used for their determinations. Numerous reports (1-4) indicate that the technical problems in radioimmunoassay have resulted in erroneous results and lack of reproducibility. We, too, have encountered such problems when using commercial kits with either 3H- or $^{131}$I-labeled digoxin. Thus, we decided to evaluate a method that involves use of $^{125}$I-labeled digoxin, lyophilized digoxin standards, and insoluble digoxin-antibody polymer. The labeled derivative is $[^{125}]$I-succinyl digoxin tyrosine, which has a high solubility and specific activity, prolonged stability, and is in a lyophilized form.

Materials and Methods

Our purpose was to (a) substantiate accuracy and reproducibility of the kit procedure (Curtis Nuclear Corp., Los Angeles, Calif. 90058), (b) validate reproducibility of a modification of this procedure involving a microtechnique, and (c) compare digoxin concentration in capillary and venous blood samples.

We used Curtis Digoxin RIA kits from nine different production lots in this study.

Blood samples for digoxin determinations were obtained from a total of 40 hospitalized patients, 45 to 83 years of age and weighing between 46 and 105 kg (104 to 233 pounds) who were under treatment for congestive heart failure. They were all maintained with single daily doses of either 0.125 mg (14 patients) or 0.25 mg (26 patients) of digoxin. All blood samples were collected at 1430-1530 hours, 6 h after ingestion of the medication, both by venipuncture of the antecubital vein and by finger prick.

Linear regression analysis was performed to test the precision of the original methodology and paired t-tests were used to determine differences between groups (6).

Procedure

The accuracy of these kits was first tested by determining the recovery of digoxin (USP grade) added to digoxin-free sera. These were primary standards and corresponded to 0, 1.2, 2.4, 4.0, and 4.8 μg of digoxin per liter. Assays were conducted according to the procedure prescribed for these kits, which contain secondary standards.

In this study we analyzed 50-μl aliquots of venous sera according to the method included with these kits and 10-μl aliquots of the same samples according to our modification of this method. We also analyzed samples of both capillary and venous sera from the same patients, collected within 1 min of one another and the sera frozen if not immediately assayed. All analyses were done in duplicate. We used a Gamma Scintillation Spectrometer, Model 5375 (Packard Instrument Co., Inc., Downers Grove, Ill. 60515), with 10-min counting times. The analytical procedure was:

All reagents and sera were brought to room temperature (15-30 °C) before use.

Each polymer tablet (from the kit) was dissolved in 0.5 ml of sodium chloride solution (9 g/liter). After the tablets were dissolved, forming the polymer tablet slurry, 100-μl aliquots were pipetted into test tubes, the solution being shaken before each aliquot to assure complete dissolution of polymer tablets. Then the samples were mixed gently by hand and left at room temperature for 10 min.

Aliquots, 10 μl, of standards and patients' sera were pipetted (Eppendorf Microliter pipets) directly into the antibody polymer slurry, mixed, and left at room temperature for 10 min.

Ten microliters of $^{125}$I-labeled digoxin tracer (no less than 1 mCi/liter can be used in this method) were pipetted directly into the tubes, mixed, and left at room temperature for 30 min.

After 30 min, 1.0 ml of the saline was pipetted into each test tube, mixed with a vortex-type mixer until the precipitate was suspended, and centrifuged (3000 rpm, 5 min) at room temperature. The saline wash was carefully decanted, and dis-
Table 1. Values Obtained with Six Lots of Curtis Nuclear Kit for Four Concentrations of USP Digoxin Added to Serum

<table>
<thead>
<tr>
<th>Lot no.</th>
<th>USP digoxin standard</th>
<th>1100</th>
<th>1100</th>
<th>1100</th>
<th>1100</th>
<th>1100</th>
<th>1100</th>
<th>Mean ((\bar{X}))</th>
<th>SD</th>
<th>CV, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>18</td>
<td>1.2</td>
<td>1.00</td>
<td>0.94</td>
<td>0.92</td>
<td>0.95</td>
<td>1.21</td>
<td>0.98</td>
<td>1.01</td>
<td>0.108</td>
<td>10.68</td>
</tr>
<tr>
<td></td>
<td>1.00</td>
<td>0.99</td>
<td>0.88</td>
<td>1.00</td>
<td>1.26</td>
<td>0.99</td>
<td>1.00</td>
<td>2.43</td>
<td>0.139</td>
<td>5.73</td>
</tr>
<tr>
<td>18</td>
<td>2.4</td>
<td>2.35</td>
<td>2.25</td>
<td>2.40</td>
<td>2.42</td>
<td>2.35</td>
<td>2.70</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>4.0</td>
<td>4.10</td>
<td>3.70</td>
<td>3.90</td>
<td>4.15</td>
<td>3.88</td>
<td>3.95</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>4.8</td>
<td>4.82</td>
<td>4.40</td>
<td>4.95</td>
<td>4.68</td>
<td>4.48</td>
<td>4.78</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.80</td>
<td>4.77</td>
<td>5.20</td>
<td>Broken tube</td>
<td>4.48</td>
<td>5.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

![Graph](image)

**Fig. 1. Standard curves prepared with 10-\(\mu\)l (X) and 50-\(\mu\)l (○) samples.**

significant correlation was obtained between primary and secondary digoxin standards. The method is linear and satisfactorily accurate.

Standard curves were constructed for each assay (Figure 1). During the course of this study, four curves were obtained for each kit, and excellent reproducibility was obtained between them. The same control serum was used as an unknown in each assay.

Serum digoxin concentrations of our patients ranged between 0.23 to 2.90 \(\mu\)g/liter. Statistical analysis of data comparing paired samples of digoxin concentrations in 10-\(\mu\)l and 50-\(\mu\)l samples of venous sera obtained in this group of patients indicated an average difference (\(\bar{X}\)) = 0.022 ± 0.3052 (SD) (t = 0.456, and \(P > 0.60 < 0.70\)).

Digoxin concentrations were compared in capillary and venous blood of 20 patients. We compared results for 10- and 50-\(\mu\)l samples of venous blood and 10-\(\mu\)l samples of capillary and venous blood. Statistical analysis indicated that for the 10- vs. 50-\(\mu\)l samples, \(\bar{X} = 0.110 \pm 0.2613 (SD)\), \(t = 1.89\) and \(P > 0.05 < 0.10\); for the 10-\(\mu\)l capillary vs. venous samples, \(\bar{X} = 0.0435 \pm 0.1975 (SD)\), \(t = 0.986\), and \(P > 0.20 < 0.40\).

**Discussion**

Our data confirm that the original radioimmunoassay kit for digoxin gives rapid, accurate results and is suitable for routine clinical use. Because special equipment and preparation of solutions required by other methods is obviated, there are fewer possible sources of laboratory error and less time is required to perform these assays. The simplification of radioimmunoassay of digoxin when \(^{125}\text{I}\)-labeled digoxin is used has been confirmed by others (6, 7).

Our micro-radioimmunoassay for digoxin depends on the specific activity of \(^{125}\text{I}\)-labeled digoxin supplied by the manufacturer being about 1 mCi/liter or greater, so that the 10-\(\mu\)l volumes we used in this method can be easily detected with a gamma counter. Our micro-method was compared to the manufacturer's prescribed method.

carded. The small packed precipitate button must not be disturbed. This step is repeated.

After the final washing, the test tubes were inverted, to drain. Each test tube was then washed thoroughly on the outside, rinsed with distilled water, dried, and the radioactivity of its contents counted.

**Results**

A line of regression was plotted from the data (Table 1) to determine the accuracy of the commercially available Curtis RIA kits. The slope was 1.1005 ± 0.0031, with an intercept of -0.49 ± 0.09. The t-test for the slope was 350.6 and for the intercept -45.7. The correlation coefficient was 0.9997 and

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There was no significant difference between capillary and venous blood concentration of digoxin.

Therefore, from the practical clinical aspect, our procedure, which requires 10 μl rather than 50 μl aliquots of sera, can be used when venipuncture is difficult or undesirable, as in cases of extreme obesity, venous thrombosis, or peripheral vascular disease, or for infants and children.

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References

Adaptation of "EMIT" Technique for Serum Phenobarbital and Diphenylhydantoin Assays to the Miniature Centrifugal Analyzer

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We used a miniature centrifugal analyzer in a spectrophotometric rate-measurement mode to determine the anticonvulsant drugs phenobarbital and diphenylhydantoin in serum, by use of a modified enzyme immunoassay ("EMIT", Syva Corp.) We decreased reagent cost per determination by at least sixfold by means of microscale techniques. Also, the analysis rate is increased by measuring multiple samples simultaneously. Our method requires only 3 μl of serum for duplicate determinations. Replicate analyses of sera containing phenobarbital and diphenylhydantoin gave reaction rates with a CV of 1.5%. Run-to-run CV was 15%. Analytical recovery for drug-supplemented serum samples was 98%, and results for a series of samples compared well with results obtained by gas chromatography (for phenobarbital, \( r = 0.95 \); for diphenylhydantoin, \( r = 0.91 \)).

Routine monitoring of phenobarbital and diphenylhydantoin is a valuable aid in the management of patients with generalized epileptic seizures (1). Gas-liquid chromatography is the most widely used method for measuring anticonvulsants (2). This technique is reasonably sensitive, accurate, and reliable, but has several inherent disadvantages. It is relatively time-consuming. Usually, several milliliters of serum are required, which may not be in the best interest of the patient if day-to-day monitoring is desired. At least one sample pre-treatment step must be performed, and the quality of results often strongly depends on the skill of the laboratory personnel. Finally, the overall cost of the determination is frequently high.

The recently introduced Enzyme Multiplicated Immunoassay Technique ("EMIT", Syva Corp., Palo Alto, Calif. 94303) provides an alternative to conventional methods (3–8). EMIT assays are based on a binding process between antibody and antigen (drug). Initially, an enzyme-labeled drug is bound to binding sites on the antibody, and the activity of free enzyme is low. Free drug present in the sample competes with an enzyme-labeled drug for antibody binding sites, enzyme is correspondingly released, and enzymic activity of the sample increases. In the present analysis, free enzyme then catalyzes the conversion of the substrate, NAD⁺, to NADH, which is reflected in an increased absorbance at 340 nm. The rate of increase is related to the concentration of free drug in the sample.

The technique is rapid, specific, reliable, and sensitive, and no sample pretreatment is required. The principal disadvantage, the high cost of reagents, can be offset by use of microscale techniques made possible by the miniature centrifugal analyzer. Here, we show that good quantitative results can be obtained at lower cost, at a higher rate, and with a smaller sample than with the usual EMIT procedure.

Materials and Methods

Apparatus

Hardware. The instrumental system, based on the original ORNL design (9), but incorporating extensive modifications, is described elsewhere (10). An automatic pipette (Model

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