is reused, renders the rest of the kit unusable. This increases the variability of the assay. In this laboratory, standard curves from such runs were in error by 20–50% for the 5 mg/L standard and by about 25% for the 20 mg/L standard; comparable errors for the RIA were 3–5%. Because of the greater immunity of the RIA to this type of analyst error, I prefer the RIA procedure.

The RIA is quite stable as reflected in the low interassay CV for the Lederle control serum; the month during which it was assayed covers the lifetime of the reconstituted reagents. Results by RIA compare well with both liquid chromatography and EMIT. No elaborate instrumentation is required except for a gamma scintillation counter. Finally, its simplicity facilitates its incorporation into the laboratory routine.

References

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Evaluation of a New Commercially Available (Ortho) Digoxin Radiolabimmunoassay Kit

To the Editor:

We evaluated a newly available digoxin radioimmunoassay (RIA) kit ("Ortho Digoxin RIA (PEG) Kit"; Ortho Diagnostics Inc., Raritan, NJ 08869) to see if it performed satisfactorily in an in vitro evaluation of patients' sera in comparison with an established digoxin RIA kit ("Quantitone 125I-Digoxin RIA Kit"; Kallestad Laboratories Inc., Chaska, MN 55318). In doing so we used 100 specimens, as follows:

10 from apparently normal, healthy individuals with no history of cardiac glycoside treatment.
30 with serum digoxin concentrations <1.0 μg/L.
30 with serum digoxin concentrations of 1.0 to 2.5 μg/L.
30 with serum digoxin concentrations >2.5 μg/L.

Samples were arbitrarily placed in the above categories based on results with the Kallestad kit.

All patients' samples were assayed in duplicate, on the same day, by the same technologist, with both digoxin RIA kits.

Briefly, in the Ortho digoxin RIA an iodinated tracer and an antibody are used, with polyethylene glycol separation of the bound fraction. A 50-μL sample is necessary for each tube. In the Kallestad digoxin RIA, an iodinated tracer is also used, but a precomplexed double-antibody step is included, which eliminates the separate addition of a separation agent. A 100-μL sample is necessary for each tube. Each kit method includes a 30-min incubation.

Table 1 summarizes our results. If a serum digoxin value exceeding 2.0 μg/L is accepted as an index of toxicity, results for only one specimen disagreed between kits, and this disagreement was modest (2.2 μg/L with the Ortho kit, 2.0 μg/L with the Kallestad kit).

In this short study, run-to-run variation was as follows (μg/L):

<table>
<thead>
<tr>
<th>Kit</th>
<th>Therapeutic concn.</th>
<th>n</th>
<th>SD</th>
<th>CV%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ortho</td>
<td>0.97 0.03 2.9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kallestad</td>
<td>0.76 0.07 9.2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Toxic concn.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ortho</td>
<td>3.03 0.16 5.4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kallestad</td>
<td>2.62 0.20 7.6</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

We conclude that there are negligible differences between results obtained with these two kits.

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Karen Warren
H. E. Sine

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Falsely Positive Results for Bilirubin with Ames' "Clini-Tek"

To the Editor:

In our laboratory, we recently began using the Ames Clini-Tek reader for routine urinalysis. We obtained several positive bilirubin results on normally colored urines with the Clini-Tek. These results were rechecked by the IctoTest method and found to be negative for bilirubin. We also obtained positive bilirubin results on some highly colored urines with the Clini-Tek. By the IctoTest method, these were all found to be negative for bilirubin. Since the IctoTest method is more sensitive for bilirubin than the N-Multistix, we are getting false-positive bilirubin results on the Clini-Tek. These false positives occur in about one urine sample out of 40 or 50 samples, or about 2%.

According to a recent evaluation of the Ames Clini-Tek (1), it gave 0% false-positive bilirubins. Since we have found a 2% incidence of false-positive bilirubins with the Clini-Tek, we now check all positive bilirubin results with the IctoTest method. Our instrument has been returned to the company and this has been verified not to be an electronic problem. For this reason we recommend that all users of the Clini-Tek verify all positive bilirubins by an alternative method.

Reference

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An author of ref. 1 above (R.G.) says that they have not encountered this problem. No hyperchromic urines were included in their study. The Ames Co.

1 "Clini-Tek," "IctoTest," and "N-Multistix" are registered trademarks of Ames Co., Division of Miles Laboratories, Inc., Elkhart, IN 46514.

Table 1. RIA Results for Digoxin, as Measured with Two Kits

<table>
<thead>
<tr>
<th>Digoxin</th>
<th>Ortho (OR) mean</th>
<th>Kallestad (K) mean</th>
<th>Regression equations</th>
</tr>
</thead>
<tbody>
<tr>
<td>µg/L</td>
<td>µg/L</td>
<td>µg/L</td>
<td></td>
</tr>
<tr>
<td>Normals (n = 10)</td>
<td>0.005</td>
<td>0.06</td>
<td>K = 1.0(OR) + 0.06</td>
</tr>
<tr>
<td>0.1 to 1.0 (n = 30)</td>
<td>0.60</td>
<td>0.68</td>
<td>K = 0.86(OR) + 0.17</td>
</tr>
<tr>
<td>1.0 to 2.5 (n = 30)</td>
<td>1.59</td>
<td>1.63</td>
<td>K = 1.0(OR) + 0.04</td>
</tr>
<tr>
<td>&gt;2.5 (n = 30)</td>
<td>3.58</td>
<td>3.60</td>
<td>K = 0.95(OR) + 0.18</td>
</tr>
<tr>
<td>All groups (n = 100)</td>
<td>1.74</td>
<td>1.77</td>
<td>K = 0.97(OR) + 0.09  (r² = 0.98)</td>
</tr>
</tbody>
</table>
was invited to comment, and the following response was received.

To the Editor:
Ames Company recognizes that occasionally a urine will give a positive bilirubin result on the Clini-Tek® and a negative IctoTest® result. This discrepancy is usually caused by the presence of a substance in the urine that interferes with the reagent-strip reaction. For example, indoxyl sulfate or indican will react with the bilirubin-reagent area to form an atypical yellowish or gold color. This atypical color is generally quite apparent and will be recognized by the Clini-Tek as atypical, and the instrument will “read through” the color to correctly interpret the strip reaction. Sometimes, however, a urine may contain a small amount of indoxyl sulfate, which in combination with other chemicals or pigments causes a color to appear on the strip that very closely matches the expected color reaction of the reagent strip. In such instances, the Clini-Tek, or a visual reader for that matter, is unable to determine that the color is atypical and reads the result as positive because it most closely matches the color of the positive color block. Only IctoTest, which “filters out” the atypical colors can differentiate between a true positive and negative specimen when this occurs. Unfortunately, technology is not available that would allow us to make a strip test for the detection of bilirubin in urine that would be totally free from such interferences. IctoTest, which should be used to confirm the presence or absence of bilirubin, is recommended whenever the strip test is in doubt.

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A Sensitive Type of “Immunocapillary Migration” Assay That Detects Insulin

To the Editor:
An “immunocapillarymigration” assay has been reported (1) in which are used fluorescein-labeled antibodies to transferrin and transferrin antibodies adsorbed to porous strips. Antigen in concentrations of 0.1 to 0.6 g/L were quantitated by measuring the distance that the antigen migrated. This distance increased with antigen concentration.

It may be possible to shorten the assay procedure and make the assay more sensitive by use of radioiodinated or enzyme-labeled antigen tracer (1).

In an assay based on the same principle, radioiodinated insulin was used. Antibody to insulin that had been coupled to agarose (Sepharose) and placed in small plastic columns (2) was used in place of the antibody insolubilized to the plastic strips. Insulin standards were prepared in borate buffer (2). Radioiodinated insulin (Abbott Laboratories) was diluted and mixed with either buffer or insulin standard so that 0.2 mL of the mixture contained about 10 000 cpm, and 0.2 mL of these mixtures of either buffer or standard and radioiodinated insulin was put on each column. After a 40-min incubation at room temperature, 0.3 mL of buffer was applied to each column and allowed to flow by gravity. The column size was such that the total 0.5 mL (and nearly all of the radioactivity contained in the applied sample) was still retained within the column.

The top and bottom quarters of each column were counted separately. The results were expressed as a ratio of the count rate on top to that on the bottom of the column (Table 1).

The results (Table 1) indicate that, when plain Sepharose columns were used, the radioactivity was rather uniformly distributed throughout the column. Use of antibody–Sepharose columns and samples with no insulin resulted in binding of the radioactivity mostly to the top quarter of the column. When the sample contained a low concentration of insulin (25 μg/L), radioactivity was redistributed within the column so that relatively more radioactivity was bound to the bottom of the column. This had the effect of increasing the migration of the radiolabeled insulin within the column so that more was detected at the bottom. The presence of a large amount of extraneous protein did not interfere.

I thank Linda Hartzell for her help.

References
1. Glad, C., and Grubb, A. O., Immuno-

Table 1. Ratios of Count Rate in the Top Quarter of a Column to That in the Bottom Quarter

<table>
<thead>
<tr>
<th>Type of column</th>
<th>Sample</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plain Sepharose</td>
<td>buffer</td>
<td>1.0</td>
</tr>
<tr>
<td>Antibody–Sepharose</td>
<td>buffer</td>
<td>7.2</td>
</tr>
<tr>
<td>Antibody–Sepharose</td>
<td>5 ng insulin*</td>
<td>2.3</td>
</tr>
<tr>
<td>Antibody–Sepharose</td>
<td>10 mg human γ-globulin*</td>
<td>6.8</td>
</tr>
</tbody>
</table>

* In 0.2-mL volume.

Possible Sodium Contamination of Evacuated Blood-Collection Tubes

To the Editor:
Often it is necessary to collect blood by syringe from difficult patients, then transfer the blood to appropriate small evacuated collection tubes. The technologists here have maintained for some time that sodium values for heparinized plasma collected in small (2-mL) tubes are invalid. To test this, we followed this protocol: 10-mL samples of blood were collected by trauma-free venipuncture from eight healthy ambulatory volunteers into disposable plastic syringes. Immediately, 2-mL of blood was transferred to green-stoppered evacuated tubes (BD, Vacutainer lot no. 7K149, sodium heparin, 2-mL), green-stoppered evacuated tubes (Sherwood Medical Industries, Monojet lot no. 03654, lithium heparin, 5-mL), and to glass tubes for clotting. The whole blood was mixed with the heparin, then introduced into recently calibrated well-controlled blood-gas instruments (Corning) “165” and “175”). Residual heparinized blood was centrifuged and the plasma assayed within 30 min for sodium, potassium, and chloride (Technicon “Stat-Ion”). Values for pH, pCO₂, calculated bicarbonate, potassium, and chloride were comparable in blood from both sizes of collection tubes. Sodium values were invariably higher in plasma from the 2-mL tube, with differences ranging from 5 to 12 mmol/L. Results for a single typical trial showed differences only for the sodium (149.1 vs. 139.8 mmol/L) and the “anion gap” (23 mmol/L vs. 13 mmol/L). Assay of serum sodium in this trial gave a value of 141 mmol/L. A brief trial of 2-mL tubes, Vacutainer lot no. 8H031, yielded sodium values from 2 to 3 mmol/L higher than sodium values of plasma from the larger tubes. Drawing directly into evacuated tubes rather than first into a syringe yielded sodium results 7 mmol/L higher in the 2-mL tube, suggesting that syringe-drawing was not responsible.

We cannot account for the extra sodium and the unknown additional anion. We would not expect the sodium contributed by the heparin salt to be sufficient to account for the “extra” sodium (personal communication, H. F. Weisberg); also, we do not detect dif-