= 0.755\text{poly} + 2.2 (r = 0.746, n = 31). Although the plate diffusion bioassay measures the biological activity of the drug, the method has so many shortcomings (3) that we cannot recommend it for netilmicin determination.

References


A. Harmoinen
P. Vuorinen
Tampere Univ. Central Hosp.
SF-33520 Tampere
Finland

L. Melamies
Orion Diagnostica
P.O. Box 83
SF-02101 Espoo, Finland

Minimizing the Effect of Digoxin-Like Immunoreactive Substances in Immunoassays for Digoxin in Neonatal Serum

To the Editor:

Digoxin-like immunoreactive substances (DLIS) in serum from infants younger than two months can lead to falsely high values for digoxin as measured by immunoassays (1–4). Two studies (2, 5) indicate that detection of DLIS depends on a unique specificity of the antibody used in the immunoassay because a change in the antiseraum used in the RIA decreased the amount of DLIS detected in neonatal serum. From these and other studies (1, 4, 6) immunoassay kits can be classified arbitrarily as reacting strongly, moderately, or weakly with DLIS when >90%, 5–20%, and <5% of the respective digoxin-free serum specimens have apparent digoxin concentrations of >0.5 µg/L.

Seeking to minimize the interference caused by DLIS, I evaluated three immunoassay kits previously shown (1, 4) to have weak reactivity to DLIS: "Immo-Phase Digoxin 125I RIA" (Corning Medical and Scientific, Medfield, MA); 125I Digoxin RIA kit” (Diagnostic Products Corp., Los Angeles, CA); and "Quanticoat 125I Digoxin RIA kit" (Kallestad Laboratories Inc., Austin, TX). In all these kits, 50 µL of sample is used per assay tube.

All three RIAs gave similar values and similar between-run CVs for digoxin added to the control materials (Unassayed Control Material, Levels I, II, III; Beckman Instruments Inc., Brea, CA), although the mean values for digoxin in the control material were 5 to 24% higher by the Diagnostic Products' RIA than by the other two assays. For all three assays, the limit of detection was 0.1 µg/L, as determined by calculating the 95% confidence interval for repeated assays of digoxin-free serum (n = eight to 18 samples per run in two or three runs).

To perform the evaluation, I used serum left over from other laboratory tests, which had been collected from infants in the intensive-care nursery of a neighboring hospital, who were less likely to have been receiving digoxin than at my institution. Figure 1 shows my results. Six samples from four different patients had apparent digoxin values >0.5 µg/L by at least one of the immunoassays (nonnumerical symbols, Figure 1). Insufficient information on samples from three patients did not allow review of the medical records, but discrepant results for digoxin in these samples (open triangles, open inverted triangles, open circles) suggest that they contained DLIS. The other patient, a male infant with respiratory distress syndrome, had not received digoxin. Three samples from this patient were analyzed by all three RIAs (solid circles, solid squares, open squares), the lowest values being obtained with the Diagnostic Products RIA.

These results confirm earlier studies showing that different RIA kits detect different amounts of DLIS in the same serum (2, 3, 6). The Diagnostic Products kit appeared to be the most specific, confirming earlier reports (4, 7). However, one of 67 neonatal serum specimens examined for DLIS by the Diagnostic Products kit (including the 42 samples in Figure 1) had a value of 1.0 µg/L. This serum was from a three-day-old infant who had not received digoxin and was hospitalized for corrective surgery. Thus, every immunoassay kit apparently will react to some extent with DLIS in neonatal serum.

This problem of false-positive concentrations of digoxin in neonates will be resolved only when the substances responsible for DLIS activity can be isolated and characterized (8). In the interim, I suggest the following threefold approach to minimize the problem of interference by DLIS in immunoassays of neonatal serum.

First, use an immunoassay kit that has weak reactivity to DLIS. Laboratories quantifying digoxin in neonatal serum must consider re-evaluation if their current method shows moderate to strong reactivity with digoxin-free neonatal serum. Evaluate a kit by using neonatal serum that remains after routine laboratory tests have been performed. The volume of serum from individual patients can be increased by pooling samples drawn on the same day (2). If the apparent digoxin concentrations exceed >0.5 µg/L, review the medical records to determine whether the infant received digoxin.

Second, obtain from the manufacturer information regarding the supply of the antibody used in the kit. A change in the lot number of antibody does not necessarily mean a change in the antibody pool, but often only the preparation of a new dilution from the same antibody pool. Manufacturers should also provide sufficient notification when the antibody pool is changed so that the laboratory has adequate time to re-evaluate the modified kit.

Finally, inform clinicians attending neonatal patients (primarily cardiologists) of the interference caused by DLIS in immunoassays for digoxin and urge them to obtain a serum sample before administering digoxin to infants younger than two months. If the pretreatment sample contains >0.2 µg/L of apparent digoxin (DLIS), the validity of the assay for accurately quantifying the drug in the infant is questionable. Conversely, a value <0.2 µg/L indicates that DLIS will probably not interfere with the accuracy of the assay. I base this conclusion on results from previous studies in which serial changes in DLIS were monitored in
neonates during the first two postnatal weeks (1-3). These studies, involving immunoassay kits having strong reactivity to DLIS, showed that the initial concentration of DLIS detected was ≈0.3 μg/L in patients whose DLIS concentration increased temporally. Similar studies, such as reported recently (7), need to be performed with kits having weak reactivity to DLIS, to confirm the validity of this cutoff-value approach.

I thank the manufacturers of the immunoassay kits for generously providing the kits for this study and the medical technologist of the chemistry laboratory for performing the analysis.

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Robert C. McCarthy
Dept. of Pathol.
The Children's Hospital
Denver, CO 80218

Simpler Measurement of Albumin in Urine or Plasma

To the Editor:

Fielding et al. (1) recently published an enzyme immunoassay method for measuring human albumin in concentrations of 3 to 1000 μg/L. We have developed a simpler "single-sandwich" technique, in which antibody of the same species is used both for binding and for detection of the albumin. The following procedure was adopted:

1. Incubate microtiter plates overnight at 4 °C with each well containing 100 μL of goat antihuman albumin (Cappell Laboratories, Cochranville, PA) diluted 1:1000 in costing buffer (0.5 mmol/L carbonate/bicarbonate buffer, pH 9.6).

2. Empty the wells and wash three times with diluent buffer (50 mmol/L Tris HCl, pH 8.0, containing 500 μL of Tween 20 surfactant per liter).

3. Incubate the plates for 1 h at room temperature with each well containing 100 μL of albumin standard or sample (urine or plasma) in diluent buffer, then empty and wash as in step 2.

4. Incubate the plates for 1 h at room temperature with each well containing 100 μL of goat antihuman albumin IgG conjugated with peroxidase (Cappell Laboratories) diluted 1:2000 in diluent buffer; empty and wash as in step 2.

5. Incubate the plates with each well containing 100 μL of the peroxidase substrate 3,3',5,5'-tetramethylbenzidine, which is prepared by dissolving 1 mg of tetramethylbenzidine in 1 mL of dimethyl sulfoxide and adding it slowly to 100 mL of 0.1 mmol/L sodium acetate/citric acid buffer, pH 6.0, then immediately before use, adding sufficient H₂O₂ to give a final concentration of 1.3 mmol/L. In contrast to o-phenylenediamine, tetramethylbenzidine need not be incubated in the dark.

6. Stop the peroxidase reaction after exactly 10 min by adding 50 μL of 2 mol/L H₂SO₄; the blue color changes to yellow.

7. Measure the absorbance of well contents at 450 nm in a microtiter plate reader.

We assessed the characteristics of the assay with standard curves of 10 albumin concentrations, measured in triplicate. The lowest concentration that differed from zero readings by more than 2 SD was 5 μg/L. Nonspecific binding was always <2% and usually <1%. The optimum working range of the standard curve was between 5 and 300 μg/L. From the precision profile the error of dose estimates was <18% over the range 5 to 300 μg/L and <8% between 25 and 100 μg/L.

The reproducibility of the assay was satisfactory: within-run variation was estimated by assaying two urine samples, with albumin concentrations of 18.5 and 8.5 mg/L, respectively. Each was assayed 18 times, given a CV of 4.9%. The between-run precision of the enzyme immunoassay was 8.9% as calculated from 12 estimates on successive days of the albumin concentration in a human urine sample (mean 600, SD 53.4 μg/L). No cross reaction with human IgG (1 mg/L), transferrin (1 mg/L) or β₂-microglobulin (500 μg/L) was detectable.

Analytical recovery of human albumin added to undiluted urine (25, 50, and 100 μg/L) was 94 to 115%.

To test for parallelism with the standard curve, we assayed four urine samples at four dilutions (1:80, 1:160, 1:320, and 1:640), in triplicate. The standard curve, linearized by logit transformation, was compared with the lines obtained for the diluted urines. In each case the points were distributed at random within the 95% confidence limits of the linearized standard curve.

The observed absence of cross reactivity implies that plasma albumin concentrations can also be measured. Plasma samples diluted 10⁶ to 10⁷-fold were suitable.

The rate of renal albumin excretion in nine healthy men and five women, ages 21 to 62 years, ranged from 3.0 to 11.3 mg/24 h (2.1 to 10.6 mg/min). Overnight excretion rates ranged from 1.6 to 6.4 μg/min (n = 21). These results agree well with ranges measured by various other methods (2, 4-7).

Clearly, human albumin bound onto adsorbed goat antibody still possesses a substantial population of free antigenic determinants for the binding of a second goat antibody. This provided the basis of a simple assay with fewer sources of error, and with adequate sensitivity, discrimination, and specificity.

The reduction of antibody layers from three to two was associated with a working range that—although somewhat higher and narrower than the range reported in 1—was still two to three orders of magnitude below the lowest albumin concentrations found in human urine.

Other technical advantages include the fact that both the coating antiseraum and the conjugated IgG were raised in the same species, so that immune reactions between them were highly unlikely; hence the addition of inactivated serum could be omitted. Nonspecific binding was very low, even when bovine serum albumin was omitted. Possible errors due to cross reactivity with this albumin could thus be avoided.

The advantages of tetramethylbenzidine are that it can be used in daylight, and that it is claimed to be noncarcinogenic and nonmutagenic (3).

References