

mL of water followed by 4000 mL of 0.25M sodium phosphate buffer, pH 7.0. The decanted resin was resuspended in 1000 mL of the ouabain-BSA solution (from above), allowed to mix for one hour at 25° C. and then mixed with 0.66 g of sodium cyanoborohydride. After 72 hours the resin was washed thoroughly with 4000 mL of 0.1% sodium dodecyl sulfate in water, 12 L of distilled water, and then 4000 mL of 0.15M sodium phosphate buffer, pH 7.1. The final resin was slurry packed into small columns (0.5 cm×7 cm) for use in automated analysis in the DuPont aca® discrete clinical analyzer.

(3) Digoxin-BSA was prepared by dissolving 1.25 g of digoxin in 75 mL of ethanol and then combining this solution with 150 mL of water containing 1.83 g of sodium metaperiodate. After two hours at 25° C. with stirring, the oxidation was stopped by passing the mixture through a bed (100 mL) of Dowex (1-X8) anion exchange resin. The eluate was combined with a solution of bovine serum albumin (2.5 g) dissolved in 0.1M sodium phosphate, pH 8.5. Sodium cyanoborohydride (0.24 g) was then added and the mixture was mixed for 48 hours at 25° C. Free unconjugated digoxin was removed by dialysis against running distilled water for two days and then against 20 volumes of 0.015M sodium phosphate buffer, pH 7.0 at 4° C.

(4) Coupling of Digoxin-BSA to Sephadex G-10

Sephadex G-10 (50 g) was allowed to swell in 250 mL of distilled water for >1 hour. Resin fines were removed by decanting and resuspension. The resin was then oxidized by resuspension in 250 mL of water containing 5 g of dissolved sodium metaperiodate. After 10 minutes, the resin was washed with 5×250 mL of water followed by 1000 mL of 0.1M sodium phosphate buffer, pH 8.5. The decanted resin (~125 mL settled bed volume) was slurried in 125 mL of 0.1M sodium phosphate buffer, pH 8.5 containing 125 mg of sodium cyanoborohydride. After 24 hours of constant mixing the resin was washed with 3×500 mL of water, 500 mL of 0.15M sodium phosphate, pH 7.8, and then resuspended in 125 mL of 0.15M sodium phosphate, pH 8.5 at 4° C. Acetic anhydride (1.25 mL) was added to the slurry and allowed to react for 30 minutes at 4° C. with mixing. The resin was washed thoroughly with 1 L of 0.5M NaCl, 4 L of distilled water, and 2 L of 0.15M sodium phosphate, pH 7.1. The final resin was slurry packed into small columns (0.5 cm×8 cm) designed for placement into the headers of aca® discrete clinical analyzer test packs.

C. Comparison of the Digoxin-BSA-Sephadex and Ouabain-BSA-Sephadex Resins Using a Divalent Antibody Enzyme Conjugate.

Both resin lots were compared under identical conditions. F(ab')₂-β-galactosidase, synthesized in (A) above, (2.6 picomoles in 200 μL of buffer) was added to 200 μL of normal human serum calibrators containing various amounts of digoxin (0, 0.5, 1.5, 3.5, and 5.0 ng/mL). After a 10 minute incubation period at 25° C., the entire antibody-sample mixture was automatically injected into an aca® discrete clinical analyzer test pack and eluted through the column in the pack header. Sample was followed by 2 mL of 0.15M sodium phosphate, pH 7.8. The column flow rate was 34 μL/sec. The pack was then filled at needle position 2 (which bypasses the column) with an additional 2.6 mL of water. ONPG was released from breaker/mixer II approximately 3.5 minutes later. Enzymatic activity was measured at 405 nm 29 and 46 sec after addition of substrate.

FIG. 1 and Table IV compares the performance of the two optimized digoxin-BSA-Sephadex and ouabain-BSA-Sephadex resins in terms of background and slope sensitivity (FIG. 1) and precision (Table IV).

While both resin lots allowed linear dose responses with the F(ab')₂-β-galactosidase conjugate, the ouabain-resin exhibited both a lower background (32% lower) and a greater slope sensitivity (47% greater). Both a lower background and a higher slope sensitivity are preferred for better assay performance and accuracy. In addition, the assay precision was markedly better for the ouabain resin than for the digoxin resin (Table IV). Individual pack assays were run exactly as described above at two digoxin levels, 0.5 ng/mL and 1.5 ng/mL. At least 12 packs were run at each drug concentration and the mean (X), standard deviation (S.D.) and the percent coefficient of variation (C.V.) was determined.

TABLE IV

COMPARISON OF OUABAIN AND DIGOXIN RESINS FOR PRECISION		
	Ouabain Column	Digoxin Column
	<u>0.5 ng/mL Digoxin</u>	
X (ng/mL)	0.47	0.43
S.D. (ng/mL)	0.04	0.07
% C.V. (ng/mL)	8.1	14.2
	<u>1.5 ng/mL Digoxin</u>	
X (ng/mL)	1.52	1.54
S.D. (ng/mL)	0.05	0.20
% C.V. (ng/mL)	3.0	13.1

We claim:

1. A noncompetitive immunoassay for the measurement of digoxin in a test sample, said assay comprising the steps of:

(a) forming a reaction mixture by contacting a molar excess of labeled monovalent or divalent anti-digoxin antibody with the test sample, whereby a fraction of said antibody forms a complex with the digoxin and a fraction remains free; and

(b) contacting the reaction mixture with a solid phase having ouabain immobilized thereon, the ouabain being present in an amount capable of binding all of the free antibody, whereby the free antibody is bound to the solid phase; and

(c) measuring the amount of complex which elutes from the solid phase by measuring the activity of the label.

2. The immunoassay of claim 1 wherein the label is an enzyme, radioisotope, chromophore or fluorophore.

3. The immunoassay of claim 1 wherein the monovalent antibody is an Fab fragment or Fab' fragment.

4. The immunoassay of claim 1 wherein the divalent antibody is an F(ab')₂ fragment.

5. The immunoassay of claim 1 wherein the solid phase is beaded agarose, beaded dextran, polyacrylamide or glass.

6. The immunoassay of claim 5 wherein the solid phase is packed in a column.

7. The immunoassay of claim 1 wherein the solid phase is beaded dextran, the monovalent antibody is an Fab' fragment and the label is β-galactosidase.

8. The immunoassay of claim 1 wherein the solid phase is beaded dextran, the divalent antibody is an F(ab')₂ fragment and the label is β-galactosidase.

9. The immunoassay of claim 7 wherein the measurement step (c) is performed by contacting the eluted β-galactosidase labeled, Fab' anti-digoxin antibody/-