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Iodine-125-Digoxin Radioimmunoassay: Comparison of Commercial Kits

Danielle J. Battaglia and Michael L. Cianci

Oscar B. Hunter Memorial Laboratory, Washington, D.C.

Iodine-125-digoxin radioimmunoassay kits available from Abbott Diagnostics (AD), Dade Division (D), Schwarz/Mann (SM), and Clinical Assays (CA) were evaluated with respect to assay quality. The kit accuracies did not differ significantly at 2.0 ng/ml and the interassay coefficients of variation ranged from 9% (AD) to 21.4% (CA). The accuracy for all kits above 4 ng/ml is questionable, and since serum-dilution values correlated well with undiluted serum values, the dilution method of dose quantitation is preferable for levels above 4 ng/ml. Although all the kits were adequate for evaluating digoxin at the 2 ng/ml level, the Abbott kit seems to be of slightly better quality.

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The current availability of radioiodinated derivatives of digoxin has made possible convenient specific assays for serum digoxin designed to aid the clinician in cardiac therapy. Digoxin radioimmunoassay has become the most extensively used radioassay, and several kits are now available, the quality of which is predetermined by the manufacturers. Here we compare the precision, accuracy, and sensitivity of four such kits and discuss the antiserum specificity and labeled derivative used. A better understanding of the observed and potential quality of commercial kits is needed.

MATERIALS AND METHODS

The kits tested were the ^{125}I-digoxin RIA kits available from Abbott Diagnostics (AD), Dade Division (D), Schwarz/Mann (SM), and Clinical Assays (CA). The digoxin radioimmunoassay procedure is essentially the same for all these kits except for the technique used to separate antibody-bound from free digoxin. The SM kit uses Dextran-coated charcoal to adsorb unbound digoxin physiochemically. Polyethylene glycol precipitates the antibody-bound fraction in the AD kit. The D kit uses a second antibody to form a precipitating complex of antibody–digoxin–antibody and centrifugation removes the unbound digoxin before counting. The CA kit, finally, is supplied with antibody-coated tubes, and separation of the two fractions is completed by aspiration and buffer wash.

Unknown sera were evaluated according to each manufacturers' protocol, using the suggested fitting techniques for the dose–response curves (Fig. 1). A digoxin primary standard (2.0 ng/ml) was prepared in 5% ethanol by dilution of a stock solution of Lanoxin (0.05 mg/ml), stored under refrigeration (2–8°C). Digoxin-free serum was used to simulate serum protein levels in blanks, primary standards, and dilution samples. In this way, interferences from abnormal protein levels were normalized. All standards and samples were analyzed in duplicate, and the mean percent bound-to-total activity (%B/T) was reported. Rodbard's statistical methods (*1*) were used to evaluate intra- and interassay precision. Assay sensitivity (i.e., the smallest assayable dose) was determined graphically by extrapolation of the dose–response curve (*2*). The relative ability to distinguish small dose differences across the measurable

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For reprints contact: D. J. Battaglia, Nuclear Medicine Dept., Oscar B. Hunter Memorial Laboratory, 1835 Eye St. NW, Suite 808, Washington, DC 20006.

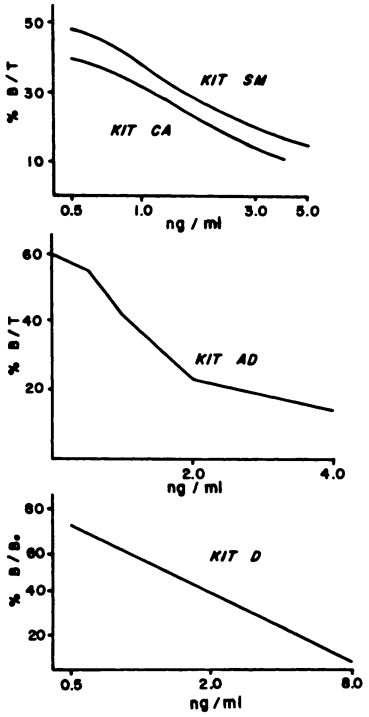

FIG. 1. Typical dose–response curves illustrating suggested curve-fitting techniques. Schwartz/Mann and Clinical Assays: semi-log, best-fit curve. Abbott Diagnostics: linear abscissa, straight lines connecting standard points. Dade Division: Logit–log transformation, best-fit straight line.

range was evaluated by an index λ, defined as the standard deviation of the quantity %B/T (from normalized dose–response curves) divided by the absolute slope of the curve at various dose levels (*3*). The numerical value of λ decreases as the assay's sensitivity to small dose differences increases.

RESULTS AND DISCUSSION

Table 1 lists the parameters of experimental assay performance. Precision and reproducibility were comparable for all kits except the CA kit, which borders on the acceptable 10% intra-assay and 20% interassay limits. The SM kit was found to measure the smallest dose of digoxin. The D kit showed good dilution correlation and the lowest intra-assay variation, but the AD kit performed better in four of the six parameters investigated.

TABLE 1. PARAMETERS OF ASSAY PERFORMANCE

Parameter	Abbott Diagnostics	Dade Division	Schwartz/Mann	Clinical Assays
Coefficient of variation (%):				
intra-assay (16 df)	4.0	3.5	4.8	9.5
interassay (3 df)	9.0	10.0	11.9	21.4
Average slope	15.7	9.9	11.8	7.1
Dilution correlation coefficient r	0.99	0.99	0.98	0.98
Sensitivity index λ	0.09	0.23	0.20	0.21
Smallest detectable dose (ng/ml)	0.25	0.20	0.10	0.30

The greatest differences among the kits showed up in their ability to distinguish small dose differences. In this instance, the AD kit was measurably superior, with the smallest sensitivity index λ. Dilution of sera does not appear to alter the sensitivity for any of the assay kits studied. The slope for all kits decreased in ranges above 4 ng/ml, indicating decreased accuracy in that range. For doses above 4 ng/ml, the dilution method of quantitation would seem to be more accurate, and therefore preferable, in all cases.

While specificity was not measured experimentally, manufacturer data give the following antiserum cross-reactivities:

1. for digitoxin as 7.4% (AD), 8.7% (SM), 5% (CA), and 25% (D);
2. for desanoside as 100% (CA and SM);
3. for digitoxigenin as 7.0% (AD);
4. for several other steroids as 0.02% with substantial interference from spironolactone and prednisone (CA).

No specific data are available regarding the chemical formula of the labeled derivative used in the D kit. However, the CA kit used a derivative of digoxigenin; AD, a digoxin triamine analog; and SM, 3-o-succinyl digoxigenin tryrosine.

The question of protein interference is of recent and substantial concern (*4–6*). The Schwartz/Mann kit states simply that high albumin levels in serum may cause falsely low digoxin estimates. The Dade kit makes no mention of the question. Abbott Diagnostics suggests an ethanol extraction technique for samples with possible protein interference, and Clinical Assays presents data showing that an additive reduces protein interference. Although the dilution studies do not indicate any problem in this area, no experiments were specifically designed to investigate the protein-interference question. Until more information is available as to the exact mechanism of the suggested digoxin-binding interference, little can be said as to the efficacy of digoxin assays in this regard.

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