Case of the Quarter

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A commercially available ^{125}I-digoxin radioimmunoassay kit had been used routinely for 2 years for determining serum digoxin levels. Suddenly, parameters recorded daily, as a means of quality control, were observed to change. Since no new techniques in handling the kit had been introduced and preparation of kit reagents was confirmed to be consistent with the manufacturer's protocol, the assay itself became suspect. Using a representative time period prior to the observed change, comparisons of data were made from complete daily records. Investigating several parameters in light of radioimmunoassay principles, a probable cause was determined.

Materials and Methods

The digoxin kit used required a 30-min incubation of ^{125}I-digoxin derivative with patient serum and antibody followed by a 5-min adsorption of unbound digoxin onto dextran-coated charcoal (DCC), centrifugation at 4,000 *g* for 10 min at 40°C, decantation, and assessment of radioactivity in the antibody-bound fraction. Computerized data handling and standard curve-fitting techniques were employed. The quality control program, including records of percent total binding (%B/T), percent bound of the 0 ng/ml standard (%B_0), and high and low digoxin level reference serums assayed daily, aided in determining the validity of each run. Toxic ranges were considered to be above 2.5 ng/ml with a minimum detectable dose of 0.4 ng/ml.

Observations

In mid-November 1974, a drop in %B/T across the assay range was noted. A mean %B_0 of 66 for the period immediately preceeding, October 20 to November 20, fell to an average 53% from November 20 to December 20. An increase in dose estimates for the low and high control pools accompanied this change in binding capacity (Table 1). The increase itself appeared more pronounced for the high control and might even perhaps be considered negligible for the low control. Most significant, however, is the loss of acceptable interassay precision. The percent coefficient of variation (%CV) was seen to double for the high control from November to December and increase significantly for the low control (Table 1). Comparison of representative dose response curves for these two periods clearly indicates a lack of parallelism with differences in slope and a decrease in total %B/T over the 0–5 ng/ml dose range of 7.0% from October/November to November/December (Fig. 1).

TABLE 1. Observed Changes in ^{125}I-Digoxin Assay

Quality control parameters	October to November	November to December
% B_0	66 ± 3.1	53 ± 4.0
% B/T (0.5 ng/ml)	51.7	44.7
Digoxin control (ng/ml)	1.2 ± 0.13	1.3 ± 0.21
% CV	10.8	16.2
Digoxin control (ng/ml)	3.4 ± 0.24	3.9 ± 0.56
% CV	7.1	14.4

All values expressed as an average ± 1 s.d.

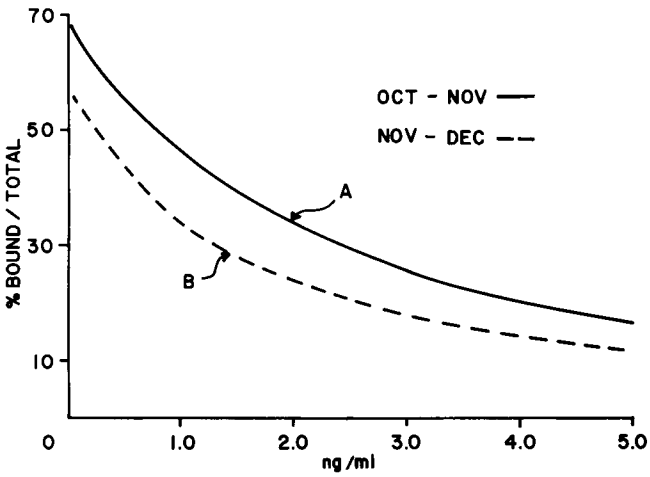

FIG. 1. Digoxin dose response curves for periods October 20 to November 20 and November 20 to December 20. A and B represent dose levels at which 50% of antibody-bound ^{125}I-digoxin derivative is displaced by digoxin.

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The possible causes of these changes include:

1. Decreased specific activity of ^{125}I-digoxin derivative.
2. Increased concentration of DCC.
3. Decreased antibody titer.
4. Increased standard concentration.
5. Decreased specificity of antibody.

Discussion

As the antibody is diluted, initial slope and sensitivity are increased (*1*) with a concomitant decrease in these two parameters occurring in the higher assay range (*2*). While from October to November half of the labeled antigen was displaced by an addition of 2.0 ng/ml, from November to December only 1.4 ng/ml was necessary to displace 50% of the ^{125}I-digoxin, thereby indicating a greater dose response curve sensitivity in the lower assay range. Furthermore, the observed loss of interassay precision and fallaciously high dose estimates evident especially at higher dose levels are suggestive of decreased slope and sensitivity in the higher assay range. Consequently, it would appear that the observed changes in assay parameters are best explained by answer 3: decreased antibody titer.

A decrease in ^{125}I-digoxin derivative specific activity would cause the dose response curve slope to decrease particularly in the low range (*3*). In this case, slope in the low range is seen to increase slightly. An increased concentration of DCC would result in consistently lower %B/T across the entire dose range, which did not occur; moreover, it would not explain the problems of irreproducibility. Changes in concentration of the supplied standard, shifting the dose response curve, would account for the increase observed in control values but would not explain the loss of precision. Decreased specificity of antibody reflected in lower dose estimates of controls would not have a significant effect on interassay variance (*4*).

References

1. Potts JT, Sherwood LM, O'Riordan JLH, et al: Radioimmunoassay of polypeptide hormones. *Adv Intern Med* 13: 183, 1967

2. Goldsmith SJ: Radioimmunoassay: Review of basic principles. *Nucl Med* 5: 125–152, 1975

3. Yalow RS, Berson SA: Radioimmunoassays. In *Statistics in Endocrinology,* McArthur JW, Colton T, eds, Cambridge, Mass, MIT Press, 1970, p 378

4. Hogan ED, Riley WJ: Radioimmunoassay of plasma digoxin with use of iodinated tracer. *Clin Chem* 19: 187–190, 1973