Radioimmunoassay

Radioimmunoassay Kit Evaluation in the Busy Nuclear Medicine Laboratory

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With the constant influx of new radioassay kits appearing on the market, the nuclear medicine technologist is continually evaluating products to be incorporated as a routine procedure in that laboratory. Although elaborate procedures exist for extensive evaluation of kits, the average nuclear medicine laboratory lacks time and personnel to perform in-depth investigative studies. This article proposes an organized, time-saving, and reliable approach to kit evaluation, adaptable to a busy clinical nuclear medicine department.

Every nuclear medicine technologist is bombarded by manufacturer's representatives who expound on the superior qualities of their products and who urge the technologist to evaluate their kits. The optimum procedure would be to investigate each one and then make a selection based on all the accumulated data. Of course, this is impossible for the average nuclear medicine laboratory owing to lack of time and personnel. Other means of discriminative selection must be used. The following discussion includes criteria for selection of the kit to be evaluated based on protocol appraisal, survey comparison, and peer consultation. Subsequent evaluation utilizing precision, accuracy, recovery, dilution, cross-reactivity, and inhibition studies is also discussed.

Protocol Appraisal

Request package inserts from all manufacturers making a kit for the assay of interest. Study the brochures and eliminate those procedures requiring unavailable equipment. Compare the methods for ease of performance, technical time required, special reagent preparation, price per tube to include standards and quality control samples, and other parameters related to individual circumstances. Note the total time required to perform the study from receipt of the specimen to calculation of the test result. Beware of oversimplified

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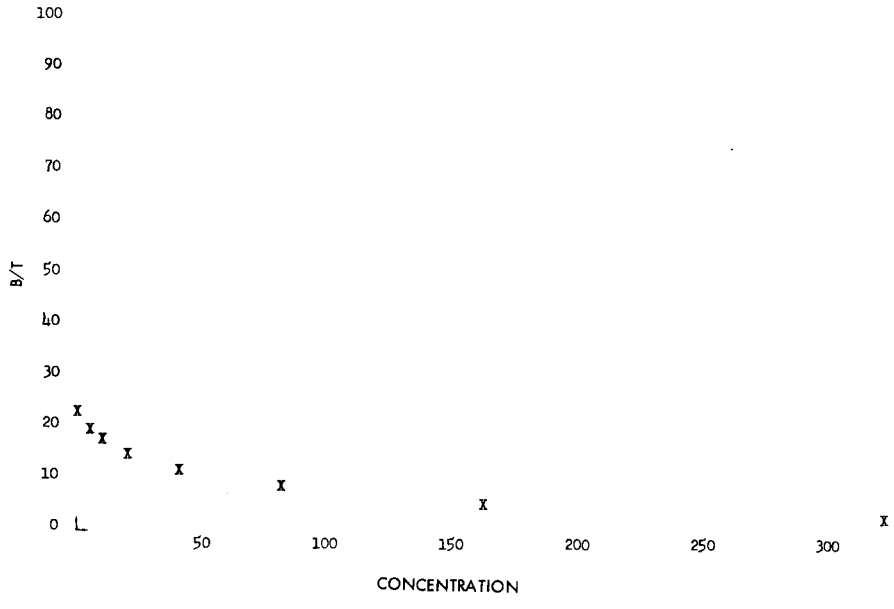

FIG. 1. Data in B/T format exhibiting low maximum binding and poor slope when displayed on linear paper.

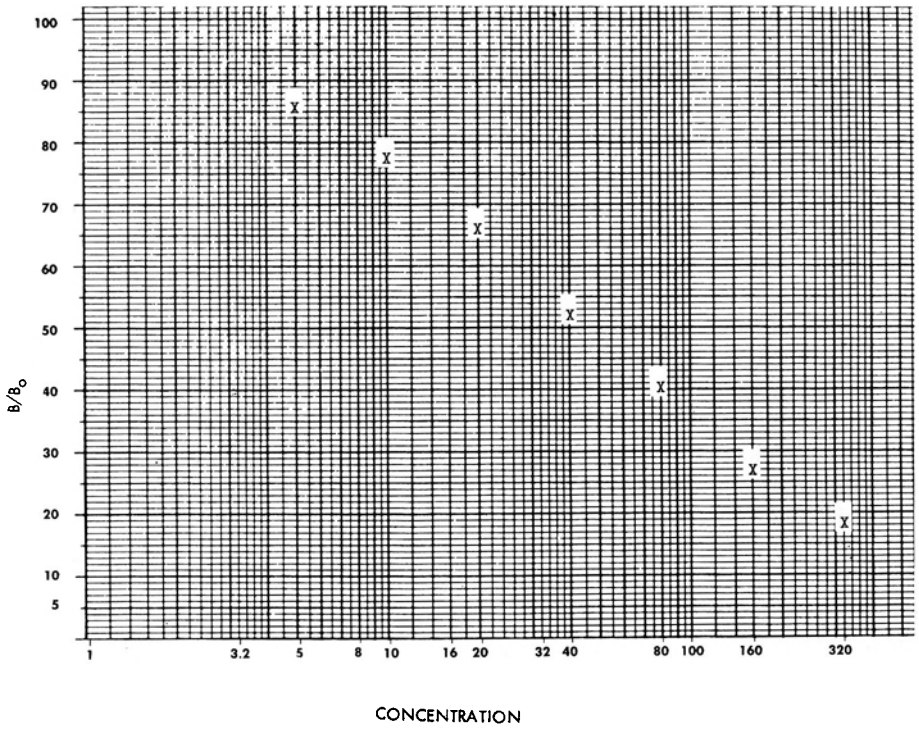

FIG. 2. B/B_0 plot on semilog paper of same raw data as in Fig. 1.

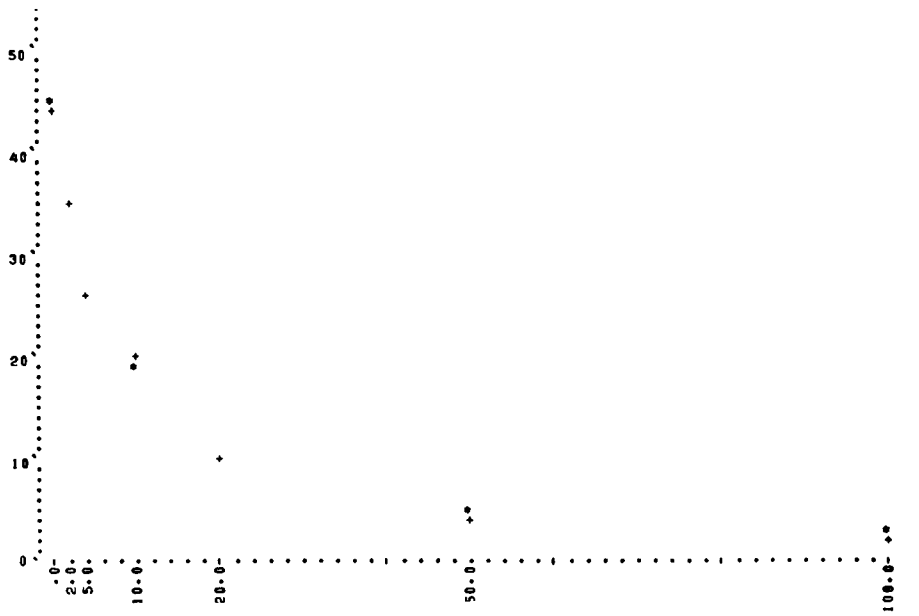

FIG. 3. Data exhibiting adequate binding and slope when plotted on linear paper.

procedures which may sacrifice precision and accuracy for ease and speed.

Most manufacturers supply a set of sample data in the protocol to demonstrate the calculation and plotting of the standard curve. From these figures, determine the maximum percent bound by dividing the net count rate of the zero standard tube (B_0) by the net count rate of the total activity (T) added into the system. This percentage (B_0/T) should be approximately 50%. If not, the sensitivity of the assay is suspect *(1)*. Take this operation further and divide the net count rate of each standard point on the curve by the net count rate of the total tube (B/T). Some protocols may suggest reducing the data in this manner while others may utilize a B/B_0 in which the net count rate of each standard point is divided by the net count rate of the maximum binding or zero standard tube. If so, transform this data into a B/T format. Plot B/T versus concentration on linear graph

paper. The resulting curve will indicate the sensitivity of the assay over the stated concentration range of the standards. A sharp slope provides greater sensitivity *(1)*. In Fig. 1, note that the slope is virtually flat and that the maximum binding is very low. The manufacturer suggests a B/B_0 plot on two- or three-cycle semilog paper (Fig. 2) to improve the appearance and functionality of the curve. It is not the intention here to imply that all manufacturers who suggest a B/B_0 plot of their kit data have something to hide; it is merely a check to assure that they do not. In fact, a B/B_0 plot in the case of hand extrapolation is often useful in minimizing technical error, and while a B/T is not always the preferred method of data reduction, it is useful when employed in this fashion to demonstrate sensitivity. Figure 3 shows an arithmetic plot of data with adequate binding and a good slope in the initial part of the curve. Sensitivity is lost, however, after a concentration of approximately 50 μU/ml. A result exceeding this limit should be reported out as "greater than 50 μU/ml" or should be diluted if an exact concentration is required. Determine whether it will be necessary to make frequent time-consuming dilutions on patient samples to produce meaningful results from the method in question.

Survey Comparison

External quality assurance programs such as the one conducted by the College of American Pathologists (CAP) may give a useful indication of kit performance in the hands of various laboratories. On a subscription basis, the CAP distributes lyophilized serum pools containing various constituents of interest to nuclear medicine laboratories. Results from all participants are tabulated and categorized according to constituent and method of determination. A mean, standard deviation, and coefficient of variation are computed for each test method with 20 responses. Inspection of these data assists the technologist in eliminating methods which significantly deviate from the mean as determined by the other methods or whose coefficient of variation is inordinately large.

Peer Consultation

Check with other laboratories performing the assay in question. Have the clinicians seemed satisfied with their results? Are they investigating another kit for the same determination, and if so, why? Do they have difficulties with delivery of the material? Is there a representative of the company who can easily be contacted to solve a technical problem? Have they modified the method in any way to improve performance or technique?

Assimilate and inspect all information gathered thus far. Make a list of the pros and cons for each kit and determine which method will be the most suitable candidate for further evaluation.

Method of Evaluation

Having selected a protocol for further testing, contact the manufacturer for sufficient gratis material to perform the evaluation. Upon receipt, store and reconstitute the reagents exactly as stated in the protocol. Run the standard curve several times for familiarization. Ascertain that the pipettes to be used are reproducible to a coefficient of variation of less than 3%. Several types of studies should then be conducted on the kit, including precision (within run and between run), accuracy, recovery or dilution, crossreactivity, and inhibition.

Precision

Purchase a sufficient quantity of the constituent to be analyzed and dilute it to the concentration of three standard curve points (low, normal, and high) in the kit. For example, if a digoxin method is being evaluated, purchase some pure digoxin. Observe the concentration in nanograms per milliliter on one of the standard vials in the kit. Determine by checking the *Merck Index* in what solution the digoxin is soluble. Also, note any precautions for special handling in the event that the material is hydroscopic, unstable, or temperature or light sensitive. Weigh out on an accurate balance a small amount and dissolve it in the appropriate solution (e.g., methanol) in a volumetric flask. Be certain that the material completely dissolves, and then determine the concentration of the solution based on the weight of the material and the volume of the solution. Serial dilutions may be necessary to bring the concentration down to the nanogram range. Prepare these dilutions with accurate pipettes and volumetric glassware to minimize errors. Add the appropriate volume either to digoxin-free serum in a large sample to be aliquoted and frozen or to individual digoxin-free samples at the time of the study. If the latter method is used, store the alcoholic working solution in an evacuated vial and draw out the daily requirement with a syringe to prevent evaporation and resulting concentration change.

If the component to be tested is a normal constituent of serum and component-free serum is not available, commercially available control serum containing the unknown is the next best choice for precision testing.

TABLE 1. Recovery Ability of Two Digoxin Kits at Different Levels

	Digoxin added (ng/ml)	Digoxin recovered (ng/ml)	Percent recovery
Kit I	0.54	0.34	63.0
	0.99	0.83	83.8
	3.96	3.59	90.7
	5.94	5.01	84.5
Kit II	0.54	0.60	110.0
	0.99	0.95	95.9
	3.96	3.90	98.9
	5.94	5.80	97.7

TABLE 2. Flowsheet for Evaluation Procedure

	Method A: 1. Component-free serum available 2. Pure component available 3. Use three levels of spiked serum	Method B: 1. Component-free serum not available 2. Pure component available 3. Use three levels of commercial serum	Method C: 1. Component-free serum not available 2. Pure component not available 3. Use three levels of commercial serum
Day 1*:	Precision (within-run) and recovery at low level: 15 component free 15 low samples Precision (between-run): 3 normal 3 high samples	Precision (within-run) at low level: 15 samples Precision (between-run): 3 normal 3 high samples	Same as method B
Day 2:	Precision (within-run) and recovery at normal and high levels: 15 normal 15 high samples Precision (between-run): 3 low samples	Same as method A	Same as method A
Day 3:	Precision (between-run): 3 low 3 normal 3 high samples	Same precision study as method A, recovery using pure component and low-level commercial serum: 3 levels in triplicate	Same precision study as method A, dilution of elevated serum by 50% and 25% each in triplicate
Day 4:	Precision (between-run): 3 low 3 normal 3 high samples Crossreactivity: 3 normal level of crossreactant 3 elevated level of crossreactant	Same as method A	Same as method A
Day 5:	Precision (between-run): 3 low 3 normal 3 high samples Inhibition: 3 normal level of inhibitor 3 elevated level of inhibitor	Same as method A	Same as method A

**A standard curve with all points in duplicate must accompany each daily run.*

However, a working solution of the unknown should still be made as described above for use in recovery studies.

Determine the values of 15 of each of the test samples in one run and calculate the mean, standard deviation, and coefficient of variation within run of each group *(2)*. Test three samples from these same pools on at least five successive days to find between-run variability. Note whether the reproducibility thus determined compares favorably with the manufacturer's advertised data. If this information is not published in the protocol, call the company and ask for their expected coefficients of variation for interassay and intraassay precision at the levels of interest.

Another method for calculation of within- and between-assay variance is by the use of duplicates and triplicates in each of several assays. This procedure and more advanced statistical data manipulation may be found in the cited reference of Rodbard *(3)*.

Accuracy and Recovery

If the method being tested produces favorable results in the precision study, additional types of studies are in order. Since few actual reference standards are available for constituents tested by radioassay, other methods to assess accuracy must be used. At the same time that the precision is being determined as described above, recovery is being checked with the pure dilution. The approximate range of the commercial preparation is also an indication of the accuracy of the method. Please note, however, that lyophilized commercial serum should be used only as a guide since the assessment of true value may be markedly different for different methods *(2)*.

Recovery studies indicate that a method can actually measure weighed amounts of constituent added at various dose levels. To perform recovery studies on serum that is not unknown free, spike the base material with known amounts of the dilution of pure chemical. Analyze in triplicate in a single run the base material alone and spiked levels covering a low, normal, and high concentration range *(2, 3)*. The results should show a rise equal to the amount of spike added in all ranges. Percent recovery is determined by dividing the concentration recovered by the concentration added, multiplied by 100. Table 1 shows recovery ability of two digoxin kits at four different levels.

Dilution

When the pure constituent is not available for recovery studies, when it is extremely difficult to handle due to deterioration, or when the solution in which it is soluble is not compatible with body fluids, dilution studies are a useful alternative to assess linearity of a method. Use kit buffer, constituent-free serum, or physiologic levels of human or bovine albumin as a diluent. Serum components may be necessary for optimum assay performance in dilution exercises; each assay should be carefully scrutinized in this regard. Test the samples in triplicate in a single run for at least three levels: 100% (no dilution), 50%, and 25%. Observe whether the resulting values are the proper multiples of each other.

Crossreactivity and Inhibition Studies

Further testing to ascertain crossreactive or inhibitive effects by given substances may be desirable. A base serum containing the material to be assayed is spiked with various physiologic levels of the substance which has the potential of interfering. Be certain that the suspected crossreactant spike is free from impurities which may cause further interference, thus generating false conclusions. The base and spikes are tested in a single run in triplicate and checked for alterations in results. For example, if spironolactone was suspected of interfering in a digoxin assay, several checks should be made. First, a digoxin-free pool spiked with an elevated physiologic level of spironolactone should be tested. If the result is greater than 0.0 ng/ml, the substance crossreacts. If not, then samples containing a selected level of digoxin (e.g., 2.0 ng/ml) should be run. Simultaneously, samples of that same pool spiked with elevated levels of spironolactone should also be tested. If a difference greater than the intraassay variability of the test exists between the two sets of samples, with the spiked sample being depressed, spironolactone would be considered an inhibitor.

Conclusion

Assessment of the accumulated data by the laboratory director may result in adopting the method for routine use or in rejection and subsequent evaluation of another kit for the same component. Although these procedures for evaluation seem cumbersome, many steps can be combined in an organized fashion to decrease the number of runs required and still fulfill the criteria for evaluation (Table 2).

The technologist must never accept a kit at face value. Blindly believing the manufacturer and running patient samples on an untried kit can lead to disastrous consequences. Complacent acceptance of published normal values can also create problems. Each laboratory should establish its own normal range by adequate population sampling under routine test conditions *(4)*.

Thorough investigation into each kit incorporated into routine use will maintain the credibility and integrity of results reported from the conscientious nuclear medicine laboratory.

References

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