

Quality Control in Radioimmunoassay

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An active quality control program is an absolute necessity to assure precision and accuracy of radioimmunoassay results. Guidelines for the initiation of an intralaboratory quality control program include a discussion of normal values and their establishment, preparation of a quality control pool, preparation and use of control charts, and the use of duplicates. The value of external surveys is also reviewed.

The purpose of this article is to concisely outline for the nuclear medicine technologist the necessity for and the initiation of a radioimmunoassay quality control program.

Competent laboratory work consists of accurate, fast results returned to the proper patient. Quality consciousness is a total environment in the laboratory for which the director is responsible. His honest desire to give good service should extend to his personnel. Each employee must participate to make it work: glassware washers must appreciate their importance to a study; typists must be aware that accuracy is paramount (10-15% of laboratory errors are in transcription) (1).

Morale is good in an atmosphere charged with modest pride, but superior work is necessary to justify that pride. Quality control then contributes positively to the environment. It is a system of insuring precision of measurement as well as a method of getting the most accurate results from the assays performed. Quality control will not improve a poor method but it will detect systematic and random error. Both new methods under study and the reliability of a procedure under regular operating conditions can be evaluated quickly by establishing quality control limits. General precision of the laboratory is improved since the technologists become control conscious and therefore more careful. An adequate program indicates to the Chief Technologist which tests need improvement or close evaluation and gives the clinician confidence in the reliability of the laboratory and its analyses. Another benefit and perhaps the most concrete of all is the fulfillment of the requirement imposed by the Joint Commission on the Accreditation of Hospitals (Standard VI of Pathology and Standard III of Nuclear Medicine) for laboratories to have a functioning quality control program.

An adequate quality control program in the radioimmunoassay laboratory encompasses both internal and external evaluations. Internal quality control is that set of precision and accuracy checks instituted by the laboratory to monitor its own studies. This is accomplished by the preparation of a known sample pool, an aliquot of which is included in every series of studies performed. The numerical value of this quality control sample should fall within a predetermined range to testify to the validity of the entire run of patient samples. External quality control takes the form of proficiency surveys conducted by various interested groups in which the individual laboratory may participate. Much useful information is derived by subscription to such a survey.

Normal Values

Normal values for a given study should be established before a quality control program is instituted for that study. Quite obviously, an abnormal value cannot be identified until the normal range is defined. It is dangerous to blindly accept a manufacturer's suggested normal values without statistical evaluation of data in the individual laboratory. Environment, equipment, and patient population may vary significantly from laboratory to laboratory, thus affecting values. An example of unchallenged acceptance of a manufacturer's published normal values was apparent in the results of a proficiency survey conducted by the College of American Pathologists in 1972, in which approximately 200 laboratories participated. Each laboratory received lyophilized serum samples and was asked to perform digoxin determinations. Table 1 compares the results of tritiated and iodinated digoxin methods as compared to the expected values of different pools. The laboratories were further requested to classify the values they determined as therapeutic, toxic, or below therapeutic.

The fact that the two methods did not report the same absolute value is much less significant than the fact that the laboratories made very different interpretations of the pool status. Had normal values

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TABLE 1. 1972 Nuclear Medicine Quality Evaluation Program

	Pool 1	Pool 2	Pool 3	Pool 4	Pool 5	Pool 6
Mean iodinated value (ng/ml)	4.1	0.7	3.5	5.1	0.8	5.3
Mean tritiated value	2.4	0.3	1.9	4.0	0.2	4.3
Expected value	3.0	0	2.0	3.0-4.0	0	3.0-4.0
	Below therapeutic		Therapeutic		Toxic	
Pool 3						
Iodinated	0		16.7%		83.3%	
Tritiated	0		84.6%		15.4%	
Pool 5						
Iodinated	53.5%		42.9%		3.6%	
Tritiated	100 %		0		0	

TABLE 2. Calculating Mean, Standard Deviation, Variance, and Coefficient of Variation

$$\bar{x} = \frac{\sum x}{n}$$

$$s^2 = \frac{\sum (x - \bar{x})^2}{n - 1}$$

$$s.d. = \sqrt{\frac{\sum (x - \bar{x})^2}{n - 1}}$$

$$CV = \frac{100s}{\bar{x}}$$

s.d. = standard deviation
 s² = variance
 Σ = sum of
 x = individual value
 \bar{x} = mean
 n = number of tests
 CV = coefficient of variation

Test no.	Value (x)	Difference from average (x - \bar{x})	Squared difference from average (x - \bar{x}) ²
1	48	2.2	4.84
2	39	6.8	46.24
3	47	1.2	1.44
4	50	4.2	17.64
5	39	6.8	46.24
6	50	4.2	17.64
7	49	3.2	10.24
8	51	5.2	27.04
9	41	4.8	23.04
10	44	1.8	3.24
$\Sigma x = 458$			197.60
$\bar{x} = 45.8$			
$s^2 = \frac{197.60}{9} = 21.955$			
$s.d. = \sqrt{\frac{197.60}{9}} = \pm 4.69$			
$CV = \frac{4.69 \times 100}{45.8} = 10.2\%$			

been established independently by each of the laboratories using iodinated digoxin, they would not have incorrectly classified the test samples. On most occasions, however, manufacturer values do coincide with laboratory studies, and until a laboratory accumulates sufficient data to determine its own normal range, published values may be used and designated "tentative."

A familiarity with basic statistical terminology is necessary to understand the derivation of normal values. Table 2 defines and demonstrates the calculations

for the mean, standard deviation, variance, and coefficient of variation (1). Table 3 briefly states definitions of commonly used terms in quality control. The curve of normal distribution (Fig. 1) is the basis for most of our statistics since this distribution of measurements around a mean value shows that 68% of the population measured is within 1 s.d., 95% is within 2 s.d., and 99.7% is within 3 s.d. This curve has been shown to hold true for all types of biologic, chemical, and physical measurements (1).

Method for Establishing Normal Values

An acceptable method for establishing normal values is to begin by measuring a series (30-40) of healthy individuals (2). Collect the normal samples over several weeks to give a reasonable approximation of geographic population, laboratory conditions, and minute method variability. Sample the total population that will later be assessed; make it representative with regard to age and sex. The sampling should be done by the same method under the same conditions. Blood samples should be drawn at the same time of day and stored in the same fashion.

Accumulate data from the individual tests and determine the mean and standard deviation of the sampling. The normal range is the numerical value of the mean \pm 3 s.d. If the results of the patient who is subsequently tested lie outside this range, he is considered abnormal.

Initiation of a Quality Control Program

The quality control sample run with every batch of radioassays is the foundation of an adequate program. The sample must be carefully prepared, stored, and tested since the assessment of the validity of patient results performed concurrently depends on its answer. If the quality control sample falls within its expected range, it is deduced that the entire batch of patient samples has also been adequately tested. Those patients, then, whose values fall outside of the previously determined normal range are in fact abnormal.

The procedure for initiating a program is briefly outlined below with amplification of each point following. (A) A large number of identical samples from the same pool are prepared and frozen. (B) The pool must resemble as closely as possible the unknown material. (C) One sample is run with each batch of unknowns. (D) The variability of the repeated analyses is measured under regular operating conditions. No special care, equipment, or technique should be utilized. (E) The mean and a \pm 3 s.d. limit is calculated, graphed, and posted. (F) A quality control sample is included in all subsequent runs. If the sample value does not fall within the known limits of inherent variability (the \pm 3 s.d.), the run of measurements is rejected and repeated. (G) A new quality control sample is used with the repeated tests.

TABLE 3. Statistical Terminology Used in Quality Control

Sample: the group of individual values actually studied; it is essential that it be representative if it is to be used for estimating the value for the entire population

Standard deviation (s.d.): a measure of the dispersion of a group of values around a mean

Coefficient of variation (CV): another way of expressing standard deviation. Expressed as a percentage, it is defined as 100 times the standard deviation divided by the mean

Range: another measure of dispersion of values and is merely the difference between the largest and the smallest of a group of measurements

Variance: the square of the standard deviation; variance can be added or subtracted, which standard deviations cannot. This process is an analysis of variance (ANOVA)

Mean: measurement of the center of a distribution

Accuracy: closeness to the true value

Precision: closeness of the results of repeated analysis performed on the same material

Curve of normal distribution: see Fig. 1

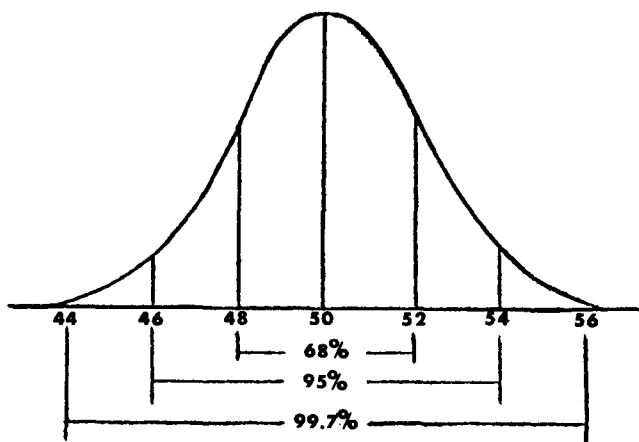


FIG. 1. Symmetrical bell-shaped curve representative of many types of numerical distributions (Gaussian curve).

Preparation of the quality control pool. There are several acceptable methods for preparing serum pool samples. One is to collect excess laboratory sera, approximately 2–3 liters. Eliminate hemolyzed, icteric, or lipemic sera and freeze in a large plastic bottle at -20°C . Add the daily excess to the bottle until the desired volume is accumulated. After collection, liquify the contents of the bottle and mix on a magnetic stirrer for 1 hr. Centrifuge at 3,000 rpm for 30 min to eliminate fibrin and other debris. (Filtering is not recommended since it takes too long, thus promoting bacterial growth). After centrifugation, mix again and aliquot out in desired volumes, either enough for 1 day or 1 run. Do not use and refreeze the samples; discard the unused portion at the end of the day. Siliconized glass test tubes with red rubber stoppers are recommended for storage.

Salvaged plasma from outdated blood may also be used for a pool, although this is not recommended.

Preparation is tedious since the plasma must be dialyzed to restore normal concentration because it has been diluted approximately 20% by anticoagulants. Also, the surplus may not be readily available to nuclear medicine personnel either because the blood bank returns outdated blood or because the surplus is consumed by the clinical laboratory.

Commercial preparations usually in the form of small vials of lyophilized sera with known approximate values may be purchased for a pool. The best approach is to buy a large amount of the same lot number so that the program will not be interrupted unduly. Reconstitute and mix the entire batch purchased and aliquot it out in the same fashion as a regular serum pool. This procedure will minimize variation due to dilution, mixing, and deterioration. Use the stated values as a guide in further determinations but do not accept them as irrefutable. Expense is an obvious disadvantage to this approach, but perhaps the ease of procurement and the aid of a predetermined approximate range would compensate. Also, the small volumes generally used in radioimmunoassay would assure a large number of individual samples per lyophilized vial.

Another pool variation particularly adaptable to the radioimmunoassay situation is to purchase a suitable amount of the pure substance in question. Weigh out and dilute the substance to equal the concentration of analyzed material used in the standard curve. This method assures that the amount of unknown to be analyzed will not exceed the maximum volume or concentration range of the system. The technologist can then easily select and prove independently points of particular interest on the curve: normal, elevated or toxic, depressed, or troublesome gray zones. Since a pure material was purchased, weighed accurately on a Mettler balance, and diluted in "A"-grade volumetric flasks, the test result should be extremely close to the expected value. This type of control can therefore prove accuracy and precision simultaneously. It is particularly useful for new procedures for which no commercial preparation is as yet available. The main disadvantage of this method is that the control does not exactly simulate a patient sample. However, in many radioimmunoassays it will be noted that the unknowns are titrated against a standard curve that does not exactly simulate a patient sample either.

Preparation and usage of the quality control chart. Make 15–25 measurements of the quality control pool that was prepared by one of the preceding methods. Do them on separate days (only one per day) under routine operating conditions. Calculate the mean and standard deviation of the measurements. Construct a graph as per Fig. 2. Plot the mean and draw the ± 3 s.d. lines. These are the "control" or "confidence" limits. Indicate days of the month, name of the measurement, and post or place in notebook. Make the charts easy to prepare and use.

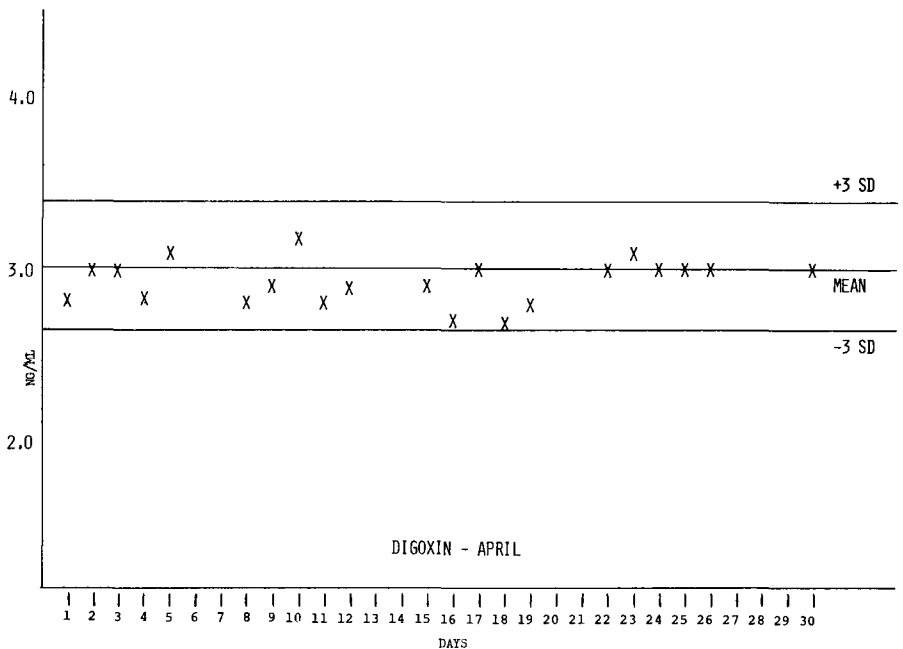


FIG. 2. Example of quality control chart for digoxin.

To effectively use the chart, plot the daily control measurement and determine whether the value falls within the confidence limits. If it does, record the value and report out the run of patient samples. A quality control sample that measures "out-of-control" indicates that the run must be repeated. First, however, check all calculations. If they are correct, thoroughly thaw and mix a new control sample and rerun it with the batch of unknowns. If the second run is out of control, a basic flaw exists in the test which must be corrected before continuing. Causes for the problem may include expired reagents, wrong temperature, inadequate mixing, insufficient centrifugation, and defective equipment. Record quality control values on a separate monthly report sheet. Find the mean, standard deviation, and coefficient of variation at the end of each month. Record out-of-control figures and their cause if determined. Do not include these values in the monthly mean since they were repeated and the samples in that run not reported.

The end-of-the-month evaluations assist the technologist in recognizing a trend of the in-control values toward one end of the range. This may be early warning of instrument, reagent, or method deficiency as well as possible deterioration of the control. Do not habitually place the control sample in the same position in the run. It will fail to detect instrument drift during a run interval. If more than one control is used, place them before and after patient samples.

Selection of the ± 3 s.d. control limits is made with the following information in mind. A ± 3 s.d. limit yields by chance alone that one pool analysis in 100 will be out of control (approximately one every 3 months). A ± 2 s.d. limit yields by chance alone that one in 20 will be out of control (approximately once a month). The

± 3 s.d. limit is generally recommended, but if the ± 2 s.d. is chosen to assure tighter control, it must be understood that approximately five times as many sets of analyses will have to be repeated for reasons of chance alone without necessarily increasing the reproducibility of the results (1).

The confidence limits selected indicate the "significant change limits." Significant change limits assist the clinician in deciding whether there is a change in the patient's status. If the difference between two measurements is greater than three times the standard deviation of the method, then there is no doubt that this represents patient change since it exceeds possible variation from the procedure (3).

Other calculations related to quality control may be derived by using analysis of variance (ANOVA). One can compute the individual contributions to total variability of the place of the sample in a run, day-to-day changes, and residual method error. These calculations are not considered generally necessary for a good quality control program but information regarding these additional statistics may be found in the literature (4).

Another type of graph used in quality control is a plot of the difference between the duplicate values of controls run with each batch. The confidence limits for this type of plot should be determined at the same time as the regular quality control limits. Simply average the differences of the duplicates and find a ± 3 s.d. Use the previously mentioned format to construct the graph. Proponents of this additional step feel that trends and drifts are detected more quickly (5).

Use of duplicates. The routine performance of analyses in duplicate was at one time considered the best method for assuring accuracy of results. It is still

useful in a procedure in which certain time-consuming or difficult manipulations are necessary so that, should a gross error in one sample occur, the test would still be salvageable. A true duplicate should start with a separate aliquot at the beginning of the analysis and must be carried through all steps of the procedure. Duplicates run right next to each other in a run are much less useful than those placed randomly. A random duplicate would at least indicate that conditions were consistent during that time period. Obviously, day-to-day analyses vary much more. If the procedure is basically inaccurate for any reason, the duplicate result will merely confirm the inaccurate value and not indicate a malfunction in the test.

External Quality Control

All controls mentioned thus far have been examples of internal quality control: that is, controls initiated by the laboratory itself. There are several programs now in existence that are indicative of external quality control. Perhaps the largest is the Quality Evaluation Program, which is a proficiency survey conducted by the College of American Pathologists. This program is one in which two vials of lyophilized sera are sent to the participants several times during the year. The subscribing laboratory reconstitutes the material according to instructions and runs an aliquot with the routine radioimmunoassays. A program is designed specifically for nu-

clear medicine and contains a section to classify the result as normal, elevated, or depressed.

Information from the participating laboratories is accumulated and analyzed according to test. The participants easily see how their results compare to the national average of the same method and to different methods for the same unknown. Surveys of this type are not infallible, but they are an asset to the nuclear medicine laboratory, in which there is constant exposure to new and untried products.

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References

1. Barnett RN: *Clinical Laboratory Statistics*. Boston, Little, Brown, 1971, pp 4-9, 20, 74, 98
2. Davidsohn I, Wells BB: *Clinical Diagnosis by Laboratory Methods*. Philadelphia, Saunders, 1963, pp 15-21
3. Copeland BE: *Quality Control Manual (rev ed)*. Chicago, American Society of Clinical Pathologists, 1967
4. Amenta JS: Analysis of variance, control charts and the clinical laboratory: A system of quality control. *Am J Clin Pathol* 49: 842-849, 1968
5. Levey S, Jennings ER: The use of control charts in the clinical laboratory. *Am J Clin Pathol* 20: 1050-1055, 1950