Quality Control for Immunologic Tests

QUALITY CONTROL FOR IMMUNOLOGIC TESTS

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PREFACE

This monograph is intended to be a general guideline to bench level technologists in establishing and maintaining an effective and efficient quality control program. It is intended as a supplement to the training manuals which indicate specific quality control procedures for specific tests. The purpose is to stimulate quality control consciousness and thinking and to provide a source of references for additional literature, methods, and standards.

The section dealing with quality control calculations has been developed to a greater extent than other sections because such information is not readily available. Even though many may not be familiar with these tests and calculations, we hope that they will learn to do them and use them in their quality control systems.

The tests in this publication include a wide range of commonly used immunologic tests. Some of the more specialized tests, especially the more recently developed cellular immunity tests, have not been included.

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I. INTRODUCTION

According to Murphy's Law, if anything can go wrong it will. The purpose of quality control is to prevent as many errors as possible and to detect those that do occur. Total quality control means that every variable that could possibly affect the quality of the test results has been controlled. Obviously such a situation is ideal rather than reasonable. However, a practical quality control system should control most of the factors that are likely to affect the test results. Determining which controls are important components of a quality control system, how often to include the controls, how many controls to include, and how to use the control results requires experience and knowledge. This monograph provides information and recommendations to laboratorians so they can intelligently design quality control systems which are appropriate for their circumstances.

Numerous sophisticated quality control recommendations are available for other clinical laboratory disciplines, but those for immunologic tests have generally been neglected. Considerable effort has been expended in developing and publishing quality control manuals for chemistry, hematology, and other clinical laboratory specialties, but the efforts in immunology have been limited and fragmentary. The fact that the distribution of results from most immunologic tests is more nearly log-normal than normal is seldom mentioned, and the implications and consequences of this fact are almost always disregarded. The application of statistical methods to immunologic data is particularly limited. We hope that this monograph provides a starting point for the development of an increased quality control consciousness among immunologists and thus a catalyst for the development and implementation of better quality control methods and programs.

This manual was developed as the result of several stimuli. One was the general atmosphere of concern about the validity of the results produced in clinical laboratories. Another factor was our experience with the results of proficiency testing programs in clinical laboratories, which includes the observations that laboratories in proficiency testing programs frequently report that they do not use the recommended control procedures, that they deviate from standardized methods, that they fail to detect product and reagent deficiencies, and that they even report test results when they have also reported that the test system is "out of control" (*108,110,111*).

In the past few years, serologic and immunologic tests have been more widely used in the clinical laboratory. As new methods and techniques are developed and adapted, adequate quality control must be developed to assure the reliability and usefulness of clinical laboratory test results. No single quality control system is appropriate for every laboratory. Such variables as the volume of testing, the qualifications and interests of the personnel, the composition of the patient population, the type of laboratory, the purpose for which the results will be used, budgetary limitations, and external factors must all be considered. The implementation of an effective program requires knowledge and experience in order to achieve the proper balance between ideal quality control requirements and the limiting factors. Since variation in test results is a composite of the variation of each step of the total procedure, prime consideration should be given to those components which contribute most to the total variation. The components of variation which are most amenable to improvement should be most carefully examined.

An effective quality control program should cover all aspects of the laboratory. Of greatest importance are personnel with adequate training and experience, since much of the quality control is a function of professional judgment (*106*). Proper specimen collection and processing of results are as important as the analytical procedure itself. Reagents must be of good quality, technique must be adequate to maintain precision and accuracy, and there must be methods for detecting errors and taking corrective steps when analyses are out of control. Preventive maintenance, continuous training of employees, documentation, and coordination of activities are also part of a comprehensive quality control program (*6,92*).

Improved accuracy and precision, smoother laboratory operation, and better morale may follow the implementation of a quality control program which is easy to maintain and interpret. When possible, the program should monitor performance quantitatively rather than subjectively and should cost no more than absolutely necessary. The major purpose of the quality control program is to confirm that results are of high quality and that the physician can rely on them.

Some aspects of quality control concern prevention, whereas others concern verification of following proper procedures. Using properly evaluated and prepared reagents, maintaining and calibrating equipment, checking temperatures, and applying proper techniques are preventive measures. Control sera and reaction mixtures are used to verify technique and detect errors. When results indicate that a procedure is "out of control," the source of error must be determined and the problem corrected as efficiently as possible.

Whether it is better to have each technologist involved in quality control or to have a "quality control technologist" is a debatable question, but if a specific person is given the responsibility for establishing an effective quality control program, his or her rsponsibility should include those of collecting and summarizing data and the rapid and effective transfer of information to the laboratory director (*7,93,131*). Records of quality control that reflect proficiency testing results, routine control sample results, equipment maintenance, calibration, and reagent testing should be maintained.

Procedural manuals should be available at the bench for each procedure offered by the laboratory. Any changes in procedures should be dated and initialed in the manual by the laboratory director or his representative.

II. GENERAL QUALITY CONTROL FUNCTIONS

A. PROCEDURE MANUAL

An important element in maintaining day-to-day uniformity in laboratory results is an established procedures manual which details all phases of the laboratory's operation (including safety precautions) and is used by all laboratory personnel. It should include instructions for collecting, transporting, and storing specimens, for preparing and storing reagents, and for performing tests. In addition, the controls and calibrators to be used should be listed along with directions for their use, expected results, and instructions for corrective measures if the expected results are not obtained. Although attempting to develop new methods and trying new methods described in the literature are to be encouraged, procedures used to test patient specimens should be changed only with the approval of the director or designee after comparative studies in the laboratory have shown that the new ones are satisfactory (*17*).

B. SELECTION OF TEST OR PROCEDURE

As new tests and methods are developed for various analytes (antibodies or antigens), the most appropriate must be chosen for each laboratory's needs. A number of factors must be considered including bias, specificity, sensitivity, precision, cost, and ease of performance. Bias, specificity and sensitivity may be related. Frequently the more sensitive a test, the less specific it is (*106*). Bias may result from low specificity or sensitivity. The population to be tested may influence the decision of what test to use; e.g., screening essentially normal patients does not necessarily require the same methods appropriate for following patients with a confirmed disease.

To determine the presence of bias, the proposed method should be compared with other reliable methods, preferably with a standard method or clinical data. The same specimens should be run with both methods in the same laboratory and the results compared, although interlaboratory comparisons are also useful. If the results from the different methods do not agree one must determine the reason for the difference and then decide which result is more useful.

The specificity of a method is evaluated by testing negative samples and samples containing substances which might cause interference. Closely related or cross-reacting substances frequently found in clinical specimens should be included.

The sensitivity of the method being evaluated should be compared to that of other methods, but the purpose of the test must also be considered. In general, a definitive test need not be as sensitive as a screening test. The test should distinguish between normal and abnormal levels of analyte.

The precision of a quantitative or semiquantitative test must be evaluated in light of the precision required for the clinical application of the test results. Many factors affect precision, but one that is frequently overlooked in serologic tests is the size of the dilution increments. If all other variables are held constant, serologic tests tend to become less precise as the size of the dilution increment increases. For example, it should be expected that a test based on a 4-fold dilutions would be less precise than the same test with 2-fold dilutions.

The predictive value of a positive test result is defined as the percentage of positive results that are true positives for a defined population. The predictive value of a negative test result is the percentage of negative results that are true negatives for the same defined population. The predictive value of test results is directly dependent on prevalence of the condition in the population being tested (*37*). This is an important fact to remember when selecting tests.

The efficiency of a test is determined on the basis of the two predictive values. It is the percentage of all results that are true results, i.e., the number of true positives plus the number of true negatives divided by the total number of test results, expressed as a percentage (*37*).

Peak test sensitivity is desirable when a disease is serious and its diagnosis should not be missed, the disease is treatable, and false-positive results do not lead to serious problems. Peak test specificity is desirable when a disease is serious but is not treatable, the knowledge that the disease is absent has psychological or public health value, and false-positive results can lead to serious problems. A high predictive value of a positive test result is desirable when treatment of a false positive might have serious consequences. Peak test efficiency is desirable when the disease is serious but treatable, and when false-positive and false-negative results are about equally hazardous (*37*).

C. COLLECTION OF SPECIMENS

Concern for the quality of test results begins when a particular test is requested. There must be a system for the orderly and efficient requesting of tests; collection and identification of specimens; and transporting, preparation, and storage of specimens. Nothing is more

important than having an adequate amount of an appropriate specimen in good condition for examination. If each specimen is not properly collected, labeled, and handled, or is not representative, the laboratory may do more harm than good by testing it (*22*). Some method is also needed for monitoring this total system (*121*). In many laboratories this latter process involves retrospective examination of records, but in other cases more sophisticated techniques are used (*57*).

D. CONTROL AND REFERENCE SERA

Control sera must be stable and capable of being stored for long periods without loss of activity. They must be similar in composition to patient specimens and must be subjected to the entire procedure being monitored. If sera are diluted or treated before being used as controls, their reactions must be verified to be similar to those of patients' sera. Controls should not be pretreated or diluted in bulk if such steps are included in the test procedure.

1. Source

Some control sera are available commercially. Small volumes are generally available as components in kits but are intended to be used only with a single kit. A few may be available in larger quantities.

Another way to obtain control sera is to pool patient sera. For many tests this source should not be overlooked, particularly if many samples with the desired concentration or reactivity levels are encountered during testing. However, the fact that patient samples may react with each other when pooled limits their usefulness.

Larger amounts of serum for serologic testing can sometimes be obtained from convalescent patients who have high titers and who are physically able to donate.

2. Preparation

Sera to be used as controls should be kept sterile to avoid deterioration. Sera with high concentrations or reactivity levels may be diluted with sera with lower concentrations or reactivity levels to obtain the desired levels. The normal level of the constituent to be tested should be considered in selecting the levels of the control sera. In general each procedure should have a normal control serum (negative), a strong positive control serum and another positive control serum which is reactive at the critical concentration (borderline positive). With some tests, controls with

a low concentration of analyte should be included. Controls recommended by the manufacturer of a particular test should always be used and additional control sera can be included if a test involves special problems.

The serum should be assayed repeatedly in parallel with a serum of known concentration to ensure that the new control gives consistent results. The adequacy of the new control should also be checked by testing it with other procedures. The concentration or titer of the new control can also be verified by other laboratories. The serum may be filtered through sterile filters or preserved with 0.1% sodium azide or thimerosal (Merthiolate).

3. Storage

Enough control serum should be available to last for at least 6 months of continuous monitoring of test performance. Adequate storage must be provided to prevent deterioration.

Sera for most tests can be frozen and held at −20°C for extended periods, but should not be thawed and refrozen. Because of this fact, refrigerators with automatic defrosting should not be used. Serum can be dispensed in small aliquots (sufficient for one run), sealed tightly, and frozen. Many sera can be lyophilized and stored at refrigerator temperatures if facilities are available.

4. Reference Materials

Sera to be used as standards should be standardized against international reference materials when they are available (see Section IV). "Standards" included in commercial kits are not calibrated with each other and often are not interchangeable.

E. REAGENTS

Quality reagents are necessary for quality performance. A record should be kept of any changes in reagents in case the performance of a test changes. Before new reagents are introduced into a system they should be tested in parallel with the old reagents against a panel of appropriate reference sera to be sure that consistent reactions are obtained. The results obtained with the panel should reflect the sensitivity and specificity of the reagents being compared. Reagents should be stored according to manufacturer's instructions. Improper storage is a frequent cause of loss of reagent activity (*31*). Expiration dates must be observed. The pH's of buffers should be checked before each use. Each time reagents are used they should be examined for precipitates, cloudiness, contamination, or other signs of dete-

rioration and should be discarded when there is evidence of their inadequacy.

Reagents should be labeled clearly to indicate their identity, hazards involved in their use, recommended storage conditions, and preparation and expiration dates (*19*). In some cases, such information as the dilution of stock reagents to be used in the test may also be helpful. Always follow the manufacturer's or author's directions in preparing and using the reagents.

Some manufacturers of reagents for serologic tests voluntarily submit samples of their products to CDC for the Premarket Evaluation Program which includes evaluation of reagents for rubella HI, bacterial antigens and control sera, viral antigens and antisera, and syphilis serology reagents. The reagents are tested according to CDC specifications using CDC reference reagents. On the basis of the reports they subsequently receive, laboratorians can request that manufacturers supply reagents from lots which have been confirmed as meeting CDC specifications.

Lists of some commercial sources for reagents are available (*31*). Because of the rapid changes occurring in the field of immunology, it is difficult to keep such a list completely up to date, but the Biological Products Evaluation Branch at CDC periodically issues such a list.

F. EQUIPMENT AND INSTRUMENTS

All glassware used in immunologic tests must be clean and free of detergent. Chipped or etched glassware should be discarded. Calibrated glassware should be checked for accuracy.

The user's accuracy and precision requirements should be met or exceeded when equipment is tested under working conditions. The manufacturer's specifications for performance should be checked and met. Instruments and equipment should be monitored routinely. The temperatures of water baths, incubators, refrigerators, and freezers should be checked periodically and records maintained. Maintenance should be performed and records kept on a regular basis by individuals who are trained and are familiar with the equipment.

Instruments used for measurements including spectrophotometers, spectrometers, dilutors, and automatic pipettors should be calibrated on a regular basis.

The monitoring necessary for commonly used laboratory equipment is listed in Tables 1 and 2. Recording thermometers are useful, as are centralized alarm systems for refrigerators and freezers. If such equipment is not available, other provisions should be made for routine monitoring (*31*).

Table 1. Suggested Monitoring of the Temperature of Laboratory Equipment

Item	Record Temperature	Other
Autoclave	Each run (also time recorded)	a. Record pressure once during each run. b. Use properly placed color indicator discs or strips in each run. c. Use peak temperature thermometer weekly. d. Use spore strips or spore suspensions monthly. e. If evidence of contamination is found, make sample cultures frequently (daily or weekly) until the cause is determined and eliminated.
Incubators	Continuously, with a recording thermometer (if possible); an alarm system is recommended.	a. If a recording thermometer is not used, record the temperature (a.m.) daily and before opening.
Water Baths	Daily before use.	a. Clean monthly.
Refrigerators	Daily; 2° to 8°C	a. Walk-in should have recording apparatus. b. Connect walk-in to alarm system. c. Clean monthly. d. Defrost or check refrigerator and freezer compartment every 3 months.
Hot Air Oven	Each run (also time recorded)	
Freezers	Daily	a. Connect to alarm system. b. Clean every 6 months.
Room Temperature	Each run (if applicable)	

Table 2. Suggested Monitoring of Laboratory Equipment

Item	Monitor Procedure
Analytical Balance	a. Check with certified weights at least once each week of use.
	b. Clean balances and weights monthly.
	c. Discard damaged weights which are no longer accurate.
pH Meter	a. Compensate for temperature each run.
	b. Date buffer solutions when first opened and, if possible, check monthly with another pH meter. Discard buffer solution if the pH deviates more than ± 0.4 from the manufacturer's stated pH or if it is contaminated with microorganisms.
	c. Standardize with at least one standard buffer (e.g., pH 7.0, if working in range below pH 6.0 use pH 4.0) before each test or series of tests.
	d. Inspect every 6 months for proper function.
Spectrophotometer	a. Check transmittance each day of use at a specified wavelength.
	b. Inspect every 6 months for proper function.
Centrifuge	a. Check brushes and bearings every 6 months.
	b. Check rheostat control against a tachometer at various loadings and frequently enough to assure proper gravitational fields.
	c. Performance must be evaluated often enough to assure proper performance.
	d. Oil and grease.
	e. Examine high speed heads for damage or corrosion.
Microscope	a. Clean after each use.
Filters	a. Do not sterilize filters (Millipore, etc.) above a temperature of 121°C.
	b. Carefully perform bubble point testing during each filtration run under positive pressure.