

Clinical laboratory methods

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with 497 illustrations and 16 color plates

EIGHTH EDITION

(内部交流)

The C. V. Mosby Company

SAINT LOUIS 1974

Preface

The rapid changes and advances in laboratory medicine have made it necessary to rewrite most chapters, eliminating outdated material, in this edition of *Clinical Laboratory Methods*. Chapter 13 of the seventh edition has been excluded entirely, as water and milk analyses are seldom performed in clinical laboratories. Chapter 14 of the seventh edition has also been deleted, since it duplicates much of the information contained in the Public Health Manual *Serologic Tests for Syphilis*, a copy of which must be available to all laboratories that are approved by the U. S. Public Health Department to perform syphilis serology.

In the section on urinalysis, drug interference is stressed. Newer items include selectivity of proteinuria, low molecular weight proteinuria, and the diagnosis of Bence Jones proteinuria by immunodiffusion. Thin-layer chromatography of urinary carbohydrates and amino acids is given in detail. The section on porphyrins contains a column chromatographic method for delta-aminolevulinic acid. Mention is made of the use of radioisotopes in the evaluation of kidney function and of the laboratory manifestations of renal allograft rejection.

Chapter 3 contains a section on fructose determination in seminal fluid, a section on sperm-agglutinating antibodies, and a discussion of chemical tests in the evaluation of placental function.

The section on hematology has been expanded to three chapters. It includes newer screening methods for hemoglobinopathies. The hemoglobin iron section contains a paragraph on ferrokinetics. Isotope methods

for intrinsic factor, vitamin B₁₂, and folic acid are part of the section on vitamin B₁₂. The urinary methylmalonic acid assay and electrophoretic demonstration of G-6-PD deficiency and of haptoglobin are mentioned.

Chapter 5 includes cytochemical stains, a discussion of nuclear sex dimorphism in leukocytes, and the quinacrine fluorescence method of Y-chromosome identification. Discussions on lysosomal polymorphonuclear defects and the nitro blue tetrazolium dye test complete the chapter. In the description of lymphocytes, attention has been drawn to T and B cells.

The section on white cell pathology includes a muramidase (lysozyme) assay method, paragraphs on light chain and heavy chain diseases, and mention of the sea-blue histiocyte. The role of the anti-Epstein-Barr virus antibodies in infectious mononucleosis is discussed. Colored photomicrographs of blood cells have been added.

In Chapter 7, newer methods in the investigation of coagulation disorders include tests for fibrin degradation products, such as the protamine sulfate serial dilution test and the staphylococcal clumping test. A description of platelet function tests completes the chapter.

The chapter on clinical chemistry has been extensively revised. The material formerly in this chapter has been divided into three chapters, one on general clinical chemistry, one on enzyme determinations, and one on hormones. In the latter chapter all hormone determinations, some of which were formerly in other chapters, have been included. In the chapter on clinical chemistry a num-

ber of new methods have been added and some older ones deleted. Additional material has been added on volumetric glassware, and the general material has been expanded to give a more complete discussion of the principles of photometry, flame and atomic absorption photometry, fluorometry, and chromatography. Material on the use of radioactive tracers in the clinical laboratory has also been added. The Folin-Wu method for blood glucose has been deleted and the widely used *o*-toluidine method has been added, as well as an enzymatic method. The discussion of the glucose tolerance test has been expanded. New methods have been added for the direct determination of albumin (dye binding) and globulins that together with an expanded discussion of cellulose acetate electrophoresis replace the salt fraction methods. New methods have been added for phosphorus, cholesterol, triglycerides, and total lipids. The chemical precipitation methods for sodium and potassium have been deleted. A discussion of lipoprotein electrophoresis and lipoprotein phenotyping has been included, replacing the older material on the atherogenic index. The section on the basal metabolic rate has been deleted.

The methods for enzymes have been expanded to include a new method for amylase, a method for gamma-glutamyl transpeptidase, and additional material on lactate dehydrogenase and alkaline phosphatase isoenzymes.

In the chapter on hormones a discussion of the use of competitive protein-binding methods and radioimmunoassay of the various hormones has been introduced. The section on determination of butanol-extractable iodine has been omitted, as it is now rarely used, and the discussion of the radioactive tracer methods for the determination of thyroxine and triiodothyronine has been expanded. Methods for the determination of urinary estrogens in pregnancy and a fluorometric method for cortisol have been added.

The discussion of blood bank methods has been brought up to date with sections on blood and blood components, on detection

of antipenicillin antibodies, and on the use of RhoGam.* Methods for the detection of Australia antigen include a radioimmunoassay method.

The parasitology chapter has been expanded to include the trichrome stain as permanent protozoal staining procedure and is enriched by colored photomicrographs. The laboratory diagnosis of primary amebic meningoencephalitis is included in the section on amoebae. Special emphasis is placed on the investigation of malaria, which includes a fluorescent antibody technique and two new color plates.

The section on the examination of biologic fluids has been expanded to include cerebrospinal fluid electrophoresis, radial immunodiffusion, the assessment of fetal maturity by amniotic fluid analysis, and tests for alpha-1-antitrypsin deficiency.

The single disk method for drug susceptibility of anaerobic bacteria is presented as part of the enlarged microbiology chapter, as well as newer media and methods in the investigation of anaerobic and *Neisseria* infections. Multitest systems for the identification of enteric gram-negative bacteria are mentioned. The *Limulus* test for the detection of endotoxemia is also new in this chapter.

In the chapter on toxicology the main additions are methods for urinary screening of drugs of abuse and for the detection of these drugs in other materials, such as tablets.

A short discussion of the laboratory diagnosis of cancer has been added.

A debt of gratitude is owed to many individuals who were most helpful in the preparation of the manuscript. We are especially thankful to Dr. A. S. Wiener for his critical reading of the section on blood groups. Dr. T. Nishi and Dr. K. McLaughlin unhesitatingly accepted the burden of additional work to allow the senior author to spend more time in the preparation of the manuscript. Dr. R. Gray, consulting bacteriologist, DePaul Hospital, and Ms. P. Keating,

*Ortho Diagnostic Div., Raritan, N. J.

M.T. (ASCP), bacteriologist, DePaul Hospital, willingly undertook to read Chapters 14, 16, and 17 and freely gave of their knowledge. Mrs. M. Thorsten, M.T. (ASCP), chief technologist, DePaul Hospital laboratory, critically read almost the entire manuscript and is responsible for most of the newly added hematologic and parasitologic color photomicrographs as well as the new black and white photomicrographs of fungi. The mycologic material was kindly supplied by Dr. L. Swidnicki.

We are grateful to Mrs. F. Makamson, who typed and retyped most of the manuscript, and Mr. K. C. Lewis, Chief, Department of Illustration, Washington University Medical School, who prepared the added color and black and white photographs. Ms. M. J. Harris of his department prepared all the new diagrams and charts. The black and

white pen drawings speak for the artistry of Mr. G. Humphrey.

We wish to thank Miss S. Hackman for the many suggestions and corrections she kindly made in reading Chapters 9, 10, 11, 18, and 19 and Mrs. M. Thompson for the excellent typing of these chapters.

Without the help of the librarians of the St. Louis Medical Society Library, this book could not have been written. They were ably assisted by Mrs. C. Humphrey who checked hundreds of references.

Above all, we are indebted to all the original investigators who so generously allowed us to use the results of their efforts.

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Some laboratory rules and quality control

Some laboratory rules

All requests for laboratory examinations should be made in writing and should contain the patient's full name, his hospital number, the type of specimen, the clinical diagnosis, and the specific examination desired. Knowledge of the patient's race is helpful at times, for instance, in blood banking. Three time slots on the requisition should indicate time and date when request was issued, when the result is requested, and when and by whom the test was completed.

On entering the patient's room the technologist should identify herself and state the reason for her presence (e.g., blood test ordered by the patient's physician). Prior to any procedure she must identify the patient by checking his Ident-A-Band, name, and hospital number. The patient must not be identified by bed number or by asking his name. If there is any doubt, the floor nurse should identify the patient.

Good work records are a must. This record should contain not only the patient's identification, test, and results but also the procedure used to arrive at these results. For example, in biochemistry, specify the dilution used and the spectrophotometer reading; in bacteriology, include the various media used and the reactions of each.

Each laboratory should have an accession book that lists all incoming specimens, their date of acquisition, their final disposition, the results of the test, and the date of completion. A number is assigned to each specimen. An adequate filing system must allow easy access to records.

Common errors

Quality control has as one of its objectives the elimination of errors. Errors frequently result from failing to observe very basic precautions and to adhere to basic laboratory rules. Frequent sources of such errors include the following:

1. Improper identification of patients, specimens, or both (The laboratory bulletin board should carry daily, easily visible warning notices of patients with identical names.)

2. Choice of poorly tested procedures that may be adequate in the normal range and inadequate in the pathologic range

3. Failure to adhere to established rules and procedures

4. A wrong, inadequate, or contaminated specimen

5. Lack of familiarity with basic arithmetic (The presently available inexpensive desk calculators are of great help in eliminating mathematic errors and also add speed to calculations.)

6. Reagents that are improperly labeled or stored in the wrong type of container or at the wrong temperature

7. Transcription errors in the laboratory reporting system or entering correct laboratory results on the wrong patient's record

Quality control

Quality control is conceived as an all-embracing concept that includes much more than standard deviation charts posted on the wall. By quality control we mean the sum of our efforts to achieve the highest degree of excellence, so that both the patient and physician obtain correct information in the shortest possible time and at reasonable cost.^{1,2,2a}

Quality control includes the following areas, which are first discussed generally and then focused on in the individual laboratory sections.

1. All patients, all laboratory personnel, all laboratory equipment, and all laboratory tests are involved, including patient preparation and identification, specimen collection, transportation and handling of the specimen, performance of the test, and reporting.

2. The laboratory's relation to other hospital departments should be expressed in writing and revised and updated at least once a year. The quality control program extends beyond the confines of the laboratory and must include many other areas such as the nursing service, the admitting office, the outpatient department, house-keeping, public relations, purchasing, the "store-room," the pharmacy, surgical supplies, the medical staff, and laboratory representation on appropriate committees.

3. Laboratory policies and procedures pertaining to the entire laboratory and to each subdivision should be collected in a manual to be revised at least once a year or when pro-

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cedures are changed. Copies of this manual should be available to other hospital departments.

4. A quality surveillance system should establish norms that must be met; e.g., tests must not be reported if results are outside an established limit such as ± 2 SD.

5. A correction system should be established to offer education, realization of why errors happen, and a program to remedy defects, e.g., in equipment, technique, specimen procurement, or storage of reagents.

6. Objective quality control parameters must be established to prove that corrective measures have actually brought results. Such parameters are quality control charts, calculating standard deviations, and rechecking results with internal and external controls. External standards are available from many sources such as the United States Public Health Department, the College of American Pathologists, the American Society of Clinical Pathologists, and commercial laboratory supply houses. Internal standards are prepared by dividing known patients' sera into aliquots, which are stored at -20°C and are included in the daily "run" at suitable intervals.

Quality control programs frequently use the terms "standard" and "control."

Standard: A standard is a substance of known composition, the value of which is established by an analytic procedure different from that used in the clinical laboratory. If the clinical laboratory procedure is able to duplicate the standard value, we accept this procedure as accurate, **accuracy** being defined as the closeness of test results to the true value. Accuracy implies freedom from error.

Control: Controls, physical and chemical, resemble the unknown specimen, e.g., serum controls or urine controls, and contain various substances of known concentration that are assayed by the usual clinical laboratory methods. Controls are assayed daily, together with the unknown; the results of these assays form the basis for the calculation of the mean and standard deviation of a given test. The control specimens are used to measure **precision**, which is the closeness of test results to each other and implies freedom from variation. Control specimens may vary in their composition and are not constant as are standards. (See the discussion of quality control in biochemistry.)

Neither standards nor controls are stable; they must be reassayed by reference methods. In some departments of the laboratory, standards may not be available and tests will have to be monitored by controls, e.g., control red cell and white cell suspensions used in hematology. In some laboratories, negative and positive controls are the only controls available, e.g., serologic testing for

syphilis, pregnancy, and infectious mononucleosis.

Continued education: Since laboratory medicine changes rapidly, the staff must be kept well informed through refresher courses, workshops, educational films, and seminars. An active program of interdepartmental postgraduate education is a valuable asset.

Motivation: The practice of laboratory medicine is a professional activity with the primary motivation of service to the patient. Inadequate training or knowledge must not be tolerated, since very often the patient's diagnosis and treatment depend on the results of laboratory tests. The technologist should be encouraged to verify the results not only by quality control methods but by relating them to the results of tests obtained in other sections of the laboratory—high urine sugar, high blood sugar, positive urine culture, etc.

Specific areas involved in quality control*:

Patients: Proper identification and preparation, exact timing of tests, and proper identification and dating of specimen.

Laboratory personnel: Qualifications of laboratory director, adequate time for supervision, adequate training and number of technicians, technologists, and supervisors, record of workshops and refresher courses attended, in-service training programs, personnel policy manual, and job descriptions.

Equipment: Adequate work and bench space, preventive maintenance of equipment according to written schedule, defects recorded, and corrective measures taken. Maintenance procedures should involve balances, spectrophotometers, photofluorometers, centrifuges, automated equipment, incubators, water baths, microscopes, refrigerators, thermometers, pH meters, etc.

Laboratory tests: Quality control involves factors to be considered in the choice of test: speed, economy, reliability, and ease with which it can be performed by personnel of various training levels.

Reagents: Label should spell out identity, concentration, purpose, date of purchase or preparation, manufacturer, expiration date, initials of technician who prepared reagent, standard used to check accuracy, and distilled water check.

For arithmetic and further discussion of quality control, see the discussion of quality control in clinical chemistry.

Quality control in clinical chemistry³⁻⁵

All the remarks made in the preceding pages also apply to quality control in clinical chemistry.

* Some of the following key words and phrases are taken from Commission on Laboratory Inspection and Accreditation: Standards for accreditation of medical laboratory, Chicago, 1970. College of American Pathologists.

Sources of error: In addition to errors in the actual reading of the colorimeter, there are other errors attributable to the use of the colorimeter that result from a fluctuating light source, stray light, imperfectly matched cuvetts, and nonlinearity of the response of the photocell and the millimeter. There are also many other sources of error in chemical determinations. These include errors in pipetting due to dirty pipets or improper technique, incorrect or fluctuating temperature of a water bath, and errors in preparing reagents and standards. Careful attention to all details of a given procedure will decrease the magnitude of errors but will never eliminate them completely.

In discussing errors involved in a given determination, the terms "precision" and "accuracy" are used. The terms are not synonymous. The results of repeated analyses on the same samples duplicating each other indicate **precision**. If the results of repeated analyses are all very close to each other, the method is high in precision. The results of an analysis or of the average of a series of analyses approaching the true or correct value refers to **accuracy**. Precision can be readily estimated by methods that will be mentioned later. The determination of accuracy is not always simple, since the **true value** of the concentration of a substance in a biologic system may not be known. Usually the accuracy of a method is judged by analyzing for the constituent by a number of different methods involving different types of chemical reactions and often using as reference those methods that are too long and complicated for routine use. The problem is not always simple, as illustrated by the fact that although relatively precise blood sugar methods have been in use for nearly 40 years, there is still some discussion as to the "true" level of glucose in the blood.

Precision and accuracy: In the laboratory one can check the accuracy of a method by comparing results obtained on samples of known concentrations that have been analyzed previously by competent workers or by using commercially available control sera, which are usually obtained in a lyophilized form and are quite stable under refrigeration. But the serum must be used within a short period after reconstitution. Even here, errors are possible. Care must be taken to add exactly the right amount of pure water and to make certain that the material is completely solubilized. Usually the control sera are quite reliable, but it should not always be assumed that they are absolutely correct. In cases of unexplainable discrepancies it is often advisable to try another sample from a different lot of the serum or even that of a different manufacturer.

In the laboratory there is generally more interest in the precision of a method or how well

repeated analyses are reproduced on the same sample. The accuracy of a method is often difficult to determine. The clinician may be more concerned with the precision of the result. If, for example, the blood sugar level is reported on one specimen as 100 mg/100 ml and a second specimen taken at a later date is 110 mg/100 ml, does this represent a true change in the glucose level or is the difference within the limits of laboratory error and can significance be attached to the difference in reported levels? Obviously the more precise the method, the more meaningful are small differences.

The determination of the precision of a method and of the significance of differences between determinations is carried out by determining the **mean** and **standard deviation**. This is a mathematic computation of the different values obtained in a test series and the difference from the average value.

Calculation of standard deviation

The mathematic formula for the standard deviation (SD) of a number of values is:

$$SD = \sqrt{\frac{\sum (\bar{x} - x_i)^2}{N - 1}} \quad (1)$$

Expressed in words, this means that one calculates the average value of the determinations (\bar{x}), finds the difference between \bar{x} and the separate values of (x_i), squares these differences ($\bar{x} - x_i$)², and forms the sum of these squares (\sum indicates the summation). This sum is then divided by one less than the number of values ($N - 1$) and the square root of the quotient is extracted. Except for the very simplest uses, this formula is not used in the calculations; the following equivalent formula is preferred.

$$SD = \sqrt{\frac{\sum (x_i)^2 - (\sum x_i)^2/N}{N - 1}} \quad (2)$$

Note the difference between $\sum (x_i)^2$ and $(\sum x_i)^2$. In the former, one sums the squares of individual values; in the latter, one adds the individual values and squares the final sum. The methods of calculation are illustrated in Table 1-1, which gives several variations. In column 1 are given the various individual values, x_i . Their sum, 1084, divided by 12, the number of values, gives 90.33, the average value, \bar{x} . In column 2 the differences between the average and the separate values ($\bar{x} - x_i$) are given. Since only the squares of these values are used, the sign of the difference need not be recorded. In column 3 the values for $(\bar{x} - x_i)^2$ are given. Their sum is 214.667. This value divided by $(N - 1)$, which is 11, gives 19.515. The square root of this last value is the standard deviation ($SD = 4.42$). Column 4 gives the values for $(x_i)^2$ for use in calculating the standard deviation from equa-

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Table 1-1. Calculation of standard deviation

1 x_i	2 $(\bar{x} - x_i)$	3 $(\bar{x} - x_i)^2$	4 $(x_i)^2$	5 x'_i	6 $(x'_i)^2$
87	3.33	11.0889	7569	7	49
95	4.67	21.8089	9025	15	225
88	2.33	5.4289	7744	8	64
97	6.67	44.4889	9409	17	289
91	0.67	0.4489	8281	11	121
83	7.33	53.7289	6889	3	9
94	3.67	13.4689	8836	14	196
87	3.33	11.0889	7569	7	49
96	5.67	32.1489	9216	16	256
91	0.67	0.4489	8281	11	121
86	4.33	18.7489	7396	6	36
89	1.33	1.7689	7921	9	81
1084		214.6668	98,136	124	1,496
$\bar{x} =$ 1084 12		SD = 214.667 11	$(1084)^2 =$ 12	$\bar{x}' =$ 124 12	$(124)^2 =$ 12
90.33		19.515	97,921.333	10.33	1281.333
		SD = $\sqrt{19.515} =$ 4.418	98,136.000 -97,921.333 214.667	$\bar{x} =$ 80 + 10.33 = 90.33	1,496.000 -1,281.333 214.667

tion 2. The sum of the values in this column is 98,136. From this one subtracts the square of the sum of the first column divided by the number of values, in accordance with equation 2, $(\sum x_i)^2/N$, to obtain 214.667, from which the standard deviation is calculated as before. If you have a good calculating machine, this method is simpler than using equation 1. Columns 5 and 6 of the table represent a variation that is convenient when the calculations must be done manually. From the values in column 1, one subtracts a convenient constant (in this illustration, 80) to give the small positive numbers that have been designated x'_i in column 5. Column 6 contains the squares of these numbers. The average of the numbers in column 5 is 124/12, or 10.33, which, when added to the constant factor subtracted earlier (80), gives the true average of the original numbers, 90.33. Using equation 2, one again obtains from the sums of columns 5 and 6 the figure 214.667, from which the standard deviation is calculated. Another convenient trick involves the change in decimal points. Suppose that the figures in column 1 were actually 8.7, 9.5, etc. The simplest way to proceed is to multiply all the figures by 10 to obtain the results actually shown. One then proceeds as illustrated and, after all the calculations have been made, divides the final results by 10 to give an average of 9.033 and a standard deviation of 0.442. This method reduces the chances of error due to misplaced decimal points. If one has a large number of determinations, many giving

duplicate values, the simplest procedure is illustrated in Table 1-2. In column 1 of the table are listed all the values obtained in numerical order. One then goes through the list of actual determinations and counts the number of results corresponding to each of the figures in column 1 as illustrated in column 2. These figures are given in column 3 as Arabic numerals for convenience. One then subtracts from each value in column 1 the lowest figure used (in the example, 85) to obtain the numbers in column 4. The figures in column 5 are then obtained by multiplying the figures in each row of columns 3 and 4. The figures in column 6 are then obtained by multiplying the figures in each row of columns 4 and 5, as indicated at the top of the table. The sums of columns 3, 5, and 6 then represent N , x_i , and $(x_i)^2$, respectively, from which the average and standard deviation can be calculated as illustrated in Table 1-1.

After the three sums, $N = 65$, $x_i = 475$, and $(x_i)^2 = 4063$ have been obtained by simple calculations and perhaps an adding machine, the final calculations can be done with sufficient accuracy for most purposes by the use of a good 10-inch slide rule. Using a slide rule one obtains an average of 92.32 and a standard deviation of 3.04.

The standard deviation is a measure of the spread of values; the greater the standard deviation, the greater the differences between the individual determinations and the less the precision of the method. When comparing the

Table 1-2. Calculation of standard deviation

1 x	2	3 N_i	4 $x'_i = x_i - 85$	5 $x' \times N$	6 $x' \times x' \times N$
85		1	0	0	0
86		0	1	0	0
87		2	2	4	8
88		5	3	15	45
89		4	4	16	64
90		7	5	35	175
91		6	6	36	216
92		8	7	56	392
93		10	8	80	640
94		6	9	54	486
95		7	10	70	700
96		4	11	44	484
97		3	12	36	432
98		0	13	0	0
99		1	14	14	196
100		1	15	15	225
$N = 65$			$\Sigma x'_i = 475$		$\Sigma (x')^2 = 4063$

$$\bar{x} = 475/65 = 7.31$$

$$\bar{x} = 85 + 7.31 = 92.31$$

$$\frac{475^2}{65} = 4063.0$$

$$3471.2 \quad - 3471.2$$

$$591.8$$

$$SD^2 = 591.8/64 = 9.25$$

$$SD = 9.25 = 3.04$$

results of determinations at different levels of concentration, the standard deviation may be expressed as a percentage of the mean value, $100 SD/\bar{x}$. This has been known as the **coefficient of variation (CV)**, but the preferred term is now **relative standard deviation (RSD)**.

When the standard deviation of a number of determinations (20 or more) is calculated, approximately 68% of all values will fall within ± 1 SD from the mean, approximately 95% within ± 2 SD, and 99.7% within ± 3 SD.

One major criterion of a good clinical laboratory is that the day-to-day determinations of a given constituent have a satisfactory degree of precision. This can best be checked by analyzing one or more samples of known concentrations each day and comparing the results. These samples should be similar in composition to the regular laboratory specimens and should have a definite, unvarying concentration. This criterion is best met by using a number of commercially available lyophilized control sera. Sometimes pooled serum from excess laboratory specimens can be used. However, this serum may be less stable, even when divided into small quantities and kept frozen. Only the quantity required for a day's work is thawed at one time. The levels of

some constituents, particularly enzymes, in pooled serum may not be satisfactory for quality control.

In setting up a quality control program, the control sera are analyzed each day along with the regular specimens. The control sera should have no preferential position in the order of analysis and must be treated exactly like the regular serum in manual methods (presumably this will automatically be done in automated methods). Records are kept of the daily results, and values may be plotted on a control chart such as that illustrated in Fig. 1-1. The solid horizontal line represents the average value and the dashed lines 2 SD from the mean. During days 1 to 6 at A, the values found all fall well within the normal control limits. On days 7 to 10 at B, although the values are within the normal range, they appear to be fluctuating considerably, which might indicate that somewhere in the procedure some factor such as bath temperature is fluctuating more than it should. On days 11 to 21 at C, we note that although the values are all within the normal range there appears to be a definite upward trend. This usually indicates a deterioration of one or more of the reagents or of the standard. At the point indicated by the arrow

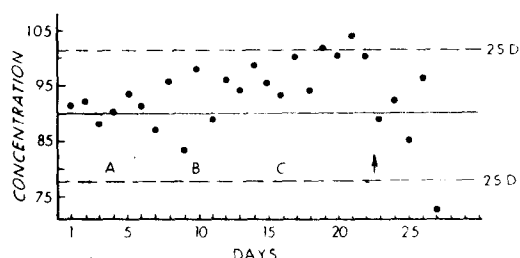


Fig. 1-1. Quality control chart.

it is assumed that new reagents were made up. One notes a return to good control. The point on day 27 is too low. This would require immediate investigation to find the cause and a repeat analysis of the samples and control serum.

The quality control program will not in itself indicate whether the method used is precise enough for ordinary purposes, i.e., whether the actually determined standard deviation is small enough. One could compare the standard deviation for a given procedure with those obtained in other laboratories, but such data are not always available. Another approach has been suggested. If, for example, the limits of normal values for fasting blood sugar are taken as 90-120 mg/100 ml and for serum sodium as 135-145 mEq/L (the actual values may vary somewhat with the method, but those mentioned will serve for illustrative purposes), then the normal range for glucose is 105 ± 15 mg/100 ml and for sodium, 140 ± 5 mEq/L. It seems reasonable that the precision should be better the smaller the normal range. The narrower the normal range, the more precise the method must be to allow a distinction between normal and abnormal values.

One proposed criterion is that the allowable variation (± 2 SD) should not exceed $\pm 1/2$ the difference between the limits of the normal range or in terms of percentage:

$$\frac{\text{Upper limit of normal range} - \text{Lower limit of normal range}}{\text{Average of normal range} \times 4} = 2 \text{ RSD}$$

with the upper limit not more than $\pm 10\%$. For the most commonly determined blood constituents this leads to ranges of 2.2% for sodium and chloride, 5% for calcium, 8% for glucose, BUN, total protein, inorganic phosphate, and potassium, and 10% for many other constituents, including most enzymes.

An additional step in a quality control program would be to run each control serum in duplicate (at different places in the series of analyses). The mean value of each pair would be plotted on a control chart such as that in Fig. 1-1. Preferably on the same chart but using a different scale is plotted the absolute value of the difference (d)

between each pair. A large difference between the two values run on a given day would indicate poor control even though their average was close to the expected value. When a considerable number of pairs has been run, one may calculate the allowable range. If d is the average of the absolute value of the differences for each pair, then the allowable differences for x (± 2 SD) should be $\pm 1.26 d$, and the limits for d (± 2 SD) should be between 0 and 2.18 d . The use of duplicates enables one to judge the precision for each daily run.

In many colorimetric determinations the standards and samples are read in a photometer, setting the instrument to zero absorbance (100% T), with the blank. Thus the absorbance of the blank is not determined. If the blank does have an appreciable absorbance, the determination is often a helpful step in quality control. The blank absorbance is read against water or another solvent. This value is recorded or plotted on a control chart. There is no good method of measuring allowable variation for a blank, but a noticeable change in the blank absorbance may be the first indication of deterioration of reagents or changes in operating conditions such as heating bath temperature.

Standards in clinical chemistry

In almost all determinations made in the laboratory by colorimetry, spectrophotometry, fluorometry, flame photometry, atomic absorption spectrometry, pH measurement, or titration, a comparison is made, directly or indirectly, between the results obtained on the samples and those obtained on a standard or standards of known composition. The accuracy of the method can then be no better than the accuracy with which the concentration of the standard is known. It is imperative, then, that the standards be as pure as possible. Very pure substances for standards can be obtained from the National Bureau of Standards (NBS). Those furnished by the NBS that are applicable to clinical chemistry include cholesterol, urea, bilirubin, creatinine, glucose, potassium chloride (for K and Cl), calcium carbonate (for Ca), potassium acid phthalate (acidimetric standard), tris(hydroxymethyl)aminomethane (alkalimetric standard), and monopotassium phosphate and disodium phosphate for the preparation of buffers of known pH. Others may be available in the future. These standards are rather expensive for daily routine use and are generally used to check the secondary standards, which are usually reagent grade chemicals or other specially purified chemicals available commercially. If NBS standards are not available, as pure a commercial product as obtainable is used.

If the standards are to be made up in solution,

they must usually be thoroughly dried and then weighed out on an analytic balance to at least 1 part in 5000. Stable materials such as inorganic salts and some organic chemicals can be dried for several hours in an oven at 110° C and then cooled in a desiccator over a good water absorbent such as anhydrous magnesium perchlorate. Other substances may be dried by placing them in a desiccator (preferably a vacuum type) over a desiccant for several days. No attempt should be made to dry bilirubin or urea. In making up aqueous solutions, a good grade of distilled or deionized water must be used as well as accurate volumetric flasks.

Many substances are available commercially as standards in solution that can be used directly or simply diluted to the proper concentration. Although such solutions have been generally found to vary less than 1% from the nominal value, they cannot always be uncritically assumed to be correct. If questionable results are obtained, the standards should always be checked against a solution made up in the laboratory or at least against a standard made by a different manufacturer.

One problem that sometimes arises with aqueous standards is that the biologic material being analyzed (such as serum) may contain interfering material not removed by preliminary treatment (such as the preparation of a protein-free filtrate) and thus may not react exactly the same as with the pure aqueous standard. There is no simple solution to this problem. Sometimes internal standards are necessary, but usually the correct level is taken to be that obtained when the determination is carefully run in comparison with the aqueous standard. The standardization of multi-channel automated instruments may present special problems. These are often standardized with commercially available lyophilized sera containing stated amounts of the desired constituents. The labeled amounts cannot always be uncritically accepted. They should be checked occasionally by running accurately prepared aqueous standards through the instrument when possible or by the analysis of the standardizing serum for the constituents by a good reference manual method.

Quality control in serology

The field of clinical serology has rapidly expanded from the initial syphilis serology and febrile agglutination tests to present-day immunology and such procedures as infectious mononucleosis test, C-reactive protein test, rheumatoid agglutination test, immunologic pregnancy tests, antistreptolysin (ASO) titer determination, and fluorescent antibody techniques.⁶

Quality control in serology is more difficult for the following reasons:

1. No standards are available, only controls.
2. Results obtained by one technique cannot be duplicated by another technique.
3. Some techniques are more sensitive than others—the microcomplement fixation technique is more sensitive than the agglutination technique, and the latter is more sensitive than the precipitation technique.
4. Various methods designed to detect the same antibody measure various properties of this antibody and thus vary in sensitivity; e.g., the antinuclear antibody of lupus erythematosus can be detected by a fluorescent method, by a clot method, and by nucleoprotein precipitation. Each method further varies according to the actual technique employed.

In serology, commercial kits are frequently used. It is mandatory to always employ negative and positive controls supplied by the manufacturer and augmented by internal positive and negative controls, which consist of previously assayed clinical specimens of which aliquots have been kept frozen.

The list of items to be checked in a quality control program in serology follows the established outline.

Equipment: Timers, centrifuges, shakers, water baths, thermometers, and fluorescence microscope (burning time of lamp).

Reagents: As commercial reagents are employed, the manufacturer's instructions regarding use, storage, and outdating must be carefully followed.

Specimens: Two sterile specimens are often required—an acute-phase specimen and a convalescent-phase specimen. Special precautions must be exercised at times in the collection of the specimen; e.g., cold agglutinins should not be stored in the refrigerator.

Personnel: Even though commercial kits are used, there should be a written procedure book available that adapts the manufacturers' instructions to the circumstances and equipment of a given laboratory.

Quality control in blood bank

Like all quality control programs, the program in blood banking can be divided into controls involving equipment, reagents, personnel, donor and recipient, and test procedures. Some features are unique to blood banks, as they deal with a biologic product.⁷⁻⁹

Equipment: Dated records should be kept of all control procedures.

Centrifuges: Most laboratories use modifications of a standardized Sero-Fuge* that only require checking of the speed and timer. The

*Clay-Adams, Parsippany, N. J.

speed should be kept at 3400 rpm and should be checked with a strobe-light tachometer. The timer should be tested against an accurate stopwatch. Vibration of the head should be checked, as it will dislodge the red cell buttons. Automated devices that wash, decant, and add Coombs serum are available but are difficult to calibrate. Coombs-positive control cells may be used to check the machine's efficiency.

View boxes: The use of the tube test is suggested for ABO and Rh typing, eliminating any worry about incorrect view box temperature or thermometer.

Refrigerators and freezers: Refrigerator temperature should be checked frequently and should remain between 2° and 6° C. The freezer temperature should also be regulated at -30° C. At -20° C the storage time of most reagents is limited to 6 months.

Water baths, heating blocks, and incubators: Certified thermometers should be used to control the temperatures and a daily log kept of the temperature readings.

Blood storage: A refrigerator should be devoted entirely to the storage of blood, blood products, and blood bank reagents. It must be equipped with an automatic temperature recorder and an alarm system tied to a hospital station that is manned day and night. The temperature must be kept at 4° C and must never exceed 10° C.

Glassware: All test tubes should be disposable glass tubes and used only once.

Reagents: The blood bank reagents (antisera, reagent test cells, enzyme preparations, etc.) are usually commercially prepared. The specificity, avidity, and potency of antisera, including Coombs sera, should be checked against specific cells. Antisera should be checked daily by the tube typing technique as follows: Anti-A₁, anti-B, group O, and absorbed anti-A sera should be tested against a panel of group A₁, A₂, B, A₂B, and O cells. Anti-A serum will react with A₁, A₂, and A₂B cells; anti-A₁ will react only with A₁ cells; group O will react with A₁, A₂, B, and A₂B cells; and anti-B will react with B and A₂B cells. Rh typing sera should be tested against Rh-positive, D⁺-positive, and Rh-negative cells. Antiglobulin serum will react with sensitized cells coated with varying amounts of anti-D. It should fail to react with nonsensitized cells.

All blood banks should have at least two sets of typing sera and of Coombs sera produced by different manufacturers.

Reagent red cells: A and B cells, Rh-positive and Rh-negative cells, and Coombs-positive and Coombs-negative cells should be tested against the corresponding antisera and their reactions graded on a scale from 1+ to 4+ (p. 294).

The tube method is preferred for ABO and Rh typing because it prevents evaporation,

allows an optimal ratio of antigen to antibody, and permits better temperature control.

Enzymes: The activity of proteolytic enzymes can be checked by their action on the gelatin layer of x-ray films (p. 541).

Personnel: Well-trained technologists must have available an up-to-date outline of blood bank procedures and policies and a current copy of *Technical Methods and Procedures* by the American Association of Blood Banks.

Donor and recipient: A log book must be kept that accounts for every unit of blood received. The following information should be recorded: date, source and code number of donor units, result of processing (ABO and Rh typing, VDRL, atypical antibody screen result, Australia antigen, etc.), result of compatibility testing, name of recipient, date of transfusion, and result of transfusion. Sequential numbering devices are commercially available for identification of donor blood, of pilot tubes, and of the recipient's blood samples.

If "outside" blood is used by the blood bank, the blood in the plastic segments should be used for retyping (ABO and Rh typing and antibody screening). The red cells obtained from the plastic segments must be washed in saline solution to remove the ACD solution before they are typed. Australia antigen and VDRL tests do not have to be repeated.

External control systems are available from blood bank supply houses and from the American Association of Clinical Pathologists.

Quality control in urinalysis

In the urine laboratory there should be available an up-to-date procedure book that not only includes the approved procedures but also the corresponding references and source and grade of chemicals used. All chemicals and dipsticks should be appropriately stored and labeled as to date of purchase, manufacturer, and date of expiration if applicable.

Many of the erroneous results of urinalysis stem from errors in collecting and handling the urine specimen. The specimen should be an early-morning midstream sample and should be examined within 30 min of the time it was obtained. The specimen should not be subject to various delays in the patient's room, at the nurses' station, or on the laboratory bench. If delay is unavoidable, the specimen should be refrigerated. Allowing urine to stand at room temperature tends to encourage precipitation of phosphates in alkaline urine, bacterial growth with concomitant change to an alkaline pH, photodegradation, oxidation, and hydrolysis.

Before testing, mix urine specimen well. The various semiquantitative tests for urinary protein, glucose, ketones, phenylpyruvic acid, occult

blood, pH, etc. should be checked from time to time using solutions containing known quantities of these substances. A relatively stable urine control is now commercially available* that provides a means of evaluating the daily accuracy of methods used for determining urinary pH, protein, glucose, ketones, hemoglobin, bilirubin, urobilinogen, and phenylketones.

Standardization of urinometers and of refractometers used in specific gravity determination is discussed on pp. 19 and 20.

Quality control in coagulation

All clotting procedures must be standardized to a high degree, including such apparently unimportant details as frequency and degree of tilting a test tube to observe clotting. Normal controls must be run with each test, and both test and control should be performed in duplicate.

Equipment:

Water bath: The thermostatic control must maintain a constant temperature of 37° C. A thermometer should be kept in the water bath and checked frequently. The water bath must be wide enough to accommodate an adequate number of test tubes and should allow free visualization of the tubes by indirect light. The fish tank type of water bath with flat glass surfaces is preferred.

Glassware: Glassware must be scrupulously clean and should not be scratched. Disposable plastic tubes are ideal for clotting procedures. Accurate, slow-emptying pipets are important, since the average amount handled varies from 0.1-0.3 ml. Test tubes should be uniform because variation in size alone may alter the result of coagulation tests, e.g., the disintegration of a blood clot in the fibrinolytic test.

Reagents: Blood must be carefully obtained without contamination by tissue thromboplastin and without stasis. The proportion of anticoagulant to blood must be accurate. The specimen should not be exposed to 37° C temperature for longer than 5 min. It can be kept in the refrigerator as long as 2 hr. Immediate return of a blood specimen to the laboratory is imperative. Best results are obtained if the blood is drawn in the laboratory. If this is not possible, double-walled jars with ice-water jackets are taken to the patient's room.

Method for visualization of clotting: An electric clot timer is suggested. Another method which, with experience, is reproducible is 60-degree tilting of the test tube at regular intervals in the water bath until the clot forms. This method is somewhat difficult if clot formation is poor, e.g., in hypofibrinogenemia. Excessive agi-

tation must be prevented. A diffuse light source is important. The platinum wire method is reproducible and can often be mastered quickly.

Centrifugation: As the number of platelets in plasma depends on the speed and size of the centrifuge, centrifugation should be carefully standardized. For many purposes a refrigerated centrifuge is advisable, or a small centrifuge can be placed in a refrigerator.

Siliconization: To preserve the number and function of platelets, contact with a rough glass surface must be prevented by a nonwetttable surface such as provided by siliconization. Smooth plastic surfaces or paraffinized areas closely duplicate the effectiveness of siliconized material.

Technique: Details such as temperature, pH, and timing must be carefully standardized. The standard deviation of various tests should be determined after repeating the same test at least 15 times. (See discussion of quality control in clinical chemistry.)

Quality control in hematology¹⁰

The sources of error in hematology can be classified as follows:

1. Errors related to methods of sampling. Capillary blood obtained from an inadequate puncture wound may lead to counts that are lower than those obtained from venous blood because of dilution with tissue fluid when the puncture site is squeezed.

2. Errors in preservation of the specimen. Improper mixing, inadequate or excessive amounts of anticoagulant, and incorrect type of anticoagulant as well as a leaking stopper may lead to inaccurate counts and hematologic tests.

3. Technical errors related to the instruments.¹¹ Inaccurate or dirty pipets, inadequately standardized automatic pipets, unmatched or dirty cuvetts, and poor standards may all add up to inaccurate laboratory work.

Many of the errors related to a specific test such as white count, red count, and hematocrit are included in the discussion of the various tests.

Equipment such as centrifuges, cell counters, and diluters should be checked according to a definite preventive maintenance plan based on manufacturers' instructions.

Methods for detection of errors

Controls are available for hemoglobin determination, hematocrit, white blood cell count, and red blood cell count.

Celltrol* is a control for red blood cell counts and hematocrit determinations. It is available in normal and below-normal range values and serves as a control of technique as well as of equipment.

*Tek-Chek, Ames Co., Elkhart, Ind.

*Chas. Pfizer & Co., Inc., New York, N. Y.

A similar product, Leukotrol,* is a suspension of stabilized white blood cells available in normal and above-normal range values of white cell counts. Both products can be used as controls for manual and electronic counts.

Daily analysis results of hematology controls are plotted on a graph to show daily variations and to indicate any possible trend toward inaccurate results. All control tests are done in duplicate, and the standard deviation of duplicate tests should be calculated daily. (See discussion of quality control in clinical chemistry.)

The mean corpuscular hemoglobin of normal blood can also be used as a control. The mean and the standard deviation can be recorded daily.

Acuglobin† is a hemoglobin standard consisting of a solution of cyanmethemoglobin and is useful for daily standardization of hemoglobin determinations. It is suggested that the standard be diluted 50% with Aculte diluent† to include low hemoglobin values in the control setup.

Estimation of total white cell count from stained blood smear:

No./hpf	Estimated total count
2-4	4000-7000
4-6	7000-10,000
6-10	10,000-13,000
10-20	13,000-18,000

Fragile leukemic cells may disintegrate in the Coulter counter; therefore the total white count should be estimated from the slide as a check on the Coulter counter result.

Estimation of platelets: A peripheral blood smear may be used to judge the platelet count:

- Less than 1 platelet/oil-immersion field—decreased number of platelets
- Several platelets with occasional clumps—adequate supply of platelets
- More than 25 platelets—increased number of platelets

If one counts the number of platelets in 10 oil-immersion fields and multiplies the total figure by 2000, the result closely approximates the platelet count as obtained by the Rees-Ecker method.

The standard deviation of various tests should be determined and the results of normal, high, and low controls plotted daily on graph paper. (See discussion of quality control in clinical chemistry.)

Quality control in microbiology

Most of the points described in detail in this section apply, with some variation, to all phases of laboratory work.

Safety rules. Quality control begins with a properly designed bacteriology laboratory equipped with a safety hood that pulls air from the laboratory to the outside. A number of safety rules should be enforced at all times when working with bacteria, such as careful technique, the use of safety pipetting devices, and periodic washing of all equipment with 2% phenol solution. An alcohol-sand flask should be available for cleaning inoculating loops prior to flaming. All bacteriologic waste material must be autoclaved prior to leaving the department. Contaminated swabs or dressings must be wrapped before disposal.

Quality control procedures must be observed in the handling of every specimen from collection and transportation to the laboratory to handling by the technologists, plating, choice of media, recording of findings, and incorporation of the findings into the patient's record.

Collection: A representative, adequate specimen must be obtained from a suitable source or area under conditions that prevent contamination. It must be collected in sterile, labeled, covered containers that allow ready access to the material. Material may also be collected on Culturette* swabs.

The label on the specimen and the request must not only identify the patient but also state the source of the material and the time when it was obtained. The material should be sent to the laboratory immediately and not be allowed to remain at the nurses' station. After its arrival in the laboratory, it should be handled immediately.

Transportation: Transport media are not needed if the culture can be taken at the bedside or in the outpatient department and sent immediately to the laboratory for processing. When specimens are collected in areas removed from the laboratory, Culturette rayon-tipped swabs should be used, which, after the specimen is obtained, are kept moist by a modified Stuart transport medium. The medium provides a moist environment for 72 hr without supplying nutrients. If *Neisseria gonorrhoeae* is suspected, the material should be streaked directly on Transgrow medium¹²; if *Shigella* is suspected, the material should be streaked directly on XLD medium.¹³

Equipment: Written records should be kept of the CO₂ content (which should be from 5-10%) of CO₂ incubators and of their temperature (which should be 35°-37° C, 35° C being the preferred temperature). CO₂ analyzers are commercially available. Autoclaves, refrigerators, and freezers must all be kept in top working condition and monitored daily.

*Chas. Pfizer & Co., Inc., New York, N. Y.

†Ortho Pharmaceutical Corp., Raritan, N. J.

*Scientific Products, Evanston, Ill.