NINTH EDITION

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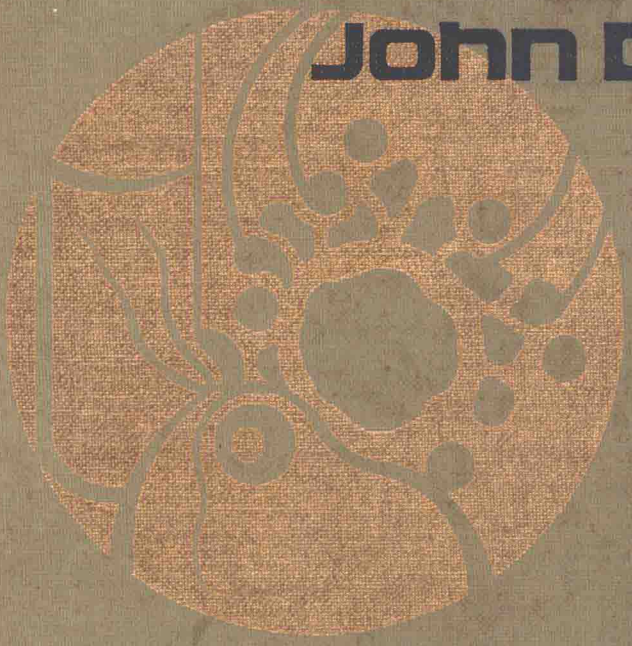
John D. Bauer



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Associate Professor of Pathology, Washington University School of Medicine; Chairman, Department of Laboratory Medicine, DePaul Community Health Center; Director of Laboratories, Faith Hospital and Central Medical Center, St. Louis, Missouri; St. Peters Community Hospital, St. Peters, Missouri

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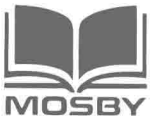

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Preface

This, the ninth edition of *Clinical Laboratory Methods*, appears 8 years after the last edition. During the intervening years there has been a tremendous growth in all phases of laboratory medicine, spearheaded by new developments in instrumentation and technic. The present edition reflects the breadth and depth of these advances, since it is updated and almost completely rewritten by a number of expert contributors. The format and the organization of the material are new; the subject matter is grouped into units that reflect typical laboratory organization. The units are divided into chapters, each one highlighted by a short introductory outline. Outdated and infrequently used tests have been eliminated, and new chapters dealing with safety, platelets, bone marrow, immunology, and serology have been added. The chapter on toxicology and therapeutic drug monitoring is entirely new.

In Unit One quality control procedures employed in each subdivision of the clinical laboratory are summarized and are then discussed in each unit in greater detail if deemed necessary. In Chapter 2 of this unit Mildred G. Hutchinson, M.T., A.S.C.P., offers suggestions and guidelines for safety in the laboratory.

In Unit Two, Hematology, I emphasize the role of automation, including automated differential counters. The discussion of hemoglobin includes a current list of hemoglobin variants and a section on glycosylated hemoglobins and their use in the evaluation of diabetic patients. The investigation of hemoglobinopathies includes electrophoresis of globin chains and isoelectric focusing. The sections dealing with disorders of red and white cells have been expanded and include a discussion of serum ferritin and the investigation of functional disorders of polymorphonuclear leukocytes. Functional disorders of lymphocytes and complement are discussed in Unit Six. Detailed instructions in how to proceed in the investigation of hemolytic anemias are contained in Units Two and Three. The descriptions of morphology, function, and pathology of the cellular components of blood are extended by electron-microscopic, phase-microscopic, and cytochemical investigations, which reveal not only structural details but also enzymatic and antigenic properties. The chapter on white cell disorders deals in detail with the leukemias and offers among other classifications the French, American, and British Cooperative Working Group classification of acute leukemias. Mention is made of the newly surfaced concept of preleukemia. Among the chronic leukemias, space is devoted to a discussion of hairy cell leukemia. The lymphoreticular disorders include Sézary syndrome and T and B cell malignancies. The monoclonal immunoglobulin disorders embrace heavy- and light-chain diseases as well as the rather common benign monoclonal gammopathy. Space is devoted to the role of the Epstein-Barr (EB) virus in the etiology of infectious mononucleosis and to its serologic expressions. The chapter on bone marrow has information on bone marrow culture and cytogenetic studies and their uses in the diagnosis of leukemia and preleukemia.

Three chapters of Unit Two are dedicated to hemostasis. They contain the latest information on the role of platelets in hemostasis, on the newer Fitzgerald and Fletcher factors, on the kallikrein-kinin system, and the enzyme nature of some of the coagulation factors and their complexes. Naturally occurring inhibitors, including antithrombin III, are contrasted with acquired inhibitors of coagulation factors. The assay procedures include single-factor assays and the latest methods using synthetic chromogenic or fluorescent substrates. The discussion of idiopathic thrombocytopenic purpura also mentions the Coombs' antiglobulin test to detect platelet-associated IgG and C3. A section on hypercoagulability follows the discussion of bleeding disorders.

Unit Three, by Kathleen S. McLaughlin,

M.D., reflects the new technology of blood banking and has been completely revised and reorganized into six chapters. Chapter 14 examines current biochemical data demonstrating basic differences between red cell antigens, allowing them to be categorized into families, or blood group systems. Basic serologic testing of the ABO and rhesus systems is again included with a greater discussion of discrepancies between ABO serum and cell testing; the problem of polyagglutination is discussed in detail with appropriate test procedures included. For the first time, a discussion of the complex system of tissue antigens is included with its relevance to human disease indicated. Chapter 15 discusses general basic transfusion practices and methods. It includes the newest anticoagulants that are available, with their impact on blood storage. Much greater emphasis is placed on cellular blood components and plasma fractions; the methods of preparation are included with the advantages and disadvantages of each. Newer concepts in transfusion practices are discussed for the first time, including the use of low–ionic strength solution, the elimination of some routine cross matching steps, the concept of a type and screen program, and the usefulness of autologous transfusions. Hepatitis testing has been updated with a discussion of third-generation test procedures. Chapter 16 is devoted to specialized test procedures, many of which are invaluable to current blood bank serology. The basic concepts of immunology, as they relate to blood banking, are found in Chapter 17. Chapter 18 deals primarily with clinical diseases as they pertain to erythrocyte antigen-antibody reactions. The discussion of isoimmune hemolytic disease of the newborn has been updated, and autoimmune hemolytic anemia is presented for the first time, as is the very difficult problem of drug-induced red cell sensitization, which can cause great difficulty in the interpretation of basic blood bank procedures. Chapter 19 pertains to the problem of adverse reactions to incompatible blood transfusions. A systematized approach to a workup of a transfusion reaction is included, and for the first time there is an extensive discussion of nonhemolytic transfusion reactions as well.

Unit Four, the section on clinical chemistry, is the result of my collaboration with Philip G. Ackermann, Ph.D., David C. Hohnadel, Ph.D., Lawrence A. Kaplan, Ph.D., Evan A. Stein, Ph.D., M.D., and Alphonse Poklis, Ph.D. The initial five chapters in this unit are the result of efforts by Drs. Ackermann and Hohnadel. Chapter 20, on laboratory instrumentation, has been updated to include an expanded discussion of the principles of gas-liquid and high-performance liquid chromatography. Chapter 21, on carbohydrates and nitrogen compounds, contains all tolerance tests and the current classification of glucose intolerance recommended by the National Diabetes Data Group. The manual methods presented have been updated to current, nonbiased methods that can easily be used in any but the largest laboratories requiring automated technic. In Chapter 22, on inorganic elements and blood gases, a calcium colorimetric method replaces the titration method and is joined by methods for ionized calcium. Also included are contemporary methods for copper, magnesium, zinc, serum iron, and iron binding. Newer electrolyte instrumentation is described, including those instruments using cesium internal standards, those using ion electrodes, and those using electrochemical half cells.

Chapter 23, on liver functions, now includes a direct spectrophotometric method for bilirubin for neonates and an enzymatic method for triglycerides followed by a procedure for high-density lipoprotein cholesterol and calculations for very low–density lipoprotein cholesterol and low-density lipoprotein cholesterol. Chapter 24, on enzymology, has been completely rewritten and reorganized, so that the assays for the various enzymes are grouped under the disease entities in the diagnosis of which they are primarily used. Sections have been added for lactate dehydrogenase (LDH), creatine phosphokinase (CPK), and amylase isoenzymes. Virtually all enzyme assays presented employ continuous monitoring kinetic technics.

Chapter 25, on hormone analysis, results from the collaboration of Drs. Ackermann and Kaplan and presents new concepts in the utilization of nonisotopic methods, such as enzyme-linked immunosorbent assays (ELISA) and enzyme inhibition assays (EIA), procedures that are also mentioned in Chapter 35, Immunology.

Dr. Poklis has written an entirely new chapter on toxicology and therapeutic drug monitoring. A systematic approach to toxicologic screening, which involves analysis of urine and blood and toxic metal determination, is presented. The qualitative urine screening procedures discussed include confirmational technics for all drugs pre-

sented and require only generally available instrumentation, such as thin-layer chromatography and ultraviolet spectrophotometric and fluorometric capabilities. Using the methods presented, even a moderately sized laboratory can offer a significant toxicology service for emergency urine drug screening. Numerous new procedures for drugs not previously considered are offered, such as for acetaminophen, benzodiazepines, antidepressants, sedatives, and hypnotics. The section on thin-layer chromatography allows the technologist to choose one of several developing and extraction systems to focus on the detection of drugs of primary interest.

Chapter 27, on urinalysis, which I revised, is introduced by a section on kidney structure and formation of urine, followed by carefully revised and expanded discussions of the tests for urinary constituents.

With the welcome assistance of Dr. Kaplan, I also revised the chapter on semen analysis, pregnancy tests, and placental hormones.

The contributors are also responsible for the chapter that deals with the examination of biologic fluids. The section on spinal fluid analysis offers new insight into the use of pH, glucose, lactic acid measurements, and the IgG-albumin index. In the amniotic fluid analysis, the tests for L/S ratio are updated and the foam stability test as a screen for fetal maturity is presented.

Chapter 30, on gastric, duodenal, and pancreatic juice analysis, by Drs. Ackermann and Stein, presents the stool guaiac test as a screening procedure for colon cancer as recommended by the American Cancer Society, the serum carotene assay as a screening procedure for fat malabsorption, the serum D-xylose procedure for carbohydrate malabsorption, and the use of stool electrolyte analysis in malabsorption syndromes. An expanded discussion on gastrin increases the value of the section on gastric and duodenal juice analysis.

Unit Five, Microbiology, is the result of my collaboration with Raymond F. Gray, Ph.D., and Richard C. Tilton, Ph.D. The discussions of quality control, *Legionella, Campylobacter,* and rapid immunochemical tests for the identification of infectious diseases have been expanded and updated. All taxonomy has been made current. Chapter 33, on mycology, has been carefully revised, and the taxonomy of fungi has been reviewed for correct usage. Chapter 34, on parasitology, was critically reviewed by Lynne Shore Garcia, A.B., M.T. (A.S.C.P.), and I am grateful for the improvements and additions she suggested. *Dientamoeba fragilis* is classified as an intestinal flagellate that may be responsible for attacks of gastroenteritis. The section dealing with free-living amebae is brought up to date as are the discussions of toxoplasmosis, *Pneumocystis,* babesiosis, and serologic methods in parasitology. A section on arthropods of medical importance completes the chapter.

Unit Six appears in this edition for the first time. Chapter 35, on clinical serology, spans the period between the earliest precipitation and agglutination tests and the most up-to-date enzyme immunoassays and laser nephelometry. Special thanks go to Alex C. Sonnenwirth, Ph.D., who permitted the use of material he published in Part XI of *Gradwohl's Clinical Laboratory Methods and Diagnosis,* ed. 8, edited by A.C. Sonnenwirth and L. Jarett. Chapter 36, on clinical immunology, by Cheng C. Tsai, M.D., and me, reflects the tremendous growth in this area of laboratory medicine. It includes the latest methods of lymphocyte typing, the harvesting of these cells, and a discussion of their disorders. Emphasis is placed on fractionation of antinuclear antibodies, quantitation of circulating immune complexes, and membrane receptor studies of frozen tissue sections.

I would like to acknowledge, with gratitude, the assistance of several persons, in particular, Carolyn Humphrey, Cathy Metz, Wendy Hoffman, and Nora Carlisle, whose efforts were essential. I would also like to thank Glenn Humphrey, who did the new line drawings for this edition.

To my wife, Marjorie, your patience and understanding are appreciated.

John D. Bauer

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COLOR PLATES

UNIT ONE

INTRODUCTION

CHAPTER 1

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, the hospital number, the type of specimen, the clinical diagnosis, the physician's name, and the specific examination desired. Knowledge of the patient's race is helpful at times (for instance, in hematology). Three time slots on the requisition should indicate time and date when request was issued, when the specimen was obtained, and the time, date, and by whom the test was completed.

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

Good work records are a must. They 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; in bacteriology, include the various media used and the reactions of each.

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

Common errors and their elimination

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

1. *Improper identification of patients or specimens.* Proper identification of the patient before obtaining samples of blood or other body fluids is essential. The name on the requisition must always be checked against the name on the patient's wristband. If the patient does not have a wristband, identification must be confirmed by a member of the nursing staff. This may not seem essential if the same technician is frequently obtaining samples from the same patient, but it is good practice to always make the check. Whenever two or more patients in the hospital have identical or very similar names, a notice should be posted in the laboratory. After collection of the specimen the tube or other container must be properly labeled with the patient's full name and room number (or some other identifying number). The labels on the tubes must be such that they cannot be easily removed or fall off. The proper type of specimen must also be obtained, with the minimal amount of hemolysis. If on return to the laboratory the specimen is to be divided into two or more aliquots for different types of tests, care must be taken to ensure proper identification and labeling.

2. *Common methodologic errors.* These may include the following:

   a. Failure to adhere to established rules and procedures
   b. Use of improperly or poorly tested procedures that appear to be satisfactory in the normal range but are inadequate in the pathologic range
   c. Use of reagents that are outdated, improperly prepared, or labeled or are stored in the wrong type of container or at the wrong temperature
   d. Failure to do regular instrumentation preventive maintenance, to properly check heating bath temperatures, spectrophotometer wavelength scales, and linearity of photometric response in the spectrophotometer, and to keep all instruments free from dirt and moisture
   e. Lack of familiarity with basic arithmetic calcula-

tions (Use of an inexpensive pocket calculator may result in greater speed and accuracy.)

f. Transcription errors, which can occur at many points in the system: in writing down the actual instrument readings, results of calculations, entries into the log book or reporting system

QUALITY CONTROL

Quality control is an all-embracing concept that includes much more than the elimination of errors or 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 a reasonable cost.[1,2]

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

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

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, housekeeping, public relations, purchasing, the "storeroom," the pharmacy, surgical supplies, the medical staff, and laboratory representation on appropriate committees.

3. Laboratory policies and procedures should be collected in a manual to be revised at least once a year or when procedures 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, technic, 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, standard deviation calculations, and comparison of results to internal and external standards. 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. External standards are available from many sources such as the U.S. Public Health Department, the College of American Pathologists, the American Society of Clinical Pathologists, and commercial laboratory supply houses.

For a more complete discussion of the preceding points the book by Dharan[1] is recommended.

Factors involved in quality 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, then this procedure is accurate. **Accuracy** is defined as the closeness of test results to the true value and implies freedom from error.

Control: Controls (both 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 being 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 (e.g., urine sugar, blood sugar, and urine culture tests) obtained in other sections of the laboratory.

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

Laboratory personnel: Board certification or the equivalence for 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; job descriptions.

Equipment: Adequate work and bench space; preventive maintenance of equipment according to written

*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.

schedule, with defects recorded and corrective measures taken. Maintenance procedures should be followed on balances, spectrophotometers, photofluorometers, centrifuges, automated equipment, incubators, water baths, microscopes, refrigerators, thermometers, and pH meters.[3]

Laboratory tests: Speed; economy; reliability; ease with which they can be performed by personnel of various training levels.

Reagents: Identity; concentration; purpose; date of purchase or preparation; manufacturer; expiration date; initials of technician who prepared reagent; standard used to check accuracy; distilled water check.[4]

Clinical chemistry

The preceding general remarks apply to all branches of the clinical laboratory and thus to the chemistry laboratory. In addition, a number of other points apply particularly to the chemistry laboratory and are discussed here.[5,6] Among the mistakes that may cause appreciable assay errors are (1) prolonged application of the tourniquet before drawing the sample, (2) undue exposure to extremes of temperature, (3) mechanical shock or bright sunlight during transport of the specimen, (4) prolonged centrifugation (which may result in undesirable heating), (5) unduly prolonged contact between the serum and clot, and (6) keeping the samples in open tubes for many hours. (Significant evaporation can occur under some circumstances from open tubes, changing the concentration of the constituents. In addition, many enzymes and some other constituents are rather unstable, and improper storage conditions may cause rapid deterioration.)

Any discussion of quality control programs in the clinical laboratory should include certain statistical concepts.[7] For example, if a large number of assays of a single sample or of supposedly identical samples are made (as is done when daily measurements are made of aliquots of the same serum), it will be found that, because of random errors, not all measurements will yield precisely the same result. The results will tend to cluster around some particular value. Most of the results will be close to this value, some will be slightly farther from the value, and a few will be even more distant. If a large number of determinations are made, the distribution of values usually tends to follow a theoretic curve, often known as the gaussian curve (Fig. 1-1). The various values found are plotted against the frequency of occurrence. The value arbitrarily labeled "100" in this plot is the one most frequently found, and values differing appreciably from this are less frequent. This is an idealized curve; in actual practice there would not be an infinite distribution of values but a discrete distribution such as that shown in Fig. 1-2. In theory this would approach the true curve as the number of measurements is increased to a very large value. It is only an assumption that the values obtained in any actual case do follow the gaussian curve, but this assumption works out fairly well in practice, at least for the type of distributions we are considering here. If we assume that the actual data do follow the theoretic curve, we can make some interesting mathematical deductions. The true gaussian curve is determined completely by only two parameters, which are conventionally designated by the two Greek letters μ and σ.

The first parameter, μ, which is the mean value, governs the position of the maximum of the curve in reference to the range of values. In Fig. 1-1 μ is arbitrarily set at 100. The best estimate of μ from the actual data is the average value of the determinations as usually calculated. This is usually designated as

$$\mu \cong \bar{x} = \sum^{n} x_i/n$$

where x_i indicates the separate values of the series of numbers, x_1, x_2, x_3, . . . x_n, or in general x_n and $\sum^{n} x_i$ denote the sum of these n numbers. The mean or average is this sum divided by the number of observations.

The other parameter, σ, is a measure of the spread of the values. In Fig. 1-1 two curves have been plotted with different values for σ (5 and 2.5 for the solid and dashed curves, respectively). The dashed curve with the smaller value for σ has a smaller spread, with very few values under 92 or over 108; however, in the distribution indicated by the solid curve ($\sigma = 5$), an ap-

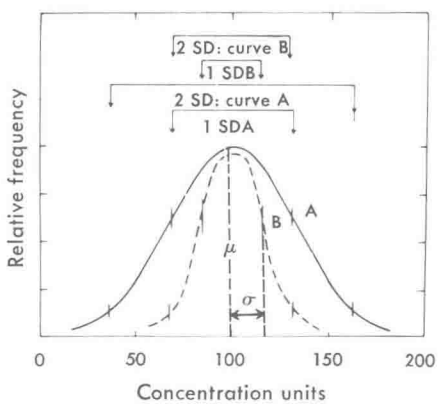

Fig. 1-1. Two typical gaussian curves. Curve A has twice the standard deviation (SD) of curve B. The ranges of 1 and 2 SD are shown for each curve.

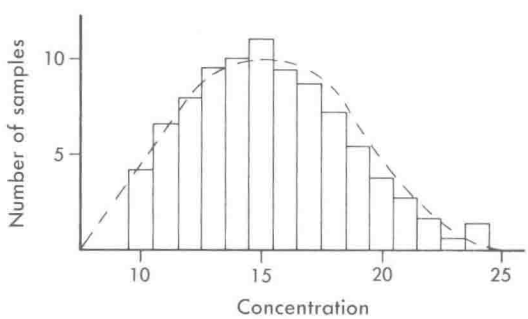

Fig. 1-2. Gaussian curve frequency histogram of hypothetical experimental results in the determination of a normal range.

preciable number of values are beyond these points. The σ value is generally termed the standard deviation (SD); an explanation of the actual method of calculating standard deviation is given later.

The Greek letters μ and σ are used for the theoretic values of the mean and standard deviation as derived from the true curve, and the English letters *m* and *s* (or *M* and *S*) refer to the calculated values from the experimental data. If the data conform closely to the theoretic curve, an average of 68.3% of the values should be within ± 1 SD of the mean and 95.5% within ± 2 SD. Furthermore, almost all of the values (99.7%) should be within ± 3 SD from the mean (Fig. 1-1). For some purposes the standard deviation is expressed as a percentage of the mean value: 100 SD/M. This is often called the *coefficient of variation* (CV), but a better term is *relative standard deviation* (RSD). The purpose of any quality control program is to check the precision of the measurements by noting whether repeated analyses of aliquots of the same material remain within certain definite limits. A limit of ± 2 SD (as calculated from a series of measurements) is often used, but other limits may be used as well.

In addition to standards for each substance being determined, a quality control program for analytic determinations requires one, or preferably two or more, control substances containing the constituent being analyzed. These are usually similar in nature to the body fluid being analyzed (serum, urine, etc.) and are analyzed with the regular samples in exactly the same way. Standards are needed whether or not a quality control program is used; therefore a discussion of standards follows.

The **standards** for use in chemical analysis may be aqueous solutions or standards in serum (or other body fluids if required). Aqueous solutions can be used for many manual testing methods (except for those testing lipids and enzymes), but the automated methods generally require standards in serum. Aqueous standards may be prepared in the laboratory or purchased, usually as stock solutions from which the actual standards can be prepared by dilution. The latter is usually preferable; however, larger laboratories may have the proper facilities for accurate weighing. Care must be taken in the dilution of aqueous stock standards. If the stock solutions are kept in the refrigerator, they must be warmed to room temperature before diluting. The stock solutions purchased are usually quite accurate, because they are prepared by weighing the proper amount of required chemical; when this is done in large batches, the error in weighing and dilution is small. Occasionally, one might suspect a stock solution to be in error. Even if the laboratory does not routinely prepare its own standards, it is helpful to be able to do so to check a purchased standard. Standard in serum are more satisfactory because they tend to compensate for the matrix elements present in the regular serum samples.

Most automated methods require a standard that is prepared in serum and contains all the constituents to be determined by the system. This is usually obtained as a lyophilized serum, which is reconstituted as needed. Most sera used for quality control are of a similar nature, and most of the following comments will apply whether the serum is used as a standard or as a quality control sample. The lyophilized material is obtained in small vials, which are stored in the refrigerator. The material usually can be obtained in several different vial sizes; the size to be used depends on the needs of the laboratory and the volume of tests requiring the serum for standard or control. The lyophilized material is reconstituted with water or, if carbon dioxide is one of the labeled constituents, with a standard ammonium bicarbonate solution. The diluting fluid should be carefully added with a volumetric pipet and the vial gently swirled; then the vial should be allowed to stand and should be occasionally swirled until the contents are completely in solution. It is not advisable to shake the vials vigorously, because this tends to inactivate some of the enzymes and the foam produced may prove troublesome. If bilirubin is one of the constituents, exposure to strong light should be avoided. The vials should be kept tightly stoppered and refrigerated when not in use. Under these conditions, many vials will be suitable for use on two consecutive days if reconstituted one morning. The stability should be checked by comparing the results from a newly reconstituted vial with one that has stood for 24-36 hr. In my experience, of the common constituents, those most likely to change under these conditions are glucose, which may decrease some 10% in value, and alkaline phosphatase, which will increase 15% or more in value. Tanishima et al.[8] have shown that reconstituting the lyophilized material with an equal-volume mixture of water and ethylene glycol will prevent changes in the level of alkaline phosphatase. This may also prevent other changes, and it might be helpful to compare the results obtained on other constituents with material reconstituted with water and with the water–ethylene glycol mixture. A serum standard prepared in a water–ethylene glycol mixture that does not require further reconstitution and is said to be quite stable under proper storage has been introduced on the market (Decision Chemistry Control Serum, Beckman Instruments, Fullerton, Calif.).

A number of different enzyme units are still in use for many of the common enzymes; when using a lyophilized serum as an enzyme standard or control, make certain that the enzyme values given on the package insert apply to the method used in the laboratory. Most of the control serums (except those made for a particular type of automated system) list the enzyme values by several different methods. Correction can be made if needed for differences in temperature, but these are not always reliable even when the appropriate factor is available.

Quality control sera can usually be purchased in different ranges: "low," "normal" "high," and "abnormal." The "high" levels are usually considerably higher than the normal values for most constituents. However, the "abnormal" values are not always higher; they may even be lower than the normal values if such lower values are of clinical significance.

When using any of the commercial control sera, a contract should be arranged with the manufacturer or

supplier to set aside a sufficient amount of one lot number of each of the types of sera desired so that the same lot number can be used for at least 1 year. Frequent changes in the lot numbers of sera used in quality control add to the difficulties in implementing a good quality control program. The billing and delivery for the sera can usually be made on a monthly or bimonthly basis.

Another type of material that has been used for quality control is a pooled serum prepared in the laboratory. Excess nonhemolyzed sera without gross hyperlipemia are collected daily in the laboratory and pooled for storage in the refrigerator. When 1-2 L has been collected, the serum is centrifuged to remove gross contamination and then filtered through rapid paper. The mixed filtrate may then be divided into 5 ml aliquots, placed in tightly stoppered tubes, and stored at $-20°$ C. The aliquots are thawed as needed and mixed well before use. This material has several disadvantages. It is not as stable as the lyophilized material and requires some effort for preparation. Also it usually does not have elevated values for any constituents and may have undesirably low values for glucose and some enzymes. (A control serum with elevated levels is desirable, because the instrument may give correct values in the normal range for a constituent but may give false low or high values in the elevated range.) Attempts can be made to concentrate the serum somewhat by partially freezing the pool and pouring off the serum from the ice crystals. In addition, the serum can be "spiked" with constituents such as glucose, urea, creatinine, and the inorganic elements; however, this is not so successful with bilirubin, cholesterol, and the enzymes. Virtually the only advantage of the serum pool is that it is relatively inexpensive. It is sometimes used as a preliminary check before using the regular control serum in some types of automated equipment.

What are the proper limits for the control serum analyses? Often the limits are taken as ± 2 SD from the average determined by the analysis of a number of control serum samples by the regular method used. However, no matter how imprecise a method may be, 95% of the results will by definition fall within 2 SD of the mean, and the question of what is a "reasonable" variation remains. One of the earlier suggestions was that of Tonks,[9] who proposed that twice the coefficient of variation for repeated analyses of a constituent should not exceed one fourth of the difference between the limits of the normal range for that constituent expressed as a percentage of the midpoint of the range. For example, if the normal range for serum calcium is taken as from 8.5-10.5 mg/dl, then twice the relative standard deviation for the control serum determinations would be

$$\frac{10.5 - 8.5}{4} \times \frac{100}{9.5} = 5.3\%$$

For a control serum having a stated value of 10.3 mg/dl, the acceptable range would be

$$10.3\ (1 + 0.0533) = 10.3 \pm 0.55$$

or from 9.75-10.85 mg/dl. If the normal range of serum sodium is from 135-145 mmole/L, then the 2 RSD for the control serum would be

$$\frac{145 - 135}{140} \times \frac{100}{140} = 1.8\%$$

and the acceptable limits for a control serum having a stated value of 138 mmole/L would be

$$138\ (1 \pm 0.018) = 138 \pm 2.5$$

or from 135.5-140.5 mmole/L. This procedure leads to a 2 SD of 1.8% for sodium and chloride, 5% for calcium, and 7% for glucose, total protein, urea nitrogen, potassium, inorganic phosphate, and albumin. Whenever the calculated limits are greater than 10%, a value of 10% is used. (If the normal range for a constituent is narrow, this constituent must be determined more precisely to give relevant information about borderline values than when the range is large.) Although this concept has not been too widely accepted, it offers some idea as to what the standard deviation should be for control purposes. The calculations break down for substances such as creatinine and bilirubin for which the normal levels are around 1 mg/dl. A range of 2 SD calculated by the above formula would be too narrow for many procedures.

Another way to determine acceptable limits for a quality control program is to examine what limits other present-day methods obtain in the analysis of survey samples. In a survey of the 1974 ASCP program,[10] the results of the analyses of 13 common constituents in sera at various levels of concentration were subjected to computer analysis and the average relative standard deviation determined for the different analyses (Table 1-1). The methods of analysis were divided into two groups: manual methods and automated methods. Since the analyses for each constituent were made at different levels of concentration, regression equations were developed relating the relative standard deviation to the concentration level. In some instances the relative standard deviation was independent of the concentration level; in others it varied, though not necessarily in a linear manner. The equations were used to calculate the relative standard deviation for round values of the concentrations as presented in Table 1-1. Since these figures are merely intended to be guides and not absolute limits, linear interpolation can be made between the concentration limits given. Thus, for example, in an automated analysis of a serum having a total protein content of 6.5 g/dl, the relative standard deviation may be estimated to be about 2.4% and the actual standard deviation to be $6.5 \times 0.024 = 0.16$ g/dl. The values calculated from Table 1-1 should give a good approximation of a desirable standard deviation, and if in any instance the actual standard deviation found was more than about 1.2 times these calculated values, the method and procedure should be carefully examined for errors or deficiencies.

After an average or "target" value for each constituent has been established, the control serum is analyzed each day along with the regular samples. The number

Table 1-1. Average relative standard deviation values found for various analyses*

Constituent	Concentration level	Manual method (%)	Automated method (%)
Albumin	2.0 g/dl	5.6	4.9
	5.0 g/dl	5.6	2.8
Bilirubin	0.7 mg/dl	18.0	15.0
	1.0 mg/dl	14.0	13.0
	1.5 mg/dl	10.0	6.3
	2.0 mg/dl	7.5	5.0
	6.0 mg/dl	5.6	4.8
BUN	10.0 mg/dl	7.5	4.9
	50.0 mg/dl	6.5	3.6
	80.0 mg/dl	5.8	4.6
Calcium	8.0 g/dl	3.4	2.6
	12.0 g/dl	3.4	2.6
Chloride	80.0 mmole/L	2.3	1.6
	120.0 mmole/L	1.7	1.6
Cholesterol	120.0 mg/dl	4.5	4.6
	220.0 mg/dl	4.5	4.6
Creatinine	1.0 mg/dl	9.2	7.9
	1.5 mg/dl	8.3	6.2
	2.0 mg/dl	7.5	5.0
	6.0 mg/dl	5.3	3.6
Glucose	70.0 mg/dl	4.4	4.0
	160.0 mg/dl	4.4	3.1
	220.0 mg/dl	4.4	3.2
Phosphorus, inorganic	3.0 mg/dl	5.6	3.9
	8.0 mg/dl	4.0	3.9
Potassium	3.0 mmole/L	2.4	2.3
	8.0 mmole/L	2.4	1.7
Protein, total	5.0 g/dl	2.9	2.7
	8.0 g/dl	2.9	2.1
Sodium	120.0 mmole/L	1.3	1.3
	160.0 mmole/L	1.3	1.3
Uric acid	4.0 mg/dl	5.7	3.2
	11.0 mg/dl	4.5	2.3

*Data from Ross, J.W., and Fraser, M.B.: Am. J. Clin. Pathol. **68**(suppl. 1):130, 1977.

of control sera to be run depends on the number of samples. If very few samples are run by a manual method, it is necessary to run only one control serum, using a normal serum unless it is anticipated that some sample results will be high. When a number of samples are run in a batch, two control sera, one normal and one abnormal, are included. The control sera are treated the same way as the regular samples. In theory the control samples should be run "blind" (i.e., the analyst should not know which samples are the control sera). In practice this is difficult to accomplish, particularly when only a few samples are run at a time. In completely automated systems this is of less importance, because the instrument will show no bias in the analyses. In any event the control sera should not always be placed in the same position in the series, such as the first or last. When many samples are run in an automated system, the various control sera should be placed at random positions in the series. In some automated systems it may be helpful to run a few control sera at the beginning to rule out gross abnormalities. The serum pool mentioned earlier can be used for this.

When the analysis results for the different constituents of the control sera are obtained, they are checked to determine if they are within the allowable limits for each constituent. If the results are all within acceptable limits for the control sera, the results for the samples are released. If the results for the control sera are not within the acceptable limits for some or all constituents, further study is required. The analysis of the control sera serves as a basis for deciding whether the analysis of the samples is accurate enough to be released.

Plotting the daily control results helps to obtain a good picture of the whole quality control program, including the day-to-day variation. A commonly used graph is the Levey-Jennings graph (Fig. 1-3).[11] The expected average value and the ± 2 SD limits (or other limits if used) are marked off as indicated and the results for each day plotted. If more than one aliquot of a control serum is run, both values or their average may be plotted. In Fig. 1-3 the results of the analyses are in good control, with all the points within the required limits. The solid horizontal line represents the average value, and the dashed lines represent 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 (e.g., bath temperature) is fluctuating more than it