CLINICAL CHEMISTRY

Laboratory Manual and Methods

". . . most human activities advance by virtue of contributions from many different types of individuals, with vastly different endowments, working at different levels. Medical investigation is no exception to this rule."

Medical Research: A Midcentury Survey
Boston, Little, Brown and Company
1955, vol. 1, p. xxxi.

CLINICAL CHEMISTRY

Laboratory Manual and Methods

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Illustrated

LONDON

HENRY KIMPTON

134 GREAT PORTLAND STREET, W.

1961

Printed in the United States of America

An Appreciation

No author writes a book alone. The content, viewpoint and expression may be uniquely his, but derived from others. It is only right that some of these be mentioned, not that they may share the responsibility for another new book, but that the author may express his appreciation for their inspiration and guidance.

Those who were especially influential upon this present effort include Professor Robert H. Hamilton, Professor and Chairman of the Department of Physiological Chemistry, Temple University School of Medicine, many of whose ideas and approaches in methodology are incorporated herein; and Dr. Howard W. Robinson, Professor of Physiological Chemistry.

The author is especially grateful to Temple University School of Medicine and Hospital for their provision for a climate of opportunity and inspiration in which it was possible to complete this effort.

To the technologists, present and past, staff and students, who contributed by their criticism and suggestions, my thanks are due. There are many others, too numerous to mention specifically.

Special thanks, too, are due to the Publishers, whose assistance is reflected in many details in the text.

J.H.B.

Preface

THE primary purpose of this text is to provide a teaching manual with clear and explicit direction to those engaged in the profession of clinical chemistry, to students and instructors alike. This has required the inclusion of not only "step-by-step" methodology, but also sufficient background and theoretical material so that the work in the laboratory can become not only accurate and precise, but "knowledgeable."

The one general principle which has guided the selection and arrangement of textual material, has been the existence of a clearly demonstrated need by the laboratory worker, whether technologist or pathologist, student or veteran in the field.

The section on *general principles of chemical analysis* will make possible, and encourage, accurate, precise and intelligent work in the clinical chemistry laboratory. A comparatively long section has been devoted to a discussion and description of *primary and secondary standards* and their preparation and use in the laboratory. While modern laboratories are increasingly compelled to rely on commercial assistance (and it is invaluable) in the preparation of pure chemicals, special enzymes, and even pre-mixed reagents, it should be within the capability of a clinical chemistry laboratory worker independently to check the reliability and accuracy of his own results. The material bearing on *clinical interpretation* is included to serve as a motivating link between the laboratory worker and the physician in their joint effort directed toward the diagnosis and treatment of disease. The section on *laboratory control and statistics* has been included to emphasize the need for recognition of random, systematic, and procedural errors in the laboratory. Only when error is not only recognized but measured is correct clinical interpretation possible. Only when error is measured is it possible to evaluate the usefulness of procedural changes.

The sections on *clinical biochemistry* serve to bring to the laboratory worker an appreciation of the factors involved in the evaluation of the clinical status of the patient. Mere technical proficiency is not enough. A section dealing with the *general principles of enzymatic assay* has been included in addition to the methodology of various enzymes. No other analytical task is so beset with traps for the unwary as is that of enzymatic measurements. Mere "cook-book" approaches are not adequate to the problem.

The illustrations of *laboratory calculations* are included to encourage complete mathematical treatment of laboratory data so that the technologist and other workers may be independent of formulæ, especially those adapted to one special procedural technic. The *theory of photometric and gasometric measurement* is developed sufficiently so that commercial instruments may become "servants" and not "masters."

It would be possible to describe methods in so general a way as to require translation into specific volumes of specific reagents before an analysis could be undertaken. It would also be possible (and it is often done) to describe a method so specifically that the effect of minor variations in technic and glassware used could not be predicted. A mixture of these two approaches combines specific description with sufficient flexibility so that each laboratory worker will be able to adapt the method to local requirements.

A clinical method *begins with the patient,* before the sample is obtained. Although our knowledge of the effect of patient variability on clinical chemistry laboratory data is rather meager, a beginning of this study has been made, and should be made in all laboratories faced with this problem. Technics required for the *collection and preservation* of the sample (blood, urine, feces, etc.) are stressed and possible or probable changes which may occur are outlined.

The analytical methods included have been chosen by a number of varying criteria. They are all in use, or have been in use, in the Temple University Hospital Clinical Chemistry Laboratory under direct supervision by the author for a period of at least several years. They are not *necessarily* the "best" method (most accurate or most precise) known to the author but those included have been subjected to scrutiny as to acceptability of results, and ease and reliability in the hands of student as well as experienced technologists. In some cases, alternate procedures are included, either for teaching purposes or because of special ease of technic or as a reference method.

The methods are arranged alphabetically, within sections. The sections are divided into General Methods, Urine Analysis, Fecal Analysis, Spinal Fluid Analysis, and Toxicology. Each method is presented as follows in outline.

1. *References* to the literature directly bearing on the analytical problem.
2. *General principle* of the method.
3. *Reagents.* An attempt is made to describe the preparation of reagents (*a*) in terms of laboratory preparation (weight and volume) and (*b*) in terms of analytical significance (normality titer).
4. *Procedure.* A step-wise outline of the procedure with details of technic emphasized whenever they are critical.
5. *Calculation.* Since this manual is designed as a teaching aid, derivations of the formulæ required are in many cases outlined. In most cases, an example of an actual laboratory analysis is included.
6. *Notes.* In any case in which experience has shown the necessity, additional clarification of the reasons for a technic or special precaution is included.
7. *Interpretations.* Normal ranges, biochemical and patho-physiological significance of variations are included in this section. To facilitate rapid reference to the significance of the results of a particular test, a special table of *Clinical Significance and Normal Ranges* has been included in the Appendix which abstracts the more complete discussion which is presented with each method.

Reference texts which have been found useful have been included in a short bibliography. This list is not meant as inclusive but only illustrative of the vast material available to the clinical chemistry investigator.

PHILADELPHIA, PENNSYLVANIA JOSEPH H. BOUTWELL, JR.

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CLINICAL CHEMISTRY

Chapter 1

Introduction

CLINICAL chemistry seeks by the analysis of biological fluids and tissues to aid the physician in the diagnosis and treatment of disease. The laboratory cannot diagnose but it can aid in diagnosis; the laboratory cannot treat but it can help to guide the physician in his therapeutic efforts. The laboratory is responsible for prompt, rapid and accurate chemical analysis of specimens submitted to it for examination. In many cases, the physician can aid the laboratory in its task by supplying certain information about the patient, such as pertinent previous medical history and the various diagnoses being considered.

ORGANIZATION IN THE LABORATORY

A clinical chemistry laboratory should be so staffed and organized that the inevitable errors of technic and of human fallibility will be immediately recognized so that steps may be taken to insure that: (*a*) the erroneous result will not be sent to the physician and (*b*) the source of the inaccuracy can be found.

Each individual must be alert to the possibilities of error and when a mistake is detected, must be willing and eager to repeat the determination. A laboratory should never be so under pressure from excessive work load that adequate measures for insuring the requisite degree of accuracy are neglected. It is *inevitable* that errors in solution preparation, analytical technic and in the calculation of results shall occur; it must be *made inevitable* that these errors be detected and remedied before they are translated into tragedy for the patient. It can never be as serious for an analyst to admit a mistake as it may be for the patient to experience the results of the mistake!

Therefore, it should be evident that the analyst must be completely honest in the obtaining and the calculation of data, not only with his fellow-workers, but also with himself. This is always difficult; the one person easiest to deceive is one's own self. Always remember: your technic may be perfect, the method or solution may be at fault. Be ready to admit mistakes!

In this laboratory, results are never given to any person other than those in immediate charge of the care of the patient; the doctors (the attending physicians) and the nurses. Any others desiring information should secure it from the physician in attendance. Also, the interpretation of results in the laboratory is not made except by repeating printed values of normal ranges which are made available. Remember—the laboratory cannot diagnose!

LABORATORY DISCIPLINE*

It should be always remembered that any consideration of medical technology just as of medicine in general must be approached from the view-point that the final goal of all work is service to the patient. The patient is the center; everything in medical work—and medical technology is a part of it—revolves around the patient.

This makes it easy to understand the need for discipline in the medical laboratory, which can be likened to a ship on the ocean. Just as the captain of the ship cannot be held responsible for his task unless there is rigid discipline, so there must be discipline in the laboratory if the pathologist is to do well his part of the medical service to the patient.

The technologist must realize the essential need of laboratory discipline; he (or she) must be ready and willing to adapt to the organization of the laboratory, although it may sometimes entail personal hardship and inconvenience. Those who cannot or will not do it should keep away from medical work of any kind.

LABORATORY EMERGENCIES*

There are occasions, rather frequent ones, when the life of a patient depends on the results of laboratory tests, on their accuracy, on the speed with which they are performed and on their interpretation. The interpretation is entirely within the scope of activities of the pathologist and the attending physicians, as has been stated previously. The accuracy of the results and the speed of performance are two aspects of laboratory work for which the medical technologist is responsible. In his (or her) training these two features must always be remembered and emphasized. Medical technologists must be trained in school and long after they have completed their undergraduate education—to be ready to face laboratory emergencies with knowledge, with mastery of technic, with a cool mind and solid judgment, and with a readiness to serve at any hour of the day or night, on Sundays and holidays, regardless of inconvenience and hardship. This is a part of the medical ethics of the medical technologist in its broad concept, just as it is a part of the medical ethics of the physician.

*** These paragraphs have been quoted by permission from "A Curriculum for Schools of Medical Technology" by Israel Davidsohn, M.D., and Kurt Stern, M.D. of Mount Sinai Hospital, Chicago, Illinois. 1953 Board of Registry, American Society of Clinical Pathologists.**

Chapter 2

Clinical Biochemistry

It is not sufficient for the clinical laboratory technologist to become merely *mechanically* proficient in the routine performance of analytical duties. A knowledge and appreciation of all of the factors bearing on the chemical investigation of the clinical status of the patient is also required. This ranges from a knowledge of the usual variations encountered in certain disease states, to an appreciation of the problems of sample collection and the sometimes rapid variability in the patient's condition.

To assist the technologist in attaining this appreciation, a knowledge of certain physiological and biochemical fundamentals is important, especially in the areas of most frequent investigation by the clinical chemistry laboratory. Further reading in these subjects may be found in the reference texts listed in the appendix (p. 345).

ACID-BASE EQUILIBRIUM AND WATER BALANCE

The clinical chemistry laboratory is increasingly depended upon to furnish the clinician with analytical data in regard to fluid and electrolyte composition and changes as they occur in patients. Changes in the distribution of water and electrolytes are of significance in patients suffering from burns, and after surgery or severe trauma; in such disorders as congestive heart disease; nephrosis, and cirrhosis; and in metabolic diseases such as diabetes, which are accompanied by serious alterations in water and electrolyte metabolism. The prevalence of the use of diuretics of many different types and the differing effects of these agents make it necessary to use the chemistry laboratory in assessing patient response to this type of therapy.

Conversely, the importance of these elements, electrolytes and water, makes it necessary to those involved in laboratory control to understand at least the basic principles of fluid and electrolyte metabolism and also some of the changes occurring in disease.

Fluid Compartments of the Body

It is useful to represent the body as being divided into three compartments or spaces. These are named (1) the vascular space, (2) the interstitial space, and (3) the intracellular space. They are separated from each other and the external environment by semipermeable membranes of various types and with varying functions.

The *vascular or plasma space* is closed off by the endothelial lining of the blood vessels and the capillaries. This endothelial lining is, at certain sites, specialized to allow a controlled flow of fluids and gases through its walls; that is, it is (selectively) more permeable at these areas. This allows easier transport of fluid and electrolytes or gases into or out of the plasma space. These sites of specialized permeability are: (*a*) the lung capillaries, which allow the transfer of oxygen into, and water and carbon dioxide out of the plasma (with the cooperation of the red blood cell, see p. 20); (*b*) the renal glomerulus, which allows the transfer out of the plasma of all of the constituents of plasma except (largely) protein and the cellular elements; (*c*) the renal tubule which may transfer material (water and dissolved substances) either back into the plasma or from the plasma into the tubular lumen; (*d*) the gastrointestinal tract, which allows the transfer of the products and agents of digestion (see p. 35) into the plasma and allows a complex process of secretion into and reabsorption from the intestinal lumen; and (*e*) the skin, across which the transfer of water and electrolytes continually occurs. At some of these sites, the capillary endothelium is almost bare (kidney and lung), at most others (*e.g.*, g.i. tract and skin) it is in contact with the fluid contained in the space between the cells, the interstitial space.

The *interstitial space* includes all of the extracellular space except the vascular compartment, and is in contact with the vascular space *via* the semipermeable endothelium of the capillaries, and with the intracellular space *via* the semipermeable membrane of the cells.

The *intracellular space* includes all of the fluid volume of the cells of the body and is in contact with the extracellular fluids *via* its semipermeable membranes. The red blood cells differ somewhat (metabolically and in chemical composition) from the general body cell and also are in contact directly with plasma instead of *via* the interstitial fluid.

In adults the plasma volume approximates 5% of the body weight, the interstitial volume about 15%, and the intracellular volume about 50% of the body weight. For a 70 kg man the plasma volume is about 3500 ml, the interstitial volume about 10,500 ml and the intracellular volume about 35,000 ml. A greater proportion of the body weight of infants is water and the distribution is different.

Body Water (% of body weight)

Compartment	*Adult*	*Infant*
Intracellular	50	25
Interstitial	15	45
Plasma	5	5
Total	70	75

Estimation of Compartment Volumes

The volume of any fluid may be estimated by adding to it a known amount of an easily identified substance which is allowed to remain until completely mixed. If the fluid is now analyzed for the concentration of the added substance, the dilution ratio can be used to calculate fluid volume. For the *in vivo* measurement of the compartment volumes, it must be shown that the added substance distributes throughout the volume to be measured; that it is not excreted or metabolized too rapidly; and it must be easily and accurately analyzed. For plasma volume, Evan's Blue (T-1824) or serum albumin labelled with radioactive iodine

(I^{131}) are commonly used. For total body water, antipyrine or heavy water (deuterium or tritium oxide) may be used. For total extracellular water (interstitial + plasma) thiocyanate may be used. From the data obtained values may be calculated for the volume of each of the three compartments of the body.

$$V_{total} = V_{plasma} + V_{intra\text{-}cellular} + V_{interstitial}$$

$$V_{extra\text{-}cellular} = V_{plasma} + V_{interstitial}$$

Exchange between Compartments

There are a number of forces or effects which influence the distribution of water and electrolytes between the compartments of the body and the absorption and excretion of substances by the body. These will be noted here but cannot be discussed fully. (See *Physical Biochemistry*, by H. B. Bull, John Wiley and Sons, Inc., New York.)

Diffusion.—Substances in solution tend to diffuse from a region of higher concentration to a region of lower concentration. The rate of net mass transfer is proportional to the differences in concentration (the concentration gradient) between the two compartments.

Permeability.—This diffusion tendency is limited by the only *partial* permeability (semipermeability) of the membranes involved in the transfer path, such as the capillary endothelium, and the cell membranes. Most, if not all, of the membranes in the body are completely permeable to water and almost so to most of the relatively small molecules dissolved therein. Proteins and larger molecules and cellular elements cannot pass through the membranes with complete freedom.

Ultra-filtration and Osmotic Pressure.—Water tends to diffuse through a membrane from the side of lesser concentration of solute to the side of greater concentration of solute. If a pressure is applied to the side of greater concentration this tendency of water to diffuse can be just balanced. The pressure needed to just balance is called the *osmotic pressure* of the solution. If the pressure on the side of higher concentration is increased above that needed to just balance, water can be forced out from the side of higher to the side of lower concentration. This process is called *ultra-filtration.*

Since all of the membranes involved in the body are permeable to water, the flow of water through the capillary wall, or through the cell membrane will depend on the *net* pressure effects. For example, at the arterial end of the capillary, the hydrostatic pressure is greater than the opposing *colloid* osmotic pressure (*oncotic pressure*) and the flow of water will be out of the capillary into the interstitial space. At the venous end of the capillary, the hydrostatic pressure becomes lower than the oncotic pressure and the net flow of water is from the interstitial fluid into the capillary. Dissolved materials (solutes) in the fluid will tend to move with the mass movement of fluid. This tendency, will, however, be modified by the semipermeability of the membrane involved and by the relative concentrations (diffusion tendencies) involved.