Standard Methods of CLINICAL CHEMISTRY

VOLUME I

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BY THE AMERICAN ASSOCIATION
OF CLINICAL CHEMISTS

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1953
ACADEMIC PRESS INC., *Publishers*
NEW YORK, N. Y.

Library of Congress Catalog Card Number: 53-7099

PRINTED IN THE UNITED STATES OF AMERICA

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Preface

This is the first volume in the series of "Standard Methods of Clinical Chemistry" published by the American Association of Clinical Chemists. The method of issuing small volumes seems to be the best way of keeping the series up to date, as it will allow revisions from time to time without waiting to reissue all the methods.

The methods used every day in the clinical laboratory are of primary importance, since a laboratory can be no better than the methods it employs. That is the reason why in the first volume we have undertaken the study of some of the methods used most frequently and have left specialized subjects for later. The choice of the most important is always a difficult problem in any selection of methods. The exact order of importance of those chosen here may be debatable, but almost all laboratories include the analysis of these constituents in their daily repertoire.

With rapid advances being made in the fields of instrumentation and methodology, one cannot say how long the method submitted will be the one of choice; so, whenever necessary, future volumes will present supplementary procedures and modifications of the published methods. Whenever the analytical principle can be carried out in a variety of ways, one of them has been selected by the Submitter. This selection is based on the consensus of clinical chemists working with these methods daily in their own laboratories; this does not imply that other modifications may not be equally satisfactory.

The procedure is as follows: first, the method is well tested in the laboratory of the *Submitter;* then it is sent to the *Checkers*, who retest it in their own laboratories for its validity and practicality. Results, suggestions, and criticisms of the Checkers are incorporated in the method published by the Submitter. Such a procedure not only requires testing of the method but also includes checking a sufficient number of blood samples from control and pathological cases. This is time-consuming, but we feel that thorough checking

of the method under practical conditions more than compensates for the time spent. After all, there are many excellent collections of biochemical procedures available, but although many of these methods yield excellent results when applied to a research problem, they may be impractical under the rigorous regime of a routine clinical chemical laboratory where speed and simplicity are next in importance to precision.

We have tried to elucidate the scientific basis of each method, its scope, and its limitations without engulfing it in unnecessary detail. The variation in the length of the chapters is due partly to the difference in the topics and partly to the treatment by the author. For example, the thymol turbidity test is comparatively new and empirical, whereas the determination of uric acid goes back as far as the beginning of biochemistry and has a real historical background.

To all members of the Editorial Committee, both past and present, I wish to convey my heartfelt thanks for their kind cooperation in a rather tedious job. I am sorry to record the untimely death of Jos Kahn, one of the staunch supporters of this project.

To Harry Sobotka and John G. Reinhold, presidents of the American Association of Clinical Chemists during the planning and writing of this book, I am deeply indebted for their generous support. It is a pleasure to thank Mrs. Peggy Richard for her assistance in typing the manuscript and Bernard H. Armbrecht for proofreading the manuscript.

This book has been written for the countless laboratory workers, chemists, and technicians who are such a vital part of the medical profession, who share in the work, but seldom in the glory. We hope that all who use this book will find it of service and will not hesitate to make suggestions. In this way we can judge the value of our project and extend its scope.

Washington, D. C.
May, 1953

MIRIAM REINER

Foreword

The present edition of "Standard Methods of Clinical Chemistry" represents one of the most cherished achievements of the American Association of Clinical Chemists. The Constitution of the Association, written in 1948, enumerates standardization and codification of methods among its foremost objectives in the interest of the public welfare. Successive Executive Committees of the Association have pondered over the best form for this task. It was decided to summarize the methods of clinical chemistry in a form similar to the "Official and Tentative Methods of Analysis" of the Association of Official Agricultural Chemists and the excellent presentation of "Organic Syntheses." It is hoped that volumes of the "Standard Methods," will accomplish this purpose by presenting individual methods, delineating the underlying principle, describing precisely the preparation of reagents and sequence of procedures, and indicating the range of normal and pathological results.

Quantitative clinical chemistry is less than a century old. One of its first exponents was J. L. W. Thudichum, that versatile scientist, who was by training a physician but who felt equally at home in the practice of wine-making, in writing the "Chemistry of the Brain" and the "Spirit of Cookery," and in studying problems of sanitation and the analytical methods of the hospital chemist. His lifetime coincides with the flourishing of cellular pathology and bacteriology, which has in turn made modern aseptic surgery possible. During this exciting period in the history of medicine, organic and physical chemistry developed their particular concepts which form the foundation of a well-organized science. The change in outlook and methodology from the secretive mystical craft of the alchemist to the logical and well-documented science of chemistry was more abrupt than the development of any other physical or biological discipline. It is little wonder that the great chemists during this heroic age considered the animal body as a source of interesting starting materials rather than as a primary object of their endeavors. Even now, half a century after biochemistry has

developed as an independent specialty, one may imagine an invisible line of demarcation between biological chemists and others who should, perhaps, properly be called chemical biologists. The former are interested for instance in the mechanisms of glycolysis or the structure of proteins *per se*, whereas the latter consider the identical problems but with a view to their significance in animal and plant life and evolution.

How did the clinical chemist enter the picture? A treatise called "Der Blutzucker" was written in 1913 by Ivar Bang, M.D., of the University of Lund in Sweden. He proposed microtechniques, comparable to Pregl's microanalytical methods, for the expeditious analysis of small quantities of blood such as could be drawn daily from patients under investigation. It is no mere coincidence that Bang was a doctor of medicine, like Ehrlich, the originator of both histochemistry and immunochemistry. But soon the impact of medicine upon chemistry became so significant that biochemists such as Folin and Benedict, van Slyke and Bloor dedicated themselves to the tasks of blood and urine analysis and created the structure of clinical chemistry.

Its methods have been described and collected in several scientific treatises which delve into the history and rationale of these procedures, and in publications by apparatus-makers who describe the techniques with reference to their specific product.

We speak to clinical chemists, technicians as well as supervisors. For historical reasons to which we have alluded, much clinical chemistry was originally carried out by doctors of medicine. For practical reasons it was natural that increasing specialization delegated the laboratory sciences to the pathologist's laboratory. The operation of flame photometers, electrophoretic equipment, microanalytical apparatus, and other machinery is gaining in importance, and this development is reflected in the steadily increasing employment by hospitals and private laboratories of competent and responsible clinical chemists. At present, much of the simpler clinical chemistry is performed by technicians, working, as it were, in isolated situations. We hope that they too will benefit from a perusal of "Standard Methods."

Considering the wide scope of clinical chemistry, its steady growth, and the progress of instrumentation, we have decided to issue this collection as a series of small volumes. This insures great flexi-

bility and facilitates as far as possible the task of Editors, Submitters, and Checkers. At the same time, it permits the issuance of handy volumes for use at the laboratory bench, and the current presentation of new and timely methods and modifications. Grouping of certain methods in each book will provide a welcome opportunity of acquiring individual volumes for those interested in a specific subject.

We hope that "Standard Methods of Clinical Chemistry" will raise the standards of clinical chemistry and will ultimately improve the medical care of the population.

New York, New York

THE EDITORIAL COMMITTEE OF THE AMERICAN ASSOCIATION OF CLINICAL CHEMISTS.

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Introduction

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Introduction

The clinical chemist is a professional member of the medical team responsible for the diagnosis of a patient's illness and for the therapy of his ailment. As such, he is responsible for the accurate performance and reporting of the laboratory procedures entrusted to him. The dosage of drugs such as dicumarol and insulin, pre- and post-operative management of fluid balance, and even the type of nutrition offered will depend on the results of chemical examination. The patient's welfare is as much the chemist's trust as that of the supervising physician.

The scope of the clinical chemist's responsibility varies in different institutions, but it may include any or all of the following:

1. Collection of specimens for analysis: This is often left to the intern; but when the chemist performs the task himself he is assured of adequate amounts and proper samples. He avoids the annoyance attendant upon receiving a request for serum potassium attached to a vial of blood preserved with potassium oxalate.

2. Performance of the test: This should be done as soon as possible after collection of the sample. The test then will not only nearly mirror the physiological state of the patient at the time of reporting the result (when it is of most use to the physician) but will also assure that the changes that occur in blood on standing have been minimized. For instance, a report of the blood sugar, serum chloride, and carbon dioxide combining power of blood tested several hours after collection will reveal a low sugar and carbon dioxide and

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a high chloride, thus presenting a false picture to the clinician, and one harmful to the patient.

3. Reporting the results to a responsible party: Telephoning laboratory reports to a ward attendant is usually tantamount to inviting a repeat test. Results of chemical examinations should be reported, preferably in writing, to the physician ordering the tests, but in the event of an "emergency" request it should be given to him or an assistant by phone.

4. Knowledge of the limitations of the methods, both chemical and clinical: These must be impressed on chemist and clinician alike. The chemist must know that fructose will not give as great a reduction as glucose in the procedure for blood sugar, and also that one need not get excited over a change in the icterus index from 120 to 85 units, as the method is hardly accurate to within 15 units in that particular range. Such knowledge may save the patient from a hasty and mistaken diagnosis.

5. Interpretation of results to the clinician and teaching of interns and residents: This requires a knowledge of the physiological, and to some extent the pathological, meaning of the results of clinical chemical procedures. Many outmoded procedures could be dropped, and newer ones properly evaluated, if clinicians and chemists discussed with each other the meanings of chemical tests, and perhaps a little of their history.

6. Training of laboratory technicians: The laboratory technicians are directly responsible for performing tests under the guidance of the clinical chemist. It is the latter's responsibility to teach the technique of performing tests, the limitations of the procedures, and the methods of calculation and reporting. Technicians should be impressed with the limits of accuracy of procedures, and they should realize that reporting a result to four (insignificant) figures does not make the tests more accurate. If a standard is good to five figures, but the photometer reading is good to only two, it is a waste of time to calculate the final answer to more than two significant figures. Care and use of instruments, which represent a substantial financial investment, must be taught the technicians.

In addition to these duties, the clinical chemist will have to choose and set up methods for use in his laboratory. The purpose of this volume is to lessen the burden of his task by providing him with a selection of tested methods suitable for use.

Collection of Blood Samples

The responsibility of the clinical chemist starts with the collection of the sample. Even if he doesn't draw the blood himself, the chemist is usually responsible for the preparation of the specimen containers, and he certainly should see that the material he receives is the proper specimen for the procedure ordered, is sufficient in quantity, and reaches the laboratory promptly.

Should the chemist be required to draw blood, there are several precautions which may aid him in collecting the specimen without violating the rules of sterility and with minimum trauma to the patient and himself.

The syringes and needles used for venipuncture should be cleaned thoroughly, then rinsed with copious amounts of distilled water. Rinsing is extremely important, especially when detergents are used. These play havoc with many tests, such as blood urea and carbon dioxide. Sterility of syringes and needles must be scrupulously maintained. They should be individually wrapped and then autoclaved for 30 minutes at 15 pounds pressure. This is the best method of assuring inactivation of the infectious hepatitis virus. Once a needle and syringe have been unwrapped, they should not be used again without resterilization.

Blood can be highly infectious, and care should be taken not to get samples onto the skin or into the mouth. If a sample is spilled on the skin or work bench, the area should be considered contaminated and washed immediately with soap and water. If blood is aspirated into the mouth, the mouth should be rinsed well with water.

Before blood is taken, the patient's arm is inspected for a suitable vein. A tourniquet is lightly applied to the upper arm. If veins are not easily palpable, it may be necessary to slap the antecubital area with the finger or to place a hot compress on the area for several minutes. This procedure usually results in the veins standing out clearly. The site of the venipuncture should be cleaned with absorbent cotton or clean gauze moistened with alcohol.

The needle should be sharp and free from burrs. If possible, the vein should be entered cleanly from above. Tissue juice may invalidate procedures such as prothrombin time. Occasionally, it will be easier to pierce the skin with a short jab, then enter the vein from the side, and thread the needle well into the lumen of the vein.

When venipuncture is successful, blood will appear in the syringe, either spontaneously or after gentle aspiration. The tourniquet is removed, and the required amount of blood is drawn into the syringe. It is probably better to use a large needle (21 or 20 gauge) when substantial quantities of blood are to be drawn, for the initial pain is often easier to bear than the annoyance of a prolonged venipuncture, and the danger of clotting is minimized. Gentle aspiration prevents foaming of the blood in the syringe.

After sufficient blood is drawn, the needle is removed from the vein, and the venipuncture site is covered with a sponge moistened with alcohol. Pressure is maintained on the sponge for 2 or 3 minutes, or the patient can elevate his arm for a short while. This will cause bleeding to stop.

The needle is taken off the syringe, and the blood is squirted gently into the proper containers. Forcing blood through a narrow needle often causes hemolysis. Containers should be labeled with the patient's name and other necessary information, such as the date and time of collection, hospital number, and type of test required. Unlabeled bottles should not be accepted in the laboratory.

Containers and Anticoagulants

Containers must be clean and dry. Those for unclotted blood must be properly prepared with the correct amounts and types of anticoagulants. The anticoagulants of choice for different procedures are given below: Lithium oxalate (or potassium or sodium oxalate)—blood alcohol, amino acids, ascorbic acid, carbon dioxide, carbon monoxide, chlorides, cholesterol, creatine, creatinine, total lipids, non-protein nitrogen, proteins, salicylates, sulfonamides, thiocynates, urea nitrogen, uric acid, (glucose). Sodium fluoride—glucose, (blood alcohol, creatinine, non-protein nitrogen). The anticoagulants are of secondary choice for those procedures enclosed in the parentheses. Sodium fluoride does have the advantage of preventing decomposition, as well as coagulation, but it interferes with enzymatic determinations.

One and one-half milligrams of lithium oxalate per milliliter of blood is recommended as anticoagulant. This is best placed in the container as an aqueous solution, which is then freed of water in an oven. After the oxalate is scraped from the sides of the bottle,

the container is ready for use. Remember that some procedures require special anticoagulants.

Receipt of Material in the Laboratory

A register of all routine and "emergency" requests should be kept. Each patient's name, and the tests requested routinely, should be entered. All "emergency" requests, when registered, should be designated in a significant manner and given special care. Each patient is then assigned a number for identifying samples of his blood. When tests are completed, the results are entered opposite the patient's name. This will serve as a permanent record of the laboratory's routine and emergency requests.

After receipt and entry in the register, specimens should be sorted according to type of sample—the clotted blood tubes placed in the centrifuge for separation of the serum, the required amount of oxalated blood removed for filtrates, and the remainder of the oxalated tubes spun to separate the plasma. Processed plasma, sera, and whole blood are then distributed to the proper work benches, and the day's tests are begun.

Use and Care of Photometers

Many laboratory procedures are adaptable to photometry. If a compound is present in solution, or if one can be formed, which will absorb light over some range of the spectrum, and if that light absorption is proportional to the concentration of the compound, then photometry may be used to help determine the quantity of compound present. This fact requires only that the compound in question absorb light in a given region of the spectrum. As dirt, finger marks, and precipitates will block passage of light, they will give rise to apparent high absorption, and therefore falsely high concentrations.

Photometers are provided with filters or monochromators to delineate the wavelengths of light to be passed through the cuvettes, with photocells to detect the light transmitted, and with galvanometers to indicate the fraction of the light started through the cuvette ("incident light") which finally reaches the photo cells. There are many types of photometers on the market, but the choice of one or more for the routine clinical laboratory will depend not only on

cost but also on the requirements as to sensitivity of the instrument, spectral range covered, stability (those without amplifier circuits are generally less prone to drift), ruggedness, adaptability to both micro and macro procedures, type of scale employed (conversion from per cent transmittance to optical density is time-consuming and may lead to error), necessity for special cuvettes (machines using matched test tubes are especially convenient for clinical procedures), and source of current (batteries are a nuisance, but they are also the only reliable source of current of constant voltage).

When photometers are used, it is generally best to run several standards along with the day's unknown samples. Because of day-to-day variations in individual technique, in laboratory conditions, and in the characteristics of photometers, calibration charts should be viewed with suspicion. If used, they must be checked frequently.

The nature of light absorption is such that it does not vary linearly with molecular concentration but increases logarithmically with an increase in the number of absorbing molecules. When light absorption increases, optical density increases, and the fraction of incident light transmitted by the solution (per cent transmission) decreases. The optical density thus varies linearly with molecular concentration and may be used to aid in determining the concentration of the compound in the cuvette.

If the particular machine used has a scale calibrated in per cent transmission, it will be necessary to convert the readings to optical density. Optical density equals the logarithm of the ratio of the incident light to the transmitted light, and the incident light intensity is taken as the 100% reading on the galvanometer. Since the logarithm of 100 is 2, then 2 minus the logarithm of the reading of the sample equals the density, which is directly proportional to the color intensity. The optical density can usually be read from a conversion chart.

To calculate the fraction of the standard represented by an unknown, the ratio (optical density of unknown/optical density of standard) is multiplied by the amount of standard substance used. Of course, any dilutions made, and the amount of sample used, must be taken into consideration. The final formula for calculating the concentration of an unknown in terms of 100 ml. of blood or plasma is

$$C_u = C_s \times \frac{D_u}{D_s} \times \frac{100 \text{ ml.}}{\text{Volume of the sample used}} \times \frac{V_u}{V_s}$$