Practical Clinical Chemistry

A GUIDE FOR TECHNICIANS

By

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PREFACE TO SECOND EDITION

Changes which appear in the second edition are introduced mainly from the point of view of further simplification of the procedures.

In the determination of non-protein nitrogen in blood a single deproteinizing solution is used in place of two solutions.

The bromsulfalein test is simplified by eliminating the necessity of using a blood sample drawn before injection of the dye.

For the determination of total cholesterol a new method is described. In this procedure the entire reaction can be carried out directly in the cuvettes, so that considerably less apparatus is required. The preliminary removal of protein is not necessary. A smaller sample of plasma or serum is used, a more stable color is developed and the analysis can be performed in less time.

Slight changes in the determination of icterus index make the method more accurate.

In the determination of sugar in blood, spinal fluid and urine, a change in one of the reagents makes the procedure less time-consuming and gives more accurate results. In making use of this modification the color developed follows Beer's law, so that one standard will suffice in place of two.

A rapid procedure for the determination of sugar in urine is added in this edition.

For the determination of uric acid a simpler, shorter procedure is described.

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PREFACE TO FIRST EDITION

This book is written mainly for technicians as an aid to the proper use of procedures in clinical chemistry and is intended to satisfy an obvious need for explicitness in details.

The procedures described have been chosen as representative analyses which are most frequently performed in a hospital laboratory of clinical chemistry. The methods have all been employed successfully over a period of several years in the laboratory of clinical chemistry at the Presbyterian Hospital of the City of Chicago.

Many of the procedures have been modified for the purpose of attaining simplicity and economy of time without sacrifice of accuracy. The photometric methods have been chosen or modified to achieve the greatest possible stability of the color reaction.

The use of the brief outlines, prepared for each method, has been found to be exceedingly helpful for checking the memory in daily routine. The methods should be learned and practiced by technicians from the description of the procedure in detail. Each outline, in some cases accompanied by a useful table, can then be placed in a cellophane protector for daily guidance in the laboratory.

Methods for the determination of urea in blood have been omitted because it is not necessary routinely to analyse blood for both urea and non-protein nitrogen. Either determination will furnish the same information and the method described for non-protein nitrogen is less time-consuming and requires less blood than an analysis of urea.

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A. H.

CONTENTS

LIST OF TABLES

PRACTICAL CLINICAL CHEMISTRY

A GUIDE FOR TECHNICIANS

CHAPTER I

GENERAL LABORATORY PROCEDURES

GENERAL PRECAUTIONS

WITH reasonably careful technique there are very few accidents in a routine laboratory of clinical chemistry. However, it is advisable to have a first-aid shelf in a convenient location so that minor accidents can be dealt with immediately. Most of the items on such a shelf can be prepared from laboratory reagents.

A simple first-aid shelf should contain the following items:

1. Sodium bicarbonate, saturated (9%) solution, which should be used to wash the mouth if an acid solution has been drawn into the mouth from a pipette and to sponge clothing on which acid or dichromate cleaning mixture has been spilled.
2. Acetic acid, about 10% solution, which can be prepared by adding 10 cc. of glacial acetic acid to 100 cc. of water. This solution should be used to wash the mouth if an alkaline solution has been drawn into the mouth from a pipette and to sponge clothing on which alkali has been spilled.
3. An eye cup.
4. Boric acid, saturated (4%) solution, which should be used to wash the eye immediately, by using it in an eye cup, when any solution or substance has entered the eye.
5. Bandages for minor cuts.
6. Any good ointment for minor burns. Burns should be treated immediately, no matter how minor they may be, to avoid blisters
7. Goggles or a face shield, which should be used whenever strong solutions are prepared, such as dichromate cleaning mixture or strong solutions of acid or alkali.

As a general precaution in preparing any acid or alkaline solutions from concentrated solutions of acids or alkalis, *always measure the acid or alkali into the water, slowly and carefully, with shaking or stirring, to avoid sudden and explosive heating. Never add water to a concentrated solution of acid or alkali.*

COLLECTION AND PREPARATION OF BLOOD SPECIMENS

As a general principle it is advisable, for chemical analyses, to use blood drawn in the post-absorptive state, before the patient has had breakfast.

For the chemical analyses described, the blood is obtained by venipuncture. Avoid stasis as much as possible during venipuncture, as stasis may lead to erroneous results in some determinations, such as plasma or serum chloride, carbon dioxide combining capacity of plasma and proteins in plasma or serum. Apply a tourniquet as lightly and briefly as possible, then release the tourniquet as soon as the needle is in the vein.

The blood is usually drawn with a sterile 20-gauge needle into an attached Luer syringe, then transferred immediately to a test tube containing an anticoagulant for the analysis of whole blood or plasma, or into a clean test tube without anticoagulant for the analysis of serum.

In transferring the blood from syringe to tubes, the blood should be handled gently to avoid hemolysis. *Do not squirt the blood from the syringe into the tube, as this will cause hemolysis.* Insert the tip of the needle into the tube as far as possible, then expel the blood *gently* down the side of the tube. If the blood is delivered into a tube containing an anticoagulant, stopper the tube immediately and invert the tube gently several times so that the blood is thoroughly mixed with the anticoagulant.

The volume of whole blood to be collected for each determination described is given in Table I, with precautions to be observed

As anticoagulants for blood, sodium citrate, potassium oxalate or heparin can be used. *Heparin is by far the best choice for an anticoagulant;* it meets all requirements and is least apt to cause hemolysis and to alter the distribution of water and electrolytes between cells and plasma. The amount of heparin required is small and there is no introduction of an electrolyte.

Preparation of tubes containing heparin. It is most convenient to prepare tubes for the collection of 10 cc. of blood. If less blood is collected, the excess heparin causes no disturbances, such as are caused by excess oxalate.

Heparin can be purchased in vials which contain 10 cc. of a solution with a concentration of 10 mg. of heparin per cc. Heparin can be obtained from Abbott Laboratories, Parke, Davis and Co. and other drug firms.

Transfer the contents of a vial of heparin, containing 100 mg.,

TABLE I

VOLUME OF WHOLE BLOOD TO COLLECT FOR EACH DETERMINATION DESCRIBED

Determination	*Volume of Whole Blood to Collect*	*Phase of Blood Used for Analysis*	*Precautions*
	cc.		
Amylase	8	Plasma or serum	Analyse immediately
Bilirubin	6	Serum	Avoid hemolysis, analyse within 2 hours
Bromsulfalein 45 mins. after injection	5	Plasma	Avoid hemolysis
Calcium	10	Serum	
Carbon dioxide capacity	8	Plasma	Avoid hemolysis, centrifuge within 30 mins.
Cephalin flocculation	5	Serum	Avoid hemolysis, analyse immediately
Cloride	4	Plasma or serum	Avoid hemolysis, centrifuge within 30 mins.
Cholesterol	5	Plasma or serum	Avoid hemolysis
Creatinine	4	Blood	
Icterus index	2	Plasma or serum	Avoid hemolysis
Non-protein nitrogen	2	Blood	
Phosphatase, acid	8	Serum	Avoid hemolysis, analyse immediately
Phosphatase, alkaline	8	Serum	Avoid hemolysis, analyse immediately
Phosphorus, inorganic	4	Plasma or serum	Avoid hemolysis, centrifuge immediately
Protein, total	2	Plasma or serum	Avoid hemolysis
Protein, A/G ratio	5	Plasma or serum	Avoid hemolysis
Sugar	3	Blood	Precipitate proteins immediately
Sulfonamides	5	Blood	
Uric acid	8	Plasma or serum	

to a 100 cc. volumetric flask. Wash the vial four times with distilled water and add the washings to the volumetric flask. Add distilled water to a volume of 100 cc. and mix thoroughly by inverting, with shaking, 10 times. The concentration of this solution is 1 mg. of heparin per cc. of the solution.