Second Edition

TECHNICAL HEMATOLOGY

Arthur Simmons

Technical Hematology

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Preface to the Second Edition

Seven years have elapsed since the writing of the first edition of Technical Hematology. During that time, laboratory hematology has changed as much as, or more than, other disciplines in Clinical Pathology. Tests that were unusual and considered esoteric several years ago have in many laboratories become commonplace. These changes have been most apparent in red cell enzyme studies and in the more recent advances in blood coagulation.

Other major changes are reflected in the concept of multiphasic patient screening. The introduction and acceptance of hematology automation has radically altered the routine work load and has brought with it, not only increases in precision and accuracy, but also the spectacle of the laboratory engineer as a potential member of the health team. This automation has enabled the hematology technologist to be freed of many mundane manual chores and has resulted in the expansion of laboratory testing to encompass a more thorough hematology investigation.

It is because of these changing concepts that the Second Edition of this text has been prepared. The addition of a large number of tests and the deletion of obsolete methods and apparatus is, then, necessary to keep this text a functional practical laboratory reference.

The most radical revisions in this text have concerned the blood bank section. Although this text is not meant to be a theoretical book on either hematology or immunohematology, it was thought necessary to revise the previous material to include sufficient theoretical aspects, so as to make the techniques that follow more understandable. In this regard, these methods have also undergone a complete revision and have been updated whenever needed. The net result is that treatment of the blood banking section has been expanded from the original single chapter to the present five chapters in the hope that this clarification will be of practical value to the working medical technologist.

I am indebted to the Medical Graphics Department of the University of Iowa and to the many student, medical technologists and the faculty of the Department of Pathology who have been helpful in this revision. Especial acknowledgements are due to Miss Judy White and Mrs. Joyce Paul for the valuable secretarial skills and patience which they showed in the preparation of the manuscript, and to Mr. George F. Stickley and to Mr. J. Stuart Freeman of the Medical Books Division of J. B. Lippincott Company.

ARTHUR SIMMONS

Preface to the First Edition

This text was written for the working graduate and the student medical technologist. Its aims are twofold: to present as completely as possible the various hematologic techniques that can be used as a diagnostic aid to the physician, and to offer as far as possible some of the alternative methods commonly used. In addition, it is hoped that the medical technologist working in the hematology laboratory will gain more of an insight into the laboratory aspect of some of the hematologic diseases.

There are many ways of determining the same hematologic value, and the method chosen is frequently only a personal preference. This is especially true with the recent surge in the type and range of automatic equipment, which allows the laboratory worker to carry out procedures in vast numbers. The ever-increasing number of laboratories being automated makes it necessary to include all of the standard equipment available on the market, although each student should be grounded in the basic manual principles of a technique before advancing to the automatic aspects.

Brief clinical data has been included, because I believe that the medical technologist, although not practising medicine, should know some of the basic medical reasons for carrying out a test. To produce hematologic data with total disregard to the patient's condition can only lead to boredom, and eventually, to lack of precision and accuracy. One might consider this aspect an accessory to quality control, which is also stressed in the later chapters.

The section on immunohematology contains more theoretical material than other sections, and endeavors to cover the more elementary aspects of this speciality.

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ARTHUR SIMMONS

Contents

Introduction

BLOOD COLLECTION

The two main methods of obtaining blood samples are finger or heel puncture (capillary blood) and venipuncture.

The first step in the collection of a blood sample is to reassure the patient. With adults it is a relatively easy matter to explain the procedure, but with children extra care should be taken and some endeavor made to win the child's confidence.

Finger Puncture Method

1. The finger is cleansed with gauze or wool moistened with 70% alcohol or with alcohol-ether mixture, and allowed to dry.
2. A quick puncture is made on the *side* of the finger with a disposable lancet. *Do not* squeeze the finger tightly, because the liberated tissue juices dilute the blood.
3. Wipe away the first drop of blood to avoid tissue juice contamination, and apply *gentle* pressure in a "milking" fashion to obtain a free flow of blood.
4. When the desired amount of blood has been obtained, a clean dry pad is applied to the wound, and the patient is instructed to apply pressure until the bleeding ceases.

Venipuncture

Blood can be obtained from any of the following sites: (1) antecubital veins of the forearm; (2) ankle veins; (3) wrist or hand veins; (4) femoral veins; and (5) scalp or jugular veins (in infants). This procedure should be carried out by a physician.

Syringe Method from Antecubital Veins

1. The area around the site is cleansed with 70% alcohol or with alcohol-ether mixture, and allowed to dry.

2. A tourniquet is applied around the upper arm, and the patient is instructed to open and close his hand to build up the blood pressure in the vein.
3. A prominent vein is located. If it is not easily seen, the technician's index finger is cleansed as in (1), and the forearm is palpated until a vein is felt.
4. The vein is fixed either by holding it with the finger or by grasping the patient's arm below the elbow and pulling the skin taut.
5. The needle is inserted in the vein obliquely with the bevel upward, and the plunger is slowly pulled back until the necessary volume of blood is obtained.
6. The tourniquet is released, and the patient is instructed to open his hand.
7. A clean pad is applied over the needle puncture and the needle quickly removed in a quick, smooth motion.
8. Pressure is applied to the puncture site with the patient's arm held straight out. *Do not* allow the patient to bend his arm at the elbow, or a large hematoma may form. This is a collection of subcutaneous blood, causing blue and then green skin coloration at the site.

Vacutainer Method

1. Procedures (1) to (4) are carried out as in the syringe method.
2. The appropriate vacutainer tube is placed in a reusable plastic holder, and a disposable needle is attached by screwing the threaded needle hub to the female end of the holder.
3. The vacutainer tube is inserted into the holder until the top of its stopper is level with the clearly marked guideline.
4. The pressure on the tube is then released, causing the tube to recede slightly.
5. The needle is inserted into the vein as in the syringe method, and the tube base is pushed forward, breaking the vacuum in the tube and enabling the sample to be collected.
6. Procedures (6) to (8) are carried out as in the syringe method.

ANTICOAGULANTS

Whole blood is necessary for most hematological investigations. The sample must, therefore, be mixed with an anticoagulant to prevent coagulation. The common hematological anticoagulants are listed below

Heller's and Paul's Mixture

(syn: double oxalate, Wintrobe's anticoagulant, balanced oxalate)

Chemical Action. This anticoagulant removes the free calcium ions from solution through the addition of ammonium and potassium oxalate. Calcium is precipitated as insoluble calcium oxalate.

Preparation

1. 1.2 g ammonium oxalate and 0.8 g potassium oxalate are dissolved in 100 ml of distilled water.
2. 0.5 ml of this solution is added to each of a series of tubes and evaporated to dryness at 37°C. Higher temperatures decompose the oxalates.

Notes

1. The dried salts from 0.5 ml of the solution is adequate to stop 5 ml of blood from clotting.
2. The mixture of blood and salts is isotonic. The ammonium salt would give a hypotonic solution, the potassium salt a hypertonic solution.
3. Blood taken into this anticoagulant is unsuitable for morphological examination. The red cells commence to crenate and the white cells exhibit bizarre nuclear patterns.

Ethylenediaminetetraacetic Acid, Dipotassium or Disodium Salt

(syn: EDTA, sequestrene, versene)

Chemical Action. This anticoagulant removes the free calcium ions by chelation.

Preparation

1. 10 g of the dipotassium salt is dissolved in 100 ml of warm distilled water.
2. 0.1 ml of this solution is added to each of a series of tubes and evaporated to dryness at 37°C.

Notes

1. The dried salt (0.01 g) from 0.1 ml of the solution is sufficient to stop the coagulation of 5 ml of blood.
2. Blood taken into this anticoagulant can be used for cellular morphology. Platelet counts can also be carried out on 24-hour-old blood.
3. The dipotassium salt is to be preferred to the disodium salt, because it is more soluble.

Heparin

Chemical Action. Heparin acts as an antithrombin and is the only naturally occurring anticoagulant used routinely in the laboratory.

Preparation

1. 0.4 g of powdered heparin is dissolved in 100 ml of distilled water.
2. 0.25 ml of this solution is added to each of a series of tubes, and evaporated to dryness at 37°C.

Notes

1. 1 mg of heparin—the amount in 0.25 ml of the solution—is sufficient to prevent the coagulation of 5 ml of blood for at least 24 hours.
2. Heparin is valuable when it is important to minimize the chance of hemolysis (e.g., in the osmotic fragility test).
3. Heparin cannot be used when differential white counts and examinations of cellular morphology are to be made, because it imparts a faint blue coloration to the background when the blood smear is stained by any Romanowsky stain.

Sodium Citrate

Chemical Action. This anticoagulant removes the free calcium ions by loosely binding them to form a calcium citrate complex.

Preparation

1. 3.8 g trisodium citrate is dissolved in 100 ml of distilled water.

Notes

1. For the investigation of coagulation disorders, 9 volumes of blood are added to 1 volume of the anticoagulant.
2. For determining sedimentation rates by the Westergren method, 4 volumes of blood are added to 1 volume of the anticoagulant.

Sodium Citrate—Citric Acid Buffer

Chemical Action. The action is identical to sodium citrate.

Preparation

1. 19.2 g of citric acid is dissolved in 100 ml of warm distilled water.
2. 29.4 g of sodium citrate is dissolved in 100 ml of warm distilled water.
3. 23.7 ml of the citric acid solution is added to 26.0 ml of the sodium citrate solution, and the mixture is diluted to approximately 90 ml with distilled water.
4. The pH of the mixture is adjusted to 4.7 and the volume made up to 100 ml with distilled water.

Notes

1. For the investigation of blood coagulation disorders, 9.9 volumes of blood are added to 0.1 volume of the buffered anticoagulant.

2. The pH of the anticoagulant is adjusted to 4.7, but when whole blood is added and the plasma is separated, the final pH should be 7.35 ± 0.05.