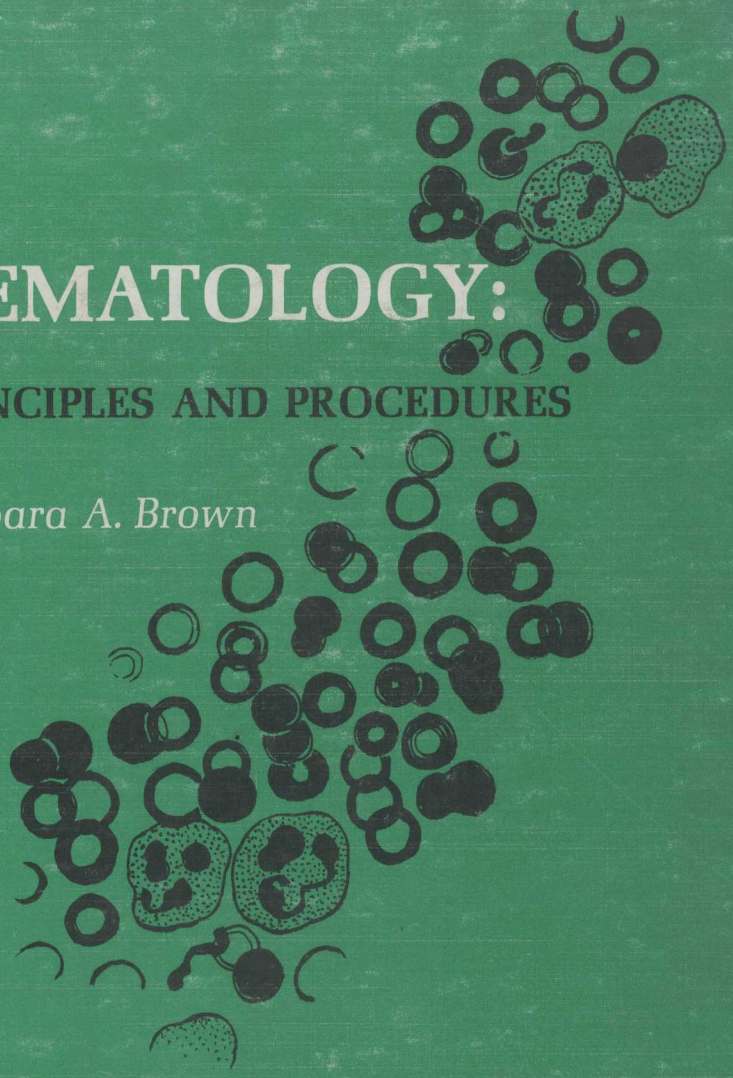

HEMATOLOGY:

PRINCIPLES AND PROCEDURES

Barbara A. Brown

THIRD EDITION

HEMATOLOGY:

Principles and Procedures

BARBARA A. BROWN

B.A., M.T. (ASCP), M.S.

Supervisor, Department of Hematology
Tufts New England Medical Center Hospital
Boston, Massachusetts

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Preface

I was very gratified to have the opportunity to write a third edition of this book. Its main concept and purpose remain the same: to acquaint the new student with a basic knowledge of hematology. Chapter 1 remains unchanged. In Chapter 2, Hematopoiesis, those areas of the section concerned with the function and physiology of the cells have been expanded to give the student a deeper insight into this area of hematology. Most of the black-and-white photographs have been replaced by newer black-and-white photomicrographs, in an attempt to show the student more nuclear and cytoplasmic detail. Perhaps, removing color (although color is very important) from a cell may encourage the student to examine nuclear chromatin patterns and cytoplasmic consistency. The use of the Unopette system has been added to Chapter 3, and a few small changes and additions have been made in the coagulation section. One new procedure has been added to the special procedures chapter, and a substitute for the Peroxidase (benzidine) stain has been added. Two diseases were added to the disease section and several diseases rewritten and updated. The Coag-A-Mates, Hema-Prep smear maker, Coulter Thrombo-fuge, and the Coulter Counter Model S Plus have been added to the automation chapter. Three new color plates have been added and one existing color plate has been changed and improved.

I appreciate the suggestions, material, and help which I received from Becton-Dickinson (Everett E. Uppwall, Sr. Advertising and Sales Promotion Supervisor), Coulter Electronics, Inc. (Pete Bono, Director of Education and Terry McCoy, Chief Instructor), General Diagnostics, Division of Warner-Lambert Co. (Mrs. Jane G. Lenahan, Manager, Educational Services), Geometric Data (Melvin N. Miller, Ph.D., President), and Streck Laboratories (Wayne L. Ryan, President).

I am indebted to Ms. Kathleen Yount of the Special Hematology Department, Charlotte Memorial Hospital, Charlotte, N.C., for the loan of many of her well-stained abnormal blood smears. I also thank Ms. Arrianne Graddick for the additional loan of abnormal smears. Many of these slides were used in making up the color plates and the new black-and-white photographs.

I appreciate the time and effort of Mr.

Steven Halpern. His expertise in photomicrography made the new black-and-white photographs possible.

My thanks and appreciation to Thomas Gould, M.D., for his suggestions and review of parts of this manuscript.

I am very appreciative of the efforts of my mother, Mrs. Nan W. Brown, who spent many hours typing the manuscript. I am also grateful to Mrs. Mary Eldridge for her additional help with the manuscript typing.

As in the first two editions, I am again indebted to Ms. Theresa Kuszaj for her many, many hours of proofreading.

I thank Mr. James J. Bonner for his art work. I am very grateful to have had his help with the illustrations for all three editions of this book.

I express my sincere appreciation to the publishing company of Lea & Febiger for their help with this book.

Boston, Massachusetts BARBARA A. BROWN

Contents

1

Basic Laboratory Techniques

Hematology is defined as the study of blood and is today concerned primarily with the study of the formed elements of the blood.

COMPOSITION OF BLOOD

The total blood volume in an adult is about 6 liters, or 7 to 8% of the body weight. Approximately 45% of this amount is composed of the formed elements of the blood: red blood cells, white blood cells, and platelets. The remaining 55% of the blood is the fluid portion, termed plasma. Approximately 90% of the plasma is water. The remaining 10% is composed of proteins (albumin, globulin, and fibrinogen), carbohydrates, vitamins, hormones, enzymes, lipids, and salts.

The blood may be thought of as a transportation system. As it circulates throughout the body, oxygen is transported from the lungs to the tissues, products of digestion are absorbed in the intestine and carried to the various tissues of the body, and substances produced in various organs are transferred to other tissues for use. Cellular elements of the blood may also be transported to fight infection or aid in blood coagulation. At the same time, waste products from the tissues are picked up by the blood to be excreted through the skin, kidneys, and lungs.

If coagulation is prevented, the formed elements of the blood can be separated from the liquid portion, or plasma. If blood is allowed to clot, the liquid portion expressed from the clot is termed serum and differs from plasma in the loss of fibrinogen, which was utilized to form the fibrin threads of the blood clot.

COLLECTION OF BLOOD

The medical technologist most often comes in contact with a patient during the process of blood collection or of bone marrow aspiration. The patient in a hospital is anxious, fearful, and in ill health. He is anxious about his physical condition; he fears because he does not know what will happen next; and he is physically uncomfortable due to his sickness or injury. He is also separated from his known surroundings and family. For these reasons, a person's mental attitude is often at its worst when he is in the hospital as a patient. It is important, therefore, for the medical technologist to show the patient, at all times, the kindness and understanding that could mean so much.

When the technologist is dealing with a child, his approach is doubly important. This may be the first time the child has had a blood test. If it turns out to be a horrendous experience, it will be remembered

and feared by the child for many years. Therefore, it is important to gain the child's confidence before proceeding with the blood collection. The child should be told what is going to happen. He should not be told that the puncture will not hurt. In doing this, the child's confidence will be lost, because what he or she is told is false.

The techniques used in obtaining blood are not learned overnight. They are an art that must be developed by study, observation, and practice, until the technologist has the necessary skill and self confidence.

Skill, patience, understanding—these are the qualities of a good phlebotomist.

Microsampling

Microsampling refers to blood collection from the finger, toe, or heel, and is frequently used for the following types of patients:

1. *Newborn infants.* The blood is generally obtained from the heel or big toe, since these two areas are larger and more accessible than the fingertip. Newborns do not have a large blood supply, and it would be dangerous to remove the volume of blood involved in a venipuncture.
2. *Young children.* If only a small amount of blood is needed, the tip of the third or fourth finger is usually punctured.
3. *Adults.* When a patient has poor veins, or when the veins are not able to be used because of intravenous (I.V.) infusions, the tip of the third or fourth finger may be used to obtain blood.

EQUIPMENT

1. Alcohol, 70% (v/v).
2. Dry gauze pads or cotton balls.
3. Sterile blood lancet (Fig. 1).
4. Appropriate pipets, diluting fluids, and tubes for microsampling.

FIG. 1. Blood lancet.

PROCEDURE

1. Rub the puncture site on the appropriate finger or toe vigorously with a gauze pad moistened well with 70% alcohol. (This cleanses the area and increases the circulation.)
2. Wipe the area dry with gauze or cotton. (Gauze is preferable since small pieces of the cotton may stick to the finger and interfere with the collection of blood.)
3. Using the sterile blood lancet, make a deep puncture on the side of the fingertip, midway between the edge and midpoint of the fingertip (Fig. 2). (A deep puncture is no more painful than a superficial one, gives a much better blood flow, and makes it unnecessary to repeat the procedure.)
4. Using dry gauze, wipe away the first drop of blood, making certain the area is completely dry.
5. Apply moderate pressure, approximately 1 cm behind the site of the puncture to obtain a drop of blood.
6. Release this pressure immediately to allow recirculation of the blood.

FIG. 2. Site of fingertip puncture.

7. Repeat steps 5 and 6 until enough blood has been collected.
8. Apply a piece of gauze to the puncture site, using slight pressure until the bleeding has stopped.

DISCUSSION

1. The finger should not be squeezed tightly. This causes tissue juice to mix with and dilute the blood.
2. When collecting blood for hematology tests, the finger must be wiped dry after each test. (Platelets clump immediately in the blood at the puncture site.)
3. The preceding procedure may be used for a heel stick, toe stick, and the rare ear lobe puncture.
4. Because of platelet adhesiveness and aggregation at the site of puncture, it is advisable to collect the platelet count and blood smears first (if requested) when samples for a number of tests are to be obtained.
5. The values for the red blood count, hematocrit, hemoglobin, and platelets are lower in capillary blood than in venous blood. Therefore, when possible and if the patient is old enough, the venipuncture is performed.

Venipuncture

A venipuncture must be performed with care. The veins of a patient are the main source of blood for testing, and the entry point for medications, intravenous solutions, and blood transfusions. Because there are only a limited number of easily accessible veins in a patient, it is important that everything be done to preserve their good condition and availability. Part of this responsibility lies with the medical technologist.

The recommended procedure is to have the patient lie down. If this is not possible, he should sit in a sturdy , comfortable chair with his arm firmly supported on a table or chair arm and easily accessible to the technologist. A patient should never stand or sit on a high stool during any process of blood collection. The technologist must be ready for the occasional patient who faints during this procedure, but this rarely occurs with hospital inpatients who are lying flat in bed.

EQUIPMENT

1. Alcohol, 70% (v/v).
2. Dry gauze pad.
3. Tourniquet.
4. Appropriate test tubes for tests ordered.
5. Vacutainer holder or syringe (Figs. 3 and 4).
6. Band-Aid.
7. Needle (Figs. 3 and 5). The choice of needle depends on the size of the vein. The most commonly used needle is the 20-gauge. The higher the gauge number, the smaller the diameter, or bore, of the needle. For small veins, a 21- or 22-gauge needle is recommended. The length of needle used is chosen by the individual technologist. The two most widely used needle lengths are 1 inch and 1½ inches. Blood may be obtained from most deep veins with a 1-inch needle.

PROCEDURE

1. *Make certain you have accurately identified the patient.* For inpatients, this may be done by checking the wristband. When collecting blood from an outpatient, ask him his name. A tube of blood mislabeled for type and cross match can end in a patient's death.
2. Prepare equipment (syringe and needle, or Vacutainer assembly).
3. Apply the tourniquet several inches above the puncture site as shown in Figs. 6 and 7, just tightly enough to be uncomfortable to the patient.

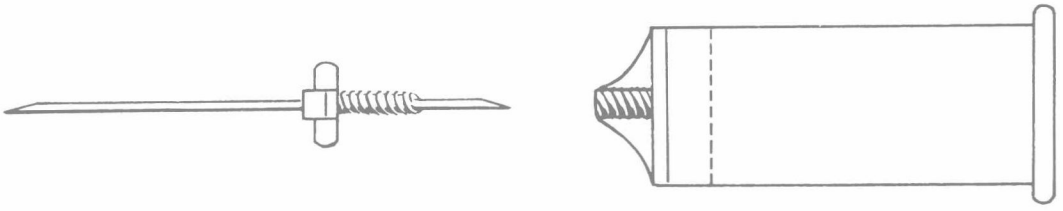

FIG. 3. Vacutainer holder and needle.

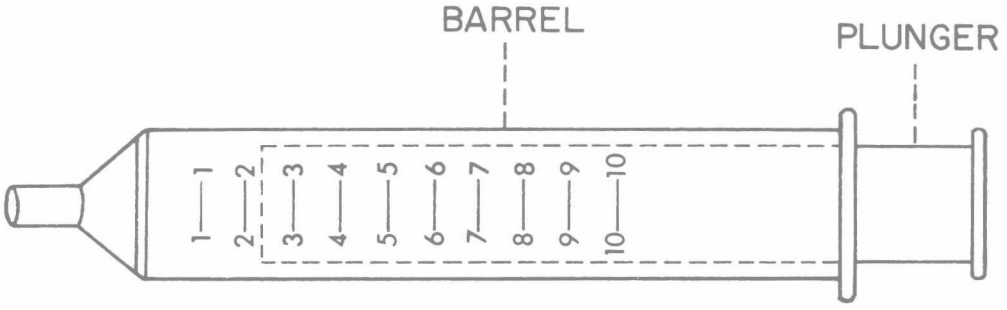

FIG. 4. Syringe.

4. Ask the patient to make a tight fist. This makes the vein more easily palpable.
5. Select a suitable vein for puncture (Fig. 8). The three main veins of the arm, which are the sites of the majority of venipunctures, are the cephalic, median cephalic, and the median basilic. (It is not necessary to learn the names of these veins. They are named here only as a method of identification for referral.) Generally, the median cephalic is the vein of choice because it is usually well anchored in tissue and does not roll when the vein is punctured. The median basilic vein, at the inner edge of the arm, has a tendency to roll in many patients. The cephalic vein is located on the edge of the outer part of the arm where the outside skin tends to be a little tougher. These points are mentioned only as a precaution. Using the index finger of your left hand, palpate the arm until you have found the best vein. It should feel similar to an elastic tube. (A frequent error made by students and by technologists is the failure to find the best vein, due to carelessness or haste.)
6. Cleanse the puncture area with 70% alcohol. Do not touch this area again with your finger or with any other unsterile object.

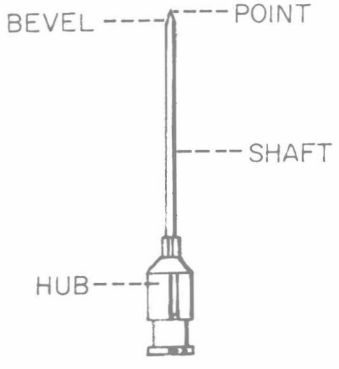

FIG. 5. Hypodermic needle.

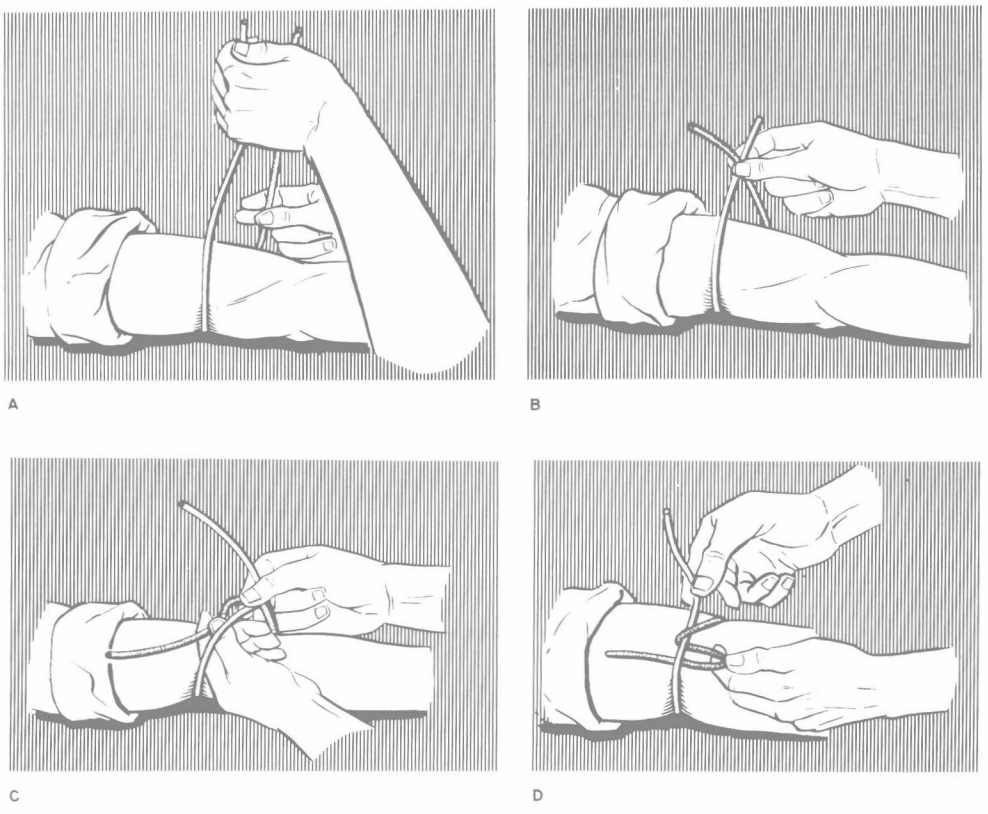

FIG. 6. Method of tourniquet application. A. Stretch the tourniquet to obtain the correct amount of tension. B. Grasp both sides of the tourniquet with the right hand while continuing to maintain the proper tension. C. With the left hand, reach through the loop and grasp the left side of the tourniquet. D. With the left hand, pull the tourniquet halfway through the loop. Release hands carefully.

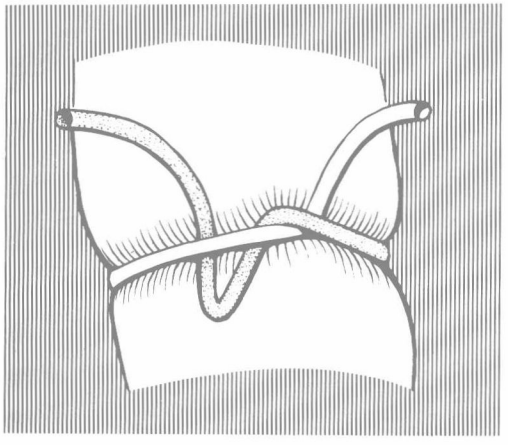

FIG. 7. Front view of tourniquet on arm. To release the tourniquet, carefully pull the end of the tourniquet on the left (shaded end).

7. If you are using a syringe, move the plunger up and down in the barrel once or twice to make sure that it does not stick. Expel all air from the syringe before proceeding.
8. Grasp the patient's arm just below the puncture site, pulling the skin tight with your thumb.
9. Hold the syringe, or Vacutainer assembly, with the opposite hand between the thumb and last three fingers. Rest the index finger against the hub of the needle to serve as a guide.
10. The needle should point in the same direction as the vein and be on a line with it at an angle approximately 15° to the arm.

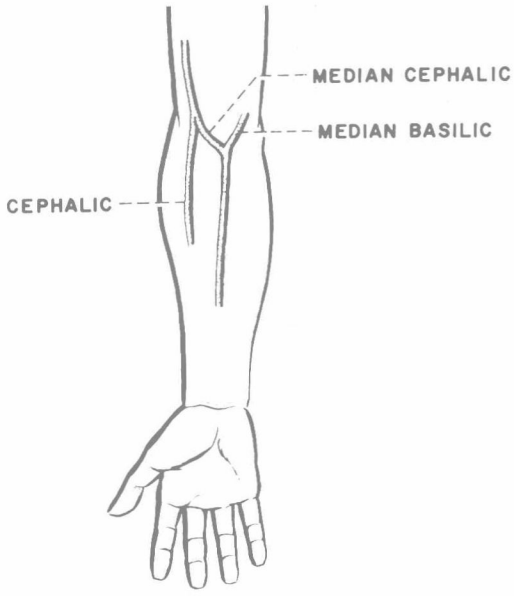

FIG. 8. Major veins of the arm.

11. The vein should be entered slightly below the area where it can be seen. In this way, there is more tissue available to serve as an anchor for the needle.
12. A prominent vein may be entered quickly with a one-step puncture of skin and vein. When the veins are deeper, or the entry more difficult, a two-step procedure should be followed. First, the skin is punctured and then, if need be, the left index finger may be used to palpate above the puncture site to confirm the exact location of the vein. The second step is to puncture the vein.
13. If a syringe is used, the blood flows into it when the needle has entered the vein. Care should be taken when pulling back on the plunger. Do not pull back with too much force because this may cause the blood to hemolyze; the force may pull the wall of the vein down on top of the bevel of the needle causing the blood flow to stop; or the needle may inadvertently be pulled out of the vein.
14. If the Vacutainer assembly is being used, as soon as the needle is in the vein, push the tube firmly but carefully in as far as it will go, ensuring that the needle is kept steady.
15. The tourniquet may be loosened as soon as the blood enters the tube or syringe, or it may be left on until the process is complete. It should be noted, however, that if the tourniquet is left on too long, the blood in this area will have an increased concentration of cells (hemoconcentration). The patient may open his fist as soon as the blood begins to flow.
16. Release the tourniquet before the needle is removed from the vein.
17. Apply a clean, dry gauze to the puncture site and quickly withdraw the needle.
18. Have the patient apply gentle pressure to the point of puncture for several minutes until the bleeding has stopped, then apply Band-Aid. The patient may also keep his arm straight, elevating it above the heart.
19. If a syringe is employed, remove the needle before expelling the blood into appropriate tubes. This process must be done quickly before the blood begins to clot.

DISCUSSION

1. In the event that you have been unable to puncture the vein immediately, use your free index finger to locate the vein. It may be that the needle has not gone deeply enough or perhaps it is slightly to the left or right of the vein. Do not attempt to puncture the vein from that location. This is painful to the patient and may cause tissue damage. Withdraw the needle until the point is almost to the surface of the skin, and then redirect the needle. This procedure is acceptable if the needle is close to the vein, but care should be taken that the pa-

tient is not caused too much pain. Sometimes a second venipuncture is necessary.

2. If a patient is receiving intravenous infusions into both arms, it is acceptable to puncture the vein 3 or 4 inches below the site of the I.V. device.
3. A technologist or student should not stick a patient more than three times. If the blood sample has not been obtained after the second attempt, it is usually advisable to call another technologist. By this time, both you and the patient have lost confidence.
4. It is important that pressure be applied to the site of the venipuncture. Failure to follow this procedure leads to a hematoma (bleeding into the tissues).
5. If the area surrounding the puncture site begins to swell while blood is being withdrawn, this usually indicates that the needle has gone through the vein or that the bevel of the needle is halfway out of the vein and there is a leakage of blood into the tissues. The tourniquet should be released and the needle withdrawn immediately, with pressure applied to the site.
6. In some instances it is almost impossible to locate a vein in the arm. In such a case, the veins of the lower arm, wrist, or hand may be used or, as a last resort, an ankle vein. The student should gain a reasonable amount of skill and confidence before attempting a venipuncture in these areas.
7. When a venipuncture must be carried out on a small child, it may be necessary to release the tourniquet when the blood starts to enter the syringe. Children's veins are small and collapse quickly due to the fact that blood is removed from the vein faster than it enters. Therefore, release the tourniquet carefully to improve the blood circulation.
8. When performing a venipuncture in the lower arm, hand, ankle, or on small children, a syringe or small Vacutainer assembly is generally used. When a venipuncture is required for a patient with poor or small veins, it is also advisable to use these. The use of standard-size Vacutainers tends to collapse these veins.
9. When disposing of the used needle, place it in a specially prepared container provided for this purpose. Never throw it directly into the wastebasket.
10. Regardless of the disease the patient has, be careful not to stick yourself with his needle. If this happens, report it to your supervisor as soon as possible on the day of the accident.

Isolation Techniques

When a patient has an infectious or communicable disease, certain safeguards must be followed to prevent further spread of the infection to hospital personnel or to other patients. Special techniques are also needed to shield or protect infection-prone patients from pathogens and other bacteria. To ensure optimal care of these patients, laboratory personnel should follow the procedures listed below, depending upon the type of illness of the patient being treated:

1. When handling patients with leukemia, severe burns, body radiation, kidney transplants, and plastic surgery, who must be protected from infection, the technologist should wear a sterile gown, cap, gloves, and mask. Shoe coverings may also be required.
2. Strict isolation is used in cases of active tuberculosis, meningococcal meningitis, rabies, diphtheria, viral encephalitis, polio, and certain infectious diseases such as measles, smallpox, and mumps. A gown should be worn by the technologist