

## CHAPTER 7

# CLINICAL LABORATORY

A basic knowledge of clinical laboratory procedures is critical for all Hospital Corpsmen, particularly those working at small dispensaries and isolated duty stations without the supervision of a medical officer. A patient's complaint may be of little value by itself, but coupled with the findings of a few easily completed laboratory studies, a diagnosis can usually be surmised and treatment initiated.

Hospital Corpsmen who can perform blood and urine tests and interpret the results are better equipped to determine the cause of illness or request assistance. Since they can provide a more complete clinical picture to the medical officer, their patients can be treated sooner.

In this chapter, we will discuss laboratory administrative responsibilities, ethics in the laboratory, the microscope, blood collection techniques, and step-by-step procedures for a complete blood count and urinalysis. Also included are basic testing procedures for bacteriologic, serologic, and fungal identification.

### THE HOSPITAL CORPSMAN AND THE CLINICAL LABORATORY

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**LEARNING OBJECTIVE:** *Recall clinical laboratory administrative procedures and ethics policy.*

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The Hospital Corpsman is not expected to make diagnoses from test findings or to institute definitive treatment based upon them. However, with the availability of modern communications facilities, having the results of these tests available will greatly assist the Corpsman in giving a clearer clinical picture to the supporting medical officer.

Needless to say, accuracy, neatness, and attention to detail are essential to obtain optimum test results. Remember also that these tests are only aids to diagnosis. Many other clinical factors must be taken into consideration before treatment may be started.

### ADMINISTRATIVE PROCEDURES AND RESPONSIBILITIES

The ability to perform clinical laboratory tests is a commendable attribute of the Hospital Corpsman. However, the entire testing effort could be wasted if proper recording and filing practices are ignored and the test results go astray. As a member of the medical team, it is your responsibility to make sure that established administrative procedures are followed with regard to accurate patient and specimen identification. It is your further responsibility to ensure laboratory reports in your department are handled and filed properly.

Since the test results are a part of the patient's clinical picture, their precision and accuracy are vital. Test results have a vital bearing upon the patient's immediate and future medical history. They are, therefore, made part of the patient's health record (inpatient or outpatient). Laboratory reports of inpatients are placed in the inpatient health record, while laboratory reports of outpatients are placed in the outpatient health record.

### Laboratory Request Forms

The armed forces have gone to great lengths to produce workable, effective laboratory forms that serve their purpose with a minimum of confusion and chance for error. These forms are standard forms (SF) in the 500 series. Their primary purpose is to request, report on, or record clinical laboratory tests. With the exception of SF-545 (*Laboratory Report Display*), SF laboratory forms are multicopied and precarbonized for convenience. The original copy of the laboratory report forms are attached to the SF-545 (located inside the patient's health record), and the carbon copy becomes part of the laboratory's master file. For a complete listing of SF forms and their purposes, refer to the *Manual of the Medical Department* (MANMED), NAVMED P-117.

SF laboratory request forms are not the only means by which healthcare providers can order laboratory tests. Many of today's naval medical facilities have computerized laboratory systems. Computerized laboratory systems enable healthcare providers to enter laboratory test requests into computers located in

their spaces. Once healthcare providers enter their test requests, patients may report immediately to the Laboratory Department, where specimens are obtained and tests are performed.

### Use of Laboratory Request Forms

Write information on the SF laboratory request forms in black or blue-black ink. Use a separate SF laboratory request form for each patient and for each test. Document the patient's full name, family member prefix and social security number, rate/rank, dependency status, branch of service, and status in the "Patient Identification" block. Also identify the ward or department ordering the test in this block. See figure 7-1 for an illustration of the *Urinalysis* request form, SF-550. Computer-generated laboratory test requests require the same patient identification data as SF laboratory requests.

Since the results of the requested laboratory test are usually closely associated with the patient's health and treatment, the requesting healthcare provider's name should also be clearly stated in the "Requesting Physician's Signature" block on the request form (fig. 7-1). The doctor requesting the urinalysis should sign in this block. Alternatively, you may type/print

the doctor's name in the block and initial the entry to authenticate it. This practice ensures that the report will get back to the provider as soon as possible.

Enter the requested test in the "Remarks" block (e.g., "Clean catch midstream to R/O urinary tract infection"). Because the data requested, the date reported, and the time of specimen collection are usually important in support of the clinical picture, these pieces of information should be clearly written on the request in the areas provided for them (fig. 7-1).

### Patient and Specimen Identification

Before accepting laboratory request forms and specimens in the laboratory, check patient identification information on both the request form and the specimen container label for completeness and legibility. Proper documentation of patient identification information on these items can prevent a great number of errors. Also, make sure the specimen(s) submitted is in fact the specimen of the patient submitting it. You need not stand over the patient while the specimen is being collected; however, keep in mind that for certain tests (such as drug or alcohol screening tests) individuals may attempt to substitute specimens.

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Figure 7-1.—SF-550, Urinalysis Request Form.

## Filing Laboratory Forms

After healthcare providers have reviewed laboratory test reports, they will initial the form. Initialing the form indicates the healthcare provider has reviewed the test results. After the healthcare provider releases the laboratory report, it should be filed in the patient's inpatient or outpatient health record, as appropriate. If a standard form is used to record test results, it should be attached **chronologically** to the SF-545, *Laboratory Report Display*, inside the patient's health record. The SF-545 functions as a display form for multiple laboratory reports. See figure 7-2. Use the preglued areas provided on the lab forms. However, since the glue is notorious for losing its grip after a while, you may use tape or staples to attach the form to the SF-545. Each SF-545 can accommodate a limited number of laboratory reports, so do not overcrowd the display form. When the SF-545 is full, add a new SF-545 to the health record and place it in front of the old SF-545. In this way, the most current lab reports will remain in chronological order.

Automated or computer-generated laboratory test reports, depending on the form's size, may be either mounted on the SF-545 or placed adjacent to the SF-545 in the health record. Keep in mind that these automated or computer-generated forms should also be filed chronologically.

## ETHICS AND GOOD PRACTICES IN THE LABORATORY

The nature of laboratory tests and their results must be treated as a confidential matter between the patient, the healthcare provider, and the performing technician. Chapter 16 of the MANMED outlines the Navy's ethics policy with regard to disclosure of the contents of a patient's medical record, including lab reports. It is good practice to prevent unauthorized access to these reports, to leave interpretation of the test results to the attending provider, and to refrain from discussing the results with the patient.

### BLOOD COLLECTION

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**LEARNING OBJECTIVE:** *Identify the correct steps to perform blood collection by the finger puncture method and venipuncture method, and recall Standard Precautions and other safety precautions that apply to blood collection.*

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There are two principal methods of obtaining blood specimens: the finger puncture method and the venipuncture method. For most clinical laboratory tests requiring a blood specimen, venous blood obtained by venipuncture is preferred. Blood collected by venipuncture is less likely to become contaminated, and the volume of blood collected is greater. Infection control practices, equipment requirements, and step-by-step instructions on performing both of these blood collection methods will be discussed in the following sections.

## STANDARD PRECAUTIONS

Under the concept of "Standard Precautions" outlined by the Centers For Disease Control and Prevention (CDC), blood and other bodily fluids should be considered as potentially infectious. To protect medical personnel from direct contact with blood during phlebotomy (blood collection), gloves are required to be worn. Gloves should be disposed of after each patient.

Needles and sharps used in the blood collection process should be handled with extreme caution and disposed of in biohazard sharps containers. Sharps containers should be conveniently located near phlebotomy work sites.

Absorbent materials, such as cotton 2 x 2's used to cover blood extraction sites, normally contain only a small amount of blood and can be disposed of as general waste. However, if a large amount of blood is absorbed, the absorbent material should be placed in a biohazard waste container and treated as infectious waste.

Clean phlebotomy work site equipment and furniture daily with a disinfectant.

## FINGER PUNCTURE

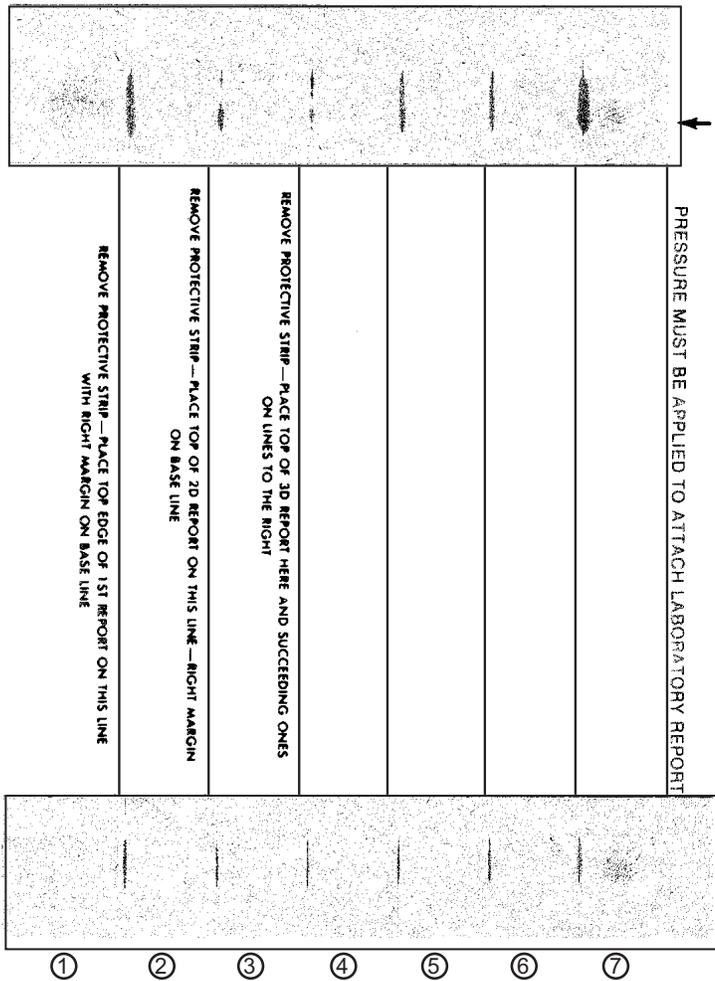
The finger puncture method is used when a patient is burned severely or is bandaged so that the veins are either covered or inaccessible. Finger puncture is also used when only a small amount of blood is needed.

### Materials Required for Finger Puncture Procedure

To perform a finger puncture, the following materials are required:

- Sterile gauze pads (2" x 2")

LABORATORY REPORT DISPLAY



PRESSURE MUST BE APPLIED TO ATTACH LABORATORY REPORT

REMOVE PROTECTIVE STRIP—PLACE TOP OF 3D REPORT HERE AND SUCCEEDING ONES ON LINES TO THE RIGHT

REMOVE PROTECTIVE STRIP—PLACE TOP OF 2D REPORT ON THIS LINE—RIGHT MARGIN ON BASE LINE

REMOVE PROTECTIVE STRIP—PLACE TOP EDGE OF 1ST REPORT ON THIS LINE WITH RIGHT MARGIN ON BASE LINE

ALIGN ALL LABORATORY REPORTS ALONG THIS BASE LINE

**INSTRUCTIONS:** This form may be used to display laboratory reports as a flow sheet to be read as a progressive table. If so, a separate sheet should be used for each type of report form. When assorted report forms are mounted on the display sheet, both test names and results should always be visible.

ENTER IN SPACE BELOW: PATIENT IDENTIFICATION—TREATING FACILITY—WARD NO.—DATE

FORMS DISPLAYED ON THIS SHEET ARE (Check one)	
<input type="checkbox"/> MOUNTED ON STRIPS 1 THROUGH 7	<input type="checkbox"/> MOUNTED ON STRIPS 1, 3, 5, AND 7
<input type="checkbox"/> CHEMISTRY I (SF 546)	<input type="checkbox"/> PARASITOLOGY (SF 552)
<input type="checkbox"/> CHEMISTRY II (SF 547)	<input type="checkbox"/> IMMUNOHEMATOLOGY (SF 556)
<input type="checkbox"/> CHEMISTRY III (SF 548)	<input type="checkbox"/> ASSORTED FORMS
<input type="checkbox"/> HEMATOLOGY (SF 549)	<input type="checkbox"/> OTHER (Specify)
<input type="checkbox"/> URINALYSIS (SF 550)	<b>MOUNTED ON STRIPS 1, 4, AND 7</b>
<input type="checkbox"/> SEROLOGY (SF 551)	<input type="checkbox"/> MICROBIOLOGY I (SF 553)
<input type="checkbox"/> SPINAL FLUID (SF 555)	<input type="checkbox"/> MICROBIOLOGY II (SF 554)
	<input type="checkbox"/> MISCELLANEOUS (SF 557)
	<input type="checkbox"/> ASSORTED FORMS

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LABORATORY REPORT DISPLAY

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Figure 7-2.—SF-545, Laboratory Report Display.

- 70% isopropyl alcohol or povidone-iodine solution pads
- Blood lancets
- Capillary tubes
- Bandages

Arrange your equipment in an orderly manner and have it within easy reach. Also, wash your hands before and after each procedure.

### Finger Puncture Procedure

To perform a finger puncture, follow the steps given below.

1. Explain the procedure to the patient.
2. Using the middle or ring finger, massage or “milk” the finger down toward the fingertip. Repeat this “milking” five or six times.
3. Cleanse the fingertip with an alcohol pad or povidone-iodine solution and let dry.
4. Take a lancet and make a quick deep stab on the side of the finger (off-center). To obtain a large rounded drop, the puncture should be across the striations of the fingertip. See figure 7-3.
5. Wipe away the first drop of blood to avoid dilution with tissue fluid. Avoid squeezing the fingertip to accelerate bleeding as this tends to dilute the blood with excess tissue fluid, but gentle pressure some distance above the puncture site may be applied to obtain a free flow of blood.
6. When the required blood has been obtained, apply a pad of sterile gauze and instruct the patient to apply pressure, then apply a bandage.

When dealing with infants and very small children, the heel or great toe puncture is the best method to obtain a blood specimen. This method is performed in much the same way.

### VENIPUNCTURE (VACUTAINER METHOD)

The collection of blood from veins is called venipuncture. For the convenience of technician and patient, arm veins are best for obtaining a blood sample. If arm veins cannot be used due to interference from bandage or IV therapy, thrombosed or hardened veins, etc., consult your supervisor for instructions on the use of hand or foot veins.

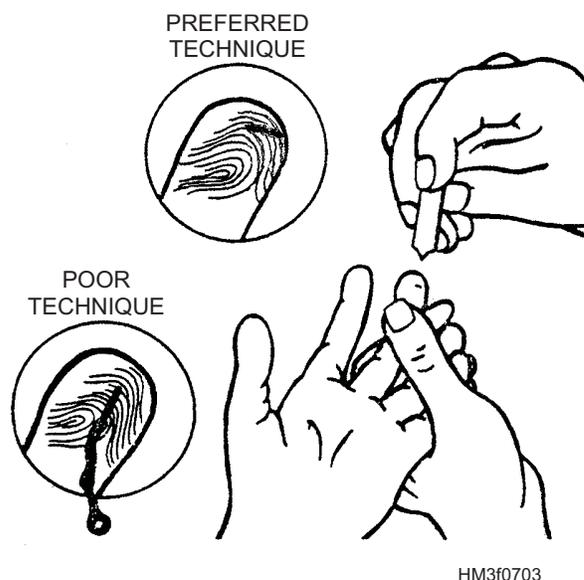


Figure 7-3.—Finger puncture.

**NOTE:** Do not draw blood from an arm with IV fluid running into it. Choose another site. The IV fluid will alter tests results.

### Materials Required for Venipuncture Procedure

To perform a venipuncture, the following materials are required:

- Sterile gauze pads (2" x 2")
- 70% isopropyl alcohol or povidone-iodine solution pads
- Tourniquet
- Vacutainer needles and holder
- Vacutainer tube appropriate for the test to be performed

Arrange your equipment in an orderly manner and have it within easy reach. Also, wash your hands before the procedure.

### Venipuncture Procedure

Position the patient so that the vein is easily accessible and you are able to perform the venipuncture in a comfortable position. Always have the patient either lying in bed or sitting in a chair with the arm propped up.

## WARNING

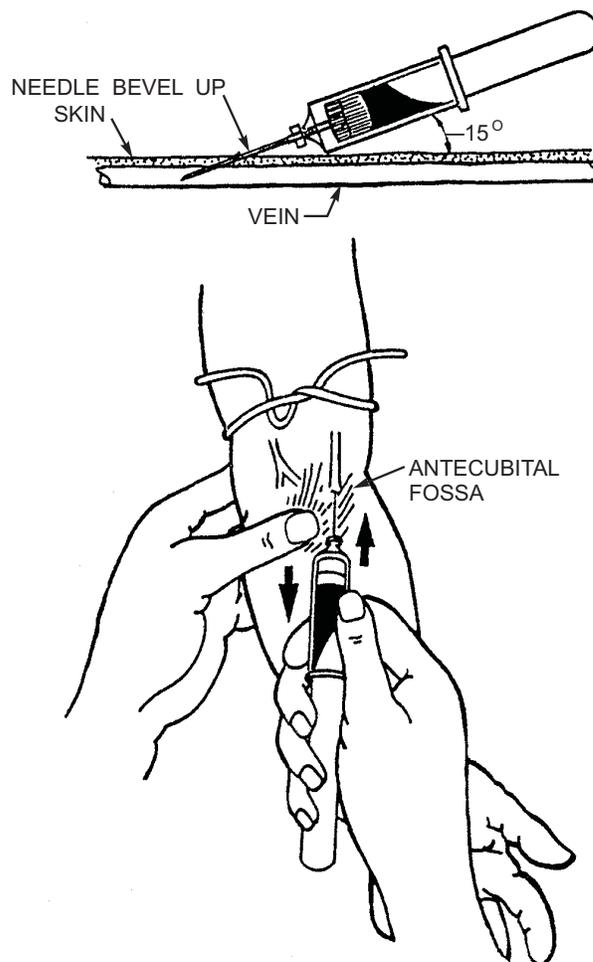
Never perform a venipuncture with the patient standing up. If patients should faint, they could seriously injure themselves. Also, safeguards should be in place to prevent patients from falling forward when they are seated.

To perform venipuncture, follow the steps given below.

1. Explain the procedure to the patient.
2. Apply tourniquet around the arm approximately 2 to 3 inches above the antecubital fossa (the depression in the anterior region of the elbow, see figure 7-4) with enough tension so that the VEIN is compressed, but not the ARTERY. A BP cuff (sphygmomanometer) may be used instead of a tourniquet if a patient is difficult to draw.
3. Position the patient's arm extended with little or no flexion at the elbow.
4. Locate a prominent vein by palpation (feeling). If the vein is difficult to find, it may be made more prominent by massaging the arm with an upward motion to force blood into the vein.
5. Cleanse the puncture site with a 70% alcohol pad or povidone-iodine solution and allow to dry.

**CAUTION:** After cleaning the puncture site, only the sterile needle should be allowed to touch it.

6. "Fix" or hold the vein taut. This is best accomplished by placing the thumb under the puncture site and exerting a slight downward pressure on the skin or placing the thumb to the side of the site and pulling the skin taut laterally (fig. 7-4).
7. Using a smooth continuous motion, introduce the needle, bevel side up, into the side of the vein at about a 15-degree angle with the skin (fig. 7-4).



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Figure 7-4.—Venipuncture.

8. Holding the vacutainer barrel with one hand, push the tube into the holder with the other hand and watch for the flow of blood into the tube until filling is completed.
9. Once all the specimens have been collected, hold the vacutainer with one hand and release the tourniquet with the other.
10. Place a sterile gauze over the puncture site and remove the needle with a quick, smooth motion.
11. Apply pressure to the puncture site and instruct the patient to keep the arm in a straight position. Have the patient hold pressure for at least 3 minutes.
12. Take this time to invert any tubes that need to have anticoagulant mixed with the blood.
13. Label specimens.
14. Reinspect the puncture site to make sure bleeding has stopped, and apply a bandage.

## THE MICROSCOPE

**LEARNING OBJECTIVE:** *Identify the parts of the microscope, and determine their functions.*

Before any attempts are made to view blood smears, urinary sediments, bacteria, parasites, etc., it is absolutely essential that beginners know the instrument with which they will be spending considerable time—the microscope. The microscope is a precision instrument used extensively in clinical laboratories to make visible objects too small to be seen by the unaided eye. Most laboratories are equipped with binocular (two-eyepiece) microscopes, but monocular microscopes are also commonly used. The type of microscope most often used in the laboratory is referred to as the **compound microscope**. See figure 7-5. A compound microscope contains a system of lenses of sufficient magnification and resolving power (ability to show, separate, and

distinguish) so that small elements lying close together in a specimen appear larger and distinctly separated. In the following sections, the compound microscope's framework, illumination system, magnification system, and focusing system will be discussed.

### FRAMEWORK

The framework of the compound microscope consists of four parts: the arm, the stage, the mechanical stage, and the base (fig. 7-5).

#### Arm

The arm is the structure that supports the magnification and focusing system. It is the handle by which the microscope is carried.

#### Stage

The stage is the platform on which a specimen is placed for examination. In the center of the stage is an aperture or hole that allows the passage of light from the condenser.

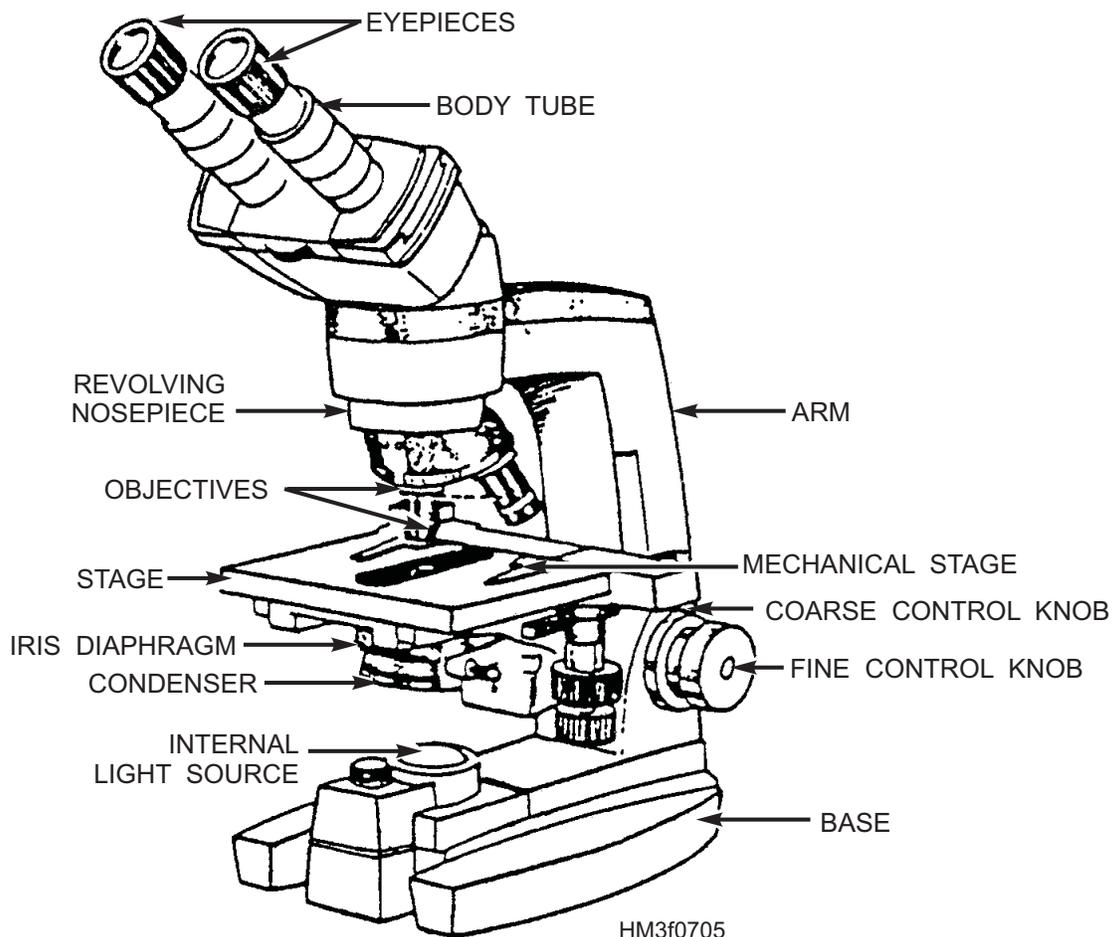


Figure 7-5.—Compound microscope.

## Mechanical Stage

The mechanical (movable) stage holds the specimen in place and is the means by which the specimen may be moved about on the stage.

## Base

The base is the structure on which the microscope rests.

## ILLUMINATION SYSTEM

Ideal illumination of a specimen viewed under the microscope requires even light distribution. The objectives must also be entirely filled with light from the condenser. To fulfill these requirements, the illumination system of the compound microscope consists of three parts: an internal light source, a condenser, and an iris diaphragm. See figure 7-5.

### Internal Light Source

The internal light source is built into the base of the microscope. It provides a precise and steady source of light into the microscope.

### Condenser

The condenser is composed of a compact lens system and is located between the light source and stage. The condenser concentrates and focuses light from the light source directly through the specimen.

### Iris Diaphragm

An iris diaphragm located on the condenser controls the diameter of the light source's beam. To improve resolution, the operator should adjust the opening of the iris diaphragm to approximately the same size as the face of the objective lens. In addition to the diaphragm on the condenser, an iris diaphragm may be located on the internal light source. This iris diaphragm controls the amount of light sent to the condenser from the internal light source.

## MAGNIFICATION SYSTEM

The magnification system of the compound microscope contains at least two lens systems. The two lens systems are mounted on either end of a tube called the body tube. The lens nearest the object is called the objective lens, and the lens nearest the eye is the ocular lens or eye piece. See figure 7-5.

## Objective Lenses

On a compound microscope, there is usually a set of three objective lenses (or "objectives"). This set of objectives is the component most responsible for the magnification and resolution of detail in a specimen. Each objective lens has a different focus distance and magnification power. A set of objectives normally consists of a low-power lens (approximate focus 16 mm, magnification 10X), a high-power lens (approximate focus 4 mm, magnification 45X), and an oil-immersion lens (approximate focus 1.8 mm, magnification 100X). Objective lenses are color coded for easy recognition: 16 mm-10X (green), 4 mm-45X (yellow), and 1.8 mm-100X (red).

### Revolving Nosepiece

The revolving nosepiece contains openings into which objective lenses are fitted, and revolves objectives into desired position.

### Body Tube

The body tube is a tube that permits light to travel from the objective to the ocular lens.

### Ocular Lenses

Ocular lenses, or eyepieces, are located on top of the body tube and usually have a magnification power of 10X. To calculate the total magnification of a specimen, you multiply the magnification power of the objective by the magnification power of the ocular lens. Examples of total magnifications are provided in table 7-1.

## FOCUSING SYSTEM

Focusing is accomplished by moving the stage up or down with the coarse and fine control knob (fig. 7-5). Whether the stage needs to be raised or lowered depends on the focal length of the objective

Table 7-1.—Examples of Total Magnifications

Objective Lens	Color Code	10X Ocular	Total Magnification
16 mm-10X	Green	10X	100X
4 mm-45X	Yellow	10X	450X
1.8 mm-100X	Red	10X	1000X

being used. For example, the high-powered objective of short focal length (4 mm) will need the stage raised so the objective is very close to the specimen, while the low-powered objective of a longer focal length (16 mm) will need the stage lowered so the objective is farther from the specimen.

The coarse control knob is used initially to bring the specimen's image into approximate focus. Once this is accomplished, the fine control knob sharpens the image.

### **Coarse Control Knob**

The coarse control knob is the larger and inner knob. Rotating the coarse control knob allows the image to appear in approximate focus.

### **Fine Control Knob**

The fine control knob is the smaller and outer knob. Rotating this control knob renders the image clear and well-defined.

## **FOCUSING THE MICROSCOPE**

The process of focusing consists of adjusting the relationship between the optical system of the microscope and the object to be examined so that a clear image of the object is obtained. The distance between the upper surface of the glass slide on the microscope stage and the faces of the objective lens varies depending upon which of the three objectives is in the focusing position. It is a good practice to obtain a focus with the low-power objective first, then change to the higher objective required to avoid accidentally damaging the objective lens, the specimen, or both. Most modern microscopes are equipped with parfocal objectives (meaning that if one objective is in focus, the others will be in approximate focus when the nosepiece is revolved). With the low-power objective in focusing position, observe the following steps in focusing.

1. Seat yourself behind the microscope, then lower your head to one side of the microscope until your eyes are approximately at the level of the stage.
2. Using the coarse adjustment knob, lower the body tube until the face of the objective is within 1/4 inch of the object. Most microscopes are constructed in such a way that the low-power (green) objective cannot be lowered and make contact with the object on the stage.

3. While you are looking through the ocular, you should use the coarse adjustment knob to elevate the body tube until the image becomes visible. Then use the fine adjustment knob to obtain a clear and distinct image. Do not move the focusing knob while changing lenses.
4. If the high-power objective (yellow) is to be used next, bring it into position by revolving the nosepiece (a distinct "click" indicates it is in proper alignment with the body tube). Use the fine adjustment knob only to bring the object into exact focus.
5. If specimen is too dark, you can increase lighting by opening the iris diaphragm of the condenser.
6. The oil-immersion objective (red) is used for detailed study of stained blood and bacterial smears. Remember that the distance between objective lens and object is very short, and great care must be employed so the specimen is not damaged. After focusing with the high-power objective and scanning for well-defined cells, raise the objective, place a small drop of immersion oil, free of bubbles, on the slide, centering the drop in the circle of light coming through the condenser. Next, revolve the nosepiece to bring the oil-immersion objective into place, and, by means of the coarse adjustment knob, slowly lower the body tube until the lens just makes contact with the drop of oil on the slide. The instant of contact is indicated by a flash of light illuminating the oil. The final step in focusing is done with the fine adjustment knob. It is with this lens in particular that lighting is important. The final focus, clear and well-defined, will be obtained only when proper light adjustment is made.

## **CARE OF THE MICROSCOPE**

The microscope is an expensive and delicate instrument that should be given proper care.

Moving or transporting microscopes should be accomplished by grasping the arm of the scope in one hand and supporting the weight of the scope with the other hand. Avoid sudden jolts and jars.

Keep the microscope clean at all times; when not in use, microscopes should be enclosed in a dustproof cover or stored in their case. Remove dust with a camel hair brush. Lenses may be wiped carefully with lens

tissue. When the oil-immersion lens is not being used, remove the oil with lens tissue. Use oil solvents (such as xylene) on lenses only when required to remove dried oil and only in the minimal amount necessary. **Never use alcohol or similar solvents to clean lenses.**

## COMPLETE BLOOD COUNT

**LEARNING OBJECTIVE:** *Identify the five parts of a complete blood count, and recognize the testing procedures for the following: Unopette® Red Blood Cell Count, Microhematocrit, Unopette White Blood Cell Count, and Differential White Blood Cell Count.*

A complete blood count consists of the following five tests:

- Total red blood cell (RBC) count
- Hemoglobin determination
- Hematocrit reading
- Total white blood cell (WBC) count
- Differential white blood cell count

The complete blood count, commonly referred to as a CBC, is used in the diagnosis of many diseases. Blood collected for these tests are capillary or peripheral blood and venous blood. CBCs may be performed either manually or by using automated hematology analyzers. The manual method is used in isolated locations and on board some naval vessels where a hematology analyzer installation is not practical. For this reason, and because machines break down on occasion, the manual method will be covered in the following sections.

## COUNTING BLOOD CELLS

To manually count red blood cells (erythrocytes) and white blood cells (leukocytes), you will need a microscope and an instrument called a **hemacytometer**. See figure 7-6. The hemacytometer is a thick glass slide with three raised parallel platforms on the middle third of the device. The central platform is subdivided by a transverse groove to form two halves, each wider than the two lateral platforms and separated from them and from each other by moats. The central platforms each contain a counting chamber and are exactly 0.1 mm lower than the lateral platforms.

Each counting chamber has precisely ruled lines etched into the glass, forming a grid. This grid or ruled area is so small that it can only be seen with the aid of a microscope. The grid used by most laboratories is the Improved Neubauer Ruling. See figure 7-7 for an example of the Improved Neubauer Ruling. The Improved Neubauer Ruling is 3 by 3 mm (9 mm<sup>2</sup>) and subdivided into nine secondary squares, each 1 by 1 mm (1 mm<sup>2</sup>).

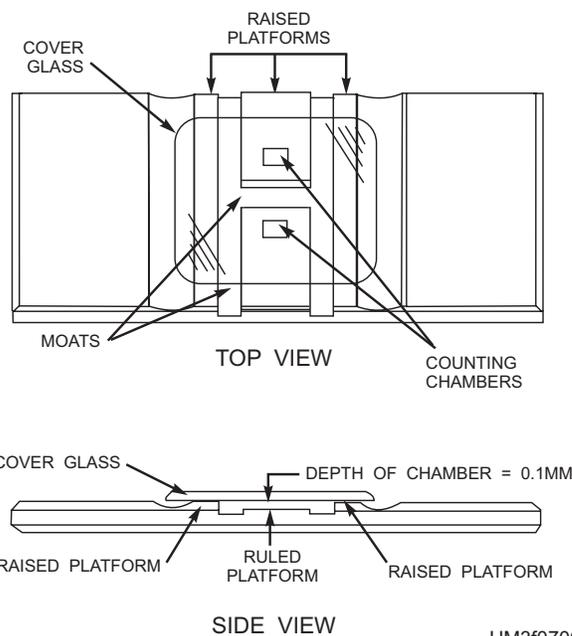
A thick cover glass, ground to a perfect plane, accompanies the counting chamber (fig. 7-6). Ordinary cover glasses have uneven surfaces and should not be used. When the cover glass is in place on the platform of the counting chamber, there is a space exactly 0.1 mm thick between it and the ruled platform.

Counts of red blood cells and white blood cells are each expressed as concentration: cells per unit volume of blood. The unit of volume for cell counts is expressed as cubic millimeters (mm<sup>3</sup>) because of the linear dimensions of the hemacytometer chamber.

## TOTAL RED BLOOD CELL COUNT

The total red blood cell (erythrocyte) count is the number of red cells in one cubic millimeter of blood. The normal red blood cell count is as follows:

Adult male.....	4.2 to 6.0 million per mm <sup>3</sup>
Adult female.....	3.6 to 5.6 million per mm <sup>3</sup>
Newborn.....	5.0 to 6.5 million per mm <sup>3</sup>



**Figure 7-6.—Top and side views of a hemacytometer.**

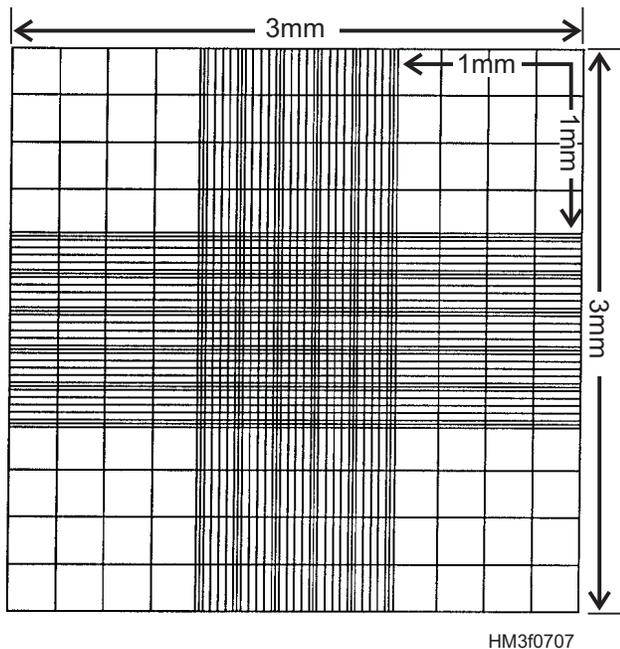


Figure 7-7.—Improved Neubauer Ruling.

As we said earlier, the red cell count is used in the diagnosis of many diseases. For example, a red cell count that drops below normal values may indicate anemia and leukemia. On the other hand, a red cell count that rises above the normal values may indicate dehydration.

The Unopette® Method is used to manually count red blood cells. Material requirements and the step-by-step procedures for performing this procedure are provided in the following sections.

### Materials Required for Unopette Procedure

The Unopette procedure consists of a disposable diluting pipette system that provides a convenient, precise, and accurate method for obtaining a red blood cell count. To perform a red blood cell count using the Unopette method, you will need to obtain the following materials:

- A disposable Unopette (see fig. 7-8) for RBC counts. The Unopette consists of
  - a shielded capillary pipette (10 microliter ( $\mu$ l) capacity), and
  - a plastic reservoir containing a premeasured volume of diluent (1:200 dilution).
- Hemacytometer and coverglass
- Microscope with light source

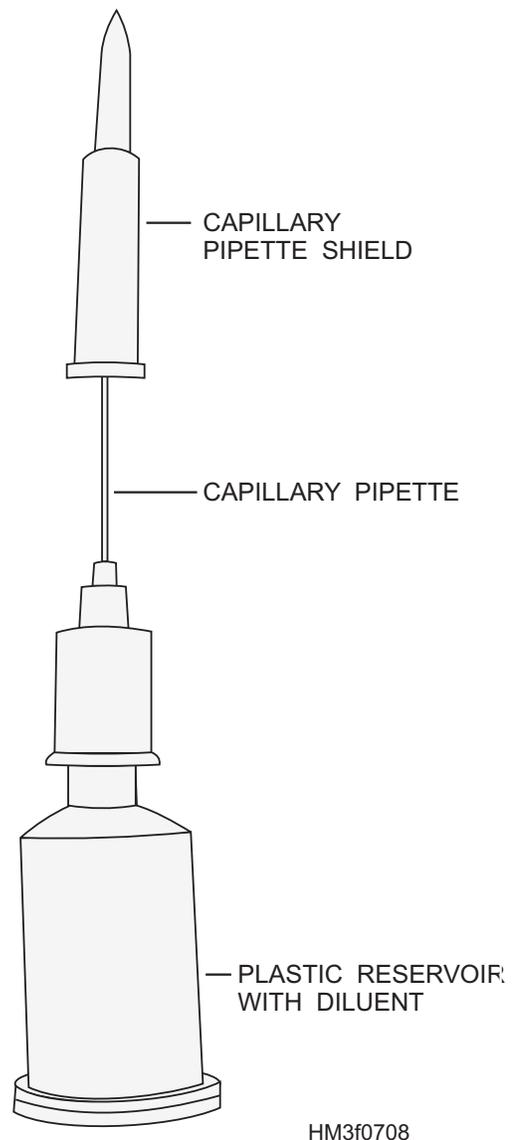


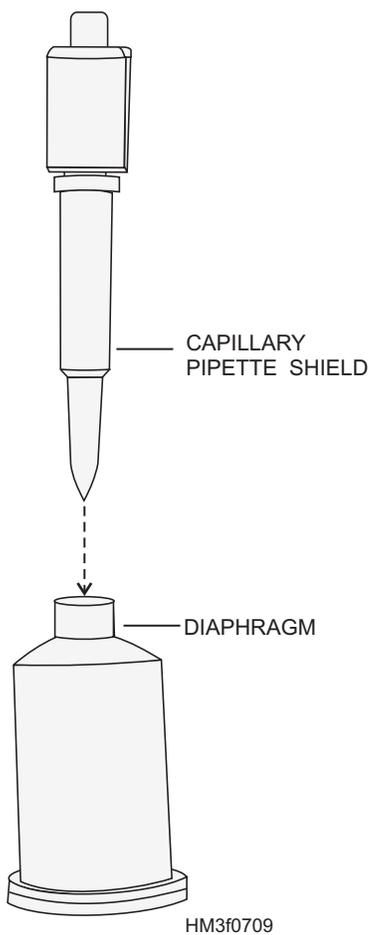
Figure 7-8.—Unopette® for RBC count.

- Hand-held counter
- Laboratory chit

### Unopette Procedure

The Unopette procedure for counting red blood cells is as follows:

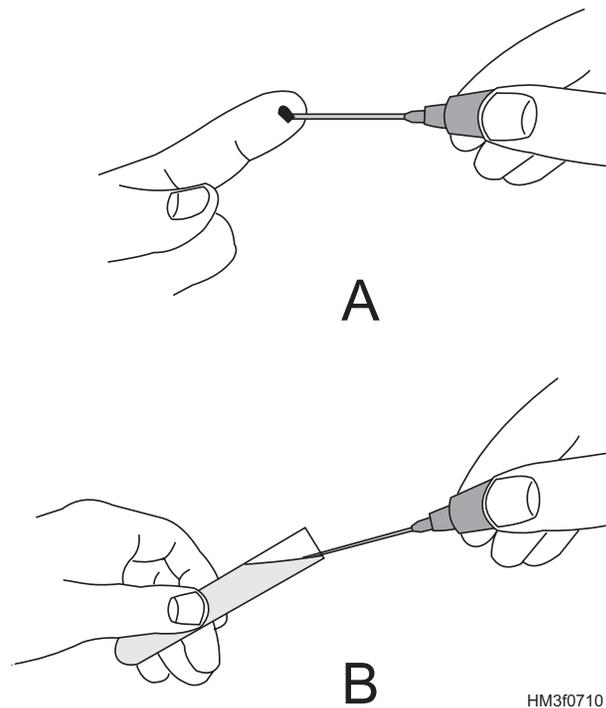
1. Puncture the diaphragm in the neck of the diluent reservoir with the tip of the capillary shield on the capillary pipette. See figure 7-9.
2. After obtaining free-flowing blood from a lancet puncture of the finger, remove the protective plastic shield from the capillary pipette. Holding the capillary pipette slightly above the horizontal, touch the tip to the blood source (see fig. 7-10, view A). The pipette will



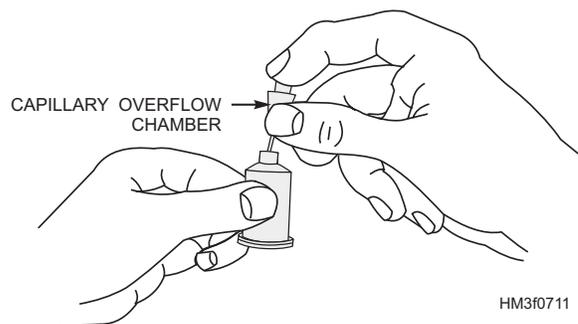
**Figure 7-9.—Puncturing the diaphragm of diluent with the capillary pipette shield.**

fill by capillary action. When blood reaches the end of the capillary bore in the neck of the pipette, filling is complete and will stop automatically. The amount of blood collected by the capillary tube is 10  $\mu$ l. Wipe any blood off the outside of the capillary tube, making sure no blood is removed from inside the capillary pipette. (An alternative source of blood is a thoroughly mixed fresh venous blood sample obtained by venipuncture. See figure 7-10, view B.)

3. With one hand, gently squeeze the reservoir to force some air out, but do not expel any diluent (fig. 7-11). Maintain pressure on the reservoir. With the other hand, cover the upper opening of the capillary overflow chamber with your index finger and seat the capillary pipette holder in the reservoir neck (see fig. 7-11).
4. Release pressure on the reservoir and remove your finger from the overflow chamber opening.



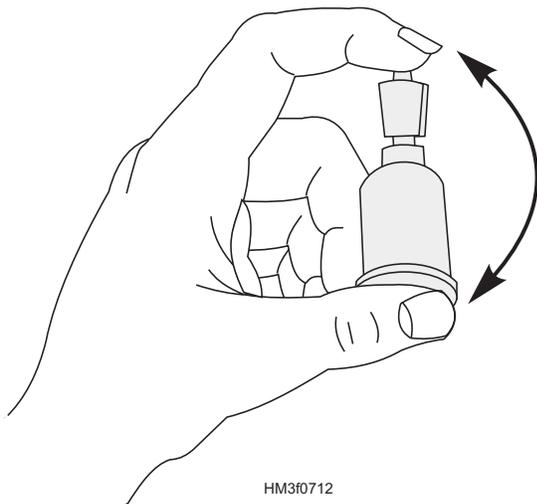
**Figure 7-10.—Drawing blood into the Unopette capillary tube: A. From a finger puncture; B. From a venous blood sample.**



**Figure 7-11.—Preparing reservoir to receive blood from the capillary tube.**

Suction will draw the blood into the diluent in the reservoir.

5. Squeeze the reservoir gently two or three times to rinse the capillary tube, forcing diluent into but not out of the overflow chamber, releasing pressure each time to return diluent to the reservoir. Close the upper opening with your index finger and invert the unit several times to mix the blood sample and the diluent. See figure 7-12.
6. For specimen storage, cover the overflow chamber of the capillary tube with the capillary shield.

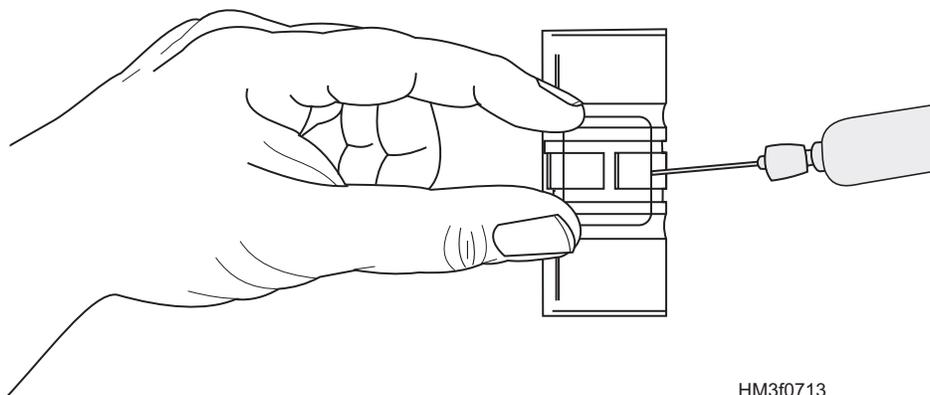


**Figure 7-12.—Mixing blood sample and diluent.**

7. Immediately prior to cell counting, mix again by gentle inversion, taking care to cover the upper opening of the overflow chamber with your index finger.
8. Place the coverglass on the hemacytometer counting chamber, making sure coverglass is clean and free of grease. (Fingerprints must be completely removed.)
9. Remove the pipette from the reservoir. Squeeze the reservoir and reseat the pipette in the reverse position, releasing pressure to draw any fluid in the capillary tube into the reservoir. Invert and fill the capillary pipette by gentle pressure on the reservoir. After discarding the first 3 drops, load (charge) the counting chamber of the hemacytometer by gently squeezing the

reservoir while touching the tip of the pipette against the edge of the coverglass and the surface of the counting chamber (fig. 7-13). A properly loaded counting chamber should have a thin, even film of fluid under the coverglass (fig. 7-14, view A). Allow 3 minutes for cells to settle. If fluid flows into the grooves (moats) at the edges of the chamber or if air bubbles are seen in the field, the chamber is flooded and must be cleaned with distilled water, dried with lens tissue, and reloaded (fig. 7-14, view B). If the chamber is underloaded, carefully add additional fluid until properly loaded.

10. Place the loaded hemacytometer into a petri dish with a piece of dampened tissue to keep the hemacytometer from drying out (fig. 7-15). Allow 5 to 10 minutes for the cells to settle.
11. Once the cells have settled, place the hemocytometer on the microscope. Use the low-power lens to locate the five small fields (1, 2, 3, 4, and 5) in the large center square bounded by the double or triple lines. See figure 7-16. Each field measures  $1/25 \text{ mm}^2$ ,  $1/10 \text{ mm}$  in depth, and is divided into 16 smaller squares. These smaller squares form a grid that makes accurate counting possible.
12. Switch to the high-power lens and count the number of cells in field 1. Move the hemacytometer until field 2 is in focus and repeat the counting procedure. Continue until the cells in all five fields have been counted. Note the fields are numbered clockwise around the chamber, with field 5 being in the center.



**Figure 7-13.—Loading the counting chamber.**

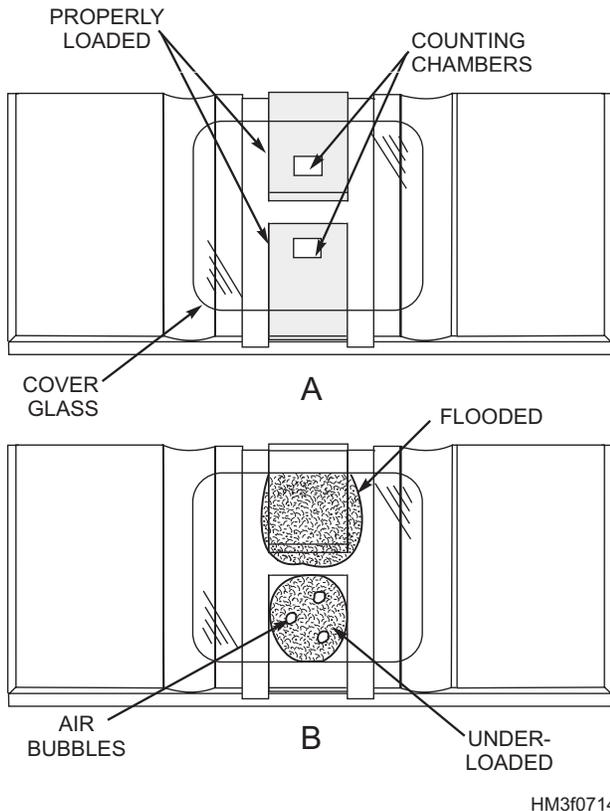


Figure 7-14.—Loading hemacytometer: A. Hemacytometer properly loaded; B. Hemacytometer improperly loaded.

Count the fields in this order. To count the cells in each field, start in the upper left small square and follow the pattern indicated by the arrow in field 1 of figure 7-16. Count all of the cells within each square, including cells touching the lines at the top and on the left. **Do not count any cells that touch the lines on the right or at the bottom.**

13. Total the number of cells counted in all five fields and multiply by 10,000 to arrive at the number of red cells per cubic millimeter of blood.

<b>Example:</b> Total number of cells counted = 423.
Multiply:
423 x 10,000 = 4,230,000
<b>Total red cell count = 4,230,000 cells/mm<sup>3</sup></b>

**NOTE:** The number of cells counted in each field should not vary by more than 20. A greater variation may indicate poor distribution of the cells in the fluid, resulting in

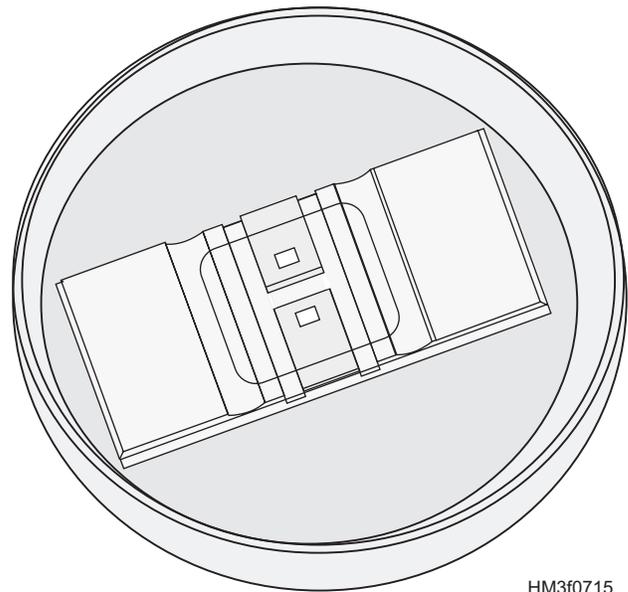


Figure 7-15—Loaded hemacytometer placed inside petri dish.

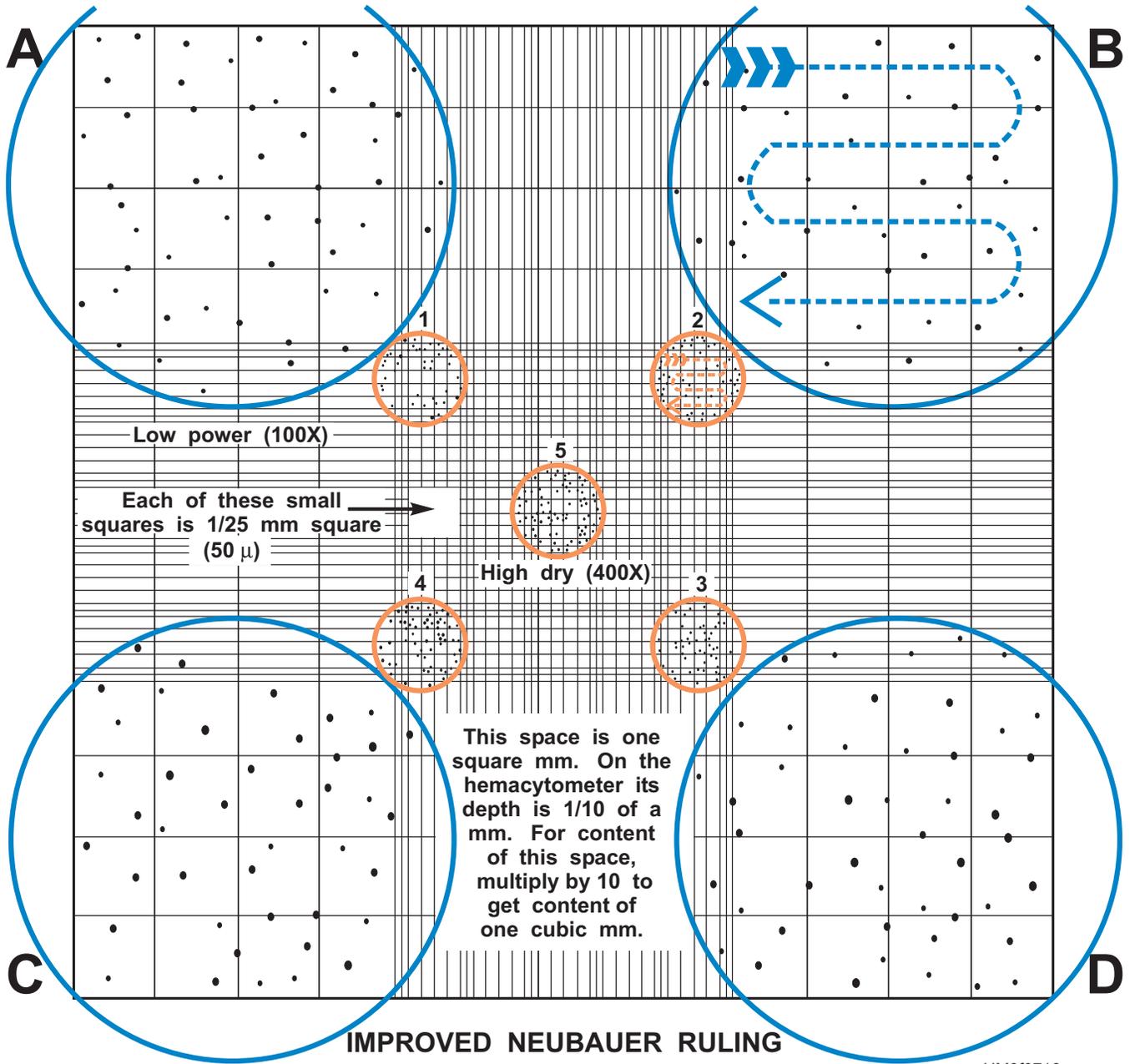
an inaccurate count. If this happens, the test must be repeated.

## HEMOGLOBIN DETERMINATION

A routine test performed on practically every patient is the hemoglobin determination. Hemoglobin determination, or hemoglobinometry, is the measurement of the concentration of hemoglobin in the blood. Hemoglobin's main function in the body is to carry oxygen from the lungs to the tissues and to assist in transporting carbon dioxide from the tissues to the lungs. The formation of hemoglobin takes place in the developing red cells located in bone marrow.

Hemoglobin values are affected by age, sex, pregnancy, disease, and altitude. During pregnancy, gains in body fluids cause the red cells to become less concentrated, causing the red cell count to fall. Since hemoglobin is contained in red cells, the hemoglobin concentration also falls. Disease may also affect the values of hemoglobin. For example, iron deficiency anemia may drop hemoglobin values from a normal value of 14 grams per 100 milliliters to 7 grams per 100 milliliters. Above-normal hemoglobin values may occur when dehydration develops. Changes in altitude affect the oxygen content of the air and, therefore, also affect hemoglobin values. At higher altitudes there is less oxygen in the air, resulting in an increase in red cell counts and hemoglobin values. At lower altitudes there is more oxygen, resulting in a decrease in red cell counts and hemoglobin values.

# HEMACYTOMETER (COUNTING CHAMBER)



A - B - C - D ARE FIELDS USED IN DOING THE WHITE BLOOD CELL COUNT.

1 - 2 - 3 - 4 - 5 ARE FIELDS USED IN DOING THE RED BLOOD CELL COUNT.

*(Letters, numbers, and arrows are not actually seen in the counting chamber. They are for illustration only. Circles depict areas seen through the microscope.)*

Figure 7-16.—Hemacytometer counting chamber.

The normal values for hemoglobin determinations are as follows:

	<u>Grams per</u> <u>100 ml blood</u>	<u>Percent</u>
Woman.....	12.5 to 15.....	83 to 110
Men.....	14 to 17.....	97 to 124
Newborn infants.....	17 to 23.....	97 to 138

Methods for hemoglobin determination are many and varied. The most widely used automated method is the **cyanmethemoglobin method**. To perform this method, blood is mixed with Drabkin's solution, a solution that contains ferricyanide and cyanide. The ferricyanide oxidizes the iron in the hemoglobin, thereby changing hemoglobin to methemoglobin. Methemoglobin then unites with the cyanide to form cyanmethemoglobin. Cyanmethemoglobin produces a color which is measured in a colorimeter, spectrophotometer, or automated instrument. The color relates to the concentration of hemoglobin in the blood.

Manual methods for determining blood hemoglobin include the **Haden-Hausse** and **Sahli-Hellige methods**. In both methods, blood is mixed with dilute hydrochloric acid. This process hemolyzes the red cells, disrupting the integrity of the red cells' membrane and causing the release of hemoglobin, which, in turn, is converted to a brownish-colored solution of acid hematin. The acid hematin solution is then compared with a color standard.

### **HEMATOCRIT (PACKED CELL VOLUME) DETERMINATION**

The hematocrit or packed cell volume (PCV) determines the percentage of red blood cells (RBCs) in whole blood.

The normal hematocrit value for men is 42% to 52%; for women, 37% to 47%; and for newborns, 53% to 65%. When hematocrit determinations are below normal, medical conditions such as anemia and leukemia may be present. Above-normal hematocrit determinations indicate medical conditions like dehydration, such as occur in severe burn cases.

Currently, automated hematology analyzers supply most hematocrits. However, when hematology analyzers are not available, hematocrit determinations can be manually performed by the microhematocrit method or macrohematocrit method. Both methods call for the blood to be centrifuged, and the percentage of packed red cells is found by calculation.

The microhematocrit method is the most accurate manual method of determining blood volume and should be used whenever feasible. Material requirements and the step-by-step procedures for performing the microhematocrit method will be covered in the following sections.

### **Materials Required for Microhematocrit Procedure**

To perform a hematocrit using the microhematocrit method, the following materials are required.

- Capillary tubes, plain or heparinized
- Modeling clay sealant
- Microhematocrit centrifuge
- Microhematocrit reader

### **Microhematocrit Procedure**

To perform the microhematocrit method, you should follow the steps listed below:

1. Fill the capillary tube two-thirds to three-quarters full with well-mixed, oxalated venous blood or fingertip blood. (For fingertip blood use heparinized tubes, and invert several times to mix.)
2. Seal one end of the tube with clay.
3. Place the filled tube in the microhematocrit centrifuge, with the plugged end away from the center of the centrifuge.
4. Centrifuge at a preset speed of 10,000 to 12,000 rpm for 5 minutes. If the hematocrit exceeds 50 percent, centrifuge for an additional 3 minutes.
5. Place the tube in the microhematocrit reader. Read the hematocrit by following the manufacturer's instructions on the microhematocrit reading device.

### **TOTAL WHITE BLOOD CELL COUNT**

The total white cell (leukocyte) count determines the number of white cells per cubic millimeter of blood. A great deal of information can be derived from white cell studies. The white blood cell count (WBC) and the differential count are common laboratory tests, and they are almost a necessity in determining the nature and severity of systemic infections. Normal WBC values in adults range from 4,500 to 11,000 cells per cubic millimeter; in children the range is from 5,000 to 15,000 cells per cubic millimeter; and in

newborns the range is from 10,000 to 30,000 cells per cubic millimeter.

White blood cell counts are performed either manually or with automated hematology analyzers. Only the manual method will be covered in this chapter. After a brief discussion on abnormal white blood cell counts, we will cover the Unopette method for manually counting white blood cells.

### Abnormal White Cell Counts

When white cell counts rise above normal values, the condition is referred to as **leukocytosis**. Leukocytosis frequently occurs when systemic or local infections (usually due to bacteria) are present. Counts for infections are highly variable. Examples of some infections and their representative white cell counts are as follows:

**Pneumonia**—20,000 to 30,000/mm<sup>3</sup>

**Meningitis**—20,000 to 30,000/mm<sup>3</sup>

**Appendicitis**—10,000 to 30,000/mm<sup>3</sup>

**Dyscrasia** (the diseased condition) of blood-forming tissues, such as occurs in leukemia (due to a malfunctioning of lymph and marrow tissues) also results in leukocytosis, with extremely high white cell counts. These white cell counts sometimes exceed 1,000,000/mm<sup>3</sup>.

Other physiological conditions that can cause leukocytosis and a white cell count as high as 15,000/mm<sup>3</sup> may occur as follows:

- Shortly after birth
- During late pregnancy
- During labor
- Accompanying severe pain
- After exercise or meals
- After cold baths
- During severe emotional upset

An abnormally low count, known as **leukopenia**, may be caused by the following conditions:

- Severe or advanced bacterial infections (such as typhoid, paratyphoid, and sometimes tularemia), or when the bacterial infection has been undetected for a period of time (as with chronic beta streptococcal infections of the throat).

- Infections caused by viruses and rickettsiae, such as measles, rubella, smallpox (until the 4th day), infectious hepatitis, psittacosis, dengue, tsutsugamushi fever, and influenza (when it may fall to 1,500/mm<sup>3</sup>, or shift to leukocytosis if complications develop).
- Protozoal infections (such as malaria) and helminthic infections (such as trichinosis). (For example, with victims of malaria, slight leukocytosis may develop for a short time during paroxysm (the sudden intensification of symptoms). Shortly thereafter, however, leukopenia ensues.)
- Overwhelming infections when the body's defense mechanisms break down.
- Anaphylactic shock
- Radiation

### Materials Required for Unopette Procedure

The Unopette method uses a disposable diluting pipette system that provides a convenient, precise, and accurate method for obtaining a white blood cell count. When the Unopette method is used, whole blood is added to a diluent. The diluent lyses (destroys) the red blood cells, but preserves the white blood cells. Once the red cells are completely lysed, the solution will be clear. The diluted blood is then added to a hemacytometer. Once the hemacytometer is loaded, the cells should be allowed to settle for 10 minutes before counting proceeds.

The following materials are required to perform a white blood cell count using the Unopette method:

- Disposable Unopette for WBC counts, which consists of
  - a shielded capillary pipette (20 microliter ( $\mu$ l) capacity), and
  - a plastic reservoir containing a premeasured volume of diluent (1:100 dilution).
- Hemacytometer and coverglass
- Microscope with light source
- Hand-held counter
- Laboratory chit

## Unopette Procedure

The Unopette disposable diluting pipette system used to count WBCs is almost identical in shape and application to the Unopette system for RBC counts. The only major difference is that the reservoir contains a different diluent and the capillary pipette capacity differs (RBC 10  $\mu\text{l}$  and WBC 20  $\mu\text{l}$ ). To assist you in performing the Unopette procedure for WBCs, we will refer to illustrations for the Unopette procedure for RBCs in this section.

The Unopette procedure for counting white blood cells is as follows:

1. Puncture the diaphragm in the neck of the reservoir with the tip of the capillary pipette shield. See figure 7-9.
2. After you obtain free-flowing blood from a lancet puncture of the finger, remove the protective plastic shield from the capillary pipette. Hold the capillary pipette slightly above the horizontal and touch the tip to the blood source (fig. 7-10, view A). The pipette will fill by capillary action. When blood reaches the end of the capillary bore in the neck of the pipette, filling is complete and will stop automatically. The amount of blood collected by the capillary tube is 20  $\mu\text{l}$ . Wipe any blood off the outside of the capillary tube, making sure no blood is removed from inside the capillary pipette. (An alternative source of blood is a thoroughly mixed fresh venous blood sample obtained by venipuncture. See figure 7-10, view B.)
3. With one hand, gently squeeze the reservoir to force some air out, but do not expel any diluent (fig. 7-11). Maintain pressure on the reservoir. With the other hand, cover the upper opening of the capillary overflow chamber with your index finger and seat the capillary pipette holder in the reservoir neck (fig. 7-11).
4. Release pressure on the reservoir and remove your finger from the overflow chamber opening. Suction will draw the blood into the diluent in the reservoir.
5. Squeeze the reservoir gently two or three times to rinse the capillary tube, forcing diluent into but not out of the overflow chamber, releasing pressure each time to return diluent to the reservoir. Close the upper opening with your index finger and invert the unit several times to mix the blood sample and diluent. See figure 7-12.
6. For specimen storage, cover the overflow chamber of the capillary tube with the capillary shield.
7. Immediately prior to cell counting, mix again by gentle inversion, taking care to cover the hole with your index finger.
8. Place the coverglass on the hemacytometer counting chamber, making sure the coverglass is clean and grease-free. (Fingerprints must be completely removed.)
9. Remove the pipette from the reservoir. Squeeze the reservoir and reseat the pipette in the reverse position. Release pressure to draw any fluid in the capillary tube into the reservoir. Invert and fill the capillary pipette by gentle pressure on the reservoir. After discarding the first 3 drops, load (charge) the counting chamber of the hemacytometer by gently squeezing the reservoir while touching the tip of the pipette against the edge of the coverglass and the surface of the counting chamber (fig. 7-13). A properly loaded counting chamber should have a thin, even film of fluid under the coverglass (fig. 7-14, view A). Allow 3 minutes for the cells to settle. If fluid flows into the grooves (moats) at the edges of the chamber or if you see air bubbles in the field, the chamber is flooded and must be cleaned with distilled water, dried with lens tissue, and reloaded (fig. 7-14, view B). If the chamber is underloaded, carefully add additional fluid until properly loaded.
10. Place the loaded hemacytometer into a petri dish with a piece of dampened tissue to keep the hemacytometer from drying out (fig. 7-15). Allow 5 to 10 minutes for the cells to settle.
11. Once the cells have settled, place the hemacytometer on the microscope. Using the high-power objective, count the WBCs in the four corner fields of the hemacytometer chamber (fields A, B, C, and D of figure 7-16). Each field is composed of 16 small squares. To count the cells in each field, start in the upper left small square and follow the pattern indicated by the arrow in field B of figure 7-16. Count all of the cells within each square, including cells touching the lines at the top and on the left. **Do not count any cells that touch the lines on the right or at the bottom.**

12. When all the cells in the 4 fields have been counted, multiply the count by 50. This will give you the total number of white cells per cubic millimeter of blood.

<b>Example:</b>	25 cells in field #1
	23 cells in field #2
	26 cells in field #3
	<u>26 cells in field #4</u>
	100 total cells in all fields
Multiply:	
100 x 50	= 5,000
<b>Total white cell count</b>	<b>= 5,000 cells/mm<sup>3</sup></b>

## DIFFERENTIAL WHITE BLOOD CELL COUNT

A total white blood cell count is not necessarily indicative of the severity of a disease, since some serious ailments may show a low white cell count. For this reason, a differential white cell count is performed. A differential white cell count consists of an examination of blood to determine the presence and the number of different types of white blood cells. This study often provides helpful information in determining the severity and extent of an infection, more than any other single procedure used in the examination of the blood.

The role of white blood cells, or leukocytes, is to control various disease conditions. Although these cells do most of their work outside the circulatory system, they use the blood for transportation to sites of infection.

Five types of white cells are normally found in the circulating blood. They are

- eosinophils,
- basophils,
- neutrophils,
- lymphocytes, and
- monocytes.

### Cell Identification

To perform a differential white cell count, you must be able to identify the different types of white cells. The ability to properly identify the different

types of white cells is not difficult to develop, but it does require a thorough knowledge of staining characteristics and morphology (the study of the form and structure of organisms). This knowledge can be gained only by extensive, supervised practice.

To acquaint you with the developmental stages of each type of leukocyte, a colorized illustration (fig. 7-17) has been provided. This illustration also displays the developmental stages of the red blood cell (erythrocyte) and the blood platelet cell (thrombocyte). To further assist you, identifying characteristics of each type of leukocyte as they appear on a stained blood smear will be covered in the following sections.

Laboratories use a **blood smear** to obtain a differential white cell count. To prepare a blood smear, a blood specimen is spread across a glass slide, stained to enhance leukocyte identification, and examined microscopically. Material requirements and the step-by-step procedure for performing a blood smear will be covered later in this chapter.

**NEUTROPHILS.**—Neutrophils account for the largest percentage of leukocytes found in a normal blood sample, and function by ingesting invading bacteria. On a stained blood smear, the cytoplasm of a neutrophil has numerous fine, barely visible lilac-colored granules and a dark purple or reddish purple nucleus (see figure 7-17). The nucleus may be oval, horseshoe, or “S”-shaped, or segmented (lobulated). Neutrophils are subclassified according to their age or maturity, which is indicated by changes in the nucleus. The subclassifications for neutrophilic cells are metamyelocyte, band, segmented, and hypersegmented.

**Neutrophilic Metamyelocyte.**—A neutrophilic metamyelocyte, also called a “juvenile” cell, is the youngest neutrophil generally reported. The nucleus is fat, indented, and is usually “bean”-shaped or “cashew nut”-shaped (fig. 7-17).

**Neutrophilic Band.**—A neutrophilic band, sometimes called a “stab” cell, is an older or intermediate neutrophil. The nucleus has started to elongate and has curved itself into a horseshoe or S-shape. As the band ages, it matures into a segmented neutrophil (fig. 7-17).

**Segmented Neutrophil.**—A segmented neutrophil is a mature neutrophil. The nucleus of a segmented neutrophil is separated into two, three, four, or five segments or lobes (fig. 7-17).

# DEVELOPMENT OF BLOOD CELLS X 1500

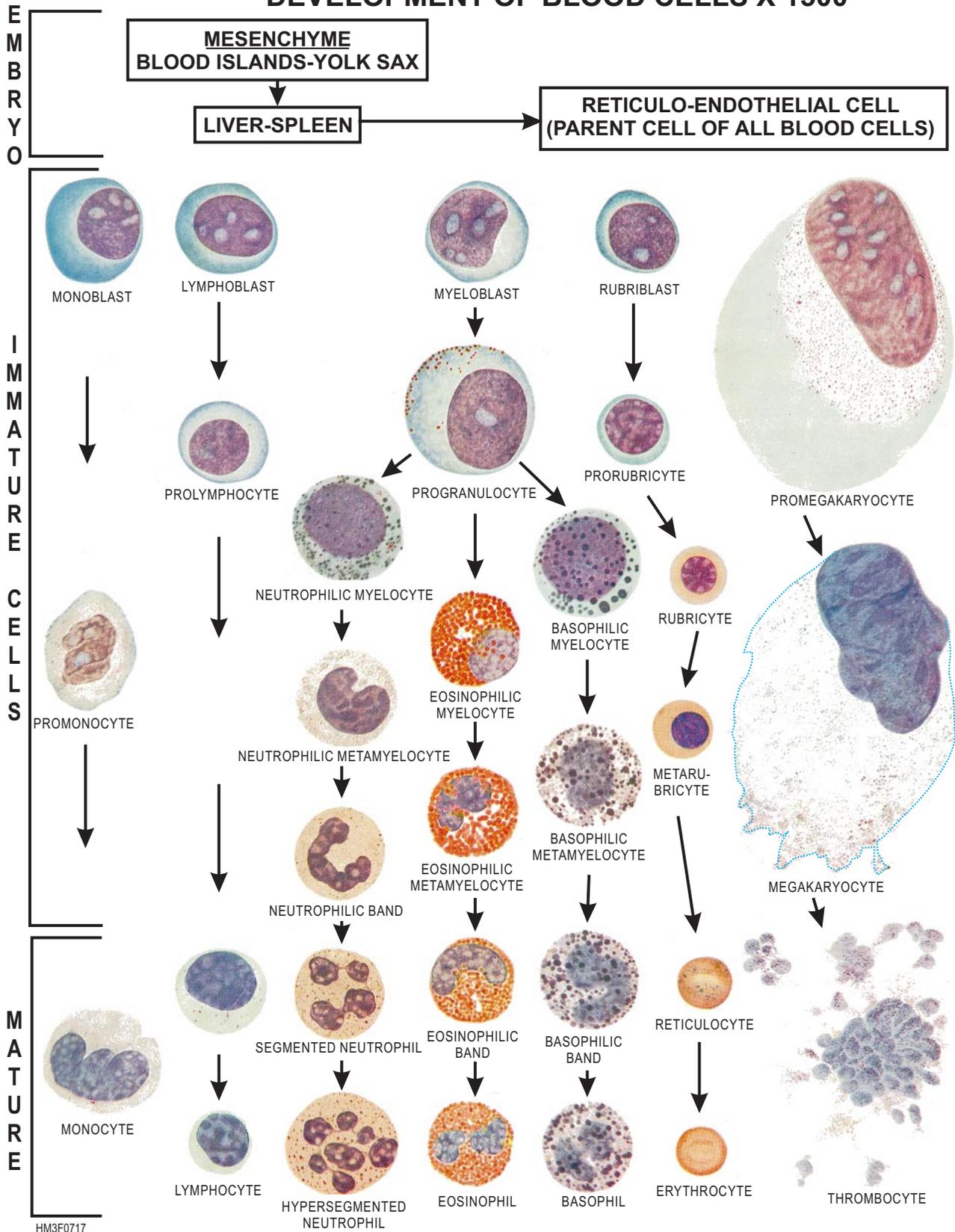


Figure 7-17.—Development of blood cells.

**Hypersegmented Neutrophil.**—A hypersegmented neutrophil is a mature neutrophil. The nucleus of a hypersegmented neutrophil is divided into six or more segments or lobes (fig. 7-17).

**EOSINOPHIL.**—Eosinophils aid in detoxification. They also break down and remove protein material. The cytoplasm of an eosinophil contains numerous coarse, reddish-orange granules, which are lighter colored than the nucleus (fig. 7-17).

**BASOPHIL.**—The function of basophilic cells is unknown. It is believed, however, that basophilic cells keep the blood from clotting in inflamed tissue. Scattered large, dark-blue granules that are darker than the nucleus, characterize the cell as a basophil (fig. 7-17). Granules may overlay the nucleus as well as the cytoplasm.

**LYMPHOCYTE.**—The function of lymphocytes is also unknown, but it is believed that they produce antibodies and destroy the toxic products of protein metabolism. The cytoplasm of a lymphocyte is clear sky blue, scanty, with few unevenly distributed, azurophilic granules with a halo around them (fig. 7-17). The nucleus is generally round, oval, or slightly indented, and the chromatin (a network of fibers within the nucleus) is lumpy and condensed at the periphery.

**MONOCYTE.**—The monocyte, the largest of the normal white blood cells, destroys bacteria, foreign particles, and protozoa. Its color resembles that of a lymphocyte, but its cytoplasm is a muddy gray-blue (fig. 7-17). The nucleus is lobulated, deeply indented or horseshoe-shaped, and has a relatively fine chromatin structure. Occasionally, the cytoplasm is more abundant than in the lymphocyte.

### Materials Required for the Differential Count Procedure

To perform a differential count, the following materials are required:

- Four plain glass microscope slides, clean and dry
- Wright-Giemsa stain solution (follow manufacturer's directions for use and storage)
- Staining containers
- Deionized or distilled water
- Microscope with light source
- Immersion oil
- Blood cell counter

### Differential Count Procedure

The procedure for the differential white cell count is done in 4 steps:

- Step 1: Making the blood smear
- Step 2: Staining the cells
- Step 3: Counting the cells
- Step 4: Reporting the count

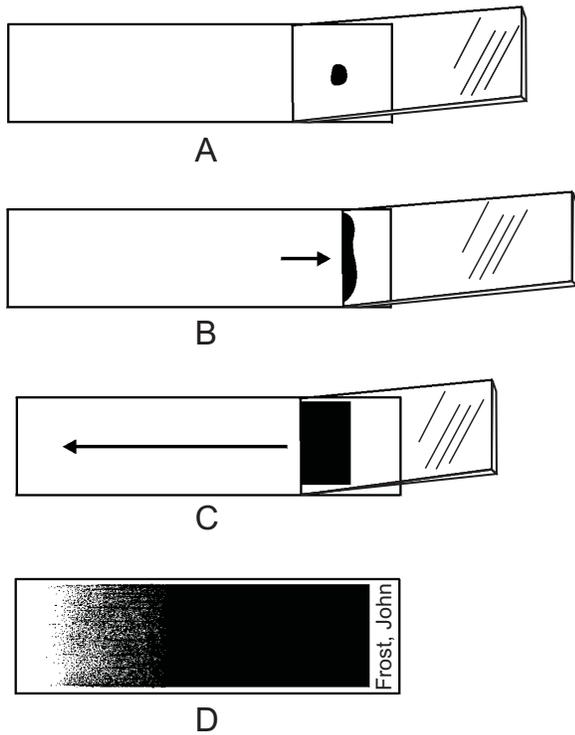
Each step of this procedure will be discussed in the following sections.

**MAKING THE BLOOD SMEAR.**—The simplest way to count the different types of white cells is to spread them out on a glass slide. The preparation is called a blood smear. There are two methods of making a blood smear: the slide method (covered in this chapter) and the cover glass method.

It is very important to make a good blood smear. If it is made poorly, the cells may be so distorted that it will be impossible to recognize them. You should make at least two smears for each patient, as the additional smear should be examined to verify any abnormal findings.

To prepare a blood smear for a differential count, follow the steps below:

1. Using a capillary tube, collect anticoagulated blood from a venous blood sample.
2. Deposit a drop of blood from capillary tube onto a clean, grease-free slide. Then place the slide on a flat surface, blood side up.
3. Hold a second slide between your thumb and forefinger and place the edge at a 23-degree angle against the top of the slide that holds the drop of blood (see figure 7-18, view A). Back the second slide down until it touches the drop of blood. The blood will distribute itself along the edge of the slide in a formed angle (see figure 7-18, view B).
4. Push the second slide along the surface of the other slide, drawing the blood across the surface in a thin, even smear (see figure 7-18, view C). If this is done in a smooth, uniform manner, a gradual tapering effect (or "feathering") of the blood will occur on the slide. This "feathering" of the blood is essential to the counting process and is the principal characteristic of a good blood smear (see figure 7-18, view D). When



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**Figure 7-18.**—Making a blood smear: A. Placing second slide at a 23° angle; B. Blood distributing itself along second slide's edge; C. Drawing blood across surface of slide; D. Example of a properly prepared blood smear.

you are making the smear, prevent blood from reaching the extreme edges of the slides. Allowing the smear to reach the edges of the slide will aggravate the tendency of large cells to stack up on the perimeter of the smear. A smear with wavy lines or blank spots should be discarded, and a new smear made.

5. Once the blood smear is made, let it dry (it will take a few minutes). Then write the patient's name in pencil on the bottom edge of the slide, as illustrated in figure 7-18, view D). Proceed to step 2, staining the cells.

**STAINING THE CELLS.**—Once a blood smear is made, it should be stained. Staining the blood smear highlights the differences among the different types of leukocytes for easier recognition during the counting process. The most popular stain used for this purpose is Wright's stain. Wright's stain is a methyl alcohol (methanol) solution of an acid dye and a basic dye. The acid dye in Wright's stain is known as eosin and is red in color. The basic dye in Wright's stain is known as methylene blue and is blue in color. Generally, white cells are identified by their affinity to the dye they prefer. For example, cells that prefer the acid dye

(eosin) are called eosinophils. Other cells that prefer the basic dye are called basophils.

### WARNING

Wright's staining solution contains methanol, which is considered a hazardous material. It is classified as flammable, a poison, and an irritant. Methanol must be kept away from heat, sparks, and open flames. Good ventilation in usage areas is paramount since exposure to vapors can irritate eyes, nose, throat, and mucous membranes of the upper respiratory tract. When not in use, methanol containers should be closed tightly and stored upright to prevent leakage. Gloves and protective clothing (e.g., lab coat or apron) and eyewear should be worn to avoid contact with the solution. Absorption through skin can cause permanent blindness. Death may result from ingestion or exposure to high vapor concentrations of methanol.

There are a variety of staining products on the market today. Some of these staining products have combined Wright's solution with other staining solutions, such as Giemsa stain. When using a new product, you should always review the manufacturer's usage and safety recommendations.

The staining process that we will cover in this chapter is known as a **quick stain**. A quick stain has very few equipment requirements and only a few procedural steps. An example of a quick stain is One Step II Wright-Giemsa Stain Solution® by Criterion Sciences. To stain a blood smear with this product, follow the steps below.

1. Prepare two staining containers by filling one with One Step II stain solution and the other with deionized or distilled water. The use of tap water instead of deionized or distilled water is not recommended since the pH of tap water varies. If tap water is used, its pH should be between 5.8 and 7.03.
2. Immerse the slide (blood smear) in the stain for 15 to 30 seconds. (To prevent debris or precipitate from contaminating the slide, do not add new stain to old.)
3. Remove the slide and allow excess stain to drain from the edge of the slide.
4. Immerse the slide in the deionized or distilled water for 5 to 15 seconds. (Change the water

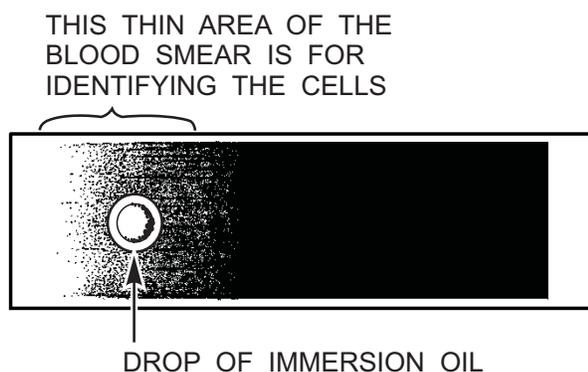
when it becomes dark blue or when film forms on the surface.) **NOTE:** Rinse time is critical and must be shorter than the stain time.

5. Drain excess water and wipe the back of the slide to reduce background color.
6. Place slide in horizontal position on table and allow to air dry. **NOTE:** Do not accelerate drying time by placing slide on a warmer or in front of a fan. The film of water on the slide is important for the color development.
7. Once the slide is dry, proceed to step 3, counting the cells.

**COUNTING THE CELLS.**—Once the blood smear has been stained, it is placed under a microscope, and the differential count is conducted.

To perform a differential white cell count, you should follow the steps listed below:

1. Place the slide under the microscope. Switch the oil immersion objective (red) (100X) into position above the stage. Turn the coarse adjustment to raise the oil immersion objective about 1 inch above the opening in the stage. Open the condenser and switch on the microscope light.
2. Place a large drop of immersion oil on the thin area of the blood smear. See figure 7-19.
3. Hold the slide so the thin area is on your left. Then fix the slide firmly in the jaws of the mechanical (movable) stage. Move the mechanical stage so the drop of oil on the slide is directly over the bright light coming up from the condenser.
4. Using the coarse control knob, you should now slowly lower oil immersion objective into the



HM3f0719

Figure 7-19.—Placement of immersion oil on blood smear.

drop of oil (on the slide). When the objective is in the drop of oil, continue turning the coarse adjustment until the objective is touching the glass slide.

5. Now, while continually looking through the eyepiece, **VERY SLOWLY** rotate the coarse adjustment toward you until you see some cells. After you have brought the cells into view with the coarse adjustment, bring the cells into perfect focus by rotating the fine adjustment. **NOTE:** Always rotate the fine adjustment back and forth when identifying cells. This step will help you see the various layers of the cell and thereby help you to identify the different types of white cells.
6. Count 100 consecutive white cells, pressing the correct key on the cell counter for each type of white cell identified. (If the cell counter is not available, record cell type and number of cells encountered on a piece of paper.) Follow path similar to one illustrated in figure 7-20 to count cells.
7. Total each type of white cell. If you count 20 lymphocytes among the 100 cells, the differential count for lymphocytes is 20%. Continue this process until your count totals 100%. This differential count is referred to as a **relative count**. Another differential count that may be requested is an **absolute count**. To perform an absolute count, multiply the total white cell count by the individual cell percentages. See the example below.

**Example:**

Patient has a total white cell count of 8,000. Differential count shows 20% leukocytes.

Multiply:

$$8,000 \times 0.20 (20\%) = 1,600$$

**Patient has 1,600 lymphocytes/mm<sup>3</sup>**



HM3f0720

Figure 7-20.—Counting path for differential count.

**NOTE:** When performing the white cell count, you may observe abnormal white cells such as distorted lymphocytes, smudge cells, and disintegrated cells.

**Distorted lymphocytes**, which appear squashed or distorted, are caused by excessive pressure on the cell during the process of making the smear. Distorted cells should be recorded as normal lymphocytes. **Smudge cells** are white cells that have ruptured and only the nucleus remains. A few smudge cells may be found in a normal blood smear. Smudge cells should not be added to the count or recorded. **Disintegrated cells** are ruptured cells, but the nucleus and cytoplasm still remain. Disintegrated cells should not be counted as one of the 100 cells, but should be recorded on the report as “disintegrated cells.”

8. Once the differential count is completed, proceed to step 4, reporting the count.

**NOTE:** If it is desirable to save a smear for reexamination, remove the immersion oil by placing a piece of lens tissue over the slide and moistening the tissue with xylene. Draw the damp tissue across the slide, and dry the smear with another piece of lens paper.

**REPORTING THE COUNT.**—When you have calculated the differential count, the report is given according to either the **Schilling classification** or **filament and nonfilament classification methods**. We will be covering the Schilling classification, since it is the simplest and most popular method.

**The Schilling Classification.**—The Schilling classification was established when Victor Schilling, a German hematologist, noticed that in many diseases there is an increase in the percentage of immature neutrophils. The blood chart he developed reported the percentages of the different neutrophilic cell types and (in part) was arranged in the following manner:

Normal %	Myelocytes	Meta-myelocytes	Band Cells	Segmented Cells
	0	0	2 to 6	55 to 75

Note that the immature cells are on the left side of the chart. If percentages of immature cell increased, Schilling referred it as a “shift to the left.” When the

shift to the left was accompanied by a low white cell count, Schilling called it a “degenerative shift to the left.” A degenerative shift to the left is seen in such diseases as typhoid fever. This shift is caused by a depression of the cell factories in the bone marrow.

When the shift to the left is accompanied by a high white cell count, it is called a “regenerative shift to the left.” A regenerative shift to the left is seen in such diseases as pneumonia. This shift is caused by a stimulus of the cell factories in the bone marrow.

A “shift to the right” implies an increase in hypersegmented neutrophils. It may be seen in pernicious anemia, an anemia caused by the malabsorption of vitamin B<sub>12</sub>.

The Schilling classification for an adult differential white cell count is provided below in table 7-2.

**NOTE:** Normal values for differential counts vary with the age of the patient. For example, children’s blood normally contains 0% to 2% basophils, 0% to 5% eosinophils, 25% to 75% neutrophils, 30% to 70% lymphocytes, and 0% to 8% monocytes. Normal values may also be adjusted by hospitals that have evaluated the normal differential value for their local population.

**General Interpretations of Leukocyte Changes.**—Together, the total white cell count and differential count aid physicians in interpreting the severity of infections. Some general interpretations of leukocyte changes are as follows:

- Leukocytosis with an increase in the percentage of neutrophils indicates a severe infection with a

**Table 7-2.—Schilling Classification of the Differential White Cell Count**

Cell	Normal %
Neutrophilic myelocytes	0
Neutrophilic metamyelocytes	0
Neutrophilic band cells	2 to 6
Neutrophilic segmented cells	55 to 75
Lymphocytes	20 to 35
Monocytes	2 to 6
Eosinophilic segmented cells	1 to 3
Basophilic segmented cells	0 to 1

good response of the bone marrow. The primary bacteria-destroying cells (known as phagocytes) are the neutrophils, and the bone marrow should supply large numbers of these to combat the infection. The greater the “shift to the left” (increase in immature neutrophils), the more severe the infection. The appearance of numerous juvenile cells (metamyelocytes) indicates irritation of the bone marrow with regeneration. If the infection continues and the patient’s resistance declines, the shift advances further to the left. If improvement ensues, the shift declines and recedes to normal.

- A falling white cell count with the number and maturity of neutrophils progressing toward normal indicates recovery.
- A continued “shift to the left” with a falling total white cell count indicates a breakdown of the body’s defense mechanism and is a poor prognosis.
- The percentage of eosinophils, lymphocytes, and monocytes generally decreases in acute infections.
- In tuberculosis, an increase in monocytes (monocytosis) indicates activity in the infected area. An increase in lymphocytes (lymphocytosis) indicates healing.
- Eosinophils increase in parasitic infections and allergic conditions.

## BACTERIOLOGY

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**LEARNING OBJECTIVE:** *Recall bacteria classifications, common bacteria, and procedural steps for making smears, Gram staining, and reading and reporting smears.*

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Bacteriology is the study of bacteria. Of primary interest to Hospital Corpsman is medical bacteriology, which deals with the bacteria that cause disease in man.

Bacteria are prokaryotic microorganisms of the kingdom Protista. They reproduce asexually by transverse binary fission in which the cell divides into two new cells. Bacteria are found almost everywhere, and the human body harbors vast numbers. Many bacteria are beneficial and essential to human life; only a few are harmful to man.

## BACTERIA CLASSIFICATION

Since there are thousands of types of bacteria, a method of classification is essential. Bacteria are classified according to their respective

- disease-producing ability,
- growth requirements,
- morphologic characteristics,
- colonial morphology,
- toxins produced, and
- Gram’s stain reaction.

### Disease-Producing Ability

The disease-producing ability of bacteria is referred to as either **pathogenic** or **nonpathogenic**. Pathogens are bacteria that cause diseases, and nonpathogens are harmless bacteria. Bacteria that are essential to our body are, in their proper environment, called common or normal flora. For example, alpha streptococcus in the throat is common flora, but when it is found elsewhere (such as in the blood stream, possibly as a result of tooth extraction), it may cause diseases such as septicemia and endocarditis.

### Growth Requirements

The four growth requirements for bacteria are

- temperature,
- oxygen,
- nutrition, and
- moisture.

**TEMPERATURE REQUIREMENTS.**—Temperature requirements are divided into the following three categories.

- **Psychrophilic**—bacteria that reproduce best at 15°C to 20°C
- **Mesophilic**—bacteria that reproduce best at 20°C to 45°C
- **Thermophilic**—bacteria that reproduce best at 50°C to 55°C

**OXYGEN REQUIREMENTS.**—The amount of oxygen needed for an organism to grow or reproduce varies with the type of organism. **Aerobes** are organisms that reproduce in the presence of oxygen. **Obligate aerobes** are organisms that grow only in the

presence of free oxygen. **Anaerobes** are organisms that do not reproduce in the presence of oxygen, and **obligate anaerobes** are organisms that grow only in the absence of free oxygen and are killed if exposed to free oxygen. **Facultative organisms** are organisms that grow in the presence of free oxygen and in an oxygen-free atmosphere. **Microaerophilic organisms** are organisms that grow only in low amounts of free oxygen.

**NUTRITION REQUIREMENTS.**—Nutrition requirements for the various types of bacteria depends on what their particular environment provides. **Autotrophic bacteria** are self-nourishing, and **heterotrophic bacteria** are not self-sustaining.

**MOISTURE REQUIREMENTS.**—Moisture is indispensable for bacterial growth.

### Morphologic Characteristics

The structural (or morphologic) characteristics of bacteria are based on three distinct shapes or categories:

- **Coccus** (*pl. cocci*)—spherical, appears singly, in pairs, chains, clusters, or packets.
- **Bacillus** (*pl. bacilli*)—rod-shaped, appears singly, in chains, or in palisades.
- **Spirillum** (*pl. spirilla*)—spiral-, corkscrew-, or comma-shaped, appearing singly only.

Three special structures, present on some bacteria, aid in the classification process of bacteria. The special structures are the capsule, the spore, and the flagellum. The **capsule** is a gummy, gelatinous, or mucoid structure surrounding certain bacteria. The **spore** is an inactive, resting, and resistant form produced within the organism, usually as a result of unfavorable environmental conditions. The third and final special structure is the **flagellum**, a hairlike structure that provides motility.

### Colonial Morphology

A colony is a cohesive mass composed of many millions of bacterial cells, growing on or in a medium (such as blood agar, a gel enriched with blood that is used in the preparation of solid culture media for microorganisms) as a result of the multiplication and division of a single cell. The size, color, shape, edge, topography, consistency, and odor of the colony vary with each organism.

### Toxins Produced

Generally, toxins produced are waste products of metabolism in a bacterial cell. Some bacteria produce toxins that attack red blood cells in a culture medium such as blood agar. Examples of toxins produced by bacteria are listed below:

- **Alpha hemolysin**—produces partial hemolysis (the disruption of the integrity of the red cell membrane causing release of hemoglobin) and changes the medium to a green color.
- **Beta hemolysin**—completely lyses the RBC, leaving a clear zone of hemolysis.
- **Endotoxin** (low potency)—comprises part of the cell wall and is released as the bacterial cell spontaneously destroys itself with self-generated enzymes (a process known as autolysis).
- **Exotoxin** (high potency)—derives from the bacteria during its growth but is found outside the bacterial cell in the surrounding medium. Exotoxins are highly poisonous, soluble, and protein in nature.

### Gram's Stain Reaction

To differentiate and identify bacteria, you must make them visible by staining. The staining procedure, devised by Dr. Hans Christian Joachim Gram, stains microorganisms such as bacteria with crystal violet, treats them with 1:15 dilution of strong iodine solution, decolorizes them with ethanol or ethanol-acetone, and counterstains them with a contrasting dye, usually safranin. Microorganisms that retain the crystal violet stain (a dark blue-black color) are said to be gram-positive, and those that lose the crystal violet stain by decolorization but stain with counterstain (a deep pink or reddish color) are said to be gram-negative.

### COMMON BACTERIA

Bacteria are named by genus and species. The first word (capitalized) indicates the genus; the second word (not capitalized) indicates the species, a subdivision of the genus. For example:

<u>GENUS</u>	<u>SPECIES</u>
Neisseria	gonorrhoeae

Table 7-3 will familiarize you with commonly encountered bacteria. This table lists the bacteria's morphologic shape, Gram stain response, genus and species, and the type of infection it produces.

### BACTERIOLOGIC METHODS

There are a variety of methods used in the laboratory to identify bacteria. However, only a few of these bacteriologic methods can be performed in isolated duty locations or on board naval vessels. One of these methods is the smear. The smear permits healthcare personnel to examine specimens microscopically. Material requirements and the step-by-step procedures for making smears is covered in the following sections.

### Smear

A smear is the procedure in which a specimen—a body fluid or a discharge—is spread across a glass slide for microscopic examination. To enhance the visualization of microorganisms on the smear, Gram staining (introduced earlier in this chapter) is used. Once the smear is stained, it is ready to be examined under the microscope. Normally, smears are examined by laboratory technicians who prepare reports of their findings.

**MATERIALS REQUIRED FOR SMEAR.**—To perform a smear, the following materials are required:

- Glass slide

Table 7-3.—Common Bacteria

COMMON BACTERIA			
Morphologic Shape	Gram-Positive or -Negative	Genus & Species	Type of Infection
Cocci	Positive	Streptococcus pneumoniae	Pneumonia
		Streptococcus pyogenes (Beta Streptococci Group A)	Strep throat
		Staphylococcus aureus	Boils, furuncles, osteomyelitis, pneumonia, septicemia, endocarditis, and impetigo
	Negative	Neisseria gonorrhoeae	Gonorrhea
		Neisseria meningitidis (meningococcus)	Meningitis
Bacilli	Positive	Corynebacterium diphtheriae	Diphtheria
		Clostridium (all are anaerobic and spore producers) <ul style="list-style-type: none"> <li>• perfringens (welchii)</li> <li>• tetani</li> <li>• botulinum</li> </ul>	Gas gangrene Tetanus Botulism
		Yersinia (Pasteurella) pestis	Bubonic plague
	Negative	Brucella abortus	Brucellosis
		Bordetella pertussis	Whooping cough

- Microscope
- Wooden applicator stick
- Saline solution
- Forceps
- Bunsen burner

**PROCEDURE FOR MAKING SMEARS.**—To prepare smears for microscopic examination, follow these steps:

1. Spread the specimen with a wood applicator stick across a slide that has been cleaned with alcohol or acetone and polished with lens paper. The smear should be thin and uniformly spread. If the smear is opaque, it is too thick and should be emulsified with a drop or two of saline.
2. Label the smear and circle the material to be stained with a diamond point pen for easier identification and location of the material after staining.
3. Let the smear air dry. Do not use forced heat drying; forced drying will distort bacterial cells and other materials.
4. Hold the smear with forceps and fix the smear by passing it through a flame (smear side up) three or four times. Avoid overheating the smear; overheating will cause cellular wall destruction.
5. Let the slide cool. Once the slide is cooled, it is ready to be stained.

### Gram's Stain

As previously explained, the most common staining procedure used in bacteriologic work is the Gram stain. This method yields valuable information and should be used on all smears that require staining. Gram's stain is also used for examining cultures to determine purity and for identification purposes.

**PRINCIPLE OF GRAM STAINING.**—As touched on previously, the crystal violet stain, the primary stain, stains everything in the smear blue. The Gram's iodine acts as a mordant, a substance that causes the crystal violet to penetrate and adhere to the gram-positive organisms. The acetone-alcohol mixture acts as the decolorizer that washes the stain away from everything in the smear except the gram-positive organisms. The safranin is the counter-stain that stains everything in the smear that has been decolorized: pus cells, mucus, and

gram-negative organisms. The gram-negative organisms will stain a much deeper pink than the pus cells and mucus will stain even lighter pink than the pus cells.

**MATERIALS REQUIRED FOR GRAM STAINING.**—To Gram stain a smear, the following materials are required:

- Gram stain kit, which consists of:
  - Crystal violet stain
  - Iodine or stabilized iodine (mordant)
  - Acetone-alcohol decolorizer
  - Safranin stain
- Staining rack
- Blotting paper or paper towel

**PROCEDURE FOR GRAM STAINING SMEARS.**—After smears have been dried, heat-fixed, and cooled, proceed as follows:

1. Place the slide on a staining rack. Then flood slide with primary stain (crystal violet). Let stand 1 minute.
2. Remove the primary stain by gently washing with cold tap water.
3. Flood the slide with mordant (iodine or stabilized iodine) and retain on slide for 1 minute.
4. Remove mordant by gently washing with tap water.
5. Tilt slide at a 45-degree angle and decolorize with the acetone-alcohol solution until the solvent that runs from the slide is colorless (30 to 60 seconds).
6. Wash the slide gently in cold tap water.
7. Flood the slide with counter-stain (safranin) and let stand for 30 to 60 seconds.
8. Wash slide with cold tap water.
9. Blot with blotting paper or paper towel or allow to air dry.
10. Examine the smear under an oil immersion objective.

### Reading and Reporting Smears

Place a drop of oil on the slide and, using the oil immersion objective of the microscope, read the

smear. All body discharges contain extraneous materials, such as pus cells and mucus. Of interest, however, are the types of bacteria that may be present. The stained smear reveals only two features: the morphology and the staining characteristics of the bacteria present. Positive identification requires cultures and further studies.

**Hospital Corpsmen should report only what they see.** For example, “Smear shows numerous gram-negative bacilli.” If two or more types of bacteria are seen in a smear, the rule is to report them in order of predominance. For example:

- “1. Numerous gram-positive cocci in clusters
2. Few gram-negative bacilli”

Gram-positive organisms are easy to see because they stain a deep blue or blue-black. Gram-negative organisms stain a deep pink, but since the background material is also pink, minute and detailed inspection is necessary before reporting the results.

In the presence of gonorrhea, the smear will reveal large numbers of pus cells with varying numbers of intracellular and extracellular gram-negative, bean-shaped cocci in pairs. Such a finding could be considered diagnostic. It is important to point out that only a few of the thousands of pus cells on the slide may contain bacteria, and sometimes it requires considerable search to find one.

## SEROLOGY

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**LEARNING OBJECTIVE:** *Recall principles and procedures for the Rapid Plasma Reagin (RPR) Card Test and the Monosticon DRI-DOT® Slide Test.*

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Serology consists of procedures by which antigens and reacting serum globulin antibodies may be measured qualitatively and quantitatively. Serologic tests have been devised to detect either antigens present or antibodies produced in a number of conditions. Most tests are based on agglutination reactions between an antigen and a specific antibody.

An **antigen** is a substance that, when introduced into an individual who does not already possess that substance, may stimulate the individual’s cells to produce specific antibodies that react to this substance in a detectable way. The five basic characteristics of an antigen are that it must be foreign to the body, it must

possess a high molecular weight, it must be structurally stable, it must be complex, and it must have a high specificity to stimulate tissues to produce a defensive protein substance called an antibody.

**Antibodies** are the specific defensive proteins produced when an antigen stimulates individual cells. Antibodies are produced by the host in response to the presence of an antigen and are capable of reacting with antigens in some detectable way.

The **antigen-antibody reaction** takes place when a reaction occurs between specific antibodies in the plasma and the antigen present on cell surfaces.

Principles and procedures of two serologic tests, the rapid plasma reagin (RPR) card test and the Monosticon DRI-DOT® Slide Test are covered in the following sections.

### RAPID PLASMA REAGIN (RPR) CARD TEST

The RPR Card test is a sensitive, easily performed screening test for syphilis. The test is performed on unheated plasma or serum. Everything needed for the test is in a kit that is available commercially. This test kit is very useful aboard ship and at small stations.

#### Principle of the RPR Card Test

In the RPR Card test method of syphilis detection, a specific antigen (carbon-particle cardiophilin) detects “reagin,” a substance present in the serum of persons who are infected with syphilis. Specimens that contain reagin cause formation of particles (called flocculation) or coagulation of the carbon particles to occur on the RPR Card antigen. Reactive specimens appear as black clumps against a white background. Nonreactive specimens appear as an even, light-gray color.

#### Materials Required for RPR Test

To perform an RPR Card test, the following materials are required:

- Serum sample—venous blood collected in tubes without anticoagulant. **NOTE:** Use clear, unhemolyzed serum that has been separated from the blood cells as soon after collection as possible.
- RPR Card Test Kit, which consists of the following components:
  - RPR Card antigen suspension

- Plastic dispensing bottle
- 20-gauge, galvanized needle, blunt cut
- Test cards
- Pipette/stirrers, 50 microliter ( $\mu$ l)
- One 1 ml tuberculin syringe
- Distilled water
- Mechanical rotator (adjusted to 100 rpm)
- RPR Card Test Control Cards (each consisting of three labeled test areas containing lyophilized (meaning a stabilized preparation of a biological substance, such as blood, that has been frozen rapidly and then dehydrated under a high vacuum) control specimens with designated patterns of reactivity: Reactive, Reactive-Minimal-to-Moderate, and Nonreactive.)

**NOTE:** RPR Card Test Antigen and Control Cards must be stored at 4°C when not in use. Both items are stable until the expiration date. Store “in use” antigen suspension in the dispensing bottle at 4°C. The antigen suspension is stable for 3 months or until the expiration date, whichever occurs first.

### Preliminary Preparations for RPR Test

The following preliminary preparations must be performed before RPR testing can begin:

1. Remove the antigen suspension vial and one control card envelope from the refrigerator. Allow the items to warm to room temperature.
2. Resuspend the contents of the vial by vigorously shaking the antigen vial.
3. Snap the neck of the vial.
4. Attach the needle (provided in the kit) to a 1 ml tuberculin syringe. Slowly draw up into the syringe from the vial approximately 1 ml of the antigen suspension.
5. Hold the syringe perpendicular to the surface and count the number of drops dispensed from a 0.5 ml volume. Allow the drops to fall into the antigen vial. **NOTE:** The needle is accurate if 30 drops, plus or minus 1 drop, are dispensed from the 0.5 ml volume.
6. Slowly expel the remainder of the antigen solution in the syringe back into the antigen vial.

7. Remove the needle from the syringe. Place the needle on the tapered fitting of the plastic dispensing bottle (provided in the kit).
8. Slowly withdraw all the contents of the antigen vial by collapsing the dispensing bottle and using it as a suction device.
9. Allow the rotator to warm up for 5 to 10 minutes; adjust to 100 rpm.

### RPR Test Procedure

To detect syphilis using the RPR Card test, follow the steps below:

1. Open the foil package and remove the control card.
2. Use a pipette/stirrer to reconstitute each control card circle with 0.5 ml of distilled water.
3. Mix solution in control card circle with pipette/stirrer until the dehydrated control specimen is dissolved. Spread specimen over entire area of circle. Use a separate pipette/stirrer for each circle.
4. Draw the patient’s sample by holding the pipette/stirrer between the thumb and forefinger near the stirring or sealed end and squeeze. Do not release pressure until the open end is below the surface of the specimen, then release pressure to draw up the sample.
5. Hold the pipette/stirrer in a vertical position, directly over the card test area where the specimen is to be delivered; squeeze pipette/stirrer, allowing 1 drop to fall onto the test area.
6. Invert the pipette/stirrer and, with the sealed end, spread specimen within the circle. Discard the pipette/stirrer when done.
7. Continue the steps above until one or two test cards are filled with patient’s samples.
8. Gently shake the antigen dispensing bottle before use. Hold the bottle in the vertical position and dispense several drops into the dispensing bottle cap to ensure the needle passage is clear. Allow 1 “free-falling” drop to fall onto each test area. Do not stir; the mixing of antigen suspension and specimen is accomplished by rotation.
9. Put the card(s) on the rotator and cover with the humidifying cover. Rotate cards for 8 minutes

at 100 rpm. To help differentiate nonreactive from reactive results, you should briefly rotate and tilt the card by hand (3 or 4 back-and-forth motions).

10. Immediately read the card macroscopically (with the unaided eye) in the “wet” state under a high-intensity lamp.
11. Compare the patient’s tests to the controls for correct interpretations. The reactive control should show small to large clumps. The nonreactive control should show no clumping or very slight roughness. The reactive-minimal-to-moderate control should show slight but definite clumping.
12. Report the test as
  - **reactive**, if agglutination or flocculation is present, or
  - **nonreactive**, if no agglutination is present.

**NOTE:** The RPR Card test is used as a screen for syphilis. If a patient’s RPR is reactive, the patient should be sent to a laboratory to have a FTA-ABS (Fluorescent Treponemal Antibody Absorption Test) performed. The FTA-ABS, a more precise test, is used to confirm primary, secondary, and late syphilis.

## MONOSTICON DRI-DOT SLIDE TEST

Mononucleosis imitates many diseases so well that diagnosis is confirmed only by selective serologic testing. The Monosticon DRI-DOT Slide Test is an accurate, 2-minute disposable test designed to detect the presence of infectious mononucleosis antibodies in serum, plasma, or whole blood.

### Principle of the Monosticon DRI-DOT Slide Test

The Monosticon DRI-DOT Slide Test consists of specially prepared, stable sheep and/or horse erythrocyte antigen (dyed) and guinea pig antigen on a disposable slide. When serum, plasma, or whole blood is mixed with these antigens on the slide, the test result for infectious mononucleosis will be positive or negative. A positive result is indicated by agglutination and a negative result is indicated by no agglutination.

## Materials Required for Monosticon DRI-DOT Slide Test

To perform the Monosticon DRI-DOT Slide Test, the following materials are required:

- Serum or plasma specimen
- Monosticon DRI-DOT Test kit, which consists of:
  - Monosticon DRI-DOT Test slides
  - Positive I.M. (infectious mononucleosis) serum control
  - Negative I.M. serum control
  - Dropper bottle
  - Dispensstirs® (designed to deliver a 0.03 ml drop)
- Distilled water
- Centrifuge
- DRI-DOT slide holder (available commercially, but not necessary to perform test)

## Controls for Monosticon DRI-DOT Slide Test

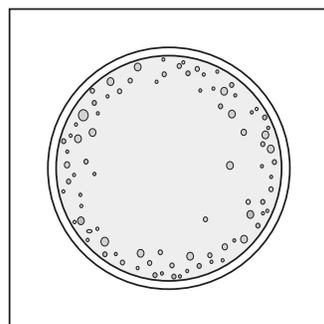
Both a positive and negative control are included in each kit to check the effectiveness of the reagents. The positive I.M. serum control (human) is a dilution of human sera (*sing.* serum) containing the specific heterophile antibody of infectious mononucleosis. The negative I.M. serum control (human) is a dilution of human sera containing no detectable antibody to infectious mononucleosis. Both controls have been dried and placed in a vial with color-coded cap and label. Since both controls are of human origin, they are potentially infectious and must be handled with care.

Both controls (positive and negative) should be tested before performing test with serum, plasma, or whole blood. Controls are prepared in the same manner as serum and plasma test described in the next section, but instead of adding serum or plasma to the slide, the control is added. Before each control is used, it must be reconstituted with 0.5 ml of distilled water. If results of the control tests are not as expected, do not use the test kit.

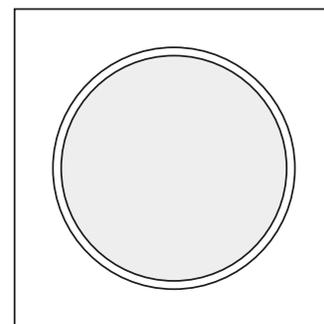
## Monosticon DRI-DOT Slide Test Procedure

To detect mononucleosis using the Monosticon DRI-DOT Slide Test, follow the steps below.

1. Centrifuge the blood specimen for 10 minutes to obtain the plasma or serum to be tested.
2. Fill the dropper bottle with distilled water.
3. Remove the disposable slide by tearing the envelope where indicated. (Remove only enough slides to perform the tests at hand.)
4. Set the slide in a holder or on a flat surface.
5. Place one drop of water from the dropper bottle **next to but not on** the blue dot within the circle on the slide.
6. Use a Dispenstir to squeeze the closed end between thumb and forefinger, and place the open end into the plasma or serum to be tested. Release pressure to draw up the specimen into the Dispenstir.
7. Hold the Dispenstir perpendicularly over the buff-colored dot (guinea pig antigen) within the circle of the slide. Place one drop of specimen onto the dot.
8. Use the flared end of the Dispenstir to mix the water, specimen, and the guinea pig antigen (buff-colored dot) thoroughly.
9. Blend this mixture thoroughly with the blue dot (horse/sheep antigen).
10. Rock the slide (or slide holder) back and forth gently in a figure-8 motion for 2 minutes so that the liquid slowly flows over the entire area within the circle.
11. After 2 minutes, read the results under a strong, glaring light.



POSITIVE - AGGLUTINATION



NEGATIVE - NO AGGLUTINATION

HM3f0721

Figure 7-21.—Illustration of positive and negative Monosticon DRI-DOT Slide Test Results.

12. Report test as

- **positive**, if agglutination is present, or
- **negative**, if no agglutination is present.

See figure 7-21 for an illustration of positive and negative test results.

**NOTE:** A positive test result usually occurs between the fourth day and the twenty-first day of illness, and may persist for several months.

## FUNGUS TEST

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**LEARNING OBJECTIVE:** *Recall how potassium hydroxide (KOH) preparation is used in the detection of fungi.*

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Fungi (*sing.* fungus) are chlorophyll-free, heterotrophic (not self-sustaining) of the same family of plants (i.e., Thyllophyta) as algae and lichens. They reproduce by spores that germinate into long filaments called **hyphae**. As the hyphae continue to grow and branch, they develop into a mat of growth called the mycelium (*pl.* mycelia). From the mycelium, spores are produced in characteristic patterns. These spores, when dispersed to new substances, germinate and form new growths. Reproduction is often asexual, usually by budding (as in yeast), but certain fungi have sexual reproduction.

Common superficial infections of the skin caused by fungi are athlete's foot and ringworm of the scalp.

A simple and frequently used method of detecting fungi is the **potassium hydroxide (KOH) preparation**. Fungi are seen in clustered round buds with thick walls, accompanied by fragments of

mycelia. Scrapings from the affected area of the skin are mounted in 10% KOH for positive laboratory diagnosis.

To detect fungi in infected tissue using the KOH preparation, follow the steps below.

1. Place skin, hair, or nail scrapings from the affected area on a glass slide and add one drop of 10% KOH. (Dissolve 10 g of KOH in 100 ml of distilled water.)
2. Place a coverslip on the preparation.
3. Warm the preparation gently over a flame, being careful not to boil it, and allow it to stand until clear. Do not allow the preparation to dry out.
4. Examine the preparation by using the high-power objective on microscope with subdued light.
  - Fungi on the skin and nails appear as refractile fragments of hyphae.
  - Fungi in the hair appear as dense clouds around the hair stub or as linear rows inside the hair shaft.

## URINALYSIS

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**LEARNING OBJECTIVE:** *Recall the three types of urine specimens, the methods used to preserve urine specimens, and the procedure for performing a urinalysis.*

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Since the physical and chemical properties of normal urine are constant, abnormalities are easily detected. The use of simple tests provides the physician with helpful information for the diagnosis and management of many diseases.

This section deals with the three types of urine specimens, methods used to preserve urine specimens, the procedure for performing a routine and microscopic examination of urine specimens, and some of the simpler interpretations of the findings.

### URINE SPECIMENS

Urine specimens for routine examinations must be collected in aseptically clean containers. Unless circumstances warrant, avoid catheterization because it may cause a urinary tract infection. Specimens of female patients are likely to be contaminated with

albumin and blood from menstrual discharge, or with albumin and pus from vaginal discharge. For bacteriologic studies, care must be taken to ensure that the external genitalia have been thoroughly cleansed with soap and water. The patient must void the initial stream of urine into the toilet or a suitable container and the remainder directly into a sterile container. All urine specimens should either be examined when freshly voided, or refrigerated to prevent decomposition of urinary constituents and to limit bacterial growth. In the following sections, we will cover three types of urine specimens: random, first morning, and 24-hour.

### Random Urine Specimen

A random urine specimen is urine voided without regard to the time of day or fasting state. This sample is satisfactory for most routine urinalyses. It is the least valid specimen, since test results may reflect a particular meal or fluid intake.

### First Morning Urine Specimen

The first morning urine specimen is the first urine voided upon rising. It is the best sample for routine urinalysis, because it is usually concentrated and more likely to reveal abnormalities. If positive results are obtained from the first morning specimen, the physician may order a 24-hour specimen for quantitative studies.

### Twenty-Four Hour Urine Specimen

The 24-hour urine specimen measures the exact output of urine over a 24-hour period. Use the following steps to collect this specimen.

1. Have patient empty bladder early in the morning and record time. Discard this urine.
2. Collect all urine voided during next 24 hours.
3. Instruct patient to empty bladder at 0800 the following day (end of 24-hour period). Add this urine to pooled specimen.

Refrigerate specimen during collection, and, depending on the test being performed, add a preservative to the first specimen voided.

The normal daily urine volume for adults ranges from 800 to 2000 ml, averaging about 1,500 ml. The amount of urine excreted in 24 hours varies with fluid intake and the amount of water lost through perspiration, respiration, and bowel activity. Diarrhea

or profuse sweating reduces urinary output; a high-protein diet tends to increase it. Daytime urine output is normally two to four times greater than nighttime output.

## PRESERVATION OF URINE SPECIMENS

To delay decomposition of urine, use the following methods of preservation:

- Refrigeration
- Preservatives
  - Hydrochloric acid
  - Boric acid
  - Glacial acetic acid

Other preservatives used include formaldehyde, toluene, and thymol. The preservative used must be identified on the label of the container. If no preservative is used, this, too, should be noted.

**NOTE:** Before adding a preservative to a urine specimen, contact the laboratory performing the test to find out what preservative to use and the quantity to add. Preservative requirements vary from laboratory to laboratory.

## ROUTINE URINE EXAMINATION

A routine urinalysis includes the examination of physical characteristics, chemical characteristics, and microscopic structures in the sediment. A sample for urinalysis (routine and microscopic) should be at least 15 ml in volume (adult), and either a random or first morning specimen. Children may only be able to provide a small volume, but 10-15 ml is preferred.

### Physical Characteristics

Physical characteristics evaluated during a routine urinalysis include color, appearance, and specific gravity.

**COLOR.**—The normal color of urine varies from straw to light amber. Diluted urine is generally pale; concentrated urine tends to be darker. The terms used to describe the color of urine follow.

- Colorless
- Light straw
- Straw
- Dark straw

- Light amber
- Amber
- Dark amber
- Red

The color of urine may be changed by the presence of blood, drugs, or diagnostic dyes. Examples are:

- **red or red-brown** (smokey appearance), caused by the presence of blood.
- **yellow or brown** (turning greenish with yellow foam when shaken), caused by the presence of bile.
- **olive green to brown-black**, caused by phenols (an extremely poisonous compound, used as an antimicrobial agent).
- **milky white**, caused by chyle. (Chyle, which consists of lymph and droplets of triglyceride, is a milky fluid taken up by lacteal vessels from the food in the intestine during digestion.)
- **dark orange**, caused by Pyridium® (a topical analgesic used in the treatment of urinary tract infections).
- **blue-green**, caused by methylene blue (used as a stain or dye for various diagnostic tests).

**APPEARANCE.**—Urine's appearance may be reported as clear, hazy, slightly cloudy, cloudy, or very cloudy. Some physicians prefer the term "turbidity" instead of "transparency," but both terms are acceptable.

Freshly passed urine is usually clear or transparent. However, urine can appear cloudy when substances such as blood, phosphates, crystals, pus, or bacteria are present. A report of transparency is of value only if the specimen is fresh. After standing, all urine becomes cloudy because of decomposition, salts, and the action of bacteria. Upon standing and cooling, all urine specimens will develop a faint cloud composed of mucus, leukocytes, and epithelial cells. This cloud settles to the bottom of the specimen container and is of no significance.

**SPECIFIC GRAVITY.**—The specific gravity of the specimen is the weight of the specimen compared to an equal volume of distilled water. The specific gravity varies directly with the amount of solids dissolved in the urine and normally ranges from 1.015 to 1.030 during a 24-hour period.

The first morning specimen of urine is more concentrated and will have a higher specific gravity than a specimen passed during the day. A high fluid intake may reduce the specific gravity to below 1.010. In the presence of disease, the specific gravity of a 24-hour specimen may vary from 1.001 to 1.060.

Specific gravity is measured with an index refractometer, available as standard equipment at most duty stations. See figure 7-22. The index refractometer may be held manually or mounted on a stand like a microscope. The specific gravity of urine is determined by the index of light refraction through solid material.

Measure the specific gravity with an index refractometer in the following manner:

1. Hold the index refractometer in one hand. Use the other hand and an applicator stick to place a drop of urine on the glass section beneath the coverglass.
2. Hold the refractometer so that the light reflects on the glass section, and look into the ocular end. Read the number that appears where the light and dark lines meet. This is the specific gravity.

### Chemical Characteristics

Chemical characteristics evaluated during a routine urinalysis include pH, protein, glucose, ketones, and blood. Some laboratories also include tests for bilirubin, urobilinogen, and nitrite, depending on the test strip used. Currently, most medical facilities use the Multistix® and Color Chart, which detects pH, protein, glucose, ketones, blood, bilirubin, and urobilinogen. The Multistix is a specially prepared multitest strip. The strip is simply dipped into the urine specimen and compared to the color values for the various tests on the accompanying chart. The color chart also indicates numerical pH values, which should be reported.

### Microscopic Examination of Urine Sediment

Microscopic examination of urine sediment is usually performed in addition to routine procedures.

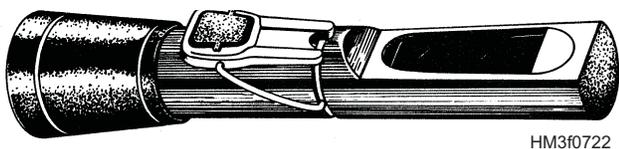


Figure 7-22.—Index refractometer.

This examination requires a degree of skill acquired through practice under the immediate supervision of an experienced technician. The specimen used for microscopic examination should be as fresh as possible. Red cells and many formed solids tend to disintegrate upon standing, particularly if the specimen is warm or alkaline.

**PREPARING SPECIMENS FOR MICROSCOPIC EXAMINATION.**—To prepare urine specimens for microscopic examination, follow the steps below.

1. Stir the specimen well.
2. Pour 15 ml of urine into a conical centrifuge tube, and centrifuge at 1,500 rpm for 5 minutes.
3. Invert the centrifuge tube and allow all of the excess urine to drain out. **Do not shake the tube while it is inverted.** Enough urine will remain in the tube to resuspend the sediment. Too much urine will cause dilution of the sediment, making an accurate reading difficult.
4. Resuspend the sediment by tapping the bottom of the tube.
5. With a medicine dropper, mount one drop of the suspension on a slide and cover it with a coverslip.
6. Place the slide under the microscope, and scan with the low-power objective and subdued lighting.
7. Switch to the high-power objective for detailed examination of a minimum of 10 to 15 fields.

**CLINICALLY SIGNIFICANT FINDINGS.**—Leukocytes, erythrocytes, and casts may all be of clinical significance when found in urine sediment.

**Leukocytes.**—Normally, 0 to 3 leukocytes per high-power field will be seen on microscopic examination. More than 3 cells per high-power field probably indicates disease somewhere in the urinary tract. Estimate the number of leukocytes present per high-power field and report it as the “estimated number per high-power field.”

**Erythrocytes.**—Red cells are not usually present in normal urine. If erythrocytes are found, estimate their number per high-power field and report it. Erythrocytes may be differentiated from white cells in several ways:

- White cells are larger than red cells.

- When focusing with the high-power lens, the red cells show a distinct circle; the white cells tend to appear granular with a visible nucleus.
- One drop of 5% acetic acid added to the urine sediment disintegrates any red cells, but it does not affect the white cells (except that the nuclei become more distinct).

**Casts.**—These urinary sediments are formed by coagulation of albuminous material in the kidney tubules. Casts are cylindrical and vary in diameter. The sides are parallel, and the ends are usually rounded. Casts in the urine always indicate some form of kidney disorder and should always be reported. If casts are present in large numbers, the urine is almost sure to be positive for albumin.

There are seven types of casts. They are as follows:

- **Hyaline casts** are the most frequently occurring casts in urine. Hyaline casts can be seen in even the mildest renal disease. They are colorless, homogeneous, transparent, and usually have rounded ends.
- **Red cell casts** indicate renal hematuria. Red cell casts may appear brown to almost colorless and are usually diagnostic of glomerular disease.
- **White cell casts** are present in renal infection and in noninfectious inflammation. The majority of white cells that appear in casts are hypersegmented neutrophils.
- **Granular casts** almost always indicate significant renal disease. However, granular casts may be present in the urine for a short time following strenuous exercise. Granular casts that contain fine granules may appear grey or

pale yellow in color. Granular casts that contain larger coarse granules are darker. These casts often appear black because of the density of the granules.

- **Epithelial casts** are rarely seen in urine because renal disease that primarily affects the tubules is infrequent. Epithelial casts may be arranged in parallel rows or haphazardly.
- **Waxy casts** result from the degeneration of granular casts. Waxy casts have been found in patients with severe chronic renal failure, malignant hypertension, and diabetic disease of the kidney. Waxy casts appear yellow, grey, or colorless. They frequently occur as short, broad casts, with blunt or broken ends, and often have cracked or serrated edges.
- **Fatty casts** are seen when there is fatty degeneration of the tubular epithelium, as in degenerative tubular disease. Fatty casts also result from lupus and toxic renal poisoning. A typical fatty cast contains both large and small fat droplets. The small fat droplets are yellowish-brown in color.

## SUMMARY

Clinical laboratory medicine is a very dynamic field of medicine, with new testing procedures and equipment being invented all the time. The goal of this chapter is to introduce you to some basic laboratory tests that do not require state-of-the-art equipment and that can be easily performed in isolated duty stations and aboard naval vessels. These tests will assist you in establishing diagnoses and will enable you to provide the best possible medical care for your patients.