Laboratory Exercise 49

Blood Testing

Materials Needed

Mammal blood other than human or contaminant-free human blood is suggested as a substitute for collected blood
Textbook
Sterile disposable blood lancets
Alcohol swabs

For Procedure A:
Heparinized microhematocrit capillary tube
Sealing clay (or Critocaps)
Microhematocrit centrifuge
Microhematocrit reader

For Procedure B:
Hemoglobinometer
Lens paper
Hemolysis applicator

For Procedure C:
Capillary tubes (nonheparinized)
Small triangular file
Timer

For Alternative Hemoglobin Content:
Tallquist test kit

Safety

- It is important that students learn and practice correct procedures for handling body fluids. Consider using either mammal blood other than human or contaminant-free blood that has been tested and is available from various laboratory supply houses. Some of the procedures might be accomplished as demonstrations only. If student blood is used, it is important that students handle only their own blood.
- Use an appropriate disinfectant to wash the laboratory tables before and after the procedures.
- Wear disposable gloves when handling blood samples.
- Clean the end of a finger with alcohol swabs before the puncture is performed.
- The sterile blood lancet should be used only once.
- Dispose of used lancets and blood-contaminated items in an appropriate container (never use the wastebasket).
- Wash your hands before leaving the laboratory.

Purpose of the Exercise

To observe the blood tests used to determine hematocrit, hemoglobin content, and coagulation time.

Learning Outcomes

After completing this exercise, you should be able to

1. Test and record the hematocrit, hemoglobin, and coagulation in a blood sample.
2. Judge the results of the blood tests compared to normal values.
3. Select the blood tests performed in this laboratory exercise that could indicate anemia.

As an aid in identifying various disease conditions, tests are often performed on blood to determine how its composition compares with normal values. These tests commonly include hematocrit (red blood cell percentage), hemoglobin content, and coagulation. A self-diagnosis should never be made as a result of a test result conducted in the biology laboratory. Always obtain proper medical exams and treatments from medical personnel.

Explore

Procedure A—Hematocrit

To determine the hematocrit (percentage of red blood cells) in a whole blood sample, the cells must be separated from the liquid plasma. This separation can be rapidly accomplished by placing a tube of blood in a centrifuge. The force created by the spinning motion of the centrifuge causes the cells to be packed into the lower end of the tube. The quantities of cells and

plasma can then be measured, and the percentage of cells (hematocrit or packed cell volume) can be calculated.

1. To determine the hematocrit in a blood sample, follow these steps:
   - **a.** Thoroughly wash hands with soap and water and dry them with paper towels.
   - **b.** Cleanse the end of your middle finger with an alcohol swab and let the finger dry in the air.
   - **c.** Remove a sterile disposable blood lancet from its package without touching the sharp end.
   - **d.** Puncture the skin on the side near the tip of the middle finger with the lancet and properly discard the lancet. Wipe away the first drop of blood with the alcohol swab.
   - **e.** Touch a drop of blood with the colored end of a heparinized capillary tube. Hold the tube tilted slightly upward so that the blood will easily move into it by capillary action (fig. 49.1*a*). To prevent an air bubble, keep the tip in the blood until filled.
   - **f.** Allow the blood to fill about two-thirds of the length of the tube. Cover the lanced finger location with a bandage.
   - **g.** Hold a finger over the tip of the dry end so that blood will not drain out while you seal the blood end. Plug the blood end of the tube by pushing it with a rotating motion into sealing clay or by adding a plastic Critocap (fig. 49.1*b*).
   - **h.** Place the sealed tube into one of the numbered grooves of a microhematocrit centrifuge. The tube's sealed end should point outward from the center and should touch the rubber lining on the rim of the centrifuge (fig. 49.1*c*).
   - **i.** The centrifuge should be balanced by placing specimen tubes on opposite sides of the moving head, the inside cover should be tightened with the lock wrench, and the outside cover should be securely fastened.
   - **j.** Run the centrifuge for 3–5 minutes.
   - **k.** After the centrifuge has stopped, remove the specimen tube. The red blood cells have been packed into the bottom of the tube. The clear liquid on top of the cells is plasma.
   - **l.** Use a microhematocrit reader to determine the percentage of red blood cells in the tube. If a microhematocrit reader is not available, measure the total length of the blood column in millimeters (red cells plus plasma) and the length of the red blood cell column alone in millimeters. Divide the red blood cell length by the total blood column length and multiply the answer by 100 to calculate the percentage of red blood cells.
2. Record the test result in Part A of Laboratory Report 49.

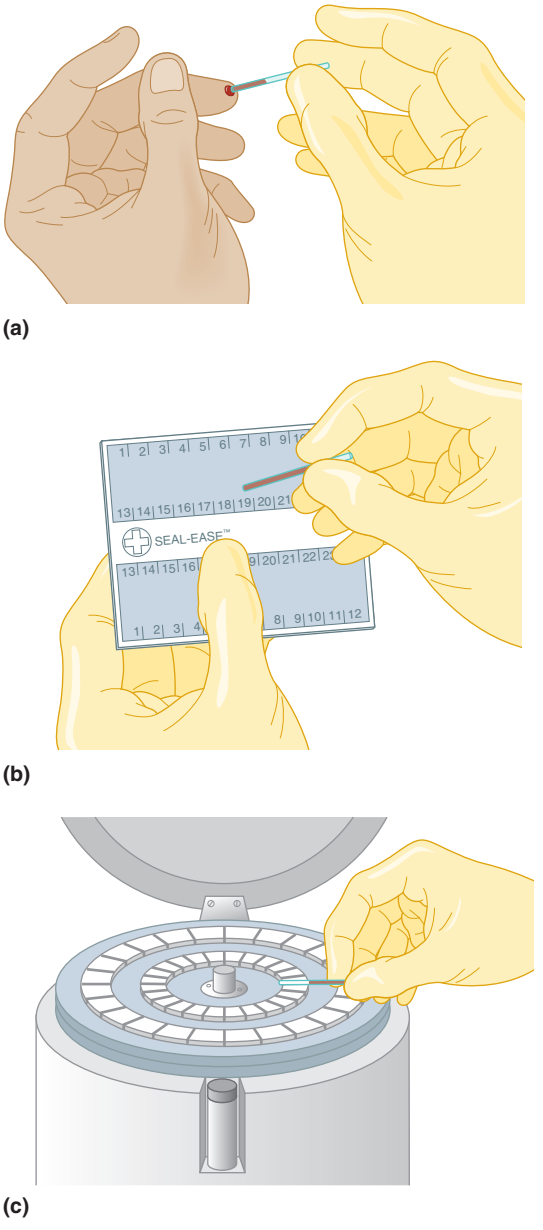


Figure 49.1 Steps of the hematocrit (red blood cell percentage) procedure: (*a*) load a heparinized capillary tube with blood; (*b*) plug the blood end of the tube with sealing clay; (*c*) place the tube in a microhematocrit centrifuge.

Explore

Procedure B—Hemoglobin Content

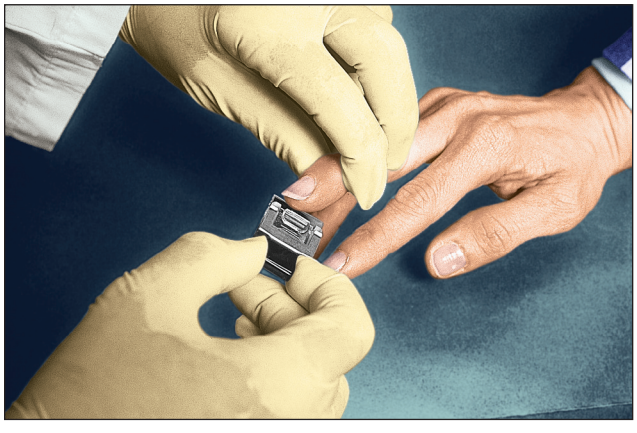
Although the hemoglobin content of a blood sample can be measured in several ways, a common method uses a hemoglobinometer. This instrument is designed to compare the color of light passing through a hemolyzed blood sample with a standard color. The results of the test are expressed in grams of hemoglobin

per 100 mL (g/dL) of blood or in percentage of normal values.

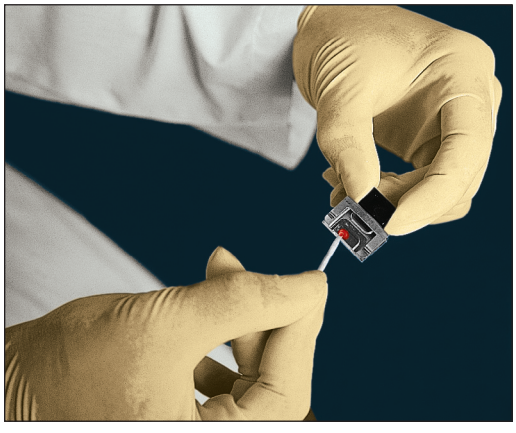
1. To measure the hemoglobin content of a blood sample, follow these steps:
   - **a.** Obtain a hemoglobinometer and remove the blood chamber from the slot in its side.
   - **b.** Separate the pieces of glass from the metal clip and clean them with alcohol swabs and lens paper. One of the pieces of glass has two broad, U-shaped areas surrounded by depressions. The other piece is flat on both sides.
   - **c.** Obtain a large drop of blood from a finger, by following the directions in Procedure A.
   - **d.** Place the drop of blood on one of the U-shaped areas of the blood chamber glass (fig. 49.2*a*).
   - **e.** Stir the blood with the tip of a hemolysis applicator until the blood appears clear rather than cloudy. This usually takes about 45 seconds (fig. 49.2*b*).
   - **f.** Place the flat piece of glass on top of the blood plate and slide both into the metal clip of the blood chamber.
   - **g.** Push the blood chamber into the slot on the side of the hemoglobinometer, making sure that it is in all the way (fig. 49.2*c*).
   - **h.** Hold the hemoglobinometer in the left hand with the thumb on the light switch on the underside (fig. 49.2*d*).
   - **i.** Look into the eyepiece and note the green area split in half.
   - **j.** Slowly move the slide on the side of the instrument back and forth with the right hand until the two halves of the green area look the same.
   - **k.** Note the value in the upper scale (grams of hemoglobin per 100 mL of blood), indicated by the mark in the center of the movable slide.
2. Record the test result in Part A of the laboratory report.

Alternative Procedure

The *Tallquist method* can also be used to determine reasonably accurate hemoglobin content. The Tallquist test kit contains a special test paper, color scale, and directions.



(a)



(b)

(c)

(d)

Figure 49.2 Steps of the hemoglobin content procedure: (*a*) load the blood chamber with blood; (*b*) stir the blood with a hemolysis applicator; (*c*) place the blood chamber in the slot of the hemoglobinometer; (*d*) match the colors in the green area by moving the slide on the side of the instrument.

Explore

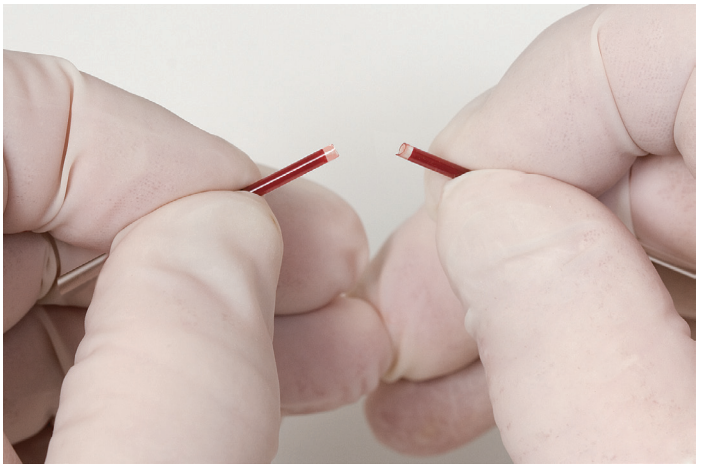
Procedure C—Coagulation

Coagulation time, often called clotting time, is the time from the onset of bleeding until the insoluble protein fibrin is formed. This happens when thrombin converts soluble fibrinogen into insoluble fibrin. This clotting time normally ranges from 2 to 10 minutes. This process is prolonged if the person has clotting deficiencies or is being treated with anticoagulants such as heparin (some is produced by basophils and mast cells), warfarin (Coumadin), or aspirin. In this laboratory exercise we will try to determine the time to the nearest minute.

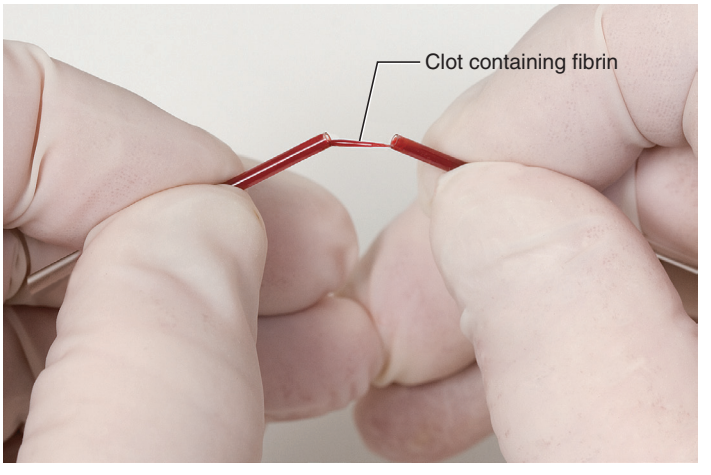
1. To determine coagulation time, follow these steps:
   - **a.** Prepare the finger to be lanced by following the directions in Procedure A. Lance the end of a finger to obtain a drop of blood. Wipe away the first drop of blood with the alcohol swab.
   - **b.** Touch the next drop of blood with one end of a nonheparinized capillary tube. Hold the tube tilted slightly upward so that the blood will easily move into it by capillary action (fig. 49.1*a*). Keep the tip in the blood until the tube is nearly filled. If the tube is nearly filled, it will allow enough tube length for breaking it several times.
   - **c.** Place the capillary tube on a paper towel. Cover the lanced finger location with a bandage. Record the time: _____
   - **d.** At 1-minute intervals, use the small triangular file and make a scratch on the capillary tube starting near one end of the tube. Hold the tube with fingers on each side of the scratch, the weakened location of the tube, and break the tube away from you, being careful to keep the two pieces close together after the break. Gently pull the two ends of the tube apart while observing carefully to see if it breaks cleanly apart. If it breaks cleanly, fibrin has not formed yet (fig. 49.3*a*).
   - **e.** Continue breaking the capillary tube each minute until fibrin is noted spanning the two parts of the capillary tube (fig. 49.3*b*). Note the time for coagulation.
2. Record the test results in Part A of the laboratory report.
3. Complete Part B of the laboratory report.

Alternative Procedure

Laboratories in modern hospitals and clinics use updated hematology analyzers (fig. 49.4) for evaluations of the blood factors described in laboratory exercise 49. The more traditional procedures performed in these laboratory activities will help you to better understand each blood characteristic.



(a)



(b)

Figure 49.3 Steps of the blood coagulation procedure: (*a*) clean break of capillary tube before any fibrin formation; (*b*) fibrin (the clot) spans the two ends of the broken capillary tube at coagulation.

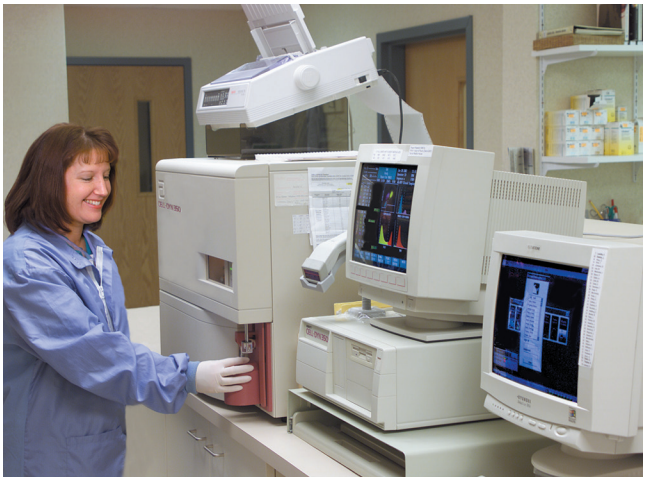


Figure 49.4 Modern hematology analyzer being used in the laboratory of a clinic. (*Note:* A tour of a laboratory in a modern hospital or clinic might be arranged.)

Name _____

Date _____

Section _____

The A corresponds to the indicated Learning Outcome(s) found at the beginning of the laboratory exercise.

Laboratory Report 49

Blood Testing

Part A Assessments

Blood test data: A1

Blood Test	Test Results	Normal Values
Hematocrit (mL per 100 mL blood)		Men: 40–54% Women: 37–47%
Hemoglobin content (g per 100 mL blood)		Men: 14–18 g/100 mL (g/dL) Women: 12–16 g/100 mL (g/dL)
Coagulation		2–10 minutes

Part B Assessments

Complete the following:

1. How does the hematocrit from the blood test compare with the normal value? A2 _____

_____

_____

2. How does the hemoglobin content from the blood test compare with the normal value? A2 _____

_____

_____

3. How does the coagulation time from the blood test compare with the normal value? A2 _____

_____

_____

Critical Thinking Application

Which blood tests performed in this lab could be used to determine possible anemia? A3