

**CHAPTER
5**



Phlebotomy Equipment

OBJECTIVES

After studying this chapter, you should be able to:

- 1.** Describe the basic units of the metric system.
- 2.** Describe the proper use of syringes in sample collection.
- 3.** State the relationship between bore size and the gauge of the needle.
- 4.** Explain the principle of the evacuated system.
- 5.** State the manner in which the following anticoagulants prevent coagulation: fluoride/oxalate, citrates, EDTA, and heparin.
- 6.** Name the anticoagulant associated with the following color-coded tubes: blue, gray, green, and lavender.
- 7.** State the anticoagulant that requires a 1:9 ratio of anticoagulant to blood.
- 8.** State the purpose of the following additives: silicon coating, silica particles, and thixotropic gel.
- 9.** Describe the three basic types of tourniquets.
- 10.** Explain how a tourniquet makes the veins more prominent.
- 11.** Define hemoconcentration.
- 12.** Describe the different type of lancets that may be used in capillary puncture.
- 13.** List the different types of microcollection equipment available.

NAACLS Competencies Relevant to Chapter 5

- ▶ Define hemostasis, and explain the basic process of coagulation and fibrinolysis.

Demonstrate knowledge of collection equipment, various types of additives used, special precautions necessary, and substances that can interfere in clinical analysis of blood constituents.

- ▶ Identify the various types of additives used in blood collection, and explain the reasons for their use.
- ▶ Identify the evacuated tube color codes associated with the additives.
- ▶ Describe substances that can interfere in clinical analysis of blood constituents and ways in which the phlebotomist can help to avoid these occurrences.
- ▶ List and select the types of equipment needed to collect blood by venipuncture and capillary and arterial puncture.
- ▶ Identify special precautions necessary during blood collections by venipuncture and capillary and arterial puncture.

KEY TERMS

| | |
|------------------------------------|---|
| Additive | Any material placed in a tube that maintains or facilitates the integrity and function of the sample. |
| Capillary Action | Adhesive molecular forces between liquid and solid materials that draw liquid into a narrow-bore capillary tube. |
| Fibrin Degradation Products (FDPs) | Fragments of the fibrin/clot found in the bloodstream. |
| Fibrinolysis | Process at the end of the clotting process that breaks down fibrin into small fragments, called fibrin degradation products, that lead to disintegration of the clot. |
| Flea | Metal rod used for mixing the blood sample that fits inside a capillary tube. |
| Hemoconcentration | Concentrating the constituents of blood by leaving the tourniquet on too long. |
| Hemostasis | Process of the formation of a blood clot when an injury occurs and then lysing of that blood clot when the injury has been repaired. |
| Palpate | To search for a vein with a pressure-and-release touch. |
| Platelet Adhesion | The process of platelet clumps adhering to an injured area to stop bleeding. |
| Platelet Aggregation | The process of platelets clumping together during hemostasis. |
| Thixotropic Separator Gel | A gel material capable of forming an interface between the cells and fluid portion of the blood as a result of centrifugation. |
| Tourniquet | Any constrictor used to facilitate vein prominence. |
| Vasoconstriction | Constriction of vessel(s) that limits blood flow. |
| Viscosity | Degree of thickness or resistance to flow of a substance. |

Developing knowledge of basic information concerning the equipment used in collection of blood samples is the focus of Chapter 5. There are multiple illustrations of the equipment used for syringe collection, evacuated system collection, butterfly (winged infusion set) collection, and microcollection. When it is appropriate to use, each type of equipment is discussed. The variations of the equipment that will be seen in different health care facilities are explained. The anticoagulants and their effect on both testing and the venipuncture technique are also covered. Explaining equipment cannot be limited to a discussion of the equipment itself. Thus the hazards and proper use of the equipment are covered to help the phlebotomist learn phlebotomy techniques in later chapters.

THE METRIC SYSTEM

Before a discussion of phlebotomy equipment can begin, a review of the metric system is essential. The metric system is a group of units used to make measurements, such as length, volume, temperature, weight, and time. To function in the health care setting, knowledge of the metric system is necessary. Most health care information and equipment used are in the metric system. The volume of blood drawn, pipette volume, urine volume, body surface area calculations, and normal (reference) values are all in the metric system. Most metric units have a prefix that describes the relationship of that unit to the basic unit. These prefixes are the same throughout the metric system and help to simplify it. Latin prefixes show divisions of the basic units. For example, *centi* means “one-hundredth” and *milli* means

METRIC CONVERSION TABLE

Length

| | |
|------------------------|----------------------------------|
| 1 inch (in.) | = 25.4 millimeters (mm) |
| 1 inch (in.) | = 2.54 centimeters (cm) |
| 1 foot (ft.) | = 30.48 centimeters (cm) |
| 1 yard (yd.) | = 91.44 centimeters or 0.9 meter |
| 39.37 inches (in.) | = 1 meter (m) |
| 0.39 inches (in.) | = 1 centimeter (cm) |
| 1,000 millimeters (mm) | = 100 centimeters (cm) |
| 100 centimeters (cm) | = 1 meter (m) |

Volume

| | |
|-------------------------|--------------------------|
| 1 ounce (oz.) | = 29.57 milliliters (mL) |
| 1 pint (pt.) | = 0.47 liter (L) |
| 1 quart (qt.) | = 0.95 liter (L) |
| 1 gallon (gal.) | = 3.78 liters (L) |
| 1 tablespoon (tbsp.) | = 15 milliliters (mL) |
| 1 teaspoon (tsp.) | = 5 milliliters (mL) |
| 1.06 quarts (qt.) | = 1 liter (L) |
| 0.03 ounce (oz.) | = 1 milliliter (mL) |
| 1 cubic centimeter (cc) | = 1 milliliter (mL) |

continued

Weight

| | |
|--------------------|-------------------|
| 1 ounce (oz.) | = 28.35 grams (g) |
| 1 pound (lb.) | = 454 grams (g) |
| 2.205 pounds (lb.) | = 1 kilogram (kg) |

Temperature

| | |
|------------------|---|
| 98.6° Fahrenheit | = 37° Celsius (normal body temperature) |
| 32° Fahrenheit | = 0° Celsius (water freezes) |
| 212° Fahrenheit | = 100° Celsius (water boils) |
| Celsius Temp. | = 5/9 (°Fahrenheit – 32) |
| Fahrenheit Temp. | = 9/5 °Celsius + 32 |

Time

| | |
|--------------------------------------|--------|
| 6:00 A.M. | = 0600 |
| 12:00 P.M. (noon) | = 1200 |
| 6:00 P.M. | = 1800 |
| 12:00 A.M. (midnight) | = 2400 |
| 12:01 A.M. (1 minute after midnight) | = 0001 |

Helpful Hint

A quick knowledge of the metric system can be helpful when working with a patient who is anxious about the amount of blood that is being collected.

For example, if you are collecting 10 mL of blood, the patient may ask, "You are taking that much blood?" You may reassure the patient with the response "It may look like a lot of blood but actually it is only 2 teaspoons of blood."

"one-thousandth" of the basic unit. Greek prefixes show multiples of the basic unit. For example, *hecto* means "100 times," *kilo* means "1,000 times," and *mega* means "1,000,000 times."

The meter (m) is the basic unit for measuring length. A meter is slightly longer than a yard. Short lengths are measured as centimeters (cm) or millimeters (mm). A physician describing the length of a person's finger describes it in terms of centimeters. If the physician was describing a tumor the size of a pea, he would describe it in terms of millimeters.

Volume measurements tell the size of a box in terms of cubic units. For example, a box 1 meter tall, 1 meter wide, and 1 meter deep has a volume of 1 cubic meter. A box measuring 1 centimeter square has a volume of 1 cubic centimeter (cc). The volume of a liquid is measured in terms of liters (L). Soft drinks often come in 1- or 2-liter bottles. One-tenth of a liter is a deciliter (dL). One-thousandth of a liter is a milliliter (mL). Liquids also can take up volume. One milliliter of liquid occupies the same volume as 1 cubic centimeter. One milliliter equals 1 cubic centimeter. A 10-milliliter (10-mL) syringe is the same size as a 10-cubic-centimeter (10-cc) syringe. Often in conversation, the terms *cubic centimeter* and *milliliter* are used interchangeably.

A kilogram is the basic unit of weight in the metric system. One kilogram (kg) equals 2.2046 pounds (lb.). A gram is for smaller weight and equals one-thousandth of a kilogram.

The metric system measures temperature in degrees Celsius (°C). Water freezes at 0° Celsius and boils at 100° Celsius. The normal human body temperature is 37° Celsius.

Time in the metric system, like all other systems, uses hours, minutes, and seconds. Much of the health care system and most countries other than the United States use 24-hour clock time (military time). Rather than A.M. and P.M., the clock goes a full 24 hours. For example, noon is 1200, 4 P.M. is 1600, 6 P.M. is 1800, and midnight is 2400.

Some formulas for calculating patient dosages require knowing the patient's weight in kilograms. A quick conversion is that 1 lb. equals 0.454 kg. Simply multiply the patient's weight in pounds by 0.454 to give the kilogram weight:

$$\text{A patient weighs 150 lb.: } 0.454 \times 150 = 68.1 \text{ kg}$$

EXERCISE 1 Fill in the Blanks

1. Metric system: Fill in the missing measurements.

| Linear | Term | Volume | Term | Weight | Term |
|---------|------|---------|------|---------------|------|
| 1/100 | | 1/1000 | | 1/1000 | |
| meter = | | liter = | | gram = | |
| | | | | 1000 × gram = | |

Metric temperature units = _____

2. Complete: 1 cc = _____ mL

10 mL = _____ teaspoons

3. What would be the dimensions of a box on each side to be able to hold 1 mL?

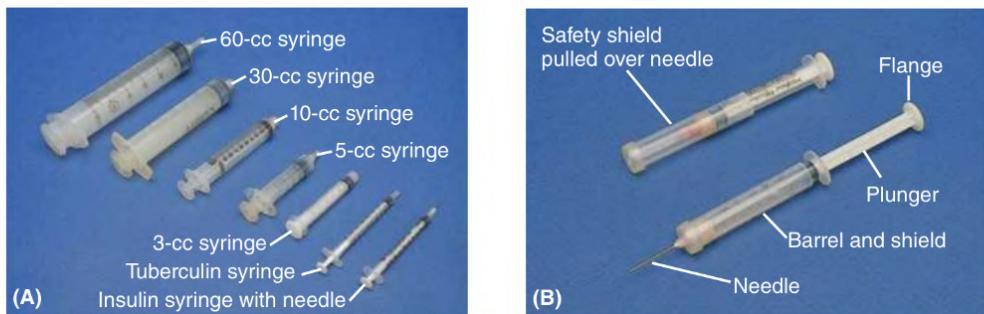
SYRINGES AND NEEDLES

All methods of venipuncture require the invasive procedure of entering a vein to obtain a blood sample. The syringe and needle method is one of the oldest methods known that does not destroy the integrity of the vein. Apparatus similar to the syringe and needle systems of today have been found in Egyptian tombs. The purpose of the system then was probably not to draw blood, but it was possibly used as a pus extractor or a miniature flame thrower. The principle and basic construction of the system have remained the same: a sleeve with a plunger that fits inside and a needle attached to the other end. Syringes are now made of either glass or plastic, with the majority being plastic. The barrel and plunger (Figure 5.1) varies in volume from 1 mL up to 60 mL. The barrel of the syringe is graduated into milliliters. Pulling on the plunger creates a vacuum within the barrel. The plunger on a syringe often sticks and is hard to pull. A technique called *breathing the syringe* needs to be done before it is used. To breathe the syringe, pull back on the plunger to about halfway up the barrel and then push the plunger back. This makes the plunger pull more smoothly and not have the tendency to jerk when first pulled. A jerking action while drawing blood both hurts the patient and possibly prevents the phlebotomist from obtaining an adequate blood sample.

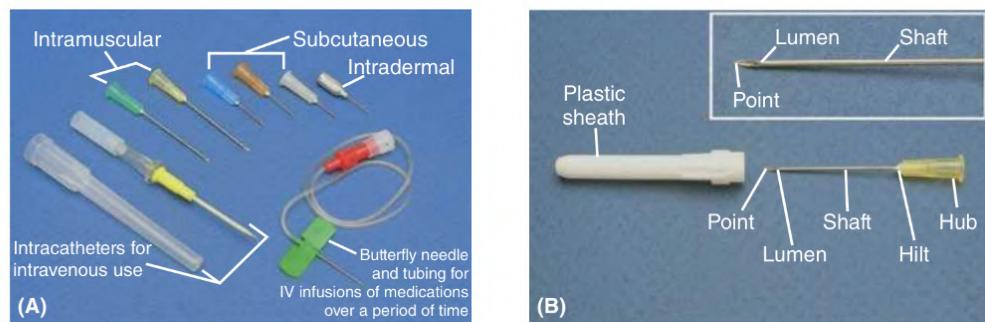
Helpful Hint

Add the total volume of blood that will be needed to fill the tubes to ensure that the syringe is capable of drawing the total volume.

The vacuum created by pulling on the plunger while a needle is in a patient's vein fills the syringe with blood. The larger the syringe, the greater the amount of vacuum obtained. Too large a vacuum has the tendency to pull too hard on the vein and collapse it. Pulling the plunger slowly and resting between pulls allows the vein time to refill with blood and prevents collapse of the vein. Generally, syringes are used for the difficult-to-draw patients who have fragile, thin, or "rolly" veins that tend to collapse when using an evacuated system. Pediatric or geriatric patients typically have these veins. The surface veins on the feet or back



▲ FIGURE 5.1 (A) Various sizes of disposable syringes. Note size difference of tuberculin and insulin syringes. (B) A type of safety syringe.



▲ FIGURE 5.2 (A) Various sizes and types of disposable needles. (B) Parts of a needle and needle sheath.

of the hands may also require the syringe technique. The use of a syringe and needle is limited by the capacity of the syringe. The use of a syringe larger than 10 to 15 mL is not recommended. If a large amount of blood is needed, a butterfly collection set should be used. This device is discussed later in the chapter. Syringes are also used in special procedures when the blood must be drawn and then transferred to a different container.

The needle used on the syringe consists of a hub, cannula (shaft), and point cut at a precise bevel (Figure 5.2). The hub of the needle attaches to the syringe. The needle is attached by sliding the hub onto the syringe or by screwing the hub into a threaded insert called a Luer lock. The recommended length of the needle is 1 inch to 1½ in. The gauge of the needle is determined by the diameter of the lumen, or the opening at the bevel end. The gauges of needles used in health care are 27, 25, 23, 22, 21, 20, 18, and 16 (from smallest to largest). The 22-, 21-, and 20-gauge needles are used for venipuncture. The 22-gauge needle is used for small veins and for pediatric patients. Use of a smaller needle may destroy red blood cells as they are pulled through the needle bore. A 23-gauge needle can be used in combination with a butterfly collection set. A 25-gauge needle cannot be used for venipuncture, because the red blood cells would be destroyed when the blood is pulled through the bore of the needle. The 27-gauge needle is used for administration of a purified protein derivative (PPD) tuberculosis skin test. The 25-gauge needle is used for intermuscular injections. The 18- and 16-gauge needles are used for the intravenous (IV) infusion of fluids or blood products or the removal of blood during the donor process (Figure 5.3).

Syringes are now required to have a safety shield to cover the needle immediately after use. This consists of an additional syringe barrel that slides over the needle (see Figure 5.1) or a device attached to the needle that slides over the needle after use (Figure 5.4). The device that slides over the needle activates by pushing the device forward with the thumb (Figure 5.5).

| Needle Gauge | Needle Use |
|--------------|--|
| 27 | PPD skin tests |
| 25 | Intermuscular injections |
| 23 | Butterfly or syringe collection |
| 22 | Syringe or evacuated system collection |
| 21 | Syringe or evacuated system collection |
| 20 | Syringe or evacuated system collection |
| 18 | IVs or blood donation |
| 16 | IVs or blood donation |

▲ FIGURE 5.3 Sizes of needles and their common uses.



▲ FIGURE 5.4 Safety syringe unengaged (top). Safety syringe engaged (bottom).



▲ FIGURE 5.5 Activating the safety device.

The bevel of the needle is the angle of the needle on the tip. The sharper the bevel, the less pain the needle produces. The length of the bevel must remain small enough to fit within the lumen of the vein. Needles are now manufactured with smooth edges and are silicon coated for easy insertion. The walls of the needle are thinner, so it has a thinner outside diameter and therefore makes a smaller hole as it enters the patient's arm. These characteristics of a syringe needle are duplicated in the needles of the evacuated tube system and the butterfly collection set.

EVACUATED SYSTEM

The evacuated system is often called the Vacutainer system. Vacutainer can be a misnomer because the term *Vacutainer* is a brand name for the evacuated system manufactured by the Becton Dickinson Company. Phlebotomists often say *Vacutainer* when they are using another company's product. This is the same as saying you are eating Jell-O when actually you are eating a generic brand of gelatin. The evacuated tube system has been manufactured since the 1940s.

The evacuated blood collection system operates on the principle that a syringe creates a vacuum when the plunger is pulled. In the evacuated system, a tube with a vacuum already in it attaches to the needle and the tube's vacuum is replaced by blood. The basic system consists of a double-pointed needle, a plastic holder, and a series of vacuum tubes with rubber stoppers.

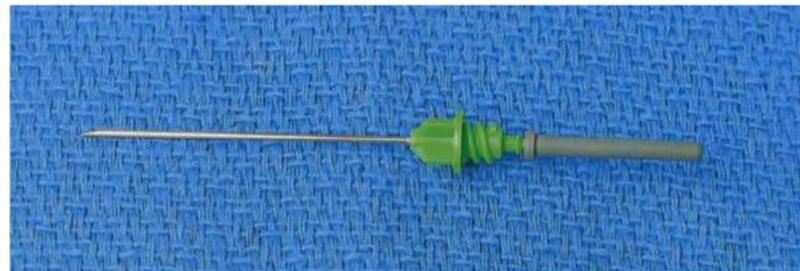
The needles are manufactured in two configurations. The basic needle is straight with no safety devices attached. Use of this needle requires the safety shield to be attached to the holder. The alternate needle has the safety device attached to it (Figure 5.6).

Helpful Hint

Visually check the point on the needle to make sure that it is not bent and does not have a burr that will hurt the patient.



▲ FIGURE 5.6 Needle with the safety device attached to the holder, Sims Portex Needle Pro holder (top). Needle with the safety device attached to the needle, Becton Dickinson Eclipse Needle (bottom).



▲ FIGURE 5.7 Evacuated blood collection system needle.

The needle is a straight hollow type with double points and a screw hub near the center. The needle extending from the holder has the proper bevel to pierce the skin and enter the vein. The needle within the holder pierces the rubber stopper on the evacuated tube. This needle has a rubber sleeve that covers it (Figure 5.7). The sleeve works as a valve that stops the flow of blood when the tube is removed. Pushing the tube into the holder compresses the rubber sleeve and exposes the needle, allowing it to enter the tube. When the tube is being removed, the sleeve slides back over the needle and stops the flow of blood. The needle entering the patient varies in length from 1 inch to 1½ inches. The gauges available are 20, 21, and 22. The 21 and 22 gauges are the most common. The gauge refers to the size of the opening (bore) in the needle shaft, also known as the lumen of the needle. One improvement has been to manufacture needles with a thinner wall. With a thinner wall, the needle still draws at a normal rate, but it hurts less because it has a smaller outside diameter. Silicon coats the needles so they slide into the skin with less resistance. The needle can be thought of as a pipeline that delivers blood from the patient to the tube. The tube is the method by which the blood is pumped from the patient. The blood fills the tube because of the vacuum of the tube.

The bevel of the needle is the slanted opening at the end of the needle. It must always be facing upward when the needle is inserted into the vein. When you look straight down on the needle as it is inserted into the skin, the opening in the needle should be visible. The bevel of the needle is cut at an angle so that when the needle is inserted into the skin the bevel angle ensures maximum blood flow through the needle. To obtain this maximum blood flow, the needle should be inserted at a 15- to 30-degree angle to the surface of the skin (Figure 5.8). The deeper the vein, the greater the angle you will need to use. A shallow vein will be accessed at a 15-degree angle, whereas a deep vein will need a 30-degree angle for the best entry.

▼ Helpful Hint

Entering at an angle less than or greater than 15 to 30 degrees will increase the chance of you missing the vein or causing nerve damage.



▲ FIGURE 5.8 Proper angle of needle insertion for venipuncture.



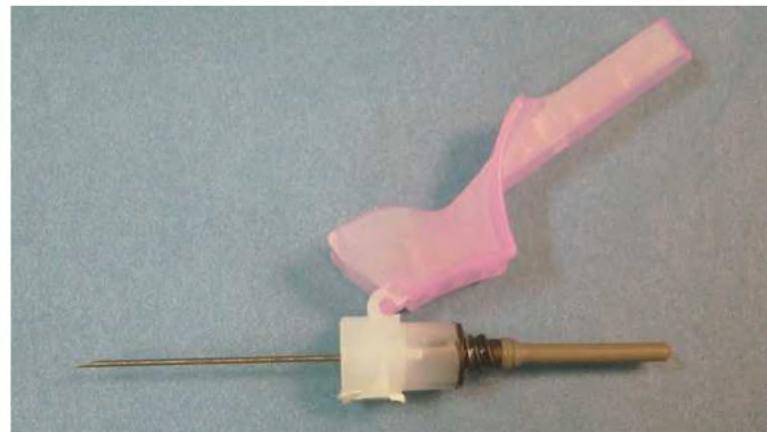
▲ FIGURE 5.9 Evacuated tube system holders: (A) adult holder and (B) pediatric holder.

The holder for the needle makes the task of collecting the blood sample easier. It gives the phlebotomist something more substantial to hold on to and a way to center the needle into the stopper of the evacuated tube. The needle screws into the holder, and the tube inserts into the other end of the holder. The holder has an indentation about $\frac{1}{4}$ inch from the hub of the needle. This indentation marks the point where the short end of the needle starts to enter the rubber stopper of the tube. If the tube is inserted past this point before you have entered the vein, the tube will fill with air and not produce a blood sample. To do a venipuncture, grasp the holder the same way you would hold the barrel of a syringe. This is discussed in detail in Chapter 6. The holders come in two sizes: one size for adult venipuncture and one size for small-diameter tubes used in pediatrics (Figure 5.9). Holders are constantly changing in an attempt to reduce needlestick injuries. As discussed in Chapter 2, most of the engineering controls adapted recently have focused on the needle or needle holder to reduce needlestick injuries. The holders have changed from the basic type to styles that cover the needle after use (Figure 5.10). These styles vary from holders that slide an outer sleeve over the contaminated needle to holders that snap a cover over the needle.

Several needles have been engineered to provide a safety feature on the needle rather than the holder. The Becton Dickinson Eclipse needle (Figure 5.11) is an example of a needle that has an attached safety feature. With this type of needle, a traditional holder may be used.



▲ FIGURE 5.10 Safety evacuated tube system holders; discard after use.



▲ FIGURE 5.11 Becton Dickinson Eclipse needle.

REUSE OF EVACUATED SYSTEM HOLDER

Many health care organizations were quick to adopt some type of safety needle. Many of the devices that slide a cover over the needle after use also protect the user from the back end of the needle. The entire device must then be discarded with the needle still attached. This action fulfills the Occupational Safety and Health Administration (OSHA) requirement:

The practice of removing the needle from a used blood-drawing/phlebotomy device is rarely, if ever required by a medical procedure. Because such devices involve the use of a double-ended needle, such removal clearly exposes employees to additional risk. Devices with needles must be used and immediately discarded after use, uncapped, into accessible sharps containers.

The intention of this OSHA paragraph is to eliminate the use of all devices in which the needle or a butterfly is removed from the needle holder. Often needles were used and then the needle was unscrewed by hand using the “keyhole” on the sharps container. This created a hazard if the needle lodged in the keyhole device that unscrewed the needle. The phlebotomist would try to push the needle back into the container with his or her hand, resulting in an injury.

Also used were devices that removed the needle from the holder by pressing the side of the holder or pushing the holder into a special sharps container. The needle would drop into the sharps container. This would save money by allowing the needle holder to be reused. However, the “back end” of the needle would create the potential for a needlestick. The thinking was that because this needle was now in a sharps container there would not

be a risk. Often in removing the needle, the needle would not drop into the sharps container and would fall to the floor or tabletop. The phlebotomist would reach to pick it up and receive an injury from the back end of the needle. This back-end needle exposure is also a concern when using a butterfly (winged infusion set).

The reuse of the needle holder has also been scrutinized for the safety reason of contamination of the holder with blood after even one use.

The cost of discarding the holder is not restricted to the additional cost of the holders but also involves the cost of filling the sharps containers more quickly. This could also result in the containers not getting changed often enough, with overfilled containers being a needlestick risk.

Some of the costs can be offset by price reductions due to increased usage. Less expensive disposable holders can also be used. Self-capping needles, such as the Eclipse, can be used, but the entire device holder and needle must be discarded intact.

SPLATTERING OF BLOOD

With all the devices there is the possibility of splattering of blood as the safety feature is activated. Holders with a sliding sleeve that covers the needle can dislodge a drop of blood as the sleeve slides over the needle. In most instances the blood droplet will be trapped in the safety device and not cause a hazard. A variation of this is the Retractable Technologies, Inc., VanishPoint holder. The VanishPoint holder automatically retracts the needle into the holder (Figure 5.12). The VanishPoint is also available in syringes and winged infusion sets.

Care must be taken when activating any safety device. There is possible splatter with the Eclipse needle and the Sims Portex Needle-Pro needle holder (see Figure 5.6). Both these devices require a shield to be flipped over the needle. There have been some instances of blood splatter as the shield is clicked into place. The Eclipse instructions illustrate the activation of the device with the employee's thumb.

The Sims Portex Needle-Pro device illustrates activation of the shield only on a surface such as a tabletop near the bed. There is still the possibility of splatter, but it will not be near a patient or employee, resulting in skin contact. Activation of the sleeve on a tabletop also reduces the possibility of the thumb missing the shield and causing a needle injury. There have been some cases where, in clicking the shield onto the Eclipse needle with the thumb, the thumb was punctured with the contaminated needle. Activating the device on a tabletop or on a solid surface and not with the thumb will reduce the exposure risk.

Helpful Hint

Removing the last tube collected from the holder before pulling the needle from the patient will reduce the chance for blood splatter from the tip of the needle.



▲ FIGURE 5.12 VanishPoint retractable needle holder.

EVACUATED COLLECTION TUBES

Evacuated collection tubes contain a vacuum with a rubber stopper sealing the tube. These tubes range in volume from 2 to 15 mL. The tubes have sterile interiors to prevent contamination of the sample and the patient. The patient can obtain an infection from a nonsterile tube if the blood flows into the tube, becomes contaminated, and then backflows into the patient. The patient will then be injected with the contaminating organism and possibly develop an infection. A sterile tube does not contaminate the blood, so any backflow of blood is inconsequential.

The tubes are glass or plastic and vary in length from 65 to 127 mm with an external diameter of 10, 13, or 16 mm. The diameter is such that it will easily slide into the evacuated system holder. The 10-mm size fits perfectly into the pediatric holder; the 13- and 16-mm sizes slide easily into the adult holder (Figure 5.13). The mechanism of the rubber stopper on the tube has changed because of the increase of blood and body fluid precautions. The traditional rubber stopper popped as the top was removed to access the sample. This created an aerosol that could be inhaled or ingested. Becton Dickinson was the first to develop a tube called Hemogard (Figure 5.14). This tube has a plastic sleeve that fits



▲ FIGURE 5.13 Assorted evacuated tubes.



▲ FIGURE 5.14 Hemogard tube.

Helpful Hint

If you use a tube past the expiration date, the laboratory will reject the sample and the patient will need to be recollected with a tube that is within the expiration time.

over the rubber stopper to contain any aerosols that might be dispersed when the cap is removed. The Hemogard cap has no benefits for the phlebotomist but is an excellent safeguard for the technologist working with the sample. Other manufacturers such as Griener Bio-One have developed a similar tube, Vacutte, that also reduces aerosols.

Tubes have an expiration date to indicate when they should no longer be used. The tubes are good until the end of the month stamped on them. After the expiration date, the vacuum will possibly not fill the tube completely and the additives are not guaranteed to perform as designed. Using tubes past the expiration date compromises the integrity of the sample and can give questionable results.

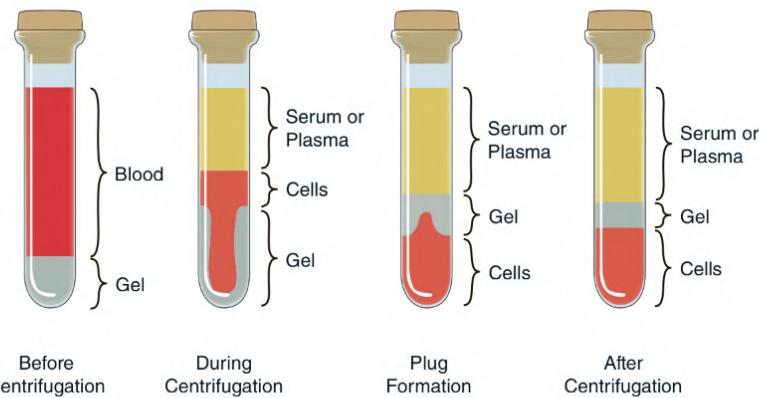
Tubes made from plastic are the primary ones used in health care settings. Before plastic tubes could be used for vacuum tubes, the difficulty of vacuum leakage had to be eliminated. Plastic tubes have now been developed that can maintain a vacuum within the tube. Plastic tubes are manufactured with or without anticoagulant.

Various additives are introduced to the tubes as part of the manufacturing process to improve the quality of the sample. These **additives** are not anticoagulants or preservatives but are used to improve sample quality or to accelerate sample processing. Most tubes have a silicon coating on the interior surface. This silicon fills the microscopically rough surface of the tube. The tube may feel smooth to the touch, but it has a rough surface that cells can stick to. The silicon fills in these cracks and crevasses and prevents the cells from adhering to the surface. This reduces the chance for hemolysis and makes the sides slicker so the cells can centrifuge to the bottom of the tube faster. These tubes have a red stopper, red/black stopper, or gold stopper (Hemogard tube), depending on the manufacturer. The glass surface of a glass tube for serum testing will activate clot formation in the tube. Some glass serum tubes have a clot activator added to speed the clotting process. A plastic tube will not activate a clot; therefore, a clot activator must be added to each tube during manufacture. This clot activator consists of silica particles on the sides of the tubes that initiate the clotting process.

One type of clot activator used for stat (emergency) testing is thrombin. The thrombin within the tube hastens the clotting process faster than the silica particles. Serum and plasma tubes can also be purchased with a **thixotropic separator gel** (Figure 5.15). This gel



▲ FIGURE 5.15 Thixotropic separator gel tubes.



▲ FIGURE 5.16 Separator gel tube: Centrifugation process.

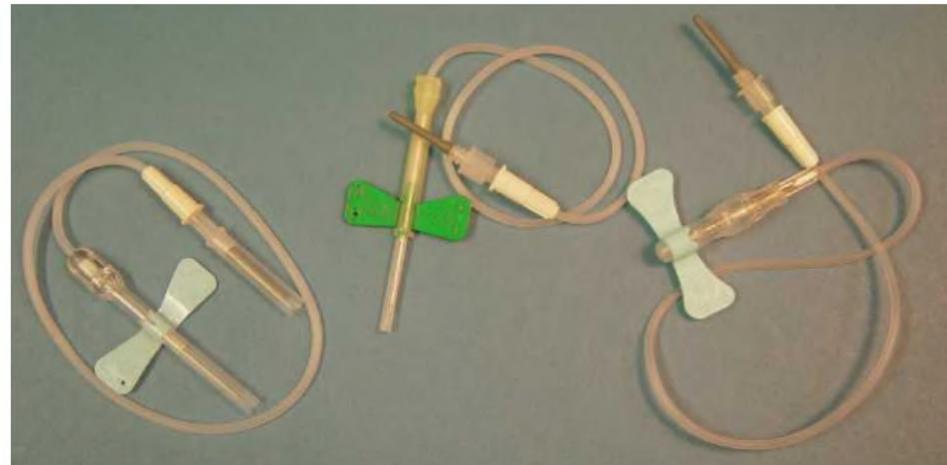
is an inert material that undergoes a temporary change in **viscosity** during centrifugation. It has a density that is intermediate to cells/clot and plasma/serum. When centrifuged, the gel moves up the sides of the tube and engulfs the cells/clot, and an interface of gel forms that separates the cells/clot from the plasma/serum (Figure 5.16).

BUTTERFLY (WINGED INFUSION SET) COLLECTION SYSTEM

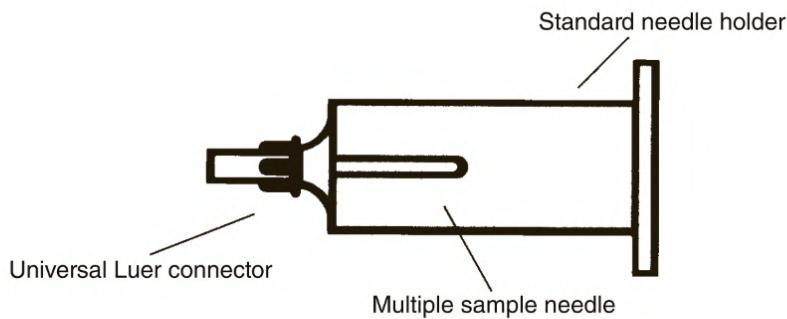
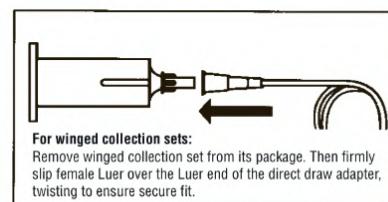
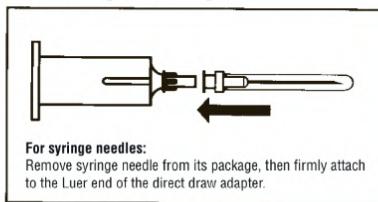
Helpful Hint

Check that the sharps container is not filled past the fill line on the container. Dropping items into a sharps container that is too full will cause needles to be pushed out of the container.

The butterfly collection system, or winged infusion blood collection set, combines the benefits of the evacuated system and the syringe system. It has a 21- or 23-gauge needle with attached plastic wings on one end. The needle should have a safety device in order to meet OSHA requirements. This device generally consists of a sleeve that slides over the needle immediately after use or a needle that retracts from the patient's arm. A piece of 3- to 12-inch tubing leads from the needle. On the other end of this tubing is a hub that can be attached to a syringe (Figure 5.17). The syringe attached to a butterfly offers some flexibility: a butterfly needle can be attached to the syringe so the needle can be anchored and the syringe pulled without disrupting the needle. Once the blood is collected with a syringe, the syringe is detached and the blood transferred to the evacuated tubes via a transfer device. The transfer device is explained in detail in Chapter 6. An alternative is to have the butterfly attached to an evacuated needle holder and *Luer adapter*. The Luer adapter (Figure 5.18) is similar to an evacuated system needle, but in place of the needle is a port to attach to the butterfly. The part of the Luer adapter that is inside the holder is the same as a standard evacuated system needle. The entire butterfly device with tubing and holder is discarded in a sharps container when the venipuncture is



▲ FIGURE 5.17 Butterfly collection set/winged infusion set.


Assembly techniques:


▲ FIGURE 5.18 Luer adapter (direct draw adapter).

completed. When discarding the butterfly device, do not push the tubing and attached holder and needle into a sharps container. The needle in the sharps container might be caught by the tubing and come back out of the sharps container. This can result in an accidental needlestick. The best way to dispose of the butterfly device is to treat it as a snake, whereby the needle end is the head of the snake. Just like holding a snake, hold the butterfly by the plastic wing and drop the head into the sharps container first. Then allow the remainder of the device to drop into the sharps container, with the holder being the last part to enter the container. With this method, the tubing curls into the sharps container and you eliminate the possibility of another needle being pushed out of the container.

The butterfly system is used for small veins that are difficult to draw with the other systems. Holding the plastic wings attached to the needle provides easy access into small surface veins on the back of the hand, the arm, or the foot. Do not enter the vein at the usual 15- to 30-degree angle. Since these veins are near the skin surface, the winged needle is inserted at approximately a 5-degree angle and then threaded into the vein. This procedure anchors the needle in the center of even a small vein. If the patient moves, the tubing is flexible so the needle stays anchored and does not pull out of the vein. The butterfly collection set works well on children who have both small veins and the tendency to move while blood is being collected. The tubing also works as a pressure relief valve. A large evacuated tube or large syringe can be attached to the tubing, and the vein will not collapse as would normally occur.

The system also offers the flexibility to start drawing blood with a syringe and then finish with the evacuated tube system. A syringe can be drawn for procedures that require a syringe sample, and then the syringe can be removed and the evacuated tube system attached for multiple tube collection. Even with all these benefits, the butterfly collection set is not used for all collection. It is much more expensive than the needle system. This additional expense is not justified for the majority of venipunctures.

Helpful Hint

Usually no more than 10 percent of the venipunctures a phlebotomist performs should require the use of a butterfly. This percentage will be greater in pediatric hospitals and oncology centers where patients' veins require the use of a smaller needle.

COAGULATION

Before an explanation of anticoagulants can begin, the process of coagulation must be explained. A simple example is given in Figure 4.9. **Hemostasis** is a coagulation process causing the formation of a blood clot when an injury occurs and then lysing of that blood clot when the injury has been repaired.

Primary hemostasis is the first step of the process. In this primary phase, the damaged vessels constrict to limit the blood flow to the injury. This **vasoconstriction** limits bleeding from the injury. Then the platelets clump in a process called **platelet aggregation**. These clumps then adhere to the injured area through the process of **platelet adhesion**. This is all that is needed to heal some injuries, such as a needle puncture of a vein. Primary hemostasis has traditionally been tested by the bleeding time test where a patient was cut with a small blade and the time to stop bleeding was determined. This was a very technique-dependent procedure that varies depending on how well trained the individual doing the testing was. As a result of the inaccuracy of this test, most health care facilities have discontinued the test. The preferred test is now the platelet function test that is performed using a blood sample rather than a time-dependent skin cut.

Secondary hemostasis is needed for more serious injuries and includes the formation of a fibrin clot. This involves a complex series of interactions in which one factor activates the next factor in a coagulation cascade. The factors are designated by Roman numerals. The coagulation cascade is divided into two separate intrinsic and extrinsic pathways that come together to form a common pathway (Figure 5.19). The intrinsic pathway begins with factor XII and is measured by the activated partial thromboplastin time (aPTT) test. The aPTT test is also used to monitor an individual's heparin therapy. The extrinsic pathway begins with factor VII and is measured by the prothrombin time (PT) test. The PT test is also used to monitor warfarin therapy.

In the common pathway, prothrombin is converted to thrombin, and fibrinogen then converts into fibrin to form a fibrin plug, which is also known as the hemostatic plug. Once the injury is repaired, **fibrinolysis** occurs to break down the fibrin clot into small fragments called **fibrin degradation products (FDPs)**.

This coagulation cascade is simplified in Figure 5.20. Blood tubes that contain an anticoagulant prevent the coagulation cascade from progressing to completion in the tube. If the required sample is a serum sample, then the tube that is used will allow the blood to clot and the coagulation cascade is completed.

This coagulation cascade is important in preventing collected blood from clotting in the tubes. Some tests require a whole blood or plasma sample and need to be drawn in a sample tube that is not allowed to clot. To prevent the blood from clotting, the tube contains a chemical anticoagulant. This anticoagulant works by stopping the clotting process by removing the calcium through the formation of calcium salts or by inhibiting the conversion of prothrombin to thrombin.

Both calcium and thrombin are part of the coagulation cascade. The coagulation cascade is like a staircase in which a ball is bounced down each step. The blood clots when the ball reaches the bottom of the stairs. If a step is taken away, the ball does not reach the bottom of the stairs and the blood does not clot. This staircase contains many steps. These steps consist of different chemicals and factors that are required for a person's blood to clot. A person with a bleeding disorder has one of the factors missing or is physically unable to use a needed chemical. A patient with this type of bleeding disorder has hemophilia. He or she can temporarily overcome this bleeding disorder by receiving an appropriate factor. The patient with hemophilia has one of the steps in the staircase missing. By giving the patient with hemophilia a factor such as factor VIII, the step is rebuilt and clotting occurs.

EXERCISE 2

Ordering/Sorting

Directions: Arrange the steps of the coagulation cascade in the correct order (1–6).

_____ Prothrombin to Thrombin

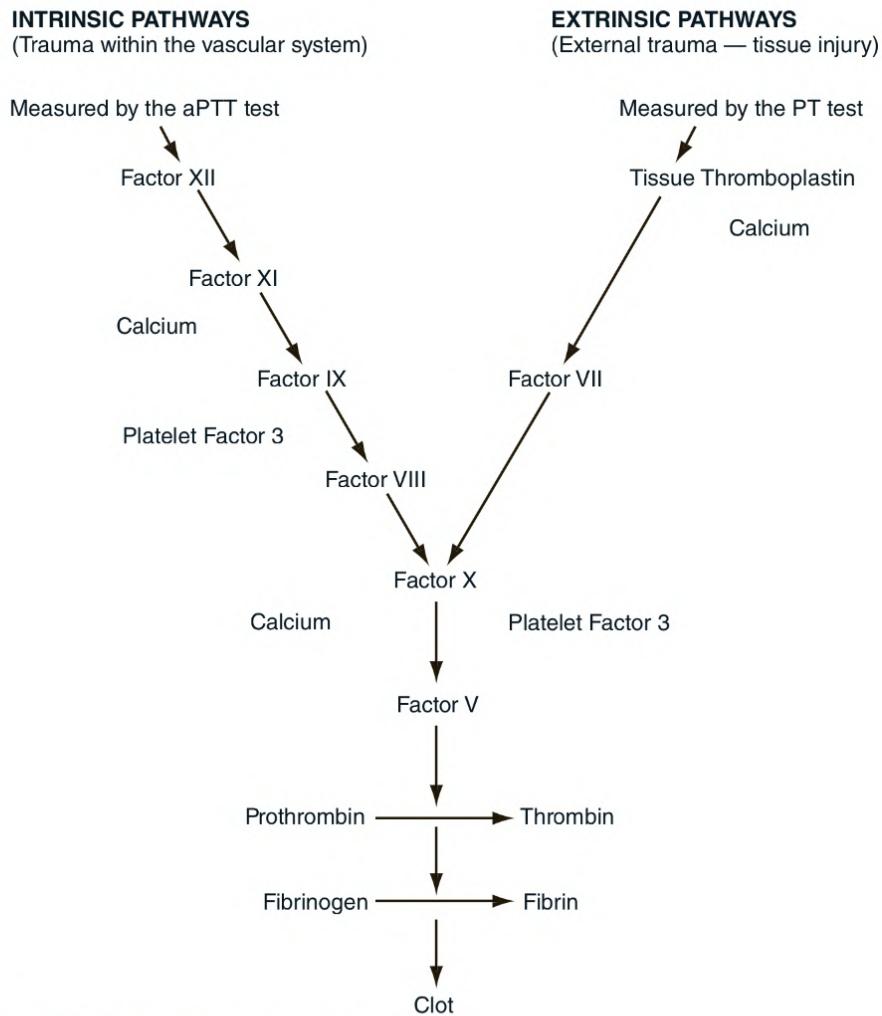
_____ Platelet Activation

_____ Factor Activation

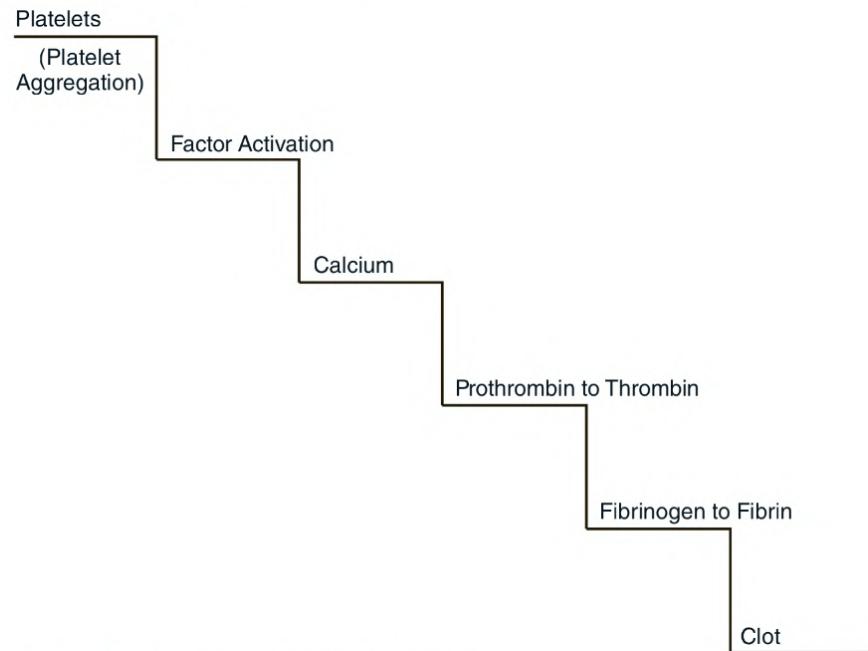
_____ Calcium

_____ Clot

_____ Fibrinogen to Fibrin



▲ FIGURE 5.19 Coagulation pathways.



▲ FIGURE 5.20 Simplified coagulation cascade.

ANTICOAGULANTS

Potassium Oxalate/Sodium Fluoride

Clotting of blood can be stopped in the test tube. A tube that has an anticoagulant removes one of the steps in the coagulation cascade, and the blood does not clot. The anticoagulant prevents clotting differently depending on the anticoagulant used. The common anticoagulants used consist of fluoride/oxalates, citrates, ethylenediaminetetraacetic acid (EDTA), and heparin.

Gray-stoppered tubes contain oxalates in combination with a weak antiglycolytic agent, sodium fluoride. The anticoagulant potassium oxalate works by precipitating out the calcium in the blood and therefore stopping the coagulation cascade. The sodium fluoride's primary function is not as an anticoagulant but as a glycolytic inhibitor. The fluoride preserves the glucose in the blood sample by inhibiting the enzymes involved in breakdown of the glucose (glycolysis). As a blood sample sits without the fluoride, the glucose is broken down at a rate of around 7 percent per hour. A blood sample that is drawn from a patient into a plain red-stoppered tube has nothing in that sample to preserve it or to keep it from clotting. As the sample sits, the glucose in the sample is used by the blood cells. The glucose molecules are broken down and the amount of glucose in the patient's sample decreases. The fluoride in the gray-stoppered tube prevents the glucose from being used by the cells.

A sample that is drawn from a patient often has a time delay before it is tested at the laboratory because the physician wants to monitor the glucose of that patient at the time the sample is drawn. If the glucose were allowed to decrease in the sample, the results at the time of testing would indicate that the patient had a glucose value less than actual. This patient could then be falsely treated for a low glucose. On the other hand, a patient who was running a high value might appear as normal. The way to prevent this is to draw samples to be tested for glucose in a gray-stoppered tube if there is to be a delay in testing.

Sodium Citrate

Light blue-stoppered tubes contain the anticoagulant sodium citrate. The sodium citrate prevents the coagulation by binding calcium in a nonionized form. The sodium citrate anticoagulant is in 3.8 percent sodium citrate (0.129 M) or 3.2 percent sodium citrate (0.105 M) concentrations. Tubes with 3.2 percent sodium citrate are the most widely used. The correct concentration of sodium citrate must be used to have consistent results for the patient. It is critical that the light-blue tubes drawn be filled to their proper level for accurate patient results. A ratio of 1 part of anticoagulant to 9 parts of blood must be maintained. If a tube is only half filled, the ratio will be off and the results will be invalid. The anticoagulant in the tube prevents the blood from clotting by binding the calcium. To test the blood for coagulation studies, the blood is centrifuged to produce a plasma sample. An aliquot of this plasma sample combines with other chemicals to restart the coagulation process and produce a clot. The time it takes for a visible clot to form is the result of the coagulation study. There are normal time ranges for this clotting to occur. A patient with coagulation problems or on anticoagulant therapy will fall outside these results. Three such tests using the light blue-stoppered tube to detect clotting problems are PT, aPTT, and fibrinogen assay. These tests give prolonged results if the tube is not filled to the proper level. These prolonged results will cause the physician to reduce the patient's medication when it was the inaccurate filling of the tube that changed the patient's results.

A citrate tube containing citrate, theophylline, adenosine, and dipyridamole (CTAD) is a light blue-stoppered tube that is used for platelet function assays and some routine coagulation determinations. Citrate is not only used in the light blue-stoppered tube. It is used extensively as the anticoagulant in routine blood donor bags. The citrate is in the form of citrate-phosphate-dextrose-adenine-1 (CPDA-1). This form of citrate both prevents the blood from clotting and preserves the viability of the erythrocytes. Weeks

after the blood is drawn from the donor, the erythrocytes are capable of transporting oxygen to the tissues. The anticoagulant is still in a 1:9 ratio with 63 mL of anticoagulant and approximately 450 mL of blood. Citrate is also found in a yellow-stoppered tube. This tube has as an additive called ACD. This additive is either solution A or solution B. The difference in the solutions depends on the concentration of trisodium citrate, citric acid, and dextrose solution. The tube has a variety of uses in testing in blood banks with tissue typing, DNA testing, and paternity testing.

Solutions A and B (ACD)

The alternate yellow-stoppered tubes are labeled as Solution A or Solution B. These two types of tubes contain variations of a mixture of trisodium citrate, citric acid, and dextrose. They are used for DNA and paternity testing, human leukocyte antigen (HLA) phenotyping, and some immunohematology studies.

Sodium Polyanethol Sulfonate (SPS)

A yellow-stoppered tube that does not contain citrate is used for collecting blood culture samples. This tube looks the same as the two previous tubes except it contains the additive sodium polyanethol sulfonate (SPS). The main function of the SPS tube is to allow bacteria to grow so they can be cultured. The SPS (1) inhibits the phagocytosis of the bacteria by the white blood cells (WBCs); (2) inhibits serum complement, which would destroy the bacteria; and (3) inhibits certain antibiotics in case a patient is already on an antibiotic. The yellow-stoppered tube can be confusing in that there are multiple types of yellow-stoppered tubes with specific uses. The phlebotomist must read the label before using one of these tubes to ensure that the proper tube with the proper additive is used.

EDTA

Helpful Hint

Heparinized plasma is often preferred over serum for testing a patient's potassium level. As blood clots the cells release potassium into the serum.

Lavender tubes contain the anticoagulant EDTA. This anticoagulant also binds the calcium to prevent coagulation. This EDTA is in the form of tripotassium EDTA or dipotassium EDTA. Tripotassium EDTA is used in glass tubes and is in the liquid form. Dipotassium EDTA is in plastic tubes in a spray-dried powder form. Dipotassium EDTA is now the anticoagulant for hematology recommended by the International Council for Standardization in Hematology (ICSH) and Clinical Laboratory Standards Institute (CLSI). Dipotassium EDTA is recommended because it preserves the cell morphology for complete blood cell counts (CBCs) and differential blood smears, and it provides stable microhematocrit results. Other anticoagulants distort the size and shape of the cells. This distortion often mimics the appearance of a disease process when it is actually the effects of the anticoagulant.

Heparin

Up to this point, all the anticoagulants discussed have prevented coagulation by precipitating or binding the calcium in the blood. The green tubes contain heparin, which stops the coagulation by inhibiting the conversion of prothrombin to thrombin and thus the following stages that lead to a clot. What has occurred is that no fibrin is formed to cause a clot. Heparin is a naturally occurring substance that is present in most of our tissues but at low levels. Thus it has the least effect of all the anticoagulants on clinical chemistry tests. It produces the least stress on erythrocytes and minimizes hemolysis.

Heparin is the anticoagulant of choice for pH determinations, electrolyte studies, and arterial blood gases. Heparin comes in three forms: lithium heparin, sodium heparin, and ammonium heparin. The lithium heparin and sodium heparin are the heparins found in evacuated tubes. Before drawing a test with a heparin tube, you must know what type of heparin is acceptable. Heparin is not acceptable for blood samples that may be stored for more than 48 hours before testing. After this time, heparinized blood will slowly begin to clot, forming small fibrin strands.

EDTA White-stoppered Tubes

White-stoppered tubes (also known as pearl top) contain EDTA as an anticoagulant and also a gel to separate the plasma from the cells. A sample for EDTA plasma can be

collected in this tube, and the tube can be centrifuged and then frozen without pouring off the plasma into a separate tube. This is especially helpful in working with human immunodeficiency virus (HIV)-positive patients. Processing the blood without the necessity of opening the tube provides greater safety for the phlebotomist. This tube is used for molecular diagnostic tests such as polymerase chain reaction (PCR) or branched DNA amplification techniques.

Trace Element Tubes

A royal blue-stoppered tube is used for trace element studies—that is, analysis of such trace elements as lead, zinc, arsenic, or copper. The patient contacts these elements through occupational or environmental exposure. Normal serum or plasma blood tubes cannot be used. In the normal manufacture of the glass and rubber contained within the tubes, the trace elements are present. If blood was drawn into normal tubes, the trace elements would leach out of the glass and rubber stopper to falsely elevate the results. The trace element tubes use specially refined glass, plastic, and rubber to avoid this. The royal blue tube comes in three varieties. One contains no anticoagulant and produces a clot sample; the other two have sodium heparin or disodium EDTA as an anticoagulant. The tube will have a green or lavender label on it to designate the anticoagulant in the tube.

All tubes that have any type of additive must be gently inverted immediately after collection. The additives do not mix with the blood as the blood enters the tube. The mixing will be complete only with five to eight gentle inversions of the tube. The tube should never be shaken. Vigorous mixing will cause hemolysis of the red blood cells. The actions of the anticoagulants and additives discussed are shown in Table 5.1.

Anticoagulant tubes that are partially filled should never be poured together to obtain a full tube. This will cause an increased amount of anticoagulant to blood in that tube. This can potentially cause erroneous test results for the patient.

Helpful Hint

Coagulation tubes must always be full to be acceptable. Check with your supervisor to determine if other types of partially filled anticoagulant tubes will be acceptable for testing.

TABLE 5.1 Actions of Anticoagulants and Additives

| Additive or Anticoagulant | Action |
|------------------------------------|--|
| Potassium oxalate | Precipitates calcium |
| Sodium fluoride | Inhibits glycolysis |
| Sodium citrate | Binds calcium |
| Sodium polyanethol sulfonate (SPS) | Binds calcium and allows bacteria to grow for culture |
| Solutions A and B (ACD) | Binds calcium |
| EDTA | Binds calcium |
| Lithium heparin | Inhibits prothrombin to thrombin |
| Sodium heparin | Inhibits prothrombin to thrombin |
| Ammonium heparin | Inhibits prothrombin to thrombin |
| Silica/glass particles | Accelerates clotting |
| Thrombin | Accelerates clotting |
| Silicon | Promotes faster centrifugation and a sample with fewer cells |
| No additive | Natural formed clot |

| Red Top | | | Light-Green Top | | |
|------------------------------------|----------------------|---|---------------------|----------------------|---|
| | Contains: | None | | Contains: | Plasma separating tube (Na heparin) |
| | Effects on Specimen: | Blood clots, and the serum is separated by centrifugation | | Effects on Specimen: | Anticoagulants with lithium heparin: plasma is separated with PST gel at the bottom of the tube |
| | Uses: | Chemistries, immunology and serology, blood bank (crossmatch) | | Uses: | Chemistries |
| Red-Gray Mottled Top ("Tiger top") | | | Lavender/Purple Top | | |
| | Contains: | Serum separating tube (SST) with clot activator | | Contains: | EDTA (liquid form) |
| | Effects on Specimen: | Forms clot quickly and separates the serum with SST gel at the bottom of the tube | | Effects on Specimen: | Forms calcium salts to remove calcium |
| | Uses: | Blood type screening and chemistries | | Uses: | Hematology (CBC) and blood bank (crossmatch); requires a full draw—invert 8 times to prevent clotting and platelet clumping |
| Gold Top | | | Light-Blue Top | | |
| | Contains: | Separating gel and clot activator | | Contains: | Sodium citrate (Na citrate) |
| | Effects on Specimen: | Serum separator tube (SST) contains a gel at the bottom to separate the blood from serum on centrifugation | | Effects on Specimen: | Forms calcium salts to remove calcium |
| | Uses: | Serology, endocrine, immunology, including HIV | | Uses: | Coagulation tests (PT, PTT, TCT), tube must be filled 100% |
| Dark-Green Top | | | Yellow Top | | |
| | Contains: | Sodium heparin or lithium heparin | | Contains: | ACD (acid-citrate-dextrose) or sodium polyanethol sulfonate (SPS) |
| | Effects on Specimen: | Inactivates thrombin and thromboplastin | | Effects on Specimen: | Complement inactivation |
| | Uses: | Ammonia, lactate, HLA typing For lithium level, use sodium heparin For ammonia level, use sodium or lithium heparin | | Uses: | Paternity testing, DNA studies |
| Dark-Blue/Royal-Blue Top | | | Tan/Brown Top | | |
| | Contains: | Sodium heparin or Na ₂ EDTA | | Contains: | Dipotassium EDTA |
| | Effects on Specimen: | Forms calcium salts Tube is designed to contain no contaminating metals | | Effects on Specimen: | Inactivates thrombin and thromboplastin |
| | Uses: | Toxicology and trace element testing (zinc, copper, lead, mercury) and drug level testing | | Uses: | Serum lead determination |
| Light Gray Top | | | Black Top | | |
| | Contains: | Sodium fluoride and potassium oxalate | | Contains: | Sodium citrate (buffered) |
| | Effects on Specimen: | Antiglycolytic agent preserves glucose up to 5 days | | Effects on Specimen: | Forms calcium salts to remove calcium |
| | Uses: | For lithium level, use sodium heparin Glucoses requires a full draw (may cause hemolysis if short draw) | | Uses: | Westergren sedimentation rate; requires a full draw |
| Orange Top | | | | | |
| | Contains: | Thrombin | | | |
| | Effects on Specimen: | Quickly clots blood | | | |
| | Uses: | STAT serum chemistries | | | |

▲ FIGURE 5.21 Collection tubes and their additives for phlebotomy.

Most tube manufacturers have tube charts available that list the tubes, color of stopper, additive, and laboratory use of the tube. These charts are helpful to post in the phlebotomy area for a quick reference if there are questions. A summary of these charts is shown in Figure 5.21.

EXERCISE 3**Matching/Identification**

Directions: Match the additive or anticoagulant with the action.

1. _____ Potassium oxalate
2. _____ Sodium fluoride
3. _____ Sodium citrate
4. _____ Sodium polyanethol sulfonate (SPS)
5. _____ Solutions A and B (ACD)
6. _____ EDTA
7. _____ Lithium heparin
8. _____ Sodium heparin
9. _____ Ammonium heparin
10. _____ Silica
11. _____ Thrombin
12. _____ Silicon
13. _____ No additive

Actions

Answers may be used more than once

- A. Precipitates/binds calcium
- B. Accelerates clotting
- C. Inhibits prothrombin to thrombin
- D. Allows bacteria to grow for culture
- E. Promotes faster centrifugation
- F. Naturally formed clot
- G. Inhibits glycolysis

TOURNIQUETS

The **tourniquet** constricts the flow of blood in the arm and makes the veins more prominent. The most commonly used tourniquet is a soft, pliable, flat strip approximately 1 inch wide by 15 to 18 inches long. This strip serves as a universal tourniquet for all conditions. Velcro strips and round rubber tubing are no longer acceptable. The flat strip is the most widely used because it can easily be released with one hand. Being about 1 inch wide, it does not cut into the patient's arm but distributes the pressure. On a patient who is obese the flat strip tourniquet will have the tendency to roll into a tube and cut into the patient's arm. Placing the tourniquet over the patient's sleeve or using a blood pressure cuff will remedy this problem.

Tourniquets should be of a nonlatex material (Figure 5.22). The nonlatex tourniquet has become the tourniquet of choice to avoid latex exposure to both the patient and the



▲ FIGURE 5.22 Nonlatex tourniquet.

phlebotomist. Many health care facilities do not stock latex tourniquets due to the concern for latex allergy. The nonlatex tourniquet must be used on patients who have latex allergies so that there will be no reaction for the patient. Latex items should never be stored with nonlatex tourniquets because the latex particles will be transferred to the nonlatex tourniquet and a patient reaction will occur.

Helpful Hint

Check with your supervisor as to what is appropriate reuse of tourniquets.

Reuse of the tourniquet is not a safety issue for the phlebotomist but is a contamination concern for the patient. In an inpatient setting, one tourniquet can be left in the patient's room and continually used on that patient until he or she is discharged. At that time a fresh tourniquet can be placed in the room for the next patient. The concern is in the outpatient setting. Should one tourniquet be reused throughout the day and used on multiple patients? Or should a new tourniquet be used with each patient? Additional cost is the limiting factor. Even with the small cost of a tourniquet, it adds to the total cost of doing the test. There are no clear standards, and directives from OSHA have not addressed this concern. The issue is interpreted differently in each institution.

There are limits to the reuse of a tourniquet. After multiple patients, the tourniquet loses its ability to stretch or will tear. Tying a tight tourniquet is extremely important for the phlebotomist to help locate a vein. If the tourniquet is not in good condition, then the ability to locate a vein will be compromised. A soiled tourniquet must be discarded and should not be cleaned. If the tourniquet is coated with blood, it must be discarded in a biohazard container.

The tourniquet is applied 3 to 4 inches above the puncture site. It is applied tight enough to slow the flow of blood in the veins but not prevent the flow of blood in the arteries. The patient should then close his or her hand to make the veins more prominent. Pumping the hand should be avoided because this can change the results of some tests. Potassium is especially affected by the pumping action. Pumping of the hand releases potassium into the bloodstream from the tissue and red blood cells, therefore elevating the potassium in the blood sample. The action of tying a tourniquet is very similar to damming a small stream. With the tourniquet applied, the arteries fill the veins with blood, pooling the blood in the veins. This pooling of blood makes the veins more prominent. The phlebotomist can then **palpate** the veins to determine their direction, depth, and size. Palpating for a vein is one of the more difficult skills a phlebotomist learns. The phlebotomist uses the same finger of the nondominant hand to feel for the vein.

Helpful Hint

Using the thumb to palpate for a vein will be less successful. The thumb is not as sensitive as the index finger.

Palpating involves using the finger to press down on top of the vein to feel for a bounce or running the finger across the arm to locate a vein as the finger runs over the "speed bump" of the vein. Often a combination of the two methods must be used to properly locate a vein. The finger is moved across the arm until a slight bump is felt, and then the phlebotomist presses down on this location to determine if there is a bounce to the vein. After the bounce is felt, direction of the vein can be determined to know the position the needle will need to be in to enter the vein.

The phlebotomist must use the same finger all the time to palpate for a vein. The finger of choice is the index finger. By using the same finger all the time, the phlebotomist builds sensitivity in that finger. Palpating with the nondominant hand is recommended. Developing a sensitivity with the nondominant hand allows the phlebotomist to be able to palpate if he or she misses and needs to redirect the needle.

The tourniquet should be on the arm no longer than 1 minute. A stream will become stagnant when it no longer flows. A tourniquet that is left on too long will cause **hemoconcentration** of the blood and increased concentration of constituents in the blood sample. The hemoconcentration will disrupt the balance of fluid in the tissue and cause potassium to be released.

A blood pressure cuff (sphygmomanometer) can be used as an alternative to a tourniquet. The wider blood pressure cuff applies pressure over a wider area than a tourniquet. This sometimes makes veins more prominent. The blood pressure cuff needs to be inflated

halfway between the diastolic and systolic reading. It should be used only when the vein is not prominent with a tourniquet. It is used frequently with obese, pediatric, or geriatric patients. The blood pressure cuff can cause greater discomfort for patients and can irritate the skin more. It is the decision of the phlebotomist as to what type of tourniquet will work best and provide the least discomfort to the patient.

MICROCOLLECTION EQUIPMENT

Blood collections sometimes require capillary puncture. For this type of collection, special microcollection equipment is needed. This equipment varies in how it is used but always makes either a puncture or a cut into the skin and through the capillary bed. The equipment used to collect the blood depends on the test being performed. The equipment consists of two parts: a method of puncturing the finger or heel and a method to collect the sample.

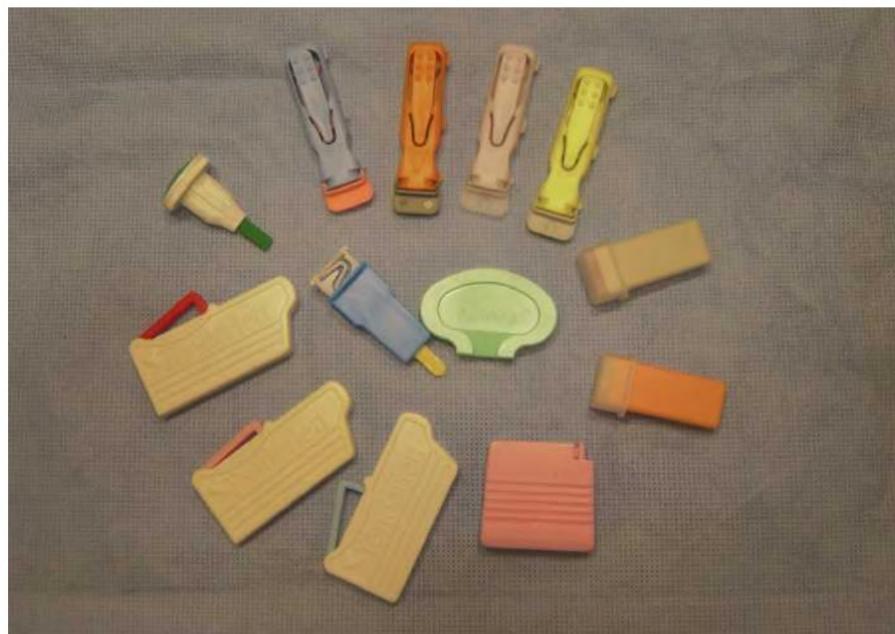
A number of different capillary puncture lancets are commercially available to puncture the skin for an adequate blood flow. Surgical blades used in the past carried the hazard of puncturing too deeply, especially in the newborn needing a heelstick or in the pediatric patient. Nonretractable lancets replaced the surgical blades and were designed for controlled depth of puncture. They consisted of a blade or needle that the phlebotomist used to manually puncture the skin. The basic handheld nonretractable blade lancets (Figure 5.23) are not used because of several disadvantages. The lancet created a hazard in that once it was used on a patient, the blade was still exposed. Because the lancet was not designed to retract into a holder, as in the newer style of lancets, the potential for an accidental puncture existed until the device was safely disposed of in a sharps container.

The nonretractable blade lancet had a stop point so the phlebotomist did not stick too deep. The tendency was to be sympathetic with the patient and not stick hard enough. If this occurred, adequate blood flow was not obtained, and the patient had to be punctured again. Another disadvantage was that the patient could see the blade coming and became more apprehensive of the stick. Patients became so apprehensive that they would pull their hand out of the phlebotomist's grasp just as the phlebotomist was ready to stick. In a case like this, the phlebotomist would possibly stick himself or herself.

The retractable puncture device is the device now used for all capillary punctures (Figure 5.24). Multiple brands are available and are too numerous to describe in this textbook. There are two types of retractable puncture devices: one type automatically punctures the



▲ FIGURE 5.23 Nonspring-loaded lancets.



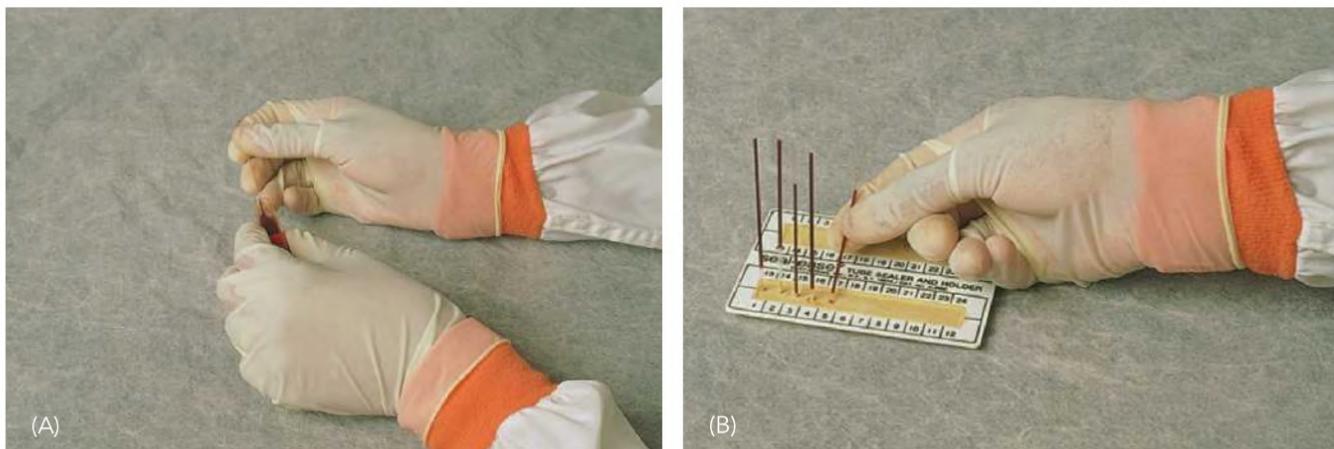
▲ FIGURE 5.24 Spring-loaded puncture devices.

patient as the device is held on the patient's skin (contact-activated device), and the other type is placed on the skin surface and the phlebotomist pushes the blade into the skin. With either device the blade then retracts after the puncture to prevent the phlebotomist from receiving an accidental puncture. These devices hide the blade in a plastic holder so the patient cannot see the blade during the puncture. The plastic device lies on the skin, and the blade punctures the skin. The blade then retracts. The rapid puncture and the invisible blade make the patient less apprehensive about skin punctures. The spring-loaded blades work by a guillotine action, or a slicing motion. Devices can be purchased that puncture no more than 0.85 mm for premature infants to 2 mm for full-term newborn heelsticks. Other devices vary in depth for fingersticks of different-age patients. What should be avoided when collecting samples for laboratory samples are the devices on the market for diabetic at-home blood glucose monitoring. These devices are excellent for their purpose but do not provide more than one or two drops of blood. This is not adequate blood flow for the sample size needed for most laboratory testing.

The devices used for collecting, processing, and transporting microcollections depend on the laboratory testing being performed. This discussion of devices here is not exhaustive. As the phlebotomist works in different laboratories, some specialized devices may also be used. Microcollection equipment is intended for one-time use, to be disposed of in a sharps container.

Disposable calibrated micropipettes are thin-bore devices much like small soda straws that draw the blood up to a certain line. The line indicates the calibration for an amount drawn from 1 to 200 microliters. They are generally used for measurement of an amount of blood and then transferred to a container or solution. The blood draws up into the tube because of **capillary action**. Capillary action is the adhesive molecular force between liquid and solid materials that draws liquid into narrow-bore capillary tubes. The capillary action of a tube is enhanced if the tube is slanted in a semihorizontal direction as the blood is being drawn into the tube.

Glass was the standard material for micropipettes, but plastic or a plastic coating over glass is now mandated by OSHA. The glass has a tendency to break and cut the phlebotomist, exposing the phlebotomist to the patient's blood. The plastic or plastic-coated micropipettes reduce the possibility of an accidental exposure.



▲ FIGURE 5.25 (A) Filling a microhematocrit tube with blood by capillary action. (B) Sealing the microhematocrit tube with sealing clay.

Similar to calibrated micropipettes are microhematocrit capillary tubes. These tubes are narrow-bore pipettes primarily intended for determining packed red cell volume in microsamples. Once filled, the tubes are sealed and then are processed in a special centrifuge that packs the formed elements of the blood. This packed red cell volume is then read on a scale that gives the packed volume as a percentage of the total. The result is the hematocrit of the patient. Because the volume is read on a special sliding scale, specific total volume in the tube is not important. The tube must be filled at least two-thirds full for accurate results. This volume required for the tube is between 50 and 75 microliters (Figure 5.25).

Blood gas collection pipettes collect skin puncture whole blood samples under anaerobic conditions for blood gas determinations. The tubes vary in size but draw 50 to 250 microliters of volume. The amount of draw needed depends on the instrument used in the blood gas testing. The tubes contain heparin to keep the blood from clotting, and generally a small magnetic stirrer, known as a **flea**, slides into the tube to maintain a mixed sample. The tubes are plugged with sealant putty or caps to maintain anaerobic conditions. Caraway or Natelson pipettes are general-purpose microcollection tubes that have a tapered end and are supplied with and without anticoagulant. They contain no markings to specify a volume and are used for the collection of skin puncture blood and the transfer to another container.

The process of collection and transfer of blood samples has been simplified with the use of noncapillary plastic microcollection devices. These devices consist of small nonsterile plastic tubes. They include a means for filling, measuring, color coding for the proper anticoagulant, stoppering, centrifugation, and storage. The color coding matches the coding on the anticoagulant tubes: lavender is EDTA, green is heparin, and red is a serum sample. The serum tubes can contain the thixotropic separator gel that separates the serum from the cells after centrifugation. Samples for bilirubin are collected in an amber tube that protects the blood from light. If a bilirubin sample is not protected from light, the bilirubin level of the blood in the tube will rapidly decrease. The key to accurate test results after the collection of all microcollection samples is a free-flowing sample. As the drop of blood forms, it is touched by the collection cap, which consists of a scoop or tubing device in the cap. The blood then flows into the bottom of the tube. The tubes hold approximately 600 microliters of blood. The tubes go by a variety of brand names, such as Microtainer and Microvette (Figure 5.26).

Patients with diabetes often do their own puncturing and collection. The patient collects a sample on a reagent strip pad, which is read by a handheld glucose meter (Figure 5.27).



▲ FIGURE 5.26 Microcollection tubes.



▲ FIGURE 5.27 Various handheld glucose analyzers are now available for home or office use.

The instrumentation for home use continues to expand. Several meters are also available to test a patient's coagulation.

The last microcollection device we discuss is blood collection on filter paper for neonatal screening programs (Figure 5.28). Neonatal screening identifies genetic defects. The newborn is punctured in the heel, and the blood is dropped onto a filter paper card. The blood is allowed to dry, and the blood-saturated filter paper is sent to the laboratory for testing. The genetic disorders the infant is tested for are phenylketonuria, galactosemia, hypothyroidism, homocystinuria, maple sugar urine disease, sickle cell disease /hemoglobinopathies, and many others. With early identification of these diseases, treatment lessens the effects of disorder and often completely prevents it.

The United States and many other countries require that newborns receive neonatal screening for metabolic disorders. The components of the required newborn screening vary from state to state. There are more than 30 different components for which a newborn's blood can be screened. Private laboratories will do testing that a state testing program does not do if the parents wish to pay to have the testing completed.

S & S® NO. 903M LOT # W-921

COMPLETELY FILL ALL CIRCLES WITH BLOOD

| | | | | | | | | | |
|---|--|---|--|--|--|---|--|---|--|
| Infant's Full Name LAST / FIRST | | 1st Protein Feed Date / / | | Lacto Formula □ Breast □ Soy Formula □ NPO □ Hyperal | | Infant's Hosp. No. Collection Date / / | | Sample # / / | |
| Birthdate / / | | Hour / | | Mother's D.O.B. / / | | Hour / | | Sex / | |
| Hour / | | Mother's D.O.B. / / | | Mother's Phone / | | Mother's Phone / | | <input type="checkbox"/> Boy <input type="checkbox"/> Girl | |
| Mother's Full Name Last / First | | Initial | | | | | | | |
| Address Number / Street | | City / State / Zip | | | | | | | |
| Other's Name | | Phone | | | | | | | |
| Hospital Number / Street | | Birth (if Different) | | | | | | | |
| Clinician Last: First | | Initial | | Phone | | | | | |
| Address Number / Street | | City / State / Zip | | | | | | | |
| BIRTHWEIGHT: <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> gms | | RACE: BLACK <input type="checkbox"/> STATUS: 1. SICK/PREMATURE <input type="checkbox"/> WHITE <input type="checkbox"/> DATE OF LAST <input type="checkbox"/> (GEST AGE <input type="checkbox"/> weeks) HISPANIC <input type="checkbox"/> TRANSFUSION: 2. ON ANTIBIOTICS <input type="checkbox"/> ASIAN <input type="checkbox"/> OTHER <input type="checkbox"/> 3. TRANSFUSED <input type="checkbox"/> OTHER <input type="checkbox"/> 4. NORMAL <input type="checkbox"/> | | | | | | | |
| BLOOD DRAWER ID | | PREVIOUS REQUISITION # T 1st test - less than 48 hrs. E 1st test - greater than 48 hrs. S 2nd test PREV. ABN. T PREV. QRS | | | | | | T ₄ = MCG/DL | |
| | | | | | | | | * F 3 6 8 0 7 * | |
| | | | | | | | | 1. DS CO 14 | |
| | | | | | | | | | |

▲ FIGURE 5.28 Newborn screen card.

EXERCISE 4 **Fill in the Blanks**
Microcollection

The device used to puncture the finger or heel is called a _____.

A name given to the type of small tube in which the blood is collected after a finger-stick or a heelstick is a _____.

The blood test that is collected on filter paper is known as _____.

SAMPLE COLLECTION TRAYS OR CARTS

Helpful Hint

Remember that the phlebotomy tray is taken from one patient room to another. Never place the tray on an area that the patients will touch and transfer any contaminants to themselves. Place a clean towel on the bedside table first and then set the tray on the towel. As you leave, remove the towel and the table will not be contaminated.

The phlebotomist needs a sample collection tray or cart to hold all the equipment necessary for proper sample collection. This tray or cart will be taken to the patient's room so the phlebotomist is prepared for whatever procedure is performed. Supplies included vary depending on the type of collections usually done or the type of hospital or laboratory where the phlebotomist works. In some cases, a tray will not be adequate, and the phlebotomist will need to have a stocked cart to roll from room to room. The tray is usually preferred because it is more portable and can be taken upstairs to avoid a long wait at the elevator. The trays come in a variety of sizes and shapes to better fit the phlebotomist's preference and needs (Figure 5.29). Since blood samples are carried on the tray, it will need to have the biohazard symbol to meet OSHA blood-borne standard precautions (refer to Figure 2.10).

The phlebotomist's tray should include at least the following:

1. Alcohol swabs
2. Gauze squares or cotton balls
3. Evacuated tube holders
4. Assorted evacuated tubes
5. Syringes
6. Syringe transfer device
7. Various-size syringe and evacuated tube needles
8. Butterfly collection sets
9. Microcollection equipment
10. Tourniquets
11. Disposable gloves
12. Sharps container
13. Marking pen



▲ FIGURE 5.29 Stocked phlebotomy tray.

It is the responsibility of the phlebotomist to keep the tray stocked and clean. Before taking the tray, all items should be checked to see if anything is expired and to ensure that there is adequate supply for the number of patients from whom the phlebotomist will collect blood. The orders for the patients should be checked before leaving with the tray to ensure that there is no special testing that would require a tube or equipment that is usually not carried on the tray. At least once per week the tray should be completely dismantled and thoroughly cleaned.

EXERCISE 5**Requisition Exercise**

Hometown Hospital USA
125 Goodcare Avenue
Small town, USA

Laboratory Requisition

| | | |
|-------------------------------------|-------------------------|------------------------------------|
| Clinic or room number: <u>1525</u> | Sample Collection | Patient Name: <u>Sam Samuelson</u> |
| Physician Name: <u>Joe Resident</u> | Date: <u>xx/xx/yyyy</u> | MRN: <u>12345678</u> |
| Diagnosis code: <u>748.16</u> | Time: _____ | DOB: <u>8/25/1965</u> |
| | Initials: _____ | Sex: <u>M</u> |

Special Instructions:

| Chemistry | Hematology | Microbiology |
|---|--|---|
| <input checked="" type="checkbox"/> Basic Metabolic Panel | <input type="checkbox"/> CBC without diff | <input type="checkbox"/> Culture, urine |
| <input type="checkbox"/> Electrolyte Panel | <input type="checkbox"/> CBC with diff | <input type="checkbox"/> Culture, throat |
| <input type="checkbox"/> Hepatic Function Panel | <input type="checkbox"/> Reticulocyte count | <input type="checkbox"/> Culture, stool |
| <input type="checkbox"/> Comprehensive Panel | <input type="checkbox"/> Sedimentation rate, ESR | <input type="checkbox"/> Ova and Parasite, stool |
| <input type="checkbox"/> Ethanol | <input type="checkbox"/> Sickle cell screen | |
| <input type="checkbox"/> Acetaminophen | <input checked="" type="checkbox"/> Protime, PT | Serology |
| <input type="checkbox"/> Salicylate | <input type="checkbox"/> APTT | <input type="checkbox"/> Syphilis RPR screen |
| <input type="checkbox"/> Glucose, Fasting | | <input type="checkbox"/> Mononucleosis screen |
| <input type="checkbox"/> Glucose Tol. _____ hr | Urine | <input type="checkbox"/> ANA screen |
| <input checked="" type="checkbox"/> Hgb A1C | <input type="checkbox"/> Urinalysis | <input type="checkbox"/> Rheumatoid factor screen |
| | <input type="checkbox"/> Urine protein | |
| Other Tests | <input type="checkbox"/> UUN, urea nitrogen | Blood Bank |
| | <input type="checkbox"/> urine electrolytes | <input type="checkbox"/> ABO |
| | <input type="checkbox"/> Creat. Clearance (24 hr.) | <input type="checkbox"/> Rh |
| | <input type="checkbox"/> Urine TOX screen | <input type="checkbox"/> Antibody Screen |
| | | <input type="checkbox"/> Type and Crossmatch |

What tubes will the phlebotomist collect for the tests listed on the requisition?

What will be the order of draw for these tubes?

REVIEW QUESTIONS

Multiple Choice

Choose the one best answer.

1. 10 milliliters (mL) is the same as
 - a. 1 liter.
 - b. 10 deciliters.
 - c. 10 cubic centimeters.
 - d. none of the above.
2. Which needle has the smallest bore?
 - a. 16-gauge needle
 - b. 20-gauge needle
 - c. 21-gauge needle
 - d. 23-gauge needle
3. A short draw with a sodium citrate tube will
 - a. lead to a prolonged aPTT and prolonged PT.
 - b. lead to a shortened aPTT and shortened PT.
 - c. lead to a prolonged aPTT and shortened PT.
 - d. have no effect on the aPTT or the PT.
4. Which of the following anticoagulants prevents coagulation of the blood by removing calcium through the formation of insoluble calcium salts?
 - a. EDTA
 - b. oxalate
 - c. sodium citrate
 - d. heparin
 - e. all of the above
 - f. a, b, and c
5. An anticoagulant is an additive placed in evacuated tubes in order to
 - a. dilute the blood prior to testing.
 - b. ensure the sterility of the tube.
 - c. make the blood clot faster.
 - d. prevent the blood from clotting.
6. A green-stoppered evacuated tube contains what kind of anticoagulant?
 - a. citrate
 - b. fluoride
 - c. heparin
 - d. no additive
7. When serum is needed for testing, blood must be collected in which of the following colored tubes?
 - a. light blue
 - b. green
 - c. lavender
 - d. red
8. Unsterile tubes may
 - a. cause false-positive blood cultures.
 - b. infect the patient through backflow.
 - c. cause short draws.
 - d. both a and b
9. The tourniquet should be applied how many inches above the proposed venipuncture site?
 - a. 1 to 2 inches
 - b. 3 to 4 inches
 - c. 4 to 5 inches
 - d. 5 to 6 inches
10. Leaving the tourniquet on a patient's arm for an extended length of time before drawing blood may cause
 - a. hemoconcentration.
 - b. sample hemolysis.
 - c. stress.
 - d. bruising.

CRITICAL THINKING

1. Explain the method used to prevent blood in the anticoagulant tubes from clotting.
2. How would a PT result be affected if the tube were only half full?

3. A physician calls and says that all the blood glucose results for his patients—from whom blood is drawn in his office and then sent to the laboratory—show low blood sugar/glucose. The physician states that there must be a problem with the laboratory instrument. What would you investigate to resolve his problem?
4. You did a fingerstick on a patient and cannot seem to get enough blood. This patient is a construction worker with large, callused hands. What could be the problem?