The primary duty of the phlebotomist is to collect blood specimens for laboratory testing. Blood is collected by several methods, including arterial puncture, capillary puncture, and venipuncture. This chapter describes general blood collection equipment and supplies commonly needed regardless of the method of collection, equipment specific to venipuncture, additives used in blood collection, and the order of draw for collecting or filling blood specimen tubes. A phlebotomist must be familiar with all the types of equipment in order to select appropriate collection devices for the type and condition of the patient’s vein and the type and amount of specimen required for the test. Choosing the appropriate tools and using them correctly helps assure the safe collection of high-quality blood specimens. (Equipment specific to capillary puncture and arterial puncture is described in Chapters 10 and 14, respectively.)

General Blood Collection Equipment and Supplies

The following paragraphs describe the equipment and supplies commonly needed for all methods of collecting blood specimens.

**BLOOD-DRAWING STATION**

A blood-drawing station is a dedicated area of a medical laboratory or clinic equipped for performing phlebotomy procedures on patients, primarily outpatients sent by their physicians for laboratory testing. A typical blood-drawing station includes a table for supplies, a special chair where the patient sits during the blood collection procedure, and a bed or reclining chair for patients with a history of fainting, persons donating blood, and other special situations. A bed or padded table is also needed if heelsticks or other procedures will be performed on infants and small children.

**PHLEBOTOMY CHAIRS**

A phlebotomy chair should be comfortable for the patient and have adjustable armrests to achieve proper positioning of either arm. Special phlebotomy chairs (Fig. 7-1) are available from a number of manufacturers. Most have adjustable armrests that lock in place to prevent the patient from falling should fainting occur.

*Figure 7-1 A phlebotomy chair. (Courtesy Lab Conco, Kansas City, MO.)*
CAUTION: In the absence of a special chair, precautions must be taken to prevent falls and ensure client safety.

EQUIPMENT CARRIERS

Equipment carriers make blood collection equipment portable. This is especially important in a hospital setting and other instances in which the patient cannot come to the laboratory.

Handheld Carriers

Handheld phlebotomy equipment carriers or trays (Fig. 7-2) come in a variety of styles and sizes designed to be easily carried by the phlebotomist and to contain enough equipment for numerous blood draws. They are convenient for “stat” or emergency situations or when relatively few patients need blood work.

Phlebotomy Carts

Phlebotomy carts (Fig. 7-3) are typically made of stainless steel or strong synthetic material. They have swivel wheels, which glide the carts smoothly and quietly down hospital hallways and in and out of elevators. They normally have several shelves to carry adequate supplies for obtaining blood specimens from many patients. Carts are commonly used for early-morning
hospital phlebotomy rounds, when many patients need lab work, and for scheduled “sweeps” (rounds that occur at regular intervals throughout the day). Carts are bulky and a potential source of nosocomial infection; they are not normally brought into patients’ rooms. Instead, they are parked outside in the hallway. A tray of supplies to be taken into the room is often carried on the cart.

**KEY POINT** Keeping carts and trays adequately stocked with supplies is an important duty of the phlebotomist.

### GLOVES AND GLOVE LINERS

The Centers for Disease Control/Healthcare Infection Control Practices Advisory Committee (CDC/HICPAC) standard precautions and the Occupational Safety and Health Administration (OSHA) Bloodborne Pathogen Standard require the wearing of gloves when performing phlebotomy. A new pair must be used for each patient and be removed when the procedure is completed. Nonsterile, disposable latex, nitrile, neoprene, polyethylene, and vinyl examination gloves are acceptable for most phlebotomy procedures. A good fit is essential. Special glove liners (Fig. 7-4A) are available for persons who develop allergies or dermatitis from wearing gloves. Barrier hand creams (Fig. 7-4B) that help prevent skin irritation and are compatible with most gloves are also available. Gloves with powder are not recommended, as the powder can be a source of contamination for some tests (especially those collected by skin puncture) and can also cause allergies in some users. In addition, powder in latex gloves can help suspend most types of particles in the air and thus pose a danger to those with latex allergy. The U.S. Food and Drug Administration (FDA) regulates glove quality.

**KEY POINT** Decontamination of hands after glove removal is essential. Any type of glove may contain defects, and some studies suggest that vinyl gloves may not provide an adequate barrier to viruses.

### ANTISEPTICS

Antiseptics are substances used to prevent sepsis, which is the presence of microorganisms or their toxic products within the bloodstream. Antiseptics prevent or inhibit the growth and development of microorganisms but do not necessarily kill them. They are considered safe to use on human skin and are used to clean the site prior to blood collection. The antiseptic most commonly used for routine blood collection is 70% isopropyl alcohol (isopropanol) in

![Figure 7-4](https://example.com/figure7-4.png) A. UltraFIT glove liners. B. SoftGUARD barrier hand cream. (Courtesy Erie Scientific Co., Portsmouth, NH.)
individually wrapped prep pads (Fig. 7-5). For a higher degree of antisepsis, the traditional antiseptic has been povidone–iodine in the form of swab sticks or sponge pads for blood culture collection and prep pads for blood gas collection. However, the use of alcohol-based preparations for these procedures is increasing because many patients are allergic to povidone–iodine. A list of antiseptics used in blood collection is shown in Box 7-1.

**KEY POINT** Cleaning with an antiseptic reduces the number of microorganisms but does not sterilize the site.

**DISINFECTANTS**

Disinfectants are chemical substances or solutions regulated by the Environmental Protection Agency (EPA) that are used to remove or kill microorganisms on surfaces and instruments. They are typically corrosive and are not safe to use on human skin. According to the CDC and HICPAC Guidelines for Environmental Infection Control in Healthcare Facilities, use of EPA-registered sodium hypochlorite products is preferred, but solutions made from generic 5.25% sodium hypochlorite (household bleach) may be used. A 1:100 dilution is recommended for decontaminating nonporous surfaces after cleaning up blood or other body fluid spills in patient-care settings. When spills involve large amounts of blood or other body fluids or occur in the laboratory, a 1:10 dilution is applied prior to cleanup. At least 10 minutes of contact time is required for disinfectants to be effective.

**KEY POINT** Fresh bleach solutions should be made daily or as needed.

**HAND SANITIZERS**

The CDC Guideline for Hand Hygiene in Health Care Settings recommends the use of alcohol-based hand sanitizers for routine decontamination of hands as a substitute for hand washing provided that the hands are not visibly soiled. If hands are heavily contaminated with

**BOX 7-1** ANTISEPTICS USED IN BLOOD COLLECTION

- 70% Ethyl alcohol
- 70% Isopropyl alcohol (isopropanol)
- Benzalkonium chloride (e.g., Zephiran chloride)
- Chlorhexidine gluconate
- Hydrogen peroxide
- Povidone–iodine (0.1%–1% available iodine)
- Tincture of iodine
organic material and hand-washing facilities are not available, it is recommended that hands be cleaned with detergent-containing wipes followed by the use of an alcohol-based hand cleanser. Alcohol-based hand cleansers are available in rinses, gels, and foams and come in various types of containers including personal-size bottles and wall-mounted dispensers (Fig. 7-6). Wall-mounted dispensers are often located just inside the doors to patient rooms and next to handwashing facilities. Phlebotomists typically carry small bottles of sanitizer in their equipment carriers.

**GAUZE PADS/COTTON BALLS**

Clean 2- by 2-inch gauze pads folded in fourths are used to hold pressure over the site following blood collection procedures. Special gauze pads with fluidproof backing are also available to help prevent contamination of gloves from blood at the site. Use of cotton balls to hold pressure is not recommended because they tend to stick to the site and reinitiate bleeding when removed because they dislodge the platelet plug that seals a puncture site.

**BANDAGES**

Adhesive bandages are used to cover a blood collection site after the bleeding has stopped. Paper, cloth, or knitted tape placed over a folded gauze square can also be used. Self-adhesive gauze placed over the gauze pad or cotton ball and wrapped around the arm is being used increasingly, especially for patients who are allergic to adhesive bandages. It is also used to form a pressure bandage following arterial puncture or venipuncture in patients with bleeding problems. Latex-free bandages are available for those with latex allergies.

**CAUTION:** Adhesive bandages should not be used on babies younger than 2 years of age because of the danger of aspiration and suffocation.
NEEDLE AND SHARPS DISPOSAL CONTAINERS

Used needles, lancets, and other sharp objects must be disposed of immediately in special containers referred to as “sharps” containers (Fig. 7-7), even if such objects contain safety features. A variety of styles and sizes of sharp containers are available. Most are red, for easy identification, but some are clear or opaque to make it easier to tell when they are full. All must be clearly marked with a biohazard symbol and be rigid, puncture-resistant, leakproof, and disposable and have locking lids to seal the contents when filled to the appropriate volume, after which they must be properly disposed of as biohazardous waste.

CAUTION: Sharps containers should not be overfilled because this creates a danger of sharps injury or other biohazard exposure (e.g., contact with infectious substances) to subsequent users.

BIOHAZARD BAGS

Biohazard bags (Fig. 7-8) are leakproof plastic bags that are commonly used to transport blood and other specimens from the collection site to the laboratory. The bags are marked with a biohazard label and often have an outside pocket in which requisitions or other forms can be placed. The bags help protect the collector and others from biohazard contamination. They will also contain a leak should a spill occur. There are various other styles and sizes of biohazard bags for biohazard trash and other biohazardous waste.

SLIDES

Precleaned 25- by 75-mm (1- by 3-in.) glass microscope slides are used to make blood films for hematology determinations. Slides are available either plain or with a frosted area at one end where the patient’s name or other information can be written in pencil.

PEN

A phlebotomist should always carry a pen with indelible (permanent) nonsmear ink to label tubes and record other patient information.

WATCH

A watch, preferably with a sweep second hand or timer, is needed to accurately determine specimen collection times and time certain tests.
The following equipment is used for venipuncture procedures in addition to the general blood collection supplies and equipment previously described.

**VEIN-LOCATING DEVICES**

There are a number of optional but useful portable transillumination devices on the market that make it easier to locate veins that are difficult to see or feel. Transillumination means to inspect an organ by passing light through its walls. These devices typically shine high-intensity LED or infrared red light through the patient’s subcutaneous tissue to highlight veins. The hemoglobin in the blood within the veins absorbs the light, causing the veins to stand out as dark lines. Most devices can be used in patients of all ages. Examples include the Venoscope II (Fig. 7-9A,B) and Neonatal Transilluminator (Venoscope, L.L.C., Lafayette, LA), and the AccuVein AV300 (Fig. 7-9C) (AccuVein LLC, Huntington, NY).

**TOURNIQUET**

A **tourniquet** (Fig. 7-10) is a device that is applied or tied around a patient’s arm prior to venipuncture to restrict blood flow. A properly applied tourniquet is tight enough to restrict venous flow out of the area but not so tight as to restrict arterial flow into the area. Restriction of venous flow distends or inflates the veins, making them larger and easier to find, and stretches the vein walls so they are thinner and easier to pierce with a needle. Restriction of blood flow can change blood components if the tourniquet is left in place for more than 1 minute, so a tourniquet must fasten in a way that is easy to release with one hand during blood collection procedures or in emergency situations, such as when a patient starts to faint or the needle accidentally backs out of the arm during venipuncture.

There are a number of different types of tourniquets, and most are available in both adult and pediatric sizes. The most common type, a strap tourniquet, is a flat strip of stretchable material, such as latex, nitrile, or vinyl that is fairly inexpensive and disposable. (This type
Figure 7-9  A. A Venoscope II transluminator device. B. A vein appears as a dark line between the light-emitting arms of the Venoscope II. (Venoscope II, LLC, Lafayette, LA.) C. AccuVein AV300 being used to locate veins on a patient’s arm. (AccuVein LLC, Huntington, NY)

Figure 7-10  Stretchable strap tourniquets. Latex tourniquet on the left. Nitrile tourniquets center and right.
is sometimes used more than once but must be discarded if dropped or contaminated with
blood or other visible contaminants.)

**KEY POINT** A blood pressure cuff may be used in place of a tourniquet. The patient’s blood pressure is taken and the pressure is then maintained below the patient’s diastolic pressure, or no greater than 40 mm Hg.

Although at present there is no regulatory requirement to dispose of tourniquets after a single use, a number of studies have shown that reusable tourniquets have the potential to transmit bacteria, including methicillin-resistant *Staphylococcus aureus* (MRSA). Consequently many facilities now use a new tourniquet for each patient. Reusable tourniquets are still in use at some facilities, however. A recent study determined that tourniquet contamination came from phlebotomists’ hands rather than patients’ skin, suggesting a need for better hand-hygiene habits on the part of phlebotomists. Although proper hand hygiene is important regardless of the type of tourniquet used, for those who reuse tourniquets, meticulous hand hygiene is crucial.

**KEY POINT** To address latex allergies and infection-control issues, more and more healthcare facilities are using disposable single-use latex-free tourniquets (Fig 7-11).

**NEEDLES**

Phlebotomy needles are sterile, disposable, and designed for a single use only. They include *multisample needles* (see “Evacuated Tube System,” below), *hypodermic needles* (see “Syringe System”), and *winged infusion (butterfly) needles* used with both the evacuated tube system and the syringe system. Multisample needles are commonly enclosed in sealed twist-off shields or covers. Hypodermic needles and butterfly needles are typically sealed in sterile pull-apart packages.

**CAUTION:** It is important to examine the packaging or seal of a needle before use. If the packaging is open or the seal is broken, the needle is no longer sterile and should not be used.

**KEY POINT** It important to visually inspect a needle prior to venipuncture. Needles are mass produced and on rare occasions contain defects such as blocked, blunt, or bent tips or rough bevels or shafts that could injure a patient’s vein, cause unnecessary pain, or result in venipuncture failure.

Specific terminology is used to refer to the parts of a needle. The end that pierces the vein is called the **bevel** because it is “beveled,” or cut on a slant. The bevel allows the needle to easily slip into the skin and vein without coring (removing a portion of the skin or vein).
The long cylindrical portion is called the **shaft**. The end that attaches to the blood collection device is called the **hub**, and the internal space of the needle is called the **lumen**. Needles are available in various sizes, indicated by gauge and length.

### Gauge

Needle **gauge** is indicated by a number that is related to the diameter of the lumen. A needle’s diameter and gauge have an inverse (opposite) relationship; that is, the higher the gauge number, the smaller the actual diameter of the needle.

Although blood typically flows more quickly through large-diameter needles, needle gauge is selected according to the size and condition of the patient’s vein, the type of procedure, and the equipment being used. Appropriate needles for the collection of most blood specimens for laboratory testing include gauges 20 through 23; however, a 21-gauge needle is considered the standard for most routine adult antecubital venipunctures. Common venipuncture needle gauges with needle type and typical use are shown in Table 7-1.

### TABLE 7-1  Common Venipuncture Needle Gauges with Needle Type and Typical Use

<table>
<thead>
<tr>
<th>Gauge</th>
<th>Needle Type</th>
<th>Typical Use</th>
</tr>
</thead>
<tbody>
<tr>
<td>15–17</td>
<td>Special needle attached to collection bag</td>
<td>Collection of donor units, autologous blood donation, and therapeutic phlebotomy.</td>
</tr>
<tr>
<td>18</td>
<td>Hypodermic</td>
<td>Used primarily as a transfer needle rather than for blood collection; safety issues have diminished use.</td>
</tr>
<tr>
<td>20</td>
<td>Multisample Hypodermic</td>
<td>Sometimes used when large-volume tubes are collected or large-volume syringes are used on patients with normal-size veins.</td>
</tr>
<tr>
<td>21</td>
<td>Multisample Hypodermic</td>
<td>Considered the standard venipuncture needle for routine venipuncture on patients with normal veins or for syringe blood culture collection.</td>
</tr>
<tr>
<td>22</td>
<td>Multisample Hypodermic</td>
<td>Used on older children and adult patients with small veins or for syringe draws on difficult veins.</td>
</tr>
<tr>
<td>23</td>
<td>Butterfly</td>
<td>Used on the veins of infants and children and on difficult or hand veins of adults.</td>
</tr>
</tbody>
</table>

Manufacturers typically color code needles by gauge for easy identification. Generally, multisample needles have color-coded caps and hubs, and syringe needles have color-coded hubs. Butterfly needles often have color-coded “wings.” Syringe and butterfly needle packaging may also contain color coding. Needle color codes vary among manufacturers; however, several large manufacturers use yellow for 20-gauge, green for 21-gauge, and black for 22-gauge needles (Fig. 7-12).

### Figure 7-12  Multisample needles with color-coded caps. A. Traditional style needles: yellow 20 gauge, green 21 gauge, and black 22 gauge. (Courtesy Greiner Bio-One International AG, Kremsmünster, Austria.) B. BD Eclipse safety needles, black 22 gauge, green 21 gauge. (Becton Dickinson, Franklin Lakes, NJ.)
Most multisample needles come in 1- or 1.5-in. lengths. Syringe needles come in many lengths; however 1- and 1.5-in. ones are most commonly used for venipuncture. Butterfly needles are typically 1/2 to 3/4 in. long. Some of the new safety needles come in slightly longer lengths to accommodate resheathing features. Length selection depends primarily upon user preference and the depth of the vein. Many phlebotomists prefer to use 1-in. needles in routine situations because it is less intimidating to the patient. Others, especially those with larger hands, feel that the 1.5-in. needle makes it easier to achieve the proper angle for entering the vein.

Safety Features

Needles are available with or without safety features. Safety features must provide immediate permanent containment and be activated using one hand, which must stay behind the needle at all times. Safety features include resheathing devices, such as shields that cover the needle after use; blunting devices; and equipment with devices that retract the needle after use. The FDA is responsible for clearing medical devices for marketing. Box 7-2 lists desirable characteristics of safety features that the FDA considers important in preventing percutaneous injury.

**KEY POINT** According to OSHA regulations, if the needle does not have a safety feature, the equipment it is used with (such as tube holder or syringe) must have a safety feature to minimize the chance of an accidental needlestick.

**EVACUATED TUBE SYSTEM**

The most common and efficient system and that preferred by the CLSI for collecting blood samples is the **evacuated tube system (ETS)** (Fig. 7-13). It is a closed system in which the patient’s blood flows through a needle inserted into a vein and then directly into a collection tube without being exposed to the air or outside contaminants. The system allows numerous tubes to be collected with a single venipuncture. Evacuated tube systems are available from several manufacturers. Although the design of individual elements may vary slightly by manufacturer, all ETS systems have three basic components: a special blood-drawing needle, a tube holder, and various types of evacuated tubes.

**KEY POINT** Unless components are specifically designed for use with multiple systems, it is recommended that all ETS components come from the same manufacturer. Mixing components from different manufacturers can lead to problems such as needles coming unscrewed and tubes popping off the needle during venipuncture.

**Multisample Needles**

ETS needles are called **multisample needles** because they allow multiple tubes of blood to be collected during a single venipuncture. They are threaded in the middle and have a beveled point on each end. The threaded portion screws into a tube holder. The end of the needle that pierces the vein is longer and has a longer bevel. The shorter end penetrates the tube stopper during specimen collection. It is covered by a sleeve that retracts as the needle goes through
the tube stopper so that blood can flow into the tube. When the tube is removed, the sleeve slides back over the needle to prevent leakage of blood. ETS needles are available with or without safety features. The color-coded needles shown in Figure 7-12A are traditional-style multisample needles without safety features. The needles shown in Figure 7-12B have safety devices used to resheath the needles after use.

CAUTION: ETS needles without safety features must be used with tube holders that have safety features.

A recent innovation in multisample needles is the Greiner Bio-One VACUETTE® VISIO PLUS Needle (Fig. 7-14). This needle has a transparent hub where blood can be seen if the venipuncture is successful. With most other multisample needles, blood flow is not evident until the tube is advanced onto the needle in the tube holder.

**Tube Holders**

A **tube holder** is a clear, plastic, disposable cylinder with a small threaded opening at one end (often also called a hub) where the needle is screwed into it and a large opening at the other end where the collection tube is placed. The large end has flanges or extensions on the sides that aid in tube placement and removal.

KEY POINT OSHA regulations require that the tube holder with needle attached be disposed of as a unit after use and never be removed from the needle and reused.

Holders are typically available in several sizes to accommodate different-sized tubes, including special sizes for large-diameter blood culture bottles, some of which have adapter inserts to narrow the diameter of the holder and allow for the collection of evacuated tubes after the blood culture specimens. Adapter inserts are also available so that small-diameter tubes can be collected in regular-size tube holders. Holders are available with and without safety features (Fig. 7-15).
**Figure 7-14** VACUETTE® VISIO PLUS needle with see-through hub attached to a safety tube holder. (Courtesy Greiner Bio-One International AG, Kremsmünster, Austria.)

**Figure 7-15** Tube holders. **A.** Traditional tube holder. **B.** JELCO Venipuncture Needle-Pro® safety tube holder with resheathing device. (Image courtesy Smiths Medical North America. JELCO, Needle-Pro is a trademark of Smiths Medical Family of Companies. All Rights Reserved.) **C.** Vanishpoint® safety tube holder with needle-retracting device attached to a traditional nonsafety needle. (Courtesy Retractible Technologies, Little Elm, TX.)
Caution: If the tube holder does not have a safety feature, the needle used with it must have a safety feature.

Safety features include shields that cover the needle and devices that manually or automatically retract the needle into the holder either before or after it is removed from the vein. Two types of holders (traditional and safety) with needles attached are shown in Figure 7-16.

A needle or tube holder that has a safety device is an example of a SESIP, which is the OSHA acronym for a sharp with engineered sharps injury protection.

### Needle and Holder Units

Needle and tube-holder devices are available permanently attached as a single unit or as both devices preassembled. Preassembled devices are often sealed in sterile packaging for use in sterile applications. An example of a single unit is the BD Vacutainer® Passive Shielding Blood Collection Needle (Fig. 7-17). The needle has a shield that releases automatically when the first tube is inserted in the holder. Upon activation, the shield rests against the patient’s arm and immediately covers the needle when it is withdrawn from the patient. This safety feature cannot be bypassed by the user, ensuring 100% compliance.

### Evacuated Tubes

Evacuated tubes (Fig. 7-18) are used with both the ETS and the syringe method of obtaining blood specimens. (With the syringe method, blood is collected in a syringe and must be immediately transferred into the tubes.) Evacuated tubes are available from a number of different manufacturers and come in various sizes and volumes ranging from 1.8 to 15 mL. Tube
selection is based on the age of the patient, the amount of blood needed for the test, and the size and condition of the patient’s vein. Most laboratories stock several sizes of each type of tube to accommodate various needs. At present, tubes are available in plastic and glass. Many laboratories have switched to plastic tubes for safety reasons. Local, state, and federal safety regulatory agencies should be consulted for current applicable regulations.

Vacuum

Evacuated tubes fill with blood automatically because there is a vacuum (negative pressure) in them. The vacuum is artificially created by pulling air from the tube. The amount of vacuum (i.e., the amount of air removed and negative pressure created) is measured precisely by the manufacturer so that the tube will draw the exact volume of blood indicated on the label. To reach its stated volume, a tube must be allowed to fill with blood until the normal

Figure 7-17  BD Vacutainer® Passive Shielding Blood Collection Needle. (Courtesy Becton Dickinson, Franklin Lakes, New Jersey)

Figure 7-18  Evacuated tubes. A. Vacutainer® Plus Plastic brand evacuated tubes. (Becton Dickinson, Franklin Lakes, NJ.) B. Vacuette® evacuated tubes. (Greiner Bio-One International AG, Kremsmünster, Austria.)
vacuum is exhausted. A tube that has prematurely lost all or part of its vacuum will fail to properly fill with blood.

**KEY POINT** Tubes do not fill with blood all the way to the stopper. When a tube is filled properly, there is always a consistent amount of head space (air) between the level of blood in the tube and the tube stopper.

Premature loss of vacuum can occur from improper storage, opening the tube, dropping the tube, advancing the tube too far onto the needle before venipuncture, or if the needle bevel becomes partially out of the skin during venipuncture. Premature loss of vacuum, removing the tube before the vacuum is exhausted, or stoppage of blood flow during the blood draw can result in an underfilled tube called a partial draw or “short draw.” Test results may be compromised in partially filled tubes that contain additives because the ratio of blood to additive has been altered. Consequently some manufacturers offer special “short draw” tubes (Fig. 7-19) designed to partially fill without compromising test results. These tubes are used in situations in which it is difficult or inadvisable to draw larger quantities of blood.

Manufacturers’ partial draw tubes are often the same size as standard-volume tubes but do not fill to the same level and may fill more slowly.

**Additive Tubes**

Most ETS tubes contain some type of additive. An additive is any substance placed within a tube other than the tube stopper. Additives have one or more specific functions, such as preventing clotting or preserving certain blood components. Blood collected in additive tubes may or may not clot, depending on the additive type. For example, if the additive prevents clotting, the result is a whole-blood specimen. Some whole-blood specimens are used directly for testing; others are centrifuged to separate the cells from the liquid portion called plasma, which is used for testing. If the additive is a clot activator, the blood will clot and the specimen must be centrifuged to obtain the fluid portion called serum. (See Chapter 6 for a discussion of serum, plasma, and whole blood.)

**KEY POINT** Plastic tubes are so smooth inside that platelet aggregation and adhesion are inhibited, resulting in delayed or incomplete clotting. Consequently, clot activators are added to plastic serum tubes. This is not a problem with glass tubes, because glass has a rough surface.

The amount of additive in a tube has been calibrated by the manufacturer to function optimally and produce accurate results with the amount of blood it takes to fill the tube to the capacity or volume indicated. Specimen quality can be compromised and lead to inaccurate results if the tube is underfilled, so it is important to allow additive tubes to fill with blood until the normal vacuum is exhausted.

*Figure 7-19* Coagulation tubes with arrows indicating fill levels; a regular draw tube (bottom) and a short draw tube (top).
CAUTION: An underfilled additive tube will have an incorrect blood-to-additive ratio, which can cause inaccurate test results.

**Nonadditive Tubes**

Very few tubes are additive-free. (Even serum tubes need an additive to promote clotting if they are plastic.) Most nonadditive plastic tubes (Fig. 7-20) that do exist are used for clearing or discard purposes only. A few glass nonadditive red-top tubes are still in existence, but most are in the process of being discontinued for safety reasons. Blood collected in a tube will clot when there is nothing in the tube to prevent it. Consequently, nonadditive tubes yield serum samples.

**Stoppers**

Tube stoppers (tops or closures) are typically made of rubber. Some tubes have a rubber stopper covered by a plastic shield designed to protect lab personnel from blood drops remaining on the stopper after the tube is removed from the needle and from aerosols (mists) and sprays of specimen when the stopper is removed from the tube. The rigidity of the plastic also prevents removal of the stopper using a “thumb roll,” a technique that has been shown to cause aerosol formation.

**Color Coding**

Tube stoppers are color-coded. Consequently, it is not unusual for evacuated tubes to be referred to as red tops, green tops, and so forth. For most tubes, the stopper color identifies a type of additive placed in the tube by the manufacturer for a specific purpose. However, for some tubes, the stopper color indicates a special property of the tube. For example, a royal-blue stopper (Fig. 7.21) indicates a tube that is as free of trace element contamination as possible.

**KEY POINT** If a royal-blue top has an additive, the color code for the additive is often displayed on the label. If not, the proper tube must be selected according to the additive listed on the label.

*Figure 7-20* Nonadditive tube used as a discard or "clear" tube.
Occasionally, there may be more than one stopper color for the same additive. In addition, although color-coding is generally universal, it may vary slightly by manufacturer. Common stopper colors, what they indicate, and what departments use them are shown in Table 7-2. For reference, two manufacturers’ tube guides are shown in Appendix F.

**Expiration Dates**

Manufacturers guarantee reliability of additives and tube vacuum until an expiration date printed on the label (Fig. 7-22), provided the tubes are handled properly and stored between 4 and 25°C. Improper handling or storage can affect additive integrity and tube vacuum, which can lead to compromised test results or improper filling, respectively.

![Figure 7-21](image-url) Royal blue top tube. The red stripe down the left side of the label indicates that it is a serum tube.

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**TABLE 7-2  Common Stopper Colors, Additives, and Departments**

<table>
<thead>
<tr>
<th>Stopper Color</th>
<th>Additive</th>
<th>Department(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Light blue</td>
<td>Sodium citrate</td>
<td>Coagulation</td>
</tr>
<tr>
<td>Red</td>
<td>None</td>
<td>Chemistry, Blood Bank, Serology/Immunology</td>
</tr>
<tr>
<td>Red</td>
<td>Clot activator</td>
<td>Chemistry</td>
</tr>
<tr>
<td>Red/light gray</td>
<td>None</td>
<td>NA (discard tube only)</td>
</tr>
<tr>
<td>Clear</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Red/black (tiger)</td>
<td>Clot activator and gel separator</td>
<td>Chemistry</td>
</tr>
<tr>
<td>Gold</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Red/gold</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Green/gray</td>
<td>Lithium heparin and gel separator</td>
<td>Chemistry</td>
</tr>
<tr>
<td>Light green</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Green</td>
<td>Lithium heparin</td>
<td>Chemistry</td>
</tr>
<tr>
<td></td>
<td>Sodium heparin</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ammonium heparin</td>
<td></td>
</tr>
<tr>
<td>Lavender (purple)</td>
<td>EDTA</td>
<td>Hematology</td>
</tr>
<tr>
<td>Pink</td>
<td>EDTA</td>
<td>Blood Bank</td>
</tr>
<tr>
<td>Gray</td>
<td>Sodium fluoride and potassium oxalate</td>
<td>Chemistry</td>
</tr>
<tr>
<td></td>
<td>Sodium fluoride and EDTA</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sodium fluoride</td>
<td></td>
</tr>
<tr>
<td>Orange</td>
<td>Thrombin</td>
<td>Chemistry</td>
</tr>
<tr>
<td>Gray/yellow</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Royal blue</td>
<td>None</td>
<td>Chemistry</td>
</tr>
<tr>
<td></td>
<td>EDTA</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sodium heparin</td>
<td></td>
</tr>
<tr>
<td>Tan</td>
<td>EDTA</td>
<td></td>
</tr>
<tr>
<td>Yellow</td>
<td>Sodium polyanethol sulfonate (SPS)</td>
<td>Microbiology</td>
</tr>
<tr>
<td>Yellow</td>
<td>Acid citrate dextrose (ACD)</td>
<td>Blood Bank/Immunohematology</td>
</tr>
</tbody>
</table>
KEY POINT Always check the expiration date on a tube before using it, and never use a tube that has expired or has been dropped. Discard it instead.

SYRINGE SYSTEM

Although the evacuated tube system (ETS) is the preferred method of blood collection, a syringe system (Fig. 7-23) is sometimes used for patients with small or difficult veins. This system consists of a sterile syringe needle called a hypodermic needle and a sterile plastic syringe with a Luer-lock tip (a special tip that allows the needle to attach more securely than a slip tip).
Syringe Needles

Syringe needles come in a wide range of gauges and lengths for many different uses. Those appropriate for phlebotomy procedures are generally gauges 21 to 23, in 1- or 1.5-in. lengths. When used to draw blood, a syringe needle must have a resheathing feature to allow it to be safely covered and removed so that a transfer device can be attached to the syringe to fill the evacuated tubes. An example of a safety needle attached to a syringe is shown in Figure 7-24A. A syringe that has a built-in safety device to cover the needle is shown in Figure 7-24B.

CAUTION: Syringe needles used for phlebotomy must have resheathing devices to minimize the chance of accidental needlesticks. Needles used for intradermal skin tests must have resheathing devices or be used with syringes that have devices that cover or retract the needle after use.

Syringes

Syringes typically come in sterile pull-apart packages and are available in various sizes or volumes. The most common volumes used for phlebotomy are 2, 5, and 10 mL. Syringe volume is selected according to the size and condition of the patient’s vein and the amount of blood to be collected.

Syringes have two parts: a barrel, which is a cylinder with graduated markings in either milliliters (mL) or cubic centimeters (cc), and a plunger, a rod-like device that fits tightly into the barrel (Fig. 7-23). When drawing venous blood by syringe, the phlebotomist slowly pulls back the plunger, creating a vacuum that causes the barrel to fill with blood.

Syringe Transfer Device

Blood collected in a syringe must be transferred into ETS tubes. In the past blood was transferred by poking the syringe needle through the tube stopper or by removing the tube stopper and ejecting blood from the syringe into the tube. Both practices are now considered unsafe. A syringe transfer device (Fig. 7-25) allows the safe transfer of blood into the tubes without using the syringe needle or removing the tube stopper. The device is similar to an ETS tube holder but has a permanently attached needle inside. After a syringe draw is completed, the needle safety device is activated and the needle is removed and discarded into a sharps container. The transfer device is then attached to the hub of the syringe. An ETS tube is placed inside it and advanced onto the needle until blood flows into the tube. Additional tubes can
be filled as long as there is enough blood in the syringe. The transfer device greatly reduces the chance of accidental needlesticks and confines any aerosol or spraying of the specimen that may be generated as tubes are removed from it.

**KEY POINT** A transfer device must be vertical when tubes are being filled in order to prevent blood in the tube from touching the needle in the transfer device. If blood mixed with additive gets on or in the needle, it could be transferred into the next tube that is filled and contaminate it.

**WINGED INFUSION SET**

A winged infusion blood collection set, or butterfly, is an indispensable tool for collecting blood from small or difficult veins such as hand veins and veins of elderly and pediatric patients as it allows much more flexibility and precision than a needle and syringe. It consists of a 1/8- to 1/4-inch stainless steel needle permanently connected to a 5- to 12-in. length of tubing with either a Luer attachment for syringe use or a multisample Luer adapter for use with the evacuated tube system (Fig. 7-26). Multisample Luer adapters are also available separately.

**KEY POINT** The first tube collected with a butterfly will underfill because of the air in the tubing. If the tube contains an additive, the blood-to-additive ratio will be affected. If an additive tube is the first tube to be collected, it is important to draw a few milliliters of blood into a nonadditive tube or another additive tube of the same type and to discard it prior to collecting the first tube. This is referred to as collecting a “clear” or discard tube and is especially critical when one is collecting coagulation tubes using a butterfly.

Plastic extensions that resemble butterfly wings (thus the name butterfly) are attached to the needle where it is joined to the tubing. During use, the needle may be held from above by gripping the “wings” together between the thumb and index finger, thus allowing the user to achieve the shallow angle of needle insertion required to access small veins.
Butterfly needles come in various gauges, although a 23-gauge needle is most commonly used for phlebotomy. In rare situations a 25-gauge needle is used by specially trained personnel to collect blood from scalp or other tiny veins of premature infants and other neonates.

**CAUTION:** Using a needle smaller than 23 gauge increases the chance of hemolyzing the specimen.

As with other blood collection needles, butterfly needles are required to have safety devices to reduce the possibility of accidental needlesticks. Butterfly safety devices include locking shields that slide over the needle, blunting devices, and needle retracting devices. See Figure 7-27 for examples of safety-winged infusion sets (butterflies).

**COMBINATION SYSTEMS**

The S-Monovette® Blood Collection System (Sarstedt, Inc., Newton, NC) shown in Figure 7-28 is a complete system for blood collection in which the blood collection tube and collection apparatus are combined in a single unit. The unit allows the specimen to be collected by either an evacuated tube or syringe system technique. The units are available with regular or butterfly-style needles. Safety devices are available to contain the needle immediately after use.

**Blood Collection Additives**

Blood collection tubes and other collection devices often contain one or more additives. There are a number of different types of additives, and each has a specific function. The type of additive required for blood collection generally depends upon the test that has been ordered. No substitution or combining of tubes with different additives is allowed.

**CAUTION:** Never transfer blood collected in an additive tube into another additive tube, even if the additives are the same. Different additives may interfere with each other or the testing process. If the additives are the same, an excess of the additive is created, which can negatively affect testing.
Figure 7-27 Examples of safety-winged infusion sets. A. BD Vacutainer® SAFETY-LOK™ Blood Collection Set for use with the evacuated tube system. (Courtesy Becton Dickinson Vacutainer Systems, Franklin Lakes, N.J.) B. Monoject Angel Wing blood collection set. (Kendall CO, LP, Mansfield, MA.) C. Vacuette® safety butterfly blood collection system (Greiner Bio-One, Kremsmünster, Austria.) D. Vacutainer® Push Button Blood Collection Set. (Courtesy Becton Dickinson, Franklin Lakes, N.J.)

Figure 7-28 Sarstedt S-Monovette® venous blood collection system. Left: System with needle attached. Upper right: System used as a syringe. Lower right: System used as ETS. (Courtesy of Sarstedt, Inc., Newton, NC.)
Additives are available in liquid, spray-dried, and powder forms. A tube with a powdered additive should be lightly tapped prior to use to settle the additive to the bottom of the tube. An additive tube must be gently inverted 3 to 10 times, depending on the type of additive and the manufacturer, immediately after collection to adequately mix the additive with the specimen. (See the tube guides in Appendix F for tube inversion information from two major tube manufacturers.)

**KEY POINT** According to tube manufacturer Becton Dickinson (BD), each inversion requires turning the wrist 180 degrees and back again.

**CAUTION:** Never shake or otherwise vigorously mix a specimen, as this can cause hemolysis, which makes most specimens unsuitable for testing.

**ANTICOAGULANTS**

The most common reason for using an additive is to prevent clotting of the specimen. Anticoagulants are substances that prevent blood from clotting (coagulating) by either of two methods: by chelating (binding) or precipitating calcium so it is unavailable to the coagulation process or by inhibiting the formation of thrombin needed to convert fibrinogen to fibrin in the coagulation process. If a test requires whole blood or plasma, the specimen must be collected in a tube that contains an anticoagulant. Anticoagulant specimens must be mixed immediately after collection to prevent microclot formation. Gentle mixing is essential to prevent hemolysis.

**KEY POINT** Because the cells are free-flowing and not clotted, a specimen collected in anticoagulant will separate through settling or centrifugation and can be resuspended by intentional or inadvertent mixing of the specimen.

There are different types of anticoagulants, each designed for use in certain types of testing. It is important to use the correct anticoagulant for the type of test collected. The most common anticoagulants are ethylenediaminetetraacetic acid (EDTA), citrates, heparin, and oxalates. Memory joggers to help you learn the most common anticoagulants are found in Table 7-3.

<table>
<thead>
<tr>
<th>TABLE 7-3 Memory Joggers for Anticoagulants</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Acronym, Mnemonic, or Acrostic</strong></td>
</tr>
<tr>
<td>ECHO</td>
</tr>
<tr>
<td>phEDTA (pronounced like fajita)</td>
</tr>
<tr>
<td>Spring Creates Colorful Light-Blue Pansies</td>
</tr>
<tr>
<td>Greenhouses Have Colorful Plants</td>
</tr>
<tr>
<td>GO (gray oxalate) Gray ox (gray oxalate)</td>
</tr>
<tr>
<td>LL (lavender last except for gray)</td>
</tr>
</tbody>
</table>
EDTA

Ethylenediaminetetraacetic acid (EDTA) is commonly available as a powdered di-potassium (K₂) or liquid tri-potassium (K₃) salt, which prevents coagulation by binding or chelating calcium.

EDTA is the additive in:
- Lavender (purple)-top tubes (Fig. 7-18)
- Microcollection containers with lavender tops
- Pink plastic-top tubes with a special blood bank patient ID label
- Pearl-top tubes with thixotropic gel separator
- Royal blue-top tubes with lavender color-coding on the label

Although EDTA is increasingly being used for blood bank tests, it is primarily used to provide whole-blood specimens for hematology tests (e.g., CBCs) because it preserves cell morphology (shape and structure) and inhibits platelet aggregation better than any other anticoagulant. EDTA specimens must be mixed immediately after collection to prevent platelet clumping and microclot formation, which can affect test results negatively. Eight to ten inversions are normally required for proper mixing.

**CAUTION:** If microclots are detected in a hematology specimen, it cannot be used for testing and must be recollected.

CLSI recommends spray-dried EDTA for most hematology tests because liquid EDTA dilutes the specimen and results in lower hemoglobin values, RBC and WBC counts, platelet counts, and packed-cell volumes. The dilutional effect is even more pronounced if the tubes are not completely filled. Therefore, it is important to fill tubes until their normal vacuum is exhausted. Either type of EDTA tube should be filled to its stated volume to maintain the correct blood-to-anticoagulant ratio.

**KEY POINT** Excess EDTA, which results when tubes are underfilled, can cause RBCs to shrink and thus change CBC results.

Citrates

Citrates prevent coagulation by binding or chelating calcium. The most common citrate is sodium citrate, which is used for coagulation tests (e.g., PT and aPTT) because it does the best job of preserving the coagulation factors. Sodium citrate tubes have light-blue stoppers (Fig. 7-18).

*Sodium citrate is also the additive in special erythrocyte sedimentation rate (ESR) tubes with black stoppers.*

Coagulation specimens require immediate mixing after collection to prevent activation of the coagulation process and microclot formation, which invalidates test results. Three to four gentle inversions are required for adequate mixing.

**CAUTION:** Vigorous mixing or an excessive number of inversions can activate platelets and shorten clotting times.

Light blue–top tubes contain a 9:1 ratio of blood to anticoagulant when filled to the stated volume and must be filled to within 90% of that volume for accurate coagulation results.
Exact fill volume is hard to tell on most tubes; however, Vacuette® sodium citrate tubes have arrows that are used to identify correct fill volume. A guide provided by the manufacturer also helps phlebotomists or specimen processors to determine whether a tube is adequately filled (Fig. 7-29).

**CAUTION:** The 9:1 ratio of blood to anticoagulant in light-blue sodium citrate tubes is critical; therefore, it is extremely important to fill them to their stated capacity. Underfilled tubes can cause artificially prolonged clotting times and visibly underfilled tubes will not be accepted for testing by most laboratories.

Coagulation tests are performed on plasma, so specimens must first be centrifuged to separate the plasma from the cells. Because sodium citrate binds calcium, calcium is added back to the specimen during the testing process so that clotting can be initiated and timed.

**Heparin**

Heparin prevents clotting by inhibiting thrombin formation. (Thrombin is an enzyme needed to convert fibrinogen into the fibrin necessary for clot formation.) Heparinized plasma is often used for some chemistry tests, especially stat tests (e.g., electrolytes) and in other rapid-response situations when a fast turnaround time (TAT) for chemistry tests is needed. Faster TAT is possible because time is eliminated that would normally be required for a specimen to clot before serum could be obtained.

**KEY POINT** Heparinized plasma is preferred over serum for potassium tests because when blood clots, potassium is released from cells into the serum and can falsely elevate results.

Heparin is the additive in:
- Green-top tubes
- Green-top and light green–top gel tubes (Fig. 7-18)
- Mottled-green and gray-top tubes
- Royal blue–top tubes with green color coding on the label
- Green-top and light green–top microtubes
- Red-banded and green-banded microhematocrit tubes
There are three heparin formulations: ammonium, lithium, and sodium heparin. Lithium heparin causes the least interference in chemistry testing and is the most widely used anticoagulant for both plasma and whole-blood chemistry tests.

**CAUTION:** It is essential to choose the right heparin formulation for the type of test. Lithium heparin must not be used to collect lithium levels. Sodium heparin must not be used to collect sodium specimens or electrolyte panels because sodium is part of the panel.

Heparinized specimens must be mixed immediately upon collection to prevent clot formation and fibrin generation. From five to ten inversions, depending on the manufacturer, are required for proper mixing. Gentle mixing is essential to prevent hemolysis. Hemolyzed specimens are unsuitable for many chemistry tests.

**Oxalates**

Oxalates prevent coagulation by precipitating calcium. **Potassium oxalate** is the most widely used. It is commonly added to tubes containing glucose preservatives (see “Antiglycolytic Agents,” below) to provide plasma for glucose testing. Potassium oxalate is most commonly found in evacuated tubes and microcollection containers with gray stoppers. Oxalate specimens must be mixed immediately upon collection to prevent clot formation and fibrin generation. Eight to ten inversions are required for proper mixing.

**CAUTION:** It is essential to fill oxalate tubes to the volume stated on the tube because excess oxalate causes hemolysis (destruction of red blood cells, or RBCs) and release of hemoglobin into the plasma.

**SPECIAL-USE ANTICOAGULANTS**

The following anticoagulants are combined with other additives and have additional properties for special-use situations.

**Acid Citrate Dextrose (ACD)**

**ACD** solution is available in two formulations (solution A and solution B) for immunohematology tests such as DNA testing and human leukocyte antigen (HLA) phenotyping, which is used in paternity evaluation and to determine transplant compatibility. The acid citrate prevents coagulation by binding calcium, with little effect on cells and platelets. Dextrose acts as an RBC nutrient and preservative by maintaining RBC viability. ACD tubes have yellow tops and require eight inversions immediately after collection to prevent clotting.

**Citrate Phosphate Dextrose (CPD)**

**CPD** is used in collecting units of blood for transfusion. Citrate prevents clotting by chelating calcium, phosphate stabilizes pH, and dextrose provides cells with energy and helps keep them alive.

**Sodium Polyanethol Sulfonate (SPS)**

**SPS** prevents coagulation by binding calcium. It is used for blood culture collection because, in addition to being an anticoagulant, it reduces the action of a protein called complement, which destroys bacteria. It also slows down phagocytosis (ingestion of bacteria by leukocytes), and reduces the activity of certain antibiotics. SPS tubes have yellow stoppers and require eight inversions to prevent clotting.

**ANTIGLYCOLYTIC AGENTS**

An **antiglycolytic agent** is a substance that prevents glycolysis, the breakdown or metabolism of glucose (blood sugar) by blood cells. If glycolysis is not prevented, the glucose concentration in a blood specimen decreases at a rate of 10 mg/dL per hour.
KEY POINT Glycolysis occurs faster in newborns because their metabolism is increased, and in patients with leukemia because of high metabolic activity of WBCs.

The most common antiglycolytic agent is sodium fluoride. It preserves glucose for up to 3 days and also inhibits the growth of bacteria. Sodium fluoride is commonly used in combination with the anticoagulant potassium oxalate to provide plasma specimens for rapid-response situations. Sodium fluoride tubes have gray stoppers and require between five and ten inversions, depending on the manufacturer, for proper mixing.

KEY POINT Sodium fluoride tubes are used to collect ethanol specimens to prevent either a decrease in alcohol concentration due to glycolysis or an increase due to fermentation by bacteria.

CLOT ACTIVATORS

A clot activator is a substance that enhances coagulation in tubes used to collect serum specimens. Clot activators include substances that provide more surface for platelet activation, such as glass (silica) particles and inert clays like Celite, and clotting factors such as thrombin. Silica particles are the clot activators in serum-separator tubes (SSTs) and plastic red-top tubes. Silica particles cause the blood to clot within 15 to 30 minutes. Blood collected in thrombin tubes generally clots within 5 minutes. Celite tubes are used with some point-of-care coagulation systems. Tubes containing clot activators require five gentle inversions for complete and rapid clotting to occur.

KEY POINT Blood in an SST tube will eventually clot without mixing; however, when it is not mixed, glass particles may become suspended in the serum and interfere in the testing process.

THIXOTROPIC GEL SEPARATOR

Thixotropic gel is an inert (nonreacting) synthetic substance initially contained in or near the bottom of certain blood collection tubes. The density of the gel is between that of the cells and the serum or plasma. When a specimen in a gel tube is centrifuged, the gel undergoes a change in viscosity (thickness) and moves to a position between the cells and the serum or plasma, forming a physical barrier between them. This physical separation prevents the cells from continuing to metabolize substances such as glucose in the serum or plasma. Serum gel-barrier tubes include Becton Dickinson (BD) tubes with gold plastic (Fig. 7-18) stoppers or tubes with mottled red/gray rubber stoppers called serum-separator tubes (SSTs); new BD tubes containing thrombin that clot in 5 minutes called Rapid Serum Tubes™ (RSTs) (Fig. 7-30); Kendall tubes with mottled red/gray rubber stoppers called Monoject Corvac tubes; and Greiner Bio-One Vacuette serum tubes with red plastic stoppers and yellow tops. Heparinized plasma gel-barrier tubes include BD tubes with light-green plastic or mottled...
gray/green rubber stoppers called **plasma-separator tubes (PSTs)** and Vacuette tubes with green plastic stoppers and yellow tops. In addition, BD has EDTA gel-barrier tubes with pearl-colored stoppers called **plasma-preparation tubes (PPTs)**.

**TRACE ELEMENT–FREE TUBES**

Although stopper colors normally indicate a type of additive in a tube, royal-blue stoppers indicate **trace element–free tubes**. These tubes are made of materials that are as free of trace element contamination as possible; they are used for trace element tests, toxicology studies, and nutrient determinations. These tests measure substances present in such small quantities that trace element contamination commonly found in the glass, plastic, or stopper material of other tubes may leach into the specimen and falsely elevate test results. Royal blue-top tubes (Fig. 7-21) contain EDTA, heparin, or no additive to meet various test requirements. Tube labels may be color-coded to indicate the type of additive, if any, in the tube.

**Order of Draw**

**Order of draw** refers to the order in which tubes are collected during a multiple-tube draw or are filled from a syringe. CLSI recommends the following order of draw for both ETS collection and in filling tubes from a syringe:

1. Sterile tube (blood culture)
2. Blue-top coagulation tube
3. Serum tube with or without clot activator, with or without gel
4. Heparin tube with or without gel plasma separator
5. EDTA tube
6. Glycolytic inhibitor tube

**Memory Jogger** For the order of draw:

- Stop (Sterile)
- Light (Lt. Blue)
- Red (Red)
- Stay (SST)
- Put (PST)
- Green (Green)
- Light (Lavender)
- Go (Gray)

The memory jogger for the order-of-draw places the red top before the SST and places the PST before the green top for convenience in memorization.

Filling tubes in the wrong order can lead to interference in testing from cross contamination of the specimen by additive carryover, tissue thromboplastin, or microorganisms. The special sequence of tube collection (order of draw) is intended to minimize these problems.

**FVI**

Order of draw may vary slightly among institutions. Consult institutional protocol before using a specific order of draw.

**CARRYOVER/CROSS-CONTAMINATION**

**Carryover** or cross-contamination is the transfer of additive from one tube to the next. It can occur when blood in an additive tube touches the needle during ETS blood collection or when blood is transferred from a syringe into ETS tubes. Blood remaining on or within the needle
can be transferred to the next tube drawn or filled, contaminating that tube with additive from the previous tube and possibly affecting test results on the specimen. Table 7-4 lists some of the most common tests affected by additive contamination.

TABLE 7-4  Common Tests Affected by Additive Contamination

<table>
<thead>
<tr>
<th>Contaminating Additive</th>
<th>Tests Potentially Affected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Citrate</td>
<td>Alkaline phosphatase</td>
</tr>
<tr>
<td></td>
<td>Calcium</td>
</tr>
<tr>
<td></td>
<td>Phosphorus</td>
</tr>
<tr>
<td>EDTA</td>
<td>Alkaline phosphatase</td>
</tr>
<tr>
<td></td>
<td>Calcium</td>
</tr>
<tr>
<td></td>
<td>Creatine kinase</td>
</tr>
<tr>
<td></td>
<td>Partial thromboplastin</td>
</tr>
<tr>
<td></td>
<td>Potassium</td>
</tr>
<tr>
<td></td>
<td>Protime</td>
</tr>
<tr>
<td></td>
<td>Serum iron</td>
</tr>
<tr>
<td></td>
<td>Sodium</td>
</tr>
<tr>
<td>Heparin (all formulations)</td>
<td>Activated clotting time</td>
</tr>
<tr>
<td></td>
<td>Acid phosphatase</td>
</tr>
<tr>
<td></td>
<td>Calcium (some test methods)</td>
</tr>
<tr>
<td></td>
<td>Partial thromboplastin</td>
</tr>
<tr>
<td></td>
<td>Protime</td>
</tr>
<tr>
<td></td>
<td>Sodium (sodium formulations)</td>
</tr>
<tr>
<td></td>
<td>Lithium (lithium formulations)</td>
</tr>
<tr>
<td>Oxalates</td>
<td>Acid phosphatase</td>
</tr>
<tr>
<td></td>
<td>Alkaline phosphatase</td>
</tr>
<tr>
<td></td>
<td>Amylase</td>
</tr>
<tr>
<td></td>
<td>Calcium</td>
</tr>
<tr>
<td></td>
<td>Lactate dehydrogenase</td>
</tr>
<tr>
<td></td>
<td>Partial thromboplastin</td>
</tr>
<tr>
<td></td>
<td>Potassium</td>
</tr>
<tr>
<td></td>
<td>Protime</td>
</tr>
<tr>
<td></td>
<td>Red cell morphology</td>
</tr>
<tr>
<td>Silica (clot activator)</td>
<td>Partial thromboplastin time</td>
</tr>
<tr>
<td></td>
<td>Protime</td>
</tr>
<tr>
<td>Sodium fluoride</td>
<td>Sodium</td>
</tr>
<tr>
<td></td>
<td>Urea nitrogen</td>
</tr>
</tbody>
</table>

KEY POINT  EDTA in tubes has been the source of more carryover problems than any other additive. Heparin causes the least interference in tests other than coagulation tests because it also occurs in blood naturally.

Remembering which tests the various additives affect can be difficult. Order of draw eliminates confusion by presenting a sequence of collection that results in the least amount of interference should carryover occur. The chance of carryover can be minimized by making certain that specimen tubes fill from the bottom up during collection and that the contents of the tube do not come in contact with the needle during the draw or in transferring blood into tubes from a syringe.

KEY POINT  According to the Center for Phlebotomy Education (www.phlebotomy.com), when using the ETS system, royal-blue tops for trace element studies should be collected separately to avoid even the smallest amount of carryover. If a syringe is being used, the transfer device should be changed if the trace element tube is filled after other tubes. (The CLSI order of draw for other tubes, including stopper colors and rationale for collection order, is summarized in Table 7-5.)
TABLE 7-5  Order of Draw, Stopper Colors, and Rationale for Collection Order

<table>
<thead>
<tr>
<th>Order of Draw</th>
<th>Tube Stopper Color</th>
<th>Rationale for Collection Order</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood cultures (sterile collections)</td>
<td>Yellow SPS</td>
<td>Minimizes chance of microbial contamination.</td>
</tr>
<tr>
<td>Coagulation tubes</td>
<td>Light blue</td>
<td>The first additive tube in the order because all other additive tubes affect coagulation tests.</td>
</tr>
<tr>
<td>Glass nonadditive tubes</td>
<td>Red</td>
<td>Prevents contamination by additives in other tubes.</td>
</tr>
<tr>
<td>Plastic clot activator tubes</td>
<td>Red</td>
<td>Filled after coagulation tests because silica particles activate clotting and affect coagulation tests (carryover of silica into subsequent tubes can be overridden by anticoagulant in them).</td>
</tr>
<tr>
<td>Serum separator tubes (SSTs)</td>
<td>Red and gray rubber Gold plastic</td>
<td></td>
</tr>
<tr>
<td>Plasma-separator tubes (PSTs)</td>
<td>Green and gray rubber Light-green plastic</td>
<td>Heparin affects coagulation tests and interferes in collection of serum specimens; it causes the least interference in tests other than coagulation tests.</td>
</tr>
<tr>
<td>Heparin tubes</td>
<td>Green</td>
<td>Responsible for more carryover problems than any other additive: elevates Na and K levels, chelates and decreases calcium and iron levels, elevates PT and PTT results.</td>
</tr>
<tr>
<td>EDTA tubes</td>
<td>lavender, pink, or purple Pearl top</td>
<td></td>
</tr>
<tr>
<td>Plasma-preparation tubes (PPTs)</td>
<td>Gray top</td>
<td>Sodium fluoride and potassium oxalate affect sodium and potassium levels, respectively. Filled after hematology tubes because oxalate damages cell membranes and causes abnormal RBC morphology. Oxalate interferes in enzyme reactions.</td>
</tr>
</tbody>
</table>

TISSUE THROMBOPLASTIN CONTAMINATION

Tissue thromboplastin, a substance present in tissue fluid, activates the extrinsic coagulation pathway and can interfere with coagulation tests. It is picked up by the needle during venipuncture and flushed into the first tube filled during ETS collection, or it is mixed with blood collected in a syringe. Although tissue thromboplastin is no longer considered to pose a significant problem for prothrombin time (PT) and partial thromboplastin time (PTT or aPTT) tests unless the draw is difficult and involves a lot of needle manipulation, it may compromise results of other coagulation tests. Therefore any time a coagulation test other than PT or PTT is the first or only tube collected, a few milliliters of blood should be drawn into a nonadditive tube or another coagulation tube before the coagulation specimen is collected. The extra tube is called a “clear” or “discard” tube because it is used to remove tissue fluid from the needle and is then thrown away.

CAUTION: A discard tube must be drawn to protect the critical 9:1 blood-to-additive ratio of a coagulation tube that is the first or only tube collected using a butterfly because air in the tubing displaces blood in the tube.

MICROBIAL CONTAMINATION

Blood cultures detect microorganisms in the blood and require special site-cleaning measures prior to collection to prevent contamination of the specimen by microorganisms that are normally found on the skin. Blood culture tubes or bottles are sterile and are collected first in the order of draw to ensure that they are collected when sterility of the site is optimal and to prevent microbial contamination of the needle from the unsterile tops of tubes used to collect other tests. Blood cultures do not often factor into the sequence of collection because they are typically drawn separately.

KEY POINT  Contamination of blood culture bottles can lead to false-positive results and inappropriate or delayed care for the patient.