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.

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.

**EDTA**

Ethylenediaminetetraacetic acid (EDTA) is commonly available as a powdered di-potassium (K$_2$) or liquid tri-potassium (K$_3$) salt, which prevents coagulation by binding or chelating calcium.

EDTA is the additive in:
- Lavender (purple)-top tubes
- 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.

*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.

**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
- 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.

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.

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.
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 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); 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 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:
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.

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.

**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.)

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.