

SPECIAL SPECIMEN COLLECTION AND HANDLING

Hemostasis/Thrombosis Specimens

To produce valid results for hemostasis/thrombosis tests and factor assays, specimen integrity is crucial and must be maintained. All specimens sent for testing must be collected and shipped in the following manner:

1. Obtain venous blood by clean venipuncture at a site away from an intravenous line. **Avoid slow-flowing draws and/or traumatic venipuncture, as either of these may result in an activated or clotted specimen. Do not use needles smaller than 23 gauge. Do not leave the tourniquet on for an extended length of time before drawing the specimen.**
2. Draw a pilot tube (non-additive or light blue-top tube) as a recommended procedure before drawing coagulation specimens (except PT/INR and aPTT) in light blue-top vacuum tubes (3.2 percent buffered sodium citrate). Discard the pilot tube. **Note:** Reference intervals have been established using 3.2 percent buffered sodium citrate.

3. Fill light blue-top tubes as far as vacuum will allow and mix by gentle inversion five to six times. Exact ratio of nine parts blood to one part anticoagulant must be maintained. Inadequate filling of the specimen tube will alter this ratio and may lead to inaccurate results.

Note: Patients who have hematocrit values above 55 percent should have the anticoagulant adjusted to maintain the nine to one ratio by removing a portion of the volume of the citrate solution. To calculate the amount of citrate required in the collection tube or syringe, use the following formula:

$$C = (1.85 \times 10^{-3}) (100 - \text{HCT}) (V_{\text{Blood}})$$

Where:

- C (mL) is the volume of 3.2% citrate remaining in the tube.
- HCT (%) is the hematocrit of the patient.
- V is the volume of blood added (if a 2.7 mL tube is used, then the volume is 2.43 mL to achieve a 9 to 1 blood to anticoagulant ratio).
- 1.85×10^{-3} is a constant.

Example calculation: Patient hematocrit is 60% and blood will be drawn into a 2.7 mL citrate tube. Calculate the adjusted citrate volume:

$$C (\text{mL}) = (1.85 \times 10^{-3}) (100 - 60) (2.43 \text{ mL}) = 0.18 \text{ mL}$$

Then calculate the amount of citrate to remove: $0.27 \text{ mL} - 0.18 \text{ mL} = 0.09 \text{ mL}$ of citrate to remove.

4. Centrifuge the specimen at 1700 x g for 15 minutes or at a speed and time required to consistently produce platelet-poor plasma (i.e., platelet count less than $10,000/\mu\text{L}$). Hemolyzed specimens will be rejected.
5. Immediately remove only the top two-thirds of the platelet-poor plasma from the specimen using a plastic transfer pipette (use of glass transfer pipettes may result in activation and/or clotting of the plasma). Place the plasma in a properly labeled standard transport tube and clearly mark the vial contents as plasma. Glass vials will be rejected.
6. Immediately freeze the plasma in a non-frost-free freezer. A frost-free freezer should not be used due to warming cycles. Specimens may be stored at -20°C or -70°C . Refer to individual test information for frozen stabilities. Specimens must remain frozen during storage and shipment. A separate standard transport tube must be submitted for each assay requested. Room temperature stabilities should be observed if a specimen cannot be frozen immediately. Specimens should be stored capped. Coagulation specimens should not be stored at refrigerated temperatures ($2-8^{\circ}\text{C}$) or on ice.
 - PT specimens, uncentrifuged, centrifuged with plasma on top of cells, or centrifuged with plasma separated from cells should be kept at $18-24^{\circ}\text{C}$ for no longer than 24 hours from the time of collection.
 - aPTT specimens (not drawn to monitor heparin therapy), uncentrifuged, centrifuged with plasma on top of cells, or centrifuged with plasma separated from cells should be kept at $18-24^{\circ}\text{C}$ for no longer than four hours from the time of collection.
 - Specimens for other coagulation tests have variable stabilities and should be stored in the same manner as aPTT specimens.
 - If testing cannot be performed within these times, platelet-poor plasma should be removed from the cells and frozen at -20°C or at -70°C . Refer to individual test information for frozen stabilities.
7. **Note:** Anticoagulant medications often interfere with coagulation studies and this should be considered when ordering and interpreting coagulation tests.