MAYO CLINIC LABORATORIES

Coagulation Guidelines for Specimen Handling and Processing

The Mayo Clinic Coagulation Laboratories have been performing coagulation factor testing on mailed-in specimens for many years. Accurate results can only be obtained on properly prepared specimens. The physician interpreting results may be misled by abnormal results obtained in mishandled specimens. To ensure the best possible specimen, follow collection requirements as closely as possible. Adherence to these guidelines will improve coagulation study results.

1. **Patient should be fasting**, if possible; for certain tests, the patient cannot be receiving anticoagulant medication:
   - Heparin
   - Warfarin/Coumadin
   - Direct thrombin inhibitor: Pradaxa (dabigatran), Acova (argatroban)
   - Direct Xa inhibitor: Xarelto (rivaroxaban), Eliquis (apixaban), Savaysa (edoxaban)
   - tPA (tissue plasmin activator)

2. **Draw blood from the patient into light blue-top (sodium citrate) evacuated tubes** containing 3.2% sodium citrate.
   a. If the patient's hematocrit is $\geq$55%, the volume of anticoagulant in the tube should be adjusted. If the hematocrit is between 55% and 65%, it is acceptable to remove 0.1 mL of the citrate anticoagulant from the tube and not perform the calculations. This will account for most of the patients with high hematocrits, since very few patients will have a hematocrit that is >65%. For those patients with hematocrit $\geq$65%, use the following formula to determine the correct anticoagulant volume:

      $C = (1.85 \times 10 - 3)(100 - HCT)(V\ Blood)$

      Abbreviations: C= volume of citrate remaining in the tube, HCT = patient's hematocrit, and V = the volume of blood to be added. (If a 5-mL tube is used, V= 4.5 mL)
   b. Blood may be drawn from a vascular access device (VAD). If possible, draw sample from a VAD lumen that has not been heparinized. If none available, the line should be flushed with 5 mL of saline, and the first 10 mL (adult) or 3 mL (pediatric) of blood discarded or used for other purposes.
   c. Tubes should be >90% filled. A clean venipuncture is essential to avoid activation of coagulation by tissue thromboplastin.
   d. Mix gently by inverting the tube end over end 5 to 6 times. Avoid vigorous mixing or additional inversion. Observe for the presence of clots. Specimens containing fibrin clots will, in most cases, be rejected.
   e. Transport at ambient temperature to the processing site or facility, and maintain at ambient temperature until processed.
   f. Sample processing ideally should take place within 1 hour of collection time; however, it must be completed within 4 hours of collection time.

3. **The specimen must be double-centrifuged to prepare a platelet-free plasma specimen (platelet count <10,000/mcL).**
   a. Immediately centrifuge specimen at 2,301 g for 10 minutes.
   b. Carefully remove plasma from cells, avoiding the platelet/buffy coat.
   c. Dispense into a plastic tube using a plastic transfer pipette. Do not pour off!
   d. Centrifuge aliquoted plasma at 2,301 g for 10 minutes.
   e. Remove the top portion of plasma, leaving approximately *250 mcL in the bottom to* ***discard***.
   f. The double-centrifuged plasma should be aliquoted (1 to 2 mL per aliquot) into clearly labeled plastic tubes. The number of tests ordered will determine the aliquots needed. Generally, a 1 mL aliquot per test is required, although test volumes may be combined up to 2 mL of plasma per aliquot. Pay particular attention to the amount of specimen required for the ordered tests. Coagulation profiles (see individual test specimen requirements) and multiple single-test orders will require multiple aliquots.
   g. After centrifugation, examine the plasma for fibrin clots and pour the cellular portion through gauze to observe for small red cell clots. Clotted specimens must be discarded and recollected.

4. **Specimens should be frozen at below -40°C**, if possible, and sent together in the same container with at least 5 lbs of dry ice. Specimens must arrive frozen.

5. **Include the requested information** (see individual test descriptions) as the testing and interpretations are dependent on clinical history in many of the more complex abnormalities.

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Reference

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