المختبر المرجعي الوطني
National Reference Laboratory
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INSTRUCTIONS FOR COLLECTION AND PROCESSING OF SAMPLES FOR COAGULATION TESTING

The accuracy of coagulation testing results is highly dependent upon the integrity of the sample. To ensure the best possible specimen, **please follow the collection requirements as closely as possible.** Adherence to these guidelines will improve the quality and reliability of the results.

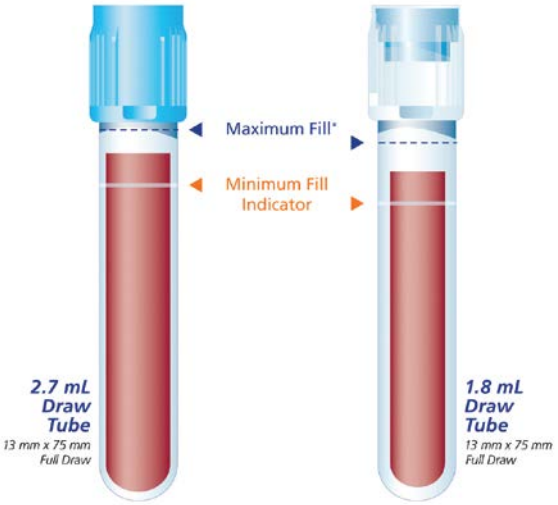
Patient Preparation:

For certain coagulation and factor assays tests, the patient should not be under therapy with the following anticoagulant medication:

- Anticoagulants: Heparin/Warfarin/Coumadin
- Direct thrombin inhibitor: Pradaxa (dabigatran), Acova (argatroban)
- Direct Xa inhibitor: Xarelto (rivaroxaban), Eliquis (apixaban)

Sample collection:

1. Collect blood into a **light blue cap Vacutainer tube** (nine parts of freshly collected blood with one part of 3.2% or 0.11 mol/L of sodium citrate). **The tubes must fill completely to the proper level** so that the correct blood-to-anticoagulant ratio is achieved.
2. A **clean venipuncture** is essential to avoid activation of coagulation by tissue thromboplastin.
3. **Invert the tube gently 4-6 times immediately** after venipuncture to ensure proper mixing of blood and anticoagulant. Excessive shaking or mixing vigorously may cause hemolysis or platelet clumping/activation, potentially leading to erroneous results.



4. If an **evacuated tube** system is used, the coagulation **sample should be the second tube** collected, unless a PT or APTT only is ordered. **Tubes with additives are NOT to be collected before the coagulation sample.**
5. If blood is drawn from an **indwelling catheter**, heparin or saline contamination may be a possibility. The line should be **flushed with 5 mL of saline and the first 5 mL of blood or six dead space volumes of the catheter discarded**.
6. If the patient's hematocrit is > 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.**

Handling Conditions:

1. Specimens **must be processed within four hours of collection**.
2. Before centrifugation, **check the whole blood specimen for clot formation** by visual evaluation or removing the cap and checking with two wooden sticks. The presence of a clot invalidates the sample and requires the sample to be re-drawn.
3. **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 test(s). Coagulation profiles (see individual test specimen requirements) and multiple single-test orders will require multiple aliquots.
4. Make arrangement such that **the specimen(s) are to be delivered to the laboratory immediately** after collection. **If specimens will not arrive within four hours, the plasma should be separated and frozen.**
5. **Please 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.

Centrifugation Technique and Processing Whole Blood Plasma for Shipping

The specimen must be double-centrifuged to prepare a platelet-free plasma specimen (platelet count <10,000/mcL).

1. **Immediately centrifuge specimen at 1,500 x G for 10 minutes** to achieve platelet-poor plasma (platelet count <10,000/µL).
2. 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.**
3. Using a plastic transfer pipette carefully **remove the top portion of plasma leaving approximately 200 uL in the bottom** to discard platelet/buffy coat. Dispense into a plastic tube using ONLY plastic transfer pipette. Do not pour off to avoid the platelet/buffy coat contamination.
4. **Repeat the second centrifugation** to ensure platelet-poor plasma.
5. **Dispense the twice centrifuged plasma** into two properly labeled plastic tube (Note that the specimen is plasma).
6. **Freeze the plasma immediately**. Specimen MUST remain frozen until the testing is performed.

Rejection:

A specimen will be rejected if it is **mislabeled**, **unlabeled**, **clotted, collected in the wrong tube**, **has visible hemolysis**, **thawed plasma**, **exceeds the stability limit**, or has **less than 90% expected fill of the collection tubes**.