Collection Protocols: Coagulation Testing

General Drawing Instructions

1. 3.2% Sodium citrate plasma shipped frozen is the only acceptable sample type for coagulation testing. All other anticoagulants (heparin, EDTA, oxalate) are NOT acceptable.
2. Proper 9:1 blood to anticoagulant ratio is required. Routine collection yields 4.5 mL blood added to 0.5 mL sodium citrate. The vacutainer tube must be completely full for this ratio to be achieved.

   Note: For patients with normal hematocrits, no anticoagulant adjustment is necessary. If the patient has an **elevated hematocrit** (as in the case of patients with Polycythemia vera), **the amount of anticoagulant in the tube must be adjusted.** Patients with a high hematocrit will have less plasma with an increased concentration of sodium citrate. The test result will be falsely prolonged in a patient with a hematocrit of 55% or greater. The amount of sodium citrate should be adjusted using the following formula:

   Formula for 5.0 mL Sodium Citrate Tubes:

   Anticoagulant vol. (X) =

   $$\frac{100 - \text{Hematocrit}}{595 - \text{Hematocrit}} \times \textbf{\#1}$$

   #1 = Total volume of anticoagulated blood required*

   Example: Patient hematocrit = 60%

   $$\frac{100 - 60}{595 - 60} \times 5.0 = .37 \text{ mL sodium citrate}$$

   *5.0 mL for standard

3. To avoid contaminating the sample with tissue thromboplastin or heparin, follow the guidelines below. These substances may alter results.

   - The venipuncture must be clean with no trauma.

   - Hemolyzed samples are not acceptable.

   - The first 5.0 mL of blood drawn from a patient should not be used for coagulation testing, unless using a vacutainer/needle assembly for Protime and APTT draw.
   - If drawn through an indwelling catheter, the first 15 mL of blood must be discarded or used for other laboratory tests before the specimen for coagulation testing can be obtained.

4. After adjusting the anticoagulant, the blood must be drawn in a syringe and the tubes filled from the syringe as the vacuum has been removed from the tube. Mix the sample gently by inverting the tube several times immediately after filling. Do not shake the tube as this will break down fibrinogen in the sample. li>

Procedure for Preparing Platelet-Poor Plasma
Follow steps 1-4 of General Drawing Instructions.

5. The fewer platelets in specimen for coagulation testing, the less interferance and greater the accuracy of results.

6. Platelet-poor plasma (PPP) should have a platelet count of less than 10×10^9 per liter. It is necessary for laboratories to employ double centrifugation in order to achieve optimal PPP. The double centrifuge technique is:

   -Spin down specimen---within 30 minutes of specimen draw time---at 3000 RPM (1600 g) for a full 10 minutes.

   - Transfer the plasma to a plastic tube with a plastic Pasteur pipet, staying away from the buffy coat layer.
   Do not use glass tubes or glass Pasteur pipets as glass can activate the clotting cascade.

   - Spin down the plasma portion again at 3000 RPM for 10 minutes. With another plastic Pasteur pipet, transfer the plasma to another plastic tube, staying clear of the bottom of the tube where the platelets lie. This will yield a sample that is as platelet free as possible. This will also give platelet-poor plasma. It is essential to remove as many platelets as possible, as any residual platelets will lyse due to the freezing process required for shipping. Platelet factors released will interfere with coagulation testing.

7. Transfer plasma to a plastic tube and quick freeze the sample. Samples must remain frozen in transit. Ship on dry ice.