Appendix E

COAGULATION
Specimen Collection and Processing

Introduction

To obtain reliable results in coagulation testing, it is imperative to begin with proper sample collection. The four primary factors necessary for a good quality sample are a trauma free collection, free flow of blood, immediate and proper mixing of blood with the anticoagulant, and gentle handling of sample after collection. A fifth factor, patient's predisposition, also is an important but is outside the control of a phlebotomist (blood collector). If one or more of the primary collection factors fail to meet ideal standards, the sample may be compromised. Test results are a direct reflection of sample integrity.

CAUSES FOR SAMPLE REJECTION: Specimens that are clotted, hemolyzed, contaminated with heparin or I.V. fluid, sample exceeded sample stability time limit, tubes under-filled or over-filled, or inappropriate anticoagulant.

Centrifugation: Double Centrifuge Technique

1. Purpose: For ALL FROZEN SAMPLES. This procedure ensures that the plasma submitted is platelet-free-plasma (<10,000 platelets/mm^3). Assays associated with lupus coagulation, hypercoagulation profile and other platelet sensitive assays require platelet free plasma.
2. Select a centrifuge with a centrifugal force of 2000-2800g.
3. Label <u>three</u> non-polystyrene, plastic aliquot tubes (e.g., polyurethane, polypropylene, or polyethylene). **<u>DO NOT USE GLASS TUBES.</u>**
4. Centrifuge the primary collection tube for 10-15 minutes.
5. Without disturbing the buffy coat layer of platelets and WBCs, transfer about ¾ of plasma to the first labeled aliquot tube.
6. Seal aliquot tube with parafilm or tube cap
7. Re-centrifuge specimen at same settings
8. While not disturbing the button, transfer equal amounts of plasma into <u>two</u> labeled aliquot tubes.
9. Identify the contents of the aliquot tubes as "FROZEN CITRATE PLASMA.
10. Double seal the aliquot tube by tightly securing tube cap and then wrapping parafilm around the cap
11. Immediately, place aliquot tubes in a freezer after processing. **<u>DO NOT FREEZE PLASMA IN GLASS TUBES.</u>**
12. DO NOT transport until sample completely frozen (i.e., frozen solid for at least 1 hours prior to shipping). DO NOT thaw sample after freezing.

Sodium citrate samples

1. **WHOLE BLOOD**: If a protime (PT) and INR is ordered as the only coagulation assay and it will arrive in the laboratory within 24-hours of collection, send the sample unopened (STOPPER seal unbroken). Store at room temperature and transport cool. Avoid refrigeration when possible. If a PT is ordered in conjunction with other coagulation tests, process as plasma.
2. **FROZEN PLASMA**: For coagulation tests requiring plasma, process samples immediately upon collection. Process samples by centrifuging the sample twice for 10-15 minutes with a centrifugal force of 2000-2800g (see Double Centrifuging Technique). Separate sample into two aliquot tubes. Freeze immediately. Transport frozen. DO NOT transport until sample completely frozen (i.e., frozen solid for at least 1 hours prior to shipping). DO NOT thaw sample after freezing.
3. **SODIUM CITRATE CONCENTRATION**: Collect all coagulation samples requiring sodium citrate with a concentration of 3.2% (0.105 M)

All coagulation tests that require serum should be drawn in plain red top collection tubes !

Primary Collection Tubes

Serum Citrate Tubes - Filled with a buffered tri-sodium citrate solution with a concentration of 3.2% (0.105 M) which mixes with blood after the tubes are gently inverted 5 - 8 times.

Serum Tubes - Do not contain an anticoagulant, but are coated with micronized silica particles that help activate clotting when the tubes are gently inverted 5 -8 times.

Coagulation Samples

Introduction - It is imperative to begin with proper sample collection to obtain reliable results in coagulation testing. The four primary factors necessary for a good quality sample are:

1. trauma free collection
2. free flow of blood
3. immediate and proper mixing of the blood with the anticoagulant
4. gentle handling of the sample after the collection

If one or more of the collection factors fails to meet the ideal standards, the sample may be compromised. Test results are a direct reflection of sample integrity.

Causes for Sample Rejection - Samples that are clotted, hemolyzed, contaminated with heparin, or IV fluid, sample exceeded the stability time limits, tubes that re over or under filled or inappropriate anticoagulant.

Whole Blood - If a Protime (PT) and INR is ordered as the only coagulation assay and it will arrive at the laboratory within 24-hours of collection, the sample may be sent unopened (Stopper seal unbroken). Transport and maintain room temperature. Avoid refrigeration. If PT is ordered in conjunction with other tests, process as frozen plasma.

Frozen Plasma - Process samples immediately upon collection. Coagulation testing requires platelet poor plasma for accurate testing. Centrifuge the specimen with a g-force of 2000 - 2900 g for 10 to 15 minutes. Separate the plasma into plastic tubes and re-spin the samples as above. Transfer the plasma into two labeled aliquots, again in plastic tubes. **DO not use glass tubes.** Freeze the tubes immediately and transport frozen. Do not allow sample to thaw after freezing.

Serum - Coagulation assays requiring serum should be transported frozen. Using a plain red top tube allow the blood to clot completely for at least 30 minutes. Centrifuge for 10 - 15 minutes. Transfer the serum to a plastic aliquot tube and freeze immediately. Transport frozen and do not allow the sample to thaw.

Hematocrit Below 20% or Above 55% - If a patient's hematocrit is below 20% or above 55%, the volume of anticoagulant to plasma will not fall into the proper 1:9 ratio. Low hematocrits shorten a clot time and patients with high hematocrits falsely prolong clot times. If a special collection tube is needed call the Special Coagulation Laboratory at (254) 724-2426.