COAGULATION SPECIMEN HANDLING AND PROCESSING

☒ St. Joseph Medical Center, Tacoma, WA
☒ St. Francis Hospital, Federal Way, WA
☒ St. Clare Hospital Lakewood, WA
☒ St. Anthony Hospital Gig Harbor, WA
☒ St. Elizabeth Hospital Enumclaw, WA
☒ Highline Medical Center Burien, WA
☒ Harrison Medical Center, Bremerton, WA
☒ Harrison Medical Center, Silverdale, WA
☒ PSC

PURPOSE

To provide instruction on how to process and handle coagulation samples being sent to the laboratory to ensure platelet poor plasma for testing.

BACKGROUND

Tests of the coagulation system are very sensitive to storage times and temperatures, concentration of anticoagulants, and surface of the containers that contain the blood. It is important to take these factors in consideration during the collection, storage and transport processes of these particular specimen types.

RELATED DOCUMENTS

CLSI H21-A5 Collection, Transport and Processing of Blood Specimens for Testing Plasma-Based Coagulation Assays and Molecular Hemostasis Assays; Approved Guideline—Fifth Edition
CLSI H3-A5 Procedures for the Collection of Diagnostic Blood Specimens by Venipuncture; Approved Standard —Sixth Edition
Anticoagulant Adjustment for High Hematocrits

SPECIMEN REQUIREMENTS

- Citrated blood (9:1 blood to anticoagulant) 3.2% sodium citrate (blue top). Follow CLSI guidelines H3-A5 and H21-A5. No other anticoagulant is acceptable.
- Samples that are short-filled (refer to B-D Vacutainer fill chart), over-filled or clotted are unacceptable for coagulation testing and will be rejected in the laboratory.
- Samples from patients receiving heparin (unfractionated heparin and/or low molecular weight heparin) should be processed immediately.
- Tubes should be labeled according to standard with at least two patient identifiers and the collection date/time.

COLLECTION NOTES

- If the collection is done using a winged butterfly set a waste tube must be drawn to displace air in the line that will cause under filling of the blue tube.

- When using collection devices other than winged butterfly set and when only coagulation studies are ordered, it is not necessary to draw a “waste” tube before drawing the citrate tube (per BD and CLSI recommendations).

- Draw the coagulation sample according to established protocol and procedures. If using a transfer device, the blood must be transferred from syringe to anticoagulated tubes within one minute to prevent clotting.

- The sample must be drawn as atraumatically as possible to avoid contamination with tissue factor, activation of clotting factors or platelets, and hemolysis. Do not leave the tourniquet on for more than one minute. Also avoid excessive pumping of the hand, or slapping to raise a vein. If a good blood flow has been established, loosen the tourniquet before drawing the coagulation samples.

- HEMOLYSIS IS UNACCEPTABLE for the more specialized coagulation tests. MARKEDLY HEMOLYZED SPECIMENS WILL BE REJECTED.

- Even though minimum **PLASMA** requirements for a test may be as little as 1.0 mL, **MINIMUM REQUIREMENT IS A FULL COAG TUBE**. There may be a line at the top of the tube that is the fill-to line. Tubes that are filled under or exceedingly over this line will be rejected. Coagulation testing and accurate test results are based on a ratio of 9 parts blood to 1 part anticoagulant and since the anticoagulant stops blood from clotting by removing a portion of the calcium from plasma, underfilling the tube removes too much calcium leading to inaccurate patient results.

- Specimen tubes from patients whose hematocrits are 55% or higher lead to smaller plasma volumes. The smaller plasma volume leads to a disproportionately higher calcium loss therefore anticoagulant volume must be adjusted for patients with high hematocrits. Contact the main laboratory for more information regarding the collection of these types of specimens.

PLATELET POOR PLASMA GUIDELINES

1. If centrifuge has been validated per policy for Platelet Poor Plasma centrifuged and test performed at facility, the double spin plasma Does Not Apply.
    - Sendouts - To obtain plasma suitable for freezing for coagulation testing, double spin coagulation samples to ensure that the plasma is platelet poor. (See below)

2. If a centrifuge has not been validated for platelet free plasma, double spin all coagulation samples to ensure that the plasma is platelet poor and suitable for freezing for coagulation testing. (See Sample Storage/Stability/Transport below)

SAMPLE STORAGE/STABILITY/TRANSPORT

See Laboratory test directory for specimen stability requirements.

Whole Blood Sample Stability/Handling/Transport

- If only a prothrombin time (PT) is ordered and the sample will reach the testing lab within 22 hours after collection, the sample must be stored and transported at room temperature.

- If only screening tests are requested (PTT, Fibrinogen, D-Dimer) and the sample will reach the testing lab within 3 hours of collection, the sample must be kept at room temperature; otherwise it must be double spun (see below) to produce platelet poor plasma, aliquoted, and frozen.

- DO NOT FREEZE A WHOLE BLOOD SAMPLE.

Plasma Stability/Handling/Transport

- All other coagulation tests (unfractionated heparin, low molecular weight heparin, and thrombin time) require that the specimen be processed for platelet poor plasma (double spun) and frozen as quickly as possible after the specimen is drawn, if testing will not be performed within established time period. The plasma must remain frozen until the test is performed.

- SPECIMENS MUST BE CHECKED FOR CLOTS. This may be done before centrifuging the specimen or after the plasma has been removed. If several tubes are drawn and the plasma is to be pooled and realiquoted, it is preferable that the tubes be checked for clots prior to centrifugation in case one of several tubes to be used for the pool is clotted. CLOTTED TUBES WILL BE REJECTED.

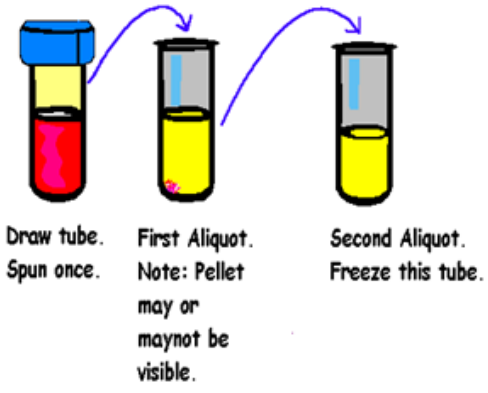
- **How to double-spin plasma:**
  To achieve platelet poor plasma without a platelet free plasma validated centrifuge a specimen must be processed by the double-spin method.

    - Centrifuge whole blood

    - Transfer the top two thirds of the plasma into a plastic aliquot tube. Maintain two patient identifiers on all aliquots.

    - Cap the aliquot tube and centrifuge the plasma again.

    - Being careful not to disturb the cells at the bottom of the tube, transfer the top two thirds of the respun plasma to a plastic aliquot tube and freeze. **NOTE**: Failure to produce platelet-poor-plasma results in residual platelets, which are a significant source of interference in coagulation testing.



TRANSPORT REQUIREMENTS

Frozen specimens: Store frozen and transport specimens on dry ice.

Test	Process
• PT only	Deliver capped whole blood sample at room temperature within 22 hours of collection, if delayed send platelet poor plasma frozen.
• PTT and/or PT • D-Dimer	Deliver capped whole blood sample at room temperature within 3 hours of collection. If delayed, send platelet poor plasma frozen.
• Fibrinogen • Heparin (UFH and LMWH) • Thrombin Time • Mixing Studies	Deliver capped whole blood sample at room temperature immediately. If delayed beyond 1 hour send platelet poor plasma frozen. Note: All other coagulation tests (ufh, lmwh, tt)—if the sample will **not** reach the testing lab within 1 hour of collection, the sample must be processed for platelet poor plasma by the double spin method.