

COAGULATION SPECIMEN HANDLING AND PROCESSING

Specimen Requirements for Blue Top Tubes (3.2% Sodium Citrate)

1. Please indicate in the order or on the laboratory requisition if the patient is on heparin or coumadin.
2. Under-filled or over-filled blue top tubes are **unacceptable** for coagulation testing.
3. Samples from patients receiving heparin should be processed immediately.
See Heparin Assay-UFH (HEPUFH) and Heparin Level-LMW (HEPLMW) sample requirements.
 - All the screening tests (Protime, PTT, Fibrinogen, D-Dimer) or any combination of the listed screening tests can be performed on a single 1.5 mL plasma aliquot.
 - Thrombin Time requires a separate aliquot.
 - Each additional test requires a separate 0.5-mL aliquot of plasma.
 - Samples that require treatment with a heparin adsorbent require a separate 1.0-mL aliquot for each test.
 - Platelet Function Analysis requires a separate blue top whole blood tube.

Labels

Tubes should be labeled with the patient's full legal name, collection date/time, and if you are not sending the sample in the primary tube label with sample type (for example blue top plasma).

Patient Information

Please note on the test request if the patient is on heparin or coumadin. Some tests (in addition to the Protime and PTT) are affected by the presence of heparin or coumadin.

****REFER TO INDIVIDUAL TEST DESCRIPTIONS FOR EXCEPTIONS TO THIS PROTOCOL****

Delivery of Samples

Test	Sample Time
NON-HEPARINIZED PATIENT	
PTT and/or Protime	Deliver capped whole blood sample at room temperature within 3-hours of collection. For delayed delivery, send platelet poor plasma frozen. Protime can be included with this collection and sample time. If a patient is on heparin see **HEPARINIZED PATIENT** lower in this table.
D-Dimer	
Fibrinogen	
Other Coag Testing	Deliver capped whole blood sample at room temperature within 3 hours. For delayed delivery send platelet poor plasma in individual frozen aliquots for each test requested.
Platelet Function Analysis	Deliver capped whole blood samples at room temperature within 3 hours. Requires separate tube.
ProTime only (no other coagulation testing requested)	Deliver capped whole blood sample at room temperature within 23-hours of collection, if delayed, send platelet poor plasma frozen.
HEPARINIZED PATIENT	
Heparin Assay or Fondaparinux (Unfractionated or low molecular weight)	**Samples from patients receiving heparin must be processed for platelet poor plasma immediately**
PTT, D-Dimer, Fibrinogen, Protime, Other Coag testing.	

Collection of Sample for Coagulation Studies

1. ANTICOAGULANT: Use blue top tube, 3.2% sodium citrate anticoagulant.
(NOTE: The majority of coagulation tests require sodium citrate anticoagulant but there are exceptions. Refer to the individual tests in the directory for specific specimen requirements.)
2. If using the Vacutette® system the blue top tube must not be the first drawn. If only coagulation specimens are being collected, draw at least 2-mL of blood into the first tube, then discard that tube.
3. If using the two-syringe technique, unscrew the safety needle and dispose of it in an approved sharps container. Screw a blood transfer device into the syringe. You can now safely fill vacuum tubes as needed, use care not to force blood into the tubes, run the blood gently down the side of the tube. Immediately after filling the tube, invert the tube GENTLY five or six times to mix. When transfer is complete, discard the entire assembly (syringe and transfer device) in an approved sharps container. Never disassemble equipment, dispose of it in its entirety. Blood must be transferred from syringe to anticoagulated tubes within one minute to prevent clotting.
4. 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 coag samples.
5. HEMOLYSIS IS UNACCEPTABLE for the more specialized coagulation tests. Screening tests (Protime, PTT, Fibrinogen, D-dimer and Thrombin Time), can be performed on slight to moderately hemolyzed specimens, for special coagulation testing any hemolyzed specimen will be rejected. **MARKEDLY HEMOLYZED SPECIMENS WILL BE REJECTED FOR ALL COAGULATION TESTING.**
6. UNDERFILLED OR OVERFILLED TUBES ARE UNACCEPTABLE. Even though minimum PLASMA requirements for a test may be as little as 0.1 mL, **MINIMUM REQUIREMENT IS A FULL COAG TUBE.** There is a black triangle located at the top of the label that is the fill-to line, tubes that are filled under or 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. For patients **whose hematocrits are 55% or higher**, a smaller plasma volume leads to a disproportionately higher calcium loss therefore anticoagulant volume must be adjusted for patients with high hematocrits. Call the Coagulation Laboratory at (802) 847-5121 for instructions.

Platelet Poor Plasma Processing

1. If only a protime is ordered and the sample will reach the lab within 23 hours after collection, the sample must be stored and transported at room temperature.

If only screening tests are requested (PTT, Fibrinogen, D-Dimer, Thrombin Time) and the sample will reach the lab within 3 hours of collection, the sample must be kept at room temperature.

DO NOT FREEZE A WHOLE BLOOD SAMPLE.

All other coagulation tests require that the specimen be processed for platelet poor plasma and frozen as quickly as possible after the specimen is drawn. The plasma must remain frozen until the test is performed.

2. **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 MUST BE REJECTED.**
3. To obtain plasma suitable for freezing for coagulation testing, the capped specimen tube must be centrifuged. It is recommended that a swing bucket rotor be used to minimize remixing of the plasma and platelets. A double-spun method is required.
4. To **double-spin plasma**: Spin whole blood and transfer the top two thirds of the plasma into a plastic aliquot tube, cap the aliquot tube and respin the plasma. 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 tube and freeze. If the plasma is for multiple tests, prepare a separate aliquot for each test. Failure to produce platelet-poor-plasma results in residual platelets, which are a significant source of interference in coagulation testing. Contact the

Hematology laboratory for further guidance ((802) 847-5121). Samples for heparin levels, tests used to detect Lupus-like inhibitors and tests on heparinized patients must be centrifuged as outlined above and frozen within one hour of collection.

5. If a delivery system other than the UVMCC courier is used, frozen specimens should be shipped in a styrofoam container with at least 5 pounds of dry ice via a courier with a guaranteed overnight delivery.

Specimen Handling for Thrombosis Panels

1. There are 2 orderable Thrombosis Panels:

- a. **Thrombosis Panel with Coumadin**, charge code TP1C. Testing includes: [Prothrombin time](#), [PTT](#), [D-Dimer](#), [Cardiolipin IgG & IgM AB](#), [APC Resistance V](#), [Antithrombin 3-Functional](#), and [Factor 8](#).

Thrombosis Panel WITHOUT Coumadin, charge code TP1. Testing includes: [Prothrombin time](#), [PTT](#), [PTT 50/50 mixing study](#), [D-Dimer](#), [Cardiolipin IgG & IgM AB](#), [Antithrombin 3 Functional](#), [Lupus Anticoagulant Cascade](#), [Activated Protein C Resistance V](#), [Protein C Functional](#) (if low, Protein C Antigen done), [Protein S Functional](#) (if low Protein S Antigen done).

Prothrombin time and PTT are subject to Medicare Local Medical Review Policy. Only order these panels if all test components are medically necessary. May only be ordered if laboratory has a signed "Physician Acknowledgement of Custom Profile" on file. Contact your Lab Outreach Specialist to obtain this form.

2. Collect and process samples for either panel in the following manner. Be sure to label all tubes with 2 patient identifiers and the date and time collected.

- a. **COLLECT:**
 - 6 Blue tops
 - 1 Serum Gel Tube

PROCESSING AND STORAGE

1. Process **blue tops** as for platelet poor plasma. Label each aliquot tube as "Blue top plasma" along with 2 patient identifiers and the date and time collected. Freeze plasma and send to the lab on dry ice

Aliquot: 2-plastic tubes with 1.0 mL each

6-plastic tubes with 0.5 mL each

2. Spin the **serum gel tube**. Send 1-mL serum refrigerated.