Porter Medical Center Laboratory
115 Porter Drive
Middlebury, VT 05753

COAGULATION SPECIMEN COLLECTION & HANDLING

Specimen Requirements

1) Please indicate on laboratory requisition if patient is on heparin or Coumadin.
2) PT with INR, APTT, and D-Dimer or any combination of these tests can be performed on a single 1.0 ml Platelet Poor Plasma aliquot.
3) Any Coagulation test that is referred to a Reference Lab requires TWO 1.0-ml aliquot of Platelet Poor Plasma.
4) ***If only a PT with INR is ordered and the sample will reach the lab within 22 hours of collection, the whole blood sample should be sent at room temperature/ambient. DO NOT refrigerate.***
5) If only screening tests are requested (APTT, Fibrinogen, D-Dimer, Thrombin Time), the patient is not on heparin, and the sample reaches the laboratory within 3 hours of collection, the whole blood sample may be sent at room temperature.
6) All other coagulation tests require that the specimen be spun and the platelet poor plasma frozen as quickly as possible after the specimen is drawn. The plasma must remain frozen until the test is performed.
7) **It is recommended that any Special Coagulation tests be collected at the laboratory.**

Collection

1) ANTICOAGULANT: Use 3.2 % sodium citrate anticoagulant.
   a) NOTE: The majority of coagulation tests require sodium citrate anticoagulant but there are exceptions**.** **Refer to the individual tests for specific specimen requirements.**
2) ***Usual blood drawing technique except for the following situations:***
   a) ***If using a vacuum system with a butterfly***, the coagulation tube must not be the first drawn.
      i) Draw at least two mL of blood into a red top tube (not serum gel tube) and then discard that tube.
   b) ***If using the syringe technique***.
      i) When adding blood to a vacuum tube from a syringe, use a transfer device.
      ii) Do not remove cap of tube.
      iii) The sample must be into the anticoagulant within one minute of drawing.
      iv) Allow the appropriate amount of blood to flow into the tube.
      v) Make sure that the syringe is held vertically so that no air bubbles from the syringe enter the tube.
      vi) Immediately after filling the tube, invert the tube GENTLY five or six times to mix.
   c) The sample must be drawn as a traumatically as possible to avoid contamination with tissue factor, activation of clotting factors or platelets, and hemolysis. Avoid leaving the tourniquet on for an extended time (over 1 minute), 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.
3) Hemolysis is unacceptable for the coagulation tests. Screening tests (PT with INR, APTT, Fibrinogen, and Thrombin Time) will be performed on a slightly hemolyzed specimen.
   a) Moderate to markedly hemolyzed specimens will be rejected.
4) **Under Filled Tubes Are Unacceptable.**
   a) Even though minimum plasma requirements for a test may be as little as 0.1 mL, minimum whole blood requirement is a full coagulation tube. Tubes that are filled with less than 90% of the stated tube volume will be rejected.
   b) 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, under filling the tube removes too much calcium leading to inaccurate patient results.
   c) This ratio must also be maintained when coagulation samples are obtained from patients whose Hematocrit are 55% or higher. The smaller plasma volume leads to a disproportionately higher calcium loss therefore anticoagulant volume must be adjusted for patients with a high Hematocrit. Call Porter Hospital Laboratory at 388-4747 for instructions if your patient has a Hematocrit >55%.

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Processing - Preparing Platelet Poor Plasma (PPP):

1) Whole blood specimen must be processed within 3 hour of draw by one of the methods below:
2) Centrifuge whole blood blue top tube for 15 minutes at 1500 x g to 2500 x g
3) Retrieve tube from centrifuge and remove the cap.
4) Without disturbing the buffy coat layer between the plasma and the red cells, transfer the top 80% of plasma with a pipette into a plastic pour-off tube.
5) Re-centrifuge the separated plasma in the pour-off tube for an additional 10 minutes.
6) Retrieve centrifuged pour-off tube from centrifuge and remove the cap.
7) Transfer the top 80% of that re-centrifuged plasma with a pipette to another plastic pour-off tube.
8) This second plastic pour-off tube now contains the processed plasma that will be used for analysis.
9) In most cases the PPP will need to be frozen.

Delivery of Samples

REFER TO INDIVIDUAL TEST DESCRIPTIONS
FOR EXCEPTIONS TO THIS PROTOCOL

Test	Sample Time
NON-HEPARINIZED PATIENT	
APTT and/or PT with INR	Deliver capped room temperature whole blood within 3-hours of collection. For delayed delivery, send frozen plasma. PT with INR can be included with this collection and sample time. **Refrigerated whole blood unacceptable**.
D-Dimer	
Fibrinogen	
Other Coag Testing	Deliver within 1-hour of collection. For delayed delivery prepare Platelet Poor Plasma and submit 2 aliquots of frozen plasma for each test requested.
Platelet Function Analysis	**MUST BE DRAWN AT PORTER LAB. REQUIRES APPOINTMENT**
PT with INR only (no other coagulation testing requested)	Deliver capped room temperature whole blood within 22-hours of collection. For delayed delivery prepare Platelet Poor Plasma and submit 2 aliquots of frozen plasma for each test requested. **Refrigerated whole blood unacceptable**.
****HEPARINIZED PATIENT****	
Heparin Assay (Unfractionated or low molecular weight)	Deliver immediately; sample must be processed as soon as possible after draw, preferably within 30 minutes. For delayed delivery prepare Platelet Poor Plasma and submit 2 aliquots of frozen plasma for each test requested. **Refrigerated whole blood unacceptable**.
APTT, D-Dimer, Fibrinogen, PT with INR, Other Coag testing.	
****Samples from patients receiving heparin must be processed immediately****	