

Collection Procedures for Coagulation and Molecular Hemostasis Testing

These guidelines cover the necessary procedures for proper collection, preparation, labeling, storage, packaging and transportation of serum and/or plasma-based coagulation and molecular hemostasis tests. As many pre-analytical variables may affect test results, phlebotomists and specimen managers must carefully adhere to all sample handling criteria. Properly following these instructions will assure specimen integrity is maintained to provide accuracy of diagnostic and therapeutic decisions made based on the coagulation results.

Specimen Collection

Prior to collecting the specimen, review the specimen requirements of each assay requested. Note the proper specimen type to be collected, volume requirements and special collection and shipping instructions. All specimens should be collected, labeled, handled, and stored in a manner which respects and protects patient privacy with adherence to the Health Insurance Portability and Accountability Act (HIPAA).

The patient and patient's specimens collected must be properly identified at the point of collection and tubes labeled in the patient's presence, with the following information:

1. patient's full name
2. unique patient identification number (not social security number)
3. date and time of collection
4. initials of person performing the collection
5. specimen type if a secondary aliquot tube is used (anticoagulant type versus serum)
6. assay requested (optional)

The information should be firmly affixed to the tube if an encoded label is used, or handwritten legibly with indelible marker.

Information for Test Request Form (TRF)

A properly completed and legible TRF must accompany each specimen submitted to the laboratory for testing. Some facilities may have the capability to submit orders electronically. The following information should be included:

1. patient's full name
2. unique patient identification number (not social security number)
3. patient's sex
4. an accessioning number if relevant to the referring facility
5. date of birth
6. collection date and time
7. specimen type submitted (whole blood, plasma, etc)
8. assay(s) requested
9. relevant clinical and laboratory information
10. health provider's name
11. billing information (copies may be separately included)
12. any other information as needed

In some instances, additional information may be required. In the case of genetic tests, some states may require a consent form or other information may be needed for the laboratory to comment on detection rates.

CLIA regulations further require that we receive physician signatures for add-on testing. Call our Client Services department at (800) 444-9111 to obtain a form with the required information for your signature and it will be sent electronically.

Specimen Collection Techniques for Plasma-Based Assays

Most hemostasis blood specimens may be obtained by routine venipuncture techniques using a blood collection system that draws the specimen directly into a glass or plastic evacuated tube containing the appropriate additive. All coagulation assays should be collected in a container with a non-activating surface.

A winged device, such as the butterfly collection devices, will allow collection to be performed directly into the evacuated tube with appropriate additive. However, when using a winged blood collection device, blood should first be drawn into a discard tube before collecting the sample into the appropriate blood collection tube. Doing so will ensure that air in the tubing of the butterfly device will not adversely affect the blood:anticoagulant ratio {from CLSI guideline sec. 5.2.1.2}. Care should be exercised with the use of needle gauge less than 25 or smaller as hemolysis may occur during collection.

Syringe draws are acceptable, however, it is important to add the blood to the appropriate volume of anticoagulant within one minute of completion of the draw and adequately mix the specimen. A small volume syringe is recommended (≤ 20 mL) as clotting may occur with larger volume syringes during the collection procedure. When transferring the blood to the appropriate evacuated containers, the needle should be removed following safety procedures, a transfer device applied to the syringe and the diaphragm of the rubber tube stopper punctured to allow the evacuated tube to flow slowly into the tube. The blood should never be forced into an evacuated tube by exerting pressure on the syringe plunger. Hemolysis may result, potentially altering the results, or the stopper may pop off, spraying blood.

All samples collected using anticoagulants should be inverted gently three to six times complete end over end inversion to assure adequate mixing of the sample and anticoagulant. Excessive shaking or mixing vigorously may cause hemolysis or platelet clumping/activation, potentially leading to erroneous results.

Green top	Sodium heparin for plasma chemistry- not acceptable for coagulation plasma-based assays or whole blood molecular assays
Light blue top	Sodium citrate for hemostasis-provides citrated plasma for plasma-based coagulation assays and may be used for molecular assays as whole blood
Red top	No preservative or anticoagulant; provides serum for chemistry; may be used for specific coagulation assays
Tiger top	Serum separator tube; no preservative or anticoagulant. Contains clot activator, provides serum for chemistry; may be used for specific coagulation assays requiring serum
Yellow top	Acid citrate dextrose (ACD); provides whole blood-may be used for whole blood molecular assays

Lavender top	Ethylenediaminetetraacetic acid (EDTA); provides whole blood; may be used for some plasma-based coagulation assays and whole blood molecular assays
--------------	---

Collection of Blood Specimens from Indwelling Catheters

When specimens are collected from an indwelling catheter, the blood collection device components (syringe, needle, connecting devices, tubing, etc) should be checked for compatibility and to avoid air leaks which may cause hemolysis and incorrect draw volumes. To prevent possible contamination or dilution of the sample from heparin, saline or other IV fluids, the line should be flushed with 5 mL of saline. The first 5 mL of blood drawn in a syringe, or six “dead space” volumes of the line must be discarded.

Centrifugation Technique and Processing Whole Blood Plasma for Shipping

Before centrifugation, check the whole blood specimen for clot formation by visual evaluation or removing the cap and checking with two wooden sticks. The presence of a clot invalidates the sample and requires the patient be re-drawn. Due to variances in manufacturer make and models with centrifuges, the use of relative centrifugal force (RCF; g-force) as compared to revolutions per minute (RPM) is recommended as RCF takes into consideration rotor size and radius. To calculate RCF:

$$RCF = 1.118 \times 10^{-5} \times r \times n^2$$

where:

r = rotating radius (cm); and

n = speed of rotation (RPM)

To obtain a plasma sample, first centrifuge the capped specimen at 1500 x g for no less than 15 minutes at room temperature. Note “g” stands for g-force or relative centrifugal force, not RPM. Then transfer the plasma from the primary tube with a plastic pipette into a polypropylene centrifuge tube, cap and re-centrifuge an additional 10 minutes at 1500 x g to obtain platelet poor plasma (platelet count <10,000/mm³). This can be achieved by transferring the plasma into a polypropylene tube using a plastic pipette taking care to not disturb the residual platelets that may have been collected at the bottom of the centrifuge tube. The double centrifugation process ensures samples are platelet-poor. Use of glass transfer pipettes or tubes may cause activation of the clotting mechanism and should be avoided. It is especially important that specimens for lupus anticoagulant, heparin, PF4 and MMP-9 activity samples are processed using this technique to be platelet free.

Samples should be processed as quickly as possible, generally in less than four hours from collection time. Transfer tubes should be frozen at -20°C or below for short term storage (less than two weeks) until shipped to the testing laboratory. The use of a household freezer with a self defrosting freezer should not be used for storage of plasma samples as the freeze-thaw cycles may adversely affect specimen integrity.

Collecting Citrated Plasma for Hemostasis Testing

The anticoagulant recommended for use in hemostasis plasma-based assays should be 3.2% trisodium citrate (0.109M), the light blue top tube. Uses of other anticoagulants, such as oxalate, heparin or EDTA, are unacceptable and may cause invalid results.

1. The proportion of blood to the liquid sodium citrate dehydrate anticoagulant volume is 9:1; inadequate filling may lead to inaccurate results. Unless specifically directed, the use of a discard tube is not necessary prior to collecting a citrate plasma sample.
2. Gently invert tubes three to six times to mix immediately after venipuncture collection. Follow specimen identification and labeling procedures as outlined.

3. Process the sample to recover citrate plasma using double centrifugation technique and store plasma at -20°C or below until shipment.
4. Hematocrit adjustment for hemostasis specimens:
The amount of anticoagulant must be adjusted for patients with hematocrits greater than 55% using the following formula:
$$C=1.85 \times 10^{-3} \times (100-H) \times V$$
Where:
C=volume of 3.2% sodium citrate in mL
H=hematocrit in percent
V=volume of whole blood in mL
5. Blood specimens from patients on anticoagulant therapy:
Anticoagulant therapy such as heparin or warfarin affects many test results and it is recommended that this information is provided to the testing laboratory and noted on the test requisition in the medication section. Heparin neutralization may be performed if requested in the “Other tests or profiles” section of the TRF if indicated.

Whole Blood for DNA Analysis

The preferred sample type is EDTA (purple top tube); other acceptable anticoagulants include ACD or citrate whole blood.

1. Fill EDTA, ACD or citrate whole blood tube completely, invert six times to mix.
2. Follow specimen identification and labeling procedures as outlined.
3. Whole blood samples may be stored at room temperature or 2-8°C, not to exceed 72 hours prior to shipment.
4. Specimens may be shipped at either ambient temperature or 2-8°C overnight; whole blood is not acceptable if frozen.
5. Buccal smears are acceptable for DNA extraction and molecular testing.
6. Rejection criteria includes:
 - a. Improperly labeled specimens
 - b. Specimens collected with incorrect anticoagulant (e.g. heparin whole blood is not an acceptable sample type)
 - c. Insufficient quantity received or incomplete filling of the tubes
 - d. Frozen samples
 - e. Significant hemolysis of the sample will result in rejection
 - f. Samples received beyond stability may yield inadequate DNA for analysis

Whole Blood for Platelet Antibody/Antigen Analysis

The preferred sample type is ACD (yellow top tube) or EDTA (purple top tube); ensure the tubes are completely filled.

1. Fill tubes completely, invert six times to mix.
2. Follow specimen identification and labeling procedures as outlined.
3. Specimens must be stored at room temperature until shipped; specimens drawn in ACD must be received within 72 hours of collection, EDTA samples must be received within 48 hours of collection.
4. Send specimens at ambient temperature overnight; whole blood is not acceptable if frozen.
5. Rejection criteria includes:
 - a. Improperly labeled specimens

- b. Specimens collected with incorrect anticoagulant (e.g. heparin whole blood is not an acceptable sample type)
- c. Insufficient quantity received or incomplete filling of the tubes
- d. Frozen samples
- e. Samples received beyond stability

Serum Specimens in Plain Red Tube

The preferred sample type is serum from the plain red top tube. Samples collected with the gel separator are acceptable (tiger top tube); the serum separator tube does not contain an anticoagulant, but does contain a clot activator.

1. Draw whole blood into the plain red top tube in an amount 2.5 times the required volume of serum so that a sufficient serum sample can be obtained. Place the collection tube upright in a rack and allow clotting for a minimum of 30 to 60 minutes.
2. After allowing a clot to form, centrifuge 1500 x g for 15 minutes, or at speed recommended by the manufacturer, using a balance tube if necessary.
3. Remove the stopper and aspirate the serum from the cells into a polypropylene transfer tube, using a plastic transfer pipette with care to not disturb the red cells at the bottom of the tube.
4. If using a serum separator tube, draw whole blood in an amount 2.5 times the required volume of serum and gently invert the gel-barrier tube five times to mix the clot activator and blood. The tube must be allowed to clot upright in the rack for no longer than 60 minutes.
5. Centrifuge 1500 x g for 15 minutes, or at recommended speed by the manufacturer, using a balance tube if necessary.
6. After centrifugation, inspect the gel barrier to ensure it has formed a solid seal around the tube between the serum and the packed cells. Transfer the serum to the polypropylene transfer tube using a plastic transfer tube, using care to not disturb the gel barrier.
7. Follow specimen identification and labeling procedures as outlined.
8. Freeze samples at -20°C or below until shipped to the testing laboratory. The use of a household freezer with a self defrosting freezer should not be used for storage of plasma samples as the freeze-thaw cycles may adversely affect specimen integrity.
9. Rejection criteria includes:
 - a. Improperly labeled samples
 - b. Specimens collected with anticoagulant
 - c. Failure to allow sample to clot completely before centrifugation
 - d. Insufficient quantity received
 - e. Samples received at ambient temperature beyond stability for the assay
 - f. Hemolysis or lipemia may be cause for rejection

Frozen Specimen Shipping for Hemostasis Specimens

Esoterix will provide shipping containers at our client's request. Please call our Client Services Department at 800-444-9111 to request a shipping container prior to collection of the specimens. Esoterix will automatically return the shipping container back to the clients who utilize them to send specimens to our testing laboratories. The client is responsible for adhering to packaging and shipping guidelines (US Department of Transportation Hazardous Materials Regulations and International Air Transport Association Dangerous Goods Regulations). It is not necessary to designate and label containers with routine patient specimens as biohazard or dangerous goods. The overnight shipper may unnecessarily delay shipments labeled in this manner. Specimens

with known infectious diseases that may affect humans (example: HIV and hepatitis B) are considered dangerous goods and must be labeled and shipped accordingly.

Items Required for Shipping Biological Specimens

1. Insulated container (inner package) with lid-container must be intact without cracks or holes. Based on federal regulations, insulated Styrofoam inserts alone are not acceptable for shipping biological specimens.
2. Cardboard shipping box (outer package)-must be of adequate strength for its capacity, mass and intended use.
3. Leak proof plastic specimen transport bag
4. Test requisition (TRF)
5. Dry ice-minimum recommended amount is 5 lb per regular size shipping container-dry ice pellets are preferred
6. Dry ice label (UN 1845)
7. Sealing tape
8. Absorbent materials, such as paper towels or absorbent pads
9. Packing material, such as newspaper, paper towels, or insulated packing pieces
10. Air bills for overnight courier shipment

Procedure for Preparing Frozen Shipments

1. Specimens must be frozen prior to shipping, unless stated otherwise in specimen collection requirements.
2. Shipments can be received Monday through Saturday. For tracking of packages shipped via Federal Express, please notify Client Services at 800-444-9111 with the air bill number, state and zip code information. For all overnight shipments to be received on Saturday, please notify Client Services on Friday. Information needed includes: facility, location, name of the overnight shipper, air bill number, state and zip code. **Remember to mark the air bill for Saturday delivery** when indicated. Place the appropriate Saturday delivery stickers on the outside of the box per individual overnight shipper guidelines.
3. Complete the top sections of the TRF with client, patient and billing information and select the ordered tests.
4. Place patient specimen vials in a leak-proof plastic specimen bag and seal the bag tightly using the zip lock closure. Place the folded TRF and associated patient information in the sleeve of the specimen bag.
5. Place approximately 2 inches of absorbent material (such as paper towels) in the bottom of the insulated container.
6. Place a layer of dry ice on top of the absorbent material. **Do not touch dry ice** with bare hands as it can burn the skin.
7. Place specimen bag(s) on top of dry ice. Cover specimens with additional dry ice (minimum 5 lbs of dry ice per container is recommended).
8. Fill top of insulated container completely with packing material. Replace cover and tape the inner packaging box with sealing tape. Dry ice deteriorates rapidly upon exposure to air, so ensure the lid is tightly secured and taped completely.
9. Place the insulated package into the rigid outer cardboard shipping box and seal the outer box top with secure packaging tape.
10. Fasten the completed air bill on top of the cardboard shipper. Specimens should be sent via Priority Overnight, Express or Next Day Air per individual shipper designations. Contact Client Services at 800-444-9111 with shipping information.

11. Fasten dry ice label (UN 1845) on side of box. Write in the amount of dry ice (in kg) on the label. Two pounds is equal to 1kg of dry ice (ie. 5 lbs. = 2.5 kg dry ice). Fill out the shipper and consignee address information on the dry ice label. Affix “Diagnostic Specimen” label to top of cardboard shipping box.
12. If shipping via Federal Express, affix pink “Priority Alert” sticker (available through your courier or Federal Express office) to the outside of the shipping container.
13. Notify overnight courier of pick-up or deliver it to the shipper.

Contact Esoterix Client Services at 800-444-9111 for questions regarding these procedures.