

Special Specimen Collection Procedures for Coagulation Testing

The accuracy of hemostasis testing depends upon the quality of the specimen submitted. Hemostasis specimens must be properly collected, labeled, stored, packaged, and transported. Phlebotomists and specimen managers must carefully adhere to all time limits. Properly following these instructions will assure specimen integrity and contribute to valid, consistent hemostasis results.

Prior to collecting the specimen, review the specimen requirements. Note the proper specimen type to be collected, amount of specimen necessary for testing and special storage and shipping instructions.

The specimen container must be properly identified with *the patient's full name, identification number, date and time of collection and specimen type.*

A properly completed requisition form must accompany each specimen. It is important that the age and/or birth date of the patient be indicated so that age-adjusted normal values can be provided.

CLIA regulations further require that we receive physician signatures on follow-up testing. Call our Client Service department at (800) 444-9111 and a form with the required information will be faxed to you for your signature.

Blood Specimen Collection

Most hemostasis blood specimens may be obtained using routine phlebotomy techniques. Some analyses require special specimen management. Check the collection methods and test menu for special instructions. Be sure to use the appropriate evacuated tube according to the following list:

Green top	Sodium heparin for plasma chemistry
Light blue top	Sodium citrate for hemostasis
Red top	No preservative or anticoagulant; provides serum for chemistry
Tiger top	Serum separator tube; no preservative or anticoagulant. Contains clot activator, provides serum for chemistry.
Yellow top	Acid citrate dextrose (ACD); provides whole blood.
Lavender top	Ethylenediaminetetraacetic acid (EDTA); provides whole blood.

Collection of Blood Specimens From Indwelling Catheters

When specimens are collected from indwelling catheters, the components of the blood collection system should be checked to insure compatibility and avoid air

leaks that may cause hemolysis and incorrect draw volumes. To prevent possible contamination or specimen dilution, the line should be flushed with 5 mL of saline. The first 5 mL of blood, or six "dead space" volumes of the catheter must be discarded.

Collecting Citrated Plasma for Hemostasis Testing

1. For hemostasis and coagulation specimens, draw blood into a light blue top tube. Blue top tubes containing 3.2% (0.109 M) sodium citrate are recommended. Use of other anticoagulants may cause invalid results. Fill to the proper level, indicated by a fill line on the tube. A "short draw" gives inaccurate results. Unless specifically directed, the use of a discard tube is not necessary prior to collecting a citrated plasma sample
2. Gently invert 6 times to mix. Process immediately
3. Centrifuge the capped tube at 2500 x g for 10 minutes. Please note that "g" stands for g-force or relative centrifugal force (RCF), not RPM. RCF is used to calculate correct RPM for individual centrifuges. Evaluate visually to make certain a clot is not present. The presence of a clot invalidates the sample and requires that the patient is re-drawn
4. Transfer plasma with a plastic pipette into a polypropylene centrifuge tube, cap and centrifuge an additional 10 minutes to obtain platelet poor plasma, that is, plasma with a platelet count <10,000/mm³. It is **especially** important that specimens for lupus anticoagulant, heparin, and protein activity testing be platelet free
5. Using care not to disturb the button at the bottom, transfer the plasma into polypropylene tubes using a plastic pipette. Use of glass transfer pipettes or tubes may cause activation of the clotting mechanism and should be avoided
6. Label the tube with patient name, identification number, and date and time of collection, then seal
7. Freeze the specimen immediately at -70°C. Specimens should **not** be frozen in an ordinary household freezer and should **never** be placed in a self-defrosting freezer for storage, as the continuous freeze-thaw cycle will adversely affect specimen integrity
8. Send frozen specimens via overnight delivery on a minimum of 5 pounds of dry ice. Specimens **must** remain frozen during transport

Hematocrit Adjustment for Hemostasis Specimens

The ratio of whole blood to anticoagulant must be 9:1 for coagulation testing. If the patient's hematocrit is greater than 55%, the amount of anticoagulant used should be adjusted according to 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

Blood Specimens from Patients on Anticoagulant Therapy

Anticoagulant therapy such as heparin or warfarin affects many test results and must be noted on the test requisition. If notation is made, Esoterix will perform heparin neutralization of the plasma when requested in those instances when the specimen is known to contain heparin that would otherwise interfere with the testing process.

Whole Blood for DNA Analysis

1. Draw blood into a lavender top EDTA tube. Be sure the tube is completely filled
2. Gently invert 6 times to mix
3. Label the tube with patient name, identification number, and date and time of collection
4. Store the specimen at 4°C until ready to ship. Specimen may be stored up to 72 hours prior to shipping
5. Send specimens at ambient temperature via overnight delivery
6. When properly stored and shipped, whole blood collected in EDTA provides stable DNA. Whole blood collected in acid citrate dextrose or heparin may also be used

Whole Blood for Platelet Antibody/Antigen Analysis

1. Draw blood into the required number of yellow top ACD or lavender top EDTA tubes. Be sure tubes are completely filled
2. Gently invert 6 times to mix
3. Label the tubes with patient name, identification number, and date and time of collection
4. Store the specimens at ambient temperature until ready to ship. Specimens drawn in ACD must be received within 72 hours of collection. Specimens drawn in EDTA must be received within 48 hours of collection
5. Send specimens at ambient temperature via overnight delivery

Collecting Serum Specimens in a Plain Red top Tube

1. Draw blood into a plain red top tube
2. Allow the blood to clot for a minimum of 30 minutes
3. Centrifuge at 2500 x g for 10 minutes. Please note that "g" stands for g-force or relative centrifugal force (RCF), not RPM. RCF is used to calculate correct RPM for individual centrifuges
4. Transfer serum into plastic tubes using a plastic pipette
5. Label the tube with patient name, identification number, and date and time of collection, then seal

6. If freezing is required, freeze the specimen immediately at -70°C . Specimens should not be frozen in an ordinary household freezer and should **never** be placed in a self-defrosting freezer for storage as the continuous freeze-thaw cycle will adversely affect specimen integrity
7. Send frozen specimens via overnight delivery on a minimum of 5 lbs. of dry ice. Specimens must remain frozen during transport

Special Hemostasis Blood Specimen Collection Guidelines
Special Instructions for the β -thromboglobulin (β -TG, 300058)
and the platelet factor 4 (PF-4, 300504) tests

1. Contact our Client Service department at (800) 444-9111 for the special collection tube (CTAD)
2. Prepare a bath of crushed ice and water deep enough to cool the entire contents of the specimen collection tube
3. Break the vacuum of the tubes containing the special anticoagulant by removing the stopper before sample collection
4. Use a loosely tied tourniquet only for locating the vein. Perform the venipuncture using a 19g butterfly needle set. If it is not possible to use a 19g needle, a 21g bore needle may be used, but no smaller than 21g. Use the two-syringe technique, first using a discard syringe and then the specimen syringe
5. Start the venipuncture and remove the tourniquet as soon as the first drops of blood appear. Discard the first syringe with 2 mL of blood, and then collect 5 mL into the second syringe
6. Complete the venipuncture; safely remove the syringe from the needle set, and place in a sharps container. Gently express 4.5 mL of blood down the side of the special collection tube
7. Cap and invert gently **three** times
8. Allow the tube to cool in the bath of crushed ice and water for at least 15 minutes
9. Within one hour, centrifuge at $2500 \times g$ for 30 minutes in a refrigerated centrifuge. Please note that "g" stands for g-force or relative centrifugal force (RCF), not RPM. RCF is used to calculate correct RPM for individual centrifuges
10. As soon as centrifugation is complete, collect one-third the volume of the plasma supernatant from the middle region of the plasma portion using a plastic pipette. Take care not to aspirate from near the top surface or the red cell layer, as platelets will enter the specimen
11. Label the tube with patient name, identification number, and date and time of collection, then seal
12. Freeze the specimen immediately at -70°C . Specimens should **not** be frozen in an ordinary household freezer and should **never** be placed in a self-defrosting freezer for storage, as the continuous freeze-thaw cycle will adversely affect specimen integrity

13. Send frozen specimens via overnight delivery on a minimum of 5 lbs. of dry ice. Specimens **must** remain frozen during transport

Special Instructions for the tissue plasminogen activity (TPA Activity, 300134) test using the Stabilyte™ tube

1. Contact our Client Service department at (800) 444-9111 for the special collection tube
2. Patient should be in a resting state and preferably drawn in the morning
3. Venipuncture should be non-traumatic and preferably without a tourniquet. If a tourniquet is necessary, it should be applied loosely and released immediately after flow begins. Draw approximately 1 mL of blood into a red top tube and discard. Collect blood into the Stabilyte™ tube and ensure that it is filled completely
4. Gently invert 6 times to mix. Process immediately
5. Centrifuge the capped tube at 2500 x g for 10 minutes. Please note that "g" stands for g-force or relative centrifugal force (RCF), not RPM. RCF is used to calculate correct RPM for individual centrifuges
6. Transfer plasma with a plastic pipette into a plastic centrifuge tube, cap and centrifuge an additional 10 minutes to obtain platelet poor plasma, that is, plasma with a platelet count <10,000/mm³
7. Using care not to disturb the button at the bottom, transfer the plasma into plastic tubes using a plastic pipette. Use of glass transfer pipettes or tubes may cause activation of the clotting mechanism and should be avoided
8. Label the tube with patient name, identification number, and date and time of collection, then seal
9. Freeze the specimen immediately at -70°C. Specimens should **not** be frozen in an ordinary household freezer and should **never** be placed in a self-defrosting freezer for storage, as the continuous freeze-thaw cycle will adversely affect specimen integrity
10. Send frozen specimens via overnight delivery on a minimum of 5 lbs. of dry ice. Specimens **must** remain frozen during transport

Instructions to Patient for ASPIRINcheck™ Specimen Collection

1. Be sure the specimen collection cup has your name on the side
 2. Remove the cover from the cup
 3. Place the open cup and cover within reach, but do not touch the inside
 4. Let a small amount of urine flow into the toilet, and then stop the urine flow
 5. Now pass your urine into the cup, replace the cover tightly, and give to the staff person
- The cup should be at least ¼ full

Instructions to Staff Member for ASPIRINcheck™ Specimen Preparation

1. Label a clean specimen collection cup with the patient's name
2. Give the cup to the patient with a copy of the above collection instructions
Do not give the patient the transport tube
3. When returned, carefully pour urine from the cup into the transport tube
Do not remove the preservative tablet
4. If no preservative is available upon collection, submit frozen urine sample
If the preservative tablet is accidentally ingested, drink milk or water
5. Fill tube to the fill line, approximately 9 mL. Do not fill past the fill line
The minimum permissible volume is 6 mL
6. Seal the tube tightly with the screw cap and label with patient's name, social security number, date and time
7. Invert the tube several times to ensure the preservative tablet is fully dissolved
8. Discard the specimen collection cup with the remainder of the urine
9. Fully complete the ASPIRINcheck test requisition

Frozen Specimen Shipping for Hemostasis Specimens

Esoterix provides shipping containers at our client's request. Please call our Client Service department at (800) 444-9111 to request a shipping container prior to collection of the specimen. Esoterix will automatically return our shipping containers back to clients who utilize them to send specimens to us.

The client is responsible for adhering to packaging and shipping guidelines. It is *not* necessary to designate routine patient specimens as biohazard or dangerous goods. The overnight shipper may unnecessarily delay shipments labeled in this manner. Specimens containing *known* infectious substances that may affect humans (example: Hepatitis B, HIV) are considered *dangerous* goods and must be labeled and shipped accordingly.

Items Required for Shipping Biological Specimens

- Insulated container (inner package) with lid - Container must be intact without any cracks or holes. Based on federal regulations, insulated inserts alone are not acceptable for shipping biological specimens
- Cardboard shipping box (outer package) - Must be of adequate strength for its capacity, mass and intended use
- Leak proof plastic specimen transport bag
- Test requisition
- Dry ice - Recommend minimum 5 lbs. per regular size shipping container (dry ice pellets preferred)
- Dry ice label (UN 1845)
- Sealing tape
- Absorbent material, such as paper towels

- Packing material, such as newspaper, paper towels or insulated packing pieces
- Airbills for overnight courier

Procedure for Preparing to Ship Frozen Specimens

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 airbill 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, and name of overnight shipper, airbill number, state and zip code. *Remember to mark the airbill for Saturday delivery.* Place the appropriate Saturday delivery stickers on the outside of the box per individual overnight shipper guidelines
3. Complete the top sections of test requisition with client, patient and billing information and select the ordered test(s)
4. Place patient specimen vials in a leak-proof plastic specimen bag and seal tightly
5. Enclose test requisition in the envelope of the specimen bag
6. Place approximately 2 inches of absorbent material (such as paper towels) in the bottom of the insulated container
7. Place layer of dry ice on top of absorbent material. Do *not* touch dry ice with bare hands as it can burn the skin
8. Place specimen bag on top of dry ice. Cover specimen with additional dry ice (minimum 5 lbs. of dry ice per container recommended)
9. Fill top of insulated container completely with packing material. Replace cover and secure with sealing tape. Dry ice deteriorates rapidly upon exposure to air, so lid should be taped on tightly and securely
10. Place insulated container in cardboard shipping box and seal top of box with sealing tape
11. Fasten completed airbill on top of cardboard shipping box. Specimens should be shipped Priority Overnight, Express or Next Day Air per individual shipper designations. Please contact Client Services at 800-444-9111 with shipping information
12. Fasten dry ice label (UN 1845) on side of box. Write the amount of dry ice (in kg) on the label. Two pounds is equal to 1 kg of dry ice (i.e. 5 lbs. = 2.5 kg dry ice) Fill out shipper and consignee address information on dry ice label. Affix Diagnostic Specimen Label to top of cardboard shipping box
13. If shipping via Federal Express, affix pink Priority Alert stickers (available through your courier or Federal Express office) to the outside of the shipping container
14. Notify overnight courier of pick-up or deliver it to the shipper

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