

General Guidelines for Hemostasis and Thrombosis

Specimen Collection Instructions: Hemostasis/Thrombosis Testing - Plasma Samples

In order to produce valid results for all hemostasis/thrombosis testing, routine and special, specimen integrity is crucial and must be maintained. All specimens sent for testing must be collected and shipped in the following manner:

1. Obtain venous blood by clean venipuncture. Avoid slow flowing draws and/or traumatic venipunctures as either of these may result in an activated or clotted sample. Do not use needles smaller than 23 gauge.
2. Always draw a discard tube, regardless of the blood collection system used, before drawing coagulation specimens in light blue, plus plastic vacuum tubes (3.2% buffered sodium citrate). **NOTE:** This reflects a change in the CLSI recommended order of draw, CLSI H21-A5, Vol 28, No 5, 7.1.1.
 - a. Preferred discard tube is clear plastic or light blue plus plastic, 3.2% sodium citrate tube. When using a winged collection set for venipuncture and a coagulation (3.2% citrate) tube is the first specimen tube to be drawn, a discard tube must be drawn to fill the blood collection set tubing's "dead space" with blood, but the discard tube does not need to be completely filled. This important step will ensure maintenance of the proper blood to anticoagulant ratio of the blood sample. Noncompliance will result in an under-filled coagulation tube, which can result in falsely prolonged coagulation results.

NOTE: Reference ranges have been established using 3.2% buffered sodium citrate.

3. Withdrawing blood from intravenous lines or indwelling catheters should be avoided if at all possible. Frequently, heparin flushes are used to maintain patency in catheters and lines. If not properly cleared of heparin before drawing blood from lines, the results of coagulation studies such as the Prothrombin Time, aPTT, Thrombin Time, dRVVT, APCR and aPTT based Protein S Assays can be **FALSELY PROLONGED**. When obtaining samples for hemostasis studies from indwelling lines that may contain heparin, the line must be flushed with 5mL of saline and the first 5mL of blood drawn must be discarded before the tube that will be used for hemostasis tests is filled.
4. Fill light blue top tubes as far as the vacuum will allow. An exact ratio of 9 parts blood to one part anticoagulant must be maintained. Mix by gentle inversion.
 - a. Samples with less than 90% fill must be redrawn. Failure to maintain an exact 9:1 ratio will interfere with accurate results.
 - i. It is unacceptable to combine the contents from separate, underfilled sodium citrate collection tubes.
 - b. Patients with hematocrits greater than 55% must be drawn in a "corrected" 3.2% sodium citrate tube. This is a tube with a portion of anticoagulant removed to compensate for the increased hematocrit but still maintains 9:1 ratio. To calculate the amount of anticoagulant to remain in the tube, see the formula below.
 - a. Formula for adjustment of 3.2% sodium citrate in tube:
$$X = \text{amount of anticoagulant to remain in the tube:}$$
$$N = 0.3, 0.2 \text{ mL of anticoagulant}$$
$$X = \frac{N(100 - \text{hct})}{55}$$

5. In order to produce accurate and valid results, all specimens must be "platelet free" (<5000/ μ L) before freezing for shipment. This residual count can be obtained by "double-spinning" the sample.
 - a. Centrifuge the specimen at no less than 1500 x g for 15 minutes (or at a speed and time required to consistently produce platelet-poor plasma, <10,000/uL) within 1 hour of blood draw.
 - b. Immediately remove only the top two-thirds of the platelet-poor plasma from the sample using a plastic transfer pipette (use of glass transfer pipettes may result in activation and/or clotting of the plasma). Place the plasma in a properly labeled plastic vial.
 - c. Re-spin this plasma at 1500 X g for 15 minutes. Remove the top two-thirds of the "platelet-free" plasma with a plastic transfer pipette being careful not to disturb any cell button at the bottom of the

- tube. Place this plasma in a properly labeled plastic vial and clearly mark the vial contents as PLASMA. **Glass vials will be rejected. Hemolyzed samples will be rejected.**
- d. Quick-freeze the samples using a -70°C freezer or a dry ice and methanol bath. **Each assay requested must be submitted in a separate vial.**
 6. Ship samples in a Styrofoam container with five pounds of block dry ice.
 7. Some assays may be performed on a priority basis if a medical emergency exists. Contact the Hemostasis/Thrombosis Laboratory to make arrangements. Please call 317-491-6000. Hours: M-F, 7AM - 4PM.
 8. All requests for coagulation assays must include a brief patient history and pertinent clinical information (i.e., medications, blood products, etc.). **NOTE:** Samples containing heparin must not be used for coagulation testing. If possible, stop heparin therapy before the draw to avoid contamination. Heparin interferes with most clotting assays.
 9. Coagulation interpretation available. Contact the Hemostasis/Thrombosis Laboratory at 317-491-6000.

Specimen Collection Instructions: Hemostasis/Thrombosis Testing - Whole Blood Samples

1. Follow the instructions for plasma samples above, steps 1-2. Several coagulation assays require a whole blood sample, i.e., Factor V Leiden, PT Mutation. Please submit a lavender or blue tube.
2. Samples for Platelet Aggregation and Ristocetin Dose Response studies must be drawn in **six, BD plus plastic, 2.7mL, 3.2% sodium citrate tubes** and sent to the Hemostasis and Thrombosis Lab. Samples must be received within 3 hours of draw. Plt aggs/Risto Dose **must be in lab by 1pm.** Tubes must be full. Please submit a short patient history and a list of medications, over-the-counter and prescribed, that the patient has been receiving for the past 7-10 days. Please call 317-491-6000 for further instructions or to obtain tubes.
3. Samples for the PFA-100 must be drawn in **2 BD plus plastic, 2.7 mL, 3.2% sodium citrate tubes** and sent to the Hemostasis and Thrombosis Lab. Tubes must be full. Samples need to be received in the lab within 3 hours of draw. PFA samples **must be in lab by 3pm.**
4. Samples for the Platelet Inhibition (P2Y12) or Aspirin assay must be drawn in **two Greiner plastic, 1.8 mL, 3.2 % sodium citrate tubes.** P2Y12/Asprin tests **must be in lab by 3pm.** Please call 317-491-6000 for more information or to obtain tubes.
5. All whole blood samples for platelet function must be sent at room temperature **and must not be sent through a pneumatic tube system.**

Acceptable Transportation Conditions: Hemostasis/Thrombosis From CLSI Guideline H21-A5, Vol. 28, No. 5, 7.1.1

Volume 28

H21-A5

Assay	Stored as Whole Blood			Processed and Plasma Aliquoted			
	Room Temp	Refrigerated	Frozen	Room Temp	Refrigerated	Frozen -20 °C**	Frozen -70 °C or colder**
PT	Up to 24 hr	Unacceptable	Unacceptable	Up to 24 hr	Unacceptable	2 wk	12 mo
APTT	Up to 4 hr	Unknown	Unacceptable	4 hr	4 hr	2 wk	12 mo
APTT- For UFH analysis	1 hr	Unknown	Unacceptable	4 hr	4 hr	*** 2 wk	*** Unknown
APTT- For VWF and VIII Analysis	4 hr	Unacceptable	Unacceptable	4 hr	4 hr	** 2 wk	** 6 mo
Other	4 hr	Unknown	Unacceptable	4 hr	4 hr	Depends on analyte; see Appendix B	

*Placing whole blood specimens directly on ice or in an ice water bath simulates refrigeration.

**Must be thoroughly mixed before testing

***Should be platelet-poor

WHOLE BLOOD MUST NEVER BE SENT ON ICE!!! ANY WHOLE BLOOD SAMPLE SENT ON ICE WILL BE REJECTED AND REQUIRE RECOLLECTION!!!

For more information call 317-491-6000

Specimen Container Labeling

See “Specimen Identification and Labeling” for detailed instructions

Primary Specimens: Primary specimens are the body fluid, tissue, or sample submitted for examination, study, or analysis. It may be within a collection tube, cup, syringe, swab, slide, data file, or other form as received by the laboratory.

Must include: At least two patient-specific identifiers, which include, but limited to: patient name, date of birth, hospital number, social security number, requisition number, accession number, unique random number.

Secondary Specimens: Any derivative of the primary specimen used in subsequent phases of testing. It may be an aliquot, dilution tube, slide, block, culture plate, reaction unit, data extract file, image, or other form during the processing or testing of a specimen. (The aliquots or images created by automated devices and tracked by internal electronic means are not secondary specimens.)

Must include: A single, unique identifier derived from the primary specimen for use in subsequent phases of testing and must provide reliable identification and be linked to the full particulars of patient identification, collection date, specimen type, etc.