

MUSC Laboratory Services Hemostasis / Coagulation Guidelines

Suggested Tests for Monitoring Anticoagulant Therapy

Anticoagulation Medication	Monitoring Assay Suggested	EPIC Lab #
Direct Thrombin Inhibitors	aPTT	LAB325
Coumadin	PT (INR)	LAB320
Hirudin	APTT	LAB325
Low Molecular Weight Heparin	Anti-Xa (Heparin, Low Molecular Weight)	LAB316
Unfractionated Heparin	aPTT	LAB325
	Anti-Xa (Heparin, Unfractionated)	LAB2815

Anti-Xa (Heparin) Assays

There are currently two chromogenic assays offered in-hospital to measure Anti-Xa (Heparin) therapy. It is imperative the correct assay is ordered for the type of anticoagulant the patient is receiving. Chromogenic measurement is possible for Unfractionated Heparin (LAB2815) and Low Molecular Weight Heparin (LAB325). At this time Rivaroxaban and Apixaban cannot be measured by current in-hospital Anti-Xa assays.

SPECIMEN COLLECTION AND STORAGE GUIDELINES

The *Clinical and Laboratory Standards Institute (CLSI) Collection, Transport, and Processing of Blood Specimens for Testing Plasma-Based Coagulation Assays and Molecular Hemostasis Assays; Approved Guideline 5th H21-A5* states "Many variables including anticoagulant amount and concentrations; specimen and sample storage; and surface of containers may affect test results".

- The following are guidelines for Collection, Transport and Storage of Coagulation Specimens as stated in CLSI H21-A5.
- Clotted specimens and specimens not collected and transported according to the guidelines below are unsuitable for testing and are rejected.

A. COLLECTION

It is recommended that blood specimens for coagulation testing be collected by venipuncture using a blood collection system that collects the specimen directly into a tube containing the anticoagulant. All specimens should be collected in a nonactivating surface container.

1. The recommended anticoagulant for coagulation assay should be 3.2 % (105 - 109 mmol/L) Sodium citrate.
2. Suitable needle gauges for coagulation tests range from 21 to 19. For the pediatric patient, a 21-23 gauge needle may be used. A winged blood collection set of the same gauge can also be used.
Note: Smaller gauge needles (25 gauge or smaller) may result in hemolysis.

3. The proportion of blood to anticoagulant volume is 9:1. Inadequate filling of the collection device will decrease this ratio, and may lead to inaccurate results. **Over/underfilled tubes will be rejected.**
4. The citrate concentration in the final blood mixture must be adjusted in patients who have hematocrit values > 55%. For hematocrits <20%, there are no current data available to support a recommendation for adjusting the citrate concentration; however, it is the recommendation of this laboratory to adjust citrate concentration for hematocrits which are <20% or >55%.
5. Immediately after the specimen is drawn, the tube should be inverted gently at least 5 times and then, as soon as possible, mixed thoroughly to avoid clotting. Avoid vigorous mixing which could result in hemolysis and/or platelet activation, leading to erroneous results.
6. Studies have shown that the PT (INR) and APTT results are not affected if tested on the first tube drawn. Proof of necessity of drawing a discard tube for other coagulation testing is circumstantial at best, but there are no current published data to suggest that this practice is unnecessary.
7. The following guidelines for syringe, winged blood collection set (e.g. butterfly), catheter or indwelling line specimens should be strictly followed to avoid clotting, hemolysis, tissue thromboplastin contamination, heparin contamination and over/under filling tube.

Transfer of blood into Vacutainer tube

Blood must be immediately added to the appropriate volume of anticoagulant (sodium citrate) and quickly but gently inverted at least 5 to 6 times end-over-end to assure thorough mixing.

- The blood should be added to the appropriate volume of anticoagulant within one minute of completion of draw.
- As soon as possible, mix thoroughly to avoid clotting.
- Avoid vigorous mixing which could result in hemolysis.
- If a syringe is used, the needle should be removed and the blood allowed to run down the side of the tube. Mixing should not be vigorous.
- The proportion of blood to anticoagulant volume is 9:1. Inaccurate filling of the collection device will alter this ratio and may lead to invalid results. **Over/under filled tubes will be rejected.**

Winged blood collection set

- Winged blood collection sets, because of their longer path length between vein and anticoagulant when used in combination with smaller-gauge needles should be used with caution to avoid platelet and coagulation activation.
- When using a winged blood collection set for venipuncture and a coagulation tube is the first tube to be drawn, a discard tube should be drawn first.
- The discard tube must be used to fill the blood collection tubing dead space and to assure maintenance of the proper anticoagulant/blood ratio; it need not be completely filled.
- The discard tube should be a non-additive or a coagulation tube. In the case of any unexpected results, a new specimen should be obtained from a different location.

Syringe

Using a hypodermic needle/syringe may have limitations because of the increased risk of hemolysis and apparent safety issues.

- **Do not collect specimen in heparinized syringe** (e.g. as used for blood gas specimens)
- With larger syringes, there is an increased chance that clotting may occur.
- A small volume syringe (≤ 20 mL) is recommended.
- If a double syringe technique is used, blood from the **second syringe** should be used for the coagulation specimen.

Vascular Access Device (VAD)

Under certain circumstances, blood specimens for coagulation testing may be drawn from a vascular access device (VAD) using a blood collection system or a syringe.

- When obtaining a blood specimen from a VAD, the components of the blood collection system (VAD, connecting device, syringe, needle, and collection device) should be checked to ensure compatibility to avoid air leaks which may cause hemolysis and incorrect draw volumes.
- Collection of the blood through lines that have been previously flushed with heparin should be avoided, if possible.
- If the blood must be drawn through a VAD, possible heparin contamination and specimen dilution should be considered. In this case the line should be flushed with 5 ml of saline and the first 5 mL of blood or six dead space volumes of the VAD discarded.
- If blood is obtained from a normal saline lock, two dead space volumes of the catheter and extension set should be discarded.

B. SPECIMEN STORAGE AND TRANSPORT

Specimens should be transported to the laboratory for testing as soon as possible adhering to the guidelines below:

PT assays ONLY

- Uncentrifuged or centrifuged with plasma remaining on top of the cells in an unopened tube kept at 18 to 24°C should be tested within 24 hours from time of specimen collection. Specimens may also be stored at 2-4 °C; however, storage at 2-4°C may result in cold activation of Factor VII and therefore alter PT results.
- If the patient is on both heparin and oral anticoagulant therapy, the PT may vary with time of storage.

APTT assays on *nonheparinized* patients

- Uncentrifuged or centrifuged with plasma remaining on top of the cells in an unopened tube kept at 2 to 4 °C or 18 to 24°F should be tested within four hours from time of specimen collection.

APTT assays suspected to contain *unfractionated heparin*

- Specimens should be kept at 2 to 4 °C or 18 to 24°C and centrifuged within one hour of collection, plasma removed from the cells, and stored at 2-4 °C.
- The plasma should be tested within four hours from time of specimen collection.

D-DIMER

- Uncentrifuged or centrifuged with plasma remaining on top of the cells in an unopened tube kept at 2 to 4 °C or 18 to 24°F should be tested within 8 hours from time of specimen collection.

Other assays

- Thrombin Time, Fibrinogen, Protein C, Protein S and Factor Assays kept at 2 to 4 °C or 18 to 24°F, should be centrifuged and tested within four hours from time of specimen collection.

Specimens which cannot be transported to the laboratory according to the guidelines above should be centrifuged to yield platelet poor plasma and the specimen sent frozen on dry ice.