

SPECIMEN COLLECTION AND HANDLING

COAGULATION TESTING

Coagulation testing is highly sensitive to a myriad of specimen collection and handling variables. Because important diagnostic and therapeutic decisions are based on the results of coagulation tests, a procedural guideline is required to fully address these variables. The following information applies to all Routine Coagulation tests (i.e. Prothrombin Time, APTT, Fibrinogen, etc.)

COLLECTION TUBES

- It is highly recommended that blood specimens for coagulation testing be collected by venipuncture using a vacuum collection device that collects the specimen directly into an evacuated tube with a non-wettable surface.
- 3.2% buffered tri-sodium citrate (light blue-top) is the proper anticoagulant. This is the anticoagulant recommended by Clinical and Laboratory Standards Institute (formerly NCCLS) H21-A3 Guidelines. This laboratory requires the use of 3.2% buffered tri-sodium citrate for all coagulation testing. No other anticoagulants are acceptable for coagulation testing.
- Light blue-top tubes (citrate) are available in a 4.5 ml full draw tube or a 2.7 ml and 1.8 ml draw to accommodate pediatric testing volumes. Partial draw tubes are not acceptable.
- These tubes are pre-calibrated to draw the specified amount of blood, resulting in the proper 9:1 ratio of blood to anticoagulant.

In the event that blood must be added to the tube via a syringe/needle (not the recommended method of collection), a good "rule of thumb" is to fill the tube to the top of the manufacturer's label.

NEEDLE SIZE

- **20-21 gauge** needles are recommended to avoid clotting or hemolysis.
- For the pediatric patient a **21- to 23-gauge** needle may be used. A butterfly blood collection set of the same gauge can also be used.
- **Syringe draws are discouraged** because of the increased risk of hemolysis. Additionally, with larger syringes, there is an increase chance that clotting may occur.
- If a syringe is used, a small volume syringe $\leq 20\text{mL}$ is recommended.
- If a hypodermic needle/syringe must be used, it is vital that the blood be **transferred to the vacutainer tube** within one minute of completion of draw.

INDWELLING CATHETER COLLECTION

- Under certain circumstances, blood specimens for coagulation testing may be drawn from an indwelling catheter. 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 catheter discarded.
- **Collection of blood through lines that have been previously flushed with heparin Should be avoided, whenever possible.** If the blood must be drawn through an indwelling catheter, possible heparin contamination and specimen dilution should be considered.

TECHNIQUE

- Specimens should be obtained from a single venipuncture with minimal tissue trauma. The blood should flow freely into the container.
- **Traumatic venipuncture and/or slow-flowing draws should be avoided.** Either may result in an activated or clotted sample. Prolonged venostasis may raise the levels of factors VIII:C and IX or it may activate the fibrinolytic system.
- Regardless of the device used for specimen collection, all tubes should be **gently inverted IMMEDIATELY at least five times to mix. DO NOT shake or mix vigorously.**
- **If only a coagulation specimen is drawn, a waste tube should be collected and discarded.** If multiple specimens are collected, the coagulation specimen should be collected into the **second or third tube.** Refer to CLSI recommendations (see below).
- If a **double syringe** technique is used, blood from the **second syringe** should be used for the coagulation specimen. The number of specimen transfers and intermediate collection devices should be kept to a minimum. Refer to CLSI recommendations (see below).

CLSI RECOMMENDED ORDER OF DRAW:

By following the following "order of draw," many pre-analytical errors and cross-contamination of additives will be avoided.

TUBE TOP COLOR		
1	BLOOD CULTURE vials	(Culture tubes/vials)
2	RED	(Non-additive tubes/serum tubes)
3	LIGHT BLUE	(Citrate tubes – Coagulation tests)
4	SPECKLED	(Gel separator tubes)
5	GREEN	(Heparin tubes)
6	LAVENDER	(EDTA tubes)
7	OTHER	(Other additives)

UNDERFILLING - Inadequate filling of the collection tube will decrease the required blood: anticoagulant ratio (9:I), and may lead to falsely prolonged results.

OVERFILLING: Overfilling will cause an incorrect blood to anticoagulant ratio which may result in slightly decreased results or the specimen may clot or contain fibrin due to inadequate anticoagulant for the blood volume drawn which will result in the specimen being rejected.

HIGH HEMATOCRIT: Results on patients with a high hematocrit ($\geq 55\%$) may be falsely prolonged. Refer to the section (below) on special handling of specimens with HIGH HEMATOCRITS.

CLOTTED SPECIMENS: Clotting will lead to erroneous results. CLSI recommends 5-10 inversions to prevent clotting. Each tube is visually examined prior to centrifugation for the presence of obvious clots. If in doubt, the specimen is checked for clots using applicator sticks.

HEMOLYSIS: Hemolysis of the RBCs causes release of hemoglobin into the plasma, which shortens the APTT test. Hemolysis also suggests the possibility of in vitro clotting, which would adversely affect ALL coagulation tests. Therefore, samples that have visible hemolysis are rejected and a redraw requested.

SPECIAL COAGULATION TESTS: CLSI guidelines recommend drawing a discard tube before specimens collected for special coagulation test.

SPECIMEN TRANSPORT

CONDITIONS of TRANSPORT and STORAGE

Coagulation tests are enzymatic procedures and as such, are subject to stringent time-frame and storage guidelines. Reaction temperatures and the pH of specimens must be controlled at all times. For best results, most sources recommend that specimens for Coagulation testing be delivered to the laboratory for testing within $\frac{1}{2}$ hour of collection. Considering the logistics and problems associated with transporting specimens, this recommendation is rarely achieved. More realistic time-frame guidelines have been established and must be adhered to. The allowable time interval between collection of the specimen and testing of the sample will depend on the transport temperature and the storage of the specimen. Specimens for coagulation testing should be processed/stored as follows:

Most specimens for routine Coagulation testing can be transported either at room temperature* (18-24°C) or refrigerated (2-4°C).

*Specimens for **Prothrombin Time** testing (PT) should be **transported at room temperature**. They should NOT be refrigerated.

- *PT assays must be performed within 24 hours of collection.*
- *APTT assays must be performed within 4 hours of collection.*

ALL other Coagulation tests must be performed within 4 hours of collection. When samples cannot be assayed within the required time frame, the plasma must be separated from the red cells and frozen within one hour of collection.

SPECIMEN PROCESSING

SPECIMEN IDENTIFICATION

*All specimens received in this laboratory must be properly identified and be accompanied by an appropriate requisition or manifest. Laboratory personnel are required to stringently check identification and test request information prior to processing specimens. **Results will NOT be reported on any patient specimen if there is any doubt as to the validity of the requisition and/or the identity of the specimen.***

SPECIMEN QUANTITY vs TUBE SIZE

- Sodium citrate tubes are pre-calibrated to draw a specific amount of blood to produce the proper 9:1 ratio of blood to anticoagulant.
- This ratio is **critical** in all methods of Coagulation tests.
- There are various sizes of tubes available for use in Coagulation. See **COLLECTION TUBES** section of this document.
- Each tube is checked for proper specimen amount prior to analysis. Specimens that do not have the proper amount of blood will be rejected.

CHECKING FOR CLOTS

- Each specimen is checked for the presence of clots.
- The tube is inspected for the presence of clots by careful observation while gently inverting the specimen.
- If a clot is suspected, the tube is uncapped, and checked with a pair of applicator sticks.
- Clotted specimens are rejected.

HIGH HEMATOCRITS

It is the responsibility of the laboratory to detect any specimens received for coagulation testing that might have an elevated hematocrit. **Specimens with extremely elevated hematocrits (>55%) require special handling for Coagulation testing due to improper plasma to anticoagulant ratio.**

Polycythemia (high hematocrits) can cause falsely prolonged results.