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DOCUMENT NUMBER:	LAMC-PPP-1340
DOCUMENT TITLE:	Specimen Requirements for Coagulation Studies
DOCUMENT NOTES:	

LOCATION:	LAMC-dft	**VERSION:**	01
DOC TYPE:	LAMC PPP	**STATUS:**	Draft

EFFECTIVE DATE:	01 Oct 2021	**NEXT REVIEW DATE:**	
RELEASE DATE:		**EXPIRATION DATE:**	

AUTHOR:		**PREVIOUS NUMBER:**	
OWNER:		**CHANGE NUMBER:**	LAMC-CR-1395

Specimen Requirements for Coagulation Studies

Purpose

This procedure provides instructions for processing blood samples for coagulation studies.

Principle

To ensure the quality of the test result, sample collection and processing must be in conformity with laboratory standards for hemostasis tests.

Scope

This procedure is to be performed by a trained Clinical Laboratory Scientist (CLS), Medical Laboratory Technician (MLT), Certified Phlebotomists, and Lab Assistants.

Policy

See SCPMG Policy for Preanalytical Processing for definitions on acceptable test orders, patient identification, labeling, and other workflows related to laboratory samples prior to testing.

Specimen sources

Citrated blood 9:1 (blood to anticoagulant) 3.2% sodium citrate. Other than citrate, no other anticoagulant is acceptable (e.g., oxalate, heparin, EDTA).

Specimen collection and processing

1. When multiple blood tubes are collected, the order of collection is important. Tubes for coagulation studies (light blue top) should be drawn before serum tubes (red top), because the clot activator in plastic serum tubes may cause interference in coagulation testing. Glass tubes that do not contain any additives (also red top) may be drawn before the coagulation tube.

2. Collect blood (9 vol.) in 0.109 M (i.e., 3.2 %) trisodium citrate anticoagulant (1 vol.). Different draw tube sizes of 3.2% sodium citrate tubes are acceptable for use. Buffered sodium citrate chelates the calcium in the sample to anticoagulate the blood and maintain the proper plasma pH. Alternatively, CTAD tubes, specially designed to prevent heparin inactivation, may be used when monitoring heparin therapy. Use sample collection tubes made of plastic or siliconized glass.
   - The ratio of blood to anticoagulant is ideally maintained at 9:1. This ratio is based on a normal hematocrit of 45%. A short sample or high hematocrit (greater than 55%) will result in an excess of anticoagulant, thereby prolonging clotting time results.
     - Sufficient volume is achieved if blood drawn falls above the minimum fill indicator (lower of the two etched lines or bottom nominal fill mark), which represents the minimum volume of blood required for appropriate analysis. Underfill of collection tube can result in prolongation of clotting times.
     - Overfill of collection tube can cause in vitro clot formation resulting in consumption of some clotting factors and elevation of others.
   - Collection of blood through lines previously flushed with heparin should be avoided, if possible. See **Limitations** for additional instructions.

3. Estimate the hematocrit of the sample. If the hematocrit is <=55%, accept the specimen. If the hematocrit of the sample is >55%, then:
   - Reject the specimen or request a redraw and inform the requesting provider of the delay, OR

Printed on 15 Sep 2021, 08:43:16 am; Printed by: H187829

Specimen Requirements for Coagulation Studies, Continued

- Redraw the sample by using a tube with adjusted anticoagulant. Adjust the anticoagulant in the container using this formula (CLSI H21-A5) to calculate the volume of citrate required in the tube for higher hematocrits:

  $C = (0.00185) \times (V) \times (100 - Hct)$

  C = volume of citrate required to be in the tube
  V = mL of whole blood (e.g., if 5 mL tube is used, volume is 4.5 mL)
  Hct = hematocrit of the patient
- Most high hematocrit samples can be adjusted as follows:

Tube size / Anticoagulant volume	Hematocrit	Anticoagulant needed	Blood volume needed	Anticoagulant to remove
5 mL / 0.5 mL	56-65%	0.3 mL	4.7 mL	0.2 mL
	>65%	0.25 mL	4.75 mL	0.25 mL
3 mL / 0.3 mL	56-65%	0.2 mL	2.8 mL	0.10 mL
	>65%	0.15 mL	2.85 mL	0.15 mL
2 mL / 0.2 mL	56-65%	0.13 mL	1.87 mL	0.07 mL
	>65%	0.10 mL	1.90 mL	0.10 mL

- Hematocrit levels of 55% or greater can result in prolongation of clotting times if the sodium citrate concentration is not properly adjusted.
- Hematocrit levels less than 23% may result in a clotted sample or shortened result, especially for factor assays.

4. Containers should immediately be gently mixed by four to six complete end-over-end inversions to assure thorough mixing of the specimen with anticoagulant. Do NOT shake or mix vigorously, as excessive mixing can cause hemolysis and/or platelet clumping and activation, leading to erroneous results. Improper mixing of anticoagulant can cause in vitro clot formation resulting in consumption of some clotting factors and elevation of others.
   - Clots may alter test results. Observe samples during inversions for any gross clot formation. If necessary, remove the cap, insert and remove two wooden sticks to detect a clot. See **Limitations** for additional instructions.
   - See also **Specimen transport/storage** for stability information on uncentrifuged whole blood samples.

5. Prior to testing, centrifuge the capped specimen tube. Centrifugation takes away platelet phospholipids from the plasma. Standard amounts of calcium and phospholipids are added to resume the clotting process in a controlled test environment.
   - The centrifugal speed and duration to consistently produce platelet-poor plasma (platelet count <10,000/uL) must be established by the laboratory and checked annually or after modification of the centrifuge.
   - Examples of centrifuge speeds and times:
     - 1500 g (RCF) for 15 minutes (CLSI)
     - 2000-2500 g (RCF) for 10-15 minutes

Specimen Requirements for Coagulation Studies, Continued

6. For samples that are frozen for later testing, double centrifugation is required to ensure the plasma is platelet-poor.
   - Use a plastic transfer pipette to transfer the plasma to a plastic tube. Do not disturb the buffy coat. Centrifuge the plasma for a second time using the validated settings. Use a transfer pipette to transfer the plasma to another plastic tube. Cap, label and freeze immediately at -20 °C or lower. Transport frozen specimens to their final destination either on dry ice or with blue frozen phase change materials (PCM) coolants.

7. Cap samples unless testing will be performed within 30 minutes.

8. It is unacceptable to combine the contents from separate, underfilled sodium citrate collection tubes.

Specimen Requirements for Coagulation Studies

Specimen transport / storage

The acceptable time delay before a sample is tested depends on the coagulation test performed. In most cases, if testing cannot occur within 4 hours, it is recommended to separate the plasma and freeze specimens.
Cold storage of citrated whole blood before processing may lead to activation of platelets, activation of Factor VII, and a time-dependent loss of Factor VIII.

Assay	**Stored as whole blood, uncentrifuged**	**Centrifuged and plasma separated within 1 hour of collection** (recommended)			
	Room temp 20 ± 5 °C *(refrigerated or frozen is unacceptable or unknown)*	**Room temp 20 ± 5 °C**	**Refrigerated 2-8 °C**	**Frozen* -20°C**	**Frozen* -70°C or colder**
PT / INR	Up to 24 hrs	Up to 24 hrs	Unacceptable	2 wks	12 months
APTT	Up to 4 hrs	4 hrs	Unacceptable	2 wks	12 months
APTT for heparin monitoring	Up to 1 hr	2 hrs	Unacceptable	2 wks	Unknown
APTT Mixing Studies	Up to 4 hrs	4 hrs	Unacceptable	2 wks	12 months
Fibrinogen	Up to 4 hrs	8 hrs	Unknown	Unknown	Unknown
DDimer	Up to 4 hrs	8 hrs	Unknown	4 wks	Unknown
Anti-Xa UFH or LMWH	Up to 1 hr	2 hrs	Unacceptable	2 wks	Unknown

Specimen Requirements for Coagulation Studies, Continued

Assay	Stored as whole blood, uncentrifuged	Centrifuged and plasma separated within 1 hour of collection (recommended)			
	Room temp 20 ± 5 °C *(refrigerated or frozen is unacceptable or unknown)*	Room temp 20 ± 5 °C	Refrigerated 2-8 °C	Frozen* -20°C	Frozen* -70°C or colder
Lupus Anticoagulant	[redacted]	[redacted]	[redacted]	2 wks*** Perform #6 under Specimen collection and processing. Verify that Platelet count is less than 10,000/uL prior to freezing.	6 months***
Factor VII	[redacted]	[redacted]	[redacted]	2 wks	6 months
Factor VIII**	4 hrs	4 hrs	Unacceptable	2 wks	6 months
vWF	4 hrs	4 hrs	Unacceptable	2 wks	6 months
Platelet Aggregation P2Y12, ARU	4 hrs				
Other	4 hrs	4 hrs	Unknown	Refer to CLSI H21-A5, Appendix B	

*Storage of citrated plasma in a frost-free freezer that is not continuously monitored can result in loss of labile Factor V and Factor VIII, and activation of Factor VII leading to overestimation of activity. Freezers that require manual defrosting should be used. A freezer with automatic freeze/thaw cycles is not acceptable, as it may result in cold activation of factor VII. Frozen plasma specimens should be rapidly thawed at 37°C water bath, then gently mixed and tested immediately. Resuspend any precipitation by thorough mixing immediately after thawing and before testing. Inadequate remixing of sample after thawing can result in spuriously low Factor VIII activity.

Specimen Requirements for Coagulation Studies

Specimen rejection

- A minimum of 90% fill is recommended. It is unacceptable to combine the contents from separate, underfilled sodium citrate collection tubes.
- Inspect coagulation specimens for clots. Specimens with grossly visible clots may have extremely low levels of fibrinogen and variably decreased levels of other coagulation proteins, so that results of the PT, aPTT, fibrinogen and other coagulation assays will be inaccurate or unobtainable.
- Use of clear plastic tubes (instead of amber) will facilitate checking integrity and condition of plasma specimens.
- Increased hematocrit value >55% (i.e. polycythemic patients) may lead to spurious coagulation results (PT, APTT, and some factor assays) due to increase plasma citrate concentration.
- Lipemia, hemolysis, or icterus may interfere in systems using photo-optical clot detection, dependent upon wavelength used in asay, and produce aberrant results.
- Hemolysis (independent of clot detection method) may prolong or shorten clotting times.

Reject specimen under the following conditions using the appropriate code:

- Improperly labeled
- Collected in wrong tube
- Clotted
- Specimen tubes filled below the etched mark fill line.
- Grossly lipemic
- Grossly hemolyzed
- Specimens with a hematocrit value >55% (i.e. polycythemic patients) should be rejected and redrawn using a reduced volume Sodium Citrate tube.
- Any condition that do not meet the stability and handling guidelines.

Materials and Supplies

- 5 mL light blue top (4.5 mL blood) – contains 0.5 mL of 3.2% (109 mM) buffered sodium citrate
- 3 mL light blue top (2.7 mL blood) – contains 0.3 mL of 3.2% (109 mM) buffered sodium citrate
- 3 mL CTAD tube (2.7 mL blood) – contains 0.3 mL of (0.11 M) buffered sodium citrate, (15 M) theophylline, (3.7 M) adenosine, and (0.198 M) dipyridamole
- 2 mL light blue top (1.8 mL blood) – contains 0.2 mL of 3.2% (109 mM) buffered sodium citrate
- Centrifuge
- Pipettes & tips

Safety

Laboratory employees are expected to maintain a safe working environment and an injury-free workplace. Laboratory employees are responsible for their own safety, the safety of others and adhering to all departmental safety policies and procedures.

Specimen Requirements for Coagulation Studies, Continued

Limitations

- **Drawing samples from IV lines:**
  1) When obtaining specimens from indwelling lines that may contain heparin, the line should be flushed with 5 mL of saline, and the first 5 mL of blood or 6 times the line volume (dead space of the catheter) be drawn off and discarded before the coagulation tube is filled.
  2) For samples collected from a normal saline lock (capped off venous port), twice the dead space volume of the catheter and extension set should be discarded.

- **Clots and serum samples:** The slightest coagulation (micro-clots) will induce considerable shortening of the times measured (autocatalytic activation of all the factors) whereas extensive coagulation will prolong the clotting times because of consumption of factors and fibrinogen. Checking for clots may be done:
  1) with applicator sticks, or
  2) by visual inspection of centrifuged plasma for small clots,
  3) by testing for Fibrinogen level. If the fibrinogen level is <25 mg/dL and a clot is not detected during PT and APTT testing, it should be suspected that the sample is actually serum.
  4) by comparing results using delta checks.

  Specimens that are clotted or determined to be probable serum samples should be rejected with the appropriate Cerner cancel codes.
- **Heparin therapy monitoring and plasma separation:** When monitoring heparin therapy, any release of platelet factor 4 (PF4) from platelets represents a major source of error, as PF4 is a potent inhibitor of heparin. For this reason, delays before centrifugation should not exceed one hour for blood collected in conventional citrate anticoagulant and within 4 hours for blood collected with CTAD tubes. Also, do not collect blood in glass, which might cause this release; collect blood in plastic, siliconized glass or CTAD tubes.
- **PT/INR and avoiding refrigerated samples:** For PT/INR testing, do not keep plasmas at 2-8 °C because in this temperature range the factor VII may be activated by the kallikrein system. Samples that will be subjected to testing of APTT- especially if this includes analysis of Factor VIII activity or vWF Factor activity- should not be stored at refrigerated temperatures (2-8°C), as cold temperatures may lead to a gradual loss of vWF and Factor VIII activity, potentially leading to a spurious diagnosis of vWD, misclassification of vWD, or spurious diagnosis of Facto VIII deficiency. Placing whole blood specimens directly on ice or in an ice water bath stimulates refrigeration and is also not recommended.

Validation of centrifuge settings for platelet-poor plasma

Annually the lab will evaluate all centrifuges used for processing coagulation testing specimens.

- Five specimens collected in sodium citrate tubes will be spun in each centrifuge
- The residual platelet count of the plasma will be measured using the hematology analyzer.
- If the average platelet count of the 5 specimens is less than $10x10^9$ /L, specimens requiring platelet-poor plasma may be spun for 5 minutes in the centrifuge.
- If the average platelet count exceeds 10x10 9 /L, modify the speed or time settings and re-test with 5 new specimens.

Specimen Requirements for Coagulation Studies, Continued

- If a centrifuge is unable to produce platelet-poor plasma and unable to modify its speed or time settings, label the centrifuge not to be used for coagulation specimens.
- Record and evaluate data using form.

Non-Controlled Documents / References

The following non-controlled documents support this procedure:

1. CLSI Document GP41: "Collection of diagnostic venous blood specimens; approved standard". Seventh edition, April 2017.

2. CLSI Document H21-A5: "Collection, transport, and processing of blood specimens for testing plasma-based coagulation assays and molecular hemostasis assays; approved guideline". Fifth edition, Vol. 28, No. 5, January 2008.

3. CLSI Document H48: "Determination of Coagulation Factor Activities Using the One-Stage Clotting Assay; approved guideline". Second edition, 2016.

4. CONTANT G., GOUAULT-HEILMANN M., MARTINOLI J.L.: "Heparin inactivation during blood storage: its prevention by blood collection in citric acid, theophylline, adenosine, dipyridamole - C.T.A.D. mixture". Thromb. Res., 31, 365-374, 1983.

5. Master Hematology and Coagulation Checklist - College of American Pathologists.

6. STA®-Neoplastine CI Plus (REF 00667) Determination of Prothrombin Time (PT) by STA® Analyzers. Diagnostica Stago Package insert, May 2019.

7. STA®-PTT A Reagent (REF 00595) Determination of Activated Partial Thromboplastin Time (APTT) by STA® Analyzers. Diagnostica Stago Package insert, May 2019.

8. STA®-Fibrinogen (REF 00674) Quantitative Determination of Fibrinogen by STA® Analyzers. Diagnostica Stago Package insert, June 2018.

9. STA®-LIATEST DDI Immuno-Turbidimetric Assay of D-Dimer (REF 00515) by STA® Analyzers. Diagnostica Stago Package insert, May 2021.

10. STA®-Liquid Anti-Xa (REF 00311 or 00322) Colorimetric Assay of Heparins (UFH and LMWH) by STA® Analyzers. Diagnostica Stago Package insert, November 2014.

Controlled Documents

Cerner Cancel Messages, SCPMG LIS
Policy for Preanalytical Processing, SCPMG PPP

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Regional Parent Document Reference Number: SCPMG-PPP-0438 Rev: 01