References:

1. Hamer M, Gibson EL, Vuononvirta R, Williams E, Steptoe A. Inflammatory and hemostatic responses to repeated mental stress: individual stability and habituation over time. Brain Behav Immun. 2006;20:456-9.
2. Blombäck M, Konkle BA, Manco-Johnson MJ, Bremme K, Hellgren M, Kaaja R; ISTH SSC Subcommittee on Women's Health Issues. Preanalytical conditions that affect coagulation testing, including hormonal status and therapy. J Thromb Haemost. 2007;5:855-8.
3. Gosselin RC, Marlar RA. Pre-analytical variables in routine coagulation testing: setting the stage for accurate results. Siemens Healthcare Diagnostics Inc. 2019. Order number 30-19-13597-01-76.
4. Lima-Oliveira G, Lippi G, Salvagno GL, Montagnana M, Picheth G, Guidi GC. Sodium citrate vacuum tubes validation: preventing preanalytical variability in routine coagulation testing. Blood Coagul Fibrinolysis. 2013;3:252-5.
5. Bowen RA, Adcock DM. Blood collection tubes as medical devices: the potential to affect assays and proposed verification and validation process for the clinical laboratory. Clin Biochem. 2016;49:1321-1330.
6. CLSI, Collection of Diagnostic Venous Blood Specimens, 7th ed. Standard GP41, 2017.
7. Uman LS, Birnie KA, Noel M, et al. Psychological interventions for needle-related procedural pain and distress in children and adolescents. Cochrane Database Syst Rev. 2013 Oct 10;(10):CD005179. doi: 10.1002/14651858.CD005179.pub3.
8. World Health Organization. WHO guidelines on drawing blood: best practices in phlebotomy. https://www.ncbi.nlm.nih.gov/books/NBK138650/pdf/Bookshelf_NBK138650.pdf
9. Favaloro EJ, Adcock Funk DM, Lippi G. Pre-analytical variables in coagulation testing associated with diagnostic errors in hemostasis. Labmedicine. 2012;43(2):1-10.
10. Mackie I, Cooper P, Lawrie A, Kitchen S, Gray E, Laffan M; British Committee for Standards in Haematology. Guidelines on the laboratory aspects of assays used in haemostasis and thrombosis. Int J Lab Hematol. 2013;35:1-13.
11. Adcock DM, Kressin DC, Marlar RA: Effect of 3.2% vs. 3.8% sodium citrate concentration on routine coagulation testing. Am J Clin Path. 1999;107:105-110.
12. Adcock DM, Kressin DC, Marlar RA. Are discard tubes necessary in coagulation studies? Lab Med. 1997;28(8):530-533.
13. Marlar RA, Potts RM, Marlar AA. Effect on routine and special coagulation testing values of citrate anticoagulant adjustment in patients with high hematocrit values. Am J Clin Path. 2006;126:400-5.
14. CLSI. Platelet function testing by aggregometry, 1st ed. H58A, 2008.
15. Glas M, Mauer D, Kassas H, Volk T, Kreuer S. Sample transport by pneumatic tube system alters results of multiple electrode aggregometry but not rotational thromboelastometry. Platelets. 2013;24:454-61.
16. Hübner U, Böckel-Frohnhöfer N, Hummel B, Geisel J. The effect of a pneumatic tube transport system on platelet aggregation using optical aggregometry and the PFA-100. Clin Lab. 2010;56:59-64.
17. Wallin O, Söderberg J, Grankvist K, Jonsson PA, Hutdin J. Preanalytical effects of pneumatic tube transport on routine hematology, coagulation parameters, platelet function and global coagulation. Clin Chem Lab Med. 2008;46:1443-9
18. van Geest-Daalderop JH, Mulder AB, Boonman-de Winter LJ, Hoekstra MM, van den Besselaar AM. Preanalytical variables and off-site blood collection: influences on the results of the prothrombin time/international normalized ratio test and implications for monitoring of oral anticoagulant therapy. Clin Chem. 2005;51:561-8.
19. Nelson S, Pritt A, Marlar RA. Rapid preparation of plasma for 'stat' coagulation testing. Arch Pathol Lab Med. 1994;118:175-6.
20. Foshat M, Bates S, Russo W, Huerta A, Albright K, Giddings K, Indrikovs A, Qian YW. Effect of freezing plasma at –20°C for 2 weeks on prothrombin time, activated partial thromboplastin time, dilute Russell viper venom time, activated protein C resistance and D-dimer levels. Clin Appl Thromb Hemost. 2015;21:41-7.
21. Gosselin RC, Dwyre DM. Determining the effect of freezing on coagulation testing: comparison of results between fresh and once frozen-thawed plasma. Blood Coagul Fibrinolysis. 2015;26:69-74.
22. Lippi G, Plebani M, Favaloro EJ. Interference in coagulation testing: focus on spurious hemolysis, icterus, and lipemia. Semin Thromb Hemost. 2013;39:258-66.
23. D'Angelo G, Villa C, Tamborini A, Villa S. Evaluation of the main coagulation tests in the presence of hemolysis in healthy subjects and patients on oral anticoagulant therapy. Int J Lab Hematol. 2015;37:819-33.
24. Lippi G, Montagnana M, Salvagno GL, Guidi GC. Interference of blood cell lysis on routine coagulation testing. Arch Pathol Lab Med. 2006;130:181-4.
25. Jahr JS, Lurie F, Gosselin R, Lin JS, Wong, L, Larkin E. Effects of a hemoglobin-based oxygen carrier (HBOC-201) on coagulation testing. Clin Lab Sci. 2000;13(4):210-14.
26. Jahr JS, Liu H, Albert OK, Gull A, Moallempour M, Lim JC, Gosselin R. Does HBOC-201 (Hemopure®) affect platelet function in orthopedic surgery: a single site analysis from a multicenter study. Am J Ther. 2010;17:140-7. Epub May 2009.

Siemens Healthineers Headquarters
Siemens Healthcare GmbH
Henkestr. 127
91052 Erlangen, Germany
Phone: +49 9131 84-0
siemens-healthineers.com

Published by
Siemens Healthcare Diagnostics
Products GmbH
Laboratory Diagnostics
Emil-von-Behring-Strasse 76
35041 Marburg, Germany

Published by Siemens Healthcare Diagnostics Inc. · Order No. 30-19-13634-01-76 · 04-2019 ·

Key Facts

Pre-analytical Variables In Routine Coagulation Testing: Setting the Stage for Accurate Results

Gosselin RC, University of California, Davis Health System, Sacramento, California, U.S.
Marlar RA, University of New Mexico Health Sciences Center Albuquerque, New Mexico, U.S.

SIEMENS Healthineers

Section	Recommendation
Patient Selection	The laboratory must obtain the proper reference interval for the populations being assessed (accreditation requirements).
	Patients should be relaxed prior to phlebotomy to avoid physiological and psychological stress that may artefactually alter coagulation tests, especially for VWF and platelet-function studies.[1,2]
	Optimal assessment of platelet-function studies should be performed using samples collected from fasting and drug-naïve patients.
	For patients that are being monitored (e.g., anticoagulation and replacement therapy), adherence to the collection time is mandatory.[3]
Specimen Collection	There are significant differences between reported PT and APTT results using collection tubes with the same citrate concentration from different manufacturers. The laboratory must validate these systems prior to implementation.[4,5]
	If the syringe technique is required, use a syringe less than 25 cc (preferably 10 cc) with a "butterfly" needle apparatus.
	Needle gauges for coagulation testing should range from 22 to 19 gauge, with higher gauges (23–25 gauge) for pediatric or difficult venous-access patients.[6,7]
	For syringe collections of blood over 30 mL, an 18 gauge needle is recommended.
	Tourniquet time should not exceed 1 minute.
	For syringe collections, blood should be carefully introduced into appropriate blood collection tubes within 1 minute of collection.
	For arterial-line collections, a two-syringe technique is required, with the first 10 mL used to clear the line and the second syringe used for blood collection.[6,8]
	For intravenous (IV)-line collections, turn off the IV line for 5 minutes. Then use the two-syringe technique, with the first 10 mL to clear the line and the second syringe for blood collection.[6,7]
	Follow manufacturer recommendations for under- or overfilling blood collection tubes. Generally both should be avoided, unless the laboratory can establish (demonstrated with supporting data) its own criteria for acceptance.
	Underfilling of blood collection tubes is the predominant cause for falsely elevated PT, INR, and APTT results.[9]
	Gentle inversion (mixing) of sodium citrates tube approximately 5–6 times is recommended. Avoid rigorous shaking or agitation.[10]
	3.2% sodium citrate is the citrate concentration of choice.[6,10,11]
	For patients who need multiple-tube collections, the specified collection sequence is required.[3,6,10]
	If only citrate collection tubes are being collected, no discard tube is necessary (unless using butterfly syringe method directly into collection tube).[12]
	Patients with elevated hematocrits (>55%) require reduced volume of citrate prior to collection.[6,10,13]
Transportation and Stability before Processing	Coagulation samples should not be transported or stored on ice.[6]
	Coagulation samples for platelet-function studies must be maintained at room temperature.[14]
	Whole-blood samples for PT testing are stable for 24 hours at room temperature.[6]
	Whole-blood samples for APTT testing are stable for 4 hours at room-temperature, unless used for UFH monitoring, in which case the room temperature stability of whole blood is 1 hour.[6]
	For other tests, unless otherwise indicated by the manufacturer, stability of whole blood is 4 hours.
	Pneumatic transport systems should not be used for samples that require platelet-function testing.[15-17]
	Samples collected outside the confines of the hospital (e.g., home health and remote facilities) should be transported in containers (e.g., insulated STYROFOAM) that ensure ambient room temperature.
	For whole-blood samples being transported distances (e.g., via automobile), the tubes should be racked and positioned upright.[18]

Section	Recommendation
Specimen Processing	Except for whole-blood testing and platelet-function studies, platelet-poor plasma (PPP) is the sample of choice.[6,10]
	PPP is defined as <10,000 platelets/µL.[6]
	Internal temperature for centrifuges for processing PPP must be room temperature (15–25°C).
	Although the recommended centrifuge force to obtain PPP is 1500 g for 10 minutes, the laboratory must verify its centrifugation speed (rpm) or force (g) to ensure PPP.[19]
	All coagulation samples must be double-centrifuged prior to freezing.
	Platelet counts from PPP processing must be verified at least annually (depending on the accreditation standard).
	Multiple tubes collected from a single patient should not be pooled prior to storage or testing.
	All primary and secondary tubes and tubes to be frozen must have multiple patient identifiers and date and time of collection.
Sample Storage	For samples not tested within the recommended room-temperature stability limits, PPP should be stored frozen in 0.5–1.0 mL aliquots in appropriately labeled polypropylene vials.
	Optimal freezing method is –70°C or colder, non-frost-free freezer, which provides PPP sample stability of 6 months.[6,10,20,21]
	PPP samples can be stored at –20°C in a non-frost-free freezer for 2 weeks.[20,21]
	Sample vials (with caps on) should be rapidly thawed in a 37°C water bath.
	Thawed PPP aliquots must be mixed prior to analysis.
Hemolysis, Icterus, Lipemia	HIL may affect the ability of optical-reading instruments to accurately assess PPP samples.[22]
	Suspected ex vivo (in vitro) hemolyzed PPP samples should be rejected.[6,10,23,24]
	Lipemic samples may be processed using ultracentrifugation methods, but a parallel, nonlipemic sample should be processed concomitantly to assure the processing method is acceptable.[22]
	Icteric samples may interfere with accurate assessment of chromogenic methods.
	Infusion of HBOC (hemoglobin-based oxygen carrier) products creates a pseudo-hemolysis appearance in the plasma and may interfere with clot- and chromogenic-based assays.[25,26]