PART 3 Sample Integrity and Preanalytical Variables

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TOPICS COVERED

- ✓ Blood Collection
- ✓ Platelet Poor Plasma (PPP)
- ✓ Interfering Substances
- ✓ Storage
- ✓ Thawing

Prior to blood collection, there are a few factors that must be taken into account. Fasting is not required prior to blood collection for most bleeding and thrombotic investigations. However, one exception is the homocysteine test which does require fasting. Exercise (Venema et al, 2017) and stress (Austin et al, 2012) can cause temporary increases in FVIII and VWF. Exercise can also affect the D-dimer test (Huskens D et al, 2016). Inflammation can affect clotting factors and other hemostatic parameters (Hardy et al, 2024). Pregnancy affects a variety of parameters including FVIII (Castaman, 2013), VWF (Delbrück et al, 2019), and D-dimer (Blombäck et al, 2007). Numerous pharmaceutical products and anticoagulants can interfere with hemostasis tests, therefore information regarding patient treatments is essential for the laboratory (Gosselin et al, 2019).

Blood Collection: Several guidelines are available describing best practices for sample collection and processing for hemostatic testing (CLSI, 2024; CLSI, 2017). Blood collection should use an evacuated collection system or plastic syringe with a 19- to 21-gauge needle (adults) or 22- to 23-gauge needle (children) (Srivastava et al, 2021). Tube types are not all the same, therefore centers should use a single tube type and generate reference intervals based on these tubes (Bowen et al, 2016). Even within a collection tube type, constitution is important; plastic and glass tubes are not interchangeable (Fiebig et al, 2005). Blood collection tubes should contain 0.105 to 0.109 M (3.2%) trisodium citrate (CLSI, 2024). The sequence of draw is important to prevent EDTA (Lima-Oliveira et al, 2015) or heparin cross contamination (Keppel et al, 2019); as such best practice in sample draw should be followed (WHO, 2010; Simundic et al, 2018). Samples require immediate anticoagulation after venipuncture, with filling to minimum 80% target volume (Kitchen et al, 2021) to achieve 9:1 blood:anticoagulant ratio. Gentle inversion (3 to 5 times) of blood tubes post phlebotomy allows suitable mixing of samples. Unwanted hemostatic changes occur in underfilled tubes (Lippi et al, 2012). Samples with hematocrit levels >55% require an adjusted citrate solution to compensate for the high packed cell volume and to obtain the correct 9:1 ratio. Significant changes in PT, APTT, and anticoagulant monitoring (INR) can be seen if this ratio is not maintained (Marlar et al, 2006). The recommend formula for readjusting citrate levels is shown below (Kitchen et al, 2021). Samples should be suitably labelled immediately pre- or post-phlebotomy following local regulatory or institutional policies.

$C = (1.85 \times 10^{-3})(100 - HCT)(V)$

C = volume of citrate in milliliters (ml) that should be added to a volume of blood (V)

HCT = is the hematocrit of the patient

V = is the volume of blood added in ml

And 1.85×10^{-3} is constant

An example using HCT of 70% and 4.5 ml of collected blood prior to addition of anticoagulant, gives the following calculation that is, 0.25 ml of citrate is mixed with 4.5 ml of blood.

$(1.85 \times 10^{-3})(100 - HCT)(V) = C$

$(1.85 \times 0.001)(100 - 70)(4.5\ ml) = 0.25\ ml\ of\ citrate$

Platelet Poor Plasma (PPP): Most coagulation tests can be performed with PPP after centrifugation at >1700g for 10 minutes (CLSI, 2024; Kitchen et al, 2021). Refrigerated centrifuges should be avoided, as cold activation of platelet factor 4 can affect heparin monitoring, platelet function tests, FVIII tests, and VWF tests (Favaloro, 2004). Some tests, such as unfractionated heparin (UFH) and Lupus anticoagulant, require double spun platelet free plasma ($<10 \times 10^9$) if testing is carried out on previously frozen samples. In this instance, plasma is removed from the spun blood tube into a secondary suitable container and re-spun with subaliquots removed for freezing. Platelet function testing requires platelet rich plasma (PRP), prepared after centrifugation at 170g for 15 minutes or 250g for 10 minutes (Gomes et al, 2021).

Interfering Substances: Hemolyzed samples should not be tested, as significant changes, particularly in APTT, can be seen (Woolley et al, 2016; Lippi et al, 2013), except when hemolysis is intravascular (Arachchillage et al, 2014). Routine assays are usually unaffected by jaundice/icterus (Woolley et al, 2016) and lipemia can be overcome by ultracentrifugation (Lippi et al, 2013, Dimeski and Jones, 2011).

Storage: Sample testing is time sensitive. Sample processing and testing should be conducted within the assay stability window after venipuncture, and samples should be stored at room temperature in the intervening time. Guidelines recommend testing within 4 hours (CLSI, 2024) for all samples, unless local data confirm extended stability for a specific tube/assay combination (Kitchen et al, 2021; Linskens et al, 2018). Higher temperature storage can lead to loss of clotting factors, such as FVIII (Omidkhoda et al, 2011). Release of platelet factor 4 can cause heparin neutralization of unfractionated heparin in samples, therefore these tubes should be centrifuged within 1 hour and analyzed within 4 hours (Baker et al, 2020). If plasma is stored for later testing, storage conditions can affect some assays. Plasma can be acceptably stored at -24°C for 3 months. However, for longer-term storage (approximately 6 months), samples should be stored -70°C (Woodhams et al, 2001; Fenclova et al, 2023).

Thawing: Frozen samples for testing should be thawed in a water bath at 37°C for 3 to 5 minutes and inverted several times prior to testing to homogenize the sample (Jo et al, 2020). Re-freezing once-thawed plasma for further testing should be avoided.

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