Quality Standards for Sample Processing, Transportation, and Storage in Hemostasis Testing

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Abstract

Samples for hemostasis testing drawn into sodium citrate anticoagulant are vulnerable to the effects of preanalytical variables associated with sample processing, transportation, and storage. These variables include the temperature at which samples are transported and stored; the stability of the samples once processed; whether maintained at room temperature, refrigerated, or frozen; methods of centrifugation; as well as the potential impact of using an automated line. Acknowledgment of these variables, as well as understanding their potential impact on assay results, is imperative to the reporting of high quality and accurate results. This article discusses the preanalytical issues associated with sample processing, transportation, and storage and also presents the ideal conditions for sample handling.

Keywords

- preanalytical variability
- coagulation testing
- prothrombin time
- activated partial thromboplastin time
- laboratory errors
- quality standards

To provide accurate and reliable laboratory test results, clinical laboratories must employ a well-developed, implemented, and monitored quality management system.[1] One of the fundamental elements of a quality management system are quality standards, such as those developed for sample processing, transportation, and storage of specimens for hemostasis testing. Quality standards must be clearly written and document not only proper procedures, but also what impact may occur to the sample and ultimately patient care, if these are not followed. Training and regular competency assessments surrounding these quality standards are also key components to a quality system.[2] Improper procedures associated with sample processing, transportation, and storage may alter the sample such that the results do not reflect the true condition of the patient, although they may be accurate for the test sample.[3] Such errors may lead to mistaken or inappropriate diagnosis with resultant detrimental impact on patient care.[4] Laboratory errors attributed to the preanalytical phase of testing can be significant and exceed those that occur in the analytical phase.[5–8] Samples for routine or specialized hemostasis testing are especially vulnerable to such errors compared with other types of laboratory samples. It is imperative, therefore, that these quality standards are closely followed and any deviations validated. This article will highlight standards that should be applied to sample processing, transportation, and storage for hemostasis assays, for those samples collected in sodium citrate anticoagulant. Sample collection standards for coagulation testing are discussed elsewhere in this issue of *Seminars in Thrombosis & Hemostasis*.[9]

Whole blood samples for plasma-based coagulation assays would, in ideal circumstances, be collected and processed to produce platelet-poor plasma (PPP) within 1 hour.[6,10] Transportation and storage of sodium citrate whole blood samples

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would occur at ambient temperature (15 to 22°C) and whole blood samples would not be placed on ice, in an iced water bath, or refrigerated. Sample analysis for routine assays would be completed within 4 hours of collection, with the notable exception that samples for prothrombin time (PT) testing are stable for 24 hours. Sample analysis for more complex hemostasis assays would also ideally be undertaken before deleterious effects of sample aging, within similar time frames (dependent on the stability of the test analyte, see ►**Table 1**).

Often, however, specimens for coagulation testing are collected at a site other than the testing facility. It is common, for example, for patient samples to be drawn in physician offices or extended care facilities. Reference or hospital laboratories also frequently utilize conveniently located patient draw stations. Each of these scenarios requires that samples then be couriered from the sites of collection to regional facilities for testing. Finally, laboratories may ship samples that require more esoteric coagulation assays to centralized specialized laboratories. Depending on the testing ordered, as well as the distance and time required between collection and analysis, samples may be transported and stored in any of the following three conditions: (1) unprocessed as sodium citrate whole blood samples, (2) centrifuged, but maintained in the primary sodium citrate tube, or (3) processed by centrifugation and plasma aliquoted into a secondary tube.

Samples must always be transported in a manner that avoids infectious potential and in accordance with institutional policies and procedures. Many regulations require that samples are transported inside of impermeable plastic bags which are sealed. Facilities must also be aware of applicable regulations regarding packaging and shipment of hazardous materials. For example, in the United States, the Centers for Disease Control, International Air Transport Association, and Department of Transportation have regulations governing the transport of clinical specimens.[10]

Transportation and Storage of Unprocessed or Processed Sodium Citrate Tubes for Plasma-Based Coagulation Assays

Samples transported from outlying facilities to central coagulation laboratories are often maintained unprocessed during transportation and storage. This has several advantages. For example, drawing sites do not require equipment (such as centrifuges) to process samples and the receiving coagulation laboratory can better evaluate primary collection tubes for certain preanalytical variables such as fill volume, presence of clots, hematocrit, and correct collection tube type. However, sample integrity for certain specific assays, such as for activated partial thromboplastin time (APTT) or anti-Xa assay in the presence of unfractionated heparin (UFH), may be enhanced if samples are centrifuged immediately after blood collection, rather than shipped unprocessed.

Specimens should arrive in the testing facility allowing sufficient time for the samples to be processed (if necessary) and analyzed in agreement with the specified time frames listed in the guidelines below. Unprocessed or processed samples collected in sodium citrate, should remain capped and maintained at ambient (room) temperature (~18 to 25°C).[10] Whole blood samples must not be refrigerated or stored on ice or in an iced water bath. Whole blood samples should be transported in a vertical rather than horizontal position and if processed, should not be agitated as to remix the plasma and cellular components.[11] The sample may be compromised and therefore develop the potential to generate erroneous results, which may then be unwittingly reported to

Table 1 Stability of Whole Blood Sample for Common Hemostasis Assays

Assay	Stability of Whole Blood Sample	
	CLSI H21 A5[10]	Other[30–35]
APTT	4 h	18–24 h
PT	24 h	24–72 h
APTT or anti-Xa assay for sample containing UFH	1 h	
APTT or Anti-Xa assay for sample containing LMWH	4 h	24 h
Factors II, VII, IX, X, and XI activities	4 h	48 h
Factors V and VIII	4 h	24 h
VWF:Ag and VWF:RCo	4 h	24–48 h
Fibrinogen	4 h	48 h to 7 d
D-dimer	4 h	48 h
Antithrombin activity	4 h	48 h to 7 d
PC activity	4 h	48 h
PS activity	4 h	4–6 h
Free PS	4 h	24 h

CLSI, Clinical and Laboratory Standards Institute; APTT, activated partial thromboplastin time; h, hours; PT, prothrombin time; UFH, unfractionated heparin; LMWH, low-molecular weight heparin; VWF:Ag, von Willebrand factor antigen; VWF:RCo, von Willebrand factor: Ristocetin cofactor; d, days; PC, protein C; PS, protein S.

the requesting clinician, because of any of the following conditions: changes in sample pH; extremes of temperature; physical trauma to the specimen; excessive delay between collection and analysis; and inappropriate condition of separation. Each of these variables and their impact on assay results is discussed below.

Sample pH

The buffering capacity of citric acid, which is a key component of commercial trisodium citrate collection tubes, maintains the sample pH between 7.30 and 7.45.[12] Maintenance of physiologic pH is critical to obtain accurate plasma-based hemostasis testing as well as platelet function studies. An increase in specimen pH occurs if samples are stored uncapped for more than ~30 minutes, as carbon dioxide diffuses from the plasma into the ambient atmosphere.[13] The use of small caliber tubes, to minimize the surface area-to-volume ratio, will help maintain pH.[13,14] Processed samples are more susceptible to change in pH than are whole blood samples due to the loss of the hemoglobin buffering capacity.[13] Increase in pH leads to clinically significant prolongations of the APTT and PT, and also affects a variety of specialized coagulation assays including loss of platelet reactivity. For example, a pH change of as little as 0.8 units may prolong the APTT of a normal sample by greater than 20 seconds, depending on the buffering capacity of the reagent used in the test system.[15]

Temperature Effects

Hemostasis factors, including procoagulant factors, naturally occurring anticoagulant factors, as well as platelets, become labile in an ex vivo environment. These components may undergo either in vitro degradation or activation that is both time and temperature dependent. For example, FVIII and protein S (PS) are particularly labile factors that are prone to degradation, such that loss of activity occurs within ~4 to 6 hours if blood is maintained at room temperature.[16,17] Degradation may be accelerated at very warm temperatures and essentially all factors will lose activity if maintained at 58°C for a period of time.[18] Conversely, platelets and FVII are activated by cold temperatures. Indeed, platelets may undergo spontaneous aggregation in the cold.[19] Cold activation of FVII can result in elevation of FVII activity by 150% or more with a resultant decrease in PT.[20] Cold activation of whole blood samples may also lead to clinically significant loss of FVIII and von Willebrand factor (VWF) leading to a mistaken diagnosis of hemophilia A or von Willebrand disease.[21] In a study published by Favaloro et al, 50% of whole blood samples demonstrated to have FVIII activity and VWF levels in the normal reference intervals when properly processed, fell below a normal reference interval of 50% when stored at ~4°C for 3.5 hours. This effect was slightly greater using a functional measure of VWF activity than the measure of antigen.[22] Whole blood samples therefore should not be stored or transported on ice or in a refrigerator. If for some reason, whole blood samples destined for VWF and FVIII analysis are stored in the cold, samples should be warmed and thoroughly mixed before processing.[23] In a further study, however, Salvagno et al failed to observe clinically significant variations of routine coagulation tests (i.e., PT, APTT, fibrinogen, and D-dimer) in whole blood samples left uncentrifuged and stored at 4°C for up to 6 hours. Significant variations were instead observed at 24 hours for APTT, but not for PT, fibrinogen, and D-dimer.[24] Extremes of temperature may occur if whole blood samples are stored in laboratory collection boxes that are maintained out of doors depending on external temperatures, or if samples are transported without adequate protection against the elements (e.g., stored in a vehicle's trunk without an insulated container).[25]

Sample Transportation

Whole blood samples should be transported in a manner that avoids significant physical trauma. If samples are transported after centrifugation but plasma is not aliquoted, centrifugation should always be repeated before analysis. Samples should be transported in an upright position. High speed pneumatic tube systems are often employed to allow rapid transport of patient samples. It has been historically reported that, due to the rapid acceleration and deceleration forces that occur in some of these systems (especially the older ones), samples may suffer trauma, resulting in platelet activation and red-cell fragmentation and/or release of adenosine diphosphate (ADP). Nevertheless, while use of pneumatic tube transport systems is generally not recommended for samples that will be subject to platelet function studies, no ill-effects have been documented for samples for plasma-based coagulation assays.[26,27] Specifically, no significant effects were demonstrated in the evaluation of paired samples (one sample hand delivered and the other transported by pneumatic tube) tested using PT and APTT assays.[28] This study also included a comparison of paired fibrin monomer results, an extremely sensitive marker of activation of the coagulation cascade, and demonstrated no significant difference. In a more recent study by Wallin et al, no preanalytical effect of pneumatic tube transport could be recorded for most coagulation parameters and Platelet Function Analyzer (PFA-100® System, Siemens Healthcare Diagnostics Inc., Marburg, Germany) analysis.[29] Only for thromboelastographic analysis did the time to clot formation exhibit a significant shortening (i.e., −16%) in samples shipped by a pneumatic transport tube system.[29] It is thereby conceivable that pneumatic transportation of specimens would only negligibly affect most coagulation tests, and it seems reasonable that only samples for platelet aggregation studies need be manually transported to the laboratory.

Guidelines for Storage and Transportation of Unprocessed or Processed Whole Blood Samples

The following guidelines for the storage and transportation of unprocessed or processed, whole blood samples in sodium citrate evacuated tubes should generally be followed, unless other conditions have been validated. Whole blood samples should be maintained capped, in an upright position, and at ambient temperatures and not be placed in refrigerated storage (from 2 to 8°C). The allowable times listed should

also encompass the time required for sample processing and analyses.

1. Specimens for APTT and most special coagulation assays (such as factor assays, assays for the detection of lupus anticoagulants (LA), and VWF assays) that do not contain UFH are stable for up to four hours from the time of specimen collection, according to the CLSI H21 guideline.[10] Limiting samples to a four-hour stability is conservative and samples for APTT and most special coagulation assays are likely stable for longer periods of time. A host of published studies have demonstrated 6 to 8 hours or longer stability of APTT-based assay samples and even 24-hour stability for some special coagulation assays.[30–33] Zürcher and colleagues demonstrated that with the exception of FV and FVIII activities and free PS antigen, several hemostasis assays are stable for 24 to 48 hours following collection (see ►**Table 1**).[31] Samples for fibrinogen and antithrombin activity have been shown to be stable for up to 7 days.[30] It has also been demonstrated that whole blood samples from patients on low-molecular weight heparin are stable for 24 hours after collection, if samples will be subject to heparin monitoring using a chromogenic anti-Xa assay.[33] Samples for most VWF-based assays are also stable for up to 24 hours when stored as whole blood at ambient temperature.[34]
2. Samples suspected or known to contain UFH that will be subjected to APTT or anti-Xa analysis, should be maintained at room temperature, and centrifuged within 1 hour of collection. If samples are left unprocessed for longer periods of time, platelet factor 4 released from platelets will neutralize the UFH present in the sample leading to clinically significant, factitiously low APTT and anti-Xa results.[35] If samples are centrifuged within 1 hour of collection, samples are stable as long as testing is completed within 4 hours.[35]
3. Samples for PT or international normalized ratio (INR) assays are stable for at least 24 hours when maintained at room temperature, unprocessed or processed, according to CLSI H21 guideline.[10] Indeed, up to 3-day stability of PT/INR determinations in patients on warfarin therapy, using a variety of thromboplastin reagents has been demonstrated.[36] Mechanical agitation of the whole blood sample should be avoided during transportation or storage, as this has been shown to lead to a spurious increase in PT/INR results due to an unknown mechanism.[11] Measured activities of the vitamin K-dependent factors (FII, FVII, FIX, and FX) have been shown to be stable for up to 24 hours if unprocessed or processed samples are stored at room temperature.[37]

Transportation and Storage of Sodium Citrate Collection Tubes for Platelet Function Studies

Samples for platelet function testing require special handling.[38] This includes determination of closure time using a or platelet aggregation studies, employing light transmittance or impedance methodologies using either platelet-rich plasma (PRP) or whole blood samples. Samples for platelet function testing should not be transported using a pneumatic tube system, especially if this has not been validated, as the potential physical trauma may lead to platelet activation and release of ADP from erythrocytes, which will blunt platelet responsiveness.[25] Platelets should always be transported in an upright position and stored at room temperature.[13] Exposure to cold temperatures causes physical alterations to the platelets including shape changes and loss of their microtubular systems.[13] Storage at refrigerated temperatures also causes spontaneous platelet activation resulting in aggregation, while storage at 37°C leads to impaired responsiveness.[13] The extent of variation in the responsiveness of platelets also depends on the time interval between venipuncture and testing, as whether the sample is tested as PRP or whole blood.

Platelet Rich Plasma

Platelets demonstrate less responsiveness for the first 30 minutes after processing and it has been suggested that this initial refractoriness is likely a result of ADP released from erythrocytes and platelets during centrifugation.[13] The platelets in PRP maintain ideal responsiveness for only 3 to 4 hours following processing and lack of response beyond this is likely a reflection of the increase in sample pH and exhaustion of energy reserves.[13,14] For this reason, testing is often performed after 30 minutes but within 3 to 4 hours of PRP preparation. Efforts to help maintain pH of the PRP should be employed such as using small caliber tubes and limiting mixing/agitation of the PRP.[13,14]

For use in platelet aggregation studies, the PRP is often adjusted using autologous PPP to achieve a standardized platelet number (typically 200×10^9/L to 300×10^9/L). This practice is discouraged by Cattaneo et al who have demonstrated that substances released by platelets and other blood cells during centrifugation necessary to obtain PPP has an inhibitor effect on platelet aggregation.[39] In support of this, Linnemann et al demonstrated that adjustment of the platelet count in PRP is time-consuming and of no advantage when performing platelet function testing and is therefore unnecessary.[40] A comparative analysis performed by Favaloro and Mohammed also noted deleterious effects of platelet count adjustment in a small case series.[41]

Whole Blood

Recommendations for handling of PRP samples should generally be applied to whole blood samples for platelet function analysis. Use of whole blood samples avoids the artifacts that may occur secondary to centrifugation, including platelet activation and release of platelet inhibitor cellular products.[42] According to manufacturer instructions, whole blood samples for testing in the PFA-100® system should sit for 10 minutes following phlebotomy. If the sample has been transported from another site, samples should sit for 30 minutes so that the platelets return to their "resting" state. Platelet function testing using whole blood samples should be completed within 3 hours of phlebotomy.[13] It may also be worthwhile to verify (e.g., by systematic centrifugation of the

specimens after analysis has been completed) whether collection and handling of whole blood samples has generated spurious hemolysis, because platelet as well as leukocyte and erythrocyte injury dramatically affects test results of PFA-100.[43]

Transportation and Storage of Plasma Aliquoted from a Sodium Citrate Collection Tube into a Secondary Aliquot Tube for Plasma-Based Coagulation Studies

If samples cannot be transported as whole blood to a facility for testing under ideal conditions or within their specified time frames, they should be processed by centrifugation and aliquoted into secondary aliquot tubes. Samples that will ultimately be aliquoted should be processed promptly, ideally within 1 hour of collection and certainly within 4 hours. Aliquot tubes must be composed on an appropriate material, consisting of a nonactivating substance such as polypropylene. Polystyrene tubes are not acceptable for coagulation studies. Once aliquoted, samples must be capped to avoid changes in pH. Aliquot tubes must be properly labeled with the appropriate patient identification and labeled as to the matrix of the sample (e.g., citrated plasma, ethylenediaminetetraacetic acid [EDTA] plasma, heparin plasma, and serum).[44]

Most hemostasis tests require citrated plasma. Moreover, EDTA plasma, heparin plasma, and serum are generally unsuitable for most hemostasis tests. Collection of samples into an incorrect type of evacuated tube accounts for ~5 to 13% of all unsuitable samples received by clinical laboratories, and up to 2% of all samples received in the coagulation laboratory.[45,46] When transported in the primary collection tube, receipt of the incorrect tube is easily determined. Once aliquoted into a secondary tube, however, serum and all types of plasma have essentially an identical appearance and it is not obvious that the sample type is inappropriate. Collection of blood for most plasma-based coagulation assays into a tube other than sodium citrate is a cause for specimen rejection. When testing is performed on the incorrect sample type, the laboratory might issue a test result that reflects the true status of the test samples provided (e.g., serum or EDTA plasma), but this would not reflect the true status of the patient under investigation.[47] The effects of these different matrices on hemostasis assays are variable and depends on the sample type received as well as the test performed (►**Table 2**).[6] While a sample that results in "no clot detected" for APTT and PT assays may be readily recognized to inappropriately be serum or lithium heparin, the effects of the different sample matrices on other test results may be much more subtle.[6,7] While patterns of test results may suggest an incorrect sample type, the application of simple algorithms incorporating the measurement of sodium, potassium, and citrate can greatly assist in classifying a sample as other than sodium citrate plasma.[47] As specifically regards citrated plasma, there is a significant decrease in potassium, chloride, calcium, and magnesium, and a substantial increase in sodium, when compared with EDTA, serum, and lithium-heparin plasma. Although two different algorithms have been developed and validated to verify the nature of the sample matrices, the simplest requires performance of only potassium and possibly sodium (►**Fig. 1**).

Separated plasma can generally be maintained at room temperature or refrigerated for a few hours without adverse effect on coagulation. If samples are stored for greater than 4 hours from collection, they should be maintained in an

Table 2 Effect of Sample Matrix on Common Hemostasis Assays

Tube Type	3.2% Citrate	EDTA	Sodium Heparin	Serum
Assay	Mean/Range			
APTT (s)	29/25–33	68/45–92	NCD	NCD
PT (s)	12.4/11.5–13.2	23/19–27	NCD	NCD
dRVVT (s)	34.6/27–43	55/45–64	NCD	NCD
FV activity (%)	113/84–142	71/39–103	81/59–103	23/13–33
FVII activity (%)	115/50–180	116/51–182	77/43–107	308/80–437
FVIII activity (%)	141/80–202	7.5/2–19	<1	4.5/1.3–7.7
FIX activity (%)	122/97–148	115/63–168	<1	350/135–565
VWF:Ag (%)	122/50–194	143/59–228	70/42–98	101/32–169
VWF:RCo (%)	114/41–188	131/46–215	37/13–60	74/25–124
PC activity (%)	111/66–155	152/100–205	<1	21.6/0–70
PS activity (%)	96/73–119	30/17–42	<1	15.3/0–39.5
Free PS Ag (%)	108/72–144	131/91–171	126/94–159	131/97–164
AT activity (%)	102/86–118	121/105–138	126/108–143	47/30–65

EDTA, ethylenediaminetetraacetic acid; APTT, activated partial thromboplastin time; s, second(s); NCD, no clot detected; PT, prothrombin time; dRVVT, dilute Russell viper venom time: F, factor; VWF:Ag, von Willebrand factor antigen; WF:RCo, von Willebrand factor: Ristocetin cofactor; PC, protein C; PS, protein S; Free PS Ag, free protein S antigen; AT, antithrombin.

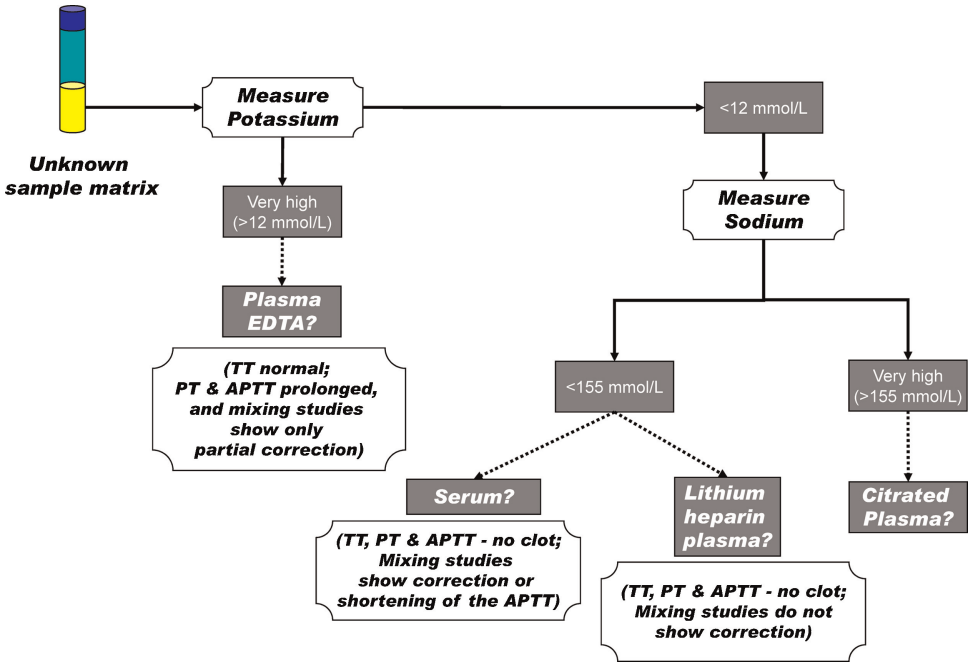

Figure 1 A recommended algorithm to assess for potentially unsuitable sample matrices. A sample that is received in a secondary tube and suspected to not be citrate plasma; can be tested for potassium level. A high level suggests the sample is an ethylenediaminetetraacetic acid plasma sample. Testing by thrombin time (TT) should be normal, but prothrombin time (PT) and activated partial thromboplastin time (APTT) would be prolonged. Mixing with normal plasma would only show partial correction. If potassium level is not high measure sodium; low sodium would suggest either serum or lithium heparin. These will both show prolonged or no clot with TT, PT, and APTT, but can be differentiated with mixing studies. High sodium would suggest a sodium citrate sample, the correct sample matrix. However, whether this sample has been appropriately processed, and whether clot-based tests will be normal or not, may require alternate and additional investigation. Figure updated and modified from reference.[47] EDTA, ethylenediaminetetraacetic acid.

appropriate freezer.[10] If a −70°C or colder freezer is not available, plasma can be stored in a −20°C freezer that does not undergo automatic freeze/thaw cycles. Frost-free freezers that have automatic defrost cycles are generally unsuitable, as they cycle freeze–thaw events to maintain the frost-free environment, and this can result in cold activation of FVII and degradation of other factors. However, the use of frost-free freezers for patient samples is acceptable when freezers are monitored by a continuous-monitoring temperature recording device, or a minimum–maximum thermometer, which enables the laboratory to show that the acceptable temperature range is never exceeded. Storage at −20°C is generally sufficient for tests that are performed within 2 weeks or less.[10] Storage for extended (greater than 2 weeks) periods of time should occur in −70°C freezer or colder.[48]

If plasma aliquots are to be shipped to another facility and the time between collection and projected analysis exceeds acceptable standards, samples should be frozen and shipped on dry ice or another means to maintain samples in a frozen state. Sufficient dry ice must be included to maintain the sample frozen for the required transportation time. Samples stored in dry ice (solid carbon dioxide, or CO_2) containers should be thawed uncapped at 37°C for at least 15 minutes, to allow equilibration with ambient air, and evaporation of plasma CO_2, before testing.[49] If CO_2 is retained in the plasma, it can cause spuriously prolonged clotting times due to increased sample pH.[12]

Sample Processing

Before centrifugation, samples for plasma-based coagulation assays must be inspected visually to ensure that the specimen has been collected into a properly labeled sodium citrate tube within its expiry date and that it is adequately filled (minimum 90% fill unless shorter collection volumes have been validated for the test being performed).[9,50,51] Specimens should always be rejected when these conditions are not met.[10] It has long been standard practice to check collection tubes for clots before centrifugation by uncapping the sample and inserting two wooden applicator sticks. This practice is no longer recommended due to the infectious potential and as it has been difficult to maintain this practice in the era of automation. While not as effective in the detection of clots as inserting wooden applicator sticks, gentle inversion of the collection tube and visual inspection may detect the presence of clots. Inspecting the sample in a back-lit setting (e.g., in front of a view box) may aid in clot detection. Small, even microclots, however, may be missed and might still interfere with results. The presence of a clot, even a microclot may lead to consumption of or possibly activation of factors and platelets. The presence of a clot, regardless of size therefore, is a cause of specimen rejection. While it may not be possible to check every sample for the presence of a clot before analysis, this possibility should certainly be investigated if the result of testing suggests such a possibility, that is, a

greatly extended APTT and/or PT. This of course can only be accomplished if the sample has remained in the primary collection tube. A recommendation to perform routine inspection of samples destined for platelet function testing, including that with the PFA-100®, can also be supported.

Once a sample has been processed, fibrin clots may be visible in the plasma. These may be seen in fresh samples, but also may appear in aliquots after a freeze–thaw cycle. Fibrin clots have the appearance of an opaque or gelatinous globule or strands of variable size (see ►**Fig. 2**). If suspected, their presence may be confirmed; for example, if present, they can often be fished out of the sample with wooden applicator sticks (see ►**Fig. 2**). These fibrin clots may affect results, and are a cause of specimen rejection of a sodium citrate plasma sample. Fibrin clots may result from activation of the sample before analysis. A shortened APTT result, below the reference range, may serve as a flag for close inspection of the sample tube for the presence of a fibrin clot.

Most coagulation-based tests, including PT, APTT, and clotting factor assays, are performed on plasma derived from once-centrifuged samples. In general, samples should be centrifuged to achieve PPP, such that the postcentrifugation plasma contains $<10 \times 10^9$ (10,000/μL).[10] The residual platelet count of processed samples can be easily verified using an automated cell counter. Adequacy of centrifugation to achieve PPP should be documented and confirmed at least annually as part of a quality assurance process. The generation of PPP can generally be achieved by centrifuging specimens at 1500 gravity (g) forces for no less than 15 minutes.[10] To prevent the remixing of plasma and reintroduction of the cellular component, a swing-out bucket (angle) rotor should be used and the brake should not be applied at the end of centrifugation. It has been documented, however, that routine coagulation assays such as APTT, PT/INR, and thrombin time, are not affected by platelet counts up to 200×10^9 (200,000/μL) when testing is performed on fresh samples.[52,53]

Centrifugation times of 15 or more minutes can create a bottleneck in the laboratory and delay result turnaround time. Shorter centrifuge times at 1500 g are acceptable for routine coagulation tests if testing is performed on fresh samples immediately postcentrifugation, but only when there are no subsequent test requirements, thereby ensuring that plasma will not be frozen or processed for additional assays.[54] Another means to reduce the time needed for centrifugation, but still achieve a PPP, is to increase the relative centrifugal force (RCF) used. Using centrifugal forces greater than 1500 g are generally discouraged as this may induce platelet activation and lysis of red blood cells.[54] To the contrary, several studies have reported no adverse effect on routine coagulation testing, such as APTT, PT, and fibrinogen, if centrifuged at high speed (i.e., 11,000 g) with short (i.e., 2 minutes) durations.[55,56] It has been cautioned, however, that samples spun in this manner should be tested within ~10 minutes if sampled from the primary tube or promptly aliquoted to a secondary tube, to avert the drift of platelets, which cling to the side of the tube at high RCF, back into the plasma.[57] It can also be noted that the latest guidelines for LA testing recommend centrifugation speeds of 2000 and ~2500 g for sample processing by double centrifugation (see also below).[58]

Centrifugation should ideally occur at ambient (room) temperatures (15 to 22°C), but this is sometimes difficult to control in laboratories that process large volumes of specimens. Nonrefrigerated centrifuges are adequate providing they do not overheat. Alternatively, refrigerated centrifuges may be used but should be set to maintain ambient temperatures, rather than low temperatures, which can lead to platelet activation and activation of select clotting factors.[53] Nevertheless, refrigerated centrifugation does not appear to affect routine coagulation tests when testing is performed soon after centrifugation.

Some samples, such as those for LA testing or UFH monitoring, should be double centrifuged ("double spun"), to

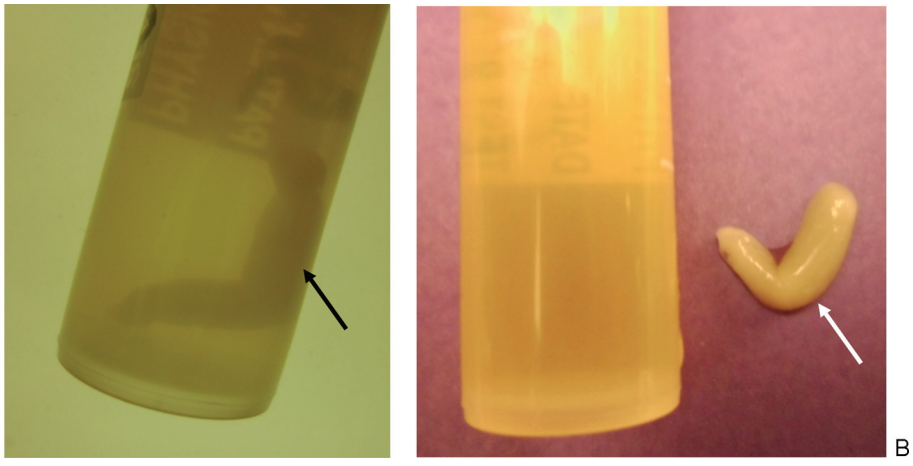

Figure 2 Fibrin clots. (A) Visual inspection of a coagulation sample shows a fibrin clot in the tube (arrow). (B) This clot (arrow) can be fished out of the tube using a wooden stick. However, this is still a cause for sample rejection; as such a clot indicates (partial) clotting has already occurred in this sample, thereby compromising performance of any subsequent clot-based assay. Subsequent nonclot–based assays may also be affected, depending on the test being performed.

ensure platelet-depleted preparations.[58] A single freeze–thaw cycle will result in lysis of any platelets present in the postcentrifuged specimen. This may alter results of certain assays, especially those where significant intraplatelet stores of the analyte exist, such as plasminogen activator inhibitor-1 antigen. In the case of LA, the freeze-thawed platelets release phospholipids which may mask any LA present in samples and lead to false-negative test results. The process of double centrifugation is performed as follows: the original specimen tube is centrifuged using standard techniques, using forces around 1500 to 2000 g.[10,58] Plasma is carefully removed using a plastic disposable pipette making certain not to disturb the buffy coat layer and transferred into a nonactivating aliquot tube. This second tube is capped and centrifuged again using standard techniques, and again using similar gravity forces. Plasma from this second tube is removed using care to not disturb any pellet of cellular material that may be present in the bottom of the tube and transferred to a nonactivating aliquot tube.[6] As all plasma-based hemostasis tests can safely be performed on "double-spun" material, it is prudent to institute this process as a general laboratory policy for any plasma that will be frozen before testing. Use of micropore filters, such as 0.2-µm filters, to achieve PPP is not recommended. The passage of plasma through a micropore filter may result in the selective removal of several factors, including VWF, FVIII, FIX, and FV.[59] Lastly, some tests require additional special differential processing (e.g., platelet function testing, as previously noted).

Following centrifugation, samples should be inspected for the presence of potentially interfering substances, such as hemolysis, icterus, or lipemia as well as the presence of fibrin clots.

Controlled Thawing of Previously Frozen Plasma Samples

Previously frozen samples should be rapidly thawed in a 37°C water bath for 5 to 10 minutes or until completely thawed.[10] Close monitoring during this time is necessary to avoid inadequate or excessive incubation at 37°C. Sample integrity may be compromised if samples are either not completely thawed or if maintained too long at 37°C. Furthermore, water baths must be properly maintained to make certain they are not inadvertently upheld at a higher temperature because this may lead to deterioration of coagulation factor activities and spurious coagulation test results. Once samples are thawed, it is imperative that they are thoroughly and adequately mixed before testing (e.g., by means of a vortex, use of a rocker, or adequate end-over-end conversions).

Sample Stability of Previously Frozen Samples

It is generally accepted that samples for coagulation testing can safely undergo one freeze–thaw cycle, but multiple freeze–thaw cycles may affect the functional portion of hemostasis proteins causing variations in activity results. Exceptions to this rule may be FVIII, which has been reported to lose activity after one such cycle and FXI which may increase activity.[60,61] It has been demonstrated that both FV and FVIII as well as PS activity, lose activity following multiple freeze–thaw cycles.[62] In an unpublished study by Gosselin, it was demonstrated that most other coagulation factors, specifically FII, FVII, FX, FIX, FXI activities, and antithrombin, protein C (PC), VWF, and plasminogen activities are stable through multiple freeze–thaw cycles.

Considerations When Using Laboratory Automation Modules or Systems

Several clinical pathology laboratories have implemented different elements or levels of laboratory automation in an effort to improve quality, reduce turnaround times, save money, and enhance staff productivity. To this end, several in vitro diagnostics manufacturers are developing or have introduced laboratory automation solutions and instrument features, with a variety of different capacities.[63] A fully automated system is one that can perform most laboratory tasks without human interaction, while a modular approach automates only targeted functions. Modular devices are especially well suited for automating front-end processes such as labeling, centrifugation, sorting, and aliquoting specimens. Due to the many preanalytical variables associated with sample collection, processing, and transportation, special care must be taken when adapting samples for hemostasis assays to automated lines.[9] Some of the following potential or realized capabilities of automated systems and instrumentation should be considered. Certain types of hemostasis samples, however, such as those for platelet function analysis, are not appropriate for automated modules or systems.

Centrifugation may be a component of some automated lines, which may allow centrifugation of 100 samples at a time. Issues surrounding on-line sample centrifugation would be the same as those discussed above, specifically using speeds to achieve PPP but prevent platelet activation and cell lysis. An additional issue to be considered in the automatic processing of primary blood tubes is the potential dishomogeneous separation of citrated plasma. Lippi et al showed that PT is significantly shortened and fibrinogen values significantly higher in the lower than in the upper part of the tube, whereas results of APTT are mostly unchanged throughout the tube.[64] As such, it seems reasonable to suggest that citrate plasma should be removed from the collection tube and appropriately mixed before analysis or aliquoting.

Some systems include a "point-in-space" arm that samples directly from the automation line rather than requiring that the sample be transported to the instrument. Point-in-space sampling is more time-efficient and can therefore hasten the time between sample processing and analysis, which may be better suited to samples with limited stability, such as samples for hemostasis testing. Cameras may be used on some lines to determine fill volume and also to detect optical interferences such as hemolysis, icterus, and lipemia. Some systems provide a liquid level sense to confirm fill volume when a primary tube is sampled. Sysmex® (Siemens Healthcare Diagnostics Inc., Marburg, Germany) has introduced a feature on their new coagulometer that allows detection of

hemolysis, icterus, and lipemia, by measuring the sample simultaneously at multiple wavelengths; 340, 405, 575, and 660 nm.[65,66] Based on the specific interference detected, analysis is performed using an appropriate wavelength to avoid the discerned interference. Instrumentation Laboratories ACL TOP® coagulation analyzer overcomes the optical interference of hemolysis, icterus, and lipemia by measuring clot detection at a wavelength above 600 nm. Sample analysis using wavelengths that do not interfere with hemoglobin, however, do not account for the shortening in clotting times that may occur in hemolyzed samples due to release of intracellular and thromboplastic substances that occur with lysis.[67]

Conclusion

Appropriately developed and implemented quality standards regarding sample processing, transportation, and storage are important components of a laboratory quality management system. Failure to recognize improperly handled samples for hemostasis testing may lead to the reporting of unreliable test results and this may compromise patient care. In the ideal circumstance, sodium citrate samples would be transported at room temperature, processed to obtain PPP, and tested within 1 to 4 hours of collection.

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