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

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Introduction

Many pre-analytical variables (PAVs) may affect the results of routine coagulation assays. To improve the precision and accuracy of laboratory testing, it is critical to identify these variables and realize their potential impact.[1,2] Additionally, advances in laboratory instrumentation have improved the reproducibility and sensitivity of the analytical phase, therefore creating greater dependence on specimen integrity.[1-5] The activated partial thromboplastin time (APTT) and prothrombin time (PT) determinations are among the most frequently ordered screening tests in the clinical laboratory. These assays are used in the evaluation of a wide variety of clinical conditions, for either diagnostic or monitoring purposes. These screening tests also form the basis of many special coagulation tests, such as factor assays and proteins C and S activity assays.

Since the introduction of coagulation assays, efforts have been made not only to automate these assays, but to better standardize testing, thus providing more-accurate results to aid in clinical assessment.[6-14] PAVs pertaining to routine coagulation testing can be classified into three major categories:

1. Specimen collection (including patient selection)

2. Specimen transportation and stability

3. Specimen processing and storage

Within each of these categories, there are a number of individual variables, each of which may have a major impact on testing. There are also a number of variables related to the analysis of the specimen, many of which are dependent on the reagent(s) and instrumentation, but variables associated with analysis are beyond the scope of this document.

Many standards for testing in the general clinical laboratory and specifically in the coagulation laboratory have been developed in an effort to improve precision and accuracy.[6-10] The Clinical and Laboratory Standards Institute (CLSI) is the primary organization for clinical laboratory standards and guidance documents in the United States, although their guidelines are also referenced and used internationally.[6] The CLSI was established in 1968 as a group of individuals representing industry, government, and professionals dedicated to the development of standards and guidance documents for clinical laboratory testing. Despite CLSI's existence, there is still a lack of practice standardization among clinical laboratories regarding specimen collection, storage, and processing for coagulation testing. Some of the procedures in practice today are apparently founded on tradition, while others are based on CLSI guidelines, with and without significant published or supporting evidence. As a result, a number of problems, inconsistencies, and erroneous results can still arise based on pre-analytical processing of the specimen, and these discrepancies may be associated with disastrous outcomes. This manuscript will review the pre-analytical variables and some analytical variables of laboratory-based coagulation testing. Point-of-care (POC) devices, which may use native or anticoagulated whole blood, are also affected by PAVs but will have limited focus in this document. Recommendations for the proper methods based on published and new data will be presented, together with recommendations for converting to these methods.

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Pre-analytical Variables In Routine Coagulation Testing: Setting the Stage for Accurate Results · **White Paper**

Table 1. Provisional guidance for testing of pharmacological effects on coagulation. Note: Laboratory must consult with primary caregiver or institutional guidance when assessing these drugs. Refer to drug-prescribing information episodically, as recommendations (if any) for assessing the pharmacodynamics (effect of coagulation) and pharmacokinetics (drug presence or amount) of the drug may change.

Drug Class	Drug	Monitoring/Measuring Test	Optimal or Desired Time of Blood Collection	Notes
Heparin	Unfractionated	APTT Anti-Xa	6 hours after dose initiation or adjustment	• Anti-Xa measurements also acceptable. • Circuit anticoagulation may require more frequent monitoring. • Circuit anticoagulation may require higher dosing that cannot be measured by APTT, so ACT may be the optimal test.
	Low-molecular-weight	Anti-Xa	4 hours after third dose	• Hybrid or LMWH calibrated anti-Xa are acceptable.
	Pentasaccharide	Anti-Xa	3 hours after dose	• Pentasaccharide-calibrated anti-Xa reported in mg/L or mg/dL.
Vitamin K Antagonists	Antagonist—oral	PT/INR (baseline PT/INR should be collected prior to initiation of therapy)	First INR is with 12–24 hours of first dose	• If outside target, consider VKORC mutation. • If within target, dose is probably acceptable. • If lower than target, probably need to adjust dose higher.
	(reversal therapy)	PT/INR	IV: 12 hours Oral: 12–24 hours	• Patient may have additional reversal agents. • More-frequent monitoring may be necessary if patient is bleeding.
Antithrombotics	Antiplatelet—oral	Platelet-function tests	1 week after initiation of therapy	• No consensus on method for measuring or whether necessary. • Methods include PFA Systems, ULTEGRA, and traditional platelet aggregation.
	Antiplatelet—intravenous	Platelet-function tests (if concomitant therapy, then ACT)	Drug-dependent Within 0.5–2 hours	• POC or platelet-function methods, including ULTEGRA, TEG, or ROTEM-based methods. Traditional platelet aggregation studies may also be suitable.
	Fibrinolytics	TT, FBG, XDP, FDP	10–15 minutes after completion of infusion	• No consensus or recommendations for monitoring available. Platelet-function tests (if concomitant therapy, then ACT) • 30 minutes for checking catheter function.
	Antifibrinolytics	PT/INR, APTT, ACT	Max. concentration at 2 hours after initiation of infusion	• No consensus or recommendations for monitoring available.
	DTI	APTT, anti-II, ECA, ECT (may require ACT if high dose for PCI), dilute thrombin time	HIT treatment: 4–6 hours PCI infusion: 5 and 45 minutes	• Drug labeling indicates APTT or ACT, but other more-specific testing may be desirable, especially when therapeutic target is not achieved.
DOAC	IIa	ECT, ECA, IIa, dTT	Trough samples (5–30 minutes prior to next dose)	• Collect just before next dose. • If peak samples are desired, then usually 2–3 hours after dose.
	Xa	Drug-calibrated anti-Xa	Trough samples (5–30 minutes prior to next dose)	• Collect just before next dose. • If peak samples are desired, then usually 2–3 hours after dose. • Specific drug-calibrated anti-Xa.
	Reversal—dabigatran	ECT, ECA, anti-IIa	10–15 minutes after completion of infusion	• No consensus or recommendations for monitoring.
	Reversal—rivaroxaban/apixaban	Anti-Xa	4 hours after infusion to reassess anti-Xa	• No consensus or recommendations for monitoring. • The drug is continuously infused. Once infusion has stopped, DOAC levels may rise. • Specific DOAC-calibrated anti-Xa.
Factor Replacement	Replacement therapy (hemophilia A or B)	Factor level	Physician-guided. PK studies may be required. Consider baseline, 30 min, 60 min, 2, 4, 8, 12, and 24 hours for PK time periods.	• Note that newer replacement therapies for hemophilia using modified (PEGylated, albumin-fused, etc.) factor replacement may require special methods. • Single-stage clotting assay or chromogenic assay may be preferred.
	DDAVP/vasopressin	VWF, FVIII	Baseline, 30 minutes, 2, 4, and 6 hours post-drug delivery	• Drug delivery is either by nasal spray or infusion.
	PCC or APCC	PT/INR, APTT	Baseline (pretreatment) and 15–30 minutes after completion of administration	• No consensus or recommendations for monitoring. • For APCC, no relationship to decreasing clotting times and bleeding outcomes.
	rVIIa	PT/INR	10–15 minutes after infusion	• No consensus or recommendations for monitoring.
	FFP, cryoprecipitate, or other plasma-based products	PT/INR, APTT	Baseline (preinfusion) and 30 minutes	• FBG, FVIII, FXIII, or other factors may be assessed if replacing for that purpose.

ACT: activated clotting time
APCC: activated prothrombin complex concentrate
APTT: activated partial thromboplastin time
DDAVP: 1-desamino-8-D-arginine vasopressin
dTT: dilute thrombin time
DOAC: direct oral anticoagulants
DTI: direct thrombin inhibitors
ECT: ecarin clotting time
ECA: ecarin chromogenic assay
FBG: fibrinogen
FDP: fibrin(ogen) degradation products
FFP: fresh frozen plasma
IIa: activated factor II (thrombin)
HIT: heparin-induced thrombocytopenia
INR: International Normalized Ratio
IV: intravenous
PCC: prothrombin complex concentrate
PCI: percutaneous coronary intervention;
PEG: polyethylene glycol
PK: pharmacokinetic
POC: point of care
PT: prothrombin time
ROTEM: rotational thromboelastometry
rVIIa: activated factor VII
TEG: thrombelastography; TT: thrombin time;
VKORC: vitamin K epoxide reductase C1
VWF: von Willebrand factor
Xa: activated factor X
XDP: D-dimer

Patient selection pre-analytical variables

Age, gender, race, blood group, and health status are all contributing PAVs for hemostasis testing, with the largest impact being on test result interpretation, to be discussed later. Therefore, each laboratory must be able to address these variables, which may require different reference intervals (RI), depending on the test and test method. This is mandatory to ensure proper result interpretation and prevention of misdiagnosis.

Numerous health issues affect the accurate measurement of hemostasis. Inflammatory conditions can lead to increased levels of fibrinogen (FBG), factor VIII (FVIII), von Willebrand factor (VWF), and plasminogen activator inhibitor-1 (PAI-1), with decreased levels of protein S (PS) activity.[15] Mental or physiological stress, including exercise, can also cause changes in hemostasis, including factitious elevation of VWF and FVIII and decreased platelet function.[15,16] As such, patients should be allowed to relax for 30 minutes prior to phlebotomy.
Other conditions (e.g., biological variation, circadian variation, etc.) that may alter coagulation results will be discussed later, as these conditions are more likely to be included in the interpretation of test results. Drugs, foods, and herbal supplements can alter platelet function, and thus optimal assessment should be performed using samples collected from fasting and drug-naïve patients.

Finally, some coagulation tests are performed for monitoring purposes. These may include anticoagulants or factor replacement therapy. For samples being collected for monitoring purposes, adherence to the physician-directed timeframes is critical for proper assessment to avoid drug dose adjustment or drug overexposure.
Table 1 provides provisional guidance for collection times for coagulation-related drug therapy, but laboratories must confirm appropriate collection times with the ordering physician.

Patient selection recommendations

- **Each laboratory must have a proper reference interval for the populations being assessed.**
- **Patients should be relaxed prior to phlebotomy to avoid physiological and psychological stress that may factitiously alter coagulation tests, especially for VWF and platelet-function studies.[15,16]**
- **Assessment of platelet-function studies should be performed using samples collected from fasting and drug-naïve patients.**
- **For patients being monitored (e.g., those on anticoagulation therapy), adherence to the collection time is mandatory (Table 1).**

Specimen Collection PAVs

After patient selection has been defined and the appropriate tests and collection times are noted, the next step is to collect the blood sample. Critical to this process are the appropriate identifiers that ensure proper patient identification and phlebotomy orders. After patient ID and orders are verified, actual blood collection follows. Personnel must be properly trained in phlebotomy practices using universal precaution techniques to ensure proper blood collection. In a published study, the authors demonstrated that trained personnel provided better-quality specimens for coagulation than untrained personnel. Samples from untrained personnel contained increased levels of coagulation activation factors (serum crosslinked fibrin [XDP], prothrombin fragment 1.2 [F1.2], and thrombin-antithrombin complexes [TAT]), indicating an activated coagulation process reflective of poorly collected samples.[17]

Phlebotomy procedural issues: whole blood versus tube collection

Whole blood is appropriate for point-of-care (POC) devices such as INR monitors or bedside APTT devices. Depending on the desired test, these POC samples may use native whole blood or require anticoagulation using evacuation tubes as noted below. Laboratories should consult the manufacturer's package insert for POC reagents and devices to confirm sample types and collection methods.

Phlebotomy procedural issues: systems and blood collection

There are several manufacturers of blood-collection tubes. Despite the similarities in citrate concentration used by these manufacturers, there are significant differences among their tubes that can potentially affect coagulation assay results. For example, differences have been found between PT and APTT results derived from tubes employing the same citrate concentration but produced by different manufacturers.[18] Because of this variability in blood-collection tubes, laboratories should validate these systems prior to implementation.[19] Laboratories that provide services outside their own region (e.g., reference laboratories), should specify to clients the preferred blood-collection tube and citrate concentration to avoid this pre-analytical bias.

Blood may be collected in individual vacuum tubes or by the syringe technique. In the syringe technique, a specimen is first drawn into an empty or coagulant-containing syringe and subsequently transferred into either individual vacuum tubes or directly into a laboratory analyzer. The syringe method has a number of limitations and should be limited to certain circumstances that require its use, such as when the phlebotomist prefers to control the vacuum force in a patient with difficult veins.[20]

If the syringe technique is necessary, use a syringe less than 25 cc (preferably 10 cc) with the correct concentration and volume of anticoagulant. All syringe drawing procedures should use a "butterfly" needle apparatus. Withdraw the blood slowly to avoid hemolysis, coagulation, and platelet activation. When multiple tubes with different anticoagulants are required, fill the syringe and quickly transfer the sample to the appropriate tube. The ICSH recommends manually mixing the citrate tube (gently inverting the tube end-over-end 5–6 times),[21] although there is some evidence to suggest that this may not be necessary with some collection systems,[22] or in patients treated with oral vitamin K antagonists (VKA).[23] Note, however, that these two published studies used a limited number of samples and collection tubes. Therefore, unless locally determined otherwise through rigorous studies, it may be prudent to use the best practice of gently mixing whole coagulation samples collected in citrate, heparin, EDTA, or other anticoagulants, as well as pediatric or volume-reduced collection. In any case, do not rigorously shake or agitate the tube.

Because the blood collected must be subsequently transferred to a specimen container, the syringe method inherently increases the phlebotomist's risk of needlestick exposure, as the receptacle container must be held in one hand while the syringe needle is guided into the tube with the other hand.[24] The syringe method also increases the risk of hemolysis if blood is forced too quickly through the hypodermic needle or against the side of the collecting tube.[24] If no anticoagulant is added to the syringe, the specimen is likely to clot if not transferred immediately (usually within 60 seconds). This likelihood increases with larger-sized syringes.[24]

Despite these shortcomings, scarce data exists to substantiate this claim. In fact, the earliest studies on collection systems for coagulation testing supported the use of the syringe technique over the vacuum tube.[25] In the controlled environment of a study, incorrect syringe technique or leaving syringe samples to clot would arguably be less likely and not reflective of real-life practices in a busy hospital.

Phlebotomy procedural issues: needle size

Needles are integral to the blood-collection process and are available in a variety of sizes (needle gauge), with increasing numbers signifying decreasing needle diameter. CLSI guidelines recommend using needle gauges ranging from 22 to 19 gauge for coagulation testing.[24] This recommendation is not referenced, but is supported by tradition in many standard textbooks concerning blood collection. For pediatric patients, higher gauges (indicating smaller needle diameter) in the range of 23–25 gauge may be necessary.[24,26] If the syringe technique is employed for collections of more than 30 mL, an 18-gauge needle is recommended to ensure adequate blood flow and reduce the chance of hemolysis.

Phlebotomy procedural issues: line collections

Arterial-line collections are acceptable if a two-syringe technique is used, with first 10 mL of line blood being cleared and the second syringe used for blood collection.[24,27] For smaller patients, if appropriate and sterilely collected, the first 10 mL that would otherwise be discarded can be replaced into the patient if the hospital has a procedure for replacement of the blood.[27] For intravenous (IV)-line collection, the IV line is ideally turned off for 5 minutes and then the two-syringe technique used as described above.[24,26] Consider point-of-care methods in patients with difficult venous or arterial access.

Phlebotomy procedural issues: tourniquet technique

Prolonged tourniquet times induce increased vessel pressure, hypoxia, and lower pH below the tourniquet, thereby potentially masking mild deficiencies in VWF, FVIII, tissue plasminogen activator (tPA), and other endothelial-associated coagulation proteins.[24,28] Many textbooks and articles on phlebotomy technique emphasize the need for both a clean, atraumatic puncture as well as avoidance of prolonged tourniquet application, but aside from the obvious logic and desire for good technique, there is actually little data to support that coagulation studies are adversely affected.

Venipuncture technique is an important pre-analytical variable in coagulation testing. Areas of concern include prolonged tourniquet application and inducing the release of procoagulant material by multiple failed attempts to draw blood (needlesticks). Application of the tourniquet for longer than 1 minute can result in hemoconcentration and endothelial cell release of proteins. The resulting venous stasis promotes anaerobic glycolysis, with an accumulation of plasma lactate and a reduction in blood pH. The lower blood pH can alter protein binding and, in the case of calcium, result in a spuriously increased level. Prolonged tourniquet application also elevates coagulation factors such as FVIII, VWF, and tPA, thereby affecting the accuracy of diagnosis. Prolonged tourniquet application may also create an acidic microenvironment, potentially leading to factitious prolongation of clotting assays.

Phlebotomy procedural issues: tube size and anticoagulant

Vacuum tubes are available in a variety of sizes to accommodate patient size and minimize blood loss and iatrogenic induction of anemia in hospitalized patients. The best-practice guidance for tube size is to use the smallest tube that can accommodate the patient and also produce enough plasma for all the tests ordered, while conserving about 50% of the plasma for add-on tests. Note that in some tubes, blood will only half-fill the tube when at the appropriate level. This sometimes poses an issue when untrained phlebotomists draw blood, as they have usually been instructed to completely fill the tube. Tubes that are over- or underfilled are unacceptable for testing and should be rejected (see below).[29] Personnel responsible for accepting tubes must be aware of the fill requirements of each tube used by the laboratory. In some hospitals, small micro-tubes have been developed to collect very small volumes from newborn infants. These tubes must be validated for use by the laboratory.[30]

Most coagulation tests are established and validated using sodium citrate as the anticoagulant. 3.2% sodium citrate is the citrate concentration of choice[21,24,31] because it is considered more tolerant for clotting-based assays. In the past, 3.8% sodium citrate was used, but it is no longer recommended because the excess sodium citrate can potentially bind calcium ions added to the clot-based assay, thereby interfering with coagulation test results. Although 3.2% sodium citrate is the recommended concentration, the sodium citrate concentration in several manufacturers' tubes is only approximately 3.2%, with variation based on the manufacturer's protocol. Note that 3.2% and 3.8% citrate collection tubes are not interchangeable in a given laboratory, as they may yield different test RI and patient results.

For some coagulation and platelet testing, other anticoagulants or no anticoagulant (generating serum) are used.[24] Tubes containing CAD (citrate, adenosine, and dipyridamol) and CTAD (citrate, theophylline, adenosine, and dipyridamol) are used to inhibit platelet activation. Tubes containing PPAK or aprotinin are used to inhibit activation of the coagulation and fibrinolytic systems respectively.[32] Lithium heparin, EDTA, or no anticoagulant (serum) are used for immunoassay tests (e.g., HIT testing and antibody testing in antiphospholipid syndrome). Specialized tubes have been created for special tests such as the fibrin degradation products (FDP) test[33] (high concentration of thrombin) and tPA assay[34] (acidified citrate), in which these tubes must be used for accurate results. If a nonstandard tube or a tube not recommended in the manufacturer's test package insert is used, the tube must be validated by the laboratory.

Phlebotomy procedural issues: order of draw

Current CLSI guidelines recommend an order for drawing of multiple samples that has become standard practice (Table 2).[21,24] Samples drawn out of sequence for coagulation studies can potentially create interference in the coagulation test and generate a false result. As an example, in an unpublished study, about 1 in 30–40 sodium citrate tubes were contaminated when a green-top tube with liquid heparin was drawn prior to drawing the sodium citrate tube; about 1 in 75–80 tubes were contaminated if a green-top tube with dried heparin was drawn prior to the sodium citrate blue-top tube. The heparin in the tube contaminates the blood, which in turn contaminates the inside and outside of the needle that is then inserted into the sodium citrate tube, thereby contaminating the patient with heparin.

If coagulation studies are the only tubes to be drawn, no discard tube is necessary.[35] This recommendation is based on the results of several studies documenting that coagulation testing may be performed on the first tube without need for a discard tube.[35] Adopting this best practice eliminates biologic waste and may yield cost savings for most laboratories.[35]

Phlebotomy procedural issues: fill volume and hematocrit adjustment

Sodium citrate (3.2%) is the most widely used anticoagulant for coagulation (clotting-based) studies. Excess citrate in the plasma sample could potentially inhibit clot formation in the clotting test by possibly binding a significant amount of the added calcium in the clotting test to artefactually affect the clotting time value.[21,24,29,31] The current guidelines dictate that the proportion of blood to anticoagulant volume be a ratio of 9:1.[21,24] However, the literature demonstrates that some underfilling can be tolerated, with the tube being filled down to 60% of the required volume for PT and 70% for PTT without a clinically significant effect.[21,24,29] Therefore, short-sampling in tubes for patients with low hematocrits may not be clinically significant unless measuring for anticoagulant effect (heparin, direct oral anticoagulants [DOAC], and direct thrombin inhibitors [DTI]), as the anticoagulant result may be artefactually decreased due to the dilution effect of low hematocrit and excess sodium citrate liquid volume.[36] Follow the manufacturer's recommendations for acceptable tolerance of both low hematocrit and underfilling of tubes.

Samples with high hematocrits (>55%, such as those found in neonates, severe dehydration, burn patients, polycythemia vera, and high-altitude visitors or residents) may result in factitiously high clotting times due to excess citrate in the sample, which will bind the calcium added to the clotting assay's calcium-testing reagent.[21,24,37] To circumvent this issue of inaccurate clotting results due to high hematocrit, the laboratory can create vacuum tubes with a reduced volume of anticoagulant. By using a very small gauge needle and removing 20% volume of the anticoagulant without removing the vacuum, labs can create sodium citrate-adjusted tubes for hematocrits greater than 55%.[37] These tubes can be stored in the laboratory and sent to the phlebotomist when necessary. Before using these modified tubes, the laboratory must validate that they work. Alternatively, the cap may be removed, the appropriate citrate amount removed, and the tube recapped. However, without vacuum, these tubes must be manually filled using the syringe technique described previously.

Specimen collection recommendations

- **Significant differences have been observed between reported PT and APTT results using collection tubes with the same citrate concentration from different manufacturers.[18] Labs must validate these systems prior to implementation.[19]**
- **If the syringe technique is to be employed, use a syringe of less than 25 cc (preferably 10 cc) with a "butterfly" needle apparatus.**
- **Needles used for coagulation testing should be 22 to 19 gauge, with higher gauges (23–25) recommended for pediatric or difficult venous-access patients.[24,26]**
- **For syringe collections of more than 30 mL of blood, 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 first 10 mL of line blood cleared and the second syringe used for blood collection.[24,27]**
- **For IV line collections, turn off the IV line for 5 minutes. The use the two-syringe technique, with the first 10 mL of line blood cleared and the second syringe used for blood collection.[24,26]**
- **Follow manufacturer recommendations for under- and overfilling of 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 of falsely elevated PT, INR, and APTT results.**
- **Gentle inversion (mixing) of sodium citrate tube approximately 5–6 times is recommended.[21] Avoid rigorous shaking or agitation.**
- **3.2% sodium citrate is the citrate concentration of choice.[21,24,31]**
- **For patients who require multiple-tube collections, follow the mandatory collection sequence (Table 2).[21,24]**
- **If only citrate tubes are being collected, no discard tube is necessary (unless using the butterfly syringe method directly into the collection tube).[35]**
- **Patients with elevated hematocrits (>55%) require tubes with a reduced volume of citrate.[21,24,37]**

Specimen Transportation and Stability PAVs

Transportation and processing of blood specimens for coagulation testing encompass a critical set of PAVs. These variables can have dramatic effects on results, which in turn can have serious consequences for patient care. The current CLSI guidelines differ on how long the sample remains stable, at what temperature, and in what condition based on the assay.[21,24] These criteria also change depending on whether the patient is anticoagulated and with what type of anticoagulant. According to the guidelines, specimens for PT assays are remarkably stable for up to 24 hours independent of processing methods (centrifuged or not), storage temperature (refrigeration or room temperature), and patient groups.[21,24] The integrity of PTT samples, unlike that of PT samples, depends on both processing conditions and the presence of anticoagulant, especially unfractionated heparin. Samples for PTT assays and anti-factor Xa (anti-FXa) monitoring

Table 2. Order of draw and corresponding vacuum tube types.

Order of Draw	Vacuum Tube Type	Color of Cap	Test Type
1	Blood culture tubes	Color varies	Blood culture
2	Sodium citrate (3.2%)	Light blue	Coagulation
3	Glass (no activator)	Red	Chemistry, immunoassays
4	SST	Gold or red/black	Chemistry, serology
5	Trace elements (no preservative)	Royal blue	Trace element toxicology
6	Sodium or lithium heparin	Green	Chemistry
7	EDTA	Lavender or pink	Hematology, blood bank
8	Sodium fluoride	Gray	Glucose
9	ACD	Yellow	Blood bank HLA testing
10	QUANTIFERON-TB	Gold	TB testing

of unfractionated heparin or DOACs are sensitive to time and processing method.[21,24,36] Samples suspected to contain unfractionated heparin must be collected and processed within 1 hour, as those containing unfractionated heparin have a clinically significant reduction of the PTT observed in noncentrifuged samples stored at room temperature.[38] This critical reduction of the PTT appears to be due to neutralization of heparin by platelet factor 4 (PF4), a high-affinity heparin-neutralizing protein secreted by stimulated platelets along with other heparin-binding proteins.[38]

The current guidelines specify that specimens for routine PTT assays on nonheparinized patients must be tested within 4 hours of specimen collection, whether centrifuged or kept as whole blood at either 2–4°C or room temperature.[21,24,38] Centrifugation improves sample stability, and once centrifuged, the stability is not substantially influenced by temperature.[38] The current guidelines stipulate that anticoagulated samples must be centrifuged within 1 hour of collection, and all other samples must be tested within 4 hours of specimen collection.[21,24] It is also recommended that specimens submitted for other assays (factor assays, protein C, VWF studies) be processed and stored within the 4-hour time limit.[21,24] DTI samples, including dabigatran, must be tested within 2 hours if using the thrombin time or a direct anti-FIIa chromogenic assay.[39,40] This guidance should be followed unless superseded by the manufacturer's package insert or other manufacturer's guidance documents. Note that there has been considerable investigation of the stability of whole-blood samples, with results that appear to be more robust than CLSI recommendations.[3] Each laboratory must investigate and document its rationale for implementing alternative sample-stability limits that exceed CLSI or manufacturer recommendations.

Home healthcare personnel must ensure that coagulation samples they draw are maintained under recommended conditions: within the room-temperature requirement, not sitting in a hot car during summer or in the cold during winter, and avoiding agitation during transport. For whole-blood samples being transported distances (e.g., via automobile), it has been demonstrated that maintaining tubes in the vertical position reduces sample agitation and preserves PT/INR result accuracy.[41]

Pneumatic tube systems can be used to move anticoagulated whole-blood specimens from hospital patient floors to the laboratory for coagulation tests, but not for platelet-function analysis, including POC platelet-function testing, and platelet aggregation studies.[42-44]

Citrated whole-blood tubes to be used for platelet-function studies (platelet aggregations) should be collected as described above but must sit at room temperature for 30 minutes to allow re-equilibration and return of platelet function but no longer than 4 hours, after which time platelet function deteriorates.[45]

Whole-blood sample transportation and stability recommendations

- **Coagulation samples should not be transported or stored on ice.[24]**
- **Coagulation samples for platelet-function studies must be maintained at room temperature.[45]**
- **Whole-blood samples for prothrombin time assays are stable for 24 hours at room temperature.[24]**
- **Whole-blood samples for APTT assays are stable for 4 hours at room temperature, unless used for unfractionated heparin (UFH) monitoring, in which case the room-temperature stability of whole blood is 1 hour.[24]**
- **For other tests, unless otherwise indicated by the manufacturer, whole-blood stability is 4 hours.[24]**
- **Pneumatic transport systems should not be used for samples that require platelet-function testing. [42-44]**
- **Samples collected outside the confines of the hospital (e.g., home healthcare) should be transported in containers (e.g., insulated STYROFOAM) that ensure ambient room temperature, and agitation of sample should be minimized.**
- **For whole-blood samples being transported distances (e.g., via automobile), the tubes should be racked and positioned upright.[41]**

Specimen Processing PAVs

Centrifugation of specimens

All blood specimens for coagulation tests (except whole-blood POC methods and platelet-function studies) must be centrifuged to yield platelet-poor plasma (PPP). PPP is defined as having <10.000 platelets/µL (10×10^9/L).[21,24,46] PPP is required because platelets, as the last cell component to be removed from the plasma, can interfere with coagulation tests as they rupture. This is especially true if the plasma sample is subsequently frozen. The centrifuge must be able to maintain room temperature (15–25°C) during use. The centrifugation parameters include the speed of the centrifuge, radius of the centrifuge arm (both speed and radius determine the g-force), and the time of centrifugation.[21,24] Therefore, each centrifuge essentially generates a different g-force, varying the time of centrifugation required to obtain the desired PPP. Usually in large standard centrifuges, the g-force is typically 1500 g (about 4500 rpm) with a centrifugation time of 10 minutes. However smaller centrifuges with higher g-forces and shorter centrifugation times (usually about 3 minutes) are also available.[21,24,47] Each laboratory must determine the centrifugal force (recommended at 1500 g) and time required to obtain the desired platelet-poor plasma, defined as <10,000/mL platelets.[21,24,48] Each lab must periodically evaluate its centrifuge parameters (both g-force and time) to ensure that samples are still considered platelet-poor. Accreditation organizations require periodic evaluation, typically ranging from annually to 12 times per year. Some guidelines state that platelet counts of 200,000/µL (200×10^9/L) are acceptable for routine testing. However, these samples cannot be used for additional or special testing and cannot be frozen.[21,24] Samples to be used for special testing usually must be frozen, in which case they should be centrifuged according to the standard laboratory procedure, the plasma removed and added to a multi-identified and labeled secondary tube, and the plasma recentrifuged to remove all residual platelets.[21,24] The platelet-free plasma is then removed from the secondary tube and added to a multi-identified and labeled storage tube and the sample immediately frozen for storage. Double-centrifugation requires periodic checks of the final plasma sample to ensure that the sample is platelet-poor.[21,24]

Light transmission platelet aggregation requires not only platelet-poor but also platelet-rich plasma (PRP, plasma enriched with a high platelet count).[45] For this testing, PPP is made as described above, and PRP is made by a slower centrifugation to remove the red blood cells and the majority of white blood cells while the smaller platelets remain in solution. The required g-force and time depend on the centrifugation instrument; the speed to remove the RBCs and WBCs is approximately 200 g.

Sample preparation and pooling

Most routine testing is performed in the primary tube after centrifugation. After initial testing, some accreditation organizations or hospital/laboratory policies may require keeping samples for a specified time. In such cases, storage of the primary tube at 4°C is recommended for up to 24 hours (note, however, that samples containing unfractionated heparin will not produce accurate results).

If the tube is small and will not fit on the instrument, or the sample is being prepared for specialized or add-on tests, the sample must be removed from the primary tube, spun a second time to make the sample platelet-free, and added to a secondary tube labeled with multiple identifiers and the time and date of collection. If more than one primary tube is obtained and the plasma samples are going to be aliquoted and frozen, each primary tube must be independently double-centrifuged and individually aliquoted into its own secondary tube. Plasma samples from different tubes should not be pooled and then aliquoted. If one of the tubes has an issue (clotted, hemolyzed, etc.), the entire specimen is considered to be unusable and must be discarded.

Sample Processing Recommendations

- **Except for whole-blood testing and platelet-function studies, platelet-poor plasma (PPP) is the sample of choice.[21,24]**
- **PPP is defined as <10,000 platelets/µL.[24]**
- **The internal temperature of centrifuges processing PPP must be room temperature (15–25°C).**
- **Although the recommended centrifuge force to obtain PPP is 1500 g for 10 minutes, each laboratory must verify its centrifugation speed (rpm) or force (g) to ensure production of PPP.[48]**
- **All coagulation samples must be double-centrifuged prior to freezing.**
- **Platelet counts from PPP processing must be verified at least annually.**
- **Multiple tubes collected from a single patient should not be pooled prior to storage or testing.**

Frozen sample storage and thawing PAVs

For all testing not completed within 24 hours for PT and 4 hours for PTT and other assays, remove the plasma from the cells and store it frozen in secondary plastic tubes with appropriate labeling.[21,24] Specimens may be stored at –20°C for up to 2 weeks or –70°C for up to 6 months.[21,24,49,50] Samples should be rapidly thawed at 37°C until all the components return to solution (shortest duration possible).[38,39]

Plasma samples that have been properly prepared and labeled should be stored in special tubes (cryo-vials or other polypropylene containers that will not crack or leak) with a cap that will remain in place even at very low temperatures.[21,24,49,50] Plasma samples should be aliquoted in either 0.5 mL or 1.0 mL aliquots to allow for optimum testing of the specialized tests. Once prepared, sample vials should be frozen in the freezer in which they will be stored. The freezer of choice is a –70 or –80°C non-frost-free ultra-low freezer in which samples can be stored for 6 months.[49,50] If the laboratory does not have an ultra-low freezer, most plasma samples can be stored at –20°C for 2 weeks,[49,50] but refer to manufacturer's package insert or other test instructions to ensure test stability at this temperature. An inventory-storage system is recommended for laboratories that receive numerous samples per week for testing or send-out. Freezing sample vials in dry ice is not recommended, as the plasma sample may undergo a change in pH that may affect the results of coagulation tests.[51]

Sample vials (with caps on) should be thawed in a 37°C water bath and gently mixed (by inverting, not extensive vortexing) for the minimal time needed to completely dissolve the plasma sample. As the sample thaws, the final component that goes into solution is the cryoprecipitate. Usually this process takes up to about 5–7 minutes. The sample should not be allowed to sit in the 37°C water bath for an extended period of time as some labile factors (FVIII, FV, etc.) will start to decay, producing erroneously low values.[21,24,49,50]

Sample Storage and Thawing Recommendations

- **PPP samples not tested within the recommended room-temperature stability limits should be stored frozen as 0.5–1.0 mL aliquots in appropriately labeled polypropylene vials.**
- **The optimal freezing method for PPP samples is a –70°C or colder non-frost-free freezer, which provides sample stability of 6 months.[21,24,49,50]**
- **PPP samples can be stored at –20°C for 2 weeks.[49,50]**
- **Sample vials (with caps on) should be rapidly thawed in a 37°C water bath.**
- **Thawed PPP aliquots must be mixed prior to analysis.**

Specimen condition PAVs: hemolysis, lipemia, and icterus

Hemolysis, lipemia, and icterus may affect the accurate reading of a coagulation assay, especially when optical methods are used. Plausible reasons for optical instrument challenges with increased levels of hemolysis and bilirubin are spectral overlap and light scatter associated with lipid particles. Analyzers that employ secondary wavelengths (>650 nm) can accurately assess hyperbilirubinemia samples and may improve readings for lipemic samples.[52] Mechanical removal of lipids (e.g., ultracentrifugation or solvents) has also been described,[52] but labs should process and run parallel, nonlipemic samples to ensure there are no biases associated with either lipid-removal process.

Icterus may interfere with chromogenic assays (e.g., antithrombin activity), and results must be interpreted with caution. Refer to reagent package inserts for further information about bilirubin levels and their effect on chromogenic assays.

Hemolysis may indicate a poorly collected sample, but labs must rule out in vitro hemolysis (e.g., alcohol toxicity, sepsis). Ex vivo hemolysis is associated with markedly elevated potassium levels and normal lactate dehydrogenase (LDH). Conversely, hemolysis that occurs in vivo is associated with normal potassium levels and markedly increased LDH. Consult the chemistry department to assess sample integrity and subsequent potassium and LDH results. PT can be affected by moderate hemolysis when using an optical device, but even marked hemolysis has minimal effect on PT and APTT when using a mechanical device.[53] Hemolysis also decreases FBG and AT while factitiously increasing D-dimer.[53] In a study creating artificial lysis, hemolysis factitiously increased PT and decreased APTT and FBG and also generated a "dimerized plasmin D fragment" (D-dimer from clot lysis).[54] Unless in vivo hemolysis is confirmed, labs should assume more-likely in vitro hemolysis and recommend sample recollection. Requesting recollection of hemolyzed samples may be problematic in neonatal or pediatric patients or when the sample is from a timed request (e.g., UFH drug monitoring), but the potential for misleading test results may lead to misdiagnoses, dose changes, and other patient mismanagement.

Finally, hemoglobin-based oxygen carriers (HBOC) are stroma-free products that may be used in patients with severe, life-threatening anemia with contraindications to traditional red blood cell replacements (e.g., religious beliefs). When transfused in a patient, the patient's plasma will mimic hemolysis, absent elevated LDH or potassium levels. This pseudo-hemolysis appearance has been demonstrated to affect the ability of optical-reading coagulation analyzers to correctly assess clot-based assays, but chromogenic-based assays may also be affected.[55,56]

Hemolysis, icterus, and lipemia recommendations

- **HIL may affect the ability of optical-reading instruments to accurately assess PPP samples.[52]**
- **Suspected ex vivo (in vitro) hemolyzed PPP samples should be rejected.[21,24,53,54]**
- **Lipemic samples may be processed using ultracentrifugation methods,[52] but a nonlipemic sample should be processed in parallel to ensure this processing method is acceptable.**
- **Icterus samples may interfere with accurate assessment of chromogenic methods.**
- **Infusion of HBOC products will create a pseudo-hemolysis appearance in the plasma and may interfere with clot- and chromogenic-based assays.[55,56]**

PAVs and Interpretation of Coagulation Test Results

As indicated earlier, age, gender, race, blood group, and health status are contributing variables for interpretation of hemostasis test results. As the liver is the last organ to fully develop and the primary organ where coagulation factors are synthesized, neonates (especially premature infants) will have different "normal" values. For most factors, infants reach adult levels by the age of 6 months.[57,58] Increasing age is associated with increases in D-dimer (XDP),[59,60] VWF, and factor VIII, as well as factors V, VII, VIII, IX, and XI (henceforth, FV, FVII, etc.).[61,62]

There are number of differences between males and females in hemostasis testing. Higher levels of factors FII, FVII, FX, FIX, FXI, and FXII are found in females as compared to males.[62] There are decreased levels of protein S (PS) and increased levels of antithrombin activity (AT) in females.[63] Males have longer closure times for the PFA-100® System* Collagen-ADP (CADP) cartridge, a test for screening platelet function defects.[64] Additional variables for females are changes in hemostasis parameters secondary to menstrual cycle, oral contraceptive use (OC), or hormone replacement therapy (HRT). Changes associated with OC depend on hormone concentration. Ethinyl estradiol increases levels of factor VII antigen, factor VIII activity, and beta-thromboglobulin.[65] The recommendation for blood collection in female patients with combined OC and HRT is for therapy to be discontinued for 2 months prior to testing, especially for PS and activated protein C resistance (APCR) testing.[16] Most studies demonstrated no menstrual cyclic variation for FBG, FXI, FXIII, tPA, PAI-1, XDP, or alpha-2-antiplasmin (A2AP). However, some studies have demonstrated that VWF, FVIII, and platelet function are at their lowest levels during the menstrual and early follicular phases, suggesting that these timeframes may be optimal for assessing female patients.[66] Factor X is higher during the follicular phase.[67] In menstruating females suspected of von Willebrand disease (VWD), the recommendation is for blood samples to be collected on cycle day 1–4.[16]

Pregnant females also have different levels of coagulation factors than those who are not pregnant. Pregnancy decreases APTT and PT, with increased XDP and soluble fibrin monomer complexes (SFMC) but no change in AT.[68] Pregnancy is also associated with increased FBG, TAT, F1.2, XDP, VWF, FVII, FVIII, FIX, FX, FXII, plasminogen (PLG), PAI-1, tPA antigen, and platelet function and decreased levels of FXIII, APCR, free PS antigen, and PS activity.[16,69,70] Given the number of alterations associated with pregnancy, the current recommendation for assessing coagulopathy is to wait 2 months postpartum, especially for VWD and PS deficiencies.[16] In addition to pregnancy altering hemostasis levels, labor increases the neonatal levels of FVIII, VWF, FIX, FXI, FXII, and PLG for vaginal deliveries as compared to neonates delivered by elective cesarean section. Meconium presence in neonates has resulted in reduced FII, FV, FVII, and FX.[71]

There are a few notable considerations regarding race and coagulation parameters, especially genetic mutations associated with thromboembolic or bleeding risk.[72-74] The majority of these risk factors are beyond the scope of this document, but readers should be aware of the potential impact when interpreting results. Relatively common occurrences are single nucleotide polymorphism of VWF in African-Americans,[75] presence of factor XI deficiencies in Ashkenazi Jews,[76] and APCR in Caucasians.[77] Sickle cell disease, most predominantly a disease in African-Americans and people of Mediterranean origin, is associated with increased FVIII, VWF, and XDP. Combined with observed increases in prothrombin fragment (F1.2) and thrombin-antithrombin complexes (TAT) and p-selectin (an indicator of platelet activation) with decreased ADAMTS-13, these data are suggestive of coagulation and platelet activation.[78]

There are notable differences in coagulation parameters among ABO blood groups, especially for FVIII and VWF. In blood group O, there are lower levels of FVIII, FIX, FXII, and VWF than in group non-O.[61-63] There is no association of Rh factor with any coagulation parameter. No ABO effect is seen for PS, protein C (PC), or AT.[63]

Exercise increases VWF, FVIII, and euglobulin lysis times (ELT), but not FXII, FV, FVII, FII, or FBG.[16] Stress activity increases VWF, CRP, and platelet activation,[15] with mental stress resulting in increases of VWF, FBG, tPA, and FVIII.[16] Phobic anxiety regarding blood collection is associated with a hypercoagulable state[79] that resolves with antidepressant therapy.[80] Prolonged mental stress leads to decreases in FV, FVIII, and FIX.[15,16]

Increased thyroid hormone levels (hyperthyroidism) are associated with increased VWF, FBG, factor VIII, and PAI-1 but decreased CEPI and CADP closure times on the PFA-100 System.*[81] Increased levothyroxine treatment is associated with increased levels of VWF, FVIII, FIX, FX, PAI-1, and ELT and concomitant decreased APTT.[82] Primary hyperparathyroidism is associated with increased levels of FVII, FX, and XDP.[83] Acidosis and, to a lesser degree, hypothermia result in altered PT and APTT testing.[84]

Assessment of the biological or circadian variance noted with coagulation-related testing is very important. The biological variation is variation noted between measurements of a given test over specified timeframes in a single individual or a population. The coagulation test with the smallest variation is prothrombin time (PT), and one of the largest variances is in VWF testing.[85] Seasonal variation has been noted with FBG, but not PT or platelet aggregation.[86] Circadian variations have been noted with heparin therapy,[87] testing for platelet function,[88] and measurements of the fibrinolytic pathway.[89] Platelet function can be affected by circadian rhythms; physiological stress (exercise, coffee, caffeine-containing products); dietary issues including flavonoids, phytoestrogens, and polyphenols; and smoking.[90-92] Fatty acids can affect platelet function as well as increase levels of PAI-1 and promote activation of FVII.[93] With these bioanalytical and seasonal variables, multiple blood collections throughout various cycles may be necessary to confirm abnormal findings.

Aside from congenital deficiencies that lead to bleeding or thrombotic risks, several disease states are associated with coagulopathy, including trauma, shock, sepsis, cancer, renal failure, liver failure, systemic lupus erythematosus (SLE) and other autoimmune disorders, surgery, and amyloidosis. Mechanical and support interventions such as extracorporeal membrane oxygenation (ECMO, also known as extracorporeal life support [ECLS]), hemodialysis, continuous venovenous/arteriovenous hemodialysis (CVVH/CAVH), blood oxygenators, and aortic balloon pumps may induce a mild consumptive coagulopathy in adults, which may be more pronounced in neonates and pediatric patients (weight-associated).[94,95] These mechanical interventions require anticoagulation that may be partially performed within the circuit, systemic (UFH), or local to the device (citrate within the device and calcium gluconate out of the device). In these acute and seriously ill patients, POC methods (e.g., activated clotting time [ACT]) are often the measuring methods of choice, but the laboratory may also be used to confirm POC findings.[95] Other mechanical assisting devices, such as left ventricular pumps, create a turbulent flow environment, causing immediate acquired type 2 VWD from mechanical shearing of the VWF protein.[96]

Numerous pharmaceutical interventions designed to alter or replace hemostatic factors in hemostasis may or may not have an impact on coagulation testing. Common anticoagulants such as vitamin K antagonists, heparins, DTIs, and DOACs may have some impact on routine or specific coagulation assays. Some of these therapies require monitoring, but others may not. Other pharmaceutical interventions that alter the hemostasis pathway include fibrinolytic therapies (e.g., tPA), defibrinating drugs, antifibrinolytics (e.g., tranexamic acid [TXA]), and antithrombotics (usually this term refers to antiplatelet therapy such as clopidogrel, aspirin, prasugrel, etc.). Drug reversal may be in the form of human products (e.g., fresh frozen plasma, cryoprecipitate, liquid-state plasma, etc.), nonspecific (3- and 4-factor prothrombin complex concentrates that may be activated), or specific to a drug (e.g., idarucizumab). Pharmaceutical interventions for a bleeding patient may include stimulating an in vivo response (e.g., nasal or infusion of desmopressin to increase circulating VWF) or infusion of an activated factor (e.g., NOVOSEVEN, a recombinant factor VIIa). Replacement therapies include specific factors (e.g., FVIII or FIX) that may be of human, recombinant, porcine, or other origin (e.g., emicizumab-kxwh, trade name HEMLIBRA). Labs should be knowledgeable about the effects of these pharmaceutical interventions on their assays. Labs should also be able to provide guidance on which assays are appropriate for assessing the drug concentration (pharmacokinetics) or effect (pharmacodynamics). For common anticoagulants, such as warfarin or UFH, historical practice uses traditional screening tests such as PT and APTT, respectively. However, even those decades-old strategies must be monitored appropriately, with blood samples collected at the correct time to avoid overexposure of the drug or inappropriate dose changes (Table 1).

**System is not available for sale outside the U.S. Product availability may vary from country to country and is subject to varying regulatory requirements. Please contact your local representative for availability.*

Newer therapies (e.g., DOACs or HEMLIBRA) and assessing the efficacy of therapies that are less well-established (e.g., fibrinolytics) may require further reading. Some provisional guidance is provided in Table 1. Patients with known congenital deficiencies (e.g., hemophilia A or B and VWD) may require pharmacokinetic (PK) studies to determine replacement therapy and potential inhibitor effect. Guidance for PK studies should be provided by drug labeling or a physician, but some provisional guidance is provided in Table 1.

Note also that some pharmaceutical interventions for which the primary target of the therapy is not related to coagulation may have an unintended impact on coagulation testing. Most commonly, antibiotic use may alter the intestinal flora to impact the utilization of vitamin K, causing a vitamin K factor deficiency (similar to warfarin treatment causing decreases in FII, FVII, FIX, and FX) or exacerbating warfarin therapy. Additionally, some lipoglycopeptides (e.g., telavancin) may interfere with coagulation assays due to their binding of the reagent phospholipid sources.[97,98] Spurious abnormal results, especially concomitant with new drug use, should be thoroughly investigated prior to intervention. Given a rise of cases in the U.S., the Centers for Disease Control (CDC) alerted clinicians about the rise of synthetic cannabis treated with rodenticides (brodifacoum), causing markedly prolonged PT and APTT.[99] Brodifacoum is a vitamin K antagonist with an extremely long half-life as compared to warfarin.[100]

Finally, laboratories must be aware of samples that may not be of human origin or may not represent "native" human plasma. As part of episodic quality-assurance (QA) measures, facilities that collect and process cryoprecipitate may require assessment of FVIII and FBG. These samples contain concentrated amounts (as compared to native plasma) of FVIII, FBG, and VWF, and thus modification of the test (dilutions) or sample (diluted with FVIII-deficient plasma) may be required prior to testing. Institutions with cell-saving devices in the operating room may require anti-Xa assessment, and the laboratory must have a system that provides an adequate lower level of detection suitable for this QA process.

Table 3. Possible causes of artifactually prolonged APTT or PT.

Category	APTT	Prothrombin Time
Patient Selection	Anticoagulants	Anticoagulants (including vitamin K antagonists)
	Liver disease	Liver disease
	Bleeding disorders (acquired or inherited)	Bleeding disorders (acquired or inherited)
Specimen Collection	Poor venipuncture	Poor venipuncture
	Inadequate anticoagulant	Inadequate anticoagulant
	Collection in wrong tube	Collection in wrong tube
	Improper fill volume	Improper fill volume
Specimen Transport	Specimen too old	Specimen too old
	Improper temperature for transport	Improper temperature for transport
	Inappropriate handling of specimen	Inappropriate handling of specimen
Specimen Processing and Storage	Inappropriate centrifugation speed and time	Inappropriate centrifugation speed and time
	Stored at warmer-than-recommended temperature	Stored at warmer-than-recommended temperature
	Stored longer than recommended	Stored longer than recommended

Table 4. Possible causes of artifactually shortened APTT or PT.

Category	APTT	Prothrombin Time
Patient Selection	Acquired active ongoing coagulation	Acquired active ongoing coagulation
Specimen Collection	Poor venipuncture	Poor venipuncture
	Incomplete mixing of tube	Incomplete mixing of tube
	Inadequate anticoagulant	Inadequate anticoagulant
	Collection in wrong tube	Collection in wrong tube
Specimen Transport	Specimen too old	Specimen too old
	Improper temperature for transport	Improper temperature for transport
	Inappropriate handling of specimen	Inappropriate handling of specimen
Specimen Processing and Storage	Inappropriate centrifugation speed and time	Inappropriate centrifugation speed and time
	Stored longer than recommended	Stored longer than recommended
	Too many residual platelets after freezing	

Animal blood, while having similar coagulation proteins, often has significantly different levels of these proteins than human blood.[101] As such, not all human-based tests may translate to animal systems, especially for factor levels, D-dimer, and platelet-function testing. This is especially true if an immunoassay is employed using animal-derived antibodies. Reagent manufacturers may be able to provide guidance on whether their reagent or method is suitable for animal testing.

As we have described, there are numerous pre-analytical variables associated with coagulation testing that may impact the diagnostic accuracy of a test result. Each laboratorian and clinician must assess and consider the impact of these variables when interpreting coagulation test data. Limitations of most published studies of these variables include small sample sizes, but more importantly, limited reagent and instrument combinations. Note the plausible causes for prolonged PT and APTT (Table 3) as well as for decreased PT and APTT clotting times (Table 4).

Conclusion

Pre-analytical variables associated with patients, specimen collection, specimen transportation, and specimen processing and storage can cause significant differences in the values obtained from routine and specialized coagulation testing. Failure to recognize or address these pre-analytical variables may create factitious test values resulting in patient mismanagement, including misdiagnosis and improper dosing and treatment. As summarized in this review, procedural errors, shortcuts, and omissions can lead to erroneous result reporting. Therefore, laboratory procedures must always comply with the most stringent recommendations and guidelines, including those from reagent and instrument manufacturers, to select and prepare specimens to optimize testing. Following established guidelines and lab procedures that specifically address pre-analytical variables may require retraining of staff members who collect, process, store, and test coagulation samples. Strict adherence to these procedures will result in more-accurate and reproducible results.

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