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. Additionally, advances in laboratory instrumentation have improved the reproducibility and sensitivity of the analytical phase, therefore creating greater dependence on specimen integrity. 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. However, despite these advancements, variability in coagulation test results remains a significant challenge for the proper methods based on published and new recommendations.

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. 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. 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 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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**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.

<table>
<thead>
<tr>
<th>Drug Class</th>
<th>Drug</th>
<th>Monitoring/Measuring Test</th>
<th>Optimal or Desired Time of Blood Collection</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heparin</td>
<td>Unfractionated</td>
<td>aPTT Anti-Xa</td>
<td>6 hours after dose initiation or adjustment</td>
<td>• Anti-Xa measurements: also acceptable.</td>
</tr>
<tr>
<td></td>
<td>Low-molecular-weight</td>
<td>Anti-Xa</td>
<td>4 hours after third dose</td>
<td>• Circuit anticoagulation may require more frequent monitoring.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pentascorbate</td>
<td>3 hours after dose initiation</td>
<td>• Circuit anticoagulation may require higher dosing that cannot be measured by aPTT, so ACT may be the optimal test.</td>
</tr>
<tr>
<td>Vitamin K Antagonists</td>
<td>PT/INR (baseline PT/INR should be collected prior to initiation of therapy)</td>
<td>First INR is with 12–24 hours of first dose</td>
<td>• If potential target, consider VKORC mutation.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(reversal therapy)</td>
<td>PT/INR</td>
<td>IV: 12 hours</td>
<td>• If within target, dose is probably acceptable.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Oral: 12–24 hours</td>
<td>• If lower than target, probably need to adjust dose higher:</td>
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<tr>
<td>Antifibrinolitics</td>
<td>Fibranalytics</td>
<td>TT, Fbg, XDP</td>
<td>10–15 minutes after completion of infusion</td>
<td>• Patient may need additional reversal agents.</td>
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<td></td>
<td></td>
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<td></td>
<td>• More-frequent monitoring may be necessary if patient is bleeding.</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>• No consensus on method for measuring or whether necessary.</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• Methods include PFA Systems, ULTEGRA, and traditional platelet aggregation.</td>
</tr>
<tr>
<td>Antiplatelet agents</td>
<td>Pharmacological effects on coagulation.</td>
<td>Pharmacological effects on coagulation.</td>
<td>Pharmacological effects on coagulation.</td>
<td>• Platelet function tests. Method: traditional platelet aggregation.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• Antiplatelet therapy.</td>
</tr>
<tr>
<td>Antiplatelet—oral</td>
<td>Pharmacological effects on coagulation.</td>
<td>Pharmacological effects on coagulation.</td>
<td>Pharmacological effects on coagulation.</td>
<td>• Antiplatelet therapy.</td>
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<td></td>
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<td></td>
<td>• Drug-dependent dosing:</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• POC or platelet function methods, including ULTEGRA, TEG, or ROTEM-based methods. Traditional platelet aggregation studies may also be suitable.</td>
</tr>
<tr>
<td>DOAC</td>
<td>Ila</td>
<td>ECT, ECA, h, ATT</td>
<td>Trough samples (5–30 minutes prior to next dose)</td>
<td>• No consensus or recommendations for monitoring.</td>
</tr>
<tr>
<td></td>
<td>Reversal-dabigatran</td>
<td>ECT, ECA, anti-Xa</td>
<td>Trough samples (5–30 minutes prior to next dose)</td>
<td>• Collect just before next dose.</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• If peak samples are desired, then usually 2–3 hours after dose.</td>
</tr>
<tr>
<td>Factor Replacement</td>
<td>Factor level (hemophilia A or B)</td>
<td>Physician-guided. PK studies may be required</td>
<td>4 hours after infusion to reassess anti-Xa</td>
<td>• No consensus or recommendations for monitoring.</td>
</tr>
<tr>
<td></td>
<td>Reversal—rivaroxaban/ apixaban</td>
<td>Anti-Xa</td>
<td>4 hours after infusion to reassess anti-Xa</td>
<td>• The drug is continuously infused.</td>
</tr>
<tr>
<td></td>
<td>Replacement therapy</td>
<td>Factor level (hemophilia A or B)</td>
<td>4 hours after infusion to reassess anti-Xa</td>
<td>• Note that newer replacement therapies for hemophilia using modified (Fpe)ated, albumin-fused, etc.) factor replacement may require special methods.</td>
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<td>(hemophilia A or B)</td>
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<tr>
<td></td>
<td>DDAVP/Pentosan</td>
<td>VWF, FVIII</td>
<td>Baseline, 30 minutes, 2, 4, and 6 hours post-drg delivery</td>
<td>• Drug delivery is either by nasal spray or infusion.</td>
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<td>• No consensus or recommendations for monitoring.</td>
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<td>• Specific drug-calibrated anti-Xa.</td>
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<td>• With concomitant therapy, then ACT.</td>
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<td></td>
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<td></td>
<td>• 30 minutes for checking catheter function.</td>
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**ACT:** activated clotting time  
**APCC:** prothrombin complex concentrate  
**aPTT:** activated partial thromboplastin time  
**DDAVP:** 1-desamino-8-D-arginine vasopressin  
**dTT:** dilute thrombin time  
**DOAC:** direct oral anticoagulants  
**PT:** prothrombin time  
**PCC:** prothrombin complex concentrate  
**PF:** polymethylmethacrylate  
**PK:** pharmacokinetic  
**POC:** point of care  
**pTT:** partial thromboplastin time  
**R:CA:** rotational thromboplastin kaolin  
**RI:** International Normalized Ratio  
**RR:** routine  
**RFV:** replacement factor VIIa  
**TT:** thromboelastography  
**VWF:** von Willebrand factor  

**Notes:**  
- AKH: anticoagulation  
- APTT: activated partial thromboplastin time  
- Anti-Xa: anti-Xa reagents  
- aPTT: activated partial thromboplastin time  
- DOAC: direct oral anticoagulants  
- INR: International Normalized Ratio  
- PK: pharmacokinetic  
- PT: prothrombin time  
- PTT: partial thromboplastin time  
- VWF: von Willebrand factor  

**Patient selection pre-analytical variables**  
Age, gender, race, blood group, and health status are all contributing factors for hemostasis testing, with the largest impact on being on test interpretation, to be discussed later. Therefore, each laboratory must be able to address these variables, which may require different reference intervals (RIs), 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 (thrombin (Fbg), factor VIII (FVIII), von Willebrand factor (VWF), and plasminogen activator inhibitor-1 (PAI-1), with decreased levels of protein S (PS) activity. Mental or physiological stress, including exercise, can also cause changes in hemostasis, including foetopituitary levels of FVIII and FVIII and decreased platelet function. As such, patients should be allowed to relax for 30 minutes prior to phlebotomy.

Other conditions (e.g., biological variation, circulation 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-naive 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 therapy drug, 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 psychological and physiological stress that may factiously alter coagulation tests, especially for VWF and platelet-function studies.  
- Assessment of platelet-function studies should be performed using samples collected from fasting and drug-naive patients.
- For patients being monitored (e.g., those on antiocoagulation therapy), adherence to the collection time is mandatory (Table 1).
Specimen Collection PAVs

After patient selection has been defined and the appropriate blood or fluid volume and types 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 identification and orders are verified, actual blood collection follows. Personnel must be properly trained in phlebotomy practices using universal precautions to ensure proper blood collection and specimen integrity. In one study, the authors demonstrated that trained personnel provided better-quality specimens for coagulation than untrained personnel. Specimens 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 specimens.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 APPT devices. Depending on the desired test, these POC samples may use native whole blood or require anticoagulation using evacuated 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. Different manufacturers use different techniques for anticoagulant production, types of anticoagulants, and anticoagulant concentrations. In some cases, the tubes are available in a variety of sizes (needle gauge), with the best-practice guidance for tube size is to use the smallest size tubes possible.26

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 of blood collection has 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.26

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 improper mixing. If multiple tubes are noted, the appropriate tubes with different anticoagulants are required, fill the syringe, and quickly transfer the sample to the appropriate tube. The Phlebotomy Advisory Committee recommends gently mixing the practice 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,26 or in patients treated with anticoagulant drugs. In one study, the authors demonstrated that two published studies used a limited number of samples and collection tubes. Therefore, unless locally determined otherwise through rigorous examination of the case, use the syringe technique 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 vigorously shake or agitate the tube.24

Because the blood collected must be subsequently transfused to a recipient container, the syringe method inherently increases the phlebotomist’s risk of needlestick exposure, as the recipient container must be held in one hand while the syringe needle is guided into the tube with the other hand.22 The syringe method also increases the risk of hemolysis if blood is forced too quickly through the hypodermic needle tip or against the side of the collecting tube.22 If no anticoagulant is added to the syringe, the specimen is likely to clot if not transferred immediately (within 30 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 versus vacuum tube.22 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 21 to 19 gauge for coagulation collection 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.21–24 If the syringe technique is employed for collections of more than 30 mL, an 18-gauge needle is recommended to reduce hematoma (blood loss) 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.21–24 For smaller patients, if appropriate and sterility collected, the first 10 mL that would otherwise be discarded can be recycled into the patient if the hospital has a policy allowing the replacement of the blood.29 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.21–24 Consider point-of-care methods in patients with difficult venous or arterial access.

Phlebotomy procedural issues: tourniquet technique

Phlebotomy personnel should be prudent to the risk of hemolysis if blood is forced too quickly through the hypodermic needle or against the side of the collecting tube. 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.22

Phlebotomy procedural issues: order of draw

Current CLSI guidelines recommend an order for drawing of multiple samples that has become standard practice (Table 2).37–39 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. A 1 mL volume of virus (e.g., fibroblasts or anticoagulant) is used to inhibit platelet activation. Tubes containing AT3 (citrate, adenine, and dipyridamol) and CTAD (citrate, theophylline, adenine, and dipyridamol) are used for nucleic acid testing of platelet concentrates.34 Tubes containing PFPK or aspirin are used to inhibit activation of the coagulation and fibrinolytic systems respectively.34,40,41 Lithium heparin, EDTA, or no anticoagulant (serum) are used for immunosassay tests (e.g., HIV testing and antibody testing in antiphospholipid syndrome). Specialized tubes have been created for specific tests such as the fibrin degradation test (FDP) test,30 where the high concentration of thrombin and IAP assay32 (acidified citrate), in which these tubes must be used for accurate results. If not standardized in the manufacturer’s test package insert is used, the tube must be validated by the laboratory.

Most coagulation tests are established and validated using sodium citrate as the anticoagulant. 3.2% sodium citrate is the citrate concentration of choice26–28 because it is coagulated more bioequivalent in test tubes. 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 blanked 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 blotted from the label 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 result and patient results. For some coagulation and platelet testing, other anticoagulants or no anticoagulant (generating serum) are used.35 Tubes containing CAC (citrate, adenine, and dipyridamol) and CTAD (citrate, theophylline, adenine, and dipyridamol) are used for nucleic acid testing of platelet concentrates.34 Tubes containing PFPK or aspirin are used to inhibit activation of the coagulation and fibrinolytic systems respectively.34,40,41 Lithium heparin, EDTA, or no anticoagulant (serum) are used for immunosassay tests (e.g., HIV testing and antibody testing in antiphospholipid syndrome). Specialized tubes have been created for specific tests such as the fibrin degradation test (FDP) test,30 where the high concentration of thrombin and IAP assay32 (acidified citrate), in which these tubes must be used for accurate results. If not standardized in the manufacturer’s test package insert is used, the tube must be validated by the laboratory.

Note, however, that these two published studies used these manufacturers, there are significant differences among their tubes that can potentially affect coagulation test results.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 in the laboratory.

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Phlebotomy procedural issues: fill volume and hematocrit adjustment
Sodium citrate (3.2%) is the most widely used anticoagulant for coagulation testing (with resulting 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 and thereby affecting the clotting time value.\(^\text{21,22}\) The current guidelines dictate that the proportion of blood to anticoagulant volume be a ratio of 9:1.\(^\text{23}\) However, the literature demonstrates that some underlying 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).\(^\text{24,25}\) Therefore, short-sampling in tubes for patients with low hematocrits may not be clinically significant unless measuring for anticoagulant effect (heparin, direct oral anticoagulants [DOACs]), and direct thrombin inhibitors (DTIs), as the anticoagulant result may be artefactually decreased due to the dilution effect of low hematocrit and excess sodium citrate liquid volume.\(^\text{26}\) 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 falsely elevated PT, INR, and APTT results.\(^\text{27,28}\) Gentle inversion (mixing) of sodium citrate tube approximately 5–6 times is recommended.\(^\text{29}\) Avoid rigorous shaking or agitation.\(^\text{30}\)

- 3.2% sodium citrate is the citrate concentration of choice.\(^\text{31,32}\)
- For patients who require multiple-tube collections, follow the mandatory collection sequence (Table 2).\(^\text{33}\)
- If only citrate tubes are being collected, no discard tube is necessary (unless using the butterfly syringe method directly into the collection tube).\(^\text{34}\)
- Patients with elevated hematocrits (>55%) may require tubes with a reduced volume of citrate.\(^\text{35,36}\)

**Specimen Transportation and Stability PAVs**

Transportation and processing of blood samples for coagulation testing encompasses 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.\(^\text{37,38}\) These criteria also change depending on whether the patient is anticoagulated and 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 (refrigerated or room temperature), and patient groups.\(^\text{37}\) The integrity of PTT samples, unlike that of PT samples, depends on both processing conditions and the type of anticoagulant, especially unfractionated heparin. Samples for PTT assays and anti-factor Xa (anti-FXa) monitoring of unfractionated heparin or DOACs are sensitive to time and processing methods.\(^\text{39-41}\) 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.\(^\text{39}\) 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.\(^\text{42,43}\)

The current guidelines stipulate 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.\(^\text{44,45}\) Centrifugation improves sample stability, and once centrifuged, the stability is not substantially influenced by temperature.\(^\text{45}\) 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.\(^\text{45}\) It is also recommended that specimens submitted for other assays (factor assays, protein C, VWF studies) be processed and stored within the 6-hour time limit.\(^\text{41}\) DTT samples, including dobiquitin, must be tested within 2 hours if using the thrombin time or a direct anti-FXa chromogenic assay.\(^\text{46}\) 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 sample samples, with results that appear to be more robust than CLSI recommendations. 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.\(^\text{41}\) 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).\(^\text{47}\,48\)

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.\(^\text{49}\)

**Whole-blood sample transportation and stability recommendations**

- Coagulation samples should not be transported or stored on ice.\(^\text{50}\)
- Coagulation samples for platelet-function studies must be maintained at room temperature.\(^\text{51}\)
- Whole-blood samples for prothrombin time assays are stable for 24 hours at room temperature.\(^\text{52}\)
- 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.\(^\text{53}\)
- For other tests, unless otherwise indicated by the manufacturer, whole-blood stability is 4 hours.\(^\text{54}\)
- Pneumatic transport systems should not be used for samples that require platelet-function testing.\(^\text{47}\,48\)
- 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 rocked and positioned upright.\(^\text{55}\)

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

<table>
<thead>
<tr>
<th>Order of Draw</th>
<th>Vacuum Tube Type</th>
<th>Color of Cap</th>
<th>Test Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Blood culture tubes</td>
<td>Color varies</td>
<td>Blood culture</td>
</tr>
<tr>
<td>2</td>
<td>Sodium citrate (3.2%)</td>
<td>Light blue</td>
<td>Coagulation</td>
</tr>
<tr>
<td>3</td>
<td>Glass (no activator)</td>
<td>Red</td>
<td>Chemistry, immunosassays</td>
</tr>
<tr>
<td>4</td>
<td>Sodium or lithium heparin</td>
<td>Green</td>
<td>Chemistry, serology</td>
</tr>
<tr>
<td>5</td>
<td>Trice elements (no preservative)</td>
<td>Royal blue</td>
<td>Trice element testology</td>
</tr>
<tr>
<td>6</td>
<td>EDTA</td>
<td>Lavender or pink</td>
<td>Hematology, blood bank</td>
</tr>
<tr>
<td>7</td>
<td>Sodium fluoride</td>
<td>Grey</td>
<td>Glucose</td>
</tr>
<tr>
<td>9</td>
<td>ACD</td>
<td>Yellow</td>
<td>Blood bank PTA testing</td>
</tr>
<tr>
<td>10</td>
<td>QUANTIFERON-TB</td>
<td>Gold</td>
<td>TB testing</td>
</tr>
</tbody>
</table>
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 (20 x 10^9/L).21,24-26 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 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.21,24-26 Each lab must periodically evaluate its centrifuge parameters (both g-force and time) to ensure that samples are still considered platelet-poor. Accreditation organizations or hospital/laboratory policies may require periodic checks of the final plasma sample for its centrifugation speed (rpm) or force (g) to ensure production of PPP.48

PPP is defined as <10,000 platelets/μL.21

• 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.44

• 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 Samples should be thawed by rapid thawing at 37°C until all the components return to solution (shortest duration possible).21,26

During testing of the specialized tests. Once prepared, sample vials should be stored in the freezer in which they will be stored. The freezer of choice is ~70–~80°C non-frost-free ultra-low freezer in which samples can be stored for 6 months.21,24 If the laboratory does not have an ultra-low freezer, most plasma samples can be stored at ~20°C for 2 weeks,21,24 but refer to manufacturer’s package insert or other test instructions to determine optimal storage.
Hemolytic, icterus, and lipemia recommendations

• HIL may affect the ability of optical-reading instruments to accurately assess PPP samples. 16
• Suspected ex vivo (in vitro) hemolyzed PPP samples should be rejected. 16,24,53
• Lipemic samples may be processed using ultracentrifugation methods, 6 but a nonlipemic sample should be prepared 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. 6,54

PAVs and Interpretation of Coagulation Test Results

As indicated earlier, age, gender, race, blood group, and health status are confounding 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.10 11 Increasing age is associated with increases in D-dimer (DD), VWF, and FVIII as well as factors V, VII, IX, and XI (henceforth, FV, FX, etc.) 12,14

There are a number of differences between males and females in hemostasis testing. Higher levels of factors FII, FV, FVIII, and FIX are found in females as compared to males.13 There are decreased levels of protein S (PS) and increased levels of antithrombin activity (AT) in females. 13 Males have longer closure times for the factors FII, FV, FVIIIA, and FIX that is for blood samples to be collected on cycle day 1-4.14 Pregnant females also have different levels of coagulation factors than those who are not pregnant. Pregnancy decreases AT and PT, while increased levels of FXF and FXF appear in the maternal circulation as a result of van Willebrand disease (VWD), the recommendation is 16

During the menstrual and early follicular phases, suggesting that these timeframes may be optimal for assessing female patients.16 Factor X is higher during the follicular phase. In menstruating females suspected of ven Willebrand disease (VWD), the recommendation is for blood samples to be collected on cycle day 1-4.14

• Decreased FV, FVIII, and FIX. 15,16
• Increased thyroid hormone levels (hyperthyroidism) are associated with increased levels of VWF, FVIII, FIX, FX, PAI-1, and ELT and concomitant decreased APTT. 72-74

Increased Factor V and VIII antigen, factor VIII activity, and beta-thromboglobulin.65

(78) Exercise increases VWF, CRP, and platelet activation, 16 with mental stress resulting in increases of VWF, FBG, IP, and FXII. 16

Increased thyroid hormone levels (hyperthyroidism) are associated with increased levels of VWF, FVIII, FIX, FX, PAI-1, and ELT and concomitant decreased APTT. 72-74

Primary hypercoagulopathy is associated with increased levels of VWF, FX, and XDP. 72 Acidosis and, to a lesser degree, hypothermia result in altered PT and APTT testing. 79

Assessment of the biological or circadian variance ratio is important. 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. 80 Seasonal variation has been noted with Fbg, but not PT or platelet aggregation. 81

VWD and PS deficiencies. 16 In addition to pregnancy altering hemostasis levels, labor increases the neonatal levels of VWF, FV, FX, FIX, and FVII, APCR, free PS antigen, and platelet function and decreased levels of FVII, FIX, FX, and FXII. 16

Increased 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 VWF, FVIII, and FIX. 16

Increased levels of FVII, FX, and XDP. 83 Acidosis and, to a lesser degree, hypothermia result in altered PT and APTT testing. 83

Increased levothyroxine treatment is may be of human, recombinant, 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-kwax, 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 (pharmacodynamic testing) 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). 81

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., fibrinolitics) may require further reading. Some provisional guidance is provided in Table 1. Patients with known congenital deficiencies (e.g., hemophilia A or B and WDD) 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 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 testing due to their binding of the reagent phospholipid sources. Spurious abnormal results, especially concomitant with new drug use, should be thoroughly investigated prior to intervention. Given a rise in the ca of the U.S., the Centers for Disease Control (CDC) alerted clinicians about the rise of synthetic cannabinoids treated with radenicides (brodaciocum), causing markedly prolonged PT and APTT. Brodaciocum is a vitamin K antagonist with an extremely long half-life compared to warfarin. 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 FIBG. These samples contain concentrated amounts (as compared to native plasma) of FIBG, FIBF, 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.

Animal blood, while having similar coagulation properties, often has significantly different levels of these proteins than human blood. As such, not all human-based tests may translate to animal systems, especially for factor levels, D-dimer, and platelet-rupture function testing. This is especially true if an immunassay 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 cloting times (Table 4).

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

<table>
<thead>
<tr>
<th>Category</th>
<th>APTT</th>
<th>Prothrombin Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient Selection</td>
<td>Anticoagulants</td>
<td>Anticoagulants (including vitamin K antagonists)</td>
</tr>
<tr>
<td>Specimen Collection</td>
<td>Inadequate anticoagulant</td>
<td>Inadequate anticoagulant</td>
</tr>
<tr>
<td>Specimen Transport</td>
<td>Improper temperature</td>
<td>Improper temperature</td>
</tr>
<tr>
<td>Specimen Processing and Storage</td>
<td>Inappropriate centrifugation speed and time</td>
<td>Inappropriate centrifugation speed and time</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Category</th>
<th>APTT</th>
<th>Prothrombin Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient Selection</td>
<td>Acquired active ongoing coagulation</td>
<td>Acquired active ongoing coagulation</td>
</tr>
<tr>
<td>Specimen Collection</td>
<td>Incomplete mixing of tube</td>
<td>Incomplete mixing of tube</td>
</tr>
<tr>
<td>Specimen Transport</td>
<td>Improper temperature for transport</td>
<td>Improper temperature for transport</td>
</tr>
<tr>
<td>Specimen Processing and Storage</td>
<td>Stressed longer than recommended</td>
<td>Stressed longer than recommended</td>
</tr>
</tbody>
</table>

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 testing. 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.

References:


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