Published online: 2019-07-10

Preanalytical Variables in Coagulation Testing: Setting the Stage for Accurate Results

Robert C. Gosselin, CLS[1] Richard A. Marlar, PhD[2]

[1] Hemostasis and Thrombosis Center, Davis Health System, University of California, Sacramento, California
[2] Department of Pathology, University of New Mexico Health Sciences Center, Albuquerque, New Mexico

Address for correspondence Robert C. Gosselin, CLS, Davis Health System, Hemostasis and Thrombosis Center, University of California, 2100 Stockton Blvd, Ticon 1 Bldg, Sacramento, CA 95817 (e-mail: rcgosselin@outlook.com).

Semin Thromb Hemost 2019;45:433–448.

Abstract

Many preanalytical variables may affect the results of routine coagulation assays. While advances in laboratory instrumentation have partially addressed the laboratory's ability to recognize some of these variables, there remains an increased reliance on laboratory personnel to recognize the three potential areas where coagulation testing preanalytical issues may arise: (1) specimen collection (including patient selection), (2) specimen transportation and stability, and (3) specimen processing and storage. The purpose of this article is to identify the preanalytical variables associated with coagulation-related testing and provide laboratory practice recommendations in an effort to improve the quality of coagulation testing and accuracy of result reporting.

Keywords
- coagulation testing
- preanalytical variables
- recommendations
- specimens

Many preanalytical variables 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) 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, either for diagnostic or monitoring purposes. These screening tests also form the basis of many special coagulation tests such as factor assays, and in some locations clot-based specialized assays (e.g., protein C [PC] and protein S [PS] activity assays, activated PC resistance [APCR]).

Since the introduction of coagulation assays, efforts have been made not only to automate them, but to better standardize such testing, thereby providing more accurate results to aid in clinical assessment.[6–14] Preanalytical variables pertaining to routine coagulation testing can be classified into three major categories: (1) specimen collection (including patient selection), (2) specimen transportation and stability, and (3) specimen processing and storage. Within each of these there are several individual variables, each of which may have a major impact on testing.

Many standards and guidance documents 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 laboratories standards and guidance documents in the United States, although their guidelines may be referenced or used internationally.[6] 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. There are several documents available from CLSI that have relevance to laboratory practitioners.[6] Despite its existence, there is still a lack of practice standardization among clinical laboratories, with regards to specimen collection, storage, and processing for coagulation testing. Some of the procedures in practice today remain founded on "tradition," while others are based on guidance documents such as provided by CLSI, with and

Issue Theme Editorial Compilation VII; Guest Editors: Emmanuel J. Favaloro, PhD, FFSc (RCPA), and Giuseppe Lippi, MD.

DOI https://doi.org/10.1055/s-0039-1692700.
ISSN 0094-6176.

Copyright © 2019 by Thieme Medical Publishers, Inc., 333 Seventh Avenue, New York, NY 10001, USA.
Tel: +1(212) 584-4662.

License terms

Table 1 CLSI available documents related to coagulation testing[6]

Standard/Guideline	Title	Issue date
H21	Collection, Transport, and Processing of Blood Specimens for Testing Plasma-Based Coagulation Assays and Molecular Hemostasis Assays, 5th Edition	1/23/2008
H30	Procedure for the Determination of Fibrinogen in Plasma, 2nd Edition	11/01/2001
H47	One-Stage Prothrombin Time (PT) Test and Activated Partial Thromboplastin Time (aPTT) Test, 2nd Edition	05/30/2008
H48	Determination of Coagulation Factor Activities Using the One-Stage Clotting Assay, 2nd Edition	03/30/2016
H54	Procedures for Validation of INR and Local Calibration of PT/INR Systems, 1st Edition	08/19/2005
H57	H57-A Protocol for the Evaluation, Validation, and Implementation of Coagulometers, 1st Edition	01/23/2008
H58	Platelet Function Testing by Aggregometry, 1st Edition	11/24/2008
H59	Quantitative D-dimer for the Exclusion of Venous Thromboembolic Disease, 1st Edition	03/31/2011
H60	Laboratory Testing for the Lupus Anticoagulant, 1st Edition	04/04/2014

without significant published or supporting evidence. In addition, many of the CLSI coagulation-related documents are outdated, and often not renewed[6,15] (►**Table 1**). As a result, many problems, inconsistencies, and erroneous results can still arise based on preanalytical processing of specimens, and these discrepancies may be associated with disastrous outcomes. This article will review the preanalytical variables associated with coagulation-related testing. Point-of-care (POC) devices, which may use native or anticoagulated whole blood, are also affected by preanalytical variables, but will have limited focus in this document. While there are also many variables related to the analysis of the specimen that also impact on testing, primarily those dependent on reagent and instrumentation, these are beyond the scope of this particular document. A recommendation for the most appropriate methods based on published and new data will be presented together with recommendations for converting these to a form of laboratory guidance document.

Specimen Collection of Preanalytical Variables

Patient Selection

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

The liver is the primary organ for synthesis of most coagulation factors. As the liver is the last organ to fully develop, neonates (especially premature infants) will have generally lower levels of coagulation factors, and thereby different "normal" values. For most coagulation factors, levels reach adult values by the age of 6 months.[16,17] Increasing age is associated with increases in D-dimer (sometimes abbreviated XDP),[18,19] von Willebrand factor (VWF), factor (F) VIII (FVIII), as well as FV, FVII, FVIII, FIX, and FXI.[20,21] Increasing age may also be associated with changes in thromboelastographic (THEG) measurements[22,23] and endogenous thrombin potential (ETP) assessment.[23] In one study, the authors indicated there were no differences between adults and pediatric patients, yet the authors recommended age-adjusted RI determination for thromboelastogram (TEG) testing to allow for more "correct interpretation of the results."[24]

There are several gender-related differences in hemostasis. Higher levels of FII, FVII, FX, FIX, FXI, and FXII are found in females as compared with males.[21] There are decreased levels of PS and increased levels of antithrombin (AT) activity in females.[25] Males have longer closure times for the PFA-100 collagen-adenosine diphosphate (CADP) cartridge, a test used for screening platelet function defects.[26] Additional variables for females are changes in hemostasis parameters secondary to menstrual cycle, oral contraceptive (OC) use, or hormone replacement therapy (HRT). Changes associated with OC use depends on hormone concentration. Ethinyl estradiol increased levels of FVII antigen, FVIII activity, and β-thromboglobulin.[27] The recommendations for blood collection for female patients with combined OC and HRT, are for therapy to be discontinued for 2 months prior to testing, especially for PS and APCR testing.[28] Most studies demonstrated no menstrual cyclic variation for fibrinogen, FXI, FXIII, tissue plasminogen activator (tPA), plasminogen activator inhibitor-1 (PAI-1), D-dimer, or α-2-antiplasmin; however, some studies have demonstrated VWF, FVIII, and platelet function are at their lowest levels during menstrual and early follicular phase, thus suggesting that these time frames may be optimal for assessing female patients for bleeding risk test parameters.[29] ETP was significantly higher in luteal phase as compared with follicular phase; and FX higher during follicular phase.[30] In menstruating females suspected of von Willebrand disease (VWD), the recommendations are for blood samples to be collected on cycle day 1 to 4.[28]

Pregnant females also have different levels of coagulation factors than those not pregnant. Pregnancy decreases aPTT, PT, and TEG parameters, with increased D-dimer and soluble fibrin monomer complexes, but causes no change in AT.[31] Pregnancy is also associated with increased fibrinogen, VWF, FVII, FVIII, FIX, FX, FXII, plasminogen, PAI-1, tPA antigen, and platelet function, with decreased levels of FXIII, APCR, free PS antigen, and activity.[28,32,33] Given the number of alterations associated with pregnancy, the current recommendations for assessing coagulopathy are to wait 2 months postpartum, especially when assessing for VWD and PS deficiencies.[28] In addition to pregnancy altering hemostasis levels, labor increases the neonatal levels of FVIII, VWF, FIX, FXI, FXII, and plasminogen for vaginal deliveries as compared with neonates delivered by elective caesarean. Meconium presence in neonates has resulted in reduced plasma levels of FII, FV, FVII, and FX.[34]

There are few, but notable differences between ethnicity/race and coagulation parameters, especially genetic mutations associated with thromboembolic or bleeding risk.[35–37] The majority of these risk factors are beyond the scope of this document, but readers should be aware of their potential impact when interpreting results. Notably, relatively common occurrences are single-nucleotide polymorphism of VWF in blacks,[38] presence of FXI deficiencies in Ashkenazi Jews,[39] and increased incidence of APCR in Caucasians.[40] Sickle cell disease, most predominantly a disease present in blacks and those with Mediterranean origins, is associated with increased FVIII, VWF, and D-dimer. Combined with noted increases in prothrombin fragment (F1.2) and thrombin–antithrombin (TAT) complexes, and P-selectin (an indicator of platelet activation) with decreased ADAMTS-13 (a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13), these data are suggestive of coagulation and platelet activation.[41]

There are notable differences in coagulation parameters according to ABO blood group, especially for FVIII and VWF. In blood group O, there are lower levels of FVIII and VWF than non-O, and also FIX and FXII.[20,21,25] There was no association of Rh factor with any coagulation parameter, and no ABO effect was seen for PS, PC, or AT.[25]

Numerous health status issues will also affect the accurate measurement of hemostasis. Inflammatory conditions can lead to increased levels of fibrinogen, FVIII, VWF, and PAI-1, with decreased levels of PS activity. Mental or physiological stress can also cause changes in hemostasis. Exercise increases VWF, FVIII, and euglobulin lysis time (ELT), but not FXII, FV, FVII, FII, or fibrinogen.[28] Stress activity increased VWF, C-reactive protein, and platelet activation[42] with mental stress resulting in increases of VWF, fibrinogen, tPA, and FVIII.[28] Phobic anxiety, merely from a blood collection, was also associated with a hypercoagulable state[43] that resolved with antidepressant therapy.[44] Prolonged mental stress may lead to decreases in FV, FVIII, and FIX.[28,42] Increased thyroid hormone levels (hyperthyroidism) are associated with increased VWF, fibrinogen, FVIII, and PAI-1, but decrease collagen/epinephrine and CADP closure times for the PFA-100.[45] In a dose escalating study in health volunteers, levothyroxine treatment was associated with increased levels of VWF, FVIII, FIX, FX, PAI-1, and ELT, and concomitant decreased aPTT.[46] Primary hyperparathyroidism is associated with increased levels of FVII, FX, and D-dimer.[47] Acidosis, and to a lesser degree hypothermia, result in altered PT and aPTT testing and TEG measurements.[48]

Of considerable importance is the assessment of the biological or circadian variance noted with coagulation-related testing. Biological variation is a change noted between measurements of a given test over specified time frames in a single individual or a population. The coagulation test with the smallest biological variation is the PT, and one with the largest variance is VWF testing.[49] Seasonal variation are also noted with fibrinogen and ETP, but not PT or platelet aggregation.[50] Circadian variations have been noted with heparin therapy,[51] testing for platelet function,[26,52] and measurements of the fibrinolytic pathway.[53] Platelet function may also be affected by physiological stress (including exercise), smoking, and dietary factors including coffee, caffeine containing products, flavonoids, phytoestrogens, and polyphenols.[54–56] Ingestion of fatty acids can also affect platelet function, as well as increase levels of PAI-1 and promote activation of FVII.[57] With these noted bioanalytical and seasonal variables, multiple blood collections throughout various cycles maybe necessary to confirm abnormal findings.

Aside from congenital deficiencies that lead to bleeding or thrombotic risks, several disease states are associated with coagulopathy. Included, but not limited to, are trauma, shock, sepsis, cancer, renal failure, liver failure, systemic lupus erythematosus, antiphospholipid antibody syndrome (APS), and other autoimmune disorders, surgery, and amyloidosis. Mechanical or support interventions such as extracorporeal membrane oxygenation (also known as extracorporeal life support), hemodialysis, continuous venovenous/arteriovenous hemodialysis, blood oxygenators, or aortic balloon pumps may induce a mild consumptive coagulopathy in adults, which may be more pronounced in neonates and pediatric patients (weight associated).[58,59] These mechanical interventions require anticoagulation which may be partially performed within the circuit, systemic (unfractionated heparin [UFH]), or local to device (citrate within device and calcium gluconate out of the device). Often, in these acute and seriously ill patients, POC methods (e.g., activated clotting time or THEG) are often measurement methods of choice, but laboratory methods may also be used to confirm POC findings.[59] Other mechanical assisting devices, such as left ventricular pumps, create a turbulent flow environment, causing an immediate acquired type 2 VWD from mechanical shearing of the VWF protein.[60]

Numerous pharmaceutical interventions that are designed to either alter or replace hemostatic factors in hemostasis may or may not have an impact on coagulation testing. Common anticoagulants, such as vitamin K antagonists (VKAs), heparins, direct thrombin inhibitors (DTIs), or direct oral anticoagulants (DOACs) may have an impact on routine and/or specific coagulation assays. Some of these therapies require monitoring, where others may not. Other pharmaceutical interventions that alter the hemostasis pathway include fibrinolytic therapies (e.g., tPA), defibrinating drugs, antifibrinolytics (e.g., tranexamic acid), and antithrombotics (usually this term refers to antiplatelet therapy such as clopidigrel, 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 which may be activated), or specific to a drug (e.g., idarucizumab for dabigatran). 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 FVIIa). Replacement therapies would include specific factors (e.g., FVIII or FIX), that may be human, recombinant, porcine, or involve other forms of treatment (e.g., emicizumab-kxwh, trade name Hemlibra). The laboratory should be knowledgeable about the effect of these pharmaceutical interventions on the impact of their assays. The laboratory should also be able to provide guidance on what assays are appropriate for assessing any drug concentration (pharmacokinetics [PKs]) or effect (pharmacodynamics) as appropriate. For common anticoagulants, such as warfarin (a VKA) or UFH, historical practice uses traditional screening tests such as the PT and aPTT, respectively. However, even these decades old anticoagulant strategies must be monitored appropriately, with blood samples being collected at the correct time to avoid overexposure of the drug or inappropriate dose changes (►**Table 2**). Newer therapies (e.g., DOAC or Hemlibra), or assessing efficacy of therapies that are less well established (e.g., fibrinolytics), may require further investigation, but some provisional guidance is provided (►**Table 2**). Patients with known congenital deficiencies (e.g., hemophilia A or B, VWD) may require PK studies to determine replacement therapy and investigation of potential inhibitor effects. The guidance for PK studies should be provided by drug labeling or physician, but some provisional guidance is provided (►**Table 2**).

It must also be noted that some pharmaceutical interventions, where the primary target of the therapy is not related to coagulation, may have 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 also interfere with coagulation assays due to their binding of the test reagent phospholipid sources.[61,62] Spurious abnormal results, especially concomitant with new drug use, should be thoroughly investigated, including drug use, prior to potentially inappropriate clinical intervention. Given a rise of cases in the United States, the Centers for Disease Control alerted clinicians about the increasing use of synthetic cannabis with added rodenticides (brodifacoum), causing clinical bleeding and markedly prolonged PT and aPTT.[63] Brodifacoum is a VKA with an extremely long half-life, as compared with warfarin.[64]

Lastly, laboratories must be cognizant of test samples that may not be of human origin, or samples that 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 fibrinogen. These samples contain concentrated amounts (as compared with native plasma) of FVIII, fibrinogen, 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-FXa assessment, and so the laboratory must have a system that provides adequate lower level of detection that is suitable for this QA process. Animal blood, while having similar coagulation proteins, often have significantly different levels of factors than humans.[65] 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, which uses animal-derived antibodies that may not react with animal proteins or may react differently than anticipated. Reagent manufacturers may be able to provide guidance on whether their reagent or method is suitable for animal testing.

Specimen Collection

Once the patient selection has been defined, and the appropriate tests and collection times are noted, the next step is in collecting the blood sample. Tantamount to this process are the appropriate identifiers that assure proper patient identification and concomitant phlebotomy orders. Once that process has been verified, the actual blood collection follows. Properly trained personnel in phlebotomy practices assure proper blood collection. In a published study, the authors demonstrated that a nurse trained in acquiring blood samples for coagulation testing provided better quality specimens for coagulation than an untrained nurse. Samples from untrained nurses contained increased levels of coagulation activation factors (D-dimer, F1.2, and TAT), indicating an activated coagulation process reflective of poorly collected samples.[66]

Phlebotomy Procedural Issues

Whole Blood versus Tube Collection

Whole blood would be appropriate for POC devices such as international normalized ratio (INR) monitors, TEG, or rotational thromboelastography (ROTEM). Depending on the desired test, these POC samples may use native whole blood or require anticoagulation using evacuation tubes as noted below. Each laboratory must consult manufacturing package insert of POC reagents and devices to confirm sample types and collection methods.

Systems and Blood Collection

There are several manufacturers of blood collection tubes. It has been noted that despite the similarities between manufacturers regarding citrate concentration, there are significant differences between these tubes and potentially the arising coagulation assay results derived from blood collected into different tubes. For example, differences in PT and aPTT results using different collection tube manufacturers with the same citrate concentration have been reported.[67] Such variability has prompted recommendations that each laboratory validate these systems, and assess/amend any associated RIs prior to implementation.[68] For laboratories

Table 2 Provisional guidance for testing of pharmacological effects on coagulation

Drug	Monitoring/ measuring test	Optimal or desired time of blood collection	Notes
Unfractionated heparin	aPTT and/or anti-FXa	6 h after dose initiation or adjustment	• Circuit anticoagulation may require more frequent monitoring • Circuit anticoagulation may require higher dosing that is unable to be measured by aPTT, so ACT may be the optimal test
Low molecular weight heparin	Anti-FXa	4 h after 3rd dose	Hybrid or LMWH calibrated anti-FXa acceptable
Pentasaccharide	Anti-FXa	3 h after dose	Pentasaccharide calibrated anti-FXa reported in mg/L or mg/dL
Vitamin K antagonists	PT/INR (note baseline PT/INR should be collected prior to initiation of therapy)	First INR is with 12–24 h of first dose	• If outside target, consider VKORC mutation • If within target dose is probably okay • If lower than target, then probably need to adjust dose higher
Vitamin K (reversal therapy)	PT/INR	IV: 12 h Oral: 12–24 h	• Patient may have additional reversal agents • More frequent monitoring may be necessary if patient bleeding
Antithrombotics			
Oral	Platelet function tests	1 wk after initiation of therapy	• No consensus on method for measuring or whether necessary • Methods include PFA, Ultegra, Multiplate, and traditional platelet aggregation
Intravenous	Platelet function tests (if concomitant therapy, then ACT)	Drug dependent Within 0.5–2 h	POC or platelet function methods to include Ultegra, TEG, or ROTEM based methods. Traditional platelet aggregation studies may also be suitable
Fibrinolytics	TT, FBG, XDP, FDP	10–15 min after completion of infusion	• No consensus or recommendations for monitoring • 30 min for checking catheter function
Antifibrinolytics	PT/INR, aPTT, ACT	Max concentration at 2 h after initiation of infusion	No consensus or recommendations for monitoring
DTI	aPTT, anti-FIIa, ECA, ECT (may require ACT if high dose for PCI), dilute thrombin time	HIT treatment: 4–6 h PCI infusion: 5 and 45 min	Drug labeling indicates aPTT or ACT, but other more specific testing may be desirable, especially in cases of failing to achieve therapeutic target
DOAC			
Anti-FIIa	ECT, ECA, anti-FIIa, dTT	Trough samples (5–30 min prior to next dose)	• Collect just before next dose • If peak samples are desired, then usually 2–3 h after dose
Anti-FXa	Drug calibrated anti-FXa	Trough samples (5–30 min prior to next dose)	• Collect just before next dose • If peak samples are desired, then usually 2–3 h after dose • Specific drug calibrated anti-FXa
DOAC reversal – dabigatran	ECT, ECA, anti-FIIa	10–15 min after completion of infusion	No consensus or recommendations for monitoring
DOAC reversal – rivaroxaban/apixaban	Anti-FXa	4 h after infusion to reassess anti-FXa	• No consensus or recommendations for monitoring • The drug is continuously infused, once infusion has stopped, DOAC levels may rise • Specific DOAC calibrated anti-FXa
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 h for PK time periods	• Note that newer replacement therapies for hemophilia using modified (PEG-lated, albumin fused, etc.) factor replacement may require special methods • Single stage clotting assay or chromogenic assay may be preferred

(*Continued*)

Table 2 (*Continued*)

Drug	Monitoring/ measuring test	Optimal or desired time of blood collection	Notes
DDAVP/vasopressin	VWF, FVIII	Baseline, 30 min, 2, 4, and 6 h postdrug delivery	Drug delivery is either by nasal spray or infusion
PCC or APCC	PT/INR, aPTT	Baseline (pretreatment) and 15–30 min 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 min after infusion	No consensus or recommendations for monitoring
FFP, cryoprecipitate or other plasma-based products	PT/INR, aPTT	Baseline (preinfusion) and 30 min	FBG, FVIII, FXIII, or other factors may be assessed if replacing for that purpose

Abbreviations: 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; ECA, ecarin chromogenic assay; ECT, ecarin clotting time; FBG, fibrinogen; FDP, fibrin(ogen) degradation products; FFP, fresh frozen plasma; FIIa, activated factor II (thrombin); FVIII/FXIII, factor VIII/XIII; FXa, activated factor X; 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; VKORC, vitamin K epoxide reductase C1; VWF, von Willebrand factor; FXa, activated factor X; XDP, d-dimer.

Note: Laboratory must consult with primary caregiver or institutional guidance for 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.

that provide services outside their own region (e.g., reference laboratories), it is may be desirable to express to client the preferred blood collection tube and citrate concentration to avoid this preanalytical bias.

Blood may be collected in individual vacuum tubes or by the syringe technique. The syringe method has several limitations and should be limited to those circumstances that require its use. If the syringe technique is to be used, then a syringe of less than 25 mL (preferably 10 mL) with the correct concentration and volume of anticoagulant should be utilized. Syringe drawing procedures should preferably utilize a "butterfly" needle apparatus. The blood should be withdrawn slowly to avoid hemolysis, and coagulation and platelet activation. When multiple tubes are needed with different anticoagulants, the syringe should be filled, and the sample quickly transferred to the appropriate tube. The British Committee for Standards in Haematology recommends manually mixing of citrate tube (gently inverting, end-over-end the tube ~5–6 times),[69] although there has been some evidence to suggest that this may not be necessary with some collection systems,[70] or in patients treated with oral VKAs.[71] However, it must be noted that these two published studies used a limited number of samples and collection tubes. As such, unless locally determined otherwise through diligent studies, it may be prudent to use best practice of gently mixing whole coagulation samples collected in citrate, heparin, ethylenediaminetetraacetic acid (EDTA), or other anticoagulants, as well as pediatric or reduced volume samples.

The syringe technique may be employed during blood collection when the phlebotomist prefers to control the vacuum force in a patient with difficult veins.[72] Use of a syringe requires that the blood collected be subsequently and quickly transferred to a specimen container. This method inherently increases the phlebotomist's risk of injection needle stick exposure, as the receptacle container must be held in one hand while the syringe needle is guided into the tube with the other hand.[73] 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 if improper technique is used.[73] If no anticoagulant is added to the syringe, then the specimen is also likely to clot if not transferred immediately (usually within 60 seconds). This likelihood increases with larger sizes of syringe.[73] Despite these logical shortcomings, scarce published data exists to substantiate these claims. In fact, the earliest studies on collection systems for coagulation testing supported the use of the syringe technique over the vacuum tube.[74] One might argue, however, that in the controlled environment of a study, incorrect syringe technique or leaving syringe samples too long and thus facilitate clotting would be less likely and not reflective of "real-life" practices in a busy hospital. 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.

Needle Size

Needles are integral to the blood collection process and come in a variety of sizes (needle gauge) with increasing numbers signifying decreasing needle diameter. CLSI guidelines recommend that needle gauges for coagulation testing range from 22 to 19 gauge.[73] 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 21 to 23 may have to be used.[73,75] If the syringe technique is employed, for collections of blood over 30 mL, an 18-gauge needle is recommended to ensure adequate blood flow and reduce the chance of hemolysis and in-syringe clotting.

Line Collections

Arterial lines collections are acceptable, if a two-syringe technique is used, with the first 10 mL of line blood being cleared and the second syringe being used for blood collection for testing.[73,76] For smaller patients, if appropriate and sterilely collected the first 10 mL that would be otherwise discarded, can be replaced back to patient if the hospital has a procedure for replacement of the blood.[76] For intravenous line collection, the intravenous line is ideally turned off for 5 minutes, then the two-syringe technique used as above.[73,75] Consideration to use of POC methods is suggested for patients with difficult venous or arterial access.

Tourniquet Technique

Prolonged tourniquet times will induce increased vessel pressure, hypoxia, and lower pH below the tourniquet, thereby potentially masking mild deficiencies in VWF, FVIII, tPA, and other endothelial associated coagulation proteins.[73,77] Venipuncture technique is an important preanalytic variable in coagulation testing. This specifically concerns prolonged tourniquet application or inducing the release of procoagulant material by multiple failed attempts to draw blood (needle sticks). 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 is also noted to elevate coagulation factors such as FVIII, VWF, and tPA, thereby affecting the accuracy of the diagnosis of coagulation defects. Prolonged tourniquet application may also create an acidic microenvironment, potentially leading to factitious prolongation of clotting assays.

Tube Size and Anticoagulant

Vacuum tubes come in a variety of tube sizes to accommodate the patient size and to minimize blood loss and iatrogenic induction of anemia in hospitalized patients. The best practice guidance for tube size is to use the smallest tube to accommodate the patient, but that will provide enough plasma for all of the tests ordered, ensuring approximately 50% of the plasma is left-over for add-on tests if needed. It should be noted that some tubes by different manufacturers will only "half fill" the tube when at the appropriate level. This has posed an issue when untrained phlebotomists draw blood as they have usually been instructed to "completely fill the tube." Tubes that are overfilled or underfilled are unacceptable for testing and should be rejected (see below).[78] The accepting laboratory personnel must also be aware of the fill requirements of each tube used by their laboratory. In some hospitals, small microtubes have been created to collect very small volumes on newborn infants; however, these tubes must be validated for use by the laboratory, as they may influence RIs and also affect anticoagulant monitoring.[79]

The majority of coagulation tests is established and validated using sodium citrate as the anticoagulant. Further, it is recommended that 3.2% (0.109 mol/L) sodium citrate is the concentration of choice.[69,73,80] The 3.2% sodium citrate is considered more tolerant for clotting-based assays. In the past, 3.8% (0.129 mol/L) sodium citrate was also used but is no longer recommended for routine coagulation studies. In the 3.8% sodium citrate tube, the excess sodium citrate that is present can potentially bind more calcium ions present, for example, as added back into the clot-based assay, thereby interfering with coagulation test results. Although the 3.2% sodium citrate is the recommended concentration, several of the manufacturer's sodium citrate concentration is only approximately 3.2%, with variation based on the manufacturer's protocol. It must be emphasized that 3.2 and 3.8% citrate collection tubes are not interchangeable in a given laboratory, as both citrate concentrations may yield different test RIs and patient results.

For some coagulation and platelet testing, other anticoagulants or no anticoagulant (generating serum) can and have been used.[73] Tubes containing citrate acid dextrose and citrate theophylline, adenosine, and dipyridamol have been used for inhibiting platelet activation. Tubes containing Phe-Pro-Arg-chloromethylketone and/or aprotinin have been used to inhibit activation of the coagulation system and the fibrinolytic systems, respectively.[81] Lithium heparin, EDTA, or no anticoagulant (to generate serum) have been used for immunoassay tests (e.g., heparin-induced thrombocytopenia testing and antibody testing for APS). Specialized tubes have been created for special tests such as the fibrin(ogen) degradation products[82] (high concentration of thrombin) and tPA assay[83] (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's package insert, then the tube must be validated by the laboratory.

Order of Draw

Current CLSI guidelines recommend on an order for drawing of multiple samples that has become standard practice (►**Table 3**).[69,73] Samples drawn out of sequence for coagulation studies can potentially create interference of the coagulation test and generate a false result. As an example, in an unpublished study, sodium citrate tubes were contaminated in approximately 1 in 30 to 40 tubes if a green top tube with liquid heparin was used prior to drawing the sodium citrate tube and approximately 1 in 75 to 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 can potentially contaminate 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 sample with heparin. Since the level of heparin in heparin-anticoagulated tubes is relatively high (∼10 U), even a small amount of heparin-contaminated blood can cause

Table 3 Vacuum tube type for the proper order of drawing specimens

Order of draw	Vacuum tube type	Color of cap	Test type
1	Blood culture tubes	Color varies Culture tubes	Blood culture
2	Sodium citrate (3.2%)	Light blue top	Coagulation
3	Glass (no activator)	Red top	Chemistry, immuno-assays
4	SST	Gold top red/Black top	Chemistry Serology
5	Trace elements (no preservative)	Royal blue top	Trace elements Toxicology
6	Sodium or lithium heparin	Green top	Chemistry
7	EDTA	Lavender top pink top	Hematology Blood bank
8	Sodium fluoride	Gray top	Glucose
9	ACD	Yellow top	Blood bank HLA testing
10	QuantiFeron	TB gold	TB testing

Abbreviations: ACD, acid citrate dextrose; EDTA, ethylenediaminetetraacetic acid; HLA, human leukocyte antigen; SST, serum separator tube; TB, tuberculosis.

false prolongation of the next sample collected into sodium citrate.

If only tubes for coagulation studies are to be drawn, no discard tube is necessary.[84] This recommendation is based on results of several studies documenting that coagulation testing may be performed on the first tube without need for a discard tube.[84] This step will eliminate a large amount of biologic waste and likely result in reasonable cost savings for most laboratories.[84] However, a discard tube may be needed if a butterfly system is used, as the first tube collected will generally be underfilled because of the residual air in the collection tube system.

Fill Volume and Hematocrit Adjustment

Sodium citrate (3.2%) is the most widely used anticoagulant for coagulation (clotting-based) studies. Fill volume of sodium citrate tubes, and ratio of sodium citrate:plasma of the patient are critical preanalytical issues associated with blood collection. When the fill volume is incomplete, or the patient hematocrit is significant elevated (> 55%), the excess citrate in the plasma sample would potentially inhibit clot formation in the clotting test. As the calcium bound by citrate is replaced within the reagents of the clotting tests (e.g., PT, aPTT), excess citrate present in the test plasma (secondary to a low fill volume or high hematocrit), would bind the reagent calcium, producing artifactually prolonged clotting time values.[69,73,78,80] Current guidelines dictate that the proportion of blood to anticoagulant volume be a ratio of 9:1.[69,73] However, the literature demonstrates that some underfilling can be tolerated with tube being filled down to approximately 80% of the required volume for PT and approximately 90% for aPTT without a clinically significant effect.[69,73,78] Thus, some short sampling in tubes in patients, especially those with low hematocrits, may not be clinically significant, unless measuring for anticoagulant effect (heparin, DOAC, DTI), as the anticoagulant result may be artifactually decreased due to the dilution effect of low hematocrit and excess sodium citrate liquid volume.[85] However, the general rule of thumb is to reject citrate anticoagulant tube fills that fall below 90%, and the laboratory must follow the manufacturer's recommendations for acceptable tolerance of both low hematocrit and underfilling of tubes. Any acceptance and testing of samples from underfilled blood collection tubes, or blood collection tubes not meeting manufacturer blood collection recommendations, must be locally validated and then approved by a pathologist, or designate, as defined by the local institution or regional regulatory protocols.

Samples with high hematocrits (> 55%, like those found in neonates, severe dehydration, burn patients, polycythemia vera, high altitude temporary stay or residents) may result in factitiously high clotting times due to excess citrate in the sample relative to the residual plasma fluid in the sample; this "excess citrate" will bind the calcium that is added to the clotting assay via the testing reagent.[69,73,86] To circumvent this issue of inaccurate clotting results due to high hematocrit, the laboratory can create vacuum tubes with a reduced volume of anticoagulant, for example, by taking a very small gauge needle and removing 20% volume of the anticoagulant (without removing the vacuum).[86] These tubes can be stored in the laboratory and sent to the phlebotomist drawing the patient when the patient has a hematocrit > 55%. Before using these modified tubes, however, the laboratory must first validate that the modified tubes work. Alternative, 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 aforementioned syringe techniques.

Specimen Transportation and Stability

The transportation and processing of blood specimens for coagulation testing encompasses a critical set of preanalytic variables. These variables can and do have dramatic effects on results, which in turn can have serious consequences for patient care. Current guidelines differ in how long the sample is stable, at what temperature, and in what condition based on the named assay.[6,69] These stability criteria also change depending on whether the patient is anticoagulated or not, and with what type of anticoagulant. The current recommendations for specimens for PT assays indicate these are remarkably stable up to 24 hours independent of processing methods (centrifuged or not), storage temperature (refrigeration or room temperature), and patient groups.[69,73] The integrity of aPTT samples, unlike the PT, is dependent on both processing conditions and the presence of anticoagulant, especially UFH. The aPTT and anti-FXa monitoring of UFH, or anti-FXa measurement of DOAC, are all sensitive to time and processing methods when used to monitor anticoagulant therapy.[69,73,85] The requirement for samples suspected to contain UFH is to collect and process these within 1 hour, as samples containing UFH showed a clinically significant shortening of the aPTT in noncentrifuged samples stored at room temperature.[87] This critical shortening of aPTT appears to be due to neutralization of heparin by platelet factor 4, a high-affinity heparin-neutralizing protein secreted by stimulated platelets along with other heparin-binding proteins.[87]

Current guidelines specify that specimens for routine aPTT assays on nonheparinized patients must be tested within 4 hours of specimen collection, whether centrifuged or kept as whole blood at either 2 to 4°C or room temperature.[69,73,87] Centrifugation improves sample stability and, once centrifuged, stability is not substantially influenced by temperature.[87] It is also recommended that specimens submitted for other assays (factor assays, PC, VWF studies) be processed and stored within the 4-hour time limit.[69,73] Testing of DTIs including dabigatran must be tested within 2 hours if using the thrombin time or a direct anti-FIIa chromogenic assay.[88,89] This guidance should be followed unless superseded by the manufacturer's package insert or other manufacturer's guidance documents or as otherwise validated by laboratories for their own situation. Of note, there has been considerable investigation of stability of whole blood samples, with results that appear to show samples may be more robust than CLSI recommendations.[3] Each laboratory must investigate and document their rationale for implementing alternative sample stability limits that exceed CLSI or manufacturer recommendations.

For all testing not completed within 24 hours for PT and 4 hours for aPTT and other assays, it is recommend removing the plasma from the cells and storing it frozen in secondary plastic tubes with appropriate labeling.[69,73] Specimens may be kept at –20°C for up to 2 weeks or –70°C for up to 6 months.[69,73,87,88] Samples should be rapidly thawed at 37°C until all of the components return to solution (shortest duration possible).[87,88]

For samples being drawn by home health care personnel, coagulation samples must be maintained at the temperatures required for all coagulation samples. Therefore, home health care personnel must ensure that the samples remain within the ambient temperature (21–24°C) requirement and not sitting in a hot car during summer or in the cold during winter. For whole blood samples being transported some distance (e.g., automobiles), it has been demonstrated that tubes maintained in a vertical position (e.g., in a rack) will reduce samples agitation and preserve PT and INR result accuracy.[90]

Pneumatic tube systems can generally be used to move anticoagulated whole blood specimens from the hospital patient floors to the laboratory for coagulation tests and standard TEG or ROTEM studies, but not for platelet function analysis, including POC platelet function testing, platelet aggregation studies, or THEG platelet function testing (e.g., TEG platelet mapping).[91–93]

Citrated whole blood tubes to be used for platelet function studies ("platelet aggregation") and associated TEG, ROTEM, and Multiplate whole blood aggregometry studies should be collected as described above, but must sit at room temperature for 30 minutes to allow re-equilibration and return of normal platelet function, but no longer than 2 to 4 hours, after which platelet function deteriorates.[94,95]

Specimen Processing and Storage

Centrifugation of Specimens

All blood specimens for coagulation tests (except whole blood POC methods, platelet function studies, and TEG or ROTEM) utilize platelet-poor plasma (PPP). PPP is defined as having $< 10{,}000$ platelets/μL (10×10^9/L).[69,73,96,97] PPP is required because platelets, as the last cell component to be removed from the plasma, can interfere with coagulation tests when they rupture or activate. This is especially true if the plasma sample is frozen and subsequently thawed for testing. The centrifugation parameters to remove the platelets depends on the speed of the centrifuge, radius of the centrifuge arm (both determine the "g" force), and the time of centrifugation.[69,73] Therefore, essentially each centrifuge generates a different *g* force varying the time of centrifugation to obtain the desired PPP. Usually in large standard centrifuges, the *g* force is 10,000 *g* with a centrifugation time of 10 minutes. However, rapid centrifugation with smaller centrifuges with higher *g* forces and short centrifugation times (e.g., around 3 minutes) are available.[69,73,98] Each laboratory must determine the centrifugal force (*g* force values) and time required to obtain the desired PPP, defined as $< 10 \times 10^9$/L platelets.[69,73] Each laboratory must periodically evaluate their centrifuge (both *g* force and time) to determine that their samples are still considered platelet-poor. Most accreditation organizations require this period evaluation from annually to 12 times per year. Some guidelines state that platelet counts of up to 200×10^9/L is acceptable for immediate routine testing; however, these samples cannot be used for additional or specialized testing or cannot be frozen for later testing.[69,73] Samples to be used for specialized testing are usually frozen, in which case the original sample should be centrifuged according to the standard laboratory procedure, the plasma removed, and added to a multi-identified labeled secondary tube and the plasma recentrifuged to remove all residual platelets.[69,73] The platelet-free plasma is then removed from the secondary tube and added to a multi-identified labeled storage tube and the sample immediately frozen for storage. This "double-centrifugation" process also requires periodic checks of the final plasma sample to ensure the sample is sufficiently platelet-poor (again, $< 10 \times 10^9$/L platelets).[69,73] Episodically (at least annually and/or after centrifuge maintenance or repair) verifying and documenting the centrifuge performance for achieving PPP by testing plasma platelet count after centrifugation process is required.

Light transmission platelet aggregation requires not only PPP but also platelet-rich plasma (PRP; plasma enriched with a high platelet count).[94] For this testing, the PRP is made by a slower centrifugation of the whole blood sample to remove the red blood cells (RBCs) and the majority of white blood cells (WBCs), while retaining the smaller platelets in solution. Again the "*g*" force and time is dependent on the centrifugation instrument and the time to remove the RBCs and WBCs. The PPP is made as per the previous paragraph and can be made from the sample in which the PRP has been removed.

Sample Preparation and Pooling

Most routine testing is performed in the primary (i.e., original) tube after centrifugation. After initial testing, some accreditation organizations or hospital/laboratory policy may require keeping samples for a specified time, either for add-on tests or for identification checking. If for add-on tests, then storage of the primary tube at 4°C is recommended for up to 24 hours (however, it should be noted that results for samples containing UFH may not be accurate).

If the tube is small and will not fit onto the instrument or the sample is being prepared for specialized tests or add-on tests, then sample must be removed from the primary tube, and spun a second time, as detailed in the preceding section, to ensure the sample is platelet-free prior to freezing. Use of secondary tubes labeled with multiple identifiers and the time and date of collection is required. If more than one primary tube is obtained and plasma samples are aliquoted and frozen, then the recommendation is that each primary tube should be independently double-centrifuged and individually aliquoted into their own secondary tube (i.e., plasma samples from different tubes should not be pooled and then aliquoted). This is since if one of the tubes has an issue (clotted, hemolyzed, etc.) then the whole specimen is considered to be unusable and must be discarded.

Frozen Sample Storage and Thawing

Plasma samples that have been properly prepared and labeled should be stored in special tubes (cryovials or appropriate plastic that will not crack or leak) with a cap that will remain on even at very low temperatures.[69,73,99,100] Plasma samples should be aliquoted in volumes that allow for optimum testing of the specialized tests (e.g., either 0.5 or 1.0 mL aliquots, as appropriate for each laboratory). 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 nonfrost-free ultra-low freezer in which samples can be stored for 6 months.[99,100] If the laboratory does not have an ultra-low freezer, plasma samples can be stored at –20°C for 2 weeks for further testing and/or send out tests,[99,100] but refer to manufacturer's package insert or other test instructions to assure test stability at this temperature. Cyclic frost-free freezers should in general not be used, as this cyclic event generates repeated freeze-thaws that adversely affect enzymes and clotting factors. Thus, test results from samples stored in such freezers will not be accurate. 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 experience a change in pH which may affect the results of coagulation tests.[101]

Sample vials (with caps on) are thawed in a 37°C water bath with gentle mixing (inverting or gentle vortex, but not extensive or vigorous 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 the thawing process takes approximately 5 to 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 and falsely prolonged clotting times.[69,73,99,100]

Specimen Condition—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 have secondary wavelength (> 650 nm) can accurately assess hyperbilirubinemia samples and may improve readings for lipemic samples.[102] Mechanical removal of lipids (e.g., ultracentrifugation or solvents) have also been described,[102] but this is time consuming, not all laboratories have access to such centrifuges/solvents, and such samples should be processed and run in parallel with native samples to assure no biases associated with the lipid removal process.

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

Hemolysis may indicate a poorly collected sample, but one must first rule out in vitro hemolysis (e.g., alcohol toxicity, sepsis). To easily differentiate, 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 ascertain sample integrity and subsequent potassium and LDH results. PT is affected by moderate hemolysis using an optical device, but even markedly hemolysis had minimal effect on both the PT and aPTT using a mechanical device.[103] Hemolysis additionally will decrease fibrinogen and AT while factitiously increasing the D-dimer.[103] In a study creating artificial lysis, hemolysis factitiously increased the PT and decreased aPTT and fibrinogen, as well as generate a "dimerized plasmin D fragment" (D-dimer from clot lysis).[104]

Unless in vivo hemolysis is confirmed, one should assume the more likely scenario of in vitro hemolysis and recommend a sample recollection. Requesting a recollection of hemolyzed samples, however, may be particular challenging in neonatal or pediatric patients, or when the sample is from a timed request (e.g., UFH drug monitoring), or an outpatient, but the potential for misleading test results may lead to misdiagnoses, dose changes, and other patient mismanagement.

Lastly, hemoglobin-based oxygen carriers are stroma-free products that may be used in patients with severe, life-threatening anemia with contraindications to traditional RBC replacements (e.g., religious beliefs). When transfused in a patient, the patient's plasma will mimic hemolysis, absent elevated LDH, or potassium levels. This pseudohemolysis 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.[105,106]

Conclusions and Recommendations

As we have described, there are numerous preanalytical 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. A limitation for most published studies on these includes small sample sizes, but more importantly, limited reagent and instrument combinations. We also note the plausible causes for prolonged PT and aPTT (►**Table 4**) as well as plausible causes for decreased PT and aPTT (►**Table 5**).

Preanalytical variables may be associated with the patient themselves, specimen collection, specimen transportation, and specimen processing and storage. These can cause significant changes in values obtained from routine and specialized coagulation testing. Failure to recognize or address these preanalytical variables may create factitious test values, resulting in patient mismanagement, including misdiagnosis, improper dosing, and improper treatment. As summarized in this review, these numerous procedural errors, shortcuts, and omissions can lead to erroneous result reporting. Therefore, laboratory procedures must always use the most stringent recommendations and guidelines, including those from reagent or instrument manufacturer, to select and prepare specimens to optimize testing (►**Table 6**). Following established guidelines and laboratory procedures that specifically address preanalytical variables may require retraining of all staff that collect, process, store, and test coagulation samples. Strict adherence to these procedures will result in more accurate and reproducible results.

Table 4 Possible causes of a prolonged aPTT or PT due to preanalytical or patient-related issues

Category	
Patient selection	Anticoagulants (including vitamin K antagonists)
	Liver disease
	Bleeding disorders (acquired or inherited)
Specimen collection	Poor venipuncture
	Inadequate anticoagulant
	Collection in wrong tube
	Improper fill volume
Specimen transport	Specimen too old
	Improper temperature for transport
	Inappropriate handling of specimen
Specimen processing & storage	Centrifugation speed and time inappropriate
	Stored at warmer than recommended temperature
	Stored longer than recommended
Other	Volume expanders (e.g., crystalloids)
	Antibiotics (lipoglycopeptides)
	Autoimmune disorders
	Hydroxy-ethyl starch, hematin, sumatin, taularidine
	Systemic fibrinolytic drugs (e.g., urokinase)

Abbreviations: aPTT, activated partial thromboplastin time; PT, prothrombin time.

Table 5 Possible causes of a shortened aPTT or PT due to preanalytical or patient-related issues

Category	
Patient selection	Acquired active ongoing coagulation
	Receiving replacement therapy (e.g., FFP, cryoprecipitate, factor replacement)
	Receiving nonreplacement therapy (e.g., HemLibra, NovoSeven)
Specimen collection	Poor venipuncture
	Incomplete mixing of tube
	Inadequate anticoagulant
	Collection in wrong tube
Specimen transport	Specimen too old
	Improper temperature for transport
	Inappropriate handling of specimen
Specimen processing & storage	Centrifugation speed and time inappropriate
	Stored at warmer than recommended temperature
	Stored longer than recommended
	Too many residual platelets after freezing (aPTT)

Abbreviations: aPTT, activated partial thromboplastin time; FFP, fresh frozen plasma; PT, prothrombin time.

Table 6 Summary of preanalytical recommendations for coagulation-related testing

Section	Recommendation	References
Patient selection	Each laboratory must obtain the proper reference interval for the populations that are being assessed	Accreditation requirements
	Patients should be relaxed prior to phlebotomy to avoid physiological and psychological stress that may artifactually alter coagulation tests, especially for VWF and platelet function studies	42–44
	Optimal assessment of platelet function studies should be performed using samples collected from fasting and drug naive patients	
	For patients that are being monitored (e.g., anticoagulation and replacement therapy), adherence to the collection time is mandatory	►**Table 1**
Specimen collection	Significant differences between reported PT and aPTT results using different collection tube manufacturers with same citrate concentration, therefore each laboratory validate these systems prior to implementation	67,68
	If the syringe technique is to be used, then a syringe of less than 25 mL (preferably 10 mL) using a “butterfly” needle apparatus	
	Needle gauges for coagulation testing should range between 22 and 19 gauge, with higher gauges (23–25 G) for pediatric or difficult venous access patients	73,76
	For syringe blood collections of blood over 30 mL, an 18 G needle is recommended	
	Tourniquet time should not exceed more than 1 min	73
	For syringe collections, blood should be carefully introduced into appropriate blood collection tubes within 1 min of collection	
	For arterial lines collections, a two-syringe technique is required, with first 10 mL to clear the line and the second syringe is used for blood collection	73,76
	For intravenous (IV) line collections, the IV line is turned off for 5 min, then use two-syringe technique is required, with first 10 mL to clear the line and the second syringe is used for blood collection	73,76
	Laboratories must follow manufacturer recommendations for underfilling or overfilling of blood collection tubes. Generally, both should be rejected, unless laboratory can establish (demonstrated with supporting data) their own criteria for acceptance	
	Underfilling of blood collection tubes is the predominant cause for falsely elevated PT, INR, and aPTT results	
	Gentle inversion (mixing) of sodium citrates tube ~5–6 times is recommended. Avoid rigorous shaking or agitation	69
	3.2% sodium citrate is the citrate concentration of choice	6,73,80
	For patients with multiple tube collections, the collection sequence is required (►**Table 2**)	69,73
	If only citrate collections tubes are being collected, no discard tube is necessary (unless using butterfly syringe method directly into collection tube)	84
	Patients with elevated hematocrits (> 55%) require reduced volume of citrate prior to collection	69,73,86
Transportation and stability before processing	Coagulation samples should not be transported or stored on ice	6,69
	Coagulation samples for platelet function studies must be maintained at room temperature	94
	The PT, in whole blood, is stable for 24 h at room temperature	6,69
	Whole blood: The aPTT is stable for whole blood kept up to 4 h at room temperature, unless used for UFH monitoring, in which case the room temperature stability of whole blood is 1 h	6,69
	For other tests, unless otherwise indicate from manufacturer, the stability in whole blood is 4 h	6,69
	Pneumatic transport systems should not be used for samples that require platelet function testing	91–93
	For samples that are collected outside the confines of the hospital (e.g., home health and remote facilities), the samples should be transported in containers (e.g., insulated Styrofoam) that assure ambient room temperature (21–24°C)	
	For whole blood samples being transported distances (e.g., automobiles), the tubes should be racked and positioned upright	90
Specimen processing	With the exception of whole blood testing and platelet function studies, platelet-poor plasma (PPP) is the sample of choice	69,73

Table 6 (*Continued*)

Section	Recommendation	References
	PPP is defined as $< 10{,}000$ platelets/μL	6,69
	Internal temperature for centrifuges for processing PPP must be at room temperature (15–25°C)	
	Although the recommended centrifuge force to obtain PPP is $1{,}500 \times g$ for 10 min, each laboratory must verify their centrifugation speed (rpm) or force (g) to assure PPP[47]	98
	Coagulation samples must have double-centrifugation process prior to freezing	6
	Platelet counts from PPP processing must be assured at least annually (depending on the accreditation standard)	
	Multiple tubes collected from a single patient should not be pooled prior to storage or testing	
	All primary, secondary and tubes to be frozen must have multiple patient identifiers and date and time of collection	
Sample storage	For samples not tested within the recommended room temperature stability limits, PPP should be stored frozen as 0.5–1.0 mL aliquots in appropriately labeled polypropylene vials	
	Optimal freezing method is at –70°C, or colder, nonfrost-free freezer with PPP sample stability of 6 mo	6,69,99,100
	PPP samples can be stored at –20°C in a nonfrost-free freezer for 2 wk	99,100
	Sample vials (with caps on) are rapidly thawed in a 37°C water bath	6,69
	Thawed PPP aliquots must be mixed prior to analysis	6,69
Hemolysis, icterus, lipemia (HIL)	HIL may affect the ability of optical reading instruments to accurately assess PPP sample	102
	Suspected ex vivo (in vitro) hemolyzed PPP samples should be rejected	6,69,103,104
	Lipemic samples may be processed using ultracentrifugation methods, but a parallel sample should be process concomitantly to assure this processing method is acceptable	102
	Icterus samples may interfere with accurate assessment of chromogenic methods	
	Infusion of HBOC products will create a pseudohemolysis appearance of the plasma and may interfere with clot and chromogenic-based assays	105,106

Abbreviations: aPTT, activated partial thromboplastin time; HBOC, hemoglobin-based oxygen carrier; INR, international normalized ratio; PT, prothrombin time; UFH, unfractionated heparin; VWF, von Willebrand factor.

Conflict of Interest

Mr. Gosselin reports personal fees from Siemens Healthcare Diagnostics, during the conduct of the study; personal fees from Grifols Diagnostic Solutions, Machaon Laboratory, Ferrer & Poiret, Garrett & Co., NovoNordisk, outside the submitted work; and Board member of International Council for Standardization in Haematology (ICSH), former board member (2018) of North American Specialized Coagulation Laboratory Association (NASCOLA), former board member (2018) of International Society for Laboratory Hematology (ISLH), former associate editor for J Laboratory Hematol (former as of February 28, 2018).

References

1 Plebani M, Favaloro EJ, Lippi G. Patient safety and quality in laboratory and hemostasis testing: a renewed loop? Semin Thromb Hemost 2012;38(06):553–558

2 Preston FE, Lippi G, Favaloro EJ, Jayandharan GR, Edison ES, Srivastava A. Quality issues in laboratory haemostasis. Haemophilia 2010;16(Suppl 5):93–99

3 Adcock Funk DM, Lippi G, Favaloro EJ. Quality standards for sample processing, transportation, and storage in hemostasis testing. Semin Thromb Hemost 2012;38(06):576–585

4 Favaloro EJ, Lippi G, Adcock DM. Preanalytical and postanalytical variables: the leading causes of diagnostic error in hemostasis? Semin Thromb Hemost 2008;34(07):612–634

5 Bonar R, Favaloro EJ, Adcock DM. Quality in coagulation and haemostasis testing. Biochem Med (Zagreb) 2010;20:184–199

6 Clinical and Laboratory Standards Institute (CLSI). Available at: https://clsi.org/. Accessed March 09, 2019

7 International Organization for Standardization (ISO) document ISO 15189: 2012. Medical laboratories— particular requirements for quality and competence. International Organization for Standardization, Geneva, Switzerland

8 United States Food and Drug Administration (FDA). Bioanalytical method validation guidance for industry. May 2018. Available at: https://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM070107.pdf. Accessed March 2019

9 Validation of analytical procedures: Text and methodology Q2(R1). ICH harmonised tripartite guideline. Available at: https://www.ich.org/products/guidelines/quality/article/quality-guidelines.html. Accessed March 2019

10 FDA Guidance for Industry Q2B Validation of Analytical Procedures: Methodology. 1996. Available at: https://www.fda.gov/downloads/drugs/guidances/ucm073384.pdf. Accessed March 2019

11 Clinical laboratory improvement amendments of 1988: Final Rule. (42 CFR Part 405) Fed Reg 57:7001-7186. February 1992

12 Thompson, Michael & Ellison, Stephen & Wood, Roger. (2002). Harmonized guidelines for single-laboratory validation of methods of analysis (IUPAC Technical Report). Pure and Applied Chemistry - PURE APPL CHEM. 74. 835-855. DOI: 10.1351/pac200274050835 [epub ahead of print]

13 Magnusson B, Örnemark U, eds. Eurachem Guide: The Fitness for Purpose of Analytical Methods – A Laboratory Guide to Method

Validation and Related Topics. 2nd ed. 2014. ISBN 978-91-87461-59-0. Available at: www.eurachem.org. Accessed May 27, 2019

14 Guideline on bioanalytical method validation EMEA/CHMP/EWP/192217/2009 Rev. 1 Corr. 2**. Available at: https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-bioanalytical-method-validation_en.pdf. Accessed March 2019

15 Favaloro EJ, Gosselin R, Olson J, Jennings I, Lippi G. Recent initiatives in harmonization of hemostasis practice. Clin Chem Lab Med 2018;56(10):1608–1619

16 Toulon P. Developmental hemostasis: laboratory and clinical implications. Int J Lab Hematol 2016;38(Suppl 1):66–77

17 Nowak-Göttl U, Limperger V, Kenet G, et al. Developmental hemostasis: a lifespan from neonates and pregnancy to the young and elderly adult in a European white population. Blood Cells Mol Dis 2017;67:2–13

18 Righini M, Nendaz M, Le Gal G, Bounameaux H, Perrier A. Influence of age on the cost-effectiveness of diagnostic strategies for suspected pulmonary embolism. J Thromb Haemost 2007;5(09):1869–1877

19 Giansante C, Fiotti N, Cattin L, Da Col PG, Calabrese S. Fibrinogen, D-dimer and thrombin-antithrombin complexes in a random population sample: relationships with other cardiovascular risk factors. Thromb Haemost 1994;71(05):581–586

20 Favaloro EJ, Soltani S, McDonald J, Grezchnik E, Easton L, Favaloro JW. Reassessment of ABO blood group, sex, and age on laboratory parameters used to diagnose von Willebrand disorder: potential influence on the diagnosis vs the potential association with risk of thrombosis. Am J Clin Pathol 2005;124(06):910–917 Erratum in: Am J Clin Pathol. 2006;125(5):796

21 Favaloro EJ, Soltani S, McDonald J, Grezchnik E, Easton L. Cross-laboratory audit of normal reference ranges and assessment of ABO blood group, gender and age on detected levels of plasma coagulation factors. Blood Coagul Fibrinolysis 2005;16(08):597–605

22 Ng KF. Changes in thrombelastograph variables associated with aging. Anesth Analg 2004;99(02):449–454

23 Schneider T, Siegemund T, Siegemund R, Petros S. Thrombin generation and rotational thromboelastometry in the healthy adult population. Hamostaseologie 2015;35(02):181–186

24 Chan KL, Summerhayes RG, Ignjatovic V, Horton SB, Monagle PT. Reference values for kaolin-activated thromboelastography in healthy children. Anesth Analg 2007;105(06):1610–1613

25 Favaloro EJ, Soltani S, McDonald J, Grezchnik E, Easton L. Laboratory identification of familial thrombophilia: do the pitfalls exceed the benefits? A reassessment of ABO-blood group, gender, age, and other laboratory parameters on the potential influence on a diagnosis of protein C, protein S, and antithrombin deficiency and the potential high risk of a false positive diagnosis. Lab Hematol 2005;11(03):174–184

26 Haubelt H, Anders C, Vogt A, Hoerdt P, Seyfert UT, Hellstern P. Variables influencing Platelet Function Analyzer-100 closure times in healthy individuals. Br J Haematol 2005;130(05):759–767

27 Lindberg UB, Crona N, Stigendal L, Teger-Nilsson AC, Silfverstolpe G. A comparison between effects of estradiol valerate and low dose ethinyl estradiol on haemostasis parameters. Thromb Haemost 1989;61(01):65–69

28 Blombäck M, Konkle BA, Manco-Johnson MJ, Bremme K, Hellgren M, Kaaja R; ISTH SSC Subcommittee on Women's Health Issues. Preanalytical conditions that affect coagulation testing, including hormonal status and therapy. J Thromb Haemost 2007;5(04):855–858

29 Knol HM, Kemperman RF, Kluin-Nelemans HC, Mulder AB, Meijer K. Haemostatic variables during normal menstrual cycle. A systematic review. Thromb Haemost 2012;107(01):22–29

30 Chaireti R, Gustafsson KM, Byström B, Bremme K, Lindahl TL. Endogenous thrombin potential is higher during the luteal phase than during the follicular phase of a normal menstrual cycle. Hum Reprod 2013;28(07):1846–1852

31 Karlsson O, Sporrong T, Hillarp A, Jeppsson A, Hellgren M. Prospective longitudinal study of thromboelastography and standard hemostatic laboratory tests in healthy women during normal pregnancy. Anesth Analg 2012;115(04):890–898

32 Ataullakhanov FI, Koltsova EM, Balandina AN, Serebriyskiy II, Vuimo TA, Panteleev MA. Classic and global hemostasis testing in pregnancy and during pregnancy complications. Semin Thromb Hemost 2016;42(07):696–716

33 Fredrik Blomqvist LR, Strandell AM, Baghaei F, Elisabet Hellgren MS. Platelet aggregation in healthy women during normal pregnancy - a longitudinal study. Platelets 2018;16:1–7

34 Kulkarni AA, Osmond M, Bapir M, et al. The effect of labour on the coagulation system in the term neonate. Haemophilia 2013;19(04):533–538

35 Tang L, Hu Y. Ethnic diversity in the genetics of venous thromboembolism. Thromb Haemost 2015;114(05):901–909

36 Bryant A, Mhyre JM, Leffert LR, Hoban RA, Yakoob MY, Bateman BT. The association of maternal race and ethnicity and the risk of postpartum hemorrhage. Anesth Analg 2012;115(05):1127–1136

37 Ho P, Ng C, Rigano J, et al. Significant age, race and gender differences in global coagulation assays parameters in the normal population. Thromb Res 2017;154:80–83

38 Flood VH, Gill JC, Morateck PA, et al. Common VWF exon 28 polymorphisms in African Americans affecting the VWF activity assay by ristocetin cofactor. Blood 2010;116(02):280–286

39 Duga S, Salomon O. Congenital factor XI deficiency: an update. Semin Thromb Hemost 2013;39(06):621–631

40 van Mens TE, Levi M, Middeldorp S. Evolution of factor V Leiden. Thromb Haemost 2013;110(01):23–30

41 Colombatti R, De Bon E, Bertomoro A, et al. Coagulation activation in children with sickle cell disease is associated with cerebral small vessel vasculopathy. PLoS One 2013;8(10):e78801

42 Hamer M, Gibson EL, Vuononvirta R, Williams E, Steptoe A. Inflammatory and hemostatic responses to repeated mental stress: individual stability and habituation over time. Brain Behav Immun 2006;20(05):456–459

43 Geiser F, Meier C, Wegener I, et al. Association between anxiety and factors of coagulation and fibrinolysis. Psychother Psychosom 2008;77(06):377–383

44 Geiser F, Conrad R, Imbierowicz K, et al. Coagulation activation and fibrinolysis impairment are reduced in patients with anxiety and depression when medicated with serotonergic antidepressants. Psychiatry Clin Neurosci 2011;65(05):518–525

45 Horacek J, Maly J, Svilias I, et al. Prothrombotic changes due to an increase in thyroid hormone levels. Eur J Endocrinol 2015;172(05):537–542

46 Van Zaane B, Squizzato A, Debeij J, et al. Alterations in coagulation and fibrinolysis after levothyroxine exposure in healthy volunteers: a controlled randomized crossover study. J Thromb Haemost 2011;9(09):1816–1824

47 Erem C, Kocak M, Hacihasanoglu A, Yilmaz M, Saglam F, Ersoz HO. Blood coagulation, fibrinolysis and lipid profile in patients with primary hyperparathyroidism: increased plasma factor VII and X activities and D-Dimer levels. Exp Clin Endocrinol Diabetes 2008;116(10):619–624

48 Ramaker AJ, Meyer P, van der Meer J, et al. Effects of acidosis, alkalosis, hyperthermia and hypothermia on haemostasis: results of point-of-care testing with the thromboelastography analyser. Blood Coagul Fibrinolysis 2009;20(06):436–439

49 de Maat MP, van Schie M, Kluft C, Leebeek FW, Meijer P. Biological variation of hemostasis variables in thrombosis and bleeding: consequences for performance specifications. Clin Chem 2016;62(12):1639–1646

50 Rudez G, Meijer P, Spronk HM, et al. Biological variation in inflammatory and hemostatic markers. J Thromb Haemost 2009;7(08):1247–1255

51 Vandiver JW, Vondracek TG. Antifactor Xa levels versus activated partial thromboplastin time for monitoring unfractionated heparin. Pharmacotherapy 2012;32(06):546–558

52 Hartley PS. The diurnal tick-tockery of platelet biology. Platelets 2012;23(02):157–160

53 Talens S, Malfliet JJ, Rudež G, et al. Biological variation in tPA-induced plasma clot lysis time. Thromb Haemost 2012;108(04): 640–646

54 Lordkipanidzé M. Platelet function tests. Semin Thromb Hemost 2016;42(03):258–267

55 Paglieroni TG, Janatpour K, Gosselin R, et al. Platelet function abnormalities in qualified whole-blood donors: effects of medication and recent food intake. Vox Sang 2004;86(01):48–53

56 Pearson DA, Paglieroni TG, Rein D, et al. The effects of flavanol-rich cocoa and aspirin on ex vivo platelet function. Thromb Res 2002;106(4-5):191–197

57 Miller GJ. Dietary fatty acids and the haemostatic system. Atherosclerosis 2005;179(02):213–227

58 Sy E, Sklar MC, Lequier L, Fan E, Kanji HD. Anticoagulation practices and the prevalence of major bleeding, thromboembolic events, and mortality in venoarterial extracorporeal membrane oxygenation: a systematic review and meta-analysis. J Crit Care 2017;39:87–96

59 Winkler AM. Managing the precarious hemostatic balance during extracorporeal life support: implications for coagulation laboratories. Semin Thromb Hemost 2017;43(03):291–299

60 Bartoli CR, Kang J, Zhang D, et al. Left ventricular assist device design reduces von Willebrand factor degradation: a comparative study between the HeartMate II and the EVAHEART left ventricular assist system. Ann Thorac Surg 2017;103(04):1239–1244

61 Gosselin R, Dager W, Roberts A, et al. Effect of telavancin (Vibativ) on routine coagulation test results. Am J Clin Pathol 2011;136 (06):848–854

62 Webster PS, Oleson FB Jr, Paterson DL, et al. Interaction of daptomycin with two recombinant thromboplastin reagents leads to falsely prolonged patient prothrombin time/International Normalized Ratio results. Blood Coagul Fibrinolysis 2008; 19(01):32–38

63 Centers for Disease Control and Prevention. Clinical action bulletin. April 2018. Available at: https://content.govdelivery.com/accounts/USCDC/bulletins/1eb9503. Accessed March 2019

64 King N, Tran MH. Long-acting anticoagulant rodenticide (Superwarfarin) poisoning: a review of its historical development, epidemiology, and clinical management. Transfus Med Rev 2015;29(04):250–258

65 Karges HE, Funk KA, Ronneberger H. Activity of coagulation and fibrinolysis parameters in animals. Arzneimittelforschung 1994; 44(06):793–797

66 Stegnar M, Cuderman TV, Bozic M. Evaluation of pre-analytical, demographic, behavioural and metabolic variables on fibrinolysis and haemostasis activation markers utilised to assess hypercoagulability. Clin Chem Lab Med 2007;45(01):40–46

67 Lima-Oliveira G, Lippi G, Salvagno GL, Montagnana M, Picheth G, Guidi GC. Sodium citrate vacuum tubes validation: preventing preanalytical variability in routine coagulation testing. Blood Coagul Fibrinolysis 2013;24(03):252–255

68 Bowen RA, Adcock DM. Blood collection tubes as medical devices: the potential to affect assays and proposed verification and validation processes for the clinical laboratory. Clin Biochem 2016;49(18):1321–1330

69 Mackie I, Cooper P, Lawrie A, Kitchen S, Gray E, Laffan M; British Committee for Standards in Haematology. Guidelines on the laboratory aspects of assays used in haemostasis and thrombosis. Int J Lab Hematol 2013;35(01):1–13

70 Lima-Oliveira G, Lippi G, Salvagno GL, et al. Processing of diagnostic blood specimens: is it really necessary to mix primary blood tubes after collection with evacuated tube system? Biopreserv Biobank 2014;12(01):53–59

71 Chuang J, Sadler MA, Witt DM. Impact of evacuated collection tube fill volume and mixing on routine coagulation testing using 2.5-ml (pediatric) tubes. Chest 2004;126(04):1262–1266

72 Giavarina D, Lippi G. Blood venous sample collection: recommendations overview and a checklist to improve quality. Clin Biochem 2017;50(10-11):568–573

73 CLSI. Collection of Diagnostic Venous Blood Specimens. 7th ed. Standard GP41. Wayne, PA: Clinical and Laboratory Standards Institute; 2017

74 Ong ME, Chan YH, Lim CS. Observational study to determine factors associated with blood sample haemolysis in the emergency department. Ann Acad Med Singapore 2008;37(09):745–748

75 Uman LS, Birnie KA, Noel M, et al. Psychological interventions for needle-related procedural pain and distress in children and adolescents. Cochrane Database Syst Rev 2013;(10):CD005179

76 World Health Organization. WHO guidelines on drawing blood: best practices in phlebotomy. Available at: https://www.ncbi.nlm.nih.gov/books/NBK138650/pdf/Bookshelf_NBK138650.pdf. Accessed March 2019

77 Kang HJ, Han CD, Jahng JS, Ko SO. Blood gas and electrolyte changes after tourniquet application in total knee replacement surgery. Yonsei Med J 1992;33(02):153–158

78 Adcock DM, Kressin DC, Marlar RA. Minimum specimen volume requirements for routine coagulation testing: dependence on citrate concentration. Am J Clin Pathol 1998;109(05):595–599

79 Phelan MP, Reineks EZ, Berriochoa JP, et al. Impact of use of smaller volume, smaller vacuum blood collection tubes on hemolysis in emergency department blood samples. Am J Clin Pathol 2017;148(04):330–335

80 Adcock DM, Kressin DC, Marlar RA. Effect of 3.2% vs 3.8% sodium citrate concentration on routine coagulation testing. Am J Clin Pathol 1997;107(01):105–110

81 Bovill EG, Terrin ML, Stump DC, et al. Hemorrhagic events during therapy with recombinant tissue-type plasminogen activator, heparin, and aspirin for acute myocardial infarction. Results of the Thrombolysis in Myocardial Infarction (TIMI), Phase II Trial. Ann Intern Med 1991;115(04):256–265

82 Kadani M, B N V S S, B M, , et al. Evaluation of plasma fibrinogen degradation products and total serum protein concentration in oral submucous fibrosis. J Clin Diagn Res 2014;8(05): ZC54–ZC57

83 Chapman SN, Mehndiratta P, Johansen MC, McMurry TL, Johnston KC, Southerland AM. Current perspectives on the use of intravenous recombinant tissue plasminogen activator (tPA) for treatment of acute ischemic stroke. Vasc Health Risk Manag 2014;10:75–87

84 Raijmakers MT, Menting CH, Vader HL, van der Graaf F. Collection of blood specimens by venipuncture for plasma-based coagulation assays: necessity of a discard tube. Am J Clin Pathol 2010; 133(02):331–335

85 Douxfils J, Ageno W, Samama CM, et al. Laboratory testing in patients treated with direct oral anticoagulants: a practical guide for clinicians. J Thromb Haemost 2018;16(02):209–219

86 Marlar RA, Potts RM, Marlar AA. Effect on routine and special coagulation testing values of citrate anticoagulant adjustment in patients with high hematocrit values. Am J Clin Pathol 2006;126 (03):400–405

87 Salvagno GL, Lippi G, Montagnana M, Franchini M, Poli G, Guidi GC. Influence of temperature and time before centrifugation of specimens for routine coagulation testing. Int J Lab Hematol 2009;31(04):462–467

88 Zürcher M, Sulzer I, Barizzi G, Lämmle B, Alberio L. Stability of coagulation assays performed in plasma from citrated whole blood transported at ambient temperature. Thromb Haemost 2008;99(02):416–426

89 Gosselin RC, Adcock DM, Bates SM, et al. International Council for Standardization in Haematology (ICSH) recommendations for laboratory measurement of direct oral anticoagulants. Thromb Haemost 2018;118(03):437–450

90 van Geest-Daalderop JH, Mulder AB, Boonman-de Winter LJ, Hoekstra MM, van den Besselaar AM. Preanalytical variables and off-site blood collection: influences on the results of the prothrombin time/international normalized ratio test and implications for monitoring of oral anticoagulant therapy. Clin Chem 2005;51(03):561–568

91 Glas M, Mauer D, Kassas H, Volk T, Kreuer S. Sample transport by pneumatic tube system alters results of multiple electrode aggregometry but not rotational thromboelastometry. Platelets 2013;24(06):454–461

92 Hübner U, Böckel-Frohnhöfer N, Hummel B, Geisel J. The effect of a pneumatic tube transport system on platelet aggregation using optical aggregometry and the PFA-100. Clin Lab 2010; 56(1-2):59–64

93 Wallin O, Söderberg J, Grankvist K, Jonsson PA, Hultdin J. Preanalytical effects of pneumatic tube transport on routine haematology, coagulation parameters, platelet function and global coagulation. Clin Chem Lab Med 2008;46(10):1443–1449

94 CLSI. Platelet Function Testing by Aggregometry. 1st ed. H58A. Wayne, PA: Clinical and Laboratory Standards Institute; 2008

95 Chapman K, Favaloro EJ. Time dependent reduction in platelet aggregation using the multiplate analyser and hirudin blood due to platelet clumping. Platelets 2018;29(03):305–308

96 Lippi G, Rossi R, Ippolito L, et al. Influence of residual platelet count on routine coagulation, factor VIII, and factor IX testing in postfreeze-thaw samples. Semin Thromb Hemost 2013;39(07): 834–839

97 Magnette A, Chatelain M, Chatelain B, Ten Cate H, Mullier F. Preanalytical issues in the haemostasis laboratory: guidance for the clinical laboratories. Thromb J 2016;14:49–53

98 Nelson S, Pritt A, Marlar RA. Rapid preparation of plasma for 'Stat' coagulation testing. Arch Pathol Lab Med 1994;118(02):175–176

99 Foshat M, Bates S, Russo W, et al. Effect of freezing plasma at -20° C for 2 weeks on prothrombin time, activated partial thromboplastin time, dilute Russell viper venom time, activated protein C resistance, and D-dimer levels. Clin Appl Thromb Hemost 2015; 21(01):41–47

100 Gosselin RC, Dwyre DW. Determining the effect of freezing on coagulation testing: comparison of results between fresh and once frozen-thawed plasma. Blood Coagul Fibrinolysis 2015;26(01): 69–74

101 Odsæter IH, Lian IA, Bratberg K, Mikkelsen G. Dry ice exposure of plasma samples influences pH and lupus anticoagulant analysis. Clin Chem Lab Med 2015;53(05):809–813

102 Lippi G, Plebani M, Favaloro EJ. Interference in coagulation testing: focus on spurious hemolysis, icterus, and lipemia. Semin Thromb Hemost 2013;39(03):258–266

103 D'Angelo G, Villa C, Tamborini A, Villa S. Evaluation of the main coagulation tests in the presence of hemolysis in healthy subjects and patients on oral anticoagulant therapy. Int J Lab Hematol 2015;37(06):819–833

104 Lippi G, Montagnana M, Salvagno GL, Guidi GC. Interference of blood cell lysis on routine coagulation testing. Arch Pathol Lab Med 2006;130(02):181–184

105 Jahr JS, Lurie F, Gosselin R, Lin JS, Wong L, Larkin E. Effects of a hemoglobin-based oxygen carrier (HBOC-201) on coagulation testing. Clin Lab Sci 2000;13(04):210–214

106 Jahr JS, Liu H, Albert OK, et al. Does HBOC-201 (Hemopure) affect platelet function in orthopedic surgery: a single-site analysis from a multicenter study. Am J Ther 2010;17(02):140–147