

PRE-ANALYTICAL VARIABLES AND THEIR IMPACT ON COAGULATION RESULTS

A CAST STUDY APPROACH

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Objectives

**1. IDENTIFY RELEVANT
PRE-ANALYTICAL
VARIABLES THAT CAN
IMPACT COAGULATION
RESULTS.**

**2. PROVIDE SOLUTIONS
TO MINIMIZE PRE-
ANALYTICAL ERRORS**

**3. ENHANCE PROBLEM
SOLVING SKILLS**

What do you want from your coagulation assays?

Precise results



Robust assays



Accurate results that can be used as an aid in diagnosis and treatment



Reproducible results

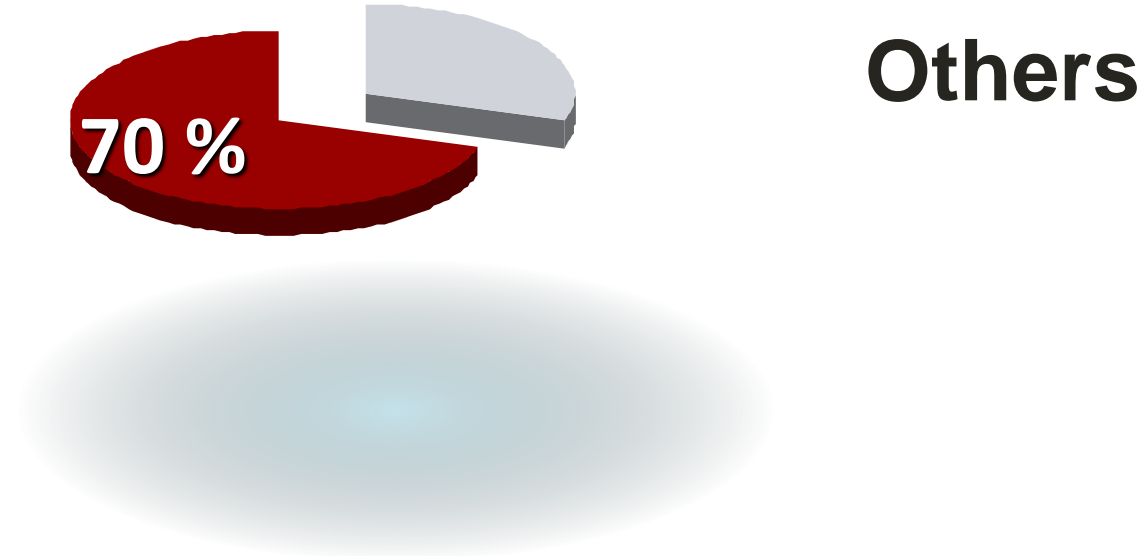
- **Laboratory medicine is typically divided into three main phases pre-analytical, analytical and post-analytical.**
- **Advancements in automation and computer applications, have raised the awareness that analytical errors are no longer the main factor influencing the quality of laboratory testing.**
- **Many preanalytical errors are a consequence of inappropriate or problematic blood sample collection, handling, storage, transport or processing.**
- **In 2013, a study estimated the average cost of a preanalytical error to be around \$200 in both European and North American institutions, representing an annual cost of \$1.2 million for a 650 bed hospital in the USA.**
- **Increased costs based on various factors, including patient management, redraws, lab investigations, blood collection consumables, and instrument downtime.**

ISSUE

Clin Biochem. 2013;46:1175-1179

Total errors in testing process

Preanalytical issues

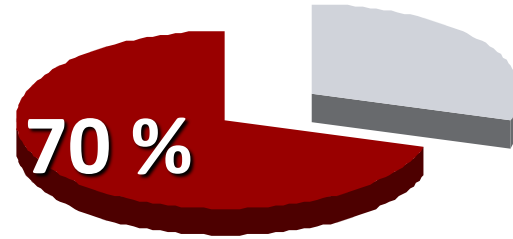


Source: Lippi et al (2008), Specimencare.com

Total errors in testing process

Preanalytical issues

- Procedures for collection
- Haemolysis
- Clotting
- Contamination
- Insufficient volume
- Inappropriate containers
- Misidentification



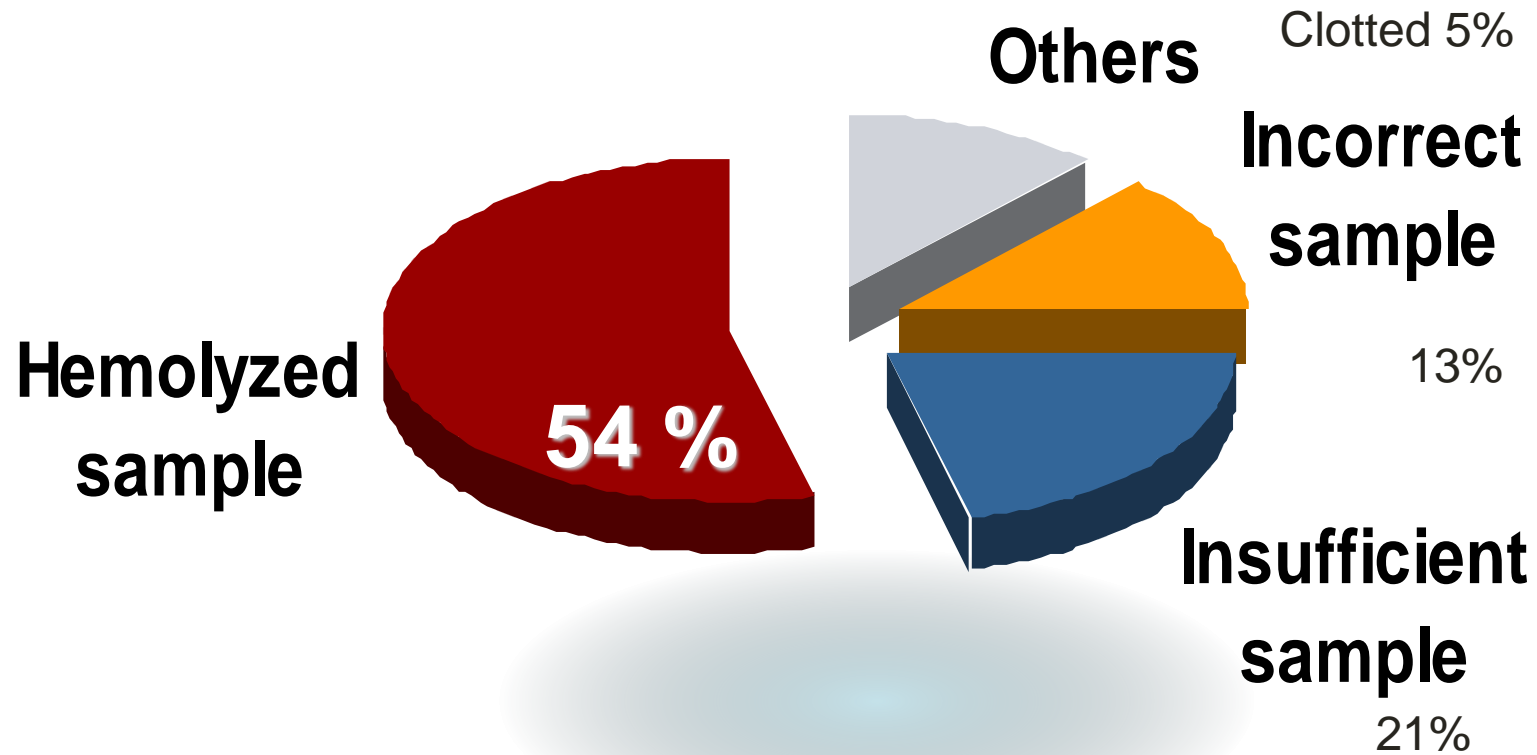
Others

- Analytical 7-13%
- Post-analytical 18-47%

Source: Lippi et al (2008), Specimencare.com

Preanalytical Errors in the Coagulation Laboratory

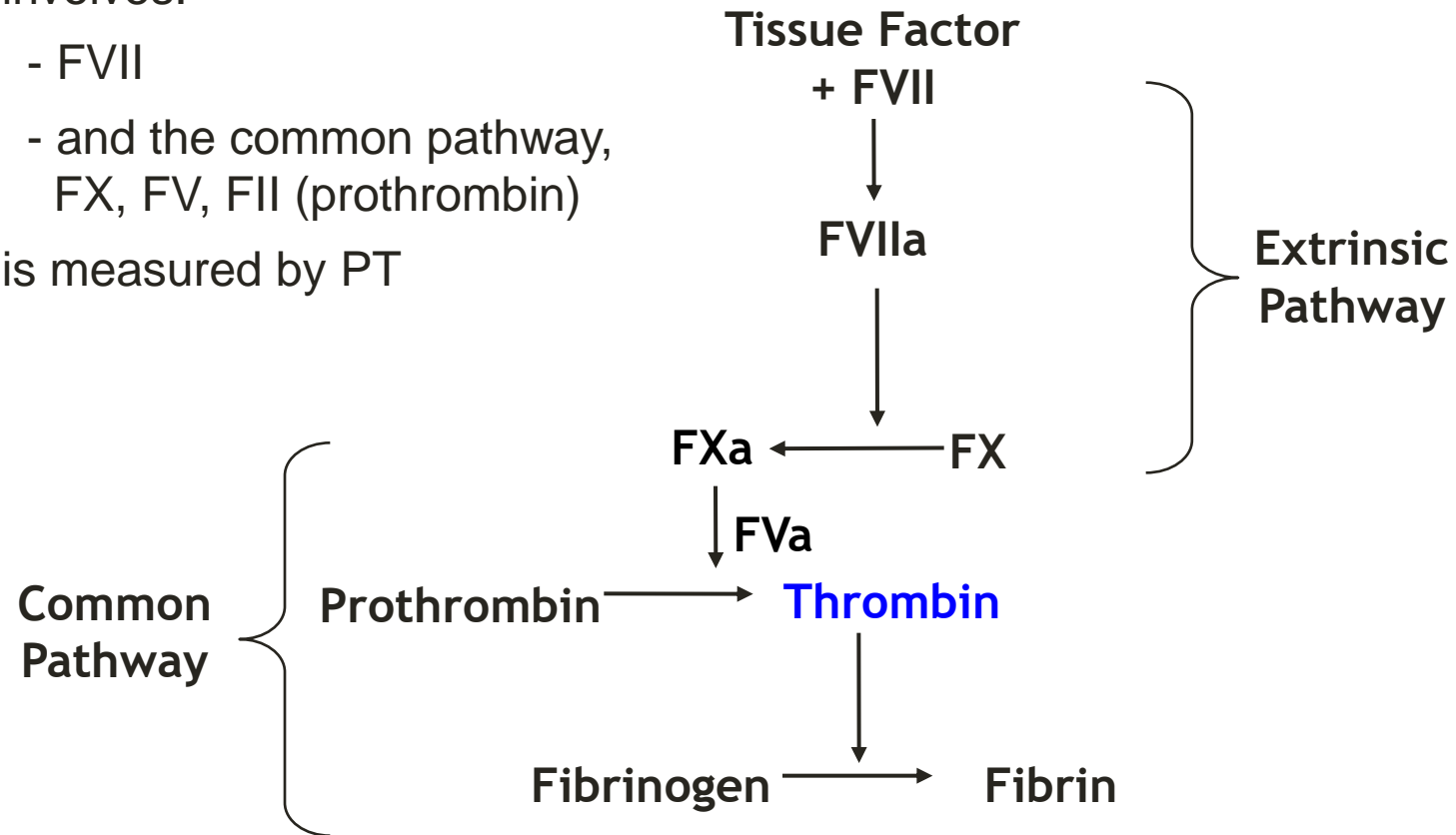
Source: Lippi et al (2006), Lippi et al (2008)



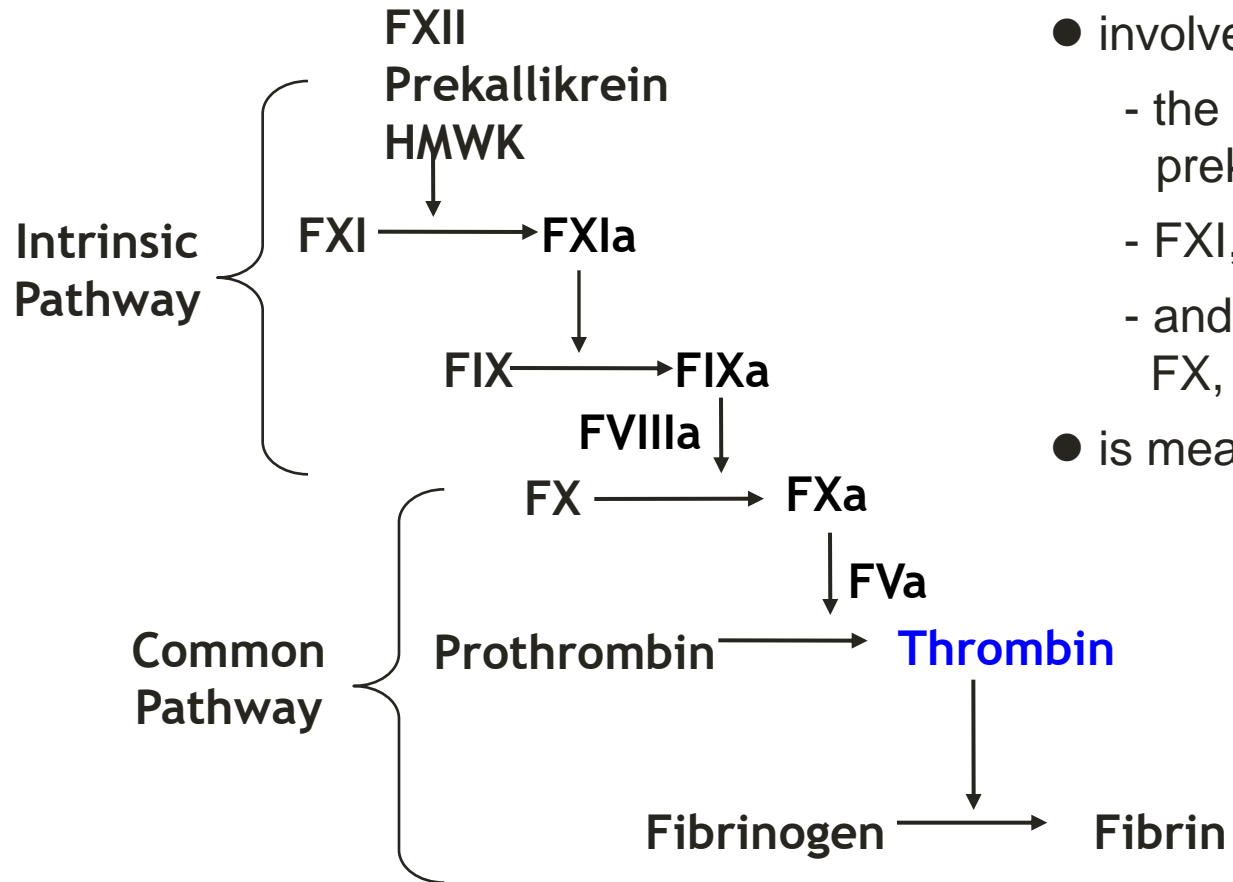
Errors are directly related to specimen collection in most cases.

Extrinsic pathway

- involves:
 - FVII
 - and the common pathway, FX, FV, FII (prothrombin)
- is measured by PT



Intrinsic pathway



- involves:
 - the contact factors FXII, prekallikrein, and HMWK
 - FXI, FIX and FVIII
 - and the common pathway, FX, FV, FII (prothrombin)
- is measured by APTT

Coagulation pathways

- Extrinsic pathway (measured by PT)

- Intrinsic pathway (measured by APTT)

Injury to blood vessel

↓
**Release of
Thromboplastin**

↓
Activation of FVII

**Contact activation of
PK, HMWK, FXII**

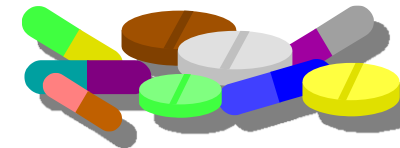
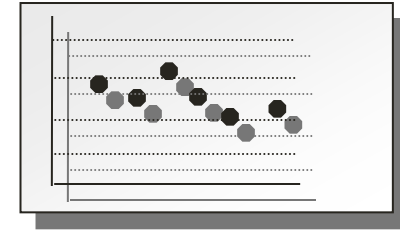
↓
**Activation of
FXI, FIX, FVIII**

↘ ↙
**Activation of FX,
FV, Prothrombin**

↓
Fibrin formation

Coagulation testing - Why?

- **Diagnosis and follow-up of coagulation disorders**
- **Monitoring and adjustment of therapy**
 - anticoagulant therapy
 - substitution therapy
- **Risk assessment**
 - bleeding risk assessment
 - thrombotic risk assessment





Case Study

**Impact of Preanalytical
Variables on
Coagulation Results**

Case Study

- A 42-year-old male presented with the following symptoms:

fatigue

difficulty breathing

tingling in the hands and feet

- Coagulation results:

PT= 25.6 seconds (11.5-13.9 sec)

aPTT=77.5 seconds (25.0-35.7 sec)

Clinician questioned the results, patient had no bleeding symptoms

Prior to additional testing:

- Reviewed additional results:
- HB= **23.5** gm/dL
- HCT= **76.2%**

- High HCT = hemoconcentrated -
- CLSI guideline and the ICSH require a 9:1 ratio of blood to anticoagulant
- This sample has too much anticoagulant in the sample due to the increased hematocrit. The result is falsely prolonged.
- Recommendation is to adjust HCT if >55%

SOLUTION:

- C=Volume of Na Citrate mm
- V=Volume of whole blood mm
- H=HCT %
- To collect 2.7 mls of blood from a patient with a HCT of 76%
- $C = (1.85 \times 10)^{-3} \times (100 - 76) \times 2.7$
- $0.00185 \times 24 \times 2.7 = 0.12 \text{ mls citrate}$

New updated CLSI H-21 A6 contains a pre-populated chart in which you adjust a standard citrated tube- Instead of making up a new tube-

Repeat sample

- **RESULTS: Collected in a new adjusted citrate volume tube:**

PT= 13.7 sec

aPTT= 35.1 sec

Both results are within normal limits

High hemoglobin and hematocrit

Additional Tests:

- **Thrombocytosis, platelet count =481,000/ μ L**
- **Leukocytosis =13,200/ μ L**
- **Increased leukocyte alkaline phosphatase (LAP) = 207**
- **Serum vitamin B-12 concentration 988 pg/mL(200-500pg/mL)**

Diagnosis:

Polycythemia Vera: Occurs more frequently in men than in women over 40

PV is usually associated with a JAK2 gene mutation

Scott LM, Tong W, Levine RL, et al. JAK2 exon 12 mutations in polycythemia vera and idiopathic erythrocytosis. N Engl J Med. 2007;356:459-468

Case Study: Sample Screened in the main laboratory for Lupus Workup

Sample run in main lab:

PT = 11.1 sec (nr= 10.5- 12.1)

APTT= **45** sec (nr= 25.2-36.5 sec)

Fibrinogen = 254 mg/dL (150-400 mg/dL)

Thrombin Time =20 seconds (18-22 sec)

Laboratory uses a Lupus Sensitive reagent.

What does that mean?

Lupus Sensitive Reagent

- aPTT reagents are phospholipid (ppl) based.
- In the laboratory, a lupus anticoagulant (LA) manifests as an antibody against phospholipid. A reagent that is sensitive to LA has a low concentration of ppl to allow the LA to prolong the aPTT. An insensitive reagent will have a high concentration of ppl which will mask the antibody and result in a normal aPTT.
- This result was prolonged using an LA sensitive reagent, so it was frozen at -80 degree C for the Special Coagulation Laboratory for a Lupus workup.
- Sample thawed the following day for 5 minutes at 37 degrees C.
- aPTT re-run in Special Coagulation the following day prior to running the Lupus workup.
- aPTT=33 sec (nr= 25.2-36.5 sec)

What happened? The result is normal Can we use this sample for Lupus testing?

Are your samples platelet poor for Special Coagulation testing?

A platelet-poor plasma (PPP) sample (platelet count $<10 \times 10^9/L$ or 10 000/mL) is critical when performing plasma-based coagulation testing especially for detection of lupus anticoagulants (LA), heparin monitoring, or when plasma is frozen and stored for subsequent testing.

The centrifugation speed and duration is determined by the laboratory to determine the optimum conditions for producing PPP, it will vary depending on the centrifuge in use.

Example is: 1500 gravity (g) forces for 15 minutes at room temperature; however, laboratories should determine that this truly produces PPP for that specific centrifuge speed and time combination.

Use of a swing-out bucket rotor minimizes contamination of the plasma with platelets and other blood cells, particularly if the plasma will be removed for other testing or frozen.

Centrifugation should occur at room temperature.

Platelet Poor Plasma (PPP)

Sample cannot be used for testing:



Platelets are a source of phospholipids



Reagents are also made of phospholipids



If platelets are present, they will react with the phospholipids and falsely shorten results



Even worse if sample is freeze/thawed, platelets will burst and greatly shorten time



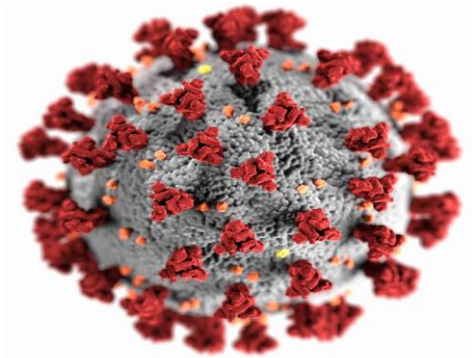
It is important when transferring plasma off of cells that the buffy coat is not disturbed, and platelets are not transferred into the sample contaminating previously platelet poor plasma.

While we are discussing Lupus: Incidence of Lupus

- The incidence of antiphospholipid syndrome (APS) is 5 new cases per 100,000 person per year with a prevalence approximately 40-50 cases per 100,000 persons.
- These antibodies are found to be positive in 13% of patients with stroke, 11% in an MI, 9.5% of patients with DVT and 6% of patients with pregnancy morbidity.
- Antibodies against cardiolipin and or β 2-GPI can be found in individuals without any clinical symptoms in up to 5.5% of healthy subjects.
- In April of 2020 our detection of lupus doubled.

Int J Mol Med, 2020 Sep;46(3):903-912

Lupus and COVID



- Covid patients present with elevated D-dimers and a prolonged aPTT.
- Patients were being tested for DRVVT and hexagonal phase neutralization (HPN).
- They also have an elevated C-reactive protein (CRP) due to the inflammatory state COVID-19 increases CRP. Correlates with disease severity and prognosis.
- An elevated CRP can interfere with LA-PTT based testing such as hexagonal phase phospholipid neutralization (HPN) assay as well as in dRVVT and the aPTT.
- An increase in LA (false positive) can be due to marked elevation of C-reactive protein which doesn't correlate with thrombotic events.
- In patients across the board, and elevated CRP may cause a falsely increased aPTT.

J Thromb Haemost. 2020;18:2065–2066

CASE STUDY

- A stat sample was sent to the laboratory for PT, aPTT, and Fibrinogen
- Sample is hemolyzed
- Can I run this sample?
- Is this in the best interest of the patient?



In-vitro vs In-vivo Hemolysis:

- **In-vitro hemolysis** is the most frequent reason for specimen rejection, prevalence as **45-60%**. Due to a generalized process of vascular and blood cell damage which causes cell membrane disruption and leakage of hemoglobin into the surrounding fluid. (blood drawing, specimen handling, specimen delivery to the laboratory, or specimen storage}
- **In- vivo hemolysis** can be further sub-characterized into intravascular or extravascular hemolysis depending on the mechanism and site of red blood cell destruction. (antigen-antibody reactions, chemical reactions, hemolytic anemias, toxins or physical agents such as artificial heart valves, particularly mechanical ones) .Prevalence is **Less than 2%**

Laga, Chevas, Am J Clin Path, 2006:126:748-755, Mitsios, J, CLN, 2018

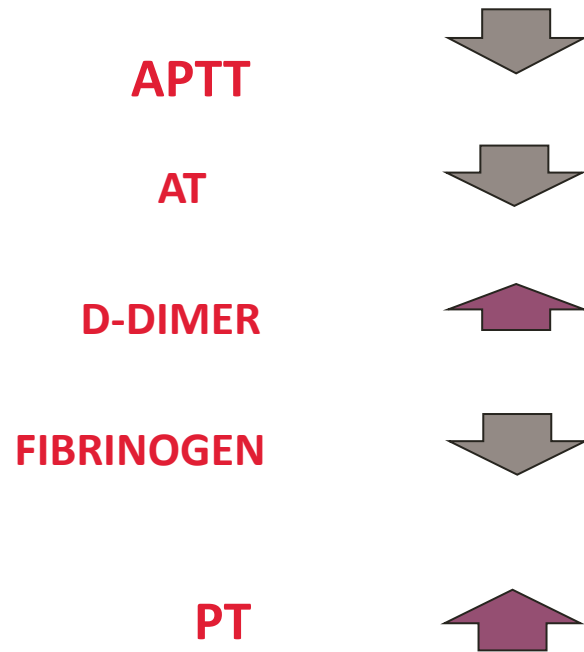
Mechanism of hemolysis and its effect on coagulation results:

- **Speculation:** Does it shorten or prolong results?
- **Shorten:** may be caused by the exposure of anionic membrane phospholipids may provide a phospholipid-rich surface to accelerate coagulation reactions.
- **Prolong:** exposure of membrane phospholipids may compete with thromboplastin for activated factor VIIa (FVIIa) reducing availability and prolong results.

Interference of Hemolysis on coagulation testing

Parameters affected by hemolysis and/or blood cell lysis due to a possible release

Of thromboplastic substances or possible analytical interference- such as in a chromogenic assay



Lippi, et al 2006 & Lippi, et al 2008

ANALYZERS:

- Photo-optical methods can be vulnerable to the presence of HIL interference, mechanical-based methods are less vulnerable.
- Hemolysis increases the spectrometric absorbance of the plasma sample and leads to high background absorbance readings this may compromise clot detection affecting accuracy of results. The use of multiple wavelengths may adjust for this background interference.
- Analyzers measure absorbance at ≥ 2 different wavelengths since HGB absorbs light at 340-440nm and 540-580nm.
- Hemolysis detection may enhance the accuracy of reportable patient test results from photo-optical instruments by flagging results when interferences are detected.

Journal of Laboratory Medicine 43:2, 2019

Concerns from Guidelines:

- **CLSI:** the quality of these specimens should be questioned due to the alterations in coagulation. Hemolysis can result in premature activation of coagulation demonstrated by shortened clotting time results or consumption of clotting factors and prolonged results.
- **ISCH:** Samples should be checked for hemolysis using an automated process to improve consistency, the aPTT should not be performed on samples that display in-vitro hemolysis due to improper collection, (moderated hemolysis has no clinical impact on PT), when in-vivo hemolysis can be confirmed, coagulation testing should proceed.
- **BSH:** Use of analyzers with automated spectrophotometric detection of hemolysis and define cutoffs to determine rejection. In-vivo hemolysis should be determined in flagged specimens. Manufacturers cutoff should be adhered to for using hemolyzed samples.

SOLUTIONS:

- Hemolysis interference was assessed by **absorbance measurement** of plasma at certain wavelengths . Interference readings are provided as value ranges on the basis of calculated results using proprietary algorithms. Manufacturer-provided cut-off values for HGB levels(eg were 5 g/L in both the PT and aPTT test).
- The methods based on **viscosity measurement** of clot formation, for example, fibrinogen and APTT, seem to be insensitive or less influenced by hemolyzed samples than the assays with chromogenic and turbidimetric detection. The three assays that turned out to be the most sensitive to hemolysis were anti-Xa (LMWH), PS, and PC.
- When samples have excess flags, results should not be tested.
- Implement process to demonstrate in-vivo hemolysis (e.g.haptoglobin).
- Disclaimer stating sample is hemolyzed, coagulation results may be impacted.

Make sure you standardize your process and make sure it is reflected in you SOP



Ann Lab Med. 2018 May 23;38(5):484–486, IJLH Volume42, Issue3, June 2020, 341-

Case Study

- Sample sent to the laboratory for a PT at 11:00 AM, the patient is on heparin, PT= 13.5 sec (nr 10.9-13.4 sec)
- At 17:00 nurse called the laboratory and wanted to add an APTT, which is the test that was really needed
- Stated it is a geriatric patient, and a hard draw
- SOP states that the aPTT is only stable for 4 hours.
- What should you do?
- Sample is run off- line- aPTT= 33.1 sec (nr 25-35 sec)
- Result is normal, tech decides to report the result out

Impact of aPTT result:

- Exceeded the stability of an aPTT
- If plasma from a heparinized sample is allowed to sit on the cells, it may cause the release of Platelet Factor IV (PF4)
- This can neutralize heparin
- Resulting in a falsely decreased aPTT
- What are the ramifications of this regarding heparin administration?

This normal result can do more harm than good; possible increase in heparin dose, putting patient at a risk for bleeding.

Case Study

- A sample was sent to the coagulation laboratory from the pediatric clinic for a hemophilia/von Willebrand work-up on an 8-year-old male. Patient presented to the clinic with bruises. It was processed and frozen on an off shift.
- Results are as follows:
 - aPTT= **37** seconds (25.0-35.7 sec)
 - FVIII= **28%** (50-150%)
 - vW antigen: 75% (50-150%)
 - vW risto cofactor= **32%** (50-150%)

What do the results show:

- **Sample shows a decreased activity Factor level, VW activity and prolonged aPTT**
- **Possible causes:**
 - 1. Patient does have von Willebrand disease- should have multimers performed to determine type of VWD.**
 - 2. If samples were placed in a refrigerated (2–8 °C) storage prior to centrifugation it can cause VWF and factor VIII values to decrease**
 - 3. Delays in transport may affect the labile factors (FV, FVIII), leading to prolonged clotting times and in vitro loss of factor activity**



Pediatric sample-ranges?



**Von Willebrand testing-
blood type impacts
levels-**



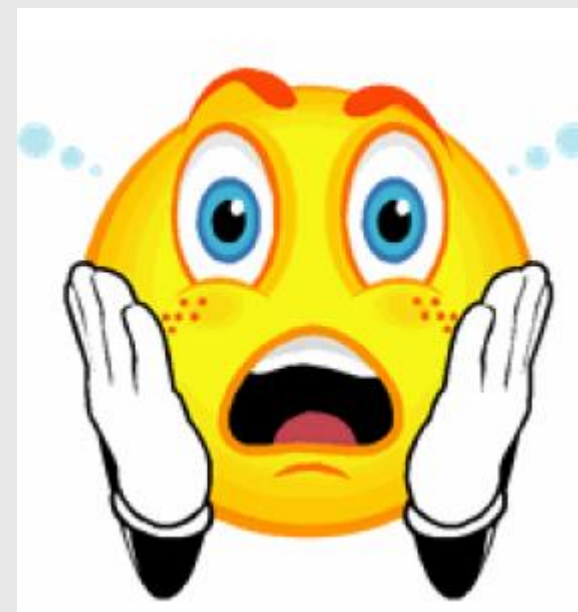
**Mild vWD? Type 2-
discordant ratio of
antigen to activity?**



**Sent from a clinic,
processed on off hours-
sample stability?**

Are these results correct?

Pre-analytical nightmare



Factor VIII:

- FVIII plays a role in endogenous and exogenous thrombin pathways
- Carrier protein for vW factor
- Heat labile
- Study showed a 23% reduction in Factor VIII after 8 hours at RT.
- Best course is to repeat study on a sample with known stability

Cardigan, R, *Transfusion* 51 Suppl 1, 50S–57S (2011).

Repeat sample:

Processed within 2 hours:

aPTT= 32.5 sec

FVIII= 80%

vW antigen = 78%

vW activity = 82%

No hemophilia, no vW disease, patient had taken aspirin and bruises were caused by defect in platelet function.

Case Study

- Sample received : PT= 15.8 sec

aPTT= 65.9sec **both prolonged**

Clinician requested factors: Factor V= 45%

Factor VIII=10%

- Clinician questioned results, no family history of bleeding, no bruising

Repeat testing requested:

PT= 12.1 sec

aPTT= 31.3 sec **both normal**

Possible reasons?

- **Incorrect patient drawn?**
- **High HGB- incorrect ratio of blood to anticoagulant?**
- **Sample contaminated with anticoagulants?**
- **Delay in sample, factors degraded?**
- **Special Coagulation had received an aliquot tube for the factors**
- **Tech went back to pull the original tube**
- **If possible, always go to the source.**

EDTA TUBE WITH A BLUE TOP:

- EDTA functions as an anticoagulant by chelating calcium ions. Similar to sodium citrate in coagulation tubes (vacutainers), calcium plays a critical role in blood clotting. By binding free calcium, K2EDTA effectively prevents the clotting cascade from initiating, thus preserving the cellular components of whole blood.
- Potential errors introduced by testing EDTA plasma
- Prolongation of clotting times
- Over-estimation of PC and PS activity (clot-based)
- Under-estimation of F5 & F8 activity
- Falsely low APCR ratio
- Mimics F8 inhibitor



Preanalytics is crucial for clinical laboratory test results.
François Depasse | May 03, 2017

How to detect EDTA

- **Wrong anticoagulant can easily be detected using routine chemistry tests**
- **EDTA plasma samples are characterized by a high potassium level (around 20mM), and very low or undetectable calcium and magnesium levels.**
- **Useful when working with previously frozen aliquots**

Case Study

- A 75-year-old male (John Doe) in the ED after an MVA presents with head trauma
- Requires emergency surgery
- No available medical history
- Needs to be cleared for surgery
- PT= **21.1** APTT=**74.7**
- Are these results due to a coagulopathy or something else?
- What information can the routine coagulation laboratory provide?

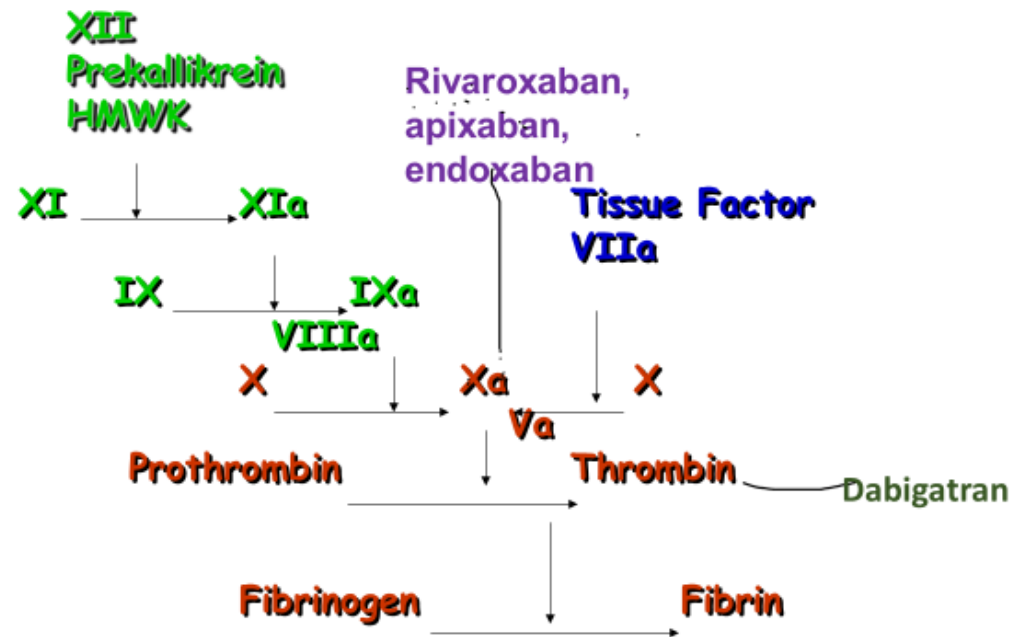
Not just heparin and warfarin anymore:

Used to prevent clotting by several different mechanisms

- Warfarin Sodium (coumadin): renders the Vitamin K factors non-functional (II, VII, IX and X)
- Heparins: Inhibits actions of IIa and Xa
- Direct Xa inhibitors- inhibits only Xa
- Direct IIa inhibitors – inhibits thrombin

PT, APTT, TT and anti-Xa not optimum for all new agents

DOAC's action on the Coagulation Cascade



3

44



Available Direct Acting Oral Anticoagulants (DOACs)

- Dabigatran
- Rivaroxaban
- Apixaban
- Edoxaban

Targeting Specific Coagulation Factors

The goal of direct oral anticoagulants is, in part, to offer more specific targeting and to afford more predictable responses than current anticoagulant therapies, such as warfarin, can offer.

- **Thrombin Inhibitors:** blocks conversion of fibrinogen to fibrin
 - **Dabigatran: pro-drug, renal clearance - twice daily**
- **FXa Inhibitors:** Prevents conversion of prothrombin to thrombin.
 - **Rivaroxaban: renal clearance - once daily**
 - **Apixaban: hepatic clearance - twice daily**
 - **Edoxaban: hepatic clearance - once daily**
- Circulation 2010;121:1523-1532

Summary Table

Parameter	Apixaban	Dabigatran	Rivaroxaban
Target Protein	Factor Xa	Thrombin (IIa)	Factor Xa
Pro-Drug	No	Yes (etexilate)	No
1° Elimination	CYP3A4/P-gp	Renal	CYP3A4/P-gp
Renal Adjustment	Avoid < 15 ml/min	↓ 15-29ml/min Avoid < 15 ml/min	Avoid < 30 ml/min
Drug-Drug Interact.	CYP3A4/P-gp	Rifampin (P-gp)	CYP3A4/P-gp
Onset of activity	3-4 hrs	1-2 hrs	2-4 hrs
t½	8-15 hrs	12-18 hrs	5-9 hrs
Dosing interval	Twice daily	Twice daily	Daily
Monitoring tests	Anti-factor Xa	ECT, TT, +/- aPTT	Anti-factor Xa
FDA Indications	Stroke prevention	Non-valvular Afib.	Non-valvular Afib. Ortho VTE Proph.
Clinical Uses	Afib Ortho VTE Proph	Afib, VTE	Afib, Ortho VTE Proph, VTE

Issues with DOACs

- Anticoagulants were FDA approved with the understanding that they did not need to be monitored.
- Because DOACs have predictable pharmacokinetic and pharmacodynamic responses at a fixed dose, they do not require monitoring.
- In certain instances, they must be monitored:
 - Patients admitted to ED with head trauma
 - Critical care patients previously on DOACs
 - Patients with renal failure
- Companies have not had luck with getting FDA approved testing for these anticoagulants.

Potential DOAC Laboratory Testing Situations

- Before **surgery** or invasive procedure
 - If the patient has taken the drug
 - in previous 24 hrs (or longer if creatinine clearance is < 50 mL / min)
- Identification of **sub- and suprathapeutic levels**
 - taking other drugs known to significantly affect pharmacokinetic
 - at extremes of body weight
 - with deteriorating renal function
- **Reversal** of anticoagulation
- Suspicion of **overdose**
- Assessment of **compliance** (If thrombosis or bleeding occurs during therapy)

Baglin T, Hillarp A, Tripodi A, Elalamy I, Buller H, Ageno W. Measuring oral direct inhibitors of thrombin and factor Xa: a recommendation from the Subcommittee on Control of Anticoagulation of the Scientific and Standardization Committee of the International Society on Thrombosis and Haemostasis. J Thromb Haemost 2013; 11: 756-60.

Issues with the DOAC's



MONITORING? 1 FDA
APPROVED TEST- HARD TO
DEMONSTRATE COMPLIANCE-



PEAK LEVELS AND TROUGH
LEVELS- CAN ONLY DETERMINE
IF THEY ARE NOT PRESENT



DO YOU WANT DETECTION OR
MONITORING?

Emergency testing of Dabigatran:

- Dabigatran:
 1. TT is normal can assume patients' risk of bleeding is low.
 2. PT/ INR is insensitive and not useful
 3. POC INR shouldn't be used
 4. APTT: high levels, no clot, prolonged 2- fold at peak levels, and 1.5- fold at 12 hours. **Very reagent dependent**

Overdose: Since this drug the last enzymatic step of the coagulation cascade it is difficult to replace.

Reversal agent **PRAXBIND (Indarucizumab)**

May not be available at all institutions

Emergency testing of rivaroxaban or apixaban:

1. PT response is assay dependent, should be prolonged
 2. anti-Xa levels using drug as a calibrator
 3. Normal levels of PT, APTT and TT qualitative indicator of absence of drug
- Overdose: approved *Andexxa* (coagulation factor Xa [recombinant]).
 - Problem is cost and availability: average dose is between \$29,700-\$59,400

Samama MM, et al. Thromb Haemost 2010; 103: 815-25; Barrett YC, Wang Z, Frost C, Shenker A. Thromb Haemost 2010; 104: 1263-71; Asmis LM, , et al. 2012; 129: 492-8; Nalezinski, S, Laboratory Medicine, 53:336-343.

Back to our patient:

- **What can we do?**
- **Perform a thrombin time?**
- **Perform an anti-Xa assay?**
- **What information can we provide?**
- **Will factor assays help?**
- **Can we help them determine if an anticoagulant has been taken? Or is the prolonged PT/aPTT due to the patient clinical picture?**

Routine Coagulation Laboratory:

Know both PT & aPTT are prolonged: common pathway

Thrombin time = > 120 sec PNP=13.8 sec normal

Anti-Xa assay 0.1 IU/mL

What does this point to?

Information:

- Rules out Heparin- anti-Xa is normal
- To determine if the prolongation is due to a factor deficiency, you can perform a mixing study
- PT= **21.1** APTT=**74.7**
- 1:1 mix = **20.9 & 72.1**
- **NO CORRECTION – looks like an inhibitor, not a deficiency-**
- **Biggest clues:**
 - **Prolonged routine screening, very prolonged TT, anti-Xa normal, mixing study confirms presence of an inhibitor**

What do direct thrombin inhibitors do?

**PT/INR, APTT
prolonged, remain
prolonged in mix**

**Acts like an inhibitor
in clotting-time
assays, under-
estimating results**

**Fibrinogen falsely
low- (reagent
dependent)**

**C & S,
overestimated**

**ATIII - has “anti-
thrombin” activity,
Increased-**

**Heparin - anti-Xa, is
okay, unless IIa**

**No clot based
testing should be
performed**

Walenga, J., (2006) Direct Thrombin Inhibitors & Laboratory Monitoring Issues, Coagulation Symposium, Indianapolis, May 5.

What information can we provide:

- We cannot monitor the level in the routine coagulation laboratory
- We can detect the presence or absence of a DOAC (peak or trough level)
- Thrombin time can help to differentiate between a IIa inhibitor and Xa inhibitor: TT should be normal in a Xa inhibitor
- An Anti-X can be used to rule out heparin as the anticoagulant and **OFF LABEL** it can also detect the presence of Xa DOAC, **not monitoring!**

ISSUES with Anti-Xa assays:

- **Werfen (IL): Only Apixaban is 510 K cleared at this time for calibrators and controls.**
- **Other companies: calibrators and controls for DOACs were never FDA cleared. There are no rivaroxaban or dabigatran or edoxaban clearances.**
- **Most likely will not change in the near future. Every submission for any new anticoagulant monitoring kit would require a de novo submission.**
- **Limited sales of these products in other markets, too little to justify resubmitting to the FDA.**
- **Can set up as an LDT.**
- **What information can be utilized from your in house anti-Xa assay?**

Relationship Between Anti-Xa & Rivaroxaban/Apixaban

Table 2. Sensitivity, Specificity, Positive Predictive Value, and Negative Predictive Value for a Safe Emergency Procedure.

	Sensitivity	Specificity	Positive Predictive Value	Negative Predictive Value
Rivaroxaban <30 ng/mL	LMWH-calibrated anti-Xa activity = 0.46 U/mL			
	100%	100%	100%	100%
Apixaban <30 ng/mL	LMWH-calibrated anti-Xa activity = 0.40 U/mL			
	100%	100%	100%	100%
Rivaroxaban <50 ng/mL	LMWH-calibrated anti-Xa activity = 0.89 U/mL			
	95.6%	86.7%	91.7%	92.7%
Apixaban <50 ng/mL	LMWH-calibrated anti-Xa activity = 0.79 U/mL			
	95.7%	100%	100%	93.3%

Abbreviation: LMWH, low-molecular-weight heparin.

In a study of 210 patients, investigators established a relationship of LMWH levels to rivaroxaban or apixaban concentration and established a LMWH level of < 0.50 IU/mL could exclude significant apixaban and rivaroxaban levels

Billoir P, Barbay V, Joly LM, Fresel M, Chrétien MH, Le Cam Duchez V. Anti-Xa Oral Anticoagulant Plasma Concentration Assay in Real Life: Rivaroxaban and Apixaban Quantification in Emergency With LMWH Calibrator. *Ann Pharmacother.* 2019;53(4):341-347.



Recommended Tests for DOACs

	Rivaroxaban	Apixaban	Edoxaban	Dabigatran
PT	Screening only*	No	Screening only*	No
aPTT	No	No	No	Screening only*
TT	No	No	No	Screening only
Dilute TT	No	No	No	Screening/ Measurement
ECA	No	No	No	Measurement
Anti-Xa	Measurement	Measurement	Measurement	No

*Due to intersubject variability of factor levels and presence of other potential interferences, along with different assay/platform sensitivities, PT and aPTT assays cannot reliably be used to rule out DOAC presence.



Expected change	Assays	Notes
Clotting time prolongation	<ul style="list-style-type: none"> • aPTT (dabigatran > direct Xa inhibitors) • PT (rivaroxaban > edoxaban > apixaban) • Thrombin time (dabigatran) 	<p>Effects on clotting times are reagent dependent.</p> <p>aPTT and PT mixing tests are expected to show incomplete correction in the presence of DOACs.</p>
False increase	<ul style="list-style-type: none"> • Clot-based protein C activity • Clot-based protein S activity • Antithrombin activity (in factor IIa–based assays with dabigatran, in factor Xa–based assays with direct Xa inhibitors) • Activated protein C resistance ratio 	<p>False increase in protein C, protein S, and antithrombin activities may result in misdiagnosis of a patient with true deficiency as normal.</p> <p>Falsely elevated activated protein C resistance ratio may result in misdiagnosis of a patient with factor V Leiden mutation as normal.</p>
False decrease	<ul style="list-style-type: none"> • aPTT-based factor assays (VIII, IX, XI, XII) • PT-based factor assays (II, V, VII, X) 	<p>Dilutions in factor assays may show nonspecific inhibitor effect.</p>
False positive (or potentially false negative)	<ul style="list-style-type: none"> • LA assays 	<p>Includes aPTT- and DRVVT-based assays, among other clotting time-based LA assays; effects are drug and reagent dependent.</p>

Summary

No change	<ul style="list-style-type: none">• Clauss fibrinogen activity (for most reagents, rare methods show false decrease in presence of high concentrations of dabigatran)• D-dimer• Chromogenic protein C activity• Free and total protein S antigen• Anticardiolipin, anti-β2GP1 ELISAs• von Willebrand activity and antigen assays• DNA-based assays (eg, factor V Leiden mutation, prothrombin G20210A mutation,	
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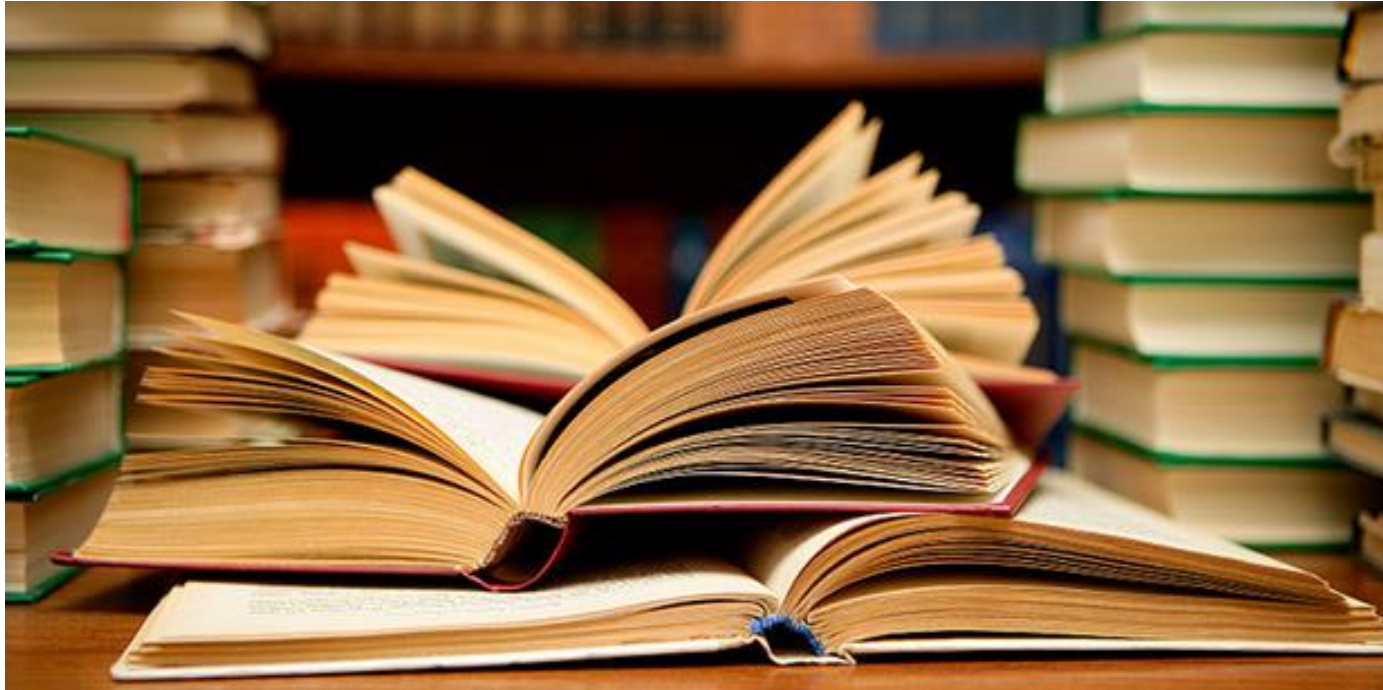
COAGULATION LABORATORY POTPOURRI | DECEMBER 10, 2021
Direct oral anticoagulant (DOAC) interference in hemostasis assays
Karen A. Moser, Kristi J. Smock Hematology ASH program

Patient outcome:

- **Based on results: looked to be the presence of a Direct Thrombin Inhibitor: Dabigatran**
- **Could they perform surgery?**
- **Peak plasma concentrations of dabigatran are reached approximately 2 hours after oral administration. The elimination half-life is 12 to 14 hours, with clearance predominantly occurring via renal excretion of unchanged drug.**
- **Can perform additional routine testing to help determine trough level**

Additional information: patient has AF and was on Dabigatran

RESOURCES FOR PRE-ANALYTICAL VARIABLES:



CLSI. Collection, Transport, and Processing of Blood Specimens for Testing Plasma-Based Coagulation Assays. 6th ed. CLSI guideline H21. Clinical and Laboratory Standards Institute; 2024.

- Recommendations provide laboratories with directions on the importance of preanalytical variables in transport and processing in coagulation testing
- Including collection,
 - labelling
 - transport
 - centrifugation
 - effect of time and temperature
 - stability of samples
 - processing
 - unacceptable samples

International Council for Standardisation in Haematology (ICSH) recommendations for collection of blood samples for coagulation testing: 27 January 2021

Guidance document that looks at:

ordering tests

sample collection tube and anticoagulant,

patient preparation,

sample collection device,

venous stasis before sample collection,

order of draw when different sample types need to be collected,

sample labelling,

blood-to-anticoagulant ratio (tube filling) and influence of hematocrit.

Guidelines on the laboratory aspects of assays used in haemostasis and thrombosis: On behalf of British Society for Hematology, Haemostasis and Thrombosis Task Force 2020

- Pre-Analytical Variables:
 - Blood Collection
 - Sample handling
 - Storage and Preparation
- Provides recommendations

Conclusion:

Take Away:



Preeanalytical variables are extremely important in coagulation testing



Can greatly impact the outcome of patient results and treatment



Need to have good quality practices in place to control what you can



Have good detective instincts and to question things you can't control!

THANK YOU FOR YOUR TIME:



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