Pre-Analytical Variables in the Coagulation Lab: Why Does It Matter?

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Pre-Analytical Variables: Objectives

1. Define Pre-Analytical variables
2. Explain how blood collection may impact test results
3. Describe best practices for sample transport and storage
4. Review sample processing procedures
5. Identify patient variables that may affect coagulation testing
What are Pre-Analytical Variables?

- Includes everything that may affect a patient specimen from the clinician ordering the test to the point of analysis.
- Some patient variables are out of the lab’s control (medications, lipemia, icterus, etc.)
- The biggest source of laboratory error - far exceeds analytical error.
Scope?

- **Up to 70% of testing errors occur in the pre-analytical phase**\(^1\)
  - It is not always clear when a sample is received in the lab that it may be unsuitable or compromised

- **Lab results lead to clinical action**
  - 70-80% of all clinical decisions regarding patient care are based on lab results

- **Coag samples are especially susceptible**
  - Sample collection initiates clotting
  - PT and PTT are complex enzymatic reactions

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Scope?

- **What are the ramifications of pre-analytical errors for the patient?**
  - Misdiagnosis
  - Inappropriate treatment

- **Diligence is required on the part of the laboratory to prevent incorrect results**
  - Must have quality indicators in place to monitor the steps of the pre-analytical phase
  - Event management where errors are investigated and corrected so that repeat events are prevented
When a sample is compromised:

• the test result may reflect the status of the sample

• *but not reflect the clinical status of the patient*
Guidelines

- In order to improve the quality of patients results, sample collection and handling should follow the CLSI guideline
  - H21-A5; 2008
Test Ordering

Is the right test ordered on the right patient?

Coag is confusing - Names sound alike!
- Factor X vs. Anti-Xa
- Factor V vs. Factor V Leiden
- aPTT vs. Anti-Xa for a patient on LMWH
- FVIII vs FXIII
- FIX vs FXI
- Prothrombin Time vs Prothrombin 20210

Can the person entering the order read the order correctly?
- Transcription errors
- Knowledge of test codes, laboratory panels
Patient/Specimen Identification

- Phlebotomist must accurately identify patient
  - Should use 2 identifiers
- Tubes MUST be labeled at time of collection with a firmly attached label that contains all pertinent information
- Minimal requirements:
  - Full name of patient and unique specimen identifier
  - Date and time of collection, name/initials of collector
Sample Collection

- **Recommended Needle Size is 19 - 22 gauge**

- **Ensure that the blood flows quickly and evenly**
  - If too slow, coagulation can take place
  - Too fast: can damage platelets

- **Optimal conditions**
  - Patient at rest, venipuncture without trauma
  - Avoid acute phase reactants. F8, Fibrinogen and vWF.
Sample Collection

- Avoid prolonged tourniquet use
- Use tourniquet for < 60 seconds
  - > 1 minute
    - ↑ Fibrinogen*
  - > 3 minutes
    - ↓ PT, aPTT, thrombin time
    - ↑ Antithrombin and F8

* Effect of tourniquet technique, Rosenson, 1996
Traumatic Sample Draw

- Avoid probing the vein with needle - causes vascular injury with release of tissue factor
- May initiate clotting and shorten PT & PTT results
- Vacutainer system is preferred over syringe
  - ≥ 20 ml should not be used to avoid clot formation
- If using syringe, blood should be added to anticoagulant tubes ASAP (within one minute)
PHLEBOTOMY ORDER OF DRAW

**Closure Color** | **Collection Tube** | **Mix by Inverting**
---|---|---
BD Vacutainer Blood Collection Tubes *(glass or plastic)*
- Blood Cultures - SPS | 8 to 10 times
- Citrate Tube* | 3 to 4 times
- BD Vacutainer SST Gel Separator Tube | 5 times
- Serum Tube *(glass or plastic)* | 5 times (plastic) none (glass)
- BD Vacutainer Rapid Serum Tube (RST) | 5 to 6 times
- BD Vacutainer PST Gel Separator Tube With Heparin | 8 to 10 times
- Heparin Tube | 8 to 10 times
- EDTA Tube | 8 to 10 times
- BD Vacutainer PPT Separator Tube K<sub>3</sub>EDTA with Gel | 8 to 10 times
- Fluoride (glucose) Tube | 8 to 10 times

*When using a winged blood collection set for venipuncture and a coagulation (citrate) tube is the first specimen tube to be drawn, a discard tube should be drawn first. The discard tube must be used to fill the blood collection set tubing’s “dead space” with blood but the discard tube does not need to be completely filled. This important step will ensure proper blood-to-additive ratio. The discard tube should be a nonadditive or coagulation tube.*

Note: Always follow your facility’s protocol for order of draw.

Remixed by ResourceNurse.org

Source: http://www.bd.com
Order of draw for multiple tubes

1. Blood culture
2. Sodium citrate tube for coagulation
3. Non-additive serum
4. Serum separator
5. Heparin
6. EDTA
7. Glycolytic inhibitor tube

Reflects change in CLSI recommended Order of Draw (H3-A5, Vol 23, No 32, 8.10.2)
Discard Tube?

- **Not necessary for routine and many special coagulation assays**
  - Blue top tube can be the 1st tube drawn – or –
  - Blue top tube should be collected after a non-additive (not a clot activator) tube

- **Recommended**
  - Platelet function studies
  - Winged (butterfly) blood collection system with tubing
Specimen Collection

- Blue top 3.2% Na citrate anticoagulant in ratio of 9 parts of blood to 1 part anticoagulant (9:1 ratio)
- Requires at least 90% fill of vacutainer; gently mixed with blood immediately and thoroughly
  - Follow tube manufacturer instructions
What constitutes a tube ‘inversion’?

- BD recommends that citrate tubes be inverted 3 to 4 times.
- An inversion is one complete turn of the wrist, 180 degrees, and back.
Why 9:1 Ratio?

- Incorrect ratio of citrate to blood
  - Under filled tube
    - Excess calcium binding plus dilutional effect
    - Falsely prolonged clotting times
      - <90% fill is unacceptable unless locally validated
      - Small volume tubes are less forgiving
      - Can’t combine 2 partially filled tubes to make 1 full tube
  - Over filled tube
    - Usually the tube has not been used according to manufacturer’s recommendations (opened tube or syringe used to draw blood)
Catheter Line Draws

- Collection of coagulation testing through intravenous lines previously flushed with heparin should be avoided.
- Line (VAD – Vascular Access Device) draws must waste sufficient amount to clear heparin in line (may require wasting 5 – 6 times the volume of line – approx 10 – 15 ml).
- Flush VAD line with 5 ml of saline and then discard first 5 ml or 6 dead volume spaces; for normal saline lock 2 dead space volumes of the catheter and extension set are discarded.
Correction for High Hematocrit (>55%)

Often needed in patients with cyanotic heart disease or polycythemia vera

- Less plasma requires less anticoagulant.
- Failure to adjust anticoagulant to plasma volume results in prolongation of clotting times.
- Excess citrate will reduce available Calcium in test and has a dilutional effect of coagulation proteins.
How to correct for High Hematocrit

Formula in CLSI guidelines H21-A5:

\[ c = (1.85 \times 10^{-3})(100-HCT)(V_{\text{blood}}) \]

Where:
- \( c \) is the volume of citrate remaining in the tube
- \( HCT \) is the hematocrit of the patient
- \( V \) is the volume of blood added
- \( 1.85 \times 10^{-3} \) is constant

Example: Patient has a hematocrit of 59% and using 3.0 mL blue-top tube.

\[ c = (1.85 \times 10^{-3})(100-59)(2.7 \text{ mL}) \]

\[ c = (.00185)*(41)*(2.7) \]

\[ c = 0.20 \text{ mL} \]

3.0 ml tube has 0.3 ml of citrate & 2.7 mL blood

So Remove: 0.10 mL of citrate. (0.3-0.2 mL)

End up with tube with 0.20 mL of citrate
How to correct for High Hematocrit

However if you make a chart:
- Using 3.0 ml tube (draws 2.7ml blood)
- Round off to one tenth
- Can remove 0.1mL for Hct’s between 55-65
- Only need to correct if Hct is > 65 which is rare

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<td>56</td>
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<td>58</td>
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<tr>
<td>64</td>
<td>0.12</td>
</tr>
<tr>
<td>65</td>
<td>0.13</td>
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</table>

Reference: Mayo Medical Labs
Case Study

- 67 y/o male with polycythemia vera with no history of bleeding.
  - Pre-op testing
    - PT = 15.7 sec
    - PTT = 42 sec
  - Physician ordered F2, F7, F8, F9, F10 and F11 ($$$) – all normal
  - Patient Hct was 62%
  - Patient was redrawn with adjusted citrate and repeated PT & PTT were normal

Average % difference in corrected vs. non-corrected samples: PT: 25%; aPTT: 19% (% exponentially increases as hematocrit increases)

Marlar, R: Effect on Routine and Special Coagulation Testing Values of Citrate Anticoagulant Adjustment in Patients With High Hematocrit Values; American Journal of Clinical Pathology 126(3):400-5 - October 2006
Pneumatic Tube Transport

Specimens must arrive in the testing facility allowing sufficient time to be processed and analysed - according to sample stability guidelines.

Not recommended for platelet function studies or samples for thromboelastography (ie. TEG, Rotem, PFA-100, Platelet aggregation, etc.)
Specimen Transport

- **Transport at room temperature!!**
  - Ideally within one hour of collection

- **Do not put on ice or refrigerate**
  - Don’t transport in cooler with ice packs
  - Elevation of F7 activity by >150%
  - Cold activation of platelets
  - Loss of F8, Fibrinogen, F13, and vWF
    - Can decrease up to 50% from baseline
    - Can cause a misdiagnosis of F8 and/or F13 deficiency or von Willebrand’s disease
Specimen Transport

- F8, F5, and Protein S are heat labile factors
  - Accelerated loss occurs at warmer temperatures
  - All Factors will lose all activity if maintained at 58C for a period of time
What about Dry Ice?

- Used for plasma aliquots shipped to another facility for testing when the time between collection & testing exceeds acceptable standards
  - Quick freeze plasma samples
  - Frozen and shipped on dry ice to maintain samples in a frozen state
Centrifugation
Know your Centrifuge!

- **Coagulation testing requires platelet poor plasma**
  - Defined as < 10,000/ul platelet count
  - Critical for frozen plasma aliquots
  - High platelet count will neutralize heparin activity
  - Lower PTT or Anti-Xa result
  - Mask presence of Lupus Anticoagulant (false negative)

- **Centrifuge @ 1500g for 15 min @ room temp**
  - RPM ≠ g

- Stat Centrifuge acceptable – but be careful

- Validate centrifuge every 6 months or after modification of centrifuge

- Double centrifugation is recommended for plasma aliquots
Platelet Poor Plasma Preparation

Double Spinning Samples for Coagulation testing

1. Centrifuge the draw tube.
2. Take plasma layer off to an aliquot tube. Be careful not to disturb the platelet layer.
3. Centrifuge the First Aliquot tube.
4. Take off plasma leaving a small amount at the bottom of the tube. Use care not to aspirate the pellet of Platelets/RBC at the bottom of the tube.
5. Transfer this platelet-free plasma to clean polypropylene aliquot tube (polystyrene is not acceptable). This is the Second Aliquot tube. Freeze this tube.
Frozen Aliquot Tubes

- Polypropylene
  - Cloudy
  - Good!!

- Polystyrene
  - Clear
  - Bad!!
Storage & Testing Intervals

- PT samples stable 24 hr if capped 18 - 24°C
- PTT samples stable 4 hr if capped 18 - 24°C
- Specimens for UFH should be centrifuged within 1 hr of collection
- LMWH is stable for 4 hr capped for anti-Xa testing
- Other assays test within 4 hr
- If this is not possible, samples should be centrifuged, plasma aliquoted, and frozen
Frozen Samples

- Frozen sample stability:
  - 2 weeks @ -20°C
  - 6 months @ -70°C

- Thaw rapidly at 37°C (5 min) in water bath – heat block is next best - *never at room temp!*

- Mix plasma immediately – consider tube rocker

- Test immediately
Also....

- Look for bubbles in sample and pour off tubes
  - May trigger false liquid level detection
- In thawed samples: look for cryo-precipitate (F8 globulin protein in specimen)
  - Looks like mucus in sample – re-centrifuge
Why capped samples??

- A key component of sodium citrate is citric acid
  - Citric Acid maintains sample pH between 7.3 & 7.45

- pH increases if samples are stored uncapped for more than 30 minutes
  - Processed samples are more susceptible to changes in pH than whole blood samples

- Increase in pH leads to clinically significant prolongations of aPTT, and affects platelet reactivity
  - A pH change of 0.8 may prolong the aPTT of a normal sample more than 20 seconds
Sample Appearance

- Hemolyzed
- Lipemic
- Icteric
- Clotted
Hemolyzed Samples

- 90% of samples with hemolysis are from bad draw technique –
  - re-draw suggested
- In vivo hemolysis (not pre-analytical)
  - Caused by AIHA, TTP, sickle cell disease, transfusion reactions
  - <1% of hemolyzed samples are in vivo type
  - In these cases the sample should be run and reported

- May interfere with photo-optical assays
- D-Dimer falsely increased
- Possible pre-activation of coag cascade
Lipemic Sample

- Milky in color
- Excess lipids (fats) in the blood
  - Inherent to the patient and cannot be avoided
  - May interfere with photo-optical assays
  - *Ultra-centrifuging not recommended* for lipemia removal – may remove other coagulation proteins

![Image of lipemic sample with L index values: 771, 413, 220, 116, 62, 31, 21, 11, 8, 8]
Icteric Sample

- Jaundice, yellowish in color
- Due to liver disease, hepatitis, & cirrhosis
  - May interfere with photo-optical assays
Clotted Sample

Causes of Clotted Samples

- Prolonged tourniquet use
- Slow fill of vacutainer
- Probing the vein with the needle
- Tube not inverted after collection
- Drawn in syringe – not transferred to blue top in time
- Drawn in red top transferred to blue top
Clotted Sample

Impact on Coagulation Samples of Clotted Samples

- Clotted samples may cause consumption of clotting factors
- Clotted samples will have low levels of fibrinogen
- If clotting time is not detected during PT & PTT testing (mMax), and Fibrinogen is $\leq 25$ mg/dl, the sample may be serum
Incorrect Specimen Submission

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
Patient Variables

- Diet
- Age
- Gender
- Drug interaction
- Disease
- Lifestyle
Patient Variables

Diet

- Food high in Vitamin K adversely affect warfarin, (INR decreases)
  - Leafy Green Vegetables:
  - Fruits: blueberries, pears, peaches, figs, currants
  - Meats: beef liver, pork liver
  - Other: mayonnaise, margarine, canola oil, soybean oil, vitamins, chili powder

Patient Variables

Increasing Age

- Some increase per decade:
  - Fibrinogen (~9mg/dL)
  - F5 (~6%)
  - F8 (~10%)
  - F9 (~10%)
  - vWF (~14%)
  - D-Dimer (~26%)

Patient Variables

❖ Decreased Age – Newborns

❖ F2, F7, F9, F10, F11, and F12 are <70% of the adult value at birth – remain 20% lower until teenage years

❖ Fibrinogen, F5, F8, vWF, F13 are >70% of adult values at birth

❖ AT vary with gestational age – 50% of adult value at birth

❖ Proteins C & S are even lower at birth – Protein C remains low throughout childhood

❖ D-Dimers – markedly elevated in newborns, remains elevated in childhood
Patient Variables

◆ **Woman Stuff**

- **Menstruation**
  - Cyclic variations occur for Fibrinogen and vWF levels
  - Testing for vWD should occur no later than day 7 of a menstrual cycle
- **Pregnancy**
  - Low grade process of intravascular coagulation
  - Progressive increase in F8 and vWF levels
  - Decrease in Protein S
- **Menopause**
  - Higher levels of Fibrinogen & F7
  - Hormone Replacement Therapy increases F7 and decreases Antithrombin & Protein S (most pronounced after initiation of drug)

Patient on Anticoagulants

- **Anticoagulants** [heparin, direct thrombin inhibitors (DTIs), Xa inhibitors]
  - UFH
    - Prolong APTT & Thrombin Time
    - False positive LA (some methods)
    - In excess (>1 IU/ml) over-estimation PS activity, prolong PT and interfere in multiple clotting assays
  - DTIs prolong all clot based tests and cause false results in many clotting tests
  - Xa inhibitors may prolong PT, APTT, DRVVT and cause false positive results in tests based on Xa inhibition
## DOAC Influence on Coagulation Assays

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<th>Dabigatran (Pradaxa)</th>
<th>Rivaroxaban (Xarelto)</th>
<th>Apixaban (Eliquis)</th>
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Influence depends on drug concentrations, reagents, assays
### DOAC Influence on Thrombophilia Assays

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*Influence depends on drug concentrations, reagents, assays*
Case Study

- Two properly labeled, citrated blood samples filled to the appropriate volume are received in the laboratory at 10:24 for coagulation testing.
- The specimens were collected at 9:47 and immediately placed on ice for transportation to the laboratory.
- The specimen requisition paperwork indicates a PT, aPTT, F8, platelet function analysis (PFA-100), and vWF antigen and activity assays are ordered.

What is the best next step with this specimen?
Case Study - Questions

1. Centrifuge tube #1 for the coagulation assays and use tube #2 for the PFA-100 assay. Report all results.
2. Reject both specimens as they have been stored on ice.
3. Reject both specimens as the pre-analytic time exceeded the recommended guidelines for testing.
4. Remove both specimens from the ice and re-warm at room temperature for 30 minutes. After rewarming, spin tube #1 one tube for coagulation assays and use the second tube for the PFA-100 assay. Report all results.
5. Freeze both whole blood sample tubes until all testing is ready to perform.
Case Study - Answers

1. Centrifuge tube #1 for the coagulation assays and use tube #2 for the PFA-100 assay. Report all results.

INCORRECT CHOICE: While this is the process a laboratory should follow for a specimen transported at room temperature, whole blood sample must not be transported on ice (or refrigerated).

Cold storage temperatures of whole blood samples can cause cold activation of F7, decrease von Willebrand and F8, disrupt platelets, and reduce platelet function. Changes such as these may result in erroneous test results.
3. Reject both specimens as the pre-analytic time exceeded the recommended guidelines for testing.

INCORRECT CHOICE: The specimen was received in the laboratory in an appropriate amount of time. Specimens must be processed into platelet poor plasma and then tested (or frozen) with 4 hours for aPTT clot-based assays and within 24 hours PT clot-based assays.

Platelet function assays must be tested within 4 hours of collection.

If a specimen is sent to measure heparin levels, the specimen must be processed to plasma within 1 hour to avoid potential heparin neutralization by platelet factor 4 (PF4).
Case Study - Answers

4. Remove both specimens from the ice and re-warm at room temperature for 30 minutes. After rewarming, spin tube #1 one tube for coagulation assays and use the second tube for the PFA-100 assay. Report all results.

INCORRECT CHOICE: Specimens used for coagulation testing must be transported at room temperature.

Cold storage temperatures of whole blood samples can cause cold activation of F7, decrease von Willebrand and factor F8, and disrupt platelets and reduce platelet function.

Changes such as these will result in erroneous test results, and re-warming specimens prior to testing will not resolve these negative effects.
5. Freeze both citrated whole blood sample tubes until all testing is ready to perform.

INCORRECT CHOICE: It is never acceptable to freeze whole blood specimens prior to testing in the coagulation laboratory.
Case Study - Answers

2. Reject the specimens as they have been stored on ice.

CORRECT CHOICE: Specimens received on ice (or refrigerated) are inappropriate for testing in the coagulation laboratory.
Questions?