Pre-analytical Variables in the Coagulation Laboratory – New CLSI Guideline

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Disclosure

- None
Outline

- Review of pre-analytical phase of testing
- Variables that may affect plasma-based testing associated with
  - Specimen Collection
    - Methods
    - Containers and Additives
  - Specimen Transport, Processing, Storage
  - Specimen Stability
  - Causes for Specimen Rejection
Collection, Transport, and Processing of Blood Specimens for Testing Plasma-Based Coagulation Assays and Molecular Hemostasis Assays; Approved Guideline – Fifth Edition

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Pre-Analytical Variables

- Variables that occur prior to the time the sample is analyzed (pre-examination)

- Include:
  - Conditions associated with the patient
    - Medications, lipemia, icterus
  - Conditions associated with specimen collection, transport, processing and/or storage
Pre-Analytical Variables

- **The** most important source of lab error
  - Exceeds analytical error
    - Improved instrument performance
    - LIS interfaces

- Plasma-based coagulation samples - especially susceptible
  - Sample procurement initiates clotting
  - Complex nature of APTT and PT reactions
  - Lability of platelets and clotting factors *in vitro*
Pre-analytical Variables

- Not only are coagulation samples especially susceptible but the effect on results can be:

  **HUGE**

- Compromise of sample integrity leading to erroneous results may cause:

  *mistaken diagnosis and patient mismanagement*
Minimizing Variables - Improves Quality

- It is not always clear when a sample referred to the laboratory is unsuitable or compromised.
- When a sample is compromised, the test result might accurately reflect the status of the sample but not accurately reflect the clinical status of the patient.
Guidelines for sample collection, transport, processing and storage must be **strictly followed and deviations avoided**, unless their impact, or lack thereof, on coagulation testing is known

Phlebotomists must be properly educated regarding proper technique
Specimen Collection

- Proper patient and sample identification is crucial
  - Specimens should be labeled in the patient’s presence
- Test request form must be properly filled out and legible
- In some states informed consent must be obtained for molecular testing
Blood Collection

- Venipuncture from peripheral vein using evacuated tube system preferred method
- Syringe draw with straight needle acceptable
  - < 20 mL size to avoid clot formation
  - Add blood to anticoagulant < one minute
  - Greater potential for hemolysis, platelet activation
- Collection from vascular access device
  - Potential for sample dilution or contamination
    - Flush with 5 ml saline and discard first 5 ml or discard 6 dead space volumes
    - Saline lock – discard 2 dead space volumes
Plasma-Based Coagulation Assays: Blood Collection - Discard Tube

- **Not necessary** for routine plasma assays*
  - Blue top tube can be the first tube drawn or
  - Blue top tube collected following a non-additive (*not clot activator*) tube
- **Recommended** for winged (butterfly) blood collection system
  - Discard amount equal to length of tubing (to displace the air in the tubing)
- **No evidence to demonstrate the need, or lack thereof, for special coagulation studies**

Components of the Collection System

- **Needle gauge**
  - Not too small (>25 gauge) and not too big (<16 gauge)
    - Either may induce hemolysis
  - 19 to 22 gauge best

- **Collection and aliquot containers for plasma-based assays must have a non activating surface**
  - Glass or plastic is acceptable
Plasma-Based Coagulation Assays: Prevent *in vitro* clot formation

- Avoid prolonged tourniquet use
- Avoid probing the vein with needle
- Encourage blood to flow freely
- Promptly and thoroughly mix anticoagulant with whole blood
  - Three to six end over end inversions
    - Avoid vigorous shaking (hemolysis)

*Clot formation is cause for specimen rejection!!*
Plasma-Based Coagulation Assays: Prevent *in vitro* clot formation

- **Impact of clot formation may cause**
  - Consumption of clotting factors
  - Loss of fibrinogen and certain factor activities, such as factors V and VIII
  - Activation of clotting
  - Shortening of the APTT and PT, elevation of factor VII
Appropriate Anticoagulant

- Sodium citrate: **Light Blue Top Tube**
  - 105 to 109 mmol/L or 3.13% to 3.2% (commonly described as 3.2%) preferred but 129 mmol/L or 3.8% acceptable
  - Standardize to one concentration within system
  - Clotting times may be longer in 3.8% vs 3.2%*

- **EDTA** (purple top) and **heparin** (green top) plasma, serum *not acceptable*

*Adcock D, et al. AJCP 1997; 107:105*
## Evacuated Tube Effect

<table>
<thead>
<tr>
<th>Tube Type Assay</th>
<th>3.2% Citrate Mean/Range</th>
<th>EDTA Mean/Range</th>
<th>Sodium Heparin Mean/Range</th>
<th>Serum Mean/Range</th>
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<tbody>
<tr>
<td>APTT (sec)</td>
<td>29/23-33</td>
<td>68/45-92</td>
<td>&gt;180</td>
<td>&gt;180</td>
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<tr>
<td>PT (sec)</td>
<td>12.4/11.5-13.2</td>
<td>23/19-27</td>
<td>&gt;60</td>
<td>&gt;60</td>
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<tr>
<td>dRVVT (sec)</td>
<td>34.6/27-43</td>
<td>55/45-64</td>
<td>&gt;150</td>
<td>&gt;150</td>
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<tr>
<td>FV Act (%)</td>
<td>113/84-142</td>
<td>71/59-103</td>
<td>81/59-103</td>
<td>23/13-32</td>
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<tr>
<td>FVII Act (%)</td>
<td>115/50-180</td>
<td>116/51-182</td>
<td>77/43-107</td>
<td>308/80-436</td>
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<tr>
<td>FVIII Act (%)</td>
<td>141/80-202</td>
<td>4.5/2-19</td>
<td>&lt; 1</td>
<td>4.5/1.3-7.7</td>
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<tr>
<td>FIX Act (%)</td>
<td>122/97-148</td>
<td>115/63-168</td>
<td>&lt; 1</td>
<td>350/135-568</td>
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<tr>
<td>VWF:Ag (%)</td>
<td>122/50-194</td>
<td>143/59-228</td>
<td>70/42-98</td>
<td>101/32-169</td>
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<tr>
<td>VWF:RCo (%)</td>
<td>114/41-188</td>
<td>131/46-215</td>
<td>37/13-60</td>
<td>74/25-123</td>
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<td>PC Act (%)</td>
<td>111/66-155</td>
<td>152/100-205</td>
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<td>15.3/0-70</td>
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<tr>
<td>PS Act (%)</td>
<td>96/73-119</td>
<td>30/17-42</td>
<td>&lt; 1</td>
<td>21.6/0-39.5</td>
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<tr>
<td>Free PS Ag (%)</td>
<td>114/89-148</td>
<td>131/91-171</td>
<td>126/94-159</td>
<td>131/97-164</td>
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<tr>
<td>AT Act (%)</td>
<td>102/86-118</td>
<td>121/105-138</td>
<td>126/108-143</td>
<td>47/30-65</td>
</tr>
</tbody>
</table>

*Valcour A, et al. LabCorp Burlington, with permission*
Evacuated Tube Effect

**EDTA Plasma**
- PT, APTT - Prolonged
- Mixing studies - Lack of correction
  - Mimic a time-dependent inhibitor
- ↓↓ FVIII, ↓ FV, ↓ PS activity
- Calcium undetectable, potassium elevated

**Serum**
- PT, APTT – no clot detected
- ↑↑ FVII, ↑↑ FIX ↓↓ FVIII, ↓ FV, ↓ PS/PC activity
Blood to anticoagulant ratio (fill volume)

- 9:1 = 9 parts blood to 1 part anticoagulant
- Under-filled tubes - prolonged clotting times
  - Prolongation may be reagent dependent
- < 90% fill unacceptable unless lower fill volume is locally validated
  - More forgiving with 3.2 vs 3.8% sodium citrate*
  - Pediatric tubes are less forgiving**
- Avoid over filling as may occur when stopper is removed and tube filled

Plasma-Based Coagulation Assays: Collection System Components

- Under-filled blue stoppered tubes are a cause for specimen rejection

- **Never** transfer blood from one tube to another to provide the required fill volume
  - Even if combining two blue stoppered tubes!
Plasma-Based Coagulation Assays: Patient Hematocrit

- **Samples with hematocrits > 55%** require adjustment of citrate concentration
  - To avoid spuriously prolonged clotting times
  - Rule of thumb – as most samples have hcts between 55 and 65% remove 0.1 mL sodium citrate from evacuated tube*

- **Samples with hematocrits < 25%** do not require citrate adjustment**

*Marlar RA. Am J Clin Pathol 2006;126:400-405
**Siegel JE. Am J Clin Pathol. 1998;110:106-110
The impact of using a higher concentration of citrate (3.8% vs 3.2%) is similar to the impact of an under-filled tube (short draw) or the impact of a sample with a hematocrit >55%.

= Excess calcium binding citrate
Plasma-Based Coagulation Assays: Sample Transport – Pre processing

- Transport at room temperature, ideally within one hour of collection
- Transport/Storage of whole blood at 2-4°C – Not Recommended
  - Loss of Factor VIII and Von Willebrand Factor *
    - Time and temperature dependent
    - Decreases up to 50% from baseline
    - VWF:RCo > VWF:Ag – can simulate type 2 VWD
- Cold activation of FVII
- Cold activation of platelets

*Favalaro E, et al. AJCP 2004;122:686
Plasma-Based Coagulation Assays: Sample Processing

- Centrifuge to obtain platelet poor plasma
  - Post centrifugation plasma platelet ct $\leq 10 \times 10^9$/L
- Confirm every 6 months or after modification of centrifuge
- Critical for frozen but not fresh plasma:
  - APTT, PT/INR and TT performed on fresh plasma samples not affected by platelet counts $\leq 200 \times 10^9$/L (200,000/$\mu$L)*
- Swing-out bucket rotor should be used to minimize contamination of the plasma with platelets and other blood cells

Plasma-Based Coagulation Assays: Sample Processing

- **“Stat-fuge”** acceptable*
  - Higher speed, shorter duration

- **Other methods to obtain platelet poor plasma**
  - Double centrifugation - recommended
  - Filtration using a 0.2 µm Millipore filter **
    - Can result in spurious prolongation of APTT and PT results due to selective removal of factors V, VIII, IX, XII and VWF

Plasma-Based Coagulation Assays: Hemolysis

- **Visible hemolysis** - reject sample
  - Potential for activation of clotting factors due to cell lysis products*
  - Controversial viewpoints in literature
- **May impair end point detection using an optical system of clot detection**
- **Samples that appear hemolyzed due to hemoglobin substitutes are not a cause of specimen rejection**
  - Test using mechanical end point detection

*Lippi G. Arch Pathol Lab Med 2006; 130:181*
Plasma-Based Coagulation Assays: Lipemia, Icterus

- Causes potential interference in optical end point detection methods
- May require mechanical or electromechanical end point detection method
- Lipemic samples may be cleared with centrifugation
  - Potential loss of fibrinogen with ultracentrifugation
Specimen Storage

- The allowable time interval between collection of the specimen and testing of the sample depends on:
  - Whether the sample is stored as whole blood or processed as plasma
  - Assay to be performed
  - Temperature

- Specimens should be stored capped
Plasma-Based Coagulation Assays: Sample Stability – PT Testing

- Stored as whole blood or processed into plasma, room temp < 24 hrs*
- Sample integrity enhanced if samples are centrifuged immediately after blood collection
- Stability of vitamin K dependent factors for 24 hours reported**

Plasma-Based Coagulation Assays: Sample Stability – APTT*

- **Non-heparin Sample**
  - Whole blood or processed, in an unopened tube at room temperature < 4 hours
  - Local validation of longer storage acceptable - normal and abnormal samples to be tested

- **Heparin Sample**
  - Centrifuge within one hour of collection, test within four hours from time of collection
  - CTAD tubes may enhance stability – 4 hours
    - Therapeutic APTT heparin ranges must be determined for CTAD matrix

Plasma-Based Coagulation Assays: Sample Stability – Other assays

- Maintain at room temperature, centrifuge and test \(\leq 4\) hours from time of collection
- Protein S, factor VIII and factor V – limited stability*
- Protein C, fibrinogen and antithrombin – stable up to 7 days*
- Vitamin K dependent factors stable 24 hours room temperature

Plasma-Based Coagulation Assays: Long Term Stability

- If the testing is not completed within 24 hours for PT specimens and 4 hours for APTT and other assay(s), plasma should be removed without disturbing the sedimented cells and frozen at -20°C or colder for short-term storage (up to two weeks) or -70°C or colder for long-term storage.

- Do not use frost-free freezers for sample storage
Frozen plasma specimens should be rapidly thawed at 37°C then gently mixed and tested immediately.

- Thorough mixing immediately after thawing and prior to testing mandatory.
- Excessive time or temperature in water bath may lead to significant loss of sample integrity.
Plasma-Based Coagulation Assays: Common Sources of Error

- Collection tube other than sodium citrate
- Incomplete filling of evacuated tube
- Inadequate mixing of evacuated tube
- Cold-activation of the whole blood sample
- Inadequate thawing and mixing of previously frozen samples
Plasma-Based Coagulation Assays: Causes for Specimen Rejection

- Plasma collected into anticoagulant other than sodium citrate
- Other than 9:1 ratio
  - Evacuated tubes under or over-filled
  - Hematocrit > 55%
- Clot evident in tube
- Hemolysis
- Improper specimen storage
Thank you!

- Question and answer period