The Top Ten Problems in Coagulation Testing

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The Fritsma Factor
Your Interactive Hemostasis Resource
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# 10 How to Monitor Platelet Function

Platelet Aggregometry
Platelet Function Testing

Collect 12–15 mL blood; 4–5.2 mL 3.2% citrate tubes
Cfg 50 g 30 ″, remove PRP
Cfg 1500 g 15 ″, PPP blank
Wait 30 ″ for “platelet shock”, dispense to cuvette
Pipette agonist, record absorbance using photometry

Light Transmittance Aggregometry

• Collect 12–15 mL blood; 4–5.2 mL 3.2% citrate tubes
• Cfg 50 g 30 ″, remove PRP
• Cfg 1500 g 15 ″, PPP blank
• Wait 30 ″ for “platelet shock”, dispense to cuvette
• Pipette agonist, record absorbance using photometry

Whole Blood Aggregometry (WBA)

• Collect 9 mL blood
  – 3 tubes 2.7 mL + 0.3 citrate
• Dilute 1:1 with saline
• Pipette agonist, timer starts
• Lower electrodes into suspension

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Top Ten Coagulation Problems

10/14/11

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10: Platelet Function Tests

1: Secretion Measured Using the “Firefly” Reaction

ATP

Chroma-lum®

firefly luciferase

PPI

Luciferin

Luciferyl:AMP

O₂

Oxyluciferin + AMP

Luminescence

Emitted light proportional to ATP release in µM

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10: Platelet Function Tests

Aggregating PLTs coat electrodes

Current impeded by platelet layer

– Impedance measured in ohms (Ω)
– 0 Ω = no aggregation
– Aggregation proportional to Ω

10: Platelet Function Tests

In Vitro Agonists and Receptors
Platelet Aggregometry

• Thrombin (IIa) or thrombin receptor-activating peptide (TRAP)
  – Binds protease activatable receptor (PAR-1) and GP V
  – Thrombin generates clot, measure secretion (release) only
  – Activates all pathways

• Arachidonic acid (AA)
  – Enters eicosanoid pathway as substrate to produce TXA₂
  – TXA₂ activates platelet by binding receptors TP₁ and TP₂

• Collagen
  – Binds receptors GP la/lla (integrin α₂β₁), GP IV, GP VI
  – Requires intact eicosanoid synthesis pathway

• ADP
  – Binds receptors P2Y₁ and P2Y₁₂
  – Epinephrine binds α-adrenergic receptor
  – Requires intact eicosanoid synthesis pathway

• Ristocetin
  – Ristocetin-induced platelet agglutination (aggregation, RIPA) tests for VWD

10: Platelet Function Tests

Whole Blood Lumi-Aggregometry (WBLA)

Reference Intervals; Mean ± 2 SD

<table>
<thead>
<tr>
<th>Agonist</th>
<th>Conc</th>
<th>Aggregation Impedance</th>
<th>Secretion: ATP Luminescence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thrombin</td>
<td>1 unit</td>
<td>N/A</td>
<td>1.0-2.0 µM</td>
</tr>
<tr>
<td>Collagen</td>
<td>5 µg/mL</td>
<td>15-31 Ω</td>
<td>0.9-1.7 µM</td>
</tr>
<tr>
<td>ADP</td>
<td>5 µM</td>
<td>1-17 Ω</td>
<td>0.0-0.7 µM</td>
</tr>
<tr>
<td></td>
<td>10 µM</td>
<td>6-24 Ω</td>
<td>0.4-1.7 µM</td>
</tr>
<tr>
<td>AA</td>
<td>0.5 mM</td>
<td>5-17 Ω</td>
<td>0.6-1.4 µM</td>
</tr>
<tr>
<td>Ristocetin</td>
<td>1 mg/mL</td>
<td>&gt; 10 Ω</td>
<td>(&gt; 70 sec lag)</td>
</tr>
</tbody>
</table>

Courtesy of Kathy Jacobs, Chronolog, Inc

10: Platelet Function Tests

Normal WBLA

Impedance

ATP Release

Full 2.0 µM Release

10 Minutes

Full 40 Ω Aggregation

0 1.0 unit thrombin or TRAP

5 Minutes

Full 40 Ω Aggregation

5 µg/mL collagen, 0.5 mM arachidonic acid, 5 µM ADP

10: Platelet Function Tests

Normal WBLA

Impedance

ATP Release

Full 1.0 µM Release

10 Minutes

Full 40 Ω Aggregation

0 1.0 unit thrombin or TRAP

5 Minutes

Full 40 Ω Aggregation

5 µg/mL collagen, 0.5 mM arachidonic acid, 5 µM ADP

10: Platelet Function Tests
**Top Ten Coagulation Problems**

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**The Fritsma Factor**

**Normal WBLA**

![Image](image1.png)

**WBLA Anticipated Aspirin or Aspirin-like Disorder (Secretion Disorder) Ranges**

<table>
<thead>
<tr>
<th>Agonist</th>
<th>Concentration</th>
<th>Aggregation</th>
<th>Secretion: ATP Release</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thrombin</td>
<td>1 unit</td>
<td>N/A</td>
<td>&gt; 0.5 μM</td>
</tr>
<tr>
<td>Collagen</td>
<td>5 μg/mL</td>
<td>21-25 Ω</td>
<td>0.3-0.7 μM</td>
</tr>
<tr>
<td>ADP</td>
<td>5 μM</td>
<td>1-13 Ω</td>
<td>&lt; 0.1 μM</td>
</tr>
<tr>
<td>AA</td>
<td>0.5 mM</td>
<td>&lt; 0.5 Ω</td>
<td>&lt; 0.1 Ω</td>
</tr>
<tr>
<td>Ristocetin</td>
<td>1 mg/mL</td>
<td>&gt; 10 Ω</td>
<td>&lt; 70 sec lag</td>
</tr>
</tbody>
</table>

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**Aspirin acetylates COX1 and reduces production of TXA2**

![Image](image2.png)

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**Whole Blood Lumiaggregometry Aspirin, Aspirin-like Disorder (Secretion Defect)**

![Image](image3.png)

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**Aspirin, Aspirin-like Disorder (Secretion Defect) Whole Blood Lumiaggregometry**

![Image](image4.png)

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**PFA-100**

![Image](image5.png)

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**www.fritsmafactor.com**
Siemens PFA-100

- Transfer 800 μL citrated whole blood to each of two cartridges, load and record interval to closure time (CT)
  - Coated membrane: agonists
- Collagen and epinephrine (Col/Epi, CEPI)
  - Initial screen cartridge
  - “Weak” normal CT 98–175s
- Collagen and ADP (Col/ADP, CADP)
  - 50 μM ADP
  - Normal CT 77–125s
  - Confirmatory

Aspirin Resistance Prevalence

<table>
<thead>
<tr>
<th>Overall</th>
<th>27.1%</th>
</tr>
</thead>
<tbody>
<tr>
<td>By method</td>
<td></td>
</tr>
<tr>
<td>PFA-100</td>
<td>29.0%</td>
</tr>
<tr>
<td>Ultegra VerifyNow</td>
<td>26.2%</td>
</tr>
<tr>
<td>LTA</td>
<td>21.3%</td>
</tr>
<tr>
<td>By population</td>
<td></td>
</tr>
<tr>
<td>CAD</td>
<td>22.9%</td>
</tr>
<tr>
<td>Stroke</td>
<td>32.1%</td>
</tr>
<tr>
<td>&lt; 100 mg/d</td>
<td>35.6%</td>
</tr>
<tr>
<td>&gt; 100 mg/d</td>
<td>18.6%</td>
</tr>
</tbody>
</table>

Aspirin, NSAIDs
- Reduced HCT or PLT count
- Mild PLT dysfunction
- Mild VWD
- Severe PLT dysfunction
- Severe VWD

Distribution of Aspirin Responses

Measured by Various Platelet Function Analyzers


Relationship of frequency and change in aggregation (%) in response to 5 umol/L ADP at 2 h, 24 h, 5 days, and 30 days after stenting

Variable Response to Plavix

Plavix

- Occupies P2Y12 ADP Receptor
- Activates platelet
- Therapeutic effect
Top Ten Coagulation Problems

Unreliable D-dimer Assay Results
Variations in reporting units and computation

D-dimer Units Are Confusing
- Fibrinogen equivalent units Vs. D-dimer units based on relative MW
  - FEU: VTE limit is ~500 ng/mL
  - DDU: VTE limit is ~250 ng/mL
  - Some laboratories conflate the two reporting systems
- Unit confusion: ng/mL, ug/mL, mg/dL, mg/L
  - Many labs compute report by hand with high error rate
  - 39% of labs report a VTE limit higher than the MFR-established limit
    - Many don’t know how they generated the limit
    - Often the limit is within the lab-generated reference range

Fibrin Degradation Products and D-dimer

D-dimer Technology Variability
- Monoclonal antibodies (MABs) vary in affinity and specificity
  - The range of MABs bind FDPs of varying MW
  - Identifying all as D-dimer
- No international standard or calibrator
- No limit established for DIC
  - Does 2000 ng/mL FEUs (1000 DDUs) indicate DIC?
  - D-dimer is an acute phase reactant that rises in inflammation
- Many labs attempt to exclude VTE using semiquantitative card test

Solutions to D-dimer Variability
A Missed-diagnosis Crisis
- Laboratories must validate reporting method, math formulas, VTE thresholds
- Need international D-dimer standard
- Need a single reporting convention
- Need electronic formulas that enable laboratories to report consistent results
PT/INR Variability

Instrument Variation
Reagent Variation
MNPT and ISI Variation

INR = (PT\textsubscript{Patient}/MNPT)^{ISI}

- Where...
  - INR = international normalized ratio
  - PT\textsubscript{Patient} = patient prothrombin time
  - MNPT = mean normal PT (computed at site)
  - ISI = international sensitivity index

- ISI computation
  - PT on ≥60 patient specimens in all ranges
  - Test all ≥ 80 using international reference preparation (IRP) or surrogate and mfr's thromboplastin
  - Regress paired results

CV\textsubscript{(INR)} = CV\textsubscript{(PT/MNPT)} \times CV\textsubscript{(ISI)}

INR Variation Among Reagents

<table>
<thead>
<tr>
<th>PT, sec</th>
<th>Rgt A</th>
<th>Rgt B</th>
<th>Rgt C</th>
<th>Rgt D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean of 9</td>
<td>16.1</td>
<td>16.9</td>
<td>13.7</td>
<td>17.5</td>
</tr>
<tr>
<td>Patient 10</td>
<td>67.3</td>
<td>81.9</td>
<td>169</td>
<td>78.3</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>INR</th>
<th>Rgt A</th>
<th>Rgt B</th>
<th>Rgt C</th>
<th>Rgt D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean of 9</td>
<td>1.5</td>
<td>1.3</td>
<td>1.2</td>
<td>1.5</td>
</tr>
<tr>
<td>Patient 10</td>
<td>31.3</td>
<td>8.7</td>
<td>15.4</td>
<td>9.3</td>
</tr>
</tbody>
</table>

Sandy Harmon and Lynne Quarles, 1997, unpublished

What Affects the INR?

- CV could be ≤15% How?
  - PT assay CV ≤5%, 205 when INR >4.5
  - MNPT local computation CV ≤5%
  - ISI manufacturers computation ≤5%. Why?

- IRP PTs are determined visually
  - Manufacturers use 2° thromboplastin to compute IsIs
  - Some are 2-3 calibration steps away from IRP

- The ISI is generalized from representative coagulometers to local instruments

- CLSI: ISI must be locally validated

Local Direct INR Calibration

- Manufacturer distributes certified plasma set
  - 1 normal, 3-5 coumadin plasmas INR 1.5 - 4.5
  - INR assigned by visual analysis from at least two labs

- Perform duplicate PTs for 3 runs
  - Compute CV% for run to run variation, reject over 5%
  - Plot mean PTs on Y scale against INR on X on log-log

- Convert PT directly to INR from the graph
  - Eliminates ISI and MNPT
  - Performed worldwide except USA
  - FDA-cleared: Beckman-Coulter calibration set prepared only for BCI instruments and methods
Comparative Regression Analysis

• Compare old and new thromboplastin
  – Assay 100 regular run plasma samples of wide PT range
  – Compute regression
  – Plot log INR of existing reagent on x-axis
  – Plot log PT of new reagent on y-axis

• Purpose
  – Verify manufacturer ISI
  – Establish local INR direct from PT
  – Verify by external quality assessment

Funk DM. Tips from the clinical experts. MLO 2009;April,38-39.

Von Willebrand Disease (VWD)

• Autosomal mucocutaneous bleeding caused WVF deficiency or dysfunction
• WVF is a 5–20 million Dalton MW glycoprotein that binds platelets to injury sites and stabilizes coagulation factor VIII
• WVF deficiency impairs platelet adhesion and reduces FVIII activity
• Prevalence 0.6–1.3% in random population

# Top Ten Coagulation Problems

## Bleeding Reported by Healthy Subjects and All Types of VWD

<table>
<thead>
<tr>
<th>Symptoms</th>
<th>Normals (%)</th>
<th>VWD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epistaxis</td>
<td>4.6–22.7</td>
<td>38.1–62.5</td>
</tr>
<tr>
<td>Menorrhagia</td>
<td>23–68.4</td>
<td>47–60</td>
</tr>
<tr>
<td>Bleeding after dental extraction</td>
<td>4.8–41.9</td>
<td>28.6–51.5</td>
</tr>
<tr>
<td>Ecchymoses</td>
<td>11.8–50</td>
<td>49.2–50.4</td>
</tr>
<tr>
<td>Bleeding from minor cuts or abrasions</td>
<td>0.2–33.3</td>
<td>36</td>
</tr>
<tr>
<td>Gingival bleeding</td>
<td>7.4–47.1</td>
<td>26.1–34.8</td>
</tr>
<tr>
<td>Postoperative bleeding</td>
<td>1.4–28.2</td>
<td>19.5–28</td>
</tr>
</tbody>
</table>


## Type 1 VWD Profile (70%)

<table>
<thead>
<tr>
<th>Assay</th>
<th>Patient</th>
<th>RI</th>
</tr>
</thead>
<tbody>
<tr>
<td>VWF:Ag</td>
<td>34%</td>
<td></td>
</tr>
<tr>
<td>VWF:RCo</td>
<td>37%</td>
<td>65–140 Units/dL</td>
</tr>
<tr>
<td>VWF:CB</td>
<td>20%</td>
<td></td>
</tr>
<tr>
<td>VWF:Imm</td>
<td>22%</td>
<td>0.94</td>
</tr>
<tr>
<td>VWF:RCo/VWF:Ag</td>
<td>1.08</td>
<td>Type 2 &lt;0.7</td>
</tr>
<tr>
<td>VWF:Imm/VWF:Ag</td>
<td>0.94</td>
<td>Type 2 &lt;0.7</td>
</tr>
<tr>
<td>VWF:CB/VWF:Ag</td>
<td>1.14</td>
<td>Type 2 &lt;0.7</td>
</tr>
<tr>
<td>Factor VIII</td>
<td>38%</td>
<td>55–150%</td>
</tr>
<tr>
<td>RIPA</td>
<td>&lt; 60%</td>
<td></td>
</tr>
<tr>
<td>VWF multimers</td>
<td>Not done</td>
<td></td>
</tr>
</tbody>
</table>

## 2004–2009 NASCOLA VWD Proficiency Test CVs

<table>
<thead>
<tr>
<th>Assay</th>
<th>Normals (7 Surveys)</th>
<th>Type 1s (7 Surveys)</th>
<th>Type 2s (3 Surveys)</th>
</tr>
</thead>
<tbody>
<tr>
<td>VWF:Ag</td>
<td>119</td>
<td>115</td>
<td>13%</td>
</tr>
<tr>
<td>VWF:RCo</td>
<td>20%</td>
<td>30%</td>
<td>42%</td>
</tr>
<tr>
<td>VWF:Imm</td>
<td>15%</td>
<td>14%</td>
<td>49%</td>
</tr>
<tr>
<td>VWF:CB</td>
<td>21%</td>
<td>14%</td>
<td>30%</td>
</tr>
<tr>
<td>VWF:RCo/VWF:Ag</td>
<td>22%</td>
<td>32%</td>
<td>49%</td>
</tr>
<tr>
<td>VWF:Imm/VWF:Ag</td>
<td>19%</td>
<td>20%</td>
<td>53%</td>
</tr>
<tr>
<td>VWF:CB/VWF:Ag</td>
<td>23%</td>
<td>12%</td>
<td>43%</td>
</tr>
</tbody>
</table>

Top Ten Coagulation Problems

**Ristocetin Cofactor Standard Curve**

VWF activity by best-fit correlation to reference dilutions

**Collagen Binding Assay: VWF:CB**


**VWF Activity Immunoassay**

- Automated 12-minute immunoassay
- Anti-VWF monoclonal Ab on latex particles
  - Directed against patient VWF GPIb receptor ligand
  - Agglutination proportional to VWF activity
  - Transmitted light scattered by agglutinates
- Compares favorably with the VWF:RCo


**Laboratory and Clinical Solutions**

- Improve VWF:Imm, VWF:CBA
- Use WHO standard for VWF:RCo
- Repeat testing to confirm, test kindred
- Physical stress, surgery, exercise, anxiety, inflammation, pregnancy, OCs raise VWF
- Lowest on days 1–4 of menstrual cycle
- Clinical consideration: 30%–50% is named “Low VWF”


**Lupus Anticoagulant Testing**

- Mix plasma with platelet-poor normal plasma (NP) and perform PTT on mix
  - 1:1 sensitive to deficiencies, may miss inhibitor
  - 1:4 sensitive to weak inhibitors, may miss mild deficiency
- Correction of PTT of the mix to within 10% of NP (Rosner index)
  - Factor deficiency
  - 1 or 2 hour incubated mixing study follow-up
- No correction: PTT remains >13% of NP
  - Inhibitor is present

The Fritsma Factor

1. If DRVVT is normal, proceed to Hepzyme®. If DRVVT is prolonged, Hepzyme is unnecessary.
2. If the Staclot LA is positive and the DRVVT is negative, assay FVIII to determine if the Staclot LA is due to a FVIII inhibitor.

PTT-LA® long Mix test plasma 1:1 with NP, incubate 2h, perform DRVVT on mix
No LA

PTT-LA® long PTT-LA mix corrects
No LA

Staclot LA

Hepzyme®

Repall PTT-LA

Pos

Neg

LA

No LA

Suspect factor deficiency

PTT-LA® long PTT-LA mix no correction

Lac Anticoagulant Testing

Table 3: APTT reagent usage among laboratories that use both low and high LAC responsive reagents: Fritsma Factor survey respondents

<table>
<thead>
<tr>
<th>Number of Laboratories*</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
</tr>
<tr>
<td>1</td>
</tr>
</tbody>
</table>

*Laboratories represent a subgroup (32%) of all survey respondents

The Fritsma Factor

Monitoring Heparin Therapy
Physician/Nurse Standard Dosage

- Standard unfractionated heparin (UFH)
- Perform "baseline" PTT to ensure patient has no deficiencies or inhibitors
- Initiate therapy: 5–10,000 U bolus
- IV 1600 U/Kg/H
- 4–24 hours after bolus, collect 2nd PTT
- Adjust dose to PTT therapeutic range
  - Traditional range: 1.5–2.5 MNR
  - Never use the traditional range

Lab Tests Used to Monitor UFH

- PTT: responds to effect of heparin-antithrombin on thrombin and Xa
- Activated clotting time (ACT, surgical suite)
  - Normal mean 120 sec
  - Angioplasty: 200–300 sec
  - Coronary bypass, heparin at 5 Units/mL: 480 sec
- Chromogenic anti-Xa heparin assay
  - Therapeutic range 0.3–0.7 Units anti-Xa heparin/mL

Anti-Xa Heparin Assay
Performance Characteristics

- May be calibrated for UFH, LMWH, or pentasaccharides
- Not affected by inhibitors, factor deficiencies, or elevated factor levels
- Reliance on patient antithrombin means test is sensitive to antithrombin deficiency

Establishing The PTT Heparin Therapeutic Range

- Collect ≥ 50 heparin patient specimens
  - All dosage ranges
  - No oral anticoagulant, PT normal
- Collect ≥ 20 normals, both sexes
- Perform PTT and anti-Xa heparin on all
- Prepare linear graph of paired results
- Correlate PTT range to the anti-Xa therapeutic range of 0.3–0.7 Units/mL
- Prophylactic range 0.1–0.4 Units/mL

HEPARN THERAPEUTIC RANGE

American Diagnostica HEPTEST®

Clot time inversely proportional to heparin concentration
Factor V or Factor V Leiden Mutation?

- Factor V activity assay is rarely ordered
  - Liver disease diagnosis
  - Congenital single factor V deficiencies
  - Bovine fibrin glue inhibitor
- Factor V Leiden mutation is often ordered
  - The factor V Leiden mutation is present in 3-8% of Caucasians, Arabs and Hispanics
  - Confers a 2x to 8x risk of thrombosis
  - 19-fold when homozygous
  - Part of a thromophilia profile
  - Screen using activated protein C resistance ratio (APCR)

Factor II or Factor II 20210 Mutation?

- Factor II activity assay is rarely ordered
  - Congenital single factor II (prothrombin) deficiencies
- The factor II 20210 mutation test is ordered often
  - The factor II 20210 (prothrombin) mutation is present in 2-3% of Caucasians, Arabs and Hispanics and confers a 2- to 4.8-fold risk of thrombosis
  - Ordered as part of a thromophilia profile, same volume as the FVL
- Or: prothrombin Vs prothrombin mutation

Protein C Activity, C-reactive Protein, or Activated Protein C Resistance?

- The protein C activity assay and the APCR are part of the thromophilia profile
  - Protein C deficiency
  - Reduced APCR predicts factor V Leiden mutation
- C-reactive protein (CRP, hsCRP) is a marker of inflammation ordered as part of a cardiovascular risk profile

Factor X or anti-Xa Heparin Assay?

- Factor X assay is rarely ordered
  - Congenital single factor X deficiencies
- The anti-Xa heparin assay often ordered
  - The anti-Xa test (anti-factor Xa, hepar-test) is used to monitor UFH therapy when the PTT is ineffective
  - The anti-Xa test is the only test that works for monitoring LMWH or pentasaccharides
- Rename heparin assay?
- Also—transpositions and deletions
  - IX and XI, VII, VIII

Coagulation Test Name Solution?

- Logical Observation Identifiers Names and Codes (LOINC®, www.LOINC.org)
  - Universal codes for identifying laboratory and clinical observations
  - Mapping terms to LOINC makes it possible to exchange and pool data from many systems for clinical care and research
- Laboratory Medicine Best Practices: CDC
  - “Appropriate Laboratory Test Selection: A Major Challenge”: CDC “Thesaurus” project
  - www.futurelabmedicine.org

www.fritsmafactor.com
PT and PTT Falsely Prolonged

- Short draw, < 2.7 mL
- Tolerances are narrow in small tubes
- Observe air space in butterfly tubes
- Failure to mix by gently inverting X6
- Blood clots form when AC and blood do not mix. Inspect all specimens for clots.
- Improper storage—too long at room temperature
- Specimen for PTT must be tested in 4h PT 24h or the plasma separated and frozen at -70ºC

PT and PTT Falsely Shortened

- Hemolysis caused by shaking
- Hemolysis and platelet activation triggers coagulation at an early stage. Reject all specimens with visible hemolysis.
- Hemolysis caused by slow collection, defective equipment
- Refrigerating or shipping on ice
- Chilling to 4ºC activates FVII and precipitates von Willebrand factor.
- Lipemia and icterus
- May shorten optical instrumentation results.

Inadequate Centrifugation

- Platelets release phospholipids, coagulation factors, and platelet factor 4
  - Phospholipids neutralize lupus anticoagulants
  - PF 4 neutralizes heparin
- Failure to produce platelet-free plasma
  - Double centrifugation, centrifuge at 2000 g
- PTT loses sensitivity for lupus anticoagulants and heparin
- Factor assays inaccurate

Recollects

- Factor V mutation (Leiden) assay may require EDTA, but blue-stoppered tube is collected or...
- Factor V mutation (Leiden) collected in citrate tube, lab erroneously centrifuges
Order of Draw

- Collect discard tube?
  - NCCLS H21-A5 directs that the first tube may be used
- Do not follow additive tube
  - Plastic red-closure tubes have particulate activator
  - Serum separator tubes have particulate activator
- Vascular access device
  - Correct-fitting syringe
  - Flush with saline
  - Discard 5 mL blood

Effective Ordering and Interpretation of Thrombophilia Profiles

- FVIII and VWF rise during infection and inflammation
- Protein C and antithrombin are consumed in sepsis and DIC
- Antithrombin drops in L-asparaginase Rx, hepatic sinusoidal veno-occlusion, nephrotic syndrome
- Acquired APC resistance in pregnancy and OCRs, raised estrogens
- Protein S drops in pregnancy, primary varicella infection
- OCRs odds ratio is 4x, in heterozygous factor V Leiden, 35x
- Obesity, smoking, and immobility have far greater impact than thrombosis risk factors

Thrombophilia Assays, Prevalence, Risk

<table>
<thead>
<tr>
<th>Assay</th>
<th>Prevalence</th>
<th>Venous Thrombosis Prevalence</th>
<th>Odds for Thrombosis</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>APCR</td>
<td>3-8% of Caucasians, Hispanics, Arabs</td>
<td>20-25%</td>
<td>Het: 2-7x, Hom: 18+</td>
<td>Confirm positives with FVL mutation test</td>
</tr>
<tr>
<td>FII20210</td>
<td>4-8%</td>
<td>4-8%</td>
<td>Het: 2-6+</td>
<td>Molecular assay only</td>
</tr>
<tr>
<td>Antithrombin deficiency</td>
<td>1 in 2-5000</td>
<td>1%</td>
<td>10-20x</td>
<td>Do not test during AC Rx or active clotting</td>
</tr>
<tr>
<td>Protein C deficiency</td>
<td>1 in 300</td>
<td>1%</td>
<td>6x</td>
<td>Perform immunocassay only when activity is consistently low</td>
</tr>
<tr>
<td>Protein S deficiency</td>
<td>Not known</td>
<td>2-10%</td>
<td>1.6-11.5x</td>
<td>Reduction does not change outcome</td>
</tr>
<tr>
<td>Homocysteinemia</td>
<td>Not known</td>
<td>Not known</td>
<td>2.5x</td>
<td></td>
</tr>
<tr>
<td>LA</td>
<td>1-2%</td>
<td>1-2%</td>
<td>35%</td>
<td>Acquired</td>
</tr>
</tbody>
</table>

Common Errors in Thrombophilia Testing

- Failure to include all relevant profile members
- Irrelevant assays
  - MTHFR C677T: No clinical correlation
  - Homocysteinemia: Reducing does not change outcome
- Ordering PC, PS, AT soon after thrombotic event, during inflammation, or while patient is on anticoagulant therapy
- Failure to confirm abnormal screens
Top Ten Coagulation Problems

10/14/11

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Top Ten Coagulation Problems

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Direct Thrombin Inhibitors (DTIs)

- Indication: heparin-induced thrombocytopenia (HIT)
  - Do not generate or bind anti-heparin-PF4
  - Platelet counts recover within three days
- Rapidly reduce thrombin production in HIT
  - Warfarin too slow
  - LMWH may cross-react
  - Fondaparinux OK


Argatroban (Novastan®)

- Raises nitric oxide, causing vasodilatation
- Metabolized and excreted by liver CYP450
- IV: 2 mcg/kg/m 5–7 d: immediate steady state
- PCI: bolus 350 mcg/kg; continuous infusion 15–40 mcg/kg/m

Argatroban Comments

- Safe to use in renal disease
- Liver disease
  - Reduce to 0.5 mcg/kg/h and monitor
  - Major bleeds 5.3%, minor 14.4%
- No antidote, but half-life is 40 minutes
- Inhibits free and fibrin-bound thrombin

Hirudin: Lepirudin

- Inhibits free, not bound thrombin
- Metabolized and excreted by kidney
  - Monitor when GFR <30 mL/min
- Immune response: anaphylaxis

Lepirudin Administration

- IV bolus: 0.4 mg/kg/h
- Infusion 0.1–0.15 mg/kg/h 11–14 d
- Steady state within 2.5 hours
- Clearance half-life 20 minutes
- Coronary bypass: 0.25 mg/kg/h
**Bivalirudin**
- Thrombin active site-directed peptide, D-Phe-Pro-Arg-Pro, linked to an analogue of the carboxy-terminal of hirudin
- Neutralizes free and bound thrombin
- FDA-cleared spring, 2008
- Reduced major hemorrhage by 41% to 61%
- Cleared for use with aspirin
- Bolus 0.75 mg/kg + 1.75 mg/kg/h infusion
- Renal excretion, 25 m half-life
- If GFR is <30 mL/minute, reduce to 1 mg/kg/h, no reduction in bolus
- If a patient is on hemodialysis, reduce infusion to 0.25 mg/kg/h

**Dabigatran (Pradaxa®)**
- Oral DTI cleared for prophylaxis in Canada and Europe 2009
  - Indication: post-surgical VTE prevention
- Cleared for prevention of stroke in atrial fibrillation in US 2010
- 110 mg/d with wide safety range
  - Immediate steady state
  - Monitoring: same reasons as LMWH
- Binds clot-bound and free thrombin
- Renal excretion 80%
  - Reduce dosage and monitor when GFR < 30 mL/min
- Half-life 12–17 hours
- No interaction with food
- Not metabolized by CYP450 pathway
- Levels raised by quinidine and verapamil
- Predictable efficacy
- No liver toxicity
- Dyspepsia

**Monitoring DTIs**
- PTT: use 1.5–3x mean of reference interval
  - Sensitivity varies by formulation
- ACT during coronary bypass
- Ecarin clotting time
- Ecarin chromogenic assay
- Thrombin inhibition assay

**Ecarin Clotting Time/Chromogenic**
- Stago Ecarin Chromogenic Assay®
  - Intensity at 405 nm is inversely proportional to DTI concentration
  - Saw-scales Viper: Echis carinatus
Top Ten Coagulation Problems

BIOPHEN DTI® Chromogenic

RUO

DTI

Intensity at 405 nm is inversely proportional to DTI concentration

BIOPHEN Hemoclot® Thrombin Inhibitors

RUO

Clot time is proportional to DTI concentration

1: New Oral A/C

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