Hemostasis Lab Challenges – Preanalytical problems in Haemostasis

- Patient ID
- Collecting blood – needle, catheters, discard tube, order of draw
- Anticoagulant, tube composition
- Sample mixing
- Sample transport
- Sample storage
- Sample processing
- Sample quality checks - haemolysis, filling

In most cases errors are directly related to specimen collection!

- Hemolyzed sample
- Insufficient sample
- Incorrect sample
- Others

Preanalytical Sample Integrity (PSI) Checks

1\textsuperscript{st} volume check

2\textsuperscript{nd} volume check

Absorbance

Wavelength (nm)

Lipemia
Hemolysis
Icterus

Hemolytic
Correct first time

Preanalysis

Analysis

Results
Preanalytical Sample Integrity (PSI) Checks

<table>
<thead>
<tr>
<th></th>
<th>405 nm</th>
<th>575 nm</th>
<th>660 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemolysis</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Icterus</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Lipemia</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

**Absorption spectrum**

- **Bilirubin-C**
- **Hemoglobin**
- **Lipid**
- **Intra Fat**
HIL - Frequency of samples rejection

Example of Haemolysis Plasma Haemoglobin
Preanalytical Sample Integrity (PSI) Checks

<table>
<thead>
<tr>
<th>Hemoglobin</th>
<th>0/10</th>
<th>1/10</th>
<th>2/10</th>
<th>3/10</th>
<th>4/10</th>
<th>5/10</th>
<th>6/10</th>
<th>7/10</th>
<th>8/10</th>
<th>9/10</th>
<th>10/10</th>
</tr>
</thead>
<tbody>
<tr>
<td>mg/dL</td>
<td>0</td>
<td>48</td>
<td>97</td>
<td>145</td>
<td>194</td>
<td>242</td>
<td>290</td>
<td>339</td>
<td>387</td>
<td>436</td>
<td>484</td>
</tr>
<tr>
<td>CS-2000i</td>
<td>0 (0.1)</td>
<td>1 (0.6)</td>
<td>1 (1.1)</td>
<td>2 (1.5)</td>
<td>3 (2.0)</td>
<td>4 (2.5)</td>
<td>4 (2.8)</td>
<td>5 (3.3)</td>
<td>5 (3.5)</td>
<td>5 (3.6)</td>
<td>5 (3.6)</td>
</tr>
</tbody>
</table>

Color tone

Check Level
HIL - What level of haemolysis is routinely encountered?

59 consecutive haemolysed samples rejected for visible haemolysis*

*Example from systems validations in the UK (Sheffield Teaching Hospitals)

Low Hb concentration are more frequent.
HIL - Effect of Haemolysis

Is the effect greater at higher levels of haemolysis?
Patient samples APTT

Δ difference H/lsed - clear APTT (sec)

Plasma Hb (g/l)

Slight hemolysis have an higher impact?
Hemostasis Lab Challenges—Preanalytical Risks to Diagnostic Accuracy

- Increasing levels of hemolysis are associated with shorter APTT results\(^1\)

- Such a shift can cause abnormal samples to produce normal results\(^1\)

The green and blue lines/symbols describe the effect of increasing levels of hemolysis on control blood samples, while the orange and maroon lines/symbols describe the effect on the same samples spiked with unfractionated heparin (0.15 IU/ml).


Reference interval of APTT Test on STA Compact System: 27.6-39.2

Reference interval of APTT Test on Sysmex CS-2100i System: 24.6-31.2

Data on file, Siemens Healthcare Diagnostics

\(^1\) Increasing levels of hemolysis are associated with shorter APTT results. Such a shift can cause abnormal samples to produce normal results.
HIL - Effect of Haemolysis

- 6 normal subjects
- Vacutainer 0.109M trisodium citrate (3.2%)
- Whole blood frozen
- Lysed cells added to plasma from same subject
- 12 different mixtures prepared
- Mean plasma haemoglobin 0 – 20 g/l (10-15% haemolysis)

**REAGENTS:**

- PT reagent
- APTT reagent
- Second APTT reagent
- TT reagent
- Fib (Clauss reagent)
- D-Dimer reagent
HIL - Effect of Haemolysis

Influence of Hemolysis on APTT results is patient dependent.
HIL - Effect of Haemolysis

Artifical haemolysis
second APTT reagent - CS series

Influence of Hemolysis on APTT results is patient and/or reagent dependent.
### HIL - Effect of Haemolysis

#### Samples falsely raised above DDimer above the VTE cutoff

(0.5 µg/ml)

<table>
<thead>
<tr>
<th>Haemolysed DDimer (µg/ml)</th>
<th>Clear DDimer (µg/ml)</th>
<th>Plasma Hb (g/l)</th>
<th>HIL check</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.95</td>
<td>0.19</td>
<td>0.5</td>
<td>1</td>
</tr>
<tr>
<td>1.53</td>
<td>0.24</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>2.7</td>
<td>0.35</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>2.98</td>
<td>0.48</td>
<td>5</td>
<td>5</td>
</tr>
</tbody>
</table>

➡️ Potentially unnecessary ultrasounds COST?
Underfilling Tubes
Case Report

Effect of under filling the tube

• First sample – PT 104 seconds (properly filled 1 ml sample)

• Second sample (no treatment) – PT 14 secs (properly filled 5ml sample)

• ? Artefact  ? Wrong patient
Underfilling Tubes
Case Report

Effect of under filling the tube

• Third sample – PT 14 seconds

• First sample
  - mixed 0.5 ml blood with 0.5ml anticoagulant in a 5 ml tube
  - this mix was added to a paediatric (1 ml) tube with further 0.1 ml anticoagulant

⇒ CITRATE excess prolonged PT from 14 sec to 104 seconds
Underfilling Tubes

APTT / CA1500
(Mean of 20 normals)

It is admitted that a threshold of 80% filling is the ultimate lower limit.
(Ray et al., 1991)

➔ Underfilling of the tube can have a significative impact on APTT time
HIL – recommendations from recent studies

Test Specific assessment of effects of haemolysis on citrated blood samples using Sysmex CS series analysers

- Issued from the collaboration between Sysmex and Royal Hallamshire Hospital Sheffield – UK
- On CS series analysers with Siemens reagents
- To assess test specific thresholds for effects of haemolysis on coagulation tests.
### HIL – recommandations from recent studies

<table>
<thead>
<tr>
<th>TEST</th>
<th>REAGENT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prothrombin Time (PT)</td>
<td>Dade Innovin Thromborel S</td>
</tr>
<tr>
<td>Activated Partial Thromboplastin Time (APTT)</td>
<td>Dade Actin FS Activated PTT Reagent Dade Actin FSL Activated PTT Reagent</td>
</tr>
<tr>
<td>Antithrombin (AT)</td>
<td>INNOVANCE Antithrombin</td>
</tr>
<tr>
<td>Protein C (PC)</td>
<td>Berichrom Protein C</td>
</tr>
<tr>
<td>D-Dimer (DD)</td>
<td>INNOVANCE D-Dimer</td>
</tr>
<tr>
<td>Factor XIII (FXIII)</td>
<td>Berichrom FXIII</td>
</tr>
<tr>
<td>Clauss Fibrinogen (CFbg)</td>
<td>Dade Thrombin Reagent</td>
</tr>
<tr>
<td>Thrombin Time (TCT)</td>
<td>Test Thrombin Reagent</td>
</tr>
</tbody>
</table>

All reagents were from Siemens Healthcare Diagnostics
HIL – recommandations from recent studies

METHODS

• Freeze thaw lysed red cells were added to plasma to establish the level of free Hb required to generate flags of 1 to 5

• Rejected haemolysed patient samples (n=20) were analysed alongside matched samples from the same subjects collected within 4 hours of the reject sample and which lacked visible haemolysis

• A series of plasma pools were spiked with haemoglobin

• The flag level required to alert when a test result was altered by >10% (Antithrombin, Protein C) or >15% (other tests) as a consequence of haemolysis was assessed for a series of tests
HIL – recommandations from recent studies
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## HIL – recommendations from recent studies

<table>
<thead>
<tr>
<th>Assay</th>
<th>Parameter</th>
<th>Acceptance Criteria</th>
<th>Recommendations</th>
<th>Hb at Flag level</th>
</tr>
</thead>
<tbody>
<tr>
<td>PT INN</td>
<td>sec</td>
<td>*+-15%</td>
<td>Level 4 or Level 5 without D-Fib</td>
<td>240 mg/dl</td>
</tr>
<tr>
<td></td>
<td>INR</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>D-Fib</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>sec</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PT THS</td>
<td>sec</td>
<td>*+-15%</td>
<td>Level 5</td>
<td>300 mg/dl</td>
</tr>
<tr>
<td></td>
<td>INR</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>D-Fib</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>APTT FS</td>
<td>sec</td>
<td>*+-15%</td>
<td>Level 1</td>
<td>40 mg/dl</td>
</tr>
<tr>
<td>APTT FSL</td>
<td>sec</td>
<td>*+-15%</td>
<td>Level 1</td>
<td>40 mg/dl</td>
</tr>
<tr>
<td>Fbg</td>
<td>g/L</td>
<td>*+-15%</td>
<td>Level 5</td>
<td>300 mg/dl</td>
</tr>
<tr>
<td>Thrombin Time</td>
<td>sec</td>
<td>*+-15%</td>
<td>Level 5</td>
<td>300 mg/dl</td>
</tr>
<tr>
<td>AT</td>
<td>%</td>
<td>*+-10%</td>
<td>Level 3</td>
<td>180 mg/dl</td>
</tr>
<tr>
<td>PC</td>
<td>%</td>
<td>*+-10%</td>
<td>Level 4</td>
<td>240 mg/dl</td>
</tr>
<tr>
<td>FXIII</td>
<td>%</td>
<td>*+-15%</td>
<td>Level 1</td>
<td>40 mg/dl</td>
</tr>
</tbody>
</table>
HIL – recommandations from recent studies

Summary

• The level of haemoglobin causing >15% changes was different in spiked samples compared to genuine patient samples for some tests.

• D-Dimer, FXIII and APTT were more tolerant of haemolysis in spiked samples.

• Taking all data into account the following flag levels were required to ensure 15% change in results (10% for AT, PC) with equivalent plasma Hb in brackets.
HIL – recommandations from recent studies

Summary

- Level 5 (>300 mg/dl) - PT (Thromborel S), Clauss Fbg, Thrombin Time;
- Level 4 (240-300 mg/dl) - PT (Innovin), Protein C;
- Level 3 (180-240 mg/dl) - Antithrombin
- Level 1 (40-120 mg/dl) - APTT (Actin FS, Actin FSL), FXIII, D-Dimer.
Preanalytical Sample Integrity (PSI) Checks Onboard—Reducing Preanalytical Risk

- Preanalytical Sample Integrity (PSI™) checks
- Multi-wavelength analysis
- Auto-verification
- Direct LIS connection

Patients and physicians

Preanalysis | Analysis | Review | Results | Accurate diagnosis

Johan Claes / Francois Richir | Laboratory Diagnostics
Now’s our time
to inspire
the future
of healthcare together