Feedback and Status of EBF Dried Blood Spot Consortium

Haematocrit Team

Stephen White on behalf of EBF DBS Consortium

EBF Open Symposium
18 November 2011
Barcelona, Spain
Content

- The team
- The challenge
- The chemical space
- The test plan
- Planning, Conduct & Deliverables
The Haematocrit Sub-Topic Team

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The Challenge

Design & conduct a test plan to answer **key questions**:

- Relationship between Haematocrit (H) and **spot homogeneity**
- Relationship between H and **spot size**
- Relationship between H and **recovery**
- Does **age** of the blood have impact on results
- Influence of **card type** on results obtained at different H
- How does all of the above relate to **chemical space**;
  - Log P; pKa; PPB; Blood/plasma distribution
- Which parameters are compound dependent

**Varying Haematocrit**
20uL Blood Spot, 6mm Punch

- 20%
- 45%
- 70%
Published Haematocrit Ranges (Human Blood)

Table 1. Typical human hematocrit levels.

<table>
<thead>
<tr>
<th>Age</th>
<th>Hematocrit levels (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth</td>
<td>42–64</td>
</tr>
<tr>
<td>Less than 1 month</td>
<td>31–67</td>
</tr>
<tr>
<td>1 month–2 years</td>
<td>28–55</td>
</tr>
<tr>
<td>2–12 years</td>
<td>34–45</td>
</tr>
<tr>
<td>12–18 years, female</td>
<td>36–46</td>
</tr>
<tr>
<td>12–18 years, male</td>
<td>37–49</td>
</tr>
<tr>
<td>Adult female</td>
<td>36–44</td>
</tr>
<tr>
<td>Adult male</td>
<td>41–50</td>
</tr>
</tbody>
</table>

Values for hematocrit measurements can vary depending on the equipment used. Data from [9–11].

Experimental test plan to focus on human blood only (range 20-70%)
Preparing Blood with Varying Haematocrit

- Mix blood and measure haematocrit in duplicate
- Calculate volume of plasma addition/removal required
  - common calculation used by each test site
- Spin blood which requires plasma removal for 10mins @ 1300g
- Add/remove plasma as required
- Gently roller mix prepared blood for 30 mins
- Measure haematocrit of prepared blood in duplicate
The Chemical Space

<table>
<thead>
<tr>
<th>Compound ID</th>
<th>Mwt (g/mol)</th>
<th>log P</th>
<th>pKa</th>
<th>PPB</th>
<th>bl/pl ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>H1</td>
<td>430</td>
<td>2.4</td>
<td>2.2 (base); 8.3 (base)</td>
<td>77%</td>
<td>0.77</td>
</tr>
<tr>
<td>H2</td>
<td>430</td>
<td>&gt; 5</td>
<td>&lt;3 (base)</td>
<td>&gt; 99.8%</td>
<td>0.7</td>
</tr>
<tr>
<td>H3</td>
<td>550</td>
<td>5.14 (pH 6)</td>
<td>1 (base); 9 (base)</td>
<td>&gt; 99.9%</td>
<td>0.65</td>
</tr>
<tr>
<td>H4</td>
<td>470</td>
<td>3.3 (pH 3); 6.0 (pH 7.4)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H5</td>
<td>750</td>
<td>&gt; 4.4</td>
<td>2.5; 5.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>H6</td>
<td>250</td>
<td>2.2</td>
<td></td>
<td></td>
<td>0.6</td>
</tr>
<tr>
<td>H7</td>
<td>470</td>
<td>3.7</td>
<td></td>
<td></td>
<td>0.7</td>
</tr>
<tr>
<td>Zolpidem</td>
<td>307</td>
<td>1.2</td>
<td>6.2</td>
<td>&gt; 99.9%</td>
<td>0.7</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>331</td>
<td>2.3</td>
<td>6.09</td>
<td>20-40%</td>
<td></td>
</tr>
</tbody>
</table>

**Lumefantrine**
- Mwt = 528.89
- Log P = 10.2 (calc)
- pKa = 8.7 (base)
- PPB = 99.9
- (published recovery approx 28%)

**Paclitaxel**
- MW: 853.9 g/mol
- Log P: 3.5
- pKa: 12.0
- PPB: 89-98%
- Blood/plasma ratio: ~1.0

**Tolbutamide**
- MW: 270.3 g/mol
- Log P: 2.2
- pKa: 5.2
- PPB: 97%
- Blood/plasma ratio: ~0.67

Plus endogenous compounds (e.g. amino acids, fatty acids, lipids)
Test Plan - 1

Card types:

- Ahlstrohm 226 untreated
- Agilent BondElut untreated
- GE DMPK-A (formerly FTA) treated
- GE DMPK-B (formerly FTA-Elute) treated

Age of (human) blood:

- Fresh blood (≤1 day old)
- Stored blood (10-14 days old blood)
Test Plan – 2

Haematocrit

- Fresh Blood
  - testing at 8 haematocrit values \( (H = 20, 30, 35, 40, 45, 50, 60, 70) \)
  - Ahlstrohm 226

- Fresh and Stored blood
  - testing at 3 haematocrit values \( (H = 20, 45, 70) \)
  - all card types

- Spot size measurement using Image J software
  - for consistency across test sites

- 3 mm punch from 25 uL blood spot
  - To reflect most common practice
Test Plan - 3

Recovery

- Fresh Blood
  - testing at 3 haematocrit values (H=20, 45, 70)
  - all card types
- Stored Blood
  - testing at 1 haematocrit value (H=45)
  - all card types

- 6 mm punch from 5 uL blood spot (punch whole spot)
- Also blank spots spiked post extraction for calculation of recovery
Test Plan - 4

Spot homogeneity

- 1 mm punch from 25 ul blood spot, both from the middle and from the edge

- Fresh Blood
  - testing at 3 haematocrit values (H=20, 45, 70)
  - all card types

- Stored Blood
  - testing at 1 haematocrit value (H=45)
  - all card types

- Spot size measurement using Image J software
  - for consistency across test sites
Test Plan - 5

Run Acceptance criteria

- One appropriate test sample injected in triplicate at beginning, middle and end of run
- %CV over 9 values ≤15%
- Mean of 3 last injections versus mean of 3 first injections difference ≤15%
Planning, Conduct & Deliverables

- **Planning**
  - 6 TC’s held between mid July and mid Nov to propose & finalize the plan

- **Conduct**
  - Experiments to be conducted between Jan & Feb 2012
  - Data review from 1st test plan Mar 2012

- **Deliverables**
  - Present 1st test plan at EBF OS (Nov 2011)
  - Circulate data from 1st test plan within EBF community (2Q12)
  - Publish findings as part of the EBF Community (2Q12)
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