

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

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- The challenge
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The Haematocrit Sub-Topic Team

Ferrer International

GSK

Janssen R&D

Nuvisan

PRA

Swiss BioQuant

TNO

Jordi Ortiz

Matthew Barfield

Ronald de Vries

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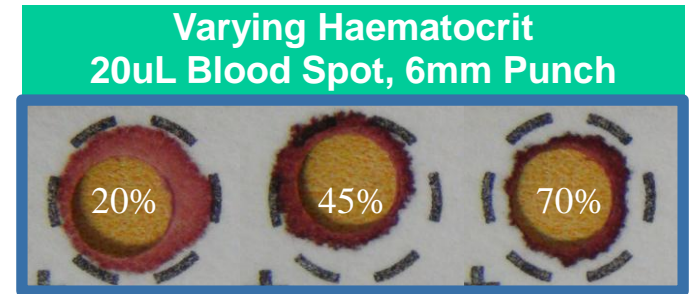
Sponsor (EBF)

Steve White

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



Published Haematocrit Ranges (Human Blood)

Table 1. Typical human hematocrit levels.

Age	Hematocrit levels (%)
Birth	42–64
Less than 1 month	31–67
1 month–2 years	28–55
2–12 years	34–45
12–18 years, female	36–46
12–18 years, male	37–49
Adult female	36–44
Adult male	41–50

*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

Compound ID	Mwt	log P	pKa	PPB	bl/pl ratio
H1	430	2.4	2.2 (base); 8.3 (base)	77%	0.77
H2	430	> 5	<3 (base)	> 99.8%	0.7
H3	550	5.14 (pH 6)	1 (base); 9 (base)	>99.9%	0.65
H4	470	3.3 (pH3); 6.0 (pH 7.4)			
H5	750	> 4.4	2.5; 5.2		
H6	250	2.2			0.6
H7	470	3.7			0.7
Zolpidem	307	1.2	6.2	92.5%	
Ciprofloxacin	331	2.3	6.09	20-40%	

Lumefantrine

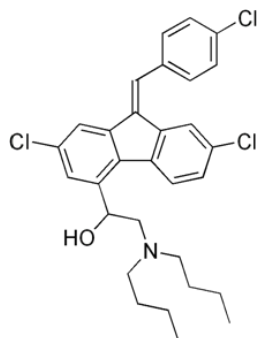
Mwt= 528.89

Log P=10.2 (calc)

pKa=8.7 (base)

PPB= 99.9

(published recovery approx 28%)



Paclitaxel

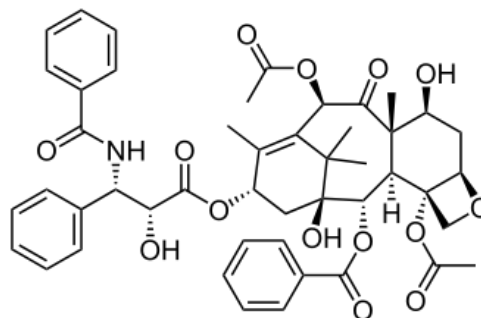
MW: 853.9 g/mol

Log P: 3.5

pKa: 12.0

PPB: 89-98%

Blood/plasma ratio: ~1.0



Tolbutamide

MW: 270.3 g/mol

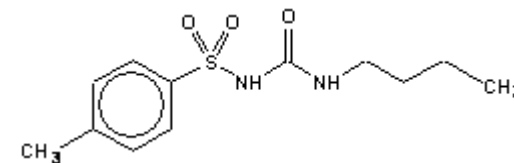
Log P: 2.2

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

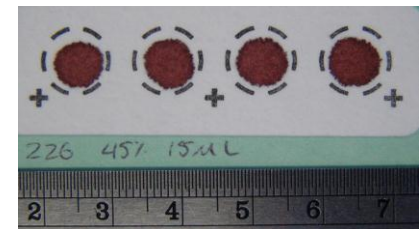
Age of (human) blood:

- 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)
 - Ahlstrom 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 accross test sites
- 3 mm punch from 25 uL blood spot
 - To reflect most common practice



Test Plan - 3

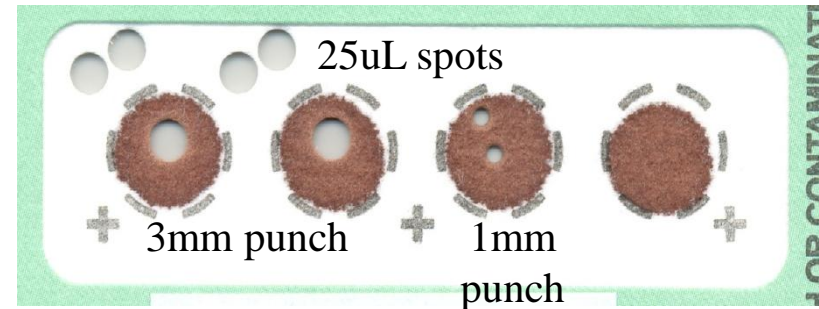
Recovery

- 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 accross 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 $\leq 15\%$
- Mean of 3 last injections versus mean of 3 first injections difference $\leq 15\%$

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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