Feedback from the EBF Liquid Microsampling Consortium

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on behalf of the EBF LMS consortium

EBF 7th Open Meeting

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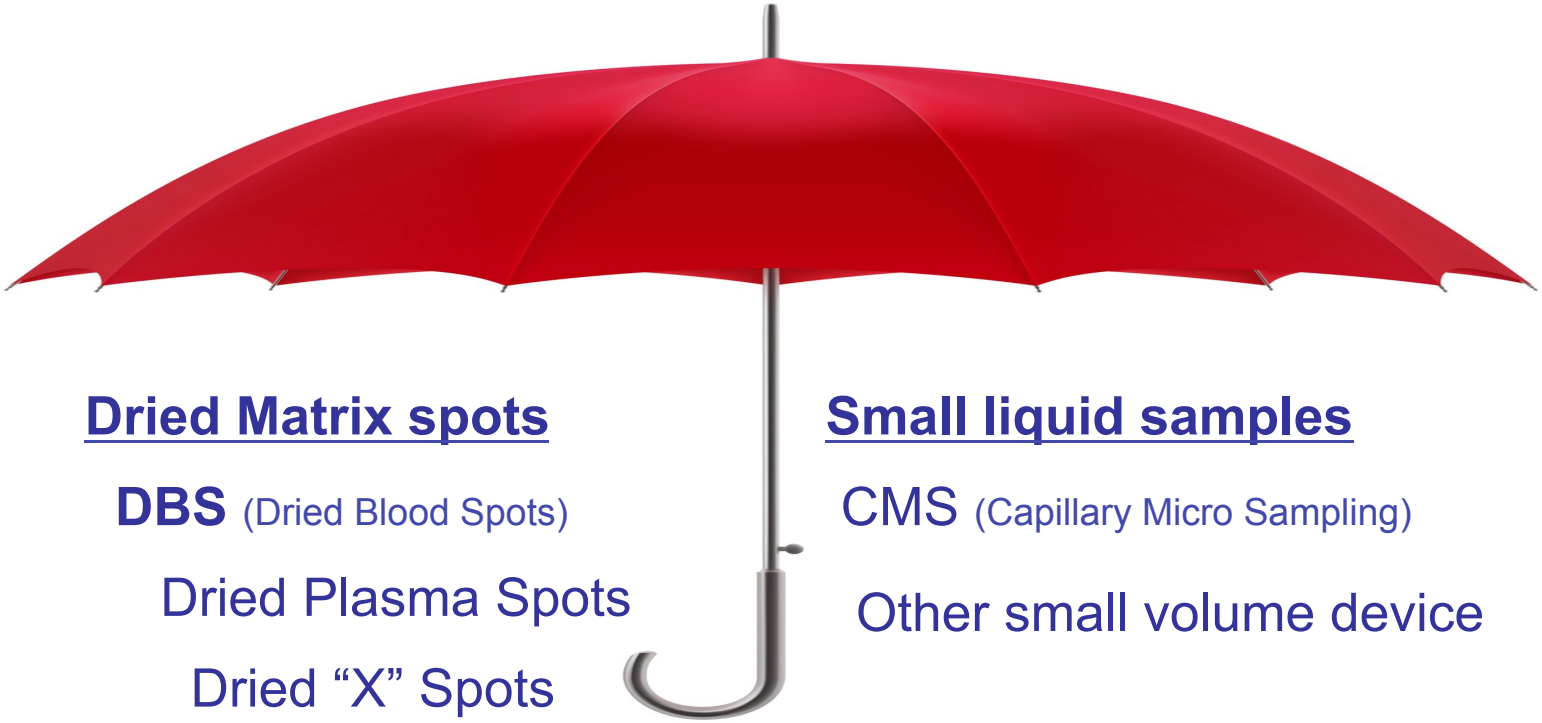
Presentation outline

- History behind EBF and LMS
- Philosophical discussions
  - Sample manipulation
  - Sample homogeneity
- Planned experiments
- Potential experiments
- Reporting back

Introduction

All volumes smaller than 20-50 µL (depending on who you talk with) belong to the ‘micro sampling’ family



Dried Matrix spots

DBS (Dried Blood Spots)

Dried Plasma Spots

Dried “X” Spots

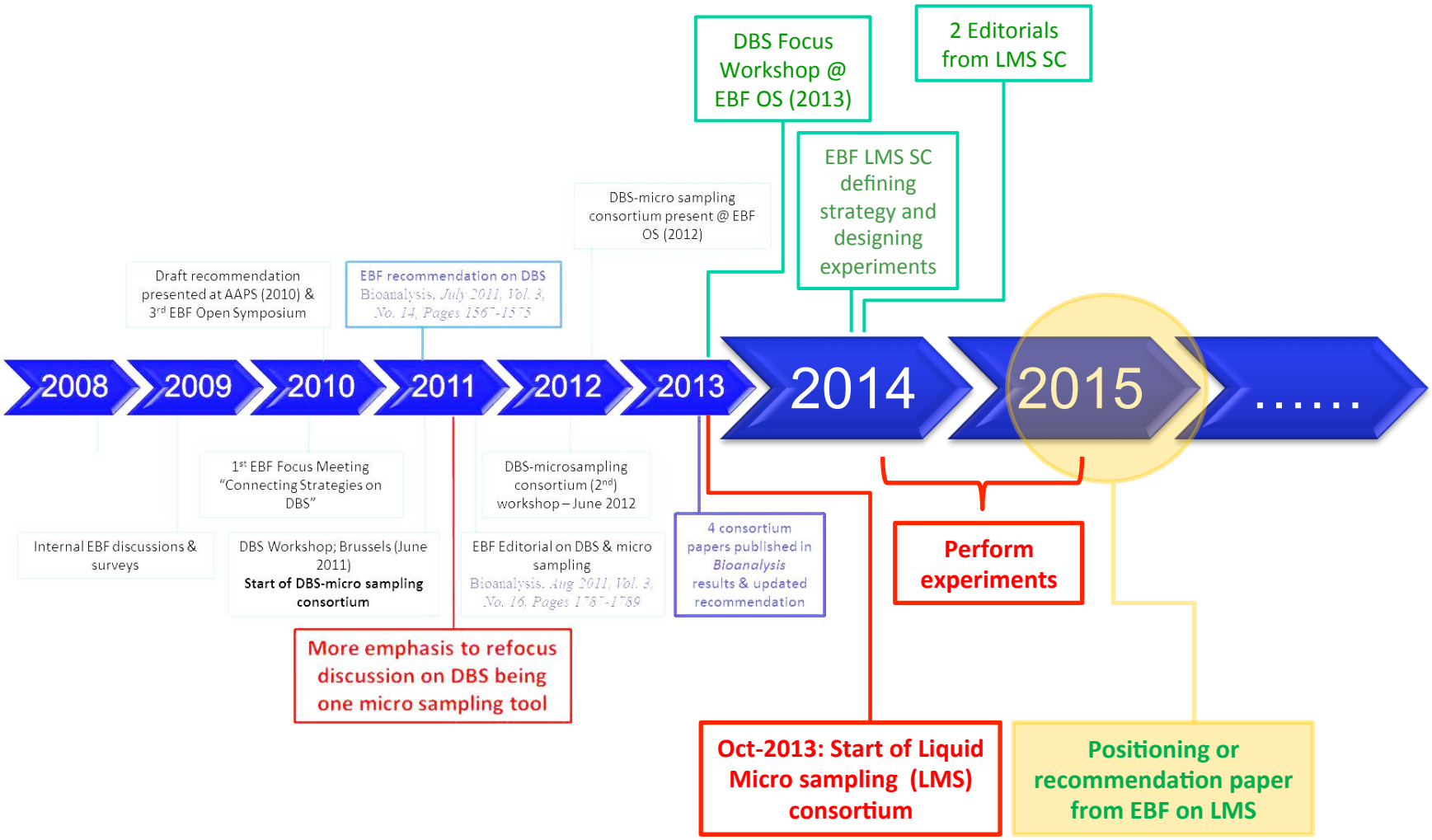
Small liquid samples

CMS (Capillary Micro Sampling)

Other small volume device

Nano tools like lab-on-a-chip, ……

EBF & LMS


DBS Focus Workshop @ EBF OS (2013)
2 Editorials from LMS SC
EBF LMS SC defining strategy and designing experiments
DBS-micro sampling consortium present @ EBF OS (2012)
Draft recommendation presented at AAPS (2010) & 3rd EBF Open Symposium
EBF recommendation on DBS Bioanalysis. July 2011, Vol. 3, No. 14, Pages 1567-1575
2008
2009
2010
2011
2012
2013
2014
2015
.....
1st EBF Focus Meeting "Connecting Strategies on DBS"
DBS-microsampling consortium (2nd) workshop – June 2012
Internal EBF discussions & surveys
DBS Workshop; Brussels (June 2011) Start of DBS-micro sampling consortium
EBF Editorial on DBS & micro sampling Bioanalysis. Aug 2011, Vol. 3, No. 16, Pages 1787-1789
4 consortium papers published in Bioanalysis results & updated recommendation
Perform experiments
More emphasis to refocus discussion on DBS being one micro sampling tool
Oct-2013: Start of Liquid Micro sampling (LMS) consortium
Positioning or recommendation paper from EBF on LMS

Action Plan To Date

- Core team
  - Publications:
    - Editorial: *European Bioanalysis Forum continued plans to support liquid microsampling, Bioanalysis (2014) 6(14), 1897–1900)*
    - Discussion paper: *Reflection on Bioanalytical Assay Requirements used to support liquid microsampling, Bioanalysis (2014) 6 (19) still in press)*
  - Prepare experimental plan for discussion with entire LMS team

- All members
  - Provide feedback on experimental plan
  - Perform agreed experiments and feedback data and finding

EBF LMS Consortium discussion

The core team started with philosophical discussions

- Scope and background
- Sample manipulation questions
- Samples homogeneity questions
- Impact on assay validation
- Blood vs. plasma
- Site of sampling

EBF LMS Consortium discussion

3 main points identified for focus and further discussion

- Impact on assay validation, additional experiments may be required to represent samples and alleviate concerns
  - Matrix stability in small volumes / capillaries
  - Matrix stability of diluted samples
  - Whole blood stability in small volumes / capillaries
- Sample manipulation – to investigate
- Sample homogeneity – to investigate

Philosophical discussion points

Sample manipulation

- Handling of low sample volumes
  - Use suitable pipettes and containers
- Dilution
  - Minimises handling issues of small volumes
  - Time of dilution and by who (sample collection or analysis)
  - Validity of diluted sample for repeat analysis
  - Impact on stability, storage and integrity of sample
- Repeat analysis: what are identical samples?
  - Plasma aliquots from same blood collection
  - Consecutive collected blood samples

Sample manipulation

Current EBF LMS consortium thinking:

- Sample integrity throughout its lifetime (collection, storage and extraction) should be supported by experiments performed during assay development / validation
- Therefore its not crucial whether diluent added on collection or analysis
- Recommend against introducing new semantics such as primary and secondary sample

- Ensure experimental evidence validates your approach
- But also think about what is practical

- Consortium will perform experiments to aid understanding

Sample homogeneity

- Is a microsample at a higher risk of inhomogeneity than a traditional sample?
  - Is this concern real or perceived?
- Factors that may impact on homogeneity
  - Surface area / volume ratios and adsorption
  - Mixing (method of)
  - Freeze-drying / evaporation

- In capillaries:
  - Is the analyte (or other components) homogeneous along length of capillary?

Sample homogeneity

Current EBF LMS consortium thinking:

- It is not yet known if homogeneity is a real or perceived concern
- Targeted experiments will give us a better insight on this topic
- Consortium hopes to generate experimental data

However:

- Experimental evidence could validate your approach
- QCs prepared in same volume and handled in the same way as samples will highlight issues

Experimental Plans

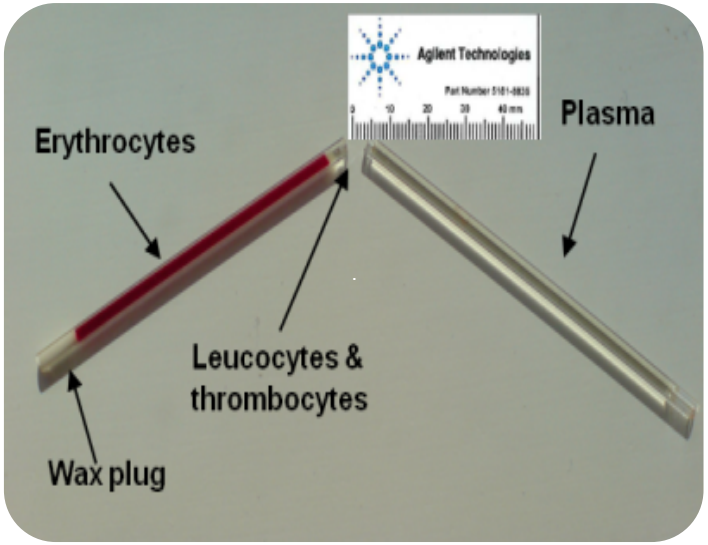
EBF

Experimental work

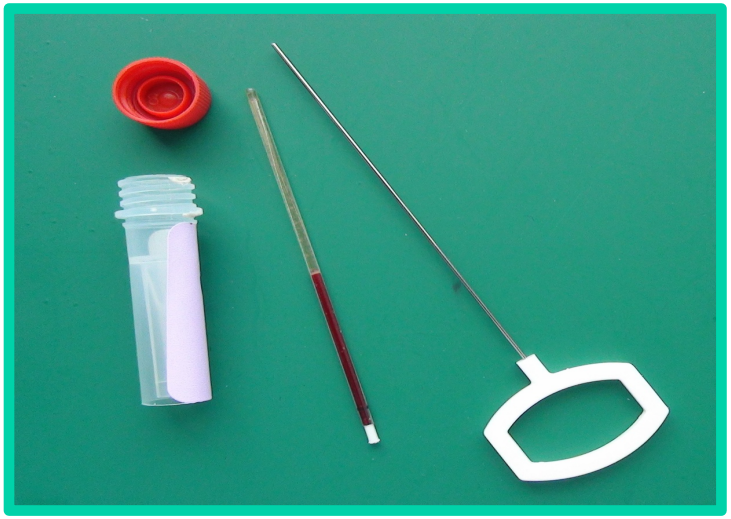
- Designed to address general non-compound specific questions
  - Expected to highlight issues that may be compound specific
- To be performed by each consortium member company
- Focused on currently used microsampling techniques
  - End to end capillaries
  - Drummond Device
  - Both typically used to sample blood and generate plasma
  - Should we look at others?

Typical microsampling techniques

End to end capillaries



Drummond Device



Question: Can we manipulate small volumes with the required precision and accuracy?

- Test using gravimetric approach using a 6 point balance
- How low can we go with current acceptance criteria?
  - Assess between 1 – 10 µL

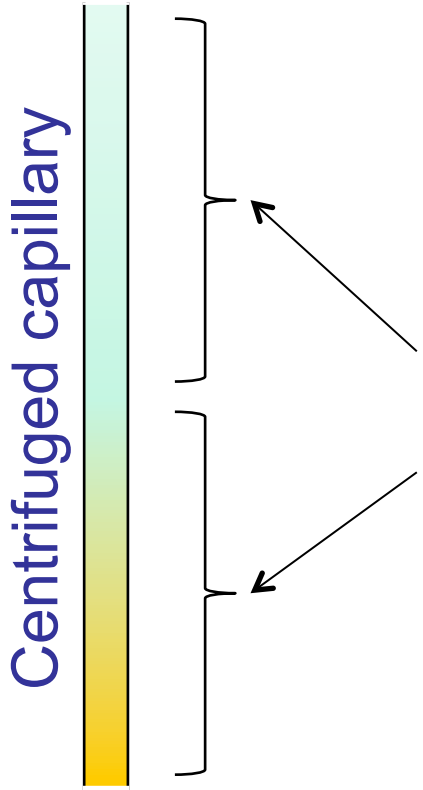
- Compare precision and accuracy over 6 replicates between
  - Water and plasma (in pipette or capillary)
  - Operators (experienced daily user and trained user)
  - Pipettes and capillaries
    - For pipettes investigate, different manufacturers, positive vs. air displacement, variable vs. fixed volume, electronic vs. manual
    - For capillaries investigate different manufacturers

Question: What affects homogeneity of capillary samples?

- Test with 2 compounds per participating company/site, to cover wide chemical space (including compounds with potential to adsorb)
  - Prepare 2 concentrations (low and high) in blood with a plasma spiking solution and a spiking volume of <2%
  - Draw spiked blood into appropriate device and take through sample preparation to generate plasma for assessment
  - Prepare plasma samples as required and freeze (neat or diluted)
  - Plasma samples are thawed and analysed neat or diluted (before or after freezing) as required
  - Include “macro” samples as a control (from 250 µL blood)

Question: What affects homogeneity of capillary samples?

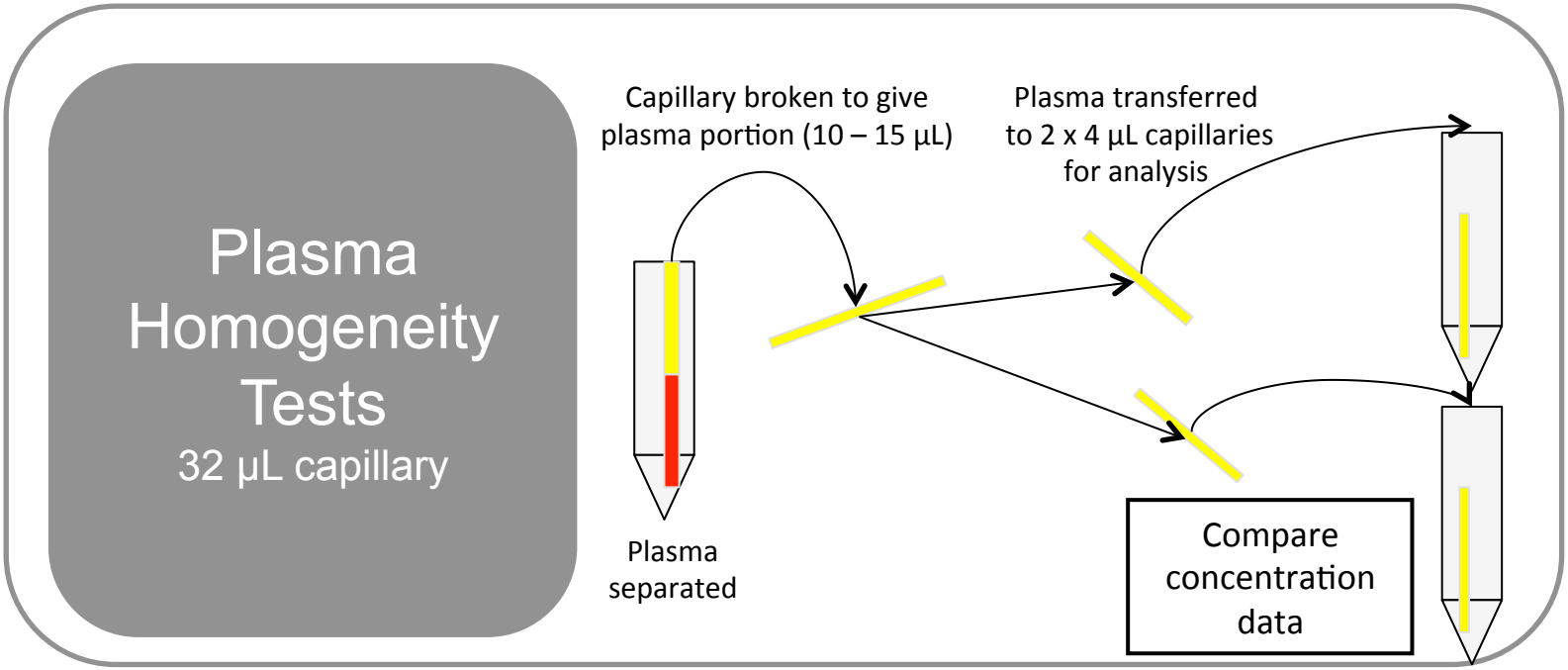
For plasma derived in end to end capillaries



Is the analyte plasma concentration the same here as it is here?

Question: What affects homogeneity of capillary samples?

- Is the derived plasma sample homogeneous along the capillary?
  - Take plasma aliquots with either 1 x 8 µL capillary or 2 x 4 µL capillary (n=6 for each) and compare data



Question: What affects homogeneity of capillary samples?

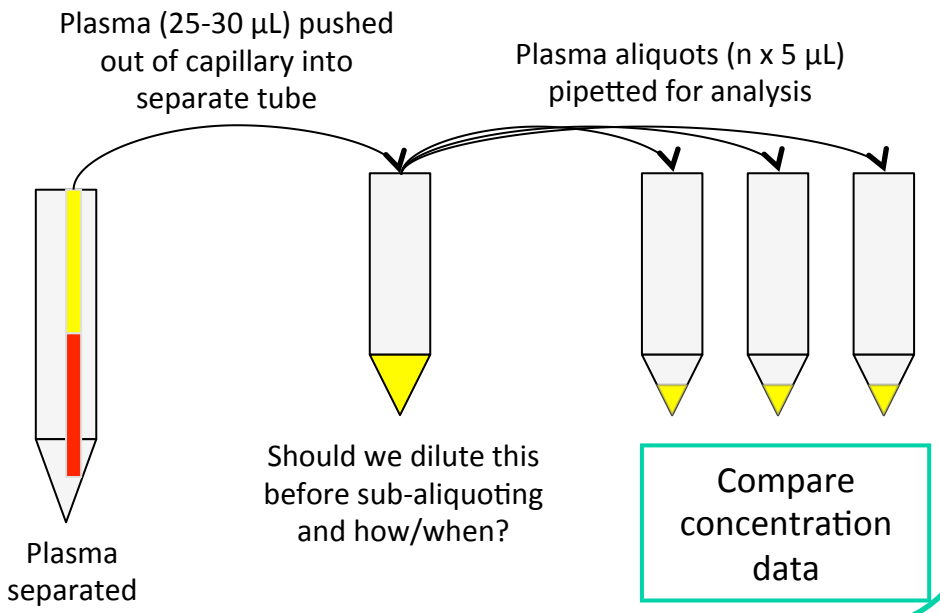
For plasma derived in end to end capillaries

- Is the derived plasma sample more homogeneous when diluted with buffer before or after freezing?
  - Dilute capillaries at least 10-fold before or after freezing and compare

Question: What affects homogeneity of capillary samples?

- Plasma derived in Drummond device

Plasma Homogeneity Tests
Drummond 70 µL capillary



Question: What affects homogeneity of capillary samples?

Plasma derived in Drummond device

- Dispense ca. 35 µL plasma into 5 x 500 µL and 5 x 1100 µL tubes per concentration per analyte
  1. Freeze directly – thaw and analyse replicate 5 µL aliquots
  2. Dilute 10-fold (25 µL + 225 µL buffer) and freeze – thaw and analyse replicate 5 µL aliquots
  3. Freeze directly – thaw, dilute 10-fold (25 µL + 225 µL buffer) and analyse replicate 5 µL aliquots
  4. Freeze directly – thaw, take 5 µL add 45 µL buffer and analyse replicate 5 µL aliquots
  5. Freeze directly – thaw, take 25 µL add 25 µL buffer and analyse replicate 5 µL aliquots

Question: What affects homogeneity of capillary samples?

Plasma derived in Drummond device

- Does container or surface area/volume ratio affect homogeneity?
  - Compare data from 1-5 in 500 µL and 1100 µL tubes
- Is sample more homogeneous when diluted with buffer before or after freezing?
  - Compare data from 2 and 3 in both tube types
- Is sample more homogeneous when diluted?
  - Compare data from 1 and 2 in both tube types
- Is sub-aliquot more representative when a bigger proportion taken?
  - Compare data from 4 and 5 in both tube types

Question: What affects homogeneity of capillary samples?

Capillary device *vs.* control

- Control diluted using same buffer / diluent and factor as in capillary method
- Loss/ gain of compound in capillary method relative to control expected to be due to freeze/thaw of small volume or adsorption.
- Increased variability in results expected to be due to inhomogeneity of sample

Other potential investigations

- Is plasma generated blood sampled in a capillary device the same as traditional plasma?
  - Assays of endogenous plasma components dismissed due to associated challenges
  - Worth considering comparing QCs prepared in both plasmas
- Is anti-coagulant (or other non analyte components) consistent across capillary and at same concentration as in traditional samples?
  - Currently not sure how to investigate
- Experiments currently focused on plasma capillary LMS
  - Should small volume non capillary LMS be investigated for blood or plasma?

Next steps

- Complete and execute experimental plan
- Publish results and recommendations
  - Scientific and Recommendation papers in peer reviewed literature (likely Bioanalysis) in 2015/2016
- Present on results and recommendations at next 8th EBF Open Symposium (Barcelona, 18-20 Nov-2015)
- Present at other international events as appropriate

Acknowledgment LMS

The EBF-LMS consortium members

Core Team

- AstraZeneca
- Charles River Laboratories
- LGC
- GlaxoSmithKline
- Janssen R&D
- Covance
- Ferring
- HLS/Harlan
- Lundbeck
- PRA Health Sciences
- QPS
- Sanofi
- TNO Triskelion

The EBF community

All of you


Questions?