Update from the EBF Liquid Microsampling Consortium

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on behalf of EBF

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A consortium is an association of two or more individuals, companies, organizations or governments (or any combination of these entities) with the objective of participating in a common activity or pooling their resources for achieving a common goal.

Consortium is a Latin word, meaning "partnership", "association" or "society" and derives from consors 'partner', itself from con- 'together' and sors 'fate', meaning owner of means or comrade.
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

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Recent Achievements & Activities

- 2013 Consortium Workshop
- European Bioanalysis Forum - Reflection on bioanalytical assay requirements used to support Liquid Microsampling. *Bioanalysis* (2014) Vol. 6, No. 19, Pages 2581-2586
- Two experimental protocols finalised
  - “Manipulation of Small Sample Volumes”
  - “Homogeneity of Small Sample Volumes”
  - Execution of these work plans is in progress

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Highlights from 2014 “philosophical” paper
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 it’s 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

European Bioanalysis Forum - Reflection on bioanalytical assay requirements used to support Liquid Microsampling. Bioanalysis (2014) Vol. 6, No. 19, Pages 2581-2586
Highlights from 2014 “philosophical” paper
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 to generate experimental data

However:

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

European Bioanalysis Forum - Reflection on bioanalytical assay requirements used to support Liquid Microsampling. Bioanalysis (2014) Vol. 6, No. 19, Pages 2581-2586
Experimental Protocols
## Small Volume Handling Protocol

### Pipettes

Evaluation of positive/air displacement pipettes and fixed/variable pipettes are to be performed, with each company investigating 2 pipettes.

Volumes evaluated will be 1, 2, 4 & 8 µL (n=6 replicates per pipette for each volume).

### Capillaries

Evaluation of end-to-end capillaries from 2 different manufacturers are to be performed by each company.

Volumes to be evaluated are 1, 2, 4 & 8 µL for Drummond and for Vitrex capillaries (n=6 replicates per capillary type for each volume).

All sites will perform the experiments using water (control) and plasma.

The experiments are to be performed by 2 operators per site (an experienced daily pipette/capillary user and a trained, but infrequent user).
Small Volume Handling Protocol
Emerging Data (lab 1)

### Lab 1 Accuracy Data - Water

![Graph showing accuracy data for water](chart1.png)

### Lab 1 Accuracy Data - Plasma

![Graph showing accuracy data for plasma](chart2.png)

### Lab 1 Precision Data - Water

![Graph showing precision data for water](chart3.png)

### Lab 1 Precision Data - Plasma

![Graph showing precision data for plasma](chart4.png)
Small Volume Handling Protocol
Emerging Data (lab 2)

Lab 2 Accuracy Data - Water

Lab 2 Accuracy Data - Plasma

Lab 2 Precision Data - Water

Lab 2 Precision Data - Plasma
Homogeneity Protocol

This experiment has been designed to understand how samples derived in this manner using two commonly employed approaches (Vitrex micro hematocrit tube and Drummond plasma capillaries) may differ from those obtained by conventional processes (controls).

Specifically, the experiment will demonstrate whether plasma derived by centrifugation of a capillary is homogenous and therefore, whether sub-aliquots taken from the sample are equivalent.
Homogeneity Protocol

Plasma Homogeneity Tests

Vitrex 32 µL micro-hematocrit tube

Capillary broken to give plasma portion (10 – 15 µL)

Plasma transferred to 2 x 4 µL capillaries for analysis

Compare concentration data

Plasma Homogeneity Tests

Drummond 70 µL plasma capillary

Plasma (25-35 µL) pushed out of capillary into separate tube

Plasma aliquots (5 µL) pipetted for analysis

Compare concentration data

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Homogeneity Protocol
Vitrex Capillaries

Spike blood with compound

Fill 18 x **Vitrex** caps (32 uL) with blood, plug, centrifuge & break

12 x 8 uL caps filled with plasma

6 caps frozen

6 caps diluted before freezing

2 x 4 µL cap filled with plasma from each of 6 Vitrex caps

6 caps frozen

6 caps frozen

6 caps frozen

2 x 4 µL cap filled with plasma from each of 6 Vitrex caps

Cap1

Cap2

Above is performed per concentration and per analyte. Total of 72 blood capillaries, 48 x 8 µL plasma capillaries and 48 x 4 µL plasma capillaries.
Homogeneity Protocol
Drummond Capillaries

Below is performed per concentration (2), per analyte (2). Total 12 Drummond devices – 3 per analyte and per concentration

Fill 3 Drummond caps (70 µL) with blood & centrifuge (per concentration, per analyte)

- Plasma collected into 500 µL Microwtube and frozen
- Plasma collected into 1100 µL Micronic and frozen
- Plasma collected into one tube and diluted 10-fold

For each of the above take 6 replicates of 5 µL for analysis

Caps= capillaries
## Test Compounds for Homogeneity Protocol

<table>
<thead>
<tr>
<th>Compounds</th>
<th>LC-MS</th>
<th>LBA</th>
<th>ICP-MS</th>
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</thead>
<tbody>
<tr>
<td><strong>Atenolol</strong></td>
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<tr>
<td>pKa= 9.43, MW=266.336, PPB=6-16%, logP=0.0965</td>
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<tr>
<td><strong>Atropine</strong></td>
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<tr>
<td>logP = 1.8, pKa = 9.4, MW = 289</td>
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<tr>
<td><strong>Buprenorphine</strong></td>
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<tr>
<td>MW=467.64, pKa=8.31/9.37, LogD=-0.27 protein binding 96%</td>
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<td><strong>Rituximab</strong></td>
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<td>Carboplatin</td>
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<tr>
<td><strong>Cabazitaxel</strong></td>
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<tr>
<td>MW=835.938, LogD=3.3, pKa=12.0&amp;12.5, PB=90-97%</td>
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<tr>
<td><strong>Clobazam</strong></td>
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<tr>
<td><strong>Diazepam</strong></td>
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<tr>
<td>logP = 2.6, pKa = 2.9, MW = 284</td>
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<tr>
<td><strong>Trastuzumab</strong></td>
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<tr>
<td>(Herceptin)</td>
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<tr>
<td><strong>Cisplatin</strong></td>
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<td><strong>Diclofenac</strong></td>
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<tr>
<td>pKa=4.15, logP=4.98, Mol wt=296.148, PB &gt;99%</td>
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<td><strong>Donepezil</strong></td>
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<td><strong>EPA</strong></td>
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<tr>
<td>(endogenous fatty acid)</td>
<td>Log D 4, MW 302</td>
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<tr>
<td><strong>Oxaliplatin</strong></td>
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<tr>
<td><strong>Fasiglifam</strong></td>
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<tr>
<td>MW=524.638, LogD=2.52</td>
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<tr>
<td><strong>Iohexol</strong></td>
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<tr>
<td>pKa n.a., log P -3.1, MW 821, PPB &lt;5%, B/P ratio 0.63</td>
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<tr>
<td><strong>Methyl Blue</strong></td>
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<tr>
<td>Log D -0.6, Quaternary amine MW 284</td>
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<tr>
<td><strong>Midazolam</strong></td>
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<tr>
<td>MW 325.0782, logP 4.13, pKb1=5.61 pKb2=4.69</td>
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<tr>
<td><strong>Norbuprenorphine</strong></td>
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<tr>
<td>MW=413.55, pKa=9.14/9.77, LogD=-1.9</td>
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<tr>
<td><strong>Omeprazole</strong></td>
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<tr>
<td>MW345.1147, logP 1.45, pKa1=13.72 pKb1=6.68 pKb2=4.04</td>
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<tr>
<td><strong>Paracetamol</strong></td>
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<tr>
<td>pKa=9.5, logP=0.49, Mol wt=151.163, PB=10-25%</td>
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<tr>
<td><strong>Rosuvastatin</strong></td>
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<tr>
<td>MW=481.54, pKa=4.25, LogD=0.89/-2.86 protein binding 88%</td>
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<tr>
<td><strong>Tolterodine</strong></td>
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<tr>
<td>pKa 9.7, Log P 5.6, MW 325</td>
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<tr>
<td><strong>Trastuzumab</strong></td>
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<tr>
<td>pI 8.5, hydrophobicity -0.4, MW 145531, PPB n.a. B/P ratio 0.40</td>
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<tr>
<td><strong>Verapamil</strong></td>
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<tr>
<td>pKa 8.92, Log P 3.79, MW 454</td>
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<tr>
<td><strong>Warfarin</strong></td>
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<tr>
<td>pKa= 4.5, MW=308.33, PPB~99%, logP=3.42</td>
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</tbody>
</table>
Emerging Data
Lab 1 - Vitrex Capillaries

Omeprazole - Vitrex Capillaries

- Control Low
- Control High
- 8 μL Dilute/Frozen (L)
- 8 μL Dilute/Frozen (H)
- 4 μL Frozen/Dilute (L)
- 4 μL Frozen/Dilute (H)

Midazolam - Vitrex Capillaries

- Control Low
- Control High
- 8 μL Dilute/Frozen (L)
- 8 μL Dilute/Frozen (H)
- 4 μL Frozen/Dilute (L)
- 4 μL Frozen/Dilute (H)

% diff from Control
CV (%)

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Emerging Data
Lab 1 - Drummond Capillaries

Omeprazole - Drummond Capillaries

Midazolam - Drummond Capillaries

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Next steps

- Complete experimental work across each member laboratory
- Consolidate and interrogate data
- Share findings within the EBF community
- Share findings with wider community via an EBF Consortium publication
Closing Remarks

- Await further experimental data before drawing conclusions on small volume manipulation & homogeneity.

- Appropriate experimental evidence during assay validation and production use will validate the sampling technique used.

- QCs prepared in same volume and handled in the same way as samples will highlight any issues (or lack of).
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Stuart McDougall – Covance
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