Update from the EBF Liquid Microsampling Consortium

Presenter: Stephen White
on behalf of EBF

EBF 8th Open Symposium
19th Nov 2015
Barcelona

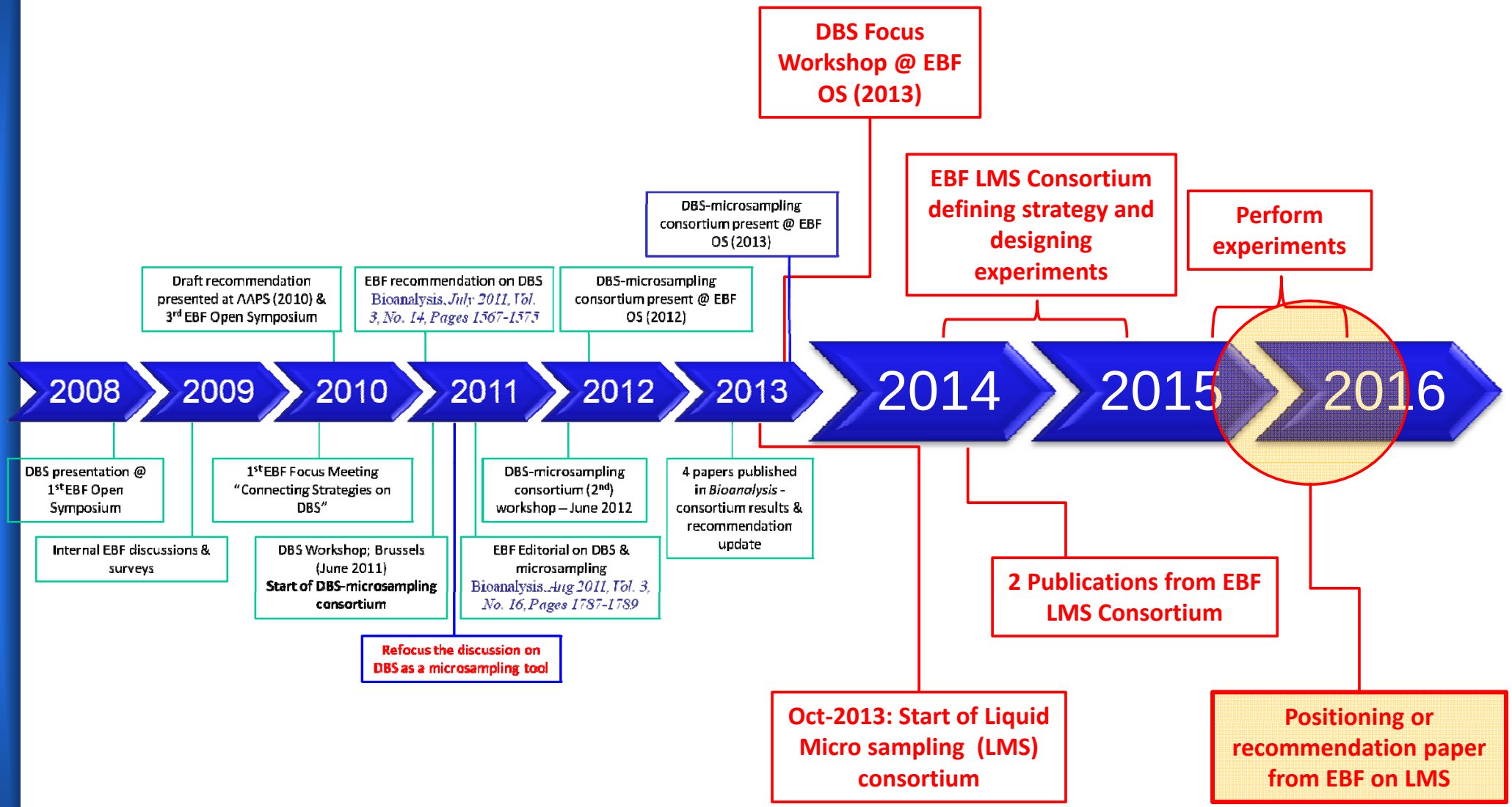
Consortium Membership

1. AstraZeneca
2. Charles River Laboratories
3. Covance
4. Envigo
5. GlaxoSmithKline
6. H Lundbeck A/S
7. Janssen R&D
8. LGC
9. PRA
10. QPS Netherlands B.V
11. Sanofi
12. TNO

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 & Liquid Microsampling



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

Recent Achievments & Activities

- 2013 Consortium Workshop
- European Bioanalysis Forum continued plans to support liquid microsampling. *Bioanalysis* (2014) Vol. 6, No. 14, Pages 1897-1900.
- 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

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 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

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).

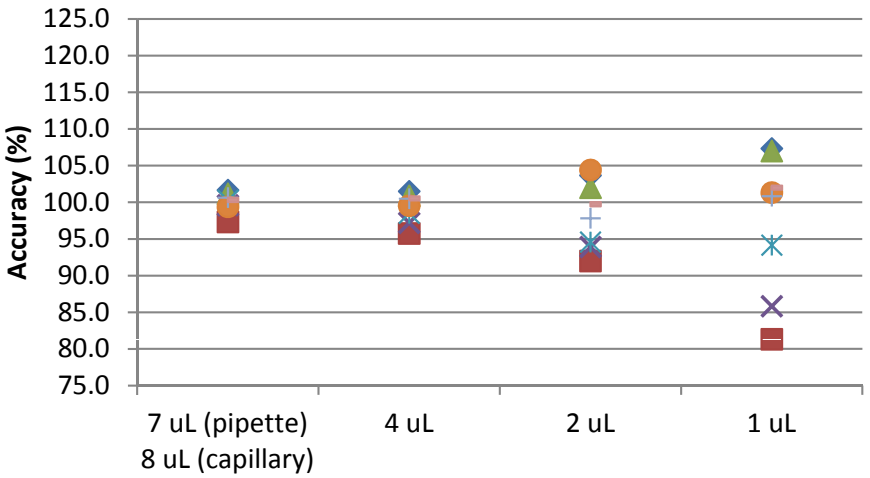
All sites will perform the experiments using water (control) and plasma.

The experiments are to be performed by 2 operators per site (an experienced daily pipette/capillary user and a trained, but infrequent user).

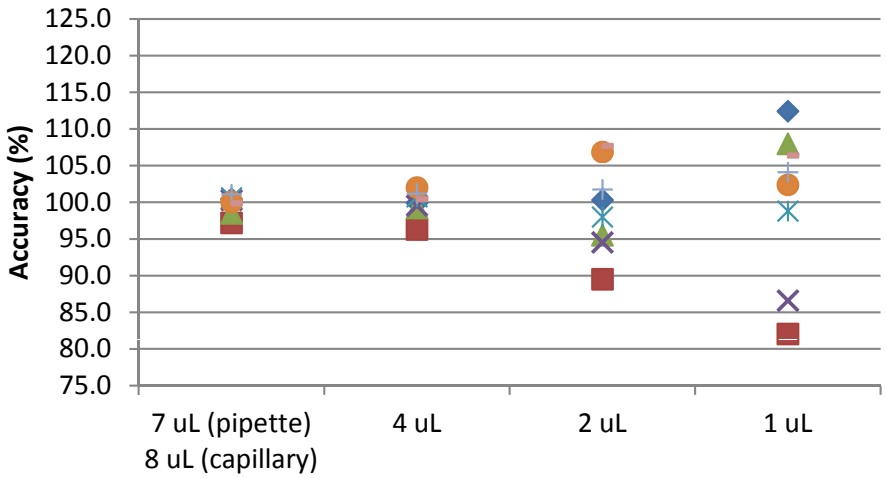
Small Volume Handling Protocol

Emerging Data (lab 1)

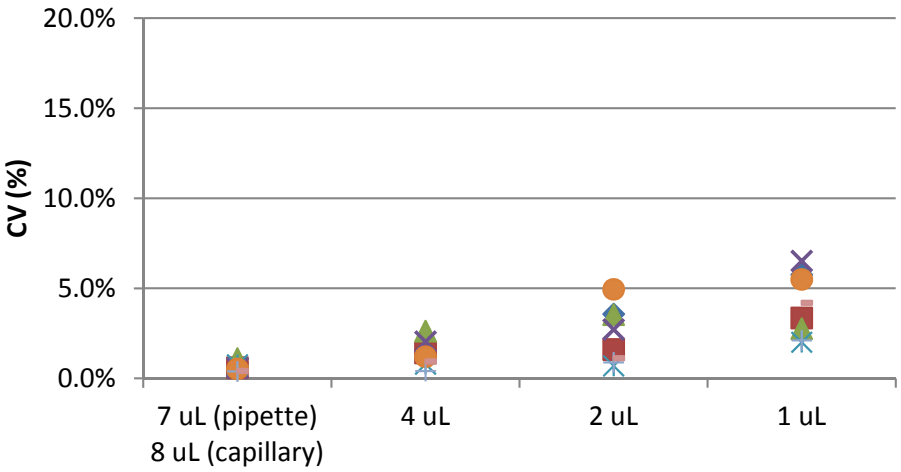
Lab 1 Accuracy Data - Water



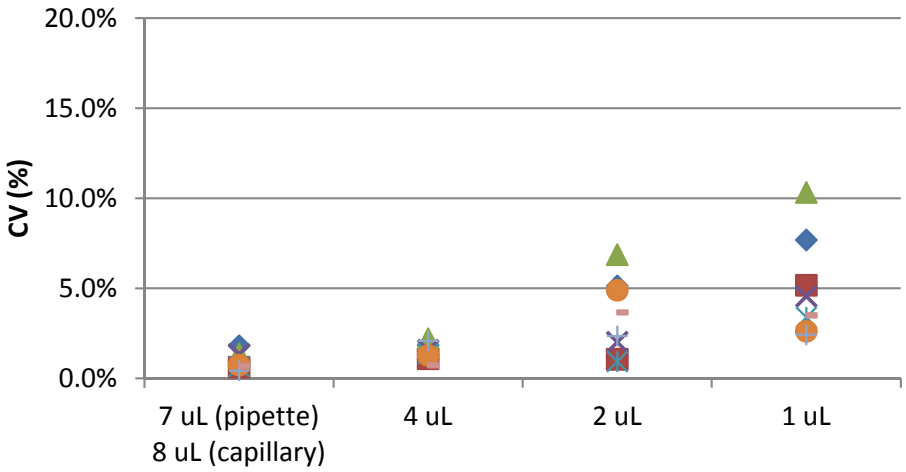
Lab 1 Accuracy Data - Plasma



Lab 1 Precision Data - Water



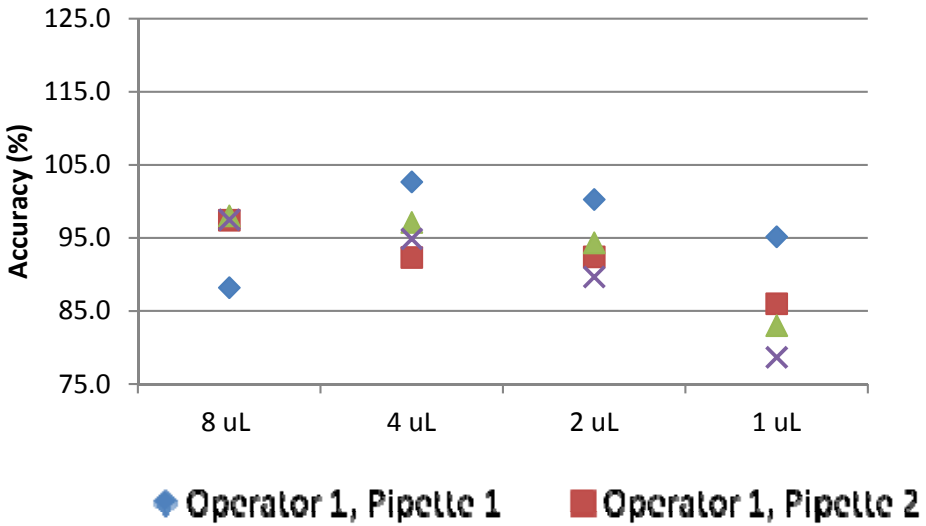
Lab 1 Precision Data - Plasma



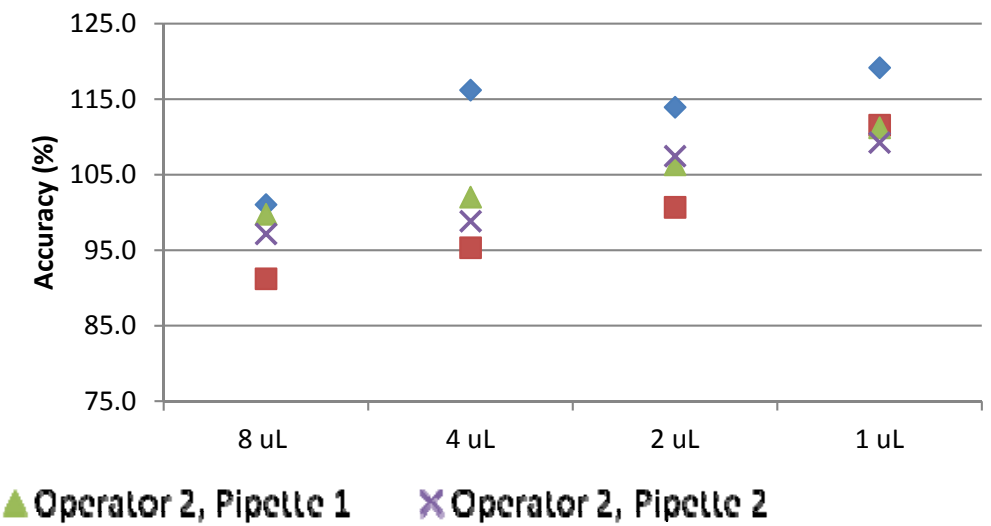
Small Volume Handling Protocol

Emerging Data (lab 2)

Lab 2 Accuracy Data - Water

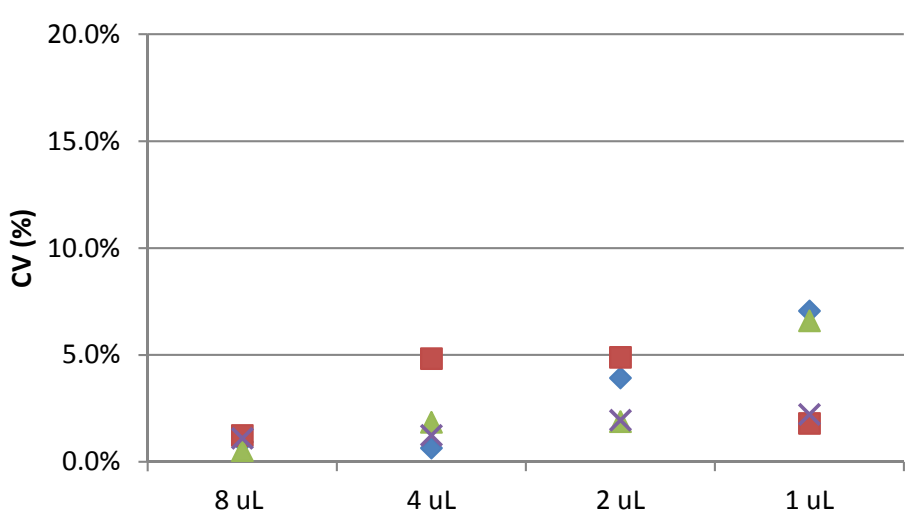


Lab 2 Accuracy Data - Plasma

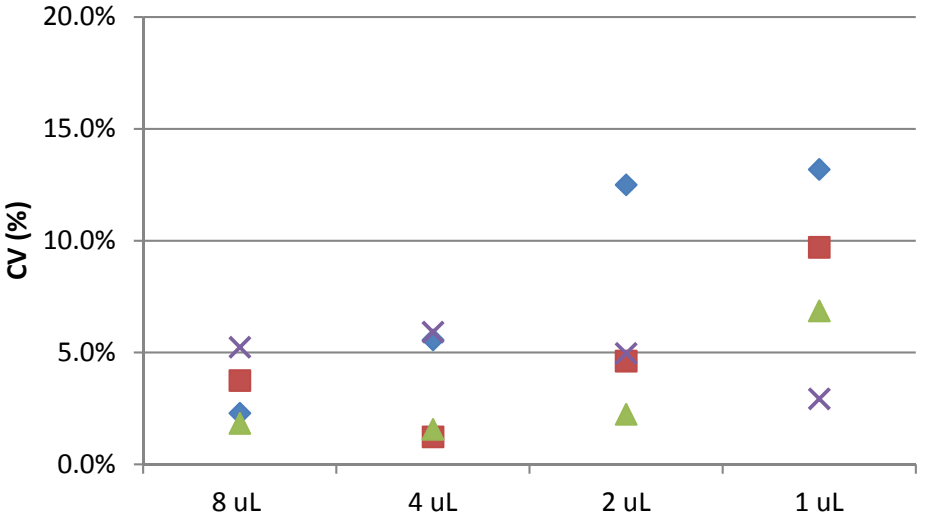


Operator 1, Pipette 1 | Operator 1, Pipette 2 | Operator 2, Pipette 1 | Operator 2, Pipette 2

Lab 2 Precision Data - Water

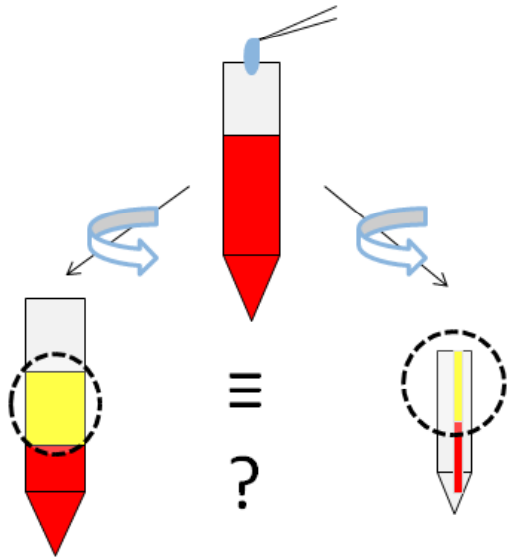


Lab 2 Precision Data - Plasma

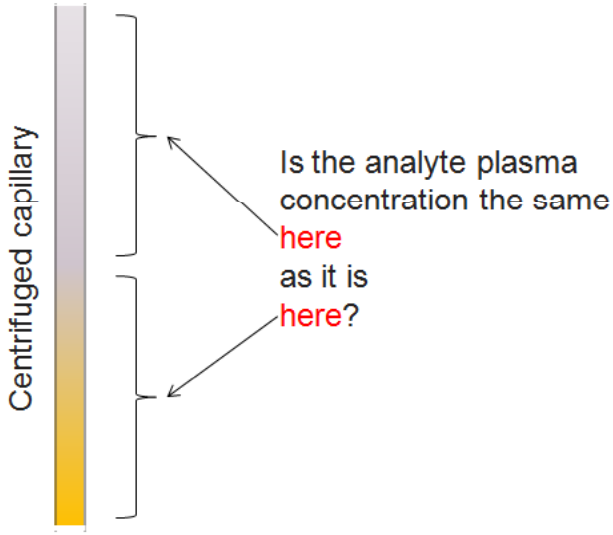


Homogeneity Protocol

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).



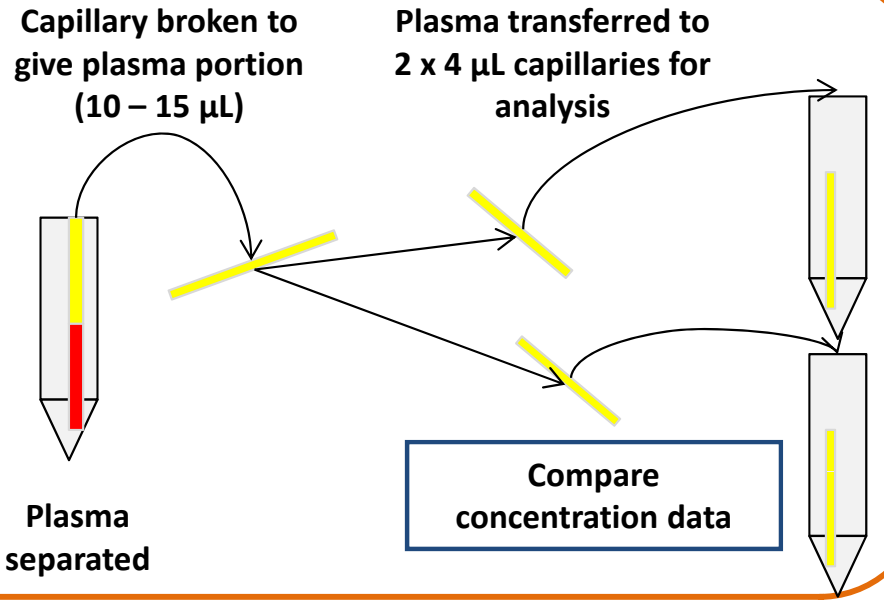
Specifically, the experiment will demonstrate whether plasma derived by centrifugation of a capillary is homogenous and therefore, whether sub-aliquots taken from the sample are equivalent.



Homogeneity Protocol

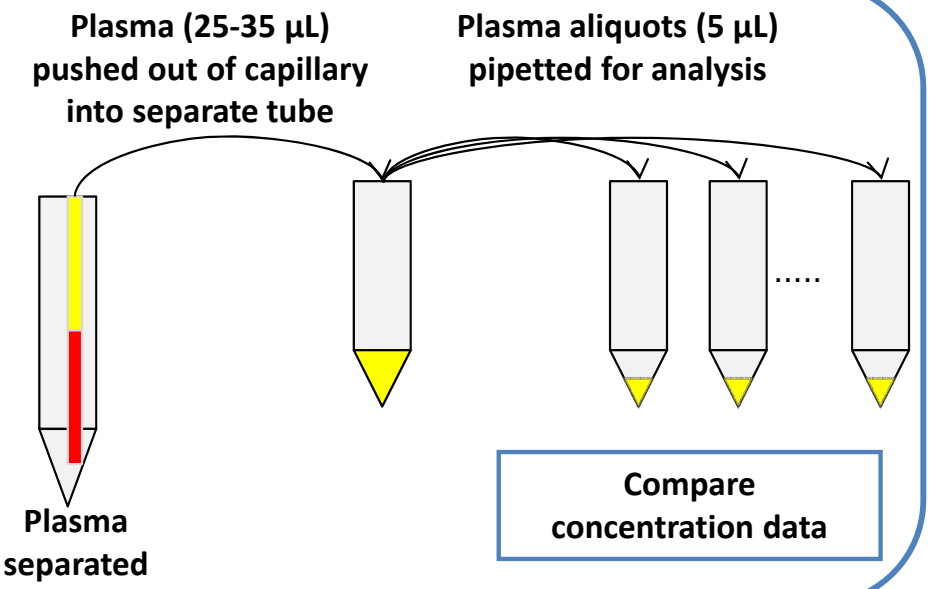
Plasma Homogeneity Tests

Vitrex 32 µL micro-hematocrit tube



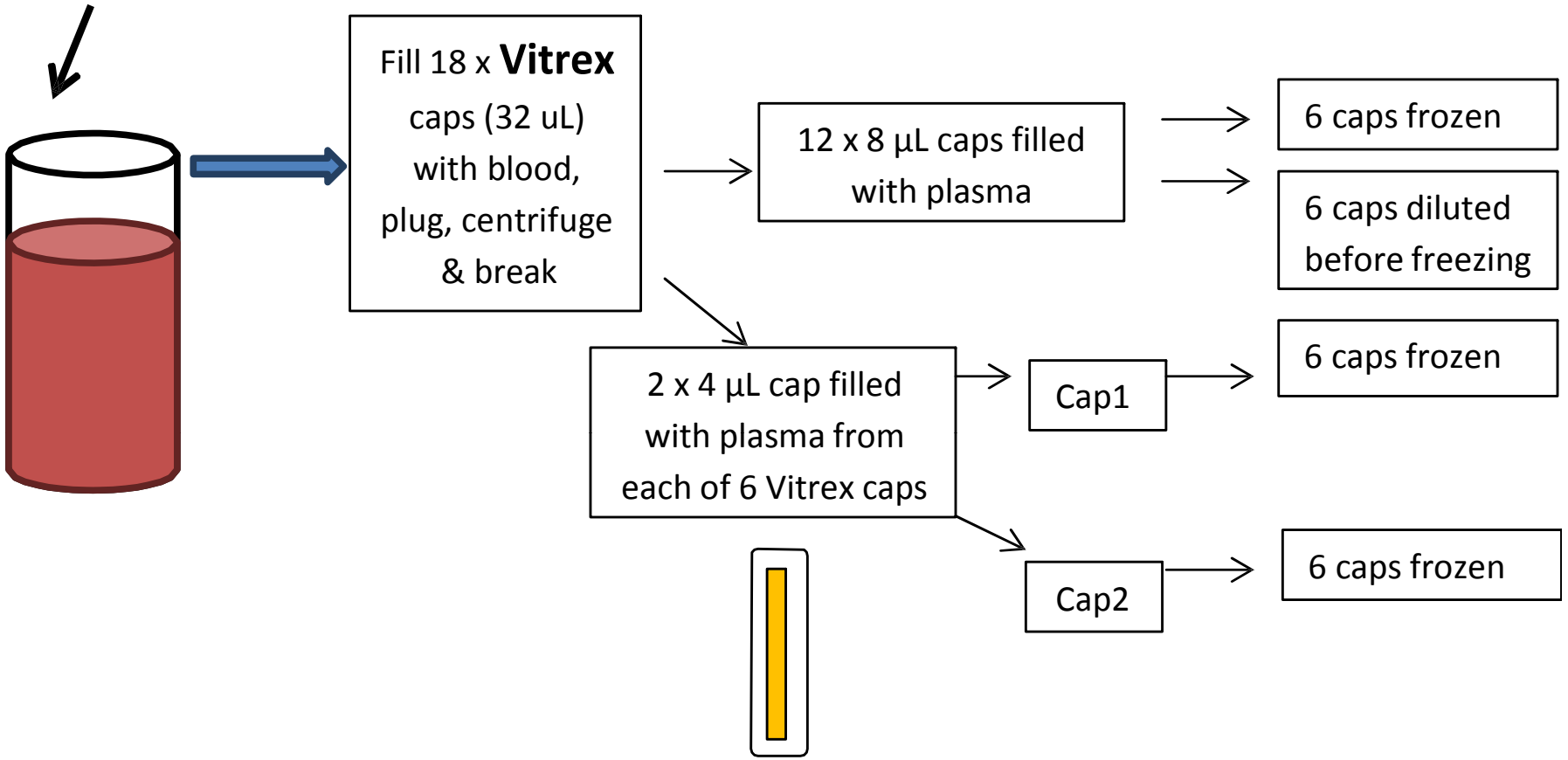
Plasma Homogeneity Tests

Drummond 70 µL plasma capillary



Homogeneity Protocol
Vitrex Capillaries

Spike blood with compound

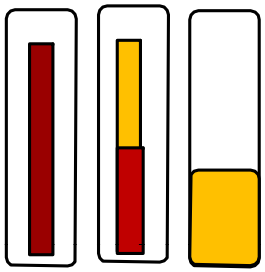


Above is performed per concentration and per analyte. Total of 72 blood capillaries, 48 x 8 µL plasma capillaries and 48 x 4 µL plasma capillaries.

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 uL) with blood & centrifuge (per concentration, per analyte)



3 collections of ca **35 µL** plasma

Plasma collected into 500 µL Micrewtube 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

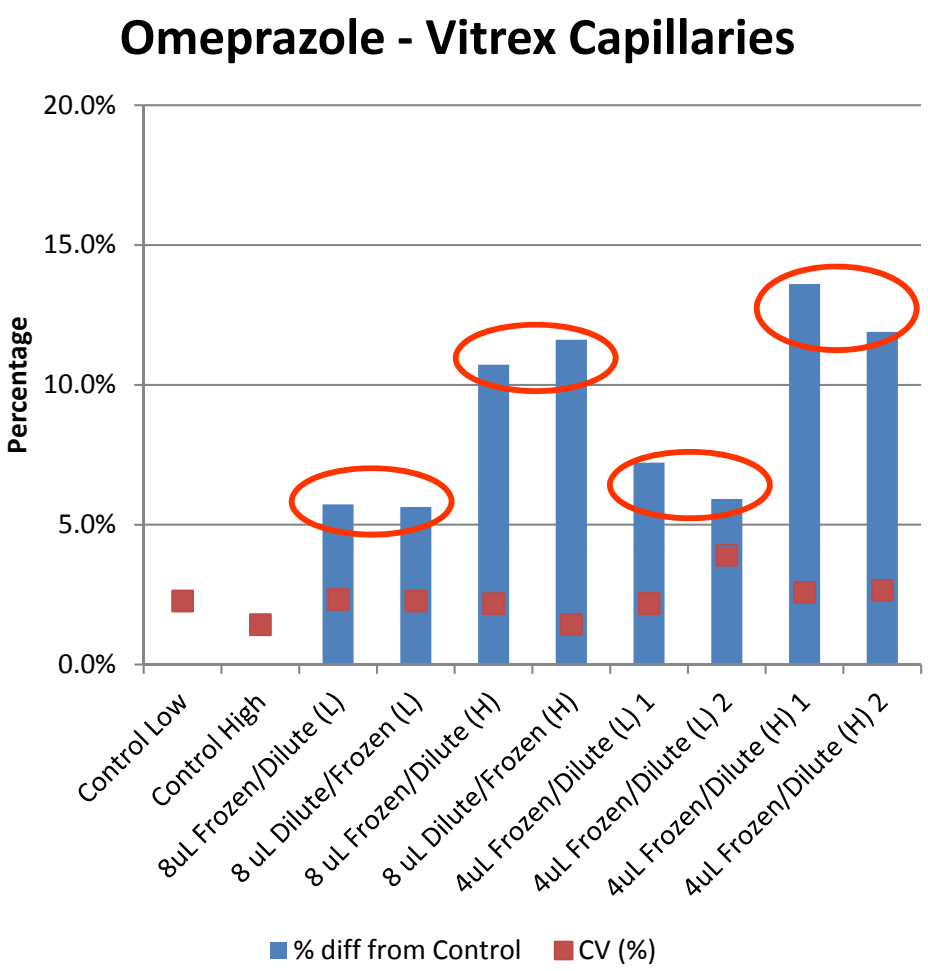
Caps= capillaries

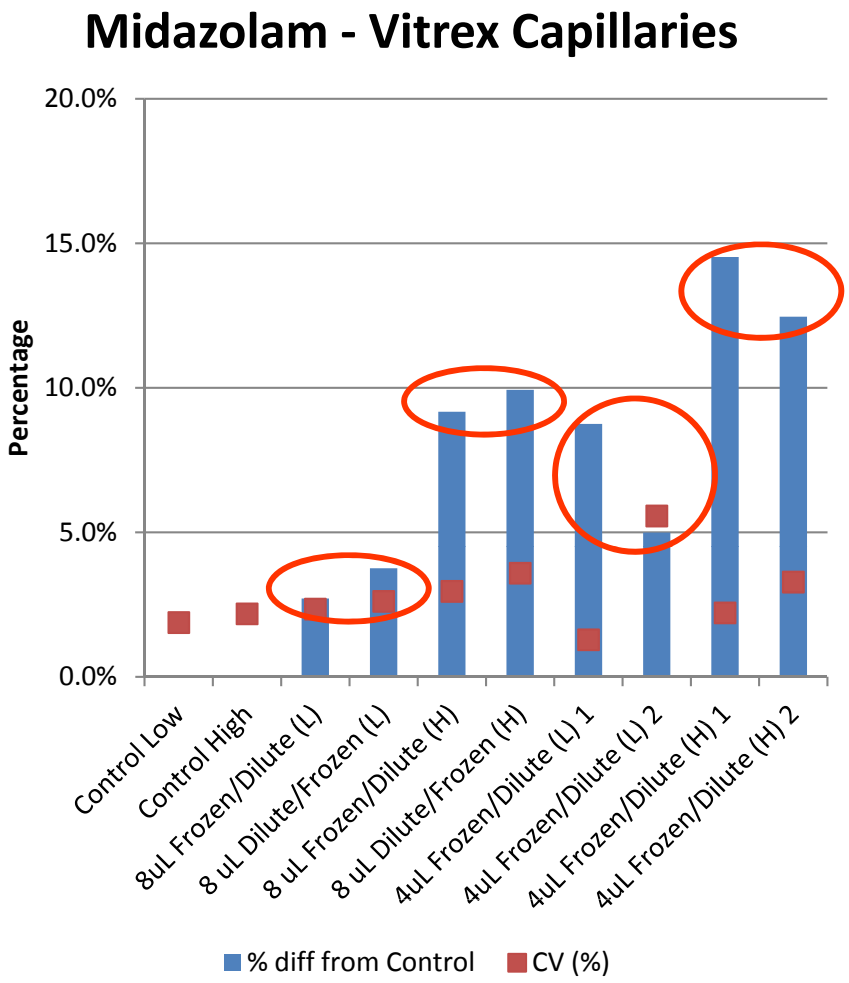
Test Compounds for Homogeneity Protocol

LC-MS (21)			LBA(3)	ICP-MS (3)
Atenolol pKa= 9.43 , MW=266.336, PPB~6-16%, logP=0.0965	**Atropine** logP = 1.8 pKa = 9.4 MW = 289	**Buprenorphine** MW=467.64, pKa=8.31/9.37, LogD=-0.27 protein binding 96%	**Rituximab**	**Carboplatin**
Cabazitaxel MW=835.938, LogD=3.3, pKa=12.0&12.5, PB=90-97%	**Clobazam**	**Diazepam** logP = 2.6 pKa = 2.9 MW = 284	**Trastuzumab** **(**Herceptin)	**Cisplatin**
Diclofenac pKa=4.15, logP=4.98, Mol wt=296.148, PB >99%	**Donepezil**	**EPA** (endogenous fatty acid) Log D 4, MW 302	**one from:** bevacizumab cetuximab adalimumab tocilizumab	**Oxaliplatin**
Fasiglifam MW=524.638, LogD=2.52	**Iohexol** pKa n.a., log P -3.1, MW 821, PPB <5%, B/P ratio 0.63	**Methyl Blue** Log D -0.6, Quaternary amine MW 284		
Midazolam, MW 325.0782, logP 4.13, pKb1=5.61 pKb2=4.69	**Norbuprenorphine** MW=413.55, pKa=9.14/9.77, LogD=-1.9	**Omeprazole** MW345.1147, logP 1.45, pKa1=13.72 pKb1=6.68 pKb2=4.04		
Paracetamol pKa=9.5, logP=0.49, Mol wt=151.163, PB=10-25%	**Rosuvastatin** MW=481.54, pKa=4.25, LogD=0.89/-2.86 protein binding 88%	**Tolterodine** pKa 9.7, Log P 5.6, MW 325		
Trastuzumab pI 8.5, hydrophobicity -0.4, MW 145531, PPB n.a. B/P ratio 0.40	**Verapamil** pKa 8.92, Log P 3.79, MW 454	**Warfarin** pKa= 4.5, MW=308.33, PPB~99%, logP=3.42		

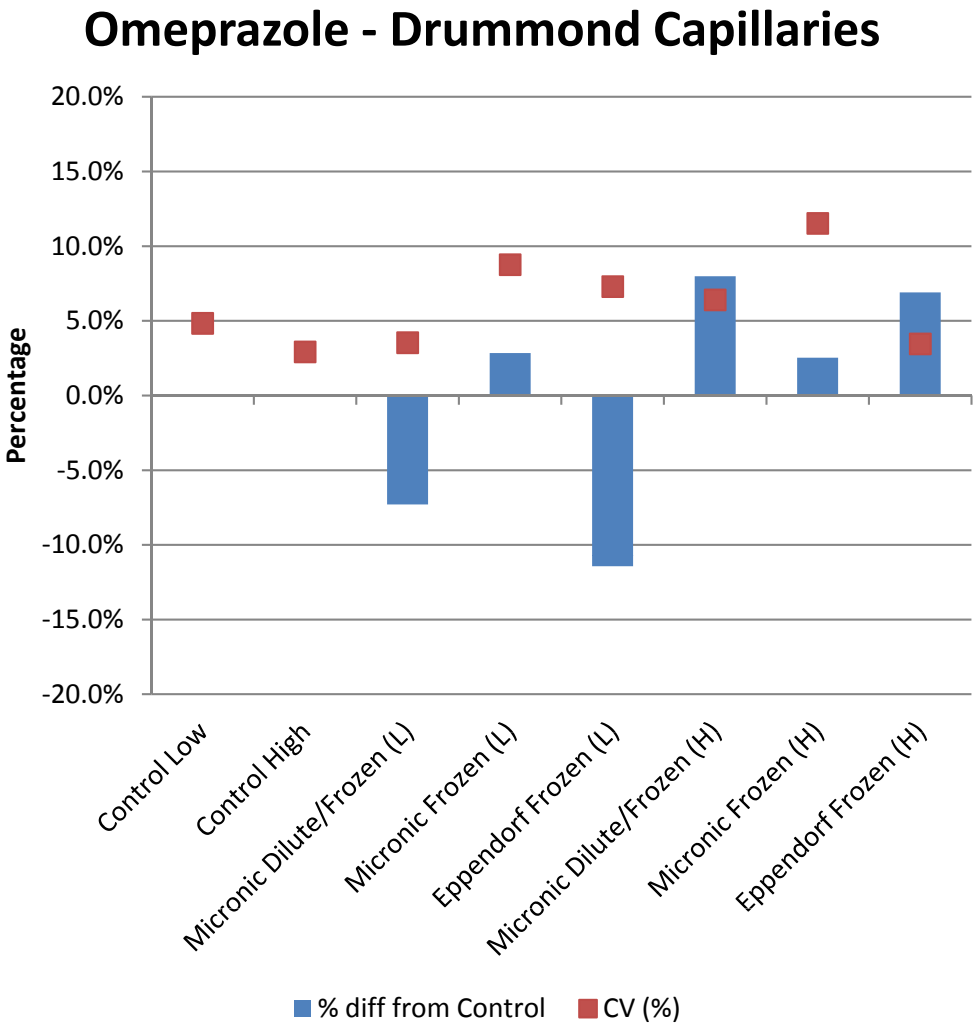
Emerging Data

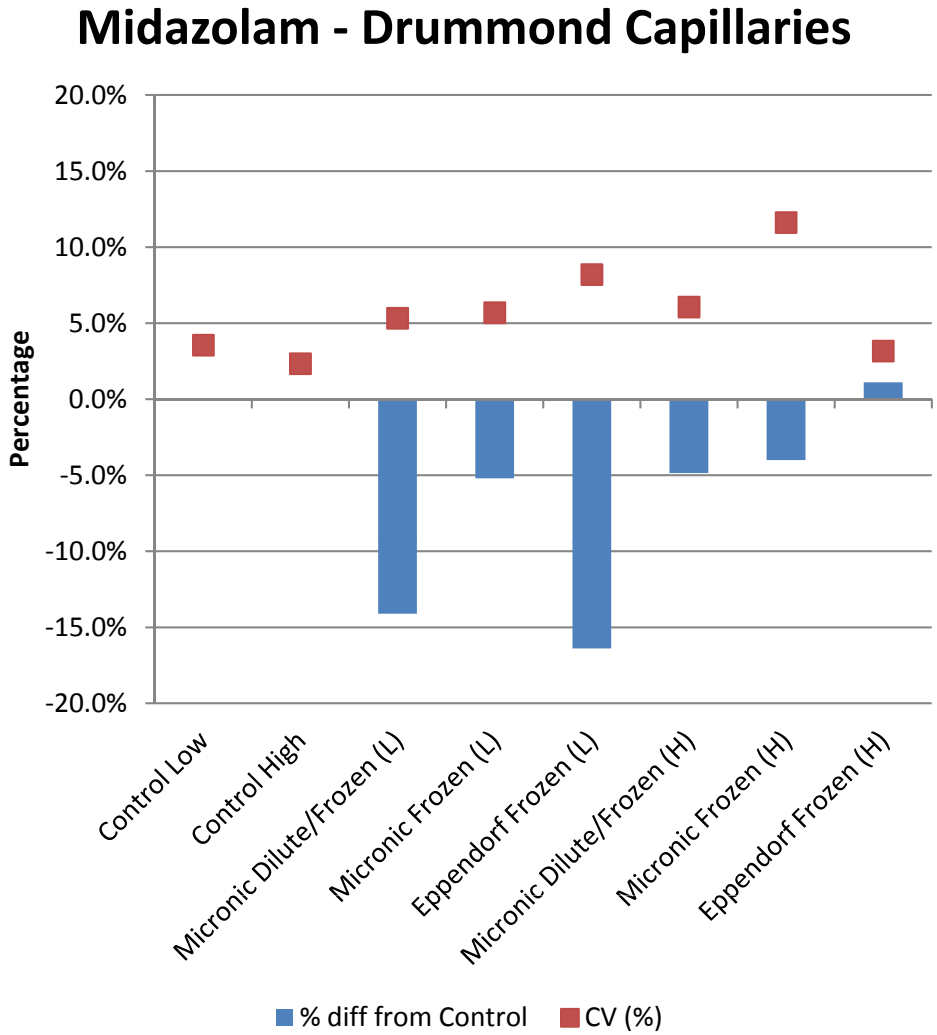
Lab 1 - Vitrex Capillaries





Emerging Data
Lab 1 - Drummond Capillaries





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)

Acknowledgements

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