

# Update from the EBF Liquid Microsampling Consortium

High level results from small volume handling  
and capillary homogeneity protocols

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on behalf of the EBF Liquid Microsampling Consortium*

EBF 9<sup>th</sup> Open Symposium  
16-18 November 2016  
Barcelona

# The story so far

- Completed experimental work across each member laboratory
- Consolidate and interrogate data
- Share findings within the BA community (today!)
- Share findings with wider BA community via an EBF Liquid Microsampling Consortium publication

<p>Editorial</p> <p>For reprint orders, please contact <a href="mailto:reprints@future-science.com">reprints@future-science.com</a></p> <h2>EBF: reflection on bioanalytical assay requirements used to support liquid microsampling</h2> <p>"As we continue to investigate further through targeted experiments, we will, as a community, gain better insight on this topic."</p> <p><b>Keywords:</b> capillary • European Bioanalysis Forum • liquid microsampling • sample homogeneity • validation</p>	<p>Bioanalysis</p> 
<p>Further to the discussions on the bioanalysis of samples generated using microsampling techniques [p-4] and more specifically the ongoing work of the European Bioanalysis Forum (EBF) Liquid Microsampling Consortium [1], this article seeks to highlight some of the 'philosophical' aspects around liquid microsampling and to introduce some of the experimental elements that will form part of future efforts by the Consortium. Discussion will be focused on three major areas: sample manipulation, homogeneity of samples and validation of assays. The scope of this manuscript will be liquid microsampling and will not focus on adsorption techniques such as dried blood spots (DBS) and solid phase microextraction. Different microsampling techniques have created great interest from toxicokinetic (TK) and pharmacokinetic (PK) scientists, since they offer the potential to reduce sample volumes for exposure assessment in bi fluids. For preclinical studies microsampling can facilitate the generation of serial profiles in rodent exposure evaluation studies, rather than working with composite designs. Microsampling has facilitated the removal of satellite animal groups leading to substantial reductions in the number of animals required and the reduction or elimination of rodent warming. Benefits in the clinical environment include the ability to take reduced sample volumes from pediatric, elderly and critically ill patient populations.</p> <p>In a recent publication the NC3R group proposes the following definition of a blood microsample: the sample should ideally not contain more than 50 µl whole blood [2]. As a consequence the subsequent plasma are 20 µl or lower. Another, methodological consideration would be that of sampling volume on the animal; for example, 50 µl of blood is considered as a 'microsample' in [3] of juvenile animals or newborn but major liquid microsampling approaches be envisaged:</p> <ul style="list-style-type: none"><li>• Liquid microsampling (non-capillary):<ul style="list-style-type: none"><li>- Blood: a low volume of blood piped into a small receptacle anticoagulant, often diluted storage;</li><li>- Plasma: a low volume of blood piped into a small receptacle anticoagulant, then centrifuged to obtain plasma. Plasma is piped into a small receptacle or storage. Dilution strategies considered;</li><li>- Serum: a low volume of blood piped into a small receptacle anticoagulant, then centrifuged to obtain serum. Serum is piped into a small receptacle or storage. Dilution strategies considered.</li></ul></li><li>• Capillary microsampling of:<ul style="list-style-type: none"><li>- Blood: blood is sampled if layered containing anticoagulant without subsequent wash/dilution at the time of sample</li></ul></li></ul>	<p>Commentary</p> <p>For reprint orders, please contact: <a href="mailto:reprints@futuremedicine.com">reprints@futuremedicine.com</a></p> <h2>European Bioanalysis Forum continued plans to support liquid microsampling</h2> <p><b>Keywords:</b> consortium • EBF • experiments • liquid microsampling • validation</p> <p>Since early 2008, the European Bioanalysis Forum (EBF) has been actively engaged in the discussion regarding the use of dried blood spot (DBS) sampling and has gradually increased their involvement by providing science-based input to the developing dialog within industry on the subject. Our contribution was built on the experience of many experts in our community who came together with the aim of providing an answer to a broader perspective on the bioanalytical challenges of DBS. The commitment of the EBF community to supporting the development of DBS was illustrated by the formation of an EBF DBS Consortium, in which we shared bioanalytical experience and agreed to perform focused DBS experiments. Furthermore, the Consortium connected different stakeholders in bioanalysis as well as in other expertise areas impacted by this technology, such as pharmacokinetics (PK) and toxicokinetics (TK) [1]. The results of the discussions and experiments performed by the Consortium were shared with industry at conferences, workshops and via publications [p-4]. This has added to the many individual contributions from the broader scientific peer community [p-9] and other industry consortia. In addition, in order to share our thoughts on a more effective strategy for the development and acceptance of the technology in those areas where it can provide immediate added value, we tried to provide context to the real or perceived hurdles for DBS and shared our vision on how this technology would fit into a wider implementation of microsampling [p-11]. One element of this vision was to ensure that the</p> <p>hurdles of DBS would not be inadvertently generalised and applied to other areas of microsampling. To emphasize the latter, one of our recommendations was to highlight DBS as 'one of the approaches' for microsampling, rather than 'the approach'. Consequently, the EBF-DBS Consortium was rebranded to reflect this commitment and continued as the 'EBF DBS-Microsampling Consortium'. After the recent publication of our updated recommendations [1], we refocused our efforts away from DBS sampling, although the Consortium offering to continue as a pivot point if needed, and regrouped around the opportunities for liquid microsampling (LMS) approaches. To reflect this, the name of the Consortium was rebranded to the current EBF Liquid Microsampling Consortium (EBF LMS Consortium).</p> <p><b>Building the EBF LMS Consortium</b></p> <p>Similarly to the initial formation of the DBS Consortium, we reached out to our members and invited them to join this new LMS Consortium. Thirteen companies with hands-on experience and vested interest in LMS signed up and declared that they were prepared to share nonproprietary data on LMS experiments and/or be available to perform bioanalytical experiments focused on LMS designed and agreed upon by the team. The team came together for the first time in a workshop on 23 October 2013 in Brussels, Belgium. The interactive discussion yielded a shortlist of the potential challenges related to LMS, in addition to some initial ideas on experiments that would be needed to further investigate some of these scientific or regulatory chal-</p> <p>Philip Timmermann<sup>1</sup>, Steve White<sup>2</sup>, Zoe Gibby<sup>3</sup>, Karen Woods<sup>4</sup>, Ronald de Vries<sup>5</sup>, Neil Spooner<sup>6</sup>, Timothy Sangster<sup>7</sup>, Lieve Dillen<sup>8</sup> &amp; Glen Haverhorst<sup>9</sup></p> <p><sup>1</sup>Nissan Research &amp; Development, Turinhouseweg, 30, 82340 Beers, Belgium <sup>2</sup>GlaxoSmithKline, Stevenage, UK <sup>3</sup>GE, Fortham, UK <sup>4</sup>Novartis, Abbey Park, Macclesfield, UK <sup>5</sup>Charles River Laboratories, Edinburgh, UK <sup>6</sup>Author for correspondence: <a href="mailto:ptimmer@dbts.pjz.com">ptimmer@dbts.pjz.com</a></p>
<p>10.4155/BIO.14.211 © 2014 Future Science Ltd</p> <p>Bioanalysis (2014) 6(10), 1887-1900</p>	<p>10.4155/BIO.14.99 © 2014 Future Science Ltd</p> <p>Bioanalysis (2014) 6(10), 1887-1900</p> <p>ISSN 1757-6180</p> <p>1887</p>

# Small Volume Handling Protocol

## Pipettes

Evaluation of positive/air displacement pipettes performed, with each company investigating 2 pipettes.

Volumes evaluated were 1, 2, 4 & 8  $\mu\text{L}$  (n=6 replicates per pipette for each volume).

## Capillaries

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

Volumes evaluated were 1, 2, 4 & 8  $\mu\text{L}$  (n=6 replicates per pipette for each volume).

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

The experiments were performed by 2 operators per site (an experienced daily pipette user and a trained, but infrequent user)

Total of 1536 data points collected

# Small volume precision and accuracy evaluation

<b>Key:</b>	< 5%	> 5%	>10%
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		Lab 6							
		Water				Plasma			
		8 uL	4 uL	2 uL	1 uL	8 uL	4 uL	2 uL	1 uL
<i>Operator 1,</i>	<b>Accuracy</b>	103.8%	106.2%	107.3%	119.8%	103.0%	105.3%	110.7%	120.2%
<i>Pipette 1</i>	<b>CV (%)</b>	2.4%	3.1%	3.9%	8.2%	1.2%	2.2%	3.2%	8.4%
<i>Operator 1,</i>	<b>Accuracy</b>	99.8%	103.7%	104.3%	115.8%	100.0%	103.0%	102.5%	112.9%
<i>Pipette 2</i>	<b>CV (%)</b>	0.5%	2.8%	2.8%	5.9%	1.4%	3.4%	7.4%	3.8%
<i>Operator 2,</i>	<b>Accuracy</b>	102.5%	103.9%	105.1%	122.7%	101.9%	105.4%	106.8%	113.2%
<i>Pipette 1</i>	<b>CV (%)</b>	2.8%	3.6%	6.9%	6.7%	1.0%	5.8%	5.7%	9.7%
<i>Operator 2,</i>	<b>Accuracy</b>	99.1%	95.5%	89.5%	86.7%	97.2%	95.7%	94.1%	82.1%
<i>Pipette 2</i>	<b>CV (%)</b>	1.7%	3.9%	2.2%	3.7%	0.6%	3.6%	5.9%	6.6%

# Small volume evaluation – Plasma Accuracy

## Pipettes

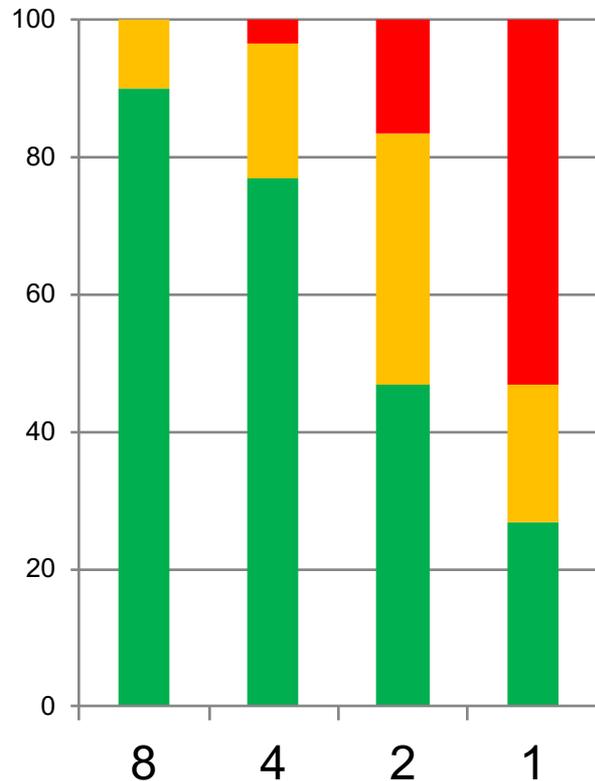
Volume (uL)			
8	4	2	1
100.5%	99.9%	100.3%	112.4%
97.2%	96.3%	89.5%	82.0%
98.5%	99.2%	95.5%	108.0%
100.3%	99.6%	94.5%	86.6%
101.0%	116.2%	113.9%	119.2%
91.2%	95.3%	100.7%	111.6%
99.8%	102.0%	106.3%	111.2%
97.2%	98.8%	107.5%	109.3%
98.0%	97.6%	90.2%	99.1%
99.5%	98.9%	94.5%	98.8%
100.7%	100.0%	101.2%	92.5%
100.7%	101.4%	105.4%	110.3%
93.4%	109.7%	114.5%	111.8%
95.0%	99.1%	82.7%	72.0%
95.8%	96.4%	96.1%	101.2%
97.0%	93.0%	103.5%	101.8%
101.1%	100.1%	102.4%	109.6%
99.8%	98.7%	98.1%	96.7%
100.8%	100.6%	99.7%	100.2%
99.8%	102.5%	98.1%	100.2%
103.0%	105.3%	110.7%	120.2%
100.0%	103.0%	102.5%	112.9%
101.9%	105.4%	106.8%	113.2%
97.2%	95.7%	94.1%	82.1%
97.8%	96.5%	93.8%	100.9%
96.7%	95.0%	91.2%	86.8%
100.1%	102.4%	102.5%	105.4%
102.2%	103.2%	102.8%	107.2%
95.6%	96.8%		
		91.3%	111.8%
107.6%	105.7%		
		100.3%	89.9%

## Capillaries

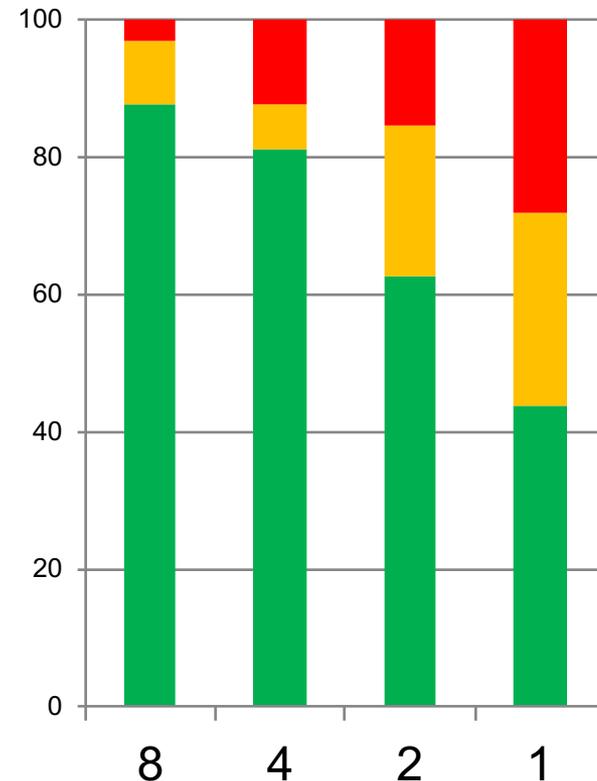
Volume (uL)			
8	4	2	1
100.6%	100.8%	98.0%	98.8%
100.1%	102.0%	106.9%	102.4%
101.1%	101.2%	101.8%	104.1%
99.9%	100.4%	107.7%	106.4%
104.4%	105.9%	114.8%	97.6%
98.8%	99.6%	96.7%	94.5%
96.0%	101.2%	102.0%	118.1%
97.5%	98.0%	113.4%	114.0%
98.3%	98.7%	97.0%	98.5%
97.7%	98.2%	98.9%	100.7%
102.4%	103.3%	103.2%	106.2%
102.6%	103.6%	103.3%	107.3%
108.8%	112.6%	124.2%	138.2%
103.4%	117.8%	162.1%	137.6%
103.6%	102.5%	101.1%	104.6%
105.9%	102.9%	104.8%	107.6%
102.0%	102.8%	103.0%	108.4%
102.3%	104.5%	105.3%	115.5%
102.8%	102.9%	97.1%	99.0%
102.9%	98.7%	101.1%	96.1%
98.9%	102.8%	92.2%	86.5%
100.2%	100.1%	100.3%	93.3%
99.0%	95.6%	90.4%	93.1%
100.6%	106.5%	107.6%	112.1%
101.2%	100.0%	98.4%	99.9%
99.1%	98.0%	100.3%	97.1%
102.3%	101.2%	100.0%	105.2%
100.9%	100.2%	101.3%	97.8%
97.8%	97.3%	101.8%	97.9%
97.6%	100.2%	102.7%	98.3%
108.2%	111.0%	107.7%	112.1%
110.0%	110.9%	117.5%	112.6%

# Small volume evaluation – Plasma Accuracy

## Pipettes



## Capillaries



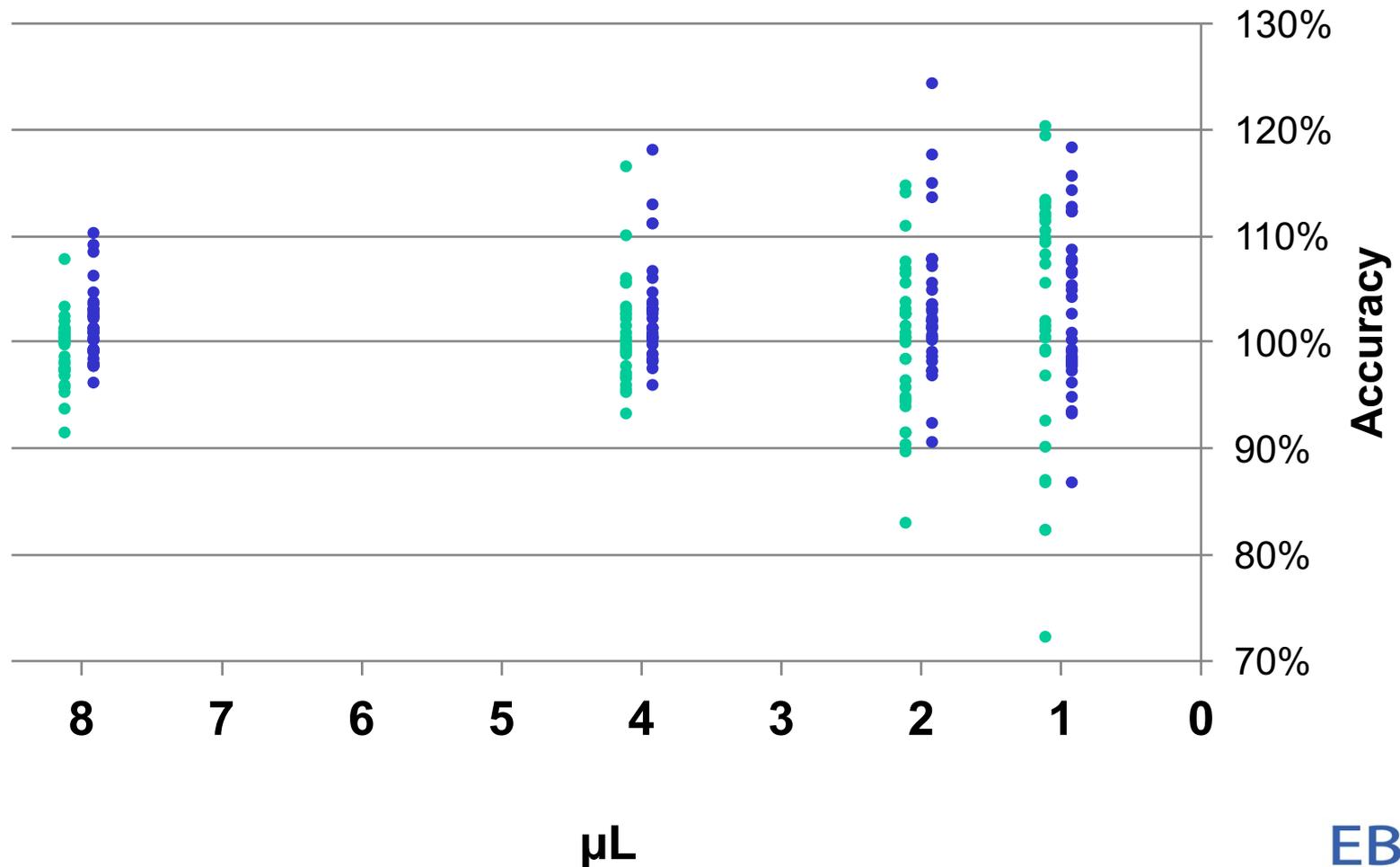
 % values red

 % values yellow

 % values green

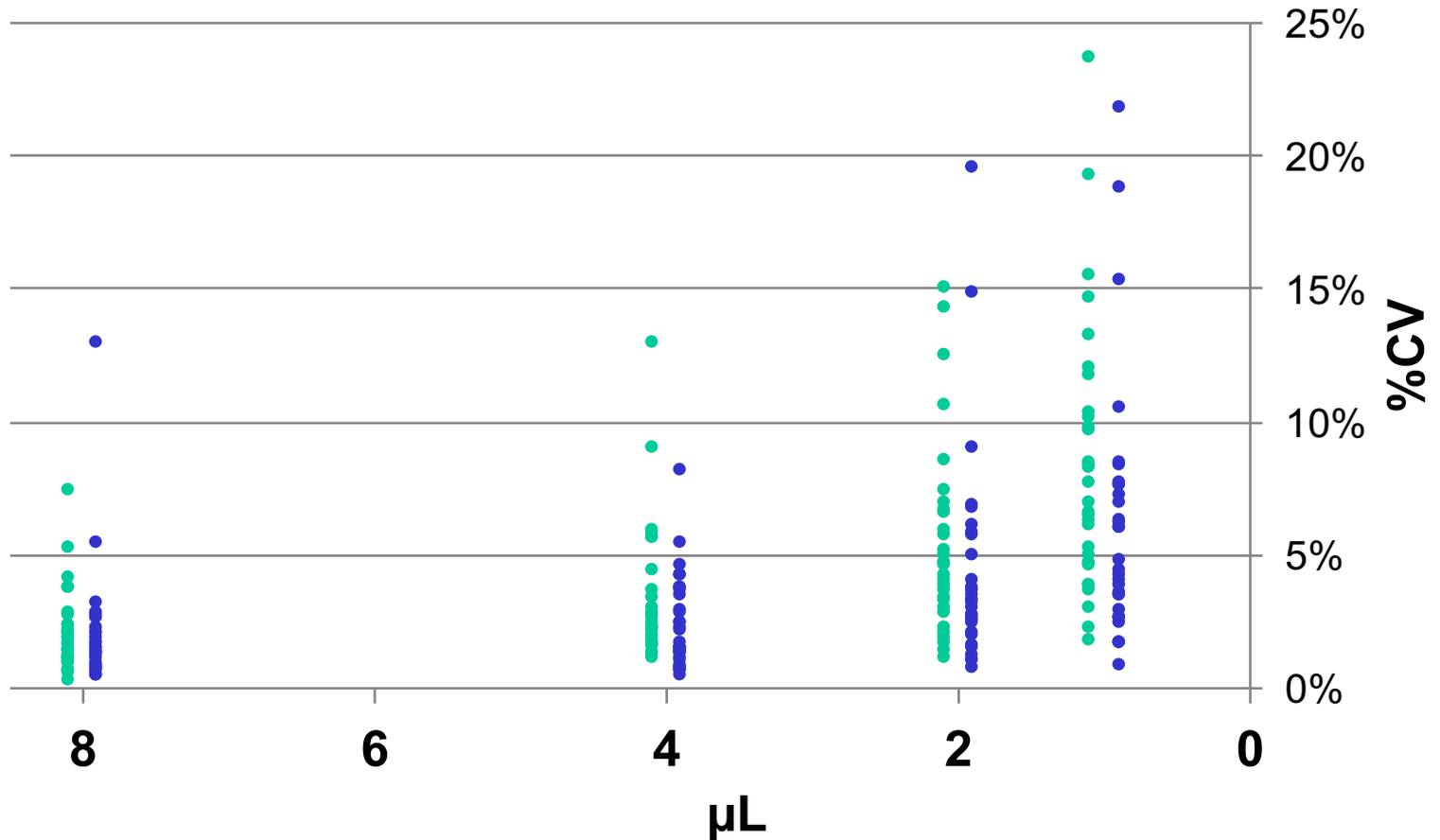
# Small volume evaluation – Accuracy

Plasma Volume **Accuracy** using **Pipettes** and **Capillaries**



# Small volume evaluation – Precision

## Plasma Volume Precision using Pipettes and Capillaries



# Small Volume Handling Protocol - conclusions

## ➤ Accuracy

- Accuracy of pipetting with capillaries is better than manual pipettes for low volume plasma handling ( $\leq 4 \mu\text{l}$ )

## ➤ Precision

- Precision of pipetting with capillaries is better than manual pipettes for low volume plasma handling ( $\leq 4 \mu\text{l}$ )

## ➤ Consequences for the assay:

- For both capillaries and pipettes, the inaccuracy and imprecisions introduced by the pipetting step for volumes  $\leq 4 \mu\text{l}$ , makes it difficult to meet current Acc&Prec. criteria for regulated BA

# Additional thoughts on small volume handling

- Data set shows what we possibly expected - worsening accuracy and precision at lower volumes
- 1 and 2 $\mu$ L are volumes generally lower than the industry currently uses
- Pipettes are generally more variable at lower volumes than capillaries

## Open questions on small volume handling

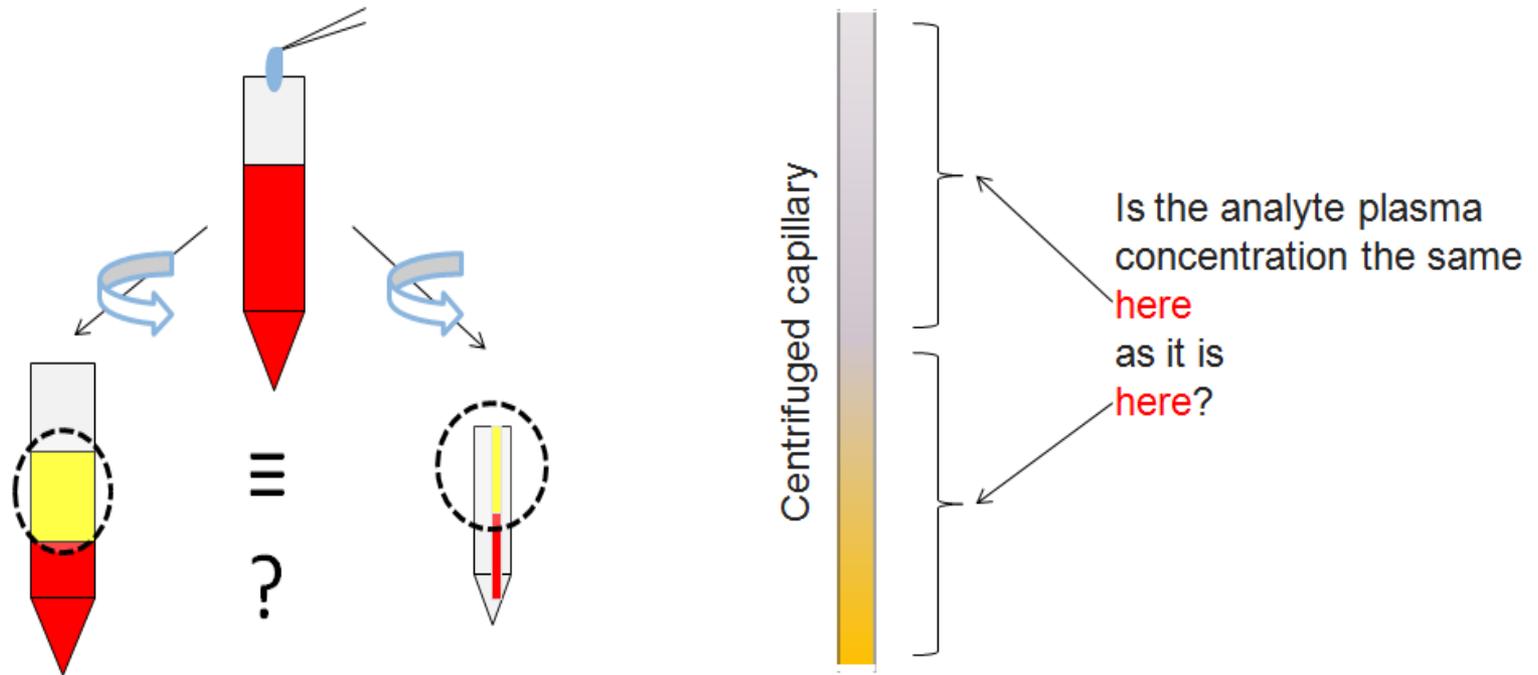
At what point (volume) is a bioanalytical assay, and the corresponding conclusions from the concentration data, impacted by low volume pipetting?

Or

What is acceptable volumetric accuracy and precision for microsampling?

# Homogeneity Protocol

'Control' plasma vs. Capillary plasma



2 capillary devices used: Vitrex and Drummond

*Images courtesy of Neil Spooner*

# Homogeneity Protocol

- Control 'classic' plasma vs. Frozen neat capillary plasma
- Homogeneity in capillary plasma – 4µL capillary #1 vs. 4 µL capillary #2

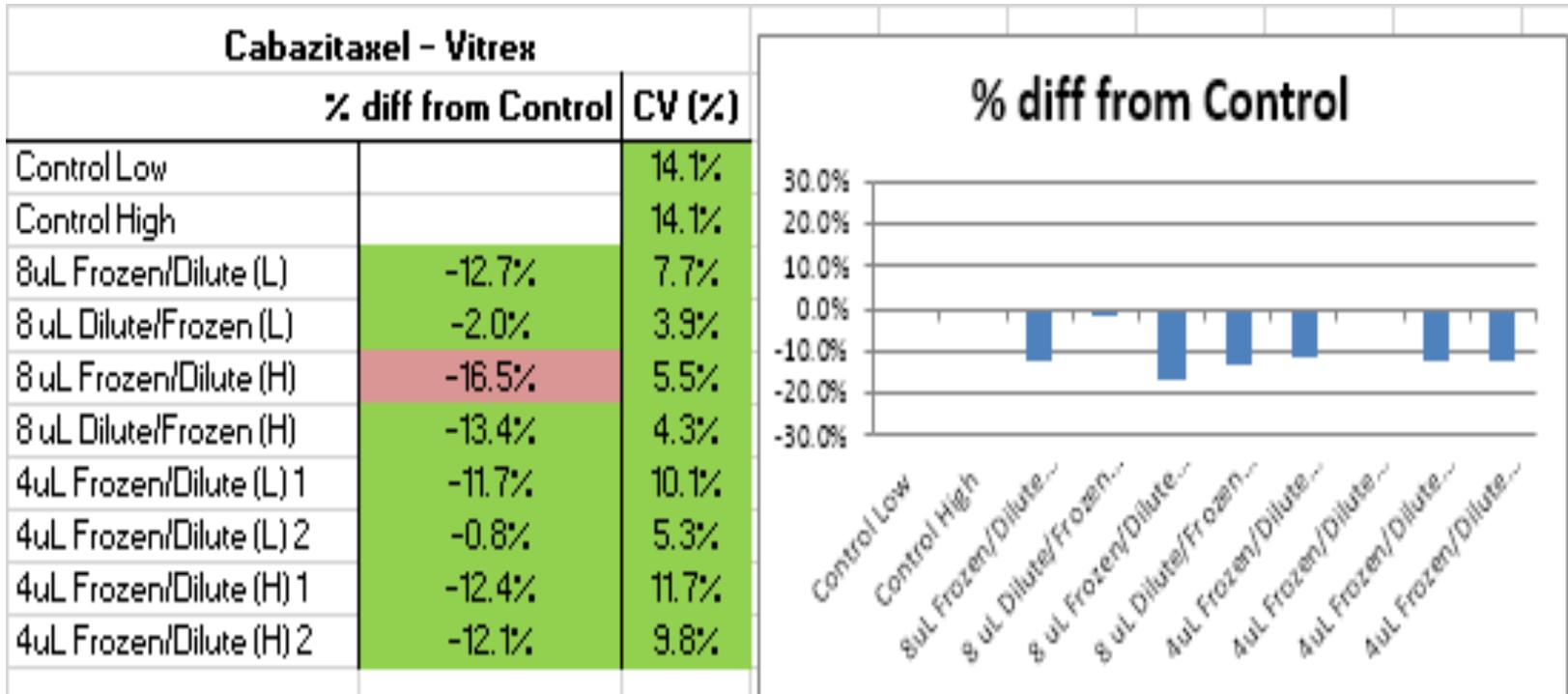
For ease of visualisation all concentration data was normalised to % of control

$$\frac{[\text{Capillary}] - [\text{Control}]}{[\text{Control}]} \times 100$$

# Homogeneity Protocol

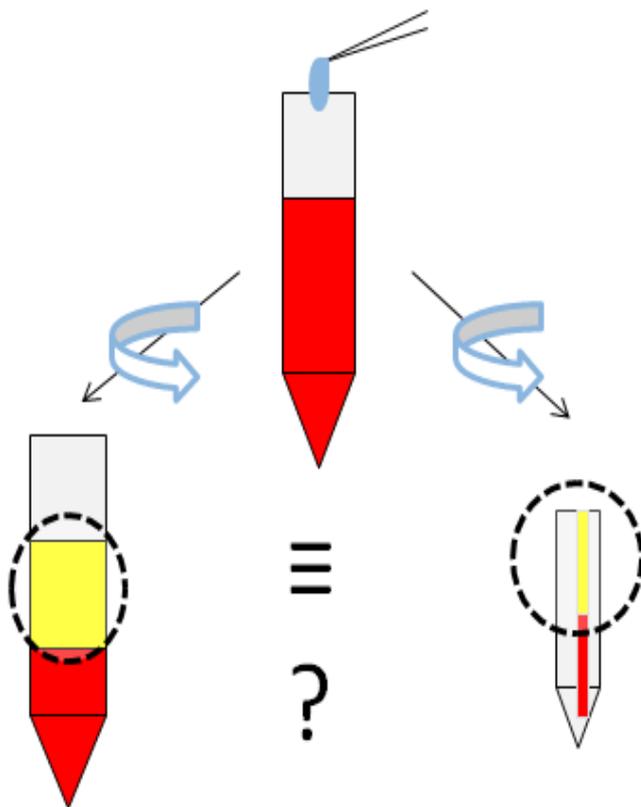
For ease of visualisation data was normalised to % of control

Key:	< 15%	>15%
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# Homogeneity conclusion 1

- Analyte measured in control plasma is the same as capillary plasma



# Homogeneity conclusion 1

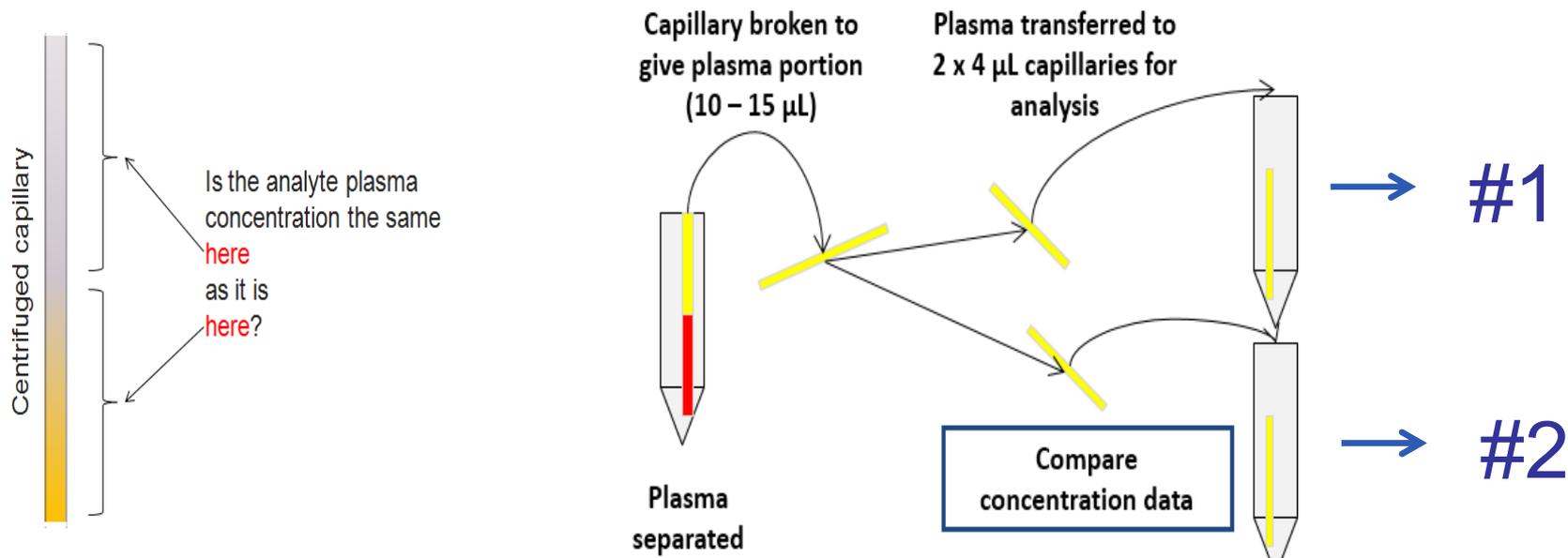
Compound	Concentration	Vitrex	Drummond
Omeprazole	Low	5.7%	2.8%
Omeprazole	High	10.7%	2.5%
Midazolam	Low	2.7%	-5.2%
Midazolam	High	9.2%	-4.0%
Cabazitaxel	Low	-12.7%	-15.0%
Cabazitaxel	High	-16.5%	-13.7%
Fasiglifam	Low	4.3%	3.3%
Fasiglifam	High	4.5%	-5.8%
Trastuzumab (Herceptin)	Low	-0.9%	-8.5%
Trastuzumab (Herceptin)	High	1.4%	-8.7%
EPA	Low	-9.4%	-3.1%
EPA	High	-4.7%	-6.9%
Buprenorphine	Low	-8.4%	-15.9%
Buprenorphine	High	-7.2%	-21.2%
Norbuprenorphine	Low	-3.9%	-0.9%
Norbuprenorphine	High	-4.1%	-10.2%
Simvastatin	Low	-6.5%	-11.0%
Simvastatin	High	-14.9%	-19.4%
Warfarin	Low	-8.1%	-11.9%
Warfarin	High	-0.9%	-4.0%
Atenolol	Low	-18.9%	-7.6%
Atenolol	High	-19.7%	-2.4%

 Capillary > control  
 Capillary < control

➤ 39/44 Determinations were within 15% of control

➤ 43/44 Determinations were within 20% of control

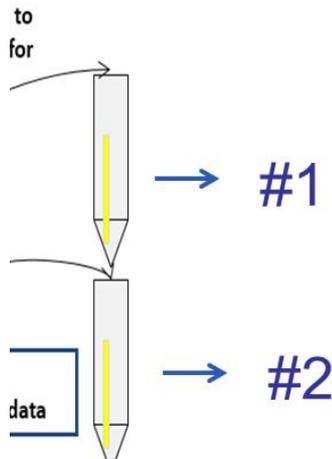
# Homogeneity conclusion 2



- No difference between '4 μL #1' and '4 μL #2'
- No difference between either of the 4uL capillaries and a single 8 uL capillary

# Comparison of 4μL #1 to 4μL #2

■ Cap 1 < Cap 2    Cap 1 > Cap 2 ■  
← →



Compound	Conc. level		
Atenolol	Low		-0.6%
Atenolol	High	■	-3.8%
Buprenorphine	Low		-0.2%
Buprenorphine	High	■	1.6%
Cabazitaxel	Low	■	-10.9%
Cabazitaxel	High		-0.2%
EPA	Low	■	0.7%
EPA	High	■	9.6%
Fasiglifam	Low	■	-0.8%
Fasiglifam	High	■	0.6%
Midazolam	Low	■	3.8%
Midazolam	High	■	2.1%
Norbuprenorphine	Low		-0.1%
Norbuprenorphine	High	■	1.9%
Omeprazole	Low	■	1.3%
Omeprazole	High	■	1.7%
Trastuzumab (Herceptin)	Low	■	-1.6%
Trastuzumab (Herceptin)	High	■	4.7%
Warfarin	Low	■	1.3%
Warfarin	High	■	-0.9%

No trend in bias

# Comparison of 4 $\mu$ L #1 and #2 to 8 $\mu$ L capillary

8  $\mu$ L < #1  
8  $\mu$ L > #1

8  $\mu$ L < #2  
8  $\mu$ L > #2

Compound	Conc. level		
Atenolol	Low		0.5%
Atenolol	High		10.7%
Buprenorphine	Low		2.2%
Buprenorphine	High		-0.7%
Cabazitaxel	Low		-1.0%
Cabazitaxel	High		-4.1%
EPA	Low		-17.5%
EPA	High		-16.0%
Fasiglifam	Low		11.9%
Fasiglifam	High		15.5%
Midazolam	Low		-6.0%
Midazolam	High		-5.4%
Norbuprenorphine	Low		2.4%
Norbuprenorphine	High		-2.0%
Omeprazole	Low		-1.5%
Omeprazole	High		-2.9%
Trastuzumab (Herceptin)	Low		6.6%
Trastuzumab (Herceptin)	High		-1.0%
Warfarin	Low		-6.8%
Warfarin	High		-0.9%

			0.0%
			6.9%
			2.0%
			0.9%
			-11.9%
			-4.3%
			-16.9%
			-6.4%
			11.1%
			16.2%
			-2.3%
			-3.3%
			2.4%
			-0.2%
			-0.2%
			-1.2%
			5.0%
			3.7%
			-5.5%
			-1.8%

All 4 $\mu$ L capillaries within 20% of concentrations determined in 8 $\mu$ L

# Additional thoughts on handling capillaries for microsampling

- The capillary diluent is important and should be assessed prior to implementing a capillary microsampling approach, for compounds with high log P recommend adding protein to diluent.
- Handling capillaries requires manual skill and is more labour intensive than conventional blood and plasma sampling approaches.
- Training and experience are key to ensure best data quality

# Conclusions

- Capillaries offer better Acc & Prec. performance in plasma than handheld pipettes
- When handling  $< 4\mu\text{L}$ , inaccuracy and imprecision introduced by the pipetting step may compromise the assay to meet current Regulated Bioanalysis Acc & Prec. acceptance criteria
  - What is acceptable volumetric accuracy and precision for microsampling?
- Homogeneity of sample does not seem to be compromised when preparing plasma using current industry used capillary volumes
- Advice: the industry would benefit from best practise handling instructions for low volume sampling devices to obtain the best quality data

# Acknowledgements

- Members of EBF Microsampling team
- Contributors to the experimental protocols
  - AstraZeneca
  - Charles River
  - GlaxoSmithKline
  - Janssen R&D
  - LGC
  - Lundbeck A/S
  - QPS
  - Sanofi
- GlaxoSmithKline and AstraZeneca for supply of protocol kits