Update from the EBF Liquid Microsampling Consortium

High level results from small volume handling and capillary homogeneity protocols

Glen Hawthorne,
on behalf of the EBF Liquid Microsampling Consortium

EBF 9th 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

Editorial

Bioanalysis

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EBF: reflection on bioanalytical assay requirements used to support liquid microsampling

"As we continue to investigate further through targeted experiments, we will, as a community, gain better insight on this topic."

EBF

Keywords: capillary • European Bioanalysis Forum • liquid microsampling • sample homogeneity • validation

Commentary

Bioanalysis

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European Bioanalysis Forum continued plans to support liquid microsampling

Keywords: consortium • EBF • experiments • liquid microsampling • validation

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Building the EBF LMS Consortium

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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 µL (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 µL (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

Key:	< 5%	> 5%	>10%

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

Small volume evaluation – Plasma Accuracy

Pipettes

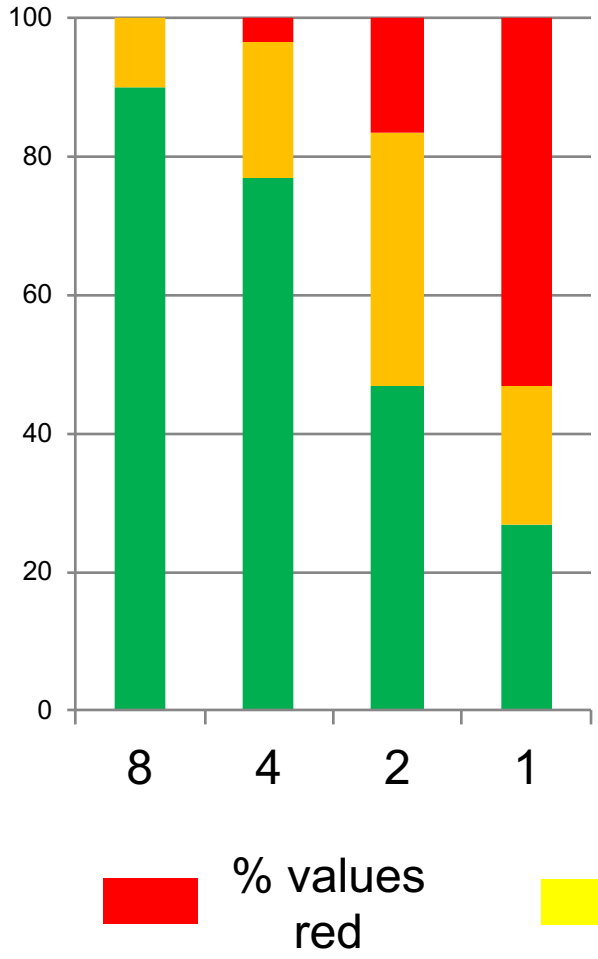
Volume (uL)			
8	4	2	1
100.5%	99.9%	100.3%	112.4%
97.2%	96.3%	89.5%	82.0%
98.5%	99.2%	95.5%	108.0%
100.3%	99.6%	94.5%	86.6%
101.0%	116.2%	113.9%	119.2%
91.2%	95.3%	100.7%	111.6%
99.8%	102.0%	106.3%	111.2%
97.2%	98.8%	107.5%	109.3%
98.0%	97.6%	90.2%	99.1%
99.5%	98.9%	94.5%	98.8%
100.7%	100.0%	101.2%	92.5%
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

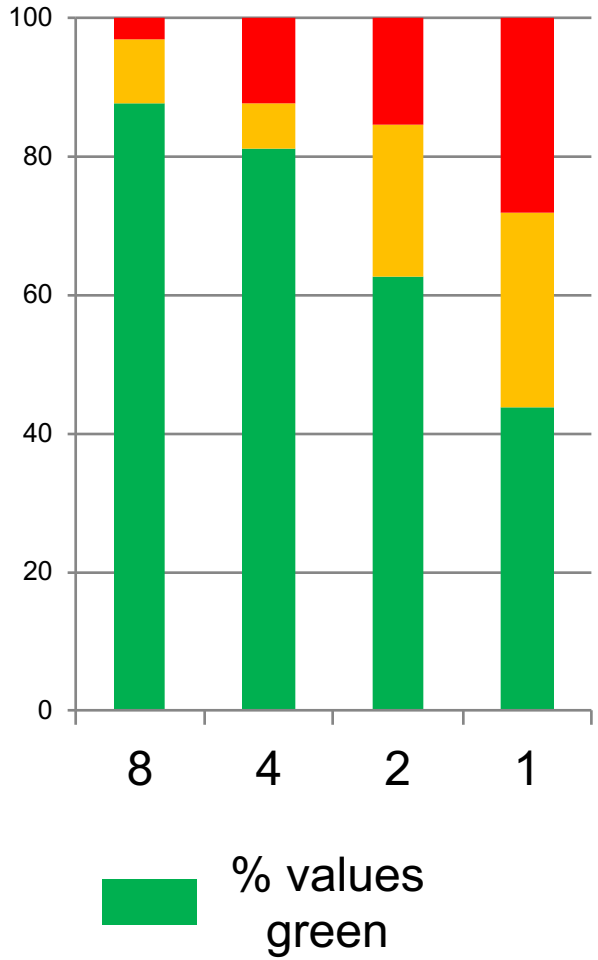
Volume (uL)			
8	4	2	1
100.6%	100.8%	98.0%	98.8%
100.1%	102.0%	106.9%	102.4%
101.1%	101.2%	101.8%	104.1%
99.9%	100.4%	107.7%	106.4%
104.4%	105.9%	114.8%	97.6%
98.8%	99.6%	96.7%	94.5%
96.0%	101.2%	102.0%	118.1%
97.5%	98.0%	113.4%	114.0%
98.3%	98.7%	97.0%	98.5%
97.7%	98.2%	98.9%	100.7%
102.4%	103.3%	103.2%	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

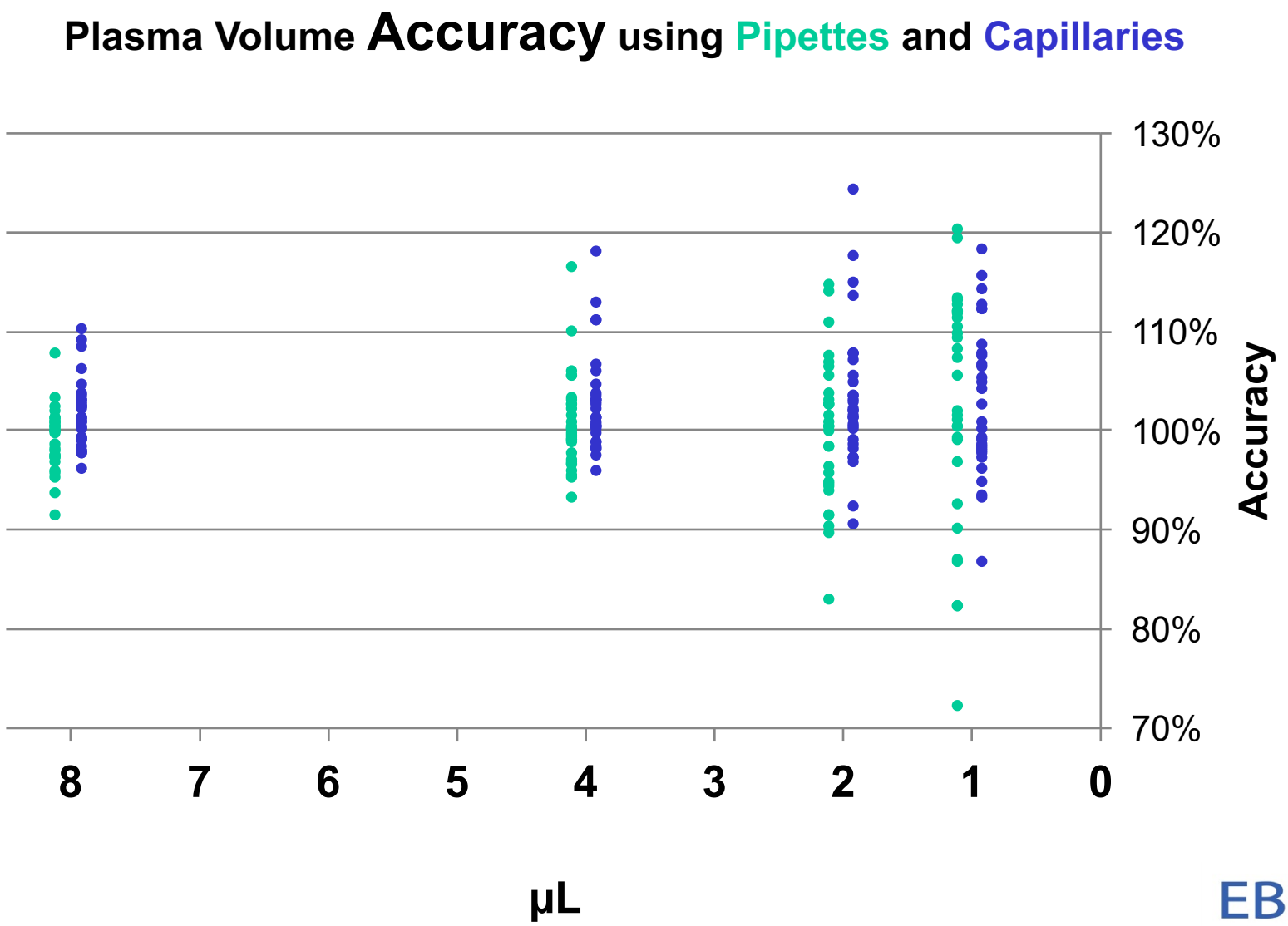


% values red

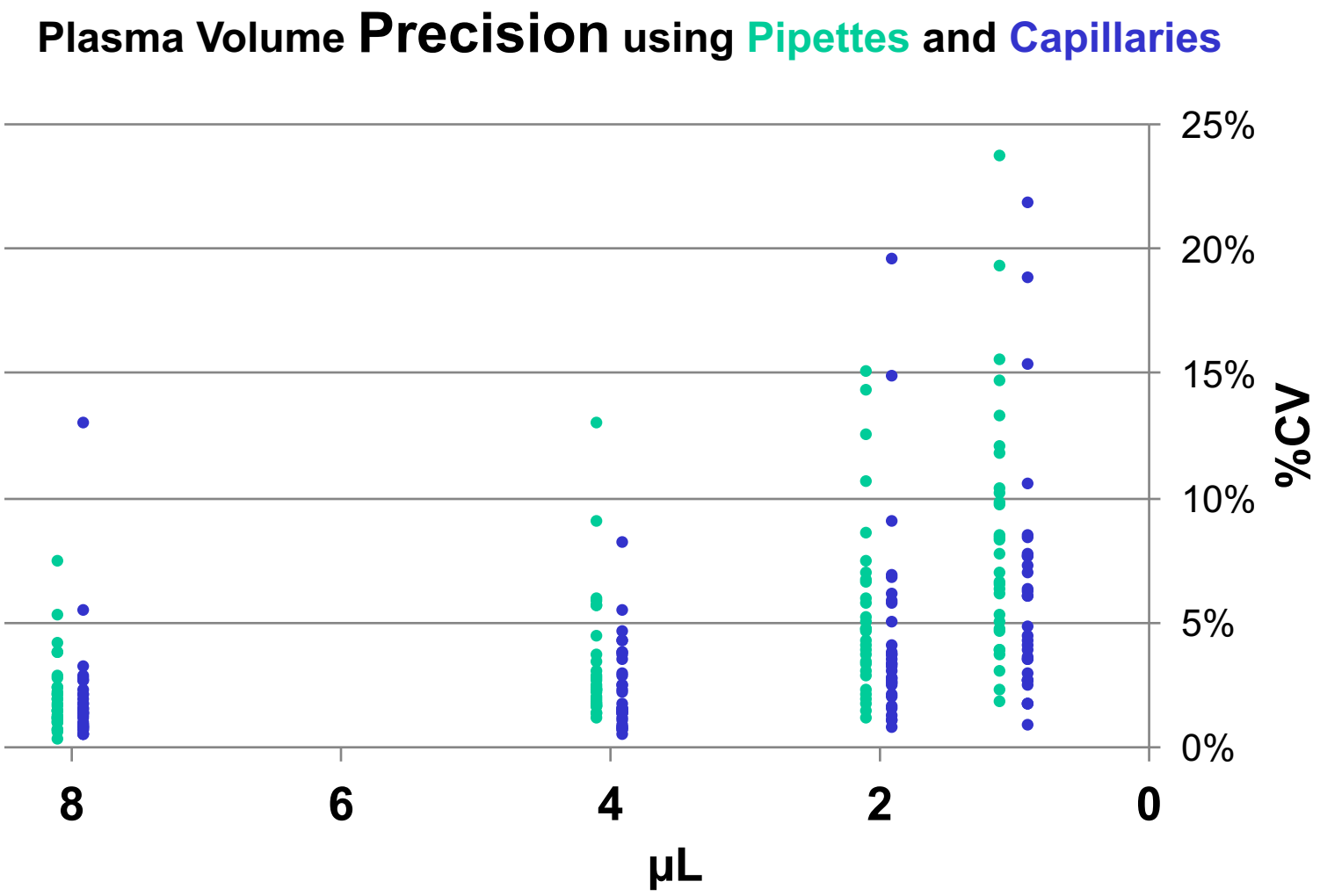
% values yellow

% values green

Small volume evaluation – Accuracy



Small volume evaluation – Precision



Small Volume Handling Protocol - conclusions

- Accuracy
  - Accuracy of pipetting with capillaries is better than manual pipettes for low volume plasma handling (≤ 4 µl)
- Precision
  - Precision of pipetting with capillaries is better than manual pipettes for low volume plasma handling (≤ 4 µl)
- Consequences for the assay:
  - For both capillaries and pipettes, the inaccuracy and imprecisions introduced by the pipetting step for volumes ≤ 4 µl, makes it difficult to meet current Acc&Prec. criteria for regulated BA

Additional thoughts on small volume handing

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

Open questions on small volume handing

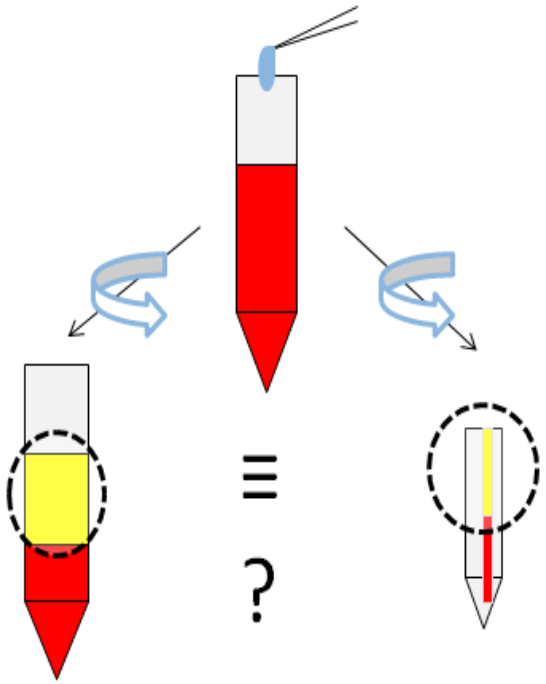
At what point (volume) is a bioanalytical assay, and the corresponding conclusions from the concentration data, impacted by low volume pipetting?

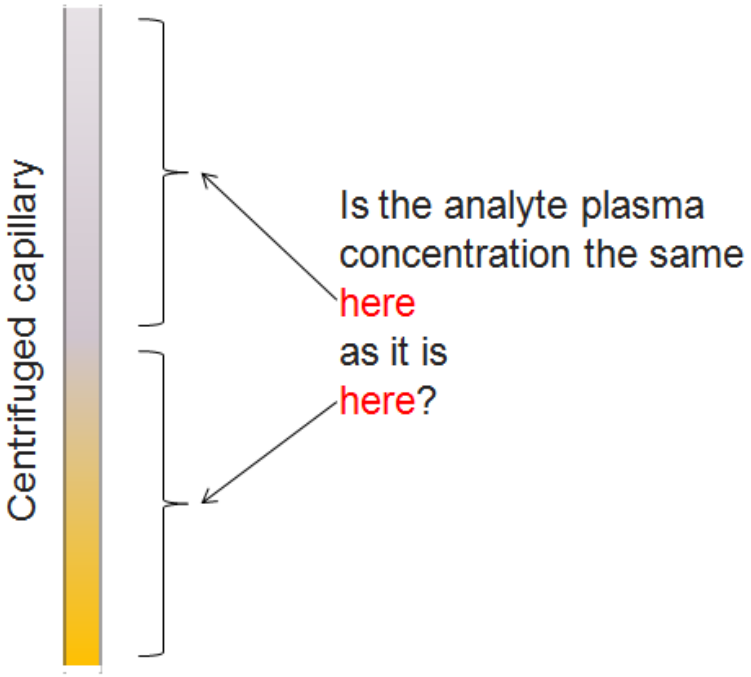
Or

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

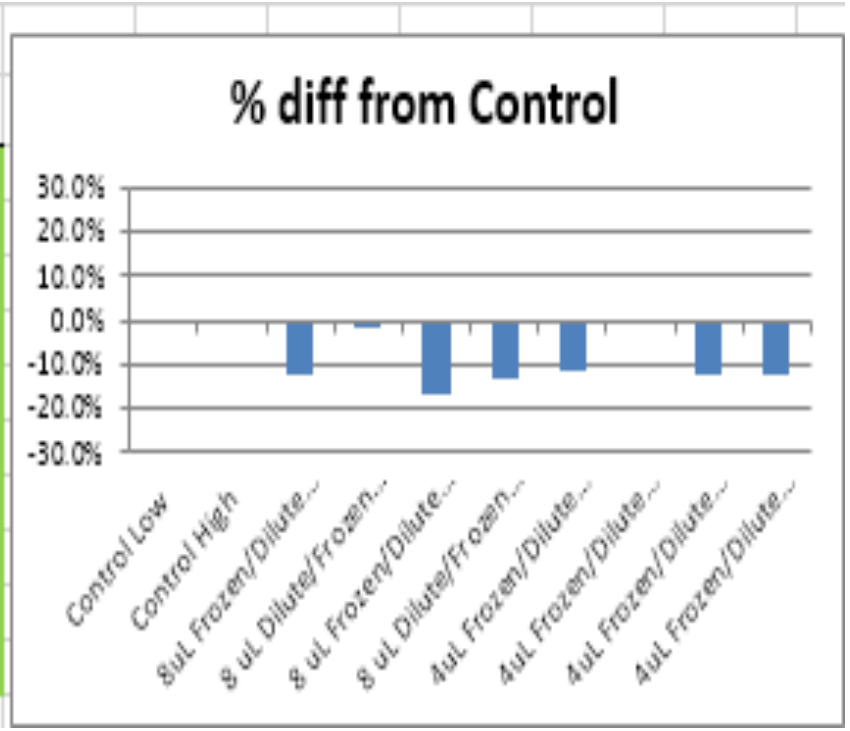
$$\frac{[\text{Capillary}] - [\text{Control}]}{[\text{Control}]} \text{ X } 100$$

Homogeneity Protocol

For ease of visualisation data was normalised to % of control

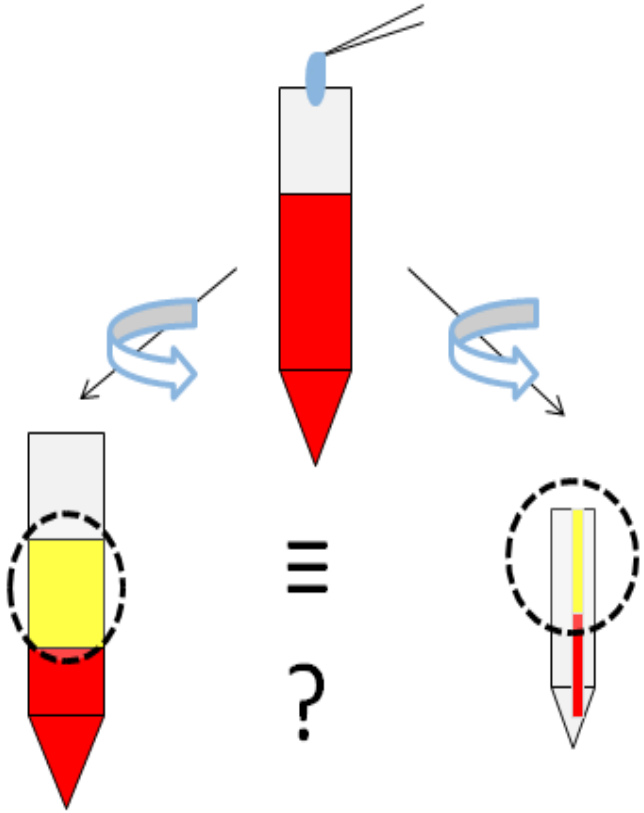
Key:	< 15%	>15%

Cabazitaxel – Vitrex		
	% diff from Control	CV (%)
Control Low		14.1%
Control High		14.1%
8uL Frozen/Dilute (L)	-12.7%	7.7%
8 uL Dilute/Frozen (L)	-2.0%	3.9%
8 uL Frozen/Dilute (H)	-16.5%	5.5%
8 uL Dilute/Frozen (H)	-13.4%	4.3%
4uL Frozen/Dilute (L) 1	-11.7%	10.1%
4uL Frozen/Dilute (L) 2	-0.8%	5.3%
4uL Frozen/Dilute (H) 1	-12.4%	11.7%
4uL Frozen/Dilute (H) 2	-12.1%	9.8%



Homogeneity conclusion 1

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



Homogeneity conclusion 1

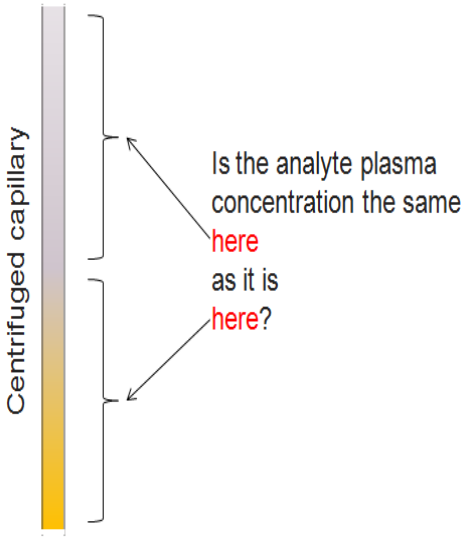
Compound	Concentration	Vitrex	Drummond
Omeprazole	Low	5.7%	2.8%
Omeprazole	High	10.7%	2.5%
Midazolam	Low	2.7%	-5.2%
Midazolam	High	9.2%	-4.0%
Cabazitaxel	Low	-12.7%	-15.0%
Cabazitaxel	High	-16.5%	-13.7%
Fasiglifam	Low	4.3%	3.3%
Fasiglifam	High	4.5%	-5.8%
Trastuzumab (Herceptin)	Low	-0.9%	-8.5%
Trastuzumab (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%

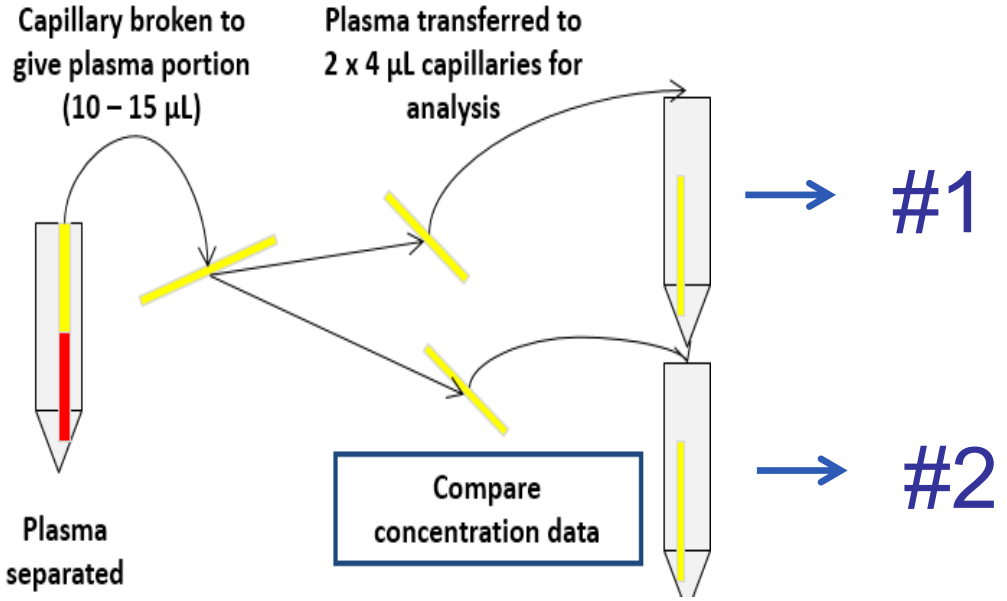
Capillary > control

Capillary < control

- 39/44 Determinations were within 15% of control
- 43/44 Determinations were within 20% of control

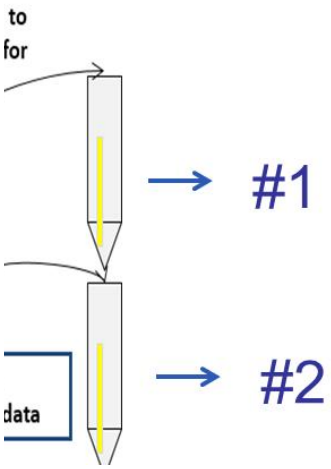
Homogeneity conclusion 2





- 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 4µL #1 and #2 to 8µL capillary

Compound	Conc. level	8 µL < #1 / 8 µL > #1	8 µL < #2 / 8 µL > #2
Atenolol	Low	0.5%	0.0%
Atenolol	High	10.7%	6.9%
Buprenorphine	Low	2.2%	2.0%
Buprenorphine	High	-0.7%	0.9%
Cabazitaxel	Low	-1.0%	-11.9%
Cabazitaxel	High	-4.1%	-4.3%
EPA	Low	-17.5%	-16.9%
EPA	High	-16.0%	-6.4%
Fasiglifam	Low	11.9%	11.1%
Fasiglifam	High	15.5%	16.2%
Midazolam	Low	-6.0%	-2.3%
Midazolam	High	-5.4%	-3.3%
Norbuprenorphine	Low	2.4%	2.4%
Norbuprenorphine	High	-2.0%	-0.2%
Omeprazole	Low	-1.5%	-0.2%
Omeprazole	High	-2.9%	-1.2%
Trastuzumab (Herceptin)	Low	6.6%	5.0%
Trastuzumab (Herceptin)	High	-1.0%	3.7%
Warfarin	Low	-6.8%	-5.5%
Warfarin	High	-0.9%	-1.8%

All 4µL capillaries within 20% of concentrations determined in 8µL

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 < 4µL, 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

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