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Evaluation of Capitainer *q*DBS Microsampling Device for Anti-Epileptic Drug Quantitation

Jeff Plomley

Scientific Director, Bioanalysis (LC-MS)

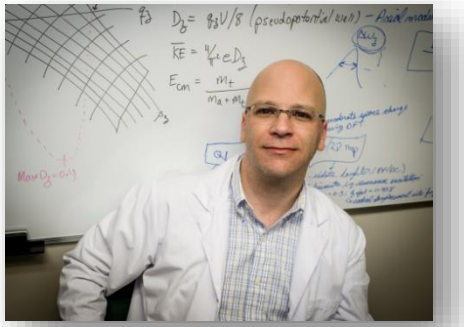
ALTASCIENCES

Session Description and Objectives

- Introduce the Capitainer qDBS patient-centric microsampling device as a novel and straightforward approach to blood collection
- Outline the mechanism of action of the qDBS device with regards to dissolvable film metering and microfluidic transport to achieve a completely self-activated system for volumetric blood deposition onto pre-cut cellulose substrate
- Describe the performance attributes of the qDBS device with presentation of experimental results from analysis of fifteen anti-epileptic drug (AED) probes
- Propose a workflow for sample preparation that mitigates potential for Ht-induced recovery bias and age-related extractability

Biography and Contact Information

- Graduate work on Applications and Scan Function development for 3D Ion traps
- Ten years in the MS instrumentation industry involved in Applications Development, Marketing and Core-research on QQQ, QTOF, QTrap and 3D ion trap platforms
- Seventeen years of regulated LC-MS assay development supporting non-clinical & clinical programs
- Numerous peer-reviewed publications, tech pubs and patents on MS instrumentation
- Currently Scientific Director for Method Development (LC-MS) at Altasciences in Canada
- Contact Info: jplomley@altasciences.com



Ideal Blood Microsampling Device

- Accurate and precise volumetric sampling, independent of Ht over a defined range
- Straightforward design that minimizes likelihood for collection error (e.g. over-sampling)
- Portable and with adequate space for sample labelling
- Allow replicate samples within the same device per fingerstick event
- Collection substrate should ideally be:
  - ✓ inert → absence of exogenous interference (e.g. matrix effect)
  - ✓ amenable to workflows that mitigate Ht-induced recovery bias
  - ✓ easily accessible to facilitate downstream BioA laboratory processing

Capitainer qDBS: Device Overview

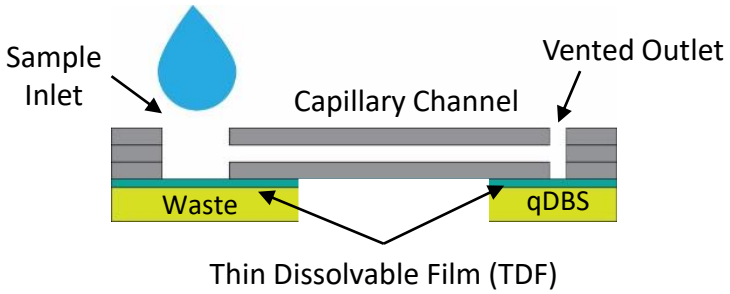
Sample labelling site

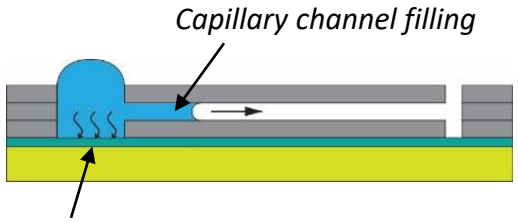
Sampling instructions

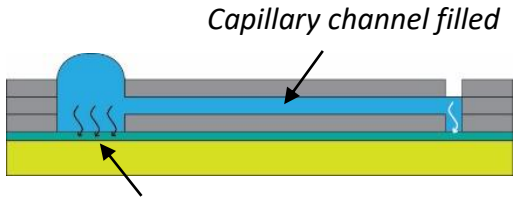
Self-indicating Collection

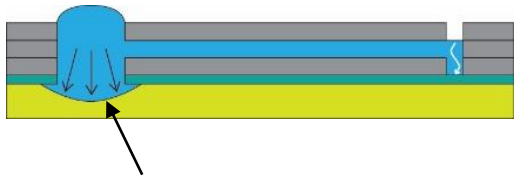
Easily removal DBS samples on pre-cut discs (2 x 10 µL)

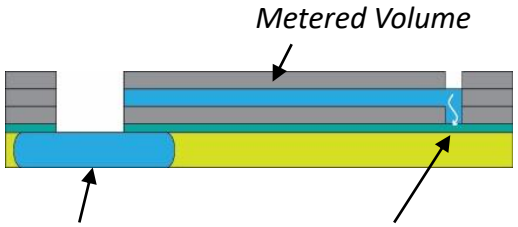
Mechanism of Volumetric Collection

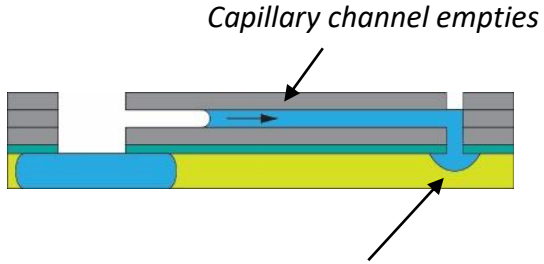




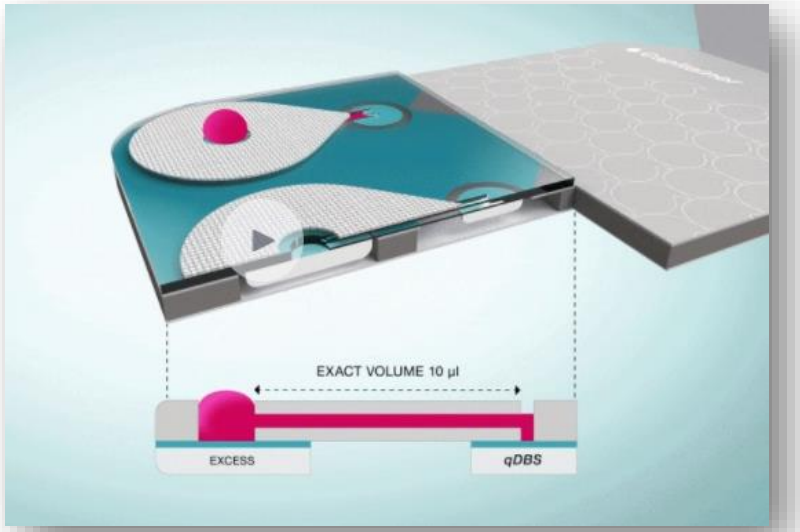


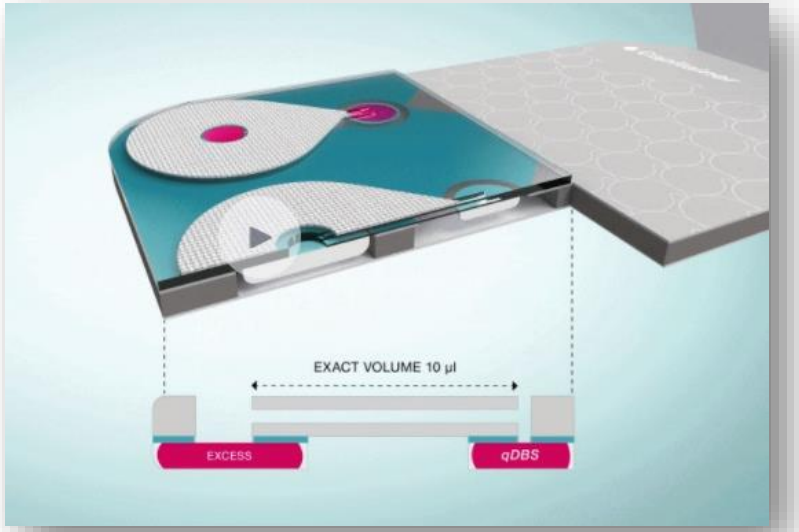






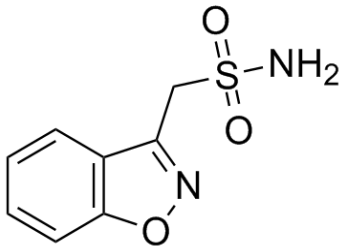
Mechanism of Volumetric Collection

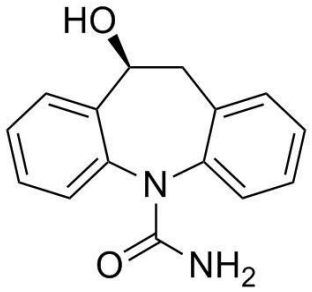


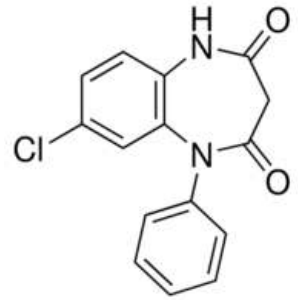


Initial Evaluation Experiments

- Leverage a panel of fifteen AEDs as probes to characterize qDBS performance
- Establish efficient workflow for consistent recovery of AEDs from Ahlstrom 222 cellulose substrate
- Determine accuracy and precision of volumetric transfer to substrate as a function of:
  - ✓ blood Ht
  - ✓ volume of blood applied at inlet sampling port
  - ✓ analyte physicochemical properties (e.g. polarity)



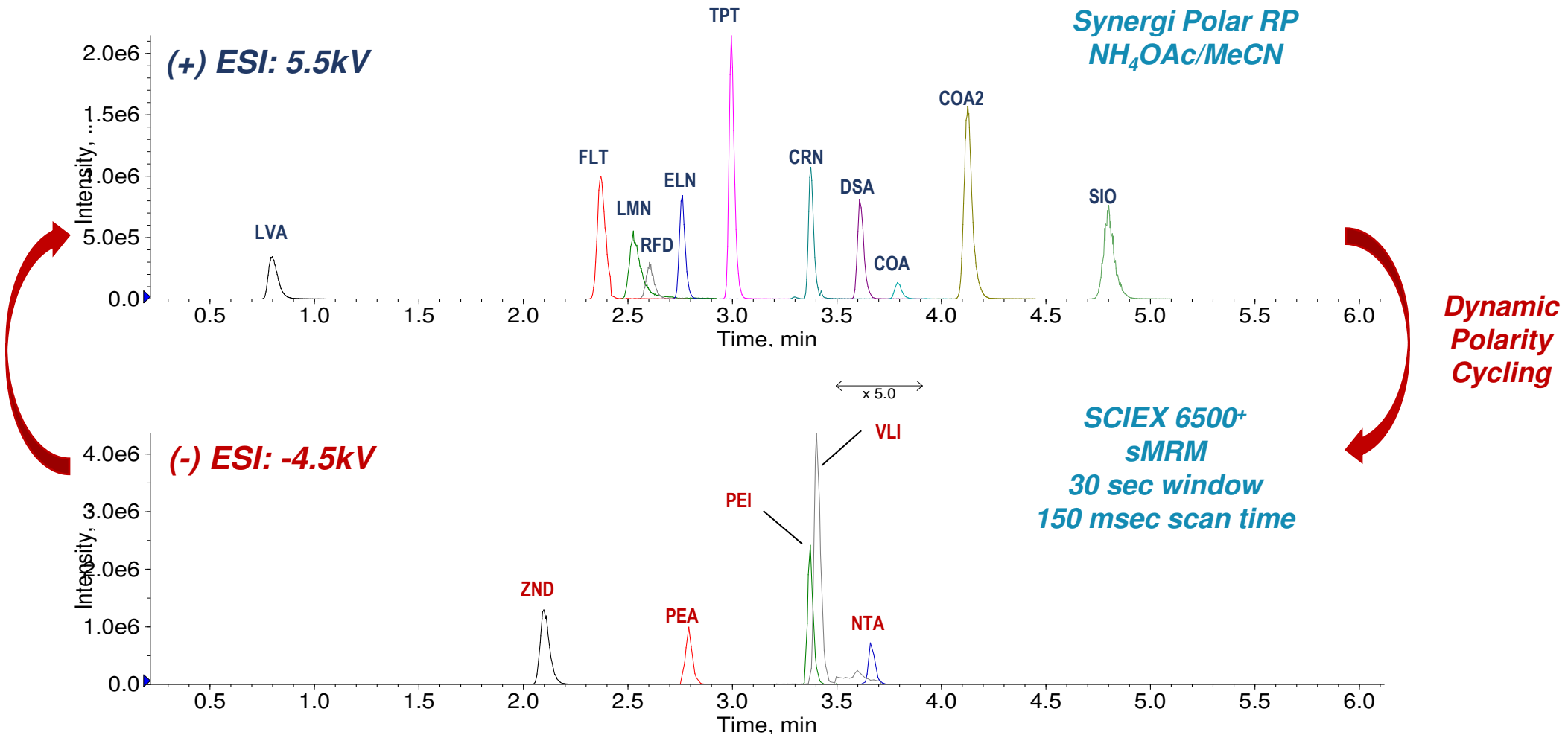




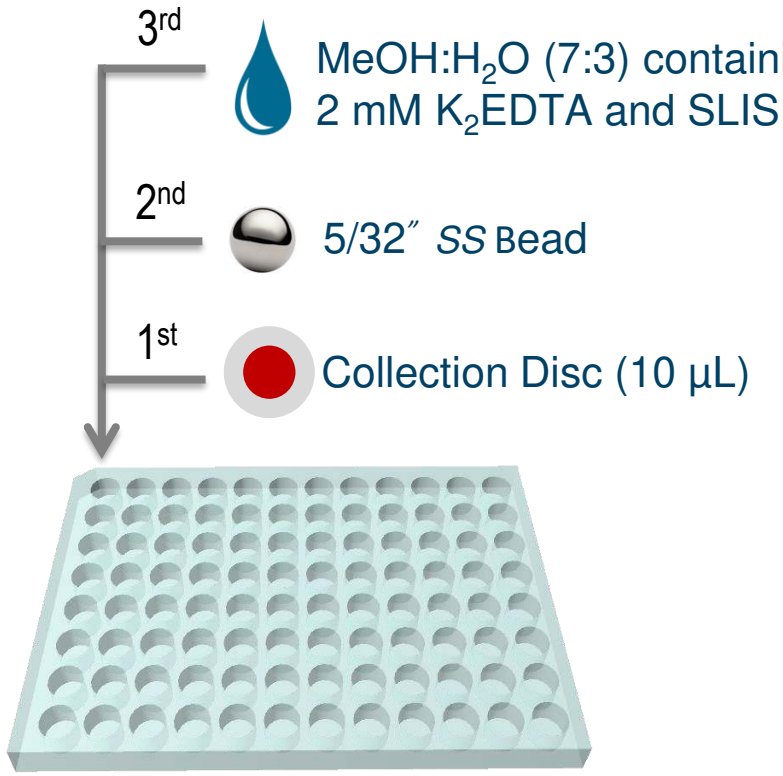
Properties of AED Probes

AED	Abbreviation	clogP	ESI Polarity	MRM Transition	t_r (min)
Clonazepam	COA	3.15	+	316.1 > 270.2	3.79
Carbamazepine	CRN	2.77	+	237.1 > 194.3	3.37
Rufinamide	RFD	1.93	+	239.3 > 221.8	2.60
Phenobarbital	PEA	2.14	-	231.1 > 42.0	2.79
Lamotrigine	LMN	1.43	+	256.0 > 211.0	2.53
Phenytoin	PEI	3.40	-	251.1 > 101.9	3.37
Stiripentol	SIO	3.12	+	217.0 > 187.0	4.80
Carbamazepine	CBM	2.77	+	237.1 > 194.3	4.13
Felbamate	FLT	0.68	+	239.2 > 117.0	2.37
Levatiracetam	LVA	0.59	+	171.1 > 126.1	0.80
N-Desmethylclobazam	DSA	**3.42**	+	287.0 > 245.1	3.61
Topiramate	TPT	0.13	+	357.3 > 264.1	3.00
S-Licarbazepine	ELN	1.73	+	255.1 >194.4	2.76
Zonisamide	ZND	**0.11**	-	211.1 > 147.0	2.10
Nitrazepam	NTA	2.55	-	280.0 > 252.2	3.66

LC-sMRM Profile of Fifteen AEDs



qDBS Collection Disc Extraction



SPEX Geno/Grinder 2010

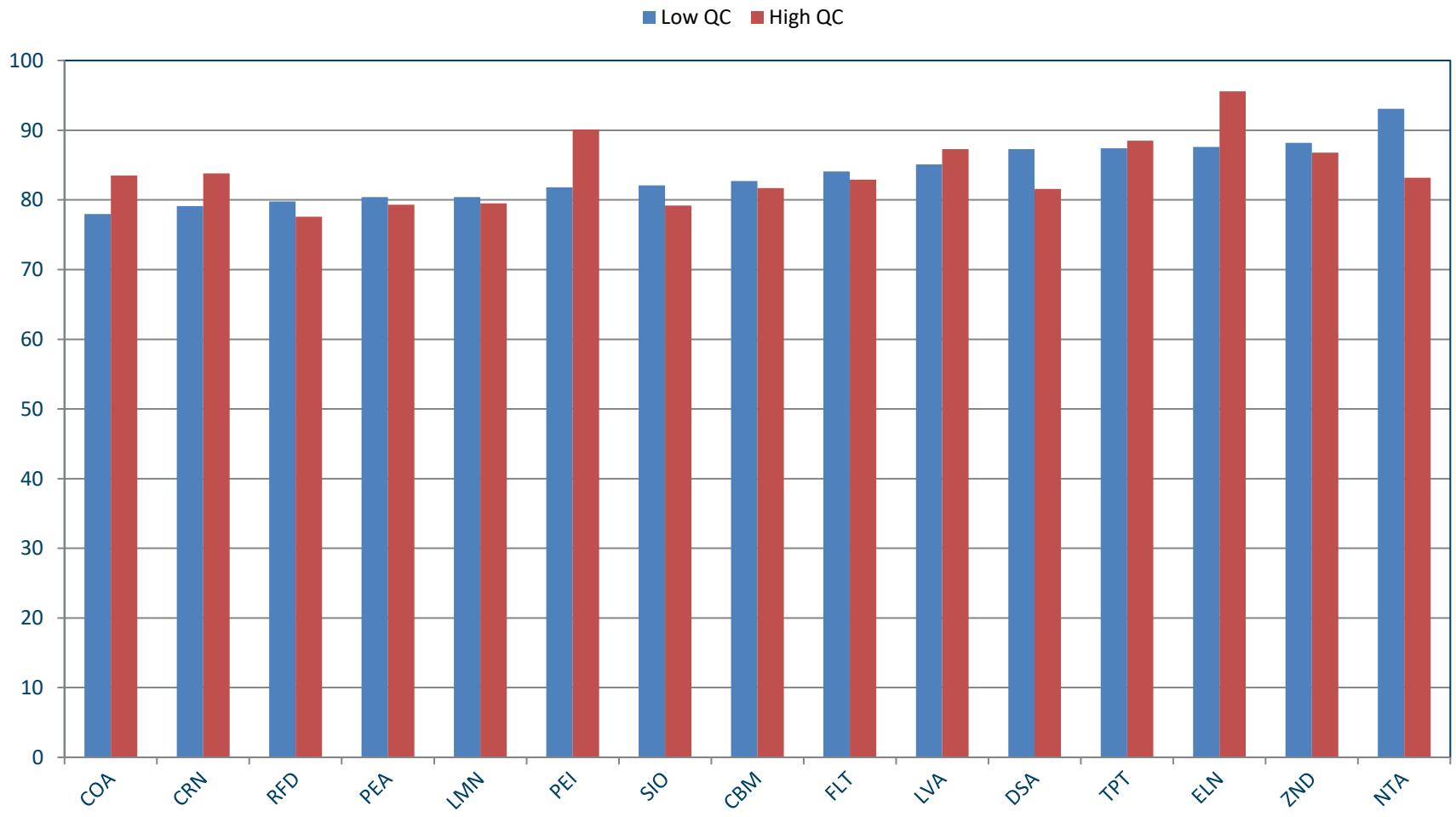
1750 strokes/min; 5 min

CF, Aliquot SN,
Dilute 3-fold

SCIEX 6500+

Scheduled MRM

Recovery by Substrate Homogenization



Impact of Inlet Sample Volume and Blood Ht

Response bias* relative to On-disc Application

AED	20 µL Inlet Sampling			50 µL Inlet Sampling
	25% Ht	*40% Ht*	*55% Ht*	*40% Ht*
COA	4.5	-0.4	-0.3	-6.6
CRN	0.7	1.6	-1.7	0.3
RFD	-1.1	-3.1	-2.8	-0.8
PEA	2.3	3.5	0.2	1.8
LMN	1.2	0.3	0.8	-0.5
PEI	-0.3	-1.6	-2.5	-1.7
SIO	-1.1	-2.4	-4.9	-0.7
CBM	1.9	-1.4	-4.7	-0.5
FLT	1.1	0.8	-2.5	0.7
LVA	2.6	4.3	4.0	0
DSA	5.5	0.2	-3.5	0.6
TPT	-6.9	-5.6	-5.7	-6.1
ELN	1.2	2.7	-0.1	0.2
ZND	-1.1	2.7	3.2	2.3
NTA	-1.7	0.6	-8.4	-8.9
Avg. Deviation	**2.1**	**2.1**	**2.6**	**2.4**

*expressed as a percent based on peak area ratio relative to 10.0 µL direct-on disc application of mid-QC at 40% Ht

Calibrants: % Deviation from Nominal

Application of 10 µL on-disc, 40% Ht; linear regression, $1/x^2$

AED	Calibrants									Range (µg/mL)	Corr. Coeff.
	P1	*P2*	*P3*	*P4*	*P5*	*P6*	*P7*	*P8*	*P9*		
COA	4.8	-2.9	-12.8	-2.6	1.0	2.5	2.2	1.1	6.7	0.0025 - 0.250	0.9955
CRN	-2.1	5.9	-1.4	-3.4	-1.4	3.5	-3.7	2.6	0.0	0.080 – 8.00	0.9985
RFD	-2.2	3.4	2.5	-1.6	3.4	-3.4	-2.9	-5.3	6.0	0.075 – 7.50	0.9979
PEA	0.1	3.3	-5.8	-0.2	-4.2	-2.3	6.5	1.4	1.3	0.080 – 8.00	0.9980
LMN	-0.9	0.5	1.3	2.6	-0.3	0.8	-4.1	-2.4	2.3	0.160 – 16.0	0.9993
PEI	2.2	-2.0	-5.6	1.2	1.5	-1.8	-0.7	6.1	-0.9	0.080 – 8.00	0.9985
SIO	-0.6	1.9	-1.0	-1.7	1.2	1.0	-2.3	0.4	1.1	0.200 – 20.0	0.9997
CBM	-0.6	1.6	-0.2	0.2	-1.0	-1.4	-2.0	0.0	3.4	0.010 – 1.00	0.9996
FLT	1.4	-3.1	-1.7	2.4	2.7	4.6	-6.7	2.8	-2.5	2.00 – 200.0	0.9981
LVA	0.1	0.7	-2.3	0.7	0.2	0.7	-0.3	1.1	-0.9	0.200 – 20.0	0.9998
DSA	-9.2	13.4	12.4	-4.7	4.2	-4.9	-5.8	-1.7	-3.6	0.010 – 1.00	0.9908
TPT	-1.3	1.8	-0.2	3.2	0.6	2.1	-1.8	-2.5	-2.0	0.200 – 20.0	0.9994
ELN	0.2	0.5	-1.7	1.7	-3.5	2.3	-4.2	2.2	2.5	0.200 – 20.0	0.9991
ZND	0.4	0.9	-3.9	-0.1	3.7	-3.3	0.1	-3.2	5.4	0.080 – 8.00	0.9986
NTA	0.4	2.5	-5.8	-0.7	-1.1	-2.6	0.9	5.4	1.0	0.001 – 0.100	0.9986

Within-Run Precision and Accuracy

Application of 20 µL at Sampling Inlet; Six QC Replicates / Level, 40% Ht

AED	QC -LOQ		Low QC		Mid QC		High QC	
	% CV	*% Nominal*	*% CV*	*% Nominal*	*% CV*	*% Nominal*	*% CV*	*% Nominal*
COA	**9.8**	97.2	8.3	**101.4**	3.8	**106.0**	4.2	**102.6**
CRN	4	96.0	5.3	96.7	4.1	100.5	3.0	100.9
RFD	6.2	90.9	5.1	**94.4**	2.6	99.2	2.7	99.4
PEA	3	95.5	5.7	97.2	3.8	98.5	4.3	101.1
LMN	3.8	98.5	3.8	98.9	2	99.9	3.1	101
PEI	8.3	99.1	4.1	98.2	3.5	99.8	3.2	101
SIO	4.3	91.0	2.7	96.7	2	97.1	1.8	99.4
CBM	2.5	97.1	1.6	97.2	2.4	98.3	1.6	99.8
FLT	4.7	91.9	4.3	94.5	4.6	99.4	3.5	98.5
LVA	4.1	96.6	2.8	97.5	3.3	100.5	2.2	100.5
DSA	6.2	**86.4**	6.4	96.4	**6.9**	**95.5**	**8.0**	98.9
TPT	5.3	100.8	4.2	96.7	3.1	99.1	2.7	101
ELN	4.3	101.2	2.4	96.4	5.1	100.8	1.7	99.8
ZND	2.8	101.1	3.6	98.1	2.9	99.8	1.9	99.5
NTA	5.5	**110.4**	**8.4**	99.7	5.3	99.1	1.8	**92.3**

Conclusions

- The Capitainer microfluidic DBS device delivered an accurate and precise volume of blood (10 µL) onto pre-cut cellulose substrate and was immune to:
  - ✓ applied blood volume (20-50 µL evaluated) → design prevents oversampling
  - ✓ Ht-bias (25-55% Ht evaluated) → complete pre-cut disc sampled
  - ✓ blood viscosity (impacting capillary flow)
  - ✓ analyte polarity and range of potential binding interactions
- Homogenization of the collection disc is expected to mitigate any potential for:
  - ✓ hematocrit-induced recovery bias
  - ✓ age-related extractability
- A straightforward patient-centric design for microsampling

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- Mikael Strom (Capitainer)

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Questions

- *jplomley@altasciences.com*
- *https://www.altasciences.com/*
- *https://www.linkedin.com/company/altasciences*
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