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Micro-managing: exploring the potential to use micro-sampling technology for quantitation of large molecules

Richard Hughes

EBF Open Symposium
2022

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Micro-managing: exploring the potential to use micro-sampling technology for quantitation of large molecules

- Types of volumetric devices available and considerations for practical use
- Investigation into the application of quantitative micro-sampling of proteins
- LBA method development perspectives

Preliminary testing of multiple volumetric devices

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What to consider?

HCT effect and recovery

P&A, Selectivity, Dilution linearity

Stability

Ease of use

Harmony with automation

Compatibility with process (LIMS etc)

Sustainability

Testing of multiple volumetric devices - Assays

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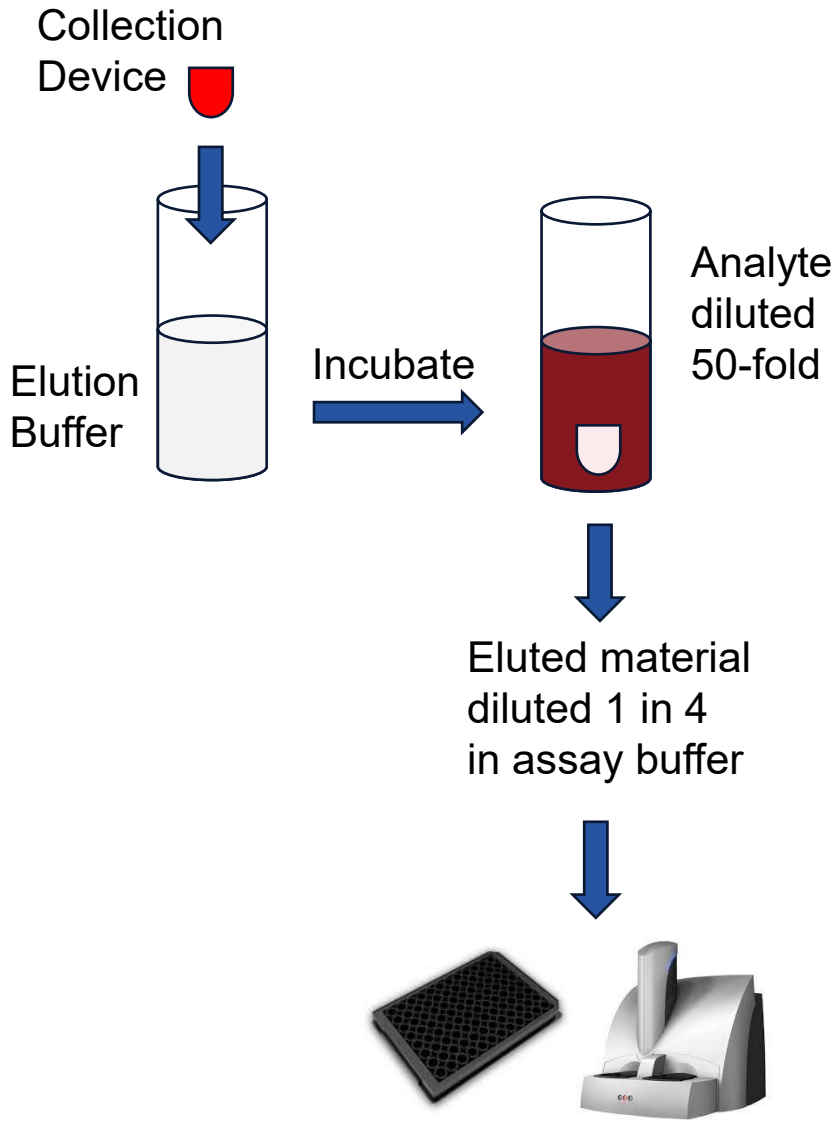
- Human full length IgG targeting soluble cytokine
- MSD endpoint assay: biotinylated anti-idiotypic mAb capture with Sulfo-tagged anti-idiotypic mAb detection
- Serum assay had an MRD of 1 in 100. This was increased to 1 in 200 to allow for elution and then a secondary dilution in assay buffer



Testing of multiple volumetric devices – initial failures

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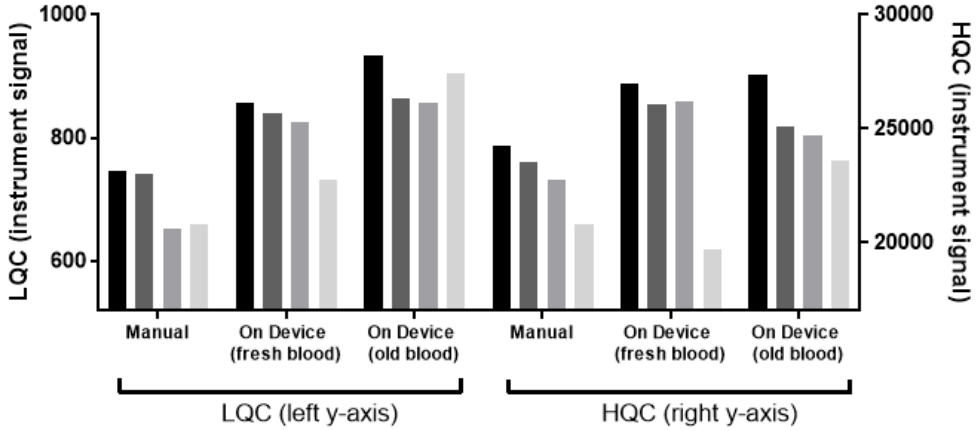
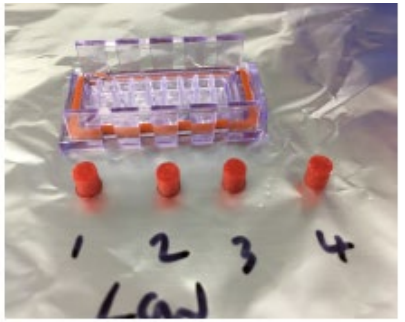
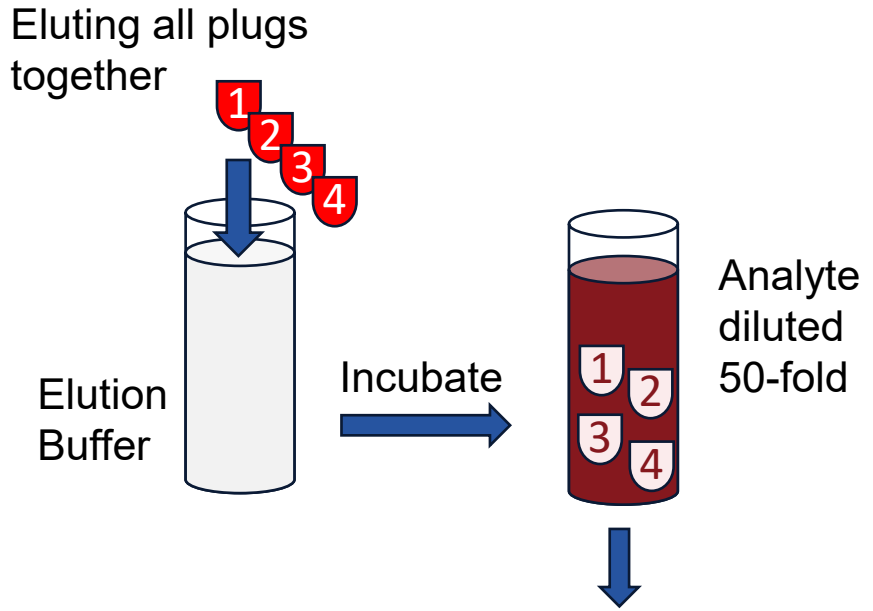
Tasso





Manual samples were prepared by removing 'clean' plugs from the device and pipetting 17.5 µL of sample directly onto the plug, independent of the cartridge.



	Tasso	*mean*	*%CV*	*%RE*
ULoQ1	22340			
ULoQ1	21929	22562	3.4	-10
ULoQ1	23419			
HQC1	20270			
HQC1	20024	20440	2.6	2
HQC1	21025			
MQC1	3275			
MQC1	3168	3291	4.0	-6
MQC1	3430			
LQC1	616			
LQC1	569	607	5.7	1
LQC1	636			
LLoQ1	203			
LLoQ1	175	187	7.7	-6
LLoQ1	183			

Testing of multiple volumetric devices – initial failures

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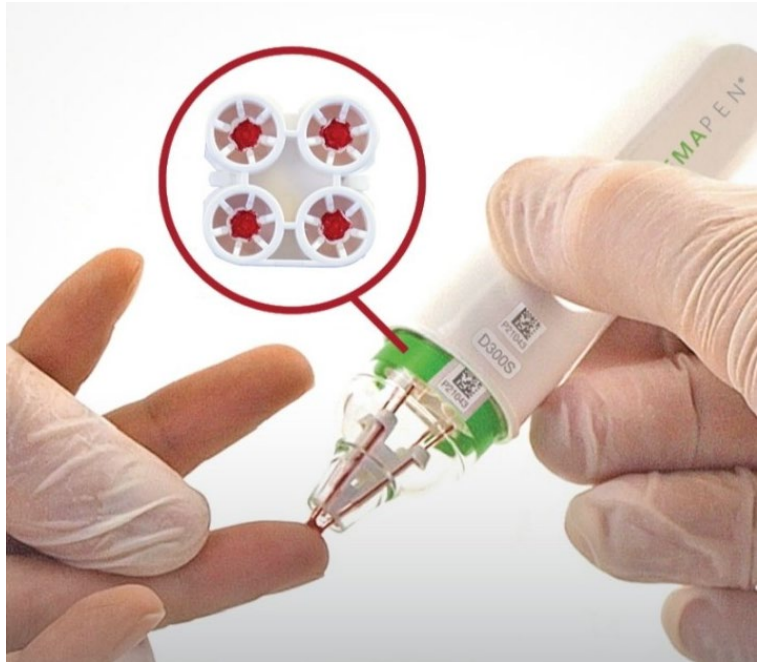
TRAJAN



	n	%CV	%RE
LLoQ	4	9.1	1.9
LQC	4	8.6	-4.7
MQC	4	5.5	-13.2
HQC	4	2.5	-15.8
ULoQ	2	4.7	-23.9

Good precision
Variable accuracy

Hemapen was not progressed because of the amount of time required to retrieve the card from the device

Focus on Capitainer and Mitra

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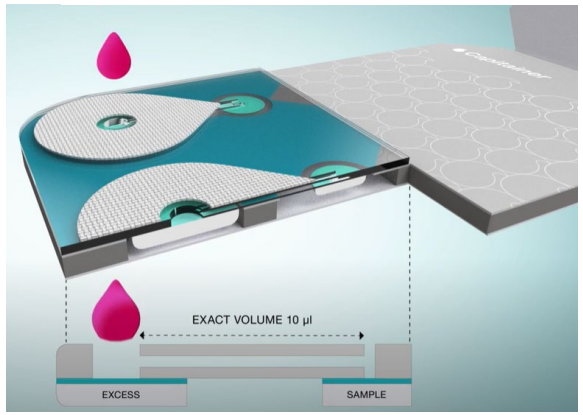
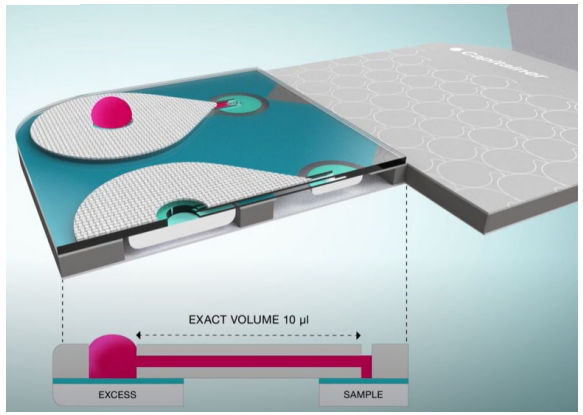
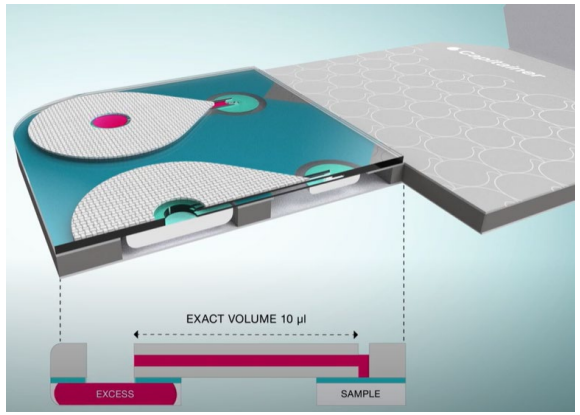
Capitainer



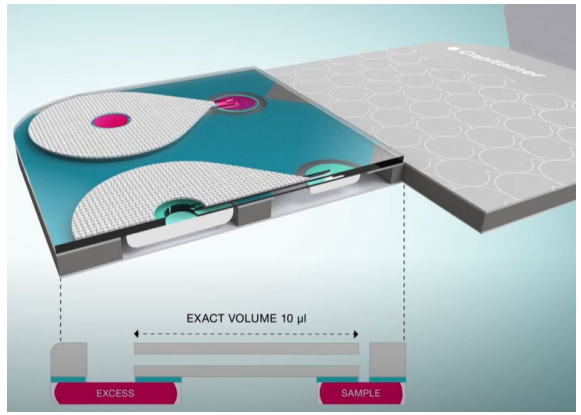
Apply droplet of blood



Blood fills microfluid metering port and excess reservoir chamber



A dissolvable membrane removes unmetered excess blood by wicking onto a waste paper disk



A second dissolvable membrane delivers the metered 10 µL of blood onto the collection filter paper disk

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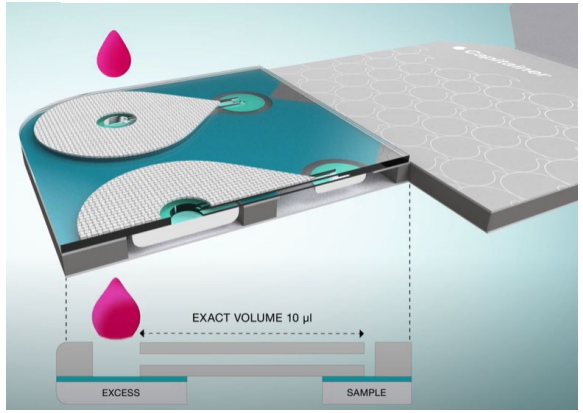
Capitainer



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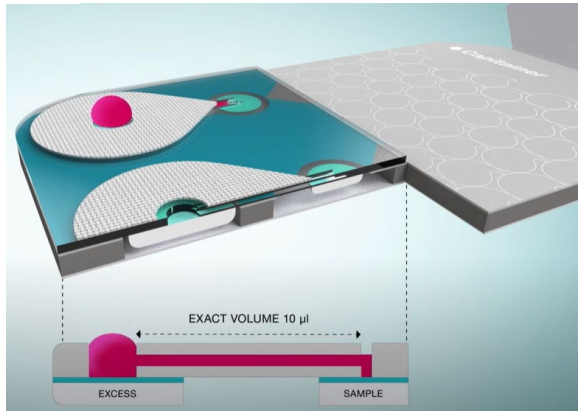

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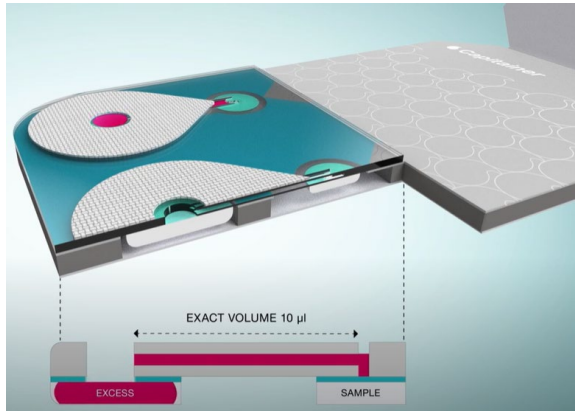

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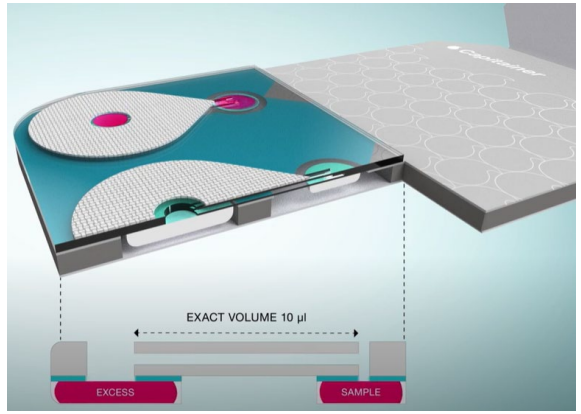

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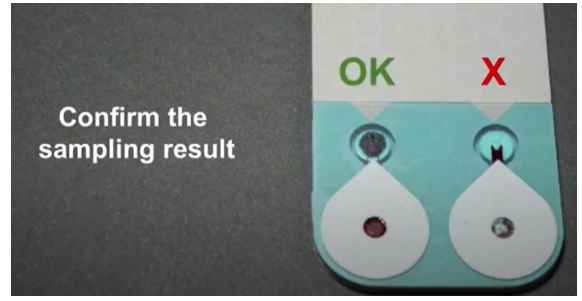

Focus on Capitainer and Mitra

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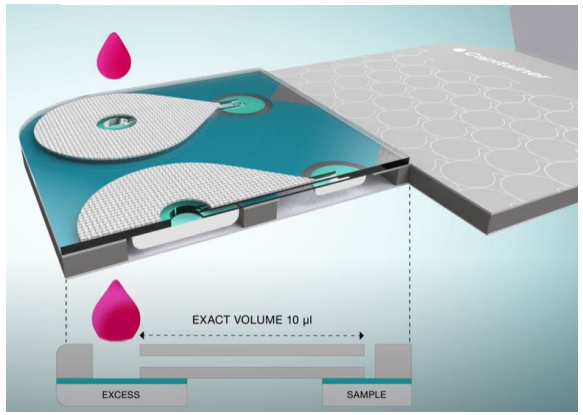
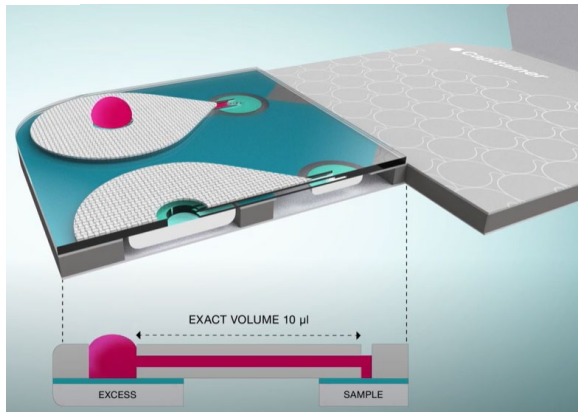
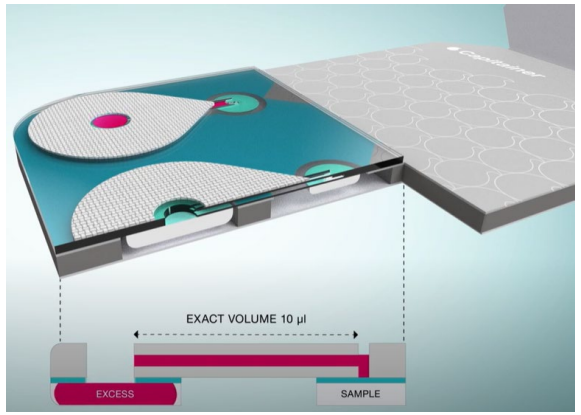
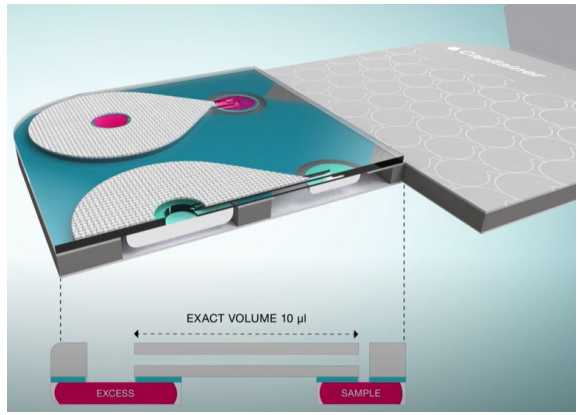
Capitainer









Manual Disk removal

Disk removal tool

Focus on Capitainer and Mitra

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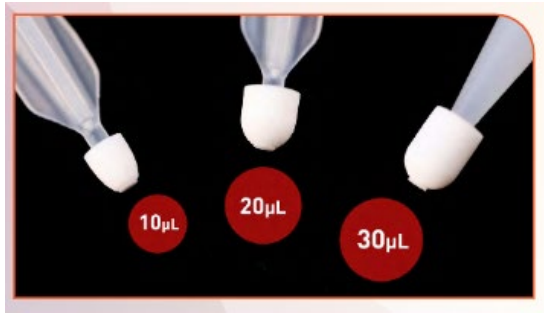
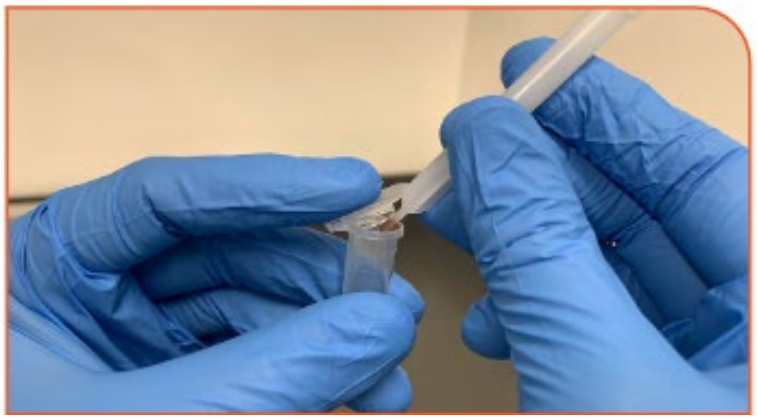
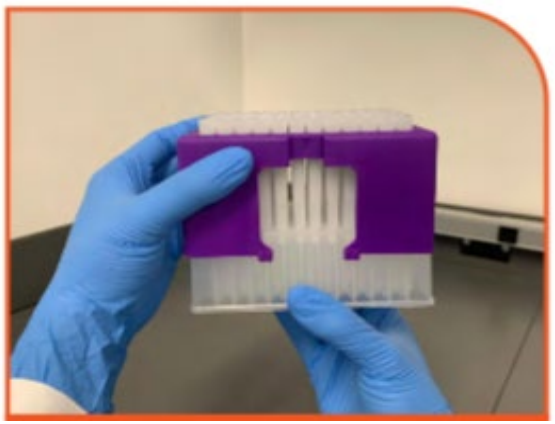
The promise of microsampling, delivered







Precision & Accuracy

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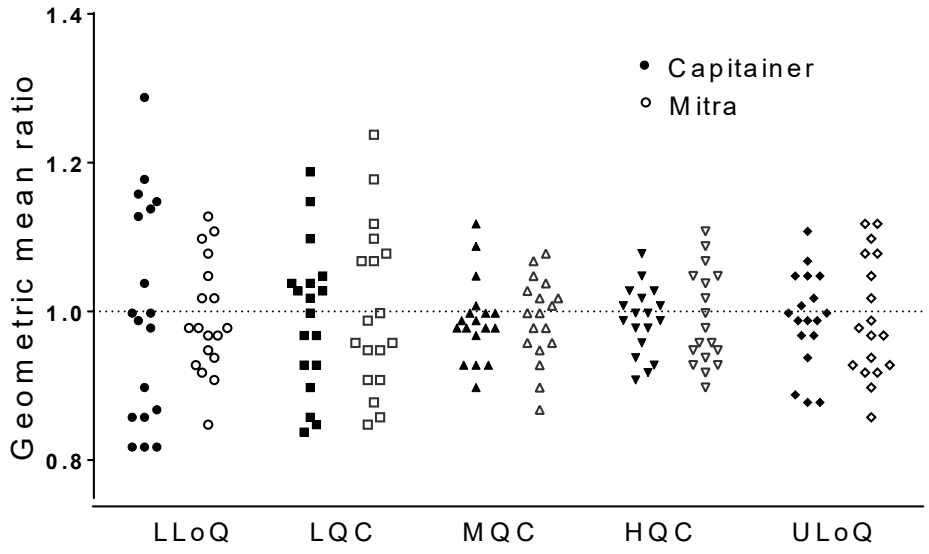
	Capitainer (CV%)		Mitra (CV%)	
	Intra	Inter	Intra	Inter
LLoQ	11.3	14.6	6.4	7.6
LOQ	9.2	9.8	8.1	11.0
MQC	5.3	5.4	5.8	5.6
HQC	3.5	4.6	3.0	6.5
ULoQ	4.2	6.5	3.6	8.2

	Capitainer (n=18)		Mitra (n=18)	
	Mean Conc. (ng/mL)	%RE	Mean Conc. (ng/mL)	%RE
LLoQ	197	-1.5	194	-3.2
LOQ	581	-3.1	587	-2.2
MQC	3530	0.9	3478	-0.6
HQC	21286	6.4	21804	9.0
ULoQ	23456	-6.2	23048	-7.8

6 reps over 3 independent runs (18 data points)
Each replicate was from an independent elution

Day 1:
Spike, collect, dry, store desiccated O/N

Day 2:
Elute, run assay



Recovery and HCT effect

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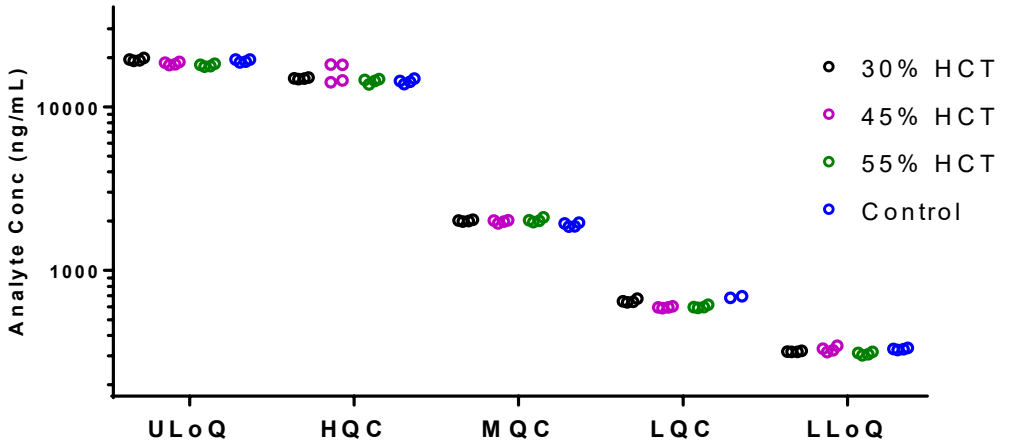
Capitainer



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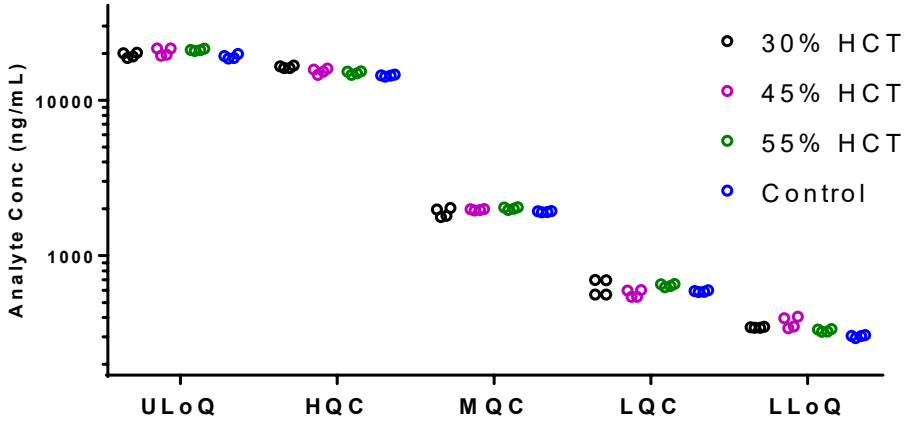
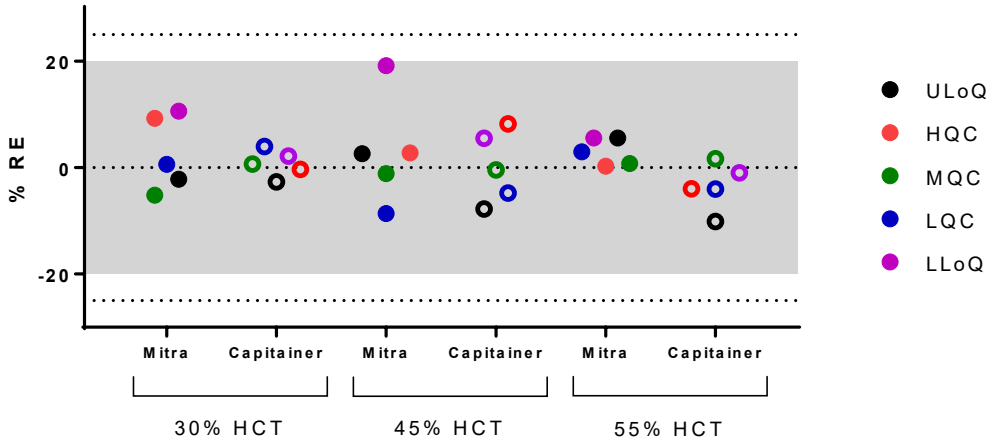
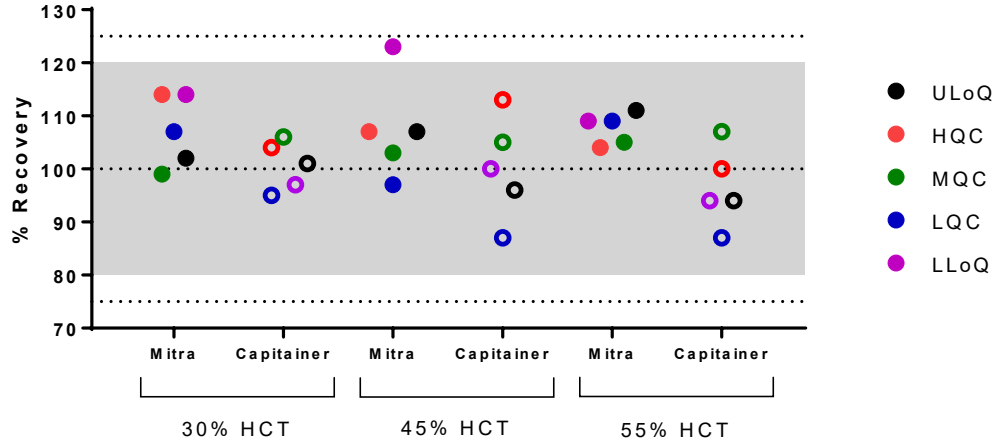


Relative Error



Extraction Recovery



Dilution linearity and selectivity

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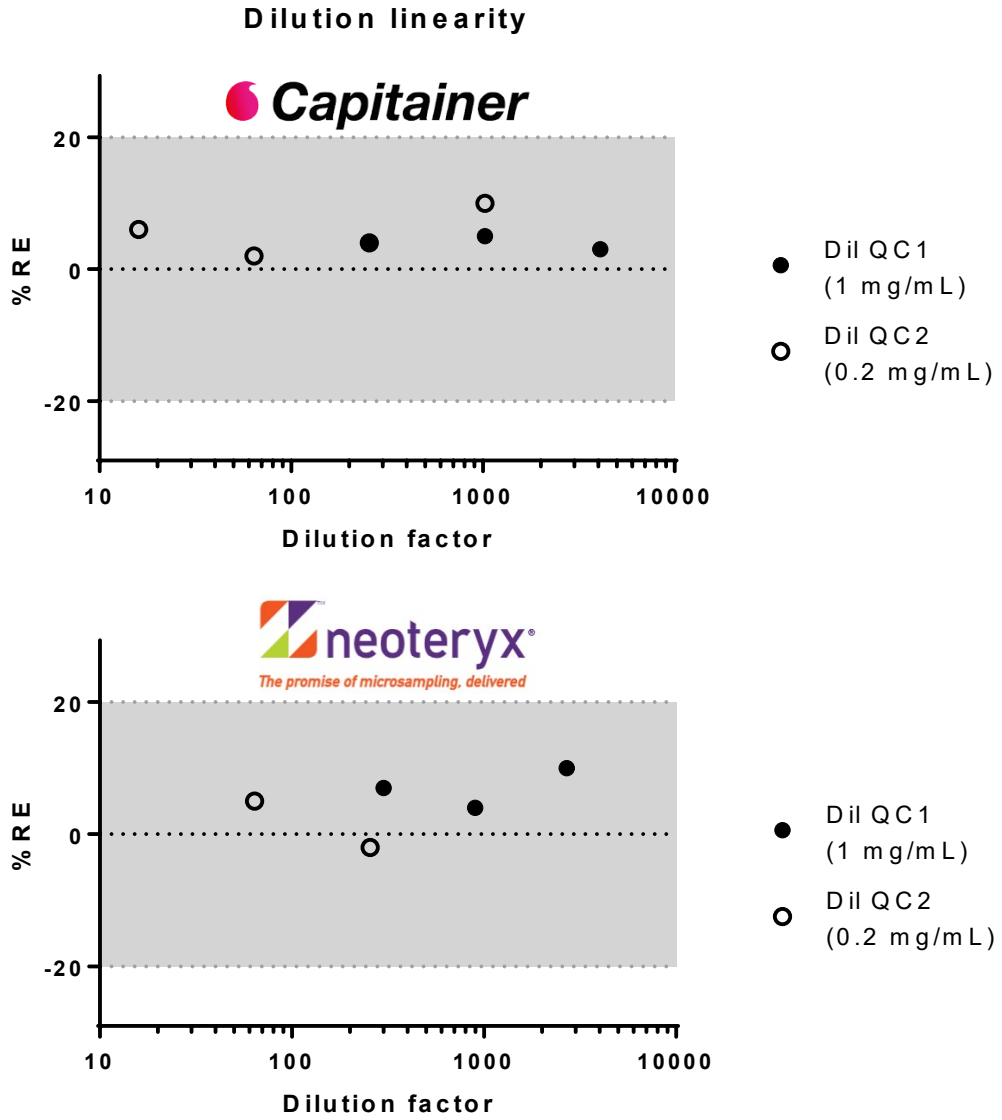

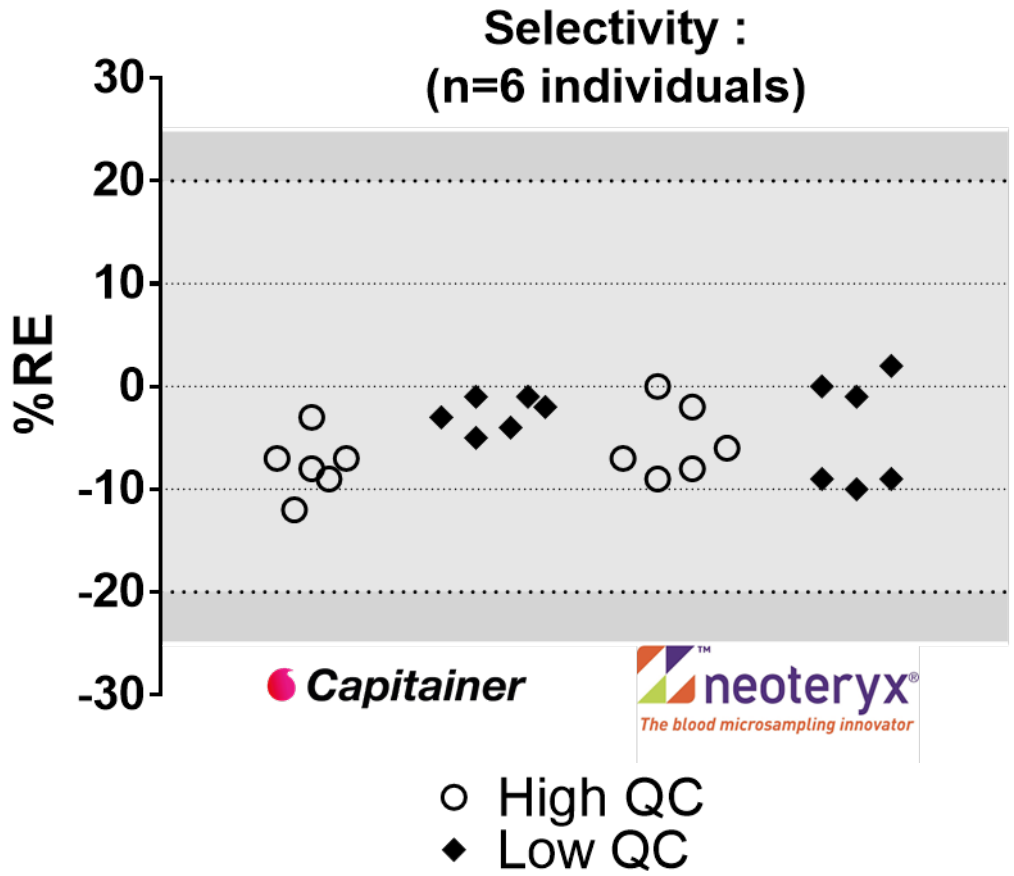

On-device Stability

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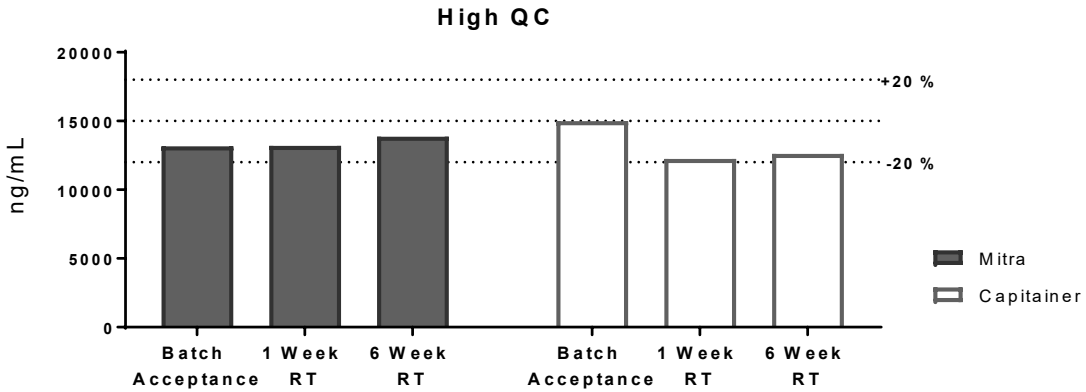

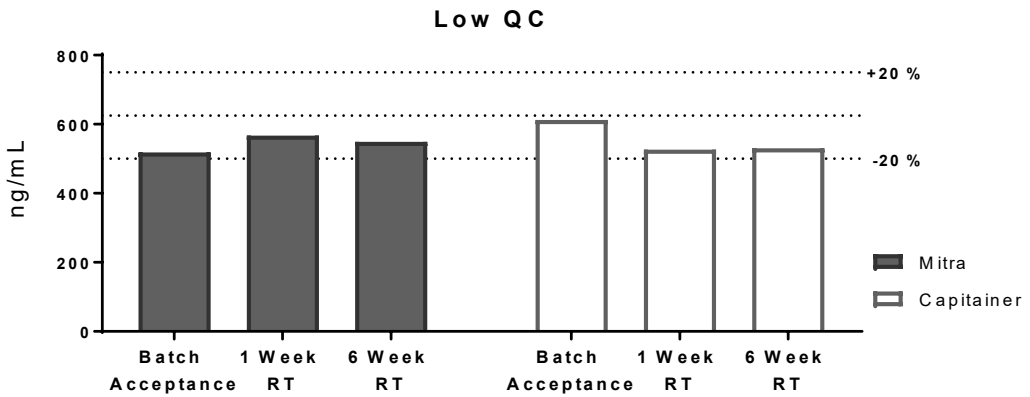

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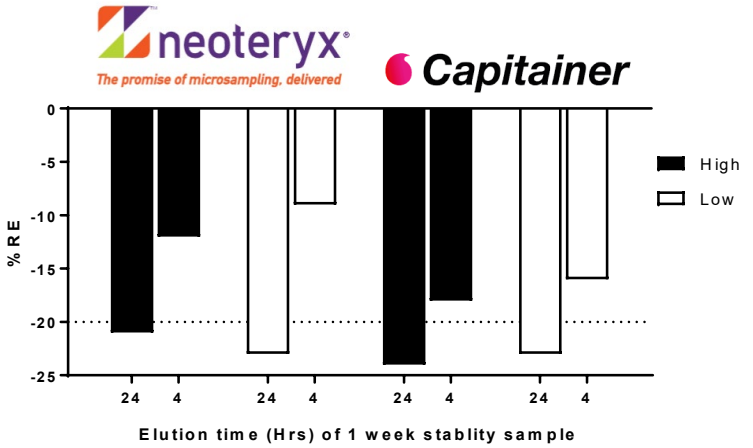
Capitainer



LBA method development perspectives

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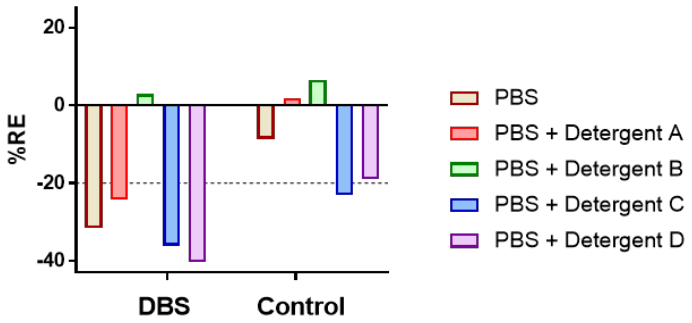
- Develop or re-purpose a method with a relatively high MRD (1 in 100 or greater)
- Check for compatibility with elution buffer, and also matrix effects in whole blood and blank eluate.
  - Ideally it should be possible to run the assay in both
- Assess different extraction techniques
  - Buffer (PBS, +/- detergent, +/- BSA)
  - Time, temperature, agitation, disruption, separation
- Assess matrix, process and extraction recoveries….across the whole range
  - Elution buffer
  - Blank WB, absorbed, eluted and then spiked
  - Spiked whole blood absorbed and eluted



mean concentration (n=4)

	C	A	B
ULoQ	18157	22806	20105
HQC	15725	20955	18310
MQC	2822	3254	3073
LQC	503	569	530
LLoQ	146	190	145

A = Spiked Elution buffer
B = Blank Eluate, spiked
C = Spiked WB Eluate

	B/A	C/B	C/A
ULoQ	0.88	0.90	0.80
HQC	0.87	0.86	0.75
MQC	0.94	0.92	0.87
LQC	0.93	0.95	0.88
LLoQ	0.76	1.00	0.77

B/A = Matrix effect
C/B = Extraction recovery
C/A = Process

LBA method development perspectives

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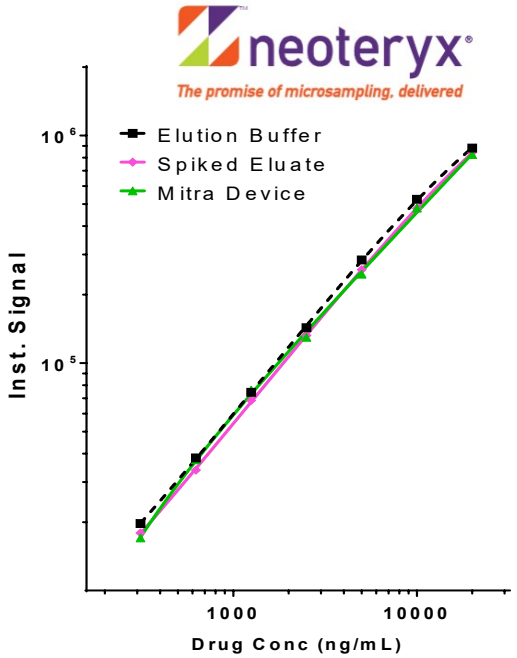
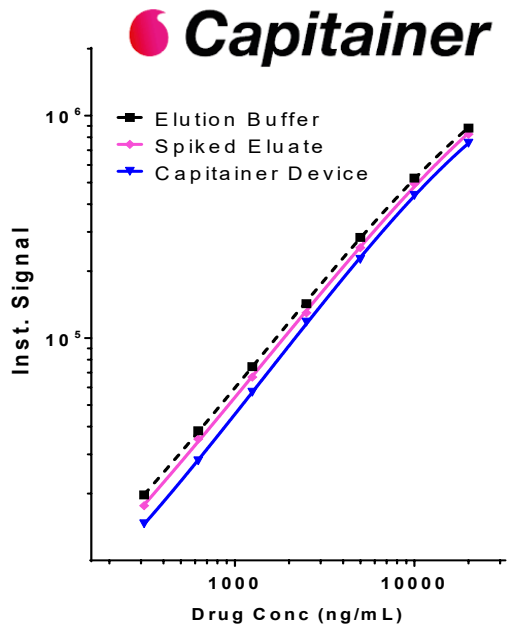
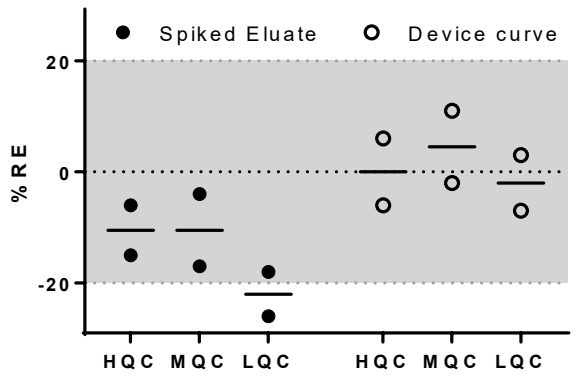
- Think about the best way to calibrate the assay
  - ➢ Spike calibrators into whole blood, collect onto device and elute
  - ➢ Absorb blank whole blood, elute, use as a blank matrix for curve preparation







Can we really use these? Which one is better?

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Yes

No one is better than the other *analytically*
It will depend on a number of variables

From a CRO perspective, the Capitainer and the Mitra tip provide the most straightforward solution for a relatively high-throughput answer

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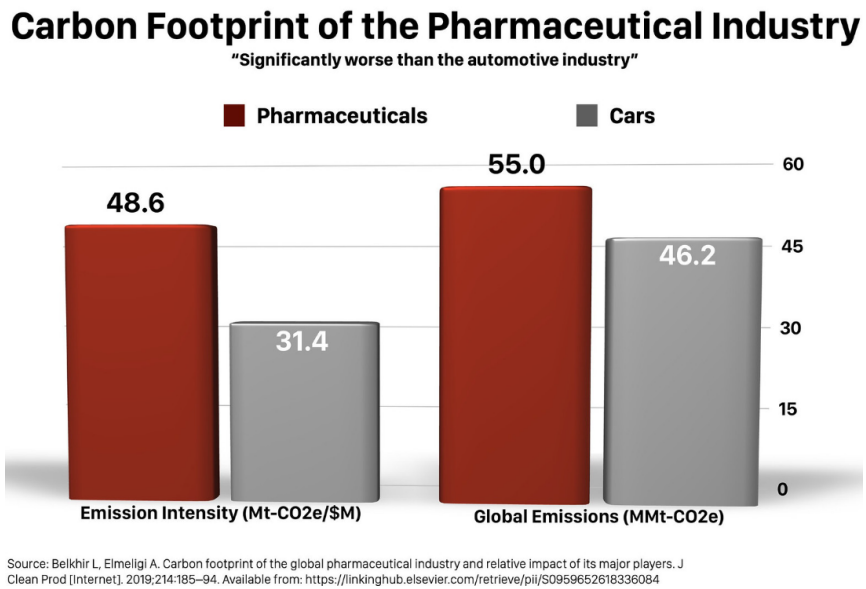
Should we be using this approach?

Absolutely

It means:

- Less dry ice usage
- Less Air Transportation
- Less -80°C storage
- Less single-use plastic



Source: Belkhir L, Elmeligi A. Carbon footprint of the global pharmaceutical industry and relative impact of its major players. J Clean Prod [Internet]. 2019;214:185–94. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0959652618336084

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Thank you for your attention

Any questions?

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