



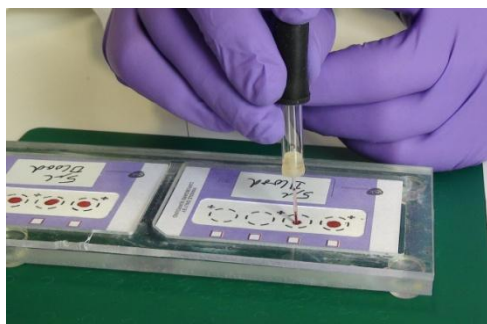
do more
feel better
live longer

Latest developments in microsampling for regulated quantitative bioanalysis

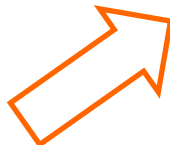
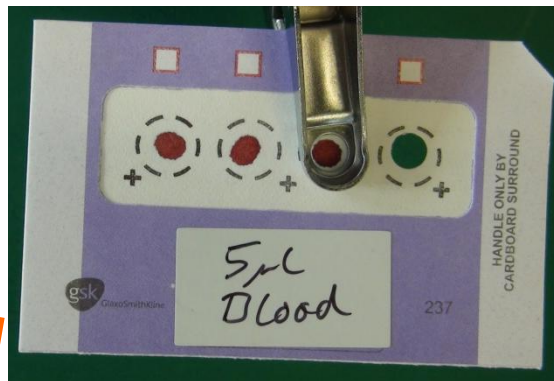
Neil Spooner
EBF 6th Open Meeting
November 2013

Dried Blood Spots

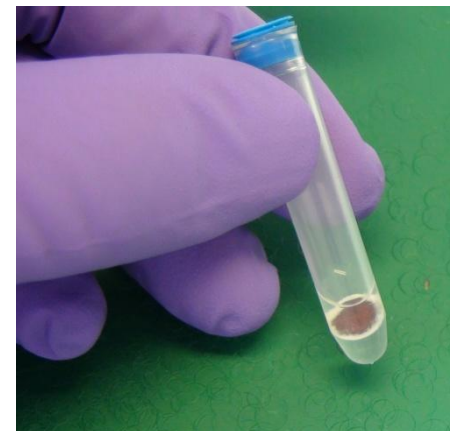
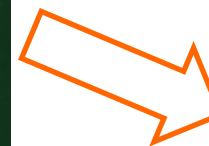
- Collect blood in small 'pot', or directly into capillary



- Spot 5 μL onto DBS card
- Dry for >2 hours
- Store at ambient



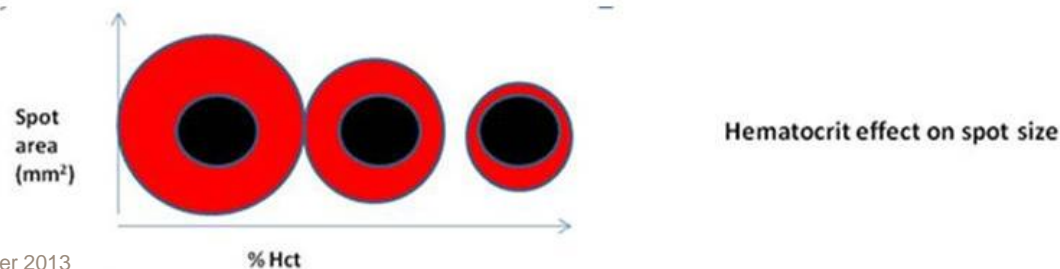
- Punch fixed diameter disc of entire spot
 - Sub-punch approach also possible



- Place disc in tube
- Extract with 200 μL solvent (60 min)

Can automate the analysis

- Understanding of the technique is continually evolving
 - Learning where the technique *is* and *is not* suitable, as well as potential hurdles
 - More difficult for BA group; advantages are to preclinical and clinical groups
 - ‘Validation’ is more extensive than for a plasma based assay
- Regulatory agencies are taking conservative approach to its use for clinical studies
 - For the time being, we are expected to employ sparse sampling approach of wet blood to compliment DBS samples and show continued concordance
- One of the biggest concerns with the technology is the hematocrit effect



Impact of Haematocrit Effect

Continuing along the Microsampling Road



Blood / Water - Capillary

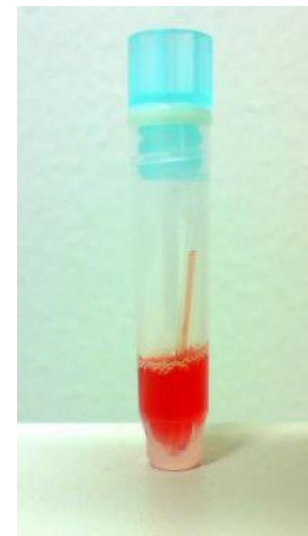
Courtesy Amanda Wilson, AZ



- Collect exact volume ($\geq 5 \mu\text{L}$) using EDTA capillary



- Place in tube

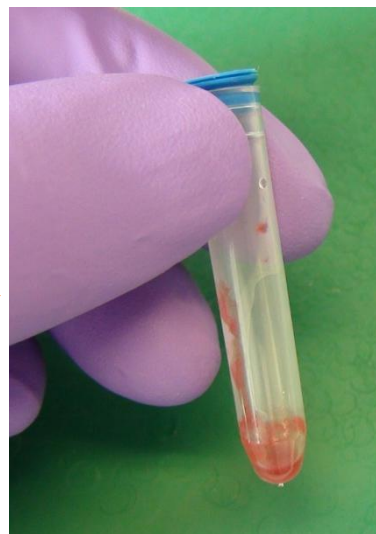
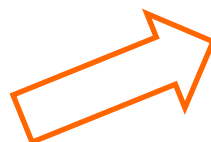


- Dilute with known volume of water
- Mix
- Freeze
- Analyse aliquot

Can automate the analysis

Jonsson *et al* (2012) *Bioanalysis* 4, 661-674

- Collect exact volume ($\geq 5 \mu\text{L}$) using pipette



- Dilute with known volume of water
- Mix
- Freeze



- Extract aliquot

Can automate the analysis

DSM – Experimental DBS Substrate

Courtesy Ynze Mengerink, DSM & Philip Denniff

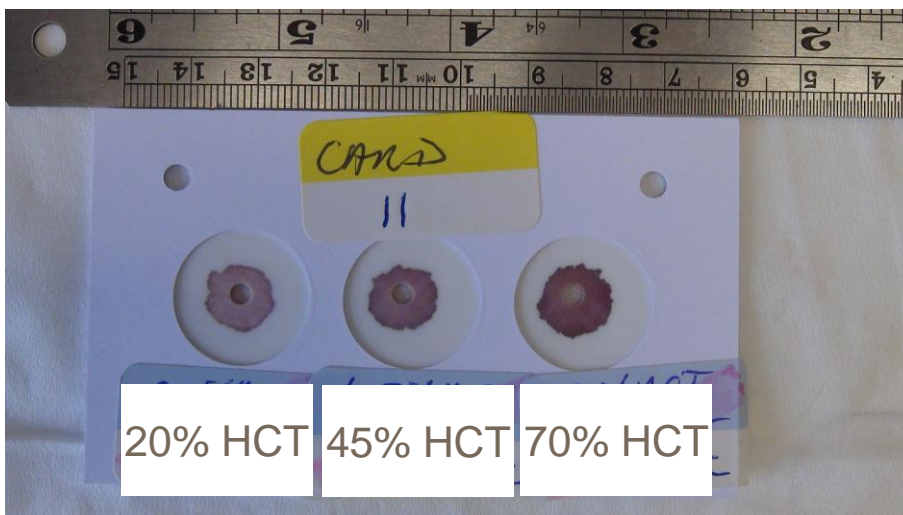


Novel substrate material

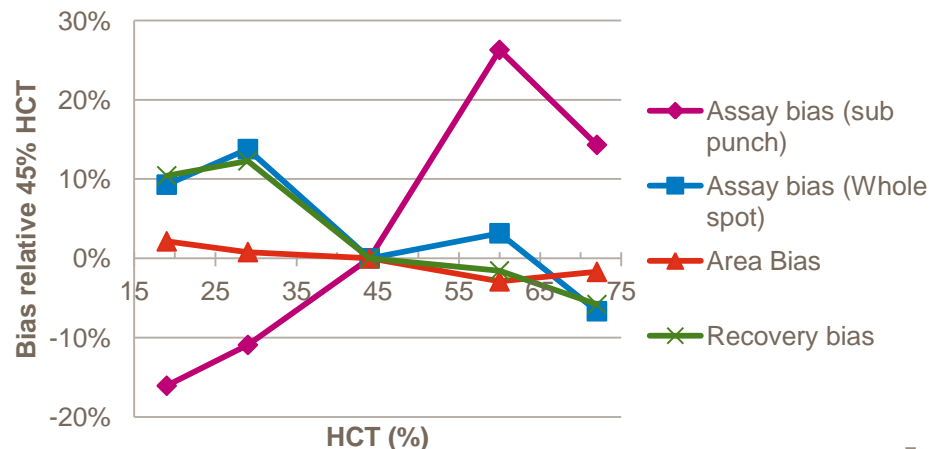
- Preliminary data for material which is still under investigation
- Minimises effect of hematocrit on spot size
- Minimises effect of hematocrit on recovery

However;

- For paracetamol a bias in the data was observed with HCT when the sub-punch approach was used
- This was not observed for codeine

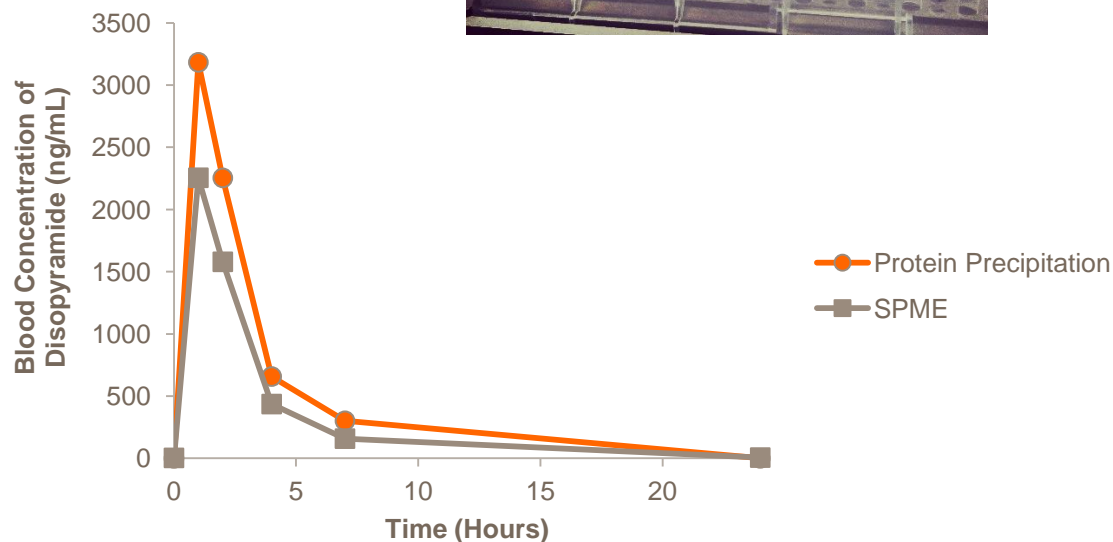
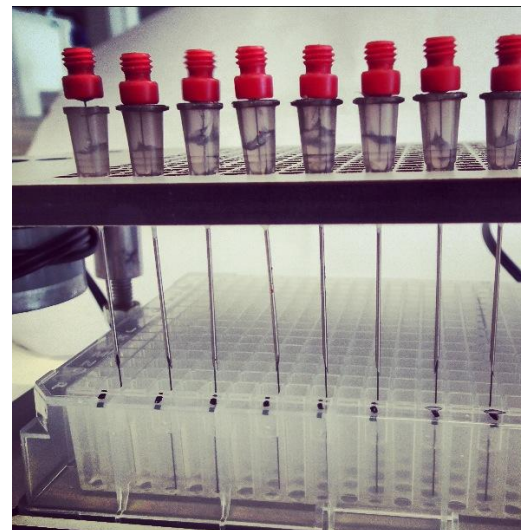
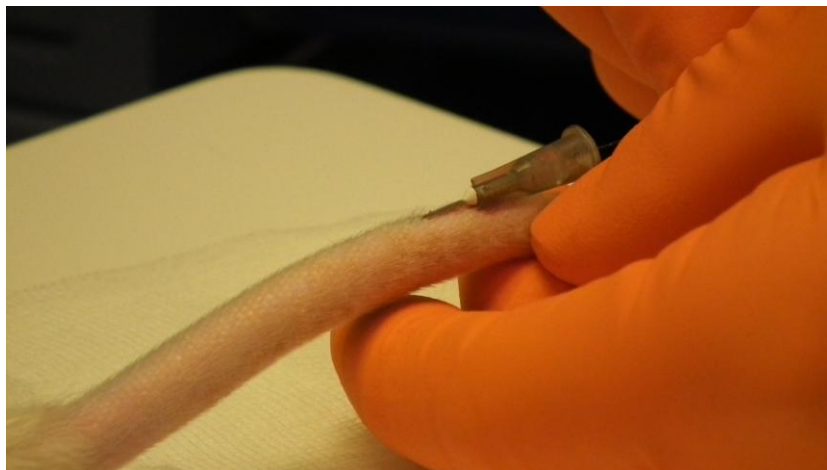


15 μ L blood containing paracetamol spotted onto DSM card



Solid Phase Microextraction

Courtesy Supelco SPME Team & Sheelan Ahmad



Disopyramide concentration in *ex vivo* samples from male beagle dog after 20 mg/kg oral dose

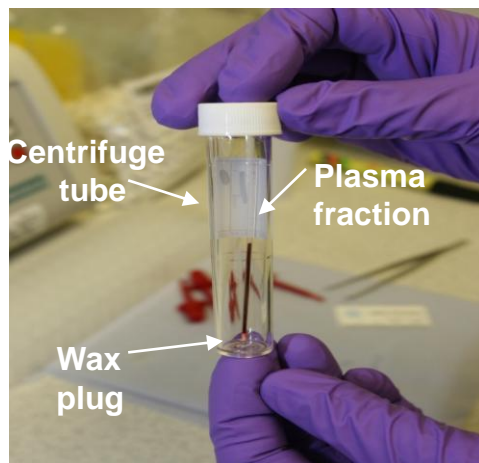
What happens if you want plasma concentrations?



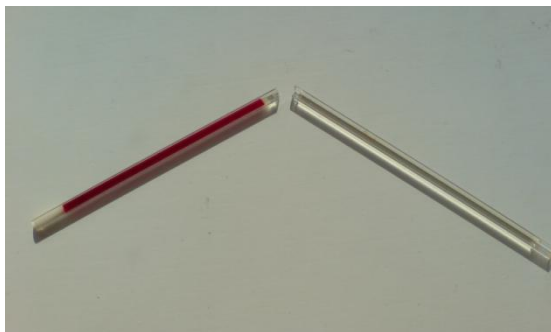
Plasma – Hematocrit Tube Approach

Courtesy Amanda Wilson, AZ

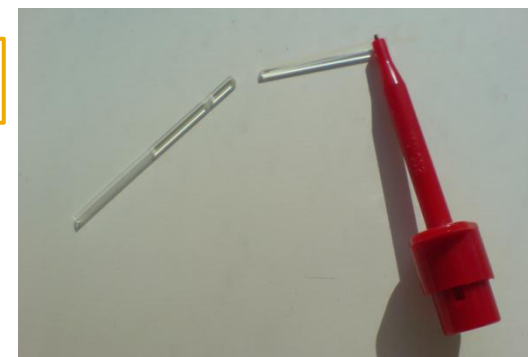
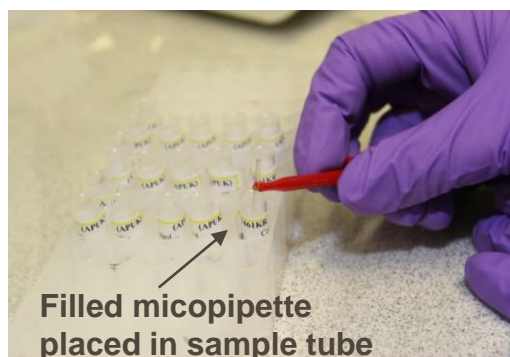
- Collect 32 μL blood in EDTA HCT tube
- Plug with wax
- Place in labelled tube
- Centrifuge 1500 $\times g$ for 10 min



- Cut using capillary cutter



Can automate the analysis



- Collect exact volume (8 μL) plasma with capillary

- Freeze
- Analyse whole aliquot

Jonsson *et al* (2012) *Bioanalysis* 4, 1989-1998

Capillary

- Glass capillary
- Mylar film coat (increased strength and safety)
- Internal EDTA coating
- Porous plug
- Thixotropic gel

Operation Overview

- Fill capillary with blood (~70 μ L) by capillary action
- Porous plug swells & seals capillary on contact with blood
- Centrifuge capillary
- Gel migrates to blood plasma interface
- Push porous plug with Wiretrol to expel plasma
- Collect plasma (20-30 μ L) & freeze
- Analyse 7 μ L aliquot



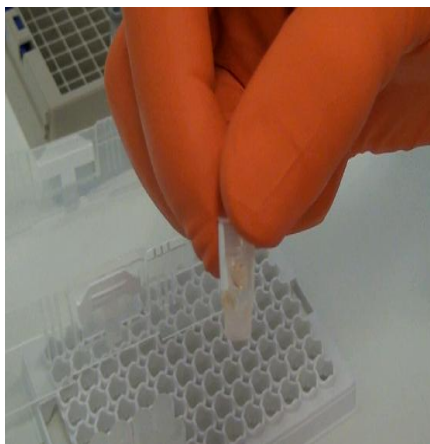
Figure 1. Novel capillary tube in various process steps. (A) Empty capillary tubes; (B) blood-filled capillary tubes; (C) centrifuged capillary tubes showing plasma separation; and (D) capillary tube fitted into a labeled Micronic tube for centrifugation.

Sample Collection & Processing

Collect blood sample into a capillary using a labelled 1.1ml tube as a holder



Centrifuge samples at 3000–4000 xg for 10 mins at ambient temp



Defrost and analyse 7 μ L aliquot



Use Wiretrol to expel the plasma from the capillary into a labelled tube and freeze

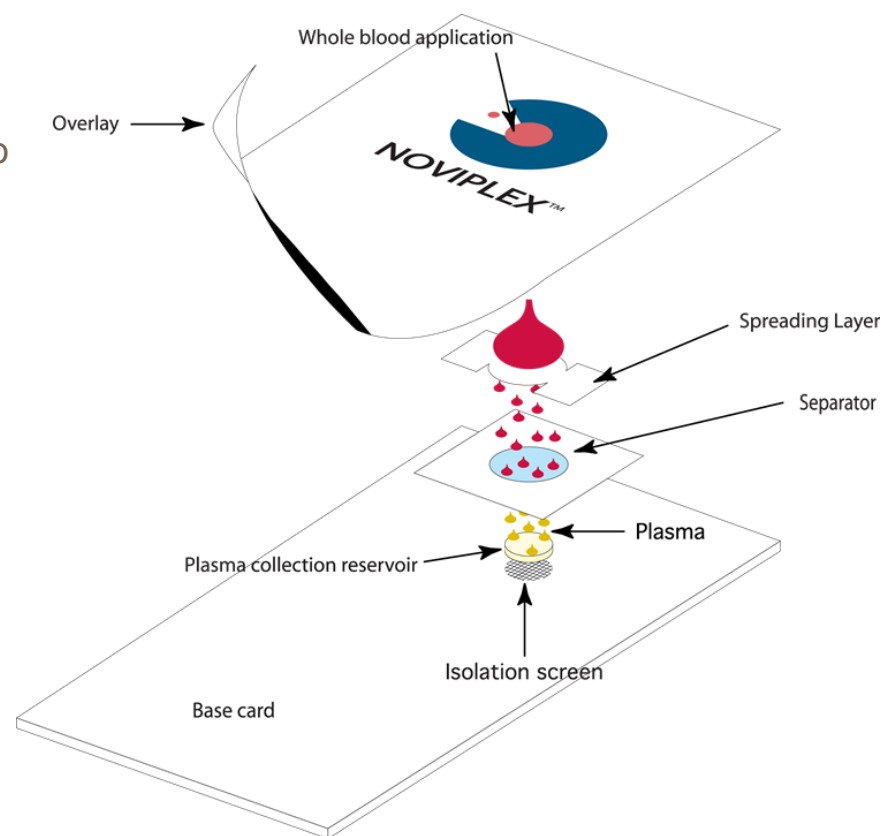


Bowen *et al* (2013) *Bioanalysis* 5(9), 1131-1135

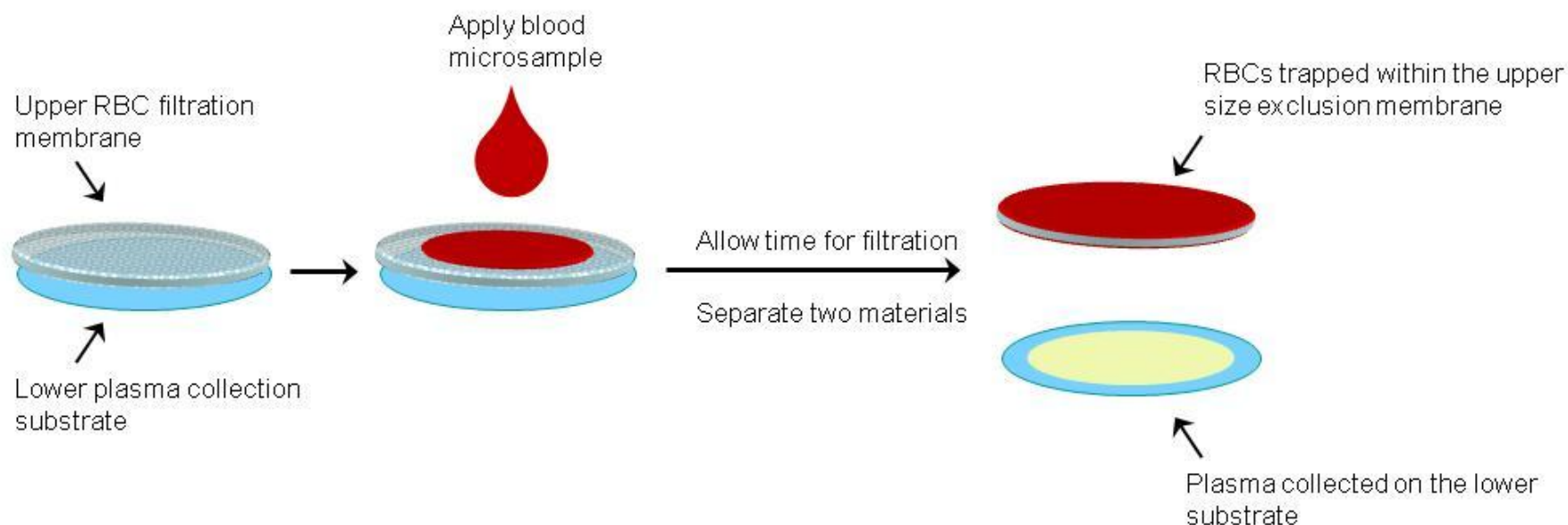
Noviplex Plasma Separator Card

Courtesy Neil Loftus, Shimadzu

- Add 20 - 75 μL whole blood to test area
 - Control spot indicates whether enough blood has been added
 - Lateral spreading layer rapidly spreads blood so it enters the filtration layer as a front
 - Filtration layer captures blood cells by a combination of filtration and adsorption
 - Collection layer loads with a specific aliquot of plasma
 - The isolation screen precludes lateral wicking along the card surface
- After 3 mins, peel back the top layer
- The collection disc contains 2.5 μL of plasma
- Air dry for 15 minutes and store/ship in original packaging with desiccant
- For analysis, remove the collection disc from the card prior to extraction



- 25 μ L blood containing SIL-IS applied to upper membrane
- Control spot indicates when upper layer can be removed (5 min after spotting)
- Lower membrane air dried for ≥ 1 hr



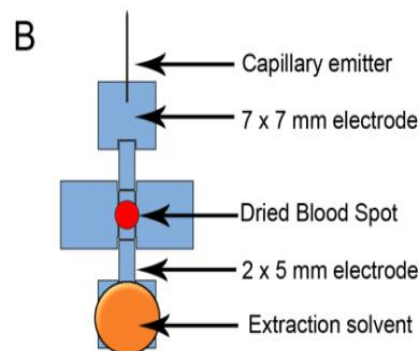
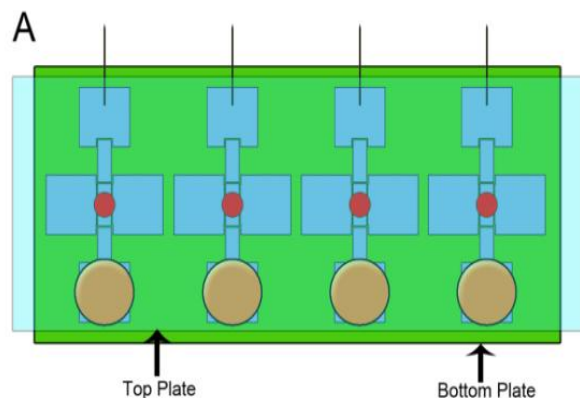
Courtesy Robert Sturm & Jack Henion, Quintiles Bioanalytical Labs and Richard Abbott & Phil Wang, Shire Pharmaceuticals

Digital Microfluidics

Courtesy Aaron Wheelers Group at University of Toronto



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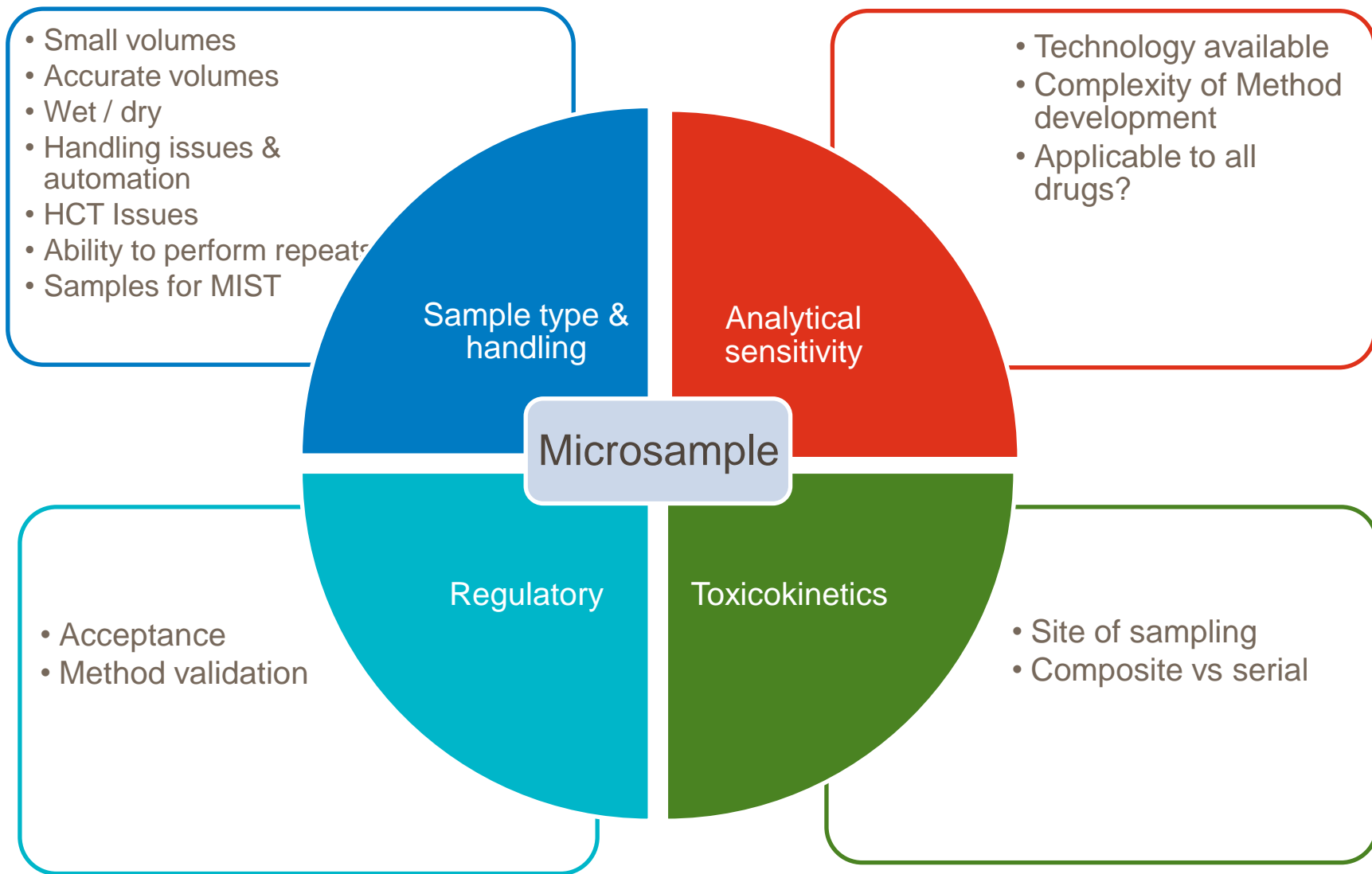


Method	Parameter	Sitamaquine	Proguanil	Benzethonium Chloride	Ibuprofen
-	[Actual Drug] ng/mL	900	450	1000	200,000
conventional	[Drug] ng/mL	978	468	1120	197,000
conventional	% Accuracy	109	104	112	98.8
conventional	%CV of Blind	3.2	2.4	3.1	5.7
conventional	No. replicates	6	6	6	6
DMF	[Drug] ng/mL	814	457	669	240000
DMF	% Accuracy	90	102	67	120
DMF	%CV of Blind	2.4	16	16	29
DMF	No. replicates	3	4	8	8

Microsampling – Issues to Consider



Courtesy Amanda Wilson, AZ



Will any of these approaches be acceptable to regulators???

Will any of these approaches be acceptable to the practitioners???

- Conservative nature of the industry
 - Are **WE** our own worst enemies for the adoption of innovative approaches???



- **Cross company high quality science as the best way forwards**

- NC3Rs
- EBF Topic Teams
- IQ Consortium

- Scientific publications
- White papers
- etc.....



The Future??!

The mould can be broken

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OUR SOLUTION / OUR TECHNOLOGY

the lab test, reinvented.

MICRO-SAMPLE
No more huge vials to fill. At Theranos, we only need a few drops.

NANOTAINDER™
1.29cm
Our nanotainer™ tube is smaller than a dime.

At Theranos, we can perform our lab tests on samples as small as 1/1,000 the size of a typical blood draw.

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OUR SOLUTION / IN WALGREENS

theranos is now in Walgreens

Theranos™ Wellness Centers will soon be located within Walgreens stores nationwide.

The Theranos™ Wellness Center, coming right to your neighborhood.

Our Lab | Theranos - Windows Internet Explorer

theranos

OUR SOLUTION / OUR LAB

One tiny drop. Thousands of answers.

Theranos can perform a full range of tests on samples as small as a few tiny drops. That's 1/1,000 the size of a typical blood draw. Our assays are validated under FDA, ICH, and WHO guidelines. And we provide the highest level of oversight, automation, and standardization in both our pre- and post-analytic processes, to realize the highest level of accuracy and precision.

Illustrative Accuracy

Three scatter plots comparing Theranos results to Reference Method results for Lymphocytes, Neutrophils, and Eosinophils. Each plot shows a strong positive correlation with a regression line. The regression equations and R-squared values are:

- Lymphocytes: $y = 0.01x + 0.11$, $R^2 = 0.98$
- Neutrophils: $y = 0.20x + 1.11$, $R^2 = 0.98$
- Eosinophils: $y = 0.10x + 0.12$, $R^2 = 0.98$

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OUR SOLUTION / DIGITAL TOOLS

the lab test goes digital.

We've been working to create a system designed to help you and your doctor know more. Because we believe the more you know about what's happening in your body, the more you can do to live the life you want to live.

We believe you have the right to your own health information.



- Sheelan Ahmad
- Paul Abu-Rabie
- Chet Bowen
- Philip Denniff
- Wesley Dopson
- Nella Rebisz
- Susan Sparrow



- Amanda Wilson



- Joe Siple



- Ynze Mengerink



- Jack Henion

- Robert Sturm



- Neil Loftus



- Richard Abbott

- Phil Wang



- SPME Team



- Aaron Wheeler

- Nelson Lafreniere



do more
feel better
live longer

Thank you

All animal studies were ethically reviewed and carried out in accordance with European Directive 2010/63/EU