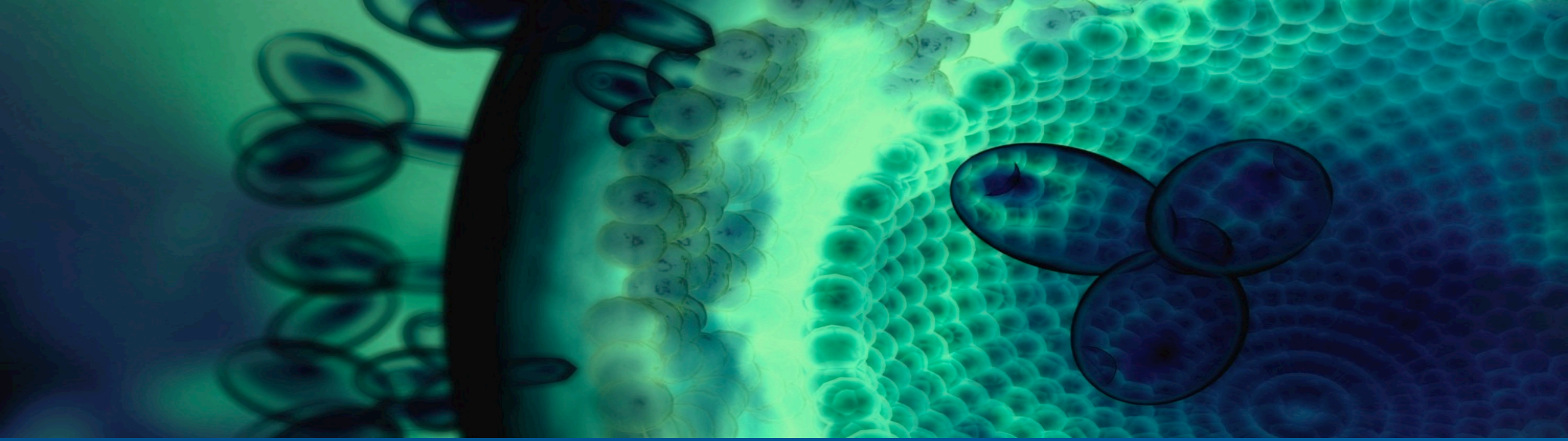


The journey of microsampling in preclinical and clinical development

Pictured above: The structure of HIV.

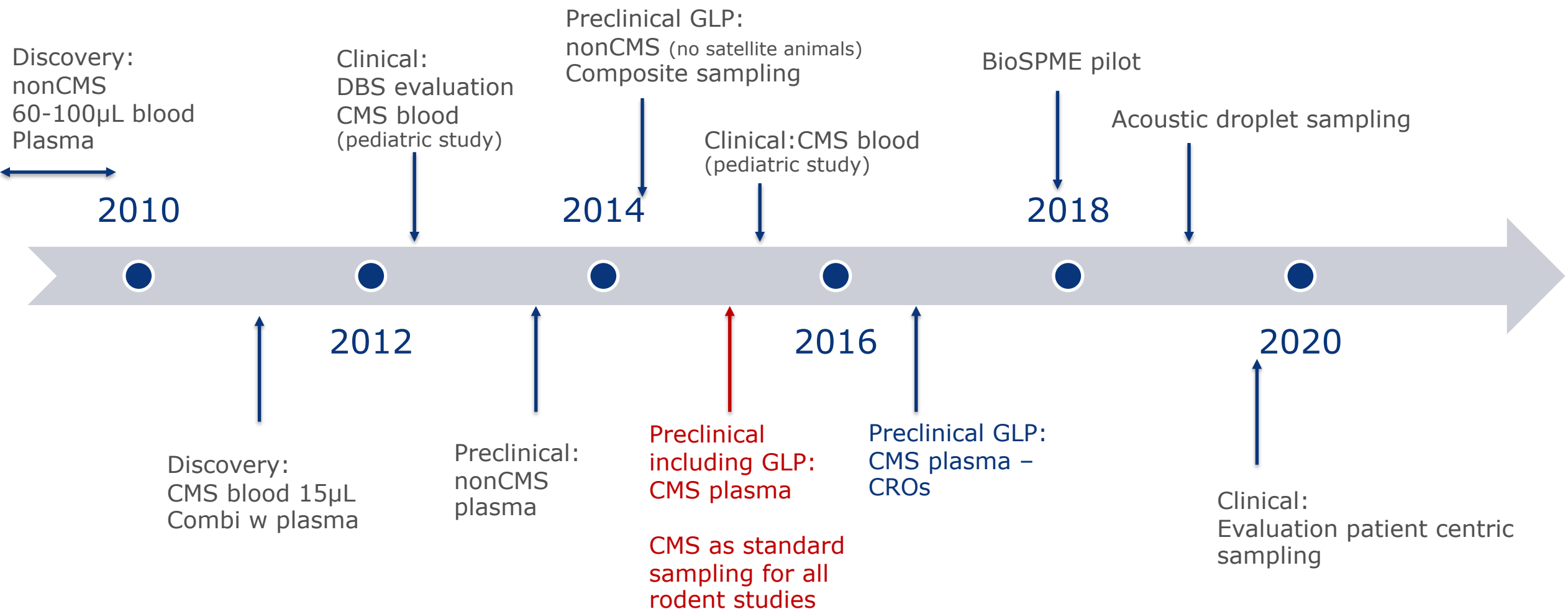
Lieve Dillen
Development Bioanalysis

Janssen | PHARMACEUTICAL COMPANIES OF Johnson&Johnson

Outline

- History of microsampling @ Janssen EU
- Different microsampling strategies
  - Non capillary microsampling (nonCMS)
    - Impact of collection device
  - DBS/VAMS
    - Considerations from a preclinical perspective
    - Experiences in clinical studies
  - Capillary microsampling (CMS)
    - Plasma CMS – application in GLP studies
    - Blood CMS in preclinical and clinical applications
    - Current preferred microsampling technique
  - BioSPME
  - Acoustic Droplet Ejection (ADE) sampling
  - Patient Centric Sampling (PCS)

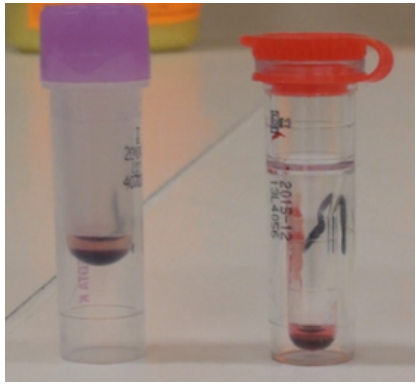
Journey of microsampling @Janssen



Non capillary microsampling

- Adapted collection devices for reduced blood collection microvette (recovery issues experienced), Kabe, Sarstedt*
- Concerns around handling of low volumes – accuracy and precision challenged (cfr. EBF LMS consortium evaluation**)
- Subsampling from low volumes of rodent plasma at time of collection in the animal facility. Accountability of accurate volume!
- Dilution of received volume for further processing (re-analysis, ISR, metabolite analysis, biomarker…)
- Applied in a few GLP studies (extra stability validation in diluted plasma)
- Automation -> Acoustic droplet sampling

microvette

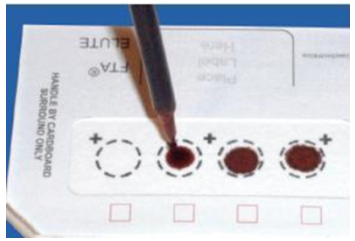


Sarstedt Kabe

* https://www.bioanalysis-zone.com/2016/09/23/microsampling-no-thing-best-technique_mcsguide/
** *Bioanalysis* 11(6), 533 (2019)

Dried blood spot (DBS) and volumetric absorptive microsampling (VAMS)

- Only blood as matrix



- DBS: volume dependent on the spot size (and on hematocrit)
  VAMS: fixed volume – not pursued in clinical space (at that time)
  samples can be shipped @ RT

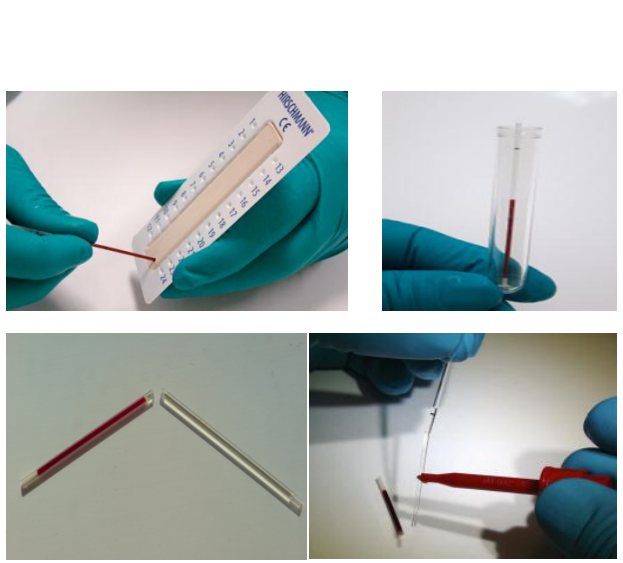
- Preclinical space: no added value identified for use of DBS or VAMS

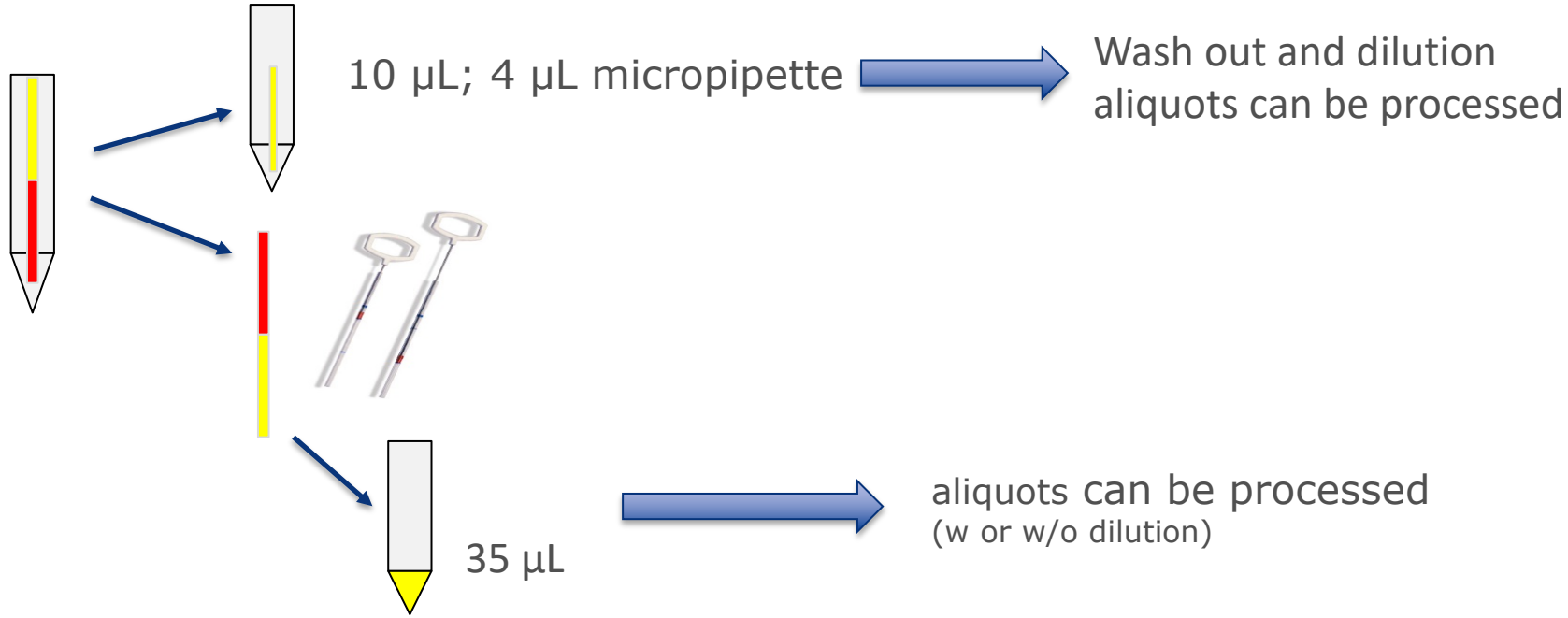
- Clinical space:
  - Study #1: FIH; full PK profiles in DBS and plasma from venous sampling
  - Study #2: relative BA; compare finger stick PK in DBS & plasma to venous PK in plasma; limited DBS sampling
  - Study #3: dose proportionality; compare venous PK in DBS and plasma; full PK profiles
  - DBS and plasma profiles correlated well after correction for blood/plasma ratio
  - Validation failures
    - difficult to prove LTS (effect of age on extraction recovery)
    - Effect of hematocrit on accuracy
    - Processing DBS in the lab is resource intensive, even with a semi-automatic puncher
    - Addition of the IS in the extraction solvent not ideal
    - Cross contamination from the puncher has been observed for some analytes

Capillary microsampling of blood with a final (capillary) plasma sample

- Anti-coagulant coated hematocrit tubes for collection of blood (15 µL for blood analysis; 32/64 µL (Vitrex) or 70 µL (Drummond) for plasma analysis)

- 2 approaches for plasma: transfer plasma into micropipette (10 µL), or collect all plasma in tube (exact volume pipetted at bioanalytical facility)



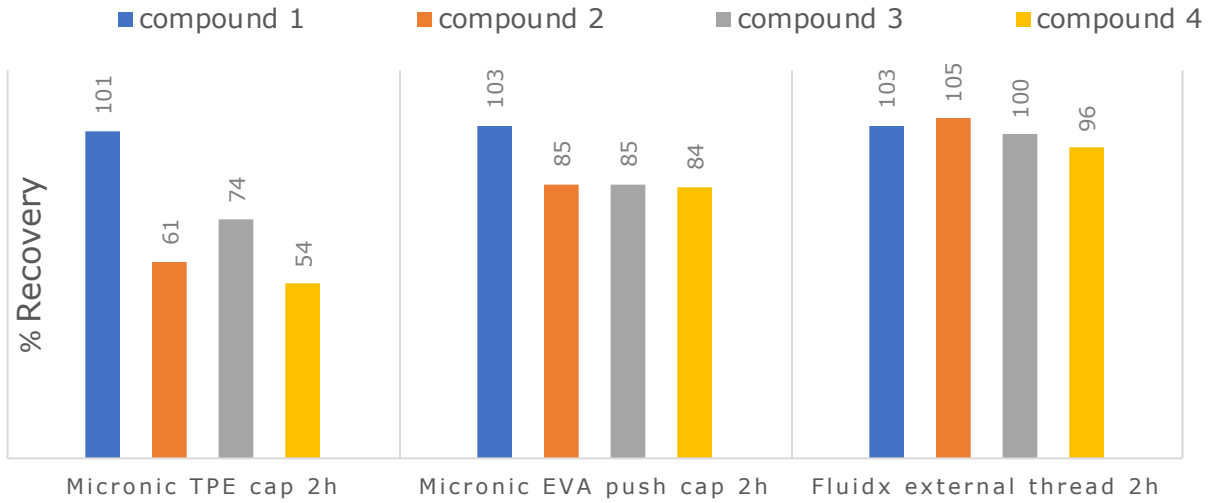


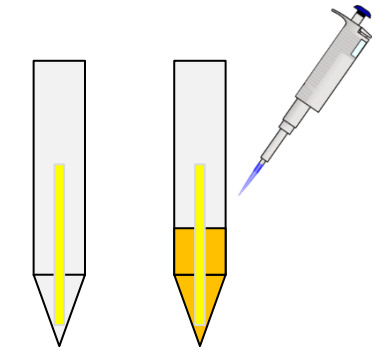
Recovery from CMS plasma following wash-out

Micronic TPE pushcaps

Micronic eva pushcaps

FluidX external thread

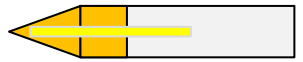




10 µL plasma

+ 100 µL Buffer (+2% BSA)

Horizontal capillary wash-out



= 110 µL of diluted plasma

Subsample analysed

Experiences with (outsourcing) preclinical studies CMS

- Impact on toxicological endpoints (CNS observations, hematopoietic system as target)
    - No effect on clinical pharmacology parameters expected*.
    - Some teams still include satellite groups in TOX studies for TK sampling

- Complex designs/multiple analytes (including metabolites & biomarkers)
    - Re-analysis, ISR, # timepoints**, composite sampling designs, stability, sensitivity

- CROs not always familiar with the sample collection technique; the assay may be validated for CMS with a traditional plasma sample delivered

- Availability of the materials (capillaries, storage tubes) (especially in USA) -> labs use other supplies (requiring additional validation efforts for BA) or sponsor needs to supply the materials.

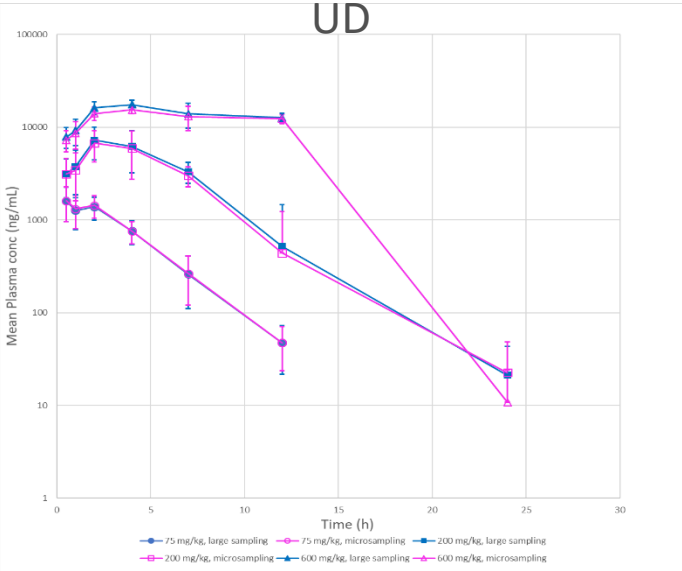
- In some studies: many samples with deviating volume; documentation and communication often not detailed enough leading to mistakes

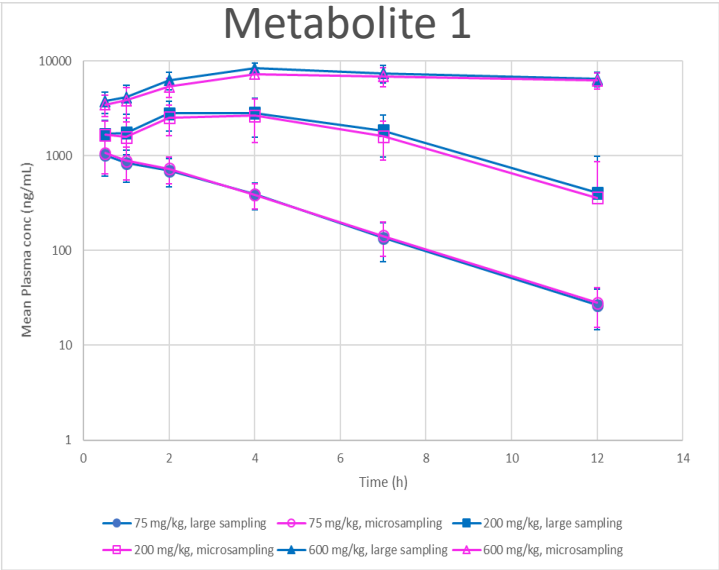
- FDA requesting bridging study when switching from traditional plasma to CMS plasma sampling within a program

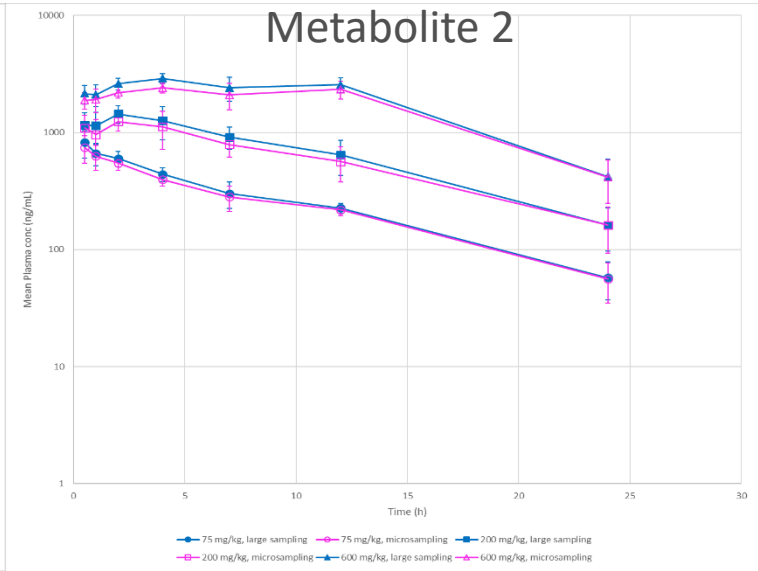
* *Bioanalysis* 4(16), 1989 (2012)
** https://www.nc3rs.org.uk/blood-sampling-general-principles

CMS: preclinical GLP – bridging study

- Development program:
  - 1 and 3 month GLP in rat with traditional sampling (300 µL blood, +/- 150 µL plasma)
  - 6 month GLP in rat with CMS (32 µL blood, 10 µL plasma)
  - 2 validated assays (3 in 1 assay; UD and 2 metabolites)
  - **Regulatory request**: prove TK parameters are comparable including both sampling approaches in 1 study
  - Sequential sampling from tail vein: first CMS followed by traditional sampling (as per request from regulators)

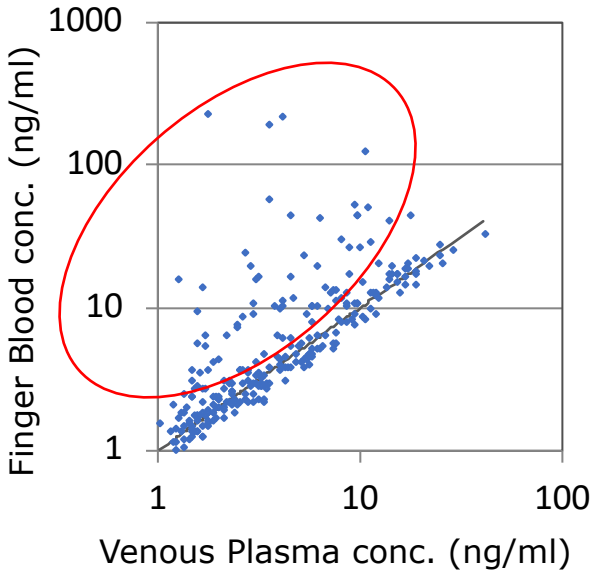


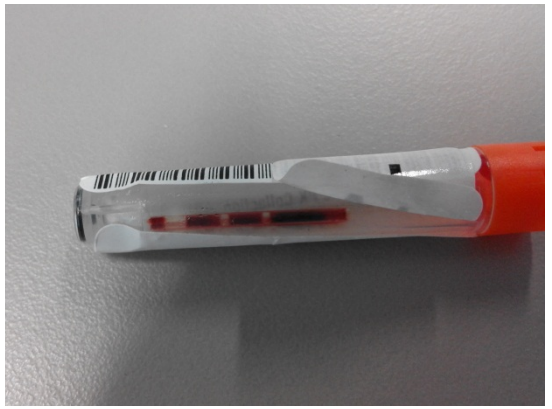




Clinical applications of CMS

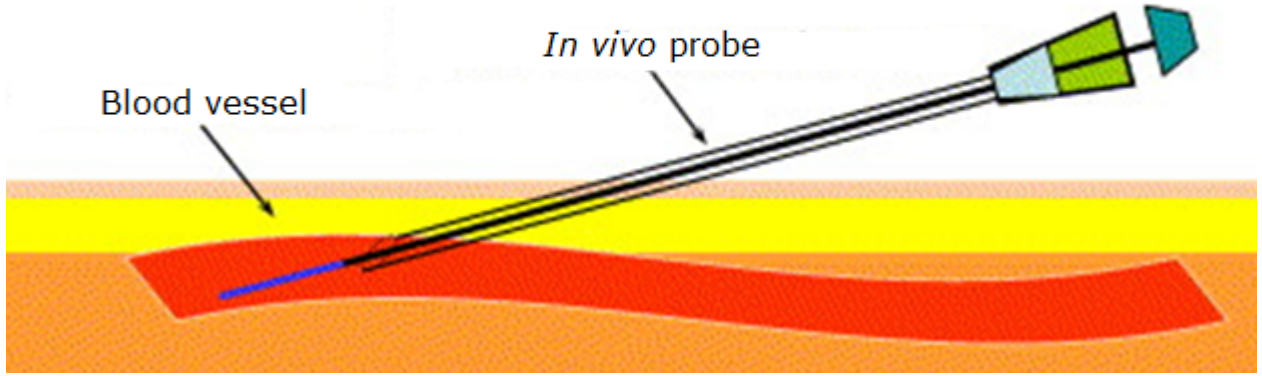
- 2 examples of **blood CMS**:
  - Phase III multisite pediatric study with fast disintegrating chewable tablet in Africa
    - **Finger prick blood microsampling** facilitates PK analysis in this study (no infrastructure to generate plasma, venous blood sampling challenging)
    - Validated LC-MS/MS method: 15 µL Vitrex capillary, wash out with 500 µL BSA/PBS, SLE of 100 µL sample
    - Bridging study: venous blood, venous plasma, finger prick blood
    - Challenges: contamination during sampling
  - Phase II: Pediatric clinical program
    - immediately in patients; complex starting dose setting (safe, yet efficacious dose?)
    - Increased concerns from Health Authorities, as the population is perceived vulnerable
    - Difficult recruitment & complex, multisite studies at an early stage
    - number and volume of samples from babies limited: 15 µL blood capillary
    - Bridging: venous/capillary blood and plasma bridging in adult HV study
    - SV assay for UD and metabolites (wash out with 150 µL BSA/PBS) – left over samples used for metID
    - Challenges: underfilled capillaries, air gaps -> mitigation weighing capillaries
    - Central labs not accepting non sterile capillaries (Sarstedt 20 µL sterile capillary proposed)



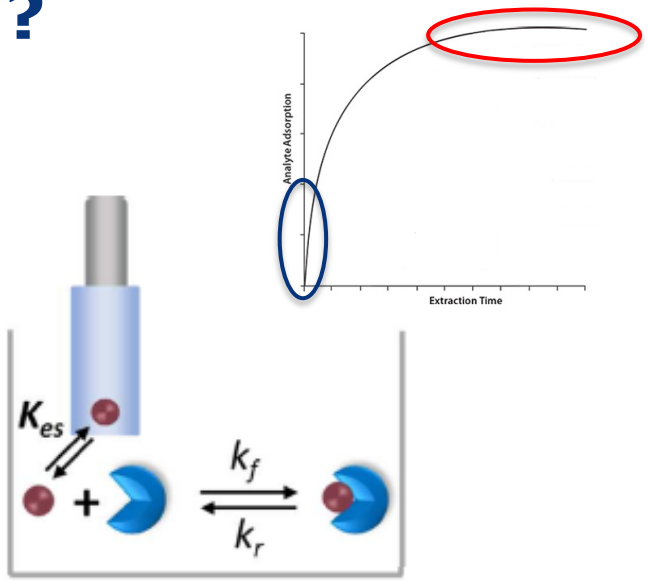


BioSPME: zero volume sampling?

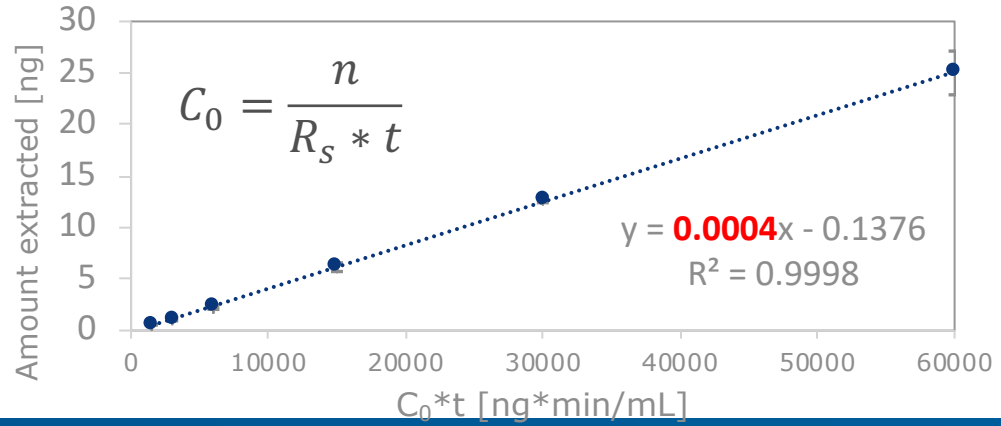
- Solid Phase Micro-Extraction
- Developed in 1989 by J. Pawliszyn
  - Environmental, fragrance and food
- C18-Coated fiber
  - *In vitro* tips and *in vivo* probe
- Insertion of probe in vein
- No blood loss



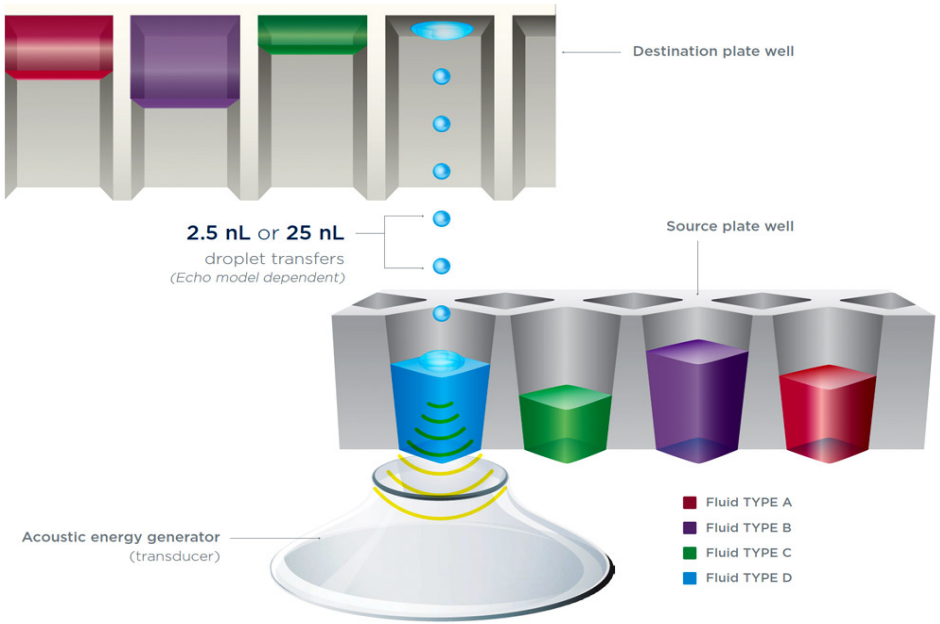
- Robustness and sensitivity remain challenge



- Free fraction analysed
- Extraction time, sampling rate



Acoustic droplet ejection (ADE) non-CMS



- An acoustic pulse is transmitted into the fluid (plasma/DMSO) in the source microwell plate, causing the fluid to form a droplet that travels upwards.
- The droplet is captured by the receiving surface (an inverted destination microwell plate). Surface tension of the fluid keeps it on the receiving surface.

- Proposed workflow:
  - Collect 50 µL blood in adapted device
  - Prepare plasma, transfer to acoustic source plate
    - 384 PP plate: 15 µL dead volume and 60 µL content
    - 384 LDV plate: 2.5 µL dead volume and max 12 µL
    - Sample tubes: 30 µL dead volume and 70 µL
    - Max volume 5 µL
    - No manual pipetting->no pipetting errors, less variability
    - No contamination
    - Fast: 200-500 droplets/s
  - organic solvent (30 µL) to precipitate can be reduced as well

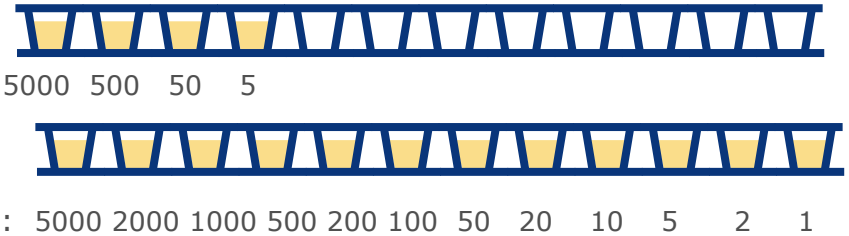
Evaluation Echo plasma calibrators + plasma QCs

Manual preparation

- **Calibrators in plasma** (mix of 4 compounds): 1 – 5000 ng/mL
- **QCs in plasma**: 5 – 50 – 500 – 5000 ng/mL
  - 6 batches of plasma: 1 lipemic batch, 1 clotted batch

Transfer with Echo

- 0.5 or 1 µL plasma (calibrator/QC)
- 2.5 nL IS
- 2 µL DMSO
- 30 µL acetonitrile (manual)



- Echo calibrators and QCs prepared in 6-fold
- **Same volumes** transferred to obtain different concentrations
- Comparison with manually prepared calibration curve

Evaluation Echo: plasma calibrators

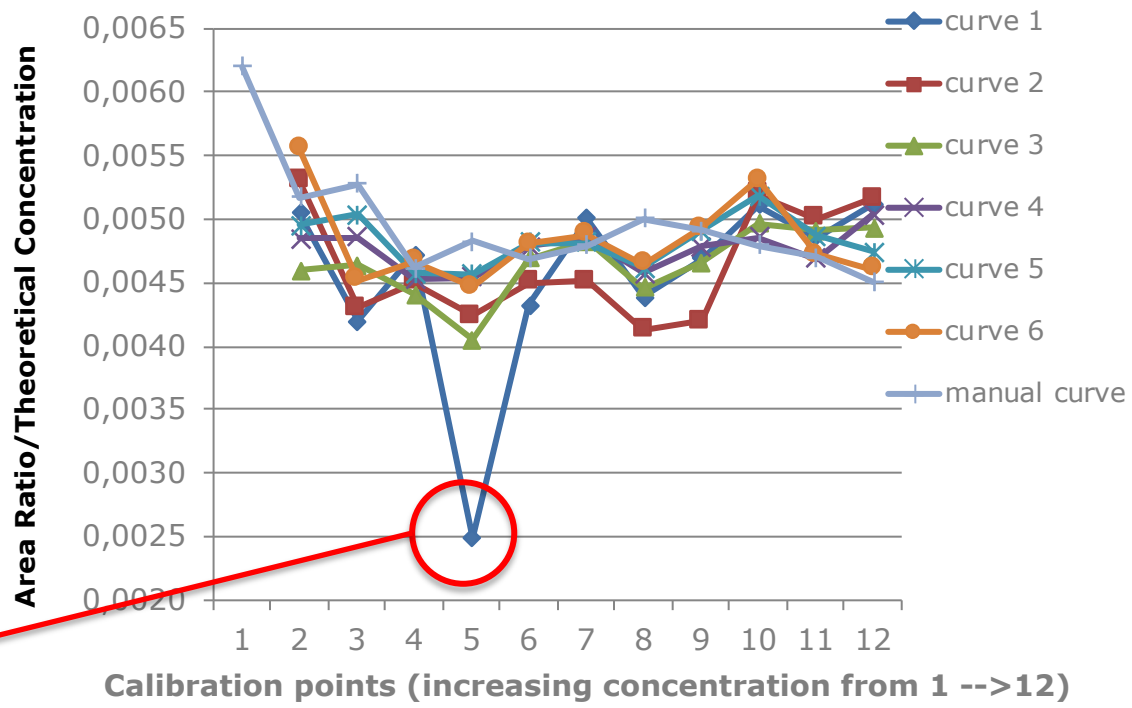
Variation **for 6 replicates** expressed as % CV

Echo compound transfer in **dog plasma** (calibrators):

Concentration (ng/mL)	compound 1	compound 2	compound 3	compound 4
1	-	-	-	-
2	9.4	6.7	-	5.0
5	5.8	7.0	5.2	6.1
10	5.3	2.5	4.5	3.4
20	17	20	19	22
50	6.1	4.3	2.3	6.0
100	8.0	3.4	8.5	5.6
200	4.6	4.3	4.8	7.5
500	4.4	5.7	5.8	5.5
1000	5.8	3.3	4.2	4.6
2000	5.0	2.5	5.6	4.0
5000	5.2	4.5	5.2	5.5

< 5 %
5 % < x ≤ 10 %
10 % < x ≤ 15 %
15 % < x

Echo calibrator curves: (**compound 1**):



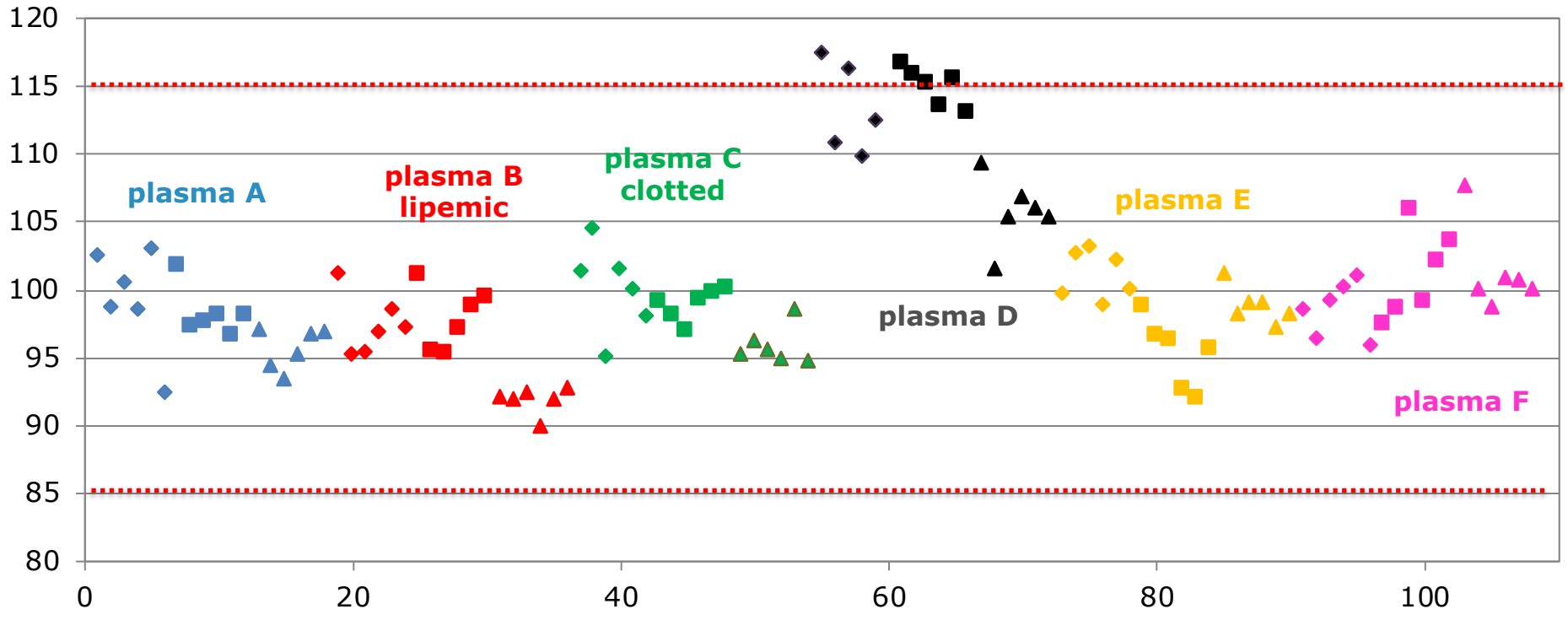
Transfer of **constant volume** (1 µL)

- **Acceptable variation, some outliers**
- **Good accuracy**

Results of QCs: 0.5 µL plasma

CV (%) of QCs prepared by Echo (results compound 1)						
Analyte (ng/mL)	plasma A	plasma B	plasma C	plasma D	plasma E	plasma F
5	**3.8**	**2.3**	**3.2**	**4.0**	**1.8**	**2.0**
50	**1.8**	**2.3**	**1.2**	**1.2**	**2.7**	**3.2**
500	**1.6**	**1.1**	**1.5**	**2.4**	**1.3**	**3.2**

Accuracy (%) compound 1: **plasma 0.5 µL**



Echo Liquid handler

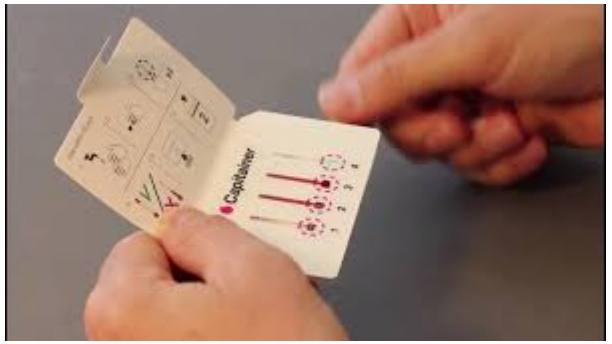
- Transfer of very small volumes (0.5 -1 µL plasma): no dilution of plasma needed (sensitivity!)
- Less organic solvent
- The transfer of plasma, calibration samples, IS added by the Echo:
  - No human bias
  - No cross-contamination
  - IS variation low

- Cost of instrument and plates
- Incompatibility with current workflow
- Lower final volumes in AS (appr. 30 µL): evaporation after injection
- Dead volume of tubes is large (30 µL) – even DV in LDV plates = 2.5 µL

Patient Centric Sampling

- Microsampling paves the way for PCS: clinical innovation team supports PCS
- Enables new sampling opportunities
  - At the time of an acute phase of diseases (migraine, epilepsy,..)
  - Fragile patients (critically ill, neonates/pediatric)
  - Patients sample in comfort @home without need to travel to hospital (facilitates recruitment)
  - Remote areas w/o centrifuge/freezer capabilities (if analytes are stable)
  - Improved monitoring of patient compliance
- Resistance in clinical teams to embrace PCS (as an extra investigational study arm) in their study
  - New collection devices are considered as medical device: CE certification required in EU
  - Venous vs capillary sampling (blood vs plasma)
  - Sample quality/integrity and compliance (documentation)
  - Training of staff/patients/HV
- Wet versus dried
  - Dried anticipated to be more convenient wrt logistics (collection and shipping)
  - Can multiple goals be achieved (combination with clinical blood parameters)
- Bioanalytical aspects
  - Extra stability evaluation/extraction of (aged) dried samples/sample management @RT
  - Preparation of calibrators/QC samples
  - Sample handling more tedious

Patient Centric Devices - blood



Capitainer

Neoteryx: MITRA

DBS System: HemaXis

7th Sense: TAP (touch activated phlebotomy) 100 µL
FDA clearance

Tasso: Hemolink 200 µL or 4 x 20 µL
(FDA approval ongoing)

Trajan: hemaPEN: 11 µL

Conclusion/summary

- Preclinical studies:
  - CMS is now standard for all studies – both GLP and non-GLP (in house and outsourced)
  - Blood sampled in capillary, centrifuged and plasma transferred to E2E 10 µL capillary
  - Wash out is standard process (to allow re-analysis and ISR)
  - Exceptions (< 10%) relate to sensitivity, complex study designs, instability of drug or metabolite
- Clinical studies:
  - At early stage DBS/VAMS explored and stopped – now revived with respect to PCS
  - Blood CMS has been accepted and implemented in pediatric clinical studies
  - Need for more robust microsampling technique
- Alternative strategies explored but currently not embraced:
  - Acoustic droplet ejection
  - BioSolidPhaseMicroExtraction
  - MS Wings in (pre)clinical space?

Acknowledgments

- Tom Verhaeghe
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