Capillary Microsampling, CMS

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EBF 4th Open Symposium, Barcelona, Nov 2011

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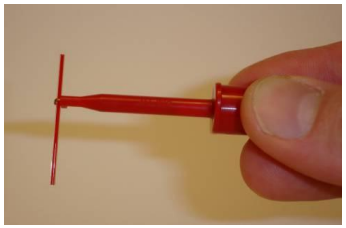
Capillary Microsampling

… is a generic method for

collection and handling of

small exact volumes of liquid matrices,

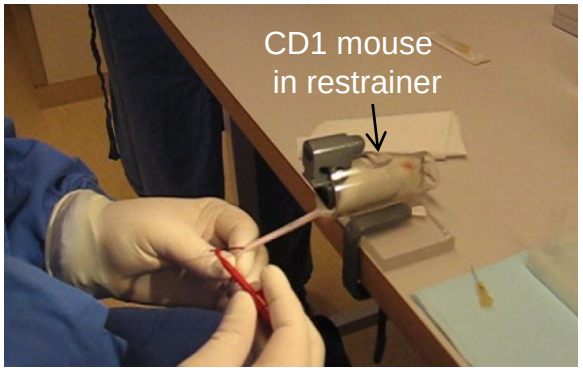
such as blood or plasma.

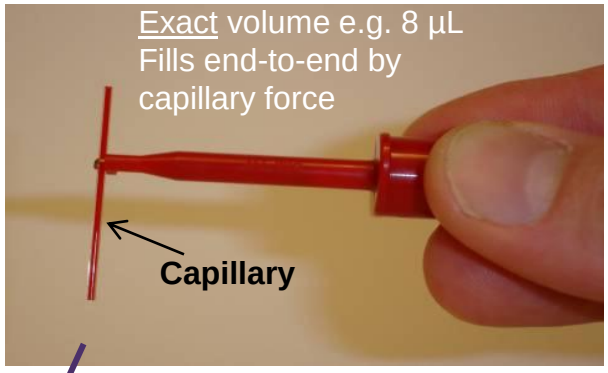


Outline

- CMS of blood
- CMS of plasma
- Validation aspects
- Automation
- Exposure in main study animals
  - Study design
  - 3R, Scientific value and Cost savings
  - Clinical pathology
  - MIST analysis
- Study examples: 1 and 6-months toxicology studies
- *Ex vivo* blood/plasma ratio
- CMS of rare matrices

CMS of blood





K_2EDTA treated micropipettes
ISO 7550 certified
Typical volumes 8-25 µL

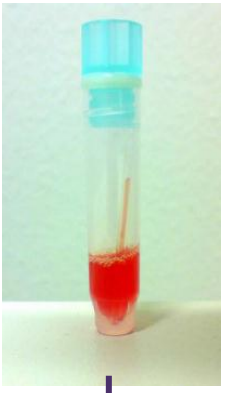
Relative error < 1%
RSD < 1%

A tail vein is penetrated with a cannula, collect e.g. 8 µL blood

Animal facility

In dry vial

…or mix with stabilizing solution



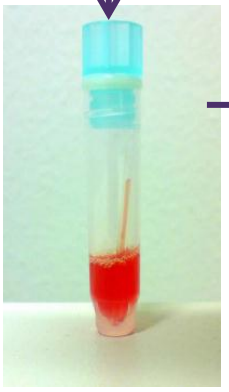
From tail to ice in 10 seconds Including stabilization

Freeze

Freeze

Bioanalysis lab

Add H_2O, buffer or internal standard. Mix!



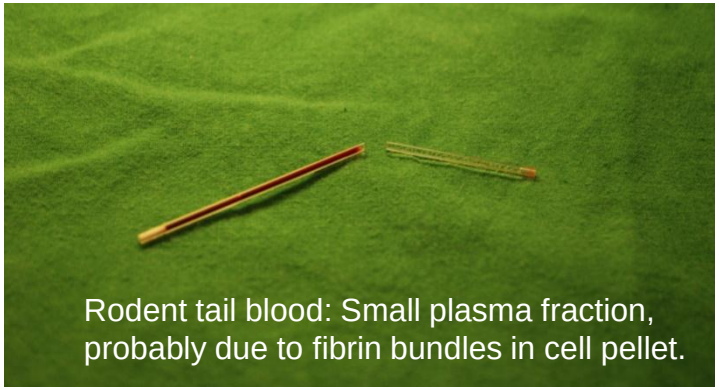
Internal standard is added to whole sample or to a fraction

An aliquot is prepared by protein precipitation, LLE, SPE or ultrafiltration and analysed by LC-MS/MS.

Sample matrix is lysed/diluted blood Frozen blood is OK for calibration and QC samples

CMS of Plasma

Currently implented globally at AstraZeneca.

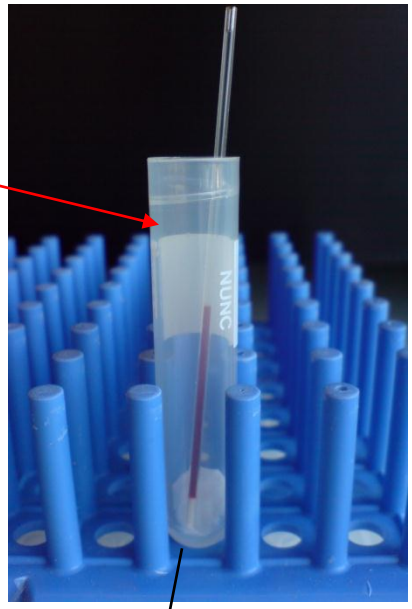


32 µL blood in (K_2EDTA) haematocrit tube

Plug with wax

Place in labelled tube

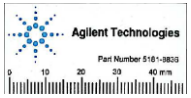
Plasma prep. 1500 g for 10 min



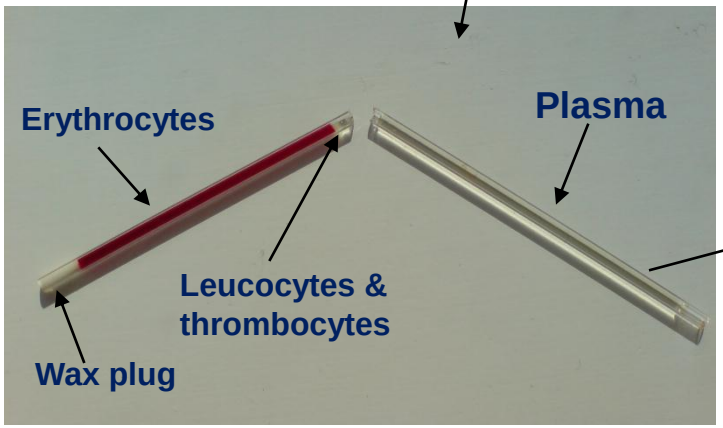
Typical volumes

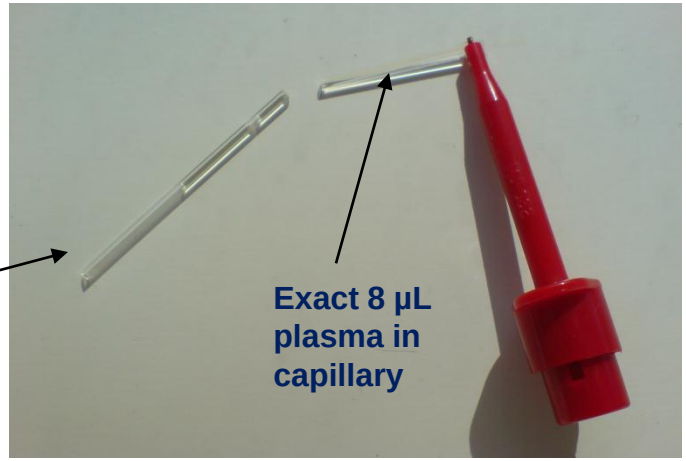
8 µL (4 µL backup) plasma from ~32 µL blood or
16 µL (8 µL backup) plasma from ~64 µL blood

Cut above the leucocyte phase using a capillary cutter.



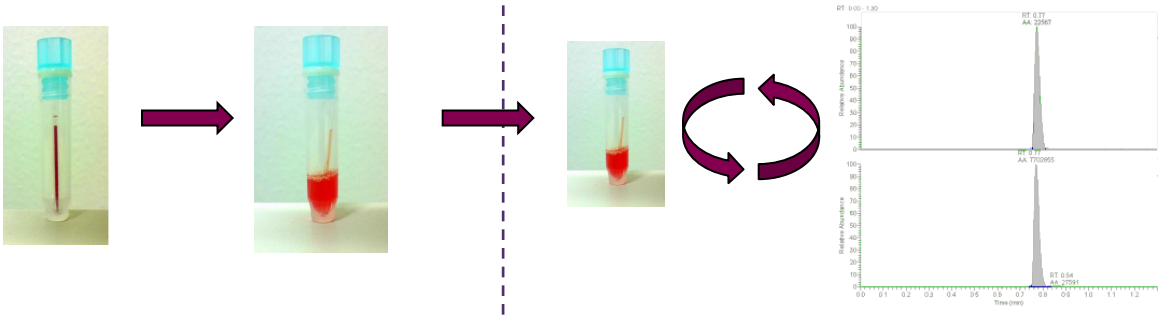
An exact volume of plasma is collected with a capillary from the end of the haematocrit tube.





Basic CMS principle

Sampling | **Dilution** | **Clean-up and analysis**



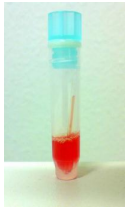
Exact volume

Dilution (Samples and QCs at the same time)

A fraction of the diluted sample is used for each analysis
Reanalysis always possible

Validation of bioanalytical methods, suggestions

•Stability in undiluted matrix inside capillary
Long term in freezer
2 freeze/thaw cycles
Room temperature



•Stability in diluted matrix
Long term in freezer
2 freeze/thaw cycles
Room temperature

•If IS is added to the whole sample:
Stability of IS in working solution and in diluted matrix.
(In this situation, the actual amount of IS becomes critical when performing dilution or reanalysis with a new standard curve)

•QCs in capillary (at least one batch)
Cals may be in diluted matrix

•Dilution with diluted blank matrix

Besides some extra stability experiments, the validation of a CMS method is very similar to the validation of conventional large volume methods.

Three days validation, example, 8 µL mouse plasma (1/3 used for one analysis)

Within and between-batch precision and accuracy of QC samples in mouse plasma

Batch	LLOQ 0.0600 µmol/L	QC L 0.150 µmol/L	QC M 9.00 µmol/L	QC H 24.0 µmol/L
Within-batch CV(%)	3.8	1.6	0.4	1.1
Within-batch Bias (%)	-12.0	-2.1	0.4	2.3
n	6	6	6	6
Within-batch CV(%)	7.8	4.6	2.0	2.1
Within-batch Bias (%)	2.7	3.5	1.1	1.9
n	6	6	6	6
Within-batch CV(%)	3.6	1.9	1.5	0.6
Within-batch Bias (%)	-1.3	2.7	-2.6	-4.8
n	6	6	6	6
Between-batch CV (%)	8.5	3.8	2.1	3.6
Between-batch Bias (%)	-3.5	1.4	-0.4	-0.2
n	18	18	18	18

Automation of CMS methods

Automation tools already available for the bioanalytical process!

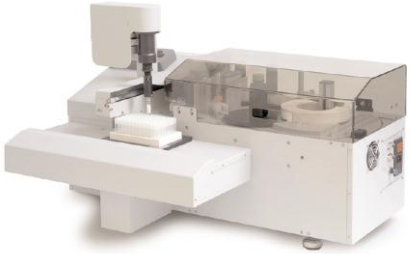
Vials in 96-format

Easily stored, 2D-coded, Roborack

Liquid handling

De-cappers! 96 tubes in 20-60 seconds

Labeling robot



Rack scanners

Exposure in main study animals!

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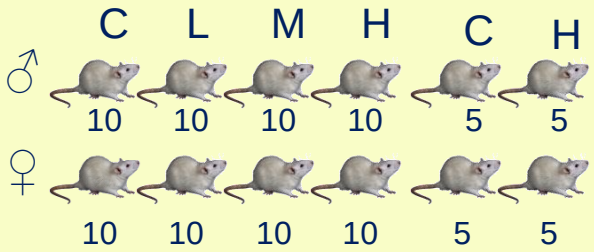
Rat study design, example 1-month toxicology study

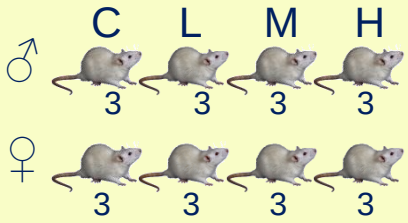
Today

Main study



Pathology
Clinical pathology

TK Satellite



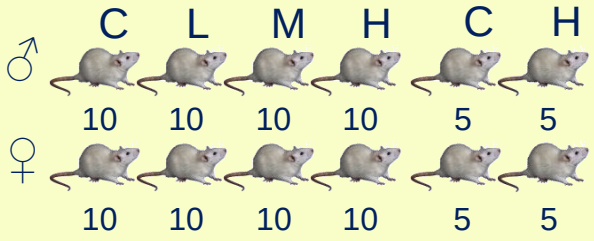
Full profile TK (rich sampling)
e.g. 5 samples/animal/day

TK based on Satellite animals
3 animals/group
15 samples/group

Scientific Value | 3R | Productivity

Future

Main study



Pathology
Clinical pathology

Composite TK from sparse sampling e.g.
2 samples/animal/day

(*Ex vivo* blood/plasma ratio, if blood TK)

TK based on Main study animals
10 animals/group
20 samples/group

Clinical pathology

Haematology and plasma chemistry

↓ Blood loss

Haemoglobin	↓
Red blood cell count	↓
Haematocrit	↓
Reticulocyte count*	↑

* Bone marrow response

Thus, the effect on clinical pathology from TK (and MIST) sampling must be controlled to enable sampling in main study animals!

We have found that

145 µL blood* collected from rats does not affect clinical pathology parameters measured two days after last TK sampling, compared to non sampled animals

*corresponds to 0.7% and 1% of circulating blood volume in male and female rats, respectively.

MIST and TK in main study animals

Problem
Microsampling volumes will in many/most cases not be enough for initial MIST investigations of disproportional metabolites

Diluted samples (CMS) not suitable for MIST

Suggested solution (under discussion)
Earmarked samples for MIST

Larger sample volumes for MIST

1. Conventional volumes, scheme designed for MIST purpose (separated from TK sampling, different day/week)
2. Pool microsamples from same time point / dose / sex

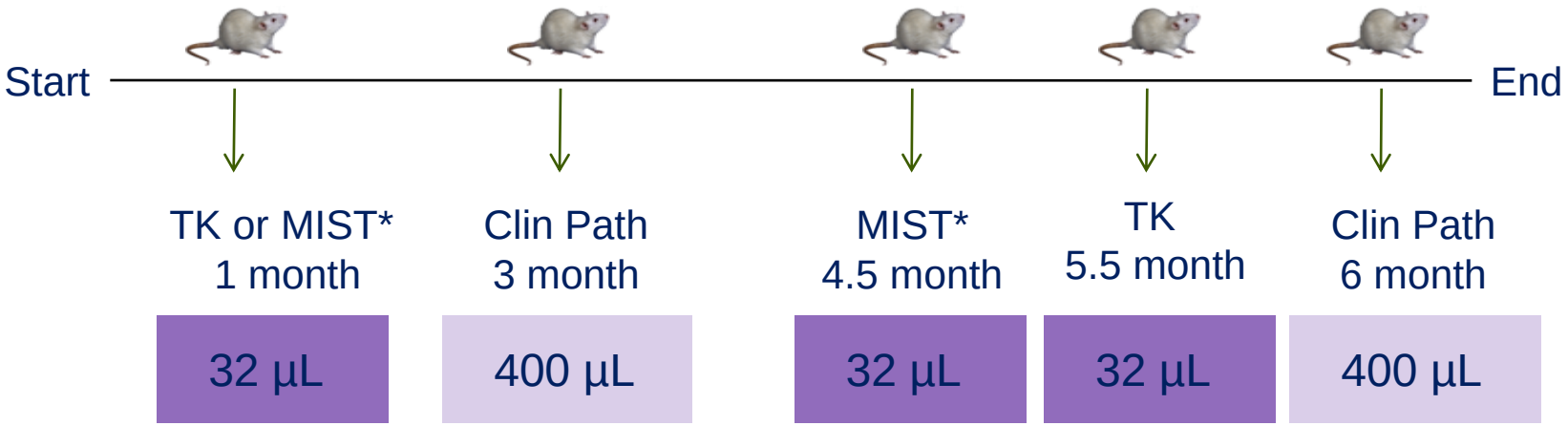
MIST performed in 3- or 6-month studies enable higher flexibility compared to 1-month study

In 1-month rat study: suggestion to collect MIST samples after 2 weeks, 1 sample/animal

Example: 6-Month toxicology study in mouse

Composite TK microsampling of plasma from the main study animals.
No satellite animals!

16M + 16F in each dose group;
control, extra low, low, mid and high



*MIST samples pooled 3 x 8 µL /timepoint/dose/sex

Productivity improvement, example

Comparison between 2 different designs of a 6 month toxicology mouse study – a conventional design and a microsampling design without satellite animals.

	Conventional design with satellite animals	Microsampling design without satellite animals	Reduction
Sample volumes for Bioanalysis	200 - 300 µl	32 µl	6 - 10 times less
Sample volume (% of blood volume in a 25 g mouse)	10-15 %	1,5 %	6 - 10 times less
Number of animals in study	238	160	78
Total no. of dosings	42 840	28 800	14 000
Hours of work (husbandry and dosing)	2100 h	1400 h	700 h

Back to blood…

Total concentration in blood… what about the free concentration?

Whole blood exposure → plasma exposure → unbound exposure

We need to know

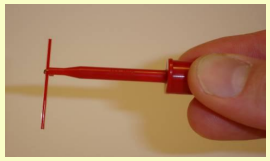
- The free fraction in plasma.
- The blood/plasma distribution ratio (B/P-ratio) and if it is dependent on:
  - Concentration?
  - Time after dose?
  - Gender/age/disease/etc?

Determination of B/P-ratio:

In vitro, incubation of spiked fresh blood

or

Ex vivo, analysis of blood and plasma study samples



8 µL blood

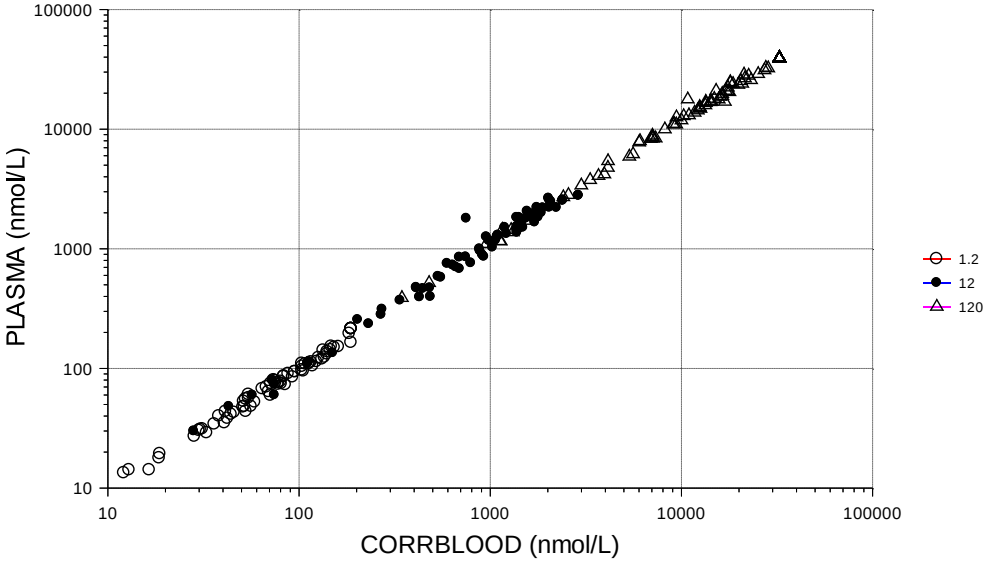
8 µL plasma

Enables estimation of B/P-ratio in rare animals such as genetically modified organisms (GMO), monkeys or juveniles, where fresh blood for *in vitro* experiments are not readily available.

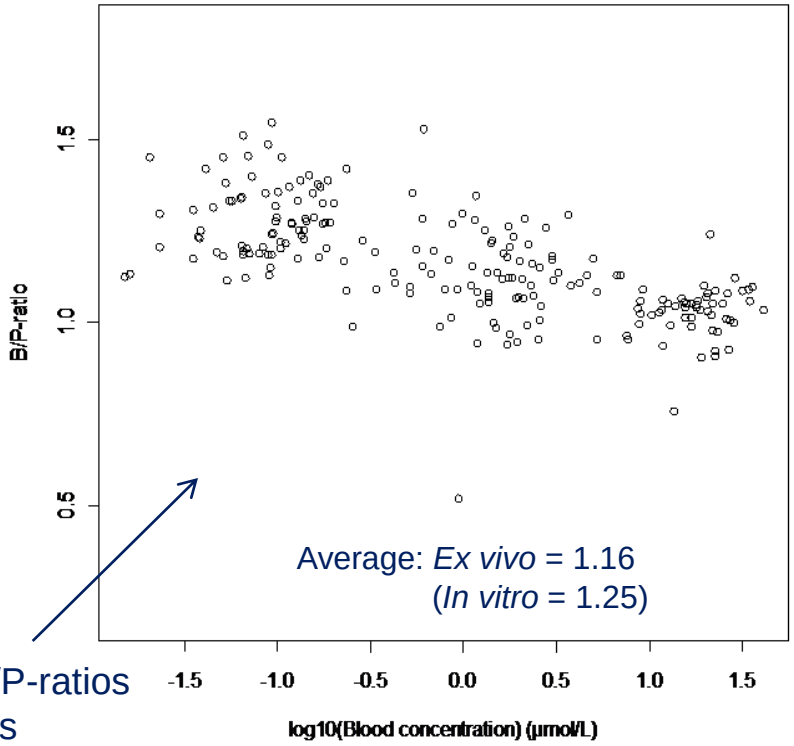
Comparison blood vs plasma, B/P-ratio from study samples, example 1-month dog study

Full data set CMS of blood and large volume plasma

Plasma vs blood (corrected for *in vitro* B/P-ratio)



Ex vivo B/P-ratio vs blood concentration



Trend towards lower B/P-ratios at higher concentrations

Opportunity to analyse rare matrices

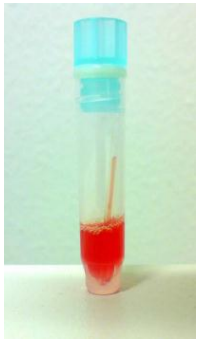
Example:
Blood/plasma from juveniles or from genetically modified animals
CSF from rodents
Interstitial fluid
Microdialysate
Sweat or tears…

Enables analysis of ”new” liquid matrices and gives the possibility to increase the scientific value from animal studies

How to validate/qualify a method in that?!

1.
Collect the blank matrix in 8 µL capillary

2.
Add dilution liquid containing the analyte.
Mix.



Volume example QC’s:
Validation batch
6x4x8 µL=192 µL
Analysis batch
2x3x8 µL = 48 µL

Minimal waste!

Stability tested in diluted matrix
Dilution at sampling site
(Or risk assessment)

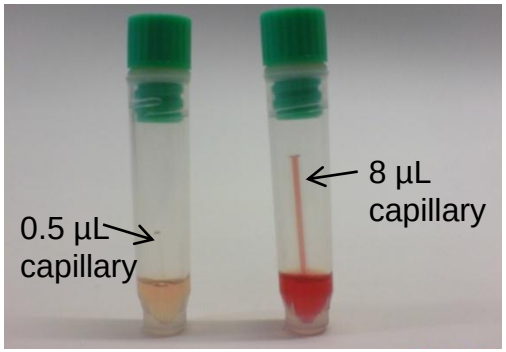
Example: Interstitial testis liquid from rat

- Interstitial testis liquid was harvested after mild centrifugation
- 8 µL was collected in capillary
- Surplus liquid from control animals (n=4) was pooled (~190 µL)
- Calibration samples (8 levels) and QC samples (3 levels n=2) were prepared, in total 112 µL
- Analysis batch was well accepted according to standard criteria
- Consistent results from left and right testis

Animal	Testis liquid (µmol/L)
R testis	0.299
L testis	0.342
R testis	0.343
L testis	0.381
R testis	0.268
L testis	0.413
R testis	0.282
L testis	0.284
R testis	9.33
L testis	9.38
R testis	8.87
L testis	9.28
R testis	14.5
L testis	11.1
R testis	15.3
L testis	16.2

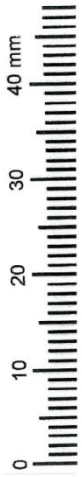
How little is “Micro”?

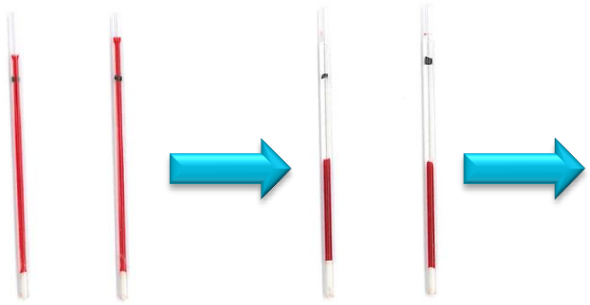
0.5 µL blood



0.5 µL and 8 µL blood mixed with 50 µL water. The diluted sample can be repeatedly analysed.

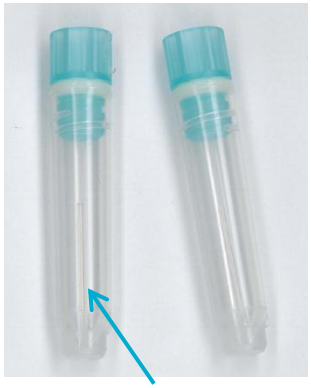
2 µL serum from 5 µL blood





5 µL blood in 6.7 µL plain glass capillary

Store 1 h at room temp. Spin!



2 µL capillary with serum. Can be repeatedly analysed.

Conclusions
Capillary microsampling (CMS)

- Blood, plasma and other biofluids
- Volumes down to a few microliter
- Exposure in main study animals

  Scientific value, 3R and Productivity hand in hand
- Exact sample volume and liquid matrix enables:

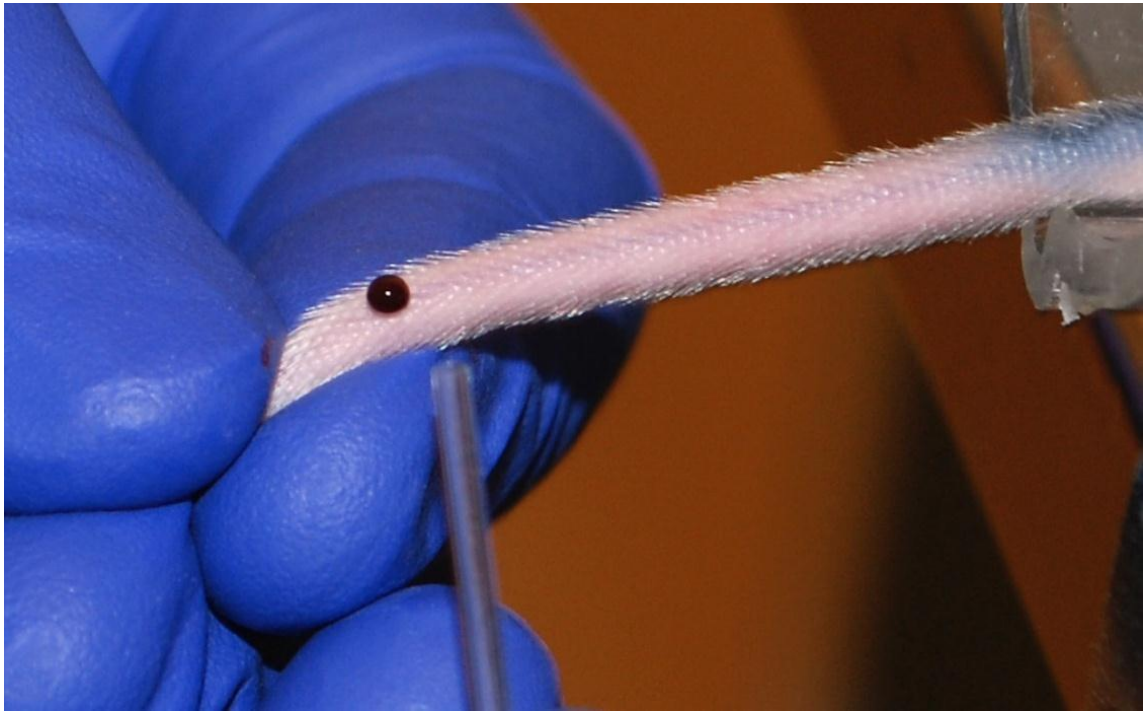
  Stabilization within 5-10 seconds (blood)

  Analysis of rare matrices
- Automation tools already available, liquid handling
- Validation of CMS method is similar to conventional method

Acknowledgement

Rodrigo Palma Villar, Marie Eriksson, Kristian Königsson, Lars B Nilsson, Carina Norsten-Höög, Anita Annas, Carl-Johan Zettervall, Lena Paulsson, **Martin Ahnoff**, Richard Grater, Victoria Holmes, Magnus Olin, Christopher Smith, Anders Olsson, Daniel Weston, Göran Eklund, Chungang Gu, Gösta Hallström, David M Higton, Kirsten Hobby, Claire Landqvist, Lars Weidolf, Patty Davis, Nicola Eraut, Jessika Weilitz, Hung Lam, John Kemp, Paul Holme, Amanda Wilson, Eva-Lena Glämsta, Liz Thomas, Patty Davis, Claes Roos, Amanda Wilson, Daniel Öhman, Ronny Fransson-Steen, Angela Sykes, Anders Samuelsson, Hans Marchner, Jacob Brogren, Magnus Halldin, Sonia Smith, Susanne Berg, Marie Persson, Ann-Sofie Sandinge, Mark Eisman, Clare Hammond, Catherine Wilkinson, Angela McDermott and Graham Ridgwell

And others...



Thanks!