Capillary Microsampling (CMS)

Better science - fewer animals

Ove Jonsson

Global DMPK, AstraZeneca R&D
Södertälje, Sweden

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AstraZeneca

ove.x.jonsson@astrazeneca.com or ovex.jonsson@gmail.com

Outline

- CMS Refresh
- Implementation in a regulatory environment
- One mouse, three PK

CMS of 4 µL blood, plasma and serum. Biomolecule on Gyros platform.

- CMS 'Rare matrix approach'
- Automation, tips and tricks

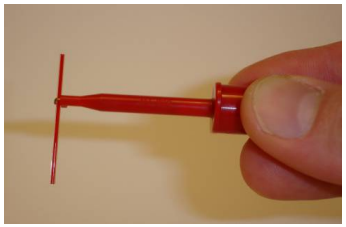
Refresh: Capillary Microsampling...

… is a generic technique for

collection and handling of

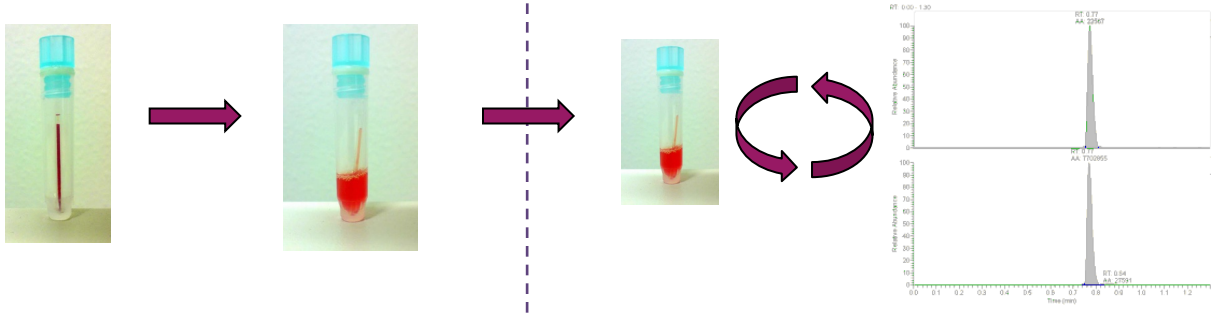
small exact volumes of liquid matrices,

such as blood, plasma or serum.



Basic CMS principle

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



Exact volume in capillary

Dilution with washout liquid
(Samples and QCs at the same time)

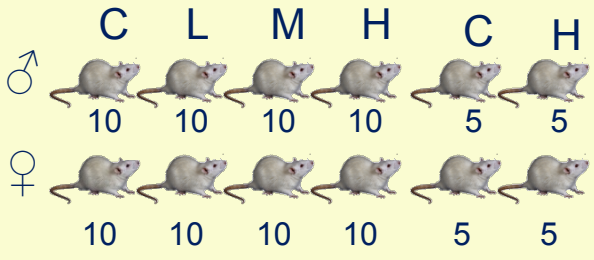
A fraction of the diluted sample is used for each analysis
Reanalysis always possible

Main goal: TK in main study animals

- Reduce and refine animal use
- Maximize scientific value
- Increase productivity
- Reduce costs

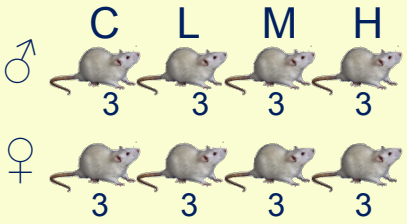
Old design

Main study



Pathology
Clinical pathology

TK Satellite

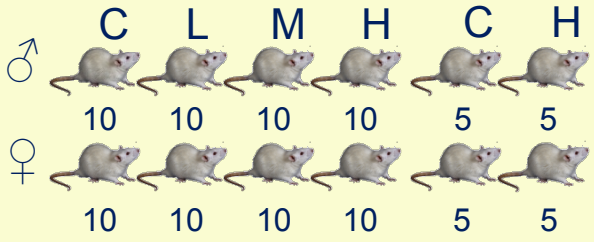


Full profile TK (rich sampling)
e.g. 6 samples/animal/day

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

New design

Main study



Pathology
Clinical pathology

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

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

CMS implementation

TK microsampling from main study rodents in general toxicology studies means the introduction of new procedures in a strictly regulated, cross-functional environment, reluctant to changes.

It will not be easily done…

Learning's and recommendations from the global implementation process at AstraZeneca

Find the passionate people within each function!
Learn about the other functions!
What needs do each function have?
What are the hurdles?
Show the good examples.
Get management support.

Present & communicate.

Present & communicate.

Present & communicate.

Present & communicate.

Never give up!

The possibility to exclude satellite animals in rodent toxicology studies will depend on:

- Species
- TK sampling design (composite sampling or full profiles from each animal),
- Total blood volume collected for TK
- Length of the study

Circulating blood volumes in different species*

The total circulating blood volume in a **25 g mouse** is approximately 1.8 mL. Withdrawal of a 32 µL blood microsample corresponds to a **2%** blood loss.

In a **250 g rat**, having a circulating blood volume of 16 mL, each microsample represents around **0.2%**.

This may be compared to the commonly used 2 mL blood withdrawal from an **8 kg dog** which corresponds to approximately **0.3%**.

*Diehl *et al*. A good practical guide to the administration of substances and removal of blood, including routes and volumes. *J. Appl. Toxicol.* 21, 15-23 (2001)

TK sampling strategy

DRF studies
Use serial sampling for full TK profiles
Rat studies: 5-6 samples from main study animals (2M+2F)
Mouse studies: 5-6 samples from satellite animals (2M+2F)

14-Day, 1-,3- and 6-Month studies in Rat:
Use composite sampling from main study animals
2 or 3 samples per animal (10M+10F or 15M+15F)

14-Day and 1-Month studies in Mouse:
Use serial sampling for full TK profiles from satellite animals (3M+3F)
5-6 samples per animal and TK day

(1-),3- and 6-Month studies in Mouse:
Use composite sampling from main study animals (10M+10F or 15M+15F)
2 or 3 samples per animal and TK day

Example of a balanced composite sampling design

(Each animal has different sampling schemes):

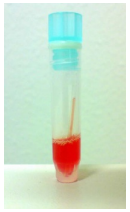
Animal No.	Sampling time point					
	#1	#2	#3	#4	#5	#6
1	x			x	x	
2	x			x		x
3		x		x	x	
4		x	x			x
5	x		x	x		
6	x		x		x	
7	x		x			x
8		x			x	x
9		x	x		x	
10		x		x		x
	n=5	n=5	n=5	n=5	n=5	n=5

Validation of bioanalytical methods

CMS specific experiments

- Stability in undiluted matrix in 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 in the washout liquid:
  Stability of IS in washout liquid 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 capillaries

- Calibration samples in capillaries or in pre-diluted matrix

- Dilution of over range samples with diluted blank matrix

MIST screening analysis

Problem
Microsampling volumes will in many/most cases not be enough for MIST screening analysis of disproportionate metabolites

Diluted samples (CMS) not suitable for MIST

Suggested solution
Earmarked samples for MIST

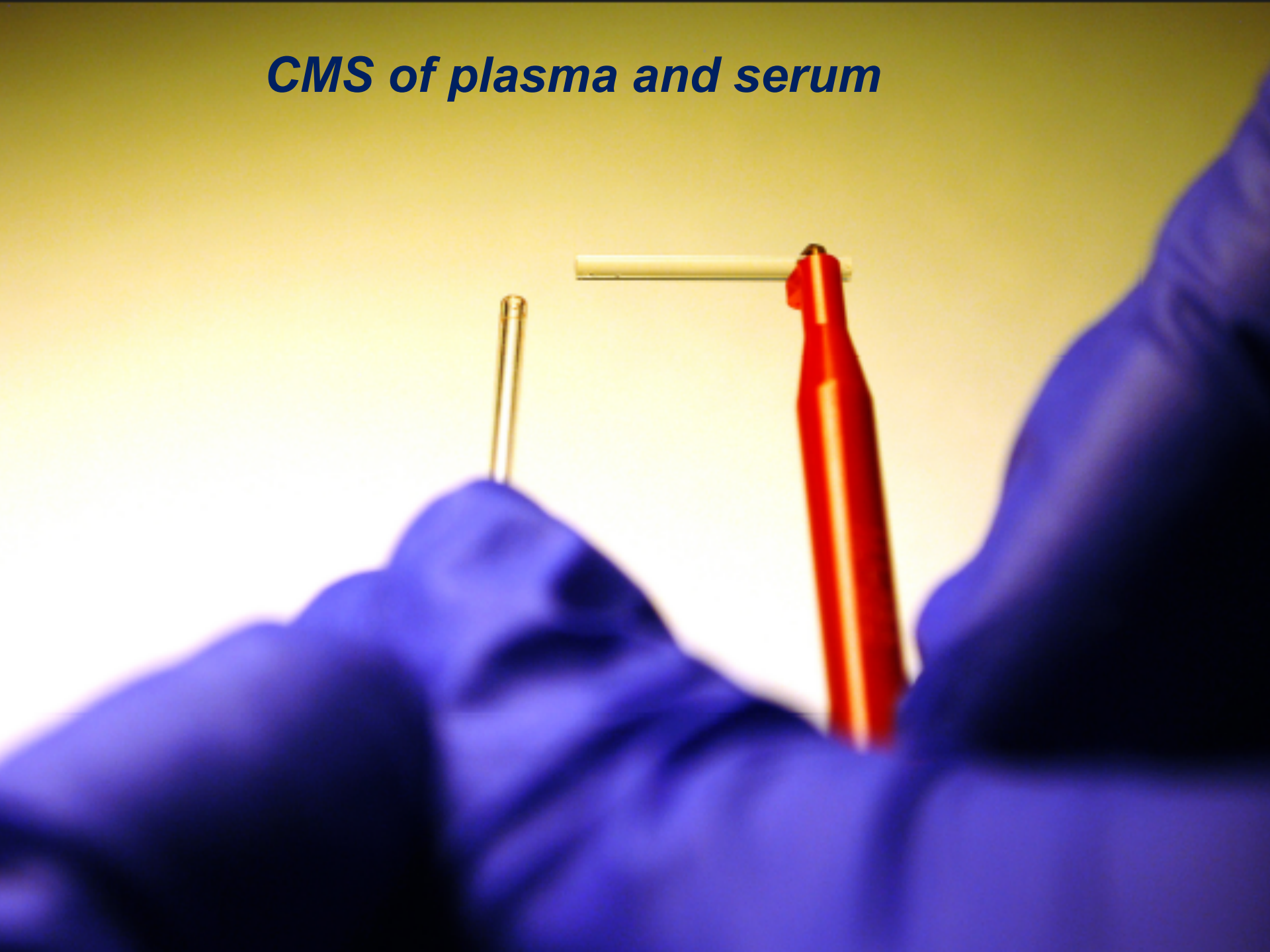
Larger sample volumes for MIST

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

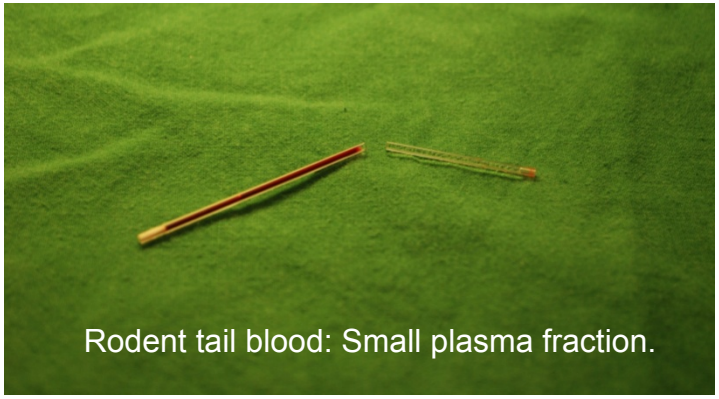
1. MIST performed in 3- or 6-month studies enables higher flexibility compared to 1-month study, better timing with MAD study.

2. Perform standalone MIST study. Only performed when project needs are known. Perfect timing with MAD study.

CMS of plasma and serum



CMS of plasma (serum)

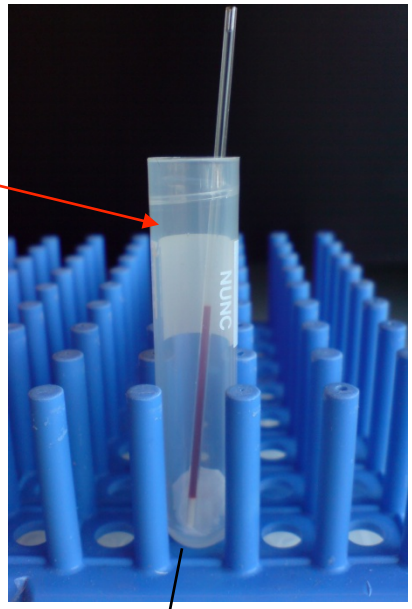


32 µL blood in K_2EDTA haematocrit tube (plain glass for serum sampling)

Plug with wax

Place in labeled tube

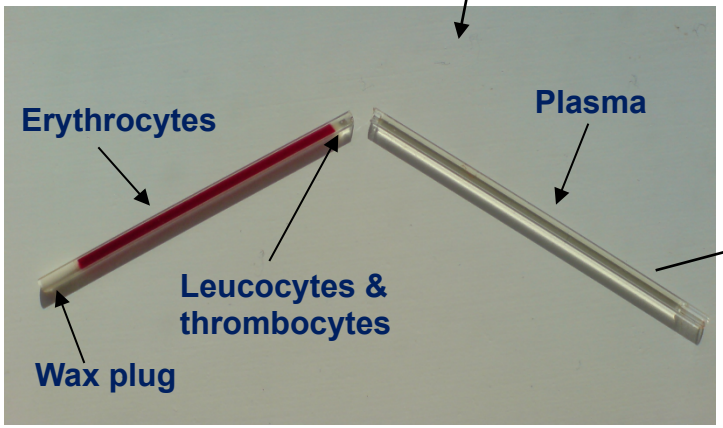
Centrifugation 1500 g for 10 min



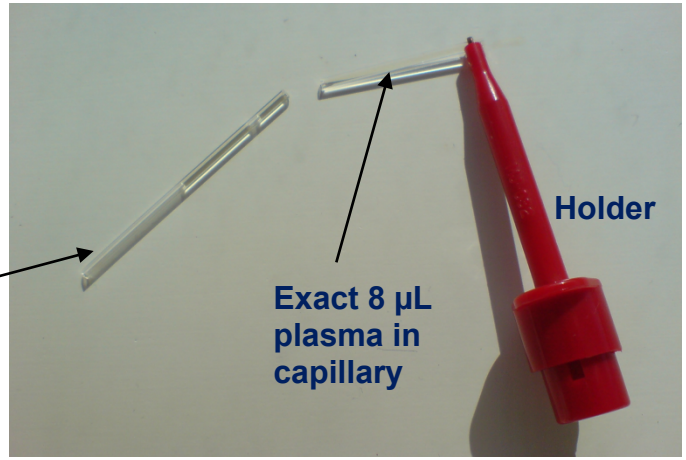
Typical volumes

8 µL (4 µL backup) plasma from ~32 µL blood

Cut above the blood cell phase using a capillary cutter.



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

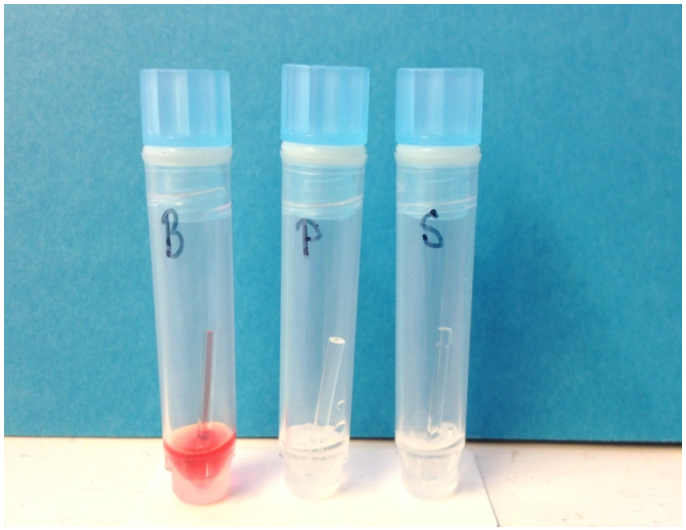


Put capillary in tube or plate

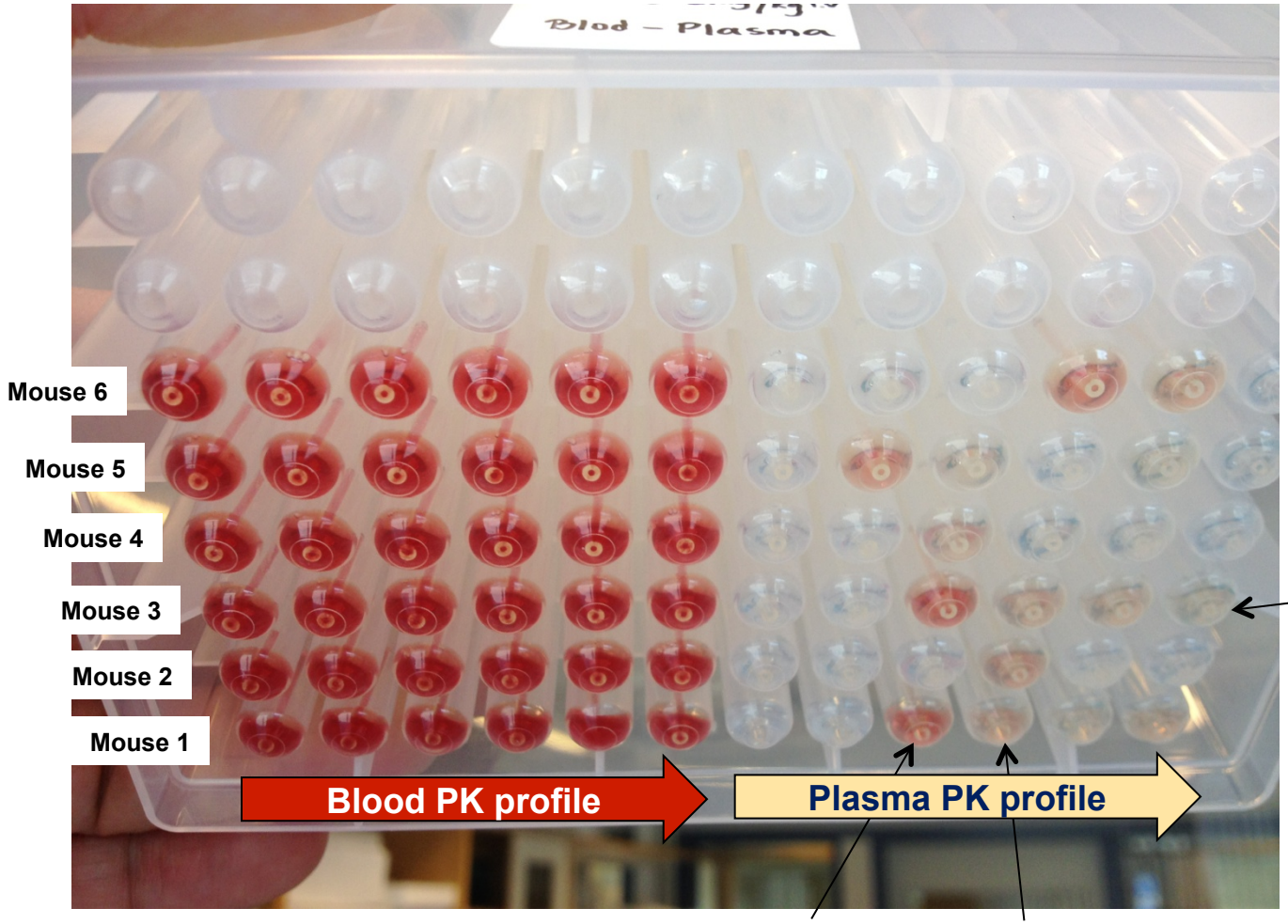
Our latest CMS application!

One mouse, three PK (blood, plasma and serum).

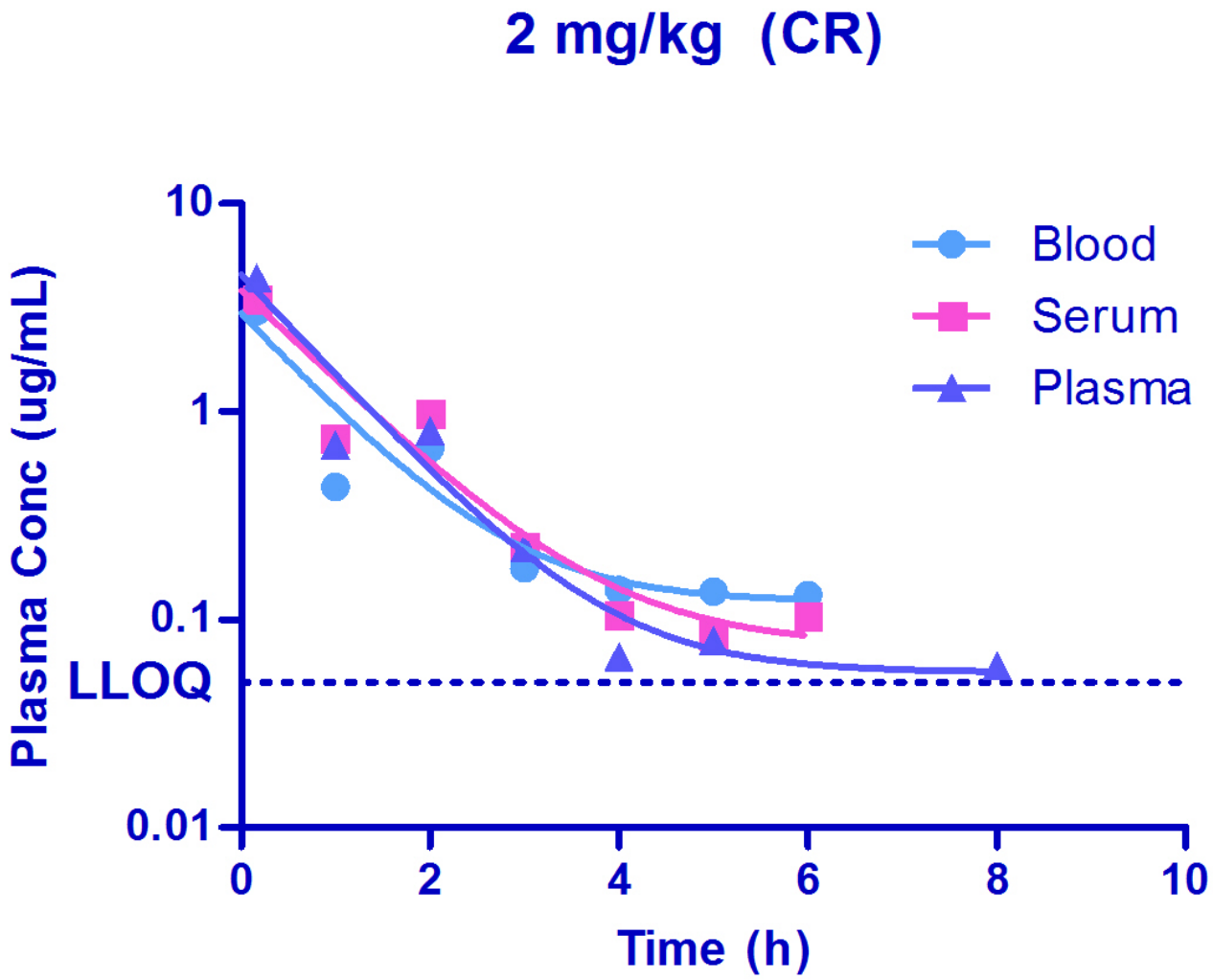
- Mice were dosed iv with a small protein
- Repeated PK sampling, 6 sampling occasions per animal
- 20 µL blood (EDTA) to give 4 µL blood and 4 µL plasma samples
- 16 µL blood (in plain glass haematocrit tubes) to give 4 µL serum
- Capillaries collected in 96 DW plate, diluted with 36 µL RexxipF buffer, Gyros
- Investigated protein measured on Gyros instrument using an antigen specific assay
- More than **600 samples** were successfully collected and analyzed



4 µL blood and plasma PK samples handled in 96 DW plate



PK in three matrices from the same animals
(Mean plasma concentrations (n=6) vs time after dose)



The CMS ‘Rare matrix approach’

A novel procedure to analyse **rare matrices** and/or determine **unstable drugs** in blood

Prepare Cals and QCs in:

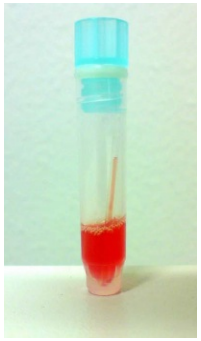
Blood/plasma from juveniles or from genetically modified animals
CSF from rodents
Interstitial fluid
Microdialysate

Prepare Cals and QCs of unstable drugs in blood:

Washout liquid that stabilizes the drug

1.
Collect the blank matrix in capillaries

2.
Add washout liquid containing the analyte.
Mix, spin.



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

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

Example: Interstitial testis liquid from rat

- Interstitial liquid was harvested after mild centrifugation of testis
- 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

Automation of CMS methods

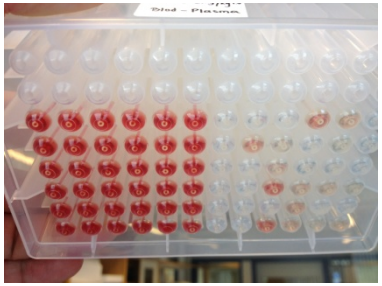
Automation tools already available for the bioanalytical process!

Vials in 96-format

Easily stored, 2D-coded, Roborack

Samples in 96 DW format

Cap mat seal



De-cappers!

96 tubes in 60 seconds

Liquid handling

Tips and tricks

1. Pipette from vial containing a capillary:

   Risk: The capillary may stick to the pipette tip.

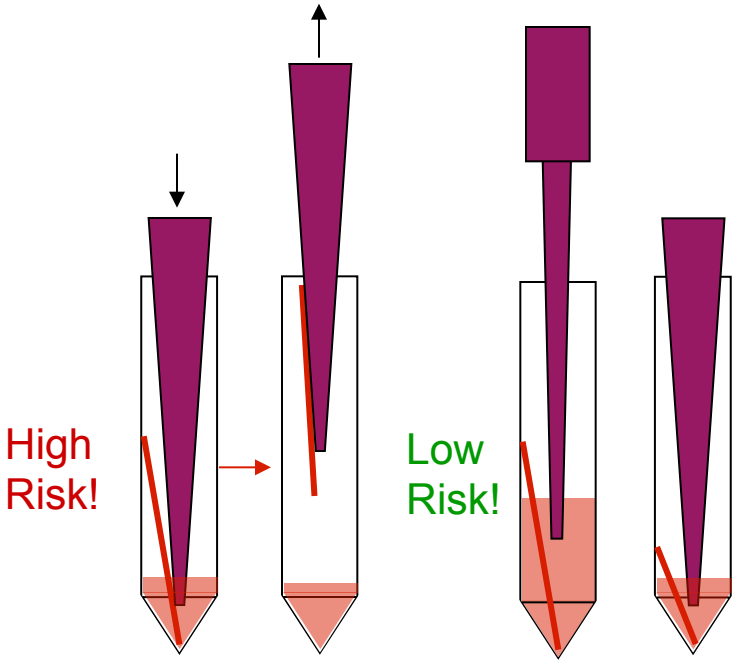
   Important parameters: Length of capillary, total sample volume, tip-shape, depth of pipetting

   Solution:

   Short capillary → Use 16 mm length rather than 24 or 29 mm

   Tip shape → Use as narrow tips as possible

   Dilution volume → Dilute in larger volume if possible



2. Collect Calibration or QC samples with a capillary from a sample tube. (‘Aliquot’ Calibs and QCs)

   Issue: A short 16 mm capillary will not reach down to the liquid in the tube.

   Solution

   1. Lean the tube, letting the plasma flow to the thread (usually works fine).

   2. Put a Pasteur pipette (without the bulb) or a plain glass haematocrit tube into the sample that will be sucked up by capillary force, collect with 8 µL capillary from the end of the Pasteur pipette (works really well!). To fill more into the Pasteur pipette, lean the tube and hold the Pasteur pipette nearly horizontal.

   3. Use very small tubes to prepare the Calibs and QCs, or transfer to low plate before taking aliquots with the capillaries.

Conclusions
Capillary microsampling (CMS)

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

  Scientific value, 3R and Productivity hand in hand
- Validation of CMS method is similar to conventional method
- Exact sample volume and the liquid matrix enables:

  Stabilization within 5-10 seconds (blood)

  Analysis of rare matrices
- Automated liquid handling tools already available

Acknowledgement

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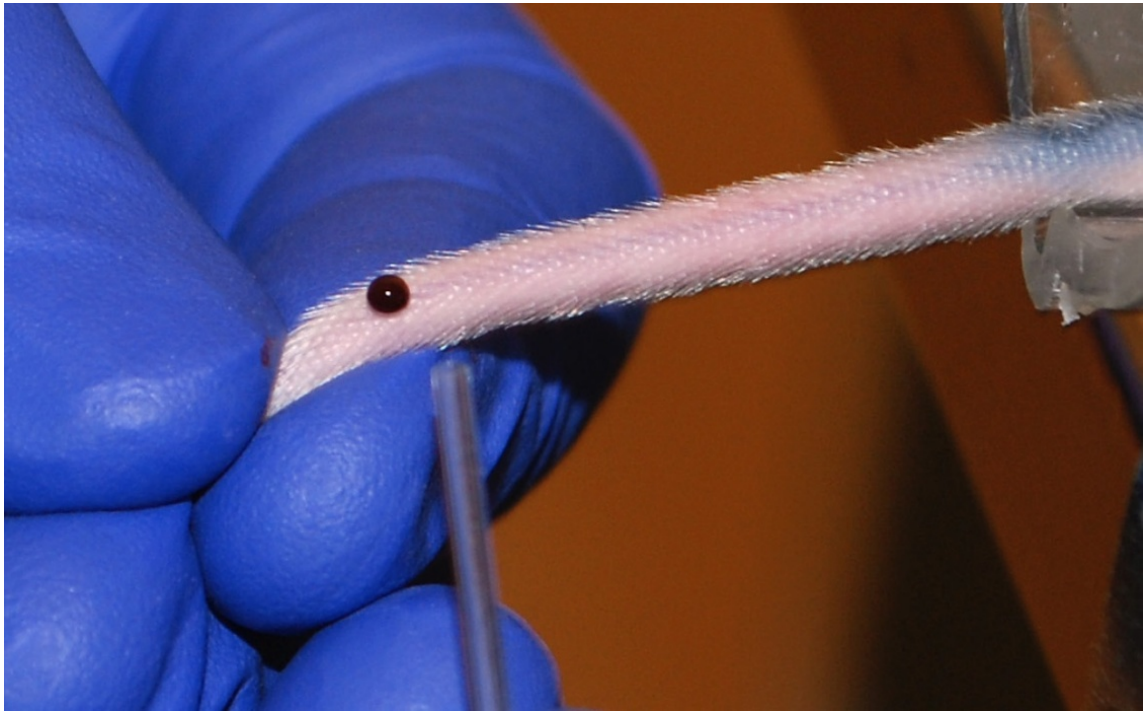
And many others...



Thank you for your attention!

Ove Jonsson
AstraZeneca