# Capillary Microsampling of Liquid Matrices

## Science – Productivity – 3R

*"Better Science – Fewer Animals"* **Ove Jonsson** **Global DMPK, AstraZeneca R&D, Södertälje, Sweden.**

## New opportunities for collection and handling of extremely small volumes of biofluids – From early screen to late clinic

### Plasma (or serum) microsampling

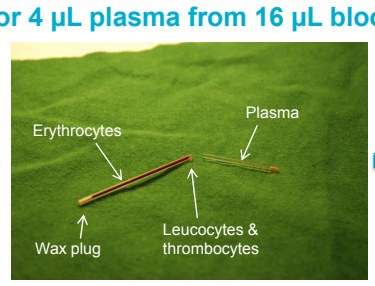
**Typical volumes: 8 µL plasma from 32 µL blood or 4 µL plasma from 16 µL blood**

**Plasma microsampling is the default sampling strategy in rodent toxicology studies at AstraZeneca.**

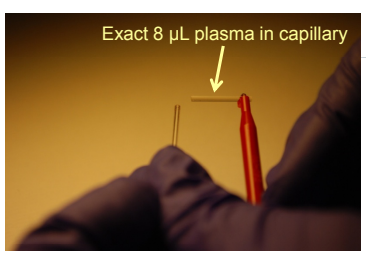
- Collect blood in a $K_2$EDTA haematocrit tube
- Plug with wax
- Place in labelled tube
- Plasma prep. 1500 g for 10 min

(Serum: blood in plain glass tube, plug, store in room temperature 30 min, spin.)

Tail vein sampling from mouse.




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. The capillary is placed in a 1 mL sample tube and frozen pending bioanalysis.

The exact volume delivered in 96-format enables rational, automated sample handling at the bioanalytical lab.

The liquid matrix offers robustness and flexibility.

Freeze

Bioanalysis

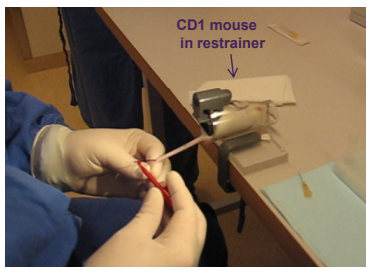
### Blood microsampling

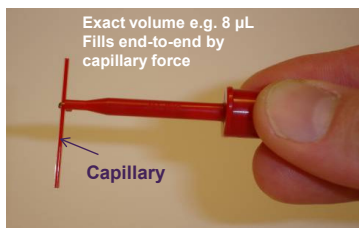
**8 µL blood**

- ✓Unstable compounds
- ✓Fastest procedure
- ✓Minimal blood loss
- ✓Juvenile tox studies

Collect 8 µL blood in $K_2$EDTA capillary (other volumes available as well, e.g. 25 µL)

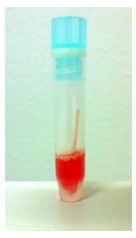
Relative error < 1%
RSD < 1%




In dry vial, or

Mix with stabilizing solution



Freeze

Bioanalysis

From tail to ice in *10 seconds* Including stabilization

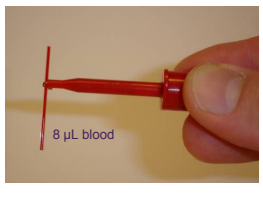
### Blood/plasma distribution

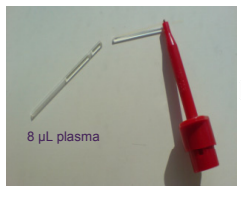
To enable translation of whole blood exposure to plasma exposure, and further to exposure to the unbound drug we need to know

- 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?

Two approaches to determine the B/P-ratio:

1. ***In vitro:*** Incubation of spiked fresh blood
2. ***Ex vivo:*** Analysis of blood and plasma study samples collected from the same animal at the same time point




*Ex vivo:* Enables estimation of B/P-ratio in rare animals such as genetically modified organisms, monkeys or juveniles, where fresh blood for *in vitro* experiments are not readily available.
Can also be used to determine **blood/serum** distribution ratio.

### Rare matrix microsampling

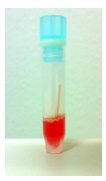
Enables analysis of "new" liquid matrices and gives the possibility to increase the scientific value from animal studies

**Example:**
**Blood/plasma/serum from foetus, juveniles or from genetically modified animals**
**CSF from rodents**
**Synovial fluid**
**Microdialysate**
**Interstitial liquid**
**Sweat or tears…**

How to validate a bioanalytical method in that?!

1. Collect the blank matrix in 8 µL capillary

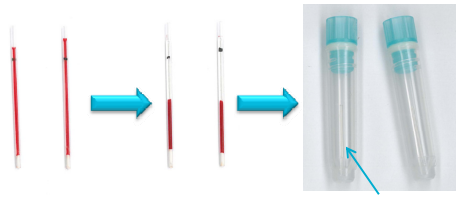
2. Add dilution liquid containing accurate amount of the drug. Mix.



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

### How small is Micro?

2 µL serum from 5 µL blood

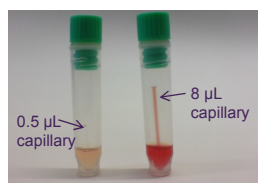


5 µL blood in 6.7 µL plain glass capillary

Store 1 h at room temp. Spin!

2 µL capillary with serum. Can be repeatedly analysed.

0.5 µL blood




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

### Scientific gain

- ✓ Exposure in main study animals
- ✓ More adequate determination of PK/TK parameters
- ✓ Improved PK/PD relationship
- ✓ Exposure in juvenile animals

### Clinical studies

- ✓ Microsampling of capillary blood or plasma from finger or heel prick, both in paediatric studies and for adult patients

### Ethics, 3R (Reduce & Refine)

- ✓ No satellite animals in rat safety studies (15% reduction)
- ✓ No satellites or reduced no. in mouse safety studies (40-50% reduction)
- ✓ Less invasive sampling routes (less impact on vessels, reduced pain?)
- ✓ Less physiological stress due to blood loss
- ✓ No heating of rats, no/reduced heating of mice

### Productivity

- ✓ Fewer animals to buy, house, dose etc
- ✓ Less amount of compound needed per study
- ✓ No plasma preparation, (blood analysis)

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*"Better Science – Fewer Animals"*

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