

# How Big Is A Microsample?

Claire Grant, Michael Spreadborough, Catherine Wilkinson, Emma Flynn, Sarah Kirk  
Alderley Park, Mereside, Alderley Park, Macclesfield, Cheshire SK10 4TG



## Abstract

Toxicokinetics (TK) is an important endpoint in studies as it allows exposure analysis and calculation of safety margins.

Within AstraZeneca rodent studies a 32  $\mu$ l microsample is used as standard for TK profiling. A TK profile usually requires at least 6 sampling timepoints within 24 h. The aim of this study was to decrease the microsample volume collected at each timepoint to 20  $\mu$ l, reducing the overall amount of blood volume taken and the stress involved in the sampling procedure.

The study has shown that a 20  $\mu$ l microsample does cause less stress to animals during sample collection and exposure analysis is comparable to larger sample volumes. This data will lead to a welfare refinement to study design without impacting on scientific interpretation.

## Introduction

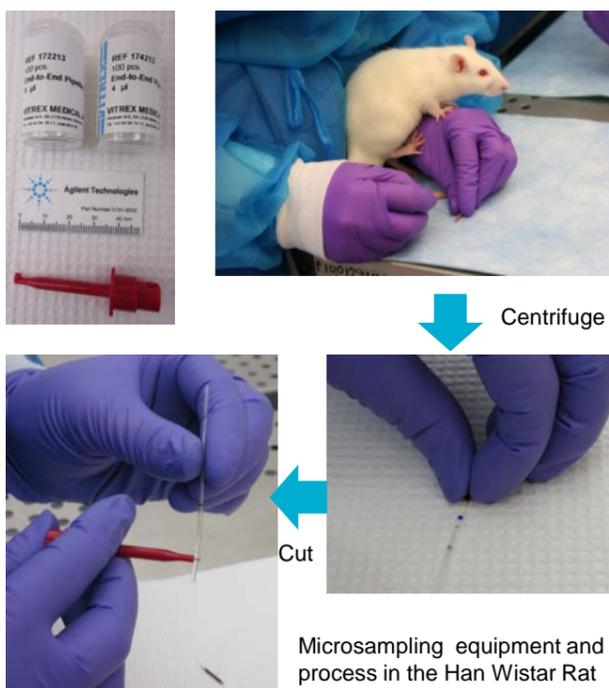
Advances in bioanalytical techniques have opened up the potential to use smaller sample volumes (microsamples) to assess drug exposure. A microsample generally refers to a sample of  $\leq 50\mu$ l. Microsampling is used in rodent studies, such as those in discovery toxicology.

AstraZeneca uses a 32  $\mu$ l sampling size as standard. The aim of this study was to show that volume can be reduced to 20  $\mu$ l.

Animals were dosed with Clonidine, a compound known to reduce heart rate and blood flow. This assessed the sampling procedure in the most difficult circumstances.

## Methods

Male Han Wistar rats were sampled collecting 32 or 20  $\mu$ l microsamples at multiple time points post-dose. Rats (n=8/gp) were dosed orally with vehicle or 0.1 mg/kg Clonidine in a cross over study design. Microsampling was performed at 0.5, 1, 2, 4, 6 and 22 h post-dose.



Animals having 32  $\mu$ l samples were placed in warming chambers for approximately 1 min. 32  $\mu$ l or 20  $\mu$ l blood samples were collected into a 75  $\mu$ l haematocrit capillary tube. Plasma was prepared by centrifugation at 1500g for 10 min at 4°C. The capillary tubes were then cut and plasma harvested using 8  $\mu$ l capillaries for 32  $\mu$ l samples or 4  $\mu$ l capillaries for 20  $\mu$ l samples and frozen at -20°C. During microsampling animals were minimally restrained by hand and blood was collected from a tail prick site near to, or at the tip of, the tail.

Animals were surgically prepared with telemetry devices (DSI® PhysioTel) and heart rate and blood pressure was recorded for 24 h. Data was analysed (an average of 5 min) before sampling (-20 and -5 min) and after sampling (5, 10, 15, 30, 60 min) as an indicator of the stress experienced by the animals and to assess recovery time.

Plasma samples were analysed for Clonidine concentrations. Following microsample dilution and protein precipitation samples were injected onto a gradient UHPLC system with tandem mass spectrometric detection.

## Results

Complete samples were collected at all planned timepoints, except in one animal at 24 h post-dose.

The plasma volume separated from the 20  $\mu$ l microsamples was assessed and at every sampling timepoint 8  $\mu$ l of plasma was collected. This indicates that an accurate 4  $\mu$ l plasma sample could be regularly achievable when collecting a 20  $\mu$ l microsample in future studies. Sampling time was quicker, tails required less milking and animals showed less distress (vocalisation and movements) during the 20  $\mu$ l TK sampling profile compared with the 32  $\mu$ l sampling.

Clonidine at 0.1 mg/kg caused the expected decrease in heart rate and blood pressure.

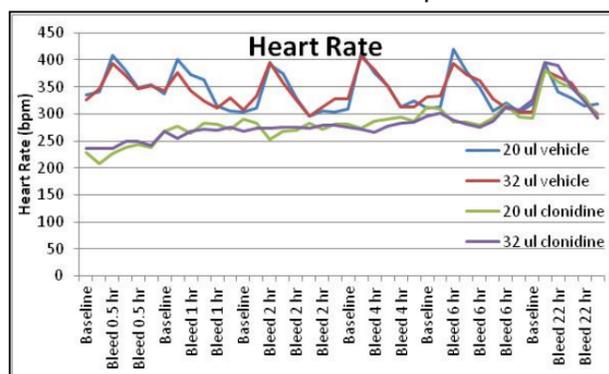


Figure 1 Graph illustrating the effects of 0.1 mg/kg Clonidine on heart rate in Han Wistar rats

Blood pressure analysis shows the pressure in animals undergoing 20  $\mu$ l microsample TK profiles was lower than in the 32  $\mu$ l microsample TK profiles, indicating the animals were less stressed.

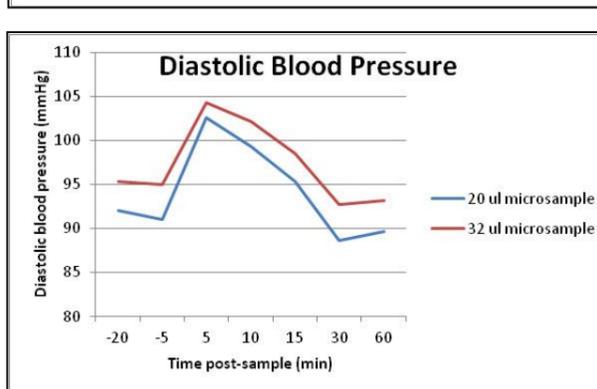
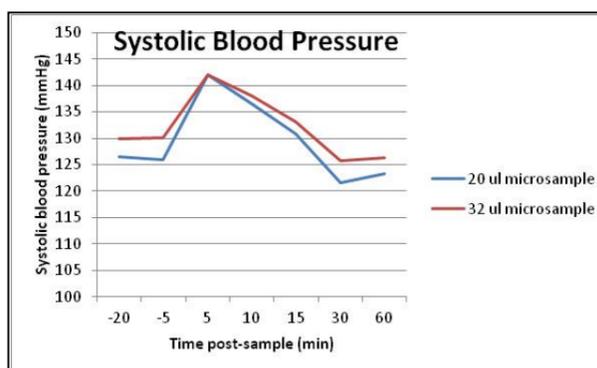


Figure 2 Graph showing impact of 20 or 32  $\mu$ l microsamples on systolic and diastolic blood pressure in vehicle rats.

Mean Clonidine exposure, following oral administration of 0.1 mg/kg Clonidine, was comparable across the sampling time-course. The TK profiles were unaffected by the sample size. Data was within the normal variability observed for this dataset.

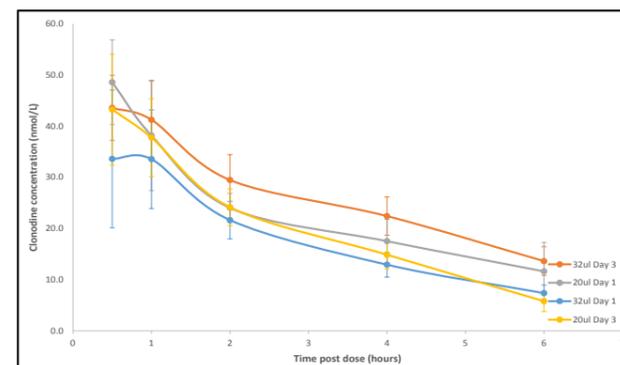


Figure 3 Graph showing Clonidine exposure data using 20 or 32  $\mu$ l sampling methods

## Conclusions

- AstraZeneca Discovery Toxicology studies will be able to use a TK blood microsample size of 20  $\mu$ l in the future.
- For a 6 sample TK profile 120  $\mu$ l of blood will be collected using 20  $\mu$ l microsamples, this is a reduction of 72  $\mu$ l per TK profile.
- This is a refinement to current standard practices as the overall blood volume taken is removed and method does not require animal warming.
- The TK exposure data is comparable to larger sampling sizes.
- More data can be obtained from the same animal, as blood could be taken for a more comprehensive TK profile, or can be used for other assessments such as haematology or clinical chemistry.
- This will lead to a large impact for mouse studies. Mice have a much smaller circulating blood volume. Only 300  $\mu$ l of blood can be collected in a 28 day period in a 25 g mouse, therefore using 20  $\mu$ l microsamples 2 TK profiles can be collected.
- In future studies a decision will be made depending on study design and in conjugation with the bioanalysis group on the microsampling volume to be used.

## Other Activities

Within the last year there have been other investigations into the use of microsampling in studies including:

- Microsampling in rat telemetry studies
- Impact of microsampling on haematology parameters
- Microsampling from subsections of groups

Activities currently being investigated including:

- Microsampling with free roaming intravenous infusions
- Microsampling from main group mice in studies investigating compound effects on reproduction

## Acknowledgements

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