

# Investigation of sample dilution on Accusampler Standard used for pharmacokinetic studies

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## Background and aims

Automated blood sampling (ABS) from laboratory animals eliminates resource demanding manual sampling and reduces stress caused by frequent handling, an important part of 3R's as well as an important contribution to high quality in vivo results. We use ABS with Accusampler Standard (fig.1) in pharmacokinetic studies in rats with sampling up to 96 hour post dosing. Some studies have special requirements regarding stabilisation of blood immediately after sampling. In this case it can be added to test tubes and the Accusampler method *SampleStandardMix*, can handle mixing of blood and stabilising agent. It is well known that sampling with Accusampler Standard gives some dilution of blood samples due to laminar flow (fig.2). The *SampleStandardMix* method is expected to give increased dilution because of repeated blood flow in tubing. We have evaluated the sample dilutions of the *SampleStandard* and *SampleStandardMix* methods compared to manually sampling to obtain values used for dilution correction of sample results.

Figure 1. Accusampler laboratory at Novo Nordisk



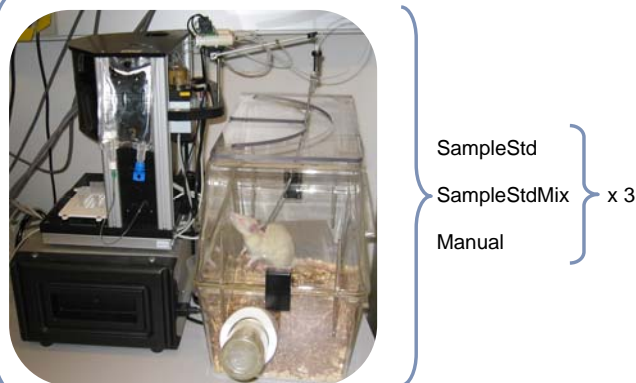
Figure 2. Laminar flow



## Study design and method

SD Rats, male, provided with permanent artery catheter 2 weeks prior to experiment, were set up in Accusampler Std (n=12). Sampling with *SampleStandard* (200µl blood) and *Sample-StandardMix* (200µl blood + 10µl anticoagulans) method together with manual sampling (200µl blood) was repeated 3 times. Manual sampling was evaluated with 100µl and 500µl waste volumen. Plasma was separated and analysed for albumine.

Figure 3. Design



## Results

We observed no difference between manual sampling with 100µl and 500µl waste volumen (paired t-test non-significant). We found 89.3% recovery of p-albumine in samples from *SampleStd* method compared to manual sampling with variation coefficient 2.5% between 12 Accusamplers (fig.4). With *SampleStdMix* method we found 79.3% recovery of p-albumine with variation coefficient 2.6% (fig.5) compared to manual sampling.

Figure 4. SampleStd Method

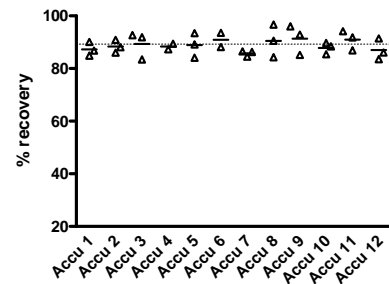


Figure 5. SampleStdMix Method

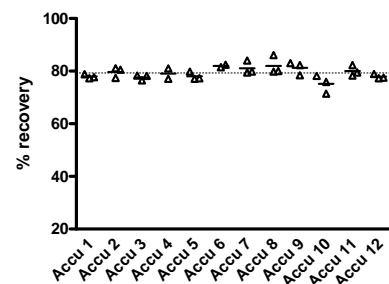


Figure 6. Results, n=12, mean ± SEM

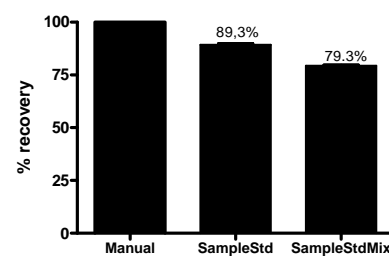


Figure 7. Sample dilution estimates

Sample volume	Catheter (Yellow line)	Waste volume	Empiric dilution % Dilab method	Empiric dilution % NN method
100 µl	293	30	31	?
100 µl	196	30	22.3	?
200 µl	196	30	14.5	?
200 µl	196	90	11.3	?
200 µl	293	30	?	12.0
200 µl + 10µl mix	293	30	?	26.1

## Conclusion

We found an increased dilution of samples by *Sample-StandardMix* method compared to *SampleStandard* method. Recovery was 89.3% for the *SampleStandard* method and 79.3% for the *SampleStandardMix* method in our Accu-sampler setup with sample blood volumes at 200µl blood ± 10µl stabilising agent.