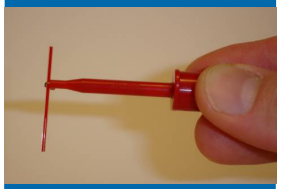

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One Mouse, One PK …. The Magic of Capillary Microsampling

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Head of Bioanalytical Sciences, Cambridge

European Bioanalysis Forum Open Meeting, Barcelona, 2014

Overview

1. Capillary Microsampling (CMS)
2. 3Rs (Reduce, Refine, Replace)
3. Pharmacokinetic Bioanalysis on Gyrolab™
4. Summary

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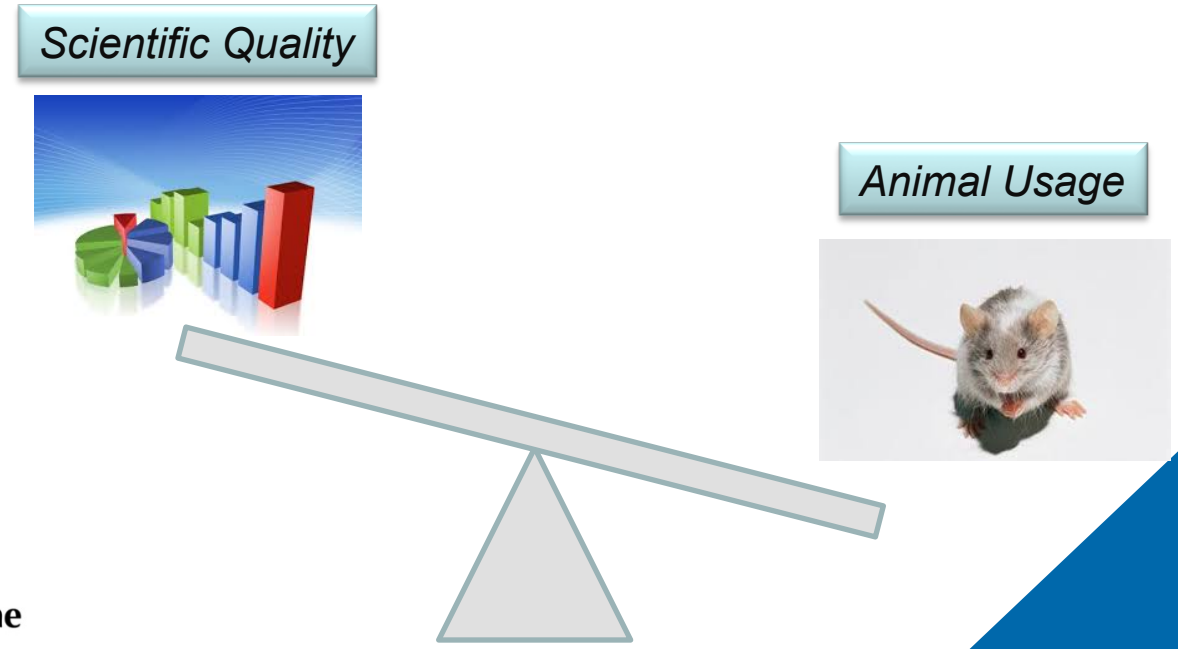
Blood Sampling is Often a Limiting Factor

- Blood sampling volumes can challenging in the preclinical setting, especially for rodent species
  - Total blood volume of 15% in any 28 day period
- In pharmacology studies, group sizes can be large to accommodate pharmacokinetic (PK) sampling time points as not all samples can be taken from one animal
- Toxicological effects cannot be correlated to exposure data in the same animal



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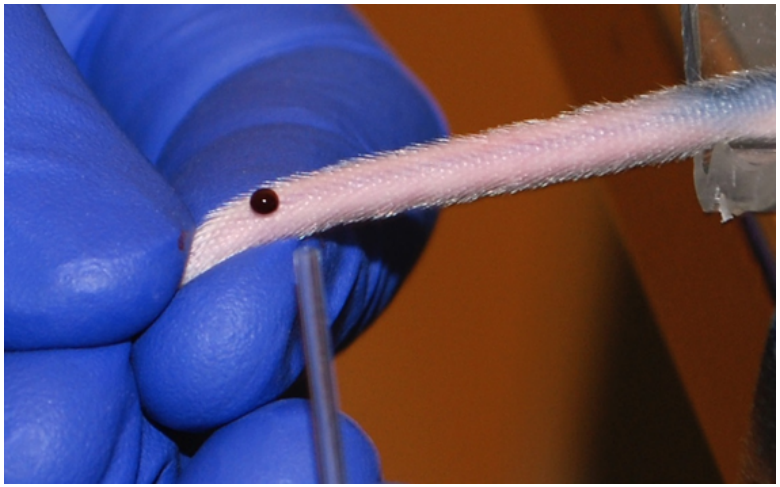
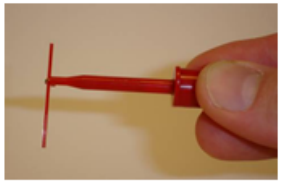
Capillary Microsampling (CMS): A Viable Alternative



… is a flexible technique for collection and handling of small exact volumes of liquid matrices, such as blood, plasma or serum.



Fastest sampling method and smallest volume
(Without surgery)

From tail to ice in 20-30 seconds

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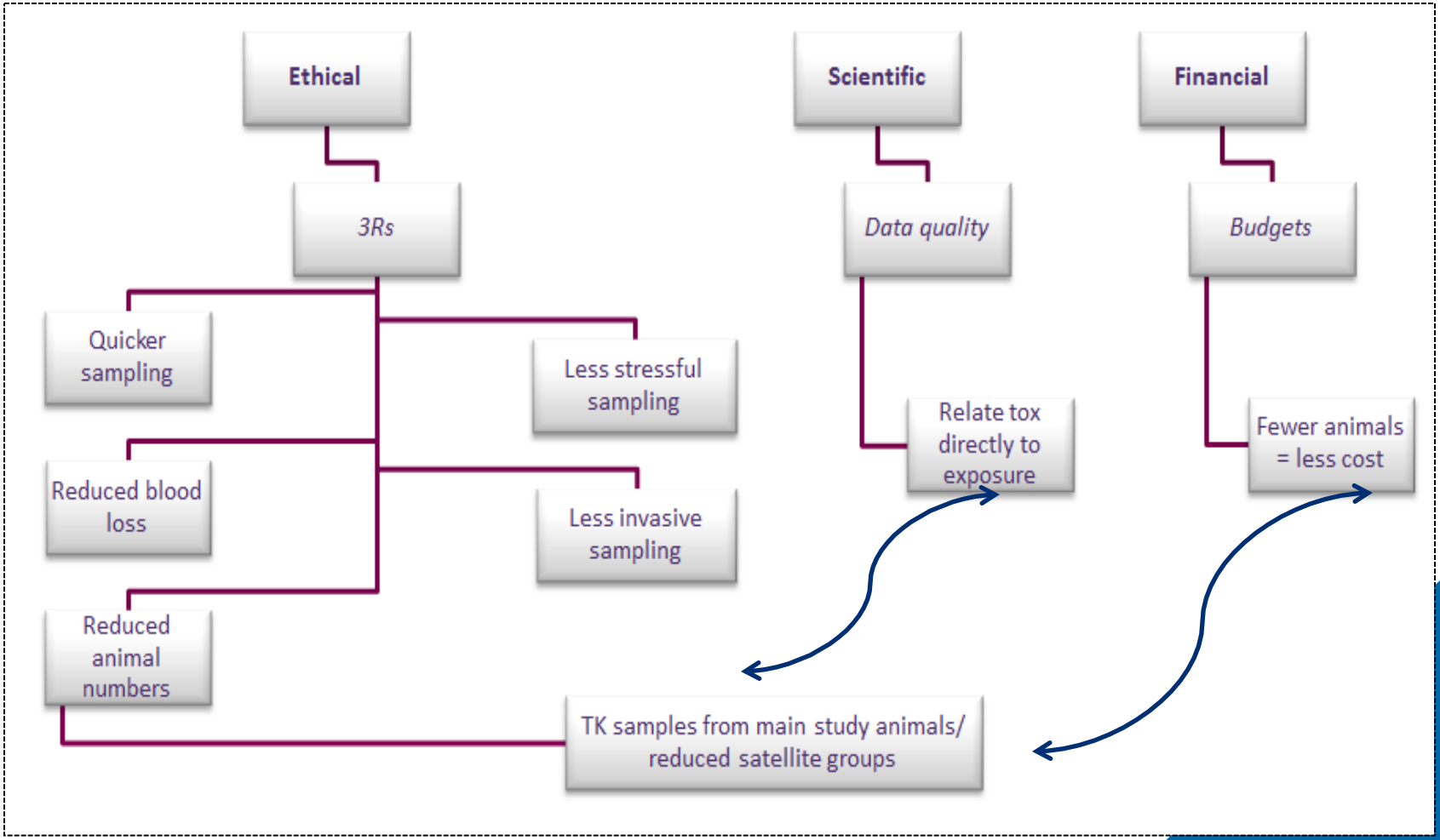
CMS Affords Benefits Beyond Ethical Considerations



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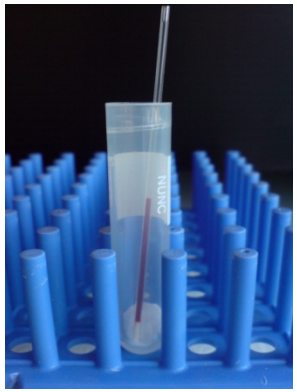
Step by Step CMS

Sampling from animal (tail vain) using plain glass or EDTA coated capillary (20 µL)



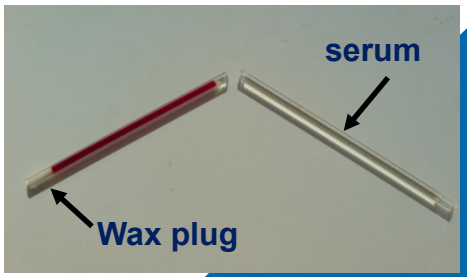
Prepare Serum/Plasma

Store sample in the freezer

CMS

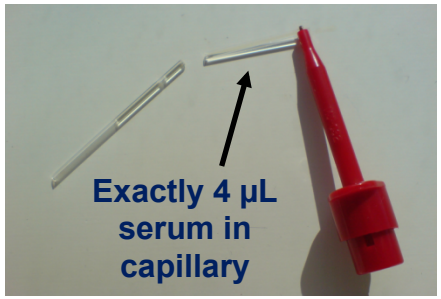
<u>Typical volumes</u>

8-10 µL serum/plasma from ~16-20 µL blood



Centrifuge glass capillary to seperate Serum/Plasma

Transfer sample into a fixed volume small plain glass capillary



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CMS was Combined with Gyrolab™

- Established technology for PK assays
- Small sample volume, ideal for CMS
- Automation
- Less manual pipetting steps
- Reduction in operator time
- Quicker assay development
- Dynamic range
- High throughput

Test established assay with CMS and Non-CMS
In combination with the Gyrolab™

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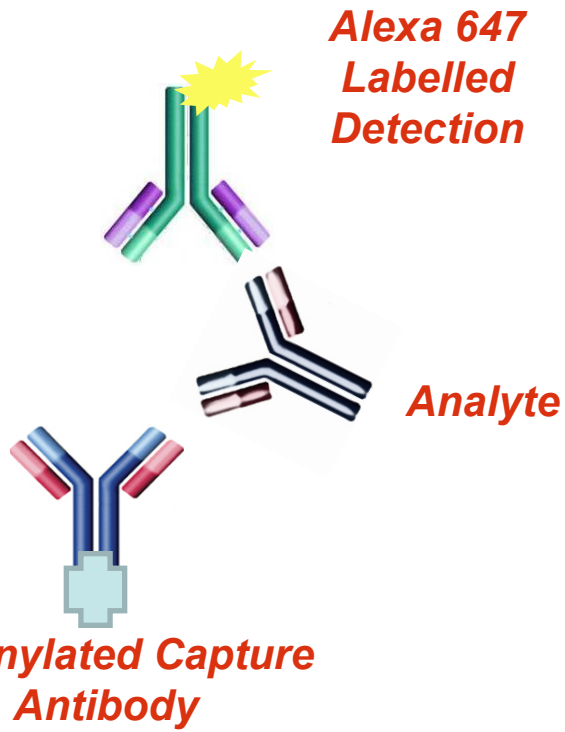
"Universal" PK Assay Allows One Format for Detection of Monoclonal Antibodies

- PK assay utilises Universal reagents to detect human immunoglobulin G (IgG) in any animal matrix
- Assay can be used for multiple analytes without the need of changing the antibody pair
- PK assay can be used for pre clinical studies
- Method available for multisite, multigroup and multiarea use (on Gyrolab™ & MSD® platforms)

Schematic of Assay Format for the Gyrolab™ platform



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Acceptable Assay Performance

Parameter	Detail
Assay Type	PK Assay: Quantitative determination of Antibody X
Matrix	Mouse Serum
Platform	Gyrolab™
Dynamic Range	50,000 – 4.29 ng/mL
MRD	1 in 10
ULOQ	50,000 ng/mL
LLOQ	68.6 ng/mL
Accuracy	≤ 20% RE
Precision	≤ 20% CV

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Study Design

Non-CMS

Traditional design

♂

To obtain a complete profile to match CMS sampling 20 mice will be required

CMS

New design

♂

Complete profile with 9 time points using 4 animals
(<15% total blood volume in 28 days)

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

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Greater Sampling in a Single Animal with CMS

Group	Sampling Time Point (hrs)								
	1	5	24	48	72	96 (Terminal for Non-CMS)	192	240	336 (Terminal for Non-CMS)
Non-CMS			x			x			
CMS	x	x	x	x	x	x	x	x	x

- Number of mice: 4 in each group
- Dose: 10 mg/kg
- Route of administration: Intravenous
- Mice: Male C57BL/6
- Matrix Serum

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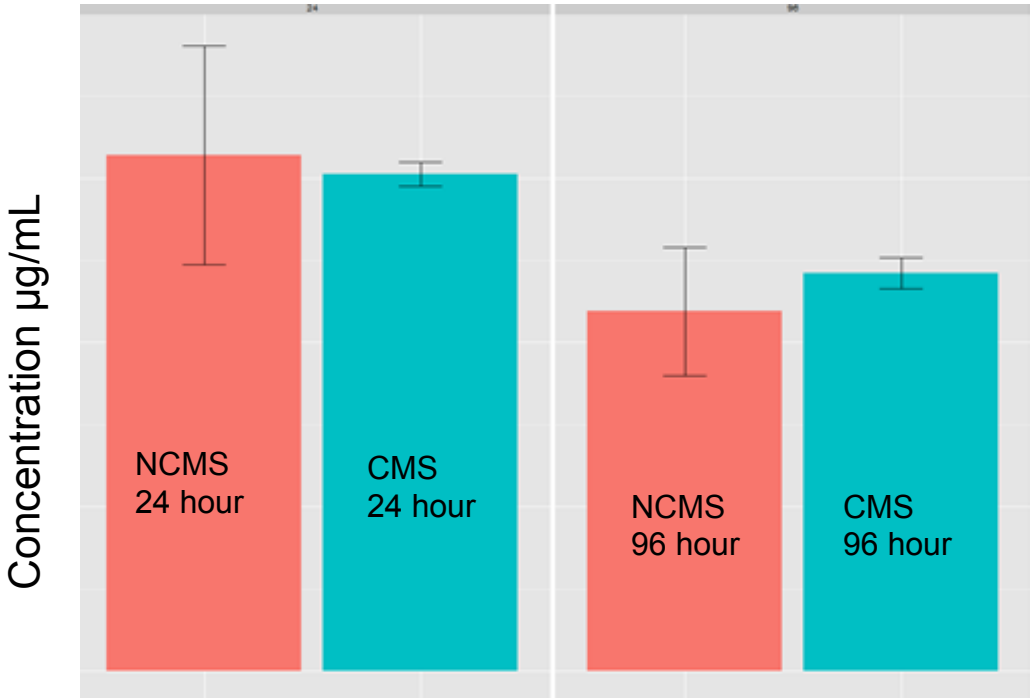
CMS Yields Comparable Data to Non-CMS



Groups CMS & NCMS at 24 and 96 hours

Error bars comparing data obtained from CMS vs Non-CMS

Group	Time	MeanResponse	StdError
NCMS	24	94.200	19.917873
NCMS	96	65.650	11.751135
CMS	24	90.725	2.254763
CMS	96	72.600	2.799107

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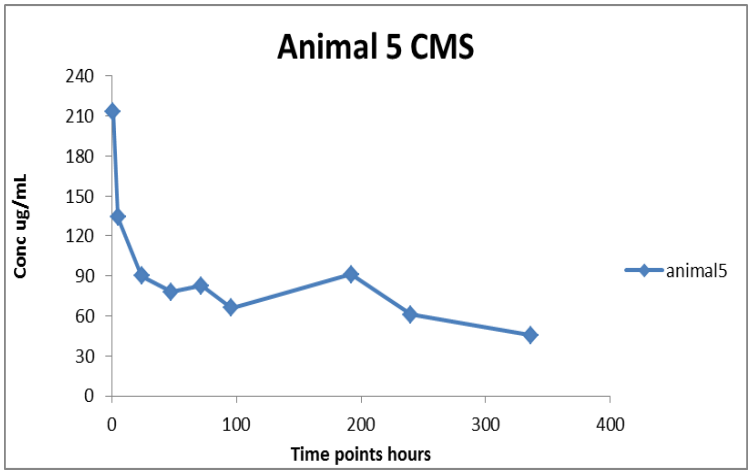
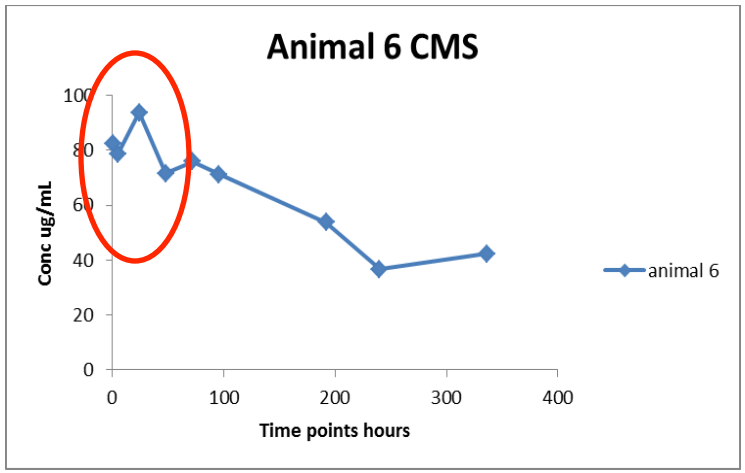
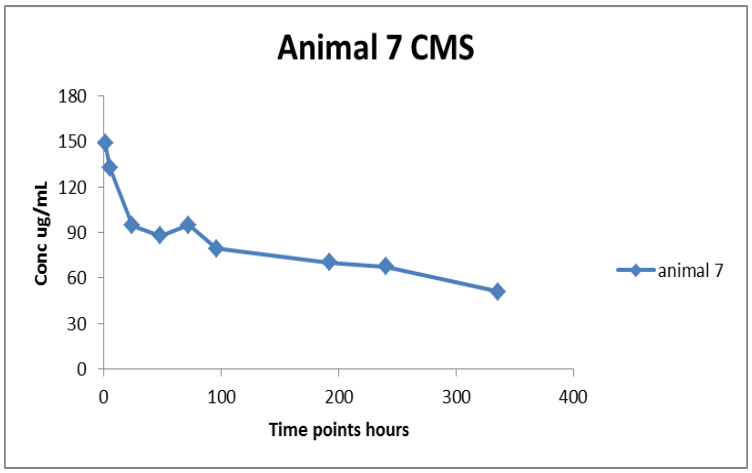
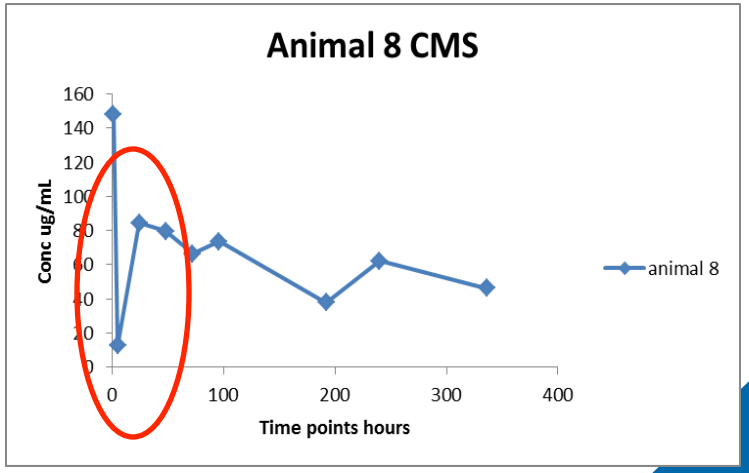
PK Profiles for Individual Animals using CMS









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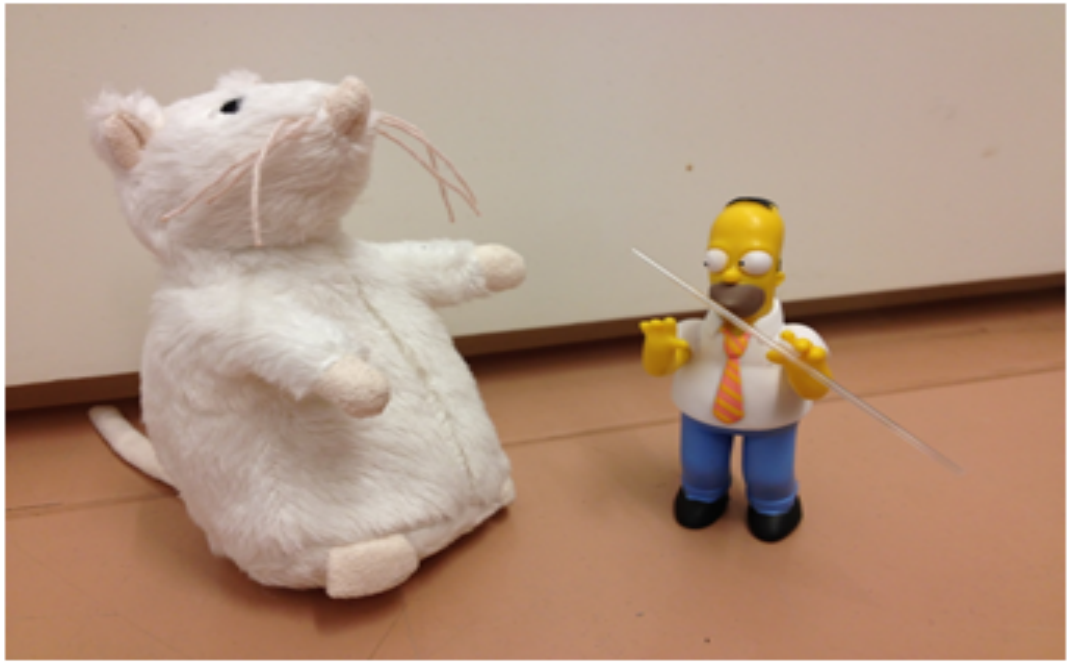
Once Mastered CMS is a Quick, Low Tech and Simple Technique



However, proper training of the in-life personnel and understanding the importance of the exact volume is crucial.

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Summary

- CMS can drastically **reduce** animal numbers
- Sampling from main study animals rather than satellite groups can strengthen data within a single animal
- The blood sampling technique is **refined**
- Possibility of generating both PK and PD data from a single sample by combining CMS with Gyrolab™

Caveats

- Limited by the use of the Gyrolab™
- Higher chance for errors when sample volume is so small; training of personnel in animal facility is key
- Should back up samples be necessary then number of time points collected in lower species may be reduced

Acknowledgements

- MedImmune: Sufyan Maqbool
- AZ: Ove Jonsson (Sweden), Michael Spreadborough (UK), Christopher Smith (UK)
- Gyros

QUESTIONS