Pushing the Boundaries of Microsampling

Realising and Understanding the Full Potential

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The AstraZeneca Journey So Far....

2007 - 2011

Work pioneered by Ove Jonsson

Blood microsampling in routine use within Discovery functions

Transitioned from blood to plasma microsampling for toxicology evaluations

2012

Definitive in-vivo data generated to support move to main study animals in GLP rodent toxicology studies

2013

Safety and DMPK leadership teams approve capillary microsampling in main study animals for use in regulatory facing non-GLP rodent toxicology studies

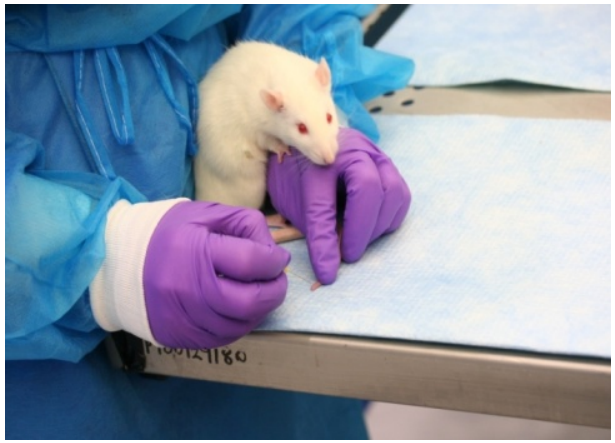
2014

TK sampling in rodents from main study animals for GLP and non-GLP studies

Recap on Benefits of Microsampling

Macrosampling

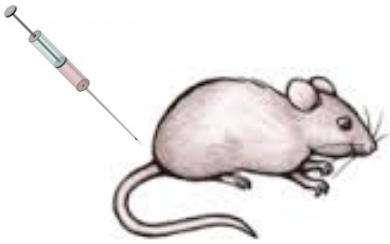
Microsampling

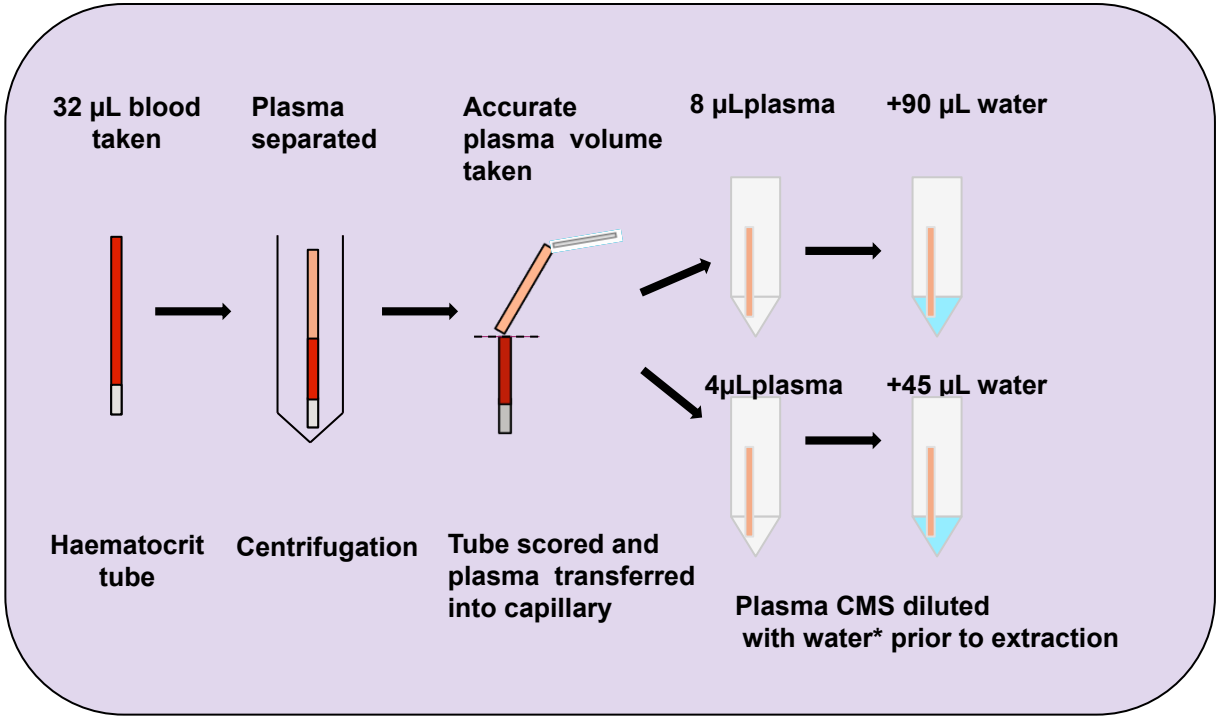


- Causes less distress to animals during sampling
- Only one technician needed for blood sampling
- Lower costs - Fewer animal required per study & less test item used
- More TK sampling time-points feasible
- TK samples taken from main group animals - improved data quality

AZ approach to capillary microsampling

Sample collection and processing





* Water used as default diluent, but stabilisation agents can be added if required

So how far can we push?

Ove Jonsson
AstraZeneca

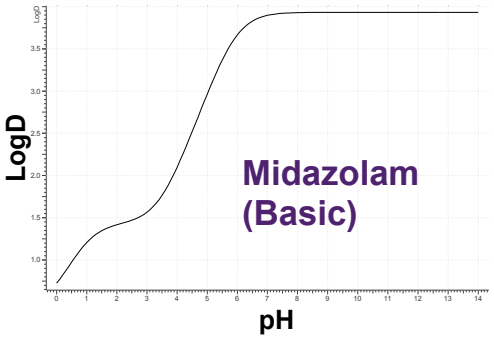
- Smaller blood volumes (< 32µL)?
- Smaller plasma volumes (< 8µL)?
- Different blood processing methods?

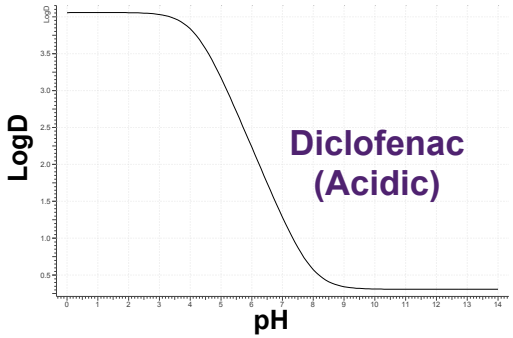
Parameters to evaluate....

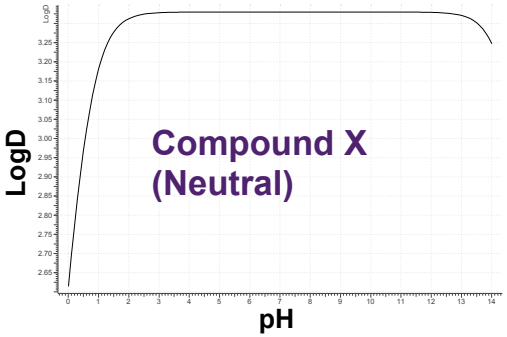
Blood volume	Sample Homogeneity	Plasma Volume	Haematocrit Level	Blood Processing
• What impact does reducing blood volume have on accuracy?	• Is analyte distribution homogeneous throughout plasma in capillary?	• Does plasma volume have an impact on quantification?	• How does haematocrit level affect quantification in a plasma microsample?	• Are alternative blood processing methods comparable to plasma microsampling

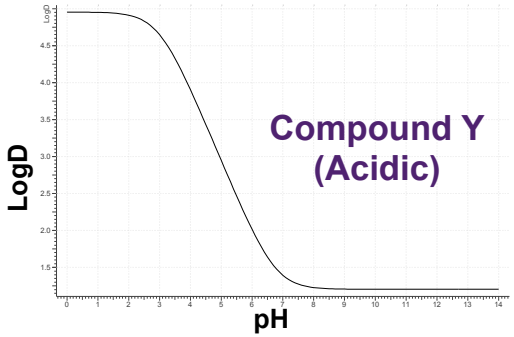
Pushing the boundaries – Evaluation

Analytes Used





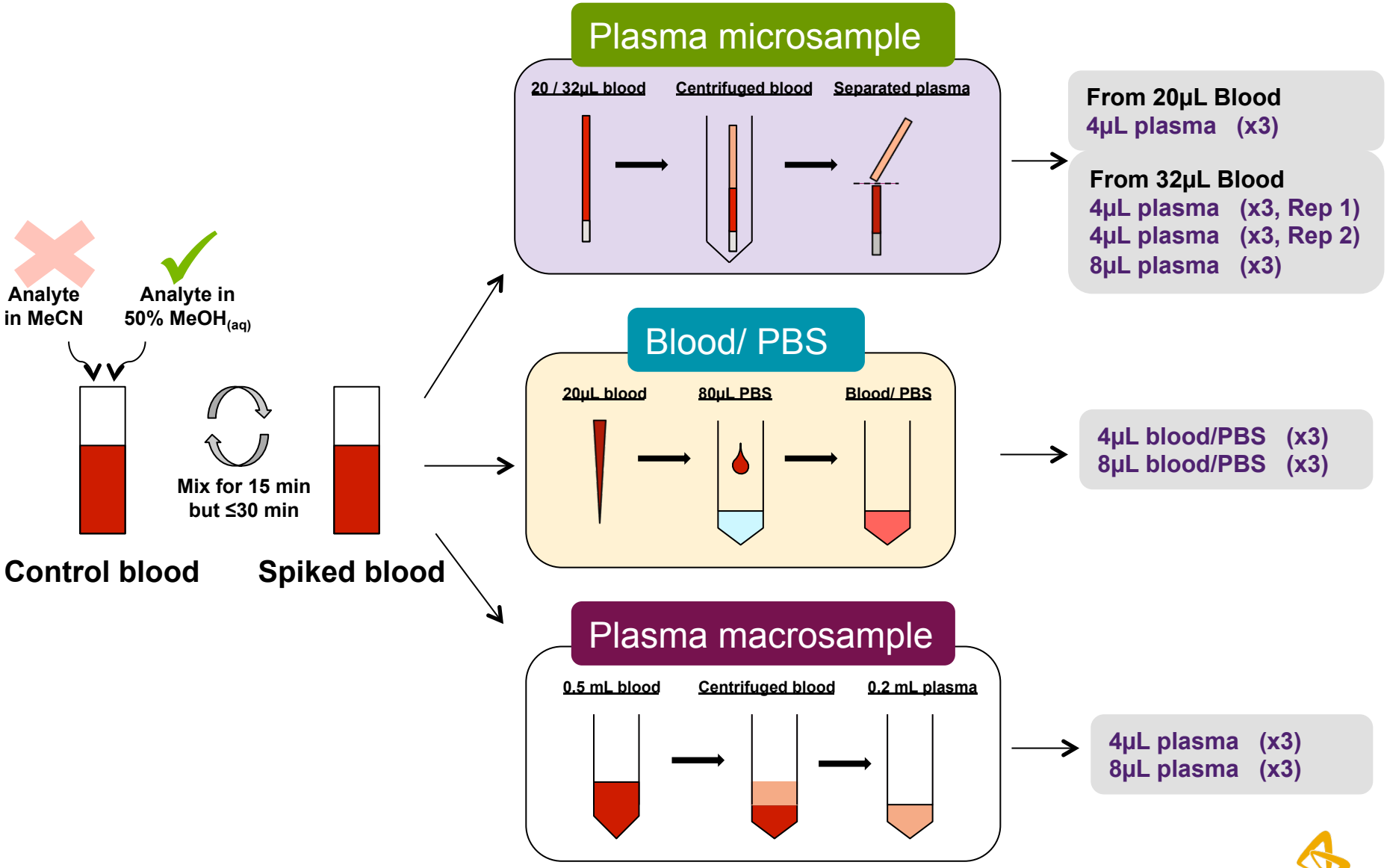




Tests carried out

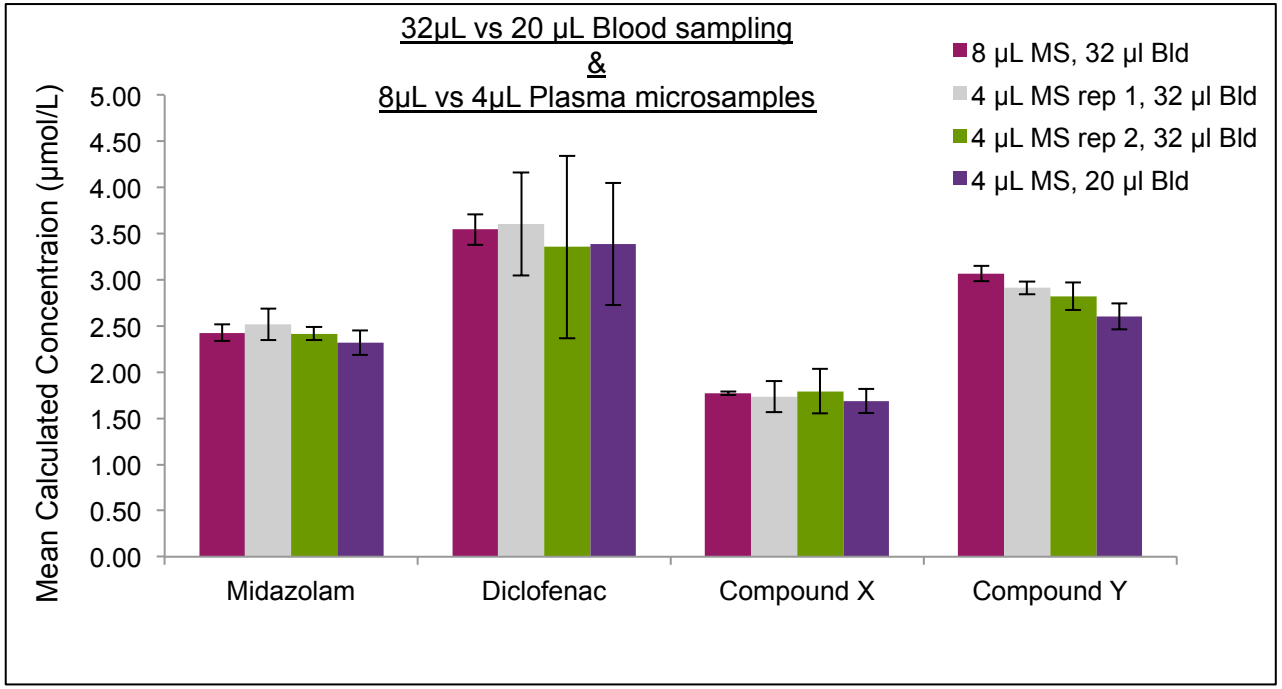
- 20 µL vs 32 µL Blood
- 2x 4 µL vs 8 µL plasma
- Microsamples vs macrosamples
- 20, 40, 50 & 60% Haematocrit
- Blood/PBS vs plasma

Sample preparation methods used



Outcome of Evaluation...

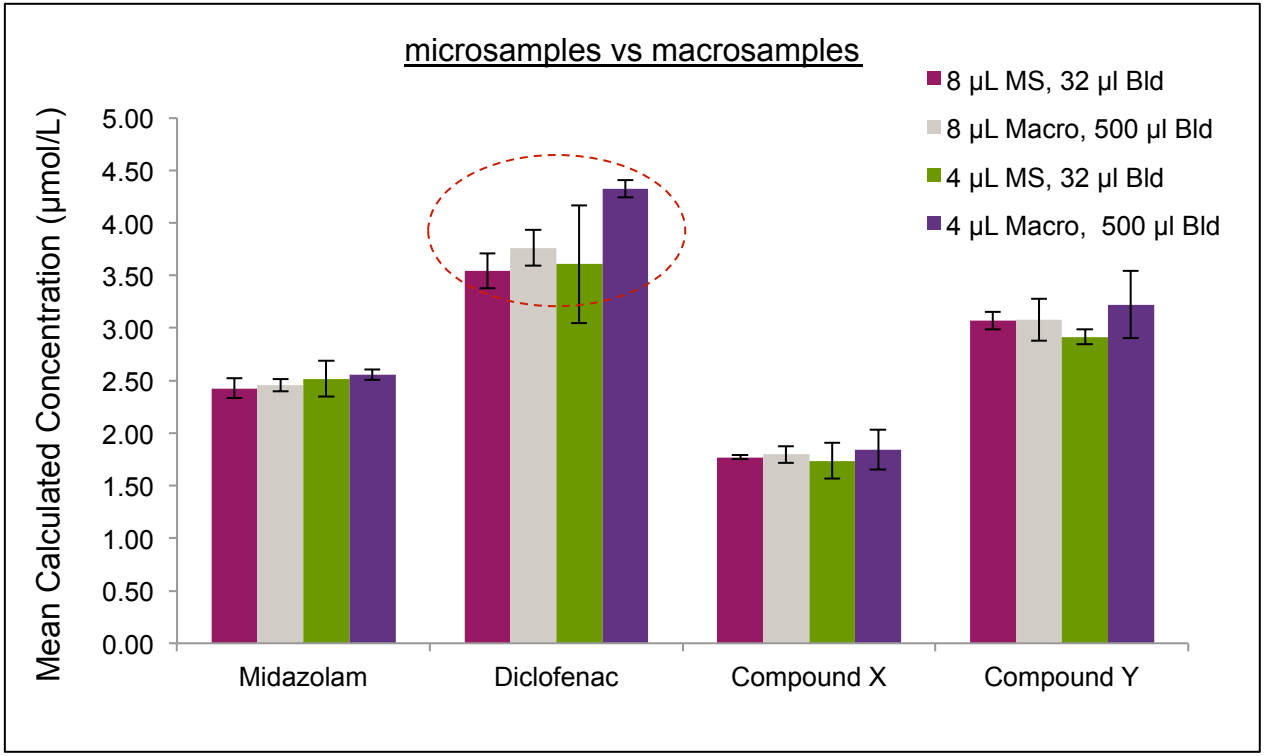
Blood Volume & Sample Homogeneity



- **Does blood volume have an impact on accuracy?**
  None observed; a 20µL vs 32µL blood sample are equivalent

- **Is analyte distribution homogeneous throughout plasma in capillary?**
  Yes; 4µL and 8µL plasma microsamples are equivalent

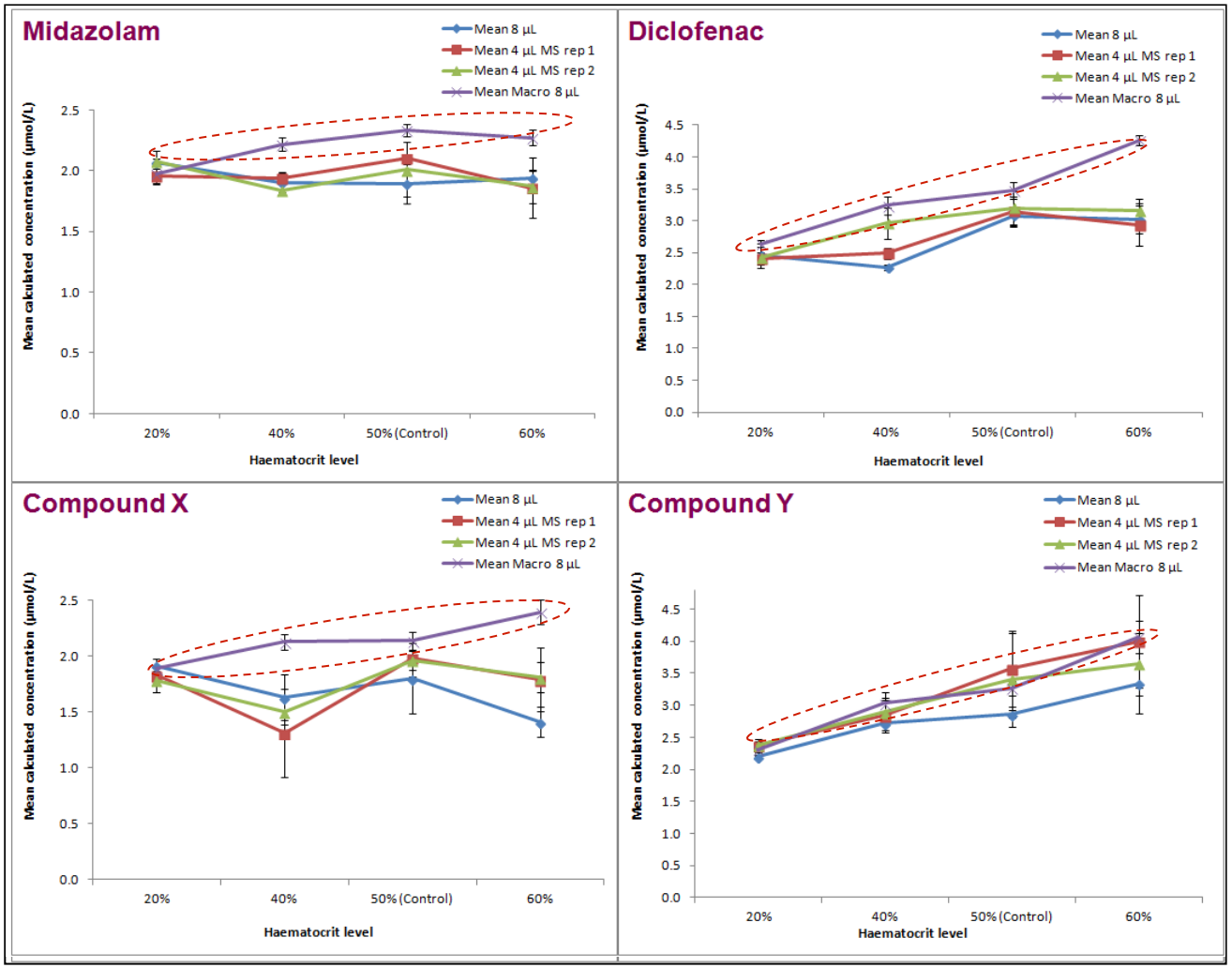
Plasma volume



- **Does plasma volume have an impact on quantification?**
  None observed; With the exception of Diclofenac, a micro and macrosample appear to be equivalent

Haematocrit Level

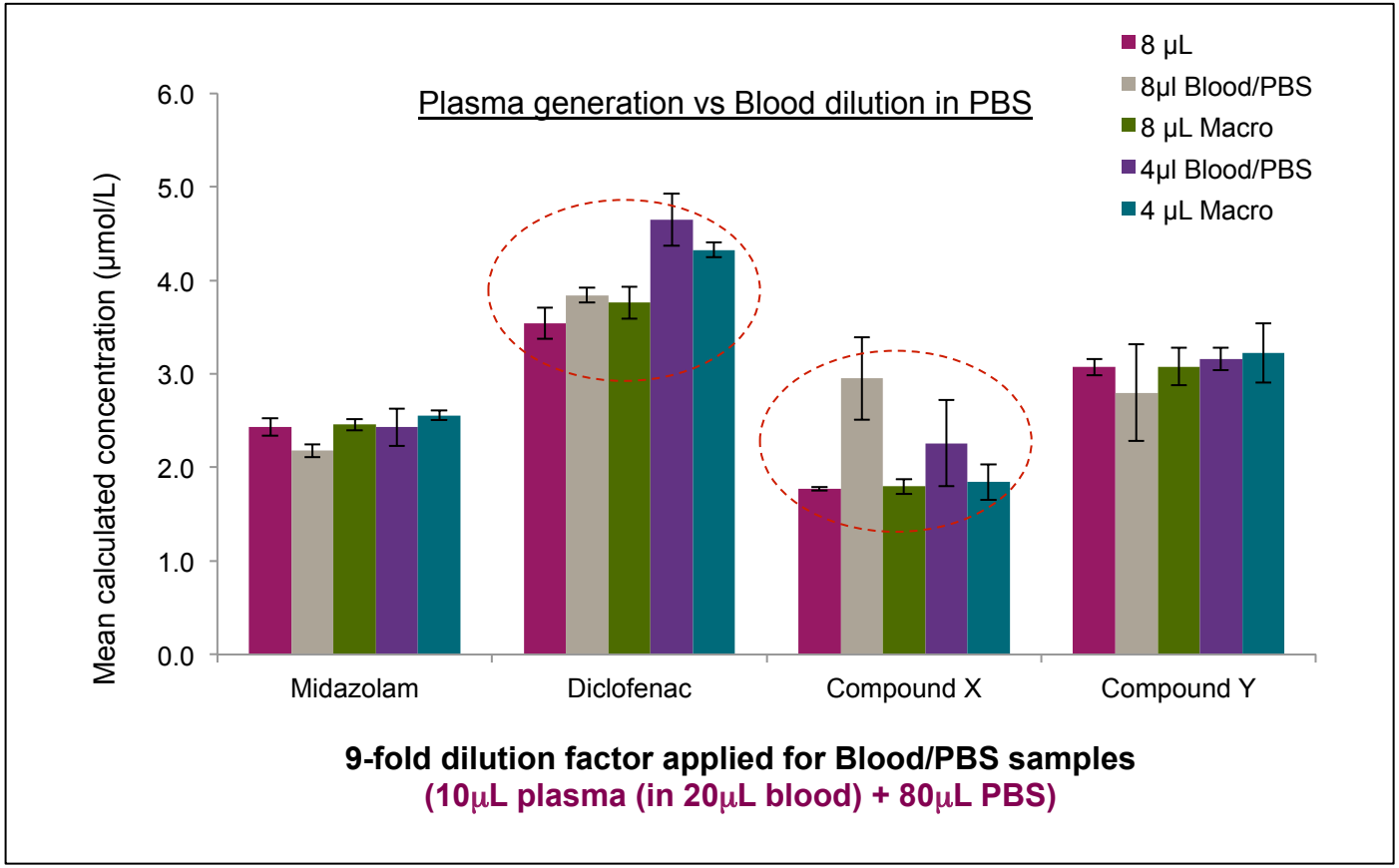
How does haematocrit level affect quantification in a plasma microsample?



Similar trends observed between micro & macro samples

Differences between haematocrit levels are more analyte-specific rather than technique related

Blood Processing



- **Are alternative blood processing methods comparable to plasma microsampling?**

Yes; there is potential to use blood dilution in place of plasma separation

Conclusions

Blood volume	Sample Homogeneity	Plasma Volume	Haematocrit Level	Blood Processing
• Use of a 20μL blood sample to generate 4μL plasma is viable (assay sensitivity dependent)	• Is not affected by using 4 or 8μL capillary microsamples as opposed to traditional macrosampes	• No significant differences in quantification between micro and macrosamles	• Similar trends seen between micro and macro plasma samples. Differences between haematocrit levels not sampling technique related	• There is potential to use PBS for dilution of a blood microvolume, but analyte blood partitioning and haematocrit level to be considered

Going forward.....

- **Perhaps carry out further tests on blood/ PBS and investigate the impact of haematocrit level as well as analyte blood/plasma partitioning properties on reproducibility?**

Acknowledgments

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Thank you for listening

Any questions?

Confidentiality Notice