



# **A Hybrid LBA-QPCR Technique (Proseek<sup>®</sup>) for Improved Assay Sensitivity**

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# Outline

- The need for microsampling
- Microsampling technique
- Proseek<sup>®</sup>

# Problems Associated with Sampling

- Require relatively high volumes of blood - limits on what is drawn
- This restricts (particularly in rodents) the endpoints and scientific investigation parameters such as:
  - biomarkers, clinical pathology, toxicokinetics, pharmacodynamics, and immunogenicity
- Use of satellite animals increases animal use by approximately a 2-fold factor - especially in mice

# Sampling Solutions

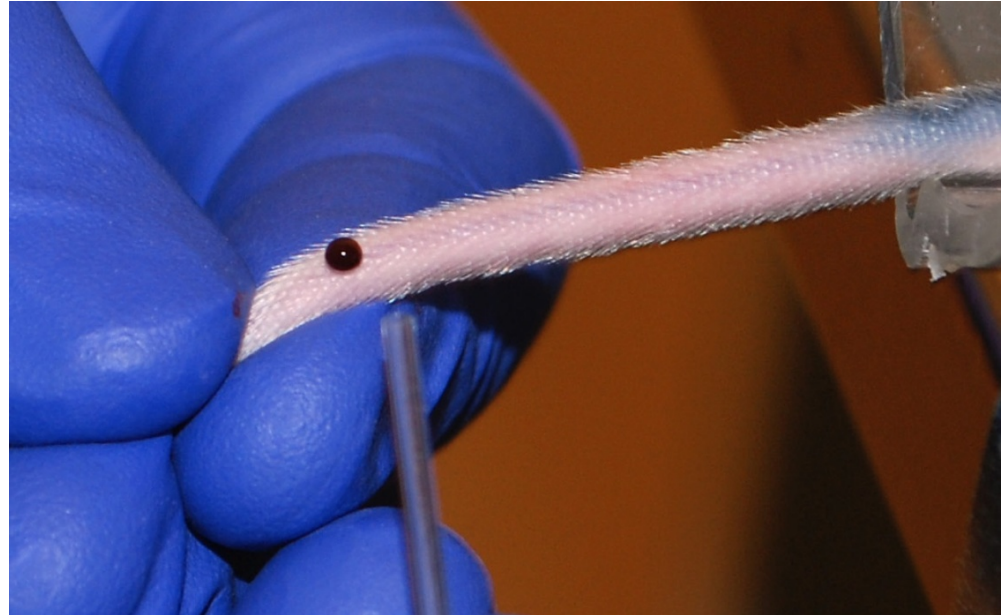
- Dried Blood Spot (DBS) – drawbacks:
  - There are haematocrit effects
  - May not be good for all drugs and biologics
  - Resistance to moving away from plasma/serum
- Capillary Microsampling – advantages:
  - Produces plasma or serum so no change in matrix, hence no regulatory hurdles to overcome
  - Minimum training required
  - Validation of existing techniques need only focus on reduced volume

# Capillary Microsampling

- Technique developed by Ove Jonsson and colleagues at AstraZeneca, Sodertalje, Sweden, during past 5 years
- Project initiated at Charles River in 2011 to bring together the requirements of toxicology to reduce animal usage and bioanalysis to work with microsamples

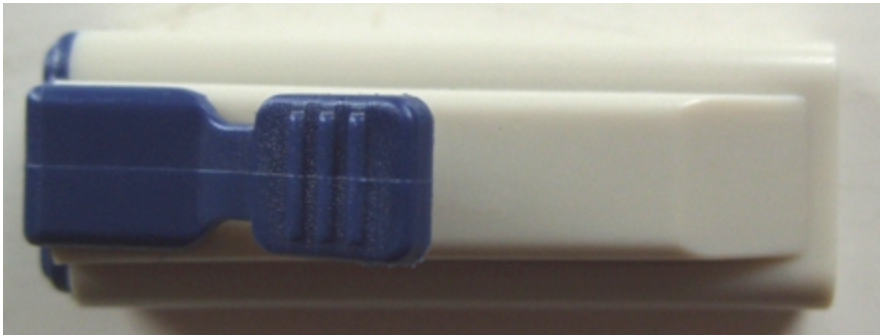
# Microsample Collection - Preclinical

- Use haematocrit/capillary tube
- With/without anticoagulant
  
- Collection from a rodent tail prick: the blood is drawn into the tube by capillary action

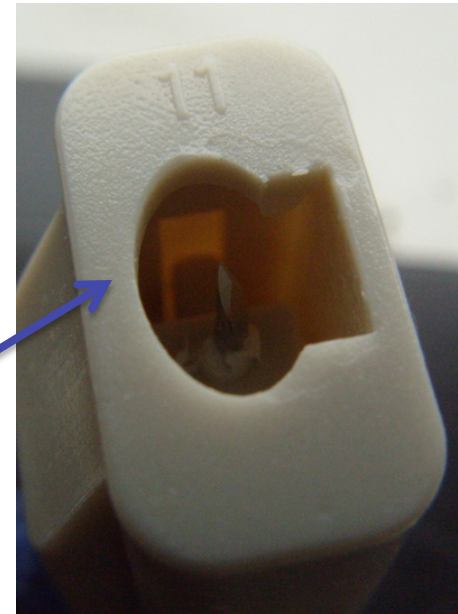


# Microsample Collection - Clinical

- Finger prick using a spring loaded lancet
- Collect sample into haematocrit/capillary tube

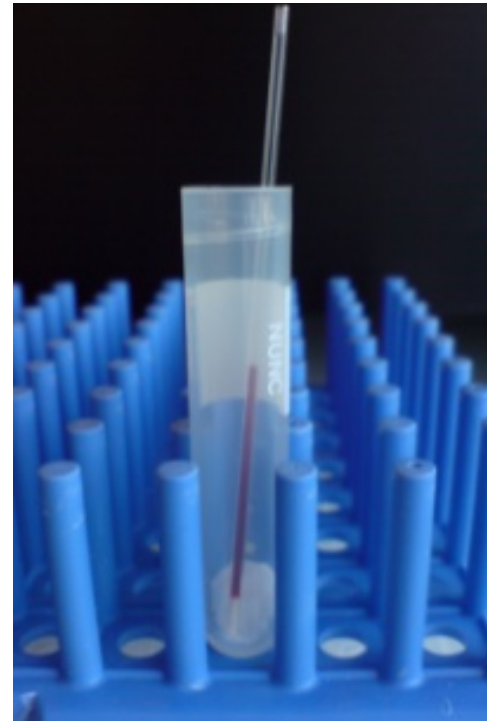


After "firing"



# Microsample Processing - Plasma

- Plasma microsample
  - Seal the end of the haematocrit tube with wax
  - Centrifuge in a container
  - Transfer plasma sample to storage container
  - Can use fixed volume capillary to get exact plasma sample volume (e.g. 4 or 8  $\mu\text{L}$ )





# Microsample Processing - Serum

- Serum microsample
  - Use plain capillary tube
  - Seal the end of the capillary tube with wax
  - Leave at room temperature for at least 60 minutes
  - Centrifuge in a container
  - Transfer serum sample to storage container



# Mouse Blood Volume Limits

- Blood volume calculation for 20 g mouse
  - Welfare/Licence limits, 25% of Total Blood Volume (1.2 mL) in 28 days
  - Therefore 300  $\mu$ L of blood available for samples
  - Microsample size  $\sim$ 30  $\mu$ L
- Microsamples can taken from every mouse
  - Saphenous or tail vein
  - 6 microsamples within 24 h

# Toxicology – Mouse Study Design

- Study design
  - Two occasions - Day 1 and Week 13
  - Control – 2 timepoints (Cmax and Cmin)
  - Treated groups – 6 timepoints/ occasion
- Standard sampling regimen
  - 3M + 3F/timepoint (bleeding each animal once)
- Microsampling regimen:
  - All animals/timepoint (assuming 32 µL blood sample)

	Control	Low	Inter	High
Main Study	10M + 10F	10M + 10F	10M + 10F	10M + 10F
Satellite Study	6M + 6F	18M + 18F	18M + 18F	18M + 18F
Total number of Satellite animals = 120				
Satellite Study	3M + 3F	3M + 3F	3M + 3F	3M + 3F
Total number of Satellite animals = 24				

# Bioanalytical Practicalities

- Assay sensitivity
  - Biggest challenge (for some dose routes it may not be suitable – dermal, inhalation)
- Sample size
  - Exact 8  $\mu\text{L}$  sample or multiple 4  $\mu\text{L}$  samples
  - Multiple samples reduce risk of sample loss
- Regulatory acceptance?
  - Plasma and serum are accepted matrices

# Proseek<sup>®</sup>

- Proseek<sup>®</sup> from Olink Bioscience
  - Protein quantification with just 1  $\mu$ L of plasma/serum
  - Comparable sensitivity to standard ELISA kits with much less sample
  - Easy workflow – little optimisation required
  - Combines the simplicity of ELISA with Q-PCR
  - Entirely liquid phase assay
  - Protein or cytokine expression can be determined in various complex sample media, including serum, EDTA plasma, citrate plasma and CSF

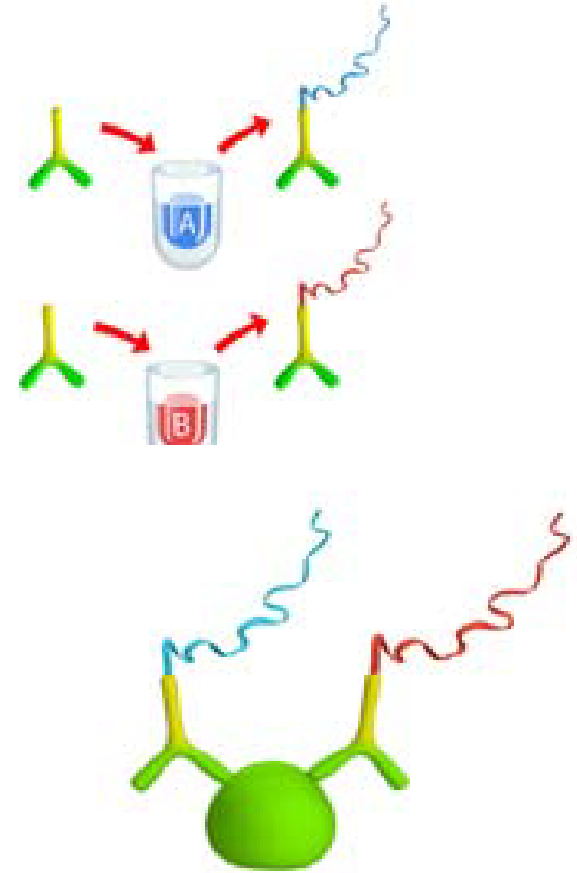
[www.olink.com](http://www.olink.com)

# Outline of Proseek® Technology

- Based on PEA - Proximity Extension Assay
  - Proseek® probes are antibodies labelled with a pair of oligonucleotides
  - Antibody probes bind to the target present in the sample in liquid phase
  - There are no washing steps
  - When the two Proseek® probes are in close proximity the homologous ends of the probes anneal
  - PCR target sequence is formed by a proximity-dependent DNA polymerization
  - The resulting target sequence is subsequently detected and quantified using standard Q-PCR

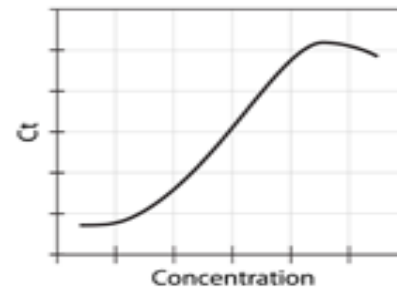
# Hybrid ELISA/QPCR (Proseek<sup>®</sup>)

- Create Proseek<sup>®</sup> probes A & B by conjugating your antibodies to complimentary Oligonucleotides A and B
- During incubation, Proseek probes will bind to the target protein



# Hybrid ELISA/QPCR (Proseek)

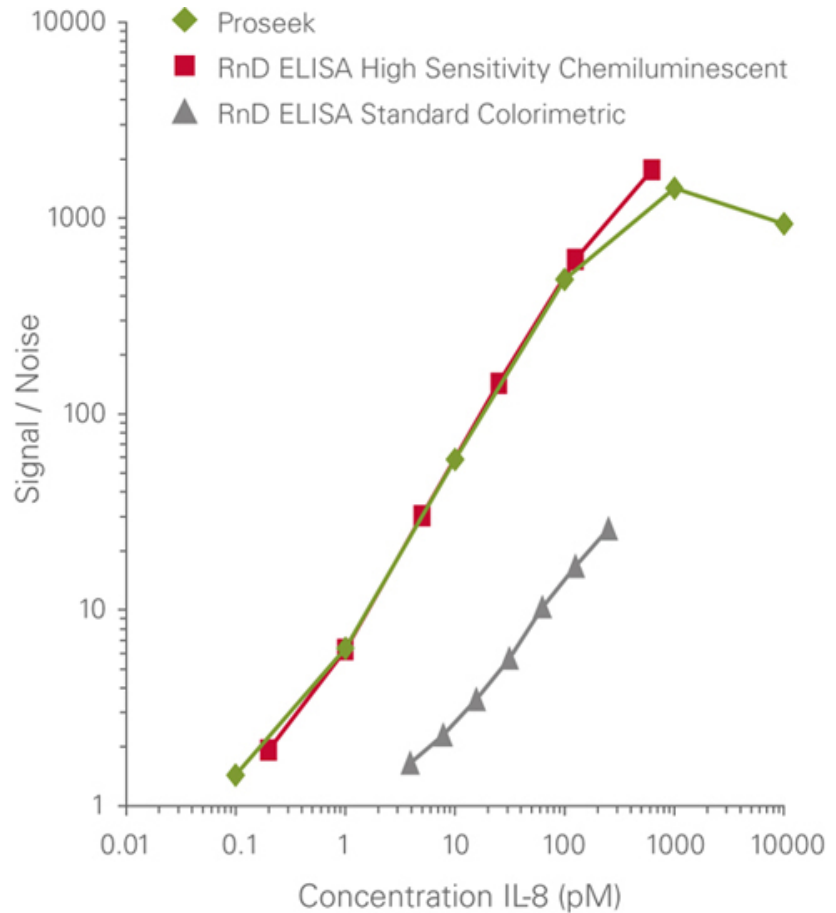
- Oligonucleotides extended through a DNA polymerisation event, creating the Q-PCR amplicon
- Amplify the DNA using standard Q-PCR instrument
- Analyse data





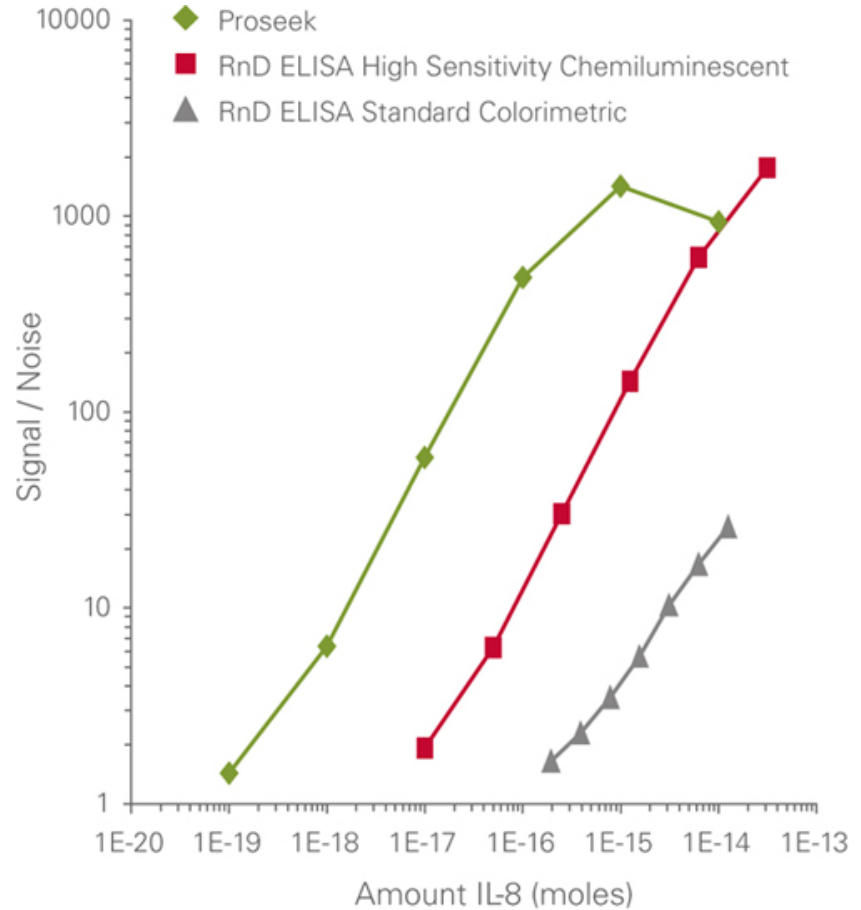
# Experimental Procedure – IL-8

- Comparison of IL-8 detection using high-sensitivity ELISA, standard ELISA and Proseek®
  - Proseek® offers same sensitivity as high-sensitivity ELISA when looking at concentration range



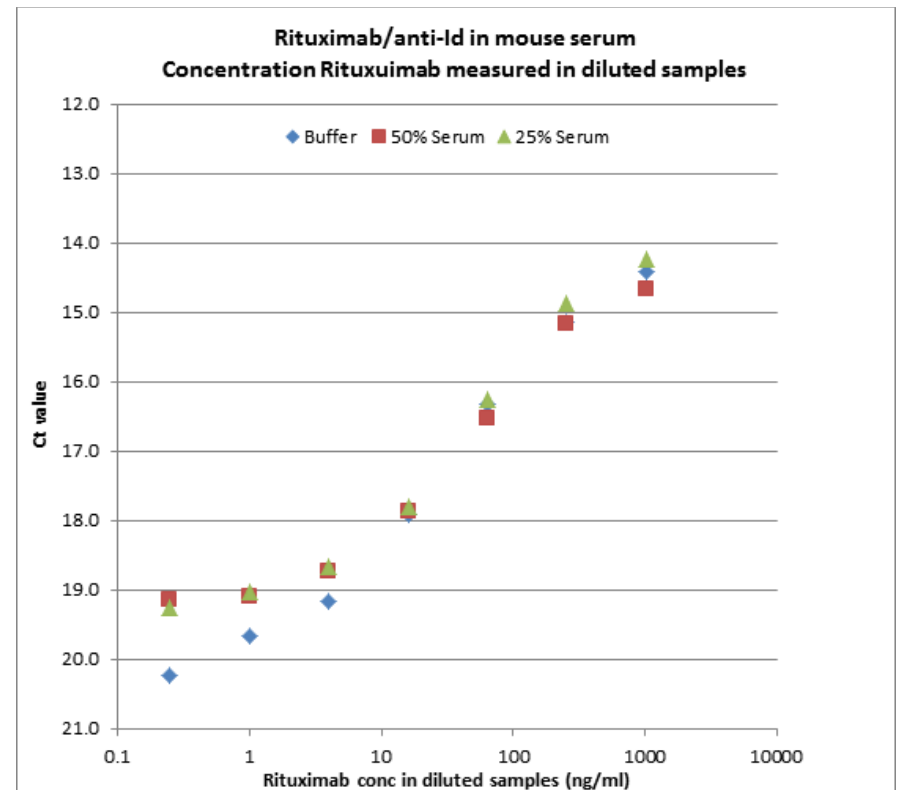
# Experimental Procedure – IL-8

- Comparison of IL-8 detection using Proseek<sup>®</sup>, standard ELISA and high sensitivity ELISA
  - Proseek<sup>®</sup> requires 100 to 1000-fold less analyte as shown by absolute amounts detected



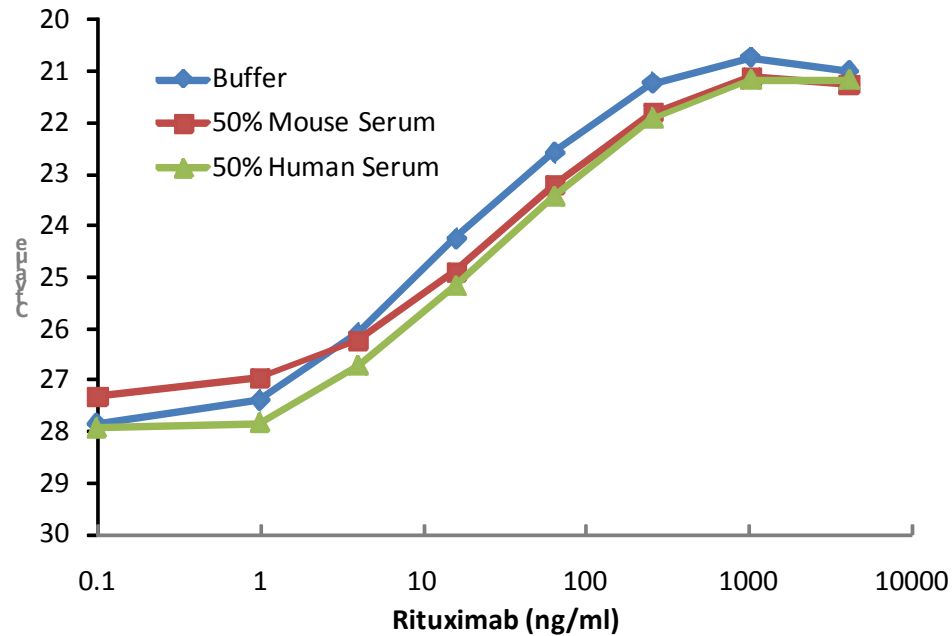
# Experimental Procedure – Rituximab

- 1  $\mu\text{L}$  sample volumes
  - Buffer
  - 50% mouse serum sample
  - 25% mouse serum sample
- Buffer shows large dynamic range
  - 0.1-1000 ng/mL
- Serum samples show matrix effect below 5 ng/mL
  - Assay usable to 1 ng/mL



# Experimental Procedure – Rituximab

- Similarity between mouse and human serum
  - Comparison of 1  $\mu\text{L}$  spiked diluted mouse and human serum to pure buffer

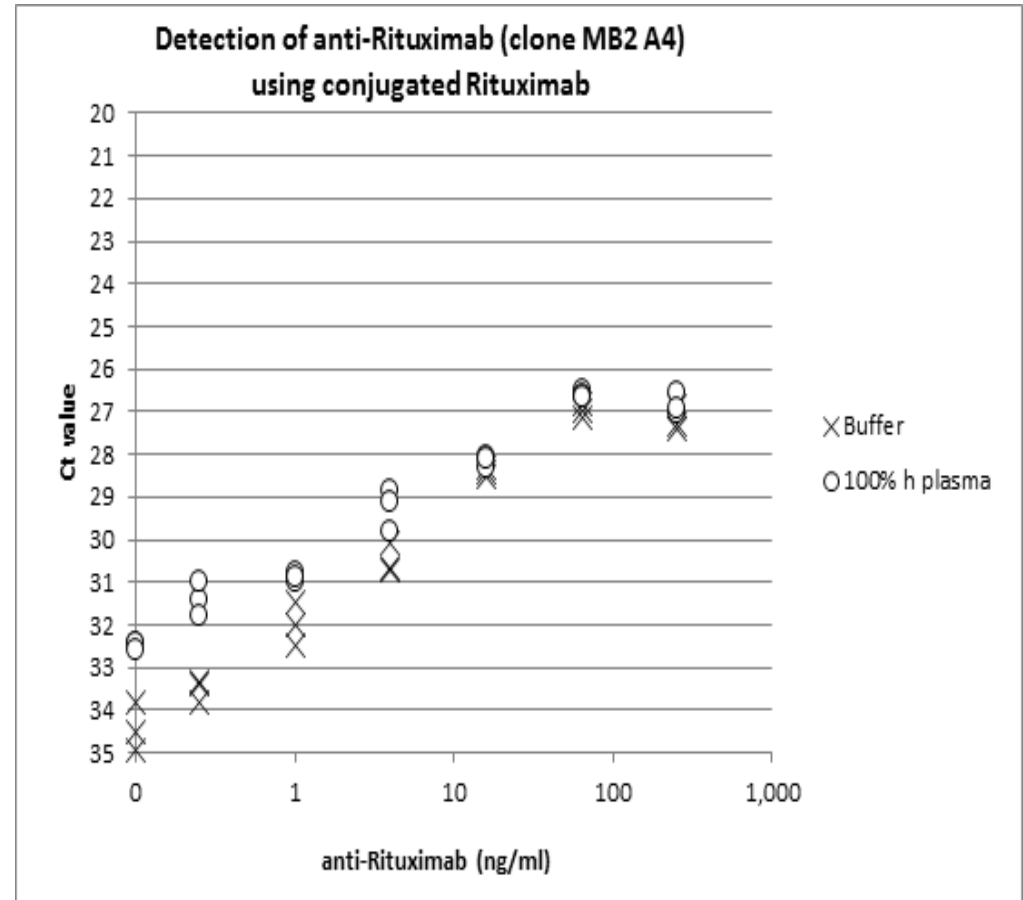


# Experimental Procedure – Rituximab

Rituximab (ng/ml)	Buffer		50% Mouse Serum		50% Human Serum	
	RE%	CV%	RE%	CV%	RE%	CV%
0	*	83	*	24	*	45
1	-0.2	16	-0.6	15	0	39
4	0.4	7	0.3	9	4	10
16	3	14	1	4	0	14
64	-6	11	-2	3	-3	13
256	16	5	4	5	9	10
1024	-10	20	6	36	-5	17
4096	-89	15	-75	68	-76	34
QC (4 ng/ml)			-5	18	11	7
QC (8 ng/ml)			-7	7	6	5

# Experimental Procedure – Anti-Rituximab in Human Plasma

- Positive Control
  - Rat Monoclonal MB2 A4 Isotype IgG2a recognises the idiotypic determinants expressed by the Rituximab humanised monoclonal antibody
  - It does not recognise other CD20 antibodies



# Proseek<sup>®</sup> Summary

- Proseek<sup>®</sup> is an easy, kit-based system for the detection of and quantification of proteins in biological matrices
- It offers the same level of simplicity as ELISA, but with some major advantages:
  - Only 1  $\mu\text{L}$  of sample is required for each assay
  - Broad dynamic range associated with Q-PCR functionality allowing rapid and easy testing of target
  - With very little optimisation required, new assays can be quickly developed

# Conclusion

- Combination of capillary microsampling and an enhanced sensitivity analytical technique can reduce animal usage without limiting the generation of TK, biomarker and immunology data



# Acknowledgements

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