

# Utility of the Gyros Gyrolab in Research and DMPK – An Automated Ligand Binding Assay Platform

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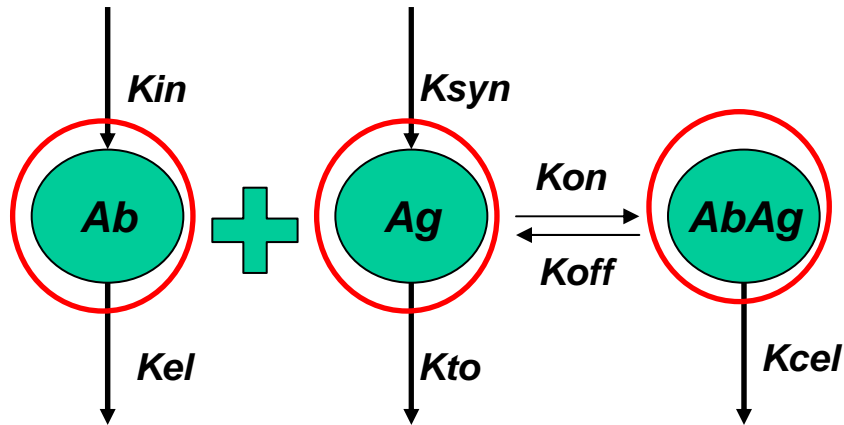
*Kevin Brady  
PDM-NBE,  
Pfizer Ltd,  
UK*

# PDM-NBE Group

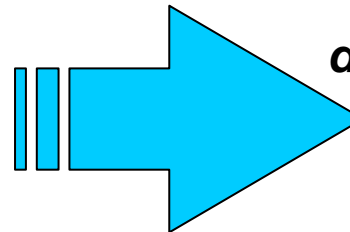


- Non-GLP studies – supporting pharmacology, PK/PD and early toxicity studies
- Ligand binding strategies for
  - MAbs
  - Recombinant proteins
  - Biomarkers
  - Soluble targets
  - Oligonucleotides
  - ADA
- We need to quantitate reliably, rapidly and efficiently
- Currently use ELISA, MSD, DELFIA, Luminex, Flow cytometry and LC-MS

# What do we want to measure and why?



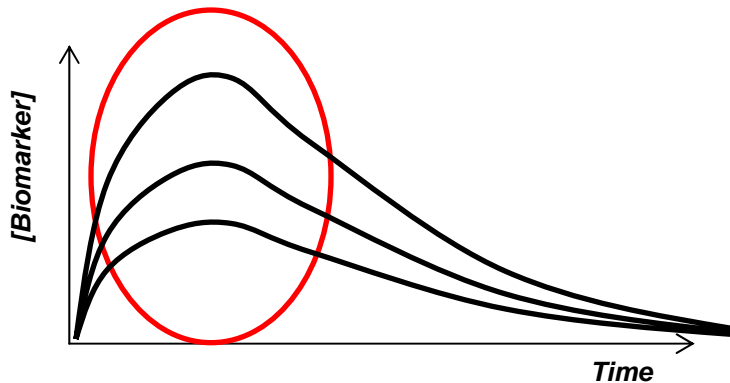
**PK**



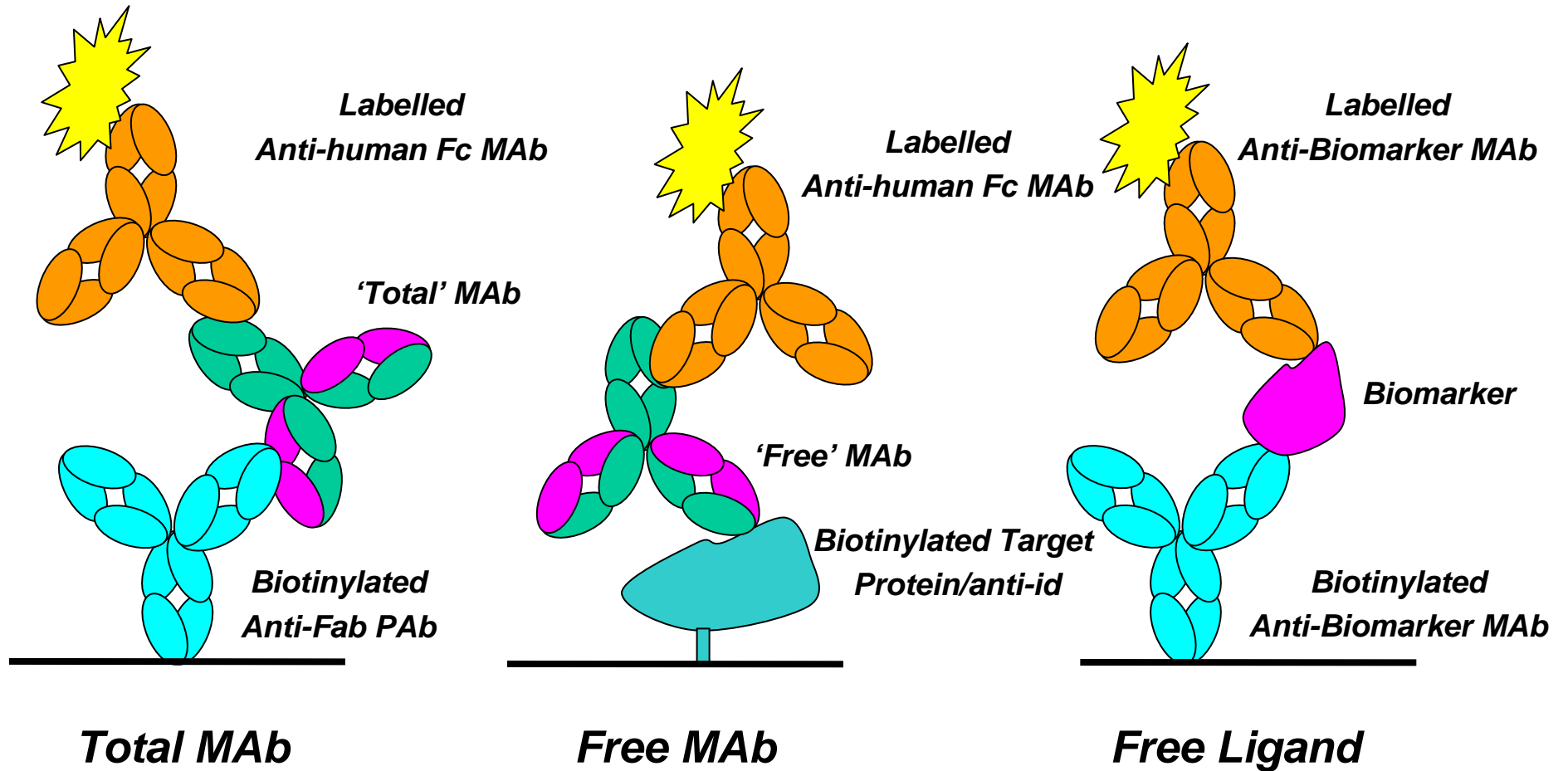
*Understanding our  
dose-response which  
leads to*

**Dose Prediction**

**PD**



# Assay formats



# Problems with standard LBAs

- Slow method development and data turn-around
- Sensitivity and range of assays are issue with some techniques (i.e. ELISA)
- Analyst time
- Reagent costs
- Assay robustness
- Technology transfers to internal/external labs
- Matrix interference
  - blocking of signal due to matrix components
- Dilution requirements
  - Often need to perform various different dilutions on the same sample set.
- Sample constraints
  - Often need sample volumes of 25-100uL

# What would be really useful?



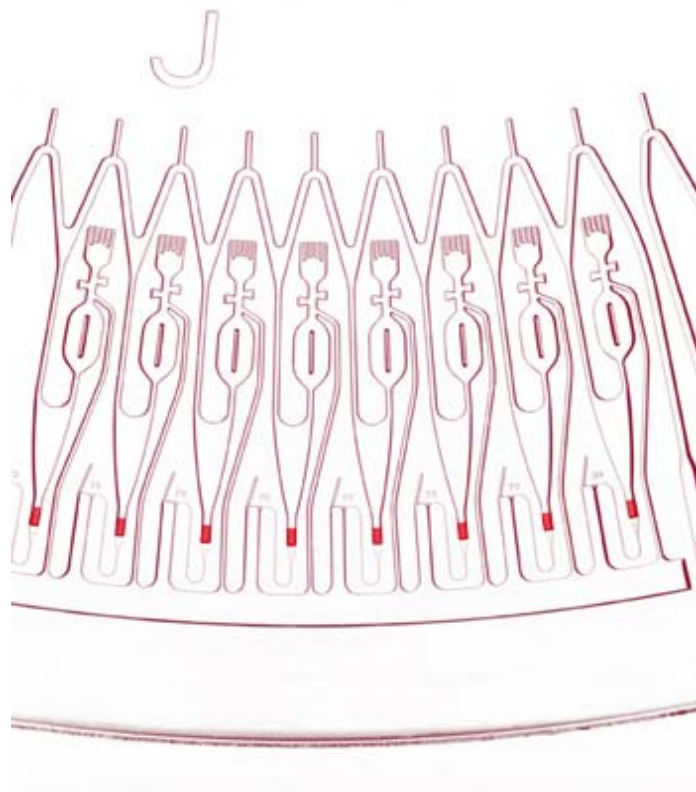
- Cut down time to run, develop and validate assays
  - Faster assay run time
  - Automated liquid additions – improved precision
- Reduced reagent requirements
- Reduced sample volumes capability
  - Mouse studies
- Large dynamic range
- Low non-specific binding
- Low dilution requirements
  - for some applications, e.g. free MAb assays

# Gyros Gyrolab<sup>®</sup>

Worldwide  
**PDM** Pharmacokinetics  
Dynamics &  
Metabolism  
*...driven by science!*



# Simultaneous processing - increase reproducibility



All datapoints processed in parallel

112 data-points (20 & 200nl CDs)

96 data-points (1000nl CD)

- no cross talk

Three Gyrolab® CDs

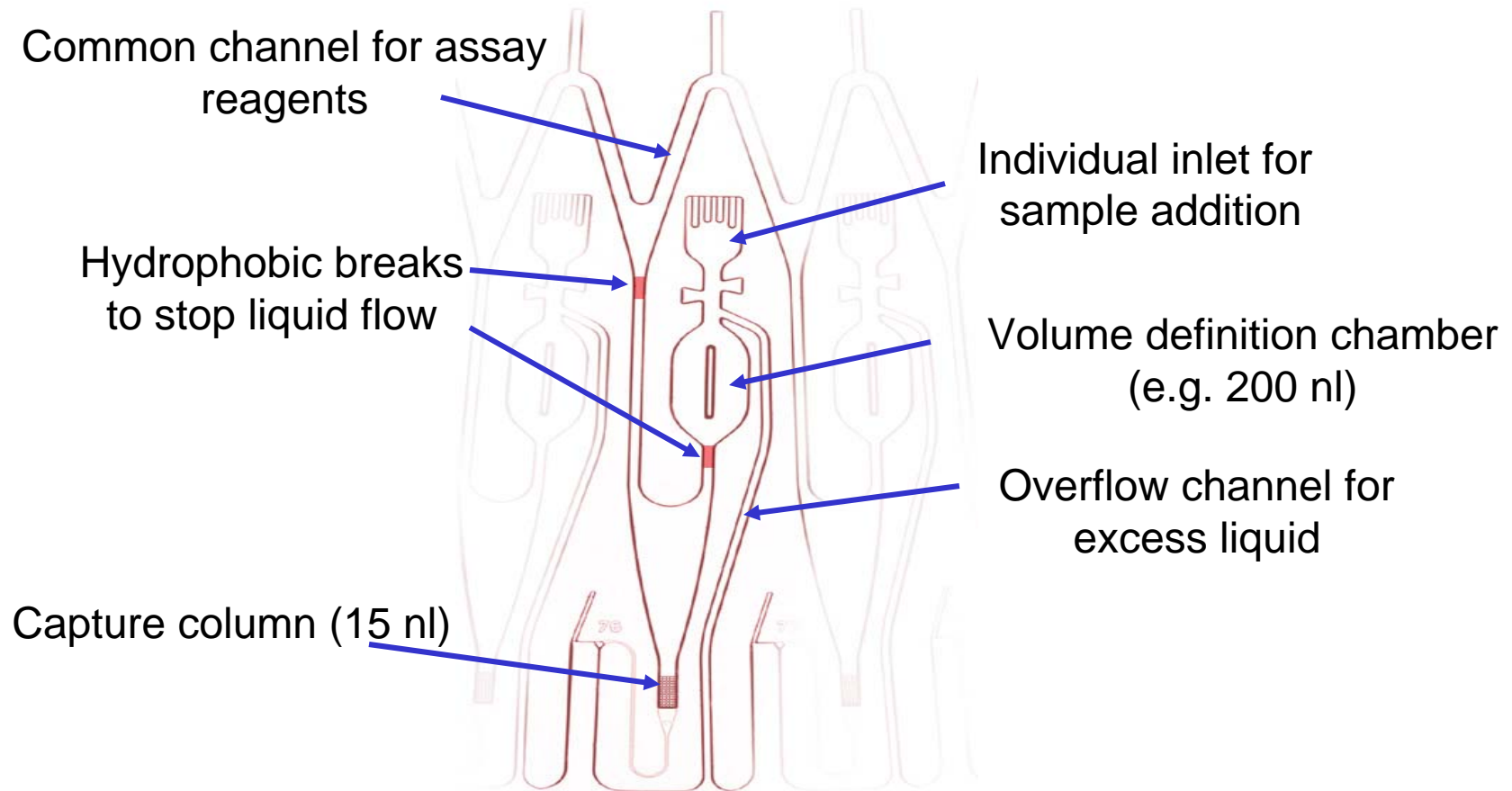
- Bioaffy 20HC (20nl)

- Bioaffy 200 (200nl)

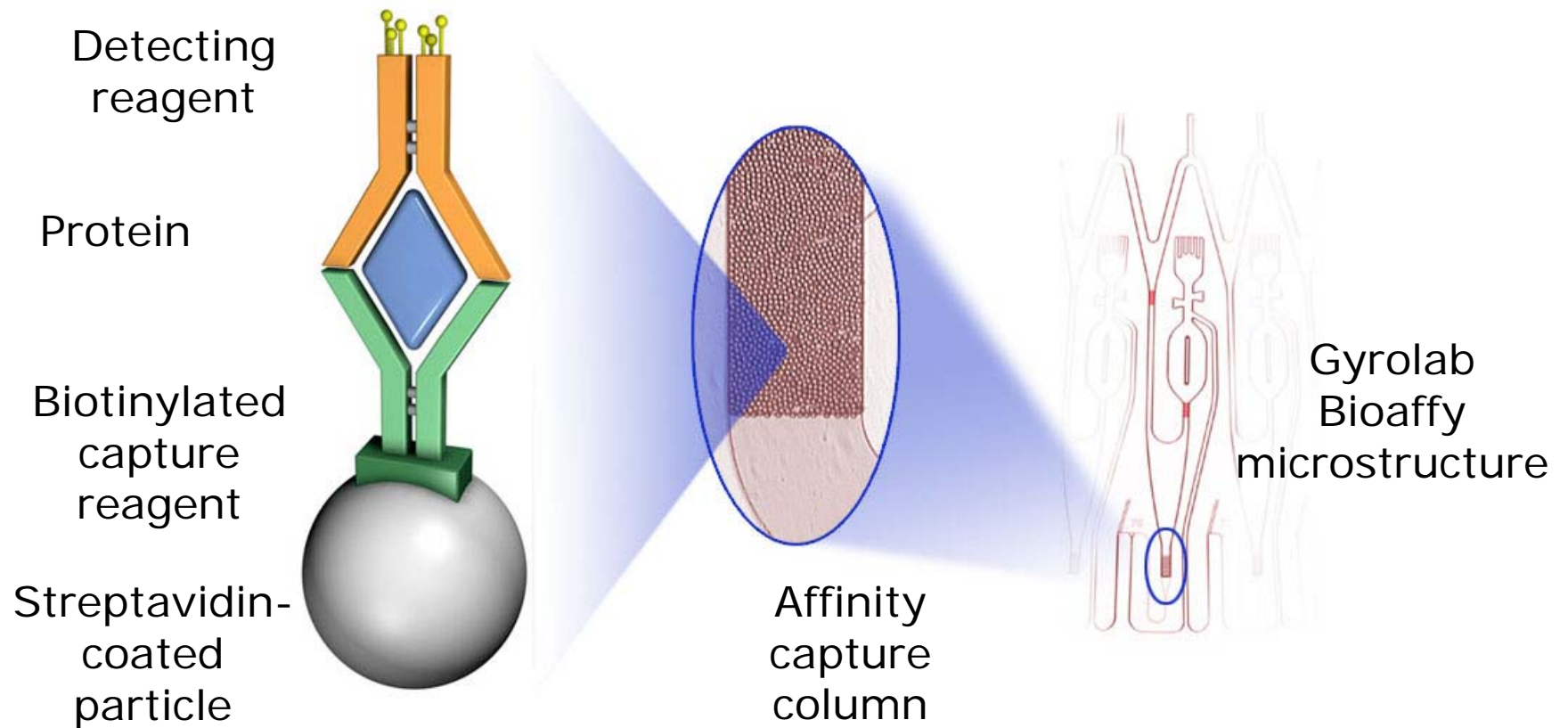
- Bioaffy 1000 (1000nl)



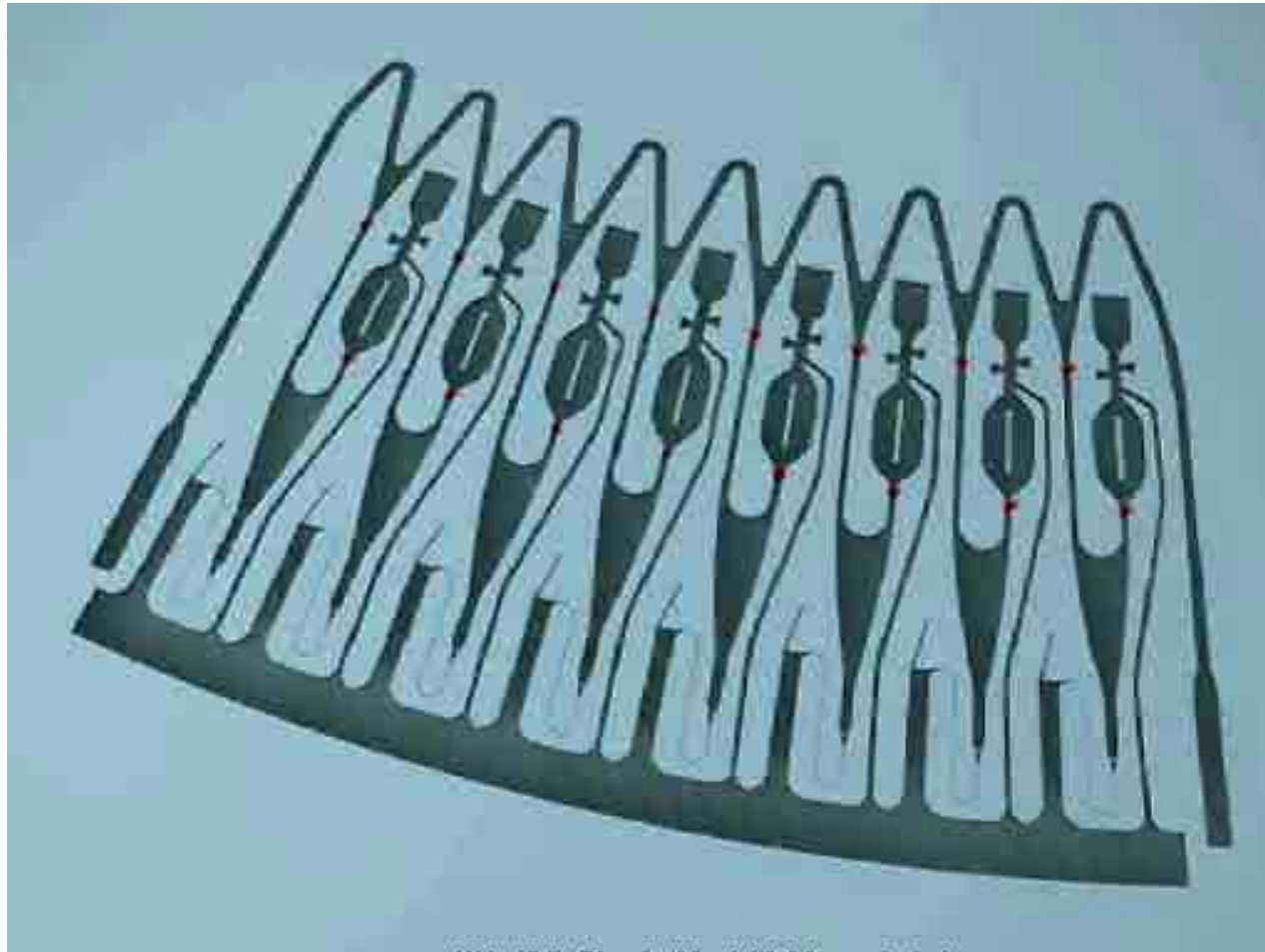
# One microstructure - one data point - no cross-talk



# Miniaturize sandwich immunoassays



# Immunoassays on Bioaffy CD



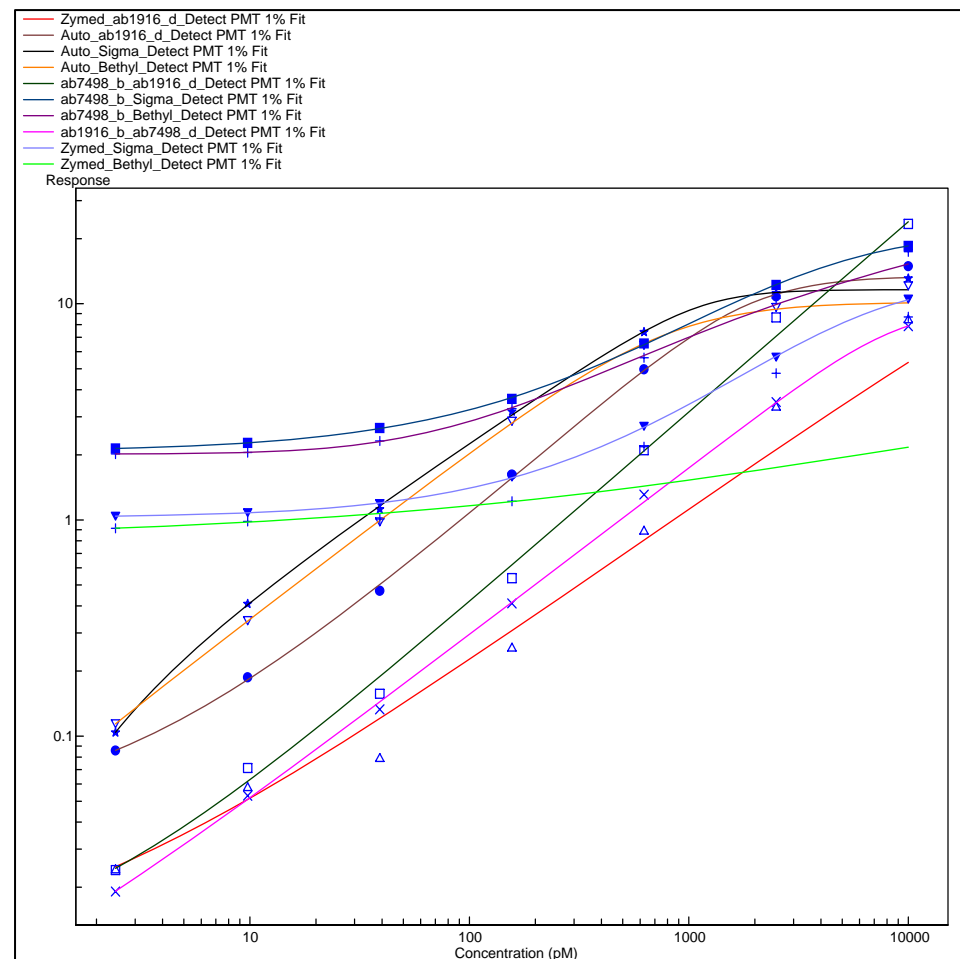
# Assay Requirements

- Biotinylated capture molecule
  - Commonly a MAb
  - Polyclonal antibodies– Use Affinity Purified vs antigen
  - Recombinant protein
  - Sulfo-NHS-LC-Biotin (Pierce) often used, followed by Zeba column clean-up
  - Pre-biotinylated reagents increasingly common
- Fluorophore-labelled detection agent
  - Usually a MAb
  - Fluorophore employed most commonly: Alexa Fluor 647
    - Could use other fluorophores e.g. DyLight 649
      - Good quantum yield
      - Excitation around 620nm
      - Emission around 650nm

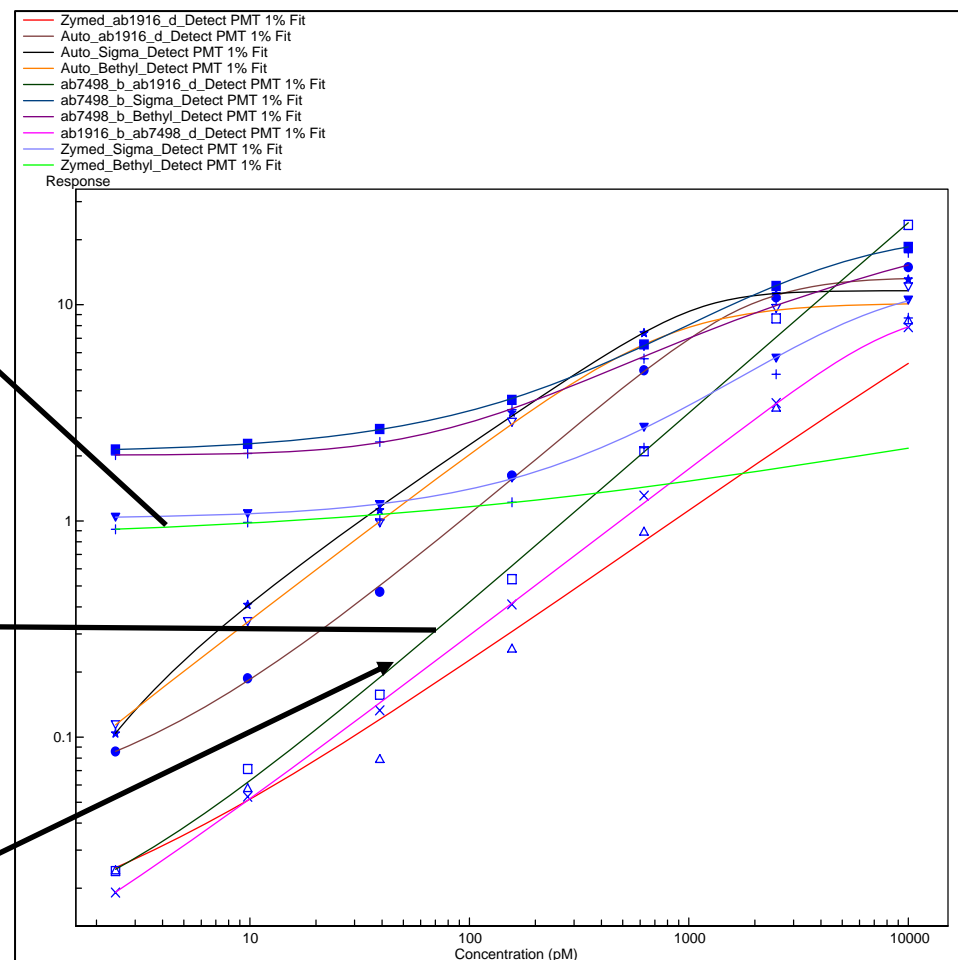
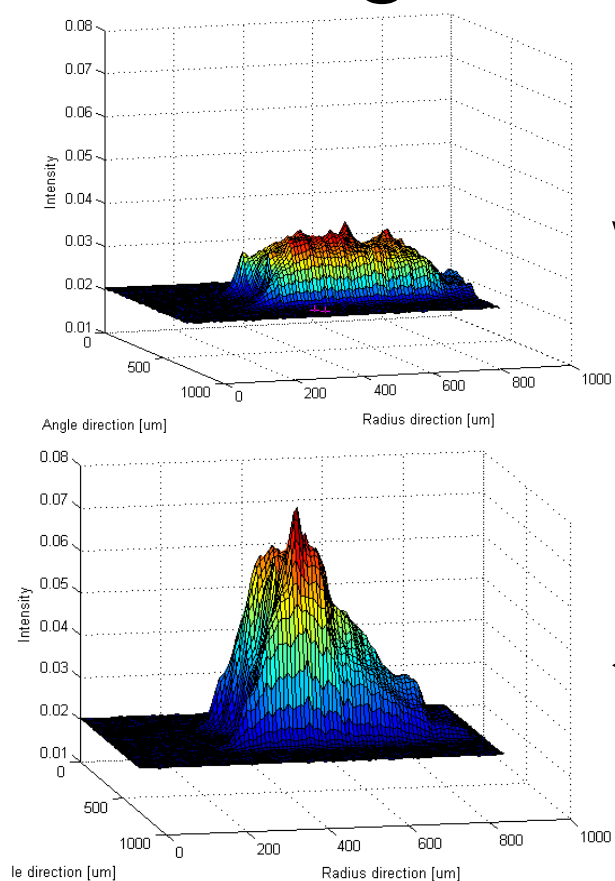
# MAb Total Assay – Reagent Screening

4 Anti-Kappa light chain MAbs  
 4 Anti-Fc MAbs  
 Capture MAbs – Biotinylated  
 Detector MAbs – Alexa Fluor 647  
 IgG<sub>2</sub> MAb - analyte  
 Used in 10 different assay combinations

Capture	AbCam1	Autogen	Zymed	AbCam2
Detector	AbCam1	Sigma	Bethyl	AbCam2

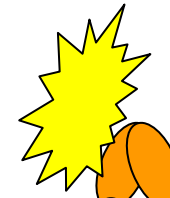
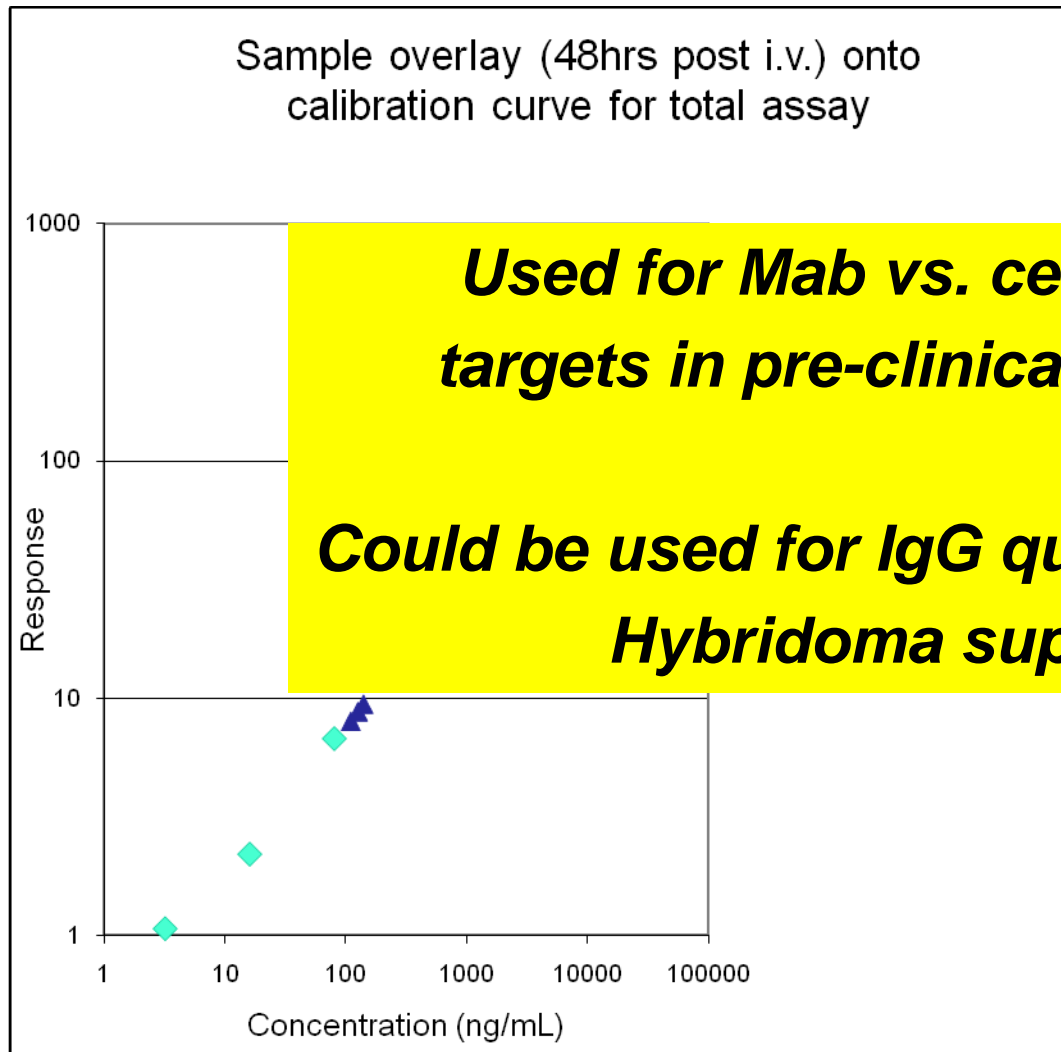


# MAb Total Assay – Reagent Screening

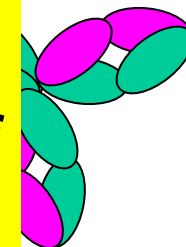


AbCam2 – Capture, AbCam1 – Detector  
Taken forward as optimal pair

# Total MAb assay



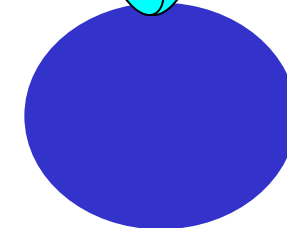
**A647 Labelled  
Anti-human MAb**



**'Total' MAb**

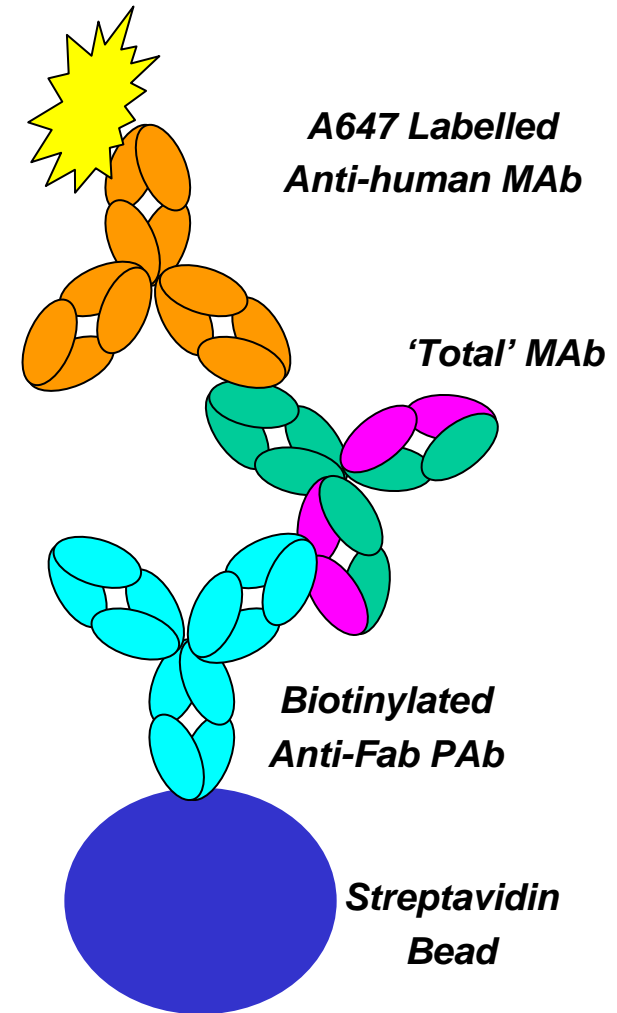
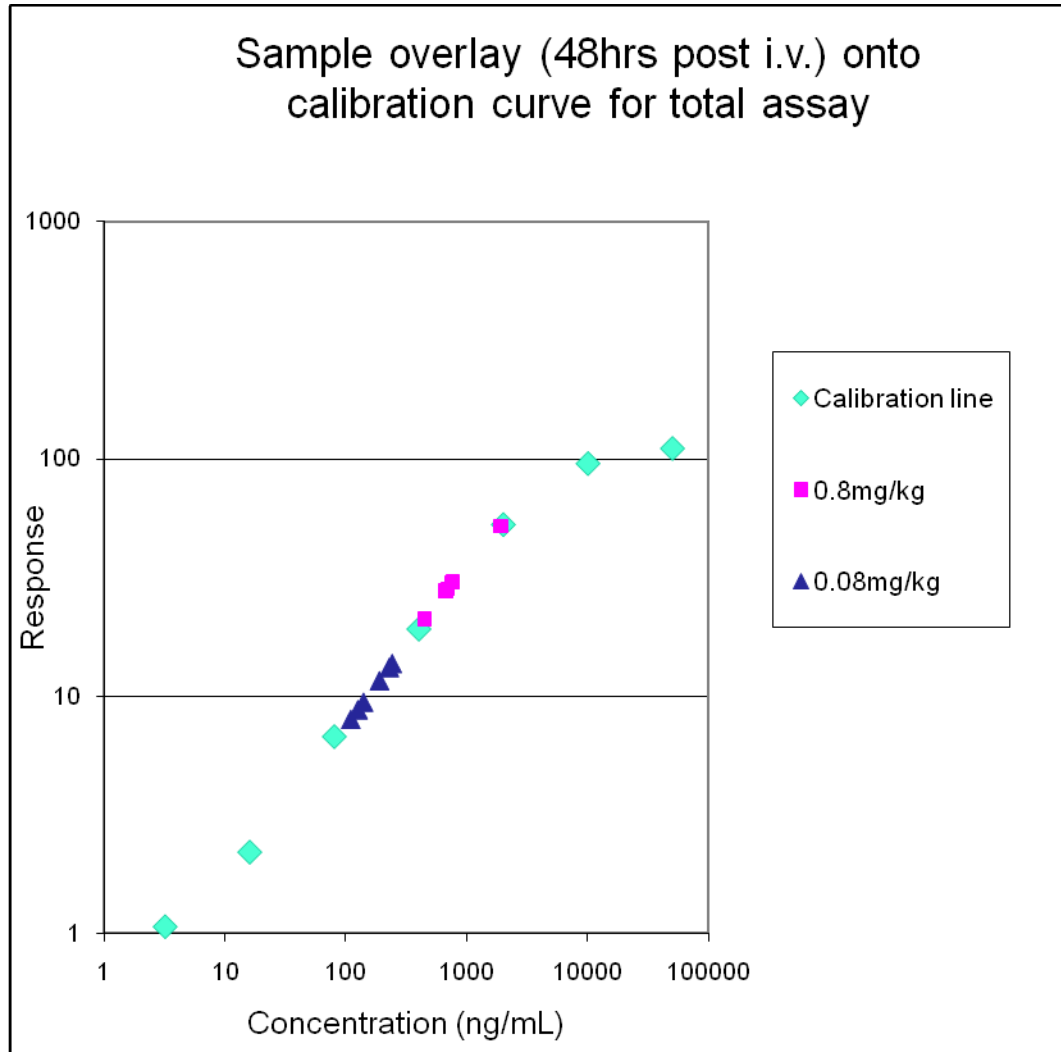


**Biotinylated  
Anti-Fab PAb**



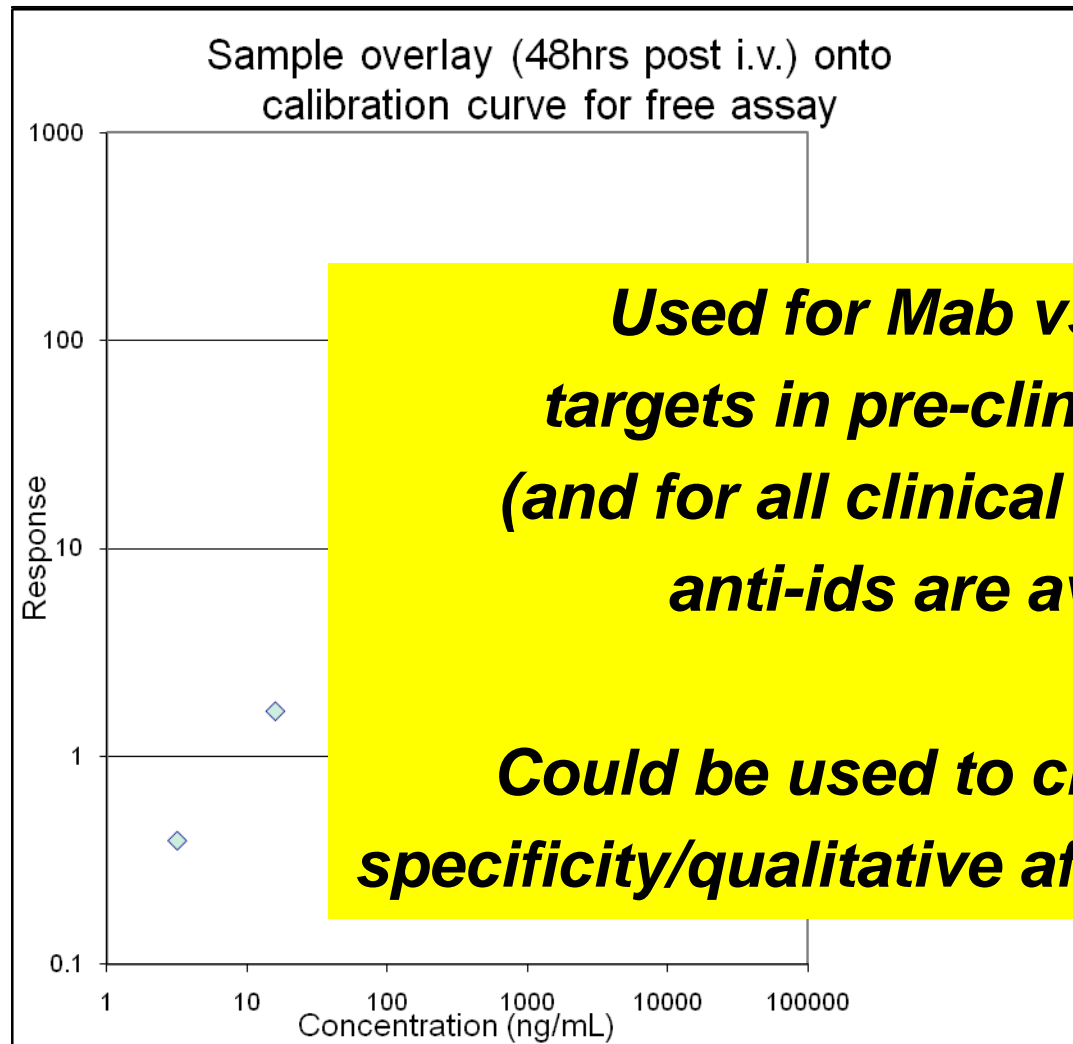
**Streptavidin  
Bead**

# Total MAb assay



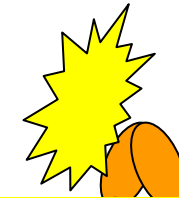


# Free MAb assay



**Used for Mab vs. soluble targets in pre-clinical studies (and for all clinical studies once anti-ids are available)**

**Could be used to check antigen specificity/qualitative affinity assessment**



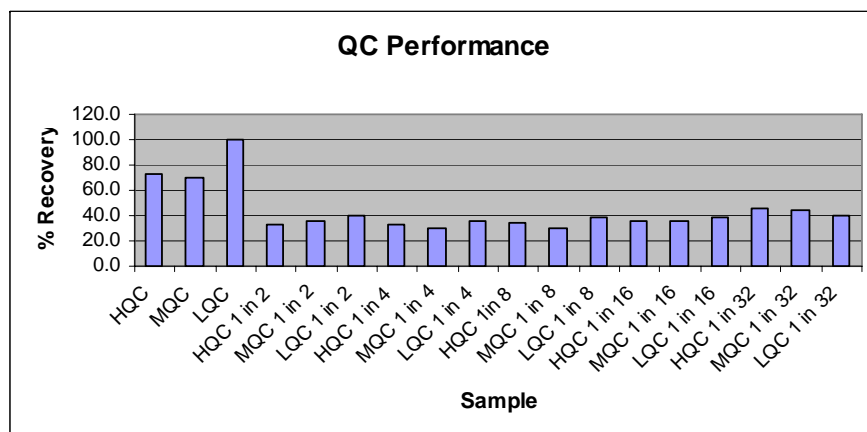
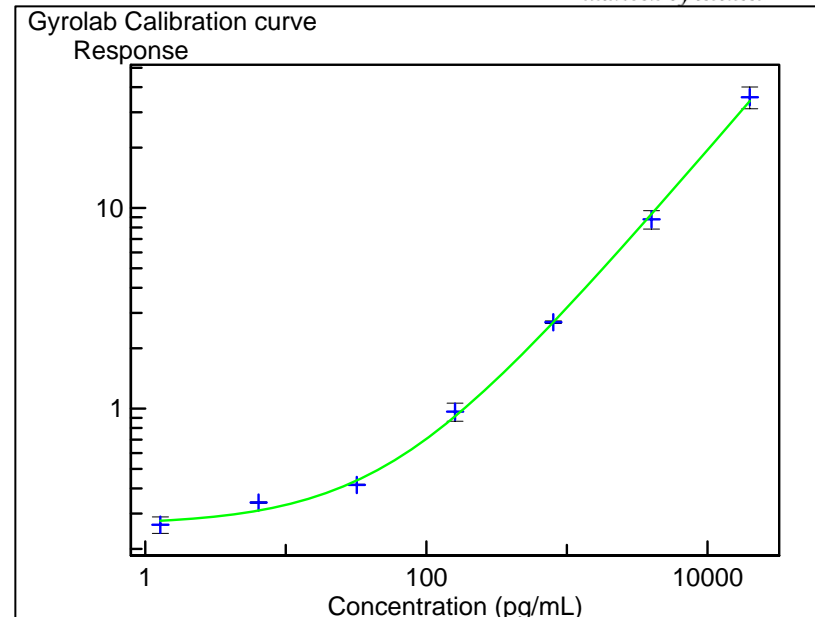
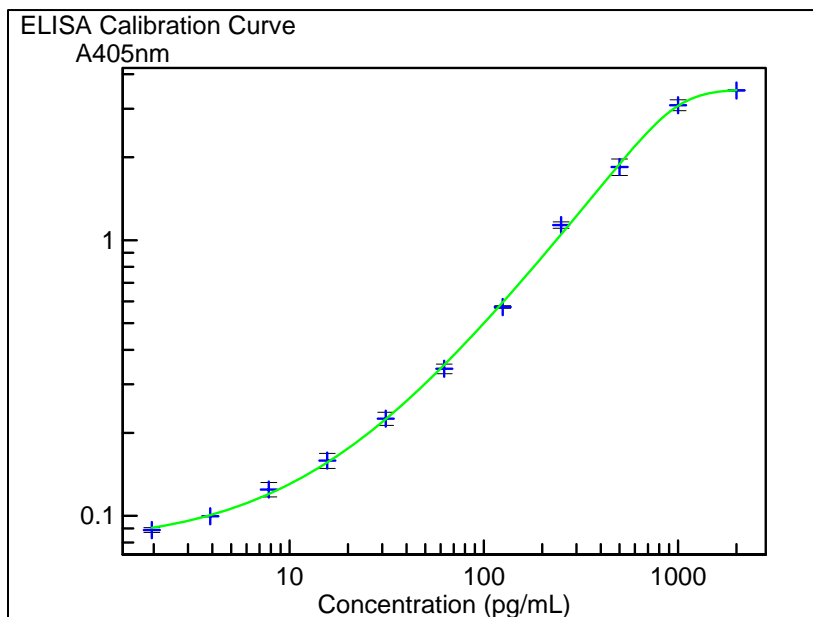
**A647 Labelled  
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**Bead**

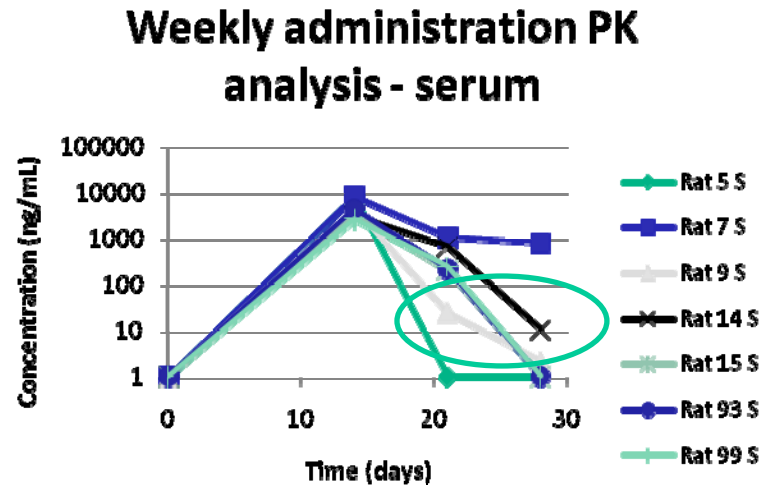
# Target Measurement



	Plasma Conc.	Measured analyte (pg/mL)	% Recovery
HQC (2000pg/mL)	50%	1951.48	<b>98</b>
MQC (200pg/mL)	50%	177.45	<b>89</b>
LQC (20pg/mL)	50%	22.16	<b>111</b>

# Anti drug antibodies

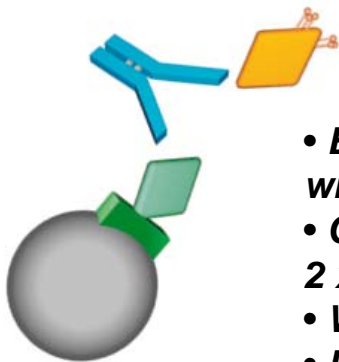
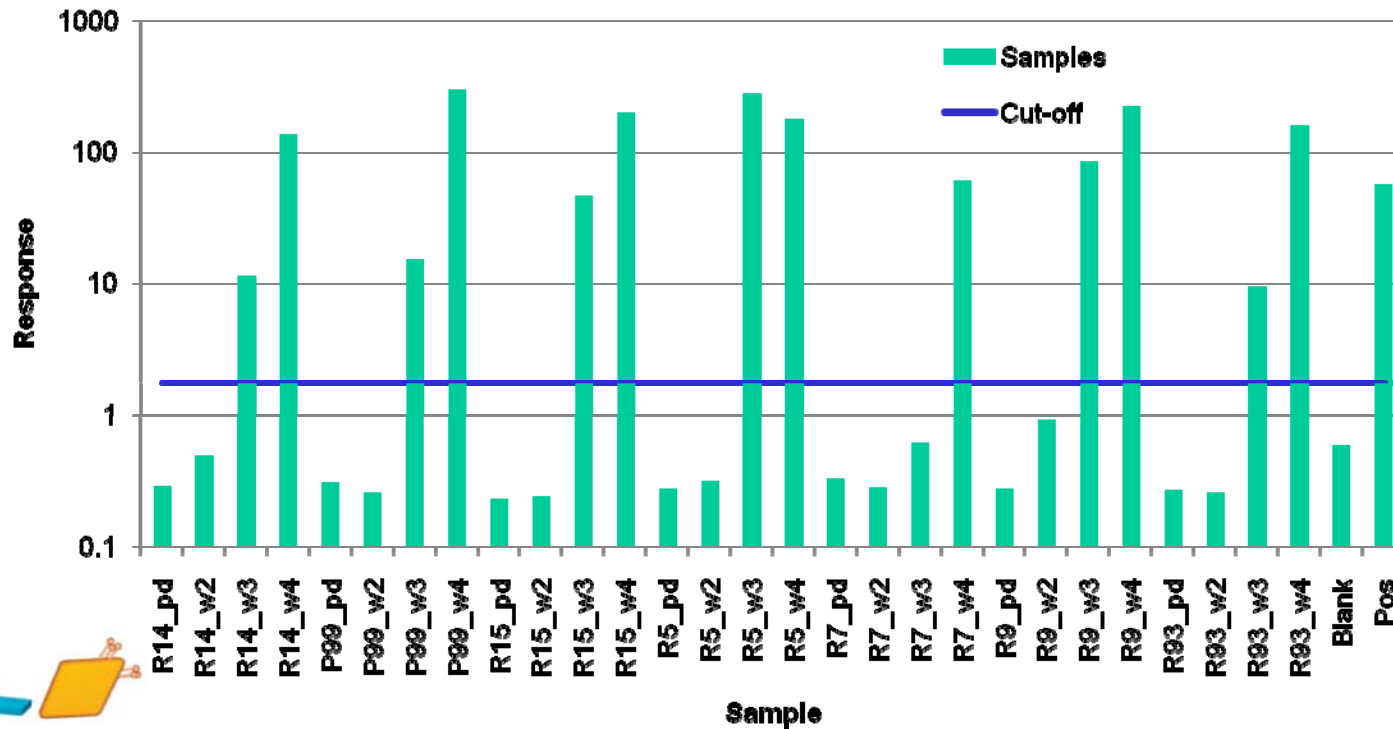
- Use the Gyrolab to run exploratory ADA studies in support of unusual PK observations in non-GLP studies



- Bridging assay format – drug-biotin and drug-AF647 incubated with samples
  - Various conditions tested

# Experimental confirmation of ADA

ADA Analysis of serum

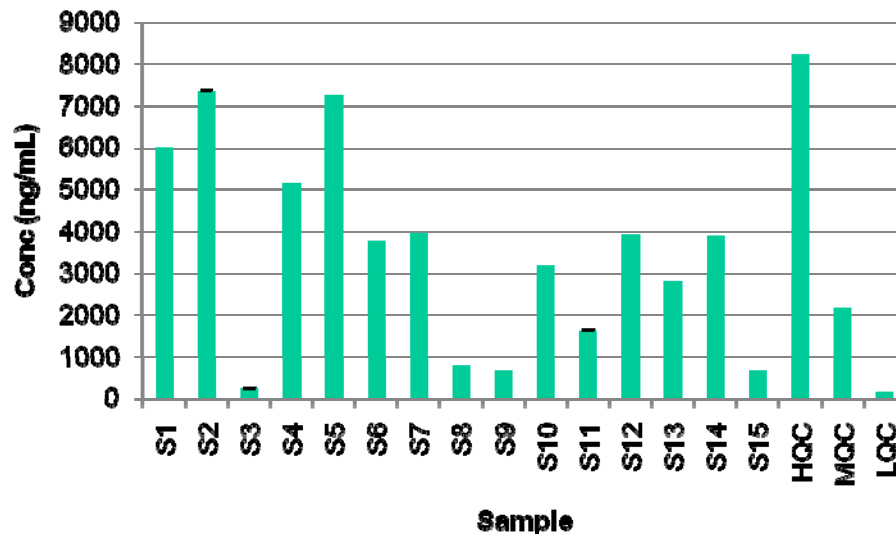
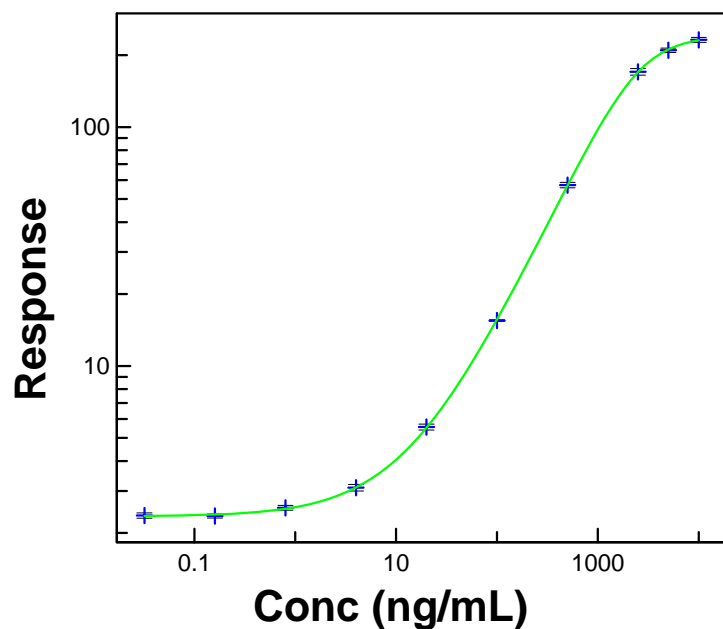


- Bridging assay involving incubation of biotin- and Alexa Fluor- conjugated drug with samples overnight.
- Cutoff defined as the average signal of all pre-dose and control animal samples + 2 x standard deviation measured for these groups
- Week 3 and 4 show significant ADA in almost all animals
- Positive control 1ug/mL anti-idiotypic antibody

# Hybridoma expression

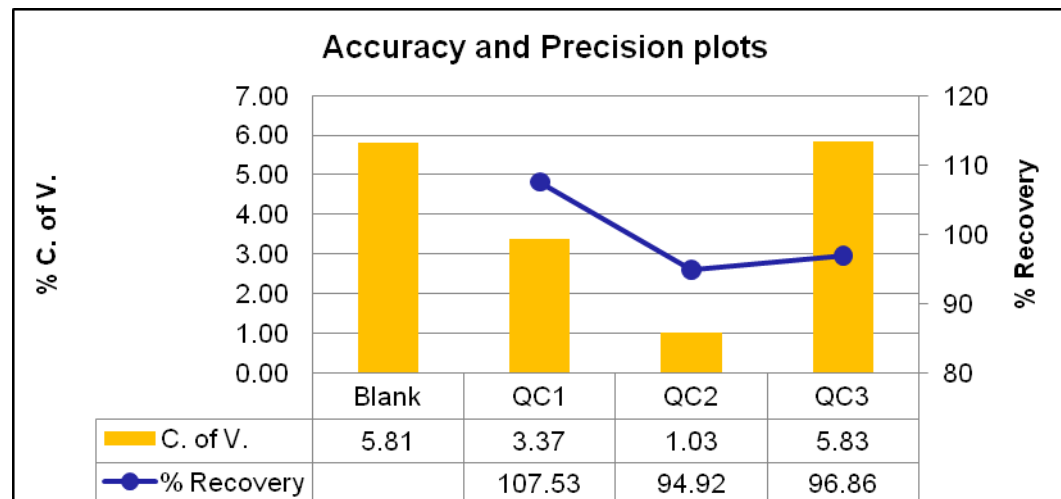
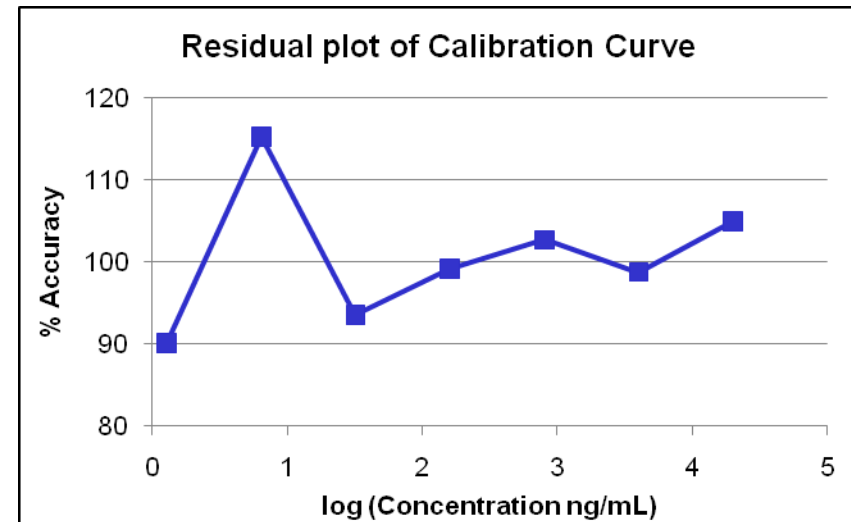
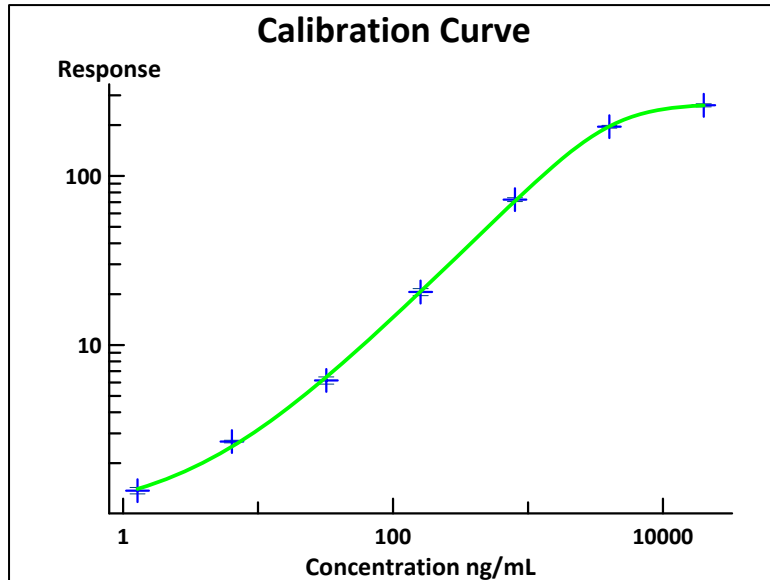
- Mouse IgG assay from hybridoma supernatants
  - Pre-labelled anti-mouse IgG Polyclonals from Jackson (biotin and Dylight 649)
- Improved performance over plate-based ELISA
  - Variability (C. of V. >20%)
- Faster data turnaround – 96 samples in 2hours (fully automated) c.f. 6 hours for ELISA

# Screening of supernatants



	Expected (ng/mL)	Calculated (ng/mL)	% Recovery
HQC	8000	8204.2	102.6
MQC	2000	2136.6	106.8
LQC	100	116.0	116.0

# Quality of data



# Cost comparison (Approx.)

	ELISA <sup>a</sup>	MSD	Gyrolab <sup>®</sup>
Plates	£0.2	£80 <sup>b</sup>	£150
Capture	£1.2	£0.3	£0.6
Detector	£20	£2	£0.5
Analyst time	£67.5 <sup>c</sup>	£67.5 <sup>c</sup>	£15
<b>TOTAL</b>	<b>£89</b>	<b>£150</b>	<b>£166</b>

**a Assumes a standard sandwich ELISA, coated onto polystyrene plastic plate**

**b Streptavidin HB plate**

**c Assumes analyst time operating assay is 4.5hours (£15/hr) – sample dilution, calibration and QC prep, plating, plate washes, reagent addition, plate reading**

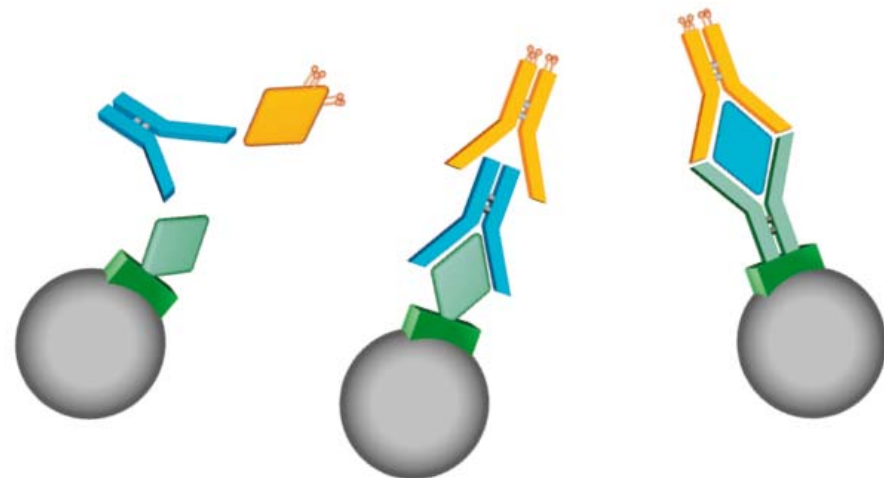


# Pros and Cons of Gyrolab

- **Pros**
- **Assay range**
  - Comparable in some cases to MSD (reagent specific)
  - Greater than ELISA (in most cases)
- **Matrix robustness**
  - Often run assays in 50% matrix
- **Automation & Quality**
  - Consistently low imprecision and inaccuracy (<10%)
- **Speed**
  - Reduced analyst time by up to 80%
  - Rapid method development/validation
- **Outsourcing very encouraging to date**
- **Cons**
- **Multiplexing currently not quite possible**
  - Still use MSD or Luminex for such assays
- **Software limitations for multiple assays within system run**
  - Possible to run 5 CDs consecutively using one method

# Future perspectives

- Anti-drug antibody analysis in non-GLP studies
- Further transition of definitive biomarkers to Gyrolab
- Move of most PK assays to Gyrolab platform
- Further side-by-side comparisons vs. other techniques
- Use bridging assay to improve understanding of qualitative affinity



# Acknowledgments

- Pfizer UK
  - Chris Grace
  - Luis Perez-Tosar
  - Tim Townend
  - Jaiesh Rawal
  - Rob Webster
- Pfizer US
  - Lindsay King
  - Jim Messamore
  - Jennifer Soung
- Gyros
  - Gary Corrigan
  - Karolina Osterland
- And you all for listening!