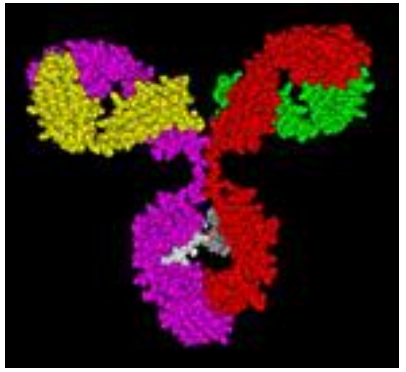
QUOTIENT BIORESEARCH

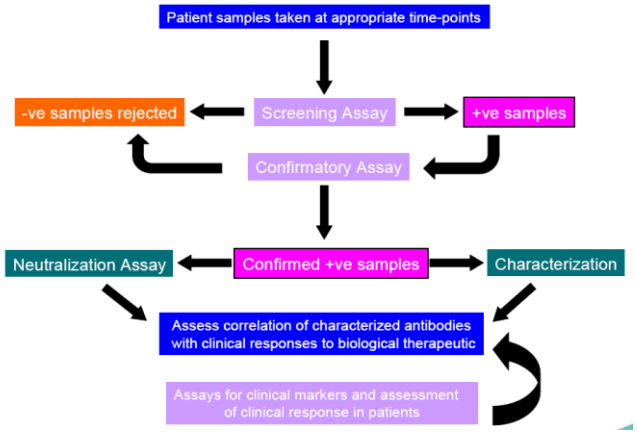
Challenges in Developing Anti-Drug Antibody Ligand Binding Assays

Answers Through Innovation

Biopharmaceuticals: Unwanted Immunogenicity

- Safety
  - Acute reactions from infusion and anaphylactic
  - Hypersensitivity
  - Cross-reactivity with endogenous counterpart
- Efficacy
  - PK/PD clearing, binding or neutralising antibodies
  - Product-related vs patient-related causes
- Regulatory authorities recommend
  - Multi-tiered approach
  - Screening, confirmatory and titre
  - Neutralising Antibodies (NABs)
  - Isotyping

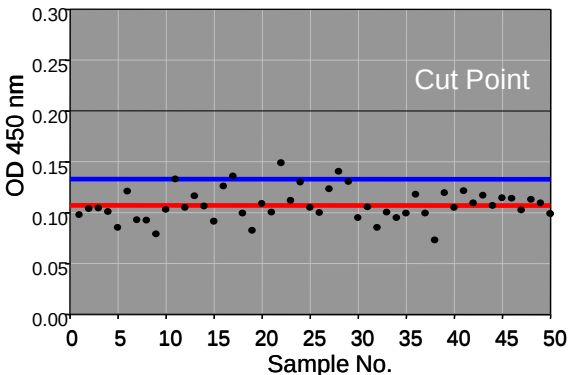


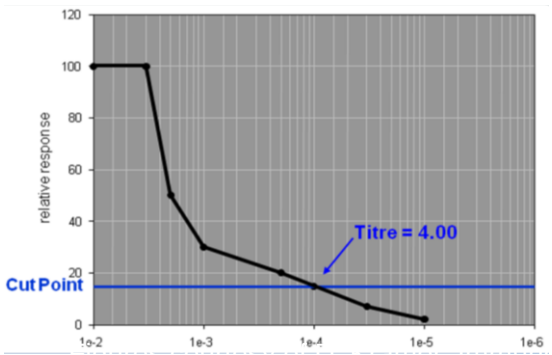


Challenges in Immunogenicity Testing

The guidelines say...

- Screening assays must be **sensitive**
- Able to detect all antibody **isotypes**
- Use relevant **positive and negative controls**
- A minimum required dilution (**MRD**) is required
- **Drug interference** must be evaluated
- A **cut point** must be set giving a low rate of false positives
- **Confirmatory** assays are used to confirm true positives
- Relative antibody responses should be reported as **titres**
- Ideally detect **low affinity** anti-drug antibodies





Semi-Homogenous Bridging Assay

Solution phase incubation of the sample/control with labelled drugs

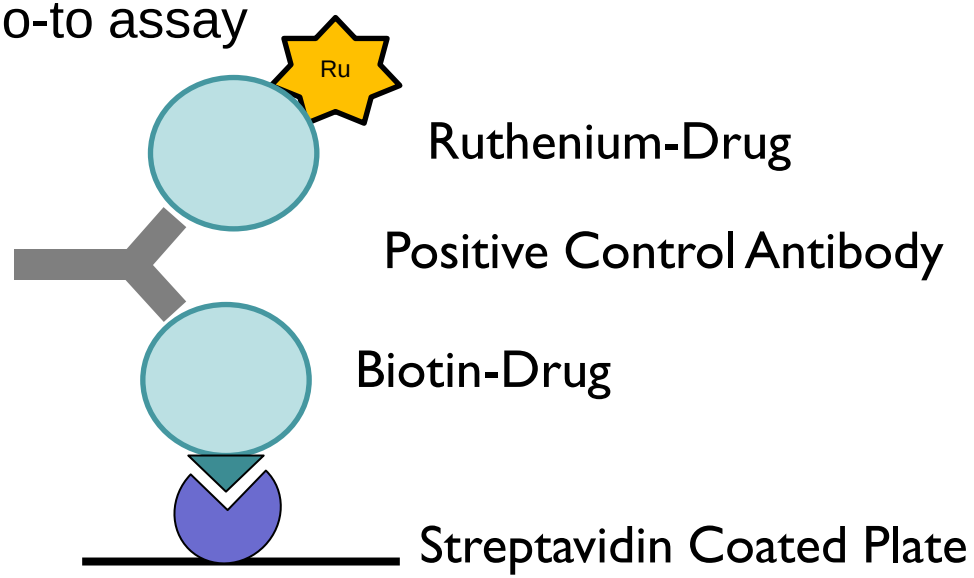
Capture to streptavidin-coated plates

Wash

Add read buffer

Read plate

Semi-homogenous format is the go-to assay

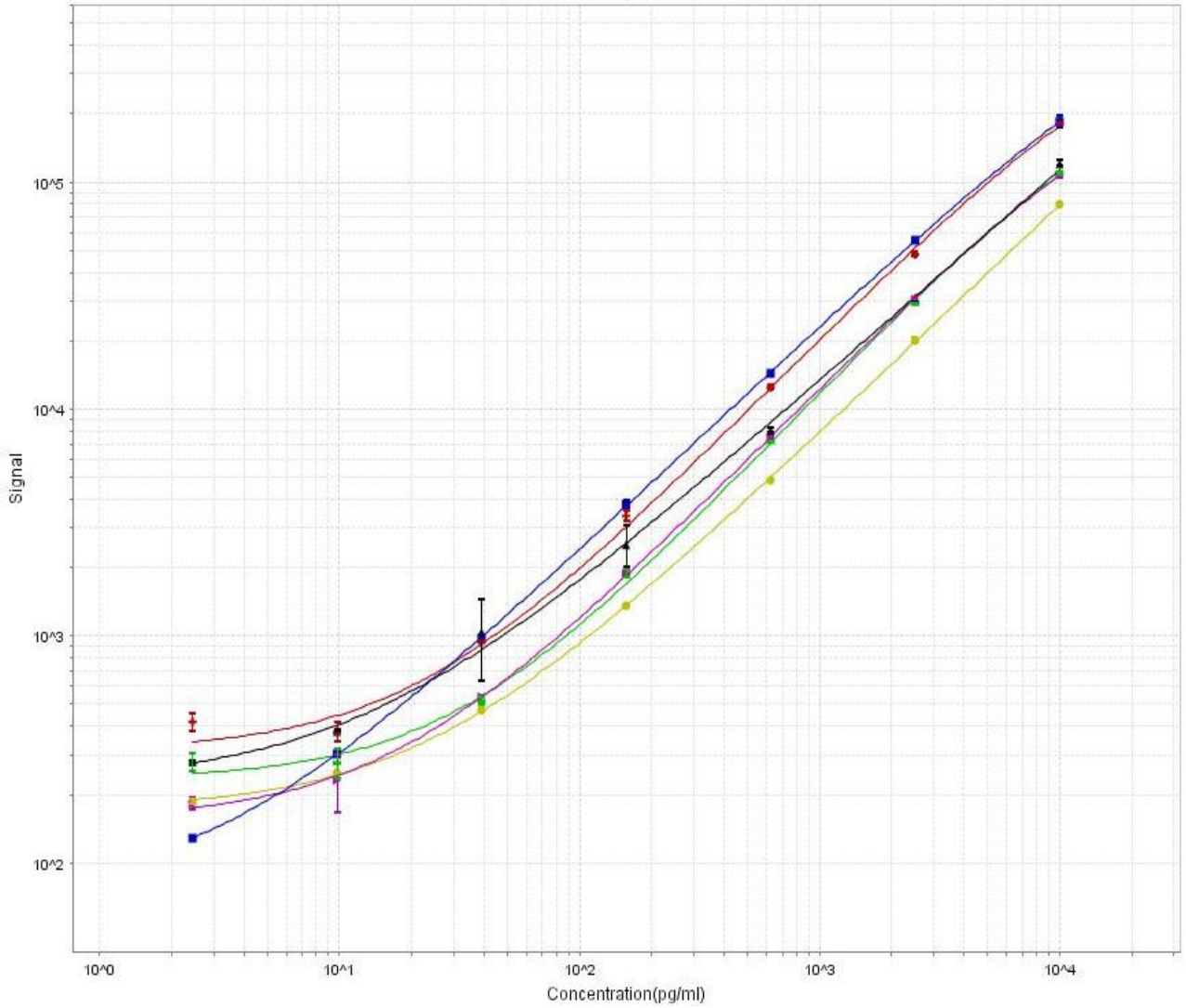


Selecting Minimum Required Dilution and Labelled Drug Ratios

Comparison of increasing labelled drugs in 100% and 25% serum

Higher signal in 25% serum

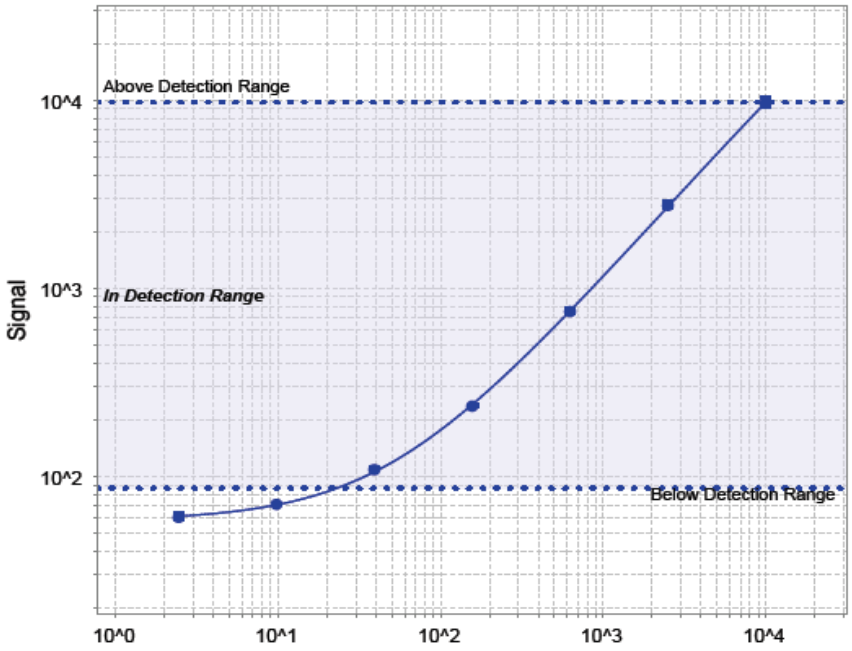
Dynamic range and signal to noise optimal with 0.25 µg/mL labelled drugs in 25% serum



Bridging Assay Pitfalls (1)
Buffer Versus Matrix

Second assay for a drug for a related target

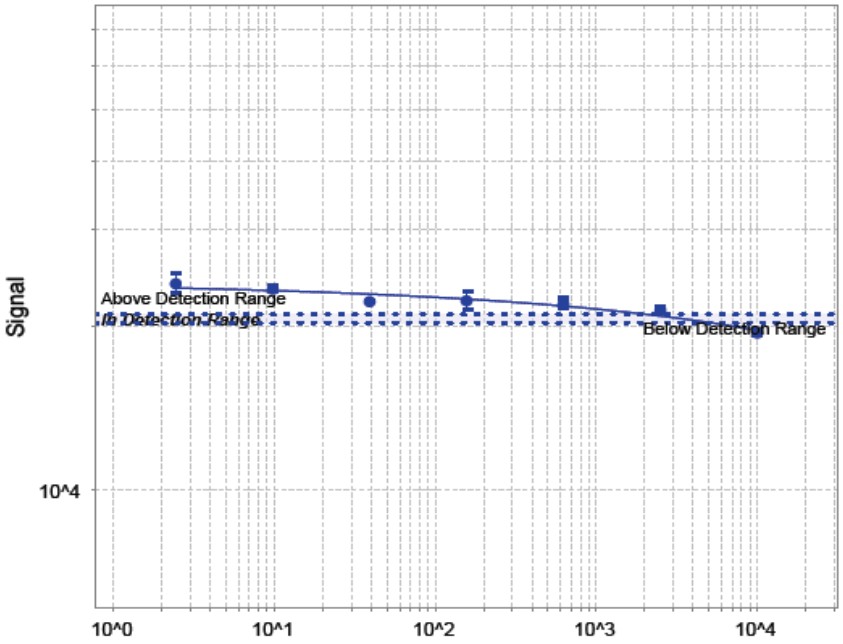
Assay works well in buffer



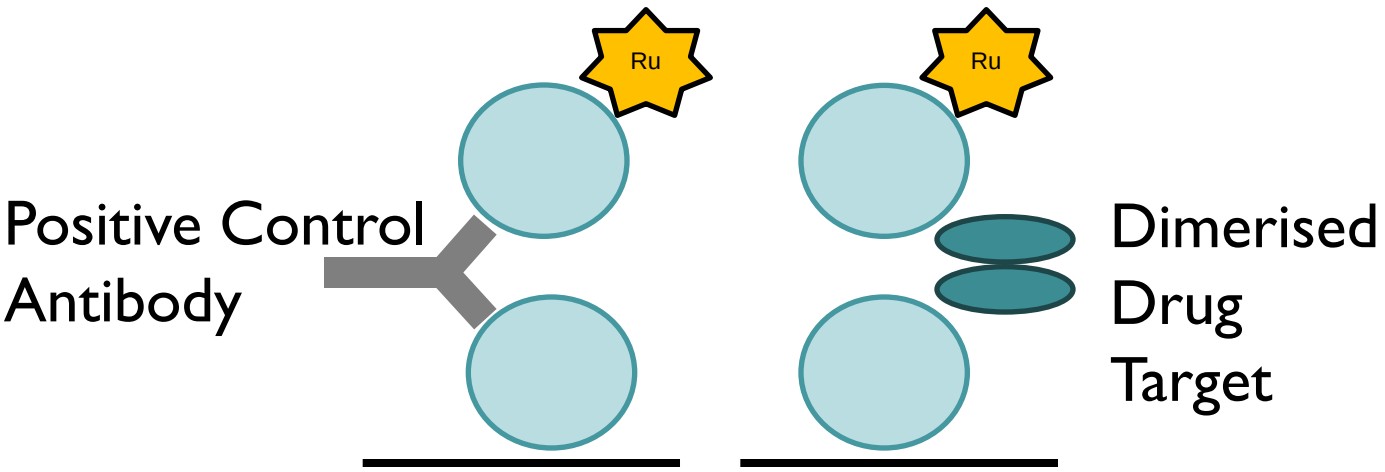
Introduce 25% serum as assay matrix

High background

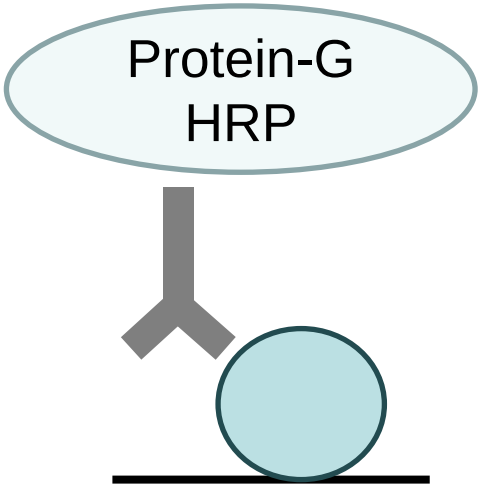
Signal reduction with increasing positive control

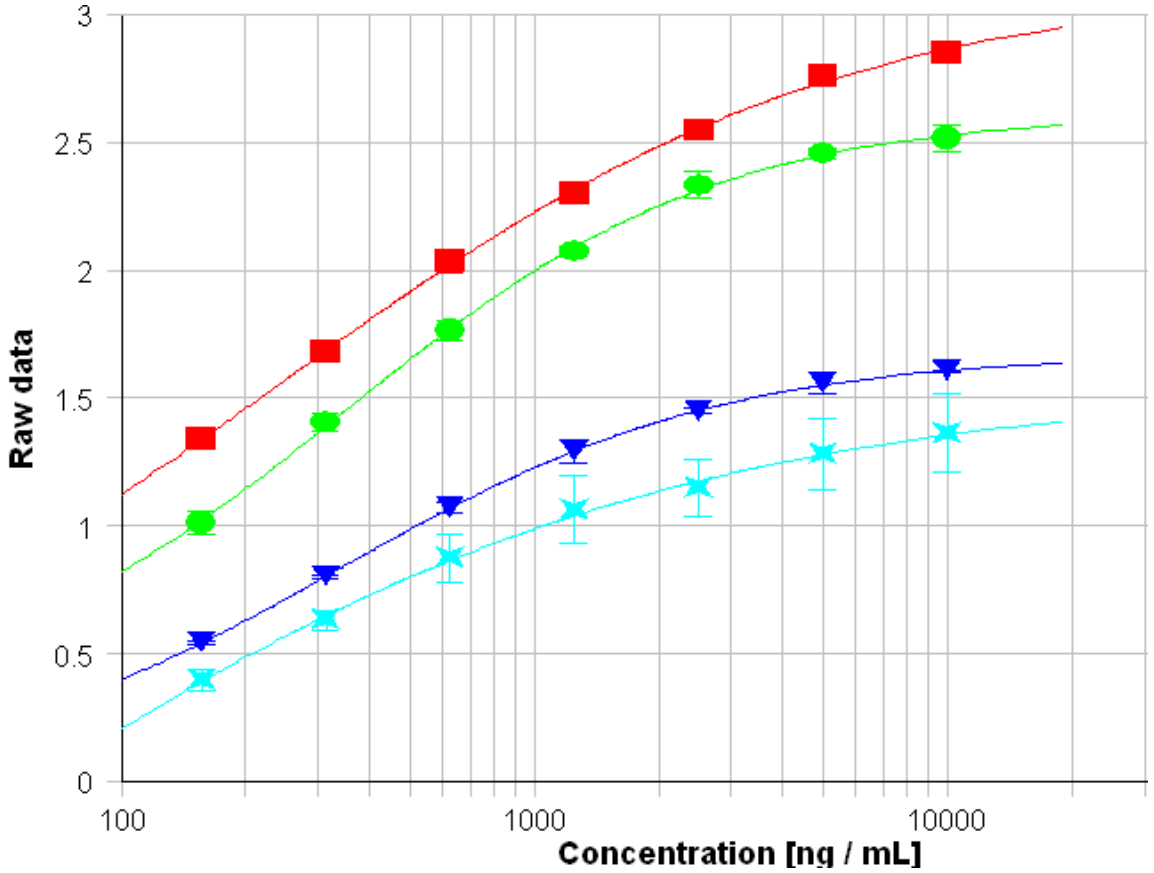


High Abundance Dimeric Drug Target?



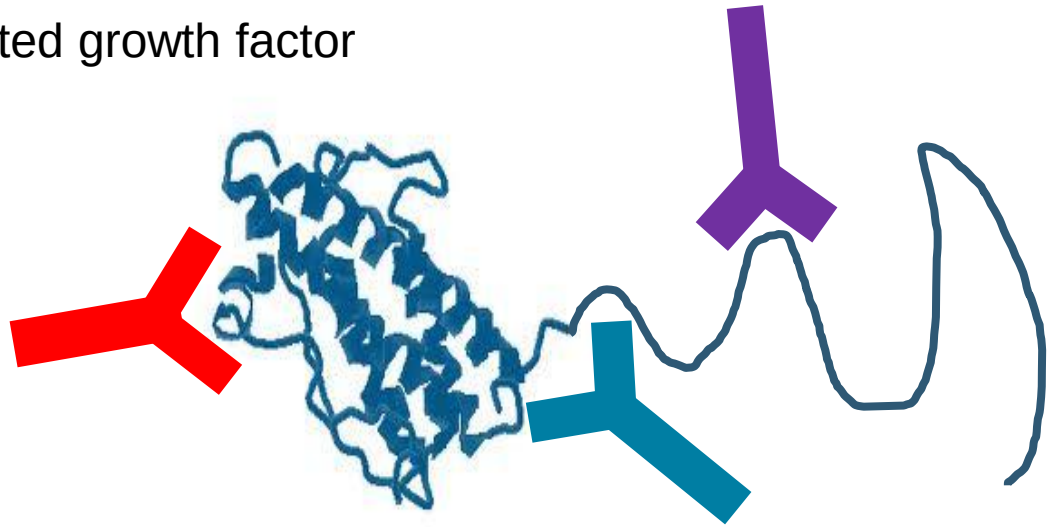
What is the Solution?
Change to Direct Binding Assay Format





Bridging Assay Pitfalls (2)
Positive Control Selection

- PEGylated growth factor
  - anti-growth factor versus
  - anti-PEG antibodies
  - Anti-PEGylated growth factor



Anti Drug Antibody Positive Control: Bridging Versus Direct Binding Assay

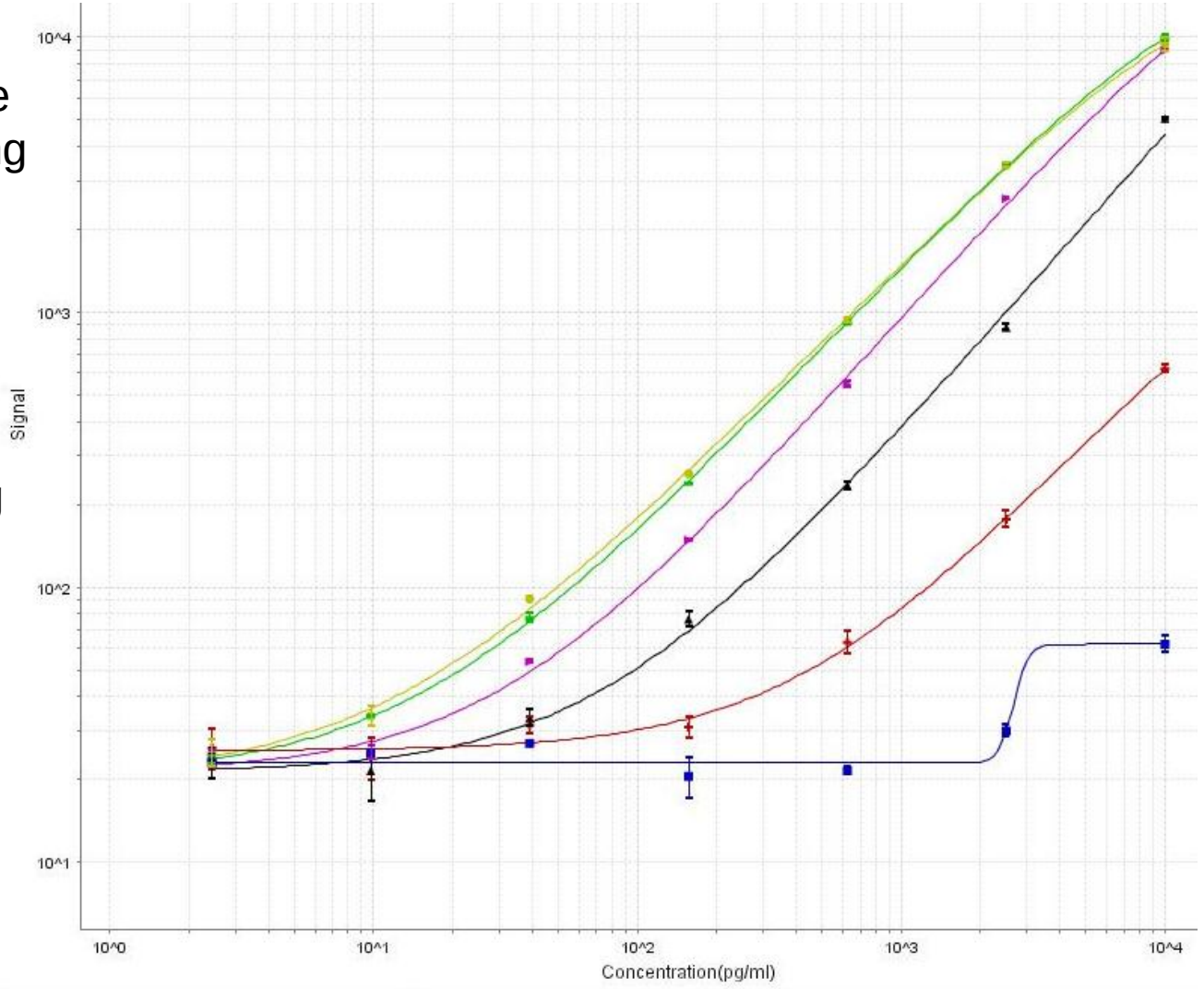
Bridging assay (MSD response units)			Direct binding assay (ELISA OD units)		
Conc (ng/mL)	Anti-growth factor detection	Anti-PEG detection	Conc (ng/mL)	Anti-growth factor detection	Anti-PEG detection
20000	28312	142	8000	2.727	2.124
5000	6959	136	4000	2.195	1.377
2500	3318	128	2000	1.524	0.865
1250	1600	141	1000	0.885	0.547
625	849	148	500	0.541	0.402
313	484	153	250	0.370	0.347
156	323	152	125	0.299	0.308
NC	157	164	0	0.207	0.308

Drug Interference:
Effect on Assay Sensitivity

Standard curve of positive control in serum containing increasing concentrations of drug

Assay sensitivity significantly affected by presence of 1 µg/mL drug

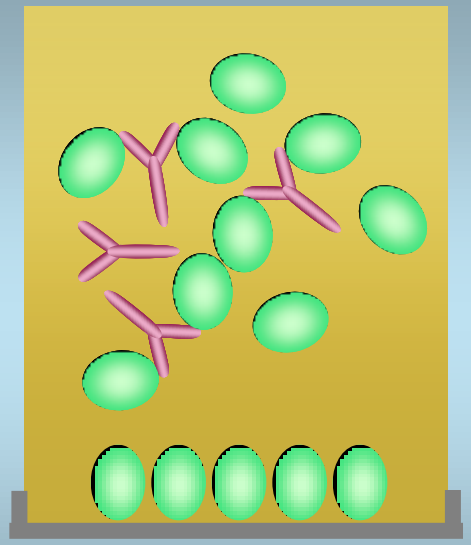
Sensitivity and Drug Interference a function of the positive control



Overcoming Drug Interference

- Major concern to all immunogenicity screening assays

High concentrations of drug in serum sample can interfere with ADA detection



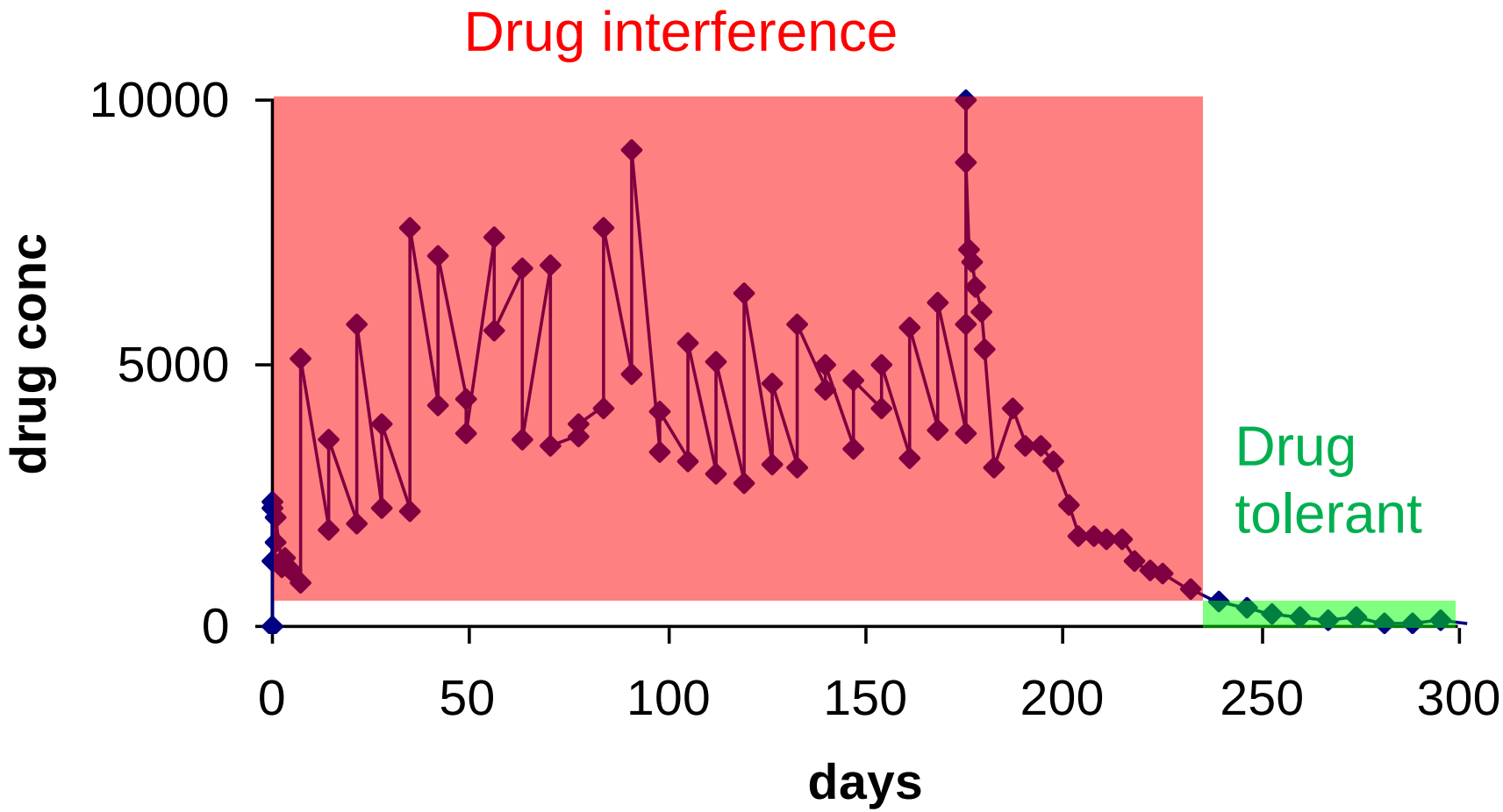
Microtitre plate with immobilised drug

Dilution of samples

Washout samples taken days/weeks after dosing

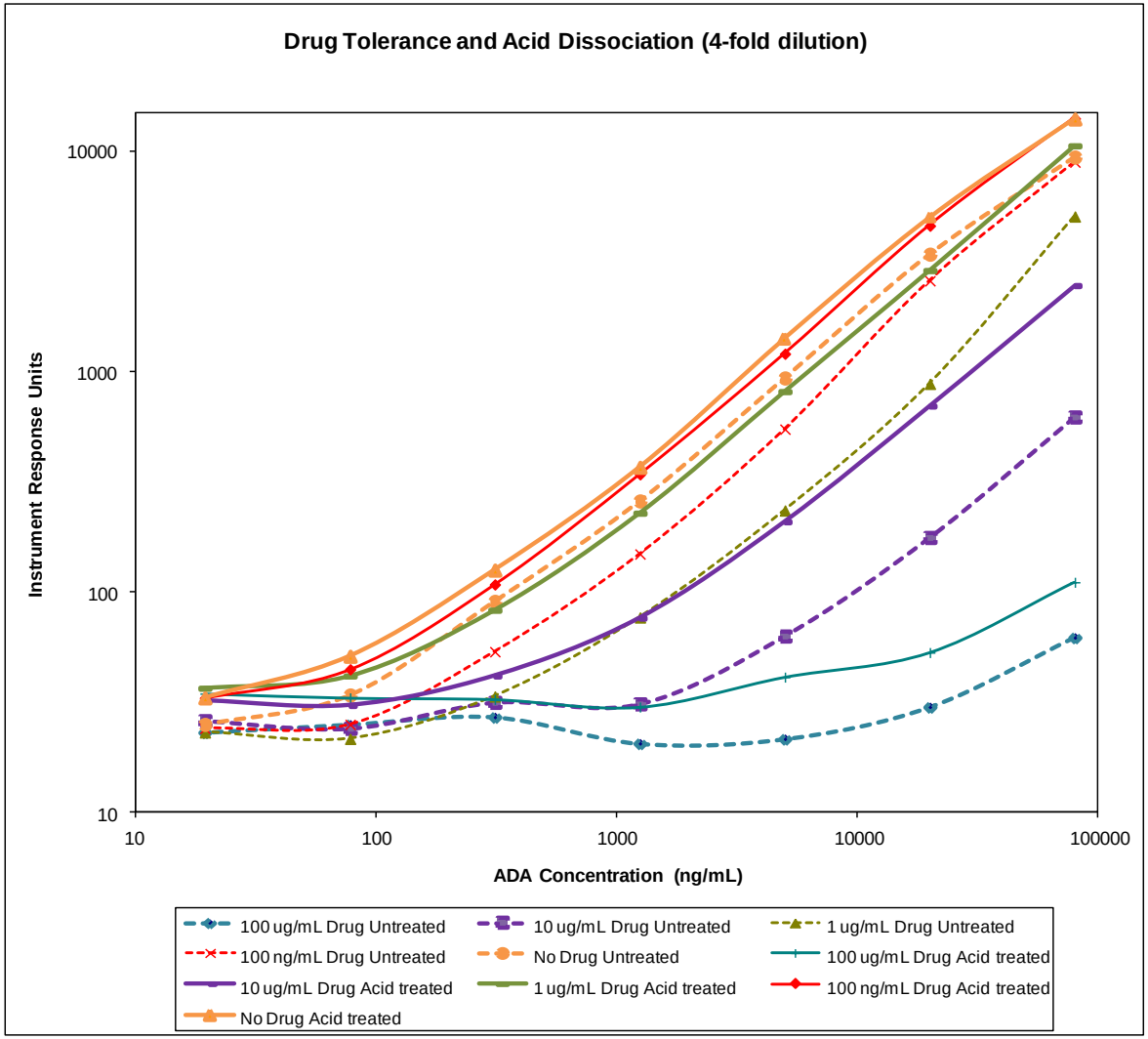
Pre-treatment to disrupt immunocomplexes (acid dissociation)

Immunogenicity Testing in Washout Period



Acid Dissociation

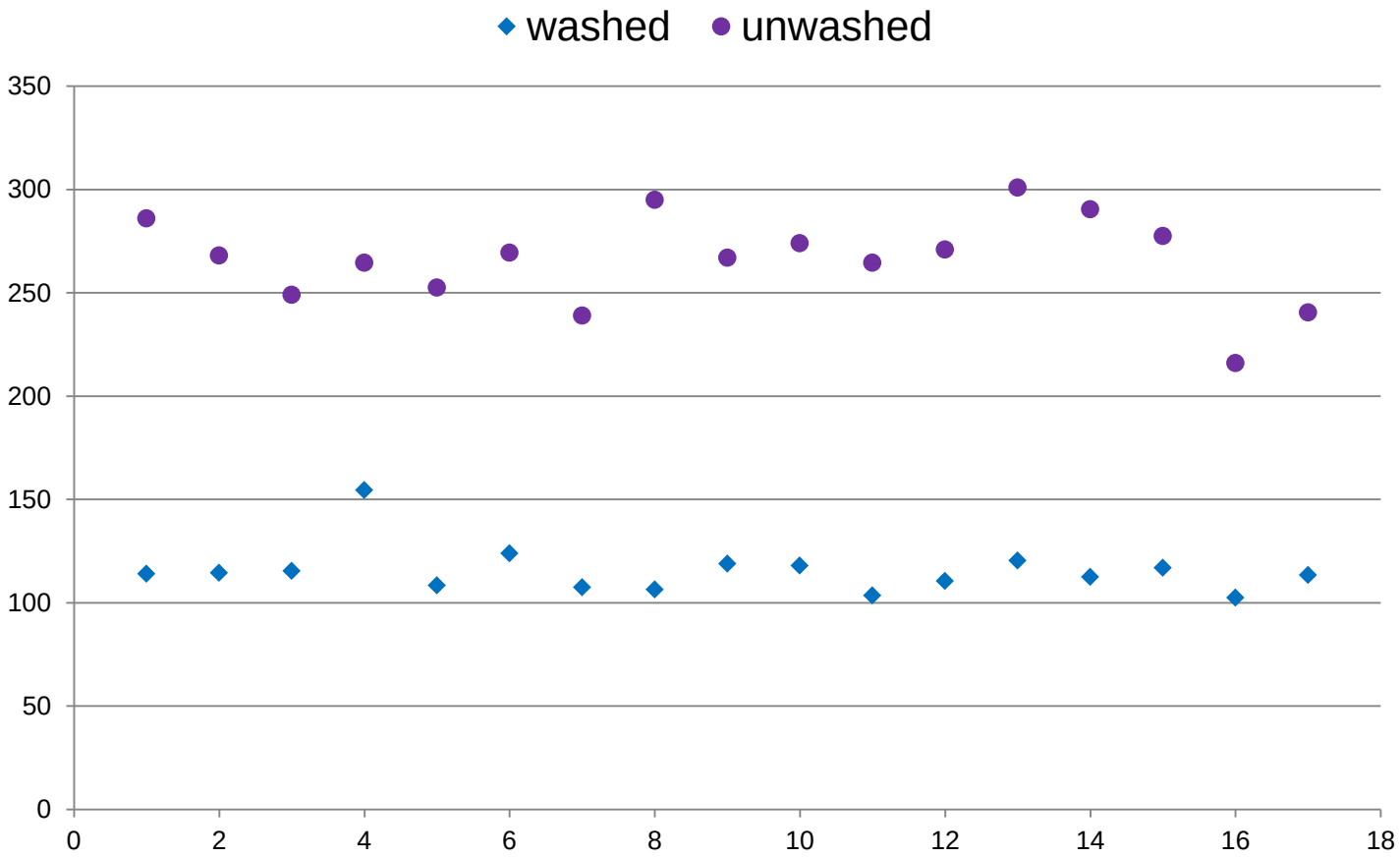
- Comparison of drug interference for samples treated with acid or PBS control
  - Treat Drug + sample with 300 mM Acetic acid 30 mins prior to assay
  - Neutralize with Trizma base containing labelled drugs
- Acid dissociation improves drug tolerance

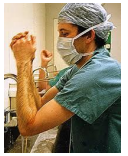


Immunogenicity and Instrument Noise

- Instrument Noise can affect cut-point determination
- Extensive washing can give low background making it difficult to determine appropriate Screening and Confirmatory Cut Points
- Reduced washing can help raise assay background
- Higher background can help produce a normal distribution

Individual Serum Responses: Washed v Unwashed

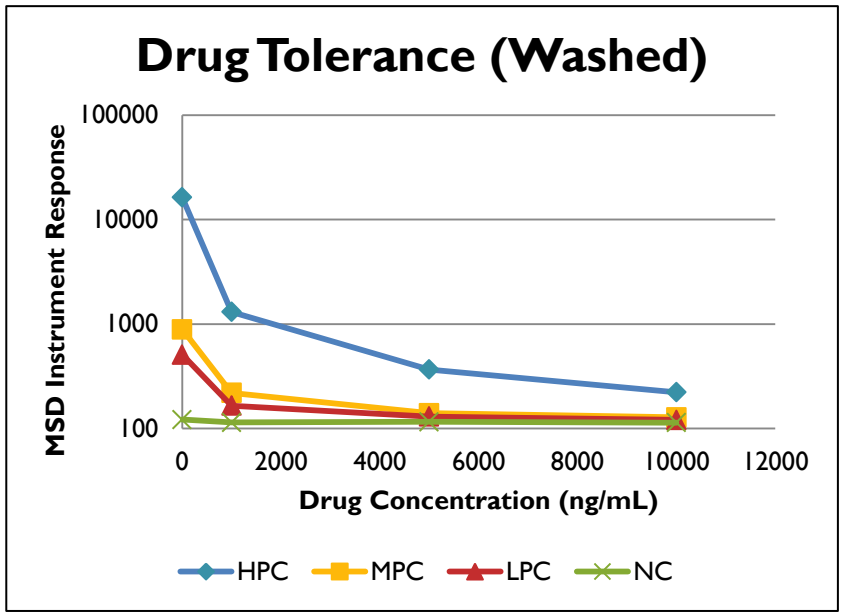


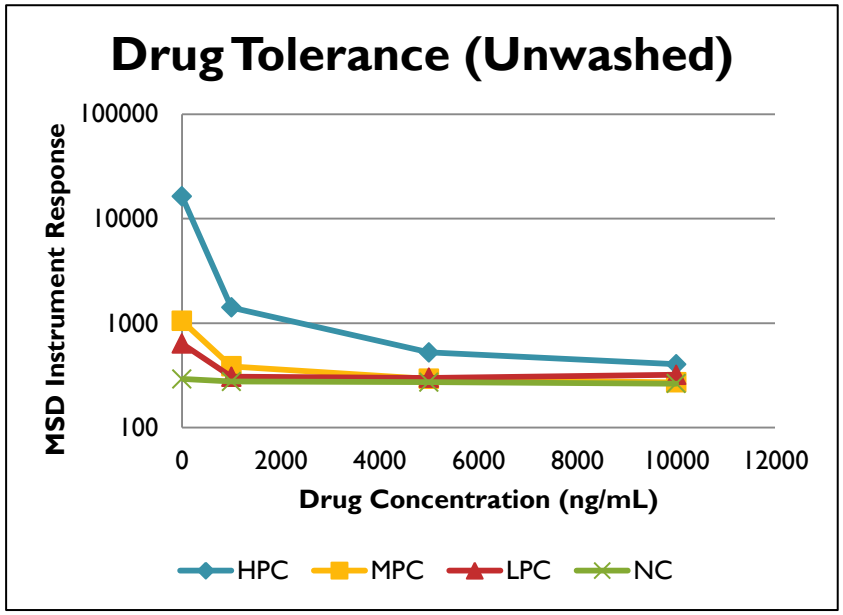


Positive Anti-Drug Antibody Control: Standard Curve

	Washed		Unwashed	
Positive control conc (ng/mL)	Instrument response	Signal:noise ratio	Instrument response	Signal:noise ratio
20000	26555	244	25990	84.7
5000	7409	68.0	7443	24.2
2500	3565	32.7	3774	12.3
1250	1713	15.7	1852	6.03
625	934	8.57	1111	3.62
313	480	4.40	655	2.13
156	283	2.59	474	1.54
Blank	109	1.00	307	1.00

Drug Tolerance: Washed versus Unwashed

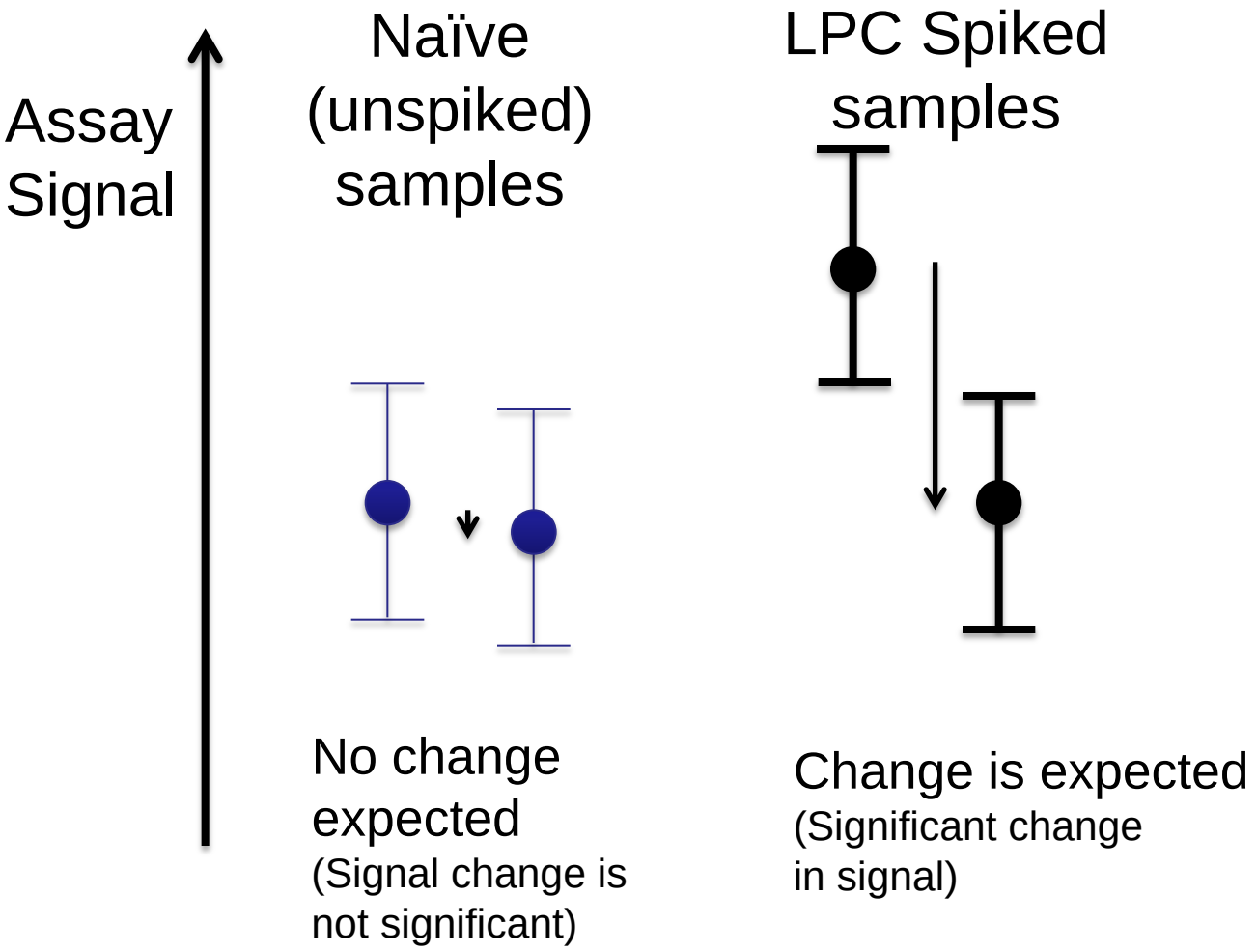




Confirmatory Cut-Point

- At least 50 individual samples
- Blank samples method
  - Naïve samples treated with free drug
  - Drug concentration is at a level sufficient to reduce the HPC to ~CP signal
  - Limit drug concentration to avoid increasing background
  - CCP = % inhibition + 2.33xSD
- LPC spike inhibition method
  - Naïve samples spiked with positive control and with free drug
  - Use upper limit of signal inhibition
  - CCP = % inhibition - 2.33xSD
- Sanity check against variance of PC samples

Naïve Aamples Versus Samples Spiked with Positive Control Anti Drug Antibody



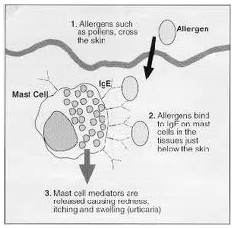
IgE Anti-Drug Antibody Assay

- Reasons to have IgE specific assay
  - Products containing non-human carbohydrate structures
  - Helping to predict products that may induce an allergic response and prepare for anaphylactic reactions in the clinic
- Background
  - Fully human antibody against a soluble target
  - In addition to anti-drug antibody assay for total response and assay was requested for IgE response
- Challenges
  - Low concentrations of IgE antibodies
  - Early and transient immune response
  - Often lack of positive control

EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

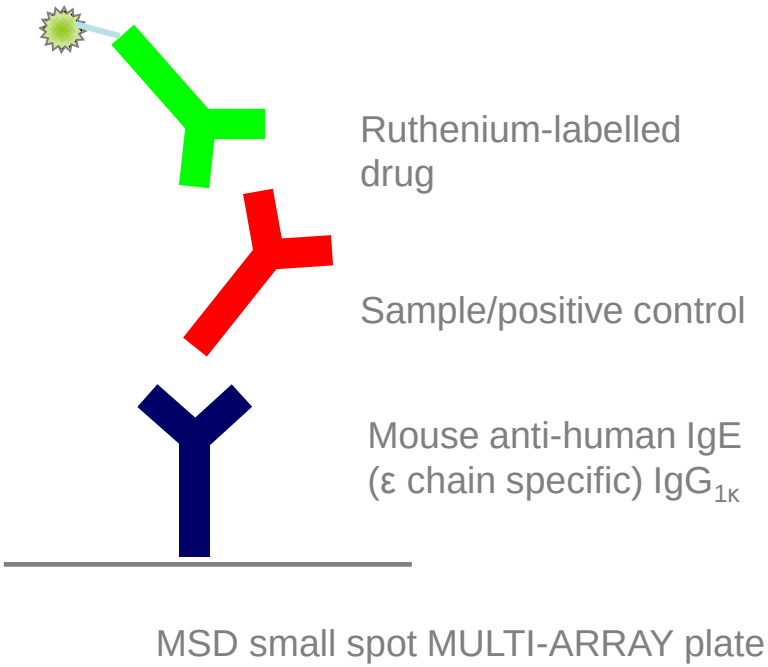
18 November 2010
EMA/CHMP/BMWP/86289/2010
Committee for Medicinal Products for Human Use (CHMP)

Guideline on immunogenicity assessment of monoclonal antibodies intended for in vivo clinical use.



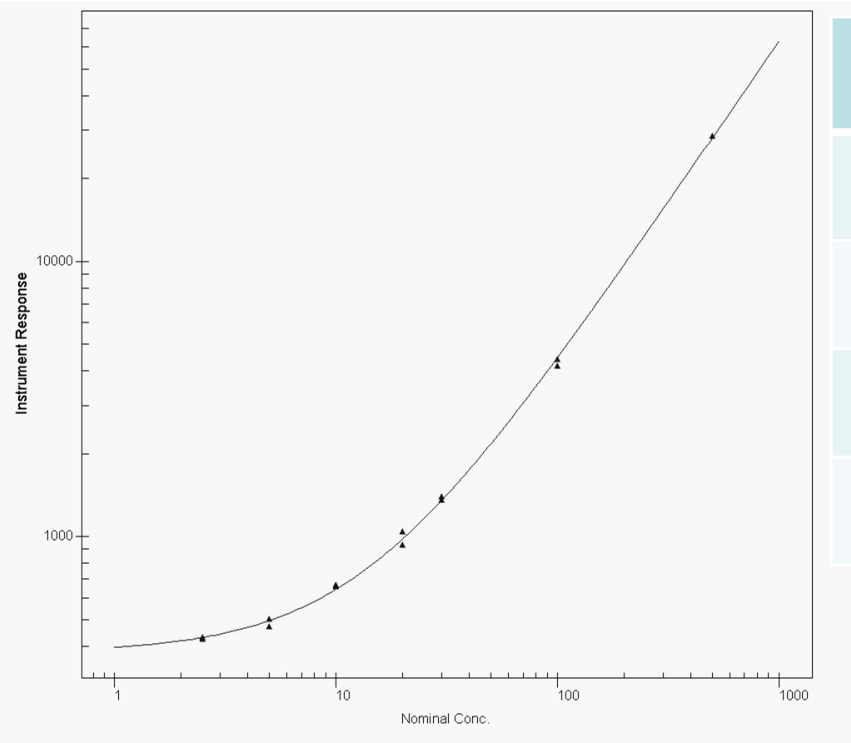
Guidance for Industry
Assay Development for Immunogenicity Testing of Therapeutic Proteins

Reverse Sandwich Assay Format for IgE Antibodies



- Coat plates with mouse anti-human IgE
- Samples at 10-fold dilution
  - Total human IgE binds
  - Positive control - human anti-drug IgE mAb
- Incubate with ruthenium labelled drug
- Add Read Buffer

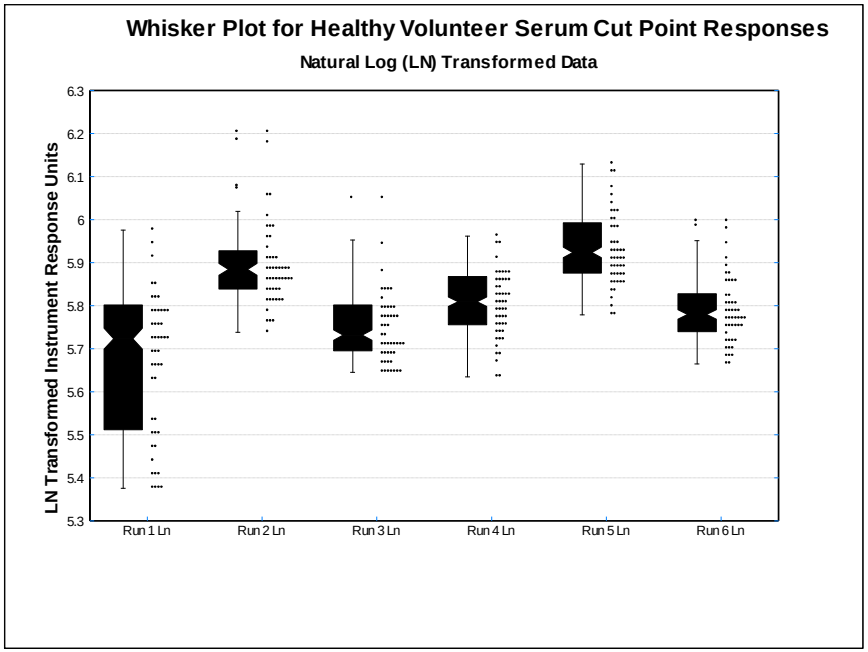
Precision and Accuracy of IgE Specific ADA Assay

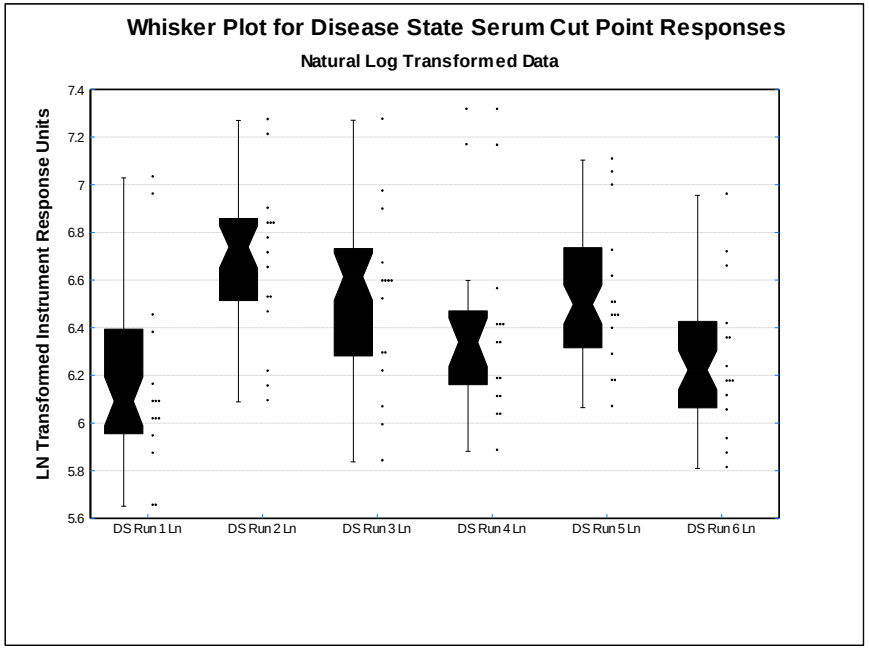


	LPC (5 ng/mL)	MPC (75 ng/mL)	HPC (300 ng/mL)
mean	4.55	62.4	248
n	64	64	64
accuracy (%CV)	59.3	13.0	16.3
precision (%RE)	-10.9	-16.8	-17.2

Cut Point Data Normal versus Disease serum

- Shapiro-Wilk test of normality indicated that the distribution sera was not normally distributed
- Log transformed data
- Cut Point Factor (CPF) calculated for each batch (95% percentile)
- Mean of the plate CPF's was reported





IgE ADA Summary

- A fit-for-purpose method for the semi-quantitative determination of IgE raised to drug in human serum has been developed and validated
- A cut point factor was established in both healthy volunteer serum samples and disease patient sera and a confirmatory cut point established
- Disease-state cut point factors will be cross-validated for new indications

Anti Drug Antibody Assay Development: Summary

- Semi-homogenous assay format on the MSD Sector 6000 platform
  - Good assay sensitivity and dynamic range for a range of target types
  - Bridging format detects all isotypes
  - Adapts well to acid dissociation, which improves drug tolerance
  - Successful on platforms other than the MSD
- Direct binding assays
  - Dimeric drugs
  - Anti-PEG response
  - Anti-IgE specific assay
- Limited washing can be beneficial
- Confirmatory cut point with naïve or positive control spiked samples
- The right positive control is critical for success

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