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Adapting Commercial Immunoassay Kits for Pre-Clinical Biomarkers: Challenges and Solutions

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Pre-Clinical Biomarkers

- Wide range of requirements for biomarkers
  - Safety/ Toxicity
  - Exploratory
  - Pharmacodynamic
- Impossible to develop specific assays
  - Range of markers
  - Number of species
- Few diagnostic kits available for tox species
  - Research use only (RUO)

Research Use Only Kits

- Species
  - Rat/ Mouse- relatively large choice
  - Cyno- not many outside cytokines/ hormones
  - Canine and Porcine Kits- very rare
- Controls
  - Only some kits come with controls
  - Recombinant material in assay buffer
- Format
  - Often Homogenous
  - Optimised for time over robustness

Challenges

- Quality and Availability
- Cross Reactive kits
- External control material
- Robustness
- Comparison of vendor kits
- Rigour of ‘fit for purpose’ Validation

RUO Kit Quality and Availability

- Research use only (RUO) kits vary wildly in quality and robustness
- Re-badging is rife- many vendors buy in kits from other suppliers and re-sell in their packaging
  - Look for common features
    - Analytical range
    - Identical product inserts/ methods
    - Performance data
- Difficult to find appropriate kit for exploratory Biomarkers
  - Cross reactivity between species
  - Adaptation of kits

Cross reactivity

- Species cross reactivity is difficult to predict
  - Homology of AA sequences
  - Distance between species
- Specificity of antibodies
  - Almost homologous analytes might not cross react
- Must use species specific external standard or endogenous samples to test
- Where an external standard is used look for linearity of dilution rather than recovery for suitability

External Control material

- Often difficult to source- especially non- rodent
  - Use best source possible and highlight compromises- fit for purpose
- Often will not get parallel response to calibrator
  - Which recombinant material is correct?
- Look at endogenous sources
  - Stimulation of PBMC's for cytokines
  - Parallelism of incurred samples
- Use kit calibrator- reconstitute in matrix for spiking experiments
- Do not expect 100% recovery when buying Material from Kit vendor

Spike recovery of ‘external’ Std

% Recovery of 160 ng/mL Spike	
Individual 1	13.8
Individual 2	11.4
Individual 3	14.0
Individual 4	13.5
Individual 5	13.3
Individual 6	12.6
Mean	***13.1***
SD	***1.0***
CV (%)	***7.5***

Kit- Rat Prolactin from Genway
Spike material- recombinant Rat Prolactin *from Genway!*

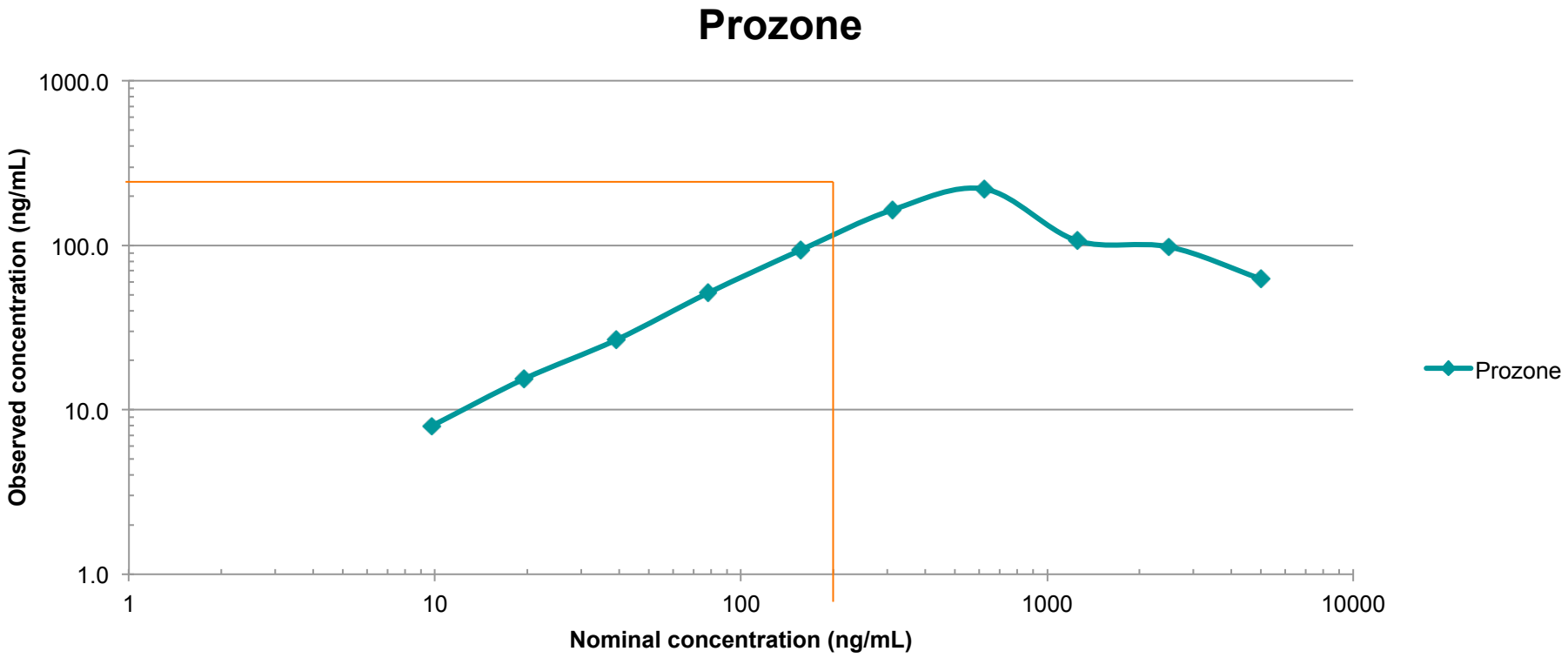
Parallelism

- Parallelism may not be required
  - Depending on the endpoint for the marker
- For discovery and exploratory endpoints
  - quasi quantitative assay
  - Distinguish between normal and high/ low results
- For PD endpoints then parallelism is required
- If kit designed for other species
  - parallelism more important
    - increases reliability of measured concentrations

Robustness

- Optimised for time
  - *Homogenous format*-Prozone
    - Split into step by step heterogenous format
    - Run samples at two dilutions
  - *Minimised incubations*-Drift
    - Extend sample and detection steps
    - Load samples from polypropylene plate
- Batch variability
  - Reserve sufficient of single lot for study
  - Use independent QC's to allow 'normalisation'

Prozone

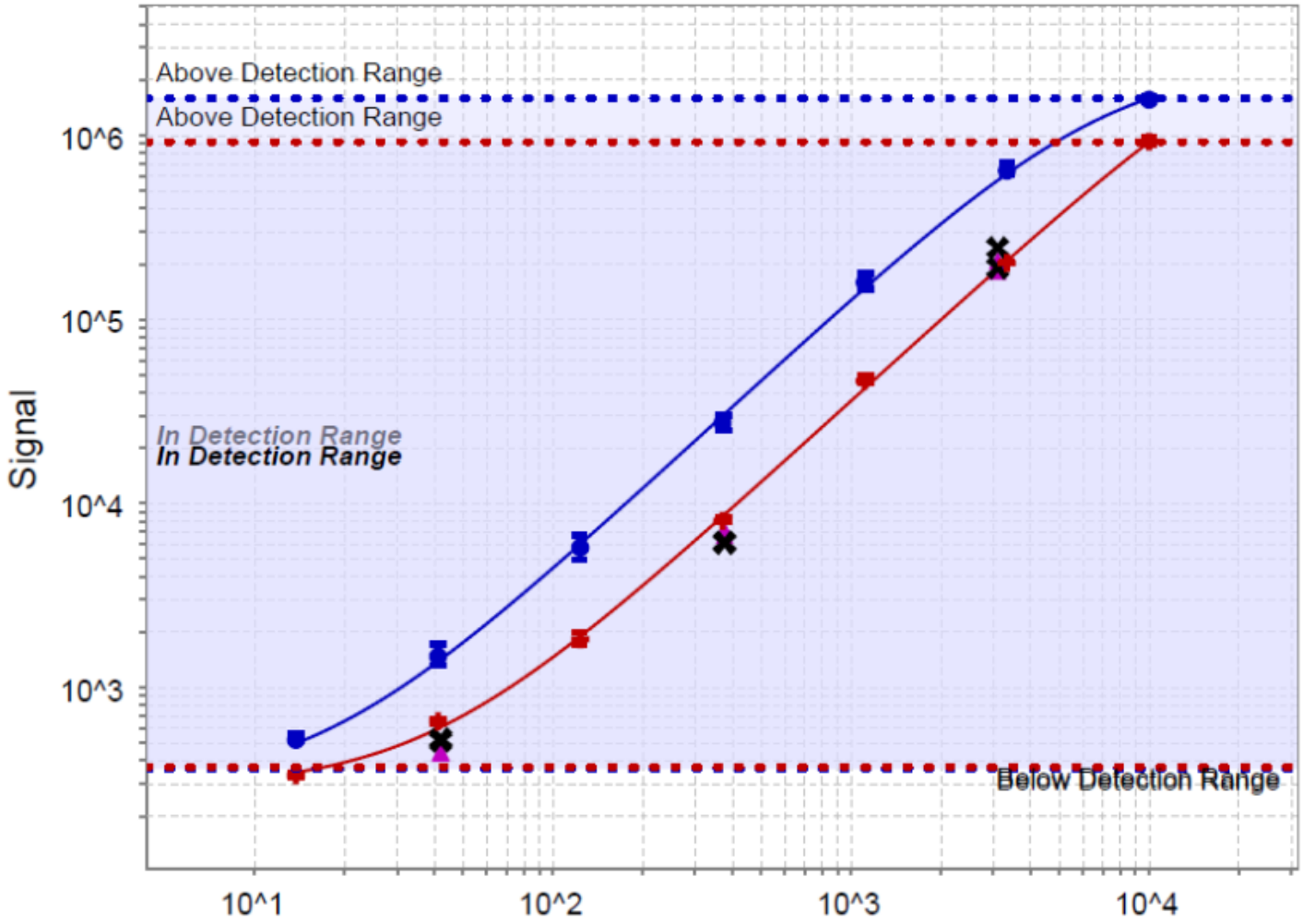


Genway Rat Prolactin

Comparison of Vendor kits

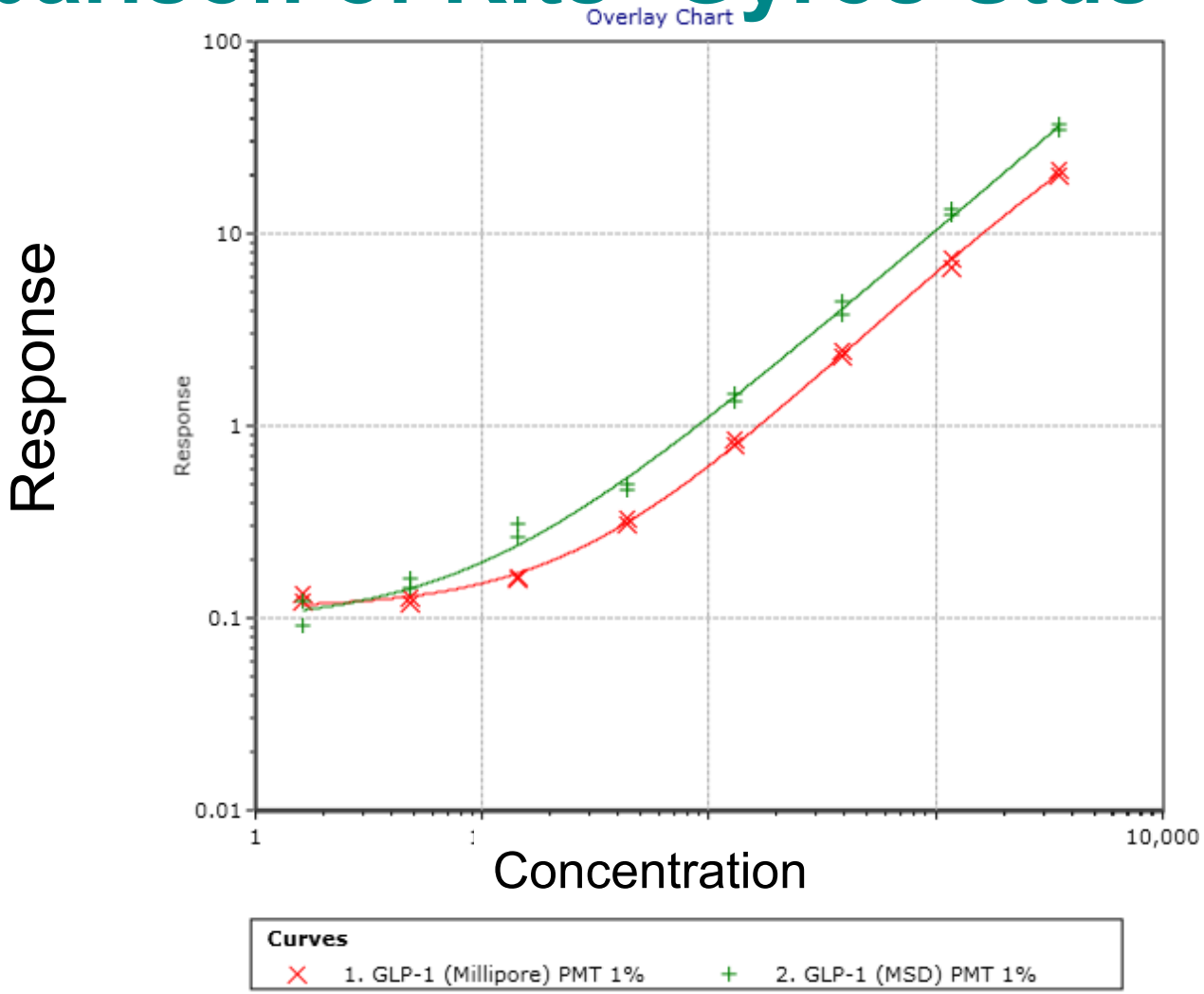
- Differing calibrator material makes comparing kit performance difficult on paper
  - Same sample can have very different results in different kits
  - Standards calibrated against external reference
    - Same material will not spike/recover as expected
    - Sensitivity cannot be reliably compared
- Run Calibrator material on both Kits
- Analyse samples in parallel with Both kits and compare results

Comparison of Kits- MSD Stds



MSD Metabolic Panel GLP-1 vs Millipore Gyros GLP-1

Comparison of Kits- Gyros Stds



Millipore Gyros GLP-1 vs MSD Metabolic Panel GLP-1

Comparison of Kits- QC

Gyros Calibrators vs. MSD Calibrators		
QC1	QC2	QC3
-77.6	-14.3	-

Gyros vs MSD		
QC1	QC2	QC3
-73.2	-50.4	-34.0

Multiplex Assays

- Compromise between analytes
- Robustness and range for increased data per sample
- Loss of quantitation counteracted by complementary data
- Sample volume critical for Pre-clinical assays
  - PK, ADA, Biomarkers, Immunotox, Cell Based Assays plus H&B!
  - Multiplexing allows reduction of animal use

Multiplex curves - working range

	Std1	Std2	Std3	Std4	Std5	Std6	Std7	Std8	Std9
G-CSF	1.52	4.57	13.7	41.2	123	370	1110	3330	10000
IFNg	1.52	4.57	13.7	41.2	123	370	1110	3330	10000
IL-1B	1.52	4.57	13.7	41.2	123	370	1110	3330	10000
IL-2	1.52	4.57	13.7	41.2	123	370	1110	3330	10000
IL-4	3.05	9.14	27.4	82.3	247	741	2220	6670	20000
IL-5	1.52	4.57	13.7	41.2	123	370	1110	3330	10000
IL-6	1.52	4.57	13.7	41.2	123	370	1110	3330	10000
IL-8	1.52	4.57	13.7	41.2	123	370	1110	3330	10000
TNFa	1.52	4.57	13.7	41.2	123	370	1110	3330	10000

Milliplex- NHP Cytokine Panel Std Curves (pg/mL)

Vendor Assessment

- Quality standards (ISO 9000, CE, 510k etc)
- Previous experiences / Industry profile
- Calibrated against international standard
- Specialist area
- Information provided in kit
  - Performance details
  - Kit specificity
  - Vendor validation packages

Vendor assessment- Tiered approach

Biomarker endpoint / Vendor Risk	Investigation	Exploratory	Safety	PD endpoint
Unknown	Limited	Partial	Full	Full
High	Limited	Partial	Full	Full
Medium	Limited	Limited	Partial	Full
Low	“Out of Box”	Limited	Partial	Full
Validated	“Out of Box”	“Out of Box”	Partial	Full

Validation Rigour

- ‘Out of the Box’- Vendor qualification
- Limited Validation (Limited)
  - 2-3 runs
  - Linearity & selectivity (MidQC spike)
  - Precision- repeat analysis of selectivity
- Partial Validation
  - 4 runs
  - Linearity and selectivity (Low and High)
  - 3 level QC Precision
- Full validation-Lee et al
- For all- Stability as appropriate

Summary

- Use Vendor assessment to ensure quality kits
- Many Markers will cross react between species
  - Ensure cross reactivity is understood
- Improve robustness by changing formats or timings
- Compare Kits by experiment
  - Do not use marketing claims of sensitivity
- Include Vendor assessment when deciding on validation Rigour

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