Advances in ADA, PK, and biomarker immunoassays to meet demands for assay speed and performance

14th EBF Open Symposium, 24-26 November 2021

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GYROS PROTEIN Technologies

AGENDA

- Singlet analysis for ADA detection
- Development of a generic ADA assay for preclinical studies
- Extending sensitivity for PK and biomarker assays

Singlet vs duplicate Gyrolab pembrolizumab immunogenicity assay - Covance

Goals of microfluidic Gyrolab ADA assay:

Automate assay using Mixing CD 96

Evaluate singlet vs duplicate analysis

Evaluate drug tolerance of assay

Research Article

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Bioanalysis

Comparing singlet and duplicate immunogenicity assay in human plasma for pembrolizumab using Gyrolab®

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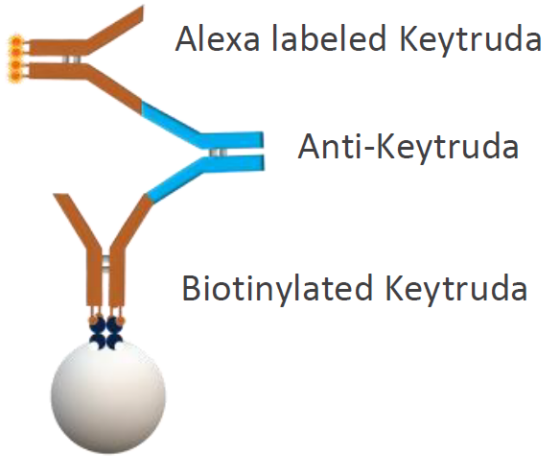
PEMBROLIZUMAB METHOD – BRIDGING ASSAY

- Capture: Biotinylated pembrolizumab
- Detect: Alexa Fluor 647-labeled pembrolizumab
- Positive control:
  Hu IgG1 anti-pembrolizumab Ab (BioRad)
- Mixing CD 96
- Gyrolab xP workstation



- Samples aliquoted and diluted 1:5 in Rexxip ADA buffer
- Method sequence: analyte, acidic buffer, master mix/neutralization

PRECISION OF SCREENING ASSAY – NO IMPROVEMENT WITH DUPLICATES

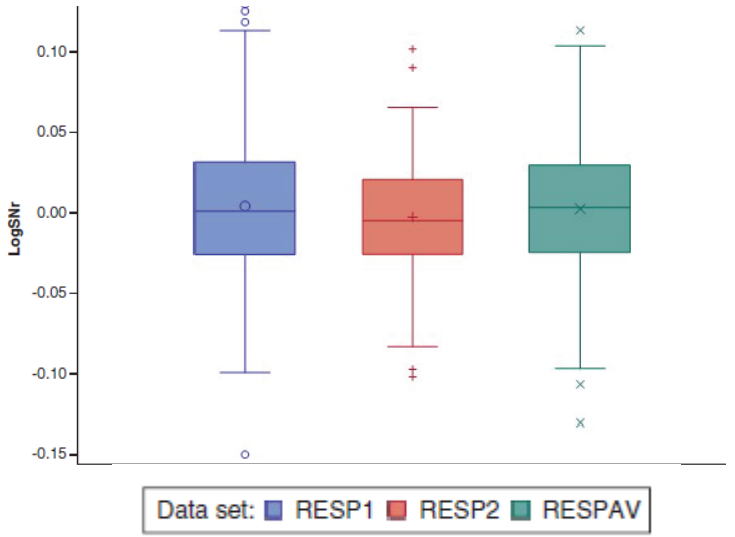
Between run precision over six runs, analyzed by three analysts.

Precision	Positive control	Run 1	Run 2	Ave
Whole CD precision	LPC HPC	7.1% 6.0%	3.4% 7.7%	4.0% 6.3%
Inter-run precision	LPC HPC	8.0% 5.5%	8.4% 6.1%	7.9% 5.6%

CD: Compact disc; HPC: High positive control; LPC: Low positive con

LPC: 50 ng/mL; HPC: 10,000 ng/mL

Box plots of screening data (outliers excluded)



Singlet and duplicate (ave. of 2 runs) values showed no significant improvements to the data quality by using the average result over a single measurement

Drug tolerance

- Assay drug tolerance calculated to be 1796 µg/mL pembrolizumab at 50 ng/mL PC
  - Tested at 50, 100, 200, 500 and 1000 µg/mL
  - Positive control at 50, 100 and 250 µg/mL
  - All response levels above cut point
- Higher drug tolerance than Gyrolab assay protocol
  - Gyrolab assay protocol drug tolerance of 640 µg/mL at 100 ng/mL PC

	Calculated drug tolerance for each cutpoint (µg/mL)		
PC level (ng/mL)	**Measurement 1 (± difference to ave)**	**Measurement 2 (± difference to ave)**	**Ave**
50	1779 (-17)	1832 (+36)	1796
100	2642 (-14)	2685 (+29)	2656
250	2616 (-5)	2632 (+11)	2621

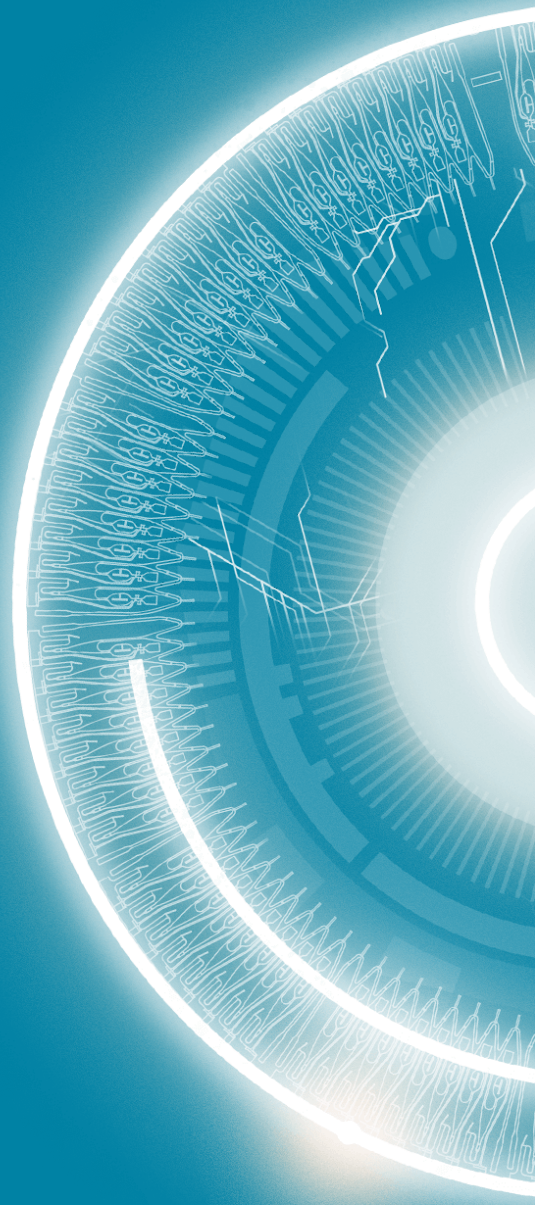
SUMMARY

- Gyrolab ADA assay using mixing CD automates acid dissociation
- High precision (<15%) for both Gyrolab assay protocol and Covance developed assays
- Drug tolerance (640 and >1000 µg/mL for Gyrolab and Covance assays, respectively) appropriate for clinical use
- Automation and use of singlet analysis increases productivity for bioanalytical laboratories
- Significant time savings

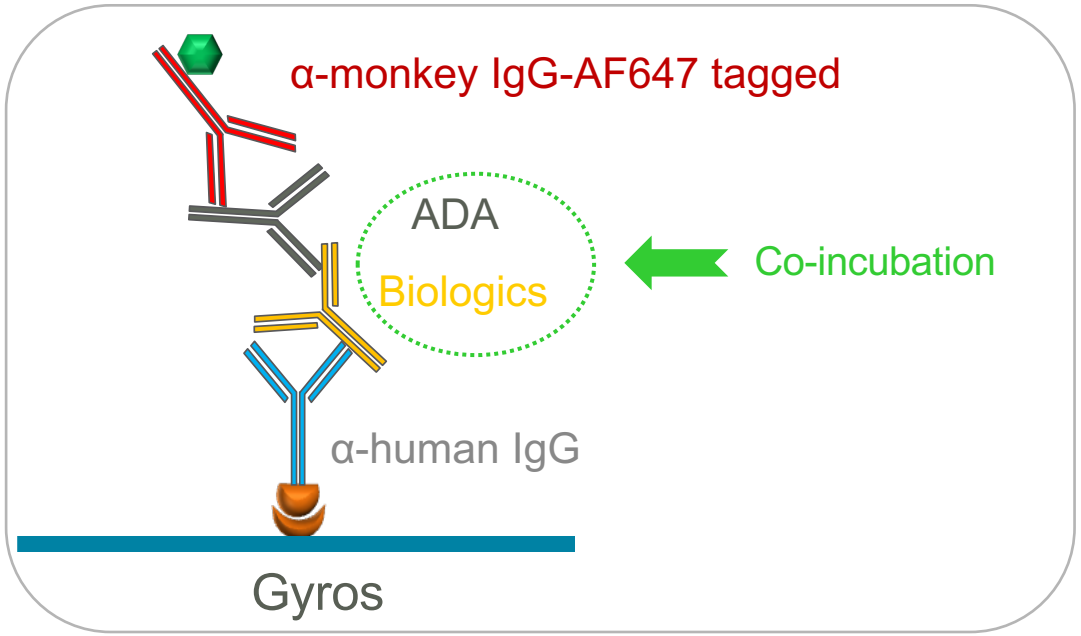
GENERIC ADA ASSAY

Developed by: Romain Gauchet and Franck Levasseur, DMPK Department – Biokinetics

Servier



ADA Generic Method: Format in Monkey



Benefits: « Plug and Play method » ➔ no need to tag each new Biologics with biotin, just need to coat biotinylated anti-human IgG to quantify all ADAs targeting the Biologics under development

Limitations: Method only works for IgG-based biologics; not working with other therapeutic protein structures (e.g hormone)

The challenge to validate the format of this generic method has been to prepare a generic positive control and to identify a suitable and reliable cut-point strategy

Generic Method vs Specific Method

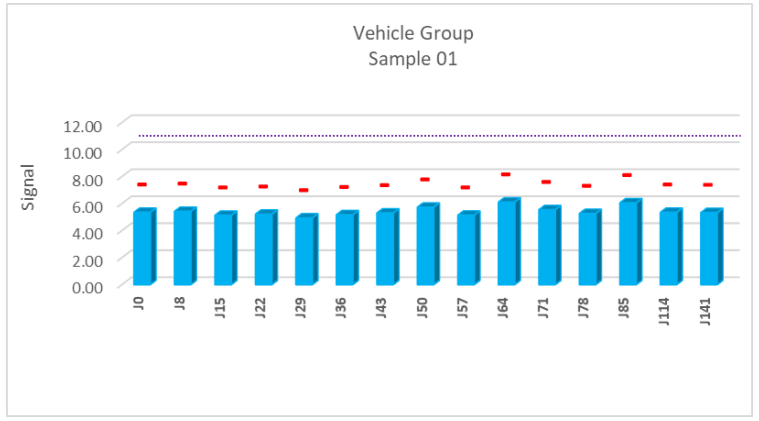
Tested Biologics :

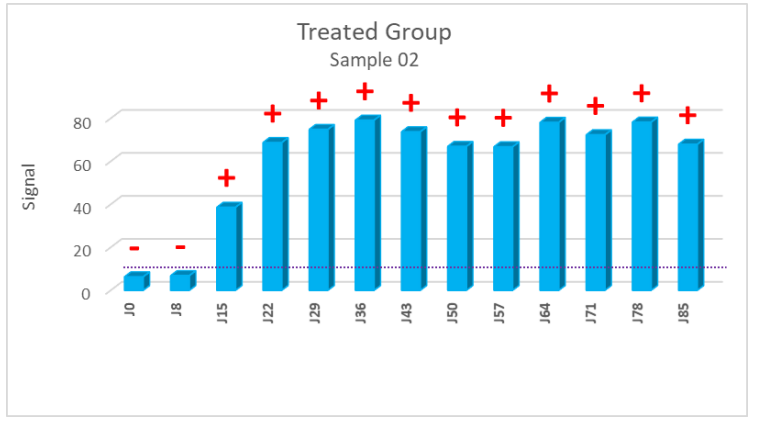
- Compound A (monoclonal Ab)
- Compound B (IgG-based compound)
- Compound C (IgG-based compound)

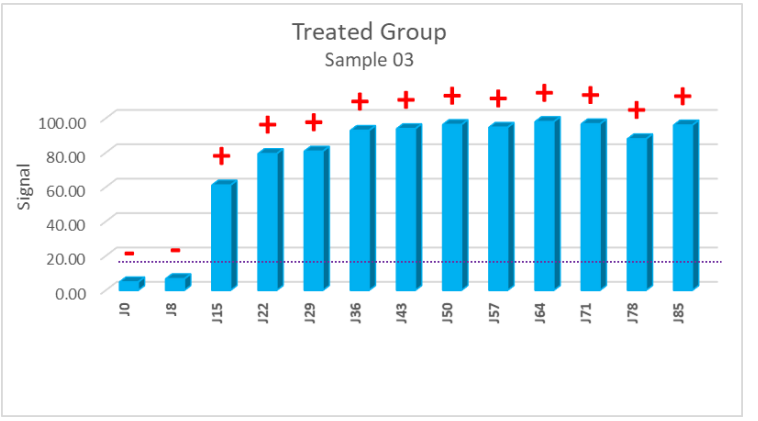
Comparison of ADA results between validated/qualified method and generic method

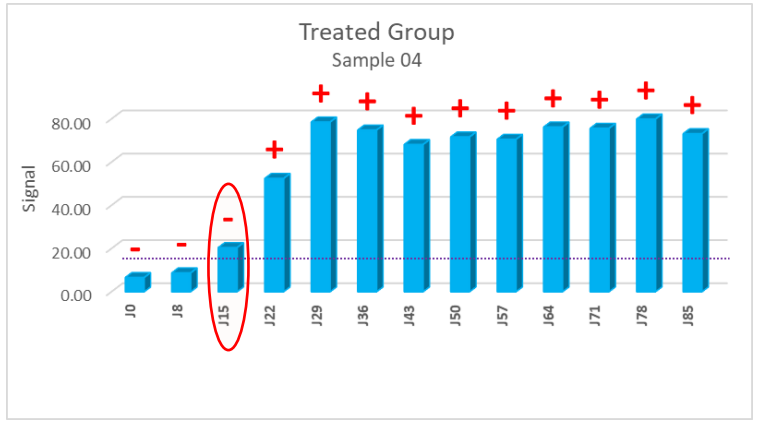
Compound A	Monoclonal antibody	IgG4	Bridging	Generic method
Compound B	IgG-based compound	IgG1	Sandwich	
Compound C	IgG-based compound	IgG1	Sandwich	

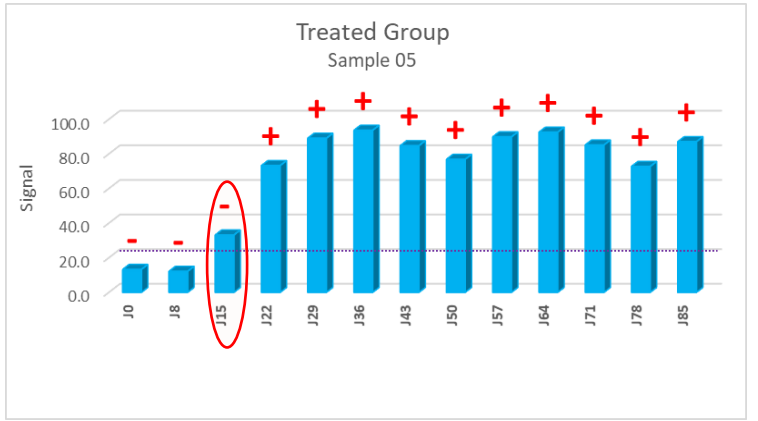
GENERIC METHOD VS SPECIFIC METHOD: COMPOUND A







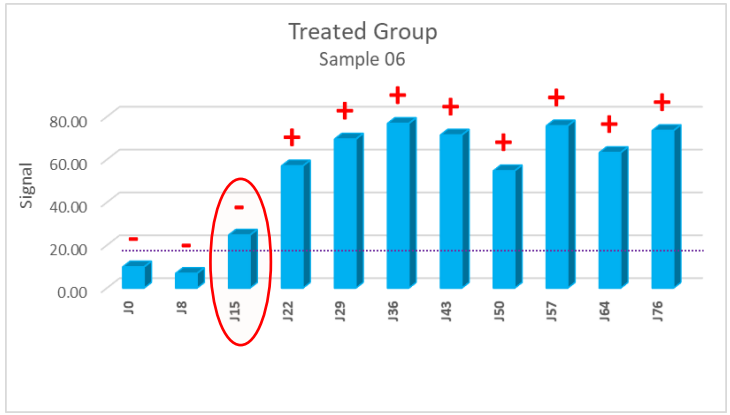


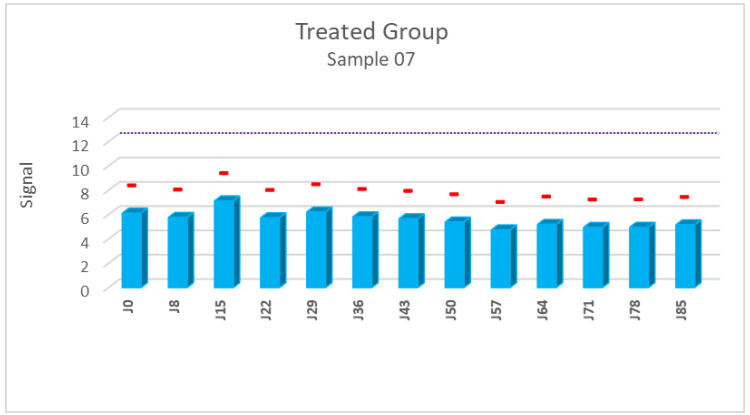


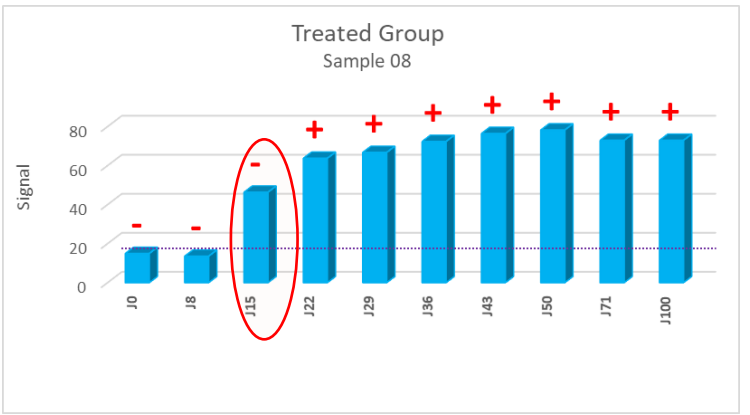
..... **Calculated Preliminary Cut-Point = 1.25* Mean NC**

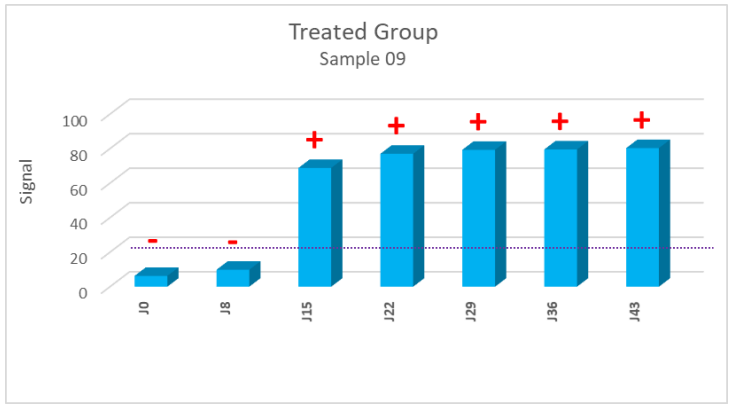
-/+ : Specific Method Results

GENERIC METHOD VS SPECIFIC METHOD: COMPOUND A







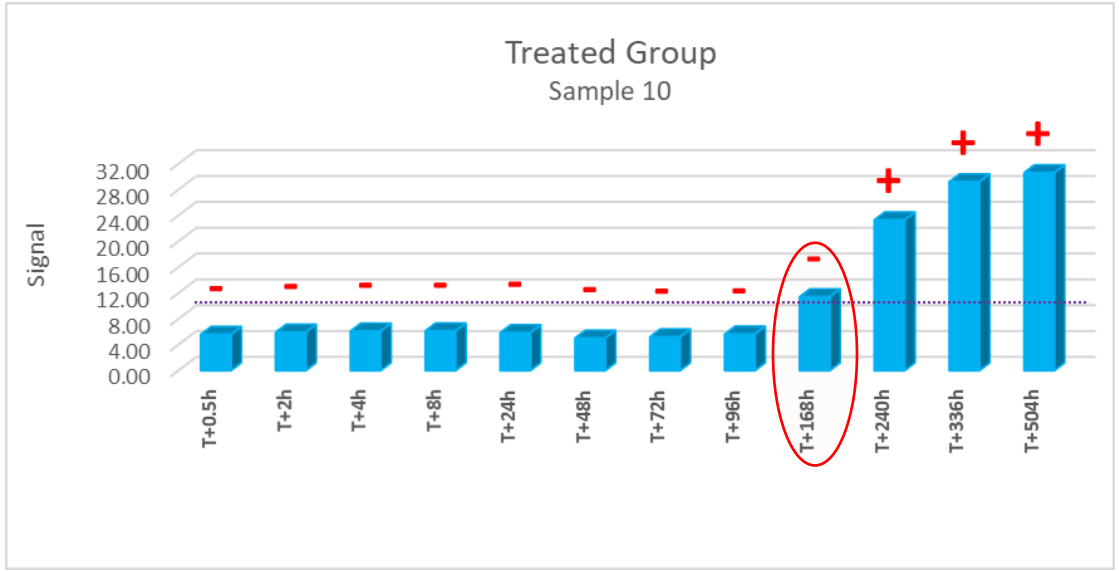


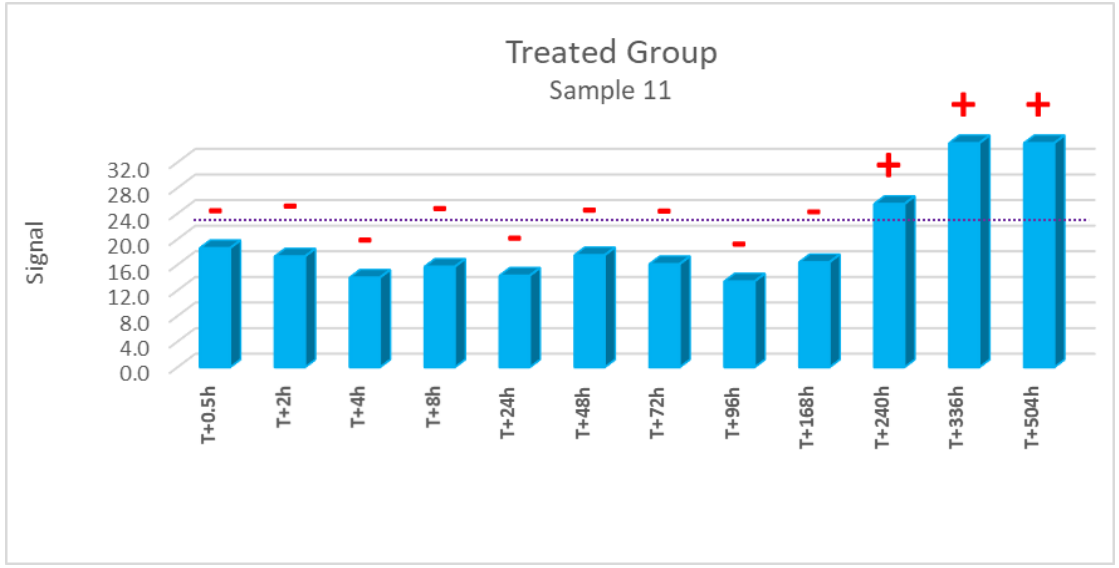
..... **Calculated Preliminary Cut-Point = 1.25* Mean NC**

-/+ : Specific Method Results

Good correlation between Specific and Generic Method
Format works well and trends of signal correlate between both methods

GENERIC METHOD VS SPECIFIC METHOD: COMPOUND B



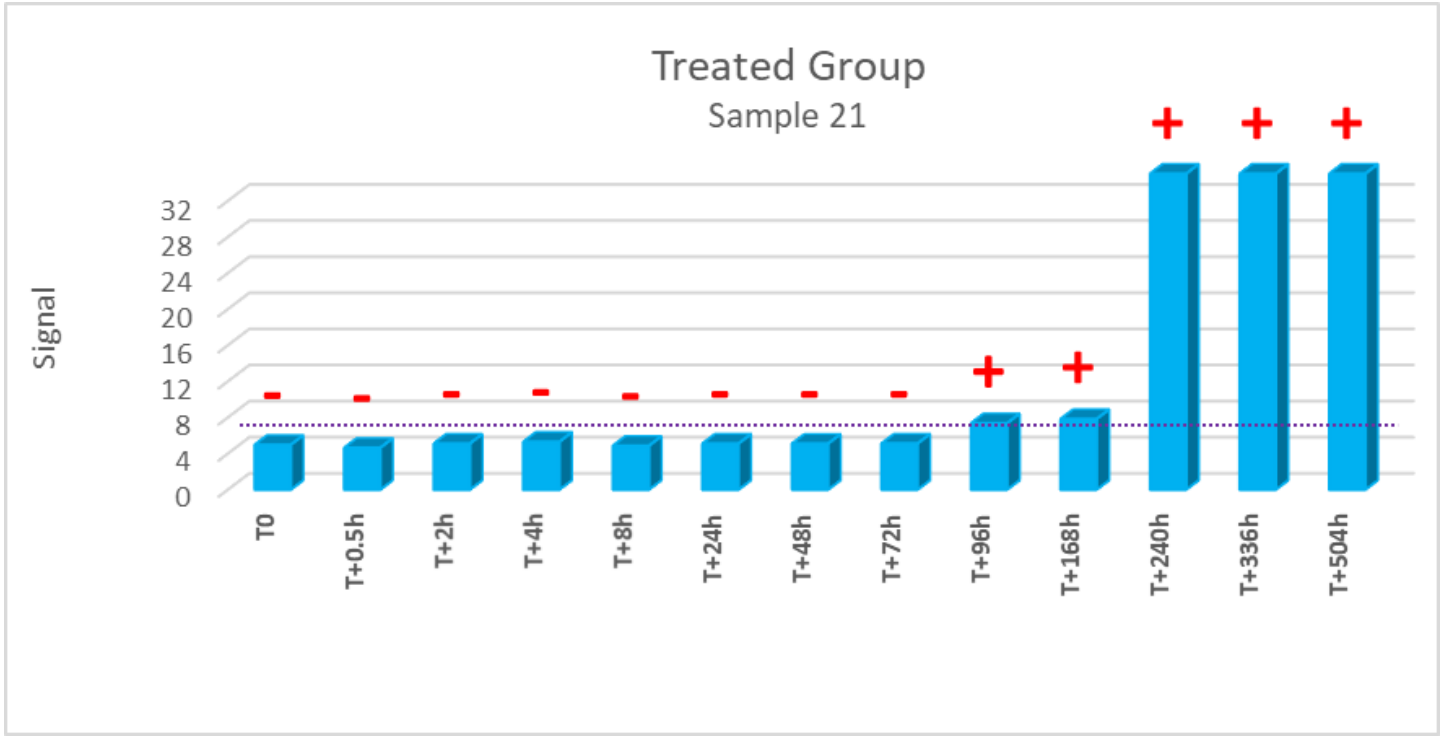


Calculated Preliminary Cut-Point = 1.25* Mean NC

-/+ : Specific Method Results

Good correlation between Specific and Generic Method
Format works well and trends of signal correlate between both methods

GENERIC METHOD VS SPECIFIC METHOD: COMPOUND C



.....Calculated Preliminary Cut-Point = 1.25* Mean NC

-/+ : Specific Method Results

Good correlation between specific and generic methods:
Identical ADA status and same trend for signal level across samples
→ Generic format performs well

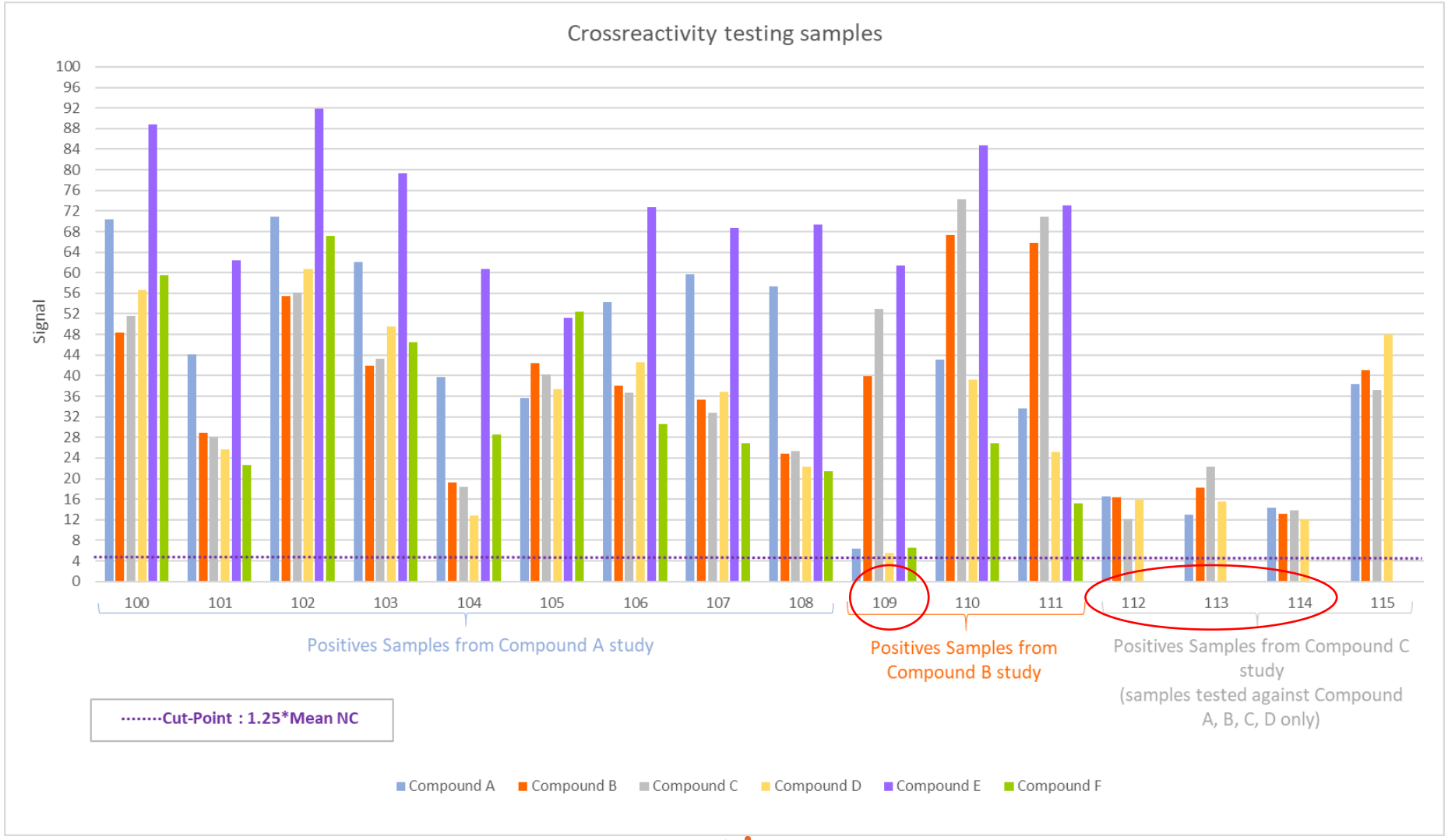
PC Production

- Selection of ADA+ samples from 3 monkey studies and cross-reactivity testing against 5 Biologics

- Selection of crossreacting ADA samples to constitute a high polyspecific positive control pool :

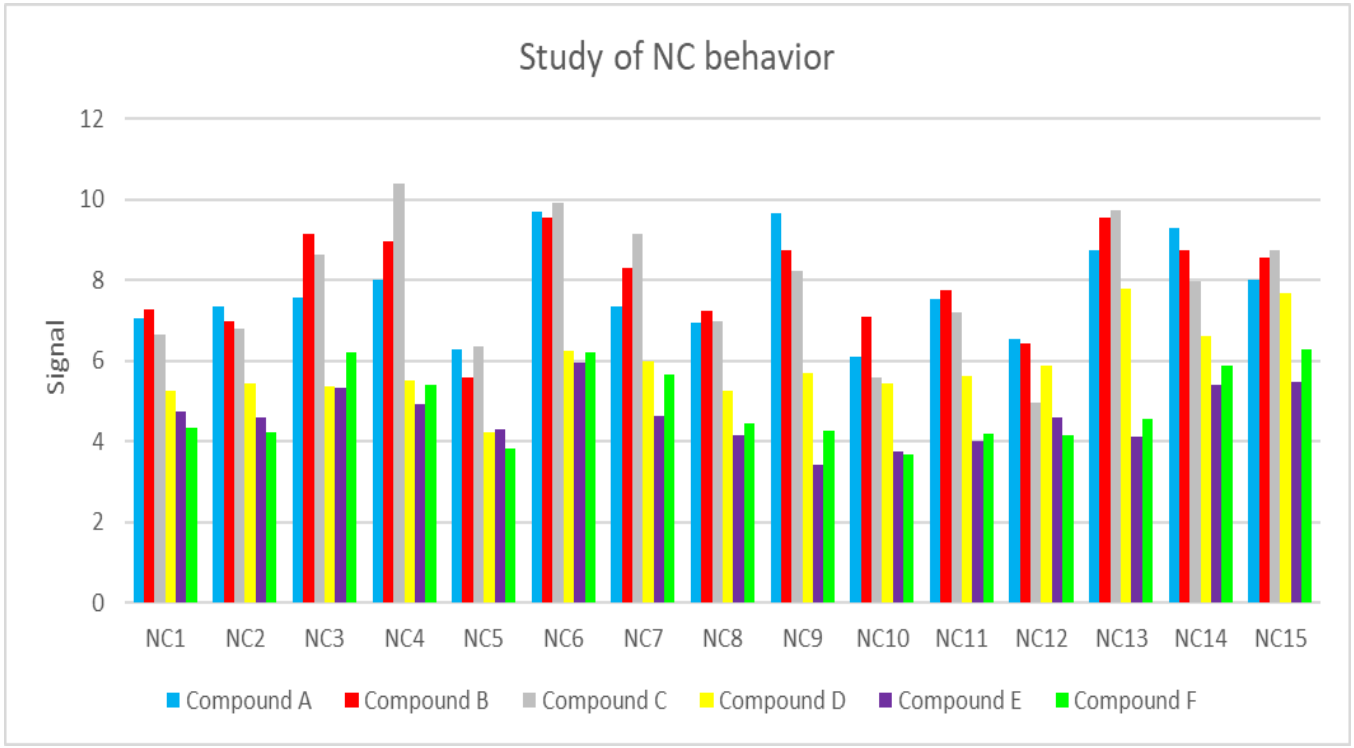
	Biologics tested for ADA cross-reaction					
	Compound A mAb IgG4	Compound B based IgG1	Compound C based IgG1	Compound D mAb IgG1	Compound E ADC	Compound F ADC
9 Positive Samples from Compound A study		9 ✓	9 ✓	9 ✓	9 ✓	9 ✓
3 Positive Samples from Compound B study	2 ✓		3 ✓	2 ✓	3 ✓	2 ✓
4 Positive Samples from Compound C study	4 ✓	4 ✓		4 ✓	Not Tested	Not Tested

PC PRODUCTION



CUT-POINT STRATEGY

- Naïve monkey samples (ADA negative) behavior against 6 different Biologics:



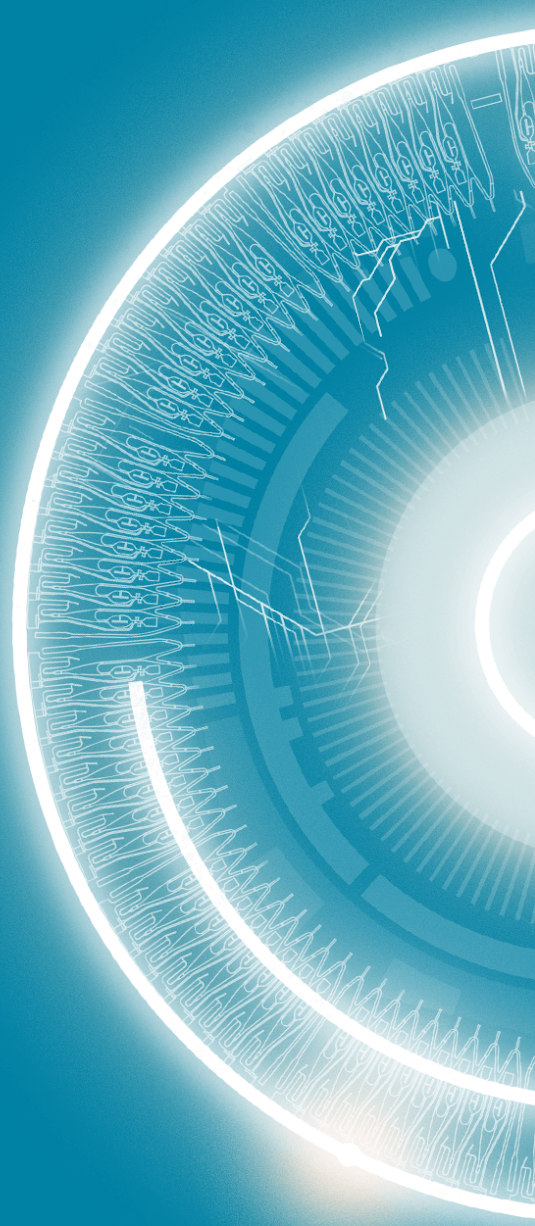
	Mean	SD	CV%
Compound A	7.75	1.16	15.0
Compound B	8.00	1.19	14.9
Compound C	7.83	1.62	20.8
Compound D	5.87	0.93	15.8
Compound E	4.63	0.70	15.0
Compound F	4.89	0.94	19.2
Compound A to F	**6.49**	**1.80**	**27.7**

Cut-Point Strategy

Conclusion:

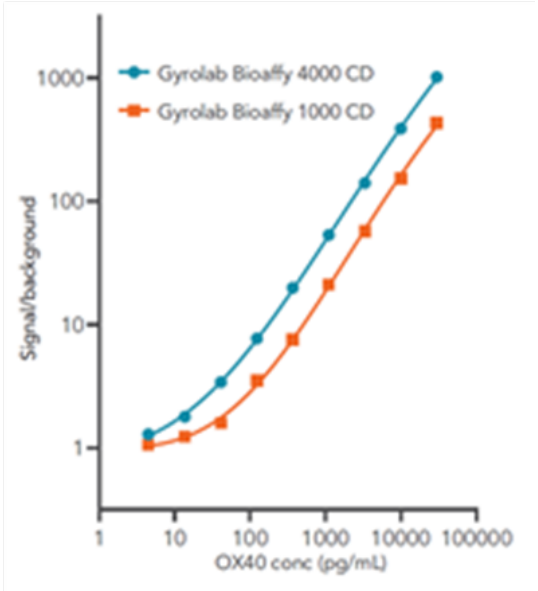
- 6 Tested Biologics → structural homology
- CV > 27% → Conservative approach applying a dynamic cut-point to reduce the risk of high variability against other Biologics
- Dynamic cut-point = mean negative control (NC) of 15 ADA negative samples + 3SD

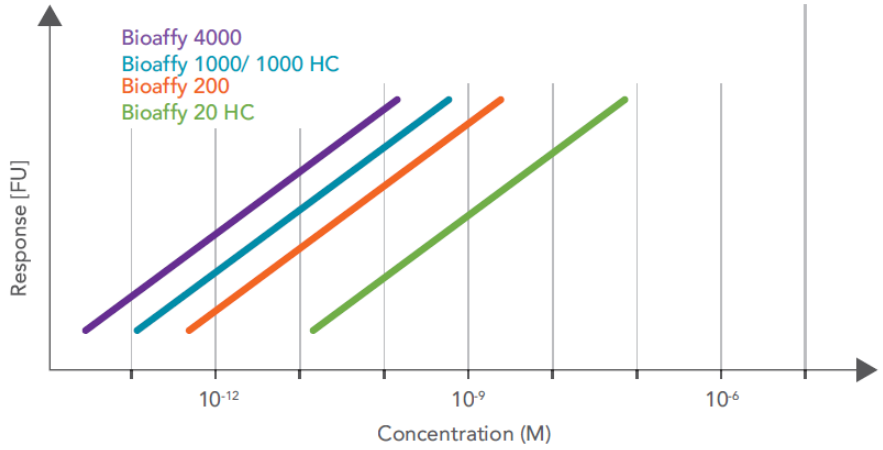
Biomarker Applications

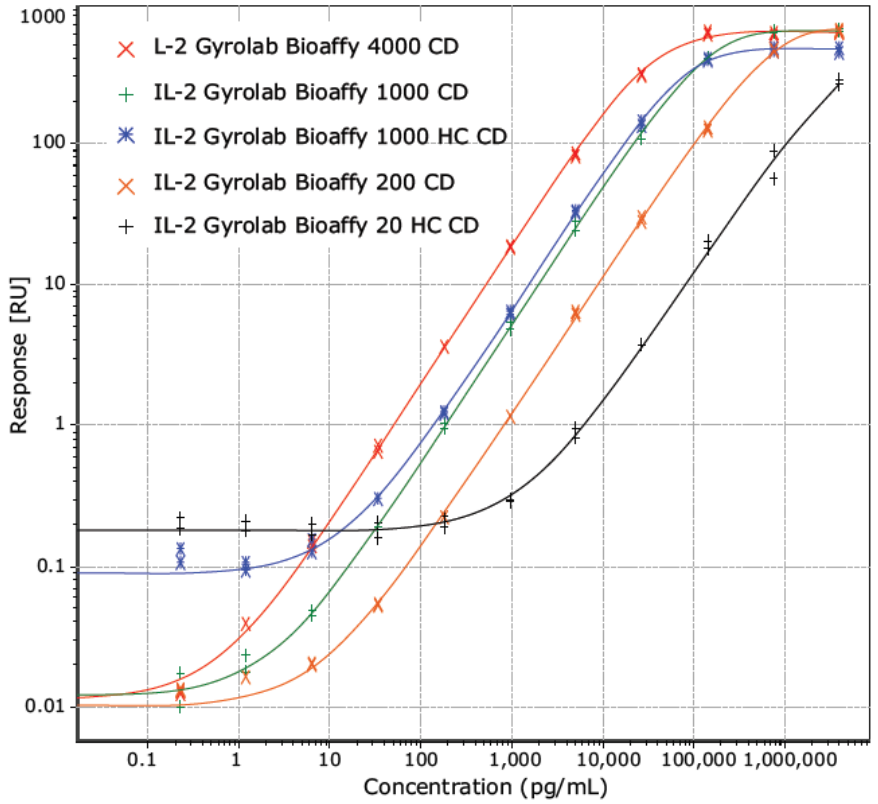


EXTENDING THE ANALYTICAL RANGE

- Combined analytical range of 6 logs
- Sample volume determines assay sensitivity
- Seamless assay transfer between CDs

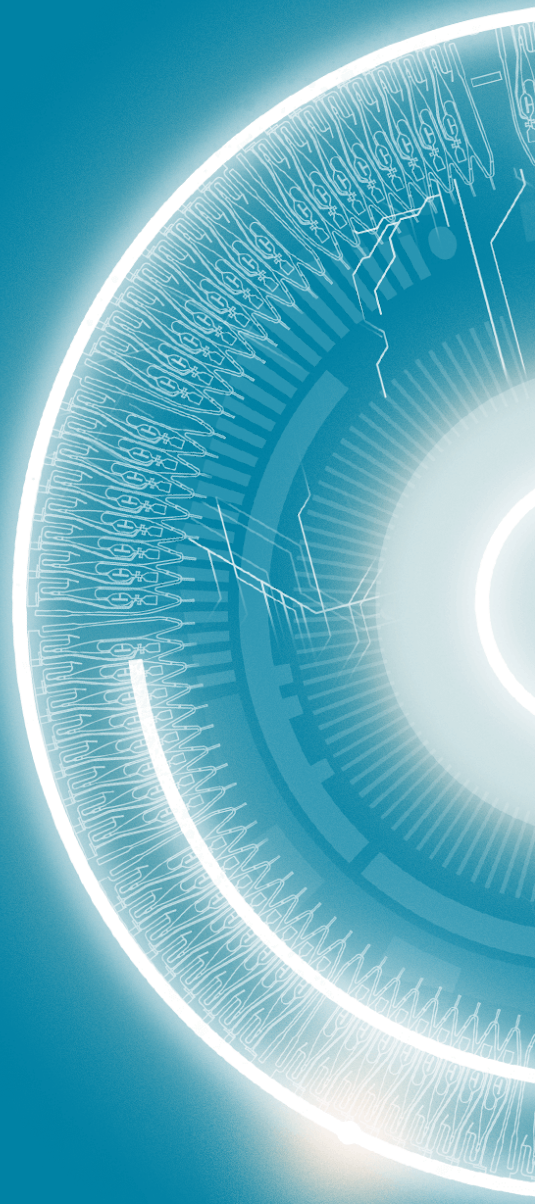


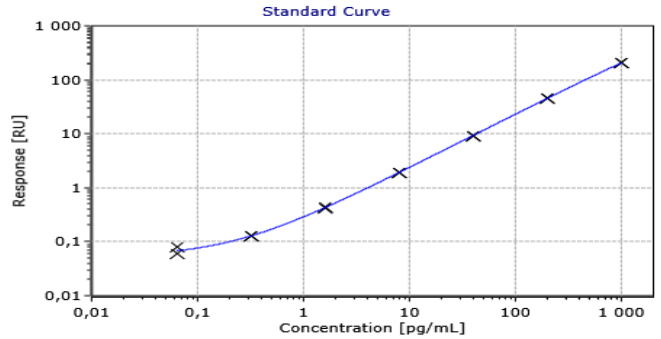




Note: The higher binding capacity of CDs using the high capacity (HC) streptavidin particle increases the upper limit of detection but may also increase background binding.

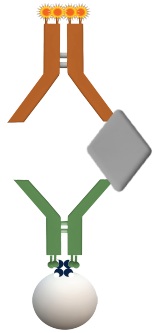
CYTOKINE ASSAYS WITH EXTENDED SENSITIVITY





Assay range	LOD (pg/mL)	LLOQ (pg/mL)	ULOQ (pg/mL)
On plate	~ 0.1	~ 0.3	~ 800
In neat serum	~ 0.2	~ 0.6	~ 1 600

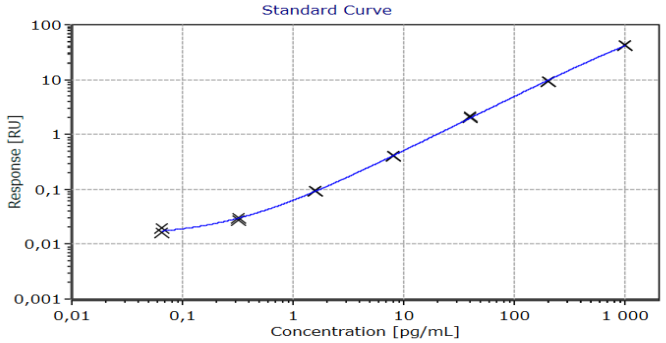
IFN- gamma



anti-human/cyno IFN-γ mAb

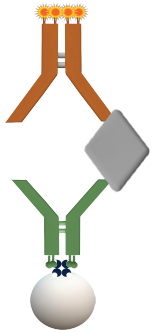
rh IFN-γ

anti-human/cyno IFN-γ mAb



Assay range	LOD (pg/mL)	LLOQ (pg/mL)	ULOQ (pg/mL)
On plate	~ 0.2	~ 0.3	~ 800
In neat serum	~ 0.4	~ 0.6	~ 1 600

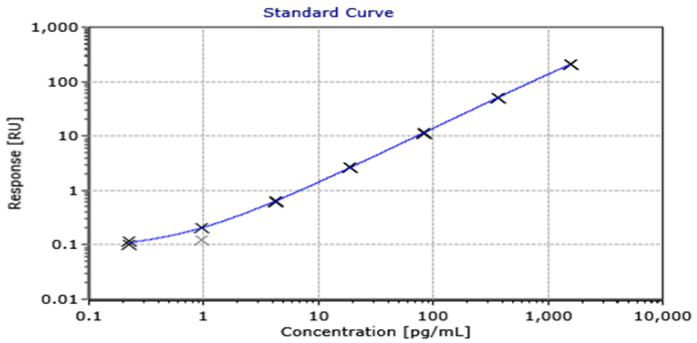
IL-4



anti-human/cyno IL-4 mAb

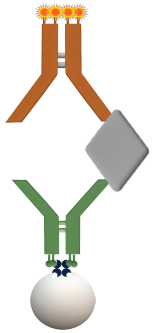
rh IL-4

anti-human/cyno IL-4 mAb



Assay range	LOD (pg/mL)	LLOQ (pg/mL)	ULOQ (pg/mL)
On plate	~ 0.6	~ 1	~ 1 200
In neat serum	~ 1.2	~ 2	~ 2 400

TNF-Alpha

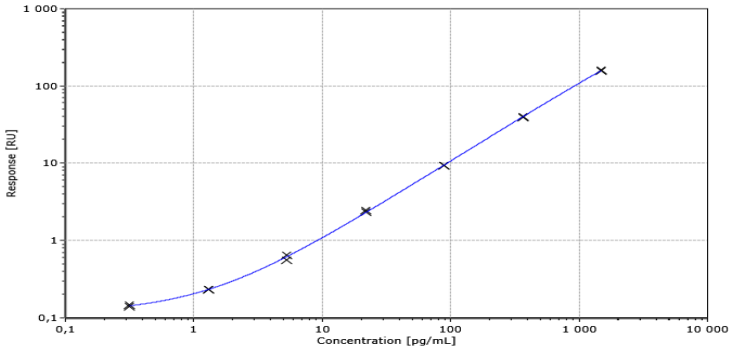


anti-human/cyno TNF-α mAb

rh TNF-α

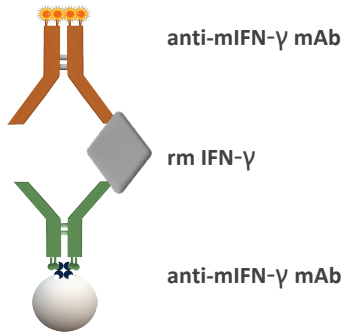
anti-human/cyno TNF-α mAb

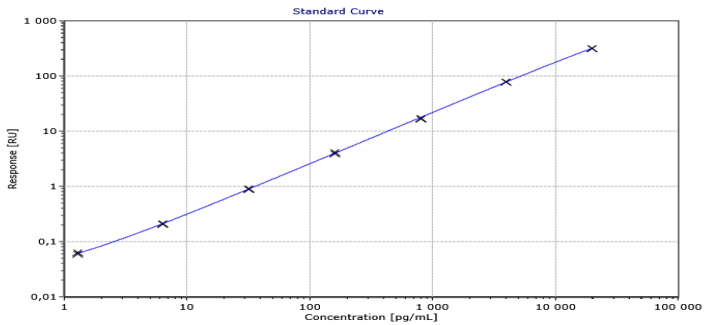
GYROS PROTEIN Technologies



Assay range	LOD (pg/mL)	LLOQ (pg/mL)	ULOQ (pg/mL)
On plate	~0.5	~1	~1 000
In neat matrix	~1	~2	~2 000

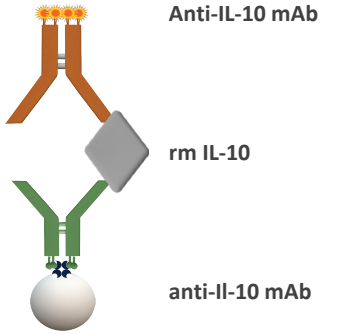
IFN- gamma

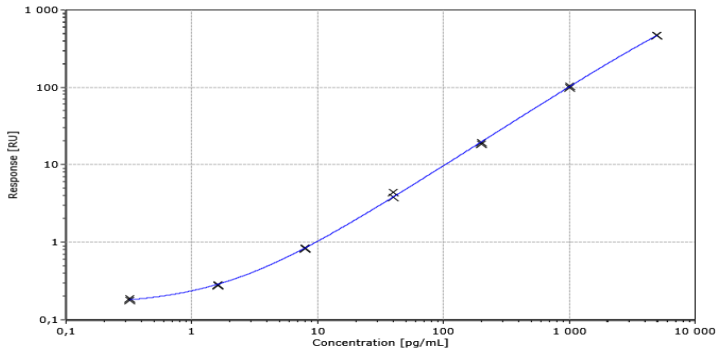




Assay range	LOD (pg/mL)	LLOQ (pg/mL)	ULOQ (pg/mL)
On plate	~0.3	~4	~15 000
In neat matrix	~0.6	~8	~30 000

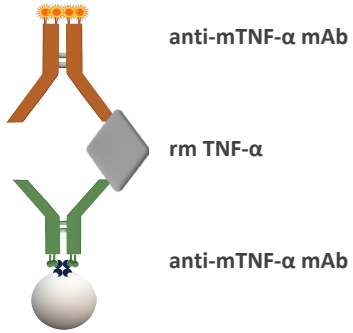
IL-10



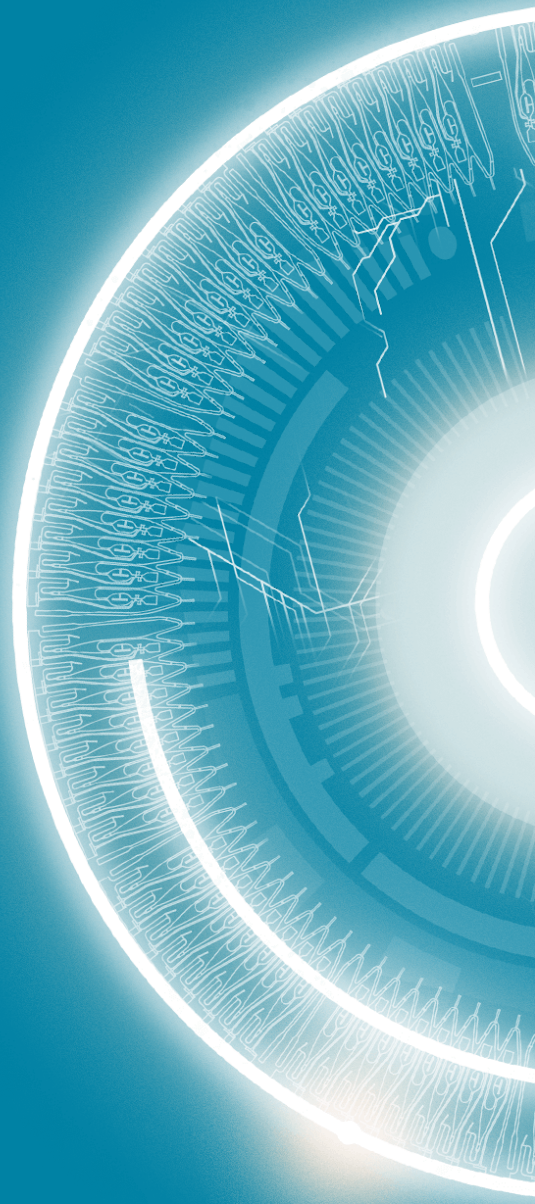


Assay range	LOD (pg/mL)	LLOQ (pg/mL)	ULOQ (pg/mL)
On plate	~0.5	~1	~4 000
In neat matrix	~1	~2	~8 000

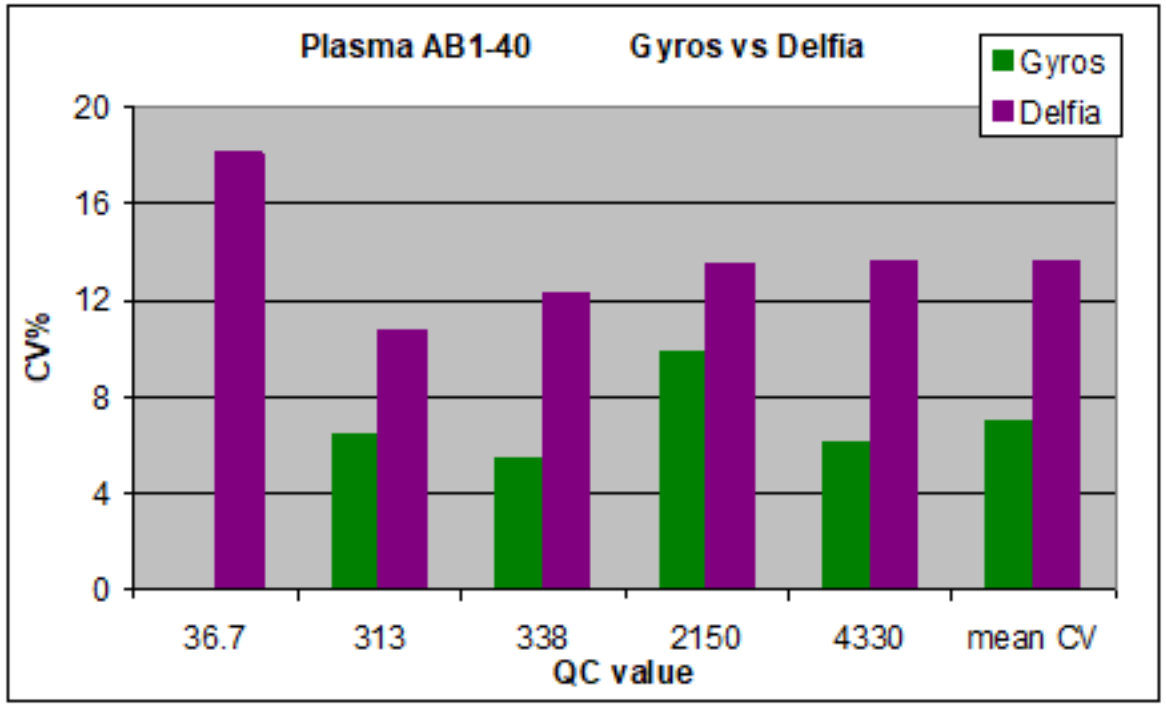
TNF-Alpha

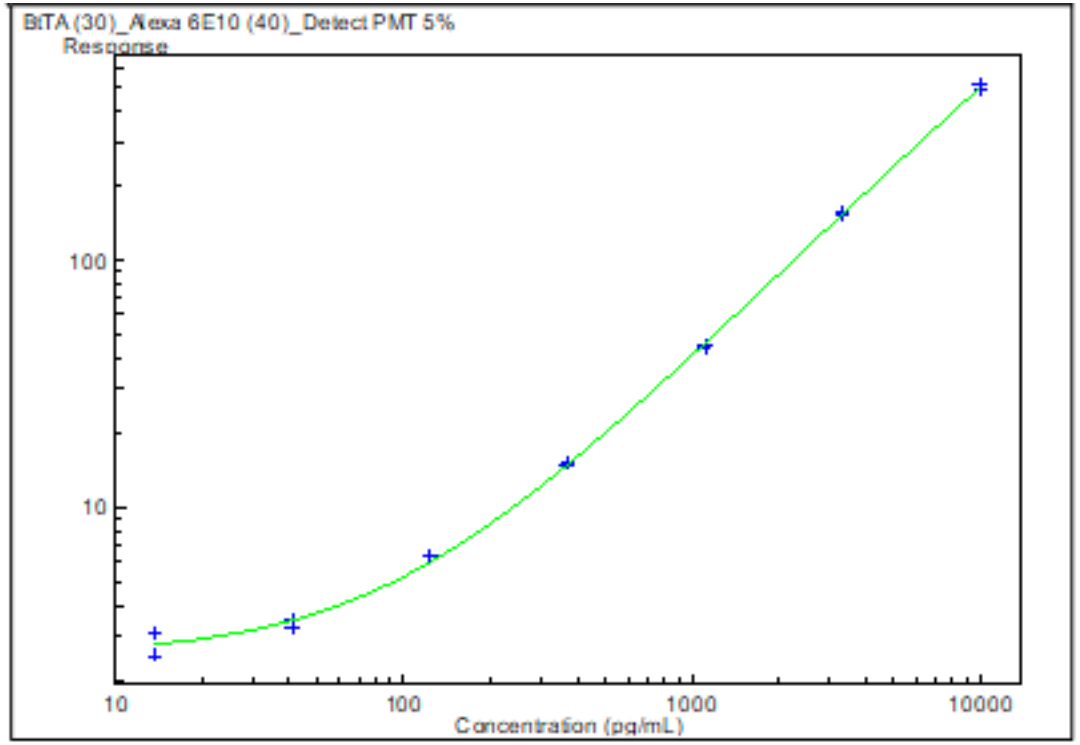


HISTORICAL ABETA AMYLOID 1-40



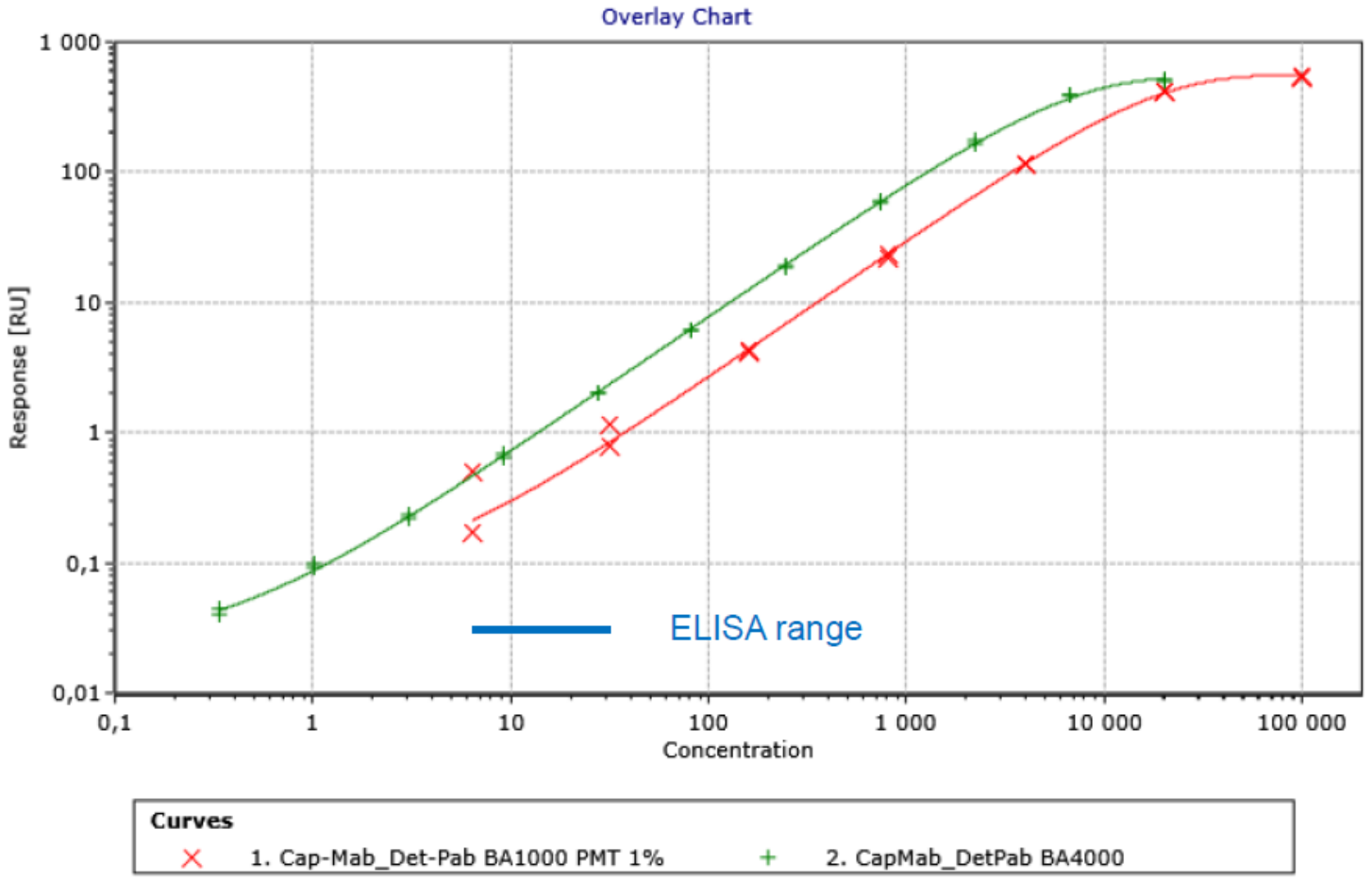
PREVIOUS ABETA AMYLOID 1-40 DATA





Courtesy of John Allinson, ICON PLC

BA 1000 vs BA 4000



BA4000 CD IMPROVED SENSITIVITY

SUMMARY

- Using the mixing CD with a Gyrolab ADA assay automates acid dissociation for significant time savings in immunogenicity assays
- High precision (<15%) for Covance pembrolizumab immunogenicity assay assays allowed singlet analysis
  - Drug tolerance 640 and >1000 µg/mL for Gyrolab (data not shown) and Covance assay, respectively appropriate for clinical use
  - Automation and use of singlet analysis increases productivity for bioanalytical laboratories with significant time and reagent savings
- Extended sensitivity for cytokine assays and high precision biomarker analysis is demonstrated by the Bioaffy 4000

Contact : John.chappell@gyrosproteintech.com