Strategies for Selecting Positive Controls for Immunogenicity Assays

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Linlin Luo, Ph.D
Regulated Bioanalysis
Merck & Co., Inc.
West Point, PA, USA
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Outline

- ✓ What are the current industry practices on selection of positive controls (PCs) for ADA assays
- ✓ What to choose as the PC, monoclonal antibody (mAb) vs. polyclonal antibody (pAb)?
- ✓ Case studies: PC selection for preclinical ADA assays
- ✓ Case studies: PC selection challenges for clinical ADA assays of pegylated proteins and bispecific molecules
- ✓ Summary

Industry Practices on PCs for ADA Assays

- ✓ PCs are used to characterize an ADA assay, such as sensitivity, drug tolerance, selectivity, prozone effect, and hemolysis/lipemia recovery.
- ✓ PCs are also used to monitor assay performance.
- ✓ Both polyclonal antibodies (pAbs) and monoclonal antibodies (mAbs) have been used as PC during method development and validation.
- ✓ Health authorities recommended that PCs should be purified and diluted to a known concentration.
- ✓ Unpurified PCs led to inaccurate assessment of assay sensitivity, misleading ADA data interpretation.
- ✓ PAbs are widely considered to best represent an endogenous ADA response and are recommended to be used for assay characterization experiments.
- ✓ MAbs may be used to prepare robust positive controls to monitor assay performance.

Ref: Myler H, et al (2019)

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Monoclonal vs. Polyclonal Antibody PCs

mAb	pAb
• With a certain binding affinity and epitope specificity; • May better mimic clinically relevant immune responses, such as neutralizing activities.	• A collection of antibodies with different binding affinities and idiotype epitope specificities; • May be a better surrogate for clinical samples that are polyclonal.
• Longer time to produce/develop the initial clone; • Relative ease of generation afterwards	• Inexpensive and relatively quick to produce; • Challenges with yield and purification
• Large quantity and minimum lot-to-lot variability	• Limited quantity and lot-to-lot variability

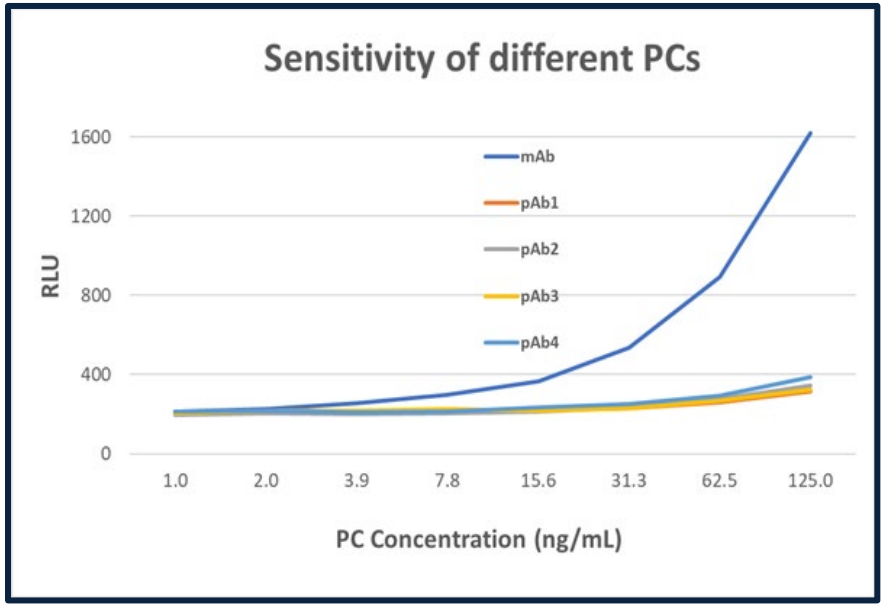
✓A panel of mAbs with diverse repertoire of different binding properties and neutralization activities can be a promising tool for assay selection and characterization, as well as data interpretation.

✓How to decide what to use as the PC, a mAb, a mixture of mAbs, or a pAb?

Mixture of mAbs vs. pAb as PC

PC type	Vendor	Sensitivity (ng/mL)	Drug tolerance[a] at PC 100 ng/mL (µg/mL)
mAb*	X	2.5	~ 500
pAb1	Y	35.8	~ 10
pAb2	Y	30.0	~ 20
pAb3	Z	33.8	~ 20
pAb4	Z	16.9	~ 50

* A mixture of 3 mAbs; used for assay development/optimization
[a] The trough level of the efficacious dose predicted to be ~ 80 µg/mL

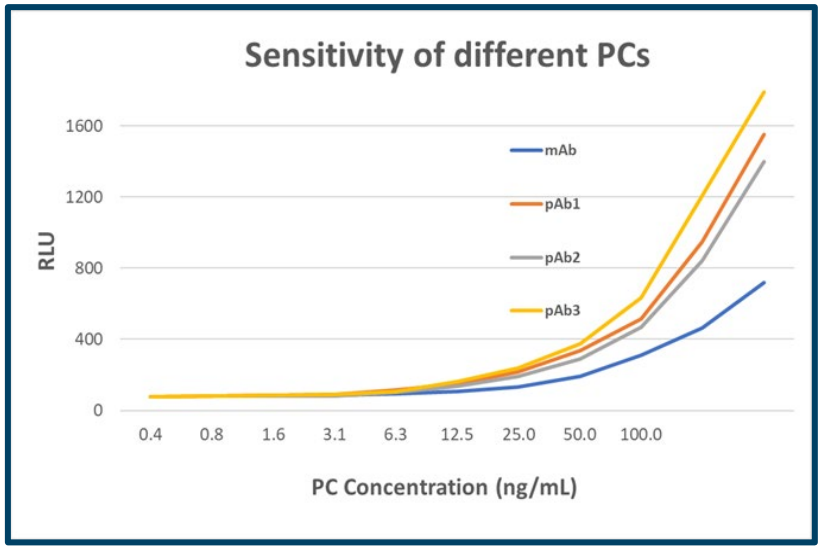


- ✓ mAbs gave better sensitivity and drug tolerance compared to pAb.
- ✓ The 4 pAbs showed lower drug tolerance than the predicted trough level of 80 µg/mL at 100 ng/mL, indicating some low levels of ADAs in clinical samples may not be detectable due to drug interference.

Single mAb vs. pAb as PC

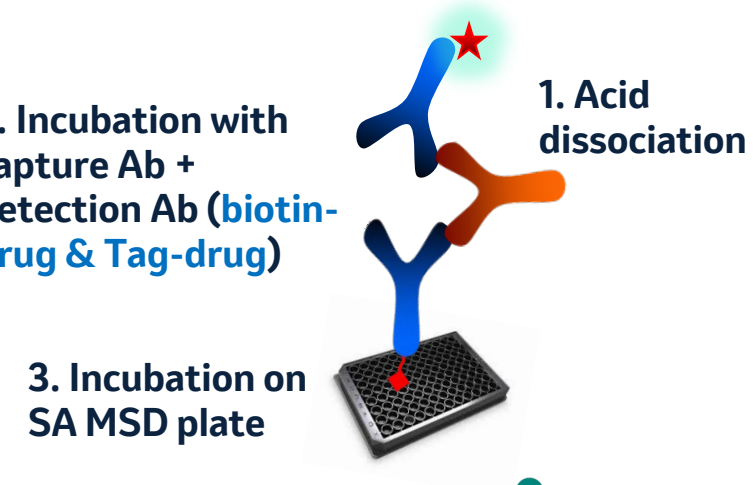
PC type	Vendor	Sensitivity (ng/mL)	Drug tolerance[a] at PC 24 ng/mL (µg/mL)	Drug tolerance[a] at PC 100 ng/mL (µg/mL)
mAb*	X	1.8	83	308
pAb1	Y	1.0	84	165
pAb2	Z	1.3	89	283
pAb3	Z	1.1	96	337

* mAb was used for assay development/optimization.
[a] The trough level of the efficacious does is predicted to be ~ 50 µg/mL.



- ✓ mAb and pAbs showed comparable sensitivity and drug tolerance.
- ✓ Either mAb or pAb may be a suitable PC for this assay.
- ✓ Drug tolerance demonstrated at low PC levels for both mAb and pAbs suggests assay's capability of detecting low ADAs in clinical samples.

- Whether the sensitivity and drug tolerance defined using a PC truly represents the assay needs to be closely looked at when evaluating clinical PK/ADA data.



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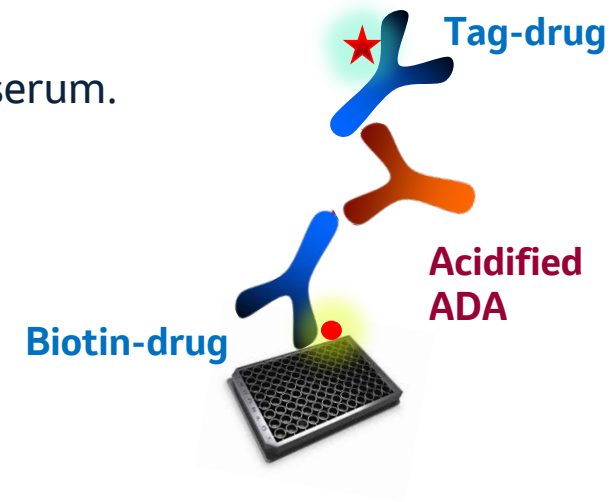
Preclinical ADA Assay for mAbs

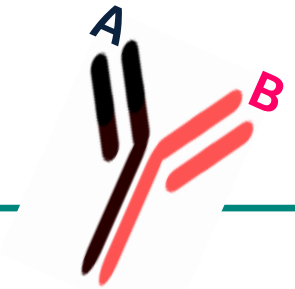
- ✓ A generic PC was used:
  - Mouse anti-Hu IgG (Ch2 domain) mAb
- ✓ An ECL bridging assay (screening tier only) with acid dissociation was qualified in rhesus serum.
- ✓ Cut Point: 1.04 (S/N)
- ✓ Parameters qualified:

Qualification Parameter	Results			
Sensitivity	1.94 ng/mL			
Precision		LPC (3 ng/mL)	MPC (500 ng/mL)	HPC (5000 ng/mL)
	Intra-assay	3.3%	13.0%	3.2%
	Inter-assay	5.8%	23.9%	23.7%
Drug Tolerance	500 ng/mL: 1509 µg/mL of drug		1000 ng/mL: > 1600 µg/mL of drug	
Selectivity	Unspiked (9/10); Spiked at LPC (10/10); Hemolyzed (5/5 for both unspiked and spiked at LPC)			
Prozone	Not observed up to 50 µg/mL			

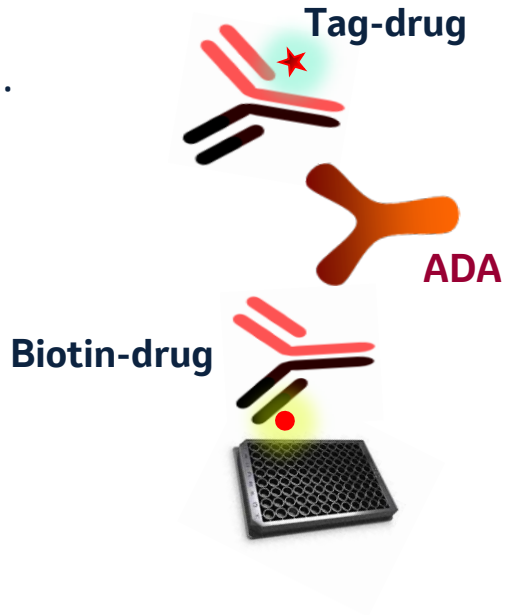


- Using generic anti-human antibodies as the PC is a common practice in non-clinical ADA assay development.

Preclinical ADA Assay for a Bispecific Molecule



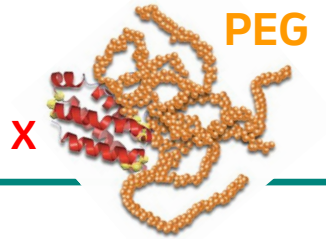
- ✓ A generic PC was used:
  - Mouse anti-Hu IgG (Ch2 domain) mAb
- ✓ An ECL bridging assay (screening tier only) with acid dissociation was qualified in cyno serum.
- ✓ Estimated cut point: ~ 1.59 (S/N)
- ✓ Parameters tested in development:



Qualification Parameter	Results		
Sensitivity	~ 24 ng/mL		
Precision (Overall)	LPC (32 ng/mL)	MPC (500 ng/mL)	HPC (5000 ng/mL)
	16%	23%	23%
Drug Tolerance	500 ng/mL: ~ 750 µg/mL of drug	100 ng/mL: < 250 µg/mL of drug	
Selectivity	Unspiked (10/10); Spiked at LPC (10/10); Hemolyzed (1/1 for both unspiked and spiked at LPC)		
Prozone	Not observed up to 100 µg/mL		

- Among different modalities, using the same generic anti-human antibody as the PC saves development effort.

Preclinical ADA Assay for Pegylated Proteins



- ✓ Two PCs
  - Anti-X: goat anti hu pAb, affinity purified
  - Anti-PEG: IgM
- ✓ An ECL bridging assay (screening tier only) qualified in rhesus serum
- ✓ Cut Point: 1.07 (S/N)
- ✓ Parameters qualified:

Qualification Parameter	Anti-X				Anti-PEG	
Sensitivity	4 ng/mL				311 ng/mL	
Precision		LPC (6 ng/mL)	MPC (100 ng/mL)	HPC (1000 ng/mL)	N/A	
	Intra-assay	3.3%	3.6%	4.1%		
	Inter-assay	8.7%	14.0%	13.1%		
Drug Tolerance	100 ng/mL: > 9.8 µg/mL of drug		1000 ng/mL: > 10 µg/mL of drug		500 ng/mL: 6.6 µg/mL of drug	1000 ng/mL: > 5 µg/mL of drug
Selectivity	Unspiked (9/10); Spiked at LPC (10/10); Hemolyzed (5/5 for both unspiked and spiked at LPC)				N/A	
Prozone	Not observed up to 10 µg/mL				Not observed up to 80 µg/mL	

- To evaluate the potential impact of anti-PEG ADAs, an anti-PEG PC is used to demonstrate assay's ability to detect ADAs against PEG, for pegylated therapeutics.

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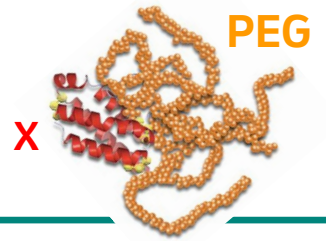
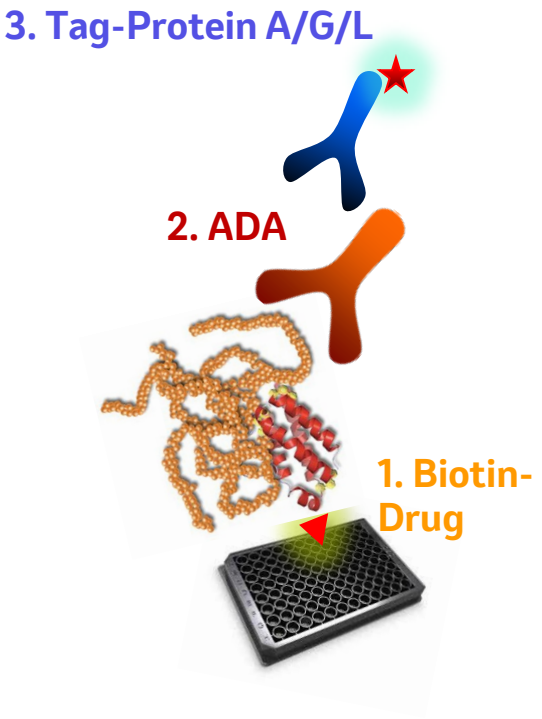
Clinical ADA Assay for a Pegylated Protein

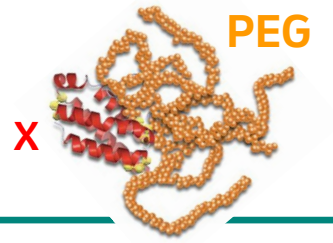


- ✓ Two PCs
  - Anti-X: Chimeric human IgG1
  - Anti-PEG: Chimeric human IgM
- ✓ A direct binding assay in development and to be validated – one assay to detect ADAs against both components, i.e., protein X and PEG
  - Screening
  - Confirmatory
  - Titer/Characterization (e.g., domain mapping) if necessary
- ✓ Challenges
  - High assay background
  - Selection of anti-PEG: IgM vs. IgG
  - Optimal assay conditions for both PCs
  - High drug concentration for confirmatory tier



Derrick Johnson et al. 2021. Sensitive assay design for detection of anti-drug antibodies to biotherapeutics that lack an immunoglobulin Fc domain. WWW.nature.com/scientificreports

Anti-PEG Antibodies: IgG vs. IgM



✓ IgGs failed to generate satisfactory signals; IgM as anti-PEG PC

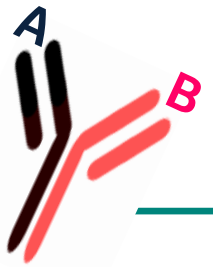
(µg/mL)	IgG #1	IgG #2	IgG #3	IgG #4	IgM
20.00	14856	38547	198	142	n/a
5.00	413	694	138	134	6358
1.25	169	185	129	112	n/a
0.31	139	159	152	144	
0.10	n/a				404
0.08	145	131	121	123	n/a
NC = 157					

- There are challenges developing a single assay able to detect ADAs against both the therapeutic protein and PEG portions with reasonable sensitivities/DTs for both.
- Esp. the anti-PEG antibody as PC needs to be carefully investigated. Our data suggest IgM is a good candidate.

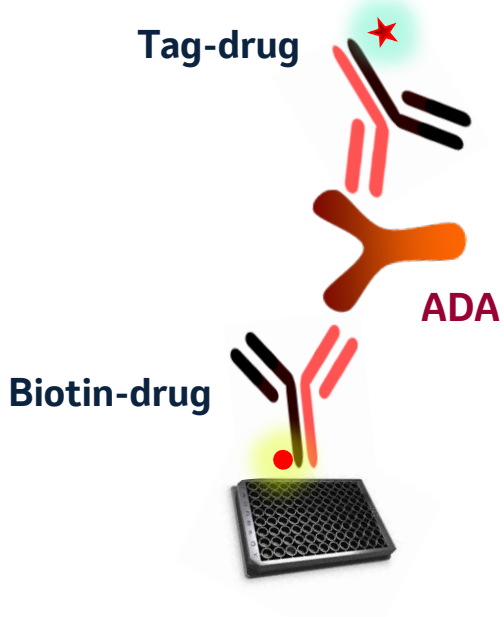
✓ Much higher drug concentration is needed for IgM signal inhibition

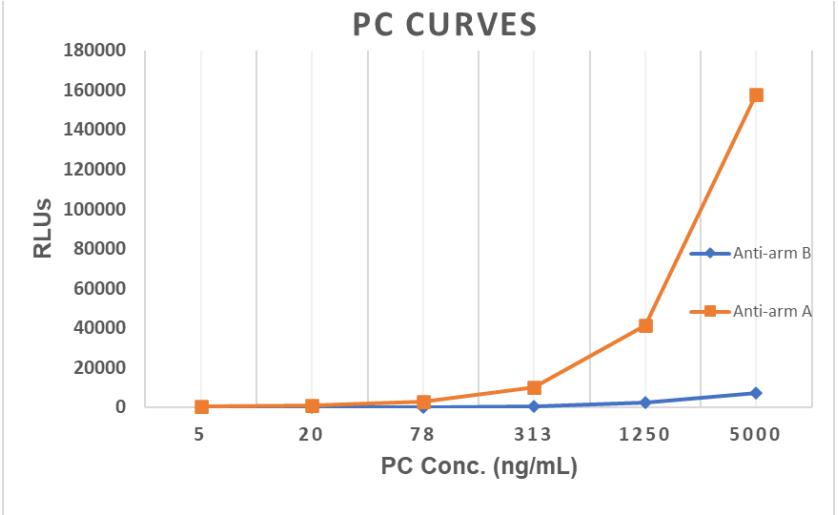
IgM (ng/mL)	Drug (µg/mL)								
	25	50	100	200	400	800	1600	25	
5000	9.6	9.7	9.7	31.1	61.8	74.6	74.6	IgG #1 5000 ng/mL	99.1
100	5.2	4.5	-0.2	15.6	23.1	30.2	30.2	IgG #2 5000 ng/mL	99.5
0 (NC)	5.4	15.0	5.4	22.9	16.3	24.9	24.9		

Clinical ADA Assay for a Bispecific Molecule

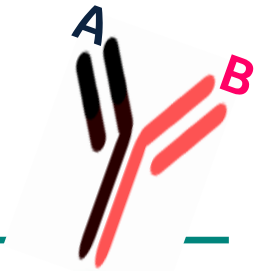


- ✓ Two PCs
  - Mouse anti-arm A IgG
    - Large scale available, to be used as assay control in production
    - To be validated for all parameters
  - Mouse anti-arm B IgG
    - Limited quantity
    - To be validated for sensitivity and drug tolerance only
- ✓ An ECL bridging assay in development and to be validated
  - Screening
  - Confirmatory
  - Titer, and if needed Characterization (e.g., domain mapping)
- ✓ Challenges
  - Significant difference of PC sensitivities
  - Searching for a suitable anti-arm B PC





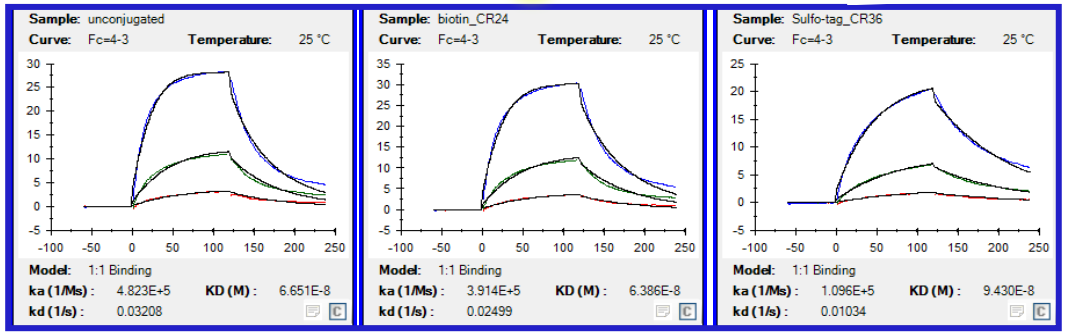
Comparison of Two Anti-arm B Antibodies: Old vs. New



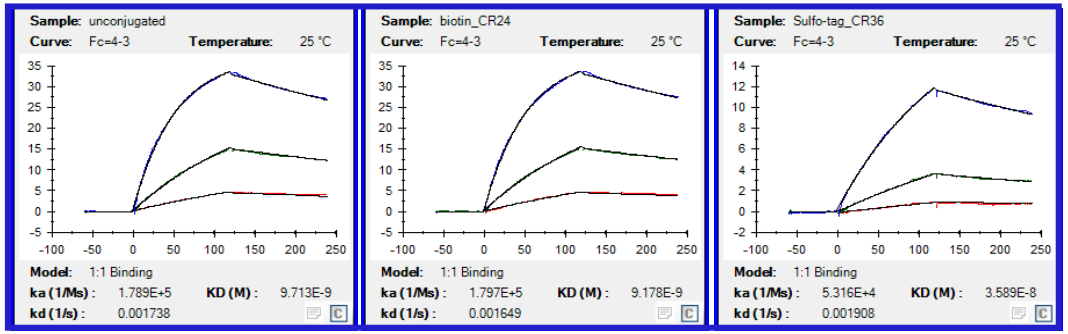
- ✓ Biacore data:
  - Old anti-arm B had high dissociation rate when binding to either unconjugated or conjugated drug (i.e., biotin & tag)
  - New anti-arm B showed slower dissociation rate
- ✓ New anti-arm B provided much improved sensitivity as PC, compared with old anti-arm B

PC Levels (ng/mL)	RLU Counts	
	Old	New
5000	2201	32082
100	142	961

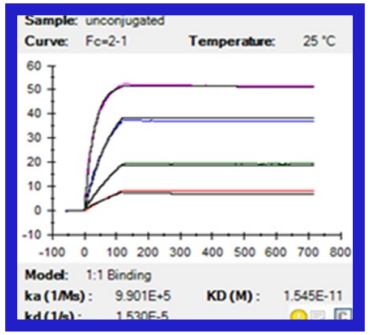
Old



New



Anti-arm A



- Proper characterization of an antibody is critical to choose an appropriate PC for an ADA assay, to help deliver more clinically relevant ADA data.

Summary

- ✓ An ADA assay needs to be properly developed in order to assess clinically relevant immunogenicity.
- ✓ Selection of representative PC enables development of suitable assays for clinical ADA assessment.
- ✓ Whether mAb or pAb is an appropriate PC is case by case and should be evaluated during method development.
- ✓ Nonclinical ADA is rarely predictive of clinical immunogenicity potential. To balance risk and benefit, a generic anti-human antibody, or commercially available specific antibody is a good choice for PC.
- ✓ For pegylated proteins, selecting an appropriate anti-PEG PC is critical for potential ADA testing against PEG. Our data demonstrated that IgM can be a good PC choice.
- ✓ For bispecific molecules, PC for each domain needs to be carefully characterized, which ensures their suitability to demonstrate the assay can sufficiently detect ADAs against each domain.
- ✓ Will the clinical outcome change when different PCs are used for clinical ADA assay development? Or will the generated ADA data correlate with clinical outcome?

Acknowledgements

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✓Yang Xu, PhD

✓Krisna Duong-Ly, PhD

Biography and Contact Information

- ✓ 16 years as a bioanalytical scientist focusing in the area of regulated bioanalytical (BA) support for biologics
- ✓ Been contributing to pharmacokinetic (PK), immunogenicity (IMG), and neutralizing antibody (NAb) assay development and validation, in support of numerous preclinical and clinical studies for many critical programs
- ✓ Currently a Director, in the Regulated Bioanalysis Group at Merck & Co., Inc., West Point, PA, USA, leading a group of scientists responsible for developing bioanalytical assays to support biotherapeutic development and licensure
- ✓ Linlin.Luo@Merck.com