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Translating Science to Medicine

Case Study: Alternative Approach of Validating ADA Assays for Bispecific Antibodies

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What Is a Bispecific Antibody?

Bispecific antibodies (BsAbs) have been engineered to contain two distinct binding domains that can bind to two antigens or two epitopes of the same antigen simultaneously.

Classifications

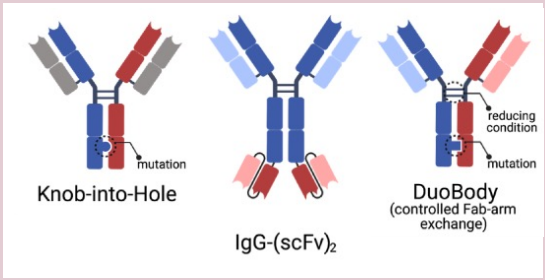
half-life *in vivo*

- **Short-lived, small (< 50 kDa) proteins**
- non-IgG-like, fragment (scFv)-based (lacking human Fc)

→ half-life of few hours

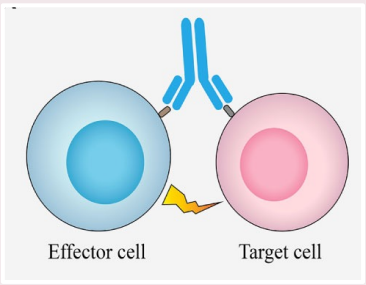
- **Long-lived, large bsAbs (> 150 KDa)**
- IgG-like (antibodies Abs)

→ half-life of up to several days

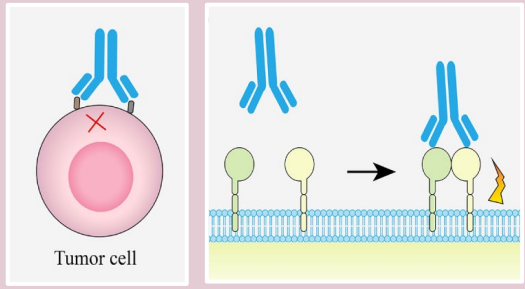


mechanism of action

- **Cell-bridging bsAbs: acting in-trans** (designed for cancer treatment by linking immune cells to malignant cells)



- **Antigen-crosslinking bsAbs, non cell-bridging: acting in-cis** (e.g. blocking signals of cell growth, activation of immune cells, co-factor mimetic)



Bispecific Antibodies as Biotherapeutics

2014
- acute lymphocytic leukemia (ALL)
- Blincyto (blinatumomab) AMGEN

2017
- Hemlibra (emacizumab) Roche
- Hemophilia A

2021
- non-small cell lung cancer (NSCLC)
- janssen Rybrevant (amivantamab)

2022
- IMMUNOCORE *Kimmtrak (tebentafusp)* — uveal melanoma
- Roche Vabysmo (faricimab) — wet age-related macular degeneration (AMD)
- janssen Tecvayli (teclistamab) — multiple myeloma
- Roche Lunsumio (mosunetuzumab) — follicular lymphoma

2023
- Roche Columvi (glofitamab) — diffuse large B-cell lymphoma
- Genmab Epkinly (epcoritamab) — diffuse large B-cell lymphoma

FDA approval

Bispecific Antibodies: Bioanalytical Challenges

- **ADA: domain specificity characterization of the immune response**
  Comparison of immunogenicity between moieties and related to novel / artificial structures (e.g. linker)

- Is domain specificity required? Regulatory recommendations:
  - FDA 2019 Guidance (Section IV.A.3):

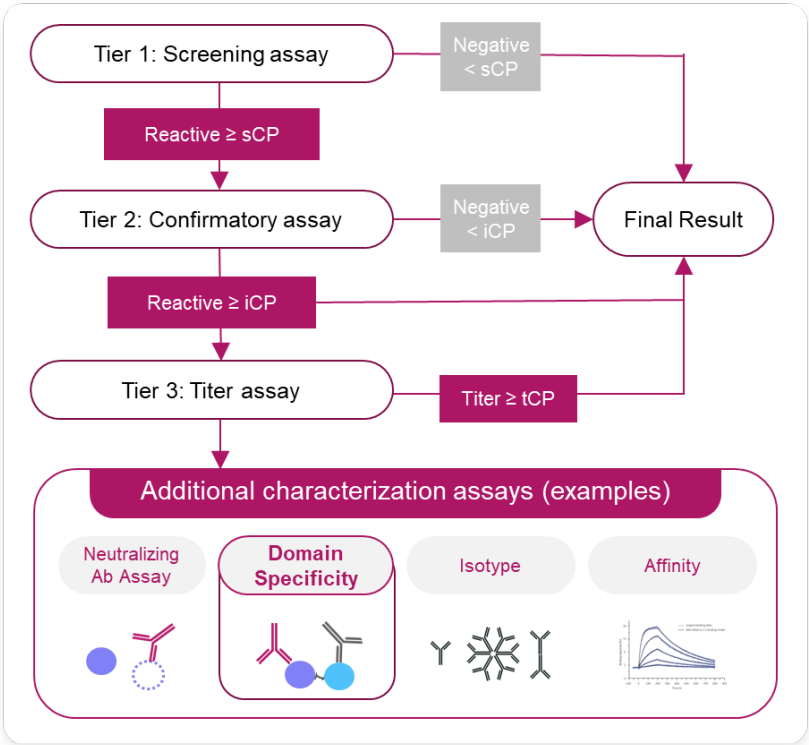
    "For multi-domain therapeutic protein products, the sponsor may need to investigate whether the ADA binds to specific clinically relevant domains in the protein."
  - EMA 2017 Guideline (Section 7.5)

    "The evaluation of this *(immune)* response, in particular, the characterization of the specificity of the induced antibodies is challenging and may require multiple assays for measuring immune responses to various moieties."

    → risk assessment (safety – moiety with endogenous counterpart; clinical phase)

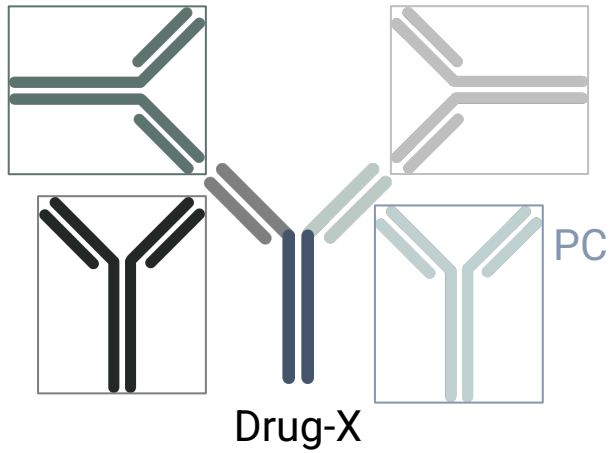
- If domain specificity characterization is required:
  - → Development of multiple domain characterization assays to measure immune responses to different domains of the molecules
  - → Additional reagent generation:
    - ❑ domain-specific positive control
    - ❑ domain-specific inhibitors for the characterisation assay
  - → Assay development and validation is more time/ resource consuming

ADA testing: the multi-tiered approach



Positive Control Strategies

Polyclonal Antibody as PC

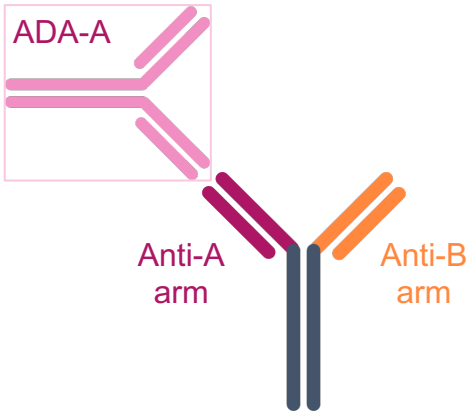


1 pAb used in all tiers

⚠ Life-cycle management of pAb PC is to be considered

Domain-specific Monoclonal Antibody as PC

Case Study 1



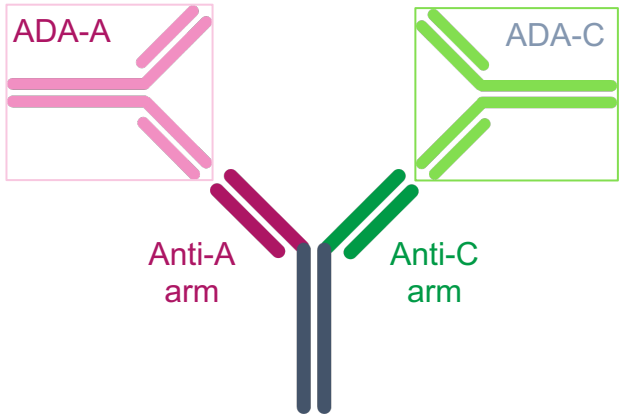
Bispecific antibody 1 (Drug-AB)

1 mAb used as main PC in all tiers

2nd mAb needed in charact. assay

→ 2 independent mAb PCs

Case Study 2



Bispecific antibody 2 (Drug-AC)

1 mAb mix used in all tiers

→ 1 Pseudo-pAb PC

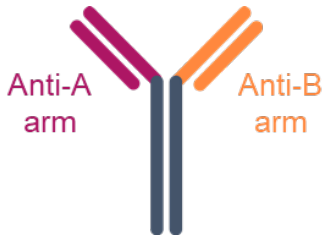
Assay Requirements and Reagent Availability

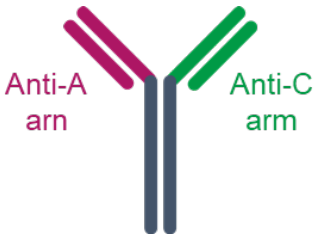
Assay requirements (Drug-AB and Drug-AC)

- ≤ 100 ng/mL senstivity
- Low free drug interference
- Likely low target interference

- Typical MSD bridging assay
- No need for sample pre-treatment like acid dissociation

Reagents	Drug-AB (Case Study 1)	Drug-AC (Case Study 2)
Positive controls	• mAb targeting anti-A arm (ADA-A) • mAb targeting anti-B arm (ADA-B)	• mAb targeting anti-A arm (ADA-A) • mAb targeting anti-C arm (ADA-C)
Capture and Detection	• Biotin- and SulfoTag-Drug-AB	• Biotin- and SulfoTag-Drug-AC
Reagents for domain characterization	• Anti-A arm • Anti-B arm	• Anti-A arm • Anti-C arm

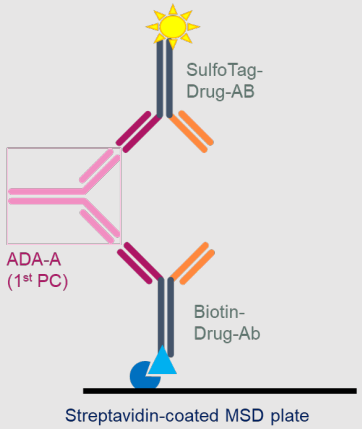




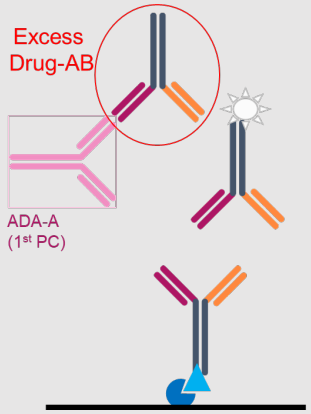
Case study 1 – Two Independent mAb Positive Controls

Drug-AB

Screening / Titer

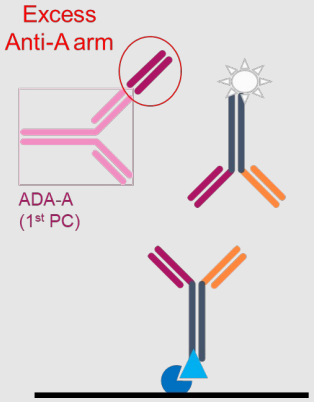


Confirmatory



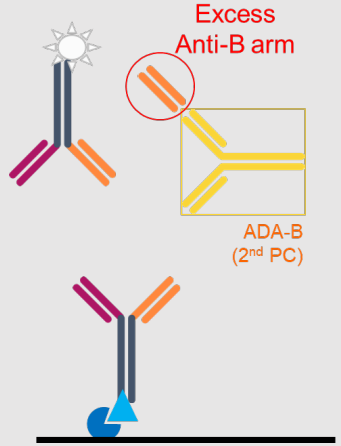
analyzed inhibited & non-inhibited

Characterization A



analyzed inhibited & non-inhibited

Characterization B



analyzed inhibited & non-inhibited

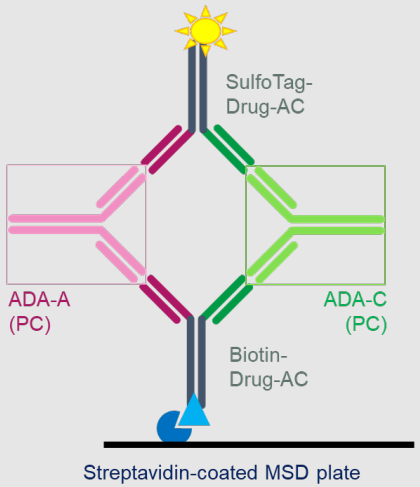
Reference item 1 (ADA-A)
→ main PC
→ LPC, MPC, HPC

Reference item 2 (ADA-B)
→ 2nd PC
→ LPC only

Case study 2 – One Pseudo-pAb Positive Control

Drug-AC

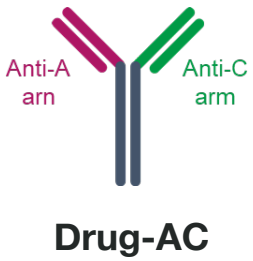
Screening / Titer

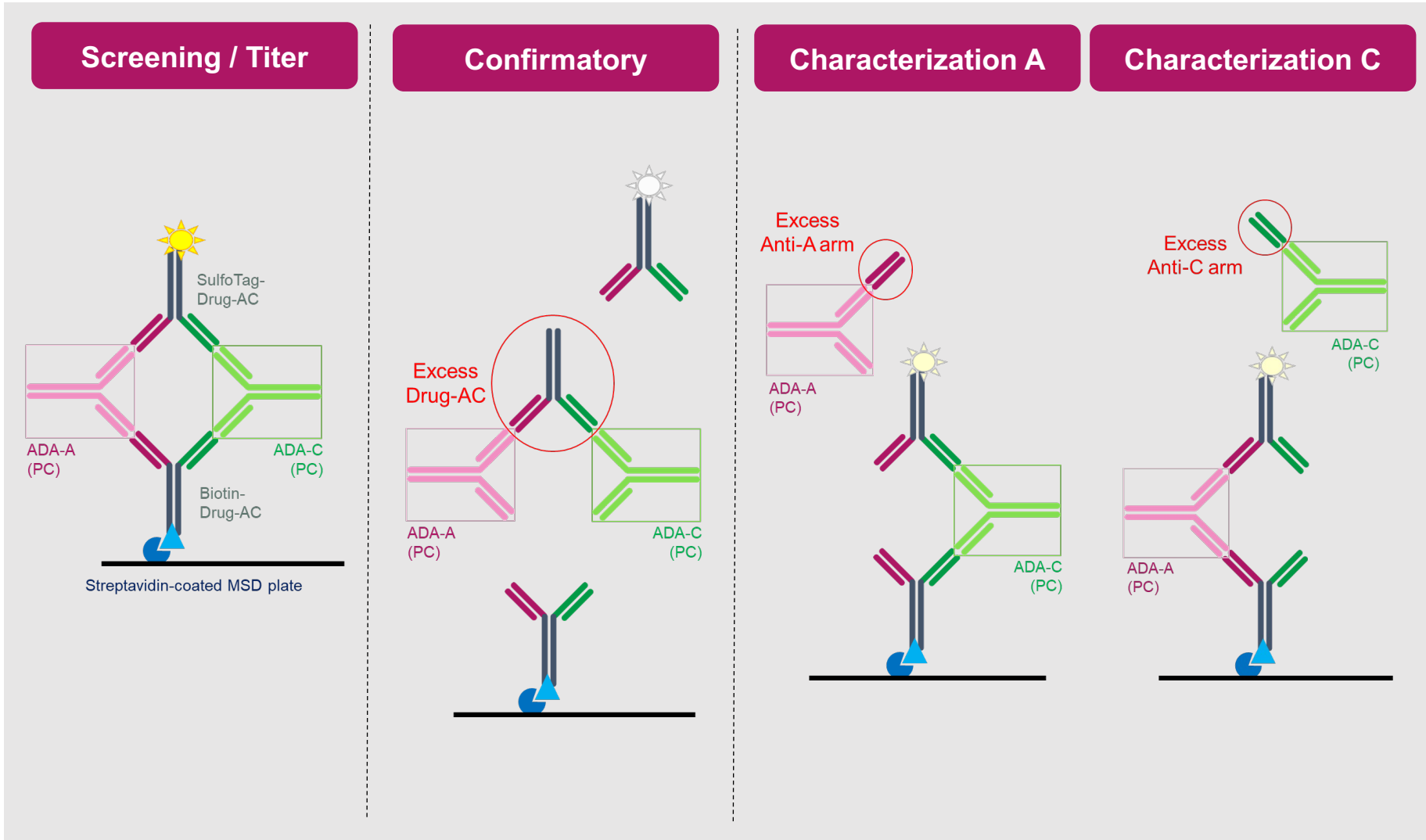


- Reference item 1 (ADA-A) and Reference item 2 (ADA-C) were mixed 1:1 to create a pseudo-polyclonal antibody as positive control
- The Ab mix was used to prepare positive control (LPC, MPC and HPC) and validation samples

  → only 1 positive control

Case study 2 – One Pseudo-pAb Positive Control





01

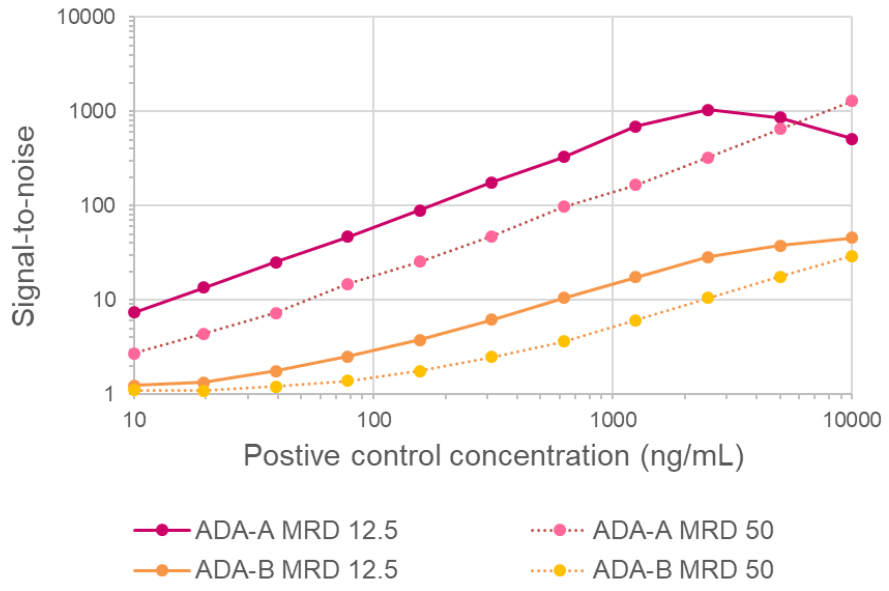
ADA Assay Development for Bispecfic Antibodies

Development Milestones for BsAb ADA Assay

1. Titration of capture and detection reagents
2. Selection of positive control
3. Testing sample pre-treatment if required (drug-tolerance)
4. Selection of MRD
5. Establishment of confirmatory and domain characterization assays
6. Evaluation of key assay performance
    - Cut points
    - Sensitivity
    - Selectivity
    - Drug (target) interference
    - (Stability)

Selection of Positive Control

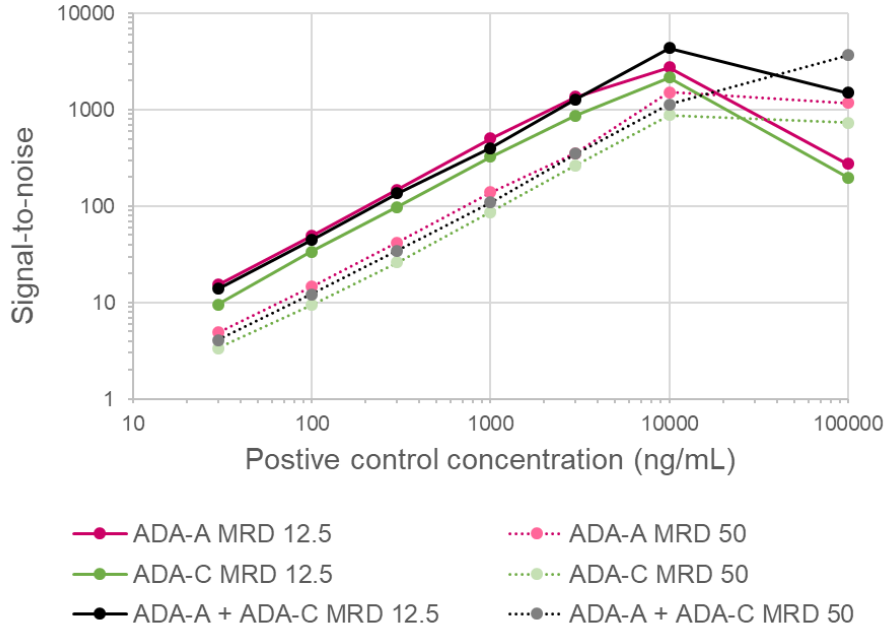
Drug-AB



Sensitivity: ADA-A >> ADA-B

→ ADA-A selected as main PC based on sensitivity (<< 100 ng/mL)

Drug-AC



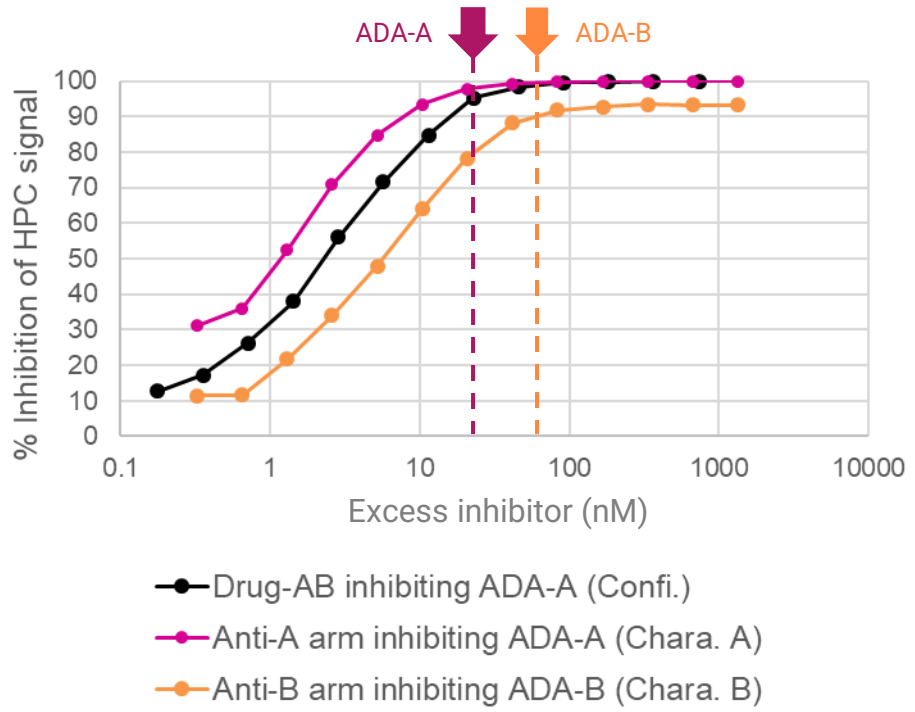
Sensitivity: ADA-A > ADA-C

→ mAb mix selected as PC based on sensitivity (<< 100 ng/mL) and dilution linearity (“hook effect” reduced)

Remark: if drug interference is expected, positive controls should also be evaluated in the presence of free drug

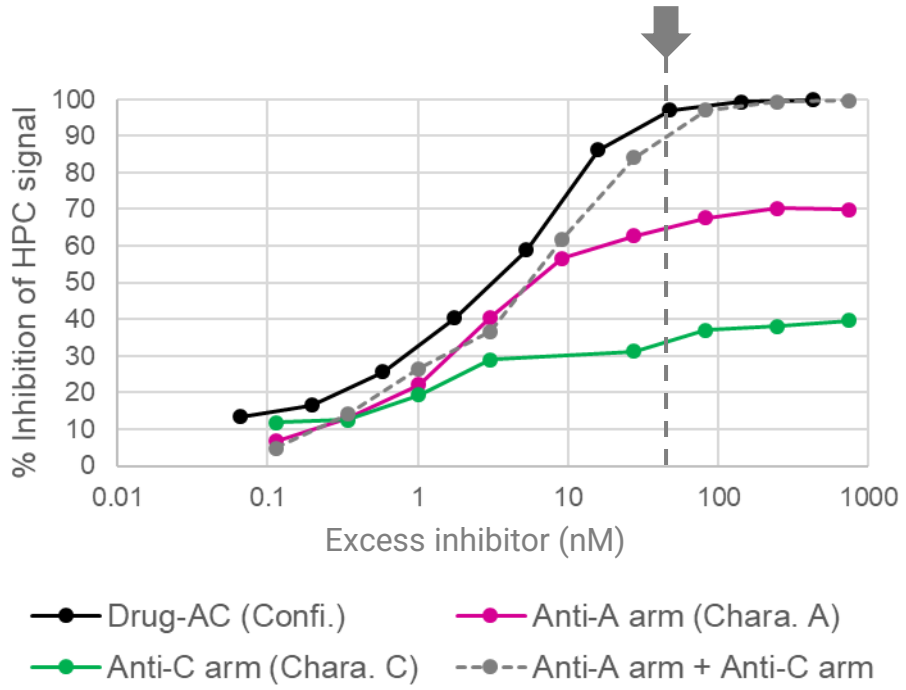
Determination of Excess Inhibitor Levels in the Confirmatory and Characterization Assays

Drug-AB



- Different PCs → different excess inhibitor conc.
- Full inhibition

Drug-AC



- Only one PC → similar excess inhibitor concentrations
- Partial inhibition (correlates with mAb binding affinities)

Final Assay Parameters

	Drug-AB	Drug-AC
Assay format	MSD bridging assay (no acid dissociation)	
Positive controls	Two positive controls strategy • ADA-A for all assay tiers but $Char_B$ • ADA-B (LPC) for $Char_B$ (LPC)	Antibody mix strategy (1 PC) • ADA-A + ADA-C (1:1 mix) for all assay tiers • Pseudo-polyclonal Ab
MRD (sample dilution)	25	50
Provisory LPC (pLPC)	$pLPC_A$: 5 ng/mL $pLPC_B$: 10 ng/mL	pLPC: 5 ng/mL

02

ADA Assay Validation for Bispecfic Antibodies

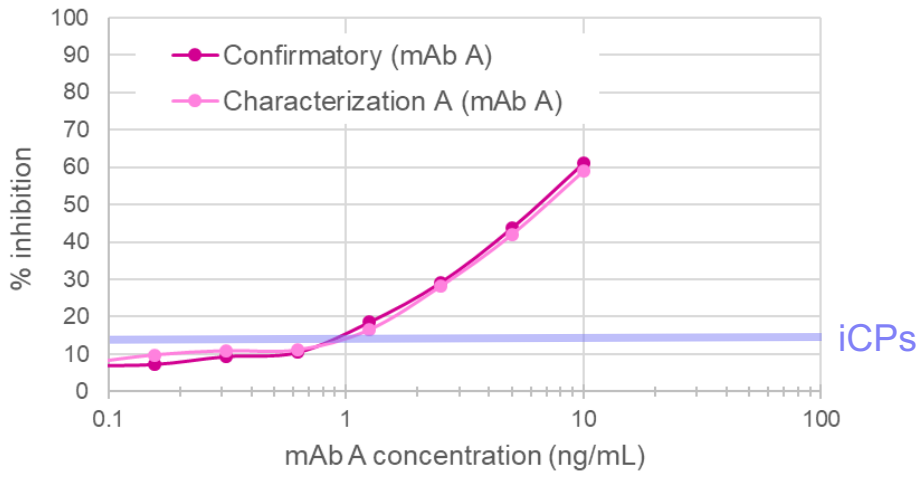
ADA Assay: Key Validation Parameters

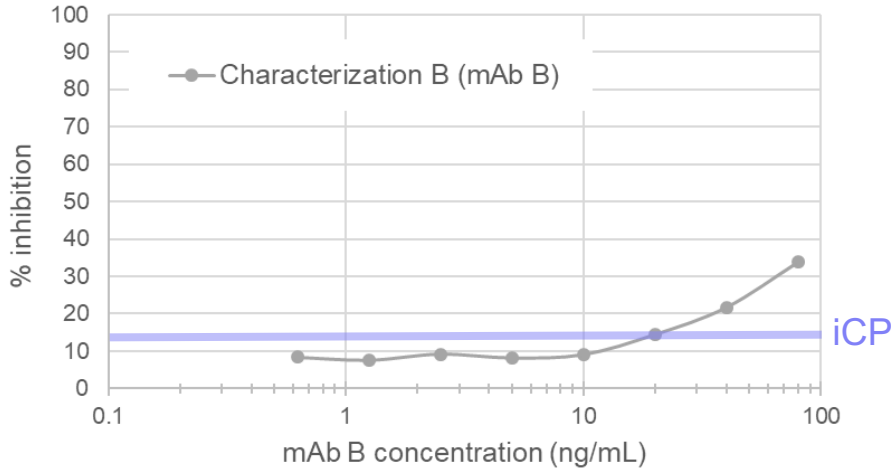
	Drug-AB		Drug-AC
Positive control evaluated	ADA-A	ADA-B	mAb mix
Screening cut point	×		×
Confirmatory cut point	×		×
Characterization cut points (2 assays)	×		×
Robustsness	×		×
Sensitivity and titer precision	×	×	×
Selectivity	×	×	×
Hemolytic / lipemic interference	×	×	×
Precision	×	×	×
Hook effect	×	×	×
Free drug interference	×	×	×
Stability	×	×	×

- With mAb mix – simplified validation as most parameters analyzed with only 1 PC

Assay Sensitivity in Confirmatory and Characterization Assays

Drug-AB





	Confi	Char A
PC	Titration of ADA-A	
Inhibitor	Drug-AB	Anti-A arm
Sensitivity	~ 1 ng/mL	

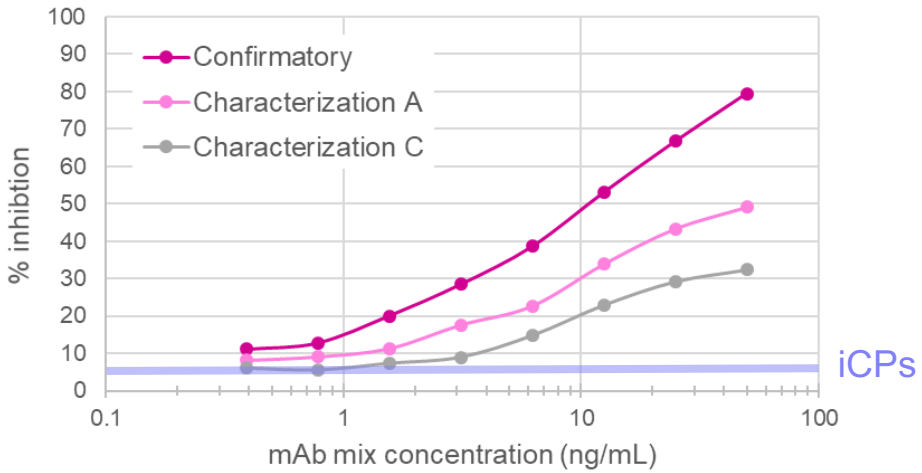
	Char B
PC	Titration of ADA-B
Inhibitor	Anti-B arm
Sensitivity	> 20 ng/mL

Two LPCs:

- LPC (ADA-A): screening / confirmatory / Char A
- LPC (ADA-B): Char B

Assay Sensitivity in Confirmatory and Characterization Assays

Drug-AC



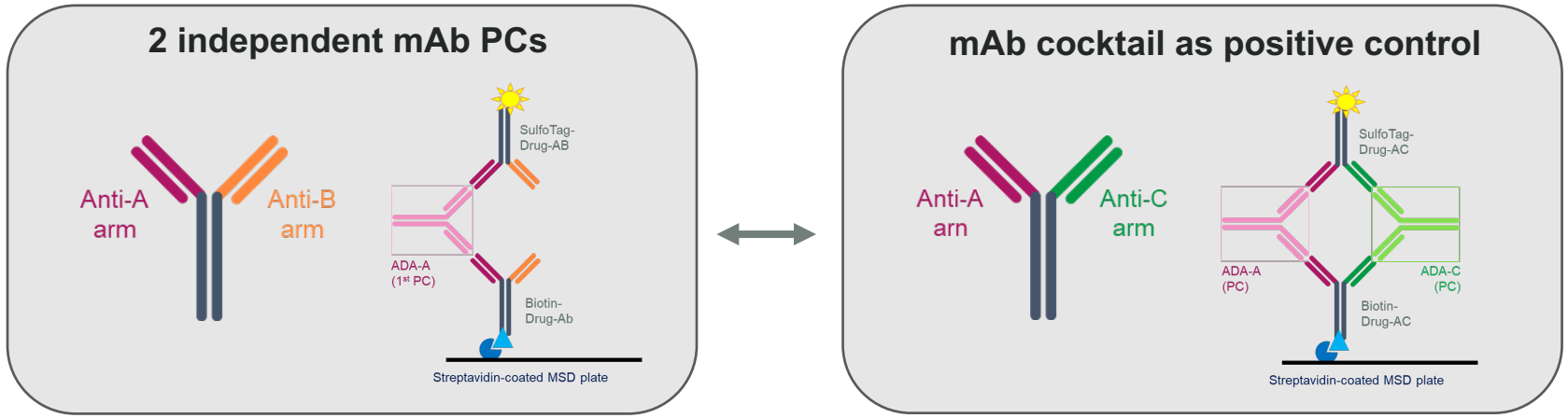
	Confi	Char A	Char C
PC	Titration of Ab mix		
Inhibitor	Drug-AC	Anti-A arm	Anti-C arm
Sensitivity	2-8 ng/mL		

Only 1 LPC

- Despite the partial % inhibition in the Characterization assays the positive control could be diluted below the inhibition cut points.
- If sensitivities in confirmatory vs. characterization assays are too different, an additional LPC level (LPC_{Char}) may be introduced in the characterization assays

03 Conclusion

Pros and Cons of Using a Reference Antibody Mix to Detect ADA Against Bispecific Biotherapeutics



Pros

- Antibody not selected solely based on performance (Ab with highest affinity)
- A cocktail of reference Ab will target both arms of the bispecific Ab thereby mimicking a polyclonal immune response
- Life-cycle management of mAb (vs pAb): easier to control batch consistency, sufficient quantity for long-term supply
- Most validation parameters (e.g. sensitivity, drug tolerance, selectivity) tested only with one positive control → reduce validation experiments, minimize spiking
- Fulfils the requirements of positive control in all tiers of ADA assays.

Cons

- If the affinities of the antibodies are too different combined with a high inhibition cut point – false negative results may occur

Conclusion

- Traditional pAb can be successfully replaced by a mAb cocktail (pseudo-pAb)
- mAb cocktail (pseudo-pAb) was also used as PC for NAb assay (case study 2 – Drug-AC)
- Can be applied more broadly to other multi-domain drugs (e.g. ADC, fusion proteins), provided:
  - The affinities of each domain-specific mAb are not too different (peggylated drug)
  - Not too many domains → partial inhibtion with narrow dynamic range (risk of false-negative)
- Positive control strategy should be considered early on as this can influence the generation of reference antibodies

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THANK YOU