AstraZeneca

The Technical Challenges of Developing Target Tolerant Immunogenicity Assays

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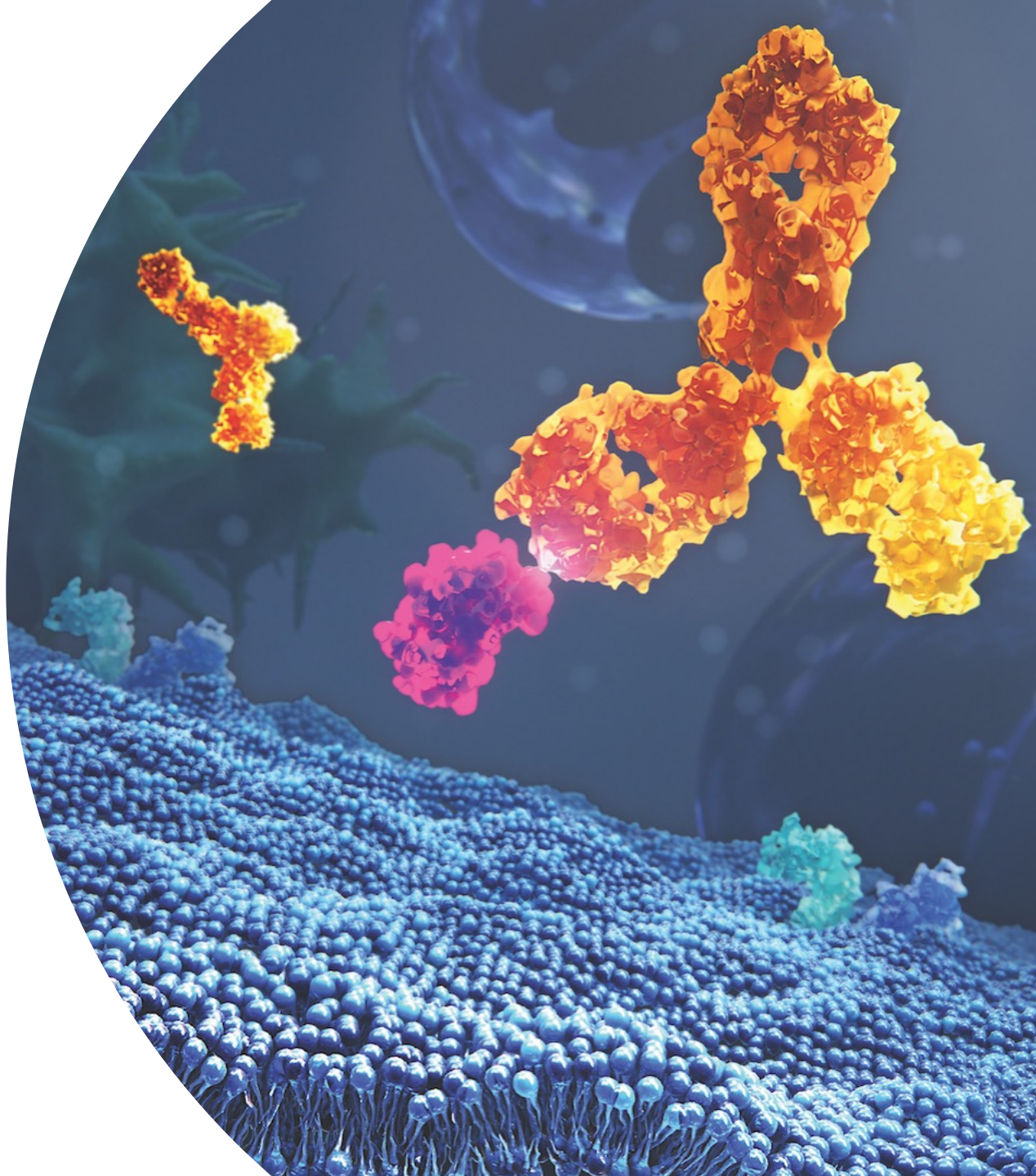


Overview

- **Immunogenicity Background**
  - Why is it important to measure immunogenicity and what are the challenges?
  - Bridging assays: advantages and disadvantages.

- **Case Study**
  - Immunogenicity assay development.
  - Approaches to overcome target interference.
  - Advantages and considerations of the strategies used.

The importance of immunogenicity measurements and achieving target and drug tolerant methods

"Immune responses to therapeutic protein products have the potential to affect product pharmacokinetics, pharmacodynamics, safety, and efficacy."

Immunogenicity Testing of Therapeutic Protein Products — Developing and Validating Assays for Anti-Drug Antibody Detection, FDA Guidance for Industry, January 2019

Challenges of Immunogenicity

Circulating interferences found in patient samples – Drug and Target.

Immunogenicity assays have to be drug and target tolerant.

Drug Tolerance

The ability of an assay to detect control antibody in the presence of drug.

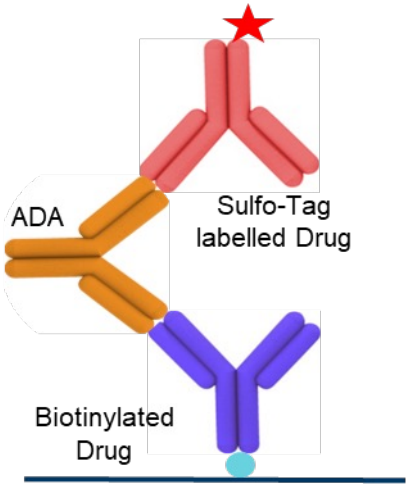
Target Tolerance

The ability of an assay to detect control antibody in the presence of target.

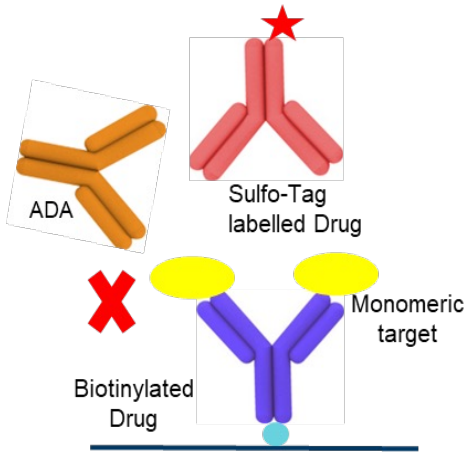
For soluble targets there is a risk the target can confound the immunoassay resulting in generation of false results.

Bridging assays are the go-to assay format for the detection of ADA

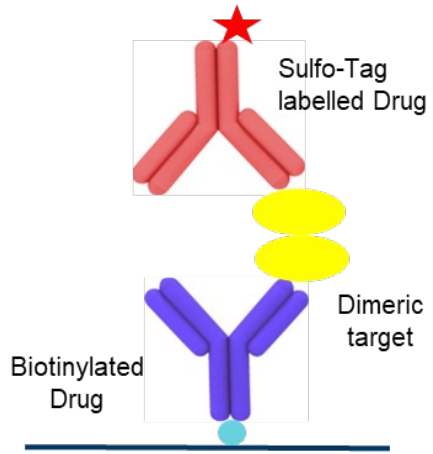
- **Bridging assays are the most common assay format for the detection of ADAs.**
  - **Advantages**
    - Capable of detecting multivalent isotypes.
    - Highly specific.
    - Good assay sensitivity.
    - Accepted by health agencies.
  - **Disadvantages**
    - Susceptible to target interference.
    - If the target is a soluble monomer – false negative results.
    - If the target is a soluble dimer – false positive results.



1. Bridging Assay



2. False Negative



3. False Positive

Immunogenicity method development required for a monoclonal antibody with a dimeric target

- Drug – Monoclonal Antibody.
- Assay format – homogenous bridging Electrochemiluminescent Immunoassay (ECLIA) on the MSD platform.
- Soluble dimeric target– risk of false positive results.

- **Limited literature available on soluble target levels in healthy and disease state samples.**
- **PK and PD data used to determine target levels however;**
  - PK and PD assays measure free drug/target respectively.
  - PD data does not represent target levels since the target bound to drug is not measured.

- **Non-clinical ADA data did not show target interference.**

Required assay performance driven by diseased state

Parameter	Requirement
Sensitivity	≤ 100 ng/mL PC
Drug Tolerance	100 µg/mL Drug + at 100 ng/mL PC
Target Tolerance	~25 ng/mL Target

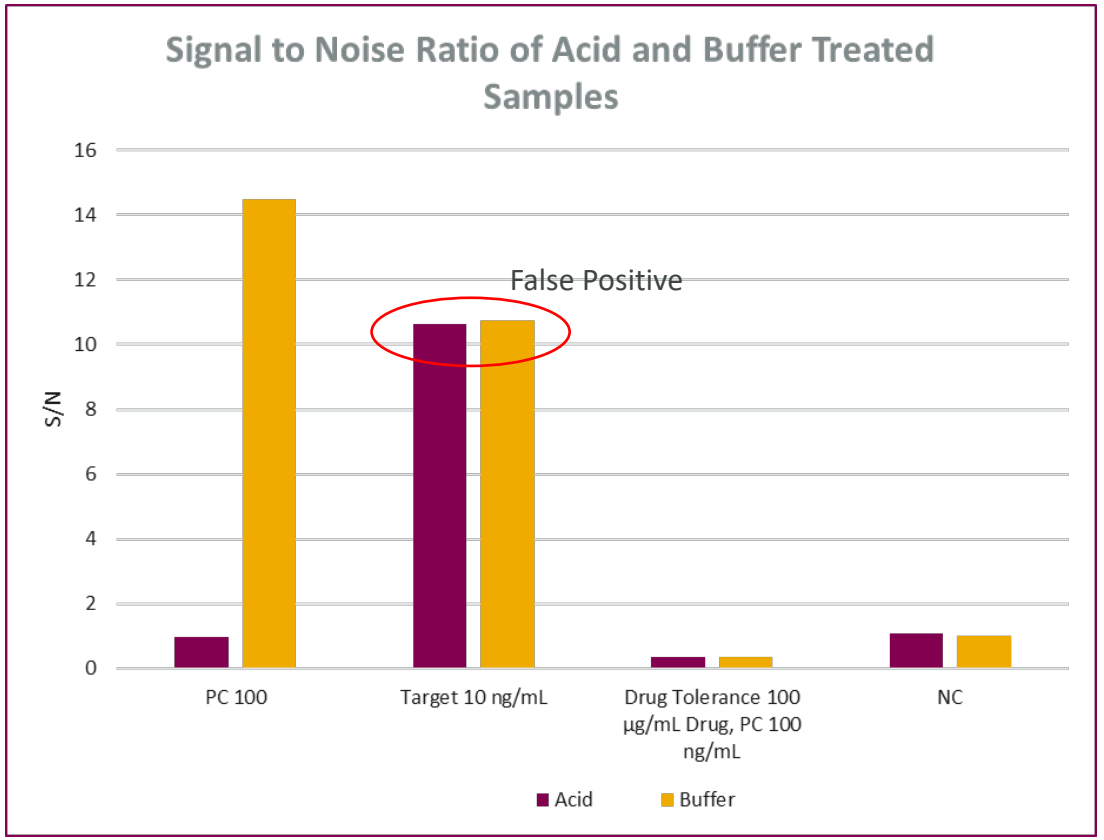
- **Positive Control (PC)**
  - Assay suitability control – used to monitor assay performance.
  - Used to establish assay sensitivity of at least 100 ng/mL.
  - However, surrogate controls may not be representative of the immune response observed in study samples.

Sample disruption using sample treatments to improve target and drug tolerance

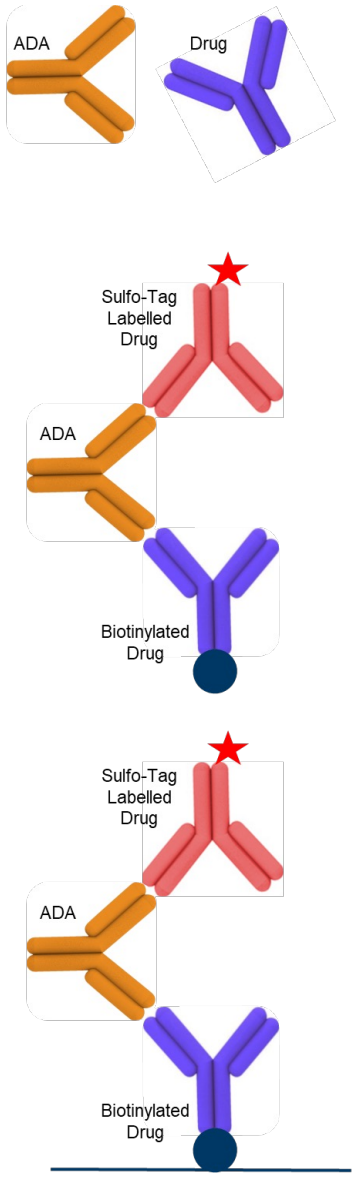
- To improve target and drug tolerance initially the assay was optimised prior to assessing alternative sample treatments:
  - Minimum Required Dilution (MRD) assessment.
  - Reagent Optimisation.

Acid Dissociation	Target Extraction	ACE: Affinity Capture Elution with Target Extraction
• Acid is used to dissociate ADA-drug complex.	• Anti-Target Antibody captures target, removing target from samples.	• Acid Dissociation of ADA-drug-target complex. • Affinity capture of ADA onto a solid phase. • Elution of captured ADA. • Target extraction.

Target and drug tolerance is not improved by acid dissociation



- PC appears acid labile.
- Method is not drug tolerant or target tolerant.



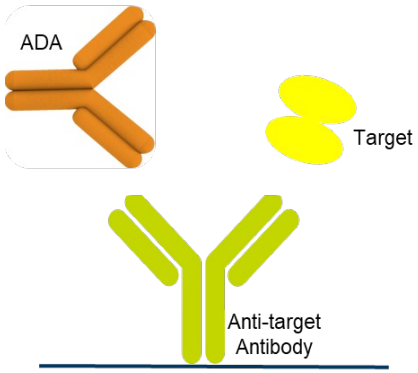
Step 1: Samples are acid dissociated. Drug-ADA complexes dissociate.

Step 2: Dissociated samples are added to a mastermix mixture. Incubated overnight.

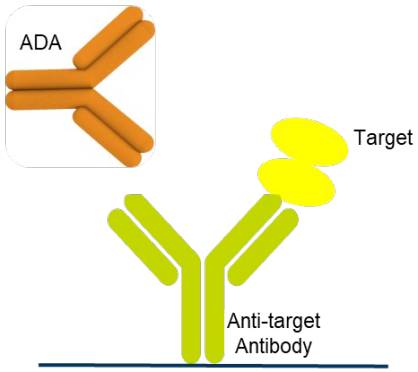
Step 3: Mixture is transferred to a streptavidin coated MSD plate the next day and incubated. After the addition of read buffer this is read on the MSD.

Target interference improvement using anti-target antibody

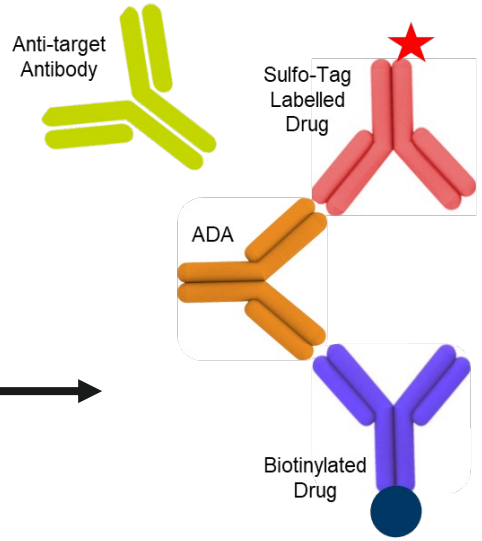
- Anti-target antibody introduced to remove target within the sample.



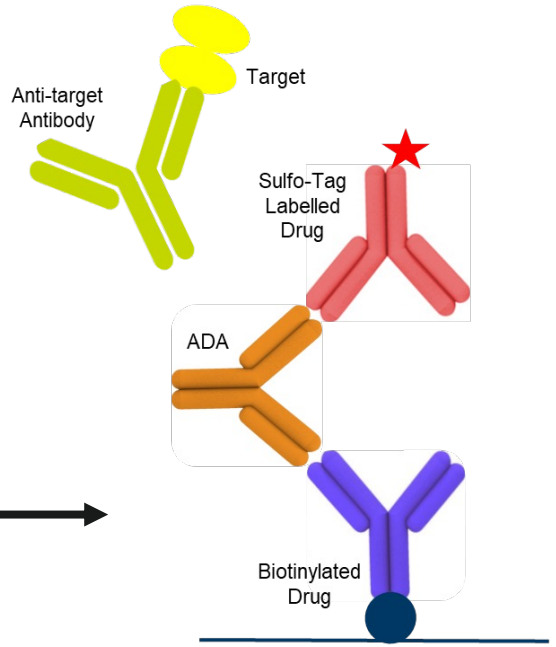
Step 1: Sample added to a high binding capacity plate coated with recombinant Anti-Target Antibody.



Step 2: Target binds Anti-Target Antibody.



Step 3: Sample transferred to mastermix solution containing labelled reagents and Anti-Target Antibody. Incubated overnight.



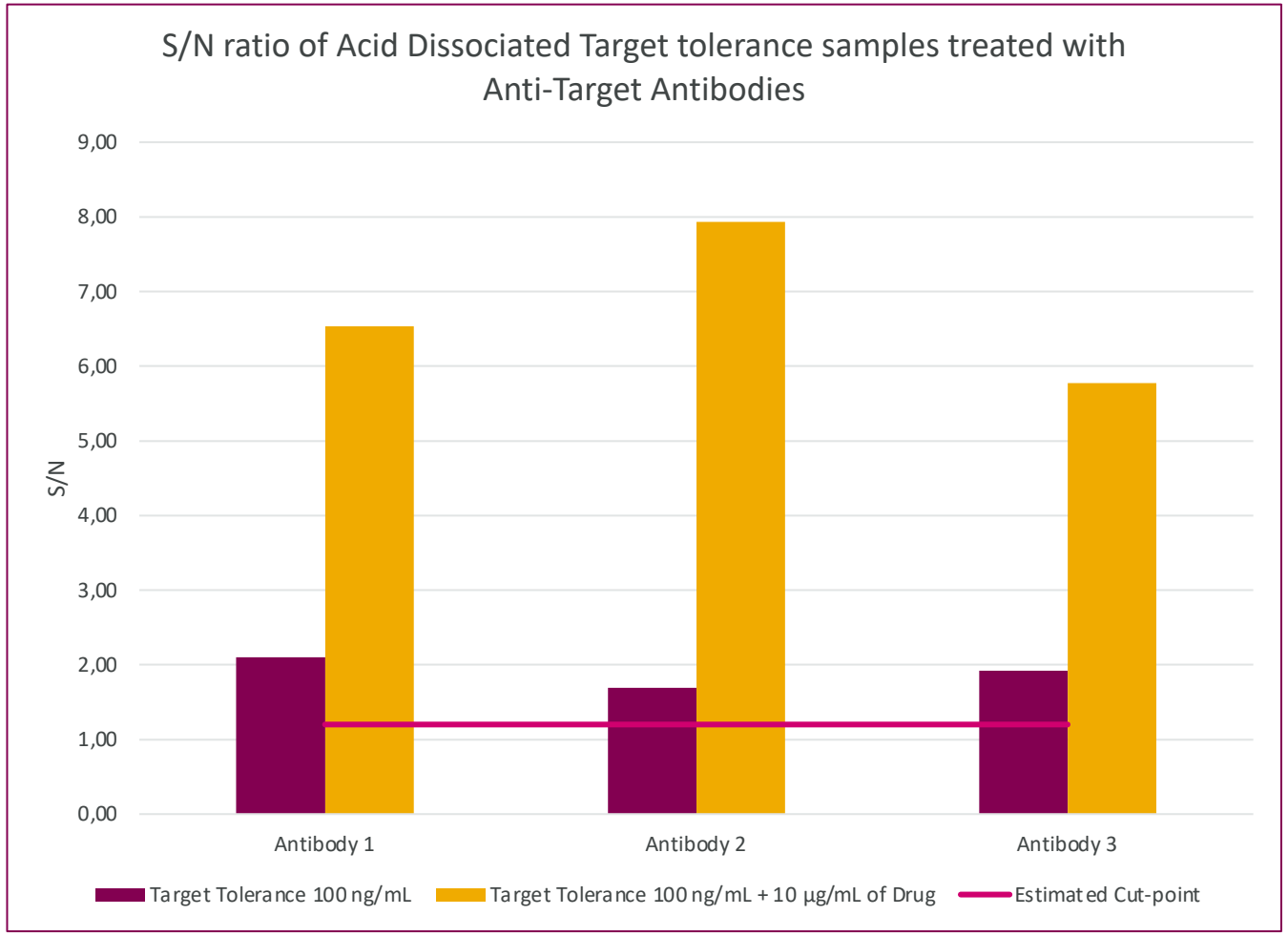
Step 4: Mixture is transferred to a streptavidin coated MSD plate the next day and incubated. After the addition of read buffer this is read on the MSD.

Target tolerance requirement updated due to emerging clinical data

- Using recombinant commercial anti-target antibody:
    - Tolerant to 25 ng/mL of target.
    - Tolerant to 200 µg/mL of drug at 100 ng/mL of PC.
    - Sensitive at 100 ng/mL.

- However, emerging clinical data showed higher levels of target was anticipated. Target tolerance of 200 ng/mL was required.

Alternative anti-target antibodies assessed to achieve target tolerance however…

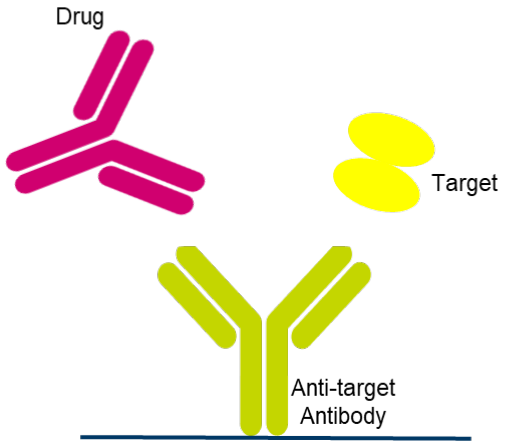


- Assessment of alternative in-house produced antibodies against the target.
  - Sample assessed with 100 ng/mL of target.
  - Sample assessed with 100 ng/mL of target + 10 µg/mL Drug.
- **When drug is added to the sample target interference increases in the presence of anti-target antibody – Why?**

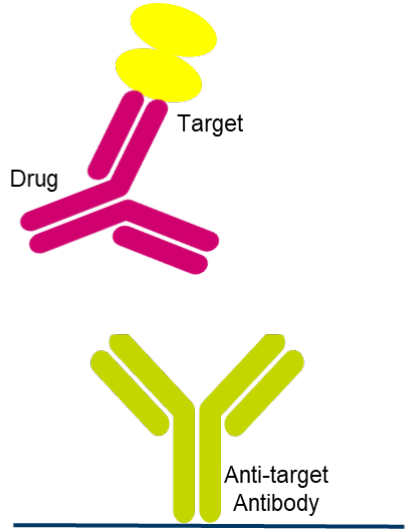
Drug interference identified due to high binding affinity of the drug to the target

- The drug has a higher binding affinity than the anti-target antibodies.
- The drug out-competes the anti-target-antibodies to bind to the target.

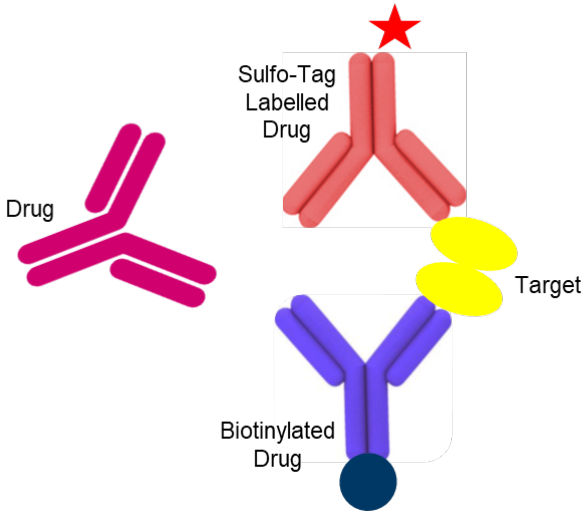
ID	K_d (nM)
Antibody 1	3.13
Antibody 2	2.35
Antibody 3	Not determined
Drug	0.159



Step 1: Target removal step: Drug and Anti-Target Antibody compete to bind target.



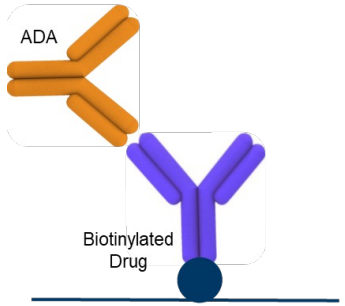
Step 2: Drug binds to the target due to higher affinity to drug than Anti-Target Antibody.



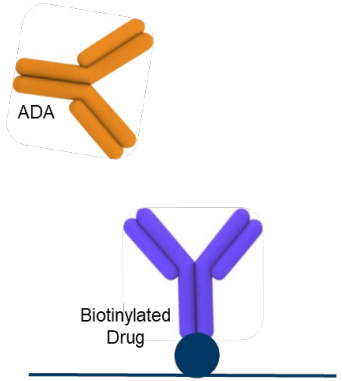
Step 3: Sample transferred to mastermix solution: drug-target complex dissociates and target binds to the labelled drug.

Drug removal step only marginally improves target tolerance

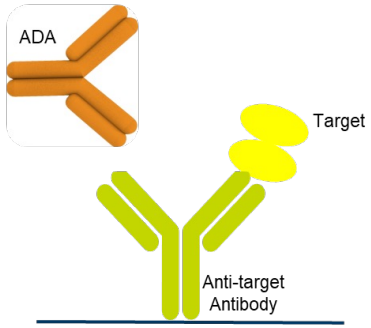
Drug removal step introduced to reduce drug interference using the method outlined below:



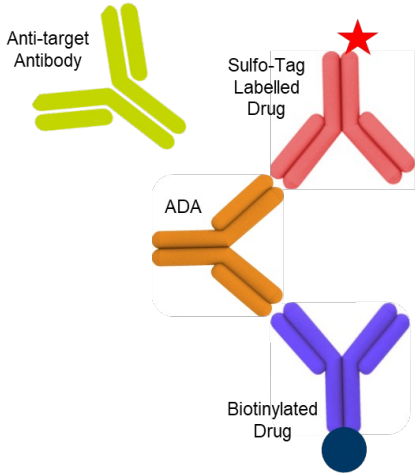
Step 1: Acid dissociated sample added to a plate coated with biotinylated drug.



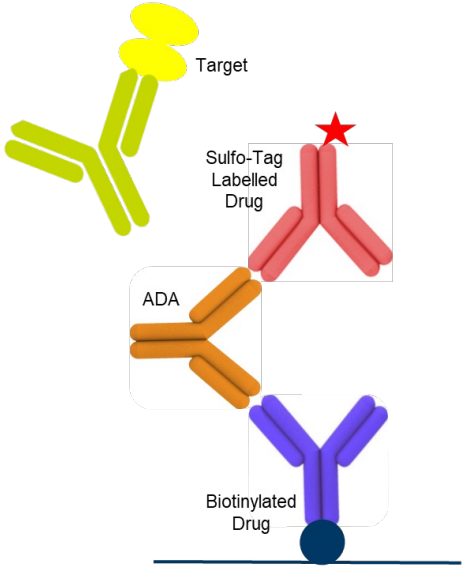
Step 2: Plate is washed to remove excess drug. Then, ADA is eluted from the plate.



Step 3: Sample is transferred to an MSD plate coated with Anti-Target Antibody and incubated.



Step 4: Sample is transferred to mastermix solution containing labelled drug and Anti-Target Antibody. This is incubated overnight.

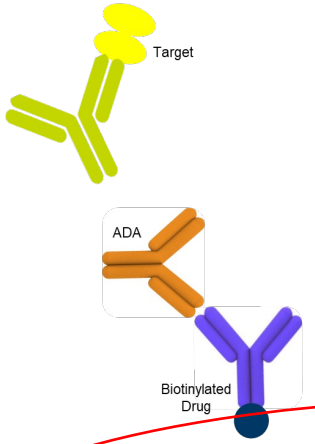


Step 5: Mixture is transferred to a streptavidin coated MSD plate and incubated. After the addition of read buffer this is read on the MSD.

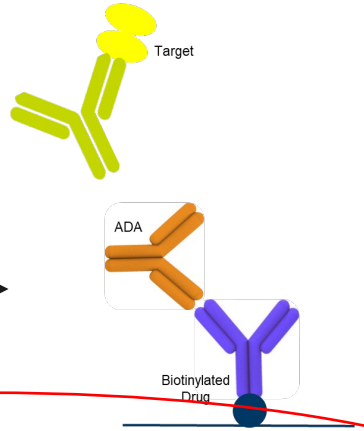
Target tolerance improved, but not to the level that was required.

Next steps: Introduce additional anti-target-antibody.

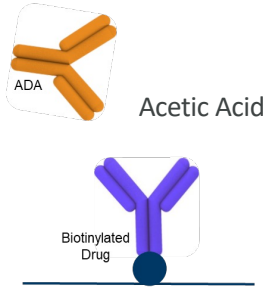
Additional anti-target antibody improves target tolerance



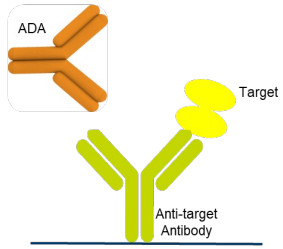
Step 1: Acid dissociated sample pre-incubated with biotinylated drug and Anti-Target Antibody.



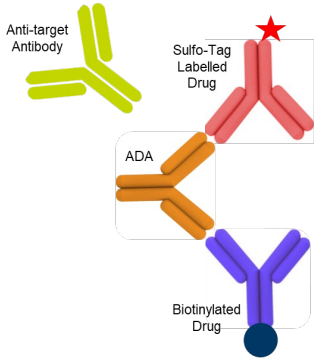
Step 2: Complex is transferred to streptavidin coated high binding capacity plate.



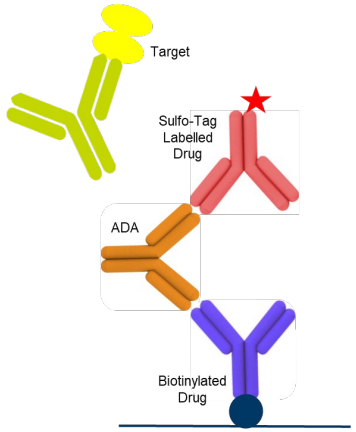
Step 3: Plate is washed to remove excess drug and target-anti-target antibody complexes. Then, ADA is eluted from the plate using acid.



Step 4: Further target removal: sample is transferred to an MSD plate coated with Anti-Target Antibody and incubated.



Step 5: Sample is transferred to mastermix solution containing labelled drug and Anti-Target Antibody. This is incubated overnight.



Step 6: Mixture is transferred to a streptavidin coated MSD plate and incubated. After the addition of read buffer this is read on the MSD.

Assay parameters achieved using additional anti-target antibody

Original assay requirements:

Parameter	Requirement
Sensitivity	≤ 100 ng/mL PC
Drug Tolerance	100 µg/mL Drug + at 100 ng/mL PC
Target Tolerance	~25 ng/mL Target

Assay parameters achieved:

Parameter	Result
Sensitivity	≤ 100 ng/mL PC
Drug Tolerance	100 µg/mL Drug + at 100 ng/mL PC
Target Tolerance	~500 ng/mL Target

Conclusion

- Soluble target can present challenges for the measurement of immunogenicity.
- This presentation highlights the challenge and importance of achieving target tolerant immunogenicity assays.
- This case study illustrates the importance of:
  - Developing a fit for purpose immunogenicity assay, using pharmacokinetic and pharmacodynamic data to determine the assay parameters.
  - Understanding the properties of the target and drug.

Thank you