Strategies for Improving Drug Tolerance in Immunogenicity Assay

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Frontage Laboratories, Inc.
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OUTLINES

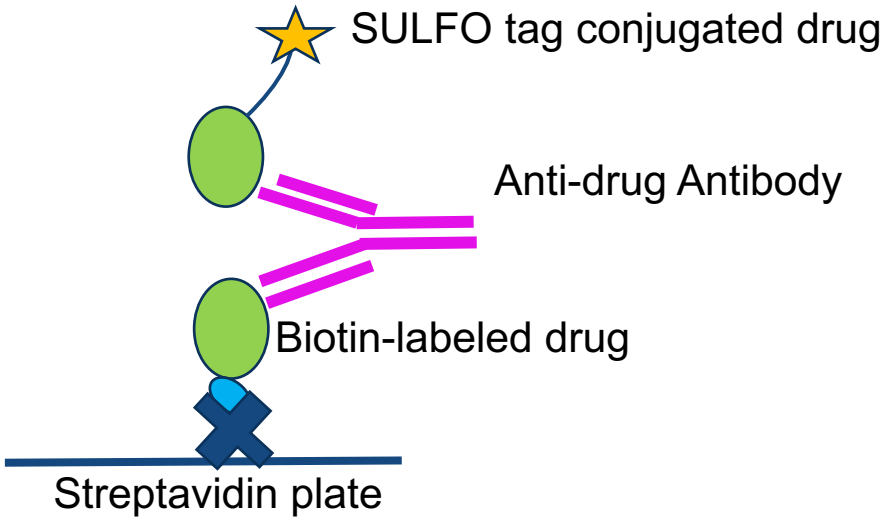
- Drug tolerance in Immunogenicity Assay
- ADA Assay
- Drug interference to ADA Assay
- NAb Assay
- Drug interference to NAb Assay
- Strategies to Mitigate Drug Interference
- Case Studies

DRUG TOLERANCE IN IMMUNOGENICITY ASSAY

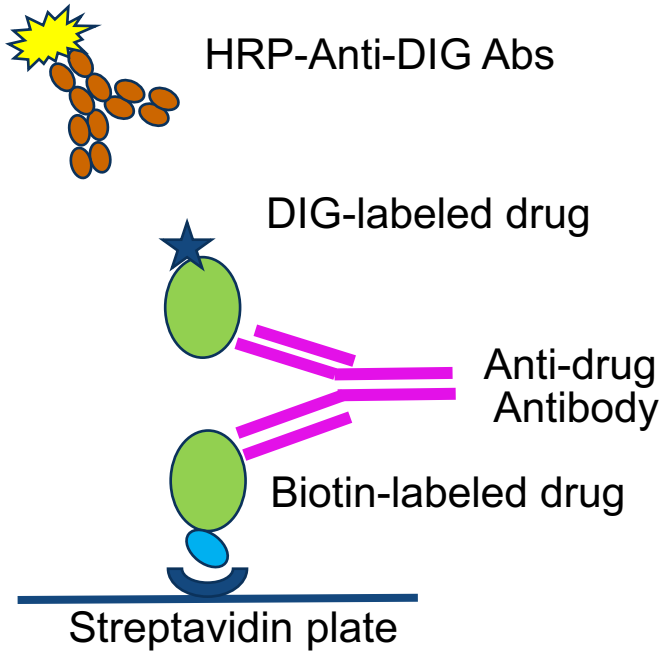
1. The therapeutic protein product present in the sample may interfere with the sensitivity of the assay. Drug tolerance refers to the maximum concentration of free drug that can be in a sample without causing false positive or false negative.
2. Regulatory guidance documents (EMA and FDA) stress the importance of evaluation of drug tolerance.
3. Drug interference is the biggest technical challenge in immunogenicity assays
4. Drug interference is especially challenging for mAb therapeutics, which are administered at high doses and have long half-lives
5. The targeted drug tolerance level is intended to exceed the concentration at the C-trough in clinical samples.

TYPICAL ADA ASSAYS

MSD
Bridge Assay

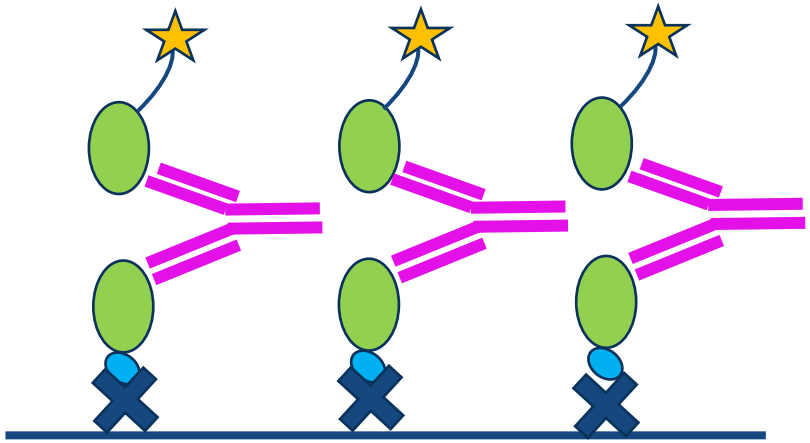


Elisa
Absorbance



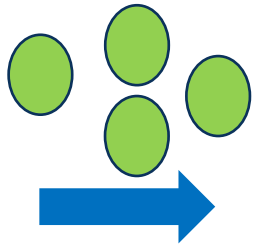
DRUG INTERFERENCE TO ADA ASSAY

MSD
Bridge Assay

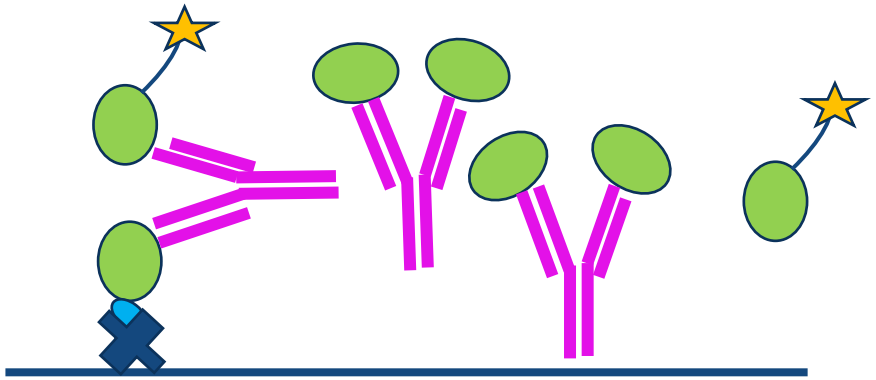


Streptavidin plate

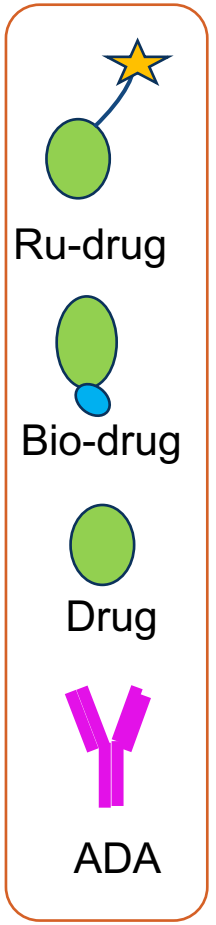
Drug
in the sample



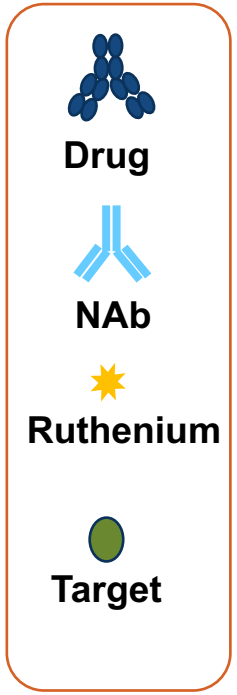
Drug in sample will compete for ADA binding with labeled drug, leading to false negative results or underestimation of titer

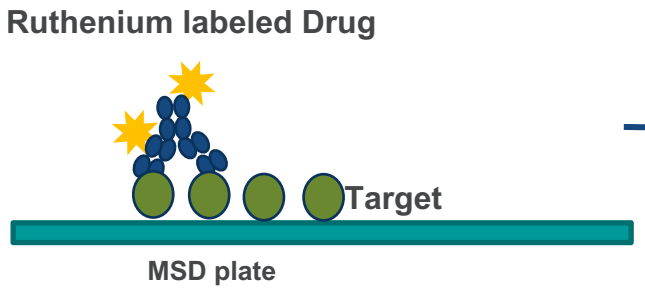


Streptavidin plate

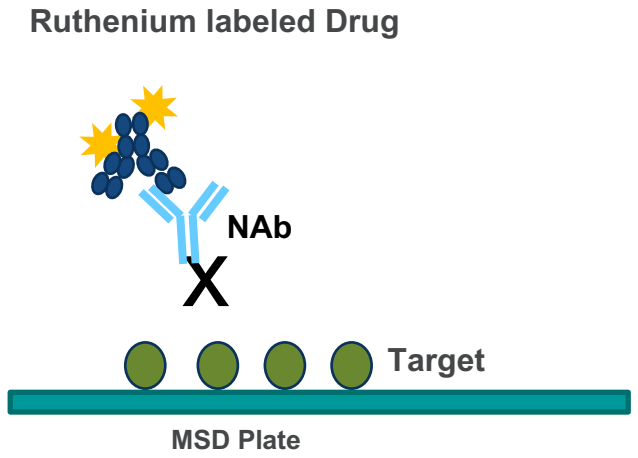


A TYPICAL NON-CELL BASED NAB ASSAY





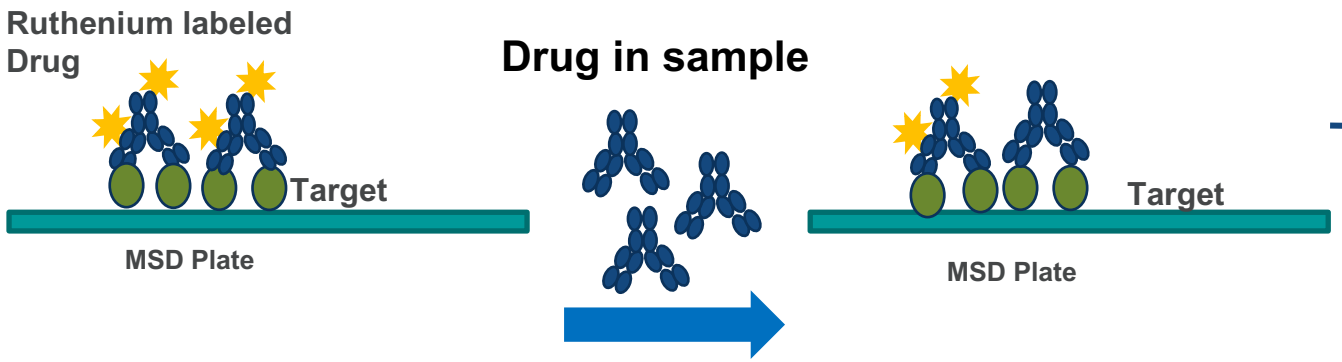
NAb negative,
High signal



NAb Positive,
NAb disrupt Drug's binding to the target,
low signal

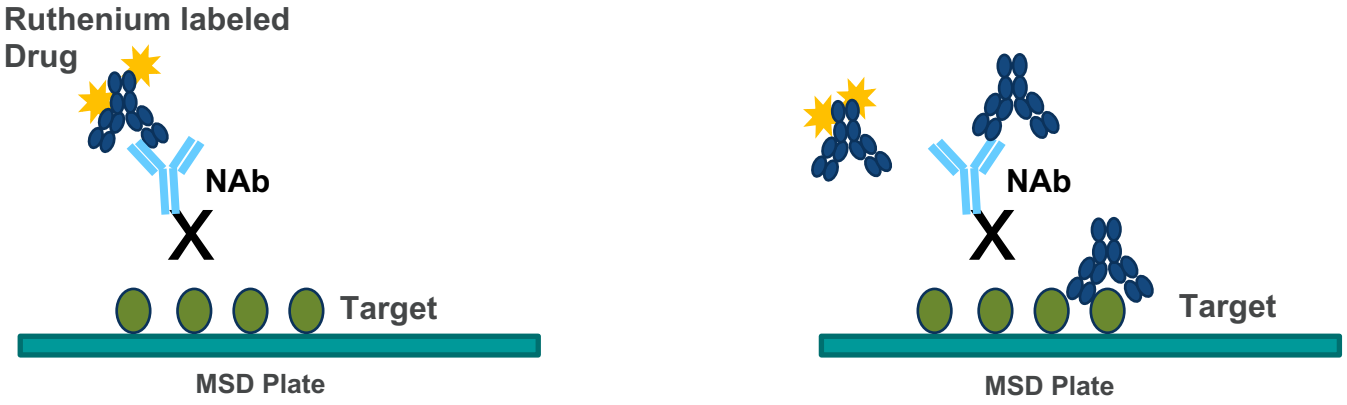
DRUG INTERFERENCE TO LBA NAB ASSAY

NAb negative



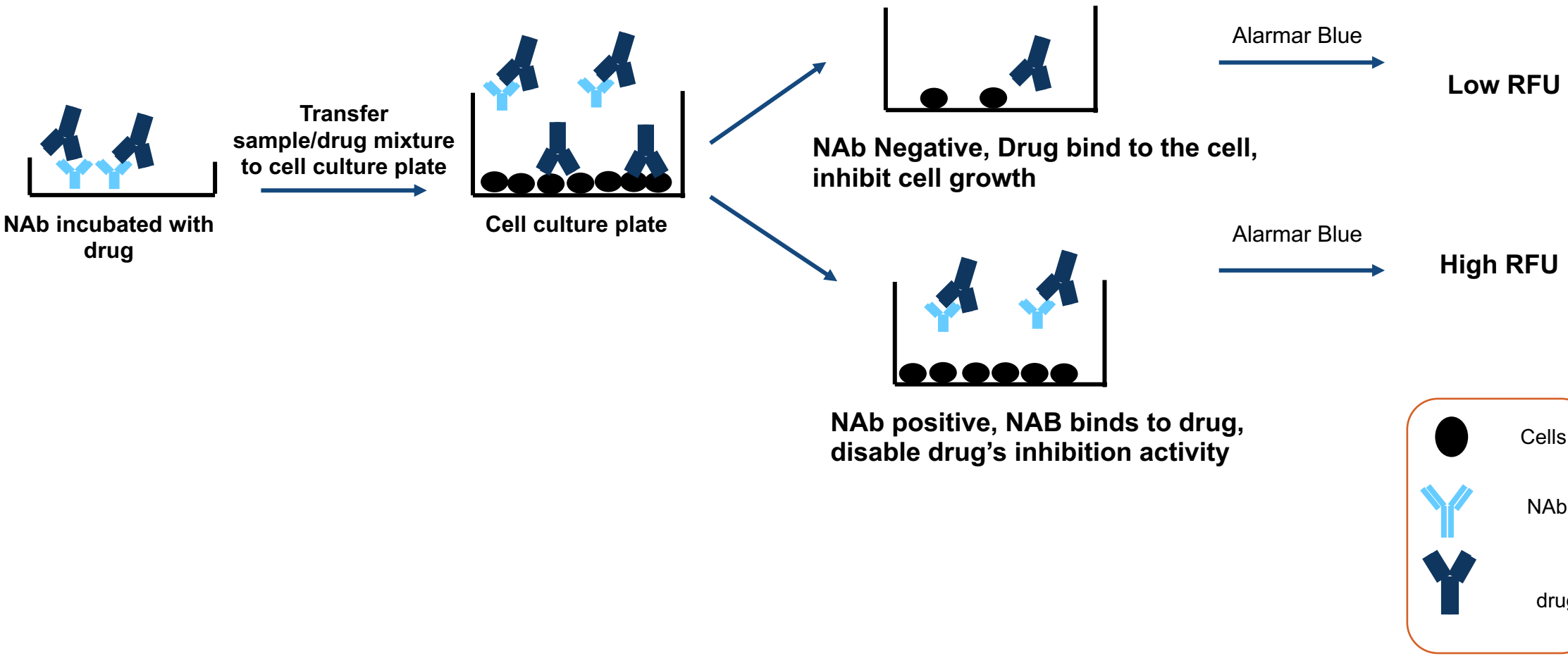
Drug in sample will compete for Target binding with labeled drug, Leading to false positive

NAb Positive



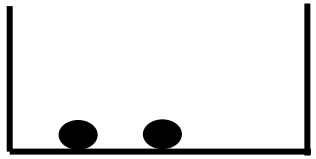
1. Drug compete for NAB binding with labeled drug,
2. Drug compete for Target binding with labeled drug,

A TYPICAL CELL-BASED NAB ASSAY: PROLIFERATION-BASED ASSAY

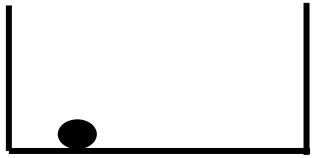


DRUG INTERFERENCE TO CELL-BASED NAB ASSAY

NAb negative

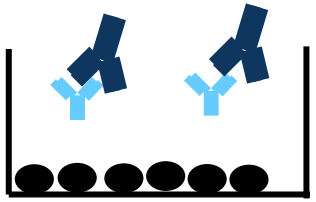


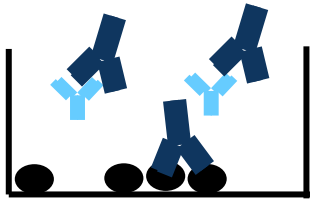
Drug in sample



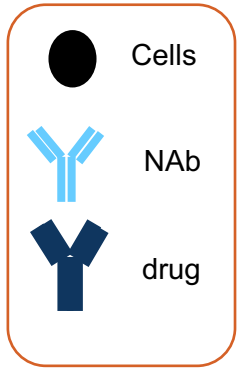
When NAb Negative, extra drug in sample will further decrease the assay signal, but negative samples are still negative

NAb positive





When NAb positive, extra drug in sample can bind/inhibit cell growth, leading to false negative



STRATEGIES TO MITIGATE DRUG INTERFERENCE

ADA Bridge assay

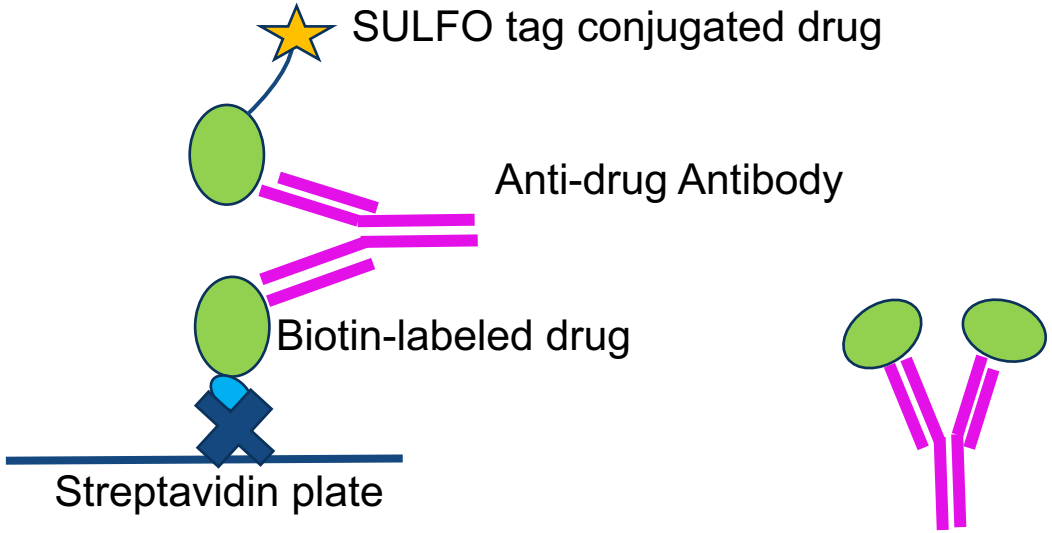
- **Increase sample dilution**
- **Increase labeled drug concentration**
- **Acid dissociation**
- **High ionic strength dissociation assay**
- **Heat-pretreatment**
- **Drug removal methods**

I. ACE (Affinity Capture and Elution)

II. SPEAD (Solid-Phase Extraction with Acid Dissociation)

III. Bead (Biotin-drug Extraction and Acid Dissociation)

IV. KF based BEAD method



STRATEGIES TO MITIGATE DRUG INTERFERENCE

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- **Increase drug/or labeled drug concentration**
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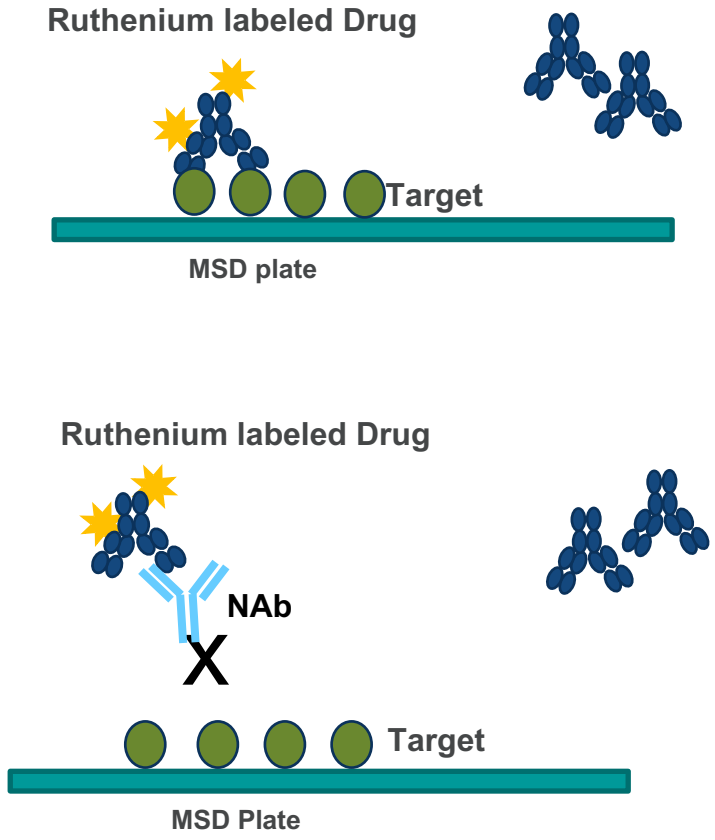
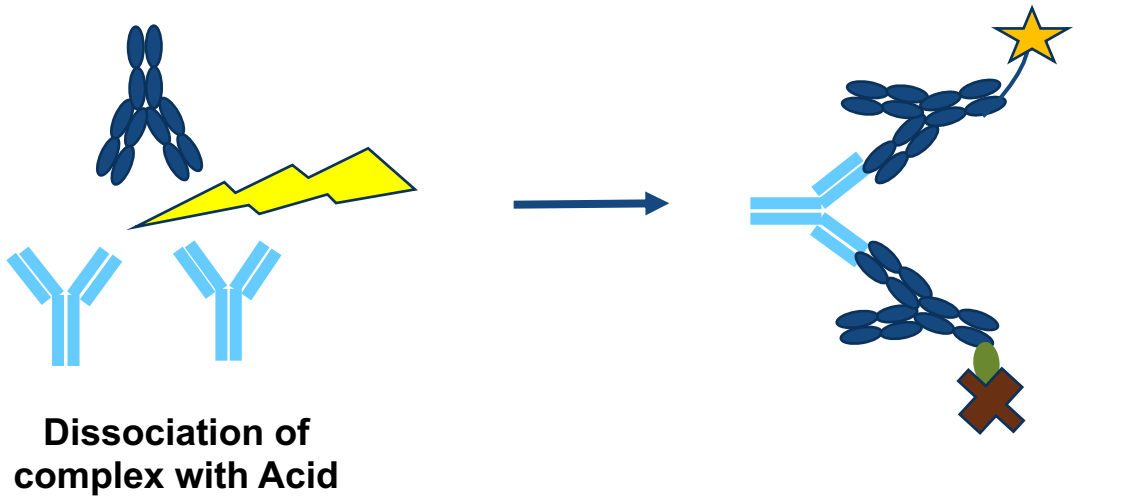


ADA CASE STUDY: CHALLENGES WITH ACID DISSOCIATION



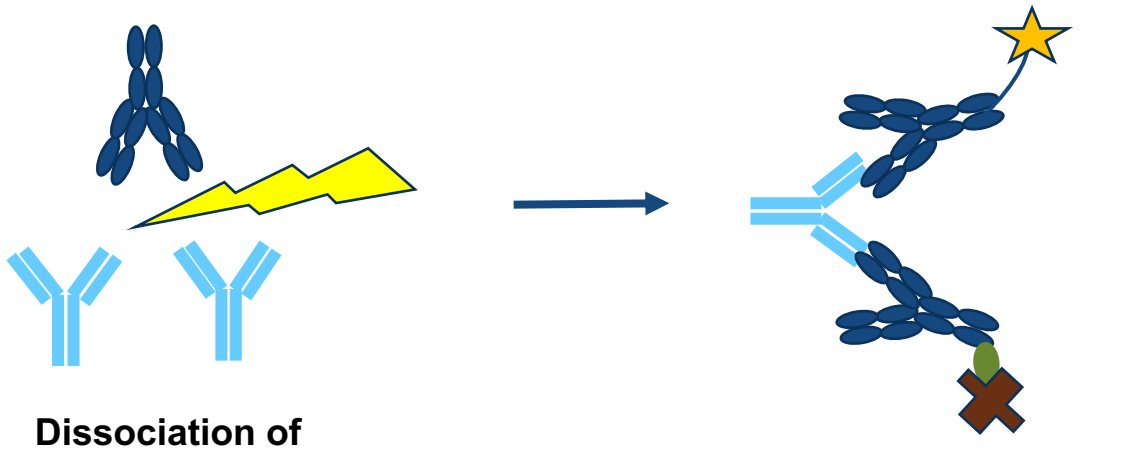
Dissociation of complex with Acid

Neutralizing with MM with base buffer

1. Drug tolerance was high with acid dissociation, > 100ug/mL at PC100 ng/mL
2. Surrogate PCs were not stable at Acid Buffer
3. Different combinations of acid/base buffer were tested, and unexpected abnormal signals persisted
4. Without Acid treatment, drug tolerance decreased to ~10 ug/mL, cannot meet the requirement
5. Heat-pretreatment cannot reduce drug interference

Standard Acid dissociate approach

ADA CASE STUDY: HIGH IONIC STRENGTH DISSOCIATION ASSAY



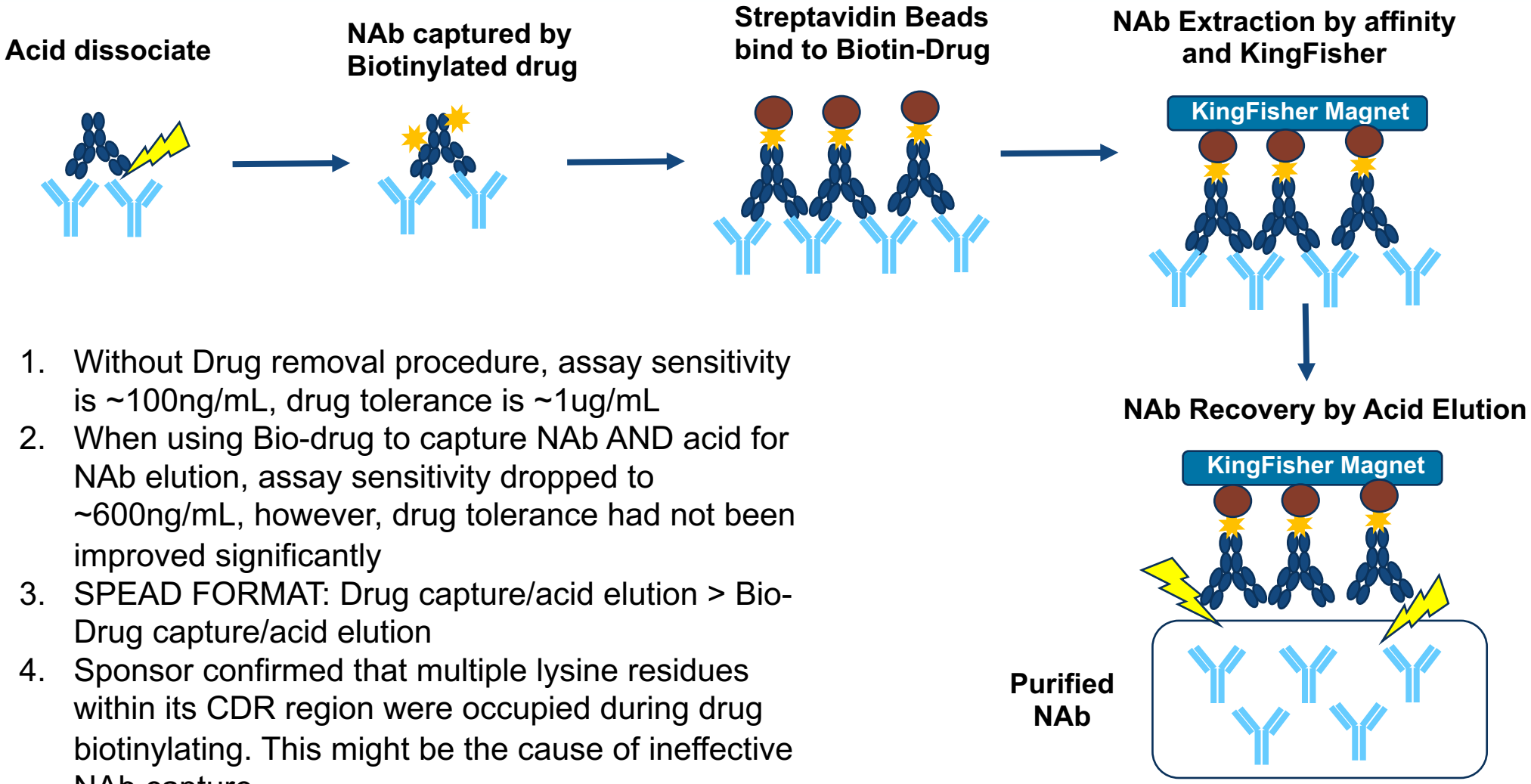
Dissociation of complex with high ionic buffer (4M MgCl2)

MgCl2 treated samples are 1:10 diluted with MM in assay buffer

1. MgCl2 solution is non-denaturing, and should cause minimal changes to serum proteins' secondary and tertiary structures
2. With High ionic strength dissociation, Drug tolerance met requirement, ~50ug/mL at PC100 ng/mL
3. Assay is stable with intra/inter assay precision CV%<10

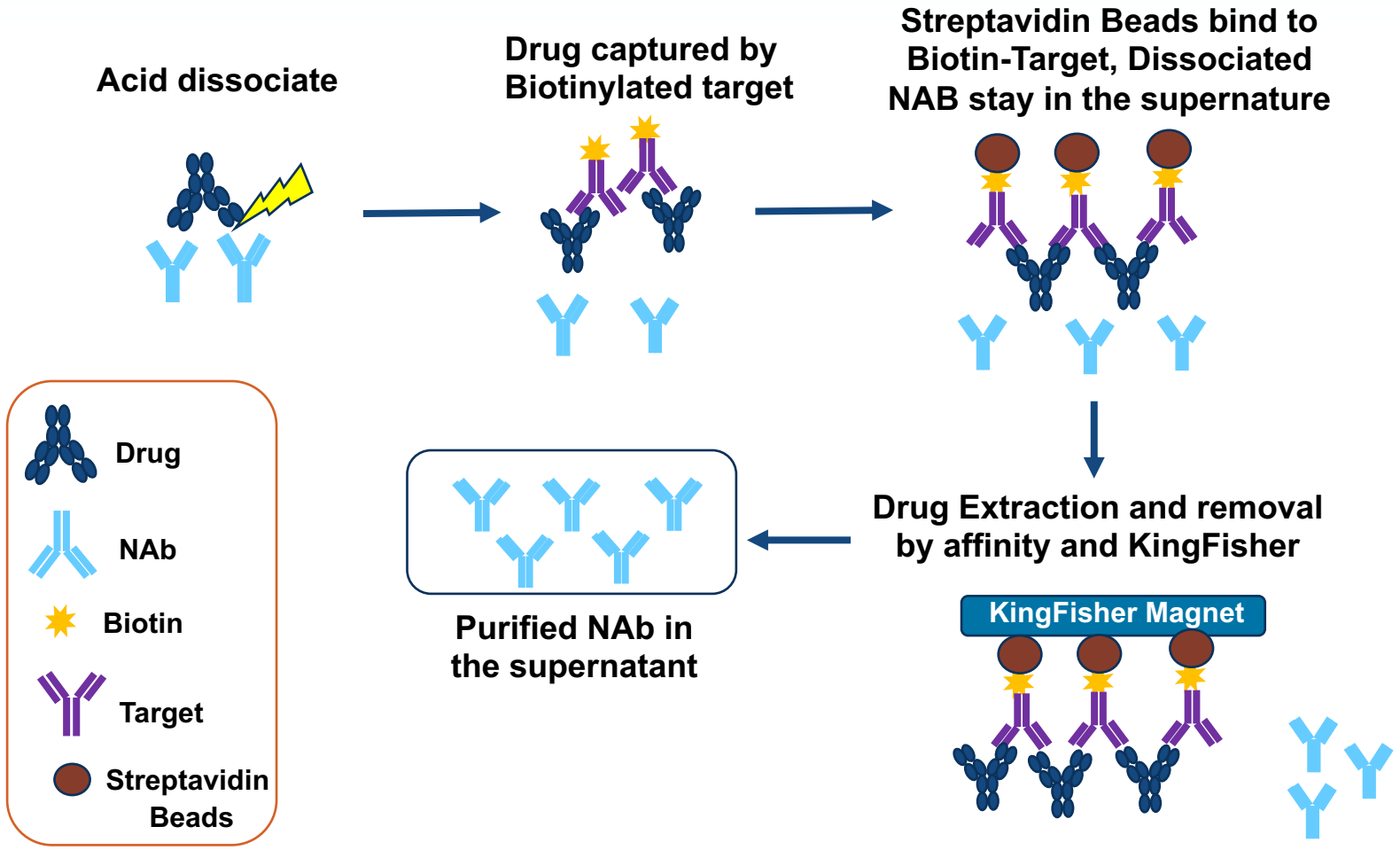
High ionic strength dissociation assay

NAB CASE STUDY: CHALLENGES WITH DRUG-CAPTURE FORMAT



1. Without Drug removal procedure, assay sensitivity is ~100ng/mL, drug tolerance is ~1ug/mL
2. When using Bio-drug to capture NAb AND acid for NAb elution, assay sensitivity dropped to ~600ng/mL, however, drug tolerance had not been improved significantly
3. SPEAD FORMAT: Drug capture/acid elution > Bio-Drug capture/acid elution
4. Sponsor confirmed that multiple lysine residues within its CDR region were occupied during drug biotinylating. This might be the cause of ineffective NAb capture

NAB ASSAY DRUG REMOVAL: TARGET CAPTURE FORMAT



1. With target capture format, Drug tolerance met requirement, ~40ug/mL at PC1000 ng/mL
2. Sensitivity is very good, ~150ng/mL
3. Assay is stable with intra/inter assay precision CV%<20
4. The assay can tolerate up to 1ug/mL of target
5. No matrix interference

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I. ACE (Affinity Capture and Elution)
II. SPEAD (Solid-Phase Extraction with Acid Dissociation)
III. Bead (Biotin-drug Extraction and Acid Dissociation)
IV. KF based BEAD method-Target capture format

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THANK YOU

FRONTAGE