

STRATEGIES FOR IMPROVING ADA ASSAY SENSITIVITY, WHEN HIGH DRUG OR TARGET CONCENTRATIONS CAUSE INTERFERENCE

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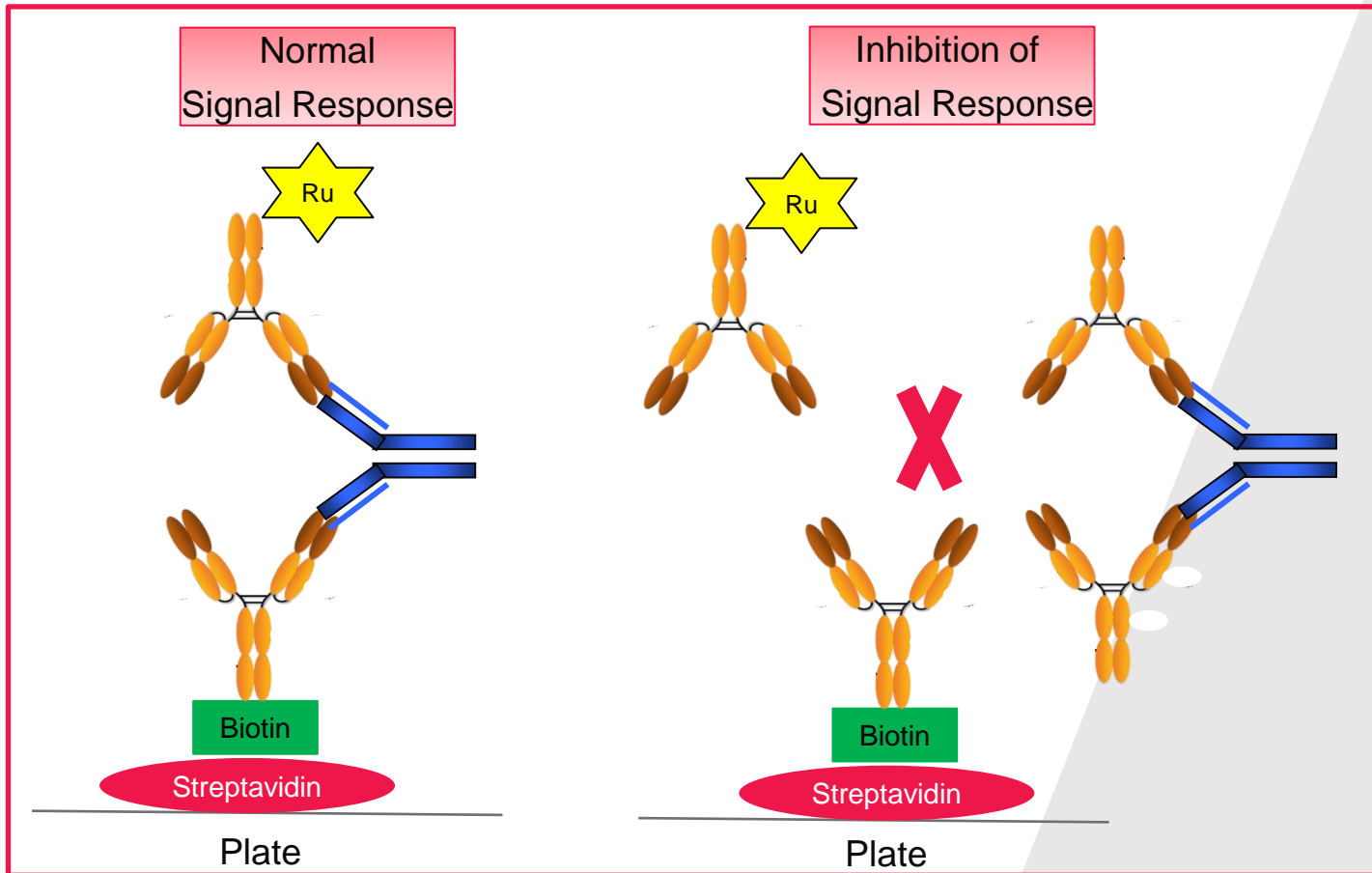
Agenda

- ▶ Introduction to why understanding ADA assay drug tolerance is important
- ▶ Strategies for improving assay sensitivity when drug tolerance is an issue
- ▶ Introduction to why understanding ADA assay target interference is important
- ▶ Strategies for improving assay sensitivity when target interference is an issue
- ▶ Conclusions

Why understanding ADA assay drug tolerance is important

Why do we need to understand ADA assay drug tolerance?

The presence of high concentrations of drug can inhibit the ability to detect anti-drug antibodies



Considerations for ADA assay drug tolerance assessment

Need to consider half life of some bio therapeutics. A high circulating concentration of drug can be present weeks after dosing for some compounds

Type of drug	Molecular Mass	Half life
Monoclonal Ab	150 KDa	≈ 14 Days
scFv	27 KDa	≈ 1 hour
Endogenous insulin	5.8 KDa	≈ 4 to 6 minutes

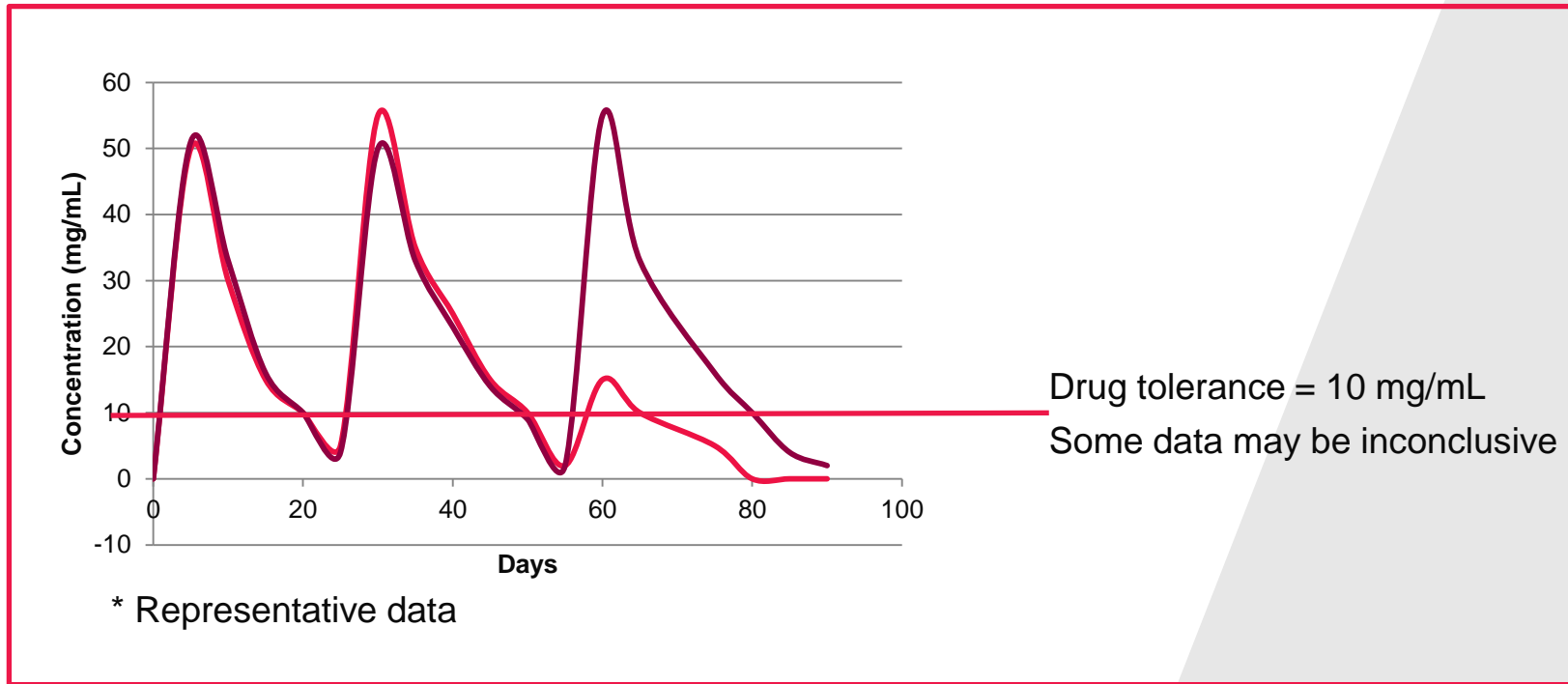
Need to consider nature of drug.

- Has it been designed for an extended half life ?
- Are there components that enable recycling of the drug back into the circulation?

Need to consider nature of study design.

- Is this a study where high doses are used?
- When are the ADA sampling time points?

Underestimation of ADA can result in poor interpretation of result



Therefore, it is important to understand when ADA can be reliably detected.

Image: Covance

Reality of drug tolerance assessment

- Recommendations for the design and optimization of immunoassays used in the detection of host antibodies against biotechnology products.
- Mire-Sluis et al. Journal of Immunological Methods. Vol 289 p1-16 (2004)
 - > Sensitivity of 250-500ng/mL in the presence of drug (Clinical)
 - > Sensitivity of 500-1000ng/mL in the presence of drug (Non-Clinical)
- FDA Draft Immunogenicity guidance > Development of specific and sensitive (including drug tolerant) assays for evaluating antibodies to therapeutic proteins down to 100ng/mL
- Every positive control will have a distinct isotype, affinity, avidity, epitope specificity, and concentration.
- All these factors in combination will affect interpretation of drug tolerance.
- The PC characteristics will be distinct from the mixture of sample characteristics.
- The PC used to determine assay sensitivity and drug tolerance is a guide to the general relevance of the assay.

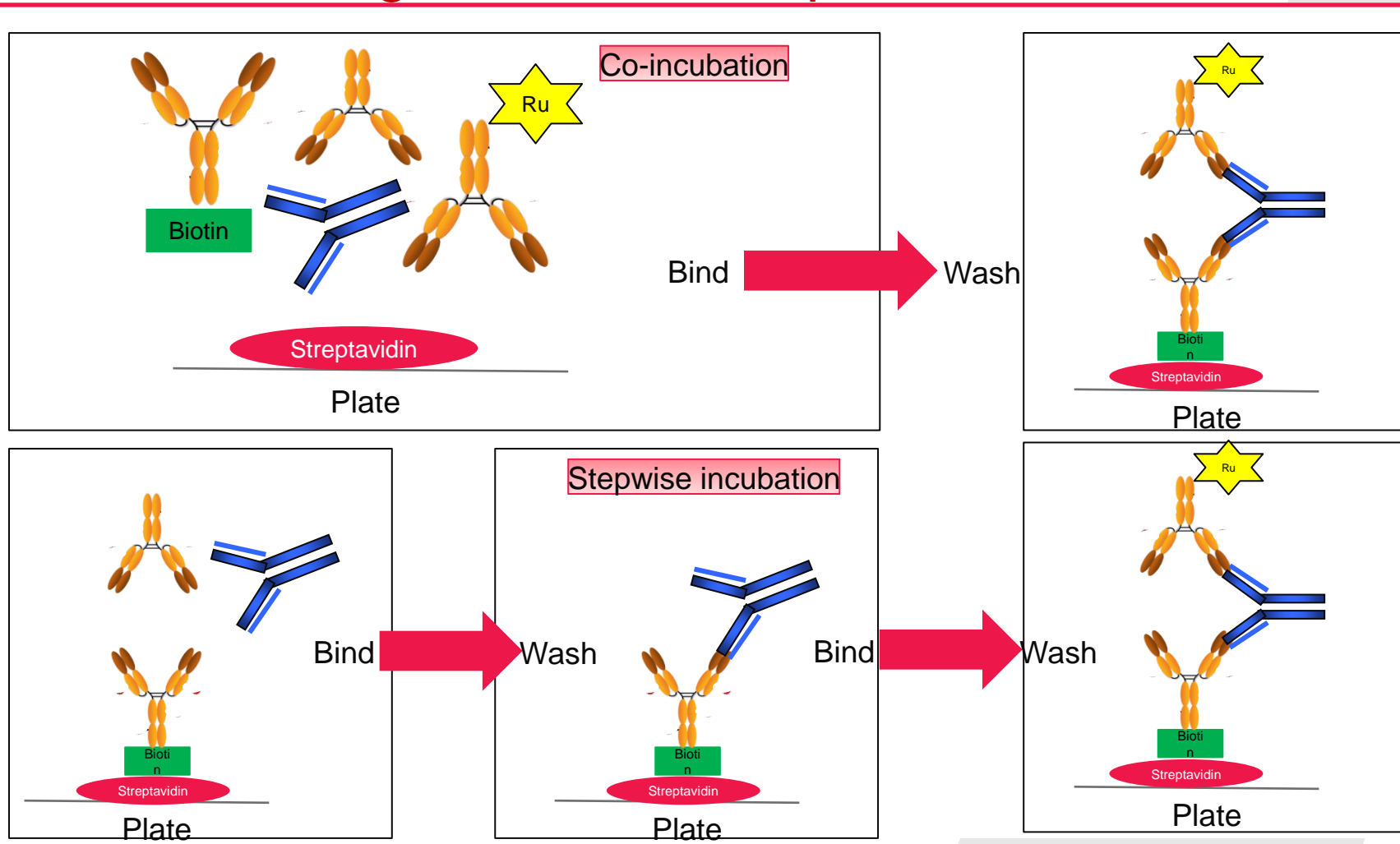
Strategies for improving assay sensitivity when drug tolerance is an issue



Pros and Cons of platforms that may facilitate the detection of drug in the presence of the ADA

Platform	Method Advantage	Method Disadvantage
Standard ELISA	Cost effective	Lower sensitivity influences drug tolerance that can be achieved
MSD platform	Higher sensitivity with low background signal increases window for drug tolerance	Proprietary equipment & cost
Gyrolab	Automated set up with acid dissociation incorporated	Proprietary equipment & cost
Immuno PCR	High dilutions can be applied to shift equilibrium to free ADA. High sensitivity enables detection	Proprietary equipment & cost

Optimisation of assay design – Co-incubation of critical reagents versus stepwise addition



Can also optimise assay for drug tolerance by increasing sample dilution or by increasing incubation times to allow critical reagents to outcompete free drug

Acid Dissociation – Patton et al 2005*

- Basic method applied to increase drug tolerance
- Low pH breaks up immune complexes allowing release of ADA from free drug

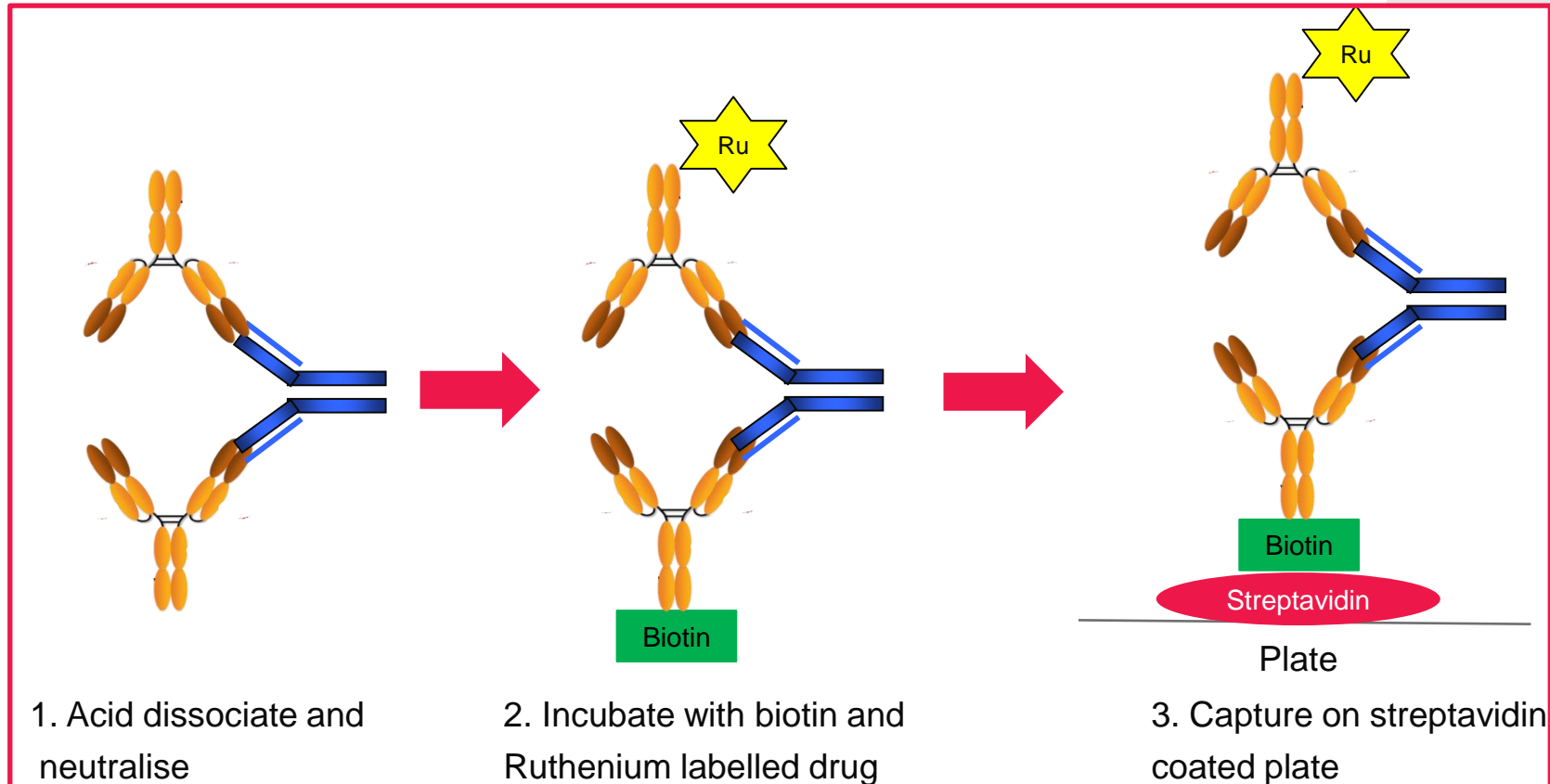
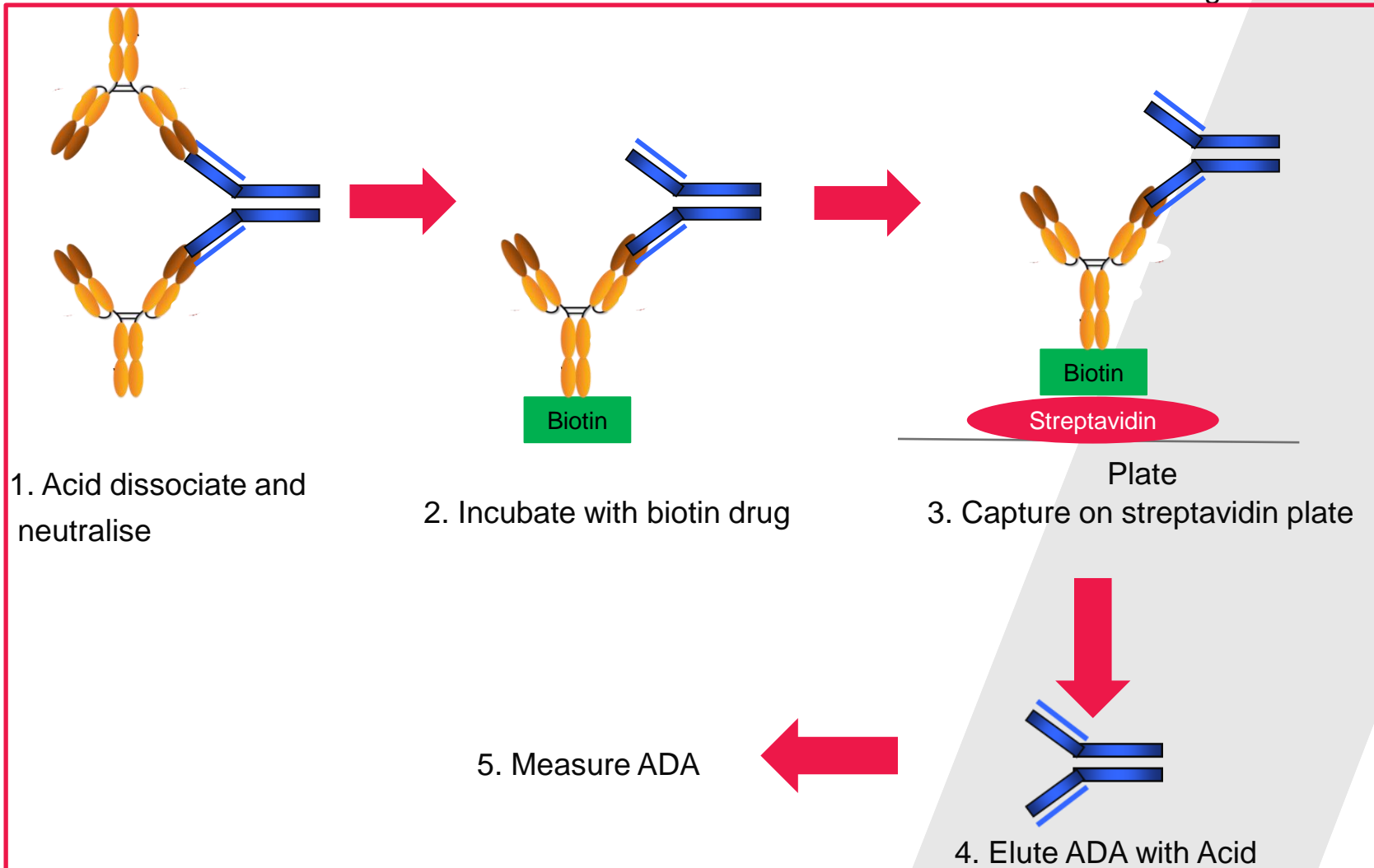


Image: Covance

* Patton A et al. J. Immunol Methods. 2005;304(1-2):41-8.

Solid-Phase Extraction with Acid Dissociation (SPEAD) Technique – Smith et al 2007*

Image: Covance



*Smith et al. Regul Toxicol Pharmacol. 2007;49(3):230-7

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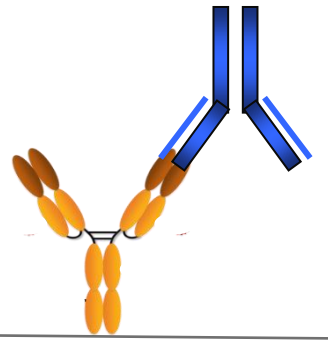
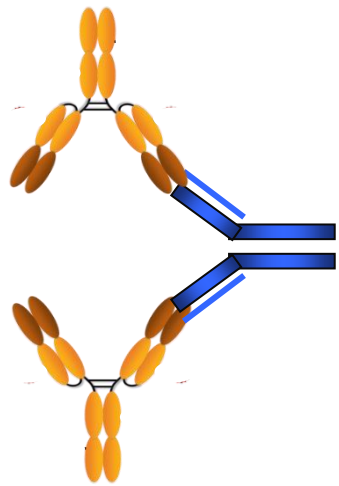
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Affinity Capture Elution (ACE) Technique

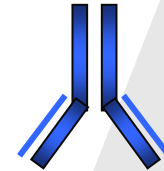
– Bourdage et al 2007*

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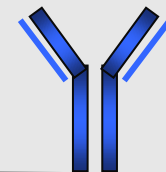


Plate

2. Capture on drug coated plate

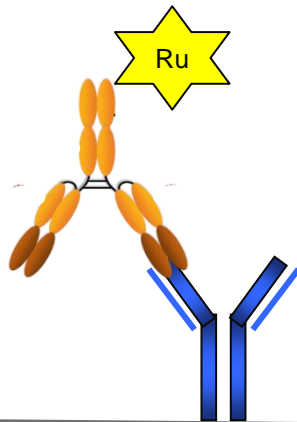


3. Elute using acid



Plate

4. Neutralise and coat on plate



Plate

5. Detect with Ruthenium labelled drug

1. Acid dissociate and neutralise

*Bourdage et al. J Immunol Methods. 2007;327(1-2):10-7.

Precision and Acid Dissociation (PandA) method – Zogbhi et al 2015*

Image not released

*Zogbhi et al. J Immunol Methods. 2015; 426:62-9.

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Covance experience with various methods for improving drug tolerance

Equipment:

MSD still tends to be the platform of choice for ADA and is generally considered to be a platform with good drug tolerance

Assay design:

Recommend a stepwise approach for assays with issues with drug tolerance

Assay format:

Most assays incorporate acid dissociation in the standard format defined by Patton et al which generally gives 10 to 100 fold improvement in drug tolerance.

However, investigating PandA method which claims over 200 fold improvement. This could be a potential game changer for overcoming issues with drug tolerance

Why understanding ADA assay target interference is important

Why do we need to understand ADA assay target interference?

Target may inhibit ADA binding at interface between drug and its target resulting in a **False negative**

OR

Multimeric target may form bridge with critical reagents used in ADA assay resulting in a **False positive**

Sources of target interference

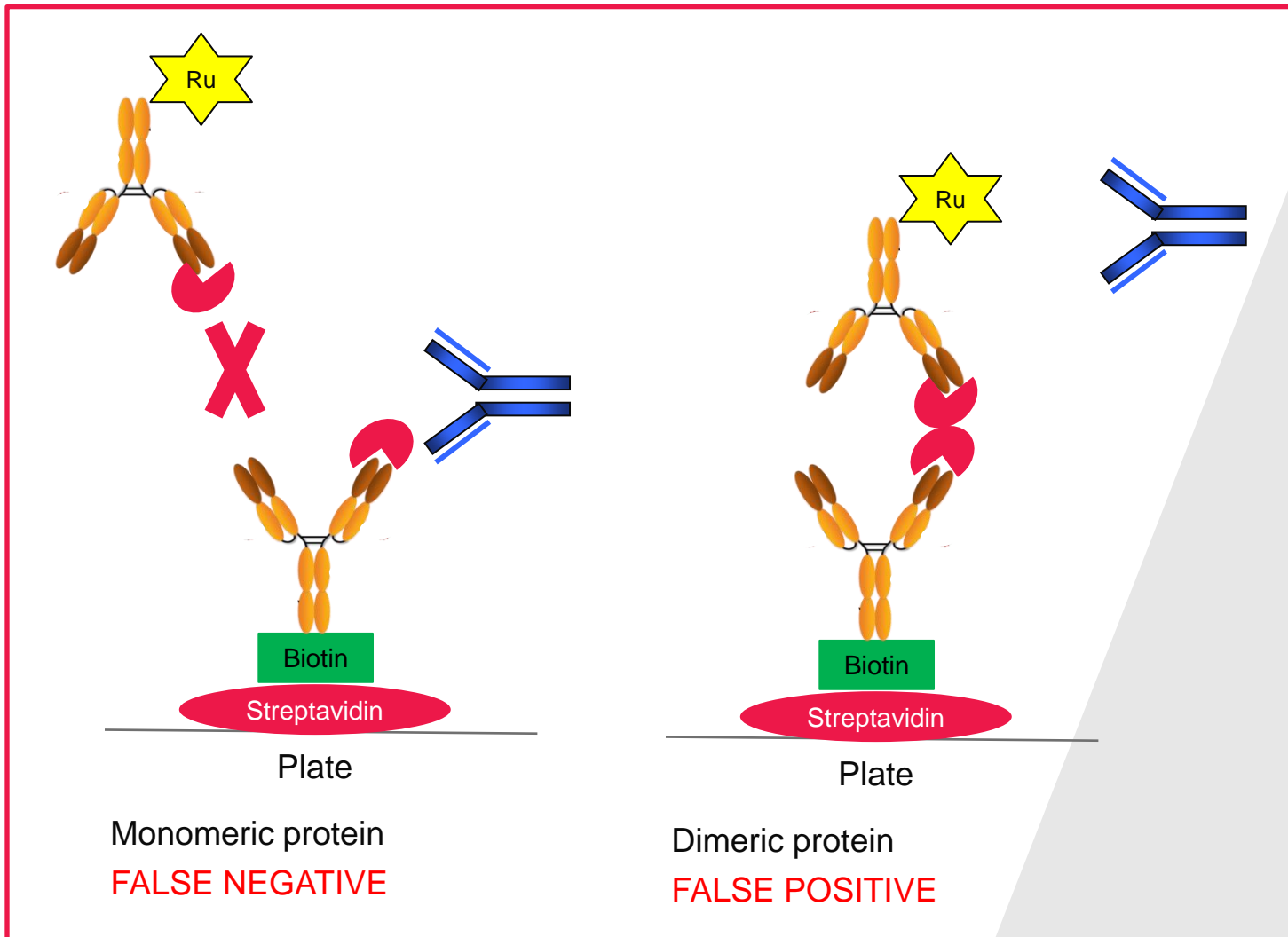


Image: Covance

Considerations for ADA assay target interference

What is the biology of the drug target?

- Is target upregulated in disease state?
- Is target monomeric or multimeric?
- How much target can your assay tolerate?
- Can samples be analysed when target is in low abundance?

Strategies for improving assay sensitivity when target interference is an issue

Strategies for removing target interference

Removal of target interference:

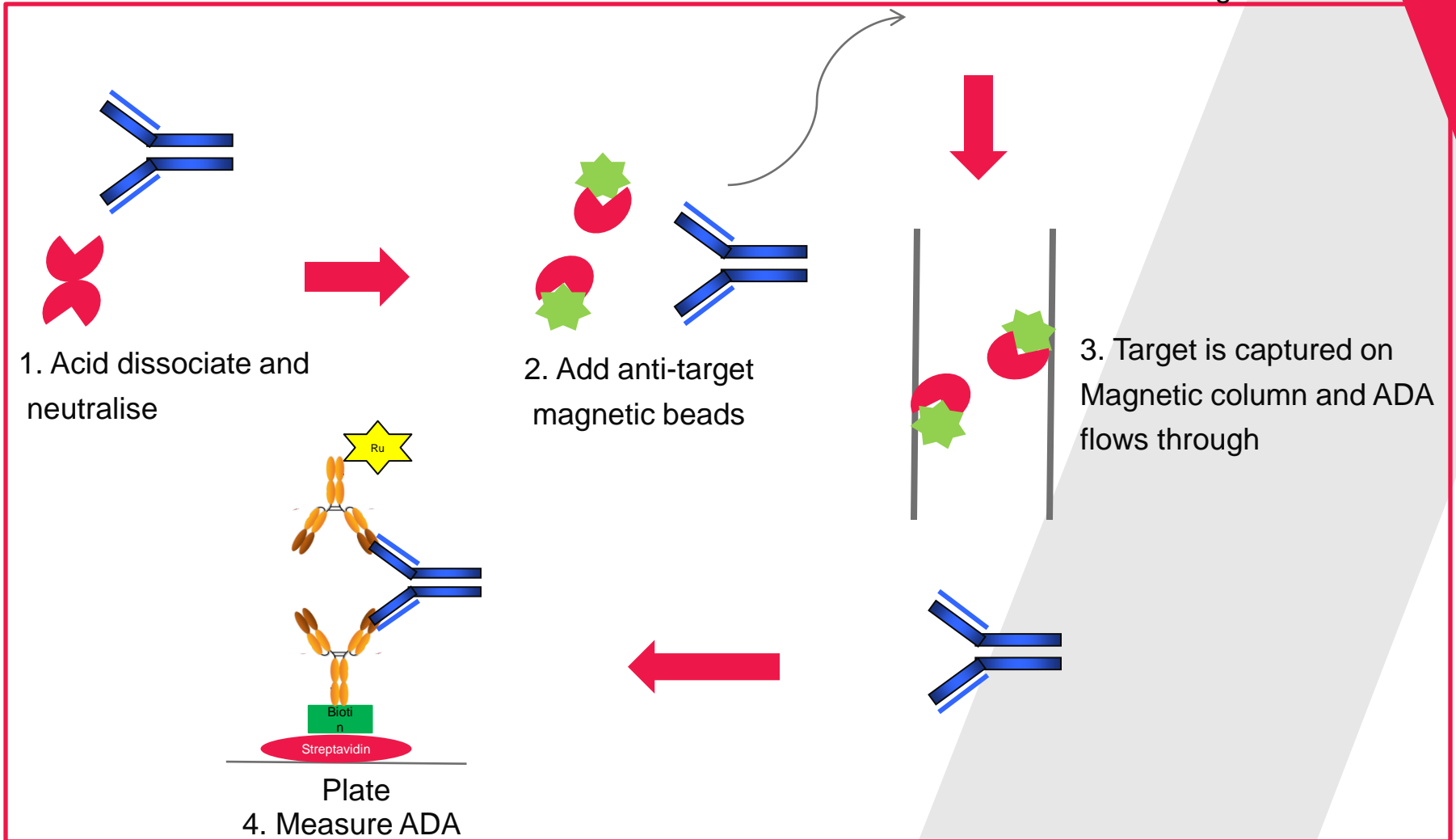
- Remove target by affinity capture techniques (e.g. magnetic beads)
- Dissociate drug/target complex by acid dissociation

Inhibit drug/target interaction:

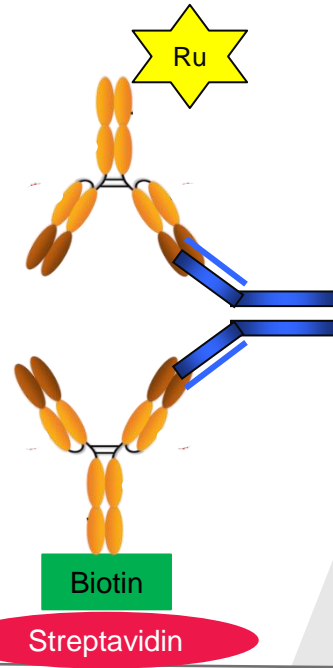
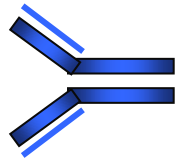
- Antibody with different CDR to drug
- Add target receptor to samples

Covance Example 1 – Magnetic bead immunodepletion

Image: Covance



Covance Example 2 – Soluble receptor interference



1. Incubate sample with target receptor

2. Measure ADA

Image: Covance

Covance experience with various methods for improving issues with target interference

- Understand the biology of the drug target and how that may change during different stages of drug development (E.g. May need different assays in different phases of development)
- Target interference can cause both false positives and false negatives depending on the nature of the target
- Target depletion techniques can be demanding and have high imprecision
- Using soluble receptors at a high concentrations can be a cost consideration

Conclusion

- ▶ Drug and Target interference need to be carefully considered to insure ADA assays give valid data for interpretation of immunogenicity
- ▶ Bespoke approaches are necessary to resolve potential issues with drug and target interference
- ▶ For drug tolerance the assay platform, assay design and assay format all influence the overall tolerance that can be achieved
- ▶ For target interference a good understanding of the drug/target interaction biology will enable successful development of ADA assays
- ▶ Broad array of assays in the analytical toolbox to build sensitive ADA assays that address the challenges of drug tolerance and target interference

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