Increase of drug tolerance
An alternative to acid dissociation

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PandA method publication

Journal of Immunological Methods 426 (2015) 62–69

Contents lists available at ScienceDirect

Journal of Immunological Methods

journal homepage: www.elsevier.com/locate/jim

A breakthrough novel method to resolve the drug and target interference problem in immunogenicity assays

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Parts of this slide deck have been presented at the 9th WRIB in Miami, Florida on April 16th, 2015

Potential Effect of Antibody Response

- Safety Considerations
  - Risk for hypersensitivity reactions
  - Potential for immune complex disease
- Efficacy Considerations
  - Antibodies may bind to drug and alter the pharmacokinetics
  - Antibodies may alter the biodistribution of the drug
  - Antibodies may bind to (or near) the active site of a drug and inhibit its activity
  - Antibodies may bind in a way that interferes with the drug binding to its receptor or ligand

Challenges in immunogenicity assays

- Biological matrix interference in detection and quantitation immunoassays remains a major challenge in the field of bioanalysis.
- Circulating drug or target may interfere with the detection of anti-drug antibodies (ADA) causing false negative (from drug) or false positive results (from target)
- Drug target, or ADA may interfere with quantitation of drug levels in PK/TK analysis.
- Monoclonal antibody drug interference, especially for human IgG4 drugs, presents an additional challenge for ADA analysis due to its longer half-life and higher dose and waiting for drug clearance is not always an acceptable solution.
- Bridging immunogenicity assays are typically used but remain susceptible to endogenous drug interference.
- Methods that use acid dissociation in bridging assays or Solid phase extraction with acid dissociation (SPEAD) or an Affinity Capture Elution Assay (ACE), have limited success due to the re-association of drug and ADA upon pH neutralization.

Decision making logistics

- What is the interference?
  - Specific: Drug, endogenous target, ADA to previous treatment with similar drugs, similar drugs, etc.
  - Non-specific: RF, human serum proteins (IgM, IgG, albumin, etc.), disease specific interference factors, etc.
  - Increase or decrease of signal?
- Can the interference be reduced (or eliminated)
  - No: Still need to understand it for better data reporting and interpretation
  - Yes: What to do and how to do it?

Technology advancement to reduce matrix interference by improving sensitivities

- What do vendors say (or don't say)?
  - What do users say?
- Some of the more mature platforms
  - MSD
  - Plasmon Resonance (Biacore, Octet)
  - Gyrolab™
- Emerging technologies, what is the user experience? (Sensitivity and specificity)
  - The Singulex® Erenna ®
  - Quanterix's SiMoa ™
  - NPX4000 Nanoparticles (ANP Technologies)
  - AQI Diagnostic's Ig PLEX™
  - Genalyte's Maverick™
- Improving sensitivity is not so ideal especially when ADA detection ends up in low ng/mL or even pg/mL levels causing higher incidence of positivity and titers.

What we know around dealing with interferences

- Is “Dilution the Solution to Pollution”?
  - Use of sample dilution (high MRD) to solve matrix interference may sometimes negatively impact assay sensitivity
  - Dilution effect for matrix interference vs. specific analyte may not go parallel (although the desired effect is to dilute interference faster than specific signal)
- Acid dissociation
  - Acid may alter analyte (and/or binding reagent) structure
  - Under neutralizing assay condition, matrix effect may reappear
- Extraction (enrichment) of target analyte
  - Extraction efficiency should be examined
  - Impact on assay throughput needs to be assessed
- Depletion or competition of unwanted interference factors
  - Evaluation of target analyte recovery is important

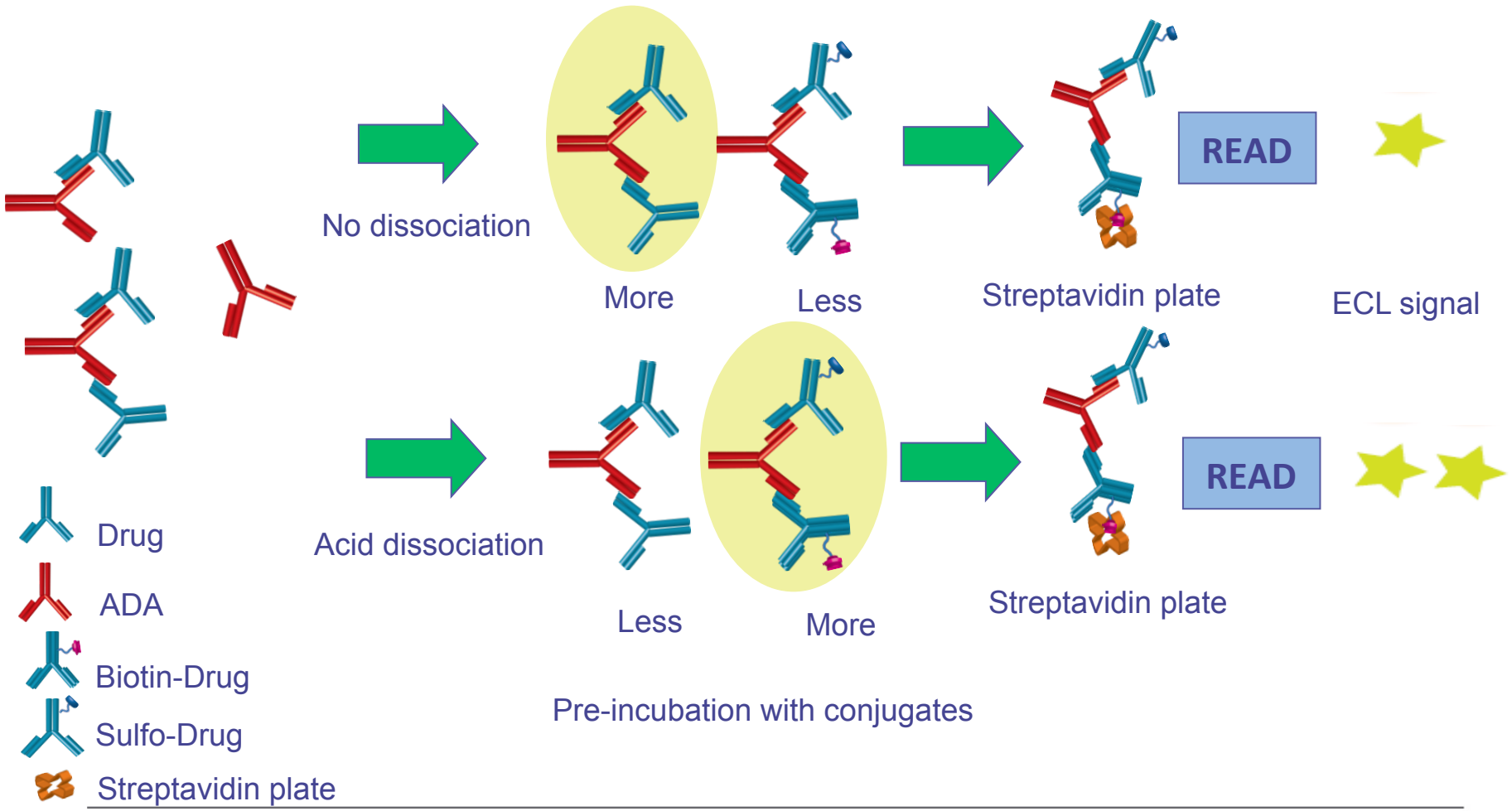
Key concepts for our new method

- Use what we learned from past experiences
- Understand why some described methods do not work that great
- If you can’t beat them, join them: use interference to your advantage

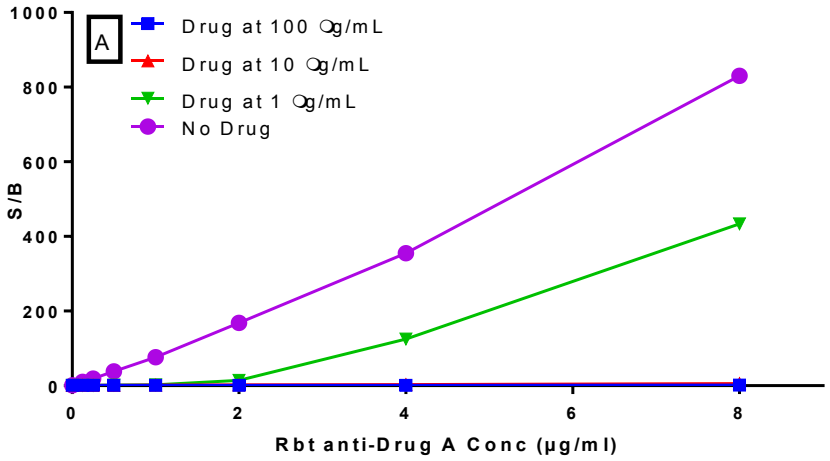
Case studies outline

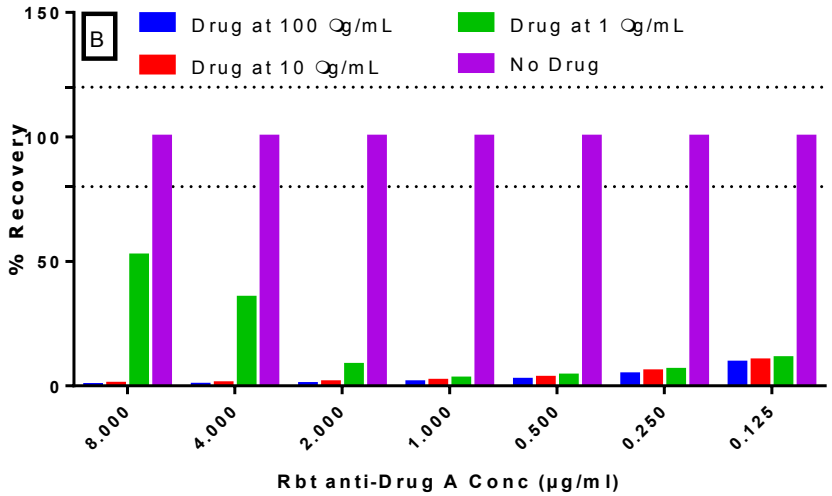
- Description and data from traditional methods
- Feasibility data shown for 2 monoclonal antibody therapeutics
  - Drug tolerance improvement for a humanized IgG1 (Drug A)
  - Proof of principle done for a new method needed for Drug B with its own challenges
  - Drug tolerance and Target interference reduction for a full human IgG4 (Drug B)

ECL Bridging Assays



ECL Bridging Assay without Acid Dissociation





- Strong dose response for ADA detection in the absence of drug.
- Inhibition is seen with as low as 1 µg/mL of Drug with percent recoveries around 10% at the 125 ng/mL of ADA.
- The assay sensitivity was reduced from 15 ng/mL in the absence of drug to 342 ng/mL with 1 µg/mL of drug and to 5143 ng/mL in the presence of 100 µg/mL of drug.

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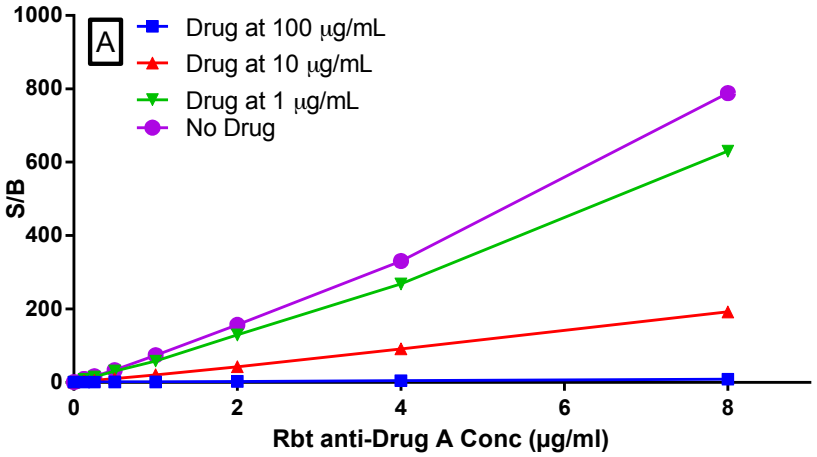
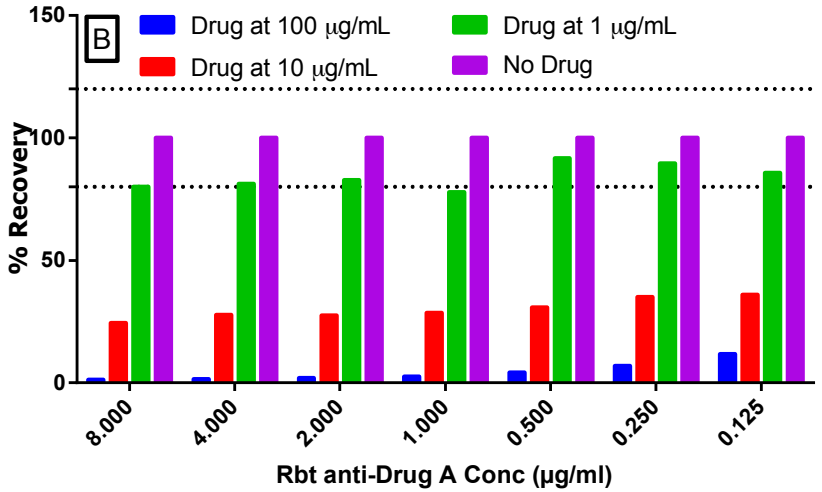
ECL Bridging Assay with Acid Dissociation





- Similar dose response for ADA detection in the absence of drug as the bridging assay without acid
- Percent recoveries are acceptable with 1 µg/mL of Drug but reduced to 35% at the 125 ng/mL of ADA with 10 µg/mL of Drug.
- The assay sensitivity was maintained for the 1 and 10 µg/mL of drug at around 15 ng/mL and reduced to 262 ng/mL in the presence of 100 µg/mL of drug.

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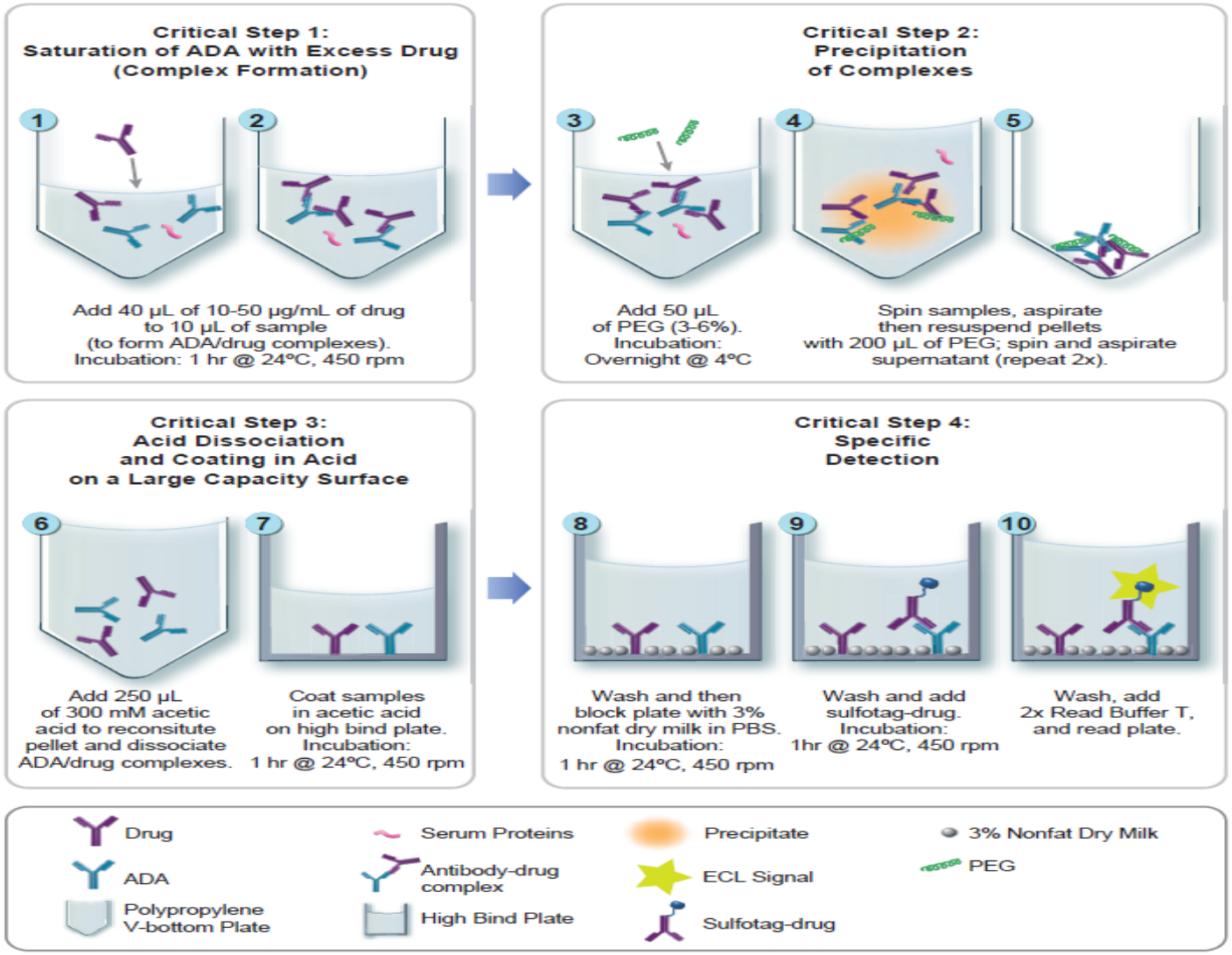
Principle of the PandA method

- Various methods have been used with limited success to address circulating drug interference with the detection of anti-drug antibodies (ADA).

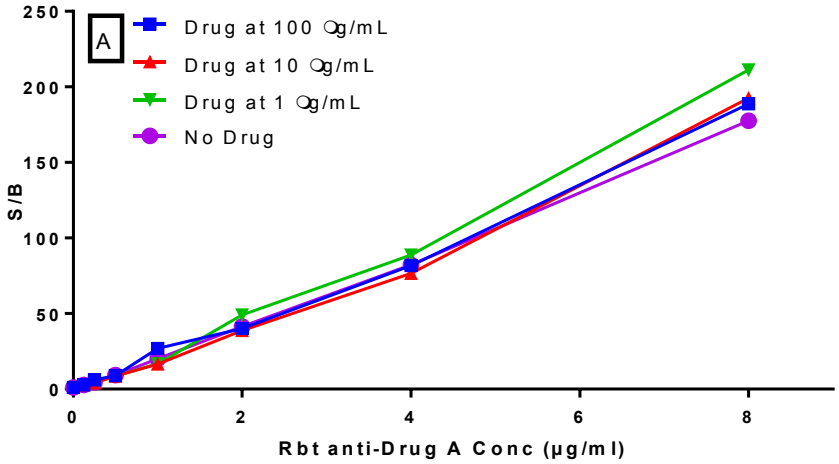
- The PandA method is effective at solving the interference problems caused by drug or target in ADA detection assays based on the following steps:

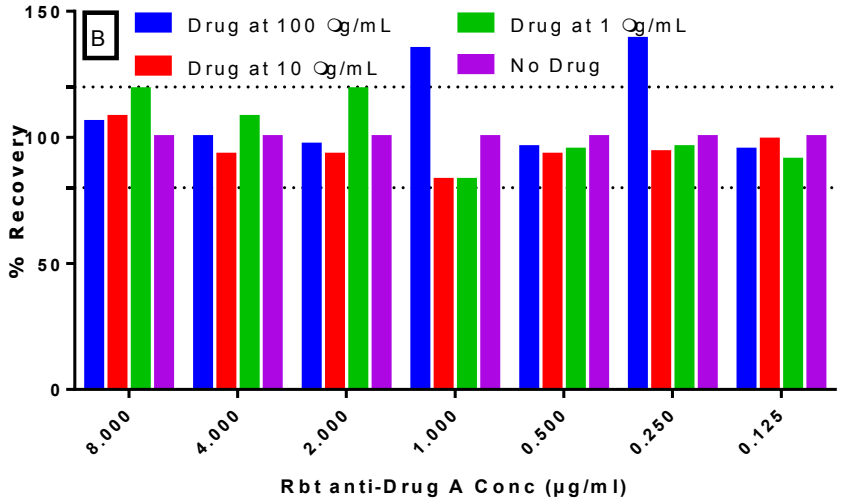
  - **Addition of excess drug material to form drug/ADA complexes.**
  - **Precipitate those complexes containing total ADA (using PEG)**
    - PEG has been introduced as a fractional precipitating agent by Polson et al. (1964)
    - The larger the molecules the lower concentration of PEG is needed
  - **Coating of reconstituted precipitate in an acidic solution on a high bind carbon plate with a large capacity to prevent reformation of ADA-drug complexes.**
  - **Specific detection of the total ADA levels using SulfoTag conjugated drug with an ECL output.**

Precipitation and Acid dissociation (PandA) Method



PandA method





- An acceptable dose response was observed for ADA detection in the absence or presence of drug in the samples.
- In most instances, the percent recoveries remained acceptable between 80-120% regardless of the drug amount present.
- The assay detection sensitivity was maintained at 9-14 ng/mL despite drug present at 100 µg/mL which is 3-4 fold higher than the expected Cmax for the therapeutic.

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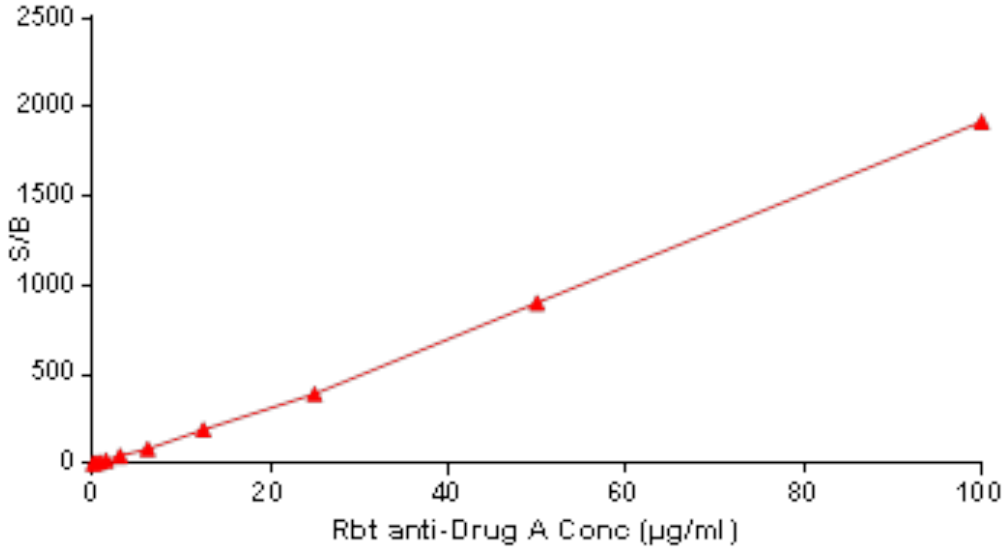
Assay Sensitivity Comparison

	Assay Sensitivity ng/mL		
Drug present µg/mL	**Bridging Assay without Acid Dissociation**	**Bridging Assay with Acid Dissociation**	**PandA Method**
0	15	15	10
1	342	8	13
10	393	16	9
100	5143	262	14

- The PandA method maintained the assay sensitivity in the bridging assays.
- In the traditional assay, sensitivity is affected at low concentrations of drug.
- The PandA method not only improved detection at high concentrations of drug but maintained sensitivity at the same levels in the presence of high amount of drug.

Dose response/capacity assessment

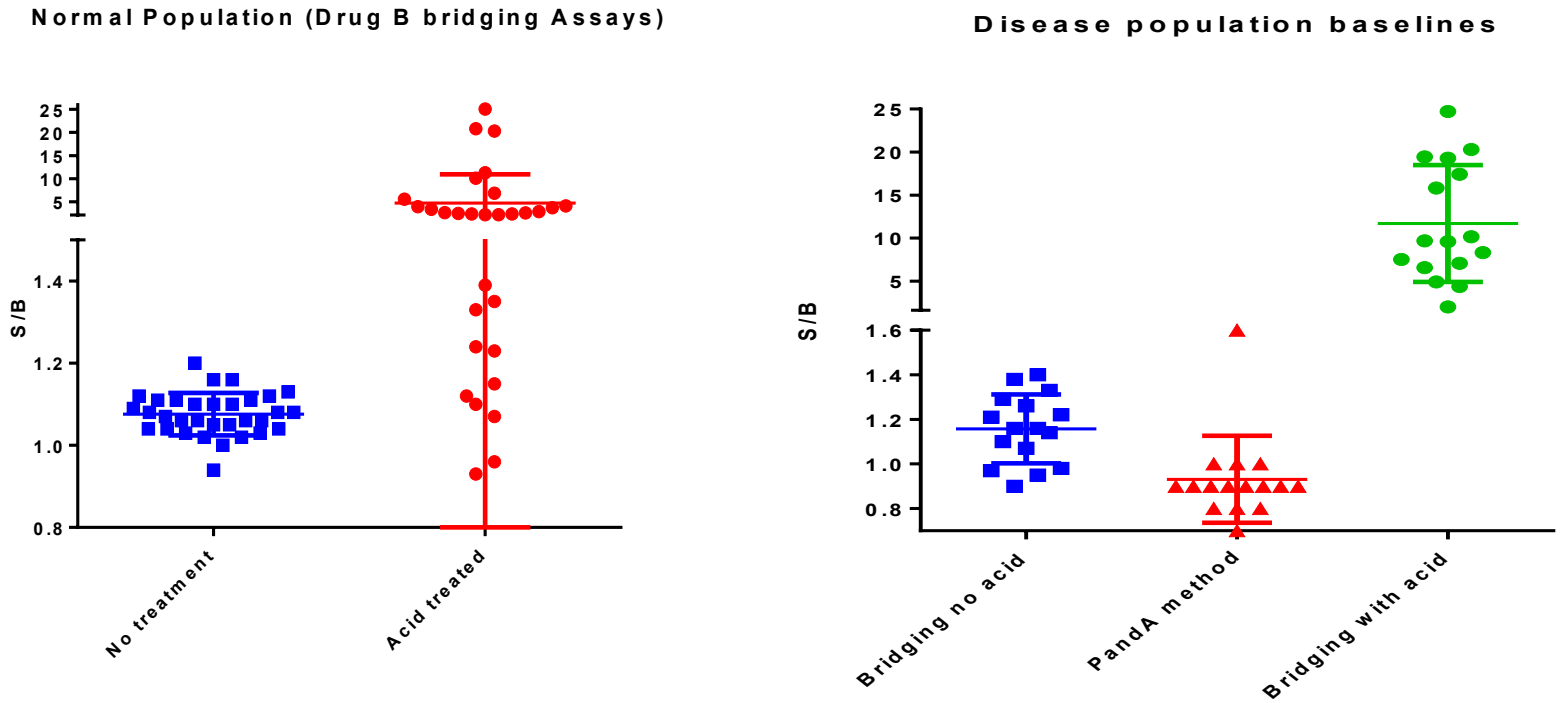


- Affinity purified rabbit anti-drug at concentrations ranging from 100 µg/mL to 100 ng/mL were prepared in pooled normal human sera and run in the method.
- This data indicates a dose dependent response and the absence of a hook effect or plate saturation.
- This data suggest that this method is feasible for detection of high titer samples.

Drug B

- Drug B is a full human IgG4 that neutralizes a soluble cytokine binding to its cell surface receptor in the target tissue for a fibrosis indication.
- It presents a specific challenge in the MSD bridging assay with acid dissociation since the target for Drug B changes from a monomer to a dimer at low pH causing false positive results.
- The dimerization effect is seen in 100% of normal serum samples and disease baseline samples in the MSD bridging assay with acid dissociation.
- IgG4 monoclonal: documented exchange of IgG half molecules or arm switching (described in IgG4 breaks all the rules (Albersee et.al, Immunology 2002, 105- 9-12)
  - **Exchange of IgG half molecules (arm switching)**

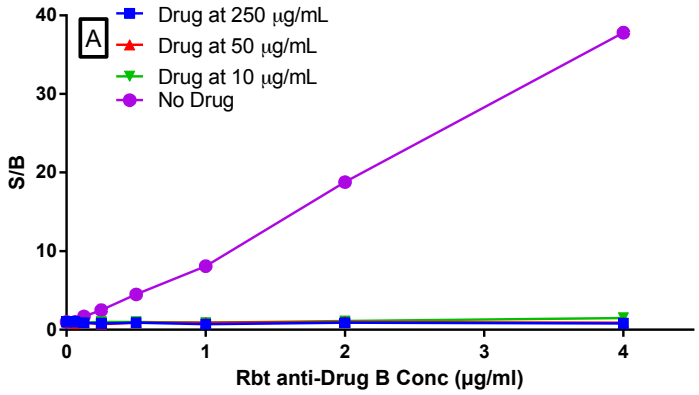
Population distributions in different methods

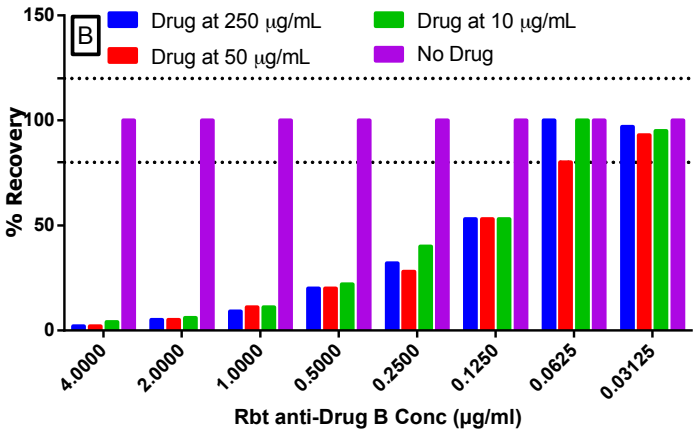


- Results were comparable between the ECL bridging without acid treatment and PandA method while the acid treatment resulted in higher S/B levels for the majority of the samples tested suggesting interference from drug target due to the dimerization effect at low pH

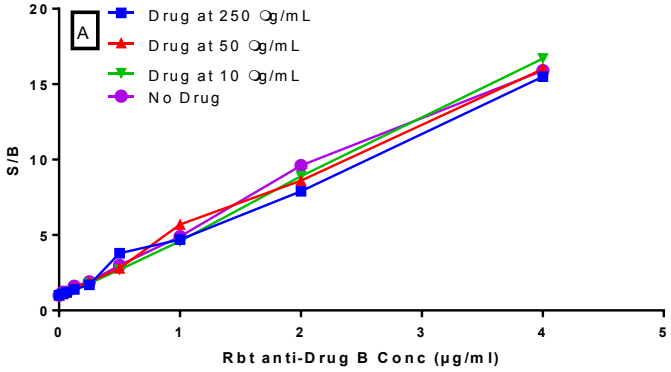
Sensitivity and Drug Tolerance Drug B

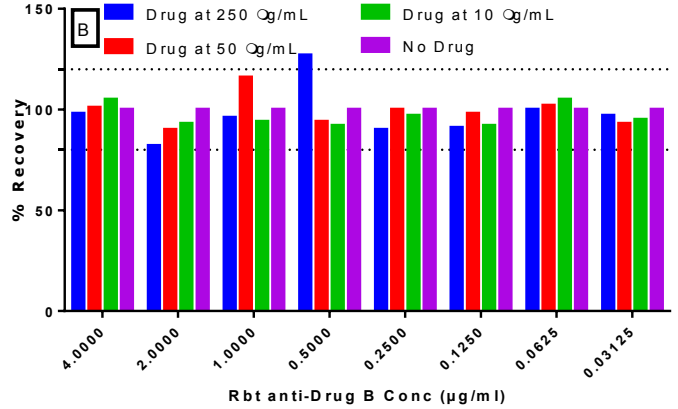
ECL Bridging





PandA





Conclusions

- The challenges of analytical interferences in immunoassays (or ligand binding assays) has long been recognized as an unmet need
- Over the years, many scientists have published techniques proven useful to overcome some of these interferences with varying success rate
- We described a novel method that has shown significant improvement for ADA detection in the presence of excess drug
- We have provided two immunogenicity case studies to demonstrate its utility
- Broader applications should be explored and method optimized accordingly
- Applications include PK assays, CIC, etc.

Acknowledgment

- Mary Brock
- Susan Richards
- Allyson Hatton (Legal)
- Alison Schroeer (Biomedical Media Services)

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Thank you for your attention!