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Pushing the Limits of Immunogenicity Assay Drug Tolerance

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Drug Development Solutions

(Part of Alliance Pharma, Inc.)

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Overview

- **Introduction**
  - Tolerance to on-board therapeutic
  - Project overview
- **Method overviews**
  - Precipitation and Acid dissociation
  - Adaptation of traditional SPEAD method
  - Adaptation of traditional Bead method
- **Comparison of method performance**

Introduction

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- The therapeutic is the most common interfering factor in an immunogenicity assay

- Assays are requiring tolerance to the onboard therapeutic in the mg/mL range
  - Clinical: drug tolerance required at 100 ng/mL of positive control
  - Pre-clinical: drug tolerance recommended at 1000 ng/mL of positive control

Introduction

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- Requirement of assays to have complex sample treatment
  - Acid dissociation
  - BEAD (Biotin-Drug Extraction and Acid Dissociation)
  - ACE (Acid, Capture, Elution)
  - SPEAD (Solid Phase Extraction and Acid Dissociation)
  - PandA (Precipitation and acid dissociation)

Development of a Immunogenicity Assay for a Covid Therapeutic

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Development of an immunogenicity assay for a covid therapeutic	The traditional bridging format did not produce a suitable assay	High tolerance to on board therapeutic required
• Clinical and pre-clinical assays required	• Other formats assessed: PandA and an adapted SPEAD assay	• Tolerance to 2 mg/mL of therapeutic was required in the pre-clinical assay

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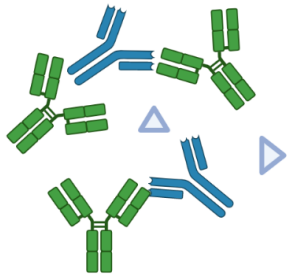
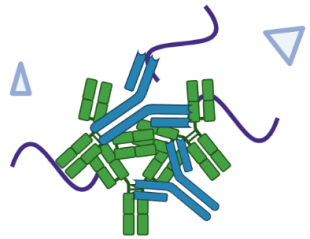
PandA (Screening)

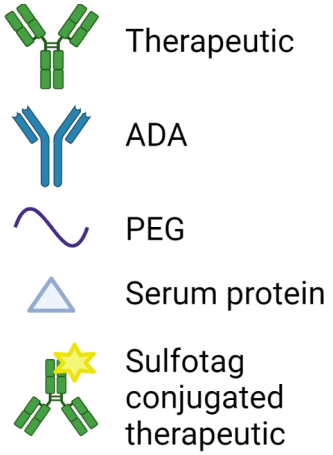
Day 1

Excess Therapeutic is added to the samples, forming complexes with the ADAs



PEG is added to the wells, causing the ADA/ therapeutic complexes to precipitate

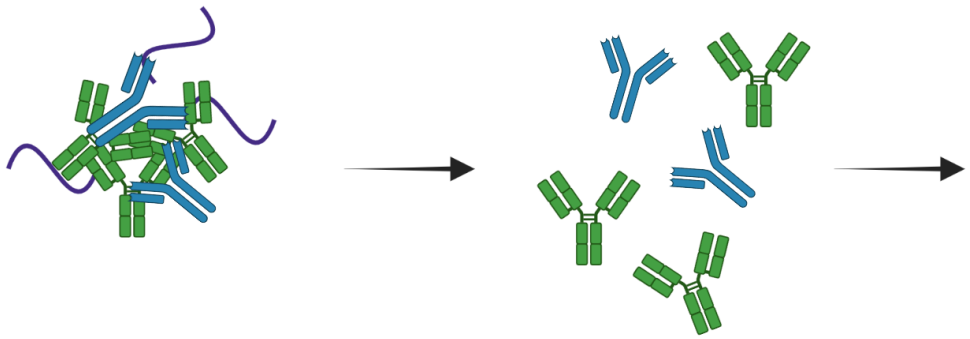




Day 2

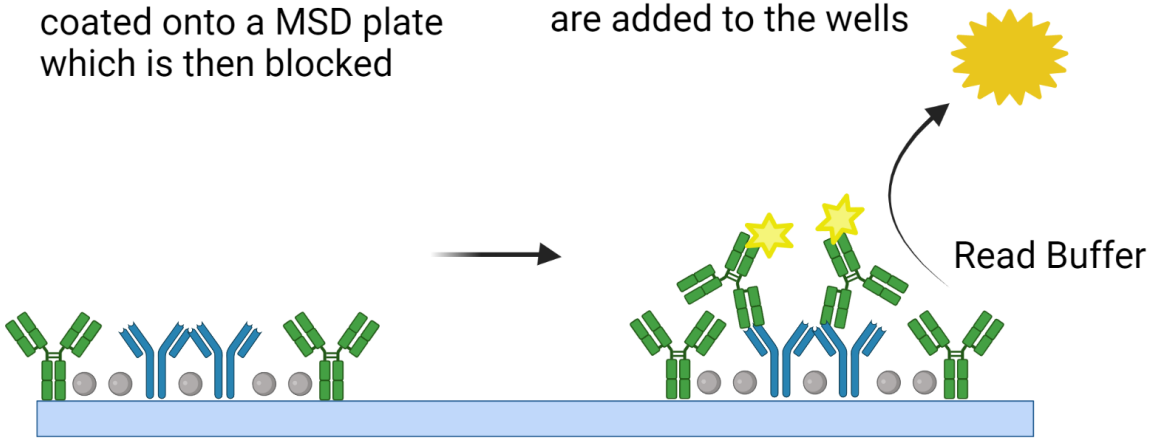
The plate is centrifuged and excess liquid removed

The pellet is re-suspended in acid, causing dissociation



Acidified solution is coated onto a MSD plate which is then blocked

Sulfotag conjugated therapeutic and unlabelled therapeutic are added to the wells



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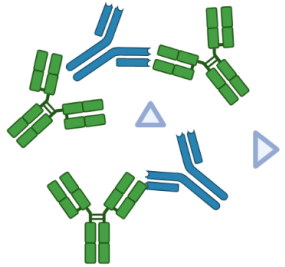
PandA (Confirmatory)

Day 1

Excess Therapeutic is added to the samples, forming complexes with the ADAs



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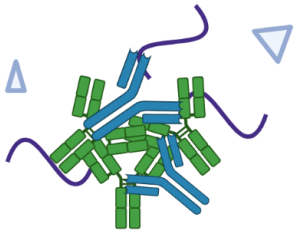

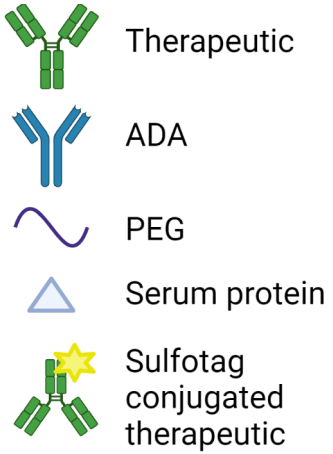

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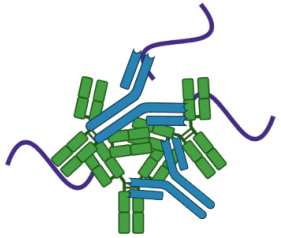

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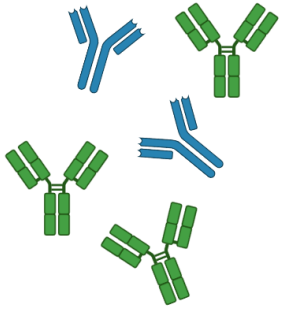

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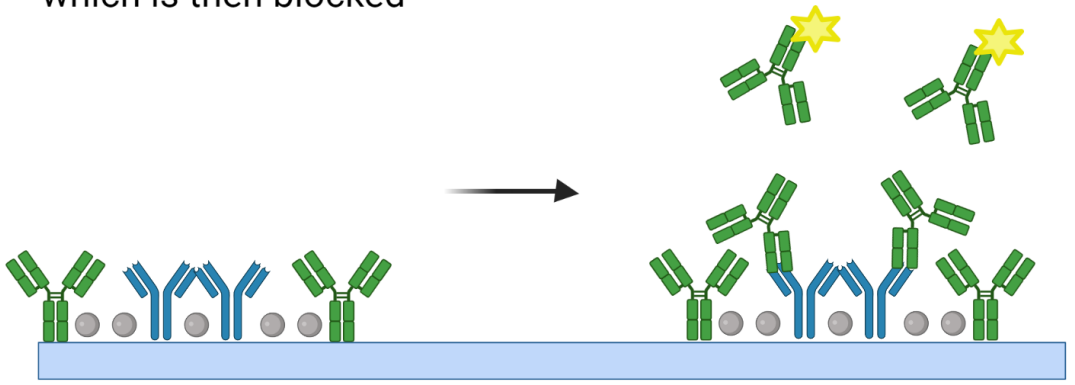

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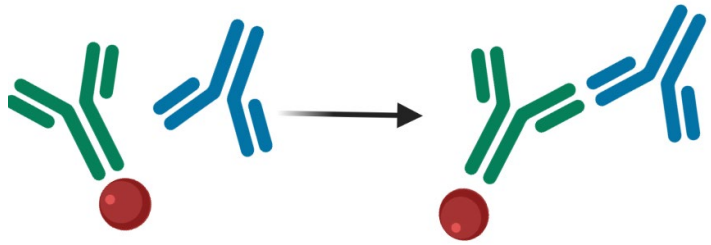
Adapted SPEAD

(Screening)

Day 1

Dissociate sample and pre-incubate with biotinylated drug (Capture)

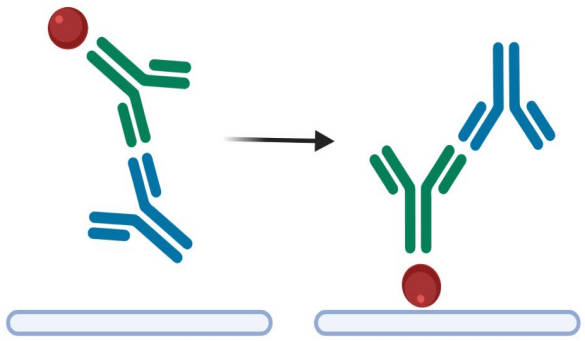


Biotinylated Therapeutic

Sulfotag Therapeutic

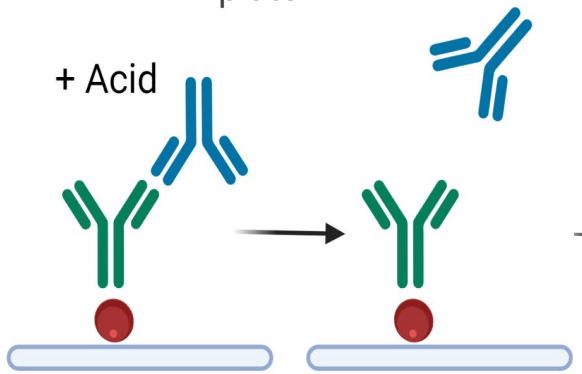
ADA

Incubate on Streptavidin plate



Dissociate from streptavidin plate

+ Acid



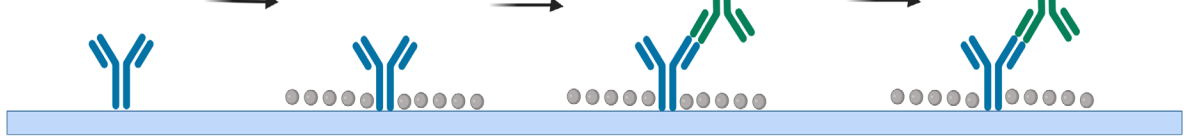
Add to assay plate

Block plate

Add sulfotagged drug (Detection)

Add read buffer and read on instrument

Read Buffer



Day 2

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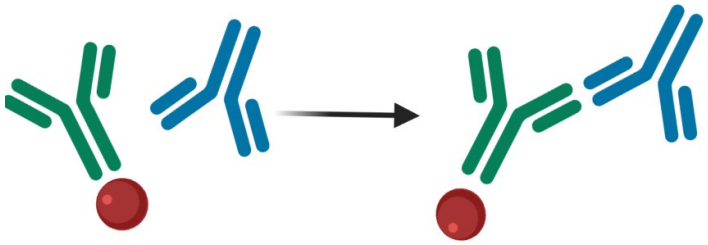
Adapted SPEAD

(Confirmatory)

Day 1

Dissociate sample and pre-incubate with biotinylated drug (Capture)

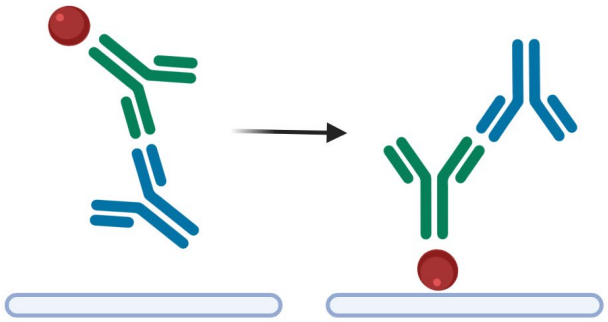


Biotinylated Therapeutic

Sulfotag Therapeutic

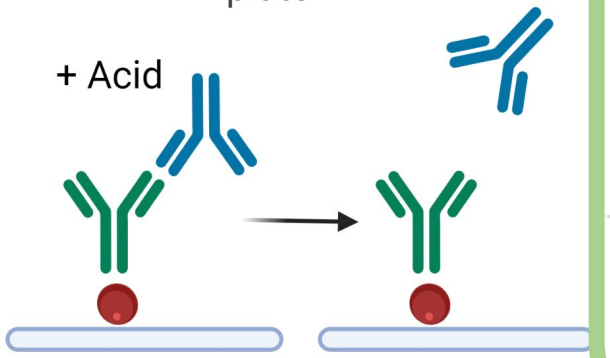
ADA

Incubate on Streptavidin plate



Dissociate from streptavidin plate

+ Acid



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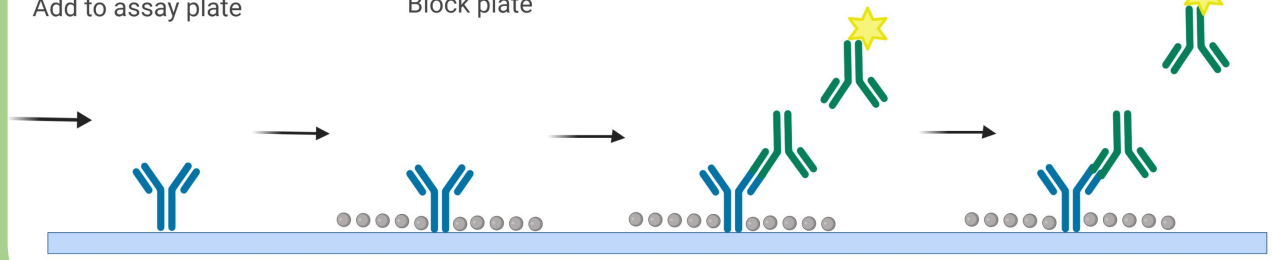

Day 2

Comparison of Validation Data

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Validation Parameter	Clinical PandA method	Clinical Adapted SPEAD method
Healthy Screening CPF	Screening CPF: 1.31 Confirmatory CP: 19.7% inhibition Titration CPF: 1.66	Screening CPF: 1.51 Confirmatory CP: 38.4% inhibition Titration CPF: 1.80
Sensitivity	Screening: 47.8 ng/mL Confirmatory: 32.5 ng/mL	Screening: 53.8 ng/mL Confirmatory: 55.4 ng/mL
Selectivity: 100 ng/mL Spike in healthy matrix	Passed	Passed
Selectivity: blank, drug naïve healthy individuals	Passed	Passed
Hook Effect	No hook effect observed up to 500,000 ng/mL	No hook effect observed up to 500,000 ng/mL
Inter and Intra assay precision	<20 % CV for all levels of PC	<20 % CV for all levels of PC

Comparison of Validation Data: Drug Tolerance

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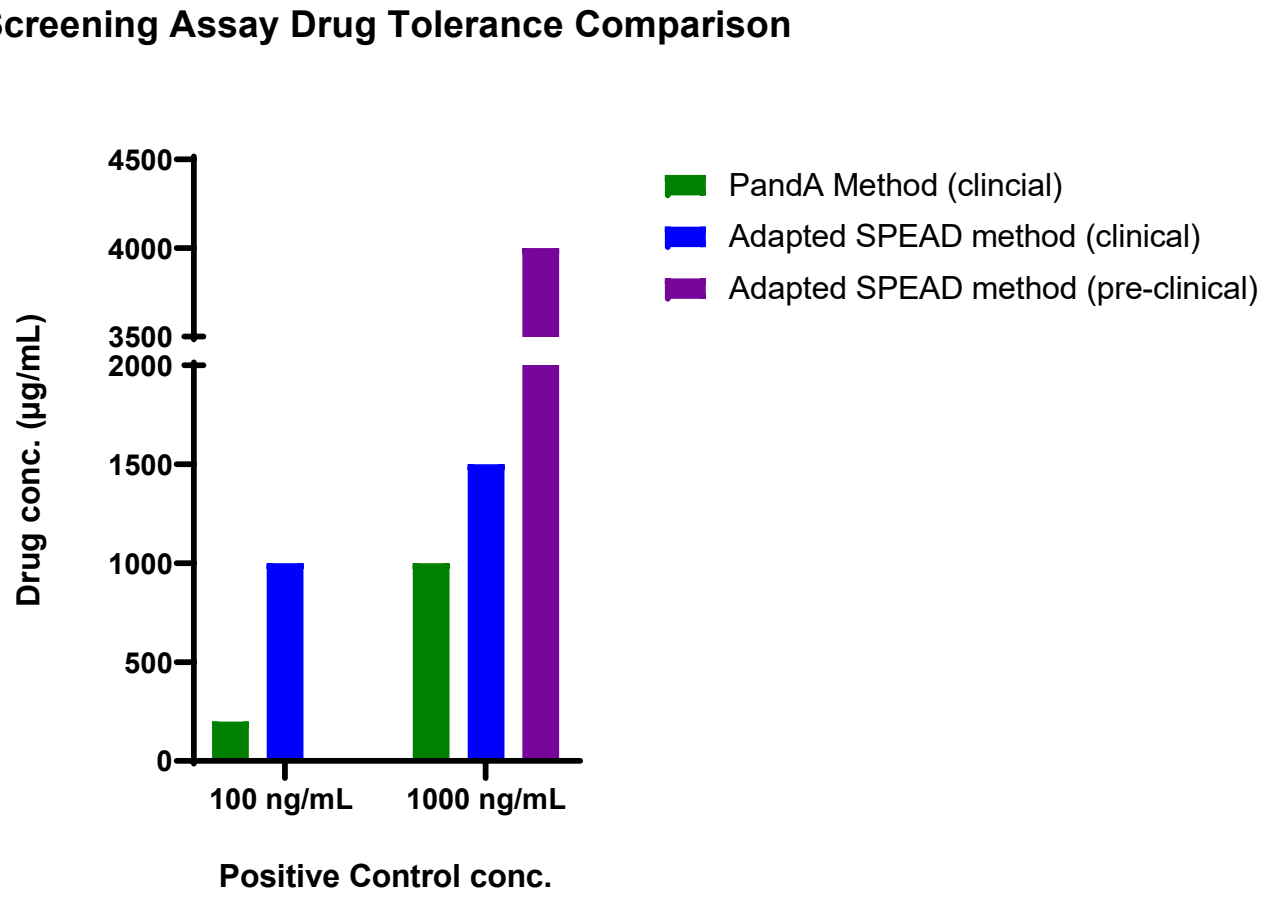

Improved Drug Tolerance in Adapted SPEAD Method

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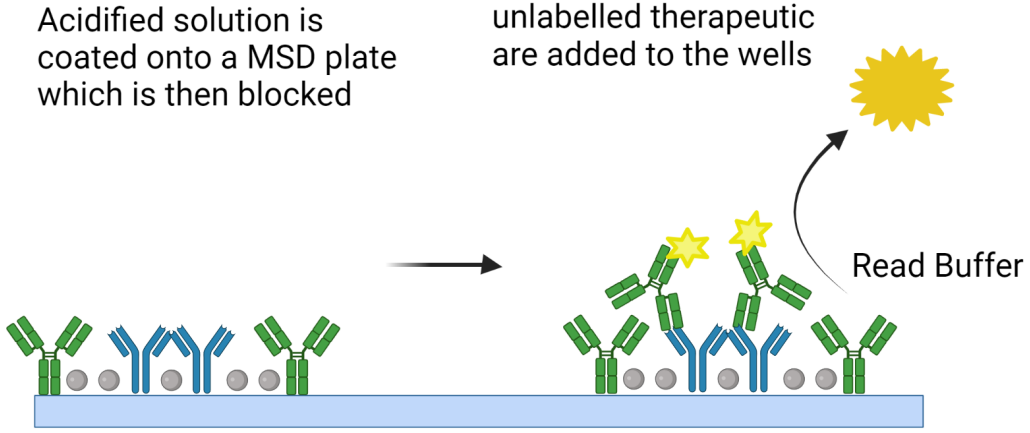
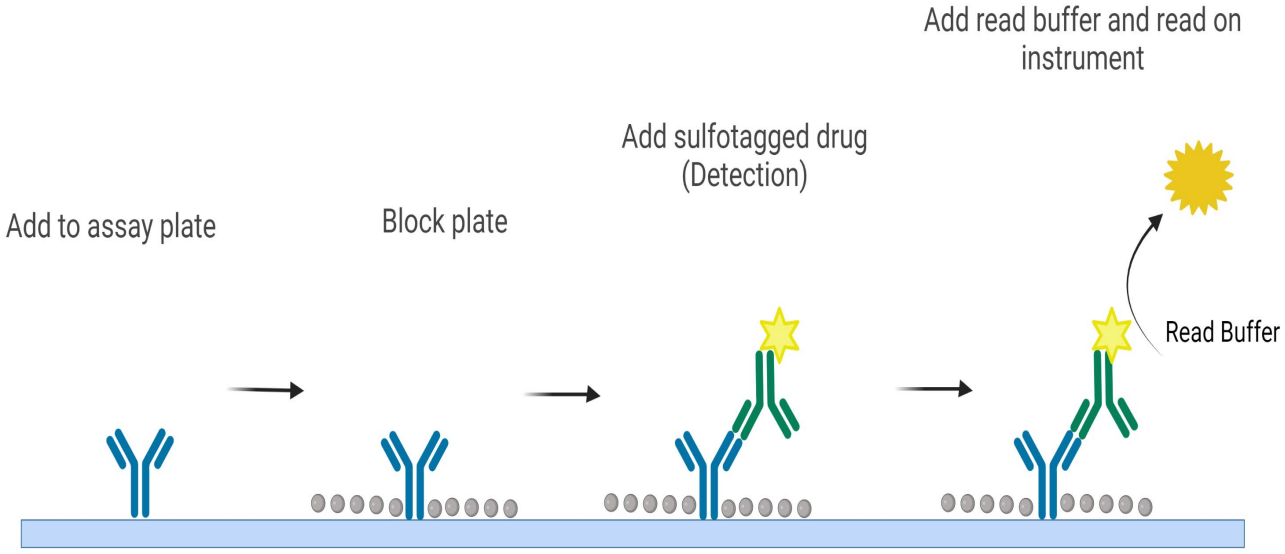
PandA



Adapted SPEAD



Comparison of Validation Data: Drug Tolerance

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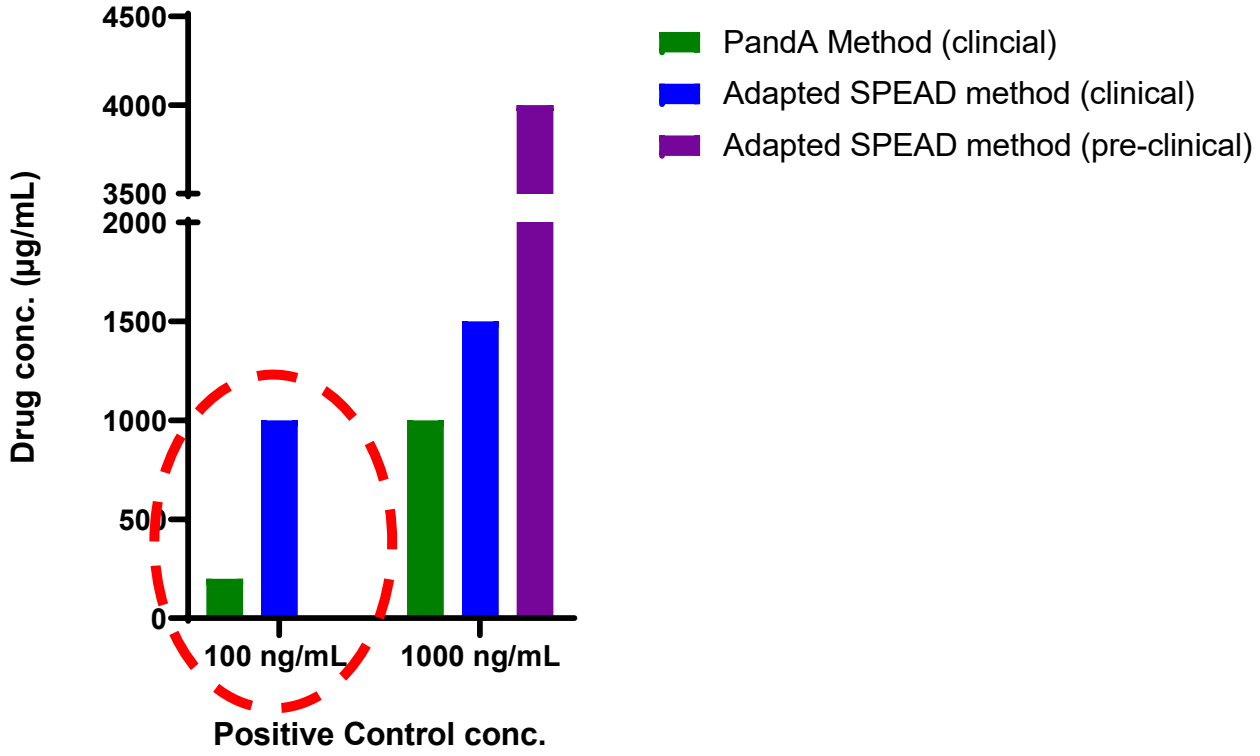
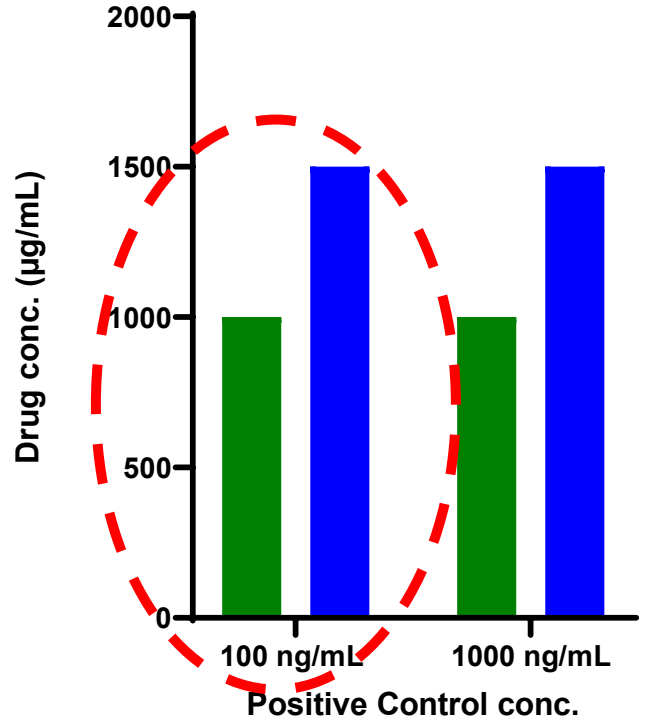
Screening Assay Drug Tolerance Comparison



Confirmatory Assay Drug Tolerance Comparison



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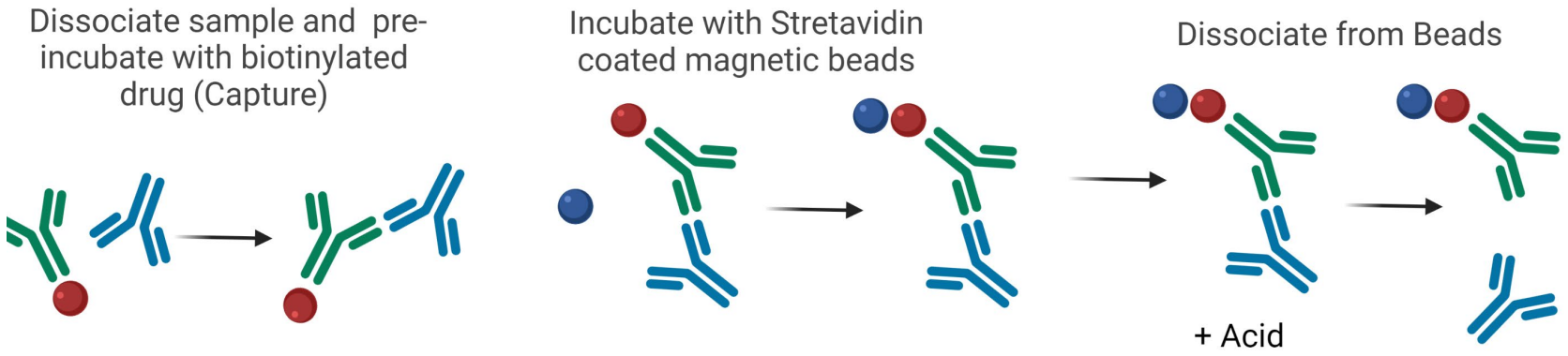
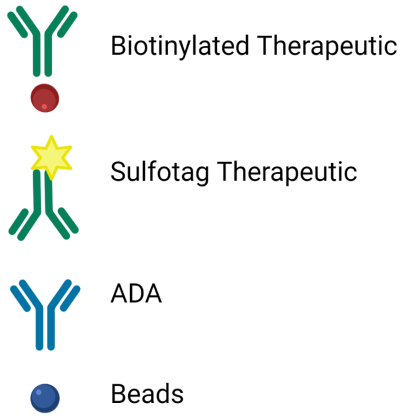
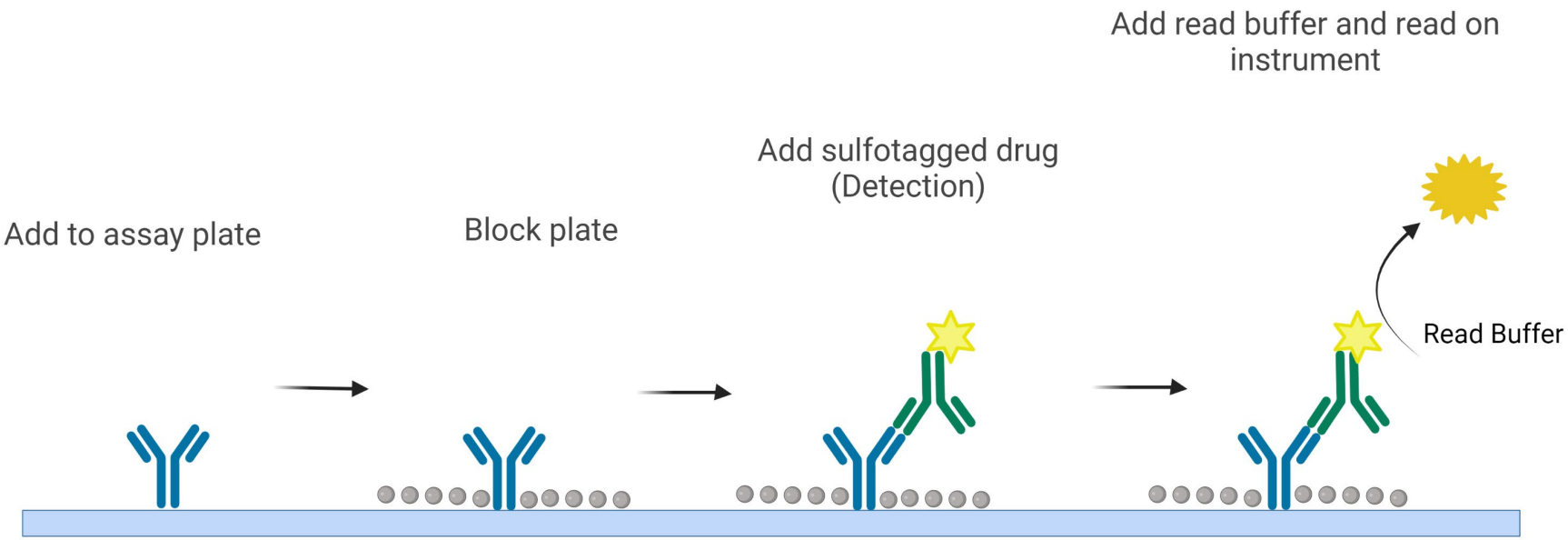
Further Adaptation of the Method: Beads







Further improved by automation:

- Electronic pipettes
- Automated pipetting
- Automated bead processing

Further Adaptation of the Method: Beads

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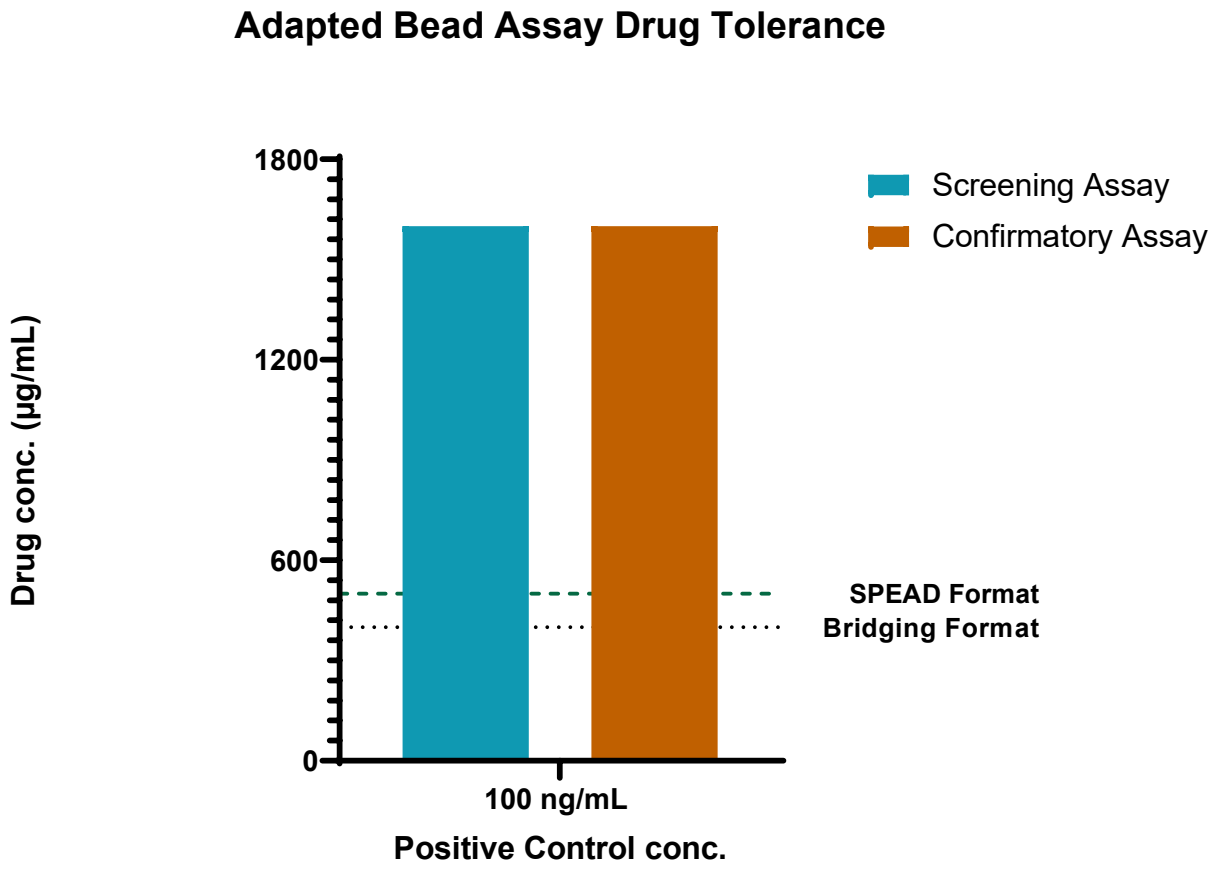

Summary

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All three methods provide an alternative to the bridging format for immunogenicity assays

Adapted SPEAD/Bead methods provide a alternative to the PandA method with the benefit of improved drug tolerance

All three methods have the potential to be more drug tolerant in the confirmatory assay due to the assay format

We continue to push the limits of immunogenicity assays to meet requirements for novel complex therapeutics, addressing both scientific and regulatory demands

Acknowledgements and References

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Acknowledgements

- DDS IA Department colleagues
- Sponsors

References

- Jad Zoghbi, Yuanxin Xu, Ryan Grabert, Valerie Theobald, Susan Richards, A breakthrough novel method to resolve the drug and target interference problem in immunogenicity assays, Journal of Immunological Methods, Volume 426, 2015, Pages 62-69
- Laurén A, Goodman J, Blaes J, Cook J, Cowan KJ, Dahlbäck M, Grudzinska-Goebel J, McManus D, Nelson R, Pihl S, Timmerman P. A strategic approach to nonclinical immunogenicity assessment: a recommendation from the European Bioanalysis Forum. Bioanalysis. 2021 Apr;13(7):537-549. doi: 10.4155/bio-2021-0028.
- Images Created on BioRender.Com

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Thank you for your attention

Any further questions?

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