Case Study

# Achieving High Drug Tolerance in ADA Testing for a Long-Acting Monoclonal Antibody

## Introduction

A company developing a high-dose, long half-life monoclonal antibody therapeutic sought to assess immunogenicity in early-phase clinical trials. Due to the molecule's pharmacokinetic profile, residual drug levels in patient samples posed a significant challenge to ADA assay sensitivity. Ardena was engaged to develop a drug-tolerant solution that met regulatory expectations for Phase 1 evaluation.

## Situation

The therapeutic in question was a fully human monoclonal antibody with a 6-month half-life and a high dosing regimen. Though immunogenic risk was expected to be low, regulatory guidance required a sensitive ADA assay capable of detecting anti-drug antibodies even in the presence of high drug concentrations. Early development work using conventional bridging assays had demonstrated significant drug interference, compromising detection of true positive samples.

## Challenge

Traditional assay pre-treatment methods such as acid dissociation and high ionic strength dissociation (HISDA) yielded insufficient drug tolerance.

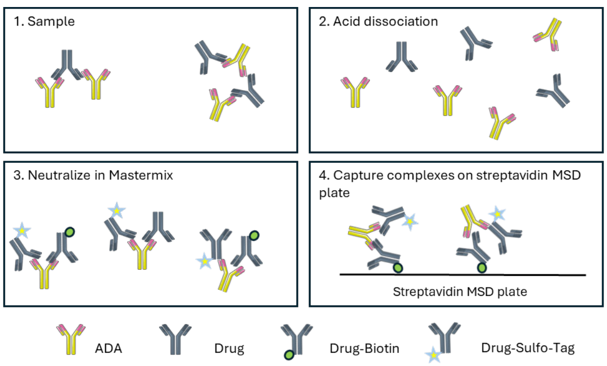


**Figure 1:** Schematic overview of acid dissociation and HISDA steps

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Despite acceptable sensitivity under drug-free conditions, both methods failed to maintain performance when drug was present—even at concentrations below the expected Cmax. The key challenge was developing an ADA assay that could reliably detect antibodies in clinical samples containing residual drug (>50.0µg/mL) while ensuring robustness, reproducibility, and transferability.

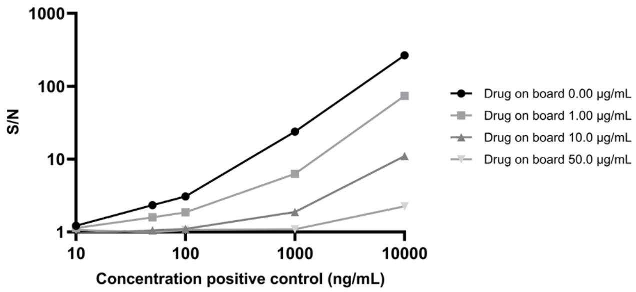


**Figure 2:** Drug tolerance using acid dissociation pre-treatment

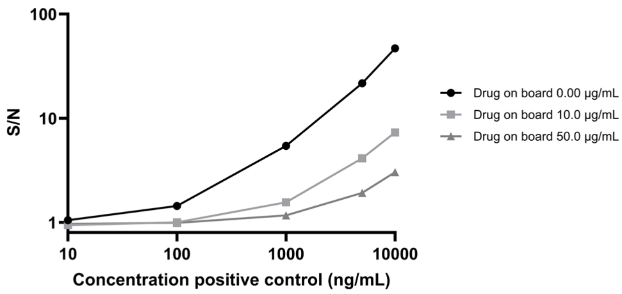


**Figure 3:** Drug tolerance using HISDA pre-treatment

## Solution

After investigating acid dissociation and HISDA, Ardena focused on optimizing the Precipitation and Acid Dissociation (PandA) method. By saturating ADAs with an excess amount of drug, ADA-drug complexes are formed. After precipitation with Polyethylene glycol (PEG), samples are centrifuged, and unbound drug and other components are washed away. After a final wash, samples are resuspended in acid, to disrupt ADA-drug complexes. The acidified sample is then coated directly onto the MSD high bind plate and subsequently detected with drug-sulfo-tag.

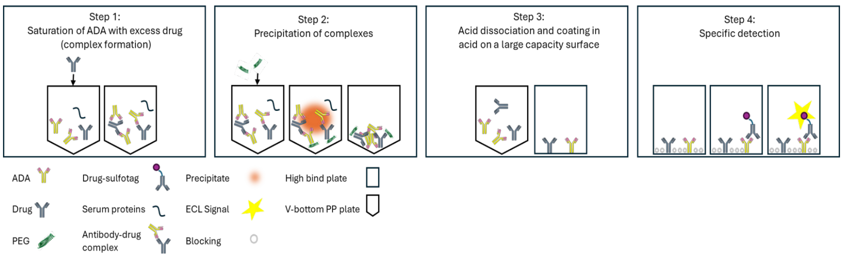


**Figure 4:** PandA sample pre-treatment steps

J. Zoghbi et al. (2015). A breakthrough novel method to resolve the drug and target interference problem in immunogenicity assays. Journal of Immunological Methods.

Optimization steps included:

- Different concentrations of PEG-8000 and the effect on assay sensitivity and drug tolerance. For this therapeutic 6.0% PEG-8000 was selected due to the better assay sensitivity and hereby drug tolerance.

- Different concentrations of saturation buffer. For this monoclonal antibody, 50.0µg/mL saturation buffer was selected due to slightly better sensitivity.

- Centrifugation optimization (3730 x g) for pellet integrity, slower centrifugation speed was tested but resulted in signal loss.

The method was refined to deliver maximum sensitivity and reproducibility. Special attention was paid to ensure the method could be transferred to additional analysts and scaled for clinical sample throughput.

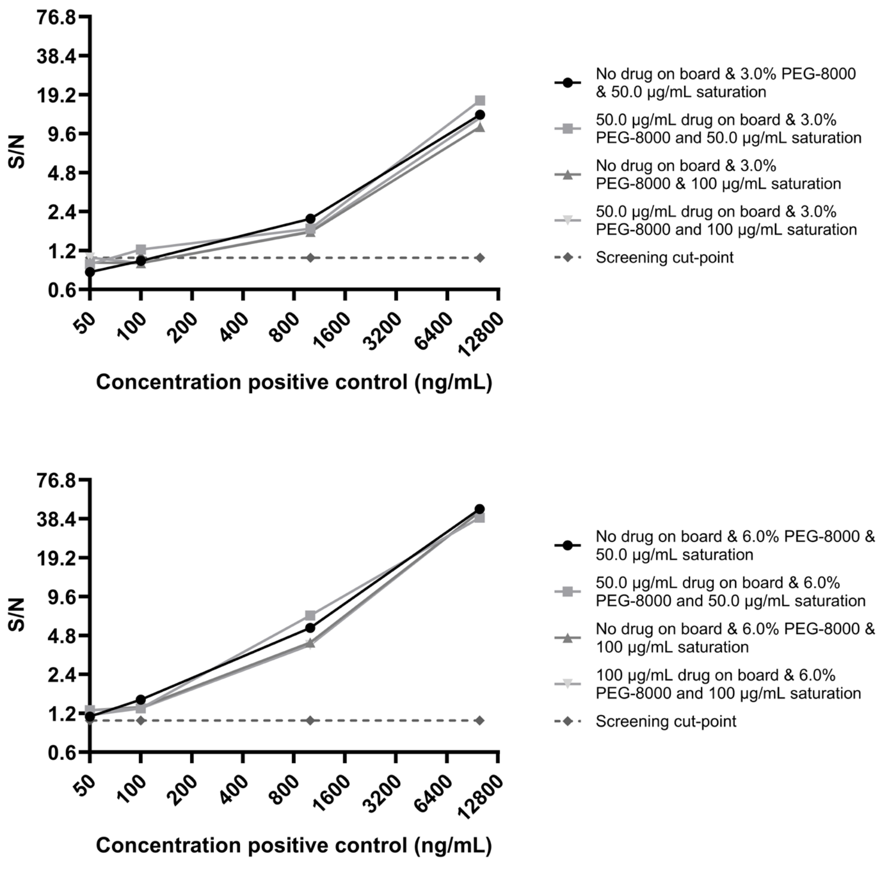


**Figure 5:** The effect of PEG-8000 and saturation concentration on assay sensitivity and drug tolerance

## Results

The PandA-based assay demonstrated outstanding drug tolerance:

- ≥500 µg/mL tolerance at 150 ng/mL ADA (LPC)
- ≥12,500 µg/mL tolerance at 25,000 ng/mL ADA (HPC)
- No loss in signal-to-noise ratios across high drug levels

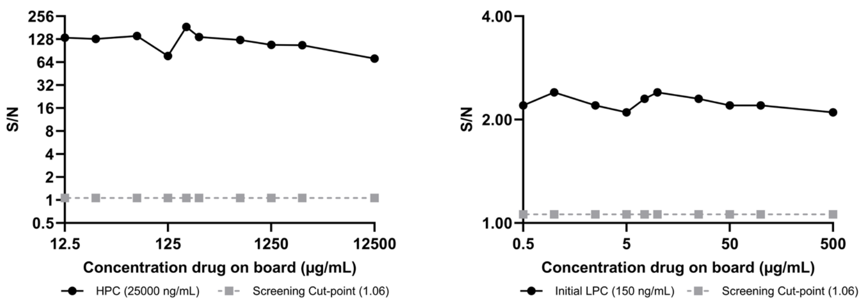


**Figure 6:** Final drug tolerance of the assay and HPC and iLPC level.

The assay was validated according to regulatory standards and proven fit-for-purpose for early-phase clinical support. The bioanalysis resulted in an expected rate of screen positives and one confirmed ADA-positive subject, which was consistent with expectations for a fully human monoclonal antibody with low immunogenic potential.

## Conclusion

By applying the PandA method, Ardena delivered a validated, highly drug-tolerant ADA assay for a challenging monoclonal antibody program. The approach ensured regulatory compliance, data integrity, and clinical relevance, reinforcing Ardena's ability to adapt complex bioanalytical strategies to meet emerging therapeutic demands.

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