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Immunogenicity Target Interference: A Novel Blocking Approach

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INTRODUCTION

The detection and characterization of anti-drug antibodies (ADAs) requires a specific, sensitive method to evaluate the potential impact of ADAs on patient safety, pharmacokinetic profile, and efficacious response to a drug. One of the main bioanalytical challenges with ADA testing is mitigating the interference encountered with a soluble drug target. While soluble targets can lead to false-positive results in ADA assays, they can also potentially mask the detection of ADAs that may bind at or near the target binding site, leading to false-negative results. Target interference can also contribute to insufficient sensitivity and greater variability in the ADA assay, thereby resulting in high confirmatory cut-points that would further contribute to false-negative results.

During the development of a solid-phase extraction with acid dissociation (SPEAD) ADA assay, it was observed that a large percentage of treatment-naïve human serum lots were showing varied basal responses against the drug, with a high level of inhibition in the confirmatory assay (≥ 40%). This would result in a high confirmatory cut-point, impacting the assay sensitivity and leading to false-negative results. Efforts were made to characterize the interference by depleting the immunoglobulin in the matrix using protein A/G. This step further confirmed that the interference was not due to pre-existing/cross-reacting antibodies, but was specific to the drug target. Various approaches were evaluated for mitigating the interference observed from the drug target while maintaining a SPEAD assay format.

Table 1. Target Interference Mitigation Approaches

Target Interference Mitigation Approaches
Method 1: Anti-target antibody sample pretreatment
Method 2: Anti-target antibody in confirmatory solution
Method 3: Anti-target antibody as additional plate-blocking reagent

The addition of anti-target antibodies has been widely used to mitigate target interference in ADA assays. Using an anti-target antibody as an additional plate-blocking reagent demonstrated superior results versus the traditional approaches of using it at the sample pretreatment or confirmatory steps.

METHOD

A typical SPEAD assay involves an overnight incubation of the controls and samples with excess biotinylated drug to promote ADA biotin-drug complex formation. These immune complexes are subsequently captured on a streptavidin plate. After washing, acid treatment is used to dissociate the ADA bound to the captured biotin-drug, allowing ADA transfer to a MSD plate where the ADA binding and neutralization occur. ECL detection is accomplished using a sulfo-tagged version of the drug.

The SPEAD assay steps that specifically prevent the binding of the target to the drug, while maintaining the drug binding properties of the ADAs, were tested by adding anti-target antibodies. In the first method tested, samples were pretreated with anti-target antibodies prior to acid dissociation and neutralization of samples (**Figure 1, Method 1**). In the second approach, the neutralization step was followed by the addition of anti-target antibodies with the confirmatory reagent so that it would bind to the carried-over target and inhibit its binding to the sulfo-tagged drug (**Figure 1, Method 2**). In the third strategy, the anti-target antibody was used as an additional plate-blocking reagent after the neutralized ADAs were coated on to the MSD plate. This succeeded in blocking the coated carried-over drug target, allowing the sulfo-tagged drug to bind only to the coated ADAs (**Figure 1, Method 3**).

Figure 1. Anti-Target Antibody Mitigation Strategies

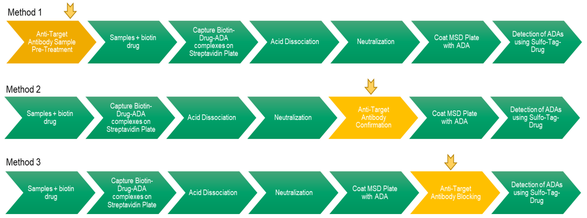
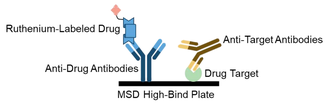


Figure 2. SPEAD Assay Format With Anti-Target Antibody Blocking



RESULTS

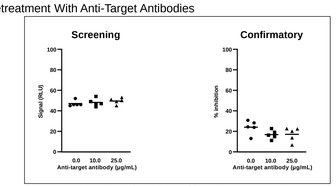
Results for interference characterization and anti-target antibody mitigation strategies are presented below.

Table 2. Interference Characterization

Sample ID		Screening		Drug Confirmation (2 µg/mL)	Drug Confirmation (5 µg/mL)
		Signal (RLU)	S/N	% Inhibition	% Inhibition
No Treatment	NC	88	-	27.3	30.7
	LPC (100 ng/mL)	184	2.1	54.9	60.3
	HPC (5000 ng/mL)	5218	59.3	81.5	90.5
IgG Depletion	NC	78	0.9	19.2	24.4
	Serum 1	99	1.3	35.4	41.4
	Serum 2	81	1.0	21.0	25.9

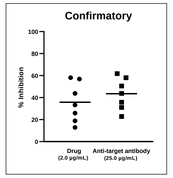
S/N is calculated on non-treated NC

Figure 3. Sample Pretreatment With Anti-Target Antibodies



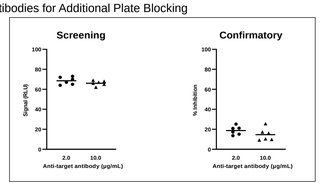
Screening and Confirmatory Results for Naïve Individual Human Serum Lots

Figure 4. Anti-Target Antibody Confirmation



Confirmatory Results for Naïve Individual Human Serum Lots

Figure 5. Anti-Target Antibodies for Additional Plate Blocking



Screening and Confirmatory Results for Naïve Individual Human Serum Lots

PRE-VALIDATION RESULTS

The SPEAD assay using an anti-target antibody as an additional plate-blocking reagent was optimized. Pre-validation results met acceptance criteria, and are presented below.

Table 3. Pre-Validation Results Summary

Evaluation	Description/Results
Plate-Screening Cut-Point	1.062
Confirmatory Cut-Point	28.5%
Titration Cut-Point	1.19
Sensitivity	<100 ng/mL
Hook Effect	No hook effect observed
Inter-Assay Precision of Screening Assay	NC signal: 11.1 % LPC S/N: 11.0 % HPC S/N: 18.0 %
Intra-Assay Precision of Screening Assay	NC signal: 7.5% to 8.0 % LPC signal: 1.9% to 5.1 % HPC signal: 2.9% to 12.1 %
Inter-Assay Precision of Confirmation Assay	ILPC % inhibition: 9.5 % IHPC % inhibition: 2.2 %
Specificity and Selectivity in Normal Human Serum	Met acceptance criteria
Specificity and Selectivity in Diseased Population of Human Serum	Met acceptance criteria
Drug Tolerance	1.0 µg/mL of drug
Combined Bench-Top and Freeze—Thaw Stability	5 freeze-thaw cycles and up to 26.2 hours at room temperature (22 °C nominal)

CONCLUSION AND CLOSING STATEMENT

Target interference is a common issue in immunogenicity assays, and one of the most difficult to overcome due to its specificity to the drug. This is especially true when the target can be presented as multimeric complexes. The addition of anti-target antibodies has been widely used to mitigate target interference. It is critical to evaluate the efficacy and appropriateness of the chosen strategy across different steps of the SPEAD assay.

The use of anti-target antibodies as an additional blocking reagent for the carried-over target in the SPEAD format was found to be an efficient method for reducing variability between individual donors in the confirmatory assay, improving assay sensitivity, and reducing the incidence of false-negative results.

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