The Rollercoaster Between Free Drug Tolerance and Target Interference in the Development of an Anti-Drug Antibody Assay

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INTRODUCTION

Assessing the immunogenicity of biotherapeutics is crucial for comprehending the impact of anti-drug antibodies (ADA) on the safety and efficacy of the drug. However, ADA testing presents challenges due to potential interference caused by circulating drug and/or soluble drug target, leading to either false-positive or false-negative results. Therefore, developing sensitive and specific ADA assays is imperative, necessitating careful consideration and scientific rigor to address these complexities.

To illustrate the challenges posed by these interferences, we present a case study where the presence of a multivalent soluble target and high drug levels resulted in inadequate assay performance. The assay requirements were outlined as follows:

- Targeted free drug tolerance (based on C_{trough}) : 400 µg/mL at 100 ng/mL positive control (PC)
- Targeted drug target tolerance: 100 ng/mL at 0 and 100 ng/mL PC

ASSAY DEVELOPMENT AND OPTIMIZATION

Bridging Assay With Acid Dissociation

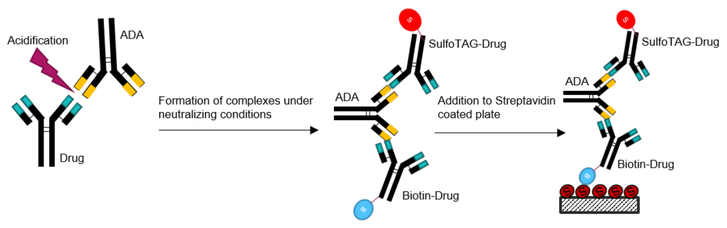


Figure 1. Assay principle of bridging assay with acid dissociation

Initially, a standard bridging electrochemiluminescence immunoassay (ECLIA), incorporating an acid dissociation step, was evaluated at a minimal required dilution (MRD) of 60 and a monoclonal antibody (mAb) as PC. However, the desired drug tolerance could not be achieved under these conditions **(Table 1)**. Increasing the MRD to 80 did not yield any improvement (data not shown), indicating the necessity for more complex sample pre-treatments.

Drug [µg/mL]	Response [RLU]	
	100 ng/mL PC	3000 ng/mL PC
500	78	81
250	81	98
120	82	139
60	92	316
30	112	606
0	560	7339

	Mean RLU	rCP (CF=1.04*)
NC	84	88

Table 1: Evaluation of free drug tolerance using a bridging ECLIA with acid dissociation. Reactive samples are shown in pink. NC: negative control. rCP: run cut point. CF: Correction factor. *CF based on screening of 50 drug-naïve individuals.

Affinity Capture Elution (ACE)

Subsequently, we assessed the ACE[1] assay format as illustrated in **Figure 2**. Remarkably, there was a lack of reproducibility in assay performance across experiments **(Table 2)** and inadequate sensitivity resulted in insufficient free drug tolerance (data not shown).

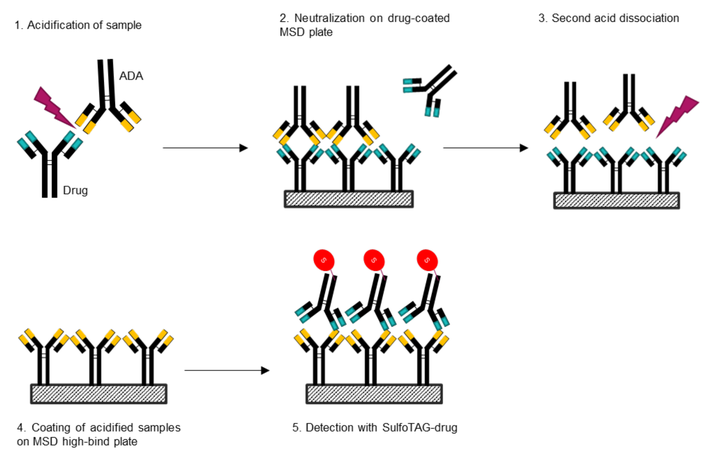


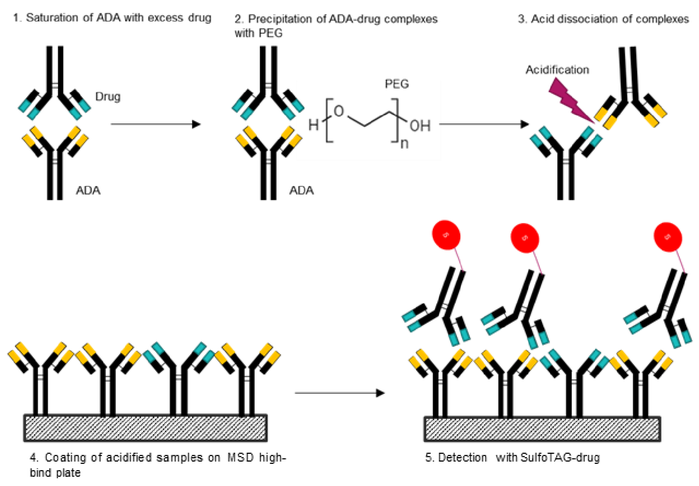
Figure 2: Assay principle of the Affinity Capture Elution.

Experiment	Response [RLU]		
	NC	100 ng/mL PC	3000 ng/mL PC
1	125	154	650
2	97	95	111

Table 2: Evaluation of sensitivity and precision.

Assay Precipitation and Acid Dissociation (PandA)

Poor assay sensitivity and reproducibility were also encountered in the PandA[2] assay, a method specifically designed to mitigate interference from high drug and/or target levels **(Figure 3)**.



Experiment	Response [RLU]		
	NC	100 ng/mL PC	3000 ng/mL PC
1	76	110	418
2	69	73	166

Figure 3: Assay principle of PandA (top) and evaluation of sensitivity and precision (bottom).

The poor sensitivity and low assay response observed in both, ACE (two acid dissociation steps) and PandA (prolonged coating of sample under acidic conditions) suggested acid sensitivity of our mAb PC. To investigate this hypothesis, six different mAb PCs were assessed in the PandA assay. Yet, none led to improved assay sensitivity (data not shown), concluding that prolonged and repeated exposure to acid should be prevented.

Optimization of Bridging Assay With Acid Dissociation

Considering the limited success of complex sample pre-treatments regarding drug tolerance, the initial bridging assay with acid dissociation was re-investigated at MRD exceeding 100. Through this optimized approach, targeted free drug tolerance could be achieved with an MRD of 150 **(Table 3, top)**. However, the presence of the target resulted in false positive results at the anticipated circulating target concentration (100 ng/mL) **(Table 3, bottom)**. Despite testing various PCs, similar assay responses were obtained (data not shown).

Free drug tolerance assessment		
PC [ng/mL]	Drug [µg/mL]	Response [RLU]
0	0	80
100	0	190
100	63	118
100	400	86

Target tolerance assessment			
PC [ng/mL]	Target [ng/mL]	Response [RLU]	
		Screening	Confirmatory
0	0	76	N/AV
0	100	128	70
0	400	263	70

Table 3: Evaluation of drug and target tolerance using an optimized bridging ECLIA with acid dissociation and MRD150. CF=1.03 based on screening of 50 drug-naïve individuals

Target Depletion Using Target-Specific Antibody

In order to reduce false-positive responses, a sample pre-treatment step was introduced to deplete the target from the matrix using biotinylated target-specific antibody immobilized on Streptavidin-coated ELISA plate **(Figure 4)**.

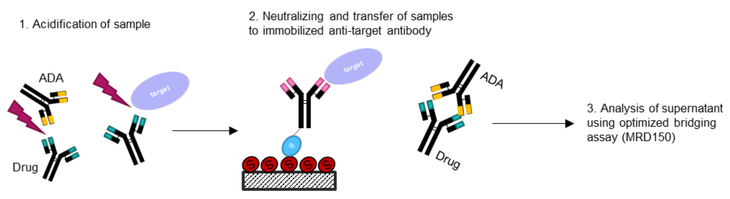


Figure 4: Assay principle of target depletion using target-specific antibody.

Although target tolerance was successfully achieved up to 200 ng/mL of target, the requested free drug tolerance of 400 µg/mL could not be reached **(Table 4)**.

Target tolerance assessment		
PC [ng/mL]	Target [ng/mL]	Response [RLU]
0	0	63
0	25	63
0	100	60
0	200	61
100	0	94
500	0	223

Free drug tolerance asessment		
Drug [µg/mL]	Response [RLU]	
	100 ng/mL PC	3000 ng/mL PC
500	61	65
400	62	68
50	61	160
10	70	319
0	93	766

Table 4: Evaluation of drug and target tolerance using target depletion pre-treatment followed by the optimized bridging ECLIA. CF = 1.08 preliminary CF calculated from screening of n = 40 individuals.

ADA Capture Followed by Reversed Direct Assay Using Protein L

Given the limitations of conventional assay formats to address both, drug and target interference, we examined a tailored assay format designed to specifically reduce interference from a non-protein-L binding drug and a non-antibody target **(Figure 5)**.

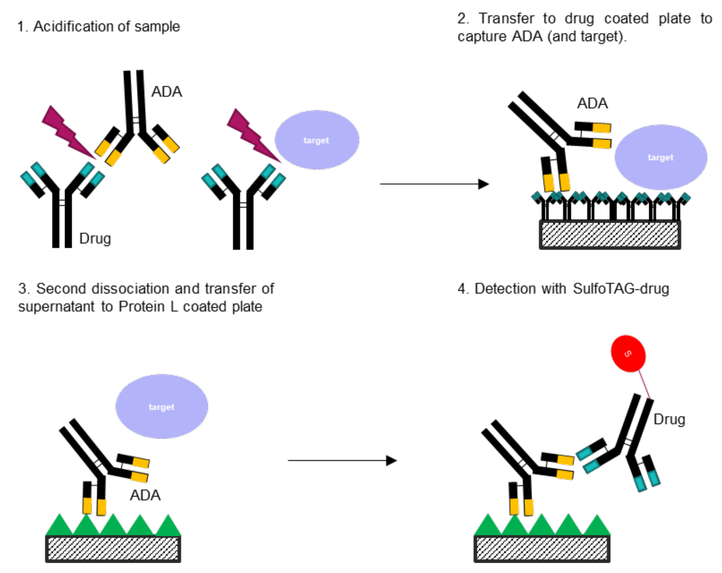


Figure 5: Assay principle of reversed direct assay using protein L with ADA capture

Through this approach, targeted free drug tolerance was achieved and no interference from drug-target was observed **(Table 5)**. However, since protein L specifically binds to the VL region of kappa light chains (human I, III, IV, predominant), but not to the VL region of lambda light chains, ADA composed of lambda light chains would not be detected using this method.

PC [ng/mL]	Drug [µg/mL]	Target [ng/mL]	Response [RLU]
0	0	0	53
100	0	0	257
3000	0	0	3658
100	200	0	59
100	400	0	58
0	0	100	53
rCP (CF = 1.08)			57

Table 5: Evaluation of critical parameters (i.e. free drug tolerance and target interference) using reversed direct assay using protein L with ADA capture. CF = 1.08 preliminary CF calculated from screening of n = 40 individuals.

ADA Capture Followed by Reversed Direct Assay Using Protein A/G/L and Fab Fragment of Drug

To broaden the detection range to various Ig classes and subclasses, the assay outlined in **Figure 5** was adapted as follows: (1) the assay plates were coated with recombinant Protein A/G/L and (2) ADA detection was performed using SulfoTAG-Fab Fragment of the drug.

Reproducible results with good sensitivity, drug AND target tolerance was obtained **(Table 6)**. However, detecting with the Fab fragment of the drug will not be able to detect ADAs against Fc domain.

Sensitivity assessment	
PC [ng/mL]	Response [RLU]
1000	2391
500	1103
250	599
125	407
50	210
25	167
10	151
5	129
1	113

Free drug tolerance asessment	
Drug [µg/mL]	Response [RLU]
10000	99
5000	100
1000	108
800	122
400	132
100	172
20	252

	Mean RLU	rCP (CF=1.1*)
NC	111	122

Target tolerance asessment		
PC [ng/mL]	Target [ng/mL]	Response [RLU]
0	0	73
0	100	75
100	0	240
3000	0	4485

Table 6: Evaluation of sensitivity, free drug tolerance and drug target interference with reversed direct assay using Protein A/G/L and Fab Fragment of drug as detection. *Arbitrary CF

DISCUSSION & CONCLUSIONS

Despite exploring various assay formats during method development, ranging from simple acid dissociation to complex sample pre-treatments, each approach encountered its own obstacles. Improving drug tolerance often resulted in reduced target tolerance and vice versa, highlighting the complexity of finding a suitable assay format.

Additional assay formats are going to be explored to encounter for both, required drug and target tolerance while not compromising the detection of potential relevant ADAs in clinical samples.

REFERENCES

[1] Chen YQ, Pottanat TG, Carter QL, Troutt JS, Konrad RJ, Sloan JH. Affinity capture elution bridging assay: A novel immunoassay format for detection of anti-therapeutic protein antibodies. J Immunol Methods. 2016 Apr;431:45-51.

[2] Zoghbi et al., 2015. A breakthrough novel method to resolve the drug and target interference problem in immunogenicity assays. Journal of Immunological Methods 426: 62-69.