A Dramatic Increase in Free Drug Tolerance Using PandA Compared to Standard Acid Dissociation

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INTRODUCTION

Drug tolerance is THE major technical challenge in immunogenicity assays. Low drug tolerance is the result of drug interference when high drug concentrations are present in samples. Of particular concern are therapeutic monoclonal antibodies with typically longer half-lives and often with the requirement of higher therapeutic doses. The safety risk of low drug tolerance is the detection of false-negative screening of low-positive samples leading to an underestimation of the potential immunogenicity of biotherapeutics. Various approaches can improve drug tolerance to assess anti-drug antibodies (ADAs), which include the standard approach of acid dissociation and more sophisticated extraction methods such Precipitation and Acid dissociation (PandA)[1].

Here, we present an example of an Electrochemiluminescence Immunoassay (ECLIA) for the detection of ADAs, making a comparison between PandA and the standard method of acid dissociation. The developed PandA method is both sensitive and remarkably tolerant to the presence of up to 800 µg/mL free drug with the capability of detecting 10 ng/mL of ADA. The standard acid dissociation presents difficulties in detecting ADAs at the same free drug concentration. The 20-fold increase in free drug tolerance with PandA highlights the potential of this method as a bioanalytical tool in studies where high drug levels will be present in patient samples.

ANALYTICAL METHODS

The aim of the PandA method is the detection of total (bound and unbound) ADA in samples in the presence of high levels of drug. This method includes four critical steps (Figure 1):

- Saturation of ADA with excess drug
- Precipitation of ADA-drug complexes using polyethylene glycol (PEG)
- Dissociation of the ADA-drug complexes with acid and transfer of acidified sample to large capacity high-bind plates to avoid re-binding
- Detection of ADA with the Sulfo-tagged drug

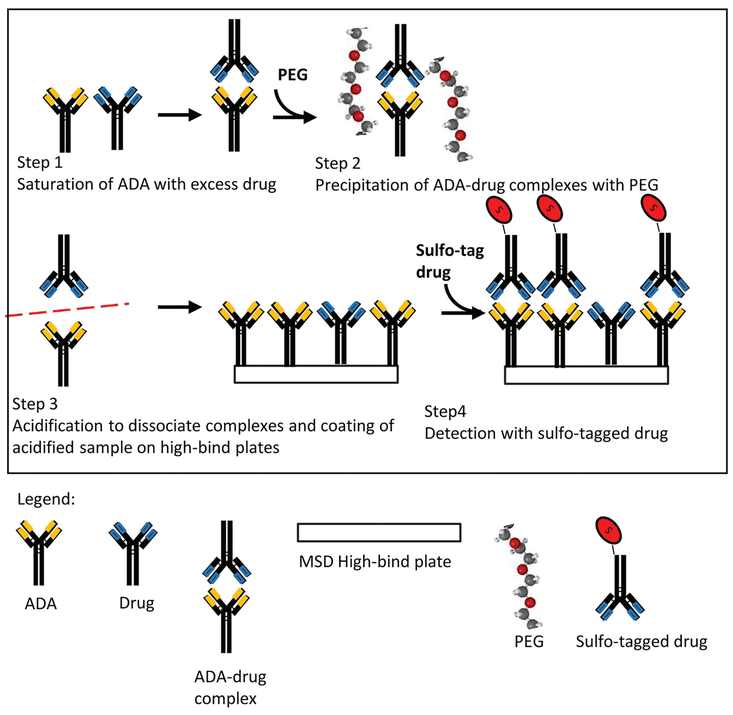

Figure 1. Schematic of the PandA pretreatment.

OPTIMIZATION OF PandA PARAMETERS

Polyethylene glycol concentration

Polyethylene glycol (PEG) was used to precipitate out ADA-drug complexes based on their molecular size (or molecular weight). The precipitation is dependent on the concentration of PEG, which was optimized during development (Figure 2). Two different PEG concentrations (low and high) were investigated of which the low PEG concentration resulted in the best signal to background noise ratio (S/N).

Excess drug concentration

Excess drug is crucial for the saturation of ADAs and the formation of ADA-drug complexes. Two different excess drug concentrations (low and high) were tested and the low excess drug concentration resulted in the best S/N ratio (Figure 2).

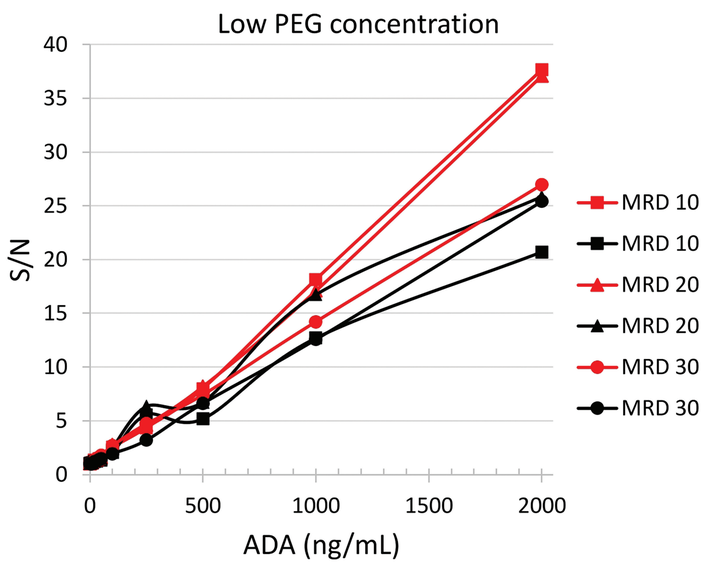

Figure 2. Optimization parameters for the PandA development. The red and black lines represent the low and high excess drug concentrations, respectively. S/N: signal to background noise ratio.

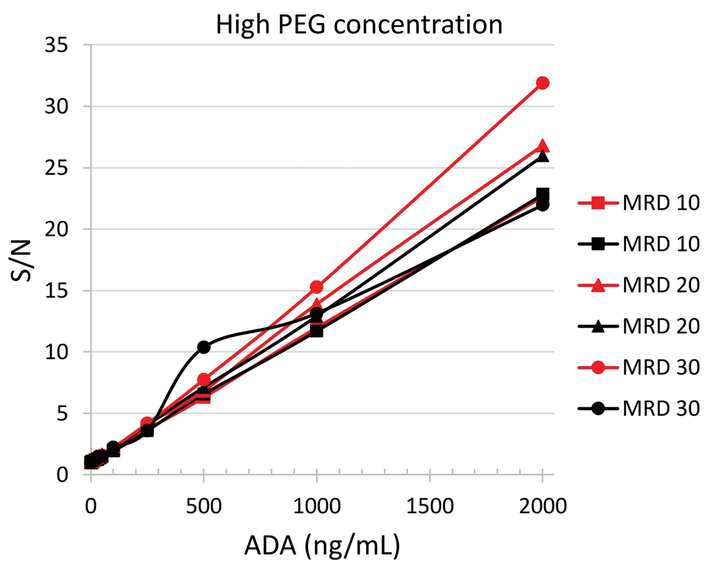

Figure 2 [continued]. Optimization parameters for the PandA development. The red and black lines represent the low and high excess drug concentrations, respectively. S/N: signal to background noise ratio.

RESULTS

The assay and results shown below have been addressed according to the international guidelines for the development and validation of immunogenicity anti-drug antibody assays[2] and show that the introduction of the PandA method did not impact the normal assay parameters.

FREE DRUG TOLERANCE

Free drug tolerance (FDT) was investigated by incubating the various PC levels with increasing concentrations of the drug (up to 800 µg/mL). We were still able to detect both levels of PC when samples contained up to 800 µg/mL of free drug with PandA. (Table 1). The standard acid dissociation method showed a lower free drug tolerance of 200 µg/mL and less depending on the minimum required dilution (MRD) applied.

Free drug [µg/mL]	Standard Acid Dissociation		PandA	
	100 ng/mL	100 ng/mL	100 ng/mL	100 ng/mL
	MRD 20	MRD 30	MRD 20	MRD 30
800	73	71	227	126
500	74	72	258	151
200	87	88	278	153
150	91	91	285	156
100	106	103	290	160
50	140	125	307	153
10	295	209	310	166
0	978	495	323	220
NC	83	75	58	63
rCP	97	87	62	70

Table 1. Free drug tolerance with standard acid dissociation and PandA at positive control concentration of 100 ng/mL. The coloured boxes indicate the Run Cut Point (rCP); green >rCP, red <rCP).

PRECISION

The precision of runs were investigated by determining the %CV between runs. The %CV of selected positive control (PC) levels are <20 %CV (Table 2).

	RLU			
	NC	LPC (50 ng/mL)	MPC (250 ng/mL)	HPC (2000 ng/mL)
Dataset 1	64	138	578	1850
Dataset 2	66	102	390	1731
Dataset 3	65	107	442	2070
Dataset 4	64	104	426	1890
Dataset 5	65	110	588	1759
Dataset 6	65	110	425	2530
Mean RLU	**65**	**112**	**475**	**1971**
%CV	**1.3**	**11.7**	**18.0**	**15.1**

Table 2. Between-run precision of the PandA method at NC and different PC concentrations.

SENSITIVITY

The sensitivity was determined between PandA and standard acid dissociation by diluting the PC at MRD 10, 20 and 30 (Figure 3). The sensitivity of the standard acid dissociation is 5-fold higher at the lowest MRD. However, the estimated sensitivity of this PandA method was ~10 ng/mL with the screening/confirmatory format showing that while overall sensitivity was somewhat reduced, ADA levels well below the target to 100 ng/mL were easily achieved (Table 3).

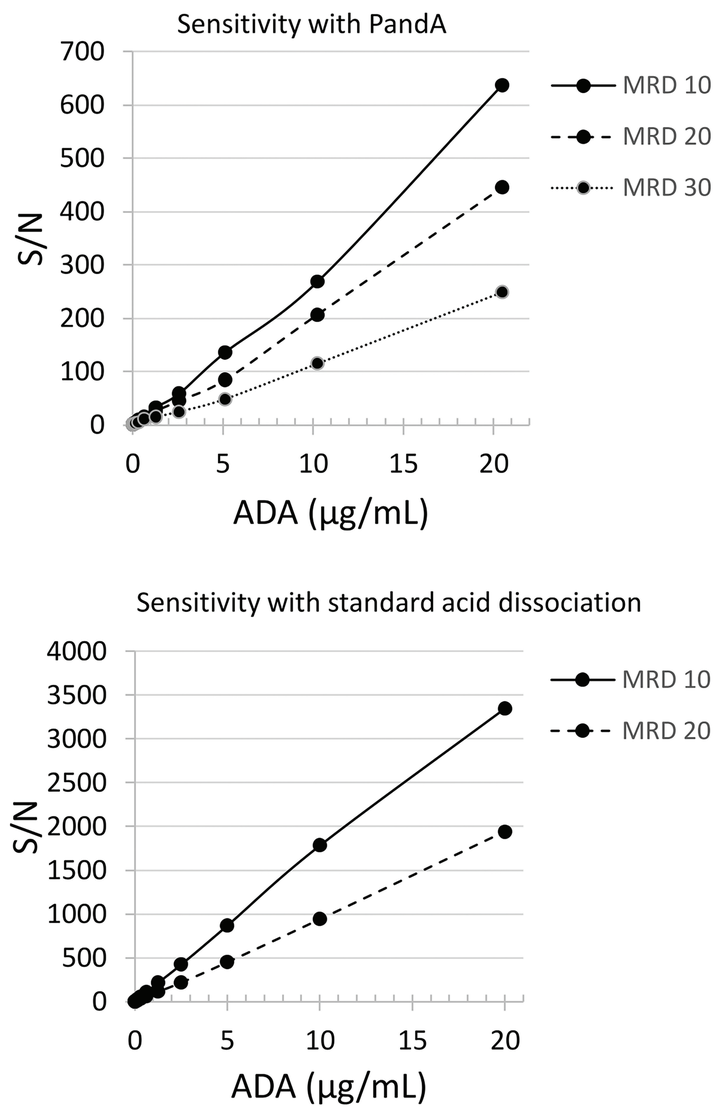

Figure 3. A comparison of sensitivity between PandA and standard acid dissociation at different MRDs.

	Conc. at rCP (ng/mL)	
	Screening	Confirmatory
Dataset 1	7.3	3.2
Dataset 2	6.9	5.7
Estimated Sensitivity	8.2	10.5

Table 3. Sensitivity of screening and confirmatory assay.

HOOK EFFECT

No hook effect was observed. The high concentration samples of up to 204 µg/mL of PC still showed a response above the HPC.

SELECTIVITY

Selectivity (recovery) was evaluated with 12 healthy individual samples spiked at LPC (50 ng/mL) and HPC level (Table 4). The selectivity run passes with only one out of 12 individuals not passing at LPC level.

Selectivity		
Unspiked	Spiked at LPC	Spiked at HPC
Pass		
12 / 12	11 / 12	12 / 12

Table 4. Recovery assessment with 12 individuals unspiked and spiked at LPC and HPC levels. No false-positives were observed.

DISCUSSION & CONCLUSIONS

In the present ADA immunoassay, we have shown the successful implementation of PandA and the increase in free drug tolerance. The PandA method shows superiority over standard acid dissociation by eliminating drug interference at high drug concentrations. With the PandA, at free drug concentrations of 800 µg/mL, we successfully measured ADA concentrations of 10 ng/mL.

These results highlight the superiority of the PandA method making it a highly useful tool for immunogenicity assays, which require a higher drug-tolerance.

REFERENCES

1. 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.
2. **Immunogenicity Testing of Therapeutic Protein Products – Developing and Validating Assays for Anti-Drug Antibody Detection. Guidance for Industry.** *US FDA* 2019.