Cutting-Edge Method for the Detection of Anti-Drug Antibodies with Record Free Drug Tolerance: Be safe with PandA

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Introduction

The use of antibody and protein therapeutics for targeting neurodegenerative diseases has become an attractive and promising approach. However due to the low exposure of the biotherapeutics across the blood-brain barrier (BBB), high dosing is often necessary.

Remarkably, the administration of biological therapeutics has the potential to induce undesirable immunogenicity, resulting in the development of anti-drug antibodies (ADA). Therefore, it is necessary to develop sensitive and specific methodology for the detection and characterisation of ADAs in order to evaluate their potential impact on the drug pharmacokinetic profile, patient safety and efficacious response to the drug.

Drug tolerance in anti-drug antibody (ADA) assays is of growing concern. The biotherapeutics with long half-life or high dosing or the targets may interfere with the detection of Anti-Drug Antibodies causing false negative (from drug) or false positive results (from target).

Goals

To overcome the above-mentioned limitations and circumvent both drug interference and matrix interfering factors, we explored several assay formats. Despite the success of those methods, their limitations led us to develop a novel method using Precipitation and Acid Dissociation (PandA)[2]. The coupling of precipitation with acid dissociation allows overcoming drug interference and improving free drug tolerance.

In the present study we developed and validated a method designed to detect antibodies in human plasma with an extraordinary high free drug tolerance (>800µg/ml) and sensitivity at 100 ng/ml.

Analytical Method

The method principle is based on four critical components for the detection of total ADA (free ADA and drug bound ADA) in the presence of drug in samples (Figure 1). Initially the samples are incubated with Excess Drug in order to saturate ADA to form drug bound ADA as drug-ADA complexes. Precipitation of the complexes then follows using Polyethylene glycol (PEG) and then the precipitated complexes are subjected to acetic acid for dissociation and free ADA (and free drug) are captured on high binding capacity plates. Following the blocking of the assay plate, the plate is incubated with the sulfo-tagged drug for the specific detection of the ADA.

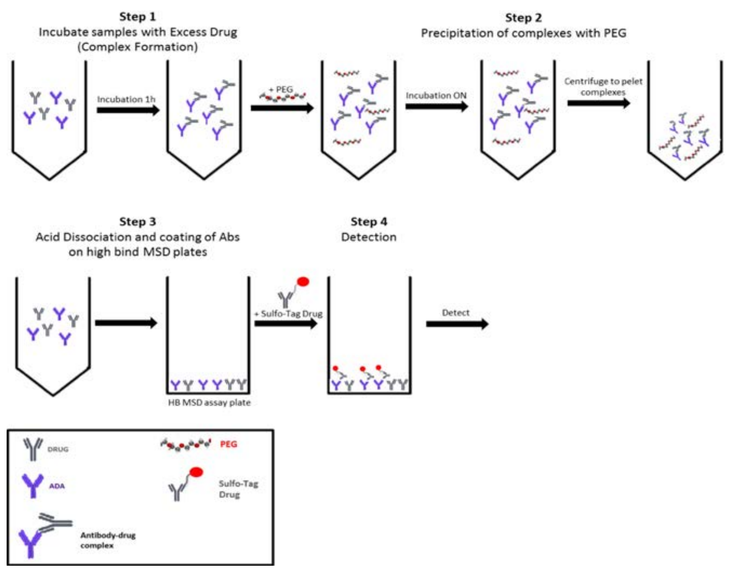
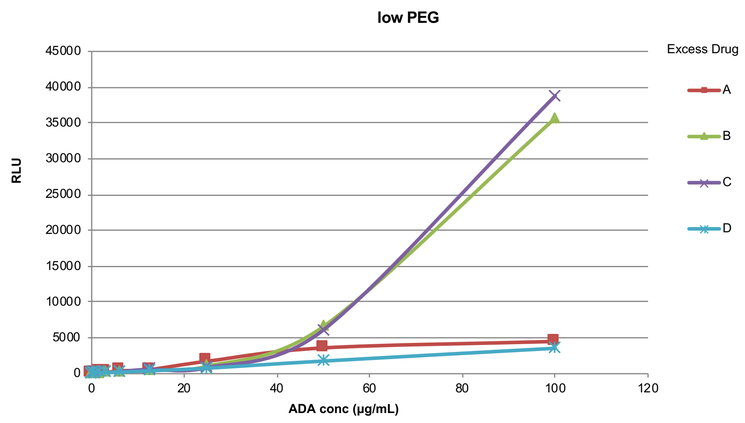


Figure 1. Principle of PandA.

Analytical Challenges and Solutions

Concentration of Excess Drug

Excess Drug is used in order to saturate ADA to form drug bound ADA as drug-ADA complexes. A range of Excess Drug (A – D) was tested during the development of the assay in order to determine the optimal concentration for complete ADA binding to the drug forming a complex. The optimal concentration of Excess Drug was chosen based on the best signal to background ratio.



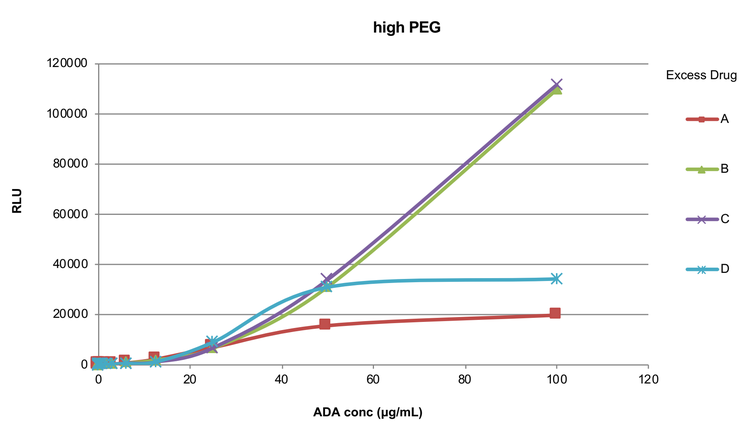


Figure 2. Excess Drug assessment in different PEG levels.

PEG Buffer

The Polyethylene glycol (PEG) precipitation is size (or molecular weight, MW) based and PEG concentration dependent. Thus for optimal performance of a sensitive and specific method, we tested several concentrations of PEG in order to minimize the amount of unbound non-specific proteins to be precipitated. Here, 4 different levels of PEG were tested. High variability was observed with the lower PEG concentrations, whereas the highest tested PEG concentration fulfils the acceptance criteria with CV% <20%.

Table 1. Comparison of different PEG concentration testing all control levels.

	PEG							
	low				low mid			
	LPC	MPC	HPC	NC	LPC	MPC	HPC	NC
	171	514	5320	74	233	492	4826	76
CV%	15.8	31.3	47.4	0.7	4.7	1.4	36.0	0.9

	PEG							
	high mid				high			
	LPC	MPC	HPC	NC	LPC	MPC	HPC	NC
	203	504	3550	78	221	505	3324	77
CV%	8.2	25.4	28.6	0.9	1.2	13.0	2.7	2.1

Method Validation

For the optimal validation of assays for Anti-Drug Antibody detection the following parameters have been addressed according to the international guidelines[1].

Table 2. Summary of Validation Results.

Assay Precision (%CV)	NC	LPC	MPC	HPC
Inter-Assay	3.20%	5.7% (250 ng/ml)	8.70%	19.60%
Inter-Assay	30.40%	6.0% (10.5 ng/mL)	7.10%	5.10%

Titer Precision	%CV
0.1% false positive rate	24.90%

Screening cut point

The screening cut point is determined by testing 50 individual samples from human healthy subjects. The vCP is calculated based on six datasets.

Table 3. Screening Cut Point Reporting Table.

Dataset	n	Mean NC	Median (ind)	nMAD	vCP
1	50	78.0	76.5	2.224	80.2
2	48	77.9	77.3	2.595	81.5
3	49	73.6	73.0	2.965	77.9
4	50	78.7	77.5	2.965	82.4
5	50	75.6	75.5	2.965	80.4
6	50	76.7	75.8	2.224	79.4

Validation screening cut point (mean vCP):	80.3
Correction factor:	+3.5

Free Drug Tolerance

Free Drug Tolerance was evaluated by incubating the LPC, HPC and a higher ADA concentration with increasing amounts of drug up to 1600 µg/mL. There is free drug tolerance at all levels of antibody tested up to 1600 µg/mL.

Table 4. Free Drug Tolerance evaluation.

Drug Tolerance	
10.5 ng/mL antibody conc. (LPC)	1600 µg/mL
5000 ng/mL antibody conc. (HPC)	1600 µg/mL
22500 ng/mL antibody conc.	1600 µg/mL

Assay Sensitivity and Specificity

Table 5. Assay sensitivity and specificity assessment.

Correction Factor (rCP)	+3.5 RLU
Sensitivity	
Screening (50% Consistency)	3.84 ng/mL
Screening (99% Consistency)	10.5 ng/mL
Confirmatory (50% Consistency)	4.79 ng/mL
Confirmatory (99% Consistency)	10.1 ng/mL

Hook Effect

There is no hook effect observed, with high dose sample response above the HPC response.

Table 6. Evaluation of hook effect.

High Dose Hook Effect	
None observed, high dose sample response is above HPC response	
HPC (5000 ng/mL)	1801.3 RLU
High Dose sample (2000 µg/mL)	232181.5 RLU

Recovery

Recovery (selectivity) was evaluated with ten individual samples spiked at the LPC (low spike) and HPC (high spike) levels.

Table 7. Recovery (selectivity) assessment.

Recovery	
at 10.5 ng/mL	10 out of 10
at 15.8 ng/mL	10 out of 10

Discussion and Conclusions

PandA, a cutting-edge method shows significant improvement over the current approaches to eliminate drug and possibly target interferences at high drug concentrations in an ADA immunoassay. In the present study, we showed the successful implementation of this novel methodology in order to meet the two challenging requirements for high free drug tolerance and a sensitive assay.

Validation of the assay demonstrated the sensitivity and robustness of the assay, with a sensitivity of 10.5 ng/ml and a free drug tolerance of 1600 µg/ml (at 100 ng/ml).

Exploring the PandA principle in bioanalytical ADA assessment has unequivocally demonstrated to be pivotal for disease indications, like neurodenerative diseases, where high drug levels are administered to patients.

References

1. Immunogenicity Testing of Therapeutic Protein Products – Developing and Validating Assays for Anti-Drug Antibody Detection. Guidance for Industry. US FDA 2019.
2. Zoghbi et al., 2015. A breakthrough novel method to resolve the drug and target interference problem in immunigenicity assays. Journal of Immunological Methods 426 (2015) 62-69.

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