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Improving Drug Tolerance in Anti-Drug Antibody (ADA) Assay using PandA Method—Case Studies

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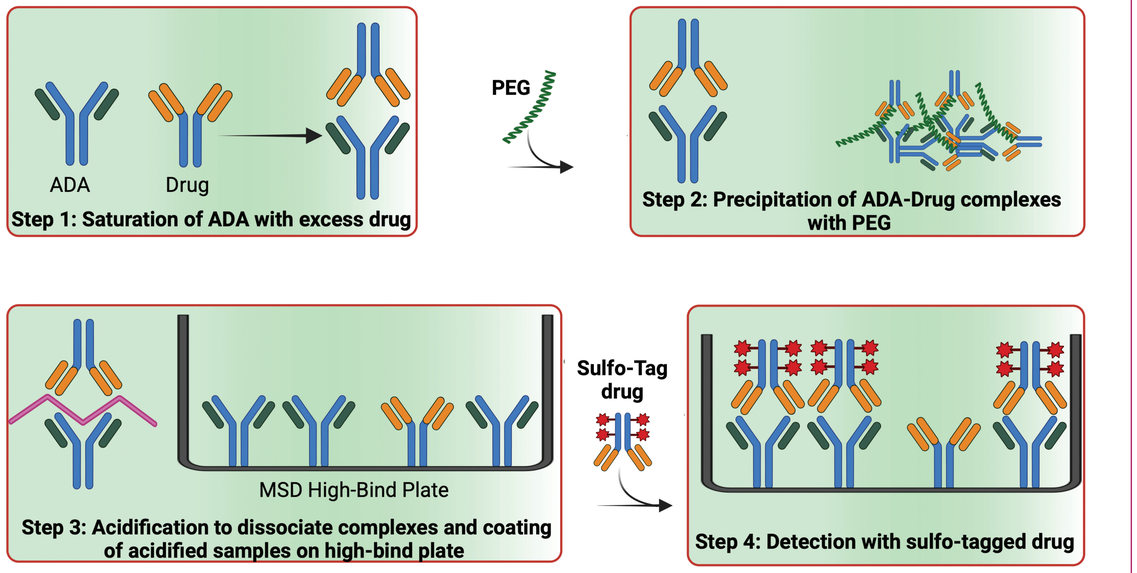
PURPOSE

A major technical challenge in developing Anti-drug Antibody (ADA) assays is to overcome drug tolerance (DT). High levels of the therapeutic drugs in matrix sample can mask ADA resulting in false negative results. This interference occurs because the drug can bind to the antibodies, forming complexes that the assay cannot detect. To overcome DT in ADA assays, sample pretreatment is needed to clear drug from the sample. Examples of pretreatment options include acid dissociation, Solid Phase Extraction and Acid Dissociation (SPEAD), Affinity Capture Elution (ACE), or Precipitation and Acid Dissociation (PandA). In this study, the DT of 3 types of protein drugs (fusion protein, naked antibody and antibody drug conjugate [ADC]) were evaluated comparing PandA and other pretreatment methods.

METHODS

The ADA sample was treated with an excess amount of drug to enable the formation of drug-ADA complexes. The complexes were then precipitated using polyethylene glycol (PEG). Following precipitation, the pellet was washed several times with a buffer containing PEG. At the end of the wash steps, the pellet was resuspended using an acid solution. This pretreatment helps remove excessive drug, thereby avoiding drug to interfere with the ADA assay. The acidified sample was directly coated onto a Meso Scale Discovery (MSD) plate and ADA was detected using a sulfotag-labeled drug as the detection reagent.

General overview of PandA method is as below:



RESULTS

The PandA method was able to overcome drug tolerance by up to 100-fold compared to other pretreatment methods such as acid dissociation or SPEAD. This method was proven to be successful for different drugs, including a fusion protein, a naked antibody, and an ADC. The tables below summarize the drug tolerance achieved with different methods.

The results clearly indicate that the PandA method substantially enhances DT across various drug types. For instance, while other methods failed to achieve any DT for the fusion protein (data not shown below), the PandA method achieved a tolerance of 50 mg/mL. Similarly, for naked antibody in this case study, the PandA method improved drug tolerance from less than 250 ug/mL to 1000 µg/mL. For the ADC molecule the improvement was from 10 µg/mL to 1000 µg/mL.

Table 1: Drug Tolerance Comparison between SPEAD method and PandA for Antibody Drug Conjugate

Drug Tolerance at 100 ng/mL of Anti-drug Antibody			
Using SPEAD Method		Using PandA	
Concentration of Drug Tested (µg/mL)	Raw Response	Concentration of Drug Tested (µg/mL)	Raw Response
500	231	1000	390
200	229	600	398
100	230	200	363
50	245	0	415
10	319		
0	319		
rCP	*267*	*rCP*	*288*

rCP = Run Cut Point

Table 2: Drug Tolerance Comparison between SPEAD method and PandA for Antibody Drug

Drug Tolerance at 100 ng/mL of Anti-drug Antibody			
Using SPEAD Method		Using PandA	
Concentration of Drug Tested (µg/mL)	Raw Response	Concentration of Drug Tested (µg/mL)	Raw Response
1000	175.5	1000	88
500	176	500	88.5
250	184	250	89
0	304	0	93
rCP	*188.7*	*rCP*	*68.75*

rCP = Run Cut Point

CONCLUSION

The PandA method was instrumental in achieving the desired DT for three different drugs. Like any sample pretreatment process, the PandA method also involves the optimization of some critical steps to achieve consistent results. Overall, the PandA method proved to be consistent in improving DT, while other methods were not as successful. This consistency and effectiveness make the PandA method a valuable tool in ADA assays, ensuring that ADAs can be detected even in the presence of high levels of therapeutic drugs.

In summary, the PandA method stands out as a robust and reliable approach to overcoming DT in ADA assays. Its ability to significantly enhance drug tolerance across various drug types highlights its potential for broader application in the field of immunogenicity testing. Future studies could further optimize and validate this method, potentially extending its use to other therapeutic drugs and improving the accuracy and reliability of ADA detection.

REFERENCES

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