Development of a clinical assay for measuring anti-drug antibodies against a monoclonal antibody drug: Overcoming soluble target interference

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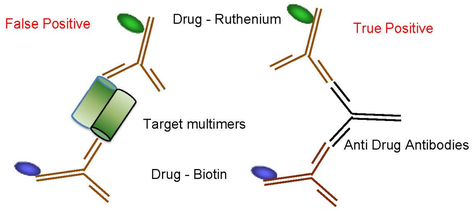
1. Abstract

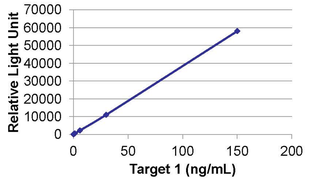
Immunogenicity assessments for biotherapeutics can be confounded by the presence of circulating target when it exists as multimers. Soluble targets that form multimers can result in false positive signals in bridging assays. This investigation provides approaches of clinical immunogenicity assays for monoclonal antibody drugs with minimal interference from the soluble target multimers found in the circulation of the intended population. For one case, a combination approach was implemented consisting of sample pretreatment to deplete the multimers with target receptor protein, followed by competing with a target specific monoclonal antibody to minimize the interference. This sample pretreatment approach was preferred over generating two independent assays with one detecting ADA against the F(ab) portion and a second assay detecting ADA against the Fc portion of the drug. Hence multiple assays would increase the complexity of assay development, sample testing and data interpretation.

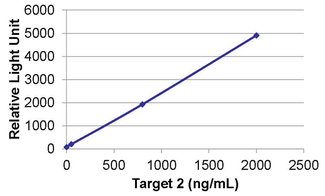
2. The Challenge

➢Soluble targets that form multimers can result in false positive signals in bridging assays. As shown in the case study figures below.

Figure 1



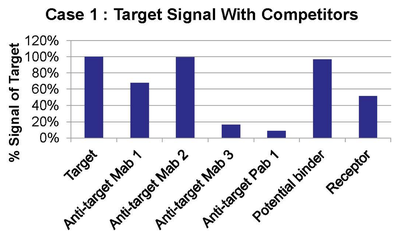


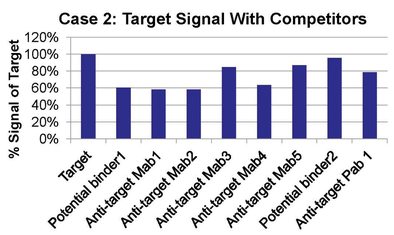


3. Strategy 1 : Compete with blocking antibodies or the receptor of the target

➢Anti target specific antibodies, receptors or binders could block the target binding to the labeled drug in the assays.

➢Efficient blocking of target interference by competitors is highly variable and should be evaluated.





Acknowledgement

- ANP Tech for providing Nanoparticle reagents.
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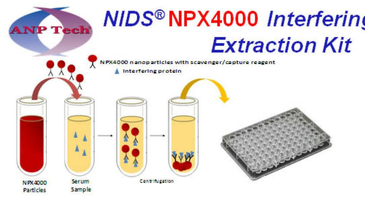
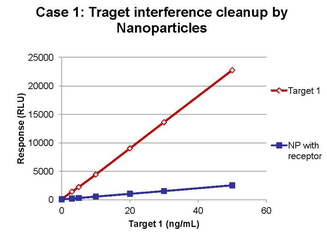
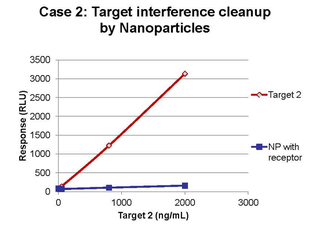
4. Strategy 2 : Remove interference by competitor-coupled Nanoparticles

Figure 2



Using this kit to attach the specific blocking antibody or receptors to activated nanoparticles and pretreating the sample, followed by a centrifugation step, enables the irreversible removal of the interference without affecting the detection of ADAs.





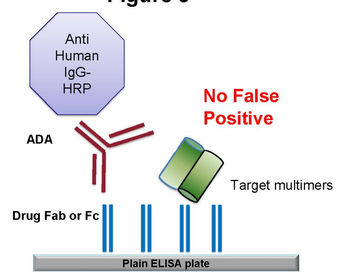
5. Strategy 3 : Non-bridging format

➢ Alternatively, non-bridging format could be used to avoid the target inference. Example format is showed in Figure 3.

➢ However, this approach will result in two separate assays, one for the F(ab) portion and the other for the Fc portion of the drug.

➢ This approach will not only increase the cost of assay development and sample testing, but also make the data interpretation process more complex.

Figure 3



6. Summary

➢ Soluble target interference is a unique challenge for developing sensitive clinical ADA assays.

➢ There is no “one-fit-for-all” method to deal with the soluble target interference for all biotherapeutics.

➢ It is important to understand the biology behind the target interference and experimentally confirm the interference in the assay.

➢ Several strategies are presented in this investigation, including anti-target antibody competition, sample cleanup by Nanoparticles and a non-bridging assay format.

➢ In some cases a single strategy is not sufficient to minimize the interference, and a combination approach can be used in order to achieve the maximum removal of the interfering target.

Table 1

Target conc (ng/mL)	Target Spiked in NC (RLU)	After treatment (RLU)
50	20400	201
30	11179	147
20	7512	125
10	3746	103
5	1871	86
3	1167	86
0	79	78
PC	3179	2940

PC: Positive Control
NC: Negative Control
RLU: Relative Light Unit
Plate cutpoint: 126 (RLU)

Table 1 data demonstrated a combination approach to minimize target interference in Case 1 study. Samples were pretreated with Nanoparticles coupled with receptor, followed by treatment with a mAb competitor. The combination treatment reduced the signal observed, from a spike of 20 ng/mL target into a serum sample, to a level below the assay cutpoint . Estimated target levels in the intended population are less than 10 ng/mL, making this technique sufficient for clinical testing.

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