Challenging the Impossible: A Highly Drug Tolerant and Sensitive Neutralizing Antibody Assay Towards a Monoclonal Antibody Therapy in Neurodegenerative Disease

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Introduction

Neurodegenerative diseases like Alzheimer are debilitating and novel potent drugs are urgently needed to offer patients better treatment options. A main safety and efficacy concern in drug development is the appearance of neutralizing anti-drug antibodies (nAbs), which could completely abrogate drug function or result in off-target effects. Therefore, it is key to have proper bioanalytical methods in place to reliably detect these nAbs.

Here we present the challenges, solutions, and special considerations regarding the development of a competitive ligand binding nAb assay against a monoclonal therapeutic antibody targeting a soluble protein in the central nervous system. The major challenge of the method development was to achieve a good sensitivity with a free drug tolerance of at least 50 μg/mL.

Detection Format Comparison

Two electrochemiluminescence formats were tested for the detection of nAbs against the antibody drug with a soluble protein target. Both formats detected the nAbs as a proportional decrease of the background signal:

1. Coating of the biotin-target on streptavidin MSD plates and detection with the SulfoTag-labeled drug (Fig.1A)
2. Coating of the biotinylated drug on streptavidin MSD plates and detection with the SulfoTag-labeled target (Fig. 1B)

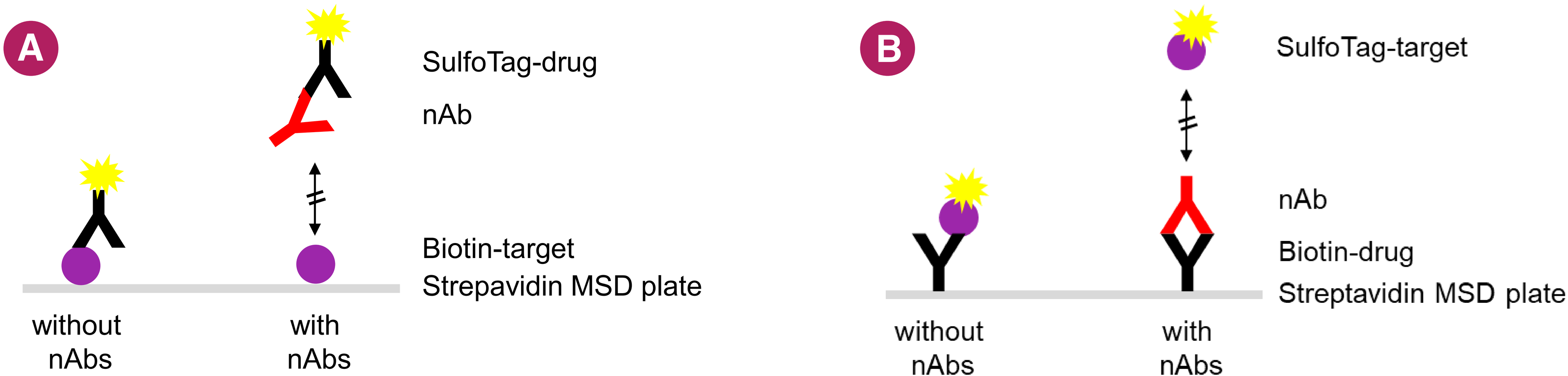

Figure 1: nAb Detection Setups: (A) Format 1 - Detection with Drug, (B) Format 2 - Detection with Target

After titration of capture and detection reagents, the preliminary sensitivity and free drug tolerance of both formats were evaluated using a polyclonal positive control at MRD 20 with acidification (Fig.2). Considering a preliminary cutpoint correction factor of 0.8, detection formats 1 and 2 had estimated sensitivities of ~500 ng/mL and ~2500 ng/mL, respectively, and both had a free drug tolerance of ~1 μg/mL. Drug concentrations above 1 μg/mL were detected as false positives in detection format 1 and as false negatives in detection format 2, highlighting the need for a sample pre-treatment step to remove excess drug. Detection format 1 was chosen for further development due to the much better sensitivity.

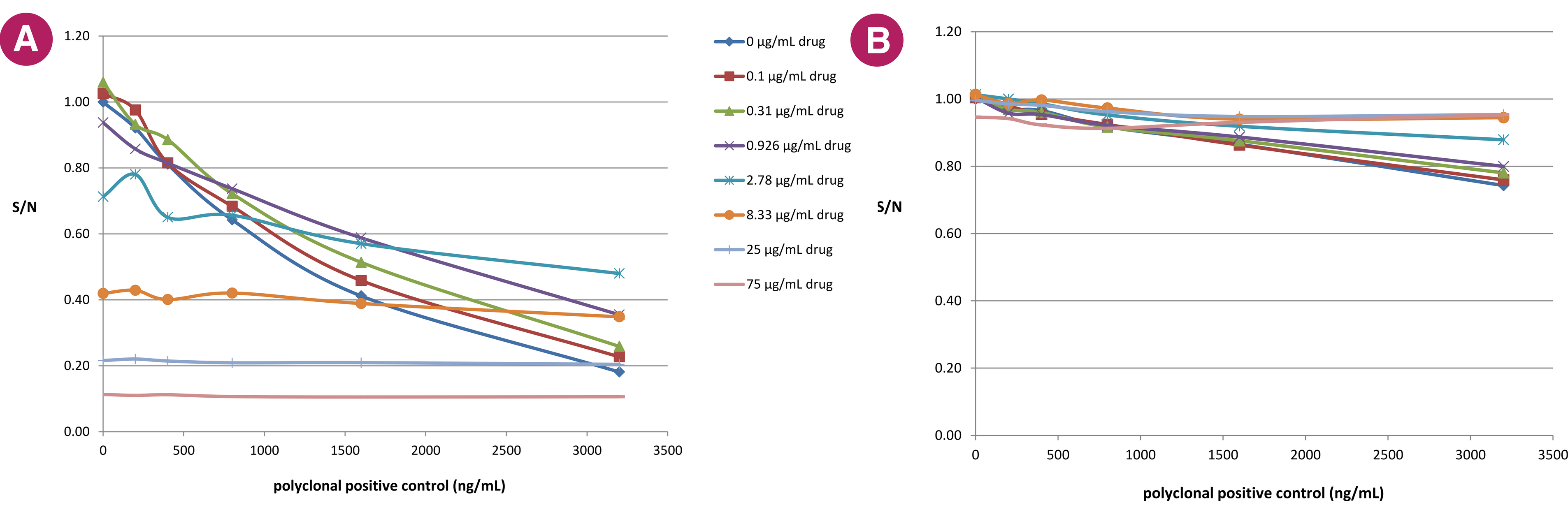

Figure 2: Sensitivity and Free Drug Tolerance of (A) Detection Format 1, (B) Detection Format 2

Drug Removal Comparison

1. PandA-nAb

Precipitation and acidification (PandA) is a novel bioanalytical method that overcomes severe drug interference in anti-drug antibody (ADA) assays. As PandA had already been successfully used in ADA assessment of the therapeutic antibody, we tried to adapt the method for nAb assessment by combining the key precipitation step with our nAb detection format 1 (Fig. 3). However, no polyclonal or monoclonal nAbs were detected regardless of the drug concentration (data not shown), most likely due to very low nAb precipitation efficiency.

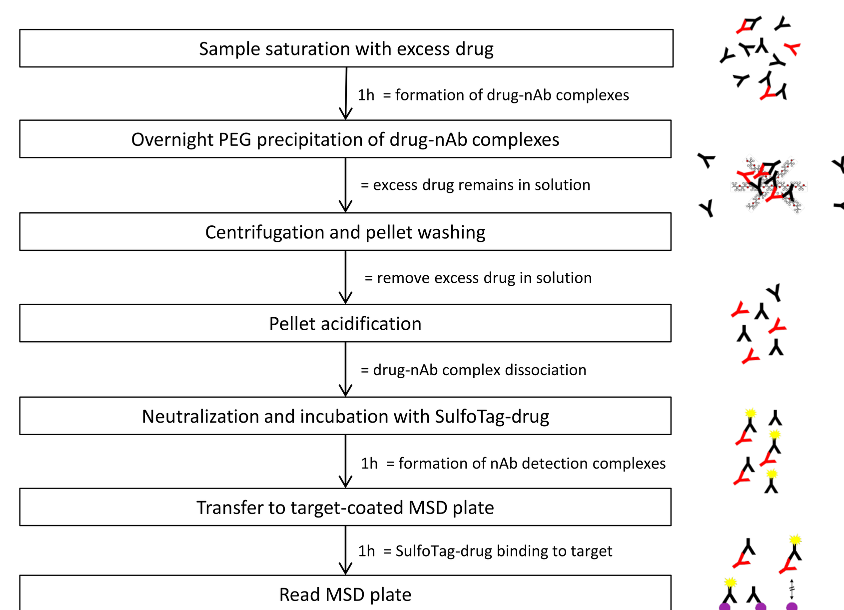

Figure 3: PandA-nAb setup

2. nAb Capture with Paramagnetic Particles

In our second approach we tried to capture sample nAbs using an excess of biotinylated drug coated on streptavidin paramagnetic particles (beads), which would outcompete the excess free drug in the sample, and which could then be physically separated from the nAbs by acidification and magnet collection (Fig. 4). However, this method only eliminated false positives without further improving the drug tolerance, and the sensitivity in the absence of free drug was decreased proportionally to the biotin-drug content, suggesting biotin-drug leakage from the coated beads (data not shown).

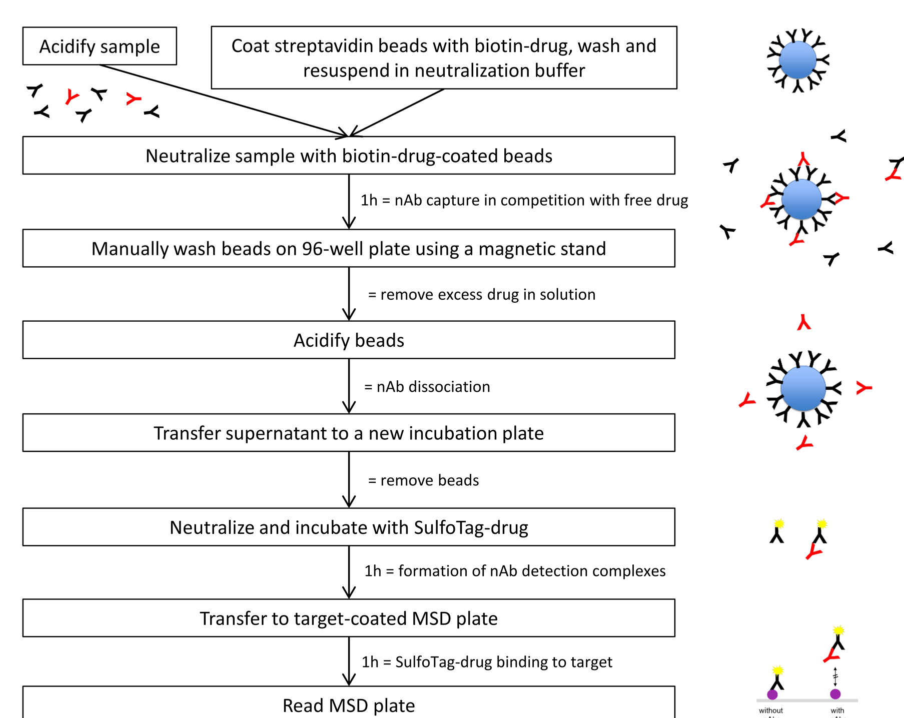

Figure 4: nAb Capture Setup

3. Drug Depletion with Paramagnetic Particles 1

Next, we tried the opposite approach where we captured and physically removed the excess free drug ***after acidification*** using an excess of biotinylated monoclonal anti-drug antibody (biotin-ADA) coated on streptavidin paramagnetic particles (Fig. 5). However, this method also only eliminated false positives without further improving the free drug tolerance (data not shown).

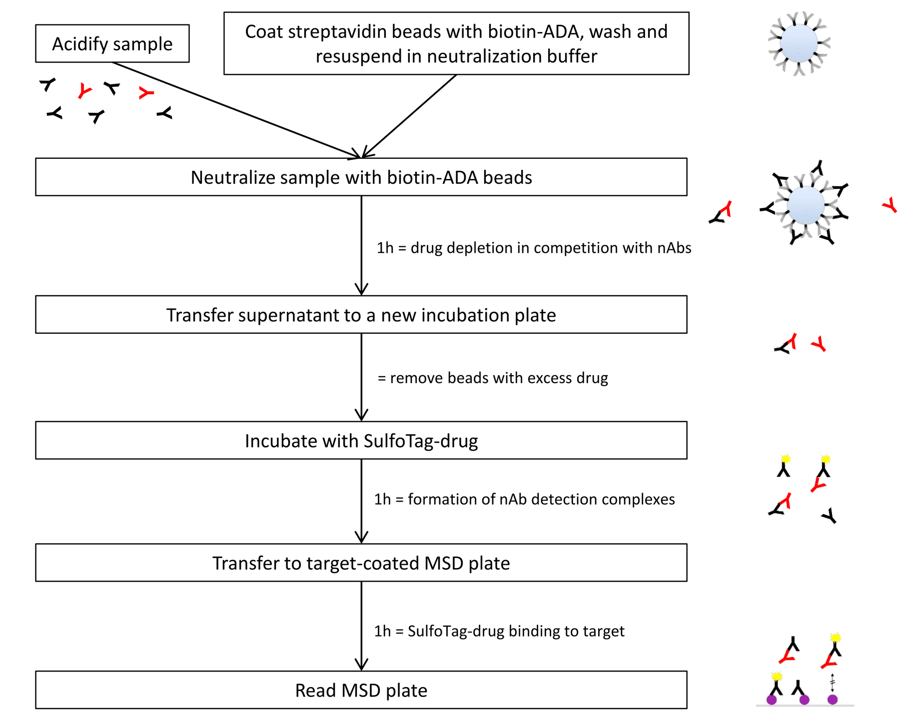

Figure 5: Drug Depletion Setup 1

4. Drug Depletion with Paramagnetic Particles 2

The final drug depletion approach that we tried was to capture and physically remove the excess free drug ***before acidification*** using an excess of biotinylated monoclonal anti-drug antibody (biotin-ADA) coated on streptavidin paramagnetic particles (Fig. 6).

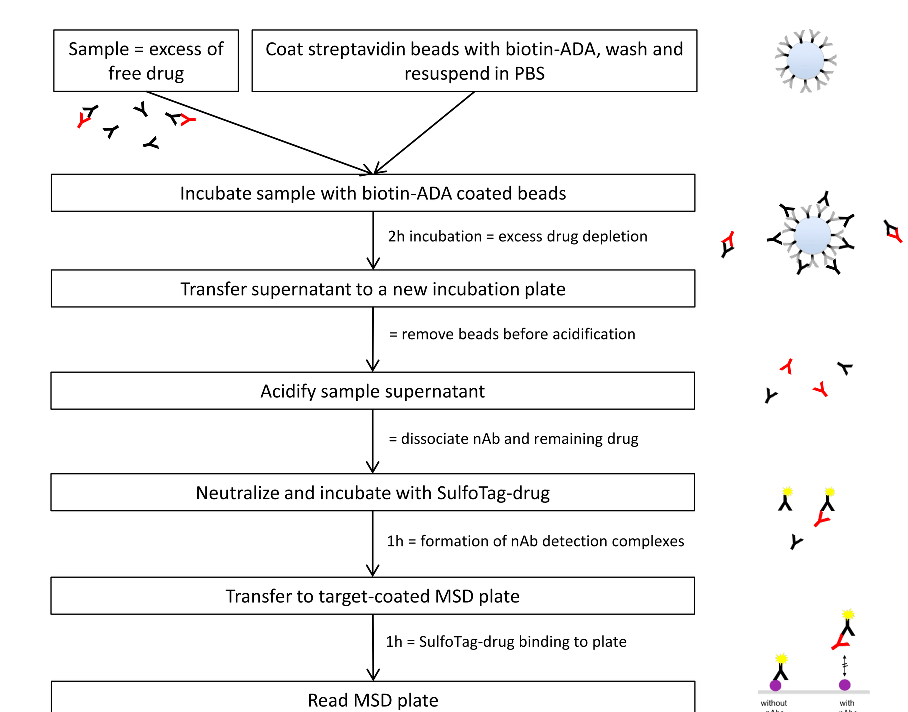

Figure 6: Drug Depletion Setup 2

After extensive optimization of MRD, coated bead amount and incubation lengths, preliminary assessment of the method considering a cutpoint correction factor of 0.8 at MRD 40 showed ~75 μg/mL free drug tolerance at ~800 ng/mL sensitivity level, with no false positives until 75 μg/mL, and no false negatives above 800 ng/mL sensitivity level (Fig. 7). The monoclonal positive control used was the same as the ADA used in the bead coating.

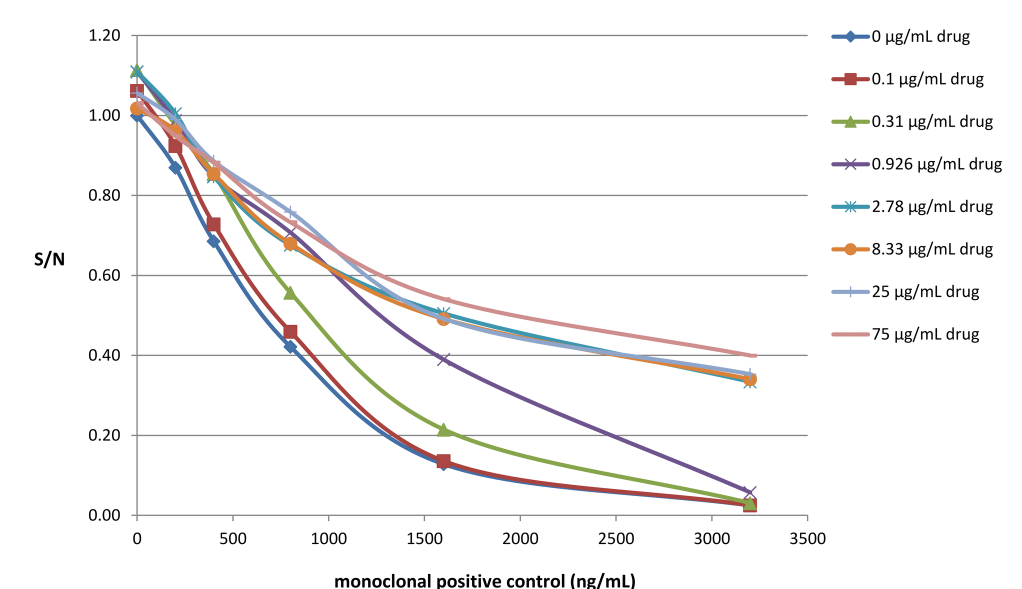

Figure 7: Sensitivity and Free Drug Tolerance of Drug Depletion Setup 2

Validation Results

The final bioanalytical method with drug depletion setup 2 was successfully validated at Celerion Switzerland AG with a sensitivity of 465 ng/mL and overall free drug tolerance of 50 μg/mL, without any false positives or false negatives. All other validation parameters such as assay precision, selectivity, and stability also passed according to regulatory criteria.

Discussion & Conclusions

The major challenge in this nAb method development was to overcome a very severe drug interference in a very sensitive nAb detection format. Three different approaches to remove excess sample drug were tested:

1. PandA, where nAb-drug complexes would be precipitated with PEG and excess free drug would remain in solution;
2. nAb enrichment, where nAbs would be captured by excess biotinylated drug coated on paramagnetic particles, and the competing excess free drug would remain in solution;
3. Drug depletion, where the excess free drug would be captured by excess biotinylated positive control coated on paramagnetic particles, and nAbs would remain in solution.

Of these, only the drug depletion approach worked after changing the order of acidification and depletion, such that first the excess free drug is depleted with the beads, and then the remaining nAb-drug complexes are dissociated by acidification and the residual free drug is outcompeted by the SulfoTag-drug during the neutralization step.

In conclusion, our study highlights the importance of extensive assay format comparison and optimization in order to achieve the impossible - a nAb assay with both high sensitivity and high drug tolerance. The developed bioanalytical method allows the reliable detection of critical nAbs and the subsequent safety and efficacy assessment of a novel drug in an area of unmet medical need.

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.