Development of an Immunogenicity Assay Using Gyrolab Platform for a Therapeutic Monoclonal Antibody (Mab)

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Purpose
Anti-Drug Antibody (ADA) assay development for therapeutic monoclonal antibodies (Mabs) against soluble targets can be challenging. Typically, ADA is assessed using a bridging immunoassay, however the target molecules may generate target mediated bridging, resulting in false positive assay responses. Identifying the right blockers to minimize such interferences can be time consuming and expensive. The purpose of this study was to explore ADA assay development using the Gyrolab technology. Our goal was to reduce the amount of target blockers used in the assay without sacrificing the sensitivity, drug tolerance (DT) and ligand tolerance (LT) when compared to an assay developed in the Mesoscale (MSD) platform.

Methods
The Gyros method uses Drug labeled with Biotin and Alexa Fluor® 647. Standards and controls (QCs) prepared using a positive control (PC) in human serum were treated with acid, then neutralized with master mix (2 µg/ml of each labeled drug + blockers) containing Tris. The samples were analyzed using the Gyros. A similar assay was developed using MSD. Raw data was analyzed using SoftmaxPro with a cut point of 1.5 to calculate sensitivity, DT and LT.

Results
The ADA assay requires two different blockers (Blockers A & B; at 100 µg/ml of each) to attain acceptable LT. With the Gyros, we were able to reduce the reaction volume 4-fold and thus reduce the amount of blockers required. The LT for the Gyros was 229 ng/ml compared to 193 ng/ml using the MSD. The DT for the Gyros platform was 457 µg/ml, compared to 1011 µg/ml using the MSD. The sensitivity for Gyros was 8.7 ng/ml compared to 1.7 ng/ml using the MSD.

Conclusion
The Gyros platform appears to be an acceptable alternative to the MSD platform for assessing immunogenicity. The LT was comparable between the two platforms. The MSD had a slight advantage over Gyros for the DT, mainly due to better sensitivity with the MSD assay, and although the Gyros assay was less sensitive compared to the MSD, it was well above the industry guidance for a human ADA assay. The major advantage of the Gyros was the reduced master mix volume. This is particularly important when expensive blocking reagents are required resulting in significant cost benefits when compared to the MSD platform.