Immunogenicity Assay Development on the Gyrolab Platform: Increasing Sensitivity and Drug Tolerance
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Purpose
One of the major challenges for immunogenicity assessment is drug interference as residual drug in study samples interferes with anti-drug antibody (ADA) detection and may lead to a false negative result. One way to reduce drug interference is to treat samples by acid dissociation prior to the ADA assay. Gyrolab is an automated micro fluidic-based nanoliter-scale platform that has transformed immunoassay development. It has a new ADA assay module that automates acid dissociation and the subsequent ADA assay. We have evaluated this new module and developed an automated ADA assay for cynomolgus monkey PK studies.

Methods
An antibody drug was labeled with biotin and DyLight-650 according to the Gyrolab protocol. The concentrations of capture and detection reagents were optimized using an affinity purified rabbit polyclonal anti-drug antibody as a positive control. Different assay parameters were further tested and optimized including pH of acidic/neutralizing buffers, dissociation and neutralization incubation time, and sample dilution factor using study samples. Both homogeneous and semi-sequential assay formats were tested.

Results
Both homogeneous and semi-sequential assay formats worked well using a positive control spiked sample. However, the semi-sequential format with an automated acid-dissociation step had a better CV (<20% vs up to 138% in the homogeneous format) with cynomolgus monkey study samples and was chosen as the final format. It reduced assay time to 1 hour from the conventional 1-2 days with a separate acid dissociation step. Compared to the bridging MSD assay, the Gyros assay could detect ADA as early as in day 10-11 cyno PK samples with drug levels about 200 ug/ml.

Conclusion
An automated ADA assay was developed on Gyrolab with both increased sensitivity and drug tolerance compared to the MSD assay. This assay was successfully used to support cynomolgus monkey PK studies and resulted in early detection of ADA.