Multiparameter Comparison of PK Immunoassay for a Therapeutic Monoclonal Antibody Implemented on Four Different Platforms
A. Kozhich, F. Zambito, Y. Zhang, B. DeSilva
Bristol-Myers Squibb Company

Purpose
Pharmacokinetic immunoassays for a therapeutic monoclonal antibody (TMA) were developed on four different platforms: Meso Scale Discovery (MSD), chemiluminescent ELISA (CLE), Gyros, and OptimizerTM (a microfluidic 96-well plate with fluorescence readout). We compared analytical performance, operational requirements and cost.

Methods
Unlabeled (OptimizerTM) or biotinylated mouse anti-idiotype mAb (MSD, CLE, Gyros) were used as capture reagents. Goat anti-ID polyclonal IgG was labeled with HRP (OptimizerTM, CLE), SulfoTAG (MSD) or Alexa 647 (Gyros) for direct detection, or with biotin for two-step detection followed by streptavidin-HRP (OptimizerTM). Standard curve and QC samples were prepared in pooled cynomolgus monkey serum. MSD and CLE assays were performed manually, OptimiserTM on a Tecan Freedom EVO automated workstation, and the Gyros assay on the Gyrolab automated workstation. Parameters were tested on each platform: coating buffer, assay buffer, minimum required dilution of samples (MRD), concentrations of capture and detection antibodies. Required assay time and cost of assay materials were approximate estimates.

Results
At 20-fold MRD, the TMA PK assay ranges in monkey serum were 20-10,000 ng/mL (MSD, CLE, Gyros) and 3.5-2500 ng/mL (OptimizerTM). Two-step detection in combination with 4X sample loading decreased the Optimizer TM LLOQ to 2.5 ng/mL. While assay precision and accuracy across standards were within expected CV of < 20% for ligand binding assays, they were higher for CLE and OptimizerTM than for MSD and Gyros. Assay times were 4-5 hr for MSD and CLE, 2 hr for OptimizerTM and 1 hr for Gyros. Reagent consumption was similar for MSD and CLE assays, but significantly less was used for Gyros and OptimizerTM assays. While the Gyros assay was completely automated, the OptimizerTM assay required manual plate reading and the MSD and CLE assays were fully manual but automatable. Materials cost was highest for Gyros, lower for MSD and OptimizerTM and the lowest for CLE.

Conclusion
Four immunoassay platforms were compared for successful TMA PK immunoassay development. Various assay parameters such as assay range, assay time, reagent consumption, material cost, automation and instrument availability should be taken into account for informed selection of assay platform.