Biomarker Quantitation on GyroLab Platform: A Case for a Two Step Assay
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Purpose
A clinical biomarker was readily quantitated on the GyroLab platform when its reference material was spiked in buffer; however, the assay had difficulty detecting endogenous analyte in matrix. Although the capture antibody’s affinity for the biomarker was much higher than that of its binding partners in matrix, it was theorized that perhaps the standard three step Gyros assay format did not allow sufficient opportunity for the antibody and biomarker to reach binding equilibrium. A two step assay, which allows for a pre-incubation of capture antibody and sample, was investigated to determine if this phenomenon was in fact driven by kinetic equilibrium.

Methods
All standard and QC samples were prepared with reference standard spiked into buffer. Subsequently all samples, including unknowns, were diluted 1:2 into sample diluent containing capture antibody at a concentration equal to that of the highest standard curve point. These sample cocktails were incubated for two hours at room temperature with gentle shaking on a loading plate. After two hours, detection antibody and wash were added to the plate and the assay was run on the GyroLab platform using Bioaffy 1000 discs to maximize sensitivity and a two step needle wash to minimize carryover. Both capture and detection antibodies were polyclonal, thereby increasing the odds of quantifying all three species of the biomarker.

Results
Endogenous biomarker was detected with the two step assay in a reproducible fashion. Evaluation of several matrix samples that had previously been BLQ on the three step assay yielded a group mean of 814 ng/mL. Qualification work showed day to day and disc to disc precision for a handful of control charted matrix samples to be less than 13%. The qualified calibration range for the assay was 31.3 ng/mL to 8000 ng/mL, with the limits of quantitation established at 62.5 ng/mL (LLOQ) and 3000 ng/mL (ULOQ).

Conclusion
Although there is never a one size fits all solution, our results show that a two step GyroLab assay can address assay equilibrium issues. Also, multiple species biomarker quantitation may be enhanced using polyclonal capture and detection antibody schemes.