Development and Validation of a Pharmacokinetic Assay on the Gyrolab Platform for Use in Phase II/III Clinical Studies

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Purpose
Pharmacokinetic sample analysis for large-scale, late phase clinical studies requires significant time and resources to perform. To maximize efficiency, we developed and validated a highly automated ligand binding assay (LBA) to measure farletuzumab (a humanized monoclonal antibody) using the Gyrolab platform, and implemented a high-throughput testing strategy requiring minimal staffing and analysis time.

Methods
The Gyrolab platform was selected due to its relatively high-throughput and level of automation. The method developed is a stepwise sandwich format with a biotin labeled anti-farletuzumab F(ab’), for capture and Alexa Fluor labeled anti-farletuzumab for detection of the free form of farletuzumab. Given the nature of the assay format and its intended use, critical factors including lot-to-lot variability of labeled detection reagents and sample carry-over were considered during development. In addition to standard LBA method validation parameters, sample stability at ambient temperature during on-instrument processing was evaluated, as well as robustness of the critical reagent labeling process.

Results

Since higher throughput was prioritized over sensitivity for a late phase clinical study, Bioaffy 200 CDs were used due their larger sample capacity. The resulting LBA has a quantification range of 0.4 -16 g/mL at an MRD of 1:10. A 5-parameter regression model was used for the standard curve and QC samples were established at 0.8, 2 and 6 g/mL. Method validation confirmed suitable assay performance for regression model fit, selectivity, accuracy, precision, MRD, quantification range, dilutional linearity, negative prozone, sample stability and assay robustness.

Validation Results

Comparison of 3 lots of Alexa-Fluor Labeled Detection Antibody. Each lot prepared by a different analyst

Sample Carry Over Evaluation

Experimental Design
Each needle samples down the plate from row A to G
Perform assay as usual with 8 standards, duplicate QCs and a blank
Alternate high and low samples for each needle.

Results
% Difference in concentration was calculated for each sample rows C to G from original sampling (Row B). No accumulation of analyte is observed

Development Considerations
Reagent Lot Variability
Reduced lot to lot variability of Alexa-Fluor labeled reagents
- Use pre-packed desalting columns to reduce column variability
- Use a BCA kit to perform protein quantification
- Verify consistent labeling by performing A280
Acceptable reagent will have 4-11 moles of dye per molecule

Methods
Once standards, QCs and samples are prepared off-line, since higher throughput was prioritized over sensitivity for a late phase clinical study, Bioaffy 200 CDs were used due their larger sample capacity. The resulting LBA has a quantification range of 0.4 -16 g/mL at an MRD of 1:10. A 5-parameter regression model was used for the standard curve and QC samples were established at 0.8, 2 and 6 g/mL.

Methods
- A 5-parameter regression model was used for the standard curve
- QC samples appear in red

Validation Results (Cont.)

Additional Platform Specific Validation Parameters
- Critical Reagent lot variability
- assay performs well with multiple lots of critical reagents
- Sample Stability during processing
- Diluted sample stability confirmed for 20 hours
- Bioaffy 200 stability
- Opened Bioaffy CD stored at 2-8°C.

Caveats:
- High throughput requires significant data processing
- Trending of standards and QCs indicate highly consistent and accurate method performance
- Over 16,000 valid sample results generated in approximately 6 months
- Good in-study intermediate precision across multiple analyst/stays, reagent lots, consumables
- Negligible analyte carry-over determined in method validation, monitored through in-study data trending

Platform Assessment

- 450 runs performed, 95.6% passing rate.
- Trending of standards and QC's indicate highly consistent and accurate method performance.
- Over 16,000 valid sample results generated in approximately 6 months
- Good in-study intermediate precision across multiple analyst/stays, reagent lots, consumables
- Negligible analyte carry-over determined in method validation, monitored through in-study data trending

Conclusion

Once standards, QCs and samples are prepared offline, approximately 200 samples can be analyzed in five hours. While the first set is being analyzed in the instrument, a second set of samples can be prepared for analysis on the same day, resulting in over 400 sample values per day. To date, assay performance has been highly consistent with an overall passing rate of 95.6% and a total of 430 valid runs, resulting in over 16,000 valid PK results. Trending analysis of the valid runs indicates good precision for the standard calibrators (≤13%).