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

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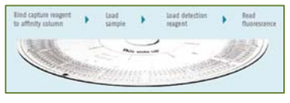
## Purpose

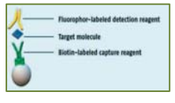
Pharmacokinetic sample analysis for large-scale, late phase clinical studies requires significant time and resources to perform. To maximize efficiency, we developed and validated a highly automated ligand binding assay (LBA) to measure farletuzumab (a humanized monoclonal antibody) using the Gyrolab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

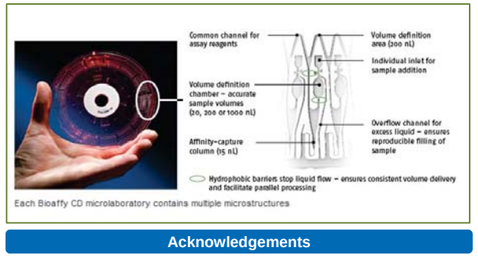
## Methods

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-farletuzamab $F(ab)_2$ for capture and Alexa Fluor labeled anti-farletuzamab 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.

## Gyrolab Platform Overview




**Acknowledgements**

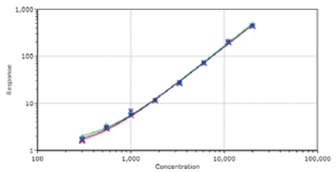
Gyrolab images courtesy of Gyros

## 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




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

### Sample Carry Over Evaluation

**Sampling Lay Out**

| Needles | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|
| A | STD 1 | STD 2 | STD 3 | STD 4 | STD 5 | STD 6 | STD 7 | STD 8 |
| B | Blank | HQC | HQC | MQC | MQC | LQC | LQC | ULOQ |
| C | ULOQ | ULOQ | ULOQ | ULOQ | ULOQ | ULOQ | ULOQ | ULOQ |
| D | LQC | LQC | LQC | LQC | LQC | LQC | LQC | LQC |
| E | ULOQ | ULOQ | ULOQ | ULOQ | ULOQ | ULOQ | ULOQ | ULOQ |
| F | HQC | HQC | HQC | HQC | HQC | HQC | HQC | HQC |
| G | Blank | Blank | Blank | Blank | Blank | Blank | Blank | Blank |

**% Difference from original sampling**

| Needles | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|
| A | | | | | | | | |
| B | | | | | | | | |
| C | -1.1 | -0.1 | -4.1 | 2.5 | 3.1 | 3.0 | 7.6 | 1.0 |
| D | -4.2 | 3.9 | 0.2 | -3.0 | -2.1 | -2.5 | -2.2 | 0.5 |
| E | -1.7 | 0.5 | -0.1 | -5.2 | 1.3 | -2.6 | 4.4 | -3.4 |
| F | -2.7 | -6.0 | -3.8 | -0.2 | -2.9 | -4.6 | -11.0 | -1.8 |
| G | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

**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**

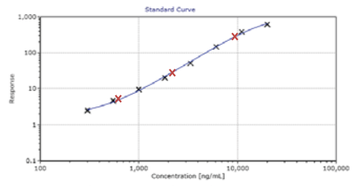
- % Difference in concentration was calculated for each sample rows C to G from original sampling (Row B)
- No accumulation of analyte is observed

## 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 -16μg/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 8μg/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

### Representative Standard Curve




QC samples appear in red

- Assay parameters include MRD of 1:10
- Assay range of 0.4μg/mL-16μg/mL
- QCs at 0.8, 2 and 8μg/mL.

**Standard Curve Analysis from 17 Validation Runs**

| | STD1 | STD2 | STD3 | STD4 | STD5 | STD6 | STD7 | STD8 |
|---|---|---|---|---|---|---|---|---|
| Nominal Conc. (μg/mL) | 20 | 10.99 | 6.04 | 3.32 | 1.8 | 1 | 0.55 | 0.3 |
| Mean Concentration | 19.2 | 11.9 | 6.1 | 3.1 | 1.8 | 1.1 | 0.6 | 0.3 |
| % Recovery of Mean | 96.1 | 108.4 | 100.7 | 94.6 | 98.9 | 106 | 110.9 | 86.7 |
| Cumulative % CV | 0.8 | 1.4 | 1 | 1 | 1.7 | 1.7 | 3.2 | 5 |

**Prozone Assessment – No Prozone Detected**

| Nominal Concentration (μg/mL) | Dilution Level | Mean Signal | ULOQ Mean Signal |
|---|---|---|---|
| 100 | 10 fold | 730 | 636 |
| 25 | 40 fold | 671 | |

**Dilutional Linearity – 1mg/mL MORAb-003 in pooled serum**

| Nominal Conc. (μg/mL) | Dilution Level | Measured. Conc. (μg/mL) | Back Calc. Conc. (μg/mL) | % Bias | Cumulative % CV |
|---|---|---|---|---|---|
| 12.5 | 80 fold | 14.1 | 1128 | 12.8 | 7.0 |
| 6.3 | 160 fold | 6.5 | 1032.0 | 3.2 | |
| 3.13 | 320 fold | 3.0 | 947.2 | -5.3 | |
| 1.56 | 640 fold | 1.5 | 966 | -3.4 | |
| 0.78 | 1,280 fold | 0.8 | 1003.5 | 0.4 | |

## Validation Results (Cont.)

**Validation Precision and Accuracy Analysis**

| Concentration (ng/mL) | n | Mean | SD | %CV | % Recovery | |
|---|---|---|---|---|---|---|
| 16,000 | 2 | 16925.0 | 272.3 | 1.7 | 105.8 | Pooled Repeatability |
| | 12 | 16925.0 | 730.0 | 4.6 | 105.8 | Intermediate |
| 8,000 | 2 | 8541.7 | 96.0 | 1.2 | 106.8 | Pooled Repeatability |
| | 12 | 8541.7 | 613.0 | 7.7 | 106.8 | Intermediate |
| 2,000 | 2 | 1942.5 | 34.0 | 1.7 | 97.1 | Pooled Repeatability |
| | 12 | 1942.5 | 157.1 | 7.9 | 97.1 | Intermediate |
| 800 | 2 | 861.8 | 8.1 | 1.0 | 107.7 | Pooled Repeatability |
| | 12 | 861.8 | 93.2 | 11.6 | 107.7 | Intermediate |
| 500 | 2 | 485.7 | 13.5 | 2.7 | 97.1 | Pooled Repeatability |
| | 12 | 485.7 | 70.4 | 14.1 | 97.1 | Intermediate |
| 400 | 2 | 386.3 | 11.0 | 2.8 | 96.6 | Pooled Repeatability |
| | 12 | 381.9 | 77.9 | 19.5 | 95.5 | Intermediate |

**Selectivity/Specificity Analysis**

| | | |
|---|---|---|
| 20 Individuals (3 disease states and normal serum) | MORAb-003 Concentration | % Passing |
| | 80μg/mL | 100 |
| | 8μg/mL | 100 |

### 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, stability confirmed for 10 days

## High Throughput Workflow


- 410 samples processed per day or 2050 samples per week.
- Developed and Validated an Excel spreadsheet for more efficient run evaluation and sample assessment

## Conclusion

Once standards, QCs and samples are prepared off-line, 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 (≤4.7%) and QC samples (≤13.1%).

## Assay Performance Statistics

**Standard Curve Analysis from 430 Runs**

| | STD1 | STD2 | STD3 | STD4 | STD5 | STD6 | STD7 | STD8 |
|---|---|---|---|---|---|---|---|---|
| Nominal Conc. (μg/mL) | 20 | 10.99 | 6.04 | 3.32 | 1.8 | 1 | 0.6 | 0.3 |
| Mean Concentration | 19.3 | 12.0 | 6.1 | 3.2 | 1.8 | 1.1 | 0.6 | 0.3 |
| % Recovery of Mean | 96.6 | 109.5 | 100.3 | 94.9 | 98.2 | 106.6 | 107.8 | 89.6 |
| Cumulative % CV | 1.3 | 2.8 | 1.9 | 1.8 | 2.2 | 2.3 | 4.7 | 4.3 |

**QC Analysis from 430 Runs**

| | HQC1 | HQC2 | MQC1 | MQC2 | LQC1 | LQC2 |
|---|---|---|---|---|---|---|
| Nominal Conc. (μg/mL) | 8 | | 2 | | 0.8 | |
| Mean Concentration | 8.4 | 8.3 | 1.9 | 1.9 | 0.84 | 0.86 |
| % Recovery of Mean | 105.3 | 104.2 | 96.5 | 96.3 | 106.1 | 106.9 |
| Cumulative % CV | 6.4 | 6.3 | 13.1 | 7.0 | 6.8 | 6.1 |

## Platform Assessment

- 450 runs performed, 95.6% passing rate.
- 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 analysts/days, reagent lots, consumables
- Negligible analyte carry-over determined in method validation, monitored through in-study data trending
- Caveats:
  - High throughput requires significant data processing
  - Requires frequent system maintenance (trained user & vendor field personnel) to sustain performance/throughput
  - Biological sample matrices require centrifugation step
  - Limited user customizations during method development
  - High cost of consumables