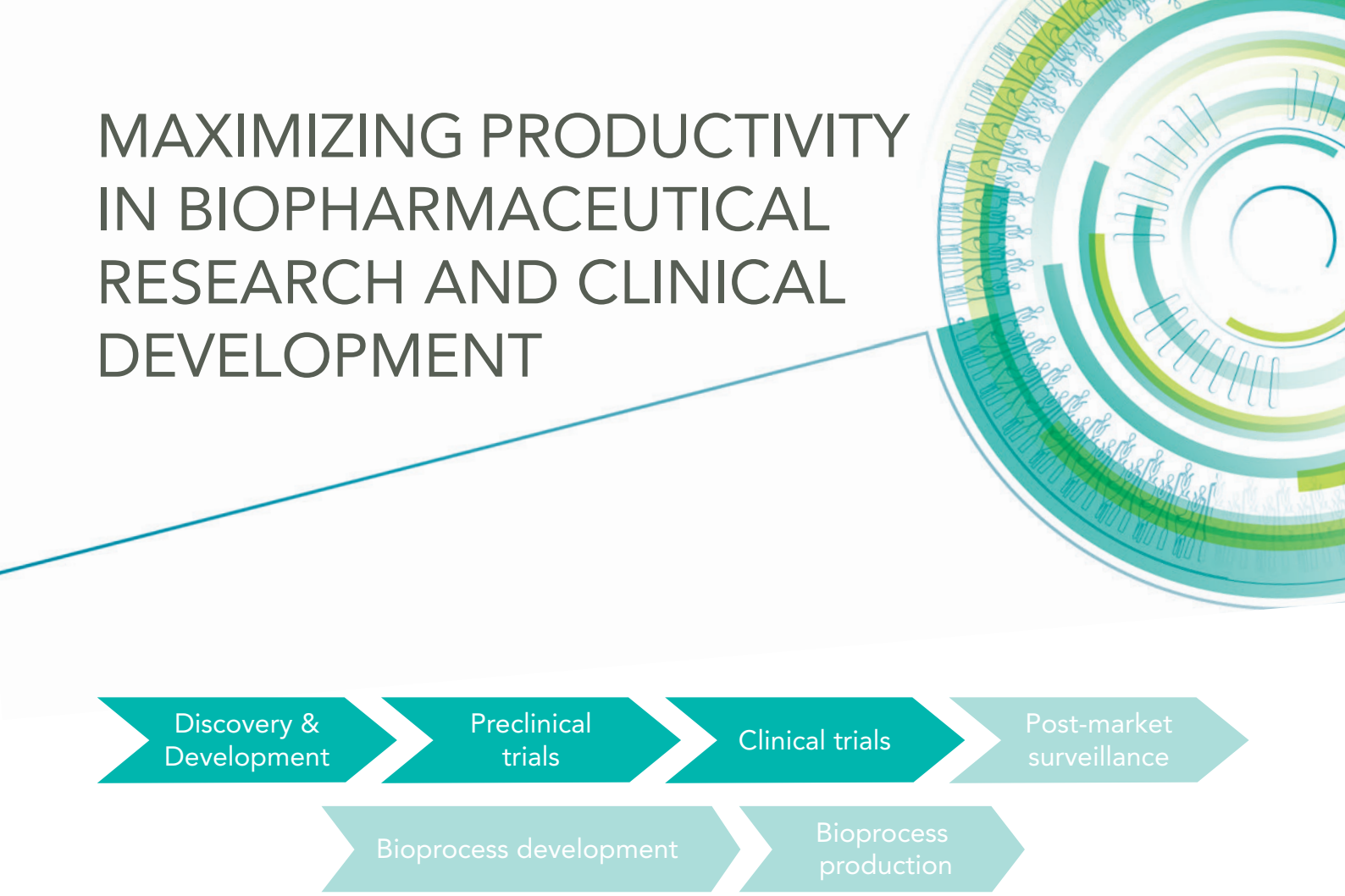
# MAXIMIZING PRODUCTIVITY IN BIOPHARMACEUTICAL RESEARCH AND CLINICAL DEVELOPMENT




## THE BIOPHARMACEUTICAL CHALLENGE

The cost and effort of bringing biopharmaceutical drugs to market has escalated dramatically. The pharma industry is therefore continually looking for new ways to identify safer, more efficacious drug candidates earlier, with less effort and cost, and advance them onto the market sooner.

## THE NEED FOR HIGH-PERFORMANCE ASSAYS

One focus area for time-to-market improvement is the development of analytical methods, such as ELISA, that deliver high quality results in a timely manner to enable rapid and confident data-driven decision-making. Some requirements of the analytical method may depend on the study phase, from discovery and preclinical to Phase II/III, but the primary requirements for analytical methods are the same throughout biopharma R&D:

- High quality data for confident decisions
- Fast turn around time to results
- Rapid assay development
- Robust assays that are readily validated to meet regulatory demands and are also easy to transfer between sites and partners (pharma and CRO)
- Low sample consumption to generate more data
- Reduced use of resources (hands-on time and reagents)
- High throughput when it counts

## THE VALUE OF AUTOMATED, NANOLITER SCALE IMMUNOASSAYS

Gyrolab® systems meet the needs of many biopharma companies that apply immunoassays throughout the development process of biotherapeutics and biosimilars. Gyrolab technology also provides rapid assay development time, automation, and a broad dynamic range that minimizes dilutions and repeats.

We present three examples that show how Gyrolab systems contribute to achieving the overall goal—to get safer, more efficacious biopharmaceutical drugs onto the market earlier and with less cost and effort.

**Case study 1:** Obtaining an entire PK profile from a single mouse

**Case study 2:** Earlier and more sensitive detection of drug-induced kidney toxicity in preclinical studies

**Case study 3:** Ramping up Phase II/III PK studies to meet critical deadlines

# **CASE STUDY 1:** OBTAINING AN ENTIRE PK PROFILE FROM A SINGLE MOUSE

Preclinical pharmacokinetic (PK) studies using mice are limited by the small sampling volume per animal. PK time courses have traditionally involved composite sampling, with individual time points taken from different animals to provide sufficient sample volume for bioanalytical analyses. The biological variability results in large data variation. In addition, this approach involves high costs, large consumption of valuable drug candidate and, not least, the ethical consequences of excessive use of research animals—an aspect that is increasingly important under the 3Rs (Reduce, Refine, Replace) initiative.

An assay that consumes nanoliter volumes provides major benefits in preclinical work by enabling serial sampling from individual mice, allowing use of fewer animals per study and generate better data for more analyses using less material. Allison Joyce and her colleagues at Pfizer tested how low sample consumption using Gyrolab technology could help them in their preclinical PK studies[1]. They compared serial sampling and composite sampling to generate a PK time concentration profile of human IgG control antibody in mice, and also tested serial sampling in two studies:

1. Ranking six potential drug candidates using PK profiles of precious drug material in the discovery phase
2. A PK study using an expensive genetically-engineered mouse model

Their serial sampling technique involved taking just 10 µL of whole blood from the tail vein of individual mice at up to 12 sequential time points. In contrast, the traditional sampling method involved either euthanasia followed by sampling 700 µL blood from the cardiac ventricle, or retro-orbital sampling under anesthetic and only once for each eye.

## COMPARABLE PK PROFILES

The study showed that serial and composite sampling methods gave comparable results for PK profiles of human IgG control antibody and that there were no matrix or sampling-site effects on drug concentrations.

## BETTER DATA

The value of serial sampling was clearly demonstrated in Studies 1 and 2, by low inter-subject variability (CV% 9–50% and 15–40%, respectively), compared to variations in the range 5–150% for PK studies in their laboratory that involved traditional composite sampling.

## FEWER ANIMALS. LESS MATERIAL. LESS TIME

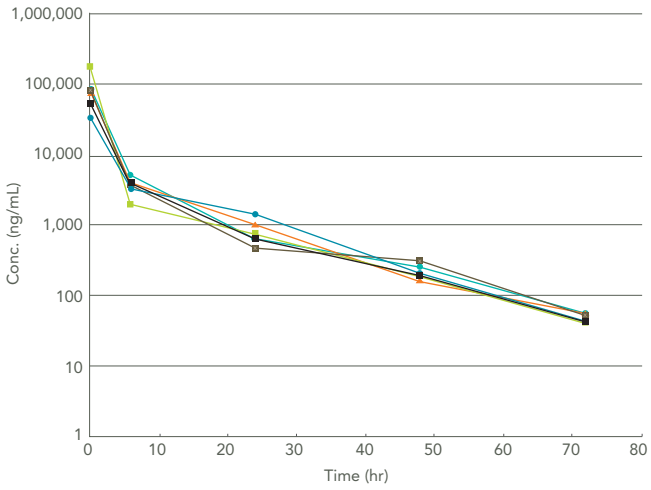
The low sample consumption required by Gyrolab system enabled serial sampling, which in turn considerably reduced the number of animals and material consumption compared to composite sampling:

**Study 1:** 36 mice instead of 180
75% savings in drug candidate
40% savings in length of time for in-life study

**Study 2:** 80% fewer individuals of a precious animal model

Finally, Joyce and her colleagues appreciated other benefits of Gyrolab systems: the short assay time, automation to save analyst time, broad dynamic range that minimized repeats, and low reagent consumption compared to traditional ELISA.

This strategy is now implemented at Pfizer for preclinical PK studies.




*Serial sampling generated very similar PK profiles for individual animals (profiles for one potential drug candidate in Study 1). Adapted from Figure 4a, Joyce et al, 2014[1]*

*[1]One mouse, one pharmacokinetic profile: quantitative whole blood serial sampling for biotherapeutics. Joyce AP, Wang M, Lawrence-Henderson R, Filliettaz C, Leung SS, Xu X, O'Hara DM. Pharm Res. 2014 Jul;31(7):1823-33*

# **CASE STUDY 2:** EARLIER AND MORE SENSITIVE DETECTION OF DRUG-INDUCED KIDNEY TOXICITY IN PRECLINICAL STUDIES

The main reason most drug candidates fail to reach the market is toxicity, not poor efficacy. Yet despite astronomical costs and high attrition rates in preclinical trials, drug approval doesn't guarantee safety—kidney damage due to adverse drug reactions remains a major cause of death and kidney disease among patients initially treated for other conditions.

In preclinical nephrotoxicity assessment, rat urinary KIM-1 and clusterin are among the most relevant biomarkers, detecting toxicity at lower levels and earlier in the onset of kidney damage than current serum standards, creatinine and blood urea nitrogen (BUN). Investigators at Merck Serono determined whether rat urinary KIM-1 and clusterin could be used to detect sub-acute drug-induced kidney damage. They measured the urinary protein biomarkers using GyroMark™ HT kits from EMD Millipore* run on Gyrolab xP workstation and performed immunohistochemistry (IHC) in a 28-day rat study using vancomycin as the toxicant.

## HIGH PERFORMANCE BIOMARKER ASSAYS

The GyroMark HT KIM-1* and clusterin kits* deliver robust data over a broad dynamic range and with low sample dilution, ensuring high sensitivity.

| | KIM-1 | CLUSTERIN |
|---|---|---|
| Standard Range (ng/mL) | 0.015-60 | 0.7-3000 |
| Sensitivity (LLOD, ng/mL) | 0.01 | 0.36 |
| Spiked Recovery | 100% | 93% |
| Dilution Linearity | 107% | 108% |
| Intra-Assay Precision | <10% | <10% |
| Inter-Assay Precision | <15% | <15% |
| Sample dilution | 1:2 | 1:10 |

*Data generated by Jehangir Mistry and colleagues at EMD Millipore*

## SETTING THE BASELINE

The baseline study included histopathology studies and IHC staining for KIM-1 on all animals to correlate and confirm kidney damage. Levels of the traditional biomarkers, blood-urea nitrogen and serum creatinine, also increased significantly in males and females after 8 days at the high vancomycin dose, including a dip in the recovery phase. No changes were seen in low dose animals.

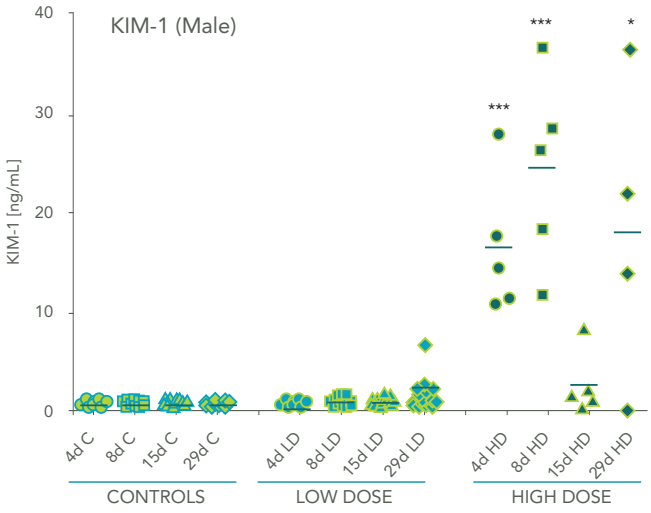
**The GyroMark HT KIM-1 and clusterin kits from EMD Millipore have been discontinued. Gyrolab Assay protocols for KIM-1 and Clusterin are available at www.gyrosproteintechnologies.com/Gyrolab Assays*

## FAST DATA GENERATION

The study design involved 120 animals and 120 one-microliter samples, run in duplicate or triplicate, thus generating hundreds of data points. Using Gyrolab xP workstation, the research team was able to analyze all the samples in a single day.

## EARLIER DETECTION AND GREATER DIAGNOSTIC VALUE

The results from assays using GyroMark HT KIM-1 and clusterin kits correlated well with levels of the traditional biomarkers for both males (see figure below) and females (results not shown), with significant changes being detected after only 4 days of exposure. Receiver-Operating Characteristic (ROC) analysis of urinary proteins confirmed that KIM-1 and clusterin indeed have great diagnostic value. The area under the curve (AUC) was used as a measure of the overall ability of the biomarker to discriminate animals without histopathological findings in kidney from those with signs of tubular damage. KIM-1 had an AUC of 0.90–0.95 and clusterin 0.94–0.98, for males and females, respectively.




*The assay using GyroMark HT KIM-1* showed significant changes after only four days of exposure of male rats to a high dose (4d HD) of vancomycin, with an expected drop during the recovery phase at day 15 (C, Control; LD, Low Dose; HD, High Dose).*

The study confirmed the high accuracy and predictivity of rat urinary KIM-1 and clusterin as biomarkers to detect sub-acute nephrotoxicity when treated with vancomycin.

# **CASE STUDY 3:** RAMPING UP PHASE II/III PK STUDIES TO MEET CRITICAL DEADLINES

The transition from Phase I into Phase II/III clinical trials generally involves significant increases in sample numbers and severe pressure to rapidly generate PK data with fully validated assays to meet tight deadlines. Immunoassays must be robust, use little sample, demand minimal hands-on, and run on a platform that can deliver the high throughput required.

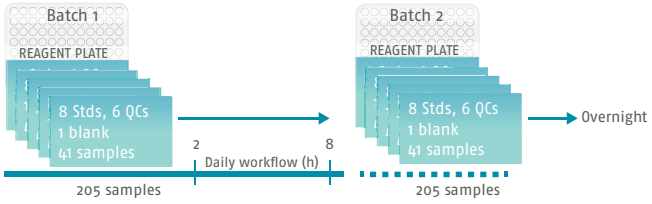
The Bioanalytical Development group at Morphotek, USA, faced the task of running a pharmacokinetic (PK) assay for an investigational therapeutic antibody on a large backlog of samples to meet the tight deadlines of a Phase III study. Their validated ELISA had limited throughput and they could see distinct advantages in transferring the assay to Gyrolab platform, which could improve sample throughput with their available resources without sacrificing assay sensitivity.

## SUCCESSFUL SYSTEM AND ASSAY VALIDATION

The team at Morphotek completed the IQ/OQ/PQ processes, and developed and validated the PK assay method. To push Gyrolab technology towards higher levels of throughput, they also made improvements to the standard protocol. The assay had a dynamic range of 0.4–16 µg/mL with a Minimum Required Dilution of 1:10, and passed their validation criteria with a %CV better than 20%, and recoveries in the range 95.5–107.7%. The assay also met their selectivity and specificity requirements when tested on 20 patient samples.

## HIGH THROUGHPUT. PASS RATE OVER 90%

The group has now performed over 650 runs and generated over 20,000 valid sample results in 12 months, with a pass rate of 94% and an Incurred Sample Reanalysis (ISR) pass rate of 96%.




*The walk-away automation of Gyrolab xP workstation enabled one analyst to run two batches of 205 samples during a working day, including an overnight run, with minimal hands-on time.*

Trending of standards and QCs indicated that the assay was highly consistent and accurate. Precision was excellent for the standard calibrators (≤5.1%) and QC samples (≤8.3%) from 664 runs (see tables). The Gyrolab assay is robust, delivering good in-study intermediate precision across multiple analysts/days, reagent lots, and Gyrolab consumables. Analyte carry-over has been negligible as determined in method development, monitored through in-study data trending.

| Standard curve Analysis from 664 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 | 19.1 | 12.1 | 6.1 | 3.1 | 1.8 | 1.1 | 0.6 | 0.3 |
| % Recovery of mean | 95.6 | 110.4 | 100.3 | 94.8 | 99.3 | 106.6 | 98.8 | 89.8 |
| Cumulative % CV | 2.9 | 2.9 | 1.2 | 1.8 | 1.8 | 2.2 | 5.1 | 4.6 |

| QC Analysis from 664 runs | | | | | | |
|---|---|---|---|---|---|---|
| | HQC1 | HQC2 | MQC1 | MQC2 | LQC1 | LQC2 |
| Nominal Conc. µg/mL | 8 | | 2 | | 0.8 | |
| Mean Concentration | 8.48 | 8.39 | 1.92 | 1.92 | 0.85 | 0.86 |
| % Recovery of mean | 106.0 | 104.9 | 96.1 | 96.1 | 106.8 | 107.1 |
| Cumulative % CV | 6.3 | 8.3 | 6.3 | 6.5 | 6.4 | 6.0 |

## GYROLAB SYSTEM MAXIMIZES EFFICIENCY

Shawn Fernando, Senior Researcher at Morphotek, summarizes their experience with Gyrolab technology: "The hands-off approach of Gyrolab technology allows us to maximize our productivity, allowing us to meet the demands of an expanding workload. With plate-based assays, you can read a plate every 90 seconds all day long, but the assays require hands-on attention by dedicated staff, which reduces our opportunities to multitask."

The automation that Morphotek experienced is one of a number of benefits of using Gyrolab technology in clinical PK studies, which also include broader dynamic range, low sample consumption, and robust assays with shorter time to results.



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