GYROS PROTEIN Technologies

How to get more reliable results with less effort

Case Study

Analytical results that you can depend on are crucial to success, especially in a regulated environment. For example, immunoassays that consistently deliver reliable data with high accuracy and precision are worth their weight in gold. ELISA has been the standard for immunoassays, but this situation is changing. We present here two case studies that show how a move from ELISA to Gyrolab™ assays is helping your colleagues to improve their results and lab workflows.

Case study #1: High performance analysis of Host Cell Protein impurities in bioprocess samples

Contamination of drug products by Host Cell Proteins (HCPs) affects drug product quality and may increase the risk of adverse effects in patients, which makes monitoring the removal of HCPs in drug product during bioprocess development a regulatory requirement. HCP analysis is challenging since HCPs are extremely diverse and complex in nature and can be present over a wide range of concentrations in a single sample. Added to that, the purification steps in process development lead to substantial changes in the concentrations of individual HCPs from initial purification to final product. Analytical methods must therefore cope with considerable dilution ranges and deliver the high sensitivity and dynamic range needed to ensure all samples fit into the quantification window.

Like most scientists involved in bioprocess development, Jun Heo and his colleagues at Merck Research Laboratories have regarded ELISA as the norm for HCP analysis, recognizing that reliable immunoassay data is a prerequisite for drug approval. But they were looking for an alternative method that could speed up their bioprocess development by solving the drawbacks of ELISA, including limited dynamic range and variable results, a laborious workflow, and consumption of large amounts of reagent. They therefore decided to evaluate the performance of Gyrolab technology and compare it with ELISA for the measurement of CHO HCPs in in-process monoclonal antibody samples from typical purification processes. The results convinced them that Gyrolab assays meet the demanding needs of HCP analysis in a regulated environment.

The assay

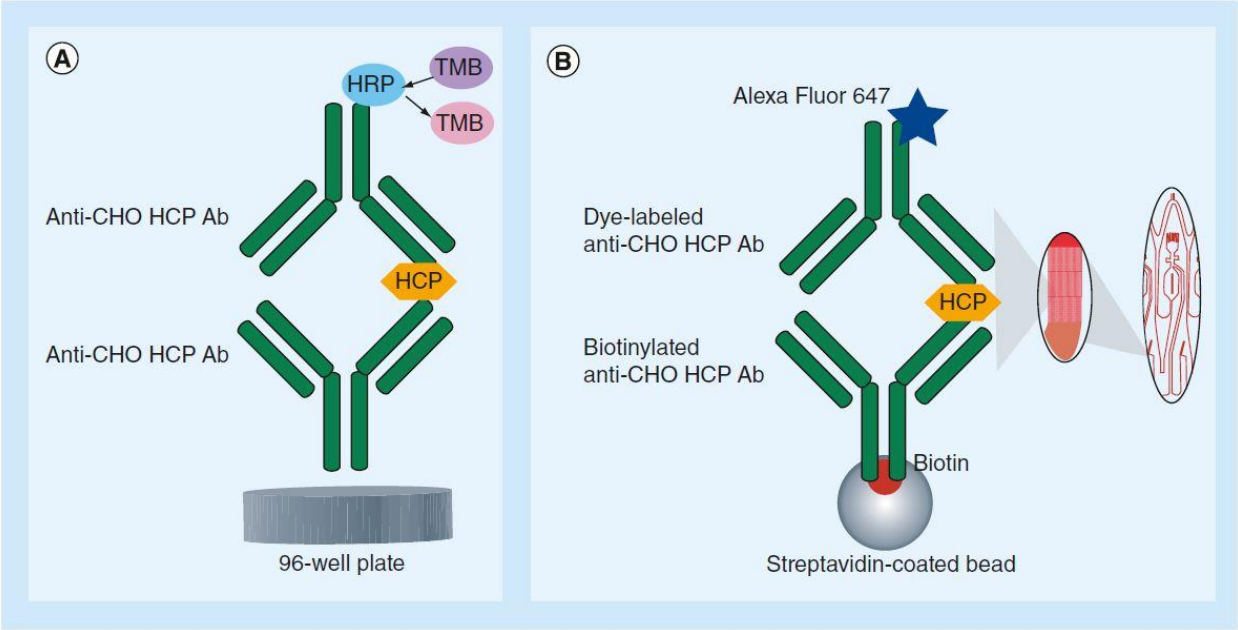

Figure 1. *Sandwich assay for ELISA (A) and Gyrolab assay (B) (Figure 1, Heo et al, 2014).*

A broad dynamic range

The dynamic range of the Gyrolab assay was 4–10,000 ng/mL, which was 100 times broader than that of their ELISA, with the Lower Limit of Quantitation (LLOQ) set at 4 ng/mL based on pass criteria of 100 ±20 % (Fig. 2). Bias was less than 20%. This broad dynamic range of the Gyrolab assay enabled the team to assay samples in purification intermediates with HCP concentrations spanning six logs.

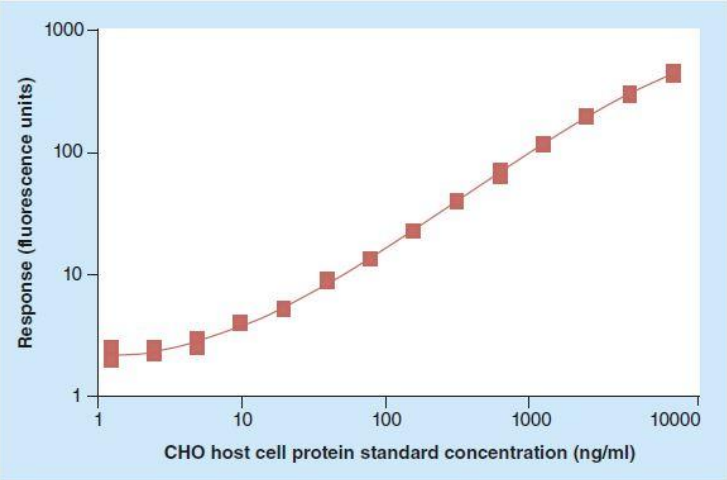

Figure 2. *The broad dynamic range of 4–10,000 ng/mL provides the flexibility needed to meet a diversity of samples for HCP analysis (Figure 5 adapted from Heo et al, 2014).*

High performance that meets regulatory demands

Regulatory authorities such as the US Food and Drug Administration (FDA) have indicated that obtaining accurate results from highly complex HCP demands assays that deliver acceptable dilutional linearity and spike recovery. In tests on dilutional linearity, samples diluted in the range x2 – x16 gave excellent results, with correlation coefficients of 0.99, and two in-process samples diluted in the range x2–x100 gave similar results (Fig. 3). Dilution-corrected HCP concentrations for all samples gave less than 10% Coefficient of Variation (CV) and the Minimum Required Dilution (MRD) could be set at 2. Spike recovery was within the variability of the assay.

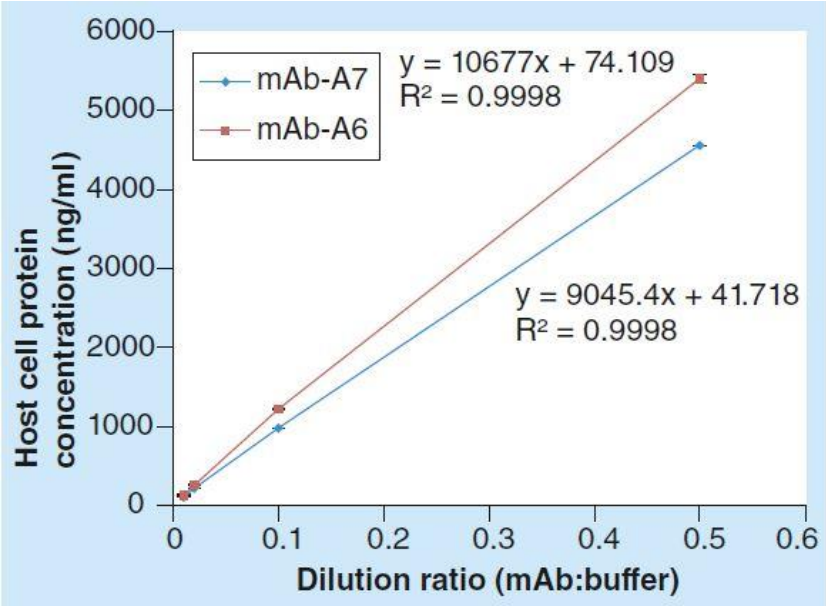

Figure 3. *The dilutional linearity of the Gyrolab assay was excellent. This figure shows results for two in-process samples diluted in the range x2–x100 (Figure 6B from Heo et al, 2014).*

As the authors pointed out, these results could be the result of the flow-through technology that minimizes matrix effects that can otherwise cause havoc when diluting samples for HCP analysis. The assay also had a high precision, with variability of less than 5% both within a CD and between CDs, and bias was better than 20%.

Comparable results with ELISA but with much less effort, reagents and sample

Confirmation that a new method under evaluation gives comparable results to the benchmark technology always instills confidence. The Gyrolab assay indeed gave comparable results to ELISA (both manual and automated) overall when tested on in-process purification samples and in fact appeared to be more sensitive in the detection of HCPs in more purified samples (Fig. 4).

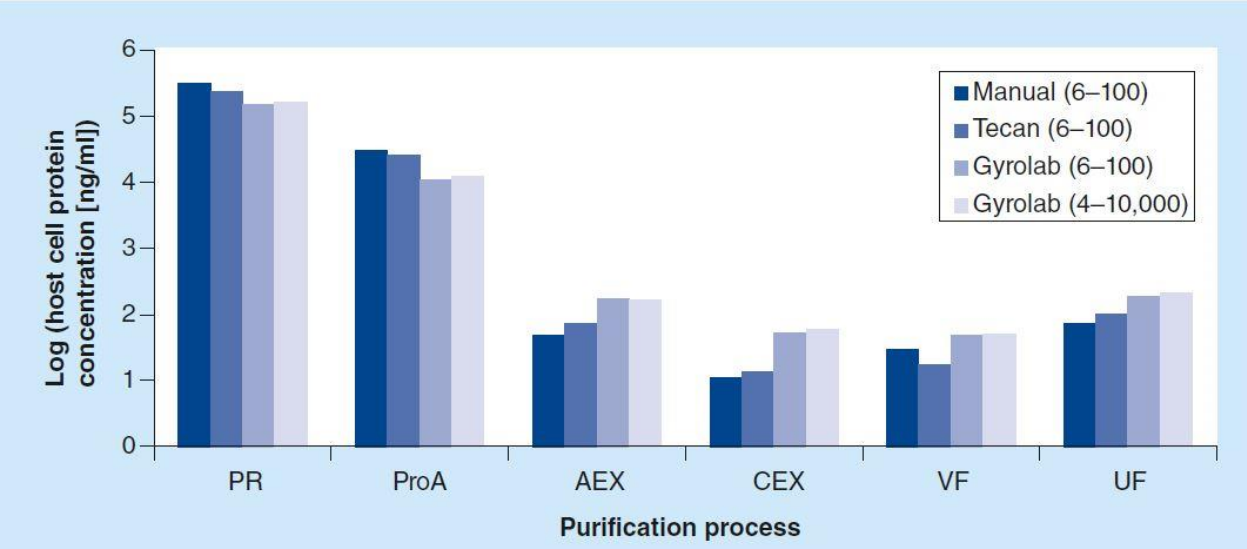

Figure 4. *The Gyrolab assay gave comparable results to manual and automated ELISA when tested on in-process samples and may be more sensitive for the detection of HCPs in highly purified samples (Figure 8 from Heo et al, 2014).*

The team confirmed that the automated Gyrolab method enabled them to ramp up sample throughput five-fold compared with ELISA, and the broader dynamic range minimized the need for dilutions and re-runs. The Gyrolab assay also consumed five times less sample and reagents, and reduced costs by half.

An assay for reliable detection of HCPs

The authors concluded that their assay demonstrated *"excellent selectivity, accuracy, and precision with in-process sample analysis. The dilutional linearity and spike recovery results suggested that Gyrolab's chromatographic mechanism minimizes the matrix effect and well-controlled microfluidics gives excellent consistency."*

The broad dynamic range (six logs) made the Gyrolab assay suitable for accurately detecting HCP levels in a large range of samples with minimum need for repeats. Compared to ELISA, the Gyrolab assay also speeded up assay development, increased throughput, reduced sample consumption, and lowered costs.

Case study #2: Fit-for-purpose analysis of therapeutic antibodies in clinical samples

ELISA has been the most commonly used immunoassay method for pharmacokinetics (PK) studies but it is often perceived as being labor intensive, consumes large amounts of critical reagents, and usually has a narrow range of quantification that results in re-runs, especially when inter-individual variability is high.

In the search for alternatives to ELISA, Liu and colleagues at QPS Delaware decided to evaluate Gyrolab technology for the determination of rituximab (MabThera®, Roche Pharmaceuticals) in human serum, including validation according to current industry guidelines for GLP-regulated immunoassays. The results gave them a lot of confidence in the assay.

The assay

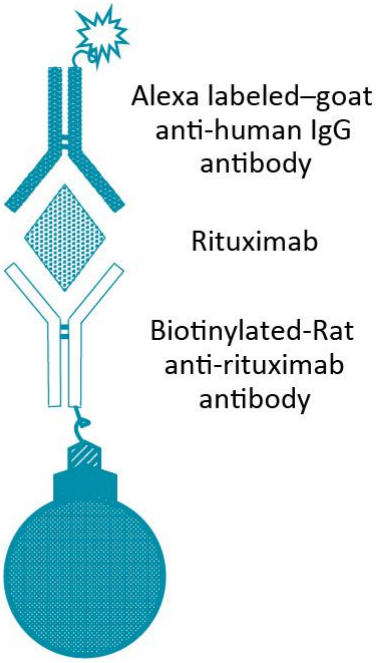

Figure 5. *The sandwich assay used by Liu and coworkers to analyze rituximab (Figure 1 from Liu et al, 2012).*

Assay performance well within acceptance limits

The Gyrolab assay delivered a dynamic range of 90–60,000 ng/mL, which was one log better than the ELISA (100–5,000 ng/mL; see Figure 6). The MRD was 30.

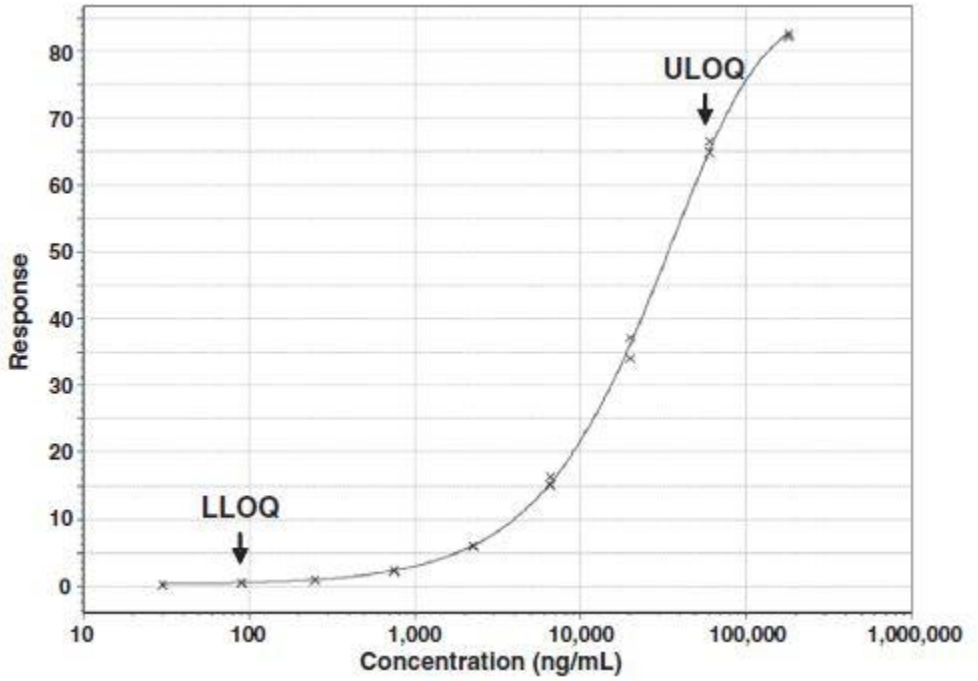

Figure 6. *The dynamic range of the Gyrolab assay was 90–60,000 ng/mL (Figure 2 from Liu et al, 2012).*

The team evaluated precision and accuracy with validation samples at five concentrations across the assay range, resulting in intra-batch precision and accuracy using spiked human serum samples of CV < 11% and mean bias < 20%. Inter-batch precision (CV) and absolute mean bias were both less than 12%, with a total error of less than 25%.

They tested the dilutional linearity of their Gyrolab assay to ensure that samples could be diluted beyond the MRD without affecting precision and accuracy. The result was a correlation coefficient of better than 0.99 after dilutions in the range 10–1,000x, and there was no hook effect (see Figure 7).

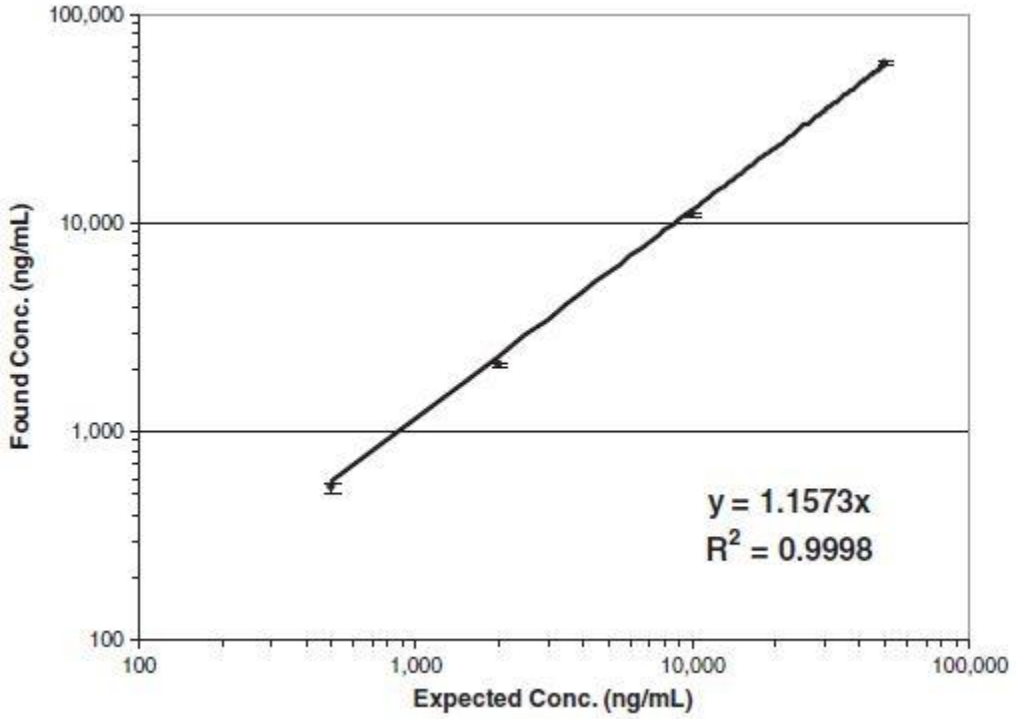

Figure 7. *The Gyrolab assay delivered excellent dilutional linearity after dilutions in the range 10–1,000 fold in human serum (Figure 4 from Liu et al, 2012).*

Assay selectivity was tested with blank sera from healthy donors and solid tumor patients. Nine of the ten healthy donors and all the tumor patient sera gave values below the LLOQ. After spiking at low and high QC levels of rituximab, eight of ten healthy sera and nine of ten patient sera gave the spike recovery value within 100±25%.

Fit-for-purpose Gyrolab assay challenges ELISA

The authors found the Gyrolab assay to be *"accurate, precise and selective, with a comparable sensitivity to the ELISA method, but provided an automated nanoscale assay with a significantly wider assay dynamic range for the determination of rituximab in human serum during pharmacokinetics/toxicokinetics studies."*

The broader dynamic range meant reduced sample dilutions and lower risk for re-assay and errors. The authors concluded that the Gyrolab assay was accurate and reliable, and met all the acceptance criteria for a fit-for-purpose assay in a 21 CFR part 11 environment.

References

A microfluidic approach to high throughput quantification of host cell protein impurities for bioprocess development. Heo J et al. Pharmaceutical Bioprocessing
April 2014, Vol. 2, No. 2, Pages 129-139.
http://www.openaccessjournals.com/articles/a-microfluidic-approach-to-high-throughput-quantification-of-host-cell-protein-impurities-for-bioprocess-development.pdf

Validation of a Gyrolab™ assay for quantification of rituximab in human serum.
Liu X et al. J Pharmacol Toxicol Methods. 2012 May-Jun;65(3):107-14.
http://www.ncbi.nlm.nih.gov/pubmed/22406436