Enhancing efficiency and economics in process development and biotherapeutics

Discover how GSK and Aragen Biosciences streamlined their processes using various techniques that produced multiple benefits, including increased throughput, improved quality control and reliable automation.

COST CONSIDERATIONS and lengthy development times for biotherapeutics have necessitated enhanced process efficiencies at all stages of drug discovery and development. Increasingly stringent regulatory requirements targeting better understanding and control of manufacturing processes are expected to positively impact product quality and performance. Quality, which begins with the determination and characterisation of critical quality attributes (CQAs) for any new biotherapeutic, is a critical step in ensuring product efficacy and safety. Once these attributes are determined, they become key parameters for assessing the quality of the product throughout its life cycle – particularly prior to commercial release and subsequent in-process testing and lot release. While a CQA should technically be dependent on the biotherapeutic in question and needs to be established specifically for the lead molecule during development, some key attributes are known to be common for most platforms. These include product specificity to target, titer, product activity or potency, cell-line inherent contaminants such as host cell proteins, and propensity to aggregation among others. The need to rapidly assess these quality attributes in a cost-effective manner therefore fuels the search for innovative analytical techniques that can provide improved performance and speed without increasing costs.

Techniques such as ELISA and high-performance liquid chromatography (HPLC) are still heavily used for different biotherapeutic attributes analysis, including titer or protein concentration analysis and aggregation profiling. They are, however, limited in some desirable capabilities such as throughput and time-to-results, among others. Biosensor-based label-free binding assays are becoming increasingly used for these quality checks as they offer some key advantages over both ELISA and HPLC.

Surface plasmon resonance- (SPR) or biolayer interferometry (BLI)-based systems allow analytical development and quality control (QC) groups to rapidly develop methods that can be used to monitor multiple attributes and easily transferred to QC and manufacturing labs to enable the monitoring and subsequent release of new lots. Octet® BLI systems, for example, have broad applications utility that include protein quantitation and functional characterisation. They also have enhanced throughput with lower requirements for sample preparation which, when combined with their ease of use and low cost of operation, provide a perfect fit for most process development and QC labs. They can also be used to rapidly determine the impact of multiple process variables at different stages of a purification process and help identify optimal conditions

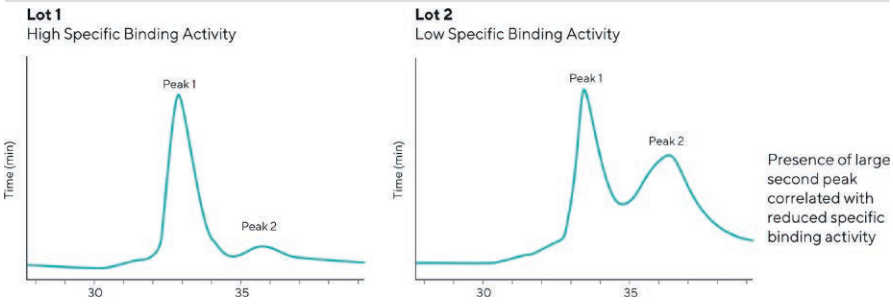
that provide products with the desired yield, binding specificity and potency. For regulated environments, optional GxP (good practice) components that ensure regulatory compliance are available. These include IQ/OQ and PQ tools and FDA 21 CFR Part 11-compliant software that meet GxP requirements. The generated data compares favourably with both HPLC and ELISA techniques in most relevant assays, with the added advantage of speed and throughput. While IgG titer has been a common application for this technology for years, Fc receptor binding, contaminant testing and activity assessments are some key applications that are becoming increasingly popular too.

Key benefits of BLI label-free technologies in QC

BLI-based systems are used in conjugation with a sample plate in a fluidics-free format. The format coupled with real-time analysis and the high-throughput readout facilitates a more rapid assessment of different assay conditions that in turn help speed up assay optimisation. Users can rely on multiple benefits:

- Parallel sample processing in the range of two to 96 samples in tandem, which leads to faster analysis across multiple samples, enabling faster decision making when assay optimisation is needed
- Direct binding assay with minimal sample preparation; the fluidic-free format avoids the sample clogging often encountered with fluidics-based label-free systems
- Automated assay without manual steps: assay setup is easy and fast; complete walkaway while experiment is running; minimal analyst intervention reduces assay errors
- QC environment-ready with few moving parts that require maintenance; fluidic-free instruments require very low maintenance. No priming is required; hence they exhibit very little downtime, making them suitable for QC environments
- A variety of ready-to-use biosensors that include chemistries for direct capture of antibodies and biosensors specifically developed for the capture of tagged ligands such the high-precision streptavidin biosensors. The different biosensor chemistries enable the Octet instrument to be used as a single platform with multiple applications.

Figure 1



HPLC spectra of Lot 1 and Lot 2 of a drug molecule. Lot 2 was made by Aragen Biosciences and had an additional peak (Peak 2) compared to the reference lot (Lot 1) provided by their customer. Data provided courtesy of Aragen Biosciences.

Table 1

Benefits of automated Octet® CHO HCP assay compared to manual ELISA summarised by GSK.

Benefit	Details
Precision	Liquid handling robot reduces pipetting variation inherent in manual pipetting
Reliability	Method performed exactly the same each time
Streamlined process	Worklist drives robotic method and creates sample plate importation files. Robotic method automatically creates and executes Octet® method file
Walk away	No analyst intervention needed to complete method after instrument loaded and diluent volumes are checked
Washing steps	No washing steps needed and plate washer integration not required
Analysts involvement	Automated Octet® ~30 minutes for 1–3 assay plates Manual ELISA ~2.5 hours per assay plate
Throughput	3 assay plates can be run in ~5 hours 38 samples/plate in duplicate wells > 108 samples in 3 plates
Antibody consumed	Re-use of coating antibody can significantly reduce consumption over multiple assay plates

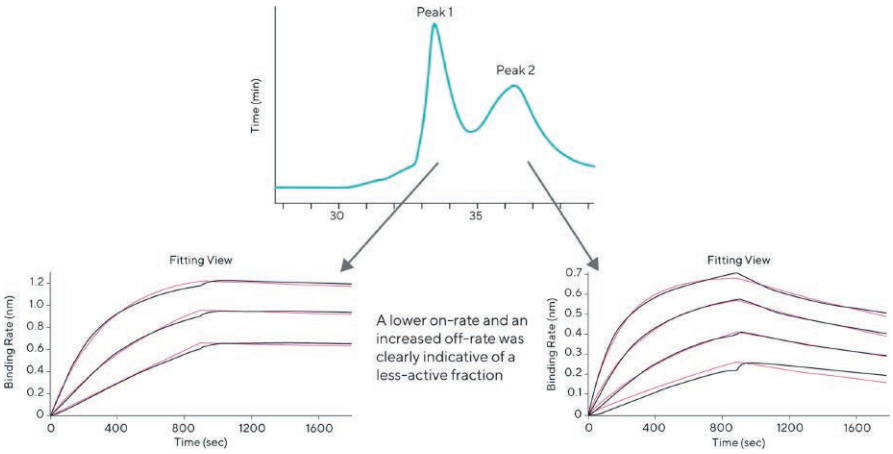
Process efficiency and product QC – case studies

A residual contaminant detection assay specifically for host cell proteins (HCP) and a product QC assay were established at GlaxoSmithKline (GSK) and Aragen Biosciences respectively.

Residual contaminants that co-purify with a biotherapeutic in development can adversely affect the safety and efficacy of the product. The detection and removal of these contaminants is therefore a key requirement during process development as well as for product release. Examples of contaminants commonly identified as CQAs include HCPs, residual Protein A (RPA) and residual DNA impurities. High sensitivity methods capable of detecting up to the allowed maximum levels for each respective contaminant are critical in both process development and QC labs.

Label-free analytical platforms are increasingly supplanting the ELISA technique in contaminant testing assays. At GSK, for example, the analytical team was able to compare the performance of a manual HCP ELISA assay with Sartorius's Octet® CHO HCP kit in an effort to streamline their workflow in process development. Their work was carried out on a 16-channel instrument capable of analysing 16 samples in parallel. The kit-based assay utilises Chinese hamster ovary (CHO) antibody pre-coated biosensors and is designed to enable HCP »

Figure 2



The Octet® binding kinetics or functional assay demonstrated that Peak 1 was the active fraction. Peak 2 was the less-active fraction, with a lower on-rate and a much faster off-rate in a binding experiment. Data provided courtesy of Aragen Biosciences.

binding response signals amplification through a detection step. The capture antibody is similar to the one used in the ELISA kit (Cygnus). The GSK team found that the automated Octet® HCP assay required minimal analyst intervention and provided more accurate and precise results than their manual ELISA assay (**Table 1**). They also showed that the hands-on time for sample preparation and processing of one to three assay plates was reduced to 30 minutes from the previous 2.5 hours with manual ELISA, and antibody consumption decreased by as much as 40 percent.

Benefits of automated Octet® CHO HCP assay compared to manual ELISA

Scientists at Aragen Biosciences, meanwhile, created a stable and scalable CHO cell line, purification platform and manufacturing process for a particular product on behalf of a client. They used the Octet platform to develop an activity assay for a quality assessment of different lots of the same product. An activity assay is generally utilised during process development, QC and manufacturing to compare various prepared lots of the drug molecule, as well as its stability. Activity assays are critical because they differentiate active protein from inactive or clipped variants, as those species will not bind the ligand. In the Aragen method, a biotinylated ligand is first captured onto Streptavidin Biosensors, followed by the binding interaction of the ligand with the protein analyte. As can be seen in **Figure 1**, sample Lot 2 contained a large second peak that was absent in Lot 1 (reference material). The second peak in Lot 2 exhibited a slower on-rate and a much faster off-rate, indicative of a less-active fraction (**Figure 2**). The activity data generated with a label-free approach were confirmed with a cell-based assay and Aragen was able to modify its production conditions to significantly reduce this second peak fraction.

Label free. Stress free.

Discover Octet® BLI technology - as versatile as a Swiss Army Knife.

It is never too soon to start thinking about GxP compliant tools for developing validated assay methods. Address a broad range of ligand-binding assays - from protein quantitation to impurity testing to kinetics characterization of biotherapeutics - on one fluidic-free, low-maintenance platform. Even in early stages, we can help to ensure your process is robust and scalable.

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