GYROS

Application Report 206

Single-system solution for quantification of monoclonal IgG and host cell proteins (HCP)

- **Switch between high- and low-concentration analysis within one system**
- **Simultaneous quantification of drug product and contaminants**
- **Automation provides a robust workflow with improved reproducibility**
- **Increased throughput (up to 112 data points in < 60 minutes)**

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Introduction

Efficient process development and optimization, and rigorous quality control are crucial elements in the successful production of protein-based drugs, such as monoclonal IgG.

Quantification of drug product

In order to generate cost effective processes for manufacturing of monoclonal IgG, significant efforts are made to optimize cell culturing conditions and purification procedures to increase overall product yields. Fast and accurate quantification of the produced material is an essential part of this process.

Current technologies, such as ELISA and HPLC, are able to deliver good quality results but have limitations in terms of throughput and measurement range. As the purification process proceeds from crude cell supernatant to final product, the IgG concentration increases significantly. At higher concentrations ELISA, in particular, increasingly requires pre-assay dilution, which is time-consuming and can introduce quantification errors.

Quantification of contaminants

A second important factor in the development of biopharmaceuticals is the quantification of protein contaminants originating from cell culturing and fermentation, which can be co-purified along with the product and potentially cause adverse reactions when the drug is administrated to patients. In crude cell supernatants and at the early stages of the purification process high concentrations of contaminants can be present. Concentrations decrease as the purification process progresses, with the aim of minimizing impurities in the final product.

ELISA is one of the most commonly used technologies for the quantification of contaminants due to its relatively high sensitivity. However, due to its narrow measurement range, ELISA requires extensive dilution when monitoring contaminants during the early stages of the purification process.

Single-system solution

The Gyros™ immunoassay platform offers a complete, automated solution to meet the needs of both applications by providing a broad measurement range. An open and flexible design facilitates quantification of any target protein for which an immunoassay can be developed, and allows experimental set-up to be designed on a day-to-day basis. Gyrolab™ Bioaffy™ CDs are used to analyze different target molecules, allowing simultaneous quantification of IgG and host cell proteins (HCP) in the same sample.

The fully automated Gyrolab workstation:

- **Bioaffy 20 HC for quantification up to the g/l range**
- **Bioaffy 200 for high sensitivity assays when detecting low concentrations**

Assay steps are integrated and miniaturized in the CD format, increasing throughput and simplifying working methods, whilst still delivering reliable, high quality results. See Figure 1 for an illustration of how the two CD microlaboratories provide analytical support in the different stages of biotherapeutic process development.

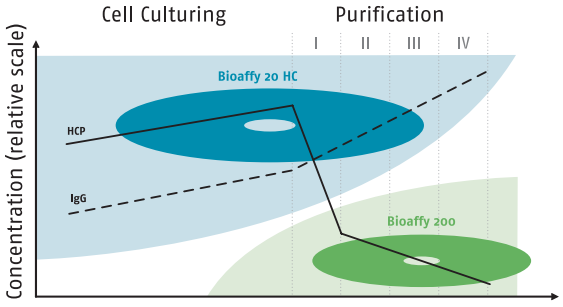

Figure 1 Illustration of the analytical support for biotherapeutic process development offered by the combination of Bioaffy 20 HC and Bioaffy 200. In the early stages, Bioaffy 20 HC can be used for simultaneous quantification of IgG and HCP. As HCP concentrations decrease high sensitivity assays are achieved using Bioaffy 200.

Case Studies

Astrazeneca, Södertälje, Sweden

The Bioprocess R&D group at AstraZeneca, Södertälje, Sweden, focuses on development of protein-based drugs for clinical studies. The group routinely uses Bioaffy 200 as a preferred alternative to HPLC and ELISA, for the quantification of monoclonal immunoglobulins and host cell proteins, due to its increased throughput and ease of use. The group analyzes samples from all stages of the purification process. As such, the introduction of Bioaffy 20 HC can provide additional benefits to their workflow by further increasing the upper limit of quantification and, thereby, reducing the need for dilution of high concentration samples.

GE Healthcare, Uppsala, Sweden

The protein separations R&D group at GE Healthcare Bio-Sciences, Uppsala, Sweden, develops processes for protein purification and performs protein quantification to monitor production of monoclonal antibodies. One of the main analytical methods used by the group is ELISA, which is both time consuming and labor intensive due to long assay times and the extensive dilution of samples required.

Gyros carried out separate collaborative projects with the two groups. Key issues for both groups included:

- **Minimizing sample dilution**
- **Improve assay sensitivity to enable quantification of host cell proteins**
- **Reduce time to result**

Quantification of monoclonal IgG in the g/l range

Quantification of monoclonal IgG was performed using Bioaffy 20 HC, designed to facilitate quantification of high concentration samples. In this CD microlaboratory, increased capacity is accomplished by equipping the microstructures with columns of high-capacity beads and through reduction of the sample volume used for quantification to 20 nl. Figure 2 shows the measurement range for quantification of monoclonal IgG using Bioaffy 20 HC. Purified recombinant IgG standard was provided by the collaborators. The combination of Bioaffy 20 HC and the selected reagents[1] resulted in a measurement range covering three orders of magnitude, allowing for quantification of monoclonal IgG up to the g/l range.

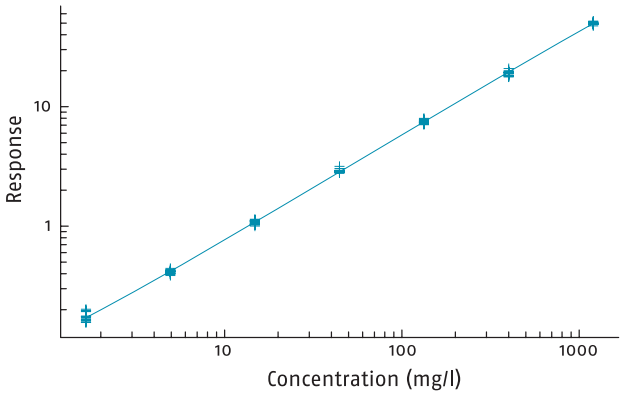

Figure 2 Standard curve for monoclonal IgG using Bioaffy 20 HC.

Assay specificity

The anti-IgG Affibody used as capture reagent is a protein known to bind to the Fc region of human IgG, excluding IgG3. The specificity of the resulting assay to the different subclasses of IgG, and to IgM, was determined on Bioaffy 20 HC. Figure 3 confirms the binding of anti-IgG Affibody to IgG1, IgG2 and IgG4. As expected there is no significant binding to IgG3 or IgM.

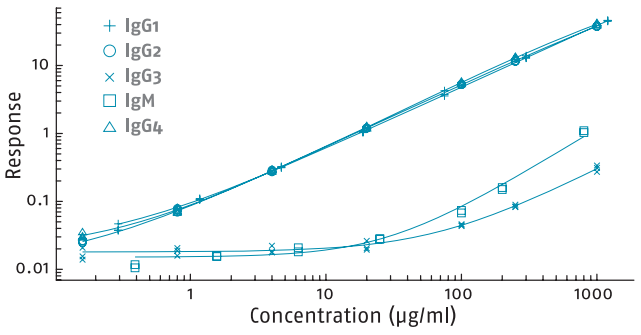

Figure 3 Specificity of F(ab)'2 specific assay using Bioaffy 20 HC, regarding subclasses of IgG, and IgM. The IgG3 and IgM preparations originate from human plasma and may be contaminated with trace amounts of other IgG subclasses.

Assay parallelism

The robustness of the IgG assay, measured as parallelism of the assay after dilution of a sample, was investigated at AstraZeneca. Serial dilutions of four samples were analyzed and plotted together with a dilution of the standard preparation. The slopes of the resulting curves were very similar, as shown in Figure 4.

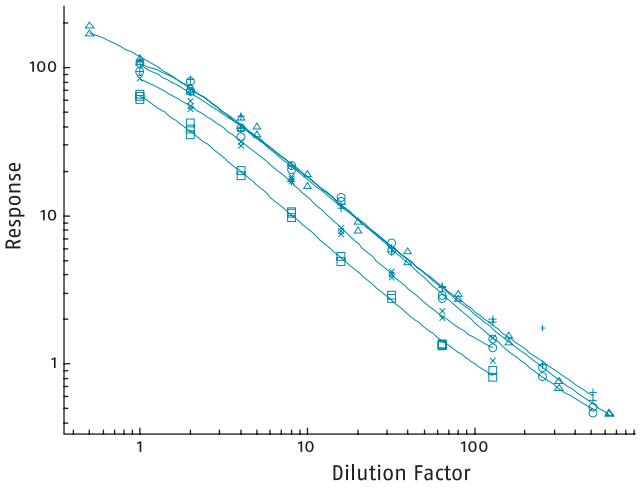

Figure 4 Dilution series of IgG standard and four cell supernatant samples respectively, provided by AstraZeneca and analyzed using Bioaffy 20 HC.

Correlation studies

Samples kindly provided by AstraZeneca were analyzed with regards to content of monoclonal IgG using Bioaffy 20 HC. Samples were diluted 1:2 or 1:4 in sample buffer to match the measurement range of the assay. All samples had previously been analyzed using affinity-based HPLC absorbance measurements at 280 nm and the correlation between the methods is shown in Figure 5.

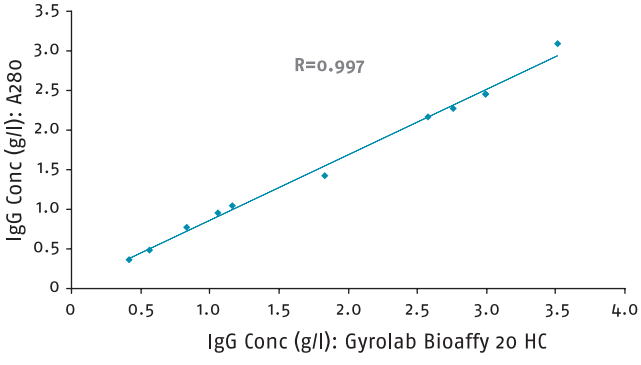

Figure 5 Correlation between Bioaffy 20 HC and HPLC absorbance measurements at 280 nm when quantifying monoclonal IgG in samples provided by AstraZeneca.

1 Anti-IgG Affibody (Affibody, Bromma, Sweden) as capture reagent and F(ab)'2 fragments of goat anti-human IgG (F(ab)'2 specific) (Jackson Immuno Research Laboratories, Suffolk, UK) as detection reagent.

Correlation between Bioaffy 20 HC and ELISA data, generated at GE Healthcare, is shown in Figure 6. The limited measurement range of the ELISA required up to several thousand times dilution of sample to perform the analysis. Sample dilution was reduced a thousand-fold when using Bioaffy 20 HC.

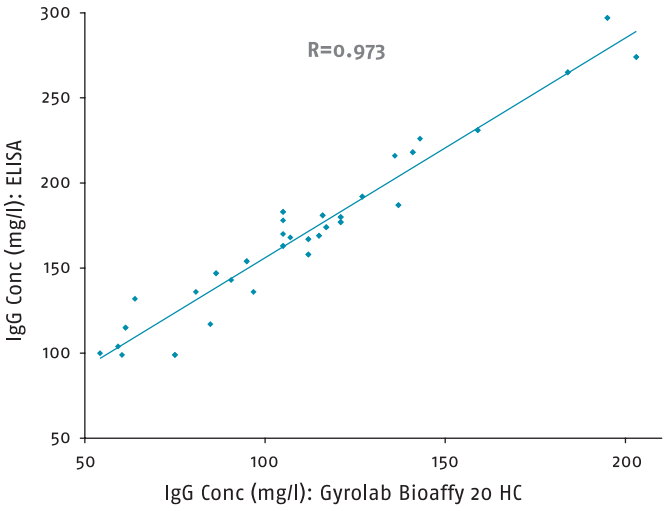

Figure 6 Correlation between Bioaffy 20 HC and ELISA data when quantifying monoclonal IgG in cell supernatant samples provided by GE Healthcare.

Broad measurement range for HCP quantification

When quantifying host cell proteins, CDs and reagents can be chosen to match the required measurement range. As illustrated in Figure 7, Bioaffy 200 can be used to achieve a high-sensitivity HCP assay[3] with a measurement range covering three orders of magnitude. If the same assay is performed using Bioaffy 20 HC the upper limit of quantification is increased by a factor of approximately 50, allowing for quantification of HCP in cell supernatants with minimal dilution required.

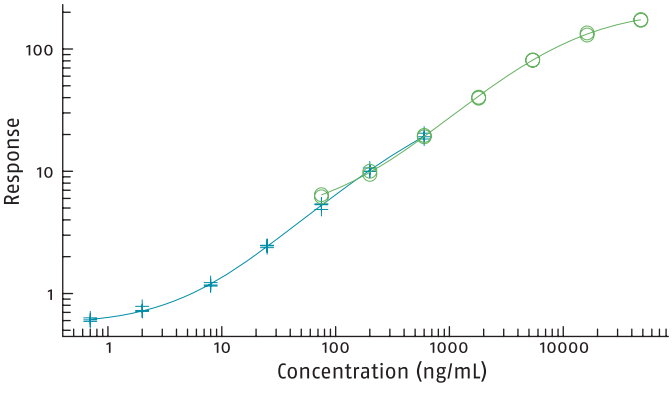

Figure 7 Extension of measurement range for HCP quantification, using Bioaffy 200 (crosses) and Bioaffy 20 HC (circles) respectively.

Monitoring cell culture development

To monitor the development of cell production over time, regarding the intended IgG molecule versus production of contaminating proteins, a series of samples taken at different times in the cell cultivation process were analyzed on Bioaffy 20 HC.

As the cultivation progresses, the concentration of IgG initially increases dramatically and then begins to reach a plateau as the productivity and viability of the cells decreases. Figure 8 shows the increase in concentration of IgG for four different clones between day 8 and day 12 of cultivation at AstraZeneca. The corresponding analysis of HCP concentration is shown in Figure 9. As expected, there is a significant increase in HCP concentration as the cultivation proceeds.

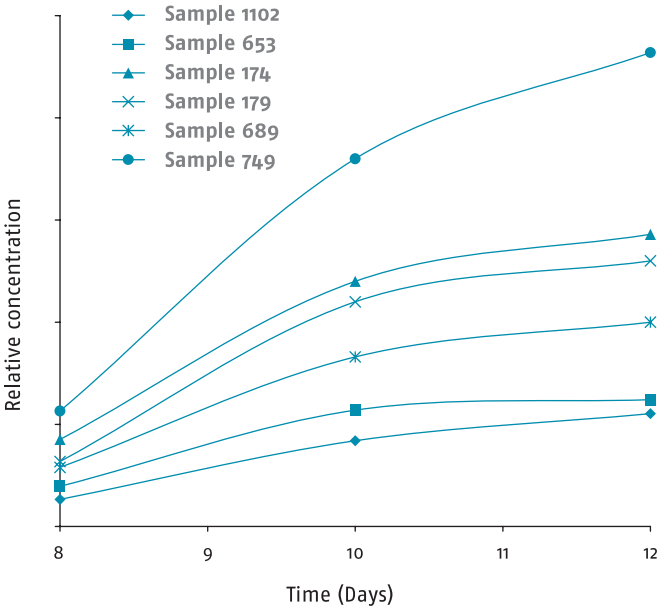

Figure 8 Quantification of monoclonal IgG in cell supernatants from six different clones from AstraZeneca after 8, 10 and 12 days of cultivation.

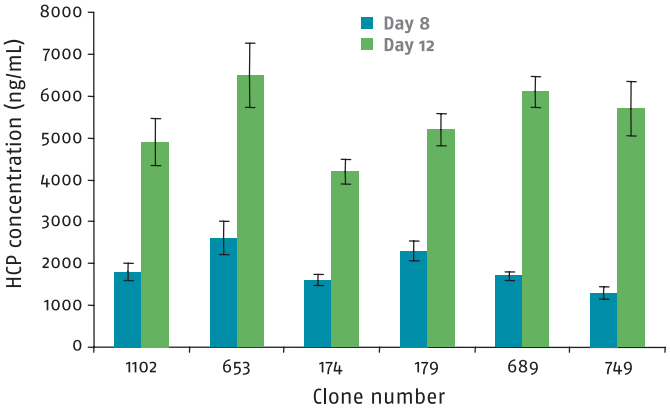

Figure 9 Quantification of HCP in cell supernatants from six different clones from AstraZeneca after 8 and 12 days of cultivation respectively (triplicate measurements).

[3] Chinese Hamster Ovary HCP (Cygnus Technologies, Southport, USA).

At GE Healthcare, a series of samples were collected to monitor production of IgG under different cell culturing conditions, such as temperature and media composition (see Figure 10). Results from the corresponding HCP quantification are shown in Figure 11.

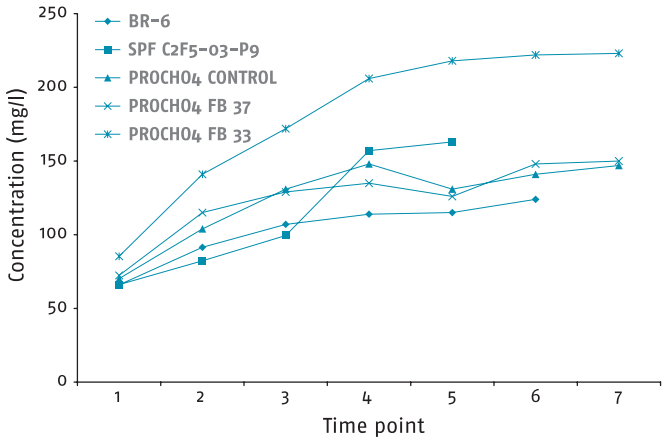

Figure 10 Quantification of IgG in cell supernatant samples from GE Healthcare collected during cultivation of cells grown under different conditions.

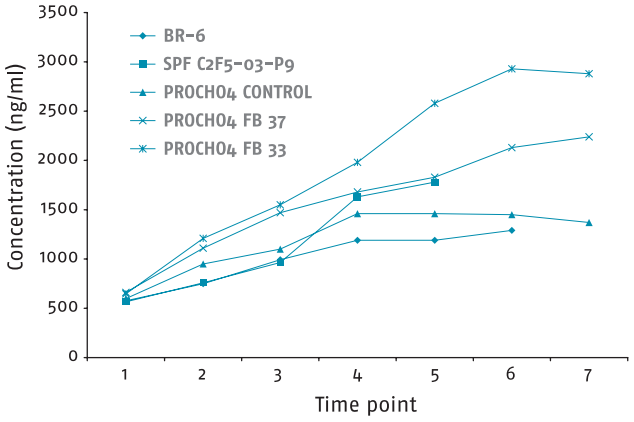

Figure 11 Quantification of HCP in cell supernatant samples from GE Healthcare collected during cultivation of cells grown under different conditions.

Excellent reproducibility

Figure 12 presents reproducibility data from IgG and HCP quantification in the GE Healthcare samples. Samples were run in duplicate and concentrations ranged between 100 and 300 mg/l of IgG and between 10 and 250 ng/ml of HCP. The coefficient of variation (CV) concentration was well below 10% in all cases.

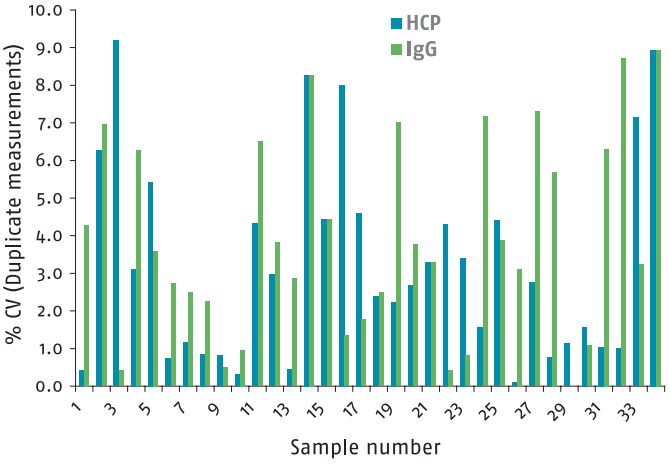

Figure 12 Precision (% CV) when quantifying HCP and IgG in cell supernatant samples (duplicate measurements).

Automation increases throughput

The fully automated system along with very small sample and reagent volumes, which result in faster reaction times, significantly shortens process times. The system generates up to 112 data points in less than 60 minutes and has the capacity to generate 560 data points in unattended operation. In addition, the integration of assay steps in the automated instrument minimizes 'hands-on' time.

Conclusions

The Gyros immunoassay platform provides an automated, flexible solution for maximizing the efficiency of biopharmaceutical process development. The open platform makes it possible to adjust assay conditions, such as CD type and reagents, to suit analytical requirements.

- **Quantification of monoclonal IgG and HCP at concentrations relevant for the entire purification process**
- **Minimizes need for pre-assay dilution**
- **Automated, easy-to-use solution reduces operator 'hands-on' time**
- **Increased throughput with up to 112 data points generated in <60 minutes**

Gyros would like to express their thanks to AstraZeneca, Södertälje, Sweden, (www.astrazeneca.com) and GE Healthcare Bio-Sciences AB, Uppsala, Sweden, (www.gehealthcare.com) for permission to show their results.

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