Increasing efficiency in biopharmaceutical process development

- Excellent assay performance
- Automation improves workflow robustness
- Increased throughput (104 data points delivered <60 minutes)
- Simple operation reduces ‘hands-on’ time and improves lab efficiency

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

The production process, as well as the methods used in process development and quality control, must be:

- Fast and flexible
- Robust enough to be easily performed
- Reproducible in production environments

Protein quantification is a commonly used method during several stages of biopharmaceutical process development and the establishment of quality control procedures. Current technologies are able to deliver good quality results. However, assay times are slow and a considerable amount of ‘hands-on’ time is required. By miniaturizing and integrating the assay in a CD microlaboratory, Gyrolab Bioaffy™ is able to overcome these problems, whilst still delivering high quality results.

**Case studies**

Gyros performed studies in collaboration with two biotechnology companies working with recombinant antibodies: Biovitrum Biopharmaceuticals, a Swedish based company offering highly qualified process development and production of new protein-based drugs (currently using ELISA); and Biogen-Idec, an international company developing targeted immunotherapies for cancer and autoimmune and inflammatory diseases (currently using HPLC).

**Results**

**FASTER PROCESS TIMES/FULL AUTOMATION**

Miniaturization reduces the cost of reagents and results in faster reaction times. This, along with parallel processing of up to 104 data points (in <60 minutes) under identical conditions meant that Gyrolab Bioaffy was able to improve workflow efficiency and speed up assay times.
By automating and integrating assay steps, ‘hands-on’ involvement in the assay was reduced, particularly compared to the HPLC method.

Figure 1 shows that Gyrolab Bioaffy provides up to a five-fold increase in throughput compared to the methods originally used at Biovitrum Biopharmaceuticals and Biogen-Idec.

EXCELLENT ASSAY PERFORMANCE
Table 1 shows a broader measurement range available with Gyrolab Bioaffy and a lower limit of detection with no increase in inter/intra assay CV% values as compared with HPLC and ELISA (based on capture with protein A).

Table 1. Comparison of performance between Gyrolab Bioaffy, HPLC and ELISA.

<table>
<thead>
<tr>
<th></th>
<th>Gyrolab Bioaffy</th>
<th>HPLC</th>
<th>ELISA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Measurement range:</td>
<td>$10^{-4}$ to $10^{-1}$</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Limit of detection (LOD):</td>
<td>70 pg/ml or 1 ng/ml depending on assay configuration.</td>
<td>1 µg/ml</td>
<td>1 ng/ml</td>
</tr>
<tr>
<td>Intra-inter assay CV%:</td>
<td>&lt;10%</td>
<td>&lt;10%</td>
<td>&lt;10%</td>
</tr>
</tbody>
</table>

Figure 2. Reference curves for human IgG assay using two different antibody combinations and signal amplification levels. Measurement range for each assay configuration covers up to four orders of magnitude.

BROAD MEASUREMENT RANGE
As Gyrolab Bioaffy allows for flexibility in both assay design and detector sensitivity, two assay configurations were developed to cover a measurement range from picograms to micrograms per milliliter as shown in Figure 2.

The assay configuration and signal amplification in Gyrolab Bioaffy can be adapted to fit the concentration range of the samples to be analyzed. At Biovitrum Biopharmaceuticals, Gyrolab Bioaffy required only two dilution steps to achieve the required measurement range, half the number of steps required by ELISA.

At the lower end of the concentration range, Biogen-Idec recognized the potential value of the low detection limit with Gyrolab Bioaffy’s more sensitive assay, which allows for detection of impurities in the produced material.

CLOSE CORRELATION
Gyrolab Bioaffy provides the improved measurement range and LOD without any loss of accuracy when compared to HPLC (see correlation coefficient in Figure 3) and ELISA (see correlation coefficient in Figure 4).
EXCELLENT REPRODUCIBILITY AND ACCURACY

Further investigation of the accuracy of the assay was performed using CHO (Chinese Hamster Ovary) cell conditioned media from Biogen-Idec spiked to two concentration levels. The IgG content was then measured on two separate days with two runs per day. The inter- and intra-assay CV-values were below 5%, see Table 2. CV values between days were 0.1 and 1.6% for the lower and higher concentration, respectively (not shown). The resulting recovery was between 95% and 120%.

<table>
<thead>
<tr>
<th></th>
<th>Day 1</th>
<th>Day 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spiked hIgG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>100 ng/ml</td>
<td>2.9 3.3 5.0</td>
<td>1.9 3.3 3.3</td>
</tr>
<tr>
<td>1000 ng/ml</td>
<td>0.7 1.84 2.4</td>
<td>0.5 1.6 1.7</td>
</tr>
</tbody>
</table>

Gyros would like to express their thanks to Biovitrum Biopharmaceuticals (www.biovitrum.se) and Biogen-Idec (www.biogen.com) for permission to show their results.

Conclusions

The successful collaborations between Gyros and both Biovitrum Biopharmaceuticals and Biogen-Idec have shown that Gyrolab Bioaffy is an effective tool for increasing efficiency in biopharmaceutical process development.

In addition to matching the current technologies used, in terms of accuracy and reproducibility, Gyrolab Bioaffy also provides:

- Full automation of the assay process and integration of steps resulting in:
  - Reduced operator ‘hands-on’ time
  - Ease of use
- Shortened assay times – less than 60 minutes, generating 104 data points per assay.
- Broad measurement range, due to a flexible platform, which allows assay configuration to be chosen according to sensitivity needs.
- Reduced consumption of reagents.
- Open system that facilitates quantification of any target protein for which a sandwich immunoassay can be designed.
Gyros uses its proprietary technology platform to miniaturize and integrate laboratory processes into application-specific microlaboratories. A Gyrolab™ microlaboratory, in the form of a compact disk, can process hundreds of samples in parallel at nanoliter scale, under the control of Gyrolab Workstation. Scientists can generate more information from less sample, improve laboratory performance and increase productivity.

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