TUHH
Hamburg University of Technology

PAIA

Optimizing cell culture processes with PAIA IgG quantification assays

Johannes Möller[1], Kirsten Koopmann[1], Kristina Lechner[2], Sebastian Giehring[2] and Ralf Pörtner[1]

[1] Hamburg University of Technology, Institute of Bioprocess and Biosystems Engineering, Hamburg
[2] PAIA Biotech GmbH, Köln

Introduction

The development of bioprocesses requires careful monitoring of numerous parameters such as cell density, viability and product titer. Developing sophisticated feeding strategies is challenging due to the high number of required experiments. Especially if multi single-use systems (e.g. shake flasks) are used, the analysis of the time course of titers leads to a high number of samples. This further increases the time for process development. PAIA antibody quantification assays are a fast and easy way to determine titers in very small sample volumes allowing for multiple sampling even in small bioreactor formats.

Cell culture supernatants can be measured without any sample preparation in a simple bead-based assay format with little hands-on time (Fig.1). One full 384-well PAIA*plate* is processed and read-out on a fluorescence reader or imager in one hour for high throughput and timely results.

In this study, the applicability of the Fc low titer assay kits (PA-104) during the development of bolus feeding strategies was tested. Furthermore, the measured concentrations were compared to ProteinA-HPLC.

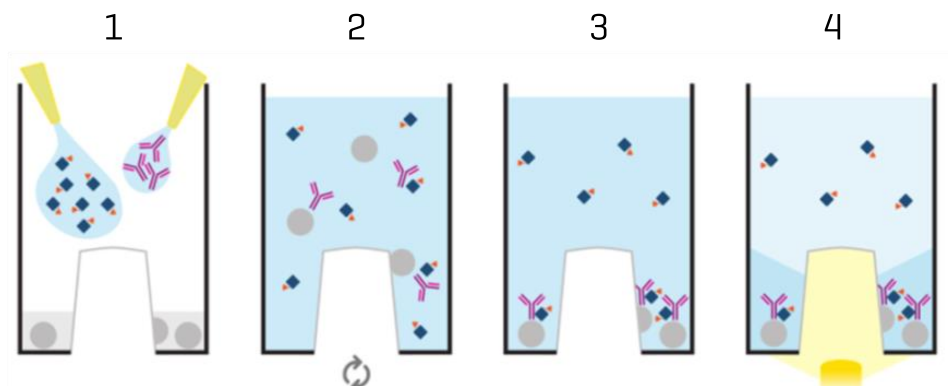

Fig. 1: General workflow of PAIA assays: 1) Buffer and analyte addition to wells with dried capture beads, 2) Assay reaction during shaking, 3) Separation of capture beads 4) measurement by fluorescence bottom reading.

Medium & Pre-culture

IL-8 antibody producing CHO DP-12 cells (kindly provided by Prof. Noll, Bielefeld University) were cultivated in the chemically defined cell culture medium TC-42 (Xell AG) supplemented with 0.1 mg mL^{-1} LONG R3 IGF-1, 200 mM methotrexate and 6 mM glutamine (all from Sigma-Aldrich). Cryocultures ($1 \cdot 10^7$ cells mL^{-1}) were thawed and cultivated in single-use Erlenmeyer baffled flasks (Corning, 40 mL working volume). The incubator (Kuhner LT-X) atmosphere was controlled at 37 °C, 5 % CO_2, 85 % humidity, 250 rpm shaking frequency and 12.5 mm shaking diameter.

PA-104 assay kit

The PAIA-104 is intended for quickly determining IgG concentrations ranging from 10-100 µg mL^{-1} in small sample volumes. The IgG in the sample releases a Fc-specific marker from capture beads, thereby producing a fluorescence signal.

In short, 54 µL of the ready-to-use assay buffer and 6 µL of crude supernatants, previously diluted 1:10 with PBS, was added to the wells. Rituximab (Mabthera™, Roche), was used to calibrate the assay. All standards were prepared in cell culture medium diluted 1:10 with PBS. Assays were measured in triplicates on a Tecan Safire reader, bottom reading (Exc. 640 nm, Em 670 nm).

Analytics: cell concentration

The total cell concentration was quantified with the Beckmann Z2 particle counter (Beckmann Coulter). Viability was measured with the DAPI method in a flow cytometer (Beckmann coulter).

Fed-batch experiments

Fed-batch experiments were performed in single-use Erlenmeyer baffled flasks with a starting volume of 30 mL. The incubator and the starting concentrations were the same as explained above. The bolus feed (Chomacs basic feed, Xell AG) was supplemented with varying concentrations of glucose and glutamine. The feeding was started at different time points and feeding was performed daily (bolus) until 50 mL working volume was reached. The different feeding strategies are listed in Table 1.

Tab. 1: Bolus feeding strategies

	Glucose [mM]	Glutamine [mM]	Start of feeding [h]
A	154	9	48
B	111	38	96
C	222	38	72
D	163	25	72

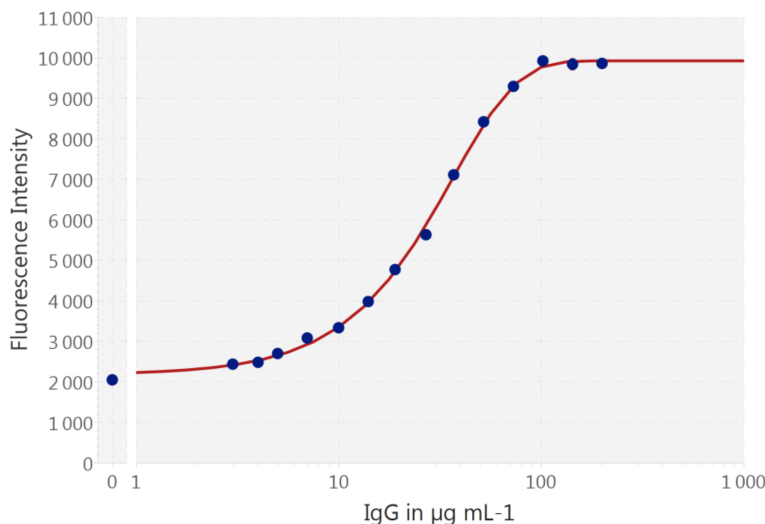

Fig. 2: Calibration curve of the PA-104 assay kit, screenshot from the PAIA Evaluation software.

HPLC

The measurements of the IgG with the PA-104 assay were compared to ProteinA-HPLC measurements. A Knauer Smartline HPLC equipped with a Poros-A column (Thermo Fisher Scientific, 0.1 mL) was used in accordance with the manufacturers protocol. Purified water containing 150 mM NaCl and 50 mM Na_2HPO_4 (pH 7) was used as the mobile phase. The samples were filtered (Cellulose filters, pore size: 0.45 µm) and 50 µL were injected. 100 mM glycin (pH 2.5) was applied to elute the antibody and the UV signal (280 nm) was measured. The system was calibrated with a standard curve of diluted Rituximab and samples were measured in duplicates.

Results

The highest antibody titer and viable cell concentration X_v (B, 260 mg L^{-1}, $35 \cdot 10^6$ cells mL^{-1}) resulted from a glutamine concentration of 38 mM, a glucose concentration of 111 mM and a starting point of feeding after 96 h. The three other conditions (A,C,D) all achieved very similar but lower cell densities. This resulted in lower IgG titers, especially in the feeding strategy D.

In general, an increase in glucose feed does not correlate with higher titers and cell densities. The starting point of feeding seems to have a stronger effect on the outcome of the shake flask cultivations.

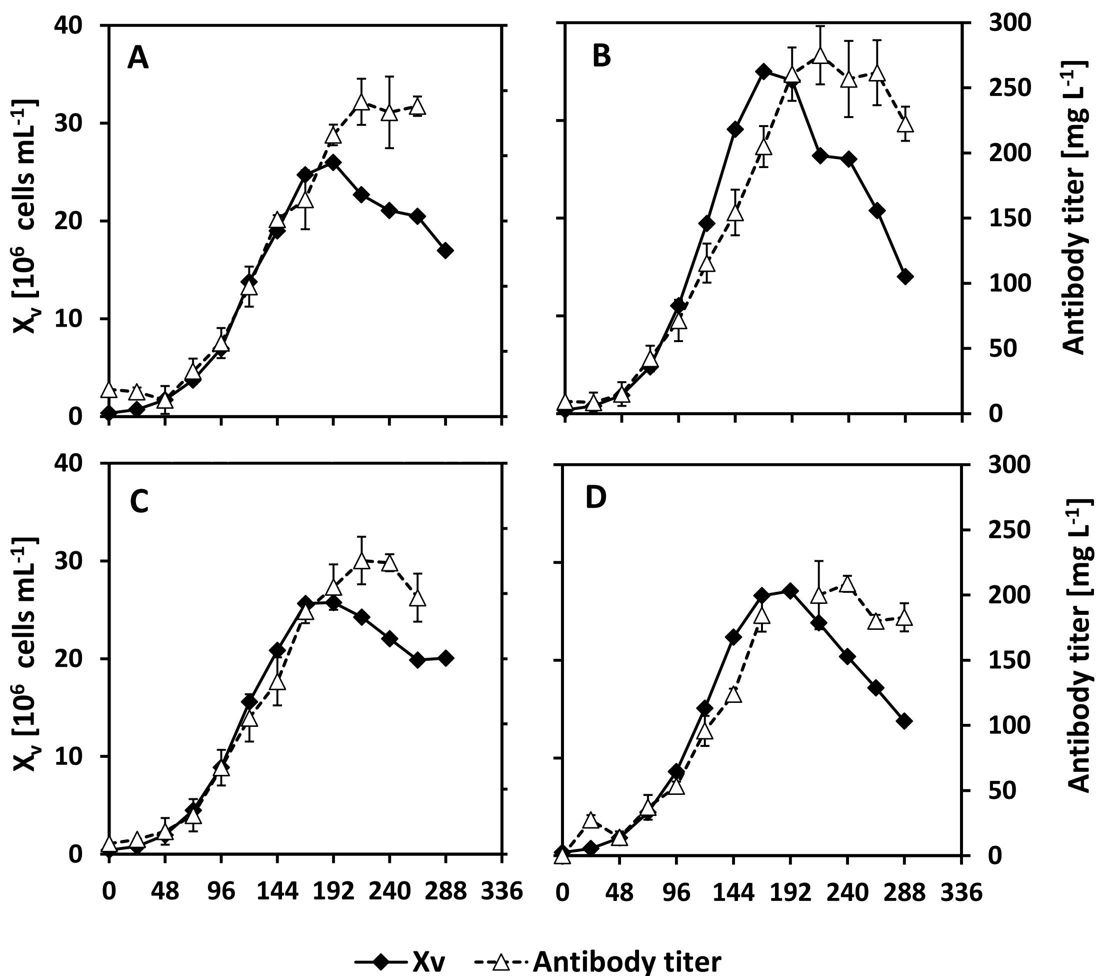

Fig. 3: Viable cell density (X_v) and antibody titer with standard deviations, measured with the PA-104 assay kit, for 4 different feeding strategies (see Tab. 1)

Comparison of PAIA vs. HPLC

A subset of the samples was analyzed with ProteinA-HPLC in parallel. Figure 4 shows the overall good correlation of both methods. Early cell culture samples could not be analyzed by HPLC because of the high detection limit of the method (100 µg mL^{-1}) compared to the PA-104 assay (10 µg mL^{-1}). PAIA assays had slightly higher CVs (three replicates) compared to the HPLC results (duplicates). Comparable titer time courses were obtained with both methods (HPLC data not shown) allowing for the optimization of cell culture parameters with either method.

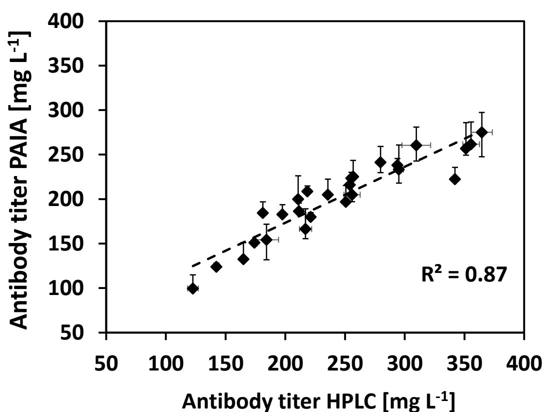

Fig. 4: Correlation of the IgG titer as determined by HPLC vs. PA-104 assay kit

The advantages of the PAIA assays are the speed of the assay (60 Mins for more than 300 samples vs. 5 min per sample for HPLC) and the small assay volume (few µL vs. approx. 200 µL for HPLC). In addition, there is no sample preparation like clarification or filtration necessary for performing PAIA assays.

Conclusion

The analysis of the results from the four different shaking flask cultivations emphasizes that the feeding strategy is a critical factor during process development. The three parameters examined in this small study (glucose and glutamine concentration and feeding start point) yielded different cell densities and product titers which enable identification of favorable conditions. The results also indicate that there may be significant potential for further optimization, e.g. in a DoE approach.

In addition, it was shown that the PA-104 IgG quantification assays are a fast and reliable method for monitoring time resolved product titers. The high throughput and the small sample volumes are especially suited to support and speed up development projects with high sample numbers and smaller bioreactors.

Material & Equipment

- PAIA Fc low titer assay kit (PA-104)
- Orbital shaker (min 1400 rpm)
- Tecan Safire reader fluorescence bottom read
- Knauer Smartline HPLC
- Poros A column 0,1 mL (Thermo)
- Cellulose filter, 0.45 µm pore size

PAIA