IgG Glycan Screening Assays In Crude Cell Culture Supernatants

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Abstract

Background and novelty: Glycosylation of antibodies is a critical quality attribute (CQA) which affects stability, aggregation, serum half-life and immunogenicity of the drug substance. The control of glycosylation during cell line development receives increasing attention because different studies have shown that small scale fed-batch cultures (e.g. in deep well plates) are not only predictive for product titers but also for glycosylation at larger scales. Since modifications of the cell culture process, e.g. by media supplements, can only change product glycosylation within the capacity of the cell line, it is critical to include glycosylation screening when selecting cell lines, especially if a defined glycosylation profile needs to be achieved (e.g. in biosimilars).
We present a novel technology that overcomes current analytical bottlenecks and allows for fast and reliable screening of non-purified cell culture samples using bead-based assays.
Experimental approach: We used the PAIA assay technology with affinity capture beads for IgG and fluorescence labeled lectins that detect different types of glycosylation. The method measures intact glycoproteins in a one-hour, high throughput and completely plate-based assay which does not require purification of the IgG.
Results and discussion: We present data examining the differences in glycosylation between IgGs or Fc-fusion proteins and case studies from screening campaigns in early cell line development and compare the results with orthogonal methods.
PAIA glycosylation assays provide a fast method to screen cell culture supernatant samples and determine relevant differences in product quality that allow cell line selection as early as in 96 deep well plates. We believe that this technology will close an important analytical gap in cell line development.

Assay principle and workflow

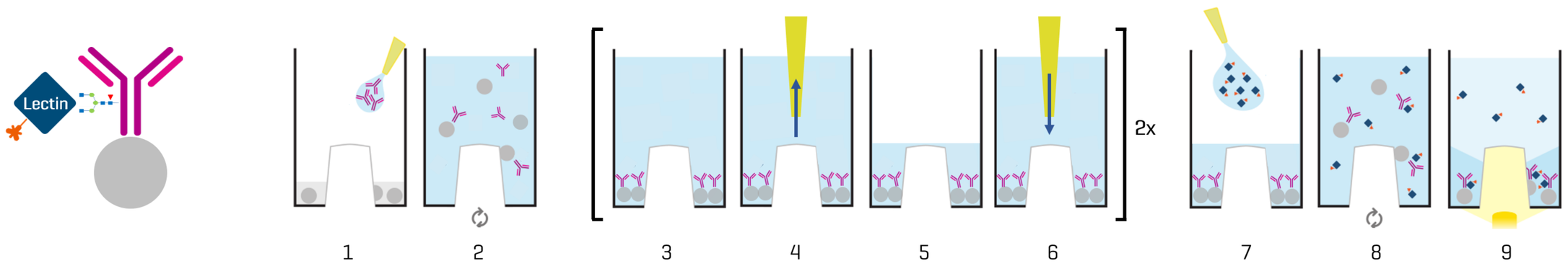

Figure 1. Workflow for glycosylation analysis in supernatants
To remove interfering components from the supernatant the protocol include a purification step which is carried out in the 384 well PAIA*plates*. At first the IgG in the sample is bound to the beads [2]. After bead settling [3], the solution can be exchanged several times [4-6], typically these steps are performed twice. The lectin markers are added for the final assay step [7]. After shaking for 45 min [8] and bead settling, the plates can be measured on a plate reader. The whole process can be easily automated and takes roughly 90 minutes. When measuring purified samples step 2-6 can be omitted and analyte and lectin markers will be added at the same time (step 1 and 7).

Lectin arrays on 384-well plates

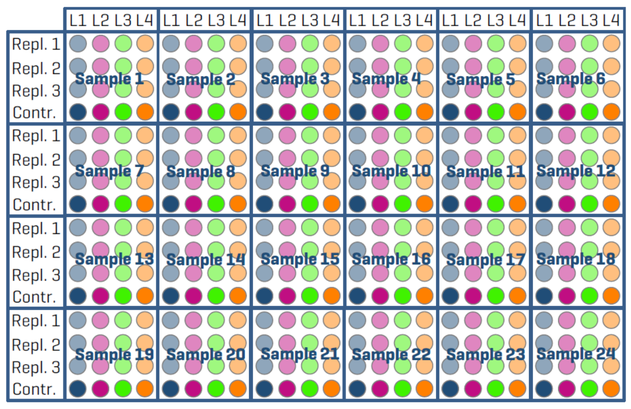

Figure 2. Plate layout for a typical glycosylation assay.
The 384-well plate is able to accommodate multiple small lectin arrays. In this case four different lectins are used. The different lectins are added to the different columns, e.g. lectin 1 to all wells colored in blue.

Glycan profiles of IgGs and Fc-fusion proteins

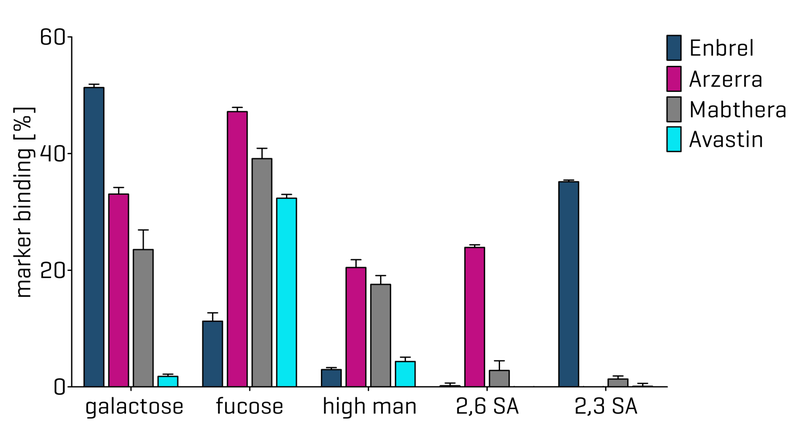

Figure 3. Glycan profiles of different analytes.
3 µg of sample was mixed with marker and shaken at RT for 45 min. High percentage of marker binding indicates high abundance of its specific glycan (High man - high mannose; 2,6 SA – 2,6 linked sialic acid; 2,3 SA – 2,3 linked sialic acid).

Sialylation Screening of Fc Fusion Proteins

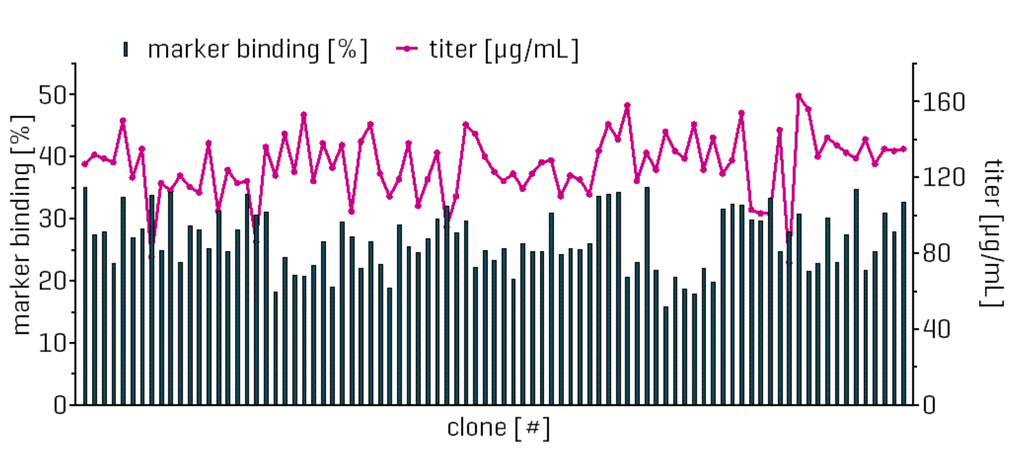

Figure 4. Sialylation screening from 24 DWP plates
Selection of cell lines expressing the product with the highest degree of sialylation based on normalized sialic acid binding lectin

Screening Crude Supernatants for High Mannose

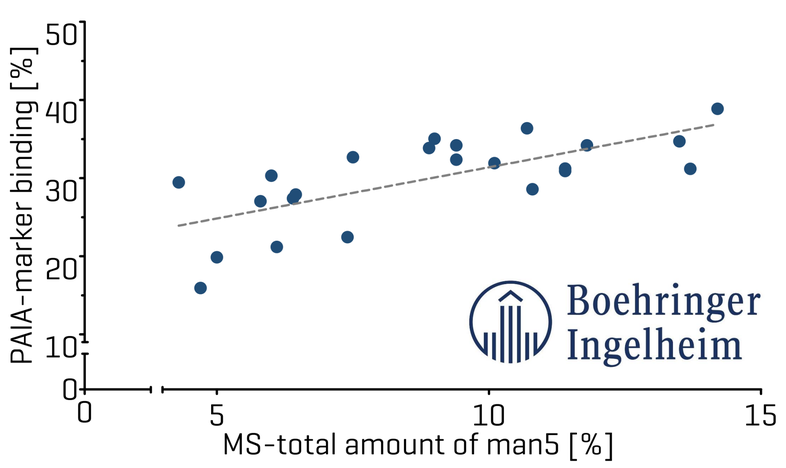

Figure 5. Clone screening for High Mannose in collaboration with Boehringer Ingelheim
Results from a clone screening project focusing on Man5 glycans. Cell lines with a tendency to produce highly mannosylated antibody can be identified and discarded.