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N-Glycan Analysis: Rapid Preparation and Screening of Biosimilar Candidates by LC/MS and CE

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Abstract

- N-Glycan profiling is becoming more common and increasingly necessary in the early stages of biotherapeutic cell line development, as N-glycan structure can play a critical role in the pharmacology of therapeutic proteins.

- Early cell culture process optimization can now include N-glycan profiling in addition to traditional screening criteria such as titer and charge variance profile.

- Using Agilent AdvanceBio Gly-X rapid sample preparation technology along with Agilent Gly-Q Glycan Analysis System, a high-throughput capillary electrophoresis platform (formerly ProZyme), biosimilar candidates for Rituximab from the Celltheon SMART platform were efficiently screened based on N-glycan profile.

- N-Glycan profiles of top biosimilar candidate molecules were further analyzed by high-resolution UHPLC.

Introduction

The structure of N-linked glycans can play a critical role in the pharmacology of therapeutic proteins, potentially affecting immunogenicity, pharmacokinetics, and pharmacodynamics, making glycan characterization an essential part of the development process.[1] N-Glycan profiling is becoming more common and increasingly necessary in the early stages of biotherapeutic cell line development. Early clone screening and cell culture process optimization allows for timeline reduction but calls for continuous assessment of the N-glycan profile. This requires significantly increased throughput for sample preparation, analytical instrumentation, data processing, and expertise in glycan characterization. These factors can cause a bottleneck when using traditional technologies.

This Application Note presents a streamlined clone and cell culture process N-glycan screening workflow using Rituximab as a model antibody (Figure 1). The Celltheon SMART Technology Platform has been used for rapid selection of high expressing stable pools (>1.0 g/L), stable clones (>3.0 g/L) with cell-specific productivity (Qp) of >50 pg/cell/day, and optimized cell culture process conditions to further match the product quality of the innovator molecule. For N-glycan-based selection of clones and cell culture process, the high-throughput Gly-Q Glycan Analysis System was used. This system combines rapid sample preparation and analysis with a simplified, user-friendly data processing approach. The sample preparation includes a five-minute deglycosylation step to release N-glycans, followed by labeling with a fluorescent dye and cleanup (Figure 2).
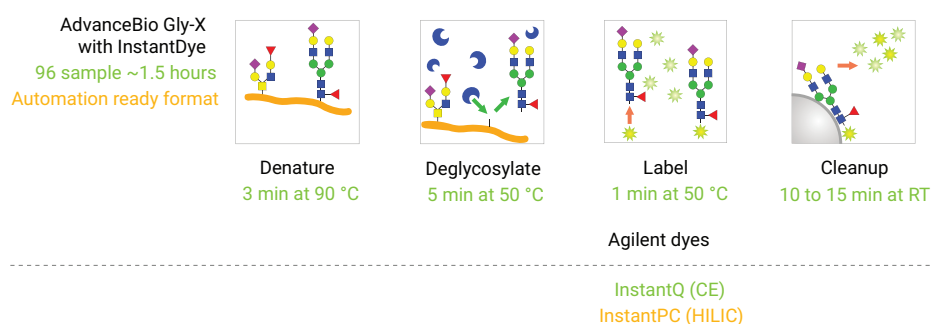
Figure 2. AdvanceBio Gly-X in-solution deglycosylation technology and dye options. Glycosylamine-reactive InstantDye workflow with PNGase F deglycosylation and labeling in solution followed by on-matrix cleanup.
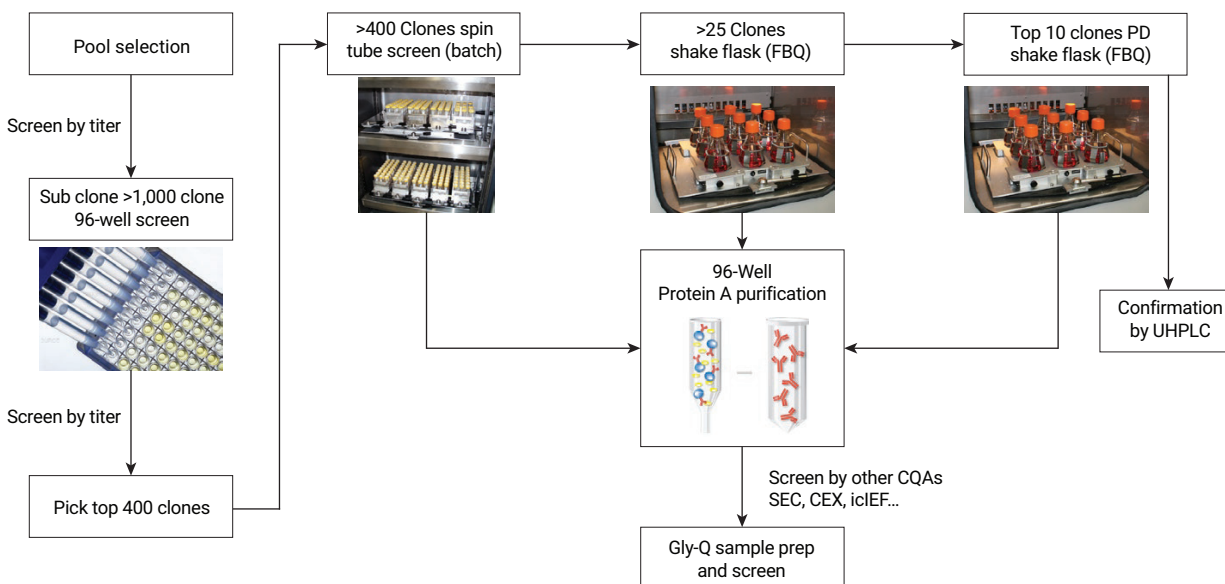
Figure 1. A high-throughout glycan development workflow. Due to the prohibitive cost and time required for N-glycan analysis, the current workflow for biosimilar development allows for only a few clones to be screened at a time. Four hundred clones were easily screened based on titer and top candidates selected for N-glycan analysis using Agilent AdvanceBio Gly-X N-Glycan Preparation along with analysis on Agilent Gly-Q. FBQ = fed-batch quantitation.

The entire workflow may be completed in less than one hour. Labeled N-glycans are separated using a small and user-friendly capillary electrophoresis (CE) instrument with a run time of two minutes per sample. This complete and focused approach allows for the preliminary screening of hundreds of single clones and cell culture conditions, enabling the selection of top candidates based on the desired N-glycan profile. Top clone candidates and optimized cell culture conditions can efficiently be selected for higher resolution profiling such as UHPLC-HILIC. This advancement in N-glycan screening capacity enables the efficient selection of multiple candidate biosimilar clones based on the N-glycan profile compared to the innovator drug substance. The ability to have multiple clone candidates allows for more flexibility in process development and higher probability of successfully matching additional key critical quality attributes (CQAs) for biosimilar development.

Results and discussion

Clones and conditions were narrowed to the top Rituximab biosimilar candidate based on titer (3 g/L), charge variance profiles (matching acidic, basic, and monomer species), aggregation profiles (>95% monomer), and similarity in N-glycan profiles. Gly-Q analysis was used in comparing N-glycan profiles of MabThera drug product and the biosimilar candidate (Figure 3).
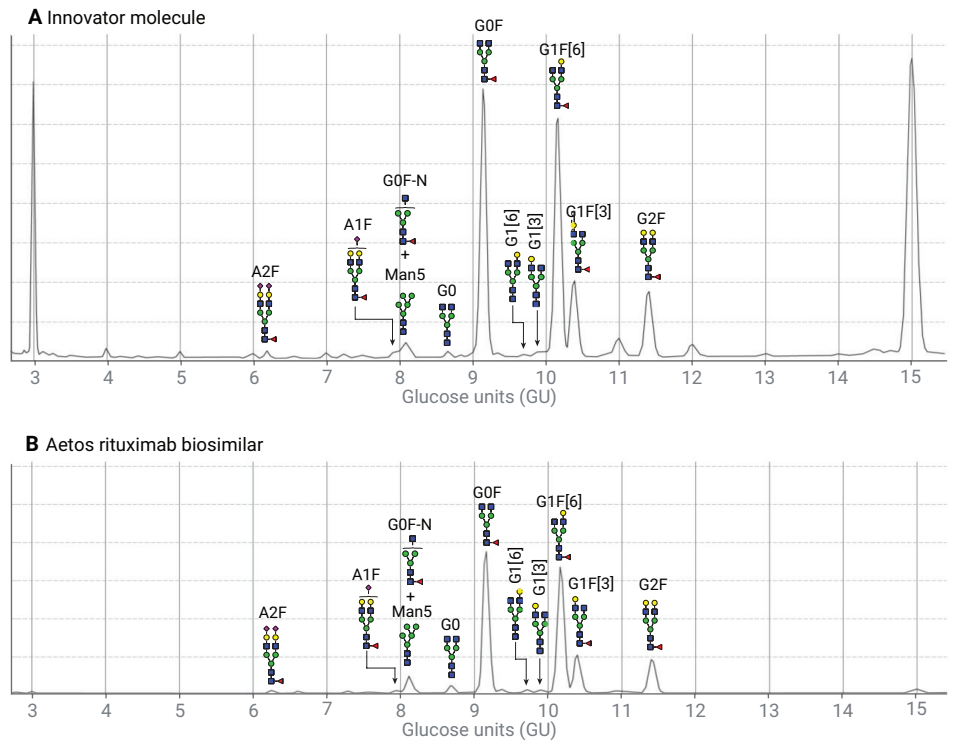

Figure 3. Agilent Gly-Q electropherograms. N-glycan profile of innovator molecule drug substance reference material (A). Selected N-glycan profile of top biosimilar candidate (B).

N-Glycan profiling with Gly-Q (Figure 4A–C) and titer measurements (Figure 4D–F) were completed at the FBQ pool stage, batch quant, and the FBQ single clone screening stage.

Based on results at the FBQ single clone screening stage, top producing candidates showing a promising N-glycan profile were selected for process optimization experiments.
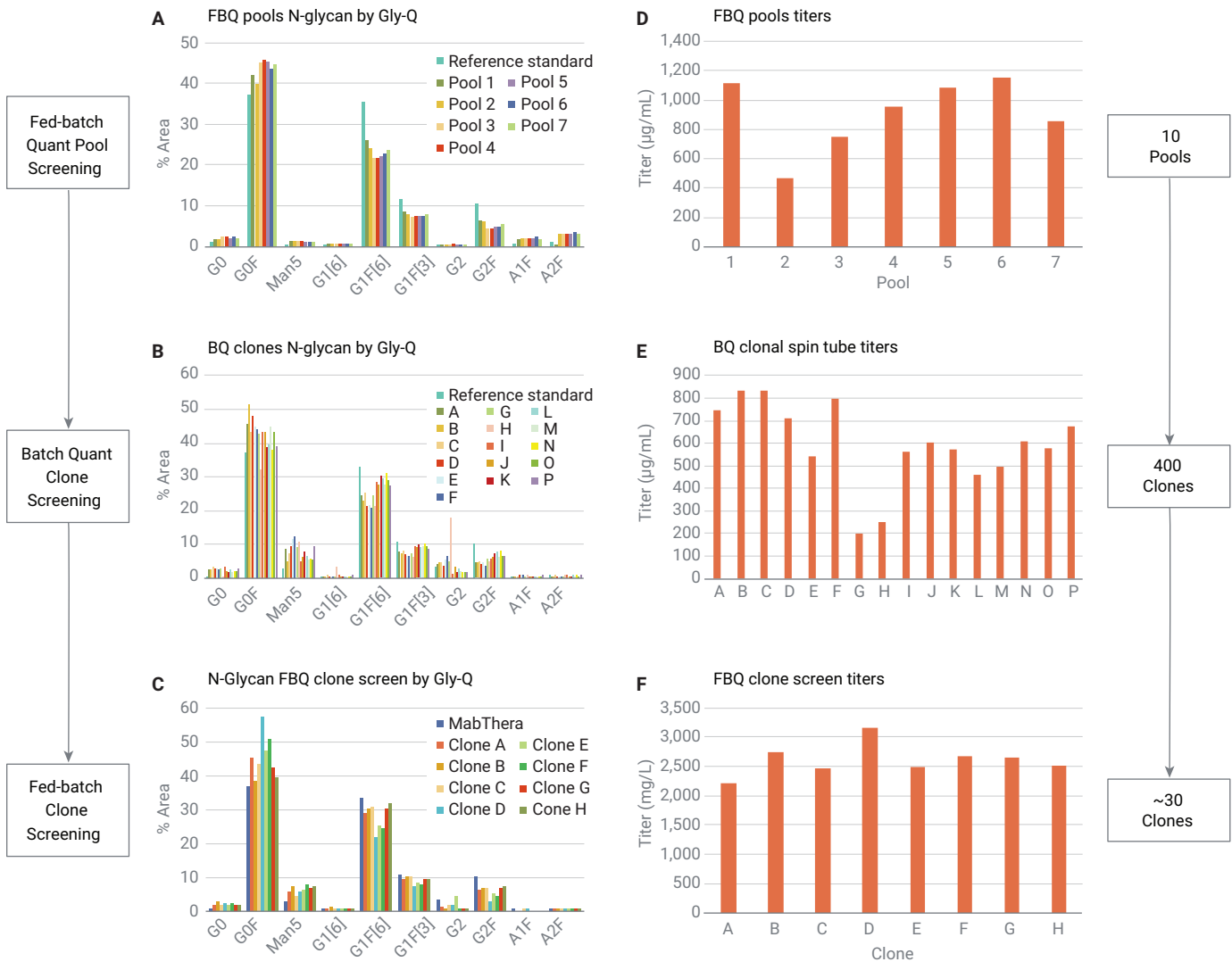

Figure 4. (A–C) Relative percent areas as reported with Agilent Gly-Q; (D–F) titers from the screening of exemplary samples during the cell line development and process development leading to the creation of a cell line capable of producing a biosimilar equivalent to Rituximab. (A) shows the screening of certain top expressing pools, where each pool differs in the applied expression technology. (B) shows N-glycan profiles of exemplary high titer clones at the batch quant (BQ) stage. (C) shows N-glycan profiles of the high titer clones at the FBQ stage; Figure 5 describes the product quality acceptance criteria at the FBQ stage in more detail.

N-Glycan profiles generated with Gly-Q of a subset of nine different cell culture conditions are shown in comparison to MabThera (Figure 5). Additional analysis was performed by UHPLC-HILIC to confirm the validity of this novel glycan assay. The UHPLC results (Figure 6) indicate that the distribution of glycan species between the Rituximab biosimilar candidate and MabThera reference material is highly similar to the glycan distribution reported by the Agilent Gly-Q Glycan Analysis System, with minor differences in relative percent area. The Aetos Biosimilar candidate compared against one lot of MabThera (Figure 6) was compared against nine additional lots of the innovator, and was deemed to be within 2.5 standard deviations (data not shown). The AdvanceBio Gly-X N-Glycan sample preparation protocol in combination with the high-throughput Gly-Q system allows for rapid, high-throughput screening of a large number of clones, effectively minimizing the burden on upstream process optimization.
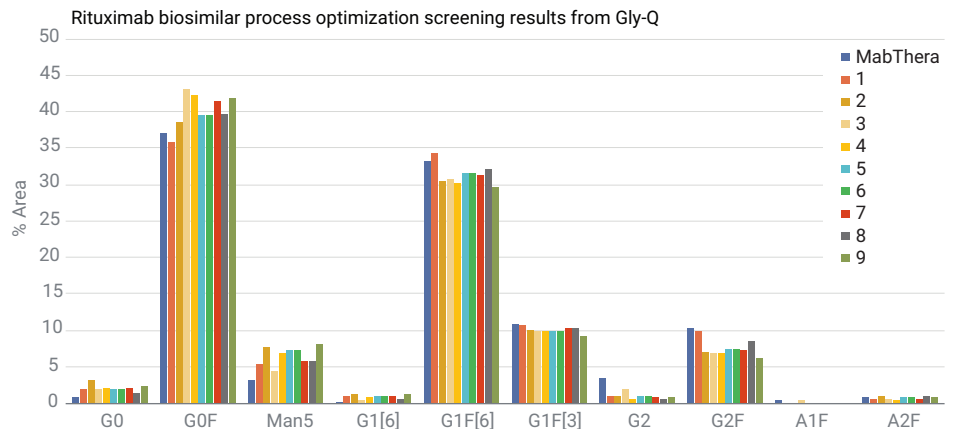

Figure 5. Rituximab biosimilar process optimization screening results from Agilent Gly-Q. Upon selection of several top clones from the FBQ stage, the first set of process optimization experiments were initiated. Data labeled 1 to 9 are a subset from several process conditions tested in this initial optimization experiment. N-Glycan profiles for all process optimization conditions were generated using Gly-Q, allowing for rapid initiation of subsequent process optimization experiments. It was determined that the N-glycan profile from condition 1 was closest to that of the MabThera innovator based on Gly-Q data output. The N-glycan profiles of Aetos' Condition 1 and MabThera were further confirmed using UHPLC-HILIC (see Figure 6).
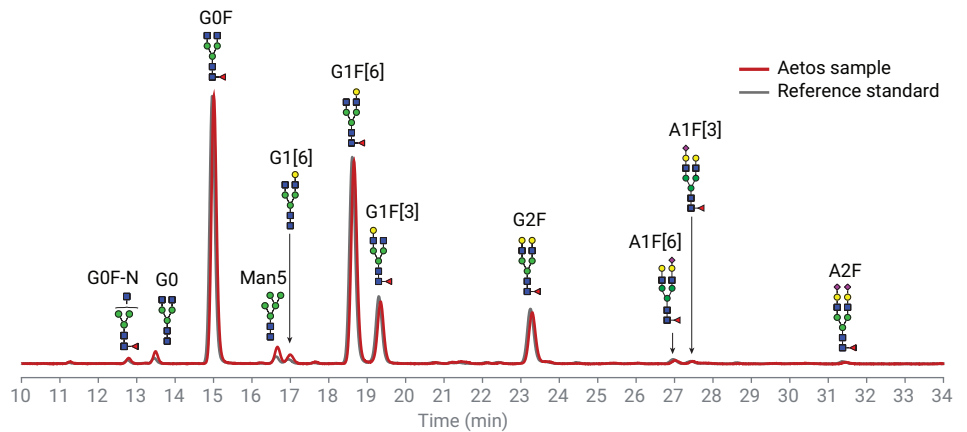

Figure 6. UHPLC-HILIC fluorescence profile of Agilent InstantPC-labeled N-glycans from the MabThera reference standard (gray) and the Aetos biosimilar candidate clone (red). The UHPLC analysis depicted here compares a single lot of the innovator molecule against the Aetos biosimilar for the purposes of analytical comparison against the Gly-Q system. Aetos has compared its biosimilar candidate against 10 lots of the innovator product, and has deemed the biosimilar glycan profile to be highly similar (within 2.5 standard deviations), data not depicted.

Conclusion

Using the Celltheon SMART Technology Platform, a novel glycoengineering workflow is presented, relying on the high-throughput methods of the AdvanceBio N-Glycan Preparation and Agilent Gly-Q Glycan Analysis System. This allowed Aetos to screen and identify top Rituximab biosimilar clone candidates and optimal processes in less than four months from transfection. The benefits of this platform and workflow are:

- Greatly reduced labor for N-glycan sample preparation and data analysis per sample

- 10-fold increase in N-glycan sample throughput due to decreased sample run and prep time compared to conventional methods of analysis such as UHPLC-HILIC

- Early, high-throughput identification of multiple high-expressing candidates not only based on titer but also CQAs

- Reduced burden, risk, and timeline on process optimization strategy by early rejection of clones that do not meet CQA acceptance criteria

- Faster development timelines: less than four months from transfection to top clones

These workflows and optimized technology have shown robust reproducibility, resulting in the development of multiple high-expressing biosimilar candidates (>4 g/L) by Aetos.

References

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