# AUTOMATING SUBUNIT MAB ATTRIBUTE SCREENING FOR IN-PROCESS MONITORING OF AN AUTOMATED HIGH-THROUGHPUT MULTI-PARALLEL BIOREACTOR

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## INTRODUCTION

**Subunit level information is highly desirable for its ability to provide product critical quality attribute (CQA) information of light chain, heavy chain and detailed glycoform level information.**

**Comparison across multiple bioreactors and conditions ensures that the desired profile can be being produced correctly under various bioreactor profiles or conditions.**

**CQA monitoring of upstream samples using LC-MS requires sample preparation that is often time consuming and potentially prone to error, so it is desirable to simplify sample processing. Automation and coupling with rapid LC-TofMS analytics enables routine monitoring of process development samples in a high throughput fashion.**

**Here we describe a simple LC-MS solution for upstream PD groups to provide key CQA analysis of in-process samples produced from high-throughput multi-parallel bioreactor samples, which includes:**

- **Automated sample processing**
- **Rapid LC-MS methodology for subunit level analysis**
- **Reporting of rich comprehensive analytics that can be used to drive PD decision making.**

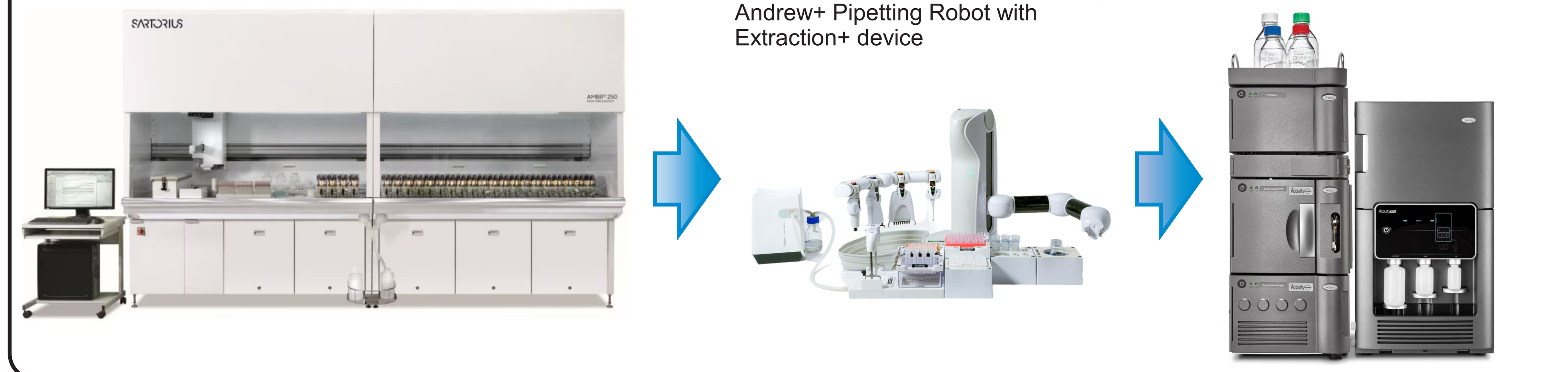


*Figure 1. Overview of sample collection, automated sample preparation and LC-MS analysis platforms*

## METHODS

Experiments were performed using the automated Ambr® 250 High Throughput Bioreactor System (Sartorius Stedim NA, USA). Five bioreactors were monitored in parallel where starting media, feed and inoculation density were varied (table 1) and samples were harvested at day 4, 6, 8, 10 and 12

Samples were processed for direct intact mass analysis, cell culture media analysis (see ASMS poster ThP 623) and also processed for subunit analysis using the Andrew+ pipetting robot system (Waters Corporation, USA).

For protein A purification, 120 uL of sample was transferred to an Andrew+ pipetting robot and loaded onto Protein A resin which had been washed and conditioned. Samples were washed, then eluted with 100mM glycine using the Andrew Extraction+ device and collected onto a 700uL plate.[1]

| Bioreactor | Media | Feed | Inoculation Density |
|---|---|---|---|
| 1a | A | 1 | Low |
| 2a | A | 1 | High |
| 5a | A | 2 | Low |
| 8a | B | 1 | Low |
| 10a | B | 2 | Low |

*Table 1. Ambr® 250 bioreactor system conditions. Samples harvested at day 4,6,8,10, and 12 for each bioreactor*

Subunit digestion was performed using FabRICATOR® (IdeS) digestion (Genovis, SE) and subsequent DTT reduction to yield mAb LC, Fd' and Fc subunits (Figure 2) and plate was transferred to a BioAccord™ LC-MS System (Waters Corporation, USA). The LC-MS analysis of the mAb subunits was performed using a 4.5 minute reversed phase gradient UPLC method with a BioResolve™ RP mAb Polyphenyl column at 60°C using 0.1% formic acid and acetonitrile mobile phase. MS data were acquired in ESI +ve from 400-7000 m/z. The Intact Mass App in the waters_connect™ LC-MS Informatics System (Waters Corporation, USA) was used for data acquisition and processing.

## RESULTS/DISCUSSION

**Fully automated sample preparation of cell culture media from 5 bioreactors (day 4 through day 12) for purification and digestion were performed in a 96-well plate format by the Andrew+ liquid handling robot (Figure 1). To ensure accurate subunit assessment of the product, affinity purification (protein A) of mAb from cell culture was performed prior to analysis.**

**The total automated sample preparation time for a 48 well plate is under 3 hours, including setup.**

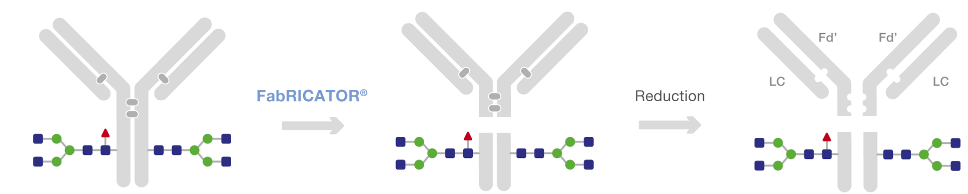


*Figure 2. FabRICATOR® enzyme produces the Fc fragment and was further reduced to generate the LC and Fd' fragments. Figure used with permission (Genovis, SE)*

**LC, Fd', and Fc/2 glycoform profiles (Figure 3) were routinely monitored using the workflow and difference in isoforms monitored throughout the campaign across multiple bioreactor conditions. Glycation profiles for LC and Fd' fragments are shown in Figure 4.**

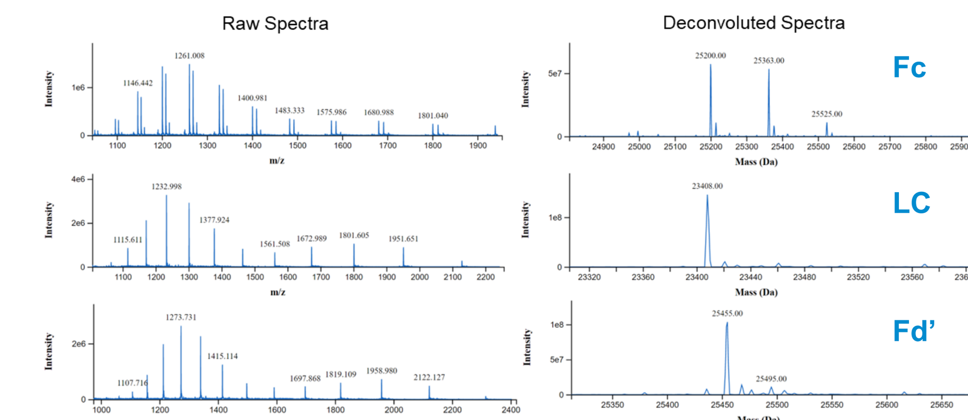


*Figure 3. Raw spectra and automatically deconvoluted spectra for Fc, LC and Fd' regions*

*Figure 4. Glycation levels for LC and Fd' per bioreactor over time*

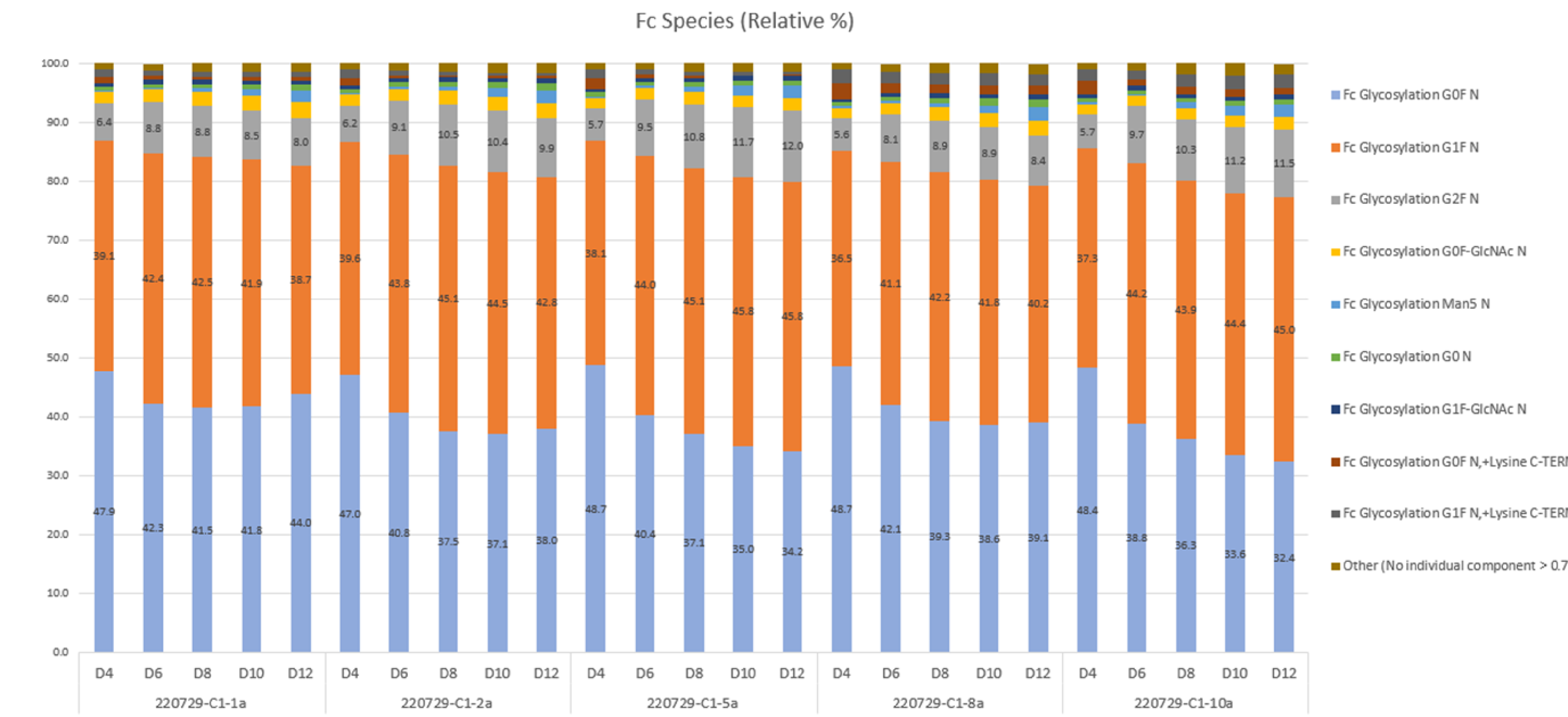


*Figure 5. Relative species stacked plot for Fc region by bioreactor and over time profiles*

**Detailed results from Fc subunit level analytics were conducted to profile glycan profiles in detail. Relative % modification values below 0.1% were successfully measured and reported as a stack plot in Figure 5. Specific quality measures were further reported as trends across bioreactors**

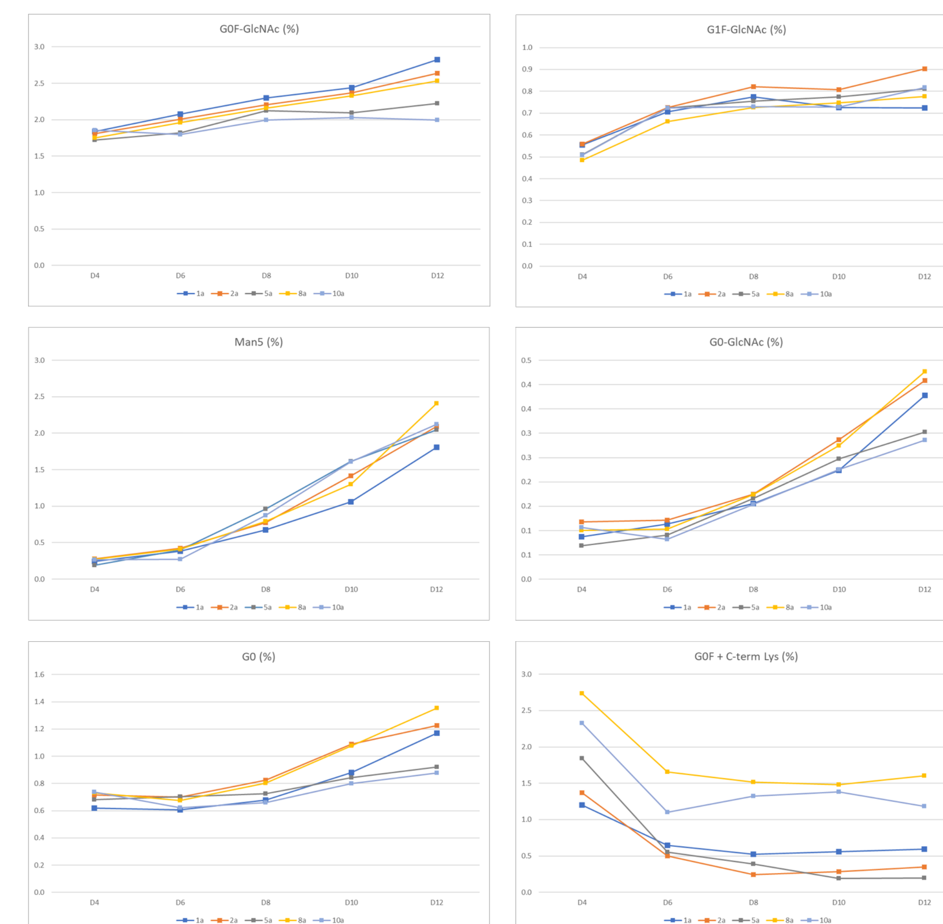


*Figure 6. Top 6 Fc glycoform species over time, showing major fucosylated, Man5 and afucosylated species and bioreactor variance*

*Figure 7. Major sialic acid species measured across varying PD media and feed conditions over time*

*Figure 8. Man 5 (mannose) levels across varying PD media and feed conditions over time*

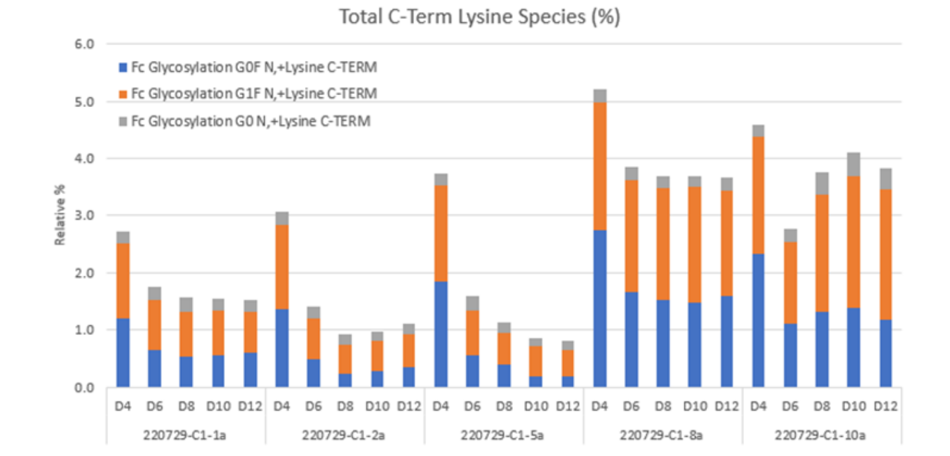


*Figure 9. Lysine processing levels across varying PD media and feed conditions over time*

**Major Fc glycoforms, including fucosylated, afucosylated, Man5 and a C-term lys product are reported (from D4 to D12) in Figure 6 showing different responses/levels across bioreactors. Major sialic acid species are reported in Figure 7 showing notable differences in bioreactor 8 and 10 vs other conditions. Man5 trends and detailed C-term lysine products are reported in Figures 8 and 9, respectively.**

**Differences in media and feed resulted in observable PQ differences in the monoclonal antibody. Media B conditions in particular resulted in increased levels of observed C-terminal lysine products and some sialic acid species.**

## CONCLUSIONS

- **Automated mAb subunit analysis sample preparation (protein A purification & digestion) of Ambr® 250 High Throughput Bioreactor System samples using the Andrew+ Pipetting Robot System demonstrated**
- **Successfully processed subunit data using waters_connect Intact Mass Application**
- **Glycan profiles as the results of subunit analysis associated the observed differences in galactose, fucosylation, mannose and lysine modifications with media and feed conditions across bioreactors**
- **Easily monitored several key CQAs during PD study**

**Reference**

1. Koza, Hanna, Jiang, Yu, "Automated HT Analytical-Scale Monoclonal Antibody Purification Using Production-Scale Protein A Affinity Chromatography Resin 2023 Waters Appnote, 720007861

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