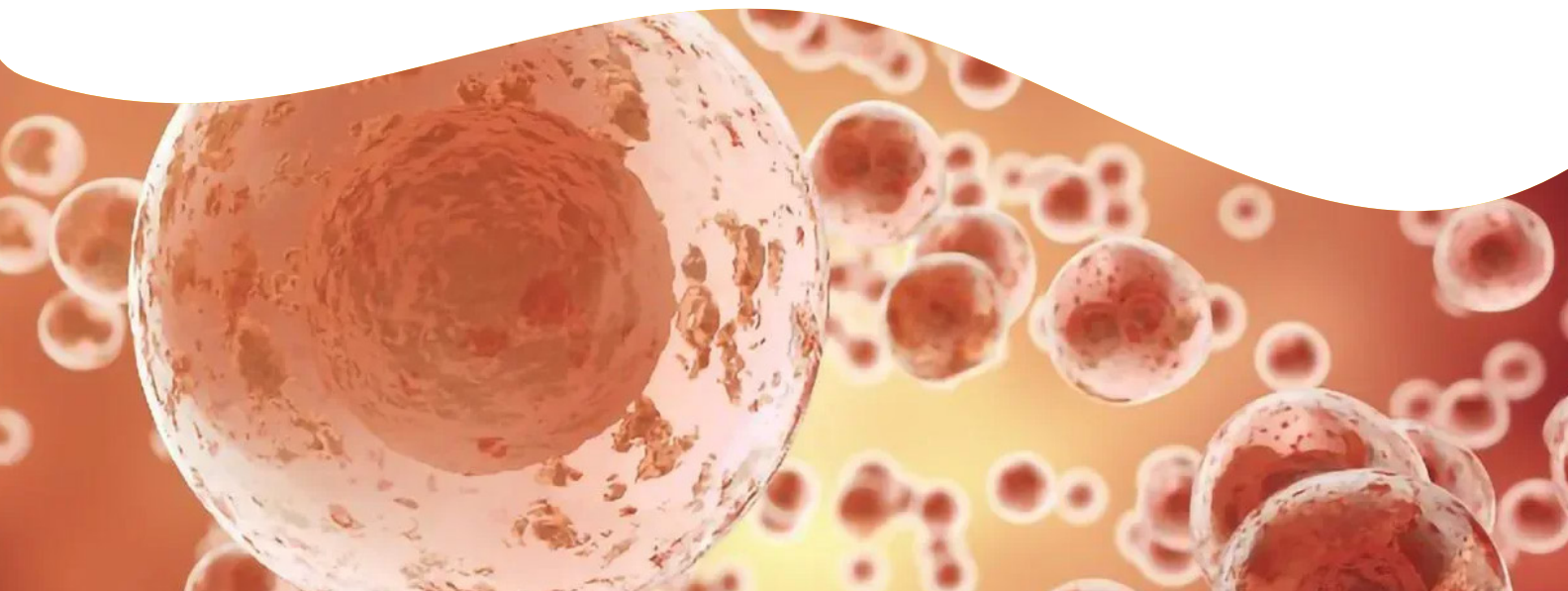


# High-throughput evaluation of product quality in cell line generation and upstream process development.

## Introduction

As advances in laboratory automation have enabled increased throughput, researchers can now take advantage of design of experiments (DoE) and Quality by Design (QbD) studies to gain faster insights into drug discovery. These studies allow researchers to glean a greater and often accelerated understanding of expression parameters, purification conditions, stability and functional aspects of their protein(s) of interest. The large number of samples produced from these experiments (e.g. monitoring the post-translational modifications from cell culture optimization or the downstream purification process) often greatly exceed the capacity of modern analytical laboratories.

Herein, we provide a case study on the use of the LabChip™ GXII Touch™ microfluidics platform to analyze antibodies produced through Rentschler's TurboCell™ fast-track cell line development platform, based on the CHO-K1-derived master TurboCell™ in-house host cell line. The multitude of microfluidic LabChip assays available provided a high-throughput workflow for data generation and analysis of sample purity and quantification with the capability to resolve low level impurities under reducing and non-reducing conditions. The combination of monitoring the charge heterogeneity variants of the proteins, as well as the upstream cell culture parameters on N-glycan patterns enables efficient and continuous process development.



## Materials and methods

Antibody producer cell lines were generated by site-directed recombinase-mediated cassette exchange. Antibody containing cell culture supernatants were purified by protein A batch purification in 96-well filter plates. Elution fractions were neutralized and analyzed by the LabChip GXII Touch HT instrument. The assays for evaluation of N-glycosylation (LabChip ProfilerPro Glycan Profiling Assay), charge heterogeneity profile (LabChip Protein Charge Variant Assay) and protein integrity (LabChip Protein Express 200 Assay) were performed in accordance to the user manuals.

## Results and discussion

### Method comparability

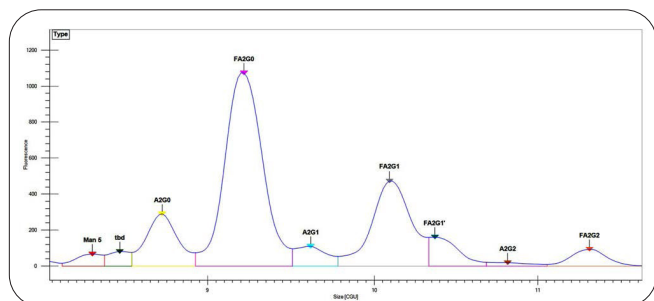


Figure 1: Electropherogram of a typical IgG glycan profile.

In contrast to most conventional methods for N-glycan determination, the LabChip system provides a high-throughput method for complete sample preparation (protein denaturation, deglycosylation by PNGase F, fluorescent labelling of the glycans) of 96 samples within four hours, followed by microchip-based CE (capillary electrophoresis) separation in 45 sec per sample. A typical electropherogram profile of a monoclonal antibody (mAb) is shown in Figure 1 using the LabChip GX Reviewer Software. Glycan relative abundance is determined as area under the curve compared to the total glycan abundance.

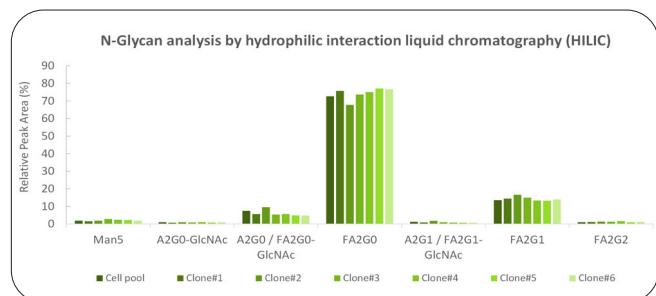


Figure 2: N-glycan expression pattern from six different IgG clones and the corresponding cell pool, as determined by hydrophilic liquid interaction chromatography (HILIC).

For evaluation of the LabChip Glycan Profiling Assay, glycosylation patterns were compared to results from traditional N-glycan analysis by 2AB-labeling and hydrophilic interaction liquid chromatography (HILIC). The glycosylation screening profiles of an early stage TurboCell™ clonal IgG1 molecule from screening are shown in Figure 2 and Figure 3.

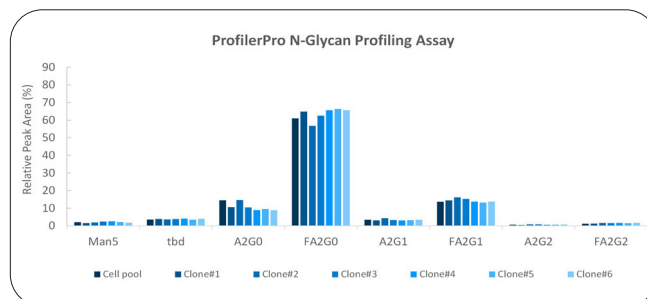


Figure 3: N-glycan expression pattern from six different IgG clones and the corresponding cell pool, as determined by the LabChip N-Glycan Profiling Assay. (tbd: to be determined)

Figures 2 and 3 illustrate highly comparable N-glycosylation profiles for an IgG1 from several TurboCell™ clones and the corresponding cell pool. An A<sub>2</sub>G<sub>0</sub>-GlcNAc standard was not available for the LabChip assay (tbd); however, similar profiling pattern was observed. Even minimum differences in glycan distribution (e.g. Clone#2) are detectable by both methods, demonstrating that the LabChip Glycan Profiling Assay has similar sensitivity as more traditional methods. Furthermore, the high-throughput setup and data generation of this assay make it highly applicable for N-glycan determination in early stage evaluation of mAb product quality.

### Monitoring modulation of N-glycosylation profile during upstream process development

In upstream process development, it might be necessary to modulate N-glycan patterns to obtain desired glycosylation profiles. Variations in the glycan profile of an IgG can be introduced with altered media and feeding strategies or changes in process parameters. Figure 4 shows the N-glycosylation profile of a selected IgG TurboCell™ cell clone in two different cultivation processes. Process 1 (orange) results in similar glycan pattern as observed earlier in the TurboCell™ clone screening (Figure 2). To increase the level of galactosylation, process parameters were modified (Process V2, blue). Monitoring these modifications of the glycan profile indicate successful scale-up from 0.25 L shake flask to 50 L single-use bioreactor growth environments, with equivalent glycan profile patterns.

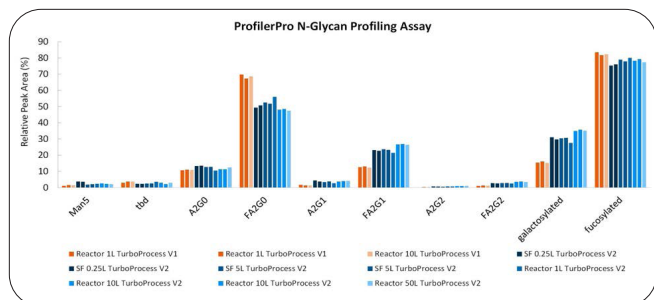


Figure 4: N-Glycan pattern comparison of a selected IgG clone for two different TurboCell™ cultivation processes. Process V1 (orange), corresponds to the cultivation process used for cell line development. A shift in the N-glycan pattern, desired increase in galactosylation, can be achieved through the alteration of process parameters (Process V2, blue). Scalability from 0.25 L shake flask (SF) to 50 L single-use bioreactor is shown.

### Controlling protein integrity and charge heterogeneity during upstream process scale-up

Besides glycosylation, additional product quality attributes like protein integrity and charge heterogeneity were monitored during process scale-up.

The LabChip Charge Variant Assay facilitates the separation of different charged protein isoforms by a microfluidic capillary zone electrophoresis. Figure 5 shows a typical mAb profile generated in 110 seconds on the LabChip system. Early eluting peaks represent the basic protein variants, followed by the main peak and the acidic isoforms on the right.

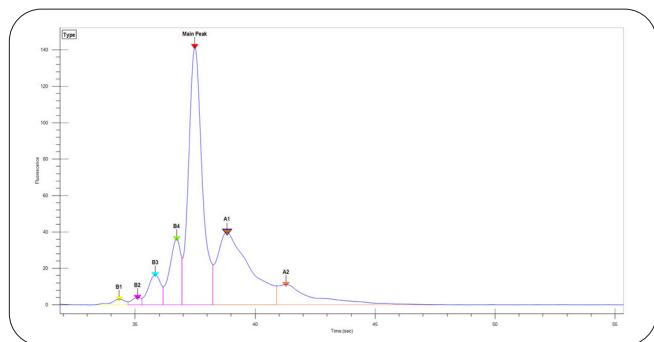


Figure 5: Electropherogram of IgG charge heterogeneity profile. Peaks eluting earlier (left) than the main peak represent basic protein variants, while acidic peaks elute after (right) the main peak.

The charge heterogeneity profile was monitored during scale-up of upstream process V2 (Figure 6). Results show comparable charge variant profiles regardless of performing the experiments in shake flasks or bioreactors.

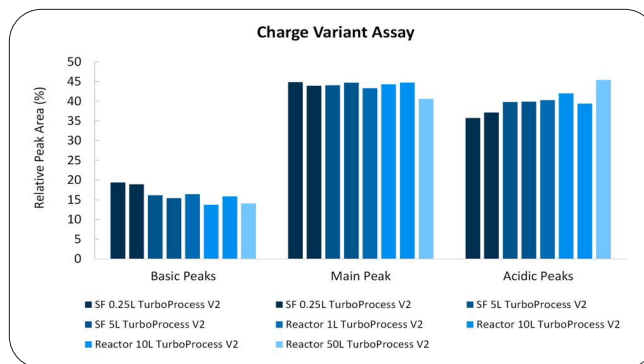


Figure 6: Monitoring of IgG charge heterogeneity pattern. High-throughput analysis using LabChip Charge Variant Assay to examine isoform pattern during process development shows comparable charge profile from shake flask (SF) to scale-up 50 L single-use bioreactors.

To assess product quality and integrity post protein A purification, proteins were separated according to their molecular weight in both reduced and non-reduced conditions on the LabChip Protein Express 200 Assay. The size (molecular weight) and purity of the intact mAb and its fragments (light chain, heavy chain, heavy-light chain, heavy-heavy chain, heavy-heavy-light chain) can be monitored for stability under non-reduced conditions (Figure 7). Consistent intact mAb purity was shown at >98% during the scaleup process (parameters V2) from 250 mL shake flask to the scale-up 50 L single-use bioreactor (Figure 8). Similarly under reduced conditions, sample purity and size assessment illustrate sufficient resolution to quantify non-glycosylated heavy chains (Figure 9) commonly referenced in process development. As referenced in Figure 8, reduced protein A purified mAb samples demonstrated > 99.6% stable and predictable purity from 0.25 L to 50 L lab scale studies.

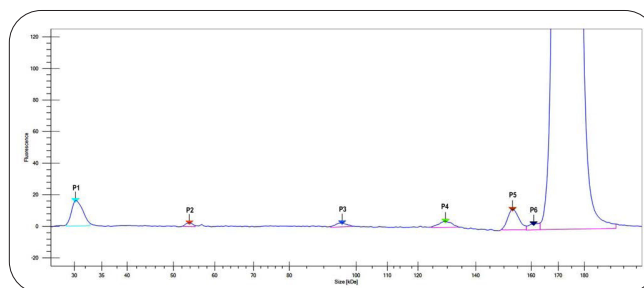


Figure 7: Non-reduced IgG electropherogram indicating various molecular weight mAb fragment peaks (P1-P6) and the desired intact mAb peak.

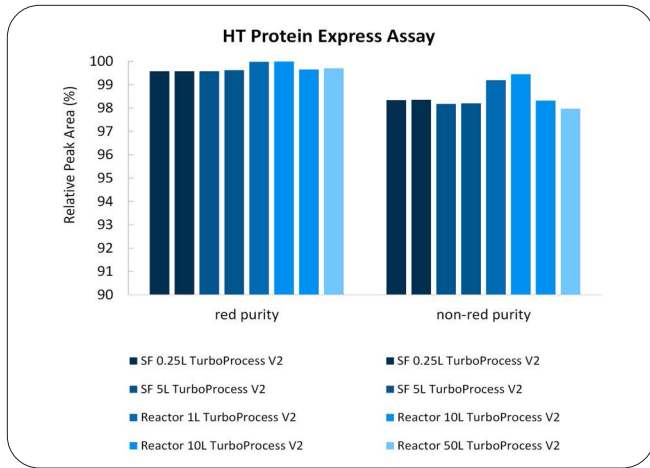


Figure 8: Monitoring IgG integrity during scale-up production with the LabChip Protein Express Assay 200. Measured intact antibody product was constantly high during scale-up with the shake flask to 50 L single-use bioreactors (reduced purity > 99.6 %; non-reduced purity > 98 %).

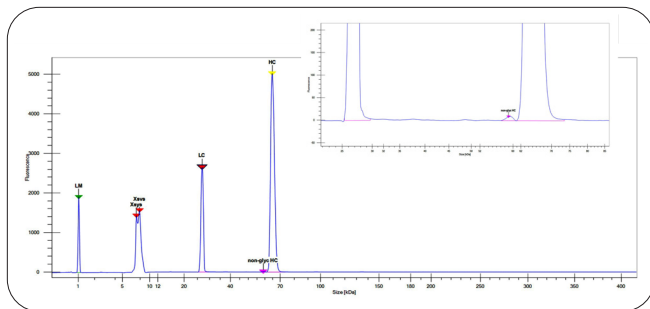


Figure 9: Reduced IgG electropherogram indicating light (LC) and heavy chain (HC) peaks, as well as non-glycosylated HC. LM = lower marker. Xsys = system peaks, not used in calculations.

## Conclusion

This case study demonstrates that the LabChip GXII Touch HT system presents a platform for high-throughput analysis of antibody product quality attributes. With a single instrument, researchers have the capability to rapidly generate and analyze data from early cell line cultivation through purification and stability with preferred product quality leading to accelerated process development.