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Application of High-Throughput methods for fast screening during Cultivation Process Development of monoclonal Antibodies

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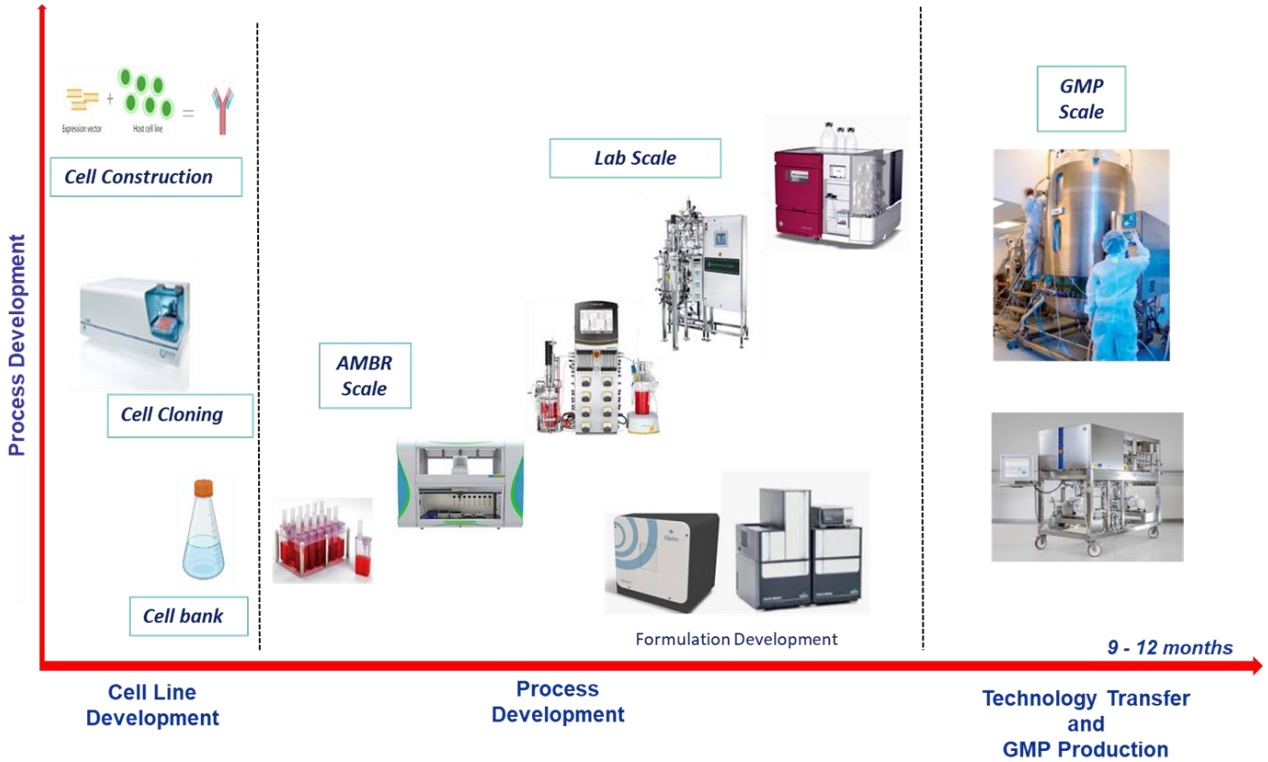
Introduction

Development of a robust and reproducible biopharmaceutical process for production of monoclonal Antibodies (mAbs) is a critical path activity in the overall process development timeline (**Figure 1**).

A platform of fast and diagnostic analytical methods is fundamental during cultivation process development for screening of different conditions to give sufficient data related to productivity and critical quality attributes and, at the same time, to make experimentation more cost effective, on tight timelines, by saving on labor, facility space, capital, media and consumables.

An overview of analytical high-throughput approaches to support cultivation process development during screening at AMBR scale is described in this article.

Figure 1: Overview of Process Development workflow



Background

During cultivation process development and optimization, the main criteria enabling the choice among different cell culture conditions are productivity and evaluation of critical quality attributes (CQAs).

Productivity in terms of mAb titer is usually determined by chromatographic method by protein A-High Performance Liquid Chromatography (HPLC).

Once titer is determined, a panel of methods to assess quality in terms of purity and identity is put in place, in particular: Size Exclusion Chromatography (SEC) by HPLC is the primary method used for aggregation analysis, Capillary Electrophoresis SDS (CE-SDS) for analysis of fragmentation, Isoelectrofocusing (IEF) or pH-gradient Ion Exchange Chromatography (IEC) to have a fingerprinting of charge variants distribution and analysis of glycans by Reverse-phase Ultra Performance Liquid Chromatography (UPLC) .

The effort directed towards the generation of a platform of faster methods keeping the diagnostic capability for screening a high number of samples, aimed to reduce time, equipments and costs.

As first, Ultra Performance Liquid Chromatography systems replaced HPLC ones, thus enabling the performance of faster chromatographic methods for both SEC [1] and IEC [2].

Then, the SEC-UPLC method was exploited as multipurpose analysis through the use of a calibration curve to allow determination of titers, in addition to aggregation [3].

A microfluidic electrophoretic separation system LabChip® GXII Touch™, coupled to different reagent kit and chips, was used as orthogonal method for determination of purity instead of capillary electrophoresis [4] and for glycan screening replacing UPLC chromatography.

Materials and Methods

A set of 34 Harvest samples containing human IgG at the end of cultivation at AMBR scale was analyzed. Combinations of different aminoacidic supplements and feeding conditions generated six different scenarios (**Table 1**) while culture medium and cultivation parameters were always the same; thus, a panel of analytical methods (**Table 2**) was tested for its capacity to determine if, respect to the reference group (standard conditions), the new combinations of cell culture conditions improved productivity and positively impacted critical quality attributes.

All analyses were performed on Harvest samples except for glycan screening that was performed on samples after one step of purification by affinity through Perkin Elmer® JANUS® G3 Bio Tx Pro Plus.

Table 1: Description of groups and respective cell culture conditions

Group	Description
EFB 3X (n=4)	Reference group in terms of amino acids concentration, feeding volume and feeding timepoints
MD (n=4)	Design of the the main feed medium as EFB 3X; all amino acid concentrations were allowed to vary between 0 (not in the medium) and twice the value in the benchmark process main feed medium
SMD (n=3)	Design of the the main feed medium as EFB 3X; all amino acid concentrations were allowed to vary between the current concentration and twice the value in the reference group.
PD (n=6)	Combined optimization of main feed medium composition as MD; optimization of feeding volumes between 0 (no feed) and the triplicate feed as compared to the reference group.
SPD (n=6)	Combined optimization of main feed medium composition as SMD; optimization of feeding volumes between 0 (no feed) and the triplicate feed as compared to the reference group.
EPD (n=10)	Combined optimization of main feed medium composition as MD; Optimization of feeding volumes between 0 (no feed) and the triplicate feed as compared to the reference group. A second instance of the main feed medium, i.e. an additional feed medium, was included that started with zero feeding volume and the same composition as EFB 3X but allowed to change in both composition and feeding volume independently from the first main feed medium

Table 2: Analytical methods

Method	System and main components	Run time 1 sample (min)
SEC-UPLC	Waters™ Acquity H-class Bio, UPLC system Waters™ Protein BEH SEC 200 Å, 4,6x150mm, 1,7µm column	**12**
IEC-UPLC	Thermo Scientific™ Vanquish™, UHPLC system Thermo Scientific™ MabPac SCX-10 RS 2.1X50 mm, 5µm, non porous (cation exchange) column	**23**
Electrophoresis by Labchip SDS	Perkin Elmer ® LabChip GXII Touch™ Perkin Elmer ® Protein Express Labchip and Protein Express Reagent kit	**<1**
Electrophoresis by Labchip for glycan assay	Perkin Elmer® LabChip GXII Touch™ Glycan release and labeling kit, HT Glycan Reagent Kit, 24 High. Resolution Labchip	**<1**

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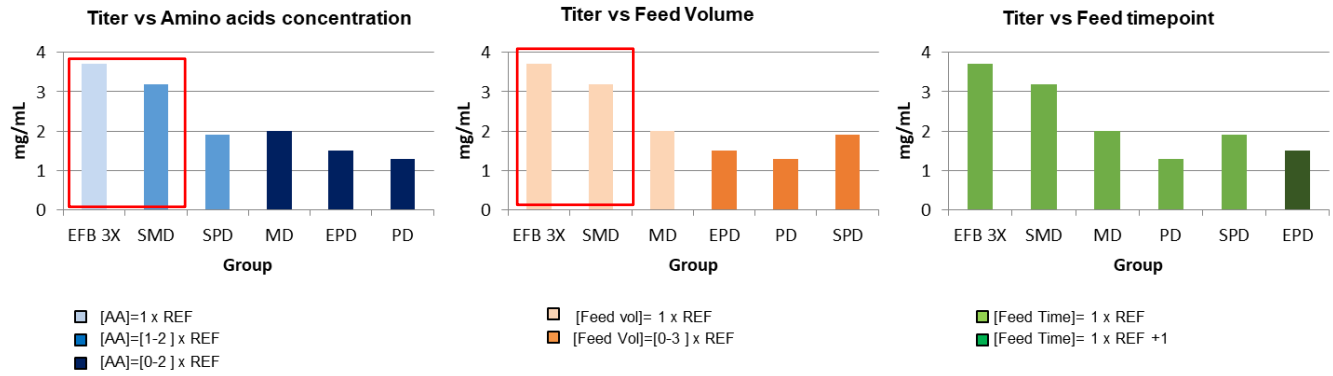
Results

For each analysis, the results shown are the mean of the samples in each group (see table 1 above).

Impact on titer

As shown in **Figure** 2, amino acids concentration combined with feed volume at least comparable to reference group have a positive impact on titer that is higher than 3 mg/mL at the end of cultivation (groups EFB 3X and SMD).

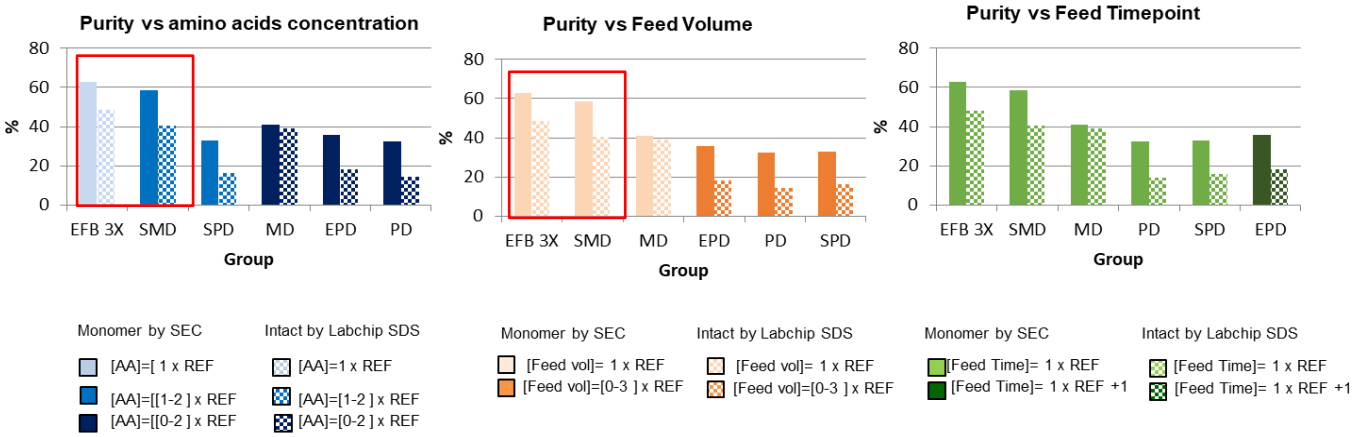
Figure 2: Impact of cell cultures variables on titer



Impact on purity

As shown in **Figure 3**, amino acids concentration combined with feed volume at least comparable to reference group have a positive impact on both aggregation and fragmentation, showing the highest % of monomer and intact IgG (groups EFB 3X and SMD).

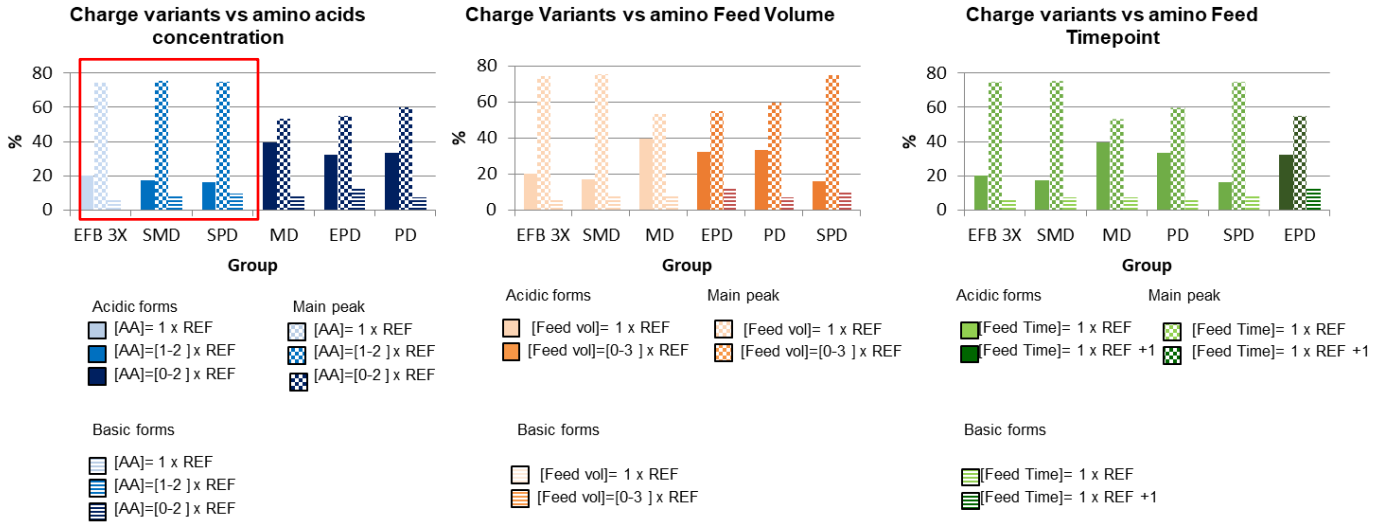
Figure 3: Impact of cell cultures variables on purity



Impact on charge variant profile and galactosylation

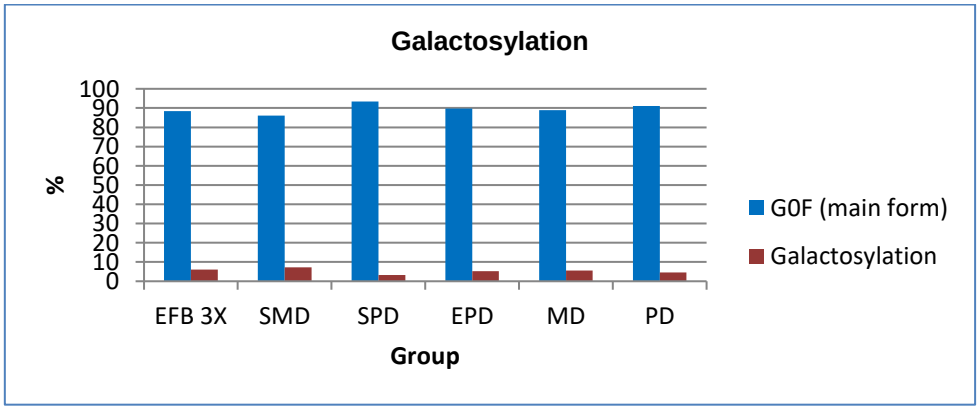
As shown in **Figure 4**, only amino acids concentration at least comparable to reference group impacts on charge variants profile, showing the highest % main peak and the lowest % of acidic forms (groups EFB 3X, SMD and SPD).

Figure 4: Impact of cell cultures variables on charge variants profile



As shown in **Figure 5**, any variable has impact on galactosylation, indeed all groups show, as main form, the one without galactose.

Figure 5: Impact of cell cultures variables on galactosylation



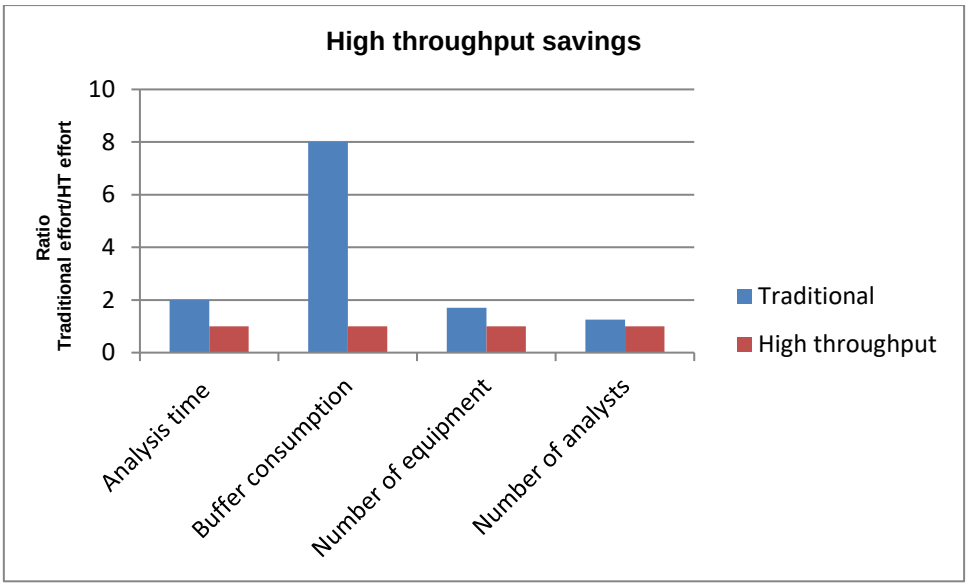
Conclusion

In this white paper, a panel of fast and high throughput methods have been applied to verify if some induced modifications in cultivation process could improve productivity or positively impact critical quality attributes.

All methods were in agreement, confirming that the most performing group remains the reference one (EFB 3X) in terms of higher titer/productivity at the end of cultivation, lowest level of aggregation and of fragmentation, highest abundance of main peak in charge-variants profile.

Finally, we quantified the effective advantages coming from the application of fast and high throughput methods instead of traditional ones (see **figure 6**).

Figure 6: High throughput methods advantages



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