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Repeatability of C100HT Biologics Analyzer a High Throughput Glycan Screening

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INTRODUCTION

The ability to screen large numbers of samples in clone selection and cell culture analysis is an important consideration to provide efficiency in choosing drug candidates. Extensive screening of potential clones for key selection criteria earlier can facilitate shorter times to identify pipeline candidates and growth conditions. Screening of glycan association is an important indicator for many molecular characteristics including potential function, immunogenicity, manufacturability and, in the case of biosimilar drugs, their bio-similarity to innovator molecule. Therefore, inclusion of glycan screening early in the selection process can assist in better identification of viable candidates for downstream analytical processes.

The C100HT Biologics Analyzer is a qualitative, multiplexed capillary electrophoresis platform that provides scientists in the bioprocessing segment the unique capability of screening glycan expression profiles for as many as five 96 well plates of glycans samples in one day.

The C100HT system consists of an assay kit with reagents to prepare up to ten 96 well plates using a validated protocol amenable to preparations both manual and/or automated. The assay kit includes a 12-capillary cartridge pre-filled with separation gel and pre-filled buffer trays making instrument set-up and run fast and easy. Intuitive system control software enables easy navigation, fast analysis, and automated data integration, providing users with corrected area %, glycan ID, and relative migration time for a full 96 well plate of samples in approximately 3 hours.
As C100HT can analyze large number of samples in an automated fashion, reliability of the system and reproducibility of data is critical. This technical note highlights the repeatability of the C100HT glycan screening assay as it relates to the reproducibility of the instrument and separation cartridge..

MATERIALS AND METHODS

The SCIEX C100HT Biologics Analyzer is equipped with LED-induced fluorescence (LED-IF) detection with excitation at 465 nm and emission at 540 nm, which allows for the detection of 1-aminopyrene-3,6,8-trisulfonate (APTS) labeled N-glycans.

The purified antibody - MAK33 - was obtained from Roche Diagnostics (Indianapolis, IN) and was diluted to 10mg/mL before sample preparation. The CCS sample was a gift from Beckman Coulter (Chaska, MN) and was concentrated to 10mg/mL prior to sample preparation. PNGase F was obtained from New England Biolabs (Boston, MA). Acetonitrile was obtained from Burdick-Jackson. A 1M solution of sodium cyanoborohydride in THF was obtained from Sigma-Aldrich (St Louis, MO). Reagents used to prepare the denaturation solution, digestion solution, and labeling solution were provided in the chemistry kit (Fig. 1) (SCIEX, PN C13787). A glycan panel was prepared by labeling and mixing 9 synthetic species (G0, G0F, G1, G1', G1F, G1'F, G2, G2F and Man5) which were obtained from Prozyme (Hayward, CA).

Briefly1, 100 µg of antibody sample is mixed to the magnetic beads. A denaturing solution is added and the mixture is heated denatured for 8 minutes at 60° C. This mixture is then digested for 20 minutes at 60° C using PNGase F. The resulting glycans were captured by the magnetic beads. The glycans were then labeled with the addition of an APTS master mix for 20 minutes at 60° C. Excess free APTS was removed using a bead-mediated cleanup process repeated 3 times. The labeled N-linked glycans were eluted from the beads by adding 50 µL of water and are ready for analysis using the C100HT Biologics Analyzer (Fig. 9). To simplify the large volume of glycan sample preparation required for this work, a Biomek i5 series liquid handler from Beckman Coulter (Indianapolis, IN) equipped with a 300 µl 96 pipetting head liquid handler platform was used.

Figure 1. C100HT Glycan Assay Kit

RESULTS

Repeatability of Corrected Area %

To showcase the repeatability between instruments and between cartridges, relative migration time and corrected area % (CA%) for the glycan species G0F, G1F and G2F was assessed. These glycan species were chosen because they are commonly associated with many therapeutic antibodies and in this sample set, represent peak corrected area % from high to low intensity. Figure 2 shows the average of corrected area % G0F, G1F and G2F observed during this study where 3 C100HT systems and 5 cartridges were used for a total of 865 runs performed.

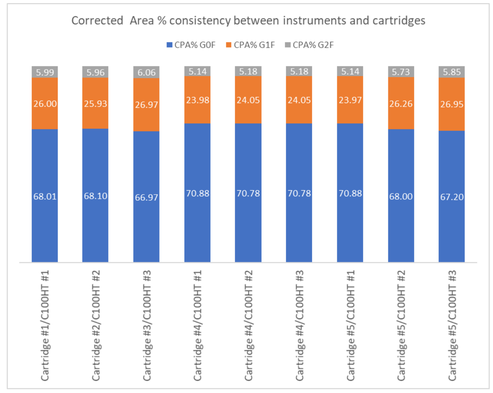

Figure 2. Corrected Area % for G0F, G1F and G2F glycans from MAK33 antibody drug product between instruments and between cartridges.

Table 1 shows the %RSD observed for the experiments illustrated in figure 2, showcasing the CA% consistency of C100HT System.

%RSD			
Scheme	CPA% G0F	CPA% G1F	CPA% G2F
Cartridge #1/Instrument #1 (N=96 runs)	0.91	1.97	1.94
Cartridge #2/Instrument #2 (N=96 runs)	0.36	0.88	0.89
Cartridge #3/Instrument #3 (N=96 runs)	1.61	3.15	4.11
Cartridge #4/Instrument #1 (N=96 runs)	0.35	0.92	1.47
Cartridge #4/Instrument #2 (N=96 runs)	0.26	0.62	1.46
Cartridge #4/Instrument #3 (N=96 runs)	0.26	0.62	1.46
Cartridge #5/Instrument #1 (N=96 runs)	0.35	0.92	1.47
Cartridge #5/Instrument #2 (N=96 runs)	0.80	1.90	1.22
Cartridge #5/Instrument #3 (N=96 runs)	0.96	1.94	2.41

Resolution Between Man5 and G0

Traditionally, the glycan species G0 and Man5 are difficult to resolve using current LC and some CE separation technologies. However, separation using the C100HT consistently can overcome this analytical challenge. Figure 3 shows a typical separation obtained for a panel of 9 glycans, and inside the red block the resolution between G0 and Man 5 peaks. The specification for resolution between G0 and Man 5 on C100HT is minimum of 1.5.

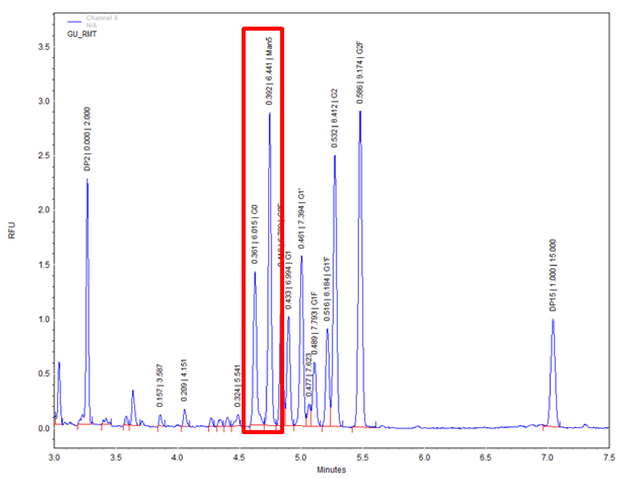

Figure 3. Typical electropherogram of a panel of 9 glycans. The red block highlights the baseline resolution between G0 and Man5.

The chart in figure 4 illustrates the consistency of the resolution between G0 and Man 5 observed in 3 cartridges and 3 C100HT instruments for a total of 768 runs. The reproducibility observed for resolution was 1.52% (n=768 runs).

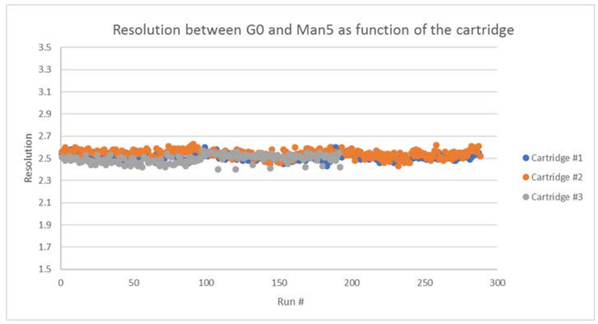

Figure. 4 Resolution observed between G0 and Man 5 across 3 cartridges and 3 instruments.

C100HT Cartridge run life

To demonstrate the run life of C100HT cartridge, one cartridge was studied for corrected area %, relative migration time and GU value assignment. Similarly, to the previous section, the released N linked glycans sample used was from a purified monoclonal antibody (MAK33) and was prepared using the Biomek i5 series liquid handler. Three sample preparations were performed in 3 different days. For each day, the sample was pooled to avoid the possible sample-to-sample variation derived from the liquid handler itself. Figures 5 through 7 show the consistency of corrected area %, GU value assignment and relative migration time across the life of the cartridge. For simplicity only the last, or tenth run representing 88-96th injection runs for a total of 960 samples respectively on the cartridge are shown.

The behavior for corrected area % shows a good consistency across the cartridge run life, the GU value assignment and relative migration time show even tighter consistency demonstrating not only excellent repeatability of the cartridge as it ages, but the outstanding precision on Glycan identification throughout the cartridge life.

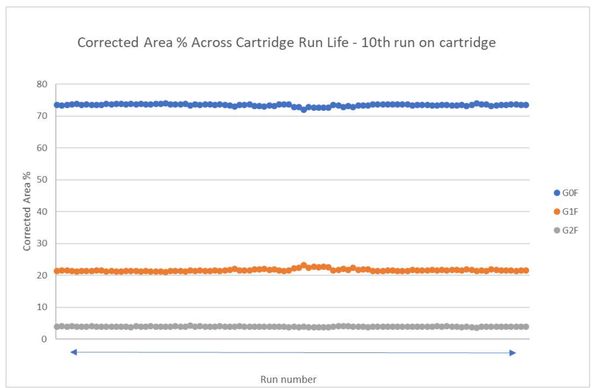

Figure 5. Corrected Area % behavior observed across the cartridge run life.

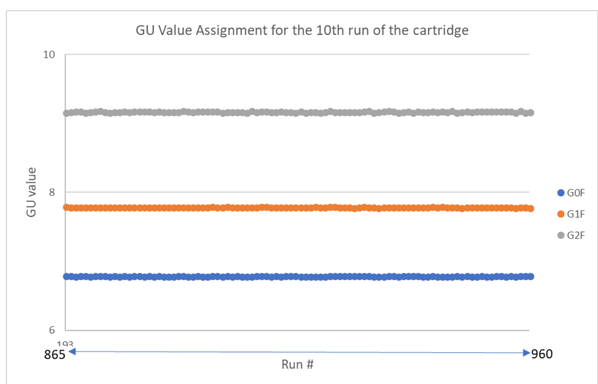

Figure 6. GU value assignment behavior observed across the cartridge run life.

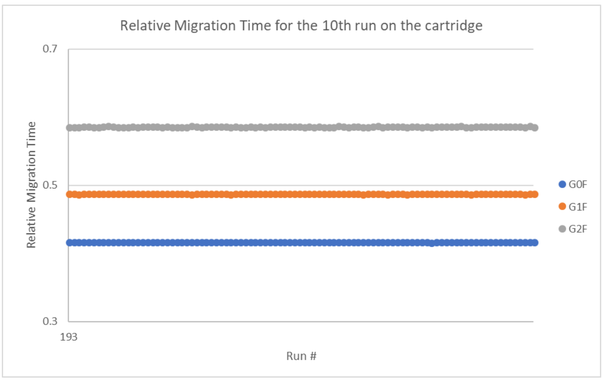

Figure 7. Relative migration time behavior observed across the cartridge run life.

CONCLUSIONS

In the bioprocessing environment, analysis of hundreds to thousands of samples is required to efficiently select pipeline candidates for further study. This process is generally a screen requiring fast time-to-result to accelerate decision making. For this reason, a reliable and robust high throughput analytical platform capable of screening this large number of samples in a short period of time is highly desirable. The workflow of C100HT Biologics Analyzer and Glycan Analysis Assay timeline (Figure 8) delivers fast separation with robust and reproducible results as illustrated by the repeatability and cartridge run life study shown in this poster.

Figure 8. C100HT Workflow timeline

Figure 9. C100HT Biologics Analyzer

REFERENCES

1 . C100HT Biologics Analyzer System - Operator Guide; RUO-IDV-05-3828-A

TRADEMARKS/LICENSING

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