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Fully Automated C100HT Biologics Analyzer Sample Preparation on a Biomek i5 Multichannel Workstation

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Introduction

Glycosylation of monoclonal antibodies affects their solubility, structural stability and clinical efficacy. As a result, there is an increasing need for high-throughput N-glycan screening in all phases of therapeutic development, especially in clone selection and cell culture optimization. The SCIEX C100HT Biologics Analyzer is a high-throughput glycan screening platform utilizing a buffer-filled multi-capillary cartridge, enabling end users to process hundreds of samples per day. However, manual glycan sample preparation is often tedious and time consuming. When sample size increases, manual sample preparation becomes less effective. The full benefit of the C100HT Biologics Analyzer in high-throughput glycan screening can be realized with automated glycan sample preparation. This technical note describes an automated, high-throughput glycan sample preparation on the Biomek i5 Multichannel Workstation that supports the high-throughput requirements of the C100HT Biologics Analyzer.

Key Features

- Fully automated glycan sample preparation

Saves time and resources
- Reproducible high-throughput glycan screening

Materials and Equipment

PNGase F (PN P0709L) was purchased from New England Biolabs (Boston, MA). Acetonitrile (PN AH015-4) was obtained from Honeywell-Burdick-Jackson (Muskegon, MI). A 1M solution of sodium cyanoborohydride in tetrahydrofuran (PN 296813) was obtained from Sigma-Aldrich (St Louis, MO).
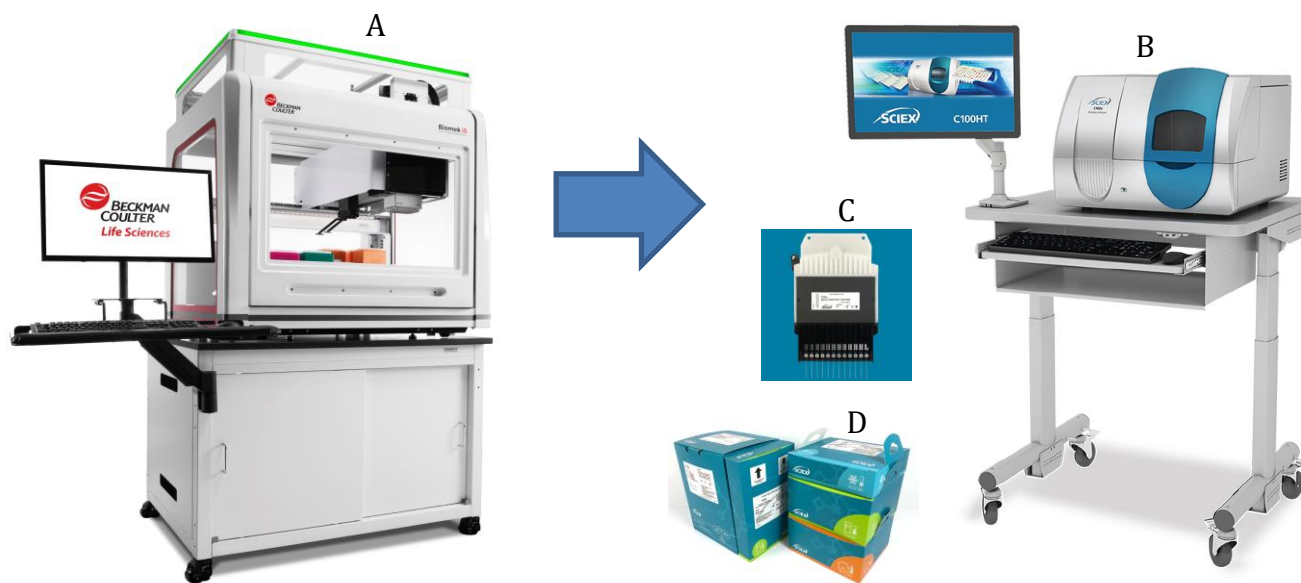

Figure 1- Beckman Coulter Biomek i5 Multichannel Workstation (A), SCIEX C100HT Biologics Analyzer (B), C100HT Glycan Labeling and Analysis Cartridge (C), and C100HT Glycan Labeling and Analysis Kit (D).

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MAK33, a commercial monoclonal antibody, was obtained from Roche Diagnostics (Indianapolis, IN). Except the reducing agent and PNGase F, all reagents needed to prepare the denaturation, digestion, and labeling solutions were provided in the C100HT Glycan Labeling and Analysis Kit (SCIEX, PN 5055078, Figure 1). The labeling dye is 1-aminopyrene-3,6,8-trisulfonate (APTS). The C100HT Glycan Labeling and Analysis Cartridge with 12 capillaries (PN 5055075, Figure 1) was from SCIEX, Framingham, MA.

The Biomek i-Series 230 µl (PN B85906) and 90 µl (PN B85881) pipette tips were from Beckman Coulter Life Science (Indianapolis, IN). The deep well plate (PN 96-6009) and Reservoir (PN 201244-100) were from Chrom Tech, Inc. (Apple Valley, MN). Magnum EX Universal Magnet Plate was from Alpaqua (PN SKU A000380).

Biomek i5 Multichannel Workstation with enclosure (PN B87583, Figure 1) along with software and hardware (Biomek software version 5.0.97, Biomek Method Launcher 3.0.0, 1x1 Static Automated Labware Positioners, 96-well Multichannel wash station: B87689, Orbital Shaker: 379448, Static Peltier: A93938) was obtained from Beckman Coulter, Indianapolis, IN.

A C100HT Biologics Analyzer (PN 5055074, Figure 1) with software version 1.0 was from SCIEX (Framingham, MA). Equipped with light emitting diode-induced fluorescence (LED-IF) detection with excitation at 465 nm and emission at 545 nm, it efficiently detects APTS labeled N-glycans.

Methods

Fully Automated Glycan Sample Preparation on Biomek i5 Multichannel Workstation

Overview of the Workflow

The fully-automated glycan sample preparation workflow is illustrated in Figure 2. Start with selecting the "SCIEX Fast Glycan Preparation" method on the Biomek i5 Multichannel Workstation, followed by entering number of samples to be processed (Figure 3), and then use the Biomek Guided Labware Setup instructions to setup the deck (Figures 4 and 5). After deck setup on the Biomek is complete, samples are denatured using on-deck Peltier to enable efficient glycan cleavage by the endoglycosidase. Then the endoglycosidase PNGase F cleaves the N-linked glycans. After adding acetonitrile, the released N-glycans are captured on magnetic beads and then labeled with

APTS. After labeling, the excess APTS is removed with 3 washes. Finally, the APTS-labeled N-glycans are eluted from magnetic beads with deionized water, ready for analysis on the C100HT Biologics Analyzer.

Launch Method

| Enter Number of Samples |

| Deck Setup |

| Denaturation (8 min at 60°C) |

| Digestion (20 min at 60°C) |

| Labeling (20 min at 60°C) |

| Excess Dye Removal |

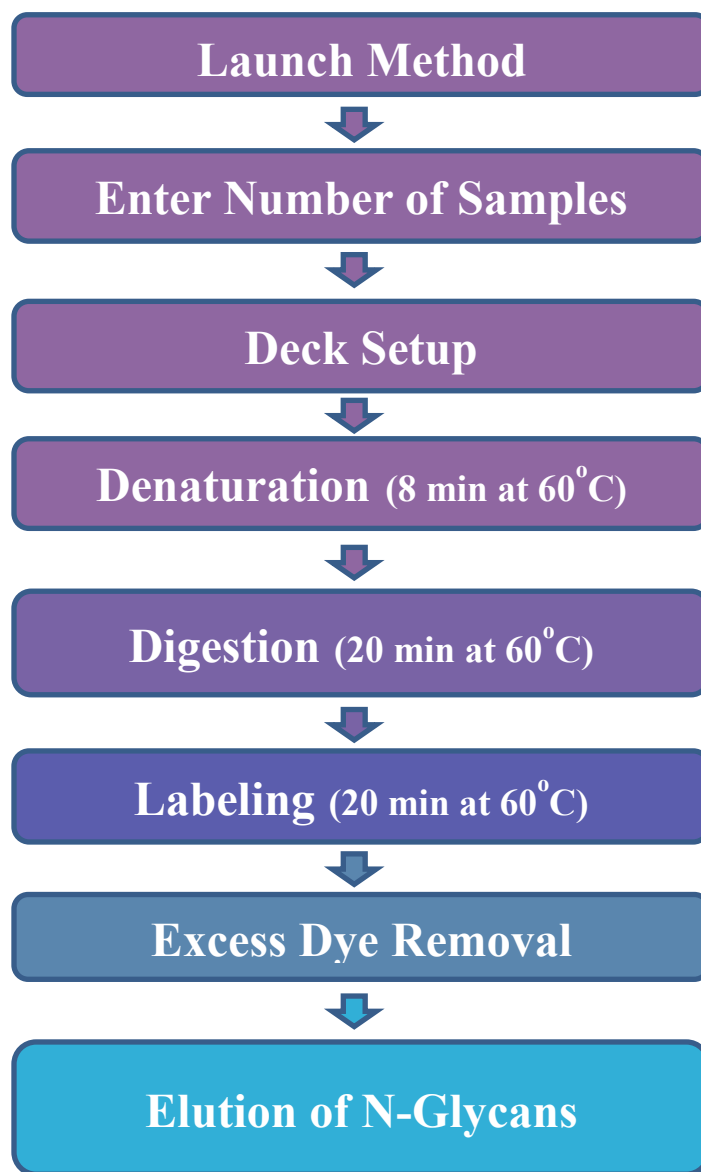| Elution of N-Glycans |

Figure 2. Workflow for fully automated glycan preparation on Biomek i5 Multichannel Workstation for C100HT Biologics Analyzer.
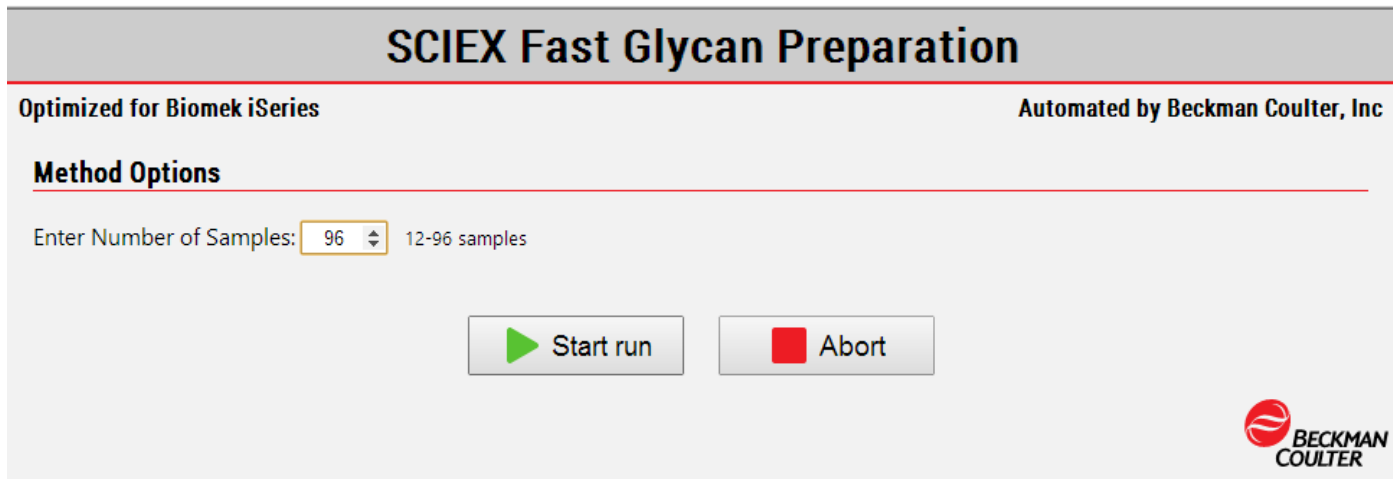
Figure 3. Biomek Method Option Selector is a user-friendly interface for entering number of samples.
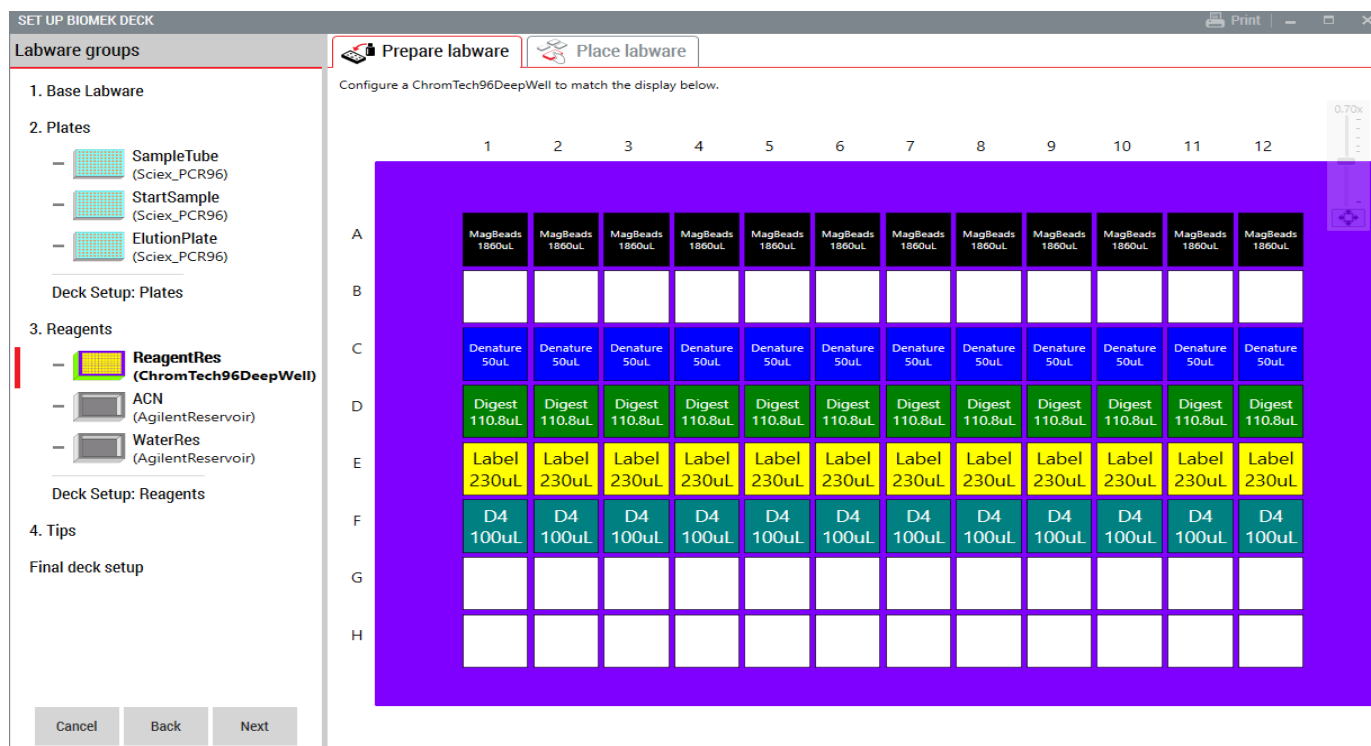

Figure 4. Biomek Guided Labware Setup indicates reagent source plate configuration and reagent volume calculations based on the Biomek Method Option Selector Input.

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Method Setup on Biomek i5 Multichannel Workstation

The fully automated SCIEX Fast Glycan Preparation method is performed through the Biomek Demonstrated Method Interface (DMI) that provides the user full instructions for Biomek deck setup. DMI consists of three modules. Module 1 is the Biomek Method Launcher (BML) for selection of the "SCIEX Fast Glycan Preparation©" method from the method folder. Second module is the Biomek Method Options Selector (MOS) with a user-friendly interface for entering the number of samples in the range of 12 to 96, as shown in Figure 3. Third moldule is the Biomek Guided Labware Setup (GLS) that automatically calculates the required volumes of

various reagents (Figure 4) and the amount of labware (Figure 5), based on number of samples to be processed. Users can determine the amount of denaturation, digestion and labeling solutions by adding 10% extra volume to compensate for loss during pipetting. These reagents are added into a 96 Deep Well reagent source plate as indicated in Figure 4. For best results, reagents should be added in the following order: denaturation solution, D4, digestion solution, labeling solution and magnetic beads. After the deck is setup by placing sample plates, reagent source plate, reagent reservoirs and tips at appropriate deck positions as shown in Figure 5, the Biomek i5 Multichannel Workstation carries out glycan sample preparation without any user intervention.
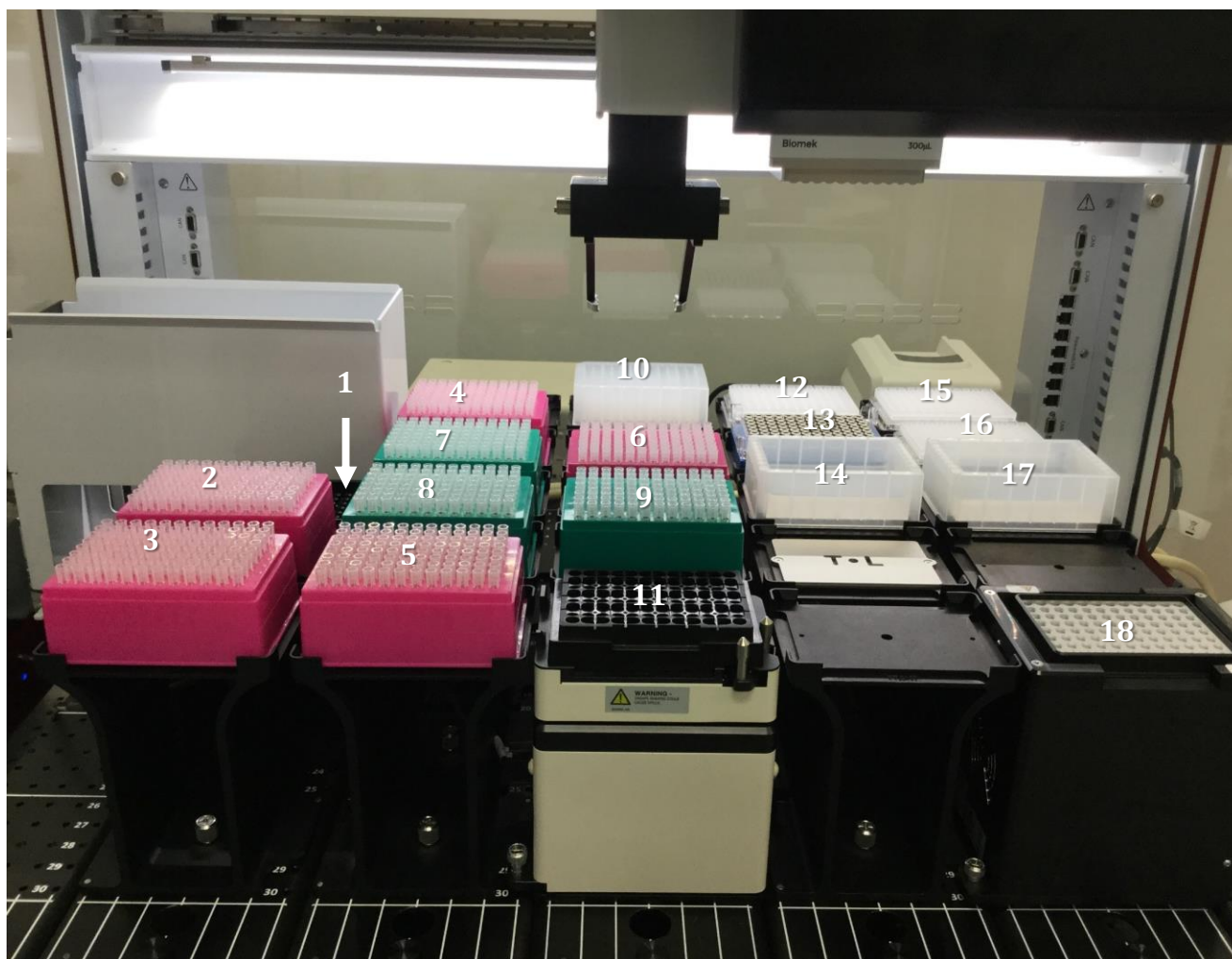

Figure 5. Deck set up on Biomek i5 Multichannel Workstation for processing 96 samples.

Notes: 1. 96 Well multichannel tip wash station; 2-6. 90 µl tip boxes; 7-9. 230 µl tip box 10. Reagent source plate; 11. Orbital shaker; 12. Reaction plate; 13. Magnum EX Universal Magnet Plate, Alpaqua; 14. Double deionized water reservoir; 15. Starting sample plate; 16. Elution plate 17. Acetonitrile reservoir; 18. Static Peltier

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Sample Handling after Glycan Elution

Once the fully automated glycan sample preparation is complete, the sample plate with eluted glycan samples is removed from the Biomek i5 Multichannel Workstation. For best results, the sample plates should be centrifuged at 1500 rpm for 5 min before being loaded onto the C100HT Biologics Analyzer (Reference 3).

Analysis of Glycan Samples on C100HT Biologics Analyzer

Setting up a sample run on C100HT Biologics Analyzer is easy and fast. First, sample plate, the bracketing standards, pre-filled buffer tray and pre-filled cartridge are loaded. Then, the appropriate pre-configured process profile available in the C100HT Biologics Analyzer software is selected. Automated electrophoretic separation is initiated upon entering sample information. Analysis of a full 96 well plate takes 170 minutes (Reference 2). Once the separation is complete, data is automatically integrated and processed in the DataReviewer Software module, which self-launches to display the results. Analysis of 96 data files is completed by DataReviwer Software in 17 minutes (Reference 2). A report containing corrected area %, GU values, glycan identities and relative migration times is generated by the analysis.
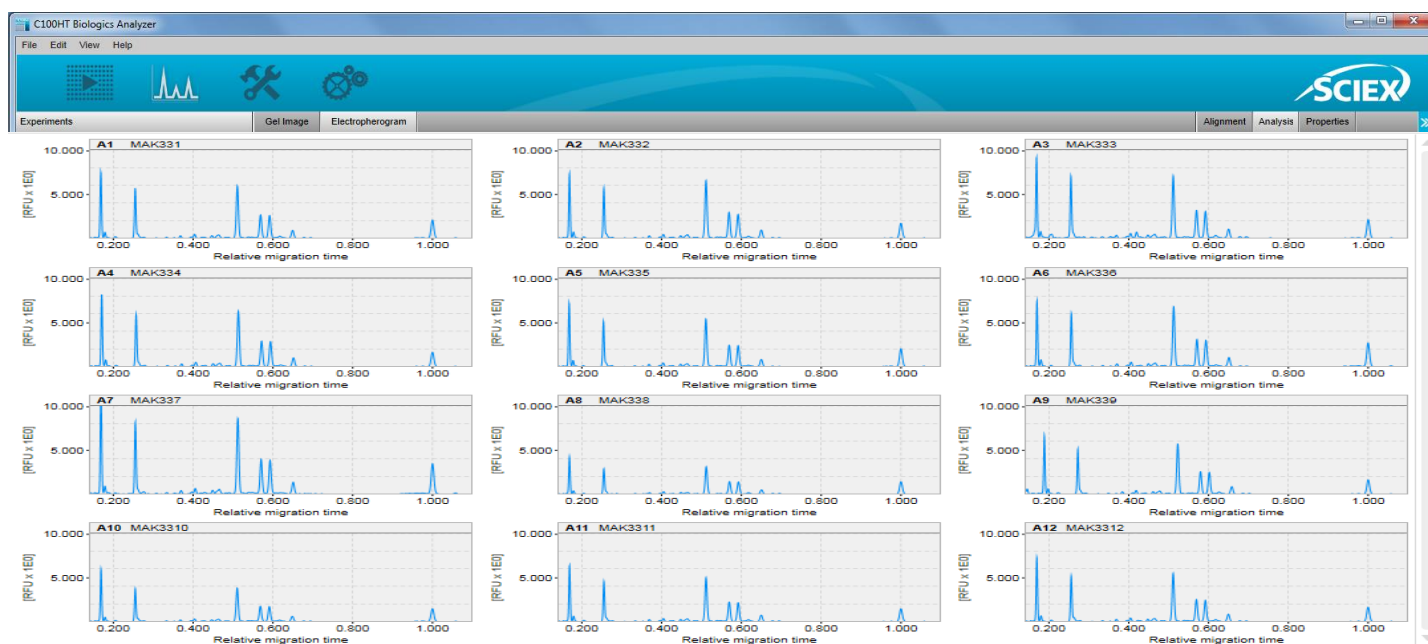

Figure 6. Reproducibility of runs on the C100HT Biologics Analyzer with MAK33 glycan samples prepared on Biomek i5 Multichannel Workstation.

Results

Verification of Reproducibility

In an experiment to verify the accuracy and reproducibility of fully automated glycan sample preparation on the Biomek i5 Multichannel Workstation, 96 glycan samples prepared from the MAK33 monoclonal antibody were analyzed on the C100HT

Biologics Analyzer. Figure 6 shows a representative stacked view of electropherograms of 12 MAK33 for glycan samples. Peak pattern for the main N-glycan species (G0, G0F, G1F, G1'F and G2F) are highly reproducible among the 12 runs. The RSD% for relative migration time for 96 samples is lower than 0.15% for all main N-glycan species. High reproducibility is also indicated by RSD% values for corrected area % for 96 MAK33 glycan samples in Figure 7.

Discussions

The fully automated glycan sample preparation method in this technical note has the following advantages over manual glycan sample preparation. First, it saves time. The total glycan sample preparation time for 96 samples is reduced from approximately 3.5 hours to 2 hours (Reference 2). The hands-on time is reduced from approximately 2.5 hours to less than one hour (Reference 2). Second, it ensures higher accuracy in pipetting. Pipetting speed, tip angle and tip depth as well as tip wash procedures on the Biomek i5 Multichannel Workstation were optimized

to minimize liquid splash and loss of reagents. High pipetting accuracy leads to high reproducibility in glycan analysis on the C100HT Biologics Analyzer, as observed in results. Third, the fully automated glycan sample preparation is more ergonomically friendly. Users only need to perform deck set up while the Biomek i5 Multichannel Workstation handles all the sample preparation tasks. Furthermore, this method is flexible. Users can employ this methodology for sample sizes from 12 to 96. Advanced users can also use this method for a sample size less than 12.
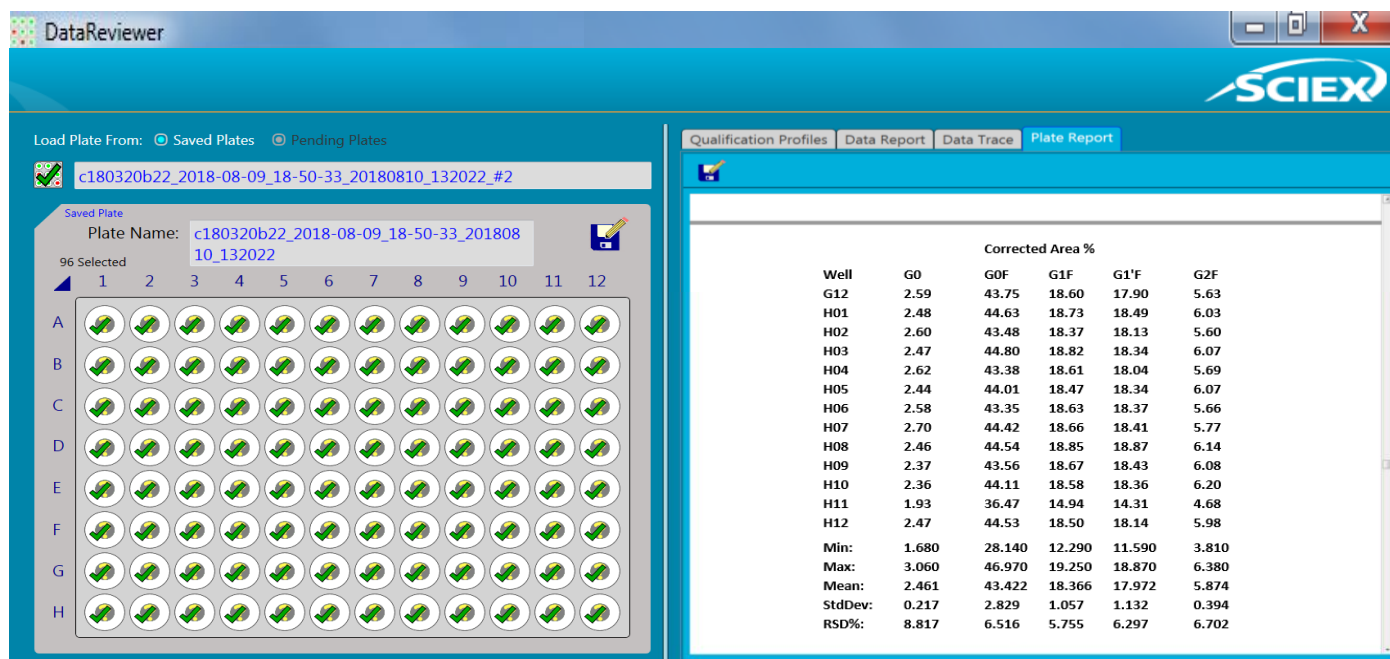

Figure 7. High reproducibility in analysis for 96 glycan samples.
In the left panel of this figure, a plate map in DataReviewer Software illustrates that all 96 samples were selected for data analysis, as indicated by the green check marks. The right panel shows the statistics section of the plate report. In this report, the corrected area % values for G0, G0F, G1F, G1'F and G2F species in each sample well are listed. The RSD% for 96 samples is below 9% for G0, and around 6% for G0F, G1F, G1'F and G2F.

Conclusions

- The fully automated glycan sample preparation method on the Biomek i5 Multichannel Workstation is a robust method that provides high efficiency and reproducibility in glycan sample preparation, freeing up time and resources which can be a challenge in fast paced biopharma environments.

- The combination of fully automated glycan sample preparation and high-throughput glycan screening using the C100HT Biologics Analyzer, provides a solution for fast N-glycosylation analysis during clone selection and cell culture optimization in the biopharmaceutical industry.

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1. M. Santos, T. Li, M. Gutierrez, A. Lou, C. Lew, R. Swart. Repeatability of C100HT Biologics Analyzer, a High Throughput Glycan Screening Platform. HPLC Poster RUO-MKT-10-8130-A.

2. M. Lies, M. Santos, T. Li, High-Throughput Glycan Screening to Remove Biopharmaceutical Development Bottlenecks, Technical Note RUO-MKT-02-7361-A

3. Based on experimental conditions identified during the development of the kit

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