Manual Automation of Rapid N-Glycan Sample Preparation – Reliable Results from Error Free Sample Tracking and Accurate Pipetting

Application Note CL-0211

Keywords

N-Glycan, glycobiology, hIgG, sample cleanup, purification, manual automation, pipette, manual automation, TOOLKIT, HPLC, glycoproteins, glycoanalysis, microchromatography

Introduction

Rapid sample preparation for N-Glycan analysis allows glycobiologists an efficient venue to speed the laborious manual process historically used for glycoanalysis. N-linked glycans are the most common glycans bound to proteins in eukaryotic cells (Apweiler, R. et al., 1999; Kronewitter, S.R., 2010). Simply defined, N-Glycans are carbohydrates linked to proteins (a.k.a. glycoproteins) that become freed glycans upon completion of the ProZyme GlykoPrep™ protocol.

Characterization of glycans is becoming increasingly important as researchers try to understand the various structures and resulting functions that glycans play for future personalized medicine and disease biomarkers (Sheridan, C. et al., 2007).
The GlykoPrep protocol uses innovative AssayMAP® cartridges, a microliter-scale analytical bind/elute chromatography, to perform sample preparation in a matter of 2-3 hours versus days. The historical bottleneck of glycoanalysis is drastically reduced to allow for faster characterization.

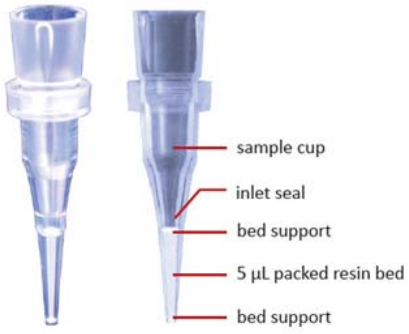

Figure 1 – AssayMAP® Cartridge

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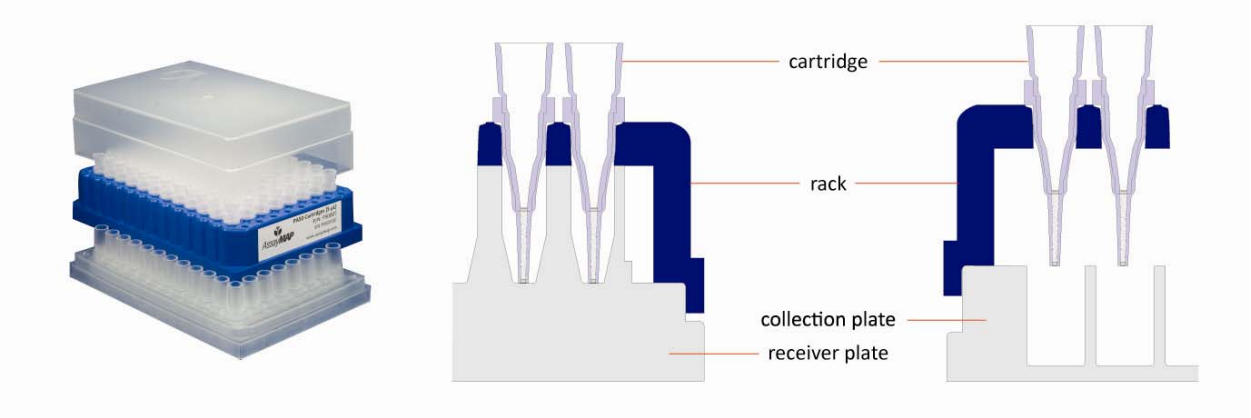
Figure 2 – AssayMAP® Technology



AssayMAP® technology scales standard chromatography practices to the micoliter range, enabling high-throughput preparation and analysis of samples with known, workable resin chemistry in microchromatography cartridges. Bind-and-elute chromatography methods are used to both purify and quantitate samples.

This application discusses benefits of manual automation of the GlykoPrep protocol using the Gilson SD TOOLKIT (Single channel Diagnostic) and Gilson MD TOOLKIT (Multichannel Diagnostic) to provide automatic sample preparation tracking and electronic protocol display using the TRACKMAN™. In combination with the ergonomic design of the Gilson PIPETMAN™ pipettes, accurate sample loading of the AssayMAP cartridges throughout the GlykoPrep protocol is further enabled with the use of the uniquely designed gradation ridges of the Gilson PIPETMAN pipette tips. This combination results is excellent reproducibility between samples run (see Table 2).

Figure 3 – Image of Gilson PIPETMAN™ Tips dispensing into AssayMAP cartridge using the rim of the tip to properly position prior to pipetting

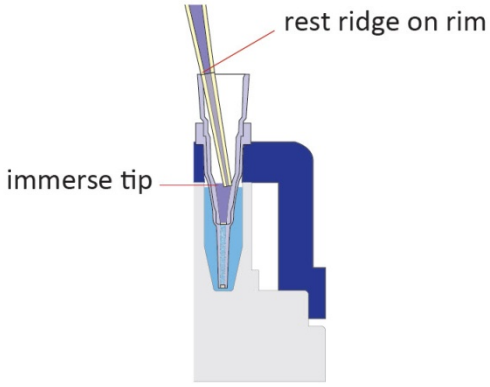


Figure 4 – SD (Single Channel Diagnostic) TOOLKIT

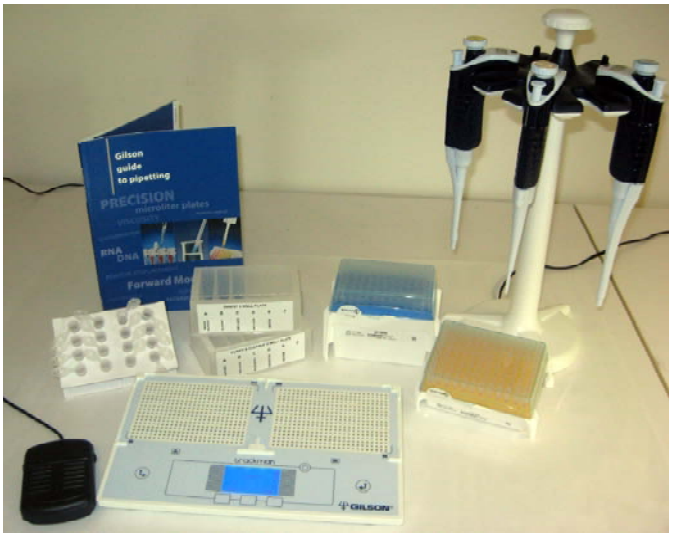


Figure 5 – MD (Multichannel Diagnostic) TOOLKIT

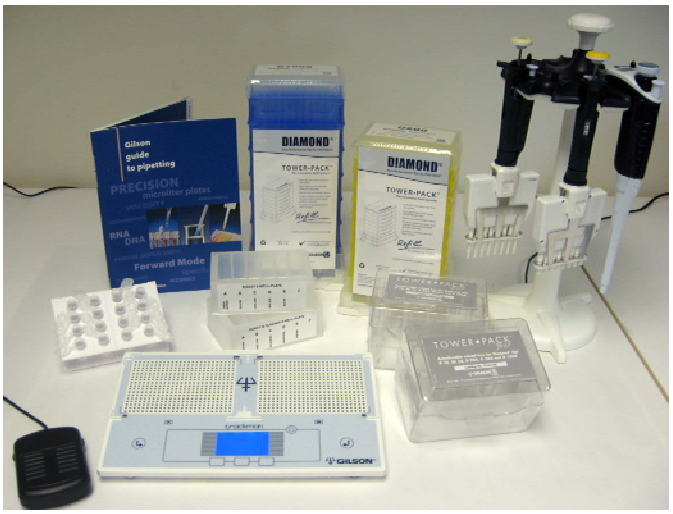
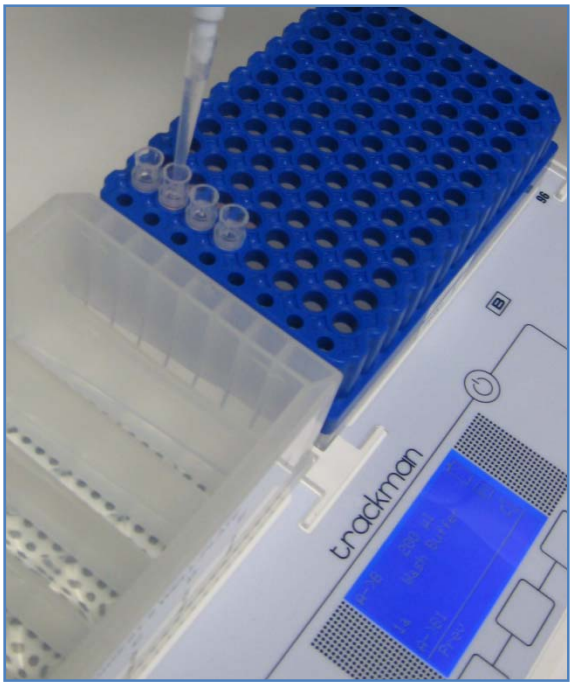


Figure 6 – Pipetting into AssayMAP cartridges



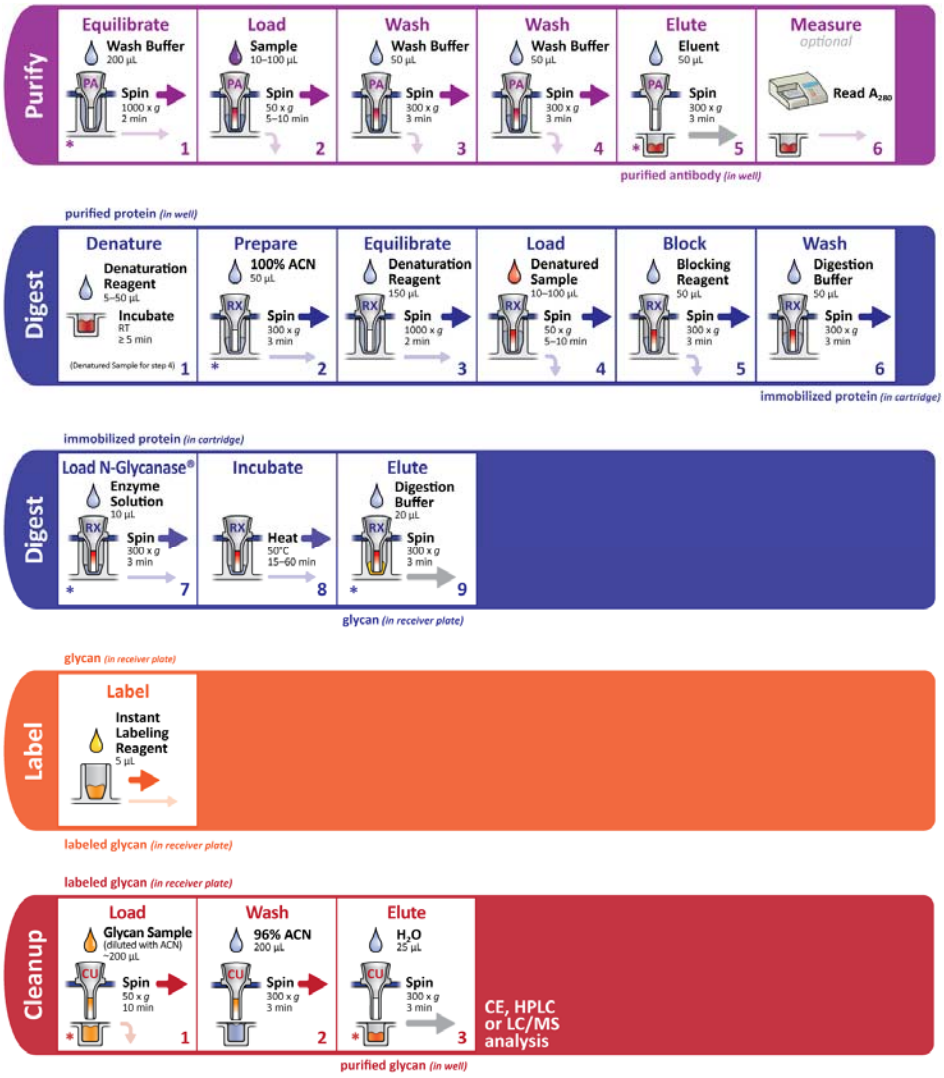
Materials & Methods

Samples & Solvents
Fetuin, from Fetal Calf Serum (Sigma, P/N F3385)
IgG from Human Serum, Technical Grade, ≥80% SDS-PAGE (Sigma, P/N 18640)
NanoPure Water
HPLC Grade Acetonitrile
Mobile Phase Buffer Preparation
    Formic Acid, 99+% - (Acros Organics, P/N 270480010)
    Ammonium Hydroxide – HPLC Grade (Fluka, P/N 17837)

Apparatus
Gilson GX-271 Liquid Handler with 402 Dual w/ Tee Dilutor
    10 mL syringe
    100 µL syringe
Gilson 306 Mobile Phase Pumps
    5 SC pump heads
Gilson 811D Dynamic Mixer
    1.5 Analytical Mixing Chamber
Gilson 805 Manometric Module
Column Heater – Set to 50°C
Jasco FP-2020 Plus Intelligent Fluorescence Detector
ProZyme GlycoSep™ N-Plus Column (P/N GKI-4730) and Guard Column

The ProZyme GlykoPrep protocol is performed according to the following steps:

Figure 7 – Rapid N-Glycan Preparation ProZyme GlykoPrep™ Protocol



Prior to starting the GlykoPrep protocol, samples of hIgG were mixed with bovine fetuin to simulate impure antibody sample loading for the Purify step. The purified hIgG antibodies were taken through the remaining GlykoPrep protocol steps 2 through 4 above. The Gilson TRACKMAN fitted with the GlykoPrep protocol was used throughout steps 1 through 4 to both track via lighting and notify via individual text screens of each protocol action between the solvent plate and the cartridge receiving plate.

The TRACKMAN GlykoPrep protocol eliminated the need to manually track each step or remember which AssayMAP cartridge was loaded for 1 to 4 or 8, 16, 24 samples. This provided additional time savings as a result of eliminating a manual tracking step. Considering there are 50 steps in the GlykoPrep protocol above, considerable time savings of 15-20 minutes are gained with the TRACKMAN versus manually picking up a pen to check off each step prior to continuing in the protocol.

Additional time savings is gained by having confidence that each sample was treated equally. The TRACKMAN also consistently held the AssayMAP cartridges in place during the pipetting process which simplified the sample and solvent loading steps.

Figure 8 – TRACKMAN illuminating the solvent plate and AssayMAP cartridges

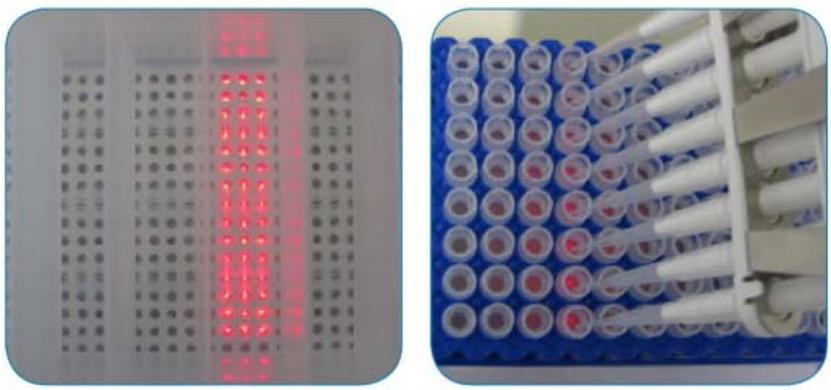
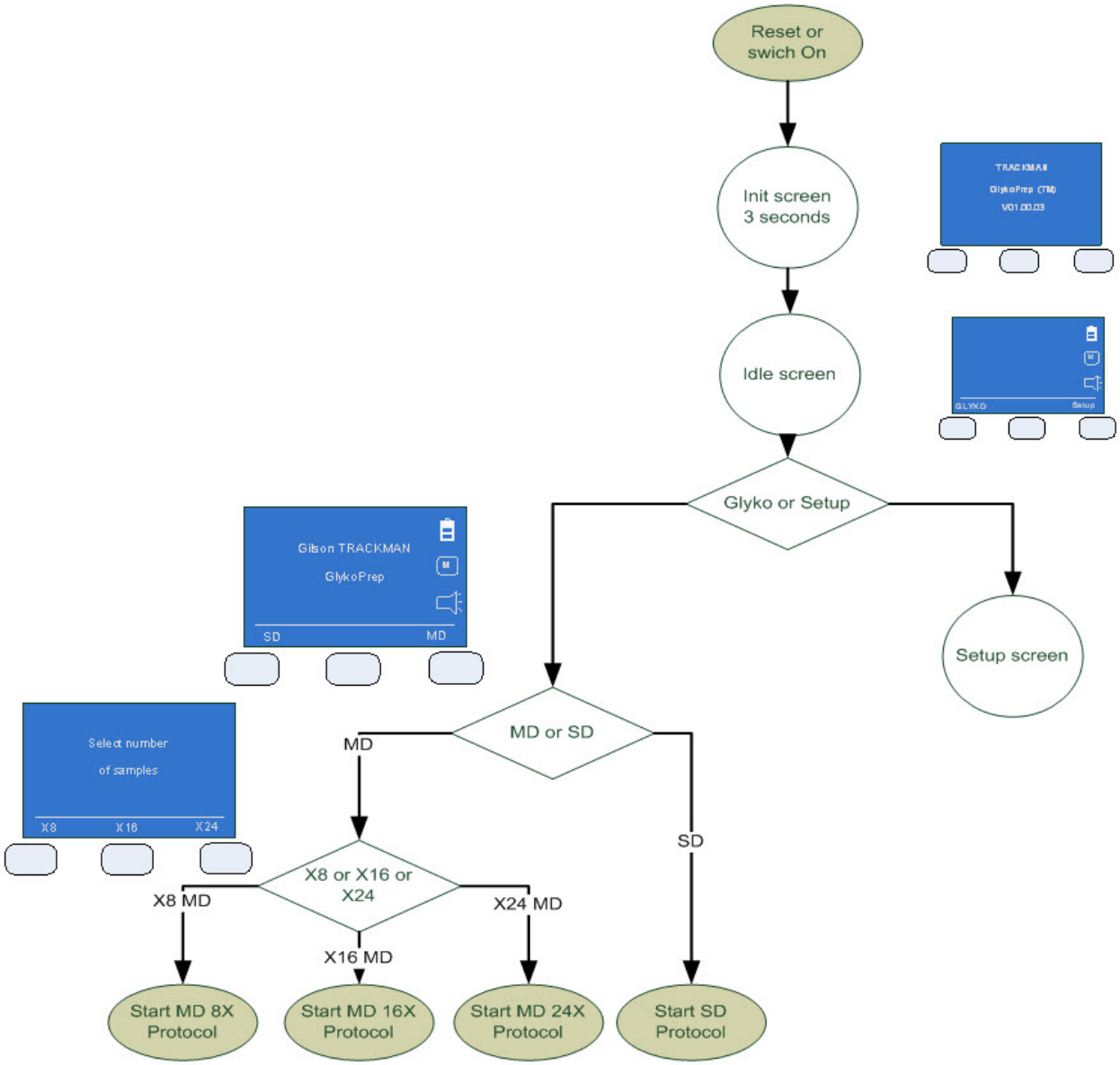
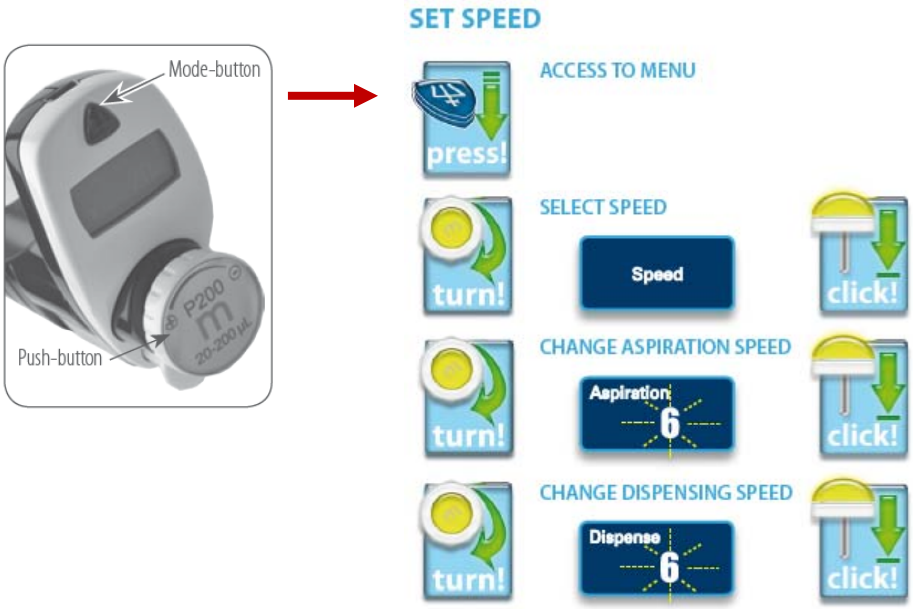


Figure 9 – TRACKMAN GlykoPrep Protocol Map

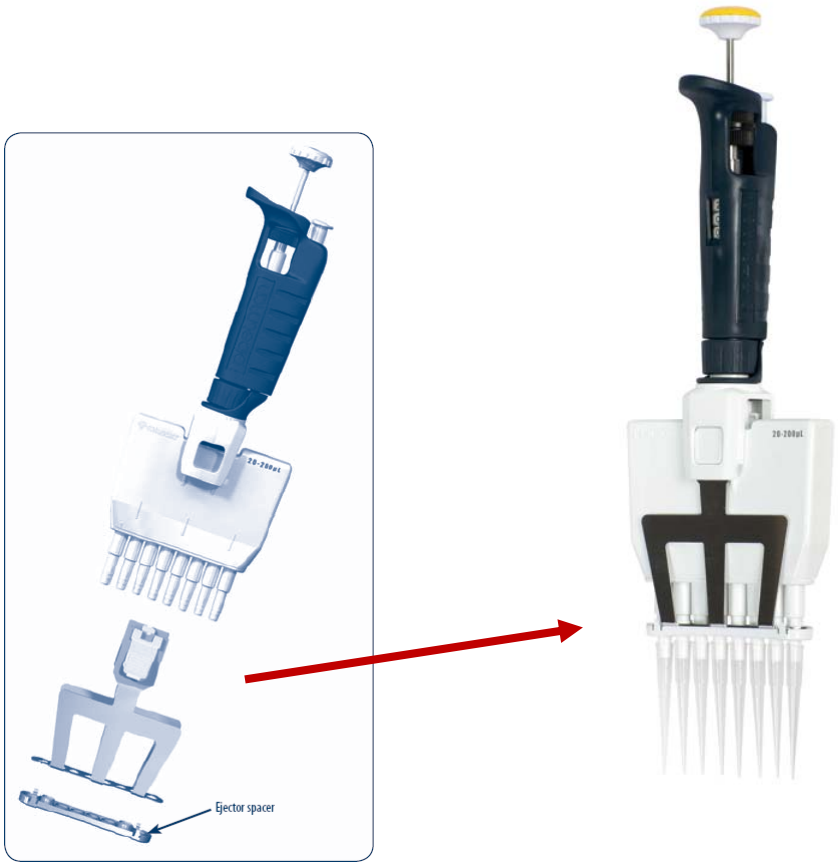


Sample loading of the AssayMAP cartridges was made more efficient with Gilson SD TOOLKIT because of the PIPETMAN M single channel fitted with PIPETMAN tips. The gradation ridge on the tips allowed for proper placement of the pipette tip into the AssayMAP cartridge, while the adjustable speed of the motorized PIPETMAN M allowed for accurate dispensing into the cartridge to eliminate air bubble formation within the cartridges. The Gilson MD TOOLKIT used the PIPETMAN Neo multichannel fitted with PIPETMAN tips to provide even and equal pressure from either side of the eight channels as a result of the unique internal Neo design. With the reduced pipetting forces of up to 50%, dispensing into the AssayMAP cartridge with the Gilson PIPETMAN tips was reliable and ergonomic.

Figures 10 & 11 – Simple Access to Set Speed on PIPETMAN M



Figures 12 & 13 – Gilson Neo Multichannel Pipette Unique Internal Tracking System



Using HILIC RP-HPLC (Hydrophilic Interaction Reverse Phase High pressure Liquid Chromatography), labeled free glycan samples and hIgG standards were separated and analyzed using total loop injection on a Gilson GX-271 Analytical HPLC System fitted with a Jasco FP-2020 Plus Intelligent Fluorescence Detector set to Excitation at 278 nm and Emission at 344 nm. Separation was performed on GlycoSep HPLC column and guard column from ProZyme. Using TRILUTION® LC version 2.1, Gilson binary mobile phase pumps were programmed to run an Acetonitrile and buffer gradient over 48 minutes.

Figure 14 – Gilson GX-271 Analytical HPLC System with Jasco FP-2020 Plus Intelligent Fluorescence Detector

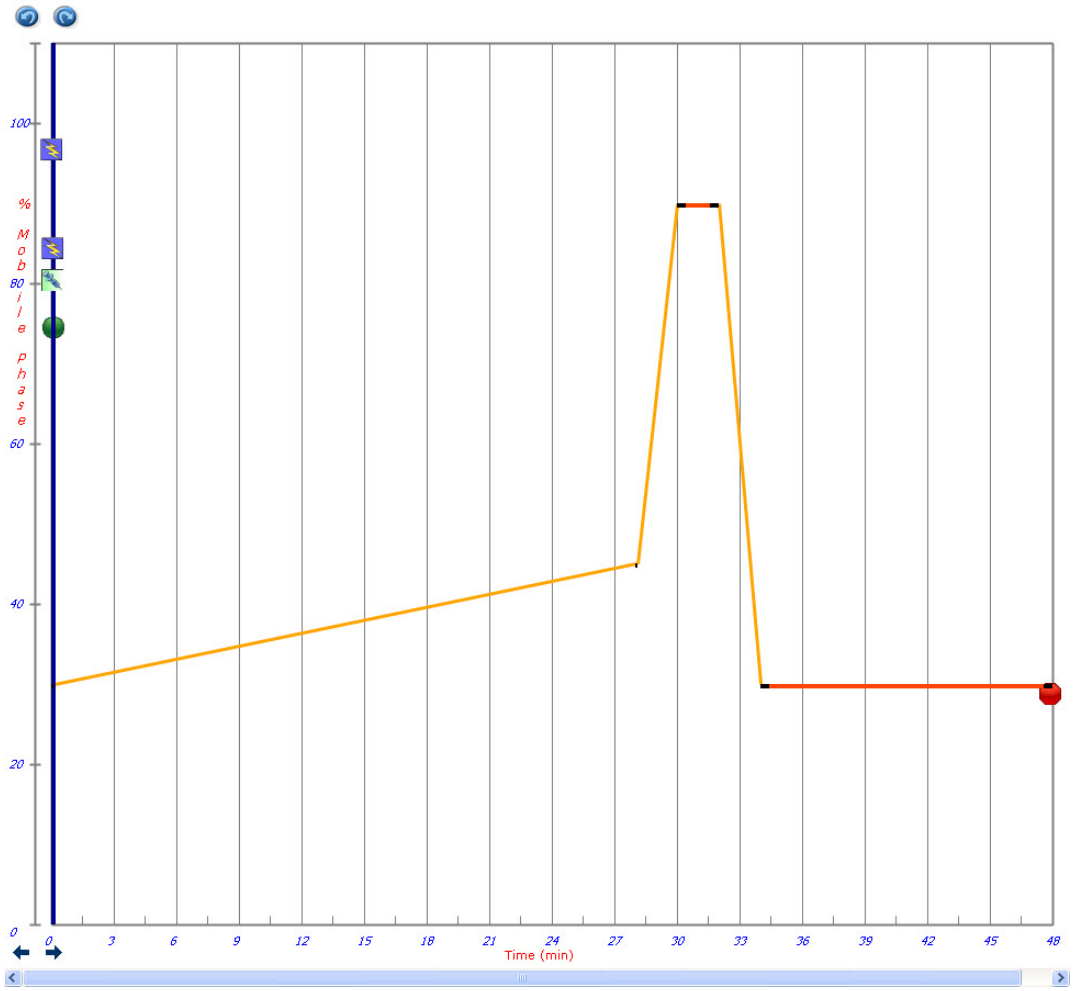


Figure 15 – TRILUTION LC GlykoPrep Analysis Method

Results

Chromatographic results show effective separation and sensitivity of the main free glycans between 12 and 23 minutes in both the independent hIgG standard and purified hIgG sample injections (Figures 16 and 17). The six major N-Glycan peaks are evident in both standard and sample injections. Overlayed chromatograms from a standard and a sample show reproducible retention times for each of these six peaks (Figure 18).

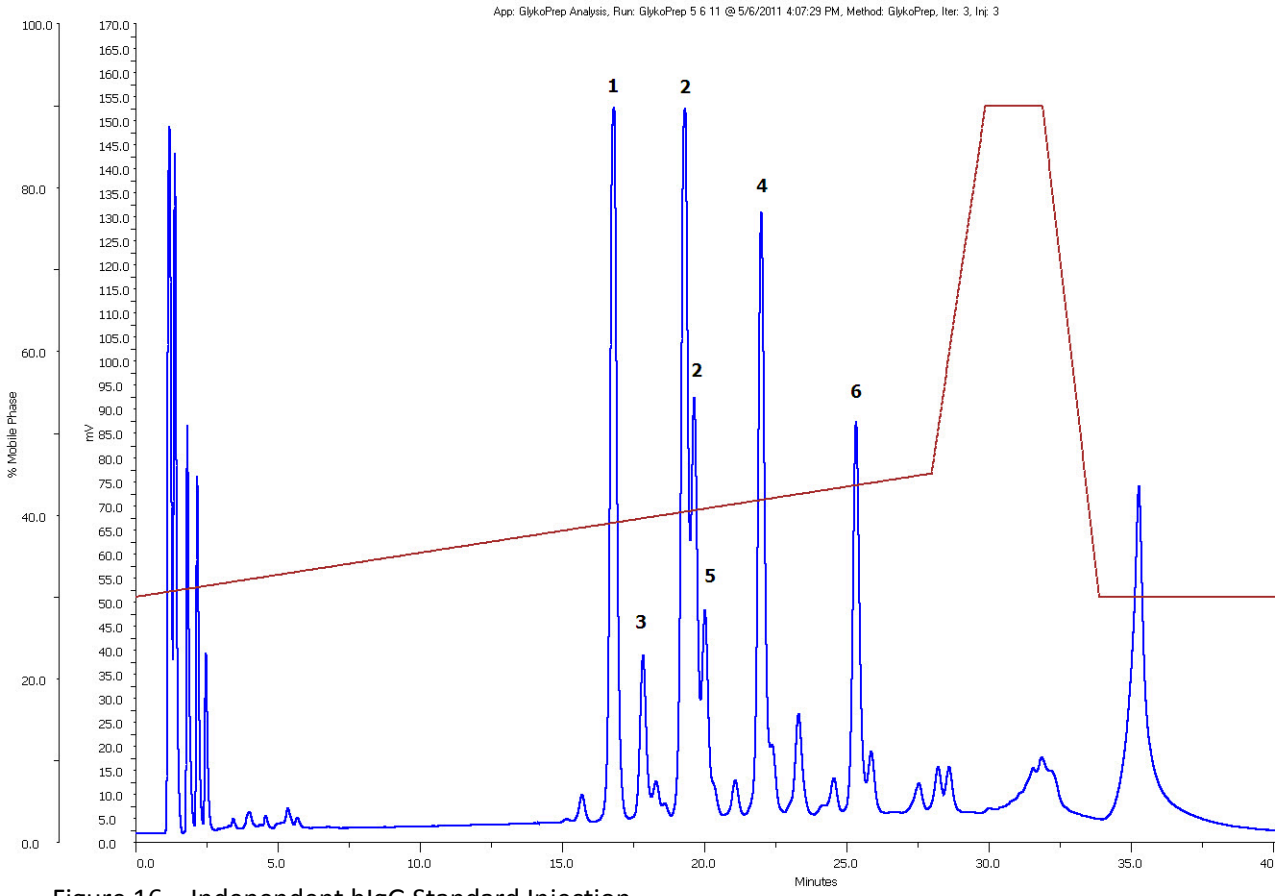


Figure 16 – Independent hIgG Standard Injection

Table 1 – Identification of Peak Names from hIgG Chromatogram

Peak Number	Peak Name
1	G0F (NGA2F)
2	G1F (NA2G1F) isomers
3	G0FB (NGA2FB)
4	G2F (NA2F)
5	G1FB (NA2F1FB)
6	G2FS1 (A1F)

Figure 17 – Impure Sample Injection – Mixed hIgG + Fetuin

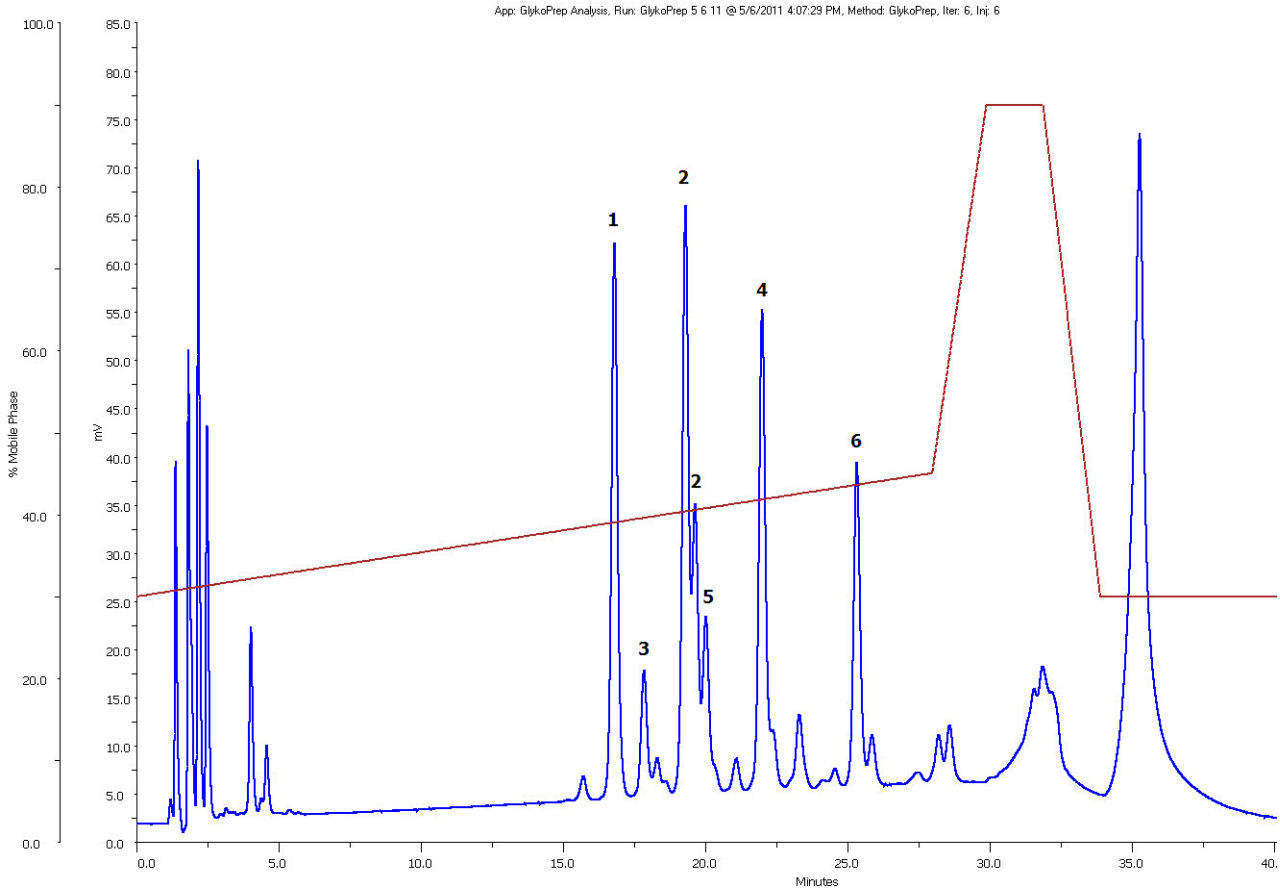
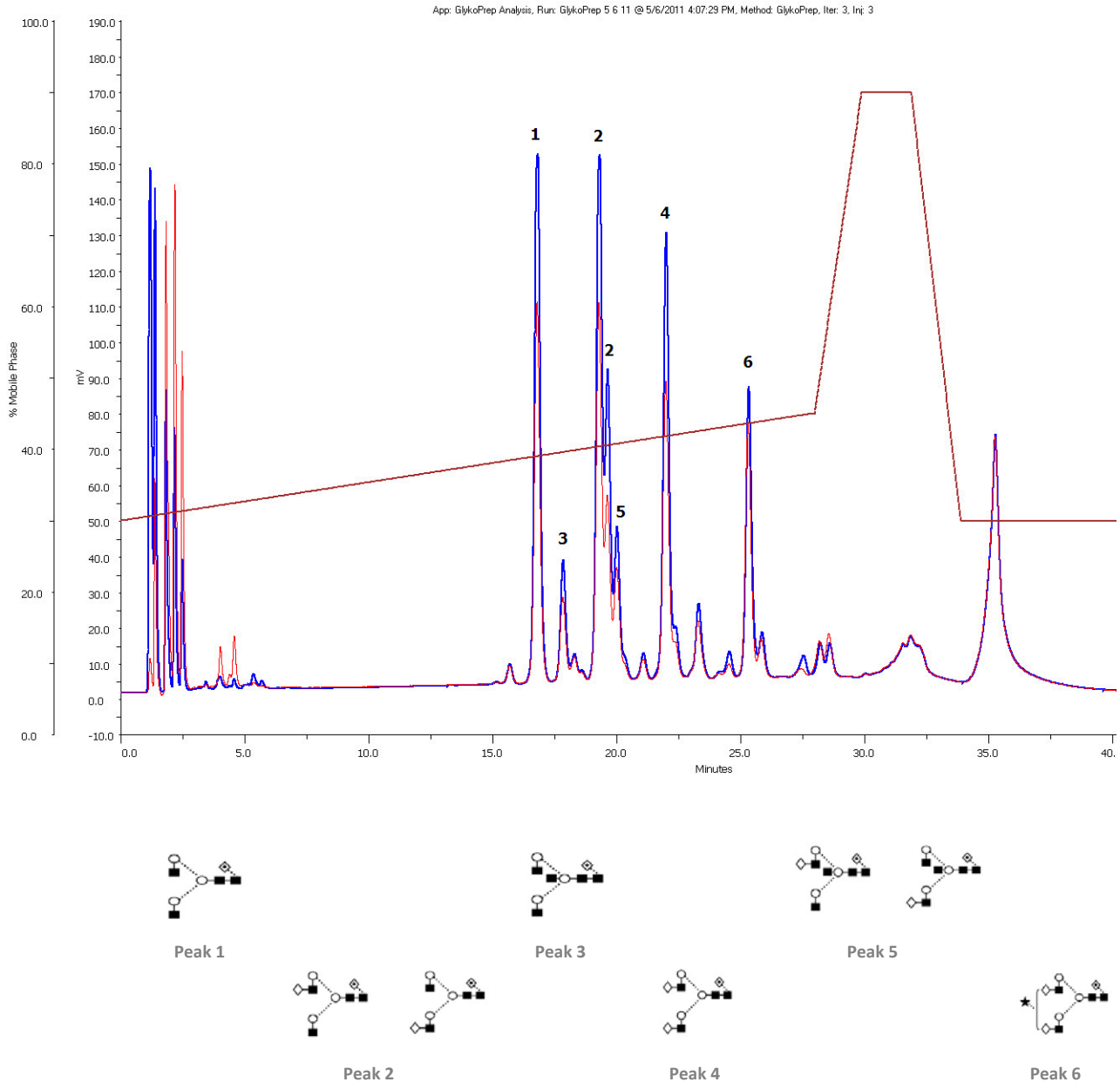


Figure 18 – Independent Standard Injection (Blue Trace) Overlayed with Mixed Impure hIgG + Fetuin Sample Injection (Red Trace)



Reproducibility data was generated for a set of 3 samples run through the entire TRACKMAN GlykoPrep Protocol using the PIPETMAN M to pipette 50 uL of hIgG mixed with 10 µL Fetuin as the starting impure sample. Percent mean peak area, standard deviation, and %CV was calculated for each of the six peaks for the set of three samples. Table 2 displays the peak area reproducibility data showing all peaks with relative standard deviation results at or less than 3%. Table 3 displays the peak retention time reproducibility for all six peaks. There is little variability for peak elution times, providing the user with confidence in identifying peaks when mass spectral analysis is not performed. Samples were stored at -20°C just after elution with water. Acetonitrile was added to each sample just prior to analysis on the Gilson GX-271 Analytical HPLC System with the Jasco FP-2020 Plus Intelligent Fluorescence Detector.

Table 2 – TRACKMAN GlykoPrep Protocol Peak Area Reproducibility (n=3)

Peak Number	% Mean Area	Standard Deviation	%CV
1	21.2609	.3101	1.458
2	34.6972	.1869	0.538
3	4.7334	.1335	2.820
4	17.2219	.3089	1.793
5	9.0760	.2529	2.786
6	13.1012	.3977	3.056

Table 3 – TRACKMAN GlykoPrep Protocol Peak Retention Time Reproducibility (n=3)

Peak Number	Mean Retention Time (min)	Standard Deviation	%CV
1	17.180	0.064	0.372
2a	19.681	0.060	0.304
2b	19.979	0.065	0.325
3	18.177	0.066	0.363
4	22.332	0.065	0.291
5	20.354	0.065	0.319
6	25.596	0.036	0.140

Summary

Rapid N-Glycan sample preparation of the GlykoPrep Protocol from ProZyme was made even more efficient, consistent, and error-free with the use of the Gilson SD TOOLKIT and Gilson MD TOOLKITs. The TRACKMAN GlykoPrep protocol allowed for accurate pipetting and effective tracking throughout the multi-step protocol using text screens and lighted wells. Chromatographic results showcased accurate sample purification with Protein A and preparation when compared with the independent standard. The use of the Gilson TOOLKITs eliminated the hassle of manually checking after each step in the protocol and further increased efficiency by letting the TRACKMAN keep track of which cartridges were loaded and processed. This saved valuable time and decreased the total time to perform the GlykoPrep protocol.

Day-to-day variation in results can be reduced with the use of the Gilson PIPETMAN disposable tips on either the PIPETMAN M or PIPETMAN Neo pipettes. The TRACKMAN GlykoPrep Protocol allows for ease in transitioning the number of samples that are run per day; from 1 to 4 and 8, 16, 24 samples. As seen by the consistency in results (See Table 2), the gradation line on the DIAMOND tips in combination with the unique features of the PIPETMAN pipettes enabled consistency in pipetting technique for a manual automated method. Analysis via HPLC with fluroescence detection can be very consistent and provide reliable results as seen by the retention time reproducibility data shown in Table 3.

References

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Scott R. Kronewitter, Carlito B. Lebrilla and other scientists in the Department of Chemistry, University of California-Davis and UC Davis Cancer Center, Sacramento, California, USA (Kronewitter, S.R. et al. (2010). www.gilson.com, **Processing Human Serum for Rapid and Reproducible N-Glycan Mass Profiling.**

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GlykoPrep™ is a trademark of ProZyme

The GlykoPrep™ platform is powered by patented AssayMAP® breakthroughput technology. AssayMAP® is a registered trademark of BioSystem Development, LLC.