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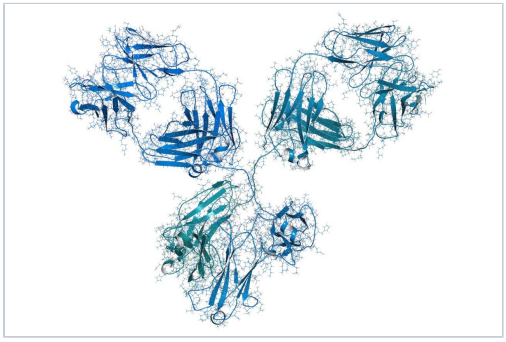
Application Note

Automated Medium and High-Throughput GlycoWorks *Rapi*Fluor-MS Preparations on the Andrew+ Pipetting Robot

Corey E. Reed, Stephan M. Koza, Steven Calciano

Waters Corporation



This is an Application Brief and does not contain a detailed Experimental section.

Abstract

Using the Andrew+ pipetting robot from Andrew Alliance, the GlycoWorks *Rapi*Fluor-MS N-glycan Labeling 24 and 48 Sample Kits were automated and verified for performance. Verification requirements included comparability to manually performed preparations of the same sample number with ≤25% deviation in total area and ≤5% deviation in relative area. Additionally, intra-preparation relative standard deviations must meet these same requirements. Ultimately, the 24-sample automated protocol showed 12.3% variation from manual preparations in terms of total area and 0.0% deviation in terms of relative area. The automated 48-sample protocol was also comparable to the 24-sample manual preparation with a 23.0% deviation in terms of total area and 0.0% deviation in terms of relative area. The automated 48-sample protocol also showed excellent intra-preparation reproducibility with 11.6% and 15.2% total area relative standard deviation over the 2 preparations.

Benefits

- Medium-throughput N-glycan analysis
- High-throughput N-glycan analysis
- Cost efficient robotics solution
- Collaborative automation
- Rapid N-glycan labeling
- Automated method transferability
- Time savings for end user
- Increased efficiency

Introduction

Glycosylation is routinely monitored as a critical quality attribute (CQA) during biopharmaceutical drug development as it is a measure of manufacturing condition uniformity, product efficacy, and product safety.[1,2] Traditional released N-glycan labeling methods can take multiple hours or days to complete and the labels often lack stability. Additionally, traditional labels do not offer strong fluorescence and mass spectrometric sensitivity at the same time, limiting the research laboratory to one detection method or the other.

Waters' introduction of the GlycoWorks *Rapi*Fluor-MS N-Glycan Labeling Kit and associated protocol provided a well-documented decrease in the time required to achieve unbiased labeling while simultaneously increasing fluorescence and mass spectrometric detection.[3] The labeling workflow along with HILIC cleanup and sample collection could be completed in under an hour depending on sample number, and the simplicity of the method leant itself to automation.[4] The primary benefits of automating this procedure are reducing the time spent by the analyst to prepare samples, reducing training and documentation burdens, and reducing potential errors due to pipetting monotony. With this in mind, the GlycoWorks *Rapi*Fluor-MS Kit was automated on the Andrew Alliance pipetting robot, Andrew, in 2018.[5]

The automated protocol underwent several rounds of optimization to ensure complete release and labeling of monoclonal antibody (mAb) N-glycans comparable to that seen when performing the protocol manually. The final protocol achieved relative standard deviation of 9-19% for major and minor glycoforms released from a murine mAb standard compared to the same sample prepared by a manual user.

In 2019, Andrew Alliance released an updated version of their pipetting robot, called Andrew+ (Figure 1). This system features web-based connected devices and an improved robotic arm compatible with single and multi-channel electronic pipettes, leading to time savings as well as the ability to handle higher sample loads. In this application brief data supporting the applicability of this automation platform for medium (24-sample) and high-throughput (48-sample) GlycoWorks *Rapi*Fluor-MS released N-glycan analysis is presented.

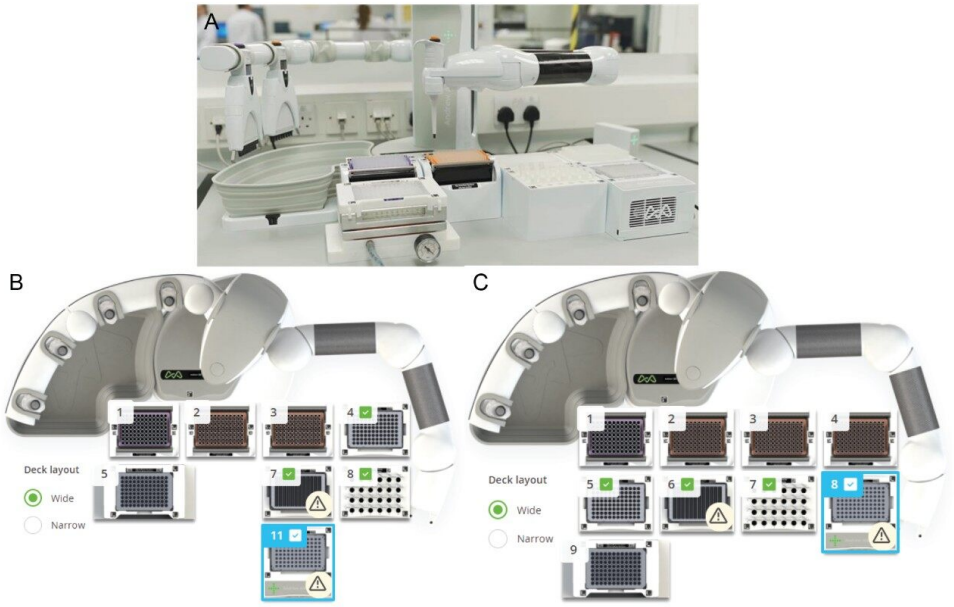


Figure 1. A) Picture of the Andrew+ set up for an 8-sample GlycoWorks RapiFluor-MS protocol.

B) OneLab top-down view of the Andrew+ setup for the 24-sample GlycoWorks RapiFluor-MS protocol. Dominos on the automation deck space include: (1) Tip Insertion System Domino fitted with 50–1,200 µL Optifit tips; (2) Tip Insertion System Domino fitted with 10–300 µL Optifit tips; (3) Tip Insertion System Domino fitted with 10–300 µL Optifit tips; (4) Storage Plate Domino fitted with QuanRecovery 700 µL 96-well plate with MaxPeak HPS from Waters; (5) Microelution Plate Vacuum+ Domino fitted with GlycoWorks HILIC µElution plate from Waters; (7) Deepwell Microplate Domino fitted with Axygen 12-well reservoir with 12-channel trough; (8) Microtube Domino fitted with Fisherbrand premium 1.5 mL conical microtubes for PNGase F Enzyme, RapiFluor-MS Labeling Reagent, and RapiGest-SF Surfactant; (11) 96-PCR Plate Peltier+ Domino fitted with an Eppendorf twin.tec 96-well skirted LoBind PCR plate containing samples for analysis.

C) OneLab top-down view of the Andrew+ setup for the 48-sample GlycoWorks RapiFluor-MS protocol. Dominos on the automation deck space include: (1) Tip Insertion System Domino fitted with 50–1,200 µL Optifit tips; (2) Tip Insertion System Domino fitted with 10–300 µL Optifit tips; (3) Tip Insertion System Domino fitted with 10–300 µL Optifit tips; (4) Tip Insertion System Domino fitted with 10–300 µL Optifit tips; (5) Storage Plate Domino fitted with QuanRecovery 700 µL 96-well plate with MaxPeak HPS from Waters; (6) Deepwell Microplate Domino fitted with Axygen 12-well reservoir with 12-channel trough; (7) Microtube Domino fitted with Fisherbrand premium 1.5 mL conical microtubes for PNGase F Enzyme, RapiFluor-MS Labeling Reagent, and RapiGest-SF Surfactant; (8) 96-PCR Plate Peltier+ Domino fitted with an Eppendorf twin.tec 96-well skirted LoBind PCR plate containing samples for analysis; (9) Microelution Plate Vacuum+ Domino fitted with GlycoWorks HILIC µElution plate from Waters.

Results and Discussion

Details of the optimization process for a low-throughput (8-sample) GlycoWorks *Rapi*Fluor-MS protocol on the Andrew+ System were presented in a previous Application Brief.[6] Due to the additional time required to process the medium and high-throughput sample preparation protocols, critical reagents used during the elution and final sample dilution steps of the protocol are not immediately added onto the automation deck. This was implemented to prevent evaporation of the reagents and prevent avoidable pipetting errors due to the reduction in volume. During development and testing of these automated protocols a rapid analytical method was used in order to expedite development. The 4 major peaks of the murine mAb were monitored for ease of processing. Figure 2 shows the verification results of the medium-throughput (24-sample) automation protocol.

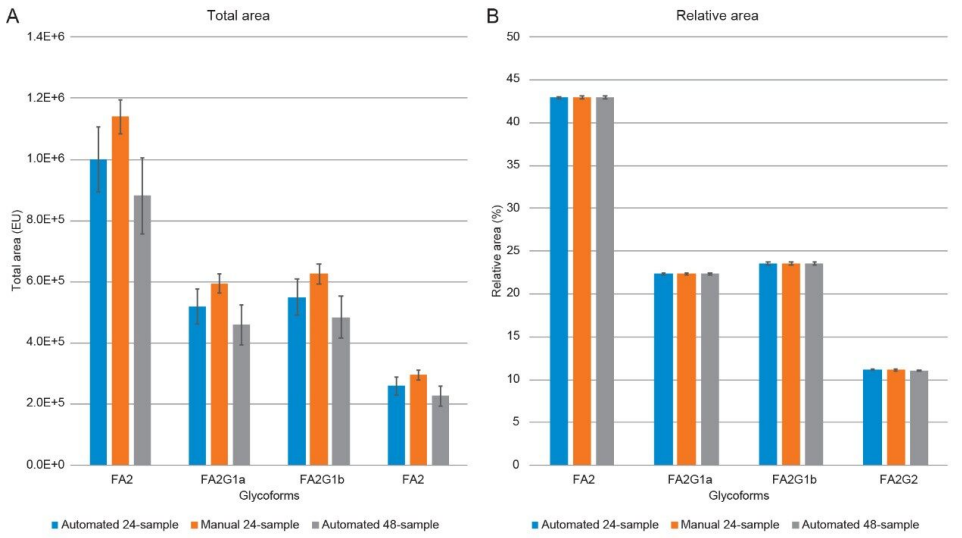


Figure 2. Manual and automated 24-sample preparations alongside automated 48-sample preparations of Intact mAb Mass Check Standard (P/N 186006552) murine IgG1 protein. Data sets are cumulative of 2 preparations on different days for a total of N = 48 for the 24-sample preparations and N = 96 for the 48-sample preparations.

A) Total area comparison of the 4 major glycoforms monitored from the chromatographic profile.

B) Relative area comparison of the same 4 glycoforms.

Automated 24-sample preparation produced results within the 25% deviation limitation set for protocol verification with the robotics platform achieving 87.7% recovery of labeled glycans compared to an experienced manual user (Table 1). Additionally, relative area comparison shows a 0% deviation compared to manual samples. Overall, the automated medium-throughput GlycoWorks *Rapi*Fluor-MS solution is comparable to manual.

	Total area										
	24-Sample preparation							48-Sample preparation			
N-Glycan	Manual 1	Manual 2	Average	Automated 1	Automated 2	Average	Comparison	Automated 1	Automated 2	Average	Comparison
FA2	1162780	1116735	1139758	1045290	954464	999877	88	840721	922032	881377	77
FA2G1a	608030	579670	593850	544854	495734	520294	88	438922	479768	459345	77
FA2G1b	638938	612132	625535	573011	524491	548751	88	463968	505430	484699	77
FA2G2	304483	287248	295865	272423	246837	259630	88	218538	237150	227844	77
						Average =	88			Average =	77

	Relative area										
	24-Sample preparation							48-Sample preparation			
N-Glycan	Manual 1	Manual 2	Average	Automated 1	Automated 2	Average	Comparison	Automated 1	Automated 2	Average	Comparison
FA2	43	43	43	43	43	43	100	43	43	43	100
FA2G1a	22	22	22	22	22	22	100	22	22	22	100
FA2G1b	24	24	24	24	24	24	100	24	24	24	100
FA2G2	11	11	11	11	11	11	100	11	11	11	100
						Average =	100			Average =	100

Table 1. Total area counts in emission units (EU) and relative area counts (%) for the automated and manual sample data shown in Figure 2 with a comparison of automated to manual samples.

Figure 2 also details the verification results of the high-throughput (48-sample) automation protocol. Initial attempts were made to compare the automated 48-sample protocol to a manual 48-sample preparation, however, after multiple attempts, an experienced manual user was unable to effectively process such a high number of samples. This, however, goes to show the benefits of automating such a high-throughput procedure on the Andrew+ robot. Averaging across two preparations, the robot produced comparable labeled N-glycan recovery to that seen with the medium-throughput method (Figures 2) with deviation from the medium-throughput manual samples of 23% for total area and 0% for relative area (Table 1).

Overall, relative standard deviations for total areas of the automated preparations were slightly higher than those achieved by an experience manual user (Table 2). The automated 24-sample preparation showed no relative standard deviation over 11.3% while the automated 48-sample preparation showed no relative standard deviation over 15.6%. For relative area, the average relative standard deviation for all preparations (automated and manual) were roughly 0.5%, again demonstrating how robust the GlycoWorks *Rapi*Fluor-MS protocol is in terms of maintaining unbiased labeling.

Intra-preparation relative standard deviations												
N-Glycan	Total area						Relative area					
	24-Sample				48-Sample		24-Sample				48-Sample	
	Autmated 1	Automated 2	Manual 1	Manual 2	Automated 1	Automated 2	Autmated 1	Automated 2	Manual 1	Manual 2	Automated 1	Automated 2
FA2	10.9	8.2	4.9	3.9	11.6	14.8	0.3	0.3	0.2	0.3	0.3	0.4
FA2G1a	11.1	8.4	5.0	4.4	11.6	15.3	0.7	0.6	0.6	0.7	0.5	0.7
FA2G1b	11.0	8.2	5.2	3.6	11.7	15.1	0.6	0.7	0.5	0.7	0.6	0.7
FA2G2	11.3	8.3	5.2	3.9	11.7	15.6	0.5	0.6	0.5	0.7	0.5	0.7
Average	11.1	8.3	5.1	4.0	11.6	15.2	0.6	0.5	0.5	0.6	0.5	0.6

Table 2. Relative standard deviation comparison of the manual and automated 24-sample and automated 48-sample GlycoWorks preparations.

Conclusion

Automated medium (24-sample) and high-throughput (48-sample) GlycoWorks *Rapi*Fluor-MS protocols were developed on the Andrew+ liquid handling platform. These automated solutions complete a portfolio of GlycoWorks scripts that are available on the OneLab Software needed to run the Andrew+ System and are readily available to download. Both scripts showed excellent comparability to manually performed protocols, fulfilling the requirement of ≤25% variation from manual samples in terms of total area and ≤5% for relative area.

References

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