AUTOMATED HIGH-THROUGHPUT N-GLYCAN PREPARATION WORKFLOW ON A COMMERCIALLY AVAILABLE BENCHTOP PIPETTING ROBOT

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OVERVIEW

◆ End-to-end RapiFluor-MS N-glycan preparation using the newly released Andrew+ pipetting robot.

◆ Reduced sample preparation time with the use of 8-channel pipettors from Sartorius.

Figure 1: The newly released Andrew + platform from Andrew Alliance. This system features Sartorius automated pipettors in both single channel and 8 channel formats.
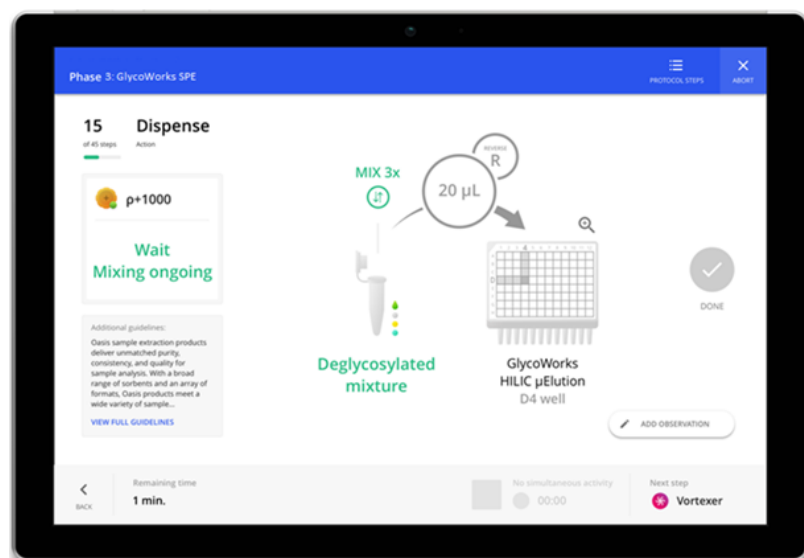

Figure 2: OneLab, a browser-based software platform, controls the Andrew+ robot for fully automated protocol execution. Easily design, edit, and share complex sample preparation protocols between OneLab connected devices.

METHODS

LC Conditions:
- Column = ACQUITY Glycan BEH Amide, 1.7 μm, 130 Å, 2.1 X 150 mm
- Sample Vial = 12x32 mm Total Recovery glass vial
- A = 50 mM Ammonium Formate in water, pH 4.4
- B = Acetonitrile

Gradient Table

Time (min)	Flow Rate (mL/min)	%A	%B
Initial	0.4	25	75
35	0.4	46	54
36.5	0.2	80	0
39.5	0.2	80	0
43.1	0.2	25	75
47.6	0.4	25	75
55	0.4	25	75

ACQUITY FLR Detector Settings:
Excitation nm = 265 nm, Emission nm = 425 nm

RESULTS

Figure 3: The OneLab workbench setup used for the automated GlycoWorks protocol. Each of the RapiFluor-MS reagents are held in a 1.5 mL tube at the start of the protocol to limit user pipetting. These are then distributed as needed to the reaction plate.
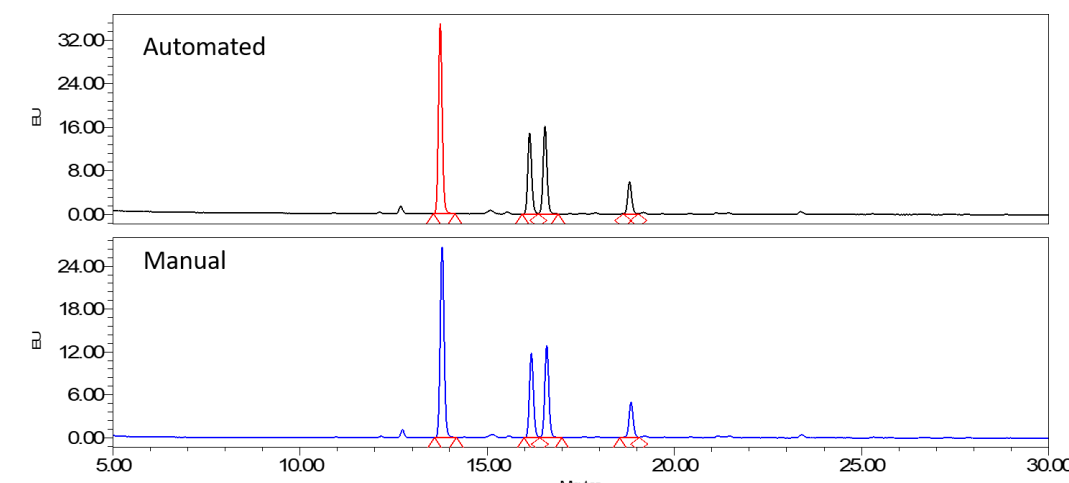

Figure 4: Reproducibility of the LC system was demonstrated by monitoring 4 major glycoforms . Sample was Waters' RFMS glycan standard. Total are was monitored across these 4 peaks through triplicate injections of 4 samples.
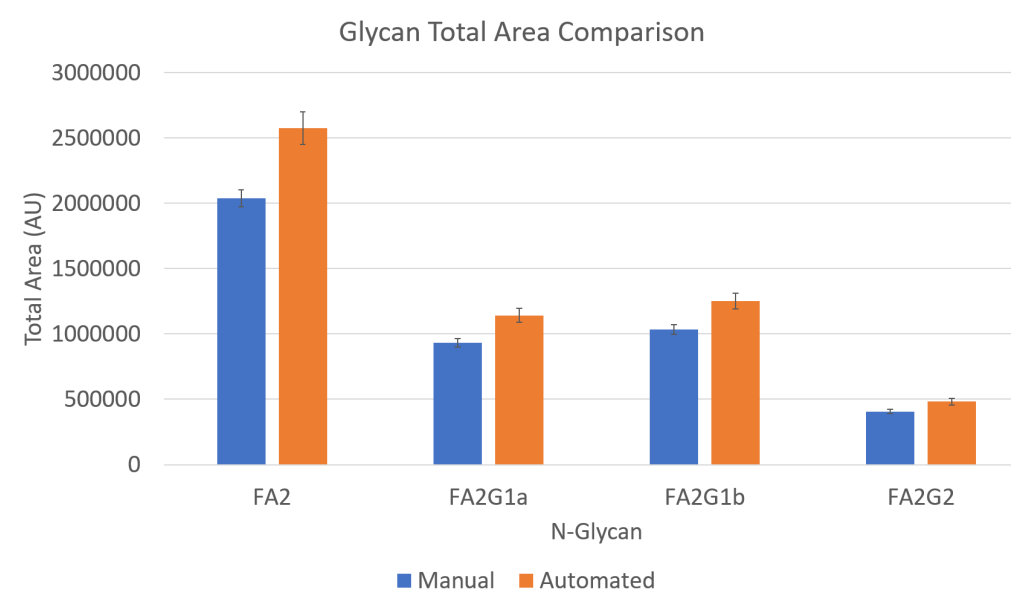

Figure 5: Total area comparison of the 4 major glycans in the Glycan Performance Test Standard. Labeled N-glycan recovery is higher in the automated samples compared to the manual samples indicating better labeling. N=12 for each N-glycan.
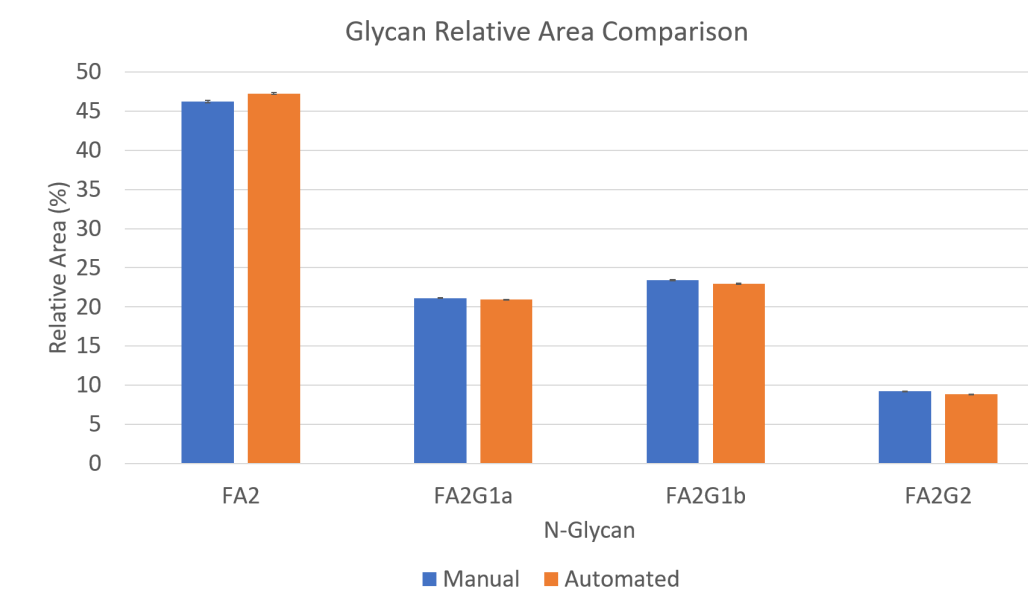

Figure 6: Relative area comparison of the 4 major glycans in the Glycan Performance Test Standard. Areas are very comparable between the manual and automated samples. N=12 for each N-glycan.

Relative Standard Deviation

N-Glycan	Retention Time Manual	Retention Time Automated	Area Manual	Area Automated	% Area Manual	% Area Automated
FA2	0.070056796	0.066985467	3.143754093	4.846256274	0.316773433	0.239064232
FA2G1a	0.059557511	0.05033689	3.459155338	4.820766521	0.176875618	0.111232875
FA2G1b	0.060950503	0.053510646	3.559594686	4.879830537	0.308561177	0.272151465
FA2G2	0.04937764	0.048925773	3.77155341	5.088781935	0.51973629	0.494913444

Table 1: Relative standard deviation comparison between automated and manual samples looking at retention time, area and relative area (% area). Manual and automated samples are similar in all cases.

CONCLUSION

- The GlycoWorks RFMS protocol on the Andrew+ automation platform has been updated to use 8-channel pipettors which reduces overall preparation time approximately two-fold.

- Preliminary data shows that samples prepared using the automated protocol are comparable to samples prepared manually.

- Relative standard deviation comparison of the retention time, total area, and relative area of 4 samples shows that the automation platform is capable of producing reliable results.

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