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응용 자료

# GlycoWorks *Rapi*Fluor-MS N-Glycan Reagent Kits: Scalable Solutions for Low- to High-throughput N-Glycan Sample Preparation Using Manual, Semi-automated or Fully Automated Workflows

Philip Lambert, Danielle Cullen, Corey Reed, Stephan M. Koza, Matthew A. Lauber, Jennifer L. Fournier, Leanne Davey

Waters Corporation

## Abstract

This application note demonstrates that the GlycoWorks *Rapi*Fluor-MS N-glycan sample preparation kit is readily scalable from manual, to semi-automated, and fully automated workflows.

## Benefits

- Throughput flexibility and scalability: Low- to high-throughput N-glycansample preparation using GlycoWorks *Rapi*Fluor-MS N-glycan reagent kit options for manual, semi-automated, or fully automated workflows.
- Automation kit includes fully validated Tecan script and detailed script development parameters.

- Reagent kit quantities are compatible with the major automated liquid handling platforms.

## Introduction

The purpose of this application note is to demonstrate the throughput flexibility and scalability of the GlycoWorks *Rapi*Fluor-MS N-glycan reagent kit options. These include; the GlycoWorks *Rapi*Fluor-MS N-Glycan Kit for low- to medium-throughput (semi-automated) sample preparation, and the GlycoWorks *Rapi*Fluor-MS N-Glycan Kit – Automation for fully automated high throughput sample preparation.

Since its launch, the GlycoWorks *Rapi*Fluor-MS N-Glycan Kit has offered scientists a fast and reproducible method for the manual preparation of glycoprotein samples for N-glycan analysis. The novel *Rapi*Fluor-MS label and associated chemistries provide marked benefits to bioanalytical scientists in terms of sample preparation efficiency and FLR and MS detection sensitivity. This manual sample preparation workflow is ideally suited to laboratories with relatively low sample throughput or laboratories which do not have access to automated liquid handling platforms.

For other laboratories, a further opportunity exists to enhance theefficiency of GlycoWorks using automation, in particular for medium-to-high-throughput requirements. In addition to gains in laboratory efficiency, the automation of complex workflows can provide a route to method simplification and standardization. Yet, a challenge for these laboratoriesis resourcing the adaptation of existing workflows to automation and the creation and validation of automation scripts.

To address these challenges, Waters has introduced the GlycoWorks *Rapi*Fluor-MS N-Glycan Kit – Automation and a range of semi-automated and fully automated workflows which combine the power of the existing rapid *Rapi*Fluor-MS and associated chemistries with the power of automation.

## Experimental

### Analytical method conditions (unless otherwise stated)

Universal N-glycan profiling method

## LC conditions

| | |
|---|---|
| LC system: | ACQUITY UPLC I-Class or ACQUITY UPLC H-Class Bio with ACQUITY UPLC FLR Fluorescence Detector |
| Column: | ACQUITY UPLC Glycan BEH Amide, 130Å, 1.7 µm, 2.1 x 150 mm (p/n: 186004742) |
| Column temp. | 60 °C |
| Sample temp. | 10 °C |
| Detection | Ex 265/Em 425 nm |
| Data rate | 10 Hz |
| Initial flow rate | 0.4 mL/min |
| Mobile phase A: | 50 mM Ammonium formate solution, pH 4.4 (p/n: 186007081) along with LC-MS-grade water [Fisher p/n W6 or equivalent] is recommended.) |
| Mobile phase B: | 100% acetonitrile (LC-MS-grade ACN is recommended, Fisher p/n A955 or equivalent) |

| | |
|---|---|
| Seal wash and LC: | 70% LC-MS grade ACN<br>(LC-MS-grade ACN is recommended,<br>Fisher p/n A955 or equivalent.),<br>30% LC-MS grade water<br>[Fisher p/n W6 or equivalent]<br>is recommended.) (v/v) |

## Auto-sampler wash

| | |
|---|---|
| Injection volume: | 10 µL suggested (=30 µL, DMF/ACN<br>diluted SPE eluate, 2.1 mm I.D. columns) |
| Injector needle: | A stainless steel needle is<br>recommended when injecting<br>samples containing DMF<br>as co-solvent |

## Gradient:

| Time (min) | Flow rate (mL/min) | %A | %B | Curve |
|---|---|---|---|---|
| 0 | 0.4 | 25 | 75 | 6 |
| 35 | 0.4 | 46 | 54 | 6 |
| 36.5 | 0.2 | 100 | 0 | 6 |

| | | | | |
|---|---|---|---|---|
| 39.5 | 0.2 | 100 | 0 | 6 |
| 43.1 | 0.2 | 25 | 75 | 6 |
| 47.6 | 0.4 | 25 | 75 | 6 |
| 55 | 0.4 | 25 | 75 | 6 |

Data management: Empower 3 CDS

## Sample description

The following protocol was executed for method comparison purposes.

Using manual, semi-automated (Andrew Alliance), and fully automated (Tecan) workflows; 15 N-glycan samples wereprepared from the same lot of glycoprotein (Waters Intact mAb Mass Check Standard (p/n: 186006552)) and using the same GlycoWorks *Rapi*Fluor-MS N-Glycan Kit – Automation lot (p/n:176004152). Samples were prepared using reagent concentrations specified in the instructions for each workflow:

- Manual: GlycoWorks *Rapi*Fluor-MS N-Glycan Kit Care and Use Manual (715004793EN)
- Semi-automated: GlycoWorks *Rapi*Fluor-MS N-Glycan Kits with the Andrew Alliance Pipetting Robot (720006197EN)
- Fully automated (Tecan): GlycoWorks *Rapi*Fluor-MS N-Glycan Kit – Automation Care and Use Manual (715005359EN)

*Rapi*Fluor-MS Glycan Performance Test Standard (p/n:186007983) and *Rapi*Fluor-MS Intact mAb Standard (p/n: 186008843) were reconstituted in 50 µL water for chromatographic benchmarking and system suitability.

All prepared samples were tested on the same ACQUITY UPLC H-Class Bio UPLC-FLR System.

# Results and Discussion

| | %CV, n = 15 | | | |
|---|---|---|---|---|
| | G0F | G1Fa | G1Fb | G2F |
| Manual | 0.2 | 0.1 | 0.3 | 0.7 |
| Tecan 48x | 1.1 | 0.6 | 0.8 | 1.7 |
| Tecan 96x | 0.8 | 0.5 | 0.6 | 1.4 |
| Andrew Alliance | 0.4 | 0.5 | 0.3 | 0.7 |

*Table 1. Precision data (%CV, n=15) for sample N-glycan area % measurements for manual, semi-automated (Andrew Alliance), and fully automated (Tecan) GlycoWorks sample preparations.*

To qualitatively compare the sample preparation methods, graphical representations of the N-glycan mean area % data for selected peaks for each sample set (n = 15) was prepared (Figures 1 and 2).

Initial statistical analyses (t-test) of the method data sets indicated that the methods were not statistically equivalent ($p<0.05$). Additional analyses indicated that the test is limited by the high precision of each data set and a simulation using only minor adjustments to a small number of data point to values yielded $p>0.05$.

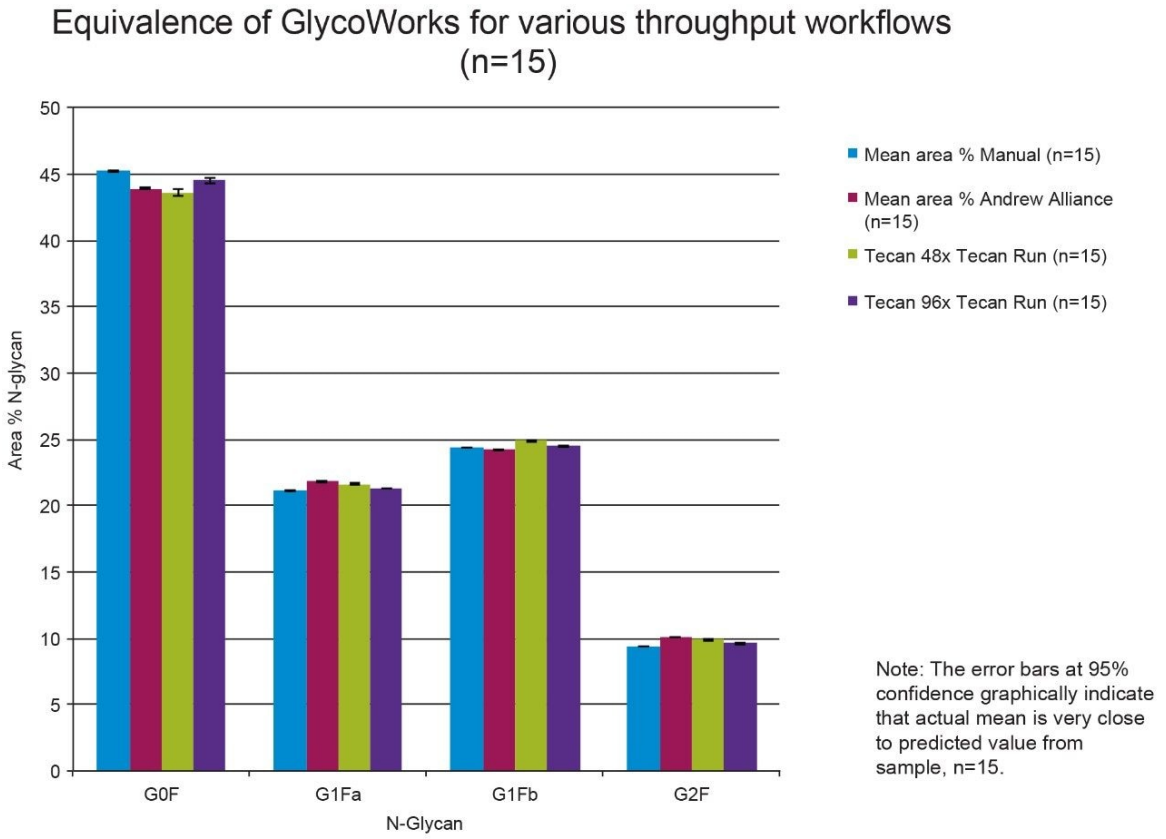


*Figure 1. Sample area % N-glycan data for manual, semi-automated (Andrew Alliance), and fully automated (Tecan) GlycoWorks sample preparations.*

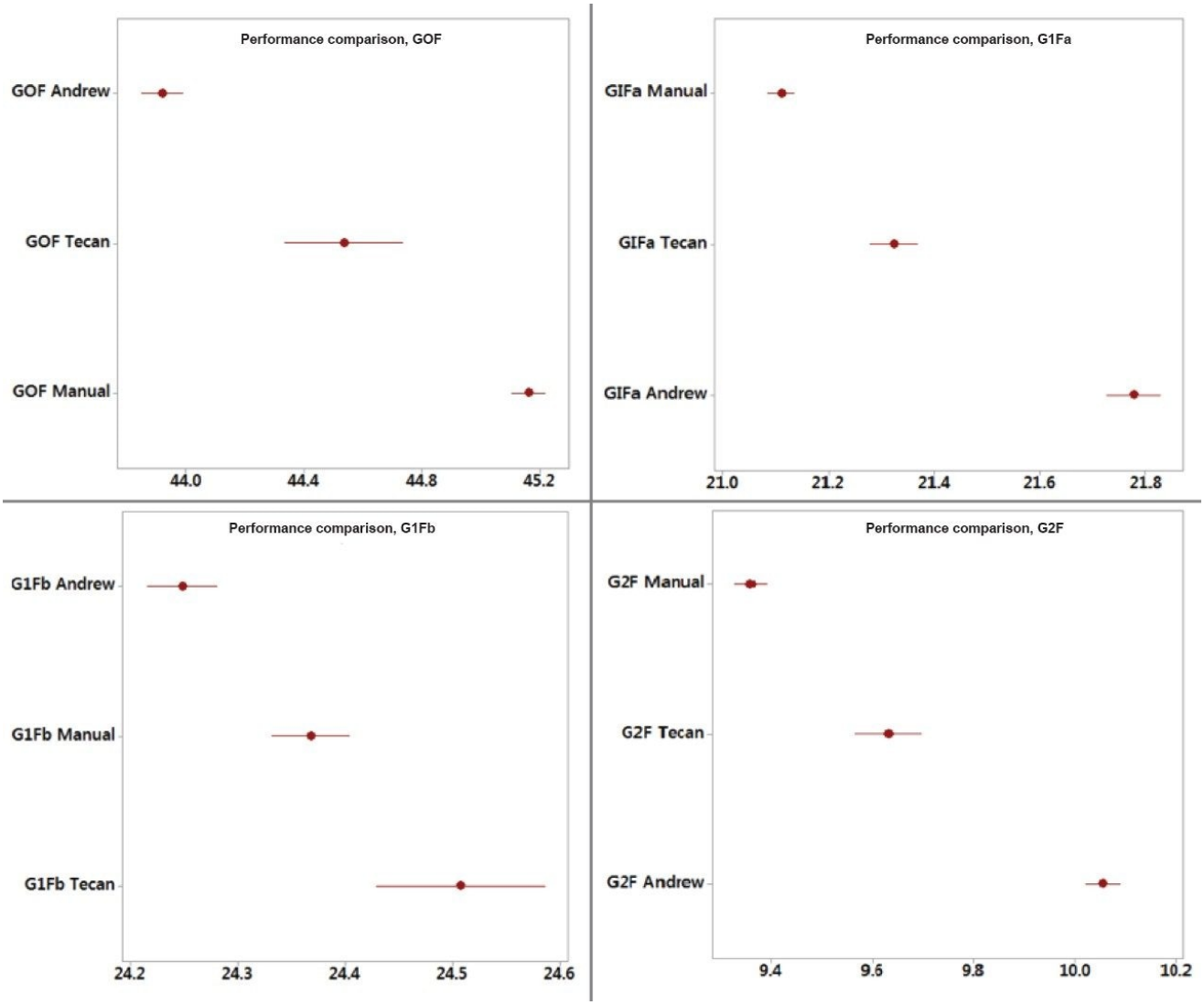


*Figure 2. Performance comparison for G0F, G1Fa, G1Fb, and G2F illustrating mean area % values for each method and precision of each measurement.*

Further statistical analyses were performed to quantify the bias for the automated methods relative to the manual method and for the semi-automated and fully automated methods. Minimum to maximum values for each N-glycan area percent data set were tabulated for evaluation as well as calculations of mean %bias as estimates and to 95% confidence intervals (Tables 2–4).

| N-glycan | Manual min-max | Tecan min-max | Pooled SD | % Bias mean differences | |
|---|---|---|---|---|---|
| | | | | Estimate | 95% CI |
| G0F | 45.030–45.320 | 44.020–45.110 | 0.402 | -0.625 | -0.849 to -0.428 |
| G1Fa | 21.080–21.160 | 21.180–21.500 | 0.213 | 0.213 | 0.151 to 0.273 |
| G1Fb | 24.230–24.510 | 24.280–24.750 | 0.141 | 0.141 | 0.061 to 0.222 |
| G2F | 9.210–9.460 | 9.420–9.820 | 0.269 | 0.269 | 0.173 to 0.366 |

*Table 2. Method comparison results for fully automated Tecan vs. manual GlycoWorks N-glycan sample preparation.*

| N-glycan | Manual min-max | Andrew Alliance min-max | SD | % Bias mean differences | |
|---|---|---|---|---|---|
| | | | | Estimate | 95% CI |
| G0F | 45.030–45.320 | 43.600–44.160 | 0.206 | -1.243 | -1.357 to -1.129 |
| G1Fa | 21.080–21.160 | 21.640–21.980 | 0.665 | 0.125 | 0.596 to 0.734 |
| G1Fb | 24.230–24.510 | 24.120–24.360 | 0.123 | -0.119 | -0.188 to -0.051 |
| G2F | 9.210–9.460 | 9.960–10.200 | 0.112 | 0.693 | 0.631 to 0.756 |

*Table 3. Method comparison results for semi-automated Andrew Alliance vs. manual GlycoWorks N-glycan sample preparation.*

| N-glycan | Andrew Alliance min-max | Tecan min-max | SD | % Bias mean differences | |
|---|---|---|---|---|---|
| | | | | Estimate | 95% CI |
| G0F | 43.600–44.160 | 44.020–45.110 | 0.430 | 0.618 | 0.380 to 0.856 |
| G1Fa | 21.640–21.980 | 21.180–21.500 | 0.146 | -0.452 | -0.533 to -0.371 |
| G1Fb | 24.120–24.360 | 24.280–24.750 | 0.187 | 0.261 | 0.157 to 0.364 |
| G2F | 9.960–10.200 | 9.730–9.950 | 0.159 | -0.424 | -0.512 to -0.336 |

*Table 4. Method comparison results for Andrew Alliance vs. Tecan GlycoWorks N-glycan sample preparation.*

The semi-automated and fully automated sample preparation workflows developed by Waters for use with the GlycoWorks *Rapi*Fluor-MS N-Glycan Kit and GlycoWorks *Rapi*Fluor-MS N-Glycan Kit – Automation provide area percent N-glycan profile results without significant bias relative to the established manual GlycoWorks *Rapi* Fluor-MS N-glycan sample preparation workflow.

A high measurement precision of <2% CV is achievable for the semi-automated and fully automated methods (Table 1). Therefore, the GlycoWorks *Rapi*Fluor-MS N-Glycan Kit –Automation can be applied to highly efficient, automated, high-throughput N-glycan sample preparation without any significant loss inprecision performance. Further statistical analysis demonstrates expected measurement ranges (minimum-maximum) and measurement bias (Tables 2 and 3) for the semi-automated and fully automated methods.

This application note provides data to help biopharmaceutical scientists evaluate the analytical performance of each method.

## Conclusion

Waters has introduced a range of validated products and automated N-glycan sample preparation workflows which enable a marked increase in efficiency and throughput flexibility for scientists. This application note demonstrates that the GlycoWorks *Rapi*Fluor-MS N-glycan sample preparation kit is readily scalable from manual, to semi-automated, and fully automated workflows.

The GlycoWorks *Rapi*Fluor-MS N-Glycan Kit – Automation is offered with a validated Tecan script for completely hands free preparation of N-glycan samples. The kit reagent quantities have been designed to be compatibl ewith the other major automated liquid handling manufacturers. The coupling of the highly efficient *Rapi*Fluor-MS label and associated chemistries ofthe GlycoWorks *Rapi*Fluor-MS N-Glycan Kit to the validated automation workflows and scripts provides an effective approach for laboratories wishing to leverage their automation resources.

The provision of these GlycoWorks automation resources completes a product family which offers maximum flexibility to suit the throughput requirements and automation resources of laboratories. Further resources for the manual workflow, semi-automated workflow on the Andrew Alliance, and the fully automated workflow on the Tecan can be found in corresponding application notes at http://wvmc.waters.com/glycans/.

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