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High Performance Analytics for Bioprocessing and Manufacturing

Global BioPharma Summit

Agenda

Introduction

- Analytical tools from Pharma Analytics group
- Fully integrated analytical solutions
- Glycan analysis
- Current Glycan Analysis Challenges

Thermo Fisher GlycanAssure Platform

- GlycanAssure system
- Sample prep kits
- CE instrumentation
- Software
- GlycanAssure Workflow

Sample Data Sets

- Repeatability of glycan separation
- Variability across instruments & arrays
- Consistency in glycan quantitation
- IgG glycan analysis
- Highly sialylated glycan analysis
- Sialylated glycan library analysis

Summary

- Take home message

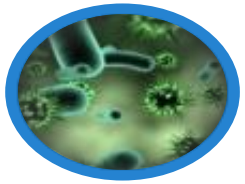
High Performance Analytics for Bioprocessing & Manufacturing

Product Safety

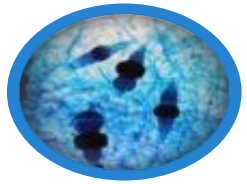
Microbial Identification and Detection

DNA Sequencing

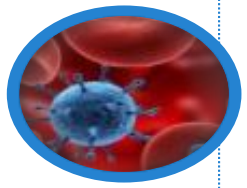
Real-Time PCR



Bacterial ID



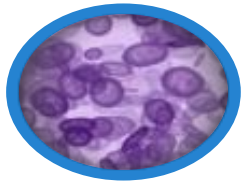
Fungal ID



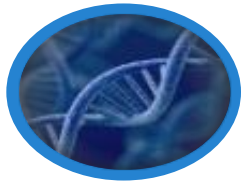
Virus Detection

Product Quality

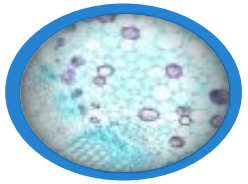
Impurity Analysis



Mycoplasma



Residual DNA



HCP

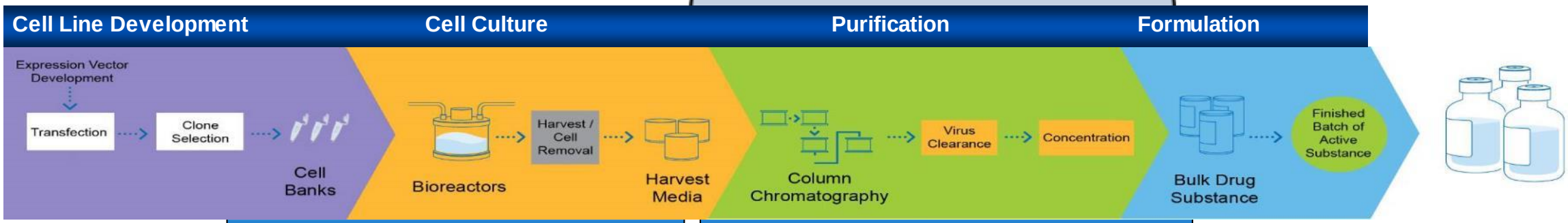
Protein A

Product Portfolio

MicroSEQ™ ID

GlycanAssure™

ProteinSEQ™



MycoSEQ™
ViralSEQ™

ResDNASEQ™

Turnkey solutions addressing critical customer needs across the Biotherapeutic production workflow

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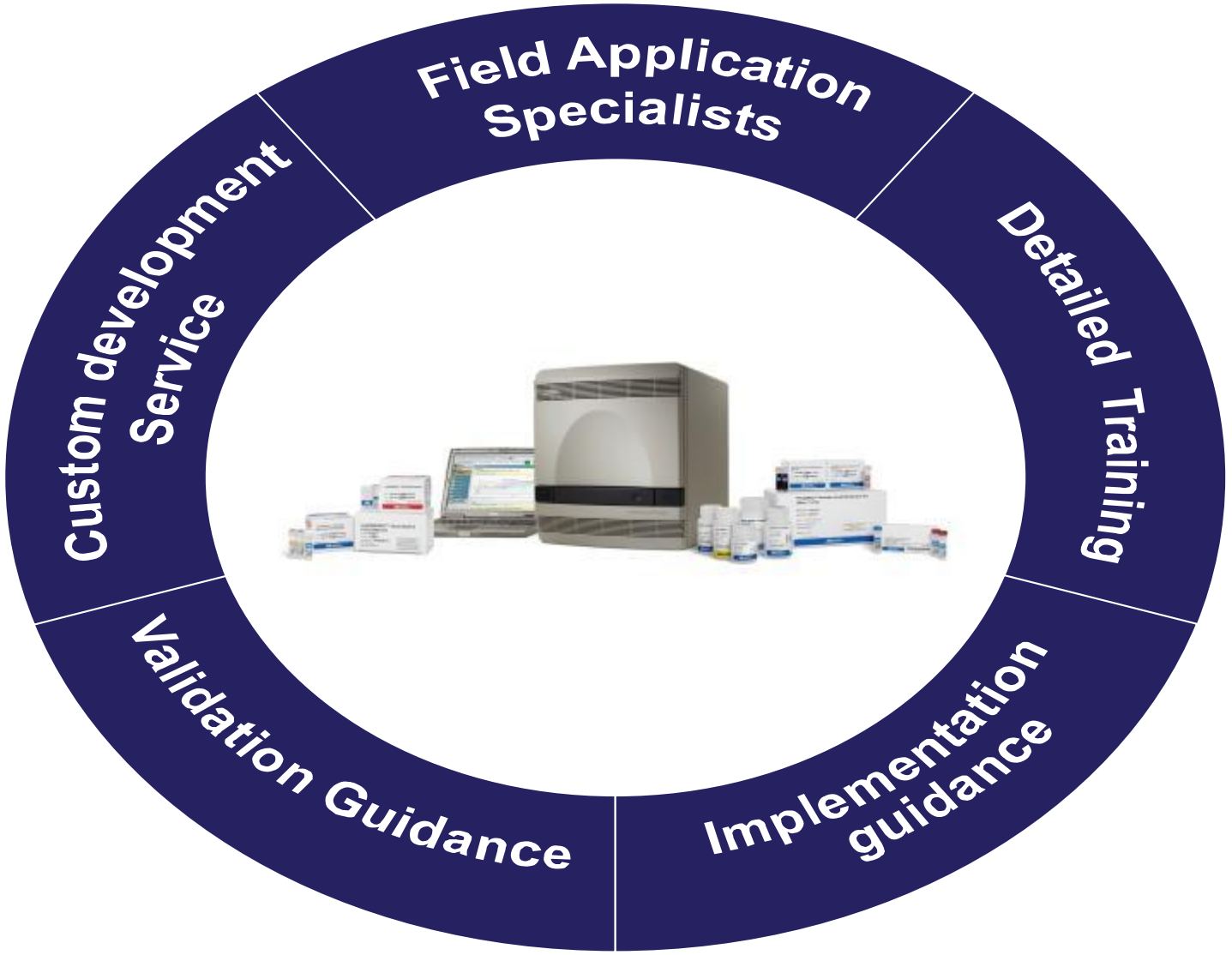
Advantages of Fully Integrated Systems

- Consistent Performance: Kit to Kit, Lot to Lot, Year to Year
- Minimizes:
  - Internal development and optimization investment
  - Procurement and qualification of reagents from multiple vendors
  - Development of SOP's for preparation of standards and controls
  - Preparation and qualification of standards and controls
- Challenging Test Samples :
  - Leverage our experience gained from solving sample preparation challenges around the world
- Customer Support:
  - Expert Field Application Scientist support eliminates development of specialized internal training program and enables rapid implementation
  - Equipment validation support, protocols and execution
  - Test method validation examples and guidance
  - Trouble shooting: Expert technical support if a problem is encountered
- Site to Site Transfers:
  - No comparability validation needed, same method and multiple sites

Worldwide support network



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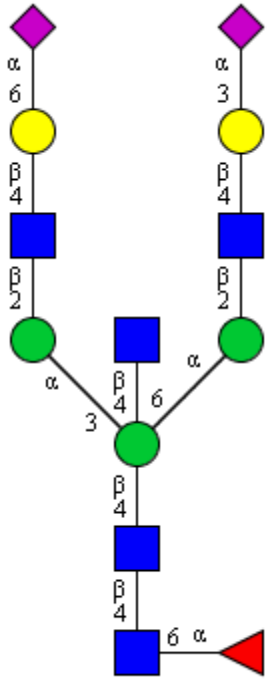
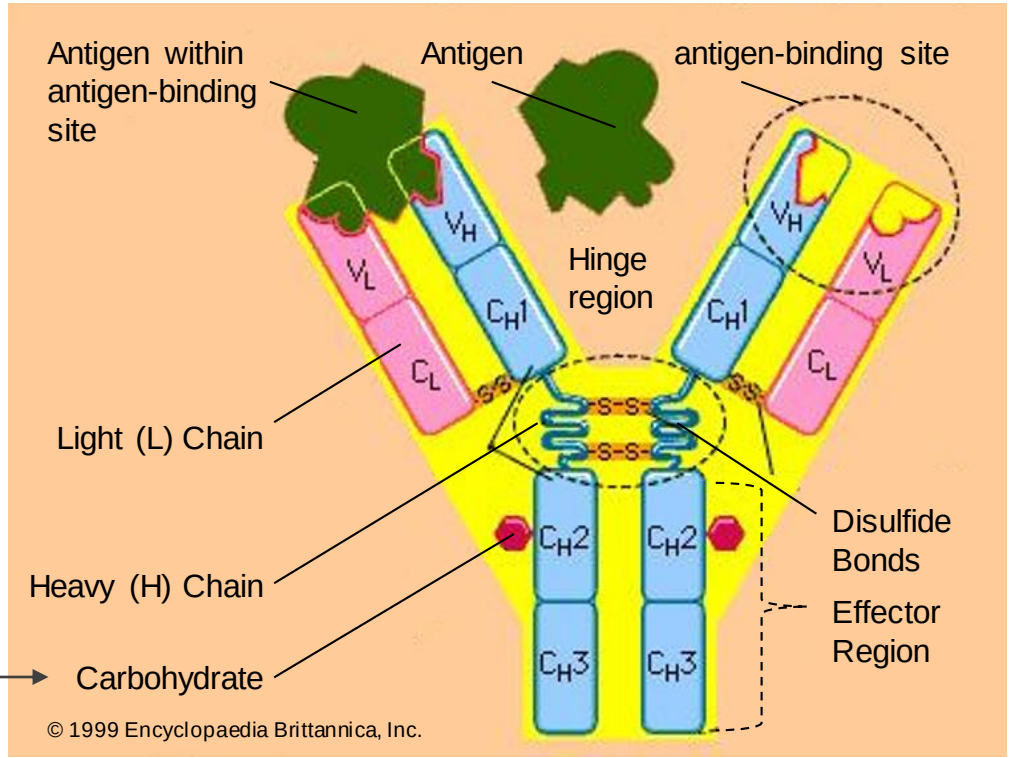
Glycan Analysis

- Gal
- Man
- Fuc
- NANA
- NGNA
- GlcNAc





Glycosylation:
Attachment of Glycans (Carbohydrates or oligosaccharides) to proteins

- Glycans are made up of **Monosaccharides**
- More than **70%** of biotherapeutics are glycosylated
- Glycans are important for **protein stability**, **folding**, **assembly**, **signaling**, etc.
- Glycosylation is a **critical quality attribute** (CQA) of biotherapeutics

Glycan Analysis Challenges

Single channel separation with low throughput

HPLC/UHPLC & single cap CE

Poor data quality of high throughput methods

Chip based instruments

Labor intensive workflow

Multiple pipetting steps, use of spin columns, etc.

Use of toxic sodium cyanoborohydride chemistry

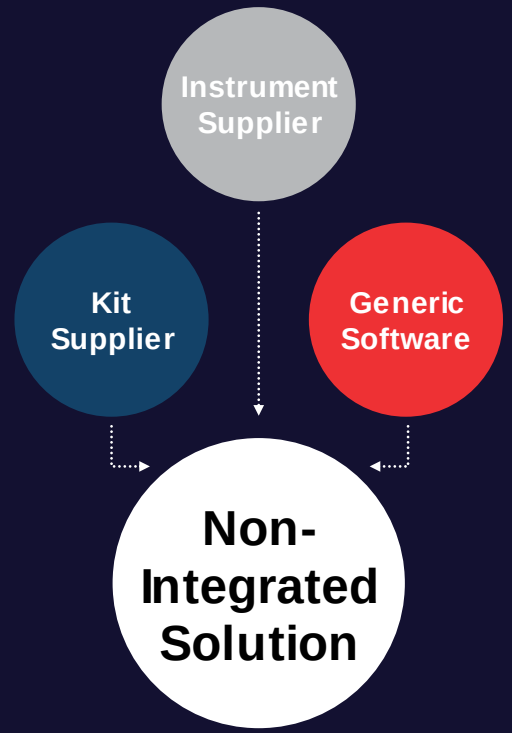
Use of lengthy vacuum centrifugation steps

Generic software taking long analysis time

Non-integrated solution from multiple vendors

Commercial sample prep kits with high cost per sample

No validation support for result/solution



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GlycanAssure System

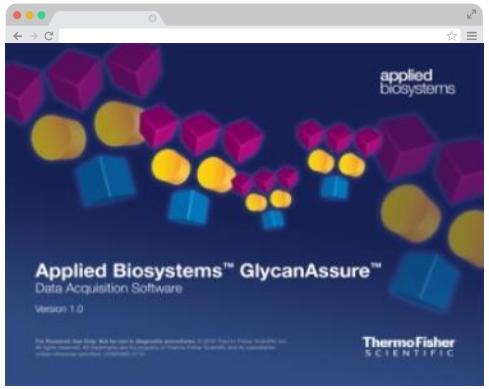
- Very high throughput (96 samples/7–9 hrs)
- No compromise in CE separation time
- Easy magnetic bead based workflow
- Fewer pipetting steps and hands-on time
- Multiple fluorescent dyes for glycan labeling
- No use of Sodium cyanoborohydride
- No vacuum centrifugation steps
- Parallel analysis of 24 samples on AB multi capillary CE (<2min/sample)
- Fit for use “app” style software
- Integrated solution with sample prep kits, CE instrument and software
- Reduced cost per sample

First fully integrated system combining throughput and data quality

Product Overview

APTS Kit
PN: A28676

Turquoise Kit
PN: A28678



Data Acquisition Software
PN: A30750

Teal Kit
PN: A28677

3500 & 3500XL
PN: A30467 & A30556

Data Analysis Software
PN: A30751

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Sample Prep Kits

- Three fluorescent dyes with distinct properties
- Traditional APTS and two Thermo Fisher Scientific proprietary dyes
- Faster glycan labeling with Teal and Turquoise dyes (30 min)
- Magnetic beads for glycan purification and excess dye removal
- No need for excess dye removal with Teal (96 samples in 7hrs)
- Each kit with PNGase-F enzyme and beads for 96 samples

APTS Kit

Teal Kit

Turquoise Kit

9-Aminopyrene- 1, 4, 6-trisulfonic acid (APTS)

CE Instrumentation

Key Features

- 8 & 24-capillary systems for medium & high throughput parallel analysis (< 2min/sample)
- Single-line, 505 nm, solid-state, long-life laser that utilizes a standard power supply and requires no heat-removal
- Powerful, integrated data acquisition and analysis software that provides real-time assessment of data
- Radio frequency identification (RFID) technology that tracks key consumables data and records administrative information
- Calibration across capillaries using internal standard
- Unrivaled application flexibility—one array and one polymer are used for most applications
- Simple setup, operation, and maintenance

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Software

Acquisition Dashboard

Quick access to instrument status and data

Run Setup

Simplified and intuitive chevron based, step by step workflow

Reports

Easily create reports with choice of templates

Library

Stores historical information for plates, experiments, methods and reference

Analysis Dashboard

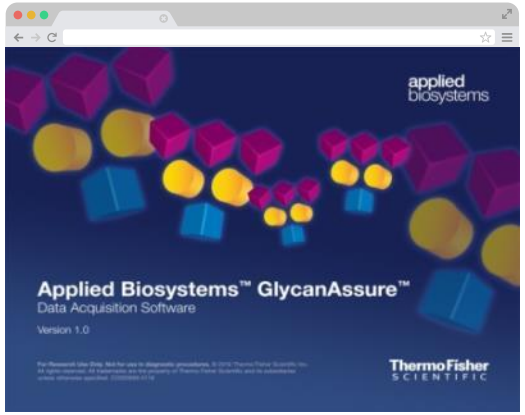
- Quick view of analyzed and un-analyzed samples. Allows to create data analysis projects
- Quick access to favorite projects

Data Processing

- Quick comparison of analysis methods, alignment, normalization, and smoothing
- Library of analysis methods and recommendations for glycan analysis

Analysis

Simplified graphics interface for manual integrations



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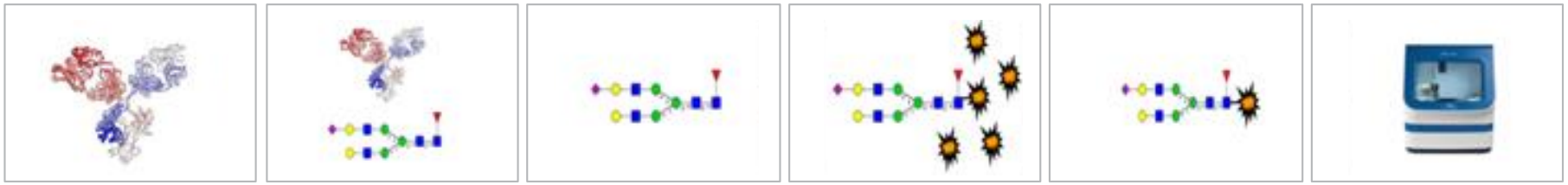
APTS Workflow



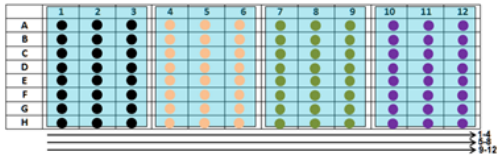
Sample	Glycan Release	Glycan Purification	Glycan Labeling	Excess Dye Removal	CE Analysis
Glycoprotein	PNGase-F	Magnetic Beads	APTS	Magnetic Beads	24 Capillary CE Analysis
	1 Hour	**30 Minutes**	**2 Hours**	30 Minutes	**3 Hours**

Time to Results:	9 Hours	96 Samples

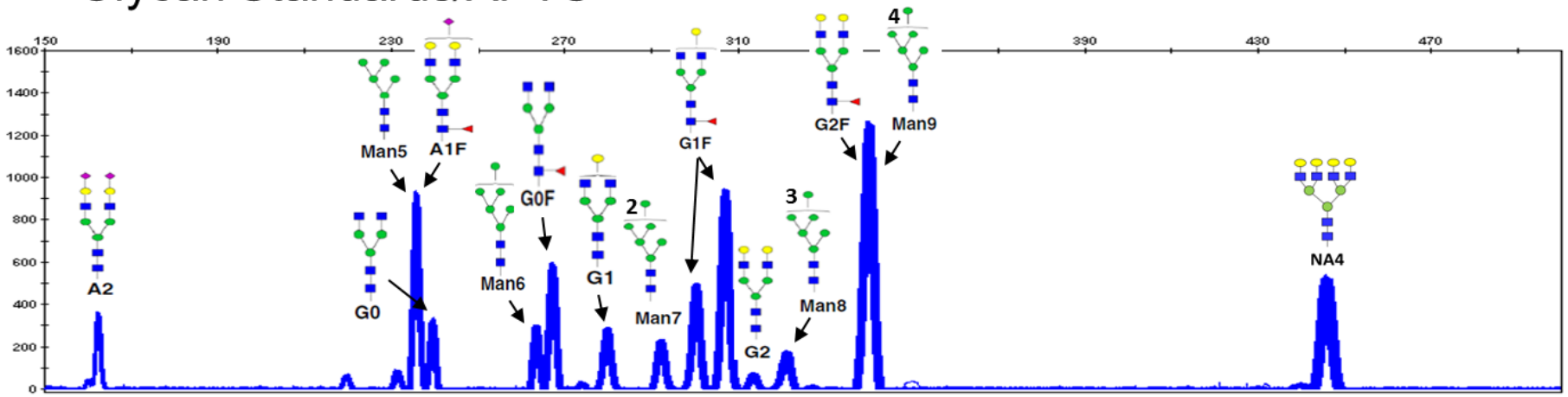
Notes:
Total 9hrs taking into account initial glycoprotein prep time and time between steps.
Total time of the workflow is only 7hrs. CE takes 45min to analyze 24 samples

Reproducibility of Glycan Separation

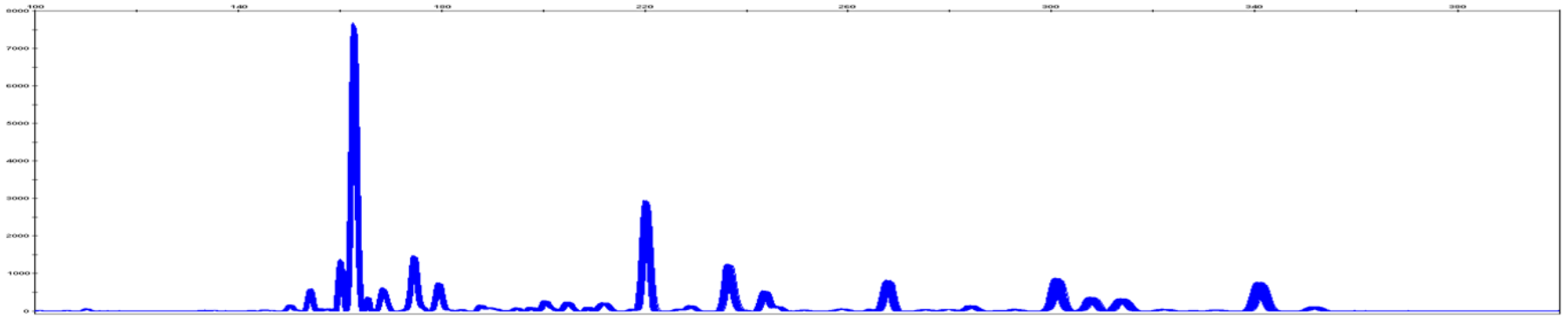
Overlay of 288 Injections



Glycan Standards/APTS



Plasma Glycans/APTS



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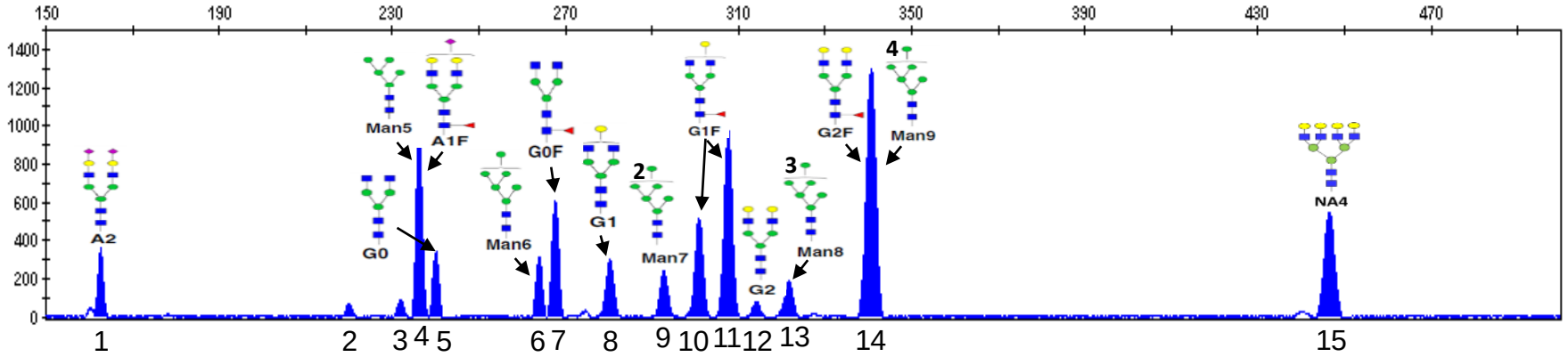
Variability Across Instruments & Arrays



	Peak	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1st 3500 32 Inj	Average	3.4%	0.8%	1.0%	10.5%	3.3%	3.6%	8.4%	4.4%	3.7%	8.0%	15.3%	1.0%	3.0%	23.5%	10.2%
	CV%	2.8%	2.2%	2.3%	1.1%	2.0%	2.8%	9.2%	1.1%	0.9%	0.5%	0.9%	3.6%	1.5%	1.0%	1.3%
2nd 3500xl 24 Inj	Average	3.3%	0.8%	1.1%	9.7%	2.8%	3.7%	8.1%	4.5%	3.7%	8.2%	15.7%	1.0%	3.1%	24.0%	10.4%
	CV%	2.0%	1.9%	1.8%	1.3%	2.3%	1.7%	0.9%	0.6%	0.7%	0.7%	0.4%	3.3%	0.8%	0.4%	1.2%
3rd 3500xl 24 Inj	Average	3.5%	0.8%	1.0%	11.1%	3.6%	3.6%	7.9%	4.4%	3.6%	7.9%	15.1%	1.0%	3.0%	23.3%	10.1%
	CV%	1.3%	3.0%	3.2%	1.2%	2.3%	1.7%	0.9%	1.1%	1.2%	0.7%	0.6%	6.1%	1.2%	0.7%	1.7%
3500xl Cap 1*	Average	3.5%	0.8%	1.0%	11.1%	3.7%	3.7%	7.9%	4.4%	3.6%	7.9%	15.1%	1.0%	3.0%	23.3%	10.0%
	CV%	1.5%	3.8%	2.7%	0.9%	1.9%	1.3%	0.6%	1.0%	1.1%	0.7%	0.3%	3.0%	1.1%	0.5%	0.9%
3500xl Cap 2*	Average	3.4%	0.8%	1.1%	10.9%	3.6%	3.8%	7.9%	4.4%	3.6%	7.9%	15.2%	1.1%	3.0%	23.3%	10.1%
	CV%	1.2%	2.4%	2.2%	0.9%	1.9%	1.8%	1.0%	0.9%	0.7%	0.5%	0.5%	1.8%	0.9%	0.4%	1.3%
3500xl Cap 3*	Average	3.4%	0.8%	1.1%	11.0%	3.6%	3.8%	8.0%	4.4%	3.6%	7.9%	15.2%	1.1%	3.0%	23.3%	9.9%
	CV%	1.5%	3.8%	2.1%	1.2%	2.3%	1.2%	0.6%	0.6%	0.8%	0.6%	0.3%	2.8%	1.2%	0.5%	0.7%

*Tight relative quantities among repetitive injections (12 Injections)

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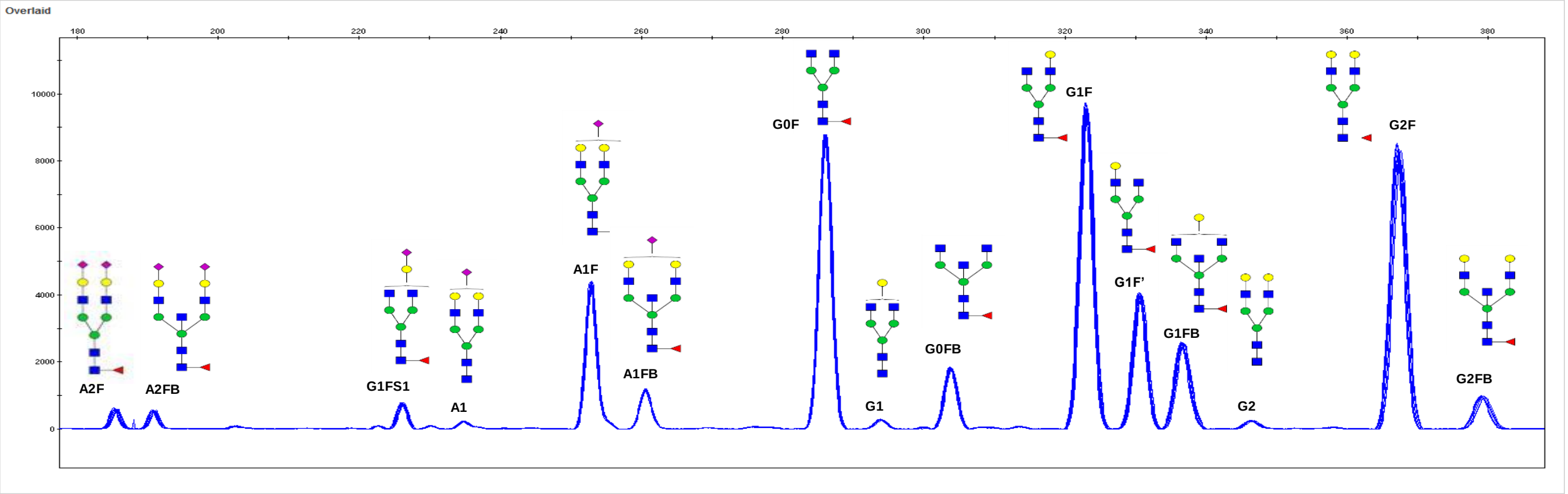
Consistent Quantitation of glycans from varying inputs of IgG

	1µg		2.5µg		5µg		25µg		50µg		100µg	
Peak #	%Area	%CV	%Area	%CV	%Area	%CV	%Area	%CV	%Area	%CV	%Area	%CV
A2F	1.11	12.27	0.96	5.23	1.03	3.60	1.08	4.48	0.93	4.99	0.89	8.25
A2FB	0.96	18.10	0.72	6.87	0.78	11.15	1.01	5.31	0.86	4.28	0.83	8.62
G1FS1	1.32	7.04	1.25	2.83	1.32	4.67	1.32	5.17	1.29	4.49	1.28	3.95
A1	0.61	14.19	0.59	14.17	0.54	13.6	0.47	11.45	0.48	5.16	0.45	3.67
A1F	9.14	4.32	8.53	2.76	8.55	3.97	9.04	1.89	8.51	3.54	8.53	3.64
A1FB	2.20	3.95	1.90	5.78	1.65	3.69	2.92	13.97	2.36	3.60	2.26	7.28
G0F	18.34	6.21	19.03	3.18	19.52	2.83	18.51	0.67	19.39	0.92	19.45	2.00
G1	0.73	12.69	0.77	3.73	0.80	2.86	0.53	2.81	0.56	4.86	0.57	6.40
G0FB	2.73	4.15	2.99	2.25	3.10	1.22	3.86	1.52	3.98	1.39	3.97	1.17
G1F	23.24	1.28	23.35	0.98	23.61	0.40	21.88	1.06	22.39	0.43	22.38	1.07
G1F’	9.66	1.19	9.71	1.82	9.66	1.41	9.21	1.62	9.30	0.83	9.29	1.10
G1FB	5.59	4.89	5.65	2.13	5.56	1.30	6.39	0.58	6.36	0.65	6.36	1.27
G2	0.49	10.45	0.49	5.55	0.46	1.54	0.60	6.16	0.57	3.34	0.57	3.50
G2F	21.83	6.3	22.03	2.02	21.62	1.77	20.49	1.00	20.60	1.19	20.59	1.37
G2FB	2.05	14.73	2.04	4.91	1.82	1.92	2.67	7.78	2.42	2.12	2.37	2.78

Three independent sample preparations were performed for every input amount - Each sample was injected three times in 3500xL CE system

Separation of N-glycans from human serum IgG



APTS label

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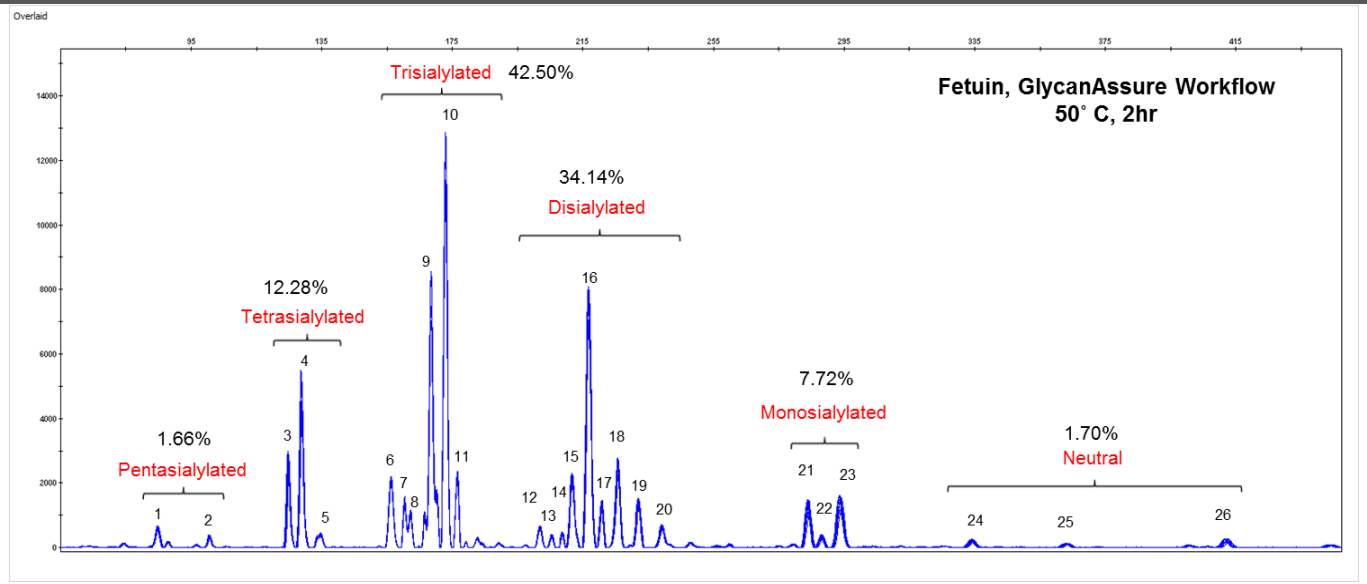
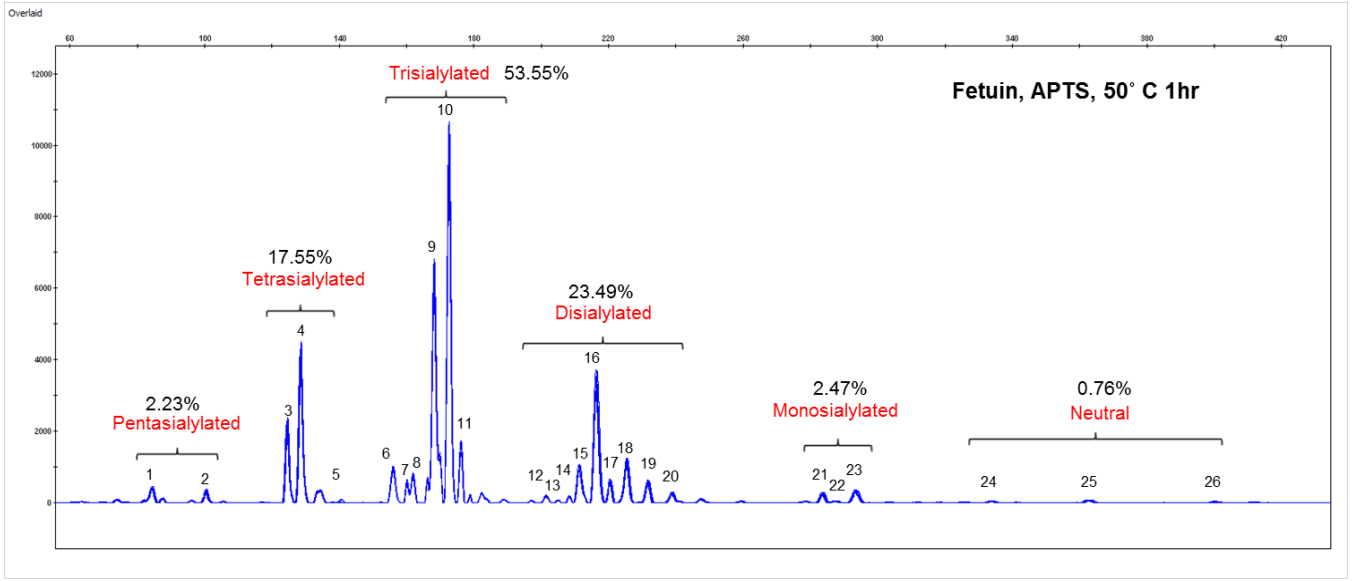
Accurate Quantitation of Highly Sialylated Glycans





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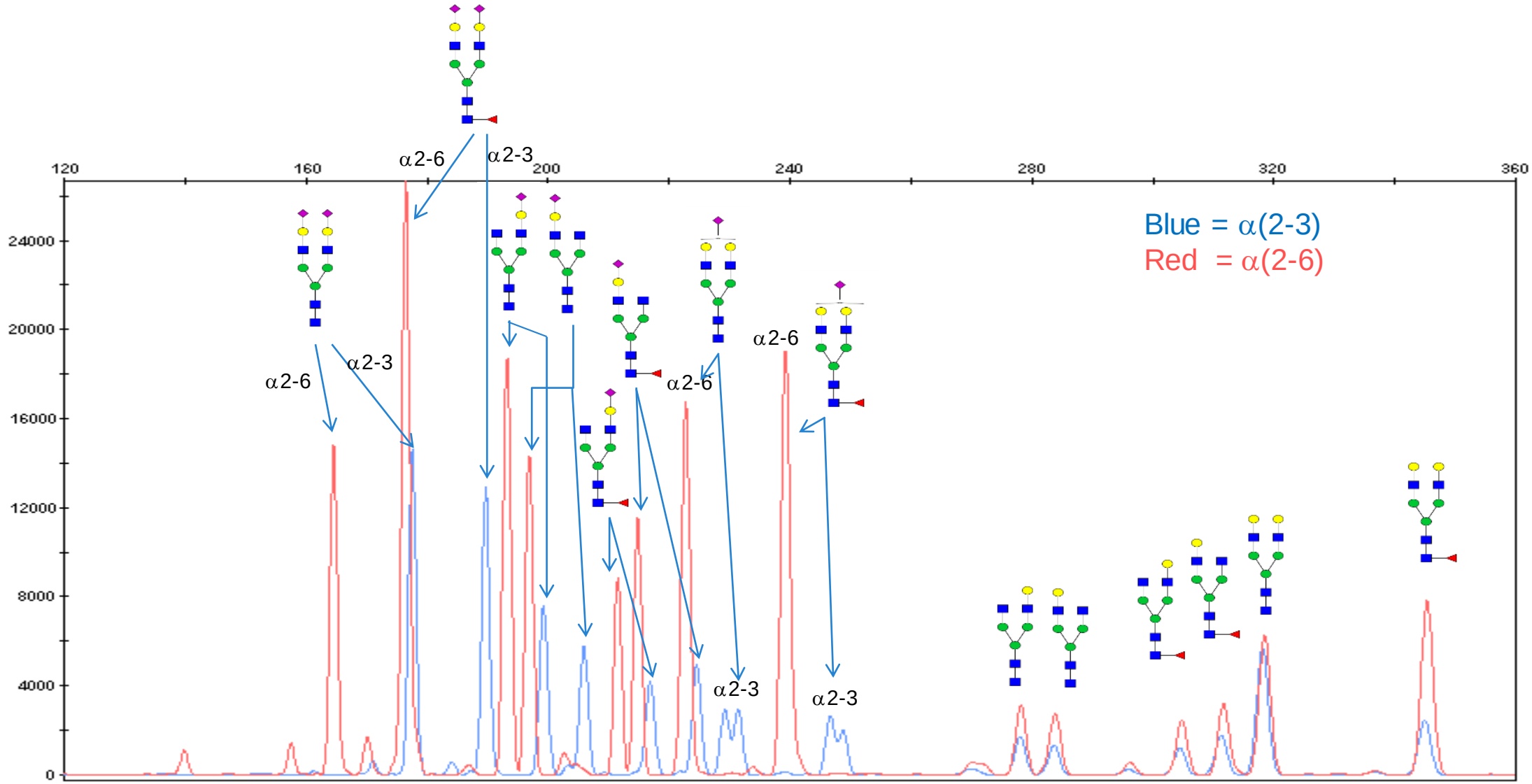
Separation of α(2-3/6) Sialylated Biantennary Glycans



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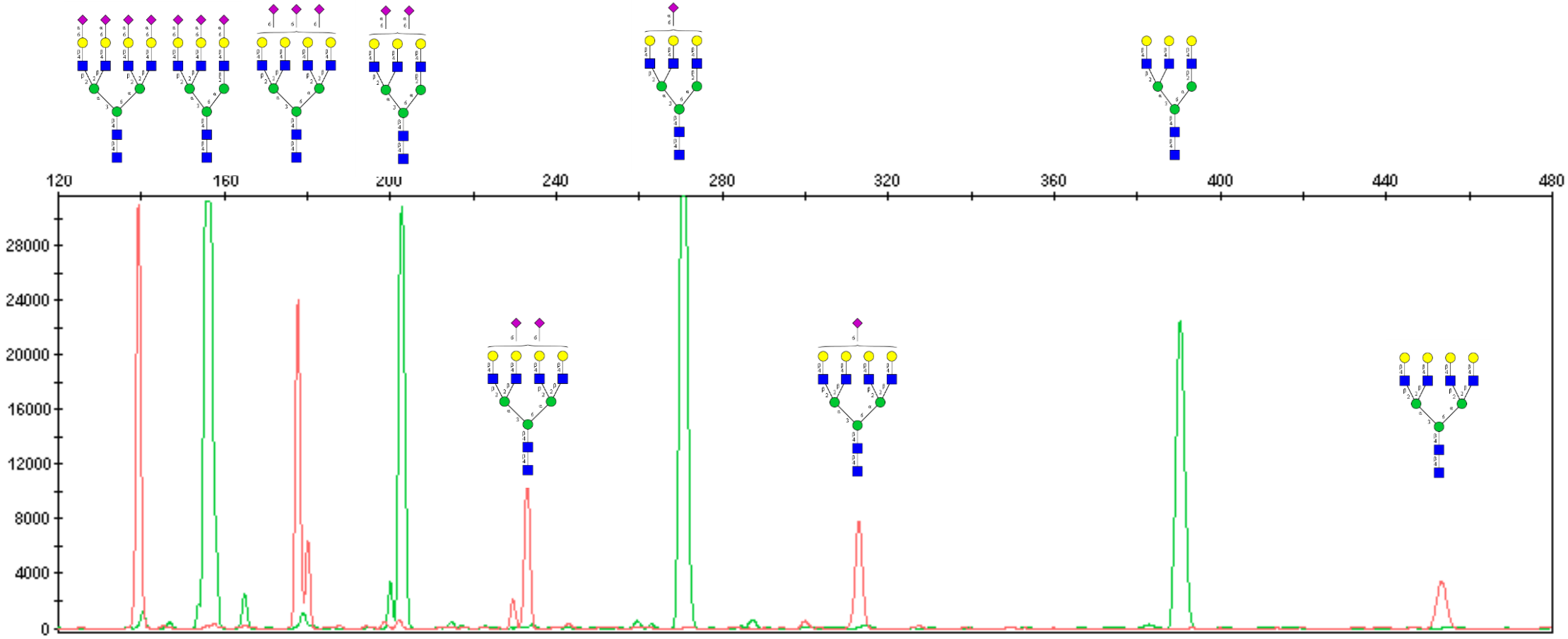
Separation of α(2-6) Sialylated Tri- and Tetraantennary Glycan Library

Green = Triantennary
Red = Tetraantennary



APTS label

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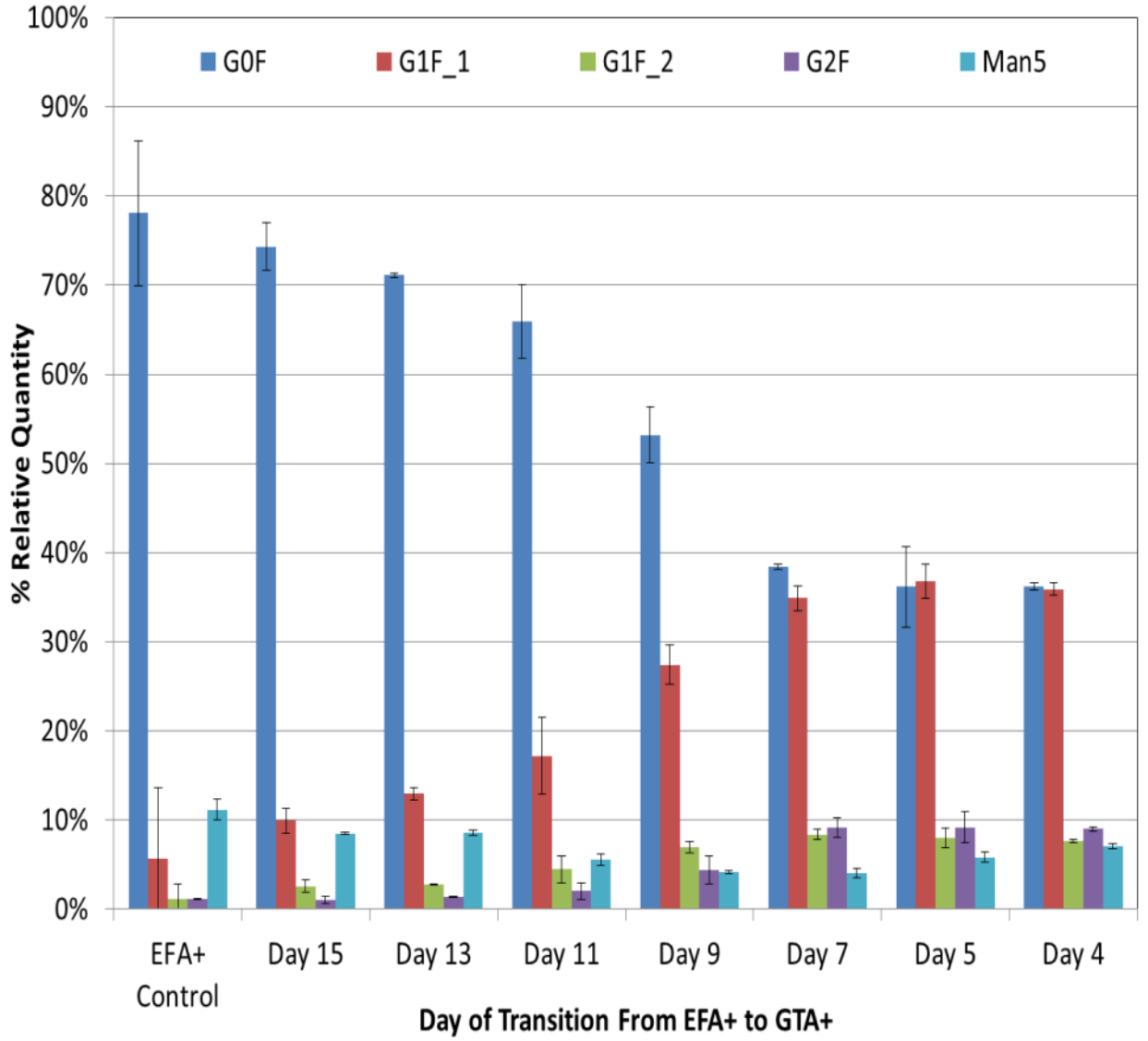
Selection of Right Clone with Right Glycosylation



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GlycanAssure: Value Proposition

General

First in class glycan analysis platform that combines both high throughput and data quality

Specific*

Faster → Simpler → High Quality → Cost-effective → Integrated Solution

* Compared to other Commercial & Homebrew Products

THANK YOU