

The Ludger GX-mAb Glycan Analysis Service

High Throughput glycan analysis service
of monoclonal antibodies (mAbs) for
drug developers and manufacturers



Who is the GX-mAb glycan analysis service for?

GX = Glycan analysis eXtra

The Ludger GX-mAb glycan analysis service is for mAb developers, both innovators and biosimilar companies, who need to analyse hundreds of mAb samples reliably, at an affordable cost and with a fast turnaround time.

Our typical GX-mAb clients are those who want to:

Demonstrate comparability of their drug's glycosylation to support submissions to regulatory authorities. This includes showing the comparability of glycosylation through out the drug lifecycle as well as biosimilarity to an innovator's drug

Optimise their drug's glycoform patterns to enhance the product's clinical performance, particularly the safety and efficacy profiles, and commercial profitability

Highlights of the GX-mAb glycan analysis service

Validated technology

Giving robust, reliable and repeatable data

Choice of analytical platforms

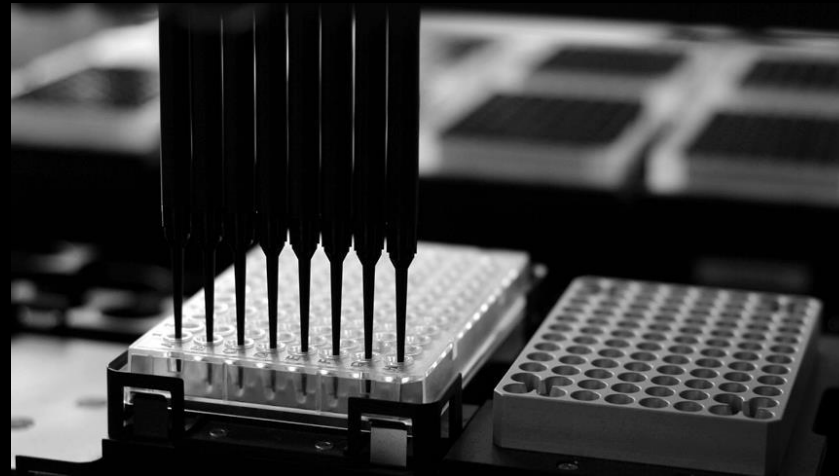
Select the analytical platform that you would like us to use giving you information on glycan relative quantitation and identification

Automated high throughput workflow

Overcoming the challenges when handling large sample numbers

Ludger experience

Using Ludger for analysing your mAbs helps to get you up and running with glycosylation analysis quickly



Cost effective

Parallel analysis of hundreds of mAb samples at an affordable cost

Quick turnaround

Results typically within two weeks of starting sample analysis

Method Transfer

Ludger methods can be transferred in house to allow you to perform the same analyses in your labs

Glycan analysis levels you need to satisfy regulatory authorities

Ludger GX-mAb is a Level 1 Analysis – the start of your journey to achieving well characterised glycosylation for your mAb

Glycan Analysis Level	Resulting Information	Detail
Level 4	Site-Specific Glycosylation: profiles for the <i>N</i> -glycans at each occupied site Site Occupancy: which <i>N</i> -glycan sites are occupied or unoccupied by glycans	*****
Level 3	Sequence and charge information on sialylation levels of <i>N</i> -glycan structures including information on sulphated and phosphorylated glycans	****
Level 2	More detailed information on <i>N</i> -glycan structures and their relative abundances including linkage and sequence information	***
Level 1 GX-mAb-P-LCMS	Adds a greater level of structural information from the MS compared to GX-mAb-AB-LC	**
Level 1 GX-mAb-AB-LC	Overall shape of glycosylation, relative abundance of each <i>N</i> -glycan structure, tentative structural assignments*	*

* Tentative structures assigned by comparing retention time values (GU values) to known glycan databases

What Data do you get with GX-mAb?

The GX-mAb service uses an automated high throughput workflow for analysing the glycosylation of hundreds of your mAbs samples. Two modules are available to choose from giving you the following data:

Level 1, GX-mAb UHPLC-MS

Mass of each glycan peak*

Data from LC-MS analysis to aid in structural assignment

N-glycan composition of each peak*

Data from LC-MS analysis to aid in structural assignment

Relative % areas of each peak

Data from UHPLC analysis for sample comparison and to see the levels of important GCQAs

GU values for each peak

Data from UHPLC analysis to compare against other samples

Level 1, GX-mAb UHPLC

Relative % areas of each peak

Data from UHPLC analysis for sample comparison and to see the levels of important GCQAs

GU values for each peak

Data from UHPLC analysis to compare against other samples and available glycan databases to assign tentative structures

* Mass of N-glycan peaks and N-glycan structures tentatively assigned using module **GX-mAb-P-LCMS**. Our glycan analysis service, Level 2 analysis module is required to fully assign N-glycan structures to peaks

Regulatory Landscape for mAb glycosylation



European Medicines Agency
Pre-authorisation Evaluation

COMMITTEE FOR MEDICINAL PRODUCTS
(CHMP)

GUIDELINE ON DEVELOPMENT, PHARMACOLOGICAL
SPECIFICATIONS FOR MONOCLONAL ANTIBODIES

AGREED BY BIOLOGICS

BY CHMP FOR REVISION

ATION (DI)

ICS W

4.3.1. Physicochemical characterisation

A physicochemical characterisation program will generally include a determination of the class, subclass, light chain composition (kappa and/or lambda chains) and primary structure of the monoclonal antibody.

The amino acid sequence should be deduced from DNA sequencing and confirmed experimentally by appropriate methods (e.g. peptide mapping, amino acid sequencing, mass spectrometry analysis). The variability of N- and C-terminal amino-acid sequences should be analysed (e.g. C-terminal lysine) and mismatch should be analysed.

Free sulphhydryl groups and disulfide bridges should be determined. Disulfide bridge integrity and carbohydrate content (neutral sugars, amino sugars and sialic acids) should be determined. In addition, the structure of the carbohydrate chains, the oligosaccharide pattern (antennary profile) glycosylation site(s) and occupancy should be analysed.

Typically, monoclonal antibodies have one N-glycosylation site on each heavy chain located in the region. The light chain is usually not glycosylated. However, additional glycosylation sites on heavy chains may occur, and thus their presence or absence should be confirmed. Glycosylation should be characterised, and particular attention should be paid to their degree of modification (often G0, G1 and G2) should be determined.

Higher-order structure of the monoclonal antibody should be characterised using appropriate physicochemical methodologies.

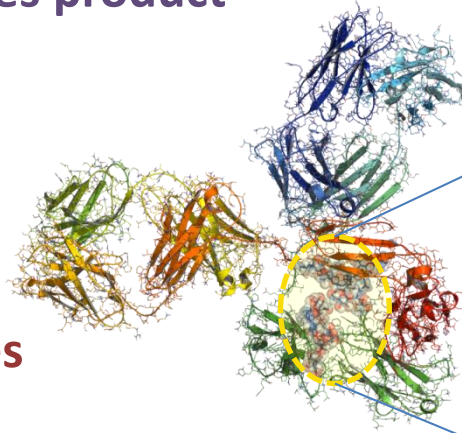
Glycans Greatly Influence the Safety and Efficacy of mAbs

Reliable measurement and control of glycosylation is essential to maintain consistent clinical performance

mAb Fc and Fab glycosylation influences product stability, activity, immunogenicity, and pharmacodynamics

Many Glycosylation Critical Quality Attributes (GCQAs) to consider

Given this, there is increasing regulatory and commercial pressure for you as a mAb producer (whether you are an innovator or biosimilar company) to properly measure, optimise and control your drug's glycosylation



GCQAs affecting ADCC:
terminal sialic acids, core fucose, bisecting N-acetylglucosamine, and mannose residues

GCQAs modulating CDC:
terminal galactose residues

GCQAs influencing anti-inflammatory activity:
sialic acid residues

Review: L. Liming, *J. Pharm. Sci.*, 2015, 1866–1884 and references therein.

Regulatory Guidelines for mAb Glycosylation – FDA and EMA

These are minimally informative – you need to do much more for QbD based drug realisation



The FDA defer to the following ICH guideline Q6B for characterisation of biopharmaceuticals:
Part 6.11(f): Carbohydrate structure



“For glycoproteins, the carbohydrate content (neutral sugars, amino sugars, and sialic acids) is determined. In addition, the structure of the carbohydrate chains, the oligosaccharide pattern (antennary profile) and the glycosylation site(s) of the polypeptide chain is analyzed, to the extent possible.”



EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

The EMA take the ICH guideline Q6B further and have written the following for monoclonal antibodies: **EMA/CHMP/BWP/157653/2007**

EMA guideline on development, production, characterisation and specifications for monoclonal antibodies and related products

“Typically, monoclonal antibodies have one N-glycosylation site on each heavy chain located in the Fc region. The light chain is usually not glycosylated. However, additional glycosylation site(s) in the heavy chains may occur, and thus their presence or absence should be confirmed. Glycan structures should be characterised, and particular attention should be paid to their degree of mannosylation, galactosylation, fucosylation and sialylation. The distribution of the main glycan structures present (often G0, G1 and G2) should be determined”

When to analyse mAb glycosylation?

For QbD you must characterise glycosylation early in your mAb's life cycle then at all key drug realisation stages

Cell line screening and Clone Selection

Gain an understanding of the glycan structures in the product

Discard lines or clones with undesired glycosylation

Process Scale-up

Demonstrate that scaling-up does not alter the structure and physicochemical properties of the product.

Provide data to demonstrate consistency of manufacture of the product

Process Optimisation

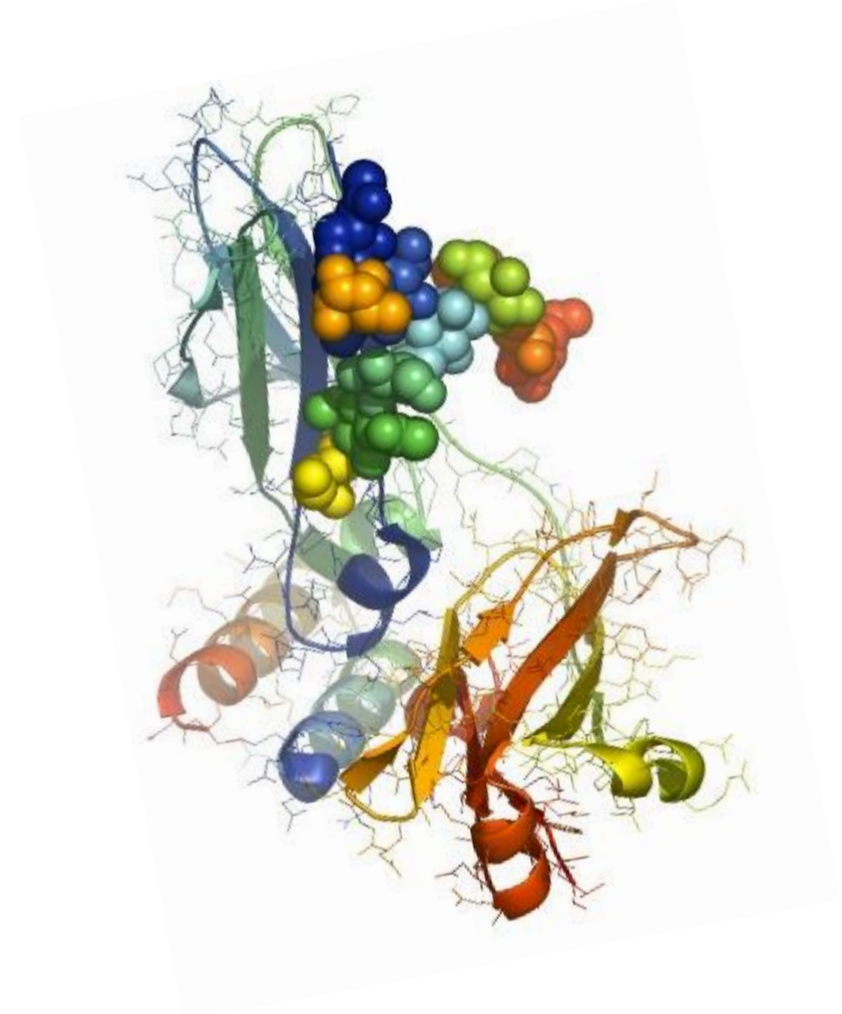
Optimise beneficial glycan structures that can impact the efficacy of the product.

Demonstrate that changes to the process do not alter the structure and physicochemical properties of product

Regulatory Submissions

Full characterisation of the final drug product

Comparison of final product with original drug or other biosimilars



Aims of the GX-mAb service

Features and benefits for drug developers

Get early answers on your mAb glycosylation

- So you can choose the best candidates and processes

Cost effective analysis of hundreds of samples

- So practical to use for QbD based development including establishment of Design and Control Spaces for mAb glycosylation

Orthogonal glycan analysis available

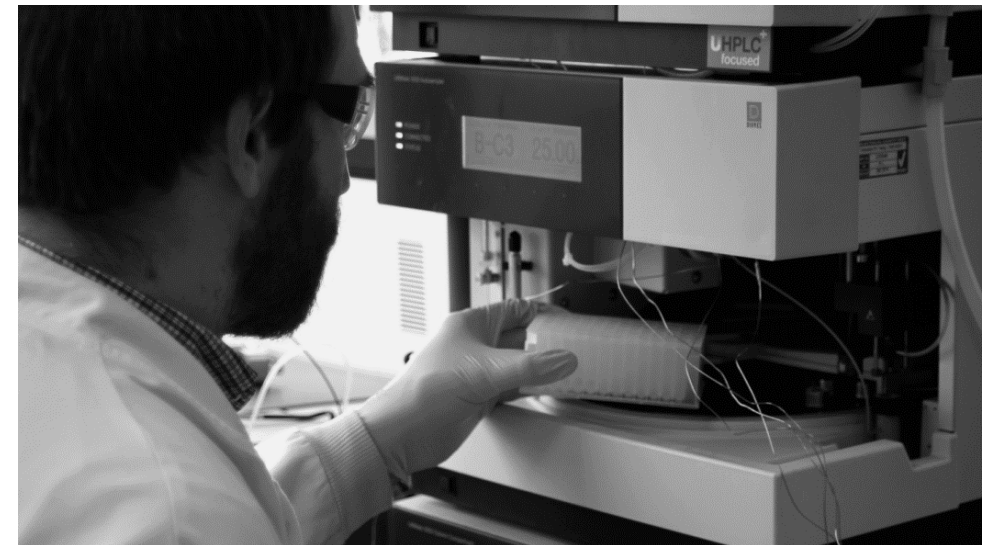
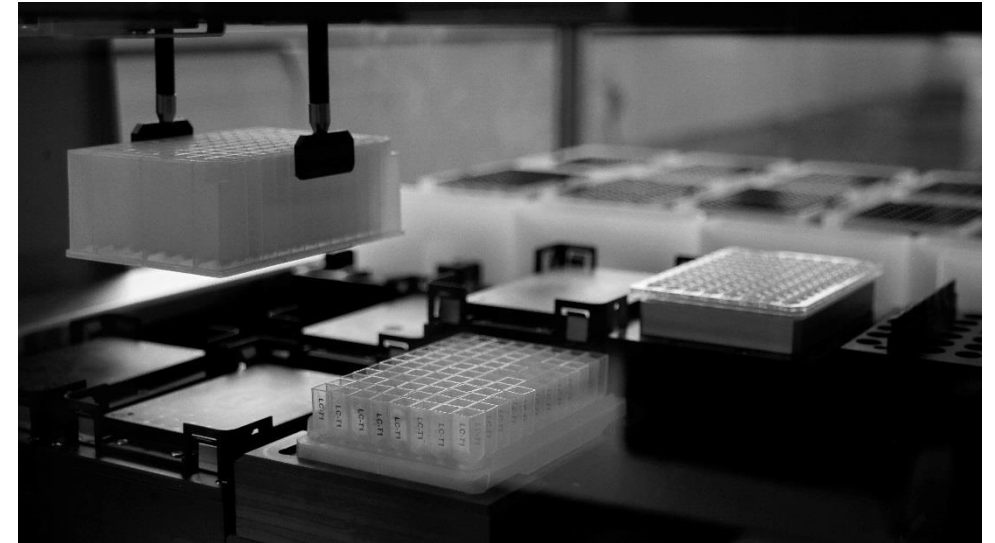
- So you get reliable glycan identification and quantitation

Validated analysis methods

- So you get reliable, repeatable data for yourself and the regulators

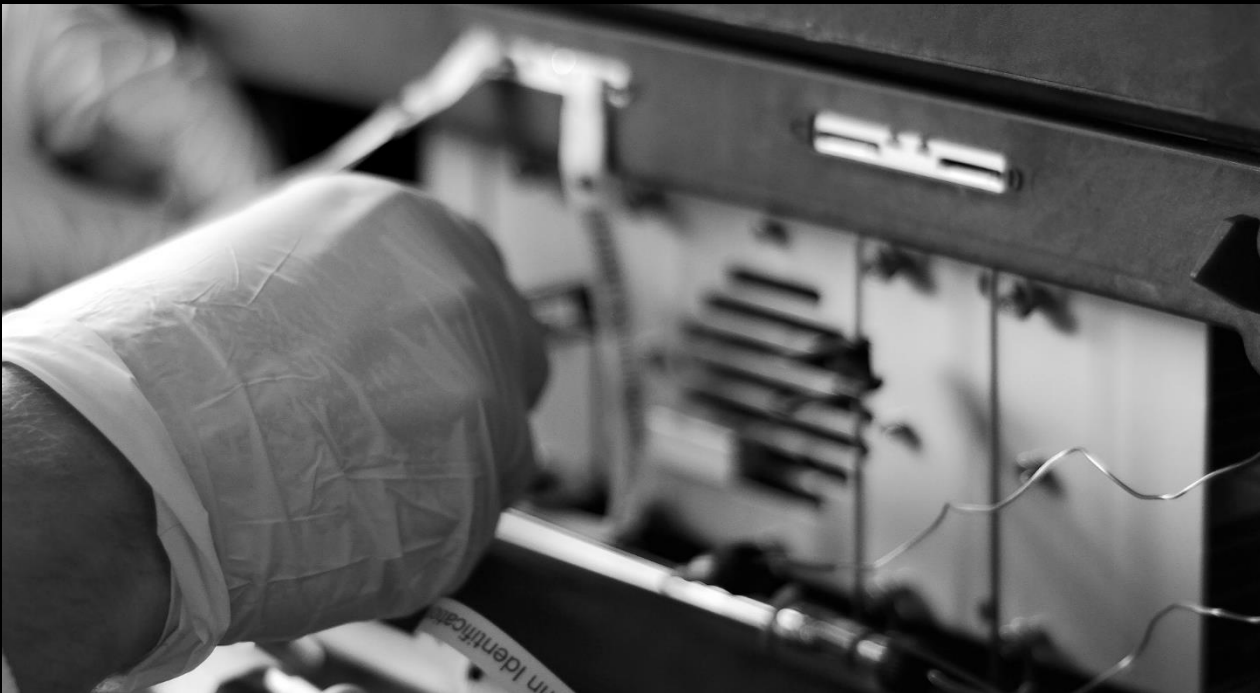
Fast turnaround time

- So you can move quickly



Orthogonal Glycoanalytical Platforms

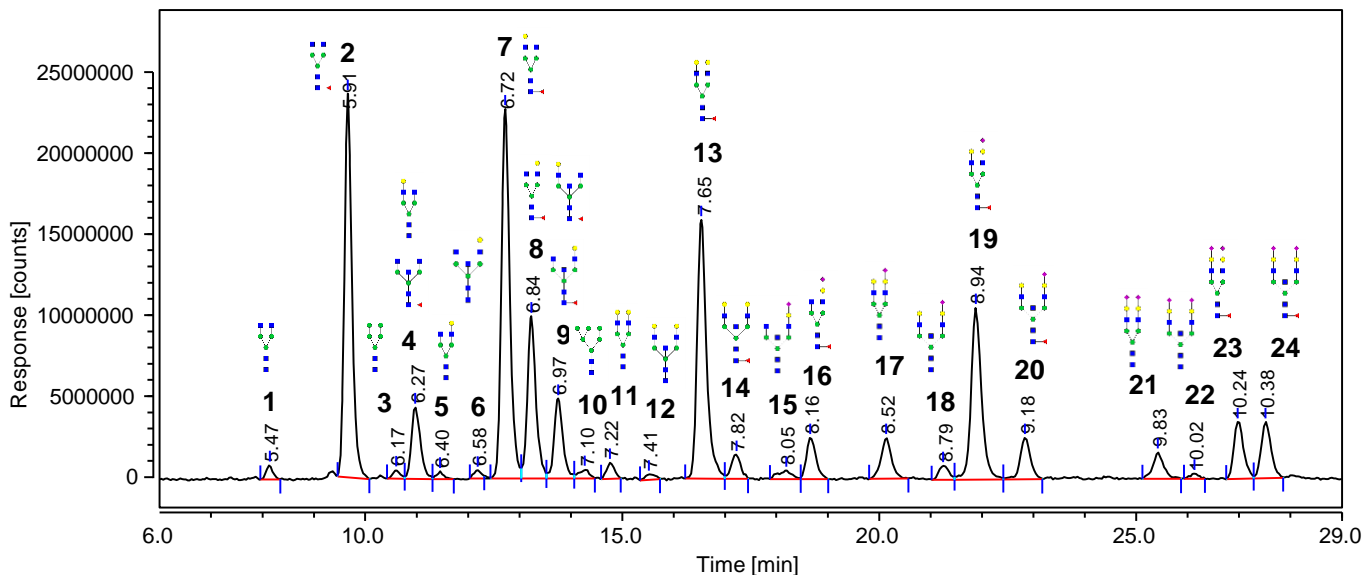
UHPLC + MS allow you to get reliable glycan structural ID and quantitation



Level 1 GX-mAb-AB-LC: HILIC UHPLC analysis of 2AB labeled mAb N-glycans

Accurate relative quantitation of N-glycans with glycan ID obtained by GU value comparison with a glycan database

PNGase F released human IgG N-glycans labeled with 2AB (LudgerTag 2AB labeling kit)



UHPLC chromatogram showing peaks numbered and labeled with their GU values and N-glycan structure*

* N-glycan structures have been assigned using orthogonal methods and are shown for illustrative purposes only. The glycan analysis service, Level 2 analysis module is required to fully assign N-glycan structures to peaks

Peak ID	GU value	Relative % area					
		hIgG S1	hIgG S2	hIgG S3	average	SD	CV
1	5.47	0.57	0.49	0.48	0.51	0.05	9.19
2	5.91	17.26	17.56	17.54	17.45	0.17	0.97
3	6.17	0.37	0.44	0.38	0.40	0.04	9.14
4	6.27	3.90	3.86	3.88	3.88	0.02	0.59
5	6.40	0.32	0.32	0.32	0.32	0.00	1.10
6	6.58	0.38	0.34	0.31	0.34	0.03	9.80
7	6.72	18.40	17.96	18.23	18.20	0.22	1.22
8	6.84	8.26	8.28	8.23	8.26	0.03	0.31
9	6.97	4.23	4.08	4.13	4.15	0.08	1.85
10	7.10	0.55	0.52	0.47	0.52	0.04	8.02
11	7.22	0.77	0.67	0.74	0.73	0.05	7.16
12	7.41	0.33	0.29	0.29	0.31	0.02	7.95
13	7.65	14.61	14.76	14.78	14.72	0.09	0.63
14	7.82	1.39	1.58	1.52	1.50	0.10	6.50
15	8.05	0.73	0.74	0.70	0.72	0.02	2.74
16	8.16	2.37	2.42	2.38	2.39	0.03	1.19
17	8.52	2.54	2.68	2.65	2.62	0.07	2.75
18	8.79	0.87	0.86	0.81	0.85	0.03	3.60
19	8.94	10.74	10.69	10.84	10.76	0.07	0.68
20	9.18	2.60	2.47	2.37	2.48	0.11	4.62
21	9.83	1.73	1.64	1.75	1.71	0.06	3.42
22	10.02	0.27	0.25	0.25	0.26	0.01	5.48
23	10.24	3.42	3.60	3.53	3.52	0.09	2.64
24	10.38	3.37	3.51	3.41	3.43	0.07	2.14

Table showing triplicate analysis of 2AB labeled human IgG N-glycans
CVs <5% highlighted green. Peaks with CVs >5% generally have a relative % area <1 %

Reporting for module GX-mAb-AB-LC

What your report will contain:

UHPLC chromatograms for all samples

including system suitability standard positive control and negative controls.

Peak numbering for peaks above the limit of quantitation LOQ.

Triplicate sample chromatograms will be overlaid to show repeatability and reliability

Relative percentage area of each peak

Obtained for peaks above the limit of quantitation LOQ

To enable comparison of samples analysed

GU values[§] for each peak¹

To enable comparison of samples analysed

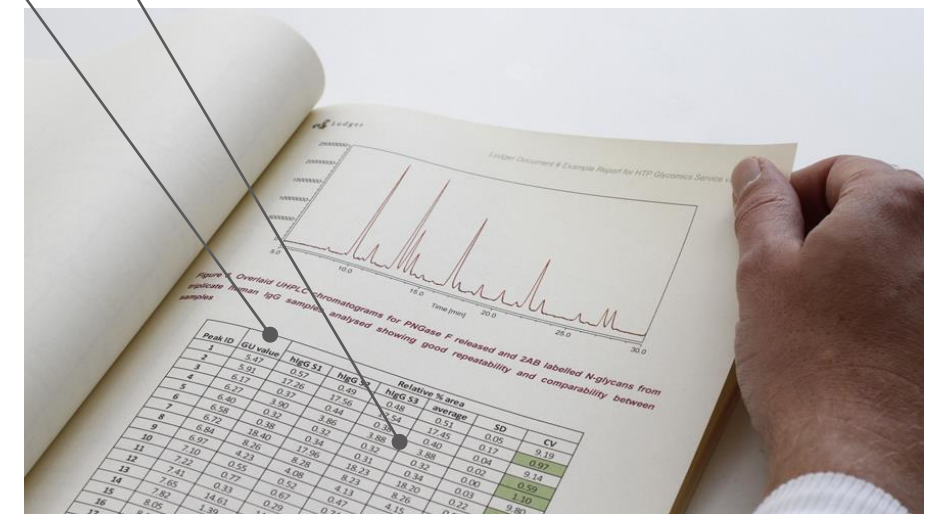
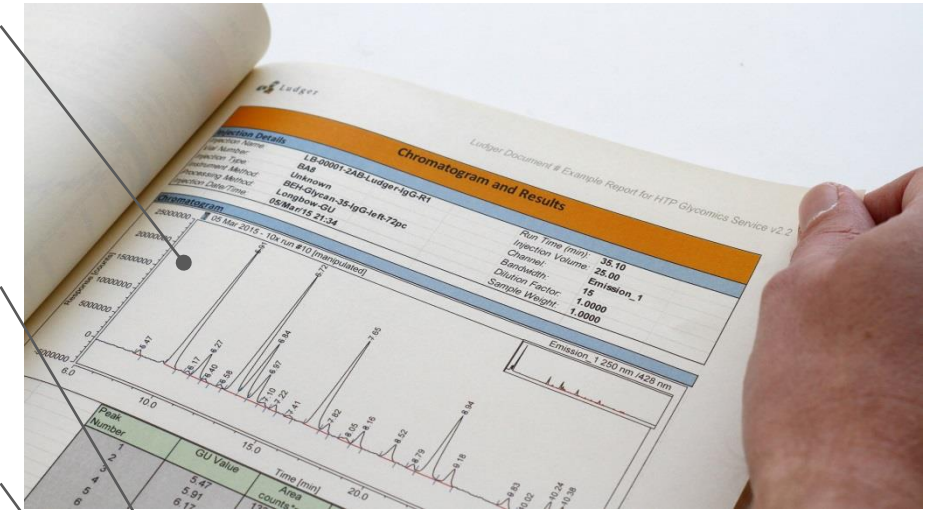
Tentative structural assignments can be made*

comparing the GU values against a database

The N-glycan profile from the Human IgG control sample can serve as a well characterised reference standard for the possible assignment of N-glycan structures*

[§] The elution times of glycans are expressed in glucose units (GU) by reference to a dextran ladder. Therefore, each glycan structure has a defined GU value independent of the instrument used to assign the GU value. On this basis, GU values can be used to predict structures

* To fully assign an N-glycan structure to each peak requires the use of orthogonal methods which is included in our glycan analysis Service, Level 2

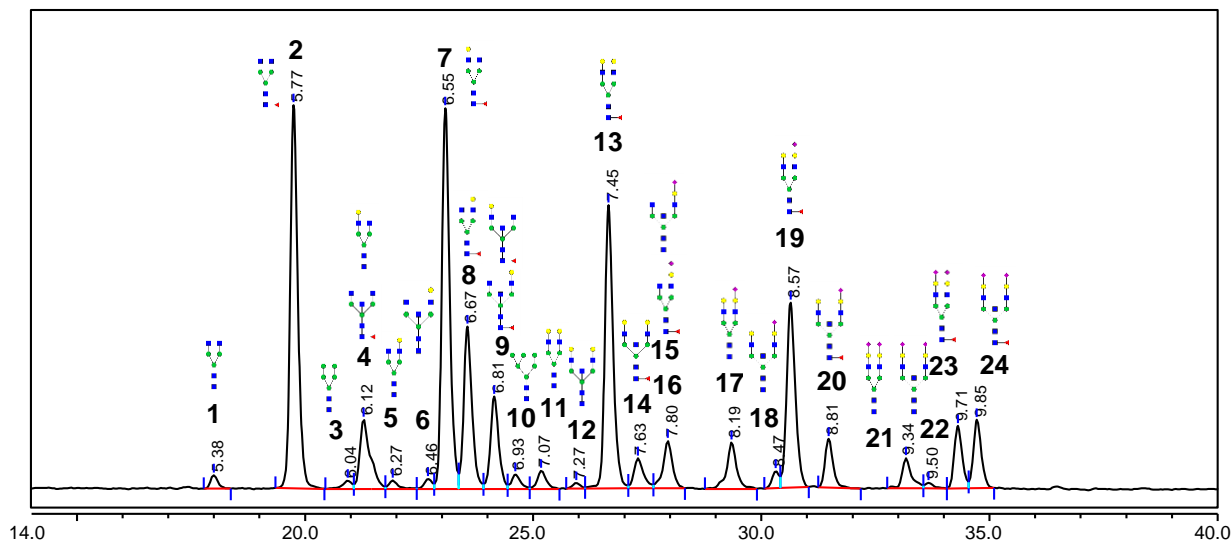


1. Royle, L. et al, *Methods Mol. Biol.* **2006**, 347, 125-143

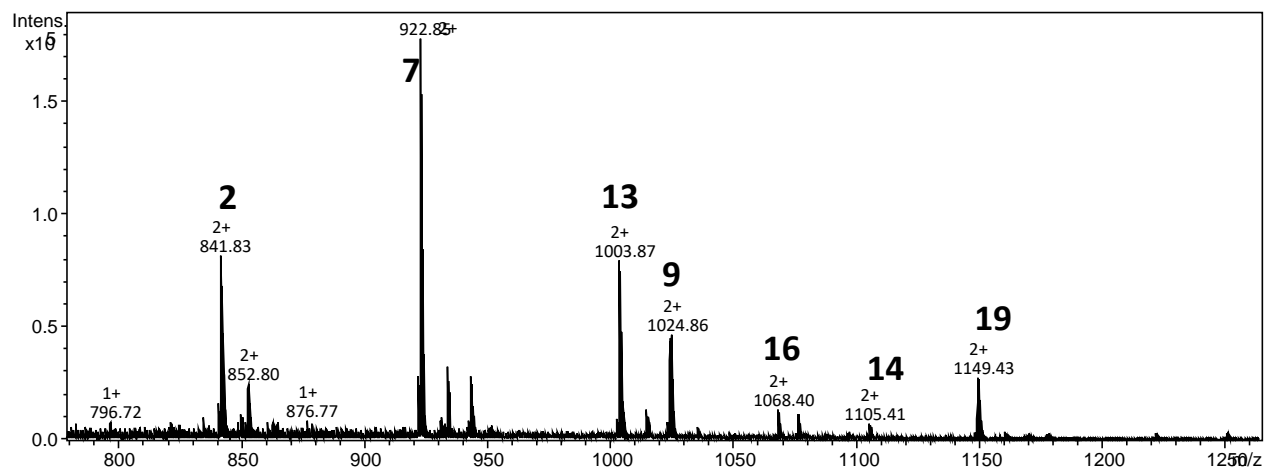
Level 1 GX-mAb-P-LCMS: HILIC UHPLC-MS analysis of procainamide labeled *N*-glycans

Accurate relative quantitation of *N*-glycans with glycan ID obtained from mass composition data from ESI-MS

PNGase F released IgG *N*-glycans labeled with procainamide (LudgerTag procainamide labeling kit)



UHPLC chromatogram showing peaks numbered and labeled with their GU values and *N*-glycan structures*



Combined MS spectrum from ESI analysis showing peaks numbered and labeled with $[M/Z]^{2+}$

Peak ID	UHPLC		ESI-LC/MS							
	GU Value	% Area	Composition				$[M/Z]^+$ calculated	$[M/Z]^{2+}$ calculated	$[M/Z]^+$ observed	$[M/Z]^{2+}$ observed
			Hex (H)	HexNAc (N)	Fuc (F)	Neu5Ac (S)				
1	5.38	0.61	3	4	0	0	1536.67	768.84	1536.87	768.96
2	5.77	17.82	3	4	1	0	1682.73	841.87	1682.80	841.99
3	6.04	0.48	5	2	0	0	1453.61	727.81	1454.67	727.92
4	6.12	4.37	3	5	1	0	1885.80	943.41	nd	943.52
			4	4	0	0	1698.72	849.86	nd	849.95
5	6.27	0.46	4	4	0	0	1698.72	849.86	nd	849.95
6	6.46	0.47	4	5	0	0	1901.80	951.40	nd	951.50
7	6.55	18.23	4	4	1	0	1844.78	922.89	nd	922.97
8	6.67	8.15	4	4	1	0	1844.78	922.90	nd	922.97
9	6.81	4.61	4	5	1	0	2047.86	1024.43	nd	1024.51
10	6.93	0.79	6	2	0	0	1616.67	808.84	nd	808.93
11	7.07	0.99	5	4	0	0	1860.77	930.89	nd	931.00
12	7.27	0.27	5	5	0	0	2063.85	1032.49	nd	1032.47
13	7.45	14.28	5	4	1	0	2006.83	1003.40	nd	1003.99
14	7.63	1.78	5	5	1	0	2209.91	1105.46	nd	1105.57
15	7.80	2.70	4	4	1	1	2135.87	1068.44	nd	1068.54
16			4	5	0	1	2192.89	1097.01	nd	1096.99
17	8.19	2.85	5	4	0	1	2151.87	1076.44	nd	1076.54
18	8.47	0.72	5	5	0	1	2354.95	1178.04	nd	1178.01
19	8.57	9.66	5	4	1	1	2297.93	1149.47	nd	1149.55
20	8.81	2.39	5	5	1	1	2501.01	1251.01	nd	1251.05
21	9.34	1.73	5	4	0	2	2442.96	1221.99	nd	1222.02
22	9.50	0.29	5	5	0	2	2646.04	1323.53	nd	1323.61
23	9.71	3.03	5	4	1	2	2589.02	1295.01	nd	1295.06
24	9.85	3.33	5	5	1	2	2792.10	1396.55	nd	1396.70

* *N*-glycan structures have been tentatively assigned using LC-MS data. The glycan analysis service, Level 2 analysis module is required to fully assign *N*-glycan structures to peaks

Reporting for module GX-mAb-P-LCMS

What your report will contain

UHPLC chromatograms for all samples

Including system suitability standard positive control and negative controls.

Peak numbering for peaks above the limit of quantitation LOQ.

Triplicate sample chromatograms will be overlaid to show repeatability and reliability

Relative percentage area of each peak

Obtained for peaks above the limit of quantitation LOQ

To enable comparison of samples analysed

GU values for each peak

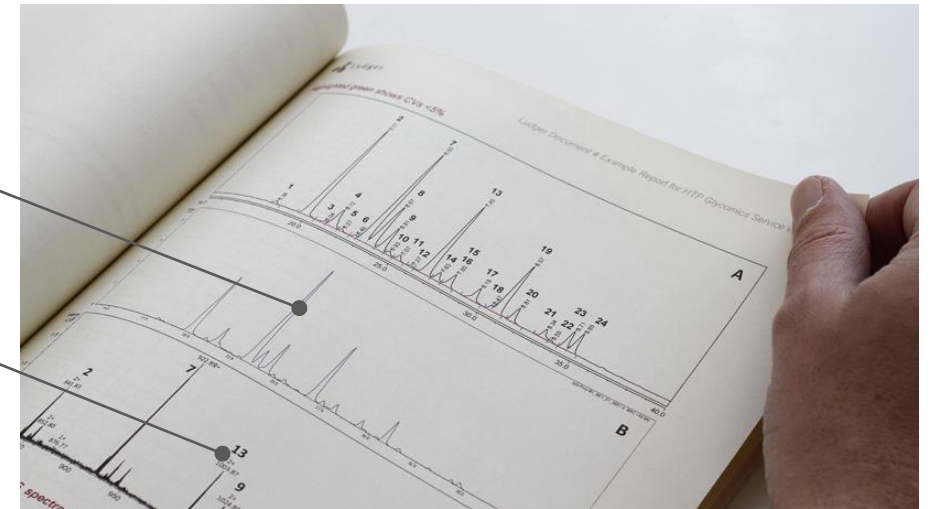
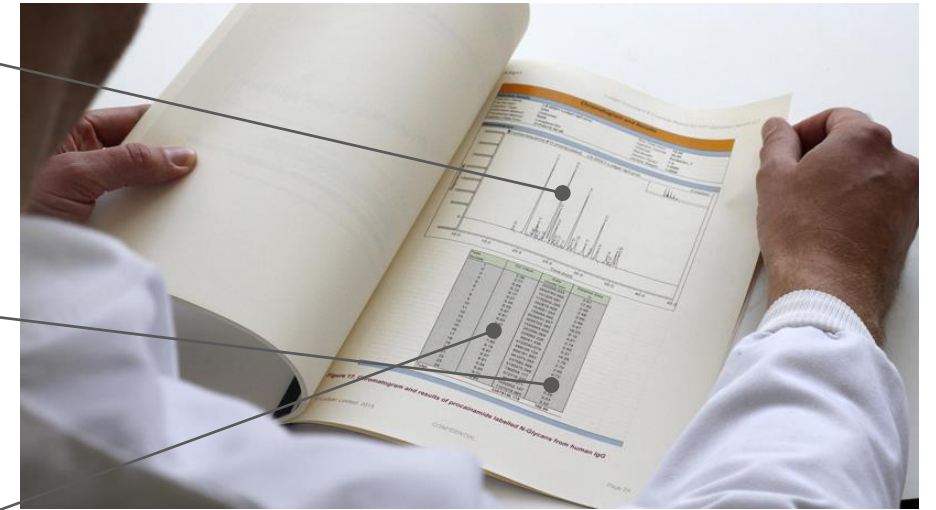
To enable comparison of samples analysed

Base peak chromatogram (BPC) of each sample

Data obtained from ESI LC-MS analysis

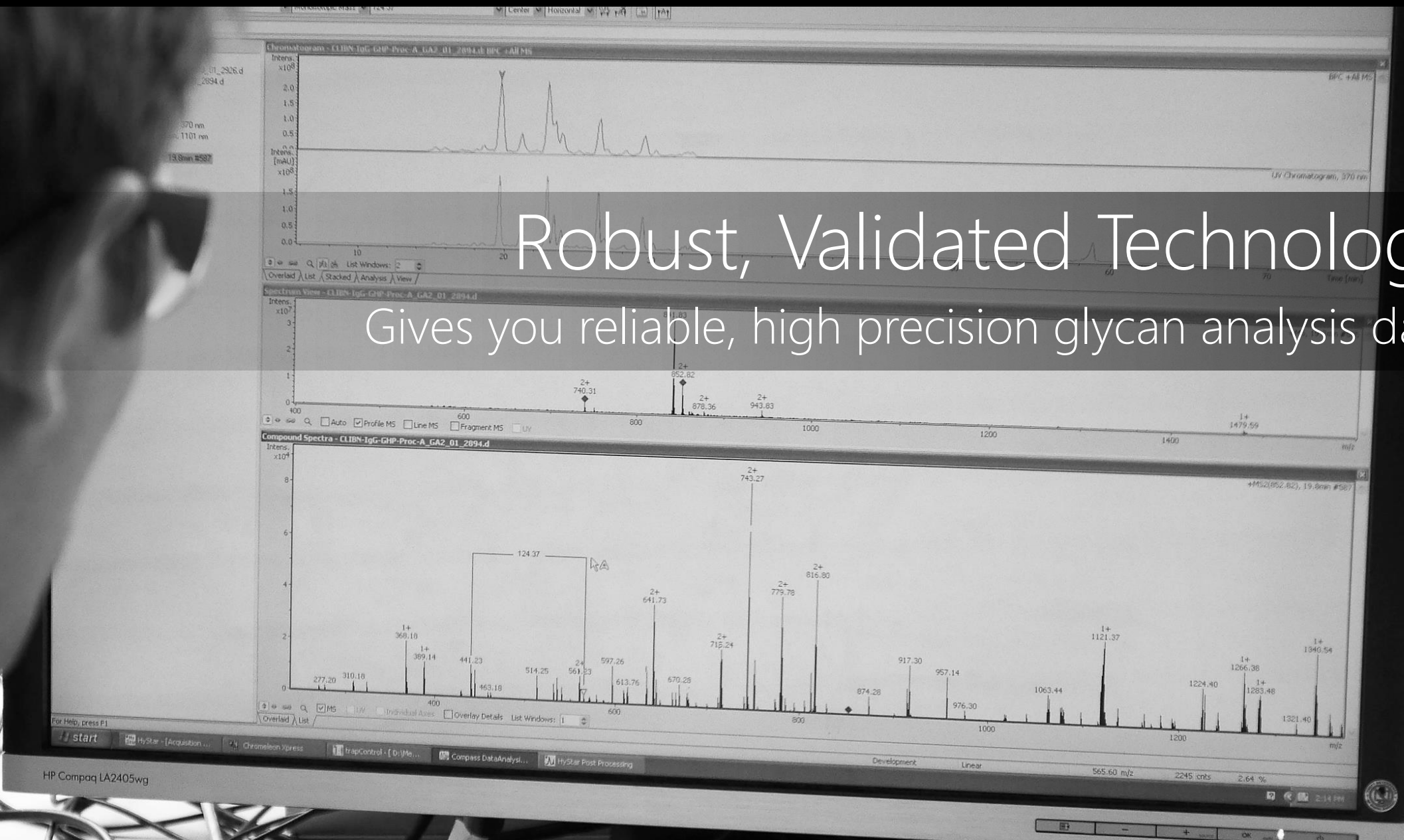
Glycan mass corresponding to each peak present in the BPC

Glycan composition* and correlation to the UHPLC chromatogram



* Glycan composition from LC-MS data is used to tentatively assign N-glycan structures. Orthogonal methods in our glycan analysis service, Level 2 analysis module are required to fully assign N-glycan structures to peaks

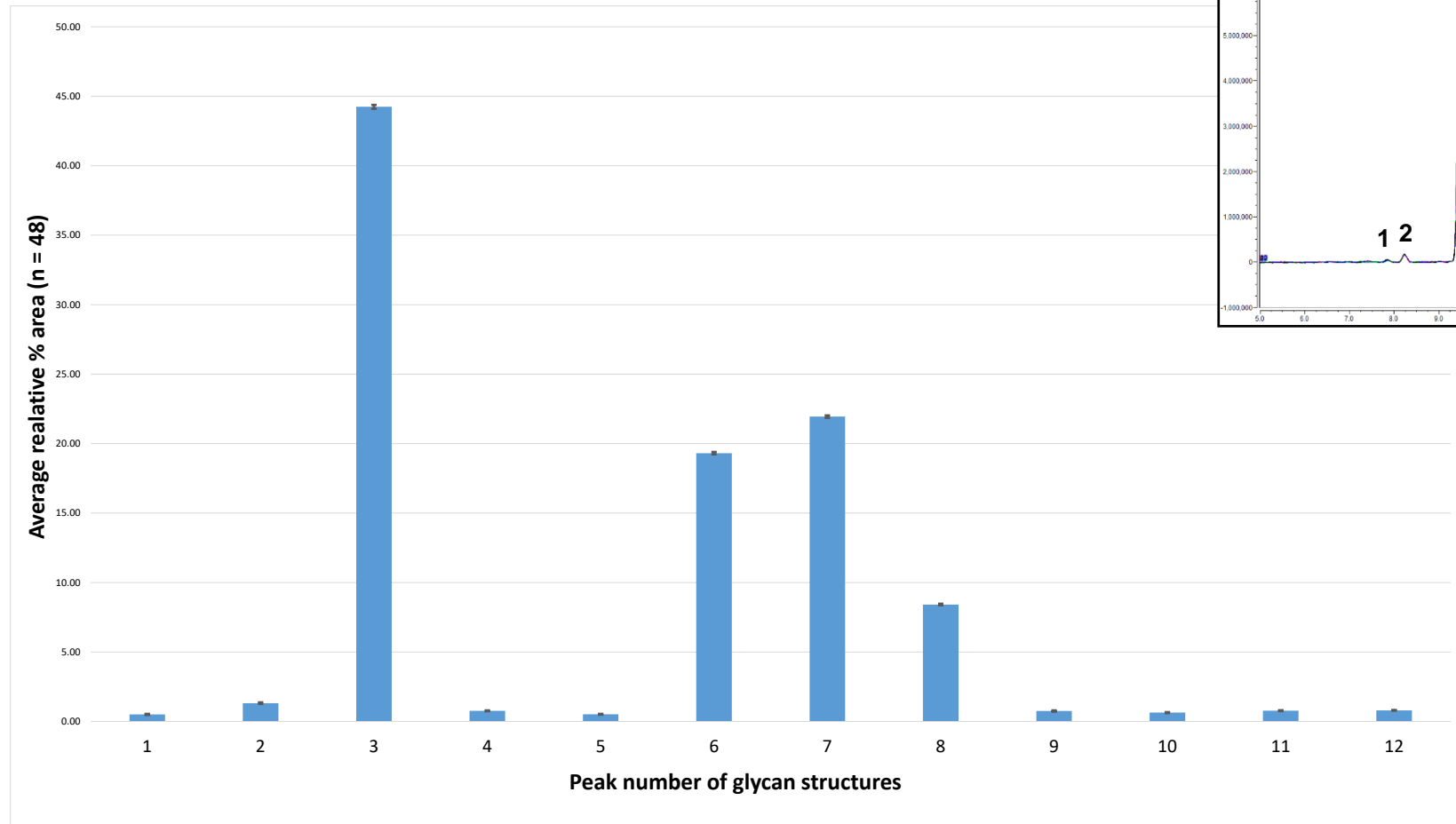
Robust, Validated Technology
Gives you reliable, high precision glycan analysis data



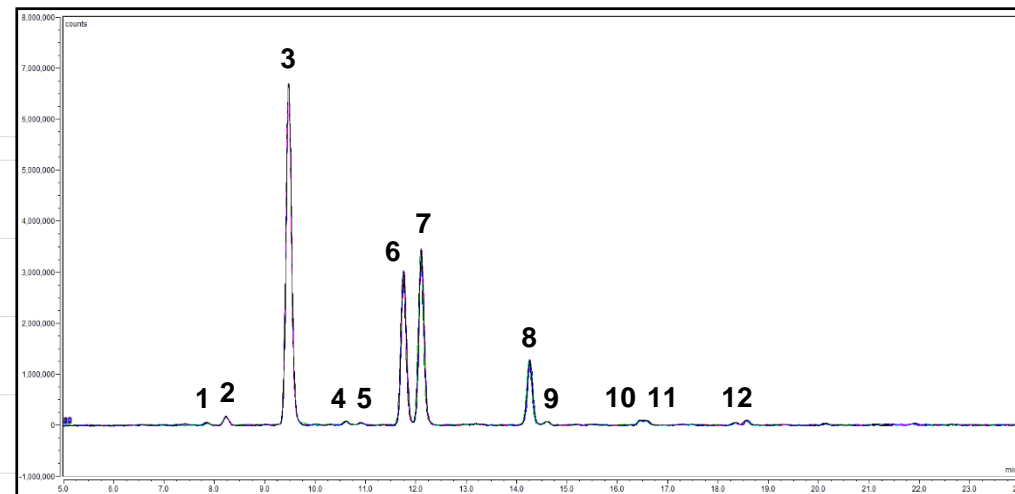
Repeatability Data for Level 1 GX-mAb-AB-LC

2AB labeled *N*-glycans released from 45 IgG1 mAb samples analysed in parallel by UHPLC

Data showing the relative % area of 2AB labeled *N*-glycans released from Waters Intact IgG1 mAb Mass Check Standard



Error bars are \pm standard deviation and show repeatability of the analysis



Column: Waters BEH Glycan 1.7 μ m, 2.1 x 150 mm

Peak ID	Relative % area		
	Average	SD	CV
1	0.51	0.04	7.93
2	1.32	0.05	4.15
3	44.24	0.14	0.31
4	0.77	0.03	3.91
5	0.52	0.03	6.03
6	19.31	0.09	0.45
7	21.95	0.09	0.39
8	8.42	0.06	0.66
9	0.75	0.05	6.58
10	0.64	0.03	5.41
11	0.78	0.03	3.49
12	0.80	0.02	2.98

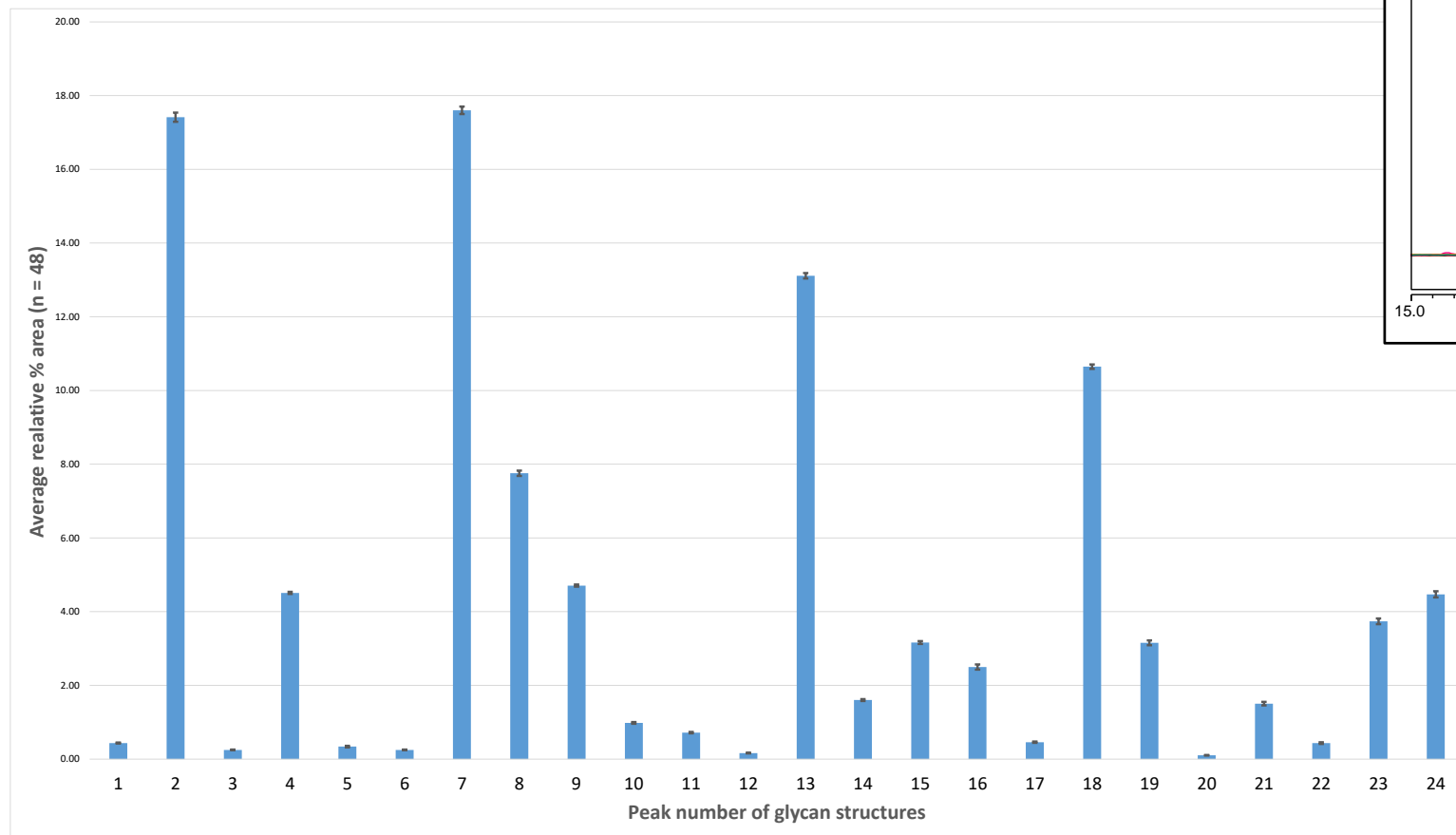
CVs <5% highlighted green.

Peaks with CVs >5% generally have a relative % area <1 %

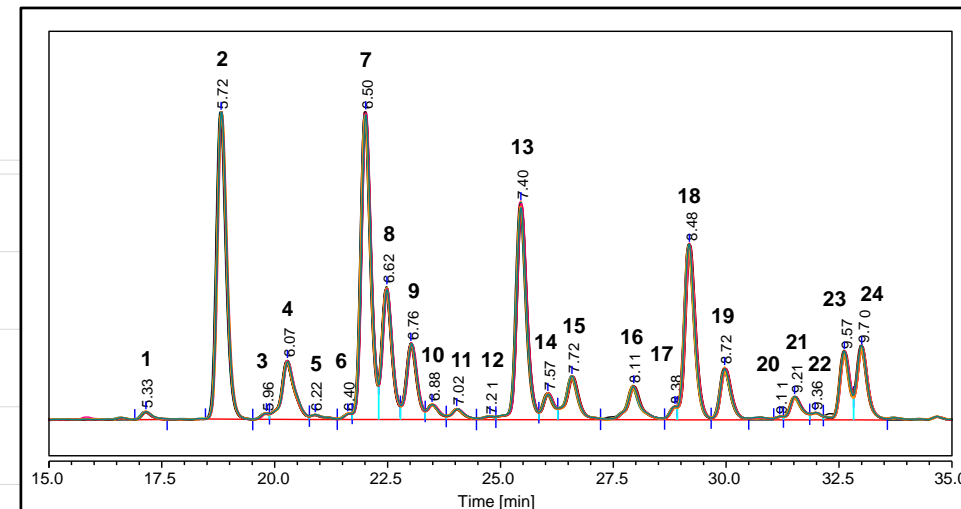
Repeatability Data for Level 1 GX-mAb-P-LCMS

Procainamide labeled *N*-glycans released from 48 human polyclonal IgG samples analysed in parallel by UHPLC

Data showing the relative % area of procainamide labeled *N*-glycans released from human polyclonal IgG



Error bars are \pm standard deviation and show repeatability of the analysis



Column: Waters BEH Glycan 1.7 μ m, 2.1 x 150 mm

Peak ID	Relative % area			Peak ID	Relative % area		
	average	SD	CV		average	SD	CV
1	0.44	0.01	3.26	13	13.11	0.07	0.57
2	17.41	0.13	0.72	14	1.60	0.03	1.57
3	0.25	0.01	3.44	15	3.16	0.04	1.28
4	4.51	0.03	0.64	16	2.50	0.07	2.82
5	0.34	0.02	6.46	17	0.46	0.02	3.53
6	0.25	0.01	4.14	18	10.65	0.06	0.55
7	17.60	0.10	0.57	19	3.15	0.07	2.10
8	7.76	0.07	0.93	20	0.10	0.01	7.67
9	4.71	0.03	0.61	21	1.50	0.05	3.21
10	0.98	0.02	2.44	22	0.43	0.02	5.19
11	0.72	0.02	2.74	23	3.74	0.08	2.10
12	0.16	0.01	5.62	24	4.47	0.08	1.87

CVs <5% highlighted green.

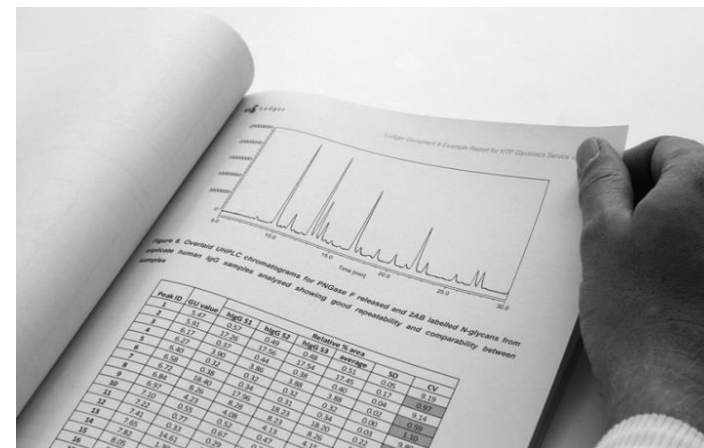
Peaks with CVs >5% generally have a relative % area <1 %



How does GX-mAb service work?

Typical Timeline for GX-mAb

We progress the analysis of your samples quickly by combining validated Ludger technology with robotic automation



---- Day 1 ----

Sample received at Ludger



---- Day 2-4 ----

Sample preparation and processing



---- Day 5-7 ----

Data Acquisition



---- Day 8-13 ----

Analysis and Reporting



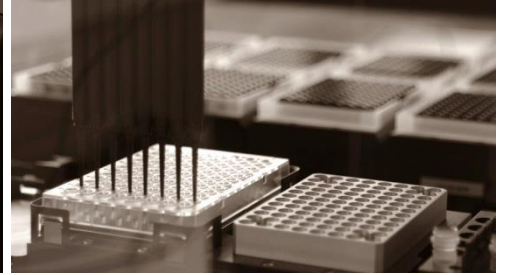
---- Day 14 ----

Report sent to you

Typical GX-mAb Workflow

We take your sample through five main stages – Two weeks from reception to results

1. Sample reception



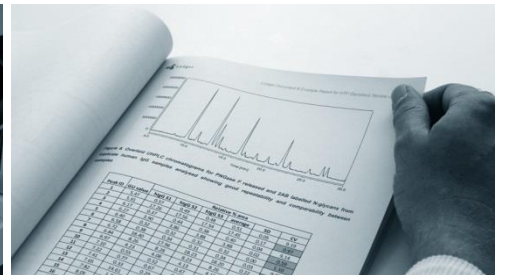
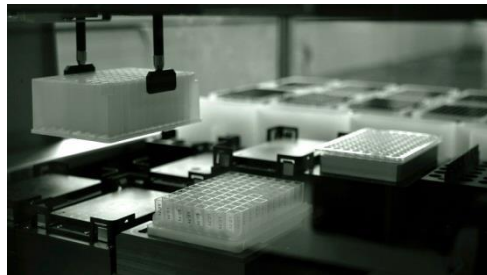
2. Glycoprotein reduction, denaturation and clean-up



3. Labeling of N-glycans



4. SPE sample clean up



5. Analysis and reporting

Next Steps...

If you have a question



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Request a quotation



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