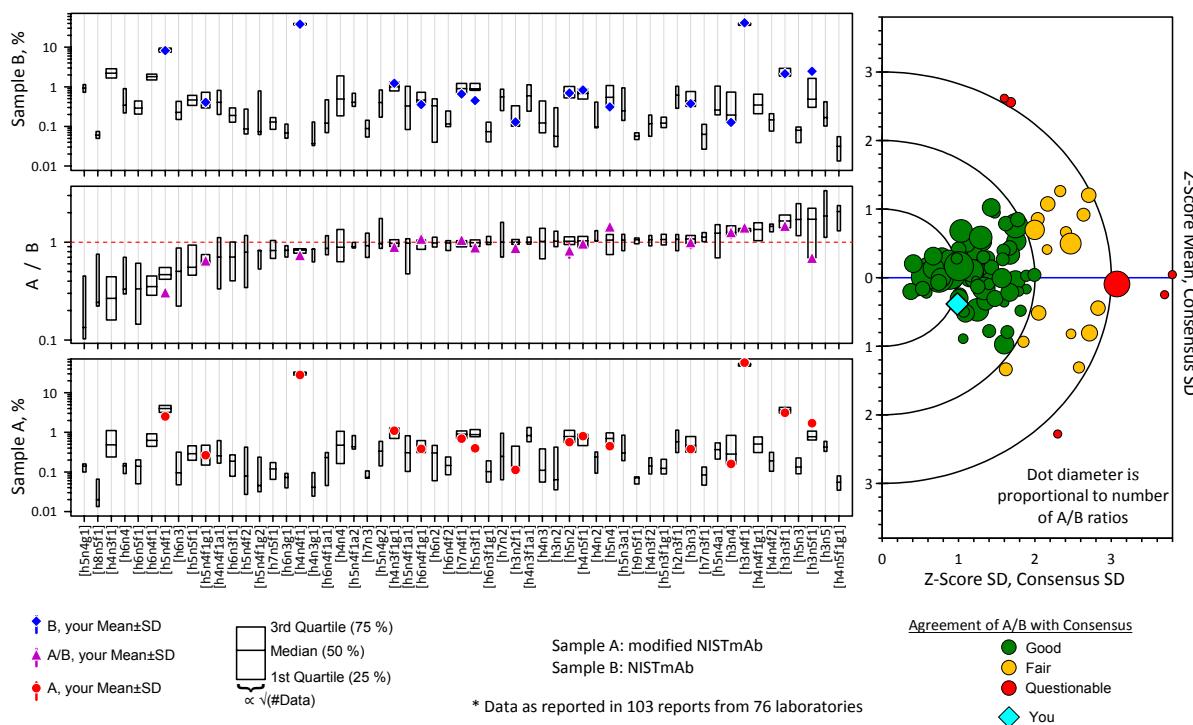


NIST Interlaboratory Study on the Glycosylation of NISTmAb, a Monoclonal Antibody Reference Material June 2015 to February 2016

Maria Lorna A. De Leoz
David L. Duewer
Stephen E. Stein

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<https://doi.org/10.6028/NIST.IR.8186>



**NIST Interlaboratory Study
on the Glycosylation of NISTmAb,
a Monoclonal Antibody Reference Material
June 2015 to February 2016**

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July 2017



U.S. Department of Commerce
Wilbur L. Ross, Jr., Secretary

National Institute of Standards and Technology
Kent Rochford, Acting NIST Director and Under Secretary of Commerce for Standards and Technology

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Abstract

The National Institute of Standards and Technology coordinated an interlaboratory study for laboratories that measure glycosylation in monoclonal antibodies. This report describes the design of and results for the NIST Interlaboratory Study on the Glycosylation of NISTmAb, a Monoclonal Antibody Reference Material from 103 reports submitted by 76 laboratories. Two materials were used in the study, 1) the Primary Standard (PS) for NIST Reference Material 8671, NISTmAb, Humanized IgG1κ Monoclonal Antibody, and 2) a material derived from the PS by treatment with galactosidase. The study was conducted in two stages: Stage 1 involved nine selected laboratories who volunteered to assist in final study design; Stage 2 was widely advertised and open to all laboratories. The materials for the study were shipped to participants in two batches: June 2015 and August to September 2015 for Stage 1 and Stage 2, respectively. Participants were requested to provide measurement results by July 30, 2015 (Stage 1) and November 6, 2015 (Stage 2).

Key words

Glycan, Glycopeptide, Glycosylation, Glycoform, Glycomics,
IgG, Monoclonal Antibody, NISTmAb, RM 8671

Disclaimer

Certain commercial entities, equipment, or materials may be identified in this document in order to describe an experimental procedure or concept adequately. Such identification is not intended to imply recommendation or endorsement by the National Institute of Standards and Technology, nor is it intended to imply that the entities, materials, or equipment are necessarily the best available for the purpose.

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Introduction

Glycosylation is a critical post-translational modification in monoclonal antibodies [1]. It can impact the safety and efficacy of the drug, including its clearance rates, effector functions, folding, immunogenicity, and solubility. However, glycosylation is inherently heterogeneous and challenging to analyze, leading to a proliferation of analysis methods [2-15]. With the advent of biosimilars and other protein-based drugs, it is important to compare the glycosylation of biologics in an accurate and precise manner [16].

The National Institute of Standards and Technology (NIST) coordinated this interlaboratory study for measurement of glycosylation in monoclonal antibodies. The goals of this study are two-fold:

- to determine measurement variability in identifying and quantifying N-glycans across the glycan measurement community and
- to aid in assigning consensus values for the glycosylation of the recombinant NIST Reference Material (RM) 8671, NISTmAb, Humanized IgG1κ Monoclonal Antibody produced in murine-derived cells [17].

Participants used their method of choice to determine glycan content in the control and study materials. Some participants submitted more than one report; each report was assigned a confidential laboratory number. Participants provided their data to NIST, where it was compiled and evaluated for consensus values, within-laboratory precision, and concordance within the glycomics community. NIST provided the participants with a technical summary of reported and derived values from all laboratories, a table of all identified glycans, and an individualized graphical analysis of their performance for the exercise. Participants who have concerns regarding their laboratory's performance were encouraged to consult with the interlaboratory coordinators.

Interlaboratory Study: Glycosylation of Monoclonal Antibodies

This interlaboratory study was done in two stages: Stage 1 was open to nine selected laboratories that helped fine-tune the study; Stage 2 was open to all laboratories.

Individual units of batch-prepared samples were distributed to each participant: two 0.5-mg (Stage 1) or 0.4-mg (Stage 2) liquid-frozen monoclonal antibody samples for analysis and one 1-mL 25 mmol/L L-Histidine pH 6.0 solution for use in reconstituting the two samples. The antibody samples were the Primary Standard (PS) for NIST RM 8671 NISTmAb and a material derived from the PS. The derived material was prepared as a 70:30 by mass mixture of unmodified PS with PS treated with β1-4 Galactosidase S (8,000 units/mL, New England Biolabs, Ipswich, MA).

Unless multiple vials were requested, participants received one vial of each sample. These samples were shipped on dry ice to 90 laboratories in June 2015 (Stage 1) or August-September 2015 (Stage 2). Participants were requested to provide measurement results by July 30, 2015 and November 6, 2015 for Stage 1 and Stage 2, respectively. However, 32 laboratories asked to delay their submission of reports. The last report was received on September 2, 2015 for Stage 1 and February 1, 2016 for Stage 2. A total of 103 reports were submitted by 76 laboratories. The communication materials included in the sample shipment and emailed to participants are provided in Appendix A.

Participants were requested to report methods and percent abundance values for all glycans found in the two samples using method and data reporting templates provided along with the samples. The

method reporting template contained drop down boxes of common methods and parameters for identification and quantification, and spaces to list other techniques or parameters. The data reporting template listed 16 glycans in Stage 1 and 54 glycans in Stage 2 and space to report other glycans not listed in the template. Not all participants reported values for all the target glycans, and many participants reported values for non-target glycans.

The final report delivered to every participant in the interlaboratory study consists of four documents, as shown in Appendix B:

- A cover letter containing a brief description of the samples and other three documents.
- An “All-Lab Report” that summarizes reported and derived values for Samples A and B, and the A/B ratio.
- A “Table of Identified Glycans” showing identifiers, compositions, and structures for all glycans reported in any data set.
- An “Individualized Report” that graphically analyzes each participant’s results for all analytes reported by at least six participants. It contains boxplots of Samples A and B, and the A/B ratio, and a target plot summary for the A/B ratio. This report also provides a graphical summary of each participant’s measurement comparability, including glycan composition counts and sums, repeatability, limits of reporting, minimum reported values, and consensus values.

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Appendix A. Shipping Package Inserts for the Interlaboratory Study

The following six items were included in each package shipped to each participant:

- Cover letter
- Instructions
- Packing List and Shipment Receipt Confirmation Form
- Data Reporting Template
- Method Reporting Template
- Comments Template

A cover letter describing the interlaboratory study, instructions, packing list, method, data and comment reporting sheets were enclosed in a sealed waterproof bag placed at the top of the shipping box, between the cardboard covering and the foam insulation. All the shipping package inserts were also sent by email as one Microsoft Excel file with multiple tabs to each participant.

Cover Letter

NIST Interlaboratory Study on Glycosylation Analysis

Welcome Packet

August 27, 2015

Name
Address
Lab #:

Dear Colleague,

Welcome to the NIST Interlaboratory Study on Glycosylation Analysis. Thank you for agreeing to participate in this endeavor. Alteration in glycosylation may significantly modify the biological activity of monoclonal antibodies. Thus, analysis of their glycoforms is essential, whether it is a batch-to-batch analysis of a drug candidate, comparison of the glycan profile of a biosimilar, or a complete glycomics profiling of a new drug. There are several published methods to quantify and identify glycoforms in proteins, but only a handful of multi-lab studies to assess the performance of these various approaches. The goals of this study are two-fold: 1) to determine measurement variability in identifying and quantifying N-glycans across laboratories and 2) to aid in assigning consensus values for the glycosylation of the NISTmAb reference material, a soon-to-be-released well-characterized monoclonal antibody reference material.

Each laboratory is assigned a laboratory number so that study results may be viewed and discussed openly in anonymity. Your lab number is shown above. Please use this number on the forms for submission of results. Your lab number remains confidential.

Sample shipment will occur on August 31, 2015. We request that the resulting data be returned on or before **November 6, 2015**. Your laboratory will receive two frozen monoclonal antibody samples, Sample A (white) and Sample B (blue), for glycosylation analysis using your own method. Both samples are humanized IgG1k expressed in murine suspension culture. The samples are "drug-like substances" not for human use. The samples are 0.4 mg each, with a concentration of 100 mg/mL. You will also receive a vial containing 25 mmol/L L-Histidine pH 6.0 solution (yellow) that you may use (optional) to reconstitute the two samples.

We ask you to perform a glycosylation analysis in triplicate using your own method for each of the two samples. The Excel file provided has two tabs called "Data Reporting" and "Method Reporting" to report your data and method, respectively. If you choose to use more than one method, we ask that you create a separate file for each. Results should be attached in an email to lorna.deleoz@nist.gov with the subject "NIST Interlab Results."

Please feel free to contact Lorna at (301) 975-6731 or lorna.deleoz@nist.gov if you have questions.

Again, many thanks for your participation.

Sincerely,

M. Lorna A. De Leoz, Ph.D.
Principal Investigator
NIST Interlaboratory Study on Glycosylation Analysis
Biomolecular Measurement Division
National Institute of Standards and Technology
Gaithersburg, Maryland, USA 20899-8362

Stephen E. Stein, Ph.D.
Group Leader
Biomolecular Measurement Division
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Gaithersburg, Maryland, USA 20899-8362

Instructions

NIST Interlaboratory Study on Glycosylation Analysis

Instructions

August 27, 2015

Dear Colleague,

Enclosed are samples for the NIST Interlaboratory Study on Glycosylation Analysis. Sample details are provided below:

Sample A: white label, frozen liquid, 0.4 mg, 100 mg/mL mAb

Sample B: blue label, frozen liquid, 0.4 mg, 100 mg/mL mAb

Buffer: yellow label, frozen liquid, 1 mL, 25 mmol/L L-Histidine, pH 6.0

The package for this study consists of two vials of monoclonal antibody samples, Sample A (white) and Sample B (blue), one vial of L-Histidine solution (yellow) which you may use to reconstitute the samples, and a welcome packet consisting of a letter, instructions, packing list/shipment receipt confirmation form, and data, method, and comment forms to report your results. **As soon as you receive the samples**, please return the filled shipment receipt confirmation form (third tab) to us.

Please perform a glycosylation analysis in triplicate using your own method for Sample A and Sample B. After completing your analysis, please enter the percent abundances of the glycans and their standard deviations in the "Data Reporting" tab. Also include the percent abundances for each replicate. If a value obtained is below your limit of detection or quantification, please indicate this result on the form as "ND" (not detected) or "NQ" (not quantified), respectively.

Please describe your method in the "Method Reporting" tab by filling in as much information as you can. The last tab, "Comments," is for your feedback on all the stages of the interlab study, including shipping, samples, data reporting, and analysis. Your input, expertise, and feedback is extremely valuable for future studies.

We request that the filled Excel sheets be returned to us as an email attachment to lorna.deleoz@nist.gov (Subject: NIST Interlab Results) on or before **November 6, 2015**. Please let me know if this schedule would pose problems for your laboratory.

Please feel free to contact Lorna at (301) 975-6731 or lorna.deleoz@nist.gov if you have questions.

Again, many thanks for your participation.

Sincerely,

M. Lorna A. De Leoz, Ph.D.
Principal Investigator
NIST Interlaboratory Study on Glycosylation Analysis
Biomolecular Measurement Division
National Institute of Standards and Technology
Gaithersburg, Maryland, USA 20899-8362

Stephen E. Stein, Ph.D.
Group Leader
Biomolecular Measurement Division
National Institute of Standards and Technology
Gaithersburg, Maryland, USA 20899-8362

Packing List and Shipment Receipt Confirmation Form

NIST Interlaboratory Study on Glycosylation Analysis Packing List and Shipment Receipt Confirmation Form

This box contains: one vial each of the following:

Please fill in only the green boxes
and return to lorna.deleoz@nist.gov
upon receipt of samples.

Vial	Form	Amount	Label Color	Description
Sample A	Liquid frozen	0.4 mg	White	100 mg/mL mAb
Sample B	Liquid frozen	0.4 mg	Blue	100 mg/mL mAb
Buffer	Liquid frozen	1 mL	Yellow	25 mmol/L L-Histidine, pH 6.0

Please:

- 1) Open the pack immediately.
- 2) Check that the box contains all of the above vials.
- 3) Check if the vials are intact.
- 4) Store the samples at -20°C or below until analysis.
- 5) Email lorna.deleoz@nist.gov the following information (Subject: NIST Interlab Shipment Receipt):

- A) Lab #
- B) Date this shipment arrived:
- C) Are all 3 vials intact? Yes / No
If "No," which one(s) were damaged?
- D) Was there any dry ice left in cooler? Yes/No
- E) Did the samples arrive frozen? Yes/No
- F) At what temperature are you storing the samples?
- G) When do you anticipate analyzing these samples?

°C

Your prompt return of this information to lorna.deleoz@nist.gov is appreciated.

Please contact Dr. M. Lorna De Leoz at (301) 975-6731 or lorna.deleoz@nist.gov if you have questions or concerns.

Thank you.

Data Reporting Template

NIST Interlaboratory Study on Glycosylation Analysis

Data Reporting Template, page 1 of 4

Lab #:

Report #:

Total # of Reports Submitted:

Please refer to the instructions before entering any data. Fill in as much of the green boxes to report your results. NOT ALL GLYCANS MAY BE PRESENT IN THE SAMPLE. Please do not reorder, edit, or delete any of the 54 glycans. If you found glycans not listed in the table, please add them below the line "List other glycans below."

Please use the glycan analysis method you normally use. If you obtained data from more than one method, please save the data and methods for each additional method in a new, renamed Excel file. If you acquired more than three replicates, you may add columns in the raw data section. If a value obtained is below your limit of detection or quantification, please indicate this result on the form as "ND" (not detected) or "NQ" (not quantified), respectively. Further guidance is shown when you hover your mouse on cells with red triangles on the upper right corner. Note that many cells are annotated.

Please double check entries into this table! It is useful to have a second person confirm data entry. If you have questions on any of the boxes, please contact Dr. M. Lorna De Leoz (lorna.deleoz@nist.gov or (301) 975-6731) for clarification.

NIST Interlaboratory Study on Glycosylation Analysis
 Data Reporting Template, page 2 of 4

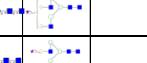
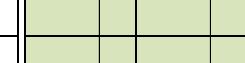
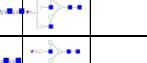
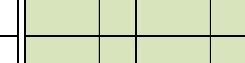
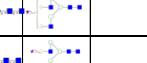
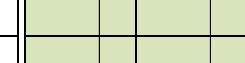
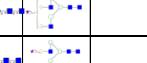
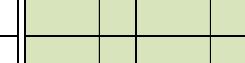
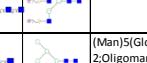
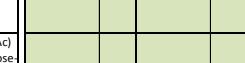
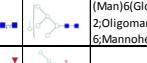
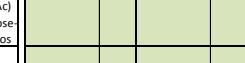
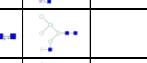
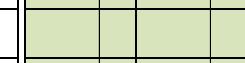
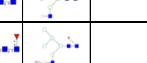
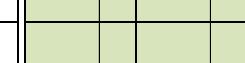
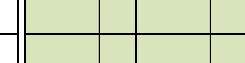
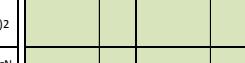
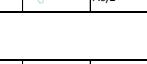
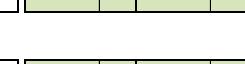
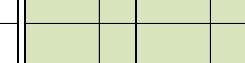
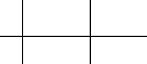
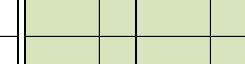
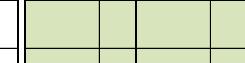
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		CFG Structure	Oxford Structure	% Abundance	SD	% Abundance	SD	Replicate 1	Replicate 2	Replicate 3	Replicate 1	Replicate 2	Replicate 3			
1	1. G0F / FA2 [h3n4f1]			NGA2F-(GlcNAc) 2Man2(Fuc)(Glc NAc)2Mannotri												
2	2. G1F / FA2G1 [h4n4f1]			NAZ2GF-Gal(Glc NAc)2(Man)3(Gl cNAc)2Fuc;Asial												
3	2a. G1F(1,6) / F(6)A2[6]G(4)1 [h4n4f1]			NAZ2GF-Gal(Glc NAc)2(Man)3(Gl cNAc)2Fuc;Asial												
3	2b. G1F(1,3) or F(6)A2[3]G(4)1 [h4n4f1]			NAZ2GF-Gal(Glc NAc)2(Man)3(Gl cNAc)2Fuc;Asial												
3	3. G2F / FA2G2 or G1F+1aGal [h5n4f1]			NAZ2F-(Gal)2(Glc NAc)2(Man)3(Gl cNAc)2Fuc;Mann												
3	3a. G2F / F(6)A2G(4)2 [h5n4f1]			NAZ2F-(Gal)2(Glc NAc)2(Man)3(Gl cNAc)2Fuc;Mann												
3	3b. G1F+1aGal / F(6)A2G1Ga1 [h5n4f1]			NAZ2F-(Gal)2(Glc NAc)2(Man)3(Gl cNAc)2Fuc;Mann												
4	4. G0F-N / FA1 [h3n3f1]															
4	4a. G0F-N(1,6) / F(6)A(6)1 [h3n3f1]															
4	4b. G0F-N(1,3) / F(6)A(3)1 [h3n3f1]															
5	5. G1F-N / FA1G1 [h4n3f1]			Core fucosylated mono antennary monogalactosyla												
5	5a. G1F-N(1,6) / FA1[6]G1 [h4n3f1]			Core fucosylated mono antennary monogalactosyla												
5	5b. G1F-N(1,3) / FA1[3]G1 [h4n3f1]			Core fucosylated mono antennary monogalactosyla												
6	6. G1F-N+1aGal / FA1G1Ga1 [h5n3f1]															
7	7. G1F2-N+1aGal / FA1F1G1Ga1 [h5n3f2]															
8	8. G0FB / FA2B [h3n5f1]															
9	9. G1FB / FA2BG1 [h4n5f1]															
10	10. G2FB / FA2BG2 [h5n5f1]															
11	11. G1F2 / FA2F1G1 [h4n4f2]															
12	12. G2F2 / FA2F1G2 [h5n4f2]															
13	13. G2F+1aGal / FA2G2Ga1 [h6n4f1]															
14	14. G2F2+1aGal / FA2F1G2Ga1 [h6n4f2]															
15	15. G2FB+1aGal / FA2BG2Ga1 [h6n5f1]															
16	16. G2F+2aGal / FA2G2Ga2 [h7n4f1]															
17	17. G2FB+2aGal / FA2BG2Ga2 [h7n5f1]															

NIST Interlaboratory Study on Glycosylation Analysis

Data Reporting Template, page 3 of 4

18	18. G0 / A2 [h3n4]			NGA2:(GlcNAc)2 Man2(GlcNAc)2; Mannotriose-di- o,monogalactosylated				
19	19. G1 / A2G1 [h4n4]			NA2G1:AGn;Asia lo,monogalactosylated				
	19a. G1(1,6) / A2[6]G1 [h4n4]			NA2G1:AGn;Asia lo,monogalactosylated				
	19b. G1(1,3) / A2[3]G1 [h4n4]			NA2G1:AGn;Asia lo,monogalactosylated				
20	20. G2 / A2G2 [h5n4]							
21	21. G0-N / A1 [h3n3]			MGn, A2G0- GlcNAc				
	21a. G0-N(1,6) / A1[6] [h3n3]			MGn, A2G0- GlcNAc				
	21b. G0-N(1,3) / A1[3] [h3n3]			MGn, A2G0- GlcNAc				
22	22. G1-N / A1G1 [h4n3]							
23	23. G1-N+1aGal / A1G1Ga1 [h5n3]							
24	24. G0B / A2B [h3n5]							
25	25. G1B / A2BG1 [h4n5]							
	25a. G1B(1,6) / A2[6]BG1 [h4n5]			NA2G1:AGn;A2G 1:Asialo, monogalactosyla				
	25b. G1(1,3) / A2[3]BG1 [h4n5]			NA2G1:AGn;A2G 1:Asialo, monogalactosyla				
26	26. G2B / A2BG2 [h5n5]							
27	27. G2+1aGal / A2G2Ga1 [h6n4]							
28	28. G2+2aGal / A2G2Ga2							
29	29. G1FS-N / FA1G1S1(NeuAc) [h4n3f1a1]							
30	30. G1FS / FA2G1S1 (NeuAc) [h4n4f1a1]							
31	31. G1FB5 / FA2BG1S1 (NeuAc) [h4n5f1a1]							
32	32. G2FS / FA2G2S1 (NeuAc) [h5n4f1a1]			A1F:NeuAc(Gal- GlcNAc)2Man3(G- lcNAc)2Fuc;1898				
33	33. G2FS2 / FA2G2S2 (NeuAc) [h5n4f1a2]			A2F:(NeuAc-Gal- GlcNAc)2Man3(F- uc)(GlcNAc)2Dis				
34	34. G2FS + 1aGal / FA2G2Ga1S1 (NeuAc) [h6n4f1a1]							
35	35. G1FS-N / FA1G1S1 (NeuGc) [h4n3f1g1]							
36	36. G1FS / FA2G1S1 (NeuGc) [h4n4f1g1]							
37	37. G1FB5 / FA2BG1S1 (NeuGc) [h4n5f1g1]							
38	38. G2FS / FA2G2S1 (NeuGc) [h5n4f1g1]							
39	39. G2FS2 / FA2G2S2 (NeuGc) [h5n4f1g2]			A2F:(NeuGc-Gal- GlcNAc)2Man3(F- uc)(GlcNAc)2Dis				
40	40. G2FS + 1aGal / FA2G2Ga1S1 (NeuGc) [h6n4f1g1]							

NIST Interlaboratory Study on Glycosylation Analysis
 Data Reporting Template, page 4 of 4

41 Complex Alditol Nucleotides	41. G2S / A2G2S1 (NeuAc) [h5n4a1]													
42	42. G2S2 / A2G2S2 (NeuAc) [h5n4a2]													
43	43. G2S / A2G2S1 (NeuGc) [h5n4g1]													
44 Complex Alditol Nucleotides	44. G2S2 / A2G2S2 (NeuGc) [h5n4g2]													
45 Neutral Monosaccharides	45. Man5 / M5 [h5n2]			(Man)5(GlcNAc) 2,Oligomannose- 5,Mannopentao										
46 Neutral Hexasaccharides	46. Man6 / M6 [h6n2]			(Man)6(GlcNAc) 2,Oligomannose- 6,Mannohexao										
47 Hybrid	47. Man5G0F hybrid / MSA1[3]S1 (NeuGc) [h5n3f1g1]													
48	48. Man5G1 hybrid / MSA1[3]G1 [h6n3]													
49 Hybrid	49. Man5G1F hybrid / M5FA1[3]G1 [h6n3f1]													
50	50. Man5G1FS hybrid / M5FA1[3]G1 [NeuGc) [h6n3f1g1]													
51	51. Man5G1+1aGal hybrid / MSA1[3]G1Ga1 [h7n3]													
52	52. Man5G1F hybrid +1aGal / M5FA1[3]G1Ga1 [h7n3f1]													
53 Fragment	53. Core / M3 [h3n2]			Man3(GlcNAc)2										
54	54. Core+F / FM3 [h3n2f1]			Man3(Fuc)(GlcNAc)2										
	List other glycans below													
55														
56														
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SUM

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Method Reporting Template

NIST Interlaboratory Study on Glycosylation Analysis

Method Reporting Template, page 1 of 3

Please describe the methods used in this reported results.

1. Identification of Data

Lab #		
Lab Classification	Select one	▼ Other/Comment:
Country	Select one	▼ Other/Comment:
Report #		
Total # of Reports Submitted		

2. Description of Measurement Method

Sample Preparation

Dates of measurements for Sample A		
Dates of measurements for Sample B		
Number of replicate measurements for Sample A		
Number of replicate measurements for Sample B		
Mass (ug) of Sample A used per run	ug	
Mass (ug) of Sample B used per run	ug	
Dilution factor (if any)		
Dilution media		
Type and number of replicates for Sample A		
Type and number of replicates for Sample B		
Proteolytic enzyme used	Select one	▼ Other/Comment:
Derivatization used	Select one	▼ Other/Comment:

Describe pre-treatment of samples (e.g. immobilization), if any

--	--	--

Describe the digestion conditions (enzyme: IgG ratio, buffer (including additives), pH, digestion time, temperature)

--	--	--

Describe purification/cleanup methods (e.g. SPE or ziptip, type, vacuum or gravity)

--	--	--

Please refer to the instructions before acquiring any data.

On this form, fill in as much of the green boxes as you can. If you have obtained another set of data using another method, please use a new sheet. You may expand the columns and rows to document additional information. Further guidance is shown when you hover your mouse on cells with red triangles on the upper right side. If you have questions on any of the boxes, please feel free to contact Dr. M. Lorna De Leoz at lorna.deleoz@nist.gov or (301) 975-6731 for clarification.

Please double check entries! It is useful to have a second person confirm data entry.

Thanks!

NIST Interlaboratory Study on Glycosylation Analysis

Method Reporting Template, page 2 of 3

Analysis

General Strategy (If other, please specify)	Select one
Other/Comment:	
Analytical Technique (If other, please specify)	Select one
Other/Comment:	
Analyte (If other, please specify)	Select one
Other/Comment:	
Identification method (If other, please specify)	Select one
Other/Comment:	
Quantification method (If other, please specify)	Select one
Other/Comment:	
Separation method (If other, please specify)	Select one
Other/Comment:	
Limit of detection	
Limit of quantification	
Dilution factor	
Dilution media	

Describe identification method (parameters, type(s) of instrument(s) used (manufacturer, model #))

Database(s) used to confirm identity

Describe quantification method, type(s) of instrument(s) used (manufacturer, model #)

Describe separation/chromatography method (gradient, parameters, type(s) of instrument(s) used (manufacturer, model #))

Describe how the percent abundances of glycans were extracted from the data

NIST Interlaboratory Study on Glycosylation Analysis

Method Reporting Template, page 3 of 3

For MS, analysis:

MS source and sample introduction

Select one

Select one

Other:

MS detector and ion mode

Select one

Select one

Other:

Tandem MS method (if used)

Select one

Select one

Other:

Resolution and mass accuracy of analysis

Specify how MS and/or tandem MS data were interpreted

For glycopeptide analysis, specify the sequence(s) of the peptide(s) used to quantify the mAb glycans

For ESI, specify how charge states were analyzed

Other information

Comments and Suggestions

NIST Interlaboratory Study on Glycosylation Analysis

Comments and Suggestions

Your input, expertise, and feedback is extremely valuable for future studies. Please write any comments and suggestions that may be relevant to the interlab study. Feel free to expand columns and rows if you need more space.

Address any questions to Dr. M. Lorna De Leoz at lorna.deleoz@nist.gov or (301) 975-6731. Thanks!

Overall:

Shipping:

Glycan Identification (current methods for describing identity of glycans):

Data Reporting:

Samples (e.g. concentration, amount, container, label...):

Sample Analysis:

Other:

Appendix B. Final Report for the Interlaboratory Study

The final report delivered to every participant in the interlaboratory study consists of four files:

- Cover letter: contains a brief description of the samples and the other three documents
- Table of Identified Glycans: list of identifiers, compositions, and structures for all glycans reported in any data set
- All-Lab Report: summarizes reported and derived values for Samples A and B, and the A/B ratio
- Individualized Report: graphically analysis of each participant's results for all glycan compositions. It contains boxplots of Samples A and B, and the A/B ratio, and a target plot summary for the A/B ratio for compositions reported by at least six participants . This report also provides a graphical summary of each participant's measurement comparability, including glycan composition counts and sums, repeatability, limits of reporting, minimum reported values, and consensus values.

Cover Letter



UNITED STATES DEPARTMENT OF COMMERCE
National Institute of Standards and Technology
Gaithersburg, Maryland 20899

Material Measurement Laboratory

June 2, 2017

Dear Colleague:

Thank you for participating in the **NIST interlaboratory study of NISTmAb Glycosylation**. Enclosed is the preliminary report of the results. Included in this report are: 1) a summary of reported and derived values for Samples A and B, and A/B ratio from all laboratories, 2) a detailed graphical analysis of your results; and 3) summary tables of the results you reported.

The NISTmAb Glycosylation study consisted of two vials of liquid-frozen monoclonal antibody samples, NISTmAb and a glycan-modified NISTmAb, and one 25 mmol/L L-Histidine pH 6.0 solution that was intended for use in reconstituting the two samples. Your overall measurement comparability is summarized in the boxplot and targetplot summaries found in page 1 of your report; this summary reflects only results for glycan compositions. We intend to follow this report with a summary of results for the unique glycoforms and glycoform isomers at a later date.

If you have concerns or questions regarding your laboratory's performance, please contact M. Lorna De Leoz at lorna.deleoz@nist.gov or +1-(301) 975-6731.

Again, many thanks for joining us in this endeavor.

Sincerely,

M. Lorna A. De Leoz, Ph.D.
Research Chemist
Biomolecular Measurement
Division

Stephen E. Stein, Ph.D.
NIST Fellow
Biomolecular Measurement
Division

David L. Duewer, Ph.D.
Chemometrician
Chemical Sciences
Division

NIST

The complete report for this study consists of three files:

Table of Identified Glycans	#Pages
Identifiers and structures for all glycans reported in any data set	17
All-Lab Report	#Pages
Summary of reported and derived values for Samples A and B, and the A/B ratio	9
Legend for the summary	1
Individualized Report	#Pages
Boxplots of Samples A and B, and the A/B ratio, and targetplot for the A/B ratio	1
Legend for the boxplots and targetplot	1
Plots summarizing measurement performance, including glycan composition counts and sums, repeatability, limits of reporting, minimum reported values, and consensus	1
Legend for measurement performance plots	1
Table of measurement summary	Variable
Legend for table of measurement summary	1
Table of derived glycan attribute quantities	1
Legend for table of derived glycan attribute quantities	1

Table of Identified Glycans

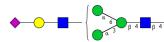
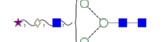
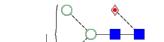
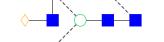
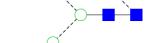
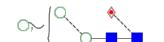
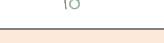
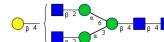
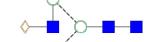
Glycans reported by at least one participant arranged in increasing monosaccharide composition. Entries highlighted in orange are unique glycan compositions; glycans having the same monosaccharide compositions are shown beneath the highlighted entry.

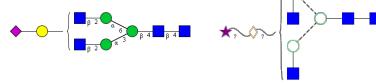
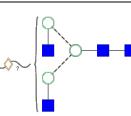
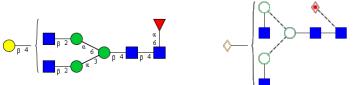
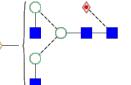
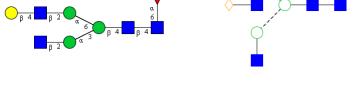
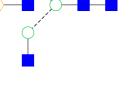
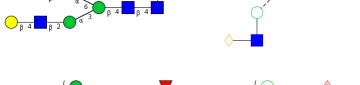
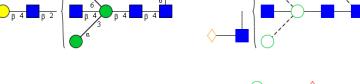
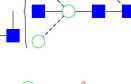
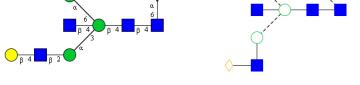
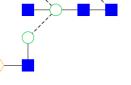
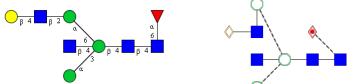
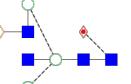
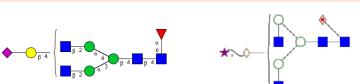
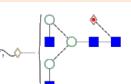
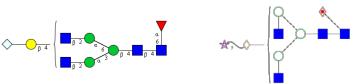
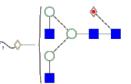
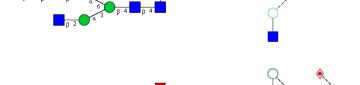
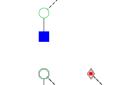
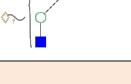
Table of Identified Glycans

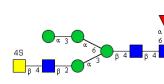
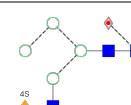
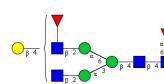
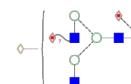
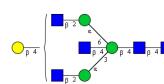
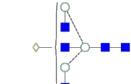
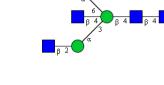
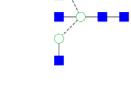
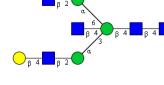
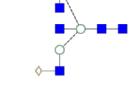
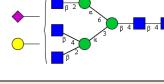
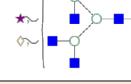
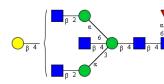
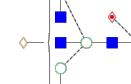
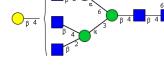
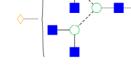
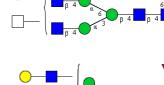
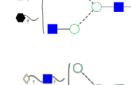
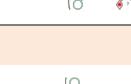
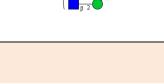
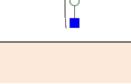
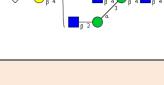
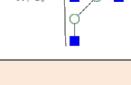
Index^a	Code^b	Measurands^c	Oxford^d	Composition^e	CFG^f	Oxford^g
42		[h2n3f1]		[h2n3f1]		
42.1		Fragment Man2F+N		[h2n3f1]		
37		[h3n2]		[h3n2]		
37.1	53	Man3	M3	[h3n2]		
19		[h3n2f1]		[h3n2f1]		
19.1	54	Man3F	FM3	[h3n2f1]		
11		[h3n3]		[h3n3]		
11.1	21	G0-N	A1	[h3n3]		
11.11	21a	G0-N(1,6)	A1[6]	[h3n3]		
11.12	21b	G0-N(1,3)	A1[3]	[h3n3]		
11.2		Man3B	M3B	[h3n3]		
4		[h3n3f1]		[h3n3f1]		
4.1	04	G0F-N	FA1	[h3n3f1]		
4.11	04a	G0F-N(1,6)	F(6)A(6)1	[h3n3f1]		
4.12	04b	G0F-N(1,3)	F(6)A(3)1	[h3n3f1]		
109		[h3n3f2]		[h3n3f2]		
109.1		Man3F2+N		[h3n3f2]		
78		[h3n3g1]		[h3n3g1]		

Index^a	Code^b	Measurands^c	Oxford^d	Composition^e	CFG^f	Oxford^g
13		[h3n4]		[h3n4]		
13.1	18	G0	A2	[h3n4]		
13.2		G0B-N	A1B	[h3n4]		
13.21		G0B-N(1,3)	A1(3)B	[h3n4]		
13.22		G0B-N(1,6)	A1(6)B	[h3n4]		
2		[h3n4f1]		[h3n4f1]		
2.1	01	G0F	FA2	[h3n4f1]		
2.2		G0FB-N	FA1B	[h3n4f1]		
2.3		Man3F+2N	FM3A2	[h3n4f1]		
79		[h3n4f1a1]		[h3n4f1a1]		
80		[h3n4f1S]		[h3n4f1S]		
80.1		G0F-N+GalNAc4Sul		[h3n4f1S]		
81		[h3n4f2]		[h3n4f2]		
81.1		G0F2-N+GalNAc		[h3n4f2]		
43		[h3n5]		[h3n5]		
43.1	24	G0B	A2B	[h3n5]		

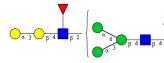
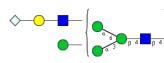
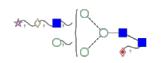
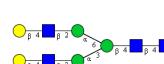
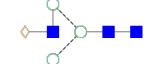
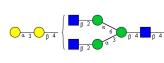
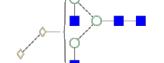
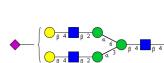
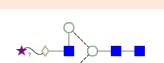
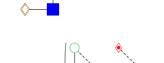
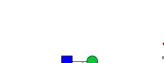
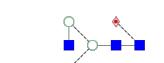
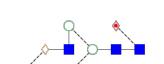
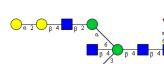
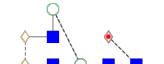
Index^a	Code^b	Measurands^c	Oxford^d	Composition^e	CFG^f	Oxford^g
43.2		G0+N (tri)	A3	[h3n5]		
18		[h3n5f1]		[h3n5f1]		
18.1	08	G0FB	FA2B	[h3n5f1]		
18.2		G0F+N (tri)	F(6)A3	[h3n5f1]		
18.3		ManF+3N		[h3n5f1]		
18.31		G0F+N		[h3n5f1]		
82		[h3n5f2]		[h3n5f2]		
83		[h3n7]		[h3n7]		
83.1		G0FB+2N (quad)	A4B	[h3n7]		
53		[h4n2]		[h4n2]		
53.1		Man4	M4	[h4n2]		
53.11		Man4D2	M4D2	[h4n2]		
64		[h4n2f1]		[h4n2f1]		
64.1		Man4F	F(6)M4	[h4n2f1]		
64.11		Man4D2F	F(6)M4D2	[h4n2f1]		
22		[h4n3]		[h4n3]		
22.1	22	G1-N	A1G1	[h4n3]		
22.2		Man4N	M4A1	[h4n3]		
22.21		Man4N[3]	M4[3]A1	[h4n3]		

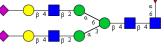
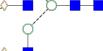
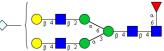
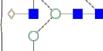
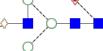
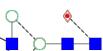
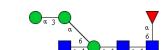
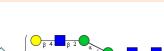
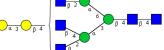
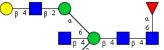
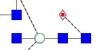
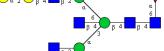
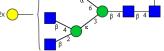
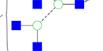
Index ^a	Code ^b	Measurands ^c	Oxford ^d	Composition ^e	CFG ^f	Oxford ^g
84		[h4n3a1]		[h4n3a1]		
84.1		G1S-N (NeuAc)	A1G1S1 (NeuAc)	[h4n3a1]		
10		[h4n3f1]		[h4n3f1]		
10.1 05	G1F-N	FA1G1		[h4n3f1]		
10.11 05a	G1F-N(1,6)	FA1[6]G1		[h4n3f1]		
10.12 05b	G1F-N(1,3)	FA1[3]G1		[h4n3f1]		
10.2	Man4FN	F(6)M4A1		[h4n3f1]		
36		[h4n3f1a1]		[h4n3f1a1]		
36.1 29	G1FS-N	FA1G1S1 (NeuAc)		[h4n3f1a1]		
12		[h4n3f1g1]		[h4n3f1g1]		
12.1 35	G1FS-N	FA1G1S1 (NeuGc)		[h4n3f1g1]		
44		[h4n3f2]		[h4n3f2]		
44.1	G1F-N+AF	FA1F1G1		[h4n3f2]		
54		[h4n3g1]		[h4n3g1]		
54.1	G1S (NeuGc)	A1G1S1 (NeuGc)		[h4n3g1]		
20		[h4n4]		[h4n4]		
20.1 19	G1	A2G1		[h4n4]		
20.11 19a	G1(1,6)	A2[6]G1		[h4n4]		
20.12 19b	G1(1,3)	A2[3]G1		[h4n4]		
20.2	G1B-N			[h4n4]		
65		[h4n4a1]		[h4n4a1]		

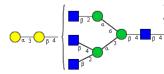
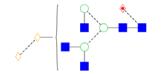
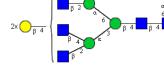
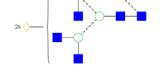
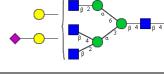
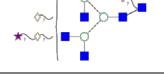
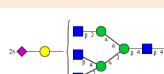
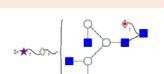
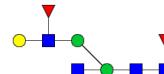
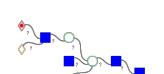
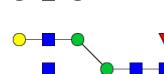
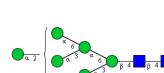
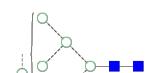
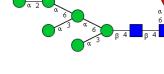
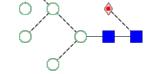
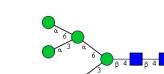
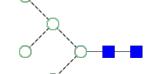
Index ^a	Code ^b	Measurands ^c	Oxford ^d	Composition ^e	CFG ^f	Oxford ^g
65.1		G1S (NeuAc)	A2G1S1	[h4n4a1]		
1		[h4n4f1]		[h4n4f1]		
1.1	02	G1F	FA2G1	[h4n4f1]		
1.11	02a	G1F(1,6)	F(6)A2[6]G(4)1	[h4n4f1]		
1.12	02b	G1F(1,3)	F(6)A2[3]G(4)1	[h4n4f1]		
1.2		G1FB-N	F(6)A1BG1	[h4n4f1]		
1.21		G1FB-N[3]	F(6)A1BG1[3]	[h4n4f1]		
1.22		G1FB-N[6]	F(6)A1BG1[6]	[h4n4f1]		
40		[h4n4f1a1]		[h4n4f1a1]		
40.1	30	G1FS (NeuAc)	FA2G1S1 (NeuAc)	[h4n4f1a1]		
14		[h4n4f1g1]		[h4n4f1g1]		
14.1	36	G1FS (NeuGc)	FA2G1S1 (NeuGc)	[h4n4f1g1]		
14.11		G1FS[6] (NeuGc)	F(6)A2[6]G(4)1S(6)1 (NeuGc)	[h4n4f1g1]		
14.12		G1FS[3] (NeuGc)	F(6)A2[3]G(4)1S(6)1 (NeuGc)	[h4n4f1g1]		
85		[h4n4f1g2]		[h4n4f1g2]		
85.1		G1FS2 (NeuGc)		[h4n4f1g2]		
86		[h4n4f1S]		[h4n4f1S]		

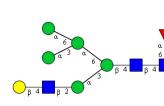
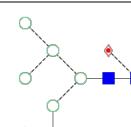
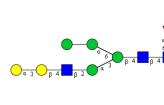
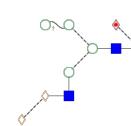
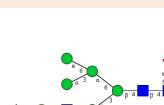
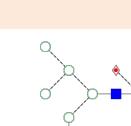
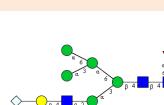
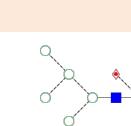
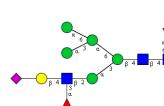
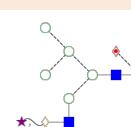
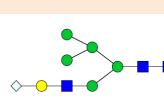
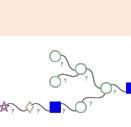
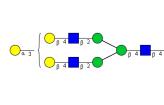
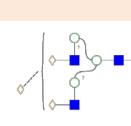
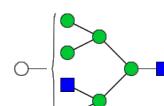
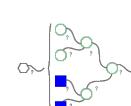
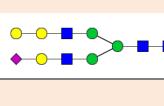
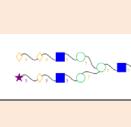
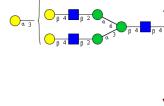
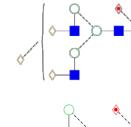
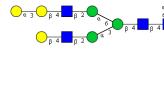
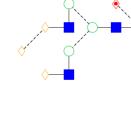
Index ^a	Code ^b	Measurands ^c	Oxford ^d	Composition ^e	CFG ^f	Oxford ^g
86.1		Man4G0F+GalNAc4 Sul hybrid		[h4n4f1S]		
38		[h4n4f2]		[h4n4f2]		
38.1 11	G1F2	FA2F1G1		[h4n4f2]		
57		[h4n5]		[h4n5]		
57.1 25	G1B	A2BG1		[h4n5]		
57.11 25a	G1B(1,6)	A2[6]BG1		[h4n5]		
57.12 25b	G1B(1,3)	A2[3]BG1		[h4n5]		
87		[h4n5a1]		[h4n5a1]		
87.1		G1S+N (NeuAc) (tri) A3G1S1		[h4n5a1]		
8		[h4n5f1]		[h4n5f1]		
8.1 09	G1FB	FA2BG1		[h4n5f1]		
8.2		NA3FG1	F(6)A3G(4)1	[h4n5f1]		
8.3		G0F+Hex+HexNAc		[h4n5f1]		
8.4		G1F+N		[h4n5f1]		
70		[h4n5f1a1]		[h4n5f1a1]		
70.1 31	G1FBS	FA2BG1S1 (NeuAc)		[h4n5f1a1]		
45		[h4n5f1g1]		[h4n5f1g1]		
45.1 37	G1FBS (NeuGc)	FA2BG1S1 (NeuGc)		[h4n5f1g1]		
5		[h5n2]		[h5n2]		

Index^a	Code^b	Measurands^c	Oxford^d	Composition^e	CFG^f	Oxford^g
5.1	45	Man5	M5	[h5n2]		
88		[h5n2f1]		[h5n2f1]		
88.1		Man5F	FM5	[h5n2f1]		
29		[h5n3]		[h5n3]		
29.1	23	G1-N+1aGal	A1G1Ga1	[h5n3]		
46		[h5n3a1]		[h5n3a1]		
46.1		Man4G1S hybrid	M4A1G(4)1S(6)1	[h5n3a1]		
46.2		Fragment G2S-CoreN (NeuAc)	A2G(4)2S(6)1	[h5n3a1]		
9		[h5n3f1]		[h5n3f1]		
9.1	06	G1F-N+1aGal	FA1G1Ga1	[h5n3f1]		
9.2		Man5GOF hybrid	FM5A1[3]	[h5n3f1]		
9.3		G1F+Man	F(6)M4A1G(4)1	[h5n3f1]		
9.31		G1F+Man(1,3)	F(6)M4A1[3]G(4)1	[h5n3f1]		
71		[h5n3f1a1]		[h5n3f1a1]		
71.1		Man4FG1FS (NeuAc)		[h5n3f1a1]		
71.2		G1FS-N+1aGal (NeuAc)	FA1G1Ga1S1	[h5n3f1a1]		
26		[h5n3f1g1]		[h5n3f1g1]		
26.1	47	Man5GOFs (NeuGc) hybrid	FM5A1[3]S1 (NeuGc)	[h5n3f1g1]		
26.2		Man4G1FS (NeuGc) hybrid	F(6)M4A1G(4)1S(6)1 (NeuGc)	[h5n3f1g1]		
58		[h5n3f2]		[h5n3f2]		

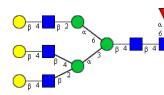
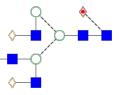
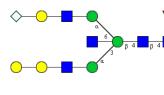
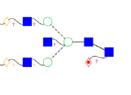
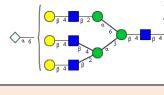
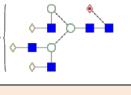
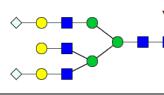
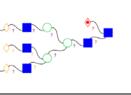
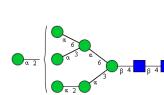
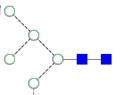
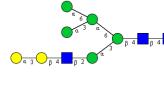
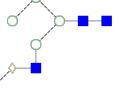
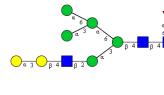
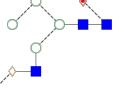
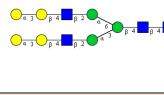
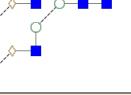
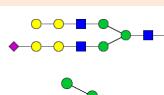
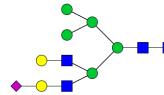
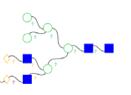
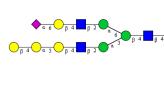
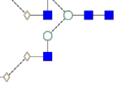
Index^a	Code^b	Measurands^c	Oxford^d	Composition^e	CFG^f	Oxford^g
58.1	07	G1F2-N+1aGal	FA1F1G1Ga1	[h5n3f2]		
66		[h5n3g1]		[h5n3g1]		
66.1		Man4G1S1 (NeuGc) hybrid		[h5n3g1]		
21		[h5n4]		[h5n4]		
21.1	20	G2	A2G2	[h5n4]		
21.2		G1+1aGal	A2G1Ga1	[h5n4]		
27		[h5n4a1]		[h5n4a1]		
27.1	41	G2S (NeuAc)	A2G2S1 (NeuAc)	[h5n4a1]		
59		[h5n4a2]		[h5n4a2]		
59.1	42	G2S2 (NeuAc)	A2G2S2 (NeuAc)	[h5n4a2]		
3		[h5n4f1]		[h5n4f1]		
3.1	03a	G2F	F(6)A2G(4)2	[h5n4f1]		
3.2	03b	G1F+1aGal	F(6)A2G1Ga1	[h5n4f1]		
3.21		G1F(1,3)+1aGal	F(6)A2[3]G(4)1Ga(3)1 [h5n4f1]			
3.22		G1F(1,6)+1aGal	F(6)A2[6]G(4)1Ga(3)1 [h5n4f1]			
3.3		G1FB-N+1aGal	FA1G1BGa(2)1	[h5n4f1]		
35		[h5n4f1a1]		[h5n4f1a1]		
35.1	32	G2FS (NeuAc)	FA2G2S1 (NeuAc)	[h5n4f1a1]		
41		[h5n4f1a2]		[h5n4f1a2]		

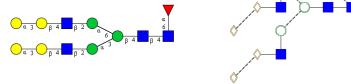
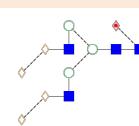
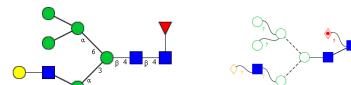
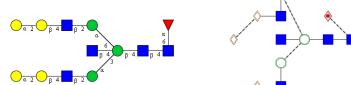
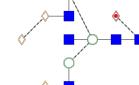
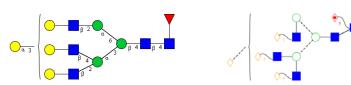
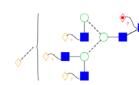
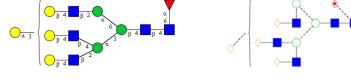
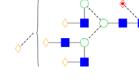
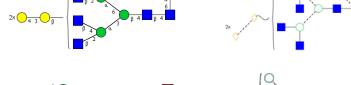
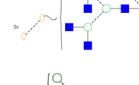
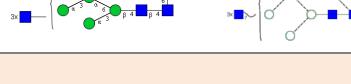
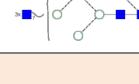
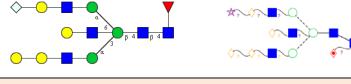
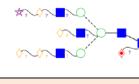
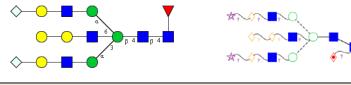
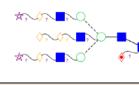
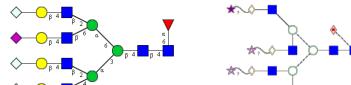
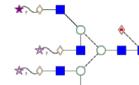
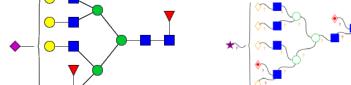
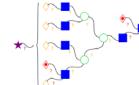
Index^a	Code^b	Measurands^c	Oxford^d	Composition^e	CFG^f	Oxford^g
41.1	33	G2FS2 (NeuAc)	FA2G2S2 (NeuAc)	[h5n4f1a2]		
16		[h5n4f1g1]		[h5n4f1g1]		
16.1	38	G2FS (NeuGc)	FA2G2S1 (NeuGc)	[h5n4f1g1]		
55		[h5n4f1g2]		[h5n4f1g2]		
55.1	39	G2FS2 (NeuGc)	FA2G2S2 (NeuGc)	[h5n4f1g2]		
47		[h5n4f2]		[h5n4f2]		
47.1	12	G2F2	FA2F1G2	[h5n4f2]		
47.2		Man4G1F2B hybrid		[h5n4f2]		
60		[h5n4g1]		[h5n4g1]		
60.1	43	G2S (NeuGc)	A2G2S1 (NeuGc)	[h5n4g1]		
48		[h5n4g2]		[h5n4g2]		
48.1	44	G2S2 (NeuGc)	A2G2S2 (NeuGc)	[h5n4g2]		
67		[h5n5]		[h5n5]		
67.1	26	G2B	A2BG2	[h5n5]		
67.2		G1+N+1aGal (tri)	A3G(4)Ga1	[h5n5]		
17		[h5n5f1]		[h5n5f1]		
17.1	10	G2FB	FA2BG2	[h5n5f1]		
17.2		G1FB+1aGal	FA2BGGa(2)1	[h5n5f1]		
17.3		G2F+N (tri)	FA3G2	[h5n5f1]		

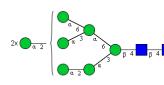
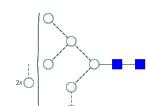
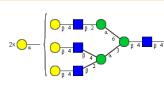
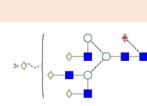
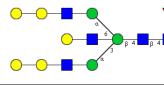
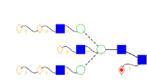
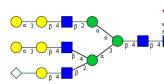
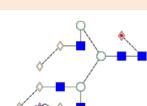
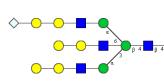
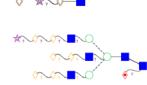
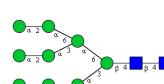
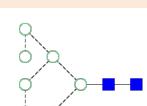
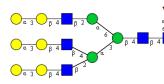
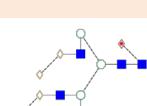
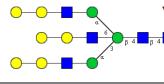
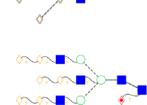
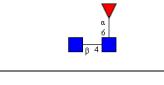
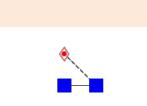
Index ^a	Code ^b	Measurands ^c	Oxford ^d	Composition ^e	CFG ^f	Oxford ^g
17.31		G1F+N+1aGal	F(6)A3G(4)Ga1	[h5n5f1]		
17.32		G2F(1,4)+N (tri)	F(6)A3G(4)2	[h5n5f1]		
17.4		G0F+2Hex+HexNAc		[h5n5f1]		
89		[h5n5f1a1]		[h5n5f1a1]		
89.1		G2FS+N (NeuAc) (tri)	FA3G2S1	[h5n5f1a1]		
90		[h5n5f1a2]		[h5n5f1a2]		
90.1		G2FS2+N (NeuAc) (tri)	FA3G2S2	[h5n5f1a2]		
91		[h5n5f1g1]		[h5n5f1g1]		
61		[h5n5f2]		[h5n5f2]		
61.1		G2F2B	FA2BG2F	[h5n5f2]		
61.2		G2F2+N (tri)	FA3F1G2	[h5n5f2]		
30		[h6n2]		[h6n2]		
30.1	46	Man6	M6	[h6n2]		
92		[h6n2f1]		[h6n2f1]		
92.1		Man6F	M6F	[h6n2f1]		
110		[h6n2g1]		[h6n2g1]		
110.1		Man5+Gal+S (NeuGc) hybrid		[h6n2g1]		
28		[h6n3]		[h6n3]		
28.1	48	Man5G1 hybrid	M5A1[3]G1	[h6n3]		
72		[h6n3a1]		[h6n3a1]		
24		[h6n3f1]		[h6n3f1]		

Index^a	Code^b	Measurands^c	Oxford^d	Composition^e	CFG^f	Oxford^g
24.1	49	Man5G1F hybrid	FM5A1[3]G1	[h6n3f1]		
24.2		Man4G1F+1aGal hybrid	F(6)M4A1G1Ga1	[h6n3f1]		
111		[h6n3f1a1]		[h6n3f1a1]		
111.1		Man5G1FS1 hybrid	F(6)M5A1G(4)1S(3)1 (NeuAc)	[h6n3f1a1]		
32		[h6n3f1g1]		[h6n3f1g1]		
32.1	50	Man5G1FS hybrid	FM5A1[3]G1 (NeuGc)	[h6n3f1g1]		
93		[h6n3f2]		[h6n3f2]		
112		[h6n3f2a1]		[h6n3f2a1]		
112.1		Man5G1F2S (NeuAc) hybrid		[h6n3f2a1]		
49		[h6n3g1]		[h6n3g1]		
49.1		Man5G1S (NeuGc) hybrid		[h6n3g1]		
50		[h6n4]		[h6n4]		
50.1	27	G2+1aGal	A2G2Ga1	[h6n4]		
50.2		Fragment Man5G1- CoreN hybrid		[h6n4]		
73		[h6n4a1]		[h6n4a1]		
73.1		G2S+1aGal (NeuAc)		[h6n4a1]		
7		[h6n4f1]		[h6n4f1]		
7.1	13	G2F+1aGal	FA2G2Ga1	[h6n4f1]		
7.11		G2F+1aGal[6]	F(6)A2[6]G2Ga1	[h6n4f1]		

Index ^a	Code ^b	Measurands ^c	Oxford ^d	Composition ^e	CFG ^f	Oxford ^g
7.12		G2F+1aGal[3]	F(6)A2[3]G2Ga1	[h6n4f1]		
51		[h6n4f1a1]		[h6n4f1a1]		
51.1	34	G2FS+1aGal (NeuAc)	FA2G2Ga1S1 (NeuAc)	[h6n4f1a1]		
51.2		Man5G1FBS (NeuAc) hybrid		[h6n4f1a1]		
15		[h6n4f1g1]		[h6n4f1g1]		
15.1	40	G2FS+1aGal (NeuGc)	FA2G2Ga1S1 (NeuGc)	[h6n4f1g1]		
31		[h6n4f2]		[h6n4f2]		
31.1	14	G2F2+1aGal	FA2F1G2Ga1	[h6n4f2]		
31.2		Man5G1F2B hybrid		[h6n4f2]		
94		[h6n4g1]		[h6n4g1]		
74		[h6n5]		[h6n5]		
74.1		G3		[h6n5]		
74.11		G3[6]	A(6)3G3	[h6n5]		
75		[h6n5a1]		[h6n5a1]		
25		[h6n5f1]		[h6n5f1]		
25.1	15	G2FB+1aGal	FA2BG2Ga1	[h6n5f1]		
25.2		G3F		[h6n5f1]		

Index^a	Code^b	Measurands^c	Oxford^d	Composition^e	CFG^f	Oxford^g
25.21	G3F[3]		F(6)A3G(4)3	[h6n5f1]		
68	[h6n5f1g1]			[h6n5f1g1]		
68.1	G2FBS+1aGal (NeuGc)			[h6n5f1g1]		
68.2	G3FS (NeuGc)		F(6)A3G(4)3S(6)1 (NeuGc)	[h6n5f1g1]		
95	[h6n5f1g2]			[h6n5f1g2]		
95.1	G3FS2(NeuGc)	FA3G3S2		[h6n5f1g2]		
96	[h6n7f4a3]			[h6n7f4a3]		
97	[h6n7f5a2]			[h6n7f5a2]		
52	[h7n2]			[h7n2]		
52.1	Man7	M7		[h7n2]		
39	[h7n3]			[h7n3]		
39.1	51	Man5G1 +1aGal hybrid	M5A1[3]G1Ga1	[h7n3]		
33	[h7n3f1]			[h7n3f1]		
33.1	52	Man5G1F hybrid +1aGal	FM5A1[3]G1Ga1	[h7n3f1]		
98	[h7n3f2]			[h7n3f2]		
99	[h7n4]			[h7n4]		
99.1	28	G2+2aGal	A2G2Ga2	[h7n4]		
62	[h7n4a1]			[h7n4a1]		
62.1	G2S+2aGal (NeuAc)			[h7n4a1]		
62.2	Man5G2S (NeuAc) hybrid			[h7n4a1]		
62.3	G2S(6)+2aGal (NeuAc)			[h7n4a1]		

Index^a	Code^b	Measurands^c	Oxford^d	Composition^e	CFG^f	Oxford^g
6		[h7n4f1]		[h7n4f1]		
6.1	16	G2F+2aGal	FA2G2Ga2	[h7n4f1]		
6.2		Man5G2F hybrid	FM5A2G2	[h7n4f1]		
23		[h7n5f1]		[h7n5f1]		
23.1	17	G2FB+2aGal	FA2BG2Ga2	[h7n5f1]		
23.2		G3F+1aGal	FA3G3Ga1	[h7n5f1]		
23.21		G(4)3F+1aGal	F(6)A3G(4)3Ga1	[h7n5f1]		
23.3		G2F+2aGal (tri)	F(6)A3G(4)2Ga2	[h7n5f1]		
23.4		Man5F+2Gal+3N	F(6)M5A3G2	[h7n5f1]		
113		[h7n5f1g1]		[h7n5f1g1]		
113.1		G2FBGS+1aGal (NeuGc)		[h7n5f1g1]		
100		[h7n5f1g2]		[h7n5f1g2]		
100.1		G2FBGS2+1aGal (NeuGc)		[h7n5f1g2]		
101		[h7n5f2]		[h7n5f2]		
114		[h7n6f1a1g3]		[h7n6f1a1g3]		
114.1		G4FS4 (3NeuGc) (1NeuAc)		[h7n6f1a1g3]		
102		[h7n6f2a1]		[h7n6f2a1]		
102.1		G4F2S (NeuAc)		[h7n6f2a1]		
103		[h7n8f3a4]		[h7n8f3a4]		
115		[h7n8f5a3]		[h7n8f5a3]		
116		[h7n8f6a3]		[h7n8f6a3]		
104		[h7n9f1a4]		[h7n9f1a4]		

Index ^a	Code ^b	Measurands ^c	Oxford ^d	Composition ^e	CFG ^f	Oxford ^g
105		[h8n10f5a3]		[h8n10f5a3]		
63		[h8n2]		[h8n2]		
63.1	Man8	M8		[h8n2]		
56		[h8n5f1]		[h8n5f1]		
56.1	G3F+2aGal	F(6)A3G(4)3Ga2		[h8n5f1]		
56.2	G2FGB+2aGal	FA2BG3Ga2		[h8n5f1]		
69		[h8n5f1g1]		[h8n5f1g1]		
69.1	G3FS+2aGal (NeuGc)	F(6)A3G(4)3Ga2S1 (NeuGc)		[h8n5f1g1]		
69.2	G2FBGS+3aGal (NeuGc)			[h8n5f1g1]		
76		[h8n5f2]		[h8n5f2]		
106		[h8n8f3a4]		[h8n8f3a4]		
77		[h9n2]		[h9n2]		
77.1	Man9	M9		[h9n2]		
34		[h9n5f1]		[h9n5f1]		
34.1	G3F+3aGal	F(6)A3G(4)3Ga3		[h9n5f1]		
34.2	G2FBG+3aGal			[h9n5f1]		
107		[n1f1]		[n1f1]		
108		[n2f1]		[n2f1]		
108.1	Fragment FN2			[n2f1]		
	G0F-N/G0F	FA1/FA2		[h3n3f1]/ [h3n4f1]		
	G0/G1F	A2/FA2G1		[h3n4]/ [h3n4f1]		
	G0F/G1F	FA2/FA2G1		[h3n4f1]/ [h4n4f1]		

Index^a	Code^b	Measurands^c	Oxford^d	Composition^e	CFG^f	Oxford^g
	G1F/G2F		FA2G1/FA2G2	[h4n4f1]/ [h5n4f1]		
	G2F/ G2F+1aGal			[h5n4f1]/ [h6n4f1]		
				[h5n5f1g1]/ [h6n5a1]		
				[h6n5a3]/ [h5n5f1a2g1]/ [h4n5f2a1g2]/ [h3n5f3g3]		
				[h6n5f1a3]/ [h5n5f2a2g1]/ [h4n5f3a1g2]/ [h3n5f4g3]		
	[Unknown]	<i>Sum of results reported for “unidentified” glycan-like signals</i>				

^a Index: Each different glycan composition is represented by an integer. Individual glycans are represented by a digit following a decimal point – isomers of these are represented by an additional digit. Indices in **bold** are glycans with complete structural assignments.

^b Code: These correspond to numbers in the data reporting template. Entries with code *Other* are glycans reported by participants but not in the data reporting template.

^c Measurands: text in square brackets correspond to monosaccharide compositions (see Composition). Common names are listed when available.

^d Oxford: Oxford naming convention: All N-glycans have two core GlcNAcs; F at the start of the abbreviation indicates a core fucose, (6) after the F indicates that the fucose is α 1-6 linked to the inner GlcNAc; Mx, number (x) of mannose on core GlcNAcs; Ax, number of antenna (GlcNAc) on trimannosyl core; A2, biantennary with both GlcNAcs as β 1-2 linked; A3, triantennary with a GlcNAc linked β 1-2 to both mannose and the third GlcNAc linked β 1-4 to the α 1-3 linked mannose; A3', triantennary with a GlcNAc linked β 1-2 to both mannose and the third GlcNAc linked β 1-6 to the α 1-6 linked mannose; A4, GlcNAcs linked as A3 with additional GlcNAc β 1-6 linked to α 1-6 mannose; B, bisecting GlcNAc linked β 1-4 to β 1-3 mannose; Gx, number (x) of linked galactose on antenna, (4) or (3) after the G indicates that the Gal is β 1-4 or β 1-3 linked; [3]G1 and [6]G1 indicates that the galactose is on the antenna of the α 1-3 or α 1-6 mannose; Gax, number (x) of linked alpha galactose on antenna; Sx, number (x) of sialic acids linked to galactose; the numbers 3 or 6 in parentheses after S indicate whether the sialic acid is in an α 2-3 or α 2-6 linkage. (Courtesy of Louise Royle, Ludder)

^e [Composition] denotes monosaccharide composition. Small letters are used to avoid confusion with elements (hydrogen, nitrogen, fluorine, etc.): h=hexose, n=N-acetylhexosamine, f=deoxyhexose (e.g. fucose), a=NeuAc, g=NeuGc. Number after the letter denotes the number of residues. For example: [h6n4f1a1] = 6 hexoses, 4 N-acetylhexosamine, 1 fucose, 1 NeuAc. For sulfonated glycans, S=sulfur.

- ^f CFG: Structure using the Consortium for Functional Glycomics (CFG) Notation: Symbol representations of glycans: galactose=  glucose= , mannose= , N-Acetylgalactosamine=  N-Acetylglucosamine=  fucose= , xylose= , N-Acetylneuraminic acid= , N-Glycolylneuraminic acid= . [1,2]
- ^g Oxford: Structure using the Oxford Glycobiology Institute (UOXF) Notation: galactose=  glucose= , mannose= , N-Acetylgalactosamine= , N-Acetylglucosamine= , fucose= , xylose= , N-Acetylneuraminic acid= , N-Glycolylneuraminic Acid= . [2,3]

References

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<https://doi.org/10.1002/pmic.200900708>.
- [2] Ceroni A, Maass K, Geyer H, Geyer R, Dell A, Haslam SM (2008) GlycoWorkbench: a tool for the computer-assisted annotation of mass spectra of glycans. *J Proteome Res* **7**(4):1650–1659.
<https://doi.org/10.1021/pr7008252>.
- [3] Harvey DJ, Merry AH, Royle L, Campbell MP, Dwek RA, Rudd PM (2009) Proposal for a standard system for drawing structural diagrams of N- and O-linked carbohydrates and related compounds. *Proteomics* **9**(15):3796-3801. <https://doi.org/10.1002/pmic.200900096>.

All-Lab Report

The All-Lab Report summarizes results of all laboratories for the two samples. It consists of:

All-Lab Report	#Pages
Summary of reported and derived values for Samples A and B, and the A/B ratio	9
Legend for the summary	1

Interlaboratory Study of NISTmAb Glycosylation

Summary of Reported and Derived Values for Samples A and B, and the A/B Ratio

Index	Composition	Common Name	Sample A, %						Sample B, %					
			#	25%	Median	75%	#	25%	Median	75%	#	25%	Median	75%
1	[14n4f1]		103	27.72	31.61	33.31	103	36.36	38.37	39.84	103	0.77	0.83	0.85
1.1	[14n4f1] Gly	G1F	103	27.72	31.61	33.31	103	36.36	38.37	39.84	103	0.77	0.83	0.85
1.11	[14n4f1] GlyIso	G1F(1,6)	58	19.04	21.21	22.06	58	27.60	28.12	29.12	58	0.71	0.76	0.77
1.12	[14n4f1] GlyIso	G1F(1,3)	59	9.38	10.38	10.72	59	9.82	10.18	10.75	59	0.93	1.02	1.03
1.2	[14n4f1] Gly	G1FB-N	2	1.06	2	1.21	2	1.21	1.21	1.21	2	0.92		
1.21	[14n4f1] GlyIso	G1FB-N[3]	2	0.64	2	0.74	2	0.74	0.74	0.74	2	1.28		
1.22	[14n4f1] GlyIso	G1FB-N[6]	2	0.43	2	0.47	2	0.47	0.47	0.47	2	0.94		
2	[13n4f1]		103	47.06	51.47	55.04	102	35.73	39.10	40.76	102	1.28	1.31	1.38
2.1	[13n4f1] Gly	G0F	103	47.06	51.47	55.04	102	35.73	39.09	40.71	102	1.28	1.31	1.38
2.2	[13n4f1] Gly	G0FB-N	1	0.77	1	0.80	1	0.80	0.80	0.80	1	0.96		
2.3	[13n4f1] Gly	Man3F+2N	2	2.26	2	1.71	2	1.71	1.71	1.71	2	1.32		
3	[15n4f1]		99	3.19	3.97	4.80	102	7.47	8.52	9.48	99	0.42	0.47	0.55
3.1	[15n4f1] Gly	G2F	46	1.00	1.42	2.77	47	5.88	7.15	7.61	45	0.17	0.26	0.51
3.2	[15n4f1] Gly	G1F+1aGal	35	1.64	2.53	3.06	32	1.27	1.60	2.20	32	0.83	1.39	1.68
3.21	[15n4f1] GlyIso	G1F(1,3)+1aGal	2	0.25	1	0.17	1	0.17	0.17	0.17	1	1.47		
3.22	[15n4f1] GlyIso	G1F(1,6)+1aGal	2	2.88	2	1.61	2	1.61	1.61	1.61	2	1.79		
3.3	[15n4f1] Gly	G1FB-N+1aGal	2	1.18	2	4.01	2	4.01	4.01	4.01	2	0.58		
4	[13n3f1]		88	3.03	3.65	4.26	89	1.87	2.13	2.93	87	1.40	1.65	1.89
4.1	[13n3f1] Gly	G0F-N	88	3.03	3.65	4.26	89	1.87	2.13	2.93	87	1.40	1.65	1.89
4.11	[13n3f1] GlyIso	G0F-N(1,6)	10	0.88	1.73	3.31	9	0.94	1.61	1.84	9	1.31	1.39	1.82
4.12	[13n3f1] GlyIso	G0F-N(1,3)	11	0.88	3.47	3.90	11	0.42	1.92	2.12	11	1.26	1.61	1.87
5	[15n2]		77	0.56	0.78	1.12	79	0.53	0.73	1.01	76	0.94	1.02	1.13
5.1	[15n2] Gly	Man5	77	0.56	0.78	1.12	79	0.53	0.73	1.01	76	0.94	1.02	1.13
6	[17n4f1]		73	0.70	0.90	1.09	71	0.68	0.90	1.22	71	0.90	0.98	1.03
6.1	[17n4f1] Gly	G2F+2aGal	71	0.69	0.87	1.05	70	0.67	0.89	1.06	70	0.89	0.98	1.03
6.2	[17n4f1] Gly	Man5G2F hybrid	1	1.82	1	1.60	1	1.60	1.60	1.60	1	1.14		
7	[16n4f1]		70	0.44	0.63	0.92	73	1.48	1.80	2.09	69	0.29	0.35	0.45
7.1	[16n4f1] Gly	G2F+1aGal	70	0.44	0.63	0.92	73	1.48	1.80	2.09	69	0.29	0.35	0.45
7.11	[16n4f1] GlyIso	G2F+1aGal[6]	2	0.49	2	1.62	2	1.62	1.62	1.62	2	0.30		

Index	Composition	Common Name	Sample A, %			Sample B, %			A/B Ratio		
			#	25%	Median	75%	#	25%	Median	75%	#
7.12	[h6n4f1] Gly so	G2F+1aGal[3]	2	0.23		0.28	2	0.89		0.80	2
8	[h4n5f1]		70	0.46	0.70	0.89	67	0.49	0.68	0.88	67
8.1	[h4n5f1] Gly	G1FB	61	0.43	0.67	0.89	57	0.49	0.67	0.87	57
8.2	[h4n5f1] Gly	NA3FG1	9	0.51	0.55	0.81	9	0.46	0.51	0.83	9
8.3	[h4n5f1] Gly	G0F+Hex+HexNAC	1		0.78		1		0.86		1
8.4	[h4n5f1] Gly	G1F+N	1		0.89		1		1.01		1
9	[h5n3f1]		69	0.78	0.88	1.17	68	0.81	0.89	1.22	67
9.1	[h5n3f1] Gly	G1F-N+1aGal	62	0.77	0.87	1.15	61	0.81	0.89	1.21	60
9.2	[h5n3f1] Gly	Man5GOF hybrid	7	0.23	0.28	0.80	5	0.22	0.83	0.88	5
9.3	[h5n3f1] Gly	G1F+Man	4	0.75	0.84	1.60	4	0.79	0.97	2.34	4
9.31	[h5n3f1] Gly so	G1F+Man(1,3)	2		2.32		2		3.62		2
10	[h4n3f1]		68	0.24	0.48	1.11	77	1.68	2.20	2.85	66
10.1	[h4n3f1] Gly	G1F-N	66	0.26	0.50	1.13	76	1.67	2.19	2.77	64
10.11	[h4n3f1] Gly so	G1F-N(1,6)	8	0.19	0.22	0.72	8	0.23	1.28	2.44	7
10.12	[h4n3f1] Gly so	G1F-N(1,3)	13	0.21	0.42	0.75	12	1.00	1.78	2.22	11
10.2	[h4n3f1] Gly	Man4FN	3	9.9E-2	0.12	0.13	2		1.50		2
11	[h3n3]		69	0.32	0.46	0.78	63	0.32	0.44	0.76	61
11.1	[h3n3] Gly	G0-N	69	0.29	0.46	0.72	63	0.32	0.43	0.76	61
11.11	[h3n3] Gly so	G0-N(1,6)	7	0.35	0.38	0.54	6	0.34	0.39	0.41	6
11.12	[h3n3] Gly so	G0-N(1,3)	4	0.10	0.34	0.80	4	7.3E-2	0.27	0.54	4
11.2	[h3n3] Gly	Man3B	2		0.48		2		0.57		2
12	[h4n3f1g1]		60	0.70	0.95	1.30	60	0.79	1.01	1.33	59
12.1	[h4n3f1g1] Gly	G1FS-N (NeuGC)	60	0.70	0.95	1.30	60	0.79	1.01	1.33	59
13	[h3n4]		63	0.16	0.28	0.84	58	0.13	0.19	0.74	57
13.1	[h3n4] Gly	G0	62	0.16	0.25	0.81	57	0.13	0.19	0.74	56
13.2	[h3n4] Gly	G0B-N	1		0.98		1		0.69		1
13.21	[h3n4] Gly so	G0B-N(1,3)	1		0.26		1		0.19		1
13.22	[h3n4] Gly so	G0B-N(1,6)	1		0.72		1		0.50		1
14	[h4n4f1g1]		53	0.31	0.51	0.77	56	0.21	0.35	0.65	52
14.1	[h4n4f1g1] Gly	G1FS (NeuGc)	53	0.31	0.51	0.77	56	0.21	0.35	0.65	52
14.11	[h4n4f1g1] Gly so	G1FS[6] (NeuGc)	5	0.13	0.27	0.29	5	0.14	0.27	0.28	5
14.12	[h4n4f1g1] Gly so	G1FS[3] (NeuGc)	4	0.42	0.48	0.48	4	0.32	0.46	0.64	4
15	[h6n4f1g1]		50	0.31	0.44	0.62	49	0.37	0.46	0.72	49
15.1	[h6n4f1g1] Gly	G2FS+1aGal (NeuGc)	50	0.31	0.44	0.62	49	0.37	0.46	0.72	49

Index	Composition	Common Name	Sample A, %			Sample B, %			A/B Ratio			
			#	25%	Median	75%	#	25%	Median	75%	#	
16	[h5n4f1g1]		50	0.15	0.27	0.47	50	0.29	0.45	0.72	48	0.58
16.1	[h5n4f1g1]Gly	G2FS (NeuGc)	50	0.15	0.27	0.47	50	0.29	0.45	0.72	48	0.58
17	[h5n5f1]		51	0.20	0.29	0.46	58	0.34	0.46	0.61	48	0.46
17.1	[h5n5f1]Gly	G2FB	44	0.19	0.28	0.46	51	0.34	0.47	0.61	42	0.47
17.2	[h5n5f1]Gly	G1FB+1aGal	1		0.46		1		0.42		1	
17.3	[h5n5f1]Gly	G2F+N (tri)	5	0.32	0.37	0.39	7	0.17	0.24	0.46	4	0.90
17.31	[h5n5f1]GlyIso	G1F+N+1aGal	2		0.29		2		0.31		1	
17.32	[h5n5f1]GlyIso	G2F(1,4)+N (tri)	4	0.27	0.35	0.48	5	0.15	0.19	0.54	3	0.75
17.4	[h5n5f1]Gly	G0F+2Hex+HexNAc	2		9.1E-2		2		0.19		2	
18	[h3n5f1]		55	0.64	0.78	1.07	46	0.30	0.49	1.63	45	0.69
18.1	[h3n5f1]Gly	G0FB	50	0.64	0.77	1.01	41	0.30	0.48	1.36	40	1.07
18.2	[h3n5f1]Gly	G0F+N (tri)	4	0.83	1.11	1.30	4	1.96	2.44	2.46	4	0.37
18.3	[h3n5f1]Gly	ManF+3N	2		1.06		2		0.44		2	
18.31	[h3n5f1]GlyIso	G0F+N	1		1.48		1		0.59		1	
19	[h3n2f1]		43	0.10	0.14	0.45	45	0.10	0.14	0.33	39	0.95
19.1	[h3n2f1]Gly	Man3F	43	0.10	0.14	0.45	45	0.10	0.14	0.33	39	0.95
20	[h4n4]		41	0.16	0.47	1.06	35	0.18	0.49	1.88	35	0.63
20.1	[h4n4]Gly	G1	40	0.15	0.52	1.08	34	0.18	0.58	1.98	34	0.61
20.11	[h4n4]GlyIso	G1(1,6)	5	0.10	0.95	1.01	5	0.18	1.38	1.60	5	0.55
20.12	[h4n4]GlyIso	G1(1,3)	7	0.13	0.25	0.53	5	7.3E-2	0.35	0.61	5	0.85
20.2	[h4n4]Gly	G1B-N	1		0.16		1		0.22		1	
21	[h5n4]		36	0.44	0.70	0.97	36	0.31	0.54	1.08	35	0.75
21.1	[h5n4]Gly	G2	36	0.38	0.63	0.94	36	0.31	0.53	1.01	35	0.71
21.2	[h5n4]Gly	G1+1aGal	1		0.91		1		0.70		1	
22	[h4n3]		26	5.6E-2	0.11	0.38	28	6.9E-2	0.12	0.44	23	0.68
22.1	[h4n3]Gly	G1-N	23	5.4E-2	9.6E-2	0.42	25	6.7E-2	0.12	0.47	20	0.63
22.2	[h4n3]Gly	Man4N	3	0.14	0.15	0.16	3	7.7E-2	8.0E-2	0.10	3	1.48
22.21	[h4n3]GlyIso	Man4N[3]	2		0.16		2		7.7E-2		2	
23	[h7n5f1]		22	6.5E-2	0.12	0.17	23	8.5E-2	0.13	0.17	20	0.68
23.1	[h7n5f1]Gly	G2FB+2aGal	18	5.7E-2	0.10	0.18	18	8.0E-2	1.0E-1	0.15	16	0.68
23.2	[h7n5f1]Gly	G3F+1aGal	3	7.6E-2	8.5E-2	0.11	3	8.0E-2	8.0E-2	0.15	3	0.72
23.21	[h7n5f1]GlyIso	G(4)3F+1aGal	1		8.5E-2		1		8.0E-2		1	
23.3	[h7n5f1]Gly	G2F+2aGal (tri)	2		0.10		2		0.12		2	
23.4	[h7n5f1]Gly	Man5F+2Gal+3N	0				1		0.17		0	

Index	Composition	Common Name	Measurand			Sample A, %			Sample B, %			A/B Ratio		
			#	25%	Median	75%	#	25%	Median	75%	#	25%	Median	75%
24	[h6n3f1]		21	7.8E-2	0.19	0.27	27	0.13	0.19	0.29	19	0.40	0.70	1.00
24.1	[h6n3f1] Gly	Man5G1F hybrid	18	6.9E-2	0.15	0.26	24	0.12	0.18	0.34	16	0.41	0.54	0.93
24.2	[h6n3f1] Gly	Man4G1F+1aGal hybrid	2	0.23		2	0.21			2		1.13		
25	[h6n5f1]		18	5.0E-2	0.14	0.20	38	0.20	0.29	0.44	18	0.14	0.33	0.61
25.1	[h6n5f1] Gly	G2FB+1aGal	13	7.0E-2	0.14	0.16	22	0.17	0.26	0.36	13	0.24	0.33	0.63
25.2	[h6n5f1] Gly	G3F	6	4.5E-2	6.0E-2	0.19	17	0.26	0.32	0.44	6	0.12	0.21	0.44
25.21	[h6n5f1] Glyso	G3F[3]	2	3.5E-2			11	0.22	0.32	0.38	2	8.8E-2		
26	[h5n3f1g1]		20	8.9E-2	0.12	0.21	20	9.3E-2	0.12	0.17	17	0.94	1.06	1.20
26.1	[h5n3f1g1] Gly	Man5G0FS (NeuGc) hybrid	13	9.6E-2	0.13	0.21	11	9.1E-2	0.14	0.17	11	1.00	1.12	1.22
26.2	[h5n3f1g1] Gly	Man4G1FS (NeuGc) hybrid	4	0.11	0.12	0.13	6	0.10	0.12	0.13	3	1.03	1.07	1.14
27	[h5n4a1]		18	0.21	0.36	0.54	19	0.20	0.26	1.04	16	0.69	1.24	1.51
27.1	[h5n4a1] Gly	G2S (NeuAc)	18	0.21	0.36	0.54	19	0.20	0.26	1.04	16	0.69	1.24	1.51
28	[h6n3]		16	4.8E-2	9.5E-2	0.31	20	0.15	0.23	0.43	16	0.22	0.50	0.87
28.1	[h6n3] Gly	Man5G1 hybrid	16	4.8E-2	9.5E-2	0.31	20	0.15	0.23	0.43	16	0.22	0.50	0.87
29	[h5n3]		17	9.0E-2	0.13	0.23	17	3.9E-2	8.0E-2	9.7E-2	15	1.17	1.71	2.48
29.1	[h5n3] Gly	G1-N+1aGal	17	9.0E-2	0.13	0.23	17	3.9E-2	8.0E-2	9.7E-2	15	1.17	1.71	2.48
30	[h6n2]		17	6.0E-2	0.30	0.47	17	4.0E-2	0.33	0.50	15	0.89	0.98	1.12
30.1	[h6n2] Gly	Man6	17	6.0E-2	0.30	0.47	17	4.0E-2	0.33	0.50	15	0.89	0.98	1.12
31	[h6n4f2]		18	8.6E-2	0.15	0.24	16	9.9E-2	0.12	0.24	15	0.82	0.98	1.04
31.1	[h6n4f2] Gly	G2F2+1aGal	17	0.11	0.16	0.24	16	9.9E-2	0.12	0.24	15	0.82	0.98	1.04
31.2	[h6n4f2] Gly	Man5G1F2B hybrid	1	2.7E-2		0					0			
32	[h6n3f1g1]		15	5.6E-2	0.10	0.19	16	4.1E-2	7.4E-2	0.13	13	0.95	1.00	1.14
32.1	[h6n3f1g1] Gly	Man5G1FS (NeuGc) hybrid	15	5.6E-2	0.10	0.19	16	4.1E-2	7.4E-2	0.13	13	0.95	1.00	1.14
33	[h7n3f1]		14	4.6E-2	8.5E-2	0.13	16	2.7E-2	6.3E-2	0.11	13	1.01	1.13	1.25
33.1	[h7n3f1] Gly	Man5G1F hybrid+1aGal	14	4.6E-2	8.5E-2	0.13	16	2.7E-2	6.3E-2	0.11	13	1.01	1.13	1.25
34	[h9n5f1]		13	5.0E-2	7.1E-2	7.5E-2	13	4.7E-2	5.7E-2	6.9E-2	13	0.96	1.06	1.10
34.1	[h9n5f1] Gly	G3F+3aGal	6	7.4E-2	7.6E-2	7.9E-2	6	6.9E-2	7.3E-2	8.1E-2	6	1.00	1.10	1.10
34.2	[h9n5f1] Gly	G2FBG+3aGal	1	5.3E-2			1	5.7E-2			1		0.93	
35	[h5n4f1a1]		16	0.10	0.31	0.82	17	8.3E-2	0.33	1.03	12	0.48	0.98	1.08
35.1	[h5n4f1a1] Gly	G2FS (NeuAc)	16	0.10	0.31	0.82	17	8.3E-2	0.33	1.03	12	0.48	0.98	1.08
36	[h4n3f1a1]		13	0.58	0.83	1.32	13	0.24	0.59	1.12	11	0.95	1.00	1.12
36.1	[h4n3f1a1] Gly	G1FS-N (NeuAc)	13	0.58	0.83	1.32	13	0.24	0.59	1.12	11	0.95	1.00	1.12
37	[h3n2]		11	3.5E-2	6.3E-2	0.42	10	3.1E-2	5.7E-2	0.30	10	0.90	1.02	1.29
37.1	[h3n2] Gly	Man3	11	3.5E-2	6.3E-2	0.42	10	3.1E-2	5.7E-2	0.30	10	0.90	1.02	1.29

Index	Composition	Common Name	Measurand			Sample A, %			Sample B, %			#	25%	Median	75%	#	25%	Median	75%
			#	25%	Median	75%	#	25%	Median	75%	#								
38	[h4n4f2]		11	0.10	0.19	0.31	13	7.7E-2	0.15	0.21	10	1.27	1.45	1.53					
38.1	[h4n4f2] Gly	G1F2	11	0.10	0.19	0.31	13	7.7E-2	0.15	0.21	10	1.27	1.45	1.53					
39	[h7n3]		10	7.0E-2	7.3E-2	0.11	10	5.4E-2	8.8E-2	0.14	10	0.70	0.92	1.23					
39.1	[h7n3] Gly	Man5G1+1aGal hybrid	10	7.0E-2	7.3E-2	0.11	10	5.4E-2	8.8E-2	0.14	10	0.70	0.92	1.23					
40	[h4n4f1a1]		9	0.17	0.26	0.62	10	0.20	0.41	0.81	7	0.33	0.70	1.11					
40.1	[h4n4f1a1] Gly	G1FS (NeuAc)	9	0.17	0.26	0.62	10	0.20	0.41	0.81	7	0.33	0.70	1.11					
41	[h5n4f1a2]		7	0.38	0.43	0.83	8	0.32	0.41	0.69	7	0.87	0.90	1.00					
41.1	[h5n4f1a2] Gly	G2FS2 (NeuAc)	7	0.38	0.43	0.83	8	0.32	0.41	0.69	7	0.87	0.90	1.00					
42	[h2n3f1]		6	0.31	0.58	1.13	6	0.29	0.62	1.03	6	0.82	1.07	1.19					
42.1	[h2n3f1] Gly	Fragment Man2F+N	6	0.33	0.42	0.60	7	0.10	0.17	0.42	6	1.12	1.85	3.36					
43	[h3n5]		6	0.31	0.58	1.13	6	0.29	0.62	1.03	6	0.82	1.07	1.19					
43.1	[h3n5] Gly	G0B	6	0.32	0.42	0.60	7	0.10	0.17	0.42	6	1.12	1.85	3.36					
43.2	[h3n5] Gly	G0+N (tri)	1		1.0E-2		1		1.0E-2		1				1		1.00		
44	[h4n3f2]		6	8.9E-2	0.14	0.23	7	5.6E-2	0.12	0.19	6	0.91	1.06	1.17					
44.1	[h4n3f2] Gly	G1F-N+AF	4	0.15	0.20	0.33	4	0.16	0.19	0.32	4	0.88	1.00	1.13					
45	[h4n5f1g1]		8	3.5E-2	5.5E-2	7.9E-2	8	1.3E-2	3.1E-2	5.5E-2	6	1.30	2.05	2.37					
45.1	[h4n5f1g1] Gly	G1FB5 (NeuGc)	8	3.5E-2	5.5E-2	7.9E-2	8	1.3E-2	3.1E-2	5.5E-2	6	1.30	2.05	2.37					
46	[h5n3a1]		7	0.19	0.30	0.84	7	0.14	0.25	0.94	6	0.82	1.05	1.20					
46.1	[h5n3a1] Gly	Man4G1S (NeuAc) hybrid	3	0.22	0.30	0.65	2		0.63		2		1.10						
46.2	[h5n3a1] Gly	Fragment G2S-CoreN (NeuAc)	1		0.18		1		0.24		1		0.76						
47	[h5n4f2]		7	2.7E-2	7.8E-2	0.42	11	6.4E-2	8.7E-2	0.27	6	0.34	0.79	1.17					
47.1	[h5n4f2] Gly	G2F2	7	2.7E-2	7.8E-2	0.42	10	7.7E-2	0.12	0.28	6	0.34	0.79	1.17					
47.2	[h5n4f2] Gly	Man4G1F2B hybrid	0				1		5.2E-2		0								
48	[h5n4g2]		8	0.14	0.33	0.60	7	0.17	0.40	0.83	6	0.86	0.96	1.74					
48.1	[h5n4g2] Gly	G2S2 (NeuGc)	8	0.14	0.33	0.60	7	0.17	0.40	0.83	6	0.86	0.96	1.74					
49	[h6n3g1]		7	4.0E-2	7.3E-2	9.2E-2	6	5.0E-2	6.8E-2	0.11	6	0.70	0.83	0.92					
49.1	[h6n3g1] Gly	Man5G1S (NeuGc) hybrid	4	7.0E-2	9.2E-2	0.32	3	8.7E-2	0.13	0.13	3	0.69	0.82	0.83					
50	[h6n4]		6	9.1E-2	0.14	0.16	6	0.22	0.34	0.90	6	0.30	0.33	0.70					
50.1	[h6n4] Gly	G2+1aGal	5	8.0E-2	0.12	0.16	5	0.27	0.42	1.06	5	0.30	0.30	0.37					
50.2	[h6n4] Gly	Fragment Man5G1-CoreN hybrid	1		0.16		1		0.20		1		0.81						
51	[h6n4f1a1]		7	4.6E-2	0.23	0.31	9	7.0E-2	0.12	0.47	6	0.74	0.86	1.16					
51.1	[h6n4f1a1] Gly	G2FS+1aGal (NeuAc)	6	2.8E-2	0.16	0.25	8	5.8E-2	0.10	0.27	5	0.74	0.77	0.96					
51.2	[h6n4f1a1] Gly	Man5G1FB5 (NeuAc) hybrid	1		0.91		1		0.57		1		1.60						
52	[h7n2]		6	6.3E-2	0.25	0.95	7	0.25	0.55	0.87	6	0.70	1.00	1.59					

Index	Composition	Common Name	Measurand			Sample A, %			Sample B, %			A/B Ratio		
			#	25%	Median	75%	#	25%	Median	75%	#	25%	Median	75%
52.1	[h7n2] Gly	Man7	6	6.3E-2	0.25	0.95	7	0.25	0.55	0.87	6	0.70	1.00	1.59
53	[h4n2]		5	9.3E-2	0.24	0.32	7	9.3E-2	9.7E-2	0.41	5	1.02	1.04	1.31
53.1	[h4n2] Gly	Man4	5	9.3E-2	0.24	0.32	7	9.3E-2	9.7E-2	0.41	5	1.02	1.04	1.31
53.11	[h4n2] Gly/so	Man4D2	1	0.70		1	0.58			1	1.21			
54	[h4n3g1]		6	2.4E-2	4.1E-2	9.6E-2	5	3.3E-2	3.7E-2	0.13	5	0.63	0.85	0.85
54.1	[h4n3g1] Gly	G1S (NeuGc)	6	2.4E-2	4.1E-2	9.6E-2	5	3.3E-2	3.7E-2	0.13	5	0.63	0.85	0.85
55	[h5n4f1g2]		7	3.2E-2	4.6E-2	0.24	5	6.3E-2	7.4E-2	0.79	5	0.53	0.82	0.83
55.1	[h5n4f1g2] Gly	G2FS2 (NeuGc)	7	3.2E-2	4.6E-2	0.24	5	6.3E-2	7.4E-2	0.79	5	0.53	0.82	0.83
56	[h8n5f1]		5	1.3E-2	2.0E-2	6.6E-2	9	5.0E-2	6.0E-2	7.5E-2	5	0.22	0.24	0.75
56.1	[h8n5f1] Gly	G3F+2aGal	2	4.0E-2		4	6.6E-2	7.2E-2	7.8E-2	2	0.50			
56.2	[h8n5f1] Gly	G2FGB+2aGal	1	4.5E-3		2	4.9E-2			1	0.12			
57	[h4n5]		4	0.30	0.37	2.87	4	9.5E-2	0.34	1.27	4	0.85	1.97	4.10
57.1	[h4n5] Gly	G1B	4	0.30	0.37	2.87	4	9.5E-2	0.34	1.27	4	0.85	1.97	4.10
57.11	[h4n5] Gly/so	G1B(1,6)	1	4.0E-2		1	5.0E-2			1	0.80			
57.12	[h4n5] Gly/so	G1B(1,3)	1	6.0E-2		1	6.0E-2			1	1.00			
58	[h5n3f2]		5	2.4E-2	6.5E-2	0.66	5	2.8E-2	0.42	0.79	4	0.75	0.84	0.87
58.1	[h5n3f2] Gly	G1F2-N+1aGal	5	2.4E-2	6.5E-2	0.66	5	2.8E-2	0.42	0.79	4	0.75	0.84	0.87
59	[h5n4a2]		5	0.20	0.27	0.74	5	0.13	0.13	0.20	4	1.26	1.44	2.50
59.1	[h5n4a2] Gly	G2S2 (NeuAc)	5	0.20	0.27	0.74	5	0.13	0.13	0.20	4	1.26	1.44	2.50
60	[h5n4g1]		7	0.10	0.14	0.16	4	0.72	0.93	1.13	4	0.10	0.13	0.45
60.1	[h5n4g1] Gly	G2S (NeuGc)	7	0.10	0.14	0.16	4	0.72	0.93	1.13	4	0.10	0.13	0.45
61	[h5n5f2]		4	1.0E-2	4.5E-2	0.25	4	1.1E-2	2.3E-2	0.21	4	1.00	1.01	1.32
61.1	[h5n5f2] Gly	G2F2B	1	1.1E-2		1	1.1E-2			1	0.98			
61.2	[h5n5f2] Gly	G2F2+N(tri)	1	1.0E-2		1	1.0E-2			1	1.00			
62	[h7n4a1]		4	0.22	0.29	0.51	4	0.20	0.27	0.47	4	1.08	1.10	1.12
62.1	[h7n4a1] Gly	G2S+2aGal (NeuAc)	1	0.33		1	0.32			1	1.05			
62.2	[h7n4a1] Gly	Man5G2S (NeuAc) hybrid	1	1.05		1	0.95			1	1.11			
62.3	[h7n4a1] Gly	G2S(6)+2aGal (NeuAc)	1	0.13		1	0.11			1	1.18			
63	[h8n2]		4	0.13	0.19	0.44	4	0.13	0.27	0.50	4	0.77	1.11	1.34
63.1	[h8n2] Gly	Man8	4	0.13	0.19	0.44	4	0.13	0.27	0.50	4	0.77	1.11	1.34
64	[h4n2f1]		3	0.16	0.18	4.92	3	0.92	1.73	7.79	3	0.40	0.70	0.95
64.1	[h4n2f1] Gly	Man4F	3	0.16	0.18	4.92	3	0.92	1.73	7.79	3	0.40	0.70	0.95
64.11	[h4n2f1] Gly/so	Man4D2F	1	0.18		1	1.73			1	0.10			
65	[h4n4a1]		3	0.37	0.50	0.70	3	0.12	0.24	0.47	3	1.16	1.29	25.65

Index	Composition	Measurand	Sample A, %			Sample B, %			A/B Ratio			
			#	25%	Median	75%	#	25%	Median	75%	#	
65.1	[h4n4a1] Gly	G1S (NeuAc)	3	0.37	0.50	0.70	3	0.12	0.24	0.47	3	1.16
66	[h5n3g1]		3	1.8E-2	2.0E-2	6.3E-2	3	2.1E-2	4.0E-2	7.5E-2	3	0.73
66.1	[h5n3g1] Gly	Man4G1S (NeuGc) hybrid	1		0.11		1		0.11		1	0.97
67	[h5n5]		4	0.27	0.48	0.99	3	1.14	1.36	1.46	3	0.43
67.1	[h5n5] Gly	G2B	3	0.44	0.67	1.32	3	1.14	1.36	1.46	3	0.43
67.2	[h5n5] Gly	G1+N+1aGal (tri)	1		0.29		0				0	
68	[h6n5f1g1]		4	9.1E-3	2.1E-2	3.7E-2	3	1.1E-2	1.3E-2	2.9E-2	3	0.82
68.1	[h6n5f1g1] Gly	G2FBs+1aGal (NeuGc)	1		1.0E-2		1		8.2E-3		1	1.22
68.2	[h6n5f1g1] Gly	G3FS (NeuGc)	1		3.2E-2		0				0	
69	[h8n5f1g1]		3	2.6E-2	3.7E-2	4.0E-2	3	2.5E-2	3.3E-2	3.8E-2	3	0.92
69.1	[h8n5f1g1] Gly	G3FS+2aGal (NeuGc)	2		4.0E-2		2		3.8E-2		2	1.07
69.2	[h8n5f1g1] Gly	G2FBs+3aGal (NeuGc)	1		1.6E-2		1		1.8E-2		1	0.85
70	[h4n5f1a1]		2		0.38		4	6.3E-2	0.49	1.48	2	1.41
70.1	[h4n5f1a1] Gly	G1FBs (NeuAc)	2		0.38		4	6.3E-2	0.49	1.48	2	1.41
71	[h5n3f1a1]		3	0.29	0.50	0.66	2		0.89		2	0.75
71.1	[h5n3f1a1] Gly	Man4FG1FS (NeuAc)	2		0.45		1		0.83		1	0.97
71.2	[h5n3f1a1] Gly	G1FS-N+1aGal (NeuAc)	1		0.50		1		0.95		1	0.53
72	[h6n3a1]		2		4.3E-2		3	1.4E-2	1.8E-2	4.0E-2	2	1.09
73	[h6n4a1]		3	9.3E-2	0.12	0.15	3	0.18	0.22	0.26	2	0.71
73.1	[h6n4a1] Gly	G2S+1aGal (NeuAc)	2		0.13		1		0.31		1	0.60
74	[h6n5]		2		0.99		2		1.55		2	0.75
74.1	[h6n5] Gly	G3	2		0.99		2		1.55		2	0.75
74.11	[h6n5] GlyIso	G3[6]	1		1.08		1		2.20		1	0.49
75	[h6n5a1]		2		7.1E-3		2		1.2E-2		2	1.05
76	[h8n5f2]		2		6.2E-3		2		1.7E-2		2	0.49
77	[h9n2]		2		7.3E-2		4	4.4E-2	6.7E-2	9.1E-2	2	1.09
77.1	[h9n2] Gly	Man9	2		7.3E-2		4	4.4E-2	6.7E-2	9.1E-2	2	1.09
78	[h3n3g1]		1		9.8E-4		1		1.0E-3		1	0.95
79	[h3n4f1a1]		1		2.17		1		2.64		1	0.82
80	[h3n4f1S]		1		6.6E-2		1		7.6E-2		1	0.87
80.1	[h3n4f1S] Gly	G0F-N+GalNAcSul	1		6.6E-2		1		7.6E-2		1	0.87
81	[h3n4f2]		1		0.18		1		0.14		1	1.37
81.1	[h3n4f2] Gly	G0F2-N+GalNAc	1		0.18		1		0.14		1	1.37
82	[h3n5f2]		1		2.18		1		0.22		1	10.08

Index	Composition	Common Name	Measurand			Sample A, %			Sample B, %			A/B Ratio		
			#	25%	Median	75%	#	25%	Median	75%	#	25%	Median	75%
83	[h3n7]			1	0.50		1	0.47			1	1.06		
83.1	[h3n7] Gly	G0FB+2N (quad)	1	0.50			1	0.47			1	1.06		
84	[h4n3a1]			1	6.0E-2		2	16.12			1	0.23		
84.1	[h4n3a1] Gly	G1S-N (NeuAc)	1	6.0E-2			2	16.12			1	0.23		
85	[h4n4f1g2]			1	2.0E-2		1	2.0E-2			1	1.00		
85.1	[h4n4f1g2] Gly	G1FS2 (NeuGc)	1	2.0E-2			1	2.0E-2			1	1.00		
86	[h4n4f1S]			1	0.13		1	8.0E-2			1	1.68		
86.1	[h4n4f1S] Gly	Man4G0F+GalNAc4Sul hybrid	1	0.13			1	8.0E-2			1	1.68		
87	[h4n5a1]			1	0.57		1	0.18			1	3.17		
87.1	[h4n5a1] Gly	G1S+N (NeuAc) (tri)	1	0.57			1	0.18			1	3.17		
88	[h5n2f1]			1	0.31		1	2.28			1	0.14		
88.1	[h5n2f1] Gly	Man5F	1	0.31			1	2.28			1	0.14		
89	[h5n5f1a1]			1	0.68		1	0.74			1	0.92		
89.1	[h5n5f1a1] Gly	G2FS+N (NeuAc) (tri)	1	0.68			1	0.74			1	0.92		
90	[h5n5f1a2]			1	0.38		1	0.74			1	0.51		
90.1	[h5n5f1a2] Gly	G2FS2+N (NeuAc) (tri)	1	0.38			1	4.5E-2			1	1.03		
91	[h5n5f1g1]			1	4.6E-2		1	0.12			1	0.39		
92	[h6n2f1]			1	4.8E-2		1	0.12			1	0.39		
92.1	[h6n2f1] Gly	Man6F	1	4.8E-2			1	2.1E-2			1	0.89		
93	[h6n3f2]			1	1.9E-2		1	2.1E-2			1	0.78		
94	[h6n4g1]			1	0.36		1	0.46			1	0.39		
95	[h6n5f1g2]			1	3.7E-2		1	6.0E-2			1	0.61		
95.1	[h6n5f1g2] Gly	G3FS2 (NeuGc)	1	3.7E-2			1	6.0E-2			1	0.61		
96	[h6n7f4a3]			1	5.7E-2		1	3.0E-2			1	1.89		
97	[h6n7f5a2]			1	4.8E-2		1	6.0E-2			1	0.80		
98	[h7n3f2]			1	3.2E-2		1	8.5E-2			1	0.38		
99	[h7n4]			2	9.0E-2		3	7.2E-2	0.10	0.23	1	0.29		
99.1	[h7n4] Gly	G2+2aGal	2	9.0E-2			3	7.2E-2	0.10	0.23	1	0.29		
100	[h7n5f1g2]			1	6.1E-3		1	6.3E-3			1	0.97		
100.1	[h7n5f1g2] Gly	G2FBGS2+1aGal (NeuGc)	1	6.1E-3			1	6.3E-3			1	0.97		
101	[h7n5f2]			1	1.9E-2		1	3.3E-2			1	0.58		
102	[h7n6f2a1]			1	0.13		1	0.10			1	1.24		
102.1	[h7n6f2a1] Gly	G4F2S (NeuAc)	1	0.13			1	0.10			1	1.24		
103	[h7n8f3a4]			1	1.8E-2		1	3.2E-2			1	0.56		

Index	Composition	Common Name	Sample A, %			Sample B, %			A/B Ratio			
			#	25%	Median	75%	#	25%	Median	75%	#	
104	[h7n6f1a4]		1	1.5E-2			1	3.6E-2			1	0.43
105	[h8n10f5a3]		1	6.5E-3			1	7.1E-3			1	0.92
106	[h8n8f3a4]		1	5.0E-3			1	2.2E-2			1	0.23
107	[n1f1]		1	4.9E-3			1	6.2E-3			1	0.79
108	[n2f1]		2	9.6E-2			1	0.13			1	1.16
108.1	[n2f1] Gly	Fragment FN2	2	9.6E-2			1	0.13			1	1.16
109	[h3n3f2]		0				1	0.20			0	
109.1	[h3n3f2] Gly	Man3F2+N	0				1	0.20			0	
110	[h6n2g1]		*			*	*					
110.1	[h6n2g1] Gly	Man5+Gal+S (NeuGc) hybrid	*				1	0.61			0	
111	[h6n3f1a1]		0				1	0.61			0	
111.1	[h6n3f1a1] Gly	Man5G1FS (NeuAc) hybrid	0				1	0.61			0	
112	[h6n3f2a1]		0				1	2.6E-2			0	
112.1	[h6n3f2a1] Gly	Man5G1F2S (NeuAc) hybrid	0				1	2.6E-2			0	
113	[h7n5f1g1]		0				2	1.8E-2			0	
113.1	[h7n5f1g1] Gly	G2FBGS+1aGal (NeuGc)	0				1	6.7E-3			0	
114	[h7n6f1a3] Gly	G4FS4 (3NeuGc) (1NeuAc)	0				1	5.48			0	
114.1	[h7n6f1a3] Gly	G4FS4 (3NeuGc) (1NeuAc)	0				1	5.48			0	
115	[h7n8f5a3]		1	2.4E-2			0				0	
116	[h7n8f6a3]		1	1.2E-2			0				0	
[Unknowns]			26	0.99	1.68	5.24	25	0.77	2.60	7.05	25	0.65
[h3n3f1]/[h3n4f1]			1	2.80			1	1.07			1	2.63
[h3n4f1]/[h3n4f1]			1	1.30			1	0.83			1	1.56
[h3n4f1]/[h4n4f1]			1	30.57			1	37.13			1	0.82
[h4n4f1]/[h5n4f1]			1	1.70			1	9.37			1	0.18
[h5n4f1]/[h6n4f1]			0				1	0.50			0	
[h5n5f1g1]/[h6n5a1]			1	0.12			1	8.0E-2			1	1.46
[h6n5a3]/[h5n5f1a2g1]/[h4n5f2a1g2]/[h3n5f3g3]			1	5.3E-2			0				0	
[h6n5f1a3]/[h5n5f2a2g1]/[h4n5f3a1g2]/[h3n5f4g3]			1	2.6E-2			0				0	

* Identified but not quantified

Interlaboratory Study of NISTmAb Glycosylation

What the Table Lists

Headings and Subheadings	Definition
Measurand	The glycan (or identified combination of glycans) for which measurement results were reported.
Index:	Decimal index of identified glycan compositions, assigned in decreasing order of number of reports. Integers denote unique compositions, tenths glycoforms, and hundredths isomers.
Composition:	Composition of the glycan in the De Leo-Z-Stein notation (see Table of Identified Glycans for details.) The result listed for a given composition is the sum of the reported results for all glycoforms of that composition, where the glycoforms are indicated by "[composition] Gly". The result listed for a given glycoform is the sum of the reported results for all isomers of that glycoform where the isomers are indicated by "[composition] GlyIso". Note: "[Unknowns]" = sum of the abundances of unidentified glycans.
Common Name:	When available, a common name of glycoforms and isomers. See Table of Identified Glycans for Oxford names.
Sample A, %	Summary statistics for results reported for sample A, a modified version of the NISTmAb material #: The number of participants reporting this measurand in this sample. 25%: The 25th percentile (1st quartile) of the distribution of the reported results. Median: The consensus median (50th percentile or 2nd quartile) of the distribution of the reported results. 75%: The 75th percentile (3rd quartile) of the distribution of the reported results.
Sample B, %	Summary statistics for results reported for sample B, the NISTmAb material. #: The number of participants reporting this measurand in this sample. 25%: The 25th percentile (1st quartile) of the distribution of the reported results. Median: The consensus median (50th percentile or 2nd quartile) of the distribution of the reported results. 75%: The 75th percentile (3rd quartile) of the distribution of the reported results.
A/B Ratio	Summary statistics for the ratio A/B when results were reported for both samples A and B. #: The number of A/B ratios. 25%: The 25th percentile (1st quartile) of the distribution of the calculated ratios. Median: The consensus median (50th percentile or 2nd quartile) of the distribution of the calculated ratios. 75%: The 75th percentile (3rd quartile) of the distribution of the calculated ratios.

Representative “Individualized Report”

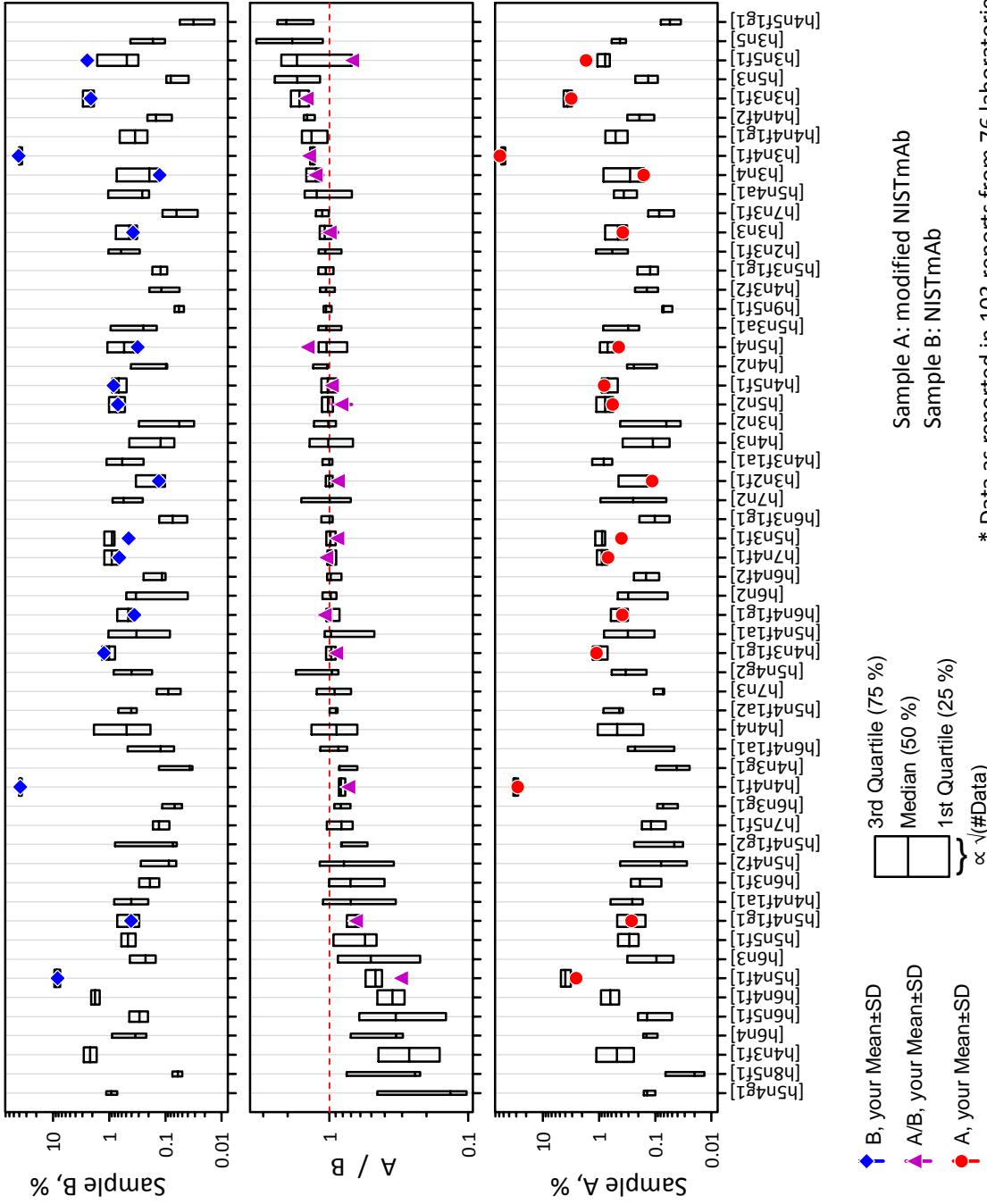
The Individualized Report contains graphical analyses of the glycan composition results:

Individualized Report	#Pages
Boxplots of Samples A and B, and the A/B ratio, and targetplot for the A/B ratio	1
Legend for the boxplots and targetplot	1
Plots summarizing measurement performance, including glycan composition counts and sums, repeatability, limits of reporting, minimum reported values, and consensus	1
Legend for measurement performance plots	1
Table of measurement summary	Variable
Legend for table of measurement summary	1
Table of derived glycan attribute quantities	1
Legend for table of derived glycan attribute quantities	1

Interlaboratory Study of NISTmAb Glycosylation

Report for "Example"

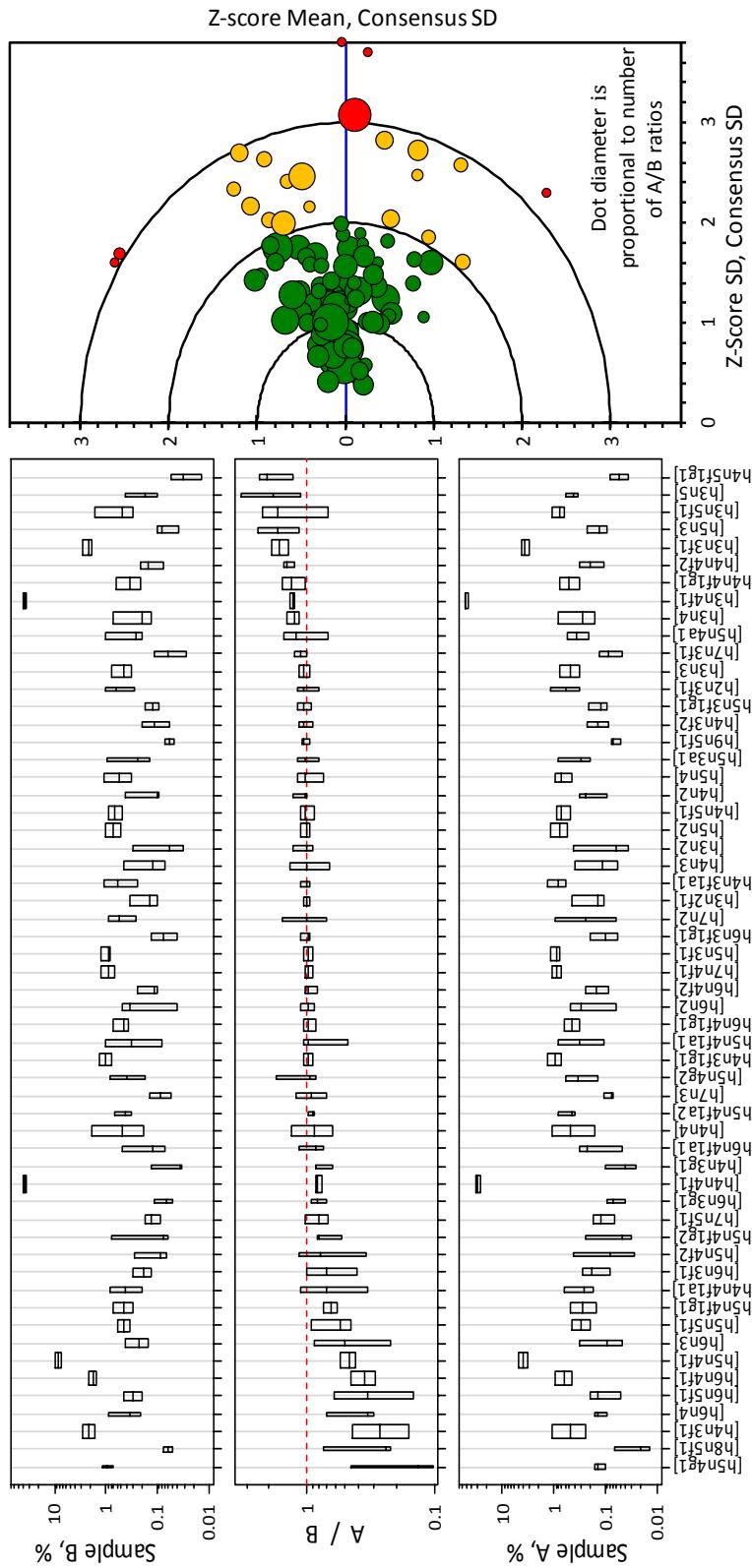
This publication is available free of charge from: <https://doi.org/10.6028/NIST.IR.8186>



* Data as reported in 103 reports from 76 laboratories

Interlaboratory Study of NISTmAb Glycosylation

What the Graphics Display

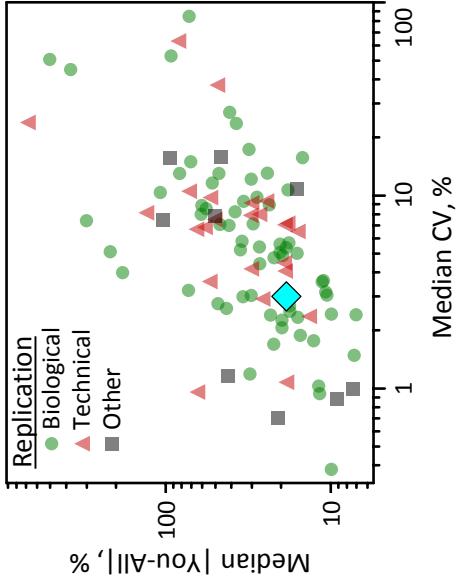
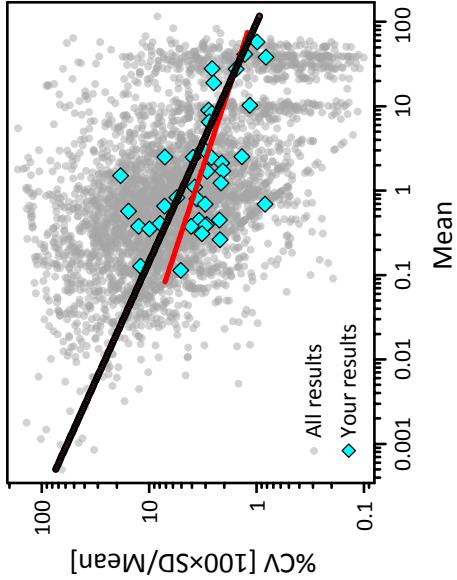
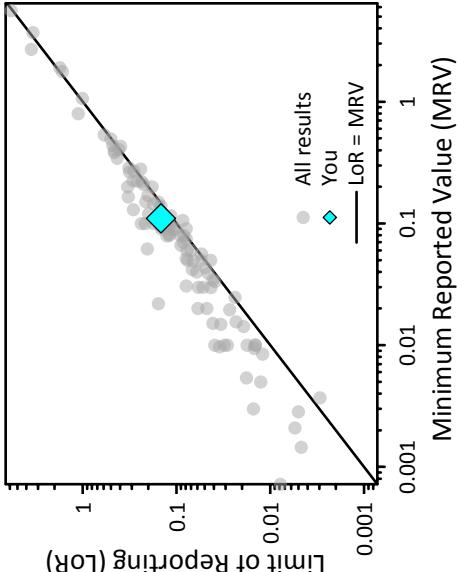
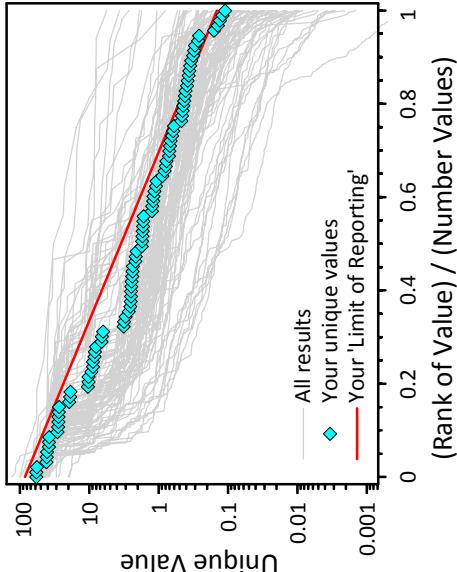
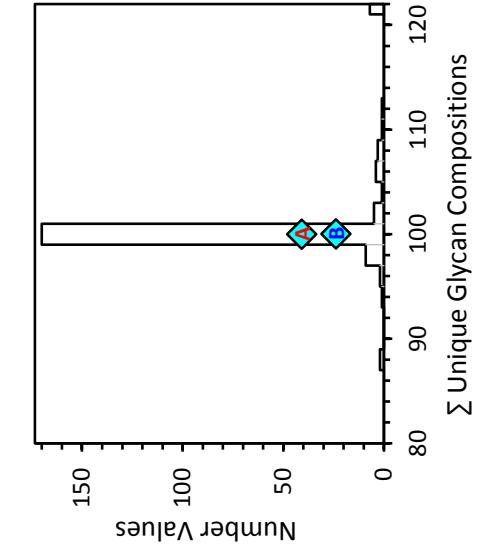
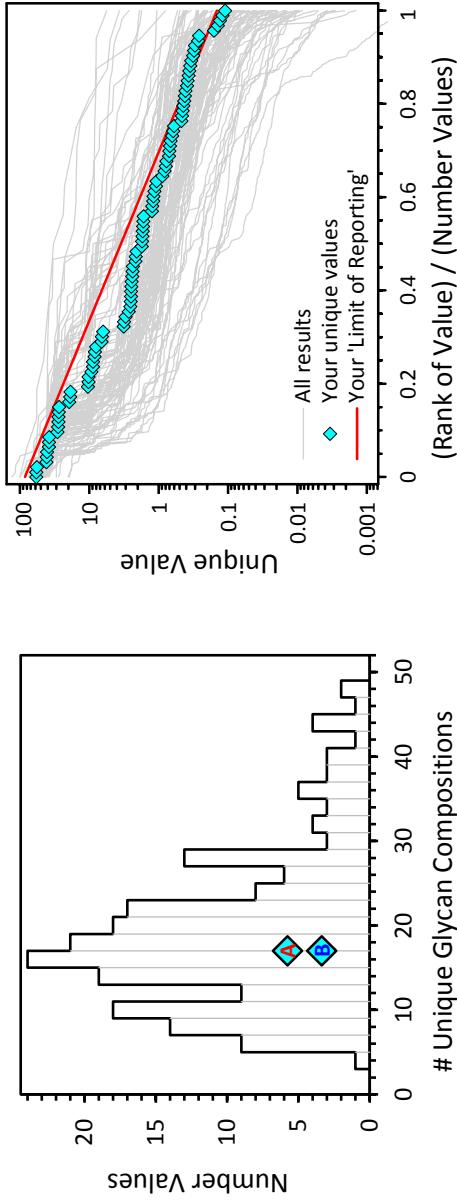


Boxplot summary of results for the 57 unique glycan compositions that were reported at least six times for one of the samples. Each box represents the distribution of the central 50 % of the mean of the reported replicate values for one glycan for sample A, B, or the A/B ratio. The horizontal middle line in each box represents the consensus median. The width of each box is proportional to the square root of the number of values defining the distribution. The dashed red line in the display of the A/B ratios denotes the expected ratio, 1.0, when a glycan result is the same in sample A as it is in B. Glycans are sorted in order of increasing A/B ratio. The light gray vertical lines are provided to facilitate associating a particular box with its glycan.

Targetplot summary of A/B ratios relative to the consensus medians. Each dot marks the summary score for one set of results. The vertical axis displays the average bias estimated as the mean of the "Z-score" values of the A/B ratios for the unique glycan compositions that they reported: $Z\text{-score} = (\text{Ratio} - \text{Median})/\text{MAD}_E$, where MAD_E is a robust estimate of standard deviation. The horizontal axis displays the variability of individual bias estimates, estimated as the standard deviation of the Z-scores. The semicircles mark one, two, and three "comparability" distances from the ideal $\{\text{Mean}, \text{SD}\}$ value of {0,0}: $\text{Distance} = \sqrt{(\text{Z-score Mean})^2 + (\text{Z-score SD})^2}$.

Interlaboratory Study of NISTmAb Glycosylation*

Report for "Example"



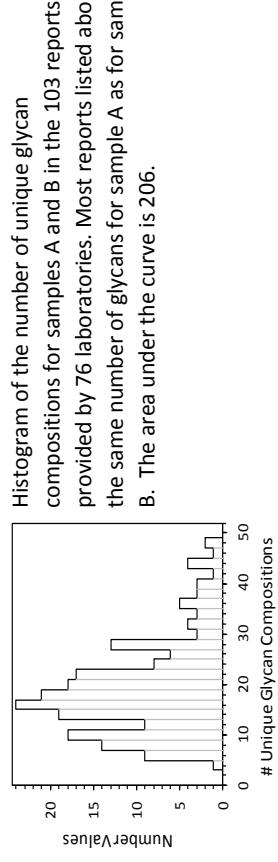
Sample A: modified NISTmAb
Sample B: NISTmAb

$\frac{\%CV}{\text{You}} = a + \text{Mean}^b$
 $a = 1.3, b = -0.26$
Consensus
 $a = 5.0, b = -0.35$

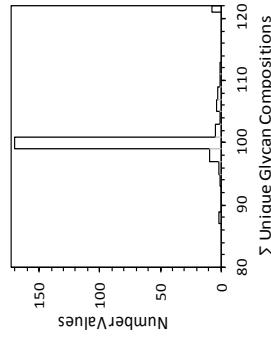
* Data as reported in 103 reports from 76 laboratories

Interlaboratory Study of NISTmAb Glycosylation

What the Graphics Display

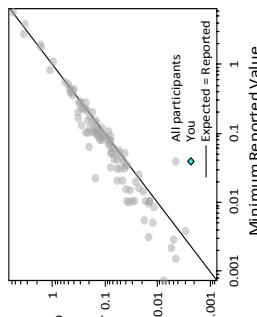


Histogram of the sum of the unique glycan composition values for samples A and B. In most, but not all, of the 103 reports the results were normalized so that the sum of the values = 100 for each sample. The area under the curve is 206.

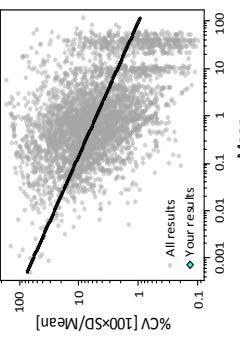


Traces of the unique non-zero values reported in each set of results, where the values are ordered by decreasing value. If the true amounts of the minor glycans are randomly distributed and all results reflect the same level of analytical effort, a best-fit line to the right-tail of the trace estimates each the "Limit of Reporting" (LoR) for that set. LoR values may be more representative of the analytical sensitivity of a measurement system than is the minimum reported value (MRV).

Most of the LoRs agree well for MRVs above about 0.05 %. Below this value, many of the sets of results contain a few values many-fold smaller than their LoR. This may reflect special interest in selected glycan components rather than reporting issues with the less-abundant glycans.



Scatterplot of the relationship between measurement repeatability, estimated as the %CV, and glycan amount, estimated as the mean of the replicates. The black line represents a simple consensus power-law: $\%CV = 5.0 * \text{Mean}^{-0.35}$ (or $SD = 0.050 * \text{Mean}^{0.65}$). Note that %CV is not constant for all glycan amounts but rather generally increases with decreasing amount. Google "Horwitz Function" for information on a similar phenomenon.



Scatterplot of the closeness to consensus of the reported values as a function of measurement repeatability estimated as %CV. "Closeness" is estimated as the relative absolute difference between a given result mean and the median of the means provided in all 103 reports: $100 * |\text{Mean}-\text{Consensus Median}| / \text{Mean}$. The symbols are coded by the user-stated nature of the reported replicates. Because of the great variability in the results for the various glycans, the summary statistics displayed in the scattergram are the respective medians taken over all (replicate) results for unique glycan compositions.

Measurement repeatability is estimated as the coefficient of variation expressed as a percentage (aka the relative standard deviation), $\%CV = 100 * SD / \text{Mean}$, of the replicate values in each report. %CVs can not be calculated for non-replicated measurements and so are not included in these plots.

Interlaboratory Study of NISTmAb Glycosylation

Measurement Summary for Results Reported by "Example"

Index	Composition	Common Name	Measurand	Sample A, % of Total Glycan						Sample B, % of Total Glycan																
				#	Mean	SD	%CV	#	25%	Median	75%	#	Mean	SD	%CV	#	25%	Median	75%	% Diff						
1 [h4n4f1]		G1F		3	28.02	0.73	2.6	103	27.72	31.61	33.31	12.8	3	38.07	0.31	0.8	103	36.36	38.37	39.84	0.8					
1.1 [h4n4f1] Gly		G1F	CFGTD2	3	28.02	0.73	2.6	103	27.72	31.61	33.31	12.8	3	38.07	0.31	0.8	103	36.36	38.37	39.84	0.8					
1.1.1 [h4n4f1] Glyso		G1F[1,6]	CFGTD2	3	19.02	0.48	2.5	58	19.04	21.21	22.06	11.5	3	27.77	0.43	1.5	58	27.60	28.12	29.12	1.2					
1.1.2 [h4n4f1] Glyso		G1F[1,3]	CFGTD2	3	9.00	0.25	2.8	59	9.38	10.38	10.72	15.3	3	10.29	0.12	1.2	59	9.82	10.18	10.75	1.1					
2 [h3n4f1]				3	57.81	0.57	1.0	103	47.06	51.47	55.04	11.0	3	41.08	0.53	1.3	102	35.73	39.10	40.76	4.8					
2.1 [h3n4f1] Gly		G0F	CFD2	3	57.81	0.57	1.0	103	47.06	51.47	55.04	11.0	3	41.08	0.53	1.3	102	35.73	39.09	40.71	4.8					
3 [h5n4f1]				3	2.53	3.5E-2	1.4	99	3.19	3.97	4.80	56.9	3	8.24	0.22	2.6	102	7.47	8.52	9.48	3.5					
3.1 [h5n4f1] Gly		G2F	CFGTD2	1	0.13			46	1.00	1.42	2.77	992.1	3	6.53	0.18	2.8	47	5.88	7.15	7.61	9.5					
3.2 [h5n4f1] Gly		G1F+1aGal	CFG LTD2	3	2.49	9.7E-2	3.9	35	1.64	2.53	3.06	1.8	3	1.71	3.5E-2	2.1	32	1.27	1.60	2.20	6.0					
3.3 [h5n4f1] Gly		G1F+1aGal	CFD	3	3.12	9.7E-2	3.1	88	3.03	3.65	4.26	17.2	3	2.13	4.5E-2	2.1	89	1.87	2.13	2.93	0.0					
4 [h3n3f1]				4	[h3n3f1]			3.12	9.7E-2	3.1	88	3.03	3.65	4.26	17.2	3	2.13	4.5E-2	2.1	89	1.87	2.13	2.93	0.0		
4.1 [h3n3f1] Gly		G0F-N	MD	3	0.57	8.9E-2	15.6	77	0.56	0.78	1.12	37.1	3	0.69	2.1E-2	3.0	79	0.53	0.73	1.01	5.6					
5 [h5n2]				5	[h5n2]			15.6	77	0.56	0.78	1.12	37.1	3	0.69	2.1E-2	3.0	79	0.53	0.73	1.01	5.6				
5.1 [h5n2] Gly		Man5	MD	3	0.57	8.9E-2	15.6	77	0.56	0.78	1.12	37.1	3	0.69	2.1E-2	3.0	79	0.53	0.73	1.01	5.6					
6 [h7n4f1]				6	[h7n4f1]			0.69	5.8E-3	0.8	73	0.70	0.90	1.09	29.3	3	0.66	4.7E-2	7.2	71	0.68	0.90	1.22	37.1		
6.1 [h7n4f1] Gly		G2F+2aGal	CFG LTD2	3	0.80	2.9E-3	0.8	71	0.69	0.87	1.05	26.1	3	0.66	4.7E-2	7.2	70	0.67	0.89	1.06	36.0					
7 [h4n5f1]				7	[h4n5f1]			3.6	70	0.46	0.70	0.89	13.1	3	0.83	4.6E-2	5.5	67	0.49	0.68	0.88	18.0				
8 [h4n5f1]				8	[h4n5f1]			3.6	9	0.51	0.55	0.81	32.0	3	0.83	4.6E-2	5.5	9	0.46	0.51	0.83	39.0				
9 [h5n3f1]				9	[h5n3f1]			2.9	69	0.78	0.88	1.17	121.8	3	0.45	1.0E-2	2.2	68	0.81	0.89	1.22	98.1				
10 [h5n3f1]				10	[h5n3f1]			2.9	62	0.77	0.87	1.15	120.2	3	0.45	1.0E-2	2.2	61	0.81	0.89	1.21	97.6				
11 [h3n3]				11	[h3n3]			CD	3	0.38	4.7E-2	12.5	69	0.29	0.46	0.72	22.3	3	0.38	1.5E-2	4.1	63	0.32	0.43	0.76	16.7
11.1 [h3n3] Gly		G0-N	CD	3	0.38	4.7E-2	12.5	7	0.35	0.38	0.54	0.9	3	0.38	1.5E-2	4.1	6	0.34	0.39	0.41	3.8					
11.1.1 [h3n3] Glyso		G0-N(1,6)	CD	3	1.10	4.2E-2	3.8	60	0.70	0.95	1.30	14.1	3	1.23	2.6E-2	2.2	60	0.79	1.01	1.33	18.0					
12 [h4n3f1g1]				12	[h4n3f1g1]			3.8	60	0.70	0.95	1.30	14.1	3	1.23	2.6E-2	2.2	60	0.79	1.01	1.33	18.0				
12.1 [h4n3f1g1] Gly		G1FS-N (NeuGc)	CFGZ	3	1.10	4.2E-2	3.8	60	0.70	0.95	1.30	14.1	3	1.23	2.6E-2	2.2	60	0.79	1.01	1.33	18.0					
13 [h3n4]				13	[h3n4]			0.0	63	0.16	0.28	0.84	76.1	3	0.13	1.5E-2	12.1	58	0.13	0.19	0.74	52.2				
13.1 [h3n4]		G0	CD2	3	0.16	0.0		62	0.16	0.25	0.81	57.4	3	0.13	1.5E-2	12.1	57	0.13	0.19	0.74	47.4					
14 [h6n4f1g1]		G2F+1aGal (NeuGc)	CFG LTD2Z	3	0.38	1.2E-2	3.0	50	0.31	0.44	0.62	14.2	3	0.35	3.5E-2	9.9	49	0.37	0.46	0.72	29.8					
15 [h6n4f1g1] Gly		G2F+1aGal (NeuGc)	CFG LTD2Z	3	0.26	5.8E-3	2.2	50	0.15	0.27	0.47	1.8	3	0.41	3.2E-2	7.9	50	0.29	0.45	0.72	11.6					
15.1 [h6n4f1g1] Gly		G2F+1aGal (NeuGc)	CFG LTD2Z	3	0.26	5.8E-3	2.2	50	0.15	0.27	0.47	1.8	3	0.41	3.2E-2	7.9	50	0.29	0.45	0.72	11.6					
16 [h5n4f1g1]		G2F+1aGal (NeuGc)	CFG LTD2Z	3	1.70	4.9E-2	2.9	55	0.64	0.78	1.07	54.4	3	2.47	6.5E-2	2.6	46	0.30	0.49	1.63	80.3					
16.1 [h5n4f1g1] Gly		G2F+1aGal (NeuGc)	CFG LTD2Z	3	1.70	4.9E-2	2.9	4	0.83	1.11	1.30	34.7	3	2.47	6.5E-2	2.6	4	1.96	2.44	2.46	1.3					

Index	Composition	Common Name	Class	Sample A, % of Total Glycan						Sample B, % of Total Glycan								
				You			All Results			You			All					
				#	Mean	SD	%CV	#	25%	Median	75%	#	Mean	SD	%CV	#		
19 [h3n2f1]	5.8E-3	5.1	43	0.11	0.10	0.14	0.45	23.4	3	0.13	0.0	45	0.10	0.14	0.33	10.3		
19.1 [h3n2f1] Gly	Man3F	MFD	43	0.11	5.8E-3	5.1	0.10	0.14	0.45	23.4	3	0.13	0.0	45	0.10	0.14	0.33	
21 [h5n4]	1.5E-2	3.4	36	0.45	0.44	0.70	0.97	57.2	3	0.31	1.0E-2	3.2	36	0.31	0.54	1.08	74.7	
21.1 [h5n4] Gly	G2	CGTD2	36	0.45	1.5E-2	3.4	0.38	0.63	0.94	41.2	3	0.31	1.0E-2	3.2	36	0.31	0.53	1.01
199 [Unknowns]	0.28	18.4	26	1.51	0.99	1.68	5.24	11.1	3	2.50	0.18	7.1	25	0.77	2.60	7.05	4.3	

Interlaboratory Study of NISTmAb Glycosylation

What the Table Lists

Headings and Subheadings		Definition	
Measurand		The glycan (or identified combination of glycans) for which measurement results were reported.	
Index		Decimal index of identified glycan compositions, assigned in decreasing order of number of reports.	
Composition		Integer values denote unique compositions, tenths glycoforms, and hundredths isomers.	
Composition		Composition of the glycan in the De Leo-Z-Stein notation (see Table of Identified Glycans for details)	
The result listed for a given composition is the sum of the reported results for all glycoforms of that composition, where the glycoforms are indicated by "[composition] Gly".		The result listed for a given glycoform is the sum of the reported results for all isomers of that glycoform, where the isomers are indicated by "[composition] GlyIso".	
Note: "[Unknowns]" = sum of the abundances of unidentified glycans.		Note: "[Unknowns]" = sum of the abundances of unidentified glycans.	
Common Name		When available, a common name of glycoforms and isomers. See Table of Identified Glycans for Oxford names.	
Class		Concatenation of single-character glycan attribute codes	
Type		C: Complex, H: Hybrid, M: High mannose	
Feature		B: Bisected, D: Desialylated, F: Fucosylated, G: Galactosylated, L: alpha-Galactosylated,	
Antenna		T: Terminal-galactosylated, Y: NeuAc-sialylated, Z: NeuGc-sialylated	
Applies to complex glycans – 2: Biantennary, 3: Triantennary, 4: Tetra-antennary			
Sample A, % of Total Glycan You		Summary statistics for results reported for sample A, a modified version of the NISTmAb material	
#:		#: The number of greater-than-zero replicate values you reported for this measurand.	
Mean:		Mean: The mean of the replicate values you reported for this measurand.	
SD:		SD: The standard deviation of the replicate values you reported for this measurand.	
%CV:		%CV: The relative standard deviation expressed in percent, $100 * SD / Mean$.	
All Results		#: The number of sets of results that reported a greater-than-zero value for this measurand in this sample.	
25%:		25%: The 25th percentile (1st quartile) of the distribution of the reported results.	
Median:		Median: The consensus median (50th percentile or 2nd quartile) of the distribution of the reported results.	
75%:		75%: The 75th percentile (3rd quartile) of the distribution of the reported results.	
% Dif :		% Dif : The relative absolute difference between your Mean and the consensus Median expressed as a percentage:	
		$100 * Mean - Median / Mean$	
Sample B, % of Total Glycan		Summary statistics for results reported for sample B, the NISTmAb material	
Other headings are as described for Sample A, above.			

Interlaboratory Study of NISTmAb Glycosylation

Derived Attribute Quantities for Measurements Reported for "Example"

Symbol	Class	Measurand	Sample A, % of Total Glycan						Sample B, % of Total Glycan									
			You			All Results			You			All Results						
#		Attribute	#	Sum	SD	#	25%	Median	75%	#	Sum	SD	#	25%	Median	75%	% Dif	
C	Type	Complex	15	97.9	0.2	43	89.9	104.6	121.3	6.4	15	96.7	0.2	43	93.3	105.5	123.1	8.3
H	Type	Hybrid	0	0	8	0.7	1.0	2.1	61.5	0	0	8	0.8	1.7	2.3			
M	Type	High mannose	2	0.7	0.1	6	0.9	1.8	3.7	61.5	2	0.8	0.0	6	1.0	1.9	3.4	57.0
B	Feature	Bisecting	0	0	7	1.7	2.4	3.4	0	0	7	1.5	2.2	3.8				
D	Feature	Desialylated	16	97.5	0.2	42	88.8	103.3	120.2	5.6	16	96.3	0.2	42	92.1	104.5	119.6	7.9
F	Feature	Fucosylated	13	97.0	0.3	40	89.0	102.8	118.4	5.7	13	96.0	0.2	40	92.0	104.0	117.7	7.6
G	Feature	Galactosylated	10	34.7	0.2	43	38.8	48.2	60.0	28.0	10	50.5	0.1	43	55.3	63.8	77.5	20.8
L	Feature	alpha-galactosylated	4	4.0	0.0	13	4.4	6.3	8.2	36.8	4	3.2	0.0	13	5.2	6.5	8.8	51.5
T	Feature	Terminal-galactosylated	9	33.6	0.2	32	36.3	44.6	53.9	24.6	9	49.3	0.1	32	53.1	60.3	70.8	18.2
Y	Feature	NeuAc-sialylated	0	0	6	1.5	2.3	4.4	0	0	6	1.1	2.1	5.0				
Z	Feature	NeuGc-sialylated	3	1.8	0.0	12	2.1	3.1	4.9	44.2	3	2.0	0.0	12	2.9	4.1	6.8	51.1
2	Antenna	Biantennary	9	90.4	0.3	30	83.2	96.1	109.2	5.9	9	89.2	0.2	30	86.5	96.9	110.5	7.9
3	Antenna	Triantennary	2	2.5	0.0	4	0.9	1.1	1.5	138.0	2	3.3	0.1	4	1.0	1.1	1.8	188.5
4	Antenna	Tetra-antennary	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	

Interlaboratory Study of NISTmAb Glycosylation

What the Table Lists

Headings and Subheadings		Definition			
Measurand Class Attribute		The quantity of a glycan attribute, estimated as the sum of the median results of all glycoforms having the attribute Nature of the attribute: Type, Feature, or Antenna			
Sample A, % of Total Glycan You		Summary statistics for results reported for sample A, a modified version of the NISTmAb material #: The number of glycoforms you reported that have this attribute in this sample			
Sum:		The mean of the replicate sums of your reported results for all glycoforms having the attribute			
SD:		The standard deviation of the replicate sums			
All Results		#: #: The number of glycoforms that have this Attribute in this sample 25%: 25th percentile (1st quartile) of the distribution of the mean replicate sums for all results			
Median:		The consensus median (50th percentile or 2nd quartile) of the distribution of the mean replicate sums			
75%: % Dif :		The 75th percentile (3rd quartile) of the distribution of the mean replicate sums % Dif : The relative absolute difference between your Mean and the consensus Median expressed as a percentage: $100 * \text{Mean} - \text{Median} / \text{Mean}$			
Sample B, % of Total Glycan		Summary statistics for results reported for sample B, the NISTmAb material Other headings are as described for Sample A, above			