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# Printed Glycan Array: a sensitive technique for the analysis of the repertoire of circulating anti-carbohydrate antibodies in small animals.

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| **Author Comments:** | This work claims to expand the use of PGA technology to investigate repertoire (specificities) and levels of circulating anti-carbohydrates antibodies, both in health and during any pathological condition. This kind of study can be extrapolated to different species (rat, primates, chickens, humans). We anticipate the potentiality that this approach may bring in the early diagnosis and derived treatment in some of the pathological conditions where antibodies seem to play an important role. As JoVE is a leading video journal, we consider it the perfect platform to disseminate the mentioned methodology to the scientific community. |
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**TITLE:**

# Printed Glycan Array: A Sensitive Technique for the Analysis of the Repertoire of Circulating Anti-Carbohydrate Antibodies in Small Animals

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**KEYWORDS:**

Pattern of natural antibodies, circulating anti-glycan antibodies, glycan specificities, glycochips, printed glycan array, PGA, mice

**SHORT ABSTRACT:**

This work shows the potential of printed glycan array (PGA) technology for the analysis of circulating anti-carbohydrate antibodies in small animals.

**LONG ABSTRACT:**

The repertoire of circulating anti-carbohydrate antibodies of a given individual is often

associated with its immunological status. Not only the individual immune condition determines the success in combating internal and external potential threat signals, but also the existence of a particular pattern of circulating anti-glycan antibodies (and their serological level variation) could be a significant marker of the onset and progression of certain pathological conditions. Here, we describe a PGA-based methodology that offers the opportunity to measure hundreds of glycan targets with very high sensitivity; using a minimal amount of sample, which is a common restriction present when small animals (rats, mice, hamster, *etc.*) are used as models to address aspects of human diseases. As a representative example of this approach, we show the results obtained from the analysis of the repertoire of natural anti-glycan antibodies in BALB/c mice. We demonstrate that each BALB/c mouse involved in the study, despite being genetically identical and maintained under the same conditions, develops a particular pattern of natural anti-carbohydrate antibodies. This work claims to expand the use of PGA technology to investigate repertoire (specificities) and the levels of circulating anti-carbohydrates antibodies, both in health and during any pathological condition.

**INTRODUCTION:**

Antibodies play a central role in our defense against invading pathogens by directly neutralizing viruses[1,2] and bacteria[2,3], by activating the complement system[4,5] and the enhancement of phagocytosis[6]. Additionally, they are essential elements in cancer targeting and elimination of malignant cells[7], and homeostasis maintenance[8,9].

Disorders of the immune system can result in autoimmune and inflammatory diseases[10] and cancer[11]. All these pathological conditions ideally demand a prompt diagnosis for an efficient treatment. In the case of autoimmune disorders, the serological presence of autoantibodies in most of the cases is a predictor for diagnostic of autoimmunity[10,12]. These antibodies react with the cell surface and extracellular autoantigens and, they are often present for many years before the presentation of autoimmune disease[10,12]. Immune deficiencies and cancer are also diagnosed with blood tests that either measure the level of immune elements such as antibodies, or their functional activity[11].

The identification of the repertoire of circulating antibodies and their serological levels are paramount to set a prognosis and evaluate the progression of all of the mentioned pathological conditions. We have previously demonstrated the potential of PGA technique for the analysis of circulating antibodies in different animal species[13-16], minimizing the use of large volumes of serological samples, avoiding the problem associated with antibodies cross-reactivity[17] and allowing high-throughput profiling of an extensive repertoire of antibodies[15].

Glycan-based immunoassays are mainly conditioned, among other factors, by the origin and production of carbohydrates, which determine the affinity and binding of ligands[15,18-21]. Glycan-based immunoassays can be developed in suspension (microspheres)[15,21,22] or in flat-activated surfaces[15,21,23,24]. The last include ELISA (the most conventional of these methods) and PGA. There is not much data comparing these methodologies in the same experimental setting[15,25-27]. We have previously compared the efficacy and selectivity of these immunoassays to profile anti-glycan antibodies in individual human plasma samples[15]. For some antibodies such as those

targeting anti-A/B blood group, all the immunoassays could detect them with statistical significance and they positively correlated with each other[15,18,21]. Meanwhile, anti-P1 antibodies were primarily detected by PGA with the highest discriminative power, and there was no correlation in the determinations made by the different glycan-based immunoassays[15,18,21]. These differences between methods were mainly related to the antibody/antigen ratio and glycan orientation[15]. ELISA and suspension arrays are more susceptible to unspecific binding than PGA because there is an excess of antigen over antibodies in these methods[15]. Additionally, the orientation of glycans in the PGA is more restricted than in ELISA and suspension arrays[15]. ELISA is convenient when the study includes a limited panel of glycans. Along with suspension arrays, ELISA offers broader flexibility regarding assay reconfiguration. PGA is exceptionally convenient for discovery approaches[15,18,21,28]. Despite these clear advantages and disadvantages, the three mentioned immunoassays could be used to study different aspects of glycan-antibody interactions. The final goal of the study is the one will guide the selection of the more suitable methodology.

The present work aims to extend the use of PGA technology for the analysis of the repertoire of circulating anti-glycan antibodies in small animals. As a representative result, we present here a detailed protocol to assess the repertoire of natural anti-carbohydrate antibodies in adult BALB/c mice by PGA.

**PROTOCOL:**

## 1. Glycochips Production

### 1.1. Microarray preparation

1.1.1. Print the glycans (50 mM) and polysaccharides (10 µg/mL) in 300 mM phosphate buffered saline (PBS, pH 8.5) at 6 replicates onto N-hydroxysuccinimide-derivatized glass slides, using non-contact robotic arrayer (drop volume ~ 900 pL). Each slide contains 4 different blocks of sub-arrays (**Figure 1A**, in colors) repeated 6 times. Every single sub-array is formed by 112 different glycan spots, including controls (8 rows × 14 columns) (**Figure 1B**).

Note: Glycan-related information is provided in the **Supplementary Table 1**. The glycan library used for printing microchips is the result of a long-term synthetic effort of the IBCh team; examples of synthesis are described in related publications[29-38]. The glycan library included blood group antigens and some of the most frequently occurring terminal oligosaccharides, as well as core motifs of mammalian N- and O-linked glycoproteins and glycolipids, tumor-associated carbohydrate antigens, and polysaccharides from pathogenic bacteria.

1.1.2. Incubate the slides in moisture box (relative humidity ~70%) at room temperature (25 °C) for 1 h.

1.1.3. Blocking microarrays: incubate the slides for 1.5 h with blocking buffer at room temperature (100 mM boric acid, 25 mM ethanolamine, 0.2% (v/v) Tween-20 in ultrapure water).

1.1.4. Wash the glycochip with ultrapure water and dry it by air.

1.2. Glycochip quality control

1.2.1. Analyze two microarrays from each batch using 1mg/mL solution of complex immunoglobulin preparation (CIP, containing IgG, IgM and IgA), 10 µg/mL solution of biotinylated goat anti-human immunoglobulins as a secondary antibody (IgM + IgG + IgA), followed by 1 µg/mL solution of the corresponding fluorescent streptavidin conjugate (via protocol described below, see step 2).

1.2.2. Scan and analyze the glycochips (see step 3, analysis of glycan array).

1.2.3. Use microarray batches with intra- and inter-chip correlation higher than 0.9.

## 2. Glycan array technique

2.1. Prepare the following aqueous solutions (in ultrapure water) and store them at room temperature (25 °C):

- Buffer-1: 1% (w/v) bovine serum albumin (BSA) in PBS, 1% (v/v) Tween-20 and 0.01% (w/v) $NaN_3$
- Buffer-2: 1% (w/v) BSA in PBS, 0.1% (v/v) Tween-20 and 0.01% (w/v) $NaN_3$.
- Buffer-3: 0.1% (v/v) Tween-20 in PBS.
- Buffer-4: 0.001% (v/v) Tween-20 in PBS.

2.2. Glycochip and sample preparation

2.2.1. Put the storage box with the slides on the table until they reach room temperature (25 °C).

Note: Use powder-free latex gloves. The glycochip must be manipulated by the bottom part of the glass slide, where the barcode is located. The barcode will help you to identify the right side, avoiding the contact with the surface where the glycans are printed.

2.2.2. Open the box, take the glycochip and place it in the incubation chamber (25 °C), already conditioned with wet filter paper to keep humidity constant inside the chamber.

2.2.3. Meanwhile, dilute the mice serum with Buffer-1 (1:20) in 1.5 mL tubes. Homogenize the serum solution (5 s) with a vortex mixer.

Note: The volume needed to totally cover a single glycochip surface is approximately 1 mL.

2.2.4. After the homogenization, incubate the diluted serum at 37 °C for 10 min in a water bath to avoid immunoglobulin aggregation. Centrifuge the tubes for 3 min at 10,000 x g and 25 °C, collect the supernatant and discard any precipitated material.

2.2.5. Place the glycochip carefully in the incubation chamber. Incubate it for 15 min at 25 °C with 1 mL of Buffer-3 to eliminate any residual material on the surface of the glycochip.

2.2.6. Hold the glycochip in a vertical position and rewash it with some drops of Buffer-3 using a plastic Pasteur pipette. Carefully remove the buffer from the glycochip surface using filter paper.

2.3. Reaction: antibodies binding

2.3.1. Place the glycochip in the incubation chamber. Spread the diluted serum sample over the glycochip surface using a micropipette. Incubate with orbital agitation (30 rpm) at 37 °C for 1.5 h. Ensure that all dry area of the glycochip surface is covered by the diluted serum sample using the tip of the pipette.

2.3.2. Remove any excess sample and immerse the glycochip for 5 min in Buffer-3 at 25 °C. Then, pass the glycochip to a container with Buffer-4 (5 min) and finally wash (5 min) the glycochip with ultrapure water. Centrifuge the glycochip for 1 min at 175 x g and 25 °C to remove the liquid.

2.4. Detection: secondary antibody

2.4.1. Place the glycochip in the incubation chamber. Spread over the glycochip surface a solution (5 µg/mL) of goat anti-mouse (IgG + IgM) conjugated to biotin in Buffer-2. Incubate with orbital agitation (30 rpm) at 37 °C for 1 h.

2.4.2. Remove the unbound fraction and repeat the washing steps.

2.4.3. After the centrifugation, incubate the glycochip in darkness at 25 °C for 45 min (30 rpm) with 2 µg/mL of the corresponding fluorochrome-labeled streptavidin solution (in Buffer-2).

2.4.4. In darkness, remove the unbound fraction and repeat the washing steps.

2.4.5. Dry the glycochip by air.

Note: Glycochip should be scanned as soon as possible. But if it's impossible to do scan immediately after staining, glycochips can be stored in a cool and dry place in darkness.

## 3. Analysis of Glycan Array

3.1. Scan the array

3.1.1. Leave the glycochip on the table until it reaches room temperature in dark. At the same time, turn on the slide scanner and the laser (excitation wavelength of 633 nm).

3.1.2. Holding the microarray, slide the glycochip into the slot until it touches the back.

3.1.3. Scan the glycochip (**run easy scan)**, and save the scan as a ".TIFF" file.

3.2. Array quantification

3.2.1. Quantify the array using a ScanArray Analysis System. Open previously scanned images, by clicking **File** in the **Configure & File group** on the Main Window (**Figure 1B-D**)

3.2.2. Load the corresponding array file template in GAL format (disposition of printed glycans on the glass slide) (**Figure 1C**).

3.2.3. Adjust GAL template by carefully aligning the array (grids) with the spots in the image and initiate quantification (**Figure 1D**).

3.2.4. Select the quantification parameters:

- Quantification type: Run Easy Quant.
- Quantification method: Fixed Circle
- Auto find Spots: unclick all options
- Normalization Method: LOWESS (Locally Weighted Scatter Plot Smoothing).

3.2.5. Save the quantified data as ".CSV" file (**Figure 1D**). Transfer this data into a common spreadsheet file using Microsoft Excel or another appropriate application.

3.2.6. Use the interquartile range (IQR) as the main statistical method: calculation of the median (Quartile 2) of all signals for each ligand and the interquartile deviation (75th and 25th percentiles, or upper and lower quartiles Q3 and Q1, respectively).

3.2.7. Perform interactive exploration of data by using a Hierarchical Clustering Explorer application.

3.2.8. Use clustering parameters: Average Linkage (UPGMA) and Euclidean distance as similarity distance measure. Perform hierarchical clustering by rows without normalization.

**REPRESENTATIVE RESULTS:**

Here, we present a summary of representative results obtained from the quantification of the repertoire of natural anti-glycan antibodies in a population of 20 BALB/c mice. The glycochips used in this study contained 419 different glycan structures. Most glycans were synthesized as –

$CH_2CH_2CH_2NH_2$ spacer-armed *O*-glycosides, in several cases as $–CH_2CH_2NH_2$ or $–NHCOCH_2NH_2$ glycosides. All glycan structures were characterized by high resolution (700- or 800 MHz) NMR spectroscopy, purified and tested by HPLC, indicating their >95% purity. We have simultaneously determined IgM + IgG anti-glycan antibodies due to a restriction in the amount of mouse serum. In the PGA, we considered values above 4,000 RFU as a positive signal of antibody binding (this value is ~10% of the top glycans RFU). The results presented in this work follow most of the guidelines for reporting glycan microarray-based data[39]. Only 17% of carbohydrate structures demonstrated ≥4,000 RFU in the PGA (**Figure 2**, in red). Most of the glycan structures exposed in the glycochips were not recognized by the repertoire of circulating anti-glycan antibodies of BALB/c mice (**Figure 2**, in blue and white)[28]. The conserved pattern of natural anti-carbohydrate antibodies of BALB/c included 12 different glycan specificities, with very high median signal intensities of antibodies binding (≥10,000 RFU **Table 1**)[28].

**Figure 1. Schematic representation (not at scale) of the glycan array configuration, printing, and analysis.** (A) Printed microchips are developed with a library of 419 different glycan structures, followed by the detection with an appropriate fluorescently labeled secondary antibody. Each slide contains 4 different blocks of sub-arrays (in colors), repeated 6 times. Every single sub-array is formed by 112 different glycan spots (8 rows × 14 columns), including controls. (B) A representative example of the images obtained from microchip scanning using a fluorescence scanner (third part of the image). (C) The process of aligning the "grid" to spots in every single sub-array (template adjustment during quantification). (D) The fluorescence is detected for each spot and results are transferred into a common spreadsheet file.

**Figure 2. Repertoire of natural circulating anti-carbohydrate antibodies of BALB/c mice**. Mouse serum (1:20) was incubated with the glycochips and scanned using a ScanArray reader. Data were analyzed with a microarray analysis system and results were expressed in relative fluorescence units (RFU) as the median ± median absolute deviation (MAD). Blue and white colors represent binding signals lower than 4,000 RFU (background); red color represents signals ≥4,000 RFU (positive binding). F, female; M, male (n = 20). This figure has been reproduced from Bello-Gil, D. *et al*.[28].

**Table 1. Top rank glycan structures recognized by natural antibodies of BALB/c mice**. Glycans with binding signals above 4,000 RFU in at least 80% of examined mice (n = 20). [b]sp means aminoethyl, aminopropyl or glycyl spacer. [c]furanose; all other monosaccharides are in a pyranose form; Fuc residue has L-configuration, all other monosaccharides - D-configuration. This table has been modified from Bello-Gil, D. *et al*.[28].

**Supplementary Table 1. List of glycans, their binding to natural circulating antibodies (IgM + IgG) of BALB/c mice (n=20), expressed in relative fluorescence units (RFU) as median ± MAD, and the number of animals exceeding *cut off* (≥ 4000 RFU).** This table has been reproduced from Bello-Gil, D. *et al*.[28].

**DISCUSSION:**

Glycan microarrays have become indispensable tools for studying protein-glycan interactions[40].

The present work describes a protocol based on PGA technology to study the repertoire of circulating of anti-carbohydrate antibodies in BALB/c mice. Since PGA offers the possibility to screen large numbers of biologically unknown glycans, it is an exceptionally convenient discovery tool[13,15,28]. The proposed method offers the possibility to measure, in the same experimental setting, hundreds of glycan structures using a reduced amount of serological sample (50 µL). This is especially critical in the case of small animals (little circulating blood volume), or when it's necessary to extract blood several times from the same experimental animal.

We demonstrated, as representative results, that genetically identical mice should not be considered as immunological equivalents; because they develop different patterns of natural anti-carbohydrate antibodies (only 12 glycan specificities were conserved). Serological levels for the rest of the repertoire of natural anti-carbohydrate antibodies varied considerably among the examined animals. Analysis of the gut microbiota of inbred animals[41] could explain this heterogeneity[42-46]. If the production of natural anti-glycan antibodies is mediated by the antigenic stimulation of microbiota, and this is different among inbred mice[41], fine specificity of these antibodies will not be identical.

The main drawback for PGA development is the access to well-defined glycan structures[40,47]. Glycans produced in biological systems are heterogeneous[40,47,48], and their biosynthesis relies on the differential expression of carbohydrate enzymes, resulting in heterogeneous mixtures of glycoforms, each with a distinct physiological activity[47]. The complex composition and configuration of the glycans present in the biological systems make their productions challenging[40,47,48]. Along with chemo-enzymatic synthesis, glycans isolated from natural sources will continue to be the major source of glycans for arrays development[40]. Low synthetic yields and the complex purification process from glycoproteins and glycosphingolipids make the efficient production of glycans at large scale difficult[40,47,48]. Hence, the availability and the prices of glycans continue being a very limiting condition to expand the use of PGA as a discovery tool.

Additionally, within the protocol, critical steps mostly relating to the correct distribution of solutions (serum, secondary antibodies) over the glycochip surface must be executed with caution. The methodology requires, at least, 1 mL of these solutions, to homogenously soak all dry areas of the glycochip surface. This is crucial to obtain minimal differences between glycan replicates and also to avoid excessive background during quantification.

Despite the mentioned limitations, PGA is a very sensitive tool for approaches related to study protein-glycan interactions[40], or to study the repertoire of anti-glycan antibodies in a particular experimental setting or condition[13,15,28]. This study can be extrapolated to different species (including human samples) [13,15,23,28], providing a versatile methodology for identifying the repertoire of circulating anti-carbohydrate antibodies.

We also anticipate the potentiality that this approach may bring in the early diagnosis and derived treatment in some of the pathological conditions where antibodies directed to glycan structures seem to play an important role.

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**DISCLOSURES:**

Nailya Khasbiullina and Alexey Nokel are employees of Semiotik LLC, who is the supplier of the glycochips used in this study.

**REFERENCES**:

1. Karlsson, G.B., Fouchier, R.A., Phogat, S., Burton, D.R., Sodroski, J., Wyatt, R.T. The challenges of eliciting neutralizing antibodies to HIV-1 and to influenza virus. *Nat Rev Microbiol*. **6**(2), 143-55, doi: 10.1038/nrmicro1819 (2008).
2. Lu, L.L., Suscovich, T.J., Fortune, S.M., Alter, G. Beyond binding: antibody effector functions in infectious diseases. *Nat Rev Immunol*. **18**(1), 46-61, doi: 10.1038/nri.2017.106 (2017).
3. Bebbington, C., Yarranton, G. Antibodies for the treatment of bacterial infections: current experience and future prospects. *Curr Opin Biotech*. **19**(6), 613-9, doi: 10.1016/j.copbio.2008.10.002 (2008).
4. Murphy, K., Travers. P., Walport, M. Janeway's Immunobiology. 7th. Garland Science; New York and London. The complement system and innate immunity, 61-80 (2008).
5. Botto, M., Kirschfink, M., Macor, P., Pickering, M.C., Wurzner, R., Tedesco, F. Complement in human diseases: lessons from complement deficiencies. *Mol Immunol*. **46**(14), 2774-83, doi: 10.1016/j.molimm.2009.04.029 (2009).
6. Borrok, M.J., *et al.* Enhancement of antibody-dependent cell-mediated cytotoxicity by endowing IgG with FcαRI (CD89) binding. *MAbs.* **7**(4), 743-51, doi: 10.1080/19420862.2015.1047570 (2015)
7. Weiner, L.M., Murray, J.C., Shuptrine, C.W. Antibody-based immunotherapy of cancer. *Cell*. **148**(6), 1081-4, doi: 10.1016/j.cell.2012.02.034 (2012).
8. Ricklin, D., Hajishengallis, G., Yang, K., Lambris, J.D. Complement: a key system for immune surveillance and homeostasis. *Nat Immunol*. **11**(9), 785-97, doi: 10.1038/ni.1923 (2010).
9. Prechl, J. A generalized quantitative antibody homeostasis model: antigen saturation, natural antibodies and a quantitative antibody network. *Clin Transl Immunology*. **6**(2), e131, doi: 10.1038/cti.2016.90 (2017).
10. Vojdani, A. Antibodies as predictors of complex autoimmune diseases. *Int J Immunopath Ph*. **21**(2), 267-78, doi: 10.1177/039463200802100203 (2008).
11. Liu, W., Peng, B., Lu, Y., Xu, W., Qian, W., Zhang, J.Y. Autoantibodies to tumor-associated antigens as biomarkers in cancer immunodiagnosis. *Autoimmun Rev*. **10**(6), 331-5, doi:

10.1016/j.autrev.2010.12.002 (2011).
12. Suurmond, J., Diamond, B. Autoantibodies in systemic autoimmune diseases: specificity and pathogenicity. *J Clin Invest*. **125**(6), 2194-202, doi: 10.1172/JCI78084 (2015).
13. Bovin, N., *et al.* Repertoire of human natural anti-glycan immunoglobulins. Do we have auto-antibodies? *Biochim Biophys Acta*. **1820**(9), 1373-82, doi: 10.1016/j.bbagen.2012.02.005 (2012).
14. de los Rios, M., Criscitiello, M.F., Smider, V.V. Structural and genetic diversity in antibody repertoires from diverse species. *Curr Opin Struc Biol*. **33**, 27-41, doi: 10.1016/j.sbi.2015.06.002 (2015).
15. Pochechueva, T., *et al.* Comparison of printed glycan array, suspension array and ELISA in the detection of human anti-glycan antibodies. *Glycoconjugate J*. **28**(8-9), 507-17, doi: 10.1007/s10719-011-9349-y (2011).
16. Shilova, N., Navakouski, M., Khasbiullina, N., Blixt, O., Bovin, N. Printed glycan array: antibodies as probed in undiluted serum and effects of dilution. *Glycoconjugate J.* **29**(2-3), 87-91, doi: 10.1007/s10719-011-9368-8 (2012).
17. Manimala, J.C., Roach, T.A., Li, Z., Gildersleeve, J.C. High-throughput carbohydrate microarray profiling of 27 antibodies demonstrates widespread specificity problems. *Glycobiology.* **17**(8), 17C-23C, doi: 10.1093/glycob/cwm047 (2007).
18. Jacob, F., *et al*. Serum anti-glycan antibody detection of non-mucinous ovarian cancers by using a printed glycan array. *Int. J. Cancer*. **130**(1), 138-46, doi: 10.1002/ijc.26002 (2012).
19. Lewallen, D.M., Siler, D., Iyer, S.S. Factors affecting protein-glycan specificity: effect of spacers and incubation time. *ChemBioChem*. **10**(9), 1486-1489, doi: 10.1002/cbic.200900211 (2009).
20. Oyelaran, O., Li, Q., Farnsworth, D., Gildersleeve, J.C. Microarrays with varying carbohydrate density reveal distinct subpopulations of serum antibodies. *J. Proteome Res.* **8**(7), 3529-3538, doi: 10.1021/pr9002245 (2009).
21. Pochechueva, T. Multiplex suspension array for human anti-carbohydrate antibody profiling. *Analyst*. **136**(3), 560-569, doi: 10.1039/c0an00758g (2011).
22. Chinarev, A.A., Galanina, O.E., Bovin, N.V. Biotinylated multivalent glycoconjugates for surface coating. *Methods Mol Biol*. **600**, 67-78, doi: 10.1007/978-1-60761-454-8_5 (2010).
23. Huflejt, M.E. Anti-carbohydrate antibodies of normal sera: findings, surprises and challenges. *Mol Immunol*. **46**(15), 3037-3049, doi: 10.1016/j.molimm.2009.06.010 (2009).
24. Buchs, J.P., Nydegger, U.E. Development of an ABO-ELISA for the quantitation of human blood group anti-A and anti-B IgM and IgG antibodies. *J Immunol Methods*. **118**(1), 37-46 (1989).
25. de Jager, W., Rijkers, G.T. Solid-phase and bead-based cytokine immunoassay: a comparison. *Methods*. **38**(4), 294-303, doi: 10.1016/j.ymeth.2005.11.008 (2006).
26. Galanina, O.E., Mecklenburg, M., Nifantiev, N.E., Pazynina, G.V., Bovin, N.V. GlycoChip: multiarray for the study of carbohydrate binding proteins. *Lab Chip*. **3**(4), 260-265, doi: 10.1039/b305963d (2003).
27. Willats, W.G., Rasmussen, S.E., Kristensen, T., Mikkelsen, J.D., Knox, J.P. Sugar-coated microarrays: a novel slide surface for the high-throughput analysis of glycans. *Proteomics*. **2**(12), 1666-1671, doi: 10.1002/1615-9861(200212)2:12<1666::AID-PROT1666>3.0.CO;2-E (2002).
28. Bello-Gil, D., Khasbiullina, N., Shilova, N., Bovin, N., Mañez, R. Repertoire of BALB/c mice

natural anti-Carbohydrate antibodies: mice vs. humans difference, and otherness of individual animals. *Front Immunol*. **8**, 1449, doi: 10.3389/fimmu.2017.01449 (2017).
29. Pazynina, G., *et al.* Synthetic glyco-O-sulfatome for profiling of human natural antibodies. *Carbohydr Res*. **445**, 23-31, doi: 10.1016/j.carres.2017.03.015 (2017).
30. Ryzhov, I.M., Korchagina, E.Y., Popova, I.S., Tyrtysh, T.V., Paramonov, A.S., Bovin, N.V. Block synthesis of A (type 2) and B (type 2) tetrasaccharides related to the human ABO blood group system. *Carbohydr Res.* **430**, 59-71, doi: 10.1016/j.carres.2016.04.029 (2016).
31. Ryzhov, I.M., *et al*. Function-spacer-lipid constructs of Lewis and chimeric Lewis/ABH glycans. Synthesis and use in serological studies. *Carbohyd Res*. **435**, 83-96, doi: 10.1016/j.carres.2016.09.016 (2016).
32. Pazynina, G.V., Tsygankova, S.V., Sablina, M.A., Paramonov, A.S., Tuzikov, A.B., Bovin, N.V. Stereo- and regio-selective synthesis of spacer armed α2-6 sialooligosaccharides. *Mendeleev Commun*. **26**(5), 380-382, doi: 10.1016/j.mencom.2016.09.004 (2016).
33. Pazynina, G.V., Tsygankova, S.V., Sablina, M.A., Paramonov, A.S., Formanovsky, A.A., Bovin, N.V. Synthesis of blood group pentasaccharides ALe$^{y}$, BLe$^{y}$ and related tri- and tetrasaccharides. *Mendeleev Commun.* **26**(2), 103-105, doi: 10.1016/j.mencom.2016.03.005 (2016).
34. Severov, V.V., Pazynina, G.V., Ovchinnikova, T.V., Bovin, N.V. The synthesis of oligosaccharides containing internal and terminal Galβ1-3GlcNAcβ fragments. *Russian J. Bioorgan. Chem.* **41**(2), 147-60, doi: 10.1134/S1068162015020120 (2015).
35. Pazynina, G.V., Tsygankova, S.V., Bovin, N.V. Synthesis of glycoprotein N-chain core fragment GlcNAcβ1-4(Fucα1-6)GlcNAc. *Mendeleev Commun*. **25**(4), 250-251, doi: 10.1016/j.mencom.2015.07.004 (2015).
36. Solís, D., *et al*. A guide into glycosciences: How chemistry, biochemistry and biology cooperate to crack the sugar code. *Biochim Biophys Acta*. **1850**(1), 186-235, doi: 10.1016/j.bbagen.2014.03.016 (2015).
37. Pazynina, G.V., *et al*. Divergent strategy for the synthesis of α2-3-Linked sialo-oligosaccharide libraries using a Neu5TFA-(α2-3)-Gal building block. *Synlett*. **24**(02), 226-230, doi: 10.1055/s-0032-1317961 (2013).
38. Blixt, O., *et al*. Printed covalent glycan array for ligand profiling of diverse glycan binding proteins*. P Natl Acad Sci USA.* **101**(49), 17033-8, doi: 10.1073/pnas.0407902101 (2004).
39. Liu, Y., *et al*. The minimum information required for a glycomics experiment (MIRAGE) project: improving the standards for reporting glycan microarray-based data. *Glycobiology*. **27**(4), 280-284, doi: 10.1093/glycob/cww118 (2017).
40. Song, X., Heimburg-Molinaro, J., Cummings, R.D., Smith, D.F. Chemistry of natural glycan microarrays. *Curr Opin Chem Biol.* **18**, 70-7, doi: 10.1016/j.cbpa.2014.01.001 (2014).
41. Hoy, Y.E., *et al.* Variation in taxonomic composition of the fecal microbiota in an inbred mouse strain across individuals and time. *PLoS One*. **10**(11), e0142825, doi: 10.1371/journal.pone.0142825 (2015).
42. D'Argenio, V., Salvatore, F. The role of the gut microbiome in the healthy adult status. *Clin Chim Acta*. **451**(Pt A), 97-102, doi: 10.1016/j.cca.2015.01.003 (2015).
43. Khasbiullina, N.R., Bovin, N.V. Hypotheses of the origin of natural antibodies: a glycobiologist's opinion. *Biochemistry (Mosc).* **80**(7), 820-35, doi: 10.1134/ S0006297915070032 (2015).

44. Butler, J.E., Sun, J., Weber, P., Navarro, P., Francis, D. Antibody repertoire development in fetal and newborn piglets, III. Colonization of the gastrointestinal tract selectively diversifies the preimmune repertoire in mucosal lymphoid tissues. *Immunology*. **100**(1), 119-30, doi: 10.1046/j.1365-2567.2000.00013.x (2000).
45. Bos, N.A., *et al.* Serum immunoglobulin levels and naturally occurring antibodies against carbohydrate antigens in germ-free BALB/c mice fed chemically defined ultrafiltered diet. *Eur J Immunol.* **19**(12), 2335-9, doi: 10.1002/eji.1830191223 (1980).
46. van der Heijden, P.J., Bianchi, A.T., Heidt, P.J., Stok, W., Bokhout, B.A. Background (spontaneous) immunoglobulin production in the murine small intestine before and after weaning. *J Reprod Immunol*. **15**(3), 217-27, doi: 10.1016/0165-0378(89)90013-2 (1989).
47. Krasnova, L., Wong, C.H. Understanding the chemistry and biology of glycosylation with glycan synthesis. *Annu Rev Biochem.* **85**, 599-630, doi: 10.1146/annurev-biochem-060614-034420 (2016).
48. Overkleeft, H.S., Seeberger, P.H. Chemoenzymatic synthesis of glycans and glycoconjugates. In: Varki, A., *et al.*, editors. Essentials of Glycobiology [Internet]. 3rd edition. Cold Spring Harbor (NY): Cold Spring Harbor Laboratory Press; 2015-2017, Chapter 54 (2017).

Figure 1

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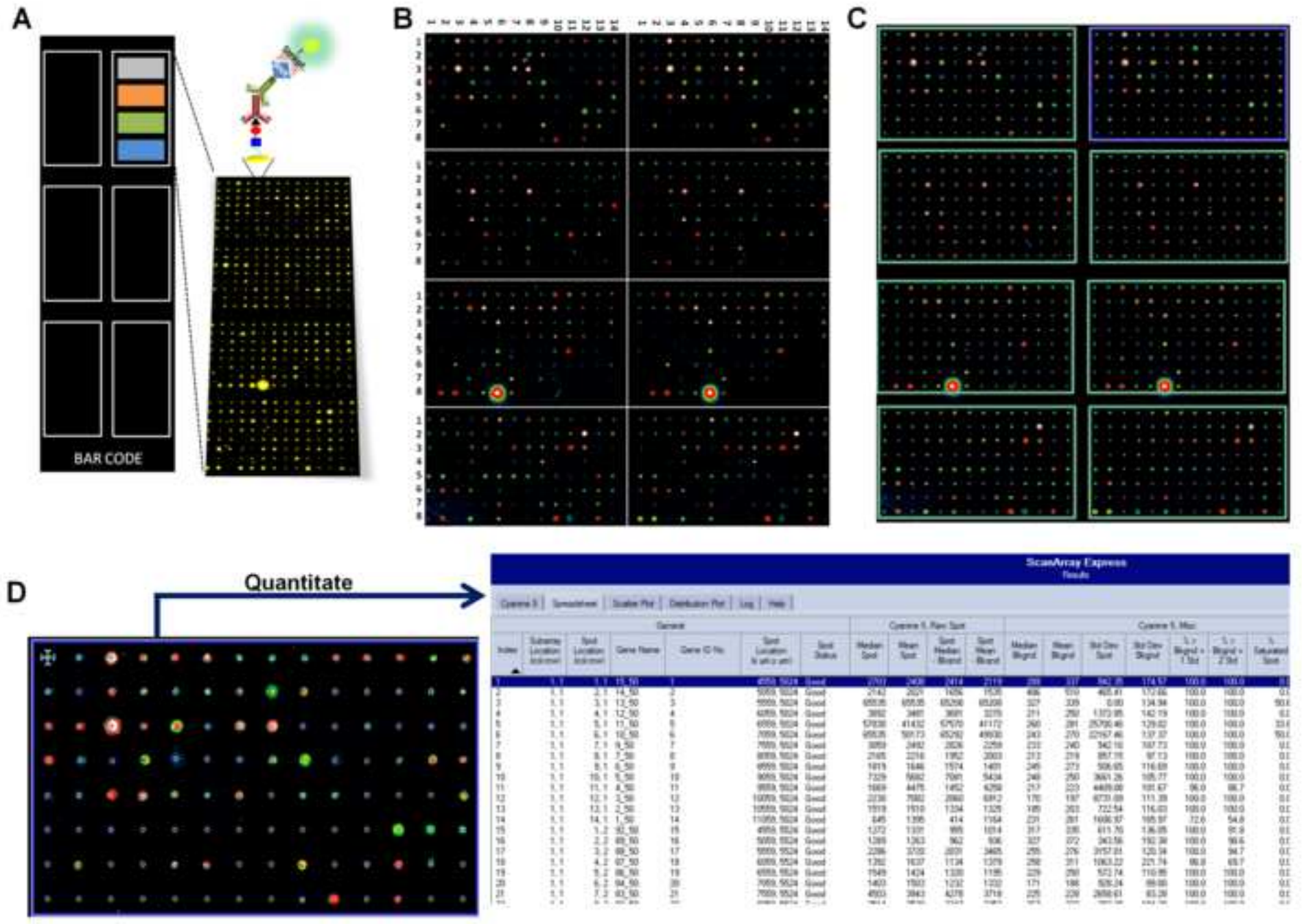

Figure 2

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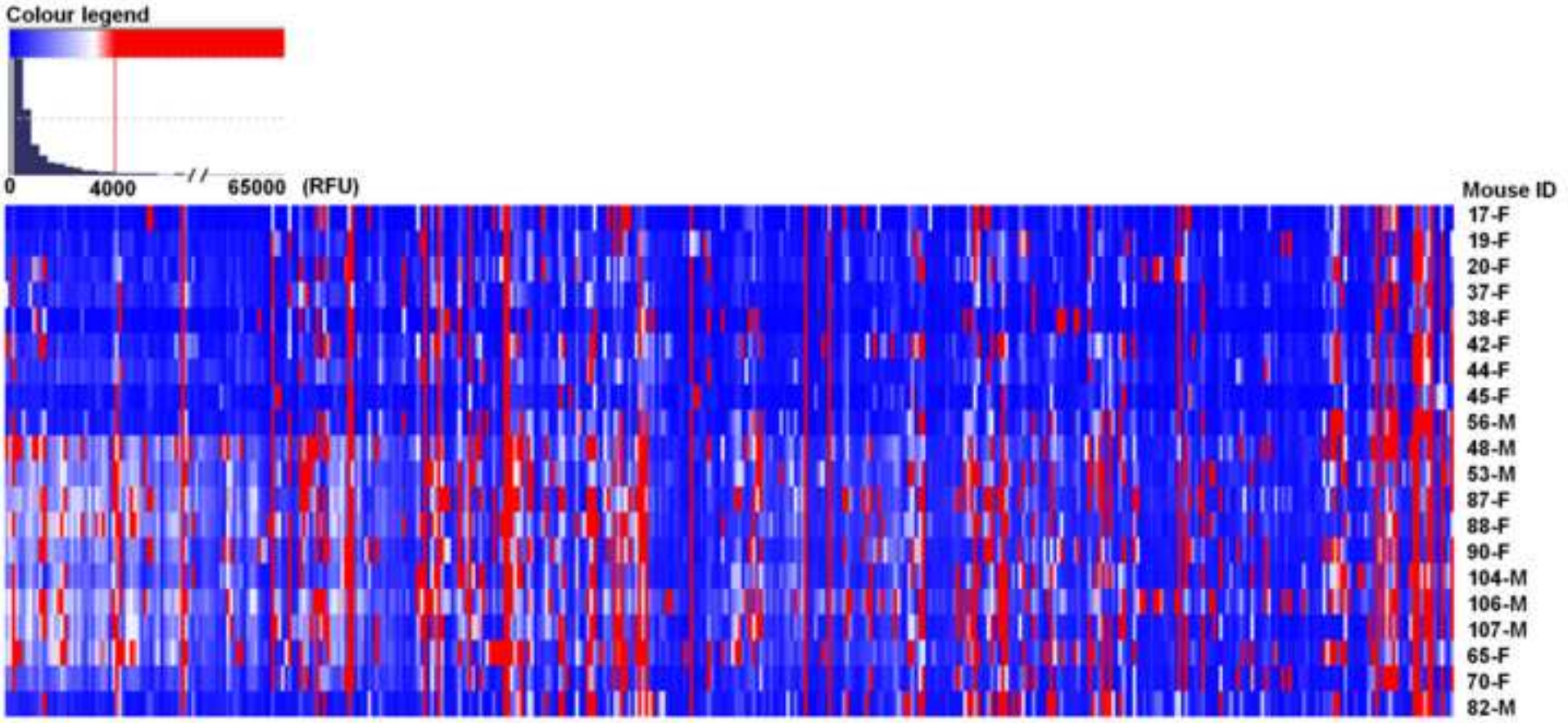

Table 1



| Glycan ID (#) | Structure |
|---|---|
| 60 | 6-O-Su-Galβ-sp[b] |
| 271 | Galβ1-6Galβ1-4Glcβ-sp |
| 802 | Galβ1-3GalNAc(fur[c])β-sp |
| 176 | 3-O-Su-Galβ1-4(6-O-Su)Glcβ-sp |
| 166 | GlcAβ1-6Galβ-sp |
| 150 | 3-O-Su-Galβ1-3GalNAcα-sp |
| 437 | GalNAcα1-3(Fucα1-2)Galβ1-3GalNAcβ-sp |
| 125 | 6-Bn-Galβ1-4GlcNAcβ-sp |
| 154 | 3-O-Su-Galβ1-3GlcNAcβ-sp |
| 177 | 3-O-Su-Galβ1-4(6-O-Su)GlcNAcβ-sp |
| 287 | 3-O-Su-Galβ1-3(Fucα1-4)GlcNAcβ-sp |
| 234 | Galβ1-4(Fucα1-3)GlcNAcβ-sp |

| Common name | Median and MAD as RFU | | Number of mice showing RFU ≥ 4000 (%) |
|---|---|---|---|
| | 61113 | 1156 | 100 |
| | 53622 | 1934 | 100 |
| | 51348 | 2324 | 100 |
| | 43008 | 9342 | 100 |
| | 39105 | 2993 | 85 |
| | 37943 | 3232 | 100 |
| A(type 4) | 33886 | 3193 | 90 |
| | 32674 | 5389 | 95 |
| | 32651 | 3954 | 100 |
| | 32496 | 7215 | 100 |
| SuLe[a] | 20063 | 4962 | 95 |
| Le[x] | 13573 | 2635 | 80 |

Materials Table

Click here to download Materials Table Table of Specific Materials-Equipment_Olivera-Ardid.xlsx

| MATERIAL/ EQUIPMENT |
|---|
| **Antibodies** |
| biotinylated goat anti-human Igs |
| biotinylated goat anti-mouse IgM + IgG |
| **Equipment** |
| Robotic Arrayer sciFLEXARRAYER S5 |
| Stain Tray (slide incubation chamber) |
| Centrifuge |
| Pipettes |
| Slide Scanner |
| Shaking incubator |
| **Biological samples** |
| BALB/c mice sera |
| Complex Immunoglobulin Preparation (CIP) |
| **Chemicals, Reagents and Glycans** |
| Glycan library |
| Bovine serum albumin (BSA) |
| Ethanolamine |
| Tween-20 |
| Phospahte buffered saline (PBS) |
| Sodium azide<br>Streptavidin Alexa Fluor 555 conjugate |
| Streptavidin Cy5 conjugate |
| **Materials** |
| N-hydroxysuccinimide-derivatized glass slides H |
| Whatman filter paper |
| 1.5 mL tubes |
| **Software and algorithms** |
| ScanArray Express Microarray Analysis System |
| Hierarchical Clustering Explorer application |

N/ A: not applicable

| SOURCE |
| --- |
| Thermo Fisher Scientific, Waltham, MA, USA |
| Thermo Fisher Scientific |
| Scienion AG, Berlin, Germany |
| Simport, Beloeil, QC, Canada |
| Eppendorf, Hamburg, Germany |
| Gilson, Middleton, WI, USA |
| PerkinElmer, Waltham, MA, USA |
| Cole-Parmer, Staffordshire, UK |
| This paper |
| Immuno-Gem, Moscow, Russia |
| Institute of Bioorganic Chemistry (IBCh), Moscow, Russia |
| Sigma-Aldrich, St. Louis, MO, |
| Sigma-Aldrich |
| Merck Chemicals & Life Science S.A., Madrid, Spain |
| VWR International Eurolab S.L, Barcelona, Spain |
| Sigma-Aldrich<br>Thermo Fisher Scientific |
| GE Healthcare, Little Chalfont, Buckinghamshire, UK |
| Schott-Nexterion, Jena, Germany |
| Sigma-Aldrich |
| Eppendorf |
| PerkinElmer |
| University of Maryland, MD, USA |

| IDENTIFIER |
| --- |
| Ref. #: 31782 |
| Ref. #: 31807 |
| http://www.scienion.com/products/sciflexarrayer/ |
| Ref. #: M920-2 |
| Ref. #: 5810 R |
| http://www.gilson.com/en/Pipette/ |
| ScanArray $G_X$ Plus |
| Ref. #: SI50 |
| N/ A |
| http://www.biomedservice.ru/price/goods/1/17531 |
| N/ A |
| Ref. #: A9418 |
| Ref. #: 411000 |
| Ref. #: 655204 |
| Ref. #: E404 |
| Ref. #: S2002 |
| Ref. #: S21381 |
| Ref. #: PA45001 |
| Ref. #: 1070936 |
| Ref. #: WHA10347509 |
| Ref. #: 6050120080 |
| http://www.per kinelmer.com/microarray |
| http://www.cs.umd.edu/hcil/hce/ |

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Author(s): Sara Olivera-Ardid, Nailya Khasbiullina, Alexey Nokel, Andrey Formanovsky, Inna Popova, Tatiana Tyrtysh, Roman Kunetskiy, Nadezhda Shilova, Nicolai Bovin, Daniel Bello-Gil, Rafael Mañez

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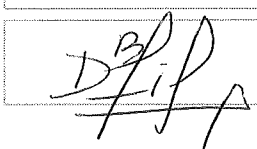
| Signature: |  Date: **30th November, 2017** |

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**Bing Wu, Ph.D.**
**Review Editor**
Journal of Visualized Experiments (JoVE)

Barcelona, January 23th, 2018

**Dear Dr. Wu,**

Please find enclosed the reviewed version of the manuscript entitled "Printed Glycan Array: a sensitive technique for the analysis of the repertoire of circulating anti-carbohydrate antibodies in small animals" by Olivera-Ardid *et al*., which we are submitting to consider for publication in JoVE.

First of all, thank you so much for your time and useful contribution. We appreciated the inputs the editorial team has given, which helped to improve our manuscript significantly.

**Specific comments from the editorial team included:**

1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues.

Many thanks.

2. Please provide a title for Supplementary Table in Figure and Table Legends.

Thanks. (see line 299).

3. Milli-Q, Streptavidin Alexa Fluor 555 conjugate, commercial language.

Thanks. It was removed and substituted by:
- Ultrapure water.
- Fluorescent streptavidin conjugate.

4. Protocol step 2.1.1: Please write this step in imperative tense.

Thanks. It was corrected (line 150).

5. 2.2.2: What's the temperature of the incubation chamber?

25 °C (line 166).

6. 2.2.4: How is homogenization performed?

Homogenize the serum solution (5 s) with a vortex mixer (line 169).

**Daniel Bello-Gil, PhD**
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7. Please revise the Discussion to explicitly cover the following in detail in 3-6 paragraphs with citations:

a) Critical steps within the protocol

Discussion line 335.

b) Any modifications and troubleshooting of the technique

Please see the notes in the protocol.

c) Any limitations of the technique

Discussion line 324.

d) The significance with respect to existing methods

Introduction line 82-102.
Discussion line 305, 341.

e) Any future applications of the technique

Discussion line 347

We look forward to hearing from you regarding our resubmission. We would be glad to respond to any further questions and comments that you may have.

Sincerely,

Daniel.

**Daniel Bello-Gil, PhD**

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# *Supplementary Material*

## **Printed Glycan Array**: a sensitive technique for the analysis of the repertoire of circulating anti-carbohydrate antibodies in small animals.

Sara Olivera-Ardid[1,*], Nailya Khasbiullina[2,3,*], Alexey Nokel[3], Andrey Formanovsky[2], Inna Popova[2], Tatiana Tyrtysh[2], Roman Kunetskiy[2], Nadezhda Shilova[2], Nicolai Bovin[2,4], Daniel Bello-Gil[1], Rafael Mañez[1,5]

*** Correspondence:** dbello@idibell.cat

**Table S1.** List of glycans, their binding to natural circulating antibodies (IgM+IgG) of BALB/c mice (n=20), expressed in relative fluorescence units (RFU) as median ± median absolute deviation (MAD), and the number of animals exceeding *cut off* (≥ 4000 RFU). This table has been reproduced from [28].

| Glycan ID (#) | Structure | Common Name | Median and MAD as RFU | | Animals showing RFU ≥ 4000 |
|---|---|---|---|---|---|
| 001 | Fucα-sp3 | | 1590 | 342 | 1 |
| 002 | Galα-sp3 | | 1522 | 316 | 0 |
| 003 | Galβ-sp3 | | 11858 | 1420 | 12 |
| 004 | GalNAcα1-OSer | $T_n$Ser | 2145 | 395 | 2 |
| 005 | GalNAcα-sp3 | $T_n$ | 1642 | 192 | 2 |
| 006 | GalNAcβ-sp3 | | 1001 | 250 | 0 |
| 007 | Glcα-sp3 | | 1242 | 222 | 0 |
| 009 | Glcβ-sp3 | | 1576 | 325 | 0 |
| 010 | GlcNAcβ-sp3 | | 4124 | 471 | 1 |
| 011 | GlcNAcβ-sp2 | | 4634 | 607 | 2 |
| 012 | GlcNAcβ-sp7 | | 2068 | 523 | 2 |
| 013 | GlcNAcβ-sp8 | | 7267 | 1117 | 9 |
| 014 | GlcN(Gc)β-sp4 | | 914 | 203 | 0 |
| 015 | $HOCH_2(HOCH)_4CH_2NH_2$ | aminoglucitol | 1374 | 316 | 0 |
| 016 | Manα-sp3 | | 1164 | 263 | 0 |
| 017 | Manα-sp4 | | 1567 | 369 | 0 |
| 018 | Manβ-sp4 | | 1842 | 319 | 2 |
| 019 | ManNAcβ-sp4 | | 4752 | 390 | 4 |
| 020 | Rhaα-sp3 | | 970 | 308 | 0 |
| 021 | Galβ-sp4 | | 1338 | 269 | 0 |
| 022 | GlcNAcβ-sp4 | | 932 | 230 | 0 |
| 023 | GalNAcβ-sp4 | | 1246 | 271 | 0 |
| 024 | GlcNAcα-sp3 | | 1463 | 260 | 2 |
| 025 | GalNAcβ-sp10 | | 848 | 209 | 0 |
| 026 | Rhaβ-sp4 | | 1098 | 227 | 0 |

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|---|---|---|---|---|---|
| 027 | 3,6-Me2Glc | DMG | 673 | 123 | 0 |
| 028 | Xylβ-sp4 | | 1411 | 302 | 0 |
| 029 | Fucβ-sp4 | | 1139 | 301 | 0 |
| 030 | Glcβ-sp4 | | 1449 | 429 | 1 |
| 031 | L-Araα-sp4 | | 1099 | 369 | 0 |
| 032 | GalNGcβ-sp3 | | 483 | 119 | 0 |
| 037 | 3-O-Su-Galβ-sp3 | | 2317 | 503 | 3 |
| 038 | 3-O-Su-GalNAcβ-sp3 | | 3657 | 689 | 7 |
| 041 | 6-O-Su-GalNAcα-sp3 | | 5504 | 1443 | 7 |
| 043 | 6-O-Su-GlcNAcβ-sp3 | | 1040 | 253 | 1 |
| 044 | GlcAα-sp3 | α-glucuronic acid | 1275 | 327 | 0 |
| 045 | GlcAβ-sp3 | β-glucuronic acid | 1671 | 335 | 1 |
| 046 | 6-$H_2PO_3$Glcβ-sp4 | β-Glc6P | 1369 | 316 | 0 |
| 047 | 6-$H_2PO_3$Manα-sp3 | α-Man6P | 1300 | 322 | 0 |
| 048 | Neu5Acα-sp3 | | 583 | 131 | 0 |
| 049 | Neu5Acα-sp9 | | 1349 | 334 | 3 |
| 050 | Neu5Acβ-sp3 | | 2184 | 526 | 3 |
| 051 | Neu5Acβ-sp9 | | 1698 | 609 | 3 |
| 052 | Neu5Gcα-sp3 | | 847 | 286 | 0 |
| 053 | Neu5Gcβ-sp3 | | 1202 | 307 | 0 |
| 054 | 9-NAc-Neu5Acα-sp3 | | 256 | 77 | 0 |
| 055 | 3-O-Su-GlcNAcβ-sp3 | | 840 | 214 | 0 |
| 056 | Galα-sp7 | | 1016 | 263 | 0 |
| 057 | Rib -sp4 | | 1153 | 248 | 0 |
| 058 | Fucβ-sp3 | | 2266 | 422 | 1 |
| 059 | Crypted | Crypted | 1360 | 389 | 0 |
| 060 | 6-O-Su-Galβ-sp3 | | 61113 | 1156 | 20 |
| 061 | 3-OSu-GalNAcα-sp3 | | 2692 | 622 | 3 |
| 071 | Fucα1-2Galβ-sp3 | $H_{di}$ | 1077 | 245 | 1 |
| 072 | Fucα1-3GlcNAcβ-sp3 | | 1424 | 300 | 2 |
| 073 | Fucα1-4GlcNAcβ-sp3 | Le | 755 | 217 | 0 |
| 074 | Fucβ1-3GlcNAcβ-sp3 | | 533 | 130 | 0 |
| 075 | Galα1-2Galβ-sp3 | | 790 | 184 | 0 |
| 076 | Galα1-3Galβ-sp3 | $B_{di}$ | 775 | 192 | 0 |
| 077 | Galα1-3GalNAcβ-sp3 | $T_{\alpha\beta}$ | 668 | 155 | 0 |
| 078 | Galα1-3GalNAcα-sp3 | $T_{\alpha\alpha}$ | 581 | 113 | 0 |
| 080 | Galα1-3GlcNAcβ-sp3 | | 384 | 76 | 0 |
| 081 | Galα1-4GlcNAcβ-sp3 | α-LN | 815 | 183 | 1 |
| 082 | Galα1-4GlcNAcβ-sp8 | α-LN | 713 | 144 | 1 |
| 083 | Galα1-6Glcβ-sp4 | melibiose | 2285 | 348 | 2 |
| 084 | Galβ1-2Galβ-sp3 | | 1332 | 301 | 1 |

| | | | | | |
|---|---|---|---|---|---|
| 086 | Galβ1-3GlcNAcβ-sp2 | $Le^C$ | 609 | 130 | 0 |
| 087 | Galβ1-3Galβ-sp3 | | 1488 | 282 | 1 |
| 088 | Galβ1-3GalNAcβ-sp3 | $T_{\beta\beta}$ | 1468 | 174 | 2 |
| 089 | Galβ1-3GalNAcα-sp3 | TF | 297 | 59 | 0 |
| 092 | Galβ1-4Glcβ-sp2 | Lactose, Lac | 889 | 182 | 0 |
| 093 | Galβ1-4Glcβ-sp4 | Lactose, Lac | 1089 | 290 | 0 |
| 094 | Galβ1-4Galβ-sp4 | | 1124 | 278 | 0 |
| 096 | Galβ1-4GlcNAcβ-sp2 | N-acetyllactosamine, LN | 2604 | 893 | 1 |
| 097 | Galβ1-4GlcNAcβ-sp3 | N-acetyllactosamine, LN | 491 | 103 | 0 |
| 098 | Galβ1-4GlcNAcβ−sp5 | N-acetyllactosamine, LN | 341 | 65 | 0 |
| 099 | Galβ1-4GlcNAcβ-sp8 | N-acetyllactosamine, LN | 357 | 65 | 1 |
| 100 | Galβ1-6Galβ-sp4 | | 18406 | 2396 | 15 |
| 101 | GalNAcα1-3GalNAcβ-sp3 | Fs-2 | 1158 | 307 | 1 |
| 102 | GalNAcα1-3Galβ-sp3 | $A_{di}$ | 1199 | 295 | 1 |
| 103 | GalNAcα1-3GalNAcα-sp3 | core 5 | 451 | 87 | 0 |
| 104 | GalNAcβ1-3Galβ-sp3 | | 603 | 144 | 0 |
| 105 | GalNAcβ1-3GalNAcβ-sp3 | para-Fs | 5975 | 1065 | 11 |
| 106 | GalNAcβ1-4GlcNAcβ-sp3 | LacdiNAc | 332 | 79 | 0 |
| 107 | GalNAcβ1-4GlcNAcβ-sp2 | LacdiNAc | 390 | 95 | 0 |
| 110 | Glcα1-4Glcβ-sp3 | maltose | 10870 | 2172 | 10 |
| 111 | Glcβ1-4Glcβ-sp4 | cellobiose | 3363 | 407 | 3 |
| 112 | Glcβ1-6Glcβ-sp4 | gentiobiose | 6968 | 1229 | 5 |
| 113 | GlcNAcβ1-3GalNAcα-sp3 | core 3 | 4852 | 620 | 3 |
| 114 | GlcNAcβ1-3Manβ-sp4 | | 1474 | 423 | 2 |
| 115 | GlcNAcβ1-4GlcNAcβ-Asn | chitobiose-Asn | 12575 | 2669 | 14 |
| 116 | GlcNAcβ1-4GlcNAcβ-sp3 | chitobiose | 1040 | 186 | 1 |
| 117 | GlcNAcβ1-4GlcNAcβ-sp4 | chitobiose | 8585 | 1418 | 11 |
| 118 | GlcNAcβ1-6GalNAcα-sp3 | core 6 | 7860 | 714 | 4 |
| 119 | Manα1-2Manβ-sp4 | | 1200 | 259 | 0 |
| 120 | Manα1-3Manβ-sp4 | | 1224 | 338 | 0 |
| 121 | Manα1-4Manβ-sp4 | | 1064 | 328 | 0 |
| 122 | Manα1-6Manβ-sp4 | | 1484 | 380 | 0 |
| 123 | Manβ1-4GlcNAcβ-sp4 | | 2103 | 428 | 2 |
| 125 | 6-Bn-Galβ1-4GlcNAcβ-sp2 | | 32674 | 5389 | 19 |
| 126 | 6-Bn-Galα1-4(6-Bn)GlcNAcβ-sp3 | | 15410 | 3185 | 15 |
| 127 | Galβ1-4Glcβ-dipeptide | | 559 | 105 | 0 |
| 128 | Galβ1-4Glcβ-dipeptide | | 1181 | 263 | 0 |
| 129 | Galβ1-3(6-O-Bn)GlcNAcβ-sp3 | | 683 | 184 | 0 |
| 130 | (6-O-Bn-Galβ1)-3GlcNAcβ-sp3 | | 378 | 104 | 0 |
| 131 | (6-O-Bn-Galβ1)-3(6-O-Bn)GlcNAcβ-sp3 | | 1908 | 494 | 2 |
| 132 | Galb1-3GalNAca-sp5 | TF | 376 | 72 | 0 |

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|---|---|---|---|---|---|
| 133 | Galβ1-4Glcβ-dipeptide | | 6816 | 465 | 4 |
| 134 | Galβ1-4Glcβ-dipeptide | | 1197 | 271 | 0 |
| 135 | Galβ1-4Glcβ-dipeptide | | 941 | 330 | 0 |
| 136 | Galβ1-4Glcβ-dipeptide | | 476 | 98 | 0 |
| 137 | Galβ1-4Glcβ-dipeptide | | 589 | 88 | 0 |
| 138 | Galβ1-4Glcβ-dipeptide | | 571 | 130 | 0 |
| 139 | Galβ1-4Glcα-sp3 | | 395 | 65 | 0 |
| 140 | Galα1-3GalNAc(fur)β-sp3 | | 608 | 117 | 0 |
| 142 | GlcNAcα1-3GalNAcβ-sp3 | | 4523 | 1339 | 4 |
| 143 | Fucα1-2(3-O-Su)Galβ-sp3 | | 468 | 138 | 0 |
| 144 | Galβ1-3(6-O-Su)GlcNAcβ-sp2 | | 297 | 73 | 0 |
| 145 | Galβ1-3(6-O-Su)GlcNAcβ-sp3 | | 388 | 76 | 0 |
| 146 | Galβ1-4(6-O-Su)Glcβ-sp2 | | 2044 | 469 | 2 |
| 149 | GlcNAcβ1-4(6-O-Su)GlcNAcβ-sp2 | | 2569 | 475 | 5 |
| 150 | 3-O-Su-Galβ1-3GalNAcα-sp3 | | 37943 | 3232 | 20 |
| 151 | 6-O-Su-Galβ1-3GalNAcα-sp3 | | 1840 | 250 | 2 |
| 152 | 3-O-Su-Galβ1-4Glcβ-sp2 | SM3 | 2564 | 413 | 2 |
| 153 | 6-O-Su-Galβ1-4Glcβ-sp2 | | 1796 | 504 | 2 |
| 154 | 3-O-Su-Galβ1-3GlcNAcβ-sp3 | | 32651 | 3954 | 20 |
| 156 | 3-O-Su-Galβ1-4GlcNAcβ-sp2 | | 2305 | 458 | 1 |
| 158 | 4-O-Su-Galβ1-4GlcNAcβ-sp2 | | 2927 | 860 | 2 |
| 159 | 4-O-Su-Galβ1-4GlcNAcβ-sp3 | | 1656 | 736 | 2 |
| 160 | 6-O-Su-Galβ1-3GlcNAcβ-sp2 | | 346 | 142 | 0 |
| 161 | 6-O-Su-Galβ1-3GlcNAcβ-sp3 | | 536 | 141 | 0 |
| 162 | 6-O-Su-Galβ1-4GlcNAcβ-sp2 | | 5615 | 1540 | 7 |
| 164 | GlcAβ1-3GlcNAcβ-sp3 | | 979 | 218 | 1 |
| 165 | GlcAβ1-3Galβ-sp3 | | 1035 | 308 | 1 |
| 166 | GlcAβ1-6Galβ-sp3 | | 39105 | 2993 | 17 |
| 167 | GlcNAcβ1-4-[HOOC($CH_3$)CH]-3-O-GlcNAcβ-sp4 | GlcNAc-Mur | 1012 | 269 | 1 |
| 168 | GlcNAcβ1-4Mur-L-Ala-D-i-Gln-Lys | GMDP-Lys | 3668 | 152 | 1 |
| 169 | Neu5Acα2-3Galβ-sp3 | GM4 | 2305 | 596 | 3 |
| 170 | Neu5Acα2-6Galβ-sp3 | | 4157 | 633 | 3 |
| 171 | Neu5Acα2-3GalNAcα-sp3 | 3-Sia$T_n$ | 844 | 168 | 1 |
| 172 | Neu5Acα2-6GalNAcα-sp3 | Sia$T_n$ | 3250 | 124 | 1 |
| 173 | Neu5Acβ2-6GalNAcα-sp3 | b-SiaTn | 3221 | 855 | 5 |
| 174 | Neu5Gcα2-6GalNAcα-sp3 | Neu5Gc-$T_n$ | 1596 | 178 | 1 |
| 175 | Neu5Gcβ2-6GalNAcα-sp3 | | 1453 | 451 | 2 |
| 176 | 3-O-Su-Galβ1-4(6-O-Su)Glcβ-sp2 | | 43008 | 9342 | 20 |
| 177 | 3-O-Su-Galβ1-4(6-O-Su)GlcNAcβ-sp3 | | 32496 | 7215 | 20 |
| 178 | 6-O-Su-Galβ1-4(6-O-Su)Glcβ-sp2 | | 3506 | 763 | 5 |
| 179 | 6-O-Su-Galβ1-3(6-O-Su)GlcNAcβ-sp2 | | 1770 | 580 | 0 |

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|---|---|---|---|---|---|
| 180 | 6-O-Su-Galβ1-4(6-O-Su)GlcNAcβ-sp2 | | 2991 | 832 | 4 |
| 181 | 3,4-O-$Su_2$-Galβ1-4GlcNAcβ-sp3 | | 2993 | 820 | 5 |
| 182 | 3,6-O-$Su_2$-Galβ1-4GlcNAcβ-sp2 | | 487 | 151 | 0 |
| 183 | 4,6-O-$Su_2$-Galβ1-4GlcNAcβ-sp2 | | 4401 | 944 | 4 |
| 184 | 4,6-O-$Su_2$-Galβ1-4GlcNAcβ-sp3 | | 1945 | 649 | 2 |
| 186 | Neu5Acα2-8Neu5Acα2-sp3 | $(Sia)_2$ | 190 | 64 | 0 |
| 187 | 3-O-Su-Galβ1-4(6-O-Su)Glcβ-sp2 | | 290 | 91 | 0 |
| 188 | Neu5Acα2-8Neu5Acβ-sp9 | | 1162 | 382 | 2 |
| 189 | 3,6-O-$Su_2$-Galβ1-4(6-O-Su)GlcNAcβ-sp2 | | 2054 | 543 | 3 |
| 190 | Galβ1-4-(6-P)GlcNAcβ-sp2 | | 1217 | 347 | 1 |
| 191 | 6-P-Galβ1-4GlcNAcβ-sp2 | | 926 | 194 | 1 |
| 192 | GalNAcβ1-4(6-O-Su)GlcNAcβ-sp3 | | 483 | 106 | 0 |
| 193 | 3-O-Su-GalNAcβ1-4GlcNAcβ-sp3 | | 3103 | 626 | 4 |
| 194 | 6-O-Su-GalNAcβ1-4GlcNAcβ-sp3 | | 2884 | 455 | 4 |
| 195 | 6-O-Su-GalNAcβ1-4-(3-O-Ac)GlcNAcβ-sp3 | | 589 | 122 | 0 |
| 196 | 3-O-Su-GalNAcβ1-4(3-O-Su)-GlcNAcβ-sp3 | | 605 | 123 | 1 |
| 197 | 3,6-O-$Su_2$-GalNAcβ1-4-GlcNAcβ-sp3 | | 287 | 54 | 0 |
| 198 | 4,6-O-$Su_2$-GalNAcβ1-4GlcNAcβ-sp3 | | 2251 | 407 | 1 |
| 199 | 4,6-O-$Su_2$-GalNAcβ1-4-(3-O-Ac)GlcNAcβ-sp3 | | 690 | 178 | 0 |
| 200 | 4-O-Su-GalNAcβ1-4GlcNAcβ-sp3 | | 466 | 134 | 0 |
| 201 | 3,4-O-$Su_2$-GalNAcβ1-4-GlcNAcβ-sp3 | | 3790 | 859 | 6 |
| 202 | 6-O-Su-GalNAcβ1-4(6-O-Su)GlcNAcβ-sp3 | | 4471 | 933 | 8 |
| 203 | Galβ1-4(6-O-Su)GlcNAcβ-sp2 | | 5424 | 1203 | 8 |
| 204 | 4-O-Su-GalNAcβ1-4GlcNAcβ-sp2 | | 753 | 120 | 1 |
| 205 | Neu5Acα2-6GalNAcβ-sp3 | | 606 | 111 | 1 |
| 206 | Neu5Gcα2-3Gal-sp3 | | 1295 | 371 | 0 |
| 207 | Neu5Acβ2-6GalNAcβ-sp3 | | 2913 | 646 | 3 |
| 208 | Galβ1-3GlcNAcβ-sp4 | $Le^C$ | 658 | 172 | 0 |
| 209 | Crypted | Crypted | 6503 | 1331 | 9 |
| 210 | Crypted | Crypted | 1743 | 558 | 1 |
| 211 | Crypted | Crypted | 8440 | 1533 | 13 |
| 212 | Crypted | Crypted | 2129 | 530 | 3 |
| 213 | Neu5Acβ2-6Galβ-sp3 | | 2672 | 827 | 3 |
| 214 | ΔGlcAβ1-3Galβ-sp3 | deltaGlcAβ3Gal | 589 | 161 | 0 |
| 215 | Fucα1-2Galβ1-3GlcNAcβ-sp3 | $Le^d$, H (type 1) | 2727 | 444 | 3 |
| 216 | Fucα1-2Galβ1-4GlcNAcβ-sp3 | H (type 2) | 10845 | 2895 | 15 |
| 217 | Fucα1-2Galβ1-3GalNAcα-sp3 | H (type 3) | 3108 | 468 | 6 |
| 219 | Fucα1-2Galβ1-4Glcβ-sp4 | H (type 6)-Gly | 8251 | 1687 | 10 |
| 220 | Galα1-3Galβ1-4Glcβ-sp2 | | 901 | 275 | 0 |
| 221 | Galα1-3Galβ1-4Glcβ-sp4 | | 1230 | 393 | 2 |
| 222 | Galα1-3Galβ1-4GlcNAcβ-sp3 | Galili (tri) | 254 | 106 | 0 |
| 223 | Galα1-4Galβ1-4Glcβ-sp2 | $P^k$, Gb3, $GbOse_3$ | 756 | 247 | 0 |

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|---|---|---|---|---|---|
| 225 | Galα1-4Galβ1-4GlcNAcβ-sp2 | $P_1$ | 573 | 165 | 0 |
| 226 | Galα1-3(Fucα1-2)Galβ-sp3 | $B_{tri}$ | 1227 | 312 | 1 |
| 227 | Galα1-3(Fucα1-2)Galβ-sp5 | $B_{tri}$-C8 | 1044 | 248 | 1 |
| 228 | Galβ1-2Galα1-3GlcNAcβ-sp3 | | 321 | 87 | 0 |
| 229 | Galβ1-3Galβ1-4GlcNAcβ-sp4 | | 323 | 81 | 0 |
| 230 | Galβ1-4Galβ1-4Glcβ-sp2 | Mc3 | 248 | 84 | 0 |
| 232 | Galβ1-4GlcNAcβ1-6GalNAcα−sp3 | | 624 | 81 | 1 |
| 233 | Galβ1-3(Fucα1-4)GlcNAcβ-sp3 | $Le^A$ | 210 | 58 | 0 |
| 234 | Galβ1-4(Fucα1-3)GlcNAcβ-sp3 | $Le^X$ | 13573 | 2635 | 16 |
| 235 | GalNAcα1-3(Fucα1-2)Galβ-sp3 | $A_{tri}$ | 586 | 306 | 1 |
| 236 | GalNAcα1-3(Fucα1-2)Galβ-sp5 | $A_{tri}$ | 498 | 262 | 1 |
| 237 | GalNHα1-3<br>Galβ-$OCH_2CH_2CH_2NHAc$<br>Fucα1-2 | | 357 | 135 | 0 |
| 239 | GalNAcβ1-3(Fucα1-2)Galβ-sp3 | | 849 | 654 | 1 |
| 240 | $(Glcα1-4)_3β$-sp4 | maltotriose | 11233 | 1567 | 6 |
| 241 | $(Glcα1-6)_3β$-sp4 | isomaltotriose | 520 | 163 | 0 |
| 242 | GlcNAcα1-3Galβ1-4GlcNAcβ-sp2 | | 278 | 90 | 0 |
| 243 | GlcNAcα1-3Galβ1-4GlcNAcβ-sp3 | | 215 | 61 | 0 |
| 245 | GlcNAcα1-6Galβ1-4GlcNAcβ-sp2 | | 3394 | 1053 | 3 |
| 246 | GlcNAcβ1-2Galβ1-3GalNAcα-sp3 | | 434 | 94 | 0 |
| 247 | GlcNAcβ1-3Galβ1-3GalNAcα-sp3 | | 321 | 63 | 0 |
| 248 | GlcNAcβ1-3Galβ1-4Glcβ-sp2 | | 822 | 179 | 0 |
| 249 | GlcNAcβ1-3Galβ1-4GlcNAcβ-sp2 | | 1652 | 322 | 2 |
| 250 | GlcNAcβ1-3Galβ1-4GlcNAcβ-sp3 | | 1942 | 488 | 2 |
| 251 | GlcNAcβ1-4Galβ1-4GlcNAcβ-sp2 | | 1067 | 216 | 1 |
| 252 | GlcNAcβ1-4GlcNAcβ1-4GlcNAcβ-sp4 | chitotriose | 1111 | 206 | 2 |
| 253 | GlcNAcβ1-6Galβ1-4GlcNAcβ-sp2 | | 2877 | 653 | 3 |
| 254 | GlcNAcβ1-6(Galβ1-3)GalNAcα-sp3 | core 2 | 2499 | 525 | 4 |
| 255 | GlcNAcβ1-6(GlcNAcβ1-3)GalNAcα-sp3 | core 4 | 5853 | 425 | 4 |
| 256 | GlcNAcβ1-6(GlcNAcβ1-4)GalNAcα-sp3 | | 5020 | 613 | 8 |
| 258 | Manα1-6(Manα1-3)Manβ-sp4 | $Man_3$ | 743 | 160 | 0 |
| 259 | Galβ1-4(Galβ1-3)GlcNAcβ-sp3 | | 372 | 58 | 0 |
| 260 | Fucβ1-4<br>GlcNAcβ-sp3<br>Galβ1-3 | | 663 | 140 | 0 |
| 261 | Fucβ1-3<br>GlcNAcβ-sp3<br>Galβ1-4 | | 299 | 62 | 0 |
| 262 | Galβ1-3GalNAcβ1-3Gal-sp4 | | 437 | 120 | 0 |
| 263 | (GalNAcβ-PEG2)3-β-DD | | 657 | 114 | 0 |
| 264 | Galβ1-4Galβ1-4GlcNAcβ-sp3 | | 461 | 87 | 0 |

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|---|---|---|---|---|---|
| 266 | Galα1-4Galβ1-4GlcNAcβ-sp3 | $P_1$ | 1017 | 227 | 0 |
| 267 | GlcNAcβ1-3Galβ1-3GlcNAcβ-sp3 | | 718 | 138 | 0 |
| 268 | GlcNAcβ1-4(Fucα1-6)GlcNAcβ-sp3 | | 1291 | 311 | 1 |
| 269 | Galβ1-3Galβ1-4Glcβ-sp4 | | 518 | 137 | 0 |
| 270 | Galβ1-4Galβ1-4Glcβ-sp4 | | 939 | 202 | 0 |
| 271 | Galβ1-6Galβ1-4Glcβ-sp4 | | 53622 | 1934 | 20 |
| 272 | Neu5Acα2-3Galβ1-4Glcβ-sp4-Cit | 3'-SL -citrullin | 236 | 60 | 0 |
| 273 | Fucβ1-2Galβ1-4GlcNAcβ-sp3 | | 242 | 60 | 0 |
| 274 | GalNAcα1-3Galβ1-4GlcNAc-sp3 | | 426 | 90 | 0 |
| 275 | GalNAcβ1-3Galβ1-4GlcNAc-sp3 | | 403 | 103 | 0 |
| 276 | GlcNAcβ1-4Galβ1-4GlcNAcβ-sp3 | | 1357 | 456 | 2 |
| 277 | GalNGcα1-3(Fucα1-2)Galβ-sp3 | N-Gc-$A_{tri}$ | 375 | 99 | 0 |
| 287 | 3-O-Su-Galβ1-3(Fucα1-4)GlcNAcβ-sp3 | 3'-OSu-$Le^A$ | 20063 | 4962 | 19 |
| 288 | 3-O-Su-Galβ1-4(Fucα1-3)GlcNAcβ-sp3 | 3'-OSu-$Le^X$ | 593 | 146 | 0 |
| 289 | Neu5Acα2-6(Galβ1-3)GalNAcα-sp3 | | 296 | 88 | 0 |
| 290 | Neu5Acα2-6(Galα1-3)GalNAcα-sp3 | | 262 | 68 | 0 |
| 291 | Neu5Acβ2-6(Galβ1-3)GalNAcα-sp3 | | 1887 | 727 | 3 |
| 292 | Neu5Acα2-3Galβ1-3GalNAcα-sp3 | | 4477 | 1186 | 4 |
| 293 | Neu5Acα2-3Galβ1-4Glcβ-sp3 | 3'SL | 1684 | 349 | 1 |
| 294 | Neu5Acα2-3Galβ1-4Glcβ-sp4 | 3'SL | 363 | 99 | 0 |
| 295 | Neu5Acα2-6Galβ1-4Glcβ-sp2 | 6'SL | 290 | 66 | 0 |
| 296 | Neu5Acα2-6Galβ1-4Glcβ-sp4 | 6'SL | 360 | 96 | 0 |
| 297 | Neu5Acβ2-6Galβ1-4Glcβ-sp2 | | 1645 | 409 | 3 |
| 298 | Neu5Acα2-3Galβ1-4GlcNAcβ-sp3 | 3'SLN | 5486 | 1855 | 3 |
| 299 | Neu5Acα2-3Galβ1-3GlcNAcβ-sp3 | 3'SiaLe$^C$ | 3968 | 1266 | 6 |
| 300 | Neu5Acα2-6Galβ1-4GlcNAcβ-sp3 | 6'SLN | 936 | 527 | 1 |
| 302 | Neu5Acβ2-6Galβ1-4GlcNAcβ-sp3 | | 1100 | 368 | 2 |
| 303 | Neu5Gcα2-3Galβ1-4GlcNAcβ-sp3 | | 2147 | 451 | 1 |
| 304 | Neu5Gcα2-6Galβ1-4GlcNAcβ-sp3 | | 792 | 191 | 0 |
| 305 | Neu5Gcβ2-6Galβ1-4GlcNAcβ-sp3 | | 601 | 112 | 0 |
| 306 | 9-NAc-Neu5Acα2-6Galβ1-4GlcNAcβ-sp3 | | 1431 | 437 | 1 |
| 307 | KDNα2-3Galβ1-3GlcNAcβ-sp2 | | 1441 | 304 | 2 |
| 308 | KDNα2-3Galβ1-4GlcNAcβ-sp2 | | 903 | 204 | 0 |
| 309 | Neu5Acα2-6(Neu5Acα2-3)GalNAcα-sp3 | | 487 | 107 | 1 |
| 310 | 3'SiaLacNAcβ-$OCH_2CH_2CH_2NH$-(3'SiaLacNAc-amide-sp3) | | 1932 | 665 | 1 |
| 315 | Neu5Acα2-3Galβ1-4-(6-O-Su)GlcNAcβ-sp3 | | 1930 | 477 | 3 |
| 316 | Neu5Acα2-3Galβ1-3-(6-O-Su)GlcNAcβ-sp3 | | 2752 | 1014 | 5 |
| 318 | Neu5Acα2-6Galβ1-4-(6-O-Su)GlcNAcβ-sp3 | | 1004 | 234 | 1 |
| 319 | Neu5Acα2-3-(6-O-Su)Galβ1-4GlcNAcβ-sp3 | | 7963 | 705 | 7 |
| 320 | 4-O-Su-Neu5Acα2-3-(6-O-Su)Galβ1-4GlcNAcβ-sp3 | | 9621 | 1580 | 10 |
| 321 | $(Neu5Ac\alpha2\text{-}8)_3$-sp3 | $(Sia)_3$ | 226 | 57 | 0 |

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|---|---|---|---|---|---|
| 322 | (Neu5Acα2-8)3β-sp3 | | 553 | 112 | 0 |
| 323 | Neu5Acα2-6Galβ1-3GlcNAc-sp3 | 6'-SiaLe$^{c}$ | 317 | 100 | 0 |
| 324 | Neu5Acα2-6Galβ1-3(6-O-Su)GlcNAc-sp3 | | 361 | 80 | 0 |
| 325 | Neu5Acα2-3Galβ1-4Glcβ-dipeptide | | 275 | 57 | 0 |
| 326 | Neu5Acα2-3Galβ1-4Glcβ-dipeptide | | 311 | 65 | 0 |
| 327 | Neu5Acα2-3Galβ1-4Glcβ-dipeptide | | 495 | 125 | 0 |
| 328 | Neu5Acα2-3Galβ1-4Glcβ-dipeptide | | 298 | 55 | 0 |
| 329 | Neu5Acα2-3Galβ1-4Glcβ-dipeptide | | 586 | 133 | 0 |
| 331 | Neu5Gcα2-3Galβ1-3GlcNAcβ-sp3 | | 2552 | 467 | 5 |
| 332 | Neu5Acα2-3Galβ1-4Glcβ-dipeptide | | 381 | 91 | 0 |
| 333 | Neu5Gcα2-3Galβ1-3-(6-O-Su)GlcNAcβ-sp3 | | 4811 | 1109 | 5 |
| 334 | Neu5Gcα2-3Galβ1-4-(6-O-Su)GlcNAcβ-sp3 | | 1639 | 274 | 2 |
| 335 | Neu5Acα2-3Galβ1-3-(6-O-Su)GlcNAcβ-sp3 | | 6401 | 1102 | 6 |
| 336 | αKdo-(2→8)-αKdo-(2→4)-αKdo-sp11 | | 5921 | 1649 | 10 |
| 337 | GalNAcα1-4Galβ1-4GlcNAcβ-sp3 | | 4219 | 657 | 7 |
| 338 | Neu5Acα2-6Galβ1-3GalNAcα-sp3 | | 306 | 59 | 0 |
| 339 | Neu5Acβ2-6Galβ1-3GalNAcα-sp3 | | 1931 | 365 | 3 |
| 340 | Galα1-3(Neu5Acβ2-6)GalNAcβ-sp3 | | 2907 | 760 | 4 |
| 341 | Neu5Acα2-3-(6-Su)Galβ1-4GlcNAcβ-sp2 | | 5964 | 1255 | 9 |
| 359 | Galα1-3(Fucα1-2)Galβ1-3GlcNAcβ-sp3 | B (type 1) | 400 | 112 | 0 |
| 360 | Galα1-3(Fucα1-2)Galβ1-4GlcNAcβ-sp3 | B (type 2) | 5071 | 644 | 6 |
| 362 | Galα1-3(Fucα1-2)Galβ1-3GalNAcα-sp3 | B (type 3) | 8283 | 2150 | 14 |
| 363 | Galα1-3(Fucα1-2)Galβ1-3GalNAcβ-sp3 | B (type 4) | 2728 | 589 | 6 |
| 364 | Galα1-3Galβ1-4(Fucα1-3)GlcNAcβ-sp3 | αGalLe$^{X}$ | 541 | 116 | 0 |
| 365 | Galα1-4(Fucα1-2)Galβ1-4GlcNAcβ-sp3 | | 962 | 361 | 1 |
| 366 | GalNAcα1-3(Fucα1-2)Galβ1-3GlcNAcβ-sp3 | A (type 1) | 351 | 71 | 0 |
| 368 | GalNAcα1-3(Fucα1-2)Galβ1-4GlcNAcβ-sp3 | A (type 2) | 2621 | 625 | 2 |
| 369 | GalNAcα1-4(Fucα1-2)Galβ1-4GlcNAcβ-sp3 | | 3246 | 374 | 3 |
| 370 | Crypted | Crypted | 2629 | 835 | 3 |
| 371 | Fucα1-2Galβ1-3(Fucα1-4)GlcNAcβ-sp3 | Le$^{B}$ | 307 | 107 | 0 |
| 372 | Fucα1-2Galβ1-4(Fucα1-3)GlcNAcβ-sp3 | Le$^{Y}$ | 3936 | 1040 | 8 |
| 373 | Galα1-3Galβ1-4GlcNAcβ1-3Galβ-sp3 | Galili (tetra) | 777 | 128 | 0 |
| 374 | Galα1-3(Galα1-4)Galβ1-4GlcNAcβ-sp3 | Galα2-3',4'LN | 280 | 72 | 0 |
| 375 | Galα1-4GlcNAcβ1-3Galβ1-4GlcNAcβ-sp3 | | 1032 | 190 | 1 |
| 377 | Galβ1-3GlcNAcβ1-3Galβ1-3GlcNAcβ-sp2 | | 827 | 166 | 1 |
| 378 | Galβ1-3GlcNAcα1-3Galβ1-4GlcNAcβ-sp3 | | 10106 | 2009 | 9 |
| 379 | Galβ1-3GlcNAcβ1-3Galβ1-4GlcNAcβ-sp3 | | 1304 | 387 | 1 |
| 380 | Galβ1-3GlcNAca1-6Galb1-4GlcNAcb-sp2 | | 2306 | 344 | 1 |
| 381 | Galβ1-3GlcNAcβ1-6Galβ1-4GlcNAcβ-sp2 | | 2581 | 827 | 3 |
| 382 | Galβ1-3GalNAcβ1-4Galβ1-4Glcβ-sp3 | GA1, asialo-GM1 | 642 | 125 | 0 |
| 383 | Galβ1-4GlcNAcβ1-3Galβ1-4Glcβ-sp4 | LNnT | 280 | 74 | 0 |

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|---|---|---|---|---|---|
| 384 | Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ-sp2 | i | 290 | 76 | 0 |
| 386 | Galβ1-4GlcNAcα1-6Galβ1-4GlcNAcβ-sp2 | | 3822 | 638 | 2 |
| 387 | Galβ1-4GlcNAcβ1-6Galβ1-4GlcNAcβ-sp2 | | 2719 | 521 | 2 |
| 388 | Galβ1-4GlcNAcβ1-6(Galβ1-3)GalNAcα-sp3 | | 229 | 77 | 0 |
| 389 | GalNAcβ1-3Galα1-4Galβ1-4Glcβ-sp3 | Gb4, P | 399 | 90 | 0 |
| 390 | $(Glc\alpha 1\text{-}4)_4\beta$-sp4 | maltotetraose | 13504 | 2947 | 10 |
| 394 | GlcNAcβ1-4<br>Galβ1-4GlcNAcβ-sp2<br>GlcNAcβ1-3 | | 2286 | 487 | 3 |
| 395 | GlcNAcβ1-6(GlcNAcβ1-3)Galβ1-4GlcNAcβ-sp2 | $T_k$ | 1249 | 316 | 0 |
| 396 | (GlcNAcβ1)3-3,4,6-GalNAcα-sp3 | | 4966 | 727 | 4 |
| 397 | $(GlcNAc\beta 1)_3$-3,4,6-GalNAcα-sp3 | | 7793 | 1480 | 9 |
| 398 | Galβ1-3GlcN(Fm)β1-3Galβ1-4GlcNAcβ-sp3 | | 557 | 106 | 0 |
| 399 | Galβ1-3GlcNAcα1-3Galβ1-3GlcNAcβ-sp2 | | 6788 | 1178 | 10 |
| 401 | Galβ1-3GlcNAcβ1-3Galβ1-3GlcNAcβ-sp3 | | 717 | 181 | 0 |
| 402 | GalNAcα1-3<br>Galα1-4GlcNAcβ-sp3<br>Fucα1-2 | | 175 | 45 | 0 |
| 403 | Galβ1-3GlcNAcβ1-3Galβ1-4GlcNAcβ-sp2 | | 2047 | 376 | 4 |
| 404 | GalNAcα1-3Galβ1-4(Fucα1-3)GlcNAcβ-sp3 | $\alpha GalNAcLe^X$ | 3611 | 1529 | 5 |
| 405 | Galα1-3(Fucα1-2)Galα1-4GlcNAcβ-sp3 | | 2929 | 227 | 1 |
| 406 | GalNAcα1-3(Fucα1-2)Galα1-3GalNAcβ-sp3 | | 304 | 60 | 0 |
| 419 | 3-O-SuGalβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ-sp3 | | 2425 | 448 | 3 |
| 420 | 4-O-SuGalβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ-sp3 | | 894 | 183 | 1 |
| 423 | Neu5Acα2-3Galβ1-4(Fucα1-3)GlcNAcβ-sp3 | $SiaLe^X$ | 1673 | 483 | 1 |
| 425 | Neu5Acα2-3Galβ1-4<br>GlcNAcβ-sp3<br>Fucβ1-3 | | 2164 | 673 | 2 |
| 426 | Neu5Acα2-3Galβ1-3(Fucα1-4)GlcNAcβ-sp3 | $SiaLe^A$ | 216 | 60 | 0 |
| 428 | Neu5Acα2-3Galβ1-4(Fucα1-3)(6-O-Su-)GlcNAcβ-sp3 | $6\text{-}OSu\text{-}SLe^x$ | 429 | 149 | 0 |
| 429 | Neu5Acα2-3(6-O-Su)Galβ1-4(Fucα1-3)GlcNAcβ-sp3 | $6'\text{-}OSu\text{-}SiaLe^x$ | 762 | 222 | 2 |
| 431 | Neu5Acα2-3Galβ1-4(2-O-Su-Fucα1-3)GlcNAcβ-sp3 | | 918 | 242 | 2 |
| 432 | Neu5Acα2-3Galβ1-4<br>GlcNAcβ-sp3<br>3-O-Su-Fucα1-3 | | 546 | 207 | 0 |
| 433 | Neu5Acα2-6(Neu5Acα2-3Galβ1-3)GalNAcα-sp3 | | 203 | 66 | 0 |
| 434 | Neu5Acα2-8Neu5Acα2-3Galβl-4Glcβ-sp4 | GD3 | 287 | 84 | 0 |
| 435 | Neu5Acα2-3Galβ1-4(2-O-Su-Fucα1-3)(6-O-Su-)GlcNAcβ-sp3 | | 425 | 133 | 0 |

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|---|---|---|---|---|---|
| 436 | 4-O-Su-Neu5Acα2-3Galβ1-4(Fucα1-3)(6-O-Su-)GlcNAcβ-sp3 | | 963 | 415 | 2 |
| 437 | GalNAcα1-3(Fucα1-2)Galβ1-3GalNAcβ-sp3 | A(type 4) | 33886 | 3193 | 18 |
| 438 | Fucβ1-2Galβ1-4(Fucα1-3)GlcNAcβ-sp3 | | 460 | 131 | 0 |
| 439 | αKdo-(2→4)-αKdo-(2→4)-αKdo-(2→6)-βGlcNAc-sp11 | | 5179 | 1212 | 9 |
| 440 | Neu5Acβ2-6(Fucα1-2)Galβ1-4GlcNAcβ-sp3 | | 2150 | 416 | 3 |
| 441 | Neu5Acα2-6(Fucα1-2)Galβ1-4GlcNAcβ-sp3 | | 719 | 160 | 1 |
| 442 | Neu5Acα2-3 (GalNAcβ1-4)Galβ1-4Glcβ-sp4 | GM2 | 499 | 111 | 0 |
| 479 | Fucα1-2Galβ1-3GlcNAcβ1-3Galβ1-4Glcβ-sp4 | LNFP I | 512 | 286 | 0 |
| 480 | Fucα1-2Galβ1-3GlcNAcβ1-3Galβ1-4GlcNAcβ-sp2 | H(type 1) penta | 3826 | 911 | 8 |
| 481 | Galα1-3Galβ1-4GlcNAcβ1-3Galβ1-4Glcβ-sp4 | Galili (penta) | 241 | 60 | 0 |
| 482 | Galα1-3(Fucα1-2)Galβ1-3(Fucα1-4)GlcNAcβ-sp3 | $BLe^B$ | 1726 | 229 | 3 |
| 483 | Galα1-3(Fucα1-2)Galβ1-4(Fucα1-3)GlcNAcβ-sp3 | $BLe^Y$ | 2689 | 293 | 3 |
| 484 | GalNAcα1-3(Fucα1-2)Galβ1-3(Fucα1-4)GlcNAcβ-sp3 | $ALe^B$ | 175 | 49 | 0 |
| 485 | Galβ1-4GalNAcα1-3(Fucα1-2)Galβ1-4GlcNAcβ-sp3 | | 2643 | 642 | 4 |
| 488 | Galβ1-4GlcNAcβ1-6(Galβ1-4GlcNAcβ1-3)GalNAcα-sp3 | | 284 | 69 | 0 |
| 489 | Galβ1-4GlcNAcβ1-3(GlcNAcβ1-6)Galβ1-4GlcNAcβ-sp2 | | 541 | 172 | 0 |
| 490 | Galβ1-4GlcNAcβ1-6(GlcNAcβ1-3)Galβ1-4GlcNAcβ-sp2 | | 527 | 119 | 0 |
| 491 | GalNAcα1-3(Fucα1-2)Galβ1-4(Fucα1-3)GlcNAcβ-sp3 | $ALe^Y$ | 213 | 65 | 0 |
| 492 | $(Glc\alpha1\text{-}6)_5\beta$-sp4 | isomaltopentaose | 1151 | 263 | 2 |
| 493 | $(GlcNAc\beta1\text{-}4)_5\beta$-sp4 | chitopentaose | 1798 | 509 | 1 |
| 495 | Manα1-6(Manα1-3)Manα1-6(Manα1-3)Manβ-sp4 | $Man_5$ | 892 | 319 | 1 |
| 496 | Fucα1-2Galβ1-3(Fucα1-4)GlcNAcβ1-3Galβ1-4Glcβ-sp4 | $Le^b$(hexa) | 223 | 72 | 0 |
| 497 | Fucα1-2Galβ1-4(Fucα1-3)GlcNAcβ1-3Galβ1-4Glcβ-sp4 | $Le^Y$(hexa) | 2057 | 212 | 4 |
| 498 | Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ-sp3 | $(LN)_3$ | 201 | 62 | 0 |
| 499 | Galβ1-4GlcNAcβ1-6(Galβ1-4GlcNAcβ1-3)Galβ1-4GlcNAcβ-sp2 | I | 200 | 45 | 0 |
| 501 | Galβ1-3GalNAcβ1-3Galα1-4Galβ1-4Glcβ-sp4 | Gb5 | 1360 | 254 | 1 |
| 502 | $(Glc\alpha1\text{-}6)_6\beta$-sp4 | maltohexaose | 3033 | 653 | 5 |
| 503 | (GlcNAcβ1-4)6-sp4 | chitohexaose | 2396 | 314 | 1 |
| 504 | $(A\text{-}GN\text{-}M)_2$-3,6-M-GN-GNβ-sp4 | 9-OS | 200 | 57 | 0 |
| 505 | $(GN\text{-}M)_2$-3,6-M-GN-GNβ-sp4 | 7-OS | 728 | 195 | 0 |
| 506 | Araf6 | Ara6 | 158 | 37 | 0 |
| 507 | GalNAcα1-3GalNAcβ1-3Galα1-4Galβ1-4Glcβ-sp3 | Fs-5 | 2021 | 988 | 3 |

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|---|---|---|---|---|---|
| 508 | GalNAcβ1-3(Fucα1-2)Galβ1-4(Fucα1-3)GlcNAcβ-sp3 | | 402 | 93 | 0 |
| 509 | Galβ1-3GalNAcβ1-4(Neu5Acα2-3)Galβ1-4Glcβ-sp4 | GM1 | 1553 | 448 | 3 |
| 527 | Neu5Acα2-3Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ-sp2 | | 179 | 48 | 0 |
| 528 | Neu5Acα2-3Galβ1-4(Fucα1-3)GlcNAcβ1-3Galβ-sp3 | SiaLe$^x$-3Gal | 203 | 49 | 0 |
| 529 | Neu5Acα2-6(Galβ1-3)GlcNAcβ1-3Galβ1-4Glcβ-sp4 | LSTb | 297 | 70 | 0 |
| 530 | (Neu5Acα2-3Galβ1)2-3,4-GlcNAcβ-sp3 | | 1529 | 352 | 1 |
| 531 | Neu5Acα2-8Neu5Acα2-3(GalNAcβ1-4)Galβ1-4Glcβ-sp2 | GD2 | 434 | 116 | 0 |
| 534 | Neu5Acα2-3Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ-sp3 | | 193 | 58 | 0 |
| 535 | Neu5Acα2-8Neu5Acα2-3(GalNAcβ1-4)Galβ1-4Glc-sp4 | GD2 | 234 | 61 | 0 |
| 536 | Neu5Aα2-3Galβ1-3GlcNAcβ1-3Galβ1-4Glcβ-sp4 | LSTa | 342 | 92 | 0 |
| 537 | Neu5Aα2-3Galβ1-4GlcNAcβ1-3Galβ1-4Glcβ-sp4 | LSTd | 202 | 61 | 0 |
| 538 | Galβ1-4(Fucα1-3)GlcNAcβ1-6(Galβ1-3GlcNAcβ1-3)Galβ1-4Glcβ-sp4 | MFLNH III | 2473 | 712 | 3 |
| 539 | Galβ1-4GlcNAcβ1-6(Fucα1-2Galβ1-3GlcNAcβ1-3)Galβ1-4Glcβ-sp4 | MFLNH I | 780 | 239 | 0 |
| 540 | Galβ1-4(Fucα1-3)GlcNAcβ1-6(Neu5Ac2-6Galβ1-4GlcNAcβ1-3)Galβ1-4Glcβ-sp4 | MSMFLNnH | 2262 | 337 | 3 |
| 541 | Galβ1-4(Fucα1-3)GlcNAcβ1-6(Fucα1-2Galβ1-3GlcNAcβ1-3)Galβ1-4Glcβ-sp4 | DFLNH (a) | 6347 | 878 | 7 |
| 542 | Galβ1-3GlcNAcβ1-3Galβ1-4(Fucα1-3)GlcNAcβ1-6(Galβ1-3GlcNAcβ1-3)Galβ1-4Glcβ-sp4 | MF(1-3)iLNO | 2867 | 344 | 6 |
| 543 | Fucα1-2Galβ1-3(Fucα1-4)GlcNAcβ1-3[Galβ1-4(Fucα1-3)GlcNAcβ1-6]Galβ1-4Glcβ-sp4 | TFLNH | 3573 | 657 | 6 |
| 545 | αKdo-(2→8)-αKdo-(2→4)-αKdo-(2→6)-βGlcNAc-(1→6)αGlcNAc-sp11 | | 3329 | 757 | 6 |
| 625 | (GlcAβ1-4GlcNAcβ1-3)$_{11\text{-}12}$-NH$_2$-ol | hyaluronic acid | 776 | 257 | 1 |
| 627 | (Sia2-6A-GN-M)$_2$-3,6-M-GN-GNβ-sp4 | 11-OS, YDS | 184 | 54 | 0 |
| 629 | Trehalose-ethanolamine | **negative control** | 323 | 71 | 0 |
| 630 | (GlcAβ1-3GlcNAcβ1-4)$_{20}$-NH(*p*-C$_6$H$_4$)CH$_2$CH$_2$NH$_2$ | hyaluronic acid , 8kDa | 921 | 236 | 1 |
| 631 | (GlcAβ1-3GlcNAcβ1-4)$_{38}$-NH(*p*-C$_6$H$_4$)CH$_2$CH$_2$NH$_2$ | hyaluronic acid, 15-30kDa | 456 | 174 | 1 |
| 632 | (GlcAβ1-3GlcNAcβ1-4)$_{13}$-NH(*p*-C$_6$H$_4$)CH$_2$CH$_2$NH$_2$ | hyaluronic acid, 4-8kDa | 481 | 109 | 0 |
| 800 | GlcNAcα1-4GlcNAcβ-sp3 | | 1697 | 257 | 1 |
| 801 | GalNAcα1-3GalNAc(fur)β-sp3 | | 3988 | 289 | 2 |
| 802 | Galβ1-3GalNAc(fur)β-sp3 | | 51348 | 2324 | 20 |
| 804 | [Galβ1-4GlcNAcβ-OCH$_2$CH$_2$]$_2$NH | LN dimer | 908 | 252 | 1 |
| 805 | GalNAcβ1-4(6-O-Bn)GlcNAcβ-sp3 | | 6754 | 1034 | 11 |
| 806 | Galα1-6Glcα-sp3 | α-melibiose | 5469 | 949 | 4 |

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|---|---|---|---|---|---|
| 807 | GlcNAcβ1-4GlcNAcα-sp4 | | 10158 | 984 | 8 |
| 808 | Galα1-6Glcβ-sp3 | melibiose | 9724 | 1131 | 7 |
| 809 | GalNAcβ1-3GalNAcα-sp3 | | 8521 | 1509 | 13 |
| 810 | GalNGcα1-3GalNAcα-sp3 | core 5(Gc) | 328 | 76 | 0 |
| 850 | Galβ1-3(6-O-Su)GalNAcα-sp3 | 6-SuTF | 341 | 102 | 0 |
| 851 | Galα1-3(6-O-Su)GalNAcα-sp3 | $6\text{-SuT}_{\alpha\alpha}$ | 267 | 86 | 0 |
| 852 | GlcNAcβ1-4-[HOOC(CH3)CH]-3-O-GlcNAcα-sp4 | GlcNAc-Murα | 1056 | 322 | 1 |
| 900 | $H\text{-(Gly)}_6\text{-NH}_2$ Gly6-amide, linear | $Gly_6$-amide, linear | 25036 | 2262 | 16 |
| 901 | biot-$CMG_2$-NH2 | **biot-$CMG_2$** | 52213 | 3728 | 20 |
| 902 | Peptide, crypted | | 5630 | 1228 | 6 |
| 903 | Peptide, crypted | | 2617 | 897 | 4 |
| 904 | Peptide, crypted | | 6111 | 1154 | 12 |
| 905 | Suc-LPWYRAPK-NH2 | RhD | 16175 | 3630 | 20 |
| 906 | Peptide, crypted | | 475 | 163 | 0 |
| 907 | Peptide, crypted | | 457 | 93 | 0 |
| 908 | Peptide, crypted | | 6687 | 2162 | 13 |
| 909 | Peptide, crypted | | 812 | 166 | 1 |
| 910 | Npentyl-Indol-linked-3-NH2 (AcOH salt) | | 686 | 168 | 0 |
| 911 | N-C5-indazole-3-CONH-PEG-NH2 (AcOH salt) | | 6037 | 1212 | 11 |

sp2 = $-O(CH_2)_2NH_2$

sp3 = $-O(CH_2)_3NH_2$

sp4 = $-NHCOCH_2NH_2$

sp5 = $-O(CH_2)_3NH\text{-}CO(CH_2)_5NH_2$

sp8 = $-(OCH_2CH_2)_6NH_2$