Supporting Information For:

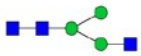
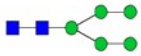
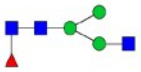
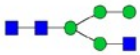
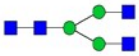
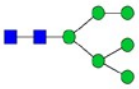
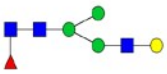
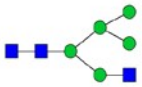
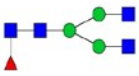
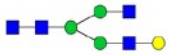
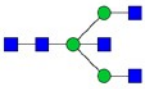
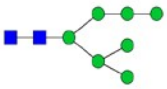
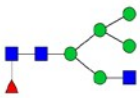
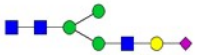
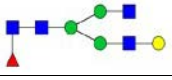
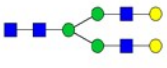
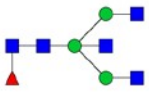
Rapid N-Glycan Profiling of Serum and Plasma by a Novel Slide Based Imaging Mass Spectrometry Workflow

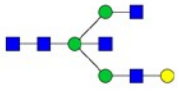
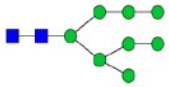
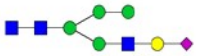
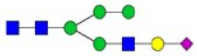
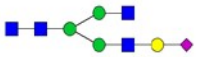
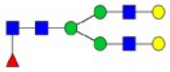
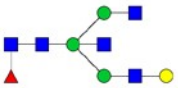
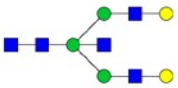
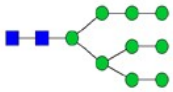
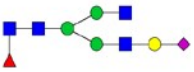
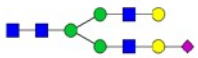
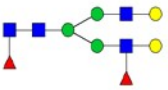
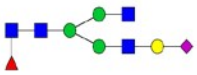
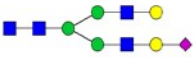
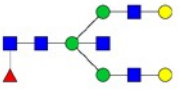
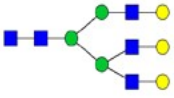
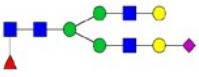
Calvin R.K. Blaschke, Alyson P. Black, Anand S. Mehta, Peggi M. Angel and Richard R. Drake

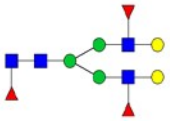
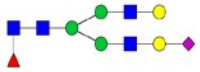
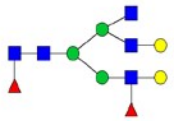
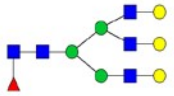
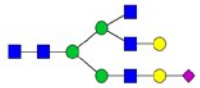
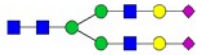
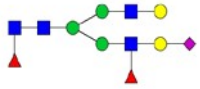
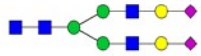
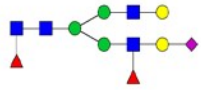
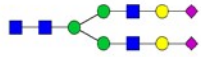
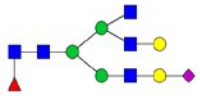
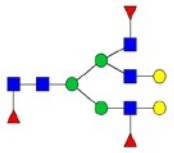
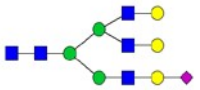
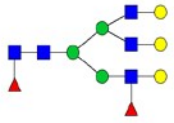
Medical University of South Carolina, Dept. of Cell and Molecular Pharmacology, Charleston, SC, 29425 South Carolina, USA

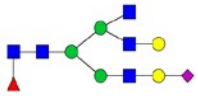
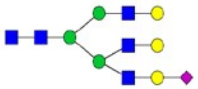
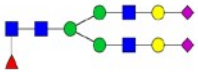
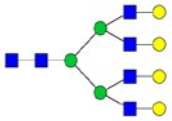
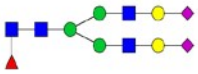
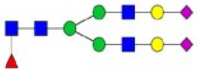
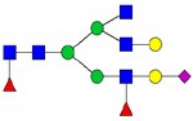
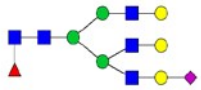
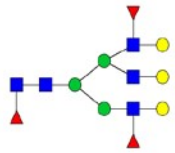
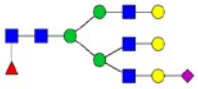
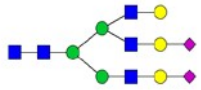
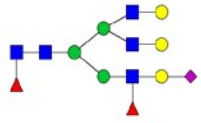
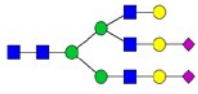
Running Title: Rapid N-Glycan Profiling of Serum and Plasma

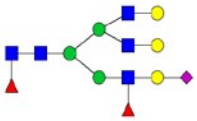
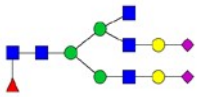
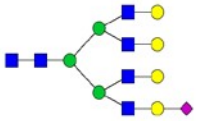
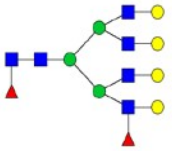
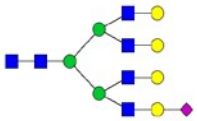
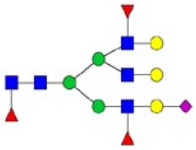
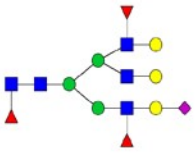
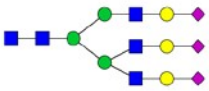
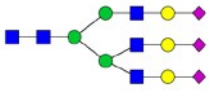
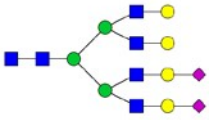
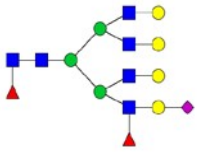
Address reprint requests to:
Richard R. Drake
Medical University of South Carolina
Dept. of Cell and Molecular Pharmacology
173 Ashley Avenue, BSB358
Charleston, South Carolina 29425, USA
843-792-4505
draker@musc.edu

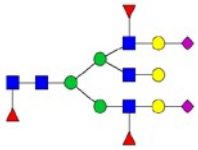
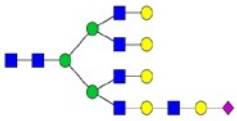
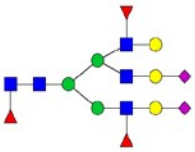
Observed Mass	Theoretical Mass	Error (ppm)	Error (Da)	Composition	Putative Structure	Glycosylation Traits
1136.3936	1136.3964	-2.46	0.0028	Hex3HexNAc3 + 1Na		
1257.4178	1257.4226	-3.82	0.0048	Hex5HexNAc2 + 1Na		Man
1282.4618	1282.4543	5.85	0.0075	Hex3dHex1HexNAc3 + 1Na		Fuc
1298.4501	1298.4492	0.69	0.0009	Hex4HexNAc3 + 1Na		
1339.4710	1339.4757	-3.51	0.0047	Hex3HexNAc4 + 1Na		Bi
1419.4712	1419.4755	-3.03	0.0043	Hex6HexNAc2 + 1Na		Man
1444.5052	1444.5071	-1.32	0.0019	Hex4dHex1HexNAc3 + 1Na		Fuc
1460.5025	1460.5020	0.34	0.0005	Hex5HexNAc3 + 1Na		
1485.5323	1485.5337	-0.94	0.0014	Hex3dHex1HexNAc4 + 1Na		Fuc, Bi
1501.5287	1501.5286	0.07	0.0001	Hex4HexNAc4 + 1Na		Bi
1542.5362*	1542.5551	-12.25	0.0189	Hex3HexNAc5 + 1Na		Bis
1581.5275	1581.5282	-0.44	0.0007	Hex7HexNAc2 + 1Na		Man
1606.5643	1606.5599	2.74	0.0044	Hex5dHex1HexNAc3 + 1Na		Fuc
1611.5181	1611.5266	-5.27	0.0085	Hex4HexNAc3NeuAc1+ 2Na		Sia
1647.5813	1647.5865	-3.16	0.0052	Hex4dHex1HexNAc4 + 1Na		Fuc, Bi
1663.5813	1663.5814	-0.06	0.0001	Hex5HexNAc4 + 1Na		Bi
1688.6115*	1688.6130	-0.89	0.0015	Hex3dHex1HexNAc5 + 1Na		Fuc, Bis

1704.6006	1704.6079	-4.28	0.0073	Hex4HexNAc5 + 1Na		Bis
1743.5810	1743.5810	0.00	0.0000	Hex8HexNAc2 + 1Na		Man
1751.5730	1751.5974	-13.93	0.0244	Hex5HexNAc3NeuAC1 + 1Na		Sia
1773.5710	1773.5872	-9.13	0.0162	Hex5HexNAc3NeuAC1 + 2Na		Sia
1792.6277	1792.6240	2.06	0.0037	Hex4HexNAc4NeuAc1 + 1Na		Sia, Bi
1809.6462	1809.6393	3.81	0.0069	Hex5dHex1HexNAc4 + 1Na		Fuc, Bi
1850.6678	1850.6659	1.03	0.0019	Hex4dHex1HexNAc5 + 1Na		Fuc, Bis
1866.6728	1866.6608	6.43	0.0120	Hex5HexNAc5 + 1Na		Bis
1905.6358	1905.6338	1.05	0.0020	Hex9HexNAc2 + 1Na		Man
1938.6922	1938.6819	5.31	0.0103	Hex4dHex1HexNAc4NeuAc1 + 1Na		Fuc, Sia, Bi
1954.6722	1954.6768	-2.35	0.0046	Hex5HexNAc4NeuAc1 + 1Na		Bi, Sia
1955.6731	1955.6972	-12.32	0.0241	Hex5dHex2HexNAc4 + 1Na		Fuc, Bi
1960.6604	1960.6638	-1.73	0.0034	Hex4dHex1HexNAc4NeuAc1 + 2Na		Fuc, Sia, Bi
1976.6556	1976.6666	-5.56	0.0110	Hex5HexNAc4NeuAc1 + 2Na		Sia, Bi
2012.7304	2012.7187	5.81	0.0117	Hex5dHex1HexNAc5 + 1Na		Fuc, Bis
2028.7138	2028.7136	0.10	0.0002	Hex6HexNAc5 + 1Na		Tri
2100.7473	2100.7347	6.00	0.0126	Hex5dHex1HexNAc4NeuAc1 + 1Na		Fuc, Bi, Sia

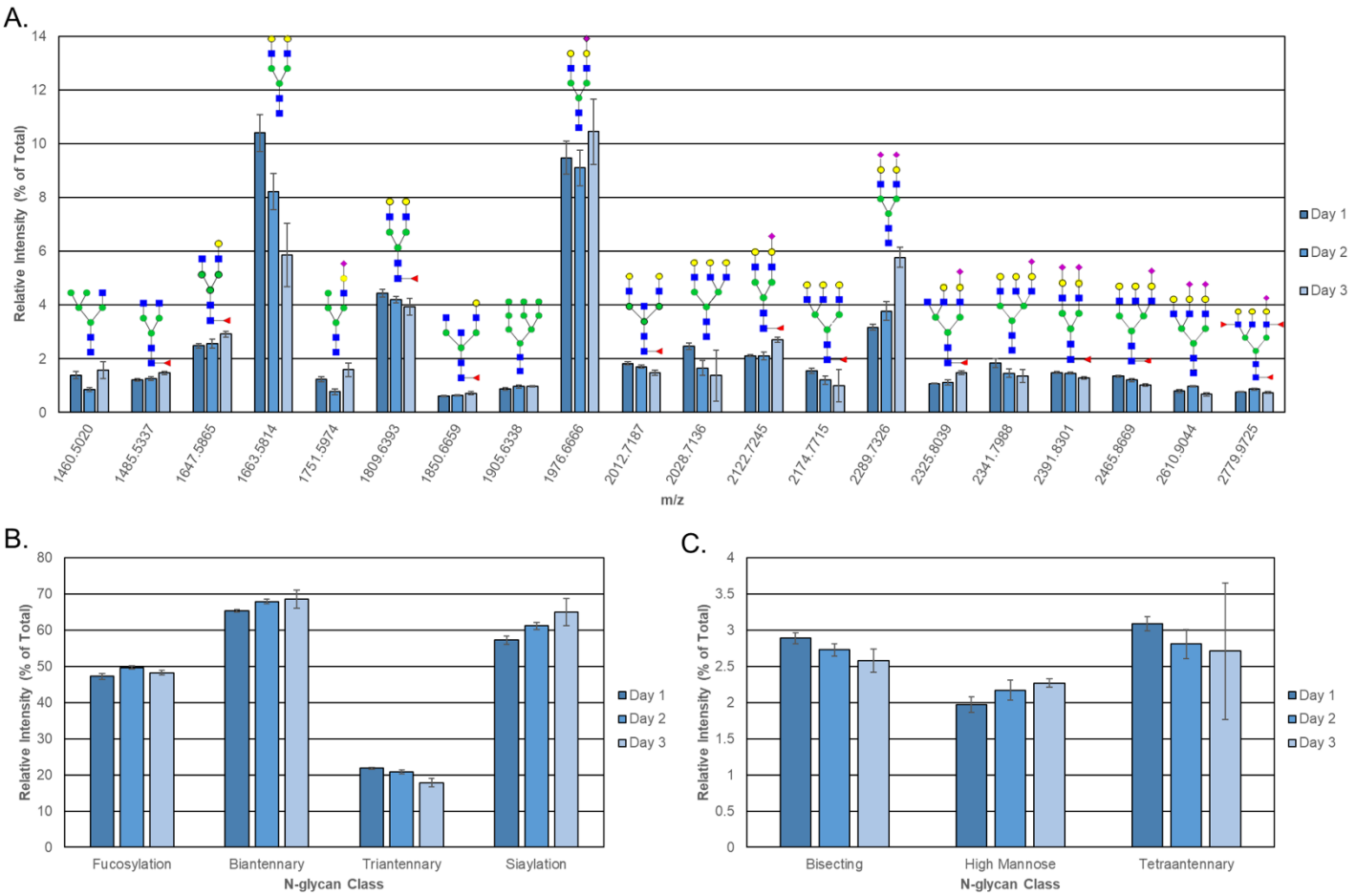
2101.7489	2101.7551	-2.95	0.0062	Hex5dHex3HexNAc4 + 1Na		Fuc, Bi
2122.7150	2122.7245	-4.48	0.0095	Hex5dHex1HexNAc4NeuAc1 + 2Na		Fuc, Sia, Bi
2158.7939	2158.7766	8.01	0.0173	Hex5dHex2HexNAc5 + 1Na		Fuc, Tri
2174.7955	2174.7715	11.04	0.0240	Hex6dHex1HexNAc5 + 1Na		Fuc, Tri
2179.7617	2179.7460	7.20	0.0157	Hex5HexNAc5NeuAc1 + 2Na		Tri, Sia
2245.7654	2245.7722	-3.03	0.0068	Hex5HexNAc4NeuAc2 + 1Na		Bi, Sia
2246.7704	2246.7926	-9.88	0.0222	Hex5dHex2HexNAc4NeuAc1 + 1Na		Fuc, Bi, Sia
2267.7636	2267.7620	0.71	0.0016	Hex5HexNAc4NeuAc2 + 2Na		Bi, SIa
2268.7652	2268.7824	-7.57	0.0172	Hex5dHex2HexNAc4NeuAc1 + 2Na		Fuc, Bi, Sia
2289.7561	2289.7326	10.26	0.0235	Hex5HexNAc4NeuAc2 + 3Na		Bi, Sia
2303.8430	2303.8141	12.54	0.0289	Hex5dHex1HexNAc5NeuAc1 + 1Na		Tri, Fuc, Sia
2304.8372	2304.8345	1.17	0.0027	Hex5dHex3HexNAc5 + 1Na		Tri, Fuc
2319.8280	2319.8090	8.19	0.0190	Hex6HexNAc5NeuAc1 + 1Na		Tri, Sia
2320.8293	2320.8294	-0.04	0.0001	Hex6dHex2HexNAc5 + 1Na		Tri, Fuc

2325.8060	2325.8039	0.90	0.0021	Hex5dHex1HexNAc5NeuAc1 + 2Na		Tri, Sia, Fuc
2341.7948	2341.7988	-1.71	0.0040	Hex6HexNAc5NeuAc1 + 2Na		Tri, Sia
2391.8211	2391.8301	-3.76	0.0090	Hex5dHex1HexNAc4NeuAc2 + 1Na		Fuc, Bi, Sia
2393.8442	2393.8458	-0.67	0.0016	Hex7HexNAc6 + 1Na		Tetra
2413.8225	2413.8199	1.08	0.0026	Hex5dHex1HexNAc4NeuAc2 + 2Na		Fuc, Bi, Sia
2435.8429	2435.8097	13.63	0.0332	Hex5dHex1HexNAc4NeuAc2 + 3Na		Fuc, Bi, Sia
2449.8932	2449.8720	8.65	0.0212	Hex5dHex2HexNAc5NeuAc1 + 1Na		Tri, Fuc, Sia
2465.8986	2465.8669	12.86	0.0317	Hex6dHex1HexNAc5NeuAc1 + 1Na		Fuc, Tri, Sia
2466.9006	2466.8873	5.39	0.0133	Hex6dHex3HexNAc5 + 1Na		Fuc, Tri
2487.8561	2487.8567	-0.24	0.0006	Hex6dHex1HexNAc5NeuAc1 + 2Na		Fuc, Tri, Sia
2610.9273	2610.9044	8.77	0.0229	Hex6HexNAc5NeuAc2 + 1Na		Tri, Sia
2611.9374	2611.9248	4.82	0.0126	Hex6dHex2HexNAc5NeuAc1 + 1Na		Fuc, Tri, Sia
2632.9301	2632.8942	13.65	0.0359	Hex6HexNAc5NeuAc2 + 2Na		Tri, Sia

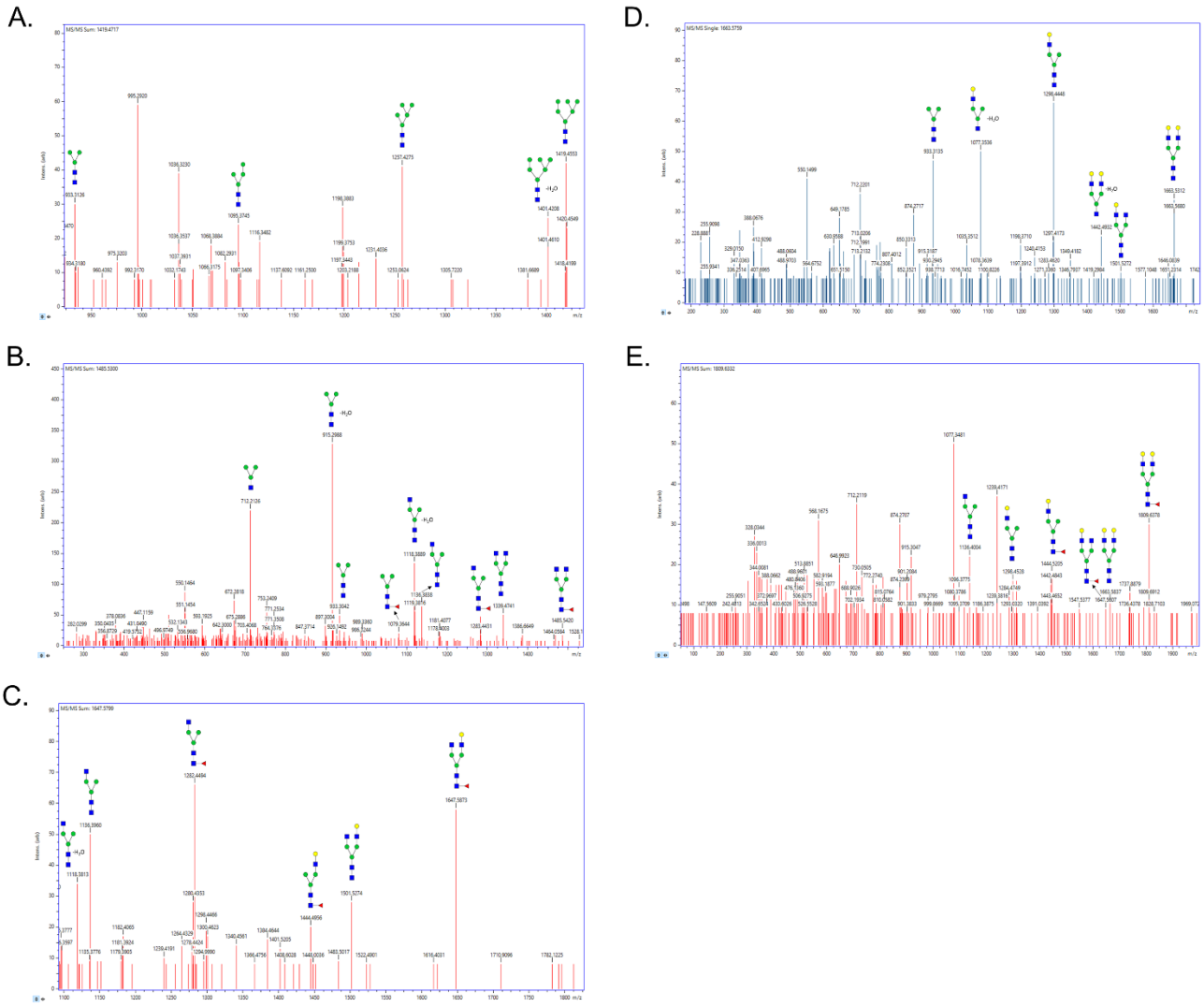
2633.9313	2633.9146	6.34	0.0167	Hex6dHex2HexNAc5NeuAc1 + 2Na		Fuc, Tri, Sia
2638.9272	2638.8891	14.44	0.0381	Hex5dHex1HexNAc5NeuAc2 + 3Na		Fuc, Tri, Sia
2684.9569	2684.9412	5.85	0.0157	Hex7HexNAc6NeuAc1 + 1Na		Tetra, Sia
2685.9739	2685.9616	4.58	0.0123	Hex7dHex2HexNAc6 + 1Na		Tetra, Fuc
2706.9205	2706.9310	-3.88	0.0105	Hex7HexNAc6NeuAc1 + 2Na		Tetra, Sia
2757.9599	2757.9827	-8.27	0.0228	Hex6dHex3HexNAc5NeuAc1 + 1Na		Tri, Fuc, Sia
2779.9401	2779.9725	-11.65	0.0324	Hex6dHex3HexNAc5NeuAc1 + 2Na		Tri, Fuc, Sia
2902.0267	2901.9998	9.27	0.0269	Hex6HexNAc5NeuAc3 + 1Na		Tri, Sia
2923.9973	2923.9896	2.63	0.0077	Hex6HexNAc5NeuAc3 + 2Na		Tri, Sia
2976.0366*	2976.0808	-14.85	0.0442	Hex7HexNAc6NeuAc2 + 1Na		Tetra, Sia
2977.0911	2977.0570	11.45	0.0341	Hex7dHex2HexNAc6NeuAc1 + 1Na		Tetra, Fuc, Sia

3049.0833	3049.0781	1.71	0.0052	Hex6dHex3HexNAc5NeuAc2 + 1Na		Tri, Fuc, Sia
3050.0908	3050.0734	5.71	0.0174	Hex8HexNAc7NeuAc1 + 1Na		Tetra, Sia
3071.0456	3071.0679	-7.25	0.0223	Hex6dHex3HexNAc5NeuAc2 + 2Na		Tri, Fuc, Sia

Supplemental Table 1. Peak list of N-glycans detected in serum and plasma. N-glycans marked with an asterisk (*) were only seen in serum samples from patients with benign lesions or breast cancer. Man = high mannose, Fuc = fucosylated, Com = complex, Bi = bi-antennary, Tri = tri-antennary, Tetra = tetra-antennary, Bis = bisected, Sia = sialylated.



Supplementary Figure 1. Repeatability of the N-glycan profiling method for plasma. The same plasma standard sample was analyzed on three successive days with a blank well that contained no serum on each run. (A) The graph shows the average relative intensities detected for the twenty most abundant N-glycans (normalized to the overall sum of N-glycan intensities) with putative structures. For determining the most abundant N-glycans, only N-glycans with no overlap with another N-glycan peak isotope were included, and only one species was selected for sialylated N-glycans with multiple sodiated adducts. Structural classes were assigned to the N-glycans, and the sum of the N-glycans for the (B) high abundance and (C) low abundance classes are displayed. Error bars indicate the standard deviation of the four replicates.



Supplementary Figure 2. MS/MS of serum N-glycans by CID done directly on the slide. A) *m/z* = 1419.4755, B) *m/z* = 1485.5337, C) *m/z* = 1647.5865, D) *m/z* = 1663.5814, and E) *m/z* = 1809.6393 N-glycan structures were confirmed with MALDI-TOF MS/MS. The collision energies and number of laser shots were individually optimized for each N-glycan.