

Factors Affecting Anti-Glycan IgG and IgM Repertoires in Human Serum

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Sample collection

Sample processing and storage by both providers were similar except for how whole blood was clotted and centrifuged to obtain serum (room temperature for Valley Biomedical Products and Services versus 4 °C for Bioreclamation LLC). Briefly Blood was allowed to clot overnight at 4 °C (Bioreclamation) or at room temperature (Valley Biomedical Products and Services). The following day the serum is centrifuged, collected, and stored at -20 °C.

The validation set (n = 90) which included samples from both providers, 60 samples from Valley Biomedical Products and Services and 30 samples from Bioreclamation LLC was evaluated for whether differences in anti-glycan antibody signals due to possible differences in how the samples were collected. To assess the comparability of data obtained for serum from both providers, the average IgG and IgM intensities for each glycan in the array were calculated from each provider. The average IgG and IgM antibody signals were similar from both providers (Figure S5). The data suggests that sample collection and handling by the two providers in this study have minimal effect on anti-glycan antibody levels.

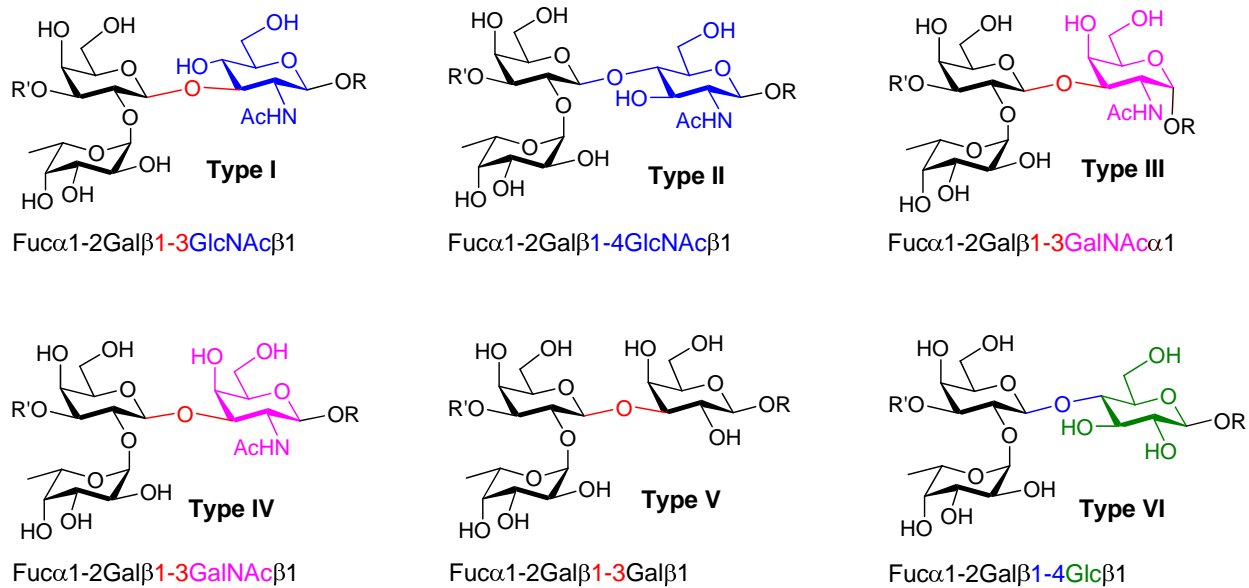
Table SI. List and description of sample characteristics from healthy individuals.

Sample #	Sex	Race	Age	Blood Type	Location
1	M	African-American	32	O	Florida
2	M	Hispanic	46	O	Florida
3	M	Caucasian	44	B	Florida
4	M	African-American	22	O	Florida
5	M	Caucasian	27	AB	Florida
6	M	African-American	43	O	Florida
7	M	African-American	25	O	Florida
8	M	Caucasian	29	AB	Florida
9	M	African-American	26	A	Florida
10	M	Hispanic	34	O	Florida
11	M	Hispanic	23	A	Florida
12	M	Caucasian	33	O	Florida
13	M	Caucasian	41	O	Florida
14	M	Caucasian	23	O	Florida
15	M	Caucasian	49	O	Florida
16	M	African-American	39	A	Florida
17	M	Hispanic	49	A	Florida
18	M	Caucasian	42	A	Florida
19	M	African-American	21	B	Florida
20	M	African-American	33	A	Florida
21	F	Caucasian	34	AB	Florida
22	F	Hispanic	34	A	Florida
23	F	African-American	50	A	Tennessee
24	F	African-American	38	AB	Florida
25	F	Hispanic	43	A	Florida
26	F	Hispanic	36	AB	Florida
27	F	Hispanic	21	AB	Florida
28	F	Caucasian	28	O	Tennessee
29	F	Caucasian	43	A	Tennessee
30	F	African-American	23	AB	Florida
31	F	Hispanic	42	AB	Florida
32	F	Hispanic	27	A	Florida
33	F	Caucasian	21	A	Tennessee
34	F	Hispanic	38	O	Florida
35	F	Hispanic	41	O	Florida
36	F	African-American	20	B	Tennessee
37	F	African-American	27	AB	Florida
38	F	Hispanic	41	AB	Tennessee
39	F	Hispanic	23	O	Florida

40	F	African-American	30	B	Tennessee
41	F	Caucasian	32	A	Tennessee
42	F	Caucasian	45	AB	Tennessee
43	F	Caucasian	36	O	Tennessee
44	F	African-American	43	AB	Florida
45	F	Caucasian	36	B	Tennessee
46	M	African-American	25	A	Florida
47	F	Hispanic	40	A	Florida
48	M	African-American	31	B	Florida
49	F	Hispanic	30	AB	Florida
50	M	Hispanic	38	O	Florida
51	F	African-American	40	B	Tennessee
52	M	Hispanic	44	O	Florida
53	F	Caucasian	32	A	Tennessee
54	M	Hispanic	24	A	Florida
55	F	Caucasian	42	A	Tennessee
56	M	Caucasian	41	B	Florida
57	F	Hispanic	22	O	Florida
58	M	Caucasian	26	O	Florida
59	F	African-American	43	O	Tennessee
60	M	African-American	40	O	Florida
61	F	Caucasian	45	O	Tennessee
62	M	Caucasian	43	O	Florida
63	F	Caucasian	23	AB	Florida
64	M	Caucasian	36	O	Florida
65	F	Caucasian	20	A	Tennessee
66	M	African-American	38	O	Florida
67	F	Caucasian	34	O	Tennessee
68	M	Hispanic	22	O	Florida
69	F	African-American	42	AB	Florida
70	M	African-American	40	A	Florida
71	F	Hispanic	39	AB	Florida
72	M	African-American	46	O	Florida
73	F	African-American	35	AB	Florida
74	M	Hispanic	45	O	Florida
75	F	Caucasian	33	A	Tennessee
76	M	Hispanic	30	A	Florida
77	F	Hispanic	37	A	Florida
78	M	African-American	23	O	Florida
79	F	Hispanic	31	AB	Florida
80	M	Caucasian	50	A	Florida

81	F	African-American	47	AB	Florida
82	M	African-American	20	B	Florida
83	F	African-American	38	AB	Florida
84	M	Hispanic	28	A	Florida
85	F	Caucasian	30	A	Tennessee
86	M	Caucasian	29	B	Florida
87	F	Hispanic	43	A	Florida
88	M	Caucasian	38	A	Florida
89	F	Caucasian	31	O	Tennessee
90	M	African-American	43	O	Florida
91	F	Hispanic	22	O	Florida
92	M	Caucasian	34	O	Florida
93	F	Hispanic	26	O	Florida
94	M	African-American	34	B	Florida
95	F	Caucasian	50	O	Tennessee
96	M	Hispanic	48	A	Florida
97	F	Caucasian	32	A	Tennessee
98	M	African-American	45	O	Florida
99	F	African-American	28	AB	Florida
100	M	Caucasian	21	O	Florida
101	F	African-American	44	A	Tennessee
102	M	Hispanic	36	A	Florida
103	F	African-American	43	AB	Florida
104	M	Hispanic	27	A	Florida
105	M	African-American	25	B	Florida
106	F	African-American	29	O	-
107	M	Hispanic	20	O	-
108	M	Hispanic	34	O	-
109	F	African-American	36	O	-
110	M	African-American	42	O	-
111	F	African-American	40	O	-
112	F	Hispanic	60	O	-
113	M	African-American	54	O	-
114	F	Hispanic	55	O	-
115	F	African-American	27	A	-
116	M	Caucasian	23	A	-
117	F	Caucasian	38	A	-
118	M	Hispanic	31	A	-
119	M	African-American	41	A	-
120	F	African-American	46	A	-
121	M	Caucasian	58	A	-

122	M	African-American	65	A	-
123	F	Hispanic	41	A	-
124	M	Caucasian	42	O	-
125	F	Hispanic	42	O	-
126	M	African-American	43	B	-
127	F	Hispanic	43	AB	-
128	F	Caucasian	43	AB	-
129	F	African-American	43	O	-
130	M	Caucasian	44	B	-
131	F	Caucasian	45	A	-
132	M	Hispanic	46	AB	-
133	M	Caucasian	49	O	-
134	M	Hispanic	49	AB	-
135	F	African-American	50	B	-



R = glycoprotein or glycolipid

R' = H for H-antigens, R' = GalNAc α 1 for A-antigens, or R' = Gal α 1 for B-antigens

Figure S1. Structures of the different types of ABH blood group antigens (I-VI).

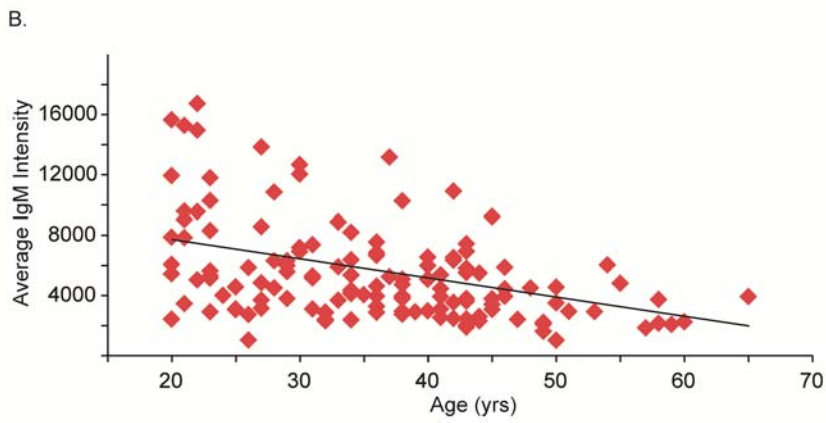
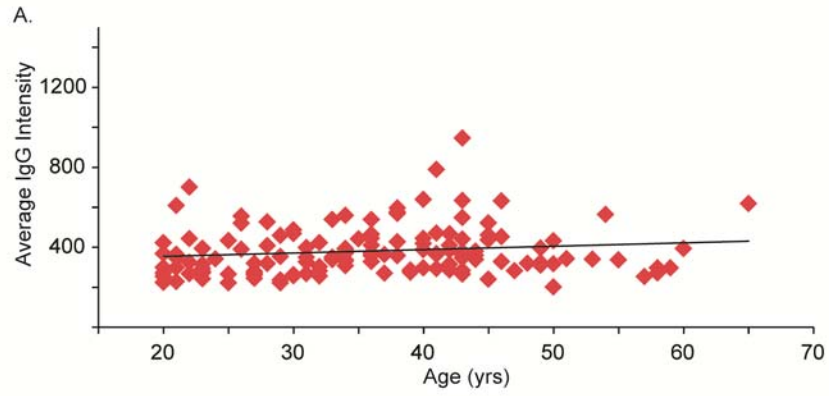


Figure S2. Age-related variations in anti-glycan antibodies in apparently healthy individuals (n = 135). **(A)** Scatter plot showing a relatively stable IgG antibody levels with age. **(B)** Scatter plot showing a drop in IgM antibody levels with age.

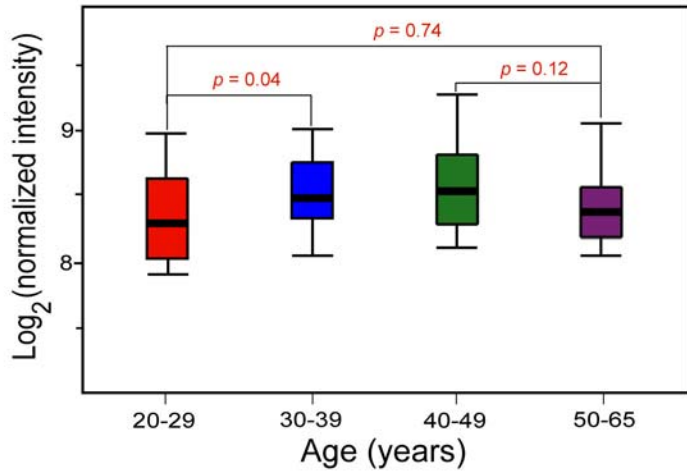


Figure S3. Box plots show minimum variation in anti-glycan IgG antibody levels with age when subjects were grouped every 10 years.

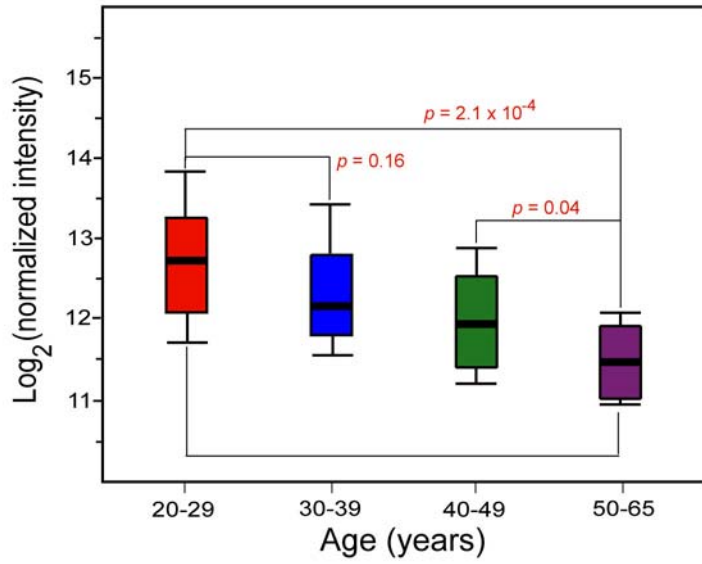


Figure S4. Box plots showing variations in anti-glycan IgM antibody levels with age when subjects were grouped every 10 years.

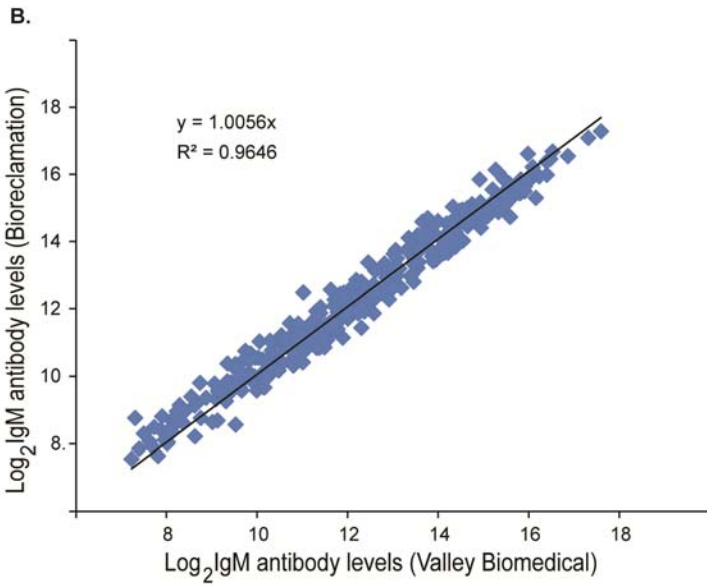
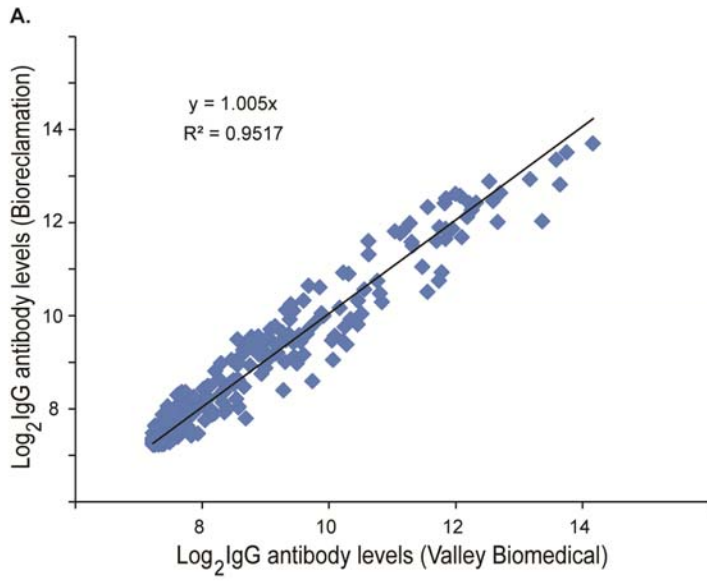


Figure S5. Scatter plots showing little variations in anti-glycan antibody levels due to sample collection and handling. **(A)** Level of IgG antibodies. **(B)** Level of IgM antibodies.

Pre-experiment images

Post-experiment images

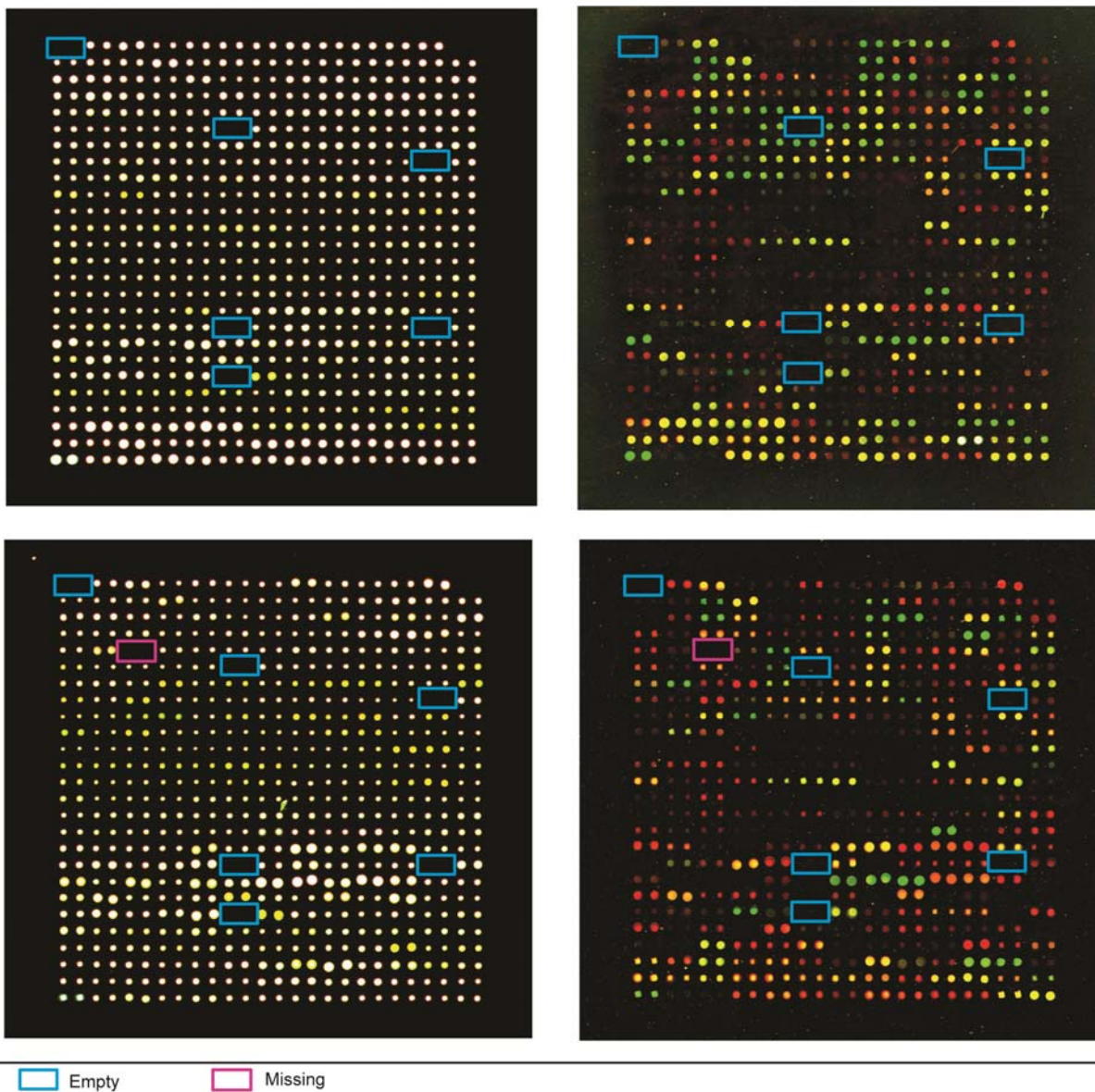


Figure S6. Representative examples of pre- and post-experimental array images. Signals in the Pre-experiment images are due to inclusion of a soluble fluorescent dye in the print buffer (the free acid of DyLight 649), which is washed away during blocking tp. Blue boxes indicate spots that were printed from empty wells, and pink boxes indicate spots that were missing during printing of the arrays.