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Multiplexed, Isotype-Specific Ultrasensitive Research-Use Bridging Serology Assays for Detection of Autoimmune Reactivities

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1 Abstract

Background: Autoimmune diseases affect over 50 million Americans and have rapidly rising incidence. The presence of multiple specific autoantibodies can often predict disease onset in at-risk individuals [e.g. type 1 diabetes (T1D), systemic lupus erythematosus, celiac disease], and assist in distinguishing disorders with similar clinical features (e.g. T1D versus type 2 diabetes). In an ongoing study to assess the feasibility of developing highly sensitive and specific multiplexed antigen-bridging serology panels for detection of autoimmune biomarkers, the MSD® MULTI-ARRAY technology is being used to produce research-use assays for detection of T1D, other organ-specific autoimmune disease, and connective tissue disorder biomarkers. First generation multiplexed assay panels developed to detect T1D autoantibodies to glutamic acid decarboxylase (GADA), insulinoma 2 (IA2A), and insulin (IAA), as well as markers relevant to celiac disease, autoimmune gastritis, and thyroid disease, were previously tested using assay proficiency evaluation samples from the Islet Autoantibody Standardization Program. In those studies, the MSD T1D-relevant assays performed comparably to existing assays, with the advantages of low sample requirements for multiplexed detection (<25 µL needed for detection of up to 10 biomarkers), high throughput, and no radioactivity [Mathew et al. (2016) Diabetes 65(Supplement 1):A431-A440, 1673P]. In the current phase of the project, next generation assays have been developed using MSD's ultrasensitive assay format to enhance multiplexed detection of autoimmune disease-related reactivities with the additional capability to discern autoantibody isotypes.

Objective: The objective of the study was to compare performance of the first and second generation MSD bridging serology assay panels in a 96-well plate, high throughput format. The assays were assessed using commercially purchased T1D and presumably normal individual sera. The multiplexing capability enabled simultaneous quantitative measurement of T1D and comorbid disease markers. The second generation assay was formatted for detection of IgG reactivities, but can easily be formatted for detection of other antibody isotypes (IgA, IgM).

Results: Preliminary data show that assay sensitivities/specificities improve from 65%/100% to 91%/96% (IAA detection), 57%/100% to 70%/92% (GADA detection), and 35%/95% to 48%/96% (IA2A detection) when comparing the first and second generation assays, respectively. Furthermore, ultrasensitive assays were included for detection of pro-insulin autoantibodies (pro-IAA) and zinc transporter 8 (ZnT8) that demonstrated initial specificity/sensitivity values of 96%/100% and 22%/92%, respectively. Two or more T1D reactivities were detected in eighteen of the twenty-four T1D samples tested and in one of the twenty-five normal samples screened.

Conclusion: The sample-sparing ultrasensitive multiplexed MSD autoantibody assays significantly enhance the ability to identify samples containing multiple autoimmune reactivities, with the capability to distinguish specific antibody isotypes, while retaining the high specificity of the bridging serology assay format.

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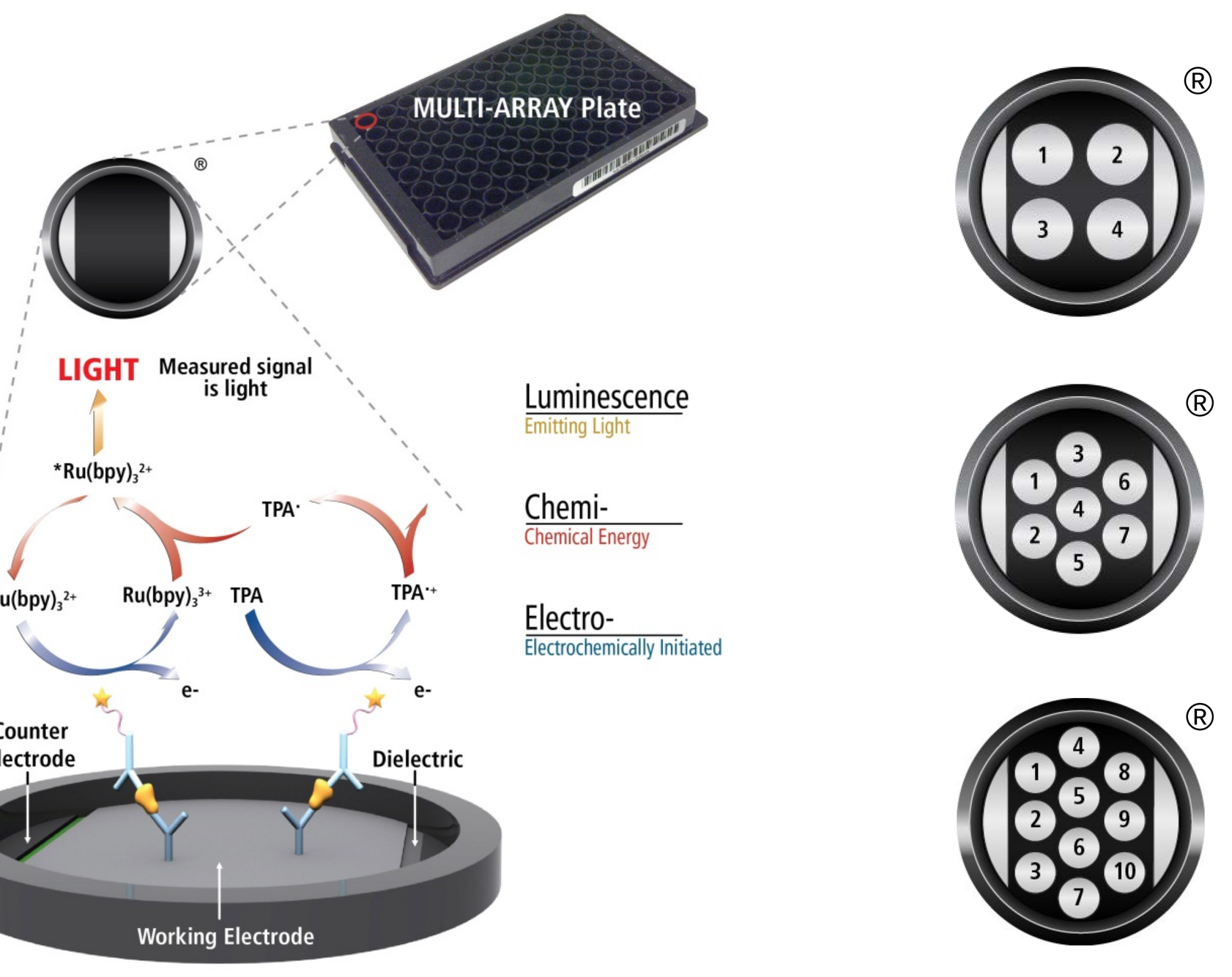
2 MSD Technology

MSD's electrochemiluminescence detection technology uses SULFO-TAG™ labels that emit light upon electrochemical stimulation initiated at the electrode surfaces of MULTI-ARRAY® and MULTI-SPOT® microplates.



Electrochemiluminescence Technology

- Minimal non-specific background and strong responses to analyte yield high signal-to-background ratios.
- The stimulation mechanism (electricity) is decoupled from the response (light signal), minimizing matrix interference.
- Only labels bound near the electrode surface are excited, enabling non-washed assays.
- Labels are stable, non-radioactive, and directly conjugated to biological molecules.
- Emission at ~620 nm eliminates problems with color quenching.
- Multiple rounds of label excitation and emission enhance light levels and improve sensitivity.
- Carbon electrode surface has 10X greater binding capacity than polystyrene wells.
- Surface coatings can be customized.

3 Methodology and Preliminary Data

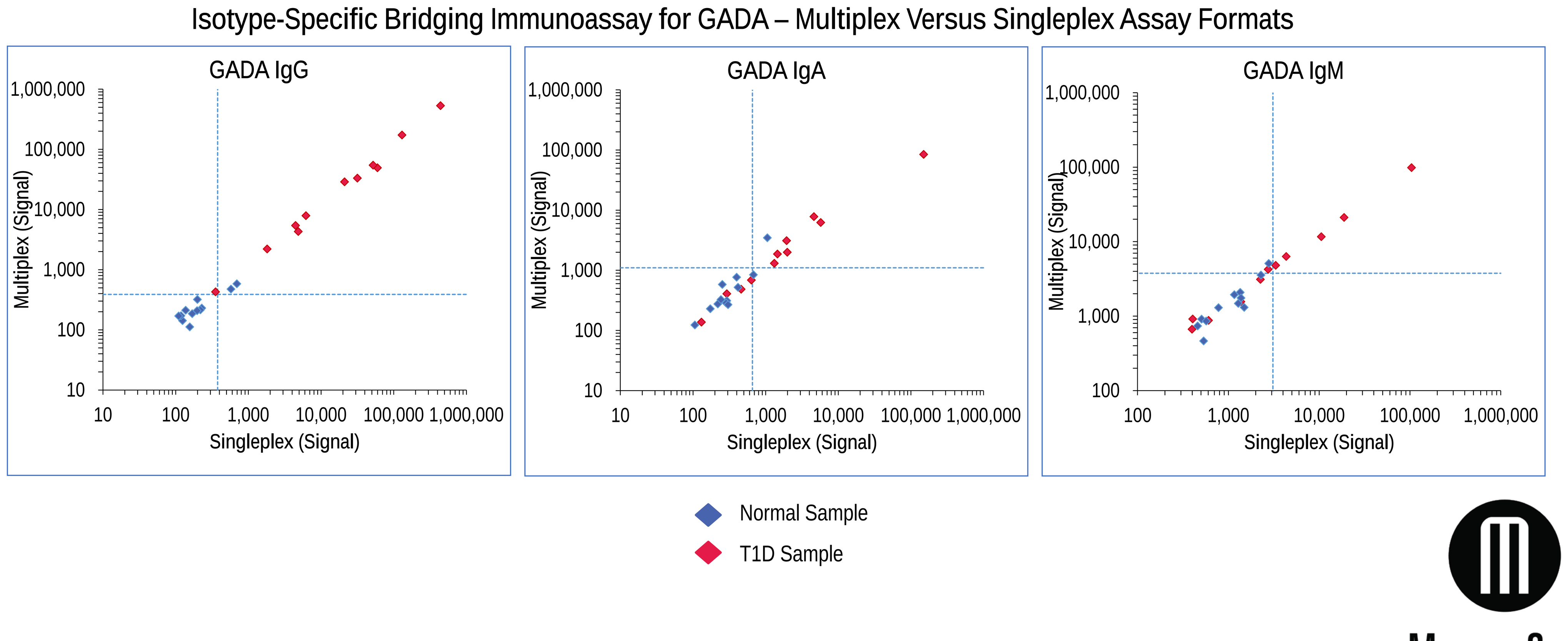
The bridging assay format is used to detect antigen-specific autoantibodies in serum samples. This method uses autoantigens labeled with either detection or capture tags. Antigen-specific antibodies must bind to both species of antigen to obtain signal, ensuring improved assay specificity. Using U-PLEX® technology, we combine the performance advantages of the bridging format with the sample-sparing advantages of MSD multiplexed assays. Simultaneous detection of multiple autoantigen reactivities minimizes the amount of sample needed. Samples are tested in duplicate, along with MSD positive control sera, where available. First generation multiplexed bridging assays for T1D-relevant biomarkers have previously been demonstrated to show excellent discrimination of positive from negative reactive samples using the IASP blinded sample cohort. The current generation of assays incorporates an optimized assay format and adds the ability to distinguish antibody isotypes. Preliminary data were generated using commercially purchased T1D and normal serum samples, prior to full assay optimization, generating the data provided in the abstract and below.

	IAA		GADA		IA2A		pro-IAA	ZnT8A
	1st Gen	2nd Gen (IgG)	1st Gen	2nd Gen (IgG)	1st Gen	2nd Gen (IgG)	2nd Gen (IgG)	2nd Gen (IgG)
Sensitivity	65%	91%	57%	70%	35%	48%	96%	22%
Specificity	100%	96%	100%	92%	95%	96%	100%	92%

Isotype-specific MSD bridging serology assays can be multiplexed with minimal effects on assay performance. Data are shown for the IgG, IgA, and IgM GADA reactivities tested in singleplex, or multiplexed with IAA, pro-IAA, and ZnT8A, using commercially purchased normal and T1D serum samples.

Dashed lines in the figures below denote assay cutoffs determined as median signal of normal samples + 2.2 x interquartile range (IQR).

Isotype-Specific Bridging Immunoassay for GADA – Multiplex Versus Singleplex Assay Formats



4 Sample Testing Results

Second generation isotype-specific bridging serology assays to detect the T1D-relevant IAA, pro-IAA, ZnT8A, GADA, and IA2A reactivities were further optimized and combined with newly developed assays to measure autoantibodies to Transglutaminase (TGA, celiac disease), Deamidated Gliadin Peptides (DGPA, celiac disease), Thyroglobulin (TGBA, autoimmune thyroid disease), 17-Hydroxylase (17-OHA, Addison's disease), Jo-1 (Jo-1A, polymyositis), and Intrinsic Factor (IFA, pernicious anemia). These assays were multiplexed in panels based on assay compatibilities and required sample dilutions. Samples were tested for IgG and IgA reactivities in duplicate, using less than 50 µL of each serum sample for all measurements.

The serum samples from the University of Florida used for testing were:

(1) 96 T1D or other autoimmune disease biomarker positive samples characterized for individual reactivities (levels of other reactivities and disease status not defined).

- 16 positive samples each for IAA (University of Florida in-house macro-IAA RIA), GADA (Kronus RIA), and IA2A (Kronus RIA)
- 18 ZnT8A positive samples (Kronus ELISA)
- 10 positive samples each for TGA (Kronus RIA), Thyroid Peroxidase (TPOA, Kronus RIA), and adrenal cell autoantibodies (ACA, LDT indirect immunofluorescence using human adrenal tissue)

(2) 64 age/gender matched normal serum samples. These samples have not been screened for T1D or other autoimmune disease markers and are hence uncharacterized.

Average sample signal data are summarized at right, with signal intensities observed spanning 3-4 orders of magnitude. Sample signals above each assay's cutoff signal value (median + 2.2 x IQR) are highlighted in red font.

The results demonstrate that 85% of the individual biomarker positive samples exhibit more than one immune reactivity, with varied combinations of markers observed. Of the provided T1D marker positive samples, 97% show multiple autoimmune reactivities, and 92% are positive for more than one T1D-specific marker. The magnitudes of the signals observed in the marker positive samples are much higher than in the normal serum samples.

Among the uncharacterized normal samples, 34% exhibit multiple reactivities, and 14% have multiple T1D-specific markers.

Samples	IAA Signal IgG	IAA Signal IgA	pro-IAA Signal IgG	pro-IAA Signal IgA	ZnT8A Signal IgG	ZnT8A Signal IgA	IA2A Signal IgG	IA2A Signal IgA	GADA Signal IgG	GADA Signal IgA	TGA Signal IgG	TGA Signal IgA	DGPA Signal IgG	DGPA Signal IgA	TGBA Signal IgG	TGBA Signal IgA	17-OHA Signal IgG	17-OHA Signal IgA	Jo1A Signal IgG	Jo1A Signal IgA	IFA Signal IgG	IFA Signal IgA
IAA Positive Samples	1,282	398	839	250	846,826	31,656	1,377,446	15,991	11,213	396	147	140	89,708	5,865	4,159	812,807	3,425	1,213	1,912	385	76	127
	39,287	3,371	4,612	506	8,680	4,062	21,608	1,477	1,160,884	11,224	6	151	259	179	145	148	9,450	5,667	361	492	379	140
	1,014	2,653	253	155	2,440	3,538	95,570	659	1,879	432	32	6	358	715	85	75	2,442	4,784	612	653	122	1,917
	148	177	78	122	8,727	1,603	239,054	2,301	590	309	44	67	70	74	89	58	1,203	717	575	1,001	83	119
	2,393	291	727	166	1,616,509	127,048	1,752,208	148,888	1,663,679	18,591	77	118	87,938	19,042	549	163,076	2,952	1,561	2,964	637	203	140
	2,102	1,578	457	1,818	3,318	3,573	909	3,600	1,171,105	62,831	38	106	788	8,242	195	645	3,407	7,226	459	869	1,210	279
	806	249	214	182	187,486	1,877	1,331,371	43,035	5,645	11,004	58	60	621	1,571	124	131	1,486	395	938	824	43	117
	1,426	223	353	115	829,106	118,730	2,410,062	638,408	2,908	481	85	60	90	123	175	93	903	884	9,993	721	37	123
	715	756	218	100	1,406	724	40,540	381	2,097	184	84	334	59	102	39	82	809	254	402	371	173	70
	7,179	1,365	411	676	9,149	2,140	4,883	355	2,069	220	64	91	60	359	40	67	335	694	257	264	123	99
	284	278	131	98	1,481	963	495	323	282	114	60	127	28	155	58	119	366	383	307	315	93	85
	1,880	321	242	388	51,521	6,915	524	165	289	109	88	404	1,172	478	52	60	428	856	348	243	135	95
	351	301	147	98	2,379	1,830	600	258	201	147	115	112	1,212	1,280	61	99	506	760	371	364	85	127
	9,236	1,159	727	187	5,120	1,411	34,073	1,370	1,194	195	99	219	55	274	2,331	297	774	763	594	366	94	89
	260	3,361	146	272	1,293	2,653	687	469	189	685	65	1,382	137	422	47	152	244	1,219	373	915	68	290
	344	4,509	121	343	1,298	3,299	544	487	336	1,809	84	1,427	112	365	59	89	355	1,428	419	1,323	132	367
ZnT8A Positive Samples	687	1,026	258	228	953,674	34,902	1,712,184	130,337	705	229	38	64	176	183	115	225	1,964	2,032	2,887	1,051	96	144
	144	203	98	64	11,087	1,627	565	350	750	160	54	48	59	105	99	72	549	1,675	539	311	83	116
	317	402	105	110	11,404	2,795	31,979	1,338	13,057	1,914	51	63	280	199	130	163	5,092	3,690	464	1,020	97	144
	214	233	109	112	22,654	1,294	875	257	4,080	418	47	56	106	227	87	74	1,737	1,108	521	992	60	88
	725	3,939	262	312	3,771	1,835	2,022	592	922,638	71,069	145,968	793	11,061	6,576	192	299	3,911	2,720	404	346	2,222,214	119,922
	199	330	123	181	10,298	2,038	164,502	2,671	316	540	74	90	70	96	62	76	960	859	462	944	85	109
	305	479	199	143	888,775	8,595	601,185	2,172	27,623	772	82	90	1,622	326	135	126	518	738	841	207	81	80
	21,164	860	2,323	462	12,890	2,175	10,606	289	2,897	169	23,695	645	205	232	174	265	6,221	1,481	534	158	65	56
	190	821	103	341	6,000	2,451	105,158	4,060	3,087	366	144	35,479	6,445	1,733	46	145	887	760	567	485	133	333
	104	164	109	150	16,283	609	1,644,767	10,760	254	153	47	155	212	21,385	11	147	489	342	2,047	318	59	69
	159	182	105	132	4,011	1,777	384,705	3,953	866	164	26	143	72	30,712	95	100	1,666	1,686	826	599	1,207	74
	177	1,663	132	172	94,377	17,900	1,233,934	50,935	1,314,815	157,037	53	214	36	233	55	115	1,359	2,750	1,387	821	771	372
	358	445	150	567	93,880	2,752	1,343,035	37,556	3,131	16,156	93	172	312	1,586	58	120	582	645	1,637	1,050	88	111
	279	1,182	217	175	526,228	19,848	602	596	308	203	133	287	506	10,441	63	129	649	1,038	409	644	91	332
	300	157	173	135	334,342	70,332	2,319,830	545,472	1,274	309	77	121	80	147	110	105	317	617	10,172	1,071	90	98
	151	130	113	161	10,345	1,783	2,404,102	76,204	35,707	1,300	111	279	87	157	69	117	274	488	6,663	565	93	126
	190	19,901	94	11,407	38,062	16,694	1,429,073	332,034	929	6,024	82	3,945	207	7,628	71	8,662	694	16,960	3,152	17,239	76	1,305
	672	980	185	797	2,764	3,091	2,126	744	344,124	24,844	161	257	563	1,287	49	104	410	589	631	786	2,111	285
IA2A Positive Samples	551	1,183	293	394	894,055	40,524	1,918,317	120,655	128,098	426	47	67	150	364	123	137	1,339	1,807	1,362	992	145	137
	904	452	382	287	653,662	27,548	1,571,722	11,933	4,434	1,336	173	148	94,088	10,842	3,686	851,149	3,220	1,192	1,496	324	282	75
	742	1,317	164	108	1,811	2,017	69,160	538	63,505	898	46	57	394	1,082	77	77	1,531	2,003	762	647	141	848
	196	161	82	108	796	1,143	1,342	145	31,680	317	66	43	108	139	207	132	644	618	313	229	96	58
	320	341	138	137	8,717	2,009	32,027	1,089	833	2,260	67	100	208	376	201	300	2,652	3,104	492	946	117	119
	114	451	76	177	17,928	1,724	893	207	1,492	549	113	93	130	252	200	133	598	1,643	667	839	109	75
	151	281	102	177	10,398	1,870	122,443	1,355	2,149	692	99	46	147	135	134	86	726	819	533	848	383	78
	1,107	321	180	117	3,314	2,323	176,841	8,887	1,948	2,573	88	72	681	3,021	137	138	795	1,137	852	344	103	58
	162	367	191	142	657,819	9,817	664,004	1,729	28,737	638	84	211	271	301	41	110	297	268	1,211	325	85	71
	3,695	658	418	471	3,124	1,702	42,753	988	14,134	1,585	115	172	80	178	13,649	497	1,015	761	664	344	84	119
	150	329	105	1,014	1,148	823	68,061	304	495	103	29	109	156	187	64	92	561	497	547	240	80	79
	206	402	117	1,062	1,461	881	80,565	348	623	89	72	114	246	271	58	107	431	668	503	233	79	98
	123	150	104	171	14,082	534	1,422,191	11,053	125	127	58	142	296	17,934	44	111	515	320	2,613	274	39	122
	141	128	105	66	1,651	491	1,079,987	7,946	376,105	12,976	92	111	401	110	64	99	902	330	1,375	345	121	83
	144	116	107	100	2,425	1,265	309,874	4,606	456	309	55	139	67	24,103	106	107	809	1,210	997	517	514	89
	586	2,069	198	197	1,295	1,021	367,286	921	2,118	160	215	3,545	850	221	72	96	285	311	1,084	245	51	169
GADA Positive Samples	142	10,538	83	959	1,939	1,733	228	92	1,216	4,848	39	121	257	269	246	271	3,323	4,788	198	197	111	135
	1,322	790	253	156	28,736	6,527	4,125	1,014	9,195	719	61	133	370	36,553	133	264	1,290	1,082	347	522	95	196
	153	112	90	62	1,862	716	345	87	1,898	786	71	99	53	149	108	147	1,727	660	200	196	140	100
	20,230	10,220	2,358	1,095	2,391	1,223	499	166	606	1,596	78	117	71	392	220	209	4,668	1,395	853	1,653	48	121
	6,616	783	390	1,042	2,586	1,288	603	287	11,792	561	50	205	524	3,219	145	313	3,530	1,293	567	647	87	81
	441	490	215	259	509,689	35,766	1,004,179	9,565	4,178	386	132	152	74,590	7,733	2,711	734,078	3,116	1,223	724	422	72	82
	320	8,796	118	104	2,673	1,558	722	246	410	440	2,063	144	902	358	150	211	8,953	2,520	437	388	70	60
	446	1,944	122	128	1,412	3,097	72,333	761	25,160	574	74	92	243	712	120	172	1,211	2,992	528	1,004	79	1,885
	1,775	1,495	670	459	2,277	3,223	1,002	437	3,739	1,270	70	192	107	105	2,103	84	1,167	1,340	586	1,058	72	169
	386	4,241	147	456	3,126	2,077	2,911	936	1,267,894	230,403	172	293	8,500	1,938	134,444	460	2,489	1,992	447	624	1,797,443	150,703
	172	14,113	93	791	3,259	3,965	1,480	493	716	245	72	138	392	131	34	120	2,742	7,194	577	687	78	118
	840	374	146	139	2,661	1,942	225,221	9,204	17,455	1,005	100	131	392	1,154	30	84	2,200	1,367	823	438	67	121
	268	433	176	120	697,841	10,559	694,516	1,607	30,203	733	107	107	600	124	25	59	738	601	1,354	273	83	112
	7,631	765	790	468	5,333	1,995	49,196	1,096	35,738	2,058	210	390	82	126	17,512	379	3,487	1,416	586	368	101	113
	221	128	104	92	2,164	734	1,093,497	4,841	757,596	6,243	157	183	297	65	52	86	2,961	777	1,246	292	400	84
	298	392	129	134	4,914	2,246	354,220	3,423	1,960	241	155	116	121	10,728	73	96	3,975	2,979	921	514	1,168	89
TGA Positive Samples	195	1,065	88	198	2,625	17,588	567	213	546	406	19,399	206	112	780	140	311	3,969	1,651	298	523	85	627
	159	332	105	127	1,619	718	504	76	146	114	338	140	87	123	87	187	2,959	994	255	200	59	105
	140	500	81	125	1,153	1,101	489	182	175	163	2,533	1,018	117	137	147	250	1,025	901	278	355	102	167
	173	2,915	104	223	1,779	2,836	346	428	252	179	560,389	7,506	435	1,622	246	332	3,487	2,318	227	721	105	161
	123	216	110	86	1,290	933	414	192	130	108	108	89	60	169	102	95	2,909	1,203	246	238	90	142
	166	158	85	134	2,083	1,294	1,040	232	121	216	229,321	941	183	286	108	130	4,791	2,233	287	290	79	133
	106	135	112	59	1,549	350	530	163	109	97	169	101	86	218	634	271	1,313	236	309	226	75	113
	118	1,618	84	90	1,836	1,398	524	275	446	294	170,982	1,352	131	1,210	4,390	530	920	627	347	336	87	576
	229	2,147	116	985	2,851	2,533	1,662	462	190	217	191	198	264	262	1,491	106	4,887	1,887	1,232	641	83	141
	95	586	68	192	934	2,327	946	305	142	166	138	213	96	164	110	108	841	1,112	645	1,059	71	134
TPOA Positive Samples	93	4,250	80	84	1,224	1,324	380	123	391	914	580	115	168	256	602	440	924	709	267	731	35	140
	62	539	60	114	1,243	924	290	75	226	273	79	94	73	149	69	132	875	588	261	224	69	113
	118	256	64	93	690	1,620	330	516	220	221	388	108	46	294	95	154	878	2,491	244	266	98	160
	155	1,383	89	327	1,081	3,543	325	1,116	175	1,630	90	165	70	1,275	97	730	1,191	2,902	183	699	33	595
	113	1,537	83	3,896	534	1,502	300	391	68	842	940	859	137	418	119	243	716	2,242	778	22,878	52	157
	62	420	43	194	675	1,068	243	323	118	475	411	79	90	451	184	2,713	711	930	252	458	84	137
	204	2,960	98	832	1,817	9,460	443	3,806	147	2,870	99	2,336	80	101,502	85	6,755	3,942	6,463	334	6,572	42	979
	91	1,263	83	180	1,460	1,122	523	771	189	496	68	86	82	121	112	112	791	1,145	413	297	41	153
	288	2,202	82	837	933	3,804	722	655	219	447	176	1,115	1,529	3,841	10,451	416	497	2,120	484	1,101	73	463
	134	1,490	114	188	1,967	1,908	2,634	367	182	639	122	148	159	1,456	1,489	141	2,020	1,344	655	458	49	90
ACA Positive Samples	161	3,761	79	678	3,163	1,923	1,066	208	401	121	263	125	51	65	106	219	581	1,031	556	352	182	193
	4,644	2,148	408	281	1,401	2,515	12,046	677	313	268	207	327	1,078	334	78	88	675	1,139	426	1,561	126	124
	103	780	72	633	1,400	860	587	111	332	105	125	92	163	71	20	57	1,250	800	370	217	105	108
	442	245	318	244	623,776	58,334	1,801,741	27,883	4,119	192	876	118,570	19,885	9,813	872	92	1,532	717	5,040	383	124	84
	161	1,769	97	98	1,836	1,200	525	140	23,950	7,699	137	137	49	80	37	85	1,797	878	363	271	146	99
	213	403	296	381	2,595	4,694	1,211	344	485	137	243	226	123	92	49	80	1,736	3,980	1,233	3,412	182	117
	1,069	762	196	350	1,161	1,473	895	198	1,337	125	99	127	169	62	80	76	779	499	391	292	169	152
	26,849	32,120	3,722	188	1,238	603	609	70	335	145	190	58	120	73	12,941	102	653	177	429	172	159	66
	160	340	96	76	746	557	6,995	190	604	136	114	126	47	105	46	103	240	143	390	266	91	114
	160	878	96	113	1,686	1,692	1,417	225	206	201	126	178	72	146	1,469	156	742	889	613	362	86	85
Uncharacterized Normal Serum Samples	187	1,731	125	103	3,753	1,091	1,064	159	782	147	45	57	387	240	120	88	2,712	1,310	394	253	95	127
	1,213	717	297	81	4,511	730	1,540	397	526	185	22	51	103	265	93	61	3,824	877	444	272	123	156
	1,124	1,618	139	161	3,415	5,735	1,127	192	309	163	52	69	235	132	120	52	1,788	20,610	505	791	93	124
	181	18,003	97	209	2,106	2,335	988	257	227	165	51	33	95	497	94	186	1,014	2,286	355	287	89	106
	303	4,419	142	92	6,121	1,413	1,645	494	491	143	50	82	264	248	134	139	3,717	1,046	491	628	92	114
	251	203	118	90	2,467	1,229	2,114	580	343	183	27	68	133	696	160	134	1,736	967	408	397	77	131
	134	3,544	123	421	2,840	3,076	1,102	326	212	189	65	92	1,502	3,140	163	186	1,310	2,788	383	317	15	177
	182	1,065	116	128	5,576	3,119	1,062	196	525	175	71	168	11,206	293,072	114	181	2,193	1,934	539	1,619	12	140
	165	336	111	145	1,928	2,799	1,132	1,422	204	183	38	66	266	1,170	160	245	699	3,379	403	395	61	100
	170	352	96	78	2,534	1,244	1,717	351	178	101	51	74	128	137	149	267	1,226	1,495	504	418	67	234
	176	332	98	121	2,121	750	1,123	608	104	110	60	84	403	325	101	132	1,768	941	331	350	51	270
	191	177	93	68	2,244	1,002	1,673	1,159	294	84	51	70	95	212	84	38	873	864	730	271	119	57
	182	734	110	182	1,844	759	738	309	223	161	60	108	70	242	98	295	906	1,006	315	637	73	131
	331	621	124	181	3,210	1,301	896	288	224	113	60	79	149	457	121	84	2,256	1,488	544	1,002	94	126
	163	205	94	71	3,024	770	968	99	169	115	60	81	273	2,390	148	73	1,732	921	296	226	81	115
	150	757	104	100	3,198	1,239	902	261	262	254	55	68	319	1,144	281	196	1,472	1,362	299	508	140	488
	162	346	75	81	1,597	2,604	517	335	86	185	1,743	223	396	1,815	201	171	1,222	1,462	268	418	132	156
	124	613	52	356	1,920	1,623	968	225	65	195	78	121	223	475	111	383	1,166	1,074	368	320	119	336
	88	245	61	305	1,358	984	1,638	3,219	82	220	85	89	75	78	63	80	1,403	1,501	311	234	136	132
	102	1,094	72	71	1,633	1,386	678	144	79	147	59	116	114	4,211	104	258	1,435	1,166	478	349	113	111
	92	204	87	65	1,264	875	558	194	58	169	56	133	948	40,822	54	122	1,593	543	280	237	119	89
	176	905	77	148	3,485	1,175	588	240	41	134	56	212	109	305	68	182	7,274	1,832	335	766	86	338
	127	15,060	62	673	1,383	6,666	689	1,333	86	7,831	70	226	79	2,366	80	2,208	1,229	5,758	402	2,320	85	294
	121	174	125	103	1,697	942	806	187	80	105	53	97	402	461	164	173	1,424	735	519	242	51	37
	114	168	83	121	1,150	806	469	306	94	202	54	81	88	217	163	225	856	597	264	363	109	245
	1,043	13,592	111	276	1,599	5,134	392	433	90	139	78	136	134	1,303	205	780	1,792	3,303	246	317	87	109
	95	1,542	78	89	1,115	716	532	225	53	143	56	89	81	786	129	186	2,352	934	229	299	137	125
	127	1,002	85	285	1,024	1,551	467	386	77	184	88	154	174,999	991	188	394	2,095	932	291	3,649	118	219
	235	748	101	163	4,069	1,369	1,022	276	133	181	53	84	196,458	829	110	213	6,286	2,333	603	411	133	111
	110	210	103	72	1,374	984	652	191	76	121	79	99	13,103	642	100	145	1,597	1,096	331	226	100	93
	6,149	1,948	96	328	1,166	1,835	566	695	68	386	145	267	60	35,458	91	215	2,155	2,363	801	848	109	183
	116	1,922	76	95	969	957	418	207	94	91	87	122	124,901	481	158	177	478	412	261	877	141	132
	119	274	89	126	885	872	915	405	267	171	79	106	56	95	47	70	244	202	399	285	151	90
	127	461	107	387	1,870	2,068	846	356	139	157	51	136	60	377	60	137	820	777	515	691	98	317
	98	435	79	98	690	538	460	138	130	127	67	97	66	616	65	112	422	274	277	215	221	419
	118	654	105	491	1,952	3,904	929	1,015	230	121	97	84	62	148	59	44	277	2,718	610	500	107	96
	137	509	108	139	1,118	1,789	1,031	482	371	298	70	82	214	138	44	90	912	993	475	973	184	228
	110	277	96	192	1,581	1,961	872	1,245	248	229	106	252	84	426	38	81	280	966	526	757	84	222
	117	167	114	114	1,734	1,359	955	280	300	715	89	144	1,082	611	51	88	459	585	545	498	88	210
	83	485	110	209	1,381	5,847	837	241	280	191	73	199	35	61	70	56	2,107	6,114	599	652	72	104
	125	140	79	216	1,425	934	1,740	160	281	97	90	158	80	129	46	94	259	292	622	154	133	105
	136	170	103	140	1,857	1,257	1,405	287	160	125	126	254	82	199	59	114	736	286	809	323	118	171
	126	382	141	1,219	2,090	1,350	994	205	270	64	104	186	206	4,504	81	90	716	484	532	462	92	177
	132	165	117	203	2,719	970	2,113	3,450	358	86	112	166	125	319	74	166	651	342	740	343	118	261
	164	594	107	156	1,671	2,268	1,141	7,697	269	102	63	148	48	123	28	125	667	1,729	529	331	81	79
	140	479	96	175	3,662	2,063	631	241	348	129	120	177	56	289	32	104	352	438	528	413	109	171
	100	309	85	316	2,059	1,430	670	194	206	1,537	53	125	84	853	58	78	497	376	445	347	53	115
	117	907	100	220	1,215	1,047	918	516	276	126	100	179	86	258	68	108	261	429	1,931	1,773	92	98
	64	926	72	488	1,281	1,914	810	391	428	290	129	290	69	175	37	95	367	533	437	1,244	124	199
	85	186	67	127	1,452	853	943	116	255	123	80	64	136	86	69	71	345	258	382	218	135	149
	690	8,439	74	547	3,085	3,511	1,502	330	371	298	92	1,508	92	557	30	198	976	1,166	761	902	177	215
	120	1,133	92	136	1,763	1,686	835	247	167	150	57	84	170	622	63	83	1,172	749	525	483	90	60
	108	368	88	615	1,301	1,341	1,079	193	336	144	102	132	2,203	3,521	60	142	713	604	645	1,140	80	145
	130	192	93	164	2,135	712	1,585	173	460	112	114	110	260	89	112,436	1,854	786	230	578	235	102	170
	165	982	89	359	1,527	1,437	1,854	252	878	195	118	144	58	113	82	79	906	357	970	581	136	141
	192	2,588	96	165	1,686	2,170	1,083	326	517	176	117	135	404	818	64	83	423	298	511	483	84	119
	106	1,147	61	219	1,698	1,602	1,294	994	284	238	599	345	239	285	53	101	759	586	698	685	136	166
	137	4,510	69	216	1,687	1,990	1,162	409	274	150	126	94	164	131	49	72	975	1,584	514	651	123	221
	84	1,068	65	285	4,082	2,223	3,066	289	2,313	244	857	140	619	306	81	91	452	833	9,198	498	163	265
	101	417	69	177	1,321	3,823	1,105	1,364	279	296	159	342	85	1,181	157	86	1,128	1,266	475	590	118	187
	98	2,159	65	68	987	1,721	1,315	259	247	171	180	175	2,010	1,182	79	93	580	569	579	383	140	91
	75	492	75	198	1,170	2,023	1,409	335	462	200	139	207	81	120	43	110	611	636	607	521	104	86
	141	158	158	99	4,175	1,117	1,757	336	533	100	112	139	78	130	79	119	1,521	477	1,056	382	132	78
	107	704	42	180	989	2,011	742	1,902	367	315	149	212	70	252	69	91	615	1,049	355	452	114	107
Cutoff Signal	276	2,424	169	454	4,426	3,776	2,206	890	738	322	201	324	692	1,929	219	337	3,213	3,025	1,000	1,173	209	366

The percent of samples positive for each marker (IgG, IgA, or combined isotypes), in each sample subset are summarized below. The percent of samples with multiple T1D markers is shown for each sample subgroup.

		Samples (Percent Above Cutoff)							
Assay	Isotype	IAA positive (n=16)	ZnT8A positive (n=18)	IA2A positive (n=16)	GADA positive (n=16)	TGA positive (n=10)	TPOA positive (n=10)	ACA positive (n=10)	Normal (n=64)
IAA	IgG	88	50	44	69	0	10	40	11
	IgA	25	11	0	31	10	20	20	13
	IgG + IgA	94	61	44	75	10	30	50	17
pro-IAA	IgG	69	39	38	44	0	0	50	2
	IgA	19	22	19	44	10	30	20	9
	IgG + IgA	69	50	50	63	10	30	70	11
ZnT8A	IgG	56	83	44	31	0	0	10	5
	IgA	31	33	19	25	10	20	20	9
	IgG + IgA	56	83	44	38	10	20	20	14
IA2A	IgG	63	72	88	56	0	10	30	2
	IgA	50	67	69	50	0	20	10	17
	IgG + IgA	69	72	88	56	0	30	30	19
GADA	IgG	63	78	75	81	0	0	30	5
	IgA	56	61	63	88	10	80	10	6
	IgG + IgA	75	83	75	94	10	80	30	11
TGA	IgG	0	11	6	13	60	40	40	5
	IgA	25	22	6	6	40	30	20	5
	IgG + IgA	25	22	6	13	60	60	40	8
DGPA	IgG	31	17	13	19	0	10	20	16
	IgA	19	28	25	31	0	20	10	14
	IgG + IgA	31	39	31	38	0	20	20	23
TGBA	IgG	19	0	13	38	40	30	30	3
	IgA	19	6	13	19	10	50	0	8
	IgG + IgA	25	6	13	38	40	60	30	9
17-OHA	IgG	19	17	6	38	40	10	0	6
	IgA	19	11	6	13	0	10	10	8
	IgG + IgA	25	22	13	44	40	10	10	14
Jo-1A	IgG	19	39	38	13	10	0	20	5
	IgA	6	6	0	6	0	20	20	8
	IgG + IgA	25	39	38	19	10	20	30	11
IFA	IgG	13	22	19	19	0	0	0	2
	IgA	13	17	6	13	20	30	0	3
	IgG + IgA	25	28	25	25	20	30	0	3
% Samples with >1 T1D Reactivity		88	100	88	94	10	50	50	16

5 Conclusion

These results show promise for the utility of the MSD second generation autoimmune disease biomarker panel in assessing multiple reactivities in small amounts of precious samples during clinical research. The bridging serology format provides greater assay specificity than traditional serology assays. The ability to detect specific antibody isotypes provides additional information relevant to understanding development of the different autoimmune disease conditions.

The plate-based multiplex format lends itself to the running of high throughput assays, for example, in prospective studies to assess levels in large sample cohorts, and for longitudinal screening of high risk subject samples to correlate with subsequent development of autoimmune diseases. Multiplexed panels will be expanded to include additional markers of interest, and samples will be screened for additional antibody isotypes, as appropriate. Further research is needed to assess the potential utility of these immunoassays in patient screening, diagnosis, and monitoring.

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