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EDTA Treatment for Overcoming the Prozone Effect and for Predicting C1q Binding in HLA Antibody Testing

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The Luminex-based single antigen bead (SAB) assay is widely used to detect HLA antibody in transplant recipients. However, one limitation of the SAB assay is the prozone effect, which occurs mostly as a result of complement interference. We investigated the efficacy of EDTA treatment for overcoming the prozone effect and predicting C1q binding of HLA antibody. We subjected 27 non-treated (naïve) and EDTA-treated serum samples from highly sensitized patients to IgG-SAB assays, and we confirmed the prozone effect in 53% and 31% of class I and class II antibody tests, respectively, after EDTA treatment. When we conducted additional assays after dithiothreitol treatment and serum dilution, EDTA was the most efficacious in eliminating the prozone effect. Reducing the prozone effect by EDTA treatment strengthened the correlation between IgG mean fluorescence intensity (MFI) and C1q MFI values (ρ=0.825) as compared with the naïve sera (ρ=0.068). Although C1q positivity was dependent on the concentration of HLA antibody in EDTA-treated sera, the correlations varied individually. Overall, our results confirmed the efficacy of EDTA treatment for overcoming the prozone effect. EDTA treatment showed a positive effect on the correlation between IgG MFI and C1q MFI values.

Key Words: Prozone effect, EDTA, Single antigen bead assay, C1q binding, HLA antibody

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Accurate assessment of HLA antibody is crucial for successful transplant management [1]. The Luminex-based single antigen bead (SAB) assay is widely used for sensitive detection of low concentrations of antibody [2-5]. In the SAB assay, antibody amount is determined semi-quantitatively and is expressed as the mean fluorescence intensity (MFI) after reaction with anti-human IgG antibody reagent labeled with a fluorescent dye. MFI values correlate with transplant-related outcomes; thus, monitoring MFI values may be useful for risk assessment after transplantation [6, 7]. In addition, SAB assays for detecting C1q-binding HLA antibodies (C1q SAB) have been introduced to better discriminate clinically relevant antibodies [8].

However, clinical application of the SAB assay is limited because of technical issues, including assay variability, complement interference, and the prozone effect [9]. Inhibition causing false-negative or falsely decreased MFI values has been observed in approximately 70% of highly sensitized patients [10]. Several studies have attempted to eliminate the prozone effect by treatment with EDTA or dithiothreitol (DTT), preheating, and/or dilution of serum [9, 11, 12]. There are multiple explanations for the prozone effect, including bead saturation [13, 14], steric hindrance from complement complex [11], and the presence of IgM HLA-specific antibodies [15]. Complement interference involves covalent binding and accumulation of C4 and C3 degradation products on the immune complex, which prevent fluorescent anti-IgG from binding to IgG. EDTA eliminates the prozone effect because, as an iron-chelating reagent, it can inhibit the formation of C1qrs complement complex and prevent com-

plement cascade activation [16]. DTT disrupts the pentameric structure of IgM and the disulfide bonds in complement molecules, thus reducing the prozone effect [15]. In this respect, SAB assays as a standard should include serum treatment to correct the prozone effect because MFI values are often used as a surrogate marker of antibody strength. Although a recent meeting report recommended EDTA and/or titration [1], there is currently no verified standard method, and different treatment methods are used across different laboratories.

We compared MFI values of SAB assays in serum samples treated with or without EDTA, DTT, or dilution. In addition, we evaluated the correlation between IgG-MFI and C1q-MFI values after EDTA treatment. To our knowledge, this is the first study to investigate the effects of EDTA on specific HLA loci in combination with C1q-MFI.

We collected leftover sera from 27 highly sensitized patients (median age=49 years, M:F=14:13). All samples contained strong HLA antibodies (MFI≥10,000), and the median panel-reactive antibody (%) was 95%. The patients had become sensitized by previous transplantation with or without transfusion or pregnancy (81.5%, N=22), previous pregnancy and transfusion (7.4%, N=2), previous pregnancy alone (7.4%, N=2), or previous transfusion alone (3.7%, N=1). The Institutional Review Board of Seoul St. Mary's Hospital, Seoul, Korea, approved this study (KC18SESI0323). LABScreen Single Antigen kits and the LABScan 3D system (One Lambda, A Thermo Fisher Scientific Brand, Canoga Park, CA, USA) were used to detect HLA antibodies (11 samples for class I only, eight samples for class II only, and eight samples for both class I and class II). For EDTA treatment, a 0.5 M EDTA solution was diluted 1:20 (final concentration of 25 mM), added to the serum, vortexed, and centrifuged at room temperature for 10 minutes before testing [10]. We defined the prozone effect as doubled MFI values, with an MFI cutoff of 1,000, or 5,000 MFI increment after serum treatment compared with the results from naïve serum, considering recent recommendations and reports [1, 12, 17].

In a comparison of naïve and EDTA-treated samples, we investigated MFI data from 3,363 beads (1,843 class I and 1,520 class II) from 35 tests (19 class I and 16 class II) (Fig. 1). The prozone effect was identified in 53% (10 out of 19) of class I antibody tests and in 31% (5 out of 16) of class II antibody tests after EDTA treatment. Of all beads, 8.3% (26.5% of positive beads) were affected by complement inhibition, with the median MFI increasing from 2,921 (range, 0–13,890) to 21,043 (1,024–27,874) after EDTA treatment. Regarding HLA specificity, prozone effect incidences were as follows: HLA-A, 28.1%; HLA-B, 43.6%; HLA-C, 3.6%; HLA-DR, 2.7%; HLA-DQ, 18.0%; and HLA-DP, <0.1% of positive beads. These findings were consistent with a previous report wherein HLA-C beads were the least affected among class I beads and HLA-DQ beads were the

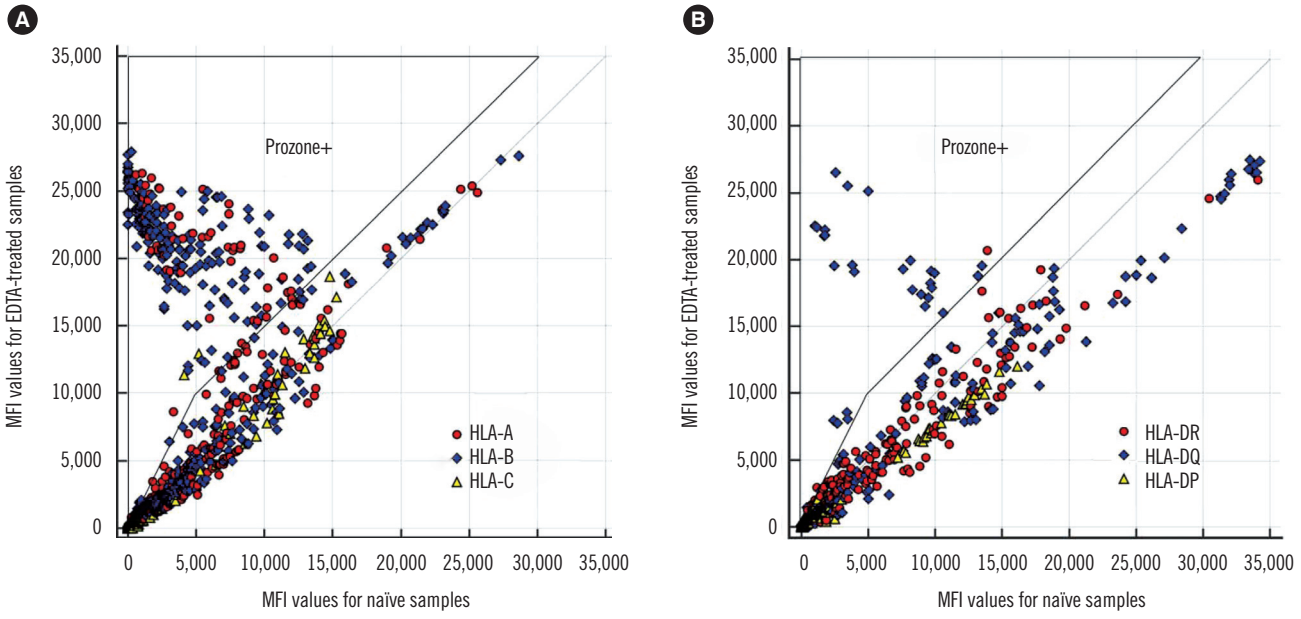

Fig. 1. Comparison of MFI values in naïve and EDTA-treated sera. (A) Class I IgG-SAB results in 19 sera (1,843 beads); (B) Class II IgG-SAB results in 16 sera (1,520 beads).
Abbreviations: MFI, mean fluorescence intensity; SAB, single antigen bead.

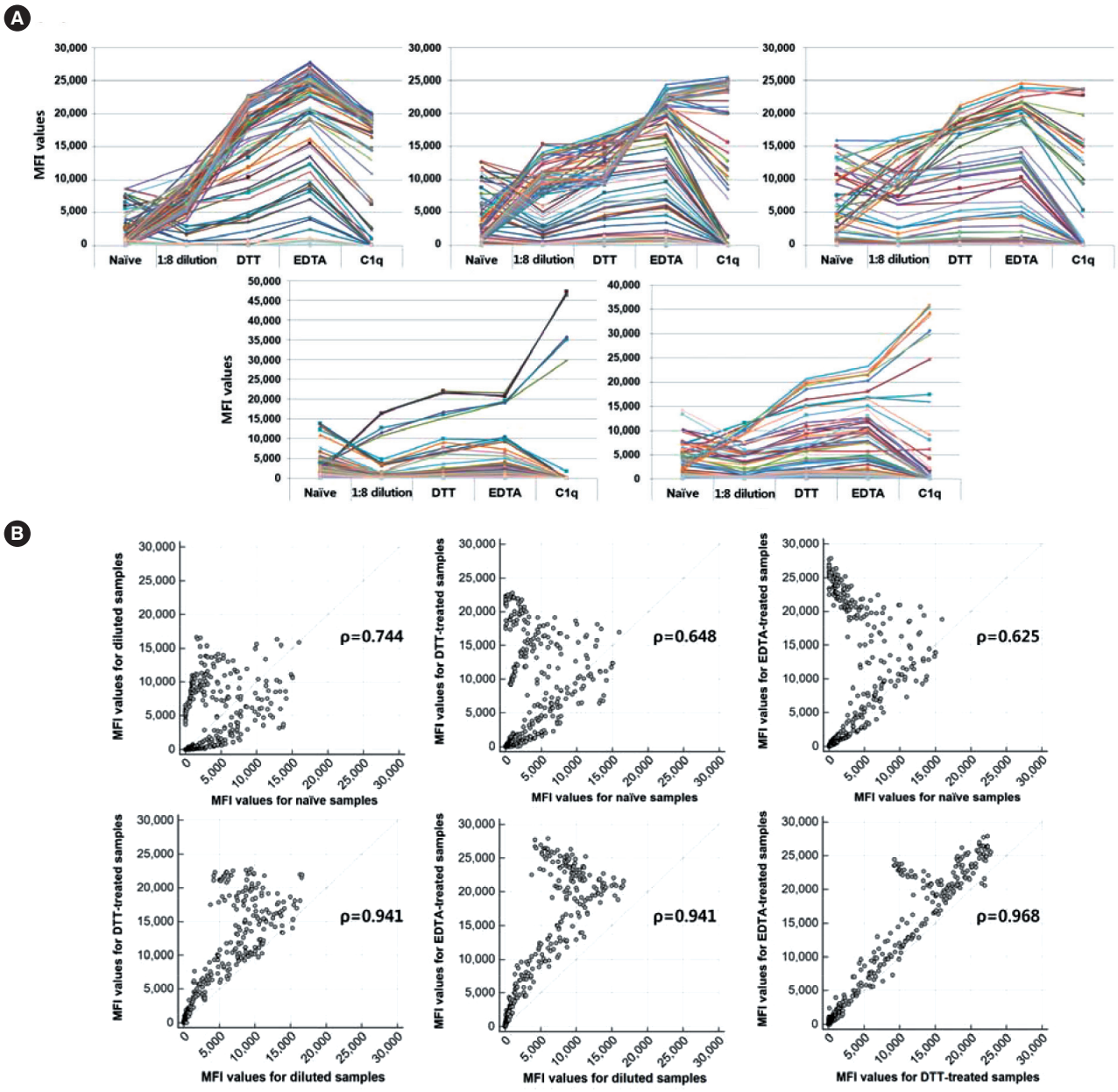

Fig. 2. MFI values for naïve samples and samples treated with 1:8 dilution, DTT, and EDTA, and C1q assay using sera from five sensitized patients. (A) MFI values for all samples with various treatments. (B) Correlations between MFI values for naïve, diluted, DTT-treated, and EDTA-treated samples.
Abbreviations: MFI, mean fluorescence intensity; DTT, dithiothreitol.

most affected among class II beads [18].

We selected five sera and additionally tested class I IgG after 1:8 dilution in phosphate-buffered saline and treatment with 5 mM DTT at 37°C for 30 minutes [19]. When we compared the three serum treatment methods, EDTA treatment was the most efficacious for overcoming prozone effect (Fig. 2). This finding was consistent with previous studies that showed the superior efficacy of EDTA for overcoming the prozone effect [12, 16]. DTT treatment and dilution resulted in small increases in MFI values in some of the beads, indicating a residual prozone effect, presumably because only IgM antibodies were removed (Supplemental Data Table S1).

We subjected 10 samples with the prozone effect (N=5 for class I and N=5 for class II HLA antibodies) to C1qScreen assays (One Lambda). We selected beads that were positive in EDTA-treated sera to calculate Spearman's correlation coeffi-

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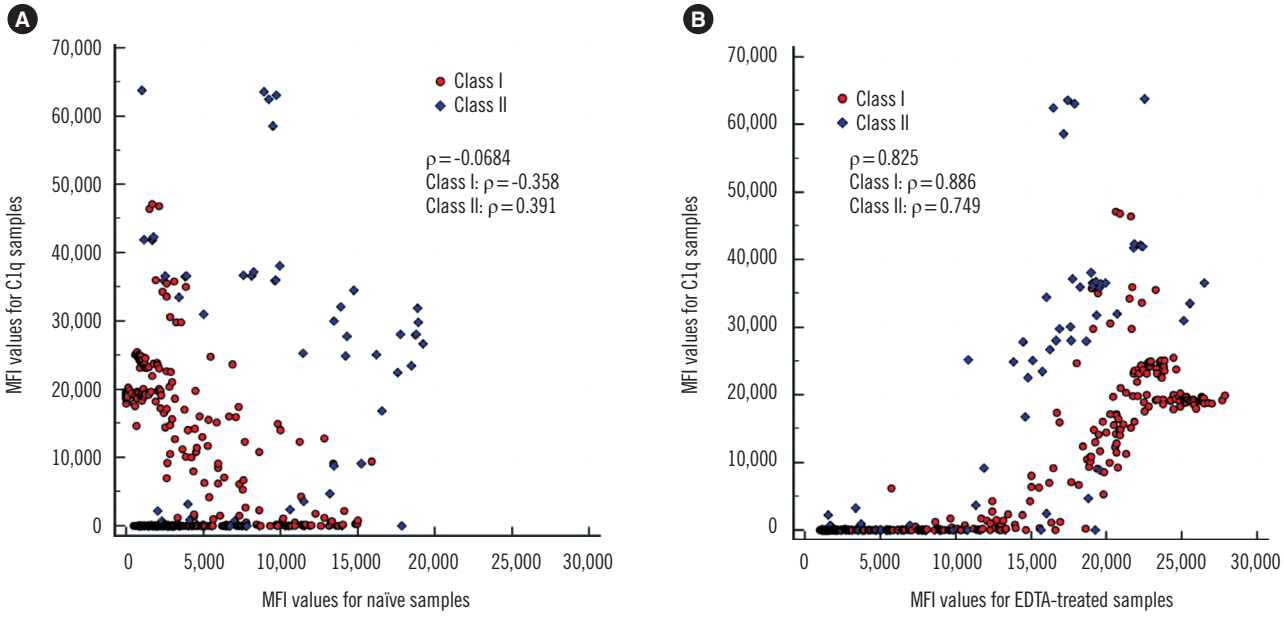

Fig. 3. Correlation of MFI values from C1q- and IgG-SAB assays using (A) naïve sera and (B) EDTA-treated sera.
Abbreviations: MFI, mean fluorescence intensity; SAB, single antigen bead.

cient (ρ). MFI values were summarized as median (range). For statistical analysis, we used MedCalc Statistical Software version 18.9 (MedCalc Software, Ostend, Belgium).

When naïve sera were used, the correlation between IgG-MFI values and C1q-MFI values was weak (ρ=-0.0684; class I: -0.358, class II: 0.391). After EDTA treatment, the correlation between IgG-MFI values and C1q-MFI values became stronger (ρ=0.825; class I: 0.886, class II: 0.749) (Fig. 3). Beads that displayed low IgG-MFI values in naïve sera, but high C1q-MFI values, showed high IgG-MFI values after EDTA treatment. In naïve sera, C1q-SAB beads with MFI≥1,000 (19.7% of all beads) had a median IgG-MFI value of 2,842 (range, 0–19,256). In EDTA-treated sera, C1q-positive beads had a median IgG-MFI value of 20,900 (1,539–27,874). These results supported previous findings that C1q binding ability depends on the concentration of HLA antibody [12]. Tambur and Wiebe [9] reported that MFI cutoffs that predicted C1q positivity varied from 6,237 to 14,154 using non-treated samples. However, not all beads that had high IgG-MFI values in EDTA-treated sera showed C1q positivity, and some high-level IgG antibodies did not bind to C1q. When we plotted IgG-MFI against C1q-MFI values from the data in 10 patients separately, we found a wide range of correlation coefficients (0.283–0.977). Therefore, it remains to be elucidated whether there is an interdependence between C1q positivity and IgG-MFI values.

Some limitations of the present study were that we could not test serial dilutions of samples, and we did not confirm the HLA-specific antibodies against intact HLA antigen on the lymphocyte. Because high-level antibodies against denatured HLA protein might also bind to C1q [20], future studies on interference by denatured HLA are needed. In addition, we selected samples with high panel-reactive antibody and strong HLA antibodies. Thus, our results may not represent the frequency of the prozone effect in SAB assays in general.

In conclusion, we confirmed the efficacy of EDTA treatment for eliminating the prozone effect. Further, EDTA treatment had a positive effect on the correlation between IgG MFI and C1q MFI values. Our study highlights the importance of method standardization to correct the prozone effect.

Authors' Disclosures of Potential Conflicts of Interest

No potential conflicts of interest relevant to this article are reported.

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Supplemental Data Table S1. MFI values of Class I 97 beads in SAB assays among naïve, dilution, DTT, and EDTA treatments using sera from five sensitized patients

Allele specificity	Sample 1				Sample 2				Sample 3				Sample 4				Sample 5			
	Naïve	Dilution	DTT	EDTA	Naïve	Dilution	DTT	EDTA	Naïve	Dilution	DTT	EDTA	Naïve	Dilution	DTT	EDTA	Naïve	Dilution	DTT	EDTA
A*01:01	398	225	173	476	3,427	705*	1,008	3,979	10,420	6,042	10,426	11,662	360	1	110	242	0	0	0	0
A*02:01	2,603	**10,939**	**18,806**	**23,628**	5,955	7,808	10,322	**15,526**	0	0	0	0	1,697	**16,341**	**21,951**	**20,609**	94	47	69	154
A*02:03	3,884	**8,599**	**15,035**	**18,954**	7,753	4,861	8,316	12,439	4	0	0	0	1,522	**16,639**	**21,891**	**21,618**	106	48	78	174
A*02:06	4,748	**13,304**	**17,832**	**21,865**	8,604	6,309	8,706	13,494	0	0	0	0	2,119	**16,546**	**21,507**	**20,909**	78	25	64	116
A*03:01	649	117	396	542	7,757	2,256	4,526	9,643	11,483	7,545	13,046	14,650	2	0	0	16	527	220	264	956
A*11:01	96	3	91	98	7,408	1,657	4,317	9,099	12,637	8,527	14,907	16,611	4,095	613*	1,318	2,395	326	95	144	545
A*11:02	0	0	0	0	3,338	1,842	5,070	**8,654**	11,378	10,133	**16,527**	**18,606**	3,385	643*	1,545	2,959	262	123	189	784
A*23:01	348	108	172	409	0	0	0	0	11,931	5,041	10,814	12,272	1,687	118*	40*	1,121	585	241	107	1,022
A*24:02	843	68	353	764	0	0	0	0	10,198	10,411	**15,444**	**16,329**	0	0	0	0	0	0	0	0
A*24:03	1,356	158*	623*	1,284	0	0	0	0	9,820	8,341	12,669	12,886	285	0	0	113	32	0	23	37
A*25:01	13	0	0	21	2,621	**8,598**	**13,329**	**20,257**	8,709	2,998	7,971	9,610	13,776	3,726	7,606	10,382	167	47	117	188
A*26:01	141	0	170	274	2,457	**7,278**	**14,464**	**20,788**	5,032	1,589	4,617	5,674	13,153	3,202	6,439	9,260	50	0	23	67
A*29:01	446	107	177	493	1,475	**8,516**	**18,162**	**24,266**	630	119	645	961	1,122	61*	313*	619*	2,735	1,367	2,237	3,779
A*29:02	707	217	314	728	1,081	**8,372**	**18,021**	**24,572**	804	155	752	1,136	1,218	70*	297*	675*	1,985	867*	1,407	2,948
A*30:01	603	132	450	559	1,009	**9,208**	**22,240**	**26,329**	476	60	444	709	0	0	0	0	16	0	9	0
A*30:02	49	0	42	46	2,284	**11,327**	**21,799**	**25,200**	383	58	407	632	0	0	0	0	1	0	13	2
A*31:01	0	0	0	0	0	**6,551**	**21,886**	**25,952**	584	70	504	801	653	23	255	325	201	66	117	327
A*32:01	187	20	112	137	2,224	**8,820**	**13,867**	**20,658**	5,580	1,423	4,539	6,169	698	68	208	391	564	114	570	784
A*33:01	4	0	4	0	1,260	**8,679**	**19,647**	**25,417**	2,504	1,169	2,922	3,475	2,843	328*	934*	1,587	57	0	45	82
A*33:03	0	0	0	0	808	**8,317**	**19,464**	**25,124**	846	210	856	1,203	3,929	488*	1,216	2,135	115	29	161	168
A*34:01	182	10	165	196	3,135	**9,654**	**18,301**	**23,785**	802	238	935	1,288	3,991	464*	1,163	2,296	22	0	2	19
A*34:02	773	143	563	678	584	**7,355**	**18,737**	**26,201**	1,705	620*	1,708	2,269	5,113	623*	1,586	3,144	24	0	25	32
A*36:01	53	0	23	74	2,025	171*	137*	2,403	2,647	2,894	3,950	4,595	229	0	51	153	101	35	65	193
A*43:01	254	54	332	482	2,842	**6,596**	**11,931**	**19,174**	6,301	2,123	5,634	6,948	6,681	1,086	2,293	3,954	7,676	5,059	5,999	9,492
A*66:01	42	0	64	138	1,112	**7,450**	**18,541**	**24,466**	6,614	2,004	5,744	6,854	13,653	3,408	6,786	9,822	117	0	18	67
A*66:02	10,671	6,154	6,517	9,830	1,599	**8,757**	**18,816**	**24,792**	1,243	349*	1,413	1,744	2,242	200*	560*	1,257	10,244	5,173	6,395	10,680
A*68:01	5,855	7,548	**18,125**	**21,625**	7,262	2,927	3,424	7,039	765	160	686	1,048	3,560	**10,696**	**15,125**	**19,141**	172	53	146	202
A*68:02	7,701	7,400	**16,826**	**20,575**	7,242	2,965	3,659	7,066	4,640	1,696	4,248	5,880	3,100	**11,594**	**16,755**	**19,037**	144	0	83	141
A*69:01	7,544	9,064	**16,814**	**19,805**	6,595	2,938	4,529	8,103	557	95	479	764	3,880	**12,663**	**16,017**	**19,436**	205	13	80	189
A*74:01	957	194	745	691	711	**7,717**	**19,304**	**25,306**	532	106	474	790	588	70	250	370	188	61	128	373

(*Continued to the next page*)

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Supplemental Data Table S1. Continued

Allele specificity	Sample 1				Sample 2				Sample 3				Sample 4				Sample 5			
	Naïve	Dilution	DTT	EDTA	Naïve	Dilution	DTT	EDTA	Naïve	Dilution	DTT	EDTA	Naïve	Dilution	DTT	EDTA	Naïve	Dilution	DTT	EDTA
A*80:01	151	68	171	111	4,090	554*	2,183	4,338	7,236	2,646	6,618	7,082	311	0	98	229	135	0	102	126
B*07:02	9,802	**14,960**	**18,646**	**20,929**	0	0	0	0	1,328	440*	1,512	1,732	5,017	704*	2,209	2,861	5,484	**10,961**	**16,473**	**18,046**
B*08:01	1,062	575*	1,164	1,045	0	0	0	157	7,770	7,883	**13,732**	**15,575**	165	0	0	114	1,305	490*	1,587	1,903
B*13:01	14,831	11,111	11,800	13,324	3,772	**7,724**	**14,183**	**20,658**	5,306	**13,521**	**16,342**	**19,563**	0	0	0	0	10,145	7,558	10,152	10,626
B*13:02	5,319	**14,007**	**17,940**	**20,492**	148	**5,741**	**18,143**	**24,893**	2,060	**15,269**	**14,032**	**22,063**	0	0	0	5	7,117	11,536	**15,146**	**16,887**
B*14:01	55	0	41	65	576	**6,382**	**18,850**	**22,545**	2,838	**11,222**	**13,861**	**18,703**	41	0	0	46	428	52	379	486
B*14:02	33	0	27	41	2,548	**8,384**	**17,389**	**20,007**	6,100	7,684	**11,902**	**13,172**	0	0	0	5	330	15	255	392
B*15:01	10,700	7,384	8,632	10,285	0	**4,994**	**21,178**	**26,986**	840	**9,061**	**9,847**	**23,570**	0	0	0	0	4,414	4,739	**9,343**	**11,715**
B*15:02	951	364	801	843	0	**4,384**	**22,332**	**26,493**	707	**7,701**	**9,197**	**23,540**	70	0	0	51	4,846	1,366	6,023	7,751
B*15:03	9,326	6,185	7,788	8,936	0	**6,011**	**22,373**	**26,368**	941	**9,375**	**10,022**	**22,923**	0	0	0	16	4,402	4,896	**9,960**	**12,050**
B*15:10	4,564	2,754	3,785	4,573	0	**5,728**	**21,771**	**26,463**	588	**7,657**	**9,291**	**23,637**	138	0	27	99	3,666	1,274	3,735	4,963
B*15:11	883	488	907	914	361	**6,912**	**22,533**	**25,753**	1,093	**10,825**	**11,751**	**21,860**	21	0	0	20	1,648	280*	1,864	2,053
B*15:12	4,939	2,607	4,193	5,043	0	**4,221**	**21,784**	**27,682**	730	**8,521**	**9,278**	**24,426**	4,507	629*	1,840	2,763	3,803	749*	3,366	4,190
B*15:13	204	107	225	253	493	**6,676**	**21,822**	**24,442**	1,688	**11,071**	**12,119**	**22,035**	539	21	50	374	159	53	225	177
B*15:16	0	0	0	0	0	**5,151**	**21,097**	**25,808**	839	**9,927**	**10,173**	**22,304**	2,683	88*	1,032	1,769	109	0	133	245
B*18:01	125	0	66	111	240	**6,039**	**22,309**	**27,874**	2,112	**13,795**	**13,362**	**22,671**	56	0	0	51	6,357	3,627	6,447	7,555
B*27:05	2,056	**9,546**	**20,644**	**23,873**	5,636	6,447	7,970	**12,309**	0	0	0	0	12,246	4,750	9,918	9,649	7,273	11,560	**14,984**	**16,692**
B*27:08	1,785	**8,840**	**21,170**	**24,630**	2,643	**8,058**	**11,848**	**16,220**	0	0	0	0	10,921	3,586	9,008	7,311	2,341	**9,538**	**19,787**	**21,511**
B*35:01	2,023	966*	1,901	1,985	81	**5,997**	**20,824**	**25,787**	1,219	**9,341**	**10,418**	**22,104**	49	0	16	49	5,473	3,298	7,024	7,893
B*37:01	6,879	11,854	**19,147**	**22,436**	0	**4,901**	**17,881**	**23,405**	3,998	**12,256**	**14,624**	**19,462**	1,960	203*	637*	1,166	7,341	3,454	5,821	5,736
B*38:01	13,296	8,629	11,114	12,638	53	**5,393**	**18,697**	**23,241**	3,149	**13,688**	**15,843**	**20,680**	5,350	859*	2,458	3,525	4,340	2,021	4,146	4,698
B*39:01	11,980	6,800	10,371	11,456	0	**3,737**	**17,517**	**22,511**	2,679	**12,535**	**15,527**	**20,745**	3,455	429*	1,472	2,376	7,316	7,773	11,646	**12,694**
B*40:01	12,864	16,318	**18,128**	**20,678**	0	0	0	0	4,590	**14,123**	**16,949**	**20,683**	2,717	337*	1,288	1,477	2,630	**10,458**	**20,708**	**23,258**
B*40:02	10,001	**15,581**	**18,104**	**20,900**	0	0	0	0	3,656	**13,658**	**15,800**	**21,309**	1,781	179*	767*	1,037	1,933	**9,295**	**19,688**	**21,703**
B*40:06	15,922	15,838	16,995	18,856	0	0	0	0	5,938	**13,029**	**16,199**	**19,842**	871	112	499	530	2,834	**10,053**	**18,491**	**20,256**
B*41:01	14,994	10,794	12,400	13,959	0	0	0	110	2,989	**15,355**	**15,297**	**21,138**	682	95	254	412	5,380	7,164	**10,806**	**12,420**
B*42:01	4,482	**13,146**	**19,135**	**21,774**	786	150	0	855	81	1	109	122	2,051	190*	677*	1,150	5,506	3,133	5,773	7,494
B*44:02	3,434	1,075	2,809	2,964	8,619	11,974	**16,266**	**18,990**	2,815	**13,869**	**13,323**	**21,298**	5,605	858*	2,027	3,360	463	136	424	518
B*44:03	6,238	2,607	5,272	5,832	1,923	**9,328**	**19,503**	**22,789**	941	**10,991**	**10,274**	**23,791**	7,644	1,370	3,508	4,998	1,154	420*	1,086	1,370
B*45:01	4,655	1,706	3,945	4,278	1,256	**8,695**	**20,114**	**23,775**	922	**10,458**	**9,773**	**22,811**	4,564	718*	1,900	2,836	7,153	5,410	8,825	10,091

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Supplemental Data Table S1. Continued

Allele specificity	Sample 1				Sample 2				Sample 3				Sample 4				Sample 5			
	Naïve	Dilution	DTT	EDTA	Naïve	Dilution	DTT	EDTA	Naïve	Dilution	DTT	EDTA	Naïve	Dilution	DTT	EDTA	Naïve	Dilution	DTT	EDTA
B*46:01	0	0	13	4	0	**5,575**	**21,683**	**25,195**	2,974	**13,798**	**14,405**	**20,933**	0	0	0	0	29	0	91	47
B*47:01	14,042	8,646	11,219	12,849	8,460	6,391	7,081	11,184	12,828	10,237	15,749	16,913	2,598	226*	616*	1,453	9,721	5,604	8,219	9,699
B*48:01	13,405	15,434	17,495	**19,415**	4,926	8,614	**15,223**	**19,252**	4,208	**13,779**	**16,608**	**20,212**	2,409	246*	958*	1,265	3,242	**10,932**	**19,226**	**21,672**
B*49:01	11,637	8,707	10,536	11,785	947	**9,445**	**22,805**	**25,531**	790	**8,046**	**9,610**	**23,851**	2,887	305*	784*	1,859	7,776	5,671	8,384	9,727
B*50:01	13,252	9,989	11,198	12,934	1,005	**9,794**	**22,058**	**24,262**	853	**8,969**	**9,926**	**23,771**	303	33	145	212	4,349	7,383	**13,193**	**15,020**
B*51:01	0	0	0	0	0	**5,899**	**22,166**	**26,356**	997	**9,389**	**10,351**	**22,787**	1,583	166*	430*	1,049	0	0	6	0
B*51:02	362	204	491	563	0	**5,381**	**21,346**	**26,417**	848	**8,343**	**9,911**	**22,950**	1,494	123*	250*	963*	58	0	91	74
B*52:01	816	271	830	802	1,016	**8,997**	**22,059**	**24,913**	1,452	**11,205**	**12,232**	**21,790**	707	0	46	448	654	394	821	1,178
B*53:01	2,355	1,048	1,995	2,021	103	**6,268**	**21,245**	**25,311**	1,252	**10,543**	**11,129**	**21,817**	1,897	138*	518*	1,182	609	202	990	841
B*54:01	11,317	9,452	13,552	14,919	4,535	**9,756**	**15,842**	**18,960**	6,514	2,285	5,769	7,683	348	0	115	237	3,698	1,515	3,810	4,359
B*55:01	15,036	10,604	12,340	13,916	7,616	10,561	**14,611**	**18,182**	7,357	3,866	7,147	8,586	792	92	315	494	3,293	1,264	3,160	3,510
B*56:01	6,685	**13,129**	**17,589**	**19,757**	1,156	**9,873**	**22,155**	**24,834**	2,209	**12,824**	**14,210**	**20,445**	600	29	259	396	4,025	2,843	6,037	7,519
B*57:01	0	0	0	0	0	**5,184**	**21,056**	**25,586**	1,329	**10,491**	**11,108**	**22,157**	4,547	1,429	6,256	5,615	228	65	450	468
B*57:03	0	0	0	0	0	**4,219**	**21,159**	**26,756**	895	**9,647**	**10,172**	**22,670**	3,033	1,512	**7,629**	**6,398**	511	200	967	**1,024**
B*58:01	0	0	0	0	720	**8,737**	**22,653**	**25,153**	1,267	**10,971**	**11,613**	**22,728**	4,886	1,428	6,018	5,547	576	168	897	968
B*59:01	7,153	3,989	6,374	6,632	5,066	7,269	**11,018**	**15,030**	6,357	11,237	**15,459**	**17,676**	1,361	79*	330*	911*	529	188	721	656
B*67:01	2,010	**10,178**	**20,079**	**23,290**	655	**7,025**	**17,373**	**20,863**	97	0	97	126	2,357	246*	967*	1,465	6,473	4,760	7,275	8,857
B*73:01	2,132	**9,505**	**20,132**	**23,358**	672	166	397	1,148	119	0	94	162	2,221	180*	936*	1,235	5,957	9,239	**14,733**	**16,464**
B*78:01	11	0	0	0	0	**5,583**	**21,542**	**26,469**	1,049	**10,026**	**10,639**	**22,900**	209	21	102	158	358	159	491	475
B*81:01	2,868	**11,109**	**20,021**	**23,651**	0	0	0	0	1,368	438*	1,434	1,683	5,007	695*	2,084	2,805	2,621	**9,525**	**20,293**	**22,345**
B*82:01	11,236	13,365	**16,253**	**18,447**	4,442	**9,965**	**17,360**	**20,610**	6,462	2,506	6,381	7,558	2,988	442*	1,287	1,908	898	245	772	832
C*01:02	514	107	377	427	0	0	0	9	30	0	71	41	171	0	25	86	26	0	46	23
C*02:02	1,351	505*	1,058	1,097	388	386	0	852	736	254	884	967	0	0	0	0	13,316	6,023	6,863	12,998
C*03:02	0	0	0	0	0	0	0	77	11,526	5,983	11,811	13,030	0	0	0	0	705	514	**1,475**	1,233
C*03:03	0	0	0	0	0	0	0	0	10,954	4,705	10,055	11,415	0	0	0	0	1,013	729*	2,022	1,522
C*03:04	2	0	2	0	0	0	0	0	9,763	4,302	9,357	10,691	0	0	0	0	571	442	**1,290**	935
C*04:01	61	0	82	87	355	50	0	625	222	42	407	535	0	0	0	0	1	0	8	0
C*05:01	0	0	52	11	0	0	0	0	724	143	789	854	0	0	0	0	18	0	75	14
C*06:02	60	0	187	67	0	0	0	192	738	142	767	868	0	0	194	0	52	0	130	78
C*07:02	38	0	130	46	0	0	0	0	173	55	468	564	1,118	45*	113*	574*	1,071	521*	1,834	1,248

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ANNALS OF LABORATORY MEDICINE

Supplemental Data Table S1. Continued

Allele specificity	Sample 1				Sample 2				Sample 3				Sample 4				Sample 5			
	Naïve	Dilution	DTT	EDTA	Naïve	Dilution	DTT	EDTA	Naïve	Dilution	DTT	EDTA	Naïve	Dilution	DTT	EDTA	Naïve	Dilution	DTT	EDTA
C*08:01	115	0	151	124	0	0	0	0	17	0	39	36	0	0	0	0	567	125	732	544
C*12:03	77	0	110	69	0	0	0	97	64	0	127	108	0	0	0	0	33	0	43	29
C*14:02	52	0	61	59	0	0	0	24	57	0	104	132	0	0	0	0	0	0	13	0
C*15:02	67	0	120	120	0	0	0	240	756	146	759	1,000	697	134	204	261	0	0	21	0
C*16:01	47	0	125	58	0	0	0	144	52	0	105	71	0	0	0	2	548	236	946	686
C*17:01	207	25	359	299	890	297	823	1,184	868	297	1,379	1,371	1,067	59*	279*	538*	14,122	7,546	9,791	14,438
C*18:02	24	0	88	31	0	0	0	229	750	152	823	828	0	0	158	0	36	0	86	31

Beads exhibiting the prozone effect are bolded and underscored.
*MFI value of bead was ≥1,000 in the naïve sample and decreased to <1,000 after serum treatment.
Abbreviations: MFI, mean fluorescence intensity; SAB, single antigen bead; DTT, dithiothreitol.

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