Experimental Modification of Non-Specific Reactivity Treatment to Reduce Unexpected Reactivity

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Abstract

Purpose: To evaluate the effect of an experimentally modified non-specific reactivity treatment on serum background and non-specific reactivity during HLA antibody testing.

Methods: Twenty-nine serum samples were analyzed before and after treatment with an experimentally modified non-specific reactivity protocol, which involved a 10-minute incubation with modified microparticles followed by serum recovery via centrifugation and magnetic separation.

Results: Treatment reduced non-specific reactivity across NC bead, DR53, DP1, and DP5 targets, with the most elevated TMFI values decreasing below the 1,000-antibody cutoff.

Introduction

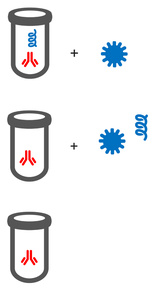
Unexpected reactivity and elevated negative control (NC) bead signals can complicate HLA antibody interpretation and lead to uncertainty during HLA antibody assignment. This study evaluated an experimentally modified treatment designed to reduce nonspecific serum reactivity before Single Antigen Bead (SAB) testing.

Materials and methods

Sample Preparation

The experimentally modified non-specific reactivity treatment involved incubating serum with modified microparticles for 10 minutes, followed by serum recovery by centrifugation and magnetic separation.

Figure 1. Treatment Overview



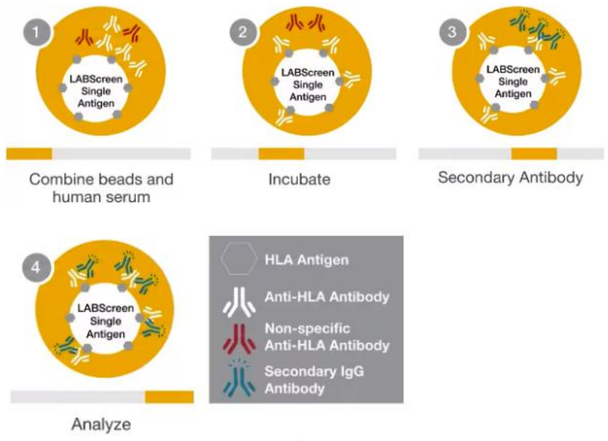
Test Method(s)

Each serum sample was tested before and after treatment using IgG, IgM, and C1q (SAB) assays.

Samples included NC bead background measurements for IgG, IgM, and C1q, as well as background reactivity against DR53, DP1, and DP5 alleles.

Materials and methods

Figure 2. SAB assay Overview



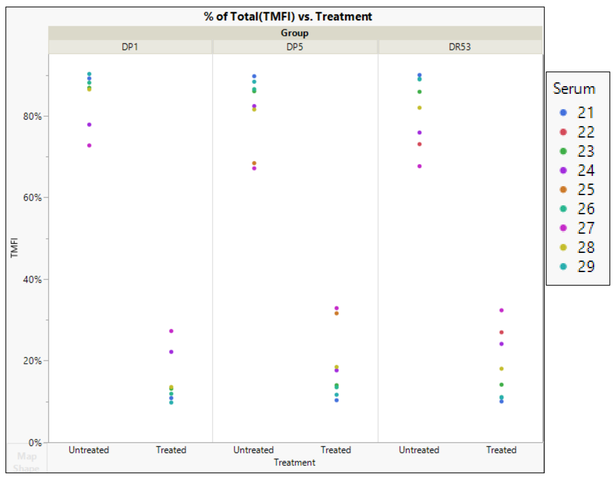
Data Analysis

A normalized trimmed mean fluorescence intensity (TMFI) cutoff of 1,000 was used for HLA antibody assignment. Raw TMFI values and HLA antibody assignments before and after treatment were compared.

Results

Alleles with untreated % of total TMFI values >60% showed a reduction to <40% following treatment.

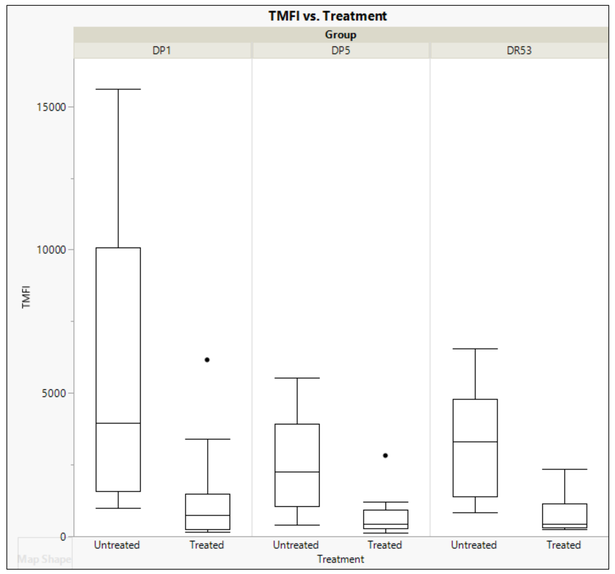
Figure 3A. Representative DR53, DP1, and DP5 SAB Reactivity Before and After Treatment



Results

Most alleles with elevated untreated TMFI values demonstrated substantial reductions, with many falling below the 1000 TMFI antibody assignment threshold.

Figure 3B. Effect of Treatment on DR53, DP1, and DP5 Reactivity



Results

Treatment consistently reduced elevated NC bead background across IgG, IgM, and C1q assays (Table 1).

Table 1. Effect of experimentally modified non-specific reactivity treatment on NC Bead Background (TMFI)

IgG (n=9)			
Serum	Untreated (TMFI)	Treated (TMFI)	**% Reduction**
1	170	20	88
2	1235	11	99
3	9909	20	100
4	11282	30	100
5	2569	2221	14
6	236	235	0
7	8947	8398	6
8	788	63	92
9	1475	1312	11
IgM (n=11)			
Serum	Untreated (TMFI)	Treated (TMFI)	**% Reduction**
10	5563	502	91
1	900	51	94
11	506	80	84
3	14558	57	100
12	752	183	76
13	9888	112	99
14	374	71	81
15	619	46	93
16	618	34	94
17	313	45	86
18	3802	18	100
C1q (n=5)			
Serum	Untreated (TMFI)	Treated (TMFI)	**% Reduction**
10	22953	852	96
13	18962	5999	68
14	8379	2069	75
19	249	325	-31
20	22824	1330	94

Conclusions

The modified non-specific reactivity treatment substantially reduced elevated background signals in IgG, IgM, and most C1q assays. Reactivity to DR53, DP1, and DP5 alleles was also reduced, with many TMFI values falling below the 1,000 TMFI antibody assignment threshold. These findings suggest that the treatment may improve SAB interpretation by reducing non-specific reactivity that can interfere with HLA antibody assignment.

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