MODIFICATION OF DOUBLE COLOR INDIRECT IMMUNOFLUORESCENT BASED PLATELET CROSSMATCH FOR PATIENTS WITH ANTI-PLATELET ANTIBODIES

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RESEARCH PROBLEMS

Patients with history of multiple blood transfusion and/or patients that are multiparous are at risk of producing anti-platelet alloantibodies resulting in platelet refractoriness. Immune-mediated refractoriness is mainly due to anti-HLA alloantibodies. So, donor HLA phenotyping and the use of HLA-matched donor is the suggested strategy for the successful platelet transfusion. Platelet crossmatch is based on a variety of principles for anti-platelet detection including solid phase red cell adherence assay (SPRCA), monoclonal antibody immobilization of platelet specific antigen (MAIPA), modified antigen capture ELISA (MACE), and platelet suspension indirect immunofluorescence (PSIFT). MAIPA is a reference method for anti-platelet detection but it is relatively time-consuming and not easily adapted for routine use. SPRCA is based on a simple hemaglutination but requires a high level of skill to perform and chloroquin treated is necessary.

The current approach differs from previous reports in that no chloroquine treatment is required to rule out anti-HLA antibodies. In the present study, platelets were fixed and blocked to prevent false negative and false positive results. Moreover, steps in the process are also adapted to avoid losing of the first signal associated with platelet alloantibodies in serum and the second signal corresponding to platelet specific antigens.

RESULTS OF THE STUDY

The result showed that pattern-3 is the best platform for platelet staining since it presents the highest signal of FITC-mouse anti-human CD41 in the lower right with the lowest background in the upper left quadrant compared to the first two patterns as observed in Figure 1C.
Comparison of treated and untreated platelets demonstrated that treated platelets provided the best results as shown in Figure 1D-1E. No false positive signal was present in the upper right quadrant. Taken together, these results indicate that platelets need to be prepared before any reaction. by firstly fixed with 1% paraformaldehyde following with AB serum in the final concentration of 20%. From all results, 2 µl/reaction of FITC-mouse anti-human CD41 antibody is recommended and 1.0 µg/ml of PE-goat F(ab')2 anti-human IgG, Fc specific is optimal to detect all primary antibodies (anti-platelets) in the serum.

Serum samples with and without anti-HLA of various panel reaction antibodies (%PRA) were tested based on identical ABO blood groups. Those twenty-six samples tested showed different patterns of signal in the upper right quadrant when identical ABO platelets were crossmatched.
Interestingly, samples with high %PRA did not always demonstrate higher MFI in the upper right quadrant compared to the low %PRA. Moreover, in some samples with low %PRA, high signal in the upper right quadrant were observed as shown in Figure 2 and Table 1, respectively. As incompatibility results was observed, some donor HLA was phenotyped. It was confirmed that most cases of incompatibility resulted from an antibody specific to donor HLA as demonstrated in Table 2.

The results also reveal that not all anti-HLA positive samples provided incompatibility. Moreover, signal in the upper right quadrant, was not related to the concentration of PRA. In addition, a donor with matched HLA may demonstrate incompatibility resulting from antibodies to non-HLA such as antibody to HPA or other membrane molecules.

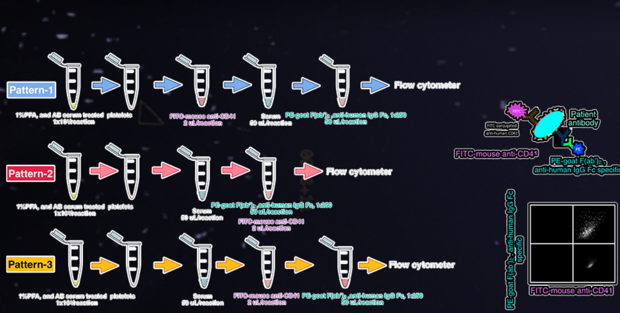

Table 1 Selection of most compatible or least incompatible platelets for patients with anti-HLA based on ABO identical blood.

Patient ID	Blood group	%PRA	Donor No.	MFI of upper right quadrant (%)			Interpretation
				Conjugated control	Donor control	Crossmatch	
KT354	A	7	1	4.8	1.2	3.8	Compatible
			2	4.9	3.2	11.3	Incompatible
			3	3.5	0.6	11.0	Incompatible
KT377	B	15	1	4.9	5.3	0.5	Compatible
			2	4.2	1.6	2.7	Compatible
			3	3.3	1.5	3.0	Compatible
KT688	B	44	1	4.9	5.3	2.2	Compatible
			2	4.2	1.6	2.6	Compatible
			3	3.3	1.5	2.1	Compatible
KT914	A	13	1	4.8	1.2	1.5	Compatible
			2	4.9	3.2	6.7	Incompatible
			3	3.5	0.6	2.3	Compatible
KT1521	O	13	1	4.6	3.1	4.0	Compatible
			2	2.0	1.4	18.8	Incompatible
			3	2.2	1.8	5.0	Least incompatible
KT1522	O	9	1	4.6	3.1	0.5	Compatible
			2	2.0	1.4	3.2	Compatible
			3	2.2	1.8	3.2	Compatible
KT1525	O	87	1	4.6	3.1	5.1	Least incompatible
			2	2.0	1.4	16.4	Incompatible
			3	2.2	1.8	2.1	Compatible
KT1544	B	7	1	4.9	5.3	3.1	Compatible
			2	4.2	1.6	0.9	Compatible
			3	3.3	1.5	1.4	Compatible

Table 2 Characteristics of three donor HLA and interpretation of platelet crossmatch based on ABO identical blood.

Patient ID	%PRA	Antidody assigned	Crossmatch with donor	HLA phenotype			MFI in UR (%)	Interpretation
				HLA-A	HLA-B	HLA-Bw		
KT354	7	B37, B47	A number 2	A*02:XX	B*38:AVDNY	4	11.3	Incompatible
				A*11:ASXFD	B*51:AYNHZ	-		
KT1521	13	B39, B38, A29	O number 2	A*24ATYXF	B*27:CFDS	4	18.8	Incompatible
				A*33:APRYA	B*44:RENE	-		
KT1525	87	B57, B67, B58, B54 B27, b13, A25, A2 B81, B63, B42, B7 B37, B49, B55, B50 A29, A3, Cw1, B11, B23 B32, B39, B56, B47, B38	O number 1	A*02:BCWPP	B*40:AVJTU	4	5.1	Least incompatible
				A*33:AXBBC	B*58:AVGCZ	6		

CONCLUSION

The present study demonstrates an effective modified flow cytometric-based method for platelet crossmatch. This system was able to crossmatch random platelets without prior antibody screening or donor HLA typing. Some background may be present in some donor autologous control due to remaining platelet surface immunoglobulins. Thus, donor control is recommended to be performed in parallel and signal in the upper right quadrant of donor control should be subtracted from the crossmatch test for interpretation. In addition, patient autologous reaction is suggested to verify the existing autoantibodies.

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