A MODIFIED PCR-SSP METHOD FOR THE IDENTIFICATION OF ABO BLOOD GROUP ANTIGENS

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Introduction

The ABO blood group antigens are carbohydrate molecules synthesised by ∀ 1-3 N-acetyl-galactosaminyl transferase encoded by the ABO gene on chromosome 9. Transplantation across the ABO barrier leads to rapid humoral graft rejection due to the presence of naturally occurring antibodies to ABO antigens.

We have recently introduced, and further developed, a method for ABO typing cadaveric organ donors by PCR-SSP.

Methods

The PCR-SSP method was based on the primer sequences described by Pearson & Hessner (1998) Br J Haematol 100, 229. Extensive modifications to eight of the primer sequences, and the inclusion of one new primer, were performed, to suit our standard HLA-A,B PCR-SSP typing technique (Darke et al., (1998) Exp Clin Immunogenet 15, 69). Details of the primer sequences together with their annealing positions are shown in Table 1.

Table 1. Primer sequences.

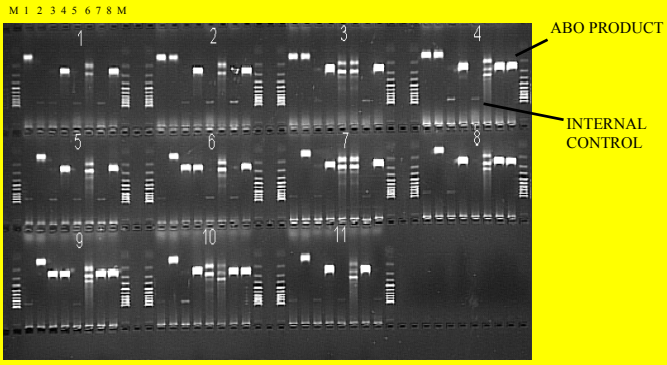
Primer name	5'-3' sequence	Orientation and annealing position(bp)
ABO5'262gTg	GAAGGATGTCCTCGTGGTG	sense 243-262 (A1, A2, O03, B)
ABO5'261gTA	GAAGGATGTCCTCGTGGTA	sense 243-261 (O1)
ABOx7CON	GATCAGTGACTTCTGCGAGCGGC	sense 573-595 (consensus)
ABO5'1061ACCg	CAGGCGGTCCGGAACCG	sense 1045-1061 (A201)
ABO5'1061CCCg	AGGCGGTCCGGAACCCG	sense 1045-1062 (A1,O1,O03,B)
ABOx6CON	ATATATATGGCAAACACAGTTAACCCAATG	antisense 345-374 (consensus)
ABO3'802Agg	CGACCCCCCGAAGAACCT	antisense 802-819 (O03)
ABO3'802ggg	CGACCCCCCGAAGAACCC	antisense 802-819 (A1, A2, O1)
ABO3'802gCg	CGACCCCCCGAAGAACGC	antisense 802-819 (B)
ABOi7CON	AATGGTGTCTCTGTGGTGGGGA	antisense 1219-1340 (consensus)
ABO3'804CgT	CCGACCCCCCGAAGAACG	antisense 803-820 (B)
ABO3'804ggT	CCGACCCCCCGAAGAACC	antisense 803-820 (A1, A2, O1, O03)

The twelve primers were used in eight mixtures to differentiate the ABO alleles into five groups: (1) A1 (all known A alleles, including A101-04, A202-04, A301, Ax01-03, Ael01-02, and A111, except A201); (2) A201; (3) O1 (all known O alleles, including O01, O02, O04-O07, O09-O13 and O0104, except O03 and O08); (4) O03; and (5) B (all known B alleles including B101-0106, B301, B(A)01, Bx01, Bel01-02 and CisAB01-02). This method detects all ABO alleles listed in the *Blood Group Antigen Gene Mutation Database* (www.bioc.aecom.yu.edu/bgmut/abo.htm).

As part of their validation process the primer mixtures were tested with DNA from 166 regular blood donors from the Welsh Bone Marrow Donor Registry. All had been previously ABO grouped, on several occasions, by standard automated serological methods.

Results

The PCR-SSP results of the 166 blood donors were in complete accord with the serological ABO grouping assignments. Eleven out of a possible fifteen genotypes were identified, examples of these are shown in Figure 1.



Donor no.	ABO genotype	Donor no.	ABO genotype
1	O1, -	7	A1, A201
2	O1, A1	8	A1, B
3	O1, A201	9	O03, B
4	O1, B	10	A201, B
5	A1, -	11	B, -
6	A1, O03		

Figure 1. Electrophoresis image of the eleven identified ABO genotypes.

ABO phenotype and maximum likelihood gene frequencies

	Phenotype frequency	Gene frequency
A1	0.41566	0.24144
A201	0.03614	0.01807
B	0.13253	0.06864
O1	0.83735	0.62057
O03	0.08434	0.04217

Hardy-Weinberg and homozygosity analysis

The observed numbers of genotypes showed a good fit to Hardy-Weinberg equilibrium (p=0.3) and the proportion of homozygotes was as expected (p=0.6).

Cadaveric donor typing

Since introducing this PCR-SSP method we have ABO typed 51 cadaver donors by PCR-SSP and our standard serological method and had no technical problems or assignment discrepancies.

Conclusions

ABO blood grouping by this PCR-SSP technique provides an accurate, simple, quick and cheap (£0.64 per sample) method of typing cadaveric organ donors. All ABO alleles are able to be detected, including rare alleles. This method works well under the same conditions as our routine HLA Class I PCR-SSP typing technique. This enables ABO typing to be carried out on the same DNA material as the HLA type thus minimising any risk of sample mix-up.