

Identification of suspected ABO subtypes by genotyping kits

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ABSTRACT

The aim of study is to use erythrocyte ABO genotyping to assist serological typing in the identification of suspected ABO subtypes. Nine samples with discrepancies in ABO serological forward and reverse typing were subjected to ABO 6–7 exon sequencing, and their respective results were cisAB01O1, Bw12O2, Ael05B, Bel03O1, Ael05O1, B(A)04O1, B(A)02O1, and two cases of O1O2 weak antibody for the reverse typing test. The ABO genotyping and serological ABO forward and reverse typing were combined for subtype identification. In the results of ABO genotyping, cisAB and B(A)04 were only found B gene in genotyping, thus the presence of A subtype B was excluded. The samples with a weak antibody for serological O type in reverse typing were tested ABO genotyping to exclude subtype A or B. We summarized a combination of ABO genotyping and serological typing can be used for identification of suspected ABO blood groups.

Keywords: ABO genotype, ABO discrepancy, ABO subtype

INTRODUCTION

Identification of ABO subgroups requires additional experiments to assist in the interpretation^[1]. Absorbed elution tests are rarely used clinically. In addition to operational errors, false positive and false negative results from experimental operations also influence accuracy. With the development of erythrocytic molecular biology, the combination of ABO genotyping and serological forward and reverse typing can detect ABO subgroups. Here, we report the serological and genotyping results of nine subjects with discrepancies between ABO forward and reverse typing.

MATERIALS AND METHODS

Subjects

Nine samples with discordant serological reac-

tions in forward and reverse typing were selected from Beijing Hospital from January 2010 to July 2016 and subjected to gene sequencing to determine subgroups and ABO genotypes. This study was approved by local IRB and all subjects assigned the informed consent.

Serological ABO forward and reverse typing

The monoclonal antibodies of anti-A, anti-B, and anti-H for ABO forward blood typing, and ABO reverse typing cells were all purchased from Jiangsu LiBio Medicine Biotechnology Co., Ltd (China). All procedures were in accordance with the standard operation code of the Beijing Hospital.

DNA extraction

Peripheral venous blood (2 mL) was placed in EDTA anti-coagulative tubes. DNA was extracted from whole blood samples using the blood genome DNA extraction kit manufactured by TIANGEN Biotechnology Co., Ltd (Beijing, China). The DNA sample was preserved at –80 °C before biological analysis.

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ABO genotyping and ABO 6–7 exon sequencing

The ABO genotyping kit (ABO Basic, RBC–Fluo–Gene series, technology transferred from Germany INNO–Train Company, Jiangsu LiBio Medicine Biotechnology Co., Ltd.) was used to detect the genotypes of the samples. The fluorescent signal reading equipment (FluoVista, technology transferred from the Germany INNO–Train Company, Jiangsu LiBio Medicine Biotechnology Co., Ltd.) was used to determine the PCR results. The automatic software (FluoGene, technology transferred from the Germany INNO–Train Company, Jiangsu LiBio Medicine Biotechnology Co., Ltd.) was used to analyze the genotypes of A1, A2, B, O1, and O2 samples. The ABO 6–7 exon sequencing kit was used to verify the subtype (ABO exon 6–7 sequence, Jiangsu LiBio Medicine Biotechnology Co., Ltd.).

RESULTS

Serotyping, genotyping, and exon sequencing results of the nine samples with discordant ABO serological forward and reverse typing are shown in (**Table 1**). Both CisAB and B (A) were found in the

Group B gene without A gene in ABO genotyping. For Ael and Bel, serological results were similar to Group O, but they have the A or B gene in ABO genotyping.

DISCUSSION

The cisAB01 is the most common cisAB subtype in China. Although forward typing is positive for both anti–A and anti–B, cisAB01 has three characteristic genetic mutations similar to Type A1^[2-4]. It contains allele B instead of allele A1 in tests using domestic registered genetic kits and the commercialized kits used in this study. Tests for other genotypes such as cisAB03 are required to determine whether other cisAB subtypes contain the A gene^[3]. Agglutination of 1+~3+ was observed on the gel for forward anti–A typing in the B(A)04 subtype. The result was negative based on visual inspection after continuous shaking at high frequency and low amplitude in the tube method. Agglutination of 4+ was observed on the gel for forward anti–A typing in the B(A)02 subtype, which can be easily confused with group AB^[5-8]. Neither B(A)02 nor B(A)04 contains A1 or A2 gene in ABO genotyping.

Table 1 Serotyping, Genotyping, and Exon Sequencing Results of Nine Samples with Discrepancies between ABO Serological Forward and Reverse Typing

Sequenced	Serotype (tube method)						ABO Genotype						
	Forward	Forward	Forward	Forward	Forward	Reverse	Reverse	A1	A2	B	O1	O2	Allele
Exon 6–7	Anti–A	Anti–B	Anti–A,B	Anti–A1	Anti–H	A cell	B cell						
cisAB01O1	4+	3+mf	3+mf	0	4+	0	w+	+	0	0	+	0	BO1
B(A)02O1	3+ [§]	4+	4+	0	4+	2+	0	0	0	+	+	0	BO1
B(A)04O1	*	4+	4+	0	4+	4+	0	0	0	+	+	0	BO1
Bw12O2	0	2+	2+	0	4+	4+	0	0	0	+	0	+	BO2
Ael05O1	0	0	0	0	4+	1+	4+	+	0	0	+	0	A1O1
Bel03O1	0	0	0	0	4+	3+	1+	0	0	+	+	0	B1O1
Ael05B	0	4+	4+	0	4+	0	0	+	0	+	0	0	A1B
O1O2	0	0	0	0	4+	1+	4+	0	0	0	+	+	O1O2
O1O2	0	0	0	0	4+	3+	0	0	0	0	+	+	O1O2

*2+ in card method, w+ and continuously and slowly diffused in tube method. [§]4+ in card method.

ing. A subtype B and B(A) can be easily distinguished by genotyping^[9].

The Ael subgroup, Bel subgroup, and O group samples with weak agglutination in reverse typing are difficult to distinguish serologically, requiring the absorption elution test. However, the absorption elution test tends to produce high levels of false positive and false negative results due to problems, such as lack of proficiency by the experimenter and use of human-derived anti–A and anti–B antibodies^[10]. ABO genotyping can be used to quickly distinguish between O group, A subgroup, and B subgroup in weak agglutination in reverse typing.

ABO genotyping can identify correct ABO subgroups, which is suitable for medical centers and laboratories of the Transfusion Department with disciplinary development potential, such as reference laboratories, etc.

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