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ORIGINAL ARTICLE

Application of RhD Blood Group to Simulate Antibody Identification Test in Immunohematology Education

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SUMMARY

Background: **Immunohematology skill education is an important part of the transfusion medicine professional training. We tried to solve the difficulty of obtaining suitable and sufficient positive samples in the immunohematology education.**
Methods: **Different identification panels and panel cells were created by RhD-positive red blood cells (RBCs) and RhD-negative RBCs, according to the underlying antibodies. Diluted anti-D reagent was used as simulated plasma for identification.**
Results: **The antibody identification of single antibody with dose-effect and two antibodies present at the same time were successfully simulated.**
Conclusions: **It is a practical and cheap method for antibody identification training to use RhD blood group, especially when positive samples are short.**
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KEYWORDS

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INTRODUCTION

Immunohematology skill education is an important part of the transfusion medicine professional training [1,2]. The detection of antibodies that are directed against red blood cell antigens is critical in the pretransfusion compatibility testing. Once an antibody has been detected with the antibody screen, additional testing, with an antibody identification panel, is necessary to identify the antibody and determine its clinical significance. Clinically significant alloantibodies are those, that can cause hemolytic transfusion reactions, fetal and neonatal hemolytic diseases, or a decrease in the transfused red blood cell survival [3]. However, it is difficult for schools and training laboratories to obtain suitable clinical samples, especially a sufficient number of positive samples to meet the antibody identification experimental teaching requirements [4]. Since red blood cell serol-

ogy essentially recognizes the reactivity of an antigen on the red blood cell membrane with its corresponding antibody, most serological experiments can be simulated, as long as the appropriate antigen-antibody pair is found. Therefore, we used the RhD antigen and anti-D to simulate antibody identification in immunohematology techniques.

MATERIALS AND METHODS

Plasma sample preparation

IgG anti-D was double-diluted and reacted with *R2R2* RBCs. The minimum dilution ratio of 2+ agglutination intensity was 512. Therefore, anti-D with this titer was chosen to simulate plasma sample containing unexpected antibodies, in subsequent experiments.

Identification panel cells fabrication

RhD-positive RBCs and RhD-negative RBCs were used to fabricate antibody identification panel cells. To simulate dose-effect and mixed antibody, we searched several RhD-positive RBCs reacting with diluted anti-D, including *R2R2*, *R1R1*, and *R0R0* RBCs. According to the agglutinative strength, *R1R1* and *R2R2* cells were chosen in subsequent experiments.

Experimental design

According to the self-made antibody identification cell panel of the simulated anti-Fy^b antibody (Table 1), RhD negative RBCs were assigned to panel cells 1, 2, 4, 6, and 8, *R2R2* cells were assigned to panel cells 5 and 7, and *R1R1* cells were assigned to panel cell 3.

The antibody identification cell panel mimicking the anti-Jk^a and anti-E mixed antibodies were made up (Table 2). Accordingly, RhD negative RBCs were assigned to panel cells 1, 2, 4, 6, and 8, *R2R2* cells were marked as panel cell 3, *R1R1* cells were numbered as panel cells 5 and 7.

Serological methodologies

The unexpected antibodies were identified with the microcolumn gel card method. The 50 μL panel cells and 25 μL simulated plasma were added to microcolumn gel card (Bio-Rad, USA) in turn, were incubated at 37℃ for 15 minutes, and were centrifuged for 10 minutes.

Analysis

Combined with the results of the simulated experiment and the self-made antibody identification cell panel, the results were analyzed with the “crossing out” method to confirm the specificity of the antibody in simulated plasma.

RESULTS

Simulated single antibody with dose effect identification exemplar reactions

An exemplar of a SAID (simulated antibody identification) panel result of anti-Fy^b reactions is shown in Figure 1. Panel cell 3, 5, and 7 had a positive agglutination reaction with simulated plasma, and the agglutination intensity of panel cell 3 was weaker than of panel cell 5 and 7, and the other cells were negative. According to the panel cell worksheet (Table 1), anti-Fy^b was identified. This verifies that this method can be used to simulate the identification of a single antibody with dose-effect.

Simulated mixed antibodies identification exemplar reactions

The exemplar of a SAID panel result of a mixture of anti-E and anti-Jk^a (Figure 2) shows the complex combination reaction pattern. Of the eight panel cells, only panel cell 3, 5, and 7 agglutinated with simulated plasma, and the agglutination intensity of panel cell 3 was stronger than of panel cell 5 and 7, which is consistent with the expected results. Combined with the self-made antibody identification worksheet (Table 2), it can be concluded that the simulated plasma in this set of experiments contains anti-E and anti-Jk^a mixed antibodies.

DISCUSSION

In this study, we designed antibody identification serological tests, by using Rh positive and negative RBC combined with a self-made antibody panel cell worksheet, successfully simulated single alloantibody of anti-Fy^b with dose-effect, as well as mixed antibodies of anti-E and anti-Jk^a. This provides a simple method to obtain teaching samples in medical colleges or clinical laboratories.

Pretransfusion tests include the patient’s ABO group and Rh type, detection of unexpected antibodies, and crossmatch. Once an antibody has been detected with the antibody screen, additional testing is necessary to identify the antibody and determine its clinical significance [5]. An antibody identification panel is a collection of 8 to 20 group O reagent RBCs with various antigen expression. The phenotypes of antigen expression should be distributed, so that single common alloantibody specificities can be clearly identified, and most others can be excluded. It is a complicated and difficult approach, so training the skill of traditional immunohematology testing is still important for medical students and for the staff of transfusion services, to some extent. However, it is difficult to obtain enough and suitable serum samples even in clinical laboratories. Therefore, we simulated antibody identification experiments by using the RhD blood group. RhD positive and negative RBCs were allocated into a series of antibody identification cell panels, according to the panel sheet and intended

Table 1. Antibody identification cell panel of the simulated anti-Fy^b antibody.

	Rh-hr						Kell						Duffy		Kidd		Lewis		P	MNS				Luther		Xg
	C	D	c	E	e	C^w	K	k	Kp^a	Kp^b	Js^a	Js^b	Fy^a	Fy^b	Jk^a	Jk^b	Le^a	Le^b	P1	M	N	S	s	Lu^a	Lu^b	Xg^a
1	0	+	+	+	0	0	0	+	0	+	0	+	+	0	+	0	0	+	+	0	+	+	0	0	+	+
2	+	0	0	+	+	+	+	0	0	+	0	+	+	0	+	0	+	0	+	+	0	0	+	0	+	+
3	+	+	0	+	0	0	0	+	0	+	0	+	+	+	0	+	0	+	0	+	0	0	+	0	+	+
4	0	+	+	0	+	0	0	+	0	+	0	+	+	0	+	0	0	+	+	0	+	+	+	0	+	+
5	+	0	0	0	+	+	+	0	0	+	0	+	0	+	0	+	+	0	+	+	+	0	+	0	+	+
6	0	0	+	0	+	0	0	+	0	+	0	+	+	0	+	0	+	0	+	+	0	0	+	0	+	+
7	+	+	+	0	+	0	+	0	0	+	0	+	0	+	0	+	0	+	+	+	0	+	0	0	+	0
8	0	0	+	0	+	0	+	0	0	+	0	+	+	0	0	+	0	+	+	+	+	+	+	0	+	0

Table 2. Antibody identification cell panel mimicking the anti-Jk^a and anti-E mixed antibodies.

	Rh-hr						Kell						Duffy		Kidd		Lewis		P	MNS				Luther		Xg
	C	D	c	E	e	C^w	K	k	Kp^a	Kp^b	Js^a	Js^b	Fy^a	Fy^b	Jk^a	Jk^b	Le^a	Le^b	P1	M	N	S	s	Lu^a	Lu^b	Xg^a
1	0	+	+	0	+	0	0	+	0	+	0	+	+	0	0	+	0	+	+	0	+	+	0	0	+	+
2	+	0	0	0	+	+	+	0	0	+	0	+	0	+	0	+	+	0	+	+	0	0	+	0	+	+
3	+	+	0	+	0	0	0	+	0	+	0	+	+	0	+	0	0	+	0	+	0	0	+	0	+	+
4	0	+	+	0	+	0	0	+	0	+	0	+	0	+	0	+	0	+	+	0	+	+	+	0	+	+
5	+	0	0	+	0	+	+	0	0	+	0	+	+	0	0	+	+	0	+	+	+	0	+	0	+	+
6	0	0	+	0	+	0	0	+	0	+	0	+	+	0	0	+	+	0	+	+	0	0	+	0	+	+
7	+	+	+	0	+	0	+	0	0	+	0	+	+	0	+	0	0	+	+	+	0	+	0	0	+	0
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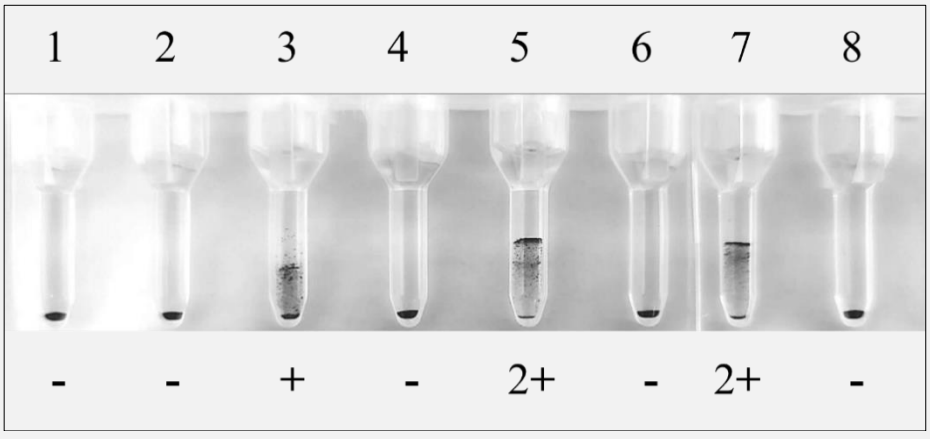

Figure 1. Simulated anti-Fy^b antibody identification exemplar reactions.

Panel cells 3, 5, and 7 had a positive agglutination reaction with simulated plasma, and the agglutination intensity of panel cell 3 was weaker than of panel cells 5 and 7, and the other cells were negative.

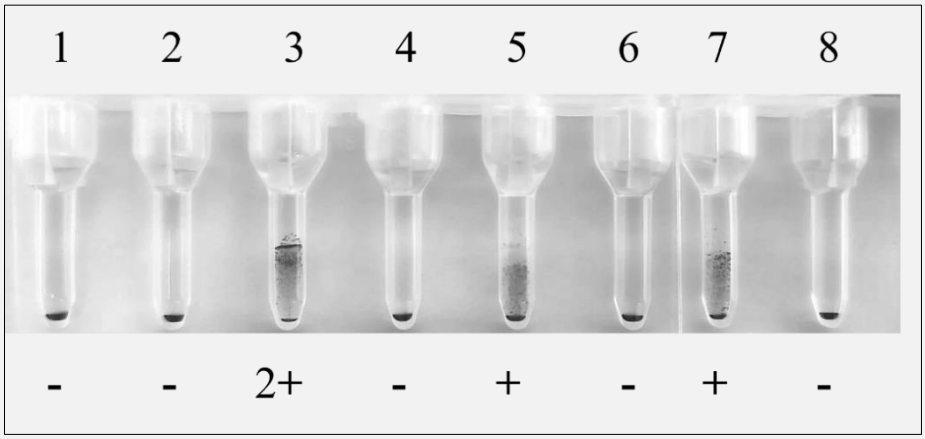

Figure 2. Simulated anti-E and anti-Jk^a mixed antibodies identification exemplar reactions.

Panel cells 3, 5, and 7 had a positive agglutination reaction with simulated plasma, and the agglutination intensity of panel cell 3 was stronger than of panel cells 5 and 7, and the other cells were negative.

antibodies. The diluted anti-D reagents simulated plasma to be identified. When anti-D reacts with Rh positive RBC, it will look like intended antibodies that react with their corresponding antigen on panel cells.

In this study, Table 1 is the antibody identification panel cell worksheet, specifically designed for anti-Fy^b. According to the experimental results (Figure 1), it can be derived, that the simulated plasma had positive agglutination reactions with the simulated identified panel cells 3, 5, and 7, while the others were negative. By negative crossing-out, the antigens of Rh, Kidd, Lewis, P, MNS, Xg blood group, K, k, Kp^b, Js^b of Kell blood group, Lu^b of Lutheran blood group, and Fy^a of Duffy blood group are probably not the antibody's target. Kp^b, Js^b of Kell blood group, Lu^a of Lutheran blood group, and Fy^b of Duffy blood group cannot be ruled out. However, Kp^b, Js^b, and Lu^a are low-prevalence antigens; the aimed antibodies may not be one of those. The most probable antibody is anti-Fy^b. On the other hand, the intensity of agglutination reaction between simulated plasma and panel cell 3 was weak. Panel cell 5 and 7 simulated the homozygote of Fy^b, and panel cell 3 simulated the heterozygote of Fy^b. Therefore, by comprehensive analysis, the unexpected antibody contained in the simulated plasma should be anti-Fy^b. In a similar way, Table 2 is designed for the antibody identification panel cell worksheet, when anti-E and anti-Jk^a were present at the same time. Based on the experimental results (Figure 2), simulated plasma reacted stronger with panel cell 3 than with panel 5 and 7. Panel cell 3, which represents both antigen E and antigen Jk^a, is positive and panel cells 5 and 7, which simulate one of the antigens, are also positive. Therefore, the simulated plasma contains both anti-E and anti-Jk^a. By altering the antibody identification panel cell worksheet, they can also be used for antibody identification of any other single antibody with a dose-effect or mixed antibodies.

Stephen M. Henry's group used Kode technology, attached a conserved viral peptide antigen sequence of SARS-CoV-2 secondarily to the RBC membrane, and then used immune plasma samples from vaccinated or COVID-19 convalescent individuals to create an antigen–antibody pairing [4]. Although more exemplars could be achieved by using the above-mentioned method, it is difficult to grasp Kode technology and obtain COVID-19 immune plasma. In contrast, RhD positive RBCs, RhD negative RBCs, and IgG anti-D reagent are easy to obtain for transfusion laboratories.

In developed countries, anti-Fy^a or other antibody reagents can be used directly as simulated plasma. In underdeveloped and developing countries, where laboratories do not have the financial capacity to provide such expensive rare reagents for antibody identification training, anti-D reagent is a more affordable option.

The limitation of this experiment is that the individual's autologous red cell phenotype cannot be identified to support the presumptive antibody identification. Determining the phenotype of an individual's autologous red cells is an important part of antibody identification, because the autologous RBCs should lack the antigen(s) to which the serum seemingly contains antibody(ies), with few exceptions (e.g., Rh-positive patient with anti-D). To sum it up, this study has effectively solved the difficulty of obtaining suitable and sufficient positive samples in immunohematology education; using RhD blood group system simulates the antibody identification test,

the materials are simple and easy to obtain, and the test results are clear and intuitive, and especially innovative in the teaching of antibody identification testing.

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Yingying Niu designed the research study and wrote the first draft of the manuscript. Yingying Niu, Honghui Long, and Danting Wang performed the research. Yuxin Wang and Jiang Wang acquired and analyzed the data. Chunyan Huang supervised the research and reviewed the manuscript.

Declaration of Interest:
The authors have no conflicts of interest to declare.

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