Evaluation of Magnetized-Erythrocyte Group Antigens to Detect ABO Antibodies

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Abstract Screening for IgM titers of anti-A and anti-B is recommended when providing ABO incompatible platelet transfusion. The life-time of reagent cells depends upon the preservative diluents. We aimed to evaluate the IgM titers of anti-A and anti-B testing with magnetized-erythrocyte group antigens (MEGA) and fresh RBCs and study the relationship of ABO antibody titers between both techniques. Altogether, 100 serum samples from group O donors at the National Blood Centre, Thai Red Cross Society, Bangkok, Thailand were included. EDTA blood from three different A and B blood group individuals was prepared as fresh reagent RBCs and MEGA. Each serum sample was tested simultaneously for IgM anti-A and anti-B titers using fresh RBCs and MEGA by standard tube technique. Antibody titers were compared between both techniques. Test for reproducibility and stability of MEGA were performed. The IgM anti-A and anti-B titers using fresh RBCs yielded higher agglutination scores than MEGA ($P < 0.001$). However, a good correlation was obtained in the agglutination titers (anti-A, $r = 0.838$ and anti-B, $r = 0.877$). The mean and standard deviation of anti-A and anti-B titers using MEGA from five sera in triplicate showed no significant difference ($P > 0.05$). Moreover, the titer test results using MEGA after dilution remained stable up to 8 h. The MEGA can be used as a

replacement for fresh RBCs to perform ABO serum grouping. It is simple to use, avoids centrifugation and provides good results in terms of stability and reproducibility.

Keywords IgM titers · Anti-A · Anti-B · Magnetized-erythrocyte group antigens

Introduction

Naturally occurring ABO antibodies are the most significant for transfusion practice, especially in providing plasma and platelet transfusions. Testing donors for IgM high titers is one of the screening methods when using platelets containing ABO incompatible plasma [1] to prevent life-threatening complications, such as acute hemolytic transfusion reactions, veno-occlusive disease and increased morbidity in allogeneic transplants [2–7]. Until now, variation in the test results of ABO titer measurements has been argued, especially in decision-making in ABO incompatible kidney transplantation; standardization of ABO titer measurement among laboratories is required [8].

Generally, reagent red blood cells (RBCs) used for routine ABO serum (reverse) grouping are suspended in saline, phosphate buffer saline (PBS) for daily use. To provide stability and similar antigenic strength of reagent RBCs, Alsever's solution has been used as a preservative solution to store RBCs. However, unexplained agglutination of stored RBCs in Alsever's solution caused by the gram-negative bacterium was reported [9].

A fully automated method of erythrocyte-magnetized technology has been previously introduced for ABO-RH-K phenotyping and antibody detection. The advantages of

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using magnetized RBCs are that centrifugation and washing phases can be avoided [10–12]. Moreover, lyophilized magnetized-erythrocyte group antigen (MEGA) can be kept at room temperature for years. A recent study reported the application of MEGA for testing MNS7 antibodies to detect antibodies with success. Among 200 human serum samples tested, the antibody detection results using MEGA and fresh GP.Mur RBCs were in agreement [13].

Importantly, rapid and accurate testing for ABO grouping including both cell and serum grouping is required in mobile blood collection units or in the rural community when serologic centrifuge is not available. Using MEGA is more challenging in determining IgM anti-A and anti-B. However, variations in IgM titers of anti-A and anti-B needed to be considered [14, 15]. In this study, the IgM titers of anti-A and anti-B testing with fresh RBCs and MEGA were compared and the relationship of the ABO antibody titers between both techniques was evaluated.

Materials and Methods

Subjects

Altogether, 100 blood samples obtained from group O Thai blood donors of the National Blood Centre, Thai Red Cross Society, Bangkok, Thailand were included in this study. They comprised 52 males and 48 females (M:F = 1.1:1) and their ages ranged from 18 to 58 years. Informed consent was obtained from each subject. This study was approved by the Committee on Human Rights Related to Research Involving Human Subjects, Thammasat University, Pathum Thani, Thailand. Two milliliters of each serum sample were separated from clotted blood within 24-h after collection and kept at -80°C until use.

Preparation of Fresh RBCs and MEGA

For fresh RBCs preparation, 5 mL of EDTA blood from three different A and B blood group individuals was prepared for a 3 % fresh RBCs suspension in saline [16]. Moreover, they were prepared for the MEGA-A and MEGA-B as previously described at the Suzhou Institute of Biomedical Engineering and Technology, Chinese Academy of Sciences, Suzhou, China [13]. Each aliquot of lyophilized MEGA-A and MEGA-B was originated from 500 μL of whole blood; however, approximately 350 μL of distilled water was added to obtain the proper size of cell buttons for reading agglutination reactions. Similar to reagent A and B cells of fresh RBCs, the MEGA-A and MEGA-B reagents were prepared from aliquots of three individuals.

IgM Anti-A and Anti-B Agglutination Titers Using Fresh RBCs and MEGA

Doubly serial two-fold dilutions of each serum sample were performed. IgM anti-A and anti-B titers were determined using the saline method with incubation at room temperature for 5 min. Each serum sample was tested simultaneously for IgM titers of anti-A and anti-B using fresh RBCs and MEGA by standard tube technique [13]. To reduce the inter-technician variation, one technician performed ABO antibody titers using fresh RBCs; while, another one performed the test using MEGA. Both tests were done in parallel. To determine IgM titers using fresh RBCs, the cell suspension was centrifuged at 1000 g for 15 s and examined for agglutination of the RBCs. In contrast, to determine IgM titers using MEGA, the migration of MEGA rapidly formed a pellet at the bottom of each tube by a magnetic bar. After being shaken, the unbound MEGA was resuspended, while agglutinated MEGA formed a button. All reactions were read macroscopically. The grading of the agglutination reactions of both techniques were scored as 12, 10, 8, 5, 3, 0 for 4+, 3+, 2+, 1+, w+ and negative, respectively [17]. A titer was determined as the highest dilution showing w+ agglutination. To increase the validity and reliability of the evaluation, the laboratory technicians were blinded for test results.

Test for Reproducibility and Stability of MEGA

The reproducibility of IgM anti-A and anti-B titers determined by MEGA-A and MEGA-B was performed using five group O serum samples. Each test was performed in triplicate. In addition, the stability of MEGA-A and MEGA-B was tested for IgM anti-A and anti-B titers with pooled group O serum samples, divided in a 1 mL aliquot and kept at -80°C until use. IgM anti-A and anti-B titers of pooled serum samples were tested with MEGA-A and MEGA-B after diluting the aliquots with distilled water and keeping at room temperature. Time interval for MEGA stability testing was divided into 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, and 24 h.

Statistical Analysis

IgM anti-A and anti-B titers obtained by using fresh RBCs and MEGA were compared. The statistical difference between paired values of the sum of agglutinations scores in two techniques was calculated by the two-tailed paired *t* test. Linear regression and Pearson's correlation were used to analyze IgM anti-A and anti-B titers between the two techniques. Moreover, reproducibility of IgM anti-A and anti-B titers using MEGA was evaluated by analysis of variance (One-way ANOVA). The analysis was performed

using SPSS (Version 15.0, SPSS Inc., Chicago, IL, USA). A P value of less than 0.05 was considered statistically significant.

Results

Determination of IgM Anti-A and Anti-B Agglutination Titers

A total of 100 group O serum samples were tested for IgM anti-A and anti-B titers using fresh RBCs and MEGA. When we compared the paired values of the sum of agglutination scores in two techniques, they were found to be significantly higher in fresh RBCs than in the MEGA for both anti-A and anti-B ($P < 0.001$), as shown in Table 1. The correlation of IgM anti-A and anti-B agglutination titers in group O serum samples testing with fresh RBCs and MEGA was analyzed. The correlation coefficient (r value) of anti-A titers between fresh RBCs and MEGA using a logarithmic scale was 0.838, $P < 0.001$ with the regression equation of $y = 0.905x + 1.1567$. Additionally, the r value of anti-B titers between two values was 0.877, $P < 0.001$ and the regression equation was $y = 0.896x + 1.0057$ (Fig. 1). When we analyzed trend differences of the agglutination titers between two values, equal titers were demonstrated in 40 samples for anti-A. MEGA titers were higher in 54 samples; whereas, they were lower in 6 samples. Regarding anti-B titers, equal titers were found among 47 samples; MEGA titers were higher in 45 samples and were lower than fresh RBCs in 8 samples.

MEGA Reproducibility and Stability Testing

To assess the reproducibility of IgM anti-A and anti-B titers using MEGA-A and MEGA-B, the agglutination titers in 5 serum samples were performed in triplicate. The mean and standard deviation (SD) values of the sum of agglutination scores were determined (Table 2). No significant difference was found between the anti-A and anti-

B agglutination scores ($P > 0.05$). Regarding the stability of MEGA-A and MEGA-B after dilution within 24-h, the agglutination titers of both anti-A and anti-B remained stable up to 8 h (Fig. 2).

Discussion

ABO blood group testing on patients and donors certainly requires both red cell and serum testing because each serves as a check on the other. The two exceptions that require only ABO testing of RBCs are testing the blood of infants less than 4 months of age and confirmation testing of ABO type in labeled donor units [16]. Regarding ABO serum grouping, various reagent A and B red cell suspension media can be used such as isotonic saline, PBS, and Alsever's solution. Unexpected agglutination reactions with reagent RBCs that are not due to blood group antibodies were found. These include IgM antibodies to hydrocortisone, antibodies to sugar, and hemagglutinating properties dependent on polycarboxyl groups [18]. In addition, unexplained hemagglutination reactions of approximately 10–20 % of different types of RBCs stored for several days in modified Alsever's solution were found. These findings were caused by the biofilm-forming, antibiotic-resistant, gram-negative bacterium and *Serratia liquefaciens* strain that was isolated from the supernatant of stored RBCs in Alsever's solutions [9].

In this study the IgM titers of anti-A and anti-B testing with fresh RBCs and MEGA were evaluated. Comparing the sum of ABO antibody agglutination scores between using fresh RBCs and MEGA, we found that the sum of agglutination scores were significantly higher in fresh RBCs than MEGA possibly due to the stronger effect of centrifugation force compared with magnetic force. However, hemagglutination titers of both anti-A and anti-B showed good correlation between the two techniques. Therefore, the use of MEGA could be accomplished as an alternative reagent RBCs for ABO serum testing. Interestingly, trend differences of the agglutination IgM ABO titers between the two values were demonstrated and the

Table 1 Comparison of paired difference in the sum of IgM agglutination scores using fresh RBCs and MEGA

Antibody	Number	Paired difference of the sum of agglutination scores					<i>P</i> value
		Mean	SD	SEM	95 % CI of the difference		
					Lower limit	Upper limit	
Anti-A (fresh RBCs score—MEGA score)	100	8.39	10.67	1.07	6.27	10.51	<0.001
Anti-B (fresh RBCs score—MEGA score)	100	7.79	8.72	0.87	6.06	9.52	<0.001

SD standard deviation, SEM standard error of mean

Fig. 1 Correlation of IgM anti-A and anti-B agglutination titers in 100 group O serum samples using fresh RBCs (a) and MEGA (b)

Table 2 Reproducibility of the sum of IgM anti-A and anti-B agglutination scores determined by MEGA-A and MEGA-B

Antibody	Number	The sum of agglutination scores					<i>P</i> value
		Mean	SD	SE	95 % CI of mean		
					Lower limit	Upper limit	
Anti-A	15	58.53	5.77	1.49	55.34	61.73	0.648
Anti-B	15	47.80	7.26	1.88	43.78	51.82	0.987

SD standard deviation, *SE* standard error

Fig. 2 IgM anti-A and anti-B agglutination titers using MEGA within 24-h

use of MEGA could provide higher titers than fresh RBCs. In general, to resolve discrepancy due to absence of expected ABO antibodies, prolonged incubation of the serum with A and B reagent RBCs at room temperature or 4 °C is recommended [16]. Hence, we suggest that the use of MEGA for ABO serum grouping could enable the correct blood group prediction in patients with hypogammaglobulinemia.

The advantages of using MEGA are first, the triplicate agglutination scores showed no significant difference. Second, working MEGA is stable at room temperature up

to 8 h, and suitable for routine testing. Third, the ABO serum testing using MEGA could be applied for mobile medical units in rural communities because of its simplicity and no centrifugation required. Fourth, validity testing of commercial anti-A and anti-B reagents using MEGA is helpful to reduce variations in antigenic strength and to provide the same standard of reagent RBCs. However, the cost required for MEGA is still not available to be considered as a commercial kit. Even though the hemolysis reaction is not found, when testing serum with MEGA compared with fresh RBCs, so it does not affect the test interpretation. Finally, the awareness of using MEGA is that after dilution of lyophilized MEGA must be used within the recommended time.

In conclusion, the MEGA can be used as a replacement for fresh RBCs to perform ABO serum grouping. It is simple to use, avoids centrifugation and provides good results in terms of stability and reproducibility.

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Compliance with Ethical Standards

Conflict of interest The authors declare that there are no conflicts of interest.

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