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Evaluation of Erythrocyte-Magnetized Technology in the Detection of Irregular Antibodies

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ABSTRACT: This study aims to evaluate the application value of erythrocyte-magnetized technology in the detection of irregular antibodies. A total of 10 113 patients requiring blood transfusion were selected for the detection of irregular antibodies by microcolumn gel test and erythrocyte-magnetized technology simultaneously. The saline solution method and in-tube indirect antihuman globulin test were used as reference comparisons. Their ability to detect low titer antibodies was evaluated. A total of 72 antibody-positive samples were detected with a detection rate of 91.67% by microcolumn gel test and 84.72% by erythrocyte-magnetized technology. IgG antibody detection ability was consistent; however, IgM antibody detection ability was significantly lower. Both of them had considerably low titer antibody detection ability. This study suggests that erythrocyte-magnetized technology could serve as a valuable platform for irregular antibody analysis in clinic.

Keywords: Erythrocyte-magnetized technology; Icrocolumn gel test; Regular antibody; Transfusion

1. Introduction

Irregular antibodies refer to blood group antibodies that do not obey the Landsteiner rule, including ABO subtype antibodies and non-ABO blood group antibodies. Irregular antibodies can be classified as immune antibodies and natural antibodies, most of which are immunogenic IgG antibodies, while a few are IgM antibodies. Irregular antibodies are mainly produced by immune stimulation such as blood transfusion or pregnancy, and the detection rate is obviously higher in people with a history of multiple pregnancies or blood transfusions [1,2]. Irregular antibodies are important factors affecting the safety and therapeutic efficacy of blood transfusion, and act as major causes of adverse transfusion reactions, hemolytic disease of the newborn (HDN), difficulties in blood group identification, difficult matching, and ineffective red blood cell transfusion [3,4]. Therefore, to ensure the safety and effectiveness of clinical blood transfusion, irregular antibody screening and identification are of great importance [5].

Irregular antibody detection methods include saline method, in-tube indirect antiglobulin test (IAT), manual polybrene test (MPT), microcolumn gel test (MGT) and erythrocyte-magnetized technology (EMT) [6,7]. The salt water method is a detection method for irregular IgM antibodies, while the in-tube indirect antiglobulin test is a classic analysis method for irregular IgG antibodies. MGT involves the aggregation of erythrocyte antigens with corresponding antibodies in a gel medium, followed by centrifugation to block the aggregated reacting erythrocytes at the top or middle of the gel column, while the non-aggregated erythrocytes are filtered through the micropores to the bottom of the microcolumn for result determination [8,9]. EMT is a novel technique for blood immunology analysis that has emerged in recent years. Based on the magnetization process of red blood cells (RBCs), the results are determined by the aggregation of RBCs due to the specific reaction of blood group antigens and antibodies, using magnetic traction instead of centrifugation [6,10,11]. Both MGT and EMT are suitable for blood immunology projects such as blood matching and irregular antibody detection in large and medium-sized medical institutions owing to their high sensitivity, fast operation, high throughput, clear interpretation of results, as well as automated and standardized operation.

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In this paper, 10 113 patients requiring clinical blood transfusion were selected, and the analytical performance and application value of EMT in clinical irregular antibody detection were evaluated by taking MGT, saline solution method, and in-tube indirect antihuman globulin test as references.

2. Materials and Methods

2.1. Objects

From March 2021 to February 2022, 10 113 patients requiring blood transfusion, including 4997 males and 5116 females, aged 9–87 years, were selected. 3965 patients had a clear history of transfusion or pregnancy, among which 695 had both transfusion and pregnancy history, 1677 had only a history of transfusion, and 1593 had only a history of pregnancy. Fresh EDTA anticoagulated venous blood of 3–5 mL was collected from all study subjects. This study was approved by the Ethics Review Committee of Shanxi Bethune Hospital.

2.2. Instruments and Reagents

The automatic blood group analysis system (Wadiana) based on MGT and the supporting Coombs microcolumn gel test cards, Coombs reagents, and antibody screening cells were from Grifols, Spain. The automatic blood group analysis system (QWALYS3) based on EMT, the matching anti human globulin accidental antibody detection kit (ScreenLys), and the accidental antibody screening red blood cell kit (HEMASCREEN) were from DIAGAST Co., France. The cells used for antibody identification were from Shanghai Blood Biomedical Co., Ltd.

2.3. Irregular Antibody Screening and Identification

Irregular antibody screening was performed in parallel by MGT and EMT on the above 10 113 samples, referring to the instrument and supporting reagent instructions for the relevant operations. In addition, the saline solution method was used as the reference to detect IgM class irregular antibodies [12]. Positive samples were identified by using a set of standard cells for antibody identification, and were further confirmed by the in-tube indirect antihuman globulin test [13].

2.4. Evaluation of Analytical Sensitivity of Irregular Antibody Detection Methods

Six cases, including anti-D, anti-E, anti-c, anti-Jk^a, anti-M, and anti-Fy^a were selected from the identified irregular antibody-positive samples and diluted in multiples using negative sera. MGT and EMT were both adopted for the test, and the negative result was repeated once to evaluate the analytical sensitivity of the assays.

2.5. Statistical Analysis

SPSS 19.0 software was used for data statistics, in which the $\chi 2$ test was applied to compare the count data between groups, and $P < 0.05$ was considered a significant difference.

3. Results

3.1. Irregular Antibody Detection

Through the microcolumn gel method and erythrocyte-magnetized technology, a total of 78 positive samples were screened out of 10 113 samples with a primary screening positivity rate of 0.77%. After antibody identification, 72 cases were determined to be positive, with a revised positive rate of 0.71%. Six cases were false positives due to hyperlipidemia, of which 2 cases were positive for EMT alone, 1 case was positive for MGT alone, and 3 cases were positive for both methods. Among 72 antibody positive samples, 34 cases (47.22%) were Rh system, 10 cases (13.89%) were MNS system, 12 cases (16.67%) were Lewis system, 4 cases (5.56%) were Kidd system, 4 cases (5.56%) were Duffy system, and 3 cases (4.17%) were compound antibody, respectively. In the other 5 samples, after analyzing the patient's medical history and laboratory test results, autoantibodies were determined in 3 cases, and 2 cases failed to identify antibodies. The Rh system was the most common, including 19 cases of anti-E, 2 cases of anti-D, 3 cases of anti-C, 6 cases of anti-c, 3 cases of anti-e, and 1 case of anti-Ec (Table 1).

Among the 72 antibody-positive samples, 66 cases were also detected by MGT, with a detection rate of 91.67%. One case of anti S IgG, one case of anti M IgM, two cases of anti N IgM, and two cases of anti Leb IgM were not

detected. 61 cases in 72 antibody-positive samples were detected by EMT with a detection rate of 84.72%. Among them, 5 cases of anti M IgM, 3 cases of anti N IgM, 1 case of anti Lea IgM, and 2 cases of anti Leb IgM were not detected. There was no significant difference between the two methods ($P > 0.05$).

Through the indirect anti human globulin test *in vitro*, 11 IgM positive and 4 IgG positive samples were tested negative, including 1 anti E, 1 anti M, 1 anti Jk^b, and 1 autoantibody. The positive rate detected by the indirect anti human globulin test method was lower than that detected by EMT, but there was no significant difference between the two methods ($P > 0.05$) (Table 1).

12 cases were confirmed IgM antibody positive by the saline method, in which 7 cases were detected by microcolumn gel method. The additional 5 cases included 1 case of anti M, 2 cases of anti N, and 2 cases of anti Le^b.

Table 1. Irregular antibody test results.

Classification	Number (%)	Antibody	Type	Result	EMT	MGT	IAT	Saline Medium
Rh system	34 (47.22)	D	IgG	2	2	2	2	
		C	IgG	3	3	3	3	
		c	IgG	6	6	6	6	
		E	IgG	19	19	19	18	
		e	IgG	3	3	3	3	
		Ec	IgG	1	1	1	1	
MNS system	10 (13.89)	M	IgM	5	0	4	0	5
		M	IgG	1	1	1	0	
		N	IgM	3	0	1	0	3
		S	IgG	1	1	0	1	
Lewis system	12 (16.67)	Le^a	IgG	7	7	7	7	
		Le^a	IgM	1	0	1	0	1
		Le^b	IgG	2	2	2	2	
		Le^b	IgM	2	0	0	0	2
Kidd system	4 (5.56)	Jk^a	IgG	3	3	3	3	
		Jk^b	IgG	1	1	1	0	
Duffy system	4 (5.56)	Fy^a	IgG	4	4	4	4	
Compound antibodies	3 (4.17)	E+Jk^a	IgG+IgG	1	1	1	1	
		c+Fy^a	IgG+IgG	1	1	1	1	
		E+M	IgG+IgM	1	1	1	1	1
Autoantibodies	3 (4.17)			3	3	3	2	
Unidentified antibodies	2 (2.78)			2	2	2	2	
Total	72			72	61 (84.72%)	66 (91.67%)	57 (79.17%)	12

EMT: erythrocyte-magnetized technology; MGT: microcolumn gel test; IAT: indirect antiglobulin test.

Among 4997 male samples, 26 were positive with a positive rate of 0.52%. Among 5116 female samples, 46 were positive with a positive rate of 0.90%, and the difference was statistically significant ($P < 0.05$) (Table 2).

Table 2. Irregular antibody test results by gender.

Gender	Sample Size	Number of Positives	Positive Rate (%)
Male	4997	26	0.52
Female	5116	46	0.90*
Total	10 113	72	0.71

* $P < 0.05$.

Among 3965 patients with a history of blood transfusion or pregnancy, 56 were positive with a positive rate of 1.41%; among 6148 patients without a history of blood transfusion or pregnancy, 16 were positive with a positive rate of 0.26% and the difference was statistically significant ($P < 0.01$). Among 695 patients with a history of blood transfusion and pregnancy, 13 were positive with a positive rate of 1.87%; among 1677 patients with only a history of blood transfusion, 21 were positive with a positive rate of 1.25%; among 1593 patients with only a history pregnancy,

22 were positive with a positive rate of 1.38%, and the difference was not statistically significant among the three ($P > 0.05$) (Table 3).

Table 3. Patient history of blood transfusion/pregnancy and irregular antibody test results.

Classification	Sample Size	Number of Positives	Positive Rate (%)
History of blood transfusion or pregnancy	3965	56	1.41
No history of blood transfusion or pregnancy	6148	16	0.26*
History of blood transfusion and pregnancy	695	13	1.87
Blood transfusion history only	1677	21	1.25
Pregnancy history only	1593	22	1.38

* $P < 0.01$.

3.2. Evaluation of Analytical Sensitivity of Irregular Antibody Detection Methods

The analytical sensitivity evaluation experiment results showed that the minimum detection ability of the microcolumn gel method and erythrocyte-magnetize technology was fundamentally the same (Table 4).

Table 4. Analytical sensitivity assessment of irregular antibody detection methods.

Gradients	Methods	Anti-D	Anti-E	Anti-c	Anti-Jk[a]	Anti-M	Anti-Fy[a]
1:2	EMT	+	+	+	+	+	+
	MGT	+	+	+	+	+	+
1:4	EMT	+	+	+	+	+	+
	MGT	+	+	+	+	+	+
1:8	EMT	+	+	+	+	+	+
	MGT	+	+	+	+	+	-
1:16	EMT	+	+	+	-	+	-
	MGT	+	+	+	-	+	-
1:32	EMT	+	+	+		-	
	MGT	+	+	+		-	
1:64	EMT	-	+	-			
	MGT	+	+	-			
1:128	EMT	-	-				
	MGT	-	-				

EMT: erythrocyte-magnetized technology; MGT: microcolumn gel test.

4. Discussion

Irregular antibodies are present in approximately 0.38% to 2.38% of the Chinese population, including Rh system, MN system, P blood group system, Lewis system, Diego system, Kell system, Duffy system, and Kidd system [4]. In this study, 72 out of 10 113 samples were positive for irregular antibodies with a positive rate of 0.71%. They included 34 cases of Rh system, 10 cases of MNS system, 12 cases of Lewis system, 4 cases of Kidd system, 4 cases of Duffy system, 3 cases of complex antibodies, 3 cases of autoantibodies, and 2 cases of failure to identify antibody types. The Rh system was the most abundant, accounting for 47.22%, and included 19 cases of anti-E, 2 cases of anti-D, 3 cases of anti-C, 6 cases of anti-c, 3 cases of anti-e, and 1 case of anti-Ec. This is consistent with other similar studies [1,7]. Rh system antibodies are usually IgG antibody produced by blood transfusion or pregnancy stimulation, and are the most common irregular antibodies responsible for immune hemolytic reaction and hemolysis of newborn. Most antibodies in MN system and Lewis system are naturally produced IgM antibodies, while a few are IgG antibodies [1]. Through the in-tube indirect antihuman globulin test, 57 cases were confirmed positive. 11 IgM positive and 4 IgG positive samples failed to be detected by IAT. The positive rate of IAT is lower than that of EMT, although with no significant difference ($P > 0.05$).

This study showed that the positive rate of irregular antibody was significantly higher in patients with a history of blood transfusion or pregnancy (1.41%) when compared with patients without a history of blood transfusion or pregnancy (0.26%) ($P < 0.01$). The positive rate was also higher in female patients (63.89%) than in male patients (36.44%) ($P < 0.05$). This is consistent with previous reports, which suggests that immune stimulation such as blood

transfusion or pregnancy is an important reason for irregular antibody production [1,3,4]. The positive rate was 1.87% in patients with a history of both transfusion and pregnancy, 1.25% in patients with a history of transfusion only, and 1.38% in patients with a history of pregnancy only, with no significant difference ($P > 0.05$). It was reported that erythrocyte blood group irregular antibodies in patients with a history of blood transfusion ranged from 2% to 9%, which was higher than the positive rate in our study [2].

EMT is the current common platform for fully automated hematology immunology assay. The most remarkable feature of this technology is that it does not require the setting up of a centrifuge. Instead of centrifugal force, a magnetic force is used to achieve rapid sedimentation of RBCs under the attraction of magnetic field, which can effectively avoid the impact of the centrifugation process on the experimental results. This ensures that the results are not interfered by fibrin, and effectively improves the detection ability of weak expression antigen, low titer antibody, or reverse typing weak reaction, thereby increasing accuracy [6,10,14].

This study showed that among 72 antibody-positive samples, 66 cases were detected by MGT with a detection rate of 91.67%, while 61 cases were detected by EMT with a detection rate of 84.72%. The two methods have considerable and similar irregular antibody detection ability ($P > 0.05$). The main difference is that EMT did not detect 5 cases of IgM antibodies, including 4 cases of anti M and 1 case of anti N. The detection results also reflected the design idea of the manufacturer. QWALYS3 system focuses on the detection of antibodies that are clinically significant, while the detection rate of antibodies that are not clinically significant is lower than other methods, with the purpose of improving the efficiency of clinical blood matching [10]. Most clinically significant antibodies are IgG type, while most IgM antibodies are naturally produced. Most IgM antibodies have no activity at 37 ℃ and rarely cause hemolytic transfusion reaction. In the ScreenLys anti human globulin accidental antibody detection kit's instruction, it is also stated that monoclonal anti human globulin is coated inside the microplate to capture IgG type antibodies that have already bound to RBC antigens. It was reported that a few IgM antibodies can be detect by MGT [7]. In this paper, 12 cases of IgM antibody positive were confirmed by brine method, while 7 cases were detected by MGT. This article also showed that irregular IgM antibodies mainly appear in MNS system and Lewis system.

Low potency antibodies are easy to be missed. The production of low potency irregular antibodies is also an important reason for ineffective blood transfusion or adverse outcomes. As the number of blood transfusions or pregnancies increases, the probability of patients producing low potency irregular antibodies also increases [15]. According to the evaluation of analytical sensitivity experiment, the minimum detection ability of microcolumn gel method and erythrocyte-magnetize technology is fundamentally the same.

Based on the RBC magnetization method, the fully automatic QWALYS3 system does not require centrifugation and pre-treatment, and its detection ability is significantly higher than other methods. Conventional blood type analysis can reach 150 samples/hour, and irregular antibody screening can reach 110 samples/hour. The detection results can be traced throughout the process, with highly reliable biological safety [10]. Due to the strong maintenance free nature of the hardware system, this system is superior to existing methods.

In addition, this study demonstrated that compared with MGT, EMT has a comparable ability to detect irregular antibodies. It can reduce the detection rate of clinically insignificant antibodies while ensuring the detection rate of clinically significant antibodies. It is further proved that the reasonable selection and cooperation of different technical platforms can make the detection of clinical irregular antibodies more accurate and comprehensive, improve the efficiency and safety of clinical blood transfusion, enhance the utilization of blood resources, and promote the in-depth implementation of the concept of accurate blood transfusion.

Author Contributions

J.Q. designed experiments, conducted research, and drafted the article. X.G. collected the data, Q.Z. analyzed and explained the data, and X.H. provided guidance on the experiments and critically reviewed the knowledge-based content of the article.

Ethics Statement

This study was conducted in accordance with the guidelines of the Helsinki Declaration and was reviewed and approved by the Clinical Research Ethics Committee of Shanxi Bethune Hospital (protocol code SBQLL-2021-155).

Informed Consent Statement

The remaining samples used in this scientific research experiment after routine testing in the transfusion department are completed, and the potential risks to the subjects do not exceed the minimum limit. It will not have a negative impact on the rights and health of the subjects, and will not collect the privacy and personal identity information of the subjects. We only analyze the test result data. During the screening and selection stage of the subjects, we will code them. The final clinical trial record table only reflects the subject's code, and it is no longer possible to find the subject, fully protecting the subject's privacy rights. The ethics committee approves an exemption from informed consent.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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