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Basic Study

Comparison of the conventional tube and erythrocyte-magnetized technology in titration of red blood cell alloantibodies

EMT in red blood cell alloantibody titration

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Abstract

BACKGROUND

Erythrocyte alloantibodies are mainly produced after immune stimulation such as blood transfusion, pregnancy and transplantation, and are the main causes of severe hemolytic transfusion reactions, and difficulty in blood grouping and matching. Antibody screening is critical to prevent and improve red cell alloantibodies. Routine tube assay is the main detection method of antibody screening. In recent years, erythrocyte-magnetized technology (EMT) has been gradually applied in clinical practice. The aim of this study is to explore the application value and effect of the conventional tube and EMT in red blood cell alloantibody titration, hoping to provide a valuable reference for clinical blood transfusion.

AIM

To investigate the application value of conventional tube and EMT in red blood cell alloantibody titration and improve the safety of clinical blood transfusion.

METHODS

A total of 1298 blood samples were selected from blood donors in the Department of Blood Transfusion of our hospital from March 2021 to December 2022. 5mL sample blood of whole blood was collected in the plastic tube, which was cut thereafter and the whole blood was put into the test tube for centrifugation, and the serum was separated. Different red blood cell blood group antibody titers were detected in parallel by tube polybrene test, tube AGT and EMT screening irregular antibody methods to determine the best test method.

RESULTS

Parallel detection was performed by tube polybrene test, tube AGT and EMT screening irregular antibody, and it was found that the irregular antibody method of EMT screening can detect all IgG and IgM irregular antibodies, and the results of manual

tube AGT are satisfactory, but the operation time is long and the equipment occupies a large empty space. Also, its activity against type O Rh (D) red blood cells was determined by EMT screening irregular antibody assay, and the results showed that it was normal. In addition, compared with the conventional tube method, the EMT screening irregular antibody method has a lower cost and significantly higher detection efficiency.

CONCLUSION

Compared with conventional tube method, EMT screening irregular antibody method can detect irregular antibody faster and more effectively, and the detection rate of IgG and IgM irregular antibody is more complete.

Key Words: Erythrocyte-magnetized technology; Conventional tube; Red blood cell alloantibodies; Transfusion reactions; Application

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Core Tip: Irregular antibody screening has long been listed as a routine blood test for blood donors in many developed countries, but only a small number of blood stations in China have tried to use saline medium for irregular antibody screening. Monoclonal anti-A (B) is a routine reagent for ABO blood grouping, but false positive or false negative reactions occur sometimes, reducing the accuracy of the test. With the development of clinical diagnostic techniques and the improvement of medical level, erythrocyte-magnetized technology (EMT) screening irregular antibody method has been gradually applied in the diagnosis of various clinical fields. Based on this, this study analyzed blood samples from voluntary blood donors in order to explore the application value and effect of the conventional tube and EMT in red blood cell

alloantibody titration, hoping to provide valuable references for the safety of clinical blood transfusion.

INTRODUCTION

Antibody screening aims to detect whether there are antibodies other than the ABO blood group in the serum or plasma of patients by serum determination, including autoantibodies, drug antibodies, and allospecific antibodies to red blood cell blood groups [1-2]. Among them, red blood cell alloantibodies refer to blood group antibodies other than anti-A and anti-B, i.e., irregular antibodies, which are mainly produced after immune stimulation such as blood transfusion, pregnancy, and transplantation and are the main causes of severe hemolytic transfusion reactions, difficulty in blood grouping, and difficult matching [3-5]. Therefore, improving the screening rate of red blood cell alloantibodies has important guiding significance for the safety of clinical blood transfusion.

Irregular antibody screening has long been included in routine blood testing tests for blood donors in many developed countries, but only a small number of blood stations in China have tried irregular antibody screening using saline media [6]. Monoclonal anti-A (B) is a routine ABO blood grouping reagent, but false positive or false negative reactions occur sometimes[7]. In recent years, Collies automatic blood grouping system has been gradually applied in clinical practice using international advanced technique - erythrocyte-magnetized technology (EMT); this technology is based on the magnetization of red blood cells and realizes the rapid sedimentation of red blood cells under the attraction of magnetic field, thus replacing the serological centrifuge [8]. It has been reported that irregular antibody screening by this instrument is an indirect antiglobulin test method using solid phase packaging antiglobulin combined with EMT to incubate the plasma to be tested with ready-to-use irregular antibody screening red blood cells, which can be sensitized by irregular antibodies in the plasma. In the presence of a magnetic field, magnetized red blood cells migrate to the bottom of the microplate and react with antiglobulin to form an evenly distributed

red blood cell layer [9-10]. Previous studies have also found that EMT can effectively avoid the impact of the centrifugation process on the experimental results, making the experimental results more reliable, and saving the cost of centrifuge calibration and maintenance [11-12].

Based on this, this study analyzed blood samples from voluntary blood donors in order to explore the application value and effect of the conventional tube and EMT in red blood cell alloantibody titration, hoping to provide valuable references for the safety of clinical blood transfusion.

MATERIALS AND METHODS

1.1 General Information

A total of 1298 blood donors (612 males and 686 females, aged 18-55 years, 897 25-40 years, accounting for 69%) were randomly selected from the Department of Blood Transfusion of our hospital from March 2021 to December 2022. 5 mL of whole blood was centrifuged in a tube and the serum was separated.

1.2 Materials and Reagents

1.2.1 Reagents and instruments: Anti-human globulin card (Jiangsu Libo Pharmaceutical Biotechnology Co., Ltd., batch number: 202208001), red blood cell antibody screening cell kit (batch number: 20187012, 20187014) and red blood cell antibody identification spectrum cells (REAGENS, batch number: 726000, 733000) were provided by Shanghai Blood Biomedicine Co., Ltd. Special centrifuge for blood immunology 2005-2 Zhuhai Bezo Biotechnology Co., Ltd. Polybrene came from reagents and instruments company of Zhuhai Bezo Biotechnology Co., Ltd. Collies 3 (QWALYS3) automatic blood analyzer (DIAGAST, France), Deakin (EvO-2) immunoassay sample adding system (TECAN, Switzerland). Shanghai Medical Equipment Co., Ltd. Digital explicit water bath, Heidolph oscillator (Titramax101) made in Germany, and HeShi centrifuge with hanging micro reaction plate in German obstetrics department.

1.2.2 11 known irregular antibodies with known specificity against D, E, C, c, e, S, Fy ', JK', M, N and PI were prepared by the Institute of Blood Transfusion Technology, Taiyuan, China.

1.2.3 The TSFT puncture kit is mainly composed of low ionic medium (LIM), polybrene application solution (PWS), neutralization solution and positive control (1gG anti-D), which are provided by Shanghai Blood Bio-Pharmaceutical Co., Ltd. and Zhuhai Bezo Biotechnology Co., Ltd., respectively.

1.3 Method

1.3.1 Procedures for antibody screening and identification of blood donors (all tube method) First, take an equal amount of 10 blood donor sera mixed; and then take the mixed sera to identify the cell reaction with antibodies, use the tube method TSPT for antibody screening. If a positive reaction showed, then re-perform antibody screening on the 10 original samples before mixing, use the traditional antiglobulin test (AGT) and identify the irregular antibody with antibody identification cells for those who are positive, and perform absorption and diffusion test when necessary to determine the antibody specificity.

1.3.2 Tube polybrene test [13]: 1 drop (50 uL) of reagent red blood cells was mixed with 2 drops (100 uL) of serum or plasma and incubated at room temperature for 3000 r/mim for 15 s. Double check and record the results, the presence of agglutination means a positive reaction, otherwise is a negative reaction.

1.3.3 Tube AGT method [14]: After observing the results with the above methods, the red blood cell serum or plasma mixture was incubated at 37 ° C for 30 min, the red blood cells were washed three times with saline, and finally the packed red blood cells were suspended with two drops (100 mL) of antiglobulin and centrifuged. The results were judged according to the same criteria as the upper water tube polybrene test method.

1.3.4 EMT screening for irregular antibodies [15]: Using the Collies automatic blood grouping system, with a special program matched with the original reagent, the instrument loads a 96-well ScreenLys microplate coated with antiglobulin, adds SONL isolation solution, diluent, 151L magnetized screening cells, 15 uL specimen plasma,

completes the closed incubation at 37 ° C after sample addition according to the set program, and completes the irregular antibody screening determination of the specimen by magnetization shaking, photography, and interpretation. The test results are shown in Figure 1.

1.3.5 Antibody Potency Assay

The irregular antibody is diluted by doubling with antibody diluent, reacts with the reagent red blood cells with positive corresponding antigens, and its titer is measured. The titer endpoint is the highest dilution with agglutination ≥ 1 +. These reagent red blood cells are also reacted with normal donor sera as a negative control test.

1.4 Statistical analysis

All data from this study were processed and analyzed using IBM SPSS 21.0 software (SPSS Inc., Chicago, IL, USA), and a t-test was used for comparison between groups, and $P < 0.05$ was considered statistically significant.

RESULTS

2.1 Screening and identification results of irregular antibodies in blood donors

1298 random blood donors were screened by commercial kit, and irregular antibody detection was performed by polybrene test. The results showed that 18 cases were positive, with a positive rate of 1.39%. After further identification, Rh, MN and other blood group system antibodies were found. See Table 1 for details.

2.2 Comparison of Sensitivity for Detecting Irregular Antibodies with Known Specificity

11 known irregular antibody titers were detected in parallel by tube polybrene test, tube AGT, and EMT screening irregular antibody methods, respectively, while negative quality control tests were performed. It was found that all IgG and IgM irregular antibodies could be detected by EMT screening irregular antibody method, and the

manual tube AGT results in the control group were satisfactory, but the operation time was long and the equipment occupied a large space (such as tubes and racks), as shown in Table 2.

Compared with the tube polybrene test, the t value of irregular antibody method for EMT screening was 2.65, $P < 0.05$, and the difference was statistically significant. Compared with the tube AGT method, the t value of the irregular antibody method for EMT screening was 2.70, $P < 0.05$, and the difference was statistically significant. The t value of the polybrene test tube method was 0.28, $p > 0.05$ compared with the AGT test tube method, and the difference was not statistically significant.

2.3 Comparison of irregular antibody screening positive samples from the blood donor

The 18 positive samples selected from the blood donor spectrum were detected in parallel by tube polybrene test, tube ACT, EMT screening irregular antibody method, and it was found that the reaction pattern of all sera and commercial screening cells or spectrum cells was consistent.

2.4 Effect of Hemolysis and Lipemia on Irregular Antibodies for EMT Screening

From the physical examination samples of blood donors, macroscopically significant severe hemolysis and lipemia samples were found, and their supernatant sera were aspirated and mixed equally with low concentrations (titer 1:8) of IgG anti-D and AB sera (negative samples), respectively. Their activities against type O Rh (D) red blood cells were determined by EMT screening irregular antibodies, and the results showed that the staining failed and the cell membrane permeability remained normal, indicating that the cell activity made normal.

2.5 Comparison of cost of reagents, equipment and manpower consumption

According to the routine screening of RBC-IAb in the above blood donors, the consumption cost (the cost used for screening irregular antibodies by different methods

divided by the corresponding number of blood donors' experiments is the consumption cost), tube polybrene test, tube ACT and EMT screening irregular antibody methods were counted. It was found that the cost of EMT screening irregular antibody method was < 0.5 RMB/blood donor, while the cost of the tube polybrene test method was > 5 RMB/blood donor, and the tube AGT method was > 5.5 RMB/blood donor, which indicated that EMT screening irregular antibody method had advanced detection technology and could also concentrate sample detection, which could significantly improve the work efficiency (Table 3).

DISCUSSION

At present, blood transfusion therapy, as a clinical treatment, is still irreplaceable in the treatment of some diseases. Although some advanced treatment options have greatly reduced the dependence on blood products in the treatment after being applied in clinical practice, hemolytic transfusion reactions and cross-matching difficulties caused by blood group antibodies other than ABO, that is, irregular antibodies, also occur from time to time [16-17]. Blood group antibodies destroy mismatched red blood cells in transfused blood or shorten their lifespan, producing hemolytic transfusion reactions, which may affect treatment outcomes or endanger the patient 's life. Studies have shown that neonatal hemolytic disease caused by prenatal irregular blood group antibodies in pregnant women, especially Rh-HDN, has severe symptoms and often leads to serious harm [18-19]. Monoclonal hyperactivity-A (B) reagent gas has been reported to react with most red blood cells with high sensitivity and specificity [20]. Normally, malformed hemolytic transfusion reactions caused by ABO blood group incompatibility are uncommon, while adverse transfusion reactions, neonatal hemolysis, and difficulty in blood grouping caused by irregular antibodies to ABO blood group accidents of red blood cells occur from time to time [21-22]. In addition, it has long been reported abroad that monoclonal reagents can lead to false agglutination or false negative phenomenon in the process of ABO blood group detection, and there have been corresponding reports in China in recent years [23]. Therefore, blood grouping

before transfusion is far from enough, and antibody screening is also necessary to ensure blood transfusion safety.

In recent years, with the development of medical technology, automatic blood grouping has been more and more widely used in the blood transfusion departments of major hospitals and blood collection institutions at all levels. It also plays an important role in blood grouping test, irregular antibody screening test and clinical cross-matching test [24]. EMT is a new technology based on red blood cell magnetization, which has been maturely applied in immunodiagnosis, cell separation, protein purification and nucleic acid extraction, and has also greatly improved the automation rate of laboratories [25-26]. Based on this, the aim of this study was to investigate the effect of conventional tube and EMT in red blood cell alloantibody titration, hoping to provide some help for clinically safe blood transfusion.

In this study, 5 mL of whole blood was collected from 1298 blood donors. Serum was centrifuged and separated. Different red blood cell blood group antibody titers were detected in parallel by tube polybrene test, tube AGT and EMT screening irregular antibody. In general, voluntary blood donor samples are often collected centrally. In order to save labor and reagents, 5-10 samples can be mixed for primary screening, and positive samples can be reexamined one by one to detect antibody-positive samples. However, because a few irregular antibodies with low titer in the mixed plasma still have the possibility of missed detection, the mixed plasma primary screening method is not suitable for the pretransfusion test of patients. In addition, it was found that all irregular antibodies of IgG and IgM nature were detected by EMT screening irregular antibody method, but irregular antibodies of low concentration were not detected by the tube polybrene test method and tube AGT method, which may be related to the lack of sensitivity of IgM active blood group antibodies at low concentration and room temperature in polybrene method and AGT.

Studies have reported that red cell alloantibodies are mainly divided into macromolecular IgM and small molecular IgG properties (27). There are many methods to detect red blood cell antibodies, but common tube methods can only detect IgM

antibodies in most cases [28-29]. The results showed that the irregular antibody method of EMT screening could significantly determine its activity against IgGO Rh (D) red blood cells, with obvious advantages. In recent years, the use of traditional *in vitro* AGT has been more common in China [30], and the test results in this paper further show that the EMT screening irregular antibody method and *in vitro* polybrene test method, *in vitro* AGT method can be used for routine screening of irregular antibodies in blood donors, but EMT screening irregular antibody method is more rapid and convenient, more efficient, and less expensive.

In summary, compared with the *in vitro* polybrene test method and *in vitro* AGT method, the Collies automatic blood group system based on EMT for irregular antibody screening has the characteristics of stronger antibody specificity, more convenient operation, more accurate results and complete lake source, which is suitable for large-scale screening of irregular antibodies in blood stations and is worthy of being widely popularized in clinical practice. Of course, more sensitive test techniques help to improve the antibody detection rate, but there may still be some antibodies that are not easy. In daily testing, the recorded history of checking past antibodies should be listed as a part of routine compatibility tests. Hospitals at all levels should actively understand the patient 's blood transfusion history, pregnancy history and current history, and perform blood group and irregular antibody detection in advance, so that the matching blood components can be selected for patients in time to ensure the safety and effectiveness of clinical blood transfusion.

CONCLUSION

Compared with the conventional tube method, the EMT-based Collies automatic blood group system for irregular antibody screening can more accurately screen its irregular antibody and has stronger antibody specificity. In addition, it is more convenient to operate, with more accurate results, lower cost consumed and other characteristics, and is worthy of being widely popularized in clinical practice.

ARTICLE HIGHLIGHTS

Research background

Magnetic red cell immunoseparation is a biomedical technique for separating and detecting small numbers of targeted cells. It has the advantages of high sensitivity, high precision, and easy operation, and has been widely used in the field of *in vitro* diagnostics and therapy.

Research motivation

Conventional separation methods can be time-consuming and involve complicated procedures, while magnetic red cell immunoseparation has the advantages of ease of use, high sensitivity, and precision. Therefore, this technology has been widely applied in *in vitro* diagnostics and therapy, attracting much attention from researchers and medical professionals alike.

Research objectives

This study aims to explore the application value of conventional test tubes and EMT in red blood cell alloantibody titration to improve the safety of clinical blood transfusion.

Research methods

Parallel detection of antibody titers for different red blood cell blood groups using *in vitro* polyene test, tube AGT and EMT screening for irregular antibodies.

Research results

The irregular antibody method for EMT screening can detect all IgG and IgM irregular antibodies, and the operation time is shorter than manual tube AGT. The EMT screening irregular antibody test was used to detect its activity on O-type R (D) red blood cells, and the results showed that it was normal. In addition, compared to the conventional tube method, the EMT screening method for irregular antibodies has lower costs and significantly higher detection efficiency.

Research conclusions

Compared with traditional *in vitro* methods, EMT screening for irregular antibodies has lower costs and significantly higher detection efficiency.

Research perspectives

The widespread application of red blood cell magnetization technology in the current medical field indicates its broad application prospects. In the future, with the continuous updates and improvements of technology, the application scope of red blood cell magnetization technology will further expand. For example, red blood cell magnetization technology can be used for early diagnosis of various diseases such as early cancer and cardiovascular diseases. In addition, this technology can be used for *in vivo* and *in vitro* research on the movement, interaction, and molecular processes of cells and pathogenic microorganisms. However, there will be a long way to go.

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