A Novel Approach toward Antibody Screening: Cells O, an Alternative for Commercial Antibody Screening Kits

Mohsen Dashti (MSc)
Blood Banking Laboratory of Ghaem Hospital, Mashhad University of Medical Sciences (MUMS), Mashhad, Iran

Afsane Bahrami (MSc)
Department of Modern Sciences and Technologies, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran

Mohammad Hadi Sadeghian (PhD)
Cancer Molecular Pathology Research Center, Mashhad University of Medical Sciences, Mashhad, Iran

Seyyede Fatemeh Shams (MSc)
Cancer Molecular Pathology Research Center, Mashhad University of Medical Sciences, Mashhad, Iran

Ahmad Ashjaee (MSc)
Blood Banking Laboratory of Ghaem Hospital, Mashhad University of Medical Sciences (MUMS), Mashhad, Iran

Zahra Arianpour (MSc)
Iranian Blood Transfusion Organization Research Center, Mashhad, Iran

Sepideh Shakeri (MSc)
Cancer Molecular Pathology Research Center, Mashhad University of Medical Sciences, Mashhad, Iran

Corresponding author: Seyyede Fatemeh Shams

Email:shams8869@yahoo.com

Tel: +989158075948

Address: Hematology and Blood Bank Department, Ghaem hospital, Shariati Square, Mashhad, Iran

ABSTRACT

Background and Objectives: Blood transfusion may induce some adverse effects on receivers. Some methods such as antibody screening and cross matching have been suggested to reduce the risk of transfusion complications. However, these methods require commercial antibody screening kits that may also need special equipment. The aim of this study was to introduce a new method for antibody screening that does not require a commercial kit, and could be used in any transfusion laboratory.

Methods: We examined 350 samples that contained alloantibody and 350 control samples without the antibody. A solution containing two O+ and one O- samples were used instead of screening cells.

Results: Sensitivity and specificity of the method were 73.32% and 45.15%, respectively. Positive predictive value and negative predictive value were 58.33% and 63.88%, respectively.

Conclusion: Our new method can be used in basic hematology laboratories with some modifications.

Keywords: Antibodies, Antigens, Coombs test.

INTRODUCTION

Blood groups were first classified by Landsteiner in 1901. He discovered blood antigens by cross contacting different anti-sera and blood cells. Until now, 30 blood group systems including more than 302 antigens have been detected (1). These antigens can induce adverse effects in blood receivers who lack the corresponding antigens. These complications are observed in 3-5% of blood receivers (2). The need for blood products has increased rapidly, especially among thalassemia and surgical patients (3,4). Approximately 85 million units of packed red blood cells are transfused annually (5). While suitable blood transfusion will not put patients at risk, mismatched blood transfusion can be a life threatening (2). It has been suggested to perform antibody screening and cross matching before blood transfusion to reduce the risk of complications (6). Some transfusion reactions are delayed, while acute hemolytic reactions, non-hemolytic fever, allergic reactions and coagulation disorders are due to exposure to foreign antigens (7-9). Different studies have introduced alloantibody screening methods based on erythrocyte agglutination (10). These methods include tube spin low-ionic-strength solution (addition method), indirect anti-globulin test (tube LISS-IAT), micro tube column agglutination techniques, affinity adherence test system, and solid-phase tests (1, 9). However, performing the mentioned methods require commercial kits. The aim of this study was to introduce a new method of alloantibody detection, which does not require commercial kits and solutions.

MATERIAL AND METHODS

This case control study was done on 700 samples, 350 of which were found positive for alloantibodies in the antibody screening test. Other 350 samples did not have alloantibodies and were used as the control group. Overall, 280 subjects with cold antibodies were included in the study. Blood samples were collected in tubes without anticoagulant. Cold antibody screening test was done for all samples according to the standard method using a screening kit (IBTO, immunohematology references laboratory, lot number t15IP3C100). First, three tubes were assigned for each patient and 100 µL of sera were added to each tube. Later, 50 µL of each antibody screening suspension wa s added to

each tube, and the tubes were shaken well. The tubes were incubated at room temperature for 30 minutes and then centrifuged at 3000 rpm for 30 seconds. The results were evaluated under a light microscope. All samples found as positive for the cold antibody were used to assess the accuracy of the new method. Moreover, 70 subjects with warm antibodies were studied. Warm antibody screening test was performed for all samples. The first four steps of this test were similar to that of the cold phase. Other steps were as follows: The tubes were incubated at 37 ^{o}C for 1 hour. The solution was washed three times by 9% normal saline solution (Samen, pharmaceutical Co.), and then dried on paper. Later, 1-2 drops of anti-human globulin (AHG, LORNE laboratory LTD, lot numbrt43584-A6) were added to each tube. The tubes were centrifuged at 3000 rpm for 30 seconds, and then shaken gently. Finally, the results were evaluated under a light microscope. The new method needs three suspensions of O cell (2 Rh Positive O and 1 Rh negative O). Three cords from blood packs along with the mentioned blood groups (0.5 ml) were placed in three separate tubes. After adding 5 ml of normal saline, the tube was centrifuged at 3000 rpm for 1 minute. The resulting clear liquid supernatant was separated. This step was performed three times for each cord. Finally, a 3-5% blood cell suspension was obtained. The new suggested method is similar to the standard method of cold and warm antibody screening, except blood cell suspensions are used instead of screening cell suspension. It is worth mentioning that the cell suspensions should be prepared from pack cords of the donors who live in the same areas as patients do. According to study of Keramati, it seems that people who live in the same area have similar erythrocyte phenotypes (11). Content of the three tubes from each patient was evaluated on slides under a microscope. Detection of erythrocyte agglutination indicated a positive result. Positive result in a tube indicated that the tube contains alloantibody. The samples that formed rouleaux agglutination were excluded from the study.

RESULTS

Sensitivity, specificity, and positive and negative predictive values of the new method are shown in table 1.

Table1- Comparison of the results obtained for detection of warm and cold alloantibodies

	Sensitivity	Specificity	Positive predictive value	Negative predictive value	Total number
Warm alloantibody	**58.82%**	**41.17%**	**50%**	**50%**	**70**
Cold alloantibody	**87.83%**	**49.21%**	**66.6%**	**77.7%**	**280**

DISCUSSION

In this study, we have proposed a new method for antibody screening. The method had some defects that limit its usage, but its benefits cannot be ignored. The superiority and disutility of this method are discussed and compared with the standard methods. Microcolumn screening method has been designed based on erythrocyte agglutination, and requires a commercial kit. However, this screening method can be performed via manual and automated testing. The results of the new method are easy to read, but their interpretation depends on skill of technicians. Test column storage for future analysis is another advantage of the microcolumn method (12), while results of the new proposed method are stable up to 24 hours after the test. The new method is as sensitive as the low-ionic-strength-salt solution (LISS) method, but agglutination is more obvious in the microcolumn screening method.

Other studies that compared different methods of antibody screening mentioned that the microcolumn-screening test is not sensitive enough for identification of insignificant antibodies (cold antibodies). The microcolumn method can be performed more quickly because it does not require many washing steps (9). Our proposed method is able to identify cold and warm antibodies with 87.83% and 58.82% sensitivity, respectively. In addition, it does not require washing step for detection of cold antibodies and only require three for detection of warm antibody, which is as same as in the LISS method.

Our method is also similar to the LISS method in terms of sequencing steps. Both methods may produce false negative results due to careless washing. The sensitivity and specificity of the LISS method have been reported to be 43.2% and 98.9%, respectively (13). Microplate method is used for erythrocyte phenotyping and antibody identification and screening. It is a highly sensitive, simple and rapid technique. The most important limitation of this method is the need for commercial kits, which also require some instruments such as a plate reader and a computer (13). Our method is convenient because it does not require a kit or instrument.

In the solid phase method, red blood cells are immobilized in wells of a microplate. This technique requires the use of commercial kits. Similar to the microcolumn method, positive results in the solid phase method are stable for two days at 2-8 $^{\circ}$C if covered. Sensitivity of the solid phase method has been reported to be 89.7% (9). Automated techniques require special equipment and a large space, and are suitable for transfusion laboratories that assess large number of samples every day (14).

CONCLUSION

Our proposed method is suitable for laboratories that are not well- equipped and modern, but some small modifications are required to improve its sensitivity and specificity.

ACKNOWLEDGMENTS

CONFLICT OF INTEREST

Authors declare that there is no conflict of interest.

REFERENCES

1. Bain BJ, Win N. *Dacie and Lewis Practical Haematology.* 11th ed. 2012; 529-30.

2. Nicholls MD. *Transfusion: morbidity and mortality.* Anaesth Intensive Care. 1993; 21(1): 15-9.

3. Azarkeivan A AS, Ahmadi MH, Hajibeigy B, Maghsudlu M, Nasizadeh S, et al. *Blood transfusion and alloimmunization in patients with thalassemia: multicenter study.* Pediatric Hematology-Oncology. 2011; 28(6): 479-85. doi: 10.3109/08880018.2011.568595.

4. Yuan S EN, Bailey D, Salvador M, Wang S. *Transfusion of multiple units of Js (b+) red blood cells in the presence of anti-Jsb in a patient with sickle beta-thalassemia disease and a review of the literature.* Immunohematology/American Red Cross. 2007; 23(2): 75-80.

5. Carson JL GB, Kleinman S, Tinmouth AT, Marques MB, Fung MK, et al. *Red blood cell transfusion: a clinical practice guideline from the AABB.Clinical TransfusionMedicine Committee of the AABB.* Ann Intern Med. 2012; 157(1): 49-58. doi: 10.7326/0003-4819-157-1-201206190-00429.

6. Lewis SSM BB, Bates I, Dacie JV. *Dacie and Lewis's practical haematology.* 10th ed. 2006.

7. Eder AF CL. *Noninfectious complications of blood transfusion.* Arch Pathol Lab Med. 2007; 131(5): 708-18.

8. Hendrickson JE HC. *Noninfectious serious hazards of transfusion.* Anesth Analg. 2009; 108(3): 759-69. doi: 10.1213/ane.0b013e3181930a6e.

9. McPherson RA, Pincus MR. *Henry's clinical diagnosis and management by laboratory methods.* 22th ed: Elsevier Health Sciences. 2011; 680.

10. GL. M. L evoluzione tecnologica in immunoematologia eritrocitaria. The Ligand Quarterly. 1993;12:466-74.

11. Keramati MR, Shakibaei H, Kheiyyami MI, Ayatollahi H, Badiei Z, Samavati M, Sadeghian MH. *Blood group antigens frequencies in the northeast of Iran.* Transfus Apher Sci. 2011; 45(2):133-6. doi: 10.1016/j.transci. 2011.07.006.

12. Hoffbrand AV, Catovsky D, Tuddenham EG. *Postgraduate haematology.* 6th ed: John Wiley & Sons. 2008; 238-9.

13. Weisbach V, Kohnhäuser T, Zimmermann R, Ringwald J, Strasser E, Zingsem J, et al. *Comparison of the performance of microtube column systems and solid-phase systems and the tube low-ionic-strength solution additive indirect antiglobulin test in the detection of red cell alloantibodies.* Transfusion Medicine. 2006; 16(4): 276-84.

14. Garozzo G, Licitra V, Criscione R, Comitini N, Noto C, Lomagno R, et al. *A comparison of two automated methods for the detection and identification of red blood cell alloantibodies.* Blood Transfusion. 2007; 5(1): 33. doi: 10.2450/2007.0022-06.