Evaluation of Erythrocytes Magnetized Technology for Measurement of ABO Isoagglutinin Titers

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Abstract

Background A variation in the measurement of ABO antibody titer has been seen among different laboratories due to lack of standardization. In our study, we aim to evaluate automated ABO isoagglutinin titer measurements by erythrocytes magnetized technology (EMT) and compare with conventional tube technique (TT).

Methods We performed ABO isoagglutinin titration on samples received in a reference laboratory during a period of 2 months. A total of 134 tests for immunoglobulin G (IgG) titer and 116 for immunoglobulin M (IgM) for anti-A or anti-B were included in the study. Samples were processed for ABO isoagglutination titers by both TT and EMT by QWALYS-3 (Diagast, France). Microsoft Excel was used to compile data, for all calculations, and to draw graphs and plots. The number and percentage of cases within ±1, ±2, or ±3 titer difference (TT-EMT) were calculated.

Results Median titers and their ranges obtained by EMT were higher or equal to those by TT for all IgM and IgG ABO-antibodies in all blood group (BGs), except anti-A IgM in (BG) O that was lower by EMT (32 [4:128]) than TT (48 [8:256]). One twenty one (121/134, 90.3%) cases of IgG titer showed an agreement by both methods (within ± one titer difference). One hundred seven cases (107/116, 92.2%) for IgM titer were within one titer difference by both the methods.

Conclusion Results of titration by EMT-based automated instrument QWALYS-3 and conventional TT may vary by one titer dilution in the majority of cases. Use of consistent method for patient management is, therefore, advised.

Keywords
- isoagglutinin titer
- erythrocytes magnetized technology
- conventional tube technique
- QWALYS-3

Introduction

ABO antibodies are naturally occurring antibodies. Individuals normally produce antibodies directed against the A and/or B antigen(s) absent from their red blood cells (RBC). The ABO antibodies are predominantly immunoglobulin M (IgM), activate complement, and react at room temperature or colder. However, there may be small quantities of immunoglobulin G (IgG) type of ABO antibodies present. The predominant immunoglobulin class of antibodies in group O serum is IgG.1 These antibodies are of clinical significance due to their ability to cause hemolytic transfusion reactions, hemolytic disease of the newborn, acute rejection in solid organ transplantation, and delay in engraftment of erythrocytes and megakaryocytes in ABO-incompatible stem cell transplantation.2-5 Therefore, ABO isoagglutinin titer

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measured by both manual conventional TT method and EMT by QWALYS-3 (Diagast, Loos Cedex, France). It is used in testing, increasing throughput in laboratories with high workload, and keeping records of patient results in form of images that can be retrieved later.

Not many studies have been done for the evaluation of isoagglutinin titers by EMT. Therefore, in this study, we evaluated automated ABO isoagglutinin titer measurements by EMT by QWALYS-3 (Diagast, Loos Cedex, France). It is used for ABO blood typing, antibody screening, identification, and cross matching. Here, we have compared the EMT-based ABO isoagglutinin titer measurements with the conventional TT in Indian scenario.

Materials and Methods

Study Population

We performed this cross-sectional study of ABO isoagglutinin titration on samples received in the Department of Hematology and Immunology at a standalone private national reference laboratory in India during a period of 2 months. A total of 134 tests for IgG titer and 116 for IgM titer for anti-A or anti-B were included in the study. The samples used were residual samples from blood grouping test done at our laboratory for whom both ethylenediaminetetraacetic acid (EDTA) samples and serum samples were available. The serum samples were processed for ABO isoagglutination titers by both manual conventional TT method and EMT by QWALYS-3 (Diagast, Loos Cedex, France). EDTA samples were used for blood grouping by QWALYS-3.

Methods for Isoagglutination Titer

Titration Using Conventional Test Tube Technique

Titers were obtained using the conventional TT as described previously. Twelve test tubes were taken and labeled (1:1, 1:2, 1:4, 1:8, 1:16, and so on). One hundred microliter normal saline was added to each of 12 test tubes except the first. One hundred microliter patient serum was added to the first tube sufficiently with a clean pipette, 100 μL of this mixture was dispensed into the next tube. Same process of mixing and sequential dilution was repeated using a clean pipette to mix and transfer each dilution. One hundred microliter of
mixture in the last tube was saved in case further dilutions were required. The red cells used were pools of known A and B blood group (BG) red cells, diluted to 5% suspension, for the corresponding antibodies. For determining the IgM titer, 50 μL of pooled red cell was added to each of the tubes containing 100 μL of serially diluted serum, and tubes were centrifuged at 1000 × g for 1 minute. For determining the IgG titer, first neutralization of the IgM present in recipient’s serum was done by heat inactivation at 63°C for 10 minutes. A dilution series was prepared using the previously neutralized serum (1:1, 1:2, 1:4, 1:8, 1:16, and so on). To each of the tube containing 100 μL of serially diluted serum, 50 μL of pooled red cell was added and incubated at 37°C for 45 minutes followed by centrifugation and observation of hemolysis and agglutination. The cells were washed three times with saline and final wash was completely decanted. Anti-human globulin (AHG; Diagast) was added to the dry cell button according to the manufacturer’s directions followed by centrifugation and observation for agglutination. The maximum dilution at which macroscopic agglutination (1+) was observed was noted and the reciprocal of the value was designated as the titer (e.g., 32, 64). If agglutination was seen in the tube containing the most dilute serum, additional serial doubling dilutions were prepared and tested. The prozone phenomenon may cause reactions to be weaker in the more concentrated serum preparations than in higher dilutions. To avoid misinterpretation of results, the tube containing the most dilute serum was examined first and then proceeded through the more concentrated samples to the undiluted specimen.

**Titration Using Erythrocyte Magnetized Technology**

All steps in the procedure of antibody titration were performed by following the recommendations of the manufacturer as provided in the technical inserts. All the steps in the procedure of antibody titration were performed by the equipment. The detection of ABO IgM isotype antibody is based on the direct agglutination of RBCs that are already magnetized, combined with a magnetic field (E.M. Technology).

Serial twofold dilution of patient’s serum is done in the D-plates (empty microplates for dilution) using DiluentLys as diluent. DiluentLys (350 μL) was added into 11 wells of D-plates. Next, 700 μL of patient serum was dispensed in the first well and mixed sufficiently and 350 μL of mixture was dispensed in the next well and diluted stepwise automatically. Furthermore, 25 μL of mixture was dispensed in a microplate, and 25 μL of 1% type A1 blood cell suspension or type B blood suspension (Mag-plate for Heamlys A1, Mag-plate for Heamlys B: Diagast, Eurasante Parc, France) was added and left for incubation at room temperature for 10 minutes (EMT IgM). This was followed by magnetization for 4 minutes and agitation for 2 minutes at 900 rpm then for 45 seconds at 450 rpm. The maximum dilution factor at which 1+ agglutination (read with camera) was read and the reciprocal of the value was designated as the agglutinin titer.

**Statistical Analysis**

Microsoft Excel (Microsoft Corporation, Redmond, Washington, United States) was used to compile data, for all calculations and to draw graphs and plots. The median, maximum, and minimum values of the agglutinin titers of IgG and IgM for each blood type obtained by both the methods were calculated. The number and percentage of cases within ±1, ±2, or ±3 titer difference (TT-EMT) were calculated. The agreement between the two methods was expressed as the percentage of cases showing within one titer difference.

**Results**

The distribution of subjects into various BGs, their mean age, and male to female ratio is given in Table 1.

Frequency and isoagglutination titers of IgG and IgM by both TT and EMT for anti-B in BG A, anti-A in BG B, and anti-A and anti-B in BG O were plotted and are shown in Fig. 1A–D and Fig. 2A–D, respectively. The distribution of the measured IgG and IgM ABO isoagglutinin titers for each BG by the tube hemagglutination technique and EMT is shown in Figs. 3A and B. The median titers of IgG, anti-B for BG A

Table 1 Distribution of subjects into various BGs, their mean age, and male to female ratio

| BG   | Number (%) | Age          | M:F | Number (%) | Age          | M:F |
|------|------------|--------------|-----|------------|--------------|-----|
| A    | 34 (25.4)  | 30.23 ± 10.87| 11/23| 36 (31)    | 32.05 ± 13.72| 13/23|
| B    | 38 (28.4)  | 26.26 ± 6.71 | 11/27| 44 (37.9)  | 27.84 ± 15.05| 17/27|
| O    | 62 (46.3)  | 29.72 ± 12.33| 20/42| 36 (31.1)  | 28.02 ± 10.91| 13/23|
| Total| 134 (100)  | 28.87 ± 10.69| 42/92| 116 (100)  | 29.20 ± 13.49| 43/93|

Abbreviations: BG, blood group; IgG, immunoglobulin G; IgM, immunoglobulin M.
and anti-A for BG B, were same by both methods: 64 (4–512) by TT and 64 (2–2,048) by EMT for anti-A in BG B; 64 (2–256) by TT and 64 (2–512) by EMT for anti-B in BG A. IgG titer was higher for both anti-A and anti-B by EMT 256 (64–2,048), 256 (32–2,048), respectively, than TT method 128 (64–1,024), 128 (16–2,048), respectively.

For IgM, the median titer for anti-B in BG A and BG O was higher by EMT than TT: 32 (0–512) by EMT and 16 (1:256) by TT in BG A; 64 (8–512) by EMT and 48 (8–256) by TT in BG O. It was equal for anti-A in BG B by both methods 16 (1:2,048) by EMT and 16 (0:512) by TT method. For anti-A IgM titer in BG O was lower by EMT than TT method 32 (4:128) by EMT and 48 (8:256) by TT.

The agreements between the two methods are shown in Table 2. One twenty one (121/134, 90.3%) cases of IgG titer showed an agreement by both methods (within ± one titer difference). All the cases for IgG titers gave results within two titer differences by both methods. One hundred seven cases (107/116, 92.2%) and one hundred fourteen cases (114/116, 98.2%) for IgM titer were within one and two titer differences, respectively, by both the methods. Fifty cases (50/134, 37.3%) in case of IgG and forty-one (41/116, 35.3%) in case of IgM titer were giving same results by both TT and EMT. For IgG, 53.7% cases gave higher titers, while only 9% gave lower titers by EMT as compared with TT. For IgM, 36.2% cases gave higher and 28.5% cases lower titers by EMT as compared with that by TT.

**Discussion**

The main findings of this study showed that the median titers and their range obtained by EMT were higher or equal to that obtained by the TT for all IgM and IgG ABO-antibodies in all BGs except anti-A IgM in BG O that was lower by EMT than TT method: 32 (4:128) by EMT and 48 (8:256) by TT. Around one-third of the cases showed similar results for IgG and IgM titer of ABO antibodies in all BGs. However, for IgG...
Another study comparing five methods for anti-ABO titration on 50 BG O healthy donors, the median titers of IgG anti-A and anti-B antibodies by SPRCA were 64 (8–2,048) and 64 (4–512), respectively. These values were lower than ours in BG O individuals.\textsuperscript{18}

The testing for isoagglutinin titers depends on the temperature differences. IgM can be measured at room temperature and IgG after an incubation at 37°C that causes complement activation. But both IgM and IgG may react at room temperature and activate complements at 37°C leading to interferences in assay methods. When plasma is not treated with DTT or heated at 63°C for 10 minutes to inactivate IgM molecules, indirect agglutination titers may be a mix of IgM and IgG antibodies, reacting at 37°C. However, both IgG and IgM antibodies are likely to be active in humoral rejection, and cold-reacting (30°C) antibodies are usually not of clinical significance.\textsuperscript{19} In a study by Nayak et al, reduction in titers by DTT treatment in nearly 50% samples tested for both anti-A and anti-B titers as compared with the gel card titers read without the use of DTT indicating that there is a good amount of IgM type of anti-A and anti-B antibodies in the serum samples, which should be avoided before reporting the titers of candidates of ABO-incompatible transplants.\textsuperscript{18} For the conventional TT tests in our study, we have not used DTT. We used heat inactivation as a method as well as monospecific anti-IgG antibodies to remove the nonspecific IgM antibodies. Other studies have also used heat inactivation as a reliable method for the estimation of IgG isoagglutinins.\textsuperscript{16,19}

Few studies have also compared the performances of other methods for anti-A and anti-B titers. There are some studies comparing TT with gel method, one of which stated that gel is more sensitive,\textsuperscript{20,21} column agglutination results being approximately two and half fold higher (one more dilution) than that of conventional TT method.\textsuperscript{20}

Another study comparing the TT, gel, card, and FCM for isoagglutinin titers showed that there were significant differences in the titers obtained by these methods and each method showed a different detection capacity for each ABO antibody depending on the BG tested. They showed that in BG O, the mean titer in gel IAT was significantly higher than that of tube IAT for anti-A. FCM with anti-IgM showed the highest titer compared with tube or gel method in all of the BGs.\textsuperscript{10}
Another study comparing five different methods for anti ABO titration concluded that due to poor agreements between the results obtained by different methods, application of consistent and uniform method for titration throughout treatment of patient was recommended.18

Since most of the methods other than TT are or can be automated, they reduce the risk of manual errors, using standardized protocols with higher precision and accuracy, and minimize manual work providing an increased throughput. Another advantage of using EMT above TT for titration is that pictorial result files are converted to quantitative results that can be compared with follow-up pictures of the patient results, thus helping to decide the course of management for the patient.

It has been established that the titers of IgG are clinically more significant.22 The titer of isoagglutinins helps in managing patients as well as determining their prognosis. The tolerable amount of isoagglutinin titer varies among hospitals or clinicians. Risk of antibody-mediated hyper-acute rejection, successful transplant by reducing anti-A and anti-B levels by giving immunosuppressant, or procedures like immunoadsorption/plasma exchange can only be determined if an adequate and consistent method for measurement is used. Also, an increase in titer levels of more than two is considered to be significant while patient is on follow-up. Therefore, the standardization of test methods and target isoagglutinin titer can contribute to increasing success rates in cases of transplantation.

In conclusion, the EMT-based automated instrument, QWALYS-3, when compared with conventional test tube method does not give similar results and may vary by one titer dilution in majority (around 90%) of the cases. Therefore, the use of consistent method along with clinical correlation of the isoagglutinin titer is a must for patient management.

Authors’ Contribution
Parul Chopra was involved in definition of intellectual content, literature search, interpretation of results, data analysis, and drafting and editing of the manuscript. Sunanda Bhardwaj interpreted results and edited the manuscript. Ajay Samkaria carried out the tests in the laboratory, analyzed the data, and edited the manuscript. Asha Amoli carried out the tests in the laboratory, interpreted the results, did data entry, and edited the manuscript. Anil Arora conceptualized the study, defined intellectual content interpretation of results, and edited the manuscript.

Ethical Approval
Since the tests were done on residual samples obtained in the laboratory for testing, ethical clearance was not required.

Conflicts of Interest
None.

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References
1. Daniels G, Bromilow I. Essential Guide to Blood Groups. 3rd edition. Chichester, West Sussex, UK: Wiley Blackwell; 2014 132
2. Li P, Pang LH, Liang HF, Chen HY, Fan XJ. Maternal IgG anti-A and anti-B titer levels screening in predicting ABO hemolytic disease of the newborn: a meta-analysis. Fetal Pediatr Pathol 2015;34(6):341–350
3. Shimamura H, Tanabe K, Ishikawa N, Tokumoto T, Takahashi K, Toma H. Role of anti-A/B antibody titers in results of ABO-incompatible kidney transplantation. Transplantation 2000;70(9):1331–1335
4. Tobian AA, Shirey RS, Montgomery RA, et al. ABO antibody titer and risk of antibody-mediated rejection in ABO-incompatible renal transplantation. Am J Transplant 2010;10(5):1247–1253
5. Worel N, Kalhs P, Keil F, et al. ABO mismatch increases transplant-related morbidity and mortality in patients given nonmyeloablative allogeneic HPC transplantation. Transfusion 2003;43(8):1153–1161
6. Lee SHK, Lee JH, Lee KH, et al. Isoagglutinin titer in major ABO incompatible bone marrow transplantation. Korean J Blood Transfus 1997;8:167–176
7. Lozano M, Heddle N, Williamson LM, Wang G, AuBuchon JP, Dumont LJ. Biomedical Excellence for Safer Transfusion Collaborative. Practices associated with ABO-incompatible platelet transfusions: a BEST collaborative international survey. Transfusion 2010;50(8):1743–1748
8. Shah BV, Rajput P, Virani ZA, Warghade S. Baseline anti-blood group antibody titers and their response to desensitization and kidney transplantation. Indian J Nephrol 2017;27(3):195–198
9. Böhmig GA, Farkas AM, Eskandary F, Wekerle T. Strategies to overcome the ABO barrier in kidney transplantation. Nat Rev Nephrol 2015;11(12):732–747
10. Kang SJ, Lim YA, Baik SY. Comparison of ABO antibody titers on the basis of the antibody detection method used. Ann Lab Med 2014;34(4):300–306
11. Knight RC. Measuring IgG anti-A/B titres using dithiothreitol (DTT). J Clin Pathol 1978;31(3):283–287
12. Bentall A, Regan F, White J, et al. No progress in ABO titer measurement: time to aim for a reference? Transplantation 2014;97(3):e19–e21
13. Bajpai M, Kaur R, Gupta E. Automation in immunohematology. Asian J Transfus Sci 2012;6(2):140–144
14. Bouix O, Ferrera V, Delamaire M, Redersdorff JC, Roubinet F. Erythrocyte-magnetized technology: an original and innovative method for blood group serology. Transfusion 2008;48(9):1878–1885
15. Roback JCM, Grossman B, Hillyer C, American Association of Blood Banks (AABB) Technical Manual. 17th edition. Bethesda, MD: American Association of Blood Banks; 2011
16. Al-Muzairai IA, Mansour M, Almajed L, Alkanderi N, Alshatti N, Samhan M. Heat inactivation can differentiate between IgG and IgM antibodies in the pretransplant cross match. Transplant Proc 2008;40(7):2198–2199
17. Shim H, Hwang JH, Kang SJ, et al. Comparison of ABO isoagglutinin titres by three different methods: tube haemagglutination, micro-column agglutination and automated immunohematology analyzer based on erythrocyte-magnetized technology. Vox Sang 2020;115(3):233–240
18. Nayak S, Makroo RN, Prakash B, et al. Comparative evaluation of five different methods of anti-ABO antibody titration: an aid for ABO-incompatible organ transplants. Ther Apher Dial 2019;23(1):86–91
19. Schweizer RT, Bartus SA, Perkins HA, Belzer FO. Renal allograft failure and cold red blood cell autoagglutinins. Transplantation 1982;33(1):77–79
20 Bhangale A, Pathak A, Pawar S, Jeloka T. Comparison of antibody titers using conventional tube technique versus column agglutination technique in ABO blood group incompatible renal transplant. Asian J Transfus Sci 2017;11(2):131–134

21 Chandak S, Prajapati A, Vanikar A. Prior ABO incompatible renal transplantation using indirect antihuman globulin test via tube incubation and gel column technique. National Journal of Laboratory Medicine 2018;7:PO12–PO15

22 Shirey RS, Cai W, Montgomery RA, Chhibber V, Ness PM, King KE. Streamlining ABO antibody titrations for monitoring ABO-incompatible kidney transplants. Transfusion 2010;50(3):631–634