RESEARCH ARTICLE

COMPARATIVE ANALYSIS OF BLOOD GROUPING IN HEALTHY BLOOD DONORS USING GEL CARD TECHNIQUE AND TUBE METHOD

Muhammad Usman1,2, Khansa Qamar1, Maeesa Sajeel1,2, Sanaullah1
1 Baqai Institute of Hematology, Baqai Medical University, Karachi, Pakistan
2 Muhammadi Institute of Hematology and Transfusion Medicine, Karachi, Pakistan
Received: October 10, 2016 Accepted: November 30, 2016

ABSTRACT
Blood grouping is a vital test in pre-transfusion testing. Both tube and gel agglutination assays are used for ABO grouping. The main object of this study was to compare ABO grouping and D typing on tube and gel agglutination assay in order to assess the efficacy of each technique. A total of 100 healthy blood donors irrespective of age and sex were included in this study. Results showed that there is no significant difference between these two techniques. However, in 10 samples it was detected that the reaction strength in serum ABO grouping by gel agglutination assay is varied by only one grade when compared to tube agglutination assay. Due to numerous positive effects of gel assay it is more beneficial to implement this technique in the setups where blood banks bear heavy routine work load.

Keywords: Blood grouping, tube agglutination assay, gel agglutination assay.

1. INTRODUCTION
A series of glycoproteins and glycolipids are present on the surface of human red blood cells (RBCs) which constitute blood group antigens. Development of these antigens is genetically controlled1. About 700 RBCs antigens are described into 30 blood group systems by the International Society of Blood Transfusion among which ABO and Rh are the most important of this blood group systems2-4.

ABO blood group has four phenotypes namely; A, B, AB and O. These are formed by three polymorphic alleles A, B and O. Blood group of an individual is recognized by the presence of A, B and O surface antigens on his/her RBCs. The surface antigens that lack on the surface of RBCs cause antibody development against that specific antigen5. ABO antibodies are formed in response to ABO-like antigenic substances. ABO blood group system is the only blood group system in which reciprocal antibodies are produced in the serum after environmental exposure6. The presence or absence of antigen and antibodies can be determined by the help of different methods such as conventional tube method, gel card technique, micro plate, etc. Many techniques have been proposed for blood group detection, out of which conventional tube test and micro column gel card technique are the most widely used. These methods are commonly used in the detection of autoimmune hemolytic anemia by direct antihuman globulin test (AHG/Coombs test) and in the detection of ABO blood groups in transfusion medicine. Conventional test tube is the standard technique for immuno-hematological studies6-7.

Since 1900s, tube agglutination method was the gold standard method for ABO grouping. Recently, due to work load column agglutination method is replaced by tube agglutination at many institutions8. Tube agglutination is a time consuming technique while column agglutination is an easy method, less time consuming and uses small volume of serum and RBCs. It does not require red cell washing. It uses gel filtration media saturated with an anti A/B reagent to induce agglutination. Owing to simplicity, reproducibility and sensitivity of this technique many
blood banks and transfusion centers are now applying this technique.\(^8\)

This study has been carried out to compare ABO grouping on test tube and gel card method in order to assess the efficacy of each technique.

2. MATERIAL AND METHODS
A total of 100 healthy blood donors irrespective of age and sex were included in the study for ABO blood grouping. This study was approved by the ethics committees of Baqai Medical University and Muhammadi Blood Bank, Karachi. Blood samples (4 ml) were collected in BD Vacutainer\(^8\) plus plastic K\(_2\) EDTA (7.2 mg) tubes for ABO and Rh blood grouping from all enrolled individuals for tube and gel card assay.

2.1. Tube Method
The tube agglutination assay was performed on all the samples for forward and reverse ABO grouping.

2.1.1. ABO Forward Typing
A 5% red cell suspension of all samples was prepared by washing three times in isotonic saline. For ABO and Rh grouping, commercial monoclonal anti-sera anti-A, anti-B, anti-AB and Rh D grouping was performed by using monoclonal/polyclonal anti-D (Bio-Rad Diacclone monoclonal IgM and Seraclone IgM anti-D, USA). A drop of anti-A, anti-B, anti-AB and anti-D was added to a 5% blood cell suspension in a clean tube and mixed well. Agglutination was recorded as a positive reaction.

2.1.2. ABO Reverse Typing
All samples were spin for 5 min. on 4000 RPM in the centrifuge (Clay Adams Sero-fuge 2001, Becton Dickenson and Company, USA) and the plasma was separated for reverse grouping. In reverse grouping antibodies were detected in all samples against 5% known ABO cells.

2.2. Gel Card Method
The gel agglutination assay was performed on all samples for forward and reverse ABO grouping by using standardized equipment (ID-Micro Typing System, Ortho-Clinical Diagnostics, Inc., USA). A 0.8% red cell suspension was prepared in low ionic strength solution diluent (ID-Diluent 2, Bio-Rad, USA). All samples were spin for 5 min. on 4000 RPM in the centrifuge.

ID-card DiaClon ABO/D+ Reverse Grouping (Bio-RAD, USA) was used that contains three microtubes monoclonal anti-A (cell line A5), anti-B (cell line G1/2) and anti-D (cell line LHM 59/20 [LDM3]+175-2) within gel matrix. The microtube ctrl was the negative control. Two microtubes with “neutral” gel served for reverse grouping. A total of 10 \(\mu L\), 0.8% cells suspension, was added in antisera and ctrl microtubes and 50 \(\mu L\) known cells were put in two reverse microtubes. Then 25 \(\mu L\) patient’s plasma was added in reverse microtubes and was spin in ID Card microtubes in ID centrifuge for 10 minutes and the results were interpreted. Agglutination was recorded as a positive reaction.

2.3. Statistical Analysis
Results were analyzed using SPSS statistical software version 17.0 (Chicago, IL, USA). Results were calculated and reported as mean, standard deviation and percentage.

3. RESULTS
ABO grouping and D typing were performed on all samples by tube and gel agglutination assay. The results of ABO grouping and D typing showed no significant difference between tube and gel agglutination assay. Results of ABO cell and serum grouping for 90 samples coincided between tube and gel agglutination assay at immediate spin. However, in gel agglutination assay 10 samples showed low grade reaction in ABO serum grouping. The result of ABO cell grouping of these 10 samples indicated that they were B and A/O in gel agglutination assay while in serum grouping they showed low reaction strengths. Stronger reaction strengths were seen in tube agglutination assay in ABO serum grouping in these samples. A summary of these results is shown in Table 1.
Table 1. Reaction strengths both in cell and serum ABO grouping by tube and gel agglutination assay.

<table>
<thead>
<tr>
<th>Tube Agglutination Assay</th>
<th>Gel Agglutination Assay</th>
<th>Blood Group</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cell Grouping</strong></td>
<td><strong>Serum Grouping</strong></td>
<td><strong>Cell Grouping</strong></td>
</tr>
<tr>
<td>Anti A</td>
<td>Anti B</td>
<td>A&lt;sub&gt;1&lt;/sub&gt; cells</td>
</tr>
<tr>
<td>–</td>
<td>4+</td>
<td>–</td>
</tr>
<tr>
<td>–</td>
<td>4+</td>
<td>–</td>
</tr>
<tr>
<td>–</td>
<td>4+</td>
<td>–</td>
</tr>
<tr>
<td>4+</td>
<td>–</td>
<td>4+</td>
</tr>
<tr>
<td>–</td>
<td>4+</td>
<td>–</td>
</tr>
<tr>
<td>–</td>
<td>4+</td>
<td>–</td>
</tr>
<tr>
<td>–</td>
<td>–</td>
<td>4+</td>
</tr>
<tr>
<td>–</td>
<td>–</td>
<td>4+</td>
</tr>
<tr>
<td>–</td>
<td>4+</td>
<td>–</td>
</tr>
<tr>
<td>4+</td>
<td>–</td>
<td>4+</td>
</tr>
</tbody>
</table>

4. DISCUSSION

ABO Blood grouping has always been a major concern in health sector. Ever since the blood groups were introduced, it has become more and more vital for the clinicians and surgeons to know the exact blood group of their patients. The importance of ABO blood group system can be estimated with the fact that so far 30 blood groups have been discovered, and only ABO and Rh blood group systems have been given prime importance in transfusion medicine. Other blood groups are only examined when clinical problems occur, which are rare. Different types of blood group antigens are present on the surface of RBCs in each individual around the globe. Blood grouping is a procedure in which testing of RBCs and serum are done to determine which type of antigens and antibodies are present on the surface of RBCs.

This study was undertaken with the primary object of assessing the comparative analysis of gel agglutination and tube agglutination assay for ABO grouping and D typing. It was postulated that tube agglutination assay is a valid and reliable technique for detection of ABO grouping. In this study no significant difference between the two techniques in testing of ABO grouping has been found. In some cases (10 samples) it has been found that reaction strength in serum ABO grouping by gel agglutination assay is varied by only one grade when compared to tube agglutination assay.

It has been reported that gel agglutination assay is more reliable and sensitive technique as compared to tube agglutination assay for the detection of ABO and D grouping. The gel agglutination assay is a FDA approved technique for detection of A, B, D, direct and indirect antiglobulin testing. In gel agglutination assay subsequent centrifugation is intended to bring the agglutination in gel column.
During centrifugation, agglutinated RBCs are trapped in the gel depending upon the strength of agglutination. If agglutination reaction does not take place between serum/antiserum and RBCs, the agglutinated cells will become pellet at the bottom of the tube\textsuperscript{6,8}.

There are numerous advantages of gel agglutination assay. The agglutination reaction in gel assay is more qualitative, well defined and stable technique as compared to tube agglutination assay, which may be permitted to save for up to 24 hours. Moreover, this technique requires small volume of sample which is beneficial for pediatric testing. This technique is also less time consuming and easy to operate with less trained personnel. Additionally, biosafety is also a valuable advantage of this technique as less hazardous waste is produced \textsuperscript{6,8,11}.

However, several drawbacks are also observed in testing of ABO grouping by gel agglutination assay. It is a costly test because it requires specified centrifugation machine and reagents like low ionic strength suspension which is not used in tube agglutination assay\textsuperscript{12}. Another disadvantage related to this procedure is that it has no O cells column for O blood group confirmation in reverse typing. Therefore, tube technique is always run side by side for confirmation of O blood group. Furthermore, this technique does not identify or rule out anomalous results. Because weak subgroups are not solved out through this type of technique, they require incubation or addition of specific reagents or elution or adsorption process which is not possible to add or perform.

5. CONCLUSION
There is no significant difference between the two test techniques. Gel as well as tube agglutination assay can both be used in blood blanks and hospitals for determination of ABO grouping D typing. Due to numerous advantageous effect of gel assay, it is more beneficial to implement this technique in those setups where blood banks bear heavy work load daily.

REFERENCES