INTRODUCTION
ABO RH grouping system is the most important test in medical laboratory and blood banking system, performed both on transfusion recipients and blood donors. The critical nature of ABO grouping stems from two characteristics of the system. First, unlike other blood group systems, antibodies of the ABO system are present in the serum of almost every person who does not have the corresponding antigen. Second, the all agglutinins of the ABO system fix complement and are capable of causing intra vascular hemolysis of incompatible red cells. For these reasons, an error in ABO grouping of a patient or donor could turn out to be fatal during blood transfusion process. While the cross-match affords an additional measure of protection, this may not be done in every case. Accurate determination of a person's ABO group requires two different test procedures: red cell grouping also called as forward grouping and serum grouping also called as reverse grouping. The individual is first assigned to one of the four ABO blood groups - A, B, AB and O based on the reaction of red cells with blood grouping sera Anti-A and Anti-B. Anti AB serum prepared from specially selected group O individuals is not a simple mixture of Anti-A and Anti B but is the component of group O serum that has the special property of reacting with weak antigens on the red cells, especially weak A antigens. Blood group is detected by various methods. ERYCARD™2.0 is blood grouping card easy to use in bed site setting or outdoor camp and easy to interpret by laboratory staff. TUBE agglutination is gold standard method for ABO blood grouping as this method gives incubation time. Therefore, the aim of the present study to compare ease of use and accuracy of ERYCARD™ 2.0 with the historical gold-standard TUBE Agglutination method.

Patients: It was a cross-sectional study done on routine samples over a period of 2 months December 2013 and January 2014. Total numbers of 300 patients were randomly investigated in OPD laboratory.

Blood samples: Under all aseptic precautions, samples were collected from the antecubital vein using a 2-ml disposable syringe with 24G needle. The
study included small (1- to 2-mL) EDTA anticoagulated blood samples from patients.

**MATERIALS AND METHODS**

**CARD method:** In this study ERYCARD™2.0 blood grouping card for ABO/Rho (D) forward grouping with autocontrol is used, which is based on principle of lateral flow guided by capillary action. Procedure: Bring the pouch and reagent buffer bottle to room temperature. Tear open the pouch just prior to the testing and remove the ERYCARD™2.0 test device. Label the test card with the patient’s ID and date. Add 5μl each of the patient’s whole blood sample to each of the sample wells indicated as ‘S’, ensuring that only the blood drop is in contact with pre-dried reagent on the sample pad and adsorbed by it. In case the micropipette tip touches the sample pad, discard the tip and use fresh tip for dispensing into next sample well. After waiting for one minute allowing the sample to react with the reagent on sample pad adds two drops of the reagent buffer to each of the reagent wells indicated as ‘R’. After addition of reagent buffer wait for 3 minutes to interpret the test results. The auto control should show a colorless patch before the results can be interpreted correctly. If the autocontrol pad has a color then the test result should not be interpreted.

**TUBE method:** to prepare a RBC suspension for the TUBE method, 1 ml of anticoagulated blood was centrifuged for 2 minutes in a centrifuge (1,000 × g at Room temperature [approx 20°C]). Plasma was collected into a separate tube, and the RBC Pellet was resuspended in 5 ml of normal saline. The suspension was then recentrifuged and resuspended 3 times and finally reconstituted to a 2% to 5% RBC suspension. In 3 tubes, 25μL of this suspension was then mixed with 50 μL of antiserum. These mixtures were incubated at room temperature for 15 minutes before centrifugation for 15 seconds at 1,000 × g. Tubes were then gently agitated, and the degree of agglutination was scored. Interpretation of the test 4+ cell button remains in one clump. 3+ cell buttons dislodges into several clumps. 2+ cell buttons dislodges into many small clumps of equal size. 1+ cell button dislodges into finely granular, but definite, small clumps. D cell button dislodges into fine granules, but not definite small clumps. Results should be recorded as doubtful. 0 Negative reaction-cell buttons dislodges into no visible clumps.

**RESULT**

Blood samples from 300 patients were included in the study. All the patients were tested by both blood grouping methods. The strengths of all test reactions (anti-A, anti-B, and anti-D) were recorded as well as the Interpreted test result for both methods. Accuracy of test methods was then calculated by comparison with the TUBE method as the standard criterion. Overall agreement between blood-typing methods was good to excellent, with identical results obtained in 293 of 300 (97.6%) patients tested with card. Details of the 7 discrepancies identified among these patients were summarized

**DISCORDANT RESULTS**

<table>
<thead>
<tr>
<th>Number of samples</th>
<th>Correct blood group</th>
<th>Blood group by ERYCARD™2.0</th>
<th>Blood group by tube agglutination method</th>
<th>Remark / reason</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>AB</td>
<td>O</td>
<td>AB</td>
<td>Hct &lt; 15% [Anemia]</td>
</tr>
<tr>
<td>2</td>
<td>B</td>
<td>AB</td>
<td>B</td>
<td>Auto clumps</td>
</tr>
<tr>
<td>2</td>
<td>A2</td>
<td>O</td>
<td>A2</td>
<td>Anti-A1 serum</td>
</tr>
<tr>
<td>2</td>
<td>A2B</td>
<td>B</td>
<td>A2B</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>O</td>
<td>-</td>
<td>-</td>
<td>Sample Hemolysed</td>
</tr>
</tbody>
</table>

Among the 300 samples examined in this study, there were 7 samples in which blood typing problems or discrepancies arose. Two of these samples were from Patients that had a recorded diagnosis of an anemia on the basis of an Hct < 15%. While the TUBE assay identified these samples to be blood type AB, card were falsely identifying these patients as blood group O. In addition, 2 of 7 samples that had auto clumps present. In this patients card showing weakly AB Positive group falsely, actually 1 of this 2 patients had B positive group and 1 patient had O positive group. Weak auto agglutination which was eliminated by washing of RBCs. 2 of 7 samples had A2 or A2B group that were not detected by card method, were
Comparison of conventional TUBE agglutination method versus ERYCARD™2.0 for the blood Grouping

detected by tube method by comparing clump size of A with O agglutination and confirmed by anti A₁ serum. 1 of 7 samples were hemolysed.

DISCUSSION

The ABO blood group system is the most clinically important blood group system in humans because ABO mismatched transfusions can cause life-threatening hemolytic reactions without prior sensitization via pregnancy or transfusion. Therefore, it is crucial that Clinicians identify a human’s blood group. In the present study, 2 ABO typing methods were compared for ease of use and accuracy. The TUBE tests are generally restricted to the laboratory and performed by specifically trained personnel, whereas the CARD is simple point-of-care kits commonly used by laboratory staff in practice. Over the past 15 to 20 years, the reagents in blood typing kits or tests have occasionally changed, and it is important to remember this when interpreting and comparing our results with those from previous Studies. In ERYCARD™2.0, the appropriate reagent are pre-dried at appropriate sample pad beneath the sample well namely Anti-A (IgM) antibodies in sample well A, Anti-B (IgM) antibodies in sample well B, Anti-D (IgM) antibodies in sample well D. The autocontrol is a negative control that does not contain any antibodies in sample well (Ctrl) and serve the validate the test results. Reagent Buffer contains sodium azide (<0.1%) as a preservative. In TUBE method, Anti A, Anti B and Anti D reagent were ready to use reagent prepared from supernatants of mouse hybridoma cell cultures. These reagents contains sodium azide (<0.1%), sodium arsenite (0.02%) and bovine albumin. However, our survey also detected a few discrepancies, with the TUBE method 99% agreement and the ERYCARD™2.0 achieving 97.6% agreement. Therefore, CARD method should be suitable for point-of-care testing in in-clinic settings when typing results are immediately needed. Results of blood typing can be affected by anemia, auto agglutination and hemolysed sample, Thereby contribute to test inaccuracies as detected in the present study. In our study, blood samples from, anemic patients had positive results for the O antigen by ERYCARD™2.0. Preparation of appropriate concentration RBC suspensions alleviates the effect of Hct TUBE assays, and for the point-of-care assays, adding more blood to test reactions when dealing with anemic patients may overcome such problems. Subjective test interpretation is a potential problem with any of the methods used in our Study but is of particular concern when agglutination is scored in an RBC suspension because test interpretation is dependent on the time of reading and degree of agitation applied by the operator. When the TUBE methods were used, the distinction between positive and negative results was clearer than those for the CARD method. This was because there were smaller numbers of 1+ and 2+ results with the TUBE methods, and such results may be confused, altering test interpretation.

CONCLUSION

Though ERYCARD™2.0 helped a lot in bedside blood grouping, on comparing the manual blood grouping methods, few discrepancies in blood grouping was noted. Card was easy to use and interpreted as compare to TUBE method but incubation could not be possible in this card method, as incubation possible TUBE agglutination method. Overall accuracy of blood group typing by ERYCARD™2.0 is as comparable as TUBE agglutination method, so it can be used as an optional method.

REFERENCE

1. Comparison of five bloodtyping methods for the AB blood group system. Seth M, Jackson KV, Giger U
4. Recommended Methods for blood Grouping Reagents Evaluation; Docket No. 845-0181
5. Importance of ABO Grouping.