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ORIGINAL ARTICLE

Study on ABO and RhD blood grouping: Comparison between conventional tile method and a new solid phase method (InTec Blood Grouping Test Kit)

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Abstract

Introduction: 'InTec Blood Grouping Test kit' using solid-phase technology is a new method which may be used at outdoor blood donation site or at bed side as an alternative to the conventional tile method in view of its stability at room temperature and fulfilled the criteria as point of care test. This study aimed to compare the efficiency of this solid phase method (InTec Blood Grouping Test Kit) with the conventional tile method in determining the ABO and RhD blood group of healthy donors. *Methods:* A total of 760 voluntary donors who attended the Blood Bank, Penang Hospital or offsite blood donation campaigns from April to May 2014 were recruited. The ABO and RhD blood groups were determined by the conventional tile method and the solid phase method, in which the tube method was used as the gold standard. *Results:* For ABO blood grouping, the tile method has shown 100% concordance results with the gold standard tube method, whereas the solid-phase method only showed concordance result for 754/760 samples (99.2%). Therefore, for ABO grouping, tile method has 100% sensitivity and specificity while the solid phase method has slightly lower sensitivity of 97.7% but both with good specificity of 100%. For RhD grouping, both the tile and solid phase methods have grouped one RhD positive specimen as negative each, thus giving the sensitivity and specificity of 99.9% and 100% for both methods respectively. *Conclusion:* The 'InTec Blood Grouping Test Kit' is suitable for offsite usage because of its simplicity and user friendliness. However, further improvement in adding the internal quality control may increase the test sensitivity and validity of the test results.

Keywords: Solid-phase method, ABO blood group, RhD blood group, blood donor, conventional tile method

INTRODUCTION

The ABO and RhD are the two most significant blood group systems in transfusion medicine.[1,2] These blood groups are being tested for all healthy blood donors as well as all patients prior to blood transfusion to ensure that the patients are given the right blood for transfusion.[3] Pre-donation blood gouping of the donor is the preliminary result which is repeated again during pre-transfusion testing from the donor unit before issuing the blood for transfusion to the patient. The current practice of ABO and RhD blood group testing include the conventional slide/tile method, glass tube method and relatively new method of microplate technique and column agglutination technique.[4] Of all these four techniques, the latter three methods are more appropriate for onsite laboratory testing of pre-donation ABO and RhD blood grouping of the donor. Besides the test reagents, these methods require additional equipment such as centrifuge for the testing procedure. Slide/tile method is the only portable and simple method that is feasible and appropriate for offsite donation drive or bedside blood group confirmation. However, this method is less sensitive compared to the other three methods mentioned earlier and drying up of the reaction mixture can cause aggregation of cells giving false positive results as well

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as weaker results are difficult to interpret.[4] Moreover, this slide/tile method though feasible, but it has its limitation i.e. it requires the testing reagents to be brought to offsite where it may not be kept in the optimal storage temperature of 2-6°C especially in the tropical countries like Malaysia, more so, the slide/tiles that have already been used for ABO and RhD testing will be contaminated with donors' blood and these will posed a risk of contamination to the operator as well as the environment.

In the recent years, a new technology of testing ABO and RhD blood group by solid-phase adherence technique has been developed.[5] This is an immunological technique where one of the reactants, either the antigen or antibody is immobilised onto a solid medium and assay for its counterpart, fluorescein or red cells may be used as the end point indicator.[6, 7] This technology has brought to the invention of a new ABO and RhD blood grouping kit (InTec Products, Inc. Xiamen) which uses the red cell as the end point indicator. This new test kit has the monoclonal anti-A, anti-B and anti-D antibodies immobilised on the porous solid carrier, and the addition of tested red cells to these antibodies will initiate the immunobinding reactions of the ABD antigens to the antibodies, thus giving rise to positive reactions symbolised by the red colour of the red cells bound to the antibodies. Otherwise the red cells will not be retained if no antigen-antibody immunoreaction happens, which indicates a negative reaction with no colour appearing. This test kit is stable at room temperature and the procedure is simple and user friendly. Besides, it does not need any additional reagents and can be kept or stored at 2 to 30°C. This method is easy to use, does not require any special equipment and the results can be read in 2 minutes. Interestingly, solid phase method when comparing with the conventional agglutination method, its end point results are stable and can be read objectively by operator.[8] It has low contamination risk to the operator and environment. It is suitable for regular ABO and RhD grouping screening test especially at outdoor such as bedside blood grouping, blood donation campaign or self-test at home. Thus, this test kit is very appropriate for field use.

In Malaysia, the ABO and RhD blood group testing performed at the mobile blood donation camp or at bedside is most commonly by the conventional tile method that has some limitation as mentioned earlier. Solid phase technique using the 'InTec Blood Grouping Test kit' can be a suitable alternative to the tile method. Therefore, this study was undertaken to evaluate the performance of the 'InTec blood grouping kit" in the Malaysian setting. The primary objective of this study was to compare the efficiency of the solid phase method with the conventional tile by using the tube method as the gold standard in determining the ABO and RhD blood group in healthy voluntary blood donors. This study also compared the sensitivity and specificity of the solid phase method with the tile method.

MATERIALS AND METHODS

This was a descriptive cross sectional study conducted over a period of two months from April to May 2014 at the Blood Bank, Penang Hospital, Malaysia. This study was approved by the UKM Medical Centre Medical Research Committee (Project Code: FF-2013-438). The blood samples were collected from the voluntary blood donors who donated blood either at the Blood Bank Centre or at mobile blood donation site. Donor selection was done following the standard procedure and a total of 760 donors were recruited in this study. All the donors were explained about the objectives of the study and the test procedures, and the written consents were obtained before the samples were taken.

Prior to the blood donation procedure, the ABO and RhD blood groups of the donors were determined by the conventional blood grouping test using the tile method as well as the 'InTec blood grouping test kit (solid-phase)' and the results were recorded. Later in the Blood Bank laboratory, all these donors' specimen collected for the double confirmation of blood grouping were tested for ABO and RhD blood groups by the gold standard tube method.

Sample collection: Blood sample was obtained by simple finger prick for the tests by tile method and the solid-phase method (InTec blood grouping kit). The tests were done by two well-trained independent medical lab personnels. As the solid phase method is operator dependent, the same trained personnel was involved to test at each time. During the blood donation procedure, a peripheral venous blood sample was collected in an EDTA tube from each donor for the confirmation of ABO and RhD grouping using the gold standard tube method.

Methods

For ABO and RhD grouping using tile method, solid-phase method and tube method, standard procedures were carried out following the

manufacturer's instructions. The test using the tile method and tube method is based on the principle of direct haemagglutination. For tile method, only forward ABO grouping was done while the gold standard tube method included both forward and reverse grouping. The tile method required monoclonal anti-A, anti-B and anti-D typing antisera for testing donors cells. The tests were performed on a water proof card for tile method. Tube method was performed in glass test tubes and monoclonal anti-A, anti-B and anti-AB (Epiclone™ Anti-A, anti-B and Anti-A,B) reagent were used in forward grouping while A1 cell, B cell and O cell (IMMUCOR) were used for reverse grouping. For RhD grouping, IgG/IgM blends of monoclonal anti-D (Epiclone™ Anti-D) was used in both tile and tube method. All the tests were performed following the manufacturer's instructions. The mixing of patient's cell/plasma with the reagent antibody anti-A, anti-B/reagent A1 cells, B cells resulted in specific antigen-antibody reaction which was visible as agglutination of the red blood cells and thus the blood group A, B, AB and RhD positive was determined. No agglutination with anti-A, anti-B, was grouped as group O while no agglutination with anti-D was grouped as RhD negative. All RhD negative samples were subsequently tested with the weak-D test to confirmed the RhD blood group.

The 'InTec blood grouping test kit (solid-phase method)' has three column labelled as A,B and D and two rows, one labelled S for sample and another row labelled D for diluent. Patient's sample and diluent were added in the respective well and the reaction was observed after 1-2 minutes. Positive reactions were indicated by red blood cell adherence over the entire surface of the wells giving red colour reaction, whereas negative reactions form discrete red blood cell buttons in the centre of the wells giving no colour.[9,10]

Data collection and analysis

The result was recorded in the data sheet and analysed. The rate of concordance between the tile method or solid phase method and the gold standard tube method were calculated. And the sensitivity and specificity of both the methods were also calculated. The results were analysed by IBM SPSS Statistics version 19.

RESULTS

Comparing the blood grouping results by tile method and tube method, both the methods have 100% concordance results for ABO blood group, but there was one sample of RhD positive that was wrongly typed as RhD negative by the tile method. On the other hand, comparing the solid-phase method and the tube method, the blood grouping concordance rate is only 99.2% in which five samples showed discrepancies of results for ABO blood group and one sample showed false negative result for RhD blood group (Table 1).

Among the ABO cases, two cases were wrongly typed as AB instead of B. There were three samples wrongly typed as A where 2 donors (A13 and A417) were actually O and one was (donor A668) actually AB. One sample (A668) did not match for RhD blood group with tube method, where tile method and solid phase method has falsely identified an RhD positive donor as RhD negative (Table 2).

The sensitivity and specificity of the tile method and solid phase method were determined. From the analysis (Table 3), the tile method has 100% sensitivity and specificity for typing of ABO blood group. However for RhD blood group, it has 99.9% sensitivity and 100% specificity. These findings showed that this method is really sensitive and specific as a screening method. For the solid-phase method, it has a lower sensitivity of 97.7% but with

TABLE 1: ABO & RhD blood grouping based on different methods

ABO & RhD blood grouping	Method, n (%)		
	Tile	Tube	Solid phase
O	298 (39.2)	298 (39.2)	296 (38.9)
A	188 (24.7)	188 (24.7)	191 (25.1)
B	230 (30.3)	230 (30.3)	228 (30.0)
AB	44 (5.8)	44 (5.8)	45 (5.9)
RhD Positive	755 (99.3)	756 (99.5)	755 (99.3)
RhD negative	5 (0.7)	4 (0.5)	5 (0.7)

TABLE 2: Samples with discrepant results

Sample No	Tile	Tube	Solid Phase
A13	O RhD Pos	O RhD Pos	A RhD Pos
A417	O RhD Pos	O RhD Pos	A RhD Pos
A257	B RhD Pos	B RhD Pos	AB RhD Pos
A596	B RhD Pos	B RhD Pos	AB RhD Pos
A668	AB RhD Pos	AB RhD Pos	A RhD Pos
A290	O RhD Neg	O RhD Pos	O RhD Neg

good specificity of 100% for ABO blood group; and similar sensitivity and specificity for RhD grouping as the tile method, i.e, 99.9% and 100% respectively.

DISCUSSION

Solid phase technique has been introduced for the last few years and in use in different countries.[11,12] This study evaluated its suitability in Malaysia using the 'InTec Blood Grouping Test kit' as a pre-donation testing for the blood donors. In this study, all the donors were tested for ABO and RhD blood group by 3 different methods. The tile method and solid-phase method were used as a screening test done at the donation area while the tube method was done as a gold standard for confirmation in the laboratory of the Blood Bank, Penang Hospital. Both the screening methods used only forward grouping that types the red cell antigens, while for the confirmation test, both the forward grouping and reverse groupings were done. Reverse grouping identified the red cell ABO antibodies and the forward and reverse grouping in the gold standard tube method aim to serves as a check for each other.[13]

Comparing the results of these two screening methods with the gold standard, it showed that the tile method has 100% concordance results for the ABO blood group with the gold standard tube method. However, for the solid-phase method, it has five discrepant results (0.66%) for the ABO blood group compared to the gold standard.

In the RhD blood group, there was one discrepant result (0.13%) noted in each method compared to the gold standard tube method, where both the tile method and solid phase method had wrongly typed it as an RhD negative sample while the confirmation test in the laboratory as well as in the referral centre

TABLE 3: Sensitivity, Specificity, Positive Predictive Value (PPV) and Negative Predictive Value (NPV) of tile method and solid phase method towards tube method

ABO & RhD blood grouping	Method	Sn (%)	Sp (%)	PPV (%)	NPV (%)
O	Tile	100.0	100.0	100.0	100.0
	Solid phase	99.3	100.0	100.0	99.6
A	Tile	100.0	100.0	100.0	100.0
	Solid phase	100.0	99.5	98.4	100.0
B	Tile	100.0	100.0	100.0	100.0
	Solid phase	99.1	100.0	100.0	99.6
AB	Tile	100.0	100.0	100.0	100.0
	Solid phase	97.7	99.7	95.6	99.9
Rh -ve	Tile	100.0	99.9	80.0	100.0
	Solid phase	100.0	99.9	80.0	100.0

Sn: Sensitivity, Sp: Specificity, PPV: Positive Predictive Value, NPV: Negative Predictive Value

(National Blood Centre, Malaysia) showed it as weak D phenotype (A290 donor) (Table 2).

Among the ABO discrepant cases, two cases of B were wrongly typed as AB. These discrepancies (sample no: A257, A596) could be due to omission or adding inadequate amount of the diluent, or delayed in adding the diluent (recommended time is < 2 minutes). As a result, red cells are attached or clotted or dried in the sample well and unable to be washed out by the diluent to give the correct blood group. Once the sample in the sample well is not washed out either due to inadequate amount of diluent or delay or failure to add in the diluent, the result will be falsely positive. It was noted that in this test kit, there was no positive or negative control indicator that can be used to ensure the operator that the diluent has been added or the amount of diluent added is adequate to justify the result of the test kit. These could be the reasons why there were 2 samples typed as blood group AB instead of B.

In this study, another 2 donors (A13 and A417) were wrongly typed as blood group A instead of blood group O by the test kit. It is possible that for these two cases, the false positive results of group A instead of group O could be explained by the similar reasons stated for the previous two discrepancies, or it could also be due to the specificity of the anti-A used to coat in the kit, which may not be really specific for the A antigen or too sensitive that it may detect antigen other than A antigen. It seems that the test kit has given false positive results on the A column namely for two donors with group O and for another two donors of group B, typed as AB. Therefore, it is suggested that the anti-A used for the test kit to be reviewed and validated further for improvement to prevent false positive reaction and to produce more specific reaction.

The last donor (A668) was wrongly typed as blood group A instead of blood group AB. It is difficult to explain the result for this finding as all the previous discrepancies showed false positive result for blood group A. However, for this donor, it is possible that the anti-B did not pick up the B antigens that were present on the surface of this red cell. This may be caused by the B antigens are weakly expressed or it is a subgroup B. However, the confirmation test did not show the reactions to suggest subgroup B. The other possible explanation for the false negative reaction could be due to the amount of blood sample used in the sample well was too little and the diluent added too much that washed away the red cells before any antigen-antibody reactions has occurred.

Besides the technical issues discussed above, wrongly typed ABO blood group could also be due to misinterpretation of blood group or clerical errors such as transcription error. Therefore, blood groups testing for donors in our centre are tested at least twice with at least one by standard methods such as glass tubes, microtiter plates and column agglutination, the results have to be checked and verified by two qualify verifiers to minimise these errors.

Although both the tile and solid phase screening methods have 100% specificity for ABO grouping, the tile method has a better sensitivity as compared to the solid phase method. There are few studies done earlier that comparing solid phase method with the agglutination method in determining ABO and RhD blood group which showed the correlation between this two techniques are between 99.6% to 100%.[5,8,10] Our findings revealed similar result with the previous studies. As we know, the tile method is a qualitative method where the positive reaction is shown as red cells agglutination. It is possible that this technique is more sensitive in view of the small agglutination present can easily be detected by naked eyes and be labelled as positive reaction. While for the solid phase method, the reaction does not show the red cell agglutination as the positive reaction but it only shows qualitative result as red colour for positive reaction or no colour as negative reaction and the mechanism of the reaction depends on many other factors such as the concentration of red cells, the amount of diluent used, the timing when the diluent is added and whether or not the diluent is added. Any missing steps in any part of the procedure, it may give rise to false positive or false negative results.

Besides, the cost evaluation was compared between both the screening methods and it was shown that the tile method is about three times cheaper than the kit method. This costing only looked at the reagent cost but not the man power and time taken to run the tests. The main limitation of the study is that the test of solid phase method (kit) was performed by one personnel only. As this method is totally manual and operator dependent, it should be performed by two independent personnel for every sample. However, in view of limited budget and staff shortage, it was only performed once for every sample.

In this study, the tile method has shown

better sensitivity and specificity in typing ABO blood grouping compared to the solid-phase method. At the same time, it is three times cheaper than the solid-phase method. Therefore, this method is more cost effective compared to solid-phase method as a screening test. The solid-phase method is technically much easier and convenient to perform, interpret, and the risk of contamination of blood sample is much less than that of the tile method and suitable for offsite use. However, its application needs further improvement before it can be considered as a good screening method for ABO and RhD grouping to replace the conventional tile method. This blood grouping kit may be further improved by adding an indicator to show the correctness of technique and thus improvement of the sensitivity and validity of the test results.

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REFERENCES

1. Dhruva G, Agravat A, Bhankhodia V. Comparison of conventional TUBE agglutination method versus ERYCARD™ 2.0 for the ABO blood grouping system-A Pilot Study. Int J Res Med. 2015; 4(1): 59-61.
2. Mitra R, Mishra N, Rath GP. Blood groups systems. Indian J Anaesth. 2014; 58(5): 524-28.
3. Bhagwat SN, Sharma JH, Jose J, Modi CJ. Comparison between conventional and automated techniques for blood grouping and crossmatching: Experience from a tertiary care centre. J Lab Physicians. 2015; 7(2): 96-102.
4. Mujahid A, Dickert FL .Blood group typing: From classical strategies to the application of synthetic antibodies generated by molecular imprinting. Sensors 2016; 16: 51
5. Sinor LT, Rachel JM, Beck ML, Bayer WL, Coenen WM, Plapp FV. Solid-phase ABO grouping and Rh typing. Transfusion. 1985; 25(1): 21-3.
6. Bajpai M, Kaur R, Gupta E. Automation in immunhematology. Asian J Transfus sci. 2012; 6: 140-4.
7. Rumsey DH, Ciesielski DJ. New protocols in serologic testing: A review of techniques to meet today's challenges. Immunohematology 2000; 16: 1317.
8. Ching E. Solid phase red cell adherence assay: A tubeless method for pretransfusion testing and other applications in transfusion science. Transfus Apher Sci 2012; 46: 287-91.
9. ABO blood grouping kit (Solid-phase method). Retrieve on 12/4/2017 from: http://1367459.en.makepolo.com/products/ABO-amp;-RhD-Blood-Grouping-Test-p79936665.html.
10. Uthemann, H., Prager, E.M, Sturmfels, L., Lenhard, V. A new solid phase method for ABO grouping, Rh phenotyping and Kell determination. Infus Ther Transfus Med. 1999; 26: 244-6.
11. Duguid JKM, Bromilow IM. New technology in hospital blood banking. J Clin Pathol. 1993; 46: 585-8.
12. Sosler SD, DeChristopher PJ. Discoveries and developments transforming blood banking and transfusion medicine—Part 1. Laboratory Medicine, 1995; 26(4): 245-251. Downloaded from https://academic.oup.com/labmed/articleabstract/26/4/245/2659382 on 02 March 2018.
13. Cooling L. ABO, H, and Lewis blood groups and structurally related antigens. In Robac JD, Grossman BJ, Harris T, Hillyer CD. Editors. *AABB Technical Manual.* 17th edition. Bethesda, Md, USA: AABB; 2011. 363-388p.