Malaysian J Pathol 2015; 37(2) : 109 – 114

ORIGINAL ARTICLE

Comparison of Abtectcell III and Diamed red cell antibody screening kit for detection of clinically significant red cells alloantibody

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Abstract

Antibody screening is important for the antenatal screening and pre-transfusion tests. This study aimed to compare the MUT/Mur kodecytesAbtectcell III (CSL Abtectcell III) red cell antibody screening kit with DiaMed ID-Dia Cell I-II-III Asia that was then used in our laboratory. In this study, 125 samples were randomly chosen, with 67 samples of known antibody specificities and 58 samples identified as negative for antibody, as the negative control. Concordant negative results were obtained in 57 out of 58 antibody negative samples. Concordant antibody positive results with both reagents were seen in 49 out of 67 samples. There were 18 discrepant results of antibody screening with CSL Abtetcell III (16/18 for vMNS antibodies). The sensitivity and specificity for CSL Abtectcell III were 73.0% and 98.3% respectively. In conclusion, the CSL Abtectcell III reagent would be an acceptable alternative for screening of red cell alloantibodies. It was able to detect all the clinically significant alloantibodies.

Keywords: blood groups, RBC antigens and antibodies, vMNS

INTRODUCTION

Antibody screening and identification of unexpected blood group antibodies is important in the immunohaematological investigations of patients during antenatal screening and pre-transfusion tests.

Difficulties are often encountered when designing suitable screening cells where knowledge of polymorphisms of blood group and the prevalence of antibody is not known or incomplete such as antibodies to variants MNS (vMNS). The detection of these antibodies is often problematic as current standard screening cells usually lack the antigens to detect these antibodies. This is particularly of concern in developing countries where almost all transfusion laboratories use commercially prepared screening cells rather than panels which are prepared from the local population. Current antibody screening reagents available for routine use have generally been manufactured in Western countries. They are less reliable in detecting antibodies common in Asian populations.

The use of current screening cells sourced from the local population is also a problem because not all antibodies detected are clinically significant and many antibodies detected are naturally occurring IgM antibodies that are unlikely to cause disease. IgG class antibodies against MUT, Vw, Mur, Hil and Mia antigens are clinically significant and capable of causing immediate and delayed Haemolytic Transfusion Reactions (HTRs) and mild to severe Haemolytic Disease of Fetus and Newborn (HDFN). It has been estimated that at least 89% of IgG clinically significant antibodies are detected against the Mia, MUT or Mur specificities.[1] These antibodies currently cannot be easily identified.

In Malaysia, the genetic heterogeneity among the multiethnic population has led to vast variation of antibody specificity. For example, antibodies to variants of MNS (vMNS) were the most common antibodies detected among patients in the UMMC.[2] A study in Taiwan also showed similar results where vMNS antibodies were the most frequent alloantibody detected.[3]

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With the emergence of new clinically significant red cell antibodies of the variant MNS system in the Asian population, there is a need for better screening cells with antigens which are specifically designed for antibody detection for our own local population. This will increase the detection rate of clinically significant antibodies and thus be able to identify suitable donor units for these patients.

At present, there are few options available. The first option is to include GP.Mur RBCs as part of a screening panel. However, antibody screening using GP.Mur RBCs could cause the problem of detecting IgM antibodies which are not clinically significant. The other option is to use genetically engineered screening cells which carry synthetic kodecytes (RBCs with peptide based antigens bearing either MUT and/or Mur antigens added using KODE Technology)[4] or other technologies such as peptide–enzyme-linked immunosorbent assay (ELISA) testing[1] which are able to detect clinically significant IgG antibodies of high titre.

The purpose of this study was to evaluate the novel MUT/Mur kodecytesAbtectcell III (0.8%) red cell reagent kit as a screening tool for antibody screening.

MATERIALS AND METHODS

Study design

This was a cross-sectional comparative study. It was carried out in the Blood Bank Unit, University Kebangsaan Malaysia Medical Centre (UKMMC). The facilities that were utilized included the currently used ID-DiaCell I-II-III Asia manufactured by BIO-RAD or previously known as Diamed ID Micro Typing System (ID-MTS) and the new Abtectcell III manufactured by Commonwealth Serum Laboratory (CSL), DiaMed ID-Card LISS/Coombs with polyspecific AHG serum microtube Column Agglutination Technology (CAT) System, gel card incubator and gel card centrifuge. The study duration was over one year and was approved by the Institutional Review Board/Ethics Committee.

Study populations

The study population was patients of UKMMC whose blood had been sent to Blood Bank UKMMC for pre-transfusion tests including Group Screen Hold (GSH) or Group Crossmatch (GXM). Samples were from clotted serum tubes or ethylenediamine tetra-acetic acid (EDTA) plasma blood collection tubes. The initial screening kit used was Diamed screening reagent, ID-DiaCell I-II-III Asia. A comparative study was then performed on 125 patient samples which were randomly chosen. There were two main study populations involved comprising 67 samples of known antibody specificities (including the clinically significant antibodies such as anti D, other Rh antibodies, anti S, Kidd and Duffy antibodies) and 58 samples identified as negative for antibody as negative control. Patients with positive direct antiglobulin test positive or known case of autoantibodies were excluded.

The antibody screening tests using the standard reagent RBCs 3-cell screening panels ID-Dia Cell I-II-III Asia and the new 3-cell screening panels Abtectcell III were performed simultaneously according to the manufacturers' instructions by indirect antiglubulin test (IAT) method, performed by gel agglutination technique using DiaMed LISS/Coombs polyspecific AHG gel card. Otherwise, we maintained constant other variables including the same gel card, gel card incubator and gel card centrifuge. The reactions were recorded and the patterns of reactions were compared with the 'Antigen Composition Sheet' provided by the manufacturer.

Statistical analysis

The estimated sensitivity and specificity of the new Abtectcell III RBCs reagent was calculated by standard formulae. These calculations were made using the results that were obtained with the 125 samples. All statistical analyses were performed using SPSS version 19 and Microsoft Office Excel 2007.

RESULTS

A total of 125 samples were analyzed. We obtained concordant negative results with the two reagent RBCs in 57 of 58 known antibody negative samples. However, there was 1 discrepant result in the antibody screening when using the two reagent RBCs due to technical error.

The majority of the samples for the antibody positive group in this study were single alloantibody (63 of 67 samples) and only four samples were of multiple antibodies. The vMNS antibodies were observed with the greatest frequency (21/67 or 31.3%) and anti-D and anti-E were the second most detected alloantibodies in this study with a frequency of (11/67 or 16.4%) each, followed by anti-M with a frequency of 5.9% (4/67). The antibodies within each of the 67 samples showed concordant positive results in

TABLE 1: Antibodies of known specificities and their reactivity (67 samples)

Specificity	Number (%) tested	Reactive with ID-DiaCell I-II-III Asia	Reactive with Abtectcell III
vMNS antibodies	21 (31.3%)	21	5
Anti-D	11 (16.4%)	11	11
Anti-E	11(16.4%)	11	11
Anti-M	4 (5.9%)	4	4
Anti-Le^a	4 (5.9%)	4	3
Anti-Le^b	2 (2.9%)	2	2
Anti-Jk^a	1 (1.4%)	1	1
Anti-Lu^a	2 (2.9%)	2	1
Anti-c	2 (2.9%)	2	2
Anti-Di^a	1 (1.4%)	1	1
Anti-Fy^b	1 (1.4%)	1	1
Anti-Jk^b	1 (1.4%)	1	1
Anti-N	1 (1.4%)	1	1
Anti-S	1 (1.4%)	1	1
Anti Le^a and Le^b	1 (1.4%)	1	1
Anti S and D	1 (1.4%)	1	1
Anti P1, Le^a and Le^b	1 (1.4%)	1	1
Anti Jk^b, Le^a and E	1 (1.4%)	1	1

antibody screening with the two reagent RBCs in 49 samples. However, we obtained 18 discrepant results in antibody screening in 16 samples for vMNS antibodies (16/18 or 88%), one anti-Lea (1/18 or 6%) and one anti-Lua (1/18 or 6%) (Table 1)

The sensitivity and specificity for CSL Abtectcell III reagent RBCs were 73.0% and 98.3% respectively (Table 2)

The antibody positive reaction strength of CSL Abtectcell III were analysed from the results of the population samples and a comparison was made with the current screening cell DiaMed ID-Dia Cell I-II-III Asia. Alloantibodies samples that showed a stronger reaction with CSL Abtectcell III included the common clinically significant antibodies (anti-E, anti-c), the less common clinically significant antibodies (anti-Jkb, anti-Fyb, anti Jka) and the naturally occurring or of low clinical significance antibodies (anti-M). However, the strength of reactions was weaker in the detection of anti-D. CSL Abtectcell III also showed negative reaction with most of the vMNS antibodies in comparison to DiaMed ID-Dia Cell I-II-III Asia, except in five cases. The two cases of false negative reactions were

TABLE 2: Diagnostic accuracy of reagent RBCs Abtectcell III

True positive	49
False positive	1
True negative	57
False negative	18
Sensitivity	73.0%
Specificity	98.3%

not included in this grading score.

Three samples of vMNS antibodies that were simultaneously received were frozen and sent to the Reference Laboratory CSL Limited in Melbourne, Australia for further antibody identification and antibody titration due to the variable reactions with CSL reagent Abtectcell III. In all three cases, the pre-transfusion testing here showed positive antibody screening with DiaMed ID Dia Cell I-II-III Asia (Cell III only which was labelled as Mia). However, the DiaMed ID Dia Panels for antibody identification were negative. DAT and autocontrol were also negative in all the three cases. Antibody screening with CSL Abtectcell III were negative for Case 1 and 2 but Case 3 showed a strong positive reaction with the MUT/Mur screening cells of the CSL Actectcell III. Further antibody identification done with extended panel of CSL Phenocell showed no reaction with Case 1. Case 2 showed weak reaction to some cells suggestive of a low incidence antibody whereas Case 3 showed a strong positive reaction on the MUT positive cell but no reaction on the Mur positive cell.

In the Australian testing, Case 1 and 2 reacted with a GP.Mur positive cell and both cases had weak antibodies with vMNS specificity. Both cases on repeat testing were non-reactive with the CSL Phenocell identification panel that has a MUT/Mur positive cell. Further testing showed Case 1 had IgG only whereas Case 2 had IgG and a component of IgM. The IgG titre was four (1:4 dilution) in both cases. Case 3 demonstrated IgG activity and was further identified as having anti-MUT specificity.

DISCUSSION

In this study, we demonstrated that we were able to detect most clinically significant alloantibodies with both reagents RBCs. The lower sensitivity of CSL Abtectcell III here was mainly due to discrepancies in the detection of vMNS antibodies. It is acknowledged in BCSH guidelines that no technique can detect all red cell antibodies.[5] Schoenfeld *et al.* 2009 also found that when comparing a new routine immunohaematology system, a suitable sensitivity is 83.3% and specificity of 92.8%.[6] However, comparison between studies is often difficult because of the variation in the methodology employed and the variable antigen compositions of the screening cells.[2,7,8]

A few technical issues should be considered when comparing these reactions. First, the characteristic of both red cell reagents were of different properties. ID-DiaCell I-II-III Asia is based on naturally-occurring phenotype positive cells and this could cause problem, as not all antibodies detected are clinically significant (many antibodies detected are naturally occurring IgM antibodies that are unlikely to cause disease). IgM antibodies reactive with GP.Mur red cells are more frequent than IgG antibodies and are likely to be clinically insignificant and naturally occurring.[9] Therefore, some of the antibodies to vMNS identified using the "Mia" DiaMed ID-Dia Cell I-II-III Asia screening red cells could well be IgM only antibodies. On the contrary, the vMNS antibodies identified using CSL Abtectcell III are more likely to be clinically significant IgG antibodies and not of IgM antibodies.

There is no current published data comparing the detection of red cell alloantibodies between ID-DiaCell I-II-III Asia and Abtectcell III red cell reagent kits. In our study, we performed Kappa agreement to determine the strength of agreement between Abtectcell III and the current standard method which found a good agreement between the two reagent red cells. The strength of agreement in Abtectcell III was considered good with a Kappa value of 0.69. This study showed that there was not much difference in the strength of positive antibodies reaction in the two reagent RBCs. However, the strength of reactions was weaker in the detection of anti-D.

CSL Abtectcell III also showed negative reaction with most of the vMNS antibodies in comparison to DiaMed ID-Dia Cell I-II-III Asia, except in 5 cases and this accounted for most of the discrepant results among antibody positive sample. These may be due to CSL Abtectcell III reagent RBCs selectively reacting with vMNS antibodies which are more likely IgG antibodies compared to DiaMed ID-Dia Cell I-II-III Asia which used GP.Mur phenotype natural screening cells that can detect vMNS antibodies of both IgM and IgG type. The other antibodies which were negative with Abtectcell III were anti-Lea and anti-Lua.

We obtained discrepant results in the antibody screening for known samples of anti-Lea with the two reagent RBCs which only reacted with ID-DiaCell I-II-III Asia reagent RBCs but not with Abtectcell III reagent RBCs. However, we detected anti-Lea when we used the antibody identification panel. There could be several explanations for this discrepancy. Firstly, the Abtectcell III reagent RBCs may show some loss of antigen reactivity during storage. It is

possible that Leb, Lea and P antigens could deteriorate during storage (insert Abtectcell III). However, the Abtectcell III reagent RBCs used for the study was still fresh and this could not explain for the discrepant result.

A retrospective study by Nadarajan *et al*. 2011 found that there was a significant difference in the detection of Lewis antibodies when using standard screening cells and MUT+Mur KODE transformed cells (kodecytes). There is no current published data about the sensitivities of KODE modified screening cells for Lewis antibodies. However, it is possible that Lewis antigen on the red cells could be disrupted during the insertion of synthetic peptides into the cells.[2] A lower sensitivity of Abtectcell III for antibodies to Lewis might be considered an advantage, because in general, antibodies against Lewis antigens are of IgM isotype and are naturally occurring, therefore not considered clinically significant.

In this study, there was one example of anti-Lua that reacted only with ID-DiaCell I-II-III Asia reagent RBCs but not with Abtectcell III reagent RBCs. This was because that particular batch of Abtectcell III reagent RBCs did not have Lua antigen represented on the Abtectcell III screening cells and consequently, anti-Lua was not able to be detected with that reagent RBCs. Lutheran antibodies can cause mild haemolytic transfusion reaction but they are generally considered to be clinically insignificant.[10]

Antibodies to vMNS were the most commonly detected antibodies in our study and yet not all vMNS antibodies detected by ID-DiaCell I-II-III Asia reagent RBCs reacted with the new reagent RBCs Abtectcell III. In our study, only five of 21 cases (24%) of vMNS antibodies showed positive reaction with CSL Abtectcell III. In fact, vMNS antibodies accounted for 88% of discrepancies (16 of 18 cases of vMNS antibodies) in the detection of antibodies. Therefore, there were significant differences in antibody detection rates when the two screening cells were tested in parallel. This can be explained by the fact that CSL Abtectcell III screening cells are created in such a way that it will selectively detect IgG class antibodies of MUT, Mur and Mia antibodies without interference of IgM antibodies.

CSL Abtectcell III screening cells has the advantage of detecting these clinically significant IgGvMNS antibodies as shown by the three cases which were sent to the Reference Laboratory in Australia. The first two cases did not react with CSL Abtectcell III due to low titre antibody (IgG of 1 in 4 dilutions) but the third case reacted strongly with CSL Abtectcell III due to presence of strong IgG anti-MUT which is considered clinically significant antibodies. Considering that vMNS antibodies have been reported to be responsible for HTR and HDFN, it is therefore important that antibody screening is capable of identifying them. In our centre, an indirect antiglobulin phase crossmatch is obligatory and that has prevented possible adverse transfusion events in which case, the antibody screening is negative but the antiglobulin phase crossmatch is incompatible.

Anti D was the second most detected antibodies in our study and the majority of the patients were female (10 of 11). Most of the anti-D detected were secondary to passive immunization by anti D prophylaxis. Only two patients were detected to have allo anti-D. We have not seen many cases of allo anti-D in pregnancy as a result of routine administration of anti-D immunoglobulin prophylaxis.

There were 58 antibody negative cases in the study, and concordance results of 98.3% (57 of 58 cases) were documented with the antibody screening using both reagent red cells ID-DiaCell I-II-III Asia and Abtectcell III. The only discrepant false positive result when tested with the new reagent Abtectcell III could be due to a technical error as the repeat antibody screening and antibody identification were both negative.

In summary, from this study, (1) both reagents were able to detect all the clinically significant alloantibodies, (2) Diamed reagents could also detected most of the clinically insignificant alloantibodies, (3) CSL reagents did not pick up most of the clinically insignificant alloantibodies, (4) although CSL reagent was able to detect anti D, the strength of the reaction however was weaker as compared to the Diamed reagent.

In conclusion, the new Abtectcell III reagent RBCs is an acceptable alternative for screening of red cell alloantibodies. It is up to each laboratory to assess the system available taking into consideration matters such as sensitivity, specificity, cost-effectiveness or what better meet the user's need.

ACKNOWLEDGEMENTS

This project is funded by National University of Malaysia Research University Grant (FF-189-2010).

SMSSA, NHH, NAM, and LCF formulated the hypothesis, SMSSA carried out experiments

assisted by NAM. SMSSA, NHH, NAM, and LCF analyzed data. NHH, SMSSA were involved in writing the paper and all authors had approved the manuscript to be submitted.

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