Performance Evaluations

MATRIX GEL SYSTEM
Gel card system for Blood Banking Applications
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<td>5.</td>
<td>Indian Society for Blood transfusion and Immunohematology, Transcon 2015</td>
<td>066</td>
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<td>6.</td>
<td>Asian Journal of Transfusion Medicine, Vol 12, Issue1, January- June 2018</td>
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<td>7.</td>
<td>Journal of Evolution of Medical and Dental Science, Vol.4, Issue 73, Sep 2015</td>
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# EXTERNAL EVALUATIONS

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<td>8.</td>
<td>Institute of Transfusion Medicine and Immunohematology, German Red Cross Center, Frankfurt Evaluation of Matrix AHG (Coombs) Test Card for antibody detection (screening) and identification.</td>
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<td>Institute of Transfusion Medicine and Immunohematology, German Red Cross Center, Frankfurt Evaluation of Matrix AHG (Coombs) Test Card for autocontrols.</td>
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Evaluation of methodology and comparative study between Spin saline tube and matrix gel card techniques for blood...

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RESEARCH ARTICLE

EVALUATION OF METHODOLOGY AND COMPARATIVE STUDY BETWEEN SPIN SALINE TUBE AND MATRIX GEL CARD TECHNIQUES FOR BLOOD COMPATIBILITY

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Key words:
Spin saline tube method, AHG, LISS, Matrix Gel card technique.

ABSTRACT

Introduction: A study on Evaluation of methodology and comparative study between Spin saline tube using without AHG, with AHG and Gel card technique for blood cross- matching on the basis of efficacy, sensitivity and specificity was undertaken on approximately 500 samples processed in Blood Bank of U.P. University of Medical Sciences, Hospital, Saifai, Etawah, India.

Material and Methods: Most commonly Spin saline tube method are used widely in blood banks. A new technique of cross matching is introduced as AHG gel card. In this study we used Matrix gel card method based on indirect coombs test (ICT) for cross match and tube method including Spin saline tube method with AHG and without AHG.

Result: five hundred samples are taken for the study and out of this 490 samples are compatible using Spin saline tube method without coombs reagent, 10 sample shows incompatibility, whereas in Spin saline tube method by using coombs reagent shows 99.2% compatibility, 06 samples show false positive and 04 samples show true positive of previously result. As per findings specificity and sensitivity is 100% of gel card and tube test using AHG, whereas Spin saline tube test specificity is 98.8 %. Spin saline tube method at room temperature, shows 98% compatibility due to 06- sample false positive and 04 sample true positive, whereas Spin saline tube with coombs reagent at 370C, shows 99.2% compatibility due to 496 sample were found compatible and 04 sample true positive. In matrix gel card also shows 99.2% compatibility.

Conclusion: The usage of Matrix Gel card in Blood Bank for cross match is easy to performed with recorded test result and more sensitive and specific then Spin saline tube method whereas indirect coombs tube method is also sensitive and specific but more time consuming as compare to Gel card but cannot recorded result and more time consuming than Spin saline and gel card method.

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INTRODUCTION

This study based on the recorded data analysis, was done in blood bank of U.P. University of Medical Sciences, Hospital, Saifai, Etawah (U.P.) with the presence and supervision of Pathologist. Matrix Gel Card a newly introduced technique for blood cross matching. The Spin saline tube method is used previously for blood cross match which is mainly Spin saline tube method (Spin saline tube method at RT) and indirect coombs tube method. Matrix gel card technique is introduced by Lapiere, which was based on controlled centrifugation of red blood cells in sephadex gel contained within microtube gel techniques (Lapiere, 1988; Letichet al., 1993).

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It also used for various test such as ABO and Rh typing, identification of alloantibodies, indirect and direct coombs test (ICT & DCT) (Mollison, 1993; Lapiere and Rigal, 1990). This study is also carried out for evaluation of methodology and comparative study between Spin saline tube using without AHG, with AHG and matrix gel card technique used for blood cross- matching on the basis of efficacy, sensitivity and specificity.

Aims and objectives of study

Evaluation of methodology and comparative study between Spin saline tube using without AHG, with AHG and matrix gel card technique for blood cross- matching on the basis of efficacy, sensitivity and specificity.
MATERIALS AND METHODS

The study based on data analysis total 500 sample randomly selected from day wise round the clock duty wise stored sample of requisition for cross matching blood in blood bank of UPUMS, Hospital, Saifai, Etawah. Most commonly Spin saline tube method are used widely in blood banks. A new technique of cross matching is introduced as AHG gel card. In this study we used Matrix gel card method based on ICT for cross matching and tube method including Spin saline tube method and indirect coombs test tube method used. Sample comes from ward with issuing form, the collection of blood from healthy donors who have >45 kg of weight with negative all serology of HIV, HbsAg, HCV, VDRL and Malaria. In this study first we follow the steps: Blood grouping of patient blood and donor blood from pilot tube by the help of antisera A, B, D. After matched of blood group we proceed to perform cross matching of blood of both donor and patient’s blood by three methods.

Spin saline tube method without AHG and with AHG reagent, other third method is Matrix Gel card method which is recently introduced in blood bank. Centrifuge the both blood samples and extract the serum and red cells from patient and donors samples, prepared cell suspension of the donor’s red cells and patient’s red cells. The method which is apply in Spin saline tube method, marking tubes as major and minor with marker, in major tube we mixing of patient’s serum and red cells of donor, in minor tube serum of donor and red cell from patient sample. After that we add the AHG reagent in spin saline tube method and kept it incubate for one hour at 37°C and then centrifuge the both tubes, see the result if clumping or agglutination or hemolysis present in both test tubes, blood bag is incompatible for patient. If clumping or agglutination not present blood is compatible for patient (Coombs, 1945 & 1946). In Gel Card technique we used Matrix Gel Card incorporated with AHG reagent (each plastic card containing six microtubes), incubator (cartridge warmer), Card centrifuge for centrifuge of Gel Card, Diluent-2 LISS, test tubes and micropipette. Firstly we prepared a 0.8% red cell suspension by adding 10µl of packed red cells of donor in 1ml Diluent-2 LISS in to clean test tube by micro pipette, after that we take a Matrix gel card, open the foil of one microtube gently and write the patient details, ID number at below part of microtube, add 50µl of 0.8% donor red cell suspension, after this add 25µl patient serum in same microtube by proper way. Incubate the Matrix gel card in card incubator (cartridge warmer) for 15 minutes at 37°C. After incubation centrifuge the Matrix gel card in card centrifuge machine for 10 minutes at preset/ 950 rpm, at the end of centrifuge read the result. If gel card shows RBCs are settled bottom of particular microtube means No agglutination (Negative result) that means Donors blood is compatible to the recipient and suitable for transfusion to patient. If RBCs are trapped or floated between upper and bottom of tube that means something is wrong and result are called Positive result and incompatible for recipient. Positive result shows grading in to 1+ to 4+. (1+ means near to bottom of micro tube and 4+ means top of micro tube). In case of 4+ reaction, indicated if a solid band of red blood cells (RBCs) on top of the gel card’s microtube, 3+ reaction displays if agglutination of RBCs in the upper half, 2+ reaction is indicated by RBCs agglutinate dispersed throughout the microtube, while a 1+ reaction shows if RBCs aggregate in mainly lower half part of the microtube with dotted structure in column.

RESULTS

Total 500 random blood sample cross matched by using Spin saline tube method with and without using AHG and Matrix Gel Card. Result are observed in Spin saline tube method without AHG, 500 sample shows 98% compatibility but in 06 sample (1.2%) shows false positive (FP) and four sample shows true positive (TP), if we Add AHG (IAT) calculated after compare the result of Spin saline tube method with AHG and Matrix Gel card method which shows 496 (99.2%) sample compatible and 04 sample (0.8%) True Positive (TP) found in observation, incompatibility of 06 samples (FP) disappear after incubation with AHG reagent at 37°C.

Fig. 1. Compatibility shows by Matrix Gel Card Technique

In table:1, five hundred samples are taken for the study and out of this 490 samples are compatible using Spin saline tube method without coombs reagent, 10 sample shows incompatibility, whereas in Spin saline method by using coombs reagent shows 99.2% compatibility, 06 sample shows false positive and 04 sample shows true positive of previously result. Sensitivity and specificity is 100% of gel card and indirect coombs tube test using AHG, whereas Spin saline tube test specificity is 98.8 %. Spin saline tube method at room temperature, shows 98% compatibility due to 06- sample false
positive and 04 sample true positive, whereas Spin saline tube with coombs reagent at 37°C, shows 99.2% compatibility due to 496 sample were found compatible and 04 sample true positive. In matrix gel card also shows 99.2% compatibility.

**DISCUSSION**

Matrix gel card technique recently introduced for blood cross-matching and ABO & Rh Blood Grouping system in India and other country. The matrix gel card test performed in various institutions and hospitals for blood cross match, matrix gel card have six microtube embedded in a plastic card (Malyska, 1994). The advantages of matrix gel card as easy reading of microtube, easily recording for a long time, handling and disposal (Malyska, 1994). In this study 0.8% sample out of 500 sample shows incompatibility (agglutination) by gel card method and also spin saline tube method using AHG. Whereas Spin saline tube method without AHG shows 98% compatibility which is not correct because 06 sample shows False Positive if we subjected to AHG. The specificity and sensitivity is 100% of both gel card and Spin saline tube method with AHG, whereas specificity of Spin saline tube without AHG is 99.2%. Matrix gel card method is better than Spin saline tube method because of its simplicity, stability of results, better handling, long time recorded, dispensation of controls with comparable sensitivity and specificity which is follow with this study (Colet et al., 2008). Matrix gel test at least assensitive as an LISS AHG tube test with a better balance of both sensitivity and specificity in blood cross-matching (Rumsey and Ciesielski, 2000). The number of non-specific antibodies and false-positive screens of results were reduced using the matrix gel test system. In antibody titers performed using the gel system were more sensitive than without AHG tube method (Bromilow, 1992). The matrix gel system was easy to use and provide reproducible and reliable results. The results of my study obtained with tube AHG same as matrix gel card method. The result shows that gel test is more sensitive than tube test for identifying clinically potentially significant of antibodies. Matrix gel card method is better than Spin saline tube method at least 99.2% compatibility. Matrix gel card also has less time consuming than tube method with AHG reagent but cost effective method. We recommended that the usage of Matrix Gel card for routine blood cross-matching, blood grouping (forward and reverse) in all blood bank.

**Acknowledgement**

We are highly thankful especially to all faculty, technical and supportive staff of department of transfusion medicine (Blood Bank), UPUMS, Saifai, Etawah.

**REFERENCES**


A comparative study of blood cross match using newly introduced gel technique and conventional tube Method

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2- Associate Professor,Department of pathology,SS.Medical college, Rewa
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Abstract:

Introduction: A comparative study of blood cross match between gel card technique and conventional tube method was undertaken on approx 1000 sample conducted in Sanjay Gandhi Memorial Hospital , Rewa associated with Shyam shah medical college Rewa.

Material & Methods: most commonly conventional tube method are used widly. Now new technique of cross matching is introduced . In this study Matrix gel card[16] method based on indirect coombs test for cross match and conventional tube method including saline tube method and indirect coombs tube method used.

Observation and result: one thousand sample is taken for the study and out of this 996 sample is compatible using indirect coombs gel card and indirect coombs tube test and 04 sample shows incompatibility of both test ,whereas in saline method without using coombs reagent shows 100% compatibility, if we use coombs indirect test, 04 sample shows false positive and 04 sample shows false negative of previously 100% compatible result. Sensitivity and specificity is 100% of gel card and indirect coombs tube test using AHG, whereas saline tube test specificity is 99.6% .And positive predictive value 100% for Gel card and indirect coombs test[5][6] using AHG and 99.6%for saline tube method if AHG not used.

Conclusion: Gel card used in blood cross match is easy to performed and recorded test and more sensitive and specific than convention saline method whereas indirect coombs tube test is sensitive and specific as Gel card but cannot record and more time consuming than saline and gel card method.

Keywords: Gel technique ; conventional tube methods

Introduction:

the current study was done in blood bank of Sanjay Gandhi memorial hospital ,Rewa, (M.P) , newly introduced a technique called Matrix Gel Card technique for blood cross matching. Previously conventional tube methods are used for blood cross match which is mainly saline tube method(Spin tube method) and indirect coombs tube method . gel technique is introduced by Lapierre, which based on controlled centrifugation of red cells though sephadex gel contained within microtube.gel techniques also used for various test like ABO and Rh typing, ide-ntification of all antibo-dies , indirect and direct antiglobulin test (IAT&DAT)Present study is carried out for comparision between gel card and conventional tube test for sensitivity and specificity , time and cost efficieny .

Aims and objectives:

Comparative study between conventional cross match and matrix gel card technique on the basis of sensitivity and specificity.

Material and methods:

1000 randomly sample selected and collected from donors attending blood bank of Sanjay Gandhi memorial Hospital ,Rewa .Donors are healthy and >47 kg of weight with negative serology of HIV,HCV, HbsAg, VDLR and
Malaria. In present study first we done the blood grouping by using Antisera A,B,D of patient blood and donors blood bag. After matching of blood group we proceeds to cross matching of the donor and recipient blood by using two methods first is Conventional tube method with AHG (IAT) and without it. Second method is Matrix Gel Card method which is newly introduced in our blood bank. Method which is apply in Conventional tube method first marking the patient and donor test tube with marker, centrifuge the both blood sample and extract the serum of patient and donor red cells, mixing of serum of patient and red cells of donor in clean test tube and after this we add the Anti Human globulin (AHG, Coombs Reagent[5][6]) and incubate in 37°C and then see the result if clumping present in test tube blood bag is incompatible. if not present blood is compatible for patient. Second method is Matrix Gel Card method in this method special machine used for centrifuge of Gel Card and also incubator for Gel card, LISS, test tubes and micropipette first we clean and ready for conducting the test Gel card technique; first prepare a 0.8% red cell suspension by adding 1ml diluents-2 in to clear test tube then add by micro pipette 10μl of packed red cells of donor to it. after this take a Matrix gel card open the foil of one micro tube gently and write the patient id no. below particular micro tube then add 50μl of 0.8 donor red cell suspension to it after this add 25μl patient serum to it. Incubate the gel card in Matrix gel card incubator for 15 minutes at 37°C. After incubation centrifuge the card in Matrix gel card centrifuge machine for 10 minutes and then read the result. If gel card result shows RBCs are settled bottom of particular micro tube means No agglutination (Negative result) that means Donors blood is compatible to the recipient and suitable for trans-fusion. If RBCs are trapped between upper and bottom of tube that means something is wrong and result are called Positive result, incompatible for recipient. Positive result are grade in to +4 to +1. (+4 means top of micro tube and +1 near to bottom of micro tube).

Observation and result:

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<tr>
<td></td>
<td></td>
<td>TN</td>
<td>FP</td>
</tr>
<tr>
<td>01 Conventional tube method without AHG</td>
<td>1000</td>
<td>992</td>
<td>04*</td>
</tr>
<tr>
<td>02 Conventional tube method with AHG(IAT)</td>
<td>1000</td>
<td>996</td>
<td>0</td>
</tr>
<tr>
<td>03 Matrix Gel Card</td>
<td>1000</td>
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</table>

*,** Result obtained only if AHG used with Conventional method otherwise it shows 100% compatible result. Table no. 01 shows 1000 random blood sample is cross matched by using conventional tube method with and without using AHG(IAT) and Matrix Gel Card. Result are observed in conventional tube method without AHG, 1000 sample shows 100% compatibility but in the table 04 sample shows false positive(FP) and 04 sample shows False Negative(FN) if we Add AHG (IAT) this is calculated after compare the result of conventional tube method with AHG and Matrix Gel card method which shows 996(99.6) sample compatible and 04 (0.4%) True Positive(TP)
Observation and result plotted in graph

**Discussion:**

In India and other countries, the gel test performed in various institutions and hospitals for blood cross-match is first introduced by Lapierre et al.[11] He gives the idea of six micro tube embedded in plastic cards. Micro-tubes filled with specific gel medium which allows testing, easy reading, recording, handling, and disposal.

In our present study, 0.4% sample shows incompatibility (agglutination) by gel card method and also conventional tube method using IAT(AHG). Where as conventional tube method (Spin tube) without IAT shows 100% compatibility which is not correct because 04 sample shows False Negative and 04 sample shows False Positive if we subjected to IAT. The specificity and sensitivity is 100% of both gel card and conventional tube method with IAT(AHG), where as specificity of conventional tube (Spin tube) without IAT is 99.6%. Swarup et al.[15] concluded that gel card method is better than conventional spin tube method because of its simplicity, stability of results, dispensation of controls, absence of wash phase with comparable sensitivity and specificity, which is agreement with this study. Rumsey DH et al.[18] proposed that the gel test at least as sensitive as an LISS IAT tube test with a better balance of sensitivity and specificity. Bromilow et al.[2][3] proposed that the number of non-specific antibodies and false-positive screens were reduced using the gel test system. Antibody titers performed using the gel system were more sensitive than with our tube IAT method. The gel system was easy to use and gave reliable, reproducible results. My study agree-ment with result but my result obtained with tube IAT same as gel card method. Noveretti MCZ et al.[14] result shows that gel test is more sensitive than tube test for identifying potentially clinically significant antibodies. Cat et al.[4] testing efficiency was improved following introduction of the gel test into routine use.

Kaur et al.[8] study shows that DiaMed gel card system easy to use and his finding suggest it proved to be more sensitive than the conventional tube agglutination technique. Nathlang et al.[17] study proposed that the gel test equal or better than conventional test tube method and simple to performed and less exposure of blood bank person
to blood specially area with HIV infection is prevalent. My study agree with above both prior study and its result. Jai prakash et al [7] concluded that gel test is better alternative to the conventional tube test for both DAT and IAT. Overall, prior study which is mention above are correlate with my present study with maximum findings.

Conclusion

Gel card is more sensitive and more specific than conventional tube methods and also less time consuming but more costly than conventional tube methods. As per result we concluded and advice for use of gel card in various blood banking services as routine test in cross matching for prior blood transfusion because of high sensitivity and specificity than conventional tube methods.

References:

DETECTION AND IDENTIFICATION OF RED CELL ALLOANTIBODIES IN MULTIPLY TRANSFUSED THALASSEMIA MAJOR PATIENTS

Jain RJ, Jain P, Choudhary NC and Mahadik VK
1C.R.G. Hospital and R. D. Gardi Medical College, Ujjain, India 2Fortis Hospital, Gurgaon, India

BACKGROUND:
Lifelong red blood transfusion remains the main treatment for β-thalassemia major patients. Transfusion therapy could be complicated with the development of anti RBC antibodies (alloantibodies and/or autoantibodies). Some alloantibodies are haemolytic and may cause haemolytic transfusion reactions and limit the availability of further safe transfusion. Alloimmunization to red cells antigens is one of the most important immunological transfusion reaction and causes delayed type of transfusion reaction.

AIM AND OBJECTIVE:
(i) To provide frequency and distribution pattern of various types of irregular red cell alloantibodies in patients with thalassemia major. (ii) To determine the mean red cell transfusion requirement and mean transfusion duration.

METHOD:
A prospective study was conducted from January 2014 to December 2014 at Transfusion Medicine and Blood Bank Dept. Seventy eight diagnosed thalassemia major patients were included in this study and samples collected and investigated for the development of alloantibody to red cell antigens by using Matrix Gel System (Tulip Diagnostics). Five to seven ml of blood was collected in plain tube serum was separated. Separated serum was taken in two aliquots, labelled properly and stored in two different boxes at -30°C in deep freezer, till the antibody screening and identification was performed. Tests for antibody screening and identification were performed on preserved sample to investigate prevalence of red cell alloimmunization by standardized laboratory techniques by the same person. Antibody screening was carried out on serum employing commercial three-cell panel (Matrix Gel System, Tulip Diagnostics) using standardized blood bank techniques. If patients were found to have irregular red cell alloantibody then the antibody identification was performed using commercial 11 cell panel cells (Matrix Gel System, Tulip Diagnostics).

RESULTS:
A total of 78 patients were included in the study. Forty eight patients were males and thirty females. Mean age was 8.2 years. Irregular red cell antibodies were found in 6 patients (7.69%). Mean age of patients who developed red cell alloantibodies was 12.48 years. Three patients developed single antibodies (50%) (2 patients anti-K and 1 patient anti-C), while other three developed multiple antibodies (50%) (anti-D and anti-E, anti-D and anti-C, anti-E and anti-K).

CONCLUSIONS:
Red cell alloimmunisation should be kept in mind in the patients receiving multiple transfusions. In present study, alloimmunisation rate was 7.69%. Mean transfusion duration in these patients was 21.80 days, probably due to the presence of alloantibody. We also suggest that red cells alloimmunisaton should not be overlooked in patients receiving regular blood transfusion. RBC alloantibody detection on regular interval and antibody negative blood transfusion is strongly recommended in transfusion depended thalassemia patients.
DETERMINATION OF THE MEAN RED CELL TRANSFUSION REQUIREMENT COMPARED ON THE BASIS OF IRON OVERLOAD AND TYPE OF CHELATION THERAPY AND DEVELOPMENT OF ALLOANTIBODIES IN MULTIPLY TRANSFUSED THALASSEMIA MAJOR PATIENTS

Jain PJ, Jain RJ, Choudhary NC and Mahadik VK
1 C.R.G. Hospital and R. D. Gardi Medical College, Ujjain, India
2 Fortis Hospital, Gurgaon, India

BACKGROUND:
Transfusion-dependant thalassemia patients, in the absence of chelation therapy, develop progressive accumulation of iron, which is responsible for tissue damage, and eventually, death. The factors which influence the iron burden are chelation therapy and mean red cell transfusion requirement. Increasing red cell transfusion requirement, iron deposit and development of alloantibodies complicate transfusion therapy in thalassemia patients.

AIM AND OBJECTIVE:
(i) To investigate the patient for the red cell transfusion requirement on the basis of iron overload and type of chelation therapy. (ii) To determine rate of development of red cell alloantibodies in thalassemia major patients.

METHOD:
A prospective study was conducted from February 2013 to December 2014. Ninety eight patients were included in this study and 3 consecutive samples collected after every 6 months and investigation for red cell requirement, compared on the basis of iron overload and type of chelation therapy. Iron overload was measured by serum ferritin levels.

RESULTS AND OBSERVATIONS:
In present study, mean red cell transfusion requirement was 206.20ml/kg/year (SD = 28.62) majority of the children in this study i.e. 40 (41.67%) were undergoing hypertransfusion therapy and transfused red cells were in the range of 208-248 ml/kg annually. It was observed that the requirement of red cell transfusion increases with the age of the patient. Out of 96 patients, 86 (89.58%) of thalassemia children were on chelation therapy. Maximum number of patients 37 (38.54%) were on oral chelation therapy and after this 35 (36.45%) of patients on combined chelation therapy. (Desferroxamine & oral chelation). Only 14 (14.58%) were on parenteral (desferroxamine) chelation therapy out of 96 patients, 10 (10.41%) patients were not taking any chelation therapy. In present study the difference of mean red cell transfusion requirement among all the chelation therapy groups when compared with each other were found highly significant (P<0.01). The mean red cell transfusion requirement were minimum in combination therapy group (combination of two iron chelators such as parenteral desferroxamine plus oral deferiprone) followed by parenteral desferroxamine chelation therapy group, oral chelation therapy group and maximum in patients those started chelation therapy but discontinued. Irregular red cell alloantibodies were found in 8 patients (8.16%). Five patients developed single antibodies, while other three patients developed multiple antibodies. (Matrix Gel, Tulip Group).

CONCLUSION:
Red cell transfusion requirement and chelation therapy should be kept in mind in the patients receiving multiple transfusions. In present study the difference of mean red cell transfusion requirement among all the chelation therapy groups when compared with each other were found highly significant (P<0.01). The mean red cell transfusion requirement was minimum in combination therapy group (combination of two iron chelators such as parenteral desferroxamine plus oral deferiprone) and maximum in patients who started chelation therapy but discontinued it and this difference was found highly significant (P<0.01). Combination of two iron chelators (such as parenteral desferroxamine plus oral deferiprone) have been shown to produce addictive and synergistic effects, may produce enhanced iron excretion, minimize side effect, decrease mean red cell transfusion requirement and improve compliance is strongly recommended in transfusion dependant thalassemia patients.
SCREEN INDIA WITH INDIAN CELL PANELS

Dr. C. Dhinesh Kumar, Dheeraj Joshi, K. Muthamil Selvi

TITLE:
Comparative evaluation of Gel Column Agglutination Technology Systems and commercial reagent red cell panel manufactured in India for antibody screening.

BACKGROUND:
The screening cells presently available and used for screening alloantibodies, are of Caucasian origin. Availability, cost and Ab indigenous to Indian groups can be missed with the Caucasian cells. Screening cells from local ethnic groups would be advantageous and increases the probability of detecting antibodies against antigens in local population.

AIMS/OBJECTIVE:
This study was to evaluate and compare the performance of CAT Systems and their antibody screening cells from
1. Indian population (Tulip)
2. Non-Indian origin cells

MATERIAL & METHODS:
In this comparative evaluation, CAT Systems and respective antibody screening cell panels from two different population were included. Matrix Gel System and Matrix ERYGEN-AS from India- Tulip Diagnostics and CAT System and cell panels manufactured from Non-Indian origin were used. Total 306 patient’s samples from multiple transfused patients, Anaemic, different pre-operative profiles and antenatal cases etc. from a Tertiary care hospital were tested for antibody screening. In positive samples antibodies were further identified for specificity.

OBSERVATION:
Out of 306 samples tested 3 patients (0.98%) were reported positive in both the systems i.e Matrix Gel System of Indian origin and by another CAT System of Non-Indian origin. The cost per test was comparatively cheaper in Matrix- Gel System.

CONCLUSION:
The study concludes that both CAT Systems are equally Sensitive and Specific. Matrix Gel System and Matrix ERYGEN-AS being manufactured in India by using cells from Indian population will give equal efficacy in detecting antibodies against antigens in local population and will be cost-effective.

KEYWORDS:
Antibody Screening, Indian Population.
An approach to incompatible cross-matched red cells: Our experience in a major regional blood transfusion center at Kolkata, Eastern India

Prasun Bhattacharya, Eeshita Samanta, Nowroz Afroza, Archana Naik, Rathindranath Biswas

Abstract:

INTRODUCTION: With the increased utilization of immunohematology (IH) analyzers in the transfusion medicine, type, and screen policy is the method of choice. Still, the importance of routine crossmatching could not be overruled. Here, we tried to understand the clinical conditions and safety of red cell transfusion and their outcomes.

MATERIALS AND METHODS: This prospective study was conducted by IH laboratory, Medical College Kolkata, Blood Bank from October 1, 2015 to March 31, 2016. A set of 3cc ethylenediaminetetraacetic acid and clotted blood samples of the patients were received according to sample acceptance criteria. Blood grouping by conventional tube technique followed by crossmatching was performed by column agglutination technology (CAT) in polyspecific (IgG + C3d) gel media. Any positive result was rechecked in duplicate with additional two group-specific donor units. The persistent incompatibility was further evaluated using direct anti-human globulin test, auto control, antibody screening, and antibody identification by CAT.

RESULTS: On the evaluation of 14,387 sets of patients’ sample, only 100 were found to be incompatible (0.69%). Incompatibility rate is higher in females (59%). Eighty-five of these patients were repeatedly transfused. Only 38% of incompatible crossmatch were positive on indirect anti-human globulin test/antibody screening. Antibody could be identified in 16 of them. Seventeen of 100 incompatible samples (17%) presented with panagglutination, were managed with Rh, Kell phenotype/best-matched red cell units. In these 16 patients, 23 alloantibodies were identified; allo anti-E was the most common.

CONCLUSION: This study showed antibody against the Rh system as the most common cause of incompatibility.

Keywords: Antibody screening, antibody identification, column agglutination test, conventional tube technique, direct anti-human globulin test, immunohematology analyzer, incompatible crossmatch, indirect anti-human globulin test, panagglutination, polyspecific (IgG + C3d) gel media

Introduction

One of the essential goals in crossmatching of red cells is that the transfused blood must be compatible with the patient to provide maximum therapeutic support and minimal red cell destruction. With the increasing utilization of automated immunohematology (IH) analyzers, the routine cross-matching is predominantly replaced by ABO and Rh type and antibody screen or type and
screen (T/S) policy. In the Eastern part of India, major cross-matching between the recipient’s serum and donor red cells by anti-human globulin is the most common practice in most of the blood banks. These tests are carried out either by the conventional tube techniques (CTT) or by the semi-automated column agglutination technology (CAT). This is due to the constraints related to trained workforce and availability of regular supply of reagents and other logistics.

It appears once the recipient’s ABO and Rh blood type is known, a transfusion of compatible blood can be given. However, in practice, donor red blood cells (RBCs) may still be incompatible as it contains other minor antigens against which the recipient is alloimmunized/sensitized. Therefore, a cross-match is done to ensure that the donor RBCs actually do match against the recipient’s serum. There are times when even after an exhaustive workup, a unit of compatible red cells becomes unavailable for the patient. The commonly observed clinical conditions and the insights obtained on how safe to transfuse the best unit of blood available was reviewed here along with their outcomes. The clinical and serologic evaluation, which allows for the transfusion of the most compatible (or “least incompatible”) blood, requires a joint effort between the clinician and the transfusion medicine physician.\[1\]

### Materials and Methods

A prospective analysis was conducted in all the incompatible cross-matched blood samples at the IH laboratory of Kolkata Medical College Hospital blood bank since October 1, 2015—March 31, 2016 (6 months). This blood bank is one of the major regional blood transfusion centers in the state of West Bengal (WB), Eastern India, with an average annual blood collection over 30,000 units. The center supplies an overall annual average of 50,000 units of blood components to the patients who were admitted in the hospital itself as well as patients referred from other hospitals located within or outside the city of Kolkata. Red cell concentrates constitute a major volume of the supplied blood components to the extent of 60% approximately. This study had been approved by the Institutional Ethics Committee.

#### Sample acceptability criteria

Any request for blood component(s) was accompanied by a duly filled and authorized blood requisition form as designed by the WB State Blood Transfusion Council along with properly labeled 3 cc ethylenediaminetetraacetic acid (EDTA) and 3 cc clotted blood samples. The samples should be freshly collected mentioning the name of the patient with patient identity number and phlebotomist’s signature. If any nonconformity were observed in the blood samples, they were referred to the blood transfusion officer (BTO) / resident doctor on duty for further decision making. Blood sample(s) showing visible evidence of gross deterioration/hemolysis were excluded from the study. If the patient had a previous history of blood transfusion, the transfusion records related to blood group and any other relevant information were verified.\[2\]

#### Preparation of the blood sample and blood grouping

The EDTA and clotted vial were centrifuged at 3000 ×g for 3 min to separate red cells and serum/plasma.\[3\] ABO and Rh typing was done by CTT using commercially available monoclonal antibodies (Tulip Diagnostics Pvt. Ltd., India). Reverse grouping was performed by CTT using freshly prepared in-house reagent pooled A, B, and O cells.\[4\] Tests were validated by a negative saline control. Any discrepancy in blood grouping results was resolved according to their type and classification, as per departmental standard operating procedures and recorded. The concerned treating facility was also intimated of their significance.

#### Cross-matching of patient’s sample

Once the blood group of the recipient’s sample was determined, a major cross-match using group-specific donor red cell units (1% donor red cell suspension in low ionic strength saline solution) was done by CAT in polyspecific (IgG + C3d) gel media (Matrix gel system, Tulip Diagnostics Pvt. Ltd., India). The tests were performed according to the manufacturer’s instructions.

A positive and negative control were run daily in parallel with the tests to validate the test results.\[5\]

#### Evaluation of an incompatible cross-matched sample

In case of any incompatible major cross-match results, a repeat cross-match with the same donor unit was performed along with two additional group specific donor units. This repetition was done to rule out any possibility of technical errors (contamination, direct anti-human globulin test [DAT] positive donor unit, mis-grouping, etc.) as well as clerical/transcriptional errors. If the incompatibility persisted on repetitions in any of these 3 units, a further evaluation of the recipient’s sample was done in the departmental IH laboratory. The IH laboratory could not routinely perform T/S policy so such an alternative protocol is chosen.

An initial workup of the recipient’s sample was done by DAT, auto-control and antibody screening using commercially available cells or in-house prepared screening cells.\[3\] Any reaction with a strength of 2+ or above was considered to be strong and below these were
weakly reactive. Antibody identification was done in antibody screen positive samples, using commercially available reagent 11 cell panel (Ortho-clinical Diagnostics Inc., USA) by CAT in Ortho BioVue system on polyspecific AHG (IgG + C3d) cassettes. The workflow of evaluation of an incompatible cross-match was shown in Figure 1. However, a detail of clinical history of the patient along with the history of medications and relevant history suggestive of alloimmunization/sensitization is recorded by the transfusion medicine resident doctor, wherever was possible.

Selection and issue of appropriate donor red cell unit
Wherever any alloantibody(s) were being detected corresponding antigen(s) negative compatible or best-matched unit was issued after consultation with the treating clinician. In situations where no specific alloantibody could be pointed, group-specific, extended Rh and Kell phenotype matched (where the patients were transfusion free for more than 3 months) red cell units were provided as emergency lifesaving resort. In situations where patients received recent transfusions (within 3 months) best-matched units (less strength than autocontrol) were provided.

Each of these transfusions was under the supervision of the treating clinician or the transfusion medicine resident. In the event of any adverse outcome, the transfusion was stopped immediately and the blood bank resident doctor was informed for further proceedings. Every successful transfusion events were monitored with a posttransfusion 24 h increase in hemoglobin (Hb) and clinical improvement of signs and symptoms. A fresh set of blood samples (EDTA and clotted) were required for each and every transfusion requisition, irrespective of the time interval between two consecutive transfusions.

Results

Demographic distribution, clinical history, and history of alloimmunization in the study population
A total of 14,387 sets of patient’s samples were accepted at the blood bank during 6 months period. Only 100 (0.69%) of these 14,387 were found to be cross-match incompatible and subjected to evaluation and selection of appropriate donor units [Figure 2]. The cross-match incompatibility was much higher in the females (59%) than the males (41%) (Figure 3). An overall distribution of incompatibility ranges from 1 to 68 years of age, with a maximum incidence of 39% (n = 39) in the 11–20 years age group. A minimum incidence of 5% (n = 5) was observed in the persons above 50 years of age (Figure 4).

On an overall 14,387 red cell demands, majority were for anemia (n = 8925), surgical procedures (n = 3455), and obstetric cases (n = 1005). The rest of the 1002 belonged to other category which was requested for miscellaneous reasons, namely, acute hemorrhage, trauma, dialysis, etc., [Figure 5]. The majority population of anemic patients were suffering from thalassemia (n = 4115, 46%),
hematological malignancies (n = 1865, 21%), autoimmune hemolytic anemia (AIHA) (n = 88, 1%), and other causes of anemia (n = 2857, 32%) [Figure 5a and b].

Out of these 100 patients, 85% (n = 85) were repeatedly transfused. Thalassemia, hematological malignancies and autoimmune anemia (primary/secondary) constitute an overall 78% (n = 78) of the total burden of cross-match incompatible samples. Comparative details of the total study population versus the incompatibility results are shown in Figure 6.

**Laboratory workup and immunohematology analysis of blood samples**

DAT was positive in 53% (n = 53) incompatible samples and 44 of these were strongly positive (more than 2 in strength/grade) [Figure 7]. Of these 44 strongly positive DAT samples, 21 weakened their strength on auto control.

A total of 38 (38%) of the incompatible cross-match blood samples were positive on indirect anti-human globulin test (IAT)/antibody screen on CAT. The causative antibody could be identified in 16 of them, with an overall antibody identification rate of 42.10% on IAT/antibody screen positive samples. In the rest 22 of these 38 patients, the specific antibody identification could not be done with the available logistics. On the other hand, 17 of the 100 samples presented with DAT positive and panagglutination, where only blood group specific, best-matched or extended Rh and Kell phenotype-matched red cells were transfused [Figure 8a and b]. The complete analysis of the rest 45 patients could not be done as either they were lost to follow-up or the patient’s blood sample was not received again.

Out of the 16 patients where antibody detection could be done, 6 of them were multiple antibodies and 10 were single. An association of c, E antibodies was observed in 5 out of 6 patients with multiple alloantibodies. The other patient with multiple alloantibody was E, S, and N.
The specificity of the alloantibody detected in 16 patients is given in Table 1. A total of 23 alloantibodies were identified in 16 patients. Majority of these antibodies identified were of the Rh system (19/23 [82.60%]) with anti-E being the most common antibody (10/23 [43.47%]).

It was also observed that 6 of these 17 patients (initially showing DAT positivity and panagglutination) who came for further follow-up after receiving best match/phenotype matched red cells transfusion along with steroid/rituximab therapy, recovered uneventfully with an appropriate rise in Hb level and became DAT negative after 3 months. An overall transfusion reaction was observed in 2 of these 17 patients (11.7%). There was no event of death due to adverse outcome.

Discussion

Incompatibility in cross-matching during pretransfusion testing is not uncommon. There is hardly any evidence-based study on frequency of incompatible cross-matched red cells and how to approach these cases for better transfusion practice from the eastern part of India till now.

In our study, we rechecked all the ABO and Rh (D) group specific incompatible cases with the same donor unit (along with two other separate donor units) to exclude clerical error, as clerical error is the most common cause of incompatibility as shown by Stainsby et al. in UK.[8] The incidence of persistent incompatible cases were 0.69%, whereas the study by Bhatt et al. in Western India showed an overall incidence of incompatibility were 0.21%.[9]

In the present study, majority of incompatible crossmatches were found in females (59%) which is comparable to the study conducted by Bhatt et al. in western part of India.[9] Incompatibility was most prevalent in the age group of 11–20 years (39%) and they were mostly thalassemic patient. A total of 58% of incompatible patients were thalassemics. The other important causes of incompatibility were AIHA (14%) and hematological malignancy (6%). This is in contrast to the study conducted by Bhatt et al. where peak incidence seen in AIHA (40%).[9] The present study had shown repeated red cell transfusion was the major factor associated with incompatible cases (85%).

On analysis of these incompatible blood samples, only 38 cases were found to be IAT/antibody screening positive. Among these 38 IAT/antibody screening positive cases alloantibody against red cell antigens was detected in 16 of them (42.1%), panagglutination (agglutination with all reagent cells) with DAT positivity was found in 17 patients (44.73%). A single alloantibody was detected

Table 1: Antibody profile in incompatible cross-match patients

<table>
<thead>
<tr>
<th>Antibody profile in patients (n=16)</th>
<th>Type specificity of antibody</th>
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<td>Patients with multiple antibodies (n=6)</td>
<td>Anti (c + E) (n=5)</td>
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<tr>
<td>Patients with single antibody (n=10)</td>
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<tr>
<td></td>
<td>Anti c (n=3)</td>
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<td></td>
<td>Anti D (n=1)</td>
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<tr>
<td></td>
<td>Anti Kell (n=1)</td>
</tr>
<tr>
<td></td>
<td>Anti JKb (n=1)</td>
</tr>
</tbody>
</table>

Figure 6: Comparative disease distribution among Anemic population and incompatible patients

Figure 7: Distribution of DAT +ve samples

Figure 8: (a) Overall serological status of incompatible samples (n = 100) 
(b) Results of further evaluation of IAT positive samples (n = 38)
in 10 patients (62.5%), and the rest 6 patients were having multiple alloantibodies (37.5%). A total of seven different types of alloantibodies were observed in these 16 patients [Table 1] having both single and multiple antibodies. Most of the alloantibody detected belonged to the Rh system (82.6%, 19 out of 23), of which anti-E (43.47%) was the most common followed by, Anti-c (34.78%), and anti-D (4.34%). This result is comparable to the study as observed by Goldfinger and Lu.[10]

**Conclusion**

This study showed the antibodies against Rh system antigens were the most common cause of incompatibility in multi-transfused patients. A significant number of incompatible cross-match were found due to AIHA, presented with positive DAT and panagglutination in antibody screening panel and were managed by best-matched red cells. The treating clinicians were informed about the type of AIHA (warm/cold/mixed) to start the definitive treatment.

A significant number of these AIHA patients were followed up for 3 months, and on follow-up, they showed clinical improvement following steroid/rituximab along with transfusion therapy.

Since the majority of alloantibodies are detected against the Rh system (82.6%), extended Rh phenotyping of the donor red cells and the recipients at the onset of initial transfusion may prevent the development of alloantibodies in the multitransfused patients.

**Acknowledgment**

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**Conflicts of interest**

There are no conflicts of interest.

**References**

A STUDY OF IRREGULAR ANTIBODIES IN 200 MULTI-TRANSFUSED PATIENTS

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HOW TO CITE THIS ARTICLE:

ABSTRACT: BACKGROUND: Alloimmunization is one of the major concern in the management of patients who required repeated blood transfusion as a lifesaving treatment. The knowledge of incidence of such alloantibodies is essential for selecting appropriate red blood cells for transfusion. AIMS: This study was carried out to get the frequency and type of unexpected red cell antibodies in the multi-transfused patient at a tertiary level government hospital in South Gujarat. MATERIALS AND METHODS: This prospective study was carried out in 200 patients who required multiple blood transfusions. The antibody screening was done with 3 & 11 commercial cell screening & identification panel by column agglutination technique (Matrix Gel System & Matrix Erygen AS-ID, Tulip Diagnostics, India) at saline & anti-human globulin phase. RESULTS: The overall prevalence of alloimmunization was 7.0%. The majority of these had a single alloantibody (11 cases, 84.62%) whereas the remaining 2 cases (15.38%) had multiple antibodies. The anti-c and anti-D antibodies comprised the most common alloantibody (27% each both) followed by, anti-N (20%), anti-C (13%), anti-e & anti-M (7%) antibodies. Gender & number of blood units were found to be risk factors of alloimmunization in transfused patients. In our study we found females (79%) are more prone to alloimmunization. Those who were transfused more than 2 units have higher frequency of alloimmunization. The highest incidence of alloimmunization was observed in obstetrics and sickle cell patients. CONCLUSIONS: The majority of alloantibodies detected in the current study were clinically significant and of mainly belonging to Rh blood group system. Thus pre-transfusion antibody screening on patients’ samples prior to cross-match needs to be initiated in India and we can at-least provide corresponding Rh antigen negative blood to ensure safe transfusion practice. KEYWORDS: Red Cell, Red cell antigen, Alloimmunization, alloantibodies, Indirect Antiglobulin Test. MESHTERMS: Erythrocyte, Isoantibodies, Coombs test.

INTRODUCTION: Alloimmunization is one of the major concern in the management of patients who required repeated blood transfusion as a lifesaving treatment. In the patients affected with haemoglobinopathies, haematologic diseases, various types of cancers, recipients of organ transplantation, and patients with renal failure, the prevalence of alloimmunization has been reported to be up to 60 per cent.[1] Alloimmunization further complicates the transfusion therapy due to difficulty in getting compatible blood & delayed haemolytic transfusion reaction.[2] The knowledge of such alloantibodies is essential for selecting appropriate red blood cells for transfusion. This study was carried out to get the frequency and type of unexpected red cell antibodies in the multi-transfused patient at a tertiary level government hospital in South Gujarat.
MATERIALS AND METHODS: The study was performed between the years 2012 to 2014 at blood bank attached to department of Immunohematology & blood transfusion of tertiary level Government Medical College and Hospital of South Gujarat after obtaining ethical committee clearance from the institute and assessed for the presence of alloantibodies. Antibody screening was carried out in 200 multi-transfused patients prior to compatibility testing. A detailed clinical and transfusion history was taken using a set performa which included the name, identification number, age, sex, diagnosis, blood group, transfusions done till date of request, transfusions during the present study period, result of serological testing like direct antiglobulin test and auto control, antibody screen tests in the study period with results and antibody identification results.

The blood requisition for these patients were received along with samples (plain & EDTA) for antibody screen testing and compatibility testing as a protocol. ABO and Rhesus blood grouping tests were done by forward and reverse grouping in all patients so as to confirm the blood group. Subsequent antibody screening was performed on all samples using a commercially available three cell panel (Matrix gel system & Matrix Erygen AS; Tulip Diagnostics, India) by the column agglutination method, using saline, antiglobulin & enzyme phase.

Antibody screening was done for antigens of blood groups which include Rh, Kell, Kidd, Duffy, Lewis, P and MNS antigens along with an auto-control. Antibody screen positive samples were further analysed for the specificity of the alloantibody with an eleven cell identification panel (Matrix gel system & Matrix Erygen ID, Tulip Diagnostics, India). Later on compatible blood at anti-human globulin phase was issued for transfusion whenever required. An auto control using the patient’s own cell and serum was tested in parallel with each screen to exclude presence of autoantibodies. The criteria for antibody screening and identification were based on the standard recommendations and Manufacturer Company.[3,4]

STATISTICAL ANALYSIS: The patient with positive screen was assessed based on gender, age, and history of transfusion, clinical diagnosis and alloantibody specificity. The two sided chi square t test & odds ratio were performed to determine the difference in antibody rate by gender and no of transfusions. P<0.05 was considered significant. The analyses and data management were performed using Epi Info software version.

RESULTS: A total of 200 patients (114 male & 86 female) were included in the present study. Different diagnosis of 200 patients was: 36(18%) of thalassemia, 30(15%) of sickle cell disease, 29(14%) with surgical illness, 23(12%) of other anaemia, 22(11%) with renal disease, 16(08%) of leukaemia, 14(07%) with GIT diseases, 10(05%) with obstetrics condition and 20(10%) with other diseases (Figure 1). Age of the patients included in the study ranged from 2 to 85 years with a mean age of 28.21±16.78 years. Among the alloimmunized cases, the age range was 18 to 35 years with a mean age of 26.61±5.3 years. Among total number of cases, 14(07%) patients were positive for different type of irregular antibodies while remaining 116(93%) patients were negative for alloimmunization. 13 patients were included in study as one patient was having auto antibody.

Among the total 13 number of patients with alloantibodies, three male patients had positive results for antibody which is 2.70% of total male patients and 23.07% of total positive cases while ten female patients were positive for alloantibody which is 13.33% of total female patients and 76.92% of total positive cases.
Female patients were significantly more positive for irregular antibodies (Chi-square test 2 tailed P value is 0.012). The odds ratio for male and female positivity was 5.4 indicating that female were 5 times more prone to develop alloantibodies in comparison to male patients. Among the positive cases, blood group distribution is shown in Figure 2. Out of 200 patients, 94(47%) patients had received ≤2 units of blood transfusion, among which 01(01.06%) had developed irregular antibodies while 106(53%) patients had received >2 units of blood transfusion, among which 12(11.32%) had developed irregular antibodies which showed significant difference between these two groups (chi-square test 2 tailed P value is 0.008). Among the 13 patients with alloantibodies, 11 patients (84.62%) had a single alloantibody, whereas two patients (15.38%) had multiple alloantibodies.

Among the total 13 patients with alloantibody/alloantibodies, four (31%) patients were having anti-c antibodies, Three (23%) patients were having anti-N antibodies, two (15%) patients were having Anti-D, two (15%) patients were found positive for both anti-D & C, one (08%) each patient was having Anti-e and anti-M (Figure 3). The adsorption & elusion study to find out possibility of Anti G antibody in two patients who were found positive screen for both anti D and anti C was not done. Among the positive cases, four (31%) cases were that of sickle cell anaemia, four (31%) cases of obstetrics, two (15%) cases of anaemia, and one each case of P. vivex (8%), hemolytic anaemia (7%) and Bernard soulier syndrome (08%).

**DISCUSSION:** It is a routine practice to perform pre transfusion compatibility testing before blood transfusion to prevent immune mediated haemolytic transfusion reactions. The steps of pre-transfusion testing involve reviewing the acceptability of blood sample, checking the ABO group and Rh D type, antibody screening test, determining the specificity of antibodies detected unexpectedly, choosing donor RBC units suitable for recipients, and carrying out cross-match.[3] As blood is routinely matched with respect to major blood group antigens i.e. ABO and Rh D antigen, there is a high probability that the donor will have minor blood group antigens not present in the recipients which will result in alloimmunization. Factors for immunization are complex and involve at least three main contributing elements. This includes RBC antigenic difference between the blood donor and the recipient, the recipient's immune status and immuno-modulatory effect of the allogenic blood transfusions on the recipient’s immune system.[5]

The development of red cell antibodies (Allo as well as autoantibodies) occurs in a variable number of multiple transfused patients. In such circumstances, transfusion therapy may become significantly complicated. Effects of alloimmunization may include difficulty in finding compatible RBC units because of the presence of clinically significant RBC antibodies, transfusion reactions, or platelet refractoriness.[6] Present study is an effort to characterize blood group alloantibody formation in the patient population.

Few studies of multiple transfused patients in India had revealed rate of alloimmunization ranging from 3 to 13% as mentioned in Table 1. The rate of present study was 07% which is similar to the studies conducted by J Shukla et al (9.87%), Pradhan et al (08%) and Gupta et al (9.48).[7,9] The studies done by Pahuja et al (3.7%) and Dhawan et al (5.64%) had lower rate while study of V Sangole et al had higher rate of 13.04 %.[10,12]

Females have been observed to be more prone to development of alloimmunization than males probably due to the fact that females, especially in developing countries, are anaemic and pregnancy is an important risk factor for alloimmunization.[13]
In present study, 11(79%) out of 14 alloimmunized patients were women, with significant association in chi-squares test (P value <0.05). This finding of the present study is in agreement with the study done by Alick et al while studies done by Bhaskar S et al & Makroo et al did not show such association with gender.[13,15] In the present study, female were five times more prone to develop alloantibodies in comparison to male patients. Clinical diagnosis of the study group may lead to a vulnerable immune status which may predispose to altered or increased immune response to various antigens. In our study, significant number of sickle cell anaemia patients developed alloantibodies. Out of 30 sickle cell cases, alloimmunization was found in four (13.33%) cases, which is comparable to study by Elliott et al (30%) and Murao et al (9.9%).[16,17]

Though antigen typing before transfusion of people with sickle cell disease and providing antigen negative units is now widely employed by sickle cell centers, the alloimmunization rate remains quite high in contemporary sickle cell populations and may be due in large part to transfusions received at institutions not providing extended matching. Two out of 21 patients of chronic anemia developed alloantibodies (9.52%), which is comparable to study by Elliott et al (05%).[16] Out of 5 patients of obstetrics, 4 (80%) patients developed alloantibodies. (Figure 3).

The specificity of most alloantibodies detected in the present study was against Rh system (85%) due to their high immunogenicity, which is similar to previous reports of Thakral et al (61%), Hmida et al (59%) and Dhawan et al (52%).[11,18,19] Anti c was detected in four patients, Anti-D in four, Anti-N in three, anti-C in two and Anti-M, Anti e in one patient each. Anti-c and anti-D (27%) were the most common antibodies in our study, which is comparable to Thakral et al (38.8%).[18] Hence, the transfusion of blood matched for Rh could prevent alloimmunization resulting in a significant difference in the alloimmunization rates, but the potential to form RBC alloantibodies to unmatched antigens will exist.[20] In our study, majority of the patients with anti-D (either singly or in combination) were multiparous females who might have formed anti-D due to previous pregnancies or transfusions. (Figure 3)

In the present study, there was an absence of anti-Kell antibody in all subjects which was similar to the findings of the study done by Thakral et al while other studies found anti Kell antibodies.[11,13,15,18] This could be due to differences in blood group antigen frequencies in different populations. According to the study on blood donors of the South Gujarat, Kell antigen positivity was found to be 6%.[21] Thus, the lower frequency of Kell antigen in donated blood might be the reason behind the lesser risk of alloimmunization from transfusion of a Kell antigen positive unit and the result was absence of anti Kell antibody in present study.

In the present study we detected single antibody in 84.62% of cases and multiple antibody in 15.38% of cases, which is comparable to study by Alick et al who found single antibody in 78.6% and multiple antibody in 21.4%.[14] Similar results was also found by Dhawan et al (22%cases had dual allo antibodies).[11] Since pre-transfusion antibody screening in patients' samples is not a routine practice in India, these patients might have received antigen mismatched blood leading to formation of multiple alloantibodies.

The risk of developing alloimmunization was very clearly associated with the number of transfusions received. In our study 11.32% patients of patient group who received more than 2 units of blood transfusion were developed alloantibodies and it is significant statistically (P<0.05). This finding is supported by some of the earlier studies done by Alick et al, Dhawan et al, Vishinski E et al & Jensen LS et al who have found a strong correlation between the numbers of blood units transfused and alloantibody formation.[11,14,20,22]
CONCLUSION: By considering the results of present and other reference Indian studies, blood banks of India should go for universal type and screen policy for finding the prevalence of alloantibodies in general patient and donor population. The indigenous development of local cell panels would be a better option to ensure adequate supplies of reagent red cells and introduction of type and screen policy for all the patients. Patients with identified alloantibodies can be flagged in a database and the information can be shared between institutions and shared with the patient in the form of report issuing to the concern person as well as patient education if possible. To avoid the effects of alloimmunization, after antibody screen and identification, corresponding antigen negative blood should be given to the patient.

The other approach to avoid alloimmunization in regularly transfused patients like sickle cell disease & thalassemia is to allot a group of 10-15 donors to such single patient. Whenever the transfusion required, donor will be selected from this group. In this way we can minimize alloimmunization as well as better safety in terms of transfusion transmitted infections also.

STUDY LIMITATION: The adsorption & elusion study to find out possibility of Anti G antibody in two patients who were found positive screen for both anti D and anti C was not done. The limitations of this study was follow up data was not available due to various reasons & phenotyping of each & every donor was not possible so only cross match compatible blood were issued.

Tables:

<table>
<thead>
<tr>
<th>Sl. No</th>
<th>Studies</th>
<th>No. of Cases</th>
<th>% of Positive Cases</th>
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<tr>
<td>1</td>
<td>J Shukla et al</td>
<td>81</td>
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Table 1: Incidence of Alloimmunization in Multi-Transfused Patients in various Studies

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<th>Studies</th>
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<tr>
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<td>R Makroo et al</td>
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<td>Thakral et al</td>
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<tr>
<td>Present study</td>
<td>anti c and anti D</td>
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Table 2: Major Antibody type in various Studies
Fig. 1: Diagnosis of Patients (n = 200)

Fig. 2: Blood Group Distribution of Positive Patient
REFERENCES:


## ORIGINAL ARTICLE

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### FINANCIAL OR OTHER COMPETING INTERESTS: None

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Date of Publishing: 08/09/2015.
EXTERNAL EVALUATIONS
Performance evaluation of Matrix™ AHG (Coombs) Test Card for antibody detection (screening) and identification.

EVALUATING CENTER:
This study was conducted at the Immunohematology Laboratory of the German Red Cross Blood Center in Frankfurt (Institute of Transfusion Medicine and Immunohematology), under the supervision of Dr. med. C. Geisen (MD). (Data on file Tulip Diagnostics Pvt. Ltd.).

SUMMARY
In this evaluation study 551 samples for antibody screening were tested in parallel with Matrix™ AHG (Coombs) Test card and DiaMed ID LISS/Coombs card using a Tecan pipetting machine. Out of 551 samples tested 42 samples showed positive results for antibody screening and were followed up by antibody identification. Overall, semi-automated system using automated pipetting system and card reading with respective Saxo card reader Matrix™ Gel System showed a 4.7% lower sensitivity and a 1.6% lower specificity with respect to antibody screening. In contrast to antibody identification Matrix™ Gel System showed a 2% higher sensitivity. Antibodies which, in one case each, were detected in the antibody identification only with Matrix™ Gel Cards included anti-Jk(a), E and K; antibodies which, in one case each, were detected only by DiaMed ID Cards included anti-c and anti-C⁺.

INTRODUCTION:
The purpose of this study was to evaluate the performance of Matrix™ AHG (Coombs) Test Card manufactured by Tulip Diagnostics Pvt. Ltd, against DiaMed ID LISS/Coombs test cards manufactured by DiaMed. Both the gel card consists of 6 microtubes prefilled with polyspecific anti-human globulin.

The performance evaluation of Matrix™ AHG (Coombs) Test Card was carried out under routine conditions for antibody detection and antibody identification. All tests were performed in parallel with both the test systems in the Immunohematology Laboratory of the German Red Cross Blood Center in Frankfurt (Institute of Transfusion Medicine and Immunohematology). The study was performed in two parts. First part of the study was performed from November 18, 2008 to December 16, 2008. Second part was performed from February 9, 2009 to February 27, 2009.

MATERIALS AND METHODS:
Blood samples used were the residual patient's blood samples which were sent for routine diagnostic testing. In first part, 404 fresh (not frozen) samples were tested: 371 random routine samples (unknown allo-antibody status) and 33 additional samples with known allo-antibody were used. In second part, 158 fresh (not frozen) random routine samples were tested. Antibody detection was performed with ID-DiaCell I-II-III in both Matrix™ AHG (Coombs) Test Card and DiaMed-ID LISS/Coombs Cards. Invitroscreen I-II-III test cells were used in combination with Matrix™ AHG (Coombs) Test Card and ID test cells were used with ID-Cards under routine testing procedure.

RESULTS OF STUDY IN PART 1:
Of the originally 404 samples, sufficient plasma was available from 398 samples. Out of 398 samples 39 samples were positive for antibody detection. On performing identification with 11 cell panel 46 Coombs reactive antibodies were detected. Data is summarized in below tables:

Table 1.1: Concordance of results of the antibody detection test on 398 routine samples tested with Matrix™ Gel System and DiaMed ID.

<table>
<thead>
<tr>
<th>Antibody Detection n=398</th>
<th>DiaMed ID</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+</td>
</tr>
<tr>
<td>Matrix™ Gel System</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>-</td>
</tr>
</tbody>
</table>
### Table 1.2: Antibody detection results for the DiaMed ID-Gel Card system:

<table>
<thead>
<tr>
<th>Antibody Detection n=398</th>
<th>Coombs reactive antibodies</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Present (39)</td>
<td>Not Present (359)</td>
</tr>
<tr>
<td>DiaMed ID Gel Card System</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td></td>
<td>38 (True positive)</td>
<td>4 (False positive)</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1 (False negative)</td>
<td>355 (True negative)</td>
</tr>
</tbody>
</table>

### Table 1.3: Antibody detection results for Matrix™ Gel System.

<table>
<thead>
<tr>
<th>Antibody Detection n=398</th>
<th>Coombs reactive antibodies</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Present (39)</td>
<td>Not Present (359)</td>
</tr>
<tr>
<td>Matrix™ Gel System</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td></td>
<td>36 (True positive)</td>
<td>5 (False positive)</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3 (False negative)</td>
<td>354 (True negative)</td>
</tr>
</tbody>
</table>

### RESULTS OF STUDY IN PART 2:

Of the originally 158 samples sufficient plasma was available in from 153 samples. Out of 153 samples 3 samples were positive for antibody detection. On performing identification with 11 cell panel 4 Coombs reactive antibodies were detected. Data is summarized in below tables:

### Table 2.1: Concordance of results of the antibody detection test on 153 routine samples tested with Matrix™ Gel System and DiaMed ID.

<table>
<thead>
<tr>
<th>Antibody Detection n=153</th>
<th>DiaMed ID</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+</td>
</tr>
<tr>
<td>Matrix™ Gel System</td>
<td></td>
</tr>
<tr>
<td></td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>-</td>
</tr>
</tbody>
</table>

### Table 2.2: Antibody detection results for the DiaMed ID-Gel Card system

<table>
<thead>
<tr>
<th>Antibody Detection n=153</th>
<th>Coombs reactive antibodies</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Present (3)</td>
<td>Not Present (150)</td>
</tr>
<tr>
<td>DiaMed ID Gel Card System</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3 (True positive)</td>
<td>2 (False positive)</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0 (False negative)</td>
<td>148 (True negative)</td>
</tr>
</tbody>
</table>
Table 2.3: Antibody detection results for Matrix™ Gel System.

<table>
<thead>
<tr>
<th>Antibody Detection n=398</th>
<th>Coombs reactive antibodies</th>
<th>Present (3)</th>
<th>Not Present (150)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Matrix™ Gel System</strong></td>
<td>+</td>
<td>3</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>(True positive)</td>
<td></td>
<td>(False positive)</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>0</td>
<td>141</td>
</tr>
<tr>
<td></td>
<td>(False negative)</td>
<td></td>
<td>(True negative)</td>
</tr>
</tbody>
</table>

CALCULATED SENSITIVITY AND SPECIFICITY OF ANTIBODY DETECTION TEST IN PART 1 & 2:
The calculated sensitivity of the antibody detection test using DiaMed ID system and Matrix™ Gel System (for part 1 & 2) were 96.7% and 92.9% respectively. The specificities of DiaMed ID system and Matrix™ Gel System (for part 1 & 2) for Coombs reactive antibodies were 98.8% and 97.2% respectively.

RESULTS OF ANTIBODY IDENTIFICATION IN STUDY PART 1:
In 39 samples 46 Coombs reactive antibodies were detected. Result data is summarized in below tables:

Table 3.1: Summary of results of antibody identification in Matrix™ Gel System and DiaMed ID.

<table>
<thead>
<tr>
<th>Confirmed antibody by identification panel n=46</th>
<th>DiaMed ID</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+</td>
</tr>
<tr>
<td><strong>Matrix™ Gel System</strong></td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>-</td>
</tr>
</tbody>
</table>

* 2 antibodies with the specificity C, c (one each) identified only by the DiaMed ID system.
** 3 Antibodies with specificity E / Jk(a) / K (one each) identified only by Matrix™ Gel System.

Table 3.2: Spectrum of 46 identified antibodies in 39 samples

<table>
<thead>
<tr>
<th>Antibody specificity</th>
<th>Total antibodies</th>
<th>Detected by DiaMed ID System</th>
<th>Detected by Matrix™ Gel System</th>
</tr>
</thead>
<tbody>
<tr>
<td>c</td>
<td>2</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>C</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>C&quot;</td>
<td>4</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>E</td>
<td>11</td>
<td>10</td>
<td>11</td>
</tr>
<tr>
<td>D</td>
<td>9</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>Jk(a)</td>
<td>3</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>K</td>
<td>5</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Le(b)</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Lu(a)</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>M</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>S</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Auto-e</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Pan agglutination</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>46</strong></td>
<td><strong>43</strong></td>
<td><strong>44</strong></td>
</tr>
</tbody>
</table>
RESULTS OF ANTIBODY IDENTIFICATION IN STUDY PART 2:
In 3 samples 4 Coombs reactive antibodies were detected. Result data is summarized in below tables:

Table 4.1: Summary of results of antibody identification in Matrix™ Gel System and DiaMed ID.

<table>
<thead>
<tr>
<th>Confirmed antibody by identification panel n=4</th>
<th>DiaMed ID</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+</td>
</tr>
<tr>
<td>Matrix™ Gel System</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>0</td>
</tr>
</tbody>
</table>

Table 4.2: Spectrum of 46 identified antibodies in 39 samples

<table>
<thead>
<tr>
<th>Antibody specificity</th>
<th>Total antibodies</th>
<th>Detected by DiaMed ID System</th>
<th>Detected by Matrix™ Gel System</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>E</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>D</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
</tbody>
</table>

CALCULATED SENSITIVITY OF ANTIBODY IDENTIFICATION IN PART 1 & 2:
Antibody identification with 11 cell ID test panel in DiaMed ID gel system and Matrix Gel System in study part 1 and 2 achieved a sensitivity of 94% and 96% respectively.

NOTE
Data on file: Tulip Diagnostics (P) Ltd.
Performance evaluation of Matrix™ AHG (Coombs) Test Card for auto-controls.

EVALUATING CENTER:
This study was conducted at the Immunohematology Laboratory of the German Red Cross Blood Center in Frankfurt (Institute of Transfusion Medicine and Immunohematology), under the supervision of Dr. med. C. Geisen (MD). (Data on file Tulip Diagnostics Pvt. Ltd.).

SUMMARY
In this evaluation study 544 samples for auto-control were tested in parallel with Matrix™ AHG (Coombs) Test card and DiaMed ID LISS/Coombs card using a Tecan pipetting machine. Out of 544 samples 146 samples showed positive auto-control and were further investigated by monospecific DAT.
Overall, semi-automated system using automated pipetting system and card reading with respective Saxo card reader Matrix™ Gel System showed a 3.1% lower sensitivity and a 1.7% lower specificity in auto-controls.

INTRODUCTION:
The purpose of this study was to evaluate the performance of Matrix™ AHG (Coombs) Test Card manufactured by Tulip Diagnostics Pvt. Ltd, against DiaMed ID LISS/Coombs test cards manufactured by DiaMed. Both the gel card consists of 6 microtubes prefilled with polyspecific anti-human globulin.

The performance evaluation of Matrix™ AHG (Coombs) Test Card was carried out under routine conditions for auto controls of patient's blood samples. All tests were performed in parallel with both the test systems in the Immunohematology Laboratory of the German Red Cross Blood Center in Frankfurt (Institute of Transfusion Medicine and Immunohematology). The study was performed in two parts. First part of the study was performed from November 18, 2008 to December 16, 2008. Second part was performed from February 9, 2009 to February 27, 2009.

The auto-control was performed routinely with both Matrix™ AHG (Coombs) Test Card and ID LISS/Coombs Test card. A positive result with either or both was followed by re-testing the sample with monospecific DAT (IgG and C3d) cards of ID and ScanGel Direct Coombs (DAT) cards.

RESULTS:
Whenever divergent results were obtained in auto-control with DiaMed ID LISS/Coombs Test Cards and Matrix™ AHG (Coombs) Test Cards, analysis was repeated in duplicate within each system. Auto-controls were scored positive when at least two of the three tests were positive; for all positive samples, monospecific DAT was performed. Of the originally tested 404 random patient's samples of the first part of the study, 386 were subjected to further analysis. Analysis of 18 samples could not be completed due insufficient sample. In second part of the study, all 158 samples were tested. Data is summarized in below tables:

RESULTS OF STUDY IN PART 1:
Table 1.1: Results of the auto-controls of 386 routine samples with Matrix™ AHG (Coombs) Test Cards and DiaMed ID LISS/Coombs Test Card:

<table>
<thead>
<tr>
<th>Auto-control n=386</th>
<th>DiaMed ID</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+</td>
</tr>
<tr>
<td>Matrix™ Gel System</td>
<td>93 84 monospecific DAT positive 9 monospecific DAT negative</td>
</tr>
<tr>
<td></td>
<td>7 6 monospecific DAT positive 1 monospecific DAT negative</td>
</tr>
</tbody>
</table>
### Table 1.2: Results of the auto-controls of 386 routine samples with DiaMed ID LISS/Coombs Test Card:

<table>
<thead>
<tr>
<th>Auto-control n=386</th>
<th>92 Confirmed by monospecific Coombs</th>
<th>294 -</th>
</tr>
</thead>
<tbody>
<tr>
<td>DiaMed ID</td>
<td>+</td>
<td>90 (true positive)</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>2 (false negative)</td>
</tr>
</tbody>
</table>

### Table 1.3: Results of the auto-controls of 386 routine samples with Matrix™ AHG (Coombs) Test Cards:

<table>
<thead>
<tr>
<th>Auto-control n=386</th>
<th>92 Confirmed by monospecific Coombs</th>
<th>294 -</th>
</tr>
</thead>
<tbody>
<tr>
<td>Matrix™ Gel System</td>
<td>+</td>
<td>86 (true positive)</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>6 (false negative)</td>
</tr>
</tbody>
</table>

### RESULTS OF STUDY IN PART 2:

**Table 2.1:** Results of the auto-controls of 158 routine samples with Matrix™ AHG (Coombs) Test Cards and DiaMed ID LISS/Coombs Test Card:

<table>
<thead>
<tr>
<th>Auto-control n=158</th>
<th>DiaMed ID</th>
<th>-</th>
</tr>
</thead>
<tbody>
<tr>
<td>Matrix™ Gel System</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>39</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>33 monospecific DAT positive</td>
<td>1 monospecific DAT positive</td>
</tr>
<tr>
<td></td>
<td>6 monospecific DAT negative</td>
<td>2 monospecific DAT negative</td>
</tr>
<tr>
<td>-</td>
<td>1</td>
<td>115</td>
</tr>
<tr>
<td></td>
<td>1 monospecific DAT positive</td>
<td></td>
</tr>
</tbody>
</table>

**Table 2.2:** Results of the auto-controls of 158 routine samples with DiaMed ID LISS/Coombs Test Card:

<table>
<thead>
<tr>
<th>Auto-control n=158</th>
<th>35 Confirmed by monospecific Coombs</th>
<th>124 -</th>
</tr>
</thead>
<tbody>
<tr>
<td>DiaMed ID</td>
<td>+</td>
<td>34 (true positive)</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>1 (false negative)</td>
</tr>
</tbody>
</table>
Table 2.3: Results of the auto-controls of 158 routine samples with Matrix™ AHG (Coombs) Test Cards:

<table>
<thead>
<tr>
<th>Auto-control n=158</th>
<th>34 Confirmed by monospecific Coombs</th>
<th>124 -</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Matrix™ Gel System</strong></td>
<td>+</td>
<td>34 (true positive)</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>1 (false negative)</td>
</tr>
</tbody>
</table>

CALCULATED SENSITIVITY AND SPECIFICITY OF ANTIBODY DETECTION TEST IN PART 1 & 2:
The calculated sensitivity of the auto-control test using DiaMed ID system and Matrix™ Gel System (for part 1 & 2) were 97.6% and 94.5% respectively. The specificities of DiaMed ID system and Matrix™ Gel System (for part 1 & 2) for auto-control were 96.2% and 94.5% respectively.

NOTE
Data on file: Tulip Diagnostics (P) Ltd.