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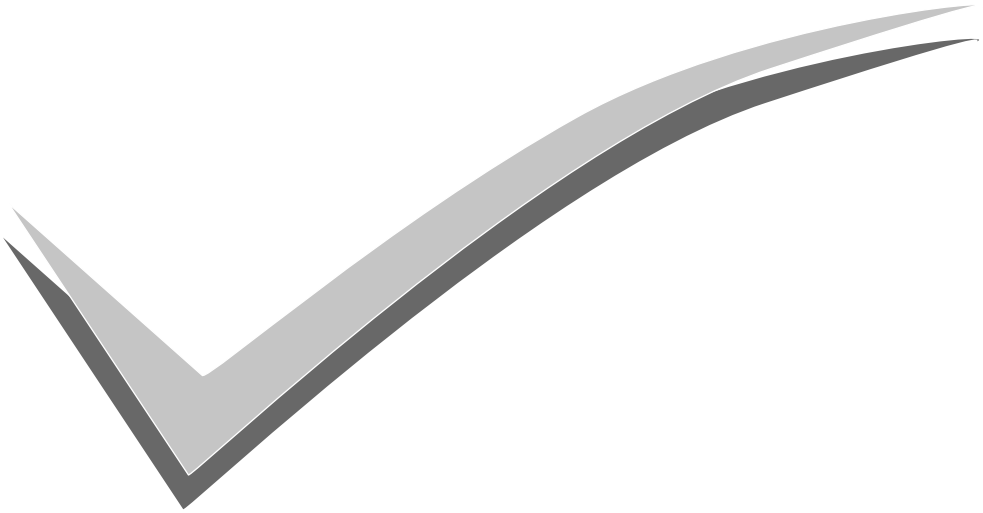

MATRIX™ GEL SYSTEM

Gel card system for Blood Banking Applications

Performance Evaluations

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EXTERNAL EVALUATIONS

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13.	Institute of Transfusion Medicine and Immunohematology, German Red Cross Center, Frankfurt Evaluation of MatrixAHG (Coombs) Test Card for autocontrols.

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The Efficiency and Specificity of Matrix Gel Method from the Forensic Point of View, in Determination of ABO Blood Grouping and Rhesus Factor

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Abstract

In today's crime investigation world determination of ABO blood grouping is still a very vital and effective precess in the field of forensic crime scenes. This investigation involves the identification of blood group, on the clothes (Accuse, Victim, injured, Complainer) collected from the scene of crime, and its cross comparision with the blood sample send by medical officer. Hence for this purpose total of 200 cases were included in the study having the samples of blood of accuse, victim, injured, complainer consisting of male and female... The determination of ABO/Rh factor was performed by conventional tube method and matrix gel card. The comparison of both techniques shows a very comparative result. As the red blood cells are sensitized with antibody will get agglutinate in the presence of anti human reagent in the matrix gel card and this will be trapped in the gel column this helps for easy analysis of blood group. However spin tube method is an operator-dependent assay, and is more susceptible to handling errors, the results are not more objective. The matrix gel card method requires Small sample volumes, and gives standardized performance with technical ease, and is with ready automation, and increased biosafety; all these factors have made this technology advantageous. In both techniques the reaction strength for ABO grouping and Rh factor is mainly govern by agglutination reaction intensity between red blood cells and anti-human reagent.

Keywords: Forensic serology; Blood group; Conventional tube technique; Haematology; Matrix gel card

Introduction

Blood grouping technique is widely used in forensic laboratories for investigation of biological fluids collected from crime scenes. In 1900, Sir Karl Landsteiner discovered the blood grouping technique known as the "ABO" system for which he was awarded with Nobel Prize in 1930 [1]. Edmond Locard a pioneer of forensic science proposed that every criminal carries some trace of evidence with him or her from the scene of crime by which he or she can be linked with the crime. The strongest evidence from the crime scene available is blood or blood stains because the source of blood and their stains help in solving the crime of violence, sexual offences, vehicular accident cases or murder. In cases of natural disasters the prime identification of body part is ABO blood grouping then later comes DNA matching. This is the most commonly followed techniques in today's forensic laboratory analysis [2,3].

The identification of biological fluids and the comparison of individual characteristics of biological evidence with known standards of its class, the biological samples can be identified. It is vital scientific evidence as it forms an important link in chain of evidence or supports circumstantial evidence. A careful investigation of blood group from the blood samples and blood stain is therefore important. Investigation of blood and blood stain gives very insight information about accused or victim which is useful in the court of law with a varying degree of reliability [4].

The presence of ABO blood group and Rhesus factor is applied to inherited antigens detected on red blood cells by specific antibodies [5,6]. Once the blood group and the Rh typing are established it remains unchanged throughout life [7,8]. The antigenic determinants on the surfaces of red blood cells are the A, B, and O blood group proteins, which are for convenience called A, B, and O antigens. Matrix gel is technique of blood group cross matching. The matrix gel card technique is introduced by lapierre, using the sephadex gel containing within micro-tube. (Lapierre1988; letich *et.al.* 1993). [9-11].

The aim of this study is to determine ABO blood group from the blood samples collected as exhibits from the crime scene and the methods used are spin tube method and matrix gel System. The comparative study for these two method was carried out by testing total 200 samples of blood in the form of clothes or different types of exhibits which having blood strains.

This study is carried out for the comparative study between spin tube method and matrix gel card method for blood group identification on the basis of efficacy sensitivity and specificity [12-14].

Material and Method

Chemicals and reagents

1. The Matrix AHG (Coombs) test card:- Matrix forward and reverse grouping card with auto control has six micro-tubes prefilled with auto control a gel in a suitable buffer. The first three micro-tubes contains Monoclonal Anti-A, Anti-B and Anti-D. The forth, five and six number Micro-tubes has neutral gel. The Forth tube is used as negative control. The fifth and sixth are used for reverse grouping.
2. Red Blood cells:- The Fresh blood samples of "A, B and O" blood group were kindly procided by blood bank of J.J. group of Hospitals, Mumbai, Maharashtra, India.Matrix card reader (Matrix Auto Max-80)
3. Matrix Diluent- LISS:- for three preparation of fresh cells.
4. Gel card centrifuge (Imperial Biotech LLP, Tulip gel card centrifuge)
5. Micropipette

Methodology

In the conventional spin tube method the ABO forward grouping is determined in the presence of the blood group antigens A, B and O by testing the RBC's with known antisera, specifically Anti-A, and Anti-B. On the other hand, the ABO reverse grouping method results into the presence of the expected ABO blood group antibodies by testing the serum or plasma with known A1 and B red blood cells. In case of matrix gel test the ABO forward grouping were performed in the Anti-A, Anti-B, micro-tubes, which contain the specific antibody incorporated into the gel.

Conventional method of ABO grouping

ABO blood group can be determined from the fresh blood samples by detection of antigens on RBC'S (forward method) or detection of antibodies in serum (reverse method). The blood samples are received in sterile bulb by forensic laboratories from the medical officers. The testing sample is taken into the tube and saline 0.2%, was added to the sample. This mixture was then centrifuged at about 3000 rpm for five minutes. The supernatant was then discarded and the suspended cells were rewashed with saline for two times. Resuspend the button of cells in fresh normal saline. After this the cell suspension was prepared in 2% saline.

Forward method

In the tile with two cavities added one drop of anti-A serum and anti-B serum in the above marked cavities A and B respectively. Added one drop of about 2% cell suspension in each cavity and mixed the contents thoroughly. The tile was roated for 5 minutes and results were examined macroscopically as well as microscopically for agglutination.

Reverse Method

Removed the serum carefully. Marked the cavities of tile as A and B. Added one drop of serum in each cavity. Added one drop of 2% cell suspension of A and B cells in the respective cavities and rotated the tile for 5 minutes results are examined macroscopically as well as microscopically for agglutination.

Matrix gel card Method

In this method ABO reverse grouping was performed in the buffer gel micro-tubes. Formation of agglutination indicates the presence of an antigen-antibody reaction, while lack of agglutination indicates the absence of an antigen-antibody reaction. Agglutinated red blood cells are trapped in the gel at various levels within the micro-tube depending on the size of the agglutinates. Free non agglutinated red cells pass through the gel and form a button of red blood cells on the bottom of the micro-tube. The control micro-tube must be negative for the results to be valid. In the gel test, the reagent red blood cells are combined with sample serum/plasma in the upper reaction chamber of the micro-tube of card. Following an incubation period to enhance antigen/antibody interaction, the sensitized red blood cells react with the antibodies incorporated in the gel of the micro-tube during a centrifugation step. Agglutination indicates the presence of an antigen/antibody reaction while lack of agglutination indicates the absence of an antigen/antibody reaction. Agglutinated red blood cells become trapped in the gel at various levels within the micro-tube, depending on the size of the agglutinates. Free non agglutinated red blood cells pass through the gel and form a button of red blood cells on the bottom of the micro-tube.

Sample preparation for forward grouping

Prepared 5% of blood cells suspension in matrix diluent LISS, by taking 0.5 mL LISS + 50 µL of whole blood or 25 µL of packed blood cells. Mixed gently and used for forward grouping.

Sample preparation for reverse grouping

Prepared 0.8% blood of cells suspension in matrix diluent LISS as follows: - Washed fresh A, B cells with 0.9% saline till the supernantant is clear. Add 1 mL of LISS in Each labelled test tubes as A and B. Used for reverse grouping.

Procedure

Pipetted 10 µL of 5% blood cell suspension in the microtubes 1 to 4 (A-B- -D- Control). Pipetted 50 µL of 0.8% known 'A' blood cell suspension in the microtube 5. Pipetted 50 µL of 0.8% Known 'B' blood cell suspension in the microtube 6. Pipetted 50 µL of serum of A and B in the microtubes 5 and 6. Allowed the card to incubate for 10 min.at room temperature. The card was removed and the results were recorded.

Agglunation in the forwarding grouping and either haemolysis or Agglunation in the reverse grouping were interpreted as a positive reaction. The results of ABO and Rh type of samples were recorded [15-17].

Results

The reaction strength may be recorded by grading of the Agglutination Reaction Intensities:-

The Red blood cells possesing the corresponding antigen will agglutinate in the presence of specific antibody, and will trapped in the gel column, and gets settle at the bottom of the microtube. The control microtube (Ctrl) must be negative to validate the forwarding results [8-10].

Positive Reaction:- A clear line on the surface of the gel column is formed by the agglutinated blood cells or sometimes it dispersed in the gel column [8-10].

Negative Reaction:- Non Agglutinated red blood cells settle at the bottom of micotube forming a compact button [8-10].

Strength of reaction Comments

G++++ (G4+):- Agglutinated red blood cells form a line at the top of the gel microtube.
G+++ (G3+):- Most agglutinated red blood cells remain in the upper half of the gel microtube.
G++ (G2+):- Agglutinated red blood cells are observed throughout the length of the microtube. A small button of red blood cells may also be visible at the bottom of the gel microtube.
G+ (G1+):- Most agglutinated red blood cells remain in the lower half of the microtube. A button of cells may also be visible at the bottom of the gel microtube.
G± Most agglutinated red blood cells are in the lower third part of the gel microtube.
Negative: - Negative all the red blood cells pass through and form a compact button at the bottom of the gel microtube.

Mixed field agglutination

Agglutinated red blood cells form a line at the top of the gel and non-agglutinated red blood cells form a compact button at the bottom of the gel microtube.

H:- Hemolysis of red blood cells

Out of 200 blood samples (which include Accuse, Victim, Suspect, injured, Deceased, Vitness etc.) cases, 5% (10 out of 200) shows "inconclusive or invalid" results.

Blood Group Type	A	B	AB	O
Samples with correct results	45	80	36	29

Table 1: Number of samples Showing blood group are as follows

Results for:- "A "Blood Group:-

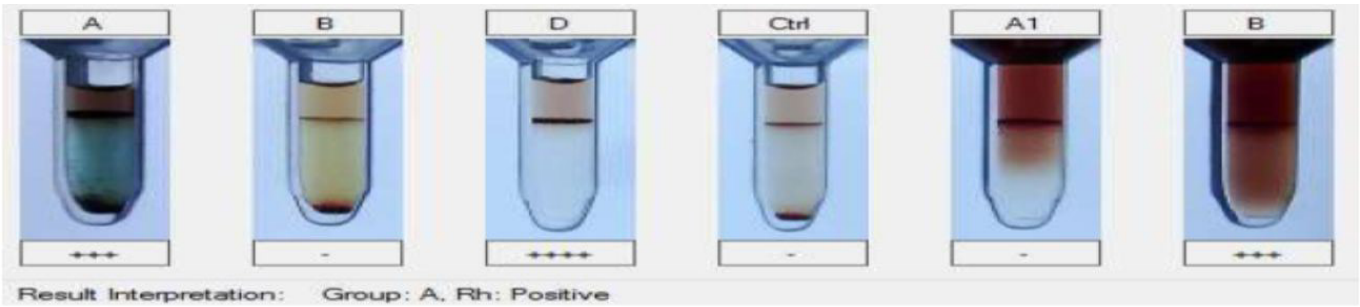

Figure 1: G (4+): Rh; G (3+): A-cells, B-Serum; Negative (-): B-cells, Ctrl, A-serum

Figure 1 shows, G (3+) agglutination reaction in forward A-cells and G (3+) agglutination reaction in reverse grouping B-serum and shows negative reaction for B-cells and A-serum. Hence the blood group is 'An' Rh+.

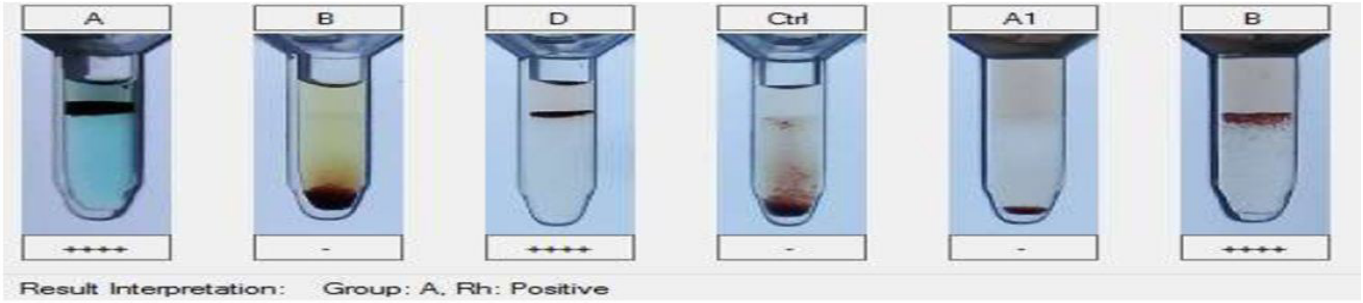

Figure 2: G (4+): A-cells, Rh, B-Serum; Negative (-): B-cells, Ctrl, A-serum

Figure 2 shows, G (4+) agglutination reaction in forward A-cells and G (4+) agglutination reaction in reverse grouping B-serum, and shows negative, reaction for B-cells and A-Serum. Hence the blood group is 'An' Rh+"

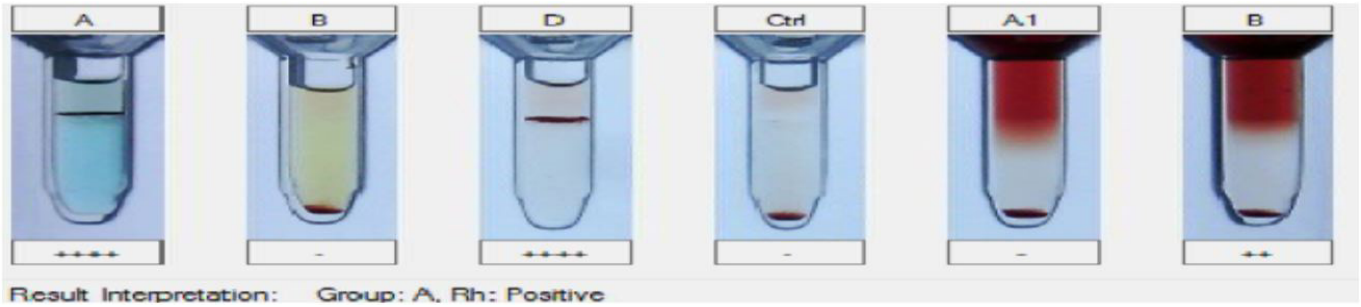

Figure 3: G (4+): A-cells, Rh; G (2+): B-Serum; Negative (-): B-cells, Ctrl, A-serum

Figure 3 shows, G (4+) agglutination reaction in forward A-cells and G (2+) agglutination reaction in reverse grouping B-serum, and shows negative, reaction for B-cells and A-Serum. Hence the blood group is 'An' Rh+"

Results for:- "B "Blood Group:-

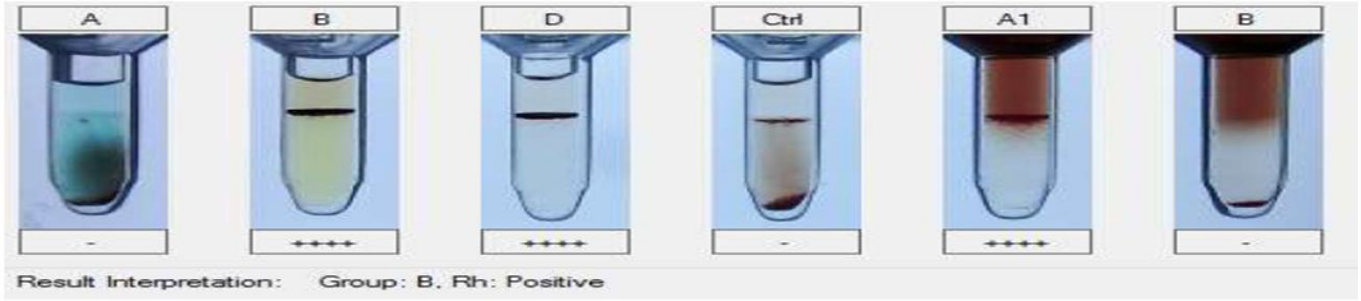

Figure 4: G (4+): B-cells, Rh, A-Serum; Negative (-): A-cells, Ctrl, B-serum

Figure 4 shows, G (4+) agglutination reaction in forward B-cells and G (4+) agglutination reaction in reverse grouping A-serum, and shows negative, reaction for A-cells and B-Serum. Hence the blood group is 'B' Rh+.

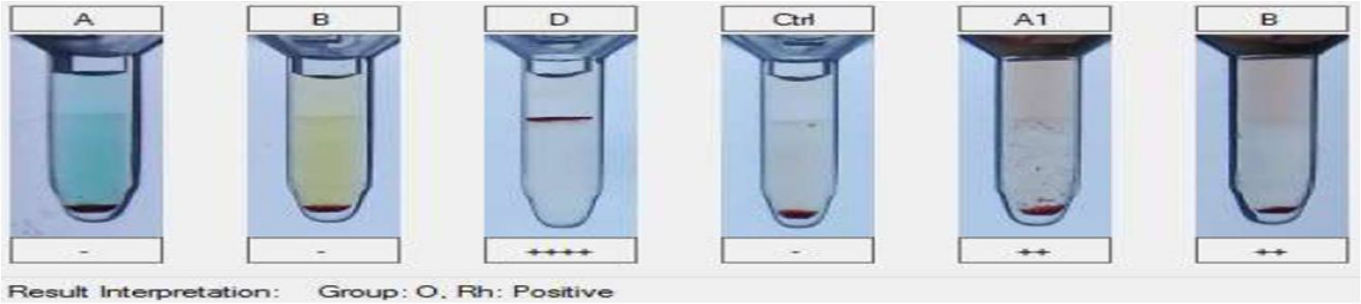

Figure 5: G (4+): B cells, Rh; G (2+): A-Serum; Negative (-): A-cells, Ctrl, B-serum

Figure 5 shows, G (4+) agglutination reaction in forward B-cells and G (2+) agglutination reaction in reverse grouping A-serum, and shows negative, reaction for A-cells and B-Serum. Hence the blood group is 'B' Rh+.

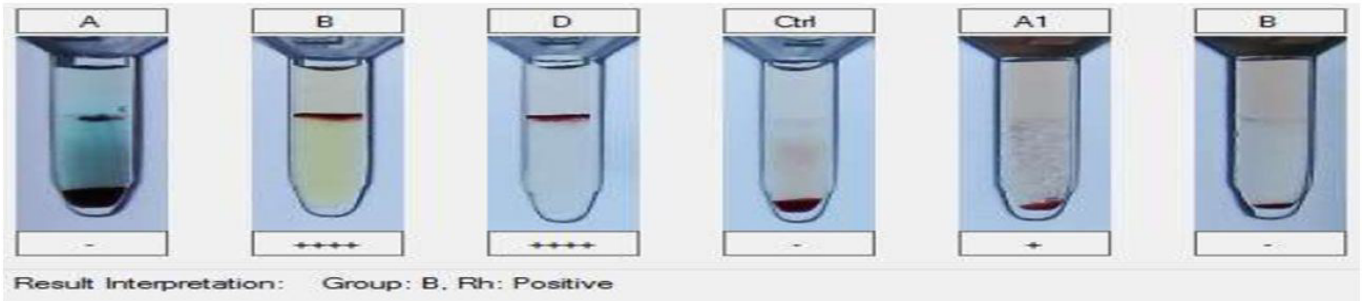

Figure 6: G (4+): B-cells, Rh; G (1+): A-Serum; Negative (-): A-cells, Ctrl, B-serum

Figure 6 shows, G(4+) agglutination reaction in forward B-cells and G (1+) agglutination reaction in reverse grouping A-serum, and shows negative, reaction for A-cells and B-Serum. Hence the blood group is 'B' Rh+.

Results for:- "AB "Blood Group:-

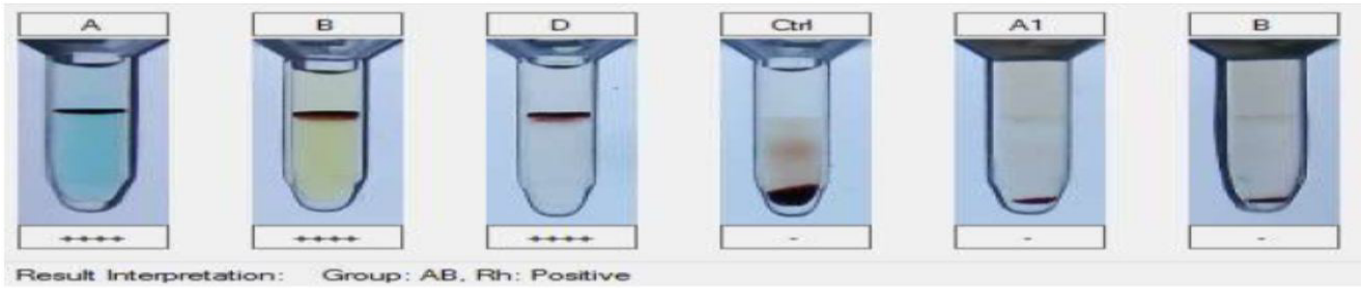

Figure 7: G (4+): A-cells, B-cells, Rh; Negative (-): A-serum, B-Serum, Ctrl

Figure 7 shows, G (4+) agglutination reaction in forward A-cells and B-cells and negative agglutination reaction in reverse grouping for A-serum and B-serum. Hence the blood group is 'AB' Rh+.

Results for:- "O "Blood Group:-

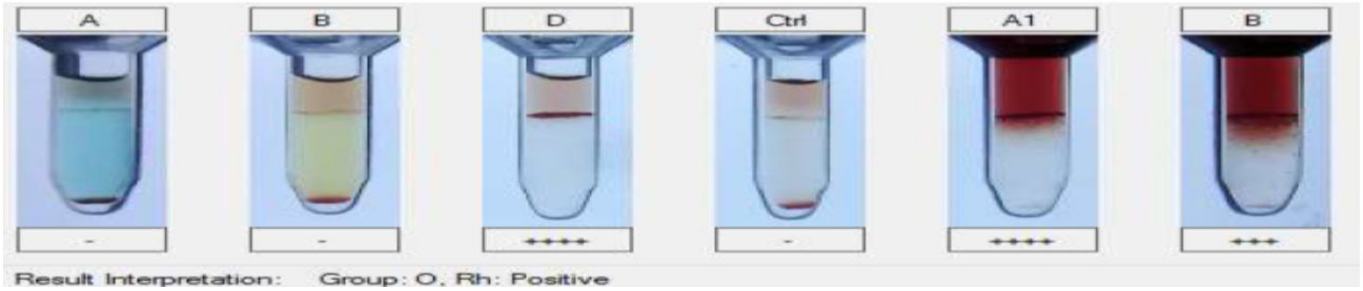

Figure 8: G (4+): Rh, A-serum; G (3+): B-Serum; Negative (-): A-cells, B-cells, Ctrl

Figure 8 shows, Negative agglutination reaction in forward A-cells and B-cells and G (4+) agglutination reaction in reverse grouping for A-serum and G (3+) agglutination reaction for B-serum. Hence the blood group is 'O' Rh+.

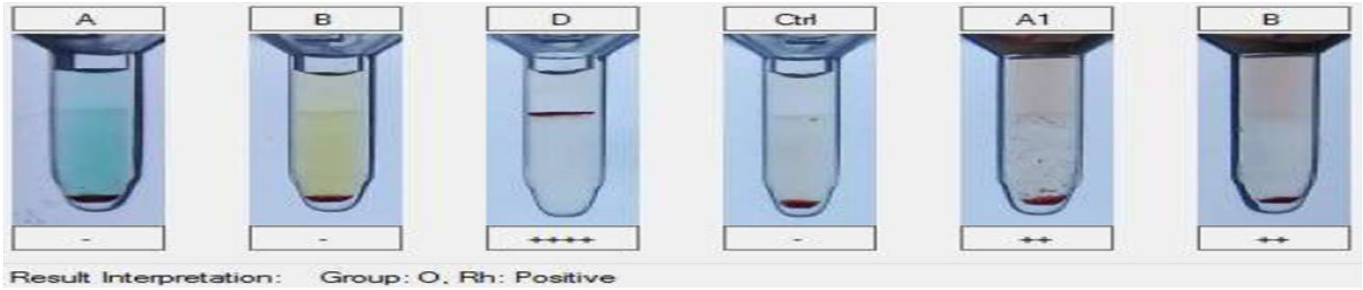

Figure 9: G (4+): Rh; G (2+): A-serum, B-Serum; Negative (-): A-cells, B-cells, Ctrl

Figure 9 shows, Negative agglutination reaction in forward A-cells and B-cells and G (2+) agglutination reaction in reverse grouping for A-serum and B-serum. Hence the blood group is 'O' Rh+.

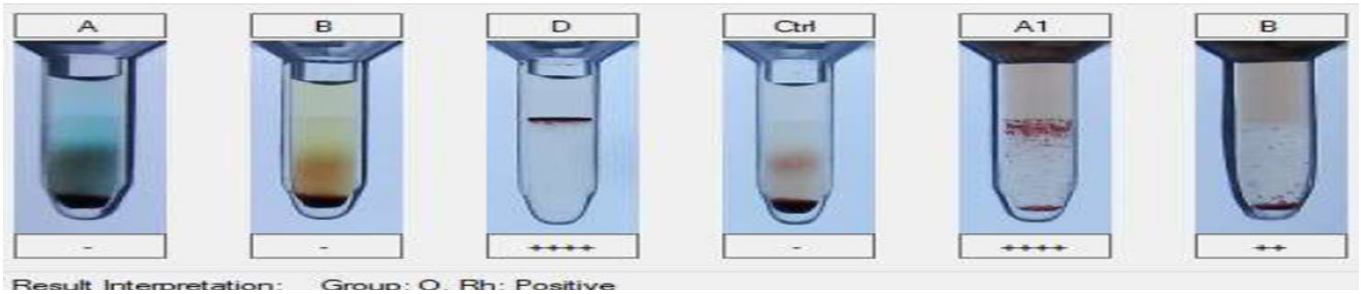

Figure 10: G (4+): Rh, A-serum; G (2+): B-Serum; Negative (-): A-cells, B-cells, Ctrl

Figure 10 shows, Negative agglutination reaction in forward A-cells and B-cells and G (4+) agglutination reaction in reverse grouping for A-serum and G (2+) agglutination reaction for B-serum. Hence the blood group is 'O' Rh+.

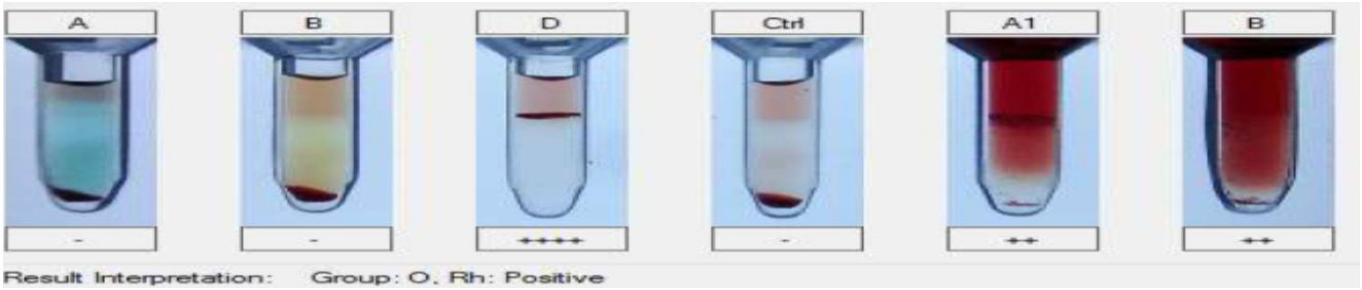

Figure 11: G (4+): Rh; G (2+): A-serum, B-Serum; Negative (-): A-cells, B-cells, Ctrl

Figure 11 shows, Negative agglutination reaction in forward A-cells and B-cells and G (2+) agglutination reaction in reverse grouping for A-serum and B-serum. Hence the blood group is 'O' Rh+.

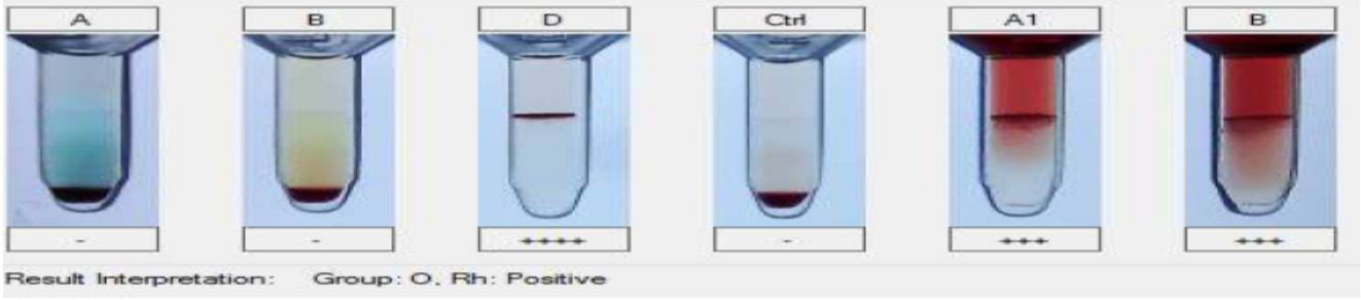

Figure 12: G (4+): Rh; G (3+): A-serum, B-Serum; Negative (-): A-cells, B-cells, Ctrl

Figure 12 shows, Negative agglutination reaction in forward A-cells and B-cells and G (3+) agglutination reaction in reverse grouping for A-serum and B-serum. Hence the blood group is 'O' Rh+.

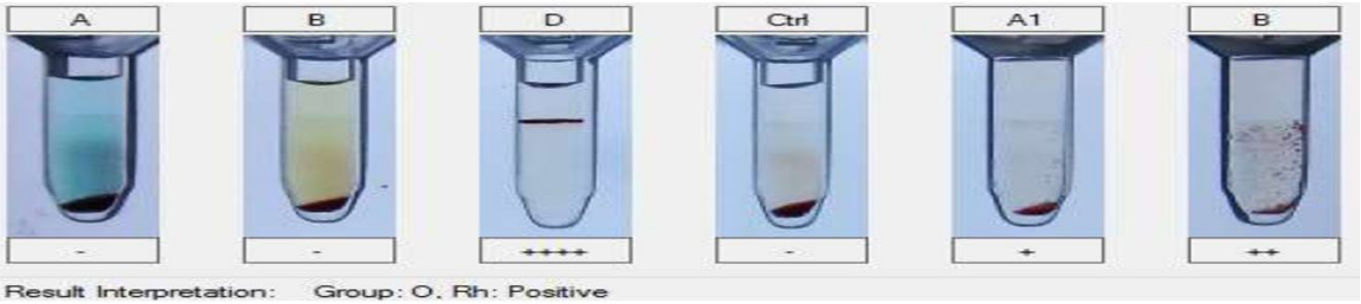

Figure 13: G (4+): Rh; G(2+): B-Serum; G(1+): A-serum; Negative(-): A-cells, B-cells, Ctrl

Figure 13 shows, Negative agglutination reaction in forward A-cells and B-cells and G (2+) agglutination reaction in reverse grouping for A-serum and G (1+) agglutination reaction for B-serum. Hence the blood group is 'O' Rh+.

Results for:- "Inconclusive "Blood Group:-

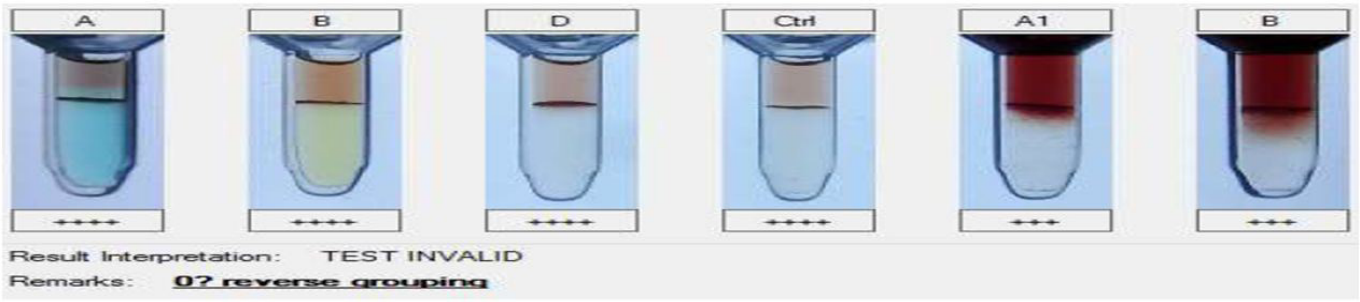

Figure 14: G (4+): A-cells, B-cells, Rh Ctrl; G (3+): A-serum, B-Serum

Figure 14 shows, G (4+) agglutination reaction in forward A-cells and B-cells and G (3+) agglutination reaction in reverse grouping for A-serum and B-serum. Hence the blood group is cannot be concluded.

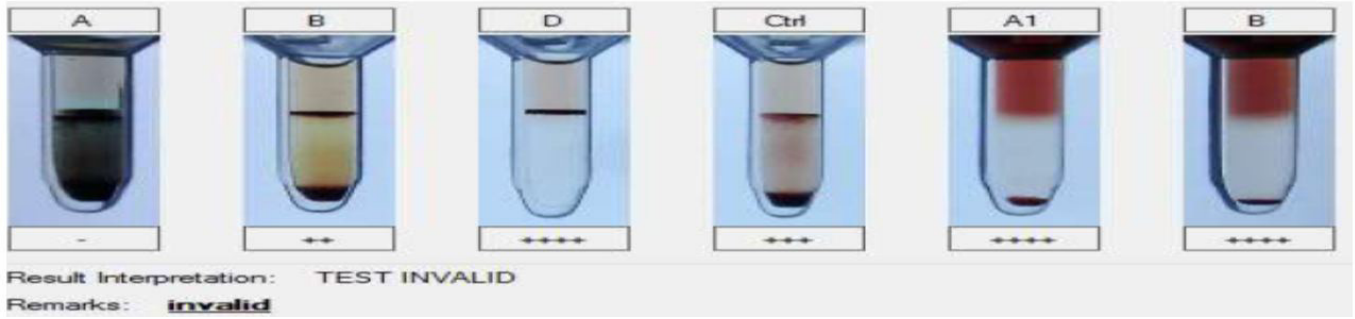

Figure 15: G (4+): Rh, A-serum, B-Serum; G (3+): Ctrl; G (2+): B-Cells; Negative(-): A-Cells

Figure 15 shows, G (2+) agglutination reaction in forward B-cells and negative agglutination reaction A-cells and G (4+) agglutination reaction in reverse grouping for A-serum and B-serum. Hence the blood group is cannot be concluded.

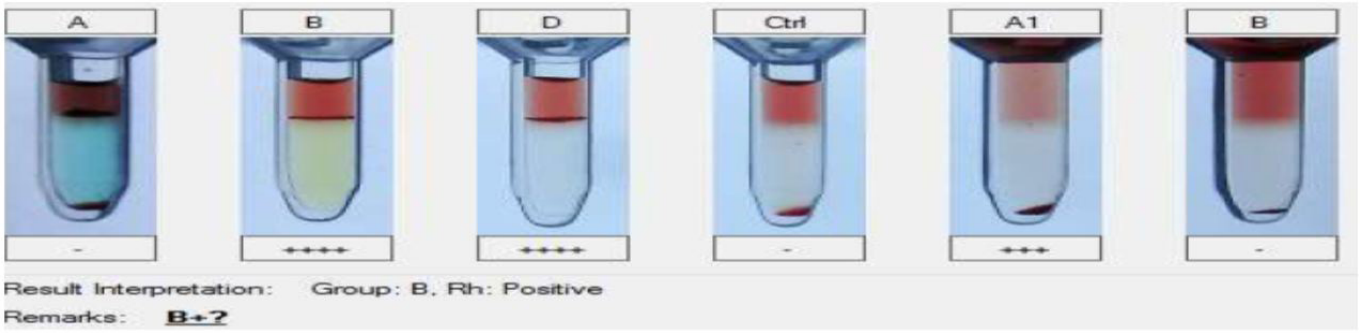

Figure 16: G (4+): B-Cells, Rh; G (3+): A Serum; Negative (-): A-Cells, Ctrl, B-serum

Figure 16 shows, G (4+) agglutination reaction in forward B-cells and negative agglutination reaction A-cells and G (3+) agglutination reaction in reverse grouping for A-serum and negative agglutination reaction for B-serum. Hence the blood group is cannot be concluded

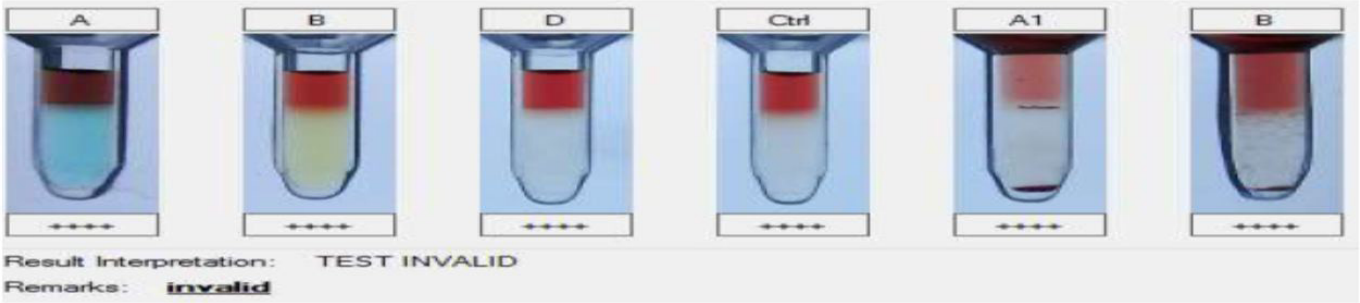

Figure 17: G (4+): A-Cells, B-Cells, Rh, Ctrl, A-serum, B-Serum

Figure 17 shows, G (4+) agglutination reaction in forward A-cells and B-cells and G (4+) agglutination reaction in reverse grouping for A-serum and B-serum. Hence the blood group is cannot be concluded

Discussion

This study presents number of advantages of blood grop cross matching by using Matrix gel card method over routine spin tube method. The hemagglutination in positive wells was strong, so that they were easily seen with the naked eye.

The ideal condition for the agglutination reaction for blood group type "A" is Figure 2, for blood group type "B" is Figure 4. For blood group type "AB" is Figure 7, for blood group type "O" is Figure 12. It was observed that Figure 1 shows G (3+), reaction and Figure 3 shows G (2+) reaction, but when these samples were preocessed, the correct reaction for reverse (Serum) grouping was some times observed difficult to conclude. Similarly the results of samples in Figure 5, Figure 6, Figure 14 shows G (2+), G (1+)... etc reaction and but when these samples were preocessed with routine spin tube method, the very poor agglutination reaction was observed.

Figure 15, Figure 16, Figure 17, and Figure 18 shows the inconclusive or invalid results by both routine spin tube method, and Matrix gel card method.

In our study it was observed that 5% (10 samples out of 200) samples which showed invalid/ Haemolysed / Inconclusive results by our routine spin tube method, was giving correct agglutination by gel card technique. Which is coated in the studies by Kaur R, Rumsey DH, and Malyska H. *et.al*?

It wass observed that Matrix gel card test 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 (Col *et al.*, 2008).

As the Matrix gel test uses an increased serum to cell ratio and there is no need of wash phase, thus reducing possibility of elution of weakly bound antibodies hence the false positive screens of results were reduced using the matrix gel test system. (Bromilow *et al*, 1992).

Matrix gel card test results remain stable within the gel, allowing rereading and it can be photocopied. The disposal of plastic cards is also very easy, which increases standardisation of laboratory techniques and introduces more objective reading of agglutination reaction. (Bromilow *et al,* 1992).

The blood group antigens for ABO and Rh factor were detected upto 12 Months. The number of non-specific anti bodies and false positive sereens of results were reduced using matrix gel system.

Conclusion

From above observations it was concluded that the Matrix gel card technique is more suitable and less time consuming. The results are more stable can be recorded after long time.The test can be carried out with very small sample. In conclusion Matrix gel card test has been shown to be very efficient in forensic fields to get the blood group from the blood samples of Accuse, Victim, injured, Complainer Consisting of Male and Female.

Acknowledgment

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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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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 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 test 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 Lapierre, which was based on controlled centrifugation of red blood cells in sephadex gel contained within microtube gel techniques (Lapierre, 1988; Letich*et 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; Lapierre 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

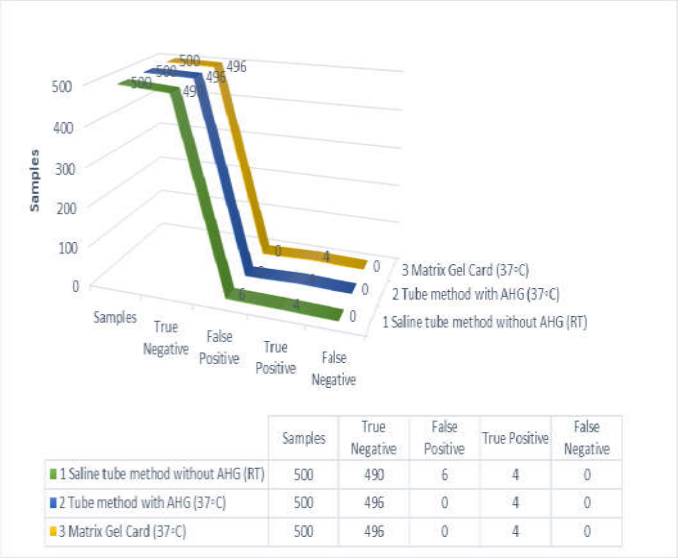
The study based on data analysis total 500 sample randomly selected from day wise round the clock duty wise stored sample of requisition for cross matching blood in blood bank of UPUMS, Hospital, Saifai, Etawah. Most commonly Spin saline tube method are used widely in blood banks. A new technique of 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 37^0C 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 37^0C. 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 37^0C.

Fig. 1. Compatibility shows by Matrix Gel card Technique



	Samples	True Negative	False Positive	True Positive	False Negative
1 Saline tube method without AHG (RT)	500	490	6	4	0
2 Tube method with AHG (37°C)	500	496	0	4	0
3 Matrix Gel Card (37°C)	500	496	0	4	0

Fig. 2. Results plotted in graph

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

Table 1. Result observation

	Method Used	Samples	Compatible		Incompatible	
			TN	FP	TP	FN
1.	Spin saline tube method without AHG (RT)	500	490	06*	04	00
2.	Spin saline tube method with AHG (37^0C)	500	496	00	04	00
3.	Matrix Gel Card (37^0C)	500	496	00	04	00

*Result obtained only if AHG not used with Spin saline tube method otherwise it shows compatible result with AHG.

positive and 04 sample true positive, whereas Spin saline tube with coombs reagent at 37^0C, 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 (Col*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 (Bromilow *et al.,* 191). The testing efficiency improved by the using of the matrix gel test into routine use (Novaretti *et al.,* 2000). It's proved that matrix gel cardsystem also easy to use and its finding suggest more sensitive than the Spin saline tube agglutination technique without AHG (Cate John and Reilly, 1999). Matrix gel card system is better than Spin saline test tube method and simple to perform and less exposure of blood bank personal to blood specially area with HIV, HbsAg and HCV infections (Nathalang *et al.,* 1993). It also concluded that matrix gel card test is better alternative to the Spin saline tube test for blood cross- matching as well as coombs tests (Direct and Indirect) (Jai prakash *et al.,* 2006). In some study author concluded that Spin saline tube method show false negative result but in my study Spin saline tube method provided six false positive, which is different from previous studies.

CONCLUSION

Matrix Gel card is more sensitive and more specific than Spin saline tube methods and also less time consuming but more costly than Spin saline tube methods. Matrix Gel Card technique is more stable and fully recordable for a long period. We can shoot the picture or scan of result and share or stored for further investigations. As per result, time consuming, recording, handling, less exposure, we concluded and advice for use of gel card in various blood banking services as routinely test performed in cross matching for blood transfusion because of high sensitivity and specificity then Spin saline tube methods. 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. The result shows that gel test is more sensitive than tube test for identifying clinically potentially significant of antibodies. Matrix gel card test also 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.

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

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Original article:

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

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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 then 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 microtu-be.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 com-parison 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 Conventio-nal 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 Reage-nt[5][6]) and incubate in 37^0C and then see the result if clumping present in test tube blood bag is incompatible. if not present blood is compa-tible 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 conduc-ting the test Gel card technique**:** first prepare a 0.8% red cell suspe-nsion 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 pati-ent 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 37^0C. After incubation centrifuge the card in Mat-rix gel card centrifuge machine for 10 min-utes 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 betw-een upper and bottom of tube that means somet-hing 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:

	Method Used	Sample Size	Compatible		Incompatible	
			TN	FP	TP	FN
01	Conventional tube method without AHG	1000	992	04*	00	04**
02	Conventional tube method with AHG(IAT)	1000	996	0	04	00
03	Matrix Gel Card	1000	996	0	04	00

*,** 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 AH-G ,1000 sample shows 100% compatibility but in the table 04 sample shows false positive(FP) and 04 sample shows False Neg-gtive(FN) if we Add AHG (IAT) this is calculated after compare the result of conventional tube meth-od with AHG and Matrix Gel card method which shows 996(99.6) sample compatible and 04 (0.4%) True Positive(TP)

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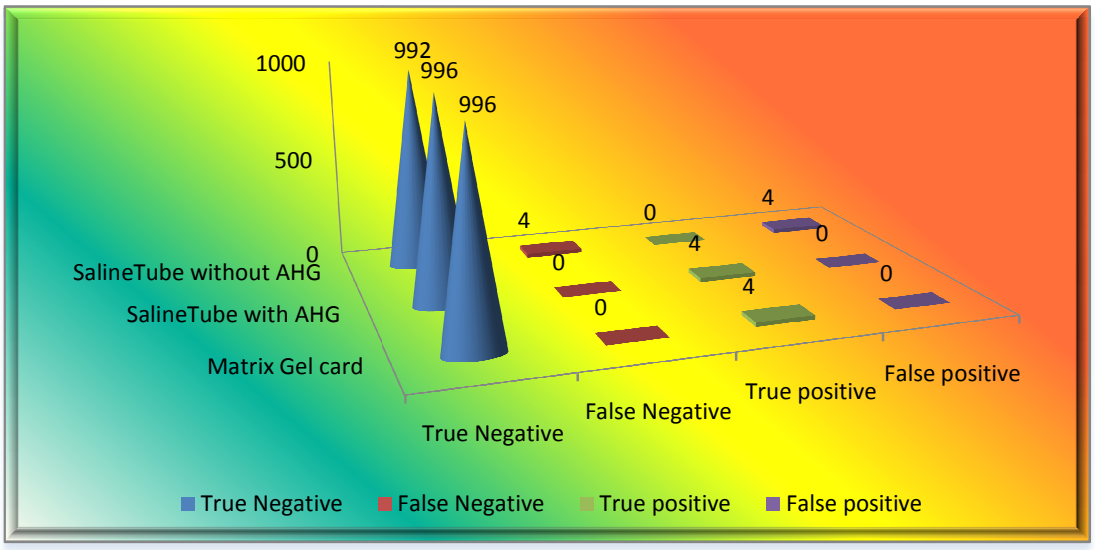

Graph No.01

Observation and result plotted in graph

Discussion:

In India and other country the gel test performed in various institutions and hospitals for blood cross match it is first introduced by **Lapierre et al.[11]** he gives the idea of six micro tube embe-dded in plastic card. Microt-ubes filled with specif-ic gel medium which allows to testing ,easy reading , recording, handling and disposal . In our present study 0.4% sample shows incomp-atibility (agglutination) by gel card method and also convent-ional 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 stu-dy.**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.**Bromillow 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 perform-ed 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 antibo-dies. **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

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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.Over all, 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 vari-ous blood banking services as routine test in cross matching for prior blood transfusion because of high sensit-ivity and specificity than conventional tube methods.

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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.

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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

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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

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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.

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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]

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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 repeatation 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.[1] 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.[6,7]

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 miscelleneous reasons, namely, acute hemorrhage, trauma, dialysis, etc., [Figure 5]. The majority population of anemic patients were suffering from thalassemia (n = 4115, 46%),

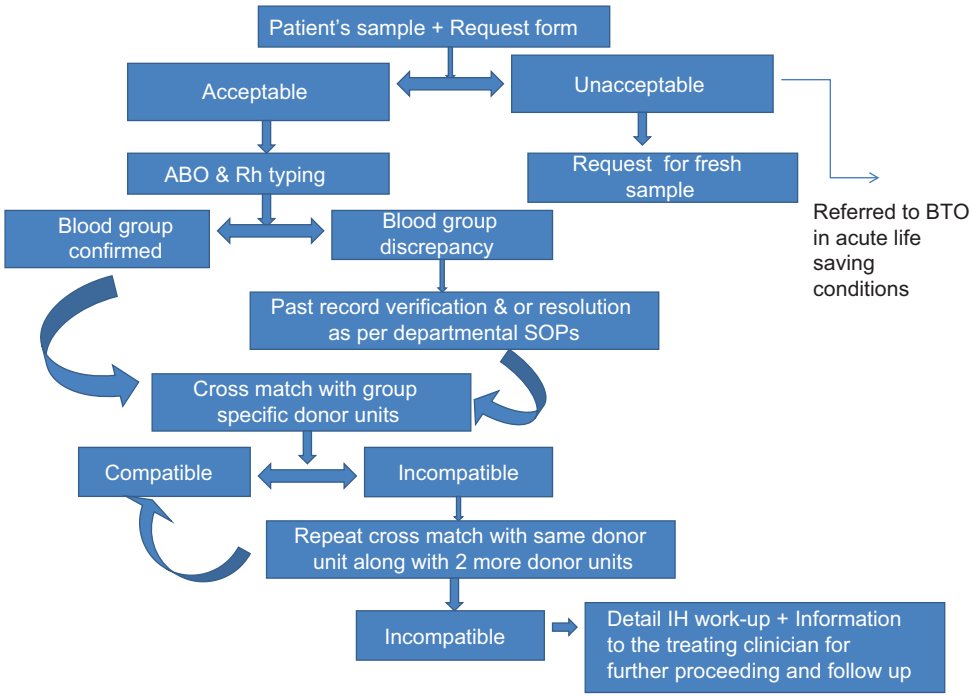

Figure 1: Evaluation of an incompatible cross-match sample

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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.

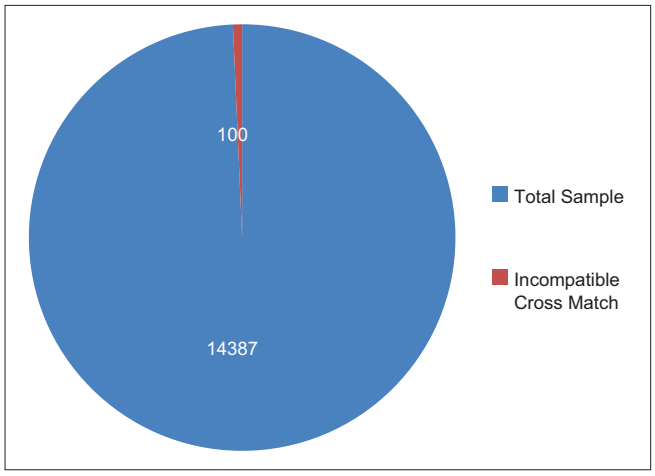


Figure 2: Total sample vs incompatible cross match

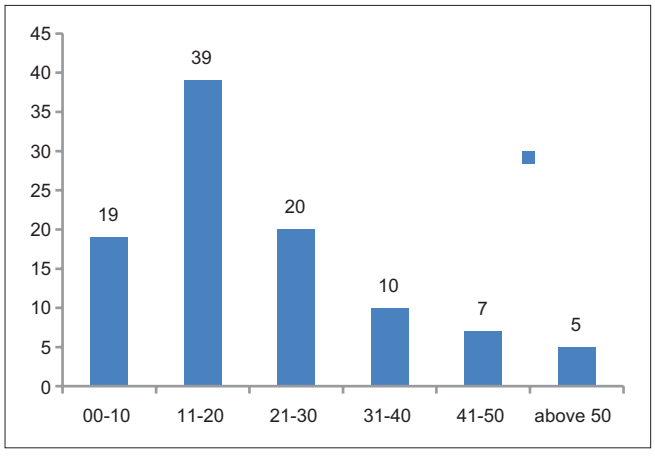
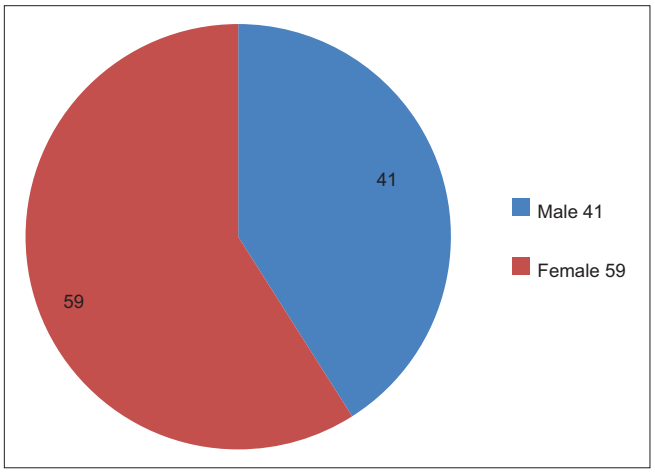


Figure 4: Overall age distribution of 100 incompatible patients



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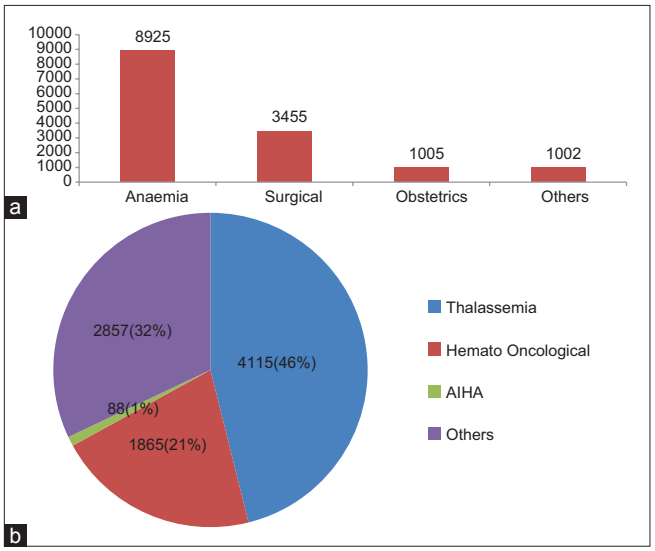

Figure 5: (a) Overall disease distribution in the study population (b) Further distribution of disease under Anemic population

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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

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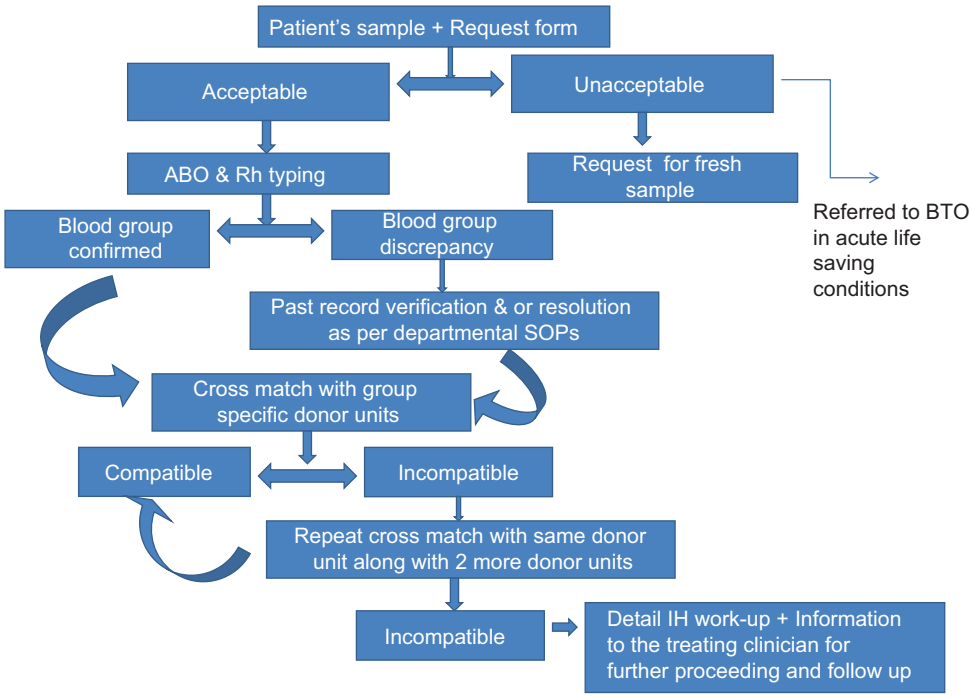

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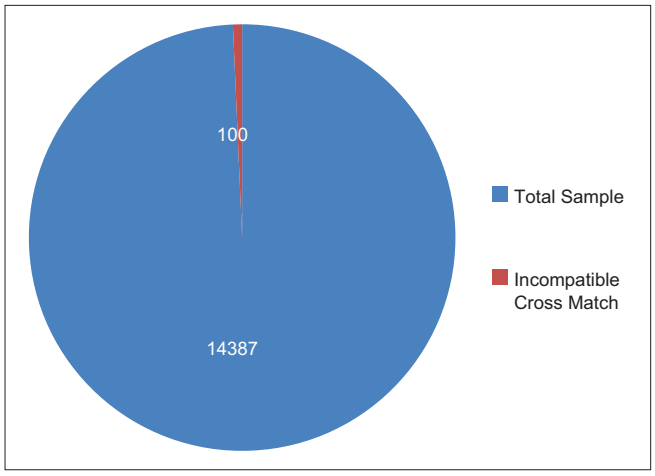


Figure 2: Total sample vs incompatible cross match

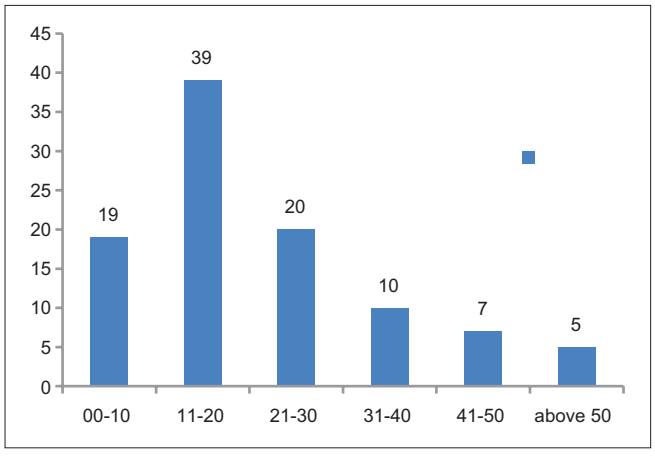
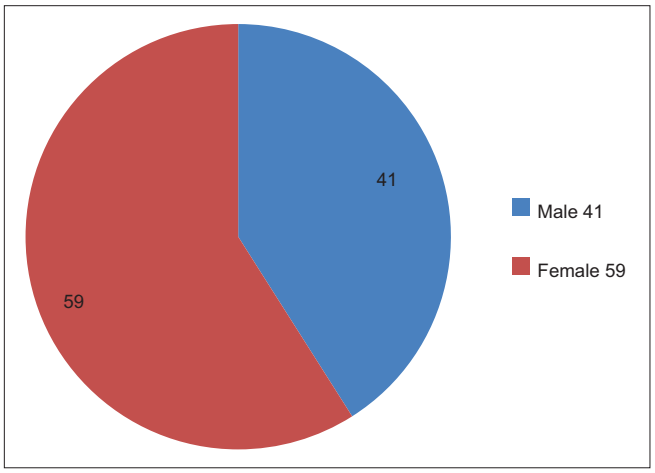


Figure 4: Overall age distribution of 100 incompatible patients



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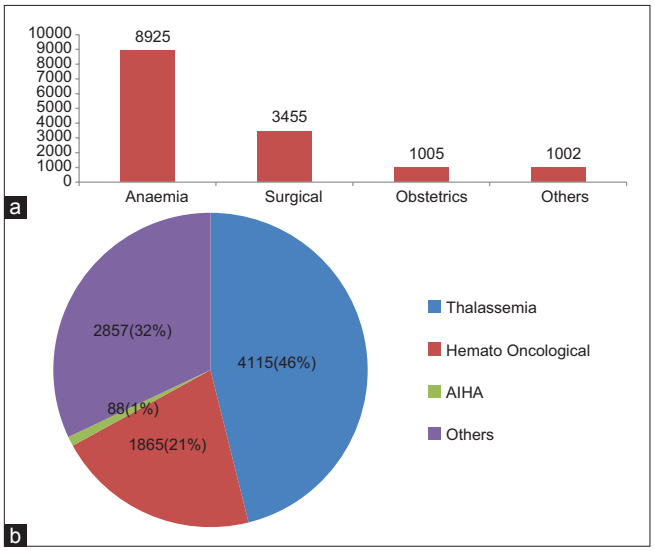

Figure 5: (a) Overall disease distribution in the study population (b) Further distribution of disease under Anemic population

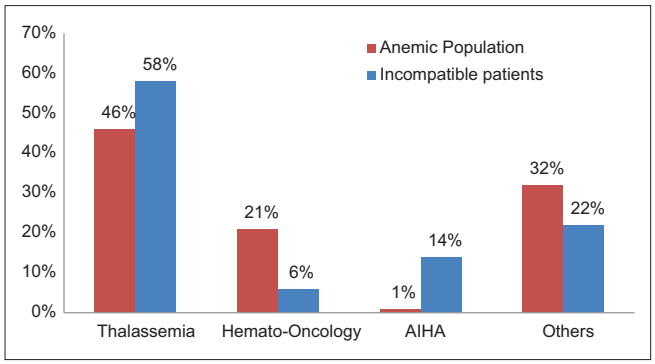

Figure 6: Comparative disease distribution among Anemic population and incompatible patients

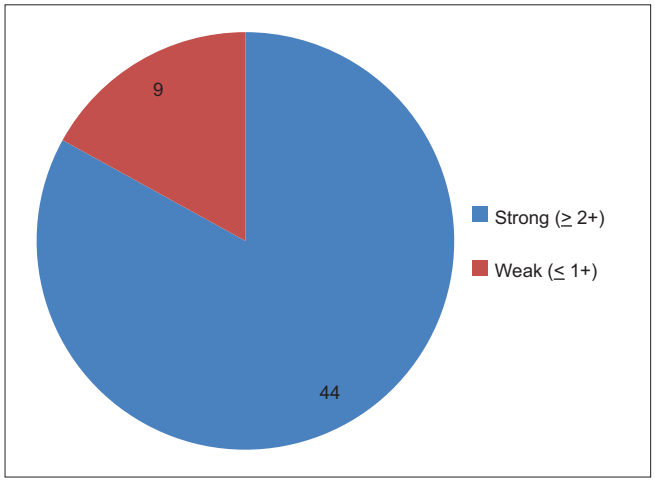

Figure 7: Distribution of DAT +ve samples

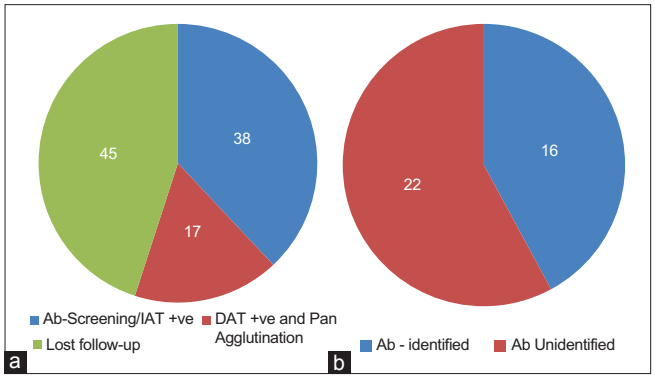

Figure 8: (a) Overall serological status of incompatible samples (*n* = 100) (b) Results of further evaluation of IAT positive samples (*n* = 38)

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/

Table 1: Antibody profile in incompatible cross-match patients

Antibody profile in patients (*n*=16)	Type specificity of antibody
Patients with multiple antibodies (*n*=6)	Anti (c + E) (*n*=5)
	Anti (E + S + N) (*n*=1)
Patients with single antibody (*n*=10)	Anti E (*n*=4)
	Anti c (*n*=3)
	Anti D (*n*=1)
	Anti Kell (*n*=1)
	Anti JK^b (*n*=1)

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

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.

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Conflicts of interest

There are no conflicts of interest.

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ORIGINAL ARTICLE

A STUDY OF IRREGULAR ANTIBODIES IN 200 MULTI-TRANSFUSED PATIENTS

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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.

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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.

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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 (8%).

DISCUSSION: It is a routine practice to perform pre transfusion compatibility testing before blood transfusion to prevent immune mediate 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]

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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]

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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 tovarious reasons & phenotyping of each & every donor was not possible so only cross match compatible blood were issued.

Tables:

Sl. No	Studies	No. of Cases	% of Positive Cases
1	J Shukla et al	81	9.87
2	Pradhan et al	100	8
3	Pahuja et al	211	3.79
4	Gupta et al	116	9.48
5	Dhawan et al	319	5.64
6	V Sangole et al	46	13.04
7	Present study	200	7

Table 1: Incidence of Alloimmunization in Multi-Transfused Patients in various Studies

Studies	Most common Antibody	%
Satyam arora et al	anti kell	35
J Shukla et al	anti C & anti E	50
R Gupta et al	anti E	36.4
B Shenoy et al	anti C & anti kell	43
R Makroo et al	anti E	37
Thakral et al	anti c	39
Present study	anti c and anti D	27

Table 2: Major Antibody type in various Studies

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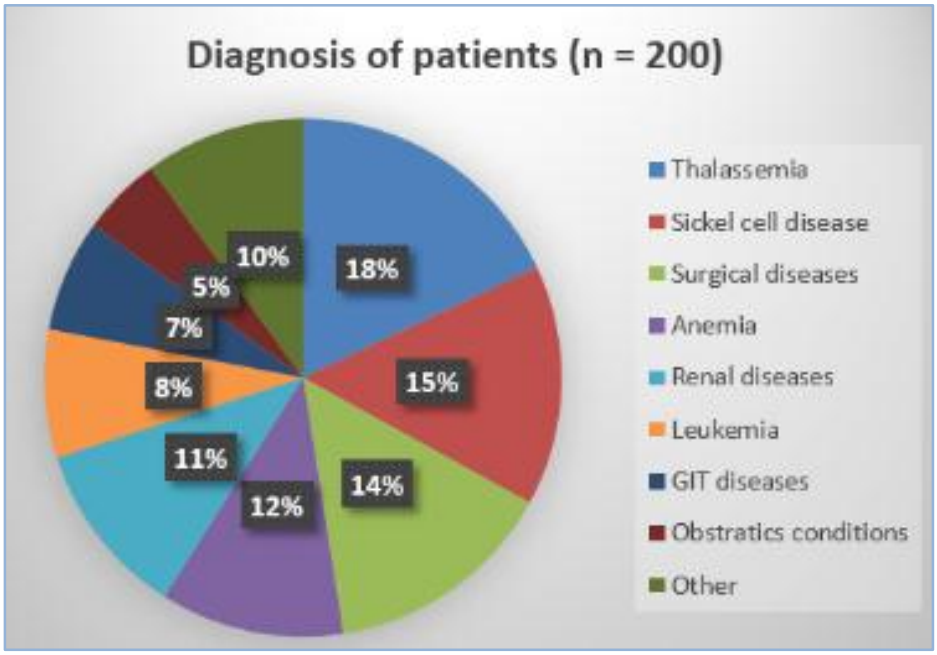

Fig. 1: Diagnosis of Patients (n = 200)

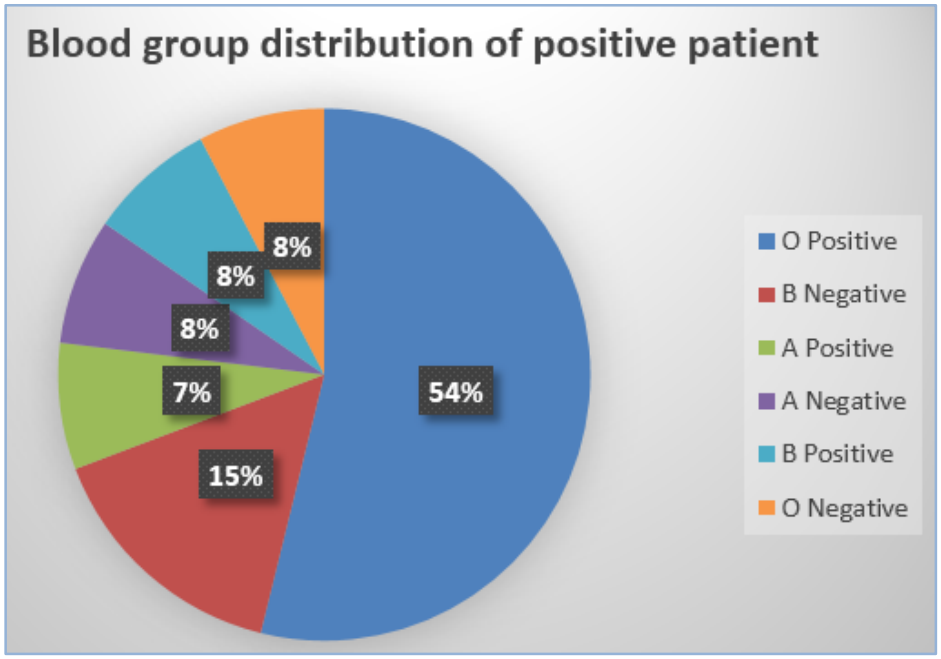

Fig. 2: Blood Group Distribution of Positive Patient

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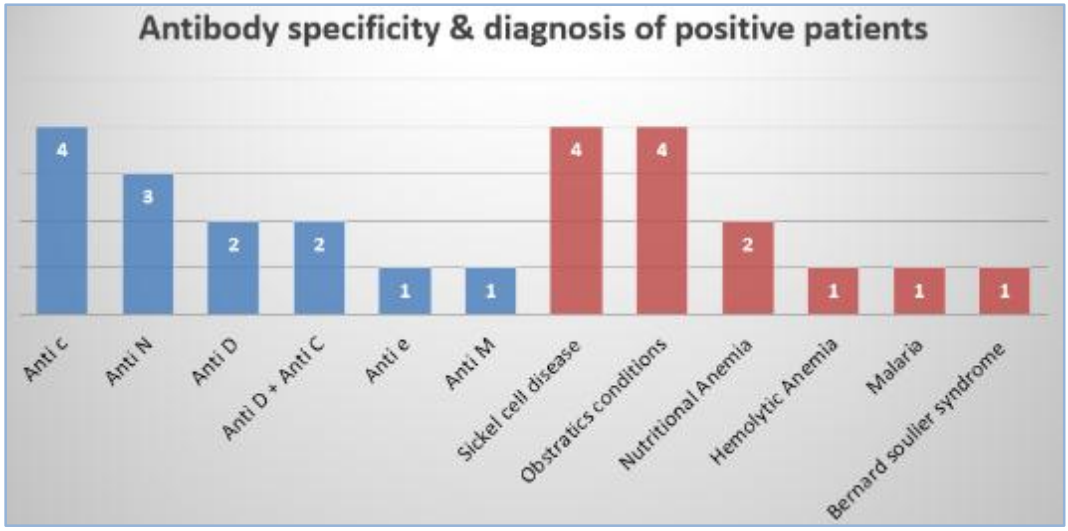

Fig. 3: Antibody specify & Diagnosis of Positive Patients

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ORIGINAL ARTICLE

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ORIGINAL ARTICLE

Gel Card and Saline Tube Techniques for Blood Cross-Matching: A Comparative Assessment Study

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Abstract

Background: Gel card technique of cross matching is one of the latest techniques in blood bank. It has high sensitivity and gives accurate results. The aim of this study is to compare the sensitivity of gel card method with the conventional saline tube method for cross matching in blood bank.

Methodology: 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 anti-human globulin (AHG) and without AHG at Mallasandra urban primary health care center for pre-transfusion cross matching on 100 blood samples.

Result: 100 samples were analyzed. 96 samples were compatible using matrix gel card method and spin saline tube method with AHG. Remaining 04 samples showed incompatibility in both methods. In saline method without AHG, compatibility was seen in 94 samples. Out of the 06 samples which were incompatible, 04 samples were true negative for incompatibility and 02 samples were false positive for incompatibility. Sensitivity and specificity of conventional tube technique (CTT) without AHG was found to be 97.9% and 100% respectively. Sensitivity and specificity of matrix gel card method was found to be 100%.

Conclusion: Matrix gel card method is simple, easy to perform and gives more stable end point result that can be recorded and photocopied. It is more sensitive and specific than CTT. A larger cohort study is necessary to analyze the efficacy of Matrix gel card method over CTT without AHG.

Keywords: Blood transfusion, Blood bank, ABO blood group system, Blood grouping and cross matching, Coombs test, Agglutination.

Introduction

ABO blood grouping system was discovered by Landsteiner during 1901 and the first anti-globulin test was performed in 1945. Since then, more specific serological methods are being developed and to avoid ABO and Rh incompatibility between blood donors and recipient[1] and also to analyze the presence of any antibody in recipient serum which can react with donor red cells and can cause serious complications after blood transfusion.[2] The purpose of cross match is to select

blood component that will have acceptable survival when transfused and will not cause any adverse reaction to the recipient.

Compatibility testing or cross matching is performed to ensure safe transfusion therapy. Cross matching is an integral part of routine pre transfusion testing. The terms "cross matching" and "compatibility test" are sometimes used interchangeably. It is necessary to perform cross match as final serological test of incompatibility even if the blood group of recipient and donor are known, because this will show if any mistakes have been done in ABO grouping, which may cause fatal hemolytic transfusion reactions.[3]

The Conventional tube technique (CTT) has been the cornerstone of compatibility testing over last 40 years, but the enhanced sensitivity of the gel card technique has made the interpretation of the tests more objective.[4] Lappiere et al. introduced the gel card method which is used for cross matching of blood along with saline tube method. The gel card is a reliable, advantageous method and suitable for routine use to detect and identify the alloantibody.[5]

Sephadex gel is used in gel cards which holds agglutinate in semisolid medium and helps in clear visualization of agglutination than that of tube method [4]. When RBCs are added on to a gel card, gel acts as a trap. RBCs which agglutinate are seen trapped in gel at the bottom of the tube, which can be seen for hours. For easy handling, reading and testing there are 6 micro tubes in a single gel card.

Aim of this study is to compare the accuracy and sensitivity of gel card technique (LISS / COOMBS) and saline tube method, also to assess the compatibility test by gel card and saline tube method with coomb's and without coomb's test.

Materials and methods

The present study was carried out at Mallasandra urban primary health care center, Bangalore Karnataka in India to evaluate the matrix gel card technique and compare the matrix gel card method with conventional tube method for pre transfusion compatibility testing.

A total of 100 samples were collected, and compatibility testing was done using CTT and gel card Method. Donor samples were collected from the pilot tubes of the blood donations collected from healthy donors with >45 kg body weight having negative serology of HIV, HBsAg, HCV, VDRL and Malaria. Patient samples were received from the ward with the blood requisition form. Cross-matching was carried out using the technique given below. Blood grouping of patient blood and donor blood with the help of anti-sera A, B, D was done. After confirmation of blood group, cross matching of both donor and patient's blood was carried out by two methods - spin saline tube method with and without AHG reagent and Matrix Gel card method.

All samples were cross matched by the following techniques:

1. Saline Tube Method: Major and minor cross match was performed using saline tube method. After preparing 5% red cell suspension, cross matching was done by adding serum and red cell suspension in the ratio of 2:1 and incubated at 37°C for 30-60 minutes. The tubes were centrifuged and observed for agglutination. The cells were washed 3-4 times to remove any unbound antibody. After adding AHG, the tubes were centrifuged, and were observed for agglutination. Check cells were added to all negative tubes for cross verification.
2. Gel card technique for Coombs test - The micro tubes of the ID-Card LISS / Coombs containing poly specific AHG, were used for cross matching. Patientserum and donor red cells were added to the micro tubes. The card was incubated at 37^0C for 15 minutes, centrifuged for 10 minutes and results were observed.

Statistical Analysis was carried out using frequency percentage and chi square test.

Results

In our study, 100 blood samples were cross-matched using Spin saline tube method with and without AHG and Matrix Gel Card. We compared both methods of cross-matching for sensitivity & specificity, the accuracy of results, and time taken. 96 (99.2%) samples were compatible, and 04 (0.8%) samples were incompatible in Gel card method. In CTT, 94 samples were compatible, and 06 samples were incompatible without AHG. In CTT with AHG 96 (99.2%) samples were compatible and 04 samples (0.8%) were incompatible.

In our study, both sensitivity and specificity of matrix gel card method was found to be 100%. Whereas sensitivity of CTT without AHG was 100% but specificity was found to be 97.9%. Further positive predictive value and negative predictive value of gel card method was 100% and positive predictive value and negative predictive value of CTT without AHG was 66.6% and 100% respectively.

Table 1: Comparison of Compatibility and Incompatibility of three methods

Technique	Compatible samples	Incompatible samples	Total samples
Spin saline tube without AHG (Room Temperature)	94	06	100
Matrix Gel Card (37°C)	96	04	100
Spin saline tube with AHG (37°C)	96	04	100

Table 2: Sensitivity and Specificity of three methods.

Technique used	Compatible		Incompatible		Total
	TN	FP	TP	FN	
Spin saline tube without AHG (Room Temperature)	94	02	04	00	100
Matrix Gel Card (37°C)	96	00	04	00	100
Spin saline tube with AHG(37°C)	96	00	04	00	100

Discussion

CTT is the most commonly used method for pre-transfusion compatibility testing, but it is associated with certain disadvantages like being laborious and time-consuming method. These are nullified in gel card method.

In our study, 94 samples showed compatibility in CTT without AHG and considered as true negative. Out of 06 samples which were incompatible, 04 samples were found to be incompatible, and 02 samples were compatible, when subjected to CTT with AHG. Hence these two samples appear to be false positive. The findings of our study are in concordance with the studies conducted by Gond SK *et al*[06], Singh DN *et al*[07], Dhariwal SK *et al*[08], Gulati P *et al*[09], Singh R *et al*[10], Sharma R *et al*[02]. The benefits of gel cards include simple micro tube reading, convenient long-term recording, handling, and disposal. Hence specificity and sensitivity of Gel card method is found to be higher than CTT without AHG. The Two false positive results can be attributed to technical insufficiency while performing the test. Results of this study is compared with other studies as shown in Table 3.

Table 3: Comparison of three methods with other studies

Technique Used	CTT		ICT		Gel Card	
	Com	Incom	Com	Incom	Comp	Incom
Our Study	94	06	96	04	96	04
Gond SK *et.al*[06] (N=1000)	992	08	996	04	996	04
Singh DN *et.al*[07] (N=500)	490	10	496	04	496	04
Dhariwal SK *et.al*[08] (N= 800)	792	08	796	04	796	04
Gulati P *et.al*[09] (N=1295)	1295	00	1288	07	1288	07
Singh R *et.al*[10] (n = 500)	497	03	497	03	497	03
Sharma R *et.al*[02] (n = 600)	600	00	597	03	597	03

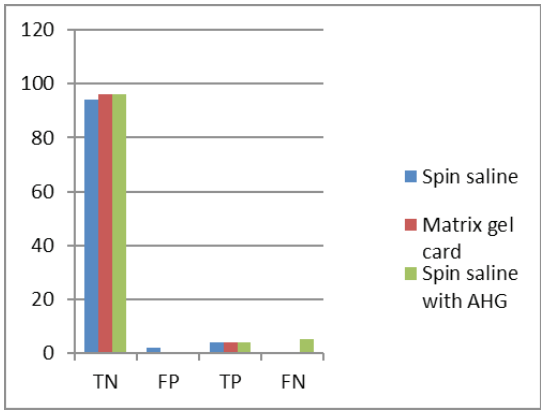

Figure 1: Sensitivity and Specificity of three methods.

Conclusion

Gel card is more sensitive and more specific than conventional tube methods and also less time consuming. The results of Gel card method is on par with results of CTT with AHG. This method is simple to perform, gives reliable, reproductive, stable end point result which can be preserved and photocopied for future record. Moreover gel cards are easy to dispose by incineration thus preventing blood bank personnel from exposure to transfusion transmitted diseases.

Gel card method of compatibility testing can be reliable

method to check for pre transfusion cross matching in health centers with high workload as it is can be carried out quickly and easily. It can also be used in suspected cases along with CTT with AHG method for confirmation of compatibility in centers which cannot afford routine gel card. As the results of our study were in concordance with previous studies, we conclude that matrix gel card method testing is more sensitive and specific. A larger cohort study is necessary to analyze the efficacy of matrix gel card method over CTT without AHG.

Limitations of the present study: Low sample size and high cost of matrix gel card when compared to CTT method.

Conflict of Interest

Nil

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COMPARISON OF BLOOD CROSS MATCH USING GEL TECHNIQUE AND CONVENTIONAL TUBE METHOD IN SSIMS, BHILAI, C.G.: CROSS SECTIONAL STUDY

Pathology

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ABSTRACT

INTRODUCTION- Conventional tube methods are used widely for blood cross match. Now a days, a new technique (Gel card technique) of cross match is introduced. In this study, matrix gel card method is compared with conventional tube method for cross match. **MATARIAL METHODS-** Comparative study of blood cross match between conventional tube method and gel card technique was undertaken on 800 sample, in Shri Shankaracharya medical college blood bank unit, bhilai, Chhattisgarh. **OBSERVATION-** Comparative cross match was done in all the 800 sample by both conventional and matrix gel card method. Out of 800 sample ,796 sample came out to be compatible by both methods, while 04 samples were found to be compatible by conventional tube cross match method, but these 04 sample's came out to be incompatible by gel matrix cross matching method. **CONCLUSION-** Gel card method is easy to perform, read, and record. Gel card method is more specific and accurate. While conventional tube method is sensitive but it has many disadvantages like it is more time consuming, results are difficult to appreciate(subjective) and this method is not suitable for future record keeping.

KEYWORDS

cross match, matrix gel card test, conventional tube method

INTRODUCTION –

The study was done in SSIMS blood bank, to implement newly introduced technique called Matrix Gel card technique for blood cross match. Previously, conventional tube methods were used for blood cross match which is mainly saline tube method (Spin tube method) and indirect coombs tube method.

Since its inception by Lapierre et al. in 1988, the gel test has revolutionized pretransfusion testing and has become a widely-used serological testing method in immunohematology laboratories worldwide. The benefits of using gel for antibody screening have been widely published and include ease of use, reduced number of procedural steps, reduced sample size, ease of reaction readability, and increased antibody detection sensitivity. [1]

According to the AABB Technical Manual, it is imperative that antibody detection systems used by each laboratory be sufficiently sensitive.[1]

Since the discovery of the ABO system and red cell agglutination by Landsteiner in 1901 and development of the antiglobulin test by Coombs in 1945, the immunohematologists are trying to establish and improve various serological investigations in human blood.[2]

Basically, these procedures try to establish the compatibility between donor and recipient ABO and Rh systems and to rule out the existence of antibodies in the recipient's serum that could react with transfused red cells. To establish the ABO and Rh compatibility between donor and recipient, both the recipient and the blood to be transfused are typed to rule out the existence of antibodies (other than anti-A or anti-B).[3]

Crossmatch is done to ensure that particular unit of blood may be safely transfused to a patient, normally, group specific blood, ABO and Rh (D). The purpose of cross match is to select blood components that will have acceptable survival when transfused and will not cause harm to the recipient. Compatibility (pre transfusion) testing is done to ensure safe transfusion therapy.4 The pretransfusion compatibility testing is performed to select red blood cell (RBC) units for transfusion to decrease the incidence of immune-mediated hemolytic reactions.5

Gel card is more sensitive and more specific than conventional tube methods and also less time consuming but more costly than conventional tube methods.[6]

Cross matching of blood is routinely done using tube method, which involves washing steps and takes 60 min (1hour). In gel card technique washing steps are not required and the time taken is only 30 min 7. The gel test is a reliable and advantageous technique and is appropriate for routine use for detection and identification of all-antibodies in blood bank service.[8]

The gel card technique is now considered better and has been introduced as a replacement to conventional tube technique on an automation platform. Though Conventional tube technique (CTT) is still considered gold standard in pretransfusion testing, it still has various disadvantages and depends on accurate hand to eye work of the laboratory personnel.

Therefore, this paper aims at comparing the accuracy and sensitivity of gel card technique and saline tube method which can be achieved with the following objective:

I. To assess the compatibility test by matrix gel card method and saline tube method with and without AHG by its sensitivity and specificity.
ii. To support matrix gel card test related studies.

Materials and Method:

Type of study: cross sectional

Place of study: SSIMS blood bank unit of SSIMS, Bhilai

Duration of study: 1 year (01 June 2018 to 31 May 2019)

Study subjects: A total of 800 random sample selected and collected from donors attending SSIMS blood bank unit of SSIMS, Bhilai

Sample size: 800

Statistical method: data was collected, compared, and analyzed using appropriate test

Inclusion criteria: Donors were healthy, have >45 kg weight, with >12.5 gm% Hb and with negative serology for HIV, HCV, HbsAg, VDRL and Malaria.

Methodology: In present study, first blood grouping was performed by using Antisera A, B, D of patient blood and donors blood bag. After

matching of blood group, cross matching of the donor and recipient blood was done by using two methods, first was Conventional tube method with AHG (IAT) and without it. Second method was Matrix Gel Card method which has been newly introduced in our blood bank. In Conventional tube method, first mark the patient and donor test tube with marker , then centrifuge both blood sample to extract the serum of patient and donor red cells, following which mixing of patient serum and red cells of donor in clean test tube was done and then in that add the Anti Human globulin (AHG, Coombs Reagent) and incubate at 37oc and then finally read the result. If clumping was present in test tube, blood bag was incompatible, if not present than blood was compatible for patient. Second method used was Matrix Gel Card method. In this method special machine was used for centrifuge of Gel Card and also incubator for Gel card, LISS, test tubes and micropipette. As per procedure; first prepare a 0.8% red cell suspension by adding 1ml diluents-2 in to clear test tube then add 10µl of packed red cells of donor by micropipette 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-degree C. After incubation, centrifuge the card in Matrix gel card centrifuge machine for 10 min and then read the result. If gel card results show, RBCs were settled at bottom of particular micro tube means [no agglutination (Negative result)] 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.

Observation and Results:

This study was done in Shri Shankaracharya Institute of Medical Sciences Blood Bank Unit during period of 1 year from June 2018 to May 2019. A total of 800 samples were included in the study.

TABLE 1: comparative results of blood cross match by using Matrix gel card method and conventional tube method.

Method used		Sample size	Compatible		Incompatible	
			TN	FP	TP	FN
01	Conventional tube method without AHG	800	792	04	00	04
O2	Conventional tube method with AHG	800	796	00	04	00
03	Matrix gel card	800	796	00	04	00

As shown in Table no.1, 800 random blood sample were cross matched by using conventional tube method with and without AHG (IAT) and matrix gel card.

Conventional tube method without AHG showed 100% compatibility, but when AHG (IAT) was added then 04 samples showed false positive (FP) and 04 samples were false negative (FN). This was calculated by comparing the observation of conventional method with AHG and Matrix gel method which showed 796 samples compatible while 4 samples to be true positive i.e. incompatible.

DISCUSSION:

Lapierre et al. was the one who first introduced Gel test by giving idea of six microtube filled with specific gel embedded in plastic card. This has made test easy to read, record, handle and even easy to dispose. In, India, this gel card test has already taken over conventional method.[9,10]

In this study, 0.5% of samples shows agglutination (incompatible) by conventional tube method using IAT and also by using Gel card method. Whereas tube method (spin tube) without IAT showed 100% compatibility which is considered false result after performing other two test (tube test with IAT and Matrix gel method). Sensitivity and specificity of tube test with IAT and Matrix gel card method found to be 100% in this study. In study done by Rumsey DH et al. and Kaur et al., concluded that gel card method is as sensitive as IAT tube test with better sensitivity and specificity, which is similar to this study. [11,12]

Cate et al. showed testing efficiency was improved following introduction of gel test into routine use.[13]

Bromillow IM et al. concluded that false positive results were reduced using gel card test. He also concluded that antibody titers of gel card test were more sensitive than tube IAT method, but our study results showed similar sensitivity of both tube IAT method and gel card test method. This requires further investigation.14,15Overall, this study is found to be in agreement with previous studies.[16,17,18,19]

CONCLUSION:

Gel card method is more sensitive and specific than conventional tube methods. It is also less time consuming, easy to perform, has reproducible results, more user friendly and easy to interpretate. Thus, gel card method is recommended over conventional tube method.

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Original Research Article

A comparative study between gel card method and manual method for Coomb's test

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ABSTRACT

Background: The main objective of this study is to compare the Gel Card method and the Conventional Tube method for Coomb's test. The standard procedures were being followed while performing the above mentioned two test. Based on an 8 months study, Gel card method was proven to be more reliable in concordance with its calculated p-value and the sensitivity. The advantages and disadvantages of which have been mentioned in the following.

Materials and Methods: For Gel Card method, the principle of saphadex gel as a semi-solid medium is being used to trap any agglutination. For the Conventional Tube method, the SOP was being followed involving cell washing and confirmation via microscopy for any micro-clumps. The use of polyspecific antiglobulin was implemented for both the above test.

Results: The study showed p-value for Gel Card method to be < 0.05 which proves to be significant and the sensitivity of Gel Card method was also better compared to the Conventional Tube method.

Conclusion: Our study showed that gel card is more sensitive, easy to perform and lesser time consumption, lesser sample volume plus standardized reporting. Results of Gel card can be preserved for 3-4 days. Avoidance of interobserver variability is also an added benefit due to the standard grading system. It is therefore an excellent method for detecting agglutination compared to the Conventional Tube method.

1. Introduction

Currently, the immunohematologists are trying to establish as well as improve majority of the serological investigations, after the discovery of ABO system and RBC agglutination by Landsteiner in 1900 and by Coombs et al. in 1945, respectively.[1]

The principle of the Coomb's test is demonstration of antibodies or complement coating red cells using Coombs reagent or Antihuman globulin.[2]

Technically various modifications have been made to bring about added sensitivity including the use of more specific reagents like monospecific AHGs.[3]

A study done by Lapierre et al. in 1990, showed improved reliability of Gel Card when correlated with Conventional tube results for detecting a variety of clinically significant known antibodies.[2]

Historically, for immuno-haematological studies, like DCT and screening of antibodies in transfusion medicine, conventional tube method was used as the standard technique.[4]

But it is time-consuming, in need of an experienced personnel to interpret the results which makes it difficult to automate and involves many cell washing steps.

Gel Card is however an easy and sensitive technique that surpasses the above disadvantages and induces agglutination by the uses gel filtration media impregnated with an antihuman globulin reagent.[1]

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2. Aim of the Study

To compare the test results of Coomb's test done by gel card method and by manual method.

3. Objective

To study the findings of Coomb's test done by Gel Card method and Conventional tube method and to compare the result of the above two methods.

3.1. Indications[5,6]

1. Hemolytic anemia
2. Erythroblastosis fetalis (hemolytic disease of the newborn
3. Infectious mononucleosis
4. Chronic lymphocytic leukemia or similar disorder
5. Mycoplasmal infection
6. Systemic lupus erythematosus
7. Syphilis
8. Transfusion reaction, such as one due to improperly matched units of blood

4. Materials and Methods

A comparative study was done in the Blood Bank of BVDU (Deemed to be University) Medical College and Hospital, Sangli, India, for a duration of 8 months from March 2022 to October 2022, where in all the samples subjected for Coomb's test, either DCT or ICT or both were considered in this study. Both Direct and Indirect Coomb's test performed by Gel Card method and Conventional tube method.

1. **For Gel card method**: blood sample, plastic microtube with 6 wells, micro pipette as per volume, LISS diluent, incubator (card warmer), timer, centrifuge machine, card reader.
2. **For Conventional tube method**: blood sample, isotonic saline, clean dropper, clean test tubes, already prepared 'O' red cell suspension, incubator, Anti-human globulin, centrifuge machine, timer, microscope.
3. Clotted samples, insufficient quantity, wrong bulb sample and more than 24 hours sample (without refrigeration) were some of the exclusion criteria considered in our study.

4.1. Procedure by gel card method

Sample preparation for Direct and Indirect Coomb's test.
Prepare 0.8% red cell suspension in LISS as follows:

1. Dispense 1 ml of LISS in a clean labelled test tube.
2. Add 10 microL packed cells and mix gently.

A. Gel Card Method for DCT:-

1. Observe the card for appearance of gel and label with patient's details and remove the foil seal as instructed.
2. Pipette 50 microL of patient's red cell suspension (0.8%) into the labelled microtube.
3. Centrifuge the car in Matrix Cerd Centrifugr for 1 cycle (10 minutes)
4. Read the reaction.

B. Gel Card Method for ICT:-

1. Observe the card for appearance of gel and label with patient's details and remove the foil seal as instructed.
2. Pipette 50microL of suspension (0.8%) od fresh pooled and washed "O" cells into the labelled microtube. Pipette cell suspension at 45 degrees angle.
3. Add 25 microL of patient's serum/plasma into the microtube at 90 degrees angle.
4. Incubate at 37 degrees for 15 minutes in Matrix Card Warmer.
5. Centrifuge the card in Matric Card Centrifuge for 1 cycle (10 minutes).
6. Read the reaction.

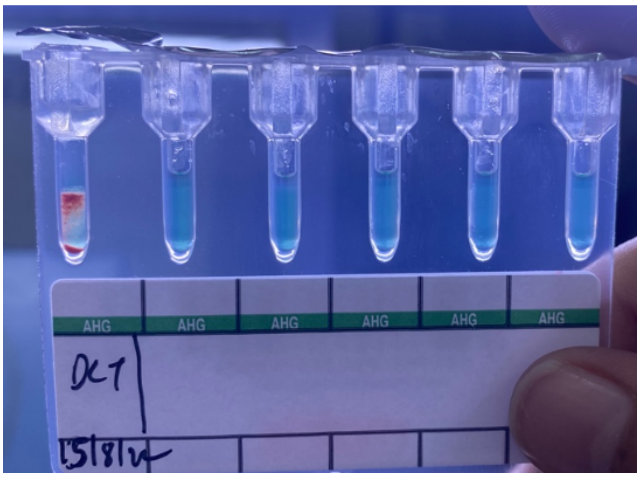

Fig. 1: Gel card showing 6 wells, with + 2 grade for DCT on the first well

Table 1: Grading for gel cardmethod[7]

Grading	Agglutination	Interpretation
0	No agglutination	Compatible
+ 1	Agglutination of red cells in the lower half of the gel card	Incompatible
+ 2	Agglutination of red cells through the entire length	
+ 3	Agglutination of red cells in the upper half of the gel card	
+ 4	Agglutination of red cells in the lower half of the gel card	
Hemolysis	-	Invalid

4.2. *Procedure by conventional tube method*

A. Conventional Tube Method for DCT :-

1. Label three test tubes as T (test serum), PC (positive control) and NC (negative control)
2. Positive control – 1 drop of Rh positive cells + 1 drop anti – D
3. Negative control – 1 drop of Rh positive cells + 1 drop of Bovine albumin
4. Test – take two drops of blood to be tested in a clean labelled tube.
5. Wash the red cells 3 – 4 times in a large volume of saline to remove free globulin molecules. Discard off all the supernatant after each cell wash including the final cell wash.
6. Add 2 drops of polyspecific AHG serum to 1 drop of washed red cells.
7. Mix and centrifuge at 1000 rpm for 1 minute immediately.
8. Gently shake the tube to dislodge the cell button and see for agglutination.
9. Record the result.
10. Add 1 drop of IgG coated red cells to NC test tube. Mix and centrifuge at 1000 rpm for 1 minute. Look for agglutination. If there is no agglutination, the test result is invalid and the whole test is repeated. If agglutination is obtained the result is valid.

B. Conventional Tube Method for ICT:

1. Label three test tubes as T (test serum), PC (positive control) and NC (negative control)
2. In the tube labelled as "T", "PC" and "NC" add two drops of test serum, Anti D serum and Bovine Serum albumin respectively.
3. Add 1 drop of 5% suspension of pooled O Rh positive red cells in each tube.
4. Incubate all three tubes at 37 degrees for 30 to 45 minutes.
5. Wash cells three times in large volume of saline. Discard supernatant with each cell wash completely.
6. Add 2 drops of AHG serum to each test tube.
7. Mix and then centrifuge at 1000 rpm for 1 minute.
8. Gently shake the tubes to dislodge the button and examine for agglutination.
9. Add 1 drop of IgG coated red cells to NC test tube. Mix and centrifuge at 1000 rpm for 1 minute. Look for agglutination. If there is no agglutination, the test result is invalid and the whole test is repeated. If agglutination is obtained the result is valid.

Interpretation of result by manual method:-

1. **Positive Result** – if agglutination is present in test tube labelled as "T".
2. **Negative Result** – if no agglutination seen in test tube labelled as "T".

Positive: Clumping (agglutination) of the blood cells. This means there are presence of antibodies either on the red blood cells (DCT) or in the serum (ICT) which induce hemolysis.[5]

Negative: No clumping of cells (no agglutination). This means there are no antibodies bound to red blood cells or in the serum.

5. Results

A total of 80 samples taken for this 8 months study, out of which 60 were evaluated for DCT and 40 evaluated for ICT. From the 60 samples for DCT, 42 showed positivity for Gel card method and 30 for Conventional tube method. And from 40 samples for ICT, 24 showed positivity for Gel card method and 18 showed positivity for Conventional tube method.

Sensitivity and specificity and p-values were calculated for the positive results by Gel Card and Conventional tube method.

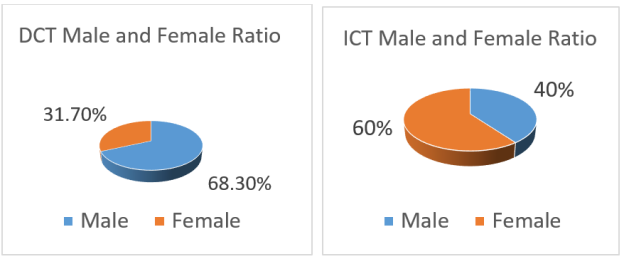

Fig. 2: Gender wise distribution for DCT and ICT

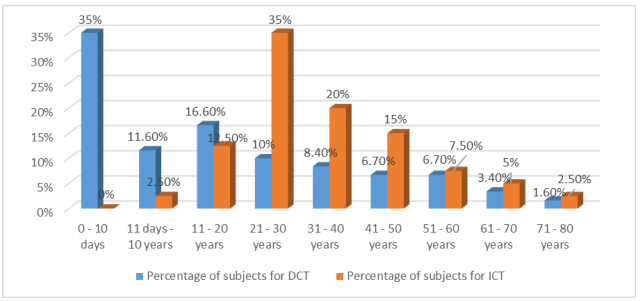

Fig. 3: Age wise distribution for DCT and ICT

6. Discussion

Conducted a comparative study in the blood bank of BVDU (Deemed to be University) Medical College and Hospital, Sangli, India, for 8 months, from March to October of 2022, where in all the samples for Coomb's test, both

Table 2: Gender wise distribution showing positive result by Gel card method and conventional tube method for DCT

Sex	Number of subjects	Gel Card DCT	Positive result %	Conventional method DCT	Positive result %
		Positive		Positive	
Male	41	20	62.5%	16	69.5%
Female	19	12	37.5%	7	30.5%
Total	60	32		23	

Table 3: Comparing coomb's test, DCT and ICT based on p-value, sensitivity and specificity

Coomb's Test	p – value	Sensitivity		Specificity	
		Gel Card	Conventional	Gel Card	Conventional
DCT	0.0495	52%	45%	49%	56%
ICT	0.0359	53%	43%	48%	58%

Table 4: Comparative table for DCT and ICT based on p-value, sensitivity, specificity and the positive and negative results, with three other studies

S.No	Comparative studies	DCT / ICT	Sensitivity		Specificity		Positive		Negative	
			Gel Card	Tube Method	Gel Card	Tube Method	Gel Card	Tube Method	Gel Card	Tube Method
1.	Present Study	DCT	52	45	49	56	53.3%	38.3%	46.7%	61.7%
		ICT	53	43	48	58	55%	35%	45%	65%
2.	ISHTM, 2011	DCT	83.1	66.03	60.4	97.67				
3.	JRMDC, 2014	DCT					40%	33%	60%	67%

Direct and Indirect were implemented by both Gel card and Conventional method simultaneously.

For a better understanding, the principle, indications, advantages and disadvantages will be discussed in the following. As we all know, Coomb's test is a laboratory investigation done for the detection of either in-vivo (Direct Coomb's test) or in-vitro (Indirect Coomb's test) antibodies directed towards RBC's.[8] For the detection of antibodies present on the surface of the red cells, Direct Coomb's test is the diagnostic tool used.[3]

As mentioned before, the Gel technique has been proven to be more efficacious and simplified technique as well as the interpretation of results along with a better reliability, reproducibility, stability and increased sensitivity.[3] Initially introduced in Thailand, 1993, and has become popular and used worldwide in several blood banks.[3] For Gel Card test we use specific microtubes which are being prepared using standard reagents. This method has been widely used for cross matching, the detection of antigen, alloantibody screening/identification.[3] Since the tube method was the first technique used, some of the blood bank personnels still prefer the Conventional Tube method despite aving many drawbacks like skilled technical expertise especially in the cell washing step (which may lead to false positive results) and also the intervariability complex.[3]

On the other hand, the benefits of Gel technology have surpassed the Conventional Tube method – quick, safe, elimination of cell washing step, technician friendly, less handling of samples, lesser interpretive errors, lesser protocol errors does not require special skills for performance.[3,9] It also has a clear cut grading system giving a uniform interpretation by the observers when get for a weeks time due to the stability of the agglutinates. The duration of 1 week gives another advantage in certain medico legal cases. Another most important point to be noted is the high sensitivity towards IgG coated cells, making it a better technique compared to the Conventional tube method.[3] Despite the above mentioned advantages, there are certain unavoidable disadvantages as well – cost, false positive reactions (macrocytosis, marked leucocytosis and increased ESR), the possibility of missing C3d coated red cells.[3]

Principle of Gel Card Method: The basic principle of the gel test is, instead of a glass test tube, the serum and cell reaction takes place in a microtube having 6 wells embedded in a plastic card, which allows easy testing, reading as well as handling and disposal. Saphadex gel is used in gel cards which holds agglutinate in semisolid medium, this helps in clear visualization of agglutination than that of the tube method.[2]

This method introduced by Lappiere et al., was firstly used for the cross matching of blood.[7] As stated earlier, the tube technique aka conventional technique has been the cornerstone for Coomb's testing over last 4 decades, but the enhanced sensitivity Gel card technique has made the interpretation more reliable.

Advantages of Gel card method:[3,10–12]

1. Simple, reliable, rapid, reproducible and sensitive
2. Greater uniformity amongst repeat test

3. Less volume of specimen required
4. Standardized reporting, grading system
5. No cell washing required
6. More consistent and reproducible interpretation of results
7. Higher sensitivity with IgG coated cells

Disadvantage of Gel card method: [3,10–12]

1. Expensive
2. Requires special incubator and centrifuge machine

Advantages of conventional method: [3,10–12]

1. Cheaper
2. Detection of C3 complement

False positive results: [3]

1. Overcentrifugation
2. Increased ESR, rouleaux formation
3. Macrocytosis
4. Leucocytosis
5. Inappropriate washing, inadequate resuspension of cell button
6. Hypergammaglobilinemia

False negative results: [3]

1. AHG reagent failure
2. Improper or inadequate or delayed washing
3. Low serum/cell ratio
4. Resuspension of cell button too vigorously

Table 5: Drugs associated with positive direct Coomb's test or hemolysis due to drug induced autoantibodies

Reported mechanism	Drug
Drug independent autoantibody induction	Levodopa, mefenamic acid, metyldopa
Drug dependent	Amoxicillin, erythromycin, insulin, penicillin, tetracyclin, tolbutamide, amphotericin B, ceftriaxone, naproxen
Nonimmunologic protein adsorption	Clavulanate potassium, diglycoaldehyde, sulbactam sodium, tazobactum sodium
Combined mechanism	Ampicillin, carbimazole, cefixime, cefotaxime, chlorpromazine, cisplatin, isoniazid, pipercillin, quinidine, ranitidine, rifampicin.

7. Conclusions

Our study showed that gel card is easier to use, more sensitive and less time-consuming with more standardized result and less sample needed for the test. Results of Gel card can be preserved for 3-4 days and this can be interpreted by various observers and compare it with the standardized grading system. Gel card assay appears to be an excellent method for detecting agglutination better than conventional tube method and easy to read weak agglutination and it can also detect ABO incompatibility. The performance of saline tube technique requires more experience and highly accuracy due to its long stages and multiple washing. But one disadvantage of gel card method is that gel cards are costly and require separate incubator and centrifuge.

8. Source of Funding

None.

9. Conflict of Interest

None.

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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.

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

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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.

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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

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[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 of 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 allo0antibodies 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.

Performance Evaluations

CE

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EXTERNAL EVALUATIONS

MATRIX™ GEL SYSTEM

Gel card system for Blood Banking Applications

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^w.

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.

Antibody Detection n=398		DiaMed ID	
		+	-
Matrix™ Gel System	+	38	3
	-	4	353

Table 1.2: Antibody detection results for the DiaMed ID-Gel Card system:

Antibody Detection n=398		Coombs reactive antibodies	
		Present (39)	**Not Present (359)**
DiaMed ID Gel Card System	**+**	38 (True positive)	4 (False positive)
	-	1 (False negative)	355 (True negative)

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

Antibody Detection n=398		Coombs reactive antibodies	
		Present (39)	**Not Present (359)**
Matrix™ Gel System	**+**	36 (True positive)	5 (False positive)
	-	3 (False negative)	354 (True negative)

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.

Antibody Detection n=153		DiaMed ID	
		+	**-**
Matrix™ Gel System	**+**	3	9
	-	2	139

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

Antibody Detection n=153		Coombs reactive antibodies	
		Present (3)	**Not Present (150)**
DiaMed ID Gel Card System	**+**	3 (True positive)	2 (False positive)
	-	0 (False negative)	148 (True negative)

Table 2.3: Antibody detection results for Matrix™ Gel System.

Antibody Detection n=398		Coombs reactive antibodies	
		Present (3)	**Not Present (150)**
Matrix™ Gel System	**+**	3 (True positive)	9 (False positive)
	-	0 (False negative)	141 (True negative)

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.

Confirmed antibody by identification panel n=46		DiaMed ID	
		+	**-**
Matrix™ Gel System	**+**	41	3**
	-	2*	0

* 2 antibodies with the specificity C^w, 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

Antibody specificity	Total antibodies	Detected by DiaMed ID System	Detected by Matrix™ Gel System
c	2	2	1
C	2	2	2
C^w	4	4	3
E	11	10	11
D	9	9	9
Jk(a)	3	2	3
K	5	4	5
Le(b)	1	1	1
Lu(a)	3	3	3
M	1	1	1
S	1	1	1
Auto-e	2	2	2
Pan agglutination	2	2	2
Total	**46**	**43**	**44**

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.

Confirmed antibody by identification panel n=4		DiaMed ID	
		+	-
Matrix™ Gel System	+	4	0
	-	0	0

Table 4.2: Spectrum of 46 identified antibodies in 39 samples

Antibody specificity	Total antibodies	Detected by DiaMed ID System	Detected by Matrix™ Gel System
C	1	1	1
E	2	2	2
D	1	1	1
Total	**4**	**4**	**4**

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:

Auto-control n=386		DiaMed ID	
		+	-
Matrix™ Gel System	+	93 84 monospecific DAT positive 9 monospecific DAT negative	3 2 monospecific DAT positive 1 monospecific DAT negative
	-	7 6 monospecific DAT positive 1 monospecific DAT negative	139

Table 1.2: Results of the auto-controls of 386 routine samples with DiaMed ID LISS/Coombs Test Card:

Auto-control n=386		**92** Confirmed by monospecific Coombs	**294** **-**
DiaMed ID	**+**	90 (true positive)	10 (false positive)
	-	2 (false negative)	284 (true negative)

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

Auto-control n=386		**92** Confirmed by monospecific Coombs	**294** **-**
Matrix™ Gel System	**+**	86 (true positive)	15 (false positive)
	-	6 (false negative)	279 (true negative)

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:

Auto-control n=158		**DiaMed ID**	
		+	**-**
Matrix™ Gel System	**+**	39 33 monospecific DAT positive 6 monospecific DAT negative	3 1 monospecific DAT positive 2 monospecific DAT negative
	-	1 1 monospecific DAT positive	115

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

Auto-control n=158		**35** Confirmed by monospecific Coombs	**124** **-**
DiaMed ID	**+**	34 (true positive)	6 (false positive)
	-	1 (false negative)	117 (true negative)

Table 2.3: Results of the auto-controls of 158 routine samples with Matrix™ AHG (Coombs) Test Cards:

Auto-control n=158		**34** Confirmed by monospecific Coombs	**124** **-**
Matrix™ Gel System	**+**	34 (true positive)	8 (false positive)
	-	1 (false negative)	115 (true negative)

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.

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