A Dissertation on

COMPARATIVE STUDY OF BLOOD CROSSMATCHING USING GEL AND CONVENTIONAL TUBE TECHNIQUE

Dissertation submitted to

THE TAMIL NADU DR. M.G.R. MEDICAL UNIVERSITY

In partial fulfillment of the regulations

for the award of the degree of

M.D. BRANCH - XXI

IMMUNOHAEMATOLOGY &
BLOOD TRANSFUSION

DEPARTMENT OF TRANSFUSION MEDICINE
THE TAMIL NADU DR. M.G.R. MEDICAL UNIVERSITY
CHENNAI, INDIA

APRIL 2012
ABSTRACT

BACKGROUND AND OBJECTIVES

Coombs cross-matching is used to detect the presence of alloantibodies in a cross matching procedure. These alloantibodies can cause hemolytic reaction in a transfused patient. Tube technique is the most commonly used method for Coombs cross matching in India. Tube has been the gold standard technique in crossmatching of blood. The aim of the study is to compare between the conventional tube technique and the gel technique in crossmatching of blood.

METHODS

Patients’ blood samples are sent to our blood bank for routine cross matching procedure. Both Tube and Gel technique were used for Coombs cross matching and their results were compared and analyzed.

RESULTS

This prospective study was conducted on 1200 blood samples to compare conventional tube and gel techniques in crossmatching of blood. The number of Coombs Crossmatch incompatible samples by
tube technique was 14 and by gel technique was 18. The prevalence of the tube and gel technique in detection of irregular antibodies was 1.2 % and 1.5 % respectively. The sensitivity and specificity of the gel technique with respect to tube technique were 100 % and 99.7 % respectively.

CONCLUSION

The gel technique has definite advantages over the tube technique because of being able to detect more positive samples than tube and its shorter duration for the crossmatching procedure. However, in a developing country like India, the gel technique if applied widely, cost factor would come down and could be considered as a better alternative to the conventional tube technique to ensure successful transfusion.

KEY WORDS

Coombs Crossmatching, Antihuman globulin, Alloantibody, Tube, Gel, Agglutination.
## CONTENTS

<table>
<thead>
<tr>
<th>S.No</th>
<th>TITLE</th>
<th>PAGE NO.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>2.</td>
<td>AIM AND OBJECTIVES</td>
<td>6</td>
</tr>
<tr>
<td>3.</td>
<td>REVIEW OF LITERATURE</td>
<td>7</td>
</tr>
<tr>
<td>4.</td>
<td>MATERIALS AND METHODS</td>
<td>37</td>
</tr>
<tr>
<td>5.</td>
<td>RESULTS</td>
<td>44</td>
</tr>
<tr>
<td>6.</td>
<td>DISCUSSION</td>
<td>58</td>
</tr>
<tr>
<td>7.</td>
<td>SUMMARY</td>
<td>66</td>
</tr>
<tr>
<td>8.</td>
<td>CONCLUSION</td>
<td>68</td>
</tr>
<tr>
<td>9.</td>
<td>BIBLIOGRAPHY</td>
<td>i - v</td>
</tr>
<tr>
<td>10.</td>
<td>ANNEXURES</td>
<td></td>
</tr>
</tbody>
</table>
INTRODUCTION
INTRODUCTION

Compatibility or pre transfusion testing involves the crossmatching of selected donor blood of appropriate ABO, Rh and of patient requiring a blood transfusion. The donor blood selected is considered compatible if there is no observable reaction in the compatibility tests between the blood of donor and blood of patient. The assumption can then be made that when transfused, the blood will survive normally in the recipient.\(^1\)

Crossmatching is a relatively simple process but if not carried out correctly can lead to the wrong blood being transfused into the patient with possible disastrous consequences. The most important steps during the process are correct identification of patient and selecting blood of correct ABO group for the patient. The crossmatching of blood is done to minimize the risk of patients’ receiving incompatible blood.

Crossmatch is of two types: Major and Minor.

The Major cross-match involves testing the serum or plasma of the intended recipient with the saline-suspended red cells of each unit of blood selected for transfusion. This is considered most important because it tests whether or not the red cells of the blood donation selected for crossmatching contain antigens against antibodies in the intended recipient. If the crossmatch is positive- signifying a reaction between donor red cells and recipient
antibodies, then the donation is incompatible and would be destroyed or have a reduced lifespan if transfused.¹

The Minor crossmatch involves testing the red cells of the intended recipient with the plasma from each unit of blood selected for transfusion. The minor crossmatch is seldom performed unless investigating an adverse reaction to the transfusion of whole blood or plasma products and to determine whether donor plasma contained antibodies directed toward a red cell antigen in the patient.

Major Crossmatching is more critical for a safer transfusion than the minor one. For the detection of red cell alloantibody the indirect antiglobulin test is the best single test available. It is capable of detecting virtually all clinically significant antibodies.²

In the major crossmatch, both saline ‘Immediate Spin’ and Indirect Antiglobulin Test (IAT) methods are used for compatibility testing. The immediate or rapid spin result is designed to check that blood selected is ABO compatible and to show any incompatibility with an irregular IgM and saline reacting IgG antibody at room temperature. The same crossmatch test is continued to the IAT phase to detect any additional incompatibility with IgG antibodies at 37°C.³

IAT phase is performed to detect IgG antibodies that may cause clinically significant transfusion reactions and is used for the antibody
screening procedure using screening red cell panels and for the crossmatch using donor cells.

The primary objective of a major crossmatch test is to detect the presence of antibodies in the recipients serum including anti A and anti B that could destroy the transfused RBC and to ensure that enough red blood cells carrying selected red cell antigens will survive when transfused for sufficient length of time. A positive result in the major IAT crossmatch test due to unexpected antibodies or any other reasons requires further explanation and the patient should not receive transfusion until the cause of incompatibility has been determined.

The causes of positive result in Major Crossmatch are ABO mismatching error, positive DAT, alloantibody in the recipient to low incidence antigen on donor red cells, polyagglutinable red cells and contaminant in the test system can all result in incompatible crossmatch with the antibody screen being negative.\(^4\)

In India, pretransfusion testing involves determining the ABO and Rh types of both patient and donor and performing a major crossmatch.

The most widely used technique for crossmatching of blood is the tube technique. The tube technique along with the inclusion of indirect antiglobulin test is able to detect ABO incompatibility and also the presence of clinically significant antibodies.
A qualified blood bank person is needed to read the result and correctly identify the relative strength of agglutination. So in order to overcome the practical difficulty of Tube technique, the gel technique was introduced by Lapierre in 1990. The gel test was initially developed to standardize agglutination reactions and to fix the agglutinates to allow a simple and reliable reading. The results produced can be stored for longer periods.

There have been various studies comparing tube technique and the gel method with respect to Blood grouping, Rh typing DAT, IAT, antibody screening, detection of various other antibodies and complements specific to certain conditions.

This study was mainly carried out to compare between the tube and gel in major crossmatching of blood. India is an economically developing country where the newer and advanced technologies are gradually replacing the older ones in each field. This gradual shift is also occurring in the field of blood banking where the slide method has been replaced by the tube method in blood grouping and typing. Crossmatching which was done only in saline ‘immediate spin’ phase proceeded to the IAT phase. This study will help to analyze whether the new ‘gel technique’ will be a good alternative to the conventional tube technique. One of the major drawbacks being put forth for gel technique is the cost factor, however if it has been widely implemented it will be cost effective. This study will take into account the labour, time and
the cost factors which play a predominant role in an emerging economy such as India.

In most of the developed countries blood transfusion service has reached the stage of automation in blood banking for easy data retrieval, lessened clerical error and foolproof vein to vein successful transfusion.
AIM AND OBJECTIVES
AIM AND OBJECTIVES

AIM

The aim of the study is to compare between the conventional tube technique and the gel technique in crossmatching of blood.

OBJECTIVES

1. To find out which technique is better at detecting irregular antibodies in the major cross matching of blood.

2. To compare the sensitivity and specificity of the gel technique with respect to the tube technique.

3. To analyze the time and cost effectiveness between the tube and gel technique.
REVIEW OF LITERATURE
REVIEW OF LITERATURE

Karl Landsteiner’s discovered ABO blood groups in 1901. However, the practice of blood transfusion without blood typing was often carried out throughout the world till early 20th century.

It was Otterberg who developed the modern day crossmatch. The knowledge about pre transfusion testing was limited and most surgeons preferred to give blood without any crossmatching of blood. As a result of that, Otterberg proved immune hemolysis occurred in transfused red cells.\(^5\)

In 1940, the discovery of Rh system put the crossmatch test in the spotlight. This Rh incompatibility (Rh negative persons who receive Rh positive blood) could not be detected with the usual crossmatching technique. This was mainly because of IgG sensitized red cells which did not agglutinate without any additional enhancement.

By the early 1940s there were reports of serious transfusion reactions in case where the donor and recipient was ABO compatible. It became clear that not all incompatible reactions could be prevented by ABO compatibility alone.

ANTIHUMAN GLOBULIN TEST (AHG)

In 1945, Coombs and associates described the use of antiglobulin test for the detection of weak and non agglutinating Rh antibodies in serum. In
1946, Coombs and coworkers described the use of AHG to detect in vivo sensitization of RBC of babies suffering from hemolytic disease of newborn (HDN). Although Coombs and associates were instrumental in introducing the antiglobulin test, the principle of test had in fact been described by Moreschi in 1908. Moreschi studies involved the use of rabbit anti goat serum to agglutinate rabbit RBC that were sensitized with low non agglutinating dose of goat anti rabbit RBC serum. Coombs procedure involved the injection of human serum into rabbit to produce antihuman serum. After absorption, the AHG serum still retained sufficient antibody activity to permit cross linking of adjacent RBC sensitized with IgG antibodies. The cross linking of sensitized RBC by AHG produces hemagglutination indicating that RBC had been sensitized by antibody that had reached with an antigen present on cell surface.\textsuperscript{6}

The antihuman globulin test is also referred to as Coombs test in honour of R.R.A Coomb who rediscovered it. Moreschi published two interesting papers describing enhancement of agglutination with antiserum to serum. However, incomplete antibodies were unknown at the time and general acceptance or use of this procedure never resulted. Later, Dr.Coombs paid tribute to Moreschi in one of his lectures for his contribution.
WASHING ANTIGLOBULIN TESTS

When using the AHG technique, cell washing is carried out to remove any unbound globulin that may neutralize the AHG reagent resulting in a false negative result. A DAT involves no incubation period and is thus washed at the outset, an IAT has an incubation period and washing is performed after incubation is completed.

Raman et al suggested that the following general precautions should be undertaken when washing AHG tests-

1. Tubes should not be overfilled with saline as this leads to a loss of red cells during centrifugation and could also cause cross contamination with other tests in the batch.

2. Sufficient centrifugation time should be allowed during each wash for the cells to be completely sedimented at the bottom of the tube to avoid loss of cells when decanting supernatant saline.

3. At the end of each wash, saline should be thoroughly decanted off cell buttons.

4. Cell buttons should be fully resusupended before adding saline for the next wash to ensure that no unbound globulin remain
trapped amongst the cells, which may then neutralize the AHG and lead to false negative results.

5. As much saline as possible should be removed from the tube after the final wash to avoid false negative results by dilution of AHG reagent.

The laboratory management team should decide whether or not to use anti IgG AHG for compatibility testing or whether the AHG reagent should be a blend of anti IgG and anti C3. A blend will detect both IgG and complement dependent antibodies.\(^7\)

**ANTIBODY SCREENING**

Testing the patient’s serum or plasma against known reagent screening red cells in addition to the donor cells is considered an integral part of crossmatch procedure. An appropriate set of group O reagent red cells is selected that together contain the common antigens within the population to enable the detection of antibodies of clinical significance.\(^1\)

**ABRIDGED CROSSMATCHING**

‘Abridged’ means shortened or abbreviated crossmatching. It has value in busy laboratories handling many specimens on a daily basis and applies to crossmatches without serological complications. The objective is to
provide suitable blood quickly and most cost effectively without compromising patients’ safety.

1. ABO and Rh D type is performed on patients sample and blood units selected.

2. An immediate spin test is performed, using serum / plasma from the patient and cells from the blood units, to check for ABO compatibility

3. The IAT phase of crossmatch using the donor cells is omitted

4. The patients serum / plasma is tested against the reagent screening cells by the same IAT method as would have been used in full crossmatch.

The abridged crossmatch is only suitable if no irregular antibodies have been detected in the patients’ serum / plasma. When the antibody screen tests are positive or the patient has a history of irregular antibodies of clinical significance, then compatibility testing using the donor cells by IAT must be performed.

**ELECTRONIC CROSSMATCHING**

The electronic or computer crossmatching of blood have been introduced into busy crossmatching laboratories that have the appropriate infrastructure. The donor blood is matched electronically with the patient; no
serological crossmatch is performed. The sample from the patient must be typed for ABO group, Rh and serum / plasma must be tested for the presence of IgG antibodies by screening by IAT.

Provided that antibody screening tests carried out on the patient are negative and no anomalies results are obtained in the grouping and there is no history of irregular antibodies, blood of the same ABO and Rh can be selected and issued using a validated computer program.\(^1\)

**TUBE TEST METHODS**

Tube test methods have been the long standing method for antibody screening since before the introduction of newer technologies for antibody detection such as gel test and solid phase testing. Various methods such as the use of saline with two drops of serum saline with increased serum to cell ratios (4-8 drops of serum to 1 drop of 2-4% reagent RBCs) and enhancing medias such as bovine serum albumin (BSA), Low Ionic Strength Saline (LISS) RBC suspension and LISS additive methods, polybrene, Poly Ethylene Glycol (PEG) and PEG-LISS additive methods and proteolytic enzymes (ficin, papain and bromelin) have been used for antibody detection.

The saline tube test is easy to use and low in cost but has poor sensitivity, particularly when used in protocols with shorter incubation times. BSA methods contributed to an improvement in detection of antibodies, mainly those that are direct agglutinating, but only minimally improved the
sensitivity of indirect agglutinating antibodies. The BSA method continued to be challenged because of the longer incubation times needed to detect antibodies of weaker variety and its ability to detect unwanted reactivity.\textsuperscript{8}

The specificity of both BSA and LISS test was made unsure by the use of polyspecific antihuman globulin (AHG) reagents that contributed to the detection of unwanted reactivity. Switching to a monospecific antiglobulin reagent such as anti IgG improved specificity but produced a decrease in sensitivity of BSA test. A less frequently used enhancement, polybrene offered speed and sensitivity but took some significant technique adjustment for laboratories to become proficient in its use.

During the 1980s LISS based methods became increasingly popular and they remain even today the most frequently used tube test method for antibody detection.

Low and Messeters introduction of low ionic strength saline method and the resultant LISS based additives that came to market contributed the first significant improvement in antibody detection by allowing an increased rate of antibody uptake in a shorter time frame.\textsuperscript{8}

In 1987, Nance and Garraty introduced PEG as a new potentiator for the detection of RBC antigen antibody reactions. A PEG/LISS method using this additive gained popularity as a routine antibody screen in some institution and as a routine or adjunct method for antibody identification in many others.
The use of proteolytic enzymes combined with another method capable of detecting enzyme sensitive antibody specificities as a test for routine antibody screen test never gained much favour in the United States but practised in European and Asian pacific countries.

SOLID PHASE RBC ADHERENCE TEST

The SPRCA was detected in the early 1980s through the work of Plapp et al. The principle of this test is based on the ability of an antigen or antibody to be bound to the solid matrix of a plastic micro well. When the appropriate reactant is added to the well, an antigen antibody reaction occurs resulting in adherence of RBCs or tagged RBCs to well. Both gel and SPRCA test produce easily defined end points that allowed for their transition to an automated platform.

TRANSITION TO NEWER TECHNOLOGIES

Overall, tube testing is considered to be the most labour intensive of the procedures used for antibody detection from the perspective of the number of steps and trained personnel required to perform the test.

Tube tests reagents are generally lower in cost as compared with the newer technologies. However, overall costs associated with skill required hands on time and non value added activity time are higher with tube test methods. The transformation of antibody detection test methods into newer
technologies and the innovation of automation capability with these technologies have provided transfusion services with a pathway to efficiency and standardization with a well balanced capability in antibody detection testing.

The new found capability along with the difficulty in finding experienced staff to fill up the ever increasing vacancies have propelled transfusion services to select tests and automation to fill the gaps created by the challenges. The automation of these methods has eliminated costs linked to human intervention and manipulation required of tube tests.

There is a downward trend in the use of saline and albumin tube test methods with LISS based methods having the highest percentage of usage.

The comparisons of methods for sensitivity and for specificity generally do not start from the same point. Many studies are done retrospectively comparing these two techniques in different time periods. Other studies compare the new test method with what has been previously detected by the established method. These types of studies are not ideal because the cohort of stored samples is inherently biased towards the method of original detection. Ideally prospective studies using consecutive samples are best. Obtaining sufficient numbers of samples is usually achievable for the specificity (true antibody negative population) aspect of the study but obtaining enough samples to be able to demonstrate statistical significance
and breadth of reactivity of specific antibodies for understanding the sensitivity of the new test is another matter. Therefore laboratories and the manufacturers are left with the usual choice of selecting previously positive stored samples as the way to evaluate sensitivity of new test.  

**GEL TECHNOLOGY**

In 1985, the gel test was developed by Dr. Yves Lapierre of Lyon, France (harmening 293). Gel particles was the ideal material for trapping agglutinates and this led to the patented process for separation of red blood cell agglutination reactions. The antiglobulin testing could be performed without multiple saline washes to remove unbound immunoglobulin and reduces variability associated with physical resuspension of RBC buttons after centrifugation and the interpretation of hemagglutination reactions.  

**PRINCIPLE OF GEL TEST**

The gel test is performed in a specially designed micro tube which consists of dextran acrylamide gel. The gel particles reflect the size of red cell agglutinates that form during centrifugation. Unagglutinated cells pass through the entire length of tube to its tip but larger agglutinates remain suspended in the gel. LISS and AHG are already present in the gel and so, no washing is required. The reaction can be read immediately after centrifugation. The Gel technique consists of six micro tubes present in a gel card. The incubation period is 15 minutes in a special incubator at 37 degree
Celsius. The centrifugation of the gel card takes place in a centrifuge at 900 rpm for 10 minutes.

Gel technology is approved for ABO forward and reverse grouping, Rh typing, DAT, antibody screen, antibody identification and compatibility testing. Cards of micro columns containing a matrix of gel or glass micro beads are available commercially for micro column techniques.\(^4\)

The system can be manual, semi automated or automated. The gel technology uses cards of micro column preloaded with a specific gel, for example a neutral gel or a gel containing AHG or a gel containing specific reagents.

It is not necessary to wash the red cells post incubation before adding the AHG. The centrifugation stage allows only the red cells and not the serum / plasma into the gel matrix, where the AHG and sensitized cells will react. Unsensitized cells pass through the matrix forming a button at the base.\(^5\)

**STUDIES COMPARING TUBE WITH GEL**

Ira A. Shulman et al did a survey on the pre transfusion testing practices in the North American laboratories from 2001 to 2004. He found that the blood banks currently favoured the tube methods when performing ABO Grouping, Rh typing, antibody screening and crossmatching. However there has been a significant increase in the use of gel based methods in recent
years especially for antibody detection and crossmatching. The selection of a particular crossmatch method is determined by the alloimmunisation history and current status of patient. The immediate spin cross match is most commonly used crossmatch method (40.4%) for patients who are not alloimmunised but the laboratories are increasingly using a gel AHG crossmatch method (30%) by the end of this survey (2004). Similarly for alloimmunized patients the preferred crossmatch is the tube test method. But there has also been a downward trend in the use of tube test for crossmatching of alloimmunized patients (from 84.9% to 68.8%) with a corresponding upward trend in the use of gel AHG crossmatching (from 14.7% to 30.3%).

John.C.Cate et al did both a retrospective and prospective study to evaluate the gel test for indirect antiglobulin testing. In the blinded retrospective study 16 antibodies were detected by the tube method while all the 18 antibodies were detected and identified by gel method. In the prospective study out of 121 samples 120 samples were identified as negative and one was positive by tube method. By the gel method 116 samples were identified as negative and five were positive. False positive reactions can occur due to fibrin present in fresh serum trapped at top of gel. When switched to EDTA plasma, false positive reactions became infrequent. Similarly cell suspensions more concentrated than 0.8% can affect the sensitivity of gel test and may result in false positive results.
The smaller sample volume used in gel testing has contributed to the efficiency of specimen procurement and processing for AHG testing. Smaller sample size is an advantage when phlebotomizing pediatric, oncology and dialysis patients with smaller or sclerotic veins. It has almost eliminated requests for repeat phlebotomies due to insufficient sample quantity.\textsuperscript{10}

Tube has been the gold standard technique in crossmatching of blood. Rumsey et al in his article ‘New protocols in serologic testing’ explains some of the disadvantages of gel method. The complexity of antibody identification usually requires multiple runs of selected cells. Although a 0.8% panel of RBC is commercially available, samples that need to be tested should have a 0.8% concentration in the appropriate diluent prior to use. This is difficult when compared to adding the cells from a vial into a test tube. Similarly rouleaux and incompletely clotted samples can cause false positive reactions.\textsuperscript{11}

Novaretti et al, in his comparative study of tube and gel for antibody identification found out that gel is more sensitive (p< 0.1) than the tube for identifying potentially clinical significant antibodies.\textsuperscript{12}

Nathalang et al used gel test in direct antiglobulin testing and antibody identification in patients with Thalasaemia, Autoimmune hemolytic anaemia and HDN and found that it had a sensitivity and specificity of 93.5\% and 88.6\% respectively. He also did a study on diagnosis of
paroxysmal nocturnal hemoglobinuria (PNH) to detect the PNH RBC populations compared with the standard acid serum test. The sensitivity and specificity were 100%.

The gel test is a highly sensitive system for most serological tests with many advantages. It is easy to read the reactions and has a clear cut grading system. Grades 4+, 3+, 2+, 1+ and can be distinguished by naked eyes. The agglutination reactions can be re-read so there is the possibility for a second or repeated reading since the reaction patterns are stable for hours. The photocopy of the positive or negative reactions can be made and kept as firm laboratory data. The gel tests are easy to handle and reduce the problem of broken glass or glass cleaning.\\(^\text{13}\)\\

Washing phase is not needed in gel because plasma proteins are less dense than gel. This property and relative stability of the reaction end point give column agglutination methods a degree of simplicity and reliability not achieved by other methods.\\(^\text{14}\)\\

Arumugam et al in his study of 50 serum samples of multitransfused thalassaemic patients’ using both gel and tube method found gel method could identify alloantibody in one of the samples missed by conventional tube technique which supports its technological superiority.\\(^\text{15}\)\\

The micro column affinity test (MCAT) is a new method suitable for the detection or identification of R.B.C antibodies, crossmatching and DAT.
A study was conducted to compare the gel test and the MCAT with respect to their specificity and sensitivity in detecting R.B.C antibodies in a routine screening procedure. A total of 3000 random serum samples were screened for R.B.C antibodies with MCAT and gel. A total of 154 antibodies (5.1%) were detected, 149 by gel test and 147 by MCAT. The overall sensitivity and specificity of gel test was 96.8 and 96.5% and of MCAT 95.5 and 97.2%.\textsuperscript{16}

Sudipta Das, Chaudary et al did a study on the comparison of conventional tube test and gel technique in evaluation of direct antiglobulin test. Both techniques were compared using in house control cells. Gel was able to detect in vitro sensitization of red cells till 1:128 dilutions while tube could detect at 1:32 dilution. Polyspecific DAT was performed on 170 blood samples. The positive samples were further tested for monospecific IgG and C3d. 100 samples were negative and 64 were positive with both methods. Five were positive by gel which was negative with tube test while 1 sample was positive exclusively with tube test. The sensitivity, specificity, positive predictive value and negative predictive value of gel compared to tube were 98.4, 95.2, 92.7 and 99%. Of the 70 samples that were DAT positive by either method, 34 were finally diagnosed as Auto immune hemolytic anaemia, while the remaining 36 were auto immune disorders such as systemic lupus erythematosus, Rheumatoid arthritis and Hashimotos thyroiditis. The strength of reaction (agglutination) evaluated by gel was either more or equal to that of tube technique. The gel provided more information on immunoglobulin and
complement binding on the red cells as compared to tube. However, the occurrence of false positive reactions of gel could not be ruled out.\textsuperscript{17}

Studies were done on gel test with respect to their ability to estimate the quantity of IgG on RBCs and the determination of the IgG subclass IgG1 and IgG3. Lynen et al did a study on 65 patients with a positive DAT. The amount of IgG1 and IgG3 on RBC was examined by use of gel cards and flow cytometry. The results were correlated with the presence or absence of hemolysis. In addition, D+ RBCs were studied after sensitization with anti D sera from 22 alloimmunized pregnant women. He found out that the amount of IgG on the RBCs as determined by gel dilution cards correlated with flow cytometry. IgG subclass results as determined by gel subclass cards were confirmed by flow cytometry in 14 cases. Gel test data obtained by IAT with anti D sera were concordant with flow cytometry.\textsuperscript{18}

Sharma et al did a study on 100 samples of Thalassaemia major patients for indirect Coombs crossmatch. He compared both gel and tube and found tube technique showing positive in 6 samples and gel showing positivity in 12 samples which included the above 6 samples. These 12 samples were positive by antibody screening using 3 three cell panels, remaining 88 were negative. Gel technique showed sensitivity of 100\% and specificity of 100\% while tube test showed sensitivity of 50\% and specificity of 100\%.\textsuperscript{19} The gel cards have a shelf life of one year and can be stored at temperature between 18\textdegree C to 25\textdegree C.\textsuperscript{20}
The gel assay was approved by the FDA for the detection of red cell antigens as well as for ABO serum testing, IAT and DAT. The tube agglutination assay has traditionally been used for the detection of A, B and D. The gel assay performed as well as the tube agglutination assay when used for the detection of A, B and D and it exhibited greater reaction strength than the tube assay. The gel assay performed better than the tube agglutination assay when used to detect weak D and performed marginally less well than the tube assay in detecting ABO subgroups. Detection of isohemagglutinins appeared to be less sensitive in the gel assay than the tube assay and the reaction strength were weaker in the gel assay. Of the 100 donor and 100 patient specimen tested, the gel assay when compared to the tube assay did not detect the B isohemagglutination in seven specimens. There are numerous advantages to the gel system. The gel assay incorporates standardized pipetting of reagents and specimens and reading of agglutination reactions. The required volume of specimen is substantially less which is advantageous of pediatric testing. Small samples volumes create less hazardous waste as does the elimination of saline washes in the performance of IAT.

Biosafety is also enhanced in the gel system by elimination of glass tubes. The gel system does require a higher degree of dexterity when small volume are pipetted into small reaction chambers but this should not be a concern to trained technologists. The gel technology can be implemented in
laboratories of all sizes and expertises levels and lend itself well to incorporation with automated systems.\textsuperscript{21}

A study was conducted by Victoria A.Greco et al to compare the gel micro column to the tube IAT using anti IgG for the detection of antibodies eluted from RBCs. Acid eluates were prepared from both peripheral blood and umblical cord blood samples. The gel assay was better at detecting antibodies eluted from RBC samples of patients with AIHA. Eluates from two of 11 patients with AIHA were positive in gel assay but negative in tube assay. The tube agglutination assay appeared to be better at detecting ABO antibodies eluted from umblical cord RBCs. Eluates from two of the 35 umblical cord blood samples were positive in the tube assay but were negative in gel assay. Gel micro column DAT were positive and tube agglutination DAT were negative in 3 of 52 samples. They tested RBCs known to have reactive anti IgG tube agglutination DATs but negative eluates in tube agglutination assays to determine whether these results were caused by an antibody that is better detected in gel assays. However, all seven eluates were negative in both assays suggesting that tube agglutination DAT results were false positive because of an IgG antibody that neither assay could detect in eluate.

Because both tube agglutination and gel assay detect antibodies by agglutination reactions, it is not surprising that the results of testing eluates in the two assays were similar. There were some differences in the sensitivity
and specificity of the two assays, but the differences were slight. Because of its practical advantages, it is likely that many laboratories will replace tube agglutination with gel micro column assay for evaluating eluates.\textsuperscript{22}

A study was conducted to compare the tube agglutination DAT to gel micro column affinity and flow cytometric DAT. RBCs from 84 patients were assessed by tube agglutination DAT, one gel micro column DAT and two affinity micro column DAT. The assays were also compared by using D+ RBCs sensitized with serially adjusted concentration of anti D. Both tube and gel micro column DAT were positive in 49 patient samples, both assays were negative in 20 samples and results were discordant in 15. Gel micro column DAT were more likely than were tube agglutination DAT to detect IgG on RBC. Affinity micro column DAT were less likely than gel micro column or tube agglutination DAT to detect IgG on RBC. Flow cytometry results were the same as gel micro column results in 12 of 15 patient samples and the same as tube agglutination results in 13 of 15. Tube agglutination and both affinity micro column assays reacted with RBC coated with anti D that was diluted one in 100. The gel micro column and flow cytometry assays reacted with RBC coated with anti D diluted one in 400.

The testing by flow cytometry suggests that DAT by flow cytometry assays are as sensitive as DAT by gel micro column assay for the detection of RBC bound IgG. Gel micro column and flow cytometric assays detected D sensitized RBC by using less anti D to coat the cells than tube agglutination
assays used. The gel micro column assay detected IgG on a larger proportion of patient samples than the tube agglutination assay, but there is a probability that some samples were false positive in gel micro column assay. Larger prospective studies that correlate flow cytometric, tube agglutination and gel assay results with clinical hemolysis are also needed.\textsuperscript{23}

In a comparative study, 172 sera previously demonstrated to contain red cell alloantibodies were tested in parallel by the tube LISS-IAT and three micro columns agglutination technique (Diamed, Ortho Biovue and Sanofi Pasteur Scangel) and one affinity adherence test system. The rate of detection of clinically significant alloantibodies (n = 154) in micro tube column system was very similar. One hundred forty one sera (91.6\%) reacted in the Diamed, 139 (90.3\%) in the ReACT, 139 (90.3\%) in the Biovue and 142 (92.2\%) in the Scangel, Only 117 (76.0\%) of these sera reacted in the tube LISS-IAT. Antibody reactivity on determined by titre and score was very similar in all micro tube column system and higher in these systems than in the tube LISS-IAT. There was no relative difference in the rate of detection of clinically significant antibodies in the four micro tube column systems. There have been reports that the frequency of positive crossmatch results and autocontrols is greater when micro tube column system is used. This has been ascribed to the detection of small amounts of immunoglobulins and immune complexes adsorbed onto donors and patients RBCs. Such findings are considered to be of doubtful clinical significance and might contribute to laboratory workload.
without providing benefit for patients who are to receive transfusions. The sensitivity of all four micro tube column system in the detection of clinically significant red cell antibodies was similar and markedly superior to that of tube LISS-IAT. An individual cost benefit analysis should be performed in every institution to decide whether a micro tube column system should be applied.\(^\text{24}\)

Studies were done to assess the performance of red blood phenotyping using gel method versus the traditionally accepted tube technique. A total of 520 tests were performed on ten of common antigens. The gel technique showed complete concordance with the tube. The gel reaction remained more stable and the validity of the reaction allowed for future review of gel cards.\(^\text{25}\)

Figueriredo et al. did a study between tube and gel in identifying A\(_1\) and A\(_2\) subgroups. He found out that gel failed to distinguish between A\(_1\) and A\(_2\) when they used Lectin A\(_1\) cards while subtyped A\(_1\) and A\(_2\). In the compatibility tests no differences were observed between the results obtained by the gel and tube which were performed on 197 blood samples.\(^\text{26}\)

Philips et al. did a study on the antibody detection by comparing tube and gel. He found that the AHG gel test is less sensitive than the tube LISS-IAT technique in the detection of the antibodies with both red cell samples having heterozygous and a homozygous expression of corresponding antigen. The poorer performance of gel test may be due to suboptimal dilution of
reagent within gel matrix. He concluded that AHG gel tests are satisfactory for phenotyping using potent, assured, blood grouping reagents and for the detection of more potent antibodies but it should be used with caution for the detection of weak antibodies particularly with test red cells having heterozygous expression of antigens that show dosage.\textsuperscript{27}

The gel method has proved its usefulness in also identifying drug dependent hemolytic reaction. Salama et al did a study on detection of cell drug (hapten) antibody complexes by the gel and compared it with the tube test. The study found that in cases in which the drug had bound firmly to RBCs there was no difference between the two tests. But in cases where the binding was weak, the drug dependent antibodies were completely removed from the cells by the conventional wash procedure prior to the final testing with antiglobulin serum. However, the gel identified all the drug dependent antibodies since the RBCs are separated from the incubation mixture only by centrifugation and the antiglobulin test is performed without washing the cells.\textsuperscript{28}

Studies were conducted to compare the sensitivity, specificity and reproducibility of the Scangel, Diamed, In house solid phase antiglobulin test (SPAT) and tube LISS IAT for antibody detection. Of 1107 antenatal patient samples screened, Scangel, Diamed and SPAT showed specificity of 99.1\%, 98.46\% and 94.4\%. 26, 25, 15 and 13 antibodies were detected by tube LISS IAT, SPAT, Diamed and Scangel respectively. Based on the titration studies
and testing the panel of 102 known antibodies, the tube LISS IAT and SPAT were more sensitive than the column agglutination technology.

Weisbach et al compared the performance of seven different test system in the detection of erythrocyte antibodies. He tested in parallel 368 sera samples with antibody that are assumed to be clinically significant and 78 sera samples with antibody that are of minor clinical significance. He compared it with tube LISS-IAT, Dia Med-ID, Ortho Biovue, BioRad Scangel, affinity adherence test system and two solid phase tests (Biotest solid and Immunocor Capture –R Ready Screen. He reported the detection of clinically significant antibody in which the sensitivity of micro column, affinity adherence and solid phase test system was markedly superior compared to the conventional tube LISS-IAT. Specificity was considerably higher in the tube LISS-IAT.\textsuperscript{29}

Stroncek did a study on the comparison of flow cytometry and tube agglutination assays in detecting red blood cells associated IgG and C3d. In samples with anti IgG, 12 of 20 samples reactive in both, six of 20 non reactive in both, two of 20 discordant with a reactive tube and non reactive flow cytometric assay. Anti C3d results showed nine of 20 reactive in both and 11 of 20 discordant with a non reactive tube and a reactive flow cytometry. The study concluded that the flow cytometry may be able to detect the onset of immune mediated hemolysis prior to the tube agglutination assay. The C3d levels that characterize clinically significant results in patients at risk.
of hemolysis has to be determined and whether the detection of low levels of RBC associated C3d is useful in assessing patients with other clinical disorders.\textsuperscript{30}

Reis et al did a study of 228 samples for red cell antibodies with two AHG reagents (one containing only IgG and other anti IgG, antiC3d) using both tube and bead technology. After initial testing, there was 94\% agreement between column agglutination and tube. An important feature of the column agglutination technology is the viscosity provided by the addition of polymers to the diluent which affects the speed at which red cells and serum components travel through the column during centrifugation. The difference in the specific gravity of red cells and serum components allows the red cells to pass through the column quickly than the less dense serum proteins do. The need to wash red cells in the traditional manner is eliminated because red cells are exposed to the AHG reagents well before the AHG reagents are exposed to soluble IgG and complement. In addition, polymers in the diluent potentiate agglutination. Another advantage is a significant decrease in the time required to train technologists to read and interpret results.\textsuperscript{31}

Lillevang et al did a study on antibody screening test based on the antiglobulin gel technique, pooled test cells and plasma. He found that the most common cause of false positive reaction in gel is an ‘edge’ on top of the gel that by microscopy proves to be rouleaux or fibrin clots. Dispensing test RBCs at an angle onto the wall of reaction chamber instead of directly on top
of / into the gel reduces the number of ‘edges’. He used EDTA plasma as test material throughout the study after an initial pilot trial of serum versus plasma. The theoretical disadvantage of using plasma is that the effective stop in complement activation brought about EDTA will hinder the detection of some complement activating antibodies. Some antibodies especially those belonging to the Kidd and Duffy systems are reportedly detected more readily through anticomplement component of AHG reacting with surface bound complement. This observation applies to serum samples and may not necessarily be true for plasma samples. The binding of large numbers of complement components to and around a bound antibody may sterically hinder the detection of anti IgG in the AHG. If plasma is used no complement is bound and the anti IgG will have ready access to bound IgG molecules. If specific type of AHG (monospecific with only anti IgG) is used plasma may be used instead of the serum.32

Fabinjinska et al evaluated by micro column technology for pre transfusion testing in multiple myeloma patients. In about five of the IAT, positive results were noticed in the tube because of rouleaux formation. The lack of false positive results in gel test was due to the fact during the centrifugation; unagglutinated red cells could pass through a gel even if they form rouleaux. In spite of negative conventional tube DAT in all our patients, the column DAT with an anti IgG and anti C3d was positive in about 40% of them. Hence red cells from multiple myeloma patients were coated with
immunoglobulin in a non specific way, different from that of antigen / antibody binding. The non specific binding of IgG was related to the level of monoclonal proteins in the patients sera. The limiting sensitivity of the tube did not pick up these monoclonal proteins. Careful attention should be given to false positive auto control during the antibody screening and cross matching in the gel test.\(^{33}\)

There have also been studies which have pointed out the disadvantages in a gel method. Neppert et al demonstrated unsatisfactory detection of an in vivo hemolytic anti Vel by the gel test which could be detected by the tube test. He suggested that this is probably due to the inclusion of EDTA in the low ionic strength saline solution supplied with the Dia Med kit. The EDTA which will chelate calcium results in inhibiting complement activation. So the gel test was neither able to identify the specificity nor in recognizing the complement activating property of antiVel.\(^{34}\)

A study was conducted to see if the increase in antibody detection by gel was actually due to detection of warm antibodies of undetermined specificities (WU). For an antibody to be called a WU there has to be a positive antibody screen followed by nonspecific reactivity in the antibody panels. However, the number of WU’s as a percentage of total samples was statistically the same in both methods (test 18% and 17.4%). The increase in
sensitivity did not translate into an increase in the rate of new WU’s being detected when compared to the tube method.  

Gautam SR et al reported that the gel technique had picked up more cases of blood cross match incompatibility as compared to tube technique. Swarup et al in his study of crossmatching 1000 samples using tube and gel reported that gel was a rapid and reliable procedure without controls. He concluded that gel technique is a better substitute for spin method.  

A retrospective study of comparison of antibody detection between tube and gel was conducted in a hospital during the period 1996-2006. During the study period all the patients were tested by the IAT. The positive results were further investigated by the use of panel test erythrocytes including the antiglobulin phase and the use of enzyme treated cells (papain) at 37 degree Celsius. From 1996-2000 (period 1) tube method was performed. From 2001-06 (period 2) all tests were made using Diamed gel. During the period 1, 240 of 3478 (6.9%) tests of IAT were found to be positive. 248 antibodies had been detected in 74 patients. During the period, 568 of 6563 (8.65%) tests of IAT were found to be positive. About 582 antibodies have been detected in 258 patients. The gel resulted in an increase in overall antibody detection rate (8.87% versus 7.13%) as compared to tube. This study confirms higher sensitivity, specificity and clinical relevance of gel techniques in transfusion services.
Elution studies are commonly carried out by performing a modified tube antiglobulin test. A study was conducted to compare the gel to the manual tube when testing acid eluates prepared from DAT positive RBCs. Gel and tube were in agreement with 44 of 51 (86%) acid eluates. Five eluates reacted weakly in tube and failed to react by gel. Similarly one eluate was negative by tube but reacted weakly in gel.³⁹

A retrospective study was carried out on samples in both tube and gel. Plasma tests in gel on post transfusion samples appear to be adequately sensitive for detecting new alloantibodies associated with delayed serological transfusion reactions. However, in pre transfusion samples, there was evidence for increased sensitivity of PEG IAT versus gel for the detection of IgG antibodies. It has to be confirmed whether the antibodies that were detectable in PEG caused clinical hemolysis after the patient received blood not typed for the particular antigen.⁴⁰

Weiss et al did a study on the gel testing for antibody screening and identification for three years. He found out that gel resulted in an increase in overall antibody detection rate (4.05%) as compared to the tube (3.2%). There was a relatively higher rate of detection of all antibodies and an increase in detection of potentially significant antibodies by the gel method. Sensitivity for potentially significant antibodies was 64% for tube and 95.5% for the gel method.⁴¹
It is well known that spin tube IATs require skilled expertise of hand and eye and that this is the major weakness of this technology. Nevertheless, a well performed spin tube IAT is the standard which any new system must be compared.\textsuperscript{42}

Claims for increased sensitivity of new system should be considered with caution as many such claims have been marred by unwanted occurrence of an increased number of false positives. Tests for false positive are therefore an essential part of validation of a new system. Useful new systems that offer cost effective sensitive antibody detection are now a fact of the life as several new technologies are finding their place in routine blood transfusion work. If very well performed, the standard tube spin IAT even in saline is still considered an acceptable technique for alloantibody detection. 1+ or 2+ reaction may be easily missed in spin tube IAT due to inappropriate reading particular in large scale screening. A weak reaction can be easily disrupted if tube is shaken too vigorously. When the IAT is used for testing large number of samples that give mostly negative results it is difficult to maintain a consistently accurate reading performance.\textsuperscript{43}

**USE OF GEL METHOD IN OTHER FIELDS**

Immunoglobulins A antibodies (anti IgA) are rare but can cause transfusion associated anaphylaxis. The detection of anti IgA has traditionally been performed using a labour intensive hemagglutination assay. Dia med
assays correctly identified patients who had significant IgA deficiency and anti IgA.\textsuperscript{44}

Gel card was evaluated for the detection of HbS and compared with both electrophoresis and solubility test. There was no discrepancy in all 103 sample results assay by three methods. In the dilutional assay, the gel test card showed higher sensitivity than the other two methods.\textsuperscript{45}

Das et al in his study of estimating fetomaternal hemorrhage compared gel and Kleihauer Betke technique. He reported that gel technique can be introduced as a screening method for fetomaternal hemorrhage.\textsuperscript{46}
MATERIALS AND METHODS
MATERIALS AND METHODS

STUDY METHOD

The prospective study to compare conventional tube technique and the gel technique in crossmatching of blood was carried out over a period of one year from 2010-2011 in the Department of Transfusion Medicine, The Tamilnadu Dr.M.G.R. Medical University, Guindy, Chennai. The blood samples from a total of 1200 patients were selected by the convenience sampling method. The study was approved by the ethical committee of the Tamilnadu Dr.M.G.R Medical University, Chennai.

SAMPLE COLLECTION

Patients’ blood samples are sent to our blood bank for routine crossmatching procedure. Both tube and gel technique were used for coombs crossmatching and their results were compared and analyzed.

TUBE METHOD

MATERIALS

Glass Tubes, 0.9% saline, Pasteur pipette, Centrifuge, 37° C incubator, polyspecific AHG reagent.
PROCEDURE

1. Add two drops of serum to the properly labeled tube.

2. Add one drop of 2 to 5% saline suspended donor red cells to the tube and mix.

3. Centrifuge at 1000 rpm for 1 minute and observe for any hemolysis and agglutination.

4. Incubate at 37°C for 30 minutes.

5. Centrifuge at 1000 rpm for 1 minute and observe for hemolysis and agglutination.

6. Wash the red cells three times with saline and completely decant the final wash.

7. Add two drops of polyspecific AHG to the dry red cell button and mix well.

8. Centrifuge at 1000 rpm for 1 minute and observe for agglutination. Grade and record the results.

9. Confirm the validity of negative results by adding IgG coated red cells.47
Interpretation

1. The presence of agglutination or hemolysis after incubation at $37^\circ C$ constitutes a positive test results.

2. The presence of agglutination after addition of AHG constitutes a positive test results.

3. Antiglobulin test results are negative when no agglutination is observed after initial centrifugation followed by agglutination with the addition of IgG coated red cells and centrifugation. If the IgG coated red cells are not agglutinated, the negative results is invalid and test must be repeated.  

<table>
<thead>
<tr>
<th>Macroscopically observed findings</th>
<th>Grade</th>
</tr>
</thead>
<tbody>
<tr>
<td>One solid agglutinate</td>
<td>4+</td>
</tr>
<tr>
<td>Several large agglutinates</td>
<td>3+</td>
</tr>
<tr>
<td>Medium sized agglutinates with clear background</td>
<td>2+</td>
</tr>
<tr>
<td>Very small agglutinates with turbid background</td>
<td>1+</td>
</tr>
<tr>
<td>No agglutination</td>
<td>0</td>
</tr>
<tr>
<td>Hemolysis</td>
<td>H( Positive reaction)</td>
</tr>
</tbody>
</table>
GRADING OF REACTION IN TUBE

Fig (a)
GEL METHOD

MATERIALS

DiaMed - ID LISS/Coombs Cards (AHG anti IgG and anti C3d), LISS reagent, micro pipette, Dia Med – ID Centrifuge, Dia Med- ID Incubator 37°C.

PROCEDURE

Preparing 0.8 % red cell suspension

- Dispense 1 ml of ID- Diluent 2 (Modified LISS for red cell suspension) into a clean tube.
- Add 10 μl of packed red cells and mix gently
  1. Pipette 50 μl of 0.8 % donor red cell suspension to the appropriate microtube.
  2. Add 25 μl of patients’ plasma or serum to the microtube.
  3. Incubate the ID card for 15 minutes at 37°C in the ID- Incubator
  4. Centrifuge the ID- card for 10 minutes in the ID- centrifuge.
  5. Read and record the results.
Interpretation

Positive – Agglutination cells forming a red line on the surface of the gel or agglutinates dispersed in the gel.

Negative – Compact button of cells on the bottom of the microtube.

Grading of Reaction

<table>
<thead>
<tr>
<th>Macroscopically observed findings</th>
<th>Grade</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solid band of agglutinated red cells at the top of the gel column..</td>
<td>4+</td>
</tr>
<tr>
<td>Red cell agglutinates predominantly observed toward the top of the gel column.</td>
<td>3+</td>
</tr>
<tr>
<td>Red cell agglutinates dispersed throughout the gel column.</td>
<td>2+</td>
</tr>
<tr>
<td>Red cell agglutinates predominantly observed in the lower half of the gel column.</td>
<td>1+</td>
</tr>
<tr>
<td>Red cells forming a pellet at the bottom of the gel column.</td>
<td>Negative</td>
</tr>
<tr>
<td>Red cell agglutinates at the top of the gel accompanied by pellet of unagglutinated cells in the bottom of gel column.</td>
<td>Mixed - Field</td>
</tr>
</tbody>
</table>
STATISTICAL ANALYSIS

Qualitative data will be given in frequencies with their percentage. Statistical tool used will be Chi square test. P < 0.05 will be taken as significant. Sensitivity and specificity of the test will be calculated. All the statistical analysis was carried out using SPSS software.

DIAMED - ID LISS/COOMBS CARD

Fig(b)
RESULTS
RESULTS

Table 1

GENDER DISTRIBUTION

<table>
<thead>
<tr>
<th>SEX</th>
<th>frequency</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>MALE</td>
<td>684</td>
<td>57.1</td>
</tr>
<tr>
<td>FEMALE</td>
<td>516</td>
<td>42.9</td>
</tr>
<tr>
<td>TOTAL</td>
<td>1200</td>
<td>100.0</td>
</tr>
</tbody>
</table>

Fig 1
Table 2

INDICATION FOR TRANSFUSION

<table>
<thead>
<tr>
<th>INDICATION</th>
<th>FREQUENCY</th>
<th>PERCENTAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>TRAUMA</td>
<td>206</td>
<td>17.2</td>
</tr>
<tr>
<td>SURGERY</td>
<td>336</td>
<td>28.0</td>
</tr>
<tr>
<td>O.G</td>
<td>301</td>
<td>25.1</td>
</tr>
<tr>
<td>Associated Medical Condition</td>
<td>357</td>
<td>29.8</td>
</tr>
</tbody>
</table>

INDICATION FOR TRANSFUSION (Fig 2)
### Table 3

**NUMBER OF TRANSFUSIONS**

<table>
<thead>
<tr>
<th>Number of transfusions</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>489</td>
<td>40.8</td>
</tr>
<tr>
<td>1</td>
<td>165</td>
<td>13.8</td>
</tr>
<tr>
<td>&gt; 1</td>
<td>546</td>
<td>45.5</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>1200</td>
<td>100.0</td>
</tr>
</tbody>
</table>

**NUMBER OF TRANSFUSIONS**

**Fig 3**

![Pie chart showing transfusion frequency]

- **0**
- **1**
- **>1**
Table 4

HISTORY OF PREVIOUS PREGNANCY OR ABORTION

<table>
<thead>
<tr>
<th>Previous pregnancy or abortion</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>YES</td>
<td>450</td>
<td>87.2</td>
</tr>
<tr>
<td>NO</td>
<td>66</td>
<td>12.8</td>
</tr>
<tr>
<td>TOTAL</td>
<td>516</td>
<td>100.0</td>
</tr>
</tbody>
</table>

PREVIOUS PREGNANCY OR ABORTION

Fig 4
Table 5

PREVIOUS TRANSFUSION

<table>
<thead>
<tr>
<th>Previous transfusion</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>YES</td>
<td>713</td>
<td>59.3</td>
</tr>
<tr>
<td>NO</td>
<td>487</td>
<td>40.6</td>
</tr>
<tr>
<td>TOTAL</td>
<td>1200</td>
<td>100.0</td>
</tr>
</tbody>
</table>

PREVIOUS TRANSFUSION

Fig 5
### Table 6

**ASSOCIATED MEDICAL CONDITION**

<table>
<thead>
<tr>
<th>Associated medical condition</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>THALATHALASSAEMIA</td>
<td>35</td>
<td>2.9</td>
</tr>
<tr>
<td>AUTOIMMUNE DISEASE</td>
<td>39</td>
<td>3.3</td>
</tr>
<tr>
<td>HEMATO-ONCOLOGY RELATED</td>
<td>156</td>
<td>13.0</td>
</tr>
<tr>
<td>RENAL FAILURE</td>
<td>101</td>
<td>8.4</td>
</tr>
<tr>
<td>OTHERS</td>
<td>340</td>
<td>28.3</td>
</tr>
<tr>
<td>NO ASSOCIATED MEDICAL CONDITION</td>
<td>529</td>
<td>44.1</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td><strong>1200</strong></td>
<td><strong>100.0</strong></td>
</tr>
</tbody>
</table>

---

**Fig 6**

![Bar Chart](chart.png)
Table 7

TUBE CROSSMATCH

<table>
<thead>
<tr>
<th>Samples</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>POSITIVE</td>
<td>14</td>
<td>1.2</td>
</tr>
<tr>
<td>NEGATIVE</td>
<td>1186</td>
<td>98.8</td>
</tr>
<tr>
<td>TOTAL</td>
<td>1200</td>
<td>100.0</td>
</tr>
</tbody>
</table>

Table 8

GEL CROSSMATCH

<table>
<thead>
<tr>
<th>Samples</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>POSITIVE</td>
<td>18</td>
<td>1.5</td>
</tr>
<tr>
<td>NEGATIVE</td>
<td>1182</td>
<td>98.5</td>
</tr>
<tr>
<td>TOTAL</td>
<td>1200</td>
<td>100.0</td>
</tr>
</tbody>
</table>

POSITIVE IN TUBE AND GEL (Fig 7)
### Table 9

**COMPARISON OF TUBE AND GEL CROSSMATCH**

<table>
<thead>
<tr>
<th>Comparison of Tube and Gel Crossmatch</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>BOTH POSITIVE</td>
<td>14</td>
<td>1.2</td>
</tr>
<tr>
<td>GEL POSITIVE AND TUBE NEGATIVE</td>
<td>4</td>
<td>0.3</td>
</tr>
<tr>
<td>BOTH NEGATIVE</td>
<td>1182</td>
<td>98.5</td>
</tr>
<tr>
<td>TOTAL</td>
<td>1200</td>
<td>100.0</td>
</tr>
</tbody>
</table>

### Table 10

**HISTORY OF PREVIOUS TRANSFUSION and POSITIVITY**

**IN TUBE and GEL**

<table>
<thead>
<tr>
<th>PREVIOUS TRANSFUSION</th>
<th>TUBE POSITIVE</th>
<th>GEL POSITIVE</th>
</tr>
</thead>
<tbody>
<tr>
<td>YES</td>
<td>11 (1.5 %)</td>
<td>14 (2.0 %)</td>
</tr>
<tr>
<td>NO</td>
<td>3 (0.6 %)</td>
<td>4 (0.8 %)</td>
</tr>
</tbody>
</table>

$P > 0.05$
### Table 11

**HISTORY OF PREVIOUS PREGNANCY and POSITIVITY IN TUBE and GEL**

<table>
<thead>
<tr>
<th>PREVIOUS PREGNANCY OR ABORTION</th>
<th>TUBE POSITIVE</th>
<th>GEL POSITIVE</th>
</tr>
</thead>
<tbody>
<tr>
<td>YES</td>
<td>7 (1.5%)</td>
<td>8 (1.7%)</td>
</tr>
<tr>
<td>NO</td>
<td>0</td>
<td>1 (1.5%)</td>
</tr>
</tbody>
</table>

*P > 0.05*

### Table 12

**INDICATION FOR TRANSFUSION and POSITIVITY IN TUBE and GEL**

<table>
<thead>
<tr>
<th>INDICATION FOR TRANSFUSION</th>
<th>TUBE POSITIVE</th>
<th>GEL POSITIVE</th>
</tr>
</thead>
<tbody>
<tr>
<td>TRAUMA</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>SURGERY</td>
<td>4 (1.2%)</td>
<td>4 (1.2%)</td>
</tr>
<tr>
<td>O.G</td>
<td>6 (2.0%)</td>
<td>6 (2.0%)</td>
</tr>
<tr>
<td>Associated Medical Condition</td>
<td>4 (1.1%)</td>
<td>8 (2.2%)</td>
</tr>
</tbody>
</table>

*P > 0.05*
Table 13

NUMBER OF TIMES TRANSFUSED AND POSITIVITY

IN TUBE and GEL

<table>
<thead>
<tr>
<th>NO. OF TIMES TRANSFUSED</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>TUBE POSITIVE</td>
<td>3</td>
</tr>
<tr>
<td>GEL  POSITIVE</td>
<td>4</td>
</tr>
<tr>
<td>TOTAL</td>
<td>489</td>
</tr>
</tbody>
</table>

P < 0.05

Table 14 (Fig 8)

PARAMETERS ASSOCIATED WITH INCOMPATIBLE CROSSMATCH:

<table>
<thead>
<tr>
<th>PARAMETERS</th>
<th>TUBE</th>
<th>GEL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total incompatible samples</td>
<td>14</td>
<td>18</td>
</tr>
<tr>
<td>Previous H/O Pregnancy/Abortion</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td>Previous H/O Transfusion</td>
<td>11</td>
<td>14</td>
</tr>
<tr>
<td>Number of Transfusions (&gt;1)</td>
<td>11</td>
<td>14</td>
</tr>
</tbody>
</table>
Tube technique detected 14 incompatible positive samples out of the total 1200 crossmatched samples (Table 7). Gel technique detected 18 incompatible positive samples out of the total 1200 crossmatched samples which included the 14 samples detected by the tube technique (Table 8). The tube however gave a positive reaction when IgG coated cells were added to all the four negative samples confirming the validity of the reaction.

The prevalence was 1.2 % for tube and 1.5 % for gel technique in the detection of irregular antibodies. In cases where the patient had been previously transfused the prevalence rate increased to 1.5 % for tube and 2 % for gel technique (Table 10). In cases with a history of pregnancy or abortion, the prevalence rate was 1.5 % for tube and 1.7 % for gel technique (Table 11). The tube technique found out 6 positive cases related to Obstetrics and
Gynaecology (2% prevalence), 4 positive cases related to surgery (1.2%) and 4 positive cases related to medical conditions (1.1%). The gel technique similarly found out 6 positive cases related to Obstetrics and Gynaecology, 4 positive cases related to surgery but 8 positive cases related to associated medical conditions (2.2%) (Table 12). The tube identified 11 positive cases that were multiple transfused in comparison to gel which identified 14 positive cases (Table 13).

Using Chi Square test, when comparing the number of transfusions with positivity in tube and gel, p value was found to be statistically significant.

**Multiple Logistic Regression**

<table>
<thead>
<tr>
<th>METHOD</th>
<th>VARIABLE</th>
<th>Odds Ratio</th>
<th>95% confidence interval for Odds Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>TUBE</td>
<td>Number of times transfused</td>
<td>1.74</td>
<td>1.40 – 2.50</td>
</tr>
<tr>
<td>GEL</td>
<td>Number of times transfused</td>
<td>1.68</td>
<td>1.38 – 2.27</td>
</tr>
</tbody>
</table>

Using backward logistic regression for tube and gel technique separately, the above Odds Ratio is tabulated. In this model, clinically significant variables were included. Finally, both the techniques did not remove the variable number of times transfused and were found to be statistically significant.
SENSITIVITY AND SPECIFICITY OF THE GEL WITH RESPECT TO TUBE

<table>
<thead>
<tr>
<th>GEL</th>
<th>TUBE</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>POSITIVE</td>
<td>NEGATIVE</td>
</tr>
<tr>
<td>POSITIVE</td>
<td>14</td>
<td>4</td>
</tr>
<tr>
<td>NEGATIVE</td>
<td>0</td>
<td>1182</td>
</tr>
<tr>
<td>TOTAL</td>
<td>14</td>
<td>1186</td>
</tr>
</tbody>
</table>

SENSITIVITY = \( \frac{14}{14} \times 100 = 100\% \)

SPECIFICITY = \( \frac{1182}{1186} \times 100 = 99.7\% \)

POSITIVE PREDICTIVE VALUE = \( \frac{14}{18} \times 100 = 77.8\% \)

NEGATIVE PREDICTIVE VALUE = \( \frac{1182}{1182} \times 100 = 100\% \)

The sensitivity and specificity of the gel were 100 % and 99.7 % with respect to the tube technique.

MEASUREMENT OF AGREEMENT

The agreement between the tube and gel technique was 87.3 % (kappa = 0.873)
THREE CELL PANEL

Missed out Samples = 4 samples which were positive in gel and negative in tube were tested again in Three cell panel (ID- DiaCell I-II-III). This test was carried out to find out whether the four samples detected in gel were genuinely false positive or the tube failed to detect the four samples.

All the four samples tested reacted in the three cell panel confirming the presence of an irregular or unexpected antibody.

DURATION OF THE TUBE AND GEL TECHNIQUES

The average time taken for one Tube Crossmatch was 52 minutes and the time taken for one Manual Gel Crossmatch was 28 minutes.

<table>
<thead>
<tr>
<th>TECHNIQUE</th>
<th>CELL-SERUM PREPARATORY PHASE (min.)</th>
<th>INCUBATION PHASE (min.)</th>
<th>CELL WASHING PHASE (min.)</th>
<th>CENTRIFUGATION PHASE (min.)</th>
<th>READING PHASE (min.)</th>
<th>TOTAL TIME (min.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TUBE</td>
<td>5</td>
<td>30</td>
<td>10</td>
<td>5</td>
<td>2</td>
<td>52</td>
</tr>
<tr>
<td>GEL</td>
<td>2</td>
<td>15</td>
<td>-</td>
<td>10</td>
<td>1</td>
<td>28</td>
</tr>
</tbody>
</table>

COST EFFECTIVENESS BETWEEN TUBE AND GEL TECHNIQUE

In our study, Gel technique cost three times more than the conventional tube technique for a single Coombs Crossmatching Procedure.
DISCUSSION
DISCUSSION

The study was undertaken to compare between the conventional tube technique and the gel technique in crossmatching of blood. 1200 patients’ serum samples were tested in conventional tube as well as by the gel technique.

In our study, the tube technique had a prevalence rate of 1.2 % while the gel had a prevalence of 1.5 % in detecting irregular antibodies. Weiss et al found that gel resulted in an increase in overall antibody detection rate (4.05%) as compared to the tube (3.2%). They have also stated that there was a relatively higher rate of detection of all antibodies and an increase in detection of potentially significant antibodies by the gel method.41

The sensitivity and specificity of the gel with respect to tube in our study was 100 % and 99.7 % respectively. Various studies given below have proven that sensitivity of the gel technique is equal to or even better than tube technique in relation to antibody detection, DAT and IAT:

<table>
<thead>
<tr>
<th>STUDIES</th>
<th>GEL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sensitivity</td>
</tr>
<tr>
<td>Weisbach et al (in Antibody Screening)</td>
<td>93.5 %</td>
</tr>
<tr>
<td>Swarup et al (in IAT)</td>
<td>100 %</td>
</tr>
<tr>
<td>Sudipta, Chaudary et al (in DAT)</td>
<td>98.4 %</td>
</tr>
<tr>
<td>Sharma et al (in IAT)</td>
<td>100 %</td>
</tr>
</tbody>
</table>
The agreement between the two methods by Sudipta et al in DAT was 95.7%. However, in our study in Cross Matching it was 87.3%.

The gel technique picked 18 incompatible positive samples which included the 14 detected by the tube method. Both techniques detected six incompatible O.G related cases and four surgery related cases. The grading strength was also similar in both the techniques. Tube detected four positive cases while gel detected eight positive cases which had a history of the following medical conditions other than Surgery and O.G related cases:

<table>
<thead>
<tr>
<th>MEDICAL CONDITION</th>
<th>POSITIVE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TU</td>
</tr>
<tr>
<td>THALASSAEMIA</td>
<td>1</td>
</tr>
<tr>
<td>AUTO IMMUNE DISEASE</td>
<td>1</td>
</tr>
<tr>
<td>HEMATO-ONCOLOGY DISEASE</td>
<td>1</td>
</tr>
<tr>
<td>RENAL FAILURE</td>
<td>1</td>
</tr>
</tbody>
</table>

Out of the 4 cases missed by tube, two were Hemato-oncology related, one was a case of autoimmune disease and the other was a case of Thalasaemmia, excepting the case of autoimmune disease all the other three cases had the history of previous transfusion with more than one unit of blood. In Gautam et al study, Hematological diseases were the most common medical condition associated with irregular antibody detection followed by
renal, liver and autoimmune diseases. The gel had a 1+ reaction in all the four cases missed by the tube.

1+ POSITIVE REACTION IN GEL (MISSED IN TUBE)

Fig 9

Three cell panel was done in all the four cases to find if they were false positive cases or if there was a presence of an irregular antibody. The serum of the four positive cases reacted with the cell panels confirming the presence of an unexpected antibody. It could have been a weakly bound antibody since the gel gave only a 1+ reaction in all the four crossmatched samples. This weakly bound antibody could have been washed off during the cell washing phase or the number of IgG bound molecules per cell would have been lower for the tube for it to identify a positive reaction. A minimum
of 100 to 200 IgG bound molecules per cell is needed for positivity in tube.\textsuperscript{49} Dissociation of antibody can occur due to prolonged period of incubation of serum and red cells, prolonged centrifugation and delay in reading the results.\textsuperscript{7}

However, statistically there was no significant difference (P>0.05) between indication for transfusion or history of associated medical conditions and positivity in tube and gel. Similarly, there was no statistically significant difference (P > 0.05) in cases of history of previous pregnancy or abortion and positivity in tube and gel.

When compared to the number of times transfused, there was a statistically significant difference for both tube (P =0.016) and for gel (P = 0.036). The tube picked up 11 incompatible positive cases which had a history of more than one time transfusion while the gel picked 14 incompatible positive cases which had a similar history of more than one time transfusion. The odds ratios were 1.74 for tube and 1.68 for gel which were statistically significant.

With regard to the expenditure, the cost required to do a gel crossmatch is thrice the amount required to do a conventional tube crossmatch. This cost can be brought down if the gel technique is introduced on a large scale. Automation of the technique can result in excellent tracking systems for quality control purposes as each step is validated and controlled, give faster result output and avoid any human errors.
The duration to do a complete crossmatching procedure takes about 52 minutes for a conventional tube technique while it takes only a maximum of 28 minutes to do a gel technique. This is mainly due to the cell washing phase which takes an additional 10 minutes and the prolonged incubation phase which takes a minimum of half an hour for a conventional tube technique. Nathalang et al explains the washing procedure in which faulty or poor techniques may adversely affect the resulting outcome. The removal of washing procedures in the gel technique has eliminated the formation of aerosols and minimized the risk of contamination by washing fluids. Therefore, the risk of contracting blood transmitted diseases such as hepatitis or HIV infection will decline.\textsuperscript{13}

From a technical point of view, the gel appears to be a better technique because of its greater uniformity between repeat tests, lesser sample volume, easy interpretation and a simple procedure. The gel though, has its disadvantages – special enhancing medium like LISS is needed, buffer evaporation can occur and gel column can get disturbed in a subtropical country like India which we observed in some gel cards. This can cause some variation in reading the strength of the reaction. False positive reactions can occur due to fibrin present in fresh serum trapped at top of gel.\textsuperscript{10} . Cell suspensions more concentrated than 0.8% can affect the sensitivity of gel test and may result in false positive results. Neppert et al demonstrated unsatisfactory detection of an in vivo hemolytic anti Vel by the gel test which could be detected by the tube test.\textsuperscript{34}
The selection of the test method should be based on the balance of sensitivity and specificity. One should consider the time, resources and cost of these newer techniques that can detect high number of antibodies along with the potential impact of these factors on patient treatment.

**Stepwise technical differences between Tube and Gel are given below:**

<table>
<thead>
<tr>
<th></th>
<th>TUBE</th>
<th>GEL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red cell suspension</td>
<td>2-5 %</td>
<td>0.9 %</td>
</tr>
<tr>
<td>Suspending medium</td>
<td>Saline</td>
<td>LISS</td>
</tr>
<tr>
<td>Cell Washing</td>
<td>Necessary</td>
<td>Not necessary</td>
</tr>
<tr>
<td>Incubation time</td>
<td>30 – 45 minutes</td>
<td>15 minutes</td>
</tr>
<tr>
<td>Reaction</td>
<td>Occurs in whole tube</td>
<td>Occurs in reaction chamber</td>
</tr>
<tr>
<td>Centrifugation</td>
<td>Usual</td>
<td>Controlled centrifugation</td>
</tr>
<tr>
<td>Reaction Medium</td>
<td>No medium is used</td>
<td>Dextran Gel</td>
</tr>
<tr>
<td>Interpretation</td>
<td>Subjective</td>
<td>Relatively objective</td>
</tr>
<tr>
<td>Reading the reaction</td>
<td>Read immediately</td>
<td>Stored and kept for documentation</td>
</tr>
<tr>
<td>Procedure</td>
<td>Technical expertise is needed</td>
<td>Simple and easy procedure</td>
</tr>
<tr>
<td>Time taken</td>
<td>Longer time (45 – 60 minutes)</td>
<td>Shorter time (25 -30 minutes)</td>
</tr>
<tr>
<td>Cost</td>
<td>Less costly</td>
<td>Three times more costly than tube</td>
</tr>
</tbody>
</table>
The advantages and disadvantages of Tube are given below:

<table>
<thead>
<tr>
<th>S.No</th>
<th>ADVANTAGES</th>
<th>DISADVANTAGES</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Standard and conventional technique</td>
<td>When compared to other techniques, more number of IgG bound molecules per cell is needed to identify a positive reaction.</td>
</tr>
<tr>
<td>2.</td>
<td>No need for any special enhancing medium (Saline is used)</td>
<td>Sample volume is more compared to gel and Time consuming procedure</td>
</tr>
<tr>
<td>3.</td>
<td>Cheap compared to other techniques</td>
<td>Cell washing is required which can dislodge weakly bound antibodies</td>
</tr>
<tr>
<td>4.</td>
<td>Hemolysis can be detected. Immediate spin phase can rule out any ABO incompatibility which can occur due to clerical error.</td>
<td>Rouleaux phenomenon can give false positive reaction.</td>
</tr>
<tr>
<td>5.</td>
<td>Non biodegradable substance (plastics) are not used</td>
<td>Results are subjective and to be read quickly</td>
</tr>
</tbody>
</table>
The advantages and disadvantages of Gel are given below:

<table>
<thead>
<tr>
<th>S.No</th>
<th>ADVANTAGES</th>
<th>DISADVANTAGES</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Sample volume is less compared to tube</td>
<td>Costly technique</td>
</tr>
<tr>
<td>2.</td>
<td>No cell washing is required and ideal for automation.</td>
<td>Plastic cards used is not biodegradable</td>
</tr>
<tr>
<td>3.</td>
<td>Person to person variation in results is absent and a less time consuming procedure.</td>
<td>Buffer evaporation can occur and gel column can be disturbed if not properly stored.</td>
</tr>
<tr>
<td>4.</td>
<td>Mixed field reaction can be detected and cold antibody can be avoided</td>
<td>Haemolysis can be misinterpreted and fibrin can give false positive results</td>
</tr>
<tr>
<td>5.</td>
<td>Since gel has already been coated with AHG, there is no separate step of adding Antiglobulin reagents.</td>
<td>Need special enhancing medium and instruments for this procedure</td>
</tr>
</tbody>
</table>
SUMMARY
SUMMARY

- This prospective study was conducted on 1200 blood samples to compare conventional tube and gel techniques in crossmatching of blood.

- The number of Coombs Crossmatch incompatible samples by tube technique was 14 and by gel technique was 18. The prevalence of the tube and gel technique in detection of irregular antibodies was 1.2 % and 1.5 % respectively.

- The sensitivity and specificity of the gel technique with respect to tube technique were 100 % and 99.7 % respectively.

- Three cell panel was done on the 4 samples which were positive by gel and negative by tube. All the four samples reacted positive with the three cell panel confirming the presence of an irregular antibody.

- The Grading Reaction of all the four samples which were negative by tube and positive by gel was 1+.

- The number of positive samples with a history of previous transfusion was 11 for tube and 14 for gel. Similarly, the number of positive samples with a history of previous pregnancy or abortion was 7 for tube and 8 for gel.
The number of transfusions with the positivity in tube and gel were statistically significant with “P” values of 0.016 and 0.036 respectively. The percentage of agreement between the two techniques was 87.3%.

The average time taken for a single Coombs Crossmatching with tube technique was 52 minutes and for a gel technique (manual) was 28 minutes.

Gel technique cost three times more than the conventional tube technique for a single Coombs Crossmatching Procedure.
CONCLUSION
CONCLUSION

The gel technique has definite advantages over the tube technique because of being able to detect more positive samples than tube and its shorter duration for the crossmatching procedure. Considering the cost factor, since there is a definite association with the parameters like previous history of transfusion, pregnancy and number of transfusions, the gel technique could be recommended for such cases. However, in a developing country like India, the gel technique if applied widely, cost factor would come down and could be considered as a better alternative to the conventional tube technique to ensure successful transfusion.
REFERENCES


ANNEXURES
ABBREVIATIONS

1. IAT - Indirect Antiglobulin Test.
2. AHG - Anti Human Globulin.
3. DAT - Direct Antiglobulin Test.
4. LISS - Liss Ionic Strength Saline.
5. BSA - Bovine Serum Albumin.
6. PEG - Poly Ethylene Glycol.
7. EDTA - Ethylene Diamine Tetra Acetic acid.
8. Ig - Immunoglobulin.
9. PNH - Paroxysmal Nocturnal Haemoglobinuria.
10. MCAT - Micro Column Affinity Test.
11. AIHA - Auto Immune Hemolytic Anaemia.
# INSTITUTIONAL ETHICS COMMITTEE

**Address of Ethics Committee:** The Tamilnadu Dr MGR Medical University
Chennai, India

**Principal Investigator:** Dr R Rajharath, MBBS

**Protocol title:** Comparative study of blood crossmatching using Gel and conventional tube technique (ECMGR0309001)

<table>
<thead>
<tr>
<th>Documents filed</th>
<th>✔</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical trial protocol</td>
<td>✔</td>
</tr>
<tr>
<td>Informed consent documents</td>
<td>✔</td>
</tr>
</tbody>
</table>
pinion of the institutional Ethics Committee-PLEASE CHECK ONE

- Approval
- Modification required prior to approval (please specify on the space below)
- Disapproval

Date of review:

Signed: [Signature] (please print name) [Name]

The research proponent is hereby informed that the Institutional Ethics Committee will require the following:

1) All adverse drug reaction (ADRs) that are both serious and unexpected to be reported promptly to the IEC within 7 working days.

2) The progress report to be submitted to the IEC at least annually.

3) Upon completion of the study, a final study status report to submitted to the IEC.
QUESTIONNAIRE

1. Patient name:

2. Age:

3. Sex:

4. IP No:

5. Donor ID:

6. Address:

7. Phone no:

8. Blood group: O / A / B / AB / Oh   Rh: POSITIVE / NEGATIVE

9. Indication for blood transfusion

10. Is there any history of pregnancy? Yes/No

11. Is there any history of abortion? Yes/No

12. Is there any history of previous transfusion? Yes/No
   
   a. If YES, then what was the indication?

   b. How many times had you been transfused?

13. Is there any associated medical condition?
CONSENT FORM

- I confirm that I read and understood the information about the above research study dated ______________ and I received chance to ask the questions.

- My participation in this study is voluntary and I know that I am free to withdraw from the study at any time, without giving any reason and without affecting of my legal rights.

- I agree to this access. I know that my identification will not be revealed in any details that is released to third persons or published.

- I agree not to restrict or interfere any data or results that are obtained from this study.

- I agree to participate in this research study for the above listed purpose.

Patient's name : 

Signature : Date :

Signature of the person who obtains consent : Date :

patient ID Number :