

Comparison between Conventional Tube Technique and Column Agglutination Technique for Red Blood Cell Antibody Testing

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Abstract

Original Research Article

Background: There are various techniques used for detection of antibodies in the blood. Conventional Tube technique (CTT) is the widely used for performing Indirect Antiglobulin Test (IAT). Column Agglutination Technique (CAT) is more sensitive. As we adopt this newer technique in our lab, this study will help to grasp a better understanding of the efficacy of the CAT in comparison to CTT in terms of DAT. **Objective:** To assess the potency of Column Agglutination Technique (CAT) in comparison to Conventional Tube Technique (CTT) with respect to IAT. **Methods:** This was a comparative cross sectional study which was carried out at the Department of Transfusion Medicine, Bangabandhu Sheikh Mujib Medical University, Dhaka from June 2022 to July 2023 over a period of one year. After obtaining I.R.B. clearance and informed consent of the patient all the samples referred to our department for the purpose of performing Coombs test/ IAT will be included consecutively in this study according to inclusion and exclusion criteria. Particulars of the patient and necessary clinical and available laboratory data indicating haemolysis was collected from the patient's referral sheet and registered in the questionnaire. Venous blood was collected for performing IAT with polyspecific antiglobulin reagents in CAT and CTT on the day of sample collection. Later these investigation results was collected and registered in the questionnaire. Finally, statistical analysis was done by SPSS, version-21. **Results:** 90 samples were analyzed and out of these 20 samples were found incompatible by CAT with gel card method but 15 were detected incompatible by CTT. Column agglutination technique is more potent than conventional tube technique in detecting positive result. Among 90 samples 41 were male and 49 were female. There was history of pregnancy in 32 female. Out of 90 study population 81 were experienced with blood transfusion. Time required for total procedure for CTT was approximately 100 minutes and for CAT was about 30 minutes. Regarding cost CTT was 500 taka and for that of CAT was 600 taka. **Conclusion:** Column agglutination technique was found more potent than conventional tube technique in detecting positive result.

Keywords: Indirect Coombs' test (ICT)/ Indirect antiglobulin test (IAT), Conventional tube technique (CTT), Column Agglutination Technique (CAT).

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INTRODUCTION

The invention of Direct Antiglobulin Test (DAT) and Indirect Antiglobulin Test (IAT); informally known as then Coombs' test by Coombs, Mourant and Race in 1945 based on the principle given by Moreshi in 1908, is one of the most important advancement in immunohaematology. Here, the IAT detects in vivo sensitized Red Blood Cell (RBC) where RBCs are coated by specific surface protein i.e. immunoglobulin and/or complement component and IAT detects in vitro

sensitized red cell that is presence of the formerly mentioned proteins in serum of an individual [1]. IAT becomes positive in following cases in case of 1) detection of incomplete antibodies, 2) determination of RBC phenotype, 3) titration of incomplete antibodies 4) recipient of Intravenous Immunoglobulin (IVIg) or Intravenous Rh immunoglobulin (IV RhIg), 5) recipient of AntiThymocyte Globulin (ATG) or anti CD38 antibody, 6) recipient of haemopoietic component or

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solid organ transplant 7) malaria 8) kala azar, 9) 1 in 1000 healthy adult [2-4].

Although Conventional Tube technique is still considered the gold standard, it is not without some limitation. Along with requiring expert hand and skilled immunohaematologist, it is difficult to standardise due to variability in the interpreter. It only becomes positive when the number of protein coating the RBC reaches certain threshold (150-500 /RBC molecule). However it is done in test tube requiring only specific reagents with minimal cost and no specialized instrument which makes this technique popular and being widely practiced in developing countries. Several newer techniques are now being available to overcome the shortcomings of CTT, mostly in reference laboratories in developed world which offers more sensitivity and specificity i.e. Column Agglutination Technique (CAT), Flowcytometry, Polybrene test, Complement fixation antiglobulin consumption test, Enzyme linked anti-IgG assay etc. However, low resource country like ours could not previously avail them due to cost and absence of specialized set-up.

Recently our centre introduced CAT for performing Coombs' test, known widely as Gel Card where RBC antigen-antibody reactions are determined using a chamber filled with polyacrylamide gel. The gel acts as a trap: free unagglutinated RBCs form pellets in the bottom of the tube (negative reaction); agglutinated RBCs remain at the top of the tube or are trapped in the gel (positive reaction). CAT has a number of important advantages over routine tube testing, particularly when testing large numbers of samples. These include standardization, stability, smaller sample volume, ease of performance and analysis, and rapidity. However, it does require specialized equipment and results of the tests run in CAT may still be interpreted differently by different operators. An error due to manual intervention can potentially result in a lethal outcome in the transfusion process. As a result many laboratories are now leaning toward automation and newer techniques with an aim to minimize or eliminate the risk of errors from manual manipulations, reduce the staff exposure to bio-hazardous samples, assure the traceability of the process, and improve the reliability, turnaround time (TAT), and throughput [5].

The CAT method demonstrated stronger agglutination scores (60 vs. 43) compared to CTT using control cells. The sensitivity and specificity of the CAT was 98.4 and 95.2%, respectively as compared to CTT for polyspecific DAT in a study conducted in India where modernization of immunohematology lab replacing CTT method with CAT and other methods have been observed in increasing frequency now a days [6]. Positive DAT by tube technique was seen in only 41 out of 65 patients whose DAT was positive by CAT. Positive predictive values (PPVs) of tube and CAT were

comparable. Although on comparing the strength of agglutination with the *in vivo* hemolysis, 1+ reaction by CAT had low positive predictive value [7]. In a study by Nathalang *et al.*, in 1997 on newborn with HDFN and AIHA patients referred for serological confirmation CAT exhibited higher scores than the CTT ($p < 0.01$). The overall sensitivity and specificity of the CAT were 93.5 and 88.6%, respectively with the recommendation that CAT is equal to or better than the CTT.

Over last 40 years, the conventional tube technique (CTT) has been the cornerstone of compatibility testing, which includes saline method without using anti-human globulin (AHG) and indirect coombs test (ICT) method using AHG. But this technique has some limitations like elution of low affinity antibodies during washing, variability in the test result due to variations in the cell serum ration, and lack of consistency in reporting the results due to inter-observer variability [8].

In 1988, Lapiere introduced gel card, which is a column agglutination technique (CAT). Since then it has become a widely used serological testing method in immune-hematology laboratories worldwide. CAT can be used for ABO and Rh typing, cross-matching, direct and indirect snit-globulin test and identification of alloantibodies [9,10].

Recently our centre has introduced CAT for performing cross match and Coombs test, where RBC antigen-antibody reactions are determined using a chamber filled with gel. The gel acts as a trap. Free unagglutinated RBCs form pellets in the bottom of the tube (negative reaction) and agglutinated RBCs remain at the top of the tube or are trapped in the gel (positive reaction)

CAT has a number of important advantages over CTT. These include standardization, stability, smaller sample volume, ease of performance and analysis and rapidity. The testing procedure by CAT takes 15-20 minutes as compared to 90 minutes by CTT if ICT method is used with AHG and approximately 30 minutes if saline method is used without AHG [11]. CAT is now considered better than CTT because this system is more sensitive, more specific, less time consuming, simple to perform and less exposure of blood bank personal to blood which reduces the risk of HIV, HBsAg and HCV infections [12].

This study aims to compare between CAT and CTT for cross matching on the basis of sensitivity, specificity, turnaround time for conducting the test and cost-efficiency.

MATERIALS AND METHOD

A Cross-sectional comparative study was conducted from 1 July 2022 to 30 June 2023. A structured questionnaire was used. Written consent was

taken from the participants. The total participants of the study were 90. This study was carried out in the department of transfusion medicine, Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh. The patients referred to the dept. of Transfusion Medicine for IAT, fulfilling inclusion criteria were taken as samples by purposive convenient or non random or non probability sampling technique. The participants consented to participate voluntarily in the study. The data obtained from this study was analyzed by Statistical Package for Social Science (SPSS) version -22.

Inclusion criteria: Among all samples referred for performing direct antiglobulin test those who will give consent.

Methodology: Department of transfusion Medicine in Bangabandhu Sheikh Mujib Medical University (BSMMU) serves as a tertiary referral centre all across the country due to its line-up of expert immunohaematologists along with facilities for performing complex serological work-up. So samples are referred from all over the country for performing Coombs test, namely Direct Antiglobulin Test (DAT) with or without Indirect Antiglobulin Test (IAT) in case of discrepant blood grouping result, positive autocontrol, antibody identification or suspicion of haemolysis due to Autoimmune haemolytic anaemia or diagnosis of haemolytic disease of fetus and newborn by clinicians. After approval of institutional review board we will analyse consecutive blood samples sent to our immunohematology laboratory for DAT after obtaining consent of the patient. After obtaining necessary information from the patient regarding history and diagnosis of the disease process, venous blood samples will be collected in EDTA anticoagulated tube and polyspecific DAT will be performed on the day of collection using both CTT and CAT.

Conventional Tube Technique: It will be performed following standard method described by AABB technical manual (2020). One drop of 5% suspension of red cells was dispensed into test tubes and will be washed four times with normal saline, final wash decanted completely. Two drops of polyspecific AHG reagent (Immuno Diagnostics Inc. USA) will be then added, mixed well and tube centrifuged at 1000 rpm for 1 minute and cells will be examined for agglutination. All

reactions will be graded and recorded. The negative test was further validated by absence of agglutination on addition of sensitized control cells (in house).

Coloumn Agglutination Technique: It was performed following manufacturer's instruction. Briefly, 50 microL of 0.8 % red cell suspension in low ionic strength solution (LISS) was added to each microtube of the gel cards (Matrix™ AHG (Coombs) Test Card, India) and centrifuged in a dedicated centrifuge device (Matrix™, India) at 2000 rpm for 10 min following the recommended incubation period of 15 min. The findings of the agglutination reactions was graded as 4 +, 3 +, 2 +, 1 +, weak and negative and documented accordingly.

Presence or absence of haemolysis will be determined by the presence or absence of any of the laboratory marker of haemolysis such as hemoglobin <9 gm/dl, peripheral blood film showing features of haemolysis, reticulocyte count >2%, total bilirubin >2 mg/dl and LDH >378 U/L [13,14]. These lab results was collected from the patient’s referral note or clinical records and enrolled in the questionnaire.

Utilization of study results: The study findings may guide the immunohematologists of Bangladesh and other low resource country to adopt to this newer technique with ease and more efficiently.

RESULTS

Among 90 study population male was 41(45.5%) and female was 49(54.4%). Among 49 female, 32(65%) was pregnant. Out of 90 study population 81(90%) had history of taken blood transfusion. Regarding time required for the procedure gel card method was most time efficient in between two methods which was statistically significant (p value 0.001). Gel card method was more expensive 600 taka and conventional technique was 300 taka. For diagnosis of recipient’s disease associated with incompatibility by convention and column agglutinating technique (Gel card method) was compared, similar result was found for NHL with AIHA in both technique. There was difference in Thalassemia major and SLE with AIHA. NHL with AIHA by CTT 2(13.3%) and by CAT was 2(13.3%). For Thalassemia major by CTT was 14(66.7%) and by CAT was 16(80%). In case of SLE with AIHA by CTT was 4(20%) and by CAT or Gel card method was 2(10%).

Table 1: Distribution of the Recipient according to sex and relevant history

Parameter	Number of Recipient	Percentage
Sex		
Male	41	41.0
Female	49	49.0
Female recipients with history of pregnancy (n=49)		
Yes	32	65.3
No	17	34.7
History of Blood Transfusion		
Yes	81	81.0
No	9	9.0

Table shows 41(%) recipients were male and 49(%) were female

It was observed that 32(%) of female had history of pregnancy and 81 (%) had history of blood transfusion.

Table 2: Comparison between two techniques

Variable	CTT ICT with Method	CAT with Gel card Method
Time required	100±5	30±5
Comparison between methods P value		
CTT with saline Method		0.001
CAT with Gel card Method		0.001

P value reached from independent-t test

This table shows the Gel card method is the most time efficient in between two methods

Table 3: Cost of each Method

Variable	CTT with ICT Method	CAT with Gel card Method
Cost	300tk	600tk

In this table it was observed that CAT with Gel card Method was expensive than CTT with ICT Method

Table 4: Diagnosis of recipient disease associated with incompatibility

Disease	Incompatible in CTT with ICT Method (n=20)		Incompatible in CAT with Gel card method (n=20)	
	n	%	n	%
Thalassaemia major	14	66.67	16	80
NHL with AIHA	2	13.33	2	13.33
SLE with AIHA	4	20	2	10

Table-4 Shows similar results AIHA for NHL with AIHA and these is difference in Thalassaemia major & SLE with AIHA

Table 5: Comparison of sensitivity, specificity and accuracy in between two methods

Method used	Total sample	Sensitivity	specificity	Accuracy
CTT with ICT method	90	20%	100%	85-88
CAT with Gel card method	90	100%	100%	100%

Comparison between two method sensitivity specificity Accuracy
CTT with ICT method <0.001-<0.05
CTT with ICT vs CAT with Gel Card <0.001->0.05

Difference between CTT with ICT method was statistically significant

DISCUSSION

In this comparative cross-sectional study evaluation of effectiveness of two groups regarding antibody detection was performed. One group was conventional tube technique and another was column agglutination technique. Indirect antiglobulin test was performed to detect antibody by conventional tube technique using antihumanglobulin (AHG) and by column agglutination technique and interpretation was compared.

Sensitivity, specificity and accuracy of both techniques were performed and compared.

This Study reveals that Gel card is more sensitive antibody detection. These findings align with other studies in the field conducted by [15-20] reported that the CAT method showed greater sensitivity than CTT for detecting clinically significant antibodies, suggesting that it offers improved accuracy in identifying Antibody.

The Direct and Indirect Coombs tests, used to detect unexpected antibodies either bound to or free in the bloodstream that may react with donor's red blood cells leading to incompatible cross match results. Most of these antibodies, particularly alloantibodies require special atmosphere to react with their corresponding antigens if present in the donor's red blood cells and

therefore, demands sensitive technique to detect any sorts of incompatibilities to ensure safe blood transfusion. In the presented study, the Direct Coombs test demonstrated a low positivity rate of 3.5%, which indicates that most patients did not exhibit bound antibodies on their red blood cells. In contrast, the Indirect Coombs test showed a higher positivity rate of 17.6%, identifying a greater number of patients with circulating antibodies. This finding highlights that the Coombs test is important to choose the appropriate cross-match technique for the recipients who are found positive antibody screening results to minimize hemolytic transfusion reactions [21]. The column agglutination technique (CAT) outperformed the conventional tube technique (CTT) for detecting antibodies, further supporting the use of sensitive methods like CAT. Additionally, it was found that CAT has higher sensitivity than CTT in detecting clinically significant antibodies, affirming its relevance in transfusion.

The comparison of sensitivity, specificity, and accuracy between the conventional tube technique (CTT) with saline, CTT with the indirect Coombs test (ICT), and column agglutination technique (CAT) with gel card demonstrates key differences in their ability to detect compatible and incompatible blood samples. The CTT with saline method had the lowest sensitivity (20%) and accuracy (85.88%) and failed to detect many incompatible cases, despite its 100% specificity. In contrast, CTT with ICT showed a much higher sensitivity (86.66%) and overall accuracy (97.64%), indicating a superior performance in detecting incompatibility. However, the CAT with Gel card method proved to be the most reliable, achieving 100% sensitivity, specificity, and accuracy, making it the most effective method for ensuring transfusion safety. These findings align with recent studies that confirm the superiority of CAT over traditional methods in terms of sensitivity and accuracy. In another study, it was found that [22].

The diagnoses associated with incompatible results across three techniques (CTT with saline, CTT with ICT, and CAT with gel card) revealed distinct patterns in a sample of 15 recipients. Thalassemia major was the most prevalent condition among the study cases associated with incompatibility, particularly in the CAT with gel card method (80% incompatibility), followed by CTT with ICT (60%). This emphasizes the high sensitivity of these methods in detecting incompatibilities among the recipients who may have developed alloantibodies in their serum in response to repeated blood transfusion, such as, Thalassemia patients. Notably, no cases of incompatibility were detected by CTT with saline method in thalassemia cases, suggesting its limitations in identifying such issues in complex cases. Recipients having auto antibodies in their serum like, Non-Hodgkin's lymphoma (NHL) with

autoimmune hemolytic anemia (AIHA) and systemic lupus erythematosus (SLE) with AIHA showed consistent incompatibility rates of 6.7% and 13.3%, respectively, across all methods, suggesting that, auto-antibodies with wide thermal range can cause incompatible cross match results in all three methods. These findings are consistent with recent literature that emphasizes the enhanced detection capabilities of CAT for complex transfusion cases, particularly in conditions like Thalassemia major, which often require frequent transfusions and are prone to allo-immunization due to chronic transfusion needs [23]. It was noted that Thalassemia major patients frequently develop alloantibodies.

The comparison of crossmatch costs using CTT with saline, CTT with ICT, and CAT with Gel card reveals significant differences in expenses, with CAT being the most expensive option (600 Tk) and CTT with saline being the most economical (300 Tk). Although CAT offers superior accuracy and shorter processing time, its higher cost raises considerations for healthcare institutions, especially in resource-limited settings.

As the advantages of gel card method outweighs the few limitations, we can recommend its usage for routine blood group serology in transfusion centres of all hospitals. We can also use this method along with CTT with AHG method.

Overall, this study results are found to be in agreement with previous studies

CONCLUSION

This Study shows there is significant difference in sensitivity, accuracy & efficiency in between column Agglutination Technique & Conventional Tube Technique (CTT) for Indirect coombs test to detect antibody. It is found that CAT is superior to CTT in sensitivity, accuracy & time efficiency. But CAT is expensive ICT by CTT method is laboratory, time consuming process, requires wash phase, depends on technicians technique, there is inter-observer variations during result interpretation and result can't be preserved on reviewed in contrast, CAT is more sensitivity accurate reliable and less time consuming, early to perform Interpret, result can be reproduce & reviewed. A large consort study is necessary to enhance the efficacy of CAT over CTT. It is concluded and recommended that column agglutination technique can be practiced as a standard procedure for ICT to detect antibody.

Recommendation: To enhance the study's Comprehensiveness future research could be consider multi center collaboration.

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Conflict of interest: None declared

Ethical Approval: This Study was approved by institution review Board, BSMMU

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