GEL CARD AND CONVENTIONAL DIRECT COOMBS TEST IN THE DIAGNOSIS OF AUTOIMMUNE HAEMOLYTIC ANEMIA

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ABSTRACT

Background: The prognosis of autoimmune hemolytic anemia (AIHA) needs concern of hemolysis and demonstration of autoantibodies next to red cells. Most laboratories use the conventional Coomb's test for the demonstration of the autoantibodies. The present study aims to compare the efficacy of Gel card Coomb's test with conventional Coomb's test in the diagnosis of AIHA. **Material and Methods:** The present study was carried at the Department of Pathology and the blood bank in the Integral institute of medical sciences and research, Lucknow, Uttar Pradesh from January 2016 to October 2016. Written informed consent was obtained from all subjects prior to the performance of any study-related procedure. The study was approved by the ethical committee of the institute.MS Excel was used for data entry and descriptive statistics. **Result:** DCT Positive in 11 cases done by Conventional Methods and DCT Positive in 13 cases done by gel card method. The gel card selected the antibodies in all the cases spotted positive by the conventional test. The sensitivity and specificity of the gel card Direct Coomb's test (DCT) as compared to the conventional method test for DCT was found to be 100% & 95% and 100% & 92% respectively. **Conclusion:** Reliable and speedy method for detection of autoantibodies, antibodies against RBCs.Considering the high sensitivity, specificity, and simplicity of the method (Gel Method) may be effectively used for diagnosis of AIHA.

Keywords: Auto-Immune hemolytic anemia, Gel Card, Direct Coomb's test

INTRODUCTION:

Autoimmune hemolytic anemia (AIHA) is specified as the increased destruction of red blood cells (RBCs) in the existence of anti-RBC autoantibodies and/or complement. The annual occurrence of AIHA ranges from 1 to 3 in 100,000 to 1 in 25,000 individuals. **(1,2)** The inconsistency reflects the use of dissimilar criteria for the diagnosis of AIHA that frequently confounds comparison among studies. AIHA affects different age groups, more adults than children. **(3)** Signs and symptoms and physical outcomes reflect the premature demolition of RBCs with insufficient compensation of bone marrow and the secondary effect of hemolysis. **(1,4)** The diagnosis of AIHA is generally simple and based on the existence of hemolytic anemia and the serological sign of anti-RBC autoantibodies noticed by the direct antiglobulin test (DAT). **(1)**Immune hemolytic anemia may be either isoimmune or autoimmune in nature. Autoimmune hemolytic anemia (AIHA) is immune trouble triggered by circulating antibodies in contradiction of antigens on the red cell (RBCs) membrane ensuing in shortened RBC life span. **(5)** They are categorized as primary (Idiopathic) and

secondary (drug-induced). The antibodies explaining the RBCs are diverse. The common form of AIHA is characterized by the presence of a "warm" type of autoantibodies, which are IgG type and react optimally at 37°C, causing RBC destruction extravascular by tissue macrophages. One more antibody includes Donath Landsteiner antibodies and "cold" agglutinins. (**6**) The foundation stone of prognosis of AIHA is a Coombs test or positive Anti-human globulin, for the incidence of hemolysis. (**5**) Sign for DCT is to notice in vivo coating of red cells with IgG and/or complement which happens in Haemolytic disease of the newborn (HDN), Autoimmune hemolytic anaemia (AIHA), Drug-induced hemolytic anaemia (DIHA) &Hemolytic transfusion reaction. (**7**) Laboratories use the conventional Coomb's test for the presentation of the autoantibodies. Nevertheless, in about 2-6% of the patients having the clinical and hematological features of AIHA, the direct agglutination test is negative on frequent testing. Efforts are being made to classify a test that could be more sensitive than the conventional test. However, simplicity and cost-effectiveness of the test. (**8**) This study aims to carry out the comparison and estimate the efficacy of the novel gel card test has been differentiating with the conventional Coomb's test for finding autoantibodies in 100 clinically suspected cases to have haemolytic anemia.

MATERIAL AND METHODS:

This clinical study was performed between January 2016 to October 2016 at the Department of Pathology and the blood bank in the Integral Institute of Medical Sciences and Research, Lucknow, Utter Pradesh. Written informed consent was obtained from all subjects prior to the performance of any study-related procedure.

The study was approved by the ethical committee of the institute.

Materials: Using EDTA Test Tube for venous blood of patients. Coombs reagent (Polyclonal Anti IgG Coomb's Gel card and Gel card centrifuge machine + Monoclonal anti c3d)

Procedure: Antiglobulin Test:- Washed red cells coated with IgG and/or components C3b or C3d will explain agglutination with broad-spectrum AHG serum. The procedure of DAT by the conventional method. (**7**)

Sample of blood sample must be within 24 hours, if not, the sample would be taken in EDTA (1.5 mg EDTA for 1 ml of blood) to defend the approval of accompaniment. Only two to three drops of blood to be tested in a fresh labelled tube. Fully wash the red cells three to four times in a large mass of saline to eliminate free globulin molecules. Adding two drops of polyspecificAHG serum in 1 drop of cleaned red cells or 1 drop of 3-5 percent suspension of cells immediately. Mixing, centrifuge at 1000 rpm for 1 minute instantly. Slightly move back and forth the tube to dislocate the cell button for agglutination, use optical aid if required. Adding 1 drop of IgG-coated red cells to a negative test. Amalgamating, centrifuge at 1000 rpm for 1 minute, and promptly look for agglutination. If a negative outcome (no agglutination) appeared the test ensue is unacceptable and a complete test must be performed again. If agglutination appeared, the outcome is valid. (**9**)

DCT by Gel Card: Innovative approach to red cell serology. Invented by Dr.Yves Lapierre of France in 1988. In which, 0.8% Suspension of the patient's red cell is set up. (1 ml LISS + 10 µl Patient Packed cell) and 50 µl of Cell Suspension in Reaction Chamber, It centrifuge for 10 minutes. (**10**)

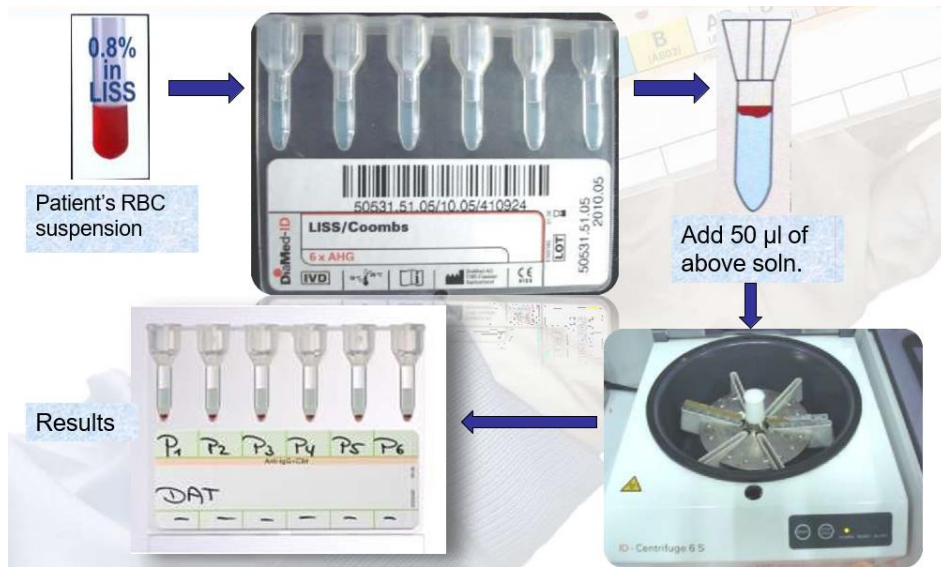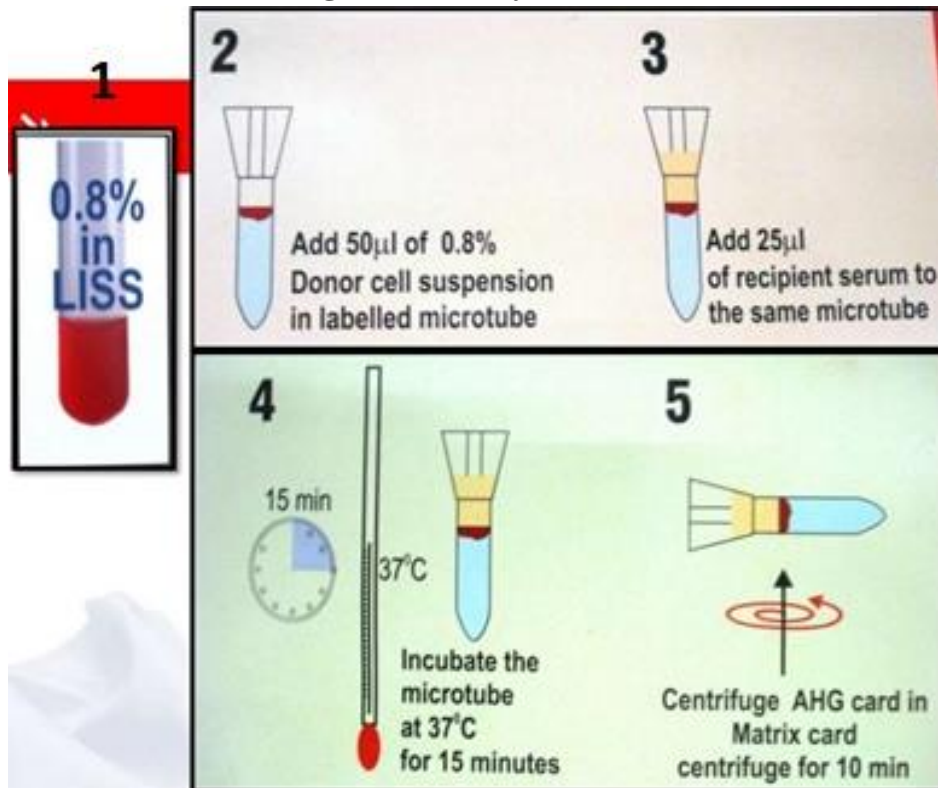**Figure: 1 Direct Coomb's Test (DCT)**

Figure2: DCT by Gel Card

The detailed history along with clinical features taken for all patients and physical examination was also done. Patients with Rh and ABO incompatibility and blood transfused patient in the last six months were excluded from this study. After performing tests, laboratory investigations like CBC, reticulocyte count,

peripheral smear examination, serum LDH estimation, and bilirubin estimation were done.

RESULTS

A total of 100 samples were studied and Direct Coomb's Test was done on their venous blood sample. Out of the total,38% of patients aged less than 20 years. The majority of patients belonged to the age group 20 to 40 years, followed by 40 to 60 years. Minimum percentages of patients were included in the age group 60 and above. The mean age was found to be 28 years and 45 patients were female and 55 were males. (Table 1)

Table 1: Age sex wise distribution of patients (n=100).

Variables	Number (N)	Percentage (%)
Age (years)		
<20	38	38.0
20-40	44	44.0
40-60	13	13.0
≥60	5	5.0
Sex	100	100.0
Male	55	55.0
Female	45	45.0

Table 2 shows that the distribution of clinical characteristics and results of physical investigation, in which, autoimmune hemolytic anemia the common presentation was dyspnea on exertion and fatigue. Out of a total thirty-eight studied patients showed pallor except. Splenomegaly was more common. (table 2) Figure 3 depicts that the frequency distribution of parameters like reticulocytosis, nucleated RBC, Polychromasia, etc. was tabulated, in which, majority of patients suffering

from increased bilirubin followed by reticulocytosis. The minimum number of patients had polychromasia. (figure 3)

Table 2: Clinical Characteristic and Physical InvestigationOutcomes

Variables	Number (N)	Percentage (%)
Symptoms		
Fatigue	21	35.0
Dyspnea on exertion	11	18.3
Joint pain	6	10.0
Bleeding	9	15.0
Weight loss	5	8.3
Dizziness	4	6.7
Fever	4	6.7
Physical Examination		
Pallor	38	54.3
Splenomegaly	32	45.7

Figure 4 shows a comparison of results of Direct Coomb's Test Done by Conventional Method and Gel card Method. out of 100 patients 32 patients were suspected as having hemolytic anaemia by examination of increased unconjugated bilirubin levels, peripheral smear examination, and elevated serum lactate dehydrogenase levels. DCT was found positive in eleven patients by Conventional Methods and DCT found positive in thirteen patients by gel card procedure. The gel card selected the antibodies in all the patients, noticed positively by conventional test. (figure 4)

Figure 3: Frequency distribution of various parameters in autoimmune hemolytic anemia
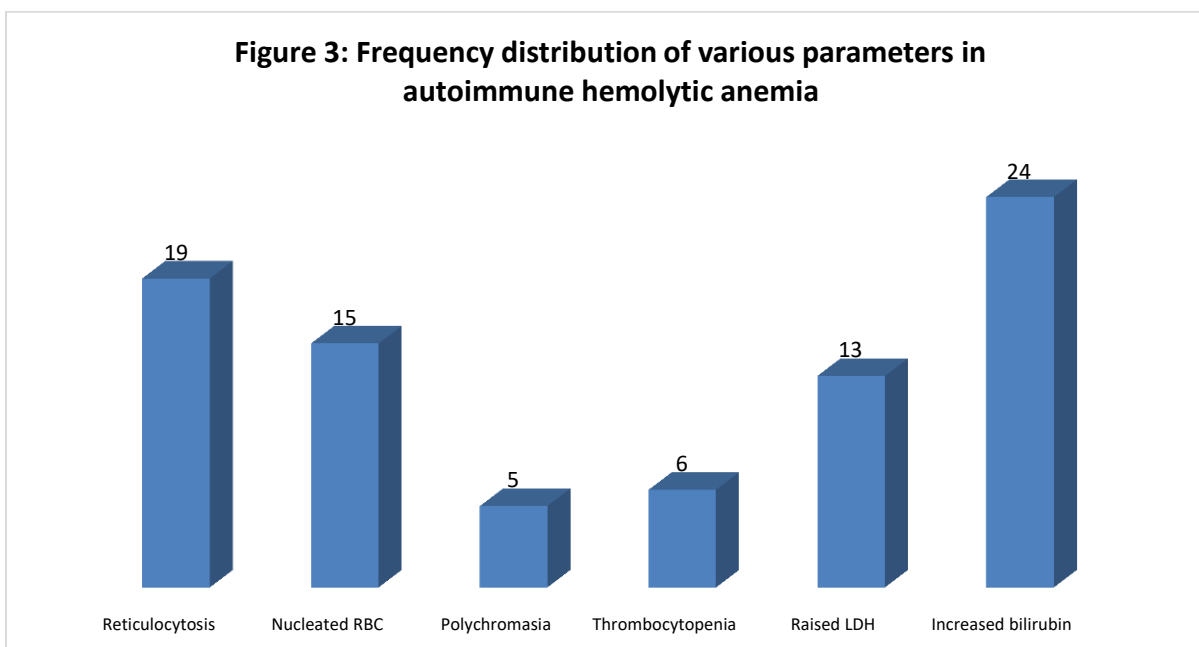

Figure 4 : Comparison of result of Direct Coomb's Test Done by Conventional Method and Gel card Method
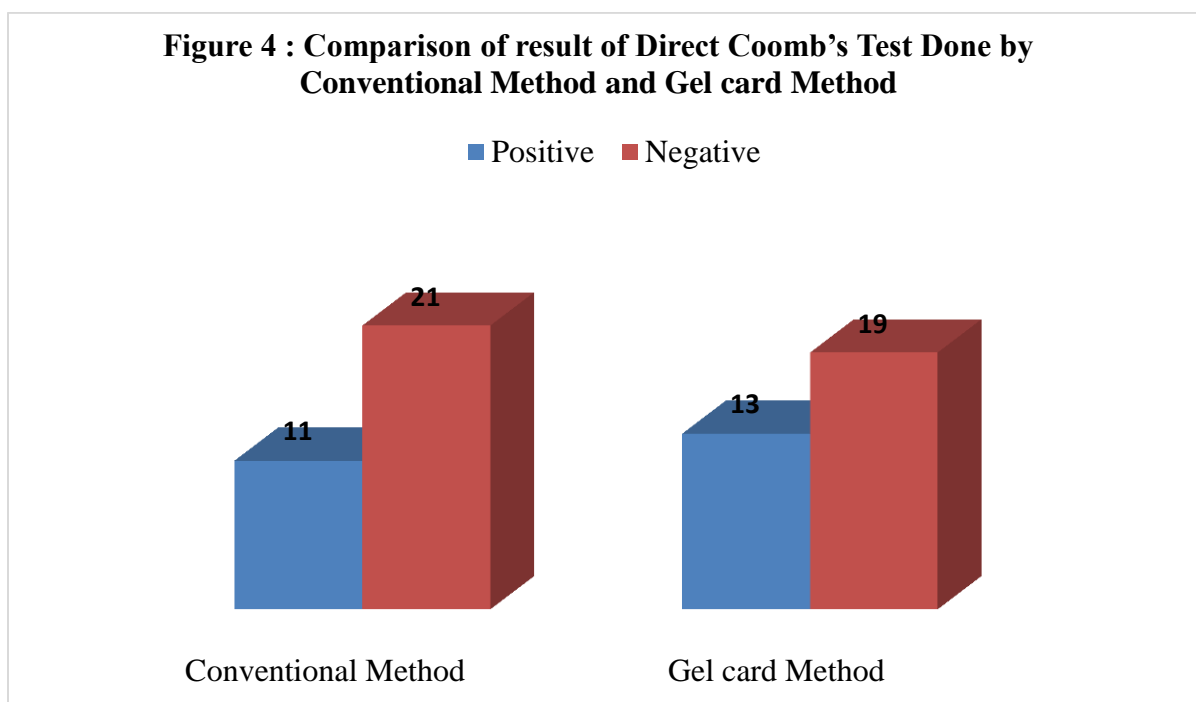
Table 3 shows the sensitivity and specificity of the gel card and conventional direct Coomb's methods. Gel card selected tests which were negative by the conventional method. sensitivity and specificity calculated for gel card Direct Coomb's test (DCT) as also for conventional method test for DCT was to be 100% & 95% and 100% & 92% respectively. Because of the high sensitivity and specificity and the ease of

method, this test may be effectively used for diagnosis of AIHA.(table 3)

Table 3: Sensitivity and Specificity of gel card and conventional direct Coomb's methods

DCT	Sensitivity	Specificity	NPV
Gel card Method	100	95	100
Conventional Method	100	92	100

DISCUSSION:

As hemolysis happens in autoimmune hemolytic anemia, positive Coomb's test (Direct Coomb's test) is the diagnostic test for it. **(5)** Coomb's et al. in 1945, displayed that the AHG test valuable in detecting antibodies to human globulin &complement that coat RBC in hemolytic anemia **(11)**. The clinical features include: dyspnea on exertion weakness, jaundice, fatigue, splenomegaly, Pallor, faintness, illness, bleeding, weight loss, abdominal pain, etc. **(12,13)** In Choudhary et al. evaluated that common most clinical feature was pallor followed by fever and jaundice. In the present study fatigue, Dyspnea on exertion, and bleeding were more frequent than other features. Splenomegaly was observed in 81% by Choudhary et al.**(14)** whereas in the present study 45.7 percent of patients suffering from splenomegaly. In contrast to the present study, Serrano J reported that warm antibodies were identified in 74.5% while cold antibodies were identified in 19%. **(15)** DAT was positive in 18(78%) and 37.7% patients by conventional method and 19 (83%) and 88.4% patients by Gel Card method by Oytip et al and Zouxl et al respectively. **(16,17)** Garg et al reported that the DAT was positive in 10(33%) patients by

conventional method and 12 (40%) patients by Gel Card method. **(18)** Similar findings are shown in the present study that, DCT positive in 11 patients by Conventional Methods and DCT Positive in 13 patients by gel card method. The gel card finds out the antibodies in all the patients spotted to be positive by the conventional test. In the present study, a newly developed gel card test has been differentiating from the conventional Coomb's test in terms of efficacy, we found that the sensitivity and specificity were found to be 100%& 95% and 100% & 92% respectively. Some studies showed similar findings, such as, Kaur et al at the experience in testing with the use of the DiaMed micro typing system which is based on the gel technology. **(19)** For one year since this technology was introduced in our blood bank, we noticed a startling 65 fold rise (p<0.0001) in the stated number of unfitting units in one year which rose from a paltry 4 (0.02%) to 260 (1.6%). Observed the DiaMed system easy to use and the outcome suggests it proved to be more sensitive than the conventional tube agglutination technique. **(19)**

Jaiprakash et al reported that the IAT by the GT showed 6.6% positivity as compared to 5.4% positivity by CTT. Sensitivity and specificity were 100% and 97.7%, and the PPV and NPV were 81.4% and 100% respectively. Suggested in their study, that the GT is a preferable substitute to the CTT for both DAT and IAT. The GT is extremely recommended to be executed as a routine method of testing in all zonal/regional blood transfusion centers. **(20)** Dutta et al reported that the sensitivity and specificity were found to be 100% and 95.1% for the gel card Direct Coomb's test when it is compared to the conventional tube test for DCT. Sensitivity and specificity for the Indirect Coomb's test (ICT) was 100% and 92.5% respectively. Given that, high sensitivity and specificity and the simplicity

of the procedure, this test may be effectively used for diagnosis of AIHA. **(8)**

CONCLUSION:

Taking consideration of age, sex, clinical features, physical examination, and DCT with laboratory examination outcome help in the diagnosis of AIHA. Reliable and speedy method for detection of autoantibodies, antibodies against RBCs. Higher sensitivity and specificity reflect that the method (Gel Method) may be effectively used for the diagnosis of AIHA.

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