

Original Article

COMPARISON BETWEEN GEL CARD AND CONVENTIONAL DIRECT COOMBS TEST FOR DIAGNOSIS OF AUTOIMMUNE HAEMOLYTIC ANAEMIA

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ABSTRACT

Background: The diagnosis of autoimmune hemolytic anemia (AIHA) requires the establishment of hemolysis and demonstration of autoantibodies against red cells. Most laboratories use the conventional Coomb's test for the demonstration of the autoantibodies. However, in approximately 4-8% of the patients who present with the clinical and haematological features of AIHA, the direct agglutination test is negative on repeated testing. Attempts are therefore being made to identify a test which could be more sensitive than the conventional test, yet retaining the simplicity and cost effectiveness of the test.

Aims & Objective: Comparison between Gel card and Conventional Direct Coombs Test for diagnosis of autoimmune haemolytic anemia.

Material and Methods: Direct Coomb's test done by conventional methods and Gel card methods. In the present study, the efficacy of the newly developed gel card test has been compared with the conventional Coomb's test for detection of autoantibodies in 100 cases clinically suspected to have haemolytic anemia in Guru Gobind Singh Government Hospital, Jamnagar.

Results and Conclusion: The gel card picked up the antibodies in all the cases that were detected to be positive by the conventional test. In addition the gel card also picked up 2 tests which were negative by the conventional method. In view of the high sensitivity, specificity and the simplicity of the procedure this test (Gel Method) may be effectively used for diagnosis of AIHA.

Key Words: Auto Immune hemolytic anemia, Direct coomb's test, Gel Card

INTRODUCTION

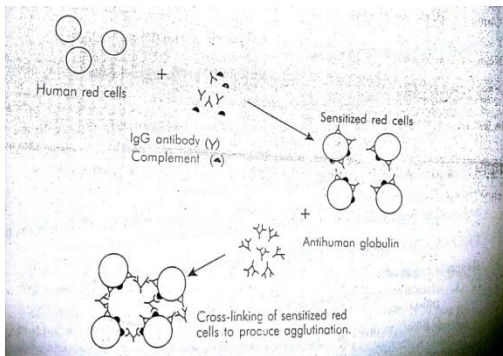
Immune hemolytic anemia is a condition in which the life-span of red cells is shortened due to presence of a humoral antibody in the circulation that is reactive with an antigen on the red cell. Broadly the immune hemolytic anemia can be classified into alloimmune & autoimmune [1].

In certain diseases or conditions an individual's blood may contain IgG antibodies that can specifically bind to antigens on the red blood cell (RBC) surface membrane, and their circulating red blood cells (RBCs) can become coated with IgG alloantibody and/or IgG autoantibodies. Complement proteins may

subsequently bind to the bound antibodies. The Coombs test is used to detect these antibodies or complement proteins that are bound to the surface of red blood cells. Both IgM and IgG antibodies bind strongly with their antigens. IgG antibodies are most reactive at 37°C. IgM antibodies are easily detected in saline at room temperature as IgM antibodies are able to bridge between RBC's owing to their large size, efficiently creating what is seen as agglutination. IgG antibodies are smaller and require assistance to bridge well enough to form a visual agglutination reaction. Reagents used to enhance IgG detection are referred to as potentiators. RBCs have a net negative

charge called zeta potential which causes them to have a natural repulsion for one another. Potentiators reduce the zeta potential of RBC membranes. Common potentiators include low ionic strength solution (LISS), albumin, polyethylene glycol (PEG), and proteolytic enzymes [2].

Fig. 1: Agglutination of the sensitized red cell by anti-human globulin molecule



Immune hemolytic anemia can be either Isoimmune or Autoimmune in nature. Autoimmune hemolytic anemia (AIHA) is an immune disorder caused by circulating antibody against antigen on red cell (RBCs) membrane resulting in shorted RBC life span. Etiologically they are classified as Primary (Idiopathic) and secondary (Co-existing with another disease or drug induced) [3].

Indication for DCT is to detect in vivo coating of red cells with IgG and/or complement which occurs in Haemolytic disease of newborn (HDN), Autoimmune hemolytic anaemia (AIHA), Drug induced hemolytic anaemia (DIHA) & Hemolytic transfusion reaction [1].

Most laboratories use the conventional Coomb's test for the demonstration of the autoantibodies. However, in approximately 2-6% of the patients who present with the clinical and haematological features of AIHA, the direct agglutination test is negative on repeated testing. Attempts are therefore being made to identify a test which could be more sensitive than the conventional test, yet retaining the simplicity and cost effectiveness of the test [4].

AIMS AND OBJECTIVE:

- To perform a comparison between gel card test with the conventional Direct Coomb's Test.
- To rule out the presence of autoimmune diseases in clinically suspected patients.

- To diagnose cases of Autoimmune Haemolytic Anaemia (AIHA) more precisely.

MATERIAL AND METHODS

The present study was carried at the Department of pathology and the blood bank in G.G. Govt. hospital, Jamnagar during the period of June 2012 to Nov 2012.

Materials:

Venous blood of patients in EDTA Test Tube.
Coombs reagent (Monoclonal anti c3d + Polyclonal Anti IgG
Coomb's Gel card and Gel card centrifuge machine

Methods:

Principle of Antiglobulin Test:- Washed red cells coated with IgG and / or components C3b or C3d will show agglutination with broad spectrum AHG serum.

Procedure of DAT by conventional method [1]:

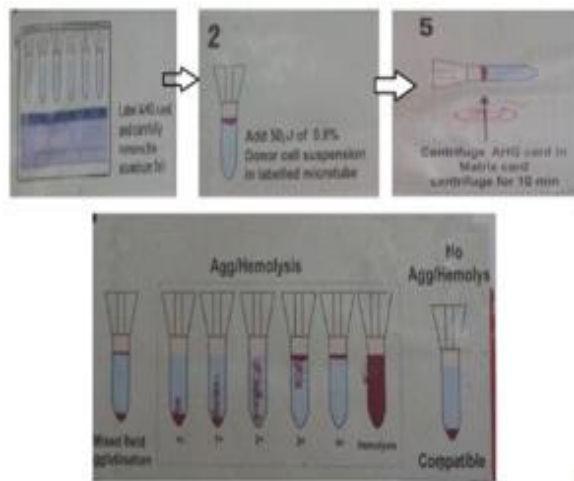
- Blood Sample - It should be as fresh as possible not more than 24 hours old, otherwise, the sample should be taken in EDTA (1.5 mg EDTA for 1 ml of blood) to protect the uptake of complement-
- Take 2-3 drops of blood to be tested in a clean labeled tube.
- Wash the red cells 3-4 times in a large volume of saline to remove free globulin molecules.
- Add 2 drops of polyspecific AHG serum in 1 drop of washed red cells or in 1 drop of 3-5 % suspension of cells immediately.
- Mix, Centrifuge at 1000 rpm for 1 minute immediately.
- Gently shake the tube to dislodge the cell button and see for agglutination, use optical aid if needed. Record the result.
- Add 1 drop of IgG coated red cells to a negative test. Mix, centrifuge at 1000 rpm for 1 min. immediately look for agglutination.
- If a negative result (no agglutination) is obtained the test result is invalid and whole test should be repeated. If agglutination is obtained, the result is valid [2].

DCT by Gel Card:

- A 0.8% Suspension of the patient's red cell is prepared (1 ml LISS + 10 µl Patient Packed cell)
- 50 µl of Cell Suspension in Reaction Chamber
- Centrifuge For 10 min

- See the Result

Fig. 2: DCT by Gel Card



In the present study, the efficacy of the newly developed gel card test has been compared with the conventional Coomb's test for detection of autoantibodies in 100 cases clinically suspected to have haemolytic anemia.

RESULTS

Total 100 samples were studied and Direct COOMB'S Test was done on their venous blood sample. As much as 42% of study subjects belonged to 21-40 years followed by 1-20 years (40%). Fig.1 shows out of a 100 patients 30 patients were suspected as having hemolytic anaemia on the basis of Peripheral smear examination, increased unconjugated bilirubin levels and elevated serum lactate dehydrogenase levels. Table 2 shows that out of 30 suspected cases of hemolytic anaemia, DCT Positive in 10 cases done by Conventional Methods and DCT Positive in 12 cases done by gel card method. The gel card picked up the antibodies in all the cases detected to be positive by the conventional test.

Table 1: Age distribution of patients

Age Distribution	No. of Patients (%)
1-20 yrs	40
21-40 yrs	42
41-60 yrs	10
61-80 yrs	08

Table 2: Comparison of result of Direct Coomb's Test Done by Conventional Method and Gel card Method

Direct Coomb's Test	Conventional Method	Gel card Method
Positive	10(33%)	12 (40%)
Negative	20(67%)	18 (60%)

Fig. 3: Number of patients suspected as cases of hemolytic anaemia on the basis of peripheral smear examination, unconjugated bilirubin and elevated LDH levels

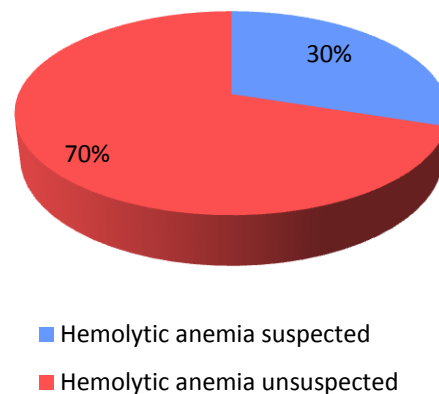
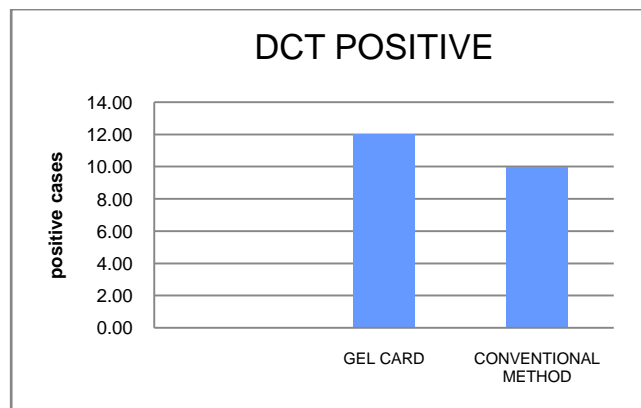


Fig. 4: Comparison of gel card v/s conventional direct Coomb's methods



DISCUSSION

Present study has been compared with two other studies which were quite acceptable. DAT was positive in 18(78%) and 37.7% cases by conventional method and 19 (83%) and 88.4% cases by Gel Card method by Oytip et al [5] and Zouxl et al [6] respectively, while In present study, it was found that DAT was positive in 10(33%) cases by conventional method and 12 (40%) cases by Gel Card method.

Advantage of gel technology [1]:

- Gel technique is simple, reliable, rapid to use, reproducible and very sensitive [4]
- Greater uniformity between repeat tests.
- Decrease specimen volume is needed to perform a large number of tests.
- No tube shaking or resuspension of red cells button leading to variation among technologists in reading and grading the agglutination
- In AHG test no wash steps and no need to use sensitized red cells in negative AHG test
- More objective consistent and reproducible interpretation of the result.

Disadvantage of gel technique:

- Expensive
- Special apparatus required

Advantage of conventional method:

Cheap

CONCLUSION

We conclude that Gel Card is better alternative to Conventional Test for detecting red cell bound antibodies in various clinical conditions and Gel card method for detection of autoimmune hemolytic anemia should be used where finance is not the obstacle.

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