ORIGINAL RESEARCH ARTICLE

Gel Tube Method and Manual Method for Coomb's Test- Study of Pros and Cons.

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Abstract:

Background: DCT and ICT Coomb's test for detection of in vivo coating of red cells by antibody and/or complement is detected using various sensitive techniques, however most hospitals even today rely on the conventional tube technique (CTT). We compared the performance of the CTT and recently introduced gel test (GT) in the evaluation of direct anti-globulin test (DAT) and ICT.

Objective: To compare the Gel tube method and manual method in direct and Indirect Coomb's test and also to find out the accurate results of complement and antibody coated RBCs. To detect Rh incompatibility in mother and to rule out Hemolytic disease of newborn and autoimmune haemolytic anemia.

Methods: Both direct and indirect Coomb's test performed by Gel tube method and manual method. In the present study, the efficacy of newly developed gel tube method has been compared with manual method for the detection of auto antibodies in MGM Medical college and Hospital in the period of De2013 to Nov 2015 in 100 cases.

Conclusion: All the cases positive by gel tube method were negative by manual method. The test is also positive in IgG and complement. The gel tube demonstrated stronger agglutination scores compared to manual method using control cells. The sensitivity and specificity of gel tube was 100% as compared to manual method for polyspecific Coomb's reagent. In view of this and simplicity of the gel tube method procedure, this test (Gel method) may be effectively used for diagnosis of Coomb's test.

Key words: Coomb's test, Gel method, manual method, direct Coomb's test.

Introduction:

Since the discovery of the ABO system and red cell agglutination by Landsteiner in 1900 and development of the antiglobulin test by Coombs et al. in 1945, the immunohematologists are tyring to establish and improve various serological investigations in human blood [1].

The principle of the Coomb's test is to demonstrate antibodies or complement coating red cells invivo by using Antihuman globulin (AHG) or Coombs reagent. Till date it remains the hallmark for the diagnosis of immune hemolytic anemias [2,3]. Technically various modifications in the procedure have been described to bring about added sensitivity to the Coomb's test. Some of these changes include; use of more specific reagents like *monospecific AHGs* (e.g. against IgG or C3) and *Enzyme elution* of the antibodies from the red cells [2]. In 1990, Lapierre et al reported that the gel test showed improved reliability when correlated with tube results for detecting a variety of clinically significant known antibodies [4,5].

Historically, conventional tube method has been used as the standard technique for immuno - haematological studies, such as in the direct antihuman globulin test for the diagnosis of autoimmune haemolytic anaemia and also in screening for specific antibodies in transfusion medicine. But it is time consuming, involves many cell washing steps and requires relatively experienced personnel to interpret the results. It is also difficult to automate and standardize [6,7].

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Gel test is an easy and sensitive technique that requires no red cell washing and uses gel filtration media impregnated with an antihuman globulin reagent to induce agglutination [6].

Materials and methods:

Both direct and indirect Coomb's test performed by Gel tube method and manual method. In the present study, the efficacy of newly developed gel tube method has been compared with manual method for the detection of auto antibodies. Control prepared in the blood bank and applied to both techniques.

Method of DCT by manual method:

- Make 3-5% suspension of sample
- Take 2 drops of 3-5% cell suspension and add 2 drops of AHG
- Incubation for 30 minutes
- Mix and centrifuge for 1 minute
- Resuspend the cell button gently, examine for agglutination and record the results
- Method of ICT by manual method:
- Place 2-3 drops of test serum in tube
- Add 1 drop of 3-5% suspension of washed o +ve red cells to the serum
- Add 1-2 drops of 22% bovine Albumin
- Mix and incubate for 37^0 C for 15 minutes
- Centrifuge at 1000 rpm for 1 minute

Examine for agglutination

- If no agglutination is seen, wash the cells 3-4 times in large volume of saline. Decant the supernatant.
- Add 2 drops of AHG serum to washed cells
- Mix and centrifuge for 1 minute at 1000 rpm
- 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.

Method of DCT by gel tube method:

- Preparation of cell suspension (3.5%)
- Add 50 micro litre of packed RBC cells of test sample in 1 ml normal saline solution and mix uniformly
- Open the wells of cassette
- Add 10 microlitre of 3.5% RBC suspension
- Centrifuge the cassette for 5 minutes
- Read the results

Method of ICT by gel tube method:

- Preperation of 3.5% RBC cell suspension
- Open the wells of cassette
- Add 50 ul of BLISS to each column
- Add 10 ul of 3.5% RBC suspension
- Add 40 microlitre of test serum
- Incubate for 10 min at 37^0 C
- Centrifuge the cassette for 5 min
- Read the results

All the samples were subjected to polyspecific antisera.

Observations & Results:

This is a retrospective study done in the blood bank of MGM Medical College Aurangabad [MS], India. All the patients of Coomb's test direct and indirect are subjected to manual method and gel tube method as a routine. Control was positive in manual method and inbuilt control was used in gel tube method.

Total of 100 samples were studied out of which 84 samples were for DCT (84%) and 16 were ICT(16%).

Table 1 : Number of DCT and ICT done in Male and Female

	ICT		DCT	
	No. of Subjects	Percentage	No. of Subjects	Percentage
Male	1	6.25%	45	53.57%
Female	15	93.75%	39	46.42%
Total	16	100%	84	100%

Out of 84 samples of DCT, male subjects were 53.57% and female subjects were 46.42%. Out of 16 samples of ICT, 93.75% were female samples anf 6.25% were male samples. Total number of male subjects were 46 and female subjects were 54. Male to female ratio was 0.85:1.

Table 2- Age distribution of Subjects

Age group	No. Of Subjects	Percentage
0-1 day	76	76.0%
1-2 days	4	4.0%
10-20 yrs	8	8.0%
20-30 yrs	12	12.0%
Total	100	100%

76% of the study subjects belonged to age group of 0 to 1 day, 4 Subjects was of 1 to 2 days, 8 and 12 subjects were of 10 to 20 years and 20 to 30 years respectively.

Table 3 : Clinical presentation of the subjects

Clinical presentation	No. Of Subjects	Percentage
AIHA	6	6%
Sensitization	15	15%
HDN	79	79%
Total	100	100%

Out of 100 subjects, 79 % were suspected to be having haemolytic disease of newborn, 15 % cases were Rh negative mothers and 6 % cases were suspected to be having Autoimmune haemolytic anemia.

Table 4 : Positive results by Gel technique

	DCT		ICT		Total	
	No. of Subjects	Percentage	No. of Subjects	Percentage	No. of Subjects	Percentage
Male	3	60.0%	0	00	03	42.86%
Female	2	40.0%	2	100%	04	57.14%
Total	5	100%	2	100	07	100%

In the present study, out of 100, 7% samples were positive by gel technique. Out of 7% samples, 5% samples were DCT positive and 2% samples were ICT positive. Out of the positive cases, 3 cases of HDN were found DCT positive, 1 case of HDN was weakly DCT positive, 2 cases of sensitization were ICT positive and 1 case of AIHA was DCT positive. Same was conducted with manual method also, showed positive control tests but test results were negative in all the cases.

Table 5 – Comparison of results by conventional and gel tube method

Test	Conventional method	Gel Tube method
Positive	0 (0%)	7 (7%)
Negative	100 (100%)	93 (93%)

All the tests i.e. DCT and ICT Coomb's tests were positive by gel tube method were negative by conventional method.

Discussion:

The basic principle of the gel test is that instead of a test tube, the serum and cell reaction takes place in a microtube. Six of such microtubes are embedded in a plastic card to allow ease of handling, testing, reading and disposal [5].

Fig. 1- Gel card tests showing one positive and others negative tests

In the present study, maximum number of cases were in the age group of 0-1 day i.e. 76% followed by 12% of cases in the age group of 20-30 years. In the study done by Garg S et al [3], 42% samples were in the age group of 21-40 years and 40% samples were in the age group of 1-20 years.

In this study, 79% of the newborn samples were suspected to be having Hemolytic disease of newborn on the basis of their smear studies and clinical presentation out of which 4% of samples were found to be positive by Gel tube method and none was positive by manual method. Out of 15% cases of Rh negative mothers, 2 cases were positive by gel method and negative by manual method. Out of 6% cases of suspected AIHA, 1% cases was positive by gel method and again negative by manual method.

In the study done by Garg S et al [3], 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. In the study done by Das S et al [1], out of 170 samples, 34 samples were diagnosed as AIHA.

Out of 100 samples, 7 samples (7%) were found positive by gel tube method and were negative by manual method. None of the sample was positive by manual method in the present study. Out of 7%

samples, 5% were DCT positive and 2% were ICT positive.

In the study done by Das S. et al1, positive results were found in 40.6% of samples by gel tube method and 38.2% samples were positive by manual method. In the study done by Alwar V. et al [2], 63.5% samples were positive by gel method whereas by manual method only 37.5% samples were positive.

Conclusion:

All the cases positive by gel tube method were negative by manual method. The test is also positive in IgG and complement. The gel tube demonstrated stronger agglutination scores compared to manual method using control cells. The sensitivity and specificity of gel tube was 100% as compared to manual method for polyspecific Coomb's reagent. In view of this and simplicity of the gel tube method procedure, this test (Gel method) may be effectively used for diagnosis of Coomb's test. We were unable to rule out false positive cases.

We conclude that further prospective studies will be required with complete clinical and other investigative data although gel tube method is a convenient and simple method for both coomb's test.

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