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Original Research Article

A comparative study between gel card method and manual method for Coomb's test

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ABSTRACT

Background: The main objective of this study is to compare the Gel Card method and the Conventional Tube method for Coomb's test. The standard procedures were being followed while performing the above mentioned two test. Based on an 8 months study, Gel card method was proven to be more reliable in concordance with its calculated p-value and the sensitivity. The advantages and disadvantages of which have been mentioned in the following.

Materials and Methods: For Gel Card method, the principle of saphadex gel as a semi-solid medium is being used to trap any agglutination. For the Conventional Tube method, the SOP was being followed involving cell washing and confirmation via microscopy for any micro-clumps. The use of polyspecific antiglobulin was implemented for both the above test.

Results: The study showed p-value for Gel Card method to be < 0.05 which proves to be significant and the sensitivity of Gel Card method was also better compared to the Conventional Tube method.

Conclusion: Our study showed that gel card is more sensitive, easy to perform and lesser time consumption, lesser sample volume plus standardized reporting. Results of Gel card can be preserved for 3-4 days. Avoidance of interobserver variability is also an added benefit due to the standard grading system. It is therefore an excellent method for detecting agglutination compared to the Conventional Tube method.

1. Introduction

Currently, the immunohematologists are trying to establish as well as improve majority of the serological investigations, after the discovery of ABO system and RBC agglutination by Landsteiner in 1900 and by Coombs et al. in 1945, respectively.[1]

The principle of the Coomb's test is demonstration of antibodies or complement coating red cells using Coombs reagent or Antihuman globulin.[2]

Technically various modifications have been made to bring about added sensitivity including the use of more specific reagents like monospecific AHGs.[3]

A study done by Lapierre et al. in 1990, showed improved reliability of Gel Card when correlated with Conventional tube results for detecting a variety of clinically significant known antibodies.[2]

Historically, for immuno-haematological studies, like DCT and screening of antibodies in transfusion medicine, conventional tube method was used as the standard technique.[4]

But it is time-consuming, in need of an experienced personnel to interpret the results which makes it difficult to automate and involves many cell washing steps.

Gel Card is however an easy and sensitive technique that surpasses the above disadvantages and induces agglutination by the uses gel filtration media impregnated with an antihuman globulin reagent.[1]

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2. Aim of the Study

To compare the test results of Coomb's test done by gel card method and by manual method.

3. Objective

To study the findings of Coomb's test done by Gel Card method and Conventional tube method and to compare the result of the above two methods.

3.1. Indications[5,6]

1. Hemolytic anemia
2. Erythroblastosis fetalis (hemolytic disease of the newborn
3. Infectious mononucleosis
4. Chronic lymphocytic leukemia or similar disorder
5. Mycoplasmal infection
6. Systemic lupus erythematosus
7. Syphilis
8. Transfusion reaction, such as one due to improperly matched units of blood

4. Materials and Methods

A comparative study was done in the Blood Bank of BVDU (Deemed to be University) Medical College and Hospital, Sangli, India, for a duration of 8 months from March 2022 to October 2022, where in all the samples subjected for Coomb's test, either DCT or ICT or both were considered in this study. Both Direct and Indirect Coomb's test performed by Gel Card method and Conventional tube method.

1. **For Gel card method**: blood sample, plastic microtube with 6 wells, micro pipette as per volume, LISS diluent, incubator (card warmer), timer, centrifuge machine, card reader.
2. **For Conventional tube method**: blood sample, isotonic saline, clean dropper, clean test tubes, already prepared 'O' red cell suspension, incubator, Anti-human globulin, centrifuge machine, timer, microscope.
3. Clotted samples, insufficient quantity, wrong bulb sample and more than 24 hours sample (without refrigeration) were some of the exclusion criteria considered in our study.

4.1. Procedure by gel card method

Sample preparation for Direct and Indirect Coomb's test.
Prepare 0.8% red cell suspension in LISS as follows:

1. Dispense 1 ml of LISS in a clean labelled test tube.
2. Add 10 microL packed cells and mix gently.

A. Gel Card Method for DCT:-

1. Observe the card for appearance of gel and label with patient's details and remove the foil seal as instructed.
2. Pipette 50 microL of patient's red cell suspension (0.8%) into the labelled microtube.
3. Centrifuge the car in Matrix Cerd Centrifugr for 1 cycle (10 minutes)
4. Read the reaction.

B. Gel Card Method for ICT:-

1. Observe the card for appearance of gel and label with patient's details and remove the foil seal as instructed.
2. Pipette 50microL of suspension (0.8%) od fresh pooled and washed "O" cells into the labelled microtube. Pipette cell suspension at 45 degrees angle.
3. Add 25 microL of patient's serum/plasma into the microtube at 90 degrees angle.
4. Incubate at 37 degrees for 15 minutes in Matrix Card Warmer.
5. Centrifuge the card in Matric Card Centrifuge for 1 cycle (10 minutes).
6. Read the reaction.

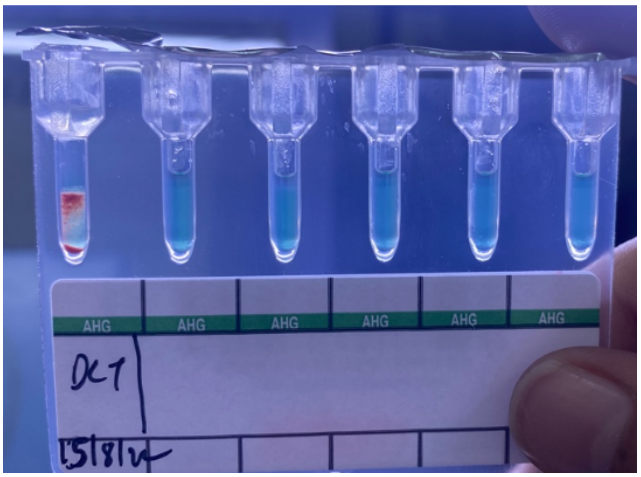

Fig. 1: Gel card showing 6 wells, with + 2 grade for DCT on the first well

Table 1: Grading for gel cardmethod[7]

Grading	Agglutination	Interpretation
0	No agglutination	Compatible
+ 1	Agglutination of red cells in the lower half of the gel card	Incompatible
+ 2	Agglutination of red cells through the entire length	
+ 3	Agglutination of red cells in the upper half of the gel card	
+ 4	Agglutination of red cells in the lower half of the gel card	
Hemolysis	-	Invalid

4.2. *Procedure by conventional tube method*

A. Conventional Tube Method for DCT :-

1. Label three test tubes as T (test serum), PC (positive control) and NC (negative control)
2. Positive control – 1 drop of Rh positive cells + 1 drop anti – D
3. Negative control – 1 drop of Rh positive cells + 1 drop of Bovine albumin
4. Test – take two drops of blood to be tested in a clean labelled tube.
5. Wash the red cells 3 – 4 times in a large volume of saline to remove free globulin molecules. Discard off all the supernatant after each cell wash including the final cell wash.
6. Add 2 drops of polyspecific AHG serum to 1 drop of washed red cells.
7. Mix and centrifuge at 1000 rpm for 1 minute immediately.
8. Gently shake the tube to dislodge the cell button and see for agglutination.
9. Record the result.
10. Add 1 drop of IgG coated red cells to NC test tube. Mix and centrifuge at 1000 rpm for 1 minute. Look for agglutination. If there is no agglutination, the test result is invalid and the whole test is repeated. If agglutination is obtained the result is valid.

B. Conventional Tube Method for ICT:

1. Label three test tubes as T (test serum), PC (positive control) and NC (negative control)
2. In the tube labelled as "T", "PC" and "NC" add two drops of test serum, Anti D serum and Bovine Serum albumin respectively.
3. Add 1 drop of 5% suspension of pooled O Rh positive red cells in each tube.
4. Incubate all three tubes at 37 degrees for 30 to 45 minutes.
5. Wash cells three times in large volume of saline. Discard supernatant with each cell wash completely.
6. Add 2 drops of AHG serum to each test tube.
7. Mix and then centrifuge at 1000 rpm for 1 minute.
8. Gently shake the tubes to dislodge the button and examine for agglutination.
9. Add 1 drop of IgG coated red cells to NC test tube. Mix and centrifuge at 1000 rpm for 1 minute. Look for agglutination. If there is no agglutination, the test result is invalid and the whole test is repeated. If agglutination is obtained the result is valid.

Interpretation of result by manual method:-

1. **Positive Result** – if agglutination is present in test tube labelled as "T".
2. **Negative Result** – if no agglutination seen in test tube labelled as "T".

Positive: Clumping (agglutination) of the blood cells. This means there are presence of antibodies either on the red blood cells (DCT) or in the serum (ICT) which induce hemolysis.[5]

Negative: No clumping of cells (no agglutination). This means there are no antibodies bound to red blood cells or in the serum.

5. Results

A total of 80 samples taken for this 8 months study, out of which 60 were evaluated for DCT and 40 evaluated for ICT. From the 60 samples for DCT, 42 showed positivity for Gel card method and 30 for Conventional tube method. And from 40 samples for ICT, 24 showed positivity for Gel card method and 18 showed positivity for Conventional tube method.

Sensitivity and specificity and p-values were calculated for the positive results by Gel Card and Conventional tube method.

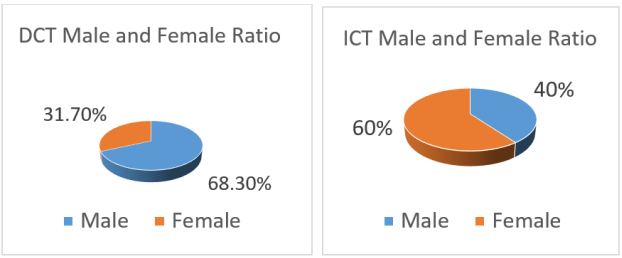

Fig. 2: Gender wise distribution for DCT and ICT

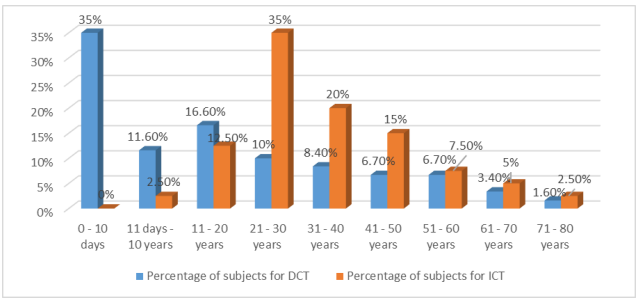

Fig. 3: Age wise distribution for DCT and ICT

6. Discussion

Conducted a comparative study in the blood bank of BVDU (Deemed to be University) Medical College and Hospital, Sangli, India, for 8 months, from March to October of 2022, where in all the samples for Coomb's test, both

Table 2: Gender wise distribution showing positive result by Gel card method and conventional tube method for DCT

Sex	Number of subjects	Gel Card DCT	Positive result %	Conventional method DCT	Positive result %
		Positive		Positive	
Male	41	20	62.5%	16	69.5%
Female	19	12	37.5%	7	30.5%
Total	60	32		23	

Table 3: Comparing coomb's test, DCT and ICT based on p-value, sensitivity and specificity

Coomb's Test	p – value	Sensitivity		Specificity	
		Gel Card	Conventional	Gel Card	Conventional
DCT	0.0495	52%	45%	49%	56%
ICT	0.0359	53%	43%	48%	58%

Table 4: Comparative table for DCT and ICT based on p-value, sensitivity, specificity and the positive and negative results, with three other studies

S.No	Comparative studies	DCT / ICT	Sensitivity		Specificity		Positive		Negative	
			Gel Card	Tube Method	Gel Card	Tube Method	Gel Card	Tube Method	Gel Card	Tube Method
1.	Present Study	DCT	52	45	49	56	53.3%	38.3%	46.7%	61.7%
		ICT	53	43	48	58	55%	35%	45%	65%
2.	ISHTM, 2011	DCT	83.1	66.03	60.4	97.67				
3.	JRMDC, 2014	DCT					40%	33%	60%	67%

Direct and Indirect were implemented by both Gel card and Conventional method simultaneously.

For a better understanding, the principle, indications, advantages and disadvantages will be discussed in the following. As we all know, Coomb's test is a laboratory investigation done for the detection of either in-vivo (Direct Coomb's test) or in-vitro (Indirect Coomb's test) antibodies directed towards RBC's.[8] For the detection of antibodies present on the surface of the red cells, Direct Coomb's test is the diagnostic tool used.[3]

As mentioned before, the Gel technique has been proven to be more efficacious and simplified technique as well as the interpretation of results along with a better reliability, reproducibility, stability and increased sensitivity.[3] Initially introduced in Thailand, 1993, and has become popular and used worldwide in several blood banks.[3] For Gel Card test we use specific microtubes which are being prepared using standard reagents. This method has been widely used for cross matching, the detection of antigen, alloantibody screening/identification.[3] Since the tube method was the first technique used, some of the blood bank personnels still prefer the Conventional Tube method despite aving many drawbacks like skilled technical expertise especially in the cell washing step (which may lead to false positive results) and also the intervariability complex.[3]

On the other hand, the benefits of Gel technology have surpassed the Conventional Tube method – quick, safe, elimination of cell washing step, technician friendly, less handling of samples, lesser interpretive errors, lesser protocol errors does not require special skills for performance.[3,9] It also has a clear cut grading system giving a uniform interpretation by the observers when get for a weeks time due to the stability of the agglutinates. The duration of 1 week gives another advantage in certain medico legal cases. Another most important point to be noted is the high sensitivity towards IgG coated cells, making it a better technique compared to the Conventional tube method.[3] Despite the above mentioned advantages, there are certain unavoidable disadvantages as well – cost, false positive reactions (macrocytosis, marked leucocytosis and increased ESR), the possibility of missing C3d coated red cells.[3]

Principle of Gel Card Method: The basic principle of the gel test is, instead of a glass test tube, the serum and cell reaction takes place in a microtube having 6 wells embedded in a plastic card, which allows easy testing, reading as well as handling and disposal. Saphadex gel is used in gel cards which holds agglutinate in semisolid medium, this helps in clear visualization of agglutination than that of the tube method.[2]

This method introduced by Lappiere et al., was firstly used for the cross matching of blood.[7] As stated earlier, the tube technique aka conventional technique has been the cornerstone for Coomb's testing over last 4 decades, but the enhanced sensitivity Gel card technique has made the interpretation more reliable.

Advantages of Gel card method:[3,10–12]

1. Simple, reliable, rapid, reproducible and sensitive
2. Greater uniformity amongst repeat test

3. Less volume of specimen required
4. Standardized reporting, grading system
5. No cell washing required
6. More consistent and reproducible interpretation of results
7. Higher sensitivity with IgG coated cells

Disadvantage of Gel card method: [3,10–12]

1. Expensive
2. Requires special incubator and centrifuge machine

Advantages of conventional method: [3,10–12]

1. Cheaper
2. Detection of C3 complement

False positive results: [3]

1. Overcentrifugation
2. Increased ESR, rouleaux formation
3. Macrocytosis
4. Leucocytosis
5. Inappropriate washing, inadequate resuspension of cell button
6. Hypergammaglobilinemia

False negative results: [3]

1. AHG reagent failure
2. Improper or inadequate or delayed washing
3. Low serum/cell ratio
4. Resuspension of cell button too vigorously

Table 5: Drugs associated with positive direct Coomb's test or hemolysis due to drug induced autoantibodies

Reported mechanism	Drug
Drug independent autoantibody induction	Levodopa, mefenamic acid, metyldopa
Drug dependent	Amoxicillin, erythromycin, insulin, penicillin, tetracyclin, tolbutamide, amphotericin B, ceftriaxone, naproxen
Nonimmunologic protein adsorption	Clavulanate potassium, diglycoaldehyde, sulbactam sodium, tazobactum sodium
Combined mechanism	Ampicillin, carbimazole, cefixime, cefotaxime, chlorpromazine, cisplatin, isoniazid, pipercillin, quinidine, ranitidine, rifampicin.

7. Conclusions

Our study showed that gel card is easier to use, more sensitive and less time-consuming with more standardized result and less sample needed for the test. Results of Gel card can be preserved for 3-4 days and this can be interpreted by various observers and compare it with the standardized grading system. Gel card assay appears to be an excellent method for detecting agglutination better than conventional tube method and easy to read weak agglutination and it can also detect ABO incompatibility. The performance of saline tube technique requires more experience and highly accuracy due to its long stages and multiple washing. But one disadvantage of gel card method is that gel cards are costly and require separate incubator and centrifuge.

8. Source of Funding

None.

9. Conflict of Interest

None.

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