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Comparison between the Manual Method of Indirect Coombs *via* Gel Technology and Solid Phase Red Cell Adherence

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ABSTRACT

Objectives: *An experiment was conducted to define the principle of the conventional tube technique, gel card technology, and solid-phase technology of blood transfusion. The study also highlights the test reactions and various methods of grading reactions for each technology. It further discusses the automated equipment available for each technology and compares the equipment, tests reactions, procedures, and sensitivity of these techniques.*

Materials and methods: *This cross-sectional study was conducted on blood samples of 40 patients with positive indirect antiglobulin test at the Padmashree Diagnostics Center and the Bangalore Medical Services Trust, India. Tube and gel card method and solid-phase red cell adherence assay (SPRCA) were evaluated.*

Results: *The results revealed SRPCA of 1+ in nine samples, 2+ in 15 samples, 3+ in 13 samples and 4+ in three samples, while the manual method yielded 1+ in 14 samples, 2+ in 13 samples, 3+ in 13 samples and 4+ in one sample.*

Conclusion: *Solid-phase red cell adherence assay is more precise and capable of detecting red cell adherence assay than tube method and indirect Coombs test gel technology.*

Keywords: SPRCA, indirect Coombs test gel technology, solid phase, IgM.

INTRODUCTION

IgM antibodies was the only marker investigated prior to the emergence of the antiglobulin test (1). Detection of non-agglutinating IgG antibodies propelled antiglobulin test to the forefront and enabled scientists to discover and characterize many new blood group systems (2). Coombs *et al* (3) detected the weak and non-agglutinating antibodies of Rh when they applied the antiglobulin test and injected human serum into rabbits to generate antihuman serum (AHG). Hetero-specific antibodies are removed to purify AHG. The AHG is diluted to avoid prozone. Diluted AHG serum

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antibodies are potent enough to permit cross-linking of adjacent red blood cells (RBCs) sensitized with IgG antibodies. Hemagglutination is the cross-linking of sensitized RBCs in AHG (4). Sensitized RBCs are applied to develop IgG alloantibodies, IgG autoantibodies and complement proteins which can be analyzed *via* antiglobulin test (1). RBC sensitization is achieved *in vitro* or *in vivo*. Indirect antiglobulin test (IAT) is an *in vitro*, two-stage RBC sensitization technique (5), whereas the direct antiglobulin test (DAT) is an in vivo, one-stage sensitization technique (6). IAT and DAT are the two dominant approaches in blood group serology. IAT is superior and more reliable for detecting clinically notable antibodies (3, 7, 8).

Lapierre *et al* (1990) proffered that the gel test was more reliable in detecting variety of clinically significant antibodies compared to the manual tube technique. Bromilow *et al* (1991) and Lillevang *et al* (1994) corroborated these findings in two detailed trials. Gels utilized in gel tests are either neutral gels or reagent-containing gels and the choice depends on the nature of the test to be performed. Reagents incorporated into gels are serum antiglobulin, anti-A, anti-B and anti-D. RBC suspension alone (for typing or direct antiglobulin testing) or a combination of RBCs and serum (for reverse ABO typing or antibody characterization) are produced under strict protocols and centrifuged in the gel. In negative reactions, RBCs migrate through the gel and pellet to the lower part of the tube, whereas in positive reactions RBCs are stuck in the gel. Positive reactions are stable for several hours. Gel tests are rapid, highly sensitive, and the results are easily reproducible. Further antiglobulin tests can be performed on the RBC and gel mixture. Derr Dickerson (9) purports the gel test is as sensitive as or more sensitive than PEG-IAT. New approaches such as column agglutination technique (CAT), solid-phase red cell adherence assay (SPRCA) and erythrocyte magnetized technique (EMT) have attempted to resolve the limitations and to improve the consistency of Derr *et al*'s findings. The gel test has application in ABO and Rh printing, cross-matching of antibodies in direct and indirect antiglobulin tests (DAT and IAT) and in the detection of alloantibodies (10-13).

Historically, the gel technique was pioneered in Thailand in 2003 to simplify and improve blood transfusion technology. In recent times, the gel technique is a popular choice in many blood banks across the world (14). Several experiments, including an analysis done in Pune, India, substantiate the benefits of the DCT and indirect Coombs test gel card technique, such as simplicity, durability, reproducibility, stability and improved sensitivity. Lately, gel card systems are gaining popularity in antigen detection, alloantibody screening/ identification, and cross-matching tests (14-16). The gel technology is susceptible to false positive readings. Applying the gel technology in macrocytosis, marked leukocytosis, and elevated ESR require correlating test results with clinical data for accuracy of test results. The odds of undetected CD3 coated red cells is higher with gel cards. Solid-phase technology uses test tubes or microplates in antiglobulin experiments and employs several different techniques (17, 18). Incorporating microplate readers into solid-phase technology permits semi-automation of the transfusion procedure. The solid-phase approach can be used to run direct and indirect experiments. In direct experiments, the antibody is attached to a microplate well and RBCs are added. If the antibody is specific to the antigen on the RBCs, a suspension appears at the bottom of the well (positive reaction). If no specificity exists between the antibody and the RBCs, the latter will settle at the bottom of the well (negative reaction). In indirect experiments, specific RBCs are bound to a well that has been treated with glutaraldehyde or poly L-lysine. Test serum is delivered into the RBC-coated wells and if the serum antibody is unique to the fixed RBC antigen, a suspension appears at the bottom of the well (positive reaction). The RBCs will settle at the bottom of the well in negative reaction. Immucor Incorporated produces a solid-phase device for the detection and characterization of alloantibodies. In this device, RBC membranes treated with Group O reagent and polystyrene are attached to the surfaces of microtitration strip wells. A patient's serum IgG antibodies are attached to the membrane antigens and the setup is incubated. After incubation the unbound immunoglobulins are rinsed from the wells and the RBC suspension is delivered into the wells. An anti-IgG-coated RBC indicator is brought into contact with the antibodies connected to the reagent RBC membranes through centrifugation. In negative test outcome

the bottom of the wells forms a pellet of indicator RBCs. In positive test outcome anti-IgG-IgG complexes and a second immobilized RBC layer are formed.

The aim of this study is to define the principle of the conventional tube technique, gel card technology and solid-phase technology. This study also highlights the test reactions and the various methods of grading reactions for each technology. It further discusses the automated equipment available for each technology and compares the equipment, tests reactions, procedures, and sensitivity of these techniques. ❑

MATERIAL AND METHODS

Blood samples of 40 patients with positive indirect antiglobulin test were collected at the Indian Padmashree Diagnostics Center and the Bangalore Medical Services Trust (TTK Blood Bank).

Tube method

Coombs reagent contains antibodies (antiglobulins) against human IgG/IgM/Complement. If RBCs coated with incomplete antibody or complement are treated with Coombs reagent, the antiglobulins in the reagent do not induce agglutination of the RBCs. Standard Coombs reagent cannot detect IgA or IgE antibodies.

Materials for the tube method were patients' serum, O Rh-positive RBCs, Coombs reagent, normal saline, and test tubes (12×75). For analysis of the sample in the tube (tube size 12×75 mm), 80 µL of sera were pipetted using asix6-inch polyethylene plastic transfer pipette. Forty µL of 5% red cell suspension and 80 µL of low-ionic saline solution buffer were added to the pipetted sera. The setup was incubated at 37°C for 10 minutes. Test reactants were washed three times before adding 80 µL of anti-IgG. Another 80 µL of Coombs reagent was added to the test tube and incubated again at 37°C for 10 minutes. The sample was then centrifuged at 2 000 rpm for one minute and examined for agglutination. One positive control and one negative control was run for each test set. For the indirect antiglobulin test, RBC agglutination indicated the presence of antibodies and these tests were reported as positive whereas uniform suspension of the test reactants indicated the absence of antibodies and these test were reported as negative.

Gel card method

ID-MTS anti-IgG is the available anti-human IgG for the gel card method. It is employed in indirect antiglobulin antibody tests. Gel card technique operates on the principle of column agglutination. Column agglutination technology relies on agglutination as the indicator for red cell antigen/antibody reaction. When agglutinates are centrifuged through a microcolumn of definite pore size, the size of the agglutinates determine the rate of travel of the agglutinates. In this case, instead of a test tube, the reaction between the patients' serum and cell occurs in a microtube. This microtube is composed of a narrow reaction chamber and contains 15 mm long and 4 mm wide columns. 35 µL of a dextran acrylamide gel prepared in a buffered solution of LISS or saline is placed in these columns. The gel may contain sodium azide as preservative, bovine serum albumin as sedimenting agent, and some specific reactants such as anti IgG or other RBC-specific antisera (ABO and D).

To perform the antibody screening or IAT or Coombs cross match, 50 µL of BLISS, 10 µL of 3-5% reagent red blood cell (Ocells) or donor's red blood cells and 40 µL of test serum/plasma were pipetted into each of the respective columns that all contained AHG. This was followed by an incubation step at 37°C for 10-30 minutes to allow antibodies present in the serum/plasma to react with the red cells. After incubation, the cassettes were moved directly into the centrifuge and spun for five minutes at two different speeds: low speed at ±800 rpm for two minutes, and high speed at ±1 500 rpm for three minutes. At the low speed spun, the red cell suspension present in the incubation chamber was forced down to the top of the density barrier. These sensitized red cells migrated into the density barrier because of their higher density and the serum/plasma and BLISS mixture remained above the density barrier because they were lighter. This also prevented the unbound serum human globulin present in the serum from neutralizing the AHG reagent. Red cells with human IgG bound to their surfaces agglutinated when they came in contact with the AHG. This was recorded as positive reaction. Lack of agglutination was recorded as negative reaction. At high speed spun, the

agglutinated red cells were separated from the non-agglutinated red cells. The agglutinated cells were trapped by the glass bead column and either stayed on top of the bead column or partially migrated back into the column (weaker reaction). In the event of a negative reaction, the red cell travelled to the bottom of the column. After the analysis, the assigned grading was as follows:

- 4+ reaction: All cells formed a band at the top of the column.
- 3+ reaction: Most cells were in the upper half of the column.
- 2+ reaction: Cells were present throughout the column.
- 1+ reaction: Most cells were in the lower half of the column.
- 0 reaction: All cells formed a button at the bottom of the column.

Solid-phase red cell adherence assay (SPRCA)

Red blood cell membranes were attached to the surface of polystyrene microwells and dried. The membrane antigens were used to capture target antibodies from patients' or donor serum or plasma. The wells were incubated for a short time. The unbound residual immunoglobulin was rinsed from the wells, which were then supplemented with an anti-IgG coated red blood cell predictor suspension. The setup was centrifuged to bring the red cell indicator into contact with antibodies attached to the red cell membranes of the reagent. The temperature of all reagents and specimens were maintained at 18-30°C prior to the test. IgG were detected with Capture-R@ Ready-Screen. A humidity indicator strip was removed from its protective pouch and was used to carefully examine the Capture-R@ Ready-Screen. The remaining humidity indicators were maintained in their protective pouches and used to examine the Capture-R@ Ready-Screens. All strips detected to contain moisture were disposed. The bottom tab of the strip was checked to be intact, then the strip was placed in the frame holder. Two drops (100+/- 10 µL) of Capture-LISS were added to each test and control wells; one drop (50±5 µL) of Capture-R positive control serum (weak) was added to one well, and one drop (50±5 µL) of Capture-R negative control serum was added to another well. Then one drop (50±5 µL) of the test serum/plasma was added to the separate wells. The strips were incubated at 36-38°C for 15 minutes. Next, the sample-LISS mixture was decanted from the wells. The wells were washed with manual and automated techniques. One drop (50 +/- 5 µL) of capture-R Ready Indicator Red cells was added to each well. The strips were immediately centrifuged for 1-3 minutes at 450-600×g. For each centrifuge used, the corresponding g forces (or RPMs) and centrifuge time were independently calculated. The strip was then positioned on an illuminated surface and inspected for adherence of IgG to the red cell indicator.

Capture-R positive and negative controls were used to monitor the quality and validity of all the test systems. Each valid test had a positive control wherein red cells adhered to all or part of the Capture-R Ready indicator strip surface and a negative control wherein red cells settled at the bottom of the Capture-R Ready Indicator strip surface. Capture-R Control Sera reactions that did not meet these two requirements were re-run and reported only after they had met the validity criteria. This quality control was included in each setup before centrifuging to eliminate mechanical errors and reagent failures. Degraded Capture-R Ready-Screen test reagents and lack of consistency in test protocol accounted for recurrent failure of repeated control tests. ❑

RESULTS

This study compared the conventional tube method, SPRCA, and IAT column agglutination methods of blood transfusion at the Padmashree Diagnostics Center and the Bangalore Medical Services Trust (TTK Blood Bank), Indiranagar, Bangalore, India. The ages of male patients ranged from 25-69 years and the mean age was 42.57±13.68 years (Table 1, Figure 1). Among females, the ages ranged from 26-63 years and the mean age was

TABLE 1. Age and gender distribution of patients

S no	Gender	No. (%)	Age (years)		
			Range	Mean±SD	p-value
1	Male	21 (52.5)	25-69	42.57±13.68	0.767^{ns}
2	Female	19 (47.5)	26-63	41.37±11.55	
3	Total	40 (100)	25-69	42.00±12.55	

ns=not significant at 5% level (p >0.05).

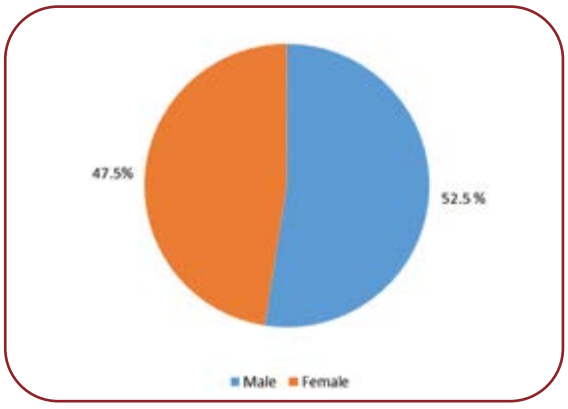

FIGURE 1. Age and gender distribution of patients

41.37±11.55 years. So, the total age range was 25-69 years and the total mean age was 42.00±12.5 years. The t-value was 0.94 whereas the p-value was 0.767. The p-value was statistically insignificant.

The screening results of the manual method and SPRCA reveal SRPCA of 1+ in nine samples, 2+ in 15 samples, 3+ in 13 samples and 4+ in three samples whiles the manual method displays 1+ in 14 samples, 2+ in 13 samples, 3+ in 13 samples and 4+ in one sample (Table 2). In this analysis, the chi-square value obtained was 46.96, which was significant at p=0.01.

The screening results of the column agglutination and SPRCA revealed that out of 40 positive IAT samples, The results revealed SRPCA of 1+ in nine samples, 2+ in 15 samples, 3+ in 13 samples and 4+ in three samples and the column agglutination method shows 1+ in 11 samples, 2+ in 16 samples, 3+ in 12 samples and 4+ in one sample (Table 3). The chi-square value obtained in this analysis was 67.13 and this was significant at p=0.01.

Sensitivity: P(T+|D+) = TP / (TP+FN)
Sensitivity of manual method
= 28/ (28+12)
= 0.70 (probability) or 70.0 (percentage).
Sensitivity of column agglutination
= 33/ (33+7)
= 0.825 (probability) or 82.5 (percentage).

Manual method	SPRCA				Total	Chi-square value
	1+	2+	3+	4+	No. (%)	
1+	9 (100)	5 (33.3)	0 (0)	0 (0)	14 (35.0)	46.96*, df=9, p=0.000
2+	0 (0)	10 (66.7)	3 (23.1)	0 (0)	13 (32.5)	
3+	0 (0)	0 (0)	9 (69.2)	3 (100)	12 (30.0)	
4+	0 (0)	0 (0)	1 (7.7)	0 (0)	1 (2.5)	
Total	9 (22.5)	15 (37.5)	13 (32.5)	3 (7.5)	40 (100)	

*Significant p <0.05

TABLE 2. Screening results of the manual method and SPRCA

Column agglutination	SPRCA				Total	Chi-square value
	1+	2+	3+	4+	No. (%)	
1+	9 (100)	2 (13.3)	0 (0)	0 (0)	11 (27.5)	67.13*, df=9, p=0.000
2+	0 (0)	13 (86.7)	3 (23.1)	0 (0)	16 (40.0)	
3+	0 (0)	0 (0)	10 (76.9)	2 (66.7)	12 (30.0)	
4+	0 (0)	0 (0)	0 (0)	1 (33.3)	1 (2.5)	
Total	9 (22.5)	15 (37.5)	13 (32.5)	3 (7.5)	40 (100)	

*Significant p <0.05

TABLE 3. Screening results of column agglutination and SPRCA

TABLE 4. Sensitivity of manual method and column Agglutination with SPRCA

	SPRCA			SPRCA	
Manual method	Positively diagnosed	28 (70.0%)	Column agglutination	Positively diagnosed	33 (82.5%)
	Negatively diagnosed	12 (30.0%)		Negatively diagnosed	7 (17.5)
	Total	40 (100%)		Total	40 (100)

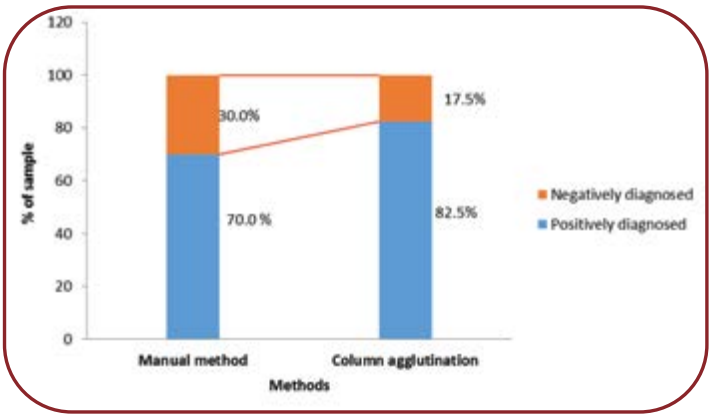

FIGURE 2. Sensitivity of manual method and column agglutination with SPRCA

The sensitivity results of the different methods studied by us showed that SPRCA was more precise and could accurately detect 82.5% of patients (Table 4, Figure 2). ❑

DISCUSSION

The conventional manual tube technology is still the gold standard for assessing blood compatibility in transfusion medicine (19). But there is a need to minimize the disadvantages associated with this technique.

Direct antiglobulin test (DAT) detects immunoglobulin or complement proteins bound to the surface of red blood cells (RBCs). It has significant application in the evaluation of patients with suspected immune-mediated hemolysis (20). In DAT, a positive result for IgG is obtained when antisera specific for IgG bound to RBCs (21). These antibodies can be eluted from the RBCs by various techniques. The specificity of the IgGs can then be determined through antibody identification studies using a panel of reagent RBCs of known phenotypes (22).

Lately, gel microcolumn (GMC) and solid-phase red cell adherence (SPRCA) systems are increasingly being applied in the transfusion laboratory for serologic testing, including antibody detection and identification (23). Generally, both methods have shown similar or better sensitivity and specificity when compared to the traditional manual tube testing method. They offer many practical advantages to the transfusion laboratory (23). Both require smaller sample and reagent volumes, involve less subjective interpretation, and yield stable results which facilitate consultation and secondary review. Another significant advantage of these methods is their potential for automation.

Elution procedures allow for the removal of IgG bound to a patient's RBCs and to produce a concentrated sample of these antibodies. Antibody identification studies performed on eluates yield useful information to aid in the diagnosis of immune-mediated hemolysis caused by alloantibodies and warm autoantibodies, especially when such antibodies cannot be demonstrated in the patient's plasma. We compared the sensitivity and specificity our study with the study conducted by Vanamala Alwar *et al* in 2011.

Table 5 shows a similar sensitivity of each of the three tests in both studies; moreover, the two studies SPRCA had 100% sensitivity in the two studies. ❑

TABLE 5. Comparison of the sensitivity of gel card technique, manual tube technique and SPRCA between two studies

Study	Vanamala Alwar et al (2011)	Our study (2016)
Gel card technique sensitivity	83.01%	82.5%
Manual tube technique sensitivity	66.03%	70%
SPRCA sensitivity	100%	100%

CONCLUSION

Although our study was limited in sample size and scope of alloantibodies detected, our data suggested that the automated SPRCA assay was an appropriate method for identifying antibodies in acid eluates prepared from the peripheral blood of both recipient and donor samples when compared to the manual tube and gel card methods. ❑

Conflicts of interest: none declared.
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References

1. **Parker V, Tormey CA.** The direct antiglobulin test: indications, interpretation, and pitfalls. *Arch Pathol Lab Medicine* 2017;2:305-310.
2. **Green RE, Hughes VC.** The antiglobulin test. *Modern Blood Banking and Transfusion Practices* 2005:93.
3. **Coombs RR, Mourant AE, Race RR.** A new test for the detection of weak and "incomplete" Rh agglutinins. *British J Experiment Pathol* 1945;4:255.
4. **Segel GB, Lichtman MA.** Direct antiglobulin ("Coombs") test-negative autoimmune hemolytic anemia: a review. *Blood Cells, Molecules, and Diseases* 2014;4:152-160.
5. **Mishra S.** *Role of Coombs' Test in analysis of Immunohematological Cases.* 2014;3.
6. **Shah N, Phulpagar M, Puranik G.** Utility of direct antiglobulin test (Direct Coombs Test) - an urban public hospital experience. *International Journal of Scientific Research* 2020;1.
7. **Coombs RR, Mourant AE, Race RR.** Detection of weak and "incomplete" Rh agglutinins: a new test. In: *Rhesus haemolytic disease*. Springer, 1945, pp 88-88.
8. **Coombs RR.** Historical note: past, present and future of the antiglobulin test. *Vox Sanguinis* 1998;2:67-73.
9. **Derr D, Dickerson S, Steiner E.** Implementation of gel column technology, including comparative testing of Ortho ID-MTS with standard polyethylene glycol tube tests. *Immunohematology* 1998;2:72.
10. **Klein HG, Anstee DJ.** *Mollison's blood transfusion in clinical medicine.* John Wiley & Sons, 2014
11. **Lapierre Y, et al.**, The gel test: a new way to detect red cell antigen-antibody reactions. *Transfusion* 1990;2:109-113.
12. **Novaretti M, Jeus E.** Evaluation of a gel test system for the detection of transplacental haemorrhage. *Transfusion* 1994;34(Suppl):S110.
13. **Kaur R, Kakkar N, Dhanoa J.** Use of the gel-based DiaMed-ID microtyping system for crossmatching enhances sensitivity. *Indian Journal of Pathology & Microbiology* 2003;4:617-620.
14. **Nathalang O, et al.** A preliminary study of the gel test for cross matching in Thailand. *J Med Tech Assoc Thai* 1993;21:101-106.
15. **Bromilow I, et al.** Evaluation of the ID-gel test for antibody screening and identification. *Transfusion Medicine* 1991;3:159-161.
16. **Kuśnierz-Alejska G, Fabijańska-Mitek J.** Use of microtest in gel for compatibility in serological testing before transfusion. *Acta Haematologica Polonica* 1993;1:49-56.
17. **Rosenfield R, Kochwa S, Kaczera Z.** Solid phase serology for the study of human erythrocytic antigen-antibody reactions. In: Proccedings of the 12th Congress of the International Society of Haematology and the 15th *Congress of the International Society of Blood Transfusion*, 1978.
18. **Moore H.** Automated reading of red cell antibody identification tests by a solid phase antiglobulin technique. *Transfusion* 1984;3:218-221.
19. **Yoo J, et al.** Evaluation of the automated immunohematology analyzer DAYMATE M. *Laboratory Medicine Online* 2017;4:163-169.
20. **Kavitha G.** Evaluation of Direct Antiglobulin Test Positive Cases by Elution Study. The Tamilnadu Dr. MGR Medical University, Chennai, 2019.
21. **Berzuini A, et al.** Red cell–bound antibodies and transfusion requirements in hospitalized patients with COVID-19. *Blood* 2020;6:766-768.
22. **Scharberg E, et al.** The impact of using genotyped reagent red blood cells in antibody identification. *Transfusion Medicine and Hemotherapy* 2018;4:218-224.
23. **Finck R, et al.** Performance of an automated solid-phase red cell adherence system compared with that of a manual gel microcolumn assay for the identification of antibodies eluted from red blood cells. *Immunohematology* 2011;1:1-5.