

UTILITY OF DIRECT ANTIGLOBULIN TEST (DIRECT COOMBS TEST) - AN URBAN PUBLIC HOSPITAL EXPERIENCE

Pathology

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ABSTRACT

Introduction and Aims-The Direct Antiglobulin Test (DAT) is the test that detects immunoglobulin and/or complement on surface of red blood cells. Most of the laboratory use conventional tube technique but in 2-6% of cases of patients with clinical symptoms of hemolysis turn out coombs test negative on repeated testing. Hence we conducted this study to know more sensitive, simpler and cost effective technique of performing direct coombs test.

Materials and Methods-A prospective study was performed over period of 18 months 500 cases of various indications were studied February 2018 to August 2019.

Results-Sensitivity of gel technique was 100%, specificity was 96.12%, positive predictive value is 84.62% and negative predictive value was 100% in our study. There was 95% agreement between the results of gel card method and conventional tube method.

Conclusion- In present study it was observed that Gel Technique was easier and quicker with better sensitivity than Conventional Tube Technique.

KEYWORDS

Direct Antiglobulin Test, Conventional tube technique, gel technique

INTRODUCTION

The most crucial step in laboratory evaluation of intravascular hemolysis in patients is DAT (Direct Antiglobulin Test). The Direct Antiglobulin Test (DAT) is the test that detects immunoglobulin and/or complement on surface of red blood cells^{1,2}. The utility of this test is to distinguish immune and non-immune etiology of hemolysis. The sensitization of red cells in vivo is detected by one stage technique, the Direct Antiglobulin Test. The DAT results need to be analyzed along with clinical history and other laboratory investigations.

The principle of the DAT being, Anti-Human Globulin (AHG) agglutinates or forms clumps with antibody coated cells. There are 3 different reagents that may be used - Monospecific IgG, Monospecific complement (C3), Polyspecific reagent for IgG and C3^{1,2}.

The Direct Anti globulin Test (DAT) was first reported by Moreschi in 1908 and widespread notoriety was found by Direct Antiglobulin Test (DAT) after being described by Coombs et al in 1945¹. In 1990, Lappierre introduced the novel 'GEL' method to demonstrate antibodies or complement coated red cells. The gel system is based on the principle - the Sephadex gel matrix acts as a sieve, through which agglutinates of RBCs are very large to pass through and remain entrapped in the gel, based on their size³. This technique was easy to perform as it did not require technical skills to perform and reduced practical difficulty to perform test as compared to conventional technique.

Most of the laboratory use conventional tube technique but sometimes in 2-6% of cases of patients with clinical symptoms of hemolysis turn out coombs test negative on repeated testing⁴. Hence we conducted this study to know more sensitive, simpler and cost effective technique of performing direct coombs test.

AIM AND OBJECTIVE

To compare between gel card and conventional tube technique of performing Direct Coombs Test.

MATERIALS AND METHODS

A prospective study was performed over period of 18 months 500 cases of various indications were studied February 2018 to August 2019. Prior institutional ethics committee clearance was obtained for study. Informed consent was obtained from the subjects or from parents/guardian for inclusion of their test result in study.

Inclusion Criteria-

1. All the adult, pediatric samples and cord blood samples of newborns requested for direct antiglobulin test by clinicians.
2. All thalassemia patients undergoing multiple transfusions were

considered.

Exclusion Criteria-

1. Those samples which were not requested by clinicians for direct antiglobulin testing except for multiple transfused thalassemia patients where all samples were considered.
2. Inadequate and hemolysed samples were not considered.

Procedure of DAT

Blood sample – Fresh sample (less than 24 hours old), sample were taken in EDTA (1.5 mg EDTA for 1 ml of blood) to protect the uptake of complement.

Conventional Tube Technique (CTT)^{4,5}

2-3 drops of blood to be tested was taken in a clean labeled tube. Red cells were washed 3 – 4 times with large volume of saline to remove free globulin molecules. Removed all supernatant after each wash and the final supernatant wash was completely removed. 2 drops of polyspecific AHG serum was added in 1 drop of sensitized washed red cells or in 1 drop of 3-5% suspension of sensitized red cells immediately. Mixed and centrifuged at rate of 1000 rpm for 1 min. The tube was shaken to dislodge the cell button and see for agglutination. Results were recorded. 1 drop of IgG coated red cells were added to a negative test. Mixed and centrifuged at rate of 1000 rpm for 1 min. Agglutination was checked. [If a negative result (no agglutination) is obtained the test result becomes invalid and whole test should be repeated. If agglutination is obtained, the result is valid.

Gel Column Agglutination Technique (GT)^{4,5}

ID cards were prepared before use. Cell suspension was prepared with normal saline and was centrifuged it to get cell button / packed cells. To one micro tube add 500 microliter of LISS (Low Ionic Strength Solution) ID diluent and 5 microliter of packed cells were added. After mixing well 50 microliter of this cell suspension (0.8%) was transferred to ID card which was labeled properly at an angle of 45 degree. ID card was centrifuged for 10 minutes in the ID centrifuge and reading of the reaction were done according to the gradations

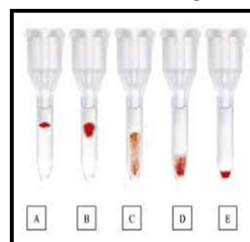


Diagram of gel test reaction patterns and grading. Reactions are graded from 0 to 4+. (A) 4+ reaction is indicated by a solid band of red cells on the top of the gel; (B) 3+ reaction displays agglutinated red cells in the upper half of the gel column; (C) 2+ reaction is characterized by red cell agglutinates through the length of the column; (D) 1+ reaction is indicated by red cell agglutinates mainly in the lower half of the gel column with some unagglutinated red cells pelleted at the bottom; and (E) Negative reactions display a pellet at the bottom and no agglutinates in the matrix of the gel column. A mixed field reaction may be observed⁶

Results

Total 500 cases were studied as they underwent testing by direct coombs test by both gel and conventional tube technique from the samples received and results obtained were as follows

Table No.1- Comparison of results of DCT by gel technique and conventional tube technique with grading.

DCT Result (Gel card Technique)	DCT Result -Conventional Tube Technique					Total
	Negative	1+	2+	3+	4+	
Negative	No. 396	0	0	0	0	396
	% 100.0%	0.0%	0.0%	0.0%	0.0%	100.0%
1+	No. 15	18	2	0	0	35
	% 42.9%	51.4%	5.7%	0.0%	0.0%	100.0%
2+	No. 1	2	22	2	0	27
	% 3.7%	7.4%	81.5%	7.4%	0.0%	100.0%
3+	No. 0	0	3	13	0	16
	% 0.0%	0.0%	18.8%	81.3%	0.0%	100.0%
4+	No. 0	0	0	0	26	26
	% 0.0%	0.0%	0.0%	0.0%	100.0%	100.0%
Total	No. 412	20	27	15	26	500
	% 82.4%	4.0%	5.4%	3.0%	5.2%	100.0%

DISCUSSION

Total 500 cases were studied; common age group was 15 or more than 15 years which were about 53.6%. There were 56.6% female and 43.4% male cases. Most common indication for which coombs test requested was anemia, in about 56.2% case. When both gel card and conventional tube methods were compared 396 cases out of 500 (79.2%) were negative by both methods, 18 cases showed DCT result as 1+, 22 showed result as 2+, 13 cases as 3+ and 26 cases as 4+ by both conventional and gel card method. There was 95% (475 out of 500) agreement between the results of gel card method and conventional tube method. It was similar to findings by Das S et al⁵. where agreement between results of DCT by gel and conventional tube technique was found as 96.4%.

Incidence of DCT positivity in hospital population studied with various clinical indication of performing the test was 20.8% (104 out of 500) by gel card technique and 17.6% (88 out of 500) by conventional tube technique. Judd W.J. et al⁷ found incidence of DAT positivity as 15% (1830 out of 12187) of total samples studied. 79.2% cases were negative by gel card technique. All these cases were also negative by conventional tube technique, but in addition to it 3.2% cases which were observed to be negative with conventional tube method were positive with gel card method. None of the cases showed coombs test result as negative by gel method and positive by conventional tube technique.

Sensitivity of gel technique was 100%, specificity was 96.12%, positive predictive value is 84.62% and negative predictive value was 100% in our study. Comparison with other was as mentioned in table no.2

Table No. 2

Parameters	Our study	Alwar V et al(2012) ³	DeLafor-Weiss E et al(2005) ⁶	Nathalng O et al(1997) ⁸	Fayek M.H. et al(2015) ⁹	Dutta P et al(2005) ¹⁰	Tissot J-D et al(1999) ¹¹
Sensitivity	100%	93.5%	95.5%	93.5%	92%	100%	75.4%
Specificity	96.12%	88.6%	-----	88.6%	-----	95.1%	95.9%

Thus using gel card method can be recommended as effective complementary test to the traditional tube method especially in public or rural health setups where interrupted supply of reagents and kits is common. In the gray area of 2-6% cases where there are clinical symptoms of hemolysis but coombs test is repeatedly negative, gel

technique used along with conventional method gives confidence to blood bank to issue correct result and best match blood required for treatment.

CONCLUSION

In present study it was observed that Gel Technique was not only easier and quicker method with better sensitivity than Conventional Tube Technique but also effective complementary test especially in public or rural health setups where interrupted supply of reagents and kits is common. Polyspecific DAT (Direct Antiglobulin Test) by gel method can be used as good primary test and further classification can be done by monospecific DAT for specific antibodies and / or complements. Gel technique used along with conventional method gives confidence to blood bank to issue correct result and best match blood required for treatment.

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