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A comparison of conventional tube test and gel technique in evaluation of direct antiglobulin test

SUDIPTA S. DAS, RAJENDRA CHAUDHARY, & DHEERAJ KHETAN

Department of Transfusion Medicine, Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow 226 014, India

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Abstract

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 antiglobulin test (DAT).

The CTT and GT were first compared using in-house prepared control cells. The polyspecific DATs were performed simultaneously by CTT and GT on 170 consecutive blood samples. Positive samples were further tested for monospecific IgG and C3d by both techniques.

GT demonstrated stronger agglutination scores (60 vs. 43) compared to CTT using control cells. The sensitivity and specificity of the GT was 98.4 and 95.2%, respectively as compared to CTT for polyspecific DAT. Discordance between the two test systems was seen in 6/170 patients. Of these, 5 were missed by CTT while GT failed to detect *in vivo* coating in only 1 case. The agreement between two methods of DAT was 96.4% ($\kappa = 0.926$) using polyspecific AHG and 95.7% ($\kappa = 0.379$) with monospecific anti-IgG. We conclude that GT is a better alternative to CTT for detecting red cell bound antibodies in various clinical conditions.

Keywords: *Immunohematology, autoimmune hemolytic anemia, direct antiglobulin test, gel test, autoantibody*

Introduction

The first description of the ABO system and red cell agglutination by Landsteiner in 1900 and subsequent development of the antiglobulin test by Coombs et al. in 1945 enabled the immunohematologists to establish and improve various serological investigations in human blood [1–3]. The direct antiglobulin test (DAT) used to investigate autoimmune hemolytic anemia (AIHA) is a diagnostic procedure to demonstrate *in vivo* antibodies and/or complement coating red blood cells. This procedure uses polyspecific antihuman globulin (AHG) reagent containing antibodies to IgG and C3d component [4]. Majority of the blood banks in the developing countries perform routine DAT using the conventional tube technique (CTT). This traditional method is not without problems and demand skilled personnel and meticulous washing of red cells [3]. All these necessitated the establishment of a new, precise, easy and sensitive technique, which led to the advent of the gel test (GT) in 1993 [5,6]. The GT requires no red cell washing and uses gel filtration media impregnated with AHG reagent to bring about agglutination [3]. Furthermore, the technique can be used with a panel of monospecific AHG reagents, such as IgG IgM, IgA, C3c or C3d to detect the immunoglobulin classes or IgG subclasses (IgG1, IgG2, IgG3, IgG4) enabling the characterization of AIHA [4].

Recently, we at our center introduced gel technology for various immunohematological investigations. The purpose of this study was to validate the GT DAT and its comparison with the already established CTT in the detection of red cell bound immunoglobullins and complements.

Correspondence: R. Chaudhary, Department of Transfusion Medicine, Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow 226014, India. Tel: 91 522 2668700. Fax: 91 522 2668017. E-mail: rkcchaud@sgpgi.ac.in

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Material and methods

Samples

We studied 170 consecutive blood samples sent to our immunohematology laboratory for DAT after obtaining consent of the patient and seeking approval of institutional review board. Blood samples were collected in K2 EDTA vials and analyzed for polyspecific DAT on the day of collection using both CTT and GT. Any sample positive with either of the techniques was further tested for monospecific DAT using anti-IgG and -C3d by both gel and CTT.

CTT

It was performed following standard method described by AABB [7]. Briefly, one drop of 2–5% suspension of red cells was dispensed into test tubes and washed three times with normal saline, final wash decanted completely. Two drops of polyspecific AHG reagent (Diaclon Coombs, Diamed, Switzerland) were then added, mixed well and tube centrifuged at 1000*g* for 30 s and cells were examined for agglutination. The polyspecific AHG reactive samples were then similarly tested using monospecific AHG reagents viz anti-IgG and -C3d (Ortho Diagnostics, USA). All reactions were graded and recorded. The negative test was further confirmed by absence of agglutination on addition of sensitized check cells (in house).

GT

It was performed following manufacturer's instruction. Briefly, 50 μl of 1% red cell suspension in low ionic strength solution (LISS) was added to each microtube of the ID cards (polyspecific AHG, LISS-Coombs card, Diamed, Switzerland) and centrifuged in a dedicated centrifuge device (Diamed, Switzerland) at 70*g* for 10 min following the recommended incubation period of 15 min. Samples reactive with polyspecific AHG were then similarly tested for monospecificity using gel ID cards (monospecific AHG, LISS-Coombs card, Diamed, Switzerland). In both the techniques, the findings of the agglutination reactions were graded as 4 + , 3 + , 2 + , 1 + , weak and negative and documented accordingly.

Comparison of GT and CTT using control cells

Serial dilutions of cold acid eluate from red cells of the 55 warm AIHA patient, DAT 4 + , ranging from 1:1 to 1:256 were prepared. "O" Rh (D) positive red cells were incubated with serial dilutions at 37°C and washed with normal saline. The sensitized cells were tested in duplicate by tube and gel methods for detection of antibodies coating the red cells for comparison across range of DAT reaction strengths. The agglutination scores for each DAT strength were calculated according to the standard guidelines [7].

Statistical analysis

The results of testing samples in the CTT and GT DAT assays were compared using paired t test. A p value of <0.05 was considered significant. The sensitivity and specificity were also calculated for GT considering CTT as the standard method. κ value was calculated as a measure of agreement using SPSS, version 9.0.

Results

Comparison of GT and CTT using control cells

The GT was able to detect *in vitro* sensitization of red cells till 1:128 dilutions with total agglutination score of 60, while CTT could detect at 1:32 dilution and total score being 43.

DAT (polyspecific) using GT and CTT

Of the 170 samples evaluated for DAT using CTT and GT, 100 (58.8%) samples were negative and 64 positive with both the methods (observed agreement = 96.4%, $\kappa = 0.926$). Discrepant results were observed in 6/170 samples: 5 were positive by GT while negative with tube test, whereas only 1 sample was positive exclusively with the tube test (Table I). The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of the GT compared to CTT were 98.4, 95.2, 92.7 and 99%, respectively, for polyspecific DAT.

Of these 70 samples that were DAT positive by either method, 34 were finally diagnosed as AIHA with evidence of *in vivo* hemolysis, while the remaining 36 samples were from patients with various autoimmune disorders such as systemic lupus erythematosus (18), rheumatoid arthritis (5), Hashimoto's thyroiditis (3) and other (10).

DAT (monospecific) using GT and CTT

Among the 70 polyspecific DAT positive samples, 4 samples showed discordant results when evaluated

Table I. Comparison of GT and CTT for polyspecific DATs in 170 subjects.

	Conventional tube test (CTT)		
Gel test (GT)	Positive	Negative	Total
Positive	64	05	69
Negative	01	100	101
Total	65	105	170

Agreement between two methods = 164/170 (96.4%, $\kappa = 0.926$).

Table II. Concordance between GT and CTT for mono-specific DAT in poly-specific DAT positive samples ($n = 70$).

GT	CTT	Anti-IgG	Anti-C3d
Positive	Positive	66	10
Positive	Negative	02	01
Negative	Positive	01	00
Negative	Negative	1	59
Total agreement		67	69
Percentage of agreement (κ)		95.7 (0.379)	98.5 (0.944)

with monospecific sera (3 with anti-IgG and 1 with anti-C3d) (Table II). Table III summarizes the discordant DAT results. Out of 5 samples negative by CTT for polyspecific DAT, 2 were from patients with AIHA having *in vivo* hemolysis. Both these samples were positive by GT. Out of 64 samples that were positive by both methods, in 96.8% the strength of reaction evaluated by GT was either more or equal to that of the CTT ($p < 0.05$). Only on two occasions the strength of DAT reaction in CTT was more than GT (Table IV).

Discussion

The GT was introduced more than a decade ago, but most blood centers rely on the CTT for evaluation of DAT for serological diagnosis of AIHA, HDN and DHTR even today [8]. Owing to the simplicity, reproducibility and sensitivity of the GT, many blood banks are now gradually adopting this technique [9]. Most cases of immune mediated hemolytic anemia can be effectively diagnosed by the CTT, but some patients, despite hemolysis, show a negative tube DAT [10–12]. We compared GT with CTT for polyspecific and monospecific DATs. We observed GT to be a good alternative to CTT in terms of sensitivity of 98.4% and specificity of 95.2%. The PPV and NPV were 92.7 and 99%, respectively. Our results are in accordance with Nathalang et al. 1997 who observed a sensitivity of 100% and specificity of 80% with the GT [4]. In 5 of the 6 patients with discordance between two test systems, red cell antibodies could not be detected by CTT using polyspecific AHG (Table I). Two of these 5 patients were finally diagnosed as AIHA with evidence of *in vivo* hemolysis. Thus, it appears from our results that the GT DAT is more reflective of clinically significant *in vivo* red cell sensitization. However, Paz et al. 2004 observed that though the GT DAT was more sensitive than CTT, the clinical significance of a DAT positive only by GT was doubtful [13]. Others also reported failure of CTT as compared to GT in the diagnosis of AIHA with evidence of hemolysis [4].

In the present study (Table II) the CTT failed while the GT succeeded in detecting autoantibodies bound to the red cells in 3 patients using monospecific DAT (anti-IgG or anti-C3d). Only on one occasion was CTT positive while GT negative with both poly and mono (IgG) DAT; however, this case was later diagnosed as SLE with a weak DAT reactivity that was not clinically significant. Thus the GT provided more information on immunoglobulin and complement binding on the red cells as compared to CTT. This is in contrast to the findings of Tissot and Colleagues in 1999 that the tube agglutination assay was more sensitive for detection of red cell bound C3d than the GT [14]. This can be explained by the advancement of gel technology over the years and use of impregnated AHG in the gel matrix rather than the application of reagents into the micro column during the test as practiced earlier. However, the occurrence of false positive reactions by the GT could not be ruled out. Thus more studies on a larger sample size including red cells with known monospecific DAT strength are needed to compare the CTT and GT in relation to the detection of red cell bound IgG or C3d.

A close agreement was observed between the two test systems using polyspecific AHG (95.7%) and monospecific anti-IgG (90.9%) (Table II). Reis et al. described close agreement between CTT and column agglutination technique using glass beads in 1993 [3]. In our study, two of the 34 patients with AIHA who were anti-IgG positive with the GT were negative with CTT. This could be attributed to increased sensitivity

Table III. Details of discordant DAT results of CTT and GT in 6 patients.

			CTT			GT		
S. No.	Diagnosis	Age (years)/sex	Poly AHG	Anti-IgG	Anti-C3d	Poly AHG	Anti-IgG	Anti-C3d
1	Primary AIHA	25/F	Neg	Neg	Neg	1 +	1 +	Neg
2	Autoimmune hepatitis	23/M	Neg	1 +	Neg	1 +	2 +	W +
3	SLE	21/F	Neg	Neg	Neg	1 +	1 +	Neg
4	NHL	70/M	Neg	1 +	W +	1 +	1 +	1 +
5	Primary AIHA	30/F	Neg	W +	Neg	1 +	W +	Neg
6	SLE	22/F	1 +	1 +	Neg	Neg	Neg	Neg

Table IV. Comparison of CTT and GT strength in 64 samples positive for polyspecific DAT by both methods.

Grade of reaction	Number of patients *n* (%)
GT > CTT	36 (56.2)
GT = CTT	26 (40.6)
GT < CTT	02(3.1)

($p < 0.05$)

of the monospecific anti-IgG gel cards or loss of loosely attached autoantibodies during vigorous washing of red cells in CTT. In addition to being sensitive and specific, the strength of DAT reaction was stronger with GT in majority (36/64, 56.2%) of the cases compared to CTT ($p < 0.05$).

The results of our study demonstrate that GT is advantageous over CTT in the evaluation of red cell bound immunoglobulins and complement. Dittmar et al. in 2001 suggested GT to be as sensitive as flow cytometry for the detection of red cell bound IgG [9]. However, it was also reported that laboratories should not rely on a single method for performing DAT and employ tests with increased sensitivity, especially in situations with clinical evidence of hemolysis and negative tube DAT. Considering the various advantages of the GT and proof of its superiority by a number of studies it would be prudent to introduce the technique in all the blood centers as an additional assay to the CTT [9,15].

References

[1] Mollison PL, Engelfriet CP, Contreras M. Blood transfusion in clinical medicine. 10th ed. Oxford: Blackwell Science Ltd; 1998.

[2] Coombs RRA, Mourant AE, Race RR. A new test for the detection of weak and incomplete Rh agglutinins. Br J Exp Pathol 1945;26:255.

[3] Reis KJ, Chachowski R, Cupido A, Davies D, Jakway J, Setcavage TM. Column agglutination technology: The antiglobulin test. Transfusion 1993;33:639–643.

[4] Nathalang O, Chuansunrit A, Prayoonwiwat W, Siripoonya P, Sriphaisal T. Comprison between conventional tube technique and gel technique in direct antiglobulin tests. Vox Sang 1997;72:169–171.

[5] Nathalang O, Kuvanont S, Suwanasit T, Yensuang K, Sriphaisal T, Krutvacho T. A preliminary study of the gel test for cross matching in Thailand. J Med Tech Assoc Thai 1993a;21:101–106.

[6] Nathalang O, O Charoen R, Sandondu N, Meesoontorn R. Application of the gel test for antibody screening and identification. Thai J Hematol Transfus Med 1993b;3: 299–306.

[7] Vengelen-Tyler V. Technical manual. 13th ed. Bethesda, MD: American Association of Blood Banks; 1999. p MD.

[8] Lapierre Y, Rigal D, Adam J, Josef D, Meyer F, Gerber S, Drot C. The gel test: A new way to detect red cell antigen–antibody reactions. Transfusion 1990;30:109–113.

[9] Dittmar K, Procter JL, Cipolone K, Njorage JM, Miller J, Stroncek DF. Comparison of DATs using traditional tube agglutination to gel column and affinity column procedures. Transfusion 2001;41:1258–1262.

[10] Gilliland BC, Baxter E, Evans RS. Red cell antibodies in acquired hemolytic anemia with negative antiglobulin serum tests. N Engl J Med 1971;285:252–256.

[11] Boccardi V, Girelli G, Perricone R, Ciccone F, Romoli P, Isacchi G. Coombs—negative autoimmune hemolytic anemia. Report of 11 cases. Hematologica 1978;63:301–310.

[12] Engelfriet CP, Overbeeke ME, von dem Borne AE. Autoimmune hemolytic anemia. Semin Hematol 1992;29: 29–33.

[13] Paz N, Itzhaky D, Ellis MH. The sensitivity, specificity and clinical relevance of gel versus tube DATs in the clinical immunology laboratory. Immunohematology 2004;20: 118–121.

[14] Tissot JD, Kiener C, Burnard B, Schneider P. Direct antiglobulin test: Still a place for tube technique? Vox Sang 1999;77(4):223–226.

[15] Fabijanska-Mitek J, Namirska-Krzton H, Seyfried H. The value of gel test and ELAT in autoimmune hemolytic anemia. Clin Lab Hematol 1995;17:311–316.