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Comparison of Conventional Tube Test Technique and Gel Microcolumn Assay for Direct Antiglobulin Test: A Large Study

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Gel microcolumn assay (GMA) is a modified serological technique that has been used for ABO and Rh typing, direct antiglobulin test (DAT), detecting alloantibodies, red cell phenotyping, and other applications. However, for DAT, the role of GMA is controversial. The purpose of this large study was to compare the performance of the conventional tube test (CTT) to GMA for detecting potentially significant antibodies coating red blood cells in vivo. From January 1996 to May 2002, we performed DATs by GMA and CTT on 9,862 blood samples submitted to our reference laboratory, using LISS/Coombs cards (DiaMed-Latino America, Lagoa Santa-MG, Brazil) for GMA and polyspecific and monospecific anti-IgG reagents for CTT. Acid eluates were prepared from all positive DAT samples. The specificity of eluates was determined by GMA. We detected nonconcordant results in 2,079 out of 3,163 positive DATs (65.7%). All of these tests were only positive in GMA. Sensitivity and specificity for DATs was 100% and 83.0% for gel, and 50.7% and 97.8% for tube, respectively. Based on this study GMA showed to be more sensitive than CTT for detecting potentially significant antibodies coating red blood cells in vivo. J. Clin. Lab. Anal. 18:255–258, 2004.

Key words: direct antiglobulin test; conventional tube test; gel microcolumn assay; gel test; alloantibodies; elution; clinically significant antibodies

INTRODUCTION

The DAT is one of the most important diagnostic tools used to determine if red cells have been coated in vivo with immunoglobulin, complement, or both. The principle of the test described by Moreschi in 1908 (1) was rediscovered and introduced into clinical medicine by Coombs et al. in 1945 (2,3). The history of the antiglobulin test has been reviewed by R.R.A. Coombs himself in 1998 (4).

A positive DAT may result from several conditions, such as autoantibodies directed to intrinsic red cell antigens, alloantibodies reacting with red cells recently transfused, antiphospholipid antibodies and maternal alloantibodies that cross the placenta and coat fetal red cells (5,6). Patients with hypergammaglobulinemia and recipients of high-dose intravenous gammaglobulin and drugs can also present positive DATs (7). Drug-induced DATs can be caused by at least three different mechanisms: adsorption, immunecomplex formation, or the production of an antibody against drugs (8).

The DAT can be performed by tube, GMA, column agglutination technology, an enzyme-linked antiglobulin test, and other techniques (9,10). Most DATs are routinely performed by conventional tube technique with a polyspecific reagent capable of detecting both IgG and C3d. If positive, tests with more specific anti-IgG and anti-complement reagents may be appropriate (11).

GMA is a modified serological technique that has been used for ABO and Rh typing, DATs, detecting alloantibodies, red cell phenotyping, and other applications (12,13). This technique has been found to be sensitive and easy to perform and may allow the reliable detection of antigen–antibody reactions (14,15). However, for DAT, the GMA role is controversial. Some authors concluded that this technique is equal to or better than the CTT (9). On the other hand, Tissot

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et al. (16) concluded that the GMA appeared to be less sensitive than the CTT when utilized for DATs.

The purpose of this study was to compare, in a large number of samples, the performance of CTT to GMA for the detection of potentially significant unexpected antibodies coating red blood cells in vivo.

MATERIALS AND METHODS

From January 1996 to May 2002, DATs were performed in parallel by GMA and CTT on 9,862 patient blood samples referred to our immunohematology reference laboratory excluding neonatal ABO incompatibilities. All blood samples were separated from plasma by centrifugation and tested within 24 hr of collection. Elution studies were performed in all positive DAT samples. Eluates were tested for antibody identification by GMA.

DATs by CTT

The DAT was performed in a standard glass tube method (10×75 mL). Red blood cells were washed three times in 0.9% saline (Halex Istar Ltda., Goiania, Brazil) and suspended to a final 3–5% suspension. A total of 50 μl of washed red blood cells suspension were added to 100 μl of anti-IgG (monospecific rabbit anti-human; ASEM-NPBI Produtos Hospitalares Ltda., Sao Paulo, Brazil) and to 100 μl of polyspecific antiglobulin reagent (monoclonal rabbit anti-human IgG and C3d; DiaMed-Latino America, Lagoa Santa, Brazil) separately. Following homogenization, the tubes were centrifuged for 15 sec at 3,400 rpm and observed for agglutination and/or hemolysis. Positive reactions were graded from 1+ to 4+ according to the American Association of Blood Banks Technical Manual (5). All negative results were controlled by adding IgG-coated red blood cells (Controcel; ASEM-NPBI Produtos Hospitalares Ltda., Brazil).

DATs by GMA Method

The patient's red blood cells were washed three times in 0.9% saline and suspended in a low ionic strength solution (ID-Diluent 2; DiaMed-Latino America, Brazil) to a final 0.8% suspension, using 1 mL of ID-Diluent 2 and 10 μl of red blood cells. In a microtube of the LISS/Coombs ID-card (monoclonal rabbit anti-human IgG and anti-C3d; DiaMed-Latino America, Brazil), 50 μl of 0.8% patient's red blood cells were dispensed and centrifuged for 10 min at 895 ± 25 rpm in an appropriate centrifuge (ID-Centrifuge 24S; DiaMed AG, Cressier sur Morat, Switzerland). Following centrifugation, the cards were examined for agglutination and/or hemolysis. All positive result samples in LISS/Coombs cards were then tested in anti-IgG, -IgA, -IgM, -C3c, and -C3d ID-cards (monoclonal DC Screening I; DiaMed AG, Cressier sur Morat, Switzerland) using 50μl of 0.8% patient's red blood cells in each microtube containing anti-IgG, -IgA, -IgM, -C3c, -C3d, and control. After centrifugation as described above, the cards were observed for agglutination and/or hemolysis. Positive reactions were graded from 1+ to 4+ according to manufacturer's instructions. In our laboratory, all reactions in gel were read by examining the front and the back of the cards.

Elution Tests

Eluates were obtained by acid elution using a commercial kit (Elu-Kit II; Gamma Biologicals, Inc., Houston, TX) according to the package insert. Eluates were tested against screening red blood cell reagents (ID-DiaCell I+II+III; DiaMed-Latino America, Brazil). In addition, an autocontrol was prepared using 10 μl of three times washed patient's cells and 1 mL of Diluent 2.

Antibody Identification in Eluates

Identification of antibodies was performed in all positive eluates by GMA (15). Briefly, 25μl of eluate and 50 μl of each 0.8% reagent red blood cells panel containing 11 vials (ID-DiaPanel; DiaMed Latino America, Brazil) suspended in Diluent 2 were dispensed in corresponding microtubes of LISS/Coombs cards and incubated 15 min at 37°C. The cards were then centrifuged for 10 min at 895 ± 25 rpm in an appropriate centrifuge. Following centrifugation, reactions in gel were observed for agglutination and/or hemolysis. Positive reactions were graded from 1+ to 4+ according to manufacturer's instructions.

Statistical Analysis

Statistical analysis of test data was performed using computer software (SAS 6.12, SAS Institute, Cary, NC). $P < 0.05$ was considered significant.

RESULTS

Results of GMA and CTT for DATs on 9,862 samples are shown in Table 1. In this series, we found 3,363 positive DATs (34.1%). Of these, only 1,084 (32.2%; $\chi < 0.001$) showed concordant positive results by CTT and GMA. All nonconcordant results were due to a positivity in gel. In contrast, we found no sample positive only by CTT. A total of 6,699 samples were negative (67.9%) by both methods. Therefore, the sensitivity and specificity of the GMA for DATs was 100% (IC = 99.8 to 100.0) and 83.0% (IC = 82.2 to 83.8), respectively. For CTT we found sensitivity of

TABLE 1. Results of a comparative study between GMA and the tube test technique for DAT*

	Tube technique		Total
Gel test	+	–	
+	1,084	2,079	3,163
–	0	6,699	6,699
Total	1,084	8,778	9,862

Observed agrement: 32.2% ($\chi < 0.001$).

50.7% (IC = 48.3 to 53.0) and specificity of 97.8% (IC = 97.5 to 98.1). The predictive value for a positive reaction was 56.7% (IC = 54.9 to 58.4) for gel and 83.9% (IC = 81.6 to 86.0) for tube, and the negative predictive value was 100% (IC = 99.9 to 100) for gel and 89.9% (IC = 89.2 to 90.6) for tube. For DAT, the efficiency of CTT was 82.6% and 89.2% for GMA.

Antibody identification was performed after acid elution testing on 3,084 out of 3,163 blood samples with positive DATs and yielding a positive antibody screening. The remaining 79 samples were positive only in C3d. We identified 678 (22%) antibodies of potential significance with respect to transfusion management, and 1,012 (32.8%) were considered harmless with respect to transfusion of incompatible blood (auto-IgG) (17,18). In two concordant DAT results, we identified anti-D in tube and anti-D and anti-C by gel. In other samples, we found anti-D and anti-C by tube and also anti-K by gel. From 2,079 nonconcordant DAT results, 357 (17.2%) potentially significant antibodies were only identified by GMA on eluates. Details of our findings (antibodies only identified by gel) are summarized in Table 2. It is of note that we identified 271 out of 357 samples with antibodies directed against the Rh system only or associated with antibodies against other systems. Specificity value for antibody identification in gel was 84.9% and positive predictive value of 72.1% on eluates.

TABLE 2. Antibodies identified only by GMA on eluates

Blood group system	n	Antibody specificity	n
Rh system	271	Anti-D	112
		Anti-D/-C	21
		Anti-D + other	06
		Anti-C	04
		Anti-C/-E	01
		Anti-C + other	08
		Anti-E	38
		Anti-E/-K	05
		Anti-E + other	09
		Anti-e	27
		Anti-e/-C	03
		Anti-c	33
		Anti-c/-E	03
		Anti-C^w	01
Kell system	33	Anti-K	32
		Anti-Kp^b	01
Kidd system	30	Anti-Jk^a	21
		Anti-Jk^a/-Fy^a	01
		Anti-Jk^b	06
		Anti-Jk^b + other	02
Duffy system	18	Anti-Fy^a	13
		Anti-Fy^a/-S	01
		Anti-Fy^b	04
MNS system	04	Anti-S	03
		Anti-s	01
Diego system	01	Anti-Di^a	01
–	448	Auto-IgG	
Negative	1195		

DISCUSSION

The reported incidence of positive DATs by CTT in healthy donors and hospitalized patients varies from 3.5% (19) to 7% (20). In our study, the incidence of positive DATs in patients was 11% for tube and 34.1% for GMA. This data can be explained because in our study the blood samples were previously tested in routine laboratories and transfusion services and referred to our immunohematology reference laboratory for further investigation.

We found that the sensitivity of the GMA is superior to the CTT in the detection of potential significant antibodies coating red blood cells in vivo ($P < 0.01$). This is consistent with several other studies that have found the DATs by GMA to be more sensitive than those tests performed by CTT at detecting red blood cell-bound IgG (9,21,22). Also, contrary to the results reported by Tissot et al. (16), gel showed to be more sensitive at detecting C3d on red blood cells in our study. Moreover, the results of efficiency test calculations confirm that gel is superior to tube for DATs.

In blood donors, GMA showed to be more sensitive than CTT for DATs (23). Other studies reported an incidence of positive DATs in healthy individuals using CTT varying from 1 per 7,000 (6) to 1 per 13,000 (24). Despite the fact that gel detected IgG on a larger proportion of patient's samples than the tube, we found false–positive results in 1,195 samples (57.5%) and in 448 samples (21.5%) we detected auto-IgG.

It was not possible to explain all false–positive results by GMA, but it may be due in part to the diluent used, as false–positive reactivity due to the low-ionic wash solution used with commercial acid-elution kits has already been reported (25).

However, 357 (17.2%) potential significant antibodies on eluate were not detected by CTT. From these, 271 antibodies have shown specificity toward the Rh system. This result is relevant since it may help in the diagnosis of hemolytic disease in newborns, or transfusion reactions that could not be confirmed if only the tube test technique had been used.

Our findings for DATs were in accordance with those already reported for alloantibodies identification in serum, which have demonstrated that gel is more sensitive than tube at detecting IgG antibodies (15,26), although nonspecific positive results were found in 12.11% of the samples tested.

Based on this study, we concluded that a predictive value for a positive reaction is greater for tube, and a predictive value for a negative reaction is greater for GMA. This data may help when interpreting DAT results. The overall test result calculations corroborate that GMA is a valuable tool for DAT investigation.

Finally, the results of this large and prospective study confirm that GMA is more sensitive than tube technique for detecting potentially significant antibodies coating red blood cells in vivo. We have also demonstrated that elution studies performed by GMA identified an expressive number of potentially significant antibodies, however false–positive results are a limitation of this method for DATs. Further studies are underway to to determine if there is a better diluent for use in GMA as well as the best elution method for GMA.

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