The sensitivity, specificity, and clinical relevance of gel versus tube DATs in the clinical immunology laboratory

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The DAT is a test used to demonstrate in vivo antibody and/or complement coating of RBCs. Typically, the DAT is performed in test tubes; however, recently a number of commercially available tests using gel-filled microtubes have become available. Few data comparing the sensitivity of these test media are available. To compare the rate of detection of a positive DAT performed in test tubes versus in gel-filled microtubes and to assess the clinical significance of the results in patients undergoing evaluation of anemia, we tested 310 consecutive EDTA-anticoagulated blood samples from adult patients. The samples were analyzed using both the conventional tube technique and a gel-based assay (DiaMed®; Cressier sur Morat, Switzerland). Test results were expressed as either positive or negative. When a positive result by either technique was encountered, the treating physician was interviewed to determine whether the result warranted further patient investigation or treatment. In 268 out of 310 cases the DAT was negative by both methods. Of the 42 patients with a positive DAT, the test was positive by both methods in 18 patients. In the remaining 24 cases the DAT was positive by the gel test only. In all cases positive by both techniques the test result affected patient management. Of the 24 cases that were positive only by gel test, 3 were judged to be clinically significant. In this study, the gel test was more sensitive than the tube technique for performance of the DAT. However, the clinical significance of a DAT positive only by a gel test is doubtful. We believe that use of the gel-based DAT should be more extensively evaluated before it is adopted as a standard technique in general clinical laboratory practice. ***Immunohematology*** **2004;20:118–121.**

Key Words: gel-based DAT test, tube DAT technique, clinical significance of a positive DAT

The DAT is used to demonstrate in vivo antibody and/or complement coating of RBCs. The principle of this test was first demonstrated in 1908 when rabbit erythrocytes, sensitized by goat anti-rabbit serum, were stongly agglutinated by the subsequent addition of rabbit anti-goat serum. However, it was not until 1945, when Coombs showed that blood group antibodies could be demonstrated in serum or on sensitized RBCs, that the test was adopted by clinical laboratories.[1] The DAT is an essential test in the evaluation of autoimmune hemolytic anemia (AIHA). The DAT also has importance in the blood bank, where it is performed as part of the evaluation of hemolytic transfusion reactions.

Typically, the DAT is performed in test tubes; however, recently a number of commercially available tests using gel-filled microtubes have become available.[2] Only limited data comparing the sensitivity of these test media and their clinical relevance are available.[3,4] In this study, we compare the rate of detection of a positive DAT performed in test tubes versus in gel-filled microtubes and assess the clinical significance of the results in patients undergoing evaluation of anemia.

Materials and Methods

Patients and samples

We studied 310 consecutive blood samples sent to our hospital's Clinical Immunology laboratory for the DAT. The samples were collected in EDTA-containing tubes (Becton Dickinson and Co., UK) and were analyzed, using both the conventional tube technique and a gel-based assay, on the day of collection.

DAT—tube technique

DATs were performed by the classic tube technique using polyspecific antihuman globulin (AHG) serum (anti-IgG + anti-C3d; Gamma Biologicals, Houston, Texas). In this technique, RBCs were washed × 3 using NaCl 0.9% and resuspended to a 3–5% saline suspension. Two drops of polyspecific AHG were added to 1 drop of the washed cells, centrifuged for 1 minute at 900 × g, and examined macroscopically for agglutination. Positive samples were further tested

with monospecific anti-IgG (Gamma Biologicals) and anti-IgA, -IgM, -C3c, and -C3d (DiaMed AG, Cressier sur Morat, Switzerland). Agglutination reactions were graded strongly positive (4+ and 3+), moderately positive (2+ and 1+), and weakly positive (W+), according to the manufacturer's directions.

DAT—gel technique

Gel tests were performed by adding 50 µL of a 0.8% RBC suspension in LISS (ID-Diluent 2) to the top of each microtube in a LISS/Coombs ID card (DiaMed AG). The cards were centrifuged at 910 rpm for 10 minutes, using the ID-Centrifuge 24S. All positive results (presence of agglutinated RBCs in the gel matrix) were re-examined using rabbit monospecific anti-IgG, -IgM, and -C3d (DC-screening monospecific Coombs sera, DiaMed AG). Negative reactions appeared as a discrete cell button at the base of the column.

Further investigation of positive DAT tests

When a positive DAT result was obtained by either the tube or the gel technique, the treating physician was interviewed by one of the investigators to determine the patient's diagnosis and whether the result warranted further patient investigation or treatment. In addition, laboratory data pertinent to possible hemolysis were abstracted from the patients' charts (serum bilirubin, lactate dehydrogenase [LDH], and, when available, haptoglobin levels and reticulocyte count). An isolated elevation of LDH was not considered to be indicative of hemolysis because of its lack of specificity for this diagnosis.

Statistical analysis

The sensitivity and specificity of the gel technique was calculated, using a result of the tube test, to be either truly positive or negative. From these calculations, the positive and negative predictive values were determined.

Results

Three hundred and ten samples were analyzed. Of these, 268 (86%) were negative by both tube and gel testing and 42 (14%) were postitive by at least one test technique. Of the 42 positive samples, 21 (50%) were positive by gel and negative by tube testing, 3 samples (7%) were positive by tube testing and negative by gel testing, and 18 (43%) were positive by both tube and gel testing (Table 1).

Table 1. Number of positive and negative DATs (using polyspecific AHG) tests by tube and gel testing

		Tube tests		
		Positive	Negative	Total
Gel tests	Positive	18	21	39
	Negative	3	268	271
	Total	21	289	310

Of the 18 samples that were positive using both techniques, the IgG test only was positive in 11, while in 7 samples both IgG and C3 were found on the RBCs. No samples were positive for C3 only. Identical results were obtained in tube and gel tests. Of the 21 samples positive using gel testing only, 20 were positive for IgG and 1 was positive for C3 only.

The sensitivity of the gel test compared to the tube test using a polyspecific AHG reagent was 85 percent and its specificity was 93 percent. When a monoclonal IgG reagent was used, the sensitivity and specificity were 95 percent and 93 percent, respectively. The positive predictive value (PPV) of a positive gel test using the polyspecific reagent was only 46 percent while the negative predictive value (NPV) was 99 percent. For the monoclonal IgG reagent, the PPV and NPV were 47 percent and 99 percent, respectively.

Clinical features of patients with a positive DAT

The clinical diagnoses of the patients with a positive DAT by both gel and tube tests are listed in Table 2. Of note is that in patients with a positive DAT by tube and gel testing, the clinical diagnosis of most of the patients is one known to be associated with a positive DAT.

Table 2. Clinical diagnoses of patients with positive DATs by both tube and gel techniques

Number of Patients	Diagnosis
7	Chronic lymphocytic leukemia
3	Systemic lupus erythematosus
3	Immune thrombocytopenic purpura
1	Non-Hodgkins lymphoma
1	Monoclonal gammopathy
1	Hypersplenism, Hepatitis C positive
2	Unknown

Laboratory parameters of patients with a positive DAT

Of the 18 patients with a positive DAT by tube and gel testing, 8 (44%) had at least 1 laboratory marker of hemolysis. However, of the 21 patients with a positive gel test only, 2 patients (9%) had positive hemolytic

parameters while none of the patients with a tube-test-only DAT had abnormal markers of hemolysis.

Effect of a positive DAT on further patient investigation or treatment

In 94 percent of the 18 patients with a positive DAT by tube and gel testing, the result elicited further diagnostic or therapeutic action, while an isolated positive gel test led to further diagnostic or therapeutic activity in only 9 percent of cases. None of the patients with a tube-test-only DAT had further tests performed nor received any treatment based on the result of the test.

Discussion

The use of gel-based microcolumn tests was introduced into the blood bank laboratory at the end of the 1980s for blood typing, antibody detection, and DATs.[5,6] Gel tests have grown in popularity because of their increased accuracy and ease of use compared to classic tube tests. Furthermore, gel tests require smaller samples of RBCs and serum for testing, a distinct advantage when testing newborn and premature infants. Gel test systems also decrease the exposure of laboratory staff to potentially hazardous blood samples and breakable glassware. Finally, the test result obtained using gel test cards remains stable for up to 48 hours and can thus be saved for comparison or consultation after the test has been performed.

A number of studies have been published demonstrating the increased sensitivity of the gel test compared to tube testing in detecting RBC alloantibodies in patients' sera.[7,8] This increased sensitivity permits the detection of alloantibodies that would otherwise have remained undiagnosed, and has been shown to have definite clinical value in preventing potential hemolytic transfusion reactions in a number of cases.[9,10]

Gel testing for the DAT compared to tube testing has been studied to a lesser extent, and the clinical relevance of a positive DAT by gel test is unknown.[3,4,11] We performed the current study to determine the sensitivity, specificity, and positive and negative predictive values of one gel test (DiaMed) compared to traditional tube testing, which until now has been considered the standard test system. We also sought to assess the immediate clinical relevance of a DAT positive by either technique by determining the treating physicians' response to the test result in terms of ordering further diagnostic tests or instituting therapy based on the test result. Our results show that of 42 samples positive by either technique using polyspecific antihuman globulin, 18 (43%) were positive by tube and gel testing, while 21 (50%) were positive by gel testing only. Three samples (17%) were positive by tube testing and negative by the gel test. Thus the sensitivity of the gel test was 86 percent. When monospecific reagents (anti-IgG and anti-C3) were used, the sensitivity increased to 95 percent for IgG and decreased to 78 percent for C3. These results are similar to those of Tissot et al.,[12] except that in their study the sensitivity of the gel test using anti-C3 was only 16 percent. This may be significant because it is known that in patients with AIHA, hemolysis is more severe when both C3 and IgG are present on the RBCs.

Our study has demonstrated that the gel test is highly specific compared to the tube test: 93 percent using polyspecific and anti-IgG reagents, and 99 percent using anti-C3. The results are concordant with those of Tissot et al.,[12] but differ from those of Nathanlang et al.[3] The latter group compared gel and tube DAT tests in newborns with suspected fetal-maternal ABO incompatibility and in adults with known AIHA and found a sensitivity of 94 percent and a specificity of 85 percent. When only the adults in the study are considered, the sensitivity and specificity were 100 percent and 80 percent, respectively. These differences may be accounted for by the fact that Nathanlang et al. studied a different patient population than ours: all of their adult patients had known AIHA and thus would be more likely to have a positive DAT by any technique, compared to our patients, who were undergoing an evaluation for anemia whose cause was undetermined at the time of DAT testing. This difference in patient population also explains the lower PPV of the gel test in our study (46%) compared to 94 percent among the adult patients in their study.

When we analyzed the clinical relevance of the positive DAT, we noted that in 17 of the 18 patients (94%) with a positive DAT by both techniques, the result impacted clinical decision making. By contrast, in only 2 of the 21 patients (9%) with a positive DAT by gel testing only did the result lead to further relevant clinical activity. A similar trend was found when laboratory markers of hemolysis were examined in patients with a positive DAT. When the DAT was positive by both techniques, hemolytic parameters were observed in 74 percent of patients, while only 9 percent of patients with a gel-only positive DAT had other laboratory evidence of hemolysis.

These results suggest that the gel test is more sensitive than the conventional tube test for performance of the DAT. The relatively low number of patients with a gel-only positive test having clinical or laboratory evidence for hemolysis suggests a high falsely positive rate for the gel test. However, we cannot exclude the possibility that some patients in our study who had a positive gel test may develop hemolytic anemia or another disease related to a positive DAT in the future. Furthermore, in this study we did not take into account the strength of agglutination obtained in the gel test. This was because, at the time that this study was performed, our hospital's immunology laboratory reported DAT test results as "positive" or "negative" and we postulated that treating physicians were likely to make clinical judgements on this basis without considering the strength of a positive test result.

In conclusion, we believe that aspects of the gel-based DAT, such as the correlation of strength of agglutination with evidence for hemolysis, should be more extensively evaluated before it is adopted as a standard technique in place of conventional tube testing in general clinical laboratory practice.

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