Evaluation of the Use of Gel Card System for Assessment of Direct Coombs Test: Weighing the Pros and Cons

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Abstract A positive direct Coombs test (DCT) is the hallmark of diagnosis of immune hemolytic anemias. The reagent used for the test is the Antihuman globulin (AHG), which may be either 'Polyspecific' or 'Monospecific'. The advent of the Gel card systems has made the procedure and interpretation of DCT simpler. Aim of this study is to evaluate three of the various techniques used for the performance and interpretation of DCT. A total of 96 EDTA samples were included in the study. DCT was performed by (i) polyspecific AHG manual tube method (ii) polyspecific AHG Gel card method and (iii) monospecific AHG (Anti IgG and Anti Complement) manual tube method. In our study we considered positivity by monospecific AHG as the standard for diagnosis. Of the total 96 samples evaluated, 44 cases positive by Gel card method, were also positive for either one or both the monospecific AHG reagents. 17 cases positive by Gel card were negative by all manual methods. These false positive cases were attributed to reasons such as increased ESR, macrocytosis and marked leucocytosis. Nine cases were negative by Gel card but were positive with the Monospecific AHG. The sensitivity of DCT done by the Gel card technique was 83.01% and the specificity was 60.46%. Use of Gel card technique to perform and interpret DCT is easier than manual tube methods, but positivity by Gel card needs to be correlated with clinical presentation of the patient and other laboratory findings. Monospecific antisera can be used to confirm cases that are positive by the Gel card systems.

Keywords Coombs test · Gel card · Monospecific antisera

Introduction

The Direct Coombs/Antiglobulin test (DCT) was originally described by Coombs, Moorant and Race in 1945 [1]. The principle of the test is to demonstrate antibodies or complement coating red cells *in vivo* by using Antihuman globulin (AHG) or Coombs reagent. Till date it remains the hallmark for the diagnosis of immune hemolytic anemias. Technically various modifications in the procedure have been described to bring about added sensitivity to the DCT. Some of these changes include; use of more specific reagents like *monospecific AHGs* (e.g. against IgG or C3) and *Enzyme elution* of the antibodies from the red cells. In practice, it was noted that DCT required technical expertise (esp steps of washing the red cells, effective centrifugation etc.) for proper interpretation. In 1990 Lappierre introduced the novel 'GEL' method to demonstrate antibodies or complement coated red cells [2]. The gel system is based on the principle that the Sephadex gel matrix acts as a sieve, through which agglutinates of RBCs are too large to pass through and remain entrapped in the gel, depending on their size. A negative reaction is seen as a clear pellet of cells settled at the bottom of the microtube. Grading of the reactions can be done according to the distribution of RBCs agglutinates through the gel column. This technique was easier to perform and did not require technical skill, thereby overcoming the practical difficulties of performing DCTs.

In this context we have tried to evaluate three of the methods used for the interpretation of DCT.

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Aim of the Study

To evaluate three of the various techniques used for the performance and interpretation of DCT.

Materials and Methods

A total of 96 EDTA samples were included in the study. These samples were referred to our Blood bank for evaluation of possible immune hemolytic etiology. Neonatal samples sent for hemolytic disease due Rh, ABO incompatibility were excluded.

All samples included were subject to DCT by the following techniques:

1. Gel card technique using polyspecific Anti Human Globulin (AHG) (GTP)

Gel card system used was Diamed Id microtyping system containing polyspecific Antihuman globulin with Anti IgG, IgA, IgM, C3c, C3d activity.

2. Conventional tube technique using polyspecific AHG (CTTp)

Reagent used was Bioclone with Anti IgG–C3d manufactured by Ortho Clinical diagnostics

3. Conventional tube technique using monospecific AHG (CTTm) against IgG and C3d

Reagents used were Eryclone (AntiC3d) and Erybank (AntiIgG) manufactured by Tulip diagnostics P Ltd.

Analysis and Statistics

Considering the DCT performed by the monospecific AHG method to be the standard, the results were analyzed and the sensitivity and specificity of the GT was calculated.

Results

Of the 96 samples evaluated; 61 were found to be positive and 35 were negative by GT. These samples were also evaluated with CTTm using Anti IgG and Anti C3d. A total of 53 cases showed positivity with the monospecific AHG (26 for IgG, six for C3 and 21 showing both IgG and C3 positivity). Forty-three cases were negative for both the mono specific AHG. With the CTTp method, 36 cases were positive and 60 were negative. Seventeen cases positive by GT were negative by all manual methods (Table 1).

Of the 61 GT positive cases evaluated, 44 showed consistent positivity with monospecific antisera (CTTm),

while nine of the 35 GT negative cases, were positive by CTTm (seven by IgG and two by C3).

Using the polyspecific AHG for the CTT (CTTp), we found only 33 of the 61 cases showing consistency with the GT positivity. On the other hand, CTTp positivity was noted in three of 35 cases that were negative for GT. When comparing CTTp with CTTm, we found, of the 36 CTTp positive cases, 35 were also positive by CTTm. Of the 60 cases negative by CTTp, 18 were positive by CTTm.

Sensitivity and Specificity

As mentioned positivity by monospecific antisera was considered gold standard and the results were evaluated. With the Gel card the sensitivity was found to be 83.01 and the specificity was 60.46. On the other hand, using the tube technique and polyspecific antisera we found lower sensitivity (66.03), but a high specificity (97.67).

Discussion

The DCT is a diagnostic procedure used to demonstrate in vivo coating of red cells by antibodies and or complement. As mentioned before, the Gel technique has been introduced to simplify the technique and interpretation. It was initially introduced in Thailand in 1993, and has now become popular in several blood banks [3]. Many studies including an Indian study done in Pune, have proved the advantages of using the Gel card technique for DCT and Indirect Coombs Test including, simplicity, reliability, reproducibility, stability and increased sensitivity [4]. The microtubes can be prepared with standard reagents for DCT. The gel card systems have now been adapted and widely used for antigen detection, alloantibody screening/identification and cross matching [3, 5, 6].

At present, majority of blood bank personnel perform the DCT by the conventional tube technique. The main problem with the CTT is that it requires skilled technical expertise not only in the procedure especially the step of washing, but also in the interpretation. For example; inappropriate washing can lead to false positive results while weak agglutination reactions by CTT require a skilled technician for correct interpretation [7]. Comparison between the GT and CTT have been evaluated by other authors.

Tissot et al. [8] evaluated 398 samples of which 185 were positive and 213 negative by GT. In their study CTT showed concomitant positivity in 178 cases with seven being false positive. The sensitivity of GT was 75.4 and specificity 95.8 (Fig. 1). These cases were further evaluated with monospecific antisera. Unlike the index study,

Table 1 Results of the 96 cases evaluated

	Cases	CTTp +ve	CTTm IgG +ve A	CTTm C3d +ve B	CTTm IgG and C3d +ve C	Total of A + B + C	CTTm IgG and C3d –ve
GTp +ve	61	33	19	4	21	44	17
GTp –ve	35	3 ^a	7	2	0	9	26
Total	96	33	26	6	21	53	43

^a Refer text **Fig. 1** Comparison of sensitivity and specificity of GT in various studies

the monospecific reagent reactions were also evaluated with GT i.e., GT with anti IgG, and GT with anti C3d.

In the context of monospecific AHG, they found the sensitivity of GT with only IgG increased to 96.8%. The drawback was C3d coated red cell detection was clearly much lower than the CTT (Sensitivity of GT with C3d: 16.3%) [8]. It has been highlighted up to 15% of autoimmune hemolytic anemias may have only the complement component on their red cells [9]. In addition up to one-third of the autoimmune hemolytic anemias have C3 on the erythrocytes in addition to either IgG, IgA, IgM or a mixture of Igs of different isotypes [10]. Further, it has been shown that cells expressing both complement receptor type 3 and Fcγ RIIB receptor mediate antibody-dependent red blood cell phagocytosis [11–13]. Thus the presence of both IgG and C3 on red cells may be important with regard to in vivo red cell destruction. In their series, Tissot et al. [8] found that the presence of C3d was frequently missed with the gel test.

The use of CTT for evaluation with monospecific antisera, in the present study was undertaken to overcome the causes of false positivity due to the physical properties of the Gel.

In another study conducted in Thailand by Nathalang et al. [14], 23 cases of autoimmune hemolytic anemias

and 52 new borns with hyperbilirubinemia were evaluated. The overall sensitivity of the GT was 93.5% and specificity was 88.6% (Fig. 1). They found GT to be very useful especially in AIHA patients (sensitivity 100%, specificity 80%).

Fabijanska-Mittek J in their study on autoimmune hemolytic anemias evaluated the GT and CTT techniques with enzyme linked antiglobulin test (ELAT) as the gold standard. They also found GT to give false positive results (18.8%) due to causes like hypergammaglobulinemia. They recommended GT as a good screening test [15]. In another study by the same author, the gel test was adapted to identify the subclass of IgG sensitizing the red cells in autoimmune hemolytic anemias. They could identify IgG subclass antibodies in all patients, including those who were ‘Coombs negative’, with less than 200 IgG molecules bound in vivo per red cell. The most frequent sensitization was by the IgG1 subclass [16].

The benefits of gel technology are multifold. The technique is quicker, safer and tech-friendly. It decreases handling of samples and technical/interpretive errors, as it does not require special skills for performance. Washing, though a simple procedure, is the most crucial step for an accurate DCT interpretation. By using Gel cards washing is not required. In addition, there is elimination of technique dependent steps and reduction in protocol errors. It provides a clear cut grading system (4+, 3+, 2+, 1+) with stability of agglutinates for up to 1 week which ensures uniform interpretation by users. The photographic recording of reactions may be useful in medico legal issues. A high sensitivity especially with IgG coated cells [8, 16] makes it a better technique in comparison with CTT.

However, the gel technology also has many drawbacks. Using this novel technique adds to the cost of a simple test. The reporting personnel must be fully aware of the possibility of false positive reactions due to reasons like macrocytosis, marked leucocytosis and increased ESR and hence test results must be interpreted with caution and clinical correlation. In addition, the possibility of missing C3d coated red cells when using the Gel cards must be borne in mind [8].

Conclusion

The present study found GT is definitely an easier, quicker technique with sensitivity better than CTTp, though the drawback of GT was low specificity. We therefore recommend GT as a good first line test, requiring confirmation with monospecific antisera especially for the diagnosis of immune hemolytic anemias.

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