Conventional Tube Agglutination with Polyethylene Glycol versus Red Cell Affinity Column Technology (ReACT®): A Comparison of Antibody Detection Methods

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Abstract. A procedure for antibody detection and identification that utilizes affinity microcolumns to isolate IgG antibodies in a gel matrix containing Protein G and Protein A was commercially available in recent years. We evaluated this method (ReACT®, Red Cell Affinity Column Technology, Immucor Co., Norcross, GA) as an alternative to standard tube agglutination testing, in an effort to minimize subjectivity and increase consistency of antibody identification in our hospital blood banks. Although the ReACT kit was withdrawn from the market soon after completion of our study, the advantages and limitations of the procedure warrant consideration should a similar product be reintroduced. The performance of the ReACT method was compared to conventional antibody detection by a standard tube agglutination technique that uses polyethylene glycol (PEG) potentiator (Dominion Biologicals Ltd., Dartmouth, Nova Scotia, Canada). Of 685 serum or plasma samples that were screened for antibodies, 96 samples were found by the PEG procedure to contain clinically significant (n = 70) and insignificant antibodies (n = 26). In contrast, 48 of the samples were found by the ReACT procedure to contain clinically significant (n = 39) and clinically insignificant antibodies (n = 9). For the ReACT method, the sensitivity was 48.8% (95% CI = 37.8%, 58.0%) and the specificity was 99.6% (95% CI = 97.5%, 99.9%), compared to the PEG procedure. While the ReACT microcolumn system was designed to limit detection of clinically insignificant antibodies, this study documents a loss of sensitivity for detection of clinically significant antibodies. (received 1 November 2002; accepted 12 November 2002)

Keywords: alloantibody, polyethylene glycol, tube agglutination, microcolumn affinity, transfusion

Introduction

Precise detection and identification of red cell antibodies is of great importance in preventing acute and delayed hemolytic transfusion reactions. The technique that is used has been shown to play a significant role in antibody identification [1]. In recent years, streamlined antibody identification methods have entered the marketplace. One such technology, the ReACT® Red Cell Affinity Column, succeeded in providing a time-efficient process that produced stable, more objective reactions. This microcolumn method employed an immunoreactive matrix that contained Protein G and Protein A. By specifically binding the Fc portion of the IgG molecule, this procedure virtually eliminated the identification of IgM antibodies, which in a great majority of instances are considered clinically insignificant. The ReACT procedure has been compared to other microcolumn techniques and a low-ionic-strength solution (LISS) tube agglutination procedure, yielding comparable results [2]. A comparison of the ReACT technology versus a PEG-based tube agglutination identification procedure has not previously been reported.
Materials and Methods

Antibody screening with subsequent identification of detected antibodies was performed on 685 serum or plasma samples using (a) a standard tube agglutination method with polyethylene glycol (PEG) potentiating agent (Dominion Biologicals, Ltd., Dartmouth, Nova Scotia, Canada) and (b) the ReACT® microcolumn method (Red Cell Affinity Column Technology, Immucor Co., Norcross, GA). Parallel testing of serums by the two procedures was performed during a 4-month period, as patient samples and proficiency survey samples (College of American Pathologists) came into the blood banks of the three hospitals in our system.

For testing by the ReACT method, 1 drop of serum or plasma and 1 drop of LISS-suspended (0.8%) red blood cells (RBCs) were added to each affinity column, which was incubated at 37°C for 15 min, and centrifuged for 3 min. Reactions were read as positive or negative on a 0 to 4+ scale. RBCs within the test microcolumn, which remained above or in the immunoreactive matrix, were considered positive. Tests in which the RBCs collected entirely at the bottom of the matrix were considered negative.

Antibody screening with PEG consisted of mixing 2 drops of sample and 1 drop of 3-5% RBC saline suspension in a tube. Two drops of PEG were added prior to incubation for 15-30 min at 37°C. The cells were washed 3 to 4 times. One to 2 drops of anti-human IgG were then added and the tube was centrifuged. Tube agglutination was graded from 0 to 4+. Guidelines for antibody identification were followed as specified in the American Association of Blood Banks’ Technical Manual [3].

For detected antibodies, the patients’ blood bank and medical records were reviewed for pertinent findings (eg, previously identified antibodies, prior transfusions, pregnancy, and other contributing factors). To verify the immunoglobulin class (IgM versus IgG) of clinically significant antibodies that were identified only by PEG, serum or plasma was sent to Immucor Co. for class determination in all cases where residual samples were available.

Sensitivity and specificity were computed by standard formulas [4], using Computer Programs for Epidemiologic Analysis (PEPI ) Version 2 [5].

Results

Antibody screening procedures (n = 685) were performed in parallel by the standard tube agglutination technique using PEG potentiator and by the ReACT microcolumn system. Ninety-six samples contained clinically significant (n = 70) and insignificant antibodies (n = 26) with the PEG method. The ReACT procedure identified 8 of the 26 clinically insignificant antibodies that were also identified by the PEG technique. One clinically insignificant antibody identified by ReACT was not found with PEG. Of the 70 clinically significant antibodies found by the PEG method, ReACT identified 39 (55%). For the ReACT procedure the sensitivity was 48.8% (95% CI = 37.8% to 58.0%) and specificity 99.6% (95% CI = 97.5% to 99.9%), compared to the PEG procedure.

The 31 clinically significant antibodies that were not identified by the ReACT method included 17 anti-E, 4 anti-K, 2 anti-C, 3 anti-c, 3 anti-Fya, and 2 anti-D antibodies. Six of the 17 anti-E antibodies were identified in multiple samples from a single patient over a 1-month period. Four additional anti-E antibodies were detected in 4 samples collected from 2 different patients over a 1-week period. In these multiple-sample cases, all of the individual samples tested positive for the antibody with PEG, but negative with ReACT. Both techniques identified 3 anti-Fya, 15 anti-E, 12 anti-K, 2 anti-Jka, 4 anti-D, 1 anti-e, 1 anti-c, and 1 warm autoantibody (Table 1). The ReACT method did

Table 1. Number of clinically significant antibodies as identified by the PEG and ReACT procedures.

<table>
<thead>
<tr>
<th>Antibody system</th>
<th>Identified by PEG</th>
<th>Identified by ReACT</th>
<th>Not detected by ReACT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rh</td>
<td>45</td>
<td>21</td>
<td>24</td>
</tr>
<tr>
<td>Kell</td>
<td>16</td>
<td>12</td>
<td>4</td>
</tr>
<tr>
<td>Kidd</td>
<td>2</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Duffy</td>
<td>6</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Warm autoantibody*</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>70</td>
<td>39</td>
<td>31</td>
</tr>
</tbody>
</table>

*Antibody directed at one zone antigen optimally reactive at body temperature.
not detect any clinically significant antibodies that were unidentified by the PEG method. The immunoglobulin class of 3 anti-E antibodies was identified as IgM.

Of the clinically insignificant antibodies, 3 anti-M, 4 anti-Lea, and 1 anti-D (Rh immune globulin) antibodies were identified by both methods. One Lua antibody was identified by ReACT, but not by PEG. The remaining 18 clinically insignificant antibodies (6 cold autoantibodies, 3 anti-M, 5 anti-Lea, 1 Leb and 3 anti-Sda), were found only by the PEG procedure.

Discussion

Temporal efficiency, stability of reactions, enhanced objectivity, and conservation of sample were clearly evident advantages of the ReACT microcolumn system. Minimizing the time spent to identify cold autoantibodies and other clinically insignificant antibodies is a highly desirable feature of an antibody screening procedure. The ReACT system provided these advantages, compared to the standard tube agglutination procedure using PEG, which is well known to be a highly sensitive technique. In an effort to limit the subjectivity and increase the consistency of antibody identification in our hospital blood banks, the ReACT system was evaluated at three hospitals and briefly implemented at one hospital within our system.

In a previous study, the ReACT kit compared favorably to the LISS and other microcolumn techniques for antibody screening [1], but the present study identified a troubling trend: when compared to PEG, ReACT demonstrated decreased sensitivity for antibodies that have traditionally been considered clinically significant (ie, E, K, C, c, Fya and D). This raised the question of whether these were newly acquired antibodies, which would be primarily IgM and hence less likely to be identified, or IgG antibodies, which should have been detected with ReACT. Three samples had IgM antibodies and the patients’ clinical histories supported recent immunization. For the other samples that were negative with ReACT, the patients’ clinical histories were consistent with remote and in some cases, repeated exposure histories, which suggested that these antibodies were most likely of the IgG class. Despite remote transfusion histories, it remains possible that some of these antibodies were of the IgM class. The clinical importance of antibodies, traditionally considered clinically significant, that are persistently of the IgM class, is to the authors’ knowledge, unclear. At our institution, patients with such antibodies detected with the PEG procedure are given units of blood that are negative for the corresponding antigen.

In 1993, a study by Issitt et al [6] of “enzyme-only” red cell alloantibodies acknowledged that only a few such antibodies are clinically significant; it also demonstrated that the PEG technique was able to detect antibodies, negative with LISS, whose identification before blood transfusion would have been advantageous. They concluded that the minimal additional work involved with PEG testing was acceptable in order to detect rare antibodies that are of clinical importance.

At our institution, based on the comparison of the PEG versus ReACT methods, we concluded the additional work involved with PEG testing is justified to maximize the detection of clinically significant red cell alloantibodies. Accordingly, we discontinued the ReACT procedure at one hospital that briefly utilized it and we opted not to employ the ReACT procedure in our transfusion services.

The manufacturer’s subsequent removal of the ReACT product from the market was due to a patent infringement suit (Product Notification Letter from Immucor Co., 31 January 2001), so the possibility of a similar product entering the market is uncertain. If such a test system becomes available in the future, it should be thoroughly compared with standard techniques to verify satisfactory performance for antibody detection and identification.

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References


