Comparison of tube and gel techniques for antibody identification


There are several methods for antibody detection and each technique has advantages and limitations. We compared the performance of the tube (polyethylene glycol-indirect antiglobulin test [PEG-IAT]) and gel test technique for antibody identification. From January to May 1999, we performed antibody screening tests by gel and tube techniques on 10,123 random blood samples submitted to our reference laboratory. Six hundred and twenty-eight (6.2%) reactive samples were tested for antibody specificity by both methods. One hundred and ninety-six were reactive only by gel: 25 anti-D, 33 anti-C, 76 anti-E, 13 anti-c, 18 anti-K, 7 anti-Jk\textsuperscript{a}, 2 anti-Di\textsuperscript{a}, 3 anti-S, 8 combination Rh antibodies (1 with anti-K), and 6 other antibody specificities. Two samples were reactive only by PEG-IAT: 1 anti-K and 1 anti-Di\textsuperscript{a}. Four hundred and thirty were positive by the two methods: 156 anti-D, 9 anti-C, 68 anti-E, 15 anti-c, 6 anti-e, 61 anti-K, 12 anti-Jk\textsuperscript{a}, 17 anti-Di\textsuperscript{a}, 5 anti-S, 73 combination Rh antibodies (2 with anti-K), and 8 other antibody specificities. Based on this study, the gel test is more sensitive ($p <.01$) than the tube test for identifying potentially clinically significant antibodies. 


Key Words: gel test, antibody detection, PEG-IAT, pretransfusion testing

The gel test has been widely used in blood banking in Europe and Latin America since 1991 for ABO and Rh typing, direct antiglobulin tests (DATs), detecting antibodies, red blood cell phenotyping, and other applications.\textsuperscript{1–4} In 1994, the Food and Drug Administration approved the gel test for use in the United States. Since then, ABO grouping, indirect antiglobulin tests (IATs), and direct antiglobulin test studies have been reported.\textsuperscript{5}

Many substances (albumin, low-ionic-strength saline [LISS], polyethylene glycol [PEG], Polyclon, etc.) have been used for enhancing the detection of antibodies.\textsuperscript{6–9} Some authors suggest that the use of PEG-IAT is equal to or superior to LISS in the detection of potentially clinically significant antibodies. It also has been demonstrated that PEG-IAT reduces detection of insignificant antibodies.\textsuperscript{10–12} The purpose of this study was to compare the gel test and the PEG-IAT in detecting and identifying RBC antibodies in a routine screening procedure.

**Material and Methods**

**Serum samples**

From January to May 1999, antibody screening tests were performed in parallel by the gel test (DiaMed AG, Switzerland) and by the PEG-IAT technique on 10,123 random blood samples submitted to our immunohematology reference laboratory. Blood samples were collected without anticoagulant. The sera were separated from coagulated whole blood by centrifugation and tested within 48 hours of collection.

**Tube testing**

Antibody screening and identification by PEG-IAT were performed (with some modification at 37°C) according to the American Association of Blood Banks Technical Manual,\textsuperscript{13} as follows: 20% PEG was prepared in-house by mixing 20g 3350 mw PEG (Sigma Chemicals, St. Louis, MO) with 100mL PBS, pH 7.3. Red blood cell (RBC) reagents for antibody screening and antibody identification were obtained from Gamma Biologicals (Duet Plus\textsuperscript{TM}, Panel Two\textsuperscript{TM}, Panel 15\textsuperscript{TM}; Houston, TX). Two volumes (100μL) of serum and one volume (50μL) of reagent RBCs were mixed, centrifuged immediately, and observed for agglutination and hemolysis. Four volumes (200 μL) of 20% PEG were then added to the tubes, followed by incubation for 15 minutes at 37°C. The tubes were washed x 3 with saline and two volumes of anti-IgG (Biostest S.A., Brazil) were added. Following centrifugation, the tubes were observed for agglutination and hemolysis. Reactions were read macroscopically and graded as previously described.\textsuperscript{13} All negative antiglobulin tests were verified with IgG-coated RBCs (Gamma Biologicals).
Gel test method

The same RBC reagents used in the tube testing were used in the gel test. Anti-IgG cards, Diluent 2, appropriate incubators, and centrifuges were obtained from the manufacturer (DiaMed-ID, Brazil) and the gel tests were performed using the anti-IgG card.

Reagent RBCs were washed 3 with saline and suspended in LISS to a final 0.8% suspension, using 1 mL of LISS (Diluent 2) and 10 μL of RBCs. In addition, an autocontrol was prepared using 10 μL of x 3 washed patient’s cells and 1 mL of LISS (Diluent 2). Antibody detection and identification were performed as recommended by the manufacturer’s instructions. Briefly, 25 μL of serum and 50 μL of 0.8% reagent RBCs suspended in a LISS diluent were dispensed in a microtube in the anti-IgG card and incubated 15 minutes at 37°C. The cards were centrifuged for 10 minutes at 895 ± 25 rpm in an appropriate centrifuge. Following centrifugation, the cards were observed for agglutination and hemolysis. Positive reactions were graded from 1+ to 4+ according to the manufacturer’s guidelines.

In our laboratory, reactions in gel were read by examining the front and the back of the cards. Doubtful reactions were evaluated using a handheld magnification lens. For each antibody identified, the patient’s RBCs were tested for the matching antigen by the tube or gel method.

Results by gel were compared with those by PEG-IAT. Discrepant results were evaluated by repeating both methods. Whenever potentially significant antibodies were missed by gel, additional tests were performed using LISS additives and adsorption-elution methods. In addition, confirmatory tests using at least three negative and three positive RBCs were performed by the same method used for antibody identification. Potentially clinical antibodies were defined based on their specificity according to the expected reactivity and clinical significance as reported elsewhere.13

Statistical analysis was performed as described elsewhere.14

Results

Antibody detection

Parallel antibody detection was performed on 10,123 blood samples, resulting in 628 (6.2%) positive results by PEG-IAT, gel, or both. Four hundred and thirty (4.24%) samples were positive by both methods. One hundred and ninety-six (1.93%) samples were positive only with gel, and 2 (0.02%) only in PEG-IAT (Table 1).

Antibody identification

Antibody identification was performed on all 628 blood samples yielding a positive antibody screen. Eighty-one blood samples showed more than one potentially clinically significant antibody. Fourteen antibodies (anti-Le a, -Leb, -P1, and cold agglutinins) that are usually considered not clinically relevant with regard to RBC transfusion were detected; six of them were found only in the gel test (Table 1).

One hundred ninety potentially clinically significant antibodies were identified only by the gel test in 190 patients. In contrast, only two potentially clinically significant antibodies (1 anti-K and 1 anti-DiP) not detected in gel were identified by PEG-IAT in two patients.

Additional tests performed using LISS additives on the two samples with antibodies missed by gel test confirmed PEG-IAT results. Four samples (0.65%) with weak positive results in the antibody detection test gave negative results in at least one RBC identification panel when the gel cards were observed by reading the front of the cards but were positive when the back of the cards were read. Those four cases were three anti-K and one anti-DiP. All four were confirmed reading the front of the gel cards when the blood samples were retested.

No positive reaction in gel was detected only by a magnification lens. Therefore, its use was not helpful in this study.

Table 1. Comparative study: PEG-IAT vs. the gel test

<table>
<thead>
<tr>
<th>Antibody (number)</th>
<th>PEG+ / Gel+</th>
<th>PEG+ / Gel−</th>
<th>PEG− / Gel+</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-D (181)</td>
<td>156</td>
<td>0</td>
<td>25</td>
</tr>
<tr>
<td>Anti-C (42)</td>
<td>9</td>
<td>0</td>
<td>33</td>
</tr>
<tr>
<td>Anti-E (144)</td>
<td>68</td>
<td>0</td>
<td>76</td>
</tr>
<tr>
<td>Anti-c (28)</td>
<td>15</td>
<td>0</td>
<td>13</td>
</tr>
<tr>
<td>Anti-e (11)</td>
<td>6</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>Anti-K (80)</td>
<td>61</td>
<td>1</td>
<td>18</td>
</tr>
<tr>
<td>Anti-Jk’ (19)</td>
<td>12</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>Anti-DiP (20)</td>
<td>17</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Anti-S (8)</td>
<td>5</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Anti-D + Anti-C (48)</td>
<td>46</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Anti-D + Anti-E (2)</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Anti-D + Anti-K (2)</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Anti-C + Anti-E (12)</td>
<td>8</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Anti-C + Anti-e (5)</td>
<td>4</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Anti-c + Anti-E (12)</td>
<td>11</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Anti-Le a (6)</td>
<td>4</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Anti-Le b (2)</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Anti-P1 (2)</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Cold agglutinins (4)</td>
<td>0</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Total (628)</td>
<td>430</td>
<td>2</td>
<td>196</td>
</tr>
</tbody>
</table>

Note: The antibodies listed above the line are considered potentially clinically significant.
Discussion

Based on this study, the sensitivity of the gel test is superior \((p < .01)\) to the conventional tube PEG-IAT in the detection of alloantibodies of potential clinical significance. Gel detected 190 potentially clinically significant antibodies not reactive by PEG-IAT, but failed to detect 2 potentially clinically significant antibodies (1 anti-K and 1 anti-D\(_{i}^\beta\)) in two patients.

A higher sensitivity for IgM antibodies like anti-Le\(\alpha\), -Le\(\beta\), -P1, and cold agglutinins might be considered a disadvantage, but there are numerous advantages to the gel system.\(^1,5\) The gel assay incorporates standardized pipeting of reagents and specimens and reading of agglutination reactions. The stable reaction endpoints may be reviewed, photographed, or photocopied at a later time. The required volume of specimen is substantially less, which is advantageous for pediatric testing. Small sample volumes create less hazardous waste, as does the elimination of saline washes in the performance of IATs.\(^1,15,16\)

In contrast to other reports, it is important to note that in our study, all RBC antibodies detected by the gel test remained reactive during antibody identification. This may be because we used only fresh samples, so attenuation of reactivity during freezing, storage, and thawing could be avoided.\(^17\)\(^−\)\(^19\) We designed a prospective study with an enormous number of sera tested in parallel in order to obtain a sufficient number of positive results. This large study shows that the gel test is more sensitive when compared to PEG-IAT.

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References


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