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Detection of Rh antibodies using two low ionic diluents: Extension of the incubation time and the number of Rh antibodies detected

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ABSTRACT

Background: Low ionic strength solution (LISS) is used to increase the rate of association of antibody to the corresponding antigen during antibody detection tests. A number of LISSs are available on the market. Aims: The efficiency of two commercial low ionic diluents, DiaMed ID-CellStab and Inverclyde LISS were assessed using the DiaMed-ID LISS Coombs microtube column system and an incubation time varying from 15 to 35 min. Materials and Methods: One-hundred samples containing five Rh antibodies (anti-D, anti-C, anti-E, anti-c and anti-e) were tested against commercial red cells using the two low ionic diluents after 15, 25 and 35 min. Results: The Inverclyde LISS detected 91, 95 and 96% of the Rh antibodies compared to 78, 79 and 83% for ID-CellStab after 15, 25 and 35 min incubation time, respectively, for both methods. Conclusions: The Inverclyde LISS is a more suitable and efficient diluent than ID-CellStab for the detection of Rh antibodies. The sensitivity of Rh antibody detection increased for the both methods as the incubation time increased.

KEY WORDS: Antibody affinity, haemagglutination, Rh antibody detection, Rh-Hr, immunoglobulin G

R

h antibodies are formed when an individual is exposed to foreign Rh antigens through transfusion or pregnancy. These antibodies are clinically significant,\[1\] react best at 37ºC and they should be considered potentially capable of causing both haemolytic transfusion reactions (HTRs) and haemolytic disease of the newborn (HDN).\[2,3\] Their detection is not always reliable because the persistence of antibodies at detectable level varies among people and even within the spectrum of Rh antibodies\[4-10\] and they have a low affinity.\[11\] The use of screening red cells with homozygous expression of rhesus antigens is recommended\[12\] to maximise antibody detection. Most laboratories use enhancement media (LISS) to decrease incubation time from 60 or 90 min to 15 or 20 min, to enhance RBC sensitization, decreased number of false-positive results\[13-17\] and increase the sensitivity of the assay. In 1953, Jerne and Skovsted\[18\] described the effect of low ionic strength solution (LISS) on the antigen antibody reaction. The value of lowering the ionic strength of the medium was also demonstrated using anti-D with D-positive red blood cells (RBMs).\[19\] However, LISS did not find favour in practical work because of the occurrence of nonspecific positive reactions. In the wake of the failure and poor performance of LISS, Low and Messeter\[14\] placed LISS in the incubation phase of the indirect antiglobulin test (IAT) on the threshold of using LISS in the routine practice. Several brands of commercial LISS are available on the market. However, controversy exists concerning the efficiency of one of these, ID-CellStab (DiaMed, Switzerland), which is widely used in detecting red cell alloantibodies.\[19-21\] The present study has two aims: first, to confirm our previous results\[21\] that compared the sensitivity of two low ionic diluents (Inverclyde LISS and ID-CellStab) in the detection of a spectrum of antibodies and second, to investigate the value of extending the incubation time up to 35 min with both diluents using DiaMed ID LISS/Coombs microcolumn system.\[22\] The incubation time was only limited to 35 min, first the samples were not sufficient and second, to avoid false positive results, as extending the incubation time longer than 40 min, may result in a loss of sensitivity.\[23\]

Materials and Methods

Subjects

Sera, known to contain anti-D, anti-C, anti-E, anti-c or anti-e antibodies, from 100 patients were tested. All antibodies under investigation were previously identified by IAT in routine antibody screening, using ID-CellStab, enzyme method, polyethylene glycol (PEG) or polybrene technique. An autocontrol was used in the original identification to avoid false positive reactions. All patients were informed about the goal of the study and consented to the use of their sera for further transfusion laboratory investigations. All samples were stored in a frozen state at -35ºC after the original identification by an expert biomedical scientist, thawed immediately before use and allowed to reach the room temperature.
Red cell samples and suspensions
Commercially available red cells (National Blood Service, Cambridge Hospital, UK) were used. For each antibody specificity tested, two positive and two negative cell suspensions were used except anti-e, where one negative cell only was used [Table 1]. The RBC suspensions were prepared using 50 µL of the commercial red cells, washed three times either with ID-CellStab (DiaMed, Switzerland) or Inverclyde LISS (Inverclyde Biologicals, Scotland) and resuspended in approximately 5 mL of the respective diluent in a clean glass tube to obtain 1%, in accordance with manufacturer’s instructions of the DiaMed technique. All RBC suspensions were prepared afresh on the day of use.

Indirect antiglobulin test
Fifty microlitres of 1% RBCs suspended in ID-CellStab or Inverclyde LISS and 25 µL of serum were added to the DiaMed-ID LISS/Coombs’ gel (containing polyspecific anti-IgG and anti-C3d) and incubated for 15, 25 and 35 min at 37°C (DiaMed-ID Incubator 37 SI) followed by centrifugation at 1000 g (DiaMed-ID-centrifuge 245 SII) for 10 min. All positive reactions were graded macroscopically from 1+ to 4+ using 1+ as an increment. All tests and interpretations were personally performed.

Controls
AB serum (NBS, Cambridge Hospital, UK): as a negative control.

Two positive and two negative cells (NBS, Cambridge Hospital, UK): for each antibody specificity with the exception of anti-e, where one negative cell suspension was used.

Results
For each antibody specificity tested, the Inverclyde LISS detected a greater number of antibodies than ID-CellStab [Table 2]. The total number of antibodies detected after 15 min incubation, using Inverclyde LISS was 91 out of 100 compared to 78 detected by CellStab [Figure 1]. The extension of the incubation time from 15 to 35 min showed an increase of 6% in the total number of antibodies detected using both low ionic diluents. For ID-CellStab, there was no increase in the antibody detection as the incubation time increased from 15 to 25 min with the exception of one case of anti-E. [Table 2]. Four additional antibodies were identified using Inverclyde LISS at 25 min incubation. Four were detected using ID-CellStab when the incubation time reached 35 min. Only one additional case of anti-E was identified using Inverclyde LISS as the incubation time was extended to 35 min. Therefore, 96% of Rh antibodies

Table 1: Rhesus antigen profile of commercial red blood cells used for Rh antibody detection

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Commercial cells used</th>
</tr>
</thead>
<tbody>
<tr>
<td>-D</td>
<td>R, R1R1, R1R2</td>
</tr>
<tr>
<td>-C</td>
<td>R, R1R1, R1R2</td>
</tr>
<tr>
<td>-E</td>
<td>R, R1R1, R1R2</td>
</tr>
<tr>
<td>-c</td>
<td>R, R1R1, R1R2</td>
</tr>
<tr>
<td>-e</td>
<td>R, R1R1, R1R2</td>
</tr>
</tbody>
</table>

Table 2: The number and percentage of each Rh antibody detected using both low ionic solutions after 15, 25 and 35 min incubation

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Incubation time (min)</th>
<th>Number of sera tested</th>
<th>Number and percentage of antibodies detected</th>
</tr>
</thead>
<tbody>
<tr>
<td>-D</td>
<td>15</td>
<td>53</td>
<td>46 CellStab No. 87 152 Inverclyde LISS No. 96</td>
</tr>
<tr>
<td>-D</td>
<td>25</td>
<td>53</td>
<td>46 CellStab No. 87 152 Inverclyde LISS No. 98</td>
</tr>
<tr>
<td>-D</td>
<td>35</td>
<td>53</td>
<td>47 CellStab No. 89 154 Inverclyde LISS No. 98</td>
</tr>
<tr>
<td>-C</td>
<td>15</td>
<td>18</td>
<td>14 CellStab No. 78 181 Inverclyde LISS No. 89</td>
</tr>
<tr>
<td>-C</td>
<td>25</td>
<td>18</td>
<td>14 CellStab No. 78 181 Inverclyde LISS No. 100</td>
</tr>
<tr>
<td>-C</td>
<td>35</td>
<td>18</td>
<td>16 CellStab No. 89 181 Inverclyde LISS No. 100</td>
</tr>
<tr>
<td>-E</td>
<td>15</td>
<td>20</td>
<td>13 CellStab No. 65 181 Inverclyde LISS No. 85</td>
</tr>
<tr>
<td>-E</td>
<td>25</td>
<td>20</td>
<td>13 CellStab No. 70 181 Inverclyde LISS No. 90</td>
</tr>
<tr>
<td>-E</td>
<td>35</td>
<td>20</td>
<td>15 CellStab No. 75 191 Inverclyde LISS No. 95</td>
</tr>
<tr>
<td>-c</td>
<td>15</td>
<td>5</td>
<td>2 CellStab No. 40 181 Inverclyde LISS No. 60</td>
</tr>
<tr>
<td>-c</td>
<td>25</td>
<td>5</td>
<td>2 CellStab No. 40 181 Inverclyde LISS No. 60</td>
</tr>
<tr>
<td>-c</td>
<td>35</td>
<td>5</td>
<td>2 CellStab No. 40 181 Inverclyde LISS No. 60</td>
</tr>
<tr>
<td>-e</td>
<td>15</td>
<td>4</td>
<td>3 CellStab No. 75 181 Inverclyde LISS No. 100</td>
</tr>
<tr>
<td>-e</td>
<td>25</td>
<td>4</td>
<td>3 CellStab No. 75 181 Inverclyde LISS No. 100</td>
</tr>
<tr>
<td>-e</td>
<td>35</td>
<td>4</td>
<td>3 CellStab No. 75 181 Inverclyde LISS No. 100</td>
</tr>
</tbody>
</table>

Figure 1: The total number of Rh antibodies detected after 15, 25 and 35 min using two different low ionic solutions (CellStab and Inverclyde LISS)
tested were detected using Inverclyde LISS after 35 min compared to 83% identified by ID-CellStab [Figure 1]. Thirteen antibodies were reactive in Inverclyde LISS, but missed in the ID-CellStab after 35 min incubation at 37°C [Table 2]. These antibodies were originally detected by the enzyme method, PEG or polybrene technique. For every antibody tested, no discrepancies were recorded in the positive or negative cells and there were no positive reactions with AB serum. No false positive reaction occurred due to a specific antibody binding after extending the incubation time up to 35 min. In addition, no discrepancy was found between the different batches of the ID-Panel. The reaction strength varies from 1+ to 2+ using Inverclyde LISS vs. ID-CellStab.

Discussion

The Inverclyde LISS detected 91, 95 and 96% of the Rh antibodies compared to 78, 79 and 83% for ID-CellStab after 15, 25 and 35 min incubation time, respectively, for both methods. The present study confirmed our previous findings on the relative merits of the two diluents[21] and demonstrated the improved sensitivity in the detection of Rh antibodies by extending the incubation period. We found in our previous study that the Inverclyde LISS detected greater number of Rh antibodies (four anti-D, four anti-E and one anti-C) than did CellStab after 15 min using the DiaMed gel test. It should be noted that in a study by Grey et al.,[19] ID-CellStab was shown to be less efficient than the two other diluents (not including Inverclyde LISS). The missing of thirteen antibodies (five anti-D, two anti-C, four anti-E, one anti-c and one anti-e) using ID-CellStab after 15 min may be attributed to the high percentage of sodium azide and/or increase of both osmolality and conductivity[18-23] of the diluent. Therefore, suboptimal binding of the Rh antibody within short incubation period occurred. The nine antibodies that were missed in both diluents may be due to insensitivity of the gel technique,[24] inappropriate volumes or percentage, specificity of the antibody, the inhibitory effect of the gel matrix[25] and the incubation time.[11] The present study adds further weight to the argument that the Inverclyde LISS is a suitable and more efficient than ID-CellStab for the detection of Rh antibodies.

Furthermore, the sensitivity of Rh antibodies detection increased as the incubation time increased.

Acknowledgments

I would like to commend for the generous assistance of Dr. David Brit, who revised grammatically and help in the preparation of the manuscript. I wish also to thank Mr. Hamed Al-Hefny, who provided the blood samples.

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