Technical methods

Staphaurex reappraised: role of protein A in false positive results and recommendations for use

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The production of free coagulase, as detected by the tube coagulase test, is considered to be the definitive means of identifying Staphylococcus aureus. This test, however, may take 24 hours before a result is obtained. Although quick, the slide coagulase test has a false negative rate between 3% and 15%. Another commonly used test, the DNase test, rarely gives false negative results but requires 24 hours' incubation.

In an attempt to overcome these problems a new latex agglutination kit (Staphaurex) has been introduced. This consists of latex particles coated with fibrinogen and IgG, which detect clumping factor and protein A, respectively. We tested this reagent with 480 human isolates of staphylococci. We also investigated the role of protein A in causing false positive results with Staphaurex.

Material and methods

Isolates were obtained from routine specimens submitted to our laboratory for analysis. Potentially clinically important isolates of staphylococci were selected and submitted to tube coagulase, slide coagulase, and DNase testing. Staphaurex testing was also performed in duplicate, once following the manufacturer's instructions and once using only 20 μl of reagent.

Organisms which gave positive results with all three reference tests were identified as S aureus. Isolates which gave negative results on tube coagulase and DNase testing and which were negative on slide coagulase testing or which autoagglutinated in saline were classified as non-S aureus staphylococci. Organisms which gave conflicting results were identified biochemically by the APIStaph system. These strains were also tested for the presence of protein A by a single, mixed tube agglutination method.

Isolates identified as S aureus were then tested for methicillin sensitivity in the routine laboratory by a disc diffusion technique.

Results

Table 1 summarises the results. Table 2 shows the sensitivity, specificity and predictive values of positive and negative results. There was complete correlation between results obtained using the different volumes of Staphaurex reagent.

Discussion

The latex test was extremely sensitive and compared favourably with other tests commonly used for identification of S aureus, both methicillin resistant and methicillin sensitive strains found in the North East Thames region. Furthermore, the Staphaurex test gave a clear cut difference between positive and negative reactions without problems of auto-agglutination.

Table 1 Comparison of Staphaurex testing with reference methods in 480 isolates of Staphylococci

<table>
<thead>
<tr>
<th>No of strains</th>
<th>Tests</th>
<th>Tube coagulase</th>
<th>Slide coagulase</th>
<th>DNase</th>
<th>Staphaurex</th>
<th>Identification</th>
<th>Protein A</th>
</tr>
</thead>
<tbody>
<tr>
<td>135</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>S aureus (Meth S)</td>
<td>+</td>
</tr>
<tr>
<td>36</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>S aureus (Meth R)</td>
<td>+</td>
</tr>
<tr>
<td>283</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Non-S aureus staphylococci</td>
<td>-</td>
</tr>
<tr>
<td>12</td>
<td>-</td>
<td>AA</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Non-S aureus staphylococci</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>S aureus (Meth S)</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>S aureus (Meth S)</td>
<td>+</td>
</tr>
<tr>
<td>1</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>S epidermidis</td>
<td>-</td>
</tr>
<tr>
<td>1</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>S hominis</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>S simulans</td>
<td>-</td>
</tr>
<tr>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>S warneri</td>
<td>-</td>
</tr>
<tr>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>S saprophyticus</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>S saprophyticus</td>
<td>-</td>
</tr>
</tbody>
</table>

+ = positive result; - = negative result.
AA = autoagglutinating strains; Meth S = methicillin sensitive; Meth R = methicillin resistant.

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Table 2  Percentage sensitivity, specificity, and predictive values of positive and negative results on Staphaurex testing compared with reference methods

<table>
<thead>
<tr>
<th></th>
<th>Tube coagulate test</th>
<th>Slide coagulate test</th>
<th>DNase test</th>
<th>Staphaurex</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>98.9</td>
<td>97.7</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Specificity</td>
<td></td>
<td></td>
<td>99.3</td>
<td>98.9</td>
</tr>
<tr>
<td>Predictive value of positive result</td>
<td>100</td>
<td></td>
<td>98.9</td>
<td>97.3</td>
</tr>
<tr>
<td>Predictive value of negative result</td>
<td>99.3</td>
<td>98.6</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

Although false positive Staphaurex reactions have been described with non-S aureus staphylococci, previous workers have not attempted to investigate this further. The production of protein A by some isolates of S saprophyticus explains some of the false positive results. Three of the five organisms which were falsely positive with Staphaurex in our study were S saprophyticus. This limits its use in testing urinary isolates.

Staphaurex reagent may be expensive. The use of 20 μl for each test instead of the 50 μl dispensed by the droppers supplied in the kit should, however, substantially reduce the cost of this useful test.

References


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Storage and recovery of small aliquots of frozen red cells for anti-D boosting

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The boosting of volunteers with frozen recovered red cells from accredited donors was recommended by the World Health Organisation in 1981. Frozen recovered red cells were successfully used in routine boosting of eight Rh D negative women previously immunised by pregnancy, and for immunising and boosting Rh negative male volunteers. In view of the lack of specific markers for non-A non-B hepatitis and the possibility of transmitting human immuno-deficiency virus to donors who volunteer to join the boosting programme, it is now even more important to implement all available safety measures. The safety of immunisation and boosting programmes could be improved by storing frozen red cells from accredited donors in small aliquots. Each volunteer could then be boosted using the same donor cells during the whole programme so as to minimise antigenic exposure.

Red cell donations were selected from a panel of R2R2 longstanding regular donors and were phenotyped for the following antigens: ABO, rhesus, M, N, S, s, k, k, P, Le\(^a\), Le\(^b\), Fy\(^a\), Fy\(^b\), Lu\(^a\), Jk\(^a\), Jk\(^b\). This allowed selection of suitable boosting cells and avoided the development of unwanted irregular antibodies. At each donation tests of liver function and for hepatitis B virus markers (hepatitis B surface antigen, hepatitis B surface antibody, and hepatitis B core antibody) were performed. Archive serum samples were used to test for antibody to human immuno-deficiency virus to ensure that all available frozen cells were negative.

We describe a method using polyolefin freezing bags for storing and recovering Rh positive red cells in small aliquots for boosting. This method satisfies safety requirements and conforms with criteria of good manufacturing practice.
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