Reliability of Latex Agglutination Tests for Identification of *Staphylococcus aureus* Resistant to Oxacillin

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Commercial latex agglutination tests (LATs) for the simultaneous detection of clumping factor and protein A are gaining increased acceptance as a means of identifying *Staphylococcus aureus*. We evaluated two LATs (Accu-Staph; Carr-Scarborough, Decatur, Ga.; Staphaurex; Wellcome, Dartford, England) with particular emphasis on their ability to correctly identify oxacillin-resistant *S. aureus*. We tested 59 oxacillin-resistant *S. aureus*, 136 oxacillin-susceptible *S. aureus*, and 92 coagulase-negative staphylococcal strains with the two LATs and with thermonuclease, slide clumping factor, tube coagulate, and protein A hemagglutination tests. Clumping factor and protein A were present in 96.9 and 82.1% of our *S. aureus* strains, respectively. Accu-Staph correctly identified 92.8% and Staphaurex correctly identified 91.3% of *S. aureus* strains. No significant difference in LAT positivity rates, presence of clumping factor, or presence of protein A was found between oxacillin-resistant and -susceptible *S. aureus*. Overall, there were 31 false-negative LATs for 20 *S. aureus* strains, 14 with Accu-Staph and 17 with Staphaurex. Ninety-five percent of these strains possessed either clumping factor or protein A or both when these factors were determined independently. There were five false-positive LATs for four strains of coagulase-negative staphylococci (three *Staphylococcus epidermidis* and one *Staphylococcus warneri*), four with Accu-Staph and one with Staphaurex. Clumping factor was present in one *S. warneri* strain. Thus, the specificities of Accu-Staph, Staphaurex, and the clumping factor test were 95.6, 98.9, and 98.9%, respectively. Our results indicated that LATs identify oxacillin-resistant and -susceptible *S. aureus* equally well; however, they offer no greater sensitivity or specificity than the clumping factor test for identification of *S. aureus*.

Rapid and reliable differentiation of *Staphylococcus aureus* from other staphylococci is important in the clinical bacteriology laboratory because of the greater pathogenic potential of *S. aureus*. Essers and Radebold (8) described a simple slide agglutination test for the simultaneous detection of protein A and clumping factor that uses latex particles coated with human plasma. Several commercial kits based on this principle are available for the rapid identification of *S. aureus*.

Although the published evaluations of these kits have shown good correlation with reference methods (1, 2, 5, 7, 9, 11, 14), there has been some suggestion (1, 4) that these tests are less reliable for the identification of *S. aureus* resistant to oxacillin than for susceptible strains. In addition, approximately 50% of oxacillin-resistant *S. aureus* strains contain either no protein A or only extracellular protein A, whereas more than 95% of oxacillin-susceptible *S. aureus* strains produce protein A (14). These reports prompted us to evaluate two kits (Accu-Staph; Carr-Scarborough, Decatur, Ga.; Staphaurex; Wellcome, Dartford, England) with particular emphasis on their ability to identify oxacillin-resistant *S. aureus*.

A total of 59 oxacillin-resistant *S. aureus* strains, 136 oxacillin-susceptible *S. aureus* strains, and 92 strains of coagulase-negative staphylococci (CNS) were included in the study. The organisms were both recent clinical isolates and frozen stock cultures. They were identified on the basis of typical colonial and microscopic morphology and a positive catalase test. All strains were subcultured three times on 5% sheep blood agar plates (BBL Microbiology Systems, Cockeysville, Md.) and incubated overnight at 35°C before testing. All staphylococci were tested for thermonuclease activity, clumping factor, and protein A and with the two latex agglutination tests (LATs). A tube coagulate test was performed on all strains that were negative in the clumping factor test. Thermonuclease- and clumping-factor- or coagulate-positive strains were identified as *S. aureus*, and thermonuclease-negative, coagulate-negative strains were called CNS. Some CNS were identified to the species level by using the Staph-Ident system (Analytab Products, Plainview, N.Y.).

Oxacillin resistance was detected by the disk diffusion method (12) and confirmed by microdilution testing in Mueller-Hinton broth supplemented with 2% NaCl (13). Oxacillin MICs for the oxacillin-resistant strains ranged from 3 to >48 µg/ml. To ensure diversity of the oxacillin-resistant organisms, only strains with unique antibiograms or phage types were included in this study when they occurred as part of a nosocomial outbreak.

Citrated rabbit plasma (Difco Laboratories, Detroit, Mich.) was used in both the clumping factor and tube coagulate tests. The clumping factor test was done by the method of Cadness-Graves et al. (6), except that the bacteria were emulsified directly in a drop of plasma. The tube coagulate test was incubated at 35°C and examined at 2 and 4 h for clot formation. Unclotted tubes were held overnight at room temperature and reexamined. Any degree of clot formation was considered a positive reaction.

The method of Barry et al. (3) was used to detect thermonuclease activity. Toluidine blue-DNA-agar plates were incubated at 35°C and examined at 24 h for a pink halo surrounding the test wells, indicative of thermonuclease activity. A microdilution modification of the procedure of

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Winblad and Ericson (15), using sheep erythrocytes sensitized with rabbit anti-sheep erythrocyte serum, was used to detect protein A. The tests were incubated at 35°C for 2 h, and the wells were examined for hemagglutination.

The two commercial LATs were performed according to the manufacturers’ instructions. Agglutination was scored as positive, equivocal, or negative. Both equivocal and frankly negative results were considered negative in the datum analysis. S. aureus ATCC 25923 and Staphylococcus epidermidis ATCC 12228 served as positive and negative controls, respectively, for the clumping factor, coagulase, and thermonuclease tests and the LATs. S. aureus Cowan 1 and A676 (10) served as positive and negative controls, respectively, for the protein A hemagglutination test.

The results of the two LATs and the independent assessment of clumping factor and protein A for the 287 staphylococci tested are shown in Table 1. Accu-Staph and Staphaurex correctly identified 92.8 and 91.3% of S. aureus, respectively. We found no significant difference in the ability of either LAT to correctly identify oxacillin-resistant and -susceptible S. aureus (P > 0.05).

Winblad and Ericson (15) reported that 47.6% of oxacillin-resistant S. aureus and 97.6% of oxacillin-susceptible S. aureus have demonstrable protein A. In contrast, we found that oxacillin-resistant organisms were as likely as oxacillin-susceptible organisms to possess protein A and that protein A was demonstrable for only 82.1% of our S. aureus strains. Regional differences in strains may account for the discrepancies between the studies. We also found no significant difference in clumping factor test positivity between resistant and susceptible strains. Clumping factor was present in 96.9% of our S. aureus strains. Since no difference existed between oxacillin-resistant and -susceptible S. aureus strains with respect to the presence of these two surface markers, it is not surprising that the LATs identified the two S. aureus populations equally well. Earlier evaluations of Accu-Staph and Staphaurex also demonstrated that they are adequate for the identification of oxacillin-resistant S. aureus (1, 4, 14).

There were 31 false-negative LATs for 20 S. aureus strains (12 oxacillin-susceptible and 8 oxacillin resistant). The 14 false-negative Accu-Staph tests consisted of 11 frank negative and 3 equivocal reactions. The 17 false-negative Staphaurex tests consisted of 7 frank negative and 10 equivocal reactions. Equivocal reactions in both LATs occurred as grainy or slightly flaky agglutination. We chose to consider equivocal results as negatives because, in practice, all such results would require confirmation by another method. The positivity rates we found for S. aureus in the Accu-Staph and Staphaurex tests were substantially lower than those reported in previous studies (1, 4, 14). The large number of equivocal LAT results we encountered may explain our lower positivity rates.

The results obtained in the clumping factor and protein A tests for the 20 S. aureus strains for which false-negative results were obtained in at least one LAT are shown in Table 2. Both clumping factor and protein A were present in 13 strains, only clumping factor was present in 5 strains, only protein A was present in 1 strain, and neither clumping factor nor protein A was present in 1 strain. Six strains were correctly identified by Accu-Staph and missed by Staphaurex, three were missed by Accu-Staph and correctly identified by Staphaurex, and eleven were missed by both LATs. The manufacturers’ claim that the LATs are superior to the clumping factor test because the former simultaneously detect the presence of clumping factor and protein A was not substantiated by our data. In fact, 19 of 20 (95%) S. aureus strains that gave a false-negative LAT had at least one of the surface markers present when these factors were determined independently.

The number of false-positives we encountered with the LATs was small (Table 1). False-positive reactions have been described for a number of different species of CNS (1, 4). The one strain of Staphylococcus warneri that was misidentified as S. aureus by both Accu-Staph and Staphaurex also possessed clumping factor. The three S. epidermidis strains positive in the Accu-Staph test did not produce clumping factor, protein A, or soluble coagulase.

### TABLE 1. Comparison of clumping factor, protein A, and latex agglutination for identification of S. aureus and CNS

<table>
<thead>
<tr>
<th>Bacteria (no. of strains)</th>
<th>Clumping factor</th>
<th>Protein A</th>
<th>Latex agglutination</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. (%) of positive tests</td>
<td>Accu-Staph</td>
<td>Staphaurex</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. aureus (195)</td>
<td>189 (96.9)</td>
<td>160 (82.1)</td>
<td>181 (92.8)</td>
</tr>
<tr>
<td>Oxacillin resistant (59)</td>
<td>58 (98.4)</td>
<td>50 (84.7)</td>
<td>53 (89.8)</td>
</tr>
<tr>
<td>Oxacillin susceptible (136)</td>
<td>131 (96.3)</td>
<td>110 (80.9)</td>
<td>128 (94.1)</td>
</tr>
<tr>
<td>CNS (92)</td>
<td>1* (1.1)</td>
<td>3* (3.3)</td>
<td>4* (4.3)</td>
</tr>
</tbody>
</table>

* S. warneri.
* S. epidermidis, S. haemolyticus, and S. sciuri.
* S. epidermidis (n = 3) and S. warneri (n = 1).
* S. warneri.

### TABLE 2. Correlation of false-negative LAT results with the presence of clumping factor and protein A for 20 S. aureus strains

<table>
<thead>
<tr>
<th>LAT results</th>
<th>No. of strains</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CF+PA+</td>
</tr>
<tr>
<td>Accu-Staph (+) Staphaurex (-)</td>
<td>4</td>
</tr>
<tr>
<td>Accu-Staph (-) Staphaurex (+)</td>
<td>3</td>
</tr>
<tr>
<td>Accu-Staph (-) Staphaurex (-)</td>
<td>6</td>
</tr>
</tbody>
</table>

* Twelve oxacillin-susceptible and eight oxacillin-resistant strains.
* CF, Clumping factor; PA, protein A.
Our data provided no explanation for these misidentifications. Three of the CNS (S. epidermidis, Staphylococcus haemolyticus and Staphylococcus sciuri) produced protein A. The specificities of the clumping factor test, protein A hemagglutination test, Accu-Staph, and Staphaurex for the identification of S. aureus were 98.9, 96.7, 96.6, and 98.9%, respectively.

We conclude that both Accu-Staph and Staphaurex can identify oxacillin-resistant and -susceptible S. aureus equally well. However, the LATs offered no advantage over the clumping factor test in terms of speed, sensitivity, or specificity. Enough false-negative results occurred in the LATs to warrant confirmation of all negative results by another test method (e.g., tube coagulase or thermonuclease). The cost per test of supplies for the Accu-Staph, Staphaurex, and clumping factor test was estimated at $0.50, $0.45, and $0.03, respectively. Based on our results and the higher cost of the LATs, the clumping factor test remains the test of choice for the rapid identification of S. aureus.


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LITERATURE CITED