Evaluation of Rapid Coagulase Methods for the Identification of *Staphylococcus aureus*

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Four rapid latex agglutination assays, StaphAurex (Wellcome Diagnostics, Research Triangle Park, N.C.), Bacto Staph (Difco Laboratories, Detroit, Mich.), SeroSTAT (Scott Laboratories, Inc., Fiskeville, R.I.), Veri-Staph (Zeus Technologies, Raritan, N.J.), and two hemagglutination tests, Staphyloslide (BBL Microbiology Systems, Cockeysville, Md.) and Hemastaph (Remel, Lenexa, Kans.), were compared with the conventional slide coagulate, tube coagulate (TC), and thermonuclease (TNase) tests for the identification of *Staphylococcus aureus*. A total of 118 clinical isolates of *S. aureus* (52 methicillin resistant), 50 *S. epidermidis*, 5 *S. capitis*, 2 *S. hominis*, 3 *S. simulans*, 6 *S. saprophyticus*, and 2 *S. warneri* were tested. The slide coagulate, TC and TNase tests detected 115 (97.5%), 117 (99.2%), and 118 (100%) of the *S. aureus* isolates, respectively. All showed 100% specificity. The StaphAurex, Veri-Staph, Staphyloslide, Hemastaph, SeroSTAT, and Bacto Staph assays correctly identified 117 (99.2%), 117 (99.2%), 116 (98.3%), 110 (93.2%), 108 (91.5%), and 107 (90.7%) of the *S. aureus* isolates, respectively. For methicillin-resistant *S. aureus* isolates, StaphAurex, Veri-Staph, Staphyloslide, Hemastaph, SeroSTAT, and Bacto Staph showed 1 (2%), 1 (2%), 2 (4%), 7 (13.5%), 7 (13.5%), and 8 (15.4%) false-negative results, respectively. All the commercial agglutination assays demonstrated false-positive results with strains of *S. capitis*, *S. saprophyticus*, and *S. warneri*. The overall accuracy of the commercial agglutination assays compared with TC and TNase ranged from 90.7 to 99.2%. We recommend that negative reactions with the rapid commercial test kits for methicillin-resistant *Staphylococcus aureus* isolates be confirmed with the TC or TNase test.

*Staphylococcus aureus* is identified primarily by the tube coagulate (TC) or slide coagulate (SC) test (8). These tests are based on the ability of two enzymes, free coagulate (TC) or bound coagulate (SC), to clot plasma. Although the TC test has been considered the reference method (3, 12, 18), lot-to-lot variations of plasma (10, 19), incubation time, and degree of clotting (16) may vary test results.

An alternative method, the thermonuclease (TNase) test, detects production of heat-stable nuclease enzymes (thermonucleases) (3, 20). Production of thermonucleases appears to be a consistent characteristic of *S. aureus* (7). The TNase test is highly reliable and comparable in results to the TC test (3, 15, 20). It is simple to perform and is not subject to variation in interpretation, since positive reactions do not revert to negative with overnight incubation, which may happen with the TC test (20). Some studies (3, 15) have suggested the simultaneous use of both TC and TNase tests for definitive identification of *S. aureus*. The major disadvantage of the TC and TNase tests is that definitive test results may be unavailable until up to 24 h after initial isolation of *S. aureus* on a culture medium.

Recently introduced commercial latex agglutination and hemmagglutination assays for the rapid detection of *S. aureus* have been reported to be as sensitive and specific as the TC and TNase tests and more accurate than the SC test (4, 13). The commercial assays detect the presence of clumping factor and protein A. They are simple to perform and interpret, with results available in as little as 15 to 60 s. Aldridge et al. (1) found that some of these commercial agglutination tests were not as sensitive as the TC or TNase test for the detection of methicillin-resistant *S. aureus*. Winbold and Ericson (17) demonstrated that some methicillin-resistant *S. aureus* strains lack protein A on their cell surface, which may account for false-negative results.

The purpose of this investigation was to compare the reliability of six commercially available rapid agglutination kits with that of the SC, TC, and TNase tests for the identification of *S. aureus* from clinical sources. This is the first report which compares the new StaphAurex kit (Wellcome Diagnostics, Research Triangle Park, N.C.) with other currently available commercial agglutination kits and includes a larger number of methicillin-resistant *S. aureus* than previously tested.

**MATERIALS AND METHODS**

A total of 186 catalase-positive, gram-positive cocci were recovered from a variety of clinical sources. Prior to testing, we subcultured each strain by passing a single colony onto a Trypticase soy agar plate supplemented with 10% sheep blood (BBL Microbiology Systems, Cockeysville, Md.) and incubating it at 35°C with 5% CO₂ for 18 to 24 h.

Strains that did not coagulate plasma or failed to produce thermosable nuclease were identified by the method of Kloos and Schleifer (8). The Sensititre system (GIBCO Diagnostics, Madison, Wis.) was used for MIC susceptibility tests as recommended by the manufacturer (6). Strains were interpreted as methicillin resistant when the MIC was greater than 8 μg/ml (14).

**SC test.** Several colonies of each organism were mixed with one drop of 0.85% saline on a microscope slide until a smooth suspension was formed. One drop of reconstituted citrated rabbit plasma (BBL) was then added. The suspension was mixed with an applicator stick. Agglutination observed within 5 to 10 s was recorded as positive. If the organism clumped in saline alone, the reaction was recorded as noninterpretable.

**TC test.** Several colonies of each organism were mixed

916

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with 0.5 ml of citrated rabbit plasma (BBL) in a sterile, plastic-capped, plastic test tube. The tube was incubated in a water bath at 37°C and examined after 4 and 24 h. Clot formation at either reading was recorded as positive.

**TNase test.** TNase activity was determined by the method of Lachica et al. (9) as modified by Barry et al. (3). The plates were incubated at 35 to 37°C and observed at 1, 2, 4, and 24 h for the formation of a pink halo 1 to 3 mm wide surrounding each test well, indicating the presence of thermostable nuclease.

**Commercial reagents.** StaphAurex (Wellcome) consists of latex particles coated with fibrinogen for the detection of clumping factor and with immunoglobulin G for the detection of protein A.

Staphyloslide (BBL) is a hemagglutination test. The kit consists of two reagents: (i) sheep erythrocytes sensitized with fibrinogen with 0.1% sodium azide preservative and (ii) nonsensitized sheep erythrocytes with 0.1% sodium azide preservative as a negative control.

Bacto Staph (Difco Laboratories, Detroit, Mich.) consists of yellow latex particles coated with plasma proteins for the detection of clumping factor and protein A. Positive and negative controls were included.

SeroSTAT (Scott Laboratories, Inc., Fiskeville, R.I.) consists of latex particles coated with plasma. Positive and negative controls were included.

Hemastaph (Remel, Lenexa, Kans.) is a hemagglutination test. Reagents included (i) formalinized, fibrinogen-sensitized sheep erythrocytes and (ii) nonsensitized sheep erythrocytes as negative controls.

Veri-Staph (Zeus Technologies, Raritan, N.J.) consists of protein-coated latex particles suspended in glycine. It detects clumping factor and protein A. Positive and negative controls are not included.

Testing with the commercial assays was done as recommended by the manufacturers. Each of the strains was coded and tested blindly with each method on the same day. Quality control was judged by blindly testing *S. aureus* ATCC 25923 and *S. epidermidis* ATCC 12228 with each run. All runs were performed by the same person (A.B.).

**RESULTS**

The results of the SC, TC, TNase, and commercial assays performed on 186 clinical strains are shown in Table 1. The SC, TC, and TNase tests correctly identified 115 (97.5%), 117 (99.1%), and 118 (100%) of the *S. aureus* isolates, respectively. Each method correctly detected all methicillin-resistant *S. aureus* isolates. The three false-negative results by the SC test were positive by both the TC and TNase tests. The one false-negative result by the TC test was positive by the TNase test.

The StaphAurex and Staphyloslide kits correctly identified all 66 (100%) methicillin-susceptible *S. aureus* isolates and 51 (98.1%) and 50 (96.2%) of the methicillin-resistant *S. aureus* isolates, respectively, each missing the same methicillin-resistant strains.

Veri-Staph, Hemastaph, SeroSTAT, and Bacto Staph correctly identified 117 (99.2%), 110 (93.2%), 108 (91.5%), and 107 (90.7%) *S. aureus* isolates, respectively.

Hemastaph and SeroSTAT each missed seven (13.5%) methicillin-resistant strains, five of which were the same isolates. Bacto Staph missed eight (15.4%) methicillin-resistant strains, four of which were the same isolates missed by SeroSTAT. Veri-Staph missed the same methicillin-resistant isolate as Bacto Staph, thought it correctly identified all of the methicillin-susceptible organisms.

False-positive results for the same *S. capitis* isolates were shown by Veri-Staph (*n* = 3), Bacto Staph (*n* = 2).

**TABLE 1. Comparison of coagulase test results for staphylococci**

<table>
<thead>
<tr>
<th>Species</th>
<th>Total no. of strains</th>
<th>SC</th>
<th>TC</th>
<th>TNase</th>
<th>StaphAurex</th>
<th>Staphyloslide</th>
<th>Veri-Staph</th>
<th>Hemastaph</th>
<th>SeroSTAT</th>
<th>Bacto Staph</th>
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</thead>
<tbody>
<tr>
<td><em>S. aureus</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Methicillin susceptible</td>
<td>66</td>
<td>63</td>
<td>65</td>
<td>66</td>
<td>66</td>
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<td>51</td>
<td>50</td>
<td>51</td>
<td>45</td>
<td>45</td>
<td>45</td>
<td>44</td>
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<tr>
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<td>0</td>
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<td>0</td>
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<td>0</td>
<td>0</td>
<td>0</td>
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<td>1</td>
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<td><em>S. hominis</em></td>
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<td>0</td>
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<td>0</td>
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<td>0</td>
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<td><em>S. simulans</em></td>
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<td>0</td>
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<td>0</td>
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<tr>
<td><em>S. saprophyticus</em></td>
<td>6</td>
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<td>0</td>
<td>0</td>
<td>2</td>
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<td>3</td>
<td>2</td>
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<td>3</td>
</tr>
<tr>
<td><em>S. warneri</em></td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
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</tr>
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</table>

**TABLE 2. Sensitivities and specificities of coagulase tests for staphylococci**

<table>
<thead>
<tr>
<th>Test</th>
<th>% Sensitivity</th>
<th>% Specificity</th>
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<tr>
<td></td>
<td>Overall</td>
<td>Methicillin-susceptible</td>
</tr>
<tr>
<td>SC</td>
<td>97.5</td>
<td>95.5</td>
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<tr>
<td>TC</td>
<td>99.2</td>
<td>98.5</td>
</tr>
<tr>
<td>TNase</td>
<td>100</td>
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</tr>
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<td>StaphAurex</td>
<td>99.2</td>
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<td>Staphyloslide</td>
<td>98.3</td>
<td>100</td>
</tr>
<tr>
<td>Veri-Staph</td>
<td>99.2</td>
<td>100</td>
</tr>
<tr>
<td>Hemastaph</td>
<td>93.2</td>
<td>98.5</td>
</tr>
<tr>
<td>SeroSTAT</td>
<td>91.5</td>
<td>95.5</td>
</tr>
<tr>
<td>Bacto Staph</td>
<td>90.7</td>
<td>95.5</td>
</tr>
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</table>
SeroSTAT \((n = 2)\), and Hemastaph \((n = 1)\). All the commercial agglutination kits demonstrated false-positive results with the same isolates of *S. saprophyticus* and *S. warneri*. None of the kits showed false-positive results for *S. epidermidis*, 25 (50\%) of which were methicillin resistant.

The StaphAurex, Staphyloslide, and Bacto Staph kits were the easiest to interpret. The SeroSTAT kit was the most difficult to read, often showing weak positive reactions for isolates which produced strong positive reactions with other kits. Test results were obtained within 10 to 60 s with all of the commercial kits.

**DISCUSSION**

The results of this study demonstrated that there is a significant variation in the sensitivity and specificity of the six commercial agglutination kits tested for the rapid identification of *S. aureus* strains.

The sensitivities of StaphAurex and Veri-Staph in this investigation (both 99.2\%) correlated best with the reference TC (99.2\%) and TNase (100\%) tests (Table 2). Both correctly identified 51 (98.1\%) of the methicillin-resistant *S. aureus* isolates and 100\% of the methicillin-susceptible *S. aureus* isolates; however, Veri-Staph showed the lowest specificity (99.4\%). The StaphAurex kit does not include a negative control. We did not consider this a limiting factor in the test procedure, because all the positive-reacting isolates produced an even suspension prior to the start of agglutination with the reagent. Positive tests were easily visualized, showing clumpy agglutination in a clear background usually within 10 to 15 s. Negative tests showed a milky appearance, occasionally with a trace of granularity but in no way resembling a positive result.

The Staphyloslide and Hemastaph kits are hemagglutination assays which detect clumping factor. Both kits correlated with the reference methods for detection of methicillin-susceptible isolates (100 and 98.4\%, respectively). Staphyloslide missed two methicillin-resistant isolates and gave three false-positive results. Hemastaph missed eight methicillin-resistant isolates and showed four false-positive results. The Staphyloslide kit utilizes nonformalinized erythrocytes. The Hemastaph kit utilizes formalinized erythrocytes. Whether the use of formalinized or nonformalinized erythrocytes may account for the differences in their sensitivities and specificities is not known.

Bacto Staph uses yellow latex particles coated with plasma. The yellow latex-coated particles are intended to make visualization and interpretation easier. We found no significant advantage in this, since all the commercial agglutination kits except for SeroSTAT were easily read. Bacto Staph showed the lowest sensitivity (90.7\%) of all the commercial kits tested.

SeroSTAT identified a lower percentage of *S. aureus* (91.5\%) in our study than those previously reported by Baker et al. (2), Doern (4), and Myrick and Elner (13), who reported positive SeroSTAT tests for 99.6, 99.4, and 98.3\% of their isolates, respectively. These researchers did not test for methicillin-resistant strains, and the higher sensitivities they reported may reflect this.

The latex agglutination assays detect clumping factor and protein A. The presence of protein A occurs in 90 to 99\% of *S. aureus* strains (5); however, the quantity may vary (11). It has been demonstrated that 50\% of methicillin-resistant strains lack protein A, whereas it is present in up to 2\% of non-*S. aureus* coagulase-negative strains (11, 17), which may account for false-negative and -positive results.

The results of this investigation demonstrated the limitations of the commercial serological kits for the identification of methicillin-resistant *S. aureus*. Although the commercial agglutination kits were comparable to the TC and TNase tests for the identification of methicillin-susceptible strains, only three of six kits were comparable to the reference methods for the identification of methicillin-resistant strains, one of which (Veri-Staph) showed a false-positive rate of 10.3\%.

Based on these results, we recommend that laboratories that choose to use commercial agglutination kits as a routine method for identifying *S. aureus* be aware that methicillin-resistant *S. aureus* may be falsely negative and that alternative testing (TC or TNase) should be performed on significant methicillin-resistant *Staphylococcus* isolates which are commercial agglutination kit negative.

**ACKNOWLEDGMENTS**

We thank Wellcome Diagnostics and Zeus Technologies for providing kits for testing.

**ADDITION**

Since this study was performed, Staph Latex (Difco) has been recalled.

**LITERATURE CITED**


