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Evaluation of Three Commercial Agglutination Tests for the Identification of *Staphylococcus aureus*

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Three commercially available rapid slide agglutination tests for the identification of *Staphylococcus aureus* were evaluated with 354 recent clinical isolates (165 strains of *S. aureus*). The test results of two latex agglutination products, SeroSTAT Staph (Scott Laboratories, Inc.) and Staphylatex (American Micro Scan), and one hemagglutination product, Staphyloslide (BBL Microbiology Systems), were compared with the results of the tube coagulase test, which was read at 4 h (4-h tube coagulase test) and, if negative, again after overnight incubation at room temperature (24-h tube coagulase test). Discrepancies between agglutination and tube coagulase identifications were resolved by use of the thermonuclease, mannitol fermentation, and slide coagulase tests. All sensitivities, specificities, predictive values of a positive result, and predictive values of a negative result for the three agglutination tests were at least 98.8% and comparable with the 4-h tube coagulase test. Best results were obtained with the 24-h tube coagulase test, which yielded one false-negative and no false-positive tests. Agglutination identifications may be performed on organisms taken directly from a primary plate when sufficient growth is present. Kit agglutination procedures yield rapid and reliable identifications and are easy to perform. This study also demonstrates the usefulness of the 24-h tube coagulase test.

Several microbial factors common to *Staphylococcus aureus*, including bound coagulase or clumping factor, free coagulase, thermostable nuclease, protein A, and enzymes for mannitol fermentation, are candidates for laboratory differentiation of *S. aureus* from non-*S. aureus Micrococcaceae* species (NSaMs). For this differentiation, most clinical laboratories utilize the slide coagulase test for the detection of clumping factor or the tube coagulase test for the detection of free coagulase. Of these, the tube test is preferred due to the lesser sensitivity of the slide test. Results of the tube test, however, may require 4 or more hours of incubation (11).

Recently, Essers and Radebold (5) described a rapid slide agglutination test with plasma-coated latex for the simultaneous detection of clumping factor and protein A. In principle, plasma contains fibrinogen, which has the capacity to bind to clumping factor, and immunoglobulin, which has the capacity to bind to protein A through its Fc fragment. Hence, the presence of either clumping factor or protein A on the bacterial cell results in coagglutination of cells and latex particles. More recently, Flandrois and Carret (6) described a rapid slide hemagglutination test with fibrinogen-sensitized sheep erythrocytes for the sole detection of clumping factor.

Since these original publications, three commercially available products have been developed: two latex agglutination tests, SeroSTAT Staph (Scott Laboratories, Inc.) and Staphylatex (American Micro Scan); and one hemagglutination test, Staphyloslide (BBL Microbiology Systems).

At this writing, three evaluations (3, 8, 12) for the conventional use of the SeroSTAT product and one evaluation for the use of this product for the identification of *S. aureus* in blood culture fluid (4) have been published. Evaluations of the Staphylatex and Staphyloslide products have not been published. The purpose of this study was to evaluate all three products by comparing the agglutination tests with conventional tests for the identification of *S. aureus*.

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MATERIALS AND METHODS

Organisms. All 354 test isolates were obtained from a variety of clinical specimens submitted to our laboratory and two area hospital laboratories for routine bacteriological culture. Organisms with colonies resembling staphylococci and characterized as gram-positive cocci in clusters with a positive catalase reaction were subcultured to a fresh veal infusion agar plate with 7% sheep blood (BAP) and incubated overnight at 35°C with CO_2. The subsequent growth was used to make a heavy suspension in stock culture medium and frozen at −70°C. All tests were performed on a primary subculture from the frozen culture to a BAP incubated overnight at 35°C under CO_2. Control organisms used were *Staphylococcus aureus* ATCC 29213 and *Staphylococcus saprophyticus* (identified by the Centers for Disease Control in Atlanta, Ga.).

Slide agglutination tests. All slide agglutination tests were performed by the procedure recommended by the corresponding manufacturer as described in the product inserts.

The SeroSTAT test was performed by placing one drop of kit-supplied physiological saline on a circle on an agglutination slide. A short sweep of confluent growth was suspended in one drop of saline to give a homogeneous suspension. One drop of kit reagent was added to the suspension and mixed with a wooden applicator stick. The slide was rotated by hand for 45 s, after which the suspension was observed for agglutination. A saline-latex reagent control was performed with each run and concurrently with any test deemed equivocal.

The Staphylatex test was performed by placing one drop of kit-supplied physiological saline on a circle on a kit-supplied agglutination card. Three to four colonies of growth were suspended in the drop of saline with a bacteriological loop to give a homogeneous suspension. One drop of kit reagent was added to the suspension and mixed with a

wooden applicator stick. The card was rotated by hand for 45 s, after which the suspension was observed for agglutination. A saline-latex reagent control was performed with each run and concurrently with any test deemed equivocal.

The Staphyloslide test was performed after carefully suspending vials of sensitized and nonsensitized erythrocytes. One drop of each suspension was placed in one of two wax pencil rings drawn on a clean microscope slide. Three to four colonies of growth were suspended in each drop with a wooden applicator stick. The slide was rocked by hand for 15 s, after which each suspension was observed for hemagglutination.

Tube coagulase test. Approximately 0.1 ml of a brain heart infusion broth (Difco Laboratories) culture incubated overnight at 35°C was added to tubes containing 0.5 ml of freshly reconstituted EDTA-rabbit plasma (Difco). The tubes were observed for clot formation after 4 h of incubation in a 37°C water bath (4-h tube coagulase test) and, when negative, again after overnight incubation at room temperature (24-h tube coagulase test). Any degree of clot formation was considered to constitute a positive test.

Slide coagulase test. A heavy, homogeneous suspension of growth was made in a drop of distilled water on a glass slide, to which was added a drop of pooled EDTA-human plasma collected from laboratory personnel the day of testing. The two drops were mixed with a bacteriological needle and observed for clumping after 15 s. Any degree of clumping was considered to constitute a positive reaction.

Thermonuclease test. Thermostable nuclease activity was determined by the method of Lachica et al. (10). In brief, the same brain heart infusion broth culture used for the tube coagulase test was heated in a boiling water bath for 15 min. A precut 3-mm well in toluidine blue-DNA agar (pH 9.0) layered in petri dishes was filled with 30 μl of the brain heart infusion culture. The plates were incubated at 35°C, and the wells were read at 4 and 24 h for the presence of pink halos indicative of nuclease activity.

Mannitol fermentation. The ability of isolates to utilize mannitol under anaerobic conditions was determined by the method recommended by the Subcommittee of Taxonomy of Staphylococci and Micrococci (13). All tubes were boiled and cooled before use to remove excess oxygen. Mineral oil was used as an overlay.

API Staph-Ident profile determinations. Staph-Ident profiles were determined by the procedure recommended by the manufacturer as described in the product insert.

Novobiocin susceptibility test. Novobiocin susceptibility testing was performed by the standard Kirby-Bauer procedure; a zone size of ≤16 mm was considered to be indicative of resistance (1).

RESULTS

Of the 354 isolates tested, 156 were identified as *S. aureus* by the three agglutination tests and by the 4- and 24-h tube coagulase tests, whereas 187 isolates were identified as NSaMs by all five tests (Table 1). Of these 187 NSaMs strains, 7 produced adherent colonies and would not suspend uniformly in saline. When mixed with agglutination reagents, these suspensions produced no additional clumping appearance and were regarded as negative for the reactions, an interpretation consistent with the tube coagulase results.

Eleven isolates yielded results with discrepancies among the five tests and were further characterized by the thermonuclease, mannitol fermentation, and slide coagulase tests (Table 1) as *S. aureus* (nine) or NSaMs (two). Isolates with positive tube coagulase and thermonuclease tests were designated as *S. aureus*, and isolates that were negative for these tests were designated as NSaMs. Four isolates which were tube coagulase negative after 4 h of incubation but turned positive after overnight incubation produced thermonuclease, mannitol fermentation, and slide coagulase reactions consistent with *S. aureus* and were designated as such. All four of these strains were properly identified by the agglutination tests. One isolate was negative for 4- and 24-h tube coagulase tests but yielded thermonuclease, mannitol fermentation and slide coagulase test results consistent with *S. aureus*. All three agglutination tests properly identified this strain. The four isolates designated as *S. aureus* and showing one or more negative agglutination reactions also yielded false-negative slide coagulase tests. The Staphyloslide test produced uninterpretable results for two strains, which were designated as NSaMs because both sensitized and nonsensitized erythrocytes agglutinated when mixed with culture. Both strains were identified as *S. saprophyticus* by the API Staph-Ident (2000 profile), supplemented with the novobiocin susceptibility test (data not shown).

Identification of the 11 isolates that yielded inconsistent agglutination and tube coagulase results as *S. aureus* and NSaMs allowed for the determination of total true- and false-positives and true- and false-negatives and, hence, the calculation of sensitivity, specificity, predictive value of a positive result (PV^+), and predictive value of a negative result (PV^-) for each test (Table 2). Of the 354 isolates tested, all three agglutination tests yielded two false-negative results each. The SeroSTAT and Staphylatex kits produced one false-positive test each. The Staphyloslide kit produced no false-positives, although two NSaMs isolates gave uninterpretable results. Both 4- and 24-h tube coagulase tests yielded no false-positive results, although the 4-h tube coagulase test produced 5 false-negatives. The 24-h tube coagulase test yielded only one false-negative test.

Sensitivity and PV^- values for the three agglutination tests were 98.8 and 98.9%, respectively, slightly superior to those of the 4-h tube coagulase test, which were 97.0 and 97.4%, respectively (Table 2). Specificity and PV^+ values for the SeroSTAT and Staphylatex tests were 99.5 and 99.4%, respectively, slightly inferior to those of the Staphyloslide and tube coagulase tests, which were 100%. Sensitivity and PV^- values for the 24-h tube coagulase test were 99.4 and 99.5%, respectively, superior to those of any other test.

DISCUSSION

The value of a rapid and reliable means of differentiating *S. aureus* from NSaMs is based upon the fact that the former is a frank pathogen that frequently causes serious acute infection, whereas the latter, although not always benign (9), is often a saprophyte. Established tests designed for this purpose are based upon the detection of microbial factors known to correlate well with this differentiation. Of these, the tube coagulase test is probably the most commonly employed.

Recently, Landau and Kaplan have suggested retaining negative 4-h tube coagulase tests for overnight room temperature incubation based on their observation that occasional isolates of *S. aureus* will not produce free coagulase at 36°C (11). Our analysis of the three agglutination products included comparison of agglutination results with the 4- and 24-h tube coagulase tests.

Of the 354 isolates tested, 11 (9 *S. aureus* and 2 NSaMs) yielded results with discrepancies among the five tests. Of these 11 isolates, 5 *S. aureus* strains exhibited false-negative 4-h tube coagulase reactions, four of which turned positive

TABLE 1. Results of tests performed on 354 *Micrococcaceae* isolates with respective strain identifications

No. of strains	Result of the following test[a]:							
	SeroSTAT	Staphylatex	Staphyloslide	Tube coagulase		Thermonuclease	Mannitol fermentation	Slide coagulase
				4 h	24 h			
S. aureus								
156	+	+	+	+	+[b]	NT	NT	NT
4	+	+	+	−	+	+	+	+
1	+	+	+	−	−	+	+	+
1	−	+	+	+	+	+	+	−
1	+	−	+	+	+	+	+	−
1	+	+	−	+	+	+	−	−
1	−	−	−	+	+	+	−	−
NSaMs								
187	−	−	−	−	−	NT	NT	NT
1	+	+	U	−	−	−	−	−
1	−	−	U	−	−	−	−	−

[a] Symbols: +, positive result; −, negative result; NT, not tested; and U, uninterpretable.
[b] Tube coagulase tests showing a positive reaction after 4 h incubation were not incubated overnight at room temperature but were regarded as positive for the 24-h tube coagulase test.

after 24 h. All five strains showed colony morphology typical of *S. aureus*, including beta hemolysis.

In light of this data, we recommended the procedure of Landau and Kaplan, that of retaining negative 4-h tube coagulase tests for overnight, room-temperature incubation and retaining reports when the colony morphology is suggestive of *S. aureus*. Alternatively, negative 4-h tube coagulase tests that are considered potentially false may be confirmed as such by performing the thermonuclease test (10). All of our 11 problem isolates encountered in this study were properly identified by the latter test. The combined use of the 4-h tube coagulase and thermonuclease tests has been suggested elsewhere (2).

One *S. aureus* strain yielded false-negative agglutination reactions for all three tests and a false-negative slide coagulase test, thus suggesting the absence of detectable clumping factor. Three additional strains of *S. aureus* yielded one false-negative agglutination reaction each and false-negative slide coagulase reactions. These strains may possess levels of clumping factor bordering on detectability. The presence of protein A would theoretically allow the latex agglutination tests to produce appropriate reactions, yet two strains produced false-negative tests in one of each latex reagent. Both of these strains gave true hemagglutination reactions. All four strains yielding false-negative agglutination reactions demonstrated typical *S. aureus* colony morphology.

One NSaMs isolate yielded false-positive latex agglutination reactions and an uninterpretable result with the hemagglutination test. All other tests were consistent with a strain identification of NSaMs. This observation may be explained by the presence of protein A on a NSaMs.

A second uninterpretable hemagglutination reaction was seen in another NSaMs; this isolate demonstrated appropriate negative latex agglutination results. Both isolates showing uninterpretable hemagglutination reactions were identified as *S. saprophyticus* by the API Staph-Ident supplemented with the novobiocin test. Hemagglutination of normal sheep erythrocytes by *S. saprophyticus* has been previously reported (7), but this phenomenon was not observed when the same strains were grown on a BAP. All of our isolates were grown on a BAP before testing.

Sensitivities, specificities, and predictive values for the five tests should be viewed in light of our isolation ratio of *S. aureus* to NSaMs, 165:189, for these values may vary with different isolation ratios. Comparison of these performance values shows few differences. All three agglutination tests performed well, with no values below 98.8%. All three kits compare well with the 4-h tube coagulase test. The most significant difference is the improved sensitivity and hence PV^- of the 24-h tube coagulase test compared with that of the 4-h tube coagulase test.

Both latex agglutination kits include positive and negative staphylococcal controls and slides for performing tests, whereas the hemagglutination kit does not. SeroSTAT reagents must be kept cold throughout testing, whereas Staphylatex reagents must be warmed to room temperature before

TABLE 2. Performance parameters for commercial agglutination and tube coagulase tests based on test results from 354 *Micrococcaceae* isolates

Test	Test result					Sensitivity (%)	Specificity (%)	PV^+ (%)	PV^- (%)
	True-positive	True-negative	False-positive	False-negative	Uninterpretable reaction				
SeroSTAT	163	188	1	2	0	98.8	99.5	99.4	98.9
Staphylatex	163	188	1	2	0	98.8	99.5	99.4	98.9
Staphyloslide	163	187	0	2	2	98.8	100	100	98.9
Tube coagulase									
4 h	160	189	0	5	0	97.0	100	100	97.4
6 h	164	189	0	1	0	99.4	100	100	99.5

use. Latex reactions are rarely questionable, but occasional weak positives require careful attention to detect. Positive hemagglutination reactions are more apparent, but erythrocytes must be carefully suspended before use, and occasional uninterpretable results may develop.

All three commercial agglutination tests yield rapid and reliable results and are easy to perform. Identifications may be performed on organisms taken directly from a primary plate when sufficient growth is present. A rapid and reliable differentiation of *S. aureus* and NSaMs not only allows for faster reporting but, in at least some cases, also eliminates unnecessary antibiotic susceptibility testing. This study also demonstrates the increased sensitivity of the tube coagulase test when tests that are negative after 4 h are retained for overnight, room-temperature incubation.

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ADDENDUM

Since submission of this manuscript for publication, additional evaluations of these agglutination tests have been published (Aldridge et al., J. Clin. Microbiol. **19**:703–704, 1984; Woolfrey et al., Am. J. Clin. Pathol. **81**:345–348, 1984).

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