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Comparison of Two Commercially Available Test Methods with Conventional Coagulase Tests for Identification of *Staphylococcus aureus*

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The API STAPHase (Analytab Products, Inc., Plainview, N.Y.) and SeroSTAT Staph (Scott Laboratories, Fiskville, R.I.) tests were compared to the conventional tube coagulase test and a slide coagulase test by using fresh isolates of members of the family *Micrococcaceae*. The 4-h, 24-h, and combined readings of the tube coagulase test detected 94.5, 99.5 and 100%, respectively, of 219 *Staphylococcus aureus* isolates. The API STAPHase, SeroSTAT Staph, and slide coagulase tests detected 95.9, 95.4 and 95.9% of the isolates of *S. aureus*, respectively. There were no false-positive results with any of the systems when tested with 103 strains of members of the family *Micrococcaceae* other than *S. aureus*. We concluded that the STAPHase and SeroSTAT Staph tests were equal in accuracy to the slide coagulase and 4-h tube coagulase tests and were suitable for use in the clinical microbiology laboratory. However, SeroSTAT Staph gave faster results than the API STAPHase, and the test was easier to perform. Also, the false-negative rate was high enough with the STAPHase, SeroSTAT Staph, and the slide coagulase tests that all negative reactions should be confirmed with a tube test.

The slide coagulase test for the detection of bound coagulase or clumping factor and the tube coagulase test for the detection of free coagulase have been the most commonly used methods for identifying *Staphylococcus aureus* and members of the family *Micrococcaceae* other than *S. aureus* in clinical laboratories (3, 7). Recently, more rapid methods of identifying *S. aureus* have been introduced. These include latex agglutination tests (2, 4, 8) and microtube coagulase tests (6). The latex slide test consists of latex particles coated with plasma that contains fibrinogen to detect clumping factor and immunoglobulin G for detection of protein A (4). The advantage of this method is that *S. aureus* can be identified immediately instead of after a 4- or 24-h tube coagulase test. The reported accuracy was also better than that previously reported for the slide coagulase test (2, 8).

Rabbit plasma containing EDTA is used with the microtube method. The reagent is lyophilized and packed in microtubes. The test is based on the same principle as the macrotube coagulase test, but its accuracy has been reported to be better than the conventional test when short incubation times are used (6). The maximum incubation time was 4 h instead of 24 h as in the macrotube method.

To determine the most accurate and reliable test procedure, we evaluated the 4- and 24-h tube coagulase tests, a slide coagulase test, a rapid latex agglutination system (SeroSTAT Staph; Scott Laboratories, Fiskville, R.I.), and a microtube coagulase test (API STAPHase; Analytab Products, Inc., Plainview, N.Y.). This is the first report of a comparison between SeroSTAT Staph and the API STAPHase tests with the same strains. This should make direct comparisons more valid.

MATERIALS AND METHODS

Collection and storage of organisms. Pure cultures of members of the *Micrococcaceae* isolated in a hospital clinical laboratory were classified as *S. aureus* or members of the *Micrococcaceae* other than *S. aureus* based on the tube coagulase test, colonial and microscopic morphology, and the catalase test. The latter group included the other species of staphylococci and any lightly pigmented or white strains of *Micrococcus* sp. which were found in the clinical material. Colonies were subcultured for additional testing within 24 h. Tests on most isolates were performed after one or two subcultures from the original isolate. Stock cultures were transferred to tryptic soy agar slants, incubated at 35°C overnight, and then covered with sterile mineral oil and stored at room temperature. Before further studies, all isolates taken from the stock cultures were streaked onto a chocolate blood agar plate and incubated at 35° overnight.

Identification of organisms. For the purpose of this study, typical catalase-positive, gram-positive cocci were identified as *S. aureus* when the tube coagulase test was positive. If there was a discrepancy between a positive tube coagulase result and the experimental test system, the identity of *S. aureus* was confirmed by using the deoxyribonuclease and mannitol fermentation tests (5). All organisms which were not *S. aureus* were designated as members of the family *Micrococcaceae* other than *S. aureus*.

Tube coagulase test. The tube coagulase test was performed as described previously (10). Rabbit plasma with EDTA (BBL Microbiology Systems, Cockeysville, Md.) was diluted as recommended by the manufacturer. Inoculation was with a drop of turbid fresh culture added to 0.5 ml of plasma in a 12-mm diameter tube. The tube was incubated at 35°C and read at 4 and 24 h. The degree of coagulation was read as described by Turner and Schwartz (11).

Slide coagulase test. The slide coagulase test was a slight variation of the test procedure described by Cadness-Graves et al (1). BBL rabbit plasma with EDTA was diluted as recommended by the manufacturer. A drop of the plasma was added to a glass slide. A loop was used to pick up a portion of a soft, moist colony and was quickly stirred and dispersed directly into the plasma. Agglutination observed within 5 to 10 s was recorded as positive.

SeroSTAT Staph. The slide agglutination reagent in the SeroSTAT Staph test consisted of latex particles coated with

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fibrinogen for detection of clumping factor and with immunoglobulin G for detection of protein A. The reagent was used in accordance with the written instructions of the manufacturer. Before beginning our study, all technical details of the SeroSTAT Staph test procedure were also confirmed by representatives of the manufacturer to ensure that the test was performed in the correct manner.

API STAPHase. Components for the API STAPHase test kit were obtained from Analytab Products, Inc. The kit was used in accordance with the written instructions of the manufacturer. Before beginning our study, all technical details of the test procedure were confirmed by contacting representatives of the manufacturer to ensure that the test was performed correctly. The direct colony technique was used.

Comparative testing. The different tests were performed on each isolate by technologists who were unaware of the other test results. Initial discrepancies were included as discrepancies in the final analysis only after the findings were confirmed by repeat testing. For all initial tests a total of 219 *S. aureus* isolates and 103 isolates of members of the *Micrococcaceae* other than *S. aureus* were evaluated.

RESULTS

In our initial comparison we studied a total of 322 isolates of members of the family *Micrococcaceae*. Organisms were placed into two groups based on the tube coagulase result, Gram stain, catalase, and typical colony morphology. A total of 219 isolates were identified as *S. aureus*. The remaining organisms were coagulase negative and were considered to be members of the *Micrococcaceae* other than *S. aureus*. The identity of an isolate was checked further when either the slide coagulase, the SeroSTAT Staph, or the API STAPHase disagreed with the tube coagulase result. In no instance did the confirmatory identification tests prove that the classification based on the tube coagulase test was incorrect. We were aware that occasional coagulase-negative strains of *S. aureus* have been found when tested with rabbit plasma (12) and that other species of staphylococci can produce coagulase (9). These organisms are relatively rare pathogens in humans but not in animals. They were not detected in any of our discrepancy checks.

In a comparison of the API STAPHase, the SeroSTAT Staph, the slide coagulase, the 4-h tube coagulase, and the 24-h tube coagulase test results, the API STAPHase and the slide coagulase tests each detected 210 (95.9%) of 219 *S. aureus* strains, whereas the SeroSTAT Staph test detected 209 (95.4%) of the isolates. The 4-h tube coagulase test detected 207 (94.5%) of the *S. aureus* isolates, the 24-h reading detected 218 (99.5%) of the coagulase-positive isolates, and the combined 4- and 24-h readings detected 219 (100%) of the isolates. All of the 103 (100%) strains of members of the *Micrococcaceae* other than *S. aureus* produced negative reactions with the API STAPHase, SeroSTAT Staph, and the slide coagulase tests. No false-positive results were detected with any of the test systems.

We extended our study of SeroSTAT Staph to include 213 additional isolates of *S. aureus* and 201 additional isolates of members of the *Micrococcaceae* other than *S. aureus*. The results of this series of tests confirmed the findings of our original studies. A total of 95.7% of the *S. aureus* strains were detected as compared to 95.4% in the first part of our study. There were no false-positive results among the 201 isolates of members of the *Micrococcaceae* other than *S. aureus* when tested by the SeroSTAT Staph test.

DISCUSSION

The results of this study differ in several respects from those of previously published studies. The SeroSTAT Staph and STAPHase tests gave slightly lower percentages of correct identification of *S. aureus* in our laboratory than those reported previously. Myrick and Ellner (8) reported a positive SeroSTAT Staph test for 98.3% of coagulase-positive strains, and Doern (2) reported that 99.4% yielded positive results. The detection of 95.4% of the coagulase-positive strains by the SeroSTAT Staph test was approximately equal to the sensitivity of 95.9% which we found for the slide coagulase test. It has been recommended that a tube coagulase test be performed on all strains found to be negative by the slide coagulase test (5, 7, 10). Since the sensitivity of SeroSTAT Staph and the slide coagulase test are equivalent, we believe that the same precaution should be applied to all SeroSTAT Staph-negative organisms.

Goldstein and Roberts reported that the API STAPHase test detected 99% of the coagulase-positive strains (6). We found that the test detected 95.9% of the *S. aureus* isolates in our series. We consider that sensitivity to be equivalent to the slide coagulase test and recommend that negative reactions be checked by the tube coagulase method.

The slide coagulase test as performed in this study gave reactions with a higher sensitivity and specificity than previously reported by others. We found a false-negative rate of 4.1% with this test, and we found no false-positive reactions. In contrast, Essers and Radebold found a false-negative rate of 4.6% and a false-positive rate of 12% (4). Myrick and Ellner found that 11.4% of their coagulase-positive strains failed to give a positive result by the clumping factor test (8). Doern found that the slide coagulase test resulted in 16.5% false-negative and no false-positive reactions (2). The slide coagulase test which we used in our study was a slight variation of the procedure described by Cadness-Graves et al (1). Instead of making a water or saline suspension of the organism before testing, the colonies were directly suspended in the rabbit plasma. Colonies that are firm or dry owing to the age of the culture or low humidity or colonies that are obviously not staphylococci should not be tested by this technique. In our study only colonies from blood agar or chocolate blood agar were tested. It cannot be assumed that organisms grown on other media, especially those with high salt concentrations, would give equivalent results.

The SeroSTAT Staph is an easily performed test that can be done in less than 1 min. The reagents are packaged in separate, small vials which require no reconstitution. One disadvantage to this system is that the reagents must be kept cold (2 to 8°C), and erroneous results may occur if the reagent is left at room temperature at any time. Unless provisions are made to keep the reagent cool at the workbench, frequent trips to a refrigerator could be anticipated. In this study, the SeroSTAT Staph and the slide coagulase tests showed comparable sensitivities of 95.4 and 95.9%, respectively. The two tests are also comparable in that similar manipulations are involved and clumping factor is detected by both procedures. The SeroSTAT Staph test purportedly detects protein A as well as clumping factor, whereas the slide coagulase test detects clumping factor only. Although we tested a total of 432 strains of *S. aureus*, this theoretical advantage over the slide coagulase test did not seem to increase the sensitivity of the SeroSTAT Staph test when compared to the conventional slide test.

The API STAPHase test has prepackaged microtubes which help to reduce reagent waste and possible contamina-

tion. However, the test is less easily performed than the SeroSTAT Staph test mainly because the microtubes require reconstitution with either sterile distilled water or a bacterial suspension in sterile distilled water. This test also requires 4 h of incubation before a negative result can be reported in contrast to the results obtained in 45 s with the SeroSTAT Staph test. The API STAPHase and the 4-h tube coagulase tests were equivalent in both sensitivity and specificity.

In conclusion, we found lower sensitivity for the API STAPHase and the SeroSTAT Staph tests than were reported by previous researchers. In a direct comparison with the same strains of staphylococci, we found that both tests were equivalent in sensitivity and specificity. However, the API STAPHase and the SeroSTAT Staph tests were no better than their conventional counterparts, the 4-h tube coagulase and the slide coagulase tests, in detecting coagulase-positive and coagulase-negative isolates of members of the family *Micrococcaceae*. Close attention to the details for performing either of these tests and a period of familiarization should allow one to confidently report *S. aureus* with a positive result. However, both tests gave enough false-negative reactions to warrant checking of negative results by a tube coagulase test.

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