

Evaluation of Various Rapid Agglutination Methods for the Identification of *Staphylococcus aureus*

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A latex agglutination test (SeroSTAT Staph; Scott Laboratories, Fiskeville, R.I.) and two hemagglutination tests (Staphyloslide; BBL Microbiology Systems, Cockeysville, Md.; and Hemastaph; Remel, Lenexa, Kans.) were compared with the slide coagulase (SC) and tube coagulase (TC) tests at room temperature (22 to 25°C) and at 37°C for the rapid identification of *Staphylococcus aureus*. A total of 380 clinical strains of staphylococci were tested. The TC test performed at room temperature yielded the largest number of TC-positive results ($n = 239$), and based on this observation 239 organisms were classified as *S. aureus* and 141 were classified as non-*S. aureus*. The SC, TC (37°C), SeroSTAT Staph, Staphyloslide, and Hemastaph tests correctly identified 210 (87.9%), 221 (92.5%), 238 (99.6%), 239 (100%), and 236 (98.7%) of the *S. aureus* isolates, respectively. Of the *S. aureus* isolates that were TC positive at room temperature 68% required 24 h of incubation before coagulase production was detected. There was one false-negative SeroSTAT Staph result and one false-negative Hemastaph result. The Staphyloslide test yielded two noninterpretable results (both organisms were later confirmed as non-*S. aureus*), whereas there were six noninterpretable results recorded with the Hemastaph test (four organisms were classified as non-*S. aureus*, and two were classified as *S. aureus*). The SeroSTAT Staph, Staphyloslide, and Hemastaph tests were all more sensitive than the conventional SC and TC (37°C) tests and were considerably more rapid than the TC test at either temperature.

Traditionally, the tube coagulase (TC) test has been used as the reference method for the identification of *Staphylococcus aureus*. However, the limitations of this method have been addressed in various reports. The type of plasma used, the length of incubation, the degree of clot formation, and false-positive and false-negative results have been indicated as sources of error (12, 16, 18, 19).

Because of these documented limitations of the TC test, other methods have been developed for the identification of *S. aureus*: mannitol fermentation (10), production of DNase (7, 8, 11, 13, 14, 21), and production of a thermostable nuclease (2, 3, 11, 13, 21). The primary disadvantage to all of these methods is that results are not always available until 24 h after initial isolation on a culture medium.

Recently, more rapid methods have become available for the identification of *S. aureus*: (i) a latex agglutination test that detects the presence of clumping factor and protein A (4, 6, 15), and (ii) a number of hemagglutination tests that detect the presence of clumping factor (1). The primary advantage to each of these methods is that test results are available within minutes after primary isolation procedures. The sensitivities of these rapid agglutination methods have been outlined in previous reports, and discrepancies have been noted (1, 4, 5, 6, 9).

To assess the accuracy of some of these recently developed tests, we compared the following for the identification of *S. aureus*: slide coagulase (SC), TC incubated at room temperature (RT) and at 37°C, a latex agglutination test (SeroSTAT Staph; Scott Laboratories, Fiskeville, R.I.), and two hemagglutination tests (Staphyloslide; BBL Microbiology Systems, Cockeysville, Md.; and Hemastaph; Remel, Lenexa, Kans.).

MATERIALS AND METHODS

Organisms. A total of 380 clinical isolates of gram-positive cocci were obtained from the microbiology laboratory of the Medical Center Hospital of Vermont. All isolates were confirmed as *Staphylococcus* species by demonstrating catalase activity and either glucose fermentation or acid production from glycerol as described by Schleifer and Kloos (17). All tests were performed on 18- to 24-h cultures obtained from a Trypticase soy agar plate (BBL) supplemented with 5% sheep blood (Krutulis Laboratories, Bridgeport, N.Y.), which were incubated at 35 to 37°C with 5% CO₂.

SC test. To perform the SC test, several colonies of each organism were mixed with 1 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, and the suspension was mixed with an applicator stick. Clumping of the plasma was interpreted as a positive SC test, whereas no observable clumping within 10 s indicated a negative SC test. If the organism clumped in the saline alone, the reaction was recorded as noninterpretable.

TC test. Two TC tests were performed simultaneously to record reactions incubated at RT (22 to 25°C) and at 37°C. Citrated rabbit plasma (0.5 ml; BBL) was added to each of two test tubes (13 by 75 mm; Fisher Scientific Co., Pittsburgh, Pa.). Several colonies of each organism tested were added to each of the tubes. One tube was incubated in a 37°C water bath, and the second tube was incubated at RT. Reactions were recorded at 4 h and at 18 to 24 h. A positive TC test was indicated by clot formation; no visible clot represented a negative TC test.

SeroSTAT Staph latex agglutination test. The SeroSTAT Staph test was performed as directed by the manufacturer. No less than five isolated colonies were initially mixed with 1 drop of saline on a test slide. After mixing, 1 drop of the SeroSTAT Staph latex reagent (latex particles coated with

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TABLE 1. Comparison of SC, TC (at RT and at 37°C), SeroSTAT Staph latex agglutination, Staphyloslide hemagglutination, and Hemastaph hemagglutination tests for 380 clinical isolates of staphylococci

Test	No. positive	No. negative	No. noninterpretable	Sensitivity (%)	Specificity (%)
SC	210	166	4 ^a	87.9	100
TC (RT ^b)					
After 4 h of incubation	76	304	NA ^c	31.8	100
After 18 to 24 h of incubation	239	141	NA ^c	100	100
TC (37°C)					
After 4 h of incubation	187	193	NA ^c	78.2	100
After 18 to 24 h of incubation	221	159 ^d	NA ^c	92.5	100
SeroSTAT Staph	238	142 ^e	0	99.6	100
Staphyloslide	239	139	2 ^f	100	100
Hemastaph	236	138 ^g	6 ^h	98.7	100

^a Two of these were later confirmed as non-*S. aureus*, and two were confirmed as *S. aureus*.

^b Between 22 and 25°C.

^c NA, Not applicable.

^d Eighteen of these were false-negative results.

^e One of these was a false-negative result.

^f Both of these were later confirmed as non-*S. aureus*.

^g One of these was a false-negative result.

^h Four of these were later confirmed as non-*S. aureus*, and two were confirmed as *S. aureus*.

plasma in phosphate-buffered saline preserved with 0.02% sodium azide) was added, and the slide was rocked for 45 s. A positive test was indicated by clumped particles in the suspension, whereas the absence of clumping represented a negative test. If the organism formed clumped particles in the presence of saline alone, the reaction was recorded as noninterpretable.

Staphyloslide test. The Staphyloslide test was performed as directed by the manufacturer. The kit consisted of two reagents: (i) sheep erythrocytes sensitized with fibrinogen with 0.1% sodium azide preservative and (ii) negative control sheep erythrocytes that were not sensitized with fibrinogen but were preserved with 0.1% sodium azide. To perform the test, one to three colonies of each organism were mixed with 1 drop of each of the two sheep erythrocyte reagents at opposite ends of a test slide. After being mixed with an applicator stock for 5 s, the slide was rocked for 15 s. The slides were examined for hemagglutination with a high-intensity incandescent lamp. A positive Staphyloslide test was indicated by visible clumping of the sensitized sheep erythrocytes and no visible clumping with the negative control sheep erythrocytes. The absence of clumping in both reagents indicated a negative test, and when clumping was observed in both reagents, the reaction was recorded as noninterpretable.

Hemastaph hemagglutination test. The Hemastaph test was performed as directed by the manufacturer. This kit consisted of: (i) reagent cells (formalinized sheep erythrocytes sensitized with human fibrinogen) and (ii) control cells (sheep erythrocytes not sensitized with human fibrinogen). To perform the test, 1 drop of each cell suspension (reagent and control) was added to opposite ends of a clean test slide. Three to five colonies of each organism were inoculated to each section of the slide and mixed for 15 s. After the slide was rocked for an additional 30 s, reactions were recorded. Agglutination in the reagent cells but no agglutination in the control cells was interpreted as a positive Hemastaph test, whereas no agglutination in both cell suspensions was interpreted as a negative test. A noninterpretable Hemastaph test

was indicated when agglutination was observed in the control cell suspension, regardless of whether there was agglutination in the reagent cell suspension.

RESULTS

The results of the SC, TC, SeroSTAT Staph, Staphyloslide, and Hemastaph tests performed on 380 clinical strains of staphylococci are shown in Table 1. The TC test performed at RT yielded the largest number of TC-positive results ($n = 239$), and based on this observation 239 (62.9%) of the organisms were classified as *S. aureus* and 141 (37.1%) were classified as non-*S. aureus*.

The SC test correctly identified 210 (87.9%) of the *S. aureus* isolates; four of the organisms clumping in saline alone yielded noninterpretable reactions. Based on the results of the TC test, two of these organisms were later identified as *S. aureus*, and the remaining two organisms were classified as non-*S. aureus*.

When performed at 37°C, the TC test correctly identified 221 (92.5%) of the *S. aureus* isolates. There were 18 false-negative TC test results at 37°C.

The SeroSTAT Staph, Staphyloslide, and Hemastaph tests correctly identified 238 (99.6%), 239 (100%), and 236 (98.7%) of the *S. aureus* isolates, respectively, and 141 (100%), 139 (98.6%), and 137 (97.2%) of the non-*S. aureus* isolates, respectively.

There was one false-negative SeroSTAT Staph result and one false-negative Hemastaph result. The Staphyloslide test yielded two noninterpretable results, whereas there were six noninterpretable results recorded with the Hemastaph test. There were no false-positive results with any of the tests performed.

DISCUSSION

The results of the SC and TC tests in this investigation emphasize the need for the development and utilization of alternate laboratory methods for the rapid differentiation between *S. aureus* and non-*S. aureus* organisms in the clinical laboratory. The SC test was 100% specific but only

87.9% sensitive for the identification of *S. aureus*. When performed at 37°C, the TC test also was 100% specific, but a lower level of sensitivity was observed (92.5%). This was influenced by the incidence of 18 (7.5%) *S. aureus* isolates that produced coagulase at RT but not at 37°C. It is important to note that the TC test at RT yielded the largest number of positive results ($n = 239$); however, 163 (68%) of the *S. aureus* isolates required 24 h of incubation before coagulase production was observed at this temperature.

The sensitivity of the SeroSTAT Staph test in this investigation (99.6%) correlates well with the previous reports of Doern (4), Doern and Robbie (5), and Myrick and Ellner (15). However, Aldridge et al. (1) and Jungkind et al. (9) have reported lower levels of sensitivity (95.1 and 95.9%, respectively). Aldridge et al. (1) demonstrated a correlation between methicillin-resistant strains of *S. aureus* and false-negative SeroSTAT Staph results when organisms were grown in the presence of oxacillin. It has been demonstrated that methicillin-resistant strains of *S. aureus* lack protein A on their cell surface (20). Consequently, when tested with the SeroSTAT Staph latex reagent, a false-negative test is observed.

Since we did not test for methicillin resistance, such a comparison cannot be made in our investigation. It is interesting to note that the one organism that yielded a false-negative SeroSTAT Staph result produced coagulase at RT but not at 37°C. This same organism yielded a noninterpretable SC test result and was correctly recorded as positive with both the Staphyloslide and Hemastaph hemagglutination methods.

The manufacturer of the SeroSTAT Staph latex agglutination test recommends rocking the slide for 45 s before reading the reaction. However, based on our observations, most positive tests were observed within 15 s. This provides a very rapid means for identifying *S. aureus*.

Both of the hemagglutination tests evaluated in this investigation were comparable in their ease of performance and interpretation of reactions. Although the manufacturers recommend different lengths of time for rocking the slide before recording the reaction (15 s for Staphyloslide and 30 s for Hemastaph), in most cases positive tests were recognized within 5 s regardless of which kit was being tested.

There were no false-positive or false-negative Staphyloslide hemagglutination test results; however, two noninterpretable reactions were observed. Based on the TC test, both of these organisms were classified as non-*S. aureus*. The sensitivity and specificity of the Staphyloslide test in this investigation (100%) agreed with a previous report (1).

Although there were no false-positive results observed with the Hemastaph hemagglutination test, there was one false-negative result recorded. This same organism produced coagulase at RT only and was SC-positive. Also, positive results were correctly recorded with both the SeroSTAT Staph and Staphyloslide methods.

One of the more interesting observations with the Hemastaph test was that of all the organisms tested, six (1.6%) yielded a noninterpretable reaction. Of these six organisms, four were classified as non-*S. aureus*, and two were classified as *S. aureus* (both of which were positive with the TC test at RT and at 37°C as well as with the SeroSTAT Staph and Staphyloslide methods). Although the Staphyloslide and Hemastaph tests are both hemagglutination procedures, the reagents are prepared differently. It is this difference in reagent preparation that may explain why the Hemastaph test produced more noninterpretable results ($n = 6$) than the Staphyloslide test did ($n = 2$). The Hemastaph test utilizes

formalized sheep erythrocytes, whereas the Staphyloslide test utilizes nonformalized sheep erythrocytes. It is possible that the Formalin caused certain strains of *S. aureus* to clump. Nevertheless, the sensitivity (98.7%) and specificity (100%) of the Hemastaph test described in this investigation are comparable to those of the other hemagglutination tests in a previous report (1).

A significant percentage of *S. aureus* isolates evaluated in this investigation (7.5%) produced coagulase at RT but not at 37°C, and a minimum of 18 to 24 h of incubation was required before coagulase production was detected at this temperature. In the pursuit of a more rapid laboratory method to differentiate accurately between *S. aureus* and non-*S. aureus* organisms, three commercially available test kits were evaluated in this investigation: SeroSTAT Staph latex agglutination, Staphyloslide hemagglutination, and Hemastaph hemagglutination. All of these methods were more sensitive than the SC and TC tests at 37°C, and all were more rapid than the TC test at either temperature. Based on these results, we recommend that any of these three commercially available kits be used in the clinical laboratory to provide the most rapid identification of *S. aureus*.

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LITERATURE CITED

1. Aldridge, K. E., C. Kogos, C. V. Sanders, and R. L. Marier. 1984. Comparison of rapid identification assays for *Staphylococcus aureus*. *J. Clin. Microbiol.* **19**:703-704.
2. Barry, A. L., R. V. F. Lachica, and F. W. Atchison. 1973. Identification of *Staphylococcus aureus* by simultaneous use of tube coagulase and thermonuclease tests. *Appl. Microbiol.* **25**:496-497.
3. Boothby, J., C. Genigeorgis, and M. J. Fanelli. 1979. Tandem coagulase/thermonuclease agar method for the detection of *Staphylococcus aureus*. *Appl. Environ. Microbiol.* **37**:298-302.
4. Doern, G. V. 1982. Evaluation of a commercial latex agglutination test for identification of *Staphylococcus aureus*. *J. Clin. Microbiol.* **15**:416-418.
5. Doern, G. V., and L. I. Robbie. 1982. Direct identification of *Staphylococcus aureus* in blood culture fluid with a commercial latex agglutination test. *J. Clin. Microbiol.* **16**:1048-1051.
6. Essers, L., and K. Radebold. 1980. Rapid and reliable identification of *Staphylococcus aureus* by a latex agglutination test. *J. Clin. Microbiol.* **12**:641-643.
7. Forsgren, A. 1970. Significance of protein A production by staphylococci. *Infect. Immun.* **2**:672-673.
8. Gidding, R. 1983. Differentiation of staphylococci on the basis of nuclease properties. *J. Clin. Microbiol.* **18**:1098-1101.
9. Jungkind, D. L., N. J. Torhan, K. E. Corman, and J. M. Bondi. 1984. Comparison of two commercially available test methods with conventional coagulase tests for identification of *Staphylococcus aureus*. *J. Clin. Microbiol.* **19**:191-193.
10. Kocur, M., and N. Mortensen. 1967. Comparison of methods for estimation of anaerobic production of acid from glucose and mannitol in staphylococci and micrococci. *Acta Pathol. Microbiol. Scand.* **71**:141-146.
11. Lachica, R. V. F., P. D. Hoeprich, and C. Genigeorgis. 1971. Nuclease production and lysostaphin susceptibility of *Staphylococcus aureus* and other catalase-positive cocci. *Appl. Microbiol.* **21**:823-826.

12. **Maloney, C. V.** 1979. Coagulase activity: plasma suitability and anticoagulants. *Lab. Med.* **10**:358-361.
13. **Menzies, R. E.** 1977. Comparison of coagulase, deoxyribonuclease (DNase) and heat-stable nuclease tests for identification of *Staphylococcus aureus*. *J. Clin. Pathol. (Lond.)* **30**:606-608.
14. **Morton, H. E., and J. Cohn.** 1972. Coagulase and deoxyribonuclease activities of staphylococci isolated from clinical sources. *Appl. Microbiol.* **23**:725-733.
15. **Myrick, B. A., and P. D. Ellner.** 1982. Evaluation of the latex slide agglutination test for identification of *Staphylococcus aureus*. *J. Clin. Microbiol.* **15**:275-277.
16. **Rayman, M. K., C. E. Park, J. Philpott, and E. C. D. Todd.** 1975. Reassessment of the coagulase and thermostable nuclease tests as means of identifying *Staphylococcus aureus*. *Appl. Microbiol.* **29**:451-454.
17. **Schleifer, K. H., and W. E. Kloos.** 1975. A simple test system for the separation of staphylococci from micrococci. *J. Clin. Microbiol.* **1**:337-338.
18. **Sperber, W. H., and S. R. Tatini.** 1975. Interpretation of the tube coagulase test for identification of *Staphylococcus aureus*. *Appl. Microbiol.* **29**:502-505.
19. **Wegrzynowicz, Z., P. B. Heczko, J. Jeljaszewicz, M. Neugebauer, and G. Pulverer.** 1979. Pseudocoagulase activity of staphylococci. *J. Clin. Microbiol.* **9**:15-19.
20. **Winbold, S., and C. Ericson.** 1973. Sensitized sheep red cells as a reactant for *Staphylococcus aureus* protein A. *Acta Pathol. Microbiol. Scand. Sect. B* **81**:150-156.
21. **Zarzour, J. Y., and E. A. Belle.** 1978. Evaluation of three test procedures for identification of *Staphylococcus aureus* from clinical sources. *J. Clin. Microbiol.* **7**:133-136.