JOURNAL OF CLINICAL MICROBIOLOGY, Feb. 1982, p. 275–277
0095-1137/82/020275-03$02.00/0

Vol. 15, No. 2

# Evaluation of the Latex Slide Agglutination Test for Identification of *Staphylococcus aureus*

BARRYETT A. MYRICK[1] AND PAUL D. ELLNER[2]*

*Clinical Microbiology Service, Columbia-Presbyterian Medical Center,[2] and Department of Microbiology, College of Physicians and Surgeons, Columbia University,[1] New York, New York 10032*

Received 9 July 1981/Accepted 25 September 1981

This study evaluated the reliability of the latex slide agglutination test for identifying *Staphylococcus aureus*. A total of 806 clinical isolates of staphylococci were tested for latex agglutination, clumping factor, and free coagulase. Positive latex tests occurred in 98.3% of coagulase-positive strains, whereas 99.6% of coagulase-negative strains gave negative latex tests. It is concluded that in most instances, the latex slide agglutination test is a reliable method for identifying *S. aureus* in the clinical laboratory.

The presence of free or bound coagulase is the criterion most generally accepted for the differentiation of *Staphylococcus aureus* from other species of staphylococci (8). Isolates that fail to give a positive slide test for clumping factor require testing for free coagulase by the tube test, a procedure that may take up to 4 h and is subject to significant variation in performance and interpretation.

Recently, Essers and Radebold (4) described a rapid agglutination test for the identification of *S. aureus*. This test utilizes latex particles coated with plasma that detect both clumping factor and protein A.

The present study was undertaken to evaluate the reliability of a commercially available latex slide agglutination reagent for the identification of *S. aureus* in the clinical laboratory. This reagent differed from that of Essers and Radebold in that the latex particles were coated with rabbit rather than human plasma. A high degree of correlation was found between the latex and coagulase reactions.

## MATERIALS AND METHODS

**Bacterial strains.** All strains of staphylococci were fresh clinical isolates obtained from specimens submitted to the Clinical Microbiology Service or to the Pediatric Bacteriology Laboratory of Columbia-Presbyterian Medical Center. Organisms were isolated on Columbia agar with 5% sheep blood (BBL Microbiology Systems, Cockeysville, Md. or Scott Laboratories, Inc., Fiskeville, R.I.) or on chocolate agar (BBL or Scott).

**Characterization of strains.** Colonies resembling staphylococci and consisting of catalase-positive, gram-positive cocci in clusters were tested for bound coagulase (clumping factor) by the slide method, using rabbit plasma (BBL; 8). Isolates that failed to give a positive slide test were tested for free coagulase by the tube method, using the same reagent as for the slide test (8). All of the coagulase-negative strains were tested for their ability to ferment glycerol in the presence of 0.4 μg of erythromycin per ml, using glycerol-erythromycin agar (Scott; 12). Strains that failed to produce acid from glycerol were tested for lysostaphin resistance with P agar containing 50 μg of lysostaphin (Sigma Chemical Co., St. Louis, Mo.) per ml. Lysostaphin-resistant strains and those that failed to grow on glycerol-erythromycin agar were considered to be *Micrococcus* species and were excluded from the study. Strains showing a discrepancy between the latex slide agglutination and coagulase tests were also examined for thermostable nuclease, as described by Zarzour and Belle (15), using DNase agar with toluidine blue (Scott) and for mannitol fermentation using the API Staph Strip (Analytab Products, Inc., Plainview, N.Y.).

**Latex slide agglutination test.** The reagent employed consisted of a suspension of latex particles coated with rabbit plasma containing EDTA (SeroSTAT Staphylococcus Test, Scott). Four to five colonies of the organism were emulsified in a drop of saline on a clean slide. One drop of the latex reagent was added, and the drops were mixed with a wooden applicator stick. The slide was tilted back and forth over a dark background. Clumping became clearly visible almost immediately with positive strains and was complete in approximately 30 s. The technologist was unaware of the coagulase or other reactions of the organisms until after the completion of the latex text.

## RESULTS

A total of 806 isolates were tested by the latex agglutination technique; 357 of these gave positive reactions. A total of 351 of the positive latex reactors gave positive tests for either clumping factor or free coagulase. Negative latex reactions were obtained with 449 strains; 447 of these were also coagulase negative. The results are shown in Table 1, and the reactions of the discrepant strains are given in Table 2. One of the latex-positive strains and eight of the latex-negative strains had characteristics of *Micrococcus* spp. as determined by lysostaphin resistance

TABLE 1. Comparison of latex agglutination and coagulase tests with 806 clinical isolates of staphylococci

| Test | No. of latex slide agglutination reactions that were: | |
|---|---|---|
| | Positive (Total, 357) | Negative (Total, 449) |
| Clumping factor (slide test) positive | 311 | 0 |
| Free coagulase positive (negative slide test) | 40 | 2 |
| Total coagulase-positive | 351 | 2 |
| Total coagulase-negative | 6 | 447 |

and failure to grow or ferment glycerol in the presence of erythromycin. These nine strains were not included in the tabulation of results.

## DISCUSSION

Properties that characterize *S. aureus* include free or bound coagulase, the production of acid anaerobically from mannitol, the presence of heat-resistant endonucleases, a cell wall containing protein A and ribitol teichoic acid, and the production of alpha toxin (1). Teichoic acid analysis is usually not possible in the clinical laboratory, nor is alpha toxin generally used as the sole distinguishing characteristic of *S. aureus*.

Forsgren (5) noted that 99% of coagulase-positive staphylococci produce protein A, whereas only 2 of 100 coagulase-negative strains produce this protein. Maxim et al. (9) described a mixed agglutination test for the detection of bound staphylococcal protein A that utilizes sheep erythrocytes sensitized with rabbit hemolysin. Essers and Radebold (3) found that this test, which can be performed in 2 h, correlates over 99% with the coagulase reaction. These workers then described a latex slide test that simultaneously detects bound coagulase and protein A (4) in less than 1 min and is more accurate for the identification of *S. aureus* than a test for protein A alone. This slide test utilizes latex particles coated with human plasma. Since plasma contains both fibrinogen and immunoglobulin G (IgG), strains of staphylococci containing bound coagulase or protein A or both cause prompt agglutination of the latex particles.

The SeroSTAT Staphylococcus Test reagent employed in this study differed somewhat from that described by Essers and Radebold in that the latex particles were coated with rabbit plasma treated with EDTA.

Ninety-nine percent of the strains we tested showed agreement between tests for coagulase and latex agglutination; 98.3% of strains giving a positive latex test were coagulase positive, and 99.6% of latex-negative strains were also coagulase negative. Eight strains showed disparity between latex and coagulase reactions. Two of these strains were considered to be false-positive latex reactors in that they failed to ferment mannitol and gave negative reactions for thermostable nuclease. Two additional strains were evaluated as giving false-negative latex reactions since they fermented mannitol and produced thermostable nuclease. One strain was determined to be a coagulase-negative *S. aureus* since positive reactions were obtained for latex agglutination, thermostable nuclease, and fermentation of mannitol. The remaining three strains were intermediate in terms of their latex, coagulase, thermostable nuclease, and mannitol reactions and could not be clearly evaluated.

The two most widely used tests for distinguishing *S. aureus* from other staphylococci are for coagulase (8) and for the production of acid from mannitol. Several species of staphylococci can produce acid from mannitol under aerobic conditions (7). *Bergey's Manual of Determinative Bacteriology* (1) indicates that only *S. aureus* can produce acid from mannitol anaerobically; it has subsequently been shown that other species, including *S. simulans* and *S. hyicus*, can also ferment this carbohydrate (2, 6). The determination of mannitol dissimilation may take 1 or more days.

Coagulase determination is subject to a number of variables that may influence results, and consequently this criterion cannot be considered totally reliable. Clumping factor (bound coagulase) cannot be determined on strains that spontaneously agglutinate. We encountered 13 such

TABLE 2. Analysis of discrepant reactions between latex agglutination and coagulase tests

| No. of strains | Latex agglutination | Clumping factor | Free coagulase | Thermostable nuclease | Mannitol | Glycerol-erythromycin | Lysostaphin |
|---|---|---|---|---|---|---|---|
| 1 | + | − | − | + | − | No growth | Sensitive |
| 1 | + | − | − | + | Acid | Growth/acid | ND[a] |
| 2 | + | − | − | − | Acid | Growth/acid | ND |
| 2 | + | − | − | − | − | Growth/acid | ND |
| 2 | − | − | − | + | Acid | Growth/acid | ND |

[a] ND, Not done.

strains in our study, all of which gave positive tests for free coagulase. Omori and Kato (10) reported enterotoxin-producing isolates of staphylococci that spontaneously agglutinated and were negative for free coagulase.

Testing for free coagulase is also subject to uncertainty; false-positive results may be associated with certain plasmas (13), and false-negative or false-positive reactions may occur if the tests are incubated for 24 h (8, 13). There may be considerable subjective variation in the interpretation of reactions other than complete clotting (13). It has been suggested that the thermostable nuclease test be performed on all cultures with doubtful coagulase reactions before classifying them as *S. aureus* (11, 15).

The coagulase test in itself is not an absolute standard. Therefore, our results may overestimate the true sensitivity of the latex agglutination test. Coagulase-negative, latex-negative (protein A-negative) *S. aureus* strains would not be detected since only those strains with discrepant latex agglutination and coagulase tests were examined for thermostable nuclease activity.

Variation in the ability of clinical isolates of antibiotic-resistant staphylococci to produce coagulase has been reported (14). These strains, which were initially coagulase negative, became coagulase positive after repeated transfer.

Our results suggest that in most instances the latex slide agglutination test can confidently be substituted for the slide and tube coagulase tests as a method for identifying *S. aureus* in the clinical laboratory. The obvious advantage of the latex test is its simplicity and rapidity. Problems relating to the source of plasma and to the subjective interpretation of partial clots associated with the tube coagulase test are not encountered with the latex test. The cost per test of the latex reagent is essentially the same as the cost of plasma for the slide and tube coagulase tests. It would appear that the latex test, based upon clumping factor and protein A, is as reliable for the identification of *S. aureus* as is the use of tests for clumping factor and free coagulase.

ACKNOWLEDGMENT

The cooperation of Katherine Sprunt and Marion Hosmer of the Pediatric Bacteriology Laboratory in providing isolates is gratefully acknowledged.

LITERATURE CITED

1. **Buchanan, R. E., and N. E. Gibbons (ed.).** 1974. Bergey's manual of determinative bacteriology, 8th ed. The Williams & Wilkins Co., Baltimore, Md.
2. **Devriese, L. A., V. Hajek, P. Oeding, S. A. Meyer, and K. H. Schleifer.** 1978. *Staphylococcus hyicus* (Sompolinsky, 1953) comb. nov. and *Staphylococcus hyicus* subsp. nov. Int. J. Syst. Bacteriol. **28**:482–490.
3. **Essers, L., and K. Radebold.** 1980. Protein A hemagglutination test, a reliable method for rapid identification of *S. aureus*. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig. Reihe A **247**:170–176.
4. **Essers, L., and K. Radebold.** 1980. Rapid and reliable identification of *Staphylococcus aureus* by a latex agglutination test. J. Clin. Microbiol. **12**:641–643.
5. **Forsgren, A.** 1970. Significance of protein A production by staphylococci. Infect. Immun. **2**:672–673.
6. **Gramoli, J. L., and B. J. Wilkinson.** 1978. Characterization and identification of coagulase-negative, heat-stable deoxyribonuclease-positive staphylococci. J. Gen. Microbiol. **105**:275–285.
7. **Kloos, W. E., and K. H. Schleifer.** 1975. Simplified scheme for routine identification of human *Staphylococcus* species. J. Clin. Microbiol. **1**:82–88.
8. **Kloos, W. E., and P. B. Smith.** 1980. Staphylococci, p. 83–87. *In* E. H. Lennette, A. Balows, W. J. Hausler, Jr., and J. P. Truant (ed.), Manual of clinical microbiology, 3rd ed. American Society for Microbiology, Washington, D.C.
9. **Maxim, P. E., H. L. Mathews, and H. F. Mengoli.** 1976. Single-tube mixed agglutination test for the detection of staphylococcal protein A. J. Clin. Microbiol. **4**:418–422.
10. **Omori, G., and Y. Kato.** 1959. A staphylococcal food poisoning caused by a coagulase-negative strain. Bilken J. **2**:92.
11. **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.
12. **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.
13. **Sperber, W. H., and S. R. Tatini.** 1975. Interpretation of the tube coagulase test for the identification of *Staphylococcus aureus*. Appl. Microbiol. **29**:502–505.
14. **Thatcher, F. S., and W. Simon.** 1956. A comparative appraisal of the properties of "staphylococci" isolated from clinical sites and from dairy products. Can. J. Microbiol. **2**:703–714.
15. **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.