Reagent Strips and Conventional Tests for Acid Production from Mannitol and Coagulase Activity of Staphylococcus: a Comparative Study

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A total of 244 Staphylococcus strains were tested simultaneously for acid production from mannitol and for coagulase activity with reagent-impregnated paper strips and with their conventional counterparts. Significant correlation was obtained with 97.9% of the strains for mannitol and with 95% for the coagulase test. The paper strip method is a combined test for both mannitol and coagulase tests, thus making it more convenient and simpler than conventional methods. The results are obtained rapidly within 6 hr by the paper strip method. However, as the paper strip method is designed for the aerobic system, the conventional tests were also carried out under aerobic conditions to compare the results.

The purpose of this investigation was to compare the results obtained by conventional methods with those obtained by using commercially available reagent paper strips to test the ability of Staphylococcus to produce acid from mannitol and to test for coagulase activity. The words "acid production" are used deliberately rather than the word "fermentation," because the paper strip method is designed to be carried out under aerobic conditions.

This investigation was essentially confined to a comparison of the results obtained by a protocol suggested by the manufacturer of the paper strips with those of conventional methods and was not intended to examine the mechanism by which the reactions are brought about.

MATERIALS AND METHODS

The conventional tube method with citrated rabbit plasma for determining coagulase activity and phenol red broth with 1% mannitol for acid production was used. The tests were carried out aerobically to be compatible with the paper strip method.

The paper strips are impregnated with standardized human plasma over a zone at one end, followed by a zone which is a water barrier. Next is a zone which is impregnated with nutrient substrates, mannitol, and phenol red indicator. Next to this zone is a water barrier followed by an untreated zone. There is a color band at the end to serve as a code.

The methodology incorporates into one procedure the tests for acid production and coagulase activity. A uniform milky suspension of the test culture is made in 0.5 ml of saline in a test tube with a sterile applicator stick. The suspension must be free from clumps. The reagent strip is then introduced into the suspension to immerse the plasma zone. The tube is immediately tilted to wet the mannitol zone and quickly brought to the upright position. A plastic cap or cotton plug is placed on the mouth of the test tube to prevent excessive evaporation. The tubes are then incubated at 37 C. The coagulase activity is detected by the formation of macroscopically visible clumps when the tube is held against a concave mirror and gently agitated. Acid production is indicated by the color change from red to yellow. A color chart is provided to interpret the reaction by comparison which includes a pale yellow as positive and pale pink as negative for weak reactions. Most positive reactions may be observed within the first 4 hr; however, negative tubes should be incubated for a period of 6 hr, which is the maximum time limit for the test. Any results obtained after 6 hr are not valid.

A total of 244 cultures, freshly isolated from clinical specimens from various hospitals in the Knoxville, Tenn., area, were used in this study. The cultures were grown on a neutral agar such as blood-agar or Trypticase soy agar for 24 hr at 37 C and stored in a refrigerator for a period not exceeding 1 week if the tests could not be run immediately. The cultures were transferred to similar agar and grown at 37 C for 18 to 24 hr before testing. All cultures were checked...
for Gram reaction, cell morphology, aggregation, and catalase activity. The tests by the conventional method and by the paper strip method were run simultaneously. A series of tests was run on 50 isolates which were negative for coagulase and mannitol as negative controls. Fifty tubes, each containing 0.5 ml of plain saline and a paper strip, were also incubated at 37°C for the test period of 6 hr.

RESULTS

The results obtained by both the methods are summarized as follows. Of 244 tests, 232 showed complete correlation between methods, the P value being 0.951 which exceeds the 95% confidence limits. The 95% confidence limit for P was 0.945 < P < 0.957. Therefore, we would expect the paper strip test results to agree with test results obtained by the conventional method more than 95.7% of the time. The pattern of correlating tests was: coagulase and mannitol detected, 169; coagulase and mannitol not detected, 32; coagulase not detected, mannitol detected, 28; coagulase detected, mannitol not detected, 3.

Table 1 shows the comparison of test results on the basis of time taken to obtain the results. The time for detection was significantly shorter for the paper strip method, both for acid production and coagulase activity. The 95% confidence interval for the paper strip test mannitol was 1.91 to 2.13 hr as against 19.2 to 24.5 hr by the conventional method, and, for coagulase activity by the paper strip method, the 95% confidence interval was 0.69 to 0.95 hr as against 1.27 to 1.55 hr by the tube method.

The 12 tests which did not agree between methods are shown in Table 2. The results were identical when repeated. If we consider only acid production, the agreement between methods-the percentage of the time as seen in Table 2 would improve the correlation somewhat. If we consider coagulase activity alone, the correlation between methods will improve. The results on negative controls are tabulated in Table 3.

DISCUSSION

The paper strip method detects only the bound coagulase or the so-called clumping factor. If one is interested in bound coagulase alone, a slide test is much more rapid than the paper strip method. In addition, the paper strip method measures only the pH shift, which may be the result of acid production and not necessarily fermentation. If seen in Table 3, 14% of the paper strips showed a nonspecific reaction with either yellow or pale yellow. About 16% of the isolates which did not show any positive reaction in either mannitol broth or mannitol agar showed a similar nonspecific positive reaction with paper strips. The frequency of the nonspecific reaction is significant and calls for proper standardization of the paper strips to ensure specificity. Otherwise they will not be dependable. Since human plasma is one of the reagents, these strips must be carefully stored at refrigeration temperatures and protected against moisture.

The paper strip employs human plasma, whereas rabbit plasma was used in the tube test; this fact is considered to be related to the dozen discrepancies listed in Table 2.

The paper strip method is a combined test for both acid production and for coagulase activity by a single procedure, thus making it a more simple and convenient method than the conventional one.

| Test                     | Positive | Negative |
|--------------------------|----------|----------|
| Coagulase tube           | 0        | 50       |
| Mannitol-broth tube      | 0        | 50       |
| Mannitol-agar stab tube  | 0        | 50       |
| Paper strip coagulase    | 0        | 50       |
| Paper strip mannitol     | 8        | 42       |
| Paper strips in plain saline for color reaction | 7 | 43 |

* Number of isolates tested was 50.

Table 1. Comparison of test results

| Test                     | Mean (hr) | 95% Confidence interval (hr) |
|--------------------------|-----------|-------------------------------|
| Conventional mannitol    | 21.8      | 19.2-24.5                     |
| Conventional coagulase   | 1.4       | 1.27-1.55                     |
| Strip mannitol           | 2.0       | 1.91-2.13                     |
| Strip coagulase          | 0.8       | 0.69-0.95                     |

Table 2. Tests with no agreement between methods

| Strain no. | Mannitol | Coagulase | Mannitol | Coagulase |
|------------|----------|-----------|----------|-----------|
|            | Conventional | Paper strip |        |           |
|            | Mannitol | Coagulase | Mannitol | Coagulase |
| 9          | +        | +         | +        | -         |
| 30         | +        | -         | +        | +         |
| 42         | +        | +         | -        | +         |
| 50         | +        | +         | +        | -         |
| 70         | -        | -         | -        | +         |
| 98         | +        | -         | -        | +         |
| 103        | +        | -         | +        | -         |
| 108        | +        | +         | +        | -         |
| 154        | +        | -         | +        | -         |
| 177        | +        | -         | +        | -         |
| 237        | +        | -         | +        | -         |
| 248        | +        | -         | +        | -         |
It is worth mentioning here that the dissimilation of D-mannitol (anaerobic metabolism-fermentation) was recommended by Evans (1, 2) and Evans and Niven (3) as an important criterion for distinguishing *S. aureus*. These authors also demonstrated that the breakdown of this polyol under aerobic conditions is nonspecific. To make use of this criterion, Mossel and Martin (5) modified the method of Hugh and Leifson (4) for determining the fermentation of mannitol and demonstrated that 97% of the coagulase-positive staphylococcal strains fermented mannitol in 4 days (94.6% in less than 2 days).

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