Evaluation of Reagent-Impregnated Coagulase-Mannitol Test Strip for Speciation of Staphylococci

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A new coagulase-mannitol reagent-impregnated strip test has been evaluated with 322 *Micrococcaceae*. Mannitol fermentation was determined accurately by this test; however, the coagulase reaction was difficult to interpret and was subject to significant error.

The development of reagent-impregnated strips for identification of gram-negative bacilli has provided a simple alternative to several of the standard taxonomic tests. This report presents an evaluation of a recently introduced coagulase-mannitol test strip (Pathotec, W-6578, General Diagnostics Division, Warner-Chilcott Laboratories, Morris Plains, N.J.).

A total of 322 *Micrococcaceae* isolated from clinical specimens in the diagnostic bacteriology laboratory of the Mayo Clinic were studied. *Micrococcaceae* were identified as described in an earlier paper from this laboratory (2). Coagulase-mannitol test strip reactions were determined on 220 strains in parallel with (i) tube coagulase activity (Difco coagulase plasma), (ii) elaboration of deoxyribonuclease (DNase test medium, BBL), (iii) fermentation of mannitol and glucose (1% mannitol or glucose semisolid agar stab-tube overlaid with 3 to 5 cm of sterile mineral oil, incubated at 37 C for 48 hr), (iv) catalase activity, and (v) reduction of nitrate. Coagulase-mannitol reagent-impregnated strips were tested by adding a loopful (3 mm) of organisms to 0.3 ml of sterile saline in a sterile test tube (13 by 100 mm) to which the strip was added. The tube was tipped to moisten the upper mannitol fermentation zone and then incubated in the upright position at 37 C for 1 hr, after which the coagulase reaction was determined. A positive reaction was indicated by the formation of clumps in the saline suspension. Absence of clumping was indicative of a negative reaction. The tube was then incubated for an additional 3 to 5 hr. Mannitol fermentation was demonstrated by the formation of a yellow color in the mannitol fermentation zone; lack of fermentation was demonstrated by formation of a pink to red color in this zone.

An additional series of 102 staphylococci were studied with 0.5 ml (instead of 0.3 ml) of sterile saline in the coagulase-mannitol test strip tube, upon recommendation by the manufacturer. Tube coagulase activity was the only test run concurrently in this series.

The relationship of 220 mannitol fermentation reactions (stab-tube method) to the tube and reagent-impregnated strip coagulase tests is shown in Table 1. The reagent-impregnated strip coagulase test missed 12% of the tube-coagulase-positive strains which fermented mannitol (false-negative reactions). Of the 106 mannitol-negative strains, 13 (12%) were coagulase-positive by the reagent-impregnated strip only. These 13 strains were deoxyribonuclease-negative also and are therefore considered to represent false-positive reactions on the strip-coagulase test.

Table 2 shows the relationship of mannitol fermentation by the stab-tube method to that demonstrated by the reagent-impregnated strip. On the basis of stab-tube results, the reagent-impregnated strip showed 1% disagreement for positive reactions and 3.7% disagreement for negative reactions.

Similar results were obtained with the second series of 102 staphylococci as shown in Table 3.

Determination of coagulase activity remains the quickest and easiest method of speciating staphylococci in the clinical bacteriology laboratory. Although Branson (1) advocated routine use of other reactions in addition to the coagulase test, work previously reported from this laboratory (2) did not substantiate this need. Use of a coagulase-mannitol reagent-impregnated strip represents an attempt to facilitate the speciation of...
TABLE 1. Relationship of mannitol fermentation (stab-tube) to tube and reagent-impregnated-strip coagulase tests: first series

| Mannitol fermentation | Coagulase result |
|-----------------------|------------------|
|                       | Tube positive, strip positive | Tube negative, strip positive | Tube positive, strip negative | Tube negative, strip negative | Total |
| Positive...           | 13               | 0                | 95                  | 6                         | 114   |
| Negative...           | 0                | 13               | 2                   | 91                        | 106   |

staphylococci. It is apparent from the results of this study that the test does not necessarily represent an improvement. By combining the two series, 10% of the strains of *Staphylococcus epidermidis* would have been called *S. aureus* on the basis of a false-positive strip-coagulase test. In addition, 13% of the strains of *S. aureus* required completion of the mannitol fermentation reaction for proper species designation, since the strip-coagulase test was negative.

Although the strip-mannitol-fermentation test was shown to be accurate, there is serious question as to the value of its companion test. In our experience, the strip-coagulase test did not always give a clear-cut result, and it should be emphasized that the observations were all made by one of us. Therefore, it is likely that when observations are made by several persons the error with the strip-coagulase test would increase. A conventional slide coagulase test may be completed in a few minutes, and the agreement between the slide and tube coagulase tests has been shown in this laboratory to be 92% (2). In nearly all instances of coagulase-positive staphylococci, the tube coagulase test was positive within 1 hr, the time at which the strip coagulase was also to be read.

Our conclusion is that the coagulase-mannitol reagent-impregnated strip test may facilitate determination of mannitol fermentation but its companion coagulase test does not provide sufficient accuracy and ease of performance to be recommended at this time.

LITERATURE CITED

1. Branson, D. 1968. Identification of *Micrococcaceae* in clinical bacteriology. Appl. Microbiol. 16:906-911.
2. Person, D. A., P. K. W. Yu, and J. A. Washington, II. 1969. Characterization of *Micrococcaceae* isolated from clinical sources. Appl. Microbiol. 18:95-97.

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### TABLE 2. Relationship of stab-tube mannitol fermentation to reagent-impregnated-strip mannitol fermentation

| Tube method | Strip method |
|-------------|--------------|
|             | Positive | Negative |
| Positive... | 112      | 2a        |
| Negative... | 4b       | 102       |

a Both strains were tube-coagulase and deoxyribonuclease positive.
b These four strains were tube-coagulase and deoxyribonuclease negative.

### TABLE 3. Relationship of mannitol fermentation (reagent-impregnated strip) to tube and reagent-impregnated-strip coagulase tests: second series

| Mannitol fermentation | Coagulase result |
|-----------------------|------------------|
|                       | Tube positive, strip negative | Tube positive, strip positive | Tube negative, strip positive | Tube negative, strip negative | Total |
| Positive...           | 8                | 1                 | 1                    | 47                   | 57    |
| Negative...           | 0                | 4                 | 40                   | 1                    | 45    |