Identification of *Staphylococcus aureus* by Simultaneous Use of Tube Coagulase and Thermonuclease Tests

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A simple system is described for efficiently detecting both thermonuclease (heat-stable nuclease) and free coagulase for the routine identification of *Staphylococcus aureus* in the clinical laboratory. Valid discrepancies between these two tests are rare enough to permit the use of one test as a control of the other.

Most clinical laboratories depend entirely upon the coagulase test for distinguishing *Staphylococcus aureus* from other *Micrococcus* species. Inaccurate results with the coagulase test would lead to an identification error which could have serious clinical implications. Such technical errors could go undetected unless the procedure is controlled by use of a separate test to confirm each identification. For this purpose, the inexpensive, simple, and rapid toluidine blue deoxyribonucleic acid (DNA) agar (TDA) technique of Lachica et al. (2) was adapted to permit detection of thermonuclease (heat-stable nuclease) at the same time that a coagulase test is performed. The TDA technique is more specific than the conventional test of DNA agar plates because the heat-labile enzymes of *Staphylococcus epidermidis* and *Micrococcus* sp. (3) are inactivated before testing.

To obtain an inoculum dense enough for both tests, an isolated colony is transferred to 0.5 ml of brain heart infusion (BHI) broth in the morning when the primary plates are first examined. After 2 to 6 h at 35 C, the subcultures are tested along with positive and negative control strains. This short period of incubation in BHI not only provides an inoculum large enough for both tests but also permits elaboration of the enzymes coagulase and nuclease (4), improving the intensity and speed of positive reactions. A 0.5-ml volume of ethylenediaminetetraacetic acid coagulase plasma (Difco) is inoculated with 2 drops of the broth culture; the culture is also transferred to a section of a blood agar plate to provide growth if retesting is necessary. The remaining BHI broth culture is heated at 100 C for 15 min and allowed to cool, and a few drops are used to fill a 3-mm well cut into a TDA plate (either ca. 10 ml of TDA in a 9-cm petri plate or 3.0 ml in an immunodiffusion slide). The TDA medium is prepared by adding 0.3 g of DNA (Difco), 10 g of agar (Difco) 1.0 ml of 0.01 M CaCl₂, 10.0 g of NaCl, and 3.0 ml of 0.1 M toluidine blue O to 1 liter of 0.05 M tris(hydroxymethyl)aminomethane buffer (pH 9.0). After 1 to 4 h at 35 C, the TDA plates are observed for a definite pink halo around each well, and the coagulase tests are read for a definite coagulum. If the work schedule of the laboratory does not permit final observation of the tests after 4 h, they may be held overnight and read the next day.

Over the past 19 months, 3,400 clinical isolates were tested in the diagnostic laboratory by the rapid techniques outlined above. In addition, the unheated broth cultures were spotted onto DNA agar (Difco) and, after 16 to 18 h at 35 C, 1 N HCl was added to precipitate unhydrolyzed DNA (1). As long as the inoculum was prepared by preincubation in BHI, there was excellent agreement between all three tests (Table 1). About 2% of the initial tests gave weak, equivocal results with one or more tests, and occasionally the tests disagreed completely. Further study with those isolates giving discrepant results almost always revealed technical difficulties with the initial tests. Most of the difficulties occurred when the cultures being tested contained bacteria other than the *Micrococcaceae*. In addition, considerable difficulty was encountered initially be-
TABLE 1. Characteristics of 3,400 Micrococcaeae recovered from clinical material

| Total no. of isolates | Results of initial tests* |
|-----------------------|---------------------------|
|                       | Tube coagulase | Heated nuclease | Unheated DNA agar |
| 1,616                 | +             | +               | +                 |
| 3                     | -a            | +               | +                 |
| 1,721                 | -             | -               | -                 |
| 60                    | -             | -               | +                 |

*a Isolates initially showing discrepant results were not included if repeat testing demonstrated technical difficulties with the initial tests.

All three strains gave positive slide coagulase tests.

cause the slide coagulase test was being relied upon too heavily. This procedure has been relegated to the role of a quick method for issuing a preliminary report in clinically urgent situations where an abundant amount of growth is available for testing from the primary plates. Also, the slide technique is used regularly for checking subcultures of those isolates which show discrepancies between the tube coagulase and thermonuclease tests.

From our present data, one could conclude that either the coagulase or thermonuclease test may be used for routine identification of *S. aureus*, providing that the inoculum is prepared by preincubation in a small volume of BHI. With the system described in the present report, very little additional effort would be required to routinely perform both tests on all isolates. Because false negative or false positive tests could not be detected with only one test, and because weak equivocal results are sometimes obtained with one or the other test, the routine use of both tests is recommended for the clinical laboratory. Occasionally some isolates will give equivocal results or discrepancies between tests, and these are the very ones which need to be examined further to rule out the possibility of technical error. In this sense, the use of two tests for identification of *S. aureus* can be viewed as a quality control measure which is unnecessary most of the time but very reassuring when difficulties are uncovered.

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