Rapid Method of Determining Coagulase Activity During Staphylococcal Bacteriophage Typing

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ABSTRACT

HIGHSMITH, ANITA K. (Communicable Disease Center, Atlanta, Ga.), and EMMETT B. SHOTTS, JR. Rapid method of determining coagulase activity during staphylococcal bacteriophage typing. Appl. Microbiol. 13:34–36. 1965—The plate test, a modification of the slide test described by Cadness-Graves was developed for the rapid identification of coagulase-positive staphylococci in conjunction with bacteriophage typing. An evaluation of 1,145 cultures by three coagulase-determination methods, the slide, tube, and plate tests, indicates that the plate test is as accurate as the slide tests, and the plate test agrees 97.7% with the tube test.

In the past decade, the epidemiologist has increasingly called upon the diagnostic laboratory to furnish confirmatory information during the investigations of epidemic situations. This aid often takes the form of the rapid processing of large numbers of specimens, and demands placed upon the laboratory often necessitate the modification of techniques to meet the need for immediate information. Such a situation prompted the modification of an existing technique for the detection of coagulase-positive staphylococci.

Coagulase activity is considered the most reliable in vitro criterion for the identification of pathogenic staphylococci (Blair and Williams, 1961). Two tests are routinely used to determine this activity: the first of these, the tube test (Smith and Hale, 1944), is considered as the confirmatory test for determining coagulase activity; the other, a more rapid test, is generally designated as the "clumping factor," or slide test (Cadness-Graves, Harper, and Miles, 1943), based on the clumping of coagulase-positive staphylococci in the presence of plasma.

This report will describe a modification of the slide test and an evaluation of this modified technique for detection of coagulase-positive staphylococci. The procedure was especially designed for use in conjunction with bacteriophage typing of large numbers of staphylococcal cultures, and will be referred to as the "plate" test. Both the phage pattern and the coagulase activity of a given culture are determined simultaneously on the typing plate.

The final identification of coagulase-positive Staphylococcus aureus strains is thereby available sooner than it would be if the tube coagulase or the slide tests were performed before bacteriophage typing.

MATERIALS AND METHODS

Specimens. The staphylococcal cultures used in the evaluation of this technique were of human origin and were isolated either during epidemic situations, or in conjunction with experimental studies conducted in the Veterinary Public Health Laboratory, Communicable Disease Center. These cultures included various pigment types. All initial isolations were made on Trypticase Soy Agar (TSA; BBL). After initial isolation, representative colonies to be typed with bacteriophage were transferred to Trypticase Soy Broth (TSB), and incubated at 37°C for 4 hr. At this time, TSA plates were inoculated from these TSB cultures to provide the "lawn" on which the phages were placed (Blair and Williams, 1961).

Coagulase tests. Three methods were used for the determination of coagulase activity: the conventional tube test, described by Smith and Hale (1944); the slide clumping factor, or slide test (Cadness-Graves et al., 1943); and the modified clumping factor, or the plate test.

Tube test. The test was performed by the addition of 0.1 ml of a 12 to 18-hr-old broth culture of staphylococci to 0.5 ml of rabbit plasma (Difco) in a sterile tube (13 x 100 mm). The tube was then incubated at 37°C for 3 hr. A positive reaction was indicated by any degree of clotting in the mixture.

Slide test. The slide test was performed as described by Cadness-Graves et al. (1943). A small portion of a staphylococcal colony was mixed in approximately 0.05 ml of water on a slide; a loopful of rabbit plasma was added and the components
RESULTS

A total of 1,145 staphylococcal cultures were examined for coagulase activity. The cultures were divided into two groups: the first group of 429 was examined by both the slide and the tube tests, and by the plate test; the remaining 716 were tested by the plate and tube methods.

Of the 429 cultures in group 1, the same results were obtained by the plate test and the tube test on 422 (98.4%), and by the plate and slide tests on 426 (99.3%) (Table 1). Two cultures were positive by the plate test, but were negative by the slide test. One culture was negative by the plate test, but positive by the slide test. This suggested that the plate and slide tests were equally sensitive, and that both might be expected to occasionally show a doubtful reading when compared with the tube test which is considered positive if any amount of clumping is obvious after a 3-hr incubation.

The second group contained 716 cultures collected during experimental studies in which recovery of a high percentage of coagulase-positive staphylococci was expected. In view of

Fig. 1. Positive plate test. Coagulase-positive staphylococcal strain nonreactive to international set of bacteriophages used for test.

were mixed thoroughly. A suspect colony was considered to have coagulase activity if clumping of the bacterial cells occurred within 5 to 20 sec. If clumping is delayed or appears doubtful, the reaction must be confirmed by the tube test.

Plate test. This test utilizes the principle of the slide test and was developed as a result of the need for a rapid technique to process large numbers of staphylococcal cultures in conjunction with bacteriophage typing. At the time the "phage" plate was inspected for the presence of lysis, it was also tested for coagulase activity in the following manner. In an area of the plate where no phage was placed, a loopful (approximately 0.05 ml) of undiluted rabbit plasma was placed on the bacterial "lawn," and the plasma was agitated with a circular motion to create a suspension of cells. A positive reaction was indicated by a distinct clumping of the plasma-bacterial mixture (Fig. 1 and 2); this should take place within 10 to 20 sec for a suspect culture to be considered coagulase-positive.

The above techniques were compared to determine the suitability of the plate method as a replacement for the tube or slide tests to determine coagulase activity.

Fig. 2. Negative plate test. Coagulase-negative staphylococcal strain nonreactive to international set of bacteriophages used for test.
the findings in the first group of cultures, only the tube and plate tests were used for comparison. Identical reactions were observed in 695 (97%) of the 716 cultures tested (Table 2). Of the 21 cultures in which results did not agree, 18 were positive only by the tube test, and 3 were plate-positive only.

When the results of tube and plate tests on both groups of 1,145 cultures were summarized and compared, 97.5% agreement was observed between the two tests.

**Table 1. Comparative findings on 429 staphylococcal cultures tested for coagulase activity by the tube, slide, and plate methods**

<table>
<thead>
<tr>
<th>Plate test results</th>
<th>Tube test results</th>
<th>Slide test results</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>Positive</td>
<td>166</td>
<td>4</td>
</tr>
<tr>
<td>Negative</td>
<td>3</td>
<td>256</td>
</tr>
</tbody>
</table>

* Percentage agreement between tube and plate tests was 422/429 (98.4%). Percentage agreement between slide and plate tests was 426/429 (99.3%).

**Table 2. Comparative findings on 716 staphylococcal cultures tested for coagulase activity by the tube and plate methods**

<table>
<thead>
<tr>
<th>Plate test results</th>
<th>Tube test results</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>Positive</td>
<td>288</td>
<td>3</td>
</tr>
<tr>
<td>Negative</td>
<td>18</td>
<td>407</td>
</tr>
<tr>
<td>Total</td>
<td>306</td>
<td>410</td>
</tr>
</tbody>
</table>

* Percentage agreement between plate and tube tests was 695/716 (97.1%).

**Discussion**

Some cultures may show very low degrees of coagulase activity. This might possibly account for the lack of correlation seen in some instances during this study. From the results obtained, it appears that the slide and plate tests are of equal merit for screening procedures, but neither is as sensitive as the tube test. However, the reaction seen in the plate method is rapid, distinct, and specific. The clumping of the cells in a positive reaction is similar to that observed in the slide test. The dilution of cells when mixed with water for the slide test often reduces the clarity of this reaction; such is not the case with the plate test. The plate test can be performed in conjunction with the phage typing operation and without the extra manipulations necessary in the slide or tube tests; this makes it very useful for processing large numbers of cultures.

To minimize the processing of cultures other than staphylococci, care should be exercised in the selection of colonies to be processed by this procedure. Particular attention to pigment production was found to be helpful in detection of staphylococcal colonies.

**Acknowledgment**

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**Literature Cited**

