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NOTES

Rapid Capillary Tube Method for Detecting Penicillin Resistance in *Staphylococcus aureus*

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A simple, rapid capillary tube test to discriminate between penicillin-resistant and -sensitive *Staphylococcus aureus* is presented. This test detects penicillinase in noninduced primary isolates from blood-agar plates.

A rapid test for *Staphylococcus aureus* susceptibility to penicillin is described. The procedure depends upon the ability of penicillin-resistant strains of *S. aureus* to produce penicillinase (EC 3.5.2.6) (3). This penicillinase can be assayed by detecting the increase in acidity produced when it converts penicillin to penicilloic acid. In a test proposed recently by Adams et al. (1), an isolated colony of *S. aureus* is grown for 5 to 6 hr in the presence of a penicillinase inducer, and then an acid-base indicator is added in a soft agar overlay. Finally penicillin is added, and a violet color develops within 1 hr with penicillinase-producing strains. In the test described here the need for inducing the enzyme has been eliminated; therefore the test is more rapid.

The test is performed in the following manner. A solution is prepared by adding 16.6 ml of water and 2 ml of 0.5% phenol red solution to a vial containing 20 million units of penicillin G (buffered potassium penicillin G for infection, U.S.P., E.R. Squibb and Sons, New York, N.Y.). Sodium hydroxide (1 M) is added dropwise (~0.5 ml) until the solution just turns violet (*p*H 8.5). This test solution is divided into portions in glass tubes. Those tubes not used on the day of preparation may be stored frozen at −20 C for as long as 1 week. Thawed tubes may be kept at 4 C for an entire working day. Longer storage periods are not advisable due to the possible hydrolysis of penicillin. As the penicillin is hydrolyzed either in the presence or absence of penicillinase, the color of the solution changes from violet or deep red (basic form of phenol red) to yellow (acid form). Thus the color of this solution is an adequate indicator of the suitability of these solutions for use after storage. A high concentration of penicillin is employed in this test because it had previously been noted that even strains of *S. aureus* that were low penicillinase producers could be detected by a similar test if the penicillin concentration was great enough (4).

The test is performed by dipping a capillary tube (blue tip coagulation capillary tubes, 0.5 to 0.9 mm ID; Sherwood Medical Industries, Inc., St. Louis, Mo.) into the test solution and allowing the liquid to flow by capillary action for a distance of 1 cm into the tube. The tip of the capillary is then scraped across a colony of *S. aureus* on a blood-agar plate, with care that no air is trapped between the bacteria and the solution, so that the bacteria form a plug in the bottom of the tube in contact with the solution. The tube is incubated at room temperature in a vertical position in such a manner that the bottom of the tube containing the plug of bacteria is not in contact with a solid surface. If the bacteria or the bacteria and solution turn yellow within 1 hr, the bacteria are penicillinase producers and are thus resistant to penicillin.

To determine the accuracy of this procedure, we subjected 330 strains of *S. aureus* and 14 strains of *S. epidermidis* to this test, the Kirby-Bauer test (2), and the Adams proce-

dure (1). The same 42 *S. aureus* and 4 *S. epidermidis* strains were sensitive to penicillin by all these procedures. The remaining 298 strains were resistant by all three procedures.

This test can also be used in the manner described by Adams et al. (1) when indeterminable results are obtained by the Kirby-Bauer technique. The test sample can be obtained from bacteria which have grown nearest to a disc of a penicillinase inducer (dicloxacillin, cephalothin, nafcillin, oxacillin, or methicillin) on the Mueller Hinton plate. In this case, since one is working with an induced culture, most resistant strains give a positive result after 5 min, though we generally still wait 1 hr before reading the results.

It should be emphasized that, although the vast majority of penicillin-resistant staphylococci owe their resistance to their ability to produce penicillinase (3), some strains can be resistant for other reasons (5). This test rapidly screens isolates of *S. aureus* for penicillinase production. However, one should always take the precaution to run a test that depends upon inhibition of growth in order to be sure one is not dealing with a rather rare methicillin-resistant strain.

This test offers a number of advantages over preceding tests (1, 2, 4) for the rapid detection of penicillin resistance in *S. aureus*. It is as accurate as the other tests but much faster, since no further growth is required after the initial isolation of the bacteria on a blood-agar plate. It is less expensive and easier to perform than the test by Adams et al. (1), since the need for petri plates and soft agar overlays is eliminated.

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