Three Rapid Methods Compared with a Conventional Method for Detection of Urease Production in Anaerobic Bacteria

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Three rapid methods (spot test, disk, and tube) for detecting urease production in anaerobic bacteria yielded results faster than the conventional method. The results were more consistent with the disk and tube methods than with the spot test. Blood agar plate growth gave more consistent results than growth from chopped-meat slants.

Conventional microbiological test tube assays are costly and time-consuming, particularly when a laboratory performs a large number of tests. On the other hand, rapid differentiation tests are cost-effective in terms of materials and labor expended per assay. The utility of these tests, however, depends on results comparable to those of traditional methods as well as on the degree of reproducibility.

The test for urea hydrolysis by anaerobes is usually performed by a test tube method in most laboratories. Because of the drawbacks of the conventional method, an attempt was made to find an alternative method. To this end, three rapid urea hydrolysis tests were examined and compared with the test tube assay.

All organisms used in this study were from the American Type Culture Collection except for a number of strains of Clostridium sordellii, which were provided by Vurus R. Dowell of the Centers for Disease Control, Atlanta, Ga. Prior to testing, organisms were grown on sheep blood agar plates and chopped-meat agar slants (2). All were incubated for 48 h at 37°C in an atmosphere of 80% N2, 10% CO2, and 10% H2. Type or neotype strains of all the species examined, with the exception of C. beijerinckii, C. pasteurianum, C. sphenoides, and C. tetanomorphum, were included in the study.

Urease activity, as determined by conventional method, was detected by measurement of the terminal pH of inoculated PY urea broth (2). A pH of 8 or higher was considered positive. The medium was prepared by adding 0.3 ml of concentrated urea broth (66 g of urea broth [Difco Laboratories, Detroit, Mich.] added to 100 ml of distilled water and filter sterilized) to 5 ml of prereduced sterile PY broth. The inoculated medium was examined at 24-h intervals for 7 days. For the spot test (3), a 10% (wt/vol) solution of urea agar base (0283; Difco) in distilled water was prepared each month and stored at 4°C. Filter paper (no. 2; Whatman Inc., Clifton, N. J.) was cut to fit a 150-mm petri dish, and 2-cm squares were drawn on the paper with a pencil; 3.3 ml of urea agar base was pipetted onto the paper, and each square was inoculated with a loop of one of the strains being tested. A change in the color of the paper from yellow to dark pink was taken as evidence of urea hydrolysis.

When Urea Differentiation Discs (1625-23; Difco) were employed, a loop of cells was suspended in 0.4 ml of sterile distilled water. A disk was placed in the suspension and incubated at 37°C for up to 4 h. A change in the color of the suspension from pale yellow to dark pink was interpreted as indicating urea hydrolysis.

The rapid tube assay employed is the method of Barry et al. (1). The urea reagent utilized contained 4.0 g of urea, 104.0 ml of 0.01% KH2PO4, and 8.0 ml of cresol red indicator. The indicator solution contained 0.1 g of cresol red, 13.1 ml of 0.02 N NaOH, and 237.0 ml of distilled water. The urea reagent was prepared each month and stored at 4°C. A loop of inoculum was placed in 0.3 ml of urea reagent in a test tube (132 by 100 mm). The sample was incubated at 55°C for 4 h. A positive reaction was indicated by a reddish to purple color; a negative reaction was indicated by yellow.

The strains of anaerobes tested in which no urease production was detected by any of the methods used are listed below. The number of strains of a species found to test negative is given in parentheses; no number is given if only one strain of a species was tested. Uniformly urease-negative strains were Acidaminococcus fermentans (three strains), Actinomyces bovis, A. israelii (two strains), Bacteroides corporis, B. ovatus, B. thetaiotaomicron, Bifidobacterium adolescentis, B. animalis, B. asteroides, B. boum, B. breve, B. catenulatum, B. chelonium, B. cuniculi, B. dentium, B. longum, Coprococcus gignivalis, C. ochracea, C. sputigena, Clostridium beijerinckii, C. bifermantans (five strains), C. ghoni, C. pasteurianum, C. perfringens (three strains), C. septicum, C. sphenoides, C. subterminale, C. tetanomorphum, Eubacterium aerofaciens, E. alactolyticum, E. biforme, E. limosum, E. moniliforme, Fusobacterium necrophorum, F. nucleatum (two strains), Leptotrichia buccalis (three strains), Megasphaera elsdenii (three strains), Peptostreptococcus anaerobius, P. asaccharolyticus, P. heliotrichreducens, P. indolicus, P. magnus (two strains), P. micros, P. productus, Veillonella atypica, V. criceti, V. dispar, V. ratti, and V. rodentium.

Some organisms that produce ammonia from peptone, notably C. perfringens, were tested, and these organisms did not give false-positives. In some instances, a pale orange color developed after the addition of the Difco Disc to the bacterial suspension, and the intensity of the color did not change. This was interpreted as a negative reaction. The three rapid methods detected urease hydrolysis in a larger number of anaerobes than does the conventional method (Table 1). The disk and rapid tube methods were found to give equivalent results and gave more consistent results than

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did the spot test. The spot test was also the most difficult to interpret because of the faintness of the resulting color. Although the tube assay and disk methods gave comparable results, the disk method is recommended because reagents need not be prepared monthly. Results presented in Table 1 also indicate that organisms grown on blood agar were more consistent in giving a positive urease response than the same bacteria grown on chopped-meat agar slants.

Two of five strains of Actinomyces viscosus and one of five strains of Bifidobacterium infantis tested were urease negative. These three negative organisms were not examined for urease activity at the time of receipt. Therefore, we do not know whether they ever possessed this activity. It is, however, uncommon for anaerobes to lose urease after isolation (4). It is also interesting to note that Wozny et al. (4) found urease in P. productus, whereas none of the methods employed in this study detected it in a strain of the same species.

The above data demonstrate that the detection of urea hydrolysis by anaerobic bacteria with Differentiation Discs and the rapid tube test is reliable. The results are yielded more quickly than by the conventional method, and the Differentiation Discs can be used as an acceptable replacement for the conventional tube test.

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