Letters to the Editor

Evaluation of Wet-Prep Motility Test for Presumptive Identification of Bacillus Species

During the month of October 2001, several cases of anthrax were discovered in the eastern part of the United States. In view of the heightened concern about bioterrorism events, our laboratory initiated procedures for the identification of suspect organisms, including Bacillus anthracis. We used a recommended algorithm for the presumptive identification of B. anthracis (http://www.asmusa.org/pcsrc/ban.asm.la.cp.102401f.pdf). The protocol outlines basic information concerning anthrax and the handling of clinical specimens, as well as about several differential tests for the presumptive identification of B. anthracis. These tests include colony morphology on 5% sheep blood agar (SBA) plates and Gram stain morphology. In addition, it is recommended that a motility test be performed. Most Bacillus species are motile, whereas B. anthracis is non-motile.

In our laboratory we are using B. subtilis ATCC 6633 (Subtilis Spore Suspension; Difco Laboratories, Detroit, Mich.) as a quality control strain because of the similarity of B. subtilis colony morphology to the B. anthracis morphology on SBA. After incubation for 15 to 24 h at 35 to 37°C in an ambient environment, the B. subtilis strain appears nonhemolytic and has a flat, irregular round colony with a ground glass appearance. The wet-prep protocol recommends that a suspicious colony from a 12- to 20-h culture be suspended in a glass tube containing approximately 0.1 ml of sterile distilled water. One drop of the suspension is transferred to the microscope slide and overlaid with the cover glass. The slide is examined under a microscope with the 40× objective. The protocol suggests that Pseudomonas aeruginosa ATCC 35032 or an equivalent strain be used as a positive control.

The bacterial suspension from an overnight SBA culture of the B. subtilis was considered immobile when prepared in distilled water as outlined in the laboratory protocol. When the suspension was made using trypticase soy broth (TSB), directional motility was clearly evident in every field. In the distilled water preparation, an occasional organism could be visualized that exhibited slight tumbling, but directional movement of individual organisms was not seen. The P. aeruginosa control strain was motile in both preparations.

Several clinical isolates of Bacillus spp. from blood cultures and American Type Culture Collection strains were also tested. The ATCC strains included Bacillus cereus ATCC 11778, B. cereus ATCC14579, and B. subtilis ATCC 9372. The last strain is used for sterility control tests in most hospitals and laboratories. None of these organisms had the classical morphology of B. anthracis after overnight incubation on SBA plates and normally would not be examined. The B. cereus isolates were motile in the distilled water and TSB, whereas the B. subtilis isolate was only motile in TSB. Of the eight miscellaneous clinical Bacillus isolates, five were motile in both suspensions, two were nonmotile in both suspensions, and one was motile in TSB only.

The fact that certain organisms are motile in broth medium but immobilized in distilled water was previously reported. Chester and Poulos (1) demonstrated that Vibrio and Campylobacter species were immobilized in distilled water but were fully motile in TSB. Because of the possibility of a false-negative result with the wet-prep motility test, we recommend that all motility tests be performed using TSB broth in place of distilled water.

Reference

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