
【ABSTRACT】We developed a new test method to determine pyrazinamide (PZA) susceptibility for Mycobacterium tuberculosis in an acidified Middlebrook 7H9 broth (pH 6.0), and evaluated in comparison with the agar proportion method of the National Committee for Clinical Laboratory Standards (NCCLS) M24–T and with pyrazinamidase assay. The test method is based on a culture in 4ml of the modified Middlebrook 7H9 broth containing 100, 200 and 400 μg PZA/ml, respectively. First, the cell suspension was adjusted to a McFarland #1 turbidity, and then diluted 1:10. After mixing, 0.1ml of the diluted cell suspension was inoculated and incubated at 36±1℃ in an ambient air. After 7 day-incubation, the test broth was read in comparison with the growth control. When a significant growth at 100 μg PZA/ml or an attenuated growth at 100 μg PZA/ml but a significant growth at 400 μg PZA/ml were observed, the test isolate was interpreted as being PZA-resistant. When PZA-susceptible and PZA-resistant ATCC reference strains were repeatedly tested, the results obtained were highly precise and accurate. A total of 65 clinical isolates were tested, the results indicating 95.4% of agreements with the agar proportion method and 90.8% with pyrazinamidase assay. There found six discrepant results of 13 resistant isolates; three were susceptible by the agar proportion and all the six were positive by pyrazinamidase assay. Accordingly, we can conclude that, in place of radiometric Bactec System, our newly developed test method is an accurate, practical, rapid and nonradiometric alternative to determine PZA susceptibility for M. tuberculosis in clinical mycobacteriology laboratories.

Key Words: pyrazinamide (ピラジナマイド), Middlebrook culture media (ミドルブルック合成培地), pyrazinamidase (ピラジナミダーゼ), antimycobacterial susceptibility (結核菌薬剤感受性試験)