The Updated MIC Determinations for Mycobacteria

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In the last three years, we developed three different antimycobacterial susceptibility tests based on broth dilutions. These test methods are presently applied to the clinical laboratories in Japan, and enable us to provide physicians with rapid and quantitative MIC determinations.

First, susceptibility test for pyrazinamide (PZA) has an inherent problem. The test requires acidic condition for antituberculosis activity of pyrazinoic acid, however acidic broth condition greatly inhibits mycobacterial growth. In the macro (4 ml) dilution test we developed, the pH of Middlebrook 7H9 broth is adjusted to 6.0, and the test isolate is incubated in the presence of 100, 200, and 400 ug/ml of PZA. The test results highly correlate with the determination of PZase.

Secondly, we developed broth microdilution antimycobacterial susceptibility test, BrothMIC MTB. Each test well contains 0.2 ml of the modified Middlebrook 7H9 broth, and the MICs against eight antimycobacterial agents are visually read after 7-day incubation. The test results are highly precise and comparable to the interpretive results by the standard agar proportion method (National Committee for Clinical Laboratory Standards; NCCLS).

Lastly, we developed BrothMIC NTM based on the microdilution test for nontuberculous mycobacteria (NTM). The test method utilized air-dried microplates containing serially diluted antimicrobial agents and the modified Middlebrook 7H9 broth. After inoculation of cell suspension in distilled water, the isolates resulted in incubation with clarithromycin at pH 7.4, and with the remaining agents at pH 6.6. The growth endpoints were visually read after 7 to 10-day incubations for slowly growing NTM, and after 3 to 5-day incubations for rapidly growing NTM, respectively. The precision of BrothMIC NTM is somewhat inferior when compared to the BrothMIC MTB due to the trailing of endpoints. However, this newly developed test method will provide a practical, rapid, quantitative means to determine susceptibility for NTM in clinical laboratories.