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Comparison of the ESP and BACTEC Systems for Testing Susceptibilities of *Mycobacterium tuberculosis* Complex Isolates to Pyrazinamide

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Fifty isolates of *Mycobacterium tuberculosis* complex were tested for susceptibility to pyrazinamide (PZA) by the ESP (Trek Diagnostic Systems, Westlake, Ohio) and BACTEC (BD Biosciences, Sparks, Md.) test systems. Initial results showed concordance for 48 of the isolates. On retest, the two discordant isolates were resolved in favor of the ESP system. The ESP Myco susceptibility test system generates rapid, reliable PZA test results.

The emergence of drug-resistant *Mycobacterium tuberculosis* in the United States led to the recommendation to use a four-drug regimen to treat active tuberculosis (1). Included in this regimen was pyrazinamide (PZA). At that time, the need for the rapid reporting of isolate identification and susceptibility test results became evident (6). Until recently, the radiometric method (BACTEC 460; BD Biosciences, Sparks, Md.) was the only rapid method available to test for PZA susceptibility among isolates of *M. tuberculosis*. Subsequently, the ESP Culture System II (Trek Diagnostic Systems, Westlake, Ohio) received Food and Drug Administration clearance for antitubercular susceptibility testing on three of the primary antitubercular drugs (isoniazid [INH], rifampin, and ethambutol). This system measures the change in gas pressure in the headspace of the culture bottle, which indicates microbial growth. Since mycobacteria utilize oxygen for metabolism, this system yielded rapid results in mycobacterial susceptibility tests. In this study, 45 isolates of *M. tuberculosis* and 5 isolates of *Mycobacterium bovis* were assayed for PZA susceptibility by using the ESP Culture System II and results were compared to those generated by the radiometric method.

Isolates used in this investigation were obtained from clinical specimens sent to the microbiology laboratory for routine mycobacterial culture. Isolates were identified as *M. tuberculosis* complex by using the AccuProbe test (Gen-Probe, San Diego, Calif.), and species were further determined by the nitrate reduction and niacin accumulation tests. Clinical isolates were identified directly from positive ESP Myco bottles as previously described (4). In addition, multidrug-resistant clinical isolates stored frozen at −70°C and isolates of *M. tuberculosis* complex contained in the College of American Pathologists and New York State proficiency tests were also included in the study. A total of 18 characterized survey isolates and 32 clinical isolates were employed in this study. The survey specimen numbers were NYS 9821, NYS 9842, NYS 9843, NYS 9876, NYS 9941, NYS 0061, NYS 0062, NYS 0063, NYS 0082, NYS 0083, NYS 0121, NYS 0124, NYS 0141, NYS 0142, NYS 0143, CAP E5 (Feb. 1998), CAP E11 (Oct. 2000), and CAP E5 (Feb. 2001). There were three isolates tested that were multidrug resistant. The resistance profiles included an isolate resistant to INH, rifampin, ethambutol, and PZA; one resistant to INH, rifampin, and PZA; and a third that was resistant to INH and PZA.

In the ESP susceptibility test system, lyophilized PZA powder (Trek Diagnostics) was rehydrated with 25 ml of PZA reconstitution fluid according to the manufacturer's instructions, and aliquots were frozen at −70°C. One milliliter of the reconstituted drug was inoculated into an ESP Myco bottle containing 1.0 ml of growth supplement (GS). The GS is supplied as a solution containing oleic acid, bovine serum albumin, dextrose, and catalase. The final concentration of PZA in the test bottle was 300 μg/ml. One milliliter of the rehydration fluid was inoculated into the drug-free control bottle. *M. tuberculosis* H37Rv was utilized as a pan-susceptible control each day of testing.

The primary ESP Myco bottle served as the inoculum source for all of the routine, nonfrozen, clinical isolates and proficiency test isolates. Positive bottles may be used as the inoculum source up to 72 h after producing a positive signal. An aliquot was removed from the ESP Myco bottle and diluted 1:10 with normal sterile saline. A 0.5-ml aliquot of this suspension was used to inoculate the drug-containing and control bottles. For frozen clinical isolates, a subculture was made onto Middlebrook 7H11 agar plates. From the resulting growth, a suspension equivalent to 1.0 McFarland standard was prepared in normal sterile saline and 0.5 ml was inoculated into an ESP Myco bottle containing GS. The bottle was incubated and monitored in the ESP instrument until it generated a positive signal. This now served as the inoculum source as outlined above.

The control and drug-containing bottles were placed into the ESP instrument, where they are monitored for changes in gas pressure. An isolate is considered resistant to PZA if the drug-containing bottle signals positive within 3 days of the control bottle becoming positive. Conversely, an isolate is reported to be susceptible to PZA if the drug-containing bottle fails to signal within 3 days of the control becoming positive. An ali-

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TABLE 1. Comparison of 50 *M. tuberculosis* complex isolates tested in the ESP and BACTEC PZA test systems

BACTEC result	No. of isolates from ESP that were:	
	PZA susceptible	PZA resistant
PZA susceptible	41	1
PZA resistant	1	7

quot from all positive bottles is smeared and stained by the Kinyoun method to confirm the presence of acid-fast bacilli and rule out contamination.

The radiometric susceptibility test method was conducted at the same time as the ESP method. All isolates were initially inoculated into a BACTEC 12B seed bottle regardless of the isolate source. The seed bottle was monitored daily for growth in the BACTEC 460 instrument. The seed bottle was used as the inoculum source when the growth index (GI) reached a value of ≥300. The test was performed as per the manufacturer's recommendations with a final PZA concentration of 100 μg/ml in the test bottle (package insert for BACTEC PZA test medium culture vials, Dec. 1998.). The bottles were assayed daily and the test was interpreted at the point when the GI in the control bottle first registered ≥200. An isolate was considered to be susceptible to PZA when the GI of the drug-containing bottle was <9% that of the control bottle. An isolate with a value of >11% was considered resistant, whereas a value between 9 and 11% was considered borderline. Any isolate yielding discordant results was retested by both methodologies.

The results of the initial testing are shown in Table 1. Of the 50 isolates of *M. tuberculosis* complex, 41 were susceptible to PZA in both test systems and 7 were resistant in both systems. Four of five isolates of *M. bovis* were PZA resistant by the radiometric method, while five of five isolates were resistant by ESP. There were two discordant results, one indicating resistance to PZA in the ESP system and, initially, susceptibility by the radiometric method and one that indicated susceptibility to PZA in the ESP system and, initially, resistance by BACTEC. On repeat testing the results generated by the ESP system yielded the same results as those initially reported, while the radiometric results were now in agreement with those generated by the ESP system.

The need to test isolates of *M. tuberculosis* for PZA susceptibility became more apparent with its increased use as first-line therapy for tuberculosis. This need has been strengthened by the reports of PZA monoresistant strains of *M. tuberculosis* in the United States (2). Susceptibility testing of *M. tuberculosis* complex isolates to PZA has always been problematic. This assay must be performed at an acid pH to retain the activity of the drug, but some isolates of *M. tuberculosis* grow poorly or not at all in an acidic environment. The agar proportion method proved inadequate, owing to the fact that the final pH of the medium (5.5) does not universally support the growth of *M. tuberculosis*. In a recent paper, Heifets and Sanchez report on a modified medium formulation that improved the reliability of this procedure (3). The radiometric method has been the only routine method available to test for PZA susceptibility among isolates of *M. tuberculosis*. However, some isolates of *M. tuberculosis* will fail to grow adequately in the media used (5). This system also requires the use of a seed bottle, increasing the time required to generate a result. The ESP Myco test system has proven reliable for testing the first-line antitubercular agents. It has a decided advantage over the radiometric system by not requiring radiolabeled compounds and by having the capability of using the primary bottle as the inoculum source. This investigation demonstrated the utility of this system for susceptibility testing of *M. tuberculosis* complex isolates to PZA. For the 50 isolates tested there were only two discordant results compared to the BACTEC method, and both discrepant results were resolved in favor of the ESP system. The final concentration of PZA tested in the ESP system was 300 μg/ml, compared to 100 μg/ml for the radiometric system. This concentration of PZA in the ESP system was determined in preclinical trials to be optimal for distinguishing between susceptible and resistant strains of *M. tuberculosis*.

In conclusion, the ESP Myco susceptibility test is a rapid reliable test system to determine PZA susceptibility among isolates of the *M. tuberculosis* complex. The ability to use the primary positive culture bottle as the inoculum source is a decided advantage over the radiometric system, as it requires less time to report results.

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