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Testing of Susceptibility of *Mycobacterium tuberculosis* to Pyrazinamide: Comparison of Bactec Method with Pyrazinamidase Assay

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The susceptibility of 428 clinical isolates of *Mycobacterium tuberculosis* to pyrazinamide was assessed by the Bactec method and the Wayne pyrazinamidase assay. The correlation between the two tests was 98.2 and 100% for susceptible and resistant strains, respectively. False resistance was seen in four (0.8%) strains with the Bactec test, and false-susceptible results occurred in two (0.5%) pyrazinamidase assays. The Bactec test is rapid and reliable, and the Bactec results correlate well with the pyrazinamidase test results, although some strains did not grow well in the test medium.

Pyrazinamide (PZA) has become one of the first-line drugs for the treatment of tuberculosis (8). It is expected that, as with other antimycobacterial drugs, increasing use of PZA will lead to the emergence of drug-resistant strains (3, 9). In order to detect such resistance, in vitro testing of the susceptibility of *M. tuberculosis* to PZA is widely advocated. Unfortunately, conventional agar-based testing for PZA susceptibility often leads to uninterpretable results because of insufficient growth in the acidified medium (13, 15). One investigator found that 61% of clinical isolates of *M. tuberculosis* failed to grow on the acidic medium required in order to perform the conventional agar tests (5). However, the addition of an albumin-dextrose-catalase supplement to the medium has been shown to decrease the number of such uninterpretable results (1). On the basis of the knowledge that PZA-susceptible *M. tuberculosis* strains deaminate PZA to pyrazinoic acid, it has been suggested that the agar method be replaced with a direct assay to detect the presence of pyrazinamidase (7). However, there are problems with the interpretation of this assay and its correlation with levels of PZA resistance (2, 11, 14).

The Bactec radiometric system for mycobacterial culture, identification, and susceptibility testing now includes a test to assay for PZA susceptibility (5, 10, 12). Lu et al. analyzed radiometric PZA susceptibility tests compared with pyrazinamidase assays and found a 98% correlation (6). Other comparisons of a radiometric assay with pyrazinamidase activity assays have also been done (4, 5). However, no study has examined a large number of both PZA-susceptible and -resistant clinical isolates of *M. tuberculosis* by means of a comparison of the current Bactec assay and a conventional method of detection of pyrazinamidase. We undertook a large prospective study to compare the Bactec PZA susceptibility test with the Wayne method of detecting pyrazinamidase for 428 strains of *M. tuberculosis* isolated from clinical specimens, including 34 known pyrazinamidase-negative strains suspected to be PZA resistant.

Four hundred twenty-eight clinical isolates of *M. tuberculosis* submitted to the Laboratoire de santé publique du Québec (Quebec Public Health Laboratory) were included in this study. Duplicate strains from the same patient were excluded. All strains underwent the Bactec PZA susceptibility test in batches, following the recommendations of the manufacturer. Briefly, actively growing cultures in Middlebrook 7H12 (Bactec 12B) medium supplemented with polyoxyethylene stearate (POES) (Becton-Dickinson Diagnostic Instrument Systems, Sparks, Md.) were appropriately inoculated into two Bactec PZA test medium bottles, one of which contained PZA (100 μg/ml) and POES and the other used as a control with POES only. Daily readings of the growth index (GI) in both bottles were done until the day of interpretation when the GI of the control vial first reached at least 200. The recommended interpretive criteria for the ratio of the GI of the drug-containing vial to the GI of the control vial were followed, and the interpretations were as follows: less than 9%, susceptible; 9 to 11%, borderline; and greater than 11%, resistant. If a GI of 200 was not obtained within 20 days in the control vial, the test was considered uninterpretable. All isolates were also tested for the presence of pyrazinamidase by the Wayne method (16, 17). A positive result of the pyrazinamidase test, indicating the presence of the enzyme, was interpreted as most probably reflective of susceptibility to the drug. All isolates with discrepant Bactec and pyrazinamidase results or uninterpretable Bactec results underwent a repeat Bactec test as well as PZA susceptibility testing by means of the conventional proportion method performed at the Centers for Disease Control and Prevention
(J. O. Kilburn), with a PZA concentration of 25μg/ml in 7H10 agar (pH 5.5) (1).

All 428 strains yielded interpretable results in the pyrazinamidase assay. However, 15 strains (3.5% of the total) produced uninterpretable results by the Bactec method, because of insufficient growth within 20 days. The mean time to achieve reportable results by the Bactec method for the 413 strains which grew adequately was 5.4 days (median, 5.0 days), with a 95% confidence interval range of 1.8 to 9.0 days.

The results of the susceptibility tests by the two methods showed that 373 isolates were susceptible by both methods, 33 were resistant by both methods, 6 were resistant by Bactec only (2 of these were also resistant by the conventional method),

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TABLE 1. Comparison of results of PZA susceptibility testing by the conventional proportion method for seven isolates of *M. tuberculosis* with discrepant results by Bactec and pyrazinamidase testing[a]

Isolate no.	Bactec test				Conventional proportion method result
	Initial		Repeat		
	GI ratio (%)[b]	Result	GI ratio (%)	Result	
9739	16	Resistant	17	Resistant	Resistant
9989	107	Resistant	3	Susceptible	Susceptible
10274	100	Resistant	100	Resistant	Susceptible
10820	23	Resistant	10	Borderline	Susceptible
11197	22	Resistant	26	Resistant	Susceptible
11830	100	Resistant	100	Resistant	Resistant
9906	10	Borderline	5	Susceptible	Susceptible

[a] For all isolates, the pyrazinamidase test gave positive (susceptible) results.
[b] GI ratio = GI of pyrazinamide-containing vial ÷ GI of control vial.

and none considered resistant by the Wayne method only. In addition, one isolate was borderline susceptible by Bactec but susceptible according to the other two assays. Therefore, 94.7% of the pyrazinamidase-positive strains and 97.1% of the pyrazinamidase-negative strains were found, respectively, susceptible and resistant to PZA by the radiometric method. The results for 15 isolates (3.5% of total) were uninterpretable by the Bactec method because of insufficient growth in the test system. For these, the results of conventional testing and the pyrazinamidase assay suggested that 14 were susceptible and that only 1 was resistant to PZA.

Seven pyrazinamidase-positive strains were found either resistant (six isolates) or borderline susceptible (one isolate) by radiometric testing. Table 1 shows the results obtained for these seven strains with three different methods: the pyrazinamidase assay, Bactec PZA method (first and repeated results), and conventional proportion method. When these seven strains were retested by the Bactec method, two strains were found to be susceptible, one displayed 10% borderline resistance, and four were again resistant, with growth ratios similar to the first Bactec result. By the conventional proportion method, only two strains were confirmed resistant to 25 μg/ml of PZA.

Until the development of the Bactec PZA susceptibility test method, susceptibility testing of *M. tuberculosis* by either the conventional proportion method or the pyrazinamidase assay relied on waiting for growth of the organism on solid media. This usually meant a delay of several weeks for results. Uninterpretable results by the conventional method, because of insufficient growth on the acidified medium, led to a greater delay or no result at all. Since the median time to obtain an interpretable Bactec result in the current study was only 5.0 days, this test is comparable in speed to the other available Bactec antituberculous susceptibility assays. The Bactec susceptibility test can be performed from growth in liquid 12B medium, and we found only 3.5% of the results to be uninterpretable because of poor growth during the test protocol. This is the first time such a quantification of uninterpretable Bactec results has been done by using a large number of clinical isolates.

By omitting the uninterpretable results from the Bactec test, the correlation was 98.2 and 100% for susceptible and resistant strains, respectively, between the Bactec result and the Wayne method of assaying for the presence of pyrazinamidase. This correlation is close to that reported by Lu et al. (6) and is higher than that reported by Fuursted (4). However, the latter study used a different method for performing the Bactec test (4). We documented false resistance in 0.8% of the strains by the Bactec test (one of which yielded susceptible results on repeat Bactec testing), but no false-susceptible results were noted with this method. In contrast, because strains highly resistant to PZA are not always pyrazinamidase negative, the pyrazinamidase assay led to two strains being falsely labeled as most probably susceptible, a potentially more serious error.

Both the Bactec system and the pyrazinamidase assay are reliable ways of assessing the susceptibility of *M. tuberculosis* to PZA. The Bactec test is much more rapid, but some strains fail to grow in the test medium and this requires further investigation.

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