Direct Colorimetric Assay for Rapid Detection of Rifampin-Resistant *Mycobacterium tuberculosis*

Getahun Abate,1,2* Abraham Aseffa,1 Alemayehu Selassie,3 Solomon Goshu,3 Bekele Fekade,3 Dawit WoldeMeskal,1 and Häkan Möörner4

Armauer Hansen Research Institute1 and St. Peter Tuberculosis Specialized Hospital,3 Addis Ababa, Ethiopia; Departments of Internal Medicine and Molecular Microbiology, Division of Infectious Diseases and Immunology, Saint Louis University Health Sciences Center, St. Louis, Missouri; and Department of Medical Microbiology, Dermatology and Infection, Lund University, Lund, Sweden4

Received 26 June 2003/Returned for modification 18 September 2003/Accepted 23 October 2003

The colorimetric 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay was standardized and evaluated using a BACTEC radiometric method as a “gold standard” for indirect detection of rifampin resistance (1, 8). MTT is a yellow tetrazolium salt that is converted into blue formazan by dehydrogenases of live cells (7). The amount of blue or purple formazan formation is proportional to the number of live mycobacteria in a sample (8). The relative optical density (OD) unit of the drug-free control. Resistance was defined as RODU (0.2). RODU values were obtained each week for samples containing susceptible isolates were compared (using the Mann-Whitney U test) with those of samples containing resistant isolates. The lowest OD value which was considered indicative of growth was determined by growing a subculture with an aliquot of vortexed broth every week before the MTT assay. The lowest OD value with a positive culture result was 0.10; therefore, the results were considered interpretable when the OD value of the control was ≥0.1.

Standard sensitivity testing. Standard biochemical tests were used to identify all isolates as *Mycobacterium tuberculosis* (5). A proportion method (5) using Middlebrook 7H10 medium was used as a reference method for rifampin susceptibility testing. Reference *M. tuberculosis* strains ATCC 35836 (rifampin susceptible) and ATCC 35838 (rifampin resistant) were used as controls.

Among the 74 samples used to evaluate the MTT assay, 5 (6.8%) were excluded, 3 because the OD values of control tubes remained below 0.1 and 2 because there was no growth on Löwenstein-Jensen medium. Of the remaining 69 samples, 5 (7.2%) were contaminated; however, for each of the five samples there was at least one noncontaminated interpretable...
result among the results of the three sets of experiments prepared for the 3 weeks. The contamination rates of 18 samples tested in the presence of PANTA (Becton Dickinson) and in the presence of a much cheaper but similar antibiotic cocktail prepared in-house were the same. There was no contamination in the presence of Löwenstein-Jensen medium.

Table 1 shows the number of interpretable results obtained in each week. In the first week, 43 of 68 (63%) of the samples gave interpretable results; the number of samples with interpretable results grew in the second (98.5%) and third (100%) weeks. The MTT assay identified 8 of 69 (11.6%) samples as containing rifampin-resistant \( M. \) tuberculosis and 61 of 69 (88.4%) as containing rifampin-susceptible \( M. \) tuberculosis. The susceptibility (sensitivity and specificity) results obtained with MTT concurred fully with findings obtained using the standard assay on 7H10 agar medium. Figure 2 shows that the RODU values of samples containing susceptible bacteria remained below 0.2 in all weeks of experiments and that the RODU values of samples containing resistant bacteria were above 0.5. The differences in the RODU values of samples

![FIG. 1. Flow chart of MTT assay. Abbreviations: BC, bacterial control without rifampin; R, tube with 2 \( \mu \)g of rifampin/ml; C, control without bacteria. LJ, Löwenstein-Jensen.](image)

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![FIG. 2. Growth patterns of rifampin-susceptible (\( n = 37 \) samples in the first week, 56 in the second week, and 58 in the third week) and rifampin-resistant (\( n = 6 \) in the first week and 8 in the subsequent weeks) strains of \( M. \) tuberculosis as reflected by RODU values (means \( \pm \) standard errors) [(RODU = \( \frac{OD_{570} \text{ of rifampin-containing medium}}{OD_{570} \text{ of drug-free medium}} \)]. The difference in RODU values in each week was statistically significant (\( P < 0.01 \) [Mann-Whitney test]).](image)

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**TABLE 1.** Contamination rate and time of interpretation of a direct MTT assay for detection of rifampin-resistant \( M. \) tuberculosis

<table>
<thead>
<tr>
<th>Assay week</th>
<th>No. of contaminated samples/total no. of samples tested (%)</th>
<th>No. of interpretable samples/total no. of samples tested (%)</th>
<th>No. of samples giving the indicated assay result$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>MTT</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>S</td>
</tr>
<tr>
<td>1</td>
<td>1/69 (1.4)</td>
<td>43/68 (63)</td>
<td>37</td>
</tr>
<tr>
<td>2</td>
<td>4/69 (5.8)</td>
<td>64/65 (98.5)</td>
<td>56</td>
</tr>
<tr>
<td>3</td>
<td>3/69 (4.3)</td>
<td>66/66 (100)</td>
<td>58</td>
</tr>
<tr>
<td>Total</td>
<td>8/207 (3.9)</td>
<td>69/69 (100)</td>
<td>61</td>
</tr>
</tbody>
</table>

$^a$ Samples were inoculated in duplicates into drug-free broth medium. The change in OD was measured with the MTT assay (1) at 1 and 2 weeks after inoculation.

$^b$ S, susceptible; R, resistant.
containing susceptible and resistant isolates were statistically significant for each week \((P < 0.01)\).

Our findings show that a direct assay based on a tetrazolium salt significantly reduces the time required to obtain reliable susceptibility results. The standard direct methods of drug sensitivity testing on solid medium take 3 to 4 weeks \((5, 6)\), and with these conventional methods, there is in addition a need to prepare appropriate dilutions of a specimen as determined on the basis of smear grading \((5)\). Our application of a direct MTT assay is not dependent on smear grading and shortens the turnaround time. Other direct rapid methods (such as the BACTEC 460 system and the mycobacterial growth indicator tube system) have a turnaround time ranging from 9 to 12 days \((3, 6)\). However, they are very expensive for routine use in most countries in which tuberculosis is endemic.

Rifampin resistance is a strong predictor of the presence of multidrug-resistant tuberculosis \((2)\). Therefore, the results of our study focusing on the direct detection of rifampin-resistant \(M.\) tuberculosis indicate the potential of this simple and inexpensive assay for control programs in countries with high levels of tuberculosis endemicity. The same assay could theoretically be used to rapidly screen for resistance to other antituberculosis drugs. Our preliminary findings indicate that the same assay could be used for reliable and rapid detection of isoniazid resistance. This new method should be evaluated under program conditions in a region with a high level of tuberculosis endemicity and optimized for program use.

This project was supported by a grant from the UNDP/WORLD BANK/WHO Special Programme for Research and Training in Tropical Diseases (TDR).

REFERENCES