Emergence of multidrug-resistant *Mycobacterium tuberculosis* strains within the last few decades has made rapid detection of these strains essential for the effective clinical management of patients (13). The proportion method using Middlebrook 7H10 agar, which is universally accepted as the “gold standard” of testing for susceptibility to antituberculosis drugs, requires a long time (generally 3 weeks) to obtain results. However, the BACTEC 460 TB and BACTEC MGIT 960 systems have been reported to provide excellent agreement with the agar proportion method within 7 days (1, 2, 3, 11, 14), while these strains within the last few decades has made rapid detection of these strains essential for the effective clinical management of patients (13). The proportion method using Middlebrook 7H10 agar, which is universally accepted as the “gold standard” of testing for susceptibility to antituberculosis drugs, requires a long time (generally 3 weeks) to obtain results. However, the BACTEC 460 TB and BACTEC MGIT 960 systems have been reported to provide excellent agreement with the agar proportion method within 7 days (1, 2, 3, 11, 14), while molecular analysis is now being introduced for antituberculosis drug susceptibility testing (6). Mutational analysis of the *rpoB* gene for rifampin resistance (8, 12) and a PCR-based reverse-hybridization line probe assay (INNO-LiPA Rif.TB; Innogenetics N.V., Ghent, Belgium) have become available for use in clinical laboratories (4, 5, 15).

The transcription and reverse-transcription concerted (TRC) reaction method can provide homogenous quantification of a target RNA sequence within 30 min by performing isothermal sequence amplification in the presence of an intercalation activating fluorescence probe and real-time monitoring of the fluorescence intensity of the reaction mixture with a dedicated instrument (7). A reduction in transcript quantity as measured with TRC may indicate dead or unviable cells. In this study, we assessed the applicability of TRC to rapid antituberculosis drug susceptibility testing.

Fifteen *Mycobacterium tuberculosis* clinical isolates were obtained from the National Minami Kyoto Hospital and Kyoto University Hospital, Kyoto, Japan. Most of the strains were resistant to at least one of the four drugs used in this study, rifampin, isoniazid, ethambutol, and streptomycin. All strains were stored on egg-based Ogawa slants until use. A suspension of a given strain was prepared at a density of 0.5 McFarland by using a homogenizing kit (Nichihi; Nippon BCG Seizou Corp., Tokyo, Japan) and diluted fivefold with distilled water. The antituberculosis drug susceptibility of the strain was then verified at Kyoto University Hospital with both the proportion method on Ogawa egg medium and the BACTEC MGIT 960 system was 100 and 87% for rifampin, 93 and 100% for isoniazid, 60 and 53% for ethambutol, and 80 and 80% for streptomycin, respectively.

RNA transcript quantification by an isothermal sequence amplification reaction was evaluated for susceptibility testing of 15 *Mycobacterium tuberculosis* strains. Agreement with the proportion method on Ogawa egg medium and the BACTEC MGIT 960 system was 100 and 87% for rifampin, 93 and 100% for isoniazid, 60 and 53% for ethambutol, and 80 and 80% for streptomycin, respectively.
Tris-HCl [pH 8.0], 0.1 mM EDTA, and 2 U/μl of RNase inhibitor [TaKaRa Bio Inc., Otsu, Japan]). Isothermal amplification of the protein antigen b (pab) transcript was performed with the TRC method as described previously (7). In brief, 5 μl of nucleic acid suspension was added to 20 μl of TRC buffer containing specific primers and the intercalation activating fluorescence probe. After addition of 5 μl of an enzyme mixture containing avian myeloblastosis virus reverse transcriptase and T7 RNA polymerase, the fluorescence intensity of the tubes, placed in the dedicated instrument TRCR-160 (Tosoh Co., Tokyo, Japan) together with tubes containing a standard pab RNA calibrator in place of the samples, was monitored in a real-time manner for 30 min at 43°C. The time to reach the cutoff value of 1.2 was used to quantify the initial number of copies in a tube based on the standard curve constructed with the times of the calibrators. Each sample was measured in duplicate, and the geometrical mean was adopted as the final value. For each strain and drug combination, a relative percentage was calculated from dividing the pab transcript copy number in drug-containing broth culture by that in the drug-free growth control. A percentage of less than 10, an arbitrarily defined cutoff value referring to the results with Mycobacterium bovis BCG of a previous report (7), was considered to indicate a significant reduction in the pab transcript. A strain not showing such a reduction with a drug was judged resistant to the drug.

Of the 15 M. tuberculosis strains tested, rifampin resistance was observed in 8 with the Ogawa medium and in 6 with the BACTEC MGIT 960 system and isoniazid resistance in 9 with Ogawa medium and in 10 with the BACTEC MGIT 960 system. Ethambutol and streptomycin resistance was detected with the Ogawa medium in, respectively, 6 and 11 strains and was detected in 5 and 11 strains with the BACTEC MGIT 960 system. Sequence analysis showed that both of the two strains which showed resistance to rifampin with the Ogawa medium and susceptibility with the BACTEC MGIT 960 system possessed a mutation in the rpoB gene, Asp516Tyr.

The number of copies of the pab transcript detected in the drug-free growth control tubes ranged from $0.2 \times 10^4$ to $56 \times 10^4$ copies/ml with a median of $11 \times 10^4$, while that in the drug-containing tubes ranged from $<0.01 \times 10^4$ to $63 \times 10^4$ copies/ml. Relative percentages of the copy numbers of the pab transcript in drug-containing broth culture compared with those in drug-free growth control were plotted as shown in Fig. 1. The relative percentages of the rifampin-susceptible strains tested with the Ogawa medium ranged from 0.1 to 4.4 with a median of 1.1, and those of the resistant strains from 13 to $>100$ with a median of 91. Those of the isoniazid-susceptible and -resistant strains ranged from 0.1 to 12 and from 18 to $>100$, respectively. Table 1 shows the correlations of susceptibility test results for the two conventional methods.

![FIG. 1. Relative percentages of the pab transcript copy numbers (copy number of drug-containing broth culture divided by that of drug-free growth control) of 15 Mycobacterium tuberculosis clinical strains determined with the TRC method after a 48-h incubation with one of four antituberculosis drugs. The relative percentages of strains with drug susceptibility detected with the two conventional antituberculosis susceptibility methods are shown by open circles (○), and those of resistant strains are indicated by open squares (■). Those of strains with discrepant susceptibility test results for the two conventional methods are shown by closed squares (■).](image)

<table>
<thead>
<tr>
<th>Drug and result with TRC method</th>
<th>No. of strains with result determined by:</th>
<th>Proportion method on Ogawa medium</th>
<th>BACTEC MGIT 960 system</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Susceptible</td>
<td>Resistant</td>
</tr>
<tr>
<td>Rifampin</td>
<td></td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>Susceptible</td>
<td></td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>Resistant</td>
<td></td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Isoniazid</td>
<td></td>
<td>1</td>
<td>9</td>
</tr>
<tr>
<td>Ethambutol</td>
<td></td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Susceptible</td>
<td></td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Resistant</td>
<td></td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Streptomycin</td>
<td></td>
<td>2</td>
<td>10</td>
</tr>
</tbody>
</table>

a The two strains possessed a mutation in the hypervariable region of the rpoB gene, Asp516Tyr.
is reportedly weaker (9). The careful selection of a target RNA transcript may well improve the sensitivity of the TRC method for the detection of growth reduction of *M. tuberculosis*.

Although the proportion method on Middlebrook 7H110 agar has been regarded as the gold standard (10), we employed two other conventional methods, the proportion method on Ogawa medium and the BACTEC MGIT 960 system, as reference methods. This may constitute one of the limitations of this study. However, the proportion method on egg-based medium is recommended in the World Health Organization/International Union Against Tuberculosis and Lung Disease guidelines (16), and the BACTEC MGIT 960 system has been reported to show strong correlation with the proportion method on 7H110 agar (1, 2, 3, 14). In fact, the agreement of these two methods was good enough for them to be considered standard methods. Both of the two strains which showed resistance to rifampin with the Ogawa medium but susceptibility with the BACTEC MGIT 960 system and possessed the *rpoB* mutation Asp516Tyr were judged resistant by the TRC method. We finally rated these two strains as resistant to rifampin. Mutations in codon 516 in the *rpoB* gene have been reported to yield low-level resistance to rifampin (17, 18), which may explain the false susceptibility indicated by the BACTEC MGIT 960 system.

In summary, the TRC method was found to provide excellent agreement with standard methods in terms of the results for rifampin and isoniazid, which were obtained within only 2 days. Although further improvement and refinement are required for it to be used in practice, this method would be a promising candidate for rapid antituberculosis drug susceptibility testing.

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REFERENCES