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# Susceptibilities of *Mycobacterium tuberculosis* to Isoniazid and Rifampin on Blood Agar

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**In this study, blood agar was used instead of 7H10 agar for the susceptibility testing of 34 clinical isolates of *Mycobacterium tuberculosis* to isoniazid (INH) and rifampin (RIF) in accordance with the NCCLS. The BACTEC 460 TB system (Becton Dickinson, Sparks, Md.) was used as a "gold standard." Results for both media were in agreement for RIF and INH at 100 and 94.1%, respectively. For INH, the specificity, sensitivity, positive predictive value, and negative predictive value were found to be 71.4, 100, 93.1, and 100%, respectively, while these values were 100% for RIF. In addition, the results of the susceptibility test performed with blood agar were obtained on day 14 of incubation. In conclusion, results were obtained much earlier with blood agar (2 weeks) than with 7H10 agar (3 weeks), and the results of this study suggest that blood agar may be used as an alternative medium for the susceptibility testing of *M. tuberculosis* to INH and RIF.**

The increasing incidence of multidrug-resistant tuberculosis (MDR-TB) produces serious problems in developed and especially in developing countries. Detecting tuberculosis and identifying MDR *Mycobacterium tuberculosis* strains by conventional methods is difficult because of the low growth rate of the causative agent. Therefore, rapid and efficient methods are needed for the control of this disease (3–6, 13).

Several manufacturers have directed considerable effort toward the development of rapid and efficient systems for the growth, detection, and susceptibility testing of mycobacteria. Two of these systems, the BACTEC 460 TB (Becton Dickinson, Sparks, Md.) and the BACTEC MGIT 960 (Becton Dickinson), have become available for the susceptibility testing of *M. tuberculosis* and are also recommended by the NCCLS. Although the time to detect *M. tuberculosis* from clinical specimens can be shortened and the results for susceptibility testing can be obtained in 4 to 7 days by the use of these systems, they are labor-intensive and expensive and generate radioactive waste (2–4, 12). Therefore, several methods based on liquid media have been developed by different investigators for the susceptibility testing of *M. tuberculosis* (2, 4–6, 10, 11, 13, 14).

Drancourt et al. (7) investigated the effectiveness of blood agar for primary isolation of *M. tuberculosis*. They reported that *M. tuberculosis* can easily grow on blood agar in 1 to 2 weeks and that this medium has been routinely used instead of egg-based medium in the inoculation of 10,000 samples in a year for the diagnosis of tuberculosis, with the same results being obtained.

In this study, we evaluated the performance of blood agar for susceptibility testing of 34 *M. tuberculosis* clinical isolates to isoniazid (INH) and rifampin (RIF) by using the proportion method.

**Bacterial isolates.** Thirty-four clinical isolates of *M. tuberculosis* were examined in this study, and H37Rv and H37Ra were also included as control strains. Drug susceptibility patterns of all isolates were previously detected by the BACTEC 460 TB system, and a standard protocol in accordance with the manufacturer's instructions was followed. Seven isolates were susceptible to INH and RIF. Eight were resistant only to INH, while the rest (19 strains) were resistant to both drugs.

**Antituberculosis drugs.** INH and RIF were purchased from Sigma. The stock solutions of INH and RIF were prepared in distilled water and methanol, respectively, filter sterilized, and kept at −80°C (12).

**Preparation of blood agar with drugs.** Blood agar-based medium (Oxoid, Hampshire, England) was used for the susceptibility testing and prepared according to the manufacturer's instructions. After sterilization, the medium was cooled to 45 to 50°C and supplemented with defibrinated sheep blood (5%, vol/vol). The appropriate volumes of diluted stock solutions were incorporated into 200-ml aliquots of sterile blood agar medium to achieve the desired final concentrations of INH (0.2 and 1 μg/ml) and RIF (1 μg/ml). Media with drugs were dispensed quickly into sterile glass plates (5 cm in diameter), allowed to solidify, and either used immediately or stored at 4°C until used. For each strain, one blood agar medium without drug was also prepared as the growth control (12).

**Proportion method with blood agar.** The proportion method was used described by the NCCLS (12), and blood agar was used instead of Middlebrook 7H10 agar. Briefly, freshly grown colonies were transferred to a tube containing 3 to 4 ml of 7H9 broth and four or five sterile glass beads. The tubes were vigorously agitated on a vortex mixer, and then clumps were allowed to settle for 30 to 45 min. Supernatants were adjusted to equal densities of a no. 1 McFarland standard with 7H9

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TABLE 1. Comparison of radiometric proportion method results and blood agar results

| Drug[a] | Result on blood agar | Results of proportion method | | | | | |
|---|---|---|---|---|---|---|---|
| | | No. of samples that were: | | Sensitivity (%) | Specificity (%) | Positive predictive value (%) | Negative predictive value (%) |
| | | Resistant | Susceptible | | | | |
| INH | Resistant | 27 | 2 | 100 | 71.4 | 93.1 | 100 |
| | Susceptible | 0 | 5 | | | | |
| RIF | Resistant | 19 | 0 | 100 | 100 | 100 | 100 |
| | Susceptible | 0 | 15 | | | | |

[a] INH at 0.2 μg/ml; RIF at 1 μg/ml.

broth and used as the standard inoculum for the proportion method. Each standard inoculum was diluted 100-fold with 7H9 broth. One hundred microliters of diluted inoculum was inoculated on blood agar medium with and without drugs. All plates were incubated at 37°C overnight, and then they were sealed, placed in plastic bags, and incubated at 37°C in 5 to 10% $CO_2$. In the first week, all of the media were examined for contamination. After 14 and 21 days of incubation, susceptibility test results were read and recorded. Resistance was defined as growth on drug-containing medium that was greater than 1% of the growth on drug-free control medium for INH and RIF (3, 12).

The results are summarized in Table 1. The results for both media were in 100 and 94.1% agreement for RIF and INH, respectively. The specificity, sensitivity, positive predictive value, and negative predictive value were 71.4, 100, 93.1, and 100% for INH, and they were all 100% for RIF. Sufficient growth was observed in all of the control plates on day 14 of incubation. For this reason, susceptibility test results for INH and RIF were recorded on day 14, but incubation was prolonged to 21 days. On day 21, the results were the same as the results obtained on day 14. For confirmation of the acid-fast bacilli, a slide was prepared from the growth on each of the blood agar media. All slides were stained by the Erlich-Ziehl-Neelsen (EZN) method and examined, and no contamination was observed. In this experiment, the results for the two strains were discordant. When the susceptibility test was repeated for INH, the results did not change. Two strains were resistant to a low concentration (0.2 μg/ml) but susceptible to a high concentration (1 μg/ml) of INH on blood agar, while they were susceptible to the drug by the radiometric proportion method. In this preliminary study, 34 clinical isolates were tested and showed that the specificity for INH was 71.4% because of the two discordant results.

Detecting *M. tuberculosis* early and improving the rapid susceptibility testing methods are important for the prevention and control of MDR-TB. Since resistance to RIF is considered a surrogate marker for the identification of MDR-TB, rapid detection of RIF resistance is important (10). In this study, the agreement was 100% for the results of RIF on blood agar, and all results were obtained in 2 weeks. The BACTEC 460 TB system and the more recently described BACTEC MGIT 960 system have some disadvantages: they are expensive, still require needles for inoculation of the media, and occupy considerable space in the laboratory (3–5, 10, 13, 14). Because of these disadvantages, they are not available to many laboratories.

Blood agar is commonly preferred in many clinical microbiology laboratories because it is inexpensive and a number of bacteria are readily grown on it. In several studies, it has been reported that blood agar could be used for the isolation of *M. tuberculosis* (1, 7, 8, 9). In addition, Drancourt et al. (7) have reported the routine use of blood agar instead of egg-based medium for the isolation of *M. tuberculosis*. Our study is the first report of susceptibility testing of *M. tuberculosis* performed with blood agar by the proportion method. In conclusion, the time needed to obtain results of susceptibility tests using blood agar is shorter (2 weeks) than that of tests using 7H10 agar (3 weeks), and the results of this study suggest that blood agar may be used as an alternative medium for the susceptibility testing of *M. tuberculosis* to INH and RIF.

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