LETTER

Evaluation of Blood Agar for Susceptibility Testing of *Mycobacterium tuberculosis* against First-Line Antituberculous Drugs: Results from Two Centers

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Tuberculosis remains one of the major unresolved global health problems and the major causes of death from a single infectious agent worldwide [1,2]. In addition, multidrug-resistant tuberculosis (MDR-TB) is still a serious public health problem in the world, especially in developing countries [3-5]. Rapid detection of MDR strains is very important to restrict their spread in the population [6]. Conventional methods for susceptibility testing of *Mycobacterium tuberculosis* are laborious and require at least 4 weeks of incubation before an isolate is reported as susceptible or resistant [6,7]. Commercial liquid-medium systems including The BACTEC 460-TB and BACTEC MGIT 960 (Becton Dickinson, Sparks, MD) require radioisotopes and an expensive machine [7]. Here, we evaluated the performance of blood agar for susceptibility testing of *M. tuberculosis* to streptomycin (STR), isoniazid (INH), rifampicin (RMP) and ethambutol (EMB) in two different centers and results are discussed.

A total of 147 strains of *M. tuberculosis* were included in this study. Of these, 47 were tested in the Mycobacteriology Unit, Department of Microbiology, Institute of Tropical Medicine, Antwerp. The other 100 strains (50 strains from Ondokuz Mayis University Medical School, Department of Microbiology and Clinical Microbiology and 50 strains from Istanbul University Istanbul Medical School, Department of Microbiology and Clinical Microbiology) were tested in Ondokuz Mayis University Medical School, Department of Microbiology and Clinical Microbiology. *M. tuberculosis* H37Rv (ATCC 27294) sensitive to first-line drugs, strains resistant to INH (ATCC 35822) and STR (ATCC 35820) were used as control strains. All strains were sub-cultured on Löwenstein-Jensen medium. Resistance profiles of *M. tuberculosis* strains used in this study are summarized in *Table 1*.

TABLE 1 - *Resistance profiles of M. tuberculosis isolates used in this study.*

	Isolates (n) (Belgium) By the proportion method	Isolates (n) (Turkey) By the BACTEC 460 TB
STR	1	5
INH	0	20
EMB	0	5
INH+RMP	0	13
INH+STR	0	4
INH+EMB	0	1
STR+INH+RMP	3	4
STR+INH+EMB	1	0
INH+RMP+EMB	0	10
STR+INH+RMP+EMB	19	5
Fully susceptible	23	33
Total	47	100

STR: streptomycin; INH: isoniazid; RMP: rifampicin; EMB: ethambutol.

Blood agar was 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). Then the appropriate volume of diluted stock solutions was incorporated into blood agar medium to achieve the critical concentrations of STR (2 and 10 µg/ml), INH (0.2 and 1 µg/ml), RMP (1 µg/ml) and EMB (5 µg/ml). Then 5 ml of media were dispensed quickly into sterile plates (5 cm in diameter). For growth control, blood agar without drug was prepared [3,4]. These media were used for susceptibility testing of 47 *M. tuberculosis* in the Mycobacteriology Unit, Department of Microbiology, Institute of Tropical Medicine, Antwerp, Belgium.

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For 100 strains which were examined in Ondokuz Mayis University Medical School, Department of Microbiology and Clinical Microbiology, blood agar was supplemented with defibrinated sheep blood as 10% vol/vol and malachite green (0.0025 g/100 ml as in Middlebrook 7H10 agar) was added to blood agar as an inhibitory agent for contamination differentiation. The same protocol was followed by two centers for the further steps of susceptibility testing of the strains.

Susceptibility testing was performed according to Clinical Laboratory Standard Institute (CLSI) recommendations [8]. Inoculum was prepared from freshly grown colonies. Bacterial suspensions adjusted to equal densities of a no. 1 McFarland standard [8] were used as the standard inoculum for the proportion method on blood agar. The inoculum was diluted 1:100 and 100 µl of diluted inoculum were inoculated on blood agar media with and without drugs. All plates were incubated at 37°C overnight. After that, they were sealed, placed in a plastic bag and incubated at 37°C. The plates were examined on the 10th, 14th and 21st days of incubation. Resistance was defined according to CLSI criteria.

The results are summarized in *Table 2*. Agreements were 93.1% for STR, 94.5% for INH, 96.5% for RMP and 87.7% for EMB. Sensitivities were 93.3, 95.5, 96.7 and 97.1% for STR, INH, RMP and EMB, respectively. Specificities were 92.8, 93.7, 96.3 and 64.2% for STR, INH, RMP and EMB, respectively. All plates were examined after the 10th, 14th and 21st days of incubation, susceptibility test results were noted on the 14th day for 100 strains whereas results were obtained on the 21st day of incubation for 47 strains. It seems that susceptibility test result on blood agar can be obtained between the 14th-21st days.

Blood agar has been used commonly in all clinical microbiology laboratories and some of its advantages are that it is inexpensive, simple to prepare, and a number of bacteria can grow readily on it. In several studies, it has been noted that blood agar, chocolate agar could be used for the isolation of tubercle bacilli [9-13]. After the evaluation of these studies Coban *et al.* [3] first evaluated the performance of blood agar for susceptibility testing of *M. tuberculosis* against INH and RMP and noted high agreement, specificity and sensitivity. One year later, Coban *et al.* [4] used blood agar instead of Middlebrook 7H10 agar for susceptibility testing of *M. tuberculosis* against first-line antituberculous drugs. They reported high agreement, specificity and sensitivity for INH, RMP and EMB, but not for STR. The positive predictive value for STR was low. In these two studies, all results were noted on the 14th day of incubation. Differently, blood agar supplemented with 10% sheep blood and malachite green was used in one of the centers in this study but there were no differences between the results of two centers and previous studies of Coban *et al.* [3,4].

Yildiz *et al.* [14] tested 60 clinical isolates of *M. tuberculosis* against INH on blood agar supplemented with 5% sheep and human blood. They found that agreements for both media were 100% for INH. In this study, the results on both agar media supplemented with sheep and human blood were obtained on the 6th day of incubation interestingly. Whereas in this study, one center noted the results on the 14th day of incubation and the other center recorded the results on the 21st day of incubation. From these results it seems that susceptibility test results on blood agar can be obtained from the 6th to 21st days of incubation.

Wanger *et al.* [15] performed Etest method for *M. tuberculosis* on 7H11 agar supplemented with blood. Thirty-one clinical isolates of *M. tuberculosis* were examined by Etest against first-line antituberculous drugs. They reported that the average time to reporting the results was 5 days for the blood containing media as compared to 8 days without blood and MIC results were all within +/- 1 dilution. When we performed Etest on blood agar for *M. tuberculosis* strains, we saw

TABLE 2 - *Comparison of reference method results and blood agar results.*

Drug	Results on blood agar	No. of samples that were: Resistant	Susceptible	Results of reference methods: Sensitivity (%)	Specificity (%)	Agreement (%)
STR	Resistant	39	7	93.3	92.8	93.1
	Susceptible	3	98			
INH	Resistant	75	3	95.5	93.7	94.5
	Susceptible	5	64			
RMP	Resistant	53	3	96.7	96.3	96.5
	Susceptible	2	89			
EMB	Resistant	27	3	97.1	64.2	87.7
	Susceptible	15	102			

STR; streptomycin, INH; isoniazid, RMP; rifampicin, EMB; ethambutol.

that susceptibility testing by Etest can be performed easily on blood agar media (unpublished data).

In this study, we evaluated the performance of blood agar in two centers and according to our results we suggest that blood agar can be used for the susceptibility testing of *M. tuberculosis* against INH and RMP. However, further studies are needed, especially for STR and EMB, before implementing the method in diagnostic laboratories.

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