SHORT COMMUNICATION

Direct Sensitivity Test of the MB/BacT System

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In order to evaluate the direct-method test of sensitivity to drugs used in the principal tuberculosis treatment regimes, in the Organon Teknika MB/BacT system, we tested 50 sputum samples positive to microscopy taken from patients with pulmonary tuberculosis and with clinical indications for an antibiogram, admitted sequentially for examination during the routine of the reference laboratory. The material was treated v/v with 23% trisodium phosphate solution, incubated for 24 h at 35°C, and neutralized v/v with 20% monosodium phosphate solution. The material was then centrifuged and the sediment inoculated into flasks containing Rifampin – 2 µg/ml, Isoniazid – 0.2 µg/ml, Pyrazinamide – 100 µg/ml, Ethambutol – 2.5 µg/ml, Ethionamide – 1.25 µg/ml, and Streptomycin – 2 µg/ml. The tests were evaluated using the indirect method in the BACTEC 460 TB (Becton Dickinson) system as the gold standard. The results showed that the Rifampin test performed best, i.e., 100% sensitivity at 95% Confidence Interval (82.2-100) and 100% specificity at 95% Confidence Interval (84.5-100), followed by Isoniazid and Pyrazinamide. In this experiment, 92% of the materials showed a final reading in 30 days; this period represents the time for primary isolation as well as the results of the sensitivity profile, and is within Centers for Disease Control and Prevention recommendations regarding time for performance of the antibiogram. The inoculated flasks showed no contamination during the experiment. The MB/BacT is shown to be a reliable, rapid, fully automated nonradiometric system for the tuberculosis antibiogram.

Key words: tuberculosis - drug resistance- laboratory diagnostics

Tuberculosis is a grave health problem, with more than 8 million persons newly infected worldwide in 1999. Brazil is one of the 23 countries that harbor 80% of all cases (WHO 2001). The most recent investigation in Brazil of resistance to the drugs used in tuberculosis treatment showed that in the 4,785 patients studied, 10.6% harbored some type of resistant strain (Braga et al. 1999).

When a patient becomes ill he undergoes a standard treatment, and an antibiogram is performed only if the physician suspects resistance to the medications and requests the test.

The majority of public health laboratories use the proportion method according to Canetti et al. (1969), with Lowenstein-Jensen medium, to perform an antibiogram. Many of these laboratories perform it by directly seeding the sputum sample into the medium containing the drug (direct test), to reduce diagnosis time and allow earlier treatment of the patient. This methodology can also be used in semi-automated systems such as the BACTEC 460 TB (Becton Dickinson Diagnostic Instrument Systems, Sparks, MD, USA), according to the maker recommendation (Siddiqui 1996).

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The MB/BacT system (Organon Teknika Corp., Durham, NC, USA) is fully automated and nonradiometric. It has been evaluated with good results for performance of the tuberculosis antibiogram, by means of a technique based on the indirect test of the proportion method, modified by the mode of operation of the apparatus (Beer et al. 1997 a,b, Brunello & Fontana 2000, Diaz-Infantes et al. 2000). In order to evaluate the direct-method test of sensitivity to the drugs used in the principal treatment regimes for tuberculosis (Rifampin – RMP, Isoniazid – INH, Pyrazinamide – PZA, Ethambutol – EMB, Ethionamide – ETH, and Streptomycin – SM), we tested 50 sputum samples positive to microscopy obtained from patients with pulmonary tuberculosis and clinical indications for an antibiogram, who were admitted sequentially for examination during the routine of the reference laboratory. The material was treated volume to volume with 23% trisodium phosphate solution and incubated for 24 h at 35°C, then neutralized volume to volume with 20% monosodium phosphate solution (Brasil 1994). After centrifugation at 3,000 x *g* for 20 min, the sediment was resuspended in sterile distilled water in sufficient volume to inoculate 0.5 ml into flasks of the MB/BacT culture medium, to which drugs (Sigma Chemical Co., St. Louis, MO, USA) had previously been added in final concentrations of: RMP – 2 µg/ml, INH – 0.2 µg/ml, PZA – 100 µg/ml, EMB – 2.5 µg/ml, ETH – 1.25 µg/ml, and SM – 2 µg/ml. As a control for growth, the flasks without drugs were seeded in the same way; one of them with the inoculum diluted 1/100 served as a standard to limit the reading time of the test (Beer et al. 1997 b). The flasks were incubated in the system's

TABLE
Correlation between BACTEC and MB/BacT results

Drugs	BACTEC (R) MB/BacT (R)	BACTEC (S) MB/BacT (R)	BACTEC (R) MB/BacT (S)	BACTEC (S) MB/BacT (S)
RMP	23	0	0	27
INH	24	1	0	25
PZA	20	0	1	29
EMB	11	1	0	38
ETH	7	1	0	42
SM	6	0	0	44

R: resistant; S: rensitive; RMP: Rifampin; INH: Isoniazid; PZA: Pyrazinamide; EMB: Ethambutol; ETH: Ethionamide; SM: Streptomycin

own incubator at 35ºC, and growth was monitored every 10 min. The flasks containing PZA were acidified with 0.5 ml of a solution of phosphoric acid in 0.2 N chlorhydric acid to obtain a final pH of 6.7 in the culture medium. The tests were evaluated, using the indirect method in the BACTEC 460-TB system as the gold standard, with the drugs in the same concentrations as the direct test (Martins et al. 1999). The isolates were identified as *Mycobacterium tuberculosis* complex using the AccuProbe® genetic probe (Gen - Probe Incorporated, San Diego, CA, USA).

We obtained 23 cultures that were sensitive to the 6 drugs tested, and 27 cultures with some type of resistance. The Table shows the results obtained with the BACTEC and the MB/BacT, used to calculate the accuracy of the direct method. These results indicate that RMP performed best in the test, with 100% sensitivity at 95% CI (82.2-100) and 100% sensitivity at 95% CI (84.5-100), followed by INH and PZA. Previous evaluations of the indirect test in the MB/BacT system reported similar results for RMP (Brunello & Fontana 2000, Diaz-Infantes et al. 2000). ETH and EMB showed, respectively, 87.5% (46.7-99.3) and 91.7% (59.8-99.6) positive predictive values, possibly because of one culture which gave false-positive results. The Centers for Disease Control and Prevention recommended periods for carrying out the isolation of *M. tuberculosis* of up to 14 days after collection of the material, and up to 30 days for the antibiogram result (Tenover et al. 1993). In this experiment, 92% of the samples grew within 30 days, including the period for primary isolation as well as for the sensitivity profile. Complete liquefaction of the sputum sample and obtaining clinical material in sufficient quantity to seed all the flasks is fundamental for the operation of the test, and in this experiment the seeded flasks showed no contamination. The majority of public health laboratories the in states' capital (Central Laboratories) perform an antibiogram for tuberculosis, and are being equipped to participate in an epidemiological study of drug resistance. The Organon-Teknika MB/BacT system is shown to be a reliable, rapid, fully automated, nonradiometric system usable for the antibiogram for tuberculosis drugs.

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