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# Evaluation of the BACTEC Radiometric Method for Recovery of Mycobacteria and Drug Susceptibility Testing of *Mycobacterium tuberculosis* from Acid-Fast Smear-Positive Specimens

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A total of 463 respiratory specimens, all smear positive for acid-fast bacteria, were inoculated onto routine solid media and into BACTEC 7H12 Middlebrook medium for detection of mycobacterial growth. Conventional drug susceptibility testing (1% proportion method) was performed on Middlebrook 7H10/7H11 medium, and radiometric susceptibility testing was performed on BACTEC 7H12 medium. The average detection times for BACTEC-positive cultures were 8.3 days for *Mycobacterium tuberculosis* and 5.2 days for mycobacteria other than tuberculosis; by conventional methods, they were 19.4 and 17.8 days, respectively. These detection times do not include time required for identification, which was done by the conventional method only. There was an excellent correlation in the recovery rates of mycobacteria by the two methods. Drug susceptibility test results of *M. tuberculosis* isolates by the two methods showed 95.1 to 100% overall agreement. The average reporting time for drug susceptibility results ranged from 4.2 to 6.9 days for the BACTEC method and 13.7 to 21 days for the conventional methods. An average of 18 days was required by the BACTEC method for complete recovery and drug susceptibility testing of *M. tuberculosis*, as compared with 38.5 days for the conventional methods.

Radiometric techniques were first introduced in mycobacteriology by Cummings et al. in 1975 (1). A major advancement was made in 1977, when Middlebrook introduced a liquid 7H12 medium containing $^{14}C$-labeled palmitic acid for radiometric detection of mycobacterial growth (2). Middlebrook first published data on primary isolation of mycobacteria from sputa, demonstrating that the radiometric technique had promise (2). Snider et al. compared drug susceptibility testing results of 300 stock cultures of *Mycobacterium tuberculosis* obtained by radiometric and conventional methods with an overall 95% agreement (contractor A [5]). Agreement among the resistant strains was not as good as agreement among strains susceptible to drugs. Siddiqi et al. later reported BACTEC drug susceptibility testing of 106 fresh isolates of *M. tuberculosis* and noted a 98% overall correlation between results obtained by two methods (4). In a recent drug susceptibility study, Vincké et al. reported a high degree of correlation (95.2 to 98.4%) between the two methods (7). Nevertheless, there has been no report on use of the BACTEC radiometric method for combined primary recovery and drug susceptibility testing of mycobacteria and its comparison with the conventional method.

This multicenter study was initiated to evaluate the BACTEC radiometric method for rapid recovery of mycobacteria from smear-positive clinical specimens. In addition, the study was conducted to evaluate the BACTEC procedure for drug susceptibility testing of *M. tuberculosis* and to compare it with a reference method conventionally used in most laboratories.

## MATERIALS AND METHODS

**Recovery of mycobacteria. (i) Conventional method.** To have a large number of positive cultures from a smaller number of samples, only respiratory specimens which were found to be smear positive for acid-fast bacteria were included in this study. For digestion and decontamination of specimens, the sodium hy-

TABLE 1. Conventional procedures employed by each institution for recovery of mycobacteria from clinical specimens

| Institution | Digestion/decontamination | | Centrifugation | | | Neutralization | Resuspension in: | Conventional media used | Size of inoculum | Incubation temp (°C) | Examination | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | NaOH[a] (%) | NALC[b] | Refrigeration temp (°C) | × g | Time (min) | | | | | | Type[c] | Schedule |
| A | 4 | Yes | NR[d] | 3,500 | 15 | No | 1 ml of 0.2% BSA[e] | LJ-2[f]<br>7H10-1[g] | 0.3 ml each | 35 | Macro and micro | Twice per week for 8 weeks |
| B | 2 | No | 30–32 | 2,500 | 20 | Yes | 5 ml of water | LJ-2<br>7H10-1<br>7H11-1 | 0.25 ml per slant<br>0.5 ml per plate | 35 | Macro | Weekly for 8 weeks |
| C | 4–6 | Yes | NR | 2,500 | 15 | No | 1–2 ml of 0.2% BSA | LJ-1<br>7H11-1 | 0.5 ml each | 37 | Macro and micro | Weekly for 6 weeks |
| D | 4 | Yes | 5–15 | 3,500 | 40 | No | 2 ml of 0.2% BSA | LJ-1<br>7H11-1<br>7H11S-1[h] biplates | 0.5 ml each | 35 | Macro and micro | Weekly for 6 weeks |
| E | 4 | Yes | NR | 2,000 | 20 | No | 3 ml of water | LJ-2<br>7H11-1 | 4 drops each | 35 | Macro and micro | Weekly for 8 weeks |

[a] Sodium hydroxide (stock solution).
[b] NALC, *N*-Acetyl-L-cysteine.
[c] Macro, Macroscopic; micro, microscopic.
[d] NR, Non-refrigerated.
[e] BSA, Bovine serum albumin.
[f] Lowenstein-Jensen slant.
[g] Plated Middlebrook 7H10 and 7H11 agar medium
[h] Plated Middlebrook 7H11 medium with antimicrobial agents.

TABLE 2. Drug concentrations used in susceptibility testing

| Institution | Method | Concns (μg/ml) of: | | | |
|---|---|---|---|---|---|
| | | Streptomycin | Isoniazid | Rifampin | Ethambutol |
| A | Conventional | 2, 10 | 0.2, 1 | 1 | 5, 10 |
| | BACTEC | 4 | 0.2 | 2 | 10 |
| B | Conventional | 2, 10 | 0.2, 1, 5 | 1, 5, 10 | 7.5, 10, 15 |
| | BACTEC | 2, 10 | 0.2, 1, 5 | 1, 5, 10 | 7.5, 10, 15 |
| C | Conventional | 2.5 | 1 | 1 | 10 |
| | BACTEC | 4 | 0.2 | 2 | 10 |
| D | Conventional | 2, 10 | 0.2, 1 | 1, 5 | 7.5, 15 |
| | BACTEC | 2, 10 | 0.2, 1 | 1, 5 | 7.5, 15 |
| E | Conventional | 2, 4 | 0.2 | 1, 2 | 5, 10 |
| | BACTEC | 2, 4 | 0.2 | 1, 2 | 5, 10 |

droxide–*N*-acetyl-L-cysteine method was used, with some modifications of the procedure recommended by the Centers for Disease Control (6). Details of procedures followed by each institution are summarized in Table 1.

**(ii) BACTEC method.** After inoculation of conventional media was completed and the smear results were ready, the remaining specimen concentrate was used to inoculate into BACTEC medium. At institutions B and C, inoculation was performed the same day. Institution D inoculated the next day, and institutions A and E refrigerated the specimen and inoculated after 1 to 16 days of specimen processing.

Each BACTEC 7H12 medium vial was supplemented with 0.1 ml of an antimicrobial mixture containing polymyxin B (1,000 U/ml); amphotericin B (100 μg/ml); carbenicillin (500 μg/ml); and trimethoprim (50 μg/ml). Concentrations of antimicrobial agents used in 7H12 medium were much lower than those recommended by Mitchison et al. (3) and used in 7H11-S medium by institution D. One vial of BACTEC medium was used for each specimen (only institution E used two vials). Smear-positive, concentrated specimen (0.1 to 0.2 ml) was inoculated into a BACTEC vial. All of these vials were then tested on a BACTEC instrument immediately to establish a 4 to 5% $CO_2$ atmosphere inside the vial. Institutions D and E used a BACTEC 301 (manual), and other institutions used the BACTEC 460 (automated) instrument. On each reading, the instrument aspirated gases out of the vial for reading of $^{14}CO_2$ and introduced fresh 4 to 5% $CO_2$ in air into the vial. All inoculated vials were incubated at 36 to 38°C and were read on alternate days for 3 weeks and weekly thereafter. When a vial showed a growth index (GI) reading of 10 or more, it was considered positive for mycobacterial growth and was read daily thereafter. After the GI reached 100 or more, smears were made and examined for acid-fast bacteria by the Ziehl Neelsen technique. Morphology of mycobacteria on smear indicated a tentative differentiation between *M. tuberculosis* and other mycobacteria, but the final identification was done by the conventional method. All vials were observed for contamination by periodic examination for turbidity and also by streaking a sample onto culture media when a vial showed an abnormal GI reading pattern.

**Drug susceptibility testing. (i) Conventional method.** Conventional drug susceptibility testing was carried out on Middlebrook 7H10 or 7H11 medium by the 1% proportion method (6). All inoculated plates were incubated at 35 to 37°C in a 5 to 10% $CO_2$ atmosphere and were checked after 2 and 3 weeks. The drug concentrations are listed in Table 2.

**(ii) BACTEC method.** Inocula for susceptibility testing originated from two sources: a positive BACTEC vial at GI 500 or more or a suspension of organisms recovered earlier on a conventional medium. The test drugs were added to the BACTEC medium in 0.1-ml quantities (final concentrations are listed in Table 2).

After being mixed well with a syringe (permanently attached needle), 0.1 ml of a positive BACTEC culture was added to each of the four vials containing the test drug. For the control, a 1:100 dilution of inoculum was made by adding 0.1 ml of inoculum to 9.9 ml of sterile special diluent (0.02% polysorbate 80, 0.2% fatty acid-free bovine albumin in water). After being mixed well, 0.1 ml of this 1:100 dilution was added to the control drug-free vial.

TABLE 3. Recovery of mycobacteria from smear-positive specimens by the BACTEC and conventional methods by different institutions

| Method and organism | No. of smear-positive specimens yielding mycobacteria at institution[a]: | | | | |
|---|---|---|---|---|---|
| | A | B | C | D | E |
| BACTEC | | | | | |
| *M. tuberculosis* | 54 (98.2) | 34 (97.1) | 82 (92.1) | 61 (100) | 14 (100) |
| MOTT[b] | 12 (92.3) | 34 (94.4) | 36 (81.8) | 20 (100) | 3 (100) |
| Conventional | | | | | |
| *M. tuberculosis* | 55 (100) | 35 (100) | 76 (85.4) | 52 (85.2) | 14 (100) |
| MOTT | 13 (100) | 35 (97.2) | 33 (75.0) | 20 (100) | 3 (100) |

[a] Numbers in parentheses indicate the number of specimens yielding mycobacteria by each method as a percentage of the total positive specimens by all methods and media combined.

[b] MOTT, Mycobacteria other than *M. tuberculosis*.

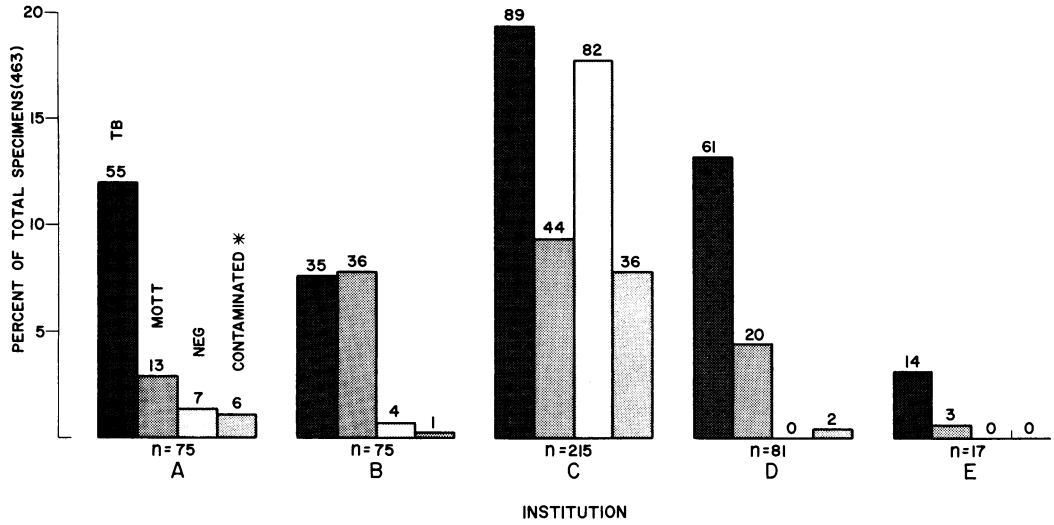


FIG. 1. Recovery of mycobacteria from 463 smear-positive specimens (both methods combined). The asterisk indicates that mycobacteria were recovered from some of the contaminated specimens.

When growth from a solid medium was used, a well-dispersed suspension (approximate to McFarland no. 1 standard) was prepared from the viable culture. These suspensions were used in the same manner as a BACTEC-positive vial described above.

After inoculation, each vial was tested on a BACTEC instrument to provide $CO_2$ in the headspace. Each vial was then tested at intervals of about 24 h. When the GI of the control read at least 30, the results were interpreted by comparing the increase in GI ($\Delta$GI) from the previous day in the control with that in the drug vial. The following formula was used to interpret results: $\Delta$GI control > $\Delta$GI drug = susceptible; $\Delta$GI control < $\Delta$GI drug = resistant.

If a clear susceptibility pattern (the difference of $\Delta$GI of control and the drug bottle) was not seen at the time the control GI was 30, the vials were read for 1 or 2 additional days to establish a definite pattern of $\Delta$GI differences.

## RESULTS

Results for primary recovery of mycobacteria and drug susceptibility testing of *M. tuberculosis*, using radiometric and conventional methods, varied slightly among institutions. The data are shown in Table 3 coded for each of the five institutions.

Of the 463 respiratory specimens processed, 370 (79.9%) were detected as culture positive by either BACTEC or conventional methods. Figure 1 shows the total number of specimens which were found to be culture negative or positive (both methods combined) by each institution and their relative contribution to the whole study. Figure 2 shows the overall recovery rate by each method separately. Of 370 culture-positive specimens by any method, the BACTEC and conventional methods detected 94.6 and 90.82% of the positive specimens, respectively. Among *M. tuberculosis* isolates, BACTEC missed 9 cultures, whereas the conventional method missed 22 cultures. For mycobacteria other than *M. tuberculosis* (MOTT bacilli), BACTEC missed 11 cultures and the conventional method missed 12 cultures. Details from individual institutions for the primary recovery are shown in Table 3.

Bacterial contamination occurred overall in 7.3 and 5.2% of the BACTEC and conventional media, respectively. However, there were instances of mycobacteria being recovered from contaminated specimens, especially on the conventional media (Fig. 1). Contamination was highest in institution C, where the rate was 16.7%; that in other institutions ranged from 0 to 8.0%.

Figure 3 shows the mean and ranges of time required to detect a positive culture by each method. The mean recovery times of *M. tuberculosis* for BACTEC and conventional methods were 8.3 and 19.4 days, respectively, and those of MOTT bacilli were 5.2 and 17.8 days, respectively. The mean times of recovery of *M. tuberculosis* varied among institutions from 6.9 to 11.0 days for the BACTEC method and from 13.6 to 22.5 days for the conventional method. Mean recovery times for all mycobacteria combined varied from 6.0 to 10.1 days and 13.2 to 22.8 days for the BACTEC and conventional methods, respectively. In general, the mean recovery time for mycobacteria was markedly shorter with the BACTEC method.

Antimicrobial susceptibility testing was started late and thus could not be performed on all isolates. We tested 126 cultures of *M. tuberculosis* recovered on conventional solid media and

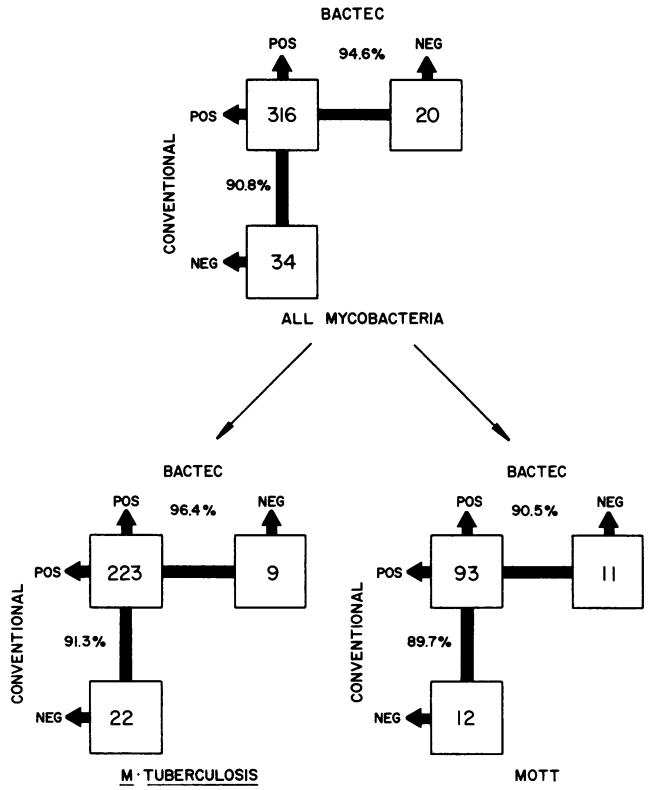


FIG. 2. Comparison of the two methods for recovery of mycobacteria from 370 culture-positive specimens.

170 cultures recovered in the BACTEC 12A medium, for drug susceptibility by the BACTEC method. The results were reported on an average of 5.1 and 6.1 days, respectively. Combined results of the two drug susceptibility tests and their comparison with results obtained by the conventional method are shown in Table 4. There were 784 drug test combinations, with a total of 20 disagreements between the BACTEC and conventional results (2.6%). There were 11 disagreements in which the cultures were susceptible by the conventional method and resistant by the BACTEC method, and 9 cultures were resistant by the conventional method and susceptible by the BACTEC method. Most of the disagreements were with results from institu-

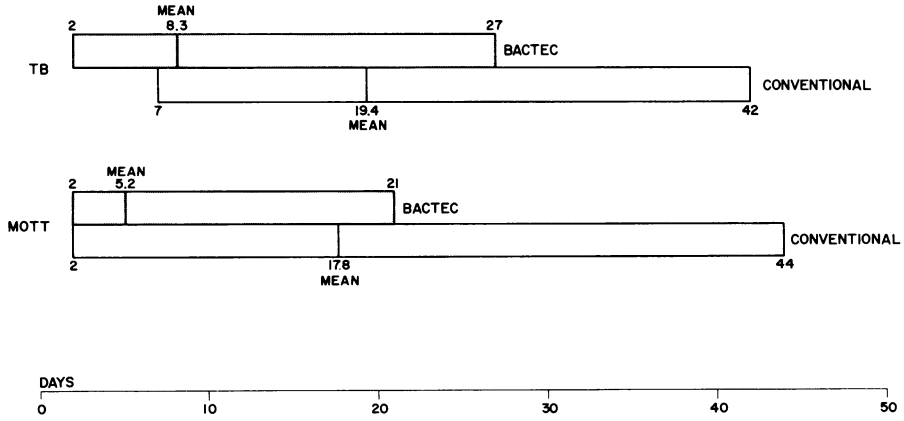


FIG. 3. Recovery times for mycobacteria from smear-positive specimens.

TABLE 4. Analysis of susceptible and resistant strains by the two methods (results from all institutions combined)

| Test drug | No. of strains with following test result[a] | | | | Parameter (%)[b] | | | |
|---|---|---|---|---|---|---|---|---|
| | Both-S (A) | Conv-S BACT-R (B) | Conv-R BACT-S (C) | Both-R (D) | Specificity | Sensitivity | Predictive value | |
| | | | | | | | Susceptibility | Resistance |
| Streptomycin | 258 | 6 | 3 | 29 | 97.7 | 90.6 | 98.8 | 82.8 |
| Isoniazid | 221 | 3 | 1 | 71 | 98.7 | 98.6 | 99.5 | 95.9 |
| Rifampin | 237 | 1 | 1 | 57 | 99.6 | 98.3 | 99.6 | 98.3 |
| Ethambutol | 283 | 1 | 4 | 8 | 99.6 | 66.7 | 98.6 | 88.9 |

[a] S, Susceptible; R, resistant; Conv, conventional method; BACT, BACTEC method.
[b] Values determined as follows: specificity, [A/(A + B)] × 100; sensitivity, [D/(D + C)] × 100; predictive value (susceptibility), [A/(A + C)] × 100; predictive value (resistance), [D/(D + B)] × 100.

tions C and D. Further analysis of the results is also included in Table 4.

Susceptibility testing of MOTT bacilli was not included in this study because of known poor correlation of results by the two methods with these mycobacteria.

The overall mean time required for the BACTEC recovery of *M. tuberculosis* and subsequent BACTEC susceptibility testing was 18.0 days, and the time required for conventional recovery and susceptibility testing was 38.5 days (Table 5). The mean times for the BACTEC method varied among institutions from 11.3 to 23.1 days, and mean times for the conventional method ranged from 33.5 to 42.1 days.

## DISCUSSION

In this study, culture results were analyzed as they became positive by the two methods. Identification of mycobacteria was carried out later by the conventional method, and the time required for identification was not included in the time to detection of a positive culture for either method.

All five study centers used their established routine methods for recovery, identification, and antimicrobial susceptibility testing of mycobacteria, and no change was made in their routines during the study. Results obtained by the BACTEC method were compared with those obtained by the routine conventional methods used in each institution. Although the basic methodology was the same, there was a wide variation in procedural details: decontamination and neutralization of specimens, number of types of media used, and method of examination of cultures.

Other important factors influencing results of the study were the type of patient population and the species of mycobacteria recovered. One institution primarily encountered patients with chronic tuberculosis whose organisms exhibited multiple drug resistance, and another acted primarily as a reference center with clinical specimens and cultures being submitted. The remaining institutions were involved with specimens from in- and outpatients seeking general medical care.

Obviously, the results varied from center to center; however, this made the study more interesting and worthwhile when the methodologies were compared. In institutions A, B, and E, both methods performed similarly for recovery of positive culture. In institutions C and D, there was a significant difference in percentage of positive cultures, and the BACTEC method performed better than the conventional method. Both of these institutions encountered chronic cases of tuberculosis, and many of the mycobacteria in specimens from this group were not recovered by conventional media but were recovered in the BACTEC medium. These were from patients on extended chemotherapy. Overall, the BACTEC system performed well and demonstrated more positive mycobacterial isolates from smear-positive specimens than did the conventional media. It is important to note that only 0.1 to 0.2 ml of the specimen was inoculated into only one BACTEC vial (institution E used two vials), whereas with conventional methods, three to four tubes and plates were inoculated with a total of approximately 1 ml of inoculum.

TABLE 5. Total time required for complete recovery and drug susceptibility testing of *M. tuberculosis*

| Institution | Total no. of cultures tested | Avg no. of days required for recovery and susceptibility testing | |
|---|---|---|---|
| | | BACTEC | Conventional |
| A | 51 | 23.1 | 37.6 |
| B | 9 | 11.3 | 33.5 |
| C | 50 | 17.7 | 42.1 |
| D | 52 | 14.7 | 37.0 |
| E | 8 | 16.4 | 35.0 |
| Mean | | 18.0 | 38.5 |

During the initial study, no difference was observed in the rate of recovery between a 0.1- and a 0.2-ml inoculum of the specimen. Therefore, at the later part of the study, most of the institutions used 0.1 ml of inoculum. Institution E used two vials throughout, but the total number of isolates was not enough to yield any conclusive data. Further studies are needed to evaluate a larger inoculum size in each vial or the use of two vials for the higher recovery of mycobacteria. Moreover, studies should be carried out to find whether the same high recovery rate of positive cultures and time-saving by the BACTEC method would hold in smear-negative specimens.

It was observed that with highly smear-positive specimens, a BACTEC culture could be reported as positive within 2 days (for *M. tuberculosis*), whereas the conventional method required at least 7 days. The time required for detecting growth by the BACTEC method varied among the five centers. Institution B had the fastest results, with an average of 6.9 days for *M. tuberculosis* and 5.1 days for MOTT bacilli. This may have been because specimens were fresh, the initial concentration of NaOH used in this institution was 2%, and the concentrated specimens were neutralized; perhaps less damage occurred to mycobacteria during the processing. On the other hand, higher concentration of NaOH may have had some adverse effect on the overall positivity of cultures with institution C. Institution A required the greatest amount of time to report a positive BACTEC culture, perhaps because most of the specimens were stored at refrigeration for some time before inoculation into 7H12 medium. Overall, there was a time savings ranging from an average of 7 to 14 days for *M. tuberculosis* and 5 to 16 days for MOTT bacilli with the BACTEC method for the primary recovery.

The reading schedule of inoculated media has a significant role in early detection of growth. Most of the institutions checked conventional media once per week for 8 weeks and checked BACTEC media three times per week for 3 weeks and weekly thereafter for another 3 weeks. The difference in the reading schedule may have some influence on the overall recovery time. More frequent reading of inoculated media would be expected to reduce the detection time.

Radiometric antimicrobial susceptibility tests, if performed by using pure culture recovered from a conventional solid medium, required approximately 5 days with 98.6% agreement with the susceptibility results obtained by the conventional plate method. These data agree with those of Siddiqi et al., who reported 98% agreement with reportable results within 5 days (4). Susceptibility testing in which inocula from positive BACTEC vials were used required, on an average, one more day than the average time required when growth from solid media was used. The agreement with the conventional results was again similar (98.1%). One center (D) had major discrepancies in results. This center had a large population of resistant cultures, with only 4% susceptible to all four drugs tested.

Analysis of the susceptibility data indicated high values for the sensitivity of the BACTEC testing, except in the case of ethambutol (67%). On the other hand, the predictive value for resistance with ethambutol was 89%. Overall, specificity, sensitivity, and predictive values in this study were similar to or, in some cases, higher than those reported earlier (4, 5).

Overall, the time savings for antimicrobial susceptibility testing of *M. tuberculosis* recovered on conventional media as well as in the BACTEC medium is significant. The reporting time was dependent on the numbers of mycobacterial cells in the inoculum; inocula with large numbers of cells required less time for reaching GI 30 in the control vial. The BACTEC technique was evaluated for a complete recovery and drug susceptibility testing, using a starting inoculum from a BACTEC vial with a GI of 500 or more. An average of 18 days was required to recover *M. tuberculosis* from specimens and provide susceptibility results. The corresponding average of 38.5 days required for the conventional method was calculated without including either (i) the time elapsed between detection and having sufficient growth for making suspension or (ii) the time for subculturing, when required. The time savings of 20 days is the minimum which can be obtained in the best conditions.

The major disadvantage of the BACTEC method is not knowing the type of mycobacteria growing in the medium. In addition, if susceptibility tests are performed directly from the BACTEC vial, many would be done unnecessarily. This deficiency could be avoided if a method were available to differentiate *M. tuberculosis* from MOTT bacilli in this liquid medium. Moreover, drug susceptibility testing of MOTT bacilli does not agree with the results obtained by the conventional method. The BACTEC method tends to detect more susceptibility to drugs (except for isoniazid) than does the conventional method. The validity of the susceptibility testing for potential pathogenic MOTT bacilli is debatable; however, the reliability and clinical relevance of the results obtained by conventional methods are also questionable. A long-range clinical trial and further laboratory studies are needed to settle this issue.

Introduction of automation has a potential of introducing standardization in mycobacteriology

procedures. At present, there is a variety of "conventional" procedures followed for recovery of mycobacteria and drug susceptibility testing. The lack of standardization in methodology sometimes creates doubts about the validity of the results. It is important that with the introduction of a new technique, a standard procedure be recommended and strictly followed.

## LITERATURE CITED

1. **Cummings, D. M., D. Ristroph, E. E. Camargo, S. M. Larson, and H. N. Wagner, Jr.** 1975. Radiometric detection of the metabolic activity of *Mycobacterium tuberculosis*. J. Nucl. Med. **16**:1189–1191.
2. **Middlebrook, G., Z. Reggiardo, and W. D. Tigertt.** 1977. Automatable radiometric detection of growth of *Mycobacterium tuberculosis* in selective media. Am. Rev. Respir. Dis. **115**:1067–1069.
3. **Mitchison, D. A., B. W. Allen, L. Carrol, J. M. Dickinson, and V. R. Aber.** 1972. A selective oleic acid albumin agar medium for tubercle bacilli. J. Med. Microbiol. **5**:165–175.
4. **Siddiqi, S. H., J. P. Libonati, and G. Middlebrook.** 1981. Evaluation of a rapid radiometric method for drug susceptibility testing of *Mycobacterium tuberculosis*. J. Clin. Microbiol. **13**:908–912.
5. **Snider, D. E., Jr., R. C. Good, J. O. Kilburn, L. F. Laskowski, Jr., R. H. Lusk, J. J. Marr, Z. Reigardo, and G. Middlebrook.** 1981. Rapid susceptibility testing of *Mycobacterium tuberculosis*. Am. Rev. Respir. Dis. **117**:631–637.
6. **Vestal, A. L.** 1975. Procedures for the isolation and identification of mycobacteria. Center for Disease Control [Department of Health, Education, and Welfare publication no. (CDC) 76-8230] 97–115.
7. **Vinckè, G., O. Yegers, H. Vanachter, P. A. Jenkins, and J. P. Butzler.** 1982. Rapid susceptibility testing of *Mycobacterium tuberculosis* by a radiometric technique. J. Antimicrob. Chemother. **10**:351–354.