Journal of Clinical Microbiology

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1991. Role of solid media when used in conjunction with the BACTEC system for mycobacterial isolation and identification.. J. Clin. Microbiol. 29(1):154-157.

JOURNAL OF CLINICAL MICROBIOLOGY, Jan. 1991, p. 154–157
0095-1137/91/010154-04$02.00/0

Vol. 29, No. 1

Role of Solid Media When Used in Conjunction with the BACTEC System for Mycobacterial Isolation and Identification

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Received 12 June 1990/Accepted 1 October 1990

This study evaluated the necessity and the contribution of solid media when used in conjunction with radiometric Middlebrook 7H12 (BACTEC 12B; Becton Dickinson, Towson, Md.) medium for recovery and complete identification of mycobacteria. Each of 1,184 digested, decontaminated respiratory specimens was inoculated into one BACTEC 12B vial, one 7H11 plate, and two Lowenstein-Jensen (LJ) slants. When the 12B vial was smear positive for acid-fast bacilli, the organisms were subcultured onto LJ slants and the BACTEC *p*-nitro-α-acetylamino-β-hydroxypropiophenone (NAP) test system was inoculated with the organisms. Niacin tests were performed by using the growth from the original LJ slants and organisms from the LJ slants subcultured from 12B or 7H11 medium. The times to achieve definitive NAP and niacin test results were recorded. Recovery of all 143 isolates found in this study could not be achieved with a single medium. Among the three media, the highest percentage (92.8%) of *Mycobacterium tuberculosis* isolate recovered was with BACTEC 12B. The use of either 7H11 medium or LJ slants along with a 12B vial increased by 4 to 6% the total percentage of *M. tuberculosis* organisms that were isolated. Isolation of the *M. tuberculosis* complex and NAP differentiation in 12B medium were completed in an average of 17 days. On average, isolation and definitive niacin test results for *M. tuberculosis* cultures were obtained in 39.3 days by a conventional procedure and in 36.3 days when 12B subcultures were used. These results support the conclusion that LJ slants contribute 4 to 6% increased recovery of *M. tuberculosis* when used in conjunction with 12B medium. Additionally, a subculture onto LJ slants from 12B medium yielded sufficient growth for niacin testing earlier than an original LJ slant did.

Use of the radiometric technique (BACTEC) for rapid isolation of mycobacteria, differentiation of the *Mycobacterium tuberculosis* complex from other mycobacteria (MOTT bacilli), and drug susceptibility testing has been well established. Several studies have indicated the high sensitivity and time savings offered by this technique (1–4). Mycobacteria are known to be difficult to grow on artificial media; thus, it is customary to use at least two different media to enhance the recovery rate of positive cultures. Most laboratories that use a radiometric system (BACTEC; Becton Dickinson, Towson, Md.) for detection of mycobacteria use a solid medium, as recommended, in conjunction with BACTEC 12B broth.

Cost-containment is an important consideration, and individuals in many laboratories may feel that elimination of the use of solid medium in conjunction with the radiometric (*M. tuberculosis*) system would reduce the cost and labor involved with testing for mycobacteria. This idea is supported by the fact that mycobacteria are detected earlier and more often in 12B medium than they are in any individual solid medium. When the radiometric broth is the only medium used, species-level identification is not possible unless isolates are subcultured onto solid medium. The only test currently available for use with 12B medium is the *p*-nitro-α-acetylamino-β-hydroxypropiophenone (NAP) test, which differentiates the *M. tuberculosis* complex from MOTT bacilli (5).

This study was initiated to assess the value of using a solid medium in conjunction with BACTEC 12B medium. Additionally, the time required for isolation and niacin testing on cultures isolated by the conventional method was compared with that required on cultures isolated in 12B medium and subcultured onto Lowenstein-Jensen (LJ) slants.

MATERIALS AND METHODS

This study was conducted at Jefferson Davis Hospital, Houston, Tex., and at the Maryland State Department of Health and Mental Hygiene, Baltimore. Sputum specimens (n = 500, Jefferson Davis Hospital; n = 684, Maryland State Department of Health and Mental Hygiene) were decontaminated and concentrated by the NaOH–*N*-acetyl-L-cysteine method (6). The final NaOH concentration was 2.0% (Jefferson Davis Hospital) or 2.5% (Maryland State Department of Health and Mental Hygiene). Approximately 0.25 ml of processed specimens was inoculated onto each of two LJ slants and 0.5 ml was inoculated onto one 7H11 agar plate. Smears were prepared and stained for acid-fast bacilli (AFB) by the fluorochrome method. The cultures were incubated at 35 to 37°C and read weekly for a maximum of 8 weeks. Once sufficient growth was obtained, identification was carried out by conventional niacin and other biochemical tests (6). The time required to obtain a definitive niacin test result was recorded. Growth was subcultured onto a fresh LJ slant if the primary culture did not yield adequate growth for a niacin test.

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TABLE 1. Isolation of *M. tuberculosis* from 1,184 specimens on different media

AFB smear results	Total no. of isolates	No. (%) of isolates in each medium			
		12B	LJ-1	LJ-2	7H11
Positive	72	71 (98.6)	60 (83.3)	65 (90.3)	66 (91.7)
Negative	40	33 (82.5)	25 (62.5)	21 (52.5)	22 (55.0)
Combined	112	104 (92.8)	85 (75.9)	86 (76.8)	88 (78.6)

The BACTEC procedure consisted of the inoculation of 0.5 ml of processed specimen into Middlebrook 7H12 medium (BACTEC 12B; Becton Dickinson). As recommended, 0.1 ml of PANTA supplement (polymyxin B, 50 U/ml; amphotericin B, 5 μg/ml; nalidixic acid, 20 μg/ml; trimethoprim, 5 μg/ml; and azlocillin, 10 μg/ml [Becton Dickinson]) was also added to 12B medium to suppress contamination. BACTEC 12B vials were initially tested on the BACTEC 460 system to establish a 5 to 10% CO_2 atmosphere. They were incubated at 37°C and read twice weekly for the first 2 weeks and weekly thereafter for 4 weeks. The amount of $^{14}CO_2$ flushed from the headspace of the vial was expressed as a growth index (GI) on a scale of 0 to 999. When the GI reached ≥10, the vial was tested daily. When the GI reached 50 to 100, a smear was prepared and examined after acid-fast staining. Specimens which demonstrated an apparently pure culture of AFB were tested by the BACTEC NAP test (Becton Dickinson). For the NAP test, positive 12B medium with growth at a GI of 50 to 100 was gently shaken, and 1 ml was transferred to a BACTEC NAP vial. The remainder of the 12B medium, which served as a positive control, and the NAP vial were incubated at 37°C and were tested daily on the BACTEC 460 system for 4 to 6 days. For a satisfactory test, the control vial needed to show a steady increase in GI values each day. In the NAP vial, a decrease or unchanging GI identified the tuberculosis complex (*M. tuberculosis*, *Mycobacterium bovis*, and *Mycobacterium africanum*). An increase in the GI indicated the presence of MOTT bacilli. A total of 0.5 ml of each positive culture with a GI of 50 to 100, after 1 additional day of incubation, was subcultured onto an LJ slant and incubated at 35 to 37°C. The LJ slant cultures were read weekly until satisfactory growth was obtained. The cultures were identified by niacin and other conventional biochemical tests. The time required for a niacin result was recorded and used as an indicator of the amount of time for complete biochemical identification.

The time required for isolation and niacin testing on cultures isolated by the conventional method was compared with that required on cultures isolated in 12B medium and subcultured onto LJ slants. Contamination rates were recorded and compared for each medium.

TABLE 2. Isolation of MOTT bacilli from 1,184 specimens on different media

Organism	Total no. of isolates	No. of isolates in each medium			
		12B	LJ-1	LJ-2	7H11
M. avium complex	20	15	10	12	16
M. fortuitum	6	4	2	3	6
M. kansasii	5	4	3	3	4

TABLE 3. Recovery of *M. tuberculosis* from 1,184 specimens in different combinations of media

Recovery of *M. tuberculosis*[a]	No. (%) of isolates recoverd from medium combinations		
	12B + LJ[b]	12B + 7H11	LJ + 7H11
Both media positive	79	84	78
12B positive only	25	20	
LJ slant positive only	7		8
7H11 positive only		4	10
Total	111 (99.1)	108 (96.4)	96 (85.7)

[a] A total of 112 isolates were recovered on all media combined.
[b] Two LJ slants were used, but only one LJ slant was used for analysis.

RESULTS

The recoveries of significant mycobacteria from 1,184 processed sputum specimens in BACTEC 12B and conventional media are summarized in Tables 1 and 2. BACTEC 12B was the most effective medium for recovery of *M. tuberculosis* from both AFB smear-positive and smear-negative specimens. There were 112 cultures positive for *M. tuberculosis* on all media combined, and 92.8% of isolates were recovered on 12B medium, while LJ slants and 7H11 performed similarly, allowing recovery of 76 to 79% of isolates (Table 1). Among the MOTT bacilli, 7H11 and 12B media allowed recovery of more of the *Mycobacterium avium* complex than LJ slants did, while 7H11 was the most effective in recovering *Mycobacterium fortuitum* (Table 2).

The recovery of *M. tuberculosis* with different combinations of media is shown in Table 3. A combination of BACTEC 12B and one LJ slant recovered 111 of 112 (99.1%) *M. tuberculosis* isolates, while BACTEC 12B and 7H11 recovered 108 of 112 (96.4%) isolates. One LJ slant and 7H11 recovered only 96 of 112 (85.7%) *M. tuberculosis* isolates.

In Table 4, data are summarized for the average time required to detect growth of mycobacteria in BACTEC 12B and conventional media. BACTEC 12B detected growth (GI, ≥10) of *M. tuberculosis* isolates in AFB smear-positive specimens in 9.3 days and in AFB smear-negative specimens in 16.8 days, which was approximately twice as fast as detection of growth on LJ slants and 7H11 medium. BACTEC 12B detected growth of MOTT bacilli in an average of 10.5 days, versus 21.8 days for 7H11 and 29.0 days for LJ medium. A positive culture was confirmed only when a smear made from a 12B vial was positive for AFB, which required an additional 1 to 3 days after initial detection.

TABLE 4. Average time required to detect growth of mycobacteria

Isolates	Avg time (days) to detect growth			
	12B Medium		Conventional method[a]	
	Detection[b]	Smear[c]	LJ	7H11
M. tuberculosis				
Smear positive	9.3	10.6	22.3	18.1
Smear negative	16.8	18.7	31.8	32.4
Combined	11.9	13.1	25.0	22.1
MOTT bacilli	10.5	12.0	29.0	21.8

[a] Macroscopic detection.
[b] GI, ≥10; tentatively positive.
[c] GI, ≥50; confirmed positive by AFB smear.

TABLE 5. Comparison of the average time required to obtain complete isolation and identification with BACTEC NAP and niacin tests

Isolates	Total time (days) to obtain isolation and BACTEC NAP results[a]	Total time (days) required to obtain isolation and niacin test results by:	
		BACTEC procedure[b]	Conventional procedure[c]
M. tuberculosis			
Smear positive	14.1	33.1	33.6
Smear negative	22.5	42.0	52.7
Combined	17.0	36.3	39.3
MOTT bacilli	14.7	37.0	48.0

[a] The BACTEC NAP test differentiated the *M. tuberculosis* complex from MOTT bacilli.

[b] The BACTEC procedure included isolation in 12B and subculture to LJ slants for the niacin test.

[c] The conventional procedure included isolation on solid medium and the niacin test for organisms from the LJ slant, including original or subcultured organisms obtained from growth on solid medium. A total of 35.4% of *M. tuberculosis* and 15.4% of MOTT bacilli required subculturing to obtain sufficient growth for the niacin test.

Comparison of average times required to obtain isolates as well as BACTEC NAP and niacin test results via the BACTEC and conventional procedures are shown in Table 5. For *M. tuberculosis*, the BACTEC NAP test results were completed in 14.1 days for AFB smear-positive specimens and in 22.5 days for AFB smear-negative specimens. The total times required to obtain a niacin test result for *M. tuberculosis* with both the BACTEC subculture and conventional procedures were approximately the same (33 days) for AFB smear-positive specimens, while for smear-negative specimens they were 42.0 and 52.7 days, respectively. For MOTT bacilli, the BACTEC NAP test results were completed in 14.7 days, and the niacin test results by the BACTEC subculture and conventional procedures were completed in 37.0 and 48.0 days, respectively.

The incidence of discarded cultures because of contamination was highest for LJ slants (10.8%), while it was lowest (5.0%) for 7H11 medium. A total of 94 (7.9%) specimens were contaminated in BACTEC 12B medium. The most notable contaminants in BACTEC 12B were as follows: fungi, 33 specimens; gram-positive rods, 35 specimens; gram-positive cocci, 23 specimens; gram-negative rods, 19 specimens. The number of cultures lost because of contamination of BACTEC 12B and all conventional media was 15. The number of cultures lost because of contamination was 21 when BACTEC 12B medium and LJ slants were used and 18 when BACTEC 12B and 7H11 media were used.

DISCUSSION

This study was done to answer the following two questions: (i) would positive cultures be lost if a solid medium was not used in conjunction with a BACTEC 12B vial, and (ii) how much time would be required, compared with the time required for the conventional method, if a positive 12B vial was subcultured onto LJ medium and this subculture was used to perform biochemical testing for identification?

It is evident from the data that no single culture medium can detect 100% of the positive specimens. If all media were combined, there were 112 *M. tuberculosis*-positive specimens of a total of 1,184. The highest recovery was in 12B medium (92.8%). The recovery rates in LJ slants and 7H11 media were approximately the same (76 to 79%). The recovery rate of *M. tuberculosis* by use of 12B medium was significantly higher with smear-negative specimens, accounting for a 20 to 30% increase in recovery over any single solid medium. The addition of one LJ slant with 12B increased the recovery rate of *M. tuberculosis* from 92.8 to 99.1%, which was about a 6% increase, while there was a 3.6% increase when 7H11 was used as the additional medium. On the other hand, LJ and 7H11 media were complementary to each other, contributing 10 and 8 additional cultures, respectively, to the total of 96 positive *M. tuberculosis* isolates.

For MOTT bacilli, the increased recovery in 12B was significant only when compared with that in an LJ slant, while in 7H11 there were three additional positive cultures, two being *M. fortuitum* and one being the *M. avium* complex.

For overall recovery of all significant mycobacteria, 12B detected 127 of 143 (88.8%) positive specimens. The recovery rate in 7H11 was 80%, which was better than that in LJ slants. The combination of 12B and either a single LJ slant or one 7H11 recovered about 94% of significant mycobacteria, an increase of 5% over that with a single 12B medium. This indicates that for an overall recovery of mycobacteria, there appears to be no difference whether an LJ slant or 7H11 is used as an additional medium in conjunction with the 12B vial. However, there may be advantages to using 7H11 plates. It yielded higher numbers of MOTT bacillus isolates, was discarded less than LJ media because of contamination, allowed easier separation of mixed isolates, and added to identification by enabling observation of colony morphology. On the other hand, for further niacin testing, growth on 7H11 agar had to be subcultured onto an LJ slant, which required additional time.

The detection of growth of *M. tuberculosis* or MOTT bacilli in 12B medium was twice as fast as detection of growth on conventional media, saving about 10 to 11 days. This observation is similar to those of earlier reports (1–4).

Another objective of this study was to establish the time required to do a complete identification by biochemical testing, with the niacin test being taken as a key test for identification. Our data indicated that with 35.4% of *M. tuberculosis* isolates and 15.4% of MOTT bacilli, growth on the original solid medium was not sufficient and a subculture had to be made in order to perform niacin tests. Overall, the average time required to complete isolation and the niacin test by conventional methods was approximately the same as the time required for isolation in 12B and biochemical testing on its subculture (36 to 39 days). For MOTT bacilli, the average time required for the conventional system was 48 days, which was 11 days longer than that for the BACTEC system.

Overall, for *M. tuberculosis* 12B vials were subcultured on an average of 11.7 days from smear-positive specimens and 19.9 days from smear-negative specimens. For MOTT bacilli, the average time was 13.3 days. Many clinical laboratories do not carry out complete identification; rather, they differentiate the *M. tuberculosis* complex from MOTT bacilli by the BACTEC NAP test. In this study, isolation and NAP test results were completed on an average of 17 days for *M. tuberculosis* and 15 days for MOTT bacilli, which is much faster than the time necessary to determine niacin results.

As for the possibility of using 12B medium as a single isolation medium, it appears from these data that for *M. tuberculosis*, which has the greatest public health significance of all mycobacteria, there is a loss of 5 to 6% of positive cultures if an additional solid medium is not used.

This percentage may be minimized by processing multiple specimens from each patient. On the other hand, 12B yielded 18 to 21% additional positive cultures within a much shorter time than any single solid medium did. Complete isolation and identification, including niacin testing, may be achieved approximately at the same time or even earlier from a 12B subculture compared with that when isolates on conventional solid media are used.

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