Journal of Clinical Microbiology

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L. Heifets, T. Linder, T. Sanchez, et al.
2000. Two Liquid Medium Systems, Mycobacteria Growth Indicator Tube and MB Redox Tube, for Mycobacterium tuberculosis Isolation from Sputum Specimens. J. Clin. Microbiol. 38(3):1227-1230.

JOURNAL OF CLINICAL MICROBIOLOGY, Mar. 2000, p. 1227–1230 Vol. 38, No. 3
0095-1137/00/$04.00+0

Two Liquid Medium Systems, Mycobacteria Growth Indicator Tube and MB Redox Tube, for *Mycobacterium tuberculosis* Isolation from Sputum Specimens

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Received 27 July 1999/Returned for modification 16 November 1999/Accepted 30 December 1999

Two manual liquid medium systems, the Mycobacteria Growth Indicator Tube (MGIT) and MB Redox tube systems, were evaluated in comparison to the radiometric BACTEC-460 semiautomated system for recovery of *Mycobacterium tuberculosis* from sputum specimens. The highest level of recovery, from a total of 77 culture-positive specimens, occurred with the BACTEC-460 system (92.2%), followed by the MB Redox tube (80.5%) and the MGIT (63.6%) systems. The shortest time to detection was observed also among the cultures in BACTEC-460: a mean of 12 days to a growth index (GI) of 10 and 15 days to a GI of 500. The mean times for the other systems were 16 days for the MB Redox tube system and 17.4 days for the MGIT system. The proportion of cultures grown after more than 3 weeks of incubation was only 2.8 or 8.4% in BACTEC-460 (for a GI of 10 or 500) but 17.7% in MB Redox and 22.5% in MGIT. Despite these differences in comparison to the BACTEC-460 system and some differences between the MGIT and MB Redox tube systems, either of the two manual liquid medium systems presents a reasonable alternative to the BACTEC-460 system, especially for laboratories with a limited workload, and a valuable element in the laboratory protocol, in conjunction with solid media, for obtaining rapid detection of growth from about 80% of culture-positive specimens and for better overall recovery of *M. tuberculosis*.

It is now mandatory in the United States that a unit of a liquid medium be used, along with solid media, for any attempt of mycobacterial culture isolation from raw specimens. The purpose of this addition is to expedite the laboratory diagnosis of tuberculosis through recovery of the early mycobacterial growth. This is especially important for the subsequent timely detection of drug resistance in the isolated cultures. Also, incorporation of an additional unit of medium increases the overall recovery of mycobacteria in cultures. For example, at the National Jewish Medical and Research Center in Denver, Colorado, four media are being used for inoculation of any raw specimen, as follows: (i) plain 7H11 agar (half of the biplate), (ii) selective 7H11 agar (second half of the biplate), (iii) Lowenstein-Jensen (L-J) egg-based medium (a slant), and (iv) 7H12 broth in a 12B BACTEC vial for radiometric detection of growth. Analysis of 243 *Mycobacterium tuberculosis* cultures isolated from 1994 to 1996 indicated that only about half of the isolates grew on all media used (Table 1). About 14% of all isolates were detected in the BACTEC broth only, and this proportion was higher for cultures isolated from smear-negative specimens (Table 1).

The BACTEC-460 system (Becton-Dickinson Microbiology Systems, Sparks, Md.) was the first semiautomated liquid medium system introduced for rapid detection of mycobacterial growth (12), and after about 20 years of successful use in many clinical laboratories it can be considered the one most frequently in standard use among such systems (5, 9, 11, 13, 17, 19, 21, 22, 25). Though the benefits of incorporation of the BACTEC-460 system into clinical protocols are well known, some problems inherent to this system have limited its broad use. Among them are (i) the need for disposal of large volumes of low-grade radiolabeled material (12B vials), (ii) labor time associated with the necessity of loading and unloading of the vials incubated separately, and (iii) manual transfer of the growth index (GI) daily readings to the laboratory worksheet.

These problems were addressed in the development of fully automated and computerized systems in which detection of growth in liquid media is based on measurement of either consumption of oxygen or release of CO_2 by other than radiometric techniques. These nonradiometric systems are BACTEC 9000MB and BACTEC MGIT 960 from Becton-Dickinson Microbiology Systems, MB/BacT from Organon-Teknica Corp. (Durham, N.C.), and ESP from Difco-AccuMed (currently Trek Diagnostic Systems, Inc., Westlake, Ohio). All of these automated systems, as well as the BACTEC-460, are quite expensive, and their implementation can be economically justified only for laboratories with a substantial volume of work. Despite the recent tendencies to have laboratory services concentrated in large laboratories, there are still a substantial number of laboratories with a relatively limited workload, and these laboratories cannot afford and/or justify the incorporation of the automated liquid medium systems into their protocols. For such laboratories, a simple and less expensive liquid medium unit to be used along with the solid media to obtain growing *M. tuberculosis* cultures in the shortest possible turnaround time became an urgent necessity.

So far, two liquid medium systems can be considered for implementation in such an environment: the Mycobacterium Growth Indicator Tube (MGIT) system from Becton-Dickinson Microbiology Systems and the MB Redox tube system from Biotest AG (Dreieich, Germany) or from Biotest Diagnostics Corporation (Danville, N.J.).

Isolation of *M. tuberculosis* in MGIT medium was evaluated in a number of reports. Some of them showed a faster turnaround time than did reports on the use of egg-based media, as well as higher recovery rates when this liquid medium was used

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TABLE 1. Isolation of *M. tuberculosis* cultures[a]

No. of AFB per field	No. of cultures	No. (%) of positive cultures on:				
		BACTEC	7H11 agar	L-J medium	7H11 and BACTEC media	All three media
None	24	29.2	12.5	8.3	33.3	16.7
<1	70	14.3	5.7	1.4	27.2	51.4
1–10	101	10.9	4.0	2.0	25.7	57.4
>10	48	14.6	2.1	2.1	18.8	62.5
All	243	14.4	4.9	2.5	25.5	52.7

[a] Cultures were isolated from sputum specimens in the Clinical Mycobacteriology Laboratory at National Jewish Medical and Research Center (1994 to 1996). AFB, acid-fast bacilli.

in conjunction with L-J or Ogawa slants (1, 14, 16, 20, 23). In other reports recovery of mycobacteria from clinical specimens in MGIT medium was compared also with that in BACTEC-460 (2, 6–8, 18). All authors concluded that MGIT medium was superior to any solid media with regard to timing, but the time to recovery and the recovery rates in MGIT medium were somehow lower than those with BACTEC-460.

MB Redox medium was evaluated only in a few studies (4, 15, 24, 27), with the conclusion that the time to recovery in this medium was significantly shorter than that on L-J medium or 7H11 agar. Only one of these studies compared MB Redox medium with MGIT medium, showing a faster turnaround time for MB Redox medium, especially from smear-negative specimens, but a slightly lower recovery rate (24).

None of the studies included comparison of the three media MGIT, MB Redox, and BACTEC-460, using the same sputum specimens. Therefore, the aim of this study was to evaluate the effectiveness of the two manual liquid media, MGIT and MB Redox, in isolation of *M. tuberculosis* from sputum specimens by comparing the recovery rates and time to growth detection with those observed with the BACTEC-460 system.

MATERIALS AND METHODS

Culture media. All three media are commercially available from the above-mentioned manufacturers. BACTEC 12B vials contain 4.0 ml of 7H12 broth, which consists of Middlebrook 7H9 broth plus bovine serum albumin plus casein hydrolysate plus catalase plus ^{14}C-labeled palmetic acid. This medium has to be supplemented for culture isolation with an antimicrobial mixture containing polymyxin B, amphotericin B, nalidixic acid, trimethoprim, and azlocillin (PANTA), 0.1 ml per vial, to prevent the growth of nonmycobacterial contaminants.

The MGIT is a 16- by 100-mm tube containing 4.0 ml of 7H9 broth with 0.25% glycerol. The bottom of this tube has a fluorescent indicator embedded in silicone. Consumption of oxygen by the growing mycobacteria activates the indicator, which produces fluorescence (bright orange colors on the bottom and the meniscus) when the tube is exposed to UV light generated by a 365-nm UV transilluminator. Before inoculation with the processed sputum specimens, the tubes have to be supplemented with 0.5 ml of OADC (oleic acid, albumin [bovine], dextrose, catalase) and 0.1 ml of PANTA.

MB Redox tubes, 16 by 125 mm, contain 4.0 ml of the modified Kirchner medium (Kirchner base medium plus glucose plus horse serum plus vitamin combination plus OADC plus catalase). This broth contains colorless tetrazolium salt, which is reduced by the mycobacterial redox system to a pink-, red-, and violet-colored formazan (3). The latter is accumulated on the cell surface in a granular form, and the growing microcolonies become visible as colored particles. MB Redox broth is a selective medium containing a mixture of antimicrobials (polymyxin B, amphotericin B, carbenicillin, and trimethoprim [PACT]) to inhibit the growth of nonmycobacterial contaminants. Unlike in the BACTEC-460 and MGIT media, this mixture of antimicrobial agents is already incorporated into the medium and does not have to be added before use. Both types of tubes, MGIT and MB Redox, have screwcap tubes, but the cap of the MB Redox tube also has a septum, which allows the choice of using either a syringe (as in BACTEC vials) or a pipette for inoculation. We have not included in this study a comparison with solid media, since there are a large number of publications stating the advantage of any liquid over solid media (7, 11, 14, 15, 18, 20, 22, 24, 25).

Specimens. Sputum specimens were obtained from patients for whom the diagnosis of tuberculosis was previously confirmed by isolation of *M. tuberculosis*. A total of 135 specimens, including 86 at the Missouri State Laboratory and 49 at the National Jewish Medical and Research Center, were included in this study. In the first phase of the study, 64 specimens were obtained during the last few months of treatment: 39 of them were smear and culture negative, 21 were smear negative but culture positive, and 4 were smear and culture positive. The second phase of the study included 71 specimens obtained during the intensive phase of therapy or at the beginning of the continuation phase, and all these specimens were smear positive, 52 of them were culture positive, and only 19 were culture negative on all media. Altogether, the analysis included 58 culture-negative specimens plus 77 specimens which produced growth on at least one culture medium unit. Specimens that produced heavy growth of contaminants on all media without any growth of *M. tuberculosis* were not included in this count.

Procedure. All sputum specimens were processed by the digestion-decontamination procedure using the standard NaOH-NALC technique, providing exposure to a final NaOH concentration of 1% for 15 min. After centrifugation of the buffered specimen at 3,000 × *g* with refrigeration, the pellets were resuspended in 0.1% bovine albumin solution to provide a sufficient volume for the subsequent procedures. Each concentrated specimen was inoculated into three medium units, 12B vial, MGIT, and MB Redox tube, 0.5 ml per each. All tubes and vials were incubated at 36.0 ± 1.0°C, and the readings were done daily for a period of 28 days and then once a week for up to 42 days.

RESULTS

Recovery of *M. tuberculosis*. From a total of 135 specimens, growth of *M. tuberculosis* was detected in 71 BACTEC 12B vials, in 62 MB Redox tubes, and in 49 MGITs (Table 2). From among the 71 BACTEC-positive cultures, the growth was also positive in 57 MB Redox tubes and in 48 MGITs. On the other hand, cultures from five specimens grown in MB Redox tubes and one grown in an MGIT did not grow in the BACTEC broth (Table 2). Direct comparison of MB Redox and MGIT medium (Table 3) indicated that the recovery rate in MB Redox medium was slightly higher than that in MGIT medium: 47 were positive in both, 15 were positive in MB Redox medium only, and 2 were positive in MGIT medium only. From a total of 77 culture-positive specimens, the recovery rates were 92.2% in the BACTEC system, 80.5% in the MB Redox tube system, and 63.6% in the MGIT system (Tables 2 and 3).

Time to positive cultures. Distribution of the positive cultures by the number of days required to detect growth in MB Redox and MGIT media was compared with that in BACTEC

TABLE 2. Recovery of *M. tuberculosis* from sputum specimens in three liquid medium systems: the MB Redox tube and MGIT systems versus the BACTEC system[a]

Result with BACTEC system	No. of cultures				
	BACTEC	MB Redox		MGIT	
		Pos.	Neg.	Pos.	Neg.
Pos.	71	57	14	48	23
Neg.	64	5	59	1	63
Total	135	62	73	49	86

[a] Pos., positive; Neg., negative.

TABLE 3. Recovery of *M. tuberculosis* from sputum specimens in two manual liquid medium systems: the MB Redox tube system versus the MGIT system[a]

Result with MB Redox medium	No. of cultures in MGIT medium that were:		Total no. of cultures
	Pos.	Neg.	
Pos.	47	15	62
Neg.	2	71	73
Total	49	86	135

[a] Pos., positive; Neg., negative.

broth by two criteria: detection of the minimal growth at a GI of 10 to 20 and at a GI of 500. It is well known that the BACTEC cultures positive at the level of the daily GI reading of about 10 to 20 are suitable for identification only if an amplification test is used (10, 26). The BACTEC cultures usually contain a sufficient number of viable bacteria to perform speciation by other methods and/or for drug susceptibility testing only when the daily GI reading exceeds 500 (which is equivalent to about 10^6 CFU per ml or more). On the other hand, the first signs of positivity in MB Redox and MGIT media appear when the content level of viable bacteria in the culture is already at this high level.

Detection of growth in the BACTEC broth at a GI of just above 10 within the first week of cultivation occurred in 15.5% of cultures, but in no cultures at this time in MGIT medium and only in 1.6% of cultures in MB Redox medium (Table 4). Subsequently, the proportion of cultures that produced growth in BACTEC broth at this level within the first 2 weeks of cultivation was substantially higher than that in the two other systems: 66.2% versus 43.5% in the MB Redox tube system and 40.8% in the MGIT system (Table 4). At the same time, the proportion of cultures that achieved a GI of 500 or greater in BACTEC broth within the first 2 weeks, 50.7%, was not so dramatically different from the results in two other systems. The proportions of cultures that turn out to be positive within the first 2 weeks of cultivation are indicators of the value of the broth systems as rapid methods compared with any solid medium used for primary culture isolation. It is also partially true for cultures in which growth is detected during the third week, days 15 to 21 (Table 4). The major role of growth detection in a liquid medium during the first 3 weeks is that it contributes to the overall recovery of *M. tuberculosis* in all media, as is illustrated from our previous experience (Table 1) and from the above-cited references. It is questionable whether the late growth detection in liquid media (after 3 weeks) can contribute to either rapid detection or the total recovery rates. The proportions of MB Redox tube and MGIT cultures that became positive after more than 3 weeks were substantially higher than that in the BACTEC system (Table 4).

DISCUSSION

There is an abundant number of reports indicating the usefulness of selective liquid media in expediting the recovery of *M. tuberculosis* from sputum and other diagnostic specimens, when such a medium is used in addition to one or two solid media. Therefore, in this report we have not addressed again the well-known issue of the effectiveness of liquid and solid media. Most of this experience in many clinical laboratories around the world has been derived from using the BACTEC-460 system along with egg-based and/or agar-based media (5, 9, 11, 13, 17, 19, 21, 22, 25). The major problem of the BACTEC-460 system, the need for disposal of a large volume of the radiolabeled material, stimulated development of alternative liquid medium systems. Two among such medium systems, the MGIT and MB Redox tube systems, require minimal or no special equipment, which makes them more affordable for tuberculosis laboratories with relatively small workloads that do not require automatization or computerization. We compared the effectiveness of these two products to the BACTEC-460 system, including recovery rates of *M. tuberculosis* and turnaround time to the positive culture. The mean time to the positive culture in MGIT medium varies in different reports depending on the proportion of specimens with low bacterial contents, from 5 to 9 days (16, 18) to 13, 15.7, 16.3 (1, 20, 23), and even up to 20.3 and 22 days for smear-negative specimens (18). Regardless of the actual range, the time to detection in MGIT medium in these reports was always shorter than that observed with egg-based media. In our study, the mean time to recovery in MGIT medium was 17.4 days, not much different from that demonstrated in some other reports, but we found that it was greater than that in the BACTEC broth (12 days at a GI of 10 and 15 days at a GI of 500) and that in the MB Redox tube (16 days). Besides the mean time to detection, the proportion of cultures grown later than within 3 weeks, which is an indicator of failure of rapid detection, was the largest for the MGIT system (22.5%) compared with the BACTEC-460 (2.8 to 8.4%) and MB Redox tube (17.7%) systems. So, the results of this study have demonstrated that both the recovery rates and time to detection in two manual liquid medium systems were less favorable than in the BACTEC-460. This difference should be considered the price for having a nonradiometric and relatively simple technique.

Comparison between the MGIT and MB Redox tube systems indicated some advantages of the MB Redox tube system in regard to both recovery rates and timing. In the only other study that compared the MB Redox tube and MGIT systems (without comparison to BACTEC) the time to recovery in these two systems was also better for the MB Redox tube system: 6.9 versus 7.2 days for smear-positive specimens, and

TABLE 4. Turnaround time of *M. tuberculosis* recovery from 77 culture-positive specimens in three liquid medium systems

System (GI)	Total no. of positive cultures[a]	No. of days to a positive culture		% of cultures with growth detected after the following no. of days:			
		Mean	Range	≤7	8–14	15–21	≥22
BACTEC (>10)	71 (92.2)	12.0	3–23	15.5	50.7	31.0	2.8
BACTEC (≥500)	71 (92.2)	15.0	8–26	1.4	49.3	40.9	8.4
MB Redox tube	62 (80.5)	16.0	8–28	1.6	41.9	38.7	17.7
MGIT	49 (63.6)	17.4	8–42	0	40.8	36.7	22.5

[a] Values in parentheses are percentages.

15.5 versus 19.5 days for smear-negative specimens (24). Despite these differences, the effectiveness of both the MGIT and the MB Redox tube systems is quite comparable to that of the BACTEC-460 system, and inclusion of any of these media into the protocol of isolation (in addition to solid media) will definitely improve the productivity of clinical laboratories. The choice between the MGIT and the MB Redox tube systems should be made by taking into account not only the effectiveness of these systems but also cost, availability, technical convenience, and other operational issues. For example, OADC and PANTA have to be added to the MGIT medium but this step is not needed for MB Redox medium. On the other hand, the shelf life of MB Redox medium is shorter, and refrigerated storage is required because OADC and PACT are included in the MB Redox tubes by the manufacturer. Neither MGITs nor MB Redox tubes should be used instead of a solid medium; rather, they should be used in addition to it. We believe, based on our experience addressed in the introduction, that the priority among solid media should be given to a combination of 7H11 plain and selective agars (biplate), since it helps to recover *M. tuberculosis* from cultures with slight contamination, and is most convenient for detection of mixed mycobacterial cultures, as well as for examination of the colonial morphology. The combined use of either MGIT or MB Redox medium with a solid medium (7H11 agar or L-J medium) improves the overall level of recovery of *M. tuberculosis* from the specimens and provides rapid detection of mycobacterial growth for up to 80% of the culture-positive specimens.

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