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Comparison of a Nonradiometric Liquid-Medium Method (MB REDOX) with the BACTEC System for Growth and Identification of Mycobacteria in Clinical Specimens

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Early identification of tuberculosis in the clinical setting is of great importance in order for specific therapy to be swiftly initiated. MB REDOX (Heipha Diagnostika), a growth-based medium without radioactive materials, was evaluated and was compared to the BACTEC system for detection of mycobacteria, including the *Mycobacterium tuberculosis* complex and atypical mycobacteria. MB REDOX consists of a Kirchner medium enriched with growth-promoting additives, antibiotic compounds, and a redox indicator which can be monitored to detect growth of mycobacteria with the naked eye. MB REDOX only detects growth and cannot differentiate the *M. tuberculosis* complex (*M. tuberculosis*, *M. bovis*, and *M. africanum*) from other species of *Mycobacterium*. Therefore, PCR-restriction fragment length polymorphism analysis (PRA) was used in this investigation to identify to the species level organisms showing positive growth with MB REDOX. Our data demonstrate the usefulness of MB REDOX for the detection of mycobacteria in clinical specimens. The rate of detection of *M. tuberculosis* complex with MB REDOX (84.3%) was higher than that with the BACTEC system (68.6%). When combined with PRA for species identification, MB REDOX is easy to perform and is suited to most clinical laboratory settings for the detection and identification of mycobacteria.

Tuberculosis remains a major health threat, and the rapid emergence of drug-resistant mycobacteria has strengthened the demand for methods for the rapid detection of mycobacteria in clinical samples. Since the prevention of tuberculosis relies on early detection and cure of infectious cases (14), current efforts are focused upon improvements in the rapidity of identification of *Mycobacterium tuberculosis*, which would allow prompt initiation of appropriate therapy. Although the PCR-based technique for the diagnosis of tuberculosis has been well established (1–6, 9, 11, 12), culture still remains the "gold standard" method. However, because *M. tuberculosis* has a doubling time of 16 to 18 h, current methodology does not allow fast detection and identification, leaving patients, contacts, and health care workers at risk. Our focus is on shortening the delay (often weeks long) for confirmation of a result by culture. Use of liquid culture media is one way of considerably shortening this delay. The BACTEC 460 system (Becton Dickinson) is recognized as a reference method, combining the advantage of liquid medium with automation. Unfortunately, this system uses a radiometric method for the detection of mycobacterial growth. Separate disposal of the radioactive waste produced presents a considerable problem in terms of increased costs. MB REDOX is a ready-to-use liquid medium produced by the company Heipha/Biotest (Heipha Diagnostika). This medium is made of a Kirchner medium (7) enriched with growth-promoting additives and an antibiotic compound, PACT (polymyxin B, amphotericin B, carbenicillin, and trimethoprim). The medium also contains a redox indicator, a colorless tetrazolium salt which is reduced to a colored formazan during the growth of mycobacteria. In this process, the pinhead-size colonies produced by *M. tuberculosis* take on a red to dark purple coloring, which is typical for organisms grown on Kirchner medium. Through the redox system of the growing mycobacteria, a colorless tetrazolium salt contained in the medium is reduced to a red- or purple-colored formazan. In this way the mycobacterial colonies acquire a coloring which can be seen with the naked eye.

The goal of this study is to compare growth and time to detection of mycobacteria with a new nonradiometric liquid medium with those with the BACTEC 460 system. MB REDOX, combined with PCR-restriction fragment length polymorphism analysis (PRA) for identification to the species level, was subjected to testing with a total of 997 clinical samples, as was the BACTEC 460 system.

MATERIALS AND METHODS

Specimen collection and processing. Specimens were collected from patients who were either suspected of having tuberculosis or who were being monitored for treatment with antituberculosis drugs at the Veterans General Hospital-Kaohsiung, Kaohsiung, Taiwan, from April to June 1997. A total of 995 specimens from 545 patients were investigated, including specimens obtained from the respiratory tract, body fluids, tissues, wound, pus, and skin. Specimens which could not be processed immediately were stored at 2 to 6°C for no longer than 72 h. All specimens were analyzed for growth of mycobacteria by using the BACTEC 460 system (Becton Dickinson Diagnostic Instrument Systems, Towson, Md.) according to the manufacturer's instructions. Specimens were decontaminated and digested with 2% NaOH in 0.5% *N*-acetyl-L-cysteine and were concentrated (8). Following digestion, decontamination, and concentration, all specimens were divided, with one-half used for modified Middlebrook medium 12B and one-half used for MB REDOX. The incubation took place over a period of 8 weeks at a temperature of 36 ± 1°C.

Smear examination. Smears of the digested and decontaminated specimens were stained with rhodamine-auramine and Ziehl-Neelsen stain and were examined by standard procedures (8) for acid-fast bacilli (AFB).

Culture and identification with BACTEC 460 system. Culture for mycobacteria was performed by inoculation of the digested, decontaminated, and concentrated material into one BACTEC 12B bottle. Growth was monitored with the BACTEC 460 instrument (Becton Dickinson Diagnostic Instrument Systems). The *p*-nitro-α-acetyl-amino-β-hydroxypropiophenone (NAP) test was performed when the bottle was detected as positive (growth index [GI], >50). A decrease or lack of change in the GI in the presence of NAP is presumptive evidence of *M. tuberculosis*.

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TABLE 1. Growth of mycobacteria as detected with BACTEC 460 system and MB REDOX

Specimen (no.)	No. (%) positive with:	
	BACTEC 460 system	MB REDOX
Respiratory (675)	86 (12.7)	84 (12.4)
Body fluids (401)	11 (3.6)	17 (5.6)
Tissue (9)	3 (33.3)	1 (11.1)
Others (10)	2 (20.0)	2 (20.0)
Total (995)	102 (10.3)	104 (10.5)

Culture and identification by MB REDOX-PRA. Initial readings by observation of red- or purple-colored particles with the naked eye were taken from MB REDOX after 24 h and then twice weekly from the 1st to the 3rd weeks. From the 4th to the 8th weeks, the readings were performed once weekly. The criteria for a positive culture was the presence of red- or purple-stained colonies. The culture was confirmed by microscopy and was identified by PRA of the *hsp65* gene (6, 12), which is present in all mycobacteria. The digested PCR products obtained by using primers common to all mycobacteria (10, 13) were evaluated by the algorithm established previously (12), thus allowing rapid identification of mycobacteria to the species level.

The contamination rate for each culture method is defined as the percentage of those cultures considered to be positive for AFB by the interpretive criteria for that method, as stated above, in which contamination with bacteria was noted.

RESULTS

Detection of growth by culture. Results of a comparison of MB REDOX with the BACTEC 460 system for the detection mycobacteria and growth for 995 specimens from 545 patients are shown in Table 1. The isolation rates were similar for both methods. One hundred two (10.3%) specimens yielded positive growth with the BACTEC 460 system, whereas 104 (10.5%) specimens were positive with MB REDOX.

The times to detection of *M. tuberculosis* complex were also similar with MB REDOX and the BACTEC 460 system (Table 2). The average and median times to detection of the *M. tuberculosis* complex were 24 and 19 days, respectively, with the BACTEC 460 system and 23 and 23 days, respectively, with MB REDOX. The range of times to detection for the BACTEC 460 system was broader, from as early as 4 days to 74 days, whereas it was 8 to 51 days for MB REDOX. The average time to detection for MB REDOX was 2 days shorter than that for the BACTEC 460 system for those *M. tuberculosis* complex-infected samples for which the initial acid-fast smear results were positive. For those samples that yielded *M. tuberculosis* complex on culture but for which the initial acid-fast smear results were negative, detection times were the same with the two media.

Identification of mycobacteria. In order to determine the species of mycobacteria detected with MB REDOX, we chose a PCR approach, PRA. The species data generated with the BACTEC 460 system linked with the NAP test were then compared to the species data generated by MB REDOX-PRA. Table 3 shows that 70 *M. tuberculosis* complex isolates, 20 isolates of *Mycobacterium* other than *M. tuberculosis* (MOTT), and 12 AFB (AFB were seen but could not be further identified) were detected with the BACTEC 460 system, while 86 *M. tuberculosis* complex isolates and 18 isolates of MOTT (5 *M. kansasii*, 5 *M. intracellulare*, 1 *M. triviale*, 1 *M. gordonae*, 3 *M. fortuitum*, and 3 *M. abscessus* isolates) were detected with MB REDOX.

Thirty-two specimens were culture positive with MB REDOX but were not detected with the BACTEC 460 system, including 26 *M. tuberculosis* complex isolates and 6 isolates of MOTT. Thirty specimens were culture positive with the BACTEC 460 system but were not detected with MB REDOX, including 14 *M. tuberculosis* complex, 12 MOTT, and 4 AFB isolates not further identified (Table 3). The isolate in one specimen identified as MOTT with the BACTEC 460 system was revealed to be an *M. tuberculosis* complex isolate by MB REDOX-PRA. The isolates in two specimens identified as *M. tuberculosis* complex by BACTEC were reported as MOTT by MB REDOX-PRA. Isolates in five and three specimens reported to be AFB positive with the BACTEC 460 system were identified as *M. tuberculosis* complex and MOTT, respectively.

One hundred thirty specimens were culture positive and were identified by either the BACTEC 460 system–NAP or MB REDOX-PRA approach. The results were compared with those of the microscopic acid-fast staining method (Table 4). The BACTEC 460 system identified 70 (68.6%) *M. tuberculosis* complex isolates, including 34 that were acid-fast positive and 36 that were acid-fast negative. The MB REDOX-PRA detected 86 (84.3%) *M. tuberculosis* complex isolates, including 40 that were acid-fast positive and 46 that were acid-fast negative. The 44 acid-fast stain-positive samples showed growth of *M. tuberculosis* complex only with the BACTEC 460 system for 4 samples and only with MB REDOX for 10 samples. The 58 acid-fast stain-negative samples showed growth of *M. tuberculosis* complex only with the BACTEC 460 system for 12 samples and only with MB REDOX for 22 samples.

The contamination rates were 7.41% with the BACTEC 460 system and 12.06% with MB REDOX. The PRA method is able to detect mycobacteria in the presence of contaminating bacteria. In the BACTEC 460 system, contamination of bacteria made the identification of mycobacteria impossible for 17 specimens, for 13 of which mycobacteria were detected with MB REDOX, including 10 *M. tuberculosis* and 3 MOTT isolates that were identified with MB REDOX but that were missed by the BACTEC 460 system due to contamination with bacteria.

TABLE 2. Times to detection of *M. tuberculosis* complex

Test and result	BACTEC 460 system				MB REDOX			
	No. of specimens	Time (days) to detection			No. of specimens	Time (days) to detection		
		Mean	Median	Range		Mean	Median	Range
Culture, positivity for Mtb[a]	70	24	19	4–74	86	23	23	8–51
Initial acid-fast smear								
Positive	34	22	15	4–74	40	20	15	8–43
Negative	36	26	22	8–53	46	26	26	8–51

[a] Mtb, *M. tuberculosis* complex.

TABLE 3. Growth and identification of mycobacteria by MB REDOX-PRA and with the BACTEC 460–NAP test system

Mycobacterium	No. of positive cultures detected with:				
	BACTEC 460 system	MB REDOX	Both systems	BACTEC 460 positive, MB REDOX negative	BACTEC 460 negative, MB REDOX positive
Mtb[a]	70	86	54	14	26
MOTT	20	18	7	12	6
AFB(+)[b]	12	0	5	4	NA[c]
No growth	893	891	861	NA	NA

[a] Mtb, *M. tuberculosis* complex.
[b] AFB(+), AFB were seen but could not be further identified.
[c] NA, not available.

DISCUSSION

This study showed that both systems have comparable abilities in terms of speed of detection and yield of mycobacteria. However, a higher rate of isolation of *M. tuberculosis* complex by MB REDOX-PRA (84.3%) in comparison with that with the BACTEC 460 system (68.6%) was found. The rate of detection of nontuberculous mycobacteria with the BACTEC 460 system was better than that by MB REDOX-PRA. However, the overall performance of MB REDOX with body fluids and tissue seems to be better than that with the BACTEC 460 system.

Further advantages of MB REDOX include the ease of handling. The antibiotic compound is already incorporated into the medium that is purchased; therefore, the extra labor for adding supplement as required for the BACTEC 12B medium is not necessary. Detection of growth is made by observation with the naked eye and does not require daily testing of the GI. No instrument is required. A further positive factor is the combination of PRA and MB REDOX, with PRA used for the identification of the mycobacteria detected with MB REDOX. We selected PRA in this investigation to identify species with positive growth on MB REDOX because of its ease and rapidity and because it may help identify numerous species of mycobacteria within a single experiment. Thus, MB REDOX has the advantages of rapidity, sensitivity, and feasibility in the presence of contaminating bacteria.

The major disadvantage of MB REDOX is that there is no complete identification procedure following detection. The medium, MB REDOX, is only able to detect growth and does not differentiate the *M. tuberculosis* complex (*M. tuberculosis*, *M. bovis*, and *M. africanum*) from other species. An external method, such as PRA, must be applied. In addition, no drug susceptibility tests following detection in MB REDOX have been adapted for use with this medium.

MB REDOX can be used in most clinical laboratory settings without expensive instruments and intensive labor. Also, use of MB REDOX avoids the need for exposure to radioactive materials and disposal of radioactive waste.

Our data demonstrate the utility of MB REDOX for the detection of mycobacteria in clinical specimens. In addition, the benefits of other approaches with molecular probes (such as those used in the BACTEC 460 system) can easily be achieved with MB REDOX. If combined with a means such as PRA for the identification of species, tests with MB REDOX are easy to perform and are suitable for most clinical laboratory settings.

TABLE 4. Analysis of 130 specimens with both systems with microscopy[a]

Organism and system	No. (%) of specimens	No. of specimens with the following acid-fast staining result:	
		Positive	Negative
Possible detection of Mtb[b]	102	44	58
BACTEC 460 system	70 (68.6)	34	36
MB REDOX	86 (84.3)	40	46
Possible detection of MOTT	31	2	29
BACTEC 460 system	20 (64.5)	1	19
MB REDOX	18 (58.1)	1	17

[a] Total number of specimens identified with either system.
[b] Mtb, *M. tuberculosis* complex.

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