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C Abe, S Hosojima, Y Fukasawa, et al.
1992. Comparison of MB-Check, BACTEC, and egg-based
media for recovery of mycobacteria.. J. Clin. Microbiol.
30(4):878-881.

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Comparison of MB-Check, BACTEC, and Egg-Based Media for Recovery of Mycobacteria

CHIYOJI ABE,* SUMIKO HOSOJIMA, YUTAKA FUKASAWA, YUKO KAZUMI,
MITSUYOSHI TAKAHASHI, KAZUE HIRANO, AND TORU MORI

Research Institute of Tuberculosis, Japan Anti-Tuberculosis Association,
Kiyose-shi, Tokyo 204, Japan

Received 10 July 1991/Accepted 2 January 1992

The rate of recovery and time to the detection of mycobacteria from clinical specimens were measured for biphasic (MB-Check; Nippon Roche Co., Ltd., Tokyo, Japan) and radiometric (BACTEC; Nippon Becton Dickinson Co., Ltd., Tokyo, Japan) liquid-based culture systems and egg-based media (3% Ogawa and Ogawa K). From the 245 sputum specimens processed, a total of 86 (35.1%) mycobacterial isolates were detected. Of these, 81 (94.2%) and 80 (93.0%) isolates were detected with the MB-Check and BACTEC systems, respectively, and 65 (75.6%) isolates were detected with the 3% Ogawa egg method. The difference in the percentages of positive cultures between the two systems based on liquid media and the 3% Ogawa egg method was significant ($P < 0.01$). This difference was even greater among smear-negative specimens. The detection time was shorter with the liquid-based systems. The mean times to the detection of the *Mycobacterium tuberculosis* complex were 19.1 days with the MB-Check system, 13.4 days with the BACTEC system, and 21.7 days with the 3% Ogawa egg method. These results indicate that both the MB-Check and the BACTEC systems, based on liquid media, are efficient for the recovery of mycobacteria.

The detection of *Mycobacterium tuberculosis* is critical for the diagnosis of pulmonary tuberculosis but is time-consuming. Laboratories today look toward more efficient systems to provide physicians and patients with faster results and to free laboratory personnel from unnecessary hours of tedious labor.

The method developed by Ogawa et al. (12)—the standard method used in Japan—is a simple one; the specimens are inoculated on 3% Ogawa egg medium without neutralization and concentration after treatment with strong alkali (final NaOH concentration, 2 to 3.2%). Since the introduction of rifampin, the problem of smear-positive and culture-negative results due to potent antimicrobial activity has become apparent, but its exact mechanisms are still unclear (11, 16, 17).

After the introduction by Middlebrook et al. (9) of the radiometric method, which greatly reduces mean detection times (5-7, 10, 13-15), the acceptance of liquid media rose rapidly. Middlebrook 7H12 medium is also more sensitive than egg-based media (1).

In this report, a comparison of the rate of recovery and time to detection of mycobacteria from clinical specimens for the newly developed biphasic culture system MB-Check and the radiometric BACTEC culture system, both of which are based on liquid media, and the egg-based media 3% Ogawa and Ogawa K was made.

MATERIALS AND METHODS

Specimens and processing methods. Sputum specimens were obtained from 245 patients admitted to Fukujiji Hospital (Kiyose-shi, Tokyo, Japan) with symptoms of pulmonary tuberculosis. These specimens consisted of 65 smear-positive and 180 smear-negative specimens.

All specimens were decontaminated by the sodium hy-

droxide method shown in Fig. 1. Two volumes of 4% NaOH were mixed with sputum specimens on a test tube mixer for digestion, and the mixtures were allowed to stand for 15 min at room temperature. From these alkali-treated samples, 0.1 ml was directly inoculated on 3% Ogawa egg medium. For the remainder of the treated samples, approximately 10 volumes of 10 mM phosphate buffer (pH 7.4) were added for dilution and the mixtures were centrifuged at $3,000 \times g$ for 20 min at 4°C. After the supernatant fluids were carefully decanted, the resulting sediments were suspended in 1 ml of the same buffer and the suspensions were inoculated into or on the other three media.

Media and culturing methods. The new biphasic culture system MB-Check (Nippon Roche Co., Ltd., Tokyo, Japan) consisted of a bottle containing the basic broth, a lyophilized supplement in a vial, and a slide enclosed in a plastic tube for subculturing. The liquid phase consisted of 20 ml of modified Middlebrook 7H9 broth in a screw-cap glass bottle. The supplement contained oleic acid, albumin, glucose, catalase, glycerol, pyridoxal hydrochloride, polyoxyethylene-40-stearate, polymyxin B, amphotericin B, nalidixic acid, trimethoprim, and azlocillin. The slide consisted of Middlebrook 7H11 agar, Middlebrook 7H11 agar with NAP (p-nitro- α -acetylamino- β -hydroxy-propionophenone) and chocolate agar. The MB-Check system is similar in design to the Septi-Check AFB in the United States. Subculturing is achieved by flooding the agar slide with the broth.

BACTEC 12B medium (Nippon Becton Dickinson Co., Ltd., Tokyo, Japan) consisted of 4 ml of Middlebrook 7H9 broth supplemented with casein hydrolysate, bovine serum albumin, catalase, ^{14}C -labeled substrate, polymyxin B, amphotericin B, nalidixic acid, trimethoprim, azlocillin, pyridoxal hydrochloride, and polyoxyethylene-40-stearate.

Two slanted solid media representing conventional culturing consisted of 3% Ogawa egg medium (12) with 1% KH_2PO_4 (final pH, 6.2), which is generally used for the isolation of mycobacteria in Japan, and Ogawa K egg medium (3) with magnesium citrate (final pH, 6.9), which is

* Corresponding author.

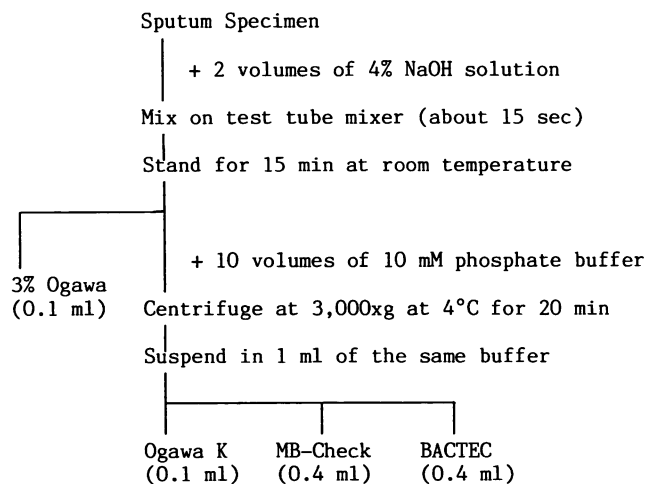


FIG. 1. Pretreatment of specimens.

inoculated after pretreatment with cetylpyridinium chloride-NaCl-succinic acid or after neutralization of the decontaminants. Both media were purchased from Kyokuto Pharmaceutical Co., Ltd., Tokyo, Japan.

All specimens were pretreated with 4% NaOH (final concentration, 2.6%), and 0.1 ml of the resulting suspension was inoculated directly without neutralization on 3% Ogawa egg medium. From the diluted and concentrated samples, 0.1 ml was inoculated on Ogawa K egg medium and 0.4 ml each was inoculated into MB-Check and BACTEC bottles. All media were incubated at 37°C and checked for the first time 3 days after inoculation. The solid media were checked thereafter once a week for up to 8 weeks. As long as no growth was visible on the slide medium, the MB-Check system was inverted once during each inspection to inoculate the slides. When mycobacterial colonies appeared on the slide medium, they were recorded as culture positive in the MB-Check system. For the BACTEC system, the threshold for a positive growth index reading was set at 20. The MB-Check and BACTEC systems were checked twice a week for 6 weeks.

Identification of mycobacterial isolates. All primary isolates were confirmed by Ziehl-Neelsen staining and differentiated and identified by an RNA-DNA hybridization assay with commercial kits for culture confirmation and identification of species belonging to the *M. tuberculosis* complex and the *Mycobacterium avium* complex (Gen-Probe, Inc., San Diego, Calif.) and by conventional culturing or biochemical testing.

Statistical analysis. The statistical significance of differences in isolation rates among the four systems was determined by the χ^2 method.

RESULTS

For primary isolation, specimens which had been pretreated with NaOH were inoculated on egg-based media (3% Ogawa and Ogawa K) and into the liquid-based MB-Check and BACTEC systems and then cultivated at 37°C.

A total of 86 (35.1%) mycobacterial isolates were obtained from 245 sputum specimens cultured by the four systems. Of these, 65 (75.6%) were detected by the 3% Ogawa egg method and 81 (94.2%) and 80 (93.0%) were detected by the MB-Check and BACTEC systems, respectively. There was

TABLE 1. Isolation of mycobacteria from 245 sputum specimens with different media

Species (no. of isolates)	No. (%) of isolates detected by the following method:			
	3% Ogawa egg	Ogawa K egg	MB-Check	BACTEC
<i>M. tuberculosis</i> complex (49)	39 (79.6)	37 (76.5)	47 (95.9)	47 (95.9)
<i>M. avium</i> com- plex (31)	24 (77.4)	26 (83.9)	28 (90.3)	30 (96.8)
Other myco- bacteria (6)	2 (33.3)	3 (50.0)	6 (100)	3 (50.0)
Total (86)	65 (75.6)	66 (76.7)	81 (94.2)	80 (93.0)

a significant difference in the percentages of positive cultures; the two liquid-based systems (MB-Check and BACTEC) outperformed the 3% Ogawa egg method ($P < 0.01$).

Of the 86 isolates, 49 were of the *M. tuberculosis* complex and 31 were of the *M. avium* complex, accounting for 93.0% of the total (Table 1). Both the MB-Check and the BACTEC systems detected 95.9% of *M. tuberculosis* isolates. On the other hand, the 3% Ogawa egg method detected 79.6% of these. The rate of recovery of the *M. avium* complex by each system was comparable to that of the *M. tuberculosis* complex.

As shown in Table 2, MB-Check detected 100% of 37 *M. tuberculosis* isolates from 65 smear-positive specimens and 83.3% of 12 isolates from 180 smear-negative specimens. The rate of isolation with BACTEC was comparable to that with MB-Check. The 3% Ogawa egg method detected 89.2% and 50.0% of *M. tuberculosis* isolates from these smear-positive and smear-negative specimens, respectively. MB-Check detected 100% of 18 MOTT (mycobacteria other than tubercle bacilli) isolates from 65 smear-positive specimens and 84.2% of 19 MOTT isolates from 180 smear-negative specimens, compared with 94.4% and 47.4% of these with the 3% Ogawa egg method, respectively. Thus, the rate of isolation from both smear-positive and smear-negative specimens was better with the two liquid-based systems. This difference was even more significant with smear-negative specimens ($P < 0.01$).

The mean times to the detection of the *M. tuberculosis* complex obtained from smear-positive and smear-negative specimens were 12.6 and 16.0 days with BACTEC and 20.9 and 26.3 days with the 3% Ogawa egg method, respectively (Table 3). The time to the detection of the *M. tuberculosis* complex with MB-Check was shorter than that with the 3% Ogawa egg method but longer than that with BACTEC. The

TABLE 2. Recovery of mycobacteria from 245 smear-positive and smear-negative specimens with different media

Isolates (n)	No. (%) of isolates detected by the following method:			
	3% Ogawa egg	Ogawa K egg	MB- Check	BACTEC
Smear-positive <i>M. tuber- culosis</i> (37)	33 (89.2)	32 (86.5)	37 (100)	36 (97.3)
Smear-negative <i>M. tuber- culosis</i> (12)	6 (50.0)	5 (41.7)	10 (83.3)	11 (91.7)
Smear-positive MOTT (18)	17 (94.4)	17 (94.4)	18 (100)	18 (100)
Smear-negative MOTT (19)	9 (47.4)	12 (63.2)	16 (84.2)	15 (78.9)

TABLE 3. Average number of days to positive cultures

Isolates (n)	Avg no. of days to positive cultures with the following method:			
	3% Ogawa egg	Ogawa K egg	MB-Check	BACTEC
Smear-positive <i>M. tuberculosis</i> (37)	20.9	22.3	18.0	12.6
Smear-negative <i>M. tuberculosis</i> (12)	26.3	27.0	23.3	16.0
Total <i>M. tuberculosis</i> (49)	21.7	22.9	19.1	13.4
Total MOTT (37)	18.6	20.5	12.2	5.1

mean times to the detection of MOTT were 5.1 days with BACTEC, 12.2 days with MB-Check, and 18.6 days with the 3% Ogawa egg method.

Contamination occurred overall in 0.4, 0.8, and 5.3% of the MB-Check, BACTEC, and 3% Ogawa egg cultures, respectively. A contamination rate of 0.8% was noted in the Ogawa K egg cultures.

DISCUSSION

The definitive diagnosis of mycobacterial disease still requires the recovery of the causative agent on culture media. In Japan, only Ogawa egg medium has been used until now for the isolation of mycobacteria from clinical specimens. The aim of this study was to compare new biphasic (MB-Check) and radiometric (BACTEC) culture systems based on liquid media with the egg-based solid media.

Two systems based on liquid media proved to be significantly better than the egg-based solid media for the isolation of mycobacteria from clinical specimens. The difference in the rates of isolation of the *M. tuberculosis* complex by these culture procedures was statistically significant (Table 1), results consistent with those of Isenberg et al. (7) and Giger and Burkardt (6). However, this result contrasted with that of Anargyros et al. (2), who could not demonstrate a significant difference in the rates of isolation of the *M. tuberculosis* complex between the BACTEC system or the MB-Check system and the conventional method. The difference in the rates of isolation of mycobacteria between two groups of media (liquid based and egg based) in our study was more remarkable with smear-negative specimens.

One explanation for an increase in sensitivity could be a larger inoculum size (2, 4, 6). As the bottle of the new biphasic system contains 20 ml of broth, it may be inoculated with 1 ml or more of the bacterial suspension. The BACTEC system may also be inoculated with a larger inoculum (up to 0.5 ml), compared with the 0.1 ml used on the egg-based slant. The striking difference between the rates of recovery of mycobacteria from clinical specimens by the liquid-based systems and the 3% Ogawa egg method may also be due to the following. (i) A liquid medium may enhance the growth of mycobacteria. (ii) In the liquid-based systems, specimens were pretreated with 4% NaOH (final concentration, 2.6%), diluted with phosphate buffer, and then concentrated: perhaps less damage occurred to the mycobacteria (especially MOTT) during processing (Tables 1 and 2).

The isolation of the *M. tuberculosis* complex by BACTEC from smear-positive and smear-negative specimens occurred 8.3 and 10.3 days earlier, respectively, than isolation by the 3% Ogawa egg method. These results were similar to those reported by Morgan et al. (10), Roberts et al. (14), and

Anargyros et al. (2). A greater difference in isolation times was observed for MOTT isolates. MOTT were detected 13.5 days earlier by BACTEC than by the 3% Ogawa egg method. The mean time to the detection of mycobacteria was shorter with MB-Check than with the 3% Ogawa egg method. It is likely that the larger inoculum and larger volume of medium in the systems based on liquid media may account for some of these differences.

There was no significant difference in the rates of recovery and times to detection between the two egg-based media, 3% Ogawa and Ogawa K.

The rate of contamination with the 3% Ogawa egg method without neutralization after treatment with NaOH was 5.3%. On the other hand, the rate of contamination with BACTEC was 0.8%, and that with MB-Check was 0.4%. The rate of contamination with the 3% Ogawa egg method was comparable to that reported by Isenberg et al. (7) and Giger and Burkardt (6). The low rates of contamination obtained with the BACTEC and MB-Check systems and the Ogawa K egg cultures may have been due to the washing by centrifugation after dilution of the specimens rather than over-decontamination.

In addition to superior ultimate yield, another practical advantage of the MB-Check system is the avoidance of radioactivity or CO₂ incubation. On the other hand, the BACTEC system is more useful for earlier detection than other systems in a laboratory in which the use of radioactive materials is possible.

ACKNOWLEDGMENTS

This work was supported in part by grants from the Ministry of Health and Welfare of Japan.

We thank Nippon Roche Co., Ltd., Nippon Becton Dickinson Co., Ltd., and Chugai Pharmaceutical Co., Ltd., for supplying MB-Check and BACTEC reagents and DNA probe kits.

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