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Inhibitory Effect of the Isolator Blood Culture System on Growth of *Mycobacterium avium-M. intracellulare* in BACTEC 12B Bottles

BENEDICT WASILAUSKAS[1]* AND ROBERT MORRELL, JR.[2]

Department of Pathology, Wake Forest University Medical Center,[1] and Clinical Microbiology Laboratory, North Carolina Baptist Hospital,[2] Winston-Salem, North Carolina 27157-1072

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The examination of 6,938 clinical specimens collected during the period January 1991 through December 1992 suggested that the Isolator blood culture system (Wampole) inhibited growth of *Mycobacterium avium-M. intracellulare* complex (MAC) in BACTEC 12B medium. Of 162 MAC blood culture isolates, 94% were recovered from Lowenstein-Jensen (LJ) medium, while only 50% were recovered from 12B medium. The time to detection with LJ medium was 18 days, while that with 12B medium was 24 days. In contrast, 62% of the 305 MAC nonblood culture isolates were recovered from the LJ medium, while 87% were found in the 12B medium. The time to detection for these cultures was also reversed, i.e., 28 days for LJ medium versus 15 days for 12B medium. Dilution studies using the lysis-anticoagulant reagent from Isolator tubes demonstrated inhibition of both clinical and American Type Culture Collection strains of MAC, even at low concentrations of lysis-anticoagulant reagent. Washing the Isolator blood sediment prior to inoculating the 12B bottles eliminated any growth inhibition. Clinical and experimental data suggest that the use of the Isolator blood culture tube with the BACTEC 12B medium is contraindicated for mycobacterial blood cultures.

The incidence of mycobacteremia, previously considered uncommon, has risen dramatically in patients with AIDS. This phenomenon has prompted laboratories to seek rapid, effective methods to detect these organisms. The combined use of lysis-centrifugation (Isolator; Wampole Laboratories, Cranbury, N.J.) and the BACTEC system (Becton Dickinson Diagnostic Instrument Systems, Sparks, Md.) has been suggested as a method for the cultivation and detection of mycobacteria (1, 3–5). Although the BACTEC bottle is expected to provide the most rapid method for detection, some investigators include a solid medium to observe colonial morphology and determine numbers of CFU (5).

The BACTEC system has been used in our hospital for the detection of mycobacteria in specimens other than blood since 1987, and the Isolator-BACTEC system has been used for mycobacterial blood cultures since 1989. During a routine evaluation of our mycobacterium-positive blood cultures, it was noted that growth in the BACTEC bottle was delayed or in some cases totally inhibited compared with growth on the solid medium that was inoculated concurrently. This was somewhat disconcerting, since we had observed that the BACTEC bottle was nearly always positive first with other types of specimens. A review of medical records of patients in whose cultures an inhibitory effect was observed in the BACTEC bottle revealed no obvious correlation with the use of antimycobacterial therapy.

In an attempt to verify these preliminary findings and determine the nature of this inhibition, the following studies were performed: (i) a retrospective examination of growth patterns of all mycobacterial cultures processed during the past 2 years, (ii) the incorporation of various amounts of Isolator lysis-anticoagulant reagent (LAR) into BACTEC bottles inoculated with clinical and American Type Culture Collection strains of *Mycobacterium avium-M. intracellulare* complex (MAC), and (iii) simulated blood cultures using Isolator-treated blood and using Isolator-treated blood that had been washed with sterile water.

MATERIALS AND METHODS

Analysis of patient specimen data. A retrospective examination of 982 culture records from January 1991 through December 1992 included the numbers of blood and nonblood specimens processed, the results of primary acid-fast smears, the number of cultures that grew mycobacteria, the medium on which growth occurred, the number of days between collection and a positive result, and the number of CFU.

Specimen processing. Blood specimens for mycobacterial culture were collected in 10-ml Isolator tubes (Wampole Laboratories). Upon receipt in the laboratory, the tubes were promptly (within 1 h) placed in a centrifuge and spun at 3,000 $\times$ *g* for 30 min. The supernatant fluid was aseptically removed, and the remaining sediment was divided equally between a BACTEC 12B bottle (Middlebrook 7H12 medium; Becton Dickinson Diagnostic Instrument Systems) and a Lowenstein-Jensen (LJ) slant. Sputum and other specimens contaminated with commensal microorganisms were digested and decontaminated with 4% NaOH and *N*-acetyl-L-cysteine and then buffered prior to preparation of smears and inoculation into BACTEC 12B bottles and LJ slants. Each BACTEC 12B bottle received 0.1 ml of the antimicrobial mixture PANTA. The PANTA mixture contained the antimicrobial agents polymyxin B, amphotericin B, nalidixic acid, trimethoprim, and azlocillin and polyoxyethylene stearate as a growth-enhancing agent. All cultures were incubated at 35°C for 6 weeks. BACTEC 12B bottles were examined with the BACTEC 460 radiometer (Becton Dickinson Diagnostic Instrument Systems) twice weekly during the first 2 weeks and then weekly thereafter. LJ

* Corresponding author. Mailing address: Department of Pathology, Bowman Gray School of Medicine of Wake Forest University, Medical Center Blvd., Winston-Salem, NC 27157-1072. Phone: (910) 716-2638. Fax: (910) 716-7595. Electronic mail address: Bwasilau@isnet.is.wfu.edu.

TABLE 1. Comparison of medium growth characteristics with 162 blood specimens positive for MAC during 1991 and 1992

Medium	No. (%) positive			Avg time (days) to positive reading
	Specified medium alone	Both media	Total	
LJ[a]	81 (50)	71 (44)	152 (94)	18
12B[b]	10 (6)	71 (44)	81 (50)	24

[a] Lowenstein-Jensen slant read weekly.
[b] BACTEC 12B bottle read twice weekly for first 2 weeks.

TABLE 2. Comparison of medium growth characteristics with 305 nonblood specimens positive for MAC during 1991 and 1992

Medium	No. (%) positive			Avg time (days) to positive reading
	Specified medium alone	Both media	Total	
LJ[a]	40 (13)	149 (49)	189 (62)	28
12B[b]	116 (38)	149 (49)	265 (87)	15

[a] LJ slant read weekly.
[b] BACTEC 12B bottle read twice weekly for first 2 weeks.

slants were examined visually once a week. A BACTEC 460 growth index (GI) of ≥20 U was considered positive, and an acid-fast smear was prepared from the 12B bottle. If no acid-fast bacilli were observed on the smear, the bottle was reincubated and read daily on the BACTEC 460 until either a negative GI or a positive smear was obtained. Acid-fast smears were prepared from any growth appearing on the LJ slant. Mycobacteria recovered from positive cultures were identified by using nucleic acid probes (Gen-Probe, Inc., San Diego, Calif.) and conventional mycobacterial methods.

Preparation of dilutions of Isolator reagent in BACTEC bottles. Undiluted LAR and serial twofold dilutions (in sterile water), ranging from 1:2 through 1:256, of the LAR were prepared in triplicate. A 0.1-ml portion of each dilution was added to a BACTEC 12B bottle. Approximately 10 CFU *M. intracellulare* ATCC 13590, contained in 0.1 ml of BACTEC 12B broth, was added to each of these dilution bottles. The bottles were incubated at 35°C, and growth was monitored daily for 6 weeks with the BACTEC 460 instrument. Growth control bottles containing only the organism were also prepared in triplicate and monitored along with the test bottles. Simplified versions of this experiment (undiluted LAR and growth controls only) were also tested with clinical isolates of *M. intracellulare* and *M. avium*, as well as with *M. avium* ATCC 25292. Smears for acid-fast staining were prepared from all bottles that showed growth and from some of the negative bottles at the end of the 6-week incubation period.

Simulated blood cultures. Approximately 60 ml of blood obtained from a human volunteer was collected in six 10-ml Isolator tubes. All tubes were processed according to the manufacturer's instructions. The sediments from three tubes were washed, reconstituted with sterile distilled water, and concentrated by centrifugation twice prior to further processing. Three sets of triplicate BACTEC 12B bottles were then set up with (i) 0.75 ml of saline (as a growth control), (ii) 0.75 ml of unwashed blood sediment, and (iii) 0.75 ml of washed blood sediment. An inoculum prepared in 0.1 ml of BACTEC 12B medium and containing approximately 100 CFU of *M. intracellulare* ATCC 13590 was added to all bottles and to three Middlebrook 7H11 agar plates to obtain a confirmatory colony count. All cultures were incubated at 35°C until the maximum GI (999) was obtained (maximum, 32 days).

RESULTS

Analysis of patient specimen data. The general growth characteristics of MAC for blood and nonblood cultures are summarized in Tables 1 and 2, respectively.

(i) Blood cultures. There were 982 blood specimens, of which 163 (17%) grew mycobacteria. MAC was recovered from 162 of these specimens. One specimen grew *Mycobacterium tuberculosis.* Of these 162 specimens, only 1 had a positive direct acid-fast smear. Ninety-four percent of all isolates grew on LJ medium, while only 50% were ever recovered from the 12B bottle. The rate of recovery from the 12B bottles rose to 64% (30 of 47) for cultures that had ≥100 colonies on the LJ medium. Of the remaining 115 blood specimens with colony counts of <100, 51 (44%) of the isolates were recovered from the 12B bottle. For 50% of all positive blood cultures, the LJ slant was the sole source of recovery, while for 6% recovery was from only the 12B bottle.

The average time to detection for blood cultures was 18 days on the LJ medium and 24 days in the 12B bottle. In those blood cultures that grew on both media, growth appeared first on the LJ medium in 68%, while the 12B bottle was positive first in only 21%.

(ii) Nonblood cultures. Of 5,956 nonblood specimens, consisting primarily of sputa, bronchial washings, and other body fluids, 434 (7%) grew mycobacteria. MAC was recovered from 305 of these specimens, and 58 were positive on the primary acid-fast smear. Recovery rates by media were largely reversed from those for blood culture specimens, with 62% recovery from LJ slants and 87% from the 12B bottle. The rate of recovery from the 12B bottle dropped slightly to 80% in the 148 nonblood cultures with LJ-documented colony counts of ≥100 and rose an equally small amount, to 93%, in the 155 cultures with colony counts of <100. The LJ medium was the sole source of recovery in only 13% of positive nonblood cultures, while the 12B bottle was the only medium positive in 38% of the positive cultures.

The average time to detection for nonblood cultures was, again, largely the reverse of that for blood cultures, with LJ slants positive in an average of 28 days and 12B bottles positive in an average of 15 days. Of those cultures for which growth was observed on both media, the 12B bottle detected growth first in 91%, while the LJ medium was positive first in only 5%.

LAR dilution studies. The results of incubating the American Type Culture Collection strain of *M. intracellulare* with various dilutions of the Isolator LAR are shown in Table 3. The growth control, containing no LAR, became positive (GI ≥ 20 U) by day 10. The GI rose to 999 U during the next 5

TABLE 3. BACTEC detection times with various dilutions of LAR

BACTEC GI (U)	Time to detection (days)[a]						
	GC	1:8	1:16	1:32	1:64	1:128	1:256
20	10	20	17	13	11	10	11
100	12	26	20	16	14	12	13
999	15	42	25	24	22	17	16

[a] The average number of days for three BACTEC bottles is shown for the indicated dilutions of LAR and for a growth control (GC) with no LAR added. Undiluted LAR and 1:2 and 1:4 dilutions of LAR showed no growth in 42 days.

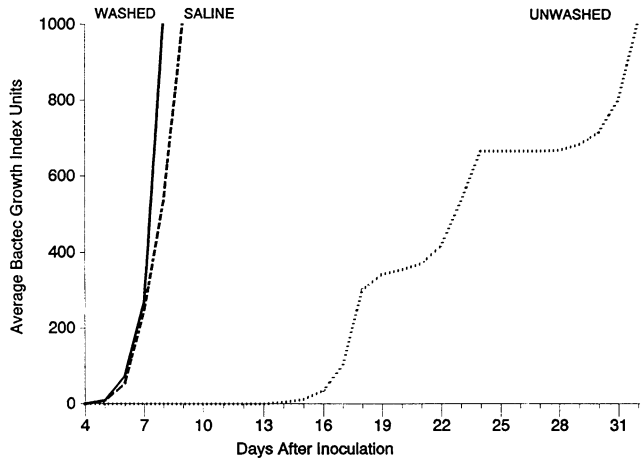

FIG. 1. Growth of MAC in BACTEC 12B bottles. Results for washed and unwashed Isolator blood sediments and for a saline control are shown.

days. Low dilutions of LAR appeared to inhibit the growth of *M. intracellulare* in the BACTEC bottles. It was only at a dilution of 1:128 that bottles became positive in nearly the same time period as observed for the growth control. An identical inhibition pattern was observed in the simplified experiments using clinical isolates of *M. intracellulare* and *M. avium* as well as *M. avium* ATCC 25291.

Organisms were never detected radiometrically in bottles with LAR dilutions of less than 1:8 during the 6-week incubation period. Acid-fast-stained smears prepared from four of these low-dilution bottles at the end of the incubation period failed to show any organisms; however, the organism was recovered from two of these negative bottles when the contents were removed and centrifuged and the pellet was placed on LJ slants. We observed a clinical parallel of this phenomenon when, during this period, two mycobacterial blood cultures from a single patient were positive on the LJ slants only. At the end of the 6-week period, the broth from the negative bottles was removed and centrifuged, and the pellet was placed on LJ slants. Visible colonies of MAC were observed within 2 weeks on one of these slants.

Simulated blood cultures. The results of the simulated blood cultures are graphically displayed in Fig. 1. The saline growth controls and the washed Isolator blood cultures became positive (GI $\geq$ 20) in the BACTEC 12B bottles within 5 days of incubation. In contrast, the Isolator sediment that was not washed required 14 days for the first 12B bottle to show positive results, 19 days for the second bottle, and 28 days for the third bottle. The 7H11 Middlebrook agar plates set up to obtain colony counts were visually positive in 8 days.

DISCUSSION

The use of the Isolator-BACTEC system for mycobacterial blood cultures has been proposed as a rapid method of recovering MAC (1, 3, 4). However, all of our data, both clinical and experimental, indicated that this is not advisable. The Isolator-BACTEC system was not as efficient or as timely as solid media for recovering these organisms, despite more frequent readings. The most conclusive evidence of this was our ability to recover living mycobacteria from clinical and experimental BACTEC bottles that were radiometrically negative for 6 weeks. This finding could have considerable significance for laboratories that rely exclusively on the Isolator-BACTEC system for recovery of blood culture mycobacteria.

It is interesting that the average time to detection for blood isolates growing on LJ medium was shorter than that for nonblood specimens with the same medium. We perceive two possible reasons for this phenomenon: medium enrichment by the blood sediment that was placed on the LJ medium or greater color contrast between the lightly colored MAC colonies and the blood-darkened LJ slants, making possible earlier visual detection by the technologist.

It is important to emphasize the distinction between blood and nonblood acid-fast cultures. Our clinical data for nonblood cultures confirm our conclusion and that of others (2) that the BACTEC system is superior to solid media in both efficiency and speed of recovery. It is only when blood is processed and cultured with the Isolator system that the BACTEC 12B bottle loses its effectiveness. Whittier et al. (7), using the Isolator system and comparing the Septi-Chek AFB biphasic system with BACTEC 12B medium, found that MAC grew in all 10 of the biphasic cultures but was detected in only 6 cultures by the BACTEC system. The use of the biphasic medium in this study most likely was responsible for the enhanced recovery of MAC and further substantiates our findings with the 12B medium.

Kiehn and Cammarata (6) compared mycobacterial blood cultures inoculated into BACTEC 13A medium with those collected in Isolator tubes and inoculated on Middlebrook 7H11 agar plates. Their findings showed no significant differences in recovery rates or times to detection. No inhibition of MAC was observed with the Isolator-7H11 plates.

We have no explanation for the discrepancies between our data and those of studies of equivalent methods (1). It should be noted, however, that our clinical data base is larger than those previously published and is substantiated with experimental data.

We conclude that the inhibitory substance is a component of the LAR of the Isolator specimen collection tubes. When inoculated into a closed liquid system such as BACTEC 12B, it inhibits the growth of MAC. No effect was observed on solid media because the component was able to diffuse into the agar and/or escape into the overlying airspace through evaporation. Further studies are needed to identify the exact cause and mechanism of this inhibition.

On the basis of our findings, the combined use of Isolator and BACTEC for recovery of mycobacteria in blood specimens is contraindicated. Because of the logistical problems in using two different collection systems for routine and mycobacterial cultures, we have elected to continue to use the Isolator collection tube but have ceased using the BACTEC system for mycobacterial blood cultures. The 12B bottle has been replaced with two 7H11 Middlebrook agar plates.

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