JOURNAL OF CLINICAL MICROBIOLOGY, Oct. 1985, p. 543–546
0095-1137/85/100543-04$02.00/0

Vol. 22, No. 4

Use of Lysis-Centrifugation (Isolator) and Radiometric (BACTEC) Blood Culture Systems for the Detection of Mycobacteremia

VEE J. GILL,[1]* CHOONG H. PARK,[2] FRIDA STOCK,[1] LINDA L. GOSEY,[1] F. G. WITEBSKY,[1] AND H. MASUR[3]

Microbiology Service, Department of Clinical Pathology[1] and Critical Care Medicine Department,[3] Clinical Center, National Institutes of Health, Bethesda, Maryland 20205, and Department of Pathology, Fairfax Hospital, Falls Church, Virginia 22046[2]

Received 20 December 1984/Accepted 15 July 1985

Patients with acquired immune deficiency syndrome may develop infection with mycobacteria, particularly *Mycobacterium avium-M. intracellulare* (MAI). These infections can frequently be associated with demonstrable mycobacteremia with the organism. In this study, we compared the sensitivity of a radiometric (BACTEC system; Johnston Laboratories, Inc., Towson, Md.) liquid medium culture system with that of conventional solid mycobacterial culture media for cultures of blood from these patients. Both systems were inoculated with blood concentrate prepared by lysis-centrifugation (Isolator; Du Pont Co., Wilmington, Del.). Of 46 acquired immune deficiency syndrome patients whose blood was cultured, 28% had cultures positive for MAI. Patients had from less than 1 to more than 100 MAI colonies per ml of blood. Lowenstein-Jensen and Middlebrook 7H11 agars were comparable in recovery of MAI. BACTEC 12A vials containing double the standard volume of medium (4 ml) were more sensitive and were positive slightly earlier than vials containing the standard volume (2 ml). Conventional media detected 98% of positive cultures; BACTEC vials containing double volumes of medium detected 94% of positive cultures, whereas single-volume vials detected 77%. BACTEC vials were positive approximately 5 to 6 days sooner than slants or plates containing conventional media. For a few cultures, the use of unconcentrated blood was compared with the use of Isolator-concentrated blood by using each of these as inocula for BACTEC vials. Results for these cultures suggested that, although the use of Isolator-concentrated blood resulted in greater sensitivity than the use of unconcentrated blood would , the use of unconcentrated blood would still result in the detection of at least 78% of positive cultures.

Until the advent of acquired immune deficiency syndrome (AIDS), *Mycobacterium avium-M. intracellulare* (MAI) was recognized primarily as an ubiquitous environmental contaminant that occasionally caused localized lung disease in individuals with chronic obstructive pulmonary disorders. Until 1979, only about 14 cases of disseminated MAI had been reported in adults. Since 1979, MAI has been recognized as a systemic infection in a surprising number of AIDS patients. Over 35% of 120 AIDS patients at the Clinical Center have had MAI cultured from at least one nonpulmonary organ or from blood. Other mycobacteria have only rarely been isolated, except for *Mycobacterium tuberculosis* in Haitian patients with AIDS (4, 5).

MAI has been found in a variety of body sites in AIDS patients at the Clinical Center. Sites include the liver, spleen, lymph node, lung, bowel wall, brain, skin, bone marrow, sputum, urine, and blood. The role that MAI plays in causing constitutional symptoms or specific organ dysfunction has been unclear. The fact that MAI can be present in impressive quantity histologically and that persistent bacteremia occurs suggests that the organism is a clinically important pathogen.

The detection of MAI infection in AIDS patients can be accomplished by the biopsy of an affected organ or tissue, particularly the bone marrow, liver, or lymph node. This procedure is not practical for repetitive surveys of a patient population that characteristically has multiple episodes of fever and constitutional signs. Blood culture is a much less invasive technique. Moreover, in assessing the efficacy of antimicrobial or immunomodulating therapy, blood cultures offer a convenient method for monitoring the patient.

* Corresponding author.

Earlier reports documented that positive blood cultures for MAI can be obtained by using a lysis-centrifugation blood culture system (3, 6), a radiometric detection system (2, 3, 6), or biphasic blood culture bottle systems for prolonged periods (6). Berlin et al. (1) recently reported on a Middlebrook 7H11 (MB) agar-brain heart infusion broth biphasic blood culture in which MAI was isolated in 6 to 15 days. Each system has relative advantages and disadvantages, but there has been no comparative study of the various techniques.

Previous work with the Isolator, a lysis-centrifugation system manufactured by the Du Pont Co., Wilmington, Del., resulted in the detection of positive cultures between 18 and 20 days, whereas BACTEC, a radiometric system manufactured by Johnston Laboratories, Inc., Towson, Md., has been documented to provide positive results at 5 to 6 days in a few instances. A present limitation of the BACTEC system is that only a small volume of inoculum can be added to each mycobacterial culture vial. Although some AIDS patients have so many organisms in their blood that a small volume of unconcentrated blood may yield a positive culture, other patients may have fewer organisms in their blood, and these might be missed when a small unconcentrated volume is cultured. This might occur early during infection or therapy or in patients who have enough immune function to provide some control of the infectious process. We evaluated the relative efficacy of inoculating Isolator concentrate into the BACTEC mycobacterial culture vials and onto routine mycobacterial culture media. For several known positive patients, we also compared the sensitivity of the BACTEC system with unconcentrated and concentrated blood specimens.

The purpose of this study was to assess the sensitivity and

speed of a radiometric detection system relative to conventional mycobacterial growth media when both are inoculated with a concentrate obtained by a lysis-centrifugation system. A rapid, sensitive test to detect mycobacteria in the blood would be a useful technique for diagnosis and for monitoring the efficacy of therapy.

MATERIALS AND METHODS

Patients cultured. AIDS patients with symptoms suggestive of mycobacterial disease were screened with blood cultures for mycobacteria. These patients usually had had prolonged unexplained fever or progressive deterioration without overt infection owing to other organisms.

Processing of blood. Approximately 7.5 ml of blood was collected into Isolator blood culture tubes. These tubes were centrifuged for 30 min at 2,800 × *g*. After removal of the supernatant, approximately 1.6 ml of concentrate was available for culture. Concentrate (0.2 ml) was inoculated into each of two BACTEC 12A (Middlebrook 7H12) mycobacterial culture vials containing 0.1 ml of BACTEC PACT supplement (polymyxin B, amphotericin B, carbenicillin, and trimethoprim). If the patient was known to be taking antimycobacterial drugs, one of the vials used was a double-volume (DV) vial prepared by transferring the entire contents of one vial (including PACT) aseptically into another vial. Cultures from treated patients thus had one DV vial and one standard or single-volume (SV) vial, each of which contained 0.2 ml of Isolator concentrate. Early in the study, some patients had either two DV or two SV vials instead of one DV and one SV vial. The rest of the concentrate (approximately 1.0 to 1.2 ml) was then distributed onto one Lowenstein-Jensen (LJ) slant (tubes [20 by 150 mm]; Regional Media Laboratories, Lenexa, Kans.) and two MB plates. Each MB plate was streaked in the standard manner recommended for Isolator plates, so that isolated colonies could be obtained and counted unless growth was confluent.

Unconcentrated blood. From nine patients known to have acid-fast bacilli from blood, 7.5 ml of blood was collected in a sterile blood collection tube containing sodium polyanetholesulfonate anticoagulant (Vacutainer; Becton Dickinson Vacutainer Systems, Rutherford, N.J.). Blood was taken directly from this tube and inoculated into BACTEC 12A vials as described above (0.2 ml into each of two vials, one DV and one SV). A separate Isolator tube was collected at the same time and processed as indicated above to compare the sensitivity of the two methods and the time required for growth to be recognized. Final colony counts on these nine cultures showed two cultures with >100 CFU, five cultures with 5 to 40 CFU, and two cultures with approximately 1 to 2 CFU/ml of blood.

Processing of cultures. BACTEC vials were measured to detect growth two to three times a week for 4 weeks. A growth index of more than 10 was considered presumptively positive. In each case this was confirmed by smear: 1 ml of the culture was removed from the vial and centrifuged, and an acid-fast stain was performed on the sediment. The growth index for MAI increased rapidly, so that within 1 to 2 days, a growth index of more than 50 was always reached, and direct smears could be done without centrifugation.

All solid media plated with Isolator concentrate were incubated at 35°C in 8% CO_2 and checked visually for growth twice weekly for 6 weeks. Growth of acid-fast bacilli was confirmed by a Kinyoun stain.

Identification of organisms. The initial isolate (from either LJ or MB medium) of each patient was identified as MAI by conventional methods (7) and was also sent to the Centers for Disease Control, Atlanta, Ga., for confirmation as MAI. Subsequent isolates from the same patient were assumed to be the same so long as colony morphology, rate of growth, and light-test characteristics were consistent with MAI. The initial isolate for each patient positive in the BACTEC system was checked by using the BACTEC *p*-nitro-α-acetylamino-β-hydroxypropiophenone inhibition test; if this test was positive, the isolate was assumed to be the same as that growing from the conventional culture media (MAI).

Design of study. The BACTEC cultures were monitored at Fairfax Hospital, and the traditional mycobacterial cultures were monitored at the National Institutes of Health. Neither laboratory was aware of the findings of the other laboratory until the conclusion of the study.

RESULTS

From February through September 1983, 46 AIDS patients were screened with one or more blood cultures for the presence of mycobacteremia. MAI grew in at least one blood culture from 13 (28%) of the 46 patients. These 13 patients subsequently had 46 follow-up cultures performed from one week to as long as six months after the initial positive blood cultures. These resulted in 34 positive follow-up cultures. Individual patients had from one to seven (average, 3.6) positive blood cultures. All of the follow-up cultures and some of the initial cultures were obtained while the patients were undergoing antimicrobial therapy for MAI.

Comparison of MB and LJ media for MAI growth after inoculation with Isolator concentrate. Forty-six positive cultures were detected on either LJ or MB agar after inoculation with Isolator concentrate. Growth was sought by the naked eye, with a magnifying hand lens used whenever the presence of pinpoint colonies was questioned. In 37 (80%) of the 46 positive cultures, MAI grew on the LJ slant, whereas in 45 (98%) of the cultures, MAI grew on either one or both of the MB plates. Because seven of the cultures that grew on MB agar were positive on only one of the two plates, the recovery on MB agar was not significantly different from the recovery on LJ agar. Detection times were determined for each culture by calculating the number of days from planting to the first observation of positivity. Results for each medium were then averaged. The average detection time was 19.5 days for the LJ medium and 16.3 days for the MB medium. There was no difference in the number of colonies recovered on the two media; 27/37 (73%) and 28/38 (74%) of the cultures had 1 to 50 colonies on LJ and MB media, respectively; the remainder had more than 50 colonies.

Sensitivity of SV versus DV BACTEC vials for the detection of MAI. The difference in recovery between DV and SV vials, each inoculated with 0.2 ml of Isolator concentrate, is shown in Table 1. Of the 22 positive cultures that were tested in parallel DV and SV vials, with concentrated blood, 22/22 (100%) were positive with the DV vial, whereas 18/22 (82%) were positive with the SV vials. The DV vials also had positive results approximately 1.7 days earlier than the SV vials.

Results for nine cultures done in parallel in DV and SV vials with concentrated and unconcentrated blood are also shown in Table 1. For these nine cultures, Isolator concentrate inoculated into DV vials resulted in 100% detection, whereas the use of SV vials resulted in 78% detection. For unconcentrated blood, 78% of the cultures were positive by DV vials compared with 89% by SV vials. There was no significant difference in detection time with concentrated versus unconcentrated blood for either DV or SV vials.

Sensitivity of BACTEC vials compared with solid media when each is inoculated with Isolator concentrate. The results of the 47 cultures done in parallel with the BACTEC vials and LJ and MB solid media inoculated with Isolator concentrate are given in Table 2. Not all of the 47 cultures had both DV and SV vials, because early in the study some patients had either two DV or two SV vials. Of the 47 cultures, 36 had at least one DV vial tested which resulted in 34/36 (94%) positive cultures. Thirty-five cultures had at least one SV vial tested, with 27/35 (77%) being positive. Of the 47 cultures, each of which contained one set of one DV and one SV, two DV, or two SV vials, 42 (89%) gave positive results. Average detection times for these cultures calculated from the earliest positive vial are also shown in Table 2. The use of conventional solid media (LJ and MB) for these 47 cultures resulted in 46 (98%) positive cultures.

Detection times by BACTEC vials compared with detection times by conventional solid LJ-MB media using Isolator concentrate as inoculum in both systems. Blood cultures containing more than 100 MAI colonies per slant (>67 CFU/ml) were usually positive by the BACTEC system between 4 and 6 days, whereas cultures with 10 to 100 colonies per slant (7 to 67 CFU/ml) were positive between 5 and 10 days. Cultures with fewer than 10 colonies per slant (<7 CFU/ml) required 8 to 29 days to be detected by the BACTEC system. With conventional solid media (LJ and MB), there was poor correlation between the number of colonies and the time needed to detect growth on solid media. The time to visually detect both 1 to 10 colonies and more than 100 colonies ranged from 7 to 22 days.

DISCUSSION

Of the 46 AIDs patients whose blood was cultured in this study, 28% had positive cultures for MAI. Their levels of bacteremia ranged from less than 1 CFU to more than 100 CFU/ml of blood.

It is generally recommended that cultures for acid-fast bacilli be done on at least two different kinds of mycobacterial media (7), the most common being LJ and Middlebrook 7H10. Sensitivity of the LT and MB were equivalent, although a detection advantage of approximately 3 days was found with the MB medium as opposed to the LJ medium. This was probably due to easier detection of some of the clear colony types of MAI on the MB medium as opposed to the LJ medium.

TABLE 1. Growth of MAI from BACTEC SV and DV vials with concentrated and unconcentrated blood as inocula[a]

Inoculum	BACTEC vial			
	SV		DV	
	No. positive/no. tested (%)	Avg detection time (days)	No. positive/no. tested (%)	Avg detection time (days)
Isolator concentrate	18/22[b] (82)	11.1	22/22 (100)	9.4
Isolator concentrate	7/9[c] (78)	10.0	9/9 (100)	8.7
Unconcentrated blood	8/9[c] (89)	10.9	7/9 (78)	8.7

[a] See the text for a description of DV vials.
[b] Twenty-two positive cultures, tested in parallel with DV and SV vials, inoculated with concentrated blood.
[c] Nine positive cultures, tested in parallel with DV and SV vials, inoculated with concentrated and unconcentrated blood.

TABLE 2. Comparison of BACTEC 7H12 vials and LJ and MB media for the detection of MAI in Isolator concentrate

Medium	No. of cultures positive/no. tested (%)	Detection time (days)	
		Avg	Range
BACTEC vials[a]			
DV	34/36 (94)	9.5	4–26
SV	27/35 (77)	11.8	5–30
All	42/47 (89)	10.4	4–26
LJ and MB[b]	46/47 (98)	16.1	7–27

[a] Each of two vials contained 0.2 ml of inoculum. Each culture had two vials; 24 cultures had one DV vial and one SV vial, 12 cultures had two DV vials, and 11 cultures had two SV vials. The earliest positive vial for each culture was used to calculate the average detection time and range.
[b] Total inoculum was 1.0 ml. Each plate or slant was inoculated with 0.3 to 0.4 ml of concentrate. The earliest positive medium for each culture was used to calculate the average detection time and range.

The BACTEC system for mycobacteria is usually used for sputum, and it is recommended that 0.2 ml of specimen be inoculated into each vial. For the detection of bacteria in routine blood cultures, it is known that the volume of blood cultured is an important factor in influencing yield. For this reason, we believed that it would be important to culture as much blood as possible without overloading a vial and without using too many vials per culture, and to keep the procedure economically practical. Because several of our patients were taking three or more antimycobacterial drugs, we were also concerned about antibiotic carry-over from the blood, which could result in the suppression of growth in the vials. For the patients being treated, we therefore used two ratios of inoculum to culture medium as described in Materials and Methods. The DV vial was an attempt to dilute out antimycobacterial effects that might be present in the inoculum. With Isolator concentrate as the inoculum for BACTEC vials, recovery was better with the DV vial (100%) than with the standard (SV) vial (82%) for cultures run in parallel. These DV vials also gave positive results approximately 1.7 days sooner than the SV vials. Overall recovery was 94% for DV vials and 77% for SV vials when all cultures were examined, with a 2.3-day advantage for the DV vials.

In the few cultures for which concentrated and unconcentrated blood was tested in parallel, 100% yielded positive results with Isolator concentrate in BACTEC DV vials compared with 78% with unconcentrated blood in BACTEC DV vials. When this comparison was done with standard (SV) vials, recovery was 78% with Isolator concentrate and 89% with unconcentrated blood. These results suggest that the use of unconcentrated blood in BACTEC vials can be reasonably effective in detecting mycobacteremia. We are accumulating more information to further clarify this point.

The comparison of MAI growth in BACTEC vials inoculated with Isolator concentrate compared with growth on conventional solid media inoculated with Isolator concentrate showed that the BACTEC system had a time advantage (10.4 compared with 16.1 days). Sensitivity with conventional media was 98%, 94% for BACTEC DV vials, 77% for BACTEC SV vials, and 89% when DV and SV results were combined. The BACTEC system thus compared well with conventional media, particularly because it received less than half the amount of inoculum used for the conventional media. It is likely that time to positivity of the BACTEC vials could be shortened even more by more frequent monitoring of the vials.

Although the average time to detect a positive culture in a BACTEC vial was 10.4 days, cultures with a high colony count were more often positive in 4 to 6 days, whereas cultures with very few organisms required from 11 to 26 days to positivity. This time difference was not as apparent when conventional slants were used. A minimum of approximately 8 to 10 days seemed necessary to visually detect colonies, even in some instances when many colonies were present.

The clinical usefulness of blood cultures for diagnosing mycobacterial infection and monitoring therapy can now be reassessed in light of technological advances in blood culture methods. The utility of obtaining blood cultures for mycobacteria in selected patients will be determined by how often the cultures help to establish an infectious etiology in otherwise undiagnosed patients. The relatively benign and noninvasive nature of the process of obtaining a blood culture compared with the process of obtaining a bone marrow aspirate makes this an attractive ancillary test. Culturing blood will be particularly useful if positive cultures can be detected in days rather than weeks.

Our results suggest that for the many laboratories which have a BACTEC system available, blood cultures for mycobacteria can be done quite effectively, perhaps even without using concentrated blood, although the use of concentrated blood may enhance recovery. Although the combination of Isolator concentrate and BACTEC vial proved optimal in these studies, it may be impractical for most laboratories to maintain both systems. We suggest a minimum of two BACTEC vials, each containing 0.2 ml of either concentrated or unconcentrated blood. Especially for patients taking antimycobacterial drugs, each vial should ideally contain twice the standard amount of culture medium. Although we used PACT antibiotic supplement in these cultures, this may be unnecessary unless the patient is known or suspected to be bacteremic with other organisms.

For those laboratories in which an Isolator system is used, this method also provides a sensitive method for performing blood cultures for acid-fast bacilli. The entire 1.6 ml of concentrate should be distributed in 0.2- to 0.3-ml portions onto a set of slants or plates of LJ or MB medium. The present study indicated that Isolator concentrate inoculated onto solid media has a slightly higher sensitivity than Isolator concentrate inoculated into BACTEC vials, although cultures in BACTEC vials were often detected as positive earlier than cultures on solid media. The difference in sensitivity may be due to the fact that a larger total inoculum can be planted on solid media.

An advantage of plating to conventional media is that the growth from the media can be used directly for standard tests, including biochemical as well as drug susceptibility tests. Plating of Isolator concentrate also provides quantitative data which may be of prognostic value or which may be used to monitor and adjust therapeutic effects.

We did not look at the effectiveness of plating unconcentrated blood directly onto conventional mycobacterial media. Based on the colony counts obtained by using Isolator concentrate, it seems likely that it would be possible to obtain positive cultures by using unconcentrated blood. However, we would anticipate, on the basis of our finding several patients with low colony counts, that overall sensitivity would be poorer than with Isolator concentrate.

ACKNOWLEDGMENTS

We appreciate the assistance of the medical and nursing staffs of the Medicine Branch of the National Cancer Institute and the National Institute of Allergy and Infectious Diseases in performing this study. We also thank the technologists of the Microbiology Service for evaluating all of the blood cultures and Dorene Dalessandro for preparing this manuscript.

LITERATURE CITED

1. **Berlin, O. G. W., P. Zakowski, D. A. Bruckner, and B. L. Johnson, Jr.** 1984. New biphasic culture system for isolation of mycobacteria from blood of patients with acquired immune deficiency syndrome. J. Clin. Microbiol. **20**:572–574.
2. **Elliott, J. L., W. L. Hoppes, M. L. Platt, J. G. Thomas, I. P. Patel, and A. Gansar.** 1983. The acquired immunodeficiency syndrome and *Mycobacterium avium-intracellulare* bacteremia in a patient with hemophilia. Ann. Int. Med. **98**:290–293.
3. **Macher, A. M., J. A. Kovacs, V. Gill, G. D. Roberts, J. Ames, C. H. Park, S. Straus, H. C. Lane, J. E. Parillo, A. S. Fauci, and H. Masur.** 1983. Bacteremia due to *Mycobacterium avium-intracellulare* in the acquired immunodeficiency syndrome. Ann. Int. Med. **99**:782–785.
4. **Masur, H., and A. M. Macher.** 1984. Acquired immune deficiency syndrome (AIDS), p. 1670–1674. *In* G. Mandell, R. Douglas, and J. Bennett (ed.), Principles and practice of infectious diseases, 2nd ed. John Wiley & Sons, Inc., New York.
5. **Pape, J. W., B. Liaufaud, F. Thomas, J. Mathurin, M. St. Amand, M. Boncy, V. Pean, M. Pamphile, A. Laroche, and W. D. Johnson.** 1983. Characteristics of the acquired immune deficiency syndrome (AIDS) in Haiti. N. Engl. J. Med. **309**:945–950.
6. **Pierce, P. F., D. R. DeYoung, and G. D. Roberts.** 1983. Mycobacteremia and the new blood culture systems. Ann. Int. Med. **99**:786–789.
7. **Vestal, A. L.** 1975. Procedures for the isolation and identification of mycobacteria, p. 21–31. U.S. Department of Health, Education, and Welfare publication no. (CDC) 79-8230. Center for Disease Control, Atlanta.