JOURNAL OF CLINICAL MICROBIOLOGY, Sept. 1984, p. 572–574
0095-1137/84/090572-03$02.00/0

Vol. 20, No. 3

New Biphasic Culture System for Isolation of Mycobacteria from Blood of Patients with Acquired Immune Deficiency Syndrome

O. GEORGE W. BERLIN,[1]* PHILLIP ZAKOWSKI,[2] DAVID A. BRUCKNER,[1] AND B. LAMAR JOHNSON, JR.[2]

Clinical Microbiology Laboratory, Department of Pathology,[1] and Department of Medicine,[2] University of California at Los Angeles Medical Center, Los Angeles, California 90024

Received 3 October 1983/Accepted 17 May 1984

A new biphasic medium consisting of a 7H11 agar slant and brain heart infusion broth liquid phase has been used for the past 10 months at the University of California at Los Angeles for isolation of *Mycobacterium avium* complex from blood. In 12 patients whose blood cultures were grown on this medium, the median time for isolation of *M. avium* complex was 7 days (range 6 to 15 days) compared with 21 to 27 days on a fungal medium and 28 to 38 days on routine blood culture medium. The system has provided a reliable and rapid way to obtain morphology and rapid identification of colonies produced from blood samples of patients with acquired immune deficiency syndrome, who are at high risk for *M. avium* complex mycobacteremia.

Routine blood cultures are probably the most important single type of specimen submitted to the microbiology laboratory for examination (6). Prompt isolation and accurate identification of the etiological agents of septicemia remains one of the most pressing functions of the laboratory. Although blood cultures are commonly used for the isolation of bacteria and fungi, they are seldom used for the isolation of mycobacteria. Although a variety of media are available for the detection of bacteremia (1, 3, 9, 10, 17, 20) and fungemia (2, 16), only recently have systems been evaluated for the isolation of mycobacteria from blood (7, 14). Requests for blood cultures for mycobacteria are seldom made because acid-fast bacteria grow slowly and mycobacteremia is rare (15).

Patients with acquired immune deficiency syndrome (AIDS), who often develop Kaposi's sarcoma, *Pneumocystis carinii* pneumonia, and other opportunistic infections, often also develop disseminated *Mycobacterium avium* complex (MAC) infections before death (5, 8, 18). Because of the high incidence of disseminated MAC infection (23), we have designed and tested a blood culture system for rapid isolation of mycobacteria from blood. No efficient system for mycobacterial blood cultures has been available before (11, 13), but recently a lysis centrifugation blood culture technique has been used to isolate mycobacteria (7). This report describes our experience with a new biphasic medium that provides rapid and reliable means of isolating MAC from the blood of patients with AIDS.

The solid phase of the new biphasic system consists of the modified 7H11 oleic acid albumin agar (4, 12), and the liquid phase is brain heart infusion (BHI) broth. For the preparation of the solid phase, 25.0 g of 7H10 agar base (Difco Laboratories, Detroit, Mich.) was mixed with 1.0 g of casein hydrolysate (Sigma Chemical Co., St. Louis, Mo.), 5.0 ml of glycerol (Mallinckrodt Chemical Works, St. Louis, Mo.), and 1.0 g of Bacto-Agar (Difco) in 1 liter of distilled water. The mixture was melted, dispensed in 70 to 75-ml portions into flat blood bottles, and autoclaved at 15 lb/in^2 for 15 min. After sterilization, 5.0 ml of sterile OADC enrichment (Difco) was aseptically added to each bottle. The resulting solution was slowly mixed and allowed to solidify as slants. The broth phase consisted of 37.0 g of BHI broth (Difco) supplemented with 5.0 ml of Polysorbate 80 (BBL Microbiology Systems, Cockeysville, Md.) in 1 liter of distilled water and autoclaved at 15 lb/in^2 for 15 min; 30 ml of the BHI broth was added aseptically to each bottle.

To evaluate the sensitivity of detection, 5 ml of blood seeded with 1, 2, 5, or 10 MAC organisms per ml isolated from an AIDS patient was inoculated into the medium; a total of five sets were used for control studies. The system was compared with a routine bacteriological blood bottle containing tryptic soy broth (Cal Scott, North Hollywood, Calif.) and a diphasic fungal bottle with BHI agar broth (Cal Scott). Blood samples (15 ml) were obtained from AIDS patients with clinically suspected mycobacteremia, and 5-ml aliquots were inoculated at the bedside into each of the three systems. In the laboratory, the bottles were vented with blood culture bottle venting units (GIBCO Laboratories, Lawrence, Mass.) incubated at 37°C, and inspected for turbidity every other day; 5 ml of the broth from bottles showing visible turbidity was aspirated into 50-ml Corning tubes and centrifuged at 3,000 × *g* for 20 min. The supernatant was decanted and slides were made from the centrifugate, dried on a slide warmer for 10 min at 65°C, stained by both the auramine-rhodamine and Ziehl-Neelsen techniques, and examined for the presence of acid-fast bacilli. Isolates were identified by standard mycobacteriological procedures (19). The blood cultures were kept for 8 weeks before being reported as negative.

When 1, 2, 5, and 10 MAC organisms per ml of blood were inoculated into the new medium, the time of recovery was inversely proportional to the number of organisms inoculated. When 10 organisms per ml were inoculated, turbidity in

TABLE 1. Growth pattern of *MAC* (control strain) in the blood culture media

No. of mycobacteria seeded per ml of blood	No. of days after inoculation that growth appeared on:				
	7H11/BHI medium		BHI biphasic fungal medium		Tryptic soy broth bacteriological medium (turbidity)
	Turbidity	Colony on slant	Turbidity	Colony on slant	
1	27–30	38–42	37–44	45–52	51–88
2	16–20	29–33	36–40	39–46	43–49
5	10–16	26–31	31–36	37–41	40–44
10	6–8	23–25	21–25	31–39	28–38

* Corresponding author.

TABLE 2. Comparison of three blood culture media in the isolation of MAC

Patient no. (age)	No. of blood cultures (no. positive)	No. of days after inoculation that growth appeared on:					Current status of patient[a]
		7H11/BHI medium		BHI biphasic fungal medium		Tryphcase soy broth, bacteriological medium (turbidity)	
		Turbidity	Colony on slant	Turbidity	Colony on slant		
1 (30)	1 (1)	ND[b]	ND	28	37	ND	D
2 (33)	3 (1)	ND	ND	ND	ND	35	D
3 (31)	2 (1)	ND	ND	ND	ND	38	D
4 (26)	9 (7)	6–7	18–23	21–26	32–38	29–33	A
5 (34)	4 (2)	7–8	20–25	23–25	30–37	30–32	D
6 (31)	2 (1)	6	18	21	34	ND	U
7 (32)	8 (5)	7–12	17–29	22–25	32–37	29–30	D
8 (36)	1 (1)	8	22	24	33	ND	A
9 (37)	2 (2)	7	20–22	22–23	33–35	28	A
10 (30)	3 (1)	15	24	27	36	34	A
11 (32)	1 (1)	20	26	ND	ND	ND	A
12 (23)	1 (1)	8	20	ND	ND	ND	D

[a] D, Deceased; A, alive; U, unknown.
[b] ND, Not done.

the fluid phase was noted within 6 to 8 days. When 1 organism per ml was inoculated, turbidity was not noted until 25 to 30 days. Under identical conditions, when 10 organisms per ml were used, the diphasic fungal bottles took at least 21 to 25 days and the bacteriological culture system took approximately 28 to 38 days for visible turbidity to develop (Table 1).

A total of 258 blood samples from 72 suspected AIDS patients were examined during this study. In addition, 16 blood samples from 9 patients with severe combined immune deficiency and other neoplastic disorders were studied. MAC was isolated from the blood of 12 male AIDS patients (Table 2). The presence of turbidity with positive acid-fast stains allowed the presumptive identification of mycobacteria in the fluid phase of the culture system within 6 to 20 days (with a mean of 6 to 8 days). Colonies of MAC began to appear on the 7H11 slant in approximately 20 days. In our experience, the appearance of acid-fast bacilli in the broth phase of the culture originating from a patient with AIDS was sufficient to make a tentative diagnosis of MAC bacteremia.

In the last two decades, the frequency of disease produced by atypical mycobacteria in general, and MAC in particular, has become more common (21). Most human cases of mycobacteriosis caused by MAC involve the lungs and widespread disseminated infection is an unusual clinical entity seen almost exclusively in immunocompromised adults and children (13, 22). Although MAC, an ubiquitous complex, is relatively avirulent in immunocompetent hosts, it is one of the unique organisms isolated from AIDS patients. The rapid isolation of MAC from the blood of these patients indicates the overwhelming nature of this infection in patients with AIDS.

Analysis of the three blood culture systems has shown that the new 7H11/BHI medium is superior to the diphasic fungal and bacteriological blood systems for the recovery of MAC. The presence of MAC in blood could usually be detected in the fluid phase of the new biphasic system in 6 to 8 days. These preliminary data suggest that the new system is a sensitive and efficient method of isolating mycobacteria from the blood. Also, colonies on the slant of the biphasic medium provides growth for biochemical identification and drug susceptibility testing. Routine subculturing is unnecessary with the biphasic system developed; this minimizes contamination and provides a level of safety.

In conclusion, the biphasic medium described for the isolation of MAC is an efficient blood culture system for the rapid detection of mycobacterial growth in blood specimens from AIDS patients. We feel that this system can be used routinely for AIDS and severe combined immune dificiency patients.

We thank Gary Kato for technical assistance and preparation of the manuscript.

ADDENDUM

Since the preparation of this manuscript, MAC has been isolated from the blood of 10 additional AIDS patients at the University of California at Los Angeles Center for Health Sciences. A total of 22 patients have now had MAC isolated from their blood by the new biphasic system.

LITERATURE CITED

1. **Babu, J. P., R. F. Schell, and J. L. Le Frock.** 1978. Evaluation of twenty-three blood culture media. J. Clin. Microbiol. **8**:288–292.
2. **Bille, J., G. D. Roberts, and J. A. Washington.** 1983. Retrospective comparison of three blood culture media for the recovery of yeasts from clinical specimens. Eur. J. Clin. Microbiol. **2**:22–25.
3. **Castaneda, M. R.** 1947. A practical method for routine blood culture for brucellosis. Proc. Soc. Exp. Biol. Med. **64**:114–115.
4. **Cohn, M. L., R. F. Waggoner, and J. F. McClatchy.** 1968. The 7H11 medium for the cultivation of mycobacteria. Am. Rev. Respir. Dis. **98**:295–296.
5. **Fainstein, F., R. Bolivar, G. Mavligit, A. Rios, and M. Luna.** 1982. Disseminated infection due to *Mycobacterium avium-intracellulare* in a homosexual man with Kaposi's sarcoma. J. Infect. Dis. **145**:586.
6. **Finegold, S. M., and W. J. Martin.** 1982. Diagnostic microbiology, 6th ed., p. 705. C. V. Mosby, St. Louis, Mo.
7. **Fojtasek, M. F., and M. T. Kelly.** 1982. Isolation of *Mycobacterium chelonei* with the lysis-centrifugation blood culture technique. J. Clin. Microbiol. **16**:403–405.
8. **Greene, J. B., G. S. Sidhu, S. Lewin, J. F. Levine, H. Masur, M. S. Simberkoff, P. Nicholas, R. C. Good, S. B. Zolla Pazner, A. A. Pollock, M. L. Tapper, and R. S. Holzman.** 1982. *Mycobacterium avium-intracellulare*: a cause of disseminated life-threatening infection in homosexuals and drug abusers. Ann.

Intern. Med. **97**:539–546.
9. **Hall, M. M., C. A. Mueske, D. M. Ilstrup, and J. A. Washington II.** 1979. Evaluation of a biphasic medium for blood cultures. J. Clin. Microbiol. **10**:673–676.
10. **Julander, I. G., M. Kalin, and L. Sjoberg.** 1983. Detection time for blood culture isolates using a biphasic system. Eur. J. Clin. Microbiol. **2**:54–55.
11. **Landau, W., J. Feczko, and R. L. Kaplan.** 1980. Radiometric detection of mycobacteria in routine blood cultures. J. Clin. Microbiol. **12**:477–478.
12. **Middlebrook, G., M. L. Cohn, W. E. Dye, W. F. Russell, and D. Levy.** 1960. Microbiologic procedures of value in tuberculosis. Acta Tuberc. Scand. **38**:66–81.
13. **Phung, N. D., and P. T. Davidson.** 1981. Increased suppressor cell activity in a patient with *Mycobacterium avium-intracellulare* pulmonary disease and hypogammaglobulinemia. Ann. Allergy **46**:204–207.
14. **Pierce, F. P., D. R. DeYoung, and G. D. Roberts.** 1983. Mycobacteria and the new blood culture media. Ann. Intern. Med. **99**:786–789.
15. **Roberts, G. D., and J. A. Washington II.** 1975. Detection of fungi in blood cultures. J. Clin. Microbiol. **1**:309–310.
16. **Rosenzweig, D. Y.** 1979. Pulmonary mycobacterial infections due to *Mycobacterium intracellulare-avium* complex. Chest **75**(2):115–119.
17. **Sliva, H. S., and J. A. Washington II.** 1980. Optimal time for routine early subculture of blood cultures. J. Clin. Microbiol. **12**:445–446.
18. **Sohn, C. C., R. W. Schroff, K. L. Kliever, D. M. Lebel, and S. Fligiel.** 1983. Disseminated *Mycobacterium avium-intracellulare* infection in homosexual men with acquired cell-mediated immunodeficiency: a histologic and immunologic study of two cases. J. Clin. Pathol. **27**:247–252.
19. **Vestal, A. L.** 1977. Procedures for the isolation and identification of mycobacteria. U. S. Department of Health, Education, and Welfare publication no. (CDC) 77-8230, 3rd ed., p. 136. Center for Disease Control, Atlanta.
20. **Washington, J. A.** 1971. Comparison of two commercially available media for detection of bacteremia. Appl. Microbiol. **22**:604–607.
21. **Wiesenthal, A. M., K. E. Powell, J. Kopp, and J. W. Splinder.** 1982. Increase in *Mycobacterium avium* complex isolates among patients admitted to a general hospital. Public Health Rep. **97**:61–65.
22. **Wolinsky, E.** 1979. Nontuberculous mycobacteria and associated diseases. Am. Rev. Respir. Dis. **119**:107–159.
23. **Zakowski, P., S. Fligiel, O. G. W. Berlin, and B. Lamar Johnson.** 1982. Disseminated *Mycobacterium avium-intracellulare* infection in homosexual men dying of acquired immunodeficiency. J. Am. Med. Assoc. **248**:2980–2982.