

Value of Terminal Subcultures From Negative BACTEC Blood Culture Bottles

GEORGE F. ARAJ,¹ ROY L. HOPFER,^{1*} MARK WENGLAR,¹ AND VICTOR FAINSTEIN²

Department of Laboratory Medicine¹ and Department of Developmental Therapeutics,² The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, Houston, Texas 77030

Received 22 January 1981/Accepted 10 June 1981

Terminal subcultures from 5,354 negative BACTEC blood culture bottles did not significantly improve the detection of positive cultures. Only 15 of the 545 total isolates were recovered from the terminal subcultures. All 15 of these isolates were either considered contaminants or had been previously detected.

An earlier study (2) evaluated the necessity for routine 7-day subcultures of previously negative cultures. No significant increase in the yield of positive cultures was found. Because this is an important finding that could affect the costs of cultures and personnel time, it prompted us to evaluate terminal subcultures of previously negative blood cultures in our institution. Since we use the BACTEC radiometric system (4, 5), we wanted to compare our findings with those of the "standard" nonradiometric two-bottle broth method of Campbell and Washington (2).

During this study, aerobic BACTEC 6B blood culture bottles were processed daily with a BACTEC 460, and anaerobic 7B bottles were processed daily on a BACTEC 225 automated radiometric blood culture system. Blind subcultures from negative 6B bottles were done from 18 to 24 h and after 7 days. Blind subcultures from 7B bottles were done only once after 72 h. A sample of blood broth mixture was withdrawn aseptically with a syringe, and approximately 0.5 ml was inoculated onto a chocolate agar plate. The plates were incubated at 37°C in 10% CO₂ and held 72 h before being discarded. When fastidious organisms or fungi were suspected, subculture plates were incubated for 7 days, and culture bottles were held for 14 days as previously described (1, 5, 6).

A total of 5,884 blood specimens (11,768 bottles) were received for culture during a 3-month period. There were 530 positive cultures and 5,354 negative cultures before the terminal subcultures. Of the 530 positive cultures, 413 were first detected by the BACTEC system. In addition, 35 and 82 positive cultures were first detected by day-2 smears and subcultures, respectively. The majority (79.3%) of these positive cultures were detected by day 3 of incubation. The routine 7-day terminal subcultures of the 5,354 previously negative BACTEC blood cul-

tures resulted in 15 positive cultures (0.3% of total subcultures) as shown in Table 1. Distribution of organisms in the 545 positive cultures included 434 bacteria, 99 yeasts, and 12 molds.

Our study showed that routine monitoring of blood cultures for 7 days yielded more than 97% of the cultures that would become positive. Radiometric monitoring by BACTEC first detected 75.9%, subcultures within the first 72 h detected 15%, and Gram-stained smears detected 6.5% of positive cultures. The 7-day terminal cultures of 5,354 previously negative blood cultures grew a *Staphylococcus epidermidis*, a *Bacillus cereus*, and a *Corynebacterium* species. All three were considered contaminants, and none of the patients were treated with antibiotics. Although eight *Cryptococcus neoformans* isolates were obtained from specimens from three patients by using the 7-day subcultures, the diagnosis of cryptococcosis had been established earlier in all three patients by other methods (India ink, cryptococcal antigen, or previous blood culture). Similarly, coccidioidomycosis had already been diagnosed in the patient with the four positive 7-day subcultures of *Coccidioides immitis*. These findings are consistent with previous reports (4, 5) from this institution. Therefore, routine terminal subculture neither improved nor increased the incidence of positive cultures. In addition, the material cost (approximately \$460 for chocolate plates and \$465 for syringes) and the time spent by our laboratory personnel in setting up and handling the 5,354 subcultures in this study could have been better spent on other areas in the clinical microbiology laboratory.

Our 7-day subculture data are consistent with and confirm the findings of Campbell and Washington (2), namely, that 7-day subcultures are of questionable value, unless fastidious bacterial or fungal organisms are suspected. The only significant isolates we obtained were fungi, and since

TABLE 1. Positive terminal subcultures from 5,354 previously negative BACTEC blood cultures

Organism	No. of isolates
<i>Staphylococcus epidermidis</i>	1
<i>Bacillus cereus</i>	1
<i>Corynebacterium sp.</i>	1
<i>Cryptococcus neoformans</i>	8 ^a
<i>Coccidioides immitis</i>	4 ^b

^a Isolated from three patients.

^b Isolated from one patient.

they were already diagnosed, those particular bottles would have been subcultured and incubated an additional 7 days.

Blood culture media, subculture methods, and time of incubation vary from one laboratory to another and depend to some extent on the type of patients admitted to the institution. However, the similarity of our findings in a cancer hospital and those of Campbell and Washington suggests that the value of terminal subcultures may be small, regardless of the hospital setting. It is important to remember that in both studies blood culture bottles were examined (visually or radiometrically) for 7 days. If such findings are repeated in numerous hospital settings, then the recommendations (1, 3) for such cultures might

be revised. Obviously, even if such recommendations are amended, each laboratory should review their terminal subculture results before making significant changes in their blood culture policy.

This investigation was supported in part by Public Health Service Grant CA-05831 from the National Cancer Institute.

LITERATURE CITED

1. Bartlett, R. C., P. D. Ellner, and J. A. Washington II. 1974. Cumitech 1, Blood cultures. Coordinating ed., J. C. Sherris. American Society for Microbiology, Washington, D.C.
2. Campbell, J., and J. A. Washington II. 1980. Evaluation of the necessity for routine terminal subcultures of previously negative blood cultures. *J. Clin. Microbiol.* **12**:576-577.
3. Commission on Laboratory Inspection and Accreditation. 1978. Inspection checklist. College of American Pathologists, Skokie, Ill.
4. Gröschel, D., R. L. Hopfer, and J. E. French. 1979. Blood cultures with the BACTEC 225 radiometric microbial growth detection system. *Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig. Reihe A* **244**:316-323.
5. Hopfer, R. L., A. Orengo, S. Chesnut, and M. Wenglar. 1980. Radiometric detection of yeasts in blood cultures of cancer patients. *J. Clin. Microbiol.* **12**:329-331.
6. Washington, J. A., II. 1978. Conventional approaches to blood culture, p. 41-87. *In* J. A. Washington II (ed.), *The detection of septicemia*. CRC Press, Inc., West Palm Beach, Fla.