JOURNAL OF CLINICAL MICROBIOLOGY, Mar. 1995, p. 784–785 Vol. 33, No. 3
0095-1137/95/$04.00+0

Letter to the Editor

Optimum Recovery of *Mycobacterium avium* Complex from Blood Specimens of Human Immunodeficiency Virus-Positive Patients by Using Small Volumes of Isolator Concentrate Inoculated into BACTEC 12B Bottles

We read with interest the article by Doern and Westerling (1) describing their observations of the Isolator-BACTEC systems for the culture of mycobacteria from blood specimens. We were pleased to see confirmation of the mycobacterial inhibition created by using the Isolator-BACTEC 12B system that we had reported earlier (2).

However, their use of a decreased volume of specimen to avoid the inhibition problem raises two issues. First, in their study they compared results obtained from 0.2 ml inoculated into the BACTEC 12B bottle with those obtained from 0.1 ml inoculated onto solid medium. Their rationale for this is that it is impractical to inoculate more than 0.1 ml of sediment onto an agar plate. Examination of their results, which appear in Table 2, reveals that when data from the two solid media are combined (bringing specimen volume up to the same 0.2 ml used in the BACTEC bottle), BACTEC and solid media both allow recovery of 32 of 42 isolates. Since many studies have indicated that greater blood specimen volume results in increased recovery, a more logical approach would have been to compare the reduced specimen volume in 12B bottles to the maximum allowable on solid media. For the past 19 months we have been inoculating approximately 0.5 ml on each of three Middlebrook 7H11 agar plates without any adverse effects, achieving a 17.7% recovery rate during this period. We allow the plates to incubate for 24 h in an upright fashion, and then they are inverted for the remainder of the incubation period.

Second, we feel that it is important to use the entire amount of Isolator sediment in the culturing process, since the organisms are not necessarily distributed homogeneously and may be missed if only a portion of the sediment is inoculated. This nonhomogeneity is evident in the data presented in Table 2 of Doern and Westerling's article in which 8 of 42 isolates grew only on the solid media and in addition, 2 of 42 required a full 1.0 ml of sediment to grow in the BACTEC 12B bottle despite the inhibition observed with that volume. However, their final recommendation utilizes only 0.7 ml of the 1.5 ml of concentrated specimen produced by Isolator processing.

Doern and Westerling describe a useful technique for reducing the inhibitory effect created by using the combined Isolator and BACTEC systems. However, optimum recovery is not likely to occur without complete utilization of the specimen.

REFERENCES

1. **Doern, G. V., and J. A. Westerling.** 1994. Optimum recovery of *Mycobacterium avium* complex from blood specimens of human immunodeficiency virus-positive patients by using small volumes of Isolator concentrate inoculated into BACTEC 12B bottles. J. Clin. Microbiol. **32:**2576–2577.
2. **Wasilauskas, B. L., and R. Morrell, Jr.** 1994. Inhibitory effect of the Isolator blood culture system on growth of *Mycobacterium avium-M. intracellulare* in BACTEC 12B bottles. J. Clin. Microbiol. **32:**654–657.

Benedict L. Wasilauskas, Ph.D.
Robert Morrell, Jr., B. S., M.B.A.
Department of Pathology
The Bowman Gray School of Medicine
Medical Center Boulevard
Winston-Salem, North Carolina 27157-1072

Author's Reply

The sole intent of our study (1) was to determine whether or not Isolator concentrate inoculated into BACTEC 12B bottles could be used to effectively recover MAC from the blood of HIV-positive individuals, *not* to define the overall best method for culture recovery of this organism group. We initiated this study largely because of concerns over the conclusions of Wasilauskas and Morrell in their earlier demonstration of inhibition of MAC growth by the lysing-anticoagulant reagent in Isolator tubes (2). In their article, they stated, "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" and "On the basis of our findings, the combined use of Isolator and BACTEC for recovery of mycobacteria in blood specimens is contraindicated."

As stated in our report, we too had recognized the inhibitory effect of the lysing-anticoagulant reagent in Isolator tubes but reasoned that this problem might be overcome by decreasing the amount of Isolator concentrate transferred to BACTEC 12B bottles. Indeed, this is precisely what we observed experimentally. To wit, more than twice as many isolates of MAC were recovered when 0.2-ml aliquots of Isolator concentrate were cultured in 12B bottles than when 1.0-ml volumes were used. In view of the shortened periods to detection with BACTEC versus solid medium, we think that this method should not be discarded but rather exploited. I would emphasize, as we stated in our report, that we have not defined the optimum volume of concentrate to be cultured in BACTEC bottles, as we only examined two different aliquot sizes, 0.2 and 1.0 ml. Studies are currently ongoing in our laboratory to address the issue of optimum volume to be cultured.

Wasilauskas and Morrell also comment that 0.5 ml of Isolator concentrate can be inoculated into Middlebrook 7H11 agar plates "without adverse effects" and that the entire Isolator concentrate should be subcultured. Although we did not attempt to address these issues in our paper, we wonder about the practicality and safety of culturing fluid samples of 0.5 ml on agar plates even when they are allowed to "incubate for 24 h in an upright fashion" before being inverted. Our experience with fluid inocula of this high volume is that they tend to leak and run all over the place. Use of the entire Isolator concentrate makes intuitive sense but is only cost justifiable if this practice is proven necessary to op-

timize recovery. We are aware of no objective studies that have systematically addressed this issue.

REFERENCES

1. **Doern, G. V., and J. A. Westerling.** 1994. Optimum recovery of *Mycobacterium avium* complex from blood specimens of human immunodeficiency virus-positive patients by using small volumes of Isolator concentrate inoculated into BACTEC 12B bottles. J. Clin. Microbiol. **32:**2576–2577.
2. **Wasilauskas, B. L., and R. Morrell, Jr.** 1994. Inhibitory effect of the Isolator blood culture system on growth of *Mycobacterium avium-M. intracellulare* in BACTEC 12B bottles. J. Clin. Microbiol. **32:**654–657.

Gary V. Doern
Judith A. Westerling
Clinical Microbiology Laboratories
University of Massachusetts Medical Center
Worcester, Massachusetts 01655