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# 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

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**Recovery of *Mycobacterium avium* complex organisms from 412 lysis-centrifugation (Isolator) concentrates of blood specimens obtained from human immunodeficiency virus-positive individuals was attempted with the following media (and Isolator concentrate inoculum volumes): BACTEC 12B broth (0.2 and 1.0 ml), Lowenstein-Jensen slants (0.1 ml), and Middlebrook 7H10/11 agar (0.1 ml). A total of 42 *M. avium* complex isolates were recovered. The highest rates of recovery and shortest detection times were noted with BACTEC 12B bottles inoculated with 0.2 ml of Isolator concentrate. Middlebrook agar was superior to Lowenstein-Jensen slants. Fluorochrome acid-fast smears performed directly upon Isolator concentrates were of no utility.**

Recognition of the *Mycobacterium avium* complex (MAC) as an important cause of bacteremia in patients with AIDS has necessitated the development of laboratory techniques that optimize recovery of this organism from blood (6–8). One such technique includes initial processing of blood specimens by lysis-centrifugation (Isolator; Wampole Laboratories, Cranberry, N.J.) with inoculation of concentrates into BACTEC 12B broth medium (Becton-Dickinson Diagnostic Instrument Systems, Sparks, Md.) for propagation and radiometric detection with a BACTEC 460 instrument. This approach is thought to combine the advantages of organism concentration in the specimen by use of the Isolator system and a shortened time to recovery by use of the BACTEC radiometric detection method (1, 3–5).

Recently, Wasilauskas and Morrell reported that among 162 MAC isolates from blood, 152 (94%) were recovered on a Lowenstein-Jensen (L-J) slant whereas only 81 (50%) were recovered in BACTEC 12B medium with a 460 detection device when both the L-J slant and 12B bottles were inoculated with equal aliquots of concentrate from an Isolator tube (9). In addition, the mean length of time to MAC detection was significantly shorter with L-J slants (i.e., 18 days) than 12B bottles (i.e., 24 days). They further demonstrated that the lysing anticoagulant solution in the Isolator tube inhibited the growth of both clinical isolates and a MAC type strain in BACTEC 12B bottles. This inhibitory effect was minimal, however, when small amounts of lysing reagent, i.e., 0.1 ml of a $\geq$1:64 dilution of reagent, was added to the bottles. They concluded that the Isolator system in conjunction with BACTEC should not be used for MAC recovery from blood.

We observed a similar effect on MAC growth in 12B bottles with blood specimens processed with the Isolator during 1990 to 1991 (unpublished observations); however, instead of discarding this technology altogether, we reasoned that by decreasing the volume of the Isolator concentrate transferred into 12B bottles, more acceptable rates of recovery could be achieved, perhaps even exceeding those achievable by inoculating the concentrate directly onto solid medium. This hypothesis was based on the notion that the larger the Isolator blood specimen concentrate aliquot transferred to BACTEC 12B bottles was, the larger the amount of carryover of lysing-anticoagulant solution, and thus the greater the chance for inhibition, would be.

To investigate this possibility, the following was done with all Isolator blood cultures ($n$ = 412) submitted for mycobacterial culture to the University of Massachusetts Medical Center Clinical Microbiology Laboratories in the 2-year period from 11 September 1991 to 14 October 1993. Isolator tubes (10 ml) were processed in accordance with the manufacturer's instructions, by using the Isostat device, usually within 1 h of specimen collection. The supernatant was discarded, and the concentrate (1.5 $\pm$ 0.1 ml) was used to inoculate two 12B BACTEC bottles, an L-J slant, and a plate containing Middlebrook agar medium aseptically. One 12B bottle received 0.2 ml of the concentrate, while the second 12B bottle received 1.0 ml. Both the L-J slant and the Middlebrook plate were inoculated with 0.1 ml of the concentrate. During the first half of this study, Middlebrook 7H10 agar was used; during the second half, Middlebrook 7H11 agar was used. All bottles, slants, and plates were incubated at 37 $\pm$ 1°C in 5 to 10% $CO_2$ and examined twice per week during the first 2 weeks and once weekly thereafter for a total of 8 weeks prior to being discarded as negative. *M. avium* group and *M. intracellulare* were identified by using commercially available rRNA probes in accordance with the manufacturer's instructions (GenProbe, La Jolla, Calif.). In addition, during the first 16 months of this study encompassing 294 specimens, a fluorochrome acid-fast smear was performed on 1 drop of Isolator concentrate (2).

Among the total of 412 specimens processed as described above, 42 specimens (10.2%) from 30 patients yielded *M. avium* group (41) or *M. intracellulare* (1) organisms. The number of isolates recovered in each culture system and the length of time to detection are shown in Table 1. Table 2 depicts the patterns of recovery. Inoculation of BACTEC 12B medium with 0.2 ml of concentrate from the Isolator tube

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TABLE 1. MAC recovery from the lysis-centrifugation concentrate of 10-ml Isolator blood culture tubes inoculated onto different media

| Medium | Inoculum vol (ml) | No. (%) of positive cultures[a] | Mean time (days) to detection (range) |
|---|---|---|---|
| BACTEC 12B bottle | 0.2 | 32 (67.2) | 11.6 (5–21) |
| BACTEC 12B bottle | 1.0 | 15 (35.7) | 24.9 (7–48) |
| L-J slant | 0.1 | 24 (57.1) | 20.2 (10–53) |
| Middlebrook agar[b] | 0.1 | 25 (59.5) | 14.2 (8–39) |

[a] A total of 42 specimens yielded MAC, i.e., 41 isolates of the *M. avium* group and 1 isolate of *M. intracellulare*.

[b] Middlebrook 7H10 agar was used during the first half of the study, and 7H11 agar was used during the second half.

provided the highest rates of recovery and shortest lengths of time to detection. The next most efficient system was inoculation of Middlebrook medium, followed by L-J slants, both inoculated with 0.1 ml of concentrate. The least useful system was 12B bottles which received 1.0-ml inocula. Fluorochrome acid-fast smears prepared directly on Isolator concentrates from the first 294 specimens were always negative despite MAC growth in 30 cases.

Our experience is consistent with the observations of Wasilauskas and Morrell (9) insofar as the lysis-anticoagulant solution in the Isolator tube appeared to inhibit MAC growth in BACTEC 12B bottles, at least when present in large amounts. Furthermore, the fact that increased yields were obtained with smaller inocula in the current study is consistent with their observations in seeded experiments that the inhibitory effect of the lysing-anticoagulant solution could be diluted away.

We believe, however, that these observations do not completely obviate the use of the Isolator system in conjunction with BACTEC 12B medium for processing of blood specimens from patients suspected of having mycobacteremia. Laboratory workers using this procedure need simply ensure that only a limited amount of lysing-anticoagulant solution is transferred into 12B bottles at the time of inoculation with the Isolator concentrate. Although the optimum concentrate volume to be inoculated into 12B bottles was not determined in this study, an inoculum of 0.2 ml seemed to perform satisfactorily. This procedure resulted in greater recovery rates and shorter periods to detection than did the use of Middlebrook agar or L-J slants. This may have been due, at least in part, to the smaller inocula employed with the solid media (0.1 versus 0.2 ml with 12B bottles); however, inoculum sizes of greater than 0.1 ml are impractical for solid medium such as L-J and Middlebrook agar in slants and plates.

Among the two solid media employed in this study, Middlebrook agar appeared to be superior to L-J slants. While recovery rates were similar on these two media, MAC organisms were recovered a mean of 6 days earlier on Middlebrook agar. This is probably explained by the facility with which even very small MAC colonies can be discerned on Middlebrook agar because of its transparency.

On the basis of the assumption that 0.1-ml aliquots of fluid specimens such as the concentrate from Isolator tubes represent the optimum specimen amount that can be reliably and conveniently cultured on solid medium, we suggest a protocol that might be used to optimize MAC recovery from blood specimens. The concentrate from a 10-ml Isolator tube is used to inoculate the following media with the following inocula: two BACTEC 12B bottles, 0.2 ml each; two Middlebrook 7H11 agar plates, 0.1 ml each; one L-J slant, 0.1 ml. Performance of acid-fast smears directly on the concentrate has no value.

TABLE 2. Patterns of recovery of 42 MAC isolates on different media with different inocula from the concentrate of 10-ml Isolator blood culture tubes

| Isolate recovery on: | | | | No. of isolates with growth pattern indicated |
|---|---|---|---|---|
| 12B medium (0.2-ml inoculum) | 12B medium (1.0-ml inoculum) | L-J slant (0.1-ml inoculum) | 7H10 or 7H11 agar (0.1-ml inoculum) | |
| + | + | + | + | 9 |
| + | + | + | − | 1 |
| + | + | − | + | 2 |
| + | + | − | − | 1 |
| + | − | + | + | 7 |
| + | − | + | − | 2[a] |
| + | − | − | + | 3 |
| + | − | − | − | 7 |
| − | − | + | + | 1 |
| − | + | − | − | 2 |
| − | − | + | − | 4 |
| − | − | − | + | 3 |

[a] One of these two isolates was *M. intracellulare*, and the remaining 41 isolates were of the *M. avium* group.

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