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NOTES

Recovery of Mycobacteria from Blood in Mycobacteria Growth Indicator Tube and Lowenstein-Jensen Slant after Lysis-Centrifugation

BRUCE A. HANNA,[1,2]* SHAUN B. WALTERS,[1] STANLEY J. BONK,[2] AND LEO J. TICK[1]

Department of Pathology, New York University School of Medicine,[1] and Bellevue Hospital,[2] New York, New York 10016

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Five hundred five blood samples for culture were processed in the Isolator lysis-centrifugation system and were then inoculated into a Mycobacteria Growth Indicator Tube (MGIT) and onto a Lowenstein-Jensen (L-J) slant. Forty-nine isolates of *Mycobacterium avium* complex and three isolates of *Mycobacterium tuberculosis* were recovered from 50 of the blood culture specimens. Forty-five isolates from 43 specimens were recovered in the MGIT, with a mean time to detection of 21 days. Forty-one isolates from 40 specimens were recovered in the L-J slants, and the mean time to detection was 36 days. Nine specimens were positive in the MGIT alone, while seven specimens were positive only in L-J medium.

Blood cultures are an essential adjunct in the diagnosis of infections caused by *Mycobacterium avium* complex (MAC) in patients with AIDS. Among the laboratory methods described for the recovery of mycobacteria from blood is the use of lysis-centrifugation and inoculation of the sediment to solid or liquid medium (1, 3–5, 7, 9).

During an 18-week period, from November 1994 to March 1995, 505 blood samples for culture were received by the mycobacteriolgy laboratory of Bellevue Hospital. For all samples, 7 to 10 ml of blood was collected into 10-ml Isolator tubes (Wampole Laboratories, Cranbury, N.J.), and the tubes were centrifuged at 1,800 × *g* for 30 min. The supernatant was removed and approximately 0.25 ml of the sediment was inoculated into a Mycobacteria Growth Indicator Tube (MGIT; Becton Dickinson Diagnostic Instrument Systems, Sparks, Md.). The MGIT contains 4.0 ml of a modified Middlebrook 7H9 broth, to which is added 0.5 ml of the MGIT OADC (oleic acid-albumin-dextrose) enrichment and 0.1 ml of the MGIT PANTA mixture of antimicrobial agents. An oxygen-sensitive fluorescence sensor embedded in silicone on the bottom of the MGIT is used to detect the presence of actively respiring mycobacteria. As the mycobacteria consume the oxygen in the MGIT the fluorescence sensor is excited and may be observed under UV illumination. A 0.25-ml aliquot from the Isolator tube sediment was also inoculated onto a Lowenstein-Jensen (L-J) slant (Becton Dickinson Microbiology Systems, Hunt Valley, Md.). MGITs were capped and incubated at 37°C, while L-J slants were loosely capped and incubated at 37°C in an atmosphere of 5% CO_2. All culture tubes were examined daily for 30 days and twice weekly thereafter for 4 weeks. Culture tubes noted to be positive were examined with an acid-fast-stained smear (Kinyoun) on the day of detection and were identified with Accu-Probes for both MAC and *Mycobacterium tuberculosis* (Gen-Probe, Inc., San Diego, Calif.) and by conventional methods.

From the 505 blood culture specimens processed in the manner described above, mycobacteria were recovered from 50 (9.9%), MAC were isolated from 49, and *M. tuberculosis* was isolated from 1, while 2 yielded MAC and *M. tuberculosis*. Forty-four (8.7%) of the MGIT cultures were positive. The time to detection for MGIT cultures ranged from 3 to 56 days, with an average of 21 days. Among the L-J cultures, 40 (7.9%) were positive. The time to detection in L-J medium ranged from 7 to 60 days, with an average of 36 days. For 32 (6.3%) blood cultures positive in both the MGITs and L-J media, the L-J medium was noted to be positive first in seven instances, with an average difference in time to positivity of 11 days, while for 25 samples, the MGIT was positive first, with an average difference in time to positivity of 17 days.

The results are summarized as a scattergram of the observations of the experiment (Fig. 1). Region I contains the results of those culture sets for which MGIT was positive before L-J medium was positive. Region II contains those for which L-J medium was positive before MGIT was positive. The points plotted on the axes are for those culture sets for which no organism was detected by one of the test methods. That is, those points on the MGIT (x) axis are those for which the L-J medium was negative, and correspondingly, those on the L-J (y) axis were negative in the MGIT.

Region I has a majority of the points ($n = 34$) and thus indicates the superiority of MGIT over L-J medium in the present study. There is considerable variability, as is to be expected. This variability occurs both within each test method as well as in the difference in the detection times for a given specimen. This experiment is of the "paired comparisons" type, and one would expect that with both test methods applied to the same specimen, the variability might be small. Since the specimen is not homogeneous, however, large differences in the number of mycobacteria in the aliquots drawn for each of the tests can occur. This will likely cause a difference in detection times, independent of the test method.

* Corresponding author. Mailing address: Department of Pathology, NYU Medical Center, Bellevue 4W1, New York, NY 10016. Phone: (212) 263-6444. Fax: (212) 263-8284. Electronic mail address: hannab@is2.nyu.edu.

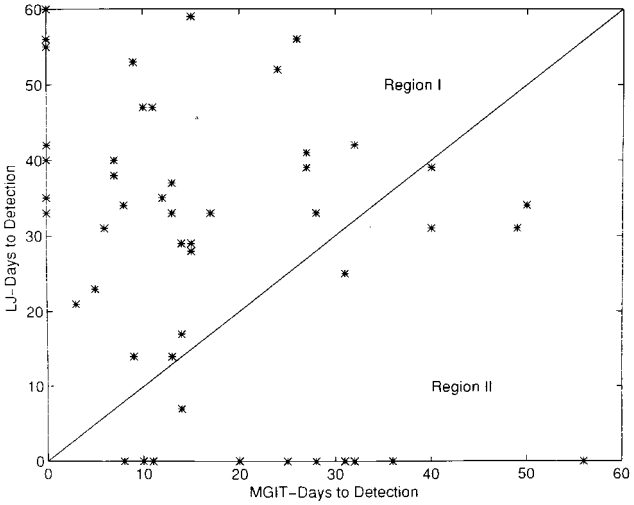

FIG. 1. Scattergram of the times to detection of mycobacteria isolated from blood in MGIT versus the times to detection of mycobacteria isolated from L-J medium.

Previous reports have compared the efficacy of culture of blood for mycobacteria following lysis-centrifugation. In a comparison of solid media versus BACTEC 12B vials, it was noted that an equal number of positive specimens were detected by the conventional media and the BACTEC vials but that positivity was detected in the BACTEC vials 5 to 6 days faster (3). In another study, blood culture specimens processed in Isolator tubes and inoculated onto solid media were compared with those collected in Vacutainer tubes with sodium polyanethol sulfonate and inoculated into BACTEC 13A vials (5). Those investigators found no significant difference in the sensitivity of or the time to detection by the two methods. Another study reported similar rates of recovery and times to detection of MAC when blood was collected in BACTEC 13A medium versus Isolator tubes and then inoculated onto Middlebrook 7H11 plates (9). Interestingly, that study began by inoculating the 0.4 ml of the Isolator tube sediment into a BACTEC 12B vial as well as onto Middlebrook 7H11 plates. During the study, however, use of the 12B vial was eliminated because although the time to detection was rapid, fewer isolates were recovered. Recently, it has been reported that blood cultures collected in the Isolator lysis-centrifugation system have an inhibitory effect on the growth of MAC in BACTEC 12B medium compared with growth in L-J medium (8). In that study, almost twice as many isolates were recovered from L-J medium than from BACTEC 12B vials. Those authors concluded the Isolator lysis-anticoagulant reagent in the BACTEC 12B vial was inhibitory to MAC but was mollified by absorption into the L-J medium. We did not observe any inhibition of MAC in the MGIT cultures compared with that in L-J medium. While the growth media and culture volumes of the MGIT and BACTEC 12B vials are similar, it should be noted that our 0.25-ml inoculum of the Isolator tube sediment was less than that used previously (8).

The inhibitory effect of the Isolator tube lysis-centrifugation system on the recovery of mycobacteria from blood inoculated into BACTEC 12B vials has been observed by others (2). In that study, the sediment was inoculated in duplicate into BACTEC 12B bottles by using 0.2- and 1.0-ml aliquots, and 0.1 ml was added to L-J and Middlebrook slants. In the BACTEC bottles inoculated with 0.2 ml, twice as many specimens were positive compared with the number of positive specimens obtained when a 1.0-ml inoculum was added. As seen in the previous study this inhibition was not apparent with the solid medium.

A recent study compared mycobacterial blood cultures collected in Vacutainer tubes with sodium polyanethol sulfonate and then incubated with sodium deoxycholate and inoculated into MGIT and onto L-J and Middlebrook 7H10 slants (6). In that study in the absence of the Isolator tube lysis reagent, the mean time to detection in the MGIT was only 11.1 days, whereas it was 38.3 days for L-J and Middlebrook 7H10 slants.

The data presented here indicate that blood cultures inoculated into MGITs will result in the more rapid detection of isolates of mycobacteria than is obtained with blood inoculated onto a conventional medium. Considering the observations of previous investigators, however, for those laboratories that use the Isolator lysis-centrifugation system, it would appear prudent to limit the volume of Isolator tube sediment inoculated into a single MGIT to 0.25 ml. Since the total Isolator tube sediment is approximately 1.5 ml, an additional culture medium should be inoculated with the remainder.

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