Comparison of BACTEC 13A Medium and Du Pont Isolator for Detection of Mycobacteremia

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BACTEC 13A medium (Johnston Laboratories, Towson, Md.) was compared with Isolator (Du Pont Co., Wilmington, Del.) for its tendency to be contaminated and for technical ease of isolation of mycobacteria from paired patient blood samples. Of 72 positive cultures, 63 were positive by both systems. Five positive cultures were detected by BACTEC 13A medium alone, and four were detected by Isolator alone. The results for the median numbers of days to positivity were 12 for BACTEC 13A medium and 14 for Isolator concentrate. BACTEC 13A medium has an advantage over the Isolator in requiring less laboratory manipulation of the specimens but has the disadvantages of not providing isolated colonies or quantitation of organisms. Some technical problems with contamination in both systems are also discussed.

Disseminated mycobacterial infection is a frequent occurrence in patients with the acquired immunodeficiency syndrome (AIDS) and can occasionally be found in patients without AIDS as well. The utility of the Isolator (Du Pont Co., Wilmington, Del.) for the detection of mycobacteremia has been previously documented (1, 3, 5, 7), and the inoculation of Isolator concentrate into BACTEC 12A medium (Johnston Laboratories, Inc., Towson, Md.) has been shown to be a particularly rapid method for the detection of mycobacteremia (1). Johnston Laboratories has recently developed BACTEC 13A medium specifically for the culture of blood for mycobacteria. In this study we evaluated the sensitivities, times to positivity, and relative merits of 13A medium and of Isolator concentrate planted on Middlebrook 7H11 plates. We also present data on the time to positivity of Isolator concentrate inoculated into BACTEC 12B bottles. We discuss a procedure for removing material from positive 13A bottles which we believe further enhances the safety of this system for laboratory personnel working with human immunodeficiency virus-positive blood samples, and we discuss the preventative strategy we currently use to guard against carry-over contamination with the BACTEC 460, with which we had a transient problem during the course of this study.

(A portion of these data was presented previously [Abstr. Annu. Meet. Am. Soc. Microbiol. 1987, U70, p. 137].)

MATERIALS AND METHODS

Patients. The patients from whom blood cultures were obtained were monitored at the George Washington University Medical Center, Washington, D.C.; the Clinical Center of the National Institutes of Health, Bethesda, Md.; and Fairfax Hospital, Fairfax, Va. The only criterion for inclusion of a patient in this study was receipt of an appropriate set of blood cultures. Positive cultures were obtained from 43 patients. Only one of these patients was known not to have AIDS. Of the total number of positive cultures, 42 were obtained while the patients were not receiving any antimycobacterial therapy, and 12 of the positive cultures were obtained while the patients were receiving antimycobacterial treatment. A variety of treatment regimens was used. The treatment histories of patients at the time the remaining 18 positive cultures were obtained were not known.

Cultures. For a blood specimen to be included in this study, a 13A bottle and an Isolator tube had to be obtained simultaneously. The culture planted from the Isolator was considered evaluable if at least one of the plates planted with Isolator concentrate remained free of contamination until the time it turned positive or for the full incubation period of at least 6 weeks if negative. The 13A culture was considered evaluable if it was initially positive with mycobacteria only or if it remained negative for the full incubation period. The study was divided into two parts. For part 1 of the study, the 13A bottle had to become positive before 10 December 1986, after which a carry-over contamination problem developed. Part 2 comprised the period after the contamination problem had been resolved.

13A. Unprocessed blood (5 ml) was inoculated directly into a 13A bottle. When the bottle was received in the laboratory, 0.5 ml of bovine serum albumin (Johnston Laboratories) was added to each bottle. The bottles were incubated at 36 ± 1°C and tested for presence of 14CO2 on a BACTEC 460 equipped with a TB hood. The amount of 14CO2 detected was represented as a Growth Index (GI) value. A gas mixture containing 10% CO2 and 90% air was used. Smears were prepared from a bottle on the day that the GI was ≥20 and stained for acid-fast bacilli with an auramine-rhodamine stain; subcultures of smear-positive bottles were performed to confirm positivity and to provide colonies for further work-up.

Isolator. A 7.5-ml Isolator was inoculated and processed in the laboratory according to the directions of the manufac-
turer for handling routine cultures. All tubes were centrifuged in a safety carrier. Concentrate (1 ml, representing 5 ml of blood) was divided equally onto three Middlebrook 7H11 plates (Remel, Lenexa, Kans.). The results from these three plates were the Isolator results for the culture. The remaining Isolator concentrate (approximately 0.4 ml) was inoculated into a 12B bottle to which neither bovine serum albumin nor antimicrobial agents were added. All planting of Isolator concentrate was performed in a biological safety cabinet. Thereafter, handling of the 12B and 13A bottles was identical. Later in the study, use of the 12B bottle was discontinued, and instead, an additional Middlebrook 7H11 plate was planted. The plates from those 12B bottles which did not have a 12B bottle were randomly numbered 1 through 4, and only plates 1 through 3 were included for purposes of this study as a part of the Isolator culture. The 7H11 plates were kept in 0101-gauge polyethylene bags except when being visually examined in a biological safety cabinet; a magnifying glass was used when necessary. Plates were incubated at 36 ± 1°C in air supplemented with 8% CO₂. The presence of mycobacteria on plates was confirmed with a Kinyoun stain.

All plates and bottles were examined during the first week (or fraction thereof) and during the following 2 full weeks on a thrice-weekly schedule, and they were examined during the final 4 weeks on a weekly schedule. All cultures, therefore, were examined for at least a full 6 weeks. For a given culture, all plates and bottles were examined on the same day. The date of positivity for bottles was the first day that a bottle with a GI ≥20 was smear positive. For plates, the date of positivity was the first day visible growth produced by acid-fast positive organisms was detected.

A flow chart summarizing the study methodology is shown in Fig. 1.

Colony counts were obtained for each plate at the end of the incubation period; for plates with more than 200 colonies, the colony count per plate could only be estimated. The colony count per milliliter of blood was determined for each culture with a positive Isolator plate. For Mycobacterium avium complex isolates only, an average colony count per milliliter was calculated for all the specimens which turned positive after a given number of days of incubation. These averages provided the datum points in Fig. 2 and 3.

**Organisms.** Organisms were identified by standard biochemical techniques (2), except for a few isolates obtained toward the end of the study. For these the Gen-Probe kit for identification of *M. avium* complex (Gen-Probe, San Diego, Calif.) from colonies, based on DNA-RNA hybridization, was used according to the directions of the manufacturer.

**RESULTS**

A total of 283 evaluable cultures were included in this study; there were 123 in part 1 and 160 in part 2. Of cultures

![Flow chart summarizing study methodology](image-url)

**FIG. 1.** Flow chart summarizing study methodology. *, Tested thrice weekly during weeks 1 through 3 and once weekly during weeks 4 through 7; ISO, Isolator; BSA, bovine serum albumin; AFB, acid-fast bacilli.
The data summarized in Table 2 indicate that the average and median times to detection of positivity for all systems were slightly longer in part 2 than in part 1. Overall average numbers of days to detection of positivity were 14.8, 16.4, and 11.2 days for the 13A, Isolator, and 12B systems, respectively; median numbers of days to detection of positivity were 12, 14, and 9 days, respectively. For cultures positive by the 13A or Isolator system alone, average times to detection of positivity were 30.4 and 25.8 days, respectively.

The distributions of number of cultures versus day to positivity for the 13A and Isolator systems, for all isolates, are shown in Fig. 4 and 5, respectively. The relationship between average colony count, calculated from Isolator plates as colonies per milliliter of blood, and day of positivity for the 13A and Isolator systems is shown in Fig. 2 and 3 for M. avium complex isolates only. A rough correlation can be seen between colony count and time to positivity.

Of the positive 13A bottles, only one had a negative smear on the first day the GI was ≥10. This bottle had a positive GI and a positive smear on the next run.

Each of the two systems detected one positive culture when the other system was inevaluable; the inevaluable Isolator was caused by mold overgrowth on all three plates, with the 13A bottle positive for M. kansasii. The inevaluable 13A bottle was caused by growth of both mold and M. avium complex in the 13A. The date of initial positivity for M. avium complex in this bottle could not be determined because of the presence of the mold. Of all the evaluable cultures positive by the Isolator system, one had only one evaluable plate, two had two evaluable plates, and all the others had all three plates evaluable.

**DISCUSSION**

The results of this study indicate that the use of the 13A system for mycobacterial culture allows detection of mycobacteremia with the same sensitivity as that provided by Isolator and may provide detection of mycobacteremia a day or two earlier than Isolator.

An advantage of the 13A system is that it involves less manipulation of specimens in the laboratory. Thus, there is
both some saving of time for the technologist and somewhat less exposure to potentially hazardous specimens. Disadvantages of the 13A system are that isolated colonies are not available and quantitation of organism load is not possible, although there is a rough correlation between organism concentration (as determined by Isolator) and time to positivity. Also, although the beta-particle-emitting 14C (5 μCi per bottle) in the 13A bottle is essentially innocuous, laboratories must comply with institutional regulations for handling such material. Isolator concentrate injected into 12B bottles appears to provide the most rapid detection of mycobacteremia; this is consistent with a previous report documenting rapid isolation of mycobacteria from blood when Isolator concentrate is injected into 12A bottles, which contained half the volume of medium in the currently available 12B (1). However, to achieve sensitivity with 12B bottles equal to that of Isolator plates or 13A bottles would probably require at least two, perhaps three, 12Bs. We think that the cost, and the specimen manipulation risks involved, do not warrant routine use of the 12B bottle now, especially in the absence of any data on the clinical significance of a delay of a few days in the diagnosis of mycobacteremia. Some might even question the utility of diagnosing mycobacteremia in patients with AIDS, given the lack of any clearly effective treatment regimen for M. avium complex organisms, the most frequent blood isolate found in patients with AIDS in the United States (6, 9). However, we believe that the attempt to diagnose mycobacteremia in patients with AIDS be warranted, as (i) the diagnosis documents one cause for their slow decline in well-being, (ii) it is sometimes elected to treat bacteremia caused by M. avium complex, and (iii) some mycobacteremias in patients with AIDS are caused by potentially more treatable organisms, such as Mycobacterium tuberculosis and M. kansasii.

We did not obtain enough specimens from patients being treated for their mycobacterial infection to assess the influence of treatment on length of time to detection of mycobacteremia. We also cannot explain the slightly prolonged times for detection of mycobacteremia in part 2 of the study compared with those in part 1. We investigated the possible influence of lower average colony counts in part 2 of the study, but we did not find a lower average colony count in the cultures from part 2 compared with the cultures from part 1. Since the completion of this study, the atmosphere placed in the 13A bottles at the time of manufacture has been changed to one that may be more favorable for mycobacterial growth for the short period between inoculation and the initial run on the BACTEC 460.

Since this study was completed, we have found a new procedure useful for handling 13A bottles with positive GI values. To remove material from the bottle, the septum is wiped with 70% isopropyl alcohol and allowed to dry, a BCB Vent/Sub Unit subculture needle (Difco Laboratories, Detroit, Mich.) is inserted, and material is carefully poured from the bottle onto a slide for staining, into tubes, or onto plates for subculturing or other studies. Some practice with this procedure allows removal of material from the bottle without having any of the contents trickle down the outside of the needle and permits handling of material from the bottle by a needle and syringe.

Studies are currently under way to examine the feasibility of identifying organisms directly from the 13A bottle by using the Gen-Probe DNA-RNA hybridization technique; preliminary studies have been encouraging. However, we expect that in the future, it will be necessary to subculture a positive 13A bottle to solid media to ensure that only one species of Mycobacterium is present. Although at least one study has demonstrated that two mycobacterial species with apparently similar colonial morphologies may be present simultaneously in a patient with AIDS and mycobacteremia (4), we think that it is not feasible now to pursue culture purity beyond the determination of colonial morphology consistent with the presence of a single species of Mycobacterium.

Contamination problems developed during the course of this evaluation in both the Isolator and 13A systems. In 36 of the 283 evaluable cultures, Isolator plates became contaminated with mold, 20 with mold on one of the three plates and 16 with mold on two plates. The most frequently identified mold was Aspergillus terreus, which had contaminated our laminar flow hoods and which required numerous episodes of cleaning to eradicate.

A more serious contamination problem occurred with the 13A bottles. Early in the course of the study there was an apparent episode of transfer of Aspergillus fumigatus among a few bottles. Later, transfer of both mycobacteria and A. fumigatus among bottles was noted, and at this point the study was temporarily discontinued until the problem was resolved. The later problem was suspected when some 13A bottles turned positive late in the course of their incubation, with high GIs developing relatively quickly, while the corresponding Isolator plates remained negative. Mycobacterial disease in some of these patients seemed unlikely on clinical grounds. In the attempt to resolve this problem, the board controlling the needle heater was found to be malfunction-
ing, although at no time did the warning light on the instrument indicate any problem with the heater. The board was replaced and then further adjusted so that both temperature and time of heating were somewhat increased. In addition, it was found that the media trap on our instrument had been on backwards for an unknown period of time. The media trap and the needle heater board were adjusted at the same time. The study was resumed (part 2), and thereafter, during the remainder of the study, no organism transfer problems appeared. Since this problem occurred, we continue to change the needles daily, and in addition, we make sure that known positive specimens (e.g., for BACTEC NAP testing or for other special studies) are run after the bottles containing patient specimens are run. Users of the BACTEC system must of course not automatically assume that a culture positive only in BACTEC medium and negative on corresponding solid media represents contamination. Such cultures may well be genuine positives detected by BACTEC medium only.

With the caveats noted above, the 13A system appears to be an excellent alternative to the Isolator system for culture of mycobacteria from blood. Because we feel that the 13A system offers sufficient advantages, we are now using it as our only mycobacterial blood-culturing system.

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LITERATURE CITED