Comparison of Three Methods for Recovery of *Mycobacterium avium* Complex from Blood Specimens

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Three methods were used for the recovery of mycobacteria from blood specimens obtained from acquired immunodeficiency syndrome patients: (i) inoculation of 7H11 agar plates with a concentrated specimen, (ii) inoculation of 7H12 BACTEC vials with a concentrated specimen, and (iii) inoculation of 7H13 BACTEC vials with a nonconcentrated specimen. In this study, we examined 255 specimens and obtained positive mycobacterial growth in 47 of them. Among these 47 cultures, 40 were found to be positive by all three methods, and the total recovery rates in relation to these culture-positive specimens were 94% for method 1, 89% for method 2, and 96% for method 3. The advantages and disadvantages of these three methods are discussed.

The rare isolation of different mycobacterial species from blood was well known in the past, but only with the development of the acquired immunodeficiency syndrome epidemic during the last few years has the isolation of mycobacteria from blood become a problem faced by clinical laboratories (3, 4, 8). The use of an isolator (Du Pont Co., Wilmington, Del.) for lysing the blood and concentrating the released mycobacteria, which was followed by the inoculation of the pellet onto 7H10 or 7H11 agar medium, yielded better recovery rates than direct inoculation with nonprocessed blood onto some conventional blood culture media (9) or into 7H12 BACTEC vials (Johnston Laboratories, Inc., Towson, Md.) (1, 6, 7). The difference was probably due to the fact that the inocula from nonprocessed specimens contained lower numbers of bacteria per unit of medium than a mycobacterial concentrate from lysed blood. The inoculation of the 7H12 vials with a blood pellet obtained by centrifugation without lysing provided the same isolation rate as the inoculation of the same vials with blood specimens processed in the Du Pont isolator (2). The recovery rates were also comparable when the 7H12 vials were inoculated with specimens processed in either a Ficol-Hypaque gradient or the Du Pont isolator (10). Routine procedures for the isolation of mycobacteria in our laboratory are based on obtaining the blood specimens in sodium polyanthelosulfonate-containing VACUTAINERs. The mycobacterial content is then concentrated by lysis of the blood with sodium deoxycholate and subsequent centrifugation before inoculation into 7H11 agar plates and 7H12 BACTEC vials (5). Johnston Laboratories recently developed a new 7H13 BACTEC medium; therefore, we compared the use of this medium with our two conventional techniques for the isolation of mycobacteria from blood (5).

**Blood specimens.** During the period from August 1986 to June 1987, a total of 255 blood specimens were obtained from 156 acquired immunodeficiency syndrome patients hospitalized at Park Plaza Hospital (Houston, Tex.). Each specimen was collected in two yellow-top VACUTAINER tubes (catalog no. 4960; Becton Dickinson Vacutainer Systems, Rutherford, N.J.) containing sodium polyanthelosulfonate as an anticoagulant. These specimens were shipped, in appropriate containers, by overnight mail to Denver, Colo.

**Media.** Three types of medium were used in this study: 7H11 agar plates; 7H12 BACTEC broth in standard vials, 4.0 ml in each (Johnston Laboratories), supplemented with PANTA drug combination (polymyxin B, amphotericin B, nalidixic acid, trimethoprim, azlocillin) to prevent contamination; and new 7H13 broth vials provided for this study by Johnston Laboratories. Each 7H13 vial contained 30.0 ml of medium, as well as sodium polyanthelosulfonate to prevent coagulation of blood, and was supplemented with bovine serum albumin, as recommended by the manufacturer.

**Procedure.** Blood (5.0 ml) from one of the two collection tubes was mixed with 30.0 ml of the 0.3% sodium deoxycholate solution (Sigma Chemical Co., St. Louis, Mo.) and incubated for 10 min at room temperature. After centrifugation for 25 min at 3,500 × g in a refrigerated centrifuge, the pellet was suspended in 0.2% bovine albumin (fraction V; Sigma) solution to make an exact volume of 4.0 ml. This step was necessary for the quantitation of CFU per milliliter of blood. A suspension made from the pellet was inoculated onto one 7H11 agar plate and into one 7H12 broth vial, 0.5 ml per medium. Blood (also 5.0 ml) from the second VACUTAINER was directly inoculated into one 7H13 broth vial. All cultures were incubated at 37°C. The growth on agar plates was checked after 7, 10, 14, 21, and 42 days of incubation, and the colonies were counted to determine the number of CFU per milliliter of blood. The radiometric growth index of the 7H12 and 7H13 vials was recorded in the BACTEC instrument (Johnston Laboratories) twice a week. A growth index of >20 was considered to indicate positive growth, and smears were made to confirm the presence of acid-fast bacilli in the culture. The isolated cultures were identified by standard tests.

**Results.** We obtained 255 specimens from 156 patients. Mycobacterial growth was recovered in 47 specimens, representing 24 patients. From 2 patients, we isolated six cultures; from 1, we isolated four cultures; and from the remaining 21, we isolated one or two cultures each. All 47 positive cultures yielded *Mycobacterium avium* complex. From one of these specimens we isolated a mixture of *M. avium* complex and *M. kansasii*. A previous specimen from the same patient contained *M. avium* complex only.

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Of the 47 culture-positive specimens, 40 cultures were positive by all three methods. The results of the isolation of these and the remaining seven cultures by different methods are shown in Table 1. The total recovery rates in regard to the number of culture-positive specimens were 94% for 7H11 agar plates and 89 and 96% for 7H12 and 7H13 vials, respectively.

The recovery times were 7 to 21 days (average, 9.6) for 7H11 agar plates, 3 to 12 days (average, 6.6) for 7H12 vials, and 5 to 40 days (average, 11.5) for 7H13 vials. The number of CFU per milliliter was determined for 41 culture-positive specimens, and 37 of these cultures were also positive in both types of liquid medium. It appears that the recovery time in liquid media, especially in 7H13 broth, was shorter for specimens with a higher number of CFU per milliliter of blood (Table 2).

No significant differences were found in recovery rates by the three methods used in this study when, for the sake of comparison, equal volumes of blood (5.0 ml) were used for direct inoculation into 7H13 broth and for lysis and concentration for the two remaining types of medium. The 5.0-ml volume is the maximum amount of blood that can be injected into a 7H13 vial, while larger volumes (about 8.5 ml from one VACUTAINER) can be used for concentration, which may increase the recovery rate. The shortest recovery time (6.6 days) found was for 7H12 vials, which was followed by the times for 7H11 plates (9.6 days) and 7H13 vials (11.5 days).

The use of 7H13 broth vials may provide an advantage in situations in which it is desirable to avoid any manipulations with blood in the laboratory: a 5.0-ml blood sample can be injected into a 7H13 vial with a syringe at the bedside. The other two methods may be preferred in a situation in which manipulations with blood (lysing and centrifugation) in the laboratory do not represent any problems, and the blood samples can be safely collected in the yellow-top VACUTAINER and mailed to the laboratory. The use of the 7H11 (or 7H10) agar plate in conjunction with either of the liquid media, when it is possible to process the blood sample, makes it possible to examine the colonial morphology (as a first step in identification), to detect mixed mycobacterial cultures, and to quantitate the live bacteria in blood when it is necessary.

| Table 1. Recovery of M. avium complex growth from 47 culture-positive blood specimens |
| No. of specimens | No. of positive cultures |
|                  | 7H11 agar | 7H12 broth | 7H13 broth |
| 40              | 40        | 40         | 40         |
| 1               | Contaminated | 1         | 1         |
| 2               | 2         | Neg.       | 2         |
| 1               | 1         | 1          | Neg.       |
| 2               | Neg.      | Neg.       | 2         |
| 1               | 1         | Neg.       | Neg.       |

*a Neg., Negative.

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**LITERATURE CITED**


