

Evaluation of the ESP Culture System II for Recovery of Mycobacteria from Blood Specimens Collected in Isolator Tubes

CINDY A. THOLCKEN, SOPHIA HUANG, AND GAIL L. WOODS*

Department of Pathology, University of Texas Medical Branch, Galveston, Texas 77555-0740

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The reliability of the ESP Culture System II (ESP II; AccuMed International, Westlake, Ohio), a continuously monitoring, nonradiometric mycobacterial culture system, for recovery of mycobacteria from sediments of blood collected in an Isolator tube was evaluated by comparing its performance to inoculation of the sediment onto Middlebrook 7H11/7H11 selective biplates. Of 1,704 blood specimens, 73 (4.3%) were positive for mycobacteria (68 *Mycobacterium avium* complex and 5 *M. tuberculosis*). Fifty-three specimens were positive by both methods; 13 were positive by ESP II only, and 7 were positive by Middlebrook agar only ($\chi^2 = 1.8$; $P > 0.05$). The mean times to positivity were 15.6 days for ESP II and 19.0 days for Middlebrook agar ($P < 0.01$). The time to detection was the same for 13 specimens; ESP II was positive first for 33, and agar plates were positive first for 7. ESP II allowed recovery of more mycobacteria (90.4% of all isolates versus 82.2% for Middlebrook agar) from sediments of blood specimens collected in Isolator tubes, and it provided significantly faster detection than did Middlebrook plates.

Disseminated mycobacterial infection, particularly disease caused by the *Mycobacterium avium* complex (MAC), is a common occurrence in patients with AIDS, especially those whose CD4⁺ lymphocyte count is less than 100/ μ l (4). For optimal diagnosis of disseminated MAC in symptomatic patients with AIDS, culture of peripheral blood for mycobacteria is recommended (4, 7). Treatment of disseminated MAC disease in patients with AIDS decreases morbidity and may prolong life; therefore, rapid and reliable detection of MAC bacteremia is important (4, 7). Several methods for detection of bacteremia with MAC or other mycobacteria are currently available. A 5-ml volume of blood may be inoculated directly into a vial of BACTEC 13A medium (Becton Dickinson, Sparks, Md.) (1, 5, 11). Alternatively, a 6- to 10-ml volume is collected in a tube containing the anticoagulant sodium polyanethanesulfonate or in an Isolator tube (Wampole Laboratories, Cranbury, N.J.), which contains lytic agents and anticoagulants. If the Isolator system is used, blood cells are lysed by the agents present in the tube, whereas if blood is collected in a sodium polyanethanesulfonate tube, blood cells must be lysed in a separate step by adding a lysing agent such as a sodium deoxycholate solution. With either tube system, the sample is centrifuged and a portion of the sediment is inoculated onto a solid medium, i.e., Middlebrook 7H10 or 7H11 agar or a Lowenstein-Jensen slant, or into a BACTEC 12B vial or an MGIT tube (Mycobacteria Growth Indicator Tube; Becton Dickinson) (1–3, 5, 9–11).

The ESP Culture System II (ESP II; AccuMed International, Westlake, Ohio), a fully automated, continuously monitoring system for growth and detection of microorganisms, including mycobacteria, recently became available for isolation of mycobacteria from clinical specimens (12). ESP II is an adaptation of the ESP blood culture system that has been available for use in the clinical laboratory for about 4 years. The technology is based on detection of pressure changes, i.e., either gas production or gas consumption resulting from microbial growth, occurring within the headspace above a broth

culture medium in a sealed bottle. The purpose of this study was to evaluate the reliability of ESP II for recovery of mycobacteria from blood.

A total of 1,704 blood specimens collected for mycobacterial culture, predominantly from patients with AIDS, and submitted to the clinical laboratory at the University of Texas Medical Branch during a 28-week period (August through September 1996 and mid-November 1996 through early February 1997) were included in the study. For all specimens, 6- to 10-ml samples of peripheral blood were collected in 10-ml Isolator tubes and transported to the microbiology laboratory, where they were processed in batches of two or more tubes several times during all shifts. Tubes were centrifuged at $1,800 \times g$ for 30 min, the supernatant was removed and discarded, and the sediment was used to inoculate media as follows: 0.5 ml was inoculated onto two Middlebrook 7H11/7H11 selective biplates (approximately 0.25 ml per plate), and 0.5 ml was inoculated into an ESP II vial, which contains 12.5 ml of broth medium. To the inoculated ESP II vial, 1.0 ml of growth supplement (Middlebrook oleic acid-albumin-dextrose) and 0.5 ml of a mixture of the antimicrobial agents polymyxin B, vancomycin, nalidixic acid, and amphotericin B were added. Vials were incubated at 35°C in the ESP II instrument for 6 weeks or until flagged as positive. For positive vials, a smear of the broth was stained with the Kinyoun stain and the broth was subcultured to a Middlebrook biplate. Middlebrook biplates were taped, incubated at 37°C in an atmosphere of 7% CO₂ for up to 6 weeks, and examined twice weekly for mycobacterial colonies (confirmed by staining a smear with the Kinyoun stain). Identification was performed by testing colonies on a solid medium with Accu-Probes (Gen-Probe, Inc., San Diego, Calif.).

The rates of recovery of mycobacteria by ESP II and Middlebrook agar were compared by using McNemar's chi square test. The paired *t* test was used to compare times to detection of mycobacterial growth by the two methods for those specimens that were positive by both ESP II and Middlebrook agar.

Of the 1,704 specimens, 73 (4.3%) were positive for mycobacteria: i.e., 68 MAC and 5 *M. tuberculosis* isolates. Fifty-

* Corresponding author. Phone: (409) 772-4851. Fax: (409) 772-5683.

three specimens were positive by both culture methods; 13 (12 MAC and 1 *M. tuberculosis*) were positive by ESP II only, and 7 (6 MAC and 1 *M. tuberculosis*) were positive by Middlebrook agar only (chi square = 1.8; $P > 0.05$). The mean time to detection for ESP II (i.e., the mean time to signal a positive from the instrument) was 15.6 days (range, 4 to 40 days; median, 14 days), compared with 19.0 days (range, 6 to 34 days; median, 19 days) for Middlebrook agar (i.e., visible colonies of acid-fast bacilli). The time to detection was the same for 13 specimens. ESP II was positive first for 33 specimens, and for these 33, the mean difference in time to positivity was 7.8 days (range, 1 to 17 days; median, 6 days). Middlebrook agar was positive first for seven specimens; for these seven, the mean difference in time to positivity was 4.0 days (range, 1 to 7 days; median, 4 days). For the 53 specimens that were positive by both methods, the mean (\pm the standard deviation) times to detection were 14.7 ± 7.68 days for ESP II and 18.8 ± 7.72 days for Middlebrook agar ($P < 0.01$). For the specimens that were positive by only one method, the mean times to mycobacterial detection were 19.1 days (range, 11 to 37 days) for ESP II and 20.9 days (range, 16 to 30 days) for Middlebrook agar.

Our data showed that the ESP II system recovered more mycobacteria (predominantly MAC) from sediments of blood collected in an Isolator tube (90.4% of all isolates for ESP II versus 82.2% for Middlebrook agar), although the difference was not statistically significant. The exact reason why one system failed to recover mycobacteria in cases when a single method was positive is not known. Based on the fact that the mean times to detection of mycobacterial growth by either system were longer when only one system was positive than when both were positive, it is possible that the failure of one system to recover the organism resulted from sampling error due to fewer mycobacteria in the initial inoculum. The similar rates of mycobacterial recovery by the two culture systems when equal volumes of Isolator sediment were inoculated also suggest that the inhibitory problems associated with the use of Isolator sediment in conjunction with the BACTEC 12B medium do not exist with the ESP II system. However, data from studies in which the issue of compatibility has been systematically investigated have not been reported.

With regard to time to detection of mycobacterial growth, ESP II provided results significantly more rapidly than did Middlebrook agar. The decreased time to detection with ESP II also has the potential for faster identification. Since our evaluation, data from two studies—one evaluating the use of AccuProbes and the other evaluating the use of latex agglutination (Myc AKT; Remel, Lenexa, Kans.)—have shown that mycobacteria can be identified by testing sediment of broth from ESP II bottles on the day when they are determined to be positive or within 3 days thereafter (6, 8).

In summary, ESP II is a reliable and rapid system for recovery of mycobacteria from blood specimens collected in an

Isolator tube. When the system is used as described here, our data suggest that inoculation of an ESP II vial only, without addition of a solid medium, is adequate. Moreover, ESP II has the advantages that it is fully automated and, thus, less labor intensive than manual examination of plates for colonies or loading and unloading of BACTEC 12B or 13A vials, it is nonradiometric, and it has a data management system that considerably simplifies tracking of results. With ESP II, however, blood cannot be inoculated directly into a culture vial, as is possible with BACTEC 13A medium. ESP II also does not allow quantitation of bacteremia, as is possible with lysis-centrifugation plus a solid medium. In general, this is not a problem because quantitative cultures are not recommended for routine clinical practice, although they are helpful in monitoring the microbiological response to antimicrobial therapy in research trials.

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