Controlled Comparison of BACTEC 13A, MYCO/F LYTIC, BacT/ALERT MB, and ISOLATOR 10 Systems for Detection of Mycobacteremia

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To compare the performance of the BACTEC 13A (Becton Dickinson, Sparks, Md.), BACTEC MYCO/F LYTIC (Becton Dickinson), BacT/ALERT MB (bioMérieux, Durham, N.C.), and ISOLATOR 10 lysis-centrifugation (Wampole Laboratories, Cranbury, N.J.) systems for detection of mycobacteremia in adults, we inoculated 5-ml aliquots of blood from patients with suspected mycobacteremia into the bottle or tube required for each system. Of 600 sets tested, 85 (14%) yielded Mycobacterium avium complex (MAC) and 9 (2%) yielded other species of mycobacteria. Of 26 complete (three bottles and one tube) adequately filled (5 ± 1 ml) sets from which MAC was recovered, BACTEC 13A was positive for 19 (73%), BACTEC MYCO/F LYTIC was positive for 21 (81%), BacT/ALERT MB was positive for 22 (85%), and ISOLATOR 10 was positive for 21 (81%). Of the six possible two-way comparisons, the mean times to detection for the recovery of MAC from each bottle in positive adequately paired sets were 15.3 days for BACTEC 13A versus 12.8 days for MYCO/F LYTIC for 33 of 340 pairs, 14.1 days for BACTEC 13A versus 11.6 days for BacT/ALERT MB for 38 of 380 pairs, 12.6 days for BACTEC 13A versus 20.0 days for ISOLATOR 10 for 26 of 261 pairs, 12.8 days for BACTEC MYCO/F LYTIC versus 11.0 days for BacT/ALERT MB for 33 of 340 pairs, 13.2 days for BACTEC MYCO/F LYTIC versus 11.0 days for ISOLATOR 10 for 24 of 230 pairs, and 9.9 days for BacT/ALERT MB versus 19.0 days for ISOLATOR 10 for 24 of 257 pairs. There were no significant differences in yields between the systems. However, the mean time to detection differed significantly among the systems. The time to detection was shortest for BacT/ALERT MB, followed by BACTEC MYCO/F LYTIC and BACTEC 13A and then ISOLATOR 10. Although the numbers were too small for statistical comparison, the time to detection was substantially shorter for MAC than for Mycobacterium tuberculosis complex in the liquid systems. The continuously monitored systems (BACTEC MYCO/F LYTIC and BacT/ALERT MB) were as sensitive and, on balance, faster for the detection of MAC bacteremia than were the heretofore standard manual ISOLATOR 10 and radiometric BACTEC 13A systems.

Disseminated Mycobacterium avium complex (MAC) infection is a common opportunistic infection in patients with advanced human immunodeficiency virus (HIV) disease that is associated with a reduced probability of survival (7). Treatment with appropriate antimicrobial therapy can significantly improve patient survival (4). Although advances in HIV management such as prophylactic antimicrobial therapy (e.g., with clarithromycin) (12) and highly active antiretroviral therapy (3) have led to reduced incidences of disseminated MAC infection among patients with access to care, timely and accurate diagnosis of the disease remains an important function of the clinical microbiology laboratory. The clinical manifestations of disseminated MAC infection are nonspecific, so clinicians rely on laboratory confirmation to secure the diagnosis. Mycobacterial blood culture is the test of first choice for the diagnosis of disseminated MAC disease (15).

Mycobacterial blood culture methods in common use include visual inspection of processed blood inoculated on a solid medium (e.g., the ISOLATOR 10 system), intermittent radiometric detection in liquid medium inoculated with blood (e.g., the BACTEC 13A system), and now, continuous nonradiometric detection in liquid medium inoculated with blood (e.g., the BACTEC MYCO/F LYTIC or BacT/ALERT MB system). We conducted a multicenter controlled study to compare the performances of the BACTEC 13A, BACTEC MYCO/F LYTIC, BacT/ALERT MB, and ISOLATOR 10 systems for the detection of mycobacteremia in adults.

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MATERIALS AND METHODS

Blood culture and collection. Samples for blood cultures were collected from patients hospitalized or seen as outpatients at the Duke University Medical Center and the Carolinas Medical Center from November 1999 through March 2002. All patients were suspected of having disseminated mycobacterial infection, determined by consultation with the infectious diseases and/or medical microbiology service. Blood cultures were performed as part of routine patient care. Venipuncture sites were disinfected with alcohol and then povidone iodine and were allowed to dry. Twenty milliliters of blood was obtained with a sterile
Candida glabrata
Candida albicans
Candida tropicalis
Histoplasma capsulatum
Cryptococcus neoformans

BACTEC 13A (Becton Dickinson, Sparks, Md.), BACTEC MYCO/F LYTIC needle and syringe, and 5 ml was distributed into a blood culture set containing 1988 CRUMP ET AL. J. CLIN. MICROBIOL.

BOTTLE 2

Mycobacterium avium complex ........................................ 85
Mycobacterium tuberculosis ...........................................  5
Mycobacterium chelonae ..............................................  3
Mycobacterium kansasii ..............................................  9
Cryptococcus neoformans .............................................  5
Histoplasma capsulatum ...............................................  5
Candida albicans ........................................................  3
Candida tropicalis ......................................................  1
Candida glabrata ........................................................  1
Total ........................................................................ 113

TABLE 1. Clinically important mycobacteria and fungi recovered from all study blood culture sets

Microorganism No. of sets in which organism was detected

BACTEC 13A bottles were placed in a 35°C incubator and radiometrically monitored two times weekly for the first 2 weeks and then weekly for the remaining 4 weeks by using the BACTEC 460 instrument. The sediment from ISOLATOR 10 tubes was plated onto two Middlebrook 7H10 solid-medium plates after centrifugation, and the plates were placed in a 35°C carbon dioxide incubator and visually inspected weekly. Bottles (BACTEC MYCO/F LYTIC, BacT/ALERT MB, BACTEC 13A) flagged by the instrument as positive were removed, and an aliquot of the blood-broth mixture was removed from the bottle with a sterile needle and tuberculin syringe. A portion was used for auramine-rhodamine staining, and the remainder was subcultured onto solid-medium plates according to the results of the staining procedures. The plates were examined weekly, and growth on primary Middlebrook 7H10 medium (with sediment from ISOLATOR 10 tubes) was sampled and prepared for Kinyoun staining. Subsequent microbial isolation, identification, and antimicrobial susceptibility testing were performed by standard techniques (11). All systems were studied for a 42-day incubation period. Negative companion bottles from positive sets were subcultured at the end of the 42-day protocol. Identification of growth was performed by standard techniques (11).

Data analysis. Comparison of recovery rates was analyzed by the McNemar chi-square test with Yates’ correction for small numbers when necessary (10). Chi-square test with Yates’s correction for means was used to compare the means of the time to detection for each system for the different isolates. Although the numbers are small, the mean time to detection was substantially shorter for MAC (14.0 days) than for the M. tuberculosis complex (23.8 days) in the liquid systems. Of the five sets yielding M. tuberculosis complex, M. tuberculosis was recovered from 4 (80%) BACTEC 13A bottles, BACTEC MYCO/F LYTIC bottles, 3 (60%) BacT/ALERT MB bottles, and 4 (80%) ISOLATOR 10 tubes. Of the adequately filled individual bottles or tubes, the mean time to detection for each system was 28.0 days (range, 16 to 40 days) for the BACTEC 13A system, 26.3 days (range, 18 to 42 days) for the BACTEC MYCO/F LYTIC system, 25.0 days (range, 24 to 26 days) for the BacT/ALERT MB system, and 22.8 days (range, 19 to 31 days) for the ISOLATOR 10 system.

DISCUSSION

In this controlled comparison of the BACTEC 13A, BACTEC MYCO/F LYTIC, BacT/ALERT MB, and ISOLATOR 10 systems for the detection of mycobacteremia, the continuously monitored systems (BACTEC MYCO/F LYTIC, BACTEC 13A, and ISOLATOR 10), and the radiometrically monitored BacT/ALERT MB system, were superior for the prompt detection of mycobacterial growth. The BacT/ALERT MB system was positive for 27 (85%) of 32 sets, BACTEC MYCO/F LYTIC system was positive for 21 (65%) of 32 sets, and ISOLATOR 10 system was positive for 15 (47%) of 32 sets. The time to detection was the shortest for the BacT/ALERT MB system, followed by the BACTEC MYCO/F LYTIC system, BACTEC 13A system, and the ISOLATOR 10 system.

The results of this study also indicate that the radiometrically monitored BacT/ALERT MB bottles were suitable for the detection of the dimorphic fungi and yeasts, but the sample size for this study was too small to permit comparisons of sensitivity or mean times to detection for these isolates.

Table 2 shows the yields of MAC for all six two-way comparisons of adequately filled pairs of bottles. There were no significant differences between any two bottles compared. Of the six possible two-way comparisons of times to detection for adequate pairs of positive blood cultures, the mean time to detection was the shortest for the BacT/ALERT MB system, followed by the BACTEC MYCO/F LYTIC and BACTEC 13A systems and then the ISOLATOR 10 system (Table 3). There were no significant differences in the times to detection for the BacT/ALERT MB system between bottles collected at the Duke University Medical Center and those collected at the Carolinas Medical Center.

DISCUSSION

In this controlled comparison of the BACTEC 13A, BACTEC MYCO/F LYTIC, BacT/ALERT MB, and ISOLATOR 10 systems for the detection of mycobacteremia, the continuously monitored systems (BACTEC MYCO/F LYTIC,
BacT/ALERT MB) were as sensitive and, on balance, faster for the detection of MAC bacteremia than the heretofore standard manual ISOLATOR 10 system (6) and radiometric BACTEC 13A system (14). Although we are not aware of previous studies that simultaneously compared the BACTEC 13A, BACTEC MYCO/F LYTIC, BacT/ALERT MB, and ISOLATOR 10 systems, our results are consistent with those of comparisons of the BACTEC MYCO/F LYTIC system with a second commercial system for the detection of mycobacteria (5, 16). However, previous studies indicate that the equivalent sensitivities of the BacT/ALERT MB system and the BACTEC 460 system and the shorter recovery times of the BacT/ALERT MB system compared with that of the BACTEC 460 system for MAC from blood cultures does not imply superior performance for the recovery of the *M. tuberculosis* complex from respiratory specimens (13).

The reasons for the faster detection times for the continuously monitored systems (BACTEC MYCO/F LYTIC, BacT/ALERT MB) could include their frequency of examination compared with the twice-weekly examination of the radiometric BACTEC 13A broth system and the weekly examination of plates inoculated with sediment from the ISOLATOR 10 system. More frequent examination of the manual systems may have led to the faster detection of growth. However, the differences observed were greater than those that could be accounted for by weekly examination. Moreover, it was our intention to assess the systems as they would be applied in routine use in the clinical microbiology laboratory, and as such, our results reflect a practical difference in time to detection of growth.

To standardize volumes, we inoculated 5 ml of blood into each system. The ISOLATOR 10 system is designed for 10 ml of blood. It is possible that the increased concentrations of lytic agent and anticoagulant in the ISOLATOR 10 system relative to the lower volume of blood obtained may have inhibited mycobacterial growth (18). However, ISOLATOR 10 tubes were processed within 8 h of collection. Furthermore, to avoid the inhibition noted with inoculation into BACTEC 12B broth, sediment from the ISOLATOR 10 tubes was plated onto solid medium (17). It should be noted that despite the longer detection times demonstrated in our study, the ISOLATOR 10 system does have the advantage of providing isolated colonies for the purposes of mycobacterial identification and antimicrobial susceptibility testing. Agitation of the continuously monitored systems may also contribute to the shorter time to positivity for the BACTEC MYCO/F LYTIC and BacT/ALERT MB systems (8).

Mycobacteria other than MAC accounted for a large proportion (10%) of the sources of mycobacterial bloodstream infections in this study. However, *M. tuberculosis* complex isolates predominated among the non-MAC isolates as sources of bloodstream infections. The substantially longer mean time to detection for *M. tuberculosis* complex than for MAC in the liquid systems is of concern because mycobacterial bloodstream infection carries a high mortality rate (1, 2) and is an important HIV-associated opportunistic infection in sub-Saharan Africa and Southeast Asia (9). Further studies in areas of endemicity will help to define the roles of these systems for the detection of other mycobacterial species.

In conclusion, clinical microbiology laboratories may shorten the times for the recovery of MAC from blood cultures without compromising the yield by changing from the more manual methods with the ISOLATOR 10 and BACTEC 13A systems to one of the continuously monitored systems studied, namely, the MYCO/F LYTIC or BacT/ALERT MB system.

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REFERENCES