Evaluation of BACTEC MYCO/F Lytic Medium for Recovery of Mycobacteria and Fungi from Blood

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The reliability of MYCO/F Lytic medium in the BACTEC 9240 blood culture system was evaluated by comparing its performance to that of the Isolator system for the recovery of fungi and to that of the ESP II system for the recovery of mycobacteria. Of 717 specimens of blood cultured for fungi, 24 were positive; 12 samples were positive with both systems, 7 samples were positive with the Isolator system only, and 5 samples were positive with MYCO/F Lytic medium only. Fourteen samples grew Histoplasma capsulatum; both systems detected H. capsulatum in seven samples but the Isolator system alone detected H. capsulatum in seven samples. The mean times to the detection of H. capsulatum were 8 days (range, 4 to 13 days) for MYCO/F Lytic medium and 9 days (range, 6 to 18 days) for the Isolator system; the mean times to identification were 20 days (range, 15 to 24 days) for isolates recovered with MYCO/F Lytic medium and 11 days (range, 6 to 18 days) for those recovered with the Isolator system (P < 0.05). Cryptococcus neoformans was isolated from 10 fungal cultures; five isolates grew in both systems, and five isolates grew in MYCO/F Lytic medium only. The mean times to detection of C. neoformans were 4 days (range, 2 to 6 days) for MYCO/F Lytic medium and 7 days (range, 5 to 7 days) for the Isolator system (P < 0.05); the mean times to identification were 15 days (range, 7 to 27 days) for isolates recovered with MYCO/F Lytic medium and 8 days (range, 7 to 11 days) for those recovered with the Isolator system. Of the 687 samples of blood cultured for mycobacteria, 64 blood samples from 42 patients grew mycobacteria (58 grew Mycobacterium avium complex, 4 grew Mycobacterium kansasii, and 2 grew Mycobacterium tuberculosis); 42 isolates were recovered with both systems, 18 were isolated with MYCO/F medium only, and 4 were isolated with the ESP II system only alone (P < 0.05). The mean time to detection of mycobacteria with MYCO/F Lytic medium was 14 days, whereas it was 17 days with the ESP II system (P < 0.05). In summary, the combination of MYCO/F Lytic medium and the BACTEC 9240 instrument is an excellent blood culture system for the growth and detection of mycobacteria. A valid assessment of MYCO/F Lytic medium with regard to fungal isolation, however, was not possible due to the small number of isolates recovered.

The detection of fungemia and mycobacteremia has become increasingly important during the past several years. Various manual, semiautomated, and fully automated culture systems may be used to recover these organisms from blood. For optimal efficiency, a fully automated system and media that are directly inoculated with blood are most desirable. A few such systems are available for the detection of fungemia, however, the Isolator system (Wampole Laboratories, Cranbury, N.J.) performs well with regard to the recovery of yeasts (2–5, 7, 8). However, the Isolator system is also detected. The MYCO/F Lytic bottle is placed into the BACTEC 9000 series of instruments for the growth of fungi and mycobacteria, although the growth of other bacteria is also detected. The MYCO/F Lytic bottle is placed into the BACTEC 9000 blood culture system instrument and is continuously incubated at 35°C with agitation for maximum recovery. Every 10 min the BACTEC 9000 instrument monitors the sensor for increasing fluorescence, which is proportional to the decrease in the oxygen concentration. Analysis of the rate of decrease of the oxygen concentration enables the instrument to determine if the bottle is positive, indicating the presumptive presence of viable microorganisms in the broth.

The goal of this study was to evaluate the BACTEC 9240 instrument and MYCO/F Lytic medium at the University of Texas Medical Branch (UTMB), a 900-bed tertiary-care medical center located in an area where H. capsulatum is endemic. UTMB provides health care for a large population of patients with AIDS, who are at high risk for disseminated histoplasmosis and disseminated Mycobacterium avium complex infection. With regard to the detection of fungemia, we hoped to evaluate this system’s ability to recover both yeast and dimorphic fungi. We were particularly concerned about the growth and detection of H. capsulatum, on the basis of data from previous studies indicating that broth culture systems are not optimal...
for the isolation of this fungus from blood (5, 7, 8). At UTMB virtually all blood samples that are culture positive for *H. capsulatum* are from patients with AIDS. The majority of blood samples that are culture positive for mycobacteria come from this same population. For these reasons, we limited our study to AIDS patients for whom blood cultures for fungi, mycobacteria, or both had been ordered.

### MATERIALS AND METHODS

#### Patient selection

Blood specimens were obtained from AIDS patients with suspected fungemia, mycobacteremia, or both.

#### Blood culture collection

Phlebotomists aseptically collected each patient’s blood in a sterile syringe. In compliance with each manufacturer’s instructions, 5 ml of blood was transferred to the MYCO/F Lytic bottle and 6 to 10 ml was transferred to a 10-ml Isolator tube. At the beginning of the study, the phlebotomists were instructed to inoculate the MYCO/F Lytic bottle first, followed by the Isolator tube. Midway through the study the order of inoculation was reversed. The MYCO/F Lytic bottles and Isolator tubes were transported to the clinical microbiology laboratory, where they were evaluated for adequate volume. The volumes in the Isolator tubes were compared to those in a standard tube with a known volume and were rejected if they contained less than 6 ml. The volume in the MYCO/F Lytic bottles was compared to a permanent mark made on each bottle prior to inoculation; this mark represented a 5-ml inoculum. Those bottles with an inoculum of less than 5 ml were excluded from the study.

#### Blood culture methods

The Isolator tubes were processed according to the manufacturer’s recommendations once on each of the three work shifts (i.e., day, evening, and night). Approximately 60 µl of the centrifuged pellet was inoculated onto one brain heart infusion agar plate (Becton Dickinson) and one BH agar plate containing 10% sheep blood, gentamicin, and chloramphenicol for the isolation of fungi. For the growth and detection of mycobacteria, 0.5 ml of the pellet was inoculated into an ESP II bottle (AccuMed International, Westlake, Ohio [formerly Difco Laboratories, Detroit, Mich.]) to which an antibiotic supplement (polymyxin B, vancomycin, nalidixic acid, and amphotericin B) had been added. The inoculated fungal media were incubated at 30°C for 42 days and were examined on days 1, 2 and 3, twice during the second week, and then once weekly for the remaining 4 weeks.

The ESP II system is a fully automated system that continuously monitors bottles for the growth of microorganisms. The system includes an algorithm that has been developed for the very slowly growing mycobacteria, in addition to the algorithm for the detection of bacteria used with the ESP blood culture system. ESP II bottles were placed into the ESP II instrument, in which they were incubated at 35°C and monitored for up to 42 days for bacterial growth, based on the detection of pressure changes (i.e., gas production or consumption) within the headspace above the broth culture medium in a sealed bottle. When an ESP II bottle gave a positive signal, 0.2 ml of broth was subcultured onto each side of one Middlebrook 7H11 selective biplate (Becton Dickinson). The plates were incubated at 37°C in an atmosphere of 5 to 10% CO2 and were examined weekly for 42 days.

The MYCO/F Lytic bottles were entered into the BACTEC 9240 instrument at the time that the corresponding Isolator tube was processed, incubated at 35°C, and continuously monitored for microbial growth for up to 42 days. The BACTEC 9000 software, version 3.60N, was used for the first 382 cultures; the BACTEC 9000 software, version 3.61F was used for the remainder of the study. For any bottle for which the BACTEC 9240 instrument gave a positive signal, broth from that bottle was used to prepare smears for staining with the Gram and the acid-fast bacteria stain. The MYCO/F Lytic bottles and Isolator tubes were transported to the clinical microbiology laboratory, where they were evaluated for adequate volume. The volumes in the Isolator tubes were compared to those in a standard tube, and the order of inoculation was reversed midway through the study. The Isolator tubes were processed according to the manufacturer’s recommendations once on each of the three work shifts (i.e., day, evening, and night). Approximately 60 µl of the centrifuged pellet was inoculated onto one brain heart infusion agar plate (Becton Dickinson) and one BH agar plate containing 10% sheep blood, gentamicin, and chloramphenicol, which was incubated at 30°C, and continuously monitored for microbial growth for up to 42 days. The bottle giving a positive signal but no organism was isolated from the subculture. For the remaining sample, a coagulase-negative *Staphylococcus* species was isolated from the MYCO/F Lytic medium. The number of specimens which grew *H. capsulatum* was too small to reliably assess the difference in the rate of recovery between the two systems. For these isolates recovered by both methods, the mean time to detection of growth of *H. capsulatum* was 8 days (range, 4 to 13 days) for MYCO/F Lytic medium and 9 days (range, 6 to 18 days) for the Isolator system (P > 0.05). The mean time to the identification of these isolates was 20 days (range, 15 to 24 days) for those recovered from MYCO/F Lytic medium, whereas it was 11 days (range, 6 to 18 days) for those recovered with the Isolator system (P < 0.05).

The remaining 10 samples (from four patients) positive for fungi by culture grew *Cryptococcus neoformans*. Five of these (from three patients) were positive with both systems, and for five samples (from three patients) *C. neoformans* was isolated from MYCO/F Lytic medium only. Because of the small number of *C. neoformans* isolates, the differences in recovery rates between the Isolator system and MYCO/F Lytic medium could not be reliably assessed. For those isolates detected by both methods, the mean times to detection of growth of *C. neoformans* were 4 days (range, 2 to 6 days) for the MYCO/F Lytic medium and 7 days (range, 5 to 7 days) for the Isolator system.

### RESULTS

A total of 717 blood cultures met the criteria for inclusion in this study. Microbial growth, including the growth of fungi, mycobacteria, and bacteria, was detected in 159 cultures (22%), and a fungus or mycobacterium was recovered from 88 cultures (12%) (Table 1).

Twenty-four specimens from 12 patients were positive for fungi; 12 specimens were positive with both systems. Seven specimens were positive with the Isolator system only, and 5 specimens were positive with MYCO/F Lytic medium only (P > 0.05). Fourteen blood samples from eight patients were positive for *H. capsulatum* by culture; seven samples (from five patients) were positive with both systems (two of these samples also grew *M. avium* complex). The other seven samples (from four patients) were positive with the Isolator system only; however, for six of those samples, the companion MYCO/F Lytic bottle gave a positive signal but no organism was isolated from the subculture. For the remaining sample, a coagulase-negative *Staphylococcus* species was isolated from the MYCO/F Lytic medium. The number of specimens which grew *H. capsulatum* was too small to reliably assess the difference in the rate of recovery between the two systems. For these isolates recovered by both methods, the mean time to detection of growth of *H. capsulatum* was 8 days (range, 4 to 13 days) for MYCO/F Lytic medium and 9 days (range, 6 to 18 days) for the Isolator system (P > 0.05). The mean time to the identification of these isolates was 20 days (range, 15 to 24 days) for those recovered from MYCO/F Lytic medium, whereas it was 11 days (range, 6 to 18 days) for those recovered with the Isolator system (P < 0.05).

The remaining 10 samples (from four patients) positive for fungi by culture grew *Cryptococcus neoformans*. Five of these (from three patients) were positive with both systems, and for five samples (from three patients) *C. neoformans* was isolated from MYCO/F Lytic medium only. Because of the small number of *C. neoformans* isolates, the differences in recovery rates between the Isolator system and MYCO/F Lytic medium could not be reliably assessed. For those isolates detected by both methods, the mean times to detection of growth of *C. neoformans* were 4 days (range, 2 to 6 days) for the MYCO/F Lytic medium and 7 days (range, 5 to 7 days) for the Isolator system.

### TABLE 1. Comparison of the organisms recovered from the Isolator and ESP II systems with MYCO/F Lytic medium

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Isolator system only</th>
<th>ESP II system only</th>
<th>MYCO/F Lytic system only</th>
<th>All systems</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fungal isolates</td>
<td>7 NA</td>
<td>5 12</td>
<td>7 5</td>
<td>NS</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td><em>H. capsulatum</em></td>
<td>7 NA</td>
<td>0 7</td>
<td>5 7</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td><em>C. neoformans</em></td>
<td>0 NA</td>
<td>5 5</td>
<td>0 5</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Mycobacterial isolates</td>
<td>NA 4</td>
<td>17 42</td>
<td>NA 3 41</td>
<td>NA 0 1</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td><em>M. avium</em> complex</td>
<td>NA 3</td>
<td>14 41</td>
<td>NA 0 1</td>
<td>NA 1 3</td>
<td></td>
</tr>
<tr>
<td><em>M. tuberculosis complex</em></td>
<td>NA 0</td>
<td>1 1</td>
<td>NA 0 1</td>
<td>NA 1 3</td>
<td></td>
</tr>
<tr>
<td><em>M. kansasii</em></td>
<td>NA 1</td>
<td>3 0</td>
<td>NA 0 7</td>
<td>NA 5 5</td>
<td></td>
</tr>
</tbody>
</table>

*NA*, not applicable.

NS, not significant (P > 0.05).
(P < 0.05). The mean times to identification were 15 days (range, 7 to 27 days) for isolates recovered in MYCO/F Lytic medium and 8 days (range, 7 to 11 days) for those recovered by the Isolator system (P > 0.05).

None of the patients whose isolate grew in only one system was known to have a fungal infection prior to collection of the blood samples for culture included in this study, nor were any of the patients being treated empirically with antifungal drugs at the time of blood collection.

Of the 717 blood samples cultured for fungi in this study, a corresponding culture for mycobacteria was done for 687 samples. Sixty-four of these grew mycobacteria; 42 samples were positive with both systems, 18 samples were positive with MYCO/F Lytic medium only, and 4 samples were positive with the ESP II system only (P < 0.05). Fifty-eight samples (from 37 patients) grew M. avium complex on culture; 41 (26 patients) were positive with both systems, 14 (11 patients) were positive with MYCO/F Lytic medium only, and 3 (2 patients) were positive with the ESP II system only. For the last three samples, bacteria (two Staphylococcus aureus isolates and one Corynebacterium jeikeium isolate) grew from the companion MYCO/F Lytic medium. Two samples (from two patients) were positive for M. tuberculosis complex; one was positive with both systems, and the other was positive with MYCO/F Lytic medium only. The remaining four positive samples (from three patients) grew M. kansasi. For three of these samples the organism was recovered from MYCO/F Lytic medium only, and for one the organism was isolated from the ESP II system only (the corresponding MYCO/F Lytic bottle grew S. aureus).

For the mycobacteria recovered with both systems, the mean times to detection were 14 days (range, 7 to 29 days) for MYCO/F Lytic medium and 17 days (range, 7 to 36 days) for the ESP II system (P < 0.05). For M. avium complex, the mean time to detection was 13 days (range, 7 to 29 days) for MYCO/F Lytic medium, whereas it was 16 days (range, 7 to 36 days) for the ESP II system (P < 0.05). The times to detection of the M. tuberculosis complex isolated with both systems were 12 days for MYCO/F Lytic medium and 17 days for the ESP II system. For the M. tuberculosis complex isolate which grew only in MYCO/F Lytic medium, the time to detection was 18 days. The mean time to detection for the M. kansasi isolates which grew only in MYCO/F Lytic medium was 22 days (range, 21 to 23 days), and growth of the M. kansasi isolate recovered with the ESP II system only was detected in 20 days.

Of the 17 patients whose mycobacterial isolate was detected with only one system, one (whose isolate grew only in the MYCO/F Lytic medium) had been diagnosed with disseminated M. avium complex disease approximately 1 month prior to the blood sample collection date and was being treated with ethambutol and azithromycin. None of the other patients was known to have a prior mycobacterial infection or was being empirically treated with antimycobacterial drugs at the time of blood collection.

The BACTEC MYCO/F Lytic system had 29 (4%) false-positive signals, whereas the ESP II system had 49 (7%) false-positive signals. No organisms were recovered from the terminal subcultures of the negative MYCO/F Lytic bottles.

**DISCUSSION**

We intentionally designed our evaluation of MYCO/F Lytic medium in the BACTEC 9240 instrument to optimize the inclusion of blood samples that would yield H. capsulatum, M. avium complex, or both. As a result of this design, i.e., blood specimens from patients without AIDS were excluded, H. capsulatum and C. neoforms were the only fungal species recovered during the study period. Our results therefore do not allow an accurate assessment of the reliability of this system for the recovery of fungi, which is a limitation of the study. However, preliminary data from a study similar to ours indicated that the MYCO/F Lytic medium performed as well as or better than the Isolator system with regard to the recovery of Candida species, although that evaluation included only 19 isolates of Candida (1).

Despite the limitation of our study, our data suggest important trends. With regard to the growth and detection of H. capsulatum, we found that more isolates were recovered with the Isolator system than with MYCO/F Lytic medium, although the numbers were too small for reliable statistical analysis. In contrast to our experience, Fuller et al. (1) found that growth and detection of H. capsulatum in the MYCO/F Lytic medium was comparable to its recovery with the Isolator system. Interestingly, for six isolates of H. capsulatum recovered with the Isolator system only in our study, the BACTEC instrument gave a positive signal for the companion MYCO/F Lytic medium. No organisms were detected in the Gram-stained smear prepared from broth from these bottles, and subsequent subcultures showed no growth; therefore, the signals were interpreted as false positive. It is possible that a more in-depth investigation of these samples would have resolved the discrepancies. For example, H. capsulatum might have been detected if smears were stained specifically for fungi, such as with a methenamine silver stain, if a larger volume of the broth had been subcultured, if more than one subculture had been performed, or if sediment from the broth had been tested with the H. capsulatum DNA probe. Unfortunately, at present, these discrepancies cannot be resolved.

In contrast to our findings with H. capsulatum, the MYCO/F Lytic medium recovered more isolates of C. neoforms than the Isolator system, although again the numbers were too small for reliable statistical analysis. Similarly, significantly more mycobacteria (predominantly M. avium complex) were recovered with the MYCO/F Lytic medium than with the Isolator and ESP II system, which we have shown results in a slightly better (although not statistically significant) recovery of mycobacteria than inoculation of the sediment from the Isolator onto two Middlebrook 7H11 selective biplates (6). Fuller et al. (1) also found that MYCO/F Lytic medium recovered more mycobacteria than the Isolator system.

With regard to times to detection, the MYCO/F Lytic medium allowed either more rapid (for C. neoforms) or comparable (for H. capsulatum) rates of detection of fungi compared with the rates for the Isolator system and allowed the more rapid detection of mycobacteria than the ESP II system. However, not unexpectedly, given the presence of colonies on a solid medium, the time to the identification of H. capsulatum was significantly shorter with the Isolator system.

In our opinion, MYCO/F Lytic medium and the BACTEC 9240 instrument offer several advantages over the available blood culture systems for the detection of fungi and mycobacteria. The blood sample is inoculated directly into the MYCO/F Lytic culture medium, which supports the growth of fungi and mycobacteria, as well as that of bacteria. The BACTEC 9240 system is nonradiometric and fully automated and provides continuous monitoring of growth of the organisms in the medium. A potential disadvantage of the medium is the absence of antimicrobial agents for the inhibition of bacterial growth. In our study, for five of the specimens that grew bacteria in the MYCO/F Lytic medium, thus potentially masking the presence of a more slowly growing organism, a fungus was recovered from the companion Isolator system or a mycobacterium was isolated from the companion ESP II bottle. Failure to recover...
mycobacteria due to the presence of a rapidly growing bacterium in the specimen was not a problem with the ESP II system, in which an antimicrobial mixture is added to the ESP II bottles.

In summary, the combination of the MYCO/F Lytic medium and BACTEC 9240 instrument is an excellent blood culture system for the detection of mycobacteremia. The few C. neoformans isolates recovered in this study and the small number of yeasts isolated in a previous study (1) do not permit definitive conclusions concerning this system’s performance with yeasts. The results of both studies suggest that the system has the potential to be at least as reliable as the Isolator system; however, additional studies should be performed to confirm these findings. Further investigation also is necessary to resolve the conflicting data concerning the growth and detection of H. capsulatum.

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REFERENCES