Utility of Paired BACTEC MYCO/F LYTIC Blood Culture Vials for Detection of Bacteremia, Mycobacteremia, and Fungemia

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In previous bloodstream infection studies in Malawi, we inoculated blood from a single venesection into a single BACTEC MYCO/F LYTIC (MFL) vial. Inoculation of one vial, however, would be expected to reduce the sensitivity of bloodstream pathogen detection with MFL vials. To ascertain the degree of this loss of sensitivity, blood was drawn from each of 228 febrile, adult inpatients in Malawi and 5 ml of each blood sample was inoculated into each of two MFL vials. Of 228 paired vials, 51 (22%) were both positive, 172 (75%) were both negative, and 5 (3%) had discordant results. Bloodstream infection would have been detected in 11 (92%) of 12 patients with mycobacteremia and 38 (92%) of 41 patients with bacteremia had only one MFL vial been inoculated. Our study shows that a second MFL vial does not significantly increase diagnostic sensitivity.

The rationale for inoculating two blood culture vials with blood drawn either from a single venepuncture and divided or from separate distinct blood draws is based on the evidence that the likelihood of recovery of bloodstream pathogens is increased if larger volumes of blood are cultured (5, 7, 8, 11). Weinstein and colleagues (14) established that, using conventional manual methods, a single set (one blood draw) of blood cultures will generally detect 91% of cases of bacteremia, while two sets would detect >99% of cases of bacteremia. Recently published data have suggested that in less developed countries where human immunodeficiency virus (HIV) infection is prevalent, valid characterization of bloodstream infections in hospitalized adults may necessitate culturing of blood for mycobacteria, bacteria, and fungi (1, 3, 4, 10). Such an endeavor often requires drawing of up to 30 ml of blood from patients. However, limited blood culture supplies or microbiology capacities in developing countries often preclude retrieval of more than one blood sample for culture per patient, inoculation of more than one vial per blood draw, or comprehensive culture for bacteria, mycobacteria, and fungi. Moreover, for cultural reasons, patients from various countries may be reluctant to part with the seemingly large volumes of blood that are necessary when culturing for these three classes of pathogens.

In a recently published report, we demonstrated that 5 ml of blood inoculated into one BACTEC MYCO/F LYTIC (MFL; Becton Dickinson Microbiology Systems) blood culture vial was a convenient method for culturing for bacteria, mycobacteria, and fungi and was as good as or superior to alternative culture systems that we evaluated (2). We proposed that the MFL vial may be a useful tool for studying (i) bloodstream infections in less developed settings where blood cultures already are part of a microbiology service repertoire or where existing resources limit blood culture service or (ii) conducting prevalence surveys by use of blood culture (3).

Although inoculation of two MFL vials has potential clinical benefit, it also could be economically unsustainable if it is recommended as part of routine blood culture services in developing countries with limited resources. Therefore, we conducted further analyses of our data to determine the utility of inoculation of blood from one draw consecutively into two MFL vials for detection of bacteremia, mycobacteremia, and fungemia in febrile, adult inpatients in Lilongwe Central Hospital (LCH) in Lilongwe, Malawi. Malawi is a developing country in sub-Saharan Africa; LCH is a 300-bed district general hospital located in the central part of the country.

The study protocol was reviewed and approved by Institutional Review Boards at the Centers for Disease Control and Prevention, Atlanta, Ga., and the Ministry of Health, Lilongwe, Malawi.

From March to May 1998 (study period), consecutive, febrile adult patients admitted to LCH were recruited into the study following receipt of informed consent from all patients. Venous blood was drawn with a needle and a syringe in a single venesecion as described previously (2), and 5 ml of each blood sample was inoculated aseptically into each of two MFL vials that were then cultured on-site at 35°C. The MFL vials were read for evidence of growth with a UV lamp (λ = 365 nm) to detect the fluorescence of the indicator at the bottom of the vial. Both MFL vials were examined twice in the first 24 h following incubation for signs of growth, daily for the next 7 days, and then weekly for 8 weeks or until growth was observed. Preliminary identification of bacteria and fungi were made on-site by standard microbiologic tests.
All blood culture vials and all bacterial, fungal, or mycobacterial isolates that were isolated at the study site were transported to the Clinical Microbiology Laboratory at Duke University Medical Center in the United States, where mycobacterial and fungal cultures were processed and the identities of bacterial and fungal isolates that had already been isolated in Malawi were confirmed. The methodologies for the identification of bacteria, mycobacteria, and fungi have been described elsewhere (2, 3). Rates of recovery of bacteria, mycobacteria, and fungi in the MFL vials were compared with each other by the McNemar modification of the chi-square test and, where appropriate, Yates’ correction for small numbers of observations.

During the study period, single blood draws from 228 study patients were inoculated into paired MFL blood culture vials; 56 (24.6%) of these study patients had clinically significant bloodstream infections: 41 caused by bacteria, 12 caused by mycobacteria, and 3 caused by fungi. The following pathogens were isolated from positive cultures of blood from febrile adults: Salmonella spp. (including three Salmonella enterica serovar Typhi isolates), n = 33 (59%); Mycobacterium tuberculosis, n = 12 (22%); other gram-negative organisms (one Acinetobacter iwoffi isolate, one Proteus mirabilis isolate, and three Escherichia coli isolates), n = 5 (9%); gram-positive organisms (one Streptococcus pneumoniae isolate and two Staphylococcus aureus isolates), n = 3 (5%); and Cryptococcus neoformans, n = 3 (5%). Salmonella spp. and Mycobacterium tuberculosis were the two most common isolates. Fifty-one (91%) of the 56 positive cultures were positive in both bottles. Of the 228 MFL vial pairs, 51 (22%) were both positive, 170 (75%) were both negative, and 5 (3%) had discordant results. Two (0.9%) of the 228 pairs of blood cultures yielded coagulase-negative Staphylococcus spp. that were considered skin contaminants and therefore were not included in the data analysis.

In a recently published report (2), we demonstrated that the results obtained with the MFL vial compared well with the results obtained with the set tested by the “gold standard” lysis-centrifugation method (6) for the detection of bacteremia, mycobacteremia, and fungemia. In the present study, we show that since nearly all (91%) of the 56 positive cultures were positive in both bottles. Of the 228 MFL vial pairs, 51 (22%) were both positive, 170 (75%) were both negative, and 5 (3%) had discordant results. Two (0.9%) of the 228 pairs of blood cultures yielded coagulase-negative Staphylococcus spp. that were considered skin contaminants and therefore were not included in the data analysis.

The data represent numbers of vials. None of the differences were statistically significant.

Although the practice of obtaining paired samples of blood from single and separate blood draws for bacterial culture has been extended to obtaining blood samples for mycobacterial culture, no published data critically evaluate these practices. Of note, the aforementioned recommendations for obtaining blood samples for culture are based on studies conducted in the United States, where rates of bloodstream infections are relatively low compared with those in settings of HIV endemicity, where bloodstream infection rates among febrile inpatients may range from 28 to 48% (1, 3, 4, 10). The prevalence rate of bloodstream infections in our study setting was 25% (95% confidence interval, 19%, 31%). Thus, there is a 95% probability that our sample size reflects the true prevalence rate of bloodstream infections in the indigenous febrile inpatient populations.

In the United States, the MFL vial is designed for use in the BACTEC 9000 series automated blood culture machines and is licensed and marketed as a system for the growth and detection of mycobacteria (2, 12). The MFL blood culture medium is not selective and will support the growth of aerobic organisms, such as mycobacteria, bacteria, and yeasts. Thus, the predominance of one organism may interfere with the recovery of other types of organisms and therefore may prevent detection of polymicrobial bloodstream infections (i.e., infections with more than one pathogen). This may explain why none of the MFL vial pairs in our study exhibited any evidence of polymicrobial bloodstream infections.

There were other limitations to this study: (i) the numbers of fungal bloodstream infections were too small to enable a valid assessment of the use of one versus two MFL vials for the detection of fungemia, and (ii) because the MFL vials were shipped to the United States for further processing, we were unable to assess and compare relative times of detection of mycobacteria for each vial.

### Table 1. Comparison of paired MFL vials for recovery of bacteria, mycobacteria, and fungi

<table>
<thead>
<tr>
<th>Organism and result for first MFL vial</th>
<th>Result for second MFL vial&lt;sup&gt;a&lt;/sup&gt;</th>
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<tbody>
<tr>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>Bacteria</td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>38</td>
</tr>
<tr>
<td>Negative</td>
<td>3</td>
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<tr>
<td>Mycobacteria</td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>11</td>
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<tr>
<td>Negative</td>
<td>0</td>
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<td>Fungi</td>
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<tr>
<td>Positive</td>
<td>2</td>
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<tr>
<td>Negative</td>
<td>1</td>
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<sup>a</sup> The data represent numbers of vials. None of the differences were statistically significant.
igration method (2). However, comparison of the MFL vial with other bottles, such as BACTEC bottles containing large volumes of resin, has not been conducted in similar settings with populations with high rates of HIV type 1 and bloodstream infections.

In summary, we found that inoculation of 5 ml of blood into one MFL vial will detect about 92% of cases of bacteremia and mycobacteremia. In addition, our data suggest that the MFL vial serves as a useful, multipurpose tool for the detection and characterization of bloodstream infections in settings of HIV endemicity in less developed countries and that inoculation of blood obtained in a single blood draw into a single MFL vial is acceptable for detection of bacteremia and mycobacteremia. A second MFL vial does not significantly increase the sensitivity of detection of these pathogens.

We are indebted to the nursing and medical staffs in the Department of Medicine at LCH, the patients who participated in the study, and the Malawi Ministry of Health and the U.S. Agency for International Development, Malawi, for facilitating the conduct of this study. The BACTEC MFL vials for this project were kindly provided by Becton Dickinson Microbiology Systems, Cockeysville, Md.

REFERENCES