Controlled Comparison of BacT/Alert MB System, Manual Myco/F Lytic Procedure, and Isolator 10 System for Diagnosis of *Mycobacterium tuberculosis* Bacteremia[▽]

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Received 20 May 2011/Accepted 27 May 2011

We compared the performance of the BacT/Alert MB system, that of the manual Bactec Myco/F Lytic procedure, and that of the Isolator 10 lysis-centrifugation system in the detection of *Mycobacterium tuberculosis* bacteremia. Mean times to detection were 16.4 days for BacT/Alert MB versus 20.0 days for Myco/F Lytic, 16.5 days for BacT/Alert MB versus 23.8 days for Isolator 10, and 21.1 days for Bactec Myco/F Lytic versus 22.7 days for Isolator 10. There were no significant differences in yields. The mean (range) magnitude of mycobacteremia was 30.0 (0.4, 90.0) CFU/ml and was correlated with the time to positivity in the BacT/Alert MB system ($r = -0.4920$). *M. tuberculosis* bacteremia was detected more rapidly in a continuously monitored liquid blood culture system, but the mean time to positivity exceeded 3 weeks.

Mycobacterium tuberculosis bloodstream infection was described within a few decades of the discovery of the tubercle bacillus (7, 14). Disseminated tuberculosis remains a major health problem in countries where generalized HIV epidemics coincide with high tuberculosis incidence rates, often causing fatal illness in patients with immunologically advanced HIV disease (1, 4–6, 15, 17, 21). In-hospital case fatality rates for bacteremic disseminated tuberculosis in the era before the widespread availability of antiretroviral therapy approached 50% (1). Early recognition and treatment are likely to be important to avoid death (13).

Although the incremental value of mycobacterial blood culture for the diagnosis of disseminated tuberculosis has been both recognized and debated (10, 16, 19), there have been few evaluations of blood culture systems for the detection of *M. tuberculosis* (2, 3). Mycobacterial blood culture methods in common use include visual inspection of processed blood inoculated on a solid medium (e.g., the Isolator 10 system) and continuous detection in liquid medium inoculated with blood (e.g., the BacT/Alert MB system or the Bactec Myco/F Lytic system). In a mycobacterial blood culture study in the United States, we noted that bottles with *M. tuberculosis* were positive after a mean of 22.8 to 28.0 days of incubation, compared with 9.9 to 20.4 days for bottles with *Mycobacterium avium* complex (MAC) (11). In order to investigate the performance of mycobacterial blood culture methods for detecting *M. tuberculosis* bacteremia, we conducted a controlled study to compare the performances of the BacT/Alert MB system, the manual Bactec Myco/F Lytic procedure, and the Isolator 10 system for the

detection of mycobacteremia among febrile patients admitted to two hospitals in northern Tanzania, a country experiencing a generalized HIV epidemic and a high incidence of tuberculosis.

Samples for blood cultures were collected from patients aged ≥ 13 years hospitalized at the Kilimanjaro Christian Medical Centre (KCMC) and Mawenzi Regional Hospital (MRH) in Moshi, Tanzania, from July 2006 through October 2009 (9). Patients with oral temperatures of $\geq 38.0^\circ\text{C}$ were invited to participate in the study. Blood from all qualifying study participants was inoculated into a bioMérieux BacT/Alert mycobacterial (MB) bottle. In addition, blood from study participants with oral temperatures of $\geq 38.0^\circ\text{C}$, HIV infection, a fever duration of >1 month, and a presumed or measured weight loss of $>10\%$ was also inoculated into a Bactec Myco/F-Lytic bottle and a Wampole Isolator 10 lysis-centrifugation tube. Blood cultures were processed at KCMC.

Blood culture bottles were assessed for volume adequacy by comparing the weights before and after inoculation with blood. A bottle was included in the primary data analysis only if it contained 4 to 6 ml of blood. A secondary analysis was performed without reference to volume adequacy.

After assessment of the adequacy of the blood volume, MB bottles were loaded into the bioMérieux BacT/Alert 3D automated microbial detection system (bioMérieux Inc., Durham, NC), where they were incubated for 42 days. Bactec Myco/F-Lytic bottles (Becton Dickinson, Franklin Lakes, NJ) were incubated at 35°C for 42 days; bottle bottoms were examined for fluorescence daily using Wood's lamp. Isolator 10 lysis-centrifugation tubes were centrifuged and processed using the Wampole Isostat/Isolator Microbial System (Inverness Medical, Princeton, NJ), plated to Middlebrook 7H10 agar, and incubated at 35°C in 5% CO_2 for 42 days. Bottles and plates with growth were processed according to standard techniques (20). AccuProbe Culture Identification

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[▽] Published ahead of print on 8 June 2011.

TABLE 1. Clinically important organisms recovered from BacT/Alert MB bottles

Microorganism	No. of sets in which organism was detected
<i>M. tuberculosis</i> complex.....	26
<i>M. sherrisii</i>	3
<i>Salmonella enterica</i> serovar Typhi.....	31
<i>Escherichia coli</i>	14
<i>Streptococcus pneumoniae</i>	12
<i>Cryptococcus neoformans</i>	7
<i>Staphylococcus aureus</i>	5
Nontyphoidal <i>Salmonella</i>	4
<i>Klebsiella</i> sp.....	2
<i>Brucella</i> sp.....	1
<i>Pseudomonas aeruginosa</i>	1

Test MTB and MAC kits (Gen-Probe Inc., San Diego, CA) were used to identify *M. tuberculosis* complex and MAC members. Mycobacteria other than *M. tuberculosis* and MAC were identified at the National Institute for Public Health and the Environment, Bilthoven, the Netherlands (12, 23). Colonies growing on Middlebrook 7H10 plates from lysis-centrifugation specimens were counted, and numbers of CFU per ml of blood were calculated. Negative companion bottles from positive sets were subcultured at the end of the 42-day protocol.

Comparison of recovery rates was analyzed by the McNemar chi-square test with Yates' correction for small numbers when necessary (18). Times to detection were compared by the paired two-sample *t* test for means after log transformation was performed to correct for the observed positively skewed (non-parametric) distributions. All analyses were done with the SAS system for Windows (release 9.1; SAS Institute, Cary, NC).

This study was approved by the KCMC Research Ethics Committee, the Tanzania National Institutes for Medical Research National Research Ethics Coordinating Committee, and an Institutional Review Board of the Duke University Medical Center.

A total of 759 BacT/Alert MB blood cultures were processed; without restriction for volume adequacy, 26 (3.4%) yielded *M. tuberculosis*, 3 (0.4%) yielded *M. sherrisii*, and 77 (10.1%) yielded other pathogens, as shown in Table 1. Sixteen (2.1%) yielded organisms classified as contaminants. Of 20 complete (two bottles and one tube), adequately filled (5 ± 1 ml) sets from which *M. tuberculosis* was recovered from at least one bottle, the BacT/Alert MB system was positive for 12 (60.0%), the Bactec Myco/F Lytic procedure was positive for 20 (100.0%), and the Isolator 10 system was positive for 9 (45.0%).

Table 2 shows the yields of *M. tuberculosis* for all three two-way comparisons of adequately filled pairs of bottles and for all bottles. For adequately filled pairs, although there were trends toward the manually read Bactec Myco/F Lytic bottle detecting *M. tuberculosis* more frequently than the BacT/Alert MB system and the Isolator 10 system, there were no significant differences between any two bottles compared. For all bottle pairs, there was also a trend toward the manually read Bactec Myco/F Lytic bottles detecting *M. tuberculosis* more frequently than the BacT/Alert MB system and the yield was significantly greater in the manually read Bactec Myco/F Lytic bottles than in the Isolator 10 system. Of 17 adequately filled

TABLE 2. Comparative yields of *M. tuberculosis* obtained with various combinations of the BacT/Alert MB system, the manual Myco/F Lytic procedure, and the Isolator 10 system

Bottle pair (bottle 1, bottle 2)	No. of bottles in which MTB was detected			P value
	Both bottles	Bottle 1 only	Bottle 2 only	
Adequately filled bottles				
BacT/Alert MB, Myco/F Lytic	6	2	5	0.4497
BacT/Alert MB, Isolator 10	7	2	0	0.4795
Myco/F Lytic, Isolator 10	6	5	0	0.0736
All bottles				
BacT/Alert MB, Myco/F Lytic	12	2	8	0.1138
BacT/Alert MB, Isolator 10	7	3	2	1.0000
Myco/F Lytic, Isolator 10	9	7	0	0.0233

MB bottles yielding *M. tuberculosis*, the mean (range) time to positivity was 22.6 (9.4 to 37.5) days. Of the three 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 continuously monitored BacT/Alert MB system, followed by the manually read Bactec Myco/F Lytic bottle and the Isolator 10 system (Table 3). When all of the bottles were considered, the time to detection was significantly shorter for the BacT/Alert MB system than for both the manually read Bactec Myco/F Lytic bottle and the Isolator 10 system (Table 3). For 3 adequately filled MB bottles yielding *M. sherrisii*, the mean (range) time to positivity was 15.2 (7.7 to 27.3) days.

For nine Isolator 10 samples yielding *M. tuberculosis*, the mean (range) magnitude of mycobacteremia was 30.0 (0.4 to 90.0) CFU/ml. For seven adequately filled BacT/Alert MB and Isolator 10 bottle pairs yielding *M. tuberculosis*, the mean (range) time to positivity for the BacT/Alert MB system was 16.5 (9.4 to 20.2) days. The mean (range) magnitude of *M. tuberculosis* bacteremia determined by the Isolator 10 system was 38.2 (0.5 to 90.0) CFU/ml. There was a trend toward a longer time to positivity in the BacT/Alert MB system for samples with lower numbers of *M. tuberculosis* CFU/ml ($r = -0.4920$, $r^2 = 0.2420$) (Fig. 1).

We have demonstrated that *M. tuberculosis* is a leading cause of bloodstream infection among febrile inpatients in northern Tanzania (9). However, even with the continuously monitored BacT/Alert MB system, the mean time to positivity exceeded 3 weeks. The mean time to positivity for the continuously monitored BacT/Alert MB system was significantly shorter than for the Isolator 10 plated to Middlebrook 7H10 solid medium considering either adequately filled bottles or all bottles. There was a nonsignificant trend toward the continuously monitored BacT/Alert MB system having shorter times to detection than the manually read Bactec Myco/F Lytic bottle for adequately filled bottles; this difference was significant when all of the bottles were considered. While there were no statistically significant differences in sensitivity between the systems when adequately filled bottles were considered, *M. tuberculosis* was detected by only one member of a bottle pair on 42% of the occasions. Furthermore, consistent with other studies (3), a comparison without respect to volume adequacy showed that the Bactec Myco/F Lytic bottle was more sensitive than the Isolator 10 plated to Middlebrook 7H10 solid medium.

TABLE 3. Comparative times to detection of *M. tuberculosis* by the BacT/Alert MB system, the manual Myco/F Lytic procedure, and the Isolator 10 system

Bottle pair (bottle 1, bottle 2)	No. of pairs	Mean (range) time (days) to positivity		<i>P</i> value
		Bottle 1	Bottle 2	
Adequately filled bottles				
BacT/Alert MB, Myco/F Lytic	6	16.4 (9.4–21.0)	20.0 (14.9–24.0)	0.2311
BacT/Alert MB, Isolator 10	7	16.5 (9.4–20.2)	23.8 (16.8–31.0)	0.0329
Myco/F Lytic, Isolator 10	6	21.1 (14.9–27.0)	22.7 (16.8–28.8)	0.5554
All bottles				
BacT/Alert MB, Myco/F Lytic	12	19.0 (9.4–32.2)	24.9 (13.0–48.0)	0.0286
BacT/Alert MB, Isolator 10	7	16.5 (9.4–20.2)	23.8 (16.8–31.0)	0.0329
Myco/F Lytic, Isolator 10	9	24.4 (14.9–33.3)	21.8 (3.0–31.0)	0.4235

Whereas *M. tuberculosis* was detected after a mean of 22.6 days by the continuously monitored BacT/Alert MB system, *M. sherrisii*, the second most frequently isolated *Mycobacterium* species in this study (12, 23), was detected after a mean of 15.2 days by the same system. The observed differences in the time to detection between *M. tuberculosis* and nontuberculous mycobacteria in liquid systems may be due to constitutional differences in the rate of replication by species, differences in the optimization of the medium for the growth of different mycobacterial species, exposure to drugs with antimycobacterial activity at the time of sample collection, and the sizes of the initial inoculums of mycobacteria placed into the blood culture bottle.

The quantitative study demonstrated a wide variation in the magnitude of *M. tuberculosis* bacteremia in this predominantly HIV-infected population (9) of <1 CFU/ml to 90 CFU/ml. We also demonstrated a trend toward longer times to detection in the continuously monitored BacT/Alert MB system among patients with a lower magnitude of mycobacteremia. The occurrence of *M. tuberculosis* bacteremia at magnitudes of <1 CFU/ml may go some way toward identifying why *M. tuberculosis* was detected in only one member of a large proportion of many bottle pairs.

Our study had a number of limitations. In order to target the use of diagnostics to a population with higher pretest probability for disseminated tuberculosis, blood was collected for mycobacterial culture in all three blood culture systems only from patients meeting the more rigorous inclusion criteria of an oral temperature of $\geq 38.0^{\circ}\text{C}$, the presence of HIV infec-

tion, a fever duration of >1 month, and a presumed or measured weight loss of >10%. However, 10 (38.5%) of 26 participants with *M. tuberculosis* bloodstream infection did not meet these criteria, which resulted in some participants with *M. tuberculosis* bacteremia having blood inoculated into a BacT/Alert MB bottle only, thus limiting our power to compare the systems. We recommend that future studies use broader inclusion criteria, for example, focused on the presence of fever and HIV infection. The lack of availability of a Bactec continuously monitored blood culture instrument at the study site meant that Bactec Myco/F Lytic bottles had to be read manually on a daily basis, placing the Bactec Myco/F Lytic procedure at a disadvantage for comparisons of times to detection relative to the BacT/Alert MB system. To standardize volumes, we inoculated 5 ml of blood into each bottle or tube, including the Isolator 10 system, which is designed for 10 ml of blood. The lower ratio of blood to lytic agent and anticoagulant in the Isolator 10 system may have inhibited mycobacterial growth (25). However, Isolator 10 tubes were processed within 8 h of collection and the sediment was plated to solid medium rather than into broth (24).

In summary, *M. tuberculosis* is a common cause of bloodstream infection in northern Tanzania. Although it is detected more rapidly in a continuously monitored liquid blood culture system than by lysis-centrifugation with plating to solid medium, a mean time to positivity exceeding 3 weeks may be too long to lead to potentially lifesaving early initiation of tuberculosis treatment. The occurrence of *M. tuberculosis* bacteremia at magnitudes of <1 CFU/ml may contribute to long times to detection in some cases and could explain, in part, the large proportion of blood culture pairs where *M. tuberculosis* was isolated from only one bottle. Future work on the detection of *M. tuberculosis* bloodstream infection should focus on optimizing both sensitivity and time to detection. Possibly strategies could include further development of *M. tuberculosis* nucleic amplification techniques on large-volume peripheral blood specimens (8, 22).

This research was supported by an International Studies of AIDS-Associated Co-Infections (ISAAC) award, a United States National Institutes of Health (NIH)-funded program (U01 AI062563). We received support from NIH ISAAC awards (J.A.C., A.B.M., H.O.R., B.N.N., V.P.M.), the AIDS International Training and Research Program (D43 PA-03-018) (J.A.C., H.O.R., B.N.N., V.P.M.), the Duke Clinical Trials Unit and Clinical Research Sites (U01 AI069484)

FIG. 1. Relationship between the number of *M. tuberculosis* CFU/ml and the time to positivity in the BacT/Alert MB system.

(J.A.C., V.P.M.), and the Center for HIV/AIDS Vaccine Immunology (U01 AI067854) (J.A.C.).

We thank Ahaz T. Kulanga for providing administrative support to this study and Pilli M. Chambo, Beata V. Kyara, Beatus A. Massawe, Anna D. Mtei, Godfrey S. Mushi, Lillian E. Ngowi, Flora M. Nkya, and Winfrida H. Shirima for reviewing and enrolling study participants. We are grateful to the leadership, clinicians, and patients of KCMC and MRH for their contributions to this research. We acknowledge the Hubert-Young Center for Global Health at Duke University for critical infrastructure support for the KCMC-Duke University Collaboration.

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