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Growth of *Mycobacterium tuberculosis* in Conventional BacT/ALERT FA Blood Culture Bottles Allows Reliable Diagnosis of Mycobacteremia

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The conventional BacT/ALERT FA blood cultures supported the ample growth of *Mycobacterium tuberculosis* in seeding experiments and appeared to perform as reliably as the BACTEC Myco/F-Lytic vials in the recovery of *M. tuberculosis* from blood in HIV-infected patients. Overall, blood cultures were positive in 39% of patients with tuberculosis.

In the 1980s, novel automated blood culture (BC) systems were investigated for their potential to diagnose mycobacteremia, especially in late-stage human immunodeficiency virus (HIV) disease. Studies, from mainly industrialized countries, described the frequent isolation of *Mycobacterium avium* complex and rather rare detection of *Mycobacterium tuberculosis* complex (MTC) (3, 7), which led to the introduction and widespread use of BC based on Middlebrook broth. Recent studies, mainly from developing countries with high tuberculosis incidence rates, reported positive mycobacterial blood cultures from 5 to more than 30% (2, 6, 8). However, little is known about the nature of mycobacteremias, especially those caused by *M. tuberculosis*, and thus little is known of the ideal conditions for the collection of blood. In HIV-infected patients, "conventional" BCs tend to be collected more frequently than mycobacterial BC based on Middlebrook broth. However, *M. tuberculosis* is thought to grow very scantly, if at all, in conventional blood culture broths (7). The aim of this study was to investigate if conventional BacT/ALERT FA (bioMerieux, Marcy-l'Etoile, France) blood cultures (nutrient-rich complex media, supplemented with 6.5% charcoal) support the growth of *M. tuberculosis* and might thus help to determine the optimal timing, number, and yield for the diagnosis of MTC mycobacteremia in patients with HIV infection in Portugal.

The study took place at a large teaching hospital in Lisbon, Portugal. To serve as a baseline for this study, all positive mycobacterial blood cultures (BACTEC Myco/F-Lytic; Becton Dickinson, Sparks, Md.), collected from January 1999 up to February 2004 at the authors' hospital, were analyzed. A total of 1,779 mycobacterial blood cultures were collected from 1,243 patients, of which 89 BCs (5%) from 78 patients (6.2%) were positive for *Mycobacterium* spp.: 74 for *M. tuberculosis* complex and 4 for *M. avium* complex.

In seeding experiments, three sets of BacT/ALERT FA bottles were inoculated in triplicate with 3 ml of a serial 10-fold dilution of three different strains of *M. tuberculosis* (range of 100,000 to 10 mycobacteria per BC vial). To one set, OADC (oleic acid, albumin, dextrose, catalase) was added, to another set, OADC and 20 ml of Middlebrook 7H9 broth were added, and one set remained without supplements. Four milliliters of fresh human blood was added to each vial. BCs were incubated for 4 weeks at 37°C and then checked for mycobacterial growth by microscopy of auramine-stained slides of the broth and by subculture to Löwenstein-Jensen slants. All positive BC bottles identified by subculture were also identified by microscopy of auramine-stained slides which revealed numerous mycobacteria (>100 to 300) per low-power field (200×). The conventional BacT/ALERT FA blood culture medium supported the ample growth of MTC and no difference was observed between the three sets of BC, with or without supplements. The lowest dilution that led to a detectable growth of mycobacteria corresponded to an inoculum of 10 to 100 mycobacteria per BC bottle.

Blood from HIV-positive patients was drawn, and 5 to 7 ml was inoculated into BACTEC Myco/F-Lytic culture vials and incubated in the BACTEC 9050 automated system during 42 days at 37°C for mycobacterial BCs. For conventional blood cultures, 5 to 7 ml of blood was inoculated into BacT/ALERT FA bottles and incubated in the BacT/ALERT automated systems for up to 10 days at 37°C (bioMerieux). All conventional BacT/ALERT FA blood cultures from HIV-infected individuals that remained negative after routine incubation (7 to 10 days) in the BacT/ALERT instrument were incubated for an additional 6 weeks, after which they were checked for the presence of mycobacteria by microscopy of auramine-stained slides of the blood culture broth. During 10 months, 457 BacT/ALERT FA blood cultures (22 positive for MTC) and 323 BACTEC Myco/F-Lytic blood cultures (19 positive for MTC) were collected from 476 patients with HIV infection. The overall results per patient are shown in Table 1. Seven patients with one or more positive blood cultures for *M. tuberculosis* had further blood cultures collected within 24 h (BacT/ALERT FA and/or BACETC Myco/F-Lytic). However, these BCs did not

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TABLE 1. Overall results of blood cultures for the detection of mycobacteremia in HIV-infected patients during a 10-month period

Patient group and result	Patients (*n*)
Patients with tuberculosis	
Negative BacT/ALERT FA/positive BACTEC[a]	3
Positive BacT/ALERT/negative BACTEC	5
Positive BacT/ALERT/positive BACTEC	7
Only positive BacT/ALERT FA or positive BACTEC[b]	8
Positive specimen/negative blood culture[c]	32
Patients without tuberculosis	421
Total	476

[a] BacT/ALERT FA, nutrient-rich complex media, supplemented with 6.5% charcoal; BACTEC, mixture of Middlebrook 7H9 and brain heart infusion broth.
[b] For eight patients only one type of blood culture bottle was received.
[c] Positive specimen was a culture of any type of specimen other than blood that yielded mycobacteria (52 specimens from 32 patients: 4 urine, 6 cerebrospinal fluid, 7 biopsy, and 35 respiratory specimens).

yield mycobacterial growth. Patients with positive blood cultures for *M. tuberculosis* in either BC system had blood collected earlier after admission (50.6% of all BCs collected within 48 h) than patients with culture-proven disseminated tuberculosis and negative BCs (18.8% collected within 48 h).

It has been shown that automated blood culture systems, essentially using some kind of Middlebrook-based broth, allow the detection of mycobacteria, including *M. tuberculosis* (2, 4, 6, 8). However, studies concluded that mycobacterial blood cultures added little to the conventional diagnostic work-up (2, 8) or were performed in areas where mycobacteria are endemic (8) and frequently in developing nations (6) where the use of expensive blood cultures is often beyond the already overstretched resources. Contrary to this, the results presented here corroborate a previous observation that blood cultures can be a useful tool for cultural confirmation of tuberculosis in HIV-infected patients in Portugal (4).

A rather surprising finding of this study was that the conventional BacT/ALERT FA culture medium did support the abundant growth of *M. tuberculosis*. Although at the beginning of the HIV pandemic studies reported that *Mycobacterium avium* complex seemed to grow and could be detected in conventional BC, it was found that *M. tuberculosis* did at best survive but did not seem to replicate in conventional automated blood culture bottles (7). Contrary to this, the conventional BacT/ALERT FA blood culture bottles supported the ample growth of *M. tuberculosis* as shown by the seeding experiments. Furthermore, they seemed to have the potential to perform as well as the BACTEC Myco/F-Lytic bottles in the recovery of *M. tuberculosis* from the blood of HIV-infected patients. It may well be possible that the BacT/ALERT instrument would allow the detection of mycobacterial growth in the BacT/ALERT FA bottles if they were incubated long enough and the detection algorithm could be adjusted accordingly. Interestingly, a recent report announcing "the end of a dogma" (1) reminded us that simple blood agar appears to be as efficient as egg-based media for the recovery of *M. tuberculosis*. Perhaps, the widely held view that liquid media have to be based on some kind of Middlebrook broth with certain supplements (like OADC) may also have to be reexamined.

Concerning the ideal timing and number of blood culture collection (5), it appears that BC for the detection of mycobacteria should be collected as soon as possible after hospital admission, as shown by the fact that patients with culture-proven disseminated tuberculosis (mainly from liver biopsy specimens) had negative mycobacterial BCs when they were collected later during their hospital stay. This interesting observation has to be confirmed with a larger number of patients.

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