Number of Days Required for Recovery of Mycobacteria from Blood and Other Samples

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From 1991 to 1996, 541 blood samples were tested for the presence of mycobacteria; 56 were positive (30 patients, 26 human immunodeficiency virus positive). The species found were Mycobacterium avium (41 samples from 18 patients), Mycobacterium tuberculosis (12 samples from 9 patients), and three other species (1 sample each). The average time to detection was 25.23 days (22.65 for M. avium and 35.33 for M. tuberculosis). For 10 patients, the blood isolate was the only mycobacterium detected (4 M. tuberculosis).

Although detection of mycobacteria in blood samples has been possible for a long time, only in recent years has this type of specimen become important in the diagnosis of diseases caused by mycobacteria. The AIDS epidemic has focused attention on mycobacteremia due to the Mycobacterium avium complex (4, 8), and the reappearance of tuberculosis as a problem in developed countries, together with evidence that disseminated disease is more prevalent in immunocompromised hosts (2), has made the detection of mycobacteria in blood samples a priority for all clinical microbiology laboratories. Many systems have been developed for mycobacterial blood cultures, with lysis-centrifugation systems being among the most highly recommended (12). Here we present our experience with the use of a lysis-centrifugation system in combination with detection of microcolonies on Middlebrook agar plates, a system that has been described for rapid detection of mycobacteria in clinical samples (13).

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We retrospectively reviewed the records of the Microbiology Department of the Fundación Jiménez Díaz for mycobacterial blood cultures performed between January 1991 and December 1996. During this period, all blood cultures for mycobacteria were processed by using the lysis-centrifugation system (ISOLATOR; Merck) according to the instructions provided by the supplier. Briefly, commercial tubes were inoculated with 10 ml of blood and sent immediately for processing. In the laboratory, the tubes were centrifuged at 3,000 rpm for 5 min and the supernatant (except 1 ml) was discarded. The sediment was resuspended by vortexing and seeded on Middlebrook 7H11 (7H11) agar plates (0.2 ml) and Lowenstein-Jensen (LJ) slants (0.2 ml), as well as in MB-Check (MB) liquid medium (BBL) (approximately 1 ml). A smear of the sediment was also made for auramine O staining. All media were incubated at 37°C for 8 weeks (7H11 and LJ in an atmosphere of 5% CO₂ for all 8 weeks or for the first 2 weeks, respectively). All media were examined weekly for evidence of microcolonies except 7H11, which was read twice weekly. Identification of the isolates was achieved by performing common biochemical tests. All isolates were also sent to the Centro Nacional de Microbiología (Majadahonda, Spain) for confirmation of their identities.

During the study period, 541 blood cultures were processed for mycobacterial culture in our department. Mycobacterial growth was detected in 56 of the cultures (10.3%), belonging to 30 patients (1.9 positive blood cultures per patient). None of the 56 blood samples was acid-fast stain positive. The species detected were M. avium (41 samples from 18 patients), Mycobacterium tuberculosis (12 samples from 9 patients), Mycobacterium kansasii (1 specimen), Mycobacterium fortuitum (1 sample), and Mycobacterium mucogenicum (1 specimen). The average length of time to detection for all mycobacteria was 25.23 days (range, 6 to 57 days). The lengths of time to detection of M. avium and M. tuberculosis for all of the different media, together with the results of statistical analyses (Student’s t test), are shown in Table 1. The sensitivities of the three media were 58.9% for 7H11, 67.9% for LJ, and 62.5% for MB. Eighteen patients with blood cultures positive for M. avium or M. tuberculosis had another type of specimen(s) which was positive for the same species, and in these patients, the time to detection was less than in those which had only a positive blood culture (Table 1), although the differences were not statistically significant (Student’s t test). However, the average time to detection of growth was less in other types of samples than in blood (Table 2), although the small number of samples prevented the determination of statistical significance. Twenty-six (86.7%) of the 30 patients were human immunodeficiency virus positive, one of them being persistently positive for M. avium bacteremia from July 1994 to February 1996. Only the two cases for which rapidly growing mycobacteria were isolated were considered as nonsignificant.

Detection of M. tuberculosis from blood has been achieved since the early years of the 20th century (6). However, it remained a rarity until the appearance of human immunodeficiency virus epidemics, when M. tuberculosis bacteremia became a more frequent finding, mainly in areas with a high prevalence of tuberculosis disease (1, 5). Other mycobacteria were also detected in blood, although this was an exceptional finding prior to the recent increase in numbers of immunosuppressed hosts, which has made this finding increasingly important (15).

Several methods have been developed for the diagnosis of mycobacteremia (12). Lysis-centrifugation systems are among the most widely employed, being useful for diagnosis of bacteremia due to the M. avium complex (10) or M. tuberculosis (11). However, the long time periods required for detection by such systems represent a disadvantage compared with the new

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blood culture techniques based on automatic detection of growth (14). Microcolony detection has been described as a useful technique for decreasing the length of time to detection (7, 9, 13), although in this study we were not able to detect statistically significant differences between times to detection with the different media, except with M. avium for 7H11 versus MB (P = 0.04). However, it seems that there is a true difference between media, and the small number of samples could be the explanation for the statistical results. In fact, a decrease in the time to detection of positives was evident for other (nonblood) samples from those patients with positive blood specimens, but the time to detection for blood samples in these cases remains long, both for M. avium and M. tuberculosis, in all patients. It is important to note that blood was the only positive mycobacterial source in 10 patients (one-third of all cases), including 4 patients with M. tuberculosis (the other 6 were positive for either M. avium [3 patients] or one of the other three species isolated). The high percentage of M. tuberculosis isolates may represent the high prevalence of this disease in Spain, as other authors have reported (3), and may be of great significance in the determination of the correct therapeutic approach for these patients.

In conclusion, we believe that detection of mycobacteremia by using lysis-centrifugation systems shows a high percentage of sensitivity, although detection time periods remain long, even when microcolony detection is used. The fact that blood was the only positive source for mycobacteria in one-third of the patients indicates the importance of considering the use of mycobacterial blood cultures as a tool for mycobacterial detection when disseminated disease is suspected.

### TABLE 1. Days to detection of positive blood cultures

<table>
<thead>
<tr>
<th>Species</th>
<th>Mean no. of days (n) to detection of positive blood cultures in²:</th>
<th>Other a,b</th>
<th>Other c</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>7H11</td>
<td>LJ</td>
<td>MB</td>
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</table>

* Results of statistical comparisons (Student’s t test) were as follows: A versus B, P = 0.12; A versus C, P = 0.04; B versus C, P = 0.67; D versus E, P = 0.26; D versus F, P = 0.64; E versus F, P = 0.58; G versus H, P = 0.07; G versus I, P = 0.12; H versus I, 0.74; J versus K, P = 0.12; L versus M, P = 0.62; N versus O, P = 0.22; and P versus Q, P = 0.10.

a Other +, blood cultures from patients from whom the same species was isolated from another source(s).

b Other −, blood cultures from patients from whom the same species was not isolated from another source(s).

### REFERENCES


