Unreliable Detection of *Mycobacterium xenopi* by the Nonradiometric Bactec MGIT 960 Culture System

Claudio Piersimoni,* Domenico Nista, Stefano Bornigia, and Giancarlo Gherardi
Department of Clinical Microbiology, United Hospitals, Ancona, Italy

Received 28 July 2008/Returned for modification 27 October 2008/Accepted 3 January 2009

From June 2006 to December 2007, 3,648 clinical specimens consecutively received for mycobacterial culture were investigated. Each processed sample was inoculated into Bactec MGIT 960 liquid medium and a Löwenstein-Jensen slant. Tubes that were flagged as positive by the instrument as well as those determined to be negative after 42 days of incubation were removed, visually inspected for growth, and checked for the presence of acid-fast bacilli. Three hundred sixty-nine mycobacterial strains were recovered; of the 44 *Mycobacterium xenopi* isolates recovered by MGIT medium, only 13 were detected by the instrument (*P* < 0.0001). Most tubes yielding *M. xenopi* exhibited a peculiar pattern of growth characterized by a scant number of round, yellow-pigmented granules instead of the fine, evenly dispersed clumps usually observed for mycobacteria. It is suggested to check all individual tubes discarded by the MGIT 960 system at the end of the incubation period to prevent a significant amount of previously undetected growth from being missed.

Despite considerable progress in the direct detection of the *Mycobacterium tuberculosis* complex (MTC) by molecular methods, the definitive diagnosis of mycobacterial infections still relies upon the isolation and identification of acid-fast bacilli (AFB). Currently, a combination of solid and liquid media is the accepted standard for the primary isolation of acid-fast bacilli (AFB). Although it has been demonstrated to support luxuriant growth of most mycobacterial species, recovery rates of AFB from clinical specimens (1, 5, 6, 9, 12, 13, 14, 17). As this phenomenon was initially correlated with the incubation period defaulted by the manufacturer at 6 weeks, it was believed that by providing a longer incubation, the sensitivity of the system would increase (12). A paper by Idigoras and colleagues (7) demonstrated that more than 60% of samples growing *M. xenopi* using MGIT 960 were shown to be consistently lower than those using Bactec 460 TB and solid media (13, 17). As this phenomenon was initially correlated with the incubation period defaulted by the manufacturer at 6 weeks, it was believed that by providing a longer incubation, the sensitivity of the system would increase (12). A paper by Idigoras and colleagues (7) demonstrated that more than 60% of samples growing *M. xenopi* (44 of 72) were undetected by the instrument and consequently discarded as negative.

In this context, we performed a prospective study to evaluate the reliability of the MGIT 960 system for the recovery and detection of *M. xenopi* and other mycobacterial species. From June 2006 to December 2007, 3,648 clinical specimens consecutively received for mycobacterial culture in our laboratory were investigated. They included 2,192 respiratory specimens (almost entirely represented by sputa and bronchial washings), 791 urine specimens, 422 sterile body fluid specimens (pleural, pericardial, synovial, cerebrospinal, and ascites), 101 tissue specimens, and 142 samples from miscellaneous sources. Blood specimens were not included in the study. Specimens were liquefied and decontaminated by the standard NaOH-N-acetyl-L-cysteine procedure (10). After decontamination, all specimens were neutralized with phosphate-buffered saline (0.067 M, pH 6.8) and centrifuged at 3,500 × g for 20 min. The pellet was used for smear preparation, resuspended in phosphate-buffered saline to a final volume of 1.5 ml, and inoculated into the culture medium. Specimens collected from sterile sites were concentrated by centrifugation without prior decontamination.

For mycobacterial culturing, a 0.5-ml portion of each processed sample was inoculated into liquid and solid media. The MGIT 960 system consists of plastic tubes containing 7 ml of Middlebrook 7H9 broth base supplemented with 0.8 ml of MGIT 960 supplement (Becton Dickinson Microbiology Systems, Sparks, MD) which includes oleic acid-albumin-dextrose-catalase growth supplement and an antibiotic mixture of polymyxin B, amphotericin B, nalidixic acid, trimethoprim, and azlocillin. After inoculation, MGIT 960 tubes were introduced into the instrument and incubated at 37°C. Cultures were monitored automatically every 60 min for 42 days, and positive cultures were flagged by visual and acoustic signals. Löwenstein-Jensen slants (BBL; Becton Dickinson Microbiology Systems, Sparks, MD) were incubated aerobically at 36°C ± 1°C in a non-CO2 incubator for 8 weeks, keeping the caps loose for the first week and then tightening them. As a quality control test (7-ml BBL MGIT package insert; Becton, Dickinson, and Co., Sparks, MD), mycobacterial strains obtained from the American Type Culture Collection (ATCC), including *M. tuberculosis H37Rv* ATCC 27294, *M. avium* ATCC 15769, *M. kansasii* ATCC 12478, and *M. fortuitum* ATCC 6841, were tested with each new batch of MGIT 960 medium.

Smears were stained with auramine-rhodamine fluorochrome to prevent a significant amount of previously undetected growth from being missed.

* Corresponding author. Mailing address: Department of Clinical Microbiology, United Hospitals, Via Conca 71, Ancona I-60020, Italy. Phone: 39-071-596-3049. Fax: 39-071-596-4184. E-mail: piersim@tin.it.

† Published ahead of print on 14 January 2009.
chrome or by the Ziehl-Neelsen method to detect mycobacteria. Smears obtained from MGIT medium were prepared from a portion of liquid medium (0.2 ml) centrifuged at 6,000 × g for 10 min at room temperature.

Tubes flagged by the MGIT 960 instrument were determined to be positive only when smears confirmed the presence of AFB. Similarly, tubes discarded because they were determined to be negative by the instrument after 42 days of incubation were removed and visually inspected for the presence of granules or other macroscopic evidence of growth using a conventional light source. Whenever detected, the color and number of the granules were evaluated. One or more of them were picked up, crushed on the surface of a microscope slide, and checked for acid fastness by Ziehl-Neelsen staining. Tubes showing acid-fast granules were subcultured onto Middlebrook 7H11 plate medium (BBL; Becton Dickinson Microbiology Systems, Sparks, MD). Finally, negative test samples growing mycobacteria upon subculturing were recorded as false negatives.

Isolates were identified by specific DNA probe assays (Accuprobe from Gen-Probe Inc., San Diego, CA, and INNO-LiPA mycobacteria from Innogenetics, Ghent, Belgium) and by conventional biochemical and cultural tests (2). Probes were chosen on the basis of AFB microscopic appearance in liquid medium (8, 11) and pellet pigmentation. Some isolates were identified by their pattern of mycolic acids or by gene sequencing (2).

The statistical significance in detection rates was determined by the chi-square test (Epi Info v6.03; CDC). P values of ≥0.05 were considered significant.

Three hundred sixty-nine mycobacterial isolates grew from 3,648 specimens. Of these, 152 (41.2%) showed AFB-positive smears and 217 (58.8%) showed negative smears. Isolates from the following mycobacterial species were included: MTC (n = 247), M. xenopi (n = 44), the M. avium complex (n = 30), M. gordonae (n = 26), M. chelonae (n = 8), M. abscessus (n = 7), M. lentiflavum (n = 3), M. fortuitum (n = 2), M. malmoense (n = 1), and the M. terrae complex (n = 1). The percentage of all specimens testing positive for any mycobacterium was 10.1%, whereas the MTC isolation rate was 6.7%. Detection rates for species recovered by the MGIT 960 system described above are summarized in Table 1. With the exception of M. xenopi and the M. avium complex, all mycobacteria grown in the liquid medium were regularly detected by the MGIT 960 instrument. Undetected strains of M. avium complex accounted for 3.3% of the total yield (1 of 30; P = 0.31). Undetected strains of M. xenopi accounted for 70% of the total yield (31 of 44; P < 0.0001). As detection by the MGIT 960 instrument relies on a fluorescence-producing ruthenium salt which is sensitive to the reduction of oxygen consumed by growing organisms, we tried to understand why this system consistently failed to work with M. xenopi. We observed that the majority of tubes from which M. xenopi isolates were recovered showed peculiar growth characterized by a scant number of round, yellow-pigmented granules (approximately 0.05 to 0.1 mm) instead of the usual microflecks or clumps uniformly dispersed on the medium. A correlation between the mean number of granules observed by visual inspection and detection by the MGIT 960 system was also attempted. Although the difference between the mean number of granules observed in the tubes that were flagged as positive (13 tubes) and that in the tubes flagged as negative (31 tubes) by the MGIT 960 instrument was significant (83 granules versus 5 granules; P < 0.01), we were unable to establish a threshold. In fact, while tubes with 7 granules (range, 7 to >100 granules) were flagged as positive by the MGIT 960 instrument, others with up to 25 granules were flagged as negative (range, 1 to 29 granules). Moreover, the pattern of growth exhibited by M. xenopi accounted for many AFB-negative smears obtained from tubes showing fewer than 10 granules (29 of 44).

Although the Bactec MGIT 960 system is a fully automated, nonradiometric, easy-to-use system approved by the Food and Drug Administration, the inability to detect a considerable number of M. xenopi isolates has been reported (7) and our data strongly confirm this finding. In our opinion, missed detection could result from the granular pattern of growth which keeps oxygen consumption below the detection threshold. Since the amount of oxygen consumption depends on the bacterial surfaces in contact with nutrients, a few large granules exhibit narrow surfaces compared to many small flecks or clumps evenly dispersed in the liquid medium. The reasons for such peculiar growth remain unknown. We hypothesize that a small bacterial load (all samples from which undetected M. xenopi isolates grew were negative according to smear microscopy and Löwenstein-Jensen medium culture), slow metabolism, and thermophilic characteristics may each play a role. In addition, mycobacterial clumping should be minimized by polyoxyethylene stearate, a surfactant agent contained in the MGIT medium (7-ml BBL MGIT package insert; Becton, Dickinson, and Co., Sparks, MD). We were also unable to explain why the detection threshold (calculated as the number of granules triggering a positive flag by the instrument) exhibited a broad range. Fortunately, most of the undetected strains had no clinical relevance (4); M. xenopi was associated with disease in two cases. In all these cases, growth in the initial and some repeat cultures was much more luxuriant, allowing easy detection by the MGIT 960 system and isolation on solid medium as well.

The MGIT 960 package insert mentions to visually check tubes determined to be negative by the instrument and stain/subculture them whenever undetected growth is suspected

### Table 1. Evaluation of the Bactec MGIT 960 system for primary growth and detection of mycobacterial clinical isolates

<table>
<thead>
<tr>
<th>Mycobacterial species</th>
<th>No. of strains grown by MGIT liquid medium</th>
<th>No. (%) of strains detected by MGIT 960 instrument</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. tuberculosis complex</td>
<td>247</td>
<td>247</td>
<td>NS</td>
</tr>
<tr>
<td>M. xenopi</td>
<td>44</td>
<td>13 (30)</td>
<td>0.0001</td>
</tr>
<tr>
<td>M. avium complex</td>
<td>30</td>
<td>29 (97)</td>
<td>NS</td>
</tr>
<tr>
<td>M. gordonae</td>
<td>26</td>
<td>26</td>
<td>NS</td>
</tr>
<tr>
<td>M. chelonae</td>
<td>8</td>
<td>8</td>
<td>NS</td>
</tr>
<tr>
<td>M. abscessus</td>
<td>7</td>
<td>7</td>
<td>NS</td>
</tr>
<tr>
<td>M. lentiflavum</td>
<td>3</td>
<td>3</td>
<td>NS</td>
</tr>
<tr>
<td>M. fortuitum</td>
<td>2</td>
<td>2</td>
<td>NS</td>
</tr>
<tr>
<td>Other</td>
<td>2</td>
<td>2</td>
<td>NS</td>
</tr>
<tr>
<td>Total</td>
<td>369</td>
<td>337 (91.3)</td>
<td>NS</td>
</tr>
</tbody>
</table>

*NS, not significant.
(7-ml BBL MGIT package insert; Becton, Dickinson, and Co., Sparks, MD). We suggest modifying the procedure described above as follows: (i) check for the presence of granules (pigmented or not) by visual inspection, and (ii) whenever detected, inspect granules for their acid fastness in addition to preparing a slide for acid-fast staining from liquid medium.

REFERENCES
2. CLSI. 2007. Laboratory detection and identification of mycobacteria: proposed guideline. CLSI M48-P. Clinical and Laboratory Standards Institute, Wayne, PA.