Mycobacteria remain an important cause of infectious diseases in developing and developed countries alike. Although amplified molecular methods are making inroads as diagnostic tools, culture-based methods are still heavily relied upon. Owing to the challenges of cultivating mycobacteria, use of at least two culture media (one liquid, one solid) is recommended to maximize recovery of clinical isolates (4, 10).

Until recently, liquid broth mycobacterial culture involved a radiometric growth detection method. Many clinical laboratories, including our own, have adopted nonradiometric detection methods, which offer several quality and safety advantages (1, 5, 8, 12, 13, 16) and can be monitored by automated instruments. One important drawback of these nonradiometric methods is that, occasionally, mycobacterial growth is present in the liquid medium culture but is not recognized by the instrumented detection system (11, 13). This problem was not frequently encountered with the radiometric approach. The device manufacturers acknowledge this drawback in their product inserts and recommend supplementary procedures to detect “instrument-negative” mycobacterial growth (Table 1) (2, 3, 17). However, it is unclear how frequently these growth detection failures occur or which mycobacterial species are most likely to produce them, although recent reports have indicated that Mycobacterium xenopi isolates are a particular problem (6, 11). Here, we address these questions by reviewing a 3-year experience with the Bactec MGIT 960 culture system in the Clinical Microbiology Laboratories at Massachusetts General Hospital.

Clinical specimens received for mycobacterial culture between January 2007 and December 2009 were processed using conventional procedures as previously described (4, 7, 9). After digestion-decontamination (if indicated) and concentration, the sediment was used to prepare an acid-fast stained smear and to inoculate both a Lowenstein-Jensen (LJ) slant and a mycobacterial growth indicator tube (MGIT; Becton, Dickinson) supplemented with an antibiotic mixture of polymyxin B, amphotericin B, nalidixic acid, trimethoprim, and azlocillin (PANTA) and growth supplement (Becton, Dickinson). MGIT broth cultures were initially incubated at 37°C for up to 42 days using automated Bactec 960 instruments, whereas LJ slants were incubated at 37°C for up to 56 days in an 8% CO2 incubator. After 42 days of incubation, all instrument-negative MGIT cultures (i.e., tubes in which growth had not been detected by the Bactec 960 instrument) were inspected visually for potential mycobacterial growth (Fig. 1), and a further workup was performed according to the algorithm described in Fig. 2. Acid-fast isolates were identified using Accu-Probe hybridization probes (Gen-Probe, San Diego, CA) for M. tuberculosis complex, M. avium complex, M. kansasii, or M. gordonae. Isolates that could not be identified by the AccuProbe assays were forwarded to a reference laboratory for identification using PCR coupled with restriction endonuclease analysis, as previously described (14, 15).

Over the 3-year study period, the laboratory received 10,263 specimens for mycobacterial culture. Among these, 1,323 (13%) were culture positive for mycobacteria (Table 2). Mycobacterial growth was detected by the Bactec 960 instruments within the routine 42-day incubation period in 1,189 of 1,323 culture-positive isolates (89%). Isolates from instrument-negative MGIT cultures included both tuberculous and nontuberculous mycobacteria.

### TABLE 1 Manufacturer-recommended supplementary procedures for detection of mycobacterial growth in instrument-negative cultures

<table>
<thead>
<tr>
<th>Manufacturer</th>
<th>Product</th>
<th>Recommended procedure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Becton, Dickinson</td>
<td>BBL MGIT</td>
<td>Perform a visual check of all instrument-negative bottles for turbidity. If bottle appears nonhomogeneous turbidity, small grains or clumps, it should be subcultured, acid-fast stained, and treated as a presumptive positive, provided the acid-fast smear result is positive (2).</td>
</tr>
<tr>
<td>bioMérieux</td>
<td>BacT/Alert MB</td>
<td>Instrument-negative cultures may be checked by smear and/or subcultured prior to discarding as negative (3).</td>
</tr>
<tr>
<td>Trek Diagnostic Systems</td>
<td>VersaTREK Myco</td>
<td>Perform a visual inspection of instrument-negative bottles for turbidity. If bottle is turbid, obtain a sample for acid-fast staining and subculture (17).</td>
</tr>
</tbody>
</table>

Mycobacterial growth in liquid culture can go undetected by automated, nonradiometric growth detection systems. In our laboratory, instrument-negative tubes from the Bactec MGIT 960 system are visually inspected for potential mycobacterial growth, which is examined by acid-fast smear analysis. A 3-year review demonstrated that ~1% of instrument-negative MGIT cultures contained mycobacterial growth and that 10% of all cultures yielding mycobacteria were instrument negative. Isolates from instrument-negative MGIT cultures included both tuberculous and nontuberculous mycobacteria.

Received 11 January 2012 Returned for modification 10 February 2012 Accepted 28 March 2012 Published ahead of print 4 April 2012 Address correspondence to John A. Branda, jbranda@partners.org. Copyright © 2012, American Society for Microbiology. All Rights Reserved. doi:10.1128/JCM.00108-12
tive specimens (90%). These cultures, designated “instrument positive,” yielded 1,266 mycobacterial isolates. Mycobacterial growth in the remaining 134 (10%) culture-positive specimens was not detected by the Bactec 960 instrument. These 134 specimens were designated “instrument negative, culture positive,” or

![FIG 1 Typical appearance of mycobacterial growth in instrument-negative broth culture. The organisms tend to form colony-like clumps (arrows) at the bottom of the tube, along the surface of the fluorescent indicator.](image)

![FIG 2 Algorithm for handling instrument-negative cultures in the Bactec MGIT 960 system. At the completion of the standard 42-day incubation protocol, supplementary procedures were applied to the MGIT broth culture if the original specimen’s primary AFB smear had been positive and/or colony-like clumps were visible at the bottom of the tube (see Fig. 1).](image)

**TABLE 2** Comparison of instrument-positive and instrument-negative specimens according to specimen type

<table>
<thead>
<tr>
<th>Specimen type</th>
<th>Instrument positive (n = 1,189)</th>
<th>LJ culture-positive (N = 18)</th>
<th>LJ culture-negative (N = 59)</th>
<th>Visual inspection positive (n = 77)</th>
<th>Visual inspection negative, LJ culture-positive (n = 57)</th>
<th>Total INCP (n = 134)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sputum</td>
<td>386 (32)</td>
<td>14 (78)</td>
<td>43 (73)</td>
<td>39 (68)</td>
<td>96 (72)</td>
<td></td>
</tr>
<tr>
<td>BAL fluid</td>
<td>241 (20)</td>
<td>2 (11)</td>
<td>9 (15)</td>
<td>9 (16)</td>
<td>20 (15)</td>
<td></td>
</tr>
<tr>
<td>Lung or pleural tissue</td>
<td>186 (16)</td>
<td>1 (6)</td>
<td>3 (5)</td>
<td>4 (7)</td>
<td>8 (6)</td>
<td></td>
</tr>
<tr>
<td>Lymph node</td>
<td>80 (7)</td>
<td>1 (6)</td>
<td>1 (2)</td>
<td>0</td>
<td>2 (1)</td>
<td></td>
</tr>
<tr>
<td>Wound</td>
<td>25 (2)</td>
<td>0</td>
<td>1 (2)</td>
<td>3 (5)</td>
<td>4 (3)</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>271 (23)</td>
<td>0</td>
<td>2 (3)</td>
<td>2 (4)</td>
<td>4 (3)</td>
<td></td>
</tr>
<tr>
<td>Total instrument-negative</td>
<td>572 (48)</td>
<td>20 (11)</td>
<td>19 (14)</td>
<td>20 (15)</td>
<td>46 (34)</td>
<td>134</td>
</tr>
</tbody>
</table>

*a* LJ, Lowenstein-Jensen; INCP, instrument negative, culture positive; BAL, bronchoalveolar lavage.

*b* Includes expectorated and induced sputa.

*c* Includes skin and subcutaneous tissue biopsy samples.

*d* Includes tissue, fluid, or abscess material from the abdomen, pelvis, central nervous system, head and neck, bones, gastrointestinal tract, skin, deep soft tissue, breast, joints, or heart/pericardium.
INCP. In 59 (44%) of 134 INCP specimens, mycobacterial growth was detected only by visual inspection of the MGIT broth tube and was not found in the cognate LJ slant culture. An additional 18 specimens were positive both by visual inspection of the MGIT and by LJ slant culture, for a total of 77 (~1%) “instrument-negative, visual inspection-positive” (INVP) specimens from 9,074 instrument-negative specimens.

When INVP specimens were stratified according to specimen type, sputum specimens were shown to be overrepresented in comparison to instrument-positive specimens. Whereas 32% of instrument-positive specimens were sputum specimens and were receiving antituberculosis agents at the time of recent diagnosis of active tuberculosis and had had other positive cultures of isolation were not found. Notably, 57 of 76 NTM isolates (75%) were not recovered in the cognate LJ slant tube and would not have been detected if instrument-negative broth cultures had not been visually inspected. Furthermore, 25 (44%) of those 57 isolates were derived from patients whose antecedent mycobacterial cultures (if any) had been negative and who had no history of mycobacterial infection.

The findings of this study present a challenge for the clinical laboratory. Only ~1% of instrument-negative broth cultures contained mycobacterial growth, but fully 10% of all positive mycobacterial cultures were instrument negative, and in some cases the
undetected organism was pathogenic (including *M. tuberculosis*) and was not recovered on solid medium. Rather than applying supplementary detection procedures to all instrument-negative broth cultures, which would be impractical, we recommend supplementary procedures for instrument-negative broth cultures containing colony-like clumps at the bottom of the tube after 42 days of incubation and for instrument-negative broth cultures of AFB smear-positive specimens.

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