Myobacteriology

Performance of the BACTEC MGIT 960 compared with solid media for detection of *Mycobacterium* in Bangkok, Thailand☆

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**Abstract**

Controlled trials have demonstrated that liquid media culture (LMC) is superior to solid media culture for diagnosis of *Mycobacterium tuberculosis* (MTB), but there is limited evidence about its performance in resource-limited settings. We evaluated the performance of LMC in a demonstration project in Bangkok, Thailand. Sputum specimens from persons with suspected or clinically diagnosed tuberculosis were inoculated in parallel on solid (Lowenstein–Jensen [LJ]) and liquid (mycobacterial growth indicator tube [MGIT 960]) media. Biochemical tests identified isolates as MTB or nontuberculosis mycobacteria (NTM). Of 2566 specimens received from October 2004 to September 2006, 1355 (53%) were culture positive by MGIT compared with 1013 (39%) by LJ. Median time to growth for MGIT was significantly less than LJ: 11 versus 27 days. Of 1417 isolates detected by at least 1 media, 1255 (86%) were identified as MTB and 162 (11%) NTM. MGIT improved speed and sensitivity of MTB isolation and drug susceptibility testing, regardless of HIV status.

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1. **Introduction**

The global pandemic of tuberculosis (TB) is estimated to have caused disease in 8 million and killed 1.6 million persons in 2006 (*World Health Organization, 2007a*). Rates of disease are highest in low- and middle-income countries of sub-Saharan Africa and Southeast Asia (*World Health Organization, 2007a*). Case finding and diagnosis remain major challenges to TB control in these countries. In high-income countries, TB diagnosis depends on culture of sputum usually on both liquid and solid media (*Steingart et al., 2006*). In contrast, TB diagnosis in low- and middle-income countries depends on microscopic examination of self-expectorated unprocessed sputum stained for acid-fast bacilli (AFB). When compared with culture, microscopy is known to be insensitive, but it remains the international standard for TB diagnosis (*Steingart et al., 2006*).

The emergence of the HIV and multidrug-resistant TB (MDR-TB) epidemics has exposed the limitations of smear
microscopy. HIV-infected TB patients frequently have negative sputum smears, and drug susceptibility testing (DST) for MDR-TB requires isolation of Mycobacterium tuberculosis (MTB) in culture (Steingart et al., 2006). As a result, in 2005, the World Health Organization (WHO) began advocating for countries to begin developing facilities for mycobacterial culture, as part of the “Second Global Plan to Stop TB, 2006–2015”. In response to the emergence of extensively drug-resistant TB, WHO is now calling for universal access to culture and DST by 2015 for all new pulmonary TB patients (Stop TB Partnership and World Health Organization, 2006).

A major public health policy question is whether low- and middle-income countries should build their laboratory infrastructure to perform conventional solid media culture (SMC) (e.g., Lowenstein–Jensen [LJ], Ogawa) or to perform both SMC and liquid media culture (LMC). Several large studies have demonstrated that LMC is superior to SMC, returning results faster and yielding more mycobacterial isolates, including nontuberculosis mycobacteria (NTM) (Cruciani et al., 2004; Hanna et al., 1999; Idigoras et al., 2000; Kanchana et al., 2000; Lee et al., 2003; Lu et al., 2002; Tortoli et al., 1999; Williams-Bouyer et al., 2000). Despite LMC’s superior performance, global health officials have been reluctant to endorse it because of the relatively high cost of supplies, equipment, and training and because there are few studies that have evaluated LMC’s performance characteristics in routine program settings of high-burden resource-limited countries (Githui, 2002; Macondo et al., 2000; Rishi et al., 2007; Rivera et al., 1997).

Thailand is a middle-income country with the 17th highest burden of TB in the world and a generalized HIV epidemic. In 2004, we began a project in Bangkok, Thailand, to evaluate the feasibility and effectiveness of LMC in diagnosis of TB and MDR-TB among patients seeking care at public TB clinics.

2. Materials and methods

2.1. Program setting

Bangkok’s public health system includes 68 public health centers for 50 districts; 19 centers serve as management units for TB diagnosis, registration, and treatment. In 2003, we established capacity at the Bangkok metropolitan administration (BMA) city laboratory to perform SMC, LMC, and DST. All 68 health centers began forwarding sputum specimens from persons suspected or diagnosed as having TB; specimens were also occasionally sent for persons already receiving TB treatment. Follow-up specimens from persons on TB treatment were excluded from the current analysis. Seven of these health centers also began collecting detailed epidemiologic and clinical data, including HIV status, about all persons diagnosed with TB in 9 districts (Varma et al., 2007). We analyzed data for specimens received at the BMA laboratory from October 2004 to September 2006.

2.2. Specimen collection and transport

At health centers, patients were asked to self-expectorate sputum into a plastic collection cup; spot and morning specimens were collected according to routine practice. No sputum specimens were induced. Patients received no incentives, and health care providers received no project-specific training in specimen collection. Specimens were transported in an ice chest, usually on the same day, to the BMA city laboratory using an established government specimen transport system. A sample of sputum was retained at the health center for direct smear microscopy. Results of smear microscopy performed at health centers were not analyzed.

2.3. Specimen processing

Upon receipt at the BMA laboratory, specimens were liquefied and decontaminated using an equal volume of 4% sodium hydroxide (NaOH), 2.9% sodium citrate solution, and N-acetyl-L-cysteine powder (final NaOH concentration, 1%) for 15 min at room temperature. After decontamination, specimens were neutralized with sterile phosphate buffer (PBS, pH 6.8) and centrifuged at 3000 × g for 15 min. The supernatant was poured off and sediment suspended in 1.5 mL of sterile PBS, pH 6.8.

2.4. Acid-fast bacilli smears

Smears were prepared using the dissolved sediment from all specimens, stained by the Ziehl–Neelsen (ZN) method, and examined for presence of AFB with a light microscope. Smears were recorded as positive if at least 10 AFB per 100 high power field (HPF) were observed (International Union Against Tuberculosis and Lung Disease [IUATLD], 2000). Smears that were initially recorded as scanty by the laboratory (IUATLD/WHO scale <10 AFB/100 HPF) were recoded as positive for analysis.

2.5. Culture systems

The suspension remaining after AFB smear preparation was inoculated in parallel into both liquid (BACTEC MGIT 960) and solid (LJ) media.

2.5.1. LJ culture

Two LJ slants were processed for each specimen with 0.1 mL of the specimen suspension inoculated onto each LJ media. LJ slants were incubated at 37 °C for 8 weeks in an incubator and observed for growth everyday for the 1st 3 days, and then once a week until 8 weeks. Time to detection was calculated as the time from the date of culture inoculation to the earliest date of visible colonies.

2.5.2. BACTEC MGIT 960 liquid media

The BACTEC MGIT 960 is a fully automated, high-volume, nonradiometric instrument that offers continuous monitoring of culture growth. Technicians inoculated 0.5 mL of specimen suspension into each 7-mL mycobacterial
growth indicator tube (MGIT) culture tube, which contained Middlebrook 7H9 broth base enriched with oleic acid, albumin, dextrose, and catalase (BBL MGIT OADC) and an antibiotic mixture of polymyxin B, amphotericin B, nalidixic acid, trimethoprim, and azlocillin (BBL MGIT PANTA). Tubes were incubated at 37 °C in the BACTEC MGIT 960 for up to 42 days. The BACTEC MGIT 960 monitors specimens hourly for an increase in fluorescence, at which time, the operator is audibly and visibly alerted to the location of tubes sensing the presence of mycobacteria. All specimens identified as positive by MGIT were confirmed using subsequent AFB smears. If no AFB were observed, the MGIT tube was returned to the instrument and incubated for a total of 42 days. Time to detection was calculated as the time between the date of culture inoculation and the earliest date the instrument recorded positive growth.

2.6. Identification of mycobacteria

All specimens found to be positive from any culture were examined by AFB staining (ZN) on the day of detection to confirm the presence of AFB. AFB-positive cultures were subcultured onto LJ slants for identification and DST. Mycobacteria were differentiated as MTB and NTM based on growth rate on solid media, colony characteristics and appearance, and biochemical tests using para-nitro-benzoid acid, the niacin production test, and the nitrate reduction test. Contaminants were not identified. A convenience sample of NTM isolates underwent high-performance liquid chromatography (HPLC) for species identification.

2.7. Drug susceptibility testing

Indirect DST was performed by the proportion method on LJ for all specimens identified as MTB from 61 health centers, and by BACTEC MGIT 960 for specimens identified from 7 health centers that participate in a related surveillance project (Canetti et al., 1969). Isolates were tested for susceptibility to 1st-line anti-TB drugs isoniazid, rifampin (RIF), streptomycin, and ethambutol.

2.8. Patient data

Basic demographic information about patients, including age, sex, and geographic location, was recorded in laboratory requisition forms. For specimens obtained from patients in the 7 health centers that were part of the Thailand Active TB Surveillance Program, health center nurses recorded more detailed data about patients on standardized surveillance and treatment outcome forms.

2.9. HIV antibody testing

Serum specimens were tested for the presence of HIV antibodies by ELISA using commercially licensed kits (Enzygnost Anti HIV 1/2 Plus [Dade Behring, Deerfield, IL], Genscreen HIV1/2 [Bio-Rad Laboratories, Hercules, CA], or Murex HIV 1.2.0 [Abbott Laboratories, Abbott Park, IL]). Nonreactive specimens were considered HIV negative. Specimens reactive using the ELISA method were subsequently tested using the gel particle agglutination (GPA) test for HIV antibodies (Serodia-HIV; Fujirebio Diagnostics, Malvern, PA). A 2nd serum sample was reevaluated by both ELISA and GPA to confirm reactivity; specimens reactive to both ELISA and GPA were considered positive. Specimens that were nonreactive to GPA were confirmed by Western blot analysis.

2.10. Data management and statistical analysis

Laboratory staff entered data into an electronic laboratory information management system. For patients from 7 health centers, health center nurses entered patient data into a standardized TB surveillance database designed for this project. Laboratory and patient records were linked using a unique patient identifier. All statistical analyses were performed using STATA version 8.0. The Wilcoxon matched pairs test was used to compare medians for time to detection. Test for trend was used to evaluate contamination rates over the observation period. Statistical significance was defined as \( P < 0.05 \).

2.11. Ethical review

The protocol for this project was reviewed by the Thailand Ministry of Public Health (MOPH), BMA, and U.S. Centers for Disease Control and Prevention and found to be surveillance and public health program implementation not requiring oversight by a human subjects research institutional review board.

3. Results

From 2004 to 2006, 2566 specimens collected from 2504 patients were received and processed for detection and
identification of mycobacteria; 1044 (41%) specimens were AFB smear positive, and 1522 (59%) were AFB smear negative. For 415 (17%) of 2504 patients, detailed patient data were available.

3.1. Recovery rates and identification of mycobacteria

Overall, the detection rate was 39% on LJ and 53% on MGIT (Table 1). In smear-negative specimens, detection rates were lower compared with smear-positive specimens; in these specimens, detection rates using MGIT were more than 2 times greater than LJ. The proportion of specimens in which mycobacteria were detected each month ranged from 44% to 73% for all specimens (P trend = 0.83), 82% to 95% for smear-positive specimens (P trend = 0.04), and 21% to 55% for smear-negative specimens (P trend = 0.80). Results were similar when the analysis was restricted to the 1st specimen obtained from each patient (n = 2504, data not shown).

Of the 1417 isolates detected by at least 1 media, 1255 (86%) were identified as MTB and 162 (11%) as NTM. Of the 1255 MTB isolates, only 79% were recovered using LJ compared with 95% recovered by MGIT. Almost all (98%) NTM isolates were detected using MGIT; only 16% of NTM isolates were detected using LJ. Identification of species for a convenience sample of 10 NTM isolates using HPLC revealed 7 Mycobacterium chelonae/abscessus, 1 Mycobacterium fortuitum/peregrinum, 1 Mycobacterium asiaticum, and 1 Mycobacterium scrofulaceum; 8 patients with NTM isolated from sputum were HIV uninfected, and 2 (both with M. chelonae/abscessus) were not HIV tested.

Of 389 persons with both HIV status and laboratory culture results, 71 were HIV infected (18%) and 318 (82%) were HIV uninfected. MGIT was more sensitive than LJ regardless of HIV status, with sensitivity almost twice that of LJ for smear-negative specimens from HIV-infected patients (Table 2). Of the 272 specimens that grew on at least 1 culture media, 256 (94%) were identified as MTB and 16 (6%) as NTM.

3.2. Contamination rates

Overall, the contamination rate was 10% for LJ and 12% for MGIT; 2% of cultures were contaminated on both media. The LJ culture contamination rate was similar for smear-positive and smear-negative specimens, but MGIT culture contamination rates were markedly higher in smear-negative specimens (15%) compared with specimens that were smear positive (9%). For LJ cultures, the proportion contaminated each month varied from 0% to 24% and showed a significant downward trend (P value for trend <0.01, Fig. 1). For MGIT cultures, the overall proportion contaminated each month ranged from 5% to 23% and also demonstrated a significant reduction in contamination over the period evaluated (P value for trend <0.01, Fig. 2). The significant trend in decreasing contamination rates over time using MGIT was evident for both smear-negative and smear-positive cultures (data not shown, both P value for trend <0.01).

3.3. Drug susceptibility

Test results for drug susceptibility to the 1st-line drugs were available for 1218 (97%) of 1255 MTB isolates. Thirteen percent of isolates were resistant to isoniazid, 4% to RIF, 12% to streptomycin, and 4% to ethambutol. Thirty-seven (3%) isolates were multidrug resistant.

3.4. Time to detection of mycobacterial growth and reporting of drug susceptibility tests

For the 951 specimens that grew on both LJ and MGIT, the median time to growth for MGIT was significantly less
than LJ: 11 (interquartile range [IQR], 7–13) versus 27 (IQR, 23–34) days for all specimens, 9 (IQR, 7–12) versus 26 (IQR, 21–33) days for smear-positive specimens, and 14 (IQR, 12–17) versus 35 (IQR, 29–42) days for smear-negative specimens (P < 0.01, all comparisons).

To compare the total turnaround time from specimen collection to DST for LJ versus MGIT, we limited our analysis to isolates that underwent culture and DST using the same method (e.g., MGIT culture and MGIT DST) and to only those isolates that were MTB. We found that the total median turnaround time was 43 days for specimens tested using MGIT compared with 58 days for specimens tested using LJ (Table 3).

<table>
<thead>
<tr>
<th>Step</th>
<th>LJ*</th>
<th>MGIT*</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Median days (IQR)</td>
<td>Median days (IQR)</td>
</tr>
<tr>
<td>Collection to AFB</td>
<td>0 (0–1)</td>
<td>0 (0–1)</td>
</tr>
<tr>
<td>Collection to culture inoculation</td>
<td>3 (1–6)</td>
<td>3 (1–6)</td>
</tr>
<tr>
<td>Culture inoculation to 1st positive b</td>
<td>27 (23–34)</td>
<td>12 (7–15)</td>
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<tr>
<td>1st positive to DST testing, including subculture and identification</td>
<td>14 (11–17)</td>
<td>14 (11–19)</td>
</tr>
<tr>
<td>DST testing to DST reading</td>
<td>29 (28–31)</td>
<td>11 (8–16)</td>
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<tr>
<td>DST reading to DST reporting</td>
<td>0</td>
<td>0</td>
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<tr>
<td>Total delay from specimen collection to DST report to physician</td>
<td>58 (54–65)</td>
<td>43 (36–51)</td>
</tr>
</tbody>
</table>

* Method used for DST.

b For all BMA, it indicates time to culture positive on any media; LJ and MGIT columns indicate time from culture inoculation to 1st positive on LJ or MGIT culture media, respectively.

4. Discussion

This study demonstrates that the BACTEC MGIT 960 LMC method performs well in a high-burden TB country, with marked increases in sensitivity and time-to-detection for culture and DST compared with LJ SMC. We also demonstrated sustained marked reductions in contamination rates from the beginning of implementation until the final analysis point, demonstrating that high-quality LMC is feasible in resource-limited program settings. The feasibility and performance of MGIT have been demonstrated in studies conducted in both resource-rich and resource-limited settings (Cruciani et al., 2004; Hanna et al., 1999; Idigoras et al., 2000; Kanchana et al., 2000; Lee et al., 2003; Lu et al., 2002; Macondo et al., 2000; Rishi et al., 2007; Rivera et al., 1997; Tortoli et al., 1999; Williams-Bouyer et al., 2000). Our study adds to the existing evidence base because it used a large number of specimens collected prospectively for routine patient care, it describes data collected over 2 years from the routine workflow of a government clinical microbiology laboratory, and it was conducted in a setting with a high burden of TB and HIV. Data such as these are essential to setting international health policy because it helps demonstrate the real-world utility of technologies that have already been proven in controlled clinical studies (World Health Organization, 2007b).

Rates of smear-negative pulmonary TB have been rising globally because of the HIV epidemic (World Health Organization, 2007c). WHO guidelines now recommend that national TB programs perform culture in all HIV-infected TB suspects that have negative sputum smears, but these guidelines do not recommend a specific culture method (Getahun et al., 2007). Our data suggest that MGIT should be used for such patients, because MGIT had twice the sensitivity compared with LJ in smear-negative HIV-infected patients. Although our sample size of known HIV-infected patients was fairly small, our data are consistent with at least 1 other small study that demonstrated enhanced performance of LMC compared with SMC in HIV-infected patients (Moore et al., 2006).

Culture is also required to confirm drug-resistant TB, but culture and DST rarely guide treatment decisions in high-burden countries because results return several months into TB treatment (Joh et al., 2007). Ideally, results should be provided to physicians as early as possible to prevent amplification of drug resistance (Cox et al., 2007). To respond to the emergence of MDR-TB in the early 1990s, the United States invested heavily in LMC and set standards for laboratories to report DST within 30 days of specimen collection (Tenover et al., 1993). Our data demonstrate that similar turnaround times can be obtained in more resource-limited settings. One substantial contributor to delay is the period between a bottle 1st signaling growth and identification of the isolate as MTB. Simple inexpensive methods for identification of mycobacteria are needed, particularly given the high rates of NTM that we found in this patient population.

In evaluating this program, we learned important lessons about the nonmicrobiologic challenges of implementing liquid culture in a resource-limited setting. There is a limited supply of trained clinical microbiologists willing to work in the public sector. Efforts to scale up culture in developing countries will require countries to increase the supply of trained personnel and to increase pay or other incentives for attracting and retaining staff. We also found that microbiologists often had little time set aside for recording and reporting data. Although we used an electronic data management system, personnel frequently reported that they only had enough time to fill out the requisite paper records for reporting results back to clinicians, not to enter data into a database. As our report demonstrates, electronic data management greatly facilitates laboratory quality evaluation. Future efforts to scale up mycobacterial culture will require tools to integrate quality monitoring and routine clinical reporting.

Our study has several important limitations. First, we relied on data collected for routine program purposes and only had patient data, such as HIV infection status, available for a subset of patients. These patients may not be
representative of the entire population. Second, detection rates were not as high as expected for both smear-positive and smear-negative specimens. Several reasons exist that may explain the low yield, including, but not limited to, a) problems in specimen transport or storage, b) overprocessing of the specimens, and c) problems with culture media. Also, the contamination rate of LJ was higher than that commonly reported in other studies (5%), which could further diminish our estimated recovery rate and overexaggerate the benefits of MGIT. Finally, we have not yet analyzed the cost-effectiveness of the BACTEC MGIT 960. Establishing mycobacterial culture facilities is resource intensive, requiring substantial investment in equipment, supplies, and personnel. These costs need to be weighed carefully against the costs of not performing culture, including treating patients when they do not have TB (e.g., NTM), delay in or failure to diagnose MDR-TB, and TB transmission from undiagnosed TB. Although we have clearly demonstrated that mycobacterial laboratory performance in a developing country can achieve that of industrialized countries, further evaluation is needed to quantify the public health return on this investment.

References


