Comparison of four culture systems for *Mycobacterium tuberculosis* in the Zambian National Reference Laboratory

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**SETTING:** National TB Reference Laboratory, Zambia.

**OBJECTIVE:** To compare four TB culture systems when used in a resource-limited setting.

**DESIGN:** Comparison of four culture systems: automated Mycobacterium Growth Indicator Tube (AMGIT) 960, manual MGIT (MMGIT) and two Löwenstein-Jensen (LJ) culture media—commercial (CLJ) and home-made (HLJ).

**RESULTS:** A total of 1916 sputum specimens were received, of which 261 (13.6%) were positive on microscopy. *Mycobacterium tuberculosis* complex (MTC) was isolated on at least one of the media in 410 (21.4%) specimens: MMMGIT recovered 336 (17.5%) MTC, AMGIT 329 (17.2%), CLJ 192 (10.0%) and HLJ 184 (9.6%). The median time to detection for smear-negative specimens was 14 days for AMGIT, 16 days for MMMGIT and 34 days for both LJ. Isolation of non-tuberculous mycobacteria (NTM) was more frequent in both MGIT systems (3.5%) than in CLJ (0.9%) and HLJ (0.8%). Contamination rates were high: 29.6% on AMGIT, 23.8% on MMMGIT, 14.9% on CLJ and 12.5% on HLJ.

**CONCLUSION:** Despite high contamination rates, either MGIT system considerably improved both the yield and the time to detection of MTC compared to LJ media. Investments in infrastructure and training are needed if culture is to be scaled up in low-income settings such as this.

**KEY WORDS:** Mycobacteria tuberculosis; mycobacteria culture; MGIT; Zambia

**THE BURDEN OF TUBERCULOSIS (TB) continues to rise in sub-Saharan Africa, with this region accounting for 23% of all reported cases of TB in 2005.**¹ There is also increasing concern about multidrug-resistant TB and the emergence of extensively drug-resistant TB.² Despite the magnitude of the problem, sensitive diagnostic tools are largely unavailable in resource-limited countries. The principal diagnostic tool is sputum smear microscopy, which is known to have low sensitivity, especially when used in areas with a high burden of human immunodeficiency virus (HIV) infection.³⁻⁵ The Stop TB Department of the World Health Organization (WHO) has recently recommended extending the use of culture for the routine diagnosis of TB in resource-limited settings.⁶ The rationale for this is to diagnose additional cases of TB among people living with HIV and to improve the availability of drug susceptibility testing (DST).

In areas of high dual TB and HIV infection, mycobacterial culture is a more sensitive and specific method of diagnosing TB.⁷ While liquid culture is the gold standard for the diagnosis of TB in high-income countries, resource-limited settings are dependent on conventional solid media for culture detection of mycobacteria, as it can be prepared locally.⁸ Recently, however, due to an increased need for more sensitive and faster culture methods, resource-limited settings have been encouraged to use the more expensive commercial liquid culture systems such as the Mycobacterium Growth Indicator Tube (BBL™ MGIT™ 960, Becton Dickinson Microbiology Systems, Sparks, MD, USA).

The BBL™ MGIT™ 960 system is a fully automated, high-capacity, non-radiometric method of liquid culture. Cultures are monitored continuously for mycobacterial growth by the use of oxygen-sensitive fluorescent sensors embedded in a silicon base.⁷ Manual reading can also be done using a semi-quantitative reader. The MGIT system has proved to be a rapid, easy-to-use technique with a high sensitivity for detection of mycobacteria directly from clinical specimens.⁷⁻¹⁰ Large-scale MGIT evaluation studies have not been performed in resource-limited settings. We conducted a four-way comparison of MGIT 960, manual MGIT and two types of Löwenstein-Jensen (LJ) culture media (commercial [CLJ] and home-made [HLJ]) on routine specimens received in a national reference laboratory in a resource-limited setting.
MATERIALS AND METHODS

Study setting
The Zambian National Reference Laboratory (NRL) for TB was established in 1973 as part of the early trials of chemotherapy for TB. It was later incorporated into the National TB Programme (NTP), and until recently it was the only public facility in the country available for mycobacterial culture. Its principal roles are to supervise quality assurance for the national microscopy network, to culture *Mycobacterium tuberculosis* in specimens referred to the laboratory and to conduct DST for clinical and surveillance purposes. The NRL has Class II safety cabinets, which are serviced every 6 months. The laboratory has a back-up generator that automatically takes over the power supply where there are power outages. Both the safety cabinets and MGIT instruments have uninterrupted power supply (UPS) systems. Before implementing the MGIT systems, staff were trained in the use of MGIT and the laboratory had to undergo renovations to include a separate room to house the MGIT 960 instruments. The cooling system was also improved by increasing the number of air conditioners.

Sample sources
Routine clinical sputum samples came from a tertiary referral hospital (University Teaching Hospital, Lusaka) and from local district health facilities in Lusaka and other parts of Zambia between August 2006 and May 2007. Samples were a mix of initial evaluation of suspected TB patients and TB follow-up patients.

Upon receipt, samples were electronically logged and assigned a laboratory number before processing. Sample processing commenced on the day of specimen arrival.

Decontamination
The samples were digested and decontaminated using the NaOH NALC method with a final NaOH concentration of 1.5% (BBL\textsuperscript{TM} MycoPrep\textsuperscript{TM} KIT, Becton Dickinson).\textsuperscript{11}

After decontamination, the sample was suspended in sterile phosphate buffer and centrifuged at 3000 × g for 15 min. Tubes were left for 5 min to allow aerosols to settle before opening to decant the supernatant, leaving only the sample pellet. The sample pellet was re-suspended in phosphate buffer to achieve a volume of 2–3 ml. Smears were obtained from the concentrated sediments after inoculation. To prevent cross-contamination, only one tube was open at a time and small portions of buffer were used.

Inoculation
Inoculation was performed according to recommended laboratory standard operating procedures.\textsuperscript{11,12} Briefly, 0.5 ml was inoculated into one automated MGIT (AMGIT) tube, one manual MGIT (MMGIT) and 0.1 ml into one CLJ and one HLJ tube. Negative and positive controls were also inoculated. Inoculated tubes were left at room temperature for 30 min before incubation, in line with the manufacturer’s standard procedures.\textsuperscript{12} The MGIT 960 tube was incubated in the MGIT 960 instrument at 37°C. Both the manual MGIT tube and the inoculated LJ media were incubated in stand-alone independent incubators at 37°C.

Quality control
Reference *M. tuberculosis* complex (MTC) strain-H37Rv stock cultures were used as positive controls for every batch and were subjected to the digestion and decontamination process. The phosphate buffer was used as a negative control. Phosphate buffer was made using distilled water, divided into 50 ml bottles and autoclaved before use. A sterility check was done on every batch of HLJ before use by placing it in an incubator for 48 h.

AMGIT cultures were monitored continuously by the MGIT 960 instrument; cultures flagged as having growth were removed from the instrument every day for reading until 42 days had elapsed. MMGIT cultures were read three times a week using the manual reader until 42 days had elapsed. CLJ and HLJ cultures were read once every week for 56 days.

Culture results were scored as positive, negative or contaminated. Once a culture was flagged as having growth by the MGIT instrument or by the manual reader or a buffy growth was observed on LJ, a Ziehl-Neelsen (ZN) stain of the isolate was made and read. If the ZN stain was positive for acid-fast bacilli (AFB), the culture was scored as positive for mycobacteria. A culture with growth was considered contaminated if the ZN stain was negative for AFB. MGIT cultures with no growth after 42 days and LJ cultures with no growth after 56 days were considered negative. The Capilia TB test (Capilia® TB, TAUNS Laboratories Inc, Shizuoka, Japan) was performed on all positive cultures to determine which mycobacterial isolates were MTC, according to the manufacturer’s instructions. Non-tuberculous mycobacteria (NTM) were not further speciated for this study.

Data collection and analysis
The primary outcome measures used for each media system were: 1) yield of MTC and NTM; 2) contamination rate; 3) time to detection (TTD); and 4) time to contamination (TTC). To calculate the sensitivity of each system, a gold standard was defined such that a specimen that was positive for MTC on at least one of the culture systems was considered positive. Secondary analysis compared the outcomes in smear-negative and smear-positive samples and the outcomes of combining different media. The χ² test was used to compare differences in proportions between yield and contamination rates. The *t*-test was used to compare the differences in median TTD. The kappa (κ) statistic was used to look for concordance between media systems.

This study did not require ethical clearance because...
it was performed in routine conditions: clinical specimens sent to the NRL for culture were used, the study did not interfere with the diagnostic process for patients, and details of patients were not known.

RESULTS

A total of 1916 samples from 1083 patients were included in the study. Of these, 411 (21.5%) grew mycobacteria on MMGIT, 397 (20.5%) on AMGIT, 210 (11.0%) on CLJ, and 200 (10.4%) on HLJ (Table 1). Of these, 336 (17.5%) isolates from MMGIT were identified as MTC, 329 (17.2%) from AMGIT, 192 (10.0%) from CLJ, and 184 (9.6%) from HLJ (Table 1).

Using the derived ‘gold standard’, 552 (28.8%) mycobacteria were isolated, of which 410 (74.3%) were MTC and 142 (25.7%) were NTM. The sensitivity of each culture system for MTC isolation in comparison to this gold standard was: MMGIT 81.9%, AMGIT 80.1%, CLJ 46.8% and HLJ 44.9%. There was no statistical significance in the difference in yield observed between the MGIT systems (P = 0.84) or between the two LJ systems (P = 0.73), but there was a significant difference in yield between either MGIT and either LJ system (P < 0.001).

The culture yield increased with increasing smear score for all media systems. The MGIT systems grew mycobacteria in >90% of the highly smear-positive samples, while the LJ systems grew mycobacteria in <50%; 96% of isolates from smear-positive specimens were identified as MTC (Figure).

Among smear-negative specimens, MGIT systems isolated one third more MTC than the LJ systems (P < 0.001).

The TTD for either MGIT was always shorter than for either LJ. The MGIT systems reduced the TTD for smear-negative samples by about 3 weeks. As expected, the TTD for AMGIT was always shorter than the TTD for MMGIT (P = 0.006), as the AMGIT is monitored continuously and read each day, whereas the MMGIT is read three times each week. The TTD for CLJ and HLJ was similar (P = 0.1590) for smear-negative isolates (Table 2).

Contamination

High contamination rates were observed on all culture systems. The AMGIT had the highest contamination rate, of 29.3% (95% confidence interval [CI] 27.2–31.4), followed by MMGIT with 23.8% (95%CI 21.9–25.8), CLJ with 14.5% (95%CI 13.3–16.6), while HLJ had the lowest rate, of 12.5% (95%CI 11.1–14.1) (Table 1).

In both MGIT systems, contamination rates were much lower for specimens that were smear-positive, whereas for LJ systems this trend was not observed (see Figure 1).
**Table 2** Median time to detection (TTD) and time to contamination (TTC) on each culture system

<table>
<thead>
<tr>
<th>Result</th>
<th>AMGIT†</th>
<th>MMGIT</th>
<th>CL J†</th>
<th>HL J</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mycobacteria</td>
<td>11 (6.8)</td>
<td>13 (7.9)</td>
<td>25 (11.9)</td>
<td>27 (11.4)</td>
</tr>
<tr>
<td>MTC</td>
<td>11 (6.0)</td>
<td>13 (7.7)</td>
<td>25 (11.7)</td>
<td>27 (11.2)</td>
</tr>
<tr>
<td>Smear-positive</td>
<td>9.5 (5.4)</td>
<td>11 (7.1)</td>
<td>21 (11.1)</td>
<td>26 (10.9)</td>
</tr>
<tr>
<td>Smear-negative</td>
<td>14 (6.2)</td>
<td>16 (7.8)</td>
<td>34 (11.0)</td>
<td>34 (10.6)</td>
</tr>
<tr>
<td>NTM</td>
<td>8 (8)</td>
<td>8 (10.7)</td>
<td>20 (14.2)</td>
<td>34 (13.8)</td>
</tr>
<tr>
<td>Contamination</td>
<td>8 (7.2)</td>
<td>10 (10.2)</td>
<td>26 (11.7)</td>
<td>25 (13.9)</td>
</tr>
</tbody>
</table>

* Paired t-test AMGIT vs. MMGIT, P < 0.001; HLJ vs. CLJ, P = 0.1590; any MGIT vs. any LJ, P < 0.001.
† AMGIT/CLJ TTD and TTC comparison on samples positive on both media.
‡ HLJ/CLJ TTD and TTC comparison on samples positive on both media.
AMGIT = automated Mycobacterial Growth Indicator Tube; MMGIT = manual Mycobacterial Growth Indicator Tube; CLJ = commercial Löwenstein-Jensen; HLJ = home-made Löwenstein-Jensen; MTC = Mycobacterium tuberculosis complex; NTM = non-tuberculous mycobacteria.

**Non-tuberculous mycobacteria**

Both MGIT systems isolated more NTM than LJ systems, with 75 (3.7%) on MMGIT, 65 (3.5%) on AMGIT, 18 (0.9%) on CLJ and 16 (0.8%) on HLJ (Table 1).

**Performance of combinations of media**

The performance of combinations of media is shown in Table 3. Compared to the gold standard derived from all four media, the combination of MMGIT/AMGIT had the highest sensitivity, of 94%.

The correlation between contaminated results was better between the two MGIT systems (κ = 0.45) than between the two LJ systems (κ = 0.26). The κ statistic for any MGIT compared with any LJ was between 0.13 and 0.19, demonstrating poor agreement between the MGIT systems and the LJ systems. As a result, the number of specimens for which all culture systems were contaminated was lower than when two systems were combined.

**DISCUSSION**

Various studies have been conducted in resource-rich countries to compare the performance of MGIT systems with that of LJ systems for the recovery of mycobacteria, and they have shown that MGIT systems have a better yield for both MTC and NTM and a quicker time to recovery than LJ media.9,10,13 Our study confirms these observations and provides additional information on the use of MGIT systems in resource-limited settings; it also provides information on the comparison of MMGIT and MGIT 960 and CL J and HL J.

Despite the high contamination rates, use of a single MGIT culture detected almost twice as many MTC isolates as an LJ system. Of the MTC isolates detected on a single MGIT system, 38% came from smear-negative specimens; MGIT would thus provide the only microbiological confirmation of the diagnosis in these cases. The MGIT systems also had a quicker time to results and reduced the time to isolation of MTC by 3 weeks in comparison to the LJ systems. The sensitivity of all systems may have been suboptimal because of the harsh decontamination protocol adopted in an effort to reduce contamination rates.

For maximum yield of mycobacteria, the US Centers for Disease Control and Prevention recommend that liquid media should be used in combination with solid media.11 Our findings confirm that when combinations of media are considered and compared to any single culture media, the mycobacterial yield increases. However, the cost and cost-effectiveness of using combination media need to be considered before deciding whether or not to use a combination of media.

Contamination rates were unacceptably high, and reducing them would be likely to make MGIT an even more attractive option for the diagnosis of TB. Although we increased the stringency of the decontamination protocol, not all of the contamination appeared to arise from the decontaminated specimen sediment. While LJ and MGIT use different approaches to reduce bacterial and fungal overgrowth, if the contamination arose directly from the decontaminated specimen sediment we would expect better concordance between the two MGIT systems and between the two LJ systems. This was not the case, and there was only moderate agreement between the MGIT systems and poor agreement between the LJ systems. The contamination process is probably influenced by a number of factors, which might be due to the type of contaminant present in the sample, inoculating and pipetting procedures, contaminants in the laboratory cabinet or individual media tubes and the sensitivity of the different inhibitors present in the media. We do not have answers for these questions, as our study was not designed to answer them and further research is needed.

**Table 3** Performance of combinations of media

<table>
<thead>
<tr>
<th>Isolates</th>
<th>AMGIT/CLJ</th>
<th>MMGIT/CLJ</th>
<th>MMGIT/HLJ</th>
<th>AMGIT/HLJ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>460 (83.3)</td>
<td>372 (90.7)</td>
<td>363 (88.5)</td>
<td>432 (78.3)</td>
</tr>
<tr>
<td>MTC</td>
<td>372 (90.7)</td>
<td>363 (88.5)</td>
<td>353 (86.1)</td>
<td>361 (88.0)</td>
</tr>
<tr>
<td>NTM</td>
<td>86 (60.6)</td>
<td>79 (55.3)</td>
<td>81 (57.0)</td>
<td>81 (57.0)</td>
</tr>
<tr>
<td>Contaminated</td>
<td>107 (102)</td>
<td>136 (136)</td>
<td>143 (143)</td>
<td>143 (143)</td>
</tr>
<tr>
<td>Negative</td>
<td>1349 (1365)</td>
<td>1348 (1331)</td>
<td>1331 (1530)</td>
<td>1530 (1530)</td>
</tr>
</tbody>
</table>

AMGIT = automated Mycobacterial Growth Indicator Tube; MMGIT = manual Mycobacterial Growth Indicator Tube; CLJ = commercial Löwenstein-Jensen; HLJ = home-made Löwenstein-Jensen; MTC = Mycobacterium tuberculosis complex; NTM = non-tuberculous mycobacteria.
to disentangle these factors. However, the implications of these findings are that: 1) increasing the stringency of the decontamination process may be counterproductive, as it will reduce the yield of MTC but may not affect non-sample-related contamination; 2) using MGIT systems requires that more attention be paid to laboratory infrastructure and microbiological techniques: the Zambian reference laboratory is old, difficult to clean and prone to dust exposure; and 3) increasing the number of cultures performed on each sample may reduce the number of samples that are worthless due to contamination.

We also noted that most of the contamination in MGIT systems occurred in smear-negative samples, with highly positive samples showing hardly any contamination: in smear-negative samples, the contamination rate approached 30% in MGIT systems compared to <5% in highly smear-positive samples. It is not immediately clear why higher contamination was experienced on smear-negative and not on smear-positive samples, but competitive growth has been suggested. Our contamination was higher than has been observed in other studies, but the heterogeneity of studies should be considered, and any comparison should consider factors such as quality of samples, sample transport systems, percentage of smear-negative samples, population from which samples have been collected and setting or laboratory infrastructure. Furthermore, no large-scale MGIT evaluation studies have been conducted in a setting similar to ours to enable comparison of results.

Both MGIT systems isolated more NTM than LJ systems, as has been reported by other studies. It is not clear if these NTM are clinically important, as this was a laboratory-based study with no other clinical information available. The NTM isolates behaved more like environmental contaminants, in that they were often not grown in both MGIT systems from the same sample.

The Capilia TB test was used to identify all mycobacteria isolates as MTC or NTM. This test has been shown to be highly sensitive and specific, and also quick to provide identification results. Conventional biochemical tests such as the niacin accumulation test can be used, although these may lead to delayed identification, as sub-cultures are required to carry out identification of liquid-culture isolates.

The use of any culture system is more expensive than microscopy. A full economic costing of this study has been done which showed that, despite a slightly higher cost, MGIT systems are cost-effective due to their higher yield.

CONCLUSION

This study provides the first large-scale data from a typical African reference laboratory using MGIT systems. When used in a high throughput laboratory in settings with a high HIV-TB burden, MGIT systems are more sensitive than LJ systems for isolation of mycobacteria. The high contamination rates highlight the challenges of using culture in this setting and reinforce the recommendation for investment in infrastructure, biosafety and training. The study provides data on the benefits that might be expected in terms of numbers of additional diagnoses and earlier diagnoses in a resource-limited setting with a high burden of HIV. These data should be used by policy makers in considering decisions about scale-up of culture.

Acknowledgements

The authors are grateful to the Zambian Ministry of Health and the staff of the NRL for their support. They thank Professor S Vermund and Dr R McNerney for commenting on the manuscript, D Milimo for the data entry, R Tembwe and all the staff of the National Reference Laboratory who assisted in laboratory work. This study was supported by the Foundation for Innovative New Diagnostics (FIND). Further support was received through a subcontract from Johns Hopkins University with funds provided by grant no. 19790.01 from the Bill and Melinda Gates Foundation. Its contents are solely the responsibility of the authors and do not necessarily represent the official views of FIND or the Bill and Melinda Gates Foundation.

References

CONTEXTE : Laboratoire national de référence de la tuberculose en Zambie.

OBJECTIF : Comparer quatre systèmes de culture TB lors de leur utilisation dans un contexte à ressources limitées.

SCHEMA : Comparaison de quatre systèmes de culture : le Mycobacterial Growth Indicator Tube (MGIT) 960 automatisé (AMGIT) et le MGIT manuel (MMGIT) ainsi que deux milieux de culture de Löwenstein-Jensen (LJ), un milieu commercial (CLJ) et un milieu fait maison (HLJ).

RÉSULTATS : On a reçu 1916 échantillons de crachats, parmi lesquels 261 (13,6%) étaient positifs à l'examen microscopique. On a isolé le complexe Mycobacterium tuberculosis (MTC) dans au moins un des milieux dans 410 échantillons (21,4%): le MTC a été découvert dans 336 cas par MMGIT (17,5%), dans 339 par AMGIT (17,2%), dans 192 par CLJ (10,0%) et dans 184 par HLJ (9,6%). La durée moyenne avant détection dans les échantillons à bacilloscopie négative a été de 14 jours pour AMGIT, de 16 jours pour MMGIT et de 34 jours pour les deux systèmes LJ. L’isolement de mycobactéries non-tuberculeuses (NTM) a été plus fréquent dans les deux systèmes MMGIT (3,5%) que dans les systèmes CLJ (0,9%) et HLJ (0,8%). Les taux de contamination ont été élevés : 29,6% sur AMGIT, 23,8% sur MMGIT, 14,9% sur CLJ et 12,5% sur HLJ.

CONCLUSION : En dépit des taux élevés de contamination, les deux systèmes MMGIT ont amélioré considérablement à la fois le rendement et la durée nécessaire à la détection du MTC par rapport aux milieux de LJ. Si l’on veut une extension des cultures dans des contextes comme celui-ci, un investissement s’impose en infrastructure et en formation.

MARCADO DE REFERENCIA : El laboratorio nacional de referencia de tuberculosis en Zambia.

OBJETIVO : Comparar cuatro sistemas de cultivo de Mycobacterium tuberculosis cuando se utilizan en entornos con recursos limitados.

MÉTODO : Se compararon los siguientes sistemas de cultivo: el método Mycobacterial Growth Indicator Tube (MGIT) 960 automatizado (AMGIT) y manual (MMGIT) y dos medios de cultivo de Löwenstein-Jensen (LJ), uno comercial (CLJ) y otro preparado en el laboratorio (HLJ).

RESULTADOS : Se recibieron 1916 muestras de esputo, de las cuales 261 (13,6%) fueron positivas en el examen microscópico. Se aislaron cepas del complejo M. tuberculosis (MTC) por lo menos en un medio en 410 muestras (21,4%). Por el método MGIT manual se recuperaron 336 cepas (17,5%) del MTC, 329 (17,2%) por el método MGIT automatizado, 192 (10,0%) en el CLJ y 184 (9,6%) en el HLJ. La mediana del lapso hasta la detección de las micobacterias en las muestras con bacilloscopía negativa fue de 14 días con el sistema AMGIT, 16 días con el MMGIT y 34 días con ambos medios de LJ. El aislamiento de micobacterias diferentes del MTC fue más frecuente con ambos sistemas MMGIT (3,5%) que con el medio CLJ (0,9%) y con el HLJ (0,8%). Las tasas de contaminación fueron altas : 29,6% con el AMGIT, 23,8% con el MMGIT, 14,9% con el CLJ y 12,5% con el HLJ.

CONCLUSIÓN : Pese a la alta tasa de contaminación, el uso de ambos sistemas MMGIT mejoró en forma considerable el rendimiento diagnóstico y el lapso de detección de cepas del MTC, en comparación con los medios LJ. Es preciso invertir en infraestructura y capacitación, si se desea extender el uso del cultivo en entornos semejantes a este.