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Evaluation of a manual identification system for detection of *Mycobacterium tuberculosis* in a primary tuberculosis laboratory in China

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Abstract

Objective: To compare the diagnostic performance of the manual BACTEC™ Mycobacteria Growth Indicator Tube (MGIT™) system (M-MGIT) with the automated BACTEC™ MGIT™ 960 system (A-MGIT) and Löwenstein-Jensen (L-J) culture method in detecting mycobacteria in sputum specimens from patients with suspected pulmonary tuberculosis (TB).

Methods: For this cross-sectional study, sputum samples were taken from patients aged ≥18 years attending a TB clinic in Beijing, China between July 2015 and October 2016. Processed sputum samples were inoculated into the MGIT systems and L-J medium for up to 6 and 8 weeks, respectively.

Results: The M-MGIT and A-MGIT methods detected significantly more *Mycobacterium tuberculosis* complex (MTC) isolates than L-J culture from the 565 sputum samples (39%, 40% and 32%, respectively). Using a positive result from any of the three culture systems as reference, the sensitivity of M-MGIT, A-MGIT and L-J methods were 92%, 94%, and 74%, respectively. The time-to-detection of mycobacteria was 12.9±4.2 days for M-MGIT, 11.8±5.2 days for A-MGIT and 24.2 ±8.7 days for L-J.

Conclusions: M-MGIT has a similar diagnostic performance to A-MGIT, and is a fast and reliable alternative to conventional culture methods in the diagnosis of pulmonary TB in a developing country.

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Introduction

Worldwide, tuberculosis (TB) causes ill-health in millions of people each year and in 2015 was one of the top 10 causes of death.[1] In 2015, there were an estimated 918,000 incident new cases of TB in China and 35,000 related deaths. [1] Therefore, addressing gaps in detection and treatment is of paramount importance. [1] Sputum smear microscopy remains the most commonly used method for diagnosing TB worldwide.[2] Developments in diagnostics over the past few years has resulted in the use of rapid molecular tests such as the Xpertw *Mycobacterium tuberculosis* (MTB)/ rifampicicn (RIF) assay and the real-time fluorescent based quantitative polymerase chain reaction (PCR) detection method. [2]However, these molecular tests are relatively expensive and their implementation is difficult in resource-limited settings.[2] Non-radiometric liquid culture methods, such as the Mycobacteria Growth Indicator Tube (MGIT ™), have been developed to speed up the isolation of slow-growing mycobacteria and have been reported to be faster and a more reliable alternative to conventional culture on Lowenstein-Jensen egg (LJ) solid medium.[3,4] Unfortunately, liquid culture systems are expensive which has precluded their widespread use, particularly in resource-limited settings.[5] However, while the BACTEC ™ MGIT ™ 960 system (Becton-Dickinson, Franklin Lakes, NJ, USA) is automated for continuous monitoring, the older manual MGIT system is more affordable for laboratories with a small budget. [4]

The present study was designed to compare the performance of the manual BACTEC MGIT system (M-MGIT) with the automated BACTEC MGIT 960 system (A-MGIT) and a conventional L-J culture method in detecting mycobacteria in sputum specimens from patients with suspected pulmonary TB.

Methods

For this cross-sectional study, sputum samples were taken from patients attending a TB clinic in Chaoyang District Centre for Disease Control and Prevention, Chaoyang District, Beijing, China between July 2015 and October 2016. Patients were $\geq$18 years of age and had a high clinical suspicion of TB according to the Chinese National Diagnostic Guidelines.[6]The study protocol was approved by the Ethics Committee of Chaoyang District Centre for Disease Control and all patients provided written informed consent.

One sputum specimen (3–5ml) was collected from each patient before treatment. Prior to processing, smears were prepared and stained with Ziehl-Neelsen stain and examined under a light microscope to confirm the presence of acid-fast bacilli (AFB). The remaining sputum was decontaminated and digested according to the N-acetyl-L-cysteine (NALC) sodium hydroxide method.[7] A portion (0.5ml) of the processed specimen was inoculated into a tube of the

M-MGIT system. The tubes were incubated at 37°C and examined daily in a 365nm wavelength UV light source fluorescence detector (BACTEC ™ MicroMGIT ™ device). For the A-MGIT system, 0.5ml of the processed specimen was inoculated into a tube which was incubated at 37°C and monitored automatically every 60 minutes for increased fluorescence. All culture tubes from both methods were incubated for 6 weeks or until they were found to be positive. A portion (0.1ml) of the remaining processed specimen was inoculated into the L-J medium and incubated at 37°C with daily examinations for eight weeks until mycobacterial colonies were detected. Typical colonies were tested for *Mycobacterium tuberculosis* complex (MTC) organisms using para nitro benzoic acid (PNB) and thiophene-2-carboxylic acid hydrazide (TCH) medium growth tests.[6]

Statistical analyses

Data were analysed using the Statistical Package for Social Sciences (SPSS®) for Windows® release 13.0 (SPSS Inc., Chicago, IL, USA) and a P-value <0.05 was considered to indicate statistical significance. The recovery and contamination rates of the three systems were compared using χ^2 test. Concordance between tests was evaluated using the *kappa* statistic and 95% confidence intervals (CI). Student t-test was used to compare the time-to-detection (TTD) in different media.

Results

Of the 565 sputum samples available from patients with presumptive pulmonary TB, 237 (42%) grew mycobacteria by the M-MGIT method, 243 (43.0%) by the A-MGIT method, and 190 (34%) by the L-J method (Table 1). There was no statistically significant difference in yield observed between the MGIT systems, but there was a statistically significant difference in yield between each MGIT system and the L-J method (χ^2=8.32 and 10.52; P <0.01).

Of the total number of isolates positive for mycobacteria, 241 (43%) were positive for MTC and 18 (3%) had non-tuberculous mycobacteria (NTM) (Table 1). To calculate the sensitivity of each system, we defined the 'gold standard' as being a specimen positive for MTC on at least one of the culture systems. Therefore, the sensitivity of each culture system for MTC isolation was

Table 1. Comparison of results from the three different culture systems in the analysis of sputum samples from 565 patients with suspected pulmonary tuberculosis

	Sputum samples			
	M-MGIT	A-MGIT	L-J	All systems
Total processed	565	565	565	565
Positive growth	237(41.9)	243 (43.0)	190 (33.6)	259 (45.8)
MTC	222 (39.3)	227 (40.2)	179 (31.7)	241 (42.6)
NTM	15 (2.6)	16 (2.8)	11 (1.9)	18 (3.2)
Contaminated	33 (5.8)	35 (6.2)	15 (2.7)	8 (1.4)†
Negative	295 (52.3)	287 (50.8)	360 (63.7)	298 (52.8)†

Data are presented as *n* or *n* (%).
†Contaminated or negative on all three media
M-MGIT™, manual Mycobacterial Growth Indicator Tube; A-MGIT, automated Mycobacterial Growth Indicator Tube (BACTEC™ MGIT™ 960); L-J, Löwenstein-Jensen culture method, MTC, *Mycobacterium tuberculosis* complex; NTM, non-tuberculous mycobacteria

as follows: M-MGIT, 92% (222/241); A-MGIT, 94% (227/241); L-J, 74% (179/241). There was no significant difference between the MGIT systems, but there was a statistically significant difference between each MGIT system and the L-J method (χ^2=27.44 and 35.99; P <0.01).

From a total of 565 sputum samples that were processed using the M-MGIT, A-MGIT and L-J methods, MTC organisms were detected in 222 (39%), 227 (40%) and 179 (32%) isolates, respectively. (Table 1). There was no significant difference between the MGIT systems but the difference between each MGIT system and L-J method was statistically significant (χ^2=7.15 and 8.86; P <0.05).

Contamination rates were statistically significantly higher for both MGIT systems compared with the L-J culture method (5.8%, 6.2% and 2.7%, respectively; χ^2=7.05 and 8.37; P <0.01) but there was no significant difference between the two MGIT systems (Table 1). Of the 565 sputum samples, 77 smears were AFB positive and 488 were AFB negative (Table 2). Statistically significantly more AFB-negative samples were found to be MTC-positive in the two MGIT systems compared with the L-J method (34%, 34%, and 25%, respectively; χ^2=8.30 and 9.50; P <0.01). There was no difference between the two MGIT systems. The sensitivities of the M-MGIT, A-MGIT and L-J methods were 92% (164/178), 94% (167/178) and 69% (123/178), respectively. Although there was no significant difference between the two MGIT methods, the difference between each MGIT method and the L-J method was statistically significant (χ^2=30.22 and 36.01; P <0.01).

With regard to AFB-positive samples, there was no significant difference between MTC-positive samples found by the two MGIT methods or the L-J method (i.e., 75%, 78% and 73%) (Table 2). In addition, there was no significant difference in the

Table 2. Overview of results from the three different culture systems in the analysis of sputum samples from 565 patients with suspected pulmonary tuberculosis

		M-MGIT			A-MGIT			L-J			All systems		
Smear		Positive growth		−ve or	Positive growth		−ve or	Positive growth		−ve or	Positive growth		−ve or
Sample	*n*	MTC	NTM	Con	MTC	NTM	Con	MTC	NTM	Con	MTC	NTM	Con
+ve AFB	77	58 (75.3)	13 (16.9)	6 (7.8)	60 (77.9)	12 (15.6)	5 (6.5)	56 (72.7)	10 (13.0)	11 (14.3)	63 (81.8)	12 (15.6)	2 (2.6)
−ve AFB	488	164 (33.6)	2 (0.4)	322 (66.0)	167 (34.2)	4 (0.8)	317 (65.0)	123 (25.2)	1 (0.2)	364 (74.6)	178 (36.5)	6 (1.2)	304 (62.3)
Total	565	222 (39.3)	15 (2.6)	328 (58.1)	227 (40.2)	16 (2.8)	322 (57.0)	179 (31.7)	11 (1.9)	375 (66.4)	241 (42.6)	18 (3.2)	306 (54.2)

Data are presented as *n* or *n* (%).
M-MGIT™, manual Mycobacterial Growth Indicator Tube; A-MGIT, automated Mycobacterial Growth Indicator Tube (BACTEC™ MGIT™ 960); L-J, Löwenstein-Jensen culture method; AFB, acid-fast bacilli; +ve, positive; −ve, negative; Con, contaminated

Table 3. Agreement between the three different culture systems in the analysis of sputum samples from 565 patients with suspected pulmonary tuberculosis

	A-MGIT				L-J			
	Positive	Negative	Con	Total	Positive	Negative	Con	Total
M-MGIT								
Positive, *n*	228	7	2	237	177	58	2	237
Negative, *n*	11	273	11	295	7	285	3	295
Contaminated, *n*	4	7	22	33	6	17	10	33
Total, *n*	243	287	35	565	190	360	15	565
Kappa, 95% CI	0.87 (0.79, 0.94)				0.69 (0.61, 0.76)			

M-MGIT ™, manual Mycobacterial Growth Indicator Tube; A-MGIT, automated Mycobacterial Growth Indicator Tube (BACTEC™ MGIT™ 960); L-J, Löwenstein-Jensen culture method; Con, contaminated; CI, confidence interval

sensitivities of the three systems (92% [58/63] for M-MGIT, 95% [60/63] for A-MGIT and 89% [56/63] for L-J).

The agreement between M-MGIT and A-MGIT was 93% (i.e., [228 + 273+22]/565; *kappa*=0.87; 95% CI 0.79-0.94), and between M-MGIT and L-J was 84% ([177 + 285+10]/565; *kappa*=0.69; 95% CI 0.61-0.76) (Table 3).

The mean ± standard deviation TTD was 12.9±4.2 (range 3-35) days for M-MGIT, 11.8±5.2 (range 4–33) days for A-MGIT and 24.2±8.7 (range 11–69) days for the L-J method. There was no significant difference between the two MGIT systems but the difference between each MGIT system and the L-J method was statistically significant ($P < 0.01$).

Discussion

The MGIT system is based on fluorescence detection of mycobacterial growth in a tube containing a modified Middlebrook 7H9 medium together with a fluorescence quenching-based oxygen sensor.[5] Consumption of the dissolved oxygen by the growing mycobacteria produces an orange fluorescence and its intensity is proportional to the number of bacteria present.[5]While the automated system (BACTEC MGIT 960) is a fully automated, continuously monitoring, high-capacity instrument, the older manual system requires tubes to be examined for fluorescence manually under a Wood's lamp or with some other long wave UV light source. [5] However, although the manual system requires more technical time than the automated system, it is less of a financial burden for low resource countries. [5]

The results of this present study showed that while the M-MGIT and A-MGIT systems had a similar yield for mycobacteria and MTC, both methods had a significantly better yield than the conventional L-J culture medium. To avoid biased estimates of the test characteristics the presence of *M. tuberculosis* was defined using a composite reference standard[8] whereby a positive culture in any medium was used as the 'gold standard'. This practice has been used in several other studies.[9–11] Using the derived 'gold standard', the sensitivity for MTC isolation was similar for the M-MGIT and A-MGIT systems (92% and 94%, respectively) and both systems were significantly higher than that of L-J culture method (74%). Although the 'gold standard' used in this present study may have been different from other studies, our findings are similar to results from several different countries. For example, in two studies where the 'gold standard' was

positive L-J cultures, the sensitivity of M-MGIT was 92% in a study from Peru[12] and 90% in a study from Malaysia.[4] However, in a study where the 'gold standard' was consistent with ours, sensitivities of M-MGIT, A-MGIT and L-J were found to be 82%, 80%, and 47% respectively.[13]

The diagnostic performance of M-MGIT was good even for smear-negative sputum samples. Indeed, among the smear-negative samples, the M-MGIT and A-MGIT methods yielded 34% MTC growth, which was significantly higher than L-J culture medium (25%). The sensitivities of the MGIT systems for MTC in smear-negative samples were similar (M-MGIT, 92% and A-MGIT, 94%) and significantly greater than L-J medium (69%). However, a study from Peru that evaluated the performance of M-MGIT in the diagnosis of 542 smear-negative samples found a yield of 24% and a sensitivity of 85%. It is important to note that sensitivities of methods may be affected by the decontamination protocol.[13] For instance, if specimens are decontaminated using excess sodium hydroxide or the decontamination time is too long, MTB may be killed, and so the positive rate will be low. By contrast, if specimens are decontaminated using an insufficient amount of sodium hydroxide or the decontamination time is short, other bacteria besides MTB may grow, and the rate of contamination will increase and the positive rate of MTB will decrease.

As a general rule, a contamination rate of 1–4% is acceptable in laboratories that receive fresh specimens,[15] and the Chinese Antituberculosis Association suggests the contamination rate should be controlled at 2–5%.[16] In the present study, the MGIT systems had similar contamination rates (6%) but they were significantly higher than for the L-J culture method (3%). A possible explanation for the higher contamination with the MGIT systems compared with the L-J culture method, is that the medium in the MGIT systems is more enriched than the L-J medium.[17]

The TTD is an important feature since early identification of the bacteria allows timely treatment and can prevent the disease from spreading. In this study, the TTD for the MGIT systems was similar (approximately 12–13 days) and significantly shorter than for the L-J culture (approximately 24 days). Our findings are similar to those from other studies (i.e. 11–13 days for M-MGIT system and 21–33 days for LJ culture medium).[4,7,13]

In the diagnosis of TB, the M-MGIT system had a similar performance, turnaround time and percentage of contaminated cultures compared with the A-MGIT system. Although in our study the costs of the M-MGIT method (70 Chinese Yuan per tube and the additional costs for a manual fluorescence reader) were higher than those of the L-J culture method (30 Chinese Yuan per tube), they were much lower than those of the A-MGIT method (70 Chinese Yuan per tube and a million- Chinese Yuan for the equipment). Therefore, in spite of the higher cost compared with the L-J culture method, the M-MGIT system appears to be more cost-effective because of its higher efficiency.

The present study was limited by the fact that the potential agreement between A-MGIT system and L-J method was not evaluated. However, this has been studied elsewhere, [18]and the focus of this study was the evaluation of the M-MGIT system. In addition, our results were obtained from one centre. Therefore, further multicentre studies are required.

In summary, the M-MGIT system has a similar diagnostic performance to the A-MGIT system, with a relatively low contamination rate and short TTD compared with the LJ method. Therefore, the M-MGIT culture system offers a fast and reliable alternative to the conventional solid

medium culture system for the diagnosis of pulmonary TB in a developing country.

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Declaration of conflicting interest

The authors declare that there are no conflicts of interest.

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