An evaluation of the recovery of mycobacteria from urine specimens using the automated Mycobacteria Growth Indicator Tube system (BACTEC MGIT 960)

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The Mycobacteria Growth Indicator Tube (MGIT 960) system was evaluated against Lowenstein–Jensen (LJ) medium and the BACTEC 460 TB system for the recovery of mycobacteria from 1393 consecutive urine specimens. The MGIT had a sensitivity of 91.3 % [95 % confidence interval (CI), 83.2–99.4] when the combination of BACTEC 460 and LJ medium was used as the reference method. The mean time for positivity for MGIT and BACTEC 460 was 19.3 days and 20 days, respectively, while that for LJ medium was 35 days. The incidence of contamination was highest for LJ medium ($n=148$), followed by MGIT 960 ($n=81$), and BACTEC 460 had the lowest incidence of contamination ($n=50$). In conclusion, the isolation of mycobacteria from urine specimens by the MGIT 960 is comparable to that of the BACTEC 460 TB system and solid media.

INTRODUCTION

The use of automated systems in laboratories has undoubtedly decreased the turnaround time and increased the sensitivity of mycobacterial culture (NICE, 2006). We have moved ahead from traditional egg-based Lowenstein–Jensen medium culture methods to using semi-automated radio-metric systems like the BD BACTEC 460, and more recently to fully automated systems like the BD BATEC Mycobacteria Growth Indicator Tube (MGIT) 960 system (hereafter referred to as MGIT 960), which function on the principle of fluorescence quenching. The MGIT 960 system had not been evaluated for mycobacterial isolation from urine specimens in the past due to lack of specimen numbers. However, a recent study by Hillemann *et al.* (2006) tested the MGIT 960 for recovering mycobacteria from 9558 extrapulmonary specimens, including 2069 urine samples. The incidence rate of tuberculosis in Singapore for the year 2006 was 26 cases per 100 000 population (according to WHO figures). In that year, we evaluated the performance of the BD MGIT 960 in isolating mycobacteria from clinical urine specimens received in a tertiary care hospital.

METHODS

Specimens. In this study, we included all consecutive urine specimens (urine from catheter excluded) submitted to the Central

Tuberculosis Laboratory at Singapore General Hospital during the year 2006.

Culture medium inoculation, incubation and test duration. The urine was first concentrated by using a 50 ml Falcon tube and centrifuged at 3000 g for 10 min. The resulting sediment was decontaminated by the standard *N*-acetyl-L-cysteine and sodium hydroxide method, with a concentration of 3 % NaOH (Master, 1992) (BBL MycoPrep).

After 30 min, the suspension was centrifuged again and the sediment suspended in 1–1.5 ml sterile phosphate buffer (pH 6.8). This final suspension was used for inoculation of the following: 0.5 ml suspension into one MGIT 960 tube (Becton Dickinson), 0.5 ml suspension into one BACTEC 12B vial (Becton Dickinson) (each containing polymyxin B, amphotericin B, nalidixic acid, trimethoprim and azlocillin – PANTA) and 0.1 ml suspension into a Lowenstein–Jensen (LJ) slant (BBL). The remaining suspension was used for auramine smears and strand displacement amplification if requested.

The MGIT 960 tubes were incubated at 37 °C and monitored by the system for increase in fluorescence once every 60 min for the next 6 weeks. BACTEC 12B vials were incubated at 37 ± 1 °C and then read by the semiautomated BACTEC 460 instrument (Becton Dickinson) twice weekly for 2 weeks and then once a week for 4 weeks thereafter. LJ slants, inoculated with 0.1 ml processed specimen, were incubated at 37 °C. The slants were examined weekly for a total of 8 weeks. All positive samples had a Kinyoun stain performed for examination of acid-fast bacilli.

Identification of mycobacteria. *Mycobacterium tuberculosis* complex colonies were identified using the Accuprobe (Gen-Probe), while non-tuberculous mycobacteria were identified with a variety of methods which included gene probes for *Mycobacterium kansasii*, *Mycobacterium goodii* and *Mycobacterium avium-intracellulare*.

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Abbreviation: CI, confidence interval.

Table 1. Isolation of mycobacteria from urine samples according to speciesMTC, *Mycobacterium tuberculosis* complex; NTM, non-tuberculous mycobacteria.

	MGIT +ve only	BACTEC 460 +ve only	LJ +ve only	MGIT and BACTEC 460 +ve only	MGIT and LJ +ve only	BACTEC 460 and LJ +ve only	All 3 +ve
MTC	8	2	–	8	5	–	20
NTM	1	–	2	2	1	–	3
<i>M. goodii</i>	–	–	–	1	–	–	–
<i>M. simiae</i>	–	–	–	1	–	–	–
<i>M. avium</i> – <i>intracellulare</i> complex	1	–	–	–	–	–	–
<i>M. fortuitum</i> group	1	–	–	–	1	–	–
Total	11	2	2	12	7	0	23

complex (Gen-Probe), and high-performance liquid chromatography (Butler *et al.*, 1996). In the event of contaminated cultures, AFB-positive specimens were reprocessed and reinoculated into BACTEC 12B vials.

RESULTS AND DISCUSSION

There were 1393 urine specimens from 751 patients. Multiple specimens were received from 363 patients (2–10 per patient). We took each individual specimen to represent an independent diagnostic event. Of the 1393 urine specimens tested, 57 (4.1 %) specimens (from 38 patients) were positive for mycobacteria. Among these 57 specimens, 43 were found to be *M. tuberculosis* complex and 14 were non-tuberculous mycobacteria. Although the study of Hillemann *et al.* (2006) had more urine isolates ($n=2069$), they had only 53 (2.6 %) positive mycobacterial urine cultures. This could be attributable to the lower incidence of TB in Germany, where the study took place (6.5 per 100 000 population in 2006 according to WHO figures).

Performance of MGIT 960 compared to BACTEC 12B and LJ medium

The comparison of culture methods showed that the MGIT 960 alone detected more mycobacterial isolates, with 11 isolates (19.3 %), than the BACTEC 460 alone, with 2 isolates (3.5 %), or solid LJ medium alone, with 2 isolates (3.5 %) (Table 1). In total, the MGIT 960 missed 4 isolates (7.02 %), while the combination of LJ medium and BACTEC 460 missed 11 isolates (19.3 %). This is in contrast to the study by Hillemann *et al.* (2006), who found that the MGIT 960 had missed 17.8 % while solid medium had missed 26.7 %.

By comparing the proportion of mycobacteria detected by MGIT 960 vs BACTEC 460 using McNemar's test (test statistic, $\chi^2=11.25$, which gives $P<0.001$ at 1 degree of freedom), it is possible to reject a null hypothesis that the two systems detect the same percentage of mycobacterial isolates in urine specimens. We estimate the difference in

percentage of isolates detected as 1.2 % (95 % CI, 0.6–1.8 %), with the MGIT 960 detecting more isolates than the BACTEC 460. Using the BACTEC 460 alone as the reference method, the MGIT 960 had a sensitivity of 94.6 % (95 % CI, 87.3–100 %) (Table 2).

Using the combination of BACTEC 460 and LJ medium as the reference method, the MGIT had a sensitivity of 91.3 % (95 % CI, 83.2–99.4 %). The MGIT was able to isolate mycobacteria in 11 cases (0.8 %) which had been negative by both the BACTEC 460 and the LJ method.

These figures are superior to a previous meta-analysis which looked at the MGIT for the isolation of mycobacteria from pulmonary and extra-pulmonary sites. In that study, the MGIT 960 had a sensitivity of 81 % (95 % CI, 76–86 %) (Cruciani *et al.*, 2004).

Instead of resorting to a fully automated system, most laboratories continue to use a combination of liquid broth and solid media for the isolation of mycobacteria. Our findings appear to support this practice for urine specimens, as the sensitivity of the MGIT was lower when a combination of LJ and BACTEC 460 was used as the reference method. However, it is plausible that unequal distribution of bacilli onto various media may influence the recovery rates and it is important to note that the

Table 2. Comparison between recovery of mycobacteria from MGIT 960 against BACTEC 460 and LJ cultures from 1393 urine samples

	Against BACTEC 460 alone		Against BACTEC 460 and LJ	
	BACTEC 460 +ve	BACTEC 460 –ve	BACTEC 460 and LJ +ve	BACTEC 460 and LJ –ve
MGIT +ve	35	18	42	11
MGIT –ve	2	1338	4	1336
Total	37	1356	46	1347

Table 3. Time to positivity

	Mean (days)	Mode (days)	Median (days)	Min. time (days)	Max. time (days)
MGIT 960	19.3	15	15	0	42
BACTEC 460	20.0	14	20	4	41
LJ slant	37.5	35	35	21	53

significance of the non-tuberculous mycobacteria isolated by the solid medium alone was not evident upon clinical review during the period of the study.

Time to positivity

The appeal of both the BACTEC 460 and the MGIT 960 is the decreased turnaround time for mycobacterial isolation. This advantage would enable clinicians to establish a definitive diagnosis, and institute appropriate therapy and infection control measures within the shortest possible time. We found that the times for positivity for MGIT 960 and BACTEC 460 were comparable, with a mean time of 19.3 days and 20.0 days, respectively ($P=0.6$) (Table 3). Not surprisingly, solid medium had a significantly longer time to positivity (mean of 37.5 days). In a previous study by Somoskovi *et al.* (2000), which looked at pulmonary specimens, the MGIT had the shortest time to positivity, followed by the BACTEC 12B and LJ medium (mean of 13.2 days vs 16.8 days and 36.2 days, respectively). A possible reason for the longer time to positivity for urine in the MGIT in our study could be the presence of inhibitory substances in urine (i.e. antimicrobials which may have been commenced before the diagnosis of renal tuberculosis). The smaller volume of specimen added could have accounted for the reduced sensitivity and increased turnaround time for the LJ method.

Contamination

Decontamination of specimens was performed using 3 % NaOH in our centre as this protocol resulted in an overall contamination rate of 2–5 %, which was consistent with previous data (Collins *et al.*, 1997) for all specimens in our laboratory. We found that the rate of breakthrough contamination (presumptive positives which were actually found to be due to non-mycobacterial organisms) were 10.6 % ($n=148$) for LJ medium (95 % CI, 9–12.24 %), 5.8 % ($n=81$) for the MGIT (95 % CI, 4.58–7.04 %) and 3.6 % ($n=50$) for the BACTEC 460 (95 % CI, 2.61–4.57 %).

This is in contrast to the study of Hillemann *et al.* (2006), which found the rate of contamination in the MGIT to be greater than that of LJ medium for urine specimens (14.9 % vs 7.2 %). In that study, 1 % NaOH was used for decontamination, which probably accounted for the higher rate of contamination in the MGIT. However, our figures were comparable with a previous meta-analysis by Cruciani *et al.*

(2004), which showed a contamination rate of 12.8 % with solid medium, 8.6 % for MGIT and 4.4 % for BACTEC 460.

Our findings remain limited by the fact that although a large number of urine specimens were included in the study, the actual number of specimens in which mycobacteria was detected remained small. Perhaps future studies should include urine specimens collected over a longer period. In summary, our study has shown that isolation of mycobacteria from urine by the MGIT 960 system is comparable if not superior to that of the BACTEC 460 system and solid medium.

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