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Use of the BACTEC Mycobacteria Growth Indicator Tube 960 Automated System for Recovery of Mycobacteria from 9,558 Extrapulmonary Specimens, Including Urine Samples▽

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The BACTEC Mycobacteria Growth Indicator Tube 960 (MGIT 960) system was applied for recovery of mycobacteria from extrapulmonary specimens and compared with solid media (Löwenstein-Jensen and Stonebrink). A total of 9,558 specimens were investigated, comprising 3,074 body fluids, 1,878 tissues, and 2,069 urine samples, from which the recovery of mycobacteria was not yet established for MGIT 960. In total, the MGIT 960 was able to detect 446 (90.3%) of the 494 isolates of *Mycobacterium tuberculosis* complex (MTBC) and 223 (86.0%) out of the 259 isolates of nontuberculous mycobacteria (NTM). In comparison to this, culture on solid medium revealed 358 (72.6%) MTBC isolates and 164 (66.8%) NTM isolates. While 136 (27.6%) of the MTBC isolates and 95 (19.2%) of the NTM isolates were recovered from the MGIT 960 only, 48 (9.7%) of the MTBC isolates and 36 (13.9%) NTM isolates grew only on solid media. Thus, the overall sensitivities for the recovery of mycobacteria from extrapulmonary specimens with MGIT 960 and solid media were 88.8% and 69.3%, respectively. However, the efficiency of the MGIT 960 system can be maximized with additional culture on solid media.

Extrapulmonary infections with *Mycobacterium tuberculosis* complex (MTBC) isolates or nontuberculous mycobacteria (NTM) remain a diagnosis that is often difficult to establish. Extrapulmonary tuberculosis in patients with active *M. tuberculosis* infection is often initially misdiagnosed as cancer (1). Obtaining material for investigation in extrapulmonary cases often requires invasive procedures and is therefore not easily repeatable. For detection of mycobacteria in a clinical specimen, smear microscopy is by far the most popular among all the methods currently employed worldwide. However, cases of extrapulmonary mycobacterial infections are more often smear negative than are pulmonary cases. In recent times, attention has been devoted to developing nucleic acid amplification diagnostic technologies owing to their rapidity and sensitivity. However, these techniques are not as sensitive as culture of mycobacteria and moreover are often hampered due to the presence of inhibitors, especially in extrapulmonary specimens (4, 18). Therefore, culture of the etiologic agent remains the accepted “gold standard” for diagnosing mycobacterial infections, inherently because of the need for cultures for susceptibility testing.

Recently, nonradiometric liquid culture media such as that used in the Mycobacteria Growth Indicator Tube (MGIT; Becton Dickinson) system have been introduced and extensively evaluated (3, 8, 11, 14). The MGIT 960 system is a fully automated system for testing simultaneously up to 960 vials via the measurement of fluorescence in each vial every 60 min by a photodetector. The amount of fluorescence is inversely proportional to the oxygen level in the culture medium, indicating the consumption of oxygen due to the growth of inoculated organisms in the vials.

The purpose of this study was to test the capability, efficiency, and reliability of the MGIT 960 system for the detection of mycobacteria in extrapulmonary specimens, especially in urine samples, for which the method had not yet been evaluated in clinical studies. The results were compared with those of the combined Löwenstein-Jensen (LJ) and Stonebrink solid media in terms of recovery and contamination rate.

MATERIALS AND METHODS

Specimens. In the study, all nonrespiratory specimens that were submitted to the German National Laboratory for Mycobacteria (NRZ) from 2000 to 2005 were included. A total of 9,558 specimens were tested, comprised of 2,069 urine samples, 1,985 gastric aspirate samples, 1,878 tissue, 1,730 pleural fluid, 1,344 cerebrospinal fluid (CSF), and 552 stool specimens.

AFB smears. After the processing of specimens, smears were prepared from all specimens other than urine and were examined at the NRZ for the presence of acid-fast bacteria (AFB). All smears were stained by the Ziehl-Neelsen (ZN) method and examined with a light microscope (6).

Culture medium inoculation, incubation, and test duration. All specimens were processed by the standard *N*-acetyl-L-cysteine and sodium hydroxide (NALC/NaOH) method, with the final concentration of 1% for NaOH (according to the Deutsches Institut für Normung guidelines [6]). After the centrifugation step, the sediment was resuspended in 1.0 to 1.5 ml of sterile phosphate buffer (pH 6.8). This suspension was used for inoculation of culture medium. Two types of culture media were inoculated: MGIT 960 and solid media.

(i) MGIT 960. The MGIT 960 culture tubes contain 7 ml of Middlebrook 7H9 broth base, to which an enrichment supplement was added according to the instructions of the manufacturer, as well as a mixture of antibiotics consisting of polymyxin B, amphotericin B, nalidixic acid, trimethoprim, and azlocillin (MGIT PANTA). After inoculation of each tube with 0.5 ml of the processed specimen, the tubes were entered into the MGIT 960 instrument. The vials were incubated at 37°C and were monitored automatically every 60 min for increase in fluorescence for a maximum of 6 weeks. Any sample, which was identified as positive, was removed from the instrument. From the positive tube, a smear was prepared for examination of AFB. Furthermore, a blood plate was inoculated and a

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TABLE 1. Distribution of MTBC and NTM isolates recovered in each culture system

Specimen type	No. of specimens tested	No. of ZN-positive specimens		No. (%) of mycobacteria recovered	Total no. (%) recovered							
					MTBC				NTM			
		MTBC	NTM		All media combined[a]	MGIT 960	Solid culture	Both methods	All media combined[a]	MGIT 960	Solid culture	Both methods
Urine	2,069	NT[c]	NT	53 (2.6)	45	37 (82.2)	33 (73.3)	25 (55.5)	8	7 (87.5)	5 (62.5)	4 (50.0)
Gastric aspirate	1,985	13	0	178 (9.0)	157	140 (89.2)	117 (74.5)	100 (63.7)	21	18 (85.7)	9 (42.9)	6 (28.6)
Tissue[b]	1,878	23	39	392 (20.9)	178	172 (96.6)	138 (77.5)	132 (74.2)	214	184 (86.0)	143 (66.8)	113 (52.8)
Pleural fluid	1,730	1	1	51 (3.0)	46	41 (89.1)	29 (63.0)	24 (52.2)	5	5 (100)	3 (60.0)	3 (60.0)
CSF	1,344	1	0	36 (2.7)	36	35 (97.2)	15 (41.7)	14 (38.9)	0	0	0	0
Stool	552	4	1	43 (7.8)	32	21 (65.6)	26 (81.3)	15 (46.9)	11	9 (81.8)	4 (36.4)	2 (18.2)
Total	9,558	42	41	753 (7.9)	494	446 (90.3)	358 (72.5)	310 (62.8)	259	223 (86.1)	164 (63.3)	128 (49.4)

[a] Either MGIT 960, the combination of solid cultures, or both.
[b] Specimen source comprised of lymph node, skin, kidney, spleen, liver, bone, lesions, and sources not specified.
[c] NT, not tested.

subculture was made on an LJ slant. Further differentiation of mycobacteria with molecular methods was performed.

(ii) Solid media. For inoculation onto conventional solid media, which included one LJ slant and one Stonebrink slant (7), 0.1 ml of the suspension was used for each. Additionally, all tissue samples were incubated on LJ, Stonebrink, and 7H10 agar slants at 31°C. Solid media were incubated at 37°C for a maximum of 8 weeks and read once weekly. An individual medium was considered positive upon appearance of colonies on the surface, confirmed by positive AFB smear and further differentiation with molecular methods. For the purpose of data analysis, the different slants were regarded as a single solid-medium system.

Identification of mycobacteria. For the identification of MTBC organisms and the differentiation of NTM from positive cultures, two commercially available DNA strip assays were used, the GenoType MTBC and the CM/AS assay (Hain Lifescience GmbH, Nehren, Germany) (15). The assays were performed according to the instructions of the manufacturer. All strains that were not undoubtedly identifiable with the MTBC or the combined CM/AS assay were further checked by DNA sequencing of the first 500 bp of the 16S rRNA gene. If identification based on differences in the 16S rRNA gene sequence was not possible, additional analysis of the internal transcribed spacer region (16) was performed. Direct sequencing of the PCR products was carried out with an ABI Prism 3100 capillary sequencer (Applied Biosystems, Foster City, CA) and the ABI Prism BigDye Terminator kit v.1.1 according to the manufacturer's instructions.

RESULTS

In this study, 9,558 specimens were included, all of which had been sent to the National Reference Center for Mycobacteria between 2000 and 2005. Among 1,878 tissue samples, the majority were from lymph nodes, but samples also included skin, kidney, spleen, liver, bone, and lesions.

Overall, 753 (7.9%) of the 9,558 specimens tested were positive for mycobacteria (Table 1). Out of these 753 positive cultures, 83 (11.0%) were also smear positive for acid-fast bacilli. Mycobacteria were recovered from the different specimen types at various quantities. The highest rates of mycobacterial isolates were recovered from the tissue specimens with 392 isolates (20.9%) comprised of 178 MTBC samples (9.5%) and 214 NTM samples (11.4%). Recovery rates differ among gastric aspirates (178 [9.0%]), stool (43 [7.8%]), pleural fluid (51 [3.0%]), and cerebrospinal fluid (36 [2.7%]). Among the urine specimens, 53 (2.6%) were positive for mycobacteria (45 [2.2%] MTBC and 8 [0.4%] NTM).

Comparison of the culture methods for the isolation of MTBC. The gold standard for the sensitivity analysis was defined as the total number of positive cultures revealed with the liquid media, solid media, and both systems combined. The detailed comparison of the culture methods showed that MGIT 960 as a single system detected more MTBC isolates with 446 isolates (90.3%) than the solid media (358 isolates [72.5%], Table 1). However, only 310 (62.8%) of the MTBC isolates were recovered with both methods, showing that MGIT 960 recovered 136 (28.0%) isolates and solid media recovered 48 (10.0%) isolates that were not detected by the respective other technique. The highest detection rate was found for the recovery of MTBC with the MGIT 960 from tissues, with 172 (96.6%) isolates recovered. The detailed analysis showed that the MGIT 960 was able to detect more MTBC isolates in each category of specimens, with the exception of stool, where the solid media were able to recover 26 (81.3%) isolates, in contrast to 21 (65.6%) isolates recovered with the MGIT 960 method. In total, the recovery rates of the MGIT 960 system ranged from 65.6% to 96.6%, whereas these rates ranged from 41.7% to 81.3% for the solid media, indicating both the broad range of recovery rates and the generally higher rates of the MGIT 960 system for recovering mycobacteria from extrapulmonary specimens.

Comparison of the culture methods for the isolation of NTM. Concerning the isolation of NTM, the same trends were noted as for MTBC. Of the 259 NTM isolates, the MGIT 960 system recovered more (223 isolates [86.1%]) than the solid media (164 isolates [63.3%]) or both (128 isolates [49.4%]). Also, the detailed analysis of isolation rates in each category of specimens showed that the rates with the MGIT 960 system were higher than with the solid media, ranging from 81.8% to 100%, in contrast to 42.9% to 66.8%. Contamination rates with the single systems were calculated as 7.5% for MGIT 960 and 4.3% for solid media (Table 2). As may be expected, urine samples and even more stool specimens exhibited a generally high contamination rate due to the high bacterial load (14.9% and 30.8% for MGIT 960 and 7.2% and 19.4% for solid media). Therefore, with the exclusion of urine and stool specimens, the average contamination rates were calculated as 2.7% for the MGIT 960 system and 1.6% with solid media.

Isolation of NTM. The total numbers of the NTM species recovered with solid and liquid media together from the different specimen types are listed in Table 3. Of the 259 NTM isolated, the majority belong to the *Mycobacterium avium* complex (130 [50.2%]). The species isolated second most often was *Mycobacterium marinum* (71 [27.4%]). By

TABLE 2. Contamination rates in MGIT and solid culture for different specimen types

Specimen type	No. of specimens tested	No. (%) of contaminated specimens	
		MGIT 960	Solid culture
Urine	2,069	308 (14.9)	149 (7.2)
Gastric aspirate	1,985	120 (6.0)	73 (3.7)
Tissue	1,878	84 (4.5)	55 (2.9)
Pleural fluid	1,730	27 (1.6)	24 (1.4)
CSF	1,344	8 (0.6)	1 (0.1)
Stool	552	170 (30.8)	107 (19.4)
Total	9,558	717 (7.5)	409 (4.3)

far, most NTM by number and species were isolated from tissue specimens.

DISCUSSION

Among mycobacterial culture detection systems, the MGIT 960 system has found its place as a standard method for the isolation of mycobacteria in recent years, allowing an annual throughput of over 6,000 specimens (2, 5, 8, 17). Since the introduction of the MGIT 960 system, it has been extensively evaluated, but most publications deal with the recovery of mycobacteria from pulmonary specimens (3, 8, 9, 12, 14). In order to test whether the MGIT 960 system is adequately applicable for the recovery of mycobacteria from extrapulmonary specimens, we compared the MGIT 960 system to a combination of conventional solid media. The obtained data illustrate clearly the better performance characteristics of the liquid MGIT 960 system compared to solid media. In particular, the MGIT 960 system detected more isolates of MTBC and NTM than did the solid media. The reasons for this could be manifold (the inoculum size, the quality of the media, and the overall system). Even though the LJ medium and the Stonebrink medium were counted together as a single system, only 522 (69%) isolates of mycobacteria were recovered, in contrast to 669 (89%) isolates with the MGIT 960 system. These results are in accordance with those of other studies dealing mainly with pulmonary specimens (8, 9, 13) and summarized in a meta-analysis which confirm the high recovery rates with the liquid MGIT 960 system (5). The authors also clearly demonstrate an increase of sensitivity when the liquid system was combined with solid media.

The higher sensitivity of the liquid system was achieved both with MTBC and NTM and from all types of specimens, with the exception of stool specimens. This is mainly due to a high frequency of breakthrough contamination (30.8%) in MGIT. To avoid a high contamination rate, some investigators propose an intensification or repetition of the decontamination process (19). All of these measures could be valuable to improve the sensitivity of the MGIT 960 system with this special kind of specimens, but has to be tested under routine conditions in the future.

Recovery of mycobacteria from urine specimens has not been included in the claims from the manufacturers of the MGIT system because not enough specimens were processed in the clinical trials. In our study, we processed more than 2,000 specimens out of which 53 were positive for mycobacteria (2.6%). Among the total that were MTBC culture positive, MGIT missed only 17.8% while solid media missed 26.7%. This indicates that MGIT can more efficiently isolate mycobacteria from urine specimens than solid media can. The higher yield was achieved despite the higher contamination rate (14.9%) in MGIT than in solid media (7.2%).

TABLE 3. Recovery of NTM from different specimen types with solid and/or MGIT cultures

NTM	No. of specimens with NTM recovered					
	Total	Tissue[a]	Pleural fluid	Stool	Urine	Gastric aspirate
M. avium complex	130	105	1	8	2	14
M. marinum	71	71				
M. chelonae complex	20	14	4	2		
M. gordonae	6	2			1	3
M. malmoense	6	5				1
M. haemophilum	5	5				
M. genavense	4	4				
M. fortuitum	4				4	
M. heckeshornense	3	3				
M. kansasii	3	1			1	1
M. bohemicum	1	1				
M. celatum	1	1				
M. interjectum	1	1				
M. terrae	1			1		
M. xenopi	1					1
M. neoaurum	1					1
Other mycobacteria[b]	1	1				
Total	259	214	5	11	8	21

[a] Specimen source comprised of lymph node, skin, kidney, spleen, liver, bone, or lesions and sources not specified.
[b] Nonvalidly published species.

In this study, the overall contamination rate in the MGIT system was 2.7%, which when stool and urine specimens are excluded was within an acceptable range, similar to the results described in previous reports for specimens, including respiratory and extrapulmonary specimens, with a range between 3.7% and 16.6% (5, 8, 10). As generally accepted, contamination of cultures is a problem which is particularly more associated with liquid media (5, 8). Despite the use of lower concentration of NaOH (1%), the contamination rate in the MGIT 960 system was only slightly higher than that obtained with the solid media for extrapulmonary specimens in this study.

Despite the advantages and the higher sensitivity of the MGIT 960 cultivation system, solid media will still play a role in the recovery of mycobacteria from extrapulmonary specimens since they increase the recovery of mycobacteria from extrapulmonary specimens significantly.

In summary, after testing of 9,558 extrapulmonary specimens, the MGIT 960 system has been proven as a dependable, highly efficient, fully automated system for the recovery of mycobacteria from extrapulmonary specimens.

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