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Evaluation of BACTEC MGIT 960 and BACTEC 460TB Systems for Recovery of Mycobacteria from Clinical Specimens of a University Hospital with Low Incidence of Tuberculosis

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Clinical samples obtained over a period of 8 months ($n = 2,624$) were processed in parallel with the BACTEC 460TB system, with the MGIT 960 system, and in Löwenstein-Jensen (LJ) medium, resulting in the recovery of 127 mycobacteria. Recovery rates in combinations of the BACTEC 460TB or MGIT 960 system with LJ were, respectively, 94.7 and 94.7% for *Mycobacterium tuberculosis* complex ($n = 57$) and 91.4 and 70.0% for nontuberculous mycobacteria ($n = 70$). Contamination rates, elevated in the MGIT 960 system, were associated with patients (cystic fibrosis) and type of material but not with transport time. Detection time was reduced in the MGIT 960 system.

The "gold standard" of mycobacterial diagnostic procedures is still cultural detection. The radiometric BACTEC 460TB system (Becton Dickinson, Heidelberg, Germany) in combination with solid media has been the benchmark for sensitivity and speed of cultural detection (8, 11); however, it is labor-intensive, bears the potential risk of cross-contamination, requires special attention regarding radioisotopes, and—as manufacture has been halted—will not be available any more in the future. The BACTEC MGIT 960 system (Becton Dickinson) is a fully automated, nonradiometric, noninvasive device for simultaneous incubation and monitoring of 960 culture tubes. In the culture vials an oxygen-sensitive fluorescent indicator is implemented, responding to the amount of consumed oxygen. Every vial is monitored hourly, and specific algorithms determine the positivity of the vials. Results of several studies with comparison to the BACTEC 460TB system indicated that the MGIT 960 system is a rapid, sensitive, and efficient method for recovery of mycobacteria from clinical specimens (2, 4, 10, 13,

TABLE 1. Isolation of mycobacteria with the BACTEC 460TB system, the MGIT 960 system, and LJ medium

Mycobacterium(a)	Total no. (%) of isolates recovered in all media	No. (%) of isolates recovered in[a]:					P^d
		460	960	LJ	460 + LJ	960 + LJ	
M. tuberculosis complex	57 (44.9)	53 (93.0)	51 (89.5)	45 (78.9)	54 (94.7)	54 (94.7)	NS
All NTM[b]	70 (55.1)	63 (90.0)	48 (68.6)	33 (47.1)	64 (91.4)	49 (70.0)	0.001
M. avium	30 (23.6)	29 (96.7)	25 (83.3)	20 (66.7)	29 (96.7)	25 (83.3)	NS
M. intracellulare	8 (6.3)	7 (87.5)	7 (87.5)	5 (62.5)	7 (87.5)	7 (87.5)	NS
M. malmoense	2 (1.6)	2 (100.0)	2 (100.0)	0 (0.0)	2 (100.0)	2 (100.0)	ND
M. marinum	1 (0.8)	1 (100.0)	1 (100.0)	1 (100.0)	1 (100.0)	1 (100.0)	ND
M. xenopi	4 (3.1)	4 (100.0)	1 (25.0)	0 (0.0)	4 (100.0)	1 (25.0)	ND
M. scrofulaceum	1 (0.8)	0 (0.0)	1 (100.0)	0 (0.0)	0 (0.0)	1 (100.0)	ND
M. fortuitum complex	9 (7.1)	7 (77.8)	2 (22.2)	2 (22.2)	8 (88.9)	3 (33.3)	NS
M. chelonae	1 (0.8)	1 (100.0)	1 (100.0)	1 (100.0)	1 (100.0)	1 (100.0)	ND
M. gordonae	11 (8.7)	10 (90.9)	6 (54.5)	3 (27.3)	10 (90.9)	6 (54.5)	NS
M. flavescens	1 (0.8)	0 (0.0)	1 (100.0)	0 (0.0)	0 (0.0)	1 (100.0)	ND
M. smegmatis	1 (0.8)	1 (100.0)	1 (100.0)	1 (100.0)	1 (100.0)	1 (100.0)	ND
Mycobacterium spp.[c]	1 (0.8)	1 (100.0)	0 (0.0)	0 (0.0)	1 (100.0)	0 (0.0)	ND
Total	127 (100.0)	116 (91.3)	99 (78.0)	78 (61.4)	118 (92.9)	103 (81.1)	0.005

[a] Abbreviations: 460, BACTEC 460TB system; 960, BACTEC MGIT 960 system; LJ, LJ medium.
[b] Sum of all nontuberculous mycobacteria recovered in this study.
[c] *Mycobacterium* spp., not further differentiated, as recovered only once out of seven different respiratory samples; the patient was well, and the strain was not *M. tuberculosis* complex.
[d] For comparison of the BACTEC 460TB or MGIT960 system with LJ medium results. Abbreviations: NS, not significant; ND, not done.

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TABLE 2. Detection of mycobacteria according to initial smear results in the BACTEC 460 TB and MGIT 960 systems

Species and smear result	Total no.	No. of isolates (% of total) recovered in[d]:		P^f
		460	960	
M. tuberculosis complex	57	53 (93.0)	51 (89.5)	NS
Positive	17	17 (100.0)	16[a] (94.1)	NS
Negative	40	36[b] (90.0)	35[b] (87.5)	NS
M. avium-M. intracellulare	38	36 (94.7)	32 (84.2)	NS
Positive	11	11 (100.0)	8[b] (72.7)	NS
Negative	27	25[b] (92.6)	24[b] (88.9)	NS
Other mycobacteria	32	27 (84.4)	16 (50.0)	0.003
Positive	3	3 (100.0)	3 (100.0)	ND
Negative	29	24[b] (82.8)	13[c] (44.8)	0.003
All mycobacteria[e]	123	112 (91.1)	96 (78.0)	0.005
Positive	30	30 (100.0)	26 (86.7)	NS
Negative	93	82 (88.2)	70 (75.3)	0.023

[a] Lost due to contamination (other samples of patient positive).
[b] No growth indicated.
[c] Three lost due to contamination; no growth indicated for 13.
[d] Abbreviations: 460, BACTEC 460TB system; 960, BACTEC MGIT 960 system.
[e] "All mycobacteria" is not equal to the sum of mycobacterial groups, as some samples had two mycobacterial species.
[f] For comparison of BACTEC systems. Abbreviations: NS, not significant; ND, not done.

14, 15, 16). However, high percentages of culture-positive specimens (9.2 to 15.1%) and high amounts of smear-positive samples (up to 72%) reported in these studies suggest that they were performed either with a collection of patients with a high incidence of infectious tuberculosis or with selected clinical specimens. One study (16) conducted in a low-incidence laboratory did not use any solid media; contamination rates were high (up to 30%), and specimen numbers were low (two study periods with 859 and 941 samples). In this study we have evaluated the reliability of the MGIT 960 system in comparison to the BACTEC 460TB system and Löwenstein-Jensen (LJ) medium in daily routine diagnostic procedures of a nonspecialized microbiology laboratory where initial screening for the presence of mycobacteria is performed. The laboratory serves a university hospital with units specialized in the treatment of cystic fibrosis patients, human immunodeficiency virus-infected patients, and transplant patients. There is no specialized tuberculosis clinic. Approximately 4,500 clinical specimens are sent annually for mycobacterial culture. Our laboratory adheres to the monitoring procedures laid down by INSTAND (Düsseldorf, Germany) in its voluntary quality control measures (twice-annual checks for sensitivity of microscopic procedures, culture procedures, and differentiation).

During an 8-month study period (August 2000 to March 2001), all specimens (except blood) sent to the mycobacterium laboratory were analyzed in parallel by staining and culture in the BACTEC 460TB system, the MGIT 960 system, and LJ medium. Specimens were processed according to standard protocols (9). Nonsterile specimens were decontaminated by *N*-acetyl-L-NaOH and concentrated by centrifugation (4,000 × *g*, 15 min). The sediment was resuspended in 2 ml of phosphate buffer (pH 6.8). Processed specimens were stained by the Ziehl-Neelsen method. Of each specimen 0.5 ml of concentrate was inoculated into the following vials: MGIT 960 tubes, containing Middlebrook 7H9 with the supplement, BBL MGIT OADC (oleic acid, albumin, dextrose, and catalase), and BBL MGIT PANTA (polymyxin B, amphotericin B [AMB], nalidixic acid, trimethoprim [TMP], and azlocillin) as recommended by the manufacturer; and BACTEC 460 12B, containing modified Middlebrook 7H12 with the antibiotic supplement PANTA (polymyxin B [2,000 U/ml], AMB [200 μg/ml], nalidixic acid [800 μg/ml], TMP [200 μg/ml], azlocillin [200 μg/ml]). In parallel, two tubes containing LJ medium—one with glycerol and one without (Biotest, Heidelberg, Germany)—were inoculated with 0.2 ml of the same specimen. LJ medium was used without antibiotics (except for respiratory specimens from cystic fibrosis patients, for which LJ medium with the antibiotic supplement PACT—polymyxin B [26 μg/ml], AMB [10 μg/ml], carbenicillin [50 μg/ml], TMP [10 μg/ml]—was used). The order of inoculation was random. All specimens were incubated at 37°C (except skin specimens [30°C]) for 8 weeks. MGIT 960 tubes were incubated and automatically monitored in the MGIT 960 instrument. BACTEC 460TB vials were monitored every 2 days during the first week and weekly thereafter. A growth index of ≥100 was considered positive. LJ cultures were inspected weekly. Nonmycobacterial overgrowth was detected by using blood agar plates. Growth of mycobacteria was verified by microscopy (Ziehl-Neelsen) and subcultivation (LJ medium). Mycobacteria were identified by nucleic acid probes (Gen-Probe, San Diego, Calif.) or by sequencing of the 16S rRNA gene (7) and conventional biochemical tests. Transport time was calculated as the difference between the sampling date and the arrival date in the laboratory, and detection time was calculated as the difference between the date the BACTEC 460TB or MGIT 960 system indicated growth and the arrival date. All statistical results were calculated using Epi Info, version 6 (3). Statistical significances of differences were determined by the χ^2 test, the

TABLE 3. Contamination rates in each culture system for different patient groups

Patient group	No. of specimens (% of total)	% Contamination in[a]:			P^c		
		460	960	LJ	460 vs 960	460 vs LJ	960 vs LJ
Without cystic fibrosis	2,382 (90.8)	3.3	6.9	5.3	<0.001	<0.001	0.021
With cystic fibrosis[b]	242 (9.2)	24.8	16.9	14.5	0.034	0.004	NS
Total	2,624 (100.0)	5.3	7.9	6.2	<0.001	0.018	NS

[a] Abbreviations: 460, BACTEC 460TB system; 960, BACTEC MGIT 960 system; LJ, LJ medium. For differences between patient groups, $P < 0.001$.
[b] From 106 cystic fibrosis patients (95.0% of specimens were sputum samples).
[c] Abbreviations: NS, not significant; ND, not done.

TABLE 4. Contamination rates in each culture system for different clinical materials from patients without cystic fibrosis

Clinical material	No. of specimens (% of total)	% Contamination in[a]:			P[c]		
		460	960	LJ	460 vs 960	460 vs LJ	960 vs LJ
Respiratory	1,218 (51.1)	3.2	6.4	4.2	<0.001	NS	0.015
Sterile body site specimen	444 (18.6)	0.5	0.7	0.2	NS	NS	NS
Urine	319 (13.4)	4.4	9.1	7.2	0.018	NS	NS
Gastrointestinal tract	251 (10.5)	8.4	21.5	19.5	<0.001	<0.001	NS
Gastric aspirate	189 (7.9)	8.5	22.2	22.2	0.002	0.002	NS
Stool	40 (1.7)	12.5	30.0	15.0	NS	NS	NS
Other[b]	150 (6.3)	1.3	0.7	2.0	NS	NS	NS
Total	2,382 (100.0)	3.3	6.9	5.3	<0.001	<0.001	0.021

[a] Abbreviations: 460, BACTEC 460TB system; 960, BACTEC MGIT 960 system; LJ, LJ medium. For differences between material groups, $P < 0.001$.
[b] Includes specimens other than those mentioned in the table.
[c] Abbreviations: NS, not significant.

χ^2-of-linearity test, or Student's *t* test, where appropriate, with a *P* of ≤0.05 considered to be significant.

From 1,188 patients, a total of 2,624 clinical specimens submitted (respiratory, 55.2%; sterile body sites, 17.1%; urine, 12.3%; gastrointestinal tract, 9.7%; others, 5.7%) were investigated. The number of samples per patient ranged from 1 to 25 (median, 1; mean ± standard deviation [SD], 2.21 ± 2.30).

In all, 4.7% of cultures were positive for mycobacteria and 2.2% were positive for *M. tuberculosis* complex. There was no statistically significant difference in recovery of *M. tuberculosis* complex (Table 1), as previously reported (2, 4, 5, 13, 14, 15). Differences in recovery of nontuberculous mycobacteria comparing the BACTEC 460TB and MGIT 960 systems have likewise been reported (5, 15). The recovery rate of *M. avium-M. intracellulare* in the MGIT 960 system was not significantly different from the recovery rate reported by Hanna et al. (4); still, we were unable to recover more *M. avium-M. intracellulare* with the MGIT 960 system. Table 2 shows recovery rates in accordance with initial smear results. In contrast to a report on the MB/BacT system (12), the MGIT 960 system was more sensitive in discovering *M. tuberculosis* complex in smear-negative samples. Still, the combination of the MGIT 960 system and LJ culture would have missed the diagnosis of tuberculosis in one patient, as only a single sputum sample was submitted for culture and the LJ culture remained negative. One smear-positive sample with subsequent growth of *M. tuberculosis* complex was contaminated in the MGIT 960 system. Tortoli et al. (15) reported on a reduced sensitivity in smear negative samples, especially for *M. gordonae* recovery, of the MGIT 960 system, similar to our result.

Contamination rates (Table 3) of samples from patients with or without cystic fibrosis for the BACTEC 460TB system and LJ cultures were comparable to those in previous reports (1, 4, 6, 12, 13, 15), as were differences in contamination rates for the BACTEC 460TB and MGIT 960 systems (2, 4, 5, 10, 13, 14, 15, 16). To our knowledge there have been no reports on contamination rates of sputa from cystic fibrosis patients cultured in the MGIT 960 system. Statistically significant differences were found for each of the three systems in different material groups (Table 4). Contamination rates of the MGIT 960 system compared to the BACTEC 460TB system were statistically significantly elevated in contaminated clinical material. Transport time has been discussed (2, 5, 10, 16) as a reason for differing contamination rates. Transport time (mean ± SD) was 0.90 ± 1.17 days (range, 0 to 13; median, 1). Calculation of contamination rates in dependency of transport time revealed no significant differences for the BACTEC 460TB system and LJ medium and no linear relation for the MGIT 960 system (χ^2 of linearity, 10.38 [$P = 0.016$, with linearity being not present when *P* was <0.05]).

The MGIT 960 system detected mycobacteria significantly earlier than the BACTEC 460TB system (Table 5), especially *M. tuberculosis* complex and *M. avium-M. intracellulare*. The results for detection time with the MGIT 960 system are similar to those in previous reports (2, 4, 5, 14, 15, 16). Kanchana et al. (5), who determined the positivity of the BACTEC 460TB system as we did, obtained comparable results for detection time in both systems. In contrast to this, studies (2, 4, 14, 15, 16) using more-intensive schemes to detect positivity in the BACTEC 460TB system were not able to show differences in detection time for the BACTEC 460 and MGIT 960 systems.

In conclusion, the MGIT 960 system is a suitable and fast nonradiometric alternative for *M. tuberculosis* complex recovery. Contamination rates were associated with underlying disease (cystic fibrosis), the material (gastric aspirate), and the detection system (MGIT 960) but not with transport time. Under our conditions the MGIT 960 system detected mycobacteria significantly earlier than the BACTEC 460TB system, especially *M. tuberculosis* complex. In the inevitable advent of nonradiometric detection systems (with their systems' inherently higher contamination rates), physicians should, whenever feasible, send respiratory samples (instead of gastric aspirate)

TABLE 5. Detection time in the BACTEC 460TB and MGIT 960 systems

Species	Detection time (mean days ± SD) in[a]:		P[b]
	460	960	
M. tuberculosis complex	18.47 ± 7.10	14.08 ± 4.70	<0.001
M. avium-M. intracellulare	14.19 ± 6.42	8.91 ± 3.37	<0.001
Other mycobacteria	24.67 ± 16.35	26.75 ± 17.42	NS
All mycobacteria	18.64 ± 10.62	14.49 ± 9.93	0.004
Contamination	18.64 ± 16.34	8.81 ± 9.47	<0.001

[a] Abbreviations: 460, BACTEC 460TB system; 960, BACTEC MGIT 960 system.
[b] For comparison of BACTEC systems. NS, not significant.

and send more repetitive samples for *M. tuberculosis* complex and nontuberculous mycobacterium recovery.

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