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Accelerated Detection and Identification of Mycobacteria with MGIT 960 and COBAS AMPLICOR Systems

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An automated cultivation system for mycobacteria, the MGIT 960 system (MGIT system), was compared in the clinical routine with two variants of Löwenstein-Jensen (L-J) medium. A total of 152 isolates were recovered from 2,015 specimens: 139 (91%) with the MGIT system and 127 (84%) with L-J media ($P = 0.05$). These included 68 isolates of *Mycobacterium tuberculosis*, of which 88% grew in the MGIT system and 93% grew in L-J media ($P = 0.389$), and 84 isolates of mycobacteria other than *M. tuberculosis* (MOTT), of which 94% grew in the MGIT system and 76% grew in L-J media ($P = 0.003$). More *M. avium* complex isolates were detected in the MGIT system ($n = 65$) than in L-J media ($n = 50$) ($P = 0.001$). Growth in the MGIT system was detected in 2 weeks for 78% of the isolates, whereas growth was detected in the two L-J media for 17 and 25% of the isolates, respectively. The mean times to detection of *M. tuberculosis* were 12 days in the MGIT system and 20 days in L-J media, and for *M. avium* complex the mean times to detection were 8 and 22 to 25 days, respectively. The contamination rates were similar (8.7 to 8.9%) in all media. A commercial amplification system (COBAS AMPLICOR) was evaluated for its ability to rapidly identify *M. tuberculosis*, *M. avium*, and *M. intracellulare* directly from 393 samples in MGIT system broth. A correct PCR result, as evaluated by culture or clinical data, was obtained for 96% of the samples, with inhibition being detected for 2% of the samples. Of the 89 results positive for *M. tuberculosis*, 91% were regarded as true positive, 8% were regarded as inconclusive, and 2% were considered false positive. For results positive for *M. avium* and *M. intracellulare*, 97 and 79%, respectively, were regarded as true positive. Increased rapidity and enhanced isolation of MOTT were obtained with the MGIT system. COBAS AMPLICOR was suitable for rapid identification of these three common pathogens from MGIT system broth.

The current epidemiology of mycobacterial infections in high- and medium-income countries is characterized by epidemic bursts of tuberculosis in high-risk minority groups (14). There has also been an increase in the number of infections caused by mycobacteria other than *Mycobacterium tuberculosis* (MOTT), which pose a particular threat to the growing populations of immunocompromised patients. Consequently, the major targets for improvement of the laboratory diagnosis of mycobacterial infections center on speeding up detection and identification of mycobacteria and also increasing the sensitivity of detection of MOTT. Molecular methods have not been able to replace cultivation for the detection of mycobacteria from clinical specimens (1), and attention is now being paid on ways to improve cultivation. When routine molecular methods for identification of mycobacteria are combined, one could predict accelerated laboratory diagnosis of mycobacterial infections. Automated blood culture systems for cultivation of mycobacteria have recently been introduced (3, 16, 17). Their major drawbacks are that they require space-demanding cabinets and the use of a needle and syringe for inoculation and sampling. A more recent approach is a modification of a previous manual technique, the mycobacteria growth indicator tube (MGIT) technique (11, 12), which has been modified to permit the use of an automated cultivation and detection system (the MGIT 960 system [MGIT system]) (2).

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The MGIT system was evaluated in the clinical laboratory routine with samples from a population in whom *M. tuberculosis* and MOTT are equally common as clinical isolates. It was compared to cultivation by using two variants of Löwenstein-Jensen (L-J) medium. It soon became evident that the recovery of mycobacteria was accelerated with the MGIT system, but this was only of partial benefit if it could not be accompanied by rapid species identification. In a subsequent study, a commercial amplification system (COBAS AMPLICOR [CA]) was evaluated for reliability of detection of *M. tuberculosis*, *M. avium*, and *M. intracellulare* directly from the MGIT system broth after the tube gave a signal for growth.

MATERIALS AND METHODS

Clinical specimens. Consecutive clinical specimens other than urine and feces submitted for cultivation of mycobacteria to the Laboratory of Clinical Microbiology, Kuopio University Hospital, Kuopio, Finland, from January to April 1998 were included in the study. Among the 2,015 specimens examined, 1,711 (85%) were sputa or bronchial secretions. The remainder included pleural fluid ($n = 95$), cerebrospinal fluid ($n = 14$), synovial, pericardial, or peritoneal fluid ($n = 19$), bone marrow aspirates ($n = 23$), biopsy specimens from soft tissues or bones ($n = 75$), and pus ($n = 78$).

Cultivation. Specimens known to contain commensal flora were decontaminated with an equal volume of NaOH (final concentration, 1%) containing *N*-acetylcysteine (MycoPrep; Becton Dickinson Microbiology Systems, Cockeysville, Md.) for 15 min. After neutralization with phosphate buffer (pH 6.8; BBL MycoPrep), 0.5 ml of the centrifuged sediment was pipetted into an MGIT system tube and 0.05 ml was pipetted into three slants of egg medium (pH 6.3) (7) by using one glycerol-supplemented tube and two pyruvate-supplemented tubes, Mycotube-1 and Mycotube-2, respectively (Orion Diagnostica, Espoo, Finland). The order of inoculation was determined each day by the toss of a coin. Specimens from normally sterile sites were similarly decontaminated, but only if they were found to be colonized after overnight culture on sheep blood agar. A spare specimen was stored at 4°C for 3 weeks and, in case of contamination of any of the culture tubes, was used for a repeat decontamination. Enrichment supplement (0.8 ml; MGIT system oleic acid-albumin-dextrose-citric acid

[OADC; BBL]) was added to each tube, and an antimicrobial supplement (MGIT system PANTA [polymyxin B, nalidixic acid, trimethoprim, and azlocillin; BBL]) was added to the tubes with decontaminated specimens. The MGIT system instrument was programmed for 7 weeks of incubation at 37°C. L-J media were incubated for 7 weeks at 35°C and were visually examined for growth once a week. Skin-associated specimens were additionally inoculated onto 7H11 agar supplemented with OADC and hemin (4) and into an MGIT system tube containing a 5-μg X-factor disk (PDM Diagnostic; AB Biodisk, Solna, Sweden) as the hemin source. Both were incubated at 30°C for 4 months (9), with the MGIT system tubes read weekly by using a manual UV light source. Whenever any of the tubes indicated growth, the parallel tubes were examined.

Processing of specimens indicating growth. Detection of growth in the MGIT system tubes is based on the consumption of oxygen. The MGIT system instrument reads each tube location once an hour and sounds an alarm if growth is indicated. An alarm signal was followed by a smear for acid-fast staining with acridine orange (5) and a subculture on 7H11 agar. If non-acid-fast microbes were detected, a repeat decontamination and inoculation were done starting with the spare specimen in storage. Species identification, done separately from isolates from the MGIT system and solid media, was based on analysis of bacterial fatty acid and alcohol compositions by gas-liquid chromatography, combined with a set of biochemical and growth characteristics, as described earlier in detail (8, 15). In this system, *M. avium* and *M. intracellulare* are classified as *M. avium* complex.

Quality control. Each batch of MGIT system tubes was controlled for support of growth by using *M. tuberculosis* ATCC 27294, *M. kansasii* ATCC 12478, and *M. fortuitum* ATCC 6841 and for inhibition by PANTA of *Pseudomonas aeruginosa* ATCC 27853 and *Staphylococcus epidermidis* 3071/94, a quality control strain. L-J media were also tested for growth of *M. intracellulare* ATCC 13950, *M. scrofulaceum* ATCC 19981, and *M. malmoense* 88/93.

Statistical analyses. Test of proportions was used to compare sensitivity of detection. A P value of ≤ 0.05 was used to indicate statistical significance. The kappa coefficient was also applied to evaluate agreement between the results obtained with the MGIT system and L-J media. A kappa value above 0.75 was regarded as an indicator of excellent agreement above chance.

Detection and identification of growth by CA system. (i) Specimens. The MGIT system broth samples used for organism identification by PCR with the CA system were collected from April to June 1998, starting during the latter part of the study with the MGIT system. An aliquot of 0.5 ml of broth was pipetted into a 1-ml screw-cap polypropylene tube from consecutive MGIT system tubes for which an alarm for growth was sounded, and the tube was deep frozen at −80°C. Otherwise, the tubes were analyzed as described above in detail. If no microbes were seen by microscopy, the MGIT system tube was reentered into the MGIT system instrument. If the tube repeatedly gave a signal for growth, a new sampling for PCR followed. The total number of samples subjected to PCR analyses was 426, and these were retrieved from 393 tubes.

(ii) Specimen processing for testing with the CA system. The samples were processed by following the manufacturer's recommendations, using the Respiratory Specimen Preparation Kit (AMPLICOR; Roche Molecular Systems, Inc., Branchburg, N.J.). In brief, a thawed sample was vortexed; and 100 μl was transferred into a 1.5-ml polypropylene tube, washed with the kit's wash solution (500 μl), and centrifuged (12,700 × *g*) for 10 min. The supernatant was discarded and the pellet was resuspended in a lysis reagent (100 μl), after which the tube was incubated in a 60°C water bath for 45 min. Finally, the lysate was neutralized with the neutralization reagent (100 μl) provided with the CA system kit.

(iii) PCR with the CA system. For PCR amplification, 50 μl of the specimen mixture was added to a tube with 50 μl of a master mixture solution containing mycobacterial primers, nucleotides, DNA polymerase, and the internal control (Roche Molecular Systems, Inc.). Negative and positive controls for *M. tuberculosis* as well as positive controls for *M. avium* and *M. intracellulare* were included in each run. The CA system amplifies the target DNA by using genus-specific biotinylated primers. After a standard number of cycles, which are automatically performed by the CA system, the amplicons were chemically denatured and hybridized with probes specific for the species *M. tuberculosis*, *M. avium*, and *M. intracellulare*. The biotin-labeled amplicons were identified by colorimetric detection (A_{600}). The CA system uses avidin-horseradish peroxidase conjugate which binds to the amplicons and which catalyzes the oxidation of 3,3′,5,5′-tetramethylbenzidine in the presence of hydrogen peroxide, forming a colored complex. The mycobacterial internal control is a plasmid with primer binding regions identical to those of the *M. tuberculosis* target sequence. The competitive amplification of the mycobacterial 16S rRNA gene and an internal control helps to identify the samples with inhibitory substances. In addition, the CA system uses the uracil-*N*-glycosylase enzyme, which is included in the master mixture, to prevent carryover of contaminating, previously amplified DNA.

RESULTS

Of all 2,015 specimens, 148 (7%) were positive for mycobacteria by culture. Two species were detected in four specimens, and thus a total of 152 isolates were recovered (Table 1). *M. tuberculosis* grew from 68 specimens, and 84 isolates of MOTT were detected from 80 specimens. The species isolated included *M. avium* complex ($n = 67$), *M. malmoense* ($n = 7$), *M. kansasii* ($n = 1$), *M. xenopi* ($n = 1$), *M. conspicuum* ($n = 1$), and *M. fortuitum* ($n = 5$). Two isolates were poorly characterizable; one was classified as an *M. simiae*-like organism, and the other was classified as a rapid grower other than *M. fortuitum* or *M. chelonae*. Four specimens positive for *M. avium* complex also grew either *M. malmoense* (one specimen) or *M. fortuitum* (three specimens). In each case, both species had been or were later recovered from the same patients' other specimens either in the same combination or separately.

TABLE 1. Recovery of mycobacteria from 2,015 samples by the MGIT system and L-J media (Mycotube-1 and Mycotube-2)

Mycobacterium isolated	Total no. of isolates	MGIT system		L-J media[a]		P[b]
		No. of isolates	%	No. of isolates	%	
M. tuberculosis	68	60 (14)[c]	88	63 (4)	94	0.389
Total MOTT	84	79 (2)	94	64 (1)	76	0.003
M. avium complex	67	65	97	50	75	0.001
Total	152	139 (16)	91	127 (5)	84	0.05

[a] Includes Mycotube-1 (one slant) and Mycotube-2 (two slants).
[b] Test of proportions was used to compare sensitivities between the MGIT system and L-J media.
[c] The number of isolates that were detected only after repeat decontamination, performed if the initial medium became contaminated (see Materials and Methods), is given in parentheses.

The MGIT system recovered 139 (91%) of the isolates, and the L-J media revealed 127 (84%) of the isolates, of which 79 (52% of all isolates) were detected in Mycotube-1 and 115 (76%) were detected in Mycotube-2. Sixty (88%) of all *M. tuberculosis* isolates were detected in the MGIT system and 63 (93%) were detected in the L-J media (Table 1), with 55 (81%) and 59 (87%) growing in Mycotube-1 and Mycotube-2, respectively. Thus, the MGIT system and the combination of L-J media used were equal in their abilities to detect *M. tuberculosis* (kappa = 0.882; 89.68% of the maximum kappa). In the MGIT system, 14 (21%) of the *M. tuberculosis* isolates became detectable only after repeat decontamination (Table 1). In Mycotube-1 and Mycotube-2, three and six isolates, respectively, were recovered only after repeat decontamination.

Of the 84 MOTT isolates, 79 (94%) were recovered in the MGIT system and 64 (76%) were recovered in L-J media, with 26 (31%) being detected in Mycotube-1 and 59 (70%) being detected in Mycotube-2. The difference in sensitivity of detection between the media was significant ($P = 0.003$). All but 2 (97%) of the *M. avium* complex isolates were detected in the MGIT system, whereas 18 (27%) and 48 (72%) isolates were detected in Mycotube-1 and Mycotube-2, respectively ($P = 0.001$). Both the MGIT system and Mycotube-2 missed two of the seven *M. malmoense* isolates, whereas only one *M. malmoense* isolate was recovered in Mycotube-1. Four (6%) of the *M. tuberculosis* isolates, 19 (28%) of the *M. avium* complex isolates, and 4 (22%) of the other MOTT isolates were exclusively detected in the MGIT system, whereas 2, 2, and 3 of these isolates, respectively, were exclusively recovered in L-J media. The specimen type had no detectable influence on the comparison of detection rates in the MGIT system and in L-J media. Contaminating microbes were detected equally often in all three medium types (8.8, 8.9, and 8.7% in the MGIT system, Mycotube-1, and Mycotube-2, respectively).

The culture medium, species, and bacterial load in the specimen, as evaluated by staining, influenced the time to initial

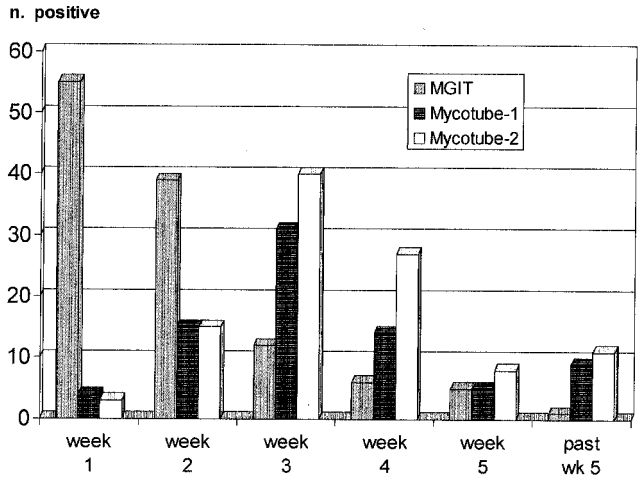

FIG. 1. Distribution of time to detection (in weeks) of mycobacteria by the MGIT system and two variants of L-J media, Mycotube-1 and Mycotube-2. The number (*n*) indicates the number of specimens found to be positive per week.

detection of mycobacterial growth. Growth was recovered within 14 days after arrival in the laboratory in 94 (78%) of the 120 specimens found to be positive with the MGIT system, in 19 (25%) of the 78 specimens positive with Mycotube-1, and in 18 (17%) of the 104 specimens positive with Mycotube-2 (Fig. 1). The specimens found to be positive for mycobacteria only after repeat decontamination have been excluded from evaluation of time to detection. The mean times to detection of *M. tuberculosis* were 8 days from smear-positive specimens and 21 days from smear-negative specimens with the MGIT system, whereas the times were 16 days (smear-positive specimens) and 23 to 25 days (smear-negative specimens) with the L-J media (Table 2). *M. avium* complex isolates were detected in 8 days in the MGIT system, whereas they were detected in 22 and 25 days in Mycotube-1 and Mycotube-2, respectively. *M. malmoense*, an exceptionally slowly growing species (6), was detected in 20 days in the MGIT system but only after 53 and 33 days in Mycotube-1 and Mycotube-2, respectively.

Among the 393 MGIT system tubes sampled for PCR identification, 128 (33%) were positive by culture for the species included in the CA identification system, i.e., *M. tuberculosis* ($n = 79$) or *M. avium* complex ($n = 49$). A total of 141 (35%) tubes were positive by PCR; 89 (23%) tubes tested positive for *M. tuberculosis*, 33 (8%) tested positive for *M. avium*, and 19 (5%) tested positive for *M. intracellulare* (Table 3). One of the tubes was PCR positive for both *M. tuberculosis* and *M. avium*, and another was positive for *M. tuberculosis* and *M. intracellulare* but none was positive for all three species. Amplification was inhibited in seven (2%) tubes.

In 362 (96%) tubes, similar results were obtained by both methods (Table 3). The results for samples from 17 tubes that were contaminated in culture or that were inhibitory to the PCR were excluded from these analyses. The same species detected by PCR was recovered in culture from 121 tubes (95% of those culture positive for *M. tuberculosis* or *M. avium* complex), and 241 tubes remained negative by PCR, which was 97% of those culture negative for *M. tuberculosis* or *M. avium* complex.

Among the samples with results discordant between PCR and culture, the following discrepancies were observed. One MGIT system tube positive by PCR for both *M. tuberculosis* and *M. intracellulare* grew only *M. tuberculosis*, and another specimen positive by PCR for both *M. tuberculosis* and *M. avium* grew only *M. avium* complex in culture. For an additional 16 samples, the positive PCR results did not match the culture results, including the results for 11 and 5 samples with *M. avium* or *M. intracellulare*. In addition, seven (2%) samples had false-negative PCR results. If a discrepancy existed between the PCR and culture results, the laboratory and clinical data available for the patient were analyzed after approximately 6 months of follow-up. Among the 23 specimens thus evaluated, the positive PCR result was regarded as true positive for 7 specimens (Table 3). Interestingly, two consecutive specimens from a patient, one positive by PCR for *M. avium* and the other positive by PCR for *M. intracellulare*, also grew two different colony types of the *M. avium* complex.

Among the PCR series, 18 specimens grew MOTT other than *M. avium* complex. These species included *M. gordonae* ($n = 3$), *M. interjectum* ($n = 1$), *M. lentiflavum* ($n = 1$), *M. malmoense* ($n = 2$), *M. xenopi* ($n = 1$), *M. abscessus* ($n = 1$), *M. fortuitum* ($n = 5$), a rapid grower other than *M. fortuitum* or *M. chelonae* ($n = 1$), and other inconclusively classifiable species ($n = 3$). Among these, 17 specimens were negative by PCR testing and 1 specimen that was culture positive for *M. fortuitum* was positive by PCR for *M. tuberculosis*. This result was regarded as false positive by clinical evaluation.

In conclusion, a positive PCR result was regarded as unequivocally truly positive for 92, 97, and 80% of the specimens infected with *M. tuberculosis*, *M. avium*, and *M. intracellulare*,

TABLE 2. Time to initial detection of mycobacterial growth in the MGIT system and L-J media (Mycotube-1 and Mycotube-2) by species isolated, excluding isolates detected only after repeat decontamination

Species isolated	MGIT		Mycotube-1		Mycotube-2	
	No. of specimens	Mean time (days) to detection (range)	No. of specimens	Mean time (days) to detection (range)	No. of specimens	Mean time (days) to detection (range)
M. tuberculosis (total)	46[a]	12 (3–33)	52[a]	20 (9–41)	53[a]	20 (9–48)
Smear positive[b]	24	8 (3–18)	27	16 (9–32)	26	16 (9–32)
Smear negative	15	21 (14–33)	17	23 (16–41)	19	25 (16–48)
M. avium complex	64	8 (3–30)	19	22 (6–41)	49	25 (5–41)
M. conspicuum	1	24	0		0	
M. kansasii	1	10	1	17	0	
M. malmoense	4	20 (15–31)	1	53	4	33 (20–53)
M. simiae-like	0	0	1	30	1	30
M. xenopi	1	49	0		0	
Rapid growers	5	7 (5–13)	4	7 (6–10)	3	6 (5–6)

[a] The total number also includes specimens lacking smear microscopy.

TABLE 3. Results of PCR with the CA system with 393 specimens from MGIT system both compared to results of culture or, in case of discrepancy, with the patients' other laboratory and clinical data

Classification[a]	No. (%) of specimens with the following PCR result[b]:		
	MTB	MAV	MIN
Total PCR positive	89 (100)	33 (100)	19 (100)
True positive	81 (91)	32 (97)	15 (79)
Inconclusive positive	6 (8)	1 (3)	3 (16)
False positive	2 (2)	0	1 (5)
False negative[c]	2 (3)	4[d] (8)	NA[e]

[a] The result was "true positive" if the same species grew in culture or the patient had received a diagnosis histopathologically verified granulomatous infection and had been successfully treated as tuberculosis. The result was "inconclusive" if three or more concurrent specimens were negative for mycobacteria by microscopy and culture but clinical evaluation could not rule out mycobacterial infection. The result was "false positive" if three or more concurrent specimens were negative for mycobacteria by microscopy and culture and mycobacterial etiology was regarded as unlikely on clinical grounds.

[b] MTB, *M. tuberculosis*; MAV, *M. avium*; MIC, *M. intracellulare*.

[c] Compared to culture of the same specimen.

[d] *M. avium* and *M. intracellulare* were classified as *M. avium* complex in culture.

[e] NA, not available.

respectively (Table 3). One (1%) of the 89 specimens positive for *M. tuberculosis*, none of those positive for *M. avium*, and 3 (16%) of the 19 specimens positive for *M. intracellulare* were regarded as false positives. For the remaining specimens, the positive PCR result was regarded as inconclusive. Some of the patients are still under observation or are receiving trial treatments.

In MGIT system tubes sampled more than once, the repeat sampling gave an identical result for 30 of the 34 samples. The first sample from one tube that was culture positive for *M. tuberculosis* was negative by PCR but became positive for *M. tuberculosis* in the PCR with the replicate sample. For the other three tubes, unexplained inconsistencies were detected, including a change from a negative result to the presence of inhibitory activity or from positivity for *M. intracellulare* or *M. avium* to negativity for the two organisms. In both cases, the initial result was evaluated as a false-positive result when the patients' other data were assessed.

DISCUSSION

The undeniable benefits of the automated MGIT system in comparison with culture on L-J media were its rapidity of primary detection of mycobacterial growth and its enhanced isolation of MOTT, particularly the *M. avium* complex. Of the *M. avium* complex isolates detected, 25% were exclusively detected by the MGIT system. Some potentially pathogenic species, such as *M. kansasii* and *M. xenopi*, which are extremely rare in Finland, could be recovered with the MGIT system. Our present experience involving a larger number of specimens further indicates that *M. xenopi* may be missed by cultivation on L-J media. *M. malmoense* is an important pathogen in the Nordic countries. It is known to grow poorly in ordinary L-J medium (6). The MGIT system supported the growth of *M. malmoense* as well as Mycotube-2 did, which is a pyruvate-supplemented L-J medium variant that was initially developed to enhance the ability to isolate *M. malmoense* and other difficult-to-grow MOTT (7).

The speed with which the MGIT system started to detect mycobacterial growth was remarkable compared to that for the L-J media. The mean time to culture verification of smear-positive tuberculosis and *M. avium* complex infection decreased to 8 days. A small bacterial load, which occurs with smear-negative tuberculosis, tripled the time to detection of *M. tuberculosis* so that it was close to those observed with the L-J medium variants used. Our earlier studies have indicated that decreased medium pH and pyruvate supplementation shorten the time to detection and also increase the rates of isolation of MOTT (7).

Rapidity of primary detection is of relative benefit only when quick identification of the species cannot be made. It is of great practical importance to differentiate *M. tuberculosis* from MOTT as quickly as possible, particularly in an epidemiological situation in which MOTT represent a considerable share of the clinical isolates. When species identification and drug susceptibility testing of *M. tuberculosis* are performed from an MGIT system subculture on solid medium, the final result is available only marginally earlier from the automated systems than by cultivation on L-J media. Rapid verification of the growth from the MGIT system as *M. tuberculosis*, *M. avium*, or *M. intracellulare* was successfully obtained by using PCR with the CA system at the first alarm signal for growth. In our experience, genetic probes (Accuprobes specific for *M. tuberculosis* and *M. avium* complex; Gen-Probe, San Diego, Calif.) have proved to be less reliable at this early stage, although they are useful after further incubation (unpublished data). From the practical point of view, the CA system proved to be easy to handle in a routine diagnostic laboratory. Inhibition was found to be a smaller problem when detecting growth from the MGIT system broth than when detecting growth from BACTEC 12B cultures (10). The spectrum of identification probes available in the CA system, however, still leaves 10 to 15% of the isolates undetected in our epidemiological situation. A reliable genus-specific probe (13) would be useful for verification of the presence of mycobacteria other than the three species included in the present study.

Recent studies have shown that drug susceptibility testing can be rapidly and directly performed from an MGIT system broth sample which is positive for MTB (12). With the MGIT system, highly infective cases of tuberculosis, i.e., smear positive, can be confirmed by culture in as little as 8 days. In this specific instance, if the CA system is used, verification of the species could be obtained by the end of the second week, with drug susceptibility results available before the end of the third week.

For only 4% of specimens did the PCR findings fail to match the culture results. Some of these were true-positive results, as verified by other laboratory and clinical data for these specimens, but others remained inconclusive. For specimens for which PCR indicated the presence of two species, it was possible that the species with slower growth rates or fewer cell numbers were overgrown and thus remained undetected by culture. However, the possibility of crossover contamination was a more likely explanation for our specimens. Among our patient material, a series of three consecutive specimens from one patient were smear positive for acid-fast bacilli. Two of them were included in the PCR series, and both MGIT system tubes proved to be PCR positive for *M. intracellulare*. None of his specimens grew mycobacteria on any solid medium, including 7H11 agar. Thus, occasional strains of *M. intracellulare* may fail to grow in common media.

When culture verification of tuberculosis was regarded as the "gold standard," broth specimens with false PCR results, either negative or positive, accounted for less than 3% of all the broth specimens analyzed. Evaluation of the results for *M. avium* complex is more obscure because harmless colonization of the respiratory tract by MOTT may be common,

and *M. avium* complex strains with exceptional growth requirements make cultivation unreliable. This was also verified in the present study. Samples from a smear-positive patient with granulomatous bronchopulmonary lesions grew acid-fast bacilli in the MGIT system and the broth was PCR positive for *M. intracellulare*. The strain has remained unculturable on any of the available solid media.

Contamination of cultures is a problem which is particularly associated with liquid media. Despite the low end concentration of NaOH used (1%), the contamination rate in the MGIT system was within an acceptable range and was similar to that obtained with the L-J media. To rescue specimens otherwise lost to contamination, repeated decontamination proved to be valuable. It is equally important to use a parallel solid medium, because contaminating microbes have varying capacities to survive in different selective media.

The MGIT system has several advantages over the widely used, semiautomated BACTEC 460 system, including decreased manual labor requirements, no radioactive waste, and no need to use a needle and syringe for inoculation and sampling, which is both cheaper and safer for laboratory personnel. One MGIT system instrument allows an annual throughput of over 6,000 specimens, four times more than the other automatic devices presently available (3, 17). An additional benefit is that one can also incubate and read MGIT system tubes manually when different incubation temperatures are needed (9).

Advances in medical technology have led to enhanced survival for an increasing number of immunocompromised individuals. Early and reliable detection of the causative organism is the key to well-targeted treatment of their infections. In the management of tuberculosis epidemics, rapid detection of infective patients and early detection of drug resistance are crucial. These goals can be achieved by improving cultivation techniques supplemented with routine molecular methods for species identification. However, there is an increase in costs compared to those of conventional cultivation techniques. This must be weighed against the benefits gained by early diagnosis and the higher sensitivity of detection.

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