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Mycobacterial Growth and Bacterial Contamination in the Mycobacteria Growth Indicator Tube and BACTEC 460 Culture Systems

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The BACTEC 460 system currently provides the most rapid detection of mycobacterial growth, but the system is radiometric and requires needles to inoculate specimens through the bottle's septum. The Mycobacteria Growth Indicator Tube (MGIT) system has a liquid medium, like the BACTEC system, and does not require needles when inoculating specimens. We compared mycobacterial growth from 510 specimens in the two systems. Average time to acid-fast bacillus (AFB) detection and identification to the species level was less with the BACTEC system, but this result was statistically significant only for AFB detection in specimens containing *Mycobacterium avium-M. intracellulare* complex. The contamination rate with MGIT was 29%; the BACTEC rate was 5%. To investigate MGIT contamination, we initiated a second study with changes in specimen processing. The MGIT contamination rate was reduced to 12%; the BACTEC rate was not significantly affected (5.5%). The most likely explanation for the contamination in MGIT is the richness of its medium compared to the BACTEC medium. Cost analysis for the two systems in a laboratory that processes 4,500 specimens a year is presented. The data suggest that the BACTEC 460 and the MGIT systems are approximately equivalent in cost and ability to support the growth of AFB. The MGIT system appears safer and easier to use and was preferred by laboratory personnel, but it cannot currently be used for blood specimens or antituberculosis susceptibility testing.

The BACTEC 460 TB System (Becton Dickinson Microbiology Systems, Cockeysville, Md.) is the broth system for detection of mycobacterial growth against which other systems are compared, but the system is radiometric and requires dedicated instrumentation and needle inoculation of specimens into the culture medium. The BBL Mycobacteria Growth Indicator Tube (MGIT) (Becton Dickinson Microbiology Systems) culture system uses test tubes whose bottoms contain silicone impregnated with an oxygen-sensitive fluorescent indicator. The large amount of oxygen initially present in the broth quenches fluorescence, but with rapid growth of mycobacteria or other organisms, the oxygen becomes consumed and the indicator fluoresces brightly under 365-nm UV light. The advantages of this system are the easy detection of growth and the lack of need for needles, radioactive materials, or specialized instruments other than a 365-nm transilluminator or a Wood's lamp.

We compared mycobacterial growth in the BACTEC 460 TB and MGIT systems in two studies. Because of a high MGIT contamination rate (29.8%) in the first study, changes in specimen processing were made in the second study, including the use of 6% NaOH for decontamination. A comparison of mycobacterial growth detection and identification in the two systems, a possible explanation for the higher MGIT contamination rate, and a cost comparison are presented in this report.

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MATERIALS AND METHODS

The first study was comprised of 238 consecutive specimens, from 137 patients, submitted to the Thomas Jefferson University Clinical Microbiology Laboratory for acid-fast bacillus (AFB) culture. Blood and bone marrow specimens were excluded because blood interferes with the detection of fluorescence. The numerical distribution of the specimens was as follows: sputum-bronchial aspirate, 143; urine, 3; stool, 11; sterile body sites, 81. The specimens from nonsterile body sites were digested, decontaminated, and concentrated for culture and microscopy by the *N*-acetyl-L-cysteine/sodium hydroxide method according to published Centers for Disease Control standards (5). A 4% concentration of NaOH was used. An auramine-rhodamine stain was applied to each concentrated specimen. Specimens from sterile body sites were inoculated directly into the culture media, and a direct smear was made for microscopy. All specimens (direct and concentrated) were inoculated into three media: a BACTEC 12B bottle (Becton Dickinson Microbiology Systems), an MGIT tube, and a Lowenstein-Jensen (LJ) slant. Inoculation and processing were carried out strictly in accordance with the manufacturers' recommendations. All media were incubated at 37°C in a 5% CO_2 incubator for up to 7 weeks. BACTEC 12B bottles were read on the BACTEC 460 twice per week for the first 2 weeks and once per week thereafter. Bottles with a growth index (GI) of 10 or greater were separated and read daily until the GI reached 100, at which time a Kinyoun smear was made and evaluated for the presence of AFB. Smear-positive bottles were reincubated until their GI exceeded 999, and then they were tested for the presence of *Mycobacterium tuberculosis* complex and *Mycobacterium avium-M. intracellulare* complex with Accuprobe test kits (Gen-Probe, Inc., San Diego, Calif.). Identification of other mycobacterial strains was accomplished by using LJ, Mycobactocel (Becton Dickinson Microbiology Systems), and 7H11 selective agar, as well as standard biochemical techniques and probes for *Mycobacterium kansasii* and *Mycobacterium gordonae* as appropriate. The MGIT tubes were observed for fluorescence five times per week (Monday through Friday) for 6 weeks with a 365-nm UV transilluminator. Fluorescing tubes were examined for the presence of AFB by using a Kinyoun stain, and smear-positive specimens were probed in the same manner as BACTEC 460 specimens. Probe procedures were performed twice per week. The BACTEC 460 specimens were tested once the GI reached 999; the MGIT specimens were tested as soon as AFB were seen on smears made from fluorescing tubes. If the Kinyoun stain revealed non-acid-fast bacteria or fungi from either BACTEC 460 or MGIT, the medium was considered contaminated. BACTEC bottles were reprocessed if AFB were found to be mixed with contaminating organisms. Contaminated MGITs received no additional workup. LJ slants were read once per week, stained with a Kinyoun stain if suspicious

TABLE 1. Growth by medium and time to AFB-positive smears

Organism	No. of isolates	Growth in:			Mean no. of days to AFB-positive smear of isolates growing in both media	
		MGIT	BACTEC	Both	MGIT	BACTEC
First study						
M. tuberculosis complex	4	0	0	4	20.8	14.5
M. avium-M. intracellulare complex	25	6	8	11	19.8	12.6
M. gordonae	7	6	1	0	28.0	20.0
Second study						
M. tuberculosis complex	0					
M. avium-M. intracellulare complex	23	6	5	11	13.7	10.7
M. gordonae	10	2	6	2	21.5	18.5
Others[a,b]	9	0	3	5	31.2	11.2

[a] One isolate grew on LJ medium only.
[b] *M. kansasii* (one), *M. marinum* (one), *M. fortuitum* (two), *M. chelonae* (one), other rapid growers (three), *M. simiae* (one).

colonies were present, and otherwise processed according to standard culture techniques.

The second study was comprised of 272 consecutive specimens (except for blood and bone marrow), from 182 patients, submitted for AFB culture. The numerical distribution of the specimens was as follows: sputum-bronchial aspirate, 127; urine, 5; stool, 9; sterile body sites, 131. Methods used for specimen digestion, decontamination, concentration, and identification were identical to those used in the first study, except that 6% NaOH was used in the decontamination process. Because of staffing and workflow changes, processing was performed and MGIT fluorescence was evaluated daily by only one medical technologist rather than by a number of technologists on a rotating basis. BACTEC bottles and MGITs which were Kinyoun smear negative for AFB but positive for bacteria or fungi were subcultured to LJ, Mycobactocel, and 7H11 selective media and a blood agar plate, and the contaminating organism was identified.

The McNemar test with Yates' correction for continuity was used to evaluate the statistical significance of differences in organism recovery in the two systems, and the Wilcoxon paired-sample test was used to evaluate differences in the amounts of time necessary for obtaining positive AFB smears and organism identification (9).

RESULTS

First study. Thirty-five of 238 specimens (14.7%) were AFB positive. The 35 specimens grew 36 AFB isolates (1 specimen grew 2 AFB): 4 *M. tuberculosis* complex, 25 *M. avium-M. intracellulare* complex, and 7 *M. gordonae* isolates (Table 1). The four specimens positive for *M. tuberculosis* complex grew isolates in both BACTEC 460 and MGIT medium. Of the 25 specimens positive for *M. avium-M. intracellulare* complex, 5 grew isolates in MGIT medium only, 8 grew isolates in BACTEC 460 medium only, and 12 grew isolates in both media. At P of <0.05, this difference in *M. avium-M. intracellulare* complex recovery was not statistically significant. Of the seven specimens positive for *M. gordonae*, six grew isolates in MGIT medium only and one grew isolates in BACTEC 460 medium only. Of the specimens which grew isolates in both MGIT and BACTEC media, the mean times to positive AFB smears were as follows: *M. tuberculosis* complex, 20.75 days in MGIT and 14.5 days in BACTEC 460 (the sample size was too small to be evaluated for statistical significance); *M. avium-M. intracellulare* complex, 19.3 days in MGIT and 12.6 days in BACTEC 460 (the difference was statistically significant at P of <0.05). The contamination rate in the MGITs was 29.8%; the contamination rate in the BACTEC bottles was 5%. Contaminating organisms were not identified.

Second study. Forty-one of 272 specimens (15.1%) were AFB positive. The 41 specimens grew 42 AFB isolates (1 specimen grew 2 AFB): 23 *M. avium-M. intracellulare* complex, 10 *M. gordonae*, and 2 *Mycobacterium fortuitum* isolates; 1 isolate each of *Mycobacterium kansasii*, *Mycobacterium marinum*, and *Mycobacterium chelonae*; 3 rapid growers that were not *M. fortuitum* or *M. chelonae*; and 1 *Mycobacterium simiae* isolate (Table 1). No specimens in this study grew *M. tuberculosis* complex.

Of the 23 specimens positive for *M. avium-M. intracellulare* complex, 6 grew isolates in MGIT medium only, 5 grew isolates in BACTEC 460 medium only, and 11 grew isolates in both media (the differences in organism recovery were not statistically significant). One *M. avium-M. intracellulare* isolate grew in LJ medium only. Of the 10 specimens positive for *M. gordonae*, 2 grew isolates in MGIT medium only, 6 grew isolates in BACTEC 460 medium only, and 2 grew isolates in both media. Of the 5 specimens positive for *M. kansasii*, *M. fortuitum*, *M. marinum*, and *M. chelonae*, all grew isolates in both MGIT medium and BACTEC 460 medium. None of the other three rapid growers grew isolates in MGIT medium; two grew isolates in BACTEC 460 medium only, and one grew isolates on LJ medium only. Contaminated specimens did not significantly influence the AFB recovery rates in the MGIT system. Two of the specimens containing *M. gordonae*, two of the specimens containing the other rapid growers, and one of the specimens containing *M. avium-M. intracellulare* complex had contamination in the MGITs. None of the specimens containing AFB had contamination in the BACTEC bottles. Although our AFB recovery rates were similar with the use of 4 and 6% NaOH, we cannot recommend 6% NaOH for processing without further study; the specimens were too small to process with both solutions.

The mean times to detection of AFB in the 10 *M. avium-M. intracellulare* complex-containing specimens were 13.7 days with MGIT and 10.7 days with BACTEC. This difference was statistically significant at P of <0.01. The mean times to identification to the species level of the 10 specimens containing *M. avium-M. intracellulare* complex were 18.9 days with MGIT and 14 days with BACTEC (not statistically significant at P of <0.05).

An unexplained growth pattern for *M. gordonae* was observed in our studies. In the first study, growth occurred almost exclusively in the MGIT system. In our second study, growth was supported best in the BACTEC 460 system.

Bacterial contamination by specimen source, the contaminating organisms, and the growth of these organisms in the MGIT and BACTEC media in the second study are outlined in Table 2. Thirty-three of 272 specimens showed contamination in the MGIT system, a rate of 12.1%. Fifteen of 272 specimens showed contamination in the BACTEC 460 system, a rate of 5.5%. This difference in contamination rates was statistically significant at P of <0.001. Forty-eight specimens in the LJ tubes were contaminated, a rate of 17.6%.

Pseudomonas species contaminated the largest number, 10, of specimens. Five of the 10 specimens were from respiratory sources; the other 5 were from stools and sterile body sites. *Pseudomonas* contamination was divided equally between MGIT and BACTEC 460. Coagulase-negative staphylococci caused contamination in eight specimens; six of the eight specimens were of respiratory origin. All 8 *Staphylococcus* spp. grew in MGIT medium only. *Corynebacterium* species also caused contamination in eight specimens; five of the eight specimens were from sterile body sites. All eight grew in MGIT

TABLE 2. Specimen sources and growth media of contaminating organisms in the second study[a]

Contaminating organism	Source of specimen			Medium		
	Respiratory source	Stool	Sterile sites	MGIT only	BACTEC 460 only	Both
Pseudomonas spp.	5	3	2	2	2	6
Coagulase-negative staphylococci	6	0	2	8	0	0
Corynebacterium spp.	2	1	5	6	0	2
Staphylococcus aureus	3	0	1	3	0	1
Streptococcus viridans	1	0	0	1	0	0
Gram-positive filamentous rods	1	0	0	0	1	0
Yeast	2	0	1	1	1	1
Mold	1	0	1	2	0	0
Serratia spp.	0	0	1	0	0	1
Enterococcus spp.	1	0	0	0	0	1
Total	22	4	13	23	4	12

[a] Some specimens were contaminated by more than one organism.

medium; two also grew in BACTEC medium. Gram-positive organisms tended to grow predominantly in the MGIT system.

DISCUSSION

Comparisons between the MGIT and BACTEC 460 culture systems in recent years (1, 3, 4, 7) have typically attested to the ease of use of MGIT and its comparability to BACTEC 460 with respect to identification of mycobacteria. Our studies confirm these observations and provide additional information on nonmycobacterial contamination.

Contamination in MGIT and BACTEC 460 by particular bacteria or fungi reflects the presence of these microorganisms in the specimens received for culture and the organisms' abilities to survive decontamination. In our studies, the most common organisms causing contamination were *Pseudomonas* species, coagulase-negative staphylococci, and *Corynebacterium* species. The most common types of specimens containing these organisms were from respiratory sources (*Pseudomonas* spp. and *Staphylococcus* spp.) and sterile body sites (*Corynebacterium* spp.). *Pseudomonas* survival was not unexpected, given the limited anti-*Pseudomonas* activity of the antibiotics contained in PANTA (polymixin, amphotericin B, nalidixic acid, trimethoprim, and azlocillin). Growth of *Pseudomonas* species was equally divided between the MGIT and the BACTEC 460 systems.

Growth of *Staphylococcus*, *Corynebacterium*, and other gram-positive organisms was distinctly favored in the MGIT system. Overall, survival of the contaminating organisms occurred more than twice as frequently in the MGIT system (12.2%) as in the BACTEC system (5.5%).

Many of the organisms which were contaminants would be expected to be resistant to the antibiotics used in the PANTA solution; however, the same lot of PANTA mixture was used in the BACTEC and MGIT media used for each specimen. A likely explanation for the increased contamination rate with MGIT is the presence of glycerol and dextrose in the enriched MGIT system (2); these substrates are not present in BACTEC 12B bottles. The listed ingredients of the BACTEC 12B (Middlebrook 7H12) vials are 7H9 broth, casein hydrolysate, bovine serum albumin, catalase, ^{14}C-substrate (palmitic acid), and deionized water (8). In their article about the formulation of media for the radiometric detection of mycobacterial growth, Middlebrook et al. (6) described omitting glucose and glycerol so the media would be more selective for mycobacterial growth. The MGIT medium contains a modified Middlebrook 7H9 broth with casein, peptone, and glycerol and is supplemented with OADC, which contains oleic acid, bovine serum albumin, dextrose and catalase (2). Presumably, the dextrose and glycerol contribute to the enhanced growth of nonmycobacterial organisms in the MGIT system.

A cost comparison of the MGIT and BACTEC 460 systems, based on 4,500 specimens analyzed per year, is outlined in Table 3. The readily measurable costs of the MGIT system, including labor and reagents, exceed those of the BACTEC 460 system by approximately $3,500.00 per year. However, the figures do not include monetary and nonmonetary costs that are more difficult to measure, e.g., laboratory time, effort, and the expense of maintaining a radiometric assay system like BACTEC 460, including proper disposal of radioactive material. In addition, laboratories will need to formulate policies governing reprocessing contaminated specimens. There is a cost associated with reprocessing each specimen as well as a cost to patient care when contaminated tubes are discarded rather than reprocessed.

Conclusion. A proper comparison of MGIT with BACTEC 460 must take several factors into account. Growth of AFB appears to be supported equally well by both systems; the amount of time necessary for mycobacterial detection and identification is generally less with the BACTEC 460 system

TABLE 3. Cost comparison of BACTEC 460 versus MGIT[a]

Item(s)	Cost ($)/yr	
	BACTEC 460	MGIT
Equipment	2,290.60[b]	
Labor	14,675.25[c]	6,906.00[d]
Reagents	13,500.00[e]	27,000.00[f]
Total	30,465.85[g]	33,906.00[h]

[a] Based on 4,500 specimens analyzed per year.
[b] Based on parts and maintenance cost of approximately $1,600.00 plus labor cost for maintenance ($23.02/h [salary and benefits] × 30 h) of $690.60 for one BACTEC instrument.
[c] Based on 8.5 min/specimen (College of American Pathologists workload units per vial) for 4,500 specimens at $23.02/h.
[d] Based on 4 min/specimen (College of American Pathologists workload units per vial) for 4,500 specimens at $23.02/h.
[e] Based on 4,500 BACTEC 12B vials and PANTA.
[f] Based on 4,500 tubes, OADC, and PANTA.
[g] Gas for BACTEC, disposal of media, and time complying with radiation laws not included.
[h] Cost of Wood's lamp or transilluminator not included.

than with MGIT. Overall bacterial contamination is less in BACTEC 460 than in MGIT, but contamination rates vary with the contaminating organisms in the specimens tested. While gram-positive bacteria tend to grow more readily in the MGIT system, *Pseudomonas* species, the most frequently isolated contaminants in our study, grew equally well in BACTEC 460 and in MGIT. When ease of use is considered, the MGIT system is superior to BACTEC 460. Dozens of MGIT tubes can be evaluated for fluorescence in a matter of minutes with simple visual inspection under a transilluminator, although fluorescence tends to be obscured by the presence of gross blood in a specimen, which is a drawback to the MGIT system. The radiometric aspect of BACTEC 460 is a major bureaucratic and disposal inconvenience, although the danger associated with the use of carbon 14 is minimal. BACTEC can be used for all specimen types and for susceptibility testing of first-line antituberculosis drugs. The laboratory technologists in our studies distinctly preferred working with the MGIT system over BACTEC 460; the need for needle inoculation of the BACTEC 460 bottles poses a real hazard that is not present in the MGIT system. For laboratories which do not receive many blood specimens and do not perform susceptibility testing, MGIT is a good alternative to the BACTEC system.

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