Reduction of Contamination of Mycobacterial Growth Indicator Tubes with a Modified Antimicrobial Combination

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Culture in the fluorimetric Mycobacteria Growth Indicator Tube (MGIT) treated with a combination of vancomycin, amphotericin B, and nalidixic acid (VAN) showed growth of most strains of 31 mycobacterial species with a less-than-1-day delay. The results were similar to those in the MGIT with polymyxin B, amphotericin B, nalidixic acid, trimethoprim, and azlocillin, but with respiratory specimens, the MGIT with VAN showed a lower contamination rate with no change in the detection rate or time.

Recent advances in molecular biology have permitted the early detection of mycobacteria in clinical specimens. However, culture is still important for making a definitive diagnosis of mycobacteriosis and for drug susceptibility testing. A broth-based system, as well as a conventional solid medium, is generally recommended for culture (12). The introduction of the radiometric semiautomated BACTEC 460TB system (BD, Franklin Lakes, N.J.) enabled the detection of tubercle bacilli in as little as 9 to 14 days instead of several weeks (12). The early pitfalls of this system, such as the need for radioactive material and the considerable labor and risk of cross-contamination, were reduced by the development of the fluorimetric Mycobacteria Growth Indicator Tube (MGIT; BD) and the BACTEC MGIT 960 system (BD) (16, 19). Many investigations have addressed the performance of both manual and automatic MGIT systems and demonstrated high contamination rates, ranging from 1.9 to 30%, probably owing to the enrichments added to the broth and the characteristics of the clinical specimens for which these tubes are used (4, 10, 12, 16, 17, 21).

In our experience, a combination of vancomycin, amphotericin B, and nalidixic acid (VAN) minimizes contamination without reducing mycobacterial recovery in a Middlebrook 7H11 agar formulation (8). We studied whether this combination would work well as a substitute for polymyxin B, amphotericin B, nalidixic acid, trimethoprim, and azlocillin (PANTA; BBL MGIT PANTA Antibiotic Mixture; BD), the manufacturer-recommended combination, for recovery of mycobacteria from clinical specimens in a manual MGIT system.

Mycobacterial cultures of isolates. Twenty-nine type strains from the American Type Culture Collection (ATCC) or the Korean Collection for Type Cultures (KCTC) and 11 clinical isolates from the Korean Institute of Tuberculosis (KIT) and the Pusan National University Hospital (PNUH) were included (Table 1). Colonies gathered from a Lowenstein-Jensen solid slant were transferred to a 15-mm tube containing 7 ml of

Middlebrook 7H9 broth and several glass beads, vortexed, and allowed to stand for 30 min at room temperature. A small amount of supernatant liquid was inoculated into 7H9 broth and incubated for as long as a week at 37°C under room air in a shaking incubator until the turbidity became 1.0 McFarland unit. Samples of 100 μ l of cultures were inoculated into two MGIT tubes after dilution to 1:10,000. In one tube, oleic acid, albumin, dextrose, and catalase (OADC; BBL MGIT OADC Enrichment; BD) and PANTA were added in accordance with the manufacturer's recommendations. In the other tube, OADC was added along with 100 μ l of a combination of VAN to establish final concentrations of 10, 10, and 25 μ g/ml, respectively. Each tube was incubated at 37°C in room air and examined daily for fluorescence in a 365-nm UV Transilluminator (Spectroline TR 365R; Spectronics Corporation, Westbury, N.Y.).

Compared with the MGIT tubes containing PANTA, the VAN-containing tubes showed growth either on the same day or 1 day later with 29 of the 42 strains, representing 31 mycobacterial species (Table 1). The strains that were detected with no or a 1-day delay included all seven *M. tuberculosis*, three of five *M. avium* complex, and all four *M. fortuitum* strains. Another six strains, which included *M. avium*, *M. chelonae*, *M. marinum*, and others that not infrequently cause human infections, showed growth 2 to 6 days later. The remaining five strains showed growth 1 to 2 weeks later.

Mycobacterial cultures of clinical specimens. All respiratory specimens submitted to the PNUH Microbiology Laboratory between July and October 2001 for mycobacterial culture were tested by the methods described below. After decontamination by standard methods with *N*-acetyl-L-cysteine (NALC)–2% NaOH, each specimen was inoculated onto one solid medium (3% Ogawa medium, an egg-based solid medium that is the primary medium most frequently used in Korea) and two MGIT tubes. Either OADC-PANTA or OADC-VAN was added to each tube. All solid and broth media were incubated at 37°C under room air and examined daily. Positive tubes were stained by the Ziehl-Neelsen method, and the species were identified by the method previously described (14). The tube was considered to be contaminated when a solid medium was liquefied or a positive-signal broth medium did not stain by

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TABLE 1. Delay in detection of type strains and clinical isolates grown in MGIT tubes with VAN versus PANTA

No or 1-day delay	2- to 6-day delay	More-than-6-day delay or no growth
Rapid growers		
<i>M. abscessus</i> ATCC 19977	<i>M. chelonae</i> KCTC 9505	<i>M. flavescens</i> ATCC 14474
<i>M. agri</i> KCTC 9502	<i>M. peregrinum</i> ATCC 14467	<i>M. mucogenicum</i> ATCC 49650
<i>M. fortuitum</i> KCTC 9510		<i>M. neoaurum</i> ATCC 25795
<i>M. fortuitum</i> KCTC 1122		<i>M. pulveris</i> KCTC 9518
<i>M. fortuitum</i> KIT 60108		
<i>M. fortuitum</i> KIT 60111		
<i>M. gilvum</i> KCTC 9512		
<i>M. phlei</i> KCTC 9087		
<i>M. porcinum</i> KCTC 9517		
<i>M. smegmatis</i> KCTC 9108		
Slow growers		
<i>M. asiaticum</i> KCTC 9503	<i>M. avium</i> KIT 41128	<i>M. nonchromogenicum</i> ATCC 19530
<i>M. avium</i> KIT 41207	<i>M. intracellulare</i> KCTC 9514	
<i>M. bovis</i> ATCC 19210	<i>M. mairium</i> ATCC 927	
<i>M. celatum</i> ATCC 51131	<i>M. terrae</i> ATCC 15755	
<i>M. gastris</i> ATCC 15754		
<i>M. gordonae</i> KCTC 9513		
<i>M. intracellulare</i> KIT 41179		
<i>M. intracellulare</i> KIT 41194		
<i>M. kansasii</i> KCTC 9515		
<i>M. malmoense</i> ATCC 29571		
<i>M. scrofulaceum</i> ATCC 19981		
<i>M. simiae</i> ATCC 25275		
<i>M. szulgai</i> KCTC 9520		
<i>M. tuberculosis</i> H37Rv		
5 <i>M. tuberculosis</i> isolates ^a		

^a Five clinical strains were randomly selected from among those isolated from our microbiology laboratory at PNUH in 2000.

the Ziehl-Neelsen method. The results of 41 sputum samples and 15 bronchial washing samples (50 acid-fast stain positive and 6 negative) from 56 patients with confirmed pulmonary or miliary tuberculosis were selected for analysis. Forty-eight samples (85.7%) were positive in at least one medium, and all contained *M. tuberculosis*. The MGIT tubes with VAN (82.1%) or PANTA (78.6%) showed significantly higher positivity rates than the Ogawa medium (62.5%) ($P = 0.003$ and 0.01 , respectively, by the McNemar modification of the chi-square test). Detection rates were not significantly different in the two series of MGIT tubes. The contamination rate was higher with PANTA (10.7%) than with VAN (5.4%), a difference that is statistically insignificant ($P = 0.13$). Among 33 samples that revealed growth on all three media, the mean time to detection was not significantly different in MGIT media with VAN and PANTA (17.5 versus 17.0 days) and in both instances was significantly shorter than that in Ogawa medium (25.2 days) ($P < 0.001$ by Student's t test).

Discussion. Specimens for mycobacterial cultures that are likely to contain normal or transient bacterial flora must be decontaminated, and the NALC-2% NaOH procedure used for MGIT cultures is probably the most popular method for this purpose. Increasing the alkalinity by using 4% instead of 2% NaOH reduces the rates of contamination, but its toxicity for mycobacteria may cause negative culture results (2, 21). Antibiotics also are frequently used to inhibit the growth of unwanted microorganisms. The manual and automated MGIT systems use the PANTA combination for that purpose. Vancomycin controls gram-positive contaminants effectively (1) and is used in combination with amphotericin B and nalidixic

acid for the cultivation of *M. paratuberculosis* from fecal and tissue specimens (20). Vancomycin probably also can be used to prevent growth of methicillin-resistant staphylococci or multiply drug-resistant *Streptococcus pneumoniae* bacteria, which are highly prevalent in Korea (9, 15). In the cultures of type strains and clinical isolates of mycobacteria, 7 of 14 rapid growers and 12 of 17 slow growers showed no or only a 1-day delay of growth in the VAN tubes compared with that in tubes containing PANTA. The time needed for detection of type strains and clinical isolates of *M. tuberculosis* complex in the tubes with VAN was not delayed more than 1 day in comparison with that in the tubes containing PANTA. Moreover, with 56 respiratory specimens, the tubes with VAN showed no significant difference in detection time and rate and the contamination rate was lower than in the tubes treated with PANTA.

Early detection of *M. tuberculosis* is an important issue for clinical mycobacteriology laboratories and has been aided by the development of radioisotope- or fluorescence-based continuous-monitoring broth culture systems, the high contamination rates of which could be controlled by the VAN combination. We also evaluated the delay in the detection of a strain of nontuberculous mycobacteria (NTM) covering most human pathogens. Most of the strains were detected within a 1-day delay. However, some NTM strains, including *M. avium* complex, *M. chelonae*, *M. marinum*, and others that not infrequently cause human infections (6), were detected with a 1- to 14-day delay. Most gram-negative bacteria, mycobacteria, and fungi are not susceptible to vancomycin (5). However, vancomycin itself has a bactericidal effect on some mycobacteria such as *M. fortuitum* (13), *M. senegalense*, *M. septicum* (18), and

unidentified strains (7). Nalidixic acid may inhibit *M. kansasii* (3). It is therefore evident that, in some instances, antibiotic suppression of contaminants might be harmful to the recovery of NTM from the specimen, not only in tubes with VAN but also in those with PANTA. However, it is inevitable that one must use some chemicals or antibiotics for decontamination of normally nonsterile specimens, and antibiotics are widely used for culturing of mycobacteria (1, 11, 20).

In conclusion, the VAN antibiotic combination could be used for recovery of *M. tuberculosis* and prudently applied for NTM cultures.

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