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Mycobacteriology

Rapid prediction of BACTEC MGIT 960 culture results by COBAS Amplicor Mycobacterium polymerase chain reaction detection

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Abstract

A combination of a rapid culture system, BACTEC MGIT 960 (MGIT), and a commercial polymerase chain reaction (PCR) system for the detection of *Mycobacterium tuberculosis* was evaluated to predict final culture results within 2 weeks. A total of 79 sputum specimens were collected from 59 tuberculosis (TB) patients before anti-TB chemotherapy. Among the 22 specimens that were smear negative and culture positive, the COBAS Amplicor nucleic amplification method for sputum resulted in 13 positives (59.1%) before culturing. In contrast, 21 liquid culture specimens (95.5%) showed positive results by COBAS Amplicor after 7 days. Similarly, 8 specimens (80%) were positive for *Mycobacterium avium* complex (MAC) based on COBAS Amplicor, and 10 liquid culture specimens (100%) showed positive results after 7 days. Among the 26 specimens that took more than 7 days to become positive by MGIT, 25 specimens (96.1%) were positive using COBAS Amplicor with 7-day-old cultures. Of the 26 positives, 21 were *M. tuberculosis*, which took 11 to 38 days to appear positive (mean, 16.6 days), and 4 were MAC, which took 8 to 10 days (mean, 8.8 days). As a result, 96.8% (31/32) of the positives could be detected by MGIT with COBAS Amplicor by day 7, and the negative predictive value was 97.9%. A combination of MGIT and COBAS Amplicor on day 7 was demonstrated as a useful method for rapid diagnosis of positives and negatives, without waiting 42 days for confirmation.

Keywords: BACTEC MGIT 960; COBAS Amplicor Mycobacterium; *Mycobacterium tuberculosis*

1. Introduction

Tuberculosis (TB) is still a life-threatening disease. Approximately 9.1 million people develop TB and 1.7 million patients die every year (World Health Organization [WHO], 2008). The WHO has initiated and expanded directly observed treatment with short-course chemotherapy and attempts to detect 70% of the acid-fast bacilli (AFB) smear-positive patients and treats 85% of them successfully with this strategy. However, the low smear positivity in TB patients with HIV has recently resulted in a critical problem for TB diagnostics (Klein et al., 1989). Culture examination has higher sensitivity than smear microscopy and could provide a powerful diagnostic tool even with HIV-coinfected TB patients.

Many mycobacterial pathogens grow slowly and take quite a long time to be detected by conventional culture methods. A rapid culturing system with liquid medium and a sensitive detection system, for example, BACTEC MGIT 960 (MGIT; Becton Dickinson, Franklin Lakes, NJ), was recently introduced and showed efficient and rapid isolation of mycobacteria from clinical specimens (Chien et al., 2000; Hanna et al., 1999; Somoskovi and Magyar, 1999; Tortoli et al., 1999). However, even by MGIT, it takes a longer time for paucibacillary specimens to be positive, and 6 weeks are needed for confirmation of negative results (Abe, 1996).

Nucleic acid amplification (NAA) is a useful method for detecting targeted bacterial sequences directly from clinical specimens and is widely used to identify mycobacterial species. NAA has dramatically shortened the time required to detect mycobacteria in clinical specimens (Aoki et al., 1994). A commercial polymerase chain reaction (PCR) test, COBAS Amplicor (Roche Diagnostics Systems, Japan), is

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recognized as a rapid and sensitive NAA test (Bodmer et al., 1997). It can detect several mycobacteria within a few hours. However, although the clinical sensitivity for diagnosing mycobacterial infections is similar to conventional culture methods, it is significantly lower with paucibacillary specimens.

Behr et al. (1999) reported that approximately 17% of new *Mycobacterium tuberculosis* infections are due to AFB smear-negative patients, and therefore, they are not negligible as a source of dissemination. Rapid detection may be crucial, especially to prevent the spread of the disease to immunocompromised people who can develop TB easily (Rieder et al., 1989; Selwyn et al., 1989). In addition, early determination of culture-negative results would reduce the number of patients who might receive anti-TB chemotherapy based only on clinical manifestations. In the present study, the advantages of MGIT and COBAS Amplicor have been combined and used for the rapid detection of *M. tuberculosis* and *Mycobacterium avium* complex (MAC) from clinical sputum specimens. The preincubation in MGIT before submitting to COBAS Amplicor may improve the sensitivity and shorten the time for examination. This idea has been verified using clinical specimens collected from TB suspects. The objective of this study was to obtain the result of culture examination as rapidly as possible.

2. Materials and methods

2.1. Sputum specimens

Sputum specimens were collected from TB suspects at Double-Barred Cross Hospital, Tokyo, Japan, from March to November 1999. The specimens were subjected to subsequent mycobacterial examinations including AFB smear microscopy, MGIT culture, and COBAS Amplicor PCR. AFB smear-positive specimens were eliminated from the study, and a total of 79 sputum specimens were collected from 59 TB suspects.

2.2. Pretreatment and culture

Each sputum specimen was digested and decontaminated using 2 volumes of 2% NaOH with *N*-acetyl-L-cysteine for 20 min at room temperature. The specimen container was filled with phosphate buffer and centrifuged at 3000 × *g* for 20 min. After decanting the buffer, the sediment was completely resuspended in 1.25 mL of phosphate buffer. The suspension was subjected to AFB smear examination (50 μL) by fluorescent microscopy, COBAS Amplicor PCR (0.1 mL), and 2 MGIT cultures (0.5 mL). If the fluorescent microscopy was positive, the sample slide was restained and confirmed by ordinary Ziehl–Neelsen method. Two MGIT tubes were incubated in BACTEC MGIT 960, and 1 was withdrawn for PCR 7 days later. The tube was mixed thoroughly with a vortex mixer and ultrasonicated for 30 s, and 1 mL of culture medium was taken for PCR and centrifuged at 15 000 × *g* for 10 min. The sediment was subjected to Amplicor PCR. The MGIT culture tube was returned to the culture drawer to observe the influences of mixing and removal of sample during incubation. The culture was incubated and observed for up to 42 days to confirm the negativity. Culture-positive specimens were identified with the AccuProbe (Gen-Probe, San Diego, CA) test for *M. tuberculosis* complex and MAC according to the manufacturer's instruction.

2.3. COBAS Amplicor Mycobacterium (PCR)

The polymerase chain amplification examination to detect *M. tuberculosis* complex, *M. avium*, and *Mycobacterium intracellulare* (MAC) was performed using the COBAS Amplicor Mycobacterium system according to the manufacturer's instructions. An internal control was used to identify any amplification errors caused by contamination of any inhibitor such as the culture medium.

2.4. Statistical analysis

Statistical analysis was performed using χ^2 or Fisher's exact test; P value of <0.05 was considered to be statistically significant.

3. Results

3.1. Detection of M. tuberculosis

A total of 22 *M. tuberculosis* strains were recovered and identified by all methods. The direct PCR detection from uncultured sputum specimens (direct PCR), MGIT culture (normal MGIT), MGIT culture with mixing and withdrawal (modified MGIT), and PCR detection after 7 days of MGIT culture (MGIT–PCR) showed positive results as indicated in Table 1. The direct PCR showed only 13 positives. Similarly, normal MGIT, modified MGIT, and MGIT–PCR combination showed 22, 22, and 21 positives at each timing, respectively. The PCR detection rate significantly (P = 0.005) increased from 59.1% (13/22) to 95.5% (21/22) after 7 days of specimen incubation by MGIT. *M. tuberculosis*

Table 1
Detection and isolation of *Mycobacterium* spp. by MGIT and COBAS Amplicor

Method	MTC	MAC	Contamination	Negative	IC negative
Direct PCR (day 0)	13	8	0	54	4[a]
MGIT–PCR (day 7)	21[b]	10[c]	0	44	1[a]
Modified MGIT (day 42)	22[b]	10	3	44	0
Normal MGIT (day 42)	22[b]	10	3	44	0

MTC = *M. tuberculosis* complex; IC = internal control.

[a] These specimens were amplified successfully with 5-fold diluted specimens.

[b] *M. tuberculosis* complex and MAC were isolated together from 1 specimen.

[c] Six of 10 specimens yielded positive results within 7 days.