

# Evaluation of Peptide Nucleic Acid Probe-based Real-time PCR for Detection of *Mycobacterium tuberculosis* Complex and Nontuberculous Mycobacteria in Respiratory Specimens

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**Background:** A peptide nucleic acid (PNA) probe-based real-time PCR (PNAqPCR™ TB/NTM detection kit; PANAGENE, Korea) assay has been recently developed for the simultaneous detection of *Mycobacterium tuberculosis* complex (MTBC) and nontuberculous mycobacteria (NTM) in clinical specimens. The study was aimed at evaluation of the performance of PNA probe-based real-time PCR in respiratory specimens.

**Methods:** To evaluate potential cross-reactivity, the extracted DNA specimens from *Mycobacterium* species and non-mycobacterial species were tested using PNA probe-based real-time PCR assay. A total of 531 respiratory specimens (482 sputum specimens and 49 bronchoalveolar washing fluid specimens) were collected from 230 patients in July and August, 2011. All specimens were analyzed for the detection of mycobacteria by direct smear examination, mycobacterial culture, and PNA probe-based real-time PCR assay.

**Results:** In cross-reactivity tests, no false-positive or false-negative results were evident. When the culture method was used as the gold standard test for comparison, PNA probe-based real-time PCR assay for detection of MTBC had a sensitivity and specificity of 96.7% (58/60) and 99.6% (469/471), respectively. Assuming the combination of culture and clinical diagnosis as the standard, the sensitivity and specificity of the new real-time PCR assay for detection of MTBC were 90.6% (58/64) and 99.6% (465/467), respectively. The new real-time PCR for the detection of NTM had a sensitivity and specificity of 69.0% (29/42) and 100% (489/489), respectively.

**Conclusions:** The new real-time PCR assay may be useful for the detection of MTBC in respiratory specimens and for discrimination of NTM from MTBC.

**Key Words:** Peptide nucleic acids, PCR, *Mycobacterium tuberculosis*, *Mycobacterium*

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## INTRODUCTION

Tuberculosis caused by the *Mycobacterium tuberculosis* complex (MTBC), which affects about 8.4 million patients and causes over 1.5 million deaths annually, is a major global health problem [1]. Nontuberculous mycobacteria (NTM) appear to be distrib-

uted widely in the environment, and about one-third of NTM species have been associated with human diseases [2, 3]. Clinical diseases caused opportunistically by NTM are being encountered with an increasing frequency in AIDS and non-AIDS populations [4, 5]. Therefore, rapid and accurate identification of mycobacteria in the clinical setting is essential for the control of the

spread of tuberculosis and for adequate antimicrobial therapy against mycobacterial infection [6]. Clinical microbiology laboratories have a central role in patient treatment and disease control, but conventional methods used in microbiology laboratories can pose major limitations. Culture methods for detecting mycobacteria in clinical specimens require long incubation times because of slow growth of the organisms [7]. Acid-fast bacilli (AFB) smear provides rapid results and is widely used in clinical laboratories. However, the AFB smear has low sensitivity for laboratory diagnosis because of the necessity for large numbers of organisms in a specimen for a positive result and yields poor positive predictive value for tuberculosis in clinical settings in which NTM is frequently isolated [8-10].

Since the introduction of nucleic acid amplification (NAA)-based tests with advances in genetic technologies in the recent decades, a remarkable improvement has occurred in the direct detection of mycobacteria [7]. A variety of NAA-based assays has been commercially developed for detection of MTBC and NTM from clinical specimens and are now widely used in clinical microbiology laboratories. In particular, the use of real-time PCR assay for the detection of microorganisms has been increasing, replacing conventional PCR that uses agarose gel electrophoresis for identification of PCR products. Real-time PCR assays using fluorescence resonance energy transfer (FRET) probes, molecular beacons, or TaqMan probes have been adapted for continuous detection of amplification products in a closed system. These assays have the advantage of a low contamination risk and simultaneous identification of multiple targets [11, 12].

Peptide nucleic acids (PNA) are artificially synthesized DNA analogues with an uncharged peptide backbone [13]. PNA have more favorable hybridization properties and chemical, thermal, and biological stability because of their uncharged nature and their peptide bond-linked backbone [14]. Because of these favorable characteristics, PNA has been widely applied as a diagnostic tool in molecular biology. Recently, a PNA probe-based real-time PCR assay (PNAqPCR™ TB/NTM detection kit; PANAGENE, Daejeon, Korea) was developed for the simultaneous detection of MTBC and NTM in clinical specimens. The aim of this study was to evaluate the performance of PNA probe-based real-time PCR assay in respiratory specimens.

## METHODS

### 1. Study design

To evaluate potential cross-reactivity, the extracted DNA speci-

mens from 6 reference strains (*M. tuberculosis* [ATCC 27294], *M. avium* [ATCC 15769], *M. intracellulare* [Korean Collection for Type Culture (KCTC) 9514], *M. fortuitum* [KCTC 1122], *Nocardia asteroides* [KCTC 9956], and *Rhodococcus equi* [KCTC 9082]) and clinically isolated strains from respiratory specimens (*Corynebacterium striatum*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Moraxella* species, *Escherichia coli*, and *Acinetobacter baumannii*) were tested by PNA probe-based real-time PCR assay.

A total of 531 respiratory specimens (482 sputum samples and 49 bronchoalveolar washing fluid specimens) were collected from 230 patients with suspected mycobacterial infection in July and August, 2011. All specimens were analyzed for the detection of mycobacteria by direct smear examination, mycobacterial culture, and the PNA probe-based real-time PCR assay.

### 2. Specimen processing, microscopic examination and culture

All respiratory specimens were liquefied and decontaminated with N-acetyl-L-cysteine-sodium hydroxide and concentrated by centrifugation at 3,000×g for 30 min. Following concentration and resuspension of the sediments in 1.5 mL of phosphate buffer, part of the sediment from each specimen was used for AFB smear and inoculated in a BACTEC™ MGIT™ 960 system (Becton Dickinson Diagnostic Instrument Systems, Sparks, MD, USA). The remaining portion of the sediment was stored at -80°C until the NAA assays were performed.

Smears were stained with the auramine-rhodamine fluorescent stain, and auramine-rhodamine-positive smears were confirmed by Ziehl-Neelsen staining. After a 500-μL aliquot of processed sediment was inoculated, the BACTEC™ MGIT™ 960 culture was incubated for 6 weeks at 36°C. A positive culture was confirmed by AFB staining, immunochromatographic assay kit (BIOLINE SD TB Ag MPT64 Rapid, Standard Diagnostics, Yongin-si, Korea), and PCR assay (Seeplex MTB/NTM ACE Detection, Seegene, Seoul, Korea).

### 3. DNA extraction

The sample DNA was extracted using an InstaGene matrix (Bio-Rad Laboratories, Hercules, CA, USA) according to the manufacturer's instructions. Decontaminated specimens were washed with Dulbecco's phosphate-buffered saline (WelGENE, Daegu, Korea). Specimens were subjected to 5 min of centrifugation at 12,000×g. The supernatant was discarded, and the sediment was resuspended in 100 μL of InstaGene Matrix and incubated at 56°C for 15 min. The mixtures were vortexed, incubated in a

dry-heat block at 100°C for 8 min, and centrifuged to sediment the matrix. Five microliters of each DNA sample was used as a template for amplification in real-time PCR.

#### 4. PNA probe-based real-time PCR assay

The PNAqPCR™ TB/NTM detection kit includes a primer set targeting the *IS6110* insertion sequence for detection of MTBC and a primer set targeting the internal transcribed spacer (ITS) sequence for detection of mycobacteria. The MTBC-specific PNA probe was labeled with Texas Red and Dabsyl (dimethyl-aminoazosulfonic acid), the mycobacteria-specific PNA probe with FAM (6-carboxyfluorescein) and Dabcyl (4,4-dimethyl-amino-azobenzene-4'-carboxylic acid), and the internal control PNA probe with HEX (4, 4, 7, 2', 4', 5', 7'-hexachloro-6-carboxy-fluorescein) and Dabcyl. The primer/probe sequences cannot be revealed because of the manufacturer's copyright policy. Generation of fluorescence signals during hybridization in real-time PCR is illustrated in Fig. 1 (provided technical data from manufacturer). PNA-based real-time PCR assay was conducted in accordance with the manufacturer's instructions using a CFX96 (Bio-Rad Laboratories). Positive and negative controls were used for every reaction. For amplification, 5 µL of extracted DNA, 10 µL of the primer/probe mixture (solution A), and 10 µL of the real-time PCR master mixture (solution B) were mixed in a 96-well plate. The cycling program was 2 min at 50°C, 15 min at 95°C, and 45 cycles of 10 sec at 95°C, 30 sec at 58°C and 15 sec at 72°C. A positive result for *IS6110* and internal control was defined as a threshold cycle ( $C_T$ ) value  $\leq 40$ , and a positive result

for ITS was defined as a  $C_T$  value  $\leq 42$ . The result of an assay was regarded as invalid if the assays for *IS6110*, ITS, and internal control all showed simultaneously negative results. When invalid results for real-time PCR assay were obtained, the assay was repeated using a 2-fold dilution of the extracted DNA. The result of an assay was interpreted as positive for MTBC and NTM according to the manufacturer's instructions. The result of an assay was considered as positive for MTBC if the assay only showed positive results for *IS6110*. When the assay only showed positive result for ITS, the result was regarded as positive for NTM. When the assay showed simultaneously positive results for *IS6110* and ITS, the result of the assay was interpreted as following: (i) if the  $C_T$  value for ITS  $>$  the  $C_T$  value for *IS6110* + 2, the result of the assay was considered as positive for MTBC (ii) if the  $C_T$  value for ITS  $\leq$  the  $C_T$  value for *IS6110* + 2, the result was considered as simultaneously positive for MTBC and NTM.

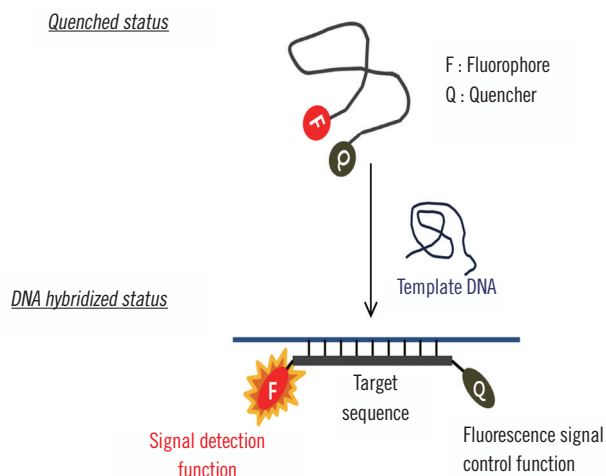
#### 5. Identification of NTM with sequencing assay

To identify the NTM species, direct sequencing of all NTM isolates was performed. Purified DNA was amplified by using a specific primer for the *Mycobacterium* genus. The cycling program was as follows: 10 min at 95°C, and 30 cycles of 30 sec at 95°C, 30 sec at 65°C and 60 sec at 72°C. The sense primer was ITS-F (5'-TGGATCCGACGAAGTCGTAACAAGG-3'), and the anti-sense primer was PAN-04R (5'-ATGCTCBCAABCACTATCCA-3') [15]. PCR products were purified by using a LaboPass™ PCR purification kit according to the manufacturer's instructions. Purified products were sequenced with selected amplification primers. The purified products were analyzed with an ABI 3730xl DNA analyzer (Applied Biosystems, Foster City, CA, USA).

#### 6. Patients' clinical evaluation and classification of specimens

For clinical assessment of tuberculosis, each patient's clinical records and chest radiography/computed tomography images were reviewed. Two categories of specimens were considered as true-positive specimens for tuberculosis: (i) culture-positive specimens for MTBC and (ii) samples that were culture-negative for MTBC but belonged to patients whose clinical history and radiography/computed tomography findings provided enough evidence of tuberculosis to initiate antituberculous chemotherapy.

Of the 531 respiratory specimens from 230 patients, 42 specimens were AFB smear positive and scored trace to 4+: (i) 11 specimens had trace levels, (ii) 3 specimens scored 1+, (iii) 5 specimens scored 2+, (iv) 19 specimens scored 3+, and (v) 4 specimens scored 4+. Among mycobacterial isolates in cultures from 102 clinical specimens, 60 were identified as MTBC and



**Fig. 1.** PNA probes that contain random coil structures undergo quenching of fluorescence. Fluorescence signals are increased by the conformational change of probes during hybridization. Abbreviation: PNA, peptide nucleic acids.

42 as NTM. In the AFB smear-positive specimens, MTBC was isolated in 36 specimens that scored trace to 4+: (i) 8 specimens had trace levels, (ii) 3 specimens scored 1+, (iii) 3 specimens scored 2+, (iv) 18 specimens scored 3+, and (v) 4 specimens scored 4+. NTM was isolated in 6 smear-positive samples that scored trace to 3+: (i) 3 specimens had trace levels, (ii) 2 specimens scored 2+, and (iii) 1 specimen scored 3+. In the smear-negative specimens, MTBC was isolated in 24 specimens and NTM was isolated in 36 specimens. Of 429 smear-negative and culture-negative specimens, 4 specimens were considered to show positive results for tuberculosis by clinical diagnosis.

## RESULTS

### 1. Cross-reactivity test

In the cross-reactivity test, extracted DNA from *M. tuberculosis* tested positive with both MTBC-specific and mycobacteria-specific PNA probes in the real-time PCR assay. Specimens extracted from *M. avium*, *M. intracellulare*, and *M. fortuitum* tested positive with the mycobacteria-specific PNA probe but negative with the MTBC-specific PNA probe in the real-time PCR assay. None of the specimens extracted from 11 non-mycobacterial strains tested positive with MTBC-specific or mycobacteria-specific PNA probes in real-time PCR assays.

### 2. Results and interpretation of the results of the PNA probe-based real-time PCR assay

Among the 531 clinical specimens, 88 yielded positive results in the PNA probe-based real-time PCR assay. Real-time PCR assays for 29 specimens only showed positive results for *IS6110* and assays for 28 specimens only showed positive results for ITS. The assays for 31 specimens showed simultaneously positive results for *IS6110* and ITS. The  $C_T$  values of real-time PCR for *IS6110* ranged from 22.7 to 37.7 with a median of 29.3 and the  $C_T$  values for ITS ranged from 26.2 to 41.5 with a median of 35.8. The interpretation of the results of the real-time PCR assay showed positive results for MTBC in 60 specimens and positive results for NTM in 29 specimens. Among those specimens, only 1 was simultaneously positive for MTBC and NTM (Table 1).

### 3. MTBC detection

Among the smear-positive and MTBC culture-positive specimens, all specimens showed MTBC positivity in the real-time PCR assay. For the 24 specimens that were smear-negative and MTBC culture-positive, the real-time PCR assay showed MTBC-positive results in 22 specimens. When MTBC culture-negative speci-

mens were tested, the real-time PCR assay showed MTBC-positive results in 2 specimens, and in these samples, NTM was isolated by culture. When the culture method was used as the gold standard test for comparison, PNA probe-based real-time PCR assay for detection of MTBC had a sensitivity and specificity of 96.7% (58/60) and 99.6% (469/471), respectively. Among the 4 specimens from patients clinically diagnosed with tuberculosis that were smear-negative and MTBC culture-negative, none showed MTBC positivity in the real-time PCR assay. Assuming the combination of culture and clinical diagnosis as the standard, the sensitivity and specificity of the PNA probe-based real-time PCR assay were 90.6% (58/64) and 99.6% (465/467), respectively (Table 2). The  $C_T$  values of real-time PCR for *IS6110* in the MTBC-positive specimens ranged from 22.7 to 37.7 with a median of 29.1. The median  $C_T$  values in smear-positive and smear-negative specimens were 27.4 and 30.7, respectively.

### 4. NTM detection

Among all respiratory specimens, 29 were positive for NTM by the PNA probe-based real-time PCR assay. NTM was isolated by culture in all PCR-positive specimens. However, among the 502 specimens that were NTM-negative by the real-time PCR assay, NTM was isolated by culture in only 13 specimens. The sensitivity and specificity of real-time PCR assay for detection of NTM were 69.0% (29/42) and 100% (489/489), respectively. When 6 specimens that were smear-positive and NTM culture-positive were tested, the real-time PCR assay showed NTM-positive results in 5 samples and an NTM-negative result in 1 sample with a trace smear (Table 2). The  $C_T$  values obtained in real-time PCR for ITS in the NTM-positive specimens ranged from 29.7 to 41.5 with a median of 37.5. The median  $C_T$  values in smear-positive and smear-negative specimens were 35.3 and 37.9, respectively. The nontuberculous mycobacterial isolates from clinical specimens and the real-time PCR results are listed in Table 3.

## DISCUSSION

In this study, MTBC- and mycobacteria-specific PNA probes were used in real-time PCR for detection of tuberculosis and NTM infection. PNA probes contain random coil structures on their ends with a fluorophore and a quencher in close proximity, and fluorescence signals are inhibited by quenching. During hybridization with template DNA, PNA probes with random coil conformations straighten, resulting in increased fluorescence signals (Fig. 1). Compared with DNA probes, PNA probes have

**Table 1.** Threshold cycle ( $C_T$ ) values of PNA probe-based real-time PCR assay for *Mycobacterium tuberculosis* complex and nontuberculous mycobacteria from respiratory specimens

Specimen type	$C_T$ value of real-time PCR			Interpretation of real-time PCR	Conventional assays		Specimen type	$C_T$ value of real-time PCR			Interpretation of real-time PCR	Conventional assays	
	IS6110	ITS	Internal control		AFB Stain	Culture		IS6110	ITS	Internal control		AFB Stain	Culture
Sputum	24.2	32.3	34.2	MTBC	Positive	MTBC	Sputum	-	40.0	34.8	NTM	Negative	NTM
Sputum	32.1	-	33.6	MTBC	Positive	MTBC	Sputum	-	38.3	37.0	NTM	Negative	NTM
Sputum	24.1	32.0	34.7	MTBC	Positive	MTBC	Sputum	28.6	38.3	35.2	MTBC	Positive	MTBC
Sputum	28.2	35.8	33.8	MTBC	Positive	MTBC	Sputum	31.5	-	36.1	MTBC	Positive	MTBC
Sputum	37.5	-	35.3	MTBC	Negative	NTM	Sputum	-	41.5	-	NTM	Negative	NTM
Sputum	29.0	36.9	33.2	MTBC	Positive	MTBC	Sputum	27.3	34.2	34.1	MTBC	Positive	MTBC
BAW	32.8	-	33.4	MTBC	Negative	MTBC	Sputum	31.7	-	33.4	MTBC	Positive	MTBC
Sputum	24.7	31.7	34.8	MTBC	Positive	MTBC	Sputum	37.7	-	33.9	MTBC	Positive	MTBC
Sputum	-	37.6	37.9	NTM	Negative	NTM	Sputum	24.6	32.8	34.9	MTBC	Positive	MTBC
Sputum	30.6	42.3	33.4	MTBC	Positive	MTBC	Sputum	27.1	33.2	33.3	MTBC	Positive	MTBC
Sputum	29.2	37.3	33.7	MTBC	Positive	MTBC	Sputum	26.0	33.2	34.4	MTBC	Positive	MTBC
Sputum	30.9	41.2	33.5	MTBC	Positive	MTBC	BAW	34.7	-	28.9	MTBC	Negative	MTBC
Sputum	27.3	-	28.6	MTBC	Negative	MTBC	Sputum	32.2	-	28.2	MTBC	Negative	MTBC
BAW	31.8	-	28.5	MTBC	Negative	MTBC	Sputum	26.1	-	27.9	MTBC	Negative	MTBC
BAW	41.2	36.9	33.3	NTM	Negative	NTM	Sputum	29.9	-	27.9	MTBC	Negative	MTBC
Sputum	-	38.6	35.4	NTM	Negative	NTM	Sputum	-	38.3	38.9	NTM	Negative	NTM
Sputum	26.2	-	28.7	MTBC	Negative	MTBC	Sputum	-	35.9	-	NTM	Negative	NTM
Sputum	28.3	-	28.9	MTBC	Negative	MTBC	Sputum	23.1	28.9	35.6	MTBC	Positive	MTBC
Sputum	22.7	29.7	34.8	MTBC	Positive	MTBC	Sputum	26.6	33.4	35.0	MTBC	Positive	MTBC
Sputum	-	40.4	35.5	NTM	Negative	NTM	Sputum	-	40.6	35.6	NTM	Negative	NTM
Sputum	23.8	26.2	27.2	MTBC	Negative	MTBC	Sputum	-	41.4	34.3	NTM	Negative	NTM
Sputum	23.5	30.1	30.9	MTBC	Positive	MTBC	Sputum	-	39.0	34.4	NTM	Negative	NTM
Sputum	34.4	-	28.4	MTBC	Negative	MTBC	BAW	25.9	31.6	33.3	MTBC	Positive	MTBC
Sputum	33.1	-	32.9	MTBC	Positive	MTBC	Sputum	30.8	33.1	28.6	MTBC	Negative	MTBC
Sputum	30.0	37.8	28.5	MTBC	Negative	MTBC	BAW	-	40.9	34.7	NTM	Negative	NTM
Sputum	31.1	-	28.6	MTBC	Negative	MTBC	Sputum	35.0	-	33.8	MTBC	Positive	MTBC
Sputum	32.3	-	31.0	MTBC	Negative	MTBC	Sputum	30.1	38.6	33.8	MTBC	Positive	MTBC
Sputum	-	30.7	38.4	NTM	Negative	NTM	Sputum	30.6	-	29.1	MTBC	Negative	MTBC
Sputum	-	32.2	38.1	NTM	Negative	NTM	BAW	-	40.5	39.6	NTM	Negative	NTM
Sputum	24.4	30.9	34.0	MTBC	Positive	MTBC	Sputum	31.4	43.8	33.6	MTBC	Positive	MTBC
Sputum	27.0	33.6	33.8	MTBC	Positive	MTBC	Sputum	-	34.4	33.7	NTM	Negative	NTM
Sputum	26.1	32.6	33.8	MTBC	Positive	MTBC	Sputum	27.4	35.3	34.9	MTBC	Positive	MTBC
Sputum	25.8	33.9	34.5	MTBC	Positive	MTBC	Sputum	31.5	-	30.6	MTBC	Negative	MTBC
Sputum	-	31.1	33.2	NTM	Positive	NTM	Sputum	-	37.1	34.1	NTM	Positive	NTM
Sputum	-	36.4	35.8	NTM	Negative	NTM	Sputum	31.2	-	29.0	MTBC	Negative	MTBC
Sputum	35.7	35.3	33.3	MTBC and NTM	Positive	NTM	Sputum	30.2	39.5	33.4	MTBC	Positive	MTBC
Sputum	-	35.1	34.7	NTM	Negative	NTM	Sputum	29.9	-	29.1	MTBC	Negative	MTBC
Sputum	35.2	-	31.0	MTBC	Negative	MTBC	Sputum	29.4	-	28.7	MTBC	Positive	MTBC
BAW	27.1	-	29.1	MTBC	Negative	MTBC	BAW	28.3	-	28.9	MTBC	Negative	MTBC
Sputum	-	29.7	34.8	NTM	Negative	NTM	Sputum	24.1	32.9	28.8	MTBC	Positive	MTBC
Sputum	-	37.5	36.5	NTM	Negative	NTM	Sputum	-	37.9	35.2	NTM	Negative	NTM
Sputum	-	39.5	34.6	NTM	Negative	NTM	Sputum	-	37.5	40.0	NTM	Positive	NTM
Sputum	26.3	32.8	33.3	MTBC	Positive	MTBC	Sputum	30.3	38.7	32.9	MTBC	Positive	MTBC
Sputum	-	37.2	37.1	NTM	Negative	NTM	BAW	-	32.7	32.7	NTM	Positive	NTM

Abbreviations: PNA, peptide nucleic acid; MTBC, *Mycobacterium tuberculosis* complex; BAW, bronchoalveolar washing fluid; NTM, nontuberculous mycobacteria; ITS, internal transcribed spacer; AFB, acid-fast bacilli.

**Table 2.** Comparison of PNA probe-based real-time PCR assay with acid-fast bacilli smear, culture, and clinical data in respiratory specimens

Category of specimens	N of specimens	N of MTBC positive by real-time PCR	N of NTM positive by real-time PCR
All tuberculosis positive	64	58	-
Smear positive and MTBC culture positive	36	36	-
Smear score 4+	4	4	-
Smear score 3+	18	18	-
Smear score 2+	3	3	-
Smear score 1+	3	3	-
Smear score Trace	8	8	-
Smear negative and MTBC culture positive	24	22	-
Smear and culture negative with clinical diagnosis of tuberculosis	4	-	-
All NTM culture positive	42	2*	29*
Smear positive and NTM culture positive	6	1*	5*
Smear score 4+	-	-	-
Smear score 3+	1	-	1
Smear score 2+	2	1*	2*
Smear score 1+	-	-	-
Smear score trace	3	-	2
Smear negative and NTM culture positive	36	1	24
Smear and culture negative, non-tuberculosis disease	425	-	-

\*One specimen was simultaneously positive for MTBC and NTM by PNA probe based real-time PCR.

Abbreviations: PNA, peptide nucleic acid; MTBC, *Mycobacterium tuberculosis* complex; NTM, nontuberculous mycobacteria.

the advantages of high affinity and sequence specificity for binding to complementary nucleic acids. An important property of PNA is its uncharged nature, because the PNA backbone is composed of repeating N-(2-aminoethyl)-glycine units linked by peptide bonds instead of the negatively charged sugar phosphate backbone of natural nucleic acids [16]. The uncharged nature of PNA allows the formation of a strong PNA/DNA duplex.

The new real-time PCR assay using PNA probes for detection of MTBC revealed high sensitivity and specificity in this study. Although the new real-time PCR assay could not detect culture-negative tuberculosis, this can be attributed to the inadequate quality of specimens in the culture-negative tuberculosis sam-

**Table 3.** Identification of nontuberculous mycobacterial isolates from clinical specimens and real-time PCR results

Species	N of specimens	N of NTM-positive specimens by real-time PCR
<i>Mycobacterium intracellulare</i>	27	22
<i>Mycobacterium abscessus</i>	5	1
<i>Mycobacterium avium</i>	2	2
<i>Mycobacterium chelonae</i>	1	-
<i>Mycobacterium goodii</i>	1	-
<i>Mycobacterium kansasii</i>	1	1
<i>Mycobacterium massiliense</i>	1	1
<i>Mycobacterium mucogenicum</i>	1	-
<i>Mycobacterium parascrofulaceum</i>	1	1
<i>Mycobacterium senegalense</i>	2	1

Abbreviation: NTM, nontuberculous mycobacteria.

ples. The performance of the new real-time PCR assay for detection of MTBC is comparable with those of other real-time PCR assays used in Korea [17]. Rapid identification of MTBC in smear-negative samples as well as in smear-positive samples is important for prevention of tuberculosis transmission, because about 17% of tuberculosis cases involve transmission from persons with negative AFB smear results [18]. Systematic reviews and meta-analyses of the performance of NAA tests for the diagnosis of tuberculosis reported that sensitivity was 96% in smear-positive samples and 66-73% in smear-negative samples [19, 20]. The sensitivity of the new real-time PCR assay in smear-negative samples is comparable with those of other NAA tests.

Unlike *M. tuberculosis*, no animal-to-human or human-to-human transmission of NTM has been reported. Infection of NTM is assumed to be acquired from environmental sources [4, 5]. There has been a significant rise in human disease caused by NTM during recent decades, with the increasing population of immunocompromised patients [21, 22]. Rapid identification and discrimination of NTM from MTBC is useful for the management of mycobacterial disease, because many NTM are resistant to the antibiotics used for the treatment of tuberculosis [15]. The sensitivity of the new real-time PCR for detection of NTM was significantly lower than that for detection of MTBC. We assume the reason for this low sensitivity is that the ITS sequence was used as the target for detection of mycobacteria, whereas the highly repetitive *IS6110* sequence, which is present in 10 to 16 copies in most MTBC members isolated from clinical specimens, was employed for detection of MTBC [7].

The detection limit of the new real-time PCR assay was not

determined in this study. Moreover, extra-pulmonary specimens were not included in our study, because a large number of specimens are required for a sufficient amount of isolated mycobacteria. Thus, further studies may be necessary to validate the new real-time PCR assay by determination of the detection limit and testing using a large number of clinical specimens in a variety of clinical settings.

In conclusion, the PNA probe-based real-time PCR assay may be useful for detection of MTBC in respiratory specimens, including smear-negative specimens, because of its high sensitivity and specificity. Further, the new real-time PCR assay may be useful for discrimination of NTM from MTBC due to its high specificity.

### Authors' Disclosures of Potential Conflicts of Interest

No potential conflicts of interest relevant to this article were reported.

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