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Evaluation of Performance of the Real-Q NTM-ID Kit for Rapid Identification of Eight Nontuberculous Mycobacterial Species

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We evaluated a multiplex real-time PCR and melting curve analysis assay (Real-Q NTM-ID kit; Biosewoom, Seoul, South Korea) for the identification of eight common nontuberculous mycobacterial species, using 30 type strains and 230 consecutive clinical isolates. The concordance rate of this assay with multigene sequence-based typing was 97.0% (223/230 isolates).

Nontuberculous mycobacteria (NTM) are increasingly significant causes of many clinical infections (1, 2). Since patterns of antibiotic susceptibilities vary among different NTM species, the goal of achieving accurate and rapid identification of multiple NTM species is one of great interest (2, 3).

Conventional biochemical tests for mycobacterial identification have mostly been replaced by molecular methods such as DNA sequencing, line probe hybridization, PCR restriction fragment length polymorphism analysis (PRA), and real-time PCR. However, DNA sequencing remains a labor-intensive approach requiring expensive instrumentation (4, 5). Moreover, line probe hybridization and PRA are prone to carryover amplicon contamination resulting from postamplification procedures (6–8). In contrast to line probe hybridization or PRA, real-time PCR and melting curve analysis can be performed in closed systems without the risk of carryover amplicon contamination and can also be easily adapted to a high-throughput format (9–11).

TABLE 1 Reference strains tested

Species	Source and strain(s)[a]
Mycobacterium	
M. abscessus	KCTC 19621
M. aubagnense	KCTC 19639
M. avium	KMRC 00136-41004
M. colombiense	KMRC 00136-86001
M. conceptionense	KCTC 19640
M. fortuitum	KCTC 9510
M. gordonae	KCTC 9513
M. intracellulare	KCTC 9514
M. kansasii	KCTC 9515
M. lentiflavum	KMRC 00200-17002
M. marinum	KMRC 00136-21108
M. massiliense	KCTC 19086
M. mucogenicum	KCTC 19088
M. neoaurum	KCTC 19096
M. nonchromogenicum	KMRC 00136-46003
M. peregrinum	KMRC 00136-34001
M. porcinum	KCTC 9517
M. scrofulaceum	KMRC 00200-30115
M. senuense	KCTC 19147
M. simiae	KMRC 00136-85001
M. smegmatis	KCTC 9108
M. szulgai	KCTC 9520
M. terrae	KCTC 9614
M. timonense	KMRC 00136-79001
M. veccae	KCTC 19087
Other *Corynebacterineae*	
Nocardia farcinica	KCTC 9958
Nocardia asteroides	KCTC 9956
Rhodococcus erythropolis	KCTC 1062
Rhodococcus equi	KCTC 9082
Tsukamurella tyrosinosolvens	KCTC 19177

[a] KCTC, Korean Collection for Type Cultures; KMRC, Korean Mycobacteria Resource Center.

In the present study, we evaluated the performance of the Real-Q NTM-ID kit (Real-Q assay; BioSewoom, Seoul, South Korea). The Real-Q assay is a multiplex real-time PCR method that incorporates melting curve analysis and is designed to target the internal transcribed spacer (ITS) region to detect and distinguish eight NTM species: *Mycobacterium abscessus*, *M. avium*, *M. intracellulare*, *M. chelonae*, *M. fortuitum*, *M. gordonae*, *M. kansasii*, and *M. massiliense*. We compared the performance of this assay to that of multigene sequence-based typing as a reference method.

This study was conducted at a tertiary-care hospital in Seoul, South Korea, and was approved by the Institutional Review Board of Samsung Medical Center. In total, 30 reference strains (25 mycobacterial and 5 other strains) and 230 consecutive clinical NTM isolates were genotyped (Table 1). Decontaminated samples were placed into a mycobacterial growth indicator tube (MGIT 960 system; Becton Dickinson, Sparks, MD) and also into 3% Ogawa agar (Shinyang, Seoul, South Korea) and cultured for 6 weeks.

For cultures grown on solid medium, DNA was prepared by suspending a loopful of bacteria in 100 μl DNA extraction buffer. For cultures grown in liquid MGIT culture medium (1 ml), the

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TABLE 2 Comparison of Real-Q NTM-ID assay results with multigene sequence-based typing results

Mycobacterial species	Total no. of isolates	No. of correct or compatible[a] results obtained with Real-Q assay	Real-Q assay result for incorrectly identified isolate(s) (no. of isolates)
Detectable by Real-Q assay			
M. avium complex			
M. avium	81	81	
M. intracellulare	55	53	Out of detection range (2)
M. abscessus-M. chelonae complex			
M. abscessus	44	44	
M. massiliense	18	18	
M. chelonae	2	2	
M. fortuitum complex			
M. fortuitum	3	1	Out of detection range (2)
M. gordonae	5	5	
M. kansasii	1	1	
M. abscessus + *M. intracellulare*	1	1	
M. avium + *M. massiliense*	1	1	
Not detectable by Real-Q assay			
M. avium complex			
M. marseillense	1	0	*M. intracellulare* (1)
M. colombiense	1	1	
M. fortuitum complex			
M. peregrinum	3	3	
M. conceptionense	3	1	*M. fortuitum* (2)
M. porcinum	1	1	
M. lentiflavum	4	4	
M. terrae complex			
M. algericum	2	2	
M. senuense	1	1	
M. mucogenicum	2	2	
M. nebraskense	1	1	
Total (%)	230 (100)	223 (97)	

[a] Compatible results are defined as "out of detection range" results from the Real-Q NTM-ID assay for mycobacterial species that the assay cannot detect.

cultures were centrifuged at 13,000 × *g* for 5 min. The pellets were resuspended in 50 μl DNA extraction buffer. Both sample types were heated in a boiling water bath for 10 min. After centrifugation, the supernatants were used in the analyses.

Real-time PCRs were performed on a 7500 Fast real-time PCR system (Applied Biosystems, Foster City, CA, USA), using the Real-Q NTM-ID kit, which comprises four reaction tubes, each containing specific primer mixtures for two NTM species. Melting curve analysis using EvaGreen dye was performed after amplification (see Table S1 and Fig. S1 in the supplemental material). Each PCR was performed in a total volume of 20 μl (8 μl of the PCR mixture, 6 μl of the primer mixture, 2 μl of distilled water, 1 μl of the internal control, and 3 μl of template DNA). Thermocycling conditions included a step at 50°C for 2 min and a step at 95°C for 10 min, followed by 35 cycles of 15 s at 94°C, 30 s at 62°C, and 15 s at 72°C. The four primer sets and their melting temperatures are shown in Table S1 in the supplemental material. The melting curve was generated by heating the reaction mixtures from 60°C to 95°C after the last cycle.

The ITS region, the *hsp65* gene, and the *rpoB* gene were sequenced according to the protocol outlined in the Clinical and Laboratory Standards Institute guideline MM18-A (12). The PCR primer sets used to amplify the ITS region, the *hsp65* gene, and the *rpoB* gene have been previously published (8, 13, 14). The amplified sequences were analyzed using GenBank. The final sequencing result was taken as the definitive identification.

The specificities of the Real-Q assay for detecting each species were evaluated using the 30 reference strains. Only the expected PCR products were amplified, except in the case of *Mycobacterium conceptionense*, which produced a peak that corresponded to that of *M. fortuitum*.

Among the 230 isolates tested, the organism most commonly identified using the multigene sequence-based typing method was *M. avium* (35.2%), followed by *M. intracellulare* (23.9%), *M. abscessus* (19.1%), *M. massiliense* (7.8%), *M. gordonae* (2.2%), and other NTM species (Table 2). Approximately 92% (211/230) of the clinical isolates were mycobacterial species that the Real-Q assay could detect. Out of the 211 isolates that could be genotyped by the assay, 207 (98.1%) were correctly identified at the species level; however, two cases of *M. intracellulare* and two cases of *M. fortuitum* were incorrectly identified as out of the detection range of the assay. One strain of *Mycobacterium marseillense* and two strains of *M. conceptionense* were misidentified as *M. intracellulare* and *M. fortuitum*, respectively. In total, 223/230 strains (97.0%) yielded the expected results (Table 2). All discrepant cases were identified as a related species, defined as a species in the same complex or group. The Real-Q assay, which targets the ITS region, could not distinguish between closely related species in the *M. avium* complex and the *M. fortuitum* complex, which might be due to sequence similarities between these two complexes.

The Real-Q assay correctly identified all 63 isolates in the *M. abscessus-M. chelonae* complex at the species level. The *M. absces-*

sus complex is frequently isolated from patients with respiratory rapidly growing mycobacteria around the world but especially in South Korea (15, 16). Koh et al. reported that the clinical impact of this species, which is classified within the *M. abscessus* complex, was different. The treatment response rates for clarithromycin-based antibiotic therapies were higher in patients with *M. massiliense* lung disease than in those with *M. abscessus* lung disease (16). Thus, species-level identification is important, especially in the *M. abscessus* complex. Differentiation between *M. abscessus* and *M. massiliense* is one advantage of the Real-Q assay compared with most other commercially available kits. The Real-Q assay could reduce the turnaround time of species identification compared with standard probe hybridization-based methods. The average turnaround time for the real-time PCR assay was less than 2.5 h. Furthermore, the Real-Q assay requires less hands-on time than do line probe assays.

However, the main limitation of this assay is its inability to identify rare isolates at the species level. Thus, additional procedures will sometimes be needed for the species-level identification of clinically important isolates. Nevertheless, the Real-Q assay can rapidly detect most clinically important NTM species with good specificity; thus, this assay could be useful as a routine method in clinical settings.

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