Performance Evaluation of Two Newly Developed Korean Mycobacteria RT-PCR Kits for the Identification of Culture Isolates

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RES-019

Background

- Korea continues to have one of the highest tuberculosis incidence rates among OECD countries.
- The incidence of nontuberculous mycobacterial (NTM) infections has also been rising significantly.
- Many RT-PCR kits for mycobacterial detection have been developed in Korea.
- RT-PCR is routinely used to simultaneously detect *Mycobacterium tuberculosis* (MTB) and NTM in both clinical specimens and mycobacterial culture isolates.
- Recently, two new RT-PCR kits have been developed in Korea, and this study aims to evaluate their diagnostic performance.

Materials and Methods

Specimens

- Culture isolates identified as MTB (n = 30) or NTM (n = 47) using the Anyplex MTB/NTM Real-time Detection kit (Seegene, Korea) during clinical diagnostic testing at Hanyang University Guri Hospital, Korea.
- Isolates were collected between August 2019 and January 2024
- Forty-seven NTM isolates were selected based on the species identification results using MolecuTech REBA Myco-ID assay (YD Diagnostics, Korea).
- The performance of two new RT-PCR kits, NextGene MTB/NTM Detection Kit (EoneBiotech, Korea) and NeoPlex TB/NTM Detection Kit (Genematrix, Korea), was evaluated.

NextGene MTB/NTM Detection Kit

- The kit was developed and CE-IVD certified in 2023.
- Primers targeting the IS6110 and mpb64 genes for the detection of MTB and primers targeting a conserved 16S rRNA region found in NTM for their detection.
- PCR amplification was carried out using the CFX96 instrument.
- Fluorescence signals were detected in separate channels: FAM for MTB, VIC for NTM, and Cy5 for the IC, with the Texas Red channel used for the PCR control.
- Cut-off Ct value for all targets are 35 cycles.

NeoPlex TB/NTM detection Kit

- The assay is developed in 2023.
- Primers targeted IS6110 for MTB, 16S rRNA for mycobacteria and proprietary markers for the five NTM species.
- FAM, Cal Red 610 and HEX fluorophore signals were used for MTB, mycobacteria, and IC, respectively.
- This assay detects MTB and NTM using RT-PCR with CFX96 system, and differentiates five NTM species through subsequent melting curve analysis.
- Cut-off Ct value for all targets are 40 cycles.

Results

- For MTB isolates (n = 30), all three RT-PCR kits yielded concordant results.

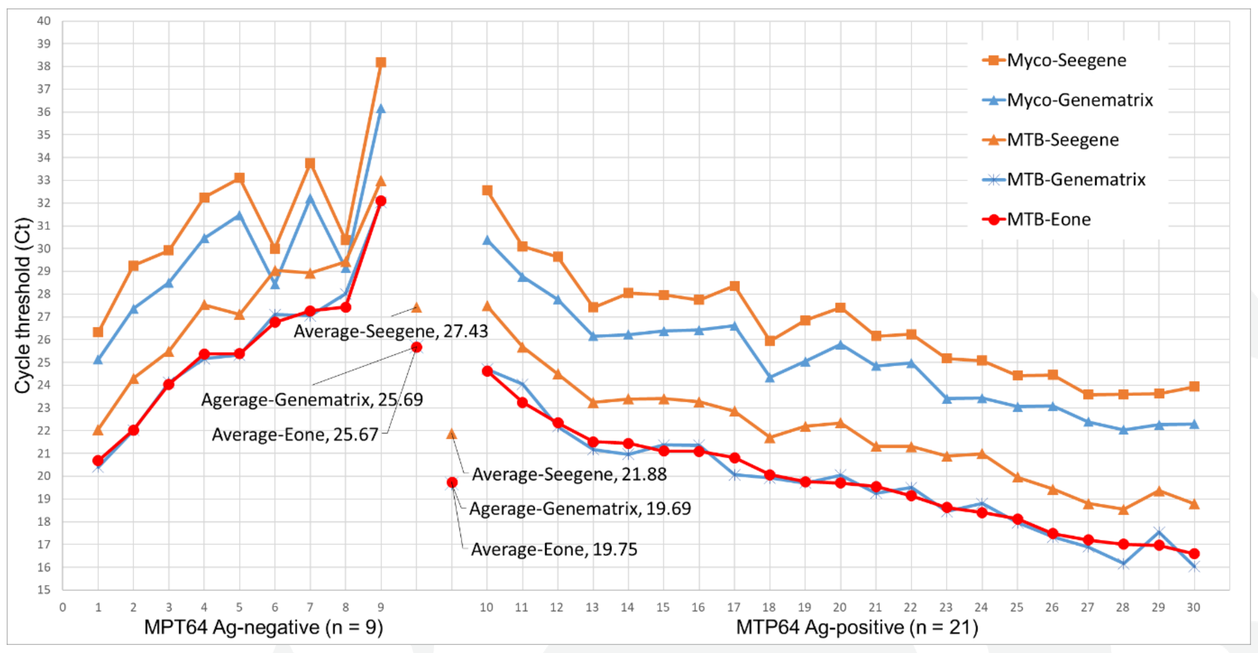

Figure 1. Comparison of Ct values among three reagent kits for 30 isolates that tested positive for MTB by the Seegene assay. The nine isolates with negative MPT64 antigen results were arranged in ascending order of Ct values, whereas the 21 isolates with positive MPT64 antigen results were arranged in descending order of Ct values.

- EoneBiotech and Genematrix assays showing similar Ct values (16.59–32.10 and 16.03–32.04, respectively), while Seegene assay exhibited slightly higher Ct values (18.79–32.99).
- For NTM isolates (n = 47), all kits provided consistent results.

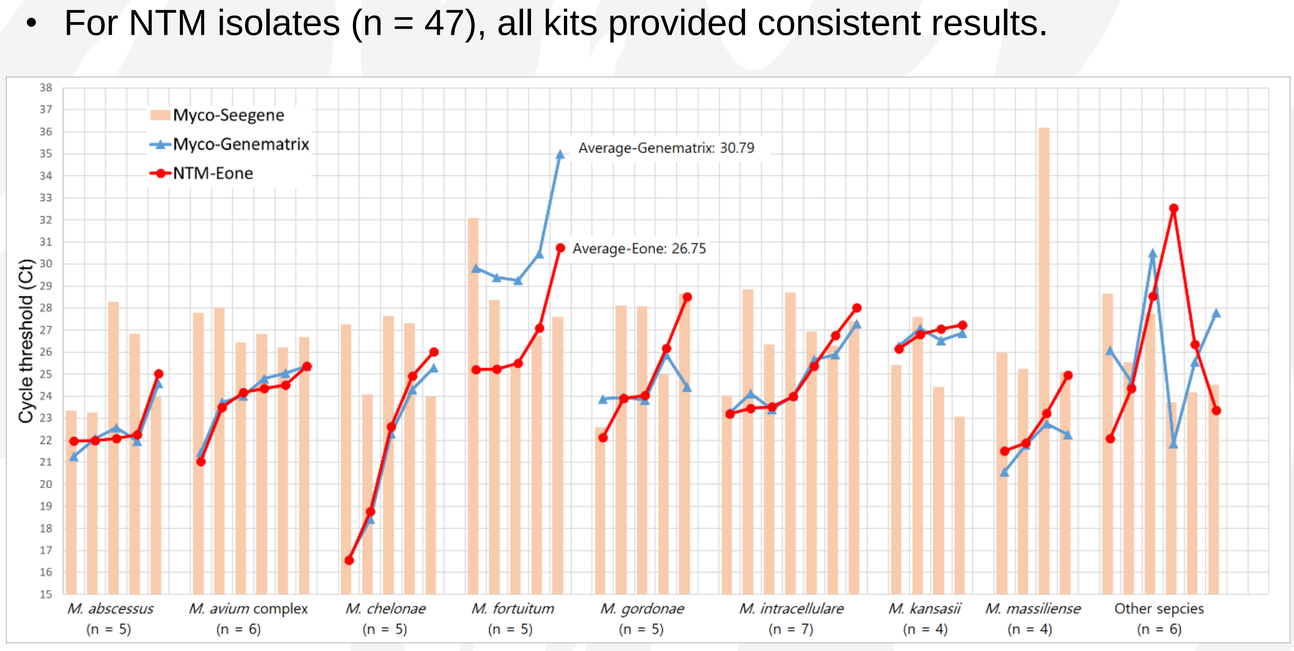

Figure 2. Comparison of Ct values among three reagent kits for 47 isolates that tested positive for NTM by the Seegene assay.

- However, for *M. fortuitum* complex, Genematrix assay produced higher Ct values (29.27–35.0) than EoneBiotech assay (25.20–30.74), with a mean Ct difference of 4.04 (range, 3.37–4.62).

Conclusions

- The newly developed RT-PCR kits demonstrated comparable performance to existing RT-PCR methods in the differential diagnosis of mycobacterial culture isolates.
- Given their additional advantages over conventional kits, these assays offer improved diagnostic capabilities: the EoneBiotech assay can independently detect and distinguish MTB and NTM, and the GeneMatrix assay provides species identification information for 5 NTM species.
- These new assays are expected to be widely adopted in clinical and diagnostic settings.

Acknowledgment

This study was supported by the clinical research fund from EoneBiotech.

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