Detection of Mycobacteria in Clinical Samples by the Newly Developed PaxView TB/NTM MPCR-ULFA Kit

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Authors' contributions

This work was carried out in collaboration among all authors. Author JHC designed the clinical experiments. Author CKK performed the clinical experiments. Author YKP performed the statistical analysis and wrote the manuscript. All authors read and approved the final manuscript.

Original Research Article

ABSTRACT

Aims: To evaluate sensitivity and specificity of the newly developed PaxView TB/NTM MPCR-ULFA Kit.
Study Design: Compared with the licensed AdvanSure TB/NTM real-time PCR.
Place and Duration of Study: SCL and PaxGenBio in Gyeonggi-do, Korea, between August 2018 and May 2019.
Methodology: In this study, 350 specimens including sputum, bronchial washing, body fluid, tissue, urine, and cerebrospinal fluid were examined to evaluate the performance of the PaxView TB/NTM MPCR-ULFA Kit compared to results of the currently licensed AdvanSure TB/NTM real-time PCR (LG Chem, Korea).
Results: Compared to the AdvanSure TB/NTM real-time PCR, the PaxView TB/NTM MPCR-ULFA Kit test was found to possess a 100% sensitivity. In other words, all 140 MTB and 61 non-tuberculous mycobacteria (NTM) specimens that tested positive with the AdvanSure TB/NTM real-time PCR also tested positive with the PaxView TB/NTM MPCR-ULFA Kit. However, the specificity of the later kit found to be 97.9% (146/149; 95% CI 95.6–100.0), meaning that out of 149

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MTB/NTM specimens that tested negative with the AdvanSure TB/NTM real-time PCR, 146 were identified as MTB/NTM-negative according to the PaxView TB/NTM MPCR-ULFA Kit. Nonetheless, the overall agreement between the two diagnostic tools was 99.1% (347/350; 95% CI 98.1– 100.0) and the kappa value was 0.982 (350; 95% CI 0.968 – 0.995), meaning that the two diagnostic tools rendered almost identical results.

Conclusion: The PaxView TB/NTM MPCR-ULFA Kit could be useful to identify MTB and NTM in resource-limited countries, as this procedure is far more cost-effective than real-time PCR and convenient than conventional gel electrophoresis approaches.

1. INTRODUCTION

Tuberculosis (TB) remains a highly contagious and widespread disease worldwide, despite substantial efforts to eradicate it. According to the 2019 Global TB Report, the WHO estimated that 1.5 million people died due to TB and 10.0 million people developed TB in 2018 [1].

Although millions of people are diagnosed and successfully treated for TB each year, a large gap remains between the reported cases and the estimated incidence. Most deaths from TB could be prevented with early diagnosis and appropriate treatment [2-3]. Therefore, early TB diagnosis is essential to reduce its worldwide lethality.

There are many kinds of TB diagnostic tools from smear microscopy developed by Robert Koch in 1882 to modern sophisticated approaches include the GeneXpert (Cepheid, USA) in the world. Each country adopts alone or in combination with other approaches, depending on personal or nation-wide socioeconomic factors. However, an optimal (i.e., effective and cost-efficient) TB diagnostic tool is yet to be developed.

Among the various existing TB diagnostic tools, molecular diagnostic tools are the fastest methods for the detection of Mycobacterium tuberculosis (MTB) in clinical specimens, and also possess satisfactory sensitivity and specificity [4]. For example, the GeneXpert MTB/RIF kit, a current leading molecular diagnostic tool, has been extensively used not only for the detection of rifampicin-resistant TB but also for primary TB case detection [5-8]. However, due to the expensive equipment, cartridges, and installation costs required by the GeneXpert approach, this testing solution is not suitable for implementation in developing countries without international monetary aid [9-10].

In house-PCR is the cheapest molecular diagnostic method, but requires cumbersome gel electrophoresis procedures. Therefore, PaxGenBio (Korea) developed the PaxView TB/NTM MPCR-ULFA Kit, which rapidly detects MTB and distinguishes between MTB and NTM without gel electrophoresis after PCR. Moreover, this procedure can be used with a conventional PCR instrument without the need for a specific expensive PCR device.

Therefore, this study sought to evaluate the potential clinical applicability of the PaxView TB/NTM MPCR-ULFA Kit for the detection of MTB and NTM in clinical samples by comparing its performance with that of the AdvanSure TB/NTM real-time PCR (LG Chem, Korea), which has been extensively used in clinical laboratories [11-12].

2. MATERIALS AND METHODS

2.1 Specimens

Seoul Clinical Laboratories (SCL, Korea) is among the largest nation-wide clinical laboratories and performs a lot of tests with various types of specimens upon the request of hospitals across South Korea. Notably, SCL routinely performs MTB detection via real-time PCR.

In this study, we utilized all hospital-requested TB diagnosis specimens collected from August of 2018 to May of 2019. The specimens were tested with the AdvanSure TB/NTM real-time PCR, which is licensed for MTB detection in South Korea. A total of 350 specimens were sampled, of which 275 were sputum, 19 bronchial washings, 26 body fluids, 20 tissues, 8 urine, and 2 cerebrospinal fluids (CSFs). All

samples were previously tested for MTB/NTM detection with the AdvanSure TB/NTM real-time PCR.

2.2 DNA Extraction from Specimens

Specimens were pretreated differently depending on sample type. Briefly, sputa and bronchial washings were mixed with an equal volume of 4% NaOH for 10 min at room temperature, tissues were homogenized, and body fluids and urine were directly processed following the AdvanSure TB/NTM real-time PCR manufacturer's protocol. Afterward, all specimens were centrifuged at 13000 rpm for 3 min. After discarding the supernatant, DNA was extracted from the resulting pellet. After the specimen pretreatment, mycobacterial DNA in the specimen was extracted with the Chemagic DNA Plasma 200 kit using the Chemagic 360 Nucleic Acid Extractor (Perkin Elmer, Germany) following the manufacturer's protocol.

2.3 AdvanSure TB/NTM Real-time PCR

Specimen-extracted DNA was amplified with the AdvanSure TB/NTM real-time PCR (the target genes were IS6110 for MTB and the internal transcribed spacer (ITS) region for NTM), following the manufacturer's protocol.

2.4 PaxView TB/NTM MPCR-ULFA Kit PCR

PCR was performed with the following protocol: 50°C for 4 min; 95°C for 10 min; 25 cycles of denaturation (95°C for 15 sec), annealing and extension (71°C for 60 sec); 20 cycles of denaturation (95°C for 15 sec), annealing (60°C for 30 sec), and extension (72°C for 30 sec).

After PCR amplification, 5 µl of PCR solution are added to the rectangular inlet at the bottom of the ULFA device, after which 50 µl of running buffer (provided by the manufacturer) are added immediately. After 5 minutes, 50 µl of washing buffer are added into the inlet and the results are then read within 20 minutes.

3. RESULTS AND DISCUSSION

The PaxView TB/NTM MPCR-ULFA Kit includes multiple primer pairs, including two for MTB-specific genes (IS6110 and mtp40), and for the mycobacteria rpoB gene. After multiplex PCR, the product identities were confirmed by universal lateral flow assay (ULFA), which is based on DNA-DNA hybridization with previously immobilized complementary DNA fragments on a nitrocellulose membrane (Fig. 1).
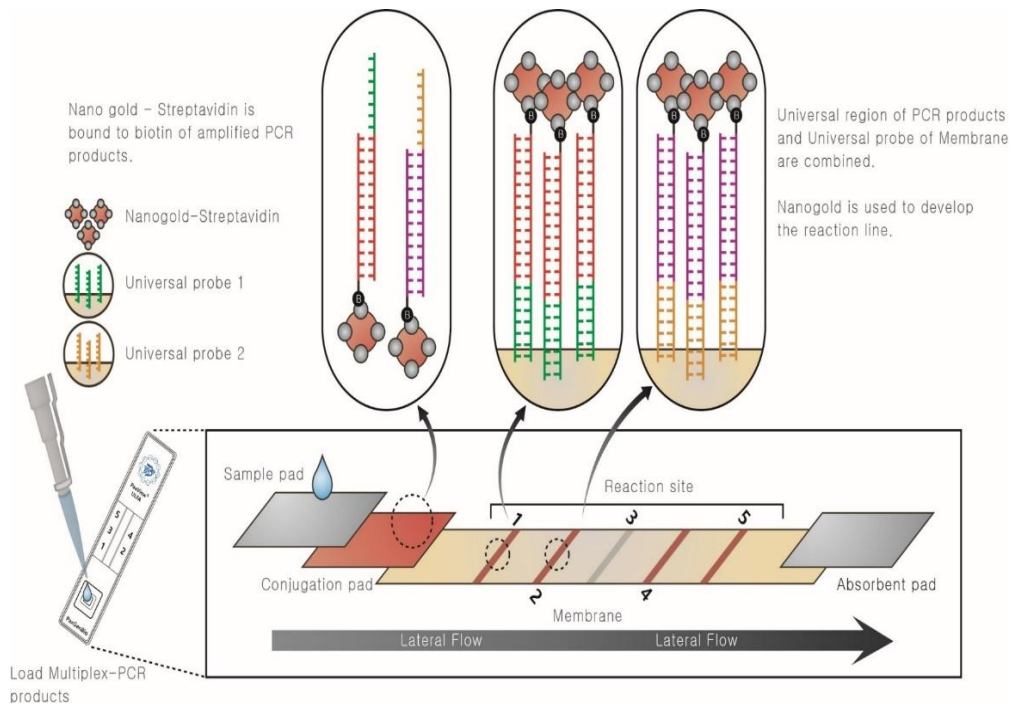

Fig. 1. Principle of PaxView TB/NTM MPCR-ULFA Kit

The PCR product amplified by a primer-attached universal probe and biotin is placed on the sample pad, the biotin of the PCR product then binds with nanogold-streptavidin on the conjugation pad. The PCR product then flows upward through the absorbent pad through the force of running buffer. During its flow, a universal probe binds with its specific complementary oligomers, which were previously immobilized on the membrane.

As a interpretation, the bands 1 and/or 2 (with or without band 3) indicate that the sample is MTB-positive, the band 3 without band 1 and 2 means that the sample is NTM-positive, the bands 4 and 5 without from band 1 to 3 indicate that the sample is MTB/NTM-negative, and the band 5 alone indicates that the test was invalid, which means that amplification process had not happened due to inappropriate template or reagents (Fig. 2).

A total of 350 clinical specimens from the Seoul Clinical Laboratory were used to compare the performance of the PaxView TB/NTM MPCR-ULFA Kit and the AdvanSure TB/NTM real-time PCR.

A total of 140 MTB-positive and 61 NTM-positive samples tested with the AdvanSure TB/NTM real- time PC were also found to be MTB- and NTM-positive with the PaxView TB/NTM MPCR-ULFA Kit, respectively (Table 1).

Moreover, out of the 149 MTB/NTM-negative specimens tested with the AdvanSure TB/NTM real-time PCR, 146 specimens rendered identical results with the PaxView TB/NTM MPCR-ULFA Kit, with only 3 exceptions in sputum samples (ID115, ID199, ID224). Out of these 3 specimens, ID115 and ID199 were identified as NTM-positive, and ID224 as MTB-positive by the PaxView TB/NTM MPCR-ULFA Kit test (Fig. 3).

Sample ID224 exhibited only an IS6110 band without rpoB or mtp40 bands in the PaxView TB/NTM MPCR-ULFA Kit test. Both the PaxView TB/NTM MPCR-ULFA Kit and the AdvanSure TB/NTM real-time PCR target the IS6110 gene for MTB detection. Therefore, both diagnostic tools exhibited similar sensitivity and specificity for MTB detection.

The Ct value threshold of the AdvanSure TB/NTM real-time Kit test is 35, whereas the PaxView TB/NTM MPCR-ULFA Kit test entails 45 cycles, including 20 cycles in the first step and 25 cycles in the second step. Therefore, small quantities of DNA extracted from bacilli may be amplified with the PaxView TB/NTM MPCR-ULFA Kit even if only a few bacilli are present in the specimens, whereas a negative result would be obtained with the AdvanSure TB/NTM real-time PCR test due to its Ct value threshold of 35. Based on these observations, it is possible that sample ID224 did in fact contain MTB bacilli, as indicated by the PaxView TB/NTM MPCR-ULFA Kit.
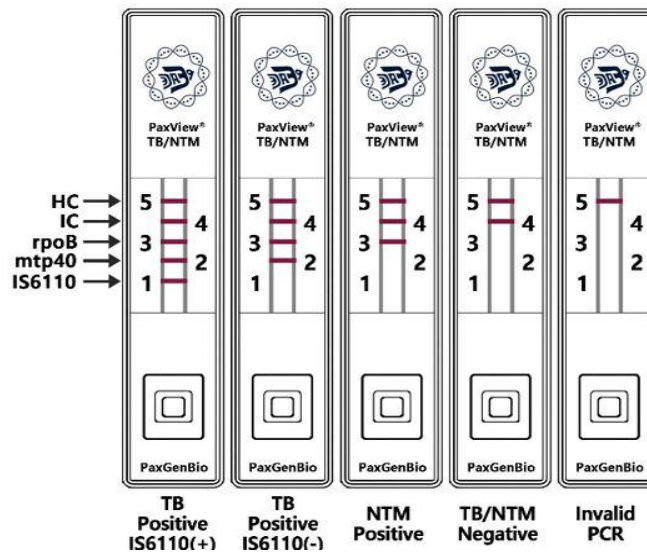

Fig. 2. Interpretation of the PaxView TB/NTM MPCR-ULFA Kit results
HC: hybridization control (band 5); IC: internal control for PCR (band 4); rpoB: mycobacteria indicator (band 3); mtp40 and IS6110: M. tuberculosis indicators (band 2 and band 1)

Table 1. Comparison of PaxView TB/NTM MPCR ULFA results and AdvanSure TB/NTM real-time PCR

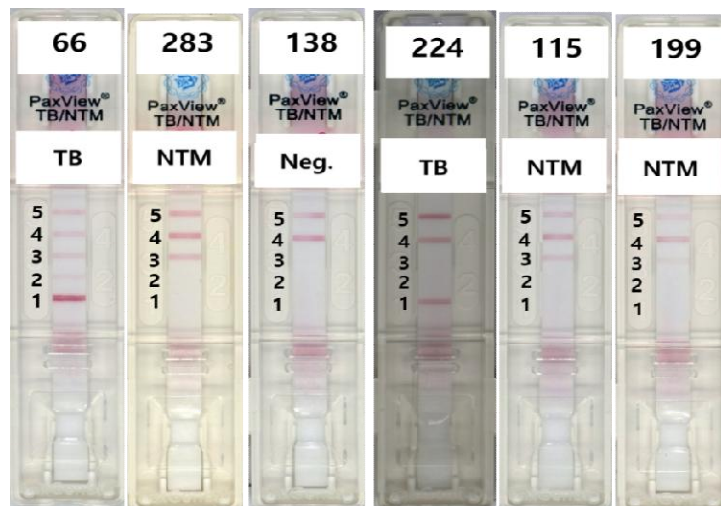|	Specimens (n=350)	PaxView TB/NTM MPCR ULFA		
		MTB positive (n=141)	NTM positive (n=63)	Negative (n=146)
AdvanSure MTB positive (n=140)	Sputum	112		
	Bronchial Washing	12		
	Body Fluid	4		
	Tissue	10		
	Urine	2		
AdvanSure NTM positive (n=61)	Sputum		55	
	Bronchial Washing		3	
	Body Fluid		3	
AdvanSure Negative (n=149)	Sputum	1	2	105
	Bronchial Washing			4
	Body Fluid			19
	Tissue			10
	Urine			6
	CSF			2

Fig. 3. Results of PaxView TB/NTM MPCR-ULFA Kit for samples
ID66: typical TB, ID283: typical NTM, ID138: typical negative, ID224: IS6110 band showed without rpoB and mtp40 bands. It may mean a paucity of DNA in the template. ID115 and ID199 revealed rpoB band. ID199: color of rpoB line was tint

MTB possesses multiple copies of the IS6110 gene, whereas the rpoB and mtp40 genes are only present as single copy in a chromosome. Particularly, Korean MTB strains are known to typically possess over 10 copies of the IS6110 gene in a single chromosome [13]. Theoretically, the occurrence of multiple copies of IS6110 within the chromosomal DNA of MTB could increase test sensitivity when only a few of bacilli are present in a specimen compared that of single-copy genes such as rpoB gene or mtp40. However, given that strains lacking IS6110 have been identified in some countries (e.g., India),

alternative PCR targets are necessary, and mtp40 can be a good candidate in such strains [14]. The mtp40 gene is exclusively present in MTB, i.e., not in M. bovis, M. bovis BCG, or NTM [15]. Therefore, the mtp40 gene is an excellent PCR target to detect MTB bacilli in specimens. Unfortunately, some MTB strains do not possess an mtp40 gene in their chromosomal DNA [16]. In these strains, the IS6110 gene can serve as an alternative PCR target. Therefore, the use of multiple complementary PCR targets for IS6110 and mtp40 is critical to increase MTB detection sensitivity and to accurately discriminate

between MTB and NTM [17]. For this reason, the PaxView TB/NTM MPCR-ULFA Kit uses these two genes as MTB detection targets.

We could not identify strains that contained exclusively mtp40 band without the co-occurrence of IS6110 band in this clinical trial, meaning that all tested strains had IS6110 copies in a given chromosome. However, the opposite happened in some strains, such as ID224. This highlights the advantage of having multiple copies of IS6110 in a given sample rather than only single-copy genes, especially when only a few bacilli are present in the tested specimen.

ID115 and ID199 tested positive for NTM in the PaxView TB/NTM MPCR-ULFA Kit test, but tested negative in the AdvanSure TB/NTM real-time PCR test. These two tools use different target genes for NTM detection. Specifically, the PaxView TB/NTM MPCR-ULFA Kit targets the rpoB gene, whereas the AdvanSure TB/NTM real-time PCR targets the ITS. Therefore, the PaxView TB/NTM MPCR-ULFA Kit may possess a wider coverage range for mycobacteria or their close relatives than the AdvanSure TB/NTM real-time PCR. However, we did not perform culture examination or further testing (e.g., sequencing) in these cases.

Furthermore, compared with the AdvanSure TB/NTM real-time PCR, the PaxView TB/NTM MPCR-ULFA Kit was found to have a 100% sensitivity. Particularly, all 140 MTB and 61 NTM specimens that tested positive with the AdvanSure TB/NTM real-time PCR also tested positive with the PaxView TB/NTM MPCR-ULFA Kit. However, the specificity of the later kit was 97.9% (146/149; 95% CI 95.6–100.0), meaning that out of 149 MTB/NTM specimens that tested negative with the AdvanSure TB/NTM real-time PCR, 146 were identified as MTB/NTM-negative, one specimen as MTB-positive, and the remaining two samples as NTM-positive, according to the PaxView TB/NTM MPCR-ULFA Kit (Table 1).

Both tools can simultaneously identify MTB and NTM. The overall agreement between the two diagnostic tools was 99.1% (347/350; 95% CI 98.1– 100.0) and the kappa value was 0.982 (350; 95% CI 0.968 – 0.995), meaning that the two diagnostic tools rendered almost identical results.

The newly-developed PaxView TB/NTM MPCR ULFA Kit entails multiple polymerase chain reactions and simplifies the result-reading process by implementing a universal lateral flow assay instead of a cumbersome electrophoresis procedure. Another advantage of the PaxView MPCR-ULFA Kit is that it only requires a standard PCR device. In contrast, the implementation of GeneXpert cartridges or real-time PCR requires specialized and expensive equipment. Finally, the PaxView TB/NTM MPCR-ULFA Kit can also discriminate between MTB- and NTM-specific PCR products, which reduces the cost associated with additional discrimination procedures.

4. CONCLUSION

The PaxView MPCR ULFA kit has the same sensitivity and specificity as the leading real-time PCR test for the detection of MTB in clinical specimens, and has the ability to detect both MTB and NTM simultaneously. Therefore, this kit could be implemented in laboratories with standard PCR equipment, bypassing the need to purchase expensive equipment. Moreover, the results of this kit can be obtained easily and quickly, without the need for unpractical electrophoresis procedures.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country.

ETHICAL APPROVAL

This study was approved by the Institutional Review Board of the Seoul Clinical Laboratory, under the project title "clinical evaluation of PaxView TB/NTM MPCR-ULFA Kit" (Approval number: 2018-60-01F).

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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