Original Article

Clinical Performance of a Molecular-based Lateral Flow Assay for Rapid and Simultaneous Detection of Tuberculosis and Non-Tuberculous Mycobacteria in Indonesia

Lidya Chaidir,[1,2] Nabilla Ghina Zavitri,[2] Adi Imam Cahyadi,[1,2] Claudia Selviyanti,[2] Dyshelly Nurkartika Pascapurnama,[2] Jessi Annisa,[3] Bachti Alisjahbana,[4,5] Prayudi Santoso[4]

[1]*Department of Biomedical Sciences, Faculty of Medicine, Universitas Padjadjaran, Bandung, Indonesia*
[2]*Center for Translational Biomarker Research, Universitas Padjadjaran, Bandung, Indonesia*
[3]*Biomedical Laboratory, Faculty of Medicine, Universitas Padjadjaran, Bandung, Indonesia*
[4]*Department of Internal Medicine, Faculty of Medicine, Universitas Padjadjaran, Hasan Sadikin Hospital, Bandung, Indonesia*
[5]*Research Center for Care and Control of Infectious Disease, Universitas Padjadjaran, Bandung, Indonesia*

Abstract

Background: Diagnostic modalities that can differ Tuberculosis (TB) and Non-Tuberculosis Mycobacteria (NTM) are urgently needed, especially in TB high burden areas, to avoid misdiagnosis. A rapid molecular approach can be utilized to accommodate easy implementation for routine diagnosis in low-resource countries to decrease the turnaround time and increase sensitivity significantly. This study evaluated the diagnostic performance of PaxView® TB/NTM, a rapid multiplex PCR assay that utilizes universal lateral flow technology for the simultaneous detection and visualization of TB and NTM.

Methods: The study was conducted in Bandung, Indonesia, using sputum samples from the outpatient TB clinic in Hasan Sadikin General Hospital. Sputum samples were collected consecutively from each patient. All the eligible samples underwent microbiological work-up and Xpert MTB/RIF testing with different sputum taken previously. The collected sputum was extracted, amplified, and then visualized using the PaxView® TB/NTM MPCR-ULFA kit.

Results: The sensitivity and specificity of the PaxView® MPCR-ULFA were 83.0% and 92.1%, respectively. The positivity rate for MPCR ULFA showed significantly higher results (43.0%) compared to culture (32.8%), smear microscopy (26.3%), and was comparable with the Xpert MTB/RIF assay (43.5%). A total of 23 confirmed NTM-positive cases were found in this study (2.6%).

Conclusion: The PaxView® MPCR-ULFA demonstrated promising performance for MTB and NTM detection. The sensitivity and specificity of this kit are comparable to those of other molecular diagnostic modalities. The simple platform using a test strip and straightforward visualization might be appropriately applied in poorly-equipped laboratories.

Keywords: nucleic acid lateral immunoflow assay, rapid molecular test, TB diagnosis

Corresponding Author:
Lidya Chaidir | Department of Biomedical Sciences, Faculty of Medicine - Center for Translational Biomarker Research, Universitas Padjadjaran, Bandung, Indonesia| lidya.chaidir@unpad.ac.id

Submitted: September 18th, 2025
Accepted: May 11th, 2026
Published: May 26th, 2026

J Respirol Indones. 2026
Vol. 46 No. 2: 112-20
https://doi.org/10.36497/jri.v46i2.1071

INTRODUCTION

In Indonesia, tuberculosis remains a serious infectious disease with high mortality. Indonesia ranks second-highest TB burden in the world after India. Based on the Global Tuberculosis Report 2024 by the World Health Organization (WHO), there were an estimated 10.800.000 TB cases in 2023, but only about 60% were notified. The 40% gap reflects a mixture of under-reporting and under-diagnosis.[1] Limited laboratory capacity contributes to the high proportion of undetected TB cases. This is important, as rapid diagnosis and treatment are essential for individual patients' health as well as for controlling the transmission of the diseases.

Sputum microscopy by Ziehl-Neelsen (ZN) staining, the cornerstone for diagnosing TB in Indonesia, only had a sensitivity of 39% compared to TB culture.[2] It was even less sensitive in extrapulmonary and Human Immunodeficiency Virus (HIV)-infected TB. Culture (liquid or solid), which is considerable as a gold standard of TB diagnosis, is available but is limited to referral laboratories and requires a long time for the results to come out.[3]

A molecular method, Xpert MTB/RIF, was introduced in Indonesia in 2012. It has contributed to

a reduction of time for primary detection, as well as for diagnosis of Rifampicin-resistant, which is a surrogate of multidrug-resistant TB.[4] Further expansion of Xpert MTB/Rif is currently progressing with donor-assisted funds. However, despite playing a crucial role in TB diagnosis, especially in increasing TB case finding and detecting drug resistance within a shorter time, performing Xpert tests remains problematic in low-resource laboratory environments.[5]

There is increasing enthusiasm for novel approaches, especially molecular rapid tests, which could replace traditional smear microscopy and be applied in resource-limited laboratories in Indonesia. These molecular methods focus on easy implementation for TB diagnosis daily in low-resource laboratories in countries with high TB burden to decrease the turnaround time and increase sensitivity significantly. Currently, there are many new TB rapid molecular tests being developed in various stages of development. Most of the tests are in early stages, a few of them are available on the market, but the accuracy data are limited.[6]

Alongside TB diagnostics, implementing an effective method that can differ TB and non-tuberculous mycobacteria (NTM) is also crucial. Concurrently, clinical concerns regarding NTM are rising, particularly in high-TB-burden regions, where NTM infection is recognized as a risk factor for post TB lung diseases.[7] Due to overlapping clinical manifestations and morphological similarities, differentiating NTM from *M. tuberculosis* remains a critical diagnostic challenge. The lack of highly sensitive and specific diagnostic modalities is urgent to differentiate TB and NTM and to prevent misdiagnosis.[8]

Non-tuberculous mycobacteria cases also face the problems of underdiagnosis and underreporting. NTM cases in most countries are not mandatory to be reported to public health authorities. Therefore, there is a lack of epidemiologic data on NTM cases, especially in Indonesia. Currently, the data rely on independent surveillance or microbiology laboratory-based results, which don't represent the actual number of the incidence.[9] A recent publication mentioned identification of significant pathogenic NTM among presumptive TB patients, where most isolates were identified as *M. abscessus*, *M. chelonae*, *M. fortuitum* group and MAC (*M. avium* complex).[10]

Within this study, the diagnostic performance of a new multi-target and rapid molecular assay, which is based on a universal lateral flow assay to visualize the amplification results (PaxView® TB/NTM MPCR-ULFA Kit), was assessed. This assay was designed to detect two specific genes of *Mycobacterium tuberculosis* (MTB): insertion element IS6110 and the *mtp40* gene, and another target in the *rpoB* gene of the *Mycobacterium* genus, by utilizing multiplex polymerase chain reaction (PCR) followed by visualization of the PCR amplicon on a universal lateral flow assay (ULFA) device. The NTM results, validated through both culture and MPCR-ULFA, underwent targeted sequencing utilizing the *hsp65* gene. This assay was evaluated in an operational condition in Dr. Hasan Sadikin Hospital, a provincial referral hospital located in Bandung City, West Java, Indonesia.

METHODS

Sputum samples were collected consecutively from subjects who came to the TB clinic in Hasan Sadikin General Hospital, Bandung, Indonesia, from 2019 to 2020. Patients (>18 years old) who came with one or more TB symptoms: cough for more than fourteen days, fever, chest or back pain, haemoptysis, weight loss, night sweat, or shortness of breath, were included in the study. Patients' data were recorded in a standard questionnaire, including demography, chief complaints, and prior TB treatment.

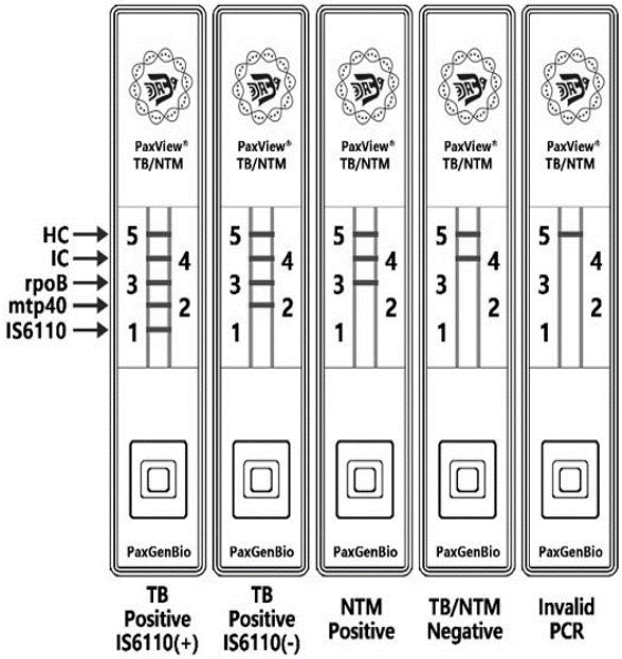
Two sputum samples were collected from each patient. The first sample was sent to the laboratory for Xpert MTB/RIF following a standard diagnostic procedure in the hospital, and the second sample was sent to the research laboratory for further microbiological work-up. Samples with insufficient volume (less than 0.5 mL) and those that contained more saliva were excluded from the study.

Mycobacteriology examinations were performed on the second sputum sample, which was subjected to smear microscopy, Mycobacterial culture, and MPCR-ULFA. Ziehl–Neelsen staining was applied for smear examination, with results graded according to the IUATLD scale. Following preparation, the sample was mixed with an equal volume of 4% NaOH, vortexed for one minute, and left to incubate at room temperature for 20 minutes. About 0.2 mL of the mixture was inoculated onto Ogawa solid medium and incubated at 37 °C. The remaining mixture was further processed for Deoxyribonucleic Acid (DNA) extraction and MPCR-ULFA. Every positive culture was subjected to the SD Bioline TB Ag MPT64 Kit (Abbott, Illinois, US) for the identification of *Mycobacterium tuberculosis* complex (MTBc).

Deoxyribonucleic acid extraction was performed on the remaining mixture as guided in the manufacturer's instructions. Briefly, 1 mL washing solution 1X PaxView® DNA Extraction Kit (PaxGenBio, Anyang, Gyeonggi, Republic of Korea) was added to the pellet and vortexed for 10 seconds, followed by centrifugation at 19,000 × g for 3 minutes. The supernatant was removed, and 100 µL of elution buffer was introduced. After mixing for 10 seconds, the sample was incubated at 95 °C for 15 minutes, centrifuged at 19,000 × g for 3 minutes, and the DNA-containing supernatant was collected for amplification.

At the DNA amplification stage, 10 µL of 2X PCR premix, 5 µl of primer mix, and 5 µl of DNA template with the PaxView® TB/NTM MPCR Kit (PaxGenBio, Anyang, Gyeonggi, Republic of Korea). The kit included positive as well as negative controls. The conventional PCR program began with a pre-denaturation step at 50 °C for 4 minutes, followed by a denaturation step at 95 °C for 10 minutes. The program continues with 25 cycles consisting of denaturation at 95 °C for 15 seconds and annealing at 71 °C for 1 minute. Then, 20 additional cycles of denaturation at 95 °C for 15 seconds, annealing at 60 °C for 30 seconds, and extension at 72 °C for 30 seconds were performed. The reaction concluded with a final extension at 72 °C for 1 minute. An amount of 5 µl of the PCR products was loaded into the sample well on the ULFA device, this was followed by the addition of 50 µl Running Buffer. After five minutes, 50 µl of Washing Buffer was added to each well.

The visualization of the amplicon on the test strip could be seen after five to fifteen minutes, which was performed on a universal lateral flow assay kit, PaxView® ULFA Kit (PaxGenBio, Anyang, Gyeonggi, Republic of Korea). The test strip consisted of five lines, including IS6110, *mtp40* gene, *rpoB* gene, internal control, and hybridization control. If bands 1 and/or 2 (with or without band 3) on the detection strip signify an MTB-positive result. If only band 3 is visible, the sample is classified as NTM-positive. The appearance of bands 4 and 5 without bands 1–3 indicates an MTB/NTM-negative result, while the presence of band 5 alone denotes an invalid test, suggesting that amplification failed due to template or reagent issues. The visualization of the results is presented in Figure 1.



Note: HC (band 5) = hybridization control; IC (band 4) = internal PCR control; rpoB gene (band 3) = mycobacteria marker; mtp40 gene and IS6110 (bands 2 and 1) = MTB markers.

Figure 1. Visualization of TB/NTM PaxView® ULFA Kit Results

All samples that showed NTM results in culture were subjected to targeted sequencing using the *hsp65* gene. The following *hsp65* primers were used: forward primer (5'-ACCAACGATGGTGTGTCCAT-

3') and reverse primer (5'-CTTGTCGAACCGCATACCCT-3'). Previously, all samples were amplified with PCR cycles of initial denaturation (95°C; 5 minutes), 35 cycles of denaturation (94°C; 30 seconds), annealing (57°C; 30 seconds), and elongation (72°C; 1 minute), followed by a final elongation at 72°C for 10 minutes.[7] All the amplicons were subjected to single-pass Sanger sequencing. The sequence results were then analyzed using BioEdit and blasted to the database of the National Center for Biotechnology Information (NCBI) to determine the NTM species of each sample.

After exclusion of samples with insufficient volume and incomplete patients' data, the positivity rates of solid culture, smear microscopy, Xpert MTB/RIF and PaxView® MPCR ULFA were expressed as percentage (%) for each TB and NTM group. The accuracy of the test was determined through calculations of sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV), each with 95% confidence intervals, using a composite reference standard (CRS). Positive CRS was determined as positive by any TB culture, Xpert MTB/RIF, and/or smear microscopy. The contamination result was excluded from the analysis.

Characteristics of the MPCR-ULFA test were assessed. Group differences were analysed using the Chi-square test, with statistical significance defined as $P<0.05$. Concordance between MPCR-ULFA and GeneXpert MTB/RIF Assay, as two molecular-based diagnostic assays at the same level, was measured using Cohen's Kappa analysis. All analyses were conducted in SPSS version 20.

The protocol for this study received approval from the Ethics Committee of the Faculty of Medicine, Universitas Padjadjaran (no. 504/UN6.KEP/EC/2019). All the participants in this study were given consent to be included in this study according to approved ethics.

RESULTS

A total of 950 subjects met the inclusion criteria and submitted sputum for this study. Twenty-six samples were excluded due to either insufficient volume for DNA extraction or incomplete patient data. Culture was contaminated in 4.8% of samples, leaving 879 samples for further analysis (Figure 2).

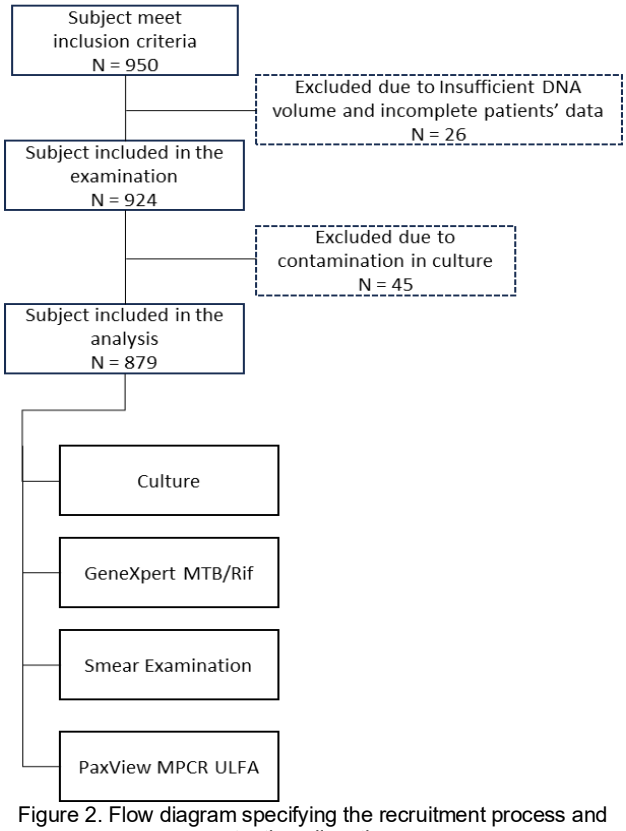


Figure 2. Flow diagram specifying the recruitment process and testing allocation

More than half of the patients recruited were male (52.9%) with a median age of 43.0 (IQR=31-57). Previous TB treatment was reported in 53.7% patients who were later grouped as multi-drug resistant (MDR) suspects. The majority of patients came with a cough for more than fourteen days (61.9%) and weight loss (41.1%). Other chief complaints included short breath (32.1%), fever (25.7%), and night sweat (26.3%). Some patients reported chest/back pain (20.1%) and hemoptysis (10,5%).

The positivity rates of MPCR-ULFA (43.0%; 95% CI=39.7-46.2%) were significantly higher than culture (32.8%; 95% CI=29.7-35.8%; $P=0.00001$) and smear microscopy (26.3%; 95% CI=23.4-29.1%; $P=0.00001$). Positivity rate of MPCR-ULFA was comparable with Xpert MTB/RIF, which was performed from different sputum samples (43.5%; 95% CI=40.2-46.7%; $P=0.96$), as shown in Table 1.

Table 1. Positivity rate of different tests for detecting tuberculosis in all samples (n=879)

Diagnostic Test	Positivity Rate (%)	95% CI
Culture	32.8%	29.7-35.8
PaxView MPCR ULFA	43.0%	39.7-46.2
Smear Examination	26.3%	23.4-29.1
GeneXpert MTB RIF Assay	43.5%	40.2-46.7

Figure 3 shows the number of positive cases in all diagnostic tests used in this study on two groups, MDR TB suspects (n=472) and TB suspects (n=407). Patients with previous TB treatment (MDR TB suspects) were more bacteriologically proven by any test compared to patients without a history of TB (TB suspects). This is seen in the positivity rate for culture (45.1%), PaxView® MPCR ULFA (58.5%), smear examination (39.4%), and Xpert MTB/RIF assay (68.8%) of the previously treated TB group higher compared than the positivity rate for culture (18.4%), PaxView® MPCR ULFA (25.6%), smear examination (11.1%), and Xpert MTB/RIF assay (18.7%) of the new TB group. As MPCR-ULFA is a molecular-based diagnostic test, similar to GeneXpert MTB/RIF Assay, the Cohen's kappa agreement of the two assays was calculated and resulted in a value of 0.7428 (87.3% of agreement), which can be concluded as substantial agreement.

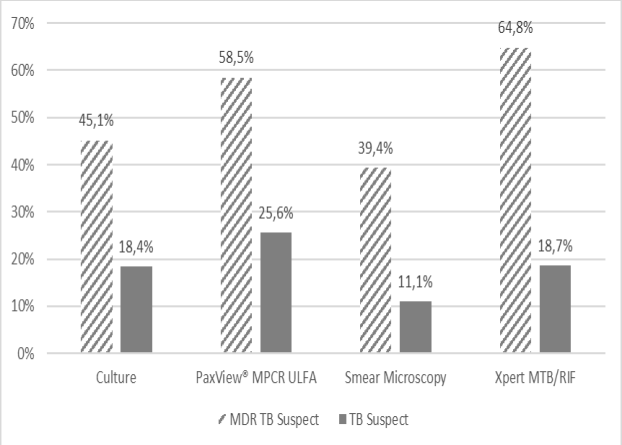


Figure 3. Comparative positivity rate of various TB diagnostic tests

Diagnostic performance of MPCR-ULFA was assessed using CRS, which included any TB test that showed positive results (culture, AFB smear microscopy, and GeneXpert MTB/RIF). While culture is the traditional gold standard, its sensitivity is known to be suboptimal in paucibacillary cases. By utilizing a CRS, aimed to capture TB cases that might have been missing by culture due to low bacterial load or the presence of non-viable bacilli that molecular assays like GeneXpert MTB/RIF and smear can still detect. Relying solely on culture would potentially decrease the specificity of MPCR-ULFA. The sensitivity of MPCR-ULFA indicated a value of 83% (95% CI=80.5-85.5%) with a fairly high specificity value at 92.1% (95% CI=90.3-93.9%) (Table 2).

Table 2. Performance of PaxView® MPCR ULFA compared with the reference standard (n=879)

PaxView® MPCR ULFA	Reference Standard*	
	Positive	Negative
Positive	342	37
Negative	70	430
Sensitivity (CI)	= 83.0% (80.5-85.5)	
Specificity (CI)	= 92.1% (90.3-93.9)	
PPV (CI)	= 90.2% (88.3-92.2)	
NPV (CI)	= 86.0% (83.7-88.3)	

Note: *Reference Standard stated as Culture, Smear Examination, and GeneXpert MTB/Rif Assay

PaxView® MPCR ULFA used a combination of two targets, IS6110 and the *mtp40* gene, to detect MTB, which maybe could be the cause of the relatively higher sensitivity result. The sensitivity of both targets showed 25.02% and for IS6110 only showed 18.20%.

Thirty-seven patients were positive only by MPCR-ULFA. Among them, fourteen patients had a history of TB treatment, with the majority of them coming with more than one clinical sign and symptom of TB. Four patients, who were further examined by the line probe assay (GenoscholarTM NTM+MDRTB II (NIPRO, Japan) in different studies, showed mixed results of TB and non-tuberculous mycobacteria. Twenty-three non-MDR patients showed a very mild disease or without any sign and symptoms of TB. They were tested for TB either because of a regular medical check-up or they were referred to TB examinations by indication from the clinician (Chest X-Ray abnormality).

The positivity rates of NTM incidences were analysed based on MPCR-ULFA-confirmed NTM cases, validated with Mycobacteria Other Than Tuberculosis (MOTT) culture identification using the MPT64 antigen test, and supplemented with results solely from MOTT culture. In this study, a total of 23 confirmed NTM-positive cases were identified,

constituting 2.6% of the study population (95% CI=1.5-3.6%). Among these cases, 10 samples (43.47%) exhibited concordant results between culture and MPCR-ULFA. Within this subset, 7 were identified as NTM rapid growers, 2 as NTM slow growers, and a *M. tuberculosis* mutant was identified through targeted sequencing of the *hsp65* gene.

Additionally, 9 samples out of the 23, which tested negative by MPCR-ULFA, were identified as MOTT by culture. This group included 6 NTM rapid growers, 2 NTM slow growers, and a *M. tuberculosis* mutant. Notably, 4 samples were identified as false TB positives by MPCR-ULFA, despite being characterized as rapid growers, slow growers, and 2 *M. tuberculosis* mutants by culture (refer to Table 3).

Table 3. NTM Positivity rate and identification based on *hsp65* sequence analysis

Culture Results	MPCR-ULFA Results	Species Identification based on *hsp65 sequence analysis*
Mycobacteria Other Than Tuberculosis (MOTT) (n=23)	NTM Positive (n=10)	a. Rapid Grower - *M. abscessus* (n=1) - *M. chelonae* (n=2) - *M. fortuitum* complex (n=1) - *M. fortuitum* (n=3) b. Slow Grower - *M. avium* complex (n=2) c. *M. tuberculosis* mutant (n=1)
	TB/NTM Negative (n=9)	a. Rapid Grower NTM - *M. abscessus* (n=2) - *M. chelonae* (n=1) - *M. fortuitum* complex (n=2) - *M. fortuitum* (n=1) b. Slow Grower NTM - *M. celatum* complex (n=1) - *M. terrae* complex (n=1) c. *M. tuberculosis* mutant (n=1)
	TB Positive (n=4)	a. Rapid Grower NTM - *M. fortuitum* (n=1) b. Slow Grower NTM - *M. gordonae* (n=1) c. *M. tuberculosis* mutant (n=2)

Moreover, among the total of 32 samples identified with NTM by MPCR-ULFA alone, 22 samples (68.75%) were found to yield false positives, with 20 exhibiting negative results in culture and 2 testing positive for TB in culture.

DISCUSSIONS

Within this work, an investigation into how well PaxView® MPCR ULFA performed as a rapid test for the detection of TB in Bandung, Indonesia. As a reference standard, we used CRS, including solid culture, smear microscopy, and GeneXpert MTB/RIF assay, which stated TB-positive results. CRS was used to maximize capturing true TB cases, reduce the likelihood of any false negative or positive results and enhance the overall validity of the evaluation as the PaxView® MPCR ULFA is a molecular assay that detects IS6110 and MPT40.

Sensitivity and specificity of PaxView® MPCR ULFA 83.0% and 92.1%, respectively. Calculation also the positivity rate for PaxView® MPCR ULFA and it displayed a significantly higher result (43.0%; 95% CI=39.7-46.2%), which was significantly higher than culture (32.8%; 95% CI=29.7-35.8%; *P*=0.00001) and smear microscopy (26.3%; 95% CI=23.4-29.1%; *P*=0.00001).

Early detection of TB is essential for the right regimen, especially in high-burden regions with a high number of local prevalence. This finding showed the local prevalence in the Bandung setting is 30%, which is in accordance with the previous data.[11] As for these findings, the previous TB treatment as TB suspect with a TB-positive result, but in the actual case, the patients could not get the proper TB regimen, as the diagnosis for TB suspect was only smear examination with lower sensitivity. The majority of TB-positive cases in this setting displayed a treatment history as MDR suspects. Rapid diagnostic tests are eventually needed for smear-negative cases and for increasing the turnaround time.

Moreover, the detection and identification of NTM in Indonesia is also hard to do daily, as it's not included in the routine testing of health services. The symptoms of NTM lung infection are similar to pulmonary TB, and currently, the diagnosis only relies on patients' characteristics that relate to the

prognosis of NTM infection and culture results. The right regimen for both infections is completely different and requires a rapid diagnostic.[12]

Based on previous studies by Saptawati et al, the prevalence of NTM in Surakarta is 15%, which is significantly higher than these findings (2.6%; 95% CI=1.5 -3.6%).[13] Although this data didn't represent the actual distribution of NTM, it still illustrates the increase in NTM incidences. In this study, founded that most of the NTM pulmonary infections were due to pathogenic rapid-growing *non-tuberculous* mycobacteria (RGM) such as *M. abscessus, M. fortuitum* group, and *M. chelonae*, followed by pathogenic slow-growing mycobacteria (SGM) such as MAC (*M. avium* complex). Non-pathogenic species were also found, that is, *M. terrae* complex, and *M. gordonae,* which common to be considered as contaminants. However, some studies also reveal that these microorganisms can lead to clinically important diseases in people regardless of whether they are immunocompetent or immunosuppressed.[10]

Currently, the approved rapid molecular diagnostic test used in reference laboratory settings is GeneXpert MTB/RIF Assay.[3,14] GeneXpert MTB/RIF Assay is known for high sensitivity (95%; CI 95%=90-97%) and specificity (98%, CI 95%, 97-99%).[14,15] This finding showed substantial agreement (Cohen's kappa agreement = 0.7428) in terms of positivity rate between PaxView® MPCR-ULFA and GeneXpert MTB/RIF.

Other than GeneXpert MTB/RIF, current rapid diagnostic molecular tests are being developed, such as EasyNAT TB, FluoroType MTB, and PURE-LAMP.[3] The performance of PaxView® MPCR ULFA is also quite comparable with these new rapid diagnostic molecular tests. EasyNAT TB, a platform based on isothermal nucleic acid amplification assay, reported sensitivity for MTB detection 84.1% and 66.7%, and specificity 97.8% and 100% in several studies.[6,16] However, the sensitivity declined when assessed solely in smear-negative yet culture-positive pulmonary TB cases, indicating the need for additional evaluation in a larger cohort.[16]

Another study using a rapid molecular diagnostic test based on Real-Time PCR and the HyBeacon fluorescence technology, the FluoroType MTB assay, documented MTB detection sensitivities of 88.1% and 95.1% in respiratory tract specimens (100% for smear-positive, 84.6% and 56.3% for smear-negative), with specificities of 96.4% and 98.9%, respectively, when compared to culture.[17,18] Meanwhile, a study on the PURE-LAMP assay demonstrated an overall sensitivity of 70.67% and a specificity of 98.32%.[19]

The high sensitivity of PaxView® MPCR ULFA results comes from the combination of two target markers, IS6110 and the *mtp40* gene, which are used in the kit. These sequence targets have been used widely in PCR-based diagnostic tests for pulmonary and extrapulmonary TB. IS6110 is known as an insertion sequence of MTBc with a high copy number and insertion sites and the *mtp40* gene is known as a conserved gene found on MTB.[20,21] In general, the PaxView® MPCR ULFA is an easily accessible diagnostic kit featuring a straightforward workflow encompassing DNA extraction, PCR, and MPCR-ULFA visualization. The entirety of the diagnostic process can be completed within approximately 3 hours, which can be considered rapid diagnostics. Nevertheless, there may be a need for improvement in the detection of NTM.

LIMITATION

In this study, the authors did not collect extensive clinical data and chest X-ray, as in the first place only wanted to evaluate PaxView® MPCR ULFA performance head-to-head with the reference standard. Therefore, the 37 cases of PaxView® MPCR ULFA positive and reference standard negative could not be retrieved from the clinical data and chest X-ray. The lack of data on recent TB treatment at the time of specimen collection was due to extensive clinical data which are not gathered properly. The low volume of sputum also might be affectedthe culture results as the load of MTB is also probably low. The authors also did not use liquid culture as a reference standard, as it is currently unavailable in these settings. Sputum specimens for

GeneXpert used from different sputum and times might affect the results.

CONCLUSION

This study showed that despite its limitations, the PaxView® MPCR ULFA showed encouraging results for detecting MTB, particularly in countries with a high disease burden. The sensitivity and specificity of this kit are comparable to those of other molecular diagnostic modalities. The simple platform using test strips and simple visualization might be appropriately applied in poor-setting laboratories.

ACKNOWLEDGMENTS

The authors express gratitude to the Faculty of Medicine, Universitas Padjadjaran, for their support and facilitation of research activities within the institution. Appreciation is also extended to our colleagues for their assistance in completing the study and for the valuable discussions throughout the research period.

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported. The authors confirm that have no connections or participation with any institution or entity that could present a financial interest (including honoraria, educational funding, speaker bureau activities, memberships, employment, consulting roles, stock or equity ownership, expert testimony, or patent/licensing agreements), nor do hold any non-financial interests (such as personal or professional relationships, affiliations, perspectives, or beliefs) related to the topic or materials presented in this manuscript.

FUNDING

This work was supported by the 2018 KOICA CTS program and RPLK (2203/UN6.3.1/PT.00/2022) of Universitas Padjadjaran. Neither the design, execution, analysis, nor reporting of this research was affected by the funding organizations.

REFERENCES

1. WHO. Global tuberculosis report 2024 [Internet]. 2025 [cited 2025 Sep 25]. Available from: https://cdn.who.int/media/docs/default-source/global-tuberculosis-report-2024/global-tb-report-2024_factsheet.pdf?sfvrsn=fee1c310_4&download=true
2. Ministry of Health. Ministry of Health [Internet]. 2020 [cited 2022 Jan 15]. The Republic of Indonesia Joint External Monitoring Mission for Tuberculosis. Available from: https://www.tbindonesia.or.id/
3. Eddabra R, Ait Benhassou H. Rapid molecular assays for detection of tuberculosis. Pneumonia. 2018;10:4.
4. van Kampen SC, Susanto NH, Simon S, Astiti SD, Chandra R, Burhan E, et al. Effects of Introducing Xpert MTB/RIF on Diagnosis and Treatment of Drug-Resistant Tuberculosis Patients in Indonesia: A Pre-Post Intervention Study. PLoS One. 2015;10(6):e0123536.
5. Handayani D, Haryanto B, Adyasiwi G, Dahlan MS, Agustin H, Wardoyo MP, et al. Diagnostic Accuracy and Clinical Utility of InaTB-Rif, Locally Developed Molecular Test for Tuberculosis, in Comparison with Xpert MTB/RIF in Indonesia. Jurnal Respirologi Indonesia. 2025;45(2):136–43.
6. Ou X, Song Y, Zhao B, Li Q, Xia H, Zhou Y, et al. A multicenter study of Cross-Priming Amplification for tuberculosis diagnosis at peripheral level in China. Tuberculosis. 2014;94(4):428–33.
7. Heidari L, Dehbidi GR, Farhadi A, Kashkooli GS, Zarghampoor F, Namdari S, et al. Simultaneous detection and differentiation of Mycobacterium tuberculosis and nontuberculous mycobacteria coexisting in patients with pulmonary tuberculosis by single-tube multiplex PCR. Iran J Microbiol. 2023;15(3):401–7.
8. Gomathy NS, Padmapriyadarsini C, Silambuchelvi K, Nabila A, Tamizhselvan M,

Banurekha VV, et al. Profile of patients with pulmonary non-tuberculous mycobacterial disease mimicking pulmonary tuberculosis. Indian Journal of Tuberculosis. 2019;66(4):461–7.

9. Park SC, Kang MJ, Han CH, Lee SM, Kim CJ, Lee JM, et al. Prevalence, incidence, and mortality of nontuberculous mycobacterial infection in Korea: a nationwide population-based study. BMC Pulm Med. 2019;19(1):140.
10. Pascapurnama DN, Zavitri N, Koesoemadinata R, Cahyadi AI, Chaidir L. Identification of Significant Pathogenic Nontuberculous Mycobacteria Species from Presumptive TB Patients Using Partial hsp65 Gene Sequencing. Infect Drug Resist. 2023;16:6923–30.
11. Rewata L, Rutherford M, Apriani L, Janssen W, Rahmadi A, Parwati I, et al. Improving diagnosis of pulmonary tuberculosis among HIV/AIDS patients: literature review and experience in a teaching hospital in Indonesia. Acta Med Indones. 2009;41(Suppl 1):57–64.
12. Saptawati L, Mashuri YA, Suryawati B, Harsono, Pradiptakirana R. Risk Factors and Prognosis of Nontuberculous Mycobacteria Infection in a High Prevalence of Tuberculosis Setting. Journal of Epidemiology and Public Health. 2020;5(1):79–87.
13. Saptawati L, Kusumo H, Suryawati B. Prevalence of Non-tuberculous Mycobacteria (NTM) in Surakarta, Indonesia: Higher Than Expected. KnE Life Sciences. 2019;4(12):132–8.
14. Pandey P, Pant ND, Rijal KR, Shrestha B, Kattel S, Banjara MR, et al. Diagnostic Accuracy of GeneXpert MTB/RIF Assay in Comparison to Conventional Drug Susceptibility Testing Method for the Diagnosis of Multidrug-Resistant Tuberculosis. PLoS One. 2017;12(1):e0169798.
15. Sah AK, Joshi B, Khadka DK, Gupta BP, Adhikari A, Singh SK, et al. Comparative Study of GeneXpert MTB/RIF Assay and Multiplex PCR Assay for Direct Detection of Mycobacterium tuberculosis in Suspected Pulmonary Tuberculosis Patients. Curr Microbiol. 2017;74(9):1026–32.
16. Mhimbira FA, Bholla M, Sasamalo M, Mukurasi W, Hella JJ, Jugheli L, et al. Detection of Mycobacterium tuberculosis by EasyNAT Diagnostic Kit in Sputum Samples from Tanzania. J Clin Microbiol. 2015;53(4):1342–4.
17. Eigner U, Veldenzer A, Holfelder M. Evaluation of the Fluorotype MTB Assay for the Rapid and Reliable Detection of Mycobacterium Tuberculosis in Respiratory Tract Specimens. Clin Lab. 2013;59(9–10):1179–81.
18. Hofmann-Thiel S, Hoffmann H. Evaluation of Fluorotype MTB for detection of Mycobacterium tuberculosis complex DNA in clinical specimens from a low-incidence country. BMC Infect Dis. 2014;14:59.
19. Ou X, Li Q, Xia H, Pang Y, Wang S, Zhao B, et al. Diagnostic Accuracy of the PURE-LAMP Test for Pulmonary Tuberculosis at the County-Level Laboratory in China. PLoS One. 2014;9(5):e94544.
20. Sinha P, Prakash P, Patne SCU, Anupurba S, Gupta S, Srivastava GN. Performance of nested multiplex PCR assay targeting MTP40 and IS6110 gene sequences for the diagnosis of tubercular lymphadenitis. Journal of Microbiology. 2017;55(1):63–7.
21. Gholoobi A, Masoudi-Kazemabad A, Meshkat M, Meshkat Z. Comparison of Culture and PCR Methods for Diagnosis of Mycobacterium tuberculosis in Different Clinical Specimens. Jundishapur J Microbiol. 2014;7(2):e8939.