Evaluation of Multiplex PCR–Universal Lateral Flow Assay for Pulmonary Tuberculosis Diagnosis: Comparison with Xpert MTB/RIF

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Abstract

Background: Global initiatives to reduce the impact of pulmonary tuberculosis (TB) depend on effective laboratory methods for detecting *Mycobacterium tuberculosis* complex (MTBC) in patient. The Multiplex PCR combined with Universal Lateral Flow Assay (MPCR-ULFA) provides molecular-based diagnostic approach that replaces gel electrophoresis with a universal lateral flow assay. **Objective:** This study purposed to assess diagnostic accuracy of MPCR-ULFA in comparison to Xpert MTB/RIF for detecting TB in suspected pulmonary TB patients. **Methods:** We compared the performance of Xpert MTB/RIF and MPCR-ULFA in TB detection at a total of 217 sputum samples from Malang isolates. We evaluate the diagnostic accuracy parameters, such as sensitivity, specificity, likelihood ratio, positive and negative predictive value, accuracy, and Youden index. To measure the agreement between Xpert MTB/RIF and MPCR-ULFA we evaluate the Kappa index. **Results:** The study results showed that MPCR-ULFA had a sensitivity and specificity of 95.74% and 93.53%, respectively. The positives likelihood ratio (LR+) was 14.80 and LR- was 0.05. Positives predictive value (PPV) was 80.36%, and the negatives predictive value (NPV) was 98.80%. The test also demonstrated an accuracy of 93.54%, a Youden index of 0.89, and a Kappa index of 0.835. **Conclussion:** By detecting multiple target genes, specifically IS6110 and mtp40, MPCR-ULFA enhances MTBC detection in sputum samples and offers a more cost-effective technique.

Keywords: Pulmonary TB, multiplex-PCR, ULFA, molecular diagnostic, diagnostic performance

INTRODUCTION

Mycobacterium tuberculosis complex (MTBC) as the causative agent of Tuberculosis (TB) has a very ancient origin that has survived for more than 70,000 years. Hippocrates described this disease by the name *phthisis*, which means consumption, while others known it as white plague, referring to the pallor of patient. For thousands of years, tuberculosis (TB) has been a significant infectious disease that affects humanity and continues to result in a large number of deaths even in the modern era.[1,2] World Health Organization (WHO) on Global TB Report highlights an alarming increase of new TB cases from 7.5 million in 2022 to 8.2 million during 2023, marking the highest figure since WHO began monitoring TB in 1995. WHO also reported that 87% of the global TB burden came from 30 low- and middle-income countries, whereas five countries: India, Indonesia, China, the Philippines, and Pakistan, responsible for 56% of cases. Indonesia alone accounts for 10% world's total TB cases and become the second-highest country of TB cases. This crisis situation emphasized the need for countries to fulfill their commitments to combat TB effectively, including implementation of good TB diagnostic tool.[3,4]

Disparities between the estimated number of persons developed TB and those who are officially reported remain challenging for decades. Thus, both the time required and the accuracy

of diagnostic tools are crucial for the early detection of TB in communities. Microscopic smear examination has low sensitivity, while culture have long turnaround times.[5,6] In 2008, WHO recommended the using of Xpert MTB/RIF (Cepheid, Sunnyvale, California) as the first diagnostic tool for individuals assumed to have multidrug-resistant TB (MDR-TB) or in patient with HIV-associated TB. The advantages of Xpert MTB/RIF include detect TB within 2 hours, identifying cases that routine testing might miss, and it can detect the rifampicin resistance. Compared to conventional culture, Xpert MTB/RIF sensitivity and specificity are 100% and 99.5%, respectively.[7] Nevertheless, implementing advanced diagnostic tools such as Xpert MTB/RIF in a resource-limited country poses significant challenges. Other than the need for specialist staff, a continuous power supply, and temperature stability, the expensive and exclusive machine and cartridges cannot be afforded without international financial support. As of 2018, rapid molecular diagnostics were used in only 12% of cases.[5,8] This situation triggered the search for other methods that had an accuracy level close to Xpert MTB/RIF but at a more cost-effective to reduce countries' dependence on global aid.

However, since Xpert MTB/RIF was introduced, molecular-based diagnostic methods have been increasingly used and developed. PCR become the cheapest molecular diagnostic tool but it needs gel electrophoresis method, which are considered impractical in clinical setting. PaxView MPCR-ULFA Kit (PaxGenBio, Korea) able to detects MTB in short time and differentiates it from NTM (Non-Tuberculosis Mycobacterium) without the need for gel electrophoresis after PCR. This kit uses multiple genes target, named IS6110 and mtp40. After MPCR procedure performed, product identities are confirmed based on DNA-DNA hybridization using a universal lateral flow assay (ULFA) on a nitrocellulose membrane.[8,9] Therefore, this study aimed to assess diagnostic accuracy of MPCR-ULFA Kit in detecting MTB in clinical specimens from pulmonary TB suspected patients and to compare its results with the Xpert MTB/RIF result.

METHODS

Study design and population

This is an analytic cross-sectional study whereas data were collected from enrolled patients from July 2019 to December 2019 at four health centers, namely RSUD Kanjuruhan Kepanjen Kabupaten Malang, RST dr. Soepraoen Malang, RSUD Lawang, and Puskesmas Rampal Celaket Malang. Enrollment criteria included age >18 years, subject has been diagnosed as suspected pulmonary TB due to clinical symptoms (cough for at least 2 weeks, hemoptysis, fever, shortness of breath, chest pain, fatigue, loss of appetite, unexplained weight loss or night sweats), and produce collected sputum minimum volume of 2 ml. Subjects were excluded if they had received TB treatment already.

Specimen collection

Subject was instructed to collect sputum with minimum volume of 2 ml. Sputum was classified as a spot collection. Sputum was stored in the refrigerator at 2–8^{0}C.

Xpert MTB/RIF

Xpert MTB/RIF test was performed according to the manufacturer's recommendations (Cepheid, Sunnyvale, CA, USA). A sample reagent is added to sputum at a ratio of 2:1 or 3:1 for decontaminated sputum pellets. The mixture is incubated at room temperature for about 15 minutes to ensure proper processing. The treated sample is transferred into a single-use plastic cartridge that contains all necessary reagents for the assay. The cartridge is then loaded into the GeneXpert

instrument, which automates the subsequent steps of the assay. Results are generated within approximately two hours and are automatically interpreted by the supporting software.

Xpert MTB/RIF result interpretation

The results of the Xpert MTB/RIF test indicate whether MTBC is detected in the sample or not. In some cases, the result is "invalid," meaning the test must be repeated. If MTBC is detected, the results will also state whether resistance to RIF is detected, not detected, or indeterminate. Indeterminate means that the test could not accurately determine the resistance, so growth-based susceptibility testing to first-line TB drugs should be conducted. In this study, the Xpert MTB/RIF results are classified as TB positive if the results show MTBC positive regardless of the resistance to rifampicin category.

MPCR-ULFA

The MPCR-ULFA test performed on the remaining sputum specimen using the PaxView MPCR-ULFA Kit (PaxGenBio, Korea). The mixture of sputum and buffer was centrifuged at 13,000 rpm for approximately 3 minutes. The supernatant then discarded and the specimen washed once again. A 100 µl of elution buffer was added and then heated at 95°C inside water bath for 15 minutes. Following centrifugation, as much as 20 µl of the supernatant was transferred to a new tube and it was used as a template. PCR were performed following the protocol. After that, 5 µl of the PCR solution was added to the inlet of ULFA kit and immediately added by 50 µl of running buffer and 50 µl of washing buffer. Results were read within 15 minutes [9].

MPCR-ULFA result interpretation

The appearance of specific lines on the lateral flow assay indicates the presence of targeted pathogens. A line for IS6110 (band 1) or mtp40 (band 2) accompanied by band 3,4, and 5 confirms the presence of *M. tuberculosis* and called as “TB Positive”. The presence of line on band 3,4, and 5 indicate “NTM positive”. The absence of lines on band 1, 2, and 3 suggested “TB/NTM negative”, while the absence of line in band 4 considered as “invalid” (Fig.1). In this study, the MPCR-ULFA results are classified as TB positive if the results show MTBC positive from both the IS6110 positive group and the mtp40 positive group. Meanwhile, positive NTM results are considered TB negative.

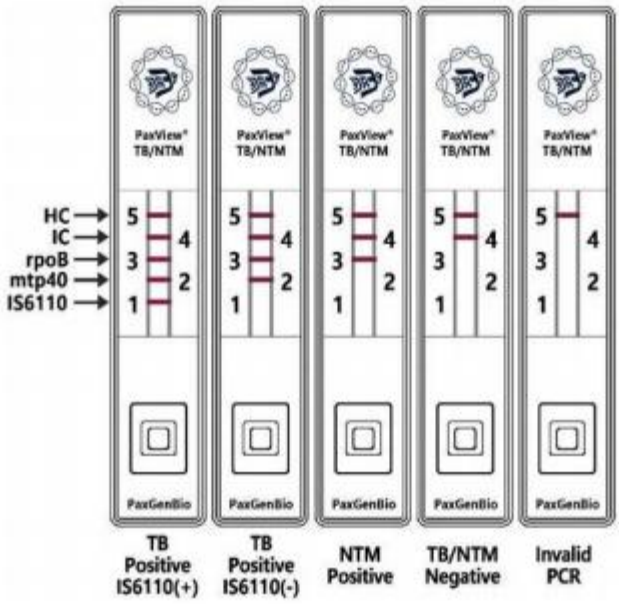

Figure 1. Interpretation of MPCR - ULFA Kit result. *M. tuberculosis* indicators bands : (band 1) IS6110 and (band 2) mtp40; mycobacteria indicator band : (band 3) rpoB band, IC: internal control band (band 4), and HC: hybridization control band (band 5). [9]

Statistical methods

Descriptive data are presented as frequencies (percentages). The results of the examination using Xpert MTB/RIF and MPCR-ULFA are presented in a 2x2 table. Diagnostic performance is analyzed through the calculation of Sensitivity, Specificity, Positives Likelihood Ratio, Negatives Likelihood Ratio, Positives Predictive Value (PPV), Negatives Predictive Value (NPV), accuracy, and Youden index. While Kappa value was measure to evaluate agreement between Xpert MTB/RIF and MPCR-ULFA in TB detection. Statistic data analysis was performed using SPSS 24.0 version.

Ethics

Ethical approve released by the Institutional Review Board of Universitas Brawijaya, under the project title "The diagnosis of pulmonary TB using MPCR-ULFA targeting IS*6110* and *mtp40*" (Number: 0208/EC/KEPK-PPDS/07/2019). This research is part of a multicenter study funded by KOICA, conducted in Malang, Surabaya, and Bandung. Some findings have been previously published.

RESULTS

A total of 217 samples were included in this study with gender distribution between male and female patient was 54.8% and 45.2%, respectively. The average age of the TB suspects was 51.4 ± 16.9 years, with patient age ranged from 18 to 85 years old (Table 1).

Table 1. Demographic distribution of suspected TB patient

	N	%
Sex		
Male	119	54.2
Female	98	45.8
Age		
18-30 y.o	33	15.2
31-45 y.o	45	20.7
46-60 y.o	68	31.3
>61 y.o	71	32.8
Mean (SD)	51.4 (± 16.9) years old	

As much as 47 samples (21.7%) were considered as positive with Xpert MTB/RIF. No samples were detected as rifampicin resistant. While 56 samples (25.8%) showed positive result with MPCR-ULFA. Five samples were considered as NTM using this test, but since this study purposed to detect the presence of MTBC, the results were included in "MPCR-ULFA negative" group (Table 2). Only 2 of the 56 positive samples were detected as mtp40 positive, while the rest were IS6110 positive.

Table 2. Test results of Xpert MTB/RIF and MPCR-ULFA from sputum of suspected TB patient

	Xpert MTB/RIF Positive	**Xpert MTB/RIF** Negative	Total
MPCR-ULFA Positive	45	11	56
MPCR-ULFA Negative	2	159	161
Total	47	170	217

Figure 2. MPCR-ULFA examination results

The Xpert MTB/RIF is a semiqualitative test with the concentration of MTB genes described as the Cycle threshold (Ct), and these results are grouped into low, medium and high (Table 3).

Table 3. Detail of Xpert MTB/RIF result

	Xpert MTB/RIF				
	Not detected	*Low*	*Medium*	*High*	Total
MPCR-ULFA Negative	159	1	1	0	161
MPCR-ULFA Positive	11	11	17	17	56
Total	170	12	18	17	217

Table 4 shows the results of the calculation of MPCR-ULFA performance in detecting MTBC in suspected pulmonary TB sputum with Xpert MTB/RIF as a reference.

Table 4. The diagnostic performance of MPCR – ULFA (Xpert MTB/RIF as reference)

	MPCR-ULFA	
	(%)	95% CI
Sensitivity	95.74	85.46-99.48
Specificity	93.53	88.72-96.73
Positives Likelihood Ratio (LR+)	14.80	8.33-26.29
Negatives Likelihood Ratio (LR-)	0.05	0.01-0.18
Positive Predictive Value (PPV)	80.36	2.44-7.33
Negative Predictive Value (NPV)	98.8	99.5-100.00
Accuracy	93.54	89.4-96.42
Youden Index	0.89	
Kappa value	0.835	

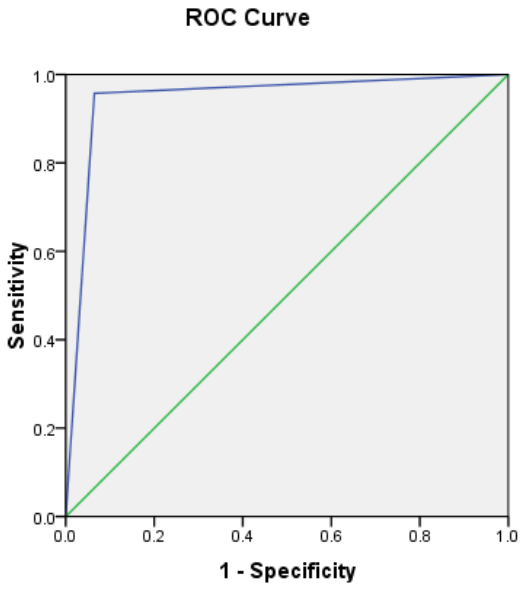

Figure 3. ROC curve of MPCR-ULFA and Xpert MTB/RIF (AUC = 0.946)

DISCUSSION

The strategies for screening tuberculosis (TB) cases in WHO Global Tuberculosis Report 2023 are designed to identify individuals at risk and facilitate early diagnosis and treatment. WHO advises that all patients, including children, who have an unexplained cough for two weeks or more, or who show unexplained abnormalities on chest X-rays suggestive of TB, should be assessed for tuberculosis. Additionally, it is necessary for all suspected TB cases to undergo a bacteriological examination to confirm the presence of TB. This examination includes analyzing smears from biological samples (such as sputum), conducting culture tests to identify *M. tuberculosis*, or using rapid diagnostic methods approved by the WHO, such as Xpert MTB/RIF [10,11]. Molecular-based identification has arisen as alternative and/or complement to conventional microbiological identification as it is more practical, faster and satisfactory accurate. Test based on nucleic acid amplifications (NAATs) offer precise and rapid identification without the need for complex biochemical tests, making them especially valuable for detecting slow-growing and fastidious microorganisms. [12–14]

Since 2010, WHO has stated that the use of molecular rapid test using Xpert MTB/RIF in TB detection is more important than conventional smear microscopy examination, sputum culture, or DST. Large-scale studies show that Xpert MTB/RIF examination has better sensitivity and specificity for TB diagnosis than microscopic examination and approaches the quality of culture examination. MTB culture in Lowenstein-Jensen medium is a time-consuming technique, which needs up to eight weeks to identify the bacteria, and an additional 4–6 weeks for detecting rifampicin resistance. The sensitivity and specificity of Xpert MTB/RIF for diagnosing adult pulmonary TB are 88% and 99%, respectively, while the sensitivity and specificity for detecting rifampicin resistance reach 95% and 98%.[15–17]

The introduction of the Xpert MTB/RIF marked a pivotal advancement in diagnosing TB and identifying rifampicin resistance worldwide. Nonetheless, it has been observed that the assay's performance falls short in certain contexts, notably in smear-negative and HIV-associated TB cases. These limitations underscore the need for continuous innovation and refinement in TB diagnostic methods to enhance accuracy and effectiveness, particularly in complex scenarios involving compromised immune systems or insufficient bacterial loads.[18] However, requirements for temperature control, specialist staffs, and sustainable power supply, the high costs of the machine and testing cartridges, become restriction of Xpert's installment in many countries [7]. In developing countries, the reagent cost of Xpert reaches 10 USD per test causing the needs of financial support, such as Global Fund, to implement it.[11]

Multiplex polymerase chain reaction (MPCR), introduced in 1988, was initially designed to identify deletions in the dystrophin gene. This technique allows for the simultaneous amplification of multiple DNA sequences in one reaction. Primer pairs must be carefully designed and optimized to function at a uniform annealing temperature. Additionally, the amplicon size, the length of their base pairs, must be different enough to form distinct bands when visualized by gel electrophoresis. [19] The newly-developed MPCR-ULFA Kit integrates multiple genes amplification using PCR and result-reading process by ULFA instead of electrophoresis procedures. ULFA simplify the process and significantly reduces the time required to read PCR products as well as eliminates the need for gel preparation and UV transillumination.[9]

In our study as much as 47 samples (21.7%) were considered as positive with Xpert MTB/RIF, while 56 samples (25.8%) showed positive result with MPCR-ULFA. Of the 170 samples that considered as MTBC not detected by Xpert MTB/RIF, 11 samples were detected as positive using MPCR -ULFA. While 2 samples were giving positive result by Xpert MTB/RIF but considered as negative using MPCR-ULFA. We performed the AFB smear microscopic examination on these discrepancy samples and obtained the agreement result with Xpert MTB/RIF. This is likely to happen because interference by non-target DNA in MPCR leading to false results.[20]

Although Xpert MTB/RIF is superior, MPCR-ULFA's ability to detect MTBC in sputum specimen is promising. This study showed MPCR-ULFA has a sensitivity of 95.74% and a specificity of 93.53%, which is also shown in the ROC curve with an Area of Under Curve (AUC) of 0.946 (Figure 3). The Positive Likelihood Ratio (LR+) and Negative Likelihood Ratio (LR-) are metrics used in diagnostic testing to quantify how much a test result shifts the probability of a disease being present or absent. Combined with sensitivity and specificity values, both provide clinically useful information about a test's performance. MPCR-ULFA showed LR + as much as 14.80 and LR- as much as 0.05. This value showed strong evidence to rule in/out TB in suspected patient (LR+>10 or LR- <0.1). In clinical settings, the actual prediction of disease is shown from the Positive Predictive Value (PPV) and Negative Predictive Value (NPV), where MPCR-ULFA has values of 80.36 (good rule-in value) and 98.8% (strong rule-out value, respectively). Accuracy measures the overall correctness of a diagnostic test by calculating the proportion of true positive (TP) and true negative (TN) of all test results. In this study, the MPCR-ULFA accuracy results were 93.54%. The Youden Index of MPCR-ULFA as much as 0.89 showed test's ability to balance sensitivity with specificity, where value closer to 1 is considered good. Kappa value (Cohen's kappa, κ) showed agreement between two methods, whereas in this study the agreement between Xpert MTB/RIF and MPCR-ULFA in TB detection with the value of 0.835 (kappa value over 0.75 categorized as excellent).

MPCR-ULFA targets IS6110 and mtp40 to detect the presence of MTBC. Insertion sequences (ISs) are one of the smallest independently transposable genetic elements. IS are prevalent in the MTBC genome and its transposition can lead to genome diversification, deletions, inversions, and duplications. The MTBC genomes contain at least 30 different IS elements, including IS6110. IS6110 DNA fingerprinting is a classical gold standard method widely used in molecular examinations. PCR targeting the IS6110 sequence has shown a sensitivity of 69.01%, which is higher than the 47.41% sensitivity of the MGIT culture. The presence of IS6110, which is considered exclusive to MTBC members, has enhanced the sensitivity of PCR in detecting both pulmonary and extrapulmonary TB. This sequence is important in term of MTB pathogenicity as it roles in transmission ability and the virulence of MTB strains.[12] In our result we found that 54 of 56 samples (96,4%) MPCR-ULFA positive were IS6110 positive, while the two rest showed mtp40 positive.

A limitation of using IS6110 is that some of *M. tuberculosis* strains lack this IS6110 sequence in their genome, rendering this method ineffective for their identification. Nevertheless, PCR targeting the IS6110 sequence has a sensitivity of 69.01%, which is higher than the 47.41% sensitivity of the MGIT culture.[16,21] The addition of genomic fragment amplification, such as mtp40, offers a more sensitive and more specific for early detection of *M. tuberculosis* strains in clinical specimens. This approach allows differentiation between *M. tuberculosis* and other

mycobacteria. This fragment is located in the plcA gene sequence and is exclusive to *M. tuberculosis,* making it particularly useful for detecting *M. tuberculosis* strains that lack the copies of IS6110 element. MPCR using IS6110 and mtp40 gene targets achieved 100% specificity for pulmonary TB and 93.9% for extrapulmonary TB. The high sensitivity of MPCR in detecting MTBC is attributed to its capability to detect target DNA in very small quantities, even in samples containing a large amount of non-target DNA.[8,19] Moreover, rpo-B gene located in band number 3 on the ULFA device is a common marker for Mycobacterium bacteria. If the test results show positive band 3 but are not accompanied by positive bands 1 or 2 can be considered as a possibility of Nontuberculous Mycobacterium (NTM) bacterial infection. This is useful for distinguishing TB infection from NTM, considering that both of which have similar symptoms but very different treatment regimens.[22]

Overall, MPCR-ULFA can be considered as the next molecular-based diagnostic method for TB with diagnostic accuracy close to Xpert MTB/RIF. The important advantage is that the test cost is only 10% of Xpert MTB/RIF, making it feasible for implementation in low-resource countries.[9] We did not perform sputum culture which is become the major limitation in this study since this examination is very important as a differentiator of performance between the two tests, especially in discordant cases. The use of AFB smear is greatly influenced by the examiner's skills, so it needs to be confirmed with culture. Moreover, this culture examination could detect the NTM as several studies reported the detection of NTM from clinical samples using MPCR.[23] Research from Raveendran and Wattal showed that out of 6 sample culture results with negative PCR results, two samples grew as NTM while the remaining four grew as MTB.[24] For future studies and work, a more comprehensive research design is needed that includes clinical data from patients, results of radiological examinations, culture testing and sequencing, as well as multicenter study with larger sample size to analyze the demographics impact. In addition, continuous development of the kit is necessary to improve its practicality in use, especially in the DNA purification stage.

CONCLUSSION

This study concluded that MPCR-ULFA has good diagnostic accuracy and is a promising alternative method for detecting MTB infection in low-resource settings. By multiple target genes detection, the sensitivity and specificity of MPCR-ULFA reach out 95.74% and 93.53%, respectively. The positives likelihood ratio (LR+) as much as 14.80 and LR- as much as 0.05. The PPV was 80.36% and NPV was 98.8%. The test also demonstrated an accuracy of 93.54%, and Youden index of 0.89. The agreement between two methods using Kappa index showed excellent value of 0.835. Additionally, MPCR ULFA has the advantage of being only 10% of the cost of the Xpert MTB/ RIF while maintaining good performance.

CONFLICT OF INTERESTS

Authors declare there is no conflict of interest. This study was supported by KOICA (Korea International Corporation Agency) through program named Creative Technology Solution and in collaboration with PaxGenBio, Republic of Korea.

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DECLARATION OF USING AI
This manuscript was created without assistance from AI tool.

AUTHOR CONTRIBUTION
PWA contributes as manuscript writer and corresponding author. AI contributes as manuscript reviewer and conceptor of this manuscript.

LIST OF ABBREVIATIONS
TB: tuberculosis; MTBC: *Mycobacterium tuberculosis complex*; MPCR-ULFA: Multiplex PCR - Universal Lateral Flow Assay; WHO: World Health Organization; PCR: Polymerase Chain Reaction; NTM: Non-tuberculosis mycobacterium; LR: Likelihood Ratio; PPV: positive predictive value; NPV: negative predictive value; IS: insertion sequence; ROC curve: receiver-operating characteristic curve; AUC: Area of Under Curve.

REFERENCES
1. Orgeur M, Sous C, Madacki J, Brosch R. Evolution and emergence of Mycobacterium tuberculosis. FEMS Microbiol Rev. 2024;48(2).
2. Barberis I, Bragazzi NL, Galluzzo L, Martini M. The history of tuberculosis: From the first historical records to the isolation of Koch's bacillus. J Prev Med Hyg. 2017;58(1):E9–12.
3. World Health Organization. Global Tuberculosis Report. Global tuberculosis report 2024. Geneva: World Health Organization; 2024.
4. Akbar PW, Rachma LN, Rahmah Z, Firdaus MI, Nanggali BV. Social support build resilence in multidrug-resistant tuberculosis patients: A cross-sectional study in MDR-TB patient support group. Jakarta: The Avicenna Medical Journal; 2024. p. 24–30.
5. Brown S, Leavy JE, Jancey J. Implementation of genexpert for tb testing in low- And middle-income countries: A systematic review. Glob Heal Sci Pract. 2021;9(3):698–710.
6. Winardi W, Wahyuni H, Hidayat M, Wirawan A, Nurwidya F, Uddin MN, et al. Challenges on tuberculosis care in health care facilities during COVID-19 pandemic: Indonesian perspective. Narra J. 2022;2(2):4–9.
7. Chaudhary R, Bhatta S, Singh A, Pradhan M, Srivastava B, Singh YI, et al. Diagnostic performance of genexpert MTB/RIF assay compared to conventional mycobacterium tuberculosis culture for diagnosis of pulmonary and extrapulmonary tuberculosis, Nepal. Narra J. 2021;1(2):1–5.
8. Iskandar A, Akbar PW, Aprilia A, Park Y kil. Comparison between the PaxView TB / NTM MPCR-ULFA Kit and current methods for Mycobacterium tuberculosis detection in Malang , East Java , Indonesia. 2021;25(5):4211–7.
9. Choo JH, Kim CK, Park YK. Detection of Mycobacteria in Clinical Samples by the Newly Developed PaxView TB/NTM MPCR-ULFA Kit. Asian J Biotechnol Bioresour Technol. 2020;6(3):11–7.
10. World Health Organization. Consolidated Guidelines On Tuberculosis Treatment. WHO consolidated guidelines on tuberculosis. Module 2: screening – systematic screening for tuberculosis disease. Geneva: World Health Organization; 2021. Licence: CC BY-NC-SA 3.0 IGO. 2024. 68 p.
11. Sharma S, Dahiya B, Sreenivas V, Singh N, Raj A, Sheoran A, et al. Comparative evaluation of

GeneXpert MTB/RIF and multiplex PCR targeting mpb64 and IS6110 for the diagnosis of pleural TB. Future Microbiol. 2018;13(4):407–13.
12. Chin KL, Sarmiento ME, Norazmi MN, Acosta A. DNA markers for tuberculosis diagnosis. Tuberculosis. 2018;113(June):139–52.
13. Procop GW. Laboratory Diagnosis and Susceptibility Testing for Mycobacterium tuberculosis. Microbiol Spectr. 2016;4(6):45–58.
14. Sali M, De Maio F, Caccuri F, Campilongo F, Sanguinetti M, Fiorentini S, et al. Multicenter evaluation of anyplex plus MTB/NTM MDR-TB assay for rapid detection of mycobacterium tuberculosis complex and multidrug-resistant isolates in pulmonary and extrapulmonary specimens. J Clin Microbiol. 2016;54(1):59–63.
15. Rocchetti TT, Silbert S, Gostnell A, Kubasek C, Widen R. Validation of a multiplex real-time PCR assay for detection of mycobacterium spp., mycobacterium tuberculosis complex, and mycobacterium avium complex directly from clinical samples by use of the BD max open system. J Clin Microbiol. 2016;54(6):1644–7.
16. Chitra A, Usharani B, Smita S, K. Vidya Raj C, Anbazhagi S, Muthuraj M. Diagnostic Performance of Polymerase Chain Reaction Targeting Insertion Sequence (IS6110) for the Detection of Extra Pulmonary Tuberculosis. Am J Infect Dis Microbiol. 2017;5(4):126–31.
17. Caulfield AJ, Wengenack NL. Diagnosis of active tuberculosis disease: From microscopy to molecular techniques. J Clin Tuberc Other Mycobact Dis. 2016;4:33–43.
18. WHO. WHO consolidated guidelines on tuberculosis. WHO Press. 2022. 98 p.
19. 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. J Microbiol. 2017;55(1):63–7.
20. Yu S, Yan L, Wu X, Li F, Wang D, Xu H. Multiplex PCR coupled with propidium monoazide for the detection of viable Cronobacter sakazakii, Bacillus cereus, and Salmonella spp. in milk and milk products. J Dairy Sci [Internet]. 2017;100(10):7874–82. Available from: http://dx.doi.org/10.3168/jds.2017-13110
21. Thabet S, Souissi N. Transposition mechanism, molecular characterization and evolution of IS6110, the specific evolutionary marker of Mycobacterium tuberculosis complex. Mol Biol Rep. 2017;44(1):25–34.
22. Akbar PW, Iskandar A, Astuti TW, Indriana K. Gambaran Infeksi Nontuberculous Mycobacteria (NTM) pada Penderita Suspek TB Paru Menggunakan Multiplex PCR (MPCR)-Universal Lateral Flow Assay (ULFA) Kit. J Kesehat Andalas. 2020;9(3):291.
23. Lodha L, Mudliar SR, Singh J, Maurya A, Khurana AK, Khadanga S, et al. Diagnostic Performance of Multiplex PCR for Detection of Mycobacterium tuberculosis Complex in Presumptive Pulmonary Tuberculosis Patients and Its Utility in Smear Negative Specimens. J Lab Physicians. 2022;14(04):403–11.
24. Raveendran R, Wattal C. Utility of multiplex real-time PCR in the diagnosis of extrapulmonary tuberculosis. Brazilian J Infect Dis. 2016;20(3):235–41.