Nasicha et al | Article 10094
J Pure Appl Microbiol. 2025;19(2):1134-1144. doi: 10.22207/JPAM.19.2.16
Received: 23 November 2024 | Accepted: 21 March 2025
Published Online: 21 May 2025

P-ISSN: 0973-7510; E-ISSN: 2581-690X

RESEARCH ARTICLE OPEN ACCESS

Xpert® MTB/RIF Ultra Accuracy in Pulmonary TB Diagnosis: Comparative Analysis with Mycobacterial Growth Indicator Tube 960 System

Arifatun Nasicha[1], Ni Made Mertaniasih[2,3,4]*, Eko Budi Koendhori[2,3], Pepy Dwi Endraswari[3,4], Ariani Permatasari[5] and Atika[6]

[1]Resident of Clinical Microbiology, Faculty of Medicine, Airlangga University, Surabaya, Indonesia.
[2]Department of Clinical Microbiology, Dr. Soetomo Hospital, Surabaya, Indonesia.
[3]Department of Clinical Microbiology and Parasitology, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia.
[4]Department of Clinical Microbiology, Universitas Airlangga Hospital, Surabaya, Indonesia.
[5]Department of Pulmonology and Respiratory Medicine, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia.
[6]Department of Public Health and Preventive Medicine, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia.

Abstract

Tuberculosis (TB) is an infectious illness induced by *Mycobacterium tuberculosis* (MTB), a principal cause of mortality globally. The incidence of TB in Indonesia is increasing every year. WHO recommends Xpert MTB/RIF Ultra, which is sensitive and specific, as an initial method of TB diagnosis that detects IS1081/IS6110 of the *Mycobacterium tuberculosis* complex (MTBC). The study intends to analyze the accuracy of Xpert Ultra with Mycobacterial Growth Indicator Tube (MGIT) 960 culture system of pulmonary TB in the Clinical Microbiology Laboratory of Dr. Soetomo Academic Hospital, Indonesia. This was an analytical, observational, and cross-sectional study that included 39 of 382 sputum samples that met the inclusion and exclusion criteria from suspected pulmonary TB patients. The result showed that 56.4% (22/39) were adults, and 41% were elderly (>60 years), 61.5% (24/39) were male, and 43% of them were smokers. Demographic factors, epidemiology, patients' clinical conditions, and chest x-ray patterns all have no significant value on suspected pulmonary TB cases ($p > 0.05$). Xpert Ultra sensitivity was 83.3%, specificity 82.5% and accuracy 84.6%. There are no statistical differences between the two diagnostic methods ($p = 0.687$, McNemar's). In conclusion, Xpert Ultra can diagnose pulmonary TB as well as MGIT 960 system culture. Although demographic factor, epidemiology, clinical symptoms, and also chest x-ray pattern cannot confirm the diagnosis of pulmonary TB, it is necessary to carry out confirmation tests under the guidelines.

Keywords: Tuberculosis, Xpert MTB/RIF Ultra, MGIT 960 Culture System, Chest X-ray

***Correspondence:** ni-made-m@fk.unair.ac.id

Citation: Nasicha A, Mertaniasih NM, Koendhori EB, Endraswari PD, Permatasari A, Atika. Xpert® MTB/RIF Ultra Accuracy in Pulmonary TB Diagnosis: Comparative Analysis with Mycobacterial Growth Indicator Tube 960 System. *J Pure Appl Microbiol.* 2025;19(2):1134-1144. doi: 10.22207/JPAM.19.2.16

INTRODUCTION

Tuberculosis (TB) is an infectious illness caused by *Mycobacterium tuberculosis* (MTB), and it is one of the top causes of death globally. The global TB incidence rate (new cases per 100,000 population) in 2023 was 134, a slight (0.2%) rise over 2022. The worldwide rise of the number of people falling ill with TB was 10.8 million in 2023, a slight rise from 10.7 million in 2022, while significantly exceeding 10.4 million in 2021 and 10.1 million in 2020.[1] There are 7,152 TB cases reported by the US Centers of Disease Control and Prevention (CDC) as of December 2024, led by California and New York.[2] Globally, Indonesia is placed second (10%) among the five nations with high-burden TB countries (India, Indonesia, China, Philippines, Pakistan) accounting for 56% of TB disease after India (26%).[1,3] Indonesia is targeting the detection of 1,035,000 tuberculosis (TB) cases by 2025.[4]

Bacteriological examination is one of the components of TB evaluation and is also very important for diagnostic enforcement. Several bacteriological examination methods carried out are acid-fast bacillus (AFB) staining, direct detection of MTB using Nucleic Acid Amplification Testing (NAAT) including Xpert MTB/RIF Ultra (Xpert Ultra), TB culture and drug susceptibility for TB diagnosis with a molecular approach.[5] AFB microscopy staining has varied sensitivity because it depends on the sputum time collection,[6] and it requires 5000-10,000 AFB/ml sputum for positive result.[7,8] AFB microscopy has low sensitivity, several reports have demonstrated the sensitivity of the staining varies between 18% and 38%.[6,9] In addition to AFB microscopy staining as a bacteriological examination of TB, a culture, which is the gold standard but consumes more time for TB detection, it needs several weeks to confirm. Therefore, for the initial diagnosis of TB, it is recommended to use molecular testing, one of which is Xpert MTB/RIF Ultra in accordance with WHO recommendations since 2017 in both children and adults.[10] Xpert Ultra is more sensitive in detecting low bacterial load of TB, and the process takes less than 80 minutes to get results.[11,12]

The Xpert Ultra detects *Mycobacterium Tuberculosis* Complex (MTBC) and rifampicin resistance simultaneously using IS1081/IS6110, which detects the presence of MTBC DNA in the specimen and amplifies specific sequences of the rpoB gene using rpo probes 1-4.[13] IS6110 and IS1810 are repeated mobile elements particular to the MTBC, frequently noted for their stability and typically comprising four to six copies within the MTBC genome. Due to this stability, several studies have used these genes as a NAAT target.[14,15] Xpert MTB/RIF Ultra offers much higher sensitivity than first-generation Xpert for negative acid-fast bacilli (AFB) smear but positive TB culture and HIV-associated TB, and it demonstrates increased accuracy for the detection of rifampicin resistance.[16] Xpert Ultra can detect with a minimal bacterial load of 12 cfu/ml[11]; the process takes less than 80 minutes. It also has a larger DNA amplification[12] reaction space to increase diagnostic accuracy and sensitivity (50 uL). However, culture is still used as the gold standard for TB diagnosis.[13] The results of the initial implementation of Xpert Ultra in Jakarta, which was carried out in 3 cities, showed that Xpert Ultra had a reasonably high examination suitability, the examination technique was the same as Xpert but had a shorter examination time of 80 minutes, the referral network from health facilities to the laboratory can be arranged using the current network arrangement mechanism, in addition, Xpert Ultra requires the exact cost as the Xpert examination.[17]

For MTBC detection can use solid media such as Lowenstein Jensen (LJ), Middlebrook 7H10 and 7H11, and can also use liquid culture with the Mycobacterial growth indicator tube (MGIT) 960 system, that contain 7H9 as the medium.[18] The MGIT 960 system is an autonomous, non-radiometric continuous monitoring system that serves as an alternative to radiometric methods for the growth and detection of Mycobacterium. The technique relies on fluorescence detection of mycobacterial growth in a tube and a fluorescence quenching-based oxygen sensor. It offers the benefits of high throughput speed within 4-13 days and facilitates easier result interpretation.[19,20] The liquid culture system detects most mycobacterial growth in 4-14 days compared to solid media, which takes 3-6 weeks.[5] Research by Lee *et al*. stated that automatic liquid culture provides twice as high and faster results than solid media.[21] To

identify MTB from MTBC, the rapid diagnosis of MPT64 antigen detection is performed. MPT64, a 24-kDa MTB protein, and a key pathogenic bacteria secretory protein, is frequently used as a diagnostic candidate protein. Capilia TB, immunochromatography (ICT), enzyme-linked immunosorbent assays (ELISA), SD Bioline, and other methods are available for detecting the MPT64 protein.[22-24]

The accurate method of establishing the diagnosis of pulmonary TB is determined by the suitability of the Xpert MTB/RIF Ultra positive results, which is MGIT 960, the gold standard reinforced with data on clinical manifestations of pulmonary TB, such as findings of specific pulmonary TB images on chest x-ray. Because of the discrepancy between Xpert Ultra results for initial pulmonary TB screening and the patients clinical symptoms in our hospital, and also there are difference result between first generation Xpert and TB culture, the study's objective is to compare the accuracy of Xpert Ultra to that of MGIT 960 culture in suspected pulmonary TB patients.

MATERIALS AND METHODS

This cross-sectional, analytical observational study aimed to determine how accurate the Xpert MTB/RIF Ultra (GXMTB/RIF-ULTRA-50, Cepheid, USA) technique was at diagnosing pulmonary TB compared to the MGIT 960 culture. This study was conducted between July and September 2024 at the Clinical Microbiology Laboratory, Dr. Soetomo Academic Hospital in Surabaya, Indonesia, a primary referral hospital in Indonesia. The total recorded sputum sent for examination was 382 specimens analyzed to identify MTBC by Xpert MTB/RIF Ultra (GXMTB/RIF-ULTRA-50, Cepheid, USA) from suspected pulmonary TB patients. All these samples were tested using cartridge Xpert MTB/RIF Ultra (Cepheid, USA). Out of 382 sputum samples, we found 39 purulent spontaneous sputum specimens from coughing and occasional blood streaks, with each having 4 ml volume and all patients had gone through chest x-ray exam. After the MGIT 960 culture (245122, BD, USA) was done, MTBC was detected, and a rapid MPT64 antigen test (08FK50, Abbot Bioline, Korea) was performed, which is specific to MTBC. We also analyzed other factors through patient electronic medical record data observing the demographic factors such as age and gender, then the patient's clinical conditions such as cough, fever, weight loss, shortness of breath, night sweats, as well as chest x-ray pattern such as the presence of infiltrates, consolidation, pleural effusion, cavities, and fibrosis on the Xpert Ultra positifity in the diagnosis of pulmonary TB. There are no age restrictions. The Ethical Committee of Dr. Soetomo General Academic Hospital has ethically approved this study (1041/KEPK/VII/2024).

Xpert MTB/RIF ultra method (Cepheid, USA)

From one volume of accurate sputum, divided into 2 ml each for Xpert Ultra and 2 ml for MGIT 960 culture system. The automated real-time polymerase chain reaction (PCR) investigation targeting IS1081 and IS6110 was performed according to the manufacturer's guidelines. For Xpert Ultra test, 2 ml sputum added with reagent following the specimen's volume (1:1), the mixture of sputum is shaken until it is homogenous.[25] Reagents can be added again if there are still visible clumps in the mixture. The mixture is left for 10 minutes at room temperature before open the specimen container. For all specimen handling steps performed at the biosafety cabinet (BSC) in biosafety-level 2 (BSL-2). After 10 minutes at room temperature, 2 ml of mixture sputum is added to the Xpert Ultra cartridge (Cepheid, USA) by pipette (up to the pipette line) to prevent bubbles that can cause errors. Then, the catridge was slowly closed and inserted into the GenXpert IV instrument. The results are interpreted using the GeneXpert Dx software 6.2 System Software, USA. Among the observed results are negative as MTB not detected, positive or MTB detected with sensitive rifampicin or resistant, and trace category that means detected MTB but has low detectable amount of MTB genetic material so that rifampicin result is indeterminate.[13,25]

MTB culture with MGIT 960 method

Decontamination and processing of the samples

Sputum from the same specimen, 2 ml, was also examined for MTB MGIT 960 liquid culture. Processing sputum specimens before culture must be done in a Biosafety Cabinet (BSC).

A 50 ml centrifuge tube (Falcon) was used to collect sputum, which was then diluted with a similar amount of Mycoprep (240862, BD, USA). This decontaminating preparation contained N-acetyl-L-cysteine-sodium hydroxide (NALC-NaOH) for the processing of mycobacterial specimens. Then, the vortex was inverted for 15-30 seconds, and the tube can be inverted to expose all tube parts to Mycoprep. Then added phosphate buffer saline (PBS) (pH 6.8) to produce the suspension's total amount of 45 ml after holding the tube for 15 minutes at room temperature, and by refrigerated centrifugation, the samples were then centrifuged at 3000 rpm for 15 minutes. Then, allow the tube to remain in place for five minutes to permit the aerosol to dissipate. Subsequently, the supernatant is discarded, leaving the sediment in the falcon tube. PBS (1-2 milliliter) is then added to the sediment.[18]

Detection of mycobacterial growth

A suspension containing a combination of the MGIT growth supplement (245124, BD, USA) and the antibiotic mixture PANTA (245144, BD, USA) as much as 0.8 ml was added to the MGIT tube (245122, BD, USA) for liquid culture. After that, half a milliliter of the concentrated and decontaminated sputum specimen suspension was added to the MGIT tube and thoroughly mixed. After the tube is closed, then inverted several times to mix the suspension evenly. The incubation is carried out until a positive sign is obtained in the form of a red light alarm as a sign of MTB growth, or a negative in the form of a green light is a sign of no MTB growth. If a positive result is obtained, the tube was removed from the machine, and then an acid-fast smear check and gram staining are carried out.[18] Tiny cords or clumps that formed at the tube's bottom during agitation are indicative of mycobacterial growth, whereas uniform turbidity throughout the tube indicates contamination.[20]

MTBC was identified by a rapid diagnostic test of the MPT64 antigen

After Mycobacterium that detected from MGIT 960 culture system, followed by immunochromatographic technique as rapid diagnostic testing using the MPT64 kit (08FK50, Abbott Bioline, Korea) that examined for the presence of the MPT64 antigen. A droplet of the positive culture is placed on the lateral flow strip. The inoculated immunochromatographic tests (ICT) cassettes were maintained at room temperature and inspected after 15-20 minutes. Two regions are called C as a control and T as a test. If the band in the T area is the same as in the C area, it is said to be a positive MPT64 test. MPT64 antigen negative is characterized by the presence of banding in C but absent in T region. No band in 'C' region was interpreted as an invalid test.[22,26]

RESULTS

We gather a 4 ml purulent with blood streak occasionally sputum specimen from suspected pulmonary TB patients as inclusion criteria, if no chest x-ray is obtained from the patient medical record, it will be excluded from this study. Out of 382 sputum specimens sent to the Clinical Microbiology Laboratory, 39 accurate spontaneous sputum samples were examined by Xpert Ultra that met the inclusion and exclusion criteria, and the remaining samples are excluded because the 343 samples had volumes less than 4 ml and the quality was not guaranteed in this study. The results of the study are displayed as a table (Table 1) containing percentage data, which were analyzed Chi-square test in the SPSS 26 application. Statistical significance was set at $p < 0.05$ significant. A total of 2.6% (1/39) were children (0-18 years), 56.4% (22/39) were adults (19-59 years) and 41% (16/39) were elderly (>60 years). The mean age was 54 (±14.6 SD), and 61.5% (24/39) of them were male. There were 10.8% with HIV co-infection. Most of the patients, 84.6% (33/39) were newly diagnosed pulmonary TB patients, 10.3% were relapsed, and 5.1% were withdrawn.

Most patients' clinical symptoms are productive cough 35 (89.7%), breathlessness 27 (69.2%) followed by weight loss 21 (53.8%). Patients had a history of tobacco smoking 17 (43%), and all of them were male, and a history of diabetes mellitus 7 (17.9%). The most common pattern on chest x-ray radiology examination was infiltrates 74.4%, and fibrotic was 64.4%. For the clinical symptoms in patients with suspected pulmonary TB such as cough, shortness of breath, weight loss, fever, night sweats and hemoptysis, all

of them have p - value > 0.05, that means it does not correlate with the incidence of pulmonary TB (p = 0.123, p = 0.151, p = 0.520, p = 1.000, p = 0.446, and p = 0.119).

Demographic factors, epidemiology, patients' clinical conditions, and also chest x-ray patterns either infiltrates, consolidations, pleural effusions, cavities and fibrosis all have no significant value on Xpert MTB/RIF Ultra, where all of them have p > 0.05 (p = 0.446, p = 0.715, p = 0.083, p = 1.000, and p = 1.000), only pleural effusion is close (p = 0.083) with chi-square statistical analysis using SPSS (Table 1).[26]

In this study, 35.9% (14/39) of the Xpert Ultra results were positive (Table 2). Of the 14 positive results, 71.4% (10/14) obtained positive MTB growth on the MGIT 960 culture culture system. At the same time, 4 had negative MTB

Table 1. Characteristics of the samples and factors associated with suspected pulmonary TB patients

Characteristic	Total (N = 39)	Xpert MTB/ RIF Ultra Detected (N = 14)	Xpert MTB/ RIF Ultra Not-detected (N = 25)	p-value
Demography dan Epidemiology				
Age				0.527
<18 years	1 (2.6%)	0 (0.0%)	1 (100%)	
19-59 years	22 (56.4%)	9 (40.9%)	13 (59.1%)	
>60 years	16 (41.0%)	5 (31.3%)	11 (68.8%)	
Gender				0.196
Male	24 (61.5%)	11 (45.8%)	13 (54.2%)	
Female	15 (38.5%)	3 (20%)	12 (80%)	
HIV Status				0.123
Yes	4 (10.3%)	3 (75%)	1 (25%)	
No	35 (97.8%)	11 (31.4%)	24 (68.6%)	
Diabetes Melitus				0.225
Yes	7 (17.9%)	4 (57.1%)	3 (42.9%)	
No	32 (82.1%)	10 (31.3%)	22 (68.8%)	
Category case				0.053
New Case	33 (84.6%)	10 (30.3%)	23 (69.7%)	
Relaps	4 (10.3%)	2 (50%)	2 (50%)	
Drop-out	2 (5.1%)	2 (100%)	0 (0%)	
Clinical Symptoms				
Cough	35 (89.7%)	11 (31.4%)	24 (68.6%)	0.123
Dyspneu	27 (69.2%)	12 (44.4%)	15 (55.6%)	0.151
Weightloss	21 (53.8%)	9 (42.9%)	12 (57.1%)	0.520
Fever	16 (41.0%)	6 (37.5%)	10 (62.5%)	1,000
Nightsweats	10 (25.6%)	5 (50.0%)	5 (50.0%)	0.446
Hemoptysis	9 (23.1%)	1 (11.1%)	8 (88.9%)	0.119
Chest X-ray				
Infiltrate	29 (74.4%)	9 (31.0%)	20 (69.0%)	0.446
Consolidation	14 (35.9%)	4 (28.6%)	10 (71.4%)	0.715
Pleural Effusion	13 (33.3%)	2 (15.4%)	11 (84.6%)	0.083
Cavity	8 (20.5%)	3 (37.5%)	5 (62.5%)	1.000
Fibrotic	29 (64.4%)	23 (63.9%)	6 (66.7%)	1.000
Microbiology				
MGIT 960 Culture				0.687
Detected	12 (30.8%)	10 (83.3%)	2 (16.7%)	
Not detected	27 (69.2%)	4 (14.8%)	23 (85.2%)	

Table 2. Sample proportion of Xpert Ultra and MGIT 960 culture system

	MGIT 960 Detected	MGIT 960 Not detected	
Xpert MTB/RIF Ultra Detected	10 (True positive) 71.4%	4 (False positive) 28.6%	14
Xpert MTB/RIF Ultra Not detected	2 (False negative) 8.0%	23 (True negative) 92%	25
	12	27	Total 39

growth, while 3 of them had trace, and 1 had very low detection. Of the total samples, 30.8% (12/39) had MTB growth on MGIT 960 culture system, while 8% (2/12) had no MTB detected by Xpert Ultra. Of the 71.4% (10/14) who were positive for MTB growth on MGIT 960, 10% (1/10) had Rifampicin resistant on Xpert Ultra, but all were Rifampicin sensitive on MGIT 960.

In comparison with the liquid culture MGIT 960 system, the 'gold standard test' for MTB, the Xpert Ultra sensitivity is 83.3% (95% CI 51.6 - 98), specificity 82.5% (95% CI 66.27 – 95.81), positive predictive value 71.43% (95% CI 49.43-86.48) and negative predictive value 92% (95% CI 76.27-97.63). For Xpert Ultra, the accuracy in lung TB detection was 84.6% (95% CI 69.4-94). The statistical differences between the two diagnostic methods, Xpert Ultra and MGIT culture, were insignificant (p = 0.687 by McNemar's test).

DISCUSSION

Tuberculosis is an infection caused by MTB bacteria that is most commonly manifested in the lungs.[27] Some of the risk factors for TB are age, smoking behavior, having a poor sanitary environment and low socioeconomic factors.[28] In this study, 56.4% of pulmonary TB suspect patients were adults (19-59 years) followed by 41% elderly (>60 years). The average age was 54 years, with a higher proportion of male than female (61.5% vs. 38.5%, respectively). However, the results of the chi-square correlation analysis of age and gender showed no statistically significant correlation between age (p = 0.527) and gender (p = 0.196) with the pulmonary TB incidens. One study states that in adults, post-primary tuberculosis is common due to endogenous reactivation or exogenous reinfection in a previously sensitized patient who has maintained some previously sensitized patients, who have maintained some level of immunity.[29] Further, several factors are thought to play a role in the mechanism of increased susceptibility in male such as smoking, which can cause lung damage and decreased immune system function, and alcohol use which has an immunosuppressive effect.[30]

Although HIV and DM are not correlated with TB incidence in this study (p = 0.123 and p = 0.225), it was found that 75% (3/4) of patients with HIV, and 57.1% (4/7) of diabetes patients had pulmonary TB. These two comorbidities are often cited as influencing the incidence of TB, suggesting that diabetes suppresses the immune response, which in turn facilitates MTB infection and/or progression to symptomatic disease. In addition, poor glycemic control adversely affects TB treatment outcomes with effects such as prolongation of culture conversion, treatment failure, relapse and death.[31] Other study states $CD4^+$ cells attacked by HIV causing the number of $CD4^+$ T cells decrease, so that the remaining T cells work excessively, resulting in exhausted T cells, where the peak of the decrease in IFN-γ and TNF-α results in increased MTB activity.[32]

In the description of clinical symptoms in patients with suspected pulmonary TB such as cough, shortness of breath, weight loss, fever, night sweats and hemoptysis, does not correlate with the incidence of pulmonary TB (p = 0.123, p = 0.151, p = 0.520, p = 1.000, p = 0.446, and p = 0.119) with chi-square analysis, although the suspected pulmonary TB patients samples in this study were mostly cough (89.7%), dyspnea (53.8%) and fever (41%). This could be due to cough and shortness of breath are also common symptoms that occur due to respiratory tract disorders, such as lung infections or non-infections[33] whether from

the lungs, heart or other causes.[34] Fever may be mediated by endogenous pyrogens (cytokines) in response to exogenous pyrogens, especially microorganisms or their products (toxins)[35] which can also be caused by organs other than the lungs.[36]

From the chest x-ray examination in this study showed that each pattern did not correlate with the incidence of pulmonary TB, either infiltrates, consolidations, pleural effusions, cavities and fibrosis ($p = 0.446$, $p = 0.715$, $p = 0.083$, $p = 1.000$, and $p = 1.000$) with chi-square correlation analysis. This could be due to the fact that chest x-ray in pulmonary TB can be in several patterns of lession. It differs from size, shape, density, and cavitation. It also can be found in mixed nodular, fibrous, pleural effusion from one person to another.[37] In addition, chest x-ray examination in pulmonary TB cannot distinguish whether it is active TB or old TB, so in diagnosing TB, only radiology examination cannot be done.[5]

In addition to Indonesia being a high-burden country contributing to the world TB incidence,[1] TB cases in Indonesia are also increasing every year,[4] so the Indonesian government is making various efforts to tackle TB cases, one of which is conducting molecular screening tests such as Xpert MTB/RIF Ultra as an initiation of early TB diagnosis in accordance with WHO recommendations.[13,38]

The Xpert MTB/RIF Ultra works by detecting MTBC and rifampicin resistance simultaneously by amplifying specific sequences of the rpoB gene using rpo probes 1-4, and the presence of one additional target gene, namely IS1081/IS6110 for detection of MTBC DNA presence in the specimen.[13] IS6110 is a unique insertion repetitive mobile element specific to the MTBC, which is widely used as a genotypic diagnostic.[15] In addition to IS6110, another gene is IS1081 of the MTBC genome, which is often reported to be very stable and consists of 4 to 6 copies. Several studies have used IS1810 as a NAAT target[14] related to conserved gene regions and multi-copies of specific genes. Xpert MTB/RIF Ultra has a larger DNA amplification reaction space to increase diagnostic accuracy and sensitivity.[12,13] Xpert Ultra has a much higher sensitivity compared to first-generation Xpert in negative acid-fast bacilli (AFB) smear but positive TB culture and HIV-TB patients and shows increased accuracy for the detection of MTBC and rifampicin resistance (RIF).[10,17]

Several studies suggest that the sensitivity of Xpert Ultra in diagnosing TB ranges from 92-95% compared to the first generation Xpert (89.40%), and specificity of 96.46% from all lung specimens in pulmonary TB suspects.[39,40] This is different from this study, where the sensitivity of Xpert Ultra was 83.3%, and specificity was 82.5% which is lower than existing literature and some studies. This could be due to there were 28.6% samples (4/14) that did not show growth in MGIT 960 culture system (2 false positive, 2 were NTM identified) that affect these sensitivity and specificity results.

The first false positive patient had received anti-tuberculosis therapy for 4 months from previous TB and then dropped out due to hepatitis B and regularly took antiretrovirals. He also has a history of diabetes mellitus and asthma that is controlled by inhaler. The patient's clinical symptoms were suggestive of TB, and the lung chest x-ray showed multiple infiltrates and cavities as well as pleural effusion. The second false positive was relapsed pulmonary TB, which received anti-tuberculosis for 6 months and was declared cured in the same year without any acid-fast staining evaluation. The patient presented with shortness of breath and cough, and the patient was also a passive smoker. A chest x-ray showed fibro-consolidation in the right lung and right pleural effusion. From these two false-positive results, could be due to the remnants of non-viable MTB bacteria after receiving therapy. There may be additional potential sources of *Mycobacterium tuberculosis* DNA in sputum if we consider Tuberculosis as a spectrum of diseases that includes subclinical and even latent stages.[41] Otherwise, the MTB DNA found in sputum can be intact with live or dead MTB, which can be obtained from the lower respiratory system.[42] Although both results were false positives, TB management was still carried out in accordance with the WHO TB diagnosis algorithm.[43]

The remaining 2 specimens showed growth on the MGIT 960 culture system but were negative for MPT64 antigen, so they were concluded to be NTM, not MTBC. The first patients is lung adenocarcinoma and multiple-site malignant lymphadenopathy, with the

main complaint of a lump in the lymph node accompanied by tightness and weight loss, without respiratory symptoms and TB history. The chest x-ray showed infiltrates in the left parahillary and pleural effusion. The second patient had a hemorrhagic stroke with the main symptoms of dysarthria, she showed respiratory symptoms after a few days of treatment. Chest x-ray radiology showed a right parahilar fibro infiltrate. Of the two patients, IS6110 and IS1081 should only be found in MTBC with positive Xpert Ultra results,[13] and none of the NTM specimens had these gene targets.[44] But one of these genes, IS6110, can also be found in NTM,[45] and another studies state that both genes can be found in NTM,[40,46] so that the Xpert Ultra results were positive and its grew on the MGIT 960 culture system. However, the research is very old, so it is necessary to re-do the research with the same idea or modified.

Of the 28.6% (4/14) patients we described above, 3 of them were trace category and 1 very low detection on Xpert Ultra. When the Xpert Ultra test comes back with a trace result, it's essential to evaluate many other aspects, according to the WHO. These include the patient's age, if they have HIV, whether they have ever taken anti-TB medication, and the possibility that the sample's location is linked to variations in bacterial load. It is necessary to reevaluate clinical symptoms and gather information on previous tuberculosis cases to follow up on the Xpert Ultra trace results. Additional Xpert Ultra tests are not recommended for adults with symptoms of pulmonary Tuberculosis with a positive initial result.[10]

In research, specificity and sensitivity may fluctuate with prevalence; thus, considerable disparities in the case between the study and actual practice might lead to spectrum bias in accuracy assessments. However, for a test to be useful, sensitivity combined with specificity should be at least 1.525, or it can be said to be 150%. In this study, we found that the sensitivity and specificity were 83.3% and 82.5%; when summed up, the result is 165.8%, which means that the Xpert Ultra method has good sensitivity and specificity in diagnosing pulmonary TB. Statistically, we conducted the McNemar test in SPSS,[26] which is a non-parametric test because the sample in this study included paired nominal data. The differences between the two diagnostic methods, Xpert Ultra and MGIT culture, were found to be insignificant ($p = 0.687$ by McNemar's test). This means that the Xpert Ultra method for diagnosing pulmonary TB is the same as the MGIT 960 culture, which is the gold standard for TB diagnosis.

In this study, the positive predictive value (PPV) and the negative predictive value (NPV) were 71.43% and 92%. We assume that the difference in PPV and NPV rates could be due to some samples not detected by culture because the patients had received previous therapy. Positive and negative predictive values are attractive because they are clinically intuitive.[47] Predictive values can differ from one setting to another for the same diagnostic test, and they are affected by the prevalence of the disease. The lower the prevalence of the disease, the higher its negative predictive value. And the higher the prevalence of the disease, the higher the positive predictive value.[48]

The fundamental metrics of a test's diagnostic accuracy are sensitivity and specificity. Additional metrics include predicted values and total accuracy. The most crucial factor in the accuracy test is the reference standard that validates the test, and there is the assumption that it is 100% accurate. So, the standard benchmark used in this study is MGIT 960 liquid culture system, the gold standard for TB diagnosis.[49] So, the closer the accuracy is to 100%, the better the result is. According to this study, the accuracy of Xpert MTB/RIF Ultra for pulmonary TB diagnosis is 84.6%.

According to this discussion, the different results between Xpert Ultra as initial TB diagnosis and culture as the gold standard must still consider clinical judgment as a guide to TB management, the small number of good-quality sputum samples obtained in the study, which can affect the overall accuracy results, is a limitation of this study. Effective coordination between doctors in charge of the patient and the medical technicians in the laboratory administering more than 1 month of anti-tuberculosis therapy can affect the results of Xpert Ultra. Many sputum specimens are sent to the laboratory for Xpert Ultra, but most are not of good quality or are insufficient in volume. Even with the quality of these specimens,

positive results can still be produced. In addition, discovering false positive results and NTM findings gives a different view of the sensitivity and specificity values. So, it is necessary to examine the diagnostic accuracy of low-quality sputum, as well as studies that can be focused on those who have never received previous therapy.

CONCLUSION

Several factor such as demography, epidemiology, clinical symptomps and also chest x-ray pattern cannot confirm the diagnosis of pulmonary TB, because in addition to being a risk factor, the clinical conditions and chest x-rays patterns of suspected pulmonary TB patients can vary, so it is necessary to carry out confirmation test in accordance with the guidelines.

According to this study, Xpert MTB/RIF Ultra has a sensitivity of 83.3%, specificity of 82.5%, and accuracy of 84.6% in diagnosing pulmonary TB compared to the MGIT 960 system culture. From these results, it can be concluded that Xpert MTB/RIF Ultra is good enough to diagnose pulmonary TB. These results are important for Dr. Soetomo's health facilities as a provincial referral hospital, so all primary and secondary health facilities conduct examination for pulmonary TB diagnosis, which are sometimes of unreliable quality and less accurate sputum, therefore, it is still necessary to conduct Xpert Ultra examination under the TB detection algorithm guideline compliance (WHO, Health ministry), starting from clinical patients as well as chest x-ray radiology.

ACKNOWLEDGMENTS

None.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

All authors listed have made a substantial, direct, and intellectual contribution to the work, and approved it for publication.

FUNDING

None.

DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

This study was approved by the Health Research Ethics Committee of Dr. Soetomo Hospital, Surabaya, vide ethical fitness number 1041/KEPK/VII/2024.

INFORMED CONSENT

Written informed consent was obtained from the participants before enrolling in the study.

REFERENCES

1. Global Tuberculosis Report 2024. Geneva: World Health Organization; 2024. Licence: CC BY-NC-SA 3.0 IGO.
2. Carlson R. Can Tuberculosis Cases Decrease in 2025. Vax-Before-Travel News. https://www.vax-before-travel.com/can-tuberculosis-cases-decrease-2025-2024-12-09 Accessed Desember 10th, 2024
3. Verma AK, Tiwari R, Chakraborty S, et al. Insights into Bovine Tuberculosis (bTB), Various Approaches for its Diagnosis, Control and its Public Health Concerns: An Update. *Asian J Anim Vet Adv.* 2014;9(6):323-344. doi: 10.3923/ajava.2014.323.344
4. Ministry of Health, Republic of Indonesia. Menkes Tegaskan Indonesia Serius Tangani TBC. Published 2024. https://kemkes.go.id/eng/menkes-tegaskan-indonesia-serius-tangani-tbc. Accessed November 2024.
5. Centers for Disease Control and Prevention (CDC). Core curriculum on tuberculosis: What the Clinician Should Know. Centers Dis Control Prev Natl Cent HIV/AIDS, Viral Hepatitis, STD, TB Prev Div Tuberc Elimin. Published online 2021:8-100. http://www.cdc.gov/tb. Accessed August 5th, 2024.
6. Schluger NW. The acid-fast bacilli smear: Hail and farewell. *Am J Respir Crit Care Med.* 2019;199(6):691-692. doi: 10.1164/rccm.201809-1772ED
7. Rasool G, Khan AM, Mohy-Ud-Din R, Riaz M. Detection of mycobacterium tuberculosis in afb smear-negative sputum specimens through MTB culture and GeneXpert® MTB/RIF assay. *Int J Immunopathol Pharmacol.* 2019;33:2058738419827174. doi: 10.1177/2058738419827174
8. Vilcheze C, Kremer L. Acid-fast positive and acid-fast negative *Mycobacterium tuberculosis*: The Koch paradox. In: WR Jacobs Jr, H McShane, V Mizrahi, IM Orme. 2nd edition, Tuberculosis and the Tubercle Bacillus, ASM Press. 2017, Chapter 23: 519-523. doi: 10.1128/9781555819569.ch23
9. Slail MJ, Booq RY, Al-Ahmad IH, et al. Evaluation of Xpert MTB/RIF Ultra for the Diagnosis of Extrapulmonary Tuberculosis: A Retrospective Analysis in Saudi Arabia.

J Epidemiol Glob Health. 2023;13(4):782-793. doi: 10.1007/s44197-023-00150-z

10. WHO consolidated guidelines on tuberculosis. Module 3: diagnosis - rapid diagnostics for tuberculosis detection, 2021 update. Geneva: World Health Organization; 2021. Licence: CC BY-NC-SA 3.0 IGO. Accessed July 7th, 2024
11. Slail MJ, Booq RY, Al-Ahmad IH, et al. Evaluation of Xpert MTB/RIF Ultra for the Diagnosis of Extrapulmonary Tuberculosis: A Retrospective Analysis in Saudi Arabia. *J Epidemiol Glob Health*. 2023;13(4):782-793.doi: 10.1007/s44197-023-00150-z
12. Kaswala C, Schmiedel Y, Kundu D, et al. Accuracy of Xpert MTB/RIF Ultra for the diagnosis of tuberculosis in adult patients: a retrospective cohort study. *Int J Infect Dis.* 2022;122:566-568. doi: 10.1016/j.ijid.2022.07.016
13. Amedeo A, Beci G, Giglia M, et al. Evaluation of trace calls by Xpert MTB/RIF ultra for clinical management in low TB burden settings. *PLoS One*. 2022;17:1-13. doi: 10.1371/journal.pone.0272997
14. Lin CR, Wang HY, Lin TW, Lu JJ, Hsieh JCH, Wu MH. Development of a two-step nucleic acid amplification test for accurate diagnosis of the *Mycobacterium tuberculosis* complex. *Sci Rep.* 2021;11(1):1-11. doi: 10.1038/s41598-021-85160-2
15. Comín J, Otal I, Samper S. In-depth Analysis of *IS6110* Genomic Variability in the *Mycobacterium tuberculosis* Complex. *Front Microbiol*. 2022;13:767912. doi: 10.3389/fmicb.2022.767912
16. Piersimoni C, Gherardi G, Gracciotti N, Pocognoli A. Comparative evaluation of Xpert MTB/RIF and the new Xpert MTB/RIF ultra with respiratory and extra-pulmonary specimens for tuberculosis case detection in a low incidence setting. *J Clin Tuberc Other Mycobact Dis*. 2019;15:100094. doi: 10.1016/j.jctube.2019.100094
17. Wang G, Huang M, Jing H, et al. The Practical Value of Xpert MTB/RIF Ultra for Diagnosis of Pulmonary Tuberculosis in a High Tuberculosis Burden Setting: a Prospective Multicenter Diagnostic Accuracy Study. *Microbiol Spectr*. 2022;10(4): e00949-22. doi:10.1128/spectrum.00949-22
18. Siddiqi SH, Rusch-Gerdes S. MGIT Procedure Manual: For BACTECTM MGIT 960TM TB System. 2006:1-52.
19. Lavrova AI, Dogonadze MZ, Sychev AV, Manicheva OA, Postnikov EB. Ensemble density-dependent synchronization of mycobacterial growth: BACTEC MGIT 960 fluorescence-based analysis and mathematical modelling of coupled biophysical and chemical processes. *AIMS Microbiol.* 2022;8(2):208-225. doi: 10.3934/microbiol.2022017
20. Hasan M, Munshi SK, Banu Momi MS, Rahman F, Noor R. Evaluation of the effectiveness of BACTEC MGIT 960 for the detection of mycobacteria in Bangladesh. *Int J Mycobacteriol*. 2013;2(4):214-219. doi: 10.1016/j.ijmyco.2013.09.001
21. Lee BH, Yoon SH, Yeo HJ, et al. Impact of implementation of an automated liquid culture system on the diagnosis of tuberculous pleurisy. *J Korean Med Sci.* 2015;30(7):871-875. doi: 10.3346/jkms.2015.30.7.871
22. Cao XJ, Li YP, Wang JY, Zhou J, Guo XG. MPT64 assays for the rapid detection of *Mycobacterium tuberculosis*. *BMC Infect Dis.* 2021;21(1):1-15. doi: 10.1186/s12879-021-06022-w
23. Neeraja D, Veeregowda BM, Rani MS, et al. Comparison of single intradermal test, gamma interferon assay and indirect ELISA for the diagnosis of tuberculosis in a dairy farm. *Asian J Anim Vet Adv.* 2014;9(9):593-598. doi: 10.3923/ajava.2014.593.598
24. Neeraja D, Veeregowda BM, Rani MS, et al. Identification of *Mycobacterium tuberculosis* complex by culture and duplex Polymerase Chain reaction in bovines. *Asian J Anim Vet Adv.* 2014;9(8):506-512. doi: 10.3923/ajava.2014.506.512
25. Guillouzouic A, Gaudart A, Tessier E, et al. Xpert MTB/RIF Ultra Trace Results: Decision Support for the Treatment of Extrapulmonary Tuberculosis in Low TB Burden Countries. *J Clin Med*. 2023;12(9). doi: 10.3390/jcm12093148
26. Armstrong DT, Pretty L, D'Agostino K, Redhead-Harper R, Parrish N. Diagnostic Accuracy of the Abbott SD Bioline MPT64 antigen test for identification of MTB Complex in a U.S. Clinical Mycobacteriology Laboratory. *Heliyon*. 2024;10(9):e30501. doi: 10.1016/j.heliyon.2024.e30501
27. Rezaei N. Tuberculosis. Integrated Studies for a Complex Disease. Vol 11. (Rezaei N, ed.). Springer Nature, Switzerland. 2023. doi: 10.1007/978-3-031-15955-8
28. Wikurendra EA, Herdiani N, Tarigan YG, Kurnianto AA. Risk factors of pulmonary tuberculosis and countermeasures: A literature review. *Open Access Maced J Med Sci.* 2021;9:549-555. doi: 10.3889/oamjms.2021.7287
29. Ray R, Das P, Gupta SD, Gahlot GP. Pathology of Tuberculosis. In: Sharma SK, ed. Textbook of Tuberculosis an Nontuberculosis Mycobacterial Disease. 3rd ed. Jaypee Brothers Medical Publishers. 2020:25-51.
30. Miller PB, Zalwango S, Galiwango R, et al. Association between tuberculosis in men and social network structure in Kampala, Uganda. *BMC Infect Dis*. 2021;21(1):1-9. doi: 10.1186/s12879-021-06475-z
31. Tulu B, Amsalu E, Zenebe Y, et al. Diabetes mellitus and HIV infection among active tuberculosis patients in Northwest Ethiopia: health facility-based cross-sectional study. *Trop Med Health.* 2021;49(1):68. doi: 10.1186/s41182-021-00358-4
32. Wong K, Nguyen J, Blair L, et al. Pathogenesis of human immunodeficiency virus-*Mycobacterium tuberculosis* co-infection. *J Clin Med.* 2020;9(11):1-23. doi: 10.3390/jcm9113575
33. Marshall BG, White V, Loveridge J. Breathlessness and cough in the acute setting. *Acute Med I.* 2020;49(2):93-97. doi: 10.1016/j.mpmed.2020.11.004
34. Baxter N, Lonergan T. The differential diagnosis of the breathless patient. *Prim Care Respir Updat*. 2020;(21):7-11.
35. El Radhi AS. Clinical Manual of Fever in Children. In: Caroll J, Klein N, eds. Clinical Manual of Fever in Children. Springer International Publishing. 2018:53-doi: 10.1007/978-3-319-92336-9
36. Balli S, Shumway KR, Sharan S. Physiology, Fever. StatPearls Publishing LLC.

37. Loddenkemper R, Lipman M, Zumla A. Clinical aspects of adult tuberculosis. *Cold Spring Harb Perspect Med.* 2016;6(1):1-25. doi: 10.1101/cshperspect.a017848
38. Lestari T, et al. The development of the national tuberculosis research priority in Indonesia: A comprehensive mixed-method approach. *PLoS ONE* 18 (2): e0281591. doi: 10.1371/journal.pone.0281591
39. Wang G, Huang M, Jing H, et al. The Practical Value of Xpert MTB/RIF Ultra for Diagnosis of Pulmonary Tuberculosis in a High Tuberculosis Burden Setting: a Prospective Multicenter Diagnostic Accuracy Study. *Microbiol Spectr.* 2022;10(4). doi: 10.1128/spectrum.00949-22
40. Mansfield M, McLaughlin AM, Roycroft E, et al. Diagnostic Performance of Xpert MTB/RIF Ultra Compared with Predecessor Test, Xpert MTB/RIF, in a Low TB Incidence Setting: a Retrospective Service Evaluation. *Microbiol Spectr.* 2022;10(3):e0234521. doi: 10.1128/spectrum.02345-21
41. Arend SM, van Soolingen D. Performance of Xpert MTB/RIF Ultra: a matter of dead or alive. *Lancet Infect Dis*. 2018;18(1):8-10. doi: 10.1016/S1473-3099(17)30695-3
42. Dorman SE, Schumacher SG, Alland D, et al. Xpert MTB/RIF Ultra for detection of *Mycobacterium tuberculosis* and rifampicin resistance: a prospective multicentre diagnostic accuracy study. *Lancet Infect Dis.* 2018;18(1):76-84. doi: 10.1016/S1473-3099(17)30691-6
43. World Health Organization (WHO). WHO consolidated guidelines on tuberculosis. Module 3: diagnosis. Tests for tuberculosis infection. Geneva: World Health Organization; 2022. Licence: CC BY-NC-SA 3.0 IGO Accessed November 10th, 2024
44. Nghiem MN, Van Nguyen B, Nguyen ST, Vo TTB, Van Nong H. A simple, single triplex PCR of IS6110, IS1081, and 23S ribosomal DNA targets, developed for rapid detection and discrimination of mycobacterium from clinical samples. *J Microbiol Biotechnol*. 2015;25(5):745-752. doi: 10.4014/jmb.1409.09089
45. Asrinawaty AN, Massi MN, Sjahril R. Detection Of Nontuberculous Mycobacteria (NTM) In Isolates From Suspected Tuberculosis Using PCR IS6110 Gene. *Nusantara Medical Science Journal*, 2016;1:10–15. doi: 10.20956/nmsj.v0i1.2203
46. Mustafa AS. Detection of Mycobacterium tuberculosis complex and non-tuberculous mycobacteria by multiplex polymerase chain reactions. *East Mediterr Helath J*. 1999;5(1):61- 70. doi: 10.26719/1999.5.1.61
47. Power M, Fell G, Wright M. Principles for high-quality, high-value testing. *Evid Based Med.* 2013;18(1):5-10. doi: 10.1136/eb-2012-100645
48. Safari S, Baratloo A, Elfil M, Negida A. Evidence Based Emergency Medicine Part 2: Positive and negative predictive values of diagnostic tests. *Emerg* (Tehran, Iran). 2015;3(3):87-88.
49. Santini A, Man A, Voidazan S. Accuracy of Diagnostic Tests. *J Crit Care Med*. 2021;7(3):241-248. doi: 10.2478/jccm-2021-0022