Feasibility and Sensitivity of Genexpert MTB/RIF Ultra on Saliva for Diagnosis of Pulmonary Tuberculosis

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KEYWORDS

Pulmonary Tuberculosis, GeneXpert MTB/RIF Ultra

Saliva, Diagnostic Specimen, Non-Invasive TB Testing

ABSTRACT:

Background: Tuberculosis (TB) remains a major global health concern, with Indonesia ranking second in TB burden, reporting 1.09 million new cases and 125,000 deaths in 2024. Despite advancements in TB diagnostics, detection gaps persist, particularly due to challenges in sputum sample collection. The GeneXpert MTB/RIF Ultra assay enhances TB detection and rifampicin resistance identification by targeting additional gene sequences (IS1081/IS6110). However, the difficulty in obtaining quality sputum specimens necessitates alternative diagnostic approaches, such as saliva-based testing, to improve accessibility and efficiency.

Objectives: This study aims to assess the feasibility and sensitivity of GeneXpert MTB/RIF Ultra using saliva for pulmonary TB diagnosis.

Methods: This cross-sectional study was conducted at Dr. Wahidin Sudirohusodo Hospital, Makassar, from August to October 2024. Suspected pulmonary TB patients were selected using consecutive sampling based on clinical symptoms, contact history, or thoracic imaging. GeneXpert MTB/RIF Ultra testing was performed on both sputum and saliva samples, with additional MTB culture examination on sputum. Diagnostic feasibility and sensitivity were analyzed.

Results: The study included 47 participants, with an average age of 48.06 ± 14.18 years. Most subjects were male (68.1%), and the predominant symptom was prolonged cough (44.7%). The test demonstrated high specificity but moderate sensitivity, indicating its reliability in confirming TB cases but limited ability to detect all infections.

Conclusion: Saliva-based GeneXpert MTB/RIF Ultra testing offers a non-invasive diagnostic alternative, particularly for patients unable to produce sputum. However, its limited sensitivity suggests the need for further optimization or complementary diagnostic methods to enhance detection rates.

1. Introduction

Tuberculosis (TB) is a chronic infectious disease caused by bacteria Mycobacterium tuberculosis (MTB). This bacterium is rod-shaped and acid-resistant, so it is often known as Acid-Resistant Bacilli (BTA) [1]. Most TB germs are often found to infect the pulmonary parenchyma (pulmonary TB), making lung disease the most common symptom. However, TB is a multi-systemic disease with a diverse presentation. The organ systems most commonly affected include the respiratory system, gastrointestinal (GI) system, lymphatic system, skin, central nervous system, musculoskeletal system, reproductive system, and liver [2].

By Global TB Report in 2022, an estimated 10.6 million people contracted TB in 2021, an increase of 4.5% from 10.1 million in 2020. A total of 1.6 million people died from TB in 2021 (including 187,000 people with HIV). The incidence rate of TB (new cases/100,000 population/year) increased by 3.6% between 2020 and 2021 [3]. Based on the 2024 Globat TB Report, Indonesia ranks second after India with an estimated burden of 1,090,000 new TB cases and 125,000 deaths. From these estimates, based on data as of January 24, 2024, a total of 872,844 (80% of the 90% target) have been notified and the number of TB cases treated has reached 89% (90% target) [4].

Over the past quarter century, the gradual improvement in TB diagnosis and treatment has reduced the mortality rate, but large gaps in detection and treatment remain that contribute to substantial ongoing morbidity and mortality. Among the several strategies available to facilitate rapid same-day TB diagnosis, sputum testing with the GeneXpert MTB/RIF Ultra (Xpert Ultra) molecular assay is the most sensitive and available approach [5]. The Xpert MTB/RIF Ultra Assay can detect complex MTB and rifampicin resistance simultaneously by amplifying rpoB gene specific sequences using rpo probes [6].

The difference in the test target in the Xpert MTB/RIF Ultra is the addition of one target gene, IS1081/IS6110, which functions to detect the presence of complex MTB DNA in the specimen. The Xpert MTB/RIF Ultra cartridge also has a Sample Processing Control (SPC) and Probe Check Control (PCC). The Xpert MTB/RIF Ultra probe can attach to wild-type and mutant sequences, in contrast to the Xpert MTB/RIF probe which can only attach to wild-type sequences [7]. The probe's ability makes MTB/RIF Ultra more sensitive in detecting Rifampicin resistance. The examination analysis of the Xpert MTB/RIF Ultra was carried out by looking at Melt Curves, which is to look specifically at the Melting Temperatures (Tm) that appear between wild type and mutant sequences. However, there are some operational challenges associated with the collection of sputum samples for the diagnosis of pulmonary TB [8].

Given the limited sputum sample caused by several factors for TB diagnosis, either due to the patient's own condition who is unable to expel sputum or the patient's lack of knowledge to collect a good sputum sample. In 2014 World Health Organization (WHO) issued guidelines for the development of tests with nonsputum samples for the diagnosis of active TB, including target product profiles indicating that acceptable nonsputum tests should have a minimum diagnostic accuracy similar to that of previous generation GeneXpert TB/RIF tests in people with BTA-negative (i.e., sensitivity > 68%, specificity > 98%) [9].

Byanyima et al. found that Xpert Ultra on a single saliva sample had a sensitivity of 90% (95% confidence interval [CI], 81-95%) compared to the reference standard based on composite sputum cultures, similar to the composite sensitivity of 87% (95% CI, 77-94%) for fluorescence microscopy (FM) for acid-resistant bacilli on two smears of sputum [10]. However, in a study conducted by Shenai et al, saliva tested with Xpert Ultra had a very low sensitivity of 39% for active TB disease compared to the reference standard of sputum liquid mycobacterial cultures.7 The study of Kim et al. also showed that of the 43 patients suspected of TB, as many as 32 people (94.1%) had a positive GeneXpert result, but only 26 people (78.8%) had a positive molecular rapid test (TCM) result and 6 other people had a negative TCM result [11].

One type of nonsputum sample that is very promising for the diagnosis of pulmonary TB is saliva, which is easily collected without coughing, thereby reducing the risk of aerosol formation and TB transmission during TB diagnostic evaluation. Although the Stop TB guidelines Partnership Salivary sputum samples were discouraged because they had lower diagnostic results for BTA when tested with smear microscopy or mycobacterial cultures, but their diagnostic results appeared to be much more promising when tested using the GeneXpert test [12]. In addition, the feasibility of using GeneXpert saliva to diagnose TB is still controversial. Therefore, it is important to understand the feasibility and sensitivity of GeneXpert saliva for the diagnosis of TB [13].

2. Objectives

The objective of this study is to evaluate the feasibility and sensitivity of the GeneXpert MTB/RIF Ultra assay using saliva samples for the diagnosis of pulmonary tuberculosis. Given the need for non-invasive, rapid, and accurate diagnostic methods, this study aims to assess whether saliva can serve as a reliable alternative specimen to sputum for detecting Mycobacterium tuberculosis, particularly in patients who have difficulty producing sputum. By analyzing the diagnostic performance of GeneXpert MTB/RIF Ultra on saliva, this research seeks to contribute to the development of more accessible and patient-friendly tuberculosis diagnostic strategies [14].

3. Methods

This study is an observational analytical study with a cross sectional approach, namely the collection of data or variables that are studied simultaneously at one time, which aims to assess the feasibility and sensitivity of GeneXpert saliva to diagnose pulmonary TB at Dr. Wahidin Sudirohusodo Hospital. The research will be conducted at the Wahidin Sudirohusodo Government General Hospital (RSUP) Makassar. Data collection, sample collection and examination of patients with Suspected Pulmonary TB in August-October 2024. The population in this study is all patients suspected of pulmonary TB either clinically, history of contact with pulmonary TB patients or based on Thoracic photos. The research sample is a population that meets the inclusion criteria and exclusion criteria from the population reached by sampling using consecutive sampling. The samples obtained were placed in the sputum room, then TCM examination was carried out with GeneXpert MTB/RIF Ultra from sputum samples and GeneXpert from salivary samples. In Sputum, Mtb culture was also examined and then the feasibility and sensitivity of GeneXpert MTB/RIF Ultra were assessed.

Research Ethics

This research was carried out after obtaining an ethical clearance certificate from the Health Research Ethics Committee, Faculty of Medicine, Hasanuddin University. The research request letter from the Scientific Writing Management Unit (UP-KTI) and SMF Pulmonology and Respiratory Hospital Dr. Wahidin Sudirohusodo Makassar to be processed and then submitted to the Director of Dr. Wahidin Sudirohusodo Hospital Makassar. For each action, written consent (informed consent) from the patient or his family is required. The subjects and their families were given an explanation of the purpose and objectives of the research as well as the expected benefits of this research. After a clear understanding, the subject or his family is asked to sign a letter of consent not to be included in this study.

4. Results

This research was conducted at Wahidin Sudirohusodo Hospital with a cross sectional design and is an observational analytical study carried out from August to October 2024. The research sample is a suspected pulmonary TB patient either clinically, a history of contact with pulmonary TB patients or based on Thoracic photos. A total of 50 people were included in this study, but during the study there were 3 people who did not have enough samples to be examined for culture, so that 47 people were left as samples in this study.

The characteristics of the subjects assessed in this study were age, gender, complaints, disease history, smoking status, and description of thoracic lesions. In table 2, the average age in this study was 48.0614.18 years, with the youngest age being 34 years and the oldest being 62 years old. A total of 32 people (68.1%) were men and 15 people (31.9%) were women. Based on complaints, as many as 21 people (44.7%) had complaints of prolonged cough, 4 people (8.5%) had complaints of coughing up blood, 3 people (6.4%) had complaints of tightness, 4 people (8.5%) had complaints of tightness, chest pain and cough, 10 people (21.3%) had complaints of tightness and cough, 1 person (2.1%) had complaints of tightness and coughing up blood, 2 people (4.3%) had complaints of tightness and chest pain, 2 people (4.3%) had no complaints. Based on complaints, subjects with a prolonged cough were the biggest complaint in this study, which was 44.7% (**Table 1**.).

Table 1. Characteristics of the Research Subject

Variable	Frequency (n=47)	Percentage (%)
Age (AverageSD)±	48,0614,18±	
Gender		
Man	32	68,1
Woman	15	31,9
Complaints		
Long cough	21	44,7
Coughing up blood	4	8,5

Variable	Frequency (n=47)	Percentage (%)
Crowded	3	6,4
Tightness + chest pain + cough	4	8,5
Tightness + cough	10	21,3
Tightness + coughing up blood	1	2,1
Tightness + chest pain	2	4,3
No complaints	2	4,3
Disease History		
DM	1	2,1
TB	11	23,4
HT	1	2,1
CKD	2	4,3
Mammae Fish	1	2,1
HT+CKD+Lung tumors	1	2,1
TB+DM	1	2,1
Hepatitis	1	2,1
COPD	1	2,1
None	27	57,4
Smoking Status		
No Smoking	19	40,4
Passive Smoker	2	4,3
Active Smoker	26	55,3
Mild	11	23,4
Moderate	10	21,3
Severe	5	10,6
Overview of Thoracic Lesions		
Extensive Lesions	23	48,9
Moderate Lesions	6	12,8
Minimal Lesions	17	36,2
Usual	1	2,1

Description: SD: Standard Deviance, HT: Hypertension, DM: Diabetes Mellitus, CKD: *Chronic Kidney Disease,* TB: Tuberculosis, COPD: Chronic Obstructive Pulmonary Disease

Table 1. present disease history, in the study subjects, 1 person (2.1%) had a history of DM, 11 people (23.4%) had a history of TB, 1 person (2.1%) had a history of HT, 2 people (4.3%) had a history of CKD, 1 person (2.1%) had a history of Ca Mammae, 1 person (2.1%) had a history of Hypertension, CKD and Lung Tumor, 1 person (2.1%) had a history of TB and Diabetes Mellitus, 1 person (2.1%) had a history of hepatitis, 1 person (2.1%) had a history of COPD, and 27 people (57.4%) had no previous history of the disease. Based on this table, subjects with a history of TB disease had the highest score, which was 23.4% compared to other disease histories. The basic characteristics of subjects with smoking status were obtained as many as 19 people (40.4%) who did not smoke, 2 people (4.3%) were passive smokers and 26 people (55.3%) were active smokers. Of the 26 people (55.3%) active smokers, as many as 11 people (23.4%) including light smokers, 10 people (21.3%) moderate smokers, and 5 people (10.6%) heavy smokers. Based on the description of thoracic lesions in table 2 which is the subject of the study, as many as 23 people, namely 48.9% have a description of extensive lesions, 6 people (12.8%) have a description of moderate lesions, 17 people (36.2%) have a description of minimal lesions and 1 person (2.1%) are normal. This table shows that the subjects that were sampled the most were subjects with a description of extensive lesions on thoracic photographs, which was 48.9%.

Table 2. Saliva Test Results

GeneXpert Ultra Saliva	Frequency	Percentage (%)
Negative	35	74,5
Positive	12	25,5
Trace	3	6,4
Very Low	1	2,1
Low	7	14,9
Medium	1	2,1

Table 2. present the results of the GeneXpert ultra examination with saliva samples, there were 12 people (25.5%) positive and 35 people (74.5%) negative. Of the 12 people who were positive, as many as 3 people (6.4%) with trace results, 1 person (2.1%) with very low results, 7 people (14.9%) low, 1 person (2.1%) medium. There were no high and very high results in the results of the GeneXpert ultra examination in saliva.

Table 3. Sputum Examination Results

GeneXpert Ultra Sputum	Frequency	Percentage
Negative	27	57,4
Positive	20	42,6
Trace	1	2,1
Very Low	3	6,4
Low	11	23,4
Medium	3	6,4
High	2	4,3

Table 3. present the results of the GeneXpret Ultra examination with sputum samples of 27 people (57.4%) were were positive. Of the 20 many as 1 person (2.1%) low, 11 people (23.4%) and 2 people (4.3%) high.

MTB Culture	Frequency	Percentage
Negative	28	59,6
Positive	19	40,4

negative, 20 people (42.6%) people who were positive, as traced, 3 people (6.4%) very low, 3 people (6.4%) medium In the GeneXpert Ultra examination with sputum samples, more positive results were obtained when compared to saliva samples with a difference of 7 samples.

Table 4. MTB Culture Examination Results

Table 4. present the MTB culture examination, from 47 samples, 28 people (59.6%) were negative and 19 people (40.4%) tested positive for the growth of Micobacterium tuberculosis germs. This study assesses the sensitivity of the GeneXpert MTB/RIF Ultra examination with saliva samples to diagnose pulmonary TB. Based on the results of the examination carried out.

Table 5. Sensitivity of GeneXpert Ultra MTB/RIF Saliva

Examination Results	MTB Culture		p-value
GeneXpert MTB/RIF Ultra Saliva	Positive	Negative	
Positive	11 (91,7%)	1 (8,3%)	<0.001*
Negative	8 (22,9%)	27 (77,1%)	
Sensitivity	57,89%		
Specificity	96,42%		
NPV	77,14%		
PPV	91,6%		

*Fisher

Table 5. present the GeneXpert MTB/RIF Ultra Saliva examination compared to MTB sputum culture had results of 11 true positives, 27 true negatives, 1 false positive and 8 false negatives. From the results of this examination, it can be seen that the results of GeneXpert MTB/RIF Ultra from saliva samples have a sensitivity of 57.89%, specificity of 96.42%, NPV of 77.14% and PPV of 91.6%.

Table 6. Gen Expert MTB/RIF Ultra Sputum Sensitivity

Examination Results	MTB Culture		p-value
GeneXpert MTB/RIF Ultra Sputum	Positive	Negative	
Positive	18 (94,7%)	2 (7,1%)	<0.001*
Negative	1 (5,3%)	26 (92,9%)	
Sensitivity	94,73%		
Specificity	92,85%		
NPV	96,29%		
PPV	90,0%		

Table 6. present the GeneXpert MTB/RIF Ultra Sputum examination had results of 18 true positives, 26 true negatives, 2 false positives and 1 false negative. This examination has a sensitivity of 94.73%, specificity of 92.85%, NPV of 94.73% and PPV of 92.85%.

Table 7. The relationship of thoracic lesions to the results of the GeneXpert MTB/RIF Ultra Saliva examination.

Thoracic lesions	GeneXpert Ultra Saliva		p-value
	Positive	Negative	
Extensive Lesions	10 (43,5%)	13 (56,5%)	0,018*
Moderate Lesions	2 (33,3%)	4 (66,7%)	
Minimal Lesions	0 (0%)	17 (100%)	
Usual	0 (0%)	1 (100%)	

*Kolmogorov Smirnov

Table 7. present a relationship between the description of thoracic lesions and the results of GeneXpert Ultra Saliva (p=0.018). The table shows that the thoracic with a normal picture and a minimum lesion of 100% has a negative result on GeneXpert MTB/RIF Ultra saliva. In moderate and extensive lesions of 33.3% and 43.5%, positive results were shown in GeneXpert MTB/RIF Ultra saliva. The results showed that the wider the thoracic lesion, the greater the positive chance of the GeneXpert MTB/RIF Ultra saliva examination.

Table 8. Wide Relationship of Thoracic Lesions to GeneXpert MTB/RIF Ultra Sputum Examination Results

Thoracic lesions	GeneXpert Ultra Sputum		p-value
	Positive	Negative	
Extensive Lesions	16 (69,6%)	7 (30,4%)	0,002*
Moderate Lesions	2 (33,3%)	4 (66,7%)	
Minimal Lesions	2 (11,8%)	15 (88,2)	
Usual	0 (0%)	1 (100%)	

*Kolmogorov-Smirnov

Table 8. present there was a relationship between the area of thoracic lesions and the results of GeneXpert MTB/RIF Ultra Sputum examination (p=0.002). A total of 16 (69.6%) in the Thoracic with extensive lesions had GeneXpert ultra sputum positive results and 7 (30.4%) negative. A total of 2 (33.3%) moderate lesions had positive and 4 (66.7%) negative GeneXpert ultra sputum results. A total of 2 (11.8%) minimal lesion areas had GeneXpert ultra sputum positive results and 15 (88.2%) negative. As many as 1 (100%) of the normal lesion area, had a negative GeneXpert ultra sputum result.

Table 9. Long Relationship of Cough with GeneXpert MTB/RIF Ultra Saliva Test Results

Duration of cough	Saliva Test Results		p-value
	Positive	Negative	
<2 weeks	11 (33,3%)	22 (66,7%)	0,158*
>2 weeks	0 (0%)	6 (100%)	

*Fisher

Table 9. present there was no association between the length of cough and the results of saliva examination (p=0.158). In patients who coughed <2 weeks, as many as 11 people (33.3%) had positive results and 22 people (66.7%) were negative. In patients who coughed >2 weeks, as many as 6 people (100%) had negative saliva results.

Table 10. Relationship between Cough Type and GeneXpert MTB/RIF Ultra Saliva Test Results

Types of Cough	Saliva Test Results		p-value
	Positive	Negative	
Coughing up phlegm	1 (8,3%)	11 (91,7%)	0,122*
Coughing up blood spots	10 (37,0%)	17 (63,0%)	

*Fisher

Table 10. present there was no relationship between the type of cough and the results of saliva examination (p=0.122). In subjects who coughed up phlegm, as many as 1 person (8.3%) had a positive result and 11 people (91.7%) had a negative result. In subjects who coughed up blood spots, as many as 10 people (37.0%) had positive saliva test results and 17 people (63.0%) had negative saliva test results.

Table 11. Relationship of GeneXpert MTB/RIF Ultra Sputum Examination Results to GeneXpert MTB/RIF Ultra Saliva Examination Results (Positive and Negative)

GeneXpert Ultra Sputum	GeneXpert Ultra Saliva		p-value
	Positive	Negative	
High	2 (100%)	0 (0%)	<0.001*
Medium	3 (100%)	0 (0%)	
Low	5 (45,5%)	6 (54,5%)	
Very Low	2 (66,7%)	1 (33,3%)	
Trace	0 (0%)	1 (100%)	
Negative	0 (0%)	27 (100%)	

*Kolmogorov Smirnov

Table 11. present there was a relationship between the results of the GeneXpert MTB/RIF Ultra Sputum examination and saliva (p<0.001). Subjects who had high, medium results in the examination of putum had a positive score of 100% in the saliva examination, while sputum that had low and very low results had positive results of 45.5% and 66.7% in the examination of saliva samples. The results showed that the higher the results of the GeneXpert MTB/RIF Ultra examination in sputum, the greater the positive chance of the GeneXpert MTB/RIF Ultra saliva examination.

5. Discussion

A total of 47 research subjects were included in this study. The average age of the subjects of this study was 48.0614.18 years old. The age group of 45-54 years was most found to suffer from pulmonary TB in the study compared to other age groups. The age group of 45-54 years is still classified as productive age. Productive age is characterized by high activity and interaction with the environment, increasing the risk of spreading TB disease. More men (68.1%) suffer from TB than women (31.9%). The characteristics of pulmonary TB, it was found that men suffer from pulmonary TB more than women (60.8% vs 39.2%). Men are more affected by pulmonary TB because it is related

to activities that can lower the body's immune system such as smoking and drinking alcohol. Based on WHO data in 2018 with the number of TB patients around 10 million people, 5.7 million of them are men [16].

Complaints of prolonged cough were the most common complaint in this study. The distribution of clinical symptoms of pulmonary TB patients. The results showed that shortness of breath, weakness, prolonged cough, fever were the most complained. Pulmonary TB has classic clinical features, including chronic cough, sputum production, loss of appetite, weight loss, fever, night sweats, and hemoptysis [17]. This study also assessed the relationship between the length of cough and the type of cough with the results of saliva examination. The results showed that there was no significant relationship. No previous studies have analyzed the length of cough and the type of cough with the results of saliva examination. The diagnostic sensitivity of Xpert was much higher in saliva samples compared to mucoid sputum samples, and blood spot sputum was associated with much lower sensitivity. There were 11 people (23.4%) who had a history of TB in this study [18]. The person can experience a relapse or relapse after consuming OAT regularly and having completed it, due to exposure to other sources of infection that have not been treated. In addition, it can be caused due to endogenous reactivation or exogenous reinfection.45 Other factors that also play a role like the existence of other diseases, in a study conducted by The diabetes increases the risk of contracting tuberculosis [19].

A total of 26 people were active smokers (55.3%) in this study. Research by Hapsari, et al (2021) examined the relationship between smoking habits and the incidence of tuberculosis. The result was that patients with smoking habits had a greater chance of getting a positive phlegm smear result compared to patients who did not smoke. Patients with moderate smoking habits compared to heavy smokers have a greater chance of getting positive phlegm smear results [20]. Smokers had about twice the risk of TB disease compared to those who did not smoke. Smoking can also affect the likelihood of progression to active disease, although it is still unclear whether the link between smoking and TB disease is due to an increased risk of infection or reactivation into active disease [21]. This interaction is further supported by the host pathogen mechanism. In the infection process, alveolar macrophages are the first line of defense against TB, which engulfs inhaled bacteria. However, the number of macrophages in the alveoli is low, and the macrophages must gather and move from other alveoli to the site of infection [22].

Twenty-two people, namely 46.8% of the sample, had extensive lesions in this study. In the description of extensive lesions, positive results were obtained of 43.50% and negative results of 56.50%. Thoracic imaging is a practical way to find tuberculosis lesions with the broad classification of lesions seen on thoracic photographs, namely minimal, moderately advanced, and far advanced lesions [23]. There are studies that also show that there is a significant relationship between the results of acid-resistant bacillus sputum examination and the overall picture of radiological lesions of pulmonary tuberculosis patients. This study showed that there was a significant relationship between the description of thoracic lesions and the results of the sputum examination. Subjects who had extensive lesions had the most positive sputum examination results [24]. The image of the Thoracic photo depicts the area of lung lesions caused by M.tb germs, while the results of the BTA sputum examination describe the number of M.tb bacteria. In theory, patients with a Thoracic photo image with a wider lesion will have a higher positivity rate on the results of the BTA sputum examination49 This study also showed that there was a significant relationship between the image of Thoracic lesions and the results of salivary examination. In subjects who had moderate and extensive lesions, some had a positive salivary examination result; while subjects who had minimal lesions had negative saliva test results [25].

The radiological picture of suspected active TB lesions is cloudy/nodular shadows in the apical and posterior segments of the posterior upper lobe and superior segments of the lower lobe, cavitations mainly more than one, surrounded by cloudy and nudular opaque shadows, mililateral patch shadows and unilateral (general) or bilateral pleural effusions (rarely). Extensive lesions are one of the signs of active TB, likely to have high levels of germs, so Mycobacterium contamination into saliva is greater

and can increase the likelihood of detecting Mycobacterium tuberculosis DNA in saliva [26]. In line with the research of Nova et al., it was shown that there was a significant relationship between the results of the examination of acid-resistant bacillus sputum and the broad picture of radiological lesions of pulmonary tuberculosis patients. Negative BTA results and +1 most radiological lesions are minimal lesions, BTA +2 most lesions are moderate advanced lesions, and BTA +3 most lesions are far advanced lesions [27]. The results of this study showed that the GeneXpert MTB/RIF Ultra saliva examination had a sensitivity of 57.89%, specificity of 96.42%, NPV of 77.14% and PPV of 91.6%. In subjects who had positive salivary GeneXpert MTB/RIF Ultra results, 11 subjects had a positive MTB cult result as well (true positive), and only 1 subject had a negative culture result (false positive). In subjects who had negative salivary GeneXpert MTB/RIF Ultra saliva results, as many as 8 subjects had positive culture results (false negative) and 27 subjects had negative cultures as well (true negative) [28]. The sensitivity results in this study were lower than that of GeneXpert MTB/RIF Ultra which was carried out using sputum. In the examination using sputum, sensitivity results were obtained of 94.73%, specificity of 92.85%, NPV of 96.29% and PPV of 90.0%. When the saliva test results are classified into medium, low, very low, trace and negative; and the results of the sputum examination were classified into high, medium, low, very low, trace and negative, the results also showed a significant relationship [29].

The quality of sputum and the diagnostic performance of GeneXpert MTB/RIF when smear results were negative, performed on patients with suspected tuberculosis. The study consisted of 1,782 adults with negative smear results who underwent evaluation for active TB, and found that salivary sputum had significantly higher diagnostic outcomes and sensitivity (66%; 95% confidence interval [CI], 53 to 77%) for culture-positive TB than mucoid sputum (52%; CI 95%, 46 to 58%) and other types of phlegm (e.g., mukopurulen, bloody), which suggests that saliva may add more diagnostic value than phlegm alone [30].

Results obtained by a 24-hour delay in examination at room temperature will result in a false positive result on the examination of the microscope. There are delays in testing, making phlegm thinner, and reducing the number of BTA findings. The diluted phlegm may be because the warmer temperature breaks down the phlegm grains, thus releasing fluid from the granules and making the phlegm thinner. This change in cosmetology makes it difficult to make preparations [31]. The consistency of liquid sputum causes the sputum to have many BTA cells that are not carried away. In addition, the sputum smear will also be more difficult to adhere to the object's glass, causing the preparation to be difficult to stain and read. As a result, the number of BTA cells counted becomes less than it should be. There are studies that have also shown that sputum examinations that are delayed for 12 hours at room temperature can result in a decrease in the number of BTAs which can lead to a false negative result [32]. This decrease in the amount of BTA can be influenced by several factors, namely nutrition, enzymatic processes in sputum and changes in sputum consistency. Just like other bacteria, acid-resistant bacteria also need nutrients to survive. In sputum, BTA can only use the nutrients in it. The number of nutrients in sputum is limited, so when the nutrients in the sputum are finally depleted due to continuous use, the BTA will lose its energy source and die. Undeterminable (negative) 62 Mtb DNA on the geneXpert assay can also be caused by statistical process control curves (SPCs) that do not show an increase in the number of amplicons, incorrect sampling processes, and inhibited PCR reactions[33].

6. Conclusion

The findings of this study indicate that the GeneXpert MTB/RIF Ultra assay using saliva as a diagnostic specimen for pulmonary tuberculosis has a limited sensitivity but a high specificity. This suggests that while the test is highly accurate in confirming positive TB cases, it may fail to detect a significant proportion of true TB infections. The high specificity highlights the reliability of positive results, reducing the likelihood of false positives. However, the moderate sensitivity underscores the need for further optimization or the use of complementary diagnostic methods to enhance detection rates, particularly in patients with low bacterial loads in their saliva. The Negative Predictive Value (NPV) suggests that while a negative test result provides reasonable confidence in ruling out TB, there

remains a possibility that some undetected cases exist. This underscores the need for clinical correlation and additional testing in suspected TB patients with negative results. Conversely, the Positive Predictive Value (PPV) confirms that when the test yields a positive result, it is highly reliable in identifying true TB cases. These findings support the potential use of saliva-based GeneXpert MTB/RIF Ultra testing as a non-invasive diagnostic tool, particularly for individuals who struggle to produce sputum, while also emphasizing the necessity of further validation studies to improve its diagnostic performance.

7. Conflict of Interest

The authors declare no conflicts of interest regarding the publication of this literature review. No financial, institutional, or personal relationships influenced the research, analysis, or conclusions presented in this manuscript.

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