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Original Research Article

# Saliva vs Sputum for Xpert MTB/RIF Ultra: A Preliminary Diagnostic Study in Suspected TB

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**Abstract**

**Background**: The World Health Organization (2022) recommends the use of Xpert MTB/RIF Ultra on nasopharyngeal aspirates (NPA), gastric aspirates, sputum, and stool specimens. However, to obtaining quality sputum are challenging, so saliva is an attractive alternative specimen due to its non-invasive nature, safety, and ease of Collection.

**Objective**: Identifying the accuracy of Xpert MTB/RIF Ultra between saliva and sputum (standard reference).

**Methods**: Preliminary prospective study of diagnostic accuracy. There were 22 subjects suspected of having TB, from the hospital's pulmonary clinic, subjects underwent the Xpert MTB/RIF Ultra test in parallel using paired sputum and saliva samples. Sensitivity, specificity, positive predictive value, negative predictive value, confidence interval, and diagnostic accuracy were calculated using 2×2 contingency analysis. The background behind this approach was chosen because of its clinical relevance, limited resources research power and time on health services.

**Results:** 11 MTB samples were true positive in saliva and sputum (TP). 5 MTB samples were positive in sputum and negative in saliva (FN), whereas 2 samples were positive for MTB in saliva, negative in sputum (FP). Where 4 samples were negative in saliva and sputum (TN). Saliva specificity (66.67%; 95% CI: 30.0-90.3) and saliva sensitivity was (68.75%; 95% CI: 44.4–85.8). PPV Value (84.62%; 95% CI:57.8-95.7) and NPV value (44.44%; 95% CI: 18.9–73.3). However, the range of confidence interval is relatively large due to the small sample size. So it can provide initial evidence that saliva can be used as a non-invasive to confirm the diagnosis of TB, especially in children in clinical cases where resources are limited.

**Conclusion**: Although it is not yet able to replace standards such as mycobacterial culture, saliva diagnostic predictions with Xpert MTB/RIF Ultra are more accurate with larger sample sizes and as an additional tool to help detect cases to eliminate TB.

**Keywords**: *Tuberculosis; Xpert MTB/RIF Ultra; Saliva; Sputum; Diagnostic Accuracy*
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## INTRODUCTION

The top five countries contribute to 55% of global TB cases, with Indonesia ranking second,

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contributing approximately 10% of cases after India (25%). It is estimated that 10.7 million individuals developed TB in 2024, with 1.23 million deaths worldwide. Therefore, effective TB control programs require rapid, accurate, and accessible diagnostic tools. The use of Xpert MTB/RIF Ultra has been recommended as a primary tool for TB diagnosis.[1,3]

The challenge to obtaining quality sputum, especially in pediatric, elderly and paucibacillary cases, are a major problem in the diagnosis of tuberculosis, causing a faster spread of infection in the community and delays in diagnosis. Xpert MTB/RIF Ultra offers exceptional speed and sensitivity by using tongue swabs and saliva as alternatives.[4,6] Confirmed by various studies (China, 2024; Uganda and Kampala, 2022), the results show varying sensitivity (45–89) and high specificity approximately 100%) of use of Xpert Ultra in children and adults compared to sputum or microbiology standards.[7-10]

Xpert MTB/RIF Ultra is very sensitive to oral swabs and saliva, e.g the results of the above findings. However, this research gap includes: very little is done, especially in countries with a high burden of TB such as Indonesia, research is carried out in referral hospitals with adequate facilities, ignores vulnerable population groups, such as paucibacillary cases, and uses different reference standards (culture versus molecular sputum), and no less important is the sampling procedure, such as saliva, tongue swab, and posterior oral swab, causing varying results that have not been standardized.

The fact that Indonesia has the highest tuberculosis rate in the world is the aim of this research. Access limitations and retrieval problems sputum causes the diagnosis is often delayed. Therefore, for to support the national TB elimination target, evaluation of the accuracy of saliva specimens compared to sputum using Xpert Ultra must be improved. This will enable diagnosis, treatment and early detection better.[2-5]

## MATERIALS AND METHODS

Preliminary, prospective, cross-sectional study of diagnostic accuracy. Conducted from early September to late September 2025, in pulmonary polyclinics and referral hospital laboratory in Jakarta. The total number of subjects was 22.

Inclusion criteria: age>18 years with suspected TB (clinical symptoms or supporting radiological images), willing to sign informed consent to take part in the study and the patient provides sputum and saliva samples on the same day. Exclusion criteria included a diagnosis of extrapulmonary TB, individuals with a history of receiving TB treatment before going to hospital, and being unable to produce saliva or collecting insufficient samples.

**Ethical clearance:** The ethics committe of Persahabatan Genaral Hospital in Jakarta, Indonesia (No.0150/KPEK-RSUPP/07/2025)

### Sample Collection Procedure

A. Preparation

Patients with suspected TB were informed about the study and provided written informed consent. Participants were instructed to refrain from eating or drinking for at least one hour prior to sample collection. Two sterile containers were provided. Saliva was collected first, followed by sputum. For saliva collection, patients were instructed to expectorate naturally without coughing or prior fluid intake, with a minimum volume of 2–3 mL. Sputum samples (minimum 4 mL) were required to be mucopurulent and viscous, without food contamination, and collected in sterile containers. Sample adequacy was assessed by nursing staff before submitted to the laboratory. Samples were processed immediately; if delayed, they were stored at 2–8°C and analyzed the following day by well-trained laboratory personnels.

B. Specimen Processing

Specimen dilution and Inactivation

1. Add Sample Reagent (SR) to the specimen at a 2:1 ratio (two parts reagent to one part specimen).
2. Securely close the container and mix gently for approximately 10–20 seconds.
3. Incubate at room temperature for 10 minutes.
4. Shake again and incubate for an additional 5 minutes (total incubation time: 15 minutes).

Cartridge Loading

1. Open the GeneXpert cartridge.
2. Using a sterile pipette, transfer 2 mL of the processed specimen into the designated cartridge chamber.
3. Close the cartridge securely.

Loading into the GeneXpert machine

1. Insert the cartridge into the GeneXpert module.
2. Run the appropriate test protocol using the GeneXpert system.

C. Protocol Selection: MTB/XDR or MTB/RIF Ultra protocol.

- The process runs automatically for 70–90 minutes.
- Result Interpretation
    - MTB Detection:
        - *Detected / Not Detected*
    - Rifampicin and Isoniazid Resistance:
        - *Susceptible / Resistant / Indeterminate*
    - Second-line Drug Resistance (XDR):
        - Fluoroquinolones (FQ): *Susceptible / Resistant*
        - Second-line injectable drugs (SLI): *Susceptible / Resistant*
    - Documentation and Reporting
        - Record all results obtained in a laboratory form or electronic system.
        - Report the results to the clinician for diagnosis and therapy.

## RESULTS

A total of 22 subjects suspected of pulmonary tuberculosis were included in this study. The majority were male (68.2%), with a mean age of 43 ± 14.1 years. The most common comorbidities were hypertension and diabetes mellitus (each 13.6%).

In this cohort (n=22), concordance of 72.7% and discordance of 27.3% were observed between saliva and sputum specimens using Xpert MTB/RIF Ultra. This indicates that the two methods differ particularly in cases with low bacillary burden (trace/paucibacillary), where saliva more frequently, fails to detect *Mycobacterium tuberculosis* compared with sputum.

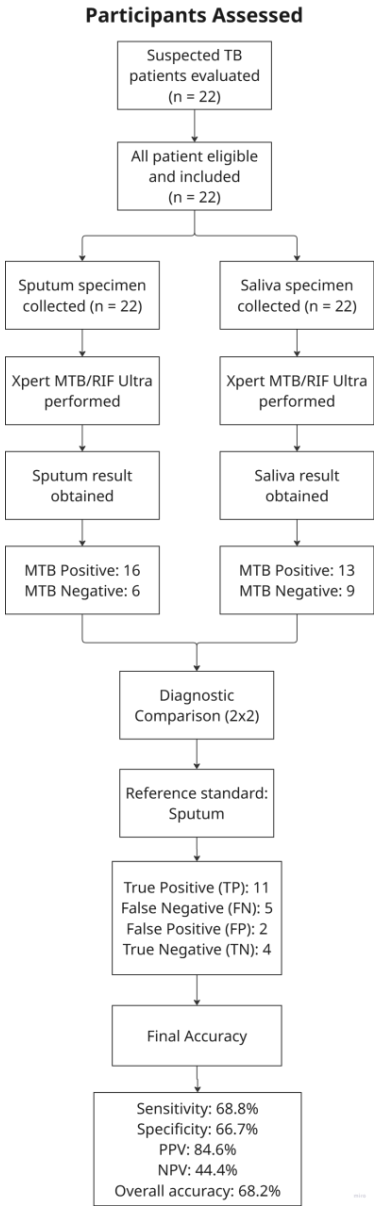



**Figure 1.** Research Framework

Although saliva may serve as an alternative specimen to sputum, it cannot yet be considered a standard diagnostic substitute (Table 1).

Chronic cough (90.9%), weight loss (81.8%), fever (72.7%), and night sweats (50%) were the most commonly reported clinical symptoms. Fibroinfiltrative lesions (45.45%) and pulmonary nodules (27.27%) were the most frequent radiological findings (Figure 1).

The diagnostic accuracy of Xpert MTB/RIF Ultra saliva was moderate, when compared with sputum as a standard. Of the 22 specimens evaluated, five results were false negative and two results were false positive. It was also found that in the saliva examination, 11 true positive results and 4 true negative results. So this finding, that there is still a discrepancy in results between saliva and sputum, will have an impact on the overall diagnosis process. (Table.2)

**Table 1.** Characteristic Subject

| Variable | n = 22 |
|---|---|
| Age (year) | 43 (±14,1) |
| Gender | |
| - Men | 15 (68,2%) |
| - Women | 7 (31,8%) |
| Comorbid disease | |
| - Hypertension | 3 (13,63%) |
| - DM tipe 2 | 3 (13,63%) |
| TCM Saliva results | |
| - MTB positive | 13 (59,1%) |
| - MTB negative | 9 (40,9%) |
| TCM sputum results | |
| - MTB positive | 16 (72,7%) |
| - MTB negative | 6 (27,3%) |
| Concordance Rifampicin | |
| - Positive-positive | |
| - Negative-negative | 12 (54,5%) |
| | 4 (18,2%) |
| Discordance Rifampicin | |
| - Sptum positive – saliva negative | 4 (18,2%) |
| - Sputum negative – saliva positive/trace | 2 (9,1%) |
| X-Ray | |
| - Atelektasis | 3 (13,63%) |
| - Destroyed lung | 3 (13,63%) |
| - Cavitas | 4 (18,18%) |
| - Fibroinfiltrat | 10 (45,45%) |
| - Nodul aru | 6 (27,27%) |
| - Consolidation inhomogen | 3 (13,63%) |
| Clinic symptoms | |
| - Chonic Cough | 20 (90,91%) |
| - Hemaptoe | 5 (22,72%) |
| - Chest pain | 4 (18,18%) |
| - Dysnoe | 10 (45,45%) |
| - Night sweats | 11 (50,0%) |
| - Febris | 16 (72,72%) |
| - Weight loss | 18 (81,81%) |
| - Effusion pleura | 2 (9,1%) |
| - Hoarse voice | 1 (4,5%) |

**Diagnostic Accuracy Analysis**

When compared with Xpert MTB/RIF Ultra sputum, saliva was clinically accurate (sensitivity

68.8% and specificity 66.7%). The high PPV value (84.6%) supports the value of saliva to confirm TB with a positive result, but the low NPV value (44.4%) limits

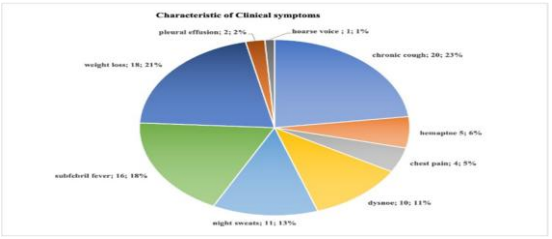



**Figure 2:** Characteristic of Clinical Symptoms

**Tabel 2.** Diagnostic contingency table

| | Positive sputum | Negative sputum | Total |
|---|---|---|---|
| Positive saliva | 11 (TP) | 2 (FP) | 13 |
| Negative saliva | 5 (FN) | 4 (TN) | 9 |
| Total | 16 | 6 | 22 |

**Table 3. Diagnostic performance of saliva as an alternative to sputum in Xpert Ultra testing**

| Variabel | Nilai (%) | 95% CI |
|---|---|---|
| Sensitivity | 68,75% | 44,44-85,8 |
| Specificity | 66,67% | 30,0-90,3 |
| PPV | 84,62% | 57,8-95,7 |
| NPV | 44,44% | 18,9-73,3 |

the ability of saliva to rule out disease with a negative result. Therefore, saliva is an alternative for screening, but cannot yet be used as an official standard for replacing sputum. All parameters have fairly large confidence intervals, thus indicating doubt in the assessment. Further studies with larger sample sizes are required to improve the accuracy and reliability of these findings (Table 3)

## DISCUSSION

This study found that all 22 subjects suspected of pulmonary tuberculosis (TB) were male, with a mean age of 43 ± 14.1 years, and 68.2% falling within this age distribution. Hypertension and diabetes mellitus each accounted for 13.6% of comorbidities (Table 1). Chronic cough, weight loss, fever, and night sweats were the most frequently reported clinical symptoms (Figure 1). These findings are consistent with a study conducted in Makassar in 2025, which reported a mean age of 48.06 ± 14.18 years, with a predominance of prolonged cough (44.7%) and male patients (68.1%). These results are in line with national tuberculosis program guidelines and the International Standards for Tuberculosis Care (ISTC).[5,10]

The most common radiological findings in this study were fibroinfiltrative lesions and pulmonary nodules. In drug-sensitive TB (DS-TB), infiltrates and ground-glass opacities are more commonly associated with active disease. In contrast, multidrug-resistant TB (MDR-TB) is more frequently associated with consolidation, cavitation, fibrosis, calcification, nodules, atelectasis, and other parenchymal abnormalities. However, atypical chest radiograph findings may still indicate active TB, even in cases with positive or negative Mycobacterium tuberculosis cultures presenting with clinical symptoms.[11] Therefore, radiological findings such as fibroinfiltrates and pulmonary nodules should be followed by confirmatory testing, including culture or Xpert MTB/RIF for drug resistance detection.

Findings in Table 1 showed that the majority of subjects were classified as positive–positive (54.5%) and negative–negative (18.2%). Based on Xpert MTB/RIF Ultra results from 22 subjects, the concordance between sputum and saliva specimens was 72.7% (16/22), indicating good agreement for both positive and negative results. Microbiology confirmation of children TB remains necessary. Xpert Ultra's sensitivity is low due to the disease's paucibacillary. In this study, saliva showed moderate accuracy with a discordance rate of 27.3% (6/22) in primarily in cases with low bacillary load, particularly sputum-positive but saliva-negative results. Therefore, it can not be used as an alternative to sputum, but it may be utilized of a multi specimen approch.[12-14]

The diagnostic performance of saliva as an alternative specimen for Xpert MTB/RIF Ultra demonstrated moderate agreement with sputum as the reference standard. This corresponds to a sensitivity of 68.75% (95% CI: 44.44–85.8) and specificity of 66.67% (95% CI: 30.0–90.3), which indicates sufficient but still limited detection ability, especially for the possibility of false results leading to discordance. The low PPV value (44.44%; 95% CI: 18.9–73.3) indicates that a positive saliva result cannot completely eliminate the disease. In contrast, the relatively high PPV value (84.62%; 95% CI: 57.8–95.7) indicates that a positive saliva result is quite reliable for confirming TB. These results are consistent with previous studies that respiratory specimens, such as saliva, are better as a confirmatory tool than single screening specimens, and have different diagnostic qualities. This mainly concerns children and paucibacillary cases. As a result, saliva still cannot be used as the main standard for Xpert MTB/RIF Ultra-based examinations.[15-17]

The main obstacle to this study is the relatively small sample size (n=22), so that estimates of diagnostic accuracy (sensitivity, specificity, and concordance–discordance) have a fairly wide confidence range and have the potential to reduce the precision of the results. These conditions may cause fluctuations in diagnostic value when applied to larger populations or with different clinical characteristics. Additionally, the use of sputum Xpert MTB/RIF Ultra as the reference standard does not fully represent the microbiological gold standard, such as Mycobacterium tuberculosis culture or a composite reference standard, introducing potential misclassification bias, particularly in paucibacillary cases.

However, in current clinical practice, culture is not widely utilized due to limited coverage under Indonesia's national health insurance system (BPJS),

higher costs, and prolonged turnaround time (several weeks), making it less practical for rapid clinical decision-making. Consequently, Xpert MTB/RIF Ultra is more commonly used as a pragmatic reference standard in routine care, despite not being an absolute gold standard. This approach remains clinically relevant in resource-limited settings and supports the exploration of saliva as a non-invasive alternative specimen for TB diagnosis.[18-22]

This study also has several strengths, particularly the use of saliva as a non-invasive alternative specimen for TB detection using Xpert MTB/RIF Ultra, which remains underexplored in the local population. This is a highly clinically relevant method that can overcome some of the limitations of sputum collection. Additionally, the study design comparing saliva with sputum directly provides a more relevant picture of performance in daily clinical practice. The findings of this research can be used quickly in health facilities with limited resources using Xpert MTB/RIF Ultra and distributed widely throughout the national TB service program.[21-22]

## CONCLUSION

This study proves the use of saliva as an alternative specimen for tuberculosis detection with Xpert MTB/RIF Ultra which has quite good diagnostic potential with adequate concordance values for sputum examination, although there are several differences that need to be considered. The results can be considered due to the very small number of subjects and no comparison with a gold standard. The accuracy of diagnostic estimates as well as the generalizability of research results can be affected by these situations.

Clinical implications: In the context of health services where access to culture is no longer routinely covered by BPJS, it is expensive, and requires a long examination time. So Xpert MTB/RIF Ultra on saliva can be a quick diagnostic option in making initial clinical decisions. However, positive results must be validated clinically and radiologically, and sputum examination does not completely replace current standards of practice.

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