**Monaldi Archives for Chest Disease**

eISSN 2532-5264 https://www.monaldi-archives.org/



Monaldi Arch Chest Dis 2025 [Online ahead of print]

*To cite this Article:*
Zaporojan N, Hodișan R, Zaha AA, et al. **Performance of conventional laboratory tests and Xpert MTB/RIF in the diagnosis of tuberculosis.** *Monaldi Arch Chest Dis* doi: 10.4081/monaldi.2025.3394

# Performance of conventional laboratory tests and Xpert MTB/RIF in the diagnosis of tuberculosis

Natalia Zaporojan,[1] Ramona Hodișan,[2] Andreea Atena Zaha,[1]
Claudiu Zaporojan,[1] Dana Carmen Zaha[1,2]

[1]Doctoral School of Biomedical Sciences, University of Oradea; [2]Department of Preclinical Disciplines, Faculty of Medicine and Pharmacy, University of Oradea, Romania

**Correspondence**: Ramona Hodișan, Department of Preclinical Disciplines, Faculty of Medicine and Pharmacy, University of Oradea, P-ta 1 December 10, 410087 Oradea, Romania. E-mail: ramona.hodisan@didactic.uoradea.ro

**Contributions**: NZ, DCZ, conceived the article; NZ, provided data; RH, AAZ, analyzed the data; NZ, CZ, wrote the manuscript; DCZ, revised the manuscript; all authors gave final approval for the version to be published.

**Conflict of interest**: the authors declare have not conflict of interest.

**Ethics approval and consent to participate**: the study protocol was approved by the Ethical Committee of the Bihor County Emergency Clinical Hospital (protocol no. 11325 dated 2024/04/15).

**Informed consent**: informed consent was obtained from all subjects involved in the study.

**Patient consent for publication**: consent for publication was obtained for this study.

**Availability of data and materials**: all data generated or analyzed during this study are included in this published article. Other data is available from the corresponding author upon request.

**Funding**: this research was funded by the University of Oradea.

**Abstract**

The laboratory diagnosis of tuberculosis (TB) represents a continuous challenge due to the variability and complexity of the required clinical samples. Although molecular technologies have considerably improved diagnostic accuracy, their combined use with traditional methods like microscopy and bacterial culture remains a subject of debate. This study aims to compare the performance of microscopy, bacterial culture on Löwenstein-Jensen medium, and the molecular Xpert MTB/RIF test in diagnosing pulmonary and extrapulmonary TB. In this retrospective study, conducted over a period from January 2016 to January 2023, data were collected from pulmonary TB and extrapulmonary TB samples of patients hospitalized in the pneumonology departments of the Bihor County Emergency Hospital. The study included 1796 patients, of whom 85.2% had samples collected from the respiratory tract. The variability of sensitivity and specificity depending on the type of sample indicates the need for a differentiated approach in diagnosis. The results show that the Xpert MTB/RIF test detected a higher number of positive cases (16%) compared to microscopy (9%) and bacterial culture (15%). Statistical analysis revealed a high sensitivity and specificity of Xpert MTB/RIF, suggesting superior accuracy compared to traditional methods. Our conclusions underline the importance of the Xpert MTB/RIF as a valuable tool in the diagnosis of TB, but it is recommended to use it in combination with other methods to ensure a complete and efficient diagnosis.

**Key words**: tuberculosis, microscopy, culture, molecular test, performance.

## Introduction

Tuberculosis (TB) remains a major global public health issue. According to the 2024 Global Tuberculosis Report by the World Health Organization, approximately 10.8 million people developed TB in 2023, corresponding to an incidence rate of 134 cases per 100,000 population [1]. Although the number of TB-related deaths decreased from 1.32 million in 2022 to 1.25 million in 2023, the disease has once again become the leading cause of death from a single infectious disease, surpassing COVID-19. Notably, 6.1% of TB cases were recorded in people living with HIV [2]. The disease typically affects the lungs but can also affect other areas. Without treatment, the mortality rate due to TB disease is high (approximately 50%) [3,4]. These data highlight the need for ongoing and intensified efforts in the prevention, diagnosis, and treatment of tuberculosis worldwide.

The COVID-19 pandemic significantly affected the TB care system, reducing testing and reporting of cases due to disruptions in services and restrictions on patient movement, which led to an increase in TB-related deaths [5]. Similarities in the behavior of the two infectious agents had serious consequences. Efforts concentrated on combating SARS-CoV-2 weakened control over TB, slowing progress in the fight against this disease [6]. New methods for diagnosing TB have reached a crossroads: their development, evaluation, and implementation have been severely affected by the diversion of resources due to SARS-CoV-2. However, promising technologies, especially non-invasive ones that do not require sputum, could facilitate efficient screening and rapid confirmation of TB at the point of care. Nevertheless, the progress of these tests is slow and will take years before they are available to patients and health workers [7].

The diagnosis of TB is based on chest radiography, smear microscopy, and bacteriological culture. Smear microscopy has variable sensitivity, detecting only 60–70% of cases. Examination of smears stained by the Ziehl-Neelsen (ZN) method, based on visualization of acid-fast bacilli, can provide rapid results in less than 30 minutes. Additionally, approximately 25% of TB cases are extrapulmonary forms, which are often missed by smear microscopy. In most laboratories, smear microscopy is the main method used for diagnosing TB. Conventional culture requires several weeks for the growth of *Mycobacterium tuberculosis*, and sputum is the preferred specimen [8]. The quality of sputum is essential for an accurate bacteriological diagnosis of pulmonary tuberculosis (PTB). A good-quality sputum sample is crucial for obtaining correct and reliable results in the diagnosis of TB and the laboratory establishes appropriate criteria for specimen collection, thus ensuring optimal test performance [4,9].

Solid medium culture using colony-forming units is widely considered to be the gold standard for determining the number of viable organisms in a specimen or experimental condition, but

it is labor-intensive and has a long turnaround time [10]. However, bacterial culture is a challenging method due to the slow growth rate of *Mycobacterium tuberculosis* and the need for laboratories with biosafety level three [11]. Modern clinical microbiology laboratories have various methods that allow rapid and accurate diagnosis of tuberculosis.

Polymerase chain reaction (PCR)-based methods developed in recent decades have significantly improved and accelerated the diagnosis of TB; however, such methods require specific configuration of the molecular diagnostic laboratory but provide results in two hours. The need for simpler PCR systems has been addressed with the real-time PCR system Xpert MTB/RIF (Cepheid, Sunnyvale, CA, USA), a simple point-of-care test that can simultaneously detect *Mycobacterium tuberculosis* and the main mutations associated with rifampicin resistance [12,13].

For certain categories of patients, Xpert MTB/RIF offers greater sensitivity in diagnosing TB such as people with HIV compared to sputum smear microscopy, reducing the time to diagnosis and treatment, which improves TB treatment outcomes [14]. This molecular diagnostic tool can identify the presence of at least 100 microbes/ml of tested suspension in vitro. However, the use of Xpert MTB/RIF to detect TB in samples that do not originate from the respiratory tract is recognized as being outside the officially established indications, a process often referred to as "off-mark" [15].

There is no standard approach for laboratory diagnosis of TB or evaluating the performance of diagnostic tests, where multiple microbiological tests are often performed on different clinical samples. In the absence of a perfect gold standard that has both high sensitivity and high specificity, there are other options to diagnose TB. We aimed to evaluate the accuracy of different laboratory tests for diagnosing TB, taking into account the type of clinical sample used, for three methods frequently used for diagnosing mycobacterial infections: ZN stained microscopic examination), cultivation on LJ medium and Xpert MTB/RIF.

## Materials and Methods

In this retrospective study, conducted from 2016 to 2023, data were collected from PTB and EPTB samples from patients hospitalized with suspected TB in the Pneumology Departments and from those who attended the specialized outpatient clinic of the Bihor County Emergency Clinical Hospital. Respiratory samples included sputum (spontaneous and induced), bronchial aspirate, bronchoalveolar lavage, and tracheal secretion. Non-respiratory samples included gastric aspirate, cerebrospinal fluid (CSF), biological fluids (pleural fluid, peritoneal fluid, synovial fluid, pericardial fluid), and purulent secretions.

Sputum samples were collected from patients in a sterile 30 ml container with a screw cap, containing 3–5 ml of sample. Between 10% and 85% of individuals suspected of pulmonary TB cannot produce sputum, depending on age and stage of the disease [16]. In cases where the patient could not expectorate, induced sputum samples were collected through inhalation of hypertonic saline aerosols (5–10% NaCl solution), which irritate the respiratory tract sufficiently to provoke coughing and expectoration [17]. The collection of bronchial aspirate samples was performed in the specialized bronchoscopy department using fiber-optic bronchoscopy by qualified personnel. For biological fluids (pleural, synovial, ascitic, and pericardial fluid), processing was per-formed on an approximate volume of 8–10 ml of aseptically collected sample [18].

For microscopic examination, the ZN staining was used. Bacterial cultures performed for *Mycobacterium tuberculosis* on LJ medium. Sputum samples were processed with a solution of N-acetyl-L-cysteine (NALC) and sodium hydroxide (NaOH) for 15–20 minutes for decontamination [19,20]. For molecular diagnosis, we used Xpert MTB/RIF, a method based on PCR.

To evaluate the general performance of microscopy, PCR, and culture according to the types of samples we measured sensitivity (SE), specificity (SP), as well as positive and negative predictive values (PPV and NPV). These measures were calculated using culture for *Mycobacterium tuberculosis* as the gold standard method for diagnosing TB in respiratory and non-respiratory samples.

Calculations were performed and analyzed with Microsoft Excel and R software. The Kappa coefficient (Kappa) was applied to evaluate the degree of agreement between the methods used. A value of the Kappa  0 indicate poor agreement, 0.01–0.20 as none to slight, 0.21–0.40 as fair, 0.41–0.60 as moderate, 0.61–0.80 as substantial, and 0.81–1.00 as almost perfect agreement [21]. To compare the difference in paired data between the tests analyzed, the McNemar test was used for correlated proportions. A McNemar value $< 0.05$ was considered to indicate statistical consistency between their results.

Area under the Receiver Operating Characteristic curve (AUC) was calculated to evaluate the performance of diagnostic methods against the reference standard using the RStudio "pROC" package. Values 0.5  AUC $< 0.6$ show very low diagnostic value, 0.6  AUC $< 0.7$ demonstrate low diagnostic value, 0.7  AUC $< 0.8$ show moderate diagnostic value, 0.8  AUC $< 0.9$ demonstrate considerable diagnostic value, and 0.9  AUC show excellent diagnostic value [22].

**Results**

Our study included 1,796 samples, which were divided into two categories: respiratory samples (n = 1,531, 85.2%) and non-respiratory samples (n = 265, 14.8%). Respiratory samples included sputum (n = 587, 32.7%), induced sputum (n = 834, 46.4%), bronchial aspirate (n = 98, 5.5%), bronchial lavage (n = 6, 0.3%), oropharyngeal secretion (n = 1, 0.1%), and tracheal secretion (n = 5, 0.3%). Non-respiratory samples were gastric aspirate (n = 173, 9.6%), CSF (n = 37, 2.1%), biological fluids (n = 44, 2.45%), and grouped purulent secretions (n = 11, 0.6%). As can be seen, most samples were included in the respiratory samples category.

Most individuals tested were men, representing 59% of total cases (1,059 patients), compared to women, who constituted 41% (737 patients). These data indicate an increased prevalence of suspected TB cases among men.

Most individuals tested for TB are adults, representing 73% of the total (n = 1,318), while 27% of patients are under 18 years old (n = 478). The average age of patients is 39 years, with a minimum age of 1 month and a maximum age of 91 years.

The evaluation by age groups and type of sample shows high positivity rates for respiratory samples, regardless of the diagnostic laboratory method. Apparently, in the [20,40) age group, the positivity rates were higher for the non-respiratory culture samples and the [80,100) group for the molecular test, but the number of tests is small (*Supplementary Table 1*).

The LJ culture results were considered the gold standard. Positive respiratory samples represented 92.67%, but also from the respiratory group a high percentage were negative (71.15%). The Xpert MTB/RIF test detected 292 positive cases (16%) and 1,504 negative cases (84%). Among the positive cases 93.83% were respiratory.

Data from *Supplementary Table 2* provides an overview of the performance of microscopic examination compared to culture for respiratory and non-respiratory samples. For all samples, the SE is relatively low (55.68%), but the SP is very high (99.41%), and the PPV and NPV values are high, indicating good performance of the microscopic test in most cases (*Supplementary Table 2*).

Among respiratory samples, sputum shows the best SE of 69.70% and a SP of 98.68%, suggesting good performance of the microscopic test. By comparison, other respiratory samples have a SE of 60% and SP of 99%, with a Kappa of 0.6821, indicating substantial agreement between the two methods.

Among non-respiratory samples, the performance of microscopic examination compared to culture showed that gastric aspiration had the best performance, with a SE of 16.67%, but

maximum SP and PPV of 100%. Other non-respiratory samples such as biological fluids had low SE (12.50%) and SP (100%), with PPV of 100% but lower NPV (83.72%).

*Supplementary Table 3* presents the performance of the Xpert MTB/RIF compared to the culture as reference method for various biological specimens. The overall accuracy is 95.82%, the Kappa indicates as almost perfect agreement agreement (0.8425), and the McNemar test shows a significant difference ($p = 0.0370$).

For other respiratory samples, although the test is highly sensitive and specific, there is a significant proportion of false-positive results. The NPV is 100%, indicating that all negative results of the Xpert MTB/RIF are accurate (*Supplementary Table 3*).

We considered culture and microscopic examination as a composite reference standard and compared the results of Xpert MTB/RIF, as shown in *Supplementary Table 4*. The overall performance for all samples shows a SE of 99.34%, SP of 91.42%, NPV of 99.93%, Kappa of 0.6401, and McNemar test with $p < 0.001$.

The performance of the Xpert MTB/RIF on various non-respiratory samples, such as CSF, showed that there are no statistically significant differences between the positive and negative results of the two tests, but these results should be interpreted cautiously due to small sample sizes.

The performance of Xpert MTB/RIF in relation to the composite reference standard varies depending on the type of sample analyzed. Gastric aspiration and respiratory samples exhibit high SE and SP, although the PPV is variable. Considering the NPV is extremely high for all types of samples (approximately 100%), the Xpert MTB/RIF proves to be particularly useful in ruling out negative patients. This aspect is important in the context of rapid clinical decisions, allowing medical staff to focus on the treatment of patients with positive results.

The AUC values for microscopic examination and Xpert MTB/RIF in relation to culture were 0.775 (moderate diagnostic) and 0.9333 respectively and the AUC for Xpert MTB/RIF in relation to composite reference standard was 0.9538 showing an excellent diagnostic (Figure 1).

## Discussion

TB primarily affects the lungs but can also involve other organs, manifesting as EPTB, continuing to represent a significant threat to public health [23]. In the context of this global challenge, TB remains a major cause of mortality, especially in resource-limited regions and the diagnosis of EPTB remains a significant challenge, given that current tests with limited accuracy in detecting this form of the disease [24].

In our study, we observed most prevalence of TB in men and in the 25–44-year age group, which represented 50.7% of cases. These findings are consistent with other studies that highlight significant variations according to sex and age [25,26]. Another study confirmed a high prevalence of TB in young people aged 3–16 years and in individuals between 31–60 years, suggesting a higher risk for these age groups [27].

The laboratory diagnosis of TB is based on a combination of different microbiological tests that can be performed on various types of clinical samples. In non-respiratory samples, which represented 14.75% of the total, gastric aspirate was the most frequent (9.6%), followed by pleural fluid and CSF (2.1% each). Many patients with non-respiratory samples were children aged 0–10 years, mainly girls, and men between 30–40 and 50–60 years.

Although the smear method with ZN staining is cheap and quick, it has reduced SE and SP, being unable to distinguish between *Mycobacterium tuberculosis* and non-tuberculous mycobacteria. The reduced SE of the smear leads to a significant number of false-negative results, especially in cases with a low bacterial load. The detection threshold is high for smear (>10,000 CFU/ml) and Xpert MTB/RIF (130–150 CFU/ml), while for culture, the threshold is low (10–100 CFU/ml) [28]. The SP of microscopic examination is very high across all types of samples, often exceeding 99%, indicating an excellent ability to correctly identify negative samples in our study. SE varies significantly depending on the type of sample, being highest in the case of sputum (69.70%) and lowest in CSF (0%). The positive predictive value is high in most cases, meaning that a positive result in microscopic examination has a high probability of being confirmed by bacterial culture. The NPV is moderately high, but the low SE in some non-respiratory samples suggests that a negative result does not always exclude the presence of infection. The Kappa indicates a substantial level of agreement between microscopic examination and bacterial culture in respiratory samples, but decreases significantly in non-respiratory samples. Due to its high SP and PPV, microscopic examination can be extremely useful in clinical scenarios that require rapid diagnosis and samples with negative results can undergo additional tests, such as culture, for confirmation. Therefore, microscopic examination is more effective when integrated into a diagnostic algorithm that includes culture confirmation. This is especially crucial for non-respiratory samples, where the SE of microscopic examination is lower, and the risk of false-negative results is higher.

Culture is considered the gold standard for diagnosing TB, having high SP but with the disadvantage of a prolonged time to obtain results and the need for specialized laboratory infrastructure, limited to reference centers [29]. In a study, 250 sputum samples from patients suspected of TB were analyzed by Xpert MTB/RIF technique and the solid culture. The results showed that 30 samples (12%) were positive by culture, while 17 samples (6.8%) were positive

by Xpert MTB/RIF and these data highlight a higher SE of the culture method compared to Xpert MTB/RIF [14]. Although the overall Kappa indicates a high concordance between Xpert MTB/RIF and the reference culture in our study, the lower value for gastric aspirate (0.7603) suggests specific challenges with this type of sample. The distribution of results across sample types shows that sputum and induced sputum have the highest number of positive results (123 and 96, respectively), which may suggest a higher prevalence of infection in these samples or increased test efficiency in detecting infections in the lower respiratory tract. This trend of positive results may influence the use and interpretation of the test, depending on the prevalence of infection in the tested population.

The Xpert MTB/RIF exhibits very high SE and SP across all types of samples. Maximum sensitivity (100%) is observed in secretions and other respiratory samples, indicating an excellent ability to detect positive cases. Both the positive and negative predictive values are high, suggesting that the test is reliable for confirming as well as excluding the diagnosis of TB. A Kappa above 0.8 in most categories indicates a substantial level of agreement between the Xpert MTB/RIF and bacterial culture.

Evaluating the performance of the three methods on different types of samples, we identified notable variations in terms of sensitivity and specificity. Our findings demonstrated that the Xpert MTB/RIF test has superior SE and SP compared to smear microscopy. In our study, the Xpert MTB/RIF demonstrated a SE of 89.74% and a SP of 96.91% for respiratory and non-respiratory samples, while microscopic examination presented a SE of 55.68% and a SP of 99.41%. These results underline the superiority of the Xpert MTB/RIF in detecting *Mycobacterium tuberculosis*, offering a greater capacity to identify positive cases compared to traditional microscopy, which can miss a significant number of infections due to its reduced SE. Our findings are generally consistent with another comparative study that evaluated the performance of the GeneXpert test, smear microscopy and conventional culture. Xpert MTB/RIF achieved an SE of 100% and a SP of 99.5%, compared to a SE of 57.3% and SP of 99.5% obtained by AFB smear microscopy [30]. Although the SE reported in our study is slightly lower for non-respiratory, the SP of the test remains high, indicating consistent reliability in avoiding false-positive results.

The performance of the Xpert MTB/RIF test for the diagnosis of tuberculous meningitis was inferior to that for PTB and inferior compared to other non-pulmonary samples. In our study, Xpert MTB/RIF presented a SE of 75% and a SP of 100% in CSF, demonstrating lower SE compared to the culture method. However, a study conducted by Mai and Thwaites highlighted that, despite its low SE, the Xpert MTB/RIF played a significant role in the diagnosis of tuberculous meningitis [31].

Xpert MTB/RIF demonstrates excellent SE (over 98%) when compared to the composite standard, being significantly more effective than microscopic examination in detecting positive cases, particularly for respiratory samples. Its SP, around 90%, reflects a strong ability to exclude negative cases; however, false-positive results remain a challenge, especially for non-respiratory samples.

There are some limitations in our study. First, data are provided from one single hospital from Romania, which may not represent other regions and we didn't follow the patients after testing. Some sample groups (e.g., CSF) were small creating interpretation difficulties.

According to type of sample, in case of extrapulmonary samples, three cases undetected by the Xpert MTB/RIF were identified through culture on LJ solid medium. Although culture is more sensitive than microscopic examination, the percentage of negative samples remains significant, underscoring the need for additional diagnostic methods for more accurate detection of EPTB.

Second, obtaining adequate samples often requires invasive procedures, and poor sample quality can limit the SE of laboratory tests [32]. The paucibacillary nature of the disease presents another challenge, as the low bacterial count in samples reduces the SE of traditional methods such as ZN staining and LJ culture [33-35]. Additionally, although Xpert MTB/RIF is an advanced technology, its sensitivity in detecting EPTB ranges from 60-80%, leading to false-negative results in cases of pleural or meningeal tuberculosis [24].

In our study, we found that SE and SP are essential factors in the diagnosis and confirmation of EPTB. The ZN microscopic examination, with a SE of 69.70% in sputum samples, proves useful for screening, identifying the majority of positive cases. Its high SP (> 99%) reduces the risk of false-positive results, making it ideal for confirming the diagnosis.

The Kappa (0.685) in respiratory samples indicates substantial agreement between microscopy and culture, supporting the use of microscopy as an initial test for rapid diagnosis. In sputum samples, this combination of SE and agreement confirms the efficiency of microscopy in identifying PTB cases. However, in non-respiratory samples, such as CSF or gastric aspirates, the low SE of microscopy limits the detection of EPTB. In these cases, complementary methods such as culture or Xpert MTB/RIF are indispensable for an accurate diagnosis.

**Conclusions**

The tests evaluated demonstrated good performance in diagnosing PTB and EPTB across various types of biological samples. Overall, Xpert MTB/RIF exhibited high SE and SP, indicating an excellent ability to correctly identify both positive and negative cases. Despite some limitations of the Xpert MTB/RIF—particularly concerning PPV for certain types of

samples—it remains a valuable tool for rapid and precise clinical diagnosis. Our findings highlight the need to adopt diagnostic methods based on the sample type. To achieve complete and accurate diagnosis and treatment of the disease, the complementary use of additional methods, such as bacterial culture and other molecular tests, is essential.

**References**


1. World Health Report. Global tuberculosis report 2024. Available online: https://www.who.int/teams/global-programme-on-tuberculosis-and-lung-health/tb-reports/global-tuberculosis-report-2024.
2. Floyd K, Glaziou P, Zumla A, Raviglione M. The global tuberculosis epidemic and progress in care, prevention, and research: an overview in year 3 of the End TB era. Lancet Respir Med 2018;6:299-314.
3. Yue X, Wang H, Jin D, et al. Healthcare data gateways: found healthcare intelligence on blockchain with novel privacy risk control. J Med Syst 2016;40:218.
4. Procop GW. Laboratory diagnosis and susceptibility testing for Mycobacterium tuberculosis. Microbiol Spectr 2016;4. doi: 10.1128/microbiolspec.TNMI7-0022-2016.
5. Cioboata R, Biciusca V, Olteanu M, Vasile CM. COVID-19 and tuberculosis: unveiling the dual threat and shared solutions perspective. J Clin Med 2023;12:4784.
6. Patra K, Batabyal S, Mandal K, et al. Tuberculosis and COVID-19: a combined global threat to human civilization. Clin Epidemiol Glob Health 2022;15:101031.
7. Abdulgader SM, Okunola AO, Ndlangalavu G, et al. Diagnosing tuberculosis: what do new technologies allow us to (not) do? Respiration 2022;101:797-813.
8. Machado D, Couto I, Viveiros M. Advances in the molecular diagnosis of tuberculosis: From probes to genomes. Infect Genet Evol 2019;72:93-112.
9. Orina F, Mwangi M, Meme H, et al. Intrinsic and extrinsic factors associated with sputum characteristics of presumed tuberculosis patients. PLoS One 2019;14:e0227107.
10. Bartolomeu-Gonçalves G, Souza JM, Fernandes BT, et al. Tuberculosis diagnosis: current, ongoing, and future approaches. Diseases 2024;12:202.
11. Boldi MO, Denis-Lessard J, Neziri R, et al. Performance of microbiological tests for tuberculosis diagnostic according to the type of respiratory specimen: a 10-year retrospective study. Front Cell Infect Microbiol 2023;13:1131241.
12. Ramachandra V, Brammacharry U, Muralidhar A, et al. Assess the diagnostic accuracy of GeneXpert to detect mycobacterium tuberculosis and rifampicin-resistant tuberculosis among presumptive tuberculosis and presumptive drug resistant tuberculosis patients. Microbiol Res 2024;15:91-108.

13. Yang Q, Han J, Shen J, et al. Diagnosis and treatment of tuberculosis in adults with HIV. Medicine 2022;101:e30405.
14. Agizew T, Chihota V, Nyirenda S, et al. Tuberculosis treatment outcomes among people living with HIV diagnosed using Xpert MTB/RIF versus sputum-smear microscopy in Botswana: a stepped-wedge cluster randomised trial. BMC Infect Dis 2019;19:1058.
15. Nadeem Z, Iqbal J, Kausar S, et al. Comparative analysis of the efficacies of the genexpert and solid culture media techniques in the diagnosis of Mycobacterium tuberculosis. Arch Razi Inst 2022;77:2065-72.
16. Gong X, He Y, Zhou K, et al. Efficacy of Xpert in tuberculosis diagnosis based on various specimens: a systematic review and meta-analysis. Front Cell Infect Microbiol 2023;13:1149741.
17. Dragonieri S, Bikov A, Capuano A, et al. Methodological aspects of induced sputum. Adv Respir Med 2023;91:397-406.
18. Metcalf T, Soria J, Montano SM, et al. Evaluation of the GeneXpert MTB/RIF in patients with presumptive tuberculous meningitis. PLoS One 2018;13:e0198695.
19. Vilchèze C, Kremer L. Acid-fast positive and acid-fast negative mycobacterium tuberculosis: the koch paradox. Microbiol Spectr 2017;5: 10.1128/microbiolspec.tbtb2-0003-2015.
20. Rodrigues da Costa R, Silva MR, Augusto CJ, Gonçalves Leite IC. Fast, simple and cheap: method modified from conventional cultivation for tuberculosis diagnosis allows seeding on Löwenstein-Jensen of any swab-embedded pulmonary samples decontaminated with sodium hydroxide. Trans R Soc Trop Med Hyg 2022;116:523-30.
21. McHugh ML. Interrater reliability: the kappa statistic. Biochem Med 2012;22:276-82.
22. Çorbacıoğlu ŞK, Aksel G. Receiver operating characteristic curve analysis in diagnostic accuracy studies: A guide to interpreting the area under the curve value. Turk J Emerg Med 2023,23:195-8.
23. Shah B, Shah SC, Kakadia P, et al. The clinician, the lab and the patient: Understanding lab diagnostics to eradicate tuberculosis. Indian J Tuberc 2023;70:42-8.
24. Elbrolosy AM, El Helbawy RH, Mansour OM, Latif RA. Diagnostic utility of GeneXpert MTB/RIF assay versus conventional methods for diagnosis of pulmonary and extra-pulmonary tuberculosis. BMC Microbiol 2021;21:144.
25. Kabir S, Parash MTH, Emran NA, et al. Diagnostic challenges and Gene-Xpert utility in detecting Mycobacterium tuberculosis among suspected cases of pulmonary tuberculosis. PLoS One 2021;16:e0251858.

26. Fernandes P, Ma Y, Gaeddert M, et al. Sex and age differences in Mycobacterium tuberculosis infection in Brazil. Epidemiol Infect 2018;146:1503-10.
27. Asare KK, Azumah DE, Adu-Gyamfi CO, et al. Comparison of microscopic and xpert MTB diagnoses of presumptive mycobacteria tuberculosis infection: retrospective analysis of routine diagnosis at Cape Coast Teaching Hospital. BMC Infect Dis 2024;24:660.
28. Rathish B, Wilson A, Pillay R, et al. A bundled approach to pulmonary tuberculosis testing: experience from a tertiary care centre in south India. Cureus 2019;11:e6042.
29. de Almeida IN, de AssisFigueredo LJ, Soares VM, et al. Evaluation of the mean cost and activity based cost in the diagnosis of pulmonary tuberculosis in the laboratory routine of a high-complexity hospital in Brazil. Front Microbiol 2017;8:249.
30. Chaudhary R, Bhatta S, Singh A, et al. Diagnostic performance of GeneXpert MTB/RIF assay compared to conventional Mycobacterium tuberculosis culture for diagnosis of pulmonary and extrapulmonary tuberculosis, Nepal. Narra J 2021;1:e33.
31. Mai NT, Thwaites GE. Recent advances in the diagnosis and management of tuberculous meningitis. Curr Opin Infect Dis 2017;30:123-8.
32. Gopalaswamy R, Dusthackeer VNA, Kannayan S, Subbian S. Extrapulmonary Tuberculosis—An Update on the Diagnosis, Treatment and Drug Resistance. J Respir 2021;1:141-64.
33. Ulain N, Ali A, Khan M, et al. Improving diagnosis of tuberculous lymphadenitis by combination of cytomorphology and MPT64 immunostaining on cell blocks from the fine needle aspirates. PLoS One 2022;17:e0276064.
34. Atnafu A, Desta K, Girma S, et al. Integration of cytopathology with molecular tests to improve the lab diagnosis for TBLN suspected patients. PLoS One 2022;17:e0265499.
35. Singh BK, Sharma SK, Sharma R, et al. Diagnostic utility of a line probe assay for multidrug resistant-TB in smear-negative pulmonary tuberculosis. PloS One 2017;12:e0182988.

Online supplementary material:
Supplementary Table 1. Distribution of patients by age groups according to product category and the results obtained from the three tests.
Supplementary Table 2. Performance of microscopic examination in relation to culture as the reference standard according to the type of sample.
Supplementary Table 3. Performance of the Xpert MTB/RIF in relation to culture (reference standard) according to the type of sample.
Supplementary Table 4. Performance of the Xpert MTB/RIF in relation to the composite reference standard according to the type of sample.

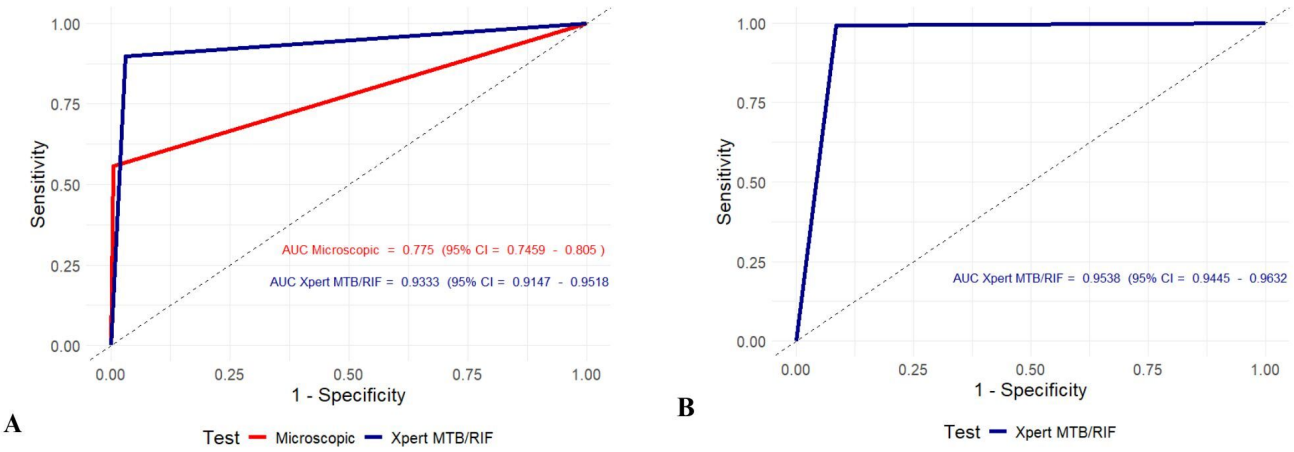


**Figure 1. ROC curve for microscopic examination, Xpert MTB/RIF analysis. A) AUC for microscopic examination, Xpert MTB/RIF relative to culture; B) AUC for Xpert MTB/RIF relative to composite reference standard.**