

## Role of Bronchoalveolar Lavage (BAL) Gene Xpert in Diagnosis of PTB in Sputum Smear Negative Patients

\*Alom KMM<sup>1</sup>, Ali MMI<sup>2</sup>, Mahmud MJ<sup>3</sup>, Ahmed MM<sup>4</sup>

### Abstract

The aim of this study was to assess the diagnostic efficacy of Xpert MTB/RIF assay in bronchoalveolar lavage (BAL) in the diagnosis of pulmonary tuberculosis in sputum-smear negative patients. This observational case series study was conducted in a tertiary base institute of Bangladesh from July 2017 to June 2019. All patients with suspected pulmonary tuberculosis who were sputum-smear negative, irrespective of age and gender were included in this study. BAL fluid thus obtained and examined the smear for acid fast bacilli (AFB) and Xpert MTB/RIF assay. The sensitivity, specificity and accuracy values were calculated for smear AFB and Xpert MTB/RIF assay. Xpert MTB/RIF was identified 33 (36.7%) of 90 patients on bronchoalveolar lavage fluid in the final analysis. AFB Fluid culture was positive in 28 (31.2%) cases. The sensitivity and specificity of Xpert MTB/RIF assay was 85.7%, and 85.5% respectively. The positive likelihood ratio was 5.90, and the negative likelihood ratio was 0.167. The accuracy was 85.56%. Xpert MTB/RIF on BAL fluid can be recommended as a fruitful diagnostic tool for mycobacterium pulmonary tuberculosis especially in sputum smear negative, and culture negative patients.

CBMJ 2023 January: vol. 12 no. 01 P: 60-66

**Keywords:** Xpert MTB/RIF assay, BAL fluid, Sputum, Pulmonary, Tuberculosis

### Introduction

Pulmonary Tuberculosis (PTB), a disease caused by contagious bacterial infection of the lungs with *Mycobacterium tuberculosis*, which causes an alarming public health problem, with high rates of morbidity and mortality all over the world.<sup>1</sup> PTB has been evaluated that there were about 10.0 million new cases of pulmonary tuberculosis in 2019, a year in which 1.2 million and 208,000 tuberculosis-related deaths occurred. Although the efforts made have reduced tuberculosis mortality by 42% since 2000 and saving the life worldwide. The strategy should be taken to reduce an annual decrease of approximately 4-5%, rather than the current 2%, would be required to reach the End PTB Strategy target of a 95% reduction by 2035. Bangladesh and Brazil are the highest 30 tuberculosis burden countries in the world. In Brazil, the estimated number of

new cases in a year was more than 90,000 in 2019.<sup>1-3</sup>

In Bangladesh, most (80%) tuberculosis is pulmonary tuberculosis that accounts for 3.6% of the global total, the estimated incidence of TB per 100,000 is 221 in Bangladesh, and the mortality rate is 24 per 100,000 population.<sup>3</sup>

1. \*Dr. K.M Monjurul Alom, Assistant Professor (Respiratory Medicine), Shaheed Ziaur Rahman Medical College, Bogura.
2. Dr. Mirza Mohammad Idris Ali, Assistant Professor (Respiratory Medicine), Cumilla Medical College, Cumilla.
3. Dr. Md. Jakaria Mahmud, Assistant Professor (Respiratory Medicine), M.A.G Osmani Medical College, Sylhet.
4. Dr. Md. Mohiuddin Ahmed, Professor & HOD (Respiratory Medicine), Dhaka Medical College, Dhaka.

**Address of Correspondence:**  
Email: mehbubkadir@gmail.com

Microbiological confirmation is achieved in only 40-60% of patients with PTB. Diagnosis based on clinical manifestation. Approximately 50% of all patients with PTB are sputum smear-negative for AFB or are unable to produce sputum samples of sufficient quantity that make the diagnosis quite challenging.<sup>1,3</sup> Fiberoptic bronchoscopy with BAL fluid is a reliable, rapid technique for collecting the useful specimens for the diagnosis of PTB.<sup>4-7</sup> Sputum smear microscopy for AFB is low sensitivity (approximately 40%). Mycobacterial culture is time consuming and the gold standard method that performs better (sensitivity 86%).<sup>8,9</sup> Therefore, Xpert MTB/RIF have been implemented to improve diagnostic accuracy in some cases, which would allow the proper treatment to be initiated early.<sup>10,11</sup> The Xpert MTB/RIF assay is a rapid ( $\leq 2$ hours) automated cartridge-based molecular test for the detection of Mycobacterium tuberculosis with its high sensitivity and specificity.<sup>12-14</sup> Currently, BAL Fluid have been shown to be suitable for analysis with the Xpert MTB/RIF assay.<sup>15-16</sup> However, there are few studies are mentioned worldwide in the literature on the analysis of BAL fluid samples by the Xpert MTB/RIF assay for the diagnosis of PTB. Therefore, the present study aimed to assess the performance of Xpert MTB/RIF assays of BALF samples from patients with suspected PTB who sputum was smear-negative or produce insufficient sputum samples. We have also evaluated the Xpert MTB/RIF assay in comparison with AFB staining, processing the BALF samples in parallel and using mycobacterial culture as the reference.

### Methodology

This observational case series study was carried out in the department of Respiratory Medicine of the National Institute of the Diseases of the Chest

and Hospital (NIDCH), Mohakhali, Dhaka, Bangladesh from the period of July 2017 to June 2019. The ethical clearance was obtained from the institutional review board (IRB) of the NIDCH.

All the patients irrespective of age and gender presented with sputum smear negative pulmonary tuberculosis despite positive clinical and radiological (Chest X-Ray) findings of PTB were selected in this study according to selection criteria. Patients with severe dyspnoea and contraindications for fibre optic bronchoscopy were excluded from this study. The flow chart of the study patients is shown in figure 1. Data were in a data sheet for each patient who had positive clinical features and high-resolution chest X-ray suggestive for pulmonary tuberculosis but sputum smear and Xpert were negative. Fibre-optic bronchoscopy (FOB) was meticulously performed via trans-nasal or trans-oral route for taking bronchoalveolar fluid lavage. BAL fluid (BALF) was sent for Xpert MTB/RIF and AFB culture in liquid culture medium by mycobacterium growth indicator tube system. Sensitivity, specificity, accuracy of Xpert MTB/RIF on BALF was analysed and compared with AFB culture. An informed written informed consent was taken from all participating patients.

### Flow chart of the study

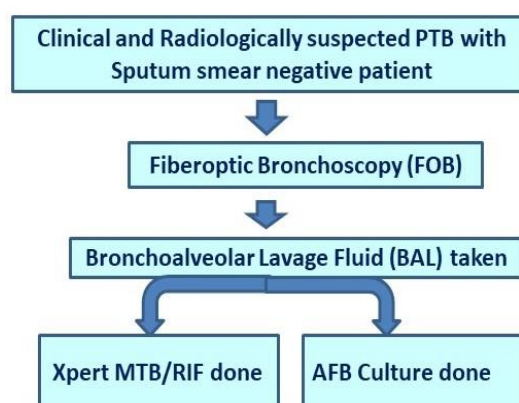


Figure 1 shows the flow chart of the study

## Results

We enrolled ninety patients in this study. Among them, Male were 59%, and female were 41%. The mean age ( $\pm$ SD) of the study subjects was 41.95 ( $\pm$ 16.48) years. The minimum age was 12 years, and the maximum age was 70 years, the median was 40 years. According to presentation of the patients, cough was the most common (100%) followed by fever (98.8%), anorexia (69%), weight loss (53.3%), sputum expectoration (34.4%), chest pain (16.7%), and haemoptysis (13.3%). The following comorbidities were associated with 22 (24.4%) patients (Table-1).

**Table-1** shows the demographic and clinical profile of the patients with sputum smear negative PTB (N=90)

Variables	Result/Value
Mean Age (Years) $\pm$ SD	41.95 $\pm$ 16.48
Median age (Years)	40
Age range (Maximum-minimum)	70-12
<b>Gender</b>	
Male	53 (59%)
Female	37 (41%)
<b>Symptoms</b>	
Cough	90 (100%)
Fever	89 (98.8%)
Anorexia	69 (76.7%)
Weight Loss	48 (53.3%)
Sputum Expectoration	31 (34.4%)
Hemoptysis	12 (13.3%)
Chest Pain	15 (16.7%)
<b>Comorbidities</b>	
<b>22 (24.4%)</b>	
COPD	07
DM	05
CKD	00
DM+CKD+COPD	04
DM +CKD	03
DM+ COPD	02
CKD+COPD	01
None	68 (75.5%)

All three comorbidities (diabetes, chronic kidney disease, and chronic obstructive pulmonary disease) were present in 4 (4.4%) patients. Table 2 shows Xpert MTB/RIF was positive in 33 (36.7%) among the ninety patients, in which AFB culture positive in 24 (26.7%) and negative was 9 (10%) patients.

Total culture positive was in 28 (31%) patients in which Xpert MTB/RIF negative was 4 (4.4%) patients. AFB culture is the gold standard diagnostic tool for PTB, Xpert MTB/RIF in Bronchoalveolar lavage fluid of the patients with sputum smear and Xpert negative PTB had sensitivity, specificity, positive likelihood ratio, and negative likelihood ratio were 85.7%, 85.48%, 5.91. and 0.17 respectively. The accuracy was 85.56%.

**Table 2** shows the profile of Xpert MTB/RIF and AFB culture in Broncho Alveolar lavage Fluid

Xpert MTB/RIF	AFB Positive	AFB Negative	Xpert MTB/RIF
Positive	24 (26.7%)	09 (10%)	33 (36.7%)
Negative	04 (4.4%)	53 (59%)	57 (63.3%)
Total	28 (31.2%)	62(68.8%)	90

## Discussion

We knew that the mycobacterial culture has long been considered the gold standard technique for the diagnosis of PTB. Still, 40-50% of cases are not diagnosed by microbiological methods due to its low sensitivity and limited specificity.<sup>1</sup> In addition, some patients do not produce significant amount of sputum for microbiological analysis.<sup>1,4</sup> The Xpert MTB/RIF is an automated rapid cartridge-based nucleic acid amplification test (NAAT) which can detect simultaneously Mycobacterium tuberculosis (MTB) and resistance to rifampicin (RIF) by polymerase

chain reaction.<sup>17</sup> The role of the Xpert MTB/RIF molecular test in BALF with sputum smear negative patients is well documented.<sup>14,18-22</sup> In this study, we found that the Xpert MTB/RIF assay is a rapid molecular test for tuberculosis which performed better in BAL fluid from suspected PTB with sputum-smear negative patients. Recently, the Brazilian government has recommended the use of the Xpert MTB/RIF assay in BAL fluid, which is obtained by fiberoptic bronchoscopy, as an alternative way to confirm the diagnosis in suspected cases of tuberculosis.<sup>2,3</sup> The Xpert MTB/RIF assay offers many advantages. It is a rapid procedure is providing results within two hours, thus allowing antituberculosis regimen to be initiated as soon as possible; high sensitivity, specificity, and overall accuracy; detecting rifampin-resistant *Mycobacterium tuberculosis* strains; and high sensitivity and specificity for the diagnosis of tuberculosis in HIV-infected patients.<sup>12,14,23</sup> Despite its advantages, it has few limitations which include a significant false-positive rate and the high costs of equipment, reagent and its maintenance. Though its limitations, the Xpert MTB/RIF assay has made an essential role as a rule-in or rule-out test for the diagnosis of tuberculosis in laboratory medicine and pulmonology practices.<sup>24,25</sup>

A study demonstrated on the comparison with mycobacterial culture, the Xpert MTB/RIF assay is superior for diagnosing *Mycobacterium tuberculosis* and rifampin resistance showing high sensitivity (91.86%) and PPV (97.53%) in patients with PTB who presented with sputum smear-negative.<sup>26</sup> Other study showed that the Xpert MTB/RIF assay is performed better in sputum and BAL fluid than did AFB staining.<sup>27</sup> The sensitivity, specificity, positive likelihood

ratio, and negative likelihood ratio were 85.7%, 85.48%, 5.91. and 0.17 respectively. The accuracy was 85.56%. Our study is consisting with a result of a recent study in India compared the Xpert MTB/RIF assay, AFB staining, and mycobacterial culture in BAL fluid samples from suspected cases of PTB.<sup>28</sup> Our data support the use of the Xpert MTB/RIF assay in BAL fluid samples is playing an interesting and suitable tool to improve the diagnosis of tuberculosis.

In the present study, the true positive of Xpert MTB/RIF was in 24 of total 33 Xpert MTB/RIF sample positive patient. AFB microbiological samples was positive in 28 (31.2%) cases among the 90 study subjects. There are nine patients (10%) in whom BAL fluid sample tested positive on the Xpert MTB/RIF assay, despite testing negative on AFB microbiological test. A study reported on two patients with the tested positive on the Xpert MTB/RIF assay in BAL fluid samples, which was testing negative on AFB staining and culture.<sup>29</sup>

Our study has some limitations including a single-center study, and small sample size. However, the study was performed at a specialized, tertiary level, referral, and teaching hospital in Bangladesh, where numerous tools are available for the investigation and accurate diagnosis of cases of tuberculosis.

## Conclusion

In BAL fluid samples, the Xpert MTB/RIF assay is performed better than AFB staining for the detecting of PTB in patients who are sputum smear-negative or unable to produce significant amount of sputum specimens. That could have a significant role on the management of such cases due to its rapid and reliable procedure for the diagnosis.

## References

1. World Health Organization [homepage on the Internet]. Geneva: World Health Organization. *Global tuberculosis report 2020*. Available from: <https://apps.who.int/iris/bitstream/handle/10665/329368/9789241565714-eng.pdf?ua=1>.
2. Brito GMX, Mafort TT, Ribeiro-Alves M, Reis LVT, Leung J, Leão RS, et al. Diagnostic performance of the Xpert MTB/RIF assay in BAL fluid samples from patients under clinical suspicion of pulmonary tuberculosis: a tertiary care experience in a high-tuberculosis-burden area. *J Bras Pneumol*. 2021;47(2): e20200581.
3. Nazneen A, Tarannum S, Chowdhury KIA, Islam MT, Islam SMH, Ahmed S, et al. (2021) Implementation status of national tuberculosis infection control guidelines in Bangladeshi hospitals. *PLoS ONE* 16(2): e0246923. <https://doi.org/10.1371/journal.pone.0246923>.
4. Shin JA, Chang YS, Kim TH, Kim HJ, Ahn CM, Byun MK. Fiberoptic bronchoscopy for the rapid diagnosis of smear-negative pulmonary tuberculosis. *BMC Infect Dis*. 2012; 12:141. <https://doi.org/10.1186/1471-2334-12-141>.
5. Nikbakhsh N, Bayani M, Siadati S. The Value of Bronchoalveolar Lavage in the Diagnosis of Sputum Smear-Negative Pulmonary Tuberculosis. *Iran J Pathol*. 2015;10(1):35-40.
6. Kalawat U, Sharma KK, Reddy PN, Kumar AG. Study of bronchoalveolar lavage in clinically and radiologically suspected cases of pulmonary tuberculosis. *Lung India*. 2010;27(3):122-124. <https://doi.org/10.4103/0970-2113.68307>.
7. Saglam L, Akgun M, Aktas E. Usefulness of induced sputum and fiberoptic bronchoscopy specimens in the diagnosis of pulmonary tuberculosis. *J Int Med Res*. 2005;33(2):260-265. <https://doi.org/10.1177/1473230005033002>
8. Steingart KR, Ng V, Henry M, Hopewell PC, Ramsay A, Cunningham J, et al. Sputum processing methods to improve the sensitivity of smear microscopy for tuberculosis: a systematic review. *Lancet Infect Dis*. 2006;6(10):664-674. [https://doi.org/10.1016/S1473-3099\(06\)70602-8](https://doi.org/10.1016/S1473-3099(06)70602-8).
9. Lange C, Mori T. Advances in the diagnosis of tuberculosis. *Respirology*. 2010;15(2):220-240. <https://doi.org/10.1111/j.1440-1843.2009.01692.x>
10. Helb D, Jones M, Story E, Boehme C, Wallace E, Ho K, et al. Rapid detection of *Mycobacterium tuberculosis* and rifampin resistance by use of on-demand, near-patient technology. *J Clin Microbiol*. 2010;48(1):229-237. <https://doi.org/10.1128/JCM.01463-09>.
11. Boehme CC, Nabeta P, Hillemann D, Nicol MP, Shenai S, Krapp F, et al. Rapid molecular detection of tuberculosis and rifampin resistance. *N Engl J Med*. 2010;363(11):1005-1015. <https://doi.org/10.1056/NEJMoa0907847>].
12. Sharma SK, Kohli M, Yadav RN, Chaubey J, Bhasin D, Sreenivas V, et al. Evaluating the Diagnostic Accuracy of Xpert MTB/RIF Assay in Pulmonary Tuberculosis. *PLoS One*. 2015;10(10): e0141011. <https://doi.org/10.1371/journal.pone.0141011>.
13. Castro AZ, Moreira AR, Oliveira J, Costa PA, Graça CLALD, Pérez MA, et al. Clinical impact and cost analysis of the use of either the Xpert MTB Rif test or sputum smear microscopy in the diagnosis of pulmonary tuberculosis in Rio de Janeiro, Brazil. *Rev Soc Bras Med Trop*. 2018;51(5):631-637. <https://doi.org/10.1590/0037-8682-0082-2018>.
14. Steingart KR, Schiller I, Horne DJ, Pai M, Boehme CC, Dendukuri N. Xpert® MTB/RIF-

- assay for pulmonary tuberculosis and rifampicin resistance in adults. *Cochrane Database Syst Rev.* 2014;2014(1):CD009593. <https://doi.org/10.1002/14651858.CD009593.pub3>.
15. Theron G, Peter J, Calligaro G, Meldau R, Hanrahan C, Khalfey H, et al. Determinants of PCR performance (Xpert MTB/RIF), including bacterial load and inhibition, for TB diagnosis using specimens from different body compartments. *Sci Rep.* 2014; 4:5658. <https://doi.org/10.1038/srep05658>.
  16. Zeka AN, Tasbakan S, Cavusoglu C. Evaluation of the GeneXpert MTB/RIF assay for rapid diagnosis of tuberculosis and detection of rifampin resistance in pulmonary and extrapulmonary specimens. *J Clin Microbiol.* 2011;49(12):4138-4141. <https://doi.org/10.1128/JCM.05434-11>.
  17. Van RA, Page-Shipp L, Scott L, Sanne I, Stevens W. Xpert MTB/RIF for point-of-care diagnosis of TB in high-HIV burden, resource-limited countries: hype or hope? *Expert Rev. Mol. Diagn.* 2010;10: 937-946.
  18. Meyer AJ, Atuheire C, Worodria W, Kizito S, Katamba A, Sanyu I, et al. Sputum quality and diagnostic performance of GeneXpert MTB/RIF among smear-negative adults with presumed tuberculosis in Uganda. *PLoS One.* 2017;12(7): e0180572. <https://doi.org/10.1371/journal.pone.0180572>.
  19. 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. <https://doi.org/10.1177/2058738419827174>
  20. Boonsarngsuk V, Suwannaphong S, Laohavich C. Combination of adenosine deaminase activity and polymerase chain reaction in bronchoalveolar lavage fluid in the diagnosis of smear-negative active pulmonary tuberculosis. *Int J Infect Dis.* 2012;16(9): e663-e668. <https://doi.org/10.1016/j.ijid.2012.05.006>.
  21. Altaf Bachh A, Gupta R, Haq I, Varudkar HG. Diagnosing sputum/smear-negative pulmonary tuberculosis: Does fibre-optic bronchoscopy play a significant role? *Lung India.* 2010;27(2):58-62. <https://doi.org/10.4103/0970-2113.63607>.
  22. Chandra TJ, Dash S, Srinivas G, Rao PV. A study on rapid confirmation of pulmonary tuberculosis in smear-negative acid-fast bacilli cases by using fiberoptic bronchoscopy, done through a trans oro pharyngeal spacer. *J Family Community Med.* 2012;19(1):43-46. <https://doi.org/10.4103/2230-8229.94014>.
  23. Lawn SD, Brooks SV, Kranzer K, Nicol MP, Whitelaw A, Vogt M, et al. Screening for HIV-associated tuberculosis and rifampicin resistance before antiretroviral therapy using the Xpert MTB/RIF assay: a prospective study. *PLoS Med.* 2011;8(7): e1001067. <https://doi.org/10.1371/journal.pmed.1001067>.
  24. Hermans S, Caldwell J, Kaplan R, Cobelens F, Wood R. The impact of the roll-out of rapid molecular diagnostic testing for tuberculosis on empirical treatment in Cape Town, South Africa. *Bull World Health Organ.* 2017;95(8):554-563. <https://doi.org/10.2471/BLT.16.185314>.
  25. Deggim V, Somoskovi A, Voit A, Böttger EC, Bloemberg GV. Integrating the Xpert MTB/RIF assay into a diagnostic workflow for rapid detection of *Mycobacterium tuberculosis* in a-

- low-prevalence area. *J Clin Microbiol.* 2013;51(7):2396-2399. <https://doi.org/10.1128/JCM.00151-13>.
26. Khalil KF, Butt T. Diagnostic yield of Bronchoalveolar Lavage gene Xpert in smear-negative and sputum-scarce pulmonary tuberculosis. *J Coll Physicians Surg Pak.* 2015;25(2):115-118.
27. Agrawal M, Bajaj A, Bhatia V, Dutt S. Comparative Study of GeneXpert with ZN Stain and Culture in Samples of Suspected Pulmonary Tuberculosis. *J Clin Diagn Res.* 2016;10(5): DC09-DC12. <https://doi.org/10.7860/JCDR/2016/18837.7755>.
28. Bashir YUI, Nahvi N, Khan S, Jahan T. Diagnostic Utility of Bronchoalveolar Lavage Xpert MTB/RIF Assay in Suspected Cases of Pulmonary Tuberculosis. *Int J Contemp Med Res.* 2019;6(6): F8-F11. <https://doi.org/10.21276/ijcmr.2019.6.6.29>.
29. Ssengooba W, Respeito D, Mambuque E, Blanco S, Bulo H, Mandomando I, et al. Do Xpert MTB/RIF Cycle Threshold Values Provide Information about Patient Delays for Tuberculosis Diagnosis? *PLoS One.* 2016;11(9): e0162833. <https://doi.org/10.1371/journal.pone.0162833>.