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

Guilherme Machado Xavier de Brito, Thiago Thomaz Mafort, Marcelo Ribeiro-Alves, Larissa Vieira Tavares dos Reis, Janaina Leung, Robson Souza Leão, Rogério Rufino, Luciana Silva Rodrigues

ABSTRACT
Objective: To assess the diagnostic performance of the Xpert MTB/RIF assay, a rapid molecular test for tuberculosis, comparing it with that of AFB staining and culture, in BAL fluid (BALF) samples from patients with clinically suspected pulmonary tuberculosis (PTB) who are sputum smear-negative or produce sputum samples of insufficient quantity. Methods: This was a retrospective study of 140 cases of suspected PTB in patients who were smear-negative or produced insufficient sputum samples and were evaluated at a tertiary teaching hospital in the city of Rio de Janeiro, Brazil. All of the patients underwent fiberoptic bronchoscopy with BAL. The BALF specimens were evaluated by AFB staining, mycobacterial culture, and the Xpert MTB/RIF assay. Results: Among the 140 patients, results for all three microbiological examinations were available for 73 (52.1%), of whom 22 tested positive on culture, 17 tested positive on AFB staining, and 20 tested positive on the Xpert MTB/RIF assay. The sensitivity, specificity, positive predictive value, negative predictive value, and overall accuracy for AFB staining were 68.1%, 96.1%, 98.2%, 87.5%, and 87.6%, respectively, compared with 81.8%, 96.1%, 90.0%, 92.4%, and 91.8%, respectively, for the Xpert MTB/RIF assay. The agreement between AFB staining and culture was 82.3% (kappa = 0.46; p < 0.001), whereas that between the Xpert MTB/RIF assay and culture was 91.8% (kappa = 0.8; p < 0.001). Conclusions: In BALF samples, the Xpert MTB/RIF assay performs better than do traditional methods, providing a reliable alternative to sputum analysis in suspected cases of PTB. However, the rate of discordant results merits careful consideration.

Keywords: Tuberculosis; Molecular diagnostic techniques; Bronchoscopy; Bronchoalveolar lavage fluid; Mycobacterium tuberculosis.

INTRODUCTION
Tuberculosis, a disease caused by infection with Mycobacterium tuberculosis, continues to be an alarming public health problem, with high rates of morbidity and mortality worldwide. It has been estimated that there were approximately 10.0 million new cases of tuberculosis in 2019, a year in which 1.2 million and 208,000 tuberculosis-related deaths occurred among non-HIV-infected and HIV-infected individuals, respectively. Although the efforts made have saved millions of lives worldwide, reducing tuberculosis mortality by 42% since 2000, an annual decrease of approximately 4–5%, rather than the current 2%, would be needed in order to reach the End TB Strategy target of a 95% reduction by 2035. In Brazil, which is one of the 30 countries with the highest tuberculosis burden, the estimated number of new cases of tuberculosis in 2019 was more than 90,000.

One major concern regarding the management of cases of tuberculosis is that microbiological confirmation is achieved in only 40-60% of patients with pulmonary tuberculosis (PTB), the main clinical manifestation and the type of tuberculosis that is the most relevant in the chain of transmission. In addition, approximately half of all patients with PTB are sputum smear-negative for AFB or are unable to produce sputum samples of sufficient quantity, making the diagnosis quite challenging. In such patients, fiberoptic bronchoscopy with BAL is a reliable, rapid method of collecting useful specimens for the diagnosis of PTB. However, conventional methods, such as Ziehl-Neelsen staining, which detects AFB, and mycobacterial cultures, have poor diagnostic yields. Sputum smear microscopy for AFB is limited by its low sensitivity (approximately 40%), whereas mycobacterial culture, which is considered the gold standard, performs better (with a sensitivity of approximately 86%),
Methods

Ethical aspects

The study was approved by the Research Ethics Committee of Pedro Ernesto University Hospital of the State University of Rio de Janeiro (Reference no. 2.013.455). All participating patients gave written informed consent.

Study design and participants

This was a retrospective analytical study of 149 patients who were sputum smear-negative, despite clinical and radiological findings suggestive of PTB. The patients were evaluated at Pedro Ernesto University Hospital, a tertiary care referral center in the city of Rio de Janeiro, Brazil, between November of 2015 and October of 2017. All of the patients underwent fiberoptic bronchoscopy. The BALF samples collected were evaluated by AFB staining, mycobacterial culture, and Xpert MTB/RIF assay. The following inclusion criteria were applied: being suspected of having PTB on the basis of clinical, physical, and radiological findings; being sputum smear-negative, producing insufficient sputum samples, or testing negative for M. tuberculosis on sputum culture; and having been referred for fiberoptic bronchoscopy. Patients who tested positive for M. tuberculosis in sputum samples (by smear microscopy or culture) were excluded, as were those with malignancy, those with fungal infection, those who were on antituberculosis regimens for more than 2 weeks, and those with a confirmed diagnosis of infection with nontuberculous mycobacteria (NTM). A confirmed diagnosis of PTB was defined as M. tuberculosis growth on mycobacterial culture of a BALF sample, which was considered the reference method. A probable diagnosis of PTB was defined as not meeting the criteria for a confirmed diagnosis of PTB but having clinical and radiological findings suggestive of tuberculosis, as well as showing a clinical response to antituberculosis treatment. Sociodemographic and clinical characteristics, such as gender, age, smoking status, history of tuberculosis, HIV status, and the presence of cavitary disease, were collected from hospital records. Smokers were defined as individuals who had smoked at least 100 cigarettes (or the equivalent) in their lifetime, and former smokers were defined as those who had quit smoking more than 12 months prior.

Patient evaluation and sample collection

In patients under intravenous sedation, clinical investigators used a flexible fiberoptic bronchoscope to perform transnasal bronchoscopy, during which there was continuous monitoring of heart rate, blood pressure, and SpO₂. In brief, after the bronchial tree had been inspected, BAL was performed by instilling sterile saline (0.9%) in serial 20-mL aliquots (up to a maximum of 200 mL). At least 50% of the total volume of the aspirate was returned, then being divided into three parts and sent to the laboratory for Ziehl-Neelsen (AFB) staining, mycobacterial culture, and Xpert MTB/RIF assay.

Smear microscopy

The BALF specimens collected from all patients were centrifuged at 3,000 × g for 15 min, after which they were submitted to microbiological tests according to Brazilian Health Regulatory Agency guidelines. The supernatant was carefully removed, after which it was processed in parallel for each subsequent test. For the AFB staining, a BALF sample was smeared on a glass microscope slide and allowed to dry, after which the staining was performed. After staining, a minimum of 100 fields were examined with a ×100 oil immersion objective and the smear was scanned systematically. The observation of at least one AFB was considered a positive result.

Solid culture

The BALF samples were decontaminated by using sodium hydroxide in a final concentration of 4%. After centrifugation, the supernatant was discarded and the precipitate was inoculated into tubes containing Löwenstein-Jensen solid medium. Cultures were
incubated at 37°C for 60 days, being examined weekly for up to 8 weeks or until growth was observed. The growth of *M. tuberculosis* was identified by using a rapid immunochromatographic MPT64 antigen testing kit (SD Bioline TB Ag MPT64 Rapid test; Standard Diagnostics Inc., Yongin-si, South Korea), according to the manufacturer’s instructions.

**Xpert MTB/RIF assay**

Sediment from the BALF samples was processed with a DNA/RNA extraction protocol, after which the Xpert MTB/RIF platform was employed according to the manufacturer’s instructions. In brief, the samples were diluted with the sample reagent provided by the manufacturer. The clinical specimens and reagent mixture were shaken in the vortex mixer for at least 10 s and left to settle at room temperature for 15 min. As recommended, all of the solutions were then transferred to the Xpert cartridge and loaded onto the GeneXpert equipment for analysis.

**Statistical analysis**

In the comparison of sociodemographic, clinical, and biochemical features between the two groups (culture-confirmed PTB and non-culture-confirmed PTB), we tested the hypothesis that the different samples in the comparison were drawn from the same distribution or distributions with the same median by using Mann-Whitney U tests for continuous variables. Fisher’s exact tests were used in order to evaluate frequencies between the two groups, testing the hypothesis of independence between the groups and the categorical variables. The kappa statistic was used in analyses of agreement among mycobacterial culture, the Xpert MTB/RIF assay, and AFB staining. For the detection of *M. tuberculosis* in BALF samples, the performance of the Xpert MTB/RIF assay and AFB staining, as well as combinations of the two, in comparison with the gold-standard (mycobacterial culture), was estimated. We used leave-one-out cross-validation to determine their accuracy, specificity, sensitivity, positive predictive value (PPV), and negative predictive value (NPV), as well as determining their rates of false-positive and false-negative results, together with the corresponding 95% confidence intervals. All statistical analyses were performed with the program R, version 3.5.2 (The R Foundation for Statistical Computing, Vienna, Austria).

**RESULTS**

**Study design and patient characteristics**

As depicted in the flow chart in Figure 1, we enrolled 149 patients who presented with clinical and radiological findings suggestive of PTB, who were sputum smear-negative or who produced sputum samples of insufficient quantity, and who underwent fiberoptic bronchoscopy. After the BALF samples had been processed and evaluated, 9 patients were excluded for receiving a final diagnosis of NTM infection. Therefore, the final study population comprised 140 patients for whom BALF test results were available. Mycobacterial culture results were available for 140 patients, *M. tuberculosis* being identified in 22 (15.7%) of those patients, compared with 31 (23.8%) of the 130 patients for whom AFB staining results were available and 20 (24.4%) of the 82 patients for whom Xpert MTB/RIF assay results were available. Results for all three examinations were available for 73 patients, of whom 22 (30.1%) tested positive on culture, 17 (23.2%) tested positive on AFB staining, and 20 (27.4%) tested positive on the Xpert MTB/RIF assay. None of the strains isolated were found to be resistant to rifampin.

Table 1 shows the demographic and clinical features of the study population, by outcome of the mycobacterial culture of the BALF samples. The median age of the patients was 56 years (IQR = 28.25 years). Seventy-eight patients (55.7%) were male, and there were no significant differences between the two groups in terms of gender distribution. All of the patients had at least one symptom suggestive of tuberculosis, the most common symptoms being weight loss (in 59.3%; p = 0.008), cough (in 57.1%), and dyspnea (in 53.6%). Cavitary disease was observed in 25 patients (17.9%). Of the 140 patients in the study population, 31 (22.1%) had previously had tuberculosis: 28 in the negative BALF culture group and 3 in the positive BALF culture group.

**Performance of AFB staining and the Xpert MTB/RIF assay in BALF samples**

Among the patients in the study population, the three methods for *M. tuberculosis* detection were used heterogeneously (Figure 1). Primarily, the test results were analyzed in different combinations (pairs) to evaluate the overall agreement among the Xpert MTB/RIF assay, AFB staining, and mycobacterial culture of BALF samples in suspected cases of PTB. As shown in Table 2, the Xpert MTB/RIF assay showed 91.78% agreement with culture (kappa = 0.8; p < 0.0001), compared with 82.31% for AFB staining (kappa = 0.46; p < 0.0001), regardless of whether we analyzed the sample as a whole (n = 140) or only those patients for whom results from all three examinations were available (n = 73). The agreement between the Xpert MTB/RIF assay and AFB staining was 82.2% (kappa = 0.53; p < 0.0001).

When we compared the Xpert MTB/RIF assay and AFB staining in terms of their individual diagnostic performance in BALF samples, using mycobacterial culture as the reference, we found that the Xpert MTB/RIF assay had an overall accuracy of 92%, a sensitivity of 81%, a PPV of 90%, and an NPV of 92%, compared with only 87%, 68%, 88%, and 87%, respectively, for AFB staining. Two different conditions ("OR" and "AND") were also analyzed. When the two methods were used in combination (Xpert MTB/RIF assay AND AFB staining) the specificity and PPV increased to 100%. However, the false-positive rate was higher when one or the other method was used (Xpert MTB/RIF assay OR AFB staining) than when the two were used in combination (Table 3).
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

To promote better identification and visualization of the overall performance of the methods evaluated, we created a Venn diagram to illustrate the outcomes of the BAL Xpert MTB/RIF assay, AFB staining, and mycobacterial culture of BALF samples in the 73 patients for whom results from all three examinations were available (Figure 2). The distribution of positive (+) and negative (−) results was as follows: Xpert MTB/RIF

---

**Table 1. Sociodemographic and clinical characteristics of the study population, by the results of mycobacterial culture of BAL fluid samples.**

| Characteristic            | Result of BALF culture | Total | p  |
|---------------------------|------------------------|-------|----|
|                           | Negative (n = 118)     |       |    |
|                           | Positive (n = 22)      |       |    |
|                           | Total (N = 140)        |       |    |
| Gender                    |                        |       |    |
| Male                      | 69 (49.3)              | 9 (6.4) | 78 (55.7) | 0.162 |
| Female                    | 49 (35.0)              | 13 (9.3) | 62 (44.3) |      |
| Age in years, median (IQR)| 56 (26.25)            | 50 (30.00) | 56 (28.25) | 0.6002|
| Smoking status            |                        |       |    |
| Never smoker              | 60 (42.9)              | 16 (11.4) | 76 (54.3) | 0.2018|
| Former smoker             | 33 (23.6)              | 4 (2.9)  | 37 (26.4) |      |
| Current smoker            | 25 (17.9)              | 2 (1.4)  | 27 (19.3) |      |
| Clinical features         |                        |       |    |
| HIV-infected              | 7 (5.0)                | 4 (2.9)  | 11 (7.9)  | 0.0718|
| Previous tuberculosis     | 28 (20.0)              | 3 (2.1)  | 31 (22.1) | 0.406 |
| Symptoms                  |                        |       |    |
| Chest pain                | 46 (32.9)              | 8 (5.7)  | 54 (38.6) | 1.00  |
| Dyspnea                   | 66 (47.1)              | 9 (6.4)  | 75 (53.6) | 0.246 |
| Fever                     | 45 (32.1)              | 6 (4.3)  | 51 (36.4) | 0.4698|
| Hemoptysis                | 49 (35.0)              | 4 (2.9)  | 53 (37.9) | 0.054 |
| Cough                     | 67 (47.9)              | 13 (9.3) | 80 (57.1) | 1.00  |
| Weight loss               | 76 (54.3)              | 7 (5.0)  | 83 (59.3) | 0.0081|
| Cavitary disease          |                        |       |    |
| Yes                       | 19 (13.6)              | 6 (4.3)  | 25 (17.9) | 0.229 |
| No                        | 99 (70.7)              | 16 (11.4) | 115 (82.1) |      |

BALF: BAL fluid. *Values expressed as n (%), except where otherwise indicated. †Determined by chest X-ray examination.

---

**Figure 1.** Flow chart of the study design and yield of the tuberculosis diagnostic tests performed in BAL fluid samples. *Performed in only 130 of the 140 patients. †Performed in only 82 of the 140 patients.
Brito GMX, Mafort TT, Ribeiro-Alves M, Reis LVT, Leung J, Leão RS, Rufino R, Rodrigues LS

assay+/culture+/AFB staining+ = 12 patients; Xpert MTB/RIF assay−/culture+/AFB staining− = 1 patient; Xpert MTB/RIF assay+/culture+/AFB staining− = 6 patients; Xpert MTB/RIF assay+/culture+/AFB staining− = 3 patients; Xpert MTB/RIF assay+/culture+/AFB staining− = 2 patients. Altogether, the Xpert MTB/RIF assay identified three more cases than did AFB staining.

**DISCUSSION**

As previously mentioned, even though mycobacterial culture has long been considered the gold standard method for the diagnosis of PTB, 40-50% of cases are not detected by microbiological methods. Sputum smear microscopy has low sensitivity and limited specificity, the latter evidenced by its incapacity to differentiate between *M. tuberculosis* and NTM species. In addition, some patients do not produce significant quantities of spontaneous or induced sputum, which makes it difficult to obtain sufficient clinical specimens for microbiological analysis. In this scenario, empirical antituberculosis treatment emerges as a possible strategy to interrupt the spread of the disease and minimize the associated mortality. In the present study, we found that the Xpert MTB/RIF assay—a rapid molecular test for tuberculosis—performed better than did AFB staining in BALF samples from patients with suspected PTB who were sputum smear-negative or produced sputum samples of insufficient quantity, in a high-tuberculosis-burden area.

Recently, the Brazilian National Ministry of Health has recommended the use of the Xpert MTB/RIF assay in

### Table 2. Agreement among the results of the Xpert MTB/RIF assay, AFB staining, and mycobacterial culture in BAL fluid samples.

| Comparator methods | Results of the comparison methods | Comparator method and results | Agreement | kappa | p  |
|--------------------|-----------------------------------|-------------------------------|------------|-------|-----|
|                    |                                   | Culture                       |            |       |     |
| Xpert MTB/RIF assay| Negative                          | Negative                      | 118 (84.3) | 22 (15.7) | 91.78% | 0.79963 | < 0.0001 |
|                    | Positive                          | Positive                      | 49 (35.0)  | 4 (2.9)   | 82.31% | 0.45892 | < 0.0001 |
| AFB staining       | Negative                          | Positive                      | 2 (1.4)    | 18 (12.9) |       |         |     |
|                    | Positive                          | Positive                      | 92 (65.7)  | 7 (5.0)   |       |         |     |
|                    | Negative                          | Positive                      | 16 (11.4)  | 15 (10.7) |       |         |     |

### Table 3. Diagnostic performance of the Xpert MTB/RIF assay and AFB staining in BAL fluid samples, in comparison with the reference method (mycobacterial culture).

| Measure          | Xpert MTB/RIF % (95% CI) | AFB staining % (95% CI) | Xpert MTB/RIF OR AFB staining % (95% CI) | Xpert MTB/RIF AND AFB staining % (95% CI) |
|------------------|--------------------------|-------------------------|------------------------------------------|------------------------------------------|
| Accuracy         | 91.78 (87.35 to 96.21)   | 87.67 (82.37 to 92.97)  | 93.15 (89.08 to 97.22)                    | 86.3 (80.76 to 91.85)                    |
| Sensitivity      | 81.82 (70.11 to 93.52)   | 68.18 (54.05 to 82.85)  | 95.45 (89.13 to 101.78)                   | 54.55 (39.43 to 69.66)                   |
| Specificity      | 96.08 (92.31 to 99.85)   | 96.08 (92.31 to 99.85)  | 92.16 (86.94 to 97.37)                    | 100.00 (100.00 to 100.00)               |
| PPV              | 90.00 (80.4 to 99.6)     | 88.74 (76.95 to 99.52)  | 84.00 (73.62 to 94.38)                    | 100.00 (100.00 to 100.00)               |
| NPV              | 92.45 (87.43 to 97.48)   | 87.50 (81.39 to 93.61)  | 97.92 (95.06 to 100.78)                   | 83.61 (77.06 to 90.16)                  |
| FPR              | 3.92 (0.15 to 7.69)      | 3.92 (0.15 to 7.69)      | 7.84 (2.63 to 13.06)                      | 0.00 (0.00 to 0.00)                     |
| FNR              | 18.18 (6.48 to 29.89)    | 31.82 (17.68 to 45.95)  | 4.55 (–1.78 to 10.87)                     | 45.45 (30.34 to 60.57)                  |

PPV: positive predictive value; NPV: negative predictive value; FPR: false-positive rate; and FNR: false-negative rate.

Test results are presented as n (%).
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

Clinical specimens other than sputum, such as BALF, which is obtained by fiberoptic bronchoscopy, as an alternative way to investigate suspected cases of tuberculosis. The Xpert MTB/RIF assay offers many advantages, such as providing results within 2 h, thus allowing antituberculosis treatment to be initiated sooner; high sensitivity, specificity, and overall accuracy; detecting rifampin-resistant *M. tuberculosis* strains; and high sensitivity and specificity for the detection of tuberculosis in HIV-infected patients. Despite its limitations, which include a high false-positive rate and the high costs of equipment maintenance and reagents/components, the Xpert MTB/RIF assay has made an essential contribution in clinical practice as a rule-in or rule-out test for the diagnosis of tuberculosis.

The performance of the Xpert MTB/RIF assay in sputum samples has been well documented, as has the value of BALF samples in the diagnosis of tuberculosis, especially in patients who are sputum smear-negative or unable to produce sufficient sputum samples; however, there have been few studies analyzing and validating the utility of molecular tests for the diagnosis of tuberculosis in BALF specimens. Khalil & Butt demonstrated that, in comparison with mycobacterial culture, the Xpert MTB/RIF assay was superior for detecting *M. tuberculosis* and rifampin resistance showing high sensitivity (91.86%) and PPV (97.53%) in patients with PTB who were sputum smear-negative or unable to produce sufficient sputum samples. In a retrospective study, Agrawal et al. also showed that the Xpert MTB/RIF assay performed better in respiratory samples (sputum and BALF) than did AFB staining. Finally, a recent study conducted at a tertiary care facility in India compared BAL the Xpert MTB/RIF assay, AFB staining, and mycobacterial culture in BALF samples from suspected cases of PTB, in a manner similar to that of the present study. Bashir et al. observed that, although the Xpert MTB/RIF assay and AFB staining had similar overall specificity, the sensitivity of the Xpert MTB/RIF assay was much higher than that of smear microscopy in BALF samples (97.1% vs. 36.7%). Together with all of that evidence, our data support the use of the Xpert MTB/RIF assay in BALF samples as an interesting and suitable tool to improve the diagnosis of tuberculosis.

In the present study, there were some discordant results among the outcomes of the Xpert MTB/RIF assay, AFB staining, and mycobacterial culture, particularly among the 73 patients for whom results from all three examinations were available. There were two patients in whom the BALF samples tested positive on the Xpert MTB/RIF assay, despite testing negative on AFB staining and culture. It is noteworthy that those samples had higher cycle threshold values (26.6-30.9; probes A-E), which implies low concentrations of *M. tuberculosis* DNA. That could explain the negative results obtained with mycobacterial culture and AFB staining. In two other patients, *M. tuberculosis* was identified only by AFB staining; one of those patients, a female, was HIV-infected. Immunosuppression can favor coinfection with other pathogens, such as NTM. However, in that case, there was no growth in mycobacterial culture, which delayed the diagnosis.

In addition, there was one case in which *M. tuberculosis*...
was identified only in culture. None of those 5 patients reported having had tuberculosis previously. Therefore, on the basis of clinical and radiological criteria, they were treated with an antituberculosis regimen. After six months, all of them showed improvement in the signs and symptoms. All of these discrepancies should be interpreted with caution, the limitations of each method and the clinical status of the patients being taken into consideration.

Our study has some limitations. First, it was a single-center study, which limits the generalizability of the data. Second, because it was a retrospective study, the clinical data were limited to those available in the medical records. Third, there was a relatively small number of cases in which results were available from all three of the methods evaluated. However, the study was performed at a specialized, tertiary, referral teaching hospital in a high-tuberculosis-burden area, where numerous tools are available for the investigation and accurate diagnosis of cases of tuberculosis. In addition, mycobacterial culture results were available for all cases, and we excluded cases of NTM infection in order to avoid bias.

In summary, in BALF samples, the Xpert MTB/RIF assay performed better than did AFB staining for the diagnosis of cases of PTB in patients who were sputum smear-negative or were unable to produce sputum specimens of sufficient quantity. That could have a significant impact on clinical practice and on the management of such cases, particularly those in which the diagnosis is challenging. When the Xpert MTB/RIF assay produces results that are discordant with those of other diagnostic tools, those discrepancies must be carefully analyzed, given that tuberculosis continues to be a significant public health problem worldwide.

ACKNOWLEDGMENTS
The authors would like to thank the administration, staff, residents, and patients of the Pedro Ernesto University Hospital for participating in this study.

AUTHOR CONTRIBUTIONS
TMM and LSR: conceived and designed the study. GMXB and RSL: performed the experiments. GMXB, MRA, and LSR: analyzed the data. RR and LSR: contributed analysis tools. GMXB, TTM, RSL, MRA, and LSR: drafted and revised the manuscript. RR and JL: supervised patient enrollment. LVT and TTM: performed the bronchoscopy procedures. LSR: coordinated the study. All authors contributed to the discussion of the data and approved the final version of the manuscript.

REFERENCES
1. World Health Organization [homepage on the Internet]. Geneva: World Health Organization [cited 2020 Nov 01]. Global tuberculosis report 2019. [Adobe Acrobat document, 297p.]. Available from: https://apps.who.int/iris/bitstream/handle/10665/323938/9789241565714-eng.pdf?ua=1
2. Brasil. Ministério da Saúde. Manual de para o Controle da Tuberculose. Brasília: Ministério da Saúde; 2019. 364 p.
3. Shin JA, Chang YS, Kim TH, Kim HJ, Ahn CM, Byun MK. Fibeceptor 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
4. Nikbakht 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.
5. Kalavat 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
6. Saglam L, Akgun M, Aktaş 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/10.1177/0300060505300215
7. Steingart KR, Ng V, Henry M, Hopewell FC, 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
8. Lange C, Mori T. Advances in the diagnosis of tuberculosis. Respiratory. 2010;11(2):220-240. https://doi.org/10.1111/j.1440-1843.2009.01692.x
9. Heib 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.01483-09
10. Boehme CC, Nabeta P, Hillermann 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/NEJMa0907847
11. Sharma SK, Kohli M, Yadav RN, Chaubey J, Bhaihn 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
12. 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
13. Steingart GR, 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(4):CD009693. https://doi.org/10.1002/14651858.CD009693.pub3
14. 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;4:9658. https://doi.org/10.1038/srep09658
15. 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 extrapolumonary specimens. J Clin Microbiol. 2011;49(12):4138-4141. https://doi.org/10.1128/JCM.05434-11
16. Brasil. Agência Nacional de Vigilância Sanitária. Microbiologia Clínica para o Controle de Infecção Relacionada à Assistência à Saúde. Brasília: a Agência; 2013.
17. 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
18. 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.16.198314
19. Deggim V, Somoskovi A, Vori 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

20. 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

21. 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

22. 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(8):e663-e668. https://doi.org/10.1016/j.ijid.2012.05.006

23. 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/10.4103/0970-2113.63867

24. 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 fibreoptic 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

25. 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.

26. 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(6):DC09-DC12. https://doi.org/10.7860/JCDR/2016/18837.775

27. 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):89-93. https://doi.org/10.21276/jcmr.2019.6.6.29

28. 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

29. Lan R, Yang C, Lan L, Ou J, Qiao K, Liu F, et al. Mycobacterium tuberculosis and non-tuberculous mycobacteria isolates from HIV-infected patients in Guangxi, China. Int J Tuberc Lung Dis. 2011;15(12):1669-1675. https://doi.org/10.5588/ijtld.11.0036

30. Akanbi MO, Achenbach C, Taiwo B, Idoko J, Ani A, Isa Y, et al. Evaluation of gene xpert for routine diagnosis of HIV-associated tuberculosis in Nigeria: A prospective cohort study. BMC Pulm Med. 2017;17(1):87. https://doi.org/10.1186/s12890-017-0430-6