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# Accuracy of a rapid molecular test for tuberculosis in sputum samples, bronchoalveolar lavage fluid, and tracheal aspirate obtained from patients with suspected pulmonary tuberculosis at a tertiary referral hospital

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## ABSTRACT

Tuberculosis continues to be a major public health problem worldwide. The aim of the present study was to evaluate the accuracy of the Xpert MTB/RIF rapid molecular test for tuberculosis, using pulmonary samples obtained from patients treated at the Júlia Kubitschek Hospital, which is operated by the Hospital Foundation of the State of Minas Gerais, in the city of Belo Horizonte, Brazil. This was a retrospective study comparing the Xpert MTB/RIF test results with those of standard culture for *Mycobacterium tuberculosis* and phenotypic susceptibility tests. Although the Xpert MTB/RIF test showed high accuracy for the detection of *M. tuberculosis* and its resistance to rifampin, attention must be given to the clinical status of the patient, in relation to the test results, as well as to the limitations of molecular tests.

**Keywords:** Tuberculosis/diagnosis; Molecular diagnostic techniques; Sputum; Bronchoalveolar lavage fluid.

Tuberculosis continues to be a major public health problem worldwide. It is estimated that there were 6.3 million new cases of tuberculosis worldwide in 2016, 1.7 million individuals having died from the disease, which is recognized by the World Health Organization (WHO) as the leading cause of death from infectious diseases worldwide.[1] In 2016, 66,796 new cases of tuberculosis were diagnosed and reported in Brazil.[2]

Early diagnosis and treatment of pulmonary tuberculosis are essential in reducing tuberculosis dissemination, morbidity, mortality, and costs.[3] In Brazil, 71.6% of all new tuberculosis cases in 2016 were confirmed by laboratory criteria, and, according to the latest WHO report, 41% of all multidrug-resistant tuberculosis cases in 2016 were diagnosed in Brazil.[1,2] However, conventional diagnostic methods have disadvantages such as low sensitivity and specificity (in the case of smear microscopy), as well as the considerable time required for obtaining test results (in the cases of culture and drug susceptibility testing).[4] Molecular diagnostic techniques have been reported as being more sensitive, specific, and rapid.[5]

In 2010, the WHO recommended the use of the Xpert MTB/RIF rapid molecular test for tuberculosis (Cepheid, Sunnyvale, CA, USA), a rapid and fully automated nucleic acid amplification test that detects *Mycobacterium tuberculosis* and its resistance to rifampin.[6] Although most studies validating the Xpert MTB/RIF test have shown promising results, showing good accuracy in sputum samples,[7] only a few have shown good accuracy in BAL fluid and tracheal aspirate (TA).[3,8-10]

The Brazilian National Ministry of Health has recently incorporated the use of the Xpert MTB/RIF test in some laboratories in Brazil, including the laboratory of the Júlia Kubitschek Hospital, which is operated by the Hospital Foundation of the State of Minas Gerais, a tertiary referral hospital for tuberculosis and drug-resistant tuberculosis in the city of Belo Horizonte, Brazil.[11] The objective of the present study was to evaluate the accuracy of the Xpert MTB/RIF test in sputum samples, BAL fluid, and TA obtained from patients with suspected pulmonary tuberculosis at the aforementioned hospital.

This was a retrospective descriptive study. We compared the Xpert MTB/RIF test results with those of standard culture for *M. tuberculosis* in a total of 534 samples in the period between December of 2014 and November of 2015. Of those samples, 238 were sputum samples, 199 were BAL fluid samples, and 97 were TA samples. Culture was considered the standard method for detecting *M.*

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*tuberculosis*. Antimicrobial susceptibility testing (AST) was considered the standard method for detecting resistance to rifampin. Samples with insufficient growth for mycobacterial species identification were excluded, as were contaminated samples and samples with growth of nontuberculous mycobacteria.

Culture was performed on Löwenstein-Jensen medium after decontamination by the sodium lauryl sulfate method.[12] Species identification and AST by the proportion method[13,14] or by an automated culture system (BACTEC Mycobacteria Growth Indicator Tube [MGIT] 960; Becton Dickinson, Sparks, MD, USA) were performed in the Ezequiel Dias Foundation state referral laboratory. The Xpert MTB/RIF test was performed in accordance with the manufacturer instructions.[15]

Sensitivity, specificity, positive predictive value, negative predictive value, accuracy, and agreement were calculated with the Minitab software, version 17 (Minitab Inc., State College, PA, USA) and the GraphPad Prism software, version 7 (GraphPad Software Inc., San Diego, CA, USA).

The study project was approved by the Research Ethics Committee of the Hospital Foundation of the State of Minas Gerais (Ruling no. 1,764,672).

Culture was positive for *M. tuberculosis* in 15.2% of the samples (81/534), and the Xpert MTB/RIF test was positive for *M. tuberculosis* in 19.9% of the samples (106/534). Table 1 shows the overall accuracy of the Xpert MTB/RIF test in detecting *M. tuberculosis*, whereas Table 2 shows the accuracy of the Xpert MTB/RIF test in detecting *M. tuberculosis* for each sample type.

AST was performed in 60 isolates from 81 cultures that were positive for *M. tuberculosis*. Of those 60 isolates, 9 were found to be resistant to rifampin by AST and the Xpert MTB/RIF test. With regard to susceptibility to rifampin, there was agreement between the two test methods for 49 of the 60 isolates and disagreement for 2 (resistance to rifampin by the Xpert MTB/RIF test and susceptibility to rifampin by AST in 1 and susceptibility to rifampin by the Xpert MTB/RIF test and resistance to rifampin by AST in 1). The accuracy of the Xpert MTB/RIF test in detecting resistance to rifampin is shown in Table 1.

In 25 patients, the Xpert MTB/RIF test results were positive but culture results were negative. Of those 25 patients, 9 had a history of tuberculosis, 4 had been receiving treatment at the time of testing, and 1 underwent testing for disease control (the test having therefore been incorrectly requested). In 4 patients, there was no history of tuberculosis and treatment was not initiated, the outcome being defined as mycobacteria other than tuberculosis. In the remaining 7 patients, it was impossible to evaluate clinical history.

Sensitivity was higher in the present study than in other studies (82-93%), whereas specificity was similar (96-100%).[3,7-9] The negative predictive value of the Xpert MTB/RIF test was found to be high, meaning that the test can rapidly rule out tuberculosis in patients suspected of having the disease.

With regard to the accuracy of the Xpert MTB/RIF test in detecting *M. tuberculosis* for each sample type, the results were similar except for the positive predictive values for sputum and BAL fluid samples, which were lower because of a higher number of discordant (false-positive) results between the two.

Of the 25 patients in whom there was disagreement between the Xpert MTB/RIF test results and culture results (i.e., positive Xpert MTB/RIF test results and negative culture results), 14 had a history of tuberculosis, with 4 being under treatment at the time of testing; this shows the importance of effective communication between the laboratory and clinical staff, given that the Xpert MTB/RIF test amplifies DNA originating from live or dead bacilli.[3] In a report published one year after the implementation of the Xpert MTB/RIF test in Brazil, 50% of the monitors stated that the test request forms used in their states lacked important information—such as whether the individual under investigation for tuberculosis has any risk factors for the disease—thus making it difficult to select the most appropriate tools for diagnosis.[16]

In patients without active disease but with dead bacilli in their lungs from previously treated active tuberculosis, the Xpert MTB/RIF test results can remain positive for up to five years; therefore, conversion to negative is not a suitable marker of treatment success. In such cases, the diagnosis of tuberculosis should be made exclusively by sputum smear microscopy and sputum culture; the Xpert MTB/RIF test can be used only to identify early resistance to rifampin.[17,18] Further studies are needed in order to quantify this better and determine predisposing factors.[17]

**Table 1.** Diagnostic accuracy of the Xpert MTB/RIF test for detecting *Mycobacterium tuberculosis* and its resistance to rifampin.[a]

| Variable | Detection of MTB | Detection of resistance to RIF |
|---|---|---|
| Sensitivity (%) | 100 (100-100) | 100 (100-100) |
| Specificity (%) | 94.5 (92.4-96.6) | 98.0 (94.2-101.8) |
| PPV (%) | 76.4 (68.3-84. 5) | 90.0 (76.0-103.1) |
| NPV (%) | 100 (100-100) | 100 (100-100) |
| Accuracy (%) | 95.3 (93.5-97.1) | 98.3 (95.1-101.6) |
| Kappa* | 0.84 (0.78-0.90) | 0.94 (0.82-1.06) |

MTB: *Mycobacterium tuberculosis*; RIF: rifampin; PPV: positive predictive value; and NPV: negative predictive value. [a]Values expressed as n (95% CI). *The criteria for kappa were as follows: < 0.20, poor; 0.21-0.40, weak; 0.41-0.60, moderate; 0.61-0.80, good; and > 0.80-1.00, very good.

**Table 2.** Diagnostic accuracy of the Xpert MTB/RIF test for different types of pulmonary samples.

| Variable | Sputum (n = 238) | Bronchoalveolar lavage fluid (n = 199) | Tracheal aspirate (n = 97) |
|---|---|---|---|
| Sensitivity (%) | 100 (100-100) | 100 (100-100) | 100 (100-100) |
| Specificity (%) | 92.8 (89.1-96.4) | 95 (91.8-98.2) | 97.5 (94.0-100.9) |
| PPV (%) | 75.9 (64.9-86.9) | 67.9 (50.6-85.2) | 90.0 (76.0-103.1) |
| NPV (%) | 100 (100-100) | 100 (100-100) | 100 (100-100) |
| Accuracy (%) | 94.1 (91.1-97.1) | 95.5 (92.6-98.4) | 97.9 (95.1-100.8) |
| Kappa* | 0.83 (0.74-0.91) | 0.78 (0.65-0.92) | 0.93 (0.84-1.02) |

PPV: positive predictive value; and NPV: negative predictive value. [a]Values expressed as n (95% CI). *The criteria for kappa were as follows: < 0.20, poor; 0.21-0.40, weak; 0.41-0.60, moderate; 0.61-0.80, good; and > 0.80-1.00, very good.

In the 4 patients who had no history of tuberculosis and who were classified as having mycobacteria other than tuberculosis in the present study, the results should be interpreted in a clinical context. In some cases, the Xpert MTB/RIF test can be more sensitive than conventional culture. The high sensitivity of the Xpert MTB/RIF test can be explained by the analytical detection limit, which is 131 colony-forming units/mL, being as high as 10 colony-forming units/mL in some samples.[9]

It has been hypothesized that false-positive results are due to residual persistent DNA from dead *M. tuberculosis* in lung tissue, expectorated because of another lung disease and thus leading to false-positive results for active tuberculosis.[17]

Of 25 positive Xpert MTB/RIF test results and negative culture results, 12 showed very low cycle threshold values (> 28 cycles) and 11 showed low values (23-28 cycles). These values represent a low concentration of *M. tuberculosis* complex DNA in our sample.[3] Further studies are needed in order to interpret these results in conjunction with patient clinical evaluation and the presence of other diseases.

Positive Xpert MTB/RIF test results and negative culture results might be related to technical manipulation issues, such as drastic decontamination procedures, temperature fluctuations in the incubator, and improper clinical sample storage.[12]

With regard to rifampin resistance, 2 samples showed disagreement between the Xpert MTB/RIF test and AST: resistance to rifampin by the Xpert MTB/RIF test and susceptibility to rifampin by AST in 1 and susceptibility to rifampin by the Xpert MTB/RIF test and resistance to rifampin by AST in 1. Resistance to rifampin is primarily due to mutations in the *rpoB* gene; however, rare mutations can occur outside the target region, and rifampin resistance cannot be detected unless 65-100% of the DNA population in the sample is mutant.[4,19] In addition, mixed infections can lead to false-negative or false-positive results. Heteroresistance is defined by the presence of susceptible and resistant populations of *M. tuberculosis* and has been reported as a possible cause of discordant AST results.[20]

One of the limitations of the present study is that sputum smear microscopy was not performed in parallel with the Xpert MTB/RIF test, because the samples were evaluated under routine laboratory conditions. In addition, it was impossible to analyze sociodemographic, clinical, and imaging data. Furthermore, it was impossible to study the impact of the Xpert MTB/RIF test on the time from diagnosis to treatment.

The results of the present study can contribute to improving the laboratory diagnosis of tuberculosis in sputum samples, BAL fluid, and TA; however, attention must be given to the clinical status of the patient, in relation to the test results, as well as to the limitations of molecular tests.[3,17,18] In addition, it is important that test request forms be filled out correctly and include information on why the test is being requested (i.e., for diagnosis or follow-up), as well as patient history of tuberculosis treatment (i.e., previous tuberculosis treatment or no previous tuberculosis treatment) and risk factors for tuberculosis.[16]

Although the Xpert MTB/RIF test showed high accuracy for the detection of *M. tuberculosis* and its resistance to rifampin, attention must be given to the clinical status of the patient, in relation to the test results, as well as to the limitations of molecular tests.[3,17,18] Further studies are needed in order to evaluate the impact of the Xpert MTB/RIF test on patients and society in different settings (primary care, secondary care, and tertiary care) in the five regions of Brazil.

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