RESEARCH ARTICLE

Comparative Evaluation of Cartridge-based Nucleic Acid Amplification Test Smear Microscopy and Conventional Culture Techniques in Laboratory Diagnosis of Tuberculosis

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Received on: 20 September 2023; *Accepted on:* 11 November 2023; *Published on:* 05 February 2024

Abstract

Aim: This study compared the nucleic acid amplification assay with smear microscopy and culture to assess the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) in patients with suspected pulmonary and extrapulmonary tuberculosis (TB) using pulmonary and extrapulmonary samples.

Methods: The information for this retrospective study was collected from the Path Kind Laboratory—a National Accreditation Board for Testing and Calibration Laboratories (NABL) accredited lab in Gurugram, Haryana, India. We evaluated 1,520 samples with suspected TB for smear microscopy, culture, and GeneXpert between January 2019 and December 2020. These samples came from the pulmonary and extrapulmonary areas. Smear microscopy and GeneXpert's diagnostic capabilities for pulmonary and extrapulmonary TB were calculated, with *Mycobacterium tuberculosis* (MTB) culture from pulmonary and extrapulmonary specimens serving as the gold standard.

Results: This study comprised 1,520 clinical samples altogether. Of these, 624 were extrapulmonary specimens, while 896 were pulmonary samples. Overall, acid-fast bacilli (AFB) smear microscopy's sensitivity, specificity, PPV, and NPV were 83.72, 91.91, 71.38, and 95.91%, respectively. Also, GeneXpert has overall values of 96.94, 81.22, 55.42, and 99.10% for sensitivity, specificity, PPV, and NPV, respectively.

Conclusion: In both pulmonary and extrapulmonary specimens, the GeneXpert assay was shown to be a quick and reliable approach for detecting *M. tuberculosis*. Because GeneXpert can detect *M. tuberculosis* and rifampicin resistance in the same 2 hours as smear microscopy and MTB culture, it offers an advantage over those methods.

Clinical significance: The early detection of TB can be greatly aided by the cartridge-based nucleic acid amplification test (CBNAAT). As an important part of the National Tuberculosis Elimination Program gene experts will certainly help in the elimination of tuberculosis from India.

Keywords: Acid-fast bacilli smear, Cartridge-based nucleic acid amplification test, Extrapulmonary, GeneXpert MTB/RIF, Pulmonary tuberculosis.

The Indian Journal of Chest Diseases and Allied Sciences (2023): 10.5005/jp-journals-11007-0089

Abbreviations used in this Article

AFB = Acid-fast bacilli; BAL = Bronchoalveolar lavages; CBNAAT = Cartridge-based nucleic acid amplification test; PPV = Positive predictive value; NABL = National Accreditation Board for Testing and Calibration Laboratories; MGIT = Mycobacteria growth indicator tube; MTB = Mycobacterium tuberculosis; NALC–NaOH = *N*-acetyl-l-cysteine sodium hydroxide; NPV = Negative predictive value; RIF = Resistance to rifampin; TB = Tuberculosis; ZN = Ziehl–Neelsen.

Introduction

Mycobacterium tuberculosis (MTB) is the causative agent of TB, a disease causing significant worldwide morbidity, and mortality.[1] About 25% of the world's cases of TB are in India. In 2019 the estimated TB incidence was 2,640,000.[2] It is possible to contract TB, an infectious illness, by coughing up microorganisms and inhaling airborne droplets.[3,4] Pulmonary TB refers to a condition that affects the lungs. Extrapulmonary tuberculosis (TB) is the term for TB infection that occurs outside of the lung.[5] Common methods used to diagnose *M. tuberculosis* include the GeneXpert MTB/resistance to rifampin (RIF) assay, conventional culture, and smear microscopy. In developing nations, smear microscopy with Ziehl–Neelsen staining for acid-fast bacilli (AFB) is a frequent method for diagnosing TB, despite its low predictive value and lack of sensitivity. Smear microscopy results are available in 2 hours.[6,7] Because there are so many smear-negative cases, it is difficult to identify TB, which further increases the disease's burden. Excitingly, a cartridge-based nucleic acid amplification test (CBNAAT) claims to be able to diagnose rifampicin resistance and TB in less than two

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How to cite this article: Naseem S, Naaz P, Rattan A, *et al.* Comparative Evaluation of Cartridge-based Nucleic Acid Amplification Test Smear Microscopy and Conventional Culture Techniques in Laboratory Diagnosis of Tuberculosis. Indian J Chest Dis Allied Sci 2023;65(3): 134–138.

Source of support: Nil

Conflict of interest: None

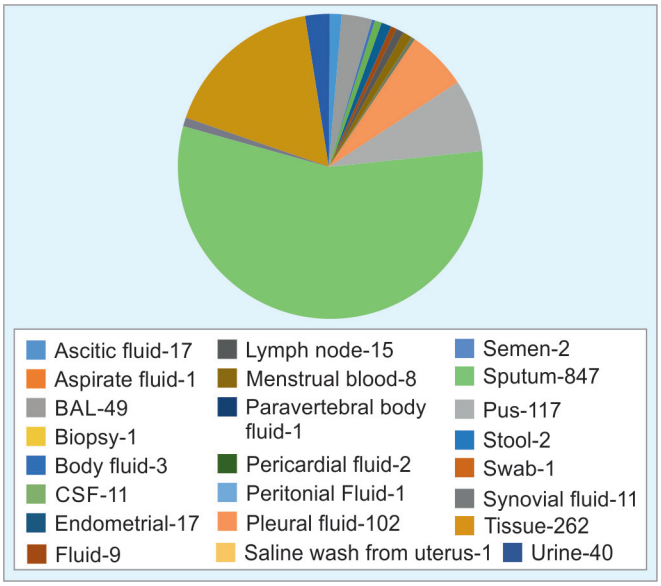

Fig. 1: Clinical specimen

hours.[8] The gold standard for diagnosing TB is a culture technique that uses Lowenstein-Jensen media for mycobacterial growth. It takes longer, usually three to four weeks, and is very delicate.[7] The use of GeneXpert MTB/RIF in national TB programs in developing countries was approved by the World Health Organization in December 2010.[9] It's the first completely automated cartridge-based nucleic acid amplification assay for TB detection.[10] It is contributing to the quick identification of TB disease and medication resistance, which is revolutionizing TB control. The test can identify rifampicin resistance and the *M. tuberculosis* complex in less than two hours.[11] The purpose of this study was to evaluate the performance of CBNAAT, smear microscopy, and conventional culture methods in the diagnosis of pulmonary and extrapulmonary TB.

Methods

This retrospective study was carried out at the Department of Paramedical Sciences, Jamia Hamdard University, New Delhi, India. A total of 1,520 clinical samples with strong clinical evidence of TB and clinical response to antitubercular treatment were received from the Path Kind Lab in Gurugram in the years 2019 and 2020. All samples were subjected to smear microscopy for early diagnosis, followed by cartridge-based nucleic acid amplification testing.

Clinical Specimens

Clinical specimens were selected based on the clinical manifestations presented by the patient. The samples included 847 sputum samples from pulmonary TB cases, 49 bronchoalveolar lavages (BAL) from pulmonary TB, 1 biopsy, 11 synovial fluids from osteoarticular TB, 40 urine [urinary tract infection (UTI)], 117 pus, 102 pleural fluid, 15 lymph node aspirates, 17 ascitic fluid, 11 cerebrospinal fluid (CSF), 17 endometrial biopsies, 8 menstrual blood, 2 semen, 3 body fluid, 9 fluid, 1 aspirate fluid, 1 paravertebral body fluid, 2 pericardial fluid, 1 peritoneal fluid, 1 saline wash from the uterus, 1 swab, 262 tissue, 2 stool samples from extrapulmonary TB cases (Fig. 1).

Sample Processing

A pulmonary and extrapulmonary sample from the centers was split into three sections and submitted to the lab for quick examination using Ziehl–Neelsen smear microscopy, followed by usage in culture and GeneXpert on the same day.[10] The *N*-acetyl-l-cysteine sodium hydroxide (NALC–NaOH) approach was used to handle nonsterile specimens.[6,12] The samples were processed as quickly as possible. In the case of a delay, they were processed after no more than 24 hours of refrigeration at 4°C.[13]

Smear Microscopy and Culture

Smears were prepared and stained with the Ziehl–Neelsen staining method.[6] Microscopically, the stained smears were examined using an oil immersion (100×) objective lens.[7] Acid-fast bacilli have a beaded look and are bright red.[13] The specimen was inoculated with the culture medium and allowed to grow at 37°C for 4 weeks.[6]

GeneXpert *Mycobacterium Tuberculosis*/Resistance to Rifampin

The CBNAAT samples were processed using the recommended WHO procedures. The assay was carried out using version 4 cartridges per the manufacturer's instructions.[6] The sputum sample was allowed to liquefy for 15 minutes after 1 mL of the sample was added to 2 mL of the sample reagent. Two milliliters of the mixture were put into the GeneXpert cartridge using the sterile dropper that came with the box. The materials required for both RIF drug resistance detection and nucleic acid amplification were included in this cartridge.[7] The systems documented the presence or absence of *M. tuberculosis* after two hours, classifying the bacterial load as very low, low, medium, or high as well as the subject's susceptibility to the antibiotic rifampicin.[13]

We obtained the values of sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) by using the formulas mentioned as follows:

Sensitivity = (True positive/True positive) + False negative × 100
Specificity = (True negative/True negative) + False positive × 100
Positive predictive value = (True positive/True positive) + False positive × 100
Negative predictive value = (True negative/True negative) + False negative × 100

Calculations of Overall from Smear Microscopy with Culture as a Gold Standard

Sensitivity = (247/247) + 48 × 100 = 83.72%
Specificity = (1126/1126) + 99 × 100 = 91.91%
Positive predictive value = (247/247) + 99 × 100 = 71.38%
Negative predictive value = (1126/1126) + 48 × 100 = 95.91%

Calculations of Overall from GeneXpert with Culture as a Gold Standard

Sensitivity = (286/286) + 9 × 100 = 96.94%
Specificity = (995/955) + 230 × 100 = 81.22%
Positive predictive value = (286/286) + 230 × 100 = 55.42%
Negative predictive value = (995/995) + 9 × 100 = 99.10%

Calculations of Pulmonary Samples from Smear Microscopy with Culture as a Gold Standard

Sensitivity = (236/236) + 31 × 100 = 88.38%
Specificity = (558/558) + 71 × 100 = 88.71%
Positive predictive value = (236/236) + 71 × 100 = 76.87%
Negative predictive value = (558/558) + 31 × 100 = 94.73%

Table 1: Overall sensitivity, specificity, PPV, and NPV values of GeneXpert and AFB with culture as a gold standard

	Sensitivity (%)	*Specificity (%)*	*PPV (%)*	*NPV (%)*
AFB	83.72	91.91	71.38	95.91
GeneXpert	96.94	81.22	55.42	99.10

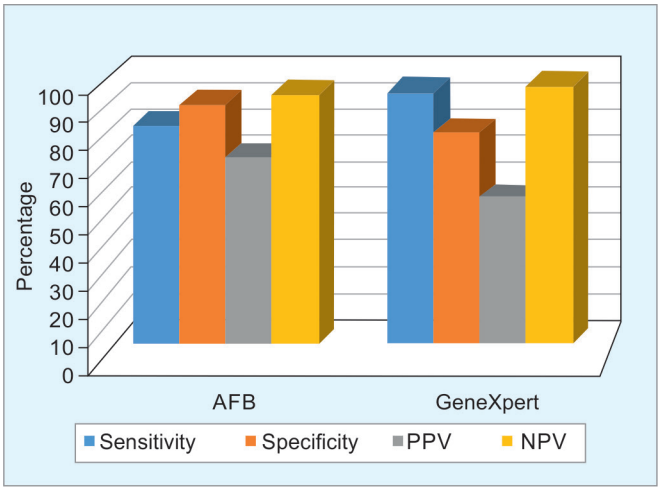

Fig. 2: Overall sensitivity, specificity, PPV, and NPV, values of GeneXpert and AFB with culture on as gold standard

Calculations of Pulmonary Samples from GeneXpert with Culture as a Gold Standard

Sensitivity = (261/261) + 6 × 100 = 97.75%
Specificity = (483/483) + 146 × 100 = 76.78%
Positive predictive value = (261/261) + 146 × 100 = 64.12%
Negative predictive value = (483/483) + 6 × 100 = 98.77%

Calculations of Extrapulmonary Samples from Smear Microscopy with Culture as a Gold Standard

Sensitivity = (11/11) + 17 × 100 = 39.28%
Specificity = (568/568) + 28 × 100 = 95.30%
Positive predictive value = (11/11) + 28 × 100 = 28.21%
Negative predictive value = (568/568) + 17 × 100 = 97.09%

Calculations of Extrapulmonary Samples from GeneXpert with Culture as a Gold Standard

Sensitivity = (25/25) + 3 × 100 = 89.28%
Specificity = (512/512) + 84 × 100 = 85.90%
Positive predictive value = (25/25) + 84 × 100 = 22.93%
Negative predictive value = (512/512) + 3 × 100 = 99.41%

Results

A total of 1,520 samples (896 pulmonary and 624 extrapulmonary samples) were examined. The overall sensitivity, specificity, PPV, and NPV of smear microscopy were, respectively, 83.72, 91.91, 71.38, and 95.91%. GeneXpert's general sensitivity, specificity, PPV, and NPV, respectively, were 96.94, 81.22, 55.42, and 99.10% (Table 1; Fig. 2).

In pulmonary and extrapulmonary samples, GeneXpert has a significantly better sensitivity than smear microscopy, while smear microscopy has a significantly higher specificity (Tables 2 and 3; Figs 3 and 4).

Table 2: Overall sensitivity, specificity, PPV, and NPV values of AFB with culture in pulmonary and extrapulmonary

	Sensitivity (%)	*Specificity (%)*	*PPV (%)*	*NPV (%)*
Pulmonary	88.38	88.71	76.87	94.73
Extrapulmonary	39.28	95.30	28.21	97.09

Table 3: Overall sensitivity, specificity, PPV, and NPV values of GeneXpert with culture in pulmonary and extrapulmonary

	Sensitivity (%)	*Specificity (%)*	*PPV (%)*	*NPV (%)*
Pulmonary	97.75	76.78	64.12	98.77
Extrapulmonary	89.28	85.90	22.93	99.41

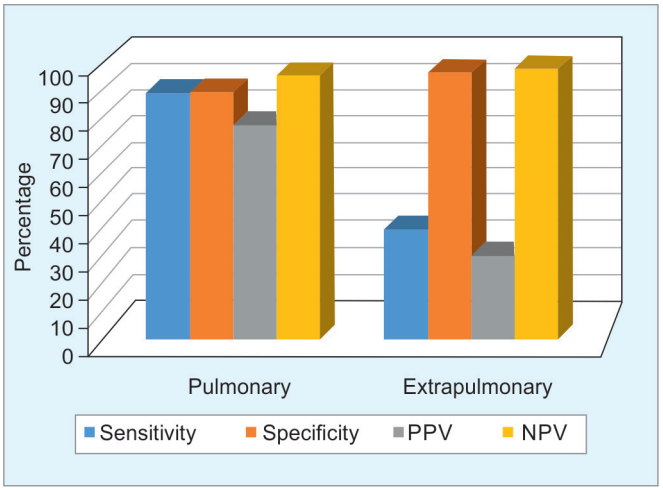

Fig. 3: Overall sensitivity, specificity, PPV, and NPV, values of AFB with culture in pulmonary and extrapulmonary

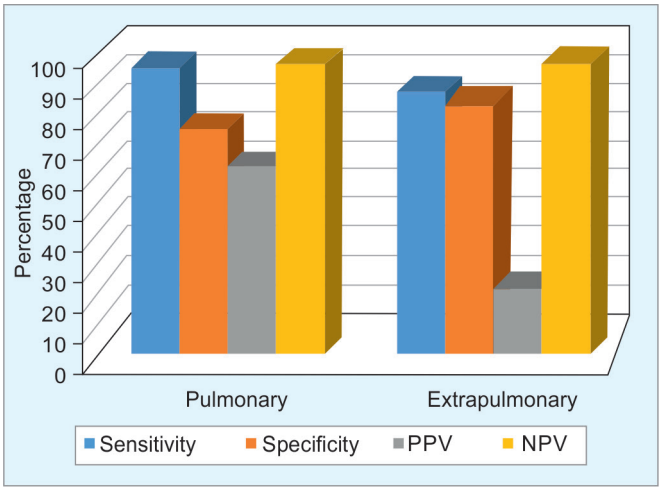

Fig. 4: Overall sensitivity, specificity, PPV, and NPV, values of GeneXpert and AFB with culture in pulmonary and extrapulmonary

The GeneXpert assay was compared to two different assays, namely smear examination and culture result, both independently and in combination, to see how sensitive it was (Table 4).

Table 4: Comparison of the sensitivity of GeneXpert MTB/RIF assay to other tests

Test result category (number)	*GeneXpert MTB/RIF*		*Sensitivity of GeneXpert MTB/RIF (%)*
	Positive	*Negative*	
Smear positive (346)	335	11	96.83
Smear negative (1,174)	181	993	15.44
Culture positive (295)	286	9	96.95
Culture negative (1,225)	230	995	18.80
Smear, culture positive (247)	244	3	98.79
Smear, culture negative (1,126)	139	987	12.36

As may be observed, the GeneXpert assay was demonstrated to be substantially more accurate than smear examination or culture. GeneXpert had a sensitivity of 96.83% in detecting *M. tuberculosis* in smear-positive samples, whereas it had a sensitivity of 15.44% in smear-negative samples. The sensitivity for detecting *M. tuberculosis* in clinical specimens that tested positive in culture was found to be 96.95% (Table 4).

When smear microscopy and culture techniques were used to confirm that clinical samples were positive for *M. tuberculosis*, GeneXpert showed a sensitivity of 98.79%. For clinical samples that tested positive for the other two methods [Ziehl–Neelsen (ZN) stain and culture], GeneXpert similarly has a sensitivity of 98.79%. In 1,126 samples that tested negative for TB by all three other methods combined, the GeneXpert assay found 139 positives (12.36%). The positive results were probably not false positives because GeneXpert had detected TB in these samples several times and they were from cases of strongly suspected TB that responded to antitubercular therapy (Table 4).

Discussion

We investigated the diagnostic yield of smear microscopy and GeneXpert for detecting *M. tuberculosis* in pulmonary and extrapulmonary samples in this retrospective study. The obtained results were compared to the gold standard, the mycobacteria growth indicator tube (MGIT) culture.[10]

Mycobacterial cultures for TB detection can be performed using either a solid (Lowenstein Jensen media) or a liquid broth technique (MGIT 960). When MGIT liquid culture media is used instead of LJ medium, the results are obtained sooner.[14,15]

The results of the MGIT 960 culture were included in our study. GeneXpert is a straightforward benchtop point-of-care diagnostic instrument that may be used with very little training. It only takes 2 hours to access the results, which is far faster than the culture, which can take several days to yield positive results.[9,16]

GeneXpert's total sensitivity for TB detection was 96.94% in our study. By comparison, GeneXpert's sensitivity to culture was 96.83% for smear-positive samples and 15.44% for smear-negative samples.[17]

The sensitivity of the GeneXpert assay was 96.94% overall, higher than that of smear microscopy (overall 83.72%). GeneXpert and smear microscopy had overall specificities of 81.22 and 91.91%, respectively, which also show good correlation with other investigations.[6,18]

In our study, the sensitivity of smear microscopy was 83.72% which correlates well with other studies.[6,18] The sensitivity was more than in the study of Arora and Dhanashree (65.7%).[18] While in our investigation, the sensitivity of smear microscopy was significantly higher than in the study conducted by Zahoor et al. (46%).[6]

The current study's specificity and NPV for smear microscopy were approximately 91.91 and 95.91%. In contrast to Zahoor et al. works (95.83%), our investigation found a low PPV of 71.38% for smear microscopy.[6] Additionally, smear microscopy's PPV was almost similar to that of the study by Arora and Dhanashree (79.3%).[18]

In the present study, the PPV of GeneXpert was quite low in the study of Zahoor et al. and Arora and Dhanashree.[6,18]

In our study, the sensitivity, specificity, PPV and NPV of GeneXpert and AFB smear microscopy are 88.38, 88.71, 76.87, and 94.73%, and 97.75, 76.78, 64.12, and 98.77% for the pulmonary sample, respectively.

A study by Agrawal M et al. For the pulmonary sample, GeneXpert's overall sensitivity, specificity, PPV, and NPV were, in stability, 86.8, 93.1, 78.5, and 96%. For the pulmonary sample, the overall sensitivity and specificity of the AFB smear microscopy were 22.2 and 78.5%, respectively.[10]

In our study, the sensitivity, specificity, PPV, and NPV of AFB smear microscopy and GeneXpert is 39.28, 95.30, 28.21, and 97.09% and 89.28, 85.90, 22.93, and 99.41% for the extrapulmonary sample respectively.

A study by Bharati et al. showed sensitivity, specificity, PPV, and NPV for GeneXpert and Smear microscopy 94.73, 96.22, 90, and 98, and 63.15, 98.11, 92.3, and 88.13% for the extrapulmonary sample, respectively. Bajrami et al. showed sensitivity, specificity, PPV, and NPV for GeneXpert and Smear microscopy 82.3, 97.6, 93.3, and 93, and 94.1, 85.7, 53.3, and 98.8% for the extrapulmonary sample respectively.[19–22]

In comparison with the culture which is used as a gold standard, sensitivity, specificity, PPV, and NPV for Smear microscopy for the pulmonary sample were recorded as 88.38, 88.71, 76.87, and 94.73%, respectively, and for the extrapulmonary sample was recorded as 39.28, 98.78, 28.20, and 95.30%, respectively. In this study, pulmonary sample sensitivity and PPV were higher than extrapulmonary samples.

GeneXpert's sensitivity, specificity, positive predictivity value, and negative predictivity value were 97.75, 76.78, 64.12, and 98.77% for the pulmonary sample, respectively, and 89.28, 85.90, 22.90, and 99.41% for the extrapulmonary sample. In this analysis, the sensitivity of extrapulmonary and pulmonary samples was comparable.

Using the GeneXpert MTB/RIF assay in routine TB diagnosis should be encouraged by the study's findings regarding the assay's sensitivity, specificity, PPV, and NPV in diagnosing *M. tuberculosis* infection. We evaluated the results of several assays for the diagnosis of TB in different clinical samples. As per our study, the GeneXpert MTB/RIF assay has the highest sensitivity in comparison to other assays.

There were some limitations to the research: For starters Since the study was conducted retrospectively, it was not possible to correlate the findings to histological or radiological findings. Second, a key advantage of the GeneXpert assay is its ability to identify Rifampicin resistance. We did not analyze the sensitivity and specificity of the GeneXpert MTB/RIF assay to determine

Rifampicin resistance in our study since we did not receive the request for Rifampicin sensitivity by phenotypic approach in all of the positive samples.

Conclusion

In conclusion, GeneXpert requires less biosafety equipment than other tests but is more sensitive for rapid TB diagnosis. Although it is the gold standard method, culture requires days to become positive and cannot simultaneously identify Rifampicin resistance. Whether smears are positive or negative, the Xpert MTB/RIF assay offers a high clinical application value for patients who are suspected pulmonary and extrapulmonary TB because of its rapid results and simultaneous detection of Rifampicin resistance. This is particularly valid for those suffering from HIV-related TB and multidrug resistance. It is necessary to assess GeneXpert's cost-effectiveness in low-income countries like India where TB is more prevalent.

Clinical Significance

The early detection of TB can be greatly aided by the cartridge-based nucleic acid amplification test. As an important part of the National Tuberculosis Elimination Program gene experts will certainly help in the elimination of TB from India.

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