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# Comparison of diagnostic efficacy of GeneXpert MTB/RIF assay with Ziehl Neelsen staining & microscopy in diagnosis of pulmonary tuberculosis

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ABSTRACT

**Introduction:** Tuberculosis (TB) is a highly communicable disease and a major health problem in many countries. An early diagnosis and treatment is important for controlling its spread. Conventional methods for TB diagnosis such as microscopy have low sensitivity. Though culture is considered as gold standard, it is time consuming. Newer molecular diagnostic methods such as GeneXpert assay are rapid and highly sensitive and are now playing a pivotal role in TB diagnosis.

**Materials and Methods:** In the present study, we compared the diagnostic efficacy of Ziehl Neelsen (Z-N) staining & microscopy with GeneXpert assay for diagnosis of pulmonary tuberculosis in case of 660 patients of suspected pulmonary TB.

**Results:** In this study, a total of 660 sputum samples were tested by both Z-N staining and microscopy and by GeneXpert assay. Of these, 23.73% of sputum samples were tested positive for acid fast bacilli (AFB) by smear and microscopic examination while 72.27% were negative for AFB. When the same sputum samples were subjected to GeneXpert assay, 34.24% samples were tested positive indicating higher sensitivity for detection of TB bacilli. 65.76% samples gave negative result in GeneXpert.

**Conclusion:** This study indicates that GeneXpert assay is more sensitive for detection of TB bacilli in sputum samples as compared to smear and microscopy alone. It should be considered as an effective tool for early diagnosis of tuberculosis especially in countries where it is prevalent.



## 1. Introduction

Tuberculosis (TB) is one of the oldest diseases known to affect humans. It is caused by bacteria belonging to *Mycobacterium tuberculosis complex*. The disease is prevalent in countries where poverty, malnutriti on and poor housing prevails. It kills about 3 million people and infects 9 million others every year.[1]

*Mycobacterium tuberculosis* usually affects the lungs causing pulmonary tuberculosis, but in about one third of cases it may affect other organs giving rise to extrapulmonary tuberculosis (EPTB).[1] In most cases of TB, the patient may be symptomatic for one to three months before diagnosis. Such delays in diagnosis may be due to low diagnostic suspicion by the medical personnel, lack of access to health services or because the patient may not acknowledge being sick or may not seek medical help due to economic or cultural reasons. An early diagnosis of TB is critical for controlling transmission of the disease in the community, especially in high risk areas such as prisons or hospitals. If diagnosis is delayed, the disease may evolve rapidly, destroying the pulmonary parenchyma.[2]

Diagnosis of pulmonary TB is usually done by Ziehl Neelsen (Z-N) acid fast staining of sputum smears and by mycobacterial culture wherever facilities are available. Though many advanced methods of TB diagnosis are available now, sputum smear and microscopy is the main and per haps the only method used for TB diagnosis in many developing and underdeveloped countries.[3] However, microscopy alone may not be able to accurately diagnose

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TB as it can be associated with errors.[4] Mycobacterial culture is the gold standard and the most sensitive method for TB diagnosis, but its use in clinical practice is limited due to a slow turnaround time of 6-8 weeks, biosafety requirements and high cost.[5] The emergence and spread of multi-drug resistance in TB (MDR-TB) bacilli is one more major challenge for health systems and TB control programs.[6] Therefore, new techniques in molecular diagnostic methods in the field of TB have been introduced into practice, which are playing an important role in early diagnosis of and prompt treatment of TB. One such method is GeneXpert MTB/RIF assay which is now widely available and being used extensively. This molecular method is more sensitive in detecting presence of TB bacilli in sputum samples than just smear and microscopic examination. It can detect both *Mycobacterium tuberculosis* DNA and genetic mutations associated with Rifampicin resistance simultaneously in a short span of just two hours using sputum samples.[7] The present study was conducted to compare the diagnostic efficacy of GeneXpert MTB/RIF assay with Ziehl Neelsen staining (smear & microscopy) for diagnosis of pulmonary tuberculosis in a tertiary care rural hospital.

## 2. Materials and Methods

### *2.1. Study Design*

This was a prospective study conducted in Department of Microbiology at a tertiary care rural hospital after approval from the institutional ethics committee, for a period of about 10 months from October 2018 to July 2019.

### *2.2. Patient inclusion criteria*

Patients clinically suspected to have pulmonary tuberculosis with symptoms such as cough with or without expectoration for >2 weeks, weight loss, fatigue, hemoptysis and loss of appetite were included in the study irrespective of their age, sex and HIV status. A total number of 660 sputum samples were tested in the study.

### *2.3. Collection of sputum samples*

Morning sputum samples were collected from these patients after giving proper instructions so as to obtain good quality sputum in a Falcon tube of 50 ml capacity. At least 5 ml of sputum sample is collected. In case of patients admitted in the hospital and who are unable to provide sputum sample, bronchoalveolar lavage (BAL) is collected by a trained personnel. The falcon tube is labelled with patient's name, registration number and other identification details and sent to tuberculosis laboratory in the department of Microbiology.

### *2.4. Processing of samples in TB laboratory*

From each sputum sample, a direct smear is prepared on a clean, grease free glass slide using a clean disposable wooden applicator stick. The slide was air dried and heat fixed by passing it through Bunsen's burner flame 3 – 4 times. The slide is then placed horizontally on a staining rack and stained by Ziehl – Neelsen (Z-N) method as per the Revised National Tuberculosis Control Program (RNTCP) guidelines.[8] Acid fast bacilli are seen as bright red or pink rods against blue background.

The same sputum samples are then tested for detection of *Mycobacterium tuberculosis* DNA using GeneXpert MTB/RIF assay. This method is more sensitive in detecting presence of TB bacilli in sputum samples than just smear and microscopic examination. It can detect both *Mycobacterium tuberculosis* DNA and genetic mutations associated with Rifampicin resistance simultaneously in a short span of just two hours using sputum samples.[9] Though conventional culture based drug sensitivity testing is considered as a gold standard investigation to detect MDR, the sensitivity and specificity of GeneXpert MTB/RIF assay is comparable with conventional methods.[10] GeneXpert MTB/RIF assay is a fully automated cartridge based molecular diagnostic test for TB. In this assay, about 3 – 5 ml of sputum sample is mixed with twice the volume of sample reagent. It is shaken vigorously and incubated at room temperature for 10 minutes. After 10 minutes it is again shaken vigorously and incubated for another 5 minutes. 2 ml of this processed sample is then added to GeneXpert cartridge which is then loaded in the device. The results are finally interpreted by the GeneXpert system based on fluorescent signals which are displayed on the system monitor after about 90 minutes.[10] The results of smear and microscopy were compared withthat of GeneXpert MTB/RIF assay using Chi square test.

## 3. Results

In this study, a total of 660 sputum samples were tested by smear and microscopic examination with Z-N staining as well as by GeneXpert MTB/RIF assay. Out of these total samples, 183 (23.73%) samples were tested positive for acid fast bacilli (AFB) by smear and microscopic examination after Z-N staining. 477 (72.27%) sputum samples were negative for AFB. Table 1 shows the result s of smear examination with Z-N staining in case of total 660 sputum samples.

**Table 1:** Results of Smear examination with Z-N staining

| Positive | Negative | Total |
|---|---|---|
| 183 (23.73%) | 477 (72.27%) | 660 |

When the same sputum samples were subjected to GeneXpert assay, in 226 (34.24%) sputum samples, *M.*

**Table 2:** Result of GeneXpert MTB/RIF test

| M. tuberculosis detected | M. tuberculosis not detected | Total |
|---|---|---|
| 226 (34.24%) | 434 (65.76%) | 660 |

**Table 3:** Combined Result ofGeneXpert testing & Sputum smear Examination

| | GeneXpert Positive | GeneXpert Negative | Total |
|---|---|---|---|
| Smear Positive | 175 | 8 | 183 |
| Smear Negative | 51 | 426 | 477 |
| Total | 226 | 434 | 660 |

*tuberculosis* DNA was detected whereas 434 (65.76%) sputum samples gave negative result in GeneXpert. Table 2 shows the result of GeneXpert testing in case of total samples.

In case of 226 sputum samples which were positive in GeneXpert assay, only 175 samples showed presence of acid fast bacilli in Z-N staining. 51 sputum samples were positive by GeneXpert but negative in smear examination. Whereas in case of 8 sputum samples, smear examination was positive and GeneXpert test was negative. The combined results of both sputum smear examination and GeneXpert testing are shown in Table **??**.

## 4. Discussion

Conventional methods such as smear microscopy for detection of *M. tuberculosis* in clinical specimens have low sensitivity. Molecular techniques, including the Cepheid GeneXpert system, have changed the field of TB with rapid diagnosis combined with high sensitivity and specificity results. In December 2010, the WHO endorsed the GeneXpert MTB/RIF assay for the rapid diagnosis of TB and MDR-TB.[6] Now a days the GeneXpert facility is available for TB diagnosis at many Government Hospitals at District and even at Taluka places under Revised National Tuberculosis Control Program (RNTCP) and is provided free of cost to all patients. It has now come up as a major revolution in the field of TB diagnosis.

In our study, out of the total 660 sputum samples from patients of suspected pulmonary tuberculosis, smear examination (Z-N staining) was positive in 183 (27.73%) patients. But when GeneXpert assay was performed on same sputum samples, it was positive for 226 (34.24%) cases. Thus GeneXpert was found to be superior for detection of *M. tuberculosis* in sputum samples as compared to microscopy and smear examination alone. (Chi square value for 5% level of significance = 6.565) Many other such studies show similar findings. In a study carried out by Chinedum OK et al. GeneXpert was positive in 65.7% cases as compared to smear examination which was positive in 38.6% cases when used to diagnose TB.[11] In another study done by Mavenyengwa R et al, 32.20% samples were found to be positive for TB by GeneXpert MTB/RIF assay, and only 24.05% were found to be positive by microscopy.[3] One more such study conducted by Bajrami R et al, GeneXpert could detect *M. tuberculosis* in 29.3% cases as compared to Z-N staining alone which was positive in 14.6% cases only.[6] All these studies including our study indicate that the GeneXpert assay is more sensitive for diagnosis of tuberculosis as compared to smear and microscopic examination.

In our study we observed that 8 sputum samples showed presence of AFB on smear and microscopic examination but these were tested negative for *M. tuberculosis* in GeneXpert assay. These acid fast bacilli may be considered as Non-tuberculous mycobacteria (NTM) because the GeneXpert assay detects DNA only in case of only *M. tuberculosis complex* and not in case of infection with NTM.[12]

Overall our study supports the fact that GeneXpert can be considered as an effective tool for early diagnosis of tuberculosis. In countries like India, where TB is quite prevalent, GeneXpert has made a huge impact. With early detection of the disease it is possible to treat it in an effective way and to prevent its spread.

## 5. C onclusion

GeneXpert MTB/RIF assay is an effective tool for diagnosis of tuberculosis and has better sensitivity than smear and microscopic examination. It is very much useful to detect more cases of smear negative TB. It can also detect rifampicin resistance in TB bacilli (Multi-drug resistant tuberculosis). Early detection and appropriate treatment of drug sensitive as well as MDR-TB is an important part of TB control activities and it will also help to control the spread of this highly communicable disease in the community.

## 6. Source of Funding

None.

## 7. Conflict of Interest

None

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