

# Revisiting Conventional Diagnosis for Pulmonary Tuberculosis vs. Latest Modalities for Resource Scarce Settings

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## Abstract

**Introduction:** Tuberculosis is an infectious disease with a high prevalence with about 9 million cases occurring annually. ZN microscopy is the most widely used technique to detect AFB, but it is less sensitive. However fluorescent microscopy is more helpful with simple diagnostic criteria. Gene Xpert® MTB/RIF assay is a rapid molecular assay that enables diagnosis of TB with simultaneous detection of rifampicin resistance.

**Methodology:** In this study the diagnostic accuracy of fluorescent microscopy and Xpert MTB/RIF for the diagnosis of TB were evaluated.

**Results:** A total of 352 respiratory specimens were tested among which 160 samples were positive by culture. Out of culture positive samples, 158 samples (98.7%) were Gene Xpert TB positive while 2 were negative. While only 49 were positive on ZN microscopy and 89 were positive on fluorescent microscopy. Out of the culture negative samples, 2 were positive with ZN microscopy, one was positive with fluorescent microscopy and 3 were positive on Gene Xpert. Sensitivity, specificity, PPV, NPV and diagnostic accuracy of ZN Smear microscopy was 39%, 99.5%, 96%, 63% and 14.5% respectively. Sensitivity, specificity, PPV, NPV and diagnostic accuracy of fluorescent smear microscopy was 55% and 99.5%, 98%, 72% and 79% respectively. Sensitivity, specificity, PPV, NPV and diagnostic accuracy of Gene XPERT was 98% and 99%, 98%, 99% and 98% respectively.

**Conclusion:** In countries like Pakistan where the disease is endemic, fluorescent microscopy and Gene Xpert PCR can help in making a timely diagnosis.

**Keywords:** Pulmonary tuberculosis; Fluorescent microscopy; Gene Xpert; Auramine phenol stain

## Introduction

Pulmonary tuberculosis (TB) is a global health burden. According to the WHO report, there were 10.4 million incident cases of TB and 1.4 million people died because of the disease [1]. Pakistan is one of the six countries which account for 60% of the global TB burden. Timely and accurate diagnosis of tuberculosis remains pivotal for controlling this disease. Ziehl Neelsen (ZN) staining is still widely used in most parts of the world as a diagnostic tool; however, this method has low sensitivity and specificity. Fluorescent microscopy is more sensitive than Ziehl Neelsen staining [2,3]. Cases of scanty bacilli which are unable to pick up on smear microscopy may lead to delay in diagnosis and subsequent treatment. Mycobacterial culture is considered to be the gold standard for diagnosis of TB; however, conventional method using Lowenstein Jensen media is time consuming [4,5].

A variety of nucleic acid amplification (NAA) methods have been developed for the rapid detection and identification of pulmonary and extra-pulmonary *Mycobacterium tuberculosis* [6]. These techniques are both, fast and accurate but require costly equipment and technical expertise. Recently, the WHO has endorsed Gene Xpert (Xpert® MTB/Rif assay) for the diagnosis of TB which aids in rapid

identification of *Mycobacterium tuberculosis* and Rifampicin resistance related mutations simultaneously in an unprocessed sample. Diagnostic accuracy of Gene Xpert for pulmonary TB is high and it is also useful to detect *M. tuberculosis* in samples with no or scanty bacilli [7,8].

The rationale of our study was to revisit the diagnostic accuracy of ZN staining, Fluorescent staining and Gene Xpert keeping culture as the gold standard for resource scarce settings.

## Materials and Methods

The study was conducted from January 2016 through December 2016 at Microbiology Department, Armed Forces Institute of Pathology, Rawalpindi, Pakistan. Ethical approval was sought from Research Ethics Review Committee. After a written informed consent, patients aged 18 to 70 years irrespective of gender with suspected TB based on history, clinical and radiological examination were registered for the study. Respiratory clinical specimens including sputum, broncho-alveolar lavage (BAL), and endobronchial washings were collected. Patients already diagnosed with pulmonary TB, repeat sample of the same patient, improperly collected samples and patients already on anti-TB treatment were excluded.

All respiratory specimens were processed by the standard N-acetyl-L-cysteine and sodium hydroxide method with final concentration of NaOH as 2%. Tube containing digested and decontaminated

specimens was centrifuged (3000 × g) for 15-20 min after which the supernatant was discarded and deposit was used for ZN staining, fluorescent staining and culture.

## ZN staining

2-3 drops from specimen were placed on a glass slide to prepare a smear before it was inoculated into MGIT 960 system. The smear was then placed into an oven at a temperature of 56° C for about 5-6 min for drying, followed by the ZN staining. The number of AFB present was reported as; 1-9 bacilli/100 fields=1+, 1-9 bacilli/10 fields=2+, 1-9 bacilli/field=3+ and more than 9 bacilli/field=4+.

## Culture

All the specimens after being digested and decontaminated processed along with MGIT growth supplement OADC (Oleic acid, Albumin, Dextrose, Catalase) and PANTA (Polymyxin-B, Amphotericin-B, Nalidixic acid, Trimethoprim and Azlocillin) were inoculated into MGIT 960 TB system (Becton Dickinson, Sparks, USA) after scanning bar code. All these specimens along with a positive control MGIT tube (containing ATCC 25177 *Mycobacterium tuberculosis*) and an un-inoculated negative control MGIT tube were incubated in MGIT 960 until the instrument showed a tube positive or negative. After a positive signal, the tube was removed from the MGIT 960 system after scanning bar code and ZN staining was done for the confirmation of the presence or absence of AFB. Mycobacteria other than tuberculosis (MOTT) were checked by rapid immunochromatographic technique using “BD MGIT™ TB Identification Test”.

## Fluorescent microscopy

2-3 drops from the specimen were placed on a glass slide to prepare a smear before it was inoculated into MGIT 960 system. The smear was then placed into an oven at a temperature of 56°C for about 5-6 min for drying, followed by the auramine phenol staining for 20 min, slide was rinsed with water followed by decolourisation by acid alcohol and counterstained with 0.1% potassium permanganate for 30 s. AFBs were visualized by using fluorescent microscope in 250X magnification power. If a sample contained 1-9 acid fast bacilli (AFB) per 10 fields it

was categorized as +1, if it contains 1-9 AFB/field this was categorized as 2+, if a sample contains 10-90 AFB/field it was be categorized as 3+, while if a sample contains >90 AFB/field it was be categorized as 4+.

## Gene Xpert PCR

One ml of the remaining deposit of clinical sample was transferred to a screw-capped tube containing 2 ml of sample reagent at a ratio of 1:2; this reagent inactivated the sample with NaOH and isopropanol. The mixture was then incubated for 15 min at room temperature and mixed every 5 min until liquefied with no visible clumps. The mixture was transferred into the Xpert MTB/RIF cartridge using the sterile pipette provided until the meniscus was above the minimum mark. The Xpert MTB/RIF cartridge includes an internal control for sample processing (DNA extraction and for PCR presence inhibitors). The inoculated cartridge was placed into the Gene Xpert instrument (GX). Results were available in less than 2 h and interpreted by the GX system automatically.

## Results

A total of 352 respiratory specimens (202 sputum, 95 EB washings and 55 BAL samples) were tested. Mean age of the patients was 42.75 ± 14.73 years. There were 258 (73.3%) male and 94 (26.7%) female patients. Out of total 352 specimens, 192 specimens were negative by all 4 modalities. 160 samples were positive by culture including 97 sputum, 26 EB washings and 37 BAL specimens. Out of the 160 that were culture positive, 158 samples (98.7%) were Gene Xpert TB positive while 2 were negative. Out of the 160 samples that were culture positive, only 49 were positive for ZN microscopy (10 specimens were detected as +1, 13 specimens were +2 and 26 specimens were +3 for AFB) and 89 specimens were positive by fluorescent microscopy. Out of the culture negative samples, 2 were positive for ZN microscopy, 1 was positive for fluorescent microscopy and 3 were positive on Gene Xpert. The specimen that was positive on fluorescent microscopy was also positive on Gene Xpert and ZN microscopy.

Sensitivity, specificity, PPV, NPV and agreement for ZN smear microscopy, Fluorescent smear microscopy and Gene Xpert is shown in Table 1.

|  |          | Culture  |          | (%)         |             |     |     |           |
|--|----------|----------|----------|-------------|-------------|-----|-----|-----------|
|  |          | Positive | Negative | Sensitivity | Specificity | PPV | NPV | Agreement |
| ZN Microscopy  | Positive | 49       | 02       | 39          | 99          | 96  | 63  | 14.5      |
|  | Negative | 111      | 190      |             |             |     |     |           |
| Fluorescent Microscopy   | Positive | 89       | 1        | 55          | 99          | 98  | 72  | 79        |
|  | Negative | 71       | 191      |             |             |     |     |           |
| Gene Xpert   | Positive | 158      | 03       | 98          | 99          | 98  | 99  | 98        |
|  | Negative | 2        | 189      |             |             |     |     |           |
| PPV: Positive Predictive Value, NPV: Negative Predictive Value |          |          |          |             |             |     |     |           |

**Table 1:** Sensitivity, specificity, PPV and NPV of different diagnostic modalities (n=352).

## Discussion

Early diagnosis and prompt treatment of TB plays a key role in breaking the chain of its transmission. The purpose of this study was to evaluate the diagnostic yield of different modalities in diagnosing tuberculosis in respiratory specimens. Sensitivity of ZN microscopy depends on factors such as smearing technique, viewer experience and number of bacilli in the specimen. Unfortunately, microscopy is not a very sensitive tool for the diagnosis of tuberculosis [9,10]. A study conducted in India concluded the sensitivity of ZN stain to be 55.5% and that of Fluorescent microscopy 71.85%. Specificity was 99.2% for both staining methods employed [11]. Sensitivity of ZN staining in our study was 39.5% whereas fluorescent stain was 55%, a lower sensitivity. However, specificity was comparable to that of the former study. Our results for ZN microscopy were in complete concordance with the study done by Agarwal et al. except our sensitivity was higher [5]. Our results for ZN microscopy were also similar to the study done by Khalil except that study had a very low negative predictive value [12].

In many developing countries where there are limited resources, the diagnosis of tuberculosis is based completely on microscopy of ZN stained smears. The fluorescent stain, auramine, however, has displayed better performance and is more sensitive than the ZN stain. Therefore, auramine may be a cost-effective step to improve the diagnosis of tuberculosis in settings where fluorescent microscope is available. Cattarmanchi et al. reported the sensitivity and specificity of fluorescent microscopy to be 72% and 81% respectively. Our results are not in concordance to this study as we have a relatively lower value for the sensitivity and higher for the specificity [13].

Results for Gene Xpert accuracy were in relative concordance with Sharma et al., Barnard et al. and Reechaipichkul et al. [9,10,14]. Khalil et al. conducted a study to evaluate the efficacy of Gene Xpert in Pakistan and results were comparable with our findings [12].

Gene Xpert allows a precise and timely diagnosis of tuberculosis. The shorter turnaround time offers an opportunity for therapeutic intervention to be done in a timely manner. This is advantageous for not only patients, but also for their contacts. Patients with false positive results may receive avoidable anti-TB therapy. False positivity of Xpert MTB/RIF assays and microscopies may be attributed to the presence of dead MTB test samples particularly among previously treated patients.

The results of our study conclude that fluorescent microscopy has better diagnostic accuracy as compared to ZN staining and is cheaper as compared to PCR. Gene Xpert PCR is a modality with very high sensitivity and specificity and can detect not only *Mycobacterium tuberculosis* but also rifampicin resistance simultaneously. In countries like Pakistan where the disease is endemic and where facilities of fluorescent microscopy and Gene Xpert PCR are available, combination of these two modalities can help in making a timely diagnosis and initiating timely treatment.

Fluorescent microscopy is a simple modality with better performance and high diagnostic yield compared with ZN microscopy. Gene Xpert MTB/RIF is a rapid, sensitive, accurate and reliable

method for diagnosis of TB along with rifampicin resistance. In a resource scarce country like Pakistan where the disease is endemic these modalities believed to be more beneficial in timely diagnosis of a highly contagious disease.

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