

# [Effectiveness of xpert mtb rif on smear negative samples](https://assignbuster.com/effectiveness-of-xpert-mtbrif-on-smear-negative-samples/)

Effectiveness of Xpert MTB/Rif on smear negative samples tested by LED Fluorescence microscopy for rapid diagnostics of TB cases in Bihar

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Abstract –

The global priorities for tuberculosis (TB) control are to improve the case detection and to detect cases earlier. The evolution of newer diagnostic technologies for TB improves the case detection and early treatment initiation. RNTCP implements the newer LED fluorescence microscopy over the ZN based microscopy for direct sample testing, as it increases the sensitivity to about 10%. In our diagnostic algorithm, the smear positive specimen goes for LPA and smear negative goes for CBNAAT for further molecular DST. Due to the high sensitivity of CBNAAT over LPA for detection of MTB, it is used for smear negative samples. In the present study, we are estimating the TB cases detected by CBNAAT which was negative on LED fluorescence microscopy to ensure the requirement of a more costly method for enhanced case finding. In our study, we have tested 513 samples on CBNAAT which was smear negative by LED fluorescence microscopy. Out of 513 samples, 371 (72. 31%) were found negative for MTBC, 108 (21. 05%) were detected positive, out of which 83 (16. 17) cases was detected rifampicin sensitive, 25 (4. 87%) cases was detected rifampicin resistant. Rest 34 (6. 62%) cases was in non conclusive results like invalid and error. This reflects that, by the use of CBNAAT the rapid detection of TB can be improved by 21%.

Key words–

Tuberculosis, RNTCP, Fluorescence microscopy, CBNAAT, MTBC

Introduction–

Tuberculosis is an infectious disease caused predominantly by Mycobacterium tuberculosis, commonly transmitted by inhalation of infectious droplet nuclei. India accounts for one fourth of the global TB burden. The estimated prevalence is about 2. 5 million. It has been observed that by the end of first few days of treatment, most of the actively dividing bacilli dies and chances of infection transmission rapidly reduced. So, the global priorities for tuberculosis (TB) control are to improve the case detection and to detect cases earlier. The evolution of newer diagnostic technologies for TB improves the case detection and early treatment initiation. In India, for a long time Ziel-Neelsen based microscopy was used for microbiological detection of TB patient. However, this technique needs 5000-10000 bacilli per ml for detection and bacilli load below this may leads to a negative result. A more sensitive technique was available, ie. Culture based test, but due to the long generation time of Mycobacterium tuberculosis, this method takes 3 to 4 weeks to yield positive result. As, culture was the only technique for drug susceptibility testing, it was not possible to early diagnose the drug resistance case microbiologically.

In present, new techniques for rapid detection of TB cases are developed and implemented. This includes Fluorescence microscopy using Auramine o fluorescence dye, Nucleic Acid Amplification test (NAAT), Reverse hybridization based Line Probe Assay (LPA), Liquid culture and DST.

Conventional fluorescence microscopy is more sensitive than Ziel-Neelsen technique and takes less time in reading slides [1], but its use has been limited by the high cost of mercury vapour light sources and need of a dark room.

However, light emitting diode (LED) fluorescence microscopy has been developed to offer the benefits of fluorescence microscopy without the associated cost. It has been observed that Use of LED-FM significantly increased the proportion of smear positive cases among presumptive TB patients [2]. WHO approves LED Fluorescence Microscopy as qualitative, operational and cost advantageous over both conventional fluorescence and Ziel-Neelsen microscopy.

On the other hand, CBNAAT (Cartridge based nucleic acid amplification test), which is manufactured by Cepheid as Xpert MTB/Rif, was developed for rapid detection of Mtb and its molecular DST with rifampicin within 2 to 3 hours. It is a fully automated real time PCR based technique using molecular beacon probe. It was validated as a more sensitive technique than reverse hybridization based Line probe Assay (LPA). LPA was used for only smear positive specimen, but CBNAAT is recommended for both smear positive and negative specimen. Presently, under RNTCP, its use is recommended for diagnosis of DRTB (Drug Resistant TB) cases in presumptive DRTB patients and TB preferentially in key population such as Pediatrics, PLHIV and Extra pulmonary TB [3]. It has been observed that using Xpert MTB/Rif assay can dramatically improve the rapid diagnosis of tuberculous meningitis and other types of extra pulmonary tuberculosis of HIV infected patients [4].

According to the WHO policy update, LED microscopy showed 84% sensitivity and 98% specificity against culture as the reference standard. Xpert MTB/RIF achieved an overall pooled sensitivity of 88% and a pooled specificity of 99% when used as an initial diagnostics test, whereas when used as an add-on test following a negative smear-microscopy result, Xpert MTB/RIF yielded a pooled sensitivity of 68% and a pooled specificity of 99% [5]. In our present work, we will study the effectiveness of Xpert MTB/Rif on smear negative samples tested by LED Fluorescence microscope. The research is based on the diagnostic algorithm under RNTCP and the lab works has been done at Intermediate Reference Laboratory, Patna, Bihar.

Material and Method–

The Presumptive MDR samples are received under Revised National TB Control Program for detection of drug resistant TB is used for the current study. We received samples from different districts of Bihar. Generally two sputum samples are taken for diagnostics. We prepared direct smears for all the samples and staining done by auramine staining method using recommended protocol.

Air dry followed with heat fixing of slides were done before staining.

Auramine staining –

* Flood the slide with filtered 0. 1 % Auramine solution.
* Keep the staining reagent for at least 20 min, make sure that the smear area is continuously covered with Auramine by adding more if needed .
* Rinse with water and drain.
* Apply decolorizing solution, 0. 5 % acid alcohol for 3 minutes.
* Gently rinse with water until the visible stain has been washed away and drained.
* Flood smear with 0. 5 % KMnO 4 for 1 minute. Time is critical because counter staining for longer time may quench the acid fast bacilli fluorescence.
* Gently rinse with water and drain.
* Air dry on a slide rack away from sunlight. If they are not read immediately place them in slide box.

Use the objective 20x for focusing and read the slide using 40 x objectives.

The slides were seen under LED fluorescence microscope. It is recommended to see at least 40 fields at 400x resolution to give a negative result. The smear negative samples tested by LED fluorescence microscopy were subjected to CBNAAT testing.

Results and Discussion –

Out of 513 samples, 371 (72. 31%) were found negative for MTBC, 108 (21. 05%) were detected positive, out of which 83 (16. 17) cases was detected rifampicin sensitive, 25 (4. 87%) cases was detected rifampicin resistant. Rest 34 (6. 62%) cases was in non conclusive results like invalid and error. The study is based on the current diagnostic algorithm under RNTCP. It was observed that by using a costlier technique like Xpert MTB/Rif can increase the rapid case detection by 21% and hence plays important role in effective patient management and TB control. The comparisons of CBNAAT results with the conventional culture test will be the future prospects of research.

Conclusion –

The implementation of using CBNAAT on smear negative sample tested by LED FM improves the rapid case detection by more than 20%. Before implementation of this technique, culture was done on smear negative presumptive TB cases, which takes more time for detection and hence cause delay in treatment initiation. By using Xpert MTB/Rif, these cases can be detected earlier and treatment can be initiated accordingly.

References –

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