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### RESEARCH ARTICLE

#### FLUORESCENCE MICROSCOPY VS TRUENAT- WHICH METHOD IS BETTER FOR DIAGNOSIS OF TUBERCULOSIS?

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#### Abstract

**Background:** Despite the advent of newer drugs, modern diagnostic techniques and improvement in management strategies, India continues to be the leader in tuberculosis burden. Several methods for diagnosis exist, such as light microscopy, fluorescent microscopy, solid culture, liquid culture (Mycobacterium growth indicator tube [MGIT] culture) to molecular methods. However, each of these methods have variable sensitivity and specificity. Very few studies have been done evaluating the role of TrueNAT with fluorescent microscopy, thereby evaluating the performance of a designated microscopy center under the national tuberculosis elimination programme.

**Methods and Materials:** This is a retrospective observational study performed in a designated microscopy center in Santhiram Medical Center, Nandyal. This is to compare the fluorescence microscopy and TrueNAT provided as part of the National Tuberculosis Elimination programme. A total of 342 patients underwent testing at this center and were diagnosed with tuberculosis.

**Results:** Majority of the study participants were males, smokers, in the age groups of 31-40 years. The mean age of males was found to be higher than that of females. (37.64 vs 32.39 years). 89.42% of patients were diagnosed on fluorescent microscopy, while 94.23% were diagnosed with TrueNAT. Sensitivity of TrueNAT was 91.61%, specificity was 87.80% and accuracy was 90.76%.

**Conclusion:** TrueNAT appears to be superior to Fluorescent microscopy in a setting of a designated microscopy center. This can be used for rapid diagnosis, with high sensitivity with specificity when compared to the traditionally accepted fluorescent microscopy.

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#### Introduction:-

The biggest number of deaths worldwide are still caused by the tremendous global burden of TB. World TB Report 2017 states that 1.6 million people die from TB each year and there are more than 9 million new cases<sup>1</sup>. Around 25% of patients who seek treatment in India's public health system do not receive a diagnosis or a course of therapy<sup>2</sup>. It is crucial to identify and treat the condition as early as possible because pulmonary TB spreads quickly and responsible for more over 80% of all TB infections. If left untreated, an active Tb patient can spread the infection through close contact to up to 15 more people. Despite the advent of newer drugs, modern diagnostic techniques and improvement in management strategies, India continues to be the leader in tuberculosis burden<sup>3</sup>. Particularly in

countries with endemic tuberculosis, like India, the need for quick, accurate diagnostic procedures to detect active tuberculosis is critical.

The most typical technique for identifying pulmonary TB is sputum smear microscopy worldwide <sup>4</sup>. Direct smear microscopy has a modest sensitivity but a good specificity in a clinical setting <sup>5</sup>. It is even lower in persons with HIV. The World Health Organization (WHO) recognises the liquid culture technique with molecular line probe as the industry standard for MDR TB fast detection <sup>6</sup>. Compared to automated techniques and biomarkers like PCR or real-time PCR, microscopy and culture are noticeably less sensitive. Several Nucleic Acid Amplification (NAA) methods have been developed for the quick identification of Mtb in clinical samples of pulmonary and extrapulmonary TB cases <sup>7,8</sup>.

A pilot study found that when compared to a Composite Reference Standard, the TrueNAT Mycobacterium tuberculosis TB test was able to diagnose TB rapidly and with good sensitivity (CRS). TrueNat produced results that were quicker and more accurate than the internal nested PCR approach. Using a CRS as the baseline, it was shown that TrueNAT had a sensitivity and specificity of 91.1% and 100%, respectively.

### Materials and Methods:-

This is a retrospective observational study performed in a designated microscopy center in Santhiram Medical Center, Nandyal. This is to compare the fluorescence microscopy (FM) and TrueNAT provided as part of the National Tuberculosis Elimination programme. A total of 342 patients of PTB underwent testing at this centre. The study included only those patients between 18-80 years that were newly diagnosed with PTB. Old patients, defaulters in treatment, pregnant women and those with HIV were excluded from the study.

For FM- slides are prepared and stacked, and then the slides are flooded with freshly filtered auramine-phenol. This is then left to air-dry for 7-10 minutes.



Figure 1:- Flooding of slide with fluorescent dye<sup>6</sup>.

For trueNAT testing- Fresh samples (sputum) from untreated persons were processed in accordance with the manufacturers' instructions in the TrueNAT kit, with a starting amount of 500 L supplied to the sample pre-treatment tube<sup>6</sup>.

The data was recorded in a master chart using a Microsoft excel spreadsheet, and correlation was checked. SPSS v 21.0 was used to statistically analyse the data. The sensitivity, specificity, PPV, NPV, and diagnostic accuracy for the diagnosis of TB was calculated for AFB smear FM and TrueNAT.

### Results:-

The study included a total of 342 patients diagnosed with PTB using ZN staining of the sputum. The mean age of the study population was 35.77 years. The male: female ratio was observed to be 3.2:1. The mean age of males was found to be higher than that of females. (37.64 vs 32.39 years). The most common presenting complaint was cough, followed by breathlessness.

36.43% of the study participants were observed to be smokers, while 24.96% patients had associated type II diabetes mellitus.

Amongst the study participants, we found that amongst those patients identified to have PTB, 89.42% of patients were diagnosed on fluorescent microscopy, while 94.23% were diagnosed with TrueNAT. When we correlated the FM with TrueNAT, we found that the sensitivity of TrueNAT was 91.61%, specificity was 87.80% and accuracy was 90.76%.

Statistic	Value	95% CI
Sensitivity	91.61%	87.77% to 94.55%
Specificity	87.80%	78.71% to 93.99%
Positive Likelihood Ratio	7.51	4.20 to 13.44
Negative Likelihood Ratio	0.10	0.06 to 0.14
Disease prevalence (*)	77.72%	73.12% to 81.87%
Positive Predictive Value (*)	96.32%	93.61% to 97.91%
Negative Predictive Value (*)	75.00%	66.98% to 81.61%
Accuracy (*)	90.76%	87.33% to 93.52%

### Discussion:-

In the present study, we compared and correlated the diagnostic potential of FM and TrueNAT in a designated microscopy center in a tertiary care hospital. We observed that TrueNAT demonstrated a high sensitivity and moderately high specificity for diagnoses of PTB when compared with FM.

In a study by Sameera Akhthar et al<sup>9</sup>, 92.6% and 7.4% of the subjects were detected positive and negative respectively with TrueNAT. The TrueNAT technique sensitivity, specificity, PPV, NPV, and accuracy were 94.05%, 42.86%, 97.53%, 23.08%, and 92.00%, respectively, which is similar to the findings of our study.

In a study by Ngangue YR et al., the sensitivity and specificity of TrueNAT for pulmonary TB was 91% and 96%, respectively<sup>10</sup>. Similarly, in a study by Penn Nicholson A et al in 2021, they observed the sensitivity and specificity of TrueNAT in a PHC setup to be 84% and 95%, respectively<sup>11</sup>. TrueNat has the advantages of lower turn-around time (two hours) for detecting drug resistant TB, high sensitivity of detection of TB with simultaneous assessment of rifampicin resistance and thus has potential to replace the gold standard culture method. However, CBNAAT is still the recommended test for sputum smear-negative specimens and the first-line of diagnostic test in MDR/RR-TB and HIV-related TB<sup>12</sup>.

The limitation of this study is that there were no cultures performed in these patients, which is the current gold standard for diagnosis in TB<sup>13</sup>. Also, this is a retrospective study, and none of the patients could be tested on follow-up to confirm the accuracy of the same or validate the test.

### References:-

1. India T. Revised national tuberculosis program annual status report. Directorate General of Health Services, Ministry of Health and Family Welfare, New Delhi, India. 2017.

2. Golub JE, Mohan CI, Comstock GW, Chaisson RE. Active case finding of tuberculosis: Historical perspective and prospects. *Int J Tuberc Lung Dis.* 2005;9(11):1183-03. PMID: 16333924; PMCID: PMC4472641.
3. Desikan P. Sputum smear microscopy in tuberculosis: Is it still relevant? *Indian J Med Res.* 2013;137(3):442-44. PMID: 23640550; PMCID: PMC3705651.
4. Pathrikar TG, Bansal VP, Mulay MV, Ghogare HS. Comparison of ZiehlNeelsen smear microscopy and AFB culture in a resource limited setting from various clinical samples. *Int J Health Sci Res.* 2020;10(4):46-51.
5. Zijenah LS. The World Health Organization Recommended TB Diagnostic Tools. In: Kayembe, J. N., editor. *Tuberculosis* [Internet]. London: IntechOpen; 2018 [cited 2022 Sep 04]. Available from: <https://www.intechopen.com/chapters/58595>. Doi: 10.5772/intechopen.73070.
6. [www.google.com](https://www.google.com). [cited 2023 Feb 28]. Available from: [https://www.google.com/url?sa=t&source=web&rct=j&url=https://tbcindia.gov.in/WriteReadData/1892s/7890638455Flourescence\\_Microscopy%2520Manual.pdf&ved=2ahUKEwj-s674uLf9AhXESGwGHXA3D-wQFnoECCEQBg&usq=AOvVaw1IyvXJTSvYK6uaZmYv3yJ9](https://www.google.com/url?sa=t&source=web&rct=j&url=https://tbcindia.gov.in/WriteReadData/1892s/7890638455Flourescence_Microscopy%2520Manual.pdf&ved=2ahUKEwj-s674uLf9AhXESGwGHXA3D-wQFnoECCEQBg&usq=AOvVaw1IyvXJTSvYK6uaZmYv3yJ9)
7. Combo Georges TA, Aissata T, Fatimata D, Abou CC, Gagne C, Moise SA, et al. Performance of Xpert MTB/RIF in comparison with light-emitting diodefluorescence microscopy and culture for detecting tuberculosis in pulmonary and extrapulmonary specimens in Bamako, Mali. *Int J Mycobacterial.* 2020;9(4):397-04. Doi: 10.4103/ijmy.ijmy\_171\_20. PMID: 33323655.
8. Nikam C, Jagannath M, Narayanan MM, Ramanabhiraman V, Kazi M, Shetty A, et al. Rapid diagnosis of Mycobacterium tuberculosis with Truenat MTB: A near-care approach. *PLoS One.* 2013;8(1):e51121.
9. Akhtar, Sameera&kaur, Mandeep& Kumar, Dinesh &Sahni, Bhavna&Chouhan, Rekha&Tabassum, Nazia& Akhtar, Sufhia& Gandhi, Sumit. (2022). Diagnostic Accuracy between CBNAAT, TrueNat, and Smear Microscopy for Diagnosis of Pulmonary Tuberculosis in Doda District of Jammu and Kashmir- A Comparative Study. *Journal of Clinical and Diagnostic Research.* 16. DC08-DC12. 10.7860/JCDR/2022/59404.17055.
10. Ngangue YR, Mbuli C, Neh A, Nshom E, Koudjou A, Palmer D, et al. Diagnostic accuracy of the Truenat MTB plus assay and comparison with the Xpert MTB/RIF assay to detect tuberculosis among hospital outpatients in Cameroon. *J ClinMicrobiol.* 2022; 60(8):e0015522. Doi: 10.1128/jcm.00155-22. Epub 2022 Jul 21. PMID: 35861529; PMCID: PMC9383115.
11. Penn-Nicholson A, Gomathi SN, Ugarte-Gil C, Meaza A, Lavu E, Patel P, et al. Truenat Trial Consortium; Members of the Truenat Trial Consortium: A prospective multicentre diagnostic accuracy study for the Truenat tuberculosis assays. *EurRespir J.* 2021;58(5):2100526. Doi: 10.1183/13993003.00526-2021. PMID: 34049948; PMCID: PMC8607906.
12. World Health Organization. Molecular line probe assays for rapid screening of patients at risk of multidrug-resistant tuberculosis (MDR-TB). Policy statement. 2008;27.
13. Ioannidis P, Papaventsis D, Karabela S, Nikolaou S, Panagia M, Raftopoulou E, et al. Cepheid GeneXpert MTB/RIF assay for Mycobacterium tuberculosis detection and rifampin resistance identification in patients with substantial clinical indications of tuberculosis and smear-negative microscopy results. *J ClinMicrobiol.* 2011;49(8):3068-70. Doi: 10.1128/JCM.00718-11. Epub 2011 Jun 15. PMID: 21677069; PMCID: PMC3147726.