Original Article

DOI: 10.7860/JCDR/2022/59404.17055

Microbiology Section

Diagnostic Accuracy between CBNAAT, TrueNat, and Smear Microscopy for Diagnosis of Pulmonary Tuberculosis in Doda District of Jammu and Kashmir- A Comparative Study

SAMEERA AKHTAR[1], AMANDEEP KAUR[2], DINESH KUMAR[3], BHAVNA SAHNI[4], REKHA CHOUHAN[5], NAZIA TABASSUM[6], SUFHIA AKHTAR[7], SUMIT G GANDHI[8]

ABSTRACT

Introduction: The need for fast, precise diagnostic tests to identify active tuberculosis is essential, mainly in endemic nations such as India. An automated real-time Polymerase Chain Reaction (PCR) method for pulmonary tuberculosis (TB) detection known as the Cartridge Based Nucleic Acid Amplification Test (CBNAAT) or GeneXpert assay shows great promise as a complement to the TrueNat and conventional sputum microscopy techniques.

Aim: To compare sensitivity, specificity, Positive Predictive Value (PPV), and Negative Predictive Value (NPV) of CBNAAT with TrueNat and smear microscopy in the detection of *Mycobacterium tuberculosis*.

Materials and Methods: A cross-sectional comparative study on 175 patients with suspected pulmonary TB was conducted from June 2021 to November 2021 in a tertiary care hospital at Government Medical College, Doda District of Jammu and Kashmir, India. The sensitivity, specificity, PPV, NPV, and diagnostic accuracy for the diagnosis of tuberculosis were calculated for Acid Fast Bacilli (AFB) smear microscopy, TrueNat, and the GeneXpert and compared with each other. Statistical analysis of the data was conducted with Statistical Package for the Social Science (SPSS) version 20.0.

Results: Out of the total 175, 168 (96%) patients were TB positive by CBNAAT, 162 (92.6%) by TrueNat, and 148 (84.6%) as per smear microscopy. Sensitivity, specificity, PPV, NPV, and accuracy of Ziehl-Neelsen (ZN) stain in the detection of pulmonary TB in sputum samples were 86.31%, 57.14%, 97.97%, 14.81%, and 85.14%, respectively. Whereas in the case of the TrueNat technique sensitivity, specificity, PPV, NPV, and accuracy were 94.05%, 42.86%, 97.53%, 23.08%, and 92.00%, respectively. In the case of CBNAAT sensitivity, specificity, PPV, NPV, and accuracy in the detection of pulmonary TB in sputum samples were 97.02%, 28.57%, 97.02%, 28.57%, and 94.29%, respectively.

Conclusion: In respiratory samples, CBNAAT is more sensitive than ZN smear microscopy and TrueNat. Positive CBNAAT, but TrueNat and AFB microscopy negative results should be read cautiously and be well correlated with the clinical and treatment history of the patient.

Keywords: Cartridge based nucleic acid amplification test, Sensitivity, Specificity, Ziehl-neelsen stain

INTRODUCTION

The global burden of TB remains enormous causing the highest number of deaths globally. According to Global TB report 2017, annually more than nine million new TB cases and 1.6 million deaths occur worldwide [1]. In India's public health system, over 25% of patients who seek care are neither diagnosed nor given a treatment regimen [2]. Because of the high rapid spread of pulmonary TB, which accounts for more than 80% of all TB infections, it is critical to diagnose and treat the disease as early as possible. If ignored, a pulmonary TB patient can infect up to 10-15 additional people through close contact. Despite highly effective therapies, TB continues to remains a major health problem worldwide, mainly because of inadequate case detection [3].

Worldwide sputum smear microscopy is the most common method for diagnosing pulmonary TB [4]. In a clinical set-up, the sensitivity of direct smear microscopy is low, while specificity is high [5]. In HIV-infected patients, it is even lower. Culture is more sensitive than direct smear microscopy. Commercially two types of broth culture systems (liquid and solid media) are available. World Health Organisation (WHO) endorses liquid culture system and molecular line probe as the current gold standard for rapid detection of Multidrug Resistance (MDR) TB [6]. But growth takes about two to six weeks to yield results and requires a highly specialised, controlled laboratory set-up and highly trained people. Microscopy and culture are notably less sensitive than automated methods and molecular markers like PCR or real-time PCR. For the rapid identification of *Mycobacterium tuberculosis* in clinical specimens of pulmonary and extrapulmonary TB cases, some Nucleic Acid Amplification (NAA) methods have been developed [7,8].

The only advantage of the rapidity of diagnosis is that these techniques can also detect even low *Mycobacterium tuberculosis* genomic copies in various specimens. The CBNAAT or GenXpert (Xpert®MTB/Rif test) has most recently received WHO approval for TB diagnosis [9]. The GeneXpert uses a DNA-PCR approach to simultaneously detect mutations linked to rifampicin resistance and *Mycobacterium tuberculosis*. It is the first fully computerised, rapid, simple benchtop CBNAAT TB detection assay that requires lesser technical expertise and incorporates all essential DNA-PCR stages including bacterial lysis, nucleic acid extraction, and amplification. Results are available in two hours. It has been reported that CBNAAT or GeneXpert has good diagnostic accuracy for pulmonary and extrapulmonary TB [10,11]. High-risk patients for TB include children with extrapulmonary cases and presumed HIV-associated TB patients, for whom an AFB smear screening is typically negative [11].

According to a pilot investigation, the TrueNat *Mycobacterium tuberculosis*, a revolutionary TB test, was able to diagnose TB quickly and with good sensitivity when compared to a Composite Reference Standard (CRS). When compared to the internal nested PCR methodology, TrueNat provided results that were more rapid

and precise. TrueNat was reported to have a sensitivity and specificity of 91.1% and 100%, respectively, using a CRS as the baseline. The widely used and WHO-approved CBNAAT or GeneXpert *Mycobacterium tuberculosis*/RIF and the reference standard for TB case detection were used in the current investigation to evaluate both methods' performance [12].

This was a hospital-based study, the first of its kind in District Doda, Jammu and Kashmir, India. The aim of the study was to evaluate the sensitivity, specificity, PPV, NPV, and diagnostic accuracy of CBNAAT (GeneXpert) using sputum samples in patients with suspected pulmonary TB and compare with TrueNat and ZN smear microscopy.

MATERIALS AND METHODS

The present cross-sectional comparative study was conducted from 1st June 2021 to 30th of November, 2021 in the Doda District of Jammu and Kashmir, India. Doda is the largest district (Geographical Area-2758.95Sq Km) in the Jammu region having a population of 409,936 (Male: 213,641, Female: 196,295), located at 33.13°N 75.57°E, at an altitude of 5000 feet above the sea level [13]. The Institutional Ethics Committee (IEC) (GMC, Doda) (GMCDoda/IEC/2021/11, Dated: 19/04/2021) had approved the study. Informed and written consent was obtained from patients enrolled in the present study.

Inclusion criteria: All the patients who were referred to the Department of Microbiology for a Mycobacteriology study by ZN staining and molecular detection of pulmonary TB were included in this study.

Exclusion criteria:

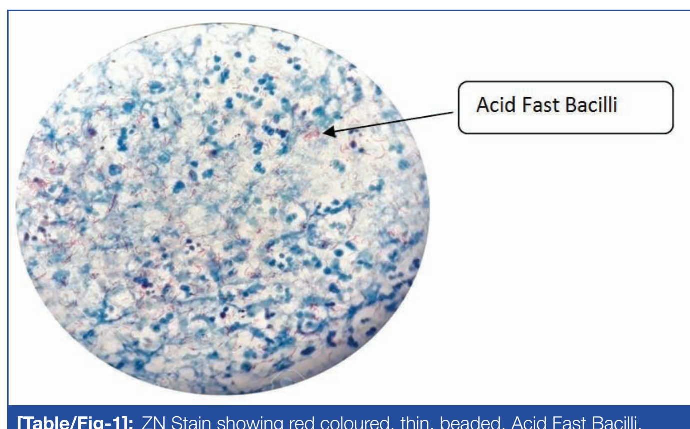
1) Samples that were obtained without a clinical history were excluded.
2) All three test samples were delivered without asking.
3) A patient who has a history of a fungal infection or lung disease.

Study Procedure

Specimen collection and processing: As necessary, sputum samples were collected under sterile conditions and also in leak-proof, sterile containers. In the shortest time possible, the samples were processed. In the case of a delay, they were kept at 4°C for no longer than 24 hours before even being processed immediately. All samples were handled in a class II A2 biosafety cabinet.

ZN staining: The ZN staining method was performed following the established technique [14]. A smear was made from each sample and spread on a label, clear glass slide. The slide was then stained by ZN stain and observed under immersion oil after that the smear had been allowed to air-dry and also fixed by heat (X100). Acid-fast bacteria had a bright red appearance and a beaded look [Table/Fig-1].

CBNAAT: As per standard protocol outlined by WHO, processing of specimens for CBNAAT was completed. By following the instructions for CBNAAT or GeneXpert *Mycobacterium tuberculosis*/standard RIF's, the sampling reagent (containing NaOH and isopropanol) was applied to the sample. It was then incubated for 10 minutes at room temperature with intermittent vigorous shaking. After that cartridge was used to load 2 mL of each of the sample and reagent mixture into the CBNAAT or GeneXpert device. The system provided data for the drug's susceptibility to rifampicin after two hours and indicated the presence or absence of *Mycobacterium tuberculosis* as well as the bacterial load as very low, low, medium, or high [14,15].



[Table/Fig-1]: ZN Stain showing red coloured, thin, beaded, Acid Fast Bacilli.

TrueNat MTB test: DNA extraction was done using Trueprep-MAG kit instructions. According to the manufacturer's instructions, fresh specimens (sputum) from untreated individuals were processed, with a starting volume of 500 μL added to the sample pretreatment tube. The Trueprep-MAG Sputum and TrueNAT *Mycobacterium tuberculosis* kits contain only proprietary master mixes for PCR as well as all buffers and reagents essential for nucleic acid extraction.

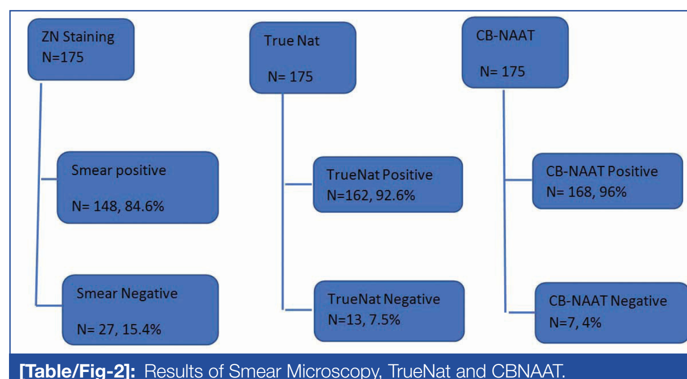
Real-time PCR on chip: A preprogrammed profile on the TrueNat *Mycobacterium tuberculosis* microchip was used to conduct real-time PCR with 5 μL of extracted DNA and lyophilised master mix. The screen displayed the results. Unique primers and an *Mycobacterium tuberculosis*-specific probe were included in the lyophilised master mix.

STATISTICAL ANALYSIS

The data was recorded in a master chart using a Microsoft excel spreadsheet, and correlation was checked. SPSS version 20.0 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 microscopy, TrueNat, and the CBNAAT or GeneXpert and compared with each other.

RESULTS

Out of the total of 175 cases of suspected TB, 108 (61.71%) were males and 67 (38.29%) were females. Of which 168 (96%) patients were found to be TB positive by CBNAAT Technique, out of which males were 98 (58.33%) whereas females were 70 (41.67%). However, in the case of TrueNat, 162 (92.6%) were positive, and in the case of ZN smear microscopy, 148 (84.6%) were positive [Table/Fig-2].



[Table/Fig-2]: Results of Smear Microscopy, TrueNat and CBNAAT.

Out of 168 TB patients who tested positive with the gold standard i.e., CBNAAT, 145 and 158 patients also tested positive for ZN stain and TrueNat, respectively and were hence labelled as true positives [Table/Fig-3] while out of seven patients who tested negative with the gold standard i.e., CBNAAT, four and three patients also gave negative result on ZN stain and TrueNat, respectively and were categorised as true negatives. However, considering the total patients who tested positive and negative, respectively using ZN staining and TrueNat, i.e., test positives and test negatives, it was observed that 3 and 23 samples turned out false positive and false negative, respectively with ZN staining while 4 and 10 patients were falsely positive and false negative on TrueNat, respectively.

Sensitivity, specificity, PPV, NPV, and accuracy of ZN stain in the detection of pulmonary TB in sputum samples were 86.31%, 57.14%, 97.97%, 14.81%, and 85.14%, respectively. Whereas

Tests	True positives (Total positive)	True negatives (Total negative)
ZN staining	145 (148)	4 (27)
TrueNat	158 (162)	3 (13)
CBNAAT (gold standard)	168	7

[Table/Fig-3]: Diagnostic accuracy of ZN stain, TrueNat in diagnosing *Mycobacterium tuberculosis* in sputum samples taking CBNAAT as a reference standard (N=175).

in the case of the TrueNat technique sensitivity, specificity, PPV, NPV, and accuracy were 94.05%, 42.86%, 97.53%, 23.08%, and 92.00%, respectively. In the case of CBNAAT sensitivity, specificity, PPV, NPV, and accuracy in the detection of pulmonary TB in sputum samples were 97.02%, 28.57%, 97.02%, 28.57%, and 94.29%, respectively [Table/Fig-4].

ZN stain			TrueNat		CBNAAT	
Statistic	Value	95% CI	Value	95% CI	Value	95% CI
Sensitivity	86.31%	80.17% to 91.12%	94.05%	89.33% to 97.11%	97.02%	93.19% to 99.03%
Specificity	57.14%	18.41% to 90.10%	42.86%	9.90% to 81.59%	28.57%	3.67 to 70.96%
Positive likelihood ratio	2.01	0.85 to 4.75	1.65	0.87 to 3.13	1.36	0.85 to 2.17
Negative likelihood ratio	0.24	0.11 to 0.50	0.14	0.05 to 0.40	0.10	0.02 to 0.45
Disease prevalence	96.00%	91.93% to 98.38%	96.00%	91.93% to 98.38%	96.00%	91.93% to 98.38%
Positive predictive value	97.97%	95.35% to 99.13%	97.53%	95.41% to 98.69%	97.02%	95.32% to 98.12%
Negative predictive value	14.81%	7.62% to 26.82%	23.08%	9.54% to 46.05%	28.57%	8.54% to 63.15%
Diagnostic accuracy	85.14%	78.99% to 90.06%	92.00%	86.94% to 95.56%	94.29%	89.74% to 97.23%

[Table/Fig-4]: Comparison between various parameters of ZN Stain, TrueNat, and CBNAAT.

DISCUSSION

In the current study, the diagnostic accuracy of GeneXpert/CBNAAT to detect *Mycobacterium tuberculosis* in pulmonary specimens was evaluated and compared with AFB smear staining by ZN staining and by TrueNat. The sensitivity of CBNAAT was 97.02% and TrueNat was 94.05% as compared to sputum smear which was 86.31%. GeneXpert/CBNAAT has been suggested for regular usage in programmatic circumstances by the WHO 2012 [14].

ZN staining of sputum sample: In the present study, 84.6% and 15.4% of the subjects were detected to be ZN stain positive and negative, respectively on sputum samples. CBNAAT was used as a gold standard to test the diagnostic efficacy of ZN staining. CBNAAT detected 168 positive specimens whereas ZN staining detected only 148 positive specimens. CBNAAT detected seven negative specimens whereas ZN staining detected 27 negative specimens. Sensitivity, specificity, PPV, NPV, and accuracy of ZN in the detection of sputum specimen was 86.31%, 57.14%, 97.97%, 14.81%, and 85.14%, respectively. Chandora AK and Chandora A, screened 100 patients for pulmonary TB observed sensitivity, specificity, PPV, NPV of Sputum microscopy was 22.22%, 78.38%, 63.64% and 37.18%, respectively [16]. Padmaja GV et al, reported the sensitivity and specificity of ZN staining on sputum samples to be 50-80% and 98%, respectively [17].

Limitation of ZN stain: Although ZN stain is quick, inexpensive, and simple, it needs atleast 10,000 bacilli per millilitre of sputum to prove TB. This test only has a sensitivity range of 20-80% and a 99% specificity. Smear microscopy's primary flaw is its inability to identify rifampicin resistance [18]. Misdiagnosis of smears could be the reason for the false positive cases. Technical mistakes such as insufficient slide preparations, poor staining technique, observational inaccuracy, etc. could be to blame for the false negative cases. A repeat sample is advised or CBNAAT should be performed to confirm the diagnosis in the event of a false positive or suspected case in order to avoid needless medical intervention, pharmacological side-effects, and stress on the patient and their family.

TrueNat of Sputum sample: In the present study, 92.6% and 7.4% of the subjects were detected positive and negative, respectively on TrueNat using sputum samples. CBNAAT was used as a gold standard to test the diagnostic efficacy of TrueNat. CBNAAT detected 168 positive specimens whereas TrueNat detected 162 positive specimens. CBNAAT detected seven negative specimens whereas TrueNat detected 13 negative specimens. Sensitivity, specificity, PPV, NPV, and Accuracy of TrueNat in the detection of sputum specimen was 94.05%, 42.86%, 97.53%, 23.08%, and 92%, respectively in the present study. Ngangue YR et al., reported the sensitivity and specificity of TrueNat for pulmonary TB on different hospitalised patients to be 91% and 96%, respectively [19]. Penn-Nicholson A et al., 2021 reported the sensitivity and specificity of TrueNat in primary healthcare to be 84% and 95%, respectively [20].

Advantages of CBNAAT and TrueNat: Compared to the above techniques, CBNAAT or GeneXpert assay and TrueNat have the advantages of less 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. CBNAAT is recommended test for sputum smear-negative specimens and the first-line of diagnostic test in MDR/RR-TB and HIV-related TB [21].

Ioannidis P et al, performed CBNAAT in 80 pulmonary samples with reported sensitivity and specificity of were 90.6% and 94.3%, respectively [22]. A total of 290 respiratory samples were included in GeneXpert study performed by Bunsow E et al., and they reported sensitivity and specificity, PPV, and NPV values of 97%, 98%, 95%, and 99%, respectively. The CBNAAT technique was said to be rapid and precise at detecting *Mycobacterium tuberculosis*, especially in respiratory samples that tested positive on smears [23]. Armand S et al., reported sensitivity of CBNAAT in 60 pulmonary samples as 79% [24]. In a study by Zeka AN et al., included 253 respiratory samples and reported the sensitivity, specificity, PPV, NPV values of CBNAAT as 86%, 99%, 96%, 98%, respectively [25]. In present study, sensitivity of CBNAAT in 168 pulmonary samples was 97.02%, respectively.

Culture technique being comparatively slow and complex, requires specialised laboratories and skilled staff. The *Mycobacterium* bacilli take days to grow in liquid media and 4-8 weeks in solid media [26].

The Xpert MTB/RIF test is a simple, rapid technique that can be performed with negligible training and results available within a few hours [27]. The expense to set-up an automated liquid culture system for TB is equal to the costs of the GeneXpert system. Though GeneXpert is commonly done to identify pulmonary TB using frozen sputum or Broncho Alveolar Lavage (BAL) samples, various studies have demonstrated that it may also be beneficial tool for identifying mycobacteria in other body fluids like Cerebrospinal Fluid (CSF), pleural, and ascitic fluid and will have wider application in the future [28].

A qualitative, nested real-time PCR in-vitro diagnostic assay termed Xpert MTB/RIF CBNAAT is used to detect rifampin-resistance-related mutations in the *rpoB* gene. Five molecular beacons are used to probe the *rpoB* gene's sequence (Probes A-E). When combined with clinical and other laboratory results, CBNAAT help in the diagnosis of pulmonary TB. It is designed to be used with samples from individuals who have a clinical suspicion of having TB and who have not had any antituberculosis treatment or have

merely received treatment for three days or less. To determine if ongoing infection control measures are necessary, the Xpert MTB/RIF Assay may be used to examine one or two sputum samples instead of performing serial acid-fast stained sputum smears.

Limitation of detection of TrueNat and CBNAAT [29]: The assay is not advised for patients receiving antituberculosis treatment, who are being monitored for bacterial cure and response to therapy. Improper or insufficient sample collection especially during transportation may affect the quality of the results giving rise to false negative results. The possibility of isolating *Mycobacterium tuberculosis*-complex from the sputum sample is not disregarded by a negative test result. Positive outcomes may not always mean that a living entity is present. The *Mycobacterium tuberculosis*-complex species are not distinguished by the Xpert *Mycobacterium tuberculosis*/RIF assay. To determine whether Mycobacteria Other Than Tuberculosis complex (MOTT) is present in addition to *Mycobacterium tuberculosis*-complex, a culture must also be done. When *M. scrofulaceum* was tested at a concentration of 10^8 CFU/mL, the Xpert *Mycobacterium tuberculosis*/RIF assay result was falsely positive. Configuration of binding sites for primer and/or probe can be altered by ongoing mutations within the target sequence leading to the failure of the amplification process. The proper annual calibration of the instrument with temperature control and an uninterrupted power supply is a must. The only major disadvantage of CBNAAT is that it cannot differentiate between active and cured TB [30].

Limitation(s)

Authors did not perform culture tests to detect *Mycobacterium tuberclosis* on Lowenstein Jensen medium as that facility was not available at our hospital setting. The topography of District Doda is hilly mountainous region, with the general population having limited access to healthcare facilities, for that reason resampling of any suspected patient was a very difficult task.

CONCLUSION(S)

To sum up, CBNAAT is one of the country's quick diagnostic tests and should be regularly utilised by both the public and private health sectors to quickly detect TB cases. GeneXpert *Mycobacterium tuberculosis*/RIF assay has proved to be a reliable method for the detection of *M. tuberculosis* and has high sensitivity, specificity, and positive predictive value compared with TrueNat and ZN-smear techniques for the detection of pulmonary TB.

It is recommended even in resource-constrained settings due to its feasibility, fast turnaround time, and minimal infrastructure requirement. It is especially helpful in areas with a highrisk of MDR-TB or HIV-associated TB. CBNAAT will help in the timely initiation of effective treatment in pulmonary TB patients and rifampicin-resistant TB cases. AFB smear and TrueNat technique though cost-effective, has low sensitivity as compared to CBNAAT.

REFERENCES

[1] Eurosurveillance editorial team. WHO publishes Global tuberculosis report 2013. Euro Surveill. 2013;18(43):20615. PMID: 24176622.

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

[3] 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.

[4] Desikan P. Sputum smear microscopy in tuberculosis: Is it still relevant? Indian J Med Res. 2013;137(3):442-44. PMID: 23640550; PMCID: PMC3705651.

[5] Pathrikar TG, Bansal VP, Mulay MV, Ghogare HS. Comparison of Ziehl Neelsen smear microscopy and AFB culture in a resource limited setting from various clinical samples. Int J Health Sci Res. 2020;10(4):46-51.

[6] 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.

[7] Umair M, Siddiqui SA, Farooq MA. Diagnostic accuracy of sputum microscopy in comparison with GeneXpert in pulmonary tuberculosis. Cureus. 2020;12(11):e11383. Doi: 10.7759/cureus.11383. PMID: 33312784; PMCID: PMC7725198.

[8] Saglam L, Akgun M, Aktas E. Usefulness of induced sputum and fibreoptic bronchoscopy specimens in the diagnosis of pulmonary tuberculosis. J Int Med Res. 2005;33(2):260-65. Doi: 10.1177/147323000503300215. PMID: 15790139.

[9] Forbes BA, Sahm DF, Weissfeld AS. Bailey and Scotts' Diagnostic microbiology 12th ed. 2007; St Louis, Mosby.

[10] Agrawal M, Bajaj A, Bhatia V, Dutt S. Comparative study of GeneXpert with ZN stain and culture in samples of suspected pulmonary tuberculosis. J Clin Diagn Res. 2016;10(5):DC09-DC12. Doi: 10.7860/JCDR/2016/18837.7755. Epub 2016 May 1. PMID: 27437212; PMCID: PMC4948388.

[11] 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 diode-fluorescence 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.

[12] 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. Doi: 10.1371/journal.pone.0051121. Epub 2013 Jan 21. PMID: 23349670; PMCID: PMC3549918.

[13] District Informatics Officer, National Informatics Centre, Ministry of Electronics & Information Technology, Government of India, District Centre, Doda-182202. https://doda.nic.in.

[14] Creswell J, Codlin AJ, Andre E, Micek MA, Bedru A, Carter EJ, et al. Results from the early programmatic implementation of Xpert MTB/RIF testing in nine countries. BMC Infect Dis. 2014;14:2. Doi: 10.1186/1471-2334-14-2. PMID: 24383553; PMCID: PMC3898850.

[15] World Health Organization. Xpert MTB/RIF implementation manual: Technical and operational 'how-to'; practical considerations. World Health Organization; 2014. Xpert MTB/RIF Implementation Manual: Technical and Operational 'How-To'; Practical Considerations. Geneva: World Health Organization; 2014. PMID: 25473699.

[16] Chandora AK, Chandora A. To assess the diagnostic accuracy of cbnaat and sputum microscopy against gold standard sputum culture among symptomatic HIV patients: An institute based study. Int J Med Res Prof. 2019;5(6):194-98. Doi: 10.21276/ijmrp.2019.5.6.042.

[17] Padmaja GV, Srujana K, Sadhana C. Comparison of Ziehl-Neelsen's stain, fluorescent stain with CBNAAT of sputum for the diagnosis of pulmonary tuberculosis. J NTR Univ Health Sci. 2019;8(4):238-43.

[18] Tiwari VD, Maqusood M, Ramakrishna G, Rastogi R. To compare the diagnostic sensitivity of ZN (Ziehl-Neelsen) staining, CBNAAT (Cartridge Based Nucleic Acid Amplification Test) and mycobacterium culture of BAL (Bronchoalveolar Lavage) fluid among sputum smear negative or non- sputum producing patients with suspected pulmonary tuberculosis. Asian J Med Res. 2020;9(1):PM14-PM19. Doi: dx.doi.org/10.47009/ajmr.2020.9.1.PM4.

[19] 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 Clin Microbiol. 2022; 60(8):e0015522. Doi: 10.1128/jcm.00155-22. Epub 2022 Jul 21. PMID: 35861529; PMCID: PMC9383115.

[20] 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. Eur Respir J. 2021;58(5):2100526. Doi: 10.1183/13993003.00526-2021. PMID: 34049948; PMCID: PMC8607906.

[21] World Health Organization. Molecular line probe assays for rapid screening of patients at risk of multidrug-resistant tuberculosis (MDR-TB). Policy statement. 2008;27.

[22] 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 Clin Microbiol. 2011;49(8):3068-70. Doi: 10.1128/JCM.00718-11. Epub 2011 Jun 15. PMID: 21677069; PMCID: PMC3147726.

[23] Bunsow E, Ruiz-Serrano MJ, López Roa P, Kestler M, Viedma DG, Bouza E. Evaluation of GeneXpert MTB/RIF for the detection of *Mycobacterium tuberculosis* and resistance to rifampin in clinical specimens. J Infect. 2014;68(4):338-43. Doi: 10.1016/j.jinf.2013.11.012. Epub 2013 Dec 1. PMID: 24296493.

[24] Armand S, Vanhuls P, Delcroix G, Courcol R, Lemaître N. Comparison of the Xpert MTB/RIF test with an IS6110-TaqMan real-time PCR assay for direct detection of *Mycobacterium tuberculosis* in respiratory and nonrespiratory specimens. J Clin Microbiol. 2011;49(5):1772-76. Doi: 10.1128/JCM.02157-10. Epub 2011 Mar 16. PMID: 21411592; PMCID: PMC3122654.

[25] Zeka AN, Tasbakan S, Cavusoglu C. Evaluation of the GeneXpert MTB/RIF assay for rapid diagnosis of tuberculosis and detection of rifampin resistance in pulmonary and extrapulmonary specimens. J Clin Microbiol. 2011;49(12):4138-41. Doi: 10.1128/JCM.05434-11. Epub 2011 Sep 28. PMID: 21956978; PMCID: PMC3232962.

[26] Rouillon A, Perdrizet S, Parrot R. Transmission of tubercle bacilli: The effects of chemotherapy. Tubercle. 1976; 57(4):275-99. Doi: 10.1016/s0041-3879(76)80006-2. PMID: 827837.

[27] Help D, Jones M, Story E, Boehme C, Wallace E, Ho K, et al. Rapid detection of Mycobacterium tuberculosis and rifampin resistance by use of on-demand, near-patient technology. J Clin Microbiol. 2010;48(1):229-37. Doi: 10.1128/JCM.01463-09. Epub 2009 Oct 28. PMID: 19864480; PMCID: PMC2812290.

[28] Tortoli E, Russo C, Piersimoni C, Mazzola E, Dal Monte P, Pascarella M, et al. Clinical validation of Xpert MTB/RIF for the diagnosis of extrapulmonary tuberculosis. European Respiratory Journal. 2012;40(2):442-47.

[29] Gogoi S, Bora I, Debnath E, Sarkar S, Jais MB, Sharma A. Perplexity vs clarity in choosing the right molecular diagnostic techniques for SARS-COV2 detection in Indian setup. J Family Med Prim Care. 2021;10(2):615-24. Doi: 10.4103/jfmpc.jfmpc_1793_20. Epub 2021 Feb 27. PMID: 34041050; PMCID: PMC8138350.

[30] Roy RD, Gupta SD. A comparative study of cartridge-based nucleic acid amplification test and Ziehl-Neelsen stain with culture on Lowenstein-Jensen media as the gold standard for the diagnosis of pulmonary tuberculosis. Indian J Respir Care. 2022;11(1):39-42.

PARTICULARS OF CONTRIBUTORS:

1. PhD Scholar, Centre for Interdisciplinary Biomedical Research, Adesh University, Bathinda, Punjab, India.
2. Professor, Department of Microbiology, Adesh Institute of Medical Sciences and Research, Bathinda, Punjab, India.
3. Professor and Head, Department of Community Medicine, GMC, Doda, Jammu and Kashmir, India.
4. Assistant Professor, Department of Community Medicine, GMC, Doda, Jammu and Kashmir, India.
5. PhD Scholar, Department of Infectious Diseases Division, CSIR Indian Institute of Integrative Medicine, Jammu and Kashmir, India.
6. Assistant Professor, Department of Pathology, GMC, Doda, Jammu and Kashmir, India.
7. Assistant Professor, Department of Microbiology, GMC, Doda, Jammu and Kashmir, India.
8. Senior Scientist, Department of Infectious Diseases Division, CSIR Indian Institute of Integrative Medicine, Jammu and Kashmir, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:

Dr. Sameera Akhtar,
PhD Scholar, Centre for Interdisciplinary Biomedical Research, Adesh University,
Bathinda, Punjab, India.
E-mail: sameerazargar@gmail.com

PLAGIARISM CHECKING METHODS: [Jain H et al.]

- Plagiarism X-checker: Aug 04, 2022
- Manual Googling: Oct 15, 2022
- iThenticate Software: Oct 17, 2022 (20%)

ETYMOLOGY: Author Origin

AUTHOR DECLARATION:

- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? Yes
- For any images presented appropriate consent has been obtained from the subjects. NA

Date of Submission: **Jul 31, 2022**
Date of Peer Review: **Aug 31, 2022**
Date of Acceptance: **Oct 18, 2022**
Date of Publishing: **Nov 01, 2022**