RESEARCH PAPER

Comparative Evaluation of Truenat Rt-Pcr, Ziehl–Neelsen Microscopy, and Lowenstein–Jensen Culture For Diagnosis of Pulmonary Tuberculosis in a Tertiary Care Hospital

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ABSTRACT

Pulmonary tuberculosis (PTB) remains a major public health challenge requiring rapid and accurate diagnostic methods for effective disease management. This study aimed to comparatively evaluate Truenat RT-PCR, Ziehl–Neelsen (ZN) microscopy, and Lowenstein–Jensen (LJ) culture for the diagnosis of pulmonary tuberculosis in a tertiary care hospital. Sputum samples from suspected PTB patients were analyzed using all three diagnostic techniques. Truenat RT-PCR demonstrated higher sensitivity and rapid turnaround time compared to ZN microscopy, while LJ culture remained the reference standard despite longer incubation periods. The findings highlight the utility of molecular diagnostics for early PTB detection and improved patient management in resource-limited healthcare settings.

Keywords: Pulmonary Tuberculosis, Truenat Rt-Pcr, Ziehl–Neelsen Microscopy, Lowenstein–Jensen Culture, Molecular Diagnosis

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Introduction

Tuberculosis (TB), primarily caused by Mycobacterium tuberculosis, remains a leading cause of infectious disease and death worldwide. According to the World Health Organization (WHO), in 2023, there were an estimated 10 million new TB cases globally, with over 1.4 million deaths attributed to the disease. India continues to bear the highest burden, accounting for approximately 27% of global Tuberculosis incidence. The resurgence of Tuberculosis, along with the emergence of drug-resistant strains, underscores the urgent need for accurate, rapid, and accessible diagnostic tools.

The genus Mycobacterium is currently the sole genus within the family Mycobacteriaceae, order Actinomycetales, and is related to other mycolic acid-containing genera. The minimal criteria for classifying a species within the genus Mycobacterium include:

Acid-alcohol fastness: Resistance to decolorization by acidified alcohol after being stained with a basic fuchsin dye. Presence of mycolic acids: These contain 60–90 carbon atoms and are cleaved by pyrolysis into C22–C26 fatty acid methyl esters.

Genomic G+C content: Ranges from 61–71 mol% (Levy-Frebault and Portaels, 1992), with the notable exception of M. leprae (>57%) (Good and Shinnick, 1998). Mycobacteria are non-motile, non-spore-forming, weakly Gram-positive, aerobic or microaerophilic, straight or slightly curved rod-shaped bacteria (0.2–0.6 × 1.0–10 μm) (Wayne and Kubica, 1986). Some species exhibit coccobacillary and branched forms. Although filamentous growth may occur, it usually fragments into rods and coccoid elements upon slight disturbance. Currently, there are about 100 known Mycobacterium species, generally classified into two major groups: slow-growing and rapid-growing mycobacteria.

In 1882, Robert Koch identified the rod-shaped bacillus Bacterium tuberculosis as the causative agent of tuberculosis and formulated Koch's postulates to establish the causal relationship

between a suspected pathogen and a disease. These species were later renamed Mycobacterium leprae and Mycobacterium tuberculosis, and were placed in the genus Mycobacterium—a name reflecting the mold-like pellicle formed by M. tuberculosis in liquid culture (Lehmann and Neumann, 1896).

At the beginning of the 20th century, M. tuberculosis was the only species routinely isolated from and associated with human disease. Over time, more environmental mycobacteria—previously termed atypical mycobacteria or mycobacteria other than tubercle bacilli (MOTT) were recognized as causative agents of human disease. These are now collectively referred to as non-tuberculous mycobacteria (NTM).

Tuberculosis (TB) remains a serious public health problem across the globe. It is a major global health challenge, with an estimated 10 million people falling ill and 1.5 million deaths attributed to the disease in 2020 [1]. The global public health response to TB has been hampered by difficulties in diagnosis and in linking individuals to appropriate care. In 2019, approximately 2.9 million TB cases were undiagnosed and unreported [2], and this number raised to over 4 million in 2020 due to the disruptions caused by the COVID-19 pandemic.

India bears the highest burden, with approximately 2.8 million TB cases annually, making it the country with the world's highest incidence of the disease [3]. Treating TB becomes particularly challenging when the bacteria which cause the disease are resistant to first-line of drugs. If the bacteria resistant to more than one drug like rifampicin and isoniazid, are reported as multidrug-resistant tuberculosis (MDR-TB). Microbiological confirmation is recommended for diagnosing both pulmonary tuberculosis (PTB) and extrapulmonary tuberculosis (EPTB) [4].

Truenat RT-PCR:

Truenat is a chip-based real-time PCR platform developed in India for the rapid detection of M. tuberculosis and rifampicin resistance. It operates on battery power, is temperature-stable, and requires minimal training, making it suitable for decentralized settings. Studies have shown that Truenat offers high sensitivity and specificity comparable to other molecular methods like GeneXpert, with a turnaround time of less than 2 hours.

History and discovery of TRUENAT: - Based on findings from a multi-centre diagnostic accuracy assessment conducted by The Foundation for Innovative New Diagnostics (FIND) and the World Health Organization (WHO) announced endorsement of the Molbio's molecular assays Truenat MTB, Truenat MTB Plus and Truenat MTB RIFDx as initial diagnostic tests of pulmonary Tuberculosis and Rifampicin Resistance through a rapid communication in January 2020. The Truenat test was incorporated in India's National Tuberculosis Elimination Programme after recommendations from the Indian Council of Medical Research (ICMR).

The Government of Andhra Pradesh was one of the first adopter of Truenat under its Revised National Tuberculosis Control Programme (RNTCP) for TB diagnosis. In October 2018, the state rolled out Truenat in Designated Microscopy Centres (DMC) across 13 districts. Truenat was found to improved TB case notification rates by 30% in Andhra Pradesh.

Study design:

Present study is prospective cross-sectional which conducted in Intermediate Reference Laboratory, Govt Medical College, Govt. Chest Disease hospital, Jammu, J&K, India during the period from December 2022 to May 2025. Sputum samples were collected from of IPD and OPD patients. These samples were collected with aseptic precautions in falcon tube. Total 384 sputum samples were processed during this period. Complete medical history of the patients, were recorded such as provisional diagnosis, surgical history and present complain.

Inclusion criteria:

- Patients with cough for three or more weeks.
- Patient complain of loss of weight.
- Patient with fever, night sweats, chills

Exclusion criteria:

1. Samples other than sputum samples.
2. Sputum sample which is only saliva. (i.e. 25or more squamous epithelial cells/ low power field).

Screening of Clinical Samples:

1. In systematic screening program Tuberculosis sputum samples were collected from Inpatient Department (IPD) and Outpatient Department (OPD).
2. we also consider age group and all Gender.

3. Sputum samples were collected aseptically in sterile wide mouth container and transport to Microbiology lab for further investigation.

Sample Collection Method:

Early morning sputum samples were collected for three consecutive days. Samples were collected in sterile wide mouth container.

Preparation before Collection

1. **Educate the Patient**:
    - Explain the purpose of the test.
    - Instruct the patient to how to **produce sputum, not saliva**.
    - Emphasize the need for deep coughing to bring up sputum from the lungs.
2. **Materials Needed**:

- Sterile, wide-mouthed, leak-proof sputum container (preferably screw-capped)
- Labels and marker
- Gloves and mask
- Tissues and waste disposal dustbin

When to Collect:

Two samples are typically collected:

- **First**: **Early morning sample**
- **Second**: ."Spot" sample (at the hospital)

Collection Procedure

1. Choose a Well-Ventilated Area.
2. Patient wears a mask until ready to expectorate.
3. Hand hygiene before and after sample collection.
4. Remove mask, take a deep breath, hold it, and then exhale slowly.
5. Repeat breathing deeply 2–3 times.
6. Cough deeply from the chest to bring up sputum.
7. Spit the sputum into the sterile container, avoiding contamination of the outside.
8. Ensure at least 2–5 mL of sputum (thick, mucoid, or purulent—not just saliva).
9. Close the container tightly.

After Collection

- Send the sample to the lab immediately.
- Store the sample at 2–8°C, if not immediately transported.

Fig 2: Sputum sample collection in falcon tube

Smear Preparation: Ziehl–Neelsen Staining

Procedure:

Sample Preparation (Lysis & DNA Extraction)

- Mix sputum with Trueprep Lysis Buffer
- Incubate for 15 minutes
- Transfer to Trueprep AUTO Sample Prep Device
    - Performs automated nucleic acid extraction
    - Takes 15–20 minutes

Real-Time PCR on Truelab Device

- Load extracted DNA onto a **Truenat MTB or MTB-RIF chip**
- Insert into the **Truelab micro-PCR analyzer**
- Select test type (MTB or MTB-RIF)

Procedure

- In the Truenat RTPCR the DNA from the patient sample is first extracted using Trueprep Cartridge Based Sample Prep Device.

- The Truenat MTB chip is placed on the chip tray of the Truelab Real Time Quantitative microPCR Analyzer.
- Six (6) µL of the purified DNA is then dispensed using the provided micropipette and tip into the microtube containing freeze dried PCR reagents and allowed to stand for 30-60 seconds to get a clear solution.
- Six (6) µL of this clear solution is then pipetted out using the same pipette and tip and dispensed into the reaction well of the Truenat MTB chip and the test is started.
- A positive amplification causes the dual labeled fluorescent probe in the Truenat MTB Chip-based Real Time PCR test to release the fluorophores in an exponential manner which is then captured by the built-in opto-electronic sensor and displayed as amplification curve on the analyzer screen, on a real time basis during the test run.
- The Cycle threshold (Ct) is defined as the number of amplification cycles required for the fluorescent signal to cross the threshold (i.e. exceed the background signal). Ct levels are inversely proportional to the amount of target nucleic acid in the sample. (i.e. the lower the Ct level the greater is the amount of target nucleic acid in the sample).
- Based on the Ct of the internal positive control (IPC), the validity of the test run is also displayed. The IPC is a full process control that undergoes all the processes the specimen undergoes - from extraction to amplification thereby validating the test run from sample to result. Absence of or shift of IPC Ct beyond a pre-set range in case of negative samples invalidates the test run. While IPC will coamplify in most positive cases also, in some specimen having a high target load, the IPC may not amplify, however the test run is still considered valid.

Results and Interpretation

- **Truenat MTB test**:
  - Detects presence of *Mycobacterium Tuberculosis*
  - If positive → proceed to MTB-RIF test
- **Truenat MTB-RIF test**:
  - Detects **rifampicin resistance**
  - Used to guide MDR- tuberculosis treatment

Total Time:

1. MTB: ~**1 hour** 2. MTB-RIF: Additional **1 hour**

Result Interpretation

Test	Result	Interpretation
Truenat MTB	MTB Detected / Not Detected	TB confirmed / TB not detected
Truenat MTB-RIF	RIF Resistance Detected	Likely MDR-TB
	RIF Resistance Not Detected	Drug-sensitive TB likely
	Indeterminate	Repeat test or confirm with another method

Figure 6: MTB detection through Truenet

Procedural precautions:

- Check all the packages before using the kit.

- Do not perform the test in the presence of reactive vapours (sod. hypochlorite, acid, alkalis) or dust
- While retrieving the Truenat MTB micro PCR chip, microtube and the tip from the pouch, ensure neither bare hands nor the gloves that are used in previous tests run are used.
- Ensure that the colour of the dessicant pouch is orange after opening in sealed Truenat chip pouch. If the colour of the dessicant pouch changes from orange to white due to moisture, do not use the content of Truenat chip pouch.

Limitations:

- Proper specimen collection, handling, storage, and transportation to the test site are necessary for this test to function at its best.
- Although extremely uncommon, mutations in the highly conserved sections of the target genome where the Truenat assay primers and/or probe bind may cause the pathogen to be under-quantified or not detected at all.
- The instrument and assay protocols are developed to reduce the possibility of PCR amplification product contamination. But it is crucial to adhere to the guidelines and good lab practices recommended.
- A specimen for which the Truenat assay reports " Not Detected" cannot be concluded to be negative for the concerned pathogen. As with any diagnostic test, result from the Truenat assay should be interpreted in the context of other clinical and laboratory findings.

Advantages of Truenat

- Portable and battery-operated → ideal for **remote areas**
- Requires **minimal lab infrastructure**
- **Fast** results (~2 hours)
- WHO recommended as a **replacement for smear microscopy**
- Automated sample prep minimizes biosafety risks

RESULTS

This present study is conducted in the Govt. Medical College, Govt. Chest Disease Hospital, Jammu, J&K. Screening of patients done in systematic way for the isolation of *Mycobacterium tuberculosis* (MTB) in clinically suspected cases. Sputum samples were collected from the both IPD (Inpatient Department) and OPD (Outpatient Department) patients of tertiary care hospital. Analyzed 384 sputum samples were subjected to smear stained by ZN stain, culture on LJ media and Truenat RTPCR.

***Mycobacterium tuberculosis* detected in sputum samples by different methods.**

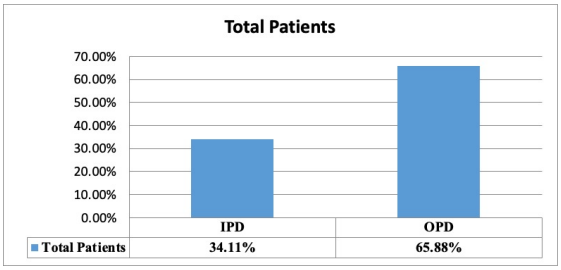
In this study total 384 sputum samples were subjected to standard microbiological test:

1. Smear microscopy with Zeihl-Neelson (ZN) stain,
2. Culture of *Mycobacterium tuberculosis* on Lowstein Jensen (LJ) media.
3. Rapid molecular diagnostic test Truenat RTPCR.

Table 1: Distribution of Patients from OPD and IPD

S.No.	Patient category	Number of sputum samples	Percentage
1.	IPD	131	34.11%
2	OPD	253	65.88 %
3	Total	384	100%

Graph 1: Distribution of Patients from OPD and IPD



Total of 384 sputum samples were collected from IPD &OPD Out of that 131 were from IPD and 253 from OPD. In our study more no. of samples is from OPD

Table 2: Gender wise distribution of Male and Female Patients

s.no.	Gender	Number of sputum samples	Percentage
1.	Male	221	57.55%
2.	Female	163	42.44%
3.	Total	384	100%

Out of total 384 sputum samples 216 were males and 164 were females

Graph 2: Gender wise distribution of male and female patients

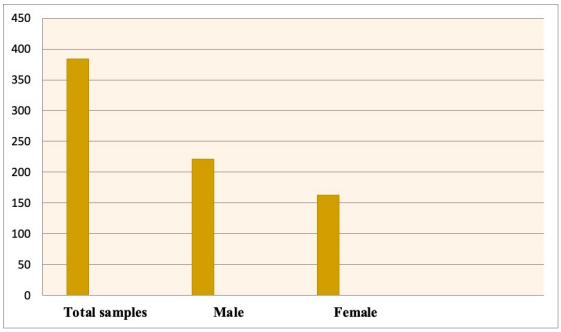


Table 3: Age wise distribution of Patients

S.No	Age group	Number of patients	Percentage
1	0-10 years	25	6.51%
2	11-20 years	42	10.93%
3	21-30 years	55	14.32%
4	31-40 years	52	13.54%
6	41-50 years	54	14.06%
7	51-60 years	63	16.40%
8	>61 years	93	24.21%
9	**Total**	**384**	**100%**

In our study maximum no. patients were from the age group >61 years 24.21% followed by the age group of 51-60 years 16.40%, followed by 14.32% from age group 21-30 years, 14.06% from the age group 41-50 years, followed by 31-40 years age group 13.54%, 10.93% from age group 11-20 years, and 6.51% from age 0-10 years as shown in table 3.

Table 4: Sputum samples positive for *Mycobacterium tuberculosis* in IPD and OPD patients with smear microscopy with Ziehl-Neelson stain, LJ culture and Truenat RTPCR.

S.No.	Patient category	No. of sputum samples	Smear positive with ZN stain	Percentage	Culture positive on LJ media	Percentage	Truenat RTPCR positive	Perce
1.	IPD	131	16	12.2%	18	13.74%	20	15.26
2	OPD	253	36	14.2%	42	16.6%	42	16.6%
3	Total	384	52		60		62	

Graph 4: Sputum samples positive for *Mycobacterium tuberculosis* in IPD and OPD patients with smear microscopy with Ziehl-Neelson stain, LJ culture and Truenat RTPCR.

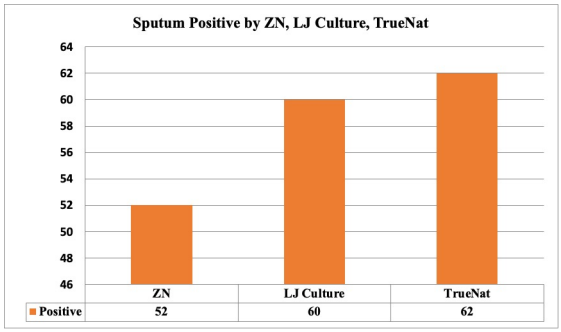


Table 5: Sputum samples positive for *Mycobacterium tuberculosis* in Male and Female patients with smear microscopy with Ziehl-Neelson stain, LJ culture and Truenat RTPCR.

S.No.	Patient category	No. of sputum samples	Smear positive with ZN stain	%	Culture positive on LJ media	Percentage	Truenat RTPCR positive	%
1.	Male	221	33	14.9%	39	17.6%	40	18.09%
2.	Female	163	19	11.7%	21	12.9%	22	13.4%
	Total	384	52		60		62	

This shows that out of 384 patients 60 were found to be culture positive with LJ media and 324 were found to be culture with LJ media negative.

Graph 5: Sputum samples positive for *Mycobacterium tuberculosis* in Male and Female patients with smear microscopy with Ziehl-Neelson stain, LJ culture and Truenat RTPCR.

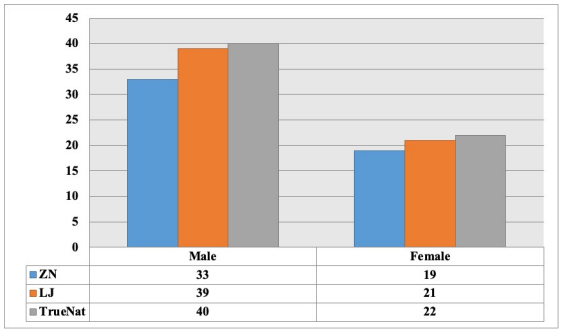


Table 6: Age wise distribution of Sputum samples positive for *Mycobacterium tuberculosis* in smear microscopy with Ziehl-Neelson stain, LJ culture and Truenat RTPCR.

S.No	Age group	Number of patients	Smear positive with ZN stain	%	Culture positive on LJ media	Percentage	Truenat RTPCR positive	%
1	0-10 years	25	0	0%	0	0%	0	0%
2	11-20 years	42	6	14.2%	6	14.2%	6	14.2%
3	21-30 years	55	10	18.2%	9	16.4%	10	18.1%
4	31-40 years	52	6	11.5%	8	15.4%	8	15.4%
6	41-50 years	54	10	18.5%	12	22.2%	12	22.2%
7	51-60 years	63	12	19%	13	20.6%	13	20.6%
8	>61 years	93	8	8.6%	12	12.9%	13	13.9%
9	Total	384	52		60		62	

This table shows that out of 384 patients 65 were found to be positive with Truenat RTPCR and 319 were found to be negative with Truenat RTPCR.

Graph 6: Age wise distribution of Sputum samples positive for *Mycobacterium tuberculosis* in smear microscopy with Ziehl-Neelson stain, LJ culture and Truenat RTPCR.

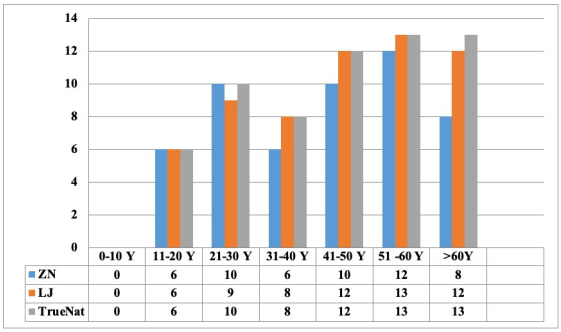


Table 7: Comparison of microbiological standard tests for confirmation of *Mycobacterium tuberculosis.*

S.no	Total number of samples	Truenat positive	Percentage	Smear Positive	Percentage	Culture Positive	Percentage
1	384	62	16.14%	52	13.54%	60	15.62%

In our study, Standard microbiological diagnostic tests i.e smear microscopy by Ziehl-Neelson stain test, solid culture on Lowstein Jensen media were compared with Truenat RTPCR test results for the diagnosis of *Mycobacterium tuberculosis* in sputum samples

A total 384 sputum samples were collected in our study, out of which 65 samples from Truenat RTPCR found positive similarly 60 samples from Lowstein Jensen culture were found positive and 52 samples from smear microscopy by Zeihl-Neelson stain were found positive.

Table 8: Comparison of ZN stain with Truenat RTPCR in Pulmonary Tuberculosis

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Test	Accuracy	Sensitivity	Specificity	PPV	NPV
Truenat	96%	89.3%	99.6%	96.1%	96%
ZN smear	75%	76.4%	80%	78.2%	80%

Graph 8: Comparison of ZN stain with Truenat RTPCR in Pulmonary Tuberculosis

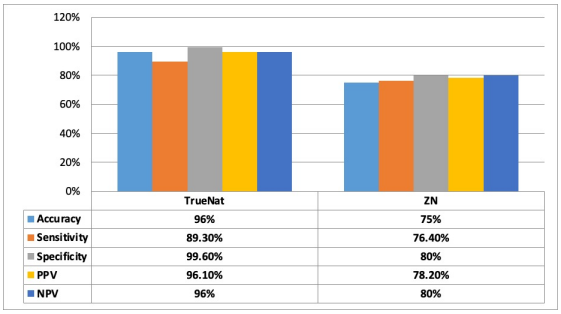
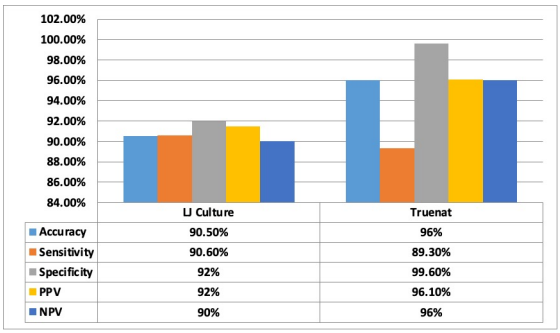


Table 9: Comparison of LJ Culture and Truenat in Pulmonary Tuberculosis

Test	Accuracy	Sensitivity	Specificity	PPV	NPV
LJ Culture	91.5%	90.6%	92%	91.5%	90%
Truenat	96%	89.3%	99.6%	96.1%	96%

Graph 9: Comparison of LJ Culture and Truenat in Pulmonary Tuberculosis



Conclusion

The present study demonstrated that Truenat RT-PCR is a rapid, reliable, and highly effective molecular diagnostic tool for the detection of *Mycobacterium tuberculosis* in sputum samples. Among the 384 clinically suspected pulmonary tuberculosis cases analyzed, Truenat RT-PCR showed higher positivity rates compared to Ziehl–Neelsen smear microscopy and Lowenstein–Jensen culture. The assay exhibited high sensitivity, specificity, positive predictive value, negative predictive value, and overall diagnostic accuracy, indicating its superior performance over conventional diagnostic methods. In addition to providing rapid results within a short turnaround time, Truenat also offers advantages such as portability, minimal infrastructure requirements, and suitability for decentralized healthcare settings. These findings support the implementation of

Truenat RT-PCR as an important diagnostic tool under the National TB Elimination Programme for early detection and timely management of tuberculosis, particularly in resource-limited regions.

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Conflict of interest

No Conflict of interest were found.

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