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Detection of Pulmonary Tuberculosis in HIV Patients: A Comparison of LJ Culture and TrueNat Methods

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ABSTRACT

Introduction:*Mycobacterium tuberculosis (MTB)* is the airborne pathogen responsible for tuberculosis (TB), a disease that primarily affects the lungs. The prevalence of active tuberculosis is significantly higher—15 to 22 times—among individuals living with HIV. Although culture on Löwenstein-Jensen (LJ) medium remains the gold standard for TB diagnosis, it is time-consuming and requires meticulous techniques to yield positive results. In contrast, the more straightforward and rapid Nucleic Acid Amplification Technique (TrueNat) not only facilitates early diagnosis of tuberculosis but also has the capability to detect drug-resistant strains, making it particularly beneficial for HIV-infected patients due to its enhanced speed and sensitivity.

Aim and Objective: present study aims to conduct a comparative analysis of LJ culture and TrueNat for the detection of pulmonary TB in sputum samples, particularly in the context of co-infection with HIV patients.

Materials and Methods: This prospective study was conducted over a 12-month period from March 2023 to March 2024 in the Department of Microbiology. A total of 100 sputum samples from individuals with suspected pulmonary tuberculosis were screened. Each sample was tested for HIV using a rapid test card, and both LJ culture and the TrueNat test were performed. Additionally, five milliliters of blood were drawn from TB patients confirmed positive and processed according to the guidelines of the National AIDS Control Organization (NACO).

Results: In this study, TrueNat detected TB in 60 (60%) of the 100 sputum samples, whereas LJ culture identified 53 (53%). The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of LJ culture were 86%, 98%, 98%, and 82%, respectively. TrueNat identified 10 patients as having multidrug-resistant TB (MDR-TB), with sensitivity, specificity, PPV, and NPV of 94%, 79%, 83%, and 93%, respectively. Among the positive pulmonary TB cases, six patients were HIV-positive.

Conclusion: While LJ culture remains a reliable and cost-effective diagnostic method, TrueNat offers superior sensitivity and rapid results for early tuberculosis diagnosis and rifampicin resistance detection, especially in HIV co-infected patients. Thus, TrueNat should be prioritized in clinical settings for its enhanced diagnostic capabilities.

Keywords- *Mycobacterium tuberculosis*, Lowenstein-Jensen, TrueNat, HIV&NACO

INTRODUCTION

Mycobacterium tuberculosis (MTB) is an acid-fast, rod-shaped bacillus responsible for tuberculosis (TB), a communicable disease that primarily affects the lungs (pulmonary TB). This infection spreads through the air from individuals with active pulmonary TB [1]. The TB Report 2022 released by the Union Health Ministry on March 20, 2022, noted a 19% increase in tuberculosis cases in India, with the total number of TB patients rising from 1,628,161 in 2020 to 1,933,381 in 2021 [2]. Early detection is crucial for effective management of this airborne infectious disease and for preventing its spread within communities [3]. Timely diagnosis is essential for positive health outcomes, but in developing countries, false-negative results and misdiagnoses are common, mainly due to the reliance on Ziehl-Neelsen (ZN) smear microscopy, which has limited sensitivity and requires multiple visits, leading to increased patient default rates. While mycobacterial culture is considered the gold standard, it is a slow process, typically taking 2 to 6 weeks for results and requiring specialized infrastructure and technical expertise.

Globally, there is a trend toward adopting rapid molecular tests, with many countries moving away from smear microscopy for diagnostics. Nevertheless, microscopy continues to be a valuable tool for monitoring treatment. Despite advancements, a significant number of TB cases reported to the WHO remain clinically diagnosed rather than confirmed bacteriologically [3]. Rapid diagnosis and timely treatment initiation are vital for controlling TB transmission.

Various Nucleic Acid Amplification (NAA) methods have been developed for the rapid detection and identification of MTB in clinical specimens, including both pulmonary and extrapulmonary cases [4]. These techniques not only facilitate quick diagnosis but also allow for the detection of low quantities of MTB genomic copies across different specimens. Although culture is recognized as the gold standard, it is slow, requiring 6 to 8 weeks, and necessitates specialized infrastructure and trained personnel [4-6]. The colonies formed during culture often appear like bread crumbs or cauliflower with a white, rough, and buff-like texture [7]. Additionally, contamination on LJ media poses ongoing challenges in Mycobacterium tuberculosis culture [8]. In many regions, smear microscopy remains the only diagnostic method for TB, achieving a detection rate of just 45% for infections [4]. Thus, there is a pressing need to develop rapid molecular TB diagnostic tools for implementation as point-of-care testing in microscopy centers. To reduce contamination rates, the WHO Laboratory Manual recommends using N-Acetyl L-cysteine (NALC) solution and the Petroff method with sodium hydroxide (NaOH) for decontamination prior to inoculation on LJ media [9].

The TrueNat MTB test utilizes a battery-operated sample preparation device known as Trueprep-MAG™, which extracts nucleic acids through an intuitive, menu-driven process optimized for sputum samples. This device integrates essential functions—heating, fluid mixing, magnet control, and timing—within a programmed microcontroller, facilitating nucleic acid isolation. The chip-based test is designed to streamline the real-time

PCR process from "sample to result," allowing laboratories with limited infrastructure to perform these tests and report PCR findings in under an hour [10].

HIV-related immunosuppression significantly heightens the risk of latent tuberculosis progressing to active TB disease, with alarmingly high mortality rates due to HIV co-infection in developing nations [11]. Additionally, MTB can activate the immune system, increasing the production of HIV virions compared to quiescent T cells [12][13].

Molbio Diagnostics, based in Bangalore, India, has developed Truenat, a novel molecular tuberculosis diagnostic tool comprising Truenat MTB, Truenat MTB Plus, and Truenat MTB-RIF Dx assays. These tests leverage chip-based real-time micro-polymerase chain reaction (PCR) technology to identify tuberculosis and RIF resistance from DNA isolated from sputum samples within an hour [10]. However, further evaluation of these assays' sensitivity and specificity is needed before clinical implementation. This study aims to compare the two diagnostic methods: Culture and TrueNat, in a tertiary care setting.

MATERIALANDMETHODS

This prospective study was conducted over a 12-month period, from March 2023 to March 2024, in the Department of Microbiology. A total of 100 sputum samples were collected from individuals clinically suspected of having pulmonary tuberculosis (PTB). These patients presented with symptoms including fever, cough, weight loss, dyspnea, fatigue, and chest pain upon their initial visit. Following a decontamination procedure, TrueNat testing was performed on all 100 samples, which were subsequently cultured on Löwenstein-Jensen (LJ) medium. The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of these two diagnostic methods were compared. Additionally, all TB-positive patients were tested for HIV co-infection using a Tridot kit.

DecontaminationprocedurebyNALC-NaOH

For the decontamination process, 5-10 mL of sputum was transferred to a Falcon tube, and an equal volume of freshly prepared digestant and decontaminants (NALC-4% NaOH) was added. After vortexing , it was allowed to digest for 15 minutes at Room Temperature (RT). Following digestion, sufficient phosphate buffer (0.067 M disodium phosphate and 0.067 M monopotassium phosphate) was added to the tube, ensuring the liquid reached within 1 cm of the top. The sample was then centrifuged at 3600 x g for 15 minutes. The sediment obtained was inoculated onto LJ media according to the National Tuberculosis Elimination Program (NTEP) guidelines.[14-15].

CultureonLowensteinJensenmedia

The sediment from the processed sample was inoculated onto LJ media and incubated aerobically at 36°C for a period of 4 to 8 weeks. Growth of *Mycobacterium tuberculosis* was indicated by the appearance of buff-colored, rough, and tough colonies. [Fig-1].

Fig1(a.):Coloniesof*Mycobacteriumtuberculosis*onLJmedium.Fig1(b.)MicroscopicExaminationof*Mycobacteriumtuberculosis* observing3plus coloniesperfield

TrueNattest(Truenat™RT-PCR,molbio)

Sputum liquefaction was initiated by adding one drop of liquefaction buffer to 1-3 mL of the sputum sample. The mixture was then thoroughly mixed and incubated for 10 minutes. Following incubation, 0.5 mL of the liquefied sputum was transferred into a lysis buffer bottle using a 1 mL transfer pipette. The entire contents of the lysis buffer were subsequently transferred to the cartridge sample chamber (indicated by the black cap) using a 3 mL transfer pipette.**[Fig-2&3]**.

Genome extraction was performed within 20 minutes using the TRUEPREP AUTO device. The chip labeled as TRUENAT™ MTB was loaded into the instrument according to the manufacturer's instructions. The Trueprep-MAG™ system utilizes a user-friendly, menu-driven process that employs a nanoparticle-based technique for nucleic acid extraction.

Once *Mycobacterium tuberculosis* was detected within 40 minutes, the same sample was further tested for rifampicin resistance using the TrueNat™ MTB RIFDx chip, which required an additional 55 minutes for completion.

Fig 2: Cartridge used in TrueNat™ RT-PCR**Fig 3:** Cartridge loaded on TRUEPREP AUTOdevice

RESULTS

In the present study a total of 100 patients suspected of TB were studied. It was observed that theratio of mlaes was more as compared to the females with 72% males and 28% females**[Fig-4]**.The mean age of positive PTB cases was observed to be 47.19±16.57. The maximum numbersofpositive PTBpatients(76%)werebetween 40-70 years ofage **[Fig-5].**

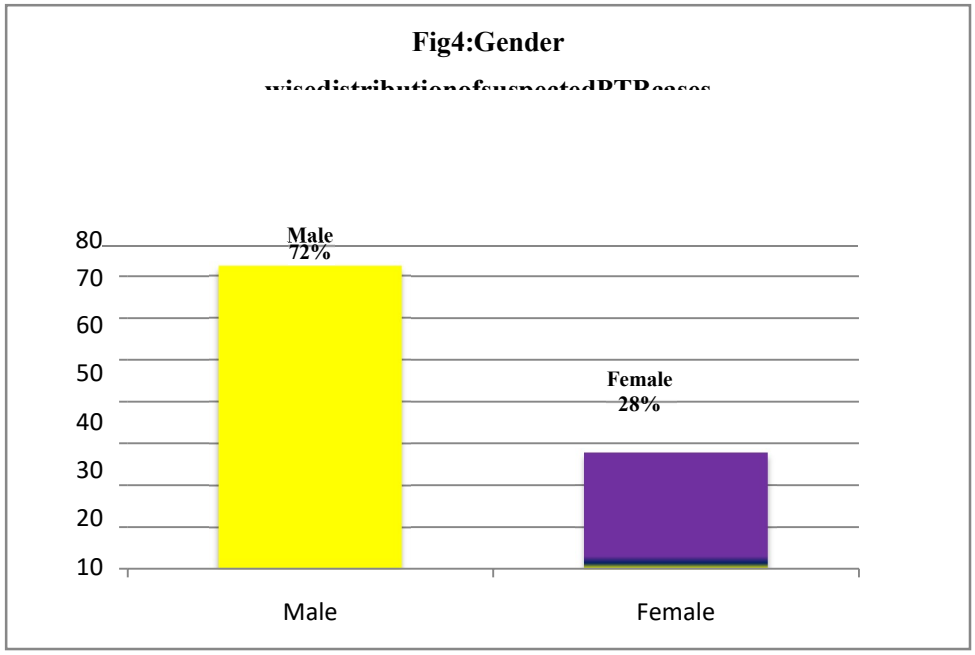
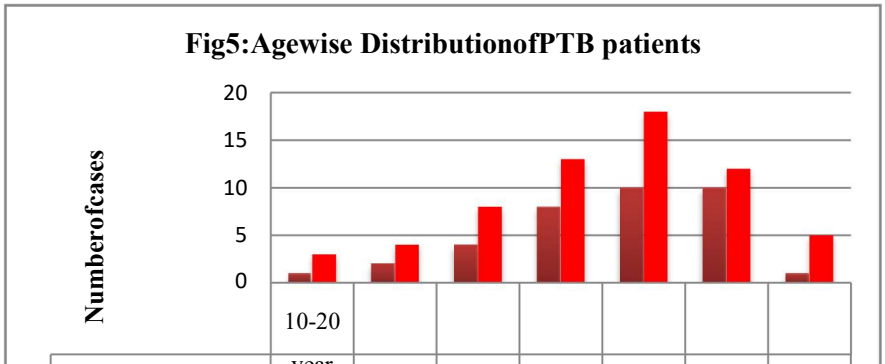


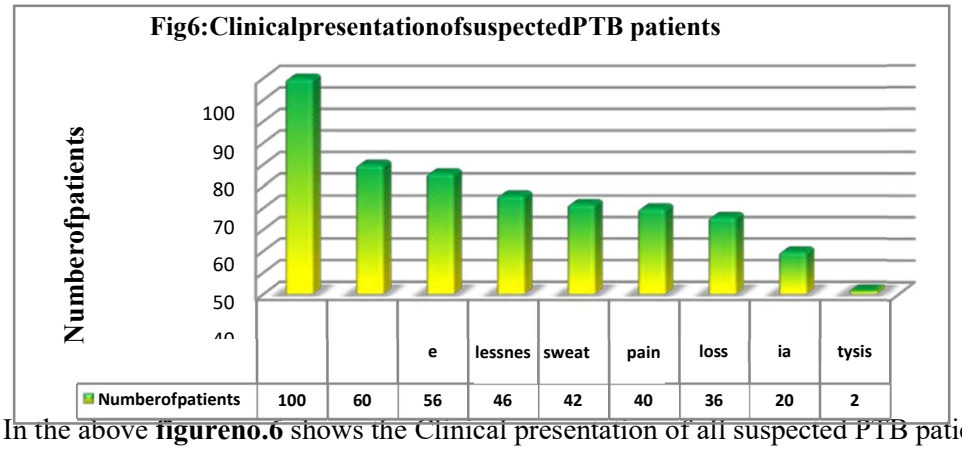
Fig-4:**ShowstheGenderwisedistributionofsuspectedPTBpatients**



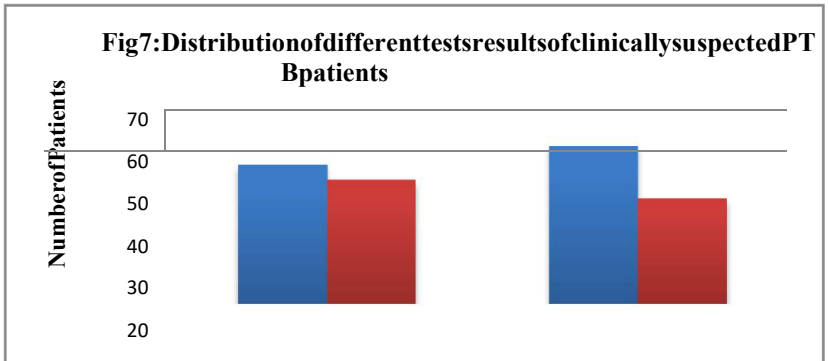
20-30	30-40	40-50	50-60	60-70	>70
2	4	8	10	10	1
4	8	13	18	12	5

Fig-5:Showstheage-wisedistributionofPTBcases.

Themaximumnumberofpatientswerehavingcough(100%),followedbyfever(60%),Fatigue(56%)andbreathlessness (46%).Hemoptosis waspresentonly in2% cases**[Fig-6]**.



In the above **figureno.6** shows the Clinical presentation of all suspected PTB patients.
Out of the100 sputum samples 53 cases were positive by LJ culture and 60 as per TrueNat **[Fig-7].** Out of 60 TrueNat positive samples 10 patients were MDR (Rifampicin Resistance) detectedbyTrueNaT^{TM}MTB RIF Dx chip.



	LJculture	TrueNat
Positive	53	60
Negative	47	40

[Fig-7]-ShowscomparisonoftwodifferenttechniquesfordetectionofPTB.

Table1:ComparisonofresultsfromCultureandTrueNattest			
Culture	**TrueNat**		
	Positive	Negative	Total
Positive	52		53
egative	8	39	47
Total	60	40	100

[Table1]Comparison of results from culture and TrueNat

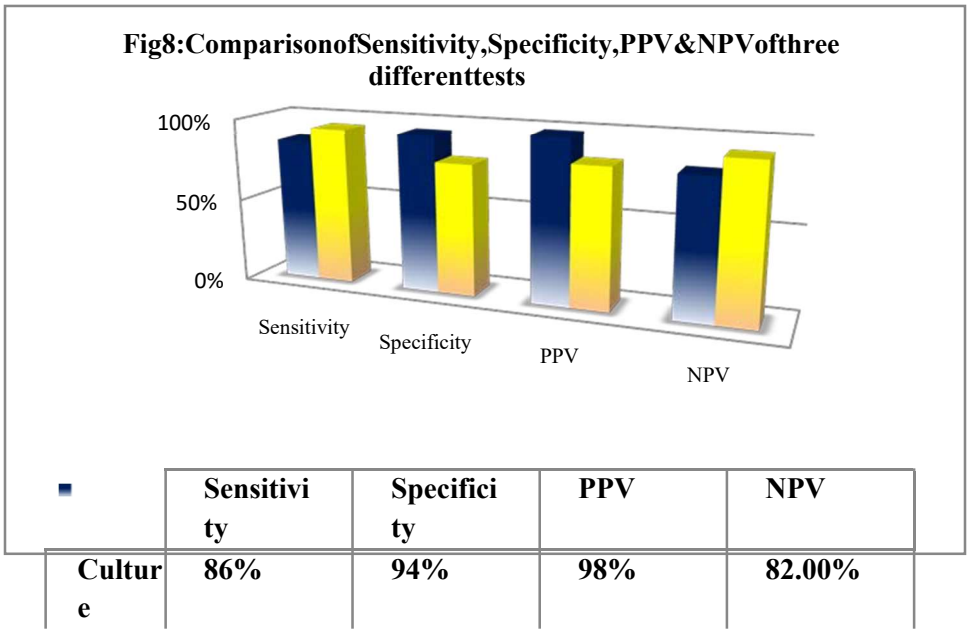
Among 100 samples, 53 were culture positive in which 52 were TrueNat positive and 1 wasnegative. Among the 47 Culture negative samples, 8 were TrueNat positive and 39 were TrueNatnegative**[Table 1].**

Table2:ComparisonofresultsfromTrueNattestandCulture			
TrueNat	**Culture**		
	Positive	Negative	Total
Positive	50	10	60
Negative	3	37	40

Total	53	47	100

[Table2]Comparison of results from TrueNat test and culture

Among 100 samples, 60 were TrueNat positive in which 50 were culture positive and 10 werenegative. Among the 40 TrueNat negative samples, 3 were culture positive and 37 were negative**[Table2].**



	Sensitivity	Specificity	PPV	NPV
Culture	**86%**	**94%**	**98%**	**82.00%**

[Figure 8] Sensitivity, specificity, positive predictive value, and negative predictive value values of Culture andTureNat with culture as gold standard

In the current study it was observed that TrueNat has a very high sensitivity (94%) in comparisonto Culture (86%) in the diagnosis of *M. tuberculosis*, On the other hand culture shows highspecificity (98%) for the detection of PTB in the sputum samples **[Fig-8].** Out of total positivePTB patients 6 HIV reactive cases were found by tridot kit and 10 patients were MDR detectedbyTrueNaTRIFchip.

DISCUSSION:

TBis a significant global public health challenge, marked by high mortality and morbidity rates. Early detection is essential for treating this airborne infectious disease and preventing its spread within populations. Despite ongoing efforts, TB remains a persistent threat worldwide, particularly in India, which is classified as a high-burden country. According to WHO statistics, approximately 2 million individuals developed TB in India, with 28% classified as smear-negative cases of pulmonary tuberculosis, 19% as extrapulmonary tuberculosis, and

6% developing drug resistance, contributing to 26% of the total TB-related deaths. The challenges posed by smear-negative TB and extrapulmonary TB, which are often paucibacillary, hinder progress toward achieving the "End TB Strategy," with inefficient diagnosis being a major obstacle. While direct smear microscopy is inexpensive and rapid, it lacks sufficient sensitivity [9,10].

Detection of Mycobacterium tuberculosis can be achieved through mycobacterial cultures using either solid (Lowenstein Jensen media) or liquid broth systems (MGIT 320). Results from the MGIT liquid culture medium are available sooner than those from the LJ medium [15]. This study aimed to compare the diagnostic yield of LJ culture with that of TrueNat.

Our research indicates that while LJ culture is reliable and cost-effective, it grows more slowly and exhibits lower sensitivity compared to TrueNat, which is significantly more sensitive. A study conducted by Devi NP et al. found that 71% of the subjects were male and 29% female [17]. This is consistent with our findings, where out of 100 sputum samples, 72% were male and 28% female, showing similar gender distribution. In our study, LJ culture and TrueNat demonstrated positivity rates of 53% and 60%, respectively. A study conducted in New Delhi reported a comparable positivity rate of 52.3% for LJ culture [16], while TrueNat showed a positivity rate of 88% [18].

The majority of TB patients in our study were aged 40-70 years, with the mean age of PTB-positive cases being 47.19±16.5. This aligns with another study that reported a mean age of 39.8 years among positive TB patients [18].We observed that the sensitivity of LJ culture was 86%, supporting findings from other researchers, such as Almazini et al., who reported a sensitivity of 72.6% [19], and similar observations from Pichitkul W. et al. and I. Afsar et al. [20,21]. Another study indicated a sensitivity of 76.1% [22]. A relative study by Somoskovi A et al. reported a sensitivity of 81.8% for LJ culture [23]. The low detection rate of PTB via LJ culture in our study may be attributed to sputum collection and quality or a higher contamination rate on LJ media [24].

TrueNat exhibited a sensitivity of 94%, specificity of 78.7%, positive predictive value (PPV) of 83.3%, and negative predictive value (NPV) of 92.5%. A study in Bangalore reported similar sensitivity (91%), specificity (100%), PPV (100%), and NPV (67%) [25]. Research in Kerala conducted by Jose Anie Reena et al. showed comparable results [26]. An evaluation of the Indian TrueNat micro RT-PCR device by Nikam C. et al. reported a sensitivity of 94.7% and a specificity of 52.8% [27]. In this study, the TrueNat MTB test provides results within 2 hours, allowing specimens to be tested promptly upon patient presentation with symptoms while simultaneously detecting Rifampicin resistance (MDR-TB). As a portable platform, it could also be employed in active case finding (ACF) programs. There is a critical need for rapid and accurate diagnostic products for early TB diagnosis in high-burden areas to curb disease transmission, making TrueNat MTB an effective point-of-care test [28].

Although culture is considered the gold standard, its lengthy positivity timeline and inability to simultaneously detect Rifampicin resistance are notable drawbacks. In contrast, GeneXpert serves as a useful diagnostic method for suspected pulmonary tuberculosis, whether AFB smear-negative or positive, due to its speed and capability for simultaneous Rifampicin resistance detection, particularly beneficial for patients with MDR and HIV-associated tuberculosis. Cost-effectiveness studies of GeneXpert in low-income countries like India, where TB prevalence is high, are essential. The prevention of TB disease through the treatment of latent TB infection (TBI) is frequently undervalued but remains a vital component of India's National Strategic Plan 2017-25 aimed at ending TB by 2025, five years ahead of the sustainable development goals. The Lancet Commission on TB asserts that diagnosis and treatment strategies will be ineffective unless TB preventive treatment (TPT) is integrated into a comprehensive strategy [29]. It is crucial to enhance the implementation of proven

interventions, including the development of effective new regimens for TPT and their efficient, rapid scale-up [30,31].

CONCLUSION:

LJ culture is a dependable and cost-effective diagnostic method; however, it is slower and less sensitive compared to TrueNat, which demonstrates significantly higher sensitivity. Therefore, TrueNat is the preferred tool for early tuberculosis diagnosis and for detecting rifampicin resistance.

LIMITATIONS:

The current study is limited by the small sample size; thus, further research with a larger cohort is needed to validate these findings.

Declarations:

Conflictsofinterest:Thereisnoanyconflictofinterestassociatedwiththisstudy

Consentto participate: We haveconsent to participate.

Consentforpublication: Wehaveconsentforthepublicationofthispaper.

Authors'contributions:Alltheauthorsequallycontributedthework.

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