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# A Comparative Study of different Diagnostic Modalities, ZN Stain, Culture and TrueNat for the detection of Pulmonary TB in Sputum Sample and To Study their Co - infection with HIV at a Tertiary Care Hospital

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**Abstract:** ***Introduction: Tuberculosis is an airborne disease caused by Mycobacterium tuberculosis (MTB) that usually affects the lungs. People having HIV infection are 15 - 22 times more likely to develop active TB. For diagnosis of TB, Sputum smear microscopy with ZN staining is the most commonly used test but it has low sensitivity, while culture on LJ media remains the gold standard is time consuming and require special procedure to give positive results. On the other side faster and simple Nucleic Acid Amplification Technique (TrueNaT) due to its rapidity and sensitivity not only help in early diagnosis of TB especially in HIV patients but also detects drug resistance TB. Aim: To evaluate the sensitivity, specificity, positive predictive value and negative predictive value of smear examination, LJ culture and TrueNat with sputum sample in patients with suspected pulmonary tuberculosis and comparison of all three different techniques and to screening the patients for Co - infection of HIV. Material and Methods: This prospective study was carried out using 100 sputum samples of suspected pulmonary TB patients. All the samples were subjected to ZN stain, LJ culture and TrueNat test. For HIV testing by rapid card, One 5 mL blood sample from confirmed TB patients have taken and processed according to the guidelines of National Aids Control Organization (NACO). Results: Out of 100 sputum samples, 36 (36%) patients were positive by ZN stain, 53 (53%) by LJ culture and 60 (60%) as per TrueNat. Sensitivity, specificity, PPV and NPV of LJ culture were 62.2%, 100%, 100% and 70.1%, respectively. Whereas ZN stain showed the sensitivity, specificity, PPV and NPV of 83.3%, 100%, 100% and 91.4%, respectively. In the case of TrueNat the sensitivity, specificity, PPV and NPV were 91%, 100%, 100% and 88%, respectively. Out of 100 patients 6 were HIV reactive and 10 patients were MDR detected by TrueNaT. Conclusion: In our study, ZN stain is rapid and inexpensive and it is better than LJ culture, while the sensitivity of TrueNat is much higher than the ZN stain and culture. So TrueNat is best tool for the early diagnosis of TB and simultaneously detect Rifampicin resistance.***

**Keywords:** *Mycobacterium tuberculosis*, Sputum, Smear microscopy, LJ Culture, TrueNat

## 1. Introduction

Tuberculosis is a communicable disease caused by bacterium *Mycobacterium tuberculosis* (MTB), acid fast rod shaped bacillus, and it usually affects the lungs (pulmonary TB) and spread by air transmission from people with active pulmonary TB [1]. According to TB Report 2022 released by the Union Health Ministry on March 2022, India reported a sharp 19% rise in cases of tuberculosis, total number of incident of TB patients during 2021 were 19, 33, 381 as opposed to that of 16, 28, 161 in 2020 [2]. Early diagnosis is imperative to cure this air borne infectious disease and prevent transmission in community [3].

Sputum smear microscopy is the most common and rapid test that is widely used for the diagnosis of pulmonary *tuberculosis* [4]. It is a simple & cheap technique that quickly identifies acid - fast bacilli (AFB) with a relatively low cost and high positive predictive value, especially when concentrated samples are used [5].

Culture considered as a gold standard but it is very slow growing and time consuming usually takes 6 - 8 weeks to grow and requires proper infrastructure and technical expertise [6]. The colonies are white, rough, tough, buffand have an appearance of bread crumbs or cauliflower [7]. Contamination on LJ media is one of the most frequent problems encountered during the culture of *Mycobacterium tuberculosis* [8]. To minimize the contamination rate Laboratory Manual of WHO recommends decontamination by N - Acetyl L - cysteine (NALC) solution and Petroff method using sodium hydroxide (NaOH) before inoculation on LJ media [9].

The TrueNat MTB test involves sputum processing using a battery - operated sample preparation device, Trueprep - MAGTM, which extracts nucleic acids by a simple menu driven process using a nanoparticle - based protocol optimized for sputum. The device integrates all operations (heating, fluid mixing, magnet control, step timing) using on a programmed micro - controller, thereby enabling nucleic acid isolation without the need for any additional equipment. The chip - based test has been designed to simplify the process of real - time PCR from 'sample to result' so that laboratories with minimal infrastructure can easily perform these tests routinely in their facilities and report PCR results in less than an hour [10].

Immunosuppressive disease HIV increases the risk of latent tuberculosis much more likely to develop TB disease, in developing Countries mortality rate due to HIV co - infection TB is high [11]. On the other hand MTB induced the immune system which leads the activation of T - cells, in turn produce more HIV virions than quiescent T cells [12]. Therefore this study was undertaken to compare the different diagnostic modalities, ZN stain, Culture and TrueNat and at a tertiary care centre Kanpur [13].

## 2. Materials and Methods

A prospective study was carried out using 100 sputum samples. All samples were collected from patients clinically suspected with pulmonary TB. These patients presented with cough, fever, weightloss, breathlessness, fatigue and chest pain. All 100 samples subjected with ZN stain, TrueNat and culture on LJ media. All these diagnostic modalities Compared for sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) in the basis of quantitative results. Each TB positive patient also tested for co - infection with HIV by Standard - Q Rapid card and reactive results confirmed by HIV Tridot test.

**ZiehlNeelsen stain**

All the samples were processed for ZN stain, examined under oil immersion (100x magnification) on light microscope. Each slide was observed for acid fast bacillus (AFB), corresponding to 200 fields examined [Fig - 1] and smear was graded according to RNTCPguidelines [14].

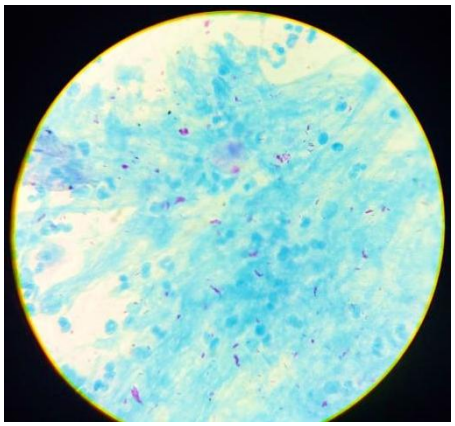


**Figure 1:** Sputum smear microscopy showing the presence of red color acid - fast bacilli

**TrueNat test (Truenat™ RT - PCR, molbio)**

Sputum liquefaction was done by adding 1 drop of liquefaction buffer for 1 - 3 ml of sputum sample, mix it, then the mixture was incubated for 10 min, then 0.5 ml of liquefied sputum sample added into lysis buffer bottle using 1 ml transfer pipette, then transferred the entire content of lysis buffer to the cartridge sample chamber (Black cap) used 3 ml transfer pipette [Fig - 2]. Genome extraction was done within 20 min by TRUEPREP AUTO device [Fig - 3].

The chip labeled as TRUENAT™ MTB was loaded into the instrument as per the as per the manufacturer's instructions. After MTB was detected over in 40 min same sample was tested for Rifamipicin resistance using Truenat™ MTB RIF Dx chip taken 55 minutes.

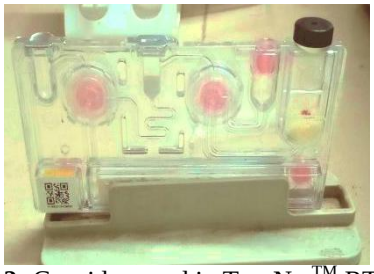


**Figure 2:** Cartridge used in TrueNat™ RT - PCR


**Figure 3:** Cartridge loaded on TRUEPREP AUTO device

**Decontamination procedure by NALC - NaOH**

5 - 10 ml of sputum was taken in a Falcon tube and added equal volume of freshly prepared digestant (NALC - 4% NaOH) after votexing and 15 mint of digestion added enough phosphate buffer (0.067M disodium phosphate and 0.067M monopotassium phosphate) to reach within 1 cm of the top of the tube then Centrifuge at 3600 x g for 15 min after that Inoculated the sediment to LJ media and prepare smear for microscopy [15].

**Culture on Lowenstein Jensen media**

Sediment of processed sample was inoculated in the LJ media and incubated aerobically at 36°C for 4 - 8 weeks. M. tuberculosis grew as buff colored, rough and tough [Fig - 4]

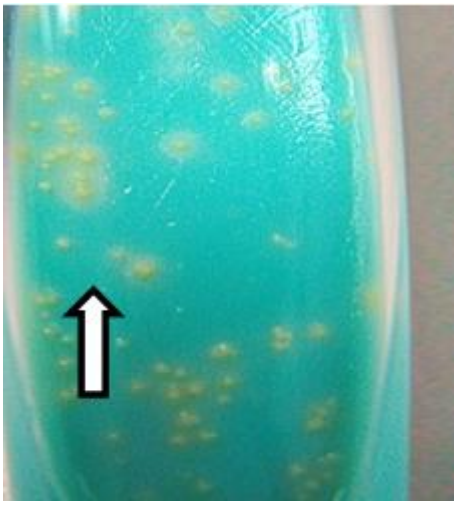


**Figure 4:** Colonies of Mycobacterium tuberculosis (arrow) on L - J medium.

## 3. Result

A total 100 sputum samples were subjected to ZN stain, LJ culture and TrueNat. Out of 100 sputum samples 72 male and 28 female [Fig - 5], 36 patients were positive by ZN stain, 53 by LJ culture and 60 as per TrueNat [Fig - 6].

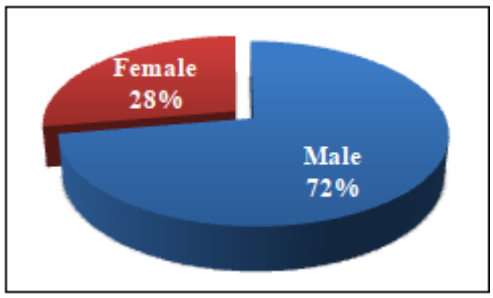



**Figure 5:** Reveal that PTB is more common in males (72%) than in females.

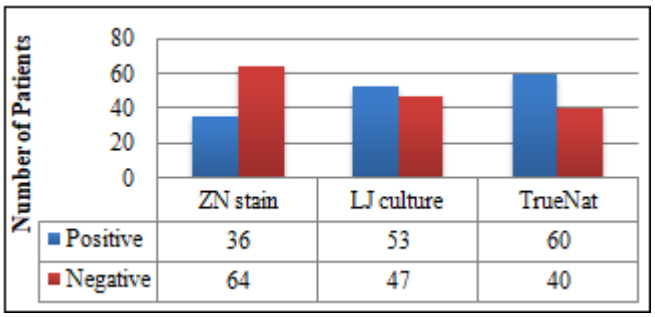



**Figure 6:** Distribution of different tests results of clinically suspected PTB patients

In the above figure no.7 shows the Clinical presentation of all suspected PTB patients.

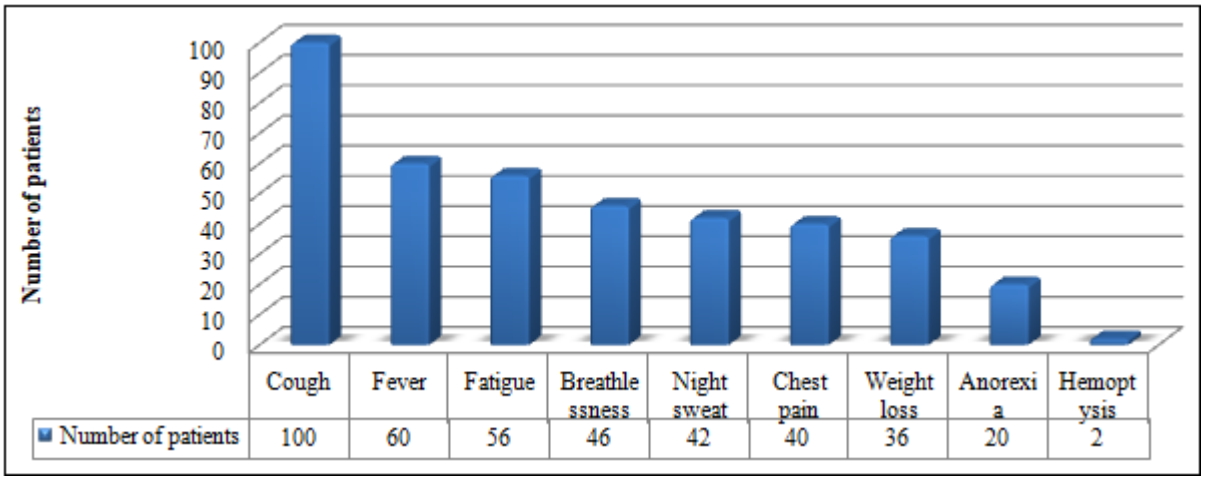



**Figure 7:** Clinical presentation of PTB patients

Fig - 8 shows the age distribution of presumptive PTB cases. The mean age of PTB positive case is 47.19±16.57. The maximum number of positive PTB patients (76%) are between 40 - 70 years of age.

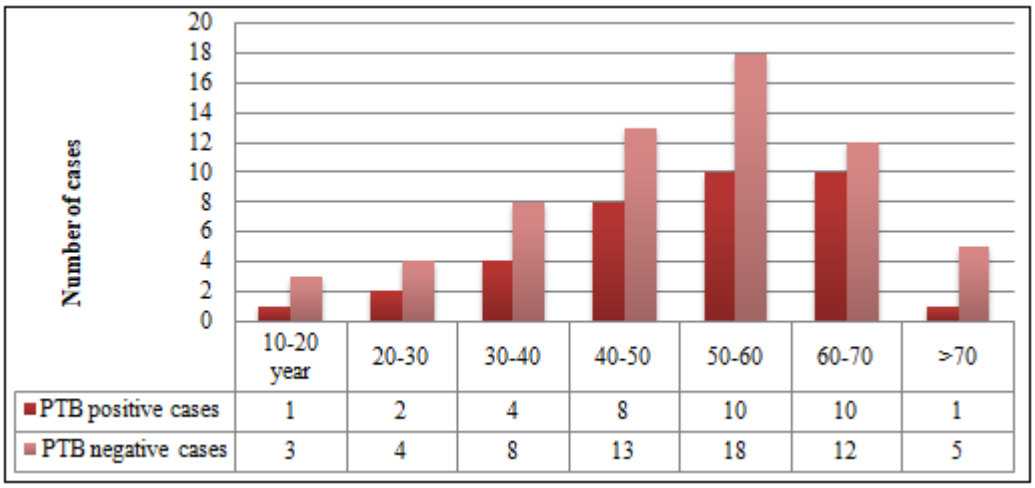



**Figure 8:** Age wise Distribution of presumptive PTB patients

**Comparison of results from ZN stain and culture**

In the present study, among the 100 samples, 36 (36%) were acid - fast stain positive, in which 30 showed growth on culture and 6 were negative. Among the 64 acid - fast negative samples, 23 showed growth on culture and 41 were negative [Table 1].

**Table 1:** Comparison of results from ZN stain and culture

| ZN stain | Culture | | |
|---|---|---|---|
| | Positive | Negative | Total |
| Positive | 30 | 6 | 36 |
| Negative | 23 | 41 | 64 |
| Total | 53 | 47 | 100 |

**Comparison of results from ZN stain and TrueNat test**

Among the 100 samples, 36were AFB positive in which 35 were TrueNat positive and 1 was negative. Among the

64AFB - negative samples, 25 were TrueNat positive and 39 were negative [Table 2].

**Table 2:** Comparison of results from ZN stain and TrueNat

| ZN stain | TrueNat | | |
|---|---|---|---|
| | Positive | Negative | Total |
| Positive | 35 | 1 | 36 |
| Negative | 25 | 39 | 64 |
| Total | 60 | 40 | 100 |

**Comparison of results from TrueNat test and culture [Table 3]**

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

**Table 3:** Comparison of results from TrueNat test and Culture

| TrueNat | Culture | | |
|---|---|---|---|
| | Positive | Negative | Total |
| Positive | 50 | 10 | 60 |
| Negative | 3 | 37 | 40 |
| Total | 53 | 47 | 100 |

**Comparison of results from culture and TrueNat [Table 4]**

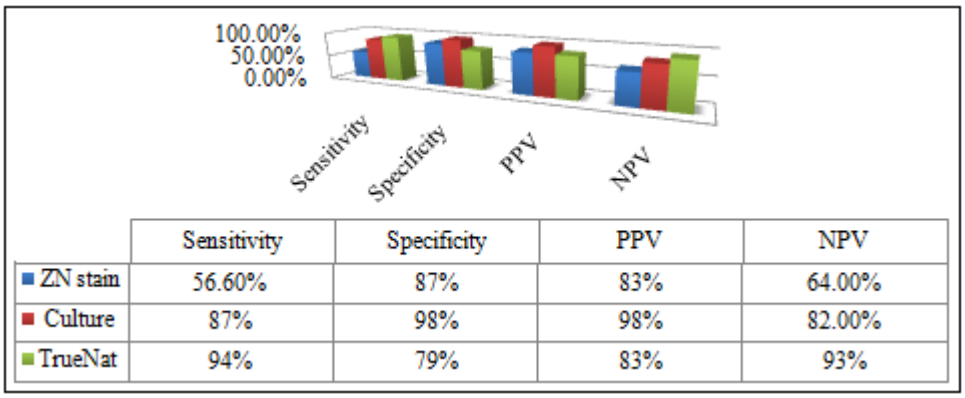
Among 100 samples, 53 were culture positive in which 52 were TrueNat positive and 1 were negative. Among the 47 Culture negative samples, 8 were TrueNat positive and 39 were TrueNat negative [Table 4].

**Table 4:** Comparison of results from Culture and TrueNat test

| Culture | TrueNat | | |
|---|---|---|---|
| | Positive | Negative | Total |
| Positive | 52 | 1 | 53 |
| Negative | 8 | 39 | 47 |
| Total | 60 | 40 | 100 |

**Sensitivity, specificity, positive predictive value, and negative predictive value values of Ziehl–Neelsenstain, Culture and TureNatwith culture as gold standard**

In my study, TrueNat has a very high sensitivity (94%) in comparison to ZN stain (56.6%) and Culture (87%) in the diagnosis of *M. tuberculosis*, On the other hand culture shows high specificity (97.5%) for the detection of PTB in the sputum samples [Fig - 9]. Out of 100 patients 6 were HIV reactive by Standard - Q rapid card and 10 patients were MDR (Rifampicin resistance) detected by TrueNaT™MTB RIF Dx chip.




| | Sensitivity | Specificity | PPV | NPV |
|---|---|---|---|---|
| ZN stain | 56.60% | 87% | 83% | 64.00% |
| Culture | 87% | 98% | 98% | 82.00% |
| TrueNat | 94% | 79% | 83% | 93% |

**Figure 9:** Comparison of different accuracy measures of ZN stain, culture and TrueNat

## 4. Discussion

The purpose of this study was to compare the diagnostic yield of ZN staining and LJ culture and TrueNat. TrueNat showed a sensitivity of 94%, specificity of 78.7%, PPV of 83.3%, and NPV of 92.5%. A study in Bangalore, India showed similar sensitivity (91%), specificity (100%), PPV (100%) and NPV (67%) [16]. A study in Kerala which is conducted by JoseAnieReena et al showed similar results [17]. In my study, One sample which was TrueNat negative [Table - 2], But it was positive in ZN stain, this was comparable to another study which compared conventional techniques to molecular [18].

A study have done on Evaluation of the Indian TrueNAT micro RT - PCR device by Nikam C. et al showed similar results the sensitivity of trueNat was 94.7% and 52.8% was the specificity [19]. In the present study, trueNat MTB test gives results within 2 hrs and TrueNat MTB is also a good point of care [20].

In our study in comparison of culture on LJ media, ZN stain showed a sensitivity of 56.6%, specificity of 87.4%, PPV of 83.3% and NPV of 64%. A study showed similar sensitivity (48%), specificity (94%), PPV (88%) and NPV (64%) of ZN stain [21]. A study by also showed Similar results by Afsar et al [22].

In this study, out of 36 ZN stain positive samples by 30 were positive by LJ culture but 6 samples were negative. The sensitivity of LJ culture was 87%. An almost similar study have done by Almazini et al. was reported sensitivity of 72.6% [23], and another one study reported 76.1% of sensitivity [24]. Most relative study by Somoskovi A, et al. reported sensitivity of 81.8% by LJ culture [25]. These variations of the results in the detection of PTB by LJ culture may be due to collection and quality of the sputum sample or higher contamination rate on LJ culture [26].

## 5. Conclusion

In our study, ZN stain is rapid and inexpensive but the sensitivity is lower than LJ culture, but culture takes 4 - 8 weeks to grow and the chances of contamination is also very high, On the other hand the sensitivity of TrueNat is much higher than the ZN stain and culture and takes less time to give results. So TrueNat is best tool for the early diagnosis of TB and simultaneously detects Rifampicin resistance.

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**Conflict of interest**
There are no conflicts of interest.

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