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# Role of line probe assay (LPA) and genexpert in diagnosis of smear-negative pulmonary tuberculosis-a prospective study

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**Abstract**
Tuberculosis still remains as a major cause of ill health worldwide causing substantial morbidity and mortality despite a decline in both incidence and prevalence. The dual challenge of low diagnositic sensitivity of Sputum Microscopy and technical challenge of performing TB cultures, particularly in SNPT delays the initiation of treatment. With India being one among 22 countries with highest TB burden globally, MDR-TB poses a global threat in TB control underscoring the importance of prompt and rapid identification of such resistant MTB strains, resulting in treatment failure and poor clinical outcome. So, there is a need for rapid, reliable molecular based tests like GeneXpert & LPA for early diagnosis and institution of treatment, thus preventing the transmission of Drug resistance TB among the vulnerable population. This study aims to compare the Diagnostic Performance of GeneXpert & LPA against the culture methods (MGIT 960) with early detection of drug resistance and its pattern among the SNPT at Dr. B.R. Ambedkar Medical College and Hospital. 100 Patients with Suspected TB with Sputum smear negative samples were randomly selected and subjected to detailed clinical examination, pathological tests, Culture methods (MGIT 960) and molecular testing-GeneXpert & LPA. Further the test results were analysed and performance of each test were compared with MGIT 960(gold standard assay).
The diagnostic performance of LPA & GeneXpert were highly sensitive and specific for early detection of MTB complex, particularly MDR strains, leading to effective management and good adherence with short duration of time. Thus LPA is a better alternative to culture with regards to detection of MDR strains and drug resistance pattern.

**Keywords:** SNPT-sputum negative pulmonary tuberculosis, T2DM-type 2 diabetes mellitus, TB-tuberculosis, TST-tuberculin skin test

## Introduction
Tuberculosis is an ancient & highly infectious disease that can potentially involve any organ or system in the body. Tuberculosis is caused by the bacterium Mycobacterium tuberculosis which primarily affects lungs but can also affect intestines, meninges, bones and joints, lymph glands, skin and other tissues of the body. The disease is usually chronic with varying clinical manifestations. Bovine tuberculosis which primarily affects cattle may also be communicated to man. Tuberculosis [TB] continues to intimidate the human race since time immemorial not only due to its effects as a medical malady, but also by its impact as a social and economic tragedy. Tuberculosis (TB) is an old disease – studies of human skeletons show that it has affected humans for thousands of years. Tuberculosis and its effects were known to mankind as early as the 6th Century BC, as evident from writings from the Assyrian empire of the time.
Although patients with sputum smear – negative, culture-positive pulmonary TB can transmit infection, they have been presumed to be far less infectious than patients who are sputum smear-positive. But patients with HIV coinfection, chronic renal diseas, neoplastic disorders and those receiving immunosuppressive therapy are more susceptible for acquiring infection from the Smear negative pulmonary tuberculosis patients. However, quantitative data are limited regarding the proportion of TB transmission that is attributable to patients with smear-negative, culture – positive disease. Our study is therefore undertaken with the objective of evaluating the diagnostic yield and durg-sensitivity profile of recovered isolates by using Line probe assay in chest-symptomatic TB suspects who have been found to be sputum smear-negative in spot and morning samples.

## Materials and Methods
The study was conducted on patients attending Out-patient department and Patient admitted to medical wards, Department of General Medicine, Dr. B.R. Ambedkar Medical College and surrounding Tuberculosis units (TU) under RNTCP. Type of study: Prospective and observational study. A prospective study is a longitudinal study that follows over time a group of individuals who differ with respect to certain factors under study, to determine how these factors affect rates of a certain outcome. Observational study draws inferences from a sample to a population where the independent variable is not under the control of the researcher because of ethical concerns or logistical constraints. Study Period: Study period was from 1/12/2017 to 31/10/2019 Study Population: Patients with suspected Tuberculosis and those who had received ATT but having symptoms were included in the study. Only those patients who had sputum smear negative (sputum microscopy negative twice) were included in the study. Sample Size: A total of 100 smear-negative sputum samples were included in the study. Ethical

Consideration: Ethical and research clearance was obtained from the Ethical committee, Dr. B.R. Ambedkar Medical College, Bangalore. The Study was approved by the The State Operational Research (OR) Committee, Government of Karnataka and was allowed to use the laboratory facilities at Bowring Hospital, National Tuberculosis Institute, Bangalore and at Intermediate Reference Laboratory at SDS-TB sanitorium, Bangalore. Informed consent was obtained from the patients before enrolment in to the study.

**Inclusion Criteria**

Age ≥ 18 years Sputum smear negative cases with radiographic signs of tuberculosis
Patients previously treated first line ATT.
New cases of tuberculosis not responding to first line ATT
Contact of an MDRTB case with symptom of pulmonary TB
Co-morbid condition like Diabetes mellitus, Alcoholic Liver Disease, Hepatitis & Cardiac failure

**Exclusion Criteria**

1. Pregnant and lactating females
2. Sputum-smear positive cases
3. A Patient with HIV, Cancer or terminal illness and extra-pulmonary tuberculosis i.e., lymph node, pleural, pericardial, neuro- TB (eg: meningeal, tuberculoma, etc), renal, genitourinary, abdominal, skeletal, skin, miliary & dissemenated TB.
4. Patient lost(i.e., continuously default for more than one month) and patient who die before being visited by investigator
5. Person not willing to participate in the study.

**Collection of Data**

1. Diagnosis of TB was confirmed as per Revised National Tuberculosis Control Programme guidelines and algorithm.
2. All the patients were subjected to detailed history, general physical examination and systemic examination.
3. Written informed consent was obtained for all patient or their legal representatives, at the time of enrollment into the study.
4. All investigation was conducted at RNTCP Unit, Dr.B.R Ambedkar Medical College, Bangalore, Bowring Hospital, National Tuberculosis Institute, Bangalore and at Intermediate Reference Laboratory at SDS-TB sanitorium, Bangalore.
5. Sputum samples from all patients suspected for TB and those receiving retreatment were subjected to smear microscopy by Ziel Neelsen staining as part of the routine diagnostic protocol and only smear negative samples were sent for Cartridge Based Nucleic Acid Amplificatin Test (CB-NAAT) [Gene Xpert] or Line Probe Assay (LPA) and Culture & Drug suspceptibility test (DST) at the above designated laboratory centres.
6. The sputum samples (about 0.5ml to 1ml) from all patients suspected for TB was collected in a 50-ml wide-mouthed sterile falcon tube according to the guidelines of the Revised National Tuberculosis Control Programme of India. The samples was decontaminated by NALC-NaOH method.
7. The sputum samples were tested by newer molecular techniques at the above designated Laboratory centres as per Diagnositic Algorithm for Pulmonary Tuberculosis under RNTCP.

**Statistical methods involved in this study**

Contingency table analysis Chi-square test Independent samples t-test
Analysis of variance (ANOVA) test
Contingency tables (also called crosstabs or two-way tables) are used in statistics to summarize the relationship between several categorical variables. A contingency table is a special type of frequency distribution table, where two variables are shown simultaneously.

**Conclusion**

This was a prospective, observational study of 100 patients of suspected Pulmonary Tuberculosis with Sputum Smear Negative samples.
All patients were aged 18 years or more, with signs and symptoms suggestive of PTB and abnormal Chest X-ray findings with Sputum smear negativity, registered under the Revised National Tuberculosis Control Programme.
In our study, a total number of 100 cases of suspected TB were subjected to GeneXpert, LPA and Culture methods-MGIT 960.
Male preponderance was observed in the study with 65% males against females who were 35% of the population. The Age distribution had varied presentation with more participants in the age group of 21-30yrs (27%), 41-50yrs(24%) and also >60yrs(17%) in the study.
Most cases of suspected cases of TB were people who belongs to lower class and lower middle class (BPL) (77%), people living in urban areas (95%) with improper/housing facilities and poor sanitation facilities, more frequent in married people (83%) and employed people (61%).
In this study, 17% of the patients were chronic smokers and 3% had ethanol dependence which were independent predictors of transmission of tuberculosis.
Most common associated Co-morbidities among the tuberculosis suspected patients was Diabetes Mellitus (20%) followed by Chronic Kidney Disease (10%) and Hypothyrodism (7%). Commonest presenting symptom among cases of TB suspects was cough with expectoration associated with fever (82%), followed by fever with chills only (6%), and significant weight loss (4cases). The radiographic patterns typical of TB were more common with patients having cough with expectorations along with fever (75%) and was suggestive of statistical significance with p-value of 0.0973. Most females in the study (20%) had low Hb (<12mg/dl), significantly lower than that of males and majority of them had high ESR value (76%). With MGIT 960 considered as the gold standard assay, both Gene Xpert and LPA had better diagnositic performance characteristic than AFB microscopy/Chest X-ray in Sputum Smear negative samples. In addition, regarding Rifampicin mono-resistance and Isoniazid mono-resistance, LPA outperformed Gene Xpert and thus was a better alternative to Culture methods with regards to detection of Drug resistance in relatively shorter duration, thus initiating early treatment for better compliance and adherence.

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