

# Diagnostic Yield of Direct Smear, Concentrated Smear, Liquid Culture, and GeneXpert by Sputum Induction in Smear-Negative Pulmonary Tuberculosis Patients

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## Abstract

**Background:** Spontaneous sputum smear gave negative results in clinically and radiologically suspected pulmonary tuberculosis (TB) patients. Hence, the study aimed to evaluate the diagnostic yield of induced sputum with direct smear, concentrated smear, liquid culture, and GeneXpert. **Materials and Methods:** This cross-sectional study was conducted on clinically and radiographically suspected TB patients who were sputum smear-negative; 88 sputum smear-negative patients underwent sputum induction, and the samples were tested for direct smear, concentrated smear, liquid culture, and GeneXpert. Descriptive analysis was carried out for frequency and proportion for categorical variables and mean and standard deviation for quantitative variables. Diagnostic yield was calculated for each test. Data analyzed using coGuide software, V.1.03. **Results:** The mean age of the study population was  $46.87 \pm 15.09$  years, and male participants were the majority. The diagnostic yield of induced sputum was 19.29% compared to a direct smear. Diagnostic yield was 59.25%, 80.70%, and 94.73% for concentrated smears, liquid culture, and GeneXpert, respectively. **Conclusion:** Induced sputum gave a high diagnostic yield in smear-negative pulmonary TB. With induced sputum, GeneXpert gave maximum yield compared to direct smear, concentrated smear, and liquid culture.

**Keywords:** Acid-fast bacilli, GeneXpert, induced sputum, tuberculosis

## INTRODUCTION

Mycobacterial culture is considered the gold standard for diagnosing tuberculosis (TB), but the final result is usually obtained in 2–6 weeks and requires technical expertise and proper infrastructure.<sup>[1-3]</sup> Acid-fast bacilli (AFB) staining by fluorescent microscopy, an essential diagnostic tool, was negative in up to 50% of active pulmonary TB (PTB).<sup>[4]</sup> Sputum induction is a noninvasive and safe method with a high diagnostic yield for PTB.<sup>[5]</sup> Hence, the aim was to evaluate the diagnostic yield of direct smear, concentrated smear, liquid culture, and GeneXpert by sputum induction in smear-negative PTB patients.

## MATERIALS AND METHODS

### Study population and study site

The study population was patients who attended the Department of Pulmonology at Government Medical College.

### Study duration

The study was conducted for 12 months from May 2018 to April 2019.

### Inclusion criteria

- Patients aged 18 years and above have a persistent cough for more than 2 weeks
- Sputum smear-negative for AFB on two samples and with chest radiographic findings favorable with the diagnosis of active PTB.

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## Exclusion criteria

- Active hemoptysis
- Recent eye surgery
- Unstable angina or arrhythmia
- Presence of pleural diseases
- Those already on antitubercular treatment
- Uncontrolled asthma or chronic obstructive pulmonary disease.

## Study design

This was a cross-sectional study.

## Sample size and sampling method

All the 100 patients who were eligible after considering the inclusion and exclusion criteria and attended during the study were recruited by universal sampling.

## Ethical considerations

Ethics committee approved the study, and participants gave informed consent.

## Data collection tools and clinical examination

### Clinical examination

Age, gender, systolic blood pressure (SBP), diastolic blood pressure (DBP), and temperature were measured. Two pulmonologists clinically examined the patients, and radiographs were analyzed during the study. The patients suspected of having TB clinically and radiographically were undergone spontaneous sputum AFB twice. Out of 100 suspected TB patients, 88 were sputum negative, and 12 did not give any result. Hence, the induced sputum samples of these 88 patients were collected and underwent further investigation.

### Induced sputum collection

Sputum induction is proposed to improve sample collection and is relatively easy to perform and generally well tolerated.<sup>[6]</sup> Eighty-eight suspected TB cases at the hospital were isolated in a well-ventilated room. A sputum collection cup was given to the patient on arrival at the hospital. Through an ultrasonic nebulizer (Beurer Ultrasonic Nebulizer IH 50), sputum induction was done using 10 ml of 3% hypertonic saline. Inhalation continued until the patient had produced a good amount of sputum. This induced sputum was used for sputum smear, concentrated smear, liquid culture, and GeneXpert.

## Statistical methods

Direct smear, induced sputum smear, concentrated smears, liquid culture, the primary outcome variable were GeneXpert. Demographic variables were considered primary explanatory variables. The diagnostic yield was calculated. Descriptive analysis was carried out by mean and standard deviation for quantitative variables, frequency, and proportion for categorical variables. Data analyzed by coGuide software, V.1.03 BDSS Corp, Bengaluru, Karnataka, India.<sup>[7]</sup>

## RESULTS

A total of 100 participants were included in the final analysis.

The mean age was  $46.87 \pm 15.09$  in the study population. The majority of 64% of participants were male. Of 100 participants, 24 (24%) had diabetes mellitus. The mean SBP, DBP, temperature, and Mantoux were  $117.50 \pm 17.07$  (mmHg),  $77.46 \pm 10.28$  (mmHg),  $99.90 \pm 0.67$  ( $^{\circ}$ F), and  $10.78 \pm 7.23$  min, respectively [Table 1].

Among the study population, the baseline was a direct smear. After two direct AFB smears, out of 100 samples, 88% were negative, and 12% could not give a result. After inducing sputum, an AFB smear was performed; 11% was found to be positive, and 5% did not provide any result. Induced sputum concentrated smears gave 32% of positive cases, liquid culture gave 46%, and GeneXpert's gave 54% of positive results [Table 2].

**Table 1: Summary of baseline parameters (n=100)**

Parameters	Summary
Age	46.87±15.09
Gender, n (%)	
Male	64 (64)
Female	36 (36)
DM	24 (24)
SBP (mmHg)	117.50±17.07 (ranged 90-150)
DBP (mmHg)	77.46±10.28 (ranged 60-100)
Temperature ( $^{\circ}$ F)	99.90±0.67 (ranged 98.8-101.4)
Mantoux (mm)	10.78±7.23 (ranged 0-22)

DM: Diabetes mellitus, SBP: Systolic blood pressure, DBP: Diastolic blood pressure

**Table 2: Summary of outcome parameters (n=100)**

Parameters	Summary, n (%)
Direct smear	
Negative	88 (88)
Not given	12 (12)
Induced sputum smear	
Positive	11 (11)
Negative	84 (84)
Not given	5 (5)
Concentrated smears	
Positive	32 (32)
Negative	63 (63)
Not given	5 (5)
Liquid culture	
Positive	46 (46)
Negative	48 (48)
Not given	5 (5)
NTM	1 (1)
GeneXpert	
Positive	54 (54)
Negative	41 (41)
Not given	5 (5)

NTM: Non-tuberculous mycobacterial

**Table 3: Diagnostic yield among the induced sputum techniques**

	Direct smear	Induced sputum smear	Concentrated smears	Liquid culture	GeneXpert	All five specimens combined
Positive	0	11	32	46	54	57
Negative	88	84	64	48	51	37
Not given	12	5	5	5	5	
NTM	0	0	0	1	0	
Diagnostic yield (%)	0	19.29	59.25	80.70	94.73	

Individual test/all five specimens combined $\times 100$ , example: diagnostic yield of induced sputum smear  $11/57 \times 100 = 19.29\%$ . NTM: Non-tuberculous mycobacterial

The induced sputum gave a diagnostic yield of 19.29% compared to a direct smear. The diagnostic yield of concentrated smears was 59.25%, liquid culture 80.70%, and GeneXpert 94.73% [Table 3].

## DISCUSSION

The diagnostic yield of induced sputum was 19.29%. GeneXpert had the highest diagnostic yield (94.73%), followed by liquid culture (80.70%) and concentrated smears (59.25%) with induced sputum. In contrast, a meta-analysis has shown that the diagnostic yield of sputum induction for the diagnosis of PTB in individual studies may range from 35% to 95%.<sup>[8]</sup> A study by Schoch *et al.* reported that the diagnostic yield for induced sputum on the spot was 46% for smears and 36% for culture.<sup>[9]</sup> The diagnostic yield for induced sputum was the same in retreatment and new cases in a study by Luhadia *et al.*; hence, they assumed that the induced sputum might help treat TB cases with scanty sputum.<sup>[10]</sup>

Sumalani *et al.* reported that sensitivity was highest for Xpert assay (34.3%), followed by AFB culture (21.6%) and Induced Sputum (IS) AFB smear (7.8%). This result showed that IS Xperts had the highest diagnostic yield for PTB among these investigations.<sup>[11]</sup> Macías *et al.* also showed a higher diagnostic accuracy for sputum Xpert assay (65%), followed by sputum culture (43.2%) and sputum smear (26.5%).<sup>[12]</sup>

Many authors reported a sensitivity of 45% to 83% for sputum smear microscopy and high specificity of 98% or above in clinical practice.<sup>[13-16]</sup> Sputum microscopy is considered the most appropriate and particular method to diagnose PTB, and culture-positive status was observed in 22%–61% of cases with smear-negative results.<sup>[17]</sup> Lack of quality of sputum sample, low bacterial load (<10,000 bacilli/ml), improper preparation examination of smears, patients with late-stage HIV disease, immune-suppressed patients, and children are the causes of smear negativity.<sup>[18]</sup> The mortality rate was reported to be 14.1% for smear-negative culture-positive cases. Thus, the early diagnosis of the active smear-negative disease is also essential.<sup>[19]</sup> When spontaneous sputum is not available, sputum induction or flexible fiberoptic bronchoscopy may be used for diagnosis.<sup>[20]</sup> In the present study, sputum induction was done.

Induced sputum smear positivity was 11% in this study. In studies by Gopathi *et al.* and Saglam *et al.*, a positivity rate of 63.3% and 47%, respectively, were reported, which is higher than our study.<sup>[20,21]</sup> The average age of the patients

was  $46.87 \pm 15.09$  years in this study, and the majority were male (64%). In a study by Schoch *et al.*, the mean age was  $38 \pm 12.5$  years, and the majority were males.<sup>[9]</sup> In contrast, in a study by Saglam *et al.*, the average age of the patients was  $35.8 \pm 17$  years, and the male-to-female ratio was almost equal.<sup>[20]</sup> One of the salient features of this study was it included various diagnostic techniques with induced sputum to compare the diagnostic yield.

## Limitations

The small sample size was used due to limited resources and practical constraints.

## CONCLUSION

Induced sputum gave a high diagnostic yield with smear-negative PTB. Hence, the sputum induction technique may be included in the Revised National Tuberculosis Control Programme diagnostic workup of PTB as a routine to improve the diagnostic yield of TB in smear-negative PTB cases to facilitate the initiation of treatment in preventing drug-resistance emergence.

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## Conflicts of interest

There are no conflicts of interest.

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