

## DIAGNOSTIC PERFORMANCE OF ZIEHL-NEELSEN & CBNAAT TECHNIQUES IN PULMONARY TUBERCULOSIS, A RETROSPECTIVE ANALYSIS AT TERTIARY CARE HOSPITAL IN JHALAWAR, RAJASTHAN.

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### Abstract

**Introduction:** Tuberculosis (TB) remains a leading cause of death among infectious diseases worldwide. The advent of the CBNAAT was a revolution in the diagnosis of tuberculosis, especially with high incidence and resource poor regions. It can be used close to the point of care by operators with minimal technical expertise, enabling diagnosis of TB and rifampicin Resistance (RIF) concurrently using unprocessed clinical specimens, in less than 2h.

**Aim:** To evaluate the diagnostic performances of Ziehl-Neelsen & CBNAAT techniques in detection of Pulmonary Tuberculosis.

**Materials and Methods:** We retrospectively reviewed the 2414 sputum samples of suspected pulmonary TB. Sputum samples were subjected for ZN staining and CBNAAT. RIF resistance was detected by CBNAAT.

**Results:** Out of 2414 samples, 751 sputum samples were positive by smear microscopy. 1127 Samples were confirmed positive by CBNAAT examination. Majority of cases, 43.57% were in 21-40 yrs age group. 24.40% were females and 75.59% were males. Sensitivity of CBNAAT was 100% for sputum positive cases and sensitivity was 22.6% for sputum negative cases. Overall RIF resistance was detected in 49 (4.4%) cases in present study.

**Conclusion:** CBNAAT provide sensitive detection of tuberculosis and rifampicin resistance directly from untreated sputum in less than 2 hours with minimal hands-on time.

**Keywords:** Cartridge-based nucleic acid amplification tests (CBNAAT), Rifampicin(RIF)

### Introduction

Despite declining global incidence and mortality, tuberculosis (TB) remains a leading cause of death among infectious diseases worldwide, with an estimated 10.0 million new cases and 1.2 million TB deaths among HIV-negative people and 251000 deaths with coexistent HIV infection in 2018.<sup>1</sup>

Detection and quantitative estimation of acid-fast bacilli (AFB) in clinical specimen by smear microscopy, a relatively simple and inexpensive screening tool, gives the first bacteriologic evidence of the presence and in assessing the patient's infectiousness. It provides the physician with a preliminary confirmation of the diagnosis. But smear microscopy is unable to differentiate drug resistant and susceptible strains of Mycobacterium tuberculosis complex (MTBC).<sup>2</sup>

Conventional methods for mycobacteriological culture and drug susceptibility testing (DST) are time consuming, cumbersome and resource-intensive requiring up to 6 and 8 weeks for final identification on liquid and solid culture media, respectively. During this time patients may be inappropriately treated and so amplification of drug resistant strains may occur. Novel technologies for rapid detection of anti-TB drug resistance have therefore become a priority in TB research and development.<sup>3</sup>

Recently introduced as a breakthrough in TB diagnostics an automated cartridge-based nucleic acid amplification test (CBNAAT), Xpert MTB/RIF assay (Cepheid, Sunnyvale, CA) is a hemi-nested real-time PCR amplifying the rpoB gene target, so capable of simultaneously detecting Mycobacterium tuberculosis complex (MTBC) and Rifampicin (RIF) resistance within 2 hours in the clinical samples; endorsed by the WHO in 2010, approved by the Food and Drug Administration (FDA) in 2013. The sample processing, nucleic acid extraction, amplification and detection of target sequences are performed in a single cartridge in this integrated system.<sup>3</sup>

### Objectives:

To evaluate the diagnostic performances of Ziehl-Neelsen & CBNAAT techniques in detection of Pulmonary Tuberculosis.

### Materials and Methods:

This retrospective study was conducted from January 2019 to October 2019 at Jhalawar Hospital after obtaining ethical approval from institutional ethical committee.

A total of 2414 sputum samples from all the patients with symptoms and signs, suggestive of pulmonary tuberculosis (PTB), as well as chest X-ray showing features of PTB, were collected from patients at the Jhalawar TB Clinic and other county medical treatment facilities, in the course of routine clinical evaluations for pulmonary TB.

**Inclusion criteria**

Patients with clinical suspicion of pulmonary tuberculosis including symptoms of cough with or without expectoration for >2 weeks, weight loss, fatigue, haemoptysis and loss of appetite.

**Exclusion Criteria**

Patients suspected to have Extra Pulmonary Tuberculosis (EPTB) were excluded from the study.

Each sputum samples received in the lab from the centers were divided;

One part was subjected for ZN staining to detect Acid Fast Bacilli (AFB). The second part was used to carry out CBNAAT for detection of *M. tuberculosis* and RIF resistance on the same day.

Using conventional ZN staining method, Acid fast bacilli were detected in the sputum samples by direct smear microscopy. Slides showing red coloured acid fast bacilli were taken as positive.

CBNAAT was performed according to the manufacturer's instructions. Samples were collected in containers provided and treated with sample reagent in a proportion of 2:1 and incubated for 15 minutes at 20-30 °C. Pipette atleast 2ml with the plastic transfer pipette from the collection container into the sample chamber of the Xpert cartridge and put into the GeneXpert instrument system and the automatically generated results were read after 90 min.

**Results:**

A total of 2414 patients were included in our study, out of which 1127 Samples were confirmed positive by CBNAAT examination. From the confirmed positive 852(75.59%) were males and 275 (24.40%) were females.

**Table 1:** Mycobacterium Tuberculosis detected by CBNAAT-Demographic Character

Males	Females
852	275

**Table 2:** Age group distribution

Age group	Males	Females
<20	95	64
21-40	359	132
41-60	308	58
>61	90	21

In our study majority of cases, 43.57% were in 21-40 yrs age group followed by 32.47% in 41-60 yrs age group

**Table 3:** Sputum smear positive versus CBNAAT positive

	No of cases examined	No of cases diagnosed	%
Smear microscopy	2414	751	31.11
CBNAAT	2414	1127	46.69

Out of 2414 samples, 751 sputum samples were positive by smear microscopy. 1127 Samples were confirmed positive by CBNAAT examination.

**Table 4:** Sputum smear positive versus CBNAAT positive in all tested cases

	Smear Positive(751)	Smear Negative(1663)
CBNAAT Positive(1127)	751	376
CBNAAT Negative(1287)	0	1287

Among 1663 ZN smear negative samples 376(22.61%) were found to be positive for MTB by CBNAAT. Out of 751 ZN smear positive, CBNAAT negative cases 0.

In our study Rifampicin (RIF) resistance was detected in 49(4.3%) cases.

**Discussion:**

Proper tuberculosis care and control averted up to 6 million deaths and cured 36 million people between 1995 and 2008.<sup>4</sup> Early diagnosis and treatment remains the cornerstone of TB control. Globally, due to rising of multidrug resistance and extensively drug-resistant tuberculosis, leading to need for dramatic expansion of culture and drug-susceptibility testing especially in endemic countries like India. Unfortunately, the infrastructure and technically skilled personnel required for such testing are available only in a limited number of reference centers. Further, the results of testing are often not available for at least 4 months, leading to dramatical reduction of its clinical utility.

The laboratory and the clinicians requesting service must be confident of the results the laboratory provides. However, in developing countries rapid and accurate diagnosis of tuberculosis is still a challenge. The main advantage of CBNAAT lies in its simplicity of use, rapid diagnostic ability and early detection of RIF resistance. In addition, molecular assays do not need viable bacilli; therefore, specimen transport conditions do not have as much impact on the test outcome as with culture, and specimens can be shipped by regular mail.<sup>5</sup>

Although the costs of instruments and tests will still be considerably higher than those for Microscopy but is less costly than implementation of culture and drug-susceptibility testing. MTB/RIF assay generates no infectious aerosols and eliminates the necessity for a biosafety cabinet.<sup>7</sup>

In this study, majority of cases, 43.57% were in 21-40 yrs age group with male preponderance (75.59%). Dewan R *et al.*<sup>6</sup> in their study found that the mean age of patients were 35±9 years and 76% were males.

In this study sensitivity of CBNAAT varied significantly between 100% in sputum smear-positive PTB and 22.6% in

sputum smear negative PTB. Saugat *et al.*<sup>8</sup> and Mohanty T *et al.*<sup>9</sup> reported sensitivity of 15% and 32% of CBNAAT in smear negative PTB, which correlates with the present study.

#### Conclusions:

Much intensified action in the form of early, accurate diagnosis and proper treatment is needed to decrease the TB incidence rate by an average of 16% per year for the next 40 years in order to eliminate TB as a public health problem (as defined by a TB incidence of less than one per million population worldwide) by 2050.<sup>4</sup>

Detection of AFB in sputum smear is a simple, rapid, inexpensive and very specific diagnostic tool for PTB. However, its major limitation is low sensitivity

This retrospective study highlights the importance of setting up CBNAAT at every district level of health care centre and all medical institutes for early and accurate detection and prompt treatment of TB.

#### Acknowledgements

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#### References:

- World Health Organisation (WHO) Global tuberculosis report-2018.
- American Thoracic Society/Centers for Disease Control and Prevention. Diagnostic standards and classification of tuberculosis in adults and children. *Am J Respir Crit Care Med.* 2000; 161: 1376–95. [https:// doi.org/10.1164/ajrccm.161.4.16141](https://doi.org/10.1164/ajrccm.161.4.16141) PMID: 10764337
- Rice JP, Seifert M, Moser KS, Rodwell TC . Performance of the Xpert MTB/RIF assay for the diagnosis of pulmonary tuberculosis and rifampin resistance in a low-incidence, highresource setting. *PLoS ONE* 12(10),2017.
- Lonroth K, Castro KG, Chakaya JM, *et al.* Tuberculosis control and elimination 2010-50: cure, care, and social development. *Lancet.* 2010; 375:1814–1829. [PubMed: 20488524]
- Parsons LM, Somoskövi Á, Gutierrez C, Lee E, Paramasivan CN, Abimiku A, *et al.* Laboratory diagnosis of tuberculosis in resource-poor Countries: Challenges and opportunities. *Clin Microbiol Rev.*, 2011; 24(2): 314–50.
- Dewan R, Anuradha S, Khanna A, Garg S, Singla S, Ish P, *et al.* Role of cartridge-based nucleic acid amplification test (CBNAAT) for early diagnosis of pulmonary tuberculosis in HIV. *J Indian AcadClin Med.* 2015;16(2):114-17.
- Banada PP, Sivasubramani SK, Blakemore R, *et al.* Containment of bioaerosol infection risk by the Xpert MTB/ RIF assay and its applicability to point-of-care settings. *J Clin Microbiol.* August18;2010 (Epub ahead of print).
- Saughat R, Modi C.S., Meena R.R. CARTRIDGE BASED NUCLEIC ACID AMPLIFICATION TEST (CBNAAT): A NEW TOOL IN DIAGNOSIS OF TUBERCULOSIS. *INDIAN JOURNAL OF RESEARCH* ; 2010;Volume - 9 ,Issue – 10.
- Mohanty T, Panigrahi SK, Pattnaik M, Panda G, Routray D, Patra JK, *et al.* Study on diagnostic modalities in smear negative pulmonary tuberculosis with special reference to sputum induction (SI CBNAAT), bronchoscopy (BAL CBNAAT and BAL culture). *J Evid Based Med Healthc.* 2017;4(47):2858-62