A Correlative And Comparative Study Between Zn Stain And Cbnaat For Detection Of Reference Resistance Detective In Tertiary Care Hospital

Dr Jafar Khan[1], Dr. Jyoti Tomar[2], Dr. Rahat Khan[3], Dr. Usman Khan[4]

[1]Ph.D. Scholar,Department of Microbiology, Pacific Institute of Medical Science, Udaipur (Rajasthan)

[2]Associate Professor, Department of Microbiology, Pacific Institute of Medical Science, Udaipur (Rajasthan)

[3] Ph.D. Scholar,Department of Pharmacology, Index Medical College, Indore (Madhya Pradesh)

[4]Associate Professor, Department of Orthopedics, Pacific Medical College and Hospital, Udaipur (Rajasthan)

Correspondence Author:
Dr Jafar Khan
zafarkhan1984@gmail.com

Received: 29-03-2023 **Accepted: 02-04-2023**

ABSTRACT

Background: For several decades smear microscopy and conventional culture techniques have been the mainstay of diagnostic testing for pulmonary tuberculosis. Culture is considered as the "gold standard" for final determination, but it is slow and may take up to 2 to 8 weeks. Although smear microscopy for acid-fast bacilli (AFB) is rapid and inexpensive, it has poor sensitivity and a poor positive predictive value (PPV). Thus, rapid identification, which is essential for earlier treatment initiation, improved patient outcomes, and more effective public health interventions, relies on nucleic acid amplification techniques

Objective: The aim of this study was comparing the two techniques (i.e., Gene Xpert and ZN stain) for detection of MTB in in smear-positive and smear-negative pulmonary and non-pulmonary clinical specimens.

Methodology: Total 700 patients of all age groups having either pulmonary or extra pulmonary tuberculosis were included in the study. All collected sputum samples will be processed by ZN staining technique and GeneXpert /CBNAAT technique. Comparisons of these techniques were done after sputum samples examined.

Result: Out of 273 total patients report positive results for ZN stain showed 271 patients showed resistance for CBNAAT and 2 of them showed negative result. 427 patients were found negative for ZN stain, out of them only 2 patients were resistant to the CBNAAT and 425 were negative to CBNAAT.

Conclusion: ZN staining and CBNAAT was found to be equally sensitive and not only in the detection of acid-fast bacilli but it could also detect rifampicin resistance.

Keywords: MTB/RIF, Gene Xpert techniques, ZN staining technique, *Mycobacterium tuberculosis* (MTB).

DOI: 10.48047/ecb/2023.12.si12.009

INTRODUCTION:

Global Tuberculosis report 2021 reveals that worldwide in 2020, 1.3 million deaths among HIV-negative people were estimated, up from 1.2 million in 2019. 214 000 deaths among HIV-positive people were also added, a small increase from 209 000 in 2019. WHO estimated that from 2000–2020, about one billion people would be newly infected, 200 million will become sick, as well as 35 million people will die from Tuberculosis.[1]

Currently, HIV infection is known as the greatest risk factor for development of latent TB infection to active Tuberculosis. Multiple eruptions of increasing death rate are caused by the co-infection HIV and Tuberculosis particularly in combination with drug resistance. Mycobacterium tuberculosis spread through airborne particles (droplet nuclei) generated when patients with pulmonary tuberculosis cough.[2]

In year 2013, Global prevalence of Tuberculosis was 159 per 100,000 populations. "Nine million people who were infected with TB in the world in 2013, India and China, both accounted for 24% and 11% of total cases respectively" [3] . Out of the estimated global annual incidence of 9.6 million Tuberculosis cases, 2.2 million were estimated to have occurred in India. i.e., approximately Twenty three percent of global annual Tuberculosis incidents occur in India making it highest Tuberculosis burden country [4].

In the global tuberculosis report (2014), "WHO reported global burden of multidrug-resistant TB (MDR-TB) was estimated to be 480,000 cases leading to estimated 210,000 deaths" [5]. Extra pulmonary TB cases being the highest reports one-fourth of total TB cases and it is more frequent in children and immune compromised people [4]. A global recurrence of TB has arisen. Straits of M. Tuberculosis immune to both isoniazid and Rifampicin with or without exposure to other medications have been referred to as multidrug resistant strains. Multidrug-resistant tuberculosis (MDR-TB) is one of the most distressing components of the antibiotic resistance pandemic, since TB patients who fail in medication are at high risk of death. Rapid diagnosis of TB significantly decreases the lag time in initiation of treatment, thereby reducing transmission rates. [6]

For several decades smear microscopy and conventional culture techniques have been the mainstay of diagnostic testing for pulmonary tuberculosis. Culture is considered as the "gold standard" for final determination, but it is slow and may take up to 2 to 8 weeks. Conventional solid culture techniques have the limitation of long time of several weeks. Liquid culture techniques were developed for early detection of MTB growth, but it is still long for a diagnostic test to be effective in curbing transmission. Such delays in diagnosis increase morbidity and mortality, predispose to secondary resistance and cause transmission of resistant strains.

Although smear microscopy for acid-fast bacilli (AFB) is rapid and inexpensive, it has poor sensitivity and a poor positive predictive value (PPV). Thus, rapid identification, which is essential for earlier treatment initiation, improved patient outcomes, and more effective public health interventions, relies on nucleic acid amplification techniques.

Sputum smear microscopy has been the main technique for pulmonary tuberculosis diagnosis in low- and middle-income countries, which account for almost 95% of TB cases and 98% of TB-related fatalities. It is an easy, quick, and affordable approach that is particularly specific in regions where tuberculosis is widely prevalent. It is widely relevant in a variety of communities with diverse socioeconomic levels and also identifies the most infectious patients. As a result, it has become a crucial component of the global TB control plan [7]. Due to its varying sensitivity, sputum smear microscopy is ineffective, especially in patients with drug-resistant TB, extrapulmonary TB, sputum smear-negative TB, and cases of HIV infection.[8-10.]

Diagnosis of pulmonary TB is usually done by ZiehlNeelsen (Z-N) acid fast staining of sputum smears and by mycobacterial culture wherever facilities are available [11]. Smear ZN stain for sputum by using light microscopy are the standard methods to identify AFB. If bacilli are present, the patient has sputum smear positive for PTB. When the MTB cannot be identified, chest X- ray is taken to assist with diagnosis.[12]Ziehl-Neelsen (ZN) smear microscopy has a low sensitivity and requires

numerous visits, which increases default. Although mycobacterial culture is the gold standard, it is sluggish and often takes two to six weeks to get a final result. It also needs the right infrastructure and technical know-how[13]. The emergence and spread of multi-drug resistance in TB (MDR-TB) bacilli is one more major challenge for health systems and TB control programs.

An additional technique to Ziehl-Neelsen (ZN) smear microscopy is fluorescent microscopy (FM), which is 8-10 % more sensitive than ZN smear microscopy and because AFB can be seen at lower magnification (40x), FM smears can be examined in a fraction (about 25%) of the time needed for ZN smears. In 2010, the WHO recommended that LED FM be phased into replace ZN microscopy for TB diagnosis. [14]

Molecular diagnostics have significantly impacted clinical medicine since the development of the polymerase chain reaction (PCR) in the early 1980s. Despite of some potential benefits, however, the use of molecular diagnostics for tuberculosis has been constrained, principally because of difficulties with DNA extraction, amplification, and identification, as well as biosafety issues related to the manipulation of Mycobacterium tuberculosis. Because it fully integrates and automates the three procedures needed for real-time PCR-based molecular testing, the GeneXpert system was introduced in 2004 and makes molecular testing simpler (i.e., specimen preparation, amplification and detection). [15]

Therefore, new techniques in molecular diagnostic methods in the field of TB have been introduced into practice, which are playing an important role in early diagnosis of and prompt treatment of TB. One such method is GeneXpert MTB/RIF assay which is now commonly available and being used widely. This molecular method is more subtle in detecting the presence of TB bacilli in sputum samples than just smear and microscopic examination. Both Mycobacterium tuberculosis DNA and genetic mutations associated with Rifampicin resistance can be detected simultaneously in a short duration of just two hours using sputum samples.[11]

The aim of this study was comparing the two techniques (i.e., Gene Xpert and ZN stain) for detection of MTB in in smear-positive and smear-negative pulmonary and non-pulmonary clinical specimens.

METHODOLOGY:

Subject: Total 700 patients of all age groups having either pulmonary or extra pulmonary tuberculosis were included in the study. The study was conducted from 2019 to 2022 at Pacific Institute of Medical Sciences (PIMS) Udaipur (Raj.), if they fulfilled the inclusion criteria. A duly filled biological form was collected with each patient along with registered Nikshay Identification Number.

Inclusive area: All smear negative cases, All Pediatric cases, All HIV positive cases (suspected of TB), All Extra Pulmonary cases were included in the study.

Exclusive area: All blood stained sputum sample and sample with presence of food particles were excluded from the study.

Data collection and Examination: All the samples were collected in well labeled falcon tubes. In pulmonary cases two sputum samples were collected: one early morning and another supervised spot specimen.

Procedure:

- **For ziehl-neelsen's staining technique:** All spot and morning sputum samples were processed. An appropriate portion of the sputum samples (from the most purulent portion) was collected with an applicator stick or a wire loop and spread on the labeled side of the microscope slide. The slides were air dried and then heat-fixed. All smears were stained by the ZN technique. All slides were examined blindly by microscope 100X objectives to scan the smear for confirming AFB.
- **For gene xpert/CBNAAT technique:** Machine contains 4 cartridges so 4 samples were processed for each run. According to the normal operating procedure, the sampling reagent (containing NAOH and isopropanol) was applied to the sample at a rate of 2:1 and held at

room temperature for 15 minutes with occasional shaking. 3 ml of the handled sample was moved to the cartridge and the cartridge was placed into the CBNAAT system module. The automated method finished the remaining assay measures, and the findings were shown on the Gene Xpert monitor after 1 hour and 50 minutes.

Comparison between genexpert MTB/RIF and ZN staining techniques: Comparisons of these techniques were done after sputum samples examined. The results of sputum samples generated from GeneXpert were compared with the results yielded by ZN staining.

RESULTS:

Out of 700 participants, 71.86% were males and 28.14% were females with mean age of 48.72 ± 17.69 years (range less than 21 years to 90 years).

Table 1: Z.N. Stain MicroscopicScreening Result of Patients sample

Z N Stain	No. of cases	Percentage
Negative	427	61.00%
Positive	273	39.00%
Total	700	100.00%

Table 1 and fig 1 shows the results of ZiehlNeelsen's stain test. According to the test 61% participants were found to be negative and remaining 39% were showing positive result for the microscopic screening of the samples.

Figure 1: Z. N. Stain Microscopic result of Patients sample

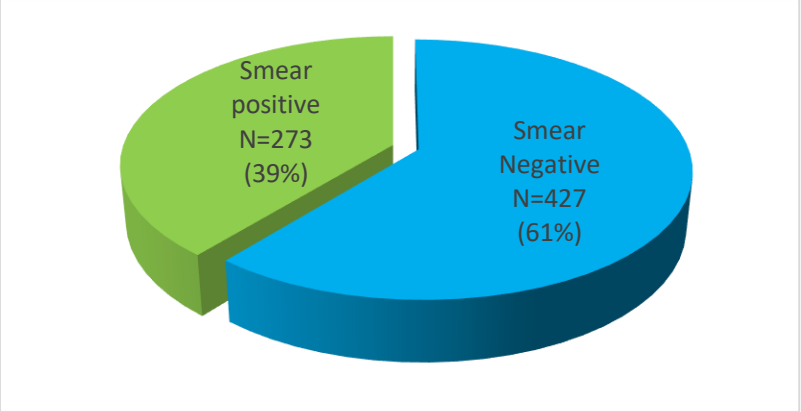
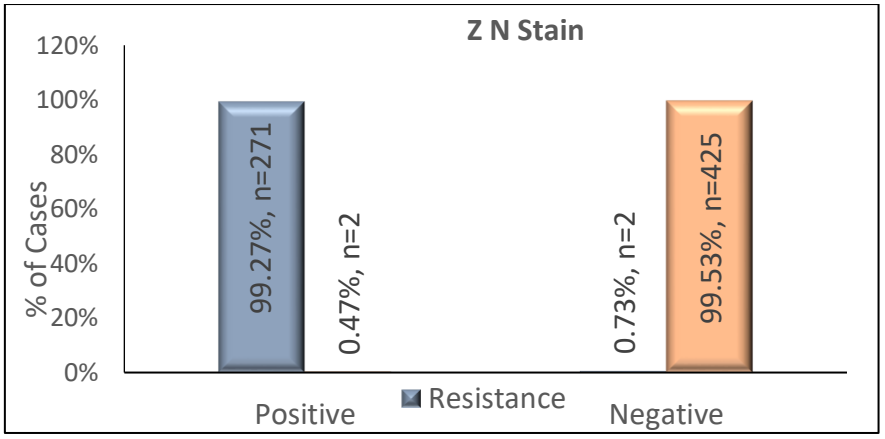


Table 2: Variation in Z N Stain and CBNAAT results of same sample of patient

	Smear Positive n=273		Smear Negative n=427	
CBNAAT Result	**No.**	**Percentage**	**No.**	**Percentage**
Positive	271	99.27%	2	0.47%
Negative	2	0.73%	425	99.53%
Total	**273**	**100%**	**427**	**100%**

P<0.001 (HS)

Figure 2: Z. N. Stain and CBNAAT wise Distribution of results of samples



Sensitivity 99.27%, Specificity 99.53%, PPV 99.27%, NPV 99.53%, Accuracy 99.43%

Table 2 and fig 2 compares the result of ZN stain and CBNAAT together. Out of 273 total patient samples which were positive by Z. N. stain, 271 patient samples were negative for CBNAAT and 2 of them showed negative result for same sample. Out of 700 total samples, 427 patient samples were found negative by ZN stain, out of them only 2 patient samples were found to be positive by CBNAAT and 425 were negative to CBNAAT.

DISCUSSION:

ZN Stain resistance wise distribution of patients

Sputum samples from all patients of suspected TB were examined for the presence of acid-fast bacilli by smear examination using staining method i.e., ZN staining. According to the test 61% patient samples were found to be negative and remaining 39% were showing positive result for the test.

CBNAAT Resistance wise Distribution of Patients

In the study CBNAAT was carried out to detect Mycobacterium tuberculosis from the sputum samples of all the patients of suspected pulmonary tuberculosis which were enrolled in the study. According to the test 61% participants were found to be negative and remaining 39% were showing positive result for the test.

Additionally, 5% to 28% more samples are detected as positive by CBNAAT when compared to ZN stain in another research. Ten samples that tested negative for CBNAAT but positive for both ZN stains could have been infected with MOTT bacteria because CBNAAT does not pick them up. Sputum samples needed to be cultured in order to confirm this, but we were unable to do so for this research. CBNAAT was used to determine the status of 63 samples that were negative by ZN staining but positive by AO staining.

Comparison of CBNAAT Results with ZN Stain

Out of 700 samples subjected to ZN and CBNAAT. In the present study all the 273 sputum samples which were positive by ZN staining were found to be positive by CBNAAT. Out of 273 total patients which produced positive results for Z N stain showed 271 patients showed resistance for CBNAAT and 2 of them showed negative result. 427 patients were found negative for ZN stain, out of them only 2 patients were resistant to the CBNAAT and 425 were negative to CBNAAT.

The existence of PCR inhibitors or insufficient nucleic acid extraction in the sputum samples may be the cause of these negative samples. Saprophytes or Non-Tuberculous Mycobacteria (NTM)/Mycobacterium other than TB (MOTT) may also be to blame for this. Because microscopy cannot distinguish between Mycobacterium avium complex (a NTM infection) and M. TB complex, diagnosing and treating NTM infections can be challenging.

In a research, Wright PW et al. came to the conclusion that ZN cannot distinguish between NTM and MTB; only culture can do so. NTM-related pulmonary infections are a growing problem, especially in Western nations where the prevalence of NTM may exceed that of tuberculosis. The recovery rates of NTM from clinical specimens with positive AFB smear results differ greatly by country, ranging from 7.3-10.6% in Korea 382, 21.1% in Spain 383, and 24.8-48.5% in the US. The prevalence of

NTM was found to be 17.4% in clinical specimens from patients with fibro cavitary disease in India, 7.4% in other clinical specimens, and 8.6% in patient sputum samples.[16]

CONCLUSION:

The present study was carried out to know the efficiency of AFB staining by Ziehl Neelsen stain and CBNAAT for the diagnosis of tuberculosis in a southern area of Rajasthan. CBNAAT was found to be sensitive and it not only detects acid-fast bacilli but it could also detect rifampicin resistance. Only limitation is that it is not providing sensitivity for isoniazid. NTM can be reported by microscopy only CBNAAT reports it negative.

REFERENCES:

1. World Health Organization. Global Tuberculosis Report, Geneva, Switzerland; World Health Organization; 2021.
2. Murray PR, Bara AJ, Jorgensen JH, Pfaller MA. and Yollan RH. Manual of Clinical Microbiology 8th Edition. 2003. New York: Wiley & Sons
3. World Health Organization. Global Tuberculosis Report, Geneva, Switzerland; World Health Organization; 2014.
4. Automated Real-Time Nucleic Acid Amplification Technology for Rapid and Simultaneous Detection of Tuberculosis and Rifampicin Resistance: Xpert MTB/ RIF Assay for the Diagnosis of Pulmonary and Extrapulmonary TB in Adults and Children: Policy Update. Geneva: World Health Organization; Issued date 2013.
5. Tuberculosis, WHO Global Tuberculosis Report 2014.http.//www.who.int/tb/publications/factsht global.pdf
6. Sharma SK, Mohan A. Multidrug-resistant tuberculosis. Indian Journal of Medical Research. 2004;120(4):354-76.
7. Desikan, P. (2013). Sputum smear microscopy in tuberculosis: Is it still relevant?. The Indian Journal of Medical Research. 2013; 137(3), 442-444.
8. Weyer K, Mirzayev F, Migliori GB, Van Gemert W, D'Ambrosio L, Zignol M, Floyd K, Centis R, Cirillo DM, Tortoli E, Gilpin C, de DieuIragena J, Falzon D, Raviglione M. Rapid molecular TB diagnosis: evidence, policy making and global implementation of Xpert MTB/RIF. European respiratory journal. 2013;42(1):252-71.
9. Xpert MTB/RIF for people living with HIV. World Health Organization October 2014. http://www.who.int/tb/challenges/hiv/Xpert_TBHIV_Information_ Note_final. Assesed on 13 Nov; 2014.
10. Walusimbi S, Bwanga F, De Costa A, Haile M1, Joloba M, Hoffner S. Meta-analysis to compare the accuracy of GeneXpert, MODS and the WHO 2007 algorithm for diagnosis of smear-negative pulmonary tuberculosis. BMC Infectious Diseases. 2013; 13:507.
11. Nakate P , Patil S , Patil S, Purohit H , Shelke Y. Comparison of diagnostic efficacy of GeneXpert MTB/RIF assay with ZiehlNeelsen staining & microscopy in diagnosis of pulmonary tuberculosis. IP International Journal of Medical Microbiology and Tropical Diseases. 2019; 5(4):218-221.
12. Zanzibar Integrated HIV (ZIHTLP), Tuberculosis and Leprosy Program. Annual Report, Zanzibar, Tanzania; 2013.
13. Agrawal M, Bajaj A, Bhatia V, and Dutt S, Comparative study of Genexpert with ZN stain and culture in samples of suspected pulmonary tuberculosis Journal of Clinical and Diagnostic Research. 2016; 10(5): 09–12
14. Salinita N, Supriya L, Usharani M, Singh HL. Detection of pulmonary tuberculosis using cartridge based nucleic acid amplification test (CBNAAT) and fluorescent microscopy. International Journal of Health Research and Medico-Legal Practice. 2018; 4:78-81.

15. Theron G, Peter J, Dowdy D, Langley I, Squire SB, Dheda K. Do high rates of empirical treatment undermine the potential effect of new diagnostic tests for tuberculosis in high-burden settings?. The Lancet infectious diseases. 2014; 14(6):527-32.
16. Wright PW, Richard J. Wallace Jr., Wright NW. Sensitivity of Fluorochrome Microscopy for Detection of Mycobacterium tuberculosis versus Nontuberculous Mycobacteria. Journal Of Clinical Microbiology. 1998: 1046–1049