Evaluation of diagnostic accuracy of Ziehl Neelson staining of sputum samples in comparison with CBNAAT for the diagnosis of Pulmonary tuberculosis at the tertiary care hospital from South Rajasthan.

Miss Neelima Varania[1], Dr Manoj Vasudeo Jani[2] , Dr Malkesh Meena[3], Dr Anjali Kulshrestha[*].

[1]Assistant Professor, Department of Microbiology, Ananta Institute of Medical Sciences & Research Center, Rajsamand, Rajasthan.

[2]Associate Professor, Department of Pulmonary Medicine, Ananta Institute of Medical Sciences & Research Center, Rajsamand, Rajasthan.

[3]Assistant Professor, Department of Pulmonary Medicine, Ananta Institute of Medical Sciences & Research Center, Rajsamand, Rajasthan.

[*]Associate Professor, Department of Microbiology, Ananta Institute of Medical Sciences & Research Center, Rajsamand, Rajasthan.

* Corresponding Author - Dr Anjali Kulshrestha.

Abstract

Tuberculosis (TB), an infectious disease is a major public health challenge costing one human life every 20 seconds globally [1]. There is wide variation in the sensitivity of ZN smear microscopy, ranging from 20% to 80% across various studies [7,9], whereas, the new molecular based methods have shown promising sensitivity in TB detection. Keeping the above points in view, also no such study was done earlier from this region. The present study was undertaken to determine the accuracy of sputum ZN smear microscopy in diagnosing pulmonary tuberculosis as compared with the sputum GeneXpert MTB/RIF assay as the reference test at our set up. In the present study, a total of 70 patients were included, in which 60(86%) were male and 10(14%) were female. A total of 65(92.9%) patients sputum samples detected MTB DNA by GeneXpert MTB/RIF and 5(7.1%) samples were negative. In these 65 positive patients, 55(85%) were male and 10(15%) were female. Older age group was most affected 24(37%) and children and young adults were least affected as only 6(9%) patients in each age group. Sputum ZN smear microscopy is a highly specific but moderately sensitive test for the diagnosis of pulmonary tuberculosis. This study recommends the sputum GeneXpert MTB/RIF test in preference to sputum ZN smear microscopy for the diagnosis of pulmonary tuberculosis.

Keywords - END TB strategy, GeneXpert MTB/RIF test, Sputum Smear Microscopy (SSM).

Introduction

Tuberculosis (TB), an infectious disease is a major public health challenge costing one human life every 20 seconds globally[1]. It is estimated that over ten million of world's population is infected with Mycobacterium tuberculosis, causative agent of TB and accounts for 1.6 million deaths annually[2]. India is one among the highest TB burden countries in the world, accounting for almost a quarter TB case. The elimination of TB requires early diagnosis followed by complete course of treatment[3].

In May 2014, World Health Organization (WHO) unanimously passed resolution to support 'END TB strategy [4]. This strategy aims to reduce TB deaths by 95%, cut the new cases up to 90% by 2035, and complete eradication of TB by 2050. For eradication of TB, the number of cases must decrease at the rate of 16% per year [4]. Identification of three million undiagnosed patients is the major focus of END-TB strategy, however new case detection rate of India is only 70% [5,6]. National Strategic Plan for TB control 11 envisions to detect 90% of TB cases by 2025.

Mycobacterial culture is the gold standard test for the diagnosis of TB, and it can detect as low as 10 bacilli/ml of the specimen [5]. However, mycobacterial culture is time-consuming and requires specialized laboratory infrastructure. A GeneXpert Mycobacterium tuberculosis/rifampicin (MTB/RIF) assay (a nucleic acid amplification test) is a sensitive but expensive test for the diagnosis of TB with a lower detection threshold of 136 bacilli/ml of the specimen [6]. Additionally, a GeneXpert MTB/RIF assay can simultaneously detect the presence of rifampicin resistance, which is a surrogate marker of multidrug resistant (MDR) TB. However, the GeneXpert MTB/RIF assay costs 10 times more than the Lowenstein Jensen (LJ) culture and 20 times as compared to ZN smear microscopy.

Acid-fast bacilli (AFB) smear microscopy is an inexpensive but less sensitive test for the diagnosis of TB with a lower detection threshold of 5,000 bacilli/ml of the specimen[5]. Ziehl-Neelsen (ZN; conventional light microscopy) and fluorochrome (light-emitting diode (LED) fluorescent microscopy) are the techniques used for AFB smear microscopy. Fluorescent microscopy is a more rapid but more expensive technique as compared to Ziehl-Neelsen (ZN) smear staining, which is less expensive and widely available[7,8] .

Sputum Smear Microscopy (SSM) continues to be a major method of TB diagnosis in primary health care settings. The AFB smear microscopy is used for the diagnosis of pulmonary TB in adult non-immunocompromised patients not at risk of drug-resistant TB and in all patients with a presumptive diagnosis of TB, where GeneXpert MTB/RIF testing is not available. . There are 14,000 designated microscopy centers in India and each caters to approximately 100,000 people in their proximity [9].

GeneXpert MTB/RIF assay is recommended after microscopy in AFB smear-positive cases and in AFB smear-negative cases, with chest X-ray changes suggestive of TB[5]. The GeneXpert MTB/RIF assay is also recommended as an initial test for the diagnosis of presumed TB in children, immunocompromised patients, suspected extrapulmonary TB, suspected drug-resistant TB, and in patients with suggestive chest X-ray changes [5]. Currently there are about 1200 CBNAAT and 200 TrueNat laboratories in the country, at the district and in some cases at a sub-district level [9].

There is wide variation in the sensitivity of ZN smear microscopy, ranging from 20% to 80% across various studies [7,10], whereas, the new molecular based methods have shown promising sensitivity in TB detection. WHO recommends sputum GeneXpert MTB/RIF testing for all suspected cases of pulmonary tuberculosis [11]. However, the upgradation of existing TB diagnostic infrastructure is a costly affair and may not be possible in the next 10 years.

Keeping the above points in view, also no such study was done earlier from this region. The present study was undertaken to determine the accuracy of sputum ZN smear microscopy in diagnosing pulmonary tuberculosis as compared with the sputum GeneXpert MTB/RIF assay as the reference test at our set up.

Material and Methods

Study design – Retrospective study .

We included all pulmonary TB patients (Suspected, Old, New, Defaulter etc) whose sputum sample was sent to Mycobacteriology Lab.

Period of study – March 2021- June 2022

We included cases of

a) Presumptive pulmonary TB cases 1. Cough >2 weeks 2. Fever >2 weeks 3. Significant

weight loss 4. Blood in sputum 5. Any abnormalities in chest radiography.

b) TB Treatment failure cases

c) Contact with known MDR TB case

d) Sputum positive and negative at diagnosis,

e) Retreatment cases

f) Follow up sputum positive cases

g) Microbiologically confirmed TB patients.

we excluded

a) Individuals not fitting in the definition of suspected pulmonary TB cases.
b) Extrapulmonary tuberculosis cases.
c) HIV TB co-infection.

CBNAAT was performed, according to manufacturer instruction maintaining all aseptic precaution though Sputum smear microscopy was done on the same sample at DMC of the college. Quantity of the sample was confirmed to be > 5 ml. MTB bacterial load was estimated in terms of High, Medium and Low. Demographical and Treatment information history was collected from the clinical notes. Smear microscopy along with CBNAAT result was compared and assessed in Excel sheet for analysis.

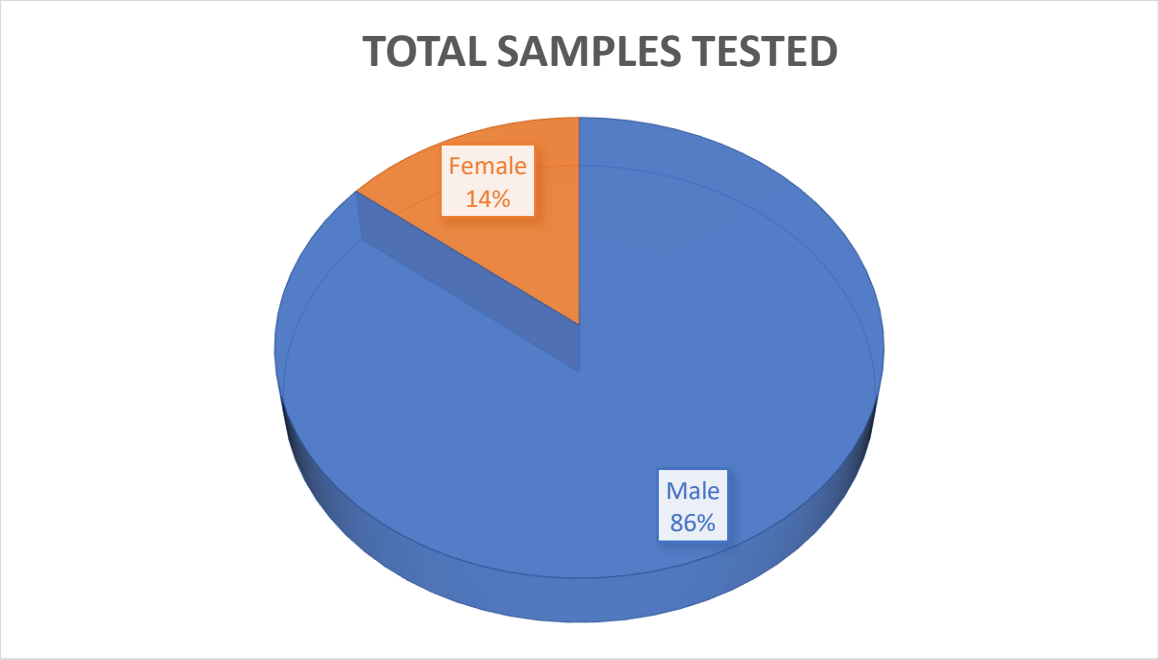
Statistical Methods: Data were checked for completeness, errors and inconsistencies prior to the data entry and were entered into raw data-base sheet (Microsoft Office Excel). Statistical testing was conducted with the statistical package for the social science system version SPSS 20.0. The diagnostic test performance of ZN smear microscopy was evaluated taking GeneXpert MTB/RIF as the reference investigation. A 2 x 2 contingency table was used for comparison. Frequencies were determined for true positives (TP), true negatives (TN), false positives (FP), and false negatives (FN). Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), positive likelihood ratio (LR+), negative likelihood ratio (LR-), and accuracy were calculated using the standard formulae. Receiver operating characteristic (ROC) curve analysis was performed and the area under curve (AUC) was determined.

Result

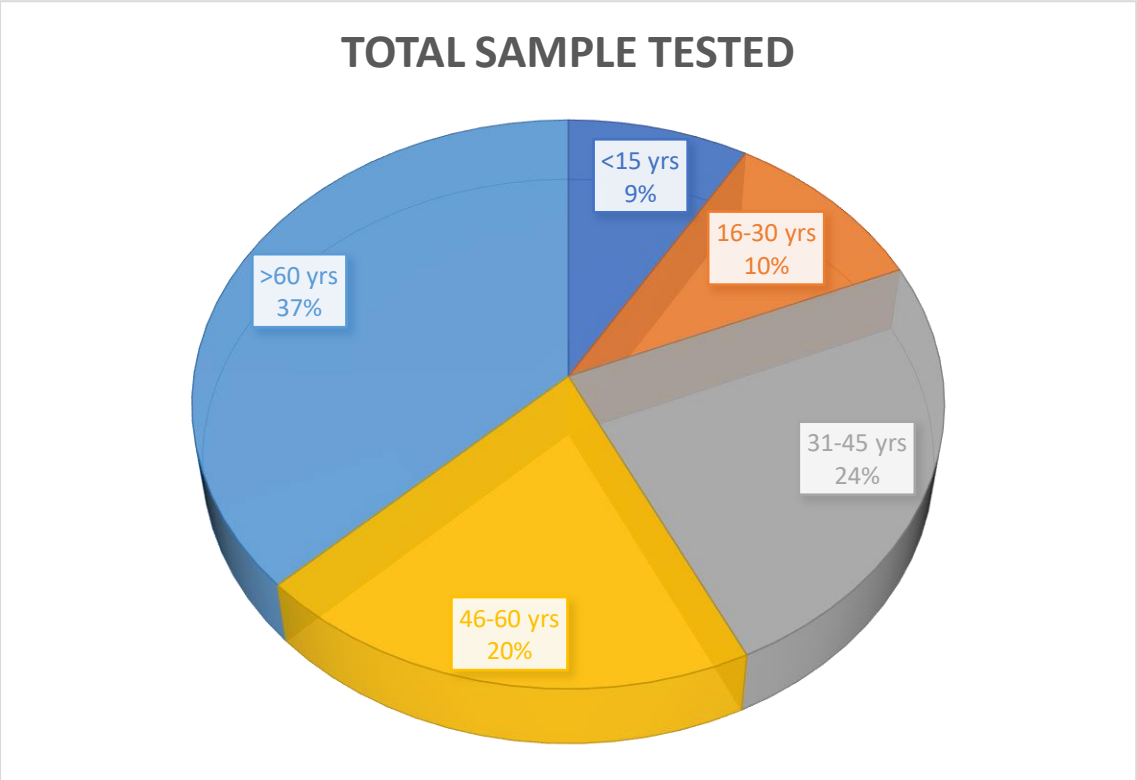
In the present study, a total of 70 patients were included in study, in which 60(86%) were male and 10(14%) were female (Graph 1). A total of 65(92.9%) patients sputum samples detected MTB DNA by GeneXpert MTB/RIF and 5(7.1%) samples were negative(Table 1). In these 65 positive patients, 55(85%) were male and 10(15%) were female (Graph 3). Older age group was most affected 24(37%) and children and young adults were least affected as only 6(9%) patients in each age group . (Graph 4).

A total of 50(71%) sputum samples were positive and 20(29%) were negative for AFB by ZN smear microscopy (Table 1). The sensitivity, specificity, positive predictive value, negative predictive value of sputum ZN smear microscopy were 76.9%, 100%,100%, 25% respectively (Table 2) taking the sputum GeneXpert MTB/RIF as reference test. Table 1 summarises the GeneXpert MTB/RIF and ZN smear microscopy test results.

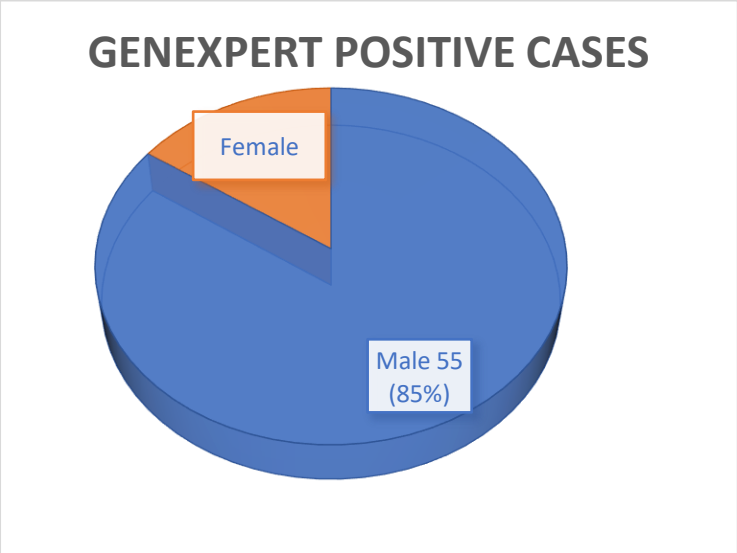
Graph 1 - Gender wise distribution of total samples tested



Graph 2 – Age wise distribution of total sample tested



Graph 3 - Gender wise distribution of GeneXpert positive cases



Graph 4 – Age wise distribution of GeneXpert positive cases

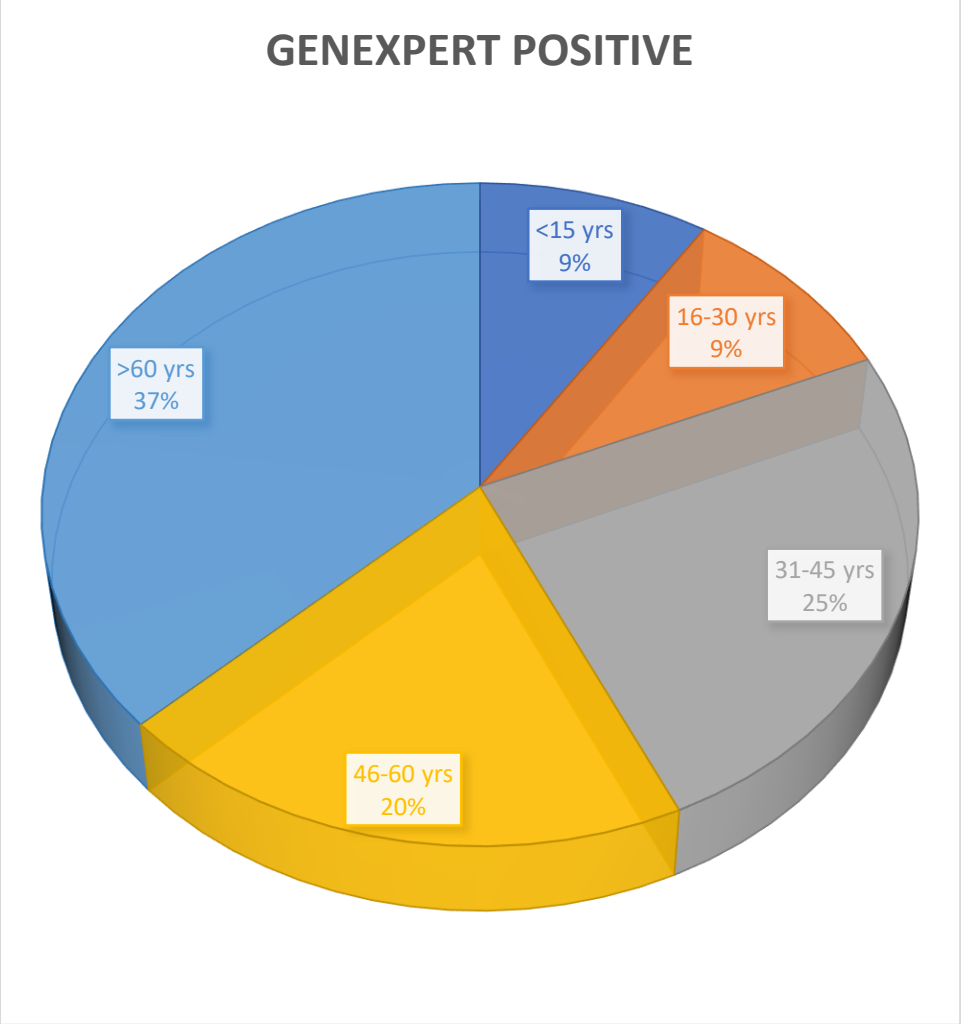


Table 1 – Summary of GeneXpert and ZN smear microscopy test results

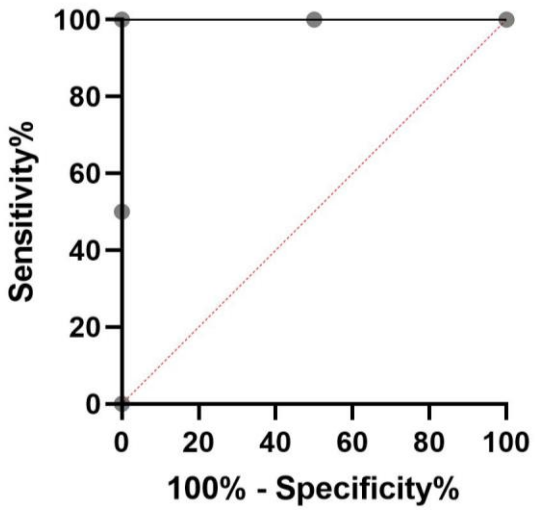
Summary of test results			
GeneXpert			
Quantification			
Positive	**65**	**Very low**	**12**
		Low	**20**
		Medium	**19**
		High	**11**
		Not available	**3**
Negative	**5**		
ZN Smear microscopy			
Quantification			
		Scanty	**13**

Positive	**50**	**1+**	**12**
		2+	**14**
		3+	**11**
Negative	**20**		

Table 2 Diagnostic accuracy of ZN staining taking GeneXpert as the reference test

Gene Xpert		
	Positive	Negative
ZN smear microscopy		
Positive	50 (TP)	0 (FP)
Negative	15 (FN)	5 (TN)
Measures of Diagnostic Accuracy of ZN Smear Microscopy		
Sensitivity		76.9%
Specificity		100%
Positive predictive value		100%
Negative predictive value		25.00%
False negative		23.08%
True negative		100.00%
Accuracy		78.57%
Positive likelihood Ratio		Infinite
Negative likelihood Ratio		0.23
AUC		1.00

FIGURE 1: ROC curve for ZN smear microscopy taking GeneXpert as the reference test



ROC: receiver operating characteristic; ZN: Ziehl-Neelsen

DISCUSSION

Tuberculosis is a major public health problem[1]. Mycobacterial culture is not recommended as an initial diagnostic investigation. However, mycobacterial culture is essential for the management of drug-resistant tuberculosis [5]. Sputum Smear Microscopy (SSM) continues to be a major method of TB diagnosis in primary health care settings[8]. This study was undertaken to determine the accuracy of sputum ZN smear microscopy in diagnosing pulmonary tuberculosis as compared with the sputum GeneXpert MTB/RIF assay as a reference test.

In our study, male patients (85%) were more affected then female patients (15%). This is similar to study conducted by Manan Bedi et al (2021) [12], Dhanaraj et al (2015) [13], and Pratibha Narang et al (2015) [14]. This gender difference possibly due to alcohol abuse, smoking, other comorbidies like lung cancer ,COPD is more in male patients compared to female patients and also may have resulted from inherent social bias, in which female patients are less likely to be visiting hospitals as compared to male patients.

In the present study, older age group was most affected 24(37%) and children and young adults were least affected as only 6(9%) patients in each age group were GeneXpert MTB/RIF positive case. This is similar to study conducted by Manan Bedi et al (2021) [12] and Masab Umair et al [15]. The older age group are more affected, may be due the reactivation of TB in the presence of a weaker immune system, delayed diagnosis and increased co-morbid conditions in this age group.

In our study, sputum Z N smear microscopy had moderate sensitivity but high specificity, positive-predictive value, and negative-predictive value taking the sputum GeneXpert MTB/RIF assay as the reference test. Masab Umair et al [15], Mavenyengwa et al.,[16] reported slightly lower sensitivity but comparable specificity, positive-predictive value, and negative-predictive value of ZN smear microscopy. The positive likelihood ratio for ZN smear microscopy was infinite in our study, suggesting the inevitable presence of pulmonary TB if the test result is positive. However, the negative likelihood ratio was 0.23; negative ZN smear microscopy reduces but does not completely eliminate the probability of the presence of pulmonary tuberculosis.

ROC curve analysis of sputum ZN smear microscopy revealed a straight diagonal line closer to the left upper corner, suggesting its ability to discriminate diseased

(sputum GeneXpert positive pulmonary TB cases) from non-diseased (sputum GeneXpert negative cases). Masab Umair et al [15], Mavenyengwa et al [16], reported a similar ROC curve but with marginally lower AUC. Sputum ZN smear microscopy missed 15 (16.4%) cases when compared with the sputum GeneXpert MTB/RIF test in our study. Other authors also had similar findings. An explanation for the lower sensitivity of AFB smear microscopy is the higher detection threshold in comparison with the GeneXpert MTB/RIF assay, i.e. 5,000 bacilli/ml vs 136 bacilli/ml of the specimen, respectively.

A missed diagnosis of pulmonary tuberculosis prolongs morbidity in the affected individual. Although smear-negative cases of pulmonary TB are considered less infectious than smear-positive cases, smear-negative cases can still transmit the disease. It has been estimated that 10%-20% of TB transmission is from smear-negative cases of pulmonary TB [17]. Furthermore, it has been estimated that the average patient cost of tuberculosis is $847, including medical expenses (20%), non-medical expenses (20%), and loss of income (60%) in low and middle-income countries [18]. On average, this cost approximates 58% of the annual individual income. Therefore, a missed diagnosis of smear-negative pulmonary TB can have significant financial implications for the individuals, families, and the country as a whole.

The GeneXpert MTB/RIF assay is a sensitive and specific test for the diagnosis of smear-negative culture-positive pulmonary tuberculosis. Although the GeneXpert MTB/RIF assay is more expensive than ZN smear microscopy, we still recommend GeneXpert testing where available to avoid prolonged morbidity in the affected individuals and to avoid the direct and indirect costs of missed diagnosis of smear-negative pulmonary TB.

Conclusions

Sputum ZN smear microscopy is a highly specific but moderately sensitive test for the diagnosis of pulmonary tuberculosis. This study recommends the sputum GeneXpert MTB/RIF test in preference to sputum ZN smear microscopy for the diagnosis of pulmonary tuberculosis to avoid a missed diagnosis of smear-negative pulmonary tuberculosis.

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