ORIGINAL ARTICLE

Evaluation of Fluorescent Microscopy and GeneXpert MTB/RIF Assay for The Detection of Mycobacterium Tuberculosis Complex in Respiratory Specimen of Patients with ZN Smear Negative Pulmonary Tuberculosis

Ahmed Khan Tareen[1], Shereen Khan[2], Abdul Rauf[3], Muhammad Naeem[4], Muhammad Umer[5]Muhammad Ali Khan[6], Amin Fahim[7]

ABSTRACT

Objective: To evaluate the diagnostic validity of the GeneXpert for quick diagnosis of TB and recognition of Rifampin resistance in Ziehl–Neelsen smear-negative Broncho-alveolar lavage and sputum specimens obtained from suspected TB patients, keeping LJ culture as "Gold Standard".

Study Design: Cross sectional study.

Place and Duration of Study: The study was conducted at Fatima Jinnah General and Chest Hospital (FJGCH), Quetta from January 2018 to December 2018.

Materials and Methods: One hundred ZN-smear negative pulmonary specimens (63 BAL and 37 sputum) were collected from suspected TB patients (34% males and 66% females; mean age 52.8±18) visiting FJGCH, Quetta. The isolates were processed for fluorescent microscopy, LJ culture and GeneXpert according to standard protocol. Efficacy of these diagnostic tests for the detection of MTB was evaluated comparatively.

Results: Out of 100 ZN smear-negative specimens; MTB was detected by FM in 18 (18%) samples while LJ culture detected MTB in 59 (59%) and GeneXpert in 55 (55%) samples.

Conclusion: We concluded that GeneXpert is an innovative assay for prompt detection of MTB in smear-negative cases having higher sensitivity and specificity with additional advantage of drug resistance detection and turnaround time of two hours. The assay facilitates early diagnosis and appropriate management of TB to minimize morbidity and mortality.

Key Words: *Fluorescent Microscopy, GeneXpert MTB/RIF Assay, Mycobacterium Tuberculosis, Respiratory Specimen, ZN Smear Negative.*

Introduction

Tuberculosis (TB) is a transmittable chronic disease and one of the leading causes of mortality worldwide. It is caused by *Mycobacterium tuberculosis* (MTB) complex which was first identified by Robert Koch in 1882.[1,2] TB mostly affects the human pulmonary system, known as pulmonary tuberculosis, however it also affects bones, joints, lymph nodes, meninges, brain, and kidneys. Usually, the sign and symptoms of TB are fever, night sweats, chill, fatigue, appetite, and weight loss.[3] TB is a treatable disease but still has higher mortality rates. World Health Organization (WHO) estimates that one third population of the worlds is infected with TB, however; only small percentage (5-10%) will convert to active TB. while remaining cases will have latent TB infection and will remain asymptomatic, but they can also get active TB disease if immune systems weaken at any stage.[4]

TB is a major public health problem in Pakistan and accounts for 300,000–500,000 cases which results in 50000 deaths annually. There is no proper disease surveillance program in Pakistan. Therefore, it is very difficult to estimate the exact incidence, prevalence, morbidity, and mortality of TB cases in Pakistan.[5,6] Based on different scarce surveys that have been conducted in various parts of the country, 518,000 TB cases are estimated to occur each year, with about 15,000 cases of multi drug resistant-TB patients.[7] Currently, there is very limited empirical data

[1,2,3,5]*Department of Pathology*
Fatima Jinnah General and Chest Hospital, Quetta
[4,6]*Center for Advanced Studies in Vaccinology and Biotechnology*
University of Baluchistan, Quetta.
[7]*Department of Pathology,*
Isra University Hyderabad

Correspondence:
Prof. Dr. Amin Fahim
Department of Pathology
Isra University Hyderabad
E-mail: draminfahim@gmail.com

Funding Source: NIL; Conflict of Interest: NIL
Received: February24, 2021; Revised: November08, 2021
Accepted: November09, 2021

available on the prevalence of TB in Pakistan.

The WHO recommends that close contacts of TB patients and other high-risk individuals should be screened out. This will help to identify the TB patients in proper and earlier time to start the needful management strategies. It is further recommended that screening should be started with proper history and clinical examination and later chest X-ray and other investigations may be carried out like sputum smear and molecular techniques using GeneExpert MTB/RIF. The findings may be confirmed by culture and sensitivity. Although the sensitivity and specificity of X-ray chest and sputum smears are low whereas culture is considered as reference standard.[8,9]

Quetta, the capital of Balochistan, is a major metropolitan city; the population belongs to several tribes and castes having different economic backgrounds. Many Afghan refugees are also residing in Quetta. The present study was designed to have samples of various ethnic groups, different tribes, and various socioeconomic backgrounds. The aim of the study was to evaluate the diagnostic validity of the GeneXpert for quick diagnosis of TB and recognition of Rifampin (RIF) resistance in Ziehl–Neelsen (ZN) smear-negative Broncho-alveolar lavage (BAL) and sputum specimens obtained from suspected TB patients, keeping LJ culture as "Gold Standard".

Materials and Methods

This cross-sectional study was conducted from January 2018 to December 2018 at Fatima Jinnah General and Chest Hospital (FJGCH), Quetta. The Broncho Alveolar Lavage (BAL) and sputum samples of one hundred (100) TB suspected patients, who were negative for the AFB on Ziehl-Neelsen (ZN) staining, were selected, based on their clinical evaluation through nonprobability convenient sampling. After taking approval from Institutional Review Board (IRB) Ref: FLS&I/BUITEMS: 249/17 dated: August 20, 2017; informed consent was taken from all the study participants. Sensitivity and specificity were confirmed using Fluorescent Microscopy and GeneXpert (MTB/RIF Assay). Sputum or Broncho Alveolar Lavage (BAL) specimens of these TB suspects against the gold standard mycobacterial culture (Lowenstein-*Jensen* Media) were carried out.[10]

All the necessary steps of the study; including samples collection, specimens processing; Zeihl-Neelsen and fluorescent stain, microscopic study, culture and GeneXpert assay were performed at the Hi-tech laboratory in FJGCH, Quetta, Pakistan. Suspects of all age groups with ZN smear-negative results and presumptive TB symptoms including chest pain, chronic coughing, fever, chills, fatigue, loss of appetite and weight, night sweats, chest X-ray abnormalities showing infiltrates and cavities or only chronic coughing for more than two weeks with or without other symptoms were recruited in the study. The patients with ZN smear positive results and those who had started anti-tuberculosis drugs were not included in study.

Various parameters viz; age, sex, signs and symptoms, body mass index (BMI), history of contact with TB patient(s) and use of anti-TB drugs (i.e., relapse, defaulter, failure, treatment completed and cured) were considered, in a standardized pre-designed questionnaire, during data collection. For the sample processing Class-II Biological Safety Cabinet (BSC) was used, moreover-acetyl-L-cysteine (NALC)-NaOH solution was used for the digestion and decontamination of the samples which was followed by concentrating the samples by spinning using the standard protocol recommended by Kent andKubica.[11] The data was subjected to analysis by SPSS version 21.0. Statistics including sensitivity, specificity, negative predictive value (NPV), positive predictive value (PPV) of GeneXpert/RIF was calculated. P value of <0.05 was taken as significant.

Results

Out of 100 ZN smear-negative clinical specimens examined for the pulmonary TB diagnostic analysis, Broncho Alveolar Lavage (BAL) and sputum specimens were 63% and 37%, respectively. The mean age of the clinically suspected TB patients was 52.8±18 years (ranging from 15 to 86 years) while the mean Body Mass Index (BMI) was 22.1±4.9 (ranging from 11 to 37). The study population included 34 (34%) males and 66 (66%) females (sex ratio 1:1.94).

Among the hundred ZN smear negative cases of TB, almost all the clinically suspected TB cases had history of cough (90%), most of the cases complained of fever (75%) and weight loss (73%), followed by night sweat (50%), and hemoptysis (21%). About 17% of the suspected cases had the history of TB contact.

Detection of tuberculosis by using three different techniques based on specimen type in ZN smear negative TB cases revealed that with fluorescence microscopy 12(19%) out of 63 cases were positive in BAL specimen whereas 6(16.2%) out of 37 cases were positive in sputum specimens (Figure-1).

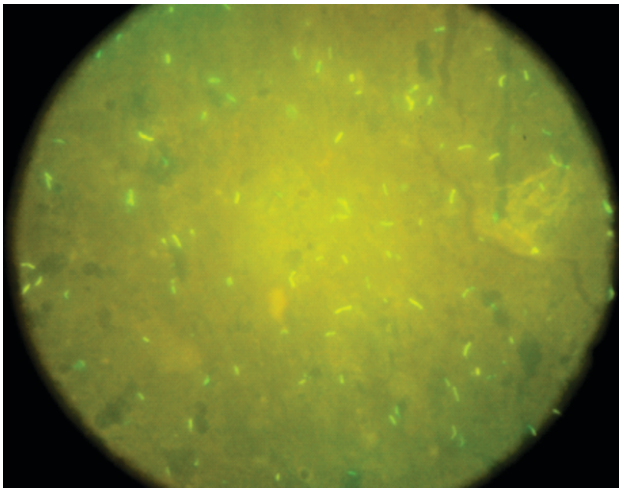

Fig.1: Fluorescent Microscopy Indicating Fluorescing AFB

The findings on GeneXpert showed 42(67%) out of 63 cases were positive in BAL specimen whereas 13(35%) out of 37 cases were positive in sputum specimens (Figure-2).

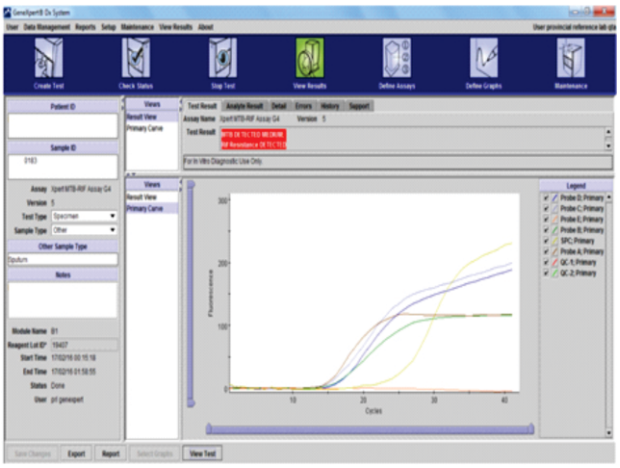

Fig. 2: Real Time PCR Curve Indicating Positive Results for The SPC Along With A, B, C And D Probes Whereas, Negative Result is shown for the Probe E. Results for Detected Rifampin Resistance and Genexpert Mycobacterium Tuberculosis/Rifampin Assay

The results of LJ culture showed 43(68%) out of 63 cases were positive in BAL specimen whereas 16(43%) out of 37 cases were positive in sputum specimens (Figure-3).

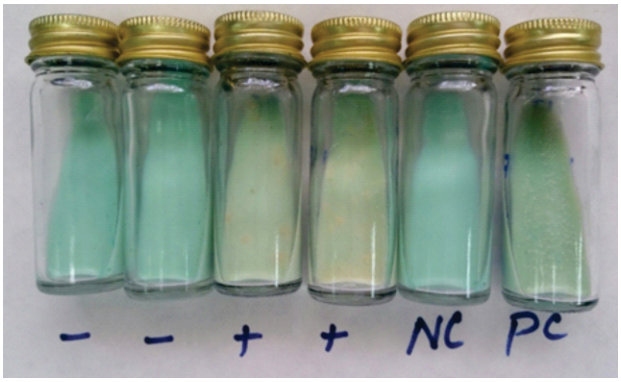

Fig. 3: Mycobacterium Growth on LJ Culture Medium. From Left to Right 1st and 2nd Slants are Negative for MTB, 3rd and 4th Slants are Positive for MTB, 5th Slant is Negative Control (NC) and 6th Slant is Positive Control (PC) for MTB

One of the most routinely used techniques in laboratory for the diagnosis of TB is FM. But FM detected the least number of positive cases 18% (18/100) in ZN smear-negative samples in contrast with mycobacterial culture with the detection rate of 59(59%) out of 100 cases. Among eighty-two FM auramine-O negative samples, half of the samples 41(50%) were found to be positive by LJ culture (Table-I). Therefore, chi-square test indicated a statistically significant difference (χ^2= 15.25, df=1, p<0.001) between FM auramine-O staining and LJ culture.

Table I: Comparison of Fluorescent Microscopy (FM) and *GeneXpert MTB/RIF assay* with LJ culture for detection of TB

Fluorescent Microscopy (FM)	LJ Culture		Total
	+ve	–ve	
FM +ve	18	0	18
FM –ve	41	41	82
Total	59	41	100
GeneXpert +ve	52	3	55
GeneXpert –ve	7	38	45
Total	59	41	100

LJ culture yielded maximum number of positive cases (59%) for the presence of AFB in BAL and sputum samples. On the other hand, GeneXpert detected 55% of the suspected cases. Among 45 GeneXpert-negative samples, 7(15.5%) samples were also found to be positive for AFB by LJ culture indicating that culture is more sensitive than GeneXpert. Chi-square test revealed a highly significant difference (χ^2= 63.84, df=1, p<0.001) in the detection rate of MTB between GeneXpert assay

and LJ culture (Table-I).

Table-II depicts the diagnostic validity of FM for the detection of MTB in the Broncho alveolar and sputum samples while considering culture as a gold standard diagnostic technique. In this study the overall sensitivity and specificity of FM using ZN smear-negative samples for the detection of AFB were found to be 30.5% and 100%, respectively, whereas 100% PPV and 50% NPV were observed for total samples.

Table II: Diagnostic Validity for detection of TB by Fluorescent Microscopy

Specimen Type	Technique		LJ Culture		Total	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
			+ve	-ve					
-ve Total	31	20	51			37.5%	100%	100 %	39.2%
	43	20	63					100 %	67.7%
+ve			6	0	6				
-ve Total	10	21	31			30.5%	100%	100 %	50%
	16	21	37						
+ve			18	0	18				
Combine	F M								
		-ve	41	41	82				
Total			59	41	100				

BAL= Bronchoalveolar lavage
FM= Fluorescent Microscopy
PPV= positive predictive value
NPV= Negative predictive value

Table-III represents the validity of GeneXpert for the detection of *M. tuberculosis* in the bronchoalveolar and sputum samples while considering culture as the "reference standard" diagnostic technique. The overall sensitivity and specificity of the GeneXpert assay for the MTB detection using ZN-smear negative samples in this study were found to be 88.1% and 92.7%, respectively, whereas the positive predictive value (PPV) and the negative predictive value (NPV) were observed 94.5% and 84.4%, respectively for total samples.

Table III: Diagnostic Validity of GeneXpert MTB/RIF for Detection of TB from ZN Smear-Negative Samples

Specimen type	Technique		LJ Culture		Total	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
			+ve	-ve					
BAL	GX	+ve	40	2	42	93.0%	90.0%	95.2%	85.7%
		-ve	3	18	21				
Total			43	20	63				
Sputum	GX	+ve	12	1	13	75.0%	95.2%	92.3%	83.3%
		-ve	4	20	24				
Total			16	21	37				
Combine	GX	+ve	52	3	55	88.1%	92.7%	94.5%	84.4%
		-ve	7	38	45				
Total			59	41	100				

BAL= Bronchoalveolar lavage
GX= GeneXpert
PPV= positive predictive value
NPV= Negative predictive value

Discussion

It was observed in the present study that the infection rate of TB was higher in females (62%) than males (53%). These results are in agreement with those reported by Shafee *et al.,* 2014.[12] However some other studies have reported that the prevalence of TB is more in males than females.[13] Present study showed 67% infected people between the age of 15-35 years, these results remained in close agreement with a descriptive study from Nairobi in which infection rate of 66.7% was reported with the age between 18-34 years.[13] Whereas a study from Pakistan in 2015; demonstrated 62% of the patients were in the age of 21-50 years.[14] However, this study found no significant association of age with TB infection (p>0.05).

Statistically, results of the present study were non-significant (p>0.05) for BMI with increased risk of TB. However, patients with a high BMI had the highest risk of TB infection followed by normal BMI as compared with low BMI. These findings were not in agreement with the results reported by another researcher that a low BMI are more at risk of TB than the higher BMI.[15]

In the present study, 55% of the ZN smear-negative TB cases were GeneXpert positive, 59% culture positive and 18% were FM smear positive. Out of 59% culture positive samples, 18% were FM smear positive and 41% were FM smear negative cases. The result of our study is almost comparable with the study from Pakistan by Iram *et al.* (2015)[16] in which MTB was detected in 49.8% pulmonary TB suspects by GeneXpert MTB/RIF test, 47.8% by culture. However unlike to our result; comparatively higher smear positivity of 40% was observed. Our finding is lower as compared with a study by Munir et al. (2015)[14] who reported 67.5% ZN smear positivity for MTB and 77.4% GeneXpert positivity.

Diagnosis of TB by smear microscopy is the mainly used apparatus in low and middle-income countries, despite of its limitations.[17] The sensitivity of fluorescent smear microscopy is higher in contrast with ZN smear microscopy however it is costly and requires a dark functioning place. Smear microscopy

for the diagnosis of TB from clinical samples has a lower sensitivity than the LJ culture because a smear requires a high bacterial load (105/ml) to be positive.[18]

In the current study the MTB was detected in 18% samples by FM that were negative on ZN staining indicating higher detection rate of FM than ZN microscopy. Similar findings were reported in studies by Ahmed S et al (2019)[19] who observed that FM has higher levels of sensitivity as compared with ZN staining for the detection of AFB in the clinical samples.

Culture using Lowenstein–Jensen medium is regarded as the "Gold standard" method in the developing countries and has high sensitivity for the detection of acid-fast MTB in the clinical samples. Overall, the highest detection rate of 59% by culture in ZN smear negative cases was found in present study proves its validity and accuracy as the reliable diagnostic method in the laboratory but it takes four to eight weeks to produce result. This finding is higher than 10.3% culture positivity in smear-negative pulmonary TB cases reported in Ethiopia by Tadesse et al. (2016)[20] and 47.8% in Pakistan by Iram et al. (2015)[16] detected in pulmonary TB cases. This high prevalence in our study is due to the inclusion of clinically significant and highly suspicious TB cases in the present settings. BACTEC and MGIT are the rapid culture methods which have greatly shortened the discovery time to 7-10 days. However the operating cost and equipment's are greatly high, continuous monitoring for numerous days, more skilled personals, and further proof of positive cultures by smear microscopy are required. [21]

MTB was detected in 55% of the pulmonary TB cases in the current study by GeneXpert MTB/RIF which is comparable to another study from Pakistan with the GeneXpert positivity of 49.8% (Iram *et al.,* 2015)[16]. In this study, GeneXpert MTB/RIF test detected all the ZN and FM smear-positive, 88.1% (52/59) culture-positive cases and 7.3% culture-negative samples from clinical pulmonary TB cases. Whereas 11.8% culture positive cases appeared negative on GeneXpert.

Using LJ culture as the reference standard, the sensitivity and specificity of GeneXpert were 88.1% and 92.7% respectively, for detection of MTB in smear-negative respiratory specimens for diagnosis of TB which is in line with the results published by Reechaipichitkul et al. (2016)[22] reporting 83.9% sensitivity and 92.1% specificity of GeneXpert assay. Compared to our study, a lower sensitivity 68.6% on GeneXpert has been observed by Zeka et al. (2011).[23] In a study by Pinyopornpanish et al. (2015)[24] the sensitivity of GeneXpert assay was observed 95.3%, specificity 86.4%, PPV 82% and NPV 96.6%.

Conclusion

We conclude from the current study that GeneXpert MTB/RIF assay was an effective and reliable technique to diagnose pulmonary TB from smear negative specimens, with more sensitivity and excellent specificity. GeneXpert has a great diagnostic value for AFB detection in smear-negative cases because it has outperformed smear microscopy as revealed in the current study. Smear-negative patients can be more benefited from GeneXpert technique especially in those areas where culture is not applicable. Prevention from fatal TB disease is essential to enhance the discovery of *Mycobacterium Tuberculosis Bacterium* (MTB) using individual or mutual laboratory techniques that in turn could avoid huge economic loss. However, since the study participants of one tertiary care institute were evaluated; more multicenter studies are recommended.

REFERENCES

1. Puchner KP, Rodriguez-Fernandez R, Oliver M, Solomos Z. Non-communicable diseases, and tuberculosis: Anticipating the impending global storm. Global public health. 2019;14(9):1372-81.
2. Martini M, Besozzi G, Barberis I. The never-ending story of the fight against tuberculosis: from Koch's bacillus to global control programs. Journal of Preventive Medicine and Hygiene. 2018;59(3): E241.
3. Shapiro AE, Ross JM, Schiller I, Kohli M, Dendukuri N, Steingart KR, Horne DJ. Xpert MTB/RIF and Xpert Ultra assays for pulmonary tuberculosis and rifampicin resistance in adults irrespective of signs or symptoms of pulmonary tuberculosis. Cochrane Database of Systematic Reviews. 2020(7).
4. Cohen A, Mathiasen VD, Schön T, Wejse C. The global prevalence of latent tuberculosis: a systematic review and meta-analysis. European Respiratory Journal. 2019;54(3):1900655.
5. Shaikh MA, Malik NA. Spatial cluster analysis of new and relapsed cases of pulmonary tuberculosis by district: pakistan 2015. Journal of Ayub Medical College Abbottabad. 2019;31(2):293-5.
6. Atif M, Bashir A, Ahmad N, Fatima RK, Saba S, Scahill S.

Predictors of unsuccessful interim treatment outcomes of multidrug resistant tuberculosis patients. BMC infectious diseases. 2017;17(1):655.

7. Jabbar A, Phelan JE, de Sessions PF, Khan TA, Rahman H, Khan SN, et al. Whole genome sequencing of drug resistant Mycobacterium tuberculosis isolates from a high burden tuberculosis region of Northwest Pakistan. Scientific reports. 2019 Oct 18;9(1):1-9.
8. Acharya B, Acharya A, Gautam S, Ghimire SP, Mishra G, Parajuli N, et al. Advances in diagnosis of Tuberculosis: an update into molecular diagnosis of Mycobacterium tuberculosis. Molecular biology reports. 2020;47(5):4065-75.
9. Saktiawati AM, Putera DD, Setyawan A, Mahendradhata Y, van der Werf TS. Diagnosis of tuberculosis through breath test: a systematic review. EBioMedicine. 2019; 46:202-14.
10. Liskina IV, Zahaba LM, Kuzovkova SD. Comparative analysis of m. Tuberculosis detection by different histological methods in lung tissue with pulmonary tuberculoma with different activity of specific inflammation. Medical Science of Ukraine (MSU). 2017 Nov 30;13(1-2):26-32.
11. Kent PT, and Kubia GP. Public Health Mycobacteriology: a guide for the level III laboratory. US Department of Health and Human Services, Public Health Service, *Centers for Disease Control*, Atlanta, GA. 1985.
12. Shafee M, Abbas F, Ashraf M, Mengal MA, Kakar N, Ahmad Z, and Ali F. Hematological profile and risk factors associated with pulmonary tuberculosis patients in Quetta, Pakistan. *Pak J Med Sci. 2014;* 30: 36-40.
13. Ndungu PW, Revathi G, Kariuki S and Ng'ang'a Z. Risk Factors in the Transmission of Tuberculosis in Nairobi: A Descriptive Epidemiological Study. *Adv Microbiol. 2013;* 3:160–165.
14. Munir MK, Rehman S, Aasim M, Iqbal R, and Saeed S. Comparison of Neelsen Microscopy with GeneXpert for Detection of MycobacteriumTuberculosis. *IOSR J Dent Med Sci. 2015;* 14(11):56-60.
15. Jurcev-Savicevic A, Mulic R, Ban B, Kozul K, Bacun-Ivcek L, Valic J, et al. Risk factors for pulmonary tuberculosis in Croatia: a matched case–control study. *BMC Public Health.* 2013; 13: 991.
16. Iram S, Zeenat A, Hussain S, Wasim Yusuf N, Aslam M. Rapid diagnosis of tuberculosis using Xpert MTB/RIF assay - Report from a developing country. Pak J Med Sci. 2015 Jan-Feb;31(1):105-10. doi: 10.12669/pjms.311.6970. PMID: 25878624; PMCID: PMC4386167.
17. World Health Organization.Global Tuberculosis Report. 2015. WHO/HTM/TB/2015.
18. Lawn SD, and Nicol MP. Xpert MTB/RIF assay: development, evaluation, and implementation of a new rapid molecular diagnostic for tuberculosis and rifampicin resistance. *FutMicrobiol. 2011;* 6(9): 1067-1082.
19. Ahmed S, Shukla I, Fatima N, Varshney SK, Shameem M, Tayyaba U. Light-emitting diode-fluorescent microscopy: Determining its sensitivity and specificity in diagnosis of pulmonary tuberculosis in a high-burden tuberculosis region and resource-limited country like India. CHRISMED Journal of Health and Research. 2019 Jan 1;6(1):44.
20. Tadesse M, Aragaw D, Rigouts L, and AbebeG. Increased detection of smear-negative pulmonary tuberculosis by GeneXpert MTB/RIF_assay after bleach concentration. *Int J Mycobacteriol. 2016;* 5(2), 211–218.
21. Chen Y, Zhang L, Hong L, Luo X, Chen J, Tang L, Chen J, Liu X, Chen Z. Rapid diagnosis of pulmonary tuberculosis and detection of drug resistance by combined simultaneous amplification testing and reverse dot blot. Journal of clinical pathology. 2018 Jun 1;71(6):498-503.
22. Reechaipichitkul W, Phetsuriyawong A, Chaimanee P, and Ananta P. Diagnostic test of sputum GeneXpert MTB/RIF for smear negative pulmonary tuberculosis.*Southeast Asian J Trop Med Public Health*. 2016; 47(3): 457-466.
23. Zeka AN, Tasbakan S, and Cavusoglu C. Evaluation of the GeneXpert MTB/RIF Assay for Rapid Diagnosis of Tuberculosis and Detection of Rifampin Resistance in Pulmonary and Extrapulmonary Specimens. *J Clin Microbiol 2011;*49(12): 4138–4141.
24. Pinyopornpanish K, Chaiwarith R, Pantip C, Keawvichit R, Wongworapat K, Khamnoi P, et al. Comparison of Xpert MTB/RIF Assay and the Conventional Sputum Microscopy in Detecting *Mycobacterium tuberculosis* in Northern.

CONFLICT OF INTEREST

Authors declared no conflicts of Interest.

GRANT SUPPORT AND FINANCIAL DISCLOSURE

Authors have declared no specific grant for this research from any funding agency in public, commercial or nonprofit sector.

DATA SHARING STATMENT

The data that support the findings of this study are available from the corresponding author upon request.

This is an Open Access article distributed under the terms of the Creative Commons Attribution- Non-Commercial 2.0 Generic License.