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Comparison of GeneXpert MTB/RIF assay and LED-FM microscopy for the diagnosis of extra pulmonary tuberculosis in Khyber Pakhtunkhwa, Pakistan



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ABSTRACT

GeneXpert is one of the recent technological instruments used to diagnose tuberculosis in a short span of time. In this study, the performance of GeneXpert MTB/RIF assay for the diagnosis of extrapulmonary tuberculosis (EPTB) was compared with light-emitting diode Fluorescent Microscopy (LED-FM) in Khyber Pakhtunkhwa, Pakistan. A total of 737 EPTB samples were collected from tuberculosis (TB) suspected patients. Out of these samples, male to female ratio was 53% ($n = 390$) to 47% ($n = 347$) respectively. The sensitivity and specificity was 73% and 100% for GeneXpert, while 40% and 100% for LED-FM microscopy. This shows that the sensitivity of GeneXpert is 40–50%, higher than LED-FM microscopy. GeneXpert also detected low number of bacilli as compared to LED-FM microscopy.

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Introduction

Mycobacterium tuberculosis complex (MTBC) is the causative agent of tuberculosis (TB). TB is the second leading cause of death after HIV worldwide. It is estimated that 9 million people develop TB and among them 1.5 million die each year out of which 360,000 people are HIV positive.¹ In Pakistan 350 cases of TB per 100,000 population are reported. Pakistan is ranked 5th among 22 high burden TB countries.² MTBC mainly infect lungs (pulmonary) and seldomly affects other parts of the body known as extra pulmonary tuberculosis (EPTB).^{3,4} Worldwide, 10% of extrapulmonary cases are reported every year.⁵ More than 50% of TB cases are reported with HIV co-infection.⁶ The reason for increased EPTB incidences in Pakistan is still vague due to lack of proper diagnosis. Microscopic observation of *M. tuberculosis* in sputum smears still remains the pillar of tuberculosis (TB) diagnosis in developing countries, despite its poor sensitivity.⁷ Fluorescence microscopy (FM) of smears have been studied as an alternative to conventional light microscopy with Ziehl Neelsen staining (ZN).⁸ On the basis of these findings, World Health Organization in 2011 replaced conventional FM by LED-FM and phase in LED-FM as an alternative to ZN microscopy.⁹

There are a number of tests available for the diagnosis of tuberculosis each having their own limitations. Conventional microscopy has low sensitivity and culture requires longer time for positivity. The commercially available automated, liquid MGIT (*Mycobacterium Growth Indicator tube*) culture system is time-consuming and requires specialized laboratories. On the other side, nucleic acid amplification techniques not only provide the advantage of rapidity of diagnosis but also detect even low genomic copies in various specimens and curtail the transmission of the disease.¹⁰

The World Health Organization (WHO) has endorsed the implementation of GeneXpert MTB/RIF assay for national tuberculosis programs in developing countries.¹¹ The Xpert MTB/RIF (Cepheid Inc.) is an automated, user friendly and rapid test based on nested real-time PCR assay and molecular beacon technology for MTB detection and RIF resistance.¹² The results are obtained within a short period of time (2 h). Moreover this technique is not prone to cross-contamination, requires minimal Biosafety facilities and has a high sensitivity in smear-negative pulmonary TB.¹³ The diagnosis of EPTB is often difficult to establish, considering that number of bacteria in specimens is often very low, a collection often requires invasive procedures, and it is not easy to obtain multiple samples. In this scenario GeneXpert is a potentially useful tool for extrapulmonary specimens.¹⁴

The current study aims to investigate the diagnostic accuracy of GeneXpert for EPTB specimens and compared with LED-FM microscopy. The percentage of enhanced case detection rate in EPTB samples was also studied.

Materials and methods

This study was conducted in Provincial TB Reference Laboratory Peshawar Khyber Pakhtunkhwa, from January 2013 to January 2015 in which two different techniques that is

GeneXpert MTB/RIF assay and LED-FM microscopy were compared. The result of both tests was evaluated against reference standard culture method.

Area/patients selection

A total of 737 samples of EPTB on the basis of clinical presentation, radiological finding and histopathological evidence were collected from TB suspects from three tertiary care hospital, 332 samples from Lady Reading Hospital (LRH), 149 and 256 samples from Khyber Teaching Hospital (KTH) and Hayatabad Medical Complex (HMC) respectively. Samples were aspirated from different body parts i.e., $n = 259$ Pleural fluids, $n = 25$ biological alveolar lavage (BAL), $n = 59$ cerebrospinal fluid (CSF), $n = 82$ pus, $n = 125$ ascetic fluid, $n = 13$ synovial fluid, $n = 47$ urine, $n = 56$ pericardial fluids, $n = 36$ bone marrow, $n = 35$ soft tissues.

Sample processing

Culture processing procedure

All samples were subjected to decontamination process according to standard protocol except bone marrow and CSF.^{15,16} Some specimens should not be decontaminated, because either they are sterile source i.e. CSF or decontamination process is a rough method and there is a risk of killing TB bacilli.¹⁷ After digestion and decontamination, samples were diluted with 50 mL sterile phosphate buffer, pH 6.8, to minimize the continuing action of NaOH and to lower the pH and specific gravity of the specimen before centrifugation. Following refrigerated centrifugation, the falcon tubes were kept for 5 min to allow aerosols settle down. Once the supernatant was decanted, 1 mL of buffer was added and vortexed to homogenized the pallets. 200ul of the sample was added to 7 mL MGIT tube supplemented already with PANTA. All the tubes were incubated in MGIT (Bactec 960, BD, USA) semi-automated liquid culture system machine for 42 days at 35–38 °C.

Post MGIT identification

The identification of MTBC was done by two different methods, including Ziehl Nelson staining (ZN) Microscopy and TBC (Becton, Dickinson and Company) device method. Staining using ZN was carried out according to standard published procedure, and slides were examined with bright-field microscopy (Olympus CX21) at 1000× magnification. Tbc is a rapid immuno-chromatographic device which was used to determine clinical isolates to the species level.

GeneXpert MTB/RIF assay and LED-FM microscopy

Smear for LED fluorescent microscopy were prepared according to standard procedures.¹¹ Smears for FM were stained using auramin O. Briefly, smears were flooded with Auramin O for 10 min, destained with acid alcohol for 2 min, and then counterstained with methylene blue for one minute. With auramine O staining, *Mycobacteria* appear as bright yellow fluorescent rods on a dark background. The slides were examined with ZEISS EXTARO 300 at 20× magnification. The presence or absence of AFB was reported using WHO/IUATLD guideline.¹⁸

The sediment samples by cytocentrifugation were processed for Xpert MTB/RIF assay. Using a fresh transfer pipette, 2 mL of the processed sample was transferred to

Table 1 – Sample wise comparison of LED-FM, GeneXpert and culture.

Types	Total sample 2015	2014	Total sample	Total number of processed sample	Positive		
					LED FM	GeneXpert	Culture
Pleural fluid	85	45	129	259	22 (8%)	30 (12%)	52 (20%)
BAL	1	1	23	25	3 (12%)	7 (28%)	8 (32%)
CSF	18	15	26	59	5 (8%)	10 (17%)	12 (20%)
Pus	31	20	31	82	4 (5%)	10 (12%)	12 (15%)
Acsetic fluid	48	20	57	125	8 (6%)	14 (11%)	14 (11%)
Synovail fluid	1	1	11	13	2 (15%)	6 (46%)	7 (54%)
Urine	15	10	22	47	4 (9%)	4 (9%)	7 (15%)
Pericardial fluid	17	10	29	56	2 (4%)	6 (11%)	7 (12%)
Bone marrow	20	15	1	36	0	3 (8%)	6 (17%)
Tissues	16	8	11	35	2 (8%)	5 (14%)	5 (14%)
Total	252	145	340	737	52 (7%)	95 (13%)	130 (18%)
				M = 390	53%		
				F = 347	47%		

the Xpert MTB/RIF cartridge 10. Load the cartridge into the GeneXpert instrument as per manufacturer's instructions according to standard protocol.^{14,19} The MTB/RIF assay interpretation is software based and not user dependent.²⁰ The sensitivity and specificity of each sample were calculated according to the following formula

Sensitivity:

$$\frac{\text{True positive}}{\text{True positive} + \text{False negative}} \times 100$$

Specificity:

$$\frac{\text{True negative}}{\text{True negative} + \text{False positive}} \times 100$$

Results

Our study was the first to show the successful implementation of LED-FM staining and Xpert MTB/RIF services in routine programmatic conditions in Khyber Pakhtunkhwa, Pakistan. A total of 737 samples were processed, 53% ($n=390$) were male and 47% ($n=347$) were female.

Detection of *M. tuberculosis* by Xpert MTB/RIF assay

Total positive on Xpert MTB/RIF assay declared 13% ($n=95$). Table 1 shows results of EPTB for GeneXpert technique. The sensitivity values obtained through GeneXpert ranges from 50 to 100% while Specificity remains high (100%) as shown in Table 2.

Detection of *M. tuberculosis* by culture method

Total positive on culture was 18% ($n=130$) (Table 1). Further, there were no samples found that have culture positive results and GeneXpert and LED-FM negative.

Detection of *M. tuberculosis* by LED fluorescence microscopy

Total positive on LED-FM microscopic technique declared 7% ($n=52$) (Table 1). The lowest sensitivity recorded by LED-FM is 0

for bone marrow while the highest is 57% for ascetic fluid and urine samples. Specificity for LED fluorescence microscopy remains high (100%) as shown in Table 2.

Discussion

In the current investigation, the diagnostic performance for EPTB cases of two different techniques were evaluated. The results were compared with reference standard culture technique. A total of 737 EPTB samples were analyzed with the detection rate of LED-FM, 7% ($n=52$), GeneXpert MTB/RIF assay 13% ($n=95$), while for reference standard culture technique 18% ($n=130$). The sensitivity and specificity for GeneXpert MTB/RIF assay was 73% and 100%, while that of LED-FM microscopy was 40% and 100% respectively. A better detection rate of GeneXpert was reported in a study when compared to smear microscopy and culture.²¹

The results revealed that the detection rate (specificity and sensitivity) of the tested samples for GeneXpert MTB/RIF assay was almost twofold to that of LED-FM microscopy (Table 2). Previous investigations showed that the specificity and sensitivity of GeneXpert MTB/RIF was 79.0% and 97.3% respectively.²² Some other studies showed specificity and sensitivity of 95% and 100% respectively in 340 positive samples.²³ This higher ratio in term of sensitivity in comparison to our results may be due to sample size.

The current conventional techniques, i.e. LED-FM and culture system not only requires the Biosafety Level III (BSLIII) and trained personnel but also take several weeks to yield results. The GeneXpert MTB/RIF is a proven technology in TB diagnosis. The GeneXpert MTB/RIF assay is independent of the user's skills and routine staff with minimal training can use the test. The efficacy of GeneXpert MTB/RIF was proved to be much higher than conventional LED-FM and comparable to that of culture. We observed a sensitivity of 73% and specificity of 100%, which was comparatively higher than the sensitivity observed in a study which consisted 52% of EPTB samples.²⁴ In another published work carried out in EPTB cases, Tortoli et al., 2012.²² reported 86.9% sensitivity and 99.7% specificity by GeneXpert, slightly higher than our results.

Table 2 – Comparison between LED-FM, GeneXpert sensitivity and specificity.

	Total		Pleural fluid		BAL		CSF		Pus		Ascetic fluid		Synovial fluid		Urine		Pericardial Fluid		Bone Marrow		Tissues.		
	GXED-FM	GXED-FM	GXED-FM	GXED-FM	GXED-FM	GXED-FM	GXED-FM	GXED-FM	GXED-FM	GXED-FM	GXED-FM	GXED-FM	GXED-FM	GXED-FM	GXED-FM	GXED-FM	GXED-FM	GXED-FM	GXED-FM	GXED-FM	GXED-FM	GXED-FM	
Sensitivity %	73	40	58	42	88	38	83	42	83	33	100	57	86	29	86	29	57	86	29	50	0	100	40
Specificity %	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
PPV	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
NPV	95	89	90	87	94	77	96	87	98	90	100	95	86	55	98	91	93	93	98	91	83	100	91

PPV, positive predictive value; NPV, negative predictive value; GX, GeneXpert; CSF, cerebrospinal fluid; LED-FM, light emitting diode fluorescent microscopy.

The sensitivity of GeneXpert was 40–50% higher than the LED-FM technique while in a previous study carried by,²⁵ the sensitivity of GeneXpert MTB/RIF assay was 45% higher than microscopy technique. If we compare our study with previous study it depicts same results regarding sensitivity and specificity (Table 2). Armand et al., reported the same results for both the techniques regarding specificity.²⁶ The positivity of EPTB increased from LED-FM to GeneXpert followed by culture gold standard. We found that the implementation of GeneXpert services in TB Diagnostic centers enhance the yield of EPTB patients. It has a shorter turnaround time and simultaneously detects rifampicin (RIF) resistance in less than 3 h. GeneXpert is preferred over the conventional techniques because of its high specificity, sensitivity and with minimal technical expertise required. Although, conventional laboratory techniques as LED-FM smear microscopy for diagnosis of tuberculosis are cost effective but less sensitive as compared to the GeneXpert because the large bacillary load (10^5 /mL) will be required for a smear to become positive.

In conclusion, specificity of GeneXpert is high in majority of EPTB cases with smear-positive non-respiratory samples. In addition, it has a high sensitivity for detecting EPTB samples like ascetic fluid and tissues. These findings support recent WHO guidelines regarding the use of GeneXpert for TB diagnosis from EPTB specimens.

Conflicts of interest

The authors declare no conflicts of interest.

REFERENCES

- World Health Organization. *Global tuberculosis report 2014*. Geneva: World Health Organization; 2015.
- WHO. *Global Tuberculosis Report 2013*. World Health Organization; 2013.
- Fanning A. Tuberculosis: 6. Extrapulmonary disease. *CMAJ Can Med Assoc J*. 1999;160:1597.
- Sharma SK, Mohan A. Extrapulmonary tuberculosis. *Indian J Med Res*. 2004;120:316.
- Rapini RP, Bologna JL, Jorizzo JL. *Dermatology: 2-Volume Set*. St. Louis: Mosby; 2007.
- Kumar V, Abbas AK, Fausto N, Mitchell R. Chapter 1. Cell injury, cell death, and adaptations. In: Kumar V, Abbas AK, Fausto N, Mitchell R, eds. *Robbins Basic Pathol*. 8th ed. Phila: Elsevier-Saunders; 2007:13–17.
- Steingart KR, Henry M, Ng V, et al. Fluorescence versus conventional sputum smear microscopy for tuberculosis: a systematic review. *Lancet Infect Dis*. 2006;6(9):570–581.
- Corbett EL, Bandason T, Cheung YB, et al. Epidemiology of tuberculosis in a high HIV prevalence population provided with enhanced diagnosis of symptomatic disease. *PLoS Med*. 2007;4(1):e22.
- Gelalcha AG, Kebede A, Mamo H. Light-emitting diode fluorescent microscopy and Xpert MTB/RIF[®] assay for diagnosis of pulmonary tuberculosis among patients attending Ambo hospital, west-central Ethiopia. *BMC Infect Dis*. 2017;17:613.
- Agrawal M, Bajaj A, Bhatia V, Dutt S. Comparative study of GeneXpert with ZN stain and culture in samples of

- suspected pulmonary tuberculosis. *J Clin Diagn Res JCDR*. 2016;10(5):DC09–DC12.
11. World Health Organization. *Fluorescent light-emitting diode (LED) microscopy for diagnosis of tuberculosis: policy statement*; 2011.
 12. Guenaoui K, Harir N, Ouardi A, et al. Use of GeneXpert *Mycobacterium tuberculosis*/rifampicin for rapid detection of rifampicin resistant *Mycobacterium tuberculosis* strains of clinically suspected multi-drug resistance tuberculosis cases. *Ann Transl Med*. 2016;4(9), <http://dx.doi.org/10.21037/atm.2016.05.09>.
 13. Álvarez-Uría G, Azcona JM, Midde M, Naik PK, Reddy S, Reddy R. *Rapid Diagnosis of Pulmonary and Extrapulmonary Tuberculosis in HIV-Infected Patients. Comparison of LED Fluorescent Microscopy and the GeneXpert MTB/RIF Assay in a District Hospital in India. Tuberculosis Research and Treatment*; 2012 <https://www.hindawi.com/journals/trt/2012/932862/>. Accessed 24.09.17.
 14. Vadwai V, Boehme C, Nabeta P, Shetty A, Alland D, Rodrigues C. Xpert MTB/RIF: a new pillar in diagnosis of extrapulmonary tuberculosis? *J Clin Microbiol*. 2011;49(7):2540–2545.
 15. Lönnroth K, Castro KG, Chakaya JM, et al. Tuberculosis control and elimination 2010–50: cure, care, and social development. *Lancet*. 2010;375:1814–1829.
 16. Parida SK. True faceTs of TB diagnosis in 2012: Hypes and realiTies. *Eur J Microbiol Immunol*. 2012;2(4):275–281.
 17. Handbook on TB laboratory diagnostic methods for the European Union. European Centre for Disease Prevention and Control. <http://ecdc.europa.eu/en/publications-data/handbook-tb-laboratory-diagnostic-methods-european-union>. Published 14.03.16. Accessed 24.09.17.
 18. WHO. The world health report 1998 – Life in the 21st century: A vision for all. WHO. <http://www.who.int/whr/1998/en/>. Accessed 24.09.17.
 19. Helb D, Jones M, Story E, et al. Rapid detection of *Mycobacterium tuberculosis* and rifampin resistance by use of on-demand, near-patient technology. *J Clin Microbiol*. 2010;48:229–237.
 20. Boehme CC, Nabeta P, Hillemann D, et al. Rapid molecular detection of tuberculosis and rifampin resistance. *N Engl J Med*. 2010;363:1005–1015.
 21. Bates M, O’Grady J, Maeurer M, et al. Assessment of the Xpert MTB/RIF assay for diagnosis of tuberculosis with gastric lavage aspirates in children in sub-Saharan Africa: a prospective descriptive study. *Lancet Infect Dis*. 2013;13(1):36–42.
 22. Tortoli E, Russo C, Piersimoni C, et al. Clinical validation of Xpert MTB/RIF for the diagnosis of extrapulmonary tuberculosis. *Eur Respir J*. 2012;40(2):442–447.
 23. Causse M, Ruiz P, Gutiérrez-Aroca JB, Casal M. Comparison of two molecular methods for rapid diagnosis of extrapulmonary tuberculosis. *J Clin Microbiol*. 2011;49:3065–3067.
 24. Zeka AN, Tasbakan S, 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.
 25. Parsons LM, Somoskövi Á, Gutierrez C, et al. Laboratory diagnosis of tuberculosis in resource-poor countries: challenges and opportunities. *Clin Microbiol Rev*. 2011;24(2):314–350.
 26. Armand S, Vanhuls P, Delcroix G, Courcol R, Lemaître N. Comparison of the Xpert MTB/RIF test with an IS6110-TaqMan real-time PCR assay for direct detection of *Mycobacterium tuberculosis* in respiratory and nonrespiratory specimens. *J Clin Microbiol*. 2011;49(5):1772–1776.