

Diagnostic validation of rapid molecular detection of *Mycobacterium tuberculosis* in pus samples by GeneXpert®

Komal Shakeel,¹ Shagufta Iram,² Mehwish Akhtar,³ Shahida Hussain,⁴ Hannana Maryam,⁵ Ambreen Anwar⁶

Abstract

Objective: To evaluate the performance of GeneXpert for detection of mycobacterium tuberculosis in pus samples and compare its results with conventional techniques in terms of validity, rapidity and rifampicin resistance.

Methods: This longitudinal, descriptive study was conducted at Jinnah Hospital, Lahore, Pakistan, from January 2012 to December 2015, and comprised pus samples of people suspected of having extra-pulmonary tuberculosis. Participants were included by using consecutive sampling technique. The pus samples were subjected to Ziehl-Neelsen smear microscopy and Lowenstein-Jensen culture as per World Health Organisation's protocol and GeneXpert as per manufacturer protocol. SPSS 17 was used for data analysis. Validity of GeneXpert and rifampicin resistance were determined and compared with Ziehl-Neelsen staining using Lowenstein-Jensen culture as the gold standard.

Results: Of the 212 pus samples, 84(39.6%) were positive on Lowenstein-Jensen culture with mean turnaround time of 20±6 days, 77(36.3%) on GeneXpert and 22(10.4%) on Ziehl-Neelsen smear. The highest detection rate of mycobacterium tuberculosis 62(80.5%) was in lymph node samples by GeneXpert. The sensitivity and specificity of GeneXpert were 91.6% and 100% respectively, while Ziehl-Neelsen smear showed a sensitivity 26.2% and specificity of 100%. Rifampicin resistance was detected in 5(6.4%) pus samples by GeneXpert.

Conclusion: GeneXpert had a higher validity compared to Ziehl-Neelsen smear microscopy.

Keywords: Mycobacterium tuberculosis (MTB), Lowenstein-Jensen (LJ) culture, Pulmonary tuberculosis (PTB), Ziehl-Neelsen (ZN) stain. (JPMA 68: 33; 2018)

Introduction

Extra-pulmonary tuberculosis (EPTB) remains a crucial challenge for the physicians with respect to timely diagnosis and treatment. It comprises about 15% to 20% of all incidences of tuberculosis (TB) in immunocompetent patients.¹ According to the World Health Organisation (WHO), 34,000 (15%) of the TB cases reported in Pakistan in 2007 were extra-pulmonary.² It can affect many body organs like lymph nodes, bones and joints, brain, spine, abdomen, pericardium and genitourinary system which occurs mostly due to lymphatic or haematogenous dissemination. The most common appearance of EPTB is tuberculous lymphadenitis with cervical lymphadenopathy being the most frequent ranging from 50-78.63%.^{1,3,4} Many EPTB patients present with pus and discharging sinuses from lymph nodes, bones and joints.

The diagnosis of EPTB is difficult as compared to pulmonary tuberculosis (PTB) due to frequent atypical clinical presentation simulating other inflammatory and

neoplastic conditions, complications associated with sample collection and processing and fewer bacteria in clinical samples.⁵ Conventionally, in developing countries Ziehl-Neelsen (ZN) smear microscopy for acid-fast bacilli (AFB) and Lowenstein-Jensen (LJ) culture has been the initial diagnostic methods for TB. However, low sensitivity (45 - 80% of positive cultures) of ZN microscopy and slow growth rate of Mycobacterium tuberculosis (MTB) on LJ culture along with requirements of infrastructure, such as biological safety lab level II/III, limit the efficacy of these methods which often leads to significant delays, compromising patients' health.^{5,6}

GeneXpert MTB/rifampicin (RIF) assay (Cepheid Inc. Sunnyvale, California, United States) has been endorsed by WHO for the diagnosis of pulmonary and extra-pulmonary TB in endemic countries, with 98% sensitivity and specificity.⁵⁻⁷ It also detects rifampicin (RIF) resistance in less than 2 hours, therefore, identifying patients with multidrug-resistant (MDR) TB.^{5,7-9} This multifunctional semi-quantitative device is a cartridge-based, automated diagnostic test which is not prone to cross-contamination, and requires minimal technical expertise and biosafety facilities.^{5,10} It works by using two real-time polymerase chain reactions, one for MTB and other one for RIF resistance along with molecular beacon technology to

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1-3,5,6Allama Iqbal Medical College, Lahore, 4Microbiology and Molecular Genetics, Punjab University, Lahore, Pakistan.

Correspondence: Komal Shakeel. Email: komal_shakeel@outlook.com

identify specific deoxyribonucleic acid (DNA) sequences.¹¹

Initially, the WHO recommended the use of Xpert MTB/RIF for testing sputum samples to diagnose pulmonary TB. But recently, its application has been expanded for the diagnosis of EPTB as well as on non-respiratory samples, promising it as a new potentially attractive tool for accurate and rapid diagnosis. However, a majority of the previous studies have been conducted on both pulmonary and extra-pulmonary samples, reporting a sensitivity of GeneXpert ranging from 52.1% to 96.7% and a specificity of 73% to 100%.¹²⁻¹⁴ Thus, inconsistency in previous literature regarding its diagnostic validity along with a dearth of studies on local level limits its use in our routine practice.

Therefore, the present study was designed to evaluate the performance of GeneXpert for diagnosing EPTB by detecting MTB in pus samples in terms of sensitivity, specificity and RIF resistance, and compare its validity with ZN smear, keeping LJ as the gold standard.

Materials and Methods

This longitudinal, descriptive study was conducted at Jinnah Hospital, Lahore, Pakistan, during January 2012 to December 2015, and comprised pus samples of people suspected of having extra-pulmonary TB on the basis of clinical history, examination and radiological findings. Approval was obtained from the institutional ethics review board. Consecutive sampling was used. Pus samples were collected from the patients using aseptic technique and standard protocol. All the samples were processed in the TB laboratory of the Pathology

Department of Allama Iqbal Medical College, Lahore. Patients with diagnosed TB, on anti-TB drugs or with previously completed treatment were excluded from the study.

The samples were processed according to WHO protocol for ZN smear microscopy and LJ culture.^{15,16} Pus samples were concentrated by cytocentrifugation at 3000g for 15 minutes and the deposit was subjected to AFB smear microscopy by ZN staining and LJ culture after decontamination. At the same time, samples were processed for detecting MTB and RIF resistance on GeneXpert according to manufacturer protocol. Pus was transferred into 15ml falcon tubes and buffer was added in 2:1 ratio. Tubes were manually agitated twice during 15 minutes incubation at room temperature. Then 2ml of material was transferred into cartridges by using disposable pipettes available in the kits and they were loaded into the machine. The interpretation of data was software based and not user dependent.⁵ Data was analysed using SPSS 17. Diagnostic validity of ZN smear and gene expert was calculated using LJ culture.

Results

A total of 212 samples were included in the study. The overall mean age of the participants was 31.6±4.7 years. Moreover, 98(46%) of them were males and 114(54%) were females. In diagnosed EPTB cases, the number of females was 120(56.6%) compared to 92(43.3%) males. Higher frequency of positive cases 176(83%) on GeneXpert according to patients' age was found in the 10-30 age group.

Table-1: Validity of GeneXpert as compared to LJ culture.

		LJ culture		Total	Sensitivity	Specificity	PPV	NPV	Accuracy
		Positive	Negative						
GeneXpert	Positive	77	0	212	91.6%	100%	100%	94.8%	96.7%
	Negative	7	128						

PPV: Positive predictive value.

NPV: Negative predictive value.

LJ: Lowenstein-Jensen.

Table-2: Validity of ZN smear as compared to LJ culture.

		LJ culture		Total	Sensitivity	Specificity	PPV	NPV	Accuracy
		Positive	Negative						
ZN smear	Positive	22	0	212	26.2%	100%	100%	67.37%	70.35%
	Negative	62	128						

PPV: Positive Predictive Value.

NPV: Negative Predictive Value

LJ: Lowenstein-Jensen

ZN: Ziehl-Neelsen.

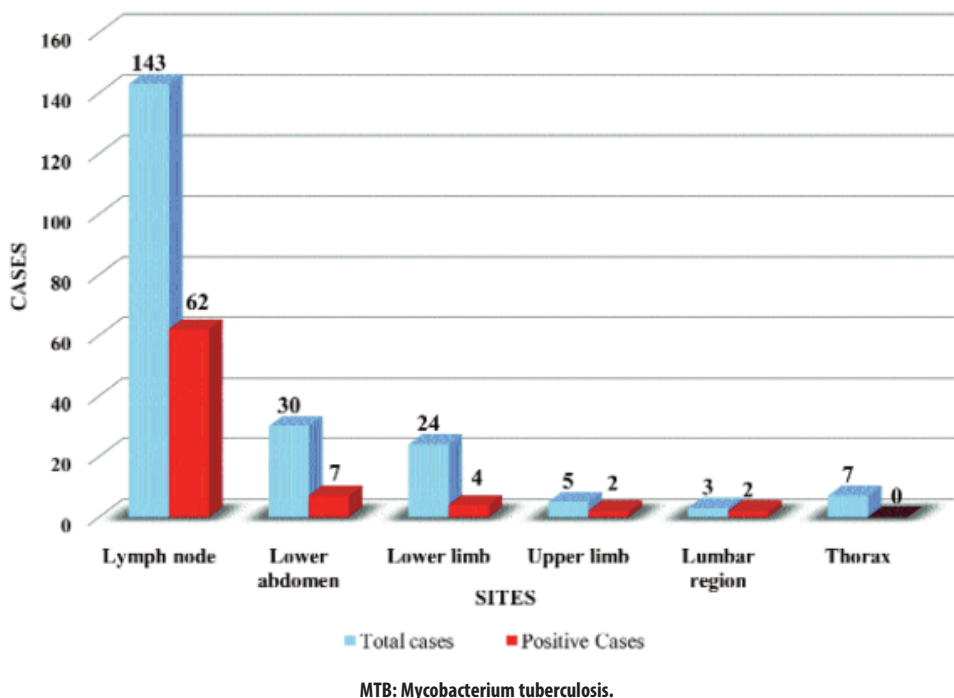


Figure: Frequency of MTB detection by GeneXpert in pus samples according to different body sites. (n=212).

According to different body sites, 143(67.5%) samples were taken from lymph nodes, 30(14.2%) from lower abdomen, 24(11.3%) from lower limb, 5(2.4%) from upper limb, 3(1.4%) from the lumbar region and 7(3.3%) from thorax (Figure).

Of all the pus samples, 84(39.6%) were positive by LJ culture followed by GeneXpert which detected 77(36.3%) positive for MTB and ZN smear which detected only 22(10.4%) positive samples. All smear positive samples were detected by GeneXpert. The sensitivity and specificity of GeneXpert were 91.6% and 100%, respectively (Table-1). In contrast, the sensitivity and specificity of ZN smear were 26.2% and 100%, respectively (Table-2).

Of the 77(36.3%) positive cases detected by GeneXpert, RIF resistance was found in 5(6.4%) pus samples.

Discussion

EPTB is a major public health concern and its rapid diagnosis is a key objective in worldwide tuberculosis control strategy. This longitudinal descriptive study is one of the few studies specifically designed to evaluate the performance of GeneXpert for detecting MTB in pus samples. These samples were collected over a period of four years from EPTB-suspected patients presenting to a tertiary care hospital which is one of the largest referral

centres. Thus, the study population can be considered as true representative of strong suspects of EPTB in far-flung TB endemic areas.

Among the 212 suspected cases of EPTB, a majority of study population was from the middle-aged group, with higher frequency of positive cases (83%) found in between the range of 10-30 years. Greater proportion of EPTB was also observed among adults rather than children and older patients in studies conducted by Tortoli et al. and Ikram et al.^{17,18} These results further strengthens the fact that TB usually involves active age group as reported by the WHO. The frequency of females (56.6%) with EPTB cases was higher than males (43.3%), a ratio consistent with other studies.^{18,19}

Out of the 212 pus samples in this study, 84 were positive on LJ culture while in 77 MTB was detected on GeneXpert and 22 were positive for AFB on ZN smear. The majority of the pus samples detected positive for MTB were from lymph node swellings (80.5%), which is in coherence with the studies conducted by Chandir et al, Zeka et al. and Iram et al. reporting tuberculous lymphadenitis as the most common entity among EPTB.^{2,12,19} On the other hand, 7 positive cases detected by LJ culture and negative on GeneXpert consisted of atypical mycobacteria confirmed by rate of growth, properties of pigment production and biochemical reactions.

The unique feature of this study is that it was conducted on a large number of pus samples. Results of the current study showed a sensitivity of 91.6% and specificity of 100% by GeneXpert which are comparable with a study conducted by Tortoli et al. that comprised 195 pus specimens and reported a sensitivity of 85.1% and specificity of 94.6%.¹⁷ A fair comparison of performance of GeneXpert cannot be made with previous studies as they have reported an overall validity in extra-pulmonary samples which included a limited number of pus samples. In accordance with the present study, Causse et al.²⁰ showed 95% sensitivity, 100% specificity

and Ligthelm et al.¹³ showed 96.7% sensitivity and 88.9% specificity. Similar results were seen by studies conducted by Malbruney et al.²¹ on both respiratory and non-respiratory samples reporting a sensitivity of 85.7% and specificity of 97.3% and Vadwai et al.¹⁴ which showed 83% sensitivity and 73% specificity. Raj et al. demonstrated the accuracy of GeneXpert in EPTB patients with a sensitivity of 81% and specificity of 99.6%.²² The results of our study are significantly higher than another study conducted by Zeka et al. who reported a low sensitivity of GeneXpert as 52.1%.¹² It can be due to the fact that it was conducted in a non-endemic area and the clinical samples included were from all types of extra-pulmonary sites in which bacterial load is generally low which strongly affect sensitivity of rapid test like GeneXpert.

As far as the diagnostic validity of ZN smear is concerned, this study showed a sensitivity and specificity of 26.17% and 100%, respectively, which is again comparable with a study conducted by Malbruney who reported it to be 28.6% and 98.7%, respectively.²¹ However, Tortoli et al. conducted a multi-centred study on a larger sample size and reported a much higher sensitivity of ZN smear as 48%.¹⁷ Ligthelm et al. reported a higher sensitivity of ZN smear (41.4%), but their study had a small sample size.¹³

Finally, not only MTB detection but also rapidly determining the patient's MDR status is of prime importance in bringing to an end of the spread of MDR-TB and decreasing mortality.^{5,7,8} In previous studies, sensitivity of GeneXpert for detecting RIF resistance was 94.4 to 100% and the specificity was 98.3 to 100%.^{5,7} Scott et al. reported RIF resistance (9.6%) in 25/260 cases.²³ In the present study, RIF resistance was detected in 5(6.4%) pus samples by GeneXpert within 2 hours. Conventional culture results take at least 3 weeks for detection of MTB with additional 2 weeks for drug susceptibility testing (DST) to detect RIF resistance.¹⁴ The average time to results for LJ culture in this research was 20±6 days. Therefore, faster methods that allow MDR regimens to be started early are urgently needed. Moreover, conventional procedures are laborious and require high-infrastructure laboratories and trained personnel that is available only in a few reference centres. Thus, GeneXpert can serve as a rapid and accurate diagnostic tool, making its high cost an offset to an extent by the rapid turnaround time, similar to that of smear microscopy, with less biohazard risk and only minimal training needed.^{5,7}

The Xpert test is a major advance in EPTB diagnostic testing, but a major limitation is that it cannot

distinguish between viable and nonviable mycobacteria, therefore, it cannot be used for monitoring patients progress and treatment efficacy. Furthermore, limited shelf-life of the diagnostic cartridges, optimum operating temperature and humidity restrictions, requirement for electricity supply, need for annual servicing and calibration of each machine are the major hindrances for its routine use in resource-limited countries.²⁴ Ensuring sustainable systems for long-term provision of servicing and consumables may be more important and challenging than initial implementation of the diagnostic equipment itself.

Conclusion

GeneXpert MTB/RIF assay appears to be a valid and accurate tool for rapid detection of MTB in pus samples making it a better choice as compared to ZN smear microscopy. By doubling the proportion of rapid detection of MTB and RIF resistance at the same time, it has a good potential for diagnosis of extra-pulmonary tuberculosis in resource-limited settings resulting in an improvement in patients' management and outcome.

Acknowledgement: We are grateful to the staff of TB laboratory for their cooperation in sample collection.

Disclaimer: None.

Conflict of Interest: None.

Source of Funding: None.

References

1. Fazal-I-wahid, Habib-Ur-Rehman, Ahmad I. Extrapulmonary tuberculosis in patients with cervical lymphadenopathy. *J Pak Med Assoc.* 2013; 63:1094-7.
2. Chandir S, Hussain H, Salahuddin N, Amir M, Ali F, Lotia I, et al. Extrapulmonary tuberculosis: a retrospective review of 194 cases at a tertiary care hospital in Karachi, Pakistan. *J Pak Med Assoc.* 2010; 60:105-9.
3. Marais BJ, Wright CA, Schaaf HS, Gie RP, Hesselning AC, Enarson DA, et al. Tuberculous lymphadenitis as a cause of persistent cervical lymphadenopathy in children from a tuberculosis-endemic area. *Pediatr Infect Dis J.* 2006; 25:142-6.
4. Ahmed P, Anwar M, Khan B, Altaf C, Ullah K, Raza S, et al. Role of isoniazid prophylaxis for prevention of tuberculosis in haemopoietic stem cell transplant recipients. *J Pak Med Assoc.* 2005; 55:378.
5. Boehme CC, Nabeta P, Hillemann D, Nicol MP, Shenai S, Krapp F, et al. Rapid molecular detection of tuberculosis and rifampin resistance. *N Engl J Med.* 2010; 363:1005-15.
6. Behr MA, Warren SA, Salamon H, Hopewell PC, De Leon AP, Daley CL, et al. Transmission of *Mycobacterium tuberculosis* from patients smear-negative for acid-fast bacilli. *Lancet.* 1999; 353:444-9.
7. Boehme CC, Nicol MP, Nabeta P, Michael JS, Gotuzzo E, Tahirli R, et al. Feasibility, diagnostic accuracy, and effectiveness of decentralised use of the Xpert MTB/RIF test for diagnosis of

- tuberculosis and multidrug resistance: a multicentre implementation study. *lancet*. 2011; 377:1495-505.
8. World Health Organization. Global tuberculosis control. Geneva, Switzerland: WHO report, 2010.
 9. Pinto LM, Udawadia ZF. Xpert MTB/RIF and pulmonary tuberculosis: time to delve deeper? *Thorax*. 2013; 68:987-8.
 10. Batz HG, Cooke GS, Reid SD. Towards lab-free tuberculosis diagnosis. Treatment Action Group, the TB/HIV Working Group of the Stop TB Partnership, Geneva: Imperial College MSF, 2011.
 11. Rie AV, Page-Shipp L, Scott L, Sanne I, Stevens W. Xpert MTB/RIF for point-of-care diagnosis of TB in high-HIV burden, resource-limited countries: hype or hope? *Expert Rev Mol Diagn*. 2010; 10:937-46.
 12. 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; 49:4138-41.
 13. Ligthelm LJ, Nicol MP, Hoek KG, Jacobson R, van Helden PD, Marais BJ, et al. Xpert MTB/RIF for rapid diagnosis of tuberculous lymphadenitis from fine-needle-aspiration biopsy specimens. *J Clin Microbiol*. 2011; 49:3967-70.
 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; 49:2540-5.
 15. International Union against Tuberculosis and Lung Disease. Sputum examination for tuberculosis by direct microscopy in low income countries. Technical Guide. 5th ed. Paris, France: IUATLD, 2000.
 16. Chihota VN, Grant AD, Fielding K, Ndibongo B, Van Zyl A, Muirhead D, et al. Liquid vs. solid culture for tuberculosis: performance and cost in a resource-constrained setting. *Int J Tuberc Lung Dis*. 2010 Aug;14(8):1024-31.
 17. Tortoli E, Russo C, Piersimoni C, Mazzola E, Dal Monte P, Pascarella M, et al. Clinical validation of Xpert MTB/RIF for the diagnosis of extrapulmonary tuberculosis. *Eur Respir J*. 2012; 40:442-7.
 18. Ikram A, Ahmed SA, Khan FA, Khadim MT, Satti L. Rapid *Mycobacterium tuberculosis* DNA detection on fine needle aspirates from extra pulmonary lymph nodes. *J Coll Phy Surg Pak*. 2015; 25:417-21.
 19. Yusuf NW, Iram S, Zeenat A, Hussain S, Aslam M. Rapid diagnosis of tuberculosis using Xpert MTB/RIF assay-Report from a developing country. *Pak J Med Sci*. 2015; 31:105-10.
 20. Causse M, Ruiz P, Gutiérrez-Aroca JB, Casal M. Comparison of two molecular methods for rapid diagnosis of extrapulmonary tuberculosis. *J clin microbiol*. 2011; 49:3065-7.
 21. Malbruny B, Le Marrec G, Courageux K, Leclercq R, Cattoir V. Rapid and efficient detection of *Mycobacterium tuberculosis* in respiratory and non-respiratory samples [Technical note]. *Int J Tuberc Lung Dis*. 2011; 15:553-5.
 22. Raj A, Singh N, Mehta PK. Gene Xpert MTB/RIF assay: a new hope for extrapulmonary tuberculosis. *IOSR J Pharma*. 2012;2:83-9.
 23. Scott LE, Beylis N, Nicol M, Nkuna G, Molapo S, Berrie L, et al. Diagnostic accuracy of Xpert MTB/RIF for extrapulmonary tuberculosis specimens: establishing a laboratory testing algorithm for South Africa. *J Clin Microbiol*. 2014; 52:1818-23.
 24. Evans CA. GeneXpert—a game-changer for tuberculosis control? *PLoS Med*. 2011; 8:e1001064.
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