10.5005/jp-journals-10036-1129

ORIGINAL ARTICLE

Utility of GeneXpert *Mycobacterium tuberculosis*/Rifampin Assay for Extrapulmonary Tuberculosis Samples

[1]Shamma Shetye, [2]Pratiksha Chheda, [3]Ashish Lad, [4]Sunmeet Matkar, [5]Priyanka Yadav

ABSTRACT

A total of 3,806 samples from suspected cases of extrapulmonary tuberculosis (EPTB) were subjected to GeneXpert *Mycobacterium tuberculosis* (MTB)/rifampin (RIF) assay. Samples consisted of body fluids, pleural fluids, pus and aspirates, lymph node (LN) tissues, and others. *Mycobacterium tuberculosis* positivity was detected in 18.10% and RIF positivity in 2.73% samples. The MTB/RIF positivity was found highest in pus and aspirates (40.38%). In this study, assay failure rate for GeneXpert MTB/RIF assay was very low (1.99%). It is concluded from this study that GeneXpert MTB/RIF is an efficient, reliable, simple, and fast technique for rapid diagnosis of EPTB in our country where incidence of tuberculosis remains high.

Keywords: Assay failure rate, Extrapulmonary tuberculosis, GeneXpert MTB/RIF assay, Positivity rate.

How to cite this article: Shetye S, Chheda P, Lad A, Matkar S, Yadav P. Utility of GeneXpert *Mycobacterium tuberculosis*/Rifampin Assay for Extrapulmonary Tuberculosis Samples. MGM J Med Sci 2017;4(1):6-9.

Source of support: Nil

Conflict of interest: None

INTRODUCTION

Tuberculosis (TB) is the world's most common infectious disease. According to the World Health Organization (WHO) report of 2015, there are 9.6 million people infected with TB. India accounted for 27% of global TB notifications in 2014.[1] Despite implementing Directly Observed Treatment Short-course strategy under the Revised National TB Control Programme in 1997, TB incidence in India continues to remain high, indicating that there could be substantial ongoing transmission.[2]

Extrapulmonary tuberculosis (EPTB) infections can affect any organ in the body. Diagnosis of EPTB infection is often difficult to establish because of the paucibacillary nature of *Mycobacterium tuberculosis* (MTB) bacilli in EPTB sites and the need for invasive procedures to secure appropriate sample.[3] Also, quick and reliable laboratory diagnostic methods for detecting tubercle bacilli in EPTB specimens are not easily available. This adds to the increased rates of morbidity and mortality in EPTB patients.[4]

In extrapulmonary samples, the WHO has recommended the use of GeneXpert MTB/RIF assay for rapid detection of MTB. The GeneXpert MTB/rifampicin (RIF) assay detects deoxyribonucleic acid (DNA) sequences specific for MTB and RIF resistance by polymerase chain reaction (PCR).[5] The GeneXpert MTB/RIF assay purifies and concentrates the MTB bacilli from extrapulmonary samples, isolates the genomic material, and amplifies the genomic DNA by PCR. The objective of our retrospective study was to evaluate the utility of GeneXpert MTB/RIF assay in the extrapulmonary TB samples.

MATERIALS AND METHODS

The study was conducted in the Department of Molecular Biology, Metropolis Healthcare, Mumbai, India.

Sample Collection

A total of 3,806 clinically suspected cases of EPTB were received in Global Reference Laboratory, Metropolis Healthcare Ltd, Mumbai, India. Extrapulmonary samples (pus and aspirates, body fluid, pleural fluid, Lymph node [LN] tissue, and others) were collected in plain universal 30 mL clear plastic container with white cap. Body fluids accounted for the largest type of specimen (n = 1,745). It was followed by pleural fluid specimen (n = 871) and pus and aspirate specimen (n = 586). The LN tissue formed least number (n = 195) of specimen type. Other specimens included biopsy specimens, brain tissue, computed tomography-guided fine-needle aspiration collection (FNAC), colon biopsy, fallopian tube, synovial tissue, and ultrasonography-guided FNAC. In total, other specimens accounted for 409 specimens (Table 1 and Graph 1).

[1]Head, [2]Senior Manager, [3]Operational Head, [4]Senior Executive [5]Scientific Officer

[1]Department of Microbiology and Molecular Biology, Metropolis Healthcare Ltd, Mumbai, Maharashtra, India

[2,4]Department of Research Development, Metropolis Healthcare Ltd, Mumbai, Maharashtra, India

[3,5]Department of Molecular Biology, Metropolis Healthcare Ltd Mumbai, Maharashtra, India

Corresponding Author: Shamma Shetye, Head, Department of Microbiology and Molecular Biology, Metropolis Healthcare Ltd Mumbai, Maharashtra, India, Phone: +9122-30840789, e-mail: shamma.shetye@metropolisindia.com

Table 1: Breakdown of EPTB samples used in the study

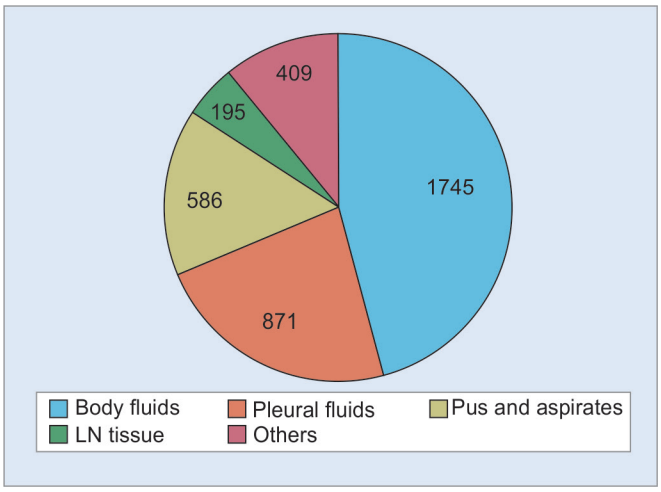
Specimen type	*Frequency*	*Percentage*
Body fluids	1745	45.84
Pleural fluids	871	22.88
Pus and aspirates	586	15.39
LN tissue	195	5.13
Others	409	10.76
Total	3806	100

Procedure for GeneXpert MTB/RIF Assay

The Xpert assay was performed as per the method described by Helb et al.[6] Sample reagent was added in a 3:1 ratio to 0.5 mL of decontaminated specimen. The closed tube was manually agitated twice during a 15-minute incubation period at room temperature before 2 mL of the inactivated sample reagent/sample mixture was transferred to the Xpert test cartridge. Cartridges were inserted into the GeneXpert device, and the automatically generated results were read after 90 minutes.

Feasibility Evaluation

The feasibility of GeneXpert MTB/RIF assay was evaluated in terms of proficiency of the assay to report a valid patient result. The absence of a valid test result for any given assay commenced was considered as a "test failure" irrespective of the underlying reason. The frequency of various reasons for the incidence of test failure was examined. The manufacturer has classified possible test failure causes as "error," "invalid," or "no result." An "error" result indicates that the GeneXpert MTB/RIF assay in a given test was aborted by internal quality control mechanisms including improper filling of the cartridge reaction tube, cartridge reagent probe integrity failure, cartridge internal pressure excess, or equipment malfunction. All "error" results are accompanied by specific error codes that provide additional information as to the underlying cause of failure (Table 2). An "invalid" result indicates that PCR has failed, usually due to the presence of PCR inhibitors. A "no result" outcome indicates that the test underway was prematurely terminated either by external or internal factors during cartridge loading process, such as power failure, manual termination of the test by the operator, or one of the equipment or cartridge component failures.[7] Under the study, for a patient, in case of "error" or "no result" outcome, repeat testing was performed on the same sample; for an "invalid" result, repeat testing was performed on a second fresh sputum sample due to concern over PCR inhibitors in the original specimen. The data from every test run were recorded by the GeneXpert software (GxAlert).



Graph 1: Diagrammatic representation of breakdown of EPTB samples used in the study

Table 2: Type of errors reported in the study

Error code	*Type of error*	*Number of cases*
5011	Postrun analysis error	22
5007		51
5006		0
2008		0
2005	Operation terminated error	0
2022		0
2025	Cartridge loading error	0
2037		0
1001		0
1002	Temperature-related errors	0
1004		0
Invalid results		03
Total		76

RESULTS

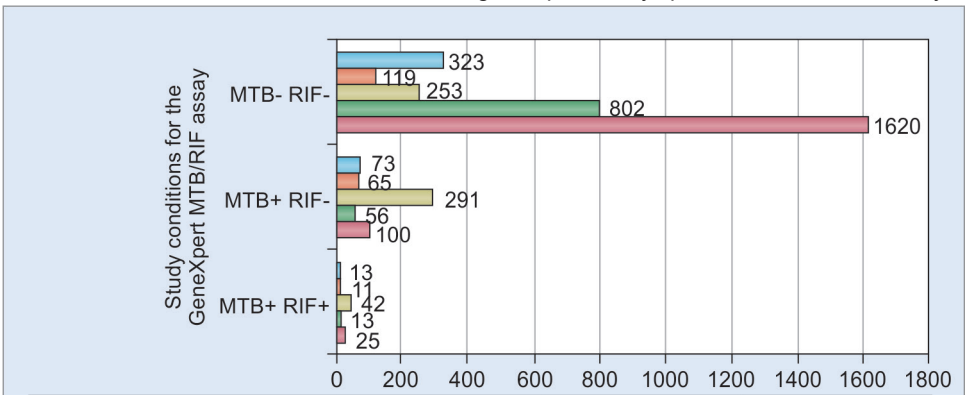
Out of the total number of extrapulmonary samples (n = 3,806) taken up for analysis, 81.89% (n = 3,117/3,806) cases were detected as MTB negative and RIF negative. Of these, body fluids (n = 1,620, 42.56%) accounted for the most of the MTB^-/RIF^- cases. This was followed by pleural fluids (n = 802, 21.07%), others (n = 323, 8.48%), pus and aspirates (n = 253, 6.64%), and LN tissue (n = 119, 3.12%) (Tables 3 and 4).

For the cases detected as MTB positive and RIF positive, the positivity rate of GeneXpert MTB/RIF assay was

Table 3: Performance of extrapulmonary specimens in GeneXpert MTB/RIF assay

	MTB^+/RIF^+	MTB^+/RIF^-	MTB^-/RIF^-
Body fluids (n = 1,745)	25	100	1620
Pleural fluids (n = 871)	13	56	802
Pus and aspirates (n = 586)	42	291	253
LN tissue (n = 195)	11	65	119
Others (n = 409)	13	73	323

Table 4: Distribution of MTB/RIF cases among extrapulmonary specimens used in the study



	MTB^+/RIF^+	MTB^+/RIF^-	MTB^-/RIF^-
Others (n = 409)	13	73	323
LN tissue (n = 195)	11	65	119
Pus and aspirates (n = 586)	42	291	253
Pleural fluids (n = 871)	13	56	802
Body (n = 1745)	25	100	1,620

recorded at 2.73% (n = 104/3,806). The maximum cases of MTB^+/RIF^+ were observed in pus and aspirates with 40.38% (n = 42/104). Second highest cases of MTB^+/RIF^+ was observed in body fluids 25.03% (n = 25/104), pleural fluids 12.50% (n = 13/104), others 12.50% (n = 13/104), and LN tissue 10.57% (n = 11/104) (Tables 2 and 3).

In the cases detected as MTB positive and RIF negative, the positivity rate of GeneXpert MTB/RIF assay was measured at around 15.37% (n = 585/3,806). Similar to the cases of MTB^+/RIF^+, the maximum cases of MTB^+/RIF^- were observed in pus and aspirates with 49.74% (n = 291/585), followed by body fluids 17.09% (n = 100/585), others 12.47% (n = 73/585), LN tissue 11.11% (n = 65/585), and pleural fluids 9.57% (n = 56/585) (Tables 2 and 3).

DISCUSSION

The study revealed that the GeneXpert MTB/RIF assay had a good diagnostic potential for specimens, such as pus and aspirates, which is difficult to diagnose by other laboratory techniques. Findings of the study supported the use of GeneXpert MTB/RIF assay in routine diagnosis for EPTB investigation, especially for pus samples.[8,9]

Similar studies were carried out by Lawn and Zumla[10] who employed the GeneXpert MTB/RIF assay for diagnosis of EPTB. Out of the total of 268 samples, the positivity rate was observed for tissue biopsies or fine-needle aspirates (35%), gastric aspirates (23%), pus (21%), urine (6%), cerebrospinal fluid (5%), and other body fluids, i.e., peritoneal, synovial, and pericardial (4%).

Test failure cause analysis: Failed tests for GeneXpert MTB/RIF assay accounted only 76/3,806 (1.99%) in our study. As per the test failure codes generated by the GeneXpert MTB/RIF assay, the leading cause of test failure was postrun analysis error, contributing to a total of 51 out of 76 test failures. The frequency of various factors contributing to test failures is described in Table 2. Literature studies[7,11] suggest that the leading cause of GeneXpert MTB/RIF assay failure results observed in the present study was due to inadequate sample processing and equipment malfunction. Like any other automated laboratory technology, GeneXpert MTB/RIF assay also require a stable electric power supply, and even a short-term interruption of power would result in test failures. The manufacturer recommends a maximum of 30°C ambient operating temperature for the operation of GeneXpert instrument. Data on the robustness of the device under prolonged periods of temperature exceeding 30°C are not available.[7] In our study, the GeneXpert MTB/RIF assay was error-free for Type error 1001, 1002, 1004, 2022, 2025, and 2037.

CONCLUSION

GeneXpert MTB/RIF assay is an efficient and reliable technique for the rapid diagnosis of EPTB. Its simplicity, sensitivity, and speed make this technique a good tool for diagnosis of MTB from extrapulmonary samples. The failure rate for GeneXpert MTB/RIF assay was acceptably low in our study. It is particularly useful for our country where the incidence of tuberculosis is high, as it provides a simple, reliable, and cost-effective diagnostic modality.

ACKNOWLEDGMENT

The authors would like to acknowledge the support and facilities provided by the management of Metropolis Healthcare Ltd, Mumbai, India.

REFERENCES

1. World Health Organization. Global tuberculosis report 2015. 20th ed. Geneva: WHO; 2015. p. 192.
2. Sreeramareddy CT, Qin ZZ, Satyanarayana S, Subbaraman R, Pai M. Delays in diagnosis and treatment of pulmonary tuberculosis in India: a systematic review. Int J Tuberc Lung Dis 2014 Mar;18(3):255-266.
3. Sajed AN, Khan S, Butt AS, Srwar A, Akhtar R, Ahmad I, Mukhtar MN, Imran S, Akbar H, Ali S, et al. Rapid detection of Mycobacterium tuberculosis and Rifampicin Resistance in extra pulmonary samples using Gene Xpert MTB/RIF assay. IOSR-JDMS 2014 Nov;13(11):50-53.
4. Hillemann D, Rüsch-Gerdes S, Boehme C, Richter E. Rapid molecular detection of extra-pulmonary tuberculosis by the automated GeneXpert MTB/RIF system. J Clin Microbiol 2011 Apr;49(4):1202-1205.
5. Boehme CC, Nabeta P, Hillemann D, Nicol MP, Shenai S, Krapp F, Allen J, Tahirli R, Blakemore R, Rustomjee R, et al. Rapid molecular detection of tuberculosis and rifampin resistance. N Engl J Med 2010 Sep;363(11):1005-1015.
6. Helb D, Jones M, Story E, Boehme C, Wallace E, Ho K, Kop J, Owens MR, Rodgers R, Banada P, et al. Rapid detection of mycobacterium tuberculosis and rifampin resistance by use of on-demand, near-patient technology. J Clin Microbiol 2010 Jan;48(1):229-237.
7. Raizada N, Sachdeva KS, Sreenivas A, Vadera B, Gupta RS, Parmar M, Kulsange S, Babre A, Thakur R, Gray C, et al. Feasibility of decentralised deployment of Xpert MTB/RIF test at lower level of health system in India. PLoS One 2014 Feb;9(2):e89301.
8. Purohit M, Mustafa T. Laboratory diagnosis of extra-pulmonary tuberculosis (EPTB) in resource-constrained setting: state of the art, challenges and the need. J Clin Diagn Res 2015 Apr;9(4):EE01-EE06.
9. Lee JY. Diagnosis and treatment of extrapulmonary tuberculosis. Tuberc Respir Dis (Seoul) 2015 Apr;78(2):47-55.
10. Lawn SD, Zumla AI. Diagnosis of extrapulmonary tuberculosis using the Xpert® MTB/RIF assay. Expert Rev Anti Infect Ther 2012 Jun;10(6):631-635.
11. Feasey NA, Banada PP, Howson W, Sloan DJ, Mdolo A, Boehme C, Chipungu GA, Allain TJ, Heyderman RS, Corbett EL, et al. Evaluation of Xpert MTB/RIF for detection of tuberculosis from blood samples of HIV-infected adults confirms Mycobacterium tuberculosis bacteremia as an indicator of poor prognosis. J Clin Microbiol 2013 Jul;51(7):2311-2316.