Original Article International Journal of Spine | 2022 January-June; 7(1): 01-06

Detection of Spinal TB Infection: A Retrospective Study Evaluating Comparative Diagnostic Efficacy of AFB Smear, Gene Expert, Histopathology, Culture Sensitivity and LPA Tests From a Biopsy Sample

Abhijith Shetty[1], Saijyot Raut[1], Manikant Anand[1], Vishal Kundnani[1], Praveen Goparaju[1], Mukul Jain[1]

Abstract

Background: Spinal Tuberculosis (TB) is one of the significant health dangers that affect the general wellbeing of an individual. It is one of the most common form of extra pulmonary tuberculosis that affects the general population. Diagnosis of tuberculosis is done using an array of techniques. The present study has compared the efficacy of these tests used for detecting the spinal TB in biopsy samples.

Material and Methods: The study was conducted on patients who suffered from spondylodiscitis and with biopsy proven spinal TB by one of the following tests and were treated in the study center. The study included a total of 150 patients with spinal TB who visited the department for further treatment. The biopsy samples of these patients were then processed for Line probe assay (LPA), Gene Xpert, liquid culture (bactec MGIT) followed by gram staining and fungal staining, and histopathological examination.

Result: In this study total of 150 patients were included who had ages ranging from 16-77 years with 93 male and 57 female patients. When the study results were compared Gene Experts showed a 100% sensitivity and 80% of specificity. When we compared the histopathology results with gene expert, we get a sensitivity of 16.7% and specificity of 50%.

Gram stain with the sensitive gene expert, the sensitivity is 45.5% and specificity is 25%. Similar analysis was done with sensitive gene and now with the resistant gene to identify their sensitivity was 0% and the specificity was at 42.9%. Gram stain when correlated with gene, the sensitivity came out to be 9.1% and specificity at 25%. Fungal stain with resistant gene, when correlated, sensitivity comes to 9.1% and specificity is at 0%.

Conclusion: This study showed that for detection of tuberculosis rather than relying on only single technique it should be done with a combination of techniques.

Keywords: Gene Xpert, LPA, Histopathological examination, Tuberculosis, Gram staining

Introduction

Tuberculosis (TB) is one of the significant health dangers that affect the general wellbeing of an individual. In combination with the human immunodeficiency infection (HIV), it is the reason for death because of irresistible maladies around the world. This disease is caused by the bacterium "Mycobacterium tuberculosis" which mainly affects the pulmonary tract in humans. Generally, TB infection spreads from an infected person through the air [1].

In a report published in 2012, WHO reported that approximately 8.6 million cases have occurred globally. In Asia and Africa, the prevalence of TB is higher. The highest incidence of TB cases was reported in India and China. In India, 2 million to 2.4 million cases were reported in 2012 [2]. Albeit a declining pattern in TB occurrence, commonness and mortality have been seen in the course of the most recent decade, disposal of the infection at the worldwide level is still distant, and a huge asset venture is as yet required. Improving access to funding and care, the fundamental prerequisites in the battle against TB is timely testing [2].

Spinal Tuberculosis is the most common form of extra pulmonary tuberculosis that affects the general population. Most of the patients of spinal TB are found in the developing countries. In common terms this particular disease is known as the Pott's spine. Interestingly, there is no specific guideline targeted towards the spinal TB diagnosis and treatment. However, it was shown that early

[1]Department of Orthopaedics, Bombay Hospital and Research Centre, Mumbai, Maharashtra, India.

Address of Correspondence
Dr. Abhijith Shetty,
Spine Surgeon, Department of Orthopaedics, Bombay Hospital and Research Centre, Mumbai, Maharashtra, India.

E-mail: abhijithshettyabhi03@gmail.com

Submitted: 28/02/2022; Reviewed: 17/03/2022; Accepted: 14/04/2022; Published: 10/06/2022

International Journal of Spine | Available on www.ijsonline.co.in | DOI:10.13107/ijs.2022.v07i01.031

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License https://creativecommons.org/licenses/by-nc-sa/4.0/ , which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

diagnosis and treatment can minimize the deformity in the spine [3].

The transformative accomplishment of Mycobacterium tuberculosis (Mtb) is reflected in its conjunction with people for more than two millennia. Smear microscopy with carbol fuchsin and fluorochromes, for example, auramine-rhodamine stays a pillar in the discovery of Mtb in clinical examples and is generally bolstered by the WHO. Fluorescence microscopy improves the affectability of Mtb identification. The nucleic acid amplification test (NAAT) offers a significant, fast, and under-used atomic apparatus in Mtb determination. These tests recognize mycobacteria with high explicitness by enhancing objective nucleic corrosive arrangements [4].

Mycobacterial culture remains the highest quality level for location and medication powerlessness testing. Fluid culture frameworks (BACTEC and MGIT (Becton Dickinson) offer an increasingly delicate and fast option in contrast to a customary strong culture and may identify development in 1–3 weeks. The mycobacterial development pointer cylinder or MGIT contains a fluorescent compound at the base of the cylinder which is extinguished by oxygen. As Mtb develops, the oxygen in the cylinder is utilized and the fluorescent compound is distinguished. At present, the WHO and the Strategic and Technical Advisory Group on TB prescribe staged execution of fluid culture where practical including low pay nations [5]. On the Other hand line probe assay is a newer technique that uses a hybridization process to differentiate between the different species of these bacteria [6].

Another rapid test that is based on nucleic acid amplification, the Xpert MTB/RIF assay detects the susceptibility of the rifampicin within 2 hours. This test is endorsed by the World Health Organization (WHO) for diagnosis of pulmonary [7]. Reither et al reported that compared with the routine culture test, the Xpert MTB/RIF assay showed an excellent specificity (100%) and average sensitivity for PTB in children. Several other studies also provided evidence that this test showed diagnostic efficacy in pulmonary and extrapulmonary cases of tuberculosis [8,9].

Different studies have reported different sensitivity and specificity for detecting the presence of Mycobacterium tuberculosis in the patient sample. Not many studies were done that have compared the efficacy of different tests in evaluating the presence of the bacterium in the patient's sample. Hence this study was conducted that have compared the efficacy of the tests used for detecting the presence of TB in patient samples.

Material and Methods

The retrospective study was conducted on patients who suffered from spondylodiscitis and biopsy proven spinal TB and were treated in the study center. The study included a total of 150 patients with spondylodiscitis whose biopsy were taken in study centre and proven to be spinal TB by one of the tests and treated in the department. Ethical clearance for the study was taken from the college ethics committee before the start of the study. The patients were described in detail about the study proforma and written consent was taken from all the patients.

Study participant recruitment

The patients were recruited based on the following inclusion parameters

Inclusion Criteria:

- All cases of spondylodiscitis with biopsy proven spinal TB by one of the following test

LPA, Gene Xpert, BACTEC MGIT, ZN staining, histopathology.

The patients were excluded based on the following criteria

Exclusion criteria

- Patients with age less than 14 years
- Patients with bacterial(aerobic/anaerobic)/ fungal culture positive biopsy samples
- A patient who was unwilling to take part in the study

Biopsy procedure

A percutaneous transpedicular approach under fluoroscopic guidance was used for procuring the biopsy samples. The procedure can be performed under a combination of local anaesthesia and intravenous sedation or under general anaesthesia. Biopsies were performed with the patient in the prone position, using the trephine biopsy kits. Preoperative CT scans and MRI images determined the side of approach. All biopsies were performed under fluoroscopic guidance using image intensifier. The puncture point was determined under fluoroscopic control. After local anaesthesia with 1% lidocaine, the thin needle of the trephine set was introduced through a small skin incision and advanced under fluoroscopic guidance until it contacted the periosteum. Fine needle replaced by the guidewire and over the guidewire graduated external sheath mounted on the handle was positioned in the middle potion of the pedicle. The handle and the guidewire removed and biopsies were performed with the cutting cannula and biopsy material collected as desired. The procedure lasted for 30-60 minutes.

Specimen processing

The biopsy samples were collected based on the guidelines of the Revised National Tuberculosis Control Programme of India [10]. One sample was collected in normal saline to be sent for LPA,GENE Xpert, BACTEC MGIT and ZN staining and another sample was collected in formalin solution to be sent for histopathological examination. The samples were further processed in a type II biosafety cabinet at a bio-safety level-3 laboratory. The samples were processed for direct smear microscopy using Ziehl–Nielsen (ZN) staining procedure [11]. After this, the samples were decontaminated by the NALC-NaOH method (final NaOH concentration, 1%) according to a previously described procedure.After the decontamination, the samples were inoculated the liquid culture media for further characterization.

ZN staining analysis

The slides were prepared for sputum analysis by Ziehl-Nielsen staining and examined under oil immersion microscopy. A positive slide was taken even for a single slide that showed positive for Mycobacterium tuberculosis in AFB/100 fields.

Liquid culture

Three media have been approved by the FDA for culturing mycobacterium species namely: the VersaTREK system, BACTEC MGIT 960 system, and the MB/BacT Alert 3D system. In the present study, we have used MGIT-960 media for growing the mycobacterium. This culture has received its name from Mycobacterial Growth Indicator Tubes and contains modified Middlebrook 7H9 broth along with a fluorescent indicator. This indicator gets quenched in the presence of oxygen within the tube and gives a signal [12].

In the present study processed sample (0.5-mL) was inoculated into the respective tube. The tubes were then kept at 37°C in an automated BACTEC MGIT-960 (Becton Dickinson) instrument for a maximum period of 42 days. The tubes were monitored regularly for the indication of mycobacterium growth. The time of the positive result was noted in positive-flagged tubes. To visualize the typical serpentine morphology of the mycobacterium tuberculosis (tightly packed rope-like aggregates) showed when they are grown on the culture medium ZN staining was performed [13]. The sterility of the culture media was also checked in brain heart infusion agar media. Drug susceptibility testing using an indirect method was also conducted using anti-TB drugs isoniazid (0.1 µg/ml) and rifampicin (1.0 µg/ml).

Xpert® MTB/RIF:

The CBNAAT was performed with 1 ml of the sputum sample. The samples were analyzed by CBNAAT on Xpert® MTB/RIF manufactured by Cepheid. This technique was endorsed by WHO in 2010. For details of the procedure, the samples were diluted three times with the reagents provided with the kit. Incubated at room temperature and loaded onto a cartridge for automated analysis. The results of this test come within 100 minutes after it is loaded. The drug sensitivity test for rifampicin was carried out in the same setting. Similarly, the detection of mycobacterium also was detected in the same process.

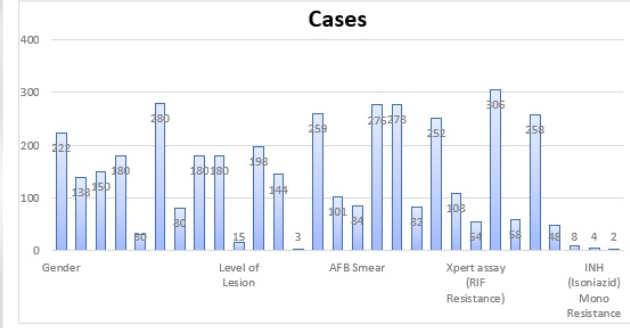
LPA

DNA of Mycobacteria was extracted using a kit base manual method. 1 mL of decontaminated sputum sample was used for DNA extraction. Polymerase chain reaction (PCR) was performed using pre-made amplification mixes. The amplification mixture A and amplification mixture B contained all the necessary components required for the PCR.

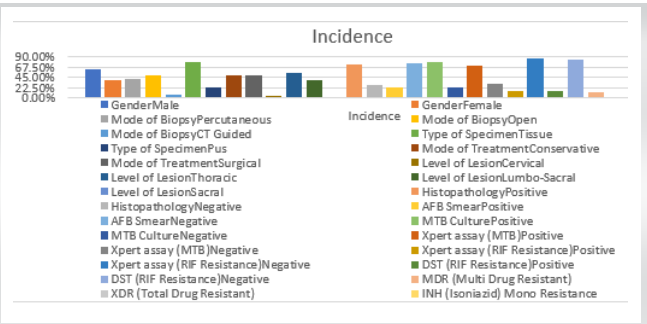
The rapid hybridization was conducted using an automated GT Blot 48 device (Hain Lifescience, Nehren, Germany). The results were noted as per the instruction manual provided by the manufacturer with the machine [14].

Statistical analysis

Statistical analysis was performed using SPSS software (statistical package for social sciences) All data are presented as a percentage. The graph was also plotted based on the results obtained from the study.



Graph 1: The general description of cases encountered in the study



Graph 2: Incidence pattern in the study population

Results

In this study total of 150 patients were included who had ages ranging from 16-77 years with 93 male and 57 female patients. The sample size comprises of 61.6% of the males and 38.3% of females.

Percutaneous mode of biopsy was performed in 41.60% of samples while open mode of biopsy was performed in 50%. In 77.7% of the tissue samples were obtained from the patients and pus was in 22.2%. Out of the total 150 patients, 75 we're managed nonsurgicaly with antitubercular therapy (ATT) and other 75 patients required surgical intervention (posterior pedicle screw fixation with transpedicular/transfacetal decompression) followed by ATT. In 4.16% cases the lesion was cervical, 55% of the cases it was thoracic, lumbo-sacral in 40% and sacral was in 0.8%.

The samples were showed positive result histopathologically in 71.90% cases and 28.05% was negative. AFB Smear was positive in 23.30% and 76.70% was negative. MTB Culture was positive for 77.20%, 22.70% was negative. Xpert assay (MIB) was found positive in 70% of the sample cases and Xpert assay (RIF resistance) was negative for 85% people and 13.33% was found Multi Drug resistant, for XDR, incidence was found in 2.20%. Isoniazid resistance was found in 1.1% of the samples and Rifampicin was found in 50% of the sample taken for the study.

When the study results were compared Gene Expert showed a 100% sensitivity and 80% of specificity. When we compared the histopathology results with gene expert, we get a sensitivity of 16.7% and specificity of 50%. The specificity study of TB MGIT is 75% and its specificities are 100% when compared or associated with Gene expert.

Gram stain with the sensitive gene expert, the sensitivity is 45.5% and specificity is 25%. Similar analysis was done with sensitive gene and now with the resistant gene to identify their sensitivity was 0%

Table 1: Sensitivity pattern				
	Sensitive			Total
		Negative	Positive	
sVAR00001 Count	40	0	0	40
% within Sensitive	100.00%	0.00%	0.00%	26.70%
Negative Count	0	10	20	30
% within Sensitive	0.00%	16.70%	40.00%	20.00%
Positive Count	0	50	30	80
% within Sensitive	0.00%	83.30%	60.00%	53.30%

Table 2: resistance pattern				
	Resistant			Total
		Negative	Positive	
VAR00001 Count	40	0	0	40
% within Resistant	100.00%	0.00%	0.00%	26.70%
Negative Count	0	20	10	30
% within Resistant	0.00%	20.00%	100.00%	20.00%
Positive Count	0	80	0	80
% within Resistant	0.00%	80.00%	0.00%	53.30%

Table 3: Histopathology result				
	Histopathology			Total
		Negative	Positive	
Negative Gene Count	30	0	10	40
% within Histopathology	42.90%	0.00%	16.70%	26.70%
Negative Count	20	10	0	30
% within Histopathology	28.60%	50.00%	0.00%	20.00%
Positive Count	20	10	50	80
% within Histopathology	28.60%	50.00%	83.30%	53.30%

Table 4: MGIT result				
	TB_MGIT			Total
		Negative	Positive	
Negative gene Count	40	0	0	40
% within TB_MGIT	50.00%	0.00%	0.00%	26.70%
Negative Count	20	0	10	30
% within TB_MGIT	25.00%	0.00%	25.00%	20.00%
Positive Count	20	30	30	80
% within TB_MGIT	25.00%	100.00%	75.00%	53.30%

Sensitive * Histopathology Crosstabulation				
	Histopathology			Total
		Negative	Positive	
Sensitive Count	30	0	10	40
% within Histopathology	42.90%	0.00%	16.70%	26.70%
Negative Count	30	0	30	60
% within Histopathology	42.90%	0.00%	50.00%	40.00%
Positive Count	10	20	20	50
% within Histopathology	14.30%	100.00%	33.30%	33.30%

Resistant * Gram_stainCrosstabulation			
	Gram_stain		Total
		Negative	
Resistant Count	30	10	40
% within Gram_stain	75.00%	9.10%	26.70%
Negative Count	10	90	100
% within Gram_stain	25.00%	81.80%	66.70%
Positive Count	0	10	10
% within Gram_stain	0.00%	9.10%	6.70%

Resistant * Histopathology Crosstabulation				
	Histopathology			Total
		Negative	Positive	
Resistant Count	30	0	10	40
% withinHistopathology	42.90%	0.00%	16.70%	26.70%
Negative Count	30	20	50	100
% within Histopathology	42.90%	100.00%	83.30%	66.70%
Positive Count	10	0	0	10
% within Histopathology	14.30%	0.00%	0.00%	6.70%

Sensitive * Gram_stainCrosstabulation			
	Gram_stain		Total
		Negative	
Sensitive Count	30	10	40
% within Gram_stain	75.00%	9.10%	26.70%
Negative Count	10	50	60
% within Gram_stain	25.00%	45.50%	40.00%
Positive Count	0	50	50
% within Gram_stain	0.00%	45.50%	33.30%

Resistant * Fungal_StainCrosstabulation			
	Fungal_Stain		Total
		Negative	
ssResistant Count	30	10	40
% within Fungal_Stain	75.00%	9.10%	26.70%
Negative Count	10	90	100
% within Fungal_Stain	25.00%	81.80%	66.70%
Positive Count	0	10	10
% within Fungal_Stain	0.00%	9.10%	6.70%

Tables

and the specificity was at 42.9%. Gram stain when correlated with gene, the sensitivity came out to be 9.1% and specificity at 25%. Fungal stain with resistant gene, when correlated, sensitivity comes to 9.1% and specificity is at 0%.

Discussion

In this present study, we have compared all the tests done for detection of TB in patient's sample including, LPA, convention culture method, Xpert MTB/RIF assay, histopathological examination, TB MGIT, gram staining, and fungal staining.

This study showed male predominance. Previous studies have also shown that TB is prevalent in males when compared to the females [15]. 13.33% was found Multi Drug resistant, for XDR, incidence was found in 2.20%. Isoniazid resistance was found in 1.1% of the samples and Rifampicin was found in 50% of the sample taken for the study.

Line probe assay is a hybridization technique that can differentiate between mycobacterium species of bacteria. It is a type of molecular assay that can detect specific DNA markers of TB associated with drug resistance from smear-positive sputum specimens after DNA extraction [13].

Xpert MTB/RIF assay is a relatively newer technology that can detect multi-drug resistance TB directly from sputum rapidly in an automated and cartridge-based molecular method. This assay is a semi-quantitative test that relies on real-time PCR for the identification of MRB complex in the patient's sample. This test can detect both MTB as well as RIF resistance. The problem of contamination between the samples is less in this technique and thus it is a more authentic method for MTB identification [10]. In the present study Xpert assay (MIB) was found positive in 70% of the sample cases and Xpert assay (RIF resistance) was negative for 85% people.

Despite the recommendations by the World Health Organization to use LPA and Xpert MTB/RIF for the identification of MTB in a patient with suspected pulmonary tuberculosis, there is not much clear about the superiority of the two techniques. Line probe assay based on a technique of reverse hybridization of DNA measures the presence of MTB along with RIF and INH resistance both on a strip. On the other hand, Xpert MTB/RIF or the cartridge-based nucleic acid amplification test relies mostly on the real-time PCR approach. The turnover time of this technique is lesser than the LPA assay. However, this technique only estimates the rifampicin resistance and hence is not ideal for the determination of multi-drug resistant TB [16].

In this present study, the maximum specificity was reported for Gene Xpert assay compared with the other techniques used in this study. TB MGIT showed higher specificity when compared with the Gene Xperts assy. It was 75% sensitive and 100% specificities were reported. However, the present study showed that some of the tests were sensitive for some samples whereas others showed positive results for the same samples. In a previous study, Rufai et al also opined that only relying on the Xpert MTB/RIF for TB detection might not prove to help detect the exact tuberculosis burden. They have suggested developing specific probes that can detect the exact burden of the disease [16].

The most important use of this technique is the lower detection time and hence this technique has gained much attention in recent time. However, the main disadvantage of this assay was its prevalence of higher false-positive results. In the past, it was reported that Xpert MTB/RIF assay showed higher false positive and negative results [17].

However, in the present study, no such incidence was reported and this study showed higher sensitivity and specificity for gene Xpert assay.

Conclusion

This study showed that for detection of tuberculosis rather than relying on a single technique detection should be done based on an array of techniques. This finding will prove to be helpful in future studies that can evaluate the efficacy of detection techniques on a larger cohort of patient samples.

Abbreviations: LPA- Liquid probe assay, MGIT- mycobacterium growth indicator tube, CBNAAT- cartridge based nucleic acid amplification test.

Declaration of patient consent : The authors certify that they have obtained all appropriate patient consent forms. In the form, the patient has given his/her consent for his/her images and other clinical information to be reported in the Journal. The patient understands that his/her name and initials will not be published, and due efforts will be made to conceal his/her identity, but anonymity cannot be guaranteed.

Conflict of Interest: None; **Source of Support:** None

References

1. Dhuria M, Sharma N, Ingle G. Impact of tuberculosis on the quality of life. Indian J Community Med Off Publ Indian AssocPrevSoc Med. 2008 Jan;33(1):58–9.
2. Sulis G, Roggi A, Matteelli A, Raviglione MC. Tuberculosis: epidemiology and control. Mediterr J Hematol Infect Dis. 2014;6(1):e2014070.
3. Garg RK, Somvanshi DS. Spinal tuberculosis: a review. J Spinal Cord Med. 2011;34(5):440–54.
4. Steingart KR, Henry M, Ng V, Hopewell PC, Ramsay A, Cunningham J, et al. Fluorescence versus conventional sputum smear microscopy for tuberculosis: a systematic review. Lancet Infect Dis. 2006 Sep;6(9):570–81.
5. Palomino JC, Martin A, Von Groll A, Portaels F. Rapid culture-based methods for drug-resistance detection in Mycobacterium tuberculosis. J Microbiol Methods. 2008 Oct;75(2):161–6.
6. Desikan P, Panwalkar N, Mirza SB, Chaturvedi A, Ansari K, Varathe R,

et al. Line probe assay for detection of Mycobacterium tuberculosis complex: An experience from Central India. Indian J Med Res. 2017 Jan;145(1):70–3.

7. Reither K, Manyama C, Clowes P, Rachow A, Mapamba D, Steiner A, et al. Xpert MTB/RIF assay for diagnosis of pulmonary tuberculosis in children: a prospective, multi-centre evaluation. J Infect. 2015 Apr;70(4):392–9.
8. Opota O, Mazza-Stalder J, Greub G, Jaton K. The rapid molecular test Xpert MTB/RIF ultra: towards improved tuberculosis diagnosis and rifampicin resistance detection. ClinMicrobiol Infect Off PublEurSocClinMicrobiol Infect Dis. 2019 Nov;25(11):1370–6.
9. Patel J, Upadhyay M, Kundnani V, Merchant Z, Jain S, Kire N. Diagnostic Efficacy, Sensitivity, and Specificity of Xpert MTB/RIF Assay for Spinal Tuberculosis and Rifampicin Resistance. Spine. 2020 Feb 1;45(3):163–9.
10. Lawn SD, Nicol MP. Xpert® MTB/RIF assay: development, evaluation and implementation of a new rapid molecular diagnostic for tuberculosis and rifampicin resistance. Future Microbiol. 2011 Sep;6(9):1067–82.
11. Buyankhishig B, Oyuntuya T, Tserelmaa B, Sarantuya J, Lucero MG, Mitarai S. Rapid molecular testing for multi-resistant tuberculosis in Mongolia: A diagnostic accuracy study. Int J Mycobacteriology. 2012 Mar;1(1):40–4.
12. Caulfield AJ, Wengenack NL. Diagnosis of active tuberculosis disease: From microscopy to molecular techniques. J ClinTubercMycobact Dis. 2016 Aug 1;4:33–43.
13. Singh BK, Sharma SK, Sharma R, Sreenivas V, Myneedu VP, Kohli M, et al. Diagnostic utility of a line probe assay for multidrug resistant-TB in smear-negative pulmonary tuberculosis. PLOS ONE. 2017 Aug 22;12(8):e0182988.
14. Crudu V, Stratan E, Romancenco E, Allerheiligen V, Hillemann A, Moraru N. First evaluation of an improved assay for molecular genetic detection of tuberculosis as well as rifampin and isoniazid resistances.J ClinMicrobiol. 2012 Apr;50(4):1264–9.
15. Zong Z, Huo F, Shi J, Jing W, Ma Y, Liang Q, et al. Relapse Versus Reinfection of Recurrent Tuberculosis Patients in a National Tuberculosis Specialized Hospital in Beijing, China. Front Microbiol. 2018;9:1858.
16. Rufai SB, Kumar P, Singh A, Prajapati S, Balooni V, Singh S. Comparison of Xpert MTB/RIF with line probe assay for detection of rifampin-monoresistant Mycobacterium tuberculosis. J ClinMicrobiol. 2014 Jun;52(6):1846–52.
17. Hillemann D, Rusch-Gerdes S, Boehme C, Richter E. Rapid Molecular Detection of Extrapulmonary Tuberculosis by the Automated GeneXpert MTB/RIF System. J ClinMicrobiol. 2011 Apr 1;49(4):1202–5.

How to Cite this Article

Shetty A, Raut S, Anand M, Kundnani V, Goparaju P, Jain M | Detection of Spinal TB Infection: A Retrospective Study Evaluating Comparative Diagnostic Efficacy of AFB Smear, Gene Expert, Histopathology, Culture Sensitivity and LPA Tests From a Biopsy Sample | International Journal of Spine | January-June 2020; 7(1): 01-06.