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A Prospective Study for Evaluation of Diagnostic Utility of Gene XPERT MTB/RIF Assay in Tuberculous Pleural Effusion

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ABSTRACT

Aim: The aim of the present study is to evaluate the diagnostic usefulness of Gene Xpert MTB/RIF assay in quick diagnosis of pleural tuberculosis, comparing it with conventional methods like pleural fluid Culture and AFB smear, histopathology of pleural biopsy tissue and determination of adenosine deaminase (ADA) in pleural fluid with lymphocytic predominance which counter to anti tubercular treatment.

Methods: This is a prospective observational study, done over a period of two years (Jan 2015 to Dec 2016) at Yashoda Super speciality Hospital, Hyderabad. After scientific and ethical committee approval, and after informed consent, the patients included were those with exudative pleural effusion and showing positive evidence in favor of pleural tuberculosis by any of these techniques (i) Pleural fluid microscopy / culture (or) (ii) Pleural biopsy and evidence of caseous necrosis / granuloma, (or) (iii) Pleural fluid ADA levels > 50 U/ml with lymphocytic predominance and response to anti tubercular treatment. And will be followed over for 3 months.

Results: In this study, the mean age of individuals is 42 years, the maximum was 85 years and minimum was 15 years. Highest numbers were among age group of 21-30 and 51-60 years, the gender distribution was approximately 2.3:1, among which 69 % were males and 31% females. The prominent symptoms were Cough (91%) with or without expectoration, fever (80%), dyspnoea (56%), anorexia and weight loss (50%), chest pain (18%), hemoptysis (3%). ZN staining was positive for Acid Fast bacilli in 4 cases (6%). Culture was not positive in any case. Histopathological examination of pleural biopsy revealed granuloma / caseous necrosis in 12 of 14 cases (85%). Gene Xpert was analysed in all cases and was positive in 12 cases of 54 patients in pleural fluid (22%) and 8 cases of 14 biopsy was positive (57%). It has specificity of 100% and Positive predictive value and negative predictive value of 100% and 68.6% respectively.

Conclusions: ADA with lymphocytosis in exudative pleural fluid remains useful diagnostic tool in clinical practice and histopathological examination of pleural tissue is useful in few uncertain cases. The yield of AFB smear and Culture is very low, making them less reliable test for accurate diagnosis. The Gene Xpert MTB/RIF assay has better yield in pleural biopsy samples compared to that in pleural fluid samples. Gene Xpert MTB/RIF assay is simple, quick and needs minimal technical expertise, but has low sensitivity but has a limited diagnostic capacity for pleural fluid samples of TB origin, which precludes its widespread implementation in this setting.

Keywords: Pleural tuberculosis; granuloma; adenosine deaminase.

INTRODUCTION

Tuberculosis is a disease of great antiquity. Tuberculous lesions have been found in the vertebrae of Neolithic men in

Europe and Egyptian mummies dating possibly as early as 3700 BC. Mycobacterium tuberculosis is a formidable pathogen and still remains a worldwide problem , despite the fact that the causative organism was discovered more than 100 years ago (by Robert Koch 1882) and effective anti tubercular drugs have been invented decades ago .Tuberculosis is not only health concern but also a complex socio economic problem that impedes human development.

In 1993 WHO took an unprecedented step of declaring tuberculosis epidemic as a global emergency. India is second most populated country of the world, and has highest burden of disease in the world, with World Health Organization giving statistical estimate of annual incidence of approximately 2.1 million cases of tuberculosis among 9 million cases worldwide and estimated prevalence of about 2.9 million cases. It is estimated about 40% of population is infected with tubercular bacilli. [1]

Although the greater parts of patients with TB have pulmonary Tuberculosis, extra pulmonary TB affecting mainly the lymph nodes and pleura serves as an initial presentation in about 25% of adults. In some countries, TB is leading cause of Pleural effusion. In western world pleural tuberculosis accounts for 1-2 % of tuberculosis cases, but in India it is probable to be around 30-40% of all cases of tuberculosis. Thus, a major factor in morbidity and mortality in India. [2]

Gene pert MTB/RIF is an automated, cartridge based nucleic acid amplification assay for the simultaneous recognition of TB and rifampicin resistance directly from sputum in less than two hours. The technology is based on the Gene Xpert platform and was developed as a partnership between the Foundation for pioneering New Diagnostic (FIND), Cepheid Inc. and University of Medicine and Dentistry of New Jersey, with support from US national institutes of Health. WHO suggested use of technology in December 2010 and is monitoring the global roll out of technology to promote coordination. [3]

MATERIALS AND METHODS

Study Design and Patient Population

This was a Prospective observational study conducted at a tertiary-care center in India between January 2015 to December 2016. Patients admitted with pleural effusion during the study period were included in the study. Patients with known Pleural fluid microscopy / culture, Pleural biopsy and evidence of caseous necrosis/ granuloma, and Pleural fluid ADA levels > 50 U/ml with lymphocytic predominance and response to anti tubercular treatment. Patient with history of anti tubercular treatment in last 2 months, Sputum positive tuberculosis, and Pleural effusions of Transudative etiology were excluded from the study. Signed inform consent forms were obtained from all the patients.

After scientific committee and ethical committee approval, the subjects included in the study would be those admitted in Yashoda Superspeciality Hospital, Hyderabad, with complains of fever, cough, pleuritic chest pain, shortness of breath or evidence of pleural effusion on physical examination and on Chest X ray.

Subjects included following informed consent, will undergo pleural fluid aspiration under sonological guidance and sent for analysis; a sample of peripheral blood will also be sent for analysis.

Pleural fluid will be analysed for total count, differential count using automated cell analyser, then sample will be centrifuged at 3000rpm for about 15 minutes and tested for Sugar level, Protein level, LDH levels, ADA levels. In microbiology department the sediment is used to prepare smear for ZN staining, Gram Staining and inoculate in culture media. Cytological examination of pleural fluid is done by preparation of cell blocks and Gene Xpert MTB/RIF assay and relevant blood investigations.

In cases where pleural fluid is inconclusive for specific etiology, or in

cases of high suspicion of Tuberculosis but not meeting criteria (ADA <50, neutrophilic predominance in pleural fluid) patient was advised to undergo pleural tissue biopsy.

ADA analysis

It is done by Guitsin and Galanti calorimetric method. ADA catalyses deamination of adenosine leading to formation of inosine and ammonia. Ammonia forms extremely blue indophenols with sodium hypochlorite and phenol in alkaline solution. Sodium nitroprusside is the catalyst. The ammonium concentration thus released by deamination by ADA is directly proportional to examination of indophenols by calorimetric method and quantified by measurement of absorbance at 628nm.

Cell count and Cytology

Part of the residue was transferred to a fresh glass slide and mixed with a part of 1% toluidine blue. After placing the cover slip, the slide was observed under the microscope for instant detection of cell morphology. Then the left over sediment was transferred with the help of a Pasteur pipette to three slides coated with albumin. One was air dried out and stained with Giemsa, the other two were fixed in 95% alcohol for a least period of 15 minutes and stained with Hematoxylin and Eosin, Papanicolaou stains.

GENE XPERT MTB/RIF assay

The assay consists of a single utilize multichambered plastic cartridge preloaded by the liquid buffer and lyophilized reagent beads essential for sample handing out, DNA extraction, and heminested real-time PCR. Clinical sample or decontaminate pellets are treat with a NaOH and isopropanol-containing sample reagent (SR). The SR is added at a 2:1 ratio to the sputum sample or sputum pellet and incubates for 15min at room temperature. The treated sample is transfer into the cartridge, the cartridge is filled to capacity into the GeneXpert instrument, and usual processes complete the remaining assay steps. The assay cartridge also contains lyophilized Bacillus globigii spores which serve as an inner sample processing and PCR manage. The spores are normally resuspended and processed throughout the sample processing step, and the resulting B. globigii DNA is amplified during the PCR step. The standard user crossing point indicate the presence or absence of M. tuberculosis, the presence or absence of RIF resistance, and a semi quantitative estimation of M. tuberculosis attentiveness (high, medium, low, and very low). Assays that are unconstructive for M. tuberculosis and also negative for the B. globigii internal control are reported as invalid.

The PCR assay amplifies a 192-bp part of the M. tuberculosis rpoB gene in a heminested real-time PCR. The inside control heminested B. globigii assay is multiplexed with the M. tuberculosis assay. M. tuberculosis is detect using five overlap molecular beacon probes (probes A to E) that are balancing to the entire 81-bp RIF resistance-determining "core" area of the wild-type rpoB gene. Mutations in the rpoB gene objective inhibit hybridization of one or additional of the rpoB-specific molecular beacons, plummeting or eliminating the indication from the corresponding probes. M. tuberculosis is recognized when at least two of the five rpoB-specific molecular beacons provide a positive signal with cycle threshold (C_T) values that are ≤38 and that be at variance by no more than two cycles. B. globigii DNA is detected when the single B. globigii molecular beacon produces a C_T of <38 cycles.

Pleural biopsy:

Patient in case of tentative results underwent Pleural Biopsy examination after informed consent, under guidance of imaging, and under appropriate anesthesia and strict aseptic precautions. Multiple biopsy samples were collected from upper margin of the rib. The sample was placed in formalin containing containers and sent for histopathological examination, ZN staining, Culture and Gene Xpert assay.

Statistical Analysis

Data entry was done by Microsoft Excel 2007 version. Data was analysed using SPSS version 22, Descriptive statistics; chi-square test was done to evaluate the sensitivity and specificity of the Gene Xpert.

Sample size calculation

$$n = \frac{Z^2\, p(1-p)}{d^2}$$

Where:

n = sample size

Z = constant (95% C.I. used, thus Z value is 1.96)

p = expected prevalence of proportion

d = precision

RESULTS

In this study, 162 subjects of pleural effusion were analysed, 68 cases were diagnosed as probable cases of tuberculous pleural effusion, remaining were non tuberculous effusions. Among 68 cases of tuberculous pleural effusion, 54 were diagnosed based on criteria of ADA >50 U/L and lymphocytosis and response to treatment; 14 were diagnosed on basis of detection of caseous necrosis on pleural biopsy or Demonstration of AFB bacilli. Among 68 cases 4 cases had AFB smear positive. In this study, the mean age of individuals is 42 years, the maximum was 85 years and minimum was 15 years. Highest numbers were among age group of 21-30 and 51-60 years (Table-1). The Gender distribution was approximately 2.3:1, among which 69 % were males and 31% females (Table-2). The prominent symptoms were Cough (91%) with or without expectoration, fever (80%), dyspnoea (56%), anorexia and weight loss (50%), chest pain (18%), hemoptysis (3%) (Table-3). The co-morbidities like diabetic (25%), hypertensive (26.4%), chronic kidney disease (CKD) (7.4%), and COPD (1.5%) (Table-4). The pleural fluid was right sided in 48%, and left sided in 40% and bilateral in 12% cases (Table-5). The average pleural fluid protein level was – 5.6 mg/dl, average pleural fluid ADA level was -89 U/L, and all cases had lymphocytosis, ZN staining was positive for Acid Fast bacilli in 4 cases (6%), culture was not positive in any case (Table-6), histopathological examination of pleural biopsy revealed granuloma / caseous necrosis in 12 of 14 cases(85%) (Table-7) and Gene Xpert was analysed in all cases and was positive in 12 cases of 54 patients in pleural fluid (22%) and 8 cases of 14 biopsy was positive (57%) (Table-8). It has specificity of 100% and Positive predictive value and negative predictive value of 100% and 68.6% respectively.

Table 1: Distribution of study population according to age group

Age Group	Frequency	Percentage
10-20	8	11.8
21-30	15	22.1
31-40	8	11.8
41-50	11	16.2
51-60	15	22.1
61-70	6	8.8
> 70 years	5	7.4
Total	68	100.0

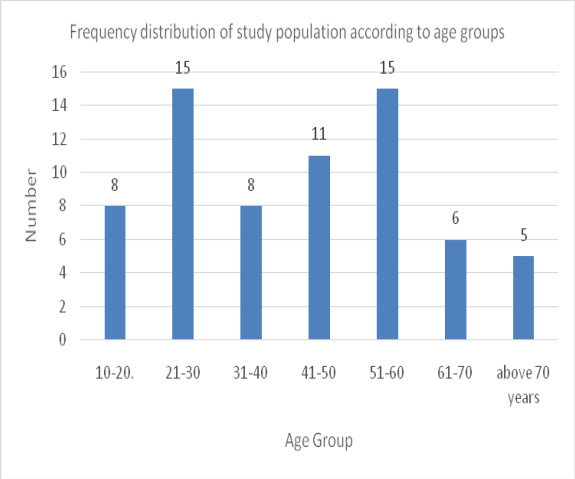


Table 2: distribution of study population according to gender

Gender	Frequency	Percentage
Male	47	69.1
Female	21	30.9

Table 3: frequency distribution according to clinical findings

Clinical Features (N=68)	Percentage
Dysponea	55.9
Cough	91.2
Expectoration	23.5
Chest Pain	17.6
Haemoptysis	3
Fever	79.4
Weight Loss	50
Anorexia	79.4

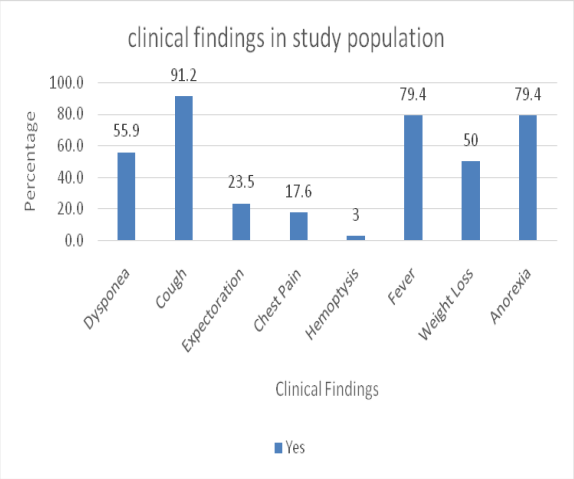


Table 4: Presence of associated co morbidities

Co - Morbidities	Yes
	Percentage
DM	25.0
HTN	26.4
CKD	7.4
COPD	1.5

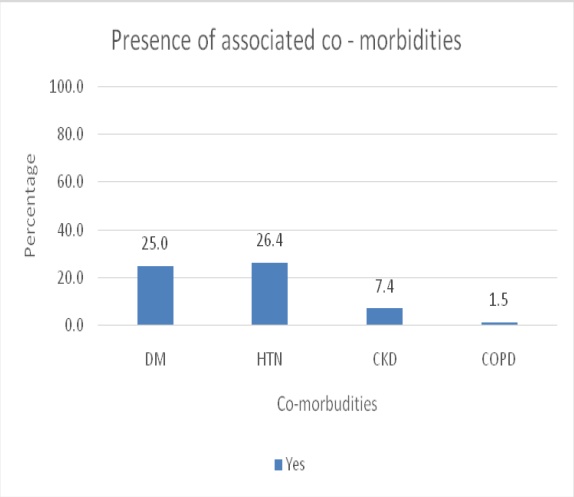


Table 5: Side of involvement

Side of involvement	Frequency	Percent
Bilateral	8	12
Left	27	40
Right	33	48.5
Total	68	100.0

Table 6: Zn Staining

ZN Stain	Frequency	Percentage
Positive	4	5.9
Negative	64	94.1
Total	68	100.0

Table 7: Histopathological examination

Histopathology	Frequency	Percentage
Not Performed	54	79.4
Granuloma	12	17.6
Normal	2	3
Total	68	100.0

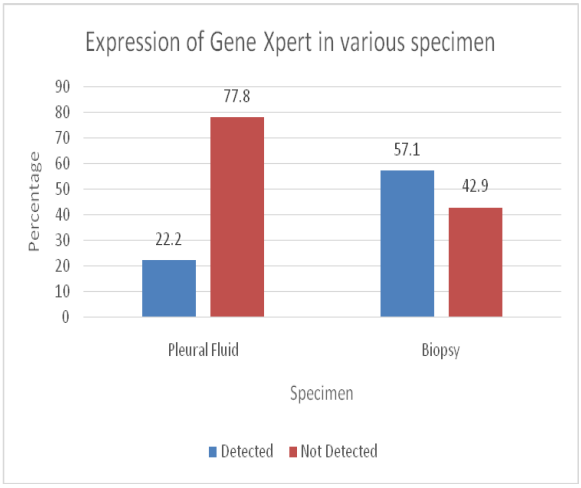
Table 8: Expression of Gene Xpert

Gene Xpert	Pleural Fluid	Biopsy	Total
Detected	12 (22.2%)	8 (57.1%)	20 (29.4%)
Not Detected	42 (77.8%)	6 (42.9%)	48 (70.6%)
Total	54 (100%)	14 (100%)	68 (100%)
Chi sq	6.53	P Value	0.011*

*-Statistically Significant (p<0.05)

Inference:

There is statistically significant difference present in the detection of disease in both the procedures with higher rate of detection in biopsy than the pleural fluid.



DISCUSSION

The Gene Xpert MTB/RIF assay, which can simultaneously detect the presence of M. tuberculosis and rifampicin resistance in sputum specimen, has revealed immense assure in the rapid analysis of TB, with an average sensitivity and specificity of 90.4% and 98.4% correspondingly. [4] It has also better the fast judgment of pulmonary TB (sensitivity of ~68%) in smear-negative patients and recently been endorsed by the Scientific and Technical Advisory Board of the WHO for use in paucibacillary samples. A recent study by Boehme et al. successfully showed the use of the Xpert test for point-of-care treatment in low-income countries for the detection of RIF resistance in pulmonary TB cases. Along with high specificity, the study showed a sensitivity of 90% for smear-negative pulmonary TB cases. Vadwai et al. concluded that Gene Xpert MTB/RIF test not only has good sensitivity and specificity for the diagnosis of TB and detection of RIF

resistance in EPTB but also perfectly fits the requirements of the Indian health care setting. [5]

The elevated quantity of pleural TB cases in the middle of patients presenting through exudative pleural effusion obtain in our study is alike to an previous report from the identical setting where 91% of patients with exudative pleural effusion had pleural TB. TB has also been reported as the commonest reason of pleural effusions in additional settings. This judgment confirms TB as the commonest source of exudative pleural effusions in the majority settings particularly anywhere the occurrence of TB is elevated.

Patients among tuberculous pleurisy tend to be younger than patients with pulmonary tuberculosis (TB), but, in industrialized countries the mean age of patients with tuberculous pleurisy tend to be older. [6] In this study, the mean age of patients was 42 years; minimum age was 15 years and maximum was 84 years. In similar study Meldau et al. had mean age of 39 years, Lusiba et al. had mean age of 32 years, Christopher et al. had median age of 46 years, Porcel et al. had mean age of 33 years. The disease was more common in males that in females (males 69%: females 31%). In previous studies Meldau et al had 40% female population, Lusiba et al had 44.8% female population, Christopher et al. had 20% female population and Porcel et al. had 63.7% of female population. The predominat symptoms were cough (91%), fever (80%) and dyspnoea (56%). In previous study by Christopher et al. also had almost all patients with this symptoms. Number of patients with co-morbidities were 25%, alike to those incorporated in study by Christopher et al (29%).

The Average ADA content of pleural fluid was 89 U/L , which well above the cut off level as mentioned in previous studies Verma et al.-36U/L, [7] Niwa et al.-38U/L, [8] Rodriguez et al.-37 U/L(15), [9] Jindal et al.-40 U/L [10] for tuberculous pleural effusion. It was positive among four patients in total 68 cases examined, similar results were observed in study by Christopher et al. [11] in which 1 case showed AFB among 33 cases examined. Culture was not found positive for Mycobacterium tuberculosis in pleural fluid or pleural biopsy tissue, similar results were also found in Hilleman et al. [12] in which pleural fluid had no positive cultures, Christopher et al. also had no positive cultures in pleural fluid though 3 pleura biopsy tissue were positive for M. tuberculosis. The reason could be (i) Paucibacillary nature of the disease, (ii) pre treatment of the sample before inoculation, (iii) inappropriate technique.

Gene Xpert MTB/RIF assay was positive in 12 pleural fluid samples among 54 cases examined, thus Sensitivity of 22.22% (C.I 12.04% to 35.60%) Specificity of 100% (C. I 96.38% to 100.00%). Similar results were also found in studies of Porcel et al. ,Meldau et al., and Christopher et al, Sharma et al. [13] sensitivity and specificity being 15% and 100%,22.5% and 98% , 13.3% and 100%, 24% and 99% respectively.

Gene Xpert MTB/RIF assay was positive in 8 pleural tissue biopsy among 14 cases examined, thus Sensitivity 57.14% (C.I 28.86% to 82.34%) and Specificity 100% (C. I 96.38% to 100.00%). In a study by Christopher et al. no cases were positive for Gene Xpert MTB among 33 cases examined. In a study by Vadwai et al. and Tortolli et al. Gene Xpert on different biopsy tissues showed sensitivity of 75% and 88.3% respectively.

The poor sensitivity of the Xpert test probably reflects the low mycobacterial load, and consequently DNA, in tuberculous pleural fluid. Although the sensitivity of Xpert is 2.5 to 5-fold that of pleural fluid smear microscopy, it is far from satisfactory from the clinical point of view. In adopting a rapid test for the identification of M. tuberculosis in pleural fluid, the simplicity, reliability and rapid results of the Xpert assay should be balanced against its comparatively high cost and the advantages of alternative tests (e.g., smear microscopy,

which is rapid and contemptible, and the report high sensitivity of viable semi-automated NAA techniques).

CONCLUSION

It has been reaffirmed that Tuberculosis is most important cause of exudative pleural effusion. ADA with lymphocytosis in exudative pleural fluid remains useful diagnostic tool in clinical practice and histopathological examination of pleural tissue is useful in few uncertain cases. The yield of AFB smear and Culture is very low, making them less reliable test for accurate diagnosis. The Gene Xpert MTB/RIF assay has better yield in pleural biopsy samples compared to that in pleural fluid samples. Gene Xpert MTB/RIF assay is simple, quick and needs minimal technical expertise, but has low sensitivity but has a limited diagnostic capacity for pleural fluid samples of TB origin, which precludes its widespread implementation in this setting.

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Conflict of interest: None

REFERENCES

1. TB Statistics India | Country wide and state statistics [Internet]. [cited 2015 Sep 29]. Available from: http://www.tbfacts.org/tb-statistics-india/
2. Udwadia ZF, Sen T. Pleural tuberculosis: an update. Curr Opin Pulm Med [Internet]. 2010 Jul [cited 2015 Sep 29];16(4):399–406. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20531085.
3. WHO | TB diagnostics and laboratory strengthening. [cited 2015 Nov 26]; Available from: http://who.int/tb/laboratory/mtbrifrollout/en
4. Chang K, Lu W, Wang J, Zhang K, Jia S, Li F, et al. Rapid and effective diagnosis of tuberculosis and rifampicin resistance with Xpert MTB/RIF assay: A meta-analysis. J Infect [Internet]. 2012 Jun [cited 2017 May 7]; 64(6):580–8. Available from: http://linkinghub.elsevier.com/retrieve/pii/S0163445312000424.
5. Vadwai V, Boehme C, Nabeta P, Shetty A, Alland D, Rodrigues C. Xpert MTB/RIF: a new pillar in diagnosis of extrapulmonary tuberculosis?. Journal of clinical microbiology. 2011 Jul 1;49 (7):2540-5.
6. Jeon D. Tuberculous pleurisy: an update. Tuberc Respir Dis (Seoul) [Internet]. 2014 Apr;76(4):153–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24851127.
7. Verma SK, Dubey AL, Singh PA, Tewerson SL, Sharma D. Adenosine Deaminase (ADA) Level in Tubercular Pleural Effusion. Lung India [Internet]. 2008 Jul [cited 2017 May 23];25(3):109–10. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20165661.
8. Niwa Y, Kishimoto H, Shimokata K. Carcinomatous and Tuberculous Pleural Effusions: Comparison of Tumor Markers. Chest. 1985;87(3):351–5.
9. Peréz Rodríguez E, Ferrando C, Flandes J. Adenosine deaminase in pleural effusion. Chest. 1992 Jul;102(1):325–6.
10. Jindal SK. Adenosine deaminases in pleural effusions. J Assoc Physicians India. 1993 Sep;41(9):620.
11. Christopher DJ, Schumacher SG, Michael JS, Luo R, Balamugesh T, Duraikannan P, et al. Performance of Xpert MTB/RIF on pleural tissue for the diagnosis of pleural tuberculosis. Eur Respir J [Internet]. 2013;42(5). Available from: http://erj.ersjournals.com/content/42/5/1427.
12. Hillemann D, Rusch-Gerdes S, Boehme C, Richter E. Rapid Molecular Detection of Extrapulmonary Tuberculosis by the Automated GeneXpert MTB/RIF System. J Clin Microbiol [Internet]. 2011 Apr 1 [cited 2017 May 7];49(4):1202–5. Available from: http://jcm.asm.org/cgi/doi/10.1128/JCM.02268-10
13. Sharma SK, Kohli M, Chaubey J, Yadav RN, Sharma R, Singh BK, et al. Evaluation of Xpert MTB/RIF assay performance in diagnosing extrapulmonary tuberculosis among adults in a tertiary care centre in India. Eur Respir J [Internet]. 2014;49(6). Available from: http://erj.ersjournals.com/content/early/2014/07/24/09031936.00059014

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