
**P-ISSN:** 2664-3685
**E-ISSN:** 2664-3693
www.paediatricjournal.com
IJPG 2021; 4(2): 15-19
Received: 10-05-2021
Accepted: 14-06-2021

**Dr. Ranjeeta Dadoria**
Assistant Professor, Department of Paediatrics, Subharti Medical College, Meerut, Uttar Pradesh, India

**Dr. Falak Bansal**
Senior Resident, Department of Paediatrics, Subharti Medical College, Meerut, Uttar Pradesh, India

**Corresponding Author:**
**Dr. Ranjeeta Dadoria**
Assistant Professor, Department of Paediatrics, Subharti Medical College, Meerut, Uttar Pradesh, India

# Role of CBNAAT in diagnosis of tuberculous meningitis in children

**Dr. Ranjeeta Dadoria and Dr. Falak Bansal**

**DOI:** https://doi.org/10.33545/26643685.2021.v4.i2a.152

**Abstract**
**Background and Objectives:** CB-NAAT is a semi-quantitative nested real-time PCR which detects both MTB and RIF resistance directly from clinical specimens. This study was conducted to measure the diagnostic yield of CBNAAT in diagnosis of tuberculous meningitis (TBM).
**Methods**: The present study was conducted among 62 children suffering from TBM in the department of Paediatrics at Teerthanker Mahaveer Medical College & Research Centre from November 2019 to July 2020. Complete physical examination was done including level of consciousness, signs of meningeal irritation (neck stiffness, Kernig's sign, Brudzinski's sign), cranial nerve involvement, etc. Following a lumbar puncture with standard and sterile method, about 10 ml CSF was obtained, transported to the laboratory within 1 h, and divided into four tubes: 1 (1-2 ml) for CSF cells, 2 protein and glucose, 3 bacterial smear and culture and 4 (8-10 ml) for TB PCR testing. Data so collected was tabulated in an excel sheet and analysed using SPSS 24.00 for window (SPSS inc, Chicago, USA).
**Results**: Out of the 62 patients of TBM, 9 patients (14.5 %) had a positive CBNAAT in CSF while only one patient (1.61 %) had positive result for Ziehl-Neelsen staining for acid fast bacilli. In our study, sensitivity, positive predictive value and diagnostic efficacy CBNAAT was 47.30%, 98.20% and 47.04% respectively.
**Conclusion**: It can be concluded from the results that CBNAAT is an efficient technique for detection of tuberculous meningitis in CSF samples. Its simplicity, speed and automation, and detection of resistance at the same time makes this technique a very attractive tool for the rapid diagnosis of TB meningitis, especially in suspected cases.

**Keywords:** TBM, CBNAAT, CSF

## Introduction
Tuberculosis (TB) remains one of the oldest and known communicable diseases. Worldwide, TB is one of the top 10 causes of death and the leading cause from a single infectious agent [1]. India is the highest TB burden country in the world, accounting for about 23.3% of the global prevalence and estimated incidence being 2.84 million cases [2]. Among various forms of extrapulmonary TB, tuberculous meningitis (TBM) is the most severe form and remains a major global health problem with the case fatality rate for untreated TBM reaching almost 100%, even after more than 100 years [3]. Early recognition of TB meningitis is of paramount importance because the clinical outcome depends greatly upon the stage at which the therapy is initiated and delay in treatment often leads to permanent neurological damage [4].
Globally the exact burden of childhood TB is not well documented, it is estimated that childhood TB constitutes about 10–20% of all TB cases, in high burden countries and TB remains one of the leading cause of childhood mortality and morbidity [5]. India accounts for 6% incidence of pediatric TB cases in a population that has 40% as estimated latent TB cases. Pediatric samples were considered as the key population in the study. TB remains the most common cause of childhood meningitis in high burden countries [6].
The cornerstone of TB control remains early diagnosis and treatment [7]. TB control has greatly benefited from the advent of newer diagnostic tests including use of liquid culture media and nucleic acid amplification tests such as line probe assay and Xpert MTB/RIF. While smear microscopy has poor sensitivity and issues related to quality control, conventional solid culture techniques have the limitation of long turnaround time of several weeks. Such delays in diagnosis increase morbidity and mortality predispose to secondary resistance and cause transmission of resistant strains [8].
The CB-NAAT is a semi-quantitative nested real-time PCR which detects both MTB and RIF resistance directly from clinical specimens.

It is the WHO-recommended method in 2010 for the diagnosis of both pulmonary and extrapulmonary TB and for diagnosing paediatric TB [9]. The advantages of this test include high sensitivity and specificity, low complexity, low cost, wide availability and less manpower involved. This test is found useful in diagnosis and management of suspected cases of pulmonary tuberculosis.

As smear-negative patients form the bulk of cases and delay in diagnosis in this subset often leads to increased morbidity and mortality, so if found superior to mycobacterial cultures that are the gold standard, CB-NAAT can rapidly detect the mycobacteria and rule out rifampicin resistance on the same day, helping in the diagnosis and management of these patients. Hence the aim of the present study was to measure the diagnostic yield of CBNAAT in diagnosis of tuberculous meningitis.

**Material & methods**

The present study was conducted in the department of Paediatrics at Teerthanker Mahaveer Medical College & Research Centre from November 2019 to July 2020.

**Sample size**

Sample size was calculated using the following formulae:

$n= z^2pq/L^2$

Where n= sample size

p= proportion in the target population estimated to have TBM (prevalence is 3%)

q= 100-p

L= allowable error (5% of p)

z= point on normal deviation (1.96) with confidence interval taken as 95%

So to calculate, prevalence was taken as 3%, therefore q=97%

On calculation "n" was equal to 44.69,

Samples taken in this study, n= 62.

**Inclusion criteria**

Patients with CSF showing features of pleocytosis, predominantly lymphocytosis, moderately decreased CSF glucose levels, high CSF protein levels and supported by the following criteria:

1. Patients having clinical features of meningitis with signs of meningeal irritation like neck stiffness and or positive Kernig's / Brudzinski's sign.
2. Patients with a sub-acute onset of symptoms (>5 days) and a positive contact history.
3. MRI brain findings suggestive of tuberculous meningitis / central nervous system tuberculosis.
4. Presence of tuberculosis elsewhere (e.g. miliary tuberculosis, pulmonary tuberculosis or cervical lymphadenopathy with histology compatible with tuberculosis).

**Exclusion criteria**

1. Patients refusing to give consent for the study.
2. Patients with bacterial meningitis including cases of partially treated pyogenic meningitis
3. Patients refusing to give consent for lumbar puncture.
4. Patients in whom ATT was started without lumbar puncture. (e.g. miliary tuberculosis)
5. Cases where CSF sample could not be processed in time due to any reason and had to be discarded.

**Materials**

1. CSF Samples after lumber puncture
2. Gastric Aspirate
3. FNAC of cervical lymph nodes

Cerebrospinal fluid culture for Mycobacterium tuberculosis was not done due to non availability of resources

**Methods**

1. Ethical approval was obtained for the study from the Ethics Committee of the institute.
2. Written informed consent was taken from the parents.
3. Every patient was interviewed and relevant history was taken
4. Detailed workup for meningitis including blood investigations, imaging (Chest X-ray, computed tomography [CT] scan and/or magnetic resonance imaging as and when indicated).
5. Following a lumbar puncture with standard and sterile method, about 10 ml CSF was obtained, transported to the laboratory within 1 h, and divided into four tubes: 1 (1-2 ml) for CSF cells, 2 protein and glucose, 3 bacterial smear and culture and 4 (8-10 ml) for TB PCR testing.
6. Complete history including past or family history of tuberculosis was taken. Complete physical examination was done including level of consciousness, signs of meningeal irritation (neck stiffness, Kernig's sign, Brudzinski's sign), cranial nerve involvement, etc.
7. Lumbar Puncture was done after ruling out papilloedema after taking consent and CSF was subjected to cytology, biochemistry, smear for AFB, culture and CBNAAT. Other investigations like TLC, DLC, blood culture, Mantoux test (Using 2TU and reading of > 10 mm, after 48 hours etc were done. Chest Xray and neuroimaging were done in relevant cases. Gram staining was done for all 62 CSF samples to rule out any pyogenic organisms. Bacterial culture in blood sample was done for all cases for identification of non mycobacterial / pyogenic organisms, as yields are expected to be positive in > 60% cases of pyogenic meningitis.

**Specimen processing**: The preferred processing method for CSF in Xpert MTB/RIF depends on the volume of sample available for testing.

**a. More than 5 ml of CSF**

1. Transfer all of the sample to a conical centrifuge tube and concentrate sample at 3000g for 15 minutes
2. Carefully pour off the supernatant through a funnel into a discard can containing 5% phenol or other mycobacterial disinfectant
3. Re-suspend the deposit to a final volume of 2ml with Xpert MTB/RIF sample reagent.
4. Label an Xpert/MTB/RIF cartridge with the sample ID
5. Using a fresh transfer pipette, transfer 2ml ml of the concentrated CSF sample to the Xpert MTB/RIF cartridge
6. Load the cartridge into the GeneXpert instrument as per manufacturer's instructions

**b. 1-5 ml of CSF (including blood-stained or xanthochromic samples)**

1. Add an equal volume of the CSF to the sample reagent
2. Add 2ml of the sample mixture directly to the Xpert MTB/RIF cartridge
3. Load the cartridge into the GeneXpert instrument as per manufacturer's instructions

**C. 0.1-1ml of CSF**

1. Re-suspend the CSF to a final volume of 2 ml with Xpert MTB/RIF sample reagent.
2. Add 2ml of the sample mixture directly to the Xpert MTB/RIF cartridge
3. Load the cartridge into the GeneXpert instrument as per manufacturer's instructions

**d. Less than 0.1ml**

1. Insufficient sample for testing in the Xpert MTB/RIF assay.

**Statistical analysis**: Data so collected was tabulated in an excel sheet, under the guidance of statistician and analysed using SPSS 24.00 for window (SPSS inc, Chicago, USA). Diagnostic tests (sensitivity, specificity and diagnostic accuracy) were used to analyse the CBNAAT efficacy and the level of significance was set at $p < 0.05$.

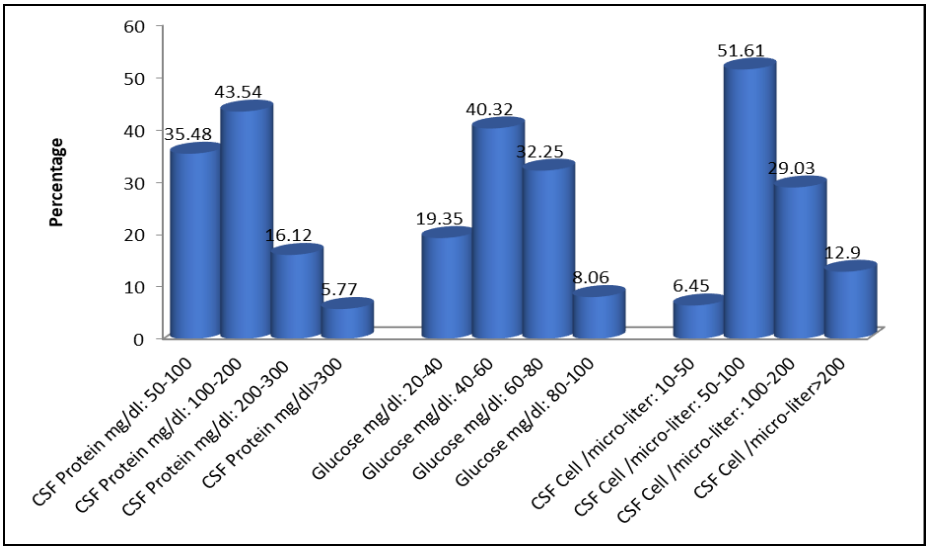
**Results**

In the demographic profile, we had (36) 58.06% male and 26 (41.93%) female cases (ratio of 1.4:1). In our study, mean±SD age was 11.55±4.39 years (range five month – 16years and 2 month). Maximum numbers of subjects were from the age group of 11-18 years (75.80%) and 6-10 years age group was least susceptible accounting for only 8.06% of cases. Fever (54 nos., 87.09%); headache (42 nos. & 67.74%) and vomiting (28nos & 45.16%), were the most common presenting symptoms. More than the half of all cases reported a history of contact with a person having tuberculosis (32 nos. and 51.61%). Other common symptoms were jerky body movements –seizures—in 24 cases (38.69%) and cough (20 nos. and 32.25%). On examination of nervous system, cranial nerve palsy (most commonly VII cranial nerve in majority of cases) was found in 24 cases (38.69%) but focal motor deficit (13 cases, 20.96%) and loss of consciousness (7 cases & 11.29%) were less common. Weight loss as reported by care givers or emaciation on examination was also fairly common (22 cases, 35.48%) as shown in table 1.

**Table 1:** Demographic Profile and clinical features of Cases

| Parameter | N (%) |
|---|---|
| **Gender** | |
| Female | 26 (41.93) |
| Male | 36 (58.06) |
| Age (years) | Mean ± SD: 11.55±4.39 (5mo – 16y 2 mo) |
| 0-5 | 10 (16.13) |
| 6-10 | 5 (8.06) |
| 11-18 | 47 (75.80) |
| **Feature** | |
| Fever | 54 (87.09) |
| Headache | 42 (67.74 ) |
| Positive Mantoux test | 41 (66.14 ) |
| History of TB contact | 32 (51.61) |
| Vomiting | 28(45.16) |
| Weight Loss | 22 (35.48) |
| Seizure | 24 (38.69) |
| Cranial Nerve Palsy | 24 (38.69) |
| Cervical Lymph-adenopathy | 15 (24.19) |
| Cough | 20 (32.25) |
| Focal Motor Defect | 13 (20.96) |
| Loss of Consciousness | 7 (11.29) |

In our study, lumbar puncture was performed in all 62 cases to obtain CSF sample. All cases had raised CSF protein and majority (more than 75%) had it between 50-200 mg/dl. CSF analysis for glucose levels revealed a reduction in about 60% cases and in 20% cases CSF sugar level was below 40mg/dl. On cytological examination, pleocytosis was found in all cases with lymphocyte predominance. Majority of patients (32, 51.61%) had cell counts in the range of 10-50 cells/micro-liter (Graph 1).




**Graph 1:** Cytology and Biochemistry Findings In (CSF)

Out of the 62 patients of TBM, 9 patients (14.5 %) had a positive CBNAAT in CSF while only one patient (1.61 %) had positive result for Ziehl -Neelsen staining for acid fast bacilli. A total of 16 cases (9: CSF; 5: Gastric Aspirate; 2:

FNAC of cervical LN) were having a positive CBNAAT out of 62 in various samples. On the other hand, only five samples (1: CSF; 4: Gastric Aspirate, 0: FNAC of cervical LN) demonstrated AFB (M. tuberculosis) on ZN staining in our study. Rifampicin resistance was detected in 1 subject (1.62%) on CBNAAT in CSF (table 2).

**Table 2:** Isolation of M. tuberculosis in various samples

| Sample | Test | Result | |
|---|---|---|---|
| | | + | - |
| CSF (n=62) | ZN Stain* | 1 | 61 |
| CSF | CBNAAT** | 9 | 53 |
| Gastric Aspirate (n=10) | ZN Stain* | 4 | 6 |
| Gastric Aspirate | CBNAAT** | 5 | 5 |
| FNAC# cervical LN (n=15) | ZN Stain* | 0 | 15 |
| FNAC of cervical LN## | CB NAAT** | 2 | 13 |

*Ziehl-Neelsen stain, ** Cartridge based nucleic acid amplification test, # Fine needle aspiration and cytology, ## Lymph Nodes

In our study, sensitivity, positive predictive value and diagnostic efficacy CBNAAT was 47.30%, 98.20% and 47.04% respectively.

## Discussion

Emphasis for one last decade has shifted towards the demonstration of M. tuberculosis for the diagnosis of TB. In children it is challenging, more so in resource-limited, tuberculosis-endemic countries. A series of meta-analyses have shown cartridge based nucleic acid amplification test (CBNAAT)/Xpert MTB/RIF to have a high specificity with variable sensitivity in different type of specimens for microbiological diagnosis [10]. Gene Xpert or CBNAAT (Cartridge Based Nucleic Acid Amplification Test) is a real time PCR test approved by WHO Policy in 2010, initially used in diagnosing MDR-TB and HIV associated TB. RNTCP policy update in 2013 expanded its uses, including for the diagnosis of TB in children, on selected specimens for the diagnosis of extra-pulmonary TB and for all individuals suspected of having pulmonary TB85. As the facility is relatively new, there are variable reports of its usefulness for diagnosis of extrapulmonary tuberculosis in children and more so in children suffering from tuberculous meningitis. There are reports that isolation of Mycobacterium tuberculosis may be as high as 59.3% to as low as 9% [11] suggesting need for more data and better understanding of what to expect from this new test.

A definitive diagnosis of tuberculous meningitis requires demonstration of Mycobacterium tuberculosis in addition to changes in CSF. Out of the 62 patients of TBM, 9 patients (14.5 %) had a positive CBNAAT in CSF while only one patient (1.61 %) had positive result for Ziehl -Neelsen staining for acid fast bacilli. A total of 16 cases (9: CSF; 5: Gastric Aspirate; 2: FNAC of cervical LN) were having a positive CBNAAT out of 62 in various samples. On the other hand, only five samples (1: CSF; 4: Gastric Aspirate, 0: FNAC of cervical LN) demonstrated AFB (M.tuberculosis) on ZN staining in our study. Rifampicin resistance was detected in 1 subject (1.62%) on CBNAAT in CSF.

Lavanya SR *et al*. [12] in their study revealed that MTB was detected in 9 CSF samples out of 100 (9%) sent for Gene Xpert technique and 1 with error. Ashi Singh reported 22 positive results for CB NAAT out of a total of 57 tuberculous meningitis cases (38.6%) Both Lavanya *et al.* [12] and Ashi singh [7] have not reported the ZN stain results in CSF. Sandip Sen *et al*. [13] in a large study reported results of a total of 497 pulmonary and 68 extra pulmonary cases. Among the pulmonary cases, CBNAAT detected 43 out of 185 sputum or induced sputum sample (23.2%), 110 out of 308 gastric lavage or aspirate sample (35.7%), 0 out of 4 samples of broncho-alveolar lavage, in total 153 pulmonary samples were detected for MTB by CBNAAT out of 497 samples (30.8%). In the extra pulmonary group, 12 out of 68 samples were detected for tuberculosis, which includes 6 out of 41 CSF samples (14.6%), 3 out of 15 pleural fluid samples (20%), 0 out of 7 ascitic fluid sample, 3 out of 5 FNAC material from lymph node (60%). They reported that Mycobacterial culture detected 140 out of 565 cases (24.8%) and ZN stain detected 116 out of 565 cases (20.5%).

Sensitivity of CBNAAT in CSF has been variable among various studies. The pooled sensitivity of CBNAAT in CSF in a meta-analysis was 80.9%16.Other studies showed sensitivity ranging from 59.3% to 9 %. In our study, sensitivity, positive predictive value and diagnostic efficacy CBNAAT was 47.30%, 98.20% and 47.04% respectively. According to Ashi Singh *et al*. [7], the sensitivity of CBNAAT was 38.6% which is comparable to our study. Sandip Sen *et al*. [13] found sensitivity, specificity, positive and negative predictive values of CBNAAT in reference to gold standard culture test were 94.3%, 89.8%, 80% and 97.3% respectively. In study by Nguyen Thi Quynh Nhu *et al*. [5], X-pert MTB/ RIF was positive in 108 (59.3%) patients with sensitivity of 59.3%and specificity 99.5%. 4 cases of RIF resistance (4/108) were identified by Xpert. Patel and colleagues report the diagnostic performance of the Gene Xpert systems Xpert MTB/RIF assay for the diagnosis of TBM assay's overall sensitivity was 62%, and specificity was 95%4. In the study conducted by Sharma Kusum *et al*. [14], multiplex PCR was positive in 84.78% cases. The overall sensitivity and specificity was 86.63% and 100 % respectively. In CSF, the pooled sensitivity from meta analysis of Xpert MTB/RIF compared against culture as a reference standard was 79.5% (95% CI, 62.0-90.2%) (16 studies, 709 specimens). Various studies conducted worldwide has varied sensitivity and specificity depending on various factors such as volume, centrifugation. Despite improved diagnostic accuracy using centrifuged CSF for Xpert compared with un-centrifuged CSF, the ideal CSF volume to collect is unknown[12].

The main limitation of our study was a small study population. The second limitation being our inability to repeat CSF samples for comparing different factors such as volume and centrifugation which could have further decreased false negative result of CSF samples study population. Therefore multi centric studies with larger sample size can be carried out to further validate the results, cost effectiveness and patient acceptability. Likewise, studies on diagnostic usefulness of CBNAAT in diagnosing TB in patients infected with HIV will be useful because of the atypical clinical presentation of TB disease and the paucibacillary nature of pulmonary disease in patients with HIV. Also studies to compare Rifampicin resistance found on Xpert MTB/RIF with that of drug susceptibility tests (DST) can be done.

Thus, CBNAAT is an efficient technique for detection of tuberculous meningitis in CSF samples. Its simplicity, speed and automation, and detection of resistance at the same time

makes this technique a very attractive tool for the rapid diagnosis of TB meningitis, especially in suspected cases.

## Conclusion

In order to reach a quick diagnosis using CSF specimens, CBNAAT should be preferentially used as rapid diagnosis and treatment is a strong prognostic indicator for reduced death and neurologic deficit. Eventhough CSF cytology gives good estimate of suspected TBM patient the test is not confirmative for bacilli demonstration. Hence CBNAAT has to be endorsed in every centres as the test gives rapid result and also detects rifampicin resistance which is the major concern for every clinician. To increase the value of this test which has gained popularities in detection of MTB and its resistance in sputum samples, a good amount as well as centrifuged CSF sample has to be considered. Clear guidance should be given by WHO regarding CBNAAT testing of CSF samples in suspected TBM patients so that this rapid test would play a major role in diagnosis and treatment of one of the most common medical emergency in India.

## References


1. Tuberculosis key facts-World Health Organisation, cited at https://www.who.int. Last accessed on 11 January 2020.
2. Global Tuberculosis report. 2018. Geneva: World Health Organisation 2018.
3. Haldar S, Sharma N, Gupta VK, Tyagi JS. Efficient diagnosis of tuberculous meningitis by detection of Mycobacterium tuberculosis DNA incerebrospinal fluid filtrates using PCR. Journal of Medical Microbiology. 2009;58:616-624.
4. Thwaites GE, van Toorn R, Schoeman J. Tuberculous meningitis: More questions, still too few answers. Lancet Neurol. 2013;12:999-1010.
5. Nhu NT, Heemskerk D, Chau TT, Mai NT, Nghia HD, Loc PP, *et al*. Evaluation of GeneXpert MTB/RIF for diagnosis of tuberculous meningitis. Journal of clinical microbiology. 2014;52(1):226-33.
6. Das PK, Ganguly SB, Mandal B. Cartridge-based nucleic acid amplification test (Xpert Mycobacterium tuberculosis/rifampicin assay): An essential molecular diagnostic test for early diagnosis and initiation of treatment in childhood tuberculous meningitis and primary multidrug-resistant cases. Biomed Biotechnol Res J. 2020;4:21-5.
7. Singh A, Shukla AK, Kaur R, Kajal NC, Kaur L, Neki NS. Role of CBNAAT in diagnosis of Tuberculous Meningitis. Int. J. Curr. Res. Med. Sci. 2018;4(2):5965.
8. WHO. Global tuberculosis report 2014. Geneva: World Health Organization, 2014. Available at http://www.who.int/tb/publications/global report/en /.
9. Oommen S, Banaji N. Laboratory diagnosis of tuberculosis: Advances in technology and drug susceptibility testing. Indian J Med Microbiol 2017;35:323-31.
10. Kumar K, Das S, Paul DK. A Study on the Role of Cartridge Based Nucleic Acid Amplification Test (CBNAAT) for Diagnosing Pediatric Tuberculosis in a Tertiary Care Hospital in Eastern India. Acad J Ped Neonatol. 2018;6(3):555745.
11. Chin JH. Tuberculous meningitis: Diagnostic and therapeutic challenges. Neurology: Clinical Practice. 2014;4(3):199-205.
12. Lavanya SR, Dave M, Gokhroo A. Role of Cartridge Based Nucleic Acid Amplification Test of Cerebrospinal Fluid in the Diagnosis of Tubercular Meningitis. Int J Cur Res Rev. 2019;11(11):11.
13. Sen S, Das AK, Sinha D. Is Cartridge Based Nucleic Acid Amplification Test (CBNAAT) Better Than Conventional Tests in Diagnosing Childhood Tuberculosis? Evidence from a Tertiary Care Hospital in Eastern India. Saudi Journal of Medical and Pharmaceutical Sciences. 2019;5(11):995-1000.
14. Sharma SK, Kohli M, Yadav RN, *et al*. Evaluating the diagnostic accuracy of Xpert MTB/RIF assay in pulmonary tuberculosis. PloS one. 2015;10(10):e0141011.