

ORIGINAL ARTICLES

Accuracy of Gene-Xpert In Diagnosis of Suspected Tuberculous Meningitis

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Abstract:

Background: The diagnosis of Extra Pulmonary Tuberculosis, especially tubercular meningitis (TBM) is challenging due to frequent atypical clinical presentation, inadequate clinical sample, and paucibacillary nature of the biological samples, which frequently results in a delay or deprivation of treatment. A semi quantitative, nested, real time PCR Gene-Xpert test is showing promising result in diagnosing pulmonary TB diagnosis, but its role in TBM is yet to be validated. **Methods:** It was a cross sectional observational study carried out on 40 clinically suspected TBM patients admitted in neuromedicine, medicine and pediatric medicine at CMCH. Clinical, radiological evaluation and conventional tests were done before PCR (Gene-Xpert) using cerebrospinal fluid. **Results:** The mean age of the study population was 28.59 (± 16.87) years. Seventeen (42.5%) were male and 23 (57.5%) were female with a male to female ratio of 1:1.35. Out of 40 study cases AFB was present in direct microscopy in only 1 (2.5%) case, positive growth on culture in 5 (12.5%) cases and positive Gene-Xpert test in 9 (22.5%) cases. Sensitivity, specificity, PPV, NPV and diagnostic accuracy of Gene-Xpert (PCR) was 44.44%, 96.77%, 80%, 85.71%, and 85.0% respectively considering culture as gold standard. Sensitivity of Gene-Xpert in CSF was 22.5% as compared to culture which was only 12.5% among the study cases. **Conclusion:** PCR (Gene-Xpert) is highly sensitive and speed in diagnosis of TBM compared to conventional methods.

Key words: Tuberculous meningitis, Gene-Xpert, Cerebrospinal fluid, Culture, Diagnostic accuracy.

Introduction:

Tuberculosis typically affects the lungs and known as pulmonary tuberculosis (PTB) but it can affect almost any organ system including the lymph nodes, central nervous system (CNS), bones/joints, genito-urinary tract, abdomen (intra-abdominal organs, peritoneum), and pericardium¹. The worldwide incidence of EPTB cases are increasing and significantly contributing to TB-related

morbidity and mortality². WHO estimates 10 million TB each year and approximately 5-15% of all of TB cases develops extrapulmonary involvement^{2,3}. CNS tuberculosis usually manifests as tuberculous meningitis (TBM) but tubercular encephalitis, intracranial tuberculoma, or a tuberculous brain abscess may occur. In TBM infection spread to the meninges and represents roughly 1% of all TB diseases. It is the most severe form of TB as it

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causes death or severe neurological defects in more than half of those affected, despite the advancements in available antituberculosis treatment⁴. Morbidity and mortality related to TBM is due to the various neurological complications of TBM like in cranial nerve palsy, constriction of internal carotid, obstruction to cerebrospinal fluid (CSF) flowing. Vasculitis that develops due to the inflammatory process is the most serious consequence of TBM^{5,6}. Numerous studies have been conducted in search of more rapid, sensitive and specific methods of diagnosis for TBM and these methods include definite microbiological confirmation such as culture, smear, Polymerase chain reaction (PCR) and supportive diagnostic methods such as radiographic assessments, cytology analysis, antibody and antigen detection, and GC-MS⁴. The identification of MTB in CSF by culture is the gold standard and various culture techniques have been evaluated for their performance and each have different advantages and disadvantages. Culture on solid medium such as Lowenstein-Jensen (L-J) enables examination of colony morphology but takes long time for the result which is about 28 - 50 days^{7,8}. Results can be obtained faster in commercially available automated systems such as Bactec MGIT 960 (Becton Dickinson, Sparks, MD, USA), radiometric Bactec 460 (Becton dickinson, Heidelberg, Germany), MBBact (OrganonTeknika, Boxtel, Netherlands) and ESP II (Difco Laboratories, Detroit, MI, USA) and they are not suitable to use for routine diagnosis due to their high cost, need for expensive lab set up. Although diagnosis based on culture is the reference standard, results are obtained only after 2-8 weeks of incubation which is too slow to aid in clinical decision making, besides the sensitivity of culture to detect *M. tuberculosis* in CSF sample is low and range from 40-60% as culture is less sensitive in paucibacillary conditions. Moreover, it also requires appropriate biological hazard containment facilities and aseptic technique that limits its use⁹. Smear microscopy with traditional Ziehl- Neelsen stain is a rapid and practical method for routine analysis due to its low cost, and high predictive value¹⁰. The sensitivity of Ziehl-Neelsen stain in detecting acid-fast bacilli

(AFB) in CSF is generally low and range from 10-60%⁹. Moreover, large volume (10-15ml) of CSF is required for a more sensitive result which is difficult to obtain in children who have a low total volume of CSF¹¹. Nucleic acid amplification technique (NAAT) such as PCR for detection of mycobacterial DNA has been reported to be more rapid, sensitive and specific^{3,13}. Studies show the sensitivity of PCR assay for TBM range from 31% to 100% and specificity from 66% to 100%. The paucibacillary nature and presence of amplification inhibitor in CSF specimen are the main challenges of applying the PCR method to detect *M. Tuberculosis*¹⁴. Moreover, the cell wall of *M. tuberculosis* is made of an impermeable complex structure that makes the lysis of the cell difficult and thus result in poor quality and low yield of nucleic acids when simple and common nucleic acid isolation procedures are used¹⁵. Physical methods of lysis such as shock treatment (freezing and heating) or Triton X-100 treatment combined with any other DNA extraction procedures are shown to improve the yield of nucleic acids isolated from *M. tuberculosis* for PCR preparation¹⁶. Several *M. tuberculosis* DNA-specific sequences such as IS6110 insertion sequence, protein antigen B, MPB64 and 65kDa have been evaluated by NAA assays¹⁷. MPB64 gene is regarded the most specific sequence for PCR assay in the detection of *M. tuberculosis* and had been used as a target sequence in many studies. The sensitivity and specificity of PCR assay targeting MPB64 gene in these studies showed a relatively good result, ranging 75-100% and 100% respectively¹⁸. Moreover, limited number of gene can be analyzed¹⁹. The Gene-Xpert MTB/RIF System (Cepheid, Sunnyvale, CA, USA) is a fully automated, single use closed-cartridge-based real-time PCR that performs sample decontamination, sonication, automated nucleic acid amplification, and fluorescence-based quantitative PCR¹⁵. It can detect MTB and rifampicin susceptibility simultaneously within two hours with high accuracy for the detection of pulmonary TB (sensitivity 89%, specificity 99%) and rifampicin resistance (sensitivity 95%, specificity 98%). Gene-Xpert MTB/RIF had been approved by WHO for *M.*

tuberculosis detection in sputum while its diagnosis value for the detection of non-respiratory TB is uncertain²⁰. A systemic meta-analysis by Denkinger and colleagues (2014) show that the sensitivity of GeneXpert for extra-pulmonary TB varied widely across different sample types in which the detection rate for TBM was only moderate²¹. Studies with extrapulmonary TB samples have been reported promising in smear positive samples compared to smear-negative specimens. It is reported by Nhu and colleagues (2014) that the sensitivity of GeneXpert MTB/RIF for diagnosing TBM was lower than smear and culture (59.3% Vs 78.6% and 66.5%)²². Larger studies to assess the usefulness of GeneXpert MTB/RIF for diagnosis of TBM are required. At present, there are few literatures regarding information on TBM diagnosis by PCR and particularly the effectiveness of GeneXpert solely on TBM, therefore the study was undertaken to evaluate the performance of GeneXpert assay in diagnosis of TBM.

Materials and methods:

This hospital based cross sectional study having both analytical and descriptive components was conducted in the department of Neurology, Department of medicine, Department of Pediatric medicine of Chattagram Medical College Hospital and Bangladesh Institute of Tropical and Infectious Disease. All the patients admitted in the above mentioned wards of CMCH with clinical diagnosis of TBM. All patients admitted to ward with suspected TBM were assessed for eligibility. Written consent from the patients or attendants was taken after explaining outcome, complications and purpose of the study and right to withdraw from the study at any stage. Patient's history including demographic information, clinical findings, results of laboratory, and neuroimaging testing was recorded in case record form. Sample of CSF was collected from every patient by a standard procedure. CSF specimens were obtained by standard lumbar puncture procedure performed by trained physician. Approximately 5ml of CSF was obtained; 2 ml of sample were used for total and differential cell count, biochemistry and smear for Gram's and acid-fast bacilli (AFB) staining and remaining 3 ml CSF was used for culture and Gene-Xpert test. Routine, gram and AFB stain,

fungal and bacterial culture was done in the Department of Microbiology of CMCH. Gene-Xpert test and culture for MTB was done in Bangladesh Institute of Tropical and Infectious Disease (BITID). All the data were checked and edited after collection. Continuous variables were reported as either means \pm SD or median (intraquartile range), and categorical variables were reported as percentages. Baseline characteristics were compared by either independent sample Kruskal Wallis test for continuous variables or the χ^2 test for categorical data among different TBM groups. For GeneXpert test, sensitivity, specificity, positive predictive value, negative predictive value and accuracy were calculated and 95% confidence intervals were estimated. For analytical comparison, mycobacteria culture was considered as gold standard. Statistical significance was defined as $P < 0.05$ and confidence interval set at 95% level.

Results and observations:

Total 40 suspected cases of tubercular meningitis were enrolled in the study. As per clinical case definition they were finally classified as follows :

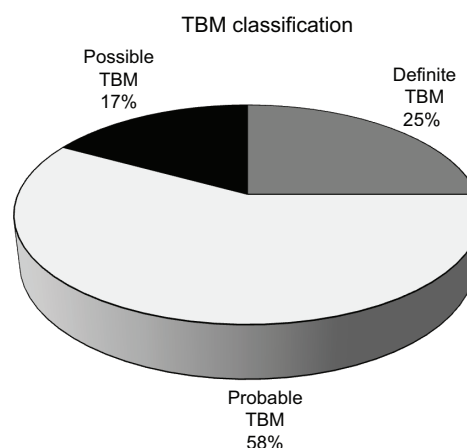


Fig.-1: Tuberculous meningitis classification of the study subjects.

Socio-demographic characteristics:

Table I shows the socio-demographic characteristics of the studied patients. Mean age is 28.59 ± 16.87 years with female predominance (male to female ratio=1:1.35). Majority was from

urban area (57.5%), and had educational qualification up to or below primary level (65.8%).

Vaccination status and closed contact with TB patients

Out of 40 suspected TBM cases, 37 (93%) were vaccinated against TB and 12 (30%) had positive history of closed contact with TB patient.

Diagnostic methods by CSF analysis

Collected CSF from the patients was subjected to AFB staining (ZN staining) and microscopy, culture by Lowenstein Jensen media and Gene-Xpert test.

Out of 40 patients AFB was seen in direct microscopy in only 1(2.5%) case, positive growth in culture in 5 (12.5%) cases and positive Gene-Xpert test in 9 (22.5%) cases (Table VIII).

Diagnostic accuracy of Gene-Xpert in comparison to AFB Culture of CSF

Table IV shows the diagnostic reliability of Gene-Xpert test considering the CSF culture test as standard. It reveals that, sensitivity, specificity, PPV, NPV and diagnostic accuracy of Gene-Xpert test is 44.44%, 96.97%, 80%, 85.71% and 85.0% respectively.

Table-I
Socio-demographic characteristics of the patients (n=40)

Variables		
Age(yrs)	<20 years	12 (30%)
	20-30 years	14 (35%)
	>30 years Mean \pm SD	14 (35%) 28.59 \pm 16.87
Gender	Male	17(42.5%)
	Female	23 (57.5%)
	Rural	17 (42.5%)
Residence	Urban	23 (57.5%)
	Illiterate	4 (10.5%)
	Primary	21 (55.3%)
Education (n=38)	Secondary	4 (10.5%)
	Higher secondary & above	9 (23.7%)

Table-II
Vaccination status and H/O TB contact of the patients (n=40)

Variables	Total (n=40)	Possible (n=7)	Probable TBM (n=23)	Definite TBM (n=10)	P value
Vaccinated	37 (93%)	7 (100%)	21 (91%)	9 (90%)	0.705
Positive contact history ^a	12 (30%)	1 (14.3%)	7 (30%)	4 (40%)	0.521

Chi-square test was performed to calculate statistical association. P<0.05 was taken as significance.

Table-III
Cerebrospinal Fluid analysis result

Diagnostic tests	Positive result
AFB stain	1 (2.5%)
Culture	5 (12.5%)
Gene-Xpert	9 (22.5%)

Data are presented as number (%).

Table-IV
Comparison of GeneXpert with ABF culture of CSF suspected TBM (n=40)

CSF culture result	GeneXpert result (n)	
	Positive	Negative
Positive (5)	4	1
Negative (35)	5	30
Total (40)	9	31

(Mc Nemar test was done)

Discussion:

CSF from 40 suspected TBM cases were subjected to PCR test detect MTB in order to see its sensitivity and specificity as Gene-Xpert is under evaluation for CSF and other body fluid specimen. The peak incidence in the present study was found in young adults, age group of 20-39 years which is similar to as reported by Sarkar et al., (2013)²⁴. In the current study incidence of TBM was higher in female than male. As the study population was small and drawn purposively from a single institute it might not reflect real scenario. However, in contrast to PTB, preponderance for EPTB is reportedly higher among females²⁵.

In the present study, history of fever is present in all of the cases (100%) which was close to study by 92% in Sarkar et al., (2013). Seizures of generalized tonic and clonic type were noted 15% of our cases while reported 8.3% in Sarkar et al., (2013)²⁶. The signs of meningeal irritation was present as neck rigidity or as Kernig's sign in 100% cases while cranial nerve palsies were observed in 43% of the cases, which is consistent with the other study from Bangladesh (42.5%) (Sarkar et al., 2013) but slightly lower than that of Indian study (Khatua et al., 1961) where it was 50%^{26, 27}. The commonest was 6th nerve palsy (12/17 cases), then 3rd nerve (3/17 cases) and other two were multiple cranial nerve palsy (2nd & 6th and 6th & 7th). In the present study, the incidence of papilloedema was 40%. Motor impairment in the form of limb weakness was noted in 20% cases, slightly higher incidence was of limb weakness in 23% cases was reported by Marais et al., (2010)²³ and median duration of the prominent symptoms in our study was 30 days (Intraquartile range: 30 to 60 days). Twenty two (60%) of our cases had CT/MRI findings compatible with TBM. The contribution of cerebral imaging to the diagnosis of tuberculous meningitis is well established, although it is not essential to establish a diagnosis of definite or probable disease²³. In our study most prevalent feature was infarction (25%), followed by tuberculoma (20%) and hydrocephalous (10%).

Out of 40 suspected TBM cases, Gene-Xpert was positive in 9 cases while L-J culture detected mycobacteria in 5 cases only. Sensitivity of Gene-

Xpert was 22.50% as compared to culture showing a sensitivity of 12.5%. Gene-Xpert could detect additional 4 (10%) cases over L-J culture. Sensitivity of Gene-Xpert in the current study is comparable to other studies who have but lower sensitivity was also reported²⁷⁻²⁹. Paucibacillary nature of TBM, low volume of CSF and small sample size may be the probable reason for low positivity of Gene-Xpert in CSF. Immunosuppression due to HIV/AIDS or age (e.g., infants, elderly) are key drivers of TBM, and immunosuppression increases the bacillary burden of MTB organisms. Studies reported higher sensitivity in CSF of TBM in HIV cases^{22,30}. Higher yield was recorded in volumes of centrifuged CSF among HIV infected persons with sensitivity of approximately 80% and associated with specificity for microbiologically confirmed TBM. But lower sensitivity of \approx 50% was recorded with uncentrifuged CSF. This difference in sensitivity associated with centrifugation was not observed in non-HIV cases. The Xpert system depends upon capture and lysis of whole bacilli, and therefore high volumes (>7 ml) of CSF are crucial to achieving high sensitivity.³¹ Bacterial loads are higher in HIV-infected TBM patients, consequently higher detection rate in all the tests for TBM. In our study no HIV-infected patient was included there was small variation from infant and elderly group. Besides only 5 ml of CSF was collected for the laboratory purpose. Rifampicin resistance was not detected among our cases which is in contrast to 3.7% resistance reported in study by Nhu et al., (2014)²². The reason for this may be due to inclusion of new cases in the current study. CSF from 12.5% cases were positive by L-J medium culture in our study and while only 2.5% sample was positive by AFB smear examination. The lower positive for AFB smear and culture in the present study is comparable with study by Poonam et al., (2007)³². This low yield of AFB smear and culture may be due to the paucibacillary nature of CSF samples; besides, inadequate volume of samples may give low positive result³³. Negative AFB smear might be due to the low concentration of mycobacteria in those samples i.e. below the detection limit of 10000 organism/ml. Culture

negative might be explained by the absence of viable mycobacteria in the samples³⁴. Nhu et al., (2014) found an exceptionally high sensitivity of smear microscopy and explained that this exceptional sensitivity depends upon the proper sample processing, meticulous examination of individual slides for 30 min by a highly skilled and experienced technician²². This may be difficult to replicate outside a dedicated research setting due to the work burden in public health laboratories of resource-limited countries like ours³¹. Similar to our finding Sarkar et al., (2013) also observed 100% negative smear microscopy²⁴.

Hillemann et al., (2011)³⁵ observed in their study that, in some cases, the GeneXpert assay result was positive but the culture remained negative. Of the seven patients with discrepant results, two patients had pulmonary TB, proven by several cultures of different specimens. Two patient had TB (culture confirmed) 1year and 2years before and were presumably still or again under treatment at the time of sampling. an indication for the resolution of the discrepancies³⁵.

TBM is the most severe form of tuberculosis where microbiological confirmation is rare, and treatment is often delayed resulting in increasing mortality and morbidity. The Gene-Xpert MTB/RIF test would be a promising diagnostic tool for early diagnosis of TBM.

Conclusion:

Our study found that Gene-Xpert had higher sensitivity compared to other diagnostic modalities currently available in our setting. High specificity of the assay explains the low false positivity achieved by this diagnostic tool, which can thus be a useful rule-in test for TBM diagnosis.

References:

1. Kruijshaar ME, Abubakar I. Increase in extrapulmonary tuberculosis in England and Wales 1999-2006. *Thorax*. 2009 Dec; 64(12): 1090-5
2. World Health Organization. (2018) Global Tuberculosis Report 2018.
3. Pehlivanoglu, F., Yasar, K.K., Sengoz, G. Tuberculous meningitis in adults: a review of

- 160 cases. *Scientific World Journal* 2012, 2012, 1-6
4. Rock, R.B., Olin, M., Baker, C.A., Molitor, T.W., Peterson, P.K. Central nervous system tuberculosis: pathogenesis and clinical aspects. *Clin Microbiol Rev*. 2008, 21(2), 243-61.
5. Thwaites, G., Chau, T.T., Mai, N.T., Drobniewski, F., McAdam, K., Farrar, J. Tuberculous meningitis. *J NeurolNeurosurg Psychiatry*, 2000, 68(3), 289-99.
6. Thwaites, G.E., van Toorn, R., Schoeman, J. Tuberculous meningitis: more questions, still too few answers. *Lancet Neurol*, 2013, 12(10), 999-1010.
7. Thakur, R., Goyal, R., Sarma, S. Laboratory diagnosis of tuberculous meningitis - Is there a scope of for further improvement? *J Lab Physicians* 2010, 2(1), 21-4.
8. Huang, Z., Xiong, G., Luo, Q., Jiang, B., Li, W., Xu, X., & Li, J. Evaluation of the performance of the microscopic bserveation drug susceptibility for diagnosis of extrapulmonary tuberculosis in China: A preliminary study. *Respirology* 2014, 19(1), 132-7.
9. Thwaites, G. Tuberculous meningitis. *Medicine* 2017, 45(11), 670-3.
10. Chen, P., Shi, M., Feng, G. D., Liu, J. Y., Wang, B. J., Shi, X. D., Ma, L., Liu, X. D., Yang, Y. N., Dai, W., Liu, T. T., He, Y., Li, J. G., Hao, X. K., Zhao, G. A highly efficient Ziehl-Neelsen stain: Identifying de novo intracellular Mycobacterium tuberculosis and improving detection of extracellular M. tuberculosis in cerebrospinal fluid. *J ClinMicrobiol*, 2012, 50(4), 1166-70.
11. Marx, G.E., Chan, E.D. Tuberculous meningitis: diagnosis and treatment overview. *Tuberculosis Research and Treatmen*, 2011, (2011), 1-9..
12. Marico, C.R., In Jameson, Fauci, Kasper, Longo, Harrison's Principle Of Internal Medicine, New York, Mcgraw Hill Education, 2018, 20th, 1244

13. Sutlas, P.N., Unal, A., Forta, H., Senol, S., Kirbas, D. Tuberculous meningitis in adults: review of 61 cases. *Infection*, 2003, 31(6), 387-91
14. Takahashi, T., Tamura, M., Takasu, T. The PCR-based diagnosis of central nervous system tuberculosis: Up to date. *Tuberc Res Treat* 2012, (3), 1-17.
15. Boulware, D.R. Utility of the Xpert MTB/RIF assay for diagnosis of tuberculous meningitis. *PLoS Med* (2013), 10(10),1-3.
16. Thakur, R., Sarma, S., Goyal, R. Comparison of DNA extraction protocols for Mycobacterium tuberculosis in diagnosis of tuberculous meningitis by Real-Time polymerase chain reaction. *J Glob Infect Dis*, 2011, 3(4), 353-6.
17. Sharma, K., Sharma, A., Singh, M., Dandora, R., Sharma, S.K., Modi, M., Prabhakar, S., Sharma, M. Evaluation of polymerase chain reaction using protein b primers for rapid diagnosis of tuberculous meningitis. *Neuro India* (2010), 58(5), 727-31.
18. Lekhak, S. P., Sharma, L., Rajbhandari, R., Rajbhandari, P., Shrestha, R., Pant, B., Evaluation of Multiplex PCR using MPB64 and IS6110 primers for Rapid Diagnosis of Tuberculous Meningitis. *Tuberculosis*, 2016, (100), 1-4 .
19. Migliori, G.B., Matteelli, A., Cirillo, D., Pai, M., Diagnosis of multidrug-resistant tuberculosis and extensively drug-resistant tuberculosis: Current standards and challenges. *Can J Infect Dis Med Microbiol* 2008; 19(2):169-172.
20. Lawn, S.D., and Nicol, M.P. Xpert® MTB/RIF assay: development, evaluation and implementation of a new rapid molecular diagnostic for tuberculosis and rifampicin resistance. *Future microbiology*, 2011, 6(9), 1067-82.
21. Denkinger, C.M., Schumacher, S.G., Boehme, C.C., Dendukuri, N. Xpert MTB/RIF assay for the diagnosis of extrapulmonary tuberculosis: a systematic review and meta-analysis. *European Respiratory Journal*, 2014, (44), 435-446
22. Nhu, N.T., Heemskerk, D., Thu, D.A, Mai, N. T. H., Nghia, H. D. T., Loc, P. P., Ha, D. T. M., Merson, L., Thinh, T. T. V., Day, J., Chau, N. V. V., Wolbers, M., Farrar, J., Caws, M., Evaluation of GeneXpert MTB/RIF for diagnosis of tuberculous meningitis. *J Clin Microbiol* 2014, 52(1), pp.226-33.
23. Marais, S., Thwaites, G., Schoeman, J.F., Török, M.E., Misra, U.K., Prasad, K., Donald, P. R., Wilkinson, R. J., Marais, B. J., Tuberculous meningitis: a uniform case definition for use in clinical research. *Lancet Infect Dis*, 2010,(10),803–12.
24. Sarkar, D.N., Hossain, M.I., Shoab, A.K.M., Quraishi, F.A. Presentation of Tuberculous Meningitis Patients: Study of 30 Cases, *Medicine Today*, 2013, 25(132), 32-5
25. Thorson, A., Gender issues in tuberculosis: Sex and Gender Differences in Infection and Treatments for Infectious Diseases, *Springer International Publishing*, 2015,(8), 231–253
26. Khatua, S.P. 'Tuberculous meningitis in children: Analysis of 231 cases', *J.Indian Med Ass*, 1961, (37), 332
27. Bhatia, R., Dayal, R., Jindal, S., Agarwal, D., Goyal, A. GeneXpert for Diagnosis of Tubercular Meningitis. *Indian J Pediatr*, 2016, 83(11), 1353–5.
28. Vadwai, V., Boehme, C., Naabeta, 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.
29. Uria, G.A., Azcona, J.M., Middle, M., Naik, P.K., Reddy, S., Reddy, R. Rapid diagnosis of pulmonary and extrapulmonary tuberculosis in HIV–infected patients. Comparison of LED fluorescent microscopy and the GeneXpert MTB/RIF assay in a district hospital in India. *Hindwai Publishing Corporation Tuberculosis Research and Treatment*, 2012, (2012). 4pages

30. Patel, V.B., Theron, G., Lenders, L., Matinyena, B., Connolly, C., Singh, R., Coovadia, Y., Ndung'u, T., Dheda, K. Diagnostic Accuracy of Quantitative PCR (Xpert MTB/RIF) for Tuberculous Meningitis in a High Burden Setting: A Prospective Study. *PLoS Med*, 2013, 10(10), 1-13.
31. Thwaites, G.E., Caws, M., Chau, T. T., Dung, N. T., Campbell, J. I., Phu, N. H., Hien, T. T., White, N. J., Farrar, J. J Comparison of conventional bacteriology with nucleic acid amplification (amplified mycobacterium direct test) for diagnosis of tuberculous meningitis before and after inception of antituberculosis chemotherapy. *J Clin Microbiol*, 2004, (42), 996–1002
32. Poonam, S.D., Kashyap, R.S., Ramteke, S.S., Nagdev, K.G., Purohit, H.J., Tiwari, G.M., Dagainawala, H.F. Evaluation of the IS6110 PCR assay for the rapid diagnosis of tuberculous meningitis. *CerebroFl Res* (2007), vol.4, pp.10
33. Chakravorty, S. Tyagi, J.S. Novel multipurpose methodology for detection of Mycobacteria in pulmonary and extrapulmonary specimens by smear microscopy, culture and PCR. *J Clin Microbiol*, 2005, 43(6), 2697-2702.
34. Yeager, H., Lacy, J., Smith, L.R., Maistre, C.A. Quantitative studies of mycobacterial population in sputum and saliva. *Am Rev Resp Dis*, 1967, (95), 998-1004
35. Hillemann, D., Rüssch-Gerdes, S., Boehme, C., Richter, E. Rapid Molecular Detection of Extrapulmonary Tuberculosis by the Automated GeneXpert MTB/RIF System. *Journal of clinical microbiology*, 2011, 49(4), 1202–1205.