



## GENE XPRT MTB/RIF: THE EVIDENCE BASED PATH BREAKING DIAGNOSTIC TOOL FOR TUBERCULOSIS DIAGNOSIS

### Community Medicine

**Debjoyoti Mohapatra\***

Senior resident, Department of Community Medicine and Family medicine AIIMS Bhubaneswar. \*Corresponding Author

**Vikas Bhatia**

Professor and Head, Department of Community Medicine and Family Medicine, AIIMS Bhubaneswar

### ABSTRACT

Tuberculosis remains one of the major killers. The prognosis is poor in patients who are diagnosed late or are suffering from any kind of drug resistance. Traditional methods of TB diagnosis such as microscopy and chest X- ray lack the desired sensitivity and thus may delay diagnosis of TB leading to increase in TB related deaths. These diagnostic modalities also cannot detect drug resistance which also leads to delay in initiating effective treatment. Culture and drug susceptibility testing is a sensitive method for diagnosing TB and detecting drug resistance but time remains a constraints. Gene Xpert provides a faster and sensitive method of diagnosing TB and detecting Multi drug resistance TB. It is of high utility in diagnosing extra-pulmonary TB and TB in HIV infected individuals. Gene Xpert has been found to be found cost effective. Many countries have already adapted this method as first line diagnostic method for TB. More countries need to expand its use for TB diagnosis and it can play a major role in achieving the goals of End TB strategy.

### KEYWORDS

Gene-xpert, TB-HIV co-infection, Extra-pulmonary TB

#### Introduction

The WHO End TB strategy aims to end the global TB epidemic, with targets to reduce TB deaths by 95% and to cut new cases by 90% between 2015 and 2035, and to ensure that no family is burdened with catastrophic expenses due to TB. It sets interim milestones for 2020, 2025, and 2030.(1)

There was an estimated 10.4 million new cases of TB in 2016. TB was the ninth leading cause of death causing about 1.3 million deaths in 2016 and worldwide was the leading cause of death due to a single infectious agent.(2) India contributes a quarter of new incidence of TB cases and about one-third of deaths due to TB.(3) The high burden in India is attributed to delay in diagnosis and treatment of the disease. Delay in diagnosis of TB increases the risk of poor treatment outcomes, including death, and enhances transmission of disease in the community.(4) In lower and lower middle income countries, the contribution of TB as a cause of death assumes much greater proportion compared to the middle and higher income countries.(2) The advent of directly observed treatment (DOTS) has seen much better treatment outcomes in diagnosed case (5–7) but case detection still remains a challenge.(8,9) Sputum microscopy and radiography have remained the backbone of diagnosis in most resource-constrained settings. The sensitivity and specificity of the two still fails to achieve desirable results or early case detection.(10) Any barrier to early case detection presents a challenge in improving treatment outcomes. More ever the rising cases of Multi drug resistance(MDR) TB. HIV-TB co-infection and TB in paediatric patient present a new challenge in TB diagnosis and treatment. The sensitivity of sputum microscopy and radiography is poor in these cases. Six lakh MDR cases occurred globally. About a quarter of it were newly detected in India alone.(11) Many MDR cases are diagnosed at advanced stage and many are left undiagnosed. Culture presents an alternate to sputum microscopy and is the gold standard for diagnosis, but the results come after a lag period of many days and therefore are not suited for early case detection. Under these circumstances Gene Xpert provides a ray of hope and shows the pathway to achieve the goals of SDG and End TB strategy.

#### What is Gene Xpert?

Gene Xpert is a Mycobacterium tuberculosis-specific automated, cartridge based nucleic acid amplification assay, which has the capability of automatic amplification and producing results within 2 hrs. It purifies concentrates, amplifies and identifies targeted nucleic acid sequences in the TB genome. Gene-Xpert, the test device platform, was launched by Cepheid in 2004. The Xpert MTB/RIF test is currently the only molecular test of its kind and uses a cartridge containing all elements necessary for the reaction, including lyophilized reagents, liquid buffers and wash solutions. Target detection and characterization is performed in real time using a six-colour laser detection device.(12)

The Foundation for Innovative New Diagnostics (FIND) had

partnered with Cepheid, Inc. (Sunnyvale, CA) to develop Xpert MTB/RIF. In December 2010, WHO recommended the use of the Xpert MTB/RIF assay. The WHO's policy statement was issued in early 2011.

#### The evidence:

In a multicountry study done by FIND, across 4 countries, Xpert MTB/RIF achieved a sensitivity of almost 98% in patients with smear-positive tuberculosis and about 73% in smear negative TB. Specificity was almost 100%. Among patients with smear-negative, culture-positive tuberculosis, the addition of a second MTB/RIF test increased sensitivity by considerable amount. As compared with phenotypic drug-susceptibility testing, MTB/RIF testing correctly identified almost all samples with rifampicin-resistant bacteria.(13)

Comparative study done in India to compare Gene Xpert with sputum microscopy found significant better sensitivity and specificity of Gene Xpert compared to sputum microscopy. The Gene Xpert had a sensitivity of around 86% and specificity of 93% compared to only 22.2% and 78.5% in sputum smear microscopy.(14)

The superiority of Gene Xpert compared to sputum microscopy has been demonstrated in a study conducted in Thailand where Gene Xpert had a sensitivity of around 95% compared to only 60% of smear microscopy.(15)

In a study conducted in one of the large tertiary care centre in India, the sensitivity and specificity of Gene Xpert was around 95% and 99% in pulmonary TB samples. The distinctive advantage of this study was evident from the finding that it was able to detect almost 80% of cases which were smear negative but culture positive.(16)

Meta-analysis of 18 studies conducted mainly in lower and middle income countries, Gene Xpert achieved a pooled sensitivity of 88% and a pooled specificity of 98%. When the same test was used as an add on test following a negative sputum smear examination, it still had 67% pooled sensitivity and a pooled specificity of 98%.(17)

#### Gene Xpert in HIV positive patients

PLHIV have a 16-27-fold increased risk of TB compared to HIV-uninfected people.(18) The detection of mycobacterium tuberculosis in HIV patient through sputum microscopy has a very low sensitivity. As a result, HIV-related TB deaths are a significant public health problem in high HIV-prevalent settings. The sensitivity and specificity of Gene Xpert has been tested under circumstances where sputum microscopy has lacked the desired sensitivity of detecting Mycobacterium, like HIV.

In a study conducted by Lawn et al among adult HIV infected patients without diagnosis of TB, Gene Xpert showed an overall sensitivity of about 73% compared to sputum microscopy which had a sensitivity of

only 28%. Culture was the gold standard in this case. The process was also able to identify rifampicin resistance in all the MDR TB case.(19)

Study conducted in two tertiary care hospital of Peru and in resource constrained settings in Ethiopia have demonstrated a distinct advantage of Gene Xpert technology in HIV positive individuals compared to sputum microscopy.(20,21) TB remains a major killer in HIV patient. Early diagnosis and treatment of TB is essential in HIV infected individuals and using Gene Xpert as first line of investigation can be a game-changer.

The superiority of Gene Xpert over other nucleic acid based test and sputum microscopy has been demonstrated in a study conducted in South Africa. Xpert based nucleic acid test was the only one which showed a sensitivity of more than 85% while rest had sensitivities restricted to only around 75%.The same study also demonstrated that it was the best nucleic acid test for HIV infected patient when compared to other nucleic acid based test.(22)

Early detection has shown to have an impact on early start of treatment of pulmonary TB especially in resource constraint setting.

In one of the large studies conducted in India, Gene Xpert was able to detect mycobacterium in significant proportion of pulmonary samples of HIV infected individuals which had not been detected by sputum.(23)

Meta-analysis has shown a pooled sensitivity of around 80% in HIV infected individuals which is much higher compared to sputum microscopy and presents a distinctive advantage.(17)

**Gene Xpert in detecting MDR TB:** Antibiotic resistance is one of the gravest dangers the world is facing today.TB is one of the notorious disease that faces the threat of drug resistance. MDR TB is assuming a gigantic proportion. In the past there has always been a struggle to deal with quick and effective diagnosis which will aid in early start of treatment. Culture was the mainstay of MDR TB diagnosis but time remained a constraining factor. Gene Xpert provides the window of opportunity providing the result of TB and rifampicin resistant within two hours.

Cochrane review based on 11 studies have demonstrated high sensitivity and specificity of Gene Xpert in detecting rifampicin resistance(94% and 98% respectively)(24)

Similar findings were found in study conducted in India (16) which also showed the huge utility of Gene Xpert in detecting rifampicin resistance with a high sensitivity (94.5%) and specificity(97.5%).

The WHO expert committee report based on 17 studies has shown a pooled sensitivity of 95% and pooled specificity of 98% in detecting rifampicin resistance. Gene Xpert thus shows a viable and feasible alternative for quick detection of MDR TB(17)

**Recommendation on extra pulmonary tuberculosis:** Extra-pulmonary TB has always posed a challenge in early diagnosis.(25) It often requires invasive and use of composite procedures and sometimes opinion of multiple consultant to come to a conclusion. The feasibility of these investigation and consultation is restricted to tertiary care centres. Moreover it is time consuming. Under the circumstances Gene Xpert provides a quick and reliable alternative. Gene Xpert has shown a good diagnostic accuracy with sensitivity of around 91% for pus and 80% for lymph node aspirate. It was low in pleural and ascitic fluid compared to culture or composite diagnostic gold standard(26). A study done in India has also shown promising result with Gene Xpert sensitivity reaching as high as 81% compared to gold standard composite reference test.(27)

In 2013 WHO expert group came up with recommendation on extra pulmonary tuberculosis based on the findings of 15 published articles and 7 unpublished articles. In lymph node aspirate, CSF, gastric fluid and pleural fluid the pooled sensitivity was 92.5%, 79.5%, 83.8% and 43.7% respectively. Based on the findings, the expert group reached the consensus that CSF and lymph node aspirate could be subjected for initial diagnostic testing for TB meningitis and TB lymphadenitis.(17) Pleural fluid lacked the desired sensitivity and should not be

considered as first line investigation for pleural TB.

**Gene Xpert in Children:** Laboratory investigation of TB is hampered by paucibacillary nature of disease and obtaining appropriate specimen.(28) Gene Xpert showed a pooled sensitivity of 66% in studies among children where expectorated sputum or induced sputum was used. Similar diagnostic sensitivity was observed in samples from gastric lavage or aspiration was used. Pooled specificity can be as high as 98%.(29) Many countries including India have already adapted Gene Xpert as a first line investigation for paediatric patient.

**Economic evaluation of Gene Xpert:** WHO did cost effectiveness review of Gene Xpert under various settings. 15 studies were selected for the review and 12 of those studies found Gene Xpert to be cost effective. Studies done in South Africa, Uganda and India found that Xpert MTB/Rif was a cost effective diagnostic strategy compared to sputum smear microscopy and clinical diagnosis combined.(30)

Two studies performed in Indian health settings also found that Xpert MTB identified fewer false positive and account of it prevented unnecessary expenditure on wrongly diagnosed TB cases. It was also found in one of the studies in India that various implementation strategies does have an effect on cost effectiveness and countries need to devise strategies for using it the most effective way.(30) Five of the fifteen studies done in high income settings also found Gene Xpert to be cost effective

#### **WHO consolidated recommendation for Gene Xpert(30)**

- Xpert MTB/RIF should be used rather than conventional microscopy, culture and DST as the initial diagnostic test in adults suspected of having MDR-TB or HIV-associated TB (strong recommendation, high-quality evidence).
- Xpert MTB/RIF should be used rather than conventional microscopy, culture and DST as the initial diagnostic test in children suspected of having MDR-TB or HIV-associated TB (strong recommendation, very low-quality evidence).
- Xpert MTB/RIF may be used rather than conventional microscopy and culture as the initial diagnostic test in all adults suspected of having TB (conditional recommendation acknowledging resource implications, high-quality evidence).
- Xpert MTB/RIF may be used rather than conventional microscopy and culture as the initial diagnostic test in all children suspected of having TB (conditional recommendation acknowledging resource implications, very low-quality evidence).

#### **For Extra-pulmonary TB (17)**

- Xpert MTB/RIF should be used in preference to conventional microscopy and culture as the initial diagnostic test for CSF specimens from patients suspected of having TB meningitis (strong recommendation given the urgency for rapid diagnosis, very low-quality evidence).
- Xpert MTB/RIF may be used as a replacement test for usual practice (including conventional microscopy, culture or histopathology) for testing specific nonrespiratory specimens (lymph nodes and other tissues) from patients suspected of having extra pulmonary TB (conditional recommendation, very low-quality evidence).

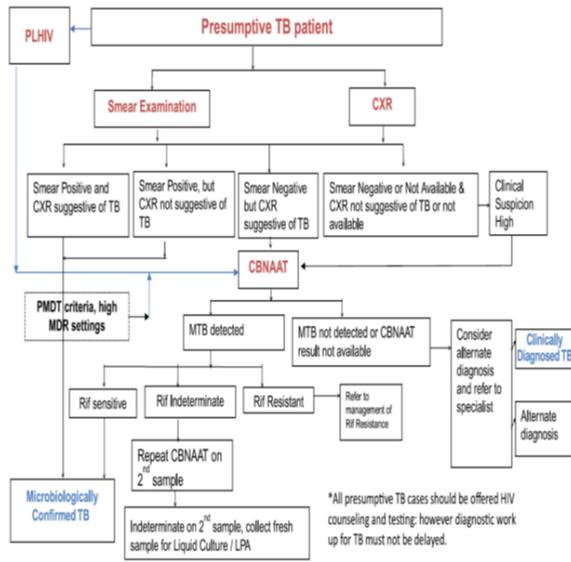
#### **Indian perspective:**

Keeping in line with the available evidence and cost effectiveness, India has strategically employed Gene Xpert as an initial testing in following case:

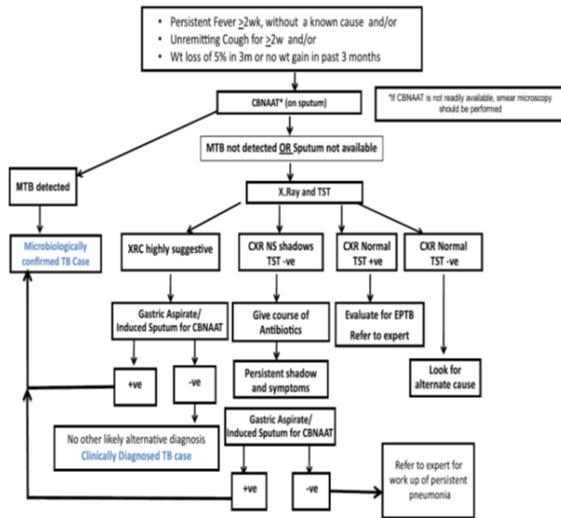
1. All people living with HIV cases.(PLHIV)
2. All paediatric TB cases.
3. All MDR contacts.
4. All private cases.
5. Extra Pulmonary TB cases with appropriate specimen
6. All CAT-2 negative cases

RNTCP programme has called for revision for diagnostic algorithm. The new algorithm for adult and paediatric patient has made way for much larger scope for use of Gene Xpert

**Revised Algorithm for TB diagnosis in a suspected adult TB: (RNTCP Technical and operational guidelines, 2016, MOHFW,GOI)**



**Revised diagnostic algorithm paediatric TB suspect: (RNTCP Technical and operational guidelines, 2016, MOHFW,GOI)**



**Extra-pulmonary TB:(31)**

**Lymph node-** TB-Xpert MTB/RIF should be used as an additional test to conventional smear microscopy, culture and cytology in fine-needle aspiration cytology (FNAC) specimens.

**TB meningitis-** Xpert MTB/RIF may be used as an adjunctive test for tuberculosis meningitis (TBM). A negative Xpert MTB/RIF result on a cerebrospinal fluid (CSF) specimen does not rule out TBM.

**Pleural TB-** Xpert MTB/RIF should not be routinely used to diagnose pleural TB.

**Conclusion:**

The Gene Xpert technology has truly revolutionised the ease with which TB is diagnosed. Not only it is time saving with results appearing within 2 hrs, but also much more sensitive than sputum microscopy. Moreover it has the added advantage of giving the result of rifampicin resistance. Culture remains the gold standard but has the disadvantage of having a great time lag between sample collection and result. Under the circumstances, Gene Xpert as a diagnostic can have a far reaching effect on dealing with the menace of Drug resistance tuberculosis. Gene Xpert also has an improvised role in diagnosing TB from specimen collected from extra pulmonary site and in paediatric TB. Gene Xpert has a definite role in achieving the National strategic plan vision of TB free India and elimination of TB in India by 2025.

**Conflict of interest:** None

**Fundings:** None

**References**

1. WHO | WHO End TB Strategy. WHO. 2015;
2. Global TB report 2017 [Internet]. 2017 [cited 2018 Jul 1]. Available from: <http://apps.who.int/iris/bitstream/handle/10665/259366/9789241565516-eng.pdf;jsessionid=D98459BCAD92785F343D2C6806ADE7C4?sequence=1>
3. World TB Day Slogans World TB Day , falling on 24 March each year , is designed to build public awareness that tuberculosis today remains an epidemic in much of the world , causing deaths of several million people each year , mostly in the third world . 2. Toman's Tuberculosis.
4. Kaur G, Goel NK, Kumar D, Janmeja AK, Swami HM, Kalia M. Treatment outcomes of patients placed on treatment under directly observed therapy short-course (dots). Lung India [Internet]. 2008 Apr [cited 2018 Jul 4];25(2):75-7. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/20165654>
5. Karanjekar V, Lokare P, Gaikwad A, Doibale M, Gujrathi V, Kulkarni A. Treatment Outcome and Follow-up of Tuberculosis Patients Put on Directly Observed Treatment Short-course Under Rural Health Training Centre, Pathan, Aurangabad in India. Ann Med Health Sci Res [Internet]. 2014 Mar [cited 2018 Jul 4];4(2):222-6. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/24761242>
6. Hoshino H, Kobayashi N. [Evaluation of effect of community DOTS on treatment outcomes by TB surveillance data]. Kekkaku [Internet]. 2006 Oct [cited 2018 Jul 4];81(10):591-602. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/17094581>
7. Lorent N, Choun K, Malhotra S, Koet P, Thai S, Khun KE, et al. Challenges from Tuberculosis Diagnosis to Care in Community-Based Active Case Finding among the Urban Poor in Cambodia: A Mixed-Methods Study. PLoS One [Internet]. 2015 [cited 2018 Jul 4];10(7):e0130179. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/26222545>
8. Sylvia S, Xue H, Zhou C, Shi Y, Yi H, Zhou H, et al. Tuberculosis detection and the challenges of integrated care in rural China: A cross-sectional standardized patient study. PLoS Med [Internet]. 2017 Oct [cited 2018 Jul 4];14(10):e1002405. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/29040263>
9. Desikan P. Sputum smear microscopy in tuberculosis: is it still relevant? Indian J Med Res [Internet]. 2013 Mar [cited 2018 Jul 4];137(3):442-4. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/23640550>
10. TB India Report 2018 :: Ministry of Health and Family Welfare [Internet]. [cited 2018 Jul 4]. Available from: <https://tbcindia.gov.in/showfile.php?lid=3314>
11. Frequently Asked Questions on Xpert MTB/RIF assay [Internet]. [cited 2018 Jul 4]. Available from: [http://www.who.int/tb/laboratory/xpert\\_faqs.pdf](http://www.who.int/tb/laboratory/xpert_faqs.pdf)
12. Lawn SD, Nicol MP. Xpert® MTB / RIF assay : development , evaluation and implementation of a new rapid molecular diagnostic for tuberculosis and rifampicin resistance. Futur Microbiol. 2011;6(9):1067-82.
13. Agrawal M, Bajaj A, Bhatia V, Dutt S. Comparative Study of GeneXpert with ZN Stain and Culture in Samples of Suspected Pulmonary Tuberculosis. J Clin Diagn Res [Internet]. 2016 May [cited 2018 Mar 8];10(5):DC09-12. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/27437212>
14. Pinyopornpanish K, Chaiwarith R, Pantip C, Keawvichit R, Wongworapat K, Khamnoi P, et al. Comparison of Xpert MTB/RIF Assay and the Conventional Sputum Microscopy in Detecting Mycobacterium tuberculosis in Northern Thailand. Tuberc Res Treat [Internet]. 2015;2015:571782. Available from: <http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=4430669&tool=pmcentrez&rendertype=abstract>
15. Sharma SK, Kohli M, Yadav RN, Chaubey J, Bhasin D, Sreenivas V, et al. Evaluating the diagnostic accuracy of xpert MTB/RIF assay in pulmonary tuberculosis. PLoS One. 2015;10(10):1-9.
16. WHO. Xpert MTB/RIF implementation manual: technical and operational 'how-to'; practical considerations. Vol. 2. 2014.
17. WHO. WHO | Tuberculosis and HIV [Internet]. WHO. World Health Organization; 2018 [cited 2018 Jul 6]. Available from: <http://www.who.int/hiv/topics/tb/en/>
18. Lawn SD, Brooks S V, Kranzer K, Nicol MP, Whitelaw A, Vogt M, et al. Screening for HIV-associated tuberculosis and rifampicin resistance before antiretroviral therapy using the Xpert MTB/RIF assay: A prospective study. PLoS Med. 2011;8(7).
19. Balcha TT, Sturegård E, Winqvist N, Skogmar S, Reepalu A, Jemal ZH, et al. Intensified tuberculosis case-finding in HIV-positive adults managed at ethiopian health centers: Diagnostic yield of xpert MTB/RIF compared with smear microscopy and liquid culture. PLoS One. 2014;9(1).
20. Carriquiry G, Otero L, González-Lagos E, Zamudio C, Sánchez E, Nabeta P, et al. A Diagnostic Accuracy Study of Xpert®MTB/RIF in HIV-Positive Patients with High Clinical Suspicion of Pulmonary Tuberculosis in Lima, Peru. PLoS One. 2012;7(9):3-9.
21. Scott LE, McCarthy K, Gous N, Nduna M, van Rie A, Sanne I, et al. Comparison of Xpert MTB/RIF with other nucleic acid technologies for diagnosing pulmonary tuberculosis in a high HIV prevalence setting: A prospective study. PLoS Med. 2011;8(7):1-11.
22. Bansal D, Avashia S, Karothiya M. A study of gene xpert in screening of sputum in hiv positive patients presenting to tertiary. 2016;7(8):657-60.
23. Steingart KR, Sohn H, Schiller I, Kloda LA, Boehme CC, Pai M, et al. Xpert® MTB/RIF assay for pulmonary tuberculosis and rifampicin resistance in adults. Cochrane database Syst Rev [Internet]. 2013;(1):CD009593. Available from: <http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=4470352&tool=pmcentrez&rendertype=abstract>
24. Solovici I, Jonsson J, Korzeniewska-Kosela M, Chiotan DI, Pace-Asciak A, Slump E, et al. Challenges in diagnosing extrapulmonary tuberculosis in the European Union. Euro Surveill [Internet]. 2011 [cited 2018 Jul 7];18(12). Available from: [www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20432](http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20432)
25. Scott LE, Beylis N, Nicol M, Nkuna G, Molapo S, Berrie L, et al. Diagnostic Accuracy of Xpert MTB/RIF for Extrapulmonary Tuberculosis Specimens: Establishing a Laboratory Testing Algorithm for South Africa. J Clin Microbiol [Internet]. 2014 [cited 2018 Jul 7];52(6):1818-23. Available from: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4042800/pdf/zjm1818.pdf>
26. Vadwai V, Boehme C, Nabeta P, Shetty A, Alland D, Rodrigues C. Xpert MTB/RIF: A new pillar in diagnosis of extrapulmonary tuberculosis? J Clin Microbiol. 2011;49(7):2540-5.
27. Rahman N, Pedersen KK, Rosenfeld V, Johansen IS. Challenges in diagnosing tuberculosis in children. Dan Med J [Internet]. 2012 Jul [cited 2018 Jul 7];59(7):A4463. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/22759843>
28. WHO. Automated Real-Time Nucleic Acid Amplification Technology for Rapid and Simultaneous Detection of Tuberculosis and Rifampicin Resistance: Xpert MTB/RIF Assay for the Diagnosis of Pulmonary and Extrapulmonary TB in Adults and Children: Policy update. World Heal Organ [Internet]. 2013;1-79. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/25473701>
29. Plasterk RHA. WHO policy statement : Xpert MTB/RIF assay for the diagnosis of TB. World Heal Organ. 2016;5(8):6-9.
30. COCHRANE. Initiative of Central TB Division Ministry of Health and Family Welfare , Government of India Convenors. Index TB Guidel. 2016;63-8.