International Journal of Medicine Research
ISSN: 2455-7404
www.medicinesjournal.com
Volume 3; Issue 2; April 2018; Page No. 01-05

Study of GeneXpert MTB/RIF for detecting mycobacterium tuberculosis along with analysis of rifampicin resistance from pulmonary and extrapulmonary specimens

Dhanya PR[1*], Shilpa K[2], Manjesh M[3]
[1, 2] Department of Microbiology, East Point College Medical Sciences and Research Centre, Avalahalli, Bangalore, Karnataka, India
[3] Department of Microbiology, Sakra World Hospital, Devarbeesanahalli, Bangalore, Karnataka, India

Abstract
Aim: To evaluate GeneXpert MTB/RIF test results performed in a tertiary care hospital and analyse the incidence of multi-drug resistance.
Method: Samples were processed by GeneXpert MTB/RIF assay which is an ultrasensitive hemi-nested PCR which simultaneously identifies *M. tuberculosis* and detect rifampicin resistance directly from clinical specimens. The samples were also subjected to ZN staining and the results were correlated.
Result: From a total of 257 samples, 33 samples were positive for GeneXpert MTB/RIF and none of them showed rifampicin resistance in the study. BAL samples predominated among the types of samples 104 (40.46%). Males were high (60.7%) in the study and samples collected were found to be the highest among age group above 60 (15.95%). Rate of positivity was high among 41-50 age group (21.21%). 16 out of 33 positive samples failed to show positive results in ZN staining while 17 positive samples were positive for smear as well.

Keywords: mycobacterium tuberculosis, gene Xpert MTB/RIF, rifampicin resistance, ZN staining

1. Introduction
Tuberculosis (TB), caused by *Mycobacterium tuberculosis* complex (MTBC), remains a major global public health concern and is the first leading cause of death from infectious diseases worldwide [1]. According to Revised National Tuberculosis Control Programme, 0.8 million new cases of extra-pulmonary TB (EPTB) were observed in 2010. Less than 5% of new and previously treated TB patients were tested for MDR-TB in most countries in 2010. In India and China, almost 50% of multidrug-resistant TB (MDR-TB) cases worldwide are estimated to occur. In India, 15 to 20% of TB cases are estimated to be cases of EPTB, which affects mainly the lymph nodes, meninges, kidney, spine, and growing ends of the bones [2]. Worldwide, large percentage of cases of MDR-TB remain undiagnosed, The conventional *Mycobacterium tuberculosis* (MTB) drug susceptibility testing is a gold standard technique but requires long period of time (8 weeks) to give definitive report. Further, it requires more sophisticated and higher bio-safety level laboratory along with the well trained staffs [3].
Growing scientific momentum in recent years, however, has fuelled a pipeline of new and improved TB diagnostics and assays to more rapidly detect drug resistance. Numbers of these assays have been endorsed by the WHO. However, one of these-the Xpert® MTB/RIF assay (Cepheid Inc., Sunnyvale, CA, USA)-has been described as a potential "game changer" for TB control. Using a prototype assay originally developed at the University of Medicine and Dentistry (NJ, USA), this new TB diagnostic was developed by the Foundation for Innovative New Diagnostics (FIND) in partnership with a commercial company, Cepheid Incorporated. This has been achieved with financial support from the US NIH and the Bill and Melinda Gates Foundation [4].

2. Materials and methods
2.1 Principle of the assay
The Gene Xpert assay is a real-time PCR test that will simultaneously identify *M. tuberculosis* and detect rifampicin resistance directly from clinical specimens. The Gene Xpert assay detects an 81-bp "core" region of the *rpoB* gene. The test utilizes five molecular beacons that detect mutations in the core region that are associated with rifampicin resistance. The analytical limit of detection of the Gene Xpert assay is reported to be 131 CFU/ml of specimen, based on spiked-sputum studies. Culture of concentrated specimens can detect very low concentrations of organisms-as low as 10 to 100 CFU/ml [5].

2.2 Samples
Both respiratory and extra-pulmonary samples were collected from the suspected patients sent to the department of microbiology at Sakra World Hospital. A total number of 257 samples were included in the study; which were collected from September 2016 to January 2018. Samples including Broncho Alveolar Lavage (BAL), sputum, Endotracheal (ET) secretion, pus and body fluids were collected and sent in sterile containers without any additives while tissue samples and lymph nodes were sent in sterile containers with normal saline. Samples sent in the day time were processed immediately but the samples received after regular working

hours or more than 4 samples received at a time were refrigerated at 2-8^{0}C and processed later.

2.3 Processing

Samples were processed by referring the guidelines of Xpert® MTB/RIF assay (Cepheid Inc., Sunnyvale, CA, USA). All samples are processed inside biosafety cabinet after wearing proper personal protective equipment.

2.3.1 Processing of lymph node and other tissue samples

1. Using sterile pair of forceps and scissors, cut the tissue specimen into small pieces in a sterile mortar (or homogenizer or tissue grinder).
2. Wash off visible blood in the sample by adding normal saline
3. Add normal saline to grind the sample to a homogenized suspension
4. Transfer 1mL of the homogenised sample to a conical screw-capped tube
5. Add 2mL of sample reagent available along with Xpert® MTB/RIF assay kit
6. Vigorously shake the tube 10 to 20 times or vortex for at least 10 seconds.
7. Incubate for 10 minutes at room temperature, and then shake the specimen vigorously again for another 10–20 times or vortex for at least 10 seconds.
8. Incubate the specimen at room temperature for an additional 5 minutes.
9. Using a fresh transfer pipette, transfer 2 ml of the processed sample to the Xpert MTB/RIF cartridge.
10. Load the cartridge into the GeneXpert instrument and start the assay

2.3.2 Processing of respiratory specimens

1. Transfer 1mL of the sample directly to a conical screw-capped tube
2. Add 2mL of sample reagent to it
3. Vigorously shake the tube 10 to 20 times or vortex for at least 10 seconds.
4. Incubate for 10 minutes at room temperature, and then shake the specimen vigorously again for another 10–20 times or vortex for at least 10 seconds.
5. Incubate the specimen at room temperature for an additional 5 minutes.
6. Using a fresh transfer pipette, transfer 2 ml of the processed sample to the Xpert MTB/RIF cartridge.
7. Load the cartridge into the GeneXpert instrument and start the assay

2.3.3 Processing of CSF and other body fluids

A. If there is more than 5mL of sample

1. Transfer all of the specimen to a conical centrifuge tube, and concentrate the specimen at 3000 g 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 2 ml by adding the sample reagent.
4. Using a fresh transfer pipette, transfer the entire content to the cartridge
5. Load the cartridge into the instrument
6. If the sample is 0.1-1mL
7. Make the sample to a final volume of 2 ml by adding the sample reagent.
8. Add 2 ml of the sample mixture directly to the Xpert MTB/RIF cartridge.
9. Load the cartridge into the GeneXpert instrument

Note: Samples less than 0.1mL are not accepted for the assay

2.4 Analysis of the data

Samples sent from September 2016 to January 2018 to the Department of Microbiology of Sakra World Hospital for GeneXpert MTB/RIF test were included in the study. The total number of samples collected was 257. All the samples were correlated with ZN staining which was done along with the test. Samples were analysed based upon the type, age, sex and the degree of positivity. Positive samples were further looked for rifampicin resistance which was detected by the instrument itself.

3. Result

Evaluation of GeneXpert MTB/RIF was based upon 257 samples collected from September 2016 to January 2018. Samples were classified according to the type and compared (Table1). Out of 257 samples, 9 (3.5%) were sputum samples in which 2 (22.2%) were positive. 104 (40.46%) were BAL in which 14 (13.36%) were positive. Pleural fluid were 38 (14.78%) in number out of which 1 (2.63%) was positive. 37 (14.39%) of tissue from different sites were sent to the lab and 7 (2.7%) among them were positive. CSF samples were 43 (16.73%) in which positive samples were 2 (4.65%). Pus samples were also from different sites with a total number of 13 (5.05%) in which MTB was detected in 4 (30.76%) samples. Ascitic fluid was 4 (1.55%) and none of them were positive. Out of 3 ET secretion samples, one (33.33%) was MTB positive. Lymph nodes were 2 (0.77%) and one (50%) was positive among them. Other samples include synovial fluid, fluid from FNAC, fluid from mediastinum and pericardial fluid which were only one each. Hence we combined all the 4 (1.55%) types together and none were positive.

The samples analysed sex-wise and the percentage was calculated. Out of 257 samples males were 156 (60.7%) and females were 101 (39.2%) (Figure 2)

Samples were then analysed by dividing them into different age group (Table 2, Figure 3). 13 (5.05%) males and 3 (1.16%) females were in 0-20 year group with a total of 16 (6.22%). 21-30 year group had 21 (8.17%) males and 19 (7.39%) females with a total number of 40 (15.56%) people. 31-40 year group had 35 (13.61%) males and 22 (8.56%) of females with a total of 57 (22.17%) people. 21 (8.17%) males and 14 (5.44%) females were in 41-50 year group and the total people in the group were 35 (13.61%). 51-60 year group was comprised of a total number of 44 (17.12%) people out of which 26 (10.11%) were males and 18 (7%) were females. Finally the people above 60 were analysed in which 41 (15.95%) were males and 24 (9.33%) were females with a total number of 65 (25.29%) people.

There were a total of 33 positive cases for Gene Xpert

MTB/RIF. Percentage of positive samples among the different age groups were then calculated (Table 3, Figure 4). 0-20 year group had 3 (9.09%) positive cases in which 2 (6.06%) were males and 1 (3.03%) was female. In 21-30 year group 6 (18.18%) were found to be positive out of which 4 (12.12%) were males and 2 (6.06%) were females. 31-40 year group comprised of 6 (18.18%) of positives in which 1 (3.03%) was male and 5 (15.15%) were females. 7 (21.21%) samples were positive in 41-50 year group in which 3 (9.09%) males and 4 (12.12%) females were present. 51-60 year group had 5 (15.15%) positives out of which 3 (9.09%) were males and 2 (6.06%) were females. 6 (18.18%) of positive samples were falling in above 60 year group in which 3 (9.09%) were males and 3 (9.09%) were females.

Positive results were appeared qualitatively in the system by categorising the results in four. MTB detected- very low, MTB detected- low, MTB detected- medium and MTB detected- High. The samples were hence analysed sex-wise according to the above mentioned 4 categories (Table 4, Figure 5). Out of 33 positive cases 10 (30.30%) people were falling under very low positive category out of which 8 (80%) were males and 2 (20%) were females. In low positive category, 9 (27.27%) were found out of which 2 (22.22%) were males and 7 (77.77%) were females. Medium positive category had 7 (21.21%) positive cases out of which 5 (71.42%) were males and 2 (28.57%) were females. High positive category had 7 (21.21%) positive cases out of which 3 (42.85%) were males and 4 (57.14%) were females.

All the samples were also subjected to ZN staining and both the results are correlated (table 5). Out of 257 samples all the negative results were negative for ZN staining also. Out of 33 positive samples 10 were very low positives and failed to show positive result in ZN staining. 9 samples showed low positive in Gene Xpert MTB/RIF. Out of them 3 samples were showed scanty AFB in ZN staining but remaining 6 were ZN negative. All the medium positive and high positive samples were also positive for ZN staining (7 medium positive samples and 7 high positive samples).

4. Discussion

With 130,000 new cases in 2015, India had the highest population of RR-TB in the world. Although two-thirds of the Indian population live in rural areas, diagnosis of RR-TB in rural settings is difficult because of the scarcity of qualified technicians and sophisticated laboratories. Although identification of *M. tuberculosis* and drug susceptibility tests remain the gold standard for TB diagnosis, pathogen culturing is time-consuming and owns a relatively high false-negative rate [6]. Our results demonstrate that the GeneXpert MTB/RIF assay can be used to diagnose RR-TB in rural settings with limited laboratory infrastructure [7].

Rapid diagnosis and detection of rifampicin (RIF) resistance is necessary for TB control, as transmission and emergence of multi-drug resistant tuberculosis (MDR-TB) cause serious health problems. Gene Xpert MTB/RIF and Geno Type MTBDR*plus* were approved by the WHO in 2011 and recommended for diagnosis of TB and MDR-TB in developing and high prevalence countries. Gene Xpert MTB/RIF is a real time PCR (RT-PCR)-based molecular assay that amplifies a specific sequence of rpo B gene in *Mycobacterium tuberculosis* (MTB) and detects rifampicin resistance mutations as a marker for MDR-TB. The sensitivity of GX is closely related to the bacilli concentration in the specimen, and subsequently depends on smear status [8].

Our study was based upon 257 samples which were taken from different sites from the suspected TB case patients in a tertiary care hospital from September 2016 to January 2018. According to the analysis of the sample type, BAL samples found out to be the most commonly sent sample for Gene Xpert assay which is 104 (40.46%) of the total samples. Surprisingly sputum samples were received very less (9 out of 257; 3.5%) which is the preferable sample for MTB detection. Samples like CSF (43 out of 257; 16.73%), pleural fluid (38 out of 257; 14.78%) and tissue samples (37 out of 257; 14.39%) were frequently received. Most number of positive cases isolated from BAL (14 out of 104) but percentage of positivity was highest in lymph node (50%) which shows the criticality of lymph node samples in the detection of MTB. It also proves than not just pulmonary TB but also extra-pulmonary TB is also prevalent in the surrounding area.

Sex-wise analysis showed that males outnumbered females by 60.7% to 39.2%. Samples were the most from the age-group above 60 years (65 out of 257; 25.29%) which is followed by 31-40 age-group (57 out of 257; 22.17%) while 0-20 year group were the least (16 out of 257; 6.22%) which shows that >60 years of age-group are suspected to be more prone to MTB while children are the least suspected group. Percentage of positivity was maximum among 41-50 year group (7 out of 33 positive cases; 21.21%) and minimum among patients below 20 (3 out of 33 positive cases; 9.09%). According to our study 41-50 year group people are more prone to MTB infection even though other age-groups were also showed considerable percentage of positivity.

There was no considerable variation among positive samples evaluated according to rate of positivity. Very low positives were marginally higher (10 out of 33; 30.30%) than other categories. But this categorisation could clearly show the upper-hand of Gene Xpert assay over ZN stain or conventional culture.

Comparison of positive results with ZN staining revealed that ZN smear is less sensitive than the GeneXpert MTB/RIF test and M960 culture because the ZN smear method requires 10^4bacilli/ml of specimen to generate a positive result. However, the Gene pert assay only requires 131 bacilli /ml of specimen and M960 culture requires as low as 10 to 100 bacilli/ml [9]. Very low positive category failed to show any positive result on the smear while medium positive samples and high positive samples were also positive for ZN smear. Only 3 out of 9 (33.33%) of low positive category showed scanty positive ZN smear while 6 out of 9 (66.66%) of low positive samples were smear negative.

In the whole study we did not come across rifampicin positive MTB strain. All the strains of MTB detected in the hospital were negative for the presence of *rpoB* gene which shows that resistance strain of MTB are not seen in the surrounding area of study.

5. Limitations

Since the rate of positivity is very low, there is only a marginal variation in the four categories of the positive result. Delay in processing samples which were received after the

normal working hours could also have affected result.

Table 1: Evaluation of the sample types with positive cases

Types of samples	No. of samples	Percentage (%)	No. of positives	% of positive samples
Sputum	9	3.5	2	22.22
BAL	104	40.46	14	13.46
Pleural fluid	38	14.78	1	2.63
Tissue	37	14.39	7	2.7
CSF	43	16.73	2	4.65
Pus	13	5.05	4	30.76
Ascitic fluid	4	1.55	0	0
ET secretion	3	1.16	1	33.33
Lymph node	2	0.77	1	50
Others	4	1.55	0	0

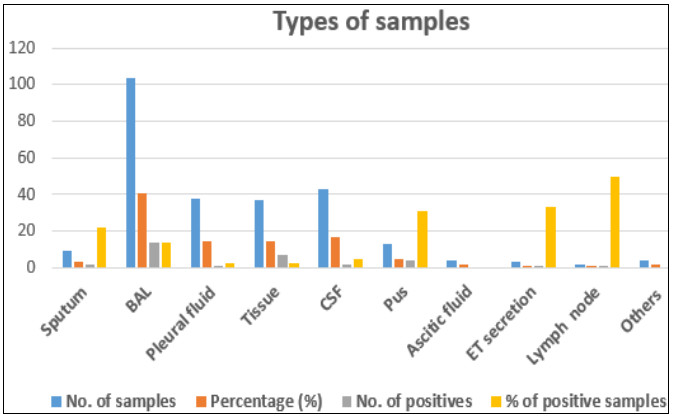

Fig 1: Distribution of sample types with positive cases

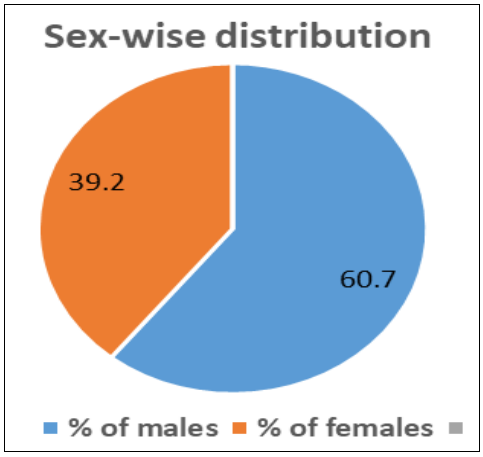

Fig 2: Sex-wise distribution of samples

Table 2: Age-wise distribution of the samples

Age group (years)	Males (%)	Females (%)	Total (%)out of 257
0-20	13 (5.05%)	3 (1.16%)	16 (6.22%)
21-30	21 (8.17%)	19 (7.39%)	40 (15.56%)
31-40	35 (13.61%)	22 (8.56%)	57 (22.17%)
41-50	21 (8.17%)	14 (5.44%)	35 (13.61%)
51-60	26 (10.11%)	18 (7%)	44 (17.12%)
>60	41 (15.95%)	24 (9.33%)	65 (25.29%)

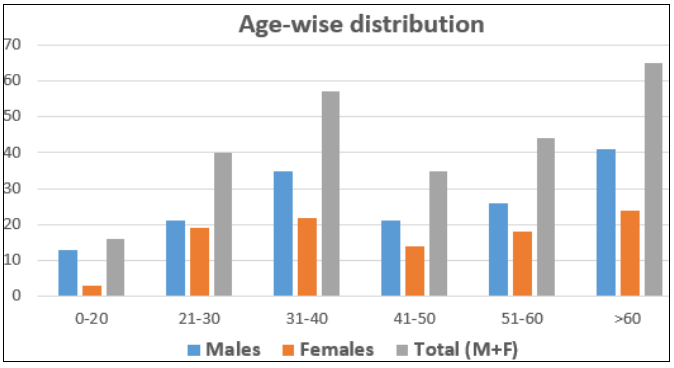

Fig 3: Age-wise analysis of samples

Table 3: Percentage of positive samples among different age groups.

Age-groups (years)	Positive Males (%)	Positive Females (%)	Total Positives (%out of N=33)
0-20	2 (6.06%)	1 (3.03)	3 (9.09%)
21-30	4 (12.12%)	2 (6.06%)	6 (18.18%)
31-40	1 (3.03%)	5 (15.15%)	6 (18.18%)
41-50	3 (9.09%)	4 (12.12%)	7 (21.21%)
51-60	3 (9.09%)	2 (6.06%)	5 (15.15%)
>60	3 (9.09%)	3 (9.09%)	6 (18.18%)

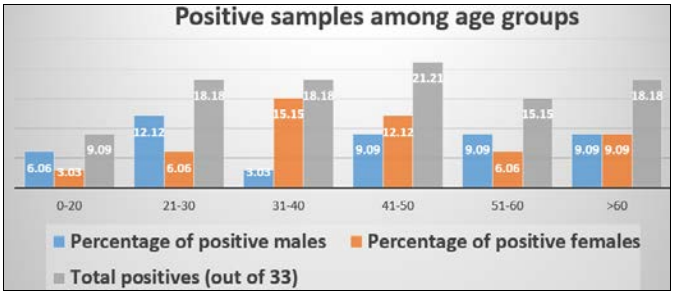

Fig 4: Distribution of positive samples among different age groups

Table 4: Sex-wise analysis of positive samples

Sex	Very low	Low	Medium	High
Male	8 (80%)	2 (22.22%)	5 (71.42%)	3 (42.85%)
Female	2 (20%)	7 (77.77	2 (28.57%)	4 (57.14%)
Total	10 (30.30%)	9 (27.27%)	7 (21.21%)	7 (21.21%)

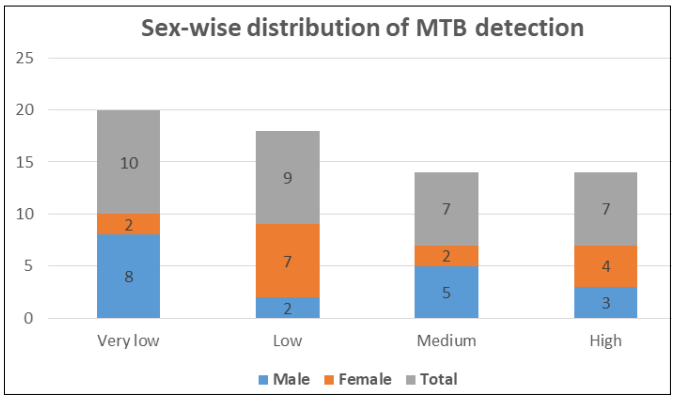

Fig 5: Sex-wise analysis of positive samples

Table 5: Comparison of GeneXpert positive samples with ZN staining

Positivity	Total samples	ZN smear positives	ZN smear negatives
Very low positive	10	0 (0%)	10 (100%)
Low positive	9	3 (33.33%)	6 (66.66%)
Medium positive	7	7 (100%)	0 (0%)
High positive	7	7 (100%)	0 (0%)

6. Conclusion

The findings of the study confirm that Gene Xpert MTB/RIF assay is an important advance in the diagnosis of TB and support the WHO guidelines [10]. Not only pulmonary TB but extra-pulmonary TB is also prevalent in the study area. 41-50 year group are found to be more prone to MTB infection. Even though very low positive category was marginally high there is no significant variation in the rate of positivity. At the same time the analysis also shows that ZN staining is less sensitive than Gene Xpert assay. While rifampicin resistance is an upcoming threat to the society, it is still not prevalent near the study area.

7. References

1. Yu Pang, Yuanyuan Shang, Jie Lu, Qian Liang, Lingling Dong, Yunxu Li, *et al*. GeneXpert MTB/RIF assay in the diagnosis of urinary tuberculosis from urine specimens. SCIeNTIfIC Reports | 7: 6181 | DOI: 10. 1038/s41598-017-06517.
2. Ankush Raj, Netrapal Singh, Promod K. Mehta. Gene Xpert MTB/RIF Assay: A New Hope for Extrapulmonary Tuberculosis. IOSR Journal of Pharmacy. 2012; 2(1):083-089.
3. Pratikshya Pandey, Narayan Dutt Pant, Komal Raj Rijal, Bhawana Shrestha, SiritaKattel, Megha Raj Banjara, *et al*. Diagnostic Accuracy of GeneXpert MTB/RIF Assay in Comparison to Conventional Drug Susceptibility Testing Method for the Diagnosis of Multidrug-Resistant Tuberculosis. Plos One y, 2017. DOI:10.1371/journal.pone.0169798.
4. Stephen D Lawn, Mark P Nicol. Xpert® MTB/RIF assay: development, evaluation and implementation of a new rapid molecular diagnostic for tuberculosis and rifampicin resistance. Future Microbiol. 2011; 6(9):1067-1082.
5. Elizabeth M. Marlowe, Susan M, Novak-Weekley, Joven Cumpio, Susan E, Sharp Michelle A. Momeny, Anna Babst, etal. Evaluation of the Cepheid Xpert MTB/RIF Assay for Direct Detection of Mycobacterium tuberculosis Complex in Respiratory Specimens. Journal of Clinical Microbiology. 2011, 1621-1623.
6. Hai Wen Pengzhi Li, Hong Ma Guohua LV. Diagnostic accuracy of Xpert MTB/RIF assay for musculoskeletal tuberculosis: a meta-analysis. Infection and Drug Resistance. 2017; 10:299-305.
7. Raghuprakash Reddy, Gerardo Alvarez-Uria. Molecular Epidemiology of Rifampicin Resistance in Mycobacterium tuberculosis using the GeneXpert MTB/RIF Assay from a Rural Setting in India. Hindawi Journal of Pathogens, 2017, 5. Article ID 6738095.
8. Tingyu Tang, Fang Liu, Xiaoling Lu, Qingdong Huang. Evaluation of GeneXpert MTB/RIF for detecting Mycobacterium tuberculosis in a hospital in China. Journal of International Medical Research. 2017; 45(2):816-822.
9. Husseiny SH Moussa, Faten S Bayoumi, Ahmed Ali M. Evaluation of Gene Xpert MTB/RIF assay for direct diagnosis of pulmonary tuberculosis. Saudi Med J, 2016; 37(10).
10. Laura Maynard-Smith, Natasha Larke, Jurgens A Peters, Stephen D Lawn. Diagnostic accuracy of the Xpert MTB/RIF assay for extrapulmonary and pulmonary tuberculosis when testing non-respiratory samples: a systematic review. BMC Infectious Diseases. 2014; 14:709.