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RESEARCH ARTICLE
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DIAGNOSTIC PERFORMANCE OF GENE XPERT MTB/RIF ASSAY IN PLEURAL FLUID: AN OBSERVATIONAL STUDY AT TERTIARY CARE CENTER

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ABSTRACT

INTRODUCTION: Tuberculosis (TB) caused by *Mycobacterium tuberculosis* is a global health concern. According to Global TB Report 2021, the incidence of tuberculosis was 19,33,381. Late diagnosis and delay in treatment causes increase in mortality and morbidity. A reliable and rapid diagnostic tool is the priority for TB control. WHO has approved Gene Xpert MTB/RIF in December 2010 which is a Cartridge Based Nucleic Acid Amplification Test (CBNAAT). A new automated diagnostic molecular test which is not only sensitive (detects 131 CFU/ml of mycobacterium tuberculosis but also a rapid test (results are available in 2 hours) in comparison to liquid culture which are time consuming and necessitates biosafety procedures and require trained laboratory staff.

OBJECTIVE: To evaluate the diagnostic yield of Gene Xpert MTB/RIF assay in pleural fluid.

MATERIAL AND METHOD: A Cross-Sectional Observational study observational study was done in Department of Microbiology, MLN Medical College, Prayagraj from January 2023 to December 2023. The GeneXpert MTB/RIF (Cepheid, Sunnyvale, CA, USA) was performed on pleural fluid along with detection of presence of *Mycobacterium tuberculosis* it also detected Rifampicin resistance.

RESULT: This study revealed the mean age of pleural fluid sample patients to be 42.0 years. GeneXpert MTB positivity was observed in 22.7% of pleural fluid samples. The diagnostic performance for pleural fluid samples showed a sensitivity of 89.47%, specificity of 100%, PPV of 100.0%, NPV of 96.55%, and an overall accuracy of 97.3%.

CONCLUSION: The Xpert MTB/RIF assay demonstrates high sensitivity and specificity in detecting *Mycobacterium tuberculosis* in CSF and pleural fluid samples. Its rapid and accurate diagnostic capabilities is a valuable tool for improving diagnosis of tuberculosis in a resource-limited areas.

KEYWORDS: MTB (Mycobacterium Tuberculosis), Gene Xpert, RIF (Rifampicin), CBNAAT (cartridge based nucleic acid amplification test).

INTRODUCTION

Tuberculosis is an important chronic infectious disease caused by mycobacterium tuberculosis which is transmitted via aerosol generated during coughing and sneezing[1]. It causes significant mortality and morbidity globally with vulnerable population such as poor, malnourished and immunosuppressed being at high risk[2]. It is considered as one of the oldest diseases recognized by mankind and leading cause of death from a single infectious agent ranking above HIV/AIDS until COVID-19 pandemic[3].

GLOBAL TB BURDEN: 10.8 million people fell ill with TB worldwide in 2023 is an increase from 10.7 million in 2022, 10.4 million in 2021. The three consecutive years of increasing global TB incidence from 2021–2023 continuing the reversal of the downward trend that had been sustained for many years up to 2020. TB burden in 2020 was 9.9 million new cases, 1.5 million deaths. 1.3 million deaths in HIV-ve and 2,14,000 deaths in HIV+ve cases. Disease is overwhelming in countries with high population density. India is the biggest contributor to global burden of TB (27% of 10 million incident cases and 27% of 1.5 million TB-related deaths annually. According to WHO's Global Tuberculosis report 2022, Oct 27. 10.6 million people become ill and 1.6 million people died in 2021(1,87,00 with HIV)[4].

DIAGNOSTIC CHALLENGES:

Diagnosis of TB offers big diagnostic challenge related to detection limit of smear microscopy requiring a concentration of 10,000 colony forming unit (CFU)/ml and only modest sensitivity and resistant although being economically easy to perform[5]. Culture being gold standard for TB diagnosis with detection of limit of 10 bacilli/ml of specimen but its long time to culture i.e 42 days causes delay in diagnosis[6]. In children who represent 30% of burden remain undiagnosed and sub-optimally treated as the disease present as non-specific clinical and radiological sign[7]. Extrapulmonary TB due to its atypical presentation, inaccessible sites, paucibacillary nature and non unform distribution of mycobacteria leading to false negative results thus making the diagnosis difficult and prolonged[6].

GOVERNMENT APPROACH:

Rapid and accurate diagnosis of TB infection is essential for starting appropriate treatment and control of infection[6]. Efforts have been made so that TB surveillance is greater and powerful together with health agencies, outline strategies to prevent, combat and develop new sensitive, specific and fast diagnostic technique[3]. Since 2003, foundation for Innovative Diagnostics (FIND) has been working for betterment of TB and has improved the access to new diagnostic tools[8]. In past decades, significant research has been made towards the expansion of new diagnostic tools comprise molecular line probe assays for multi-drugresistant TB diagnosis (Genotype MTBDR plus). Molecular detection of MTB using Xpert limits wrong use of anti-TB drugs in patient with MDR-TB[9].

GENE XPERT MTB/RIF:

In 2010 WHO implemented Gene Xpert MTB/RIF assay (Cephid, CA, USA) to assist rapid detection of MTB isolates as well as to detect the resistance against RIF with revelation of >95% sensitive for smear positive and 55% for smear-negative cases. It can be directly performed on sputum samples of individuals with presumptive TB and can deliver results within two hours. Xpert Ultra works on same principle as Xpert but with greater specificity since 2017.

NATIONAL TB ELEMINATION PROGRAMME:

The National Strategic Plan (NSP) for TB elimination 2025 aims to bring down TB incidence to 44 per 1,00,000, prevalence rate to 65 per 1,00,000 and mortality by 3 per 1,00,000 by 2025. WHO's post-2015 End TB Strategy of 80% decrease in new cases, 90% decrease in death due to TB by 2030 (from 2015 base). Building a strong coalition with civil society and community is emphasized in End TB Strategy and is a major component of pillar 2.

AIM & OBJECTIVES

Aim: To evaluate the diagnostic performance of Xpert MTB/RIF assay in pleural fluid.

Objectives:

To detect the diagnostic performance of Xpert MTB/RIF assay in pleural fluid.

To detect the resistance against Rifampicin (RIF).

MATERIAL & METHOD

A Cross-Sectional Observational study was done from January 2023 to December 2023. The patients were selected from OPD & IPD of Pulmonary Medicine of Swaroop Rani Nehru Hospital and Paediatric Department of Sarojini Naidu Children Hospital, Prayagraj. All the patients clinically suspected of pleural tuberculosis, pleural fluid samples were collected and processed in the Department of Microbiology, Moti Lal Nehru Medical College. Patients with Pulmonary Tuberculosis, Bacterial infection, Fungal infection, Lung malignancies and patients not willing to be a part of study were not included in the study.

Methodology: The study obtained the institutional ethics clearance prior to recruitment of patients. After obtaining the informed consent from patients, the detailed history of the patient was obtained including prior treatment history and presence of any co-morbid condition. The patient's pleural fluid samples (5ml) were collected by pleural tapping under full aseptic procedure and subjected for gene Xpert MTB/RIF assay. Samples were prepared according to the manufacturer's user instructions. Sample reagent was added directly into the falcon tube with pleural fluid in the ratio of 2:1. The cap was placed and the tube was vortexed 10-20 times and incubated at room temperature. After 10 minutes, the specimen was vortexed again for 10-20 times. Sample ID. Was labelled on the Cartridge and 2ml of processed specimen was transferred with the help of sterile disposable transferring pipette into it.

The Cartridge barcode was scanned. After entering the sample ID on the system, the cartridge was positioned on the Xpert machine and test was started. Result was recorded within 2 hours. Two components were noted in the result, first the detection of MTB and the second component was identification of RIF resistance.

RESULT

Total sample received from from January 2023 to December 2023 were 150.

This study revealed following results:

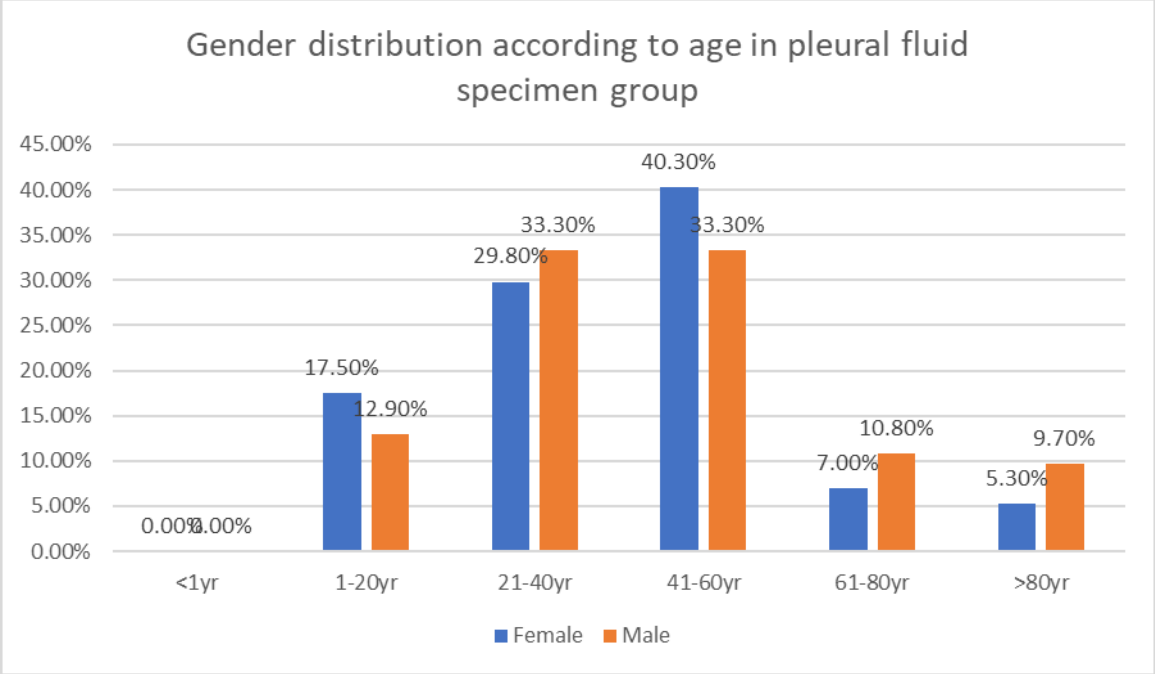
The mean age of pleural fluid specimen was 42.0yrs.

Pleural fluid	
Mean	SD
42.0	20.7

AGE-SEX DISTRIBUTION:

AGE WISE DISTRIBUTION	Female N=57 (100.0%)	Male N=93 (100.0%)
<1yr	0 (0.0%)	0 (0.0%)
1-20yr	10 (17.5%)	12 (12.9%)
21-40yr	17 (29.8%)	31 (33.3%)

41-60yr	23 (40.3%)	31 (33.3%)
61-80yr	4 (7.0%)	10 (10.8%)
>80yr	3 (5.3%)	9 (9.7%)



FINDINGS OF GENE XPERT:

GENE XPERT	Count
NEGATIVE	116
POSITIVE	34

RIF STATUS:

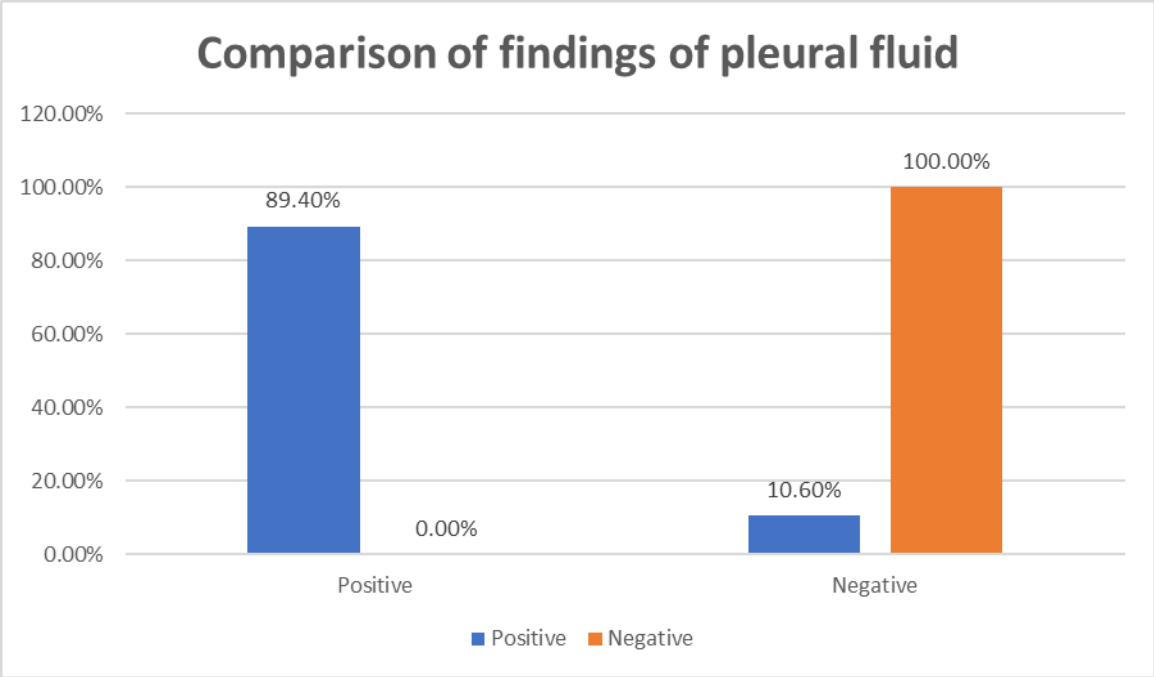
RIF	Count=150
I	1
NP	116
R	5
S	28

BIOCHEMICAL PARAMETERS:

BIOCHEMICALS	PLEURAL FLUID	
	Mean	SD
PROTEIN gm/dl	4.9	9.9
GLUCOSE mg/dl	106.4	69.9
ADA U/L	43.68	47.40
LDH IU/L	480.1	451.2

COMPARISON WITH FINAL DIAGNOSIS:

PLEURAL FLUID GENEXPERT MTB	FINAL DIAGNOSIS		
	Positive	Negative	Total
Positive	34 (89.4%)	0 (0.0%)	34
Negative	4 (10.6%)	112 (100%)	116
Total	38	112	



DIAGNOSTIC CHARACTERISTCS OF PLEURAL FUID GENE XPERT MTB:

Statistic	**Value**	**95% CI**
Sensitivity	89.47%	73.94% to 97.06%
Specificity	100%	93.81% to 100.0%
Positive Predictive Value	100.0%	89.12% to 100.0%
Negative Predictive Value	96.55%	91.74% to 98.60%
Accuracy	97.33%	91.50% to 99.27%

DISCUSSION

This study aimed to evaluate the diagnostic performance of the Xpert MTB/RIF assay in pleural fluid by assessing its sensitivity, specificity, PPV, and NPV. By comparing its performance with biochemical markers, this study seeks to establish the clinical utility of the assay in diagnosing Pleural TB in a tertiary care setting.

In present study, the mean age of pleural fluid specimen was 42.0yrs. In study by Rai A et al., 55 children (less than and equal to 18 years) with tubercular meningitis. 14 (25.4%) children were less than 5 years of age, 28 (50.9%) of 5-10 years of age; and 13 (23.7%) were 10-18 years age[10].

In study by Raj A et al., documented with out of total 831 samples included in the study, 507 (61.01%) were males, 324 (38.99%) were females. The mean age of patients was 52.01[11]. Gupta H et al., the mean age of the study group was 38.01 yrs. and the sex ratio was 2.63:1[12]. The mean age was 36.86 years in study by Aricha SA et al[13].

In pleural fluid 22.7% were positive on GeneXpert MTB. Biochemical parameters such as protein, Glucose, ADA and LDH were measured.

The most instances were found using a combination of culture and Xpert[14]. Also in study by Rice JP et al., found that Xpert MTB/RIF assay demonstrated an overall sensitivity of 89.6% for detecting MTBC, with 97.7% sensitivity in smear-positive cases and 74.5% in smear-negative cases, along with

a specificity of 97.2%. In comparison, the acid-fast bacilli (AFB) smear test showed a sensitivity of 64.9% and a specificity of 77.8%. Xpert MTB/RIF successfully identified 35 out of 47 smear-negative, culture-positive specimens and ruled out 124 of 137 smear-positive, culture-negative specimens.

In study by Brito MG et al., showed sensitivity of 81%, PPV of 90%, NPV of 92%, and accuracy of 92% compared with AFB staining 87%, 68%, 88%, and 87%, respectively[15]. The sensitivity of 94.55% and specificity of 95.97% showed by Xpert MTB/RIF in relation to culture while the accuracy was 95.69%, the PPV was 85.53, and the NPV was 98.59 in study by Belotti V et al[16].

The diagnostic characteristics of Pleural fluid showing the sensitivity of 89.47%, specificity of 100%, PPV of 100%, NPV of 96.55% and overall accuracy was found to be 97.33%.

Friedrich SO et al., found that the Xpert test in pleural fluid has a sensitivity and specificity of 25% and 100%, respectively. Pleural fluid culture confirmed all positive Xpert test results[17]. The sensitivities ranged from 34 to 51.4% and specificities ranged from 98 to 99% in pleural fluid sample in the study by Soe YS et al. various type of samples showed varied pooled sensitivity in this study. In diagnosing paediatric EPTB, Xpert displayed high specificity but moderate sensitivity[18].

The Xpert MTB/RIF assay demonstrated a 97.2% concordance rate with culture results for detecting Rifampicin resistance. The findings suggest that Xpert MTB/RIF can replace the AFB smear for diagnosing extrapulmonary tuberculosis and provides high specificity for detecting Rifampicin resistance[19]. AFB smear microscopy demonstrated a sensitivity of 72.1%, specificity of 81.3%, false-negative rate of 27.9%, and total accuracy of 78.8% for pulmonary tuberculosis (PTB), while for extrapulmonary tuberculosis (EPTB), these values were 63.2%, 70.5%, 36.8%, and 68.4%, respectively, using LJ culture as the gold standard. GeneXpert MTB/RIF outperformed AFB smear microscopy, with higher sensitivity and specificity for PTB (90.2% and 86.9%, respectively, with a 9.8% false-negative rate) compared to EPTB (81.6% sensitivity, 78.9% specificity, and an 18.5% false-negative rate)[20].

CONCLUSION

Among the various forms of Extrapulmonary tuberculosis, tuberculous pleuritis is associated with high morbidity and mortality due to delayed or inadequate diagnosis. Conventional diagnostic methods, including smear microscopy and culture, have limitations in sensitivity, turnaround time, and reliability, particularly in paucibacillary specimens such as cerebrospinal fluid and pleural fluid. Within less than 2 hours Xpert performs and incorporates the steps of bacterial lysis, DNA extraction, amplification and amplicon detection using a disposable plastic cartridge.

The Xpert MTB/RIF assay, a rapid, automated, cartridge-based nucleic acid amplification test (NAAT), has revolutionized TB diagnosis by providing results within two hours with high sensitivity and specificity. This assay detects DNA of *Mycobacterium tuberculosis* and rifampicin resistance mutations, enabling prompt initiation of appropriate treatment. Although extensively validated for pulmonary TB, its diagnostic accuracy in extrapulmonary samples, particularly pleural fluid, remains a subject of ongoing research.

The advantages of Gene Xpert MTB/RIF are:

- Minimum turn-around time
- Low complexity
- High sensitivity and specificity
- Minimum safety concerns
- Detects MTB along with Rifampicin resistance.

Pleural TB often presents with nonspecific symptoms, making its diagnosis challenging. Biochemical markers such as adenosine deaminase (ADA), lactate dehydrogenase (LDH), and protein levels in CSF and pleural fluid provide supportive diagnostic information, but their specificity varies. Therefore, a reliable molecular test like Xpert MTB/RIF is essential to improve diagnostic efficiency and patient outcomes.

Results from this study helps to bridge the gap in TB diagnosis, reporting and early and prompt treatment of positive patients. The theme of World TB Day 2021 “The clock is ticking” conveys the

urgent need of rapid diagnostic tool to tackle this disease. Gene Xpert MTB/RIF has redeemed about 53 million lives from the clutches of TB, mainly through timely diagnosis and effective treatment.

REFERENCES

1. Umair. M et al. Diagnostic accuracy of sputum microscopy in comparison with Gene Xpert in Pulmonary TB 2020 Nov 8;12(11):e11383. doi: 10.7759/cureus.11383.
2. Garba. M.A et al. Trends in paediatric tuberculosis diagnosis utilizing Xpert *Mycobacterium tuberculosis*/Rifampicin in a poor-resource, high-burden region: A retrospective, multicenter study 2023 Jan-Mar;12(1):77-81 doi: 10.4103/ijmy.ijmy_1_23.
3. Belotti. N.C.U et al. Evaluation of xpert *Mycobacterium tuberculosis* rifampicin for tuberculosis diagnosis in a reference laboratory 2022 Oct-Dec;11(4):435-441 doi: 10.4103/ijmy.ijmy_195_22.
4. Global Tuberculosis report 2022, Oct 27
5. Arora. D et al. Utility of smear microscopy and GeneXpert for the detection of *Mycobacterium tuberculosis* in clinical samples 2020 Jun 2;10(2):81-87doi: 10.18683/germs.2020.1188. eCollection 2020 Jun.
6. Poornima. M.V et al. Comparison of TrueNat Polymerase chain reaction and Mycobactrium growth indicator tube culture in the diagnosis of pulmonary and extrapulmonary tuberculosis. Journal of the academy of clinical microbiologist.
7. Zar. H.J et al. Tuberculosis diagnosis in children using Xpert ultra on different respiratory specimens. 2019 Dec 15;200(12):1531-1538 doi: 10.1164/rccm.201904-0772OC.
8. Raj. A et al. Validity of CBNAAT assay in diagnosing tuberculosis in comparison to culture: A study from an urban area of South India 2020 Oct 28:21:100198 doi: 10.1016/j.jctube.2020.100198. eCollection 2020 Dec.
9. Anand. M.K et al. Cepheid Gene Xpert MTB/RIF assay for the rapid detection of Mycobacterium tuberculosis and identification of Rifampicin resistance.
10. Rai A, Prasad R, Das BK, Anupurba S, Singh UK. Cerebrospinal fluid Gene XPERT (CBNAAT) in children with tuberculous meningitis. Journal of Clinical Tuberculosis and Other Mycobacterial Diseases. 2021 Aug 1;24:100255.
11. Raj A, Baliga S, Shenoy MS, Dhanashree B, Mithra PP, Nambiar SK, Sharon L. Validity of a CB-NAAT assay in diagnosing tuberculosis in comparison to culture: A study from an urban area of South India. Journal of Clinical Tuberculosis and Other Mycobacterial Diseases. 2020 Dec 1;21:100198.
12. Gupta H, Pandey RP, Prajapati MK, Indurkar M. CBNAAT Positivity in Sputum of Tuberculosis Patients with HIV. Journal of Evolution of Medical and Dental Sciences. 2019 Dec 16;8(50):3764-9.
13. Aricha SA, Kingwara L, Mwirigi NW, Chaba L, Kiptai T, Wahogo J, Otwabe JS, Onyango PO, Karanja M, Ayieko C, Matu SW. Comparison of GeneXpert and line probe assay for detection of Mycobacterium tuberculosis and rifampicin-mono resistance at the National Tuberculosis Reference Laboratory, Kenya. BMC infectious diseases. 2019 Dec;19:1-8.
14. Bahr NC, Tugume L, Rajasingham R, Kiggundu R, Williams DA, Morawski B, et al. Improved diagnostic sensitivity for tuberculous meningitis with Xpert® MTB/RIF of centrifuged CSF. Int J Tuberc lung Dis. 2015;19(10):1209–15.
15. Brito GM, Mafort TT, Ribeiro-Alves M, Reis LV, Leung J, Leão RS, Rufino R, Rodrigues LS. Diagnostic performance of the Xpert MTB/RIF assay in BAL fluid samples from patients under clinical suspicion of pulmonary tuberculosis: a tertiary care experience in a high-tuberculosis-burden area. Jornal Brasileiro de Pneumologia. 2021 May 17;47(02):e20200581.
16. Belotti NU, Madela NK, Nardi SM, Mariano DC, de Souza NG, Oliveira RS, Pedro HD. Evaluation of xpert Mycobacterium tuberculosis rifampicin for tuberculosis diagnosis in a reference laboratory. The International Journal of Mycobacteriology. 2022 Oct 1;11(4):435-41.

17. Friedrich SO, von Groote-Bidlingmaier F, Diacon AH. Xpert MTB/RIF assay for diagnosis of pleural tuberculosis. J Clin Microbiol. 2011 Dec;49(12):4341–2.
18. Seo YS, Kang J-M, Kim DS, Ahn JG. Xpert MTB/RIF assay for diagnosis of extrapulmonary tuberculosis in children: a systematic review and meta-analysis. BMC Infect Dis. 2020;20(1):1–10.
19. Kim S-K, Chang J, Choi S-H, Sung H, Kim M-N. Performance of Xpert MTB/RIF for the Diagnosis of Extrapulmonary Tuberculosis. Clin Lab. 2021;(2):1–4.
20. Elbrolosy AM, El Helbawy RH, Mansour OM, Latif RA. Diagnostic utility of GeneXpert MTB/RIF assay versus conventional methods for diagnosis of pulmonary and extra-pulmonary tuberculosis. BMC Microbiol. 2021;21:1–10.