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Original Article

Comparative Analysis of the Efficacies of the GeneXpert and Solid Culture Media Techniques in the Diagnosis of Mycobacterium Tuberculosis

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Abstract

Tuberculosis is one of the predominant infectious diseases causing significant deaths worldwide. Detection of *Mycobacterium tuberculosis* bacilli (MTB) using culture media was officially recognized by World Health Organization. However, there is a significant limitation in the authenticity of evaluation for its effectiveness on clinically important attributes. GeneXpert detects the presence of *Mycobacterium tuberculosis* (*M. tuberculosis*) based on the detection of nucleic acid and is able to identify the resistance of both isoniazid (INH) and Rifampicin (RIF) drugs. In this technique, DNA amplification is done using the GeneXpert instrument in the suspected sample with a specific reagent cartridge. Although GeneXpert is a rapid technique compared to other diagnostic tools for MTB identification due to false-negative results, the culture media technique is still considered the gold standard in detecting *M. tuberculosis*. The current study was designed to evaluate the comparative efficacies of GeneXpert and the solid culture media technique in identifying MTB. Sputum samples of 250 (n=250) suspected tuberculosis (TB) patients were investigated using both diagnostic techniques. The results revealed that out of the 250 suspected patients, 30 (12%) samples were positive with the culture media technique, while only 17 (6.8%) samples showed positive results with GeneXpert. Culture tests and GeneXpert are not equally efficient in detecting *M. tuberculosis.* The current study's findings showed that the culture-based detection method for *M. tuberculosis* is more efficient and reliable than GeneXpert.

Keywords: *M. tuberculosis*, GeneXpert, Culture media, Acid-fast *Bacilli*

1. Introduction

Tuberculosis (TB) is one of the deadliest diseases worldwide caused by *M. tuberculosis* (1). This communicable disease usually affects the lungs and is transmitted by the air as droplet nuclei (1–5 µm) (2). MTB is 2-4 µm in length, non-motile obligate aerobe,

while the multiplication time of these bacilli ranges from 15-20 hours. Being a major global medical issue, TB accounted for 9 million new cases and 2 million deaths consistently. TB patients with HIV co-infection often express extra-pulmonary response and rapid disease progression unless the disease is treated properly. The early detection of disease contributes to a large extent to devising an appropriate treatment strategy that works to improve efficiency, increase the rate of cure and limit disease transmission (3). However, other than that is what occurs in developing countries, and this is due to the poor diagnostic techniques, as they rely on primitive methods of treatment that result in many interventions and give false results or delay diagnosis, which ultimately affects the rate of recovery. Where the detection strategy depends on a microscopic examination using Ziehl-Neelsen (ZN) or by culture technique, but despite its quality, it requires more time, up to a month, and this, of course, will delay the diagnosis, and the condition deteriorates, and the chance of recovery is little or even non-existent (4).

Therefore, with the rapid development of scientific research and the introduction of modern techniques in order to speed up early diagnosis, the Polymerase Chain Reaction (PCR) technique is used, as it depends on the amplification of the number of DNA, which was used in the detection of *M. tuberculosis* in women with pulmonary TB. However, the PCR results must be corrected for the presence of inhibitors as well as for DNA contamination. Mycobacterium is mainly diagnosed by GeneXpert, liquid media, and different types of solid media. The most commonly used media is Lowenstein-Jensen (LJ) to detect mycobacterium. However, liquid cultures are used to prepare the inoculum to perform susceptibility of antibiotics and frequent sub-culturing. WHO recommended using the next-generation Xpert® MTB/RIF assay (called Xpert® MTB/RIF Ultra) as a replacement for the current Xpert MTB/RIF® cartridge, as this technique is used to detect the presence of the microbe *M. tuberculosis* that causes TB not only this but also to detect mutations in *M. tuberculosis* DNA developing resistance against rifamycin (5). For instance, it depends on the duplication of DNA, and this process takes place within two hours. This method proved effective, especially in people likely to be infected with TB, with the limitations of the initial diagnostic methods.

Bovine tuberculosis (TB) caused by Mycobacterium Bovis is a significant health threat to cattle and a zoonotic threat for humans in many developing countries. Rapid and accurate detection of M. Bovis is fundamental for controlling the disease in animals and humans, and adequately treating patients as one of the first-line anti-TB drugs, pyrazinamide, is ineffective against M. Bovis. Currently, there are no rapid, simplified, and low-cost diagnostic methods that can be easily integrated with many developing countries. Here, we report the development of a loop-mediated isothermal amplification (LAMP) assay for specific identification of M. Bovis by targeting the region of difference 4 (RD4), a 12.7 kb genomic region that is deleted solely in M. Bovis. The assay's specificity was evaluated using 139 isolates comprising 65 M. Bovis isolates, 40 M. tuberculosis isolates, seven M. tuberculosis complex reference strains, 22 non-tuberculous mycobacteria, and five other bacteria. The established LAMP detected only M. Bovis isolates as positive, and no false positives were observed using the other mycobacteria, and non-mycobacteria tested. Our LAMP assay detected as low as 10 copies of M. Bovis genomic DNA within 40 minutes. The procedure of LAMP is simple, with incubation at a constant temperature. Results are observed with the naked eye by a color change, and there is no need for expensive equipment. The established LAMP can detect M. Bovis infections in cattle and humans in resource-limited areas. The Xpert MTB/RIF is a DNA-based, semi-quantitative, real-time PCR to detect *M. tuberculosis* and rifampicin resistance with associated mutations within the *rpo B* gene (5). The reaction uses a (03) set of primers with five DNA probes to ensure the best results with high specificity. The sequence in the region

of Xpert MTB/RIF helps to amplify a part of the *rpoB* gene. The sequences (probes) were used as a standard to detect the associated mutations with RIF resistance. Patients with suspected HIV and pediatric pulmonary cases with negative Acid-Fast Bacillus (AFB) smear are normally recommended to be diagnosed with GeneXpert (6). GeneXpert can detect at least 100 microbes/ml in vitro suspension. However, the detection of TB in non-respiratory samples using Xpert is considered an "off-mark."

Recently, nucleic acid amplification has been used following the LAMP (Loop-Mediated Isothermal Amplification) assay, which detects the presence of *M. Bovis; M. Canetti; M. africanum;* MTB, and *M. microti* (7). The prevalence of XDR-TB in patients co-infected with HIV reveals a serious threat to TB control and its management (8). The conventional techniques to purify the proteins to detect *M. tuberculosis* infection depend upon latitude response, especially after BCG (Bacillus Calmette–Guérin) treatment (9).

Culture media is a reliable, sensitive, and specific method for detecting *M. tuberculosis.* This method provides new isolates to identify and drug response for a specific strain. Therefore, these methods are considered the gold standard for detecting *M. tuberculosis* (10). The detection based on LJ bacteriological culture to diagnose negative samples typically takes 6-8 weeks which is not acceptable because fast detection and identification are mandatory for epidemiological and medical reasons (11). The sensitivity of this method in sputum samples is mentioned as 98% (ABF +) and 72% (ABF -), with an average specificity value >99% in a previous study (12). The study was designed to compare GeneXpert's sensitivity and specificity to culture media in patients with *M. tuberculosi*s

2. Materials and Methods

2.1. Sample Collection and Processing

A total of two hundred and fifty (n= 250) sputum samples were collected from patients clinically diagnosed with MTB in different hospitals in Lahore following standard ethical procedures. The samples were decontaminated and homogenized with a 2% solution of N-acetyl-L-cysteine-sodium hydroxide (NALC-NaOH). A standard sample was added with an equal amount of both buffers (decontamination buffer and homogenization buffer) in a falcon tube (15 mL followed by shaking in an automated shaker for 25 minutes at room temperature. After that, the tube was added with an equal volume of 1X phosphate buffer, followed by vortexing and centrifuge at 3000 rpm for 15 minutes at 4°C. The pellet was resuspended by discarding the supernatant in 1 mL phosphate-buffered saline (PBS). The suspension was bifurcated into two Eppendorfs for further reaction with MODS (Microscopic Observation Drug Susceptibility Assay) culture and smear.

2.2. Preparation of Culture Media

The egg-yolk-enriched LJ media at 32°C (M168 and M511) were prepared following standard protocols and manual instructions. Both these media were tested for sterility to prevent contamination by incubating for 72 hours. Finally, the media was transferred into a tightly closed container and stored below 30°C.

The sputum sample was collected by requesting the patient to cough into a sterile container, followed by transfer of samples immediately to the Laboratory for quality screening using microscopy. The samples without any evidence of active inflammation and contamination were discarded, and fresh samples were recollected to ensure active contamination and inflammation. In the safety hood, suitable samples were shifted carefully into a 50 mL falcon tube and centrifuged at 3000 g centrifugation speed for 15 minutes. Sodium hypochlorite containing 10% chlorine treatment was used to decontaminate the supernatants before discarding, and sediments were resuspended into sterilized distilled water to use in *M. tuberculosis* culture as per the Modified Petroff's method (13). In brief, the concentrate was digested and decontaminated using sodium hydroxide (modified

Petroff). The sediment (200 µl) was inoculated on two slopes of LJ medium and incubated at 37 ℃, followed by the preparation of ZN smears from the concentrated samples. After incubation, the slopes were looked for any growth daily during the first week and then twice a week for 8 weeks. The media was observed for the growth of *M. tuberculosis* with an increased number of colonies and typical buff color. The absence of the specific growth was reported as negative, while the appearance of bacterial growth was confirmed by microscope using ZN stain and reported as a positive result.

2.3. GeneXpert Assay

The sputum samples were labeled with proper lab code, and a reagent (2:1 v/v) was added, followed by vigorous shaking for 10-20 minutes by inverting. Afterward, the samples were incubated at room temperature for 15 minutes following 10-20 vigorous shaking. The samples were further ensured to be liquefied without visible sputum clumps. The liquefied samples were aspirated using a sterile pipette to a calibrated minimum mark. The cartridge lid was opened to transfer the sample into the already open port of Xpert MTB/RIF followed by slow dispensing to minimize the risk of aerosol contamination. The reaction progress was monitored and checked by using Windows R software in association with an automated computer. Finally, the module door was opened to release the cartridge. The MTB results were very low, low, medium, and high. Finally, while the results were high, the used cartridge was discarded according to standard practices.

2.4. Statistical Analysis

The results were analyzed statistically using the statistical software SPSS21 (Online, trial version), where the results were expressed as n (%). Pearson chi-square test was used to compare results for GeneXpert and culture medium. The results were of statistical significance if the value of $p < 0.05$. Sensitivity and specificity were calculated for both GeneXpert's and culture media. The culture media method followed by ZN stain was considered the reference method to interpret the results as positive or negative. Thus the result showed positive on culture media while negative using GeneXpert's; it was considered a false negative. While in the case of the negative result by culture media and a positive by GeneXpert's was considered a false positive result.

3. Results

GeneXpert and Culture media are techniques for many microbiologists to detect *M. tuberculosis*. Both methods were analyzed and compared using a different set of samples. Out of 250 samples studied, two hundred and twenty (n= 220) (88%) samples were detected as negative, while 30 (12%) samples were positive on culture media. The GeneXpert showed two hundred and twenty-three (n= 223) out of 250 (89%) samples as negative, while 17 samples (6.8%) were detected as positive via GeneXpert. However, 5 samples were detected as positive on both culture media and GeneXpert (Table 1, Figure 1). The isolates were further confirmed for *M. tuberculosis* using biochemical procedures (Figure 2). Table 2 shows the ratios of Pearson Chi-Square, Continuity Correlation, Likelihood Ratio, Fisher's Exact Test, Linear-by-Linear Association, and the Number of valid cases. Similarly, table 3 revealed odds ratios of culture media and GeneXpert at a confidence interval of 95%. The risk estimate test revealed that odd values of culture media were 3.46 times the odds of GeneXpert (Table 3).

Table 1. Cross-tabulation of culture media and GeneXpert shows that out of 250 samples 220 samples are negative and 30 samples are positive on culture media while out of 250 samples 233 samples are negative and 17(6.8%) samples are positive on GeneXpert. 5 (16.7%) samples are positive both on culture media and GeneXpert

	GeneXpert		Total
	NO	YES	
NO Count	208	12	220
% within Culture	94.5%	5.5%	100.0%
YES Count	25	5	30
% within Culture	83.3%	16.7%	100.0%
Total Count	233	17	250
% within Culture	93.2%	6.8%	100.0%

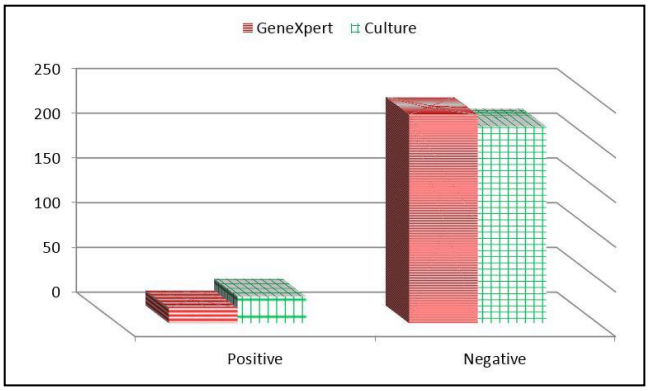

Figure 1. Comparison between cases in both culture and GeneXpert

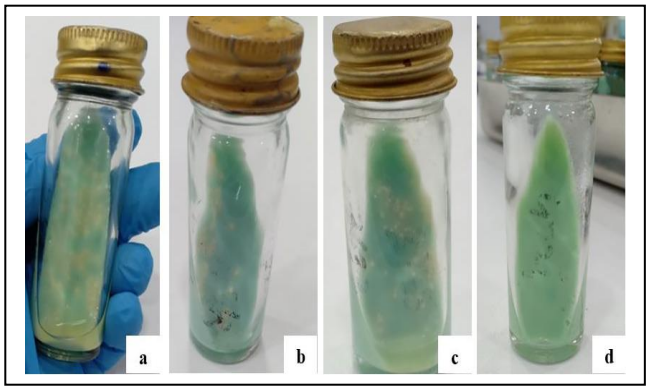

Figure 2. Growth of *Mycobacterium tuberculosis* on LJ Media after 8 weeks (Bottles a, b, and c show the growth while bottle d represents no growth (control)

Table 2. Comparison of culture media and GeneXpert assay (a. 1 cells (25.0%) have expected count less than 5. The minimum expected count is 2.04. b. Computed only for a 2x2 table. $P<0.05$ significant value)

	Value	DF	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	5.237[a]	1	0.022		
Continuity Correction	3.617	1	0.057		
Likelihood Ratio	4.041	1	0.044		
Fisher's Exact Test				.039	.039
Linear-by-Linear Association	5.216	1	0.022		
No. of Valid Cases	250				

Table 3. Comparison of OR value between culture media and GeneXpert assay. The OR of culture media was estimated to be 3.46 times the OR of GeneXpert

	Values	95% Confidence Interval	
		Lower	Upper
Odds Ratio for Culture (NO / YES)	3.467	1.128	10.654
For cohort GeneXpert = NO	1.135	0.964	1.336
For cohort GeneXpert = YES	0.327	0.124	0.864
No. of Valid Cases	250		

4. Discussion

The detection of *M. tuberculosis* using conventional methods like direct microscopy is sensitive. The culture method is the most reliable way to diagnose the bacterium despite the need for biosafety protocols with trained laboratory professionals within 8 weeks (14). However, rapid results for the detection of *M. tuberculosis* are possible with molecular techniques using GeneXpert that also targets resistance conferred by RIF. In a short time, a single system uses cell lysis, DNA extraction, amplification, and amplicon detection with fewer chances to detect the secondary microbes due to sample contamination. A MODS was an optimal alternative rapid diagnostic technique for TB and detection of MTB and multidrug-resistant (MDR)-TB in resource-limited settings; results were obtained in a median of 8 days. On the other hand, Gene Xpert was a novel integrated diagnostic PCR analysis in a single hand-free step for rapid diagnosis of TB and detection of RMP resistance in clinical specimens in 2 hours, but it was expensive.

The detection sensitivity of *M. tuberculosis* has improved with the development of different cultural media. The present study analyzed and compared two techniques, GeneXpert and culture media, for detecting

M. tuberculosis to find the most suitable technique for detecting the disease (15).

The sensitivity and specificity of GeneXpert and culture media were 6.8% and 83.3%, respectively. According to findings by Aricha, Kingwara (16), it was shown that by comparing culture medium with GeneXpert, GeneXpert 's scores for sensitivity, specificity, positive predictive value, and negative predictive value were 8.5, 64.9, 59.4, and 82.2%, respectively in diagnosing patients with TB. Mechal, Benaissa (17) reported values for sensitivity and specificity of GeneXpert as 78.2 and 90.4%, and 79. 3 and 90.3%, respectively.

Cross-tabulation of culture and GeneXpert showed that out of 250 samples, 220 samples were negative, and 30 samples were positive on culture, while out of 250 samples, 233 samples were negative, and 17 (6.8%) samples were positive on GeneXpert. 5 (16.7%) samples were observed positive both on culture and GeneXpert. This shows that culture media is more sensitive than GeneXpert and is recommended as the Gold standard for authentic diagnosis of *M. tuberculosis.* The statistical analysis using Chi-square and OR test explored significant (P<0.05) variation in the studied variables represented in tables 1 and 3. Out of all the studied samples, only 5 samples (16.7%) were observed positive on both GeneXpert and culture media (Table 1).

From the finding of molecular technique as GeneXpert, it is suggested that positive cases with GeneXpert but negative with culture should be investigated and correlated with patient history. The Chi-square values (5.237 with a P-value of 0.022 (<0.05) (Table 2) showed a significant association between solid culture media and GeneXpert for the diagnosis of *M. tuberculosis.* The statistical results showed that both techniques could not be equally accurate for diagnosing *M. tuberculosis*. The chi-square test revealed that culture media (independent variable) has the highest diagnostic rate than GeneXpert (dependent variable).

The risk estimate analysis revealed culture media to be 3.46 times the OR value of GeneXpert. Alternatively, the OR value of *M. tuberculosis* detection with the culture media was 246% higher than that of GeneXpert. The retrospective nature of the current study and its results could not be correlated with histopathological and radiological findings. Detecting the presence of RIF resistance is an important attribute of GeneXpert and culture media. This feature inhibits the irrelevant bacterial growth for significant detection of *M. tuberculosis*.

A remarkable conclusion is that GeneXpert MTB/RIF concerts, based on the total (pulmonary and extra-pulmonary) results of the microscopic examination, have 20.6% more sensitivity than the microscopic examination. This increase was highlighted for extra-pulmonary samples (30%) (18).

All the current study results are in line with the WHO recommendations on TB diagnosis, which highlights the importance of molecular research for the entire population of TB suspects, especially for high-risk groups such as suspected of MDR-TB and suspects of HIV-related TB. The current findings confirm the relevance of WHO is recommendations to make the molecular diagnosis by GeneXpert MTB/RIF as a main diagnostic approach (19).

GeneXpert MTB/RIF is a practical qualitative and quantitative test for early recognition of PTB and RIF resistance; moreover, watery sputum and AFB smear-positive cases are potent predictors of Xpert MTB/RIF detection (20).

The studies accompanied by Zetola, Shin (21) supported the false-negative results of GeneXpert MTB/RIF for RIF resistance detection with a mix of sensitive and resistant bacilli in the same sample. This state is recurrently encountered in highly endemic countries. The poor detection performance of GeneXpert MTB/RIF is also described in a study by Rufai, Kumar (22) Notably, 5% of RIF resistance is due to mutations outside the *rpoB* gene and is subsequently not distinguished by the GeneXpert MTB/RIF.

Despite the preference of GeneXpert in terms of ease, speed, availability, and inexpensiveness, as it saves time and effort and helps speed diagnosis compared to Culture Media, the results were in favor of the Culture Media technique in terms of sensitivity and specialization. This might be due to the dependence on GeneXpert performance or sample preparation; proper technical training might be required for the Laboratory scientist/ technologist who performs the analysis, the device used, and the working environment in terms of pressure, temperature and humidity, the and demographic data by gender and age. All this may affect the results.

WHO commendations for the amalgamation of GeneXpert MTB/RIF in the TB diagnosis methods are interconnected to its short time to results and established performance (sensitivity and specificity) for both pulmonary and extra-pulmonary TB diagnosis (23). The WHO has instigated a TB control approach to end the TB epidemics by 2035.

5. Conclusion

Although, molecular-based techniques also ways preferred over conventional diagnostic techniques due to their high sensitivity, specificity, rapidness, and easy performance. However, the current investigation found that the old culture media (Gold standard) technique still has more specificity and sensitivity to diagnose the *M. tuberculosis* bacteria in the sputum samples of MTB patients compared to the GeneXpert technique. However, GeneXpert may be useful to diagnose cases of tuberculosis and those in which RIF resistance occurs. It would be recommended that this method be further studied to avoid or know factors that affect it, such as age, gender, the device used, lack of experience of workers, sputum viscosity, and cartridge damage.

Authors' Contribution

J. I. developed the original idea and the protocol.
Z. N. abstracted and analyzed data.
S. K. and F. S. wrote the manuscript, and is guarantor.
M. A. analysed and interpreted the data.
A. G. B., S. N., and I. S. drafted the manuscript.
Critical revision of the manuscript for important intellectual content: P. O. O., S. A. S., A. A. A., W. F. E. and A. G.
Statistical analysis: N. M.
Administrative, technical, and material support: M. R.

Ethics

This study was approved by the Ethics Committee of the Société Francophone de Nutrithérapie et de Nutrigénétique Appliquée, Villeurbanne, France.

Conflict of Interest

The authors declare that they have no conflict of interest.

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References

1. WHO. Global tuberculosis report 2013: World Health Organization; 2013 [Available from: https://apps.who.int/iris/handle/10665/91355.
2. Leung NHL. Transmissibility and transmission of respiratory viruses. Nat Rev Microbiol. 2021;19(8):528-45.
3. Mollel E, Lekule I, Lynen L, Decroo T. Effect of reliance on Xpert MTB/RIF on time to treatment and multidrug-resistant tuberculosis treatment outcomes in Tanzania: a retrospective cohort study. Int Health. 2019;11(6):520-7.
4. Garg SK, Tiwari RP, Tiwari D, Singh R, Malhotra D, Ramnani VK, et al. Diagnosis of tuberculosis: available technologies, limitations, and possibilities. J Clin Lab Anal. 2003;17(5):155-63.
5. WHO. WHO meeting report of a technical expert consultation: non-inferiority analysis of Xpert MTB/RIF Ultra compared to Xpert MTB/RIF: World Health Organization; 2017 [Available from: https://www.who.int/publications-detail-redirect/WHO-HTM-TB-2017.04.

6. Shah D, Gupta Y. Role of Molecular Tests for Diagnosis of Tuberculosis in Children. Pediatric Oncall Journal. 2015;12(1):1-3.
7. Kapalamula TF, Thapa J, Akapelwa ML, Hayashida K, Gordon SV, Hang' Ombe BM, et al. Development of a loop-mediated isothermal amplification (LAMP) method for specific detection of Mycobacterium bovis. PLoS Negl Trop Dis. 2021;15(1):e0008996.
8. Singh A, Prasad R, Balasubramanian V, Gupta N. Drug-Resistant Tuberculosis and HIV Infection: Current Perspectives. HIV AIDS (Auckl). 2020;12:9-31.
9. Chen J, Wang S, Zhang Y, Su X, Wu J, Shao L, et al. Rv1985c, a promising novel antigen for diagnosis of tuberculosis infection from BCG-vaccinated controls. BMC Infect Dis. 2010;10:273.
10. Comas I, Coscolla M, Luo T, Borrell S, Holt KE, Kato-Maeda M, et al. Out-of-Africa migration and Neolithic coexpansion of Mycobacterium tuberculosis with modern humans. Nat Genet. 2013;45(10):1176-82.
11. Negi SS, Khan SFB, Gupta S, Pasha ST, Khare S, Lal S. Comparison of the conventional diagnostic modalities, bactec culture and polymerase chain reaction test for diagnosis of tuberculosis. Indian J Med Microbiol. 2005;23(1):29-33.
12. Held M, Laubscher M, Zar HJ, Dunn RN. GeneXpert polymerase chain reaction for spinal tuberculosis: an accurate and rapid diagnostic test. Bone Joint J. 2014;96-B(10):1366-9.
13. Tripathi K, Tripathi PC, Nema S, Shrivastava AK, Dwiwedi K, Dhanvijay AK. Modified Petroff's Method: an excellent simplified decontamination technique in comparison with Petroff's Method. Int J Recent Trends Sci Technol. 2014;10:461-4.
14. ECDC. Handbook on tuberculosis laboratory diagnostic methods in the European Union: European Centre for Disease Prevention and Control; 2018. Available from: https://www.ecdc.europa.eu/en/publications-data/handbook-tuberculosis-laboratory-diagnostic-methods-european-union-updated-2018.
15. Takarada Y, Kodera T, Kobayashi K, Nakajima C, Kawase M, Suzuki Y. Rapid detection of rifampicin-resistant Mycobacterium tuberculosis, based on isothermal DNA amplification and DNA chromatography. J Microbiol Methods. 2020;177:106062.
16. Aricha SA, Kingwara L, Mwirigi NW, Chaba L, Kiptai T, Wahogo J, et al. Comparison of GeneXpert and line probe assay for detection of Mycobacterium tuberculosis and rifampicin-mono resistance at the National Tuberculosis Reference Laboratory, Kenya. BMC Infect Dis. 2019;19(1):852.
17. Mechal Y, Benaissa E, El Mrimar N, Benlahlou Y, Bssaibis F, Zegmout A, et al. Evaluation of GeneXpert MTB/RIF system performances in the diagnosis of extrapulmonary tuberculosis. BMC Infect Dis. 2019;19(1):1069.
18. Li S, Liu B, Peng M, Chen M, Yin W, Tang H, et al. Diagnostic accuracy of Xpert MTB/RIF for tuberculosis detection in different regions with different endemic burden: A systematic review and meta-analysis. PLoS One. 2017;12(7):e0180725.
19. Sah SK, Bhattarai PR, Shrestha A, Dhami D, Guruwacharya D, Shrestha R. Rifampicin-resistant Mycobacterium tuberculosis by GeneXpert MTB/RIF and Associated Factors Among Presumptive Pulmonary Tuberculosis Patients in Nepal. Infect Drug Resist. 2020;13:2911-9.
20. El-Helbawy R, AMA T. eneXpert Mycobacterial tuberculosis/rifampicin: predictors of successful performance. Egypt J Chest Dis Tuberc. 2020;69(1):33.
21. Zetola NM, Shin SS, Tumedi KA, Moeti K, Ncube R, Nicol M, et al. Mixed Mycobacterium tuberculosis complex infections and false-negative results for rifampin resistance by GeneXpert MTB/RIF are associated with poor clinical outcomes. J Clin Microbiol. 2014;52(7):2422-9.
22. Rufai SB, Kumar P, Singh A, Prajapati S, Balooni V, Singh S. Comparison of Xpert MTB/RIF with line probe assay for detection of rifampin-monoresistant Mycobacterium tuberculosis. J Clin Microbiol. 2014;52(6):1846-52.
23. 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 extra-pulmonary TB in adults and children: policy update: World Health Organization; 2022 [Available from: https://apps.who.int/iris/handle/10665/112472.