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Original article

Role of Gene-Xpert MTB/RIF assay in detection of *Mycobacterium tuberculosis* in smear-negative sputum samples

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ABSTRACT

Background: Smear negative tuberculosis patients are usually associated with delayed or incorrect diagnosis, as well as poor treatment efficacy. This study aimed to assess the Gene-Xpert MTB/RIF assay performance in detection of *Mycobacterium tuberculosis* in smear-negative sputum samples**Methods:** The study was conducted in Al Quwayiyah General Hospital, Riyadh, Saudi Arabia from January to December 2021 involving 131 patients. Each sputum sample was examined directly by Ziehl-Neelsen stain. All sputum samples were cultured on MGIT tubes using BACTEC MGIT 960 System. GENEXPERT MTB/RIF assay was carried out for collected sputum samples after digestion-decontamination of sputum using MycoPrep. reagent.**Results**: Out of the 131 studied patients, 24 (18.32%) had positive mycobacterial cultures, of these 24 positive patients, 14 (10.68%) were culture positive smear positive tuberculosis patients, 10 (7.63%) were culture positive smear negative tuberculosis patients and 107 were culture negative patients. Twenty three (17.6%) sputa were positive by both tuberculosis culture and GeneXpert MTB/RIF assay, one sputum sample was culture positive but GeneXpert MTB/RIF assay negative. In comparison to mycobacterial culture as the gold standard, the sensitivity of the GeneXpert MTB/RIF assay in culture positive/smear positive was 100% (76.84-100%) while the sensitivity of the GeneXpert MTB/RIF technique among culture positive smear negative tuberculosis patients was 90.91% (58.72%-99.77%). The specificity of the GeneXpert MTB/RIF assay was 99.07% (94.90%-99.98%) in culture positive/smear positive patients and it was 98.15% (93.47%-99.77%) among smear culture positive smear negative tuberculosis patients. **Conclusion**: The Xpert MTB/RIF assay has better sensitivity and specificity which is nearly as same as culture while it is much faster in detection of Mycobacteria.

Introduction

Tuberculosis (TB) is a frequent infection that requires extensive treatment. Active pulmonary TB patients can transmit the infection through the air then the droplet nuclei move through upper respiratory tract and bronchi to reach the lungs alveoli. The World Health Organization (WHO) reported that 9.0 million individuals contracted tuberculosis in 2014, with 1.5 million deaths The worldwide proportion of multidrug-resistant tuberculosis (MDR-TB) new cases is believed to be

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3.5 %; however, some areas have significantly greater levels of resistance and poorer consequences [1].

It is critical to treat active pulmonary TB patients as soon as possible in order to decrease the danger of infection spreading to others. The initial stage in TB diagnosis is sputum acid fast bacillus (AFB) staining, which has an advantage of having an average turnaround time (TAT) about 24 hours. [2].

Acid fast bacillus staining requires minimally 5,000 to 10,000 bacilli per milliliter of sputum sample to detect mycobacterial tuberculosis (MTB), while a positive culture requires only 10-100 bacilli per milliliter [3]. If the AFB staining of two sputum samples is negative, the next step is mycobacterial culture and drug sensitivity testing that takes 3 to 4 weeks using liquid culture and 6 to 8 weeks by solid media [2].

Mycobacterium tuberculosis culture has advantage that it can distinguish between MTB and non-tuberculous mycobacterium (NTM) and can detect MDR-TB strains, although the time it takes to submit the report is the major restriction of using culture [2].

The GeneXpert MTB/RIF assay is a real-time polymerase chain reaction (PCR) nucleic acid amplification technique, which can simultaneously detect MTB and rifampicin (RIF) resistance in less than 2 hours. Molecular beacon technology and ultrasensitive nested PCR are the basis of GeneXpert test [4].

The GeneXpert MTB/RIF assay detectsRIF resistance which is associated to mutations in the rpoB gene the TB-specific rpoB gene using a reverse transcriptase polymerase chain reaction (RT-PCR) [5].

The Gene-Xpert MTB/RIF assay was approved by the WHO in 2010 for use in TB-endemic countries as a diagnostic test for fast detection of TB and in diagnosis of MDR-TB patients [6].

The aim of this study was to assess the Gene-Xpert MTB/RIF assay performance in detection of *Mycobacterium tuberculosis* in smear-negative sputum samples.

Materials and Methods

Study design

A cross-sectional study was performed on cases with suspected pulmonary TB. The study was conducted at chest and microbiology departments in Al Quwayiyah General Hospital, Riyadh, Saudi Arabia. The cases included in this study aged more than 18 years who had signs and symptoms indicative of pulmonary TB and/or had chest x-ray suggestive of TB and had a prior history of TB. Patients who were receiving treatment for TB for more than 2 weeks before start of the study were excluded. The study was performed from January to December 2021 involving 131 patients. Data collection was done after informing each participant the aim of the study and after giving consent.

Samples collection

Three consecutive morning sputa were obtained on 3 consecutive days from each patient. Five to 10 ml of sputum were obtained in each container. If the patient cannot expectorate a sputum, then induction could be done by the inhalation of sterile warm aerosol of 5-10% sodium chloride in water through a nebulizer. After collection, specimens for TB culture were kept refrigerated at 2-8^{o}C.

Laboratory methods

Stain

Each sputum specimen was directly examined microscopically after Ziehl-Neelsen staining. Clinical and Laboratory Standards Institute (CLSI) guidelines was used for AFB grading into four categories (1+, 2+, 3+, 4+).Smear-negative pulmonary TB was considered when patients had symptoms and signs consistent with TB [7].

GeneXpert MTB/RIF assay

Sputum specimens were washed with sample reagent which contain isopropanol and NaOH using MycoPrep. reagent,(Becton Dickinson, USA) which mixed with the sputum sample in a 2 to 1 ratio and then incubation was done at room temperature for 15 min. The washed sputum specimen was transported to the cartridge, which was then inserted into the GeneXpert machine for automatic conduction of all test processes. The results were classified as invalid, negative, or positi ve. The positive results were categorized into 4 groups (high, medium, low, very low) then were classified into susceptible or resistant to rifampicin based on the presence of rpoB gene mutations [8].

Mycobacterial culture

All collected sputum samples were placed in a cold box with an ice pack, sent to regional laboratory, Riyadh.The cultures were done after doing digestion-decontamination of sputum by MycoPrep. reagent, then inoculation in MGIT tubes and

incubation in BACTEC MGIT 960 System until the machine flagged them positive and a maximum of 6 weeks to be flagged as negative cultures [9].

Cases definition

Positive TB patients were considered if MTB culture were positive. Smear negative pulmonary TB case was described as a case has minimally 2 negative AFB smears and a positive TB culture. Non TB patients were considered when MTB cultures were negative [10].

Statistical analysis

The Gene-Xpert assay results were compared to smear and culture. The data were analyzed by SPSS version 20. Using TB culture as the gold standard. The sensitivity, specificity, positive and negative predictive values were calculated for Gene-Xpert MTB/RIF assay results. Wilcoxons's rank sum test and Fisher's exact test were used to detect the relation between continuous and categorical variables respectively. Significant*p*-values was considered if it was less than 0.05.

Results

Table 1 shows that in the current study, 131 studied patients for TB detection, 24 (18.32%) were positive by mycobacterial culture, of these 24 positive samples, 14 (10.68%) were culture positive smear positive TB patients, 10 (7.63%) were culture positive smear negative TB patients and 107 were culture negative patients.The study included 85 (64.8 %) males and 46 (35.2%) females. Among studied patients groups it was observed that males were more than females, they were 9 (64.28%),7 (70%) and 69 (64.48%) amongculture positive smear positive TB patients, culture positive smear negative TB patients and culture negative patients respectively. The participants ages were from 19 to 87 years, 21 to 85 years and 24 to 89 years amongculture positive smear positive TB patients, culture positive smear negative TB patients and culture negative patients respectively.Eighty eight (76.18%) were Saudi while 43(32.82%) were non Saudi. In regards to sex, age, and nationality, there was no statistically significant difference between the three groups.

Table 2shows the final clinical assessment of patients with smear negative sputum, out of 116 smear negative patients (you told me 107(explanation 107 include the negative AFB plus the one which is AFB false positive which is included in the non TB patients in the above result page 5), 10 (8.62%) were smear negative while MTBculture positive and 106 were smear negative with MTBculture negative which were distributed as 12 (11.21%) old pulmonary TB, 18 (16.82%) bacterial pneumonia, 9 (8.41%) bronchogenic carcinoma, 12 (11.21%) bronchiectasis, 14 (13.08%) lung abscess, 12 (11.21%) volume overload, 15 (14.01%) pulmonary hypertension, 3 (2.80%) empyema thoracis and 12 (11.21%) no definite pulmonary disease.

Table 3 shows that out of the 131 studied patients, 23/131 (17.6%) were positive by both MTB culture and GeneXpert assay, one had a positive MTB culture but negative GeneXpert assay. Two cases were negative by MTB culture while they were GeneXpert assay positive. Fifteen patients were ZN smear positive, while 116 smear negative. Out of 116 smear negative, 10 sputum samples were both culture positive and smear negative. One patient had a sputum sample which was smear positive but culture negative. Out of the 116 smear negative cases, 9 were positive for MTB by both culture and GeneXpert assay. One patient had a sputum sample which was positive by culture but negative on GeneXpert analysis. One patient had a sample that was culture negative while it was positive on Xpert testing.

GeneXpert MTB/RIF detected rifampicin resistance in 4/26 (15.38%) of TB culture positive, the 4 isolates were treated previously as pulmonary TB cases and all isolates were smear positive.

Table 4. In the current study, in comparison to mycobacterial culture as the a gold standard, the sensitivity of the GeneXpert MTB/RIF assay in culture positive smear positive tuberculosis patients was 100% (76.84-100%) while the GeneXpert MTB/RIF assay sensitivity between culture positive smear negative tuberculosis patients was 90.91% (58.72%-99.77%). The specificity of the GeneXpert MTB/RIF assay was 99.07% (94.90%-99.98%) in culture positive smear positive tuberculosis patients and it was 98.15% (93.47%-99.77%) among culture positive smear negative TB patients. The positive predictive value (PPV) was 93.33% (66.56%-98.99%) in culture positive smear positive TB patients while among culture positive smear negative TB patients the positive predictive value (PPV) was 83.33% (55.57%-95.24%).The negative predictive value (NPV) was 100% and 99.07% (94.24%-99.85%) in culture positive smear positive TB patients and culture positive smear negative TB patients, respectively. The positive likelihood ratio (LR+) was 10.7% (15.21%-

752.69%) in culture positive smear positive TB patients and 49.09% (12.28%-196.25%) in culture positive smear negative TB patients. The negative likelihood ratio (LR-) was 0.17 (95% CI: 0.08-0.40) and 0.31 (95% CI: 0.30-0.49) in culture positive smear positive TB patients and culture positive smear negative TB patients, respectively. Accuracy was 99.17% (95.48%-99.98%) in culture positive smear positive TB patients and accuracy was 97.48(92. 81%-99.48%) in culture positive smear negative TB patients

Table 1. Demographic characteristics among studied patients.

	Culture positive smear positive tuberculosis patients N =14	**Culture positive smear negative tuberculosis patients N =10**	**Culture negative patients N=107**	**Total** N=131
Sex				
Male	9 (64.28%)	7 (70%)	69 (64.48%)	85 (64.8%)
Female	5 (35.72%)	3 (30%)	38 (35.52%)	46 (35.2%)
Age	19 - 87 years	21-85 years	24-89 years	
Median age	43years	38 years	45 years	
Nationality				
Saudi	6 (24.85%)	4 (40%)	78 (72.89%)	88(76.18%)
Non Saudi	8 (57.14%)	6 (60%)	29 (27.10%)	43(32.82%)

Table 2. Final clinical assessment of patients with smear negative sputum.

Clinical diagnosis	**No. = 126**	**(%)**
culture positive smear negative tuberculosis patients	10	8.62%
Smear negative, culture negative patients	106	91.38%
Bacterial pneumonia	18	16.98%
Old pulmonary tuberculosis	12	11.32%
Bronchogenic carcinoma	9	8.49%
Bronchiectasis	12	11.32%
Lung abscess	14	13.20%
Pulmonary hypertension	15	14.15%
Volume overload	12	11.32%
Empyema thoracis	3	2.83%
No definite pulmonary disease	11	10.38%
Total smear negative patients	116	100%

Table 3. Comparison between AFB smear microscopy/culture and GeneXpert among studied patients.

TEST	GeneXpert MTB/RIF positive	GeneXpert MTB/RIF negative	Total
Smear positive/culture positive	14	0	14
Smear positive/culture negative	1	0	1
Smear negative/culture positive	9	1	10
Smear negative/culture negative	2	104	106
Total	26	105	131

Table 4. Gene Xpert MTB/RIF assay performance in detection of MTB

Aspects	Gene Xpert assay(95%CL)
Sensitivity	
Culture positive smear positive tuberculosis patients	100%(76.84-100%)
Culture positive smear negative tuberculosis patients	90% (58.72%-99.77%)
Specificity	
Culture positive smear positive tuberculosis patients	99.07% (94.90%-99.98%)
Culture positive smear negative tuberculosis patients	98.15% (93.47%-99.77%)
Positive likelihood ratio	
Culture positive smear positive tuberculosis patients	107% (15.21%-752.69%)
Culture positive smear negative tuberculosis patients	49.09% (12.28%-196.25%)
Negative likelihood ratio	
Culture positive smear positive tuberculosis patients	0.17 (95% CI: 0.08-0.40)
Culture positive smear negative tuberculosis patients	0.31 (95% CI: 0.30-0.49)
PPV	
Culture positive smear positive tuberculosis patients	93.33% (66.56%-98.99%)
Culture positive smear negative tuberculosis patients	83.33% (55.57%-95.24%)
NPV	
Culture positive smear positive tuberculosis patients	100%
Culture positive smear negative tuberculosis patients	99.07% (94.24%-99.85%)
Accuracy	
Culture positive smear positive tuberculosis patients	99.17% (95.48%-99.98%)
Culture positive smear negative tuberculosis patients	97.48(92. 81%-99.48%)

Discussion

Early diagnosis and therapy are the most effective approaches to eradicate TB.These facilitate the rapid application of infection control approaches and the beginning of appropriate treatment. [11].

In this study out of 131 studied patients for TB detection, 24 (18.32%) were positive by MTB culture, of these 24 positive samples, 14 (10.68%) were culture positive smear positive TB patients, 10 (7.63%) were culture positive smear negative TB patients and 107 were culture negative patients. **Tostmann et al.** 2008 [12] also reported that patients with smear-negative, culture-positive pulmonary TB are accountable for 10% of identified TB patients. **Also Mulualem et al.**2016 [13] found that among 185 smear-negative

pulmonary TB patient, 19 (10.3%) had culture positive TB.

The study included 85 (64.8 %) males and 46 (35.2%) females. Among studied patients groups it was observed that males were more than females, they were 9 (64.28%),7 (70%) and 69 (64.48%) amongculture positive smear positive TB patients, culture positive smear negative TB patients and culture negative patients respectively.Another study performed at the King Abdul Aziz University Hospital in Jeddah on a broad collected data to assess the link between demographical and clinical variables of EPTB which found that the male gender was shown to have a 57.5 % dominance [14]. In 2014, the global male-to-female (M:F) ratio in smear-positive pulmonary TB case reporting was 1.7 [15].

Statistically there were no significant difference among the three groups regarding sex, age and nationality. Similar findings were found in previous studies [16,17], neither nationality, gender, or age were found to be linked with TB in 622 primary TB patients investigated in Madinah Al-Munawara, Saudi Arabia. [16]. Although the autochthonous population had a comparable dominance in terms of incidence rate in another study[12], the frequency of non-Saudi was higher than that of Saudi [18], according to a previous report. The incoherence in TB incidence in different KSA regions may have been attributable to differences between studies.

Smear-negative pulmonary TB is a primary cause of undetected TB in resource poor countries. It is linked to poor therapeutic efficacy, including mortality as a result of delayed or non-diagnosis. [19]

In this study the final clinical assessment of patients with smear negative sputum showed that, out of 116 smear negative patients, 8.62% were smear negative while culture positive and 106 were smear negative withMTB culture negative which were distributed as 11.21% old pulmonary TB, 16.82% bacterial pneumonia, 8.41% bronchogenic carcinoma, 11.21% bronchiectasis, 13.08% lung abscess, 11.21%volume overload, 14.01%pulmonary hypertension, 2.80% empyema thoracis and 11.21% no definite pulmonary disease. **Colebunders and Bastian.** 2000 [20] also highlighted that infectious and noninfectious disorders are included in the differential diagnosis of smear negative pulmonary TB. In our study, bacterial pneumonia was the most common infection among non tuberculous patients, 16.82%, same as other reports by **Colebunders and Bastian.** 2000 [20] and **Nyamande et al.** 2007 [21] reported that bacterial pneumonia was the intial diagnosis in the diseases that matched with TB. **Colebunders and Bastian.** 2000[20]; **Bhatt et al.** 2012[22] also reported that bronchogenic carcinoma can be difficult to distinguish from pulmonary TB in patients with noninfectious diseases. Old pulmonary TB was also a prominent diagnosis in the current work, as the clinician suspected TB re-infection; however, there was negative sputum smear and negative MTB culture, same results were also revealed by **Colebunders and Bastian.** 2000 [20] and **Nyamande et al.** 2007 [21].

Smear negative TB cases are associated with a greater risk of transmission, delayed TB detection and mortality. Using the GeneXpert MTB/RIF assay early in the diagnostic process has the ability to increase detection and reduce laboratory turnaround time[23].

In the current study, the GeneXpert MTB/RIF assay significantly increased MTB diagnosis with greater sensitivity in smear positive patients compared to smear negative cases in comparison to MTB culture as the gold standard, the GeneXpert MTB/RIF assay sensitivity in culture positive smear positive TB patients was 100% (76.84-100%) while the GeneXpert MTB/RIF assay sensitivity among culture positive smear negative tuberculosis patients was 90.91% (58.72%-99.77%). These results were in agreement with reports from previous studies by **Ryan et al.** 2014 and **Bunsow et al.** 2014 [24,25] who assessed the GeneXpert MTB/RIF assay sensitivity in the diagnosis of smear positive pulmonary MTB revealing that it was 100%. Previous studies **Bunsow et al.** 2014 [25] reported that the sensitivity of Xpert MTB/RIF assay was 71.4% for smear negative sputum specimens, which is markedly less than the sensitivity noted in this study. This could be due to patient demographics differences or the quality of the specimen. Nonetheless, the capacity of this assay to detect smear negative AFB is of major clinical importance, as a great number of smear negative cases are being seen in TB patients, particularly in laboratories with limited resources[26]. **Maynard-Smith et al.** 2014 reported similar results as seen in this study, he found sensitivity of 98% in smear positive/culture positive TB patients and 88% in smear negative and MTB

culture positive cases. [27]. **Amany et al.** 2019 also revealed that GeneXpert showed sensitivity was of 94.1% in smear negative whereas it was 96.9%. in smear-positive, culture-positive TB patients in a study conducted in Egypt [28].

The specificity of the GeneXpert MTB/RIF assay was 99.07% (94.90%-99.98%) in culture positive smear positive TB patients and it was 98.15% (93.47%-99.77%) among culture positive smear negative TB patients. Overall 94.4% specificity in diagnosing pulmonary TB was reported by **Amany et al.** 2019 [28] while **Moussa et al.** [29] in Saudi Arabia reported specificity of 98.3% in agreement to this study. Specificity was 98.2% for AFB smear-negative specimens and 98.7% for smear-positive specimens, respectively as reported by **Chang et al.** 2012 [30].

The global prevention of TB is still a challenging problem in terms of diagnosis, detection of drug resistance and treatment opportunities. The use of fast molecular assays to determine tuberculosis bacilli and antituberculosis drug resistance is critical for a timely and effective treatment regimen [31].

GeneXpert MTB/RIF detected rifampicin resistance in 15.38% of TB culture positive, the 4 isolates were treated previously as pulmonary TB cases and all isolates were smear positive. According to previous studies, the GeneXpert MTB/RIF assay enhances the rate of RIF resistance identification and reduces unnecessary empiric treatment among pulmonary MTB patients [32,33].

Conclusion

The GeneXpert MTB/RIF approach yielded a higher percentage of positive results for tuberculosis in comparison to ZN stain method. It has better sensitivity and specificity which is nearly as same as culture while it is much faster in detection of Mycobacteria. In culture positive smear negative tuberculosis patients, early detection using the GeneXpert MTB/RIF assay and early treatment can reduce the spread of infection and the severity of the disease.

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Competing interests

The authors report no conflicts of interest.

Authorship

Both authors have worked together to complete this research. Author ESK planned and designed the study, prepared the protocol, gathered the samples, contributed in the interpretation and analysis of the findings, drafted and critically revised the paper. Author KHMA was involved in the study's planning and design, sample collection, clinical evaluation of cases and interpretation of the data. The final manuscript was reviewed and approved by both authors.

Ethical approval

Ethical approval was obtained from institutional ethics committee of Al-Quwayiyh General Hospital.

References

1-**World Health Organization (WHO).** Global tuberculosis report. Geneva: WHO, 2014

2-**American Thoracic Society (ATS).** Diagnostic standards and classification of tuberculosis in adults and children. Am J Respir Crit Care Med 2000; 161 (4): 1376-95.

3-**Hobby GL, Holman AP, Iseman MD, Jones JM.** Enumeration of tubercle bacilli in sputum of patients with pulmonary tuberculosis. Antimicrob Agents Chemother 1973; 4: 94-104

4-**Hanif SN, Eldeen HS, Ahmad S, Mokaddas E.** GeneXpert®MTB/RIF for rapid detection of Mycobacterium tuberculosis in pulmonary and extra-pulmonary samples Int J Tuberc Lung Dis 2011;15:1274–1275.

5-**Boehme CC, Nabeta P, Hillemann D,Nicol MP, Shenai S, Krapp F, et al.** Rapid molecular detection of tuberculosis and rifampin resistance. N Engl J Med 2010; 363: 1005-15.

6-**World Health Organization.** Policy Statement: Automated real-time Nucleic Acid Amplification Technology for Rapid and Simultaneous Detection of Tuberculosis and Rifampicin Resistance: GeneXpert MTB/RIF

System. Geneva, Switzerland: World Health Organization; 2011.

7-**Van Rie A, Fitzgerald D, Kabuya G, Van Deun A, Tabala M, Jarret N, et al.** Sputum smear microscopy: evaluation of impact of training, microscope distribution, and use of external quality assessment guidelines for resource-poor settings. J Clin Microbiol. 2008;46:897–901. doi: 10.1128/JCM.01553-07.

8-**Cepheid.**GeneXpert Dx System Xpert MTB/RIF assay manual: Operator Manual Software Version-4. Cepheid: 2011. Available at: http://www.cepheid.com/us/.

9-**Kontos F, Petinaki E, Gitti Z, Costopoulos C, Anagnostou S, Tselentis I, et al.** Combined use of the fully automated BACTEC MGIT 960 System and a PCR-restriction fragment length polymorphism analysis for routine detection and identification of mycobacteria from clinical samples. J Microbiol Methods 2003; 52:137-140.

10-**World Health Organization (WHO).** Treat¬ment of tuberculosis: guidelines. 4th ed. Geneva: WHO, 2010. [Cited 2015 Oct 1]. Available from: http://www.who.int/ tb/publications/2010/9789241547833/en/ World Health Organization (WHO). Global.

11-**Memish ZA, Bamgboye EA, Abuljadayel N, Smadi H, Abouzeid MS, Hakeem RF, et al**. Incidence of and risk factors associated with pulmonary and extra-pulmonary tuberculosis in Saudi Arabia (2010-2011) PLoS One. 2014;9:e95654.

12-**Tostmann A, Kik SV, Kalisvaart NA,Sebek MM, Verver S, Boeree MJ, et al.** Tuberculosis transmission by patients with smear-negative pulmonary tuberculosis in a large cohort in the Netherlands. Clin Infect Dis 2008; 47: 1135-42.

13-**Mulualem T, Dossegnaw A, Leen R, Gemeda A.**Increased detection of smear-negative pulmonary tuberculosis by GeneXpert MTB/RIF® assay after bleach concentration International Journal of Mycobacteriology; 2016; 5(2):211-218

14-**Qari FA.** The spectrum of tuberculosis among patients of the King Abdul Aziz Unveristy Hospital, Jeddah, Saudi Arabia. Southeast Asian J Trop Med Public Health 2002; 33: 331-337.

15-**World Health Organization**. Global tuberculosis report 2015. WHO/HTM/TB/2015.22. 2015 [cited 2 Aug 2016]

16-**Elhassan M, Hemeg H, Elmekki M, Turkistani K, Abdul-Aziz A.** Burden of Multidrug Resistant Mycobacterium Tuberculosis Among New Cases in Al-Madinah AlMonawarah, Saudi Arabia. Infectious DisordersDrug Targets (Formerly Current Drug TargetsInfectious Disorders) 2017; 17(1):14-23.

17-**Somily AM, Naeem T, Habib HA, Sarwar MS, Kunimoto DY, Kambal AM.** Changing Epidemiology of Tuberculosis Detected by an 8- year Retrospective Laboratory Study in a Tertiary Teaching Hospital in Central Saudi Arabia. Saudi Medical Journal 2014;35(7):691-8. 15-

18-**Abouzeid MS, Zumla AI, Felemban S, Alotaibi B, O'Grady J, Memish ZA**. Tuberculosis Trends in Saudis and Non-Saudis in the Kingdom of Saudi Arabia–a 10 year Retrospective study (2000–2009). PloS one 2012;7(6):e39478

19-**Getahun M.** Harrington, R. O'Brien, et al.Diagnosis of smear-negative pulmonary tuberculosis in people with HIV infection or AIDS in resource-constrained settings:

informing urgent policy changes Lancet, 2007; 369: 2042-2049

20-**Colebunders R, Bastian I.** A review of the diagnosis and treatment of smear-negative pulmonary tuberculosis. Int J Tuberc Lung Dis 2000; 4: 97-107.

21-**Nyamande K, Lalloo UG, John M.** TB present¬ing as community-acquired pneumonia in a setting of high TB incidence and high HIV prevalence. Int J Tuberc Lung Dis 2007; 11: 1308-13.

22-**Bhatt M, Kant S, Bhaskar R**. Pulmonary tuber¬culosis as differential diagnosis of lung cancer. South Asian J Cancer 2012; 1: 36-42.

23-**Ali M. Somily, Mazin A. Barry, Hanan A. Habib, Fawzia E. Alotaibi, Fahad A. Al-Zamil, Mohammed A.** Khan and Mohammed S. Sarwar, Evaluation of GeneXpert MTB/RIF for detection of Mycobacterium tuberculosis complex and rpo B gene in respiratory and non-respiratory clinical specimens at a tertiary care teaching hospital in Saudi Arabia. Saudi Med J. 2016 Dec; 37(12): 1404–1407.

24-**Ryan GJ, Shapiro HM, Lenaerts AJ.** Improving acid fast fluorescent staining for the detection of mycobacteria using a new nucleic acid staining approach. Tuberculosis (Edinb) 2014;94:511–518

25-**Bunsow E, Ruiz-Serrano MJ, Roa P, Kestler M, Viedma D, Bouza E.** Evaluation of GeneXpert MTB/RIF for the detection of Mycobacterium tuberculosis and resistance to rifampicin in clinical specimens. J Infect. 2014; 68:338–343.

26-**Steingart KR, Schiller I, Horne DJ, Pai M, Boehme CC, Dendukuri N.** Xpert MTB/RIF assay for pulmonary tuberculosis and rifampin resistance in adults. Cochrane Database Syst Rev. 2014;(1):CD009593.

27-**Maynard-Smith L, Larke N, Peters JA, Lawn SD.** Diagnostic accuracy of the Xpert MTB/RIF assay for extra-pulmonary and pulmonary tuberculosis when testing non-respiratory samples: a systematic review. BMC Infect Dis2014;14:709.

28-**Omar A, Elfadl AE, Ahmed Y, Hosny M.** Valuing the use of GeneXpert test as an unconventional approach to diagnose pulmonary tuberculosis. Egypt J Bronchol 2019 13:403–407

29-**Moussa SM, Bayoumi FS, Ali AM.** Evaluation of GeneXpert MTB/RIF assay for direct diagnosis of pulmonary tuberculosis. Saudi Med J 2016; 37:1076–1081.

30-**Chang K, Lu W, Wang J,Zhang K, Jia S, Li F, et al.**Rapid and effective diagnosis of tuberculosis and rifampicin resistance with Xpert MTB/RIF assay: a meta-analysis. J Infect 2012; 64: 580-8.

31-**Ling DI, Zwerling AA, Pai M.** GenoType MTBDR Assays for the Diagnosis of MultidrugResistant Tuberculosis: A Meta-Analysis. European Respiratory Journal 2008;32(5):1165- 74

32-**Seung KJ, Keshavjee S, Rich ML.** Multidrug-Resistant Tuberculosis and Extensively Drug-Resistant Tuberculosis. Cold Spring Harb Perspect Med. 2015;5(9):a017863.

33-**Kim YW, Seong MW, Kim TS, Yoo CG, Kim YW, Han SK, et al.** Evaluation of Xpert MTB/RIF assay: diagnosis and treatment outcomes in Rifampin resistant tuberculosis. Int J Tuberc Lung Dis 2015; 19:1261–1221.