DOI: https://doi.org/10.54393/mjz.v2i2.21

MARKHOR
THE JOURNAL OF ZOOLOGY
https://www.markhorjournal.com/index.php/mjz
Volume 2, Issue 2 (Jul-Dec 2021)

Original Article

Comparison of GeneXpert assay and Fluorescent Microscopy for the Diagnosis of Pulmonary Tuberculosis in Narowal region

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Keywords: Tuberculosis (TB), Fluorescence microscope (FM), Sensitivity, Specificity, Light Emitting Diode (LED), GeneXpert

How to Cite:
Amjad, A., Ansari, F., Iqbal, A., Ain, Q. ul, Ali, A., Aziz, S., & Yusuf, Z. (2021). Comparison of GeneXpert assay and Fluorescent Microscopy for the Diagnosis of Pulmonary Tuberculosis in Narowal region. *MARKHOR (The Journal of Zoology)*, *2*(2). https://doi.org/10.54393/mjz.v2i2.21

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Article History
Received: 01 Aug 2021
Accepted: 29th Aug 2021
Published: 31st December 2021

ABSTRACT

GeneXpert is attributed as one of the latest technical means for diagnosing Tuberculosis in very short period of time. **Objectives:** To evaluate the efficiency of GeneXpert and fluorescent microscopy in the detection of pulmonary tuberculosis (TB); To compare the sensitivity and specificity of GeneXpert and fluorescent microscope in the diagnosis of *Mycobacterium tuberculosis* **Methods:** In the present investigation, the diagnostic performance of GeneXpert MTB / RIF for tuberculosis was compared with the performance of light-emitting diode fluorescence microscope in TB samples from Narowal, Pakistan. For this purpose, a total of 299 TB positive specimens were obtained. Among these 54% (n = 160) were categorized to be obtained from male and 46% (n = 139) from female population. Data collected was distributed in 4 age groups; 0-20, 21-40, 41-60, and 61-80, in which the percentage and number of samples were found as 2% (n = 6), 60% (n = 179), 27% (n = 80) and 11% (n = 34), respectively. **Results:** The parameters including sensitivity and specificity calculated for GeneXpert were 73% and 100%, correspondingly, while the sensitivity and specificity calculated for LED-FM microscope were 43% and 100%, respectively. **Conclusions:** This indicates that the GeneXpert is more sensitive in detecting MTB in comparison to LED-FM technique. The GeneXpert assay was also found to detect small number of bacillus from samples in comparison to LED-FM method.

INTRODUCTION:

Mycobacterium tuberculosis (MTB) is a causal cause of tuberculosis [1]. The lung is the main organ affected by MTB, but other organs of body may also be severely affected by it [2]. In most cases, the ailment can reduce symptoms, in which case it is called drowsiness or inactive tuberculosis [3, 4]. About 10% of lethargic or inactive disease develops into dynamic disease, and when untreated, the slayer will infect more people [5]. Model indicators of dynamic tuberculosis are blood stasis, weight loss, night sweats and fever. Weight loss is what has long been called consumption. The broad combination of symptoms is caused by contamination of different organs [6]. When people with dynamic tuberculosis in the lungs cough, spit, talk or sneeze, tuberculosis spreads through airborne droplets. People with inactive tuberculosis do not spread the disease. HIV/AIDS patients and smokers are prone to active infection [7]. The diagnosis of active TB depends on X-rays of the chest, along with culture and the microscopy of various body fluids. The identification of indolent tuberculosis depends on the special skin test called tuberculin skin test (TST) or by way of blood test [8-10].

Avoidance of tuberculosis includes vaccination against BCG, increased screening hazard, timely identification and correct management of the cases [11]. High-risk group include family members, work environment, and social

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interactions with active tuberculosis patients. Treatment requires long-term use of various antibiotic agents. The main problem now devouring society is antibiotic resistance, incidence of multidrug resistant tuberculosis and extensive drug resistance tuberculosis. 33% of the total population worldwide carry the *mycobacterium tuberculosis* in dormant condition but only 1% of new infections occur in the general population. In 2016, there were more than 10 million cases of dynamic tuberculosis, resulting in 1.3 million deaths. Hence making it an important cause of death due to infection. In most of the developing countries, more than 95% of the deaths occurred more than half of these occurred in Indonesia, Pakistan, the Philippines, India and China. Since 2000, the number of fresh cases has decreased every year. Approximately 80% of the people in different African and Asian countries are tested, whereas in the United States, 5-10% of individual lumps are found to be positive by way of the tuberculin test [12].

Xpert MTB / RIF provides patients with distinct advantages such as early diagnosis and early start of appropriate treatment resulting in improved general health i.e. reducing opportunities for tuberculosis transmission, especially in developing countries [13]. The GeneXpert MTB/RIF technique is used for the diagnosis of TB and rifampicin (RIF) resistance [14]. This study was designed for the association of GeneXpert MTB/RIF assays and fluorescence microscope for rapid detection of TB versus culture.'

METHODS

It is a Cross-sectional study. The study will be conducted in the tuberculosis department at DHQ Hospital in Narowal. Sample size was 299 and sampling technique was Judgment/purposive sampling. Inclusion criteria: Patients of all ages, Patients belonging to male and female gender, Patients with active tuberculosis infection and Patients who can actively cough up sputum.

Laboratory Processing

The patient's detailed clinical parameters were recorded and the patient was guided to collect the sputum sample in a defined container. These samples were then assigned a specific laboratory number and processed further.

Preparation of Sample

Smears were prepared from samples after concentration and re-suspension of the pallet.
A drop of specimen was placed on a microscope slide. It was spread and allowed to dry.
Smear was heated and then kept for fixation at 65-75 ^{o}C for 2-3 hours.

Staining Procedure

Smear was covered with stain. After 15 minutes of washing, the stains were rinsed and the slides were immersed for 2 minutes in 0.5% decolorizing agent. Slides were washed again and covered with potassium permanganate solution. Slides were rinsed after 2 minutes, air dried and examined under UV light.

Microscopy

After staining, the slides were examined by the microscopists and me. An eyepiece with a10x amplification and an objective lens with 40x amplification was used. Quantification of acid-fast bacilli was carried out as meeting the guidelines and criteria of the Centre for Disease Control (CDC).'

200X	REPORT
No AFB in one length	Negative
1-4 AFB in one length	Confirmation required
5-49 AFB in one length	Exact number (scanty)
3-24 AFB in one field	1+
25-250 AFB in one field	2+
>250 AFB in one field	3+

Table 1: Grading of cells under FM Microscope (WHO,1998)

GENEXPERT

Requirements are GeneXpert system (GeneXpert + Computer + Barcode scanner), Sample reagent, Cartridge, Personal protective equipments (N95 mask, gloves, apron, closed shoe, face shield), Vortex, Timer

Procedure

The sample reagent and the sputum collection container lids were opened.02 volumes of sample reagent was added to 01 volume of sputum and lid was replaced.The mixture was thoroughly mixed over a vortex for at least 10 seconds.Then it was incubated for 10 minutes at room temperature and then mixed again.It was incubated for another 05 minutes.The

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sample was processed till it was perfectly liquid, if it was still viscous, a waiting time of 05-10 minutes was given.The side of the cartridge was labelled with the sample id before its lid was opened.
Sample (2ml) was slowly transferred to the sample chamber of the cartridge taking care that care that bubbles don't form.The lid was firmly closed and the test was run on GeneXpert instrument.

Culture Media

Lowenstein Jensen media was employed to detect the bacilli from samples.

Media Composition

It includes: Malachite green, Asparagine, Potato starch, Coagulated egg, Mineral salt solution (potassium dihydrogen phosphate, magnesium sulfate, sodium citrate) ,Low levels of penicillin and nalidixic acid are also present in LJ medium to inhibit growth of Gram-positive and Gram-negative bacteria and to limit growth to Mycobacterium species only. Presence of malachite green in the medium inhibits most other bacteria. It is disinfected and solidified by a process of inspissation. Presence of glycerol enhances the growth of *M. tuberculosis*. For cultivation of *M.bovis,* glycerol is omitted and sodium pyruvate is added.

Culture Inoculation

Positive and negative results of samples as found by microscopy and GeneXpert were cultured on Lowenstein Jensen media. After inoculation, the plates were incubated for at least 6 weeks at 37°C. Any visible growth was observed and recorded as MTB and MOTT**.'**

Statistical Analysis

Using the 2×2 table in the SPSS-20 software and considering the sputum culture as gold standard. The sensitivity, specificity, PPV and NPV for each assay were calculated to diagnose TB in patients. The kappa (k) test was used to assess the consistency between the tests. The receiver operating characteristic (ROC) curves were performed using SPSS-20 software.

Using the formula, the sensitivity was found as follows:

Sensitivity % = true positive (TP) / (true positive (TP) + false negative (FN)) X 100

Specificity was calculated using the formula given below:

Specificity % = true negative (TN) / (true negative (TN) + false positive (FP)) X 100

RESULTS

The current study was conducted (in Narowal, Pakistan) to compare the diagnosis of tuberculosis with GeneXpert and fluorescence microscopy. Total processed samples were 299 of which 54% (n = 160) were obtained from male and 46% (n = 139) from female population.

Data obtained was divided into 4 groups according to age as; 0-20, 21-40, 41-60, and 61-80.

	Age groups			
	0-20	**21-40**	**41-60**	**61-80**
No. of patients (n)	6	179	80	34
Percentage (%)	2	60	27	11

Table 2: Categorization of individuals across age groups

This distribution showed that 2% (n = 6), 60% (n = 179) and 27% (n = 80), 11% (n = 34) were found in the age range of 0-20, 21-40, 41-60, and 61-80 respectively (Table 2).

Selection of Samples

Samples that fulfilled the inclusion and exclusion criteria were collected and processed.

Physical Examination of Sputum Sample

Physical examination revealed that out of 299 samples 5% (n=15) were salivary, 49% (n=149) were mucoid, 36% (n=109) were purulent and 10% (n=31) were blood tinted (Table 3).

Variables	**Saliva**	**Mucoid**	**Purulent**	**Blood tinted**	**Total**
Number of samples (n)	15	149	104	31	299
Percentage (%)	5	49	36	10	100

Table 3: Physical examination of sputum sample

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Grading of Samples upon Fluorescent Microscopy

Out of 299 processed samples grading of microscopy revealed that 86% (n=256) samples were negative, 1% (n=4) were scanty, 2% (n=6) were 1+, 6% (n=18) were 2+, 5% (n=15) were 3+ (Table 4).'

Grading	Negative	Scanty	1+	2+	3+	Total
Number of samples	256	4	6	18	15	299
Percentage (%)	86	1	2	6	5	100

Table 4: Grading of processed samples analyzed on FM

Detection of Tuberculosis with FM

Fluorescence microscopy declared 18% (n=55) as positive (Table 5). The sensitivity and specificity recorded for FM were 43% and 100%, respectively. Furthermore positive predictive value (PPV) and negative predictive value (NPV) estimated for FM techniques were found to be 71% and 100%, respectively (Table 6).

Detection of Tuberculosis by GENEXPERT

Total positive samples as observed through GeneXpert were 31% (n=93) (Table 5). The sensitivity and specificity recorded for GeneXpert were 73% and 100% respectively. Furthermore PPV and NPV values estimated for GeneXpert assay were found to be 83% and 100%, respectively (Table 6).'

	Methods	
	FM	**GeneXpert**
Total positive (n)	55	93
Percentage (%)	18	31

Table 5: Comparison of diagnostic techniques in detecting tuberculosis

Comparison of FM and GENEXPERT

FM had sensitivity of 43% while GeneXpert showed 73% sensitivity. It was also revealed that GeneXpert was more sensitive as compared to Flourescence Microscopy (Table 6). The area under ROC curves demonstrated that it was greater for GeneXpert (.859) as compared to FM (.703) (Table 8, figure 1), this shows that GeneXpert is more efficient than Microscopy. The concordance value showed the moderate trend as it was noted to be .642 for GeneXpert and FM (Table 7).

	FM	GeneXpert
Sensitivity (%)	43	73
Specificity (%)	100	100
PPV	71	83
NPV	100	100

Table 6: Comparison of sensitivity and specificity of FM, GeneXpert and Culture

From the above table, it is clear that GeneXpert is more sensitive than FM considering culture as a gold standard. The specificity of GeneXpert and FM is same.

Diagnosing techniques	k value
FM x Culture	.434
GeneXpert x Culture	.741
FM x GeneXpert	.642

Table 7: Concordance for different methods by kappa test

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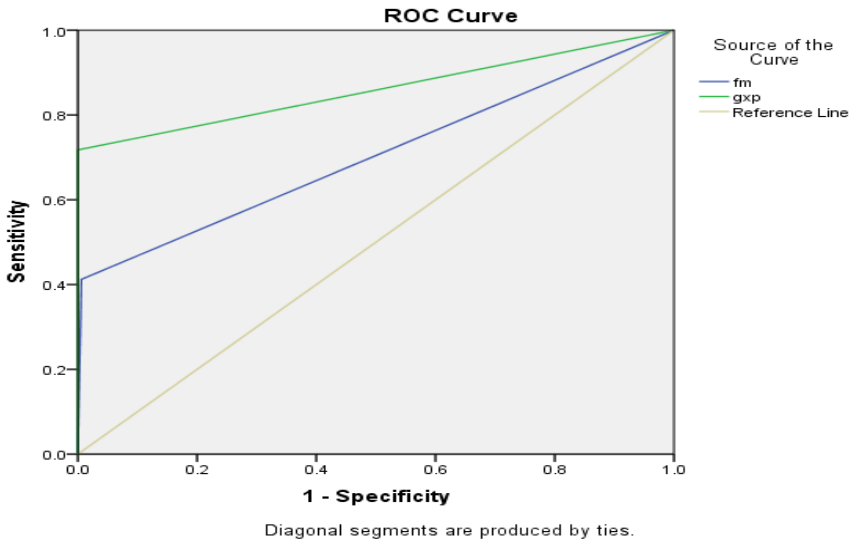

Figure 1: ROC curve

Test	Area	Significance
FM	.703	.000
GeneXpert	.859	.000

Table 8: ROC area of different methods

DISCUSSION

The purpose of investigation was to investigate diagnostic ability of three different techniques for tuberculosis cases. The findings were paralleled to standard culture techniques. Total 299 sputum samples were examined, with an FM detection rate of 18% (n = 55), GeneXpert of 31% (n = 93) and a standard culture technique of 43% (n = 128). The specificity and sensitivity of the GeneXpert assay were known to be 100% and 73%, respectively, in addition the sensitivity and specificity of the FM microscope were 43% and 100%, respectively. The findings showed culture as better than the two techniques used. This is in contrast with another study comparing the GeneXpert findings and stated GneXpert to be better [15].

Sajjad Ahmed and his colleagues studied the GeneXpert MTB / RIF assay for the detection of TB on sputum specimens. After meeting the inclusion criteria, a total of 268 participants were included in the study. Their sputum samples were collected and processed by the N-acetyl-L-cysteine-sodium hydroxide (NALC-NaOH) method and the GeneXpert MTB / RIF assay. The study determined the overall sensitivity and specificity of the MTB / RIF assays, 92.4% (86/93) and 97.1% (138/142), respectively. The sensitivity was 98.4% (60/61) in the culture proven smear positive samples, while the culture confirmed that the smear negative sample had a sensitivity of 93.7% (30/32) using the culture as a reference standard [16]. Few other studies have reported similar findings {17,18]. The result of this study are similar to our study which aimed to evaluate GeneXpert for culture and fluorescence microscopy, and GeneXpert analysis showed sensitivity and specificity of 73% and 100%, correspondingly.

In a survey conducted by *Elisabetta Walters* and her colleagues, 14 samples were analyzed by culture and GeneXpert techniques. Of the 9/14 (64%) cases confirmed by culture, 7 (78%) were positive for broncheoalveolar lavage (BAL) samples; in addition, GeneXpert also confirmed two cases with as earlier negative diagnosis (14%). Two drug-resistant cases were identified: one from BAL Xpert and the other from genotyping tests for gastric inhalation culture. All children started receiving anti-tuberculosis treatment and responded well to the treatment [19]. In our study we worked on sputum samples with a sensitivity of 18%, 31%, and 43% for FM microscopy, GeneXpert assay and culture respectively. This study is contradictory to our study which maintains that culture is a gold standard technique, and it's better than GeneXpert and fluorescent microscopy.

'*Lidya Chaidir* and her colleagues studied the application of FM technique for the purpose of diagnosing lung cancer and also HIV related tuberculosis in Indonesian hospital settings. They assessed that fluorescent microscope was more sensitive, but not as specific as ZN. The sensitivity and specificity of FM increase when sputum is concentrated before smear preparation. In people living with HIV, FM exhibit to some extent higher specificity and sensitivity than traditional

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ZN microscopes [20]. In our study, we did not compare FM microscopy with conventional ZN staining. Though FM microscopy showed 43% sensitivity and 100% specificity. On the contrary, FM takes half the time of the ZN microscopy and has a similar operating cost.

Method for diagnosing urinary tract tuberculosis in urine samples by genotoxicity Mtb/rif assay was studied by *Yu Pang*. Total 167 patients participated in the study. Out of these, 4 (2.4%) patients were omitted from the study. Therefore, 163 patients were analyzed finally, of which 44 (27%) were diagnosed with urinary tuberculosis (UTB) cases based on clinical symptoms and anti-TB treatment and 37 (22.7%) were cultured positive UTB cases. The sensitivity of acid bacillus microscopy and GeneXpert is 40.5% and 94.6% as compared to LJ culture, respectively. After using clinical diagnosis as a reference standard, the specificity and the sensitivity of AFB smears were 98.8% and 18.5% respectively. LJ culture cases are twice times higher than AFB smear cases, with sensitivity and specificity of 45.7% and 100%. In addition, from the clinically diagnosed 81 urinary tract tuberculosis cases, 51 were processed by the Xpert technique, showing the sensitivity of 63% that is considerably higher than AFB smear microscopy and LJ culture method. GeneXpert was only detected in 5 patients with RIF resistance, and all patients had a phenotypic sensitivity test with a sensitivity of 100% [21]. This study is quite similar to present study in which GeneXpert is more sensitive and specific as compared to FM.

CONCLUSIONS

MTB/RIF examinations should create faster resistance testing and, in selected groups, strengthen tuberculosis case detection. If subsidy for improved MDR TB treatment is accessible then it will be very cost effective and beneficial for rapid screening of tuberculosis patients but this influence is predictably be limited by its cost. While this progress should be celebrated and priority should be given to funding in this area, it must be seen as a shocking situation that nearly two million peoples die from tuberculosis every year and in fact some people will be protected by any type of diagnostic test. Mostly deaths occur in HIV negative individuals, most of them died from medication sensitive tuberculosis, primarily due to insufficient basic affordable healthcare services for the treatable infectious disease.

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