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Review Article

Diagnosis of Tuberculosis by GeneXpert MTB/RIF Assay Technology: A Short Review

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ABSTRACT

Mycobacterium tuberculosis remains one of the most significant causes of death from an infectious agent. *Mycobacterium tuberculosis* complex bacteria and resistance to rifampicin drug (RIF). India has the world's largest burden of tuberculosis (TB), accounting for one-fourth (24%) of the global TB incidence. There were many techniques that are used universally, but they are not able to test the resistivity of the drugs. The GeneXpert MTB/RIF assay is a diagnostic and automated technique for rapid diagnosis of Mycobacterium tuberculosis. The aim of the article is to detect the extra pulmonary Tuberculosis by GeneXpert MTB/RIF Assay with Rifampin Resistance that can be identified in the patients. This article is suggested that Gene Xpert MTB/RIF is a technique which does not require any bacteriological test, it is cheap, fast and specific test that can help us in distinguishing the specific tuberculosis. It requires less manpower but a trained person to perform this test.

INTRODUCTION

India is country which is under high threat of spreading tuberculosis. [1] There was more than one technique that was employed for confirmation of the test. [2]

The diagnosis of tuberculosis which is extrapulmonary that were always been a big challenge due to the disease shows a pleomorphic effect. While sample collected it shows paucibacillary while its microbiological diagnosis. In a result it shows a low sensitivity while in microscopic smear and before that nucleic acid amplification tests were done. [3]

The techniques that were employed for diagnosis of tuberculosis that is smear microscopic examination, Ziehl-Neelsen (ZN) staining, cultivation, many serological test, histological/ cytological test, Mantoux test and PCR test but they are not specific, These tests does not differentiate between Tuberculosis and non tuberculosis bacteria [4]

Later a new technique introduced which can be able to differentiate the tuberculosis and non tuberculosis bacteria and needs minimum handling and training. [1] The technique is Xpert MTB/RIF is based on GeneXpert platform, a highly sensitive, rapid and simple to use Nucleic acid amplification test (NAAT). The Xpert MTB/RIF purifies, concentrates, amplifies (by Real-Time PCR) and identifies targeted nucleic acid sequences in the TB genome, and provides results from unprocessed sputum samples in 90 minutes (less than 2 hours), with minimum biohazard and very small technical training needed to operate. [5]

There has been the three main aim of this article. First aim is to diagnose the Tuberculosis by Gene Xpert assay and its steps, second to illuminate the common misunderstanding about the technique and third to condense the study on tuberculosis patient diagnosis in relation to drug resistance, its treatment and its research priorities.

GENE XPERT ASSAY

Gene Xpert assay is composed of some key features. As this process is composed of PCR technique and sample is stored in disposable plastic cartridge which is all automated. Gene Xpert Assay composed of Bacterial lysis, Nucleic Acid extraction, amplification and amplicon detection. The NAAT have more sensitivity for specimens that are acid-fast bacillus (AFB) positive by microscopy but lower for AFB-negative specimens. [6]

CLINICAL SAMPLES FOR TESTS

The pulmonary and extrapulmonary samples of the patients showing symptoms of tuberculosis i.e. pulmonary samples include sputum, bronchoalveolar lavage, bronchoscopic aspirate, postbronchoscopic sputum, and gastric fluid specimens and extrapulmonary samples include pleural fluid, lymph node biopsy, disc material, ascitic fluid, cerebrospinal fluid, pericardial fluid, skin biopsy, and urine specimens. The reagent was taken in a ratio of 2:1 i.e. with discontaminated and concentrated specimen. The universal container was cleaned. After decontamination, smears were prepared by the auramine-rhodamine acid-fast staining method. [7]

Before processing of specimens, smears were performed and stained by the ZN method and examined with a light microscope for the presence of AFB. [8]

AFB positive samples, cultured on Lowenstein-Jensen (LJ) medium and strains were identified on the basis of biochemical tests such as pigment production, niacin, nitrate reduction and the 68°C catalase test[9].

CULTURE

Culture of AFB still used as gold standard method. LJ medium was used to inoculate with 0.5 mL of dissolved specimen solution. The inoculated LJ medium was then incubated at 37°C for eight weeks and examined weekly, while 0.5 mL of the specimen solution was also added to liquid medium in Mycobacterium Growth Indicator Tubes (MGIT). The MGIT tubes were then incubated in an automated MGIT 960 system at 37°C for six weeks. The remaining deposit was used for PCR investigation using the Xpert MTB/RIF assay[10].

GENE XPERT MTB/RIF ASSAY

Then take 2ml of inactivated solution was transferred in the GeneXpert test cartridge for 15 minutes. Cartridges inserted into the Gene Xpert device in which the sample is automatically filtered, washed and concentrated bacilli and inhibitors are removed. Ultrasonic lysis of filtered sample captures the organisms that to release DNA. DNA mixed with dry PCR reagents. Semi nested real time amplification and detection in integrated reaction tube employed. The process needs about 90 min result automatically generated and make the result readable[11].

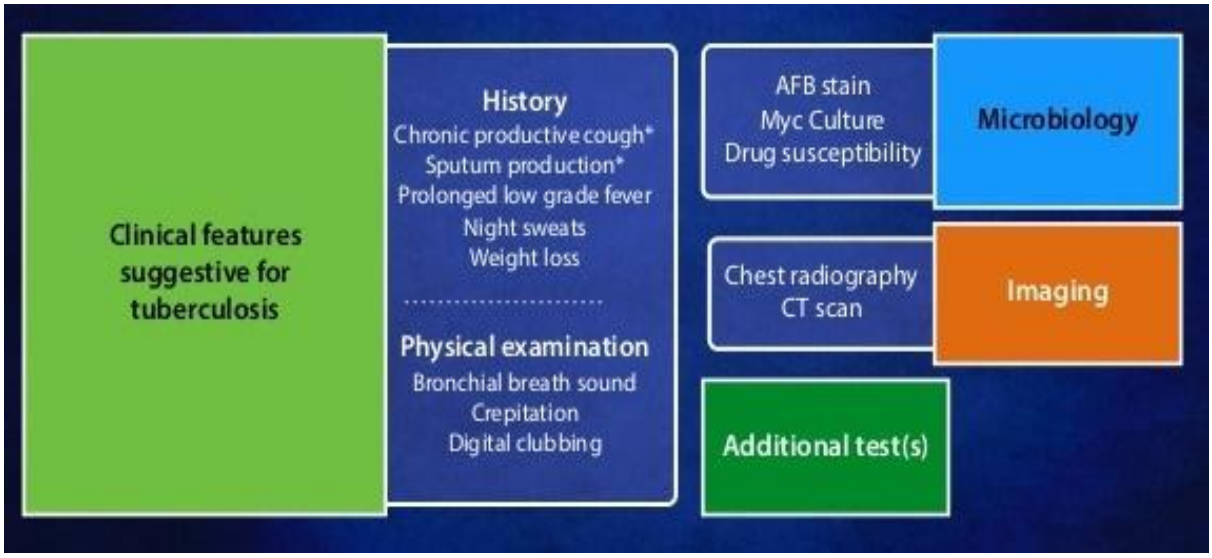

Fig.1: Showing clinical features suggestive for TB.

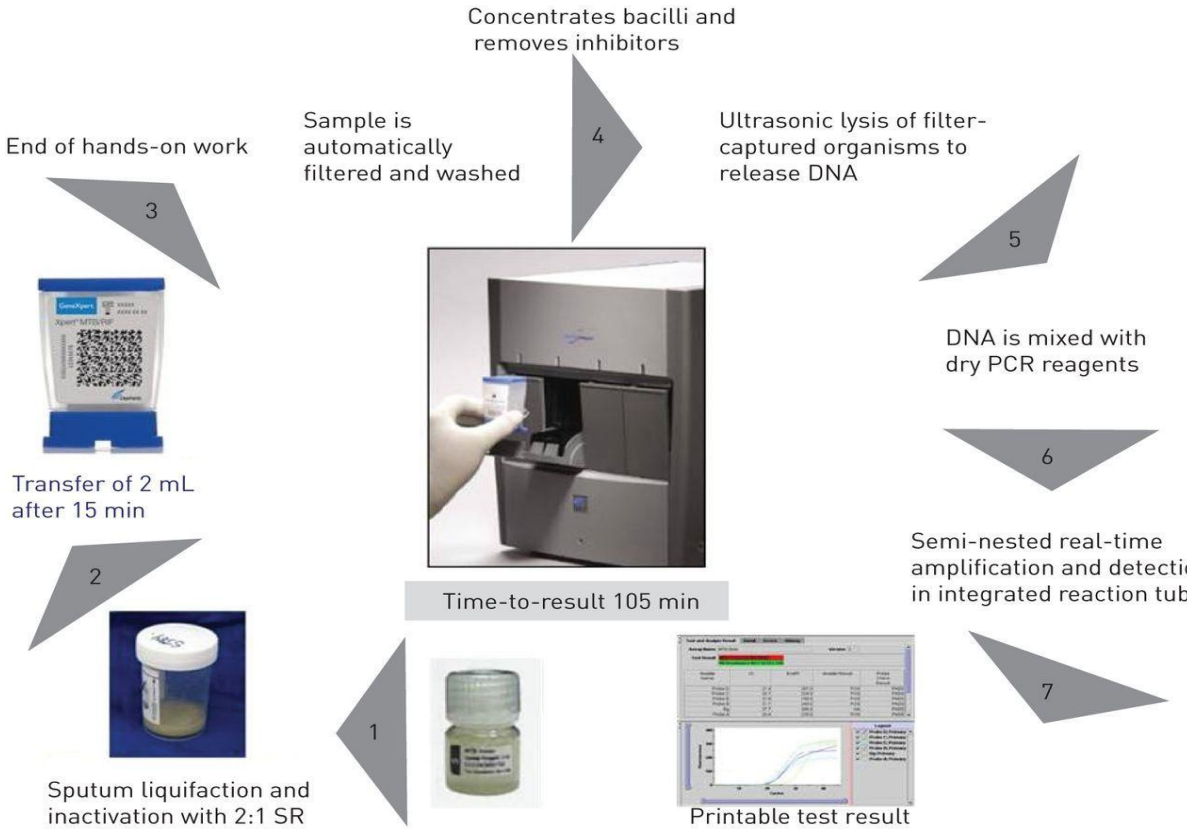

Fig.2: Showing diagrammatic representation of test procedure. [12]

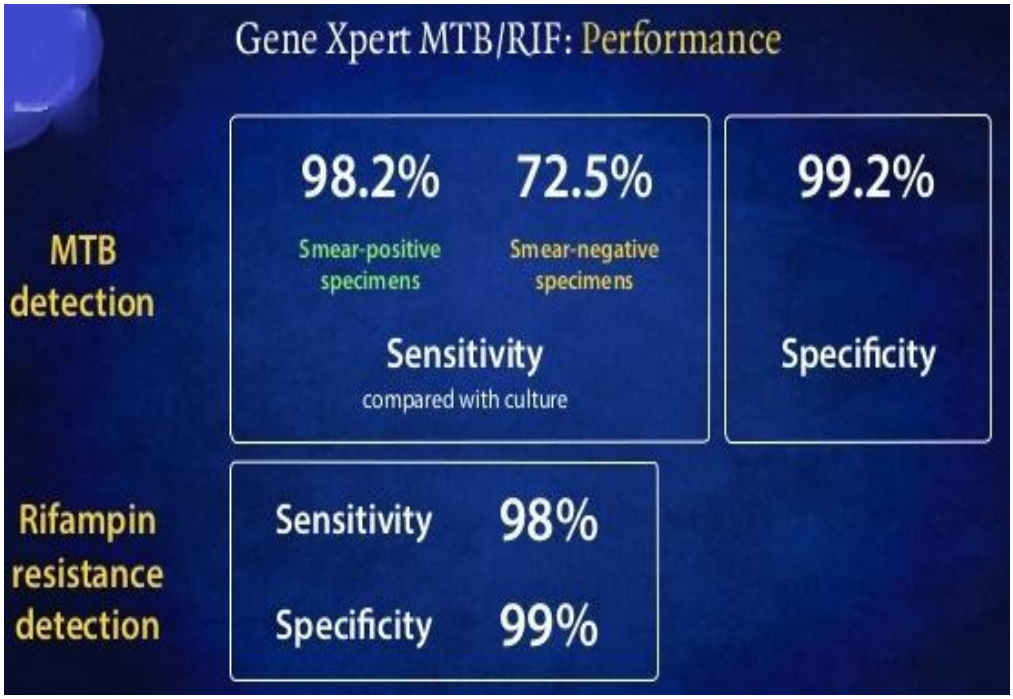

Fig.3: Showing performance of the Gene Xpert assay for detection of M. tuberculosis and rifampin drug resistance.

Advantages of GeneXpert MTB/RIF:
GeneXpert Performance in MTB detection specimen shows 98.2 % were smear positive specimens and 72.5% were smear negative specimens when their sensitivity is compared with culture and 99.2% were specific. In Rifampcin resistance detection in this 98% specimen were sensitive and 99% were specific in a study.

Gene Xpert MTB/RIF is called as a screening tool in Drug resistance technique. In TB affected patients, rifampcin resistance works as an important indicator in MDR-TB with their serious clinical implication15. Gene Xpert is much more useful technique for the early diagnosis of patients showing the clinical symptoms of pulmonary tuberculosis. It is helpful in detecting rifampicin resistance and patients with MDR and HIV associated tuberculosis. [13]

Disadvantages of GeneXpert MTB/RIF:
It requires a stable uninterruptable electrical supply is essential. Temperature does not exceed 300 C, cartridges stored at less than 280 C. Shelf life of cartridges must be matured. Security measures must be taken to avoid hustling of laptop or desktop computer. Specimens must take in a leak proof container and stored at room temperature. RT-PCR is employed that targets the *rpoB* gene hot spot region. Rifampin and isoniazid resistance are employed for the identification of mutations. [14]

CONCLUSION

Mycobacterium tuberculosis remains one of the most significant causes of death from an infectious agent. India has the world's largest burden of tuberculosis (TB), accounting for one-forth (24%) of the global TB incidence. So, finally we can say technically there is no perfect test for distinguishing and identifying Tuberculosis15. It is a type of apparatus used for distinguishing rifampcin resistance, Xpert MTB/RIF has a sensitivity of 95% and specificity of 98% when compared with phenotypic references standards. Biosafety precautions and the training needed are minimized. For smear – negative culture – positive TB, the pooled sensitivity of Xpert MTB/RIF has been found to be 68%13. Gives result in less than 2 hours and needs minimum handling with training. As the Gene Xpert is more sensitive technique than AFB smear microscopically in pulmonary samples. [13] Although the MTB/RIF test could be a useful tool for rapid identification of RIF-resistant M. tuberculosis, especially in smear-positive clinical samples. [16]

In arbitrary, I can say that Gene Xpert MTB/RIF is a technique which does not require any bacteriological test, it is a cost effective, fast and specific test that can help us in distinguishing the specific tuberculosis. It requires less manpower but a trained person to perform this test.

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