Cartridge-Based Nucleic Acid Amplification for Rapid Tuberculosis Diagnosis

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Abstract

In terms of public health, tuberculosis continues to have a significant impact on people's lives. The process of determining the underlying cause of a sickness or condition in a patient is known as clinical diagnosis. It entails the use of numerous diagnostic methods, including physical examinations, laboratory testing, and imaging procedures for medical purposes. Smear microscopy, which are frequently utilized, have poor sensitivity and lengthy turnaround times, respectively. An innovative, quick, cartridge-based molecular test called the GeneXpert (GX) assay has a well-established use for the quick identification of pulmonary and extrapulmonary Mycobacterium tuberculosis (MTB) infections. This device delivers a report indicating whether the sample tested positive or negative for TB after around two hours. The report offers details on the quantity of TB bacteria found in the sample, which can help determine treatment choices. HIV, hepatitis C, and gonorrhea are just a few of the infectious disorders that the GeneXpert system can identify. It is also used to detect the SARS-COV-2 virus-related illness known as COVID-19.

Keywords: GeneXpert, mycobacterium tuberculosis, diagnosis, infection, rapid testing technologies

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TUBERCULOSIS

Mycobacterium tuberculosis (MTB), which is an endemic and frequent cause of chronic infection-related morbidity and mortality, is the cause of tuberculosis (TB), a disease that primarily affects the lungs but can also infect the bones, kidney, and brain [1-3]. When a TB patient coughs, sneezes, or talks, tiny drops of saliva are thrown into the air nearby and transmitted from person to person. Although the guidelines for diagnosing and treating pulmonary tuberculosis are well established, managing extra-pulmonary tuberculosis remains challenging [4-5].

The symptoms of TB differ depending on the portion of the body that is afflicted. TB's most typical signs and symptoms include: [6]

- The most typical symptom is a persistent cough that lasts longer than three months.
- chest discomfort
- fatigue
- loss of weight
- the night sweats
- chills and a fever
- reduced appetite

Depending on which area of the body is being damaged by the TB bacteria, TB can also cause additional symptoms. In contrast to TB in the kidneys, which can cause blood in the urine, TB in the bones can cause joint pain and swelling. [7]

Several tests are required for the diagnosis of TB, including:-

Tuberculin skin test: During this test, the patient receives a little injection of a pure protein derivative (PPD) under the skin of the forearm. If a person has TB infection, their body will respond to the PPD by developing an induration where the injection was made. [8]

Blood test: A blood test involves identifying the antibodies produced in the blood against the TB bacterium.

X-rays of the chest can reveal whether TB is present in the lungs or other places of the body.

Sputum test: In this test, Sputum or mucus from the mouth and lungs is collected and examined under a microscope to check for TB bacteria.

If TB is found, the typical course of therapy includes two or more medicines given for six to nine months. HIV-associated TB and multidrug-resistant (MDR) TB are two elements that have changed the epidemic and made TB treatment more difficult. Delays in diagnosis cause many TB deaths, and the pandemic persists because we are unable to significantly lower transmission using the diagnostics available today. So the GeneXpert platform provides a significant improvement over these constraints.

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Gene Expert Device:

An advanced molecular diagnostic device called the GeneXpert is utilised to find the presence of infectious diseases like tuberculosis. It utilises an original technique known as cartridge-based nucleic acid amplification testing (CB-NAAT) to quickly and precisely find the DNA of the pathogenic bacteria [9]. According to research, the Xpert MTB/RIF assay has good sensitivity and specificity and can accurately detect Rifampicin resistance [10]. The World Health Organization (WHO) recommends it for the diagnosis of pulmonary tuberculosis. The utility of Xpert MTB/RIF for extra pulmonary TB can be determined by using the Xpert MTB/RIF assay, which is available quickly and automatically [9-11].

The device uses a disposable cartridge containing all the reagents required to find the target organism's DNA. Thanks to a tiny chip also included in the cartridge, the machine can understand the results and display them on a screen. The GeneXpert machine's cartridge is a tiny, throw-away container that contains all the tools and materials required to conduct a molecular diagnostic test. [9] It is made to be put into the machine, where it is processed and analysed automatically.

A pre-loaded assay, which is a collection of particular probes and primers used to find the target organism's DNA, is included in the cartridge. [8] The assay is made to identify genetic sequences that are particular to Mycobacterium tuberculosis, the bacterium that causes tuberculosis, to diagnose TB. Tuberculosis can be found with the GeneXpert machine in as little as two hours, which is far quicker than using conventional techniques, which can take up to six weeks. The device can also identify drug-resistant tuberculosis strains, which is crucial for selecting the best course of therapy.

The following steps make up the protocol for utilising the GeneXpert device to test for tuberculosis (TB):[12-13]

- **Sample collection:** The probable TB patient has a sputum sample taken. A sterile container is used to collect the sputum that is produced from the patient's lungs when they are told to cough deeply.
- **Sample preparation:** The collected sputum sample is mixed with a sample reagent that helps to break down any interfering substances that might be present in the sample. The mixture is then incubated for a short period to allow for further processing.
- **Cartridge preparation:** A disposable cartridge, which contains all the necessary reagents for the test, is prepared for use. The cartridge is labeled with the patient's information and barcode, and is inserted into the GeneXpert machine.

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Fig.no. 1. Loading Sample In cartridge

Sample loading: A small volume of the prepared sputum sample is then loaded into the cartridge using a pipette. The cartridge is then sealed and inserted into the GeneXpert machine. Loading sample in cartridge are shown in figure 1.

Machine operation: Using an automated process, the GeneXpert equipment extracts and amplifies the TB DNA present in the sample. These procedures entail dissecting the bacterial cells, separating the DNA, and employing real-time polymerase chain reaction (PCR) equipment to amplify the target DNA sequence. Loading cartridge in Gene expert machine are shown in figure 2.

Fig. no. 2 loading cartridge in Gene expert machine

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Result Interpretation:

The GeneXpert device delivers a report indicating whether the sample tested positive or negative for TB after around two hours. The report offers details on the quantity of TB bacteria found in the sample, which can help determine treatment choices. [9]

• Record-keeping: The findings are entered into the patient's medical file and distributed to the proper healthcare professionals for treatment and follow-up.

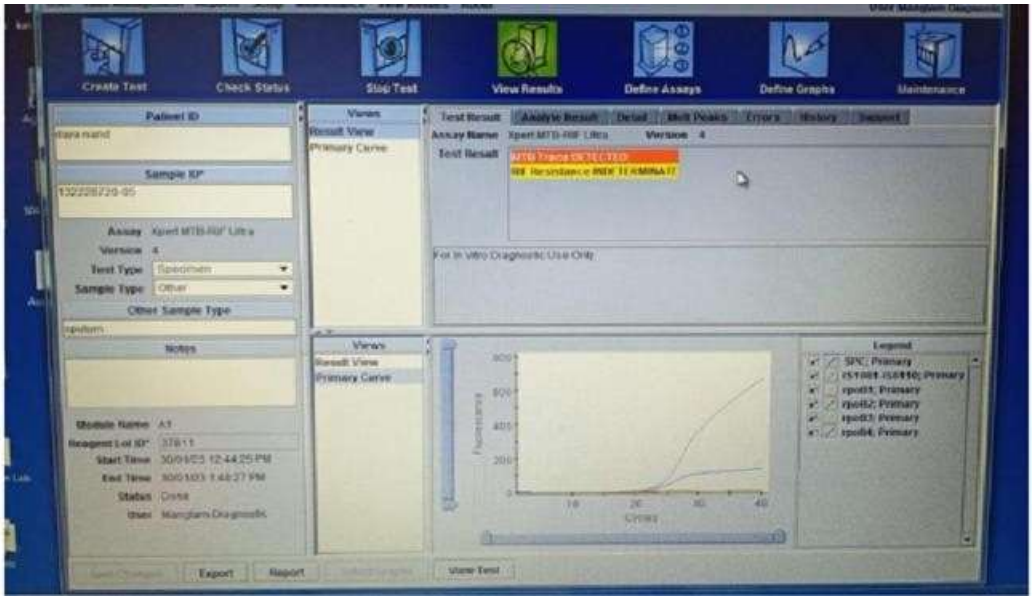

Fig. no. 3 Result of gene expert showing +ve

Overall, the GeneXpert device is a straightforward and trustworthy tool for detecting TB, and its use can assist in lowering the burden of this illness in settings with limited resources. The GeneXpert device can also be used to identify other infectious diseases like HIV, hepatitis C, and gonorrhea in addition to tuberculosis. Additionally, it is utilised to identify COVID-19, a condition caused by the SARS-COV-2 virus. Result of gene expert showing +ve in figure 3 and showing –ve in figure 4.

Particularly in environments with limited resources, the GeneXpert machine has revolutionised the diagnosis of infectious diseases. It has greatly shortened the amount of time needed for diagnosis and increased the precision of the findings. It is an essential tool in the fight against infectious diseases worldwide because of its portability and simplicity of usage.

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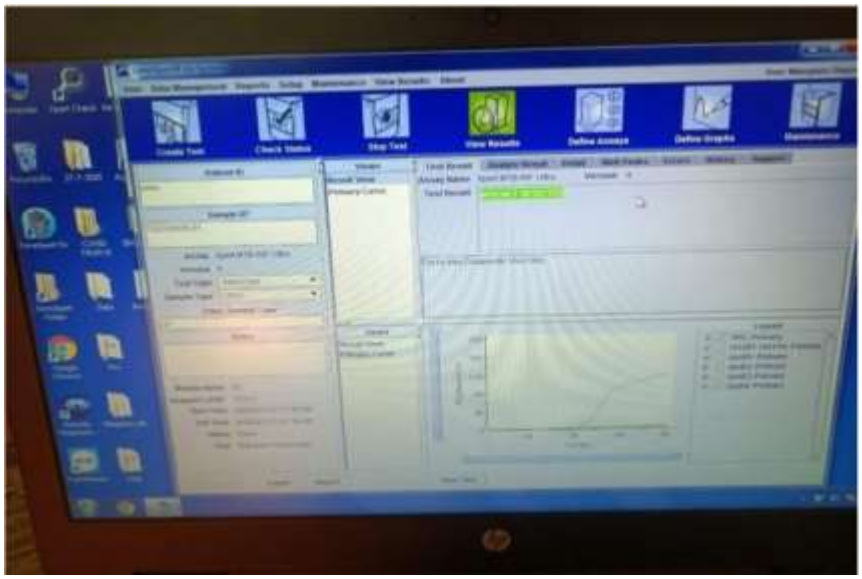

Fig. No. 4. The gene expert's result reveals TB -ve

DISCUSSION:

Healthcare workers utilise the GeneXpert machine test as a diagnostic tool to identify tuberculosis. The GeneXpert TB machine test is used to identify Mycobacterium tuberculosis, the organism that causes tuberculosis. The polymerase chain reaction (PCR) method is used in the test to amplify and find DNA sequences unique to M. tuberculosis. Sputum microscopy is a quick and affordable method for diagnosing TB, although it is frequently inaccurate. When persons are HIV positive, it is especially unreliable. So the GeneXpert machine test is a useful tool for TB diagnosis since it produces prompt and precise findings, frequently in less than two hours.

CONCLUSION:

Clinical diagnostic methods are essential for the timely and precise identification of tuberculosis (TB), a highly contagious illness brought on by Mycobacterium tuberculosis. Various diagnostic methods are available to identify TB infection, Sputum Smear Microscopy, Microscopic Examination, Culture, Nucleic Acid Amplification Tests (NAATs), Molecular Line Probe Assays, Serological Tests, Chest X-rays, Interferon-Gamma Release Assays (IGRAs), GeneXpert MTB/RIF Test. The choice of diagnostic method depends on factors such as the prevalence of TB and drug resistance in the population, the availability of resources, and the clinical context. Combining multiple diagnostic techniques can enhance accuracy and ensure comprehensive patient care. Continuous research and development aim to improve diagnostic tools for more efficient TB detection and control. These techniques aim to detect active TB disease, latent TB infection (LTBI), and drug-resistant TB strains. Using sputum samples, the GeneXpert MTB/RIF Test molecular test directly detects M. tuberculosis and rifampicin resistance. It is rapid and highly sensitive, making it valuable for both TB diagnosis and detection of drug-resistant strains.

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Conflict of interest

The author declares no conflict of interest.

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