International Journal of Biotechnology and Microbiology
Onlien ISSN: 2664-7680; Print ISSN: 2664-7672
Received: 01-11-2018; Accepted: 03-12-2018; Published: 03-01-2019
www.biotechnologyjournals.com
Volume 1; Issue 1; 2019; Page No. 01-04

Identification of mycobacterium tuberculosis in tuberculosis patients

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Abstract
Mycobacterium is the cause of tuberculosis specifically in third world countries. In this recent research some basic techniques have been used to identify the mycobacterium in T.B (Tuberculosis) patients. The mycobacterium is also called acid fast (bacilli). Different techniques were used for diagnosis of TB in the current study. By using these techniques the presence of the AFB (Acid-Fast bacilli) can be identified in the patients that showed the TB like symptoms. These different techniques for the identification of AFB are, LED microscopy auramine staining, Gen expert technique, and culture media. The sensitivity of the LED microscope is higher than light microscope comparable specificity. Result by LED microscope were obtained in crystals like formation of the AFB (Acid-Fast bacilli). The presence of the AFB showed that result is positive for diagnosis of TB. The presence of the AFB shows the patient is suffering from TB. The techniques are very use full in identification of Bacteria as well as diagnosis of TB in patients.

Keywords: tb, mycobacterium, AFB, infectious.

Introduction
Mycobacterium infection spreads through the infectious aerosol particles released from close contact. It is a contagious disease that mostly affects the lungs. Tuberculosis is an infectious bacterial disease that affects the lungs and causes the severe chest pain, fever and coughing [1, 2]. The enigmatic disease, originated from the Latin word which meaning is the rod-shaped bacillus and it was announced by the German microbiologist Robert Koch that Mycobacterium Tuberculosis causes TB in 1882 [2, 7]. The prevalence of TB were seen in young adults, health care workers, people which has weak immune systems as in patients of HIV and smokers [3, 4].
Mycobacterium infection can also spread through the infectious aerosol particles released from close contact [4, 12]. When the MTB (MTB is a new test that is revolutionizing tuberculosis (TB) control by contributing to the rapid diagnosis of TB disease and drug resistance. The test simultaneously detects Mycobacterium tuberculosis complex (MTBC) in less than 2 hours) is entered into the lungs it causes reluctance in the growth of Mycobacterium Tuberculosis, so as a result-bacteria become dormant and this condition is called the latent tuberculosis [5, 6, 8].

Material and Method
The following standard methods were used for AFB (Acid-Fast Bacilli)

1. Auramine staining
The auramine–rhodamine stain is a histological technique used to visualize acid-fast bacilli using fluorescence microscopy, notably species in the Mycobacterium genus. A cid-fast organism displays a reddish-yellow fluorescence.

2. Gen expert diagnostic assay
Gen expert test is a molecular *test* for TB which diagnoses TB by detecting the presence of TB bacteria.

3. Preparation of Lowenstein-Jensen
This media is prepared by 1000 ml of a uniform suspension of fresh eggs under aseptic conditions. Avoid whipping air into suspension during the collection and mixing. Aseptically mix the 1000 ml of egg suspension with 600 ml of the sterile Lowenstein-Jensen Medium cooled to 50 – 60°C, avoiding air bubbles.

4. Homogenized whole eggs
Pasteurized eggs are eggs that have been pasteurized in order to reduce the risk of food-borne.

5. Culture media preparation Nutrient agar
Suspend 28 g of nutrient agar powder in 1 litre of distilled water. Add 13 g of nutrient broth powder to 1 litre of distilled water.

6. Rapid device
Scientists at McGill University's Department of Bioengineering developed a device that allows early and quick detection of bacteria and an antibiotic-resistant bacterial strain

in small samples, potentially allowing clinicians to make life-saving diagnoses

Results

Findings of Auramine Staining

Auramine staining results showed the bright yellow colour colonies of the acid-fast bacilli under the fluorescence microscope and results are shown in fig.1

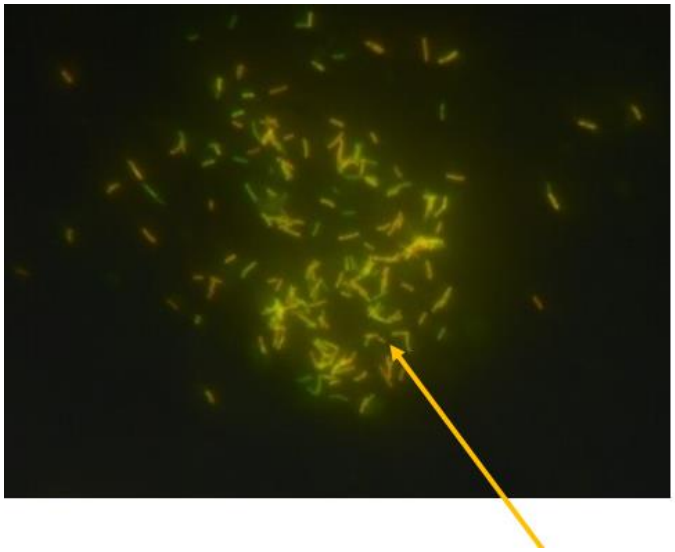

Fig 1: Result of yellow colored colonies of acid-fast bacilli.

Gene Expert results

The result of gene x pert obtained in printable form showed two kinds of results as: MTB (Mycobacterium tuberculosis) detected very low.

RIF (rifampicin) resistance detected.

Results that was positive for MTB and for RIF resistance means that the bacteria had a high probability of resistance to RIF.fig.**2**

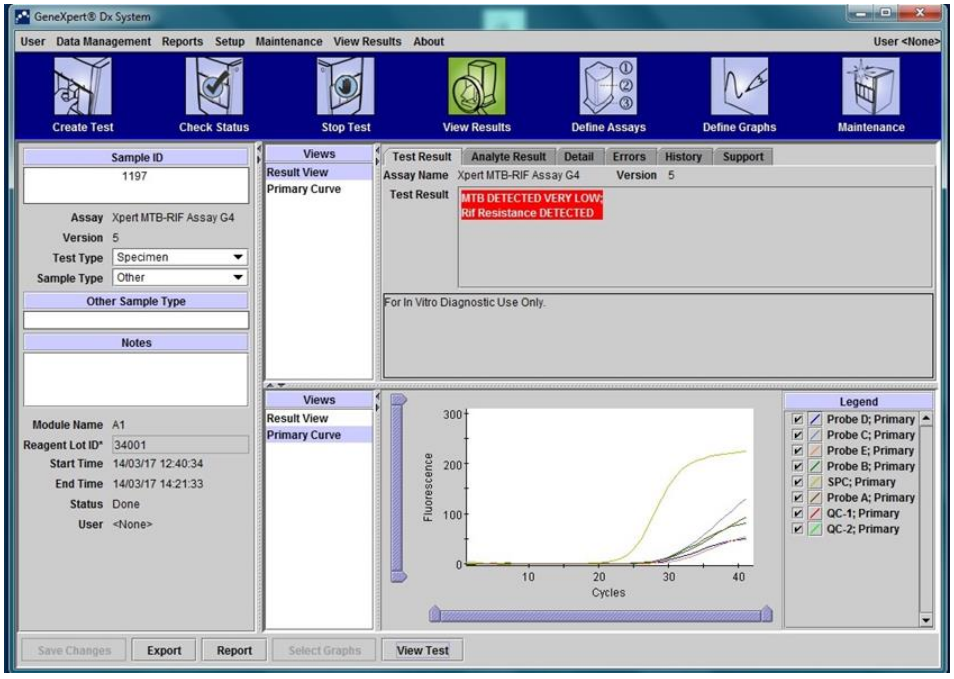

Fig 2: Different hybridization probes are used which detect the bacterial DNA in the sample

Results of Lowenstein-Jensen (LJ) Media Preparation

M. tuberculosis required aerobic condition and a protein enriched medium for culture. Löwenstein–Jensen (LJ) slopes was main solid media used to culture Mycobacteria. It contained inspissated eggs, malachite green and glycerol (or pyruvate). LJ medium contained glycerol which favoured the growth of M. tuberculosis.The prepared media pic is given below in fig.3

Fig 3: Results of prepared Lowenstein-Jensen media slopes.

Results of culture media

M. tuberculosis grew slowly *(generation time of 16 to 24 hours)* and took 3-6 weeks or longer to give visible colonies. It produced raised, dry, cream (buff) colored colonies in Löwenstein–Jensen media and result are in fig.4

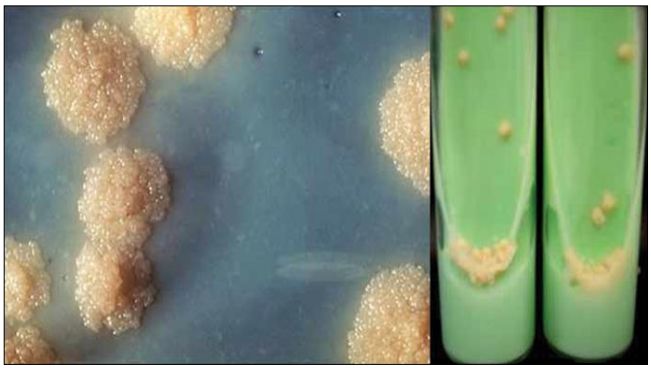

Fig 4: raised, dry, cream (buff) colored coloniesof bacteria on Lowenstein-Jensen media produced.

Results of Rapid device

When serum or plasma applied at side (A), it flew past the antigen line (B). Bound antibody detected which produced one pink line. The test considered positive when at least the antigen line and the control line (C) developed a pink color. Results are in fig.5

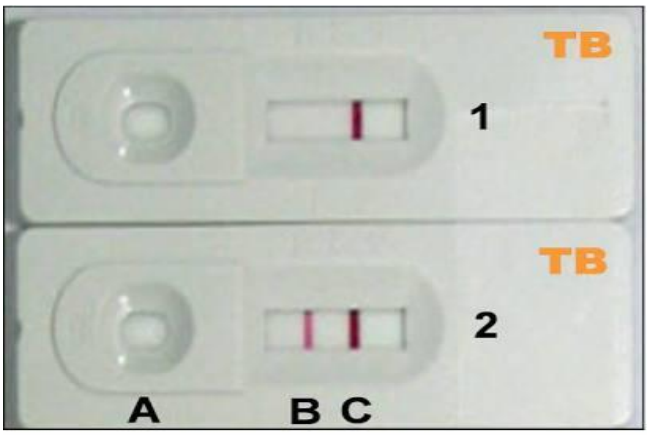

Fig 5: Results of rapid device showed for acid fast bacilli.

Discussion

Different techniques were used for diagnosis of TB in the current study. By using these techniques, the presence of the AFB can be identified in the patients that showed the TB like symptoms. These different techniques for the identification of AFB are, LED microscopy auramine staining, Gen expert technique, and culture media. The sensitivity of the LED microscope is higher than light microscope comparable specificity. Result by LED microscope were obtained in crystals like formation of the AFB. The presence of the AFB showed that result is positive for diagnosis of TB. By counting the numbers of the AFB, the stage of the TB disease is identified. But in severe case the stage of the TB cannot be described by counting the AFB. So then the other techniques are used. Under LED microscope, the absence of crystal like formation of the AFB shows negative result [9,10]. Results of gene expert technique showed that it is significantly more sensitive than other technique and give more specific good results than LED microscopy auramine staining due to its more significant sensitivity. The sensitivity of the genexpert is 130 AFB per ml. Within 1 hour and 45 minute accurate results are obtained in graphical form on the screen of the computer. By reading this graph the diagnosis of the TB can be carried out. [11, 12, 13]. This technique is more better than other two above techniques. Because it gives results within 1 to 45 min. Result showed the formation of colonies of the AFB on the culture media. Rough, circular, irregular colonies formation occurs that shows the positive result in the diagnosis of TB. The sensitivity of the culture media is 10 to 100 AFB per ml [14, 15]. Non tuberculosis mycobacterium isolated during the treatment of pulmonary tuberculosis. Rapid device is also used in TB diagnosis. The light pink colour of the strip that is present on rapid device shows positive result. This device is used to confirm the result is positive or negative that are not tested or confirm by culture media technique.

Conclusions

In our recent study it was concluded that mycobacterium Tuberculosis can easily be identified by using the above-mentioned techniques. Hence these techniques may helpful to diagnose the TB patients and this chronic disease may be eradicated. Diagnosis and appropriate treatment of TB can help in achieving the targets. TB is mainly curable, if it is detected and treated effectively, thus it is highly significant to have robust economically viable diagnosis test. The technique used in current study is used to diagnosis the TB. Appropriate results are obtained by each technique according to its sensitivity.

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