# Current Trend in Diagnosis of Tuberculosis Infection

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## Abstract

Tuberculosis is a major health issue especially in the developing world leading to chronic morbidity. Early diagnosis and successful treatment is paramount not only to avoid further complications but also to contain the spread of infection in the community. This review discusses the various diagnostic methods which include the conventional methods as well as the newer molecular methods along with their advantages and disadvantages. No particular single test can be qualified as sensitive, reliable, and rapid as well as being cost effective. A combination of tests needs to be performed to ensure a rapid and reliable diagnosis that would enable the clinician to administer an effective treatment.

**Keywords:** Tuberculosis, Infectious disease, Polymerase chain reaction (PCR)

## Introduction

Tuberculosis is still a high priority public health issue, becoming one of the main causes of death at global level. According to World Health Organization (WHO), there are approximately 10 million newly reported cases and roughly 2 million deaths are estimated to have taken place in the year 2014, with Asia and Africa being the most burden continent carrying the disease. Tuberculosis are mostly common is developing countries such as Indonesia, India and China [1]. Tuberculosis is often related as poverty disease as its more common in 3rd world countries and linked to financial deprivation. Tuberculosis is an infectious disease commonly caused by *Mycobacterium tuberculosis*. Patients with pulmonary tuberculosis present with chronic cough with excess sputum production, loss of weight and appetite and sometimes haemoptysis. Tuberculosis is divided into 3 types based on the clinical features of presentation which are latent infection where the person is infected with *M. tuberculosis* but do not have tuberculous disease, active tuberculosis where the patient is suffering from the disease and third category is drug-resistant tuberculosis [2]. The risk factors of tuberculosis are decreased immunity, low body weight due to malnutrition, diabetes mellitus, some other chronic lung diseases like silicosis, chronic renal failure, chronic alcoholism certain cancers like Hodgkin lymphoma while the most crucial risk factor is infection with human immunodeficiency virus (HIV), with high proportion of HIV death is attributable to tuberculosis [3]. Tuberculosis can be prevented and treated which makes the prevention cost effective [4]. Rapid diagnosis is an important step for tuberculosis control, this is not only to treat individual but also for public safety. The most commonly used screening method, chest x-ray is not sufficient as it has low specificity in detecting pulmonary tuberculosis [5]. Usually, infected patient who is symptomatic will be sent for microscopic examination of sputum and culture. The culture is the gold standard method for detecting presence of mycobacteria. With culture it is possible to differentiate the various strains of *Mycobacterium* genus. Culture is useful to detect the cases with low bacterial load. Also, further processing the sample for drug sensitivity testing can be done especially when the prevalence of drug resistant strain is on rise. The major drawback of this process is that it takes approximately a month for interpretation as bacteria grow very slowly on solid culture media (4 to 6 weeks). Sputum smears microscopy and special staining (Ziehl-Neelsen) for detection of acid-fast bacilli with light microscope can

be fast, easy and cost-effective method to detect *Mycobacterium tuberculosis*. Moreover, it also diagnoses the most infectious patient with presence of high number of acid-fast bacilli in the sputum. This test can be used in different population and socio-economics people. However, it has low sensitivity when the bacterial load is less. Also, it cannot detect the cases of extra pulmonary tuberculosis [6-8]. Lately, new diagnostic assay has been developed such as molecular and non-molecular technique for early screening of active tuberculosis. This method includes the detection of tuberculosis with and without drug resistance. This method is solely based on the signs and symptoms of tuberculosis [9]. Since tuberculosis is related with the immune system, the immune function detection is done to detect presence of a specific cellular immune response directed towards mycobacterial antigens in the absence of clinical infection. The drawback of this method is during clinical phase, the specific immune response won't give a direct evidence of viable bacilli. The method is composed of *ex vivo* technique where the detection of specific immune response is depending on induction of cytokines upon production of lymphocyte with mycobacterial antigens [10]. Thus, in this review paper, recent diagnosis of tuberculosis is summarized based on the type of tuberculosis, the clinical practice and the future challenge for diagnosis and prevention of tuberculosis will be recommended.

## Discussion about Diagnostic Methods

### *Radiology studies*

*Mycobacterium tuberculosis* cause chronic pulmonary and systemic disease. Pulmonary tuberculosis should be suspected when the patient presents with cough of longer duration with production of sputum, low grade fever, loss of appetite and weight, sometimes with haemoptysis and chest pain. The slender, aerobic, acid fast bacilli spread via respiratory droplets from active cases of pulmonary tuberculosis. The diagnosis of tuberculosis will be confirmed by diagnosing the causative agent with the biological specimen provided. The methods used for detection are radiological studies, culture detection method, molecular testing and immunodiagnostic test. The most common test done for pulmonary tuberculosis is chest x-ray used to view the organs of a person [11]. Although chest x-ray is useful in detecting symptomatic lesions of tuberculosis, but it is not specific and also it does produce false positive results [5]. Thus radiography test cannot be used as an independent testing method and sputum test are usually advised. Chest x-ray is being used as primary diagnostic for pulmonary tuberculosis testing, computed tomography (CT) is usually needed to diagnose small lesion which cannot be detected in chest x-ray. CT scan is efficient tool as it also provides significant information of the lesion especially when plain films are inconclusive. Although, CT can be used to clarify difficult findings, it does not provide information for tuberculosis management thus tuberculosis culture should be done prior to this test [12].

### *Culture methods*

Next up, acid fast bacilli smear microscopy and culture is another important diagnostic tool for tuberculosis detection. The most commonly used method is direct sputum smear microscopy and common in clinical setting [7]. This method requires collection of multiple samples to increase the diagnostic sensitivity. In majority of the cases, 2 samples are good enough to diagnose. This is most commonly used method in developing countries [13]. Ziehl-Neelsen stained smear are prepared from sputum are used for detecting Mycobacteria. This method is also useful when the resource or sample is limited. Also, Ziehl-Neelsen microscopy has high specificity [14]. Light emitting diodes (LED) is being used as an alternative method for conventional microscopy. Based on WHO finding, its proven that LED microscopy has higher sensitivity in results compared to conventional and fluorescence microscopy. Besides LED microscopy is cost effective compared to other methods [15].

However, despite the accuracy of microscopy smear diagnostic, this technique cannot detect drug-resistant strain. For significant drug susceptibility testing, culture confirmation of tuberculosis should be done [16]. Also, growth of tuberculosis bacilli on solid culture media can take 4-6 weeks to grow hence delaying the need of appropriate treatment. Culturing is usually done on egg based medium like Lowenstein-Jensen medium which is a time-consuming method. Various liquid media like Middlebrook 7H9 are used which are significantly faster (usually 10-14 days) in isolating the bacilli. The sensitivity of liquid media is higher compared to Lowenstein-Jensen medium [17].

***Molecular methods***

For the past 10 years, advancement in technology helped in understanding the genetical structure of *mycobacterium*. With this knowledge, gene sequence, gene probe and amplification systems for this disease has been developed [18]. *Mycobacterium* detection can be a time consuming and tedious activity by conventional methods. For rapid testing and high specificity, several type of DNA probe and ribosomal RNA method have been developed [19]. Nucleic acid of the infectious agent like virus or bacteria can be detected by molecular methods. Detection of *M. tuberculosis* can be done by polymerase chain reaction assay by using nucleotide primers to amplify the bacterial DNA fragment. These tests are called Nucleic acid amplification tests (NAATs) These test help in rapid detection of bacilli (within few hours), are highly specific and useful in direct detection of multidrug resistant tuberculosis in specific clinical setting. The sensitivity of these tests varies in smears, such as in negative cases [20,21]. The nucleic acid test principle is based on the detection of nucleic acid of infectious agent. This method differs from others as it detects genetic material instead of antibodies and antigens [22].

Apart from that, Line Prob Assay [LPA] has been developed to check drug susceptibility as this assay allows detection of specific gene marker related to rifampicin resistance (*rpo* B gene) [23]. In line probe assay, extraction of DNA from mycobacterial isolates is done and later polymerase chain reaction (PCR) amplification of resistance determining region of gene is done. This is followed by hybridization of PCR product and lastly development of colorimetric assay to which allow for lines to be seen at the location of the probes [24].

Due to high false negative results of microscopic examination, WHO recommends molecular diagnostic methods when tuberculosis is suspected. The most commercially used will be Xpert MTB/RIF test which is the most efficient and best used when the samples are small in quantity. This test can be used to detect tuberculosis along with rifampicin resistance in 120 minutes directly from the sputum itself. The GeneXpert cartridge is filled with the reagent needed for processing the sample. Then it undergoes DNA extraction, amplification and laser detection of amplified *rpo* B gene target [25,26]. When comparing with smear microscopy, its proven to have both higher specificity and sensitivity on respiratory as well as extrapulmonary specimens. Also, this test allows to identify *Mycobacterium tuberculosis* and mutation related to rifampicin resistance, hence making it a good method for multi-drug resistance strains [27,28]. Since in children, low sample is usually being obtained, the Xpert MTB/RIF test is highly recommended.

The spread of multi drug resistant strains of *mycobacterium* causing tuberculosis has poses a serious challenge. The time required for conventional method to produce results is long and during this period the patient could suffer more [29]. Thus for rifampicin, the colorimetric assay using 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) for drug susceptibility testing is suggested. The sensitivity and specificity of MTT assay for rifampicin were roughly around 90% successful while for isoniazid, the MTT assay had 100% sensitivity and specificity [30]. In short, MTT assay can diagnose resistant and susceptible strains of *Mycobacterium tuberculosis*.

***Immunodiagnostic methods***

By determining patient's immune function can contribute to diagnosis of tuberculosis. The two most common test done is tuberculin skin test (TST) and IFN-y release assay. The interpretation of the test is same for both children and adult [31]. TST test can be inconvenient to interpret as the accuracy of intradermal injection is important. The reading should be done to the nearest millimeter [mm] of the transverse diameter of induration [32]. The induration at TST site is from the migration of mononuclear cells to area of inflammation. Thus, this response can be attributable to *mycobacterium* infection [33]. However; this test cannot be used to distinguish latent Tuberculosis infection and active Tuberculosis disease. Moreover, the size of TST reaction changes depending on the strain and vaccine dose, administration route, age at the time of BCG vaccination and number of BCG doses [34]. There can be false negative results especially in children with LTBI who fail to mount an appropriate delayed-type sensitivity response especially when they are immunosuppressed [35]. In short, there are disadvantages of TST when it comes to specificity and sensitivity [36].

Another commonly used immunoassay is Interferon gamma y release assays (IGRAs). This test is *ex vivo* blood test that diagnoses interferons that are released from the patient's T-lymphocytes after stimulation with antigens that are found in *Mycobacterium tuberculosis* [37]. Same as TST, this test does not differentiate between latent TB infection and active TB disease. This test is performed with both negative and positive control where the QFT (QuantiFERON-TB Gold In-Tube assay) is an ELISA whole blood test. When the TB antigen cut off value is above 0.35 IU then the test is considered positive. There are concerns regarding the reproducibility of results on serial performance [38]. Also serial testing on patient with lesser risk to infection have many times revealed conversion of IGRA end-results to positive while reversion to negative [39]. Most of the time immunoassay is not conclusive due to antibodies and delayed type hypersensitivity response persist after subsidence of clinical disease.

## Conclusion

To conclude, diagnosis of tuberculosis remains a significant problem although there's presence of advanced diagnostic test. The disadvantage and advantage of each diagnostic test are evident, and no test has met the target in both accuracy and reliability. The cost and diagnostic time should also be counted as tuberculosis is more common is developing countries. The invention of new method remains a major challenge for scientist nowadays. For future studies, increased investments are necessary to support biomarker discovery, validation, and translation into clinical tools. Molecular methods are more encouraged such as Xpert MTB/RIF instead of conventional test like sputum and smear microscopy. .

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