



Review Article

A Review on Recent Advancement in Diagnosis of Tuberculosis

Authors

Meenaaz Fatima*, Syeda Sayema Aimen, Niloufer Tarannum,
Adeeba Tahniyath, Firdous Irrum, Summaiya Tahniyath

¹Department of Pharmacy Practice, Shadan College of Pharmacy, Hyderabad, Telangana, India

*Corresponding Author

Meenaaz Fatima

Department of Pharmacy Practice, Shadan College of Pharmacy, Hyderabad, Telangana, India

Abstract

Objective: *The main purpose of this article is to list out the most recently updated diagnostic tools for tuberculosis. Unfortunately, most of the patients are diagnosed after many weeks of infection which may lead to complications and prolongation of standard drug therapy. This might result in failure of adherence and poor health related quality of life. Sometimes, many of them are never diagnosed which results in millions of yearly death worldwide. As it is highly infectious disease, the main target should be to control the disease by developing the highly advanced diagnostic techniques for the early detection and to provide standard appropriate treatment as early as possible. This will help to reduce the transmission risk and improve the health related quality of life.*

Summary: *Since tuberculosis has been a major challenge for the early detection of the Latent Forms of TB (LTBI) and Multi Drug Resistant TB (MDR-TB) and with the high prevalence of tuberculosis in immuno-compromised patients, there is an urgent need for the rapid development and advancement in the diagnostic tools. The sensitivity and specificity of commonly used diagnostic test is limited. Further, it is very difficult to detect the infection at early stage in case of asymptomatic tuberculosis. Inaccurate diagnostic methods results in delayed management of tuberculosis and worsening of the condition. Hence newer strategies have been introduced for the rapid detection of tuberculosis so as to give the appropriate treatment at early stage and to prevent the associated complications. Thus, this article summarizes the recent advancement introduced in the diagnostic tools for the tuberculosis. This article also covers the recent updates which have been introduced for the early diagnosis of tuberculosis.*

Keywords: *Diagnosis, advance techniques, tuberculosis.*

Introduction

The World Health Organization (WHO) Global TB report 2016 states that TB killed 1.4 million people (1.2 to 1.6 million) HIV-negative and 0.39 million (0.32 million to 0.46 million) HIV-positive individuals in 2015. The 30 high TB burden countries accounted for 87% of all

estimated incident cases worldwide. Of these, China, India and Indonesia alone accounted for 45% of global cases in 2015.⁽¹⁾

Microbiological confirmation of childhood TB, unlike in adults, has not been routinely attempted particularly in primary care. This is partly due to the paucibacillary nature of childhood TB, with

most children being smear negative. Microbiological confirmation currently depends on obtaining an adequate sample for acid fast staining, culture and sensitivity.^[2]

An outline of tests used for identification of mycobacteria:⁽³⁾

Mycobacteria are classified as category three pathogens and should be processed in biological safety cabinets (class I or II) with a class III containment facility. The techniques currently utilised for the detection of mycobacterial diseases are:

Direct methods of Microscopy or culture

- Analysis of lipid composition by chromatography
- Mycobacterial antigen detection by monoclonal sera of Mycobacterial speciation by biochemical assays
- Detection of DNA or RNA of mycobacterial origin
- Indirect methods of Detection of IgG or IgM antibodies against mycobacteria cellular immunity via skin tests.

Gas chromatography⁽³⁾

The identification of tuberculostearic acid (TBSA) in clinical samples is an alternative approach available in gas chromatography. TBSA can be detected by gas chromatography-mass spectrometry. It is a cell wall fatty acid of mycobacteria and other actinomycetales such as *Nocardia*. In the diagnosis of TB meningitis, the detection of TBSA in samples of CSF is one of the good rapid approach. However, it requires special equipment and is labour intensive.

Microscopy⁽³⁾

The hallmark of detection is still direct microscopy of ziehl neelson stained slides and identification of cultured mycobacteria by biochemical tests.

Microscopy is the easiest and quickest diagnostic test for the assessment of tuberculosis but has limited sensitivity ($>10^5$ bacteria/ml) and cannot identify bacterial species. Microscopy used in ziehl neelson staining procedure is rapid cheap and easy. The sensitivity varies depending on the

source of sample and the mycobacterium involved. Best results are obtained in respiratory samples. Sensitivity ranges from 46 – 78% and the specificity is 100% centrifugation.

Fluorescent microscopy with light-emitting diodes⁽¹⁾

The quartz-halogen lamps or high-pressure mercury vapour lamps used in the Conventional Fluorescence Microscopy (FM) was expensive, vulnerable and required expert handling. Light-emitting diodes (LEDs) have advantages of FM at peripheral health-care systems as they are more robust, sustainable and user-friendly. In the newer generation fluorescent microscopes, Royal blue colour LED lamps have an extremely long life expectancy (10,000 h vs. 200 for a conventional mercury lamp). FM allows much larger area of the smear to be seen, hence it increases the sensitivity of smear microscopy resulting in more rapid examination of the specimen (up to four times faster), allowing sixty slides to be screened per day as opposed to 25 using the Ziehl–Neelsen (ZN) method. WHO recommended in 2009, the RNTCP has adopted LED microscopy to replace ZN method in its designated microscopy centres (DMCs) across India.

The international guidance on quality assurance for FM does not currently exist and is under development. The approaches of specificity, sensitivity, cost-effectiveness and cost-benefit have not yet been established adequately.

Front-loaded microscopy⁽¹⁾

The WHO definition (2009) of a smear-positive case allows patients to be diagnosed as TB on a single smear. When a patient is presented to the clinic, the first two specimens are collected and examined on the same day in front-loaded microscopy. Patients with negative smears are asked to return with a morning specimen the next day, depending on whether routine services are based on two or three specimens being examined. Thus, TB patients can be offered treatment on the first day they present.

Development of Interferon Gamma Release Assays (IGRAs):^[4]

The skin test of Tuberculin have been used worldwide for more than a century for diagnosing both LTBI and active tuberculosis. This positive TST result is associated with an increased risk for future or current active tuberculosis. However, certain limitations are associated with the use of TSTs. A valid Test of TST is required for proper administration by the Mantoux method with intradermal injection of 0.1mL of tuberculin-purified protein derivative (PPD) into the volar surface of the forearm. In addition, patients must visit to a health-care provider for test reading, and inaccuracies and bias exist in reading the test. Also, contact with nontuberculous mycobacteria or vaccination with Bacille Calmette-Guerin (BCG) results in false positive TSTs, because the TST test material (PPD) contains antigens that are also in BCG and certain nontuberculous mycobacteria.

QFT became the first IGRA approved by FDA in 2001 as an aid for diagnosing *M. tuberculosis* infection. This test used an enzyme-linked immunosorbent assay (ELISA) to measure the amount of IFN- γ released in response to PPD compared with controls. Moreover, QFT specificity was less than that of TST even after the use of *M. avium* antigen as a control for nontuberculous mycobacterial sensitization and also saline as a negative control.

Since 2005 QFT has not been available commercially.

New IGRAs were developed to improve specificity. These IGRAs assess response to synthetic overlapping peptides which represent specific *M.*

Tuberculosis proteins, such as early secretory antigenic target-6 (ESAT-6) and culture filtrate protein 10 (CFP-10). These tuberculosis proteins are present in all *M. tuberculosis* and they stimulate measurable release of IFN- γ in most infected persons, but they are absent from BCG vaccine strains and from most

Nontuberculous mycobacteria. Thus, as test antigens, these proteins offer improved test specificity compared with PPD. However, ESAT-6 and CFP-10 are present in *M. kansasii*, *M. szulgai*, and *M. marinum*, and sensitization to these organisms may also contribute to the release of IFN- γ in response to these antigens and cause false-positive IGRA results. Because ESAT-6 and CFP-10 are recognized by fewer T lymphocytes and stimulate less IFN- γ release compared with PPD, a more sensitive ELISA than was used for QFT is required to measure IFN- γ concentrations and responses to ESAT 6 and CFP-10

Tools for rapidly identifying the species of culture isolates:⁽¹⁾

Most laboratories using liquid cultures including the intermediate reference laboratories (IRLs) in India utilize this antigen detection for rapid identification of *M. tuberculosis* complex in liquid culture as per the WHO recommendation in 2007–2008.

Antigen detection:⁽¹⁾

A glycolipid which is mainly present in the outer cell wall of mycobacterial species is Lipoarabinomannan which is immunogenic and a major virulence factor promoting survival in human host. The test is available as an ELISA or dipstick method with a turnaround time (TAT) of 4–6 h or 20 min depending on the test kit used.

WHO recommends that it should not be used for the diagnosis of TB, except for HIV-positive in-patients with signs and symptoms of TB (pulmonary and/or extrapulmonary) who have a CD4 cell count ≤ 100 cells/ μ L or HIV-positive patients who are seriously ill, regardless of the CD4 count.

•Enzyme Linked Immunosorbent Assay (ELISA) for antigen detection⁽³⁾

The detection of mycobacterial antigens in sputum, CSF and pleural and ascitic fluids can be done by using double antibody sandwich procedures of ELISA. The tests lack specificity because of the use of polyclonal antibodies. The

specificity is increased by the use of monoclonal antibodies.

To capture the antigen in clinical samples, Wade et al (1990) have used rabbit polyclonal antibodies against sonicated extract of *M.tuberculosis*. The captured antigen was revealed by purified anti-*M.tuberculosis* antibody conjugated with horse radish peroxidase.

Antigen with the specificity of 97% and sensitivity of 100% in CSF and body fluids was detected in this test

Molecular detection: Nucleic acid amplification tests:⁽¹⁾

WHO endorsing molecular methods detects the nucleic acid (DNA) of both live and dead bacilli. The United States Food and Drug Administration recommends Nucleic acid amplification tests (NAATs) that were initially used widely, to assist the diagnosis of TB were polymerase chain reaction (PCR). *M. tuberculosis* complex can be rapidly detected and identified is the advantage of these methods; however, Due to issues related to sensitivity and inhibition, MTB cannot be ruled out.

In the diagnosis of tuberculous meningitis, these techniques also have an important role.

Currently, three methods exist; the first two being WHO recommended ⁽¹⁾

1. Cartridge-based NAAT (CB-NAAT)
2. Line probe assay (LPA)
3. Loop-mediated amplification (LAMP).

Cartridge-based nucleic acid amplification test:⁽¹⁾

The CB-NAAT is a semi-quantitative nested real-time PCR, used to detect both MTB and RIF resistance directly from clinical specimens. It is the method recommended by WHO for the diagnosis of both pulmonary and extra pulmonary TB and for diagnosing paediatric TB in 2010. Liquefied sputum is inactivated with a sample reagent which kills over 99.9% of TB bacilli in the specimen, and 2 ml of this material is transferred into a cartridge which is then inserted in the MTB-RIF test platform. Inside the cartridge, the sample

is automatically filled, washed, filtered, by ultrasonic lysis of the filter captured organisms to release the DNA. Specificity is ensured by the use of three specific primers and five unique molecular probes.

Issue that has be taken into consideration while using CB-NAAT is the presence of monoresistance to INH which is not detected in this test. Concerns exist regarding false-positive RIF resistance results; therefore, samples found to be resistant should be confirmed by a second Xpert MTB/RIF test or an LPA and phenotypic culture testing. In case of an indeterminate result on the first specimen, a repeat testing/ re-testing of a new specimen by CBNAAT is necessary, if the result of this is again indeterminate, testing by culture and DST or Line Probe assay is mandated.

Line Probe Assay

This strip test is used to detect TB DNA and genetic mutations that is associated with drug resistance from smear-positive culture isolates or sputum specimens after extraction of DNA and PCR amplification. It is a hybridisation assay that allows differentiation between the *Mycobacterium* species. 27 reaction zones (bands) are present on each strip.⁽¹⁾

The sensitivity of PCR is 91.5% compared to 51% for smear microscopy and 68% for sputum culture. This was because of the fact that PCR could pick up bacterial DNA even from saliva mixed sputum specimens, which are generally considered inappropriate for microbiology. The specificity of PCR (86%) was found to be lower than other diagnostic tests, the reason being the lack of a suitable gold standard to assess its efficiency^[5]

Loop-mediated amplification:⁽¹⁾

LAMP is a simple, rapid, specific and cost-effective nucleic acid amplification method solely developed by Eiken Chemical Co., Ltd, Japan. In this technique, four different primers are used which recognise six distinct regions on the target gene *gyrB* and 16S rRNA. It is a single step process which mainly involves incubation of mixture of sample, primers, DNA polymerase

with strand displacement activity and substrates at a constant temperature (about 65°C). A result can be achieved with in 35 min for a solid medium culture and in 60 min for a liquid medium culture or for a sputum specimen that contains a corresponding amount of DNA available for testing.

Updated WHO policy guidance on TB diagnostics:

A molecular assay based on loop-mediated isothermal amplification (TB-LAMP), Loopamp TM MTBC Detection Kit (Eiken Chemical Company Ltd, Japan) can be used as alternative for microscopy for detection of pulmonary TB in adults with signs and symptoms of TB. TB-LAMP may also be used as a follow-on test to microscopy in adults with signs and symptoms of pulmonary TB, especially when further testing of sputum smear-negative specimens is necessary.⁽⁶⁾

The only WRD currently available for detection of TB and rifampicin resistance is the Xpert MTB/RIF® assay. The original WHO recommendations in 2010 prioritized to consider its use as the initial diagnostic test in individuals suspected to MDR-TB. Used for the diagnosis of TB in children, on selected specimens for the diagnosis of extrapulmonary TB, and for all people suspected of having pulmonary TB as a replacement for microscopy.⁽⁷⁾

Xpert MTB/RIF Assay⁽⁸⁾

The Xpert MTB/RIF assay is a nuclear acid amplification-based test using a cartridge based on the Gene Xpert Instrument System. It is based on PCR which is used to identify DNA sequences specific to the MTB in sputum samples.

According to the WHO in 2013, a Xpert MTB/RIF assay could be used for: an add-on test following microscopic TB examination; a replacement examination for AFB smear microscopy; detection of MTB in both AFB smear-positive and smear-negative culture-positive cases; detection of MTB in pleural in pleural fluid; detection of MTB in lymph node in samples from biopsy or fine-needle aspiration; detection of MTB in gastric fluid; detection of

MTB in samples of cerebrospinal fluid; and detection of MTB in tissue samples.

In 2014, the WHO stated that the Xpert MTB/RIF assay is considered to be the initial diagnostic test in all subjects suspected on having pulmonary TB. The WHO recommends subjects who are at high risk of MDR-TB should always have their sputum checked using the Xpert MTB/RIF test.

Conclusion

The various direct and indirect methods used for the diagnosis of TB, their advancement and their developments have been briefly explained in this article. The hallmark detection method of tuberculosis i.e, the zeihl neelson method is the easiest and the quickest diagnostic tool in the diagnosis of active TB infection. The diagnosis using this method is easy rapid and cheap however less sensitive as compared to various newer and advanced techniques like IGRA and NAAT.

Identification of TBSA using gas chromatography-mass spectrometry is another method that involves intensive labour and special equipments. Conventional fluorescent microscopy has been replaced by LED that are user friendly and easy to handle. It is more sensitive and fast as compared to ZN microscopy and was recommended by WHO in 2009. Patients can be offered treatment on the day they present using front load microscopy. Development of IGRAs is aiding in the diagnosis of both latent and active TB infections.

Assays based on RD1-specific antigens, ESAT-6 or CFP-10, correlate better with intensity of exposure, and therefore are more likely than TST/purified protein derivative (PPD)-based assays to detect LTBI accurately.⁽⁹⁾

Various tools for identifying species of culture isolates have been developed that aid in diagnosis of tuberculosis. Antigen detection and molecular detection techniques have been developed. Molecular detection involves nucleic acid amplification tests (NAAT).

Compared with AFB smear microscopy, the added value of NAA testing is twofold.⁽¹⁰⁾ The three

methods currently used are cartridge based NAAT (CB-NAAT), line probe assay (LPA) and loop mediated amplification (LAMP). Of these CB-NAAT and LPA are WHO recommended. Xpert MTB/RIF assay is another nuclear acid amplification-based test that could be used as an add-on test following microscopic TB examination. WHO stated that the Xpert MTB/RIF assay could be used as the initial diagnostic test in all subjects suspected on having pulmonary TB. Further studies are being carried out to improve the diagnostic techniques for the early detection of TB, reduce its transmission, to provide the treatment as early as possible and to reduce complications that occur due to late diagnosis.

Conflicts of interest: Nil

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Abbreviations

AFB: acid fast bacilli, LAM: lipoarabinomannan, TB-SA: tuberculostearic acid, TB-LAMP: tuberculosis loop mediated amplification, WRD: WHO recommended diagnosis FM: fluorescent microscopy, LTBI: latent tuberculosis infection, ZN: zeihl-neelson, DMCs: designated microscopy centres, TST: tuberculin skin test, PPD: purified protein derivative, IGRA: interferon gamma release assay, QFT: quantiferon test, CFP-10: culture filtrate protein-10, ESAT-6: early secretory antigen target-6, BCG: bacilli calmette-guerin.